FRONTIERS OF NMR IN MOLECULAR BIOLOGY III

Organizers: Thomas L. James, Stephen W. Fesik and Peter E. Wright

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Protein Structure

LZ 001 STRUCTURE-FUNCTION STUDIES OF [2Fe-2S*] FERREDOXINS, John L. Markley,[a,b] Young-Kee Chae,[b] Hong Cheng,[a] Byung-Ha Oh,[a,*] Bin Xia,[b] Bruce L. Jacobson,[c] Hazel M. Holden,[a,b,c] George Reed,[a,b,c] and Larry E. Vickery,[d] [a]Biochemistry Department, [b]Biophysics Program, and [c]Institute for Enzyme Research, University of Wisconsin, Madison, WI 53706 and [d]Department of Biological Chemistry and Department of Physiology and Biophysics, University of California, Irvine, CA 92717.

Site-directed mutagenesis, NMR and EPR spectroscopies, and single-crystal X-ray diffraction are being used in investigations of three ferredoxin (Fd) types: the photosynthetic Fd from the vegetative form of *Anabaena* 7120,[1-6] the nitrogen-fixing Fd from the heterocyst form of *Anabaena* 7120,[7] and the respiratory Fd from human placenta.[8] The goals of these studies are to determine: *(i)* the solution and crystal structures of these proteins, *(ii)* the patterns of electron delocalization in the iron-sulfur clusters, *(iii)* factors that influence the reduction potential of the cluster, and *(iv)* determinants of cluster structure and stability. We have developed procedures for efficient overexpression of all three proteins (from *E. coli*), uniform (^{13}C, ^{15}N) and selective (^{2}H, ^{15}N) isotope labeling, and cluster reconstitution. As a test of the requirements for cluster formation, we are systematically mutating each of the four cluster ligands of the vegetative Fd from Cys to Ser, His, Tyr, Asp, or Ala. X-ray and NMR data will be compared for the wild-type vegetative and heterocyst *Anabaena* Fd's and for mutant C49S of the vegetative Fd. EPR and NMR data will be compared for several mutants including C41S, C46S, and C49S.

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LZ 002 RELAXATION EXPERIMENTS FOR STUDIES OF PROTEIN MOBILITY, Gerhard Wagner, Jeff W. Peng, Kwaku Dayie and Jean-Francois Lefèvre, Harvard Medical School, Boston.

While NMR techniques for elucidation of protein structures is far advanced, methods for studies of protein mobility are rather rudimentary, and our understanding of protein dynamics is based more on simulations than on real experimental data. Relaxation experiments can provide data on both fast and slow internal motions in proteins. There is hope that the fast motions can be compared with results from simulations. Recently, we have developed a strategy for directly mapping the spectral density functions for the rotational diffusive motions of N-H bond vectors in proteins. This strategy relies on measurements of six relaxation parameters for each NH group[1]. Previous strategies for interpretation of relaxation parameters were based on mechanistic models of internal motions, such as the "wobbling in a cone" model[2]. Alternatively, the "model-free approach" was developed[3] which makes, however, the assumption that the autocorrelation function G(t) can be described as a sum of decaying exponentials. As a consequence, the shape of the spectral density function is restricted to a sum of Lorentzians. Such assumptions about mechanistic models or about a functional dependence of $J(\omega)$ on certain parameters were necessary since the relaxation rates depend on five values of the spectral density functions, $J(0)$, $J(\omega_N)$, $J(\omega_H+\omega_N)$, $J(\omega_H)$ and $J(\omega_H-\omega_N)$, while fewer experimental ^{15}N relaxation parameters were measured, such as T_1, T_2 and the heteronuclear NOE. Thus the problem is underdetermined, and model assumptions have to be made. We added some additional experiments to be able to calculate the values of the spectral density functions[1]. These are the longitudinal relaxation rate of N_z, the transverse relaxation rates of in-phase and anti-phase coherence, $N_{x,y}$ and $2N_{x,y}H_z$, the relaxation rate of longitudinal two-spin order, $2N_zH_z$, the heteronuclear cross relaxation rate and the longitudinal relaxation rates of protons. The pulse sequences to measure these parameters for an ^{15}N enriched protein yield 2D ^{15}N-^{1}H correlated spectra in which the peak intensities can be measured as a function of a relaxation delay τ. The analysis includes relaxation by dipole-dipole interaction and CSA relaxation. Cross-correlation between dipole-dipole and CSA is minimized by appropriate decoupling sequences. A complete set of data was recorded for the protein eglin c[4]. Recently, the data set was extended to two other field strengths, and spectral densities were derived. The technique was also applied to other proteins and protein complexes. Other relaxation experiments were carried out to identify slow internal motions. Evidence for such slow motions were found in the DNA binding domain of the transcriptional activator GAL4.

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LZ 003 WHAT DOES NMR TELL US ABOUT PROTEIN STRUCTURES IN SOLUTION? Kurt Wüthrich, Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule-Hönggerberg, CH-8093 Zürich, Switzerland.

The rate at which new three-dimensional protein structures are determined both by NMR spectroscopy in solution and by X-ray diffraction in protein crystals has rapidly increased during the last decade. In 1990 and 1991 alone, over 200 new structures were published (1,2). In parallel, the quality of the structure determinations by both methods has continuously been improved, so that ever more meaningful comparisons at atomic resolution can be made between corresponding protein structures in crystals and in solution. For globular proteins such comparisons reveal close similarity between the molecular architectures in the different environments, but also quite striking differences near the surface in the protein-solvent interface. Important new insights have been obtained from NMR investigations of protein hydration in aqueous solution (3,4). On the one hand, internal water molecules that are shielded from contact with the solvent turn out to be in the same positions in the crystals and in solution, and to be an integral part of the protein architecture in both states. Nonetheless, these water molecules exchange quite rapidly with the bulk water, with upper limits for the life times in the protein hydration sites in the millisecond time range (5). Interestingly, this time scale for exchange of interior waters is comparable to that for aromatic ring flips (6), indicating that ring flips and interior water exchange manifest the same type of structure fluctuations. On the other hand, there are observations of surface hydration water (4) which are perhaps the most clearcut evidence available for important differences between the state of proteins in crystals and in solution.

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Spectral Analysis and Structural Refinement

LZ 004 NEW DEVELOPMENTS IN PROTEIN STRUCTURE REFINEMENT TECHNIQUES, David A. Case, Dept. of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

My talk will deal with methods for determination of high-resolution solution structures from nmr data, and for estimating the level of uncertainty in the results. The general procedure will be illustrated through a review of recent structural work on plastocyanin, a zinc-finger peptide, and myoglobin. I will emphasize techniques that go beyond the "single average structure" model to take some account of internal molecular motion and conformational heterogeneity. Analyses of solvated molecular dynamics simulations have provided new information about the probable inpact of internal motion on proton NOESY spectra. I will describe the approaches that can be used to model internal motion with a relatively small number of adjustable parameters, and illustrate the expected level of agreement with experiment through comparisons with observed NOESY intensities from plastocyanin. A new approach that allows simultaneous refinement into both homonuclear and heteronuclear relaxation data will be described and illustrated with results from a zinc-finger peptide. The talk will conclude with an overview of prospects for improving structure refinement through the inclusion of more experimental data, through more powerful and realistic modelling and optimization protocols, and through the joint refinement of structural and dynamical variables.

LZ 005 EXPLORING THE LIMITS OF PRECISION AND ACCURACY OF PROTEIN STRUCTURES DETERMINED BY NMR SPECTROSCOPY, G. Marius Clore, Mark A Robien and Angela M. Gronenborn, Laboratory of Chemical Physics, Building 2, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892

The effects of the number, precision and accuracy of inter-proton distance restraints, of direct refinement against NOE intensities and of the description of the non-bonded contacts on the precision and accuracy of NMR protein structure determination have been investigated. The model system employed is the 56 residue IgG binding domain of Streptococ-cal protein G for which a very high resolution NMR structure, based on a total of 1058 experimental NMR restraints, is available. It is shown that neglecting the effects of the non-bonded contacts, the maximum ensemble accuracy (as opposed to precision) that can be achieved in practice is 0.25-0.3 Å for backbone atoms. Taking into account effects of non-bonded contacts, the likely limit on accuracy is 0.4-0.6 Å for backbone, 0.8-1.1 Å for all atoms and 0.5-0.8 Å for all ordered atoms.

LZ 006 QUANTITATIVE INFORMATION FROM COMPLICATED NMR SPECTRA OF BIOLOGICAL MACROMOLECULES, Jens J. Led, Henrik Gesmar, Peter F. Nielsen, Helle B. Olsen and Frits Abildgaard, Department of Chemistry, University of Copenhagen, The H.C. Ørsted Institute, Universitetsparken 5, DK-2100 Copenhagen Ø, Denmark.

NMR spectra are, at present, the most important source of information about the three-dimensional structure and the dynamics of bio-macromolecules in solution (1). The quality of the structural and dynamical information derived from the spectra depends, however, on the accuracy by which the parameters (frequencies, linewidths and intensities) that characterize each signal can be determined. Linear Prediction (2) and non-linear Least-Squares (3) analyses of time- and frequency-domain NMR signals can provide these parameters for the individual signals with optimum accuracy, even in case of complicated NMR spectra of biological macromolecules. The application of the two methods for this purpose will be illustrated by a series of examples including a) a quantitative estimation of the exchange rates of slow exchanging amide protons in a protein (e.g. insulin) from a *single* 2D NOE spectrum, b) a quantitative estimation of the intensities of overlapping signals in 2D NMR spectra of proteins and nucleic acids by combining the two methods with an appropriate use of one dimensional row and column intensities, as first suggested by Holak et al. (4), and c) a detailed evaluation of the refolding of a protein (e.g human growth hormone) obtained from a simultaneous analysis of a series of ^{13}C NMR spectra recorded as function of pH. In all cases, the accuracy of the estimations is unaffected by spectral artifacts associated with the discrete Fourier Transformation and depends only on the quality of the experimental data.

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LZ 007 AUTOMATED ANALYSIS OF 3D NMR SPECTRA, Hartmut Oschkinat and David Croft, NMR Group, EMBL, Heidelberg, Germany.

Automated assignment of NMR spectra usually starts with the extraction of a peak list from a spectrum. This puts a heavy emphasis on good peak detection and, once it is done, the original spectrum is lost. In contrast, the manual interpretation of the spectra always involves a cyclic procedure, characterized by a recourse to the original data. The value of a single peak, for example, is always judged by comparing it to the whole spectrum. To approach the human system as closely as possible, two ideas are employed: i) we are searching for *patterns* of peaks, linked to nucleus chemical shift values, as opposed to searching for individual peaks; ii) peaks within a pattern can be detected with maximum accuracy *within the original spectrum*, using masks which have been tuned to select them. This approach has the advantage that all the knowledge of spectral assignment can be applied to the data before they are reduced to peak lists. The use of chemical shifts, for example, to identify amino acid type gram heteronuclear 3D spectra is easily possible. In order to facilitate pattern specification, a simple pattern description language has been written. Examples for different kinds of pattern will be given, e.g. for amino acids in 13C-13C-TOCSY spectra, protein backbone in triple resonance spectra and in TOCSY-NOESY spectra. The program finds patterns of interest by scanning through all possible chemical shift combinations for the nuclei in the user supplied pattern. The chemical shifts for the most strongly responding patterns are recorded. Multiple spectra can be searched simultaneously. Applications to homo- and heteronuclear 3D-spectra are presented.

Nucleic Acid Structure

LZ 008 HIGH-RESOLUTION STRUCTURES OF GENOME TARGETS, Thomas L. James, Karl D. Bishop, Forrest Blocker, William M. Egan, Anil Kumar, He Liu, Anwer Mujeeb, Uli Schmitz, Ingmar Sethson, Nicolai Ulyanov, Klaus Weisz and Yate-Ching Yuan, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446.

Fairly subtle, sequence-dependent structural variations in the DNA double helix guide protein, mutagen or drug recognition. These subtle variations demand detailed knowledge of the structure. Better resolution can be obtained with a greater number of structural constraints, e.g., internuclear distances and torsion angles, and more accurate structural constraints. The MARDIGRAS algorithm is a very efficient iterative relaxation matrix procedure which enables a large number of accurate distances to be calculated from 2D NOE peak intensities, while incorporating the effects of molecular motions and exchange. Taking into account molecular motions, signal-to-noise, exchange and fit of the final converged matrix with the experimental data, MARDIGRAS also yields logical choices for upper and lower distance bounds for each distance. Torsion angle values are obtained by fitting of simulated double-quantum-filtered cross-peaks. These constraints and bounds can be utilized in distance geometry, restrained molecular dynamics (rMD) or restrained Monte Carlo (rMC) calculations to yield structures. Efforts to obtain these structural constraints along with an appropriate assessment of their accuracy and a consideration of conformational flexibility is rewarded with a high-resolution time-averaged structure of the DNA duplex fragment. A number of genomic sequences important in recognition have been studied and their structures determined.

LZ 009 ISOTOPE-LABELLED RNAs FROM HIV-1

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The recent development of methods for the introduction of ^{13}C and ^{15}N labels into *in vitro* transcribed RNAs in this and other laboratories offers a significant improvement in the application of NMR to small and medium-sized RNA structures. Our ongoing studies focus on two RNAs involved in regulation of the HIV-1 life cycle: a 29mer derived from the TAR (*trans*-activator response) element and a 33mer fragment from stem II of the RRE (rev responsive element) sequence. Both uniform and selective labelling by residue type have been employed.

In addition to the new approaches that labelling brings to RNA resonance assignment and structure determination, we are particularly interested in conformational flexibility and the interactions of these RNAs with their cognate ligands. The interactions of labelled TAR fragment with 12-, 26- and 40-mer peptides derived from HIV-1 Tat protein serve as test cases for NMR analysis of RNA complexation. Motional behaviour correlates quite well with the importance of individual TAR residues in protein recognition. With few intermolecular NOEs, and the relative paucity of structurally-informative internal distance markers, it becomes necessary to make greater use of chemical shift changes as an analytical tool. Coordinate analysis of ^{1}H, ^{15}N and ^{13}C chemical shifts offers improved interpretation of ligand-induced conformational changes in flexible RNA regions.

Supported by the NIH, the Welch Foundation and the W.M. Keck Center for Computational Biology.

LZ 010 **Solution Structures of RNA**, Arthur Pardi, Fiona M. Jucker, Pascale Legault and Edward P. Nikonowicz, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-0215.

We have recently developed efficient methods for production of ^{13}C and ^{15}N labelled RNA (1). Having labelled molecules makes it possible to apply 3D and 4D heteronuclear magnetic resonance techniques to RNA structure determinations. Multi-dimensional NMR experiments are revolutionizing the solution structure determinations of proteins and our results show that these techniques will have a similar effect on NMR structure determinations of RNAs. The resolution of the NMR spectrum of an isotopically labelled RNA is enormously improved by application of multi-dimensional heteronuclear magnetic resonance experiments (2,3). These techniques also simplify the structure determination and therefore allow structures of larger molecules to be determined at higher resolution. These advances now make it possible to determine the solution structure of biologically active RNAs. We are currently using multi-dimensional NMR to study the structure of several catalytic RNAs including the hammerhead ribozyme, a lead-dependent self-cleaving ribozyme and the hairpin ribozyme. NMR structural studies are also being performed on RNA folding domains such are RNA hairpins containing tetranucleotide loops. Progress on structure determinations of these systems will be presented.

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New Experimental Developments

LZ 011 RECENT ADVANCES IN THE STUDY OF ISOTOPICALLY ENRICHED PROTEINS, Jacob Anglister, Ad Bax, Frank Delaglio, Stephan Grzesiek and Geerten W. Vuister, Laboratory of Chemical Physics, NIDDK, NIH, Bethesda, Maryland 20892.

A number of recently developed triple resonance experiments can simplify considerably the assignment process of uniformly isotopically enriched proteins. For proteins smaller than ~20 kDa, experiments that J correlate the backbone amide with ^{1}H and ^{13}C resonances of both the intraresidue side chain and the side chain of the preceding residue are feasible. Provided that sample concentrations are at least a few millimolar, many of the experiments can be performed rapidly by using pulsed field gradients. For larger proteins, the relatively small size of the $^{1}J_{NC\alpha}$ coupling makes it impossible to obtain the intraresidue backbone amide to sidechain J connectivities, but interresidue H^{N}/N-sidechain connectivities can be observed for proteins in the 30 kDa range.

The maximum size of proteins that can be studied by isotope-assisted NMR methods is determined primarily by the rotational correlation time which determines the resonance line width, and to a much lesser extent by the spectral complexity. Therefore, proteins that do not exhibit sufficiently short correlation times, despite their relatively small size, can be intractible by the new multi-dimensional NMR methods. Calcineurin B, a calcium binding protein which is part of the calcineurin A/B complex, is one such example. It is demonstrated that the use of mixed solvents can solve the line width problem without significantly changing the structure of the protein, permitting a detailed structural characterization.

Homonuclear and heteronuclear J couplings in proteins contain structurally useful information. The size of these frequently unresolvable couplings sometimes can be measured by E. COSY type techniques. Here an alternative procedure is described which quantitates the size of the J coupling from the relative intensity of observed correlations versus reference correlations. The method is applicable to ^{1}H-^{1}H and long-range ^{1}H-^{15}N, ^{1}H-^{13}C and ^{13}C-^{13}C J couplings and can provide remarkably accurate results that agree well with values predicted on the basis of the protein structure.

LZ 012 GRADIENT-BASED EDITING TECHNIQUES, Ralph E. Hurd[1], Daniel Plant[2] and Boban K. John[2], [1]General Electric Medical Systems, 255 Fourier Ave. Fremont, CA 94539., [2] Bruker Instruments, Inc., 255 Fourier Ave, Fremont, CA 94539.

Magnetic field gradients can be used to eliminate transverse magnetization, encode space, motion, and coherence order. All four of these basic gradient applications can be used to improve existing high resolution NMR methods. Coherence order selection has been used to eliminate phase cycling, reduce artifacts, and avoid signal loss associated with traditional water elimination methods. Differences between the diffusional motion of solvent and sample has been used as a basis for lineshape independent water elimination, regions of RF-coil inhomogeneity have been avoided using spatially selective methods, and spoiler gradients have been used to eliminate unwanted, selectively generated transverse magnetization. These NMR gradient methods have been used separately and in combination to improve the pulse sequences used in high resolution NMR spectroscopy.

There are both compelling benefits and trade-offs when using gradients in NMR. This talk will focus on recent developments in B_0-field gradient methods, and the applicability of these techniques to biomolecular NMR.

LZ 013 FREQUENCY SELECTIVE SHAPED PULSES IN PROTEIN NMR SPECTROSCOPY, Mark A. McCoy, Eastman Kodak Company, Life Sciences Research Laboratory, Rochester NY 14650 - 02158.

Double and triple resonance heteronuclear correlation experiments, made possible by the incorporation of ^{15}N and ^{13}C labels, have proven extraordinarily powerful in the determination of protein structures. In an attempt to simplify, shorten or otherwise improve these experiments selective and semi-selective pulses are used for coherence pathway selection and refocusing of heteronuclear scalar interactions during indirect evolution periods. There can be, however, severe consequences to the arbitrary use of shaped pulses for purposes for which they are not intended. Shaped pulses are typically optimized to perform net 90° or 180° rotations of equilibrium magnetization. If the off resonance magnetization is not at equilibrium, deviations in the expected frequency selectivity of the pulse occurs and can be quantified in terms of nonresonant rotations. Due to the complexity of most heteronuclear correlation experiments, selective pulses are almost always applied to nonequilibrium spin states and therefore nonresonant effects become important to understand and compensate. We discuss the nature of these effects, their consequences (phase shifts and loss of signal) and their nearly complete compensation.

In addition to selective excitation and refocusing, we have recently developed a series of selective decoupling sequences in which shaped pulses perform selective rotations within the framework developed for broadband heteronuclear composite pulse decoupling. In contrast with the use of selective refocusing pulses, the implementation of selective decoupling sequences is straightforward and the symmetry of their construction compensates for rf inhomogeneity. As with single shaped pulses, shaped decoupling sequences are designed with different sets of criteria depending upon their ultimate application. We discuss the substantial differences between selective decoupling sequences such as SEDUCE, SINC and G3 and compare their performance with low power broadband sequences for different applications in selective homo- and heteronuclear decoupling. Selective decoupling sequences are also prone to nonresonant effects including "coherence quenching", but this only occurs in special cases and, as with single selective pulses, can be easily compensated. After they have been thoughtfully implemented, shaped decoupling sequences are found to be useful in a variety of applications including carbonyl decoupling during the evolution of C_α coherence, C_α decoupling during the measurement of coupling constants and "total decoupling" where all hetero- and homonuclear couplings (C=O and C_β) are removed during C_α evolution.

Pharmaceutical Applications (Joint)

LZ 014 NMR STUDIES OF RAS P21 AND HIV PROTEASE, Sharon Campbell-Burk[1], Rich DeLoskey[1], Tom Van Aken[1], Peter Domaille[1], P. Weber[1], M. Hillman[1], Richard Yates[1], E. Laue[2], [1]Du Pont Merck Pharmaceutical Company, Wilmington, DE and [2]Cambride University, Cambridge, England.

We have been involved in NMR efforts directed toward understanding the structural and dynamic basis for protein-antagonist interactions in two systems: ras p21 and HIV protease. Both proteins are of wide spread pharmaceutical interest. The c-H-ras p21 protein is the product of the human ras proto-oncogene and is amongst the most prevalent of all oncogenes found in human tumors. HIV-1, on the other hand, is an aspartyl-protease responsible for proteolytic processing of gag and gag-pol fusion protein which is essential for HIV infectivity. Both systems have proven to be tractable NMR problems after considerable optimization. NMR progress on these systems and efforts involved in expression, purification, refolding, concentration, solubility as well as genetic engineering will be discussed.

LZ 015 HIGH RESOLUTION STRUCTURE, DYNAMICS AND FUNCTION OF THE GRAMICIDIN A CHANNEL, Timothy A. Cross, Christopher L. North, Steven M. Pascal, Randal R. Ketchem, Weidong Hu and Kwun-Chi Lee, Institute of Molecular Biophysics and Department of Chemistry, Florida State University, Tallahassee, FL 32306

The function of the gramicidin A dimer in a lipid bilayer is to selectively conduct monovalent cations. However, the atomic details of this function remain mysterious. The extent of cation solvation by the polypeptide backbone, which lines the channel, is undetermined. While the cations are in the channel they are largely stripped of their waters of hydration, however the carbonyl oxygens can play a solvating role. The location of the carbonyl oxygens relative to the channel axis is dependent upon knowing, not only the Φ and Ψ torsion angles, but also the ω torsion angle in the polypeptide backbone. Local dynamics of these carbonyl oxygens may also be critical for the functioning of the channel. For this it is important to develop a model for the local motions, rather than assuming a general model, and then solve for the local motional frequencies. Solid state NMR of isotopically labeled gramicidin channels in oriented bilayers are being used to determine a detailed model for the molecular motions of the polypeptide backbone. The axis about which local motions occur has been determined to be coincident with the axis joining adjacent alpha carbons and the amplitude which varies from one peptide plane to another has an RMSD of 12 to 17°. Furthermore, through ^2H lineshape analyses it has been clearly shown that the global correlation time is in the μsec timescale. In light of this motionally averaged model and the global correlation time it has been possible to analyze the relaxation data for ^{15}N sites at different field strengths and to show that the frequency for these local motions is in the nanosecond timescale. This raises the exciting possibility that the dynamics of the polypeptide backbone and the kinetic rate for cation transit which also occurs on the nanosecond timescale may be correlated.

Another functional question is centered on the role of the four indole rings in each gramicidin monomer. When these indole groups are replaced with phenyl groups the conductance decreases by a factor of 20. Through solid state NMR studies the orientation of the indole dipole moments have been determined and their potential import for conductance will be discussed.

LZ 016 MULTIDIMENSIONAL NMR STUDIES OF IMMUNOSUPPRESSANTS AND THEIR BINDING PROTEINS.

Stephen Fesik, Andrew Petros, Tim Logan, Liping Yu, Robert Xu, Yves Theriault, David Nettesheim, Robert Meadows, Edward Olejniczak, Jay Luly, Thomas Holzman, Earl Gubbins, and Robert Simmer. Abbott Laboratories, Pharmaceutical Discovery Division, Abbott Park, IL 60064.

Using isotope-edited and isotope-filtered NMR techniques, the conformation and active site environment of protein-bound ligands can be rapidly determined. In addition, due to recent advances in heteronuclear three- and four-dimensional NMR methods, virtually complete ^{1}H, ^{13}C, and ^{15}N assignments and high resolution three-dimensional structures of protein/ligand complexes can be obtained. In principle, this type of structural information on a small molecule bound to its target site could aid in the design of analogs which are more suitable as pharmaceutical agents than the inital lead compounds. In order to aid in the design of improved immunosuppressants, we have been studying the three-dimensional structures of several immunosuppressants bound to their target proteins by NMR spectroscopy. Cyclosporin A (CsA) and FK506, two structurally different immunosuppressants, bind to two different target proteins. CsA binds to cyclophilin (165 aa); whereas, FK506 and the structurally related immunosuppressants, ascomycin and rapamycin, bind to FKBP (107 aa). Both proteins are peptidyl-prolyl cis-trans-isomerases, and both play an important role in T cell activation. In this presentation, the conformation and active site environment of several protein-bound immunosuppressants determined from isotope-filtered NMR experiments will be described. This information is used to explain the structure/activity relationships observed for these ligands. In addition, high resolution structures of the ascomycin/FKBP and CsA/cyclophilin complexes determined from a quantitative analysis of heteronuclear 3D and 4D NOE data will be presented. Our attempts to use these 3D structures in the design of novel immunosuppressants will be discussed.

LZ 017 STRUCTURE OF HNRNP C PROTEIN AND ITS COMPLEXES TO RNA OLIGONUCLEOTIDES,

Luciano Mueller[1], Michael Wittekind[1], Mark S. Friedrichs[1], Donna Bassolino[1], Matthias Görlach[2], Gideon Dreyfuss[2], [1]Bristol-Myers Squibb Pharmaceutical Research Institute, 4000, Princeton, NJ 08543-4000, [2]Howard Hughes Medical Institute and Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104-6148.

The hnRNP C1 and C2 are abundant nuclear proteins that bind to heterogeneous nuclear RNAs and appear involved with pre-mRNA processing. We have studied the RNA-binding domain of hnRNP C protein, which contains ninety three amino acid residues. Sequential resonance assignments were performed using heteronuclear multidimensional NMR methods using uniformly [^{15}N] and [^{15}N, ^{13}C]- enriched samples at a molar concentration of 2.5 mM. Distance constraints for structure calculations were extracted from three- and four-dimensional NOESY spectra by manual and automated procedures. Homo- and heteronuclear vicinal J-couplings provided torsion angle constraints. These couplings were obtained with various heteronuclear methods. A valuable source for inter proton J-coupling information were ^{13}C-correlated proton TOCSY spectra. The protein has a compact folded structure (babbab) with the RNA-binding pocket formed by the four-stranded anti-parallel b-sheet. Besides the free protein, we also characterized its complexes to putative target molecules i.e. RNA oligo nucleotides which contain a segment of multiple uracil residues. Our observations suggest that in addition to, the beta sheet structure of the domain,both the flexible N- and C- terminal ends of the peptides are also involved in the RNA binding.

Nucleic Acid Complexes

LZ 018 STRUCTURE AND NUCLEIC ACID INTERACTIVE PROPERTIES OF THE HIV-1 NUCLEOCAPSID PROTEIN,

Michael F. Summers, Terri L. South, Brian Lee, and Paul R. Blake, Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, Maryland 21228.

The nucleic acid interactive properties of a synthetic peptide with sequence of the N-terminal CCHC zinc finger (CCHC = Cys-X_2-Cys-X_4-His-X_4-Cys; X = variable amino acid) of the HIV-1 nucleocapsid protein, Zn(HIV1-F1), have been studied by ^{1}H NMR spectroscopy. Titration of Zn(HIV1-F1) with oligodeoxyribonucleic acids containing different nucleotide sequences reveal, for the first time, sequence-dependent binding that requires the presence of at least one guanosine residue for tight complex formation. The dynamics of complex formation are sensitive to the nature of the residues adjacent to guanosine, with residues on the 3' side of guanosine having the largest influence. An oligodeoxyribonucleotide with sequence corresponding to a portion of the HIV-1 psi-packaging signal, d(ACGCC), forms a relatively tight complex with Zn(HIV1-F1) ($K_{dissoc} = 5 \times 10^{-6}$ M). Two-dimensional nuclear Overhauser effect (NOESY) data indicate that the bound nucleic acid exists predominantly in a single-stranded, A-helical conformation, and the presence of more than a dozen intermolecular NOE cross peaks enabled three-dimensional modeling of the complex. The nucleic acid binds within a hydrophobic cleft on the peptide surface. This hydrophobic cleft is defined by the side chains of residues Val^1, Phe^4, Ile^{12} and Ala^{13}. Backbone amide protons of Phe^4 and Ala^{13} and the backbone carbonyl oxygen of Lys^2 that lie within this cleft appear to form hydrogen bonds with the guanosine O6 and N1H atoms, respectively. In addition, the positively charged side chain of Arg^{14} is ideally positioned for electrostatic interactions with the phosphodiester backbone of the nucleic acid. The structural findings provide a rationalization for the general conservation of these hydrophobic and basic residues in CCHC zinc fingers, and are consistent with site-directed mutagenesis results that implicate these residues as direct participants in viral genome recognition. Ejection of zinc by novel antiviral agents leads to loss of high-affinity nucleotide binding, providing insights into the mechanism of action of these potential chemotherapeutic agents.

LZ 019 SINGLE STRAND DNA BINDING PROTEINS, F.J.M. van de Ven[1], P.J.M. Folkers[1], B.J.M. Harmsen[1], R.N.H. Konings[1], M. Nilges[2] and C.W. Hilbers[1], [1]University of Nijmegen, The Netherlands, [2]E.M.B.L., Heidelberg, Germany.

The M13 bacteriophage genome encodes for the ssDNA binding proteins G5P (gene 5 protein) and G8P. During the phage life-cycle the G5P coats the viral ssDNA in the cytoplasm, thus preventing replication, while G8P resides as an integral plasma membrane protein. Upon virus production the G5P is stripped off and replaced by G8P which then becomes the major coat protein of the phage.
We are studying structure and dynamics of these two proteins, in relation to their biological activity, by means of high resolution NMR. Here we report the assignment (^1H, ^{15}N, ^{13}C) of the NMR spectra, and the 3D structures of G5P in aqueous solution and G8P in detergent micelles, based on NOE data. Since G5P is a symmetric dimer special care had to be taken to discriminate between intra- and inter-monomer NOEs. To this end we employed both experimental techniques, via ^{13}C labelling, and computational methods. The interaction between G5P and DNA, and between G8P and lipids, has been investigated using spin-label probes.

Biomolecules Posing Special Problems

LZ 020 THE ROLES OF A CENTRAL ALPHA-HELIX IN THE FOLDING AND DYNAMICS OF T4 LYSOZYME, Jirong Lu, Victoria Feher, D. Eric Anderson and F. W. Dahlquist, Institute of Molecular Biology, University of Oregon, Eugene, Oregon.

The most buried α-helix of T4 lysozyme (residues 95-106) plays a central role in establishing the hydrophobic core of the C-terminal domain of the protein. Work in Brian Matthews' laboratory has shown that alanine substitutions for large side chains in this helix produce cavities (1,2) or crevices (E.P. Baldwin, unpublished) that bind hydrophobic ligands such as benzene with dissociation constants in the millimolar range. We have shown that the cavity formed in the mutant L99A is readily available to benzene despite the presence of at least 7Å of protein that shields the cavity from solvent. The pathway of benzene access to the cavity and implications concerning protein dynamics will be discussed.

As revealed by ^1H-^{15}N 2D NMR, combined with pulsed hydrogen exchange methods, the residues of the buried helix also form a major portion of the structured residues at early times in the folding pathway. Mutational analysis combined with pulsed hydrogen exchange measurements shows that tertiary interactions may be formed at these early times in the folding pathway. The implications of these results for the mechanism of folding of this protein will be discussed.

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Conformation and Biological Activity (Joint)

LZ 021 NEW HETERONUCLEAR NMR TECHNIQUES FOR THE DETERMINATION OF STRUCTURE AND DYNAMICS OF PEPTIDES AND PROTEINS - CONSEQUENCES FOR DRUG DESIGN, Horst Kessler, Jochen Balbach, Gerhard Müller, Dale F. Mierke, Peter Schmieder, and Stephan Seip, Organisch-Chemisches Institut, Technische Universität München, W-8046 Garching, Germany.

Surface interactions of peptides and proteins may strongly determine the molecular conformation and dynamics. This is especially important in small cyclic peptides. These effects can be studied by NMR using different solvents and performing restrained and free molecular dynamics with inclusion of the solvent. In this way the softness or rigidity within a peptide can be studied. More experimental access to conformational flexibility is obtained from homo- and heteronuclear coupling constants. For small to medium size peptides new techniques are developed to determine heteronuclear long range couplings $^3J_{CH}$, $^3J_{NH}$ with heteroatoms in natural abundance. For very large proteins, new heteronuclear techniques will be presented to determine $^3J_{HNC\alpha H}$, $^3J_{HNC\beta}$, $^3J_{NHC'}$ in uniformely labeled (^{15}N, ^{13}C) molecules. The usefulness of these techniques will be described on the 31 kDa homodimeric P13 domaine of mannose permease. For inclusion of coupling constants in MD calculations a energy penalty function is used directly based on the Karplus equations for the different coupling constants. This excludes problems arriving from the ambiguities from up to four bond angles for one coupling constants. In cases where the conformation cannot be described by one predominant conformer the method of time dependent J-restraints is used similar to the procedure previously developed for NOE derived distance restraints. Knowledge of softness and flexibility can be used to interprete selectivity and activity of cyclic RGD containing peptidic inhibitors of cell-cell recognition. In this case driven MD is used to explain the distinct differences in bioactivity and to prove the induced fit model.

LZ 022 TERNARY PEPTIDE COMPLEXES MIMIC CALCIUM SENSITIVE INTERACTIONS IN MUSCLE, Gary S. Shaw, Carolyn M. Slupsky, A. Patricia Campbell, Wendy A. Findlay, Stéphane M. Gagné, and Brian D. Sykes, Department of Biochemistry and MRC Group in Protein Structure and Function, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

In the thin filament of skeletal muscle, the binding of calcium to the calcium-binding protein troponin-C (TnC) induces a conformational change in the protein which alters its interaction with the inhibitory protein troponin-I (TnI) and ultimately leads to muscle contraction. We have focussed on several aspects of this calcium triggering including the structures of the calcium binding domains of TnC and of intact TnC, the nature of the conformational change that occurs in TnC upon calcium binding, and the structure and location of TnI binding to TnC. To accomplish this we have studied peptide fragments and domains of TnC and TnI along with the intact TnC, and binary and ternary complexes of them. These include SCIII and SCIV [34 residue peptides representing sites III and IV from the C-domain of TnC], TR_1C [a tryptic fragment (12-87) containing sites I and II from the N-domain of TnC], N-domain [a cloned fragment (1-90) containing the whole N-domain], and TnIp [the minimum inhibitory fragment (104-115) of TnI]. Both the N-domain and TnC have been cloned and expressed in *E. coli* so they have been ^{13}C and ^{15}N enriched.

Two dimensional ^1H NMR techniques have been used to show that the calcium bound forms of SCIII and SCIV each form symmetric two-site homodimers, while an equimolar mixture of SCIII and SCIV preferentially and stoichiometrically forms heterodimers. The structures of these dimers are similar to the C-domain of TnC. However, the SCIII/SCIV heterodimer is significantly more stable than the SCIII homodimer by approximately 6 kJ/mol and about 16 kJ/mol more stable than the SCIV homodimer. This is in agreement with calcium titration experiments which show that these dimers have very different calcium binding properties; SCIII/SCIV has two "high" affinity sites, the SCIII homodimer has one "high" and one "low" affinity site and the SCIV homodimer binds calcium with low affinity. It is also noteworthy that site IV is more negative overall than site III and this may influence calcium-binding to the second site. For the SCIII dimer, the binding of a second calcium may be inhibited due to a more rigid symmetric arrangement of hydrophobics at the dimer interface.

The structure of the apo TR_1C fragment of TnC has been determined using standard 2D 1H NMR techniques. The structure is similar to the N-domain in the X-ray structure and shows the lack of contacts of the C-helix with the A, B and D helices of the site I·II domain. This is the same helix that carries the extra negative charge in the III·IV heterodimer and may be crucial in metal ion binding.

The calcium saturated forms of the N-domain and intact TnC are being studied by triple resonance 3D experimentals of the type developed by Bax and co-workers. These structures will provide us with the structure of the Ca saturated state and information about domain-domain interactions.

The structure of the TnIp inhibitory peptide when bound to calcium-saturated TnC has been previously using the intramolecular transferred nOe approach [*J. Mol. Biol.* 222:405-421 (1991)]. We present evidence for the binding of this peptide to the hydrophobic pocket of the C-terminal domain of TnC, obtained from measurement of intermolecular transferred nOe's between TnC and TnIp, and observation of the perturbation of the chemical shifts of TnC residues upon TnIp binding. In particular we demonstrate that the interaction of TnIp with the C-terminal domain of TnC is identical with its interaction with the III·IV heterodimer.

Dynamics/Multiple Conformations

LZ 023 SELECTIVE MEASUREMENT OF PROTON RELAXATION IN BIOLOGICAL MACROMOLECULES, Benoit Boulat, Catherine Zwahlen, Sébastien Vincent, Stelian Nicula, Irene Burghardt, Robert Konrat, and Geoffrey Bodenhausen, Section de Chimie, Université de Lausanne, Rue de la Barre 2, CH-1005 Lausanne, Switzerland.

A variety of novel experiments are described (1-5) which make it possible to measure relaxation rates of protons in crowded NMR spectra of macromolecules such as proteins: longitudinal spin-lattice relaxation rates, transverse relaxation rates measured under conditions of free precession, transverse relaxation rates measured under conditions of spin-locking, transverse relaxation rates of double- and zero-quantum coherences. A "synchronous nutation" experiment is presented which is designed to allow one to measure the cross-relaxation rate (Overhauser effect) between two selected protons in a macromolecule, in such a manner that spin-diffusion effects involving other protons are excluded. Because of extensive overlap of proton signals in NMR spectra of biological macromolecules, it is usually necessary to combine the experimental schemes with one or several magnetization transfer steps using the doubly-selective homonuclear Hartmann-Hahn method.(1) Numerous variants of the basic methods may be conceived, depending on the extent of signal overlap and on the topology of the networks of scalar couplings through which the magnetization can be transferred. Applications are shown to the ε and δ protons of tyrosine-23, to the α, β and β' protons of cysteine-30, and to the α and β protons of alanine-24 in bovine pancreatic trypsine inhibitor (BPTI).

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LZ 024 NMR INVESTIGATIONS OF THE STRUCTURE AND DYNAMICS OF MYOGLOBIN, Mark Chiu[1], Stephen G. Sligar[1], Krishnakumar Rajarathnam [2], and Gerd N. La Mar [2], [1] University of Illinois, Urbana - Champaign and [2] University of California, Davis.

Sperm whale myoglobin (Mb) is used as a model system to examine protein - ligand interactions. The ability to generate a variety of point mutations can help develop and test new NMR probes of Mb structure and dynamics. Specifically, the role of Mb's amino acids on the stability of heme-bound water and the orientation of the heme-bound cyanide ligand have been determined. The ^1H NMR spectra of a series of high spin ferric Mbs have been analyzed to develop structural probes for the heme iron ligation state. These spectral probes help elucidate the role of the distal residues in modulating H_2O coordination in a series of E7 and E11 point mutants of sperm whale Mb. It is concluded that the hydrogen bonding by the E7 residue is the strongest but not the only influence on H_2O coordination. The magnetic axes of the wild-type metMbCN complex can be determined by using a method that utilized the crystal coordinates of the native protein for that portion of the molecule unperturbed by the point mutations and the experimental dipolar NMR chemical shifts for protons in the proximal and distal sides of the heme. The magnetic axes are calculated for a strongly perturbed (His E7 Gly) and a minimally perturbed (Arg CD3 Gly) sperm whale Mb point mutants. For the E7 Gly Mb mutant, the major magnetic axis tilt is minimally altered, but the projection of the tilt is rotated by ~45°. The CD3 Gly Mb mutant yielded a magnetic axes orientation within the range defined by the data sets of the wild-type metMbCN. Assuming that the orientation of the magnetic axes of the wild-type metMbCN can be related to the distal steric tilt of the isostructural Fe-CO unit in native MbCO, the demonstration that the magnetic axes obtained for Mb point mutants can help elucidate the effect of protein steric constraints on heme bound ligands in a variety of low spin heme proteins.

LZ 025 PROTON EXCHANGE in NUCLEIC ACIDS and their COMPLEXES, *Maurice Guéron, Ali Kettani & Jean-Louis Leroy, Groupe de Biophysique, Ecole Polytechnique & URA CNRS D1254, 91128 Palaiseau, France.*

The NMR measurement of *imino proton exchange* has provided information on *internal motions* and, indirectly, on *structural properties* of nucleic acids. This includes the concept of independent base-pair opening and the determination of its kinetics (base-pair lifetimes in the 10 ms range, with activation energies of 40-80 kJ/mole) and thermodynamics ($K_{diss} \approx 10^{-5}$) in B-DNA. Or, on the basis of its characteristic base-pair opening kinetics, the determination of sequence requirements for the formation of the B'-DNA structure which gives rise to DNA bending, and its cooperative properties (1). Or, more recently, the analysis of exchange of the imino proton of *acidic cytidine* in $C^+.C$ and $C^+.G$ pairs, which is quite different from that found in Watson-Crick pairs, but still proceeds from the general theory.

In contrast, and except for one feature derived from the documented process of *internal exchange catalysis*, the *open state geometry* remains unknown. We try to approach it with systems presenting obstacles to base-pair opening, such as *DNA-drug complexes*, where the drug can block the minor groove and/or strain the DNA backbone by intercalation, or *triple-stranded nucleic acids*, where the third strand occupies the major groove. Most drugs slow down base-pair opening, but usually by less than a factor of 10^2. We interpret this as an indication that *opening is not* towards the minor groove, and that it is not primarily dependent on backbone distortions. A remarkable exception is that of *bis-intercalating drugs*, such as luzopeptin which clasps two base pairs between the intercalating moieties: the opening rate of the tethered pairs is slowed down by four powers of 10, whereas the base pairs immediately beyond the clasp are hardly affected.

Amino protons are an integral part of the Watson-Crick base pairs and of other base associations. Their exchange processes have been less explored than those of the imino protons, because of difficulties related to their *high pK* and inconvenient spectral characteristics, to the *rotation of the amino group* and to the possibility of exchange from the closed base pair. But the properties of amino-proton exchange in Z-DNA (2), in triple-stranded DNA and in the base pairs of the acid form of $d(C)_n$ oligomers suggest that their study might prove rewarding. We have now determined all the exchange processes of the amino protons in the A, C and G monomers, as well as the rotation rate of the amino group, between pH 0 and pH 10. By comparison, information is obtained concerning exchange and rotation in the polymers such as B-DNA, triple-stranded DNA, and $d(C)_n$.

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LZ 026 ACCOUNTING FOR MOBILITY IN STRUCTURE DETERMINATION, Wilfred F. van Gunsteren, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland.

During the last decade it has become possible to derive the spatial structure of small proteins and polypeptides in solution using multi-dimensional NMR experiments and model building techniques. The NMR experiments generate different types of data: NOE intensities, J-coupling constants and chemical shifts. These data are used in a penalty function which is added to the physical interaction function that is used in molecular dynamics simulations of the molecular system of interest.

The application of time-dependent distance, J-coupling constant or chemical shift restraints will be discussed. Besides, the quality of the physical interaction function and computational procedure will be assessed by a comparison of simulated (without penalty function) with NMR or X-ray results.

Biomolecular Interactions/Complexes (Joint)

LZ 027 EXAMINING THE CONFORMATIONAL DIFFERENCES OF PEPTIDES IN SOLUTION AND IN PEPTIDE/PROTEIN COMPLEXES, Victor J. Hruby[1], Klaas Hallenga[2], Patricia Hill[1], N.R. Nirmala[2], G. Lippens[2], and K.C. Russell[1], [1]Department of Chemistry, University of Arizona, Tucson, AZ 85721, U.S.A. [2]University Libre de Bruxelles, Avenue Paul Heger, P2-CP 160, B-1050 Brussels, Belgium.

The binding of peptide ligands to biologically relevant proteins such as receptors, enzymes, antibodies, carrier proteins and other acceptors is of critical importance to many biological processes. It is believed that in general both the three dimensional structure of the peptide and of the receptor (acceptor) is changed as a results of this interaction. These changes often are important for transforming the protein into its "active" conformation. Thus an understanding of the nature of these interactions is central to any effort to understand these processes, and to develop a rational approach to peptide hormone, neurotransmitter, substrate, etc. ligand design. Oxytocin, H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2, a peptide hormone originally found in the pituitary gland, has played a central role in the development of our current understanding of peptide-receptor interactions in biological systems. Using NMR and other biophysical methods, the solution conformation of oxytocin has been well studied and recently an X-ray structure of deamino-oxytocin has been determined. The results from these studies have been discussed in terms of the relationships of conformation and dynamics to biological activity. Most recently, using primarily NMR methods and distance geometry calculations, we have determined the conformation of oxytocin bound to its carrier protein in the brain, neurophysin. These studies demonstrate that oxytocin has a different conformation in each "environment." A comparison of the different conformations and their possible origins will be discussed. The implications of these findings to peptide ligand design will be given. Despite the complexities of the situation, methods are available for rational design of peptide ligands and the insights gained from these studies can aid in further developments. The work was supported by grants from the U.S. Public Health Service and the National Science Foundation.

LZ 028 SOLUTION CONFORMATION OF TUBOCURARINE, FREE AND BOUND TO A GENETICALLY ENGINEERED ACTIVE SITE PEPTIDE OF THE ACETYLCHOLINE RECEPTOR, Gil Navon[1], Yigal Fraenkel[1] and Jonathan M. Gershoni[2], [1]School of Chemistry, [2]Department of Cell Research and Immunology, Tel Aviv University, Ramat Aviv, Tel Aviv, Israel.

The nicotinic acetylcholine receptor (AChR) is a cation channel, which is activated by the neurotransmitter acetylcholine. A variety of drugs and toxins are active through their interaction with the AChR. For a rational drug design one has to determine their conformations in the bound state. The AChR is a very large, membrane bound, glycoprotein (M.W.~300,000). Thus direct NMR determination of the structure of the receptor-ligand complex seems presently highly improbable. The technique of transfer NOE can give the required information. Indeed, using this technique we have shown that acetylcholine undergoes a major conformation change upon binding to the native Torpedo AChR[1]. From the extended conformation in free solution it acquires a bent conformation.

We have now measured the conformations of acetylcholine and of its potent inhibitor d-tubocurarine, upon binding to a genetically engineered 37 kD construct protein which contains a 17 amino acid peptide of the binding site of the Torpedo AChR (Tα184-200). We have shown previously that this protein specifically binds acetylcholine, its agonists and antagonists[2]. The conformation of the acetylcholine bound to Tα184-200 was found to be identical to that bound to the native receptor. Measurements of the conformations of free and bound d-tubocurarine indicated a dramatic conformational change upon binding. Again, as in the case of acetylcholine, it is converted from an extended to a bent conformation. In this bent geometry hydrophobic and hydrophilic domains are created. The hydrophobic domain frontiers consist of the two aliphatic portions of the two tetrahydroisoquinolinic rings which have become closer. The hydrophilic domain contains the six oxygens. The major hydrophobic domains in ACh and d-tubocurarine are unique in that they consist of methyl groups which are connected to a positively charged nitrogen. This may be denoted as a '**positively charged hydrophobic domain**'. Recently, using t- NOE experiment we have identified in the Tα184-200 sequence a tryptophan residue that interacts with acetylcholine[3]. Thus, unlike the traditional thought that there must be a negatively charged residue such as aspartate or glutamate on the AChR to complement the ammonium group, we find that 'positively charged hydrophobic domains' are attracted to aromatic residues which combine electron rich π orbitals with hydrophobicity.

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LZ 029NMR STUDIES OF LIGAND/MACROMOLECULE INTERACTIONS, David Wemmer[1], Kalle Gehring[1], Philip Williams[2], Hiromi Morimoto[2], Devendra Jaiswal[2], Mark Kubinec[1], Stefan Highsmith[3], Richard Storrs[1], Bernhard Geierstanger[4], B.Volkman[1], [1]Dept. of Chemistry, Univ. of CA, Berkeley, and Structural Biology Division, Lawrence Berkeley Lab, [2]National Tritium Labelling Facility, Lawrence Berkeley Lab, [3]Dept. of Biochemistry, Univ. of the Pacific School of Dentistry, San Francisco, [4]Dept. of Biophysics, University of CA, Berkeley.

Characterization of ligands bound to macromolecules using NMR spectroscopy has developed dramatically over the past several years. When the macromolecule is not too large, it is possible to use incorporation of stable isotope labels (^{15}N and ^{13}C) to carry out filtering experiments to determine both the bound conformation and interactions with the macromolecule. However as the macromolecules grow in molecular weight, or the lines broaden due to chemical exchange, it becomes more difficult to study the binding. As an example of molecules in the lower molecular weight limit we will compare the binding of a small drug ligand, distamycin-A, to DNA with the binding of a peptide. The peptide had previously shown to bind preferentially to A-T rich DNAs (as does distamycin), and analogies were drawn between the peptide and the drug. However the NMR data obtained (albeit complicated by the effects of chemical exchange) show clearly that the binding mode does not fit the initial model. As examples of the binding of ligands to larger proteins we will present data using tritium NMR to selectively detect the resonances from bound ligand, even in cases where the broadening due to both high molecular weight and chemical exchange lead to rather large linewidths (100 Hz and greater). The binding of maltodextrins to the maltose binding protein from the periplasmic space of *E. coli* was analyzed. With maltose it was found that there is moderate anomeric specificity (the α form binding more tightly than the β). However with longer maltose oligomers the anomeric specificity was shown to be higher, and there were two distinct binding modes found for the β anomer, which were in rapid exchange with one another. A model was developed which explains these data qualitatively through differences in interaction of the terminal sugar with the binding pocket. In this example ^{3}H NMR was an ideal probe since resonance could be detected with essentially no background signal, and the very broad lines in the complexes could be followed under a variety of conditions. Another example using ^{3}H NMR in the ATP/myosin S1 system will be presented. In this case two bound forms of ADP could be detected when bound to the 105 kD myosin S1 fragment. The lines could be followed as a function of temperature, and the presence of vanadate. There is again evidence for chemical exchange. A final example of a tritiated hapten binding to an antibody Fab fragment will be presented. In this case a tritiated nitrophenylphosphate was used as a transition state analog to induce a catalytic antibody (the work of Prof. Peter Schultz, UCB Chemistry). The resonances of the bound NPP were easily detected at low concentration.

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Late Abstract

DETERMINATION OF LIGAND EXCHANGE RATES AND THEIR EFFECT ON TRANSFERRED NOE MEASUREMENTS, Robert E. London, Michael E. Perlman, and Donald G. Davis, Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC. 27709

Bacterial purine nucleoside phosphorylase (PNP) is a 135 kD enzyme with six identical subunits (M.W. = 22.5 kD), which catalyzes the phosphorolytic cleavage of purine nucleosides and is used commercially for the preparation of nucleosides from the corresponding bases and ribose-1-phosphate. Information about the active site is currently fragmentary, and studies of the inhibitory potency of conformationally constrained nuclesides have suggested that the active site may differ substantially from that of the corresponding mammalian enzyme. The conformation of PNP-complexed tubercidin (7-deazaadenosine), an inhibitor of the enzyme with K_I = 18 μM, has been studied utilizing the transferred nuclear Overhauser enhancement approach. Transferred NOE data inconsistent with a single bound conformation led to more extensive modeling of the dependence of the TRNOE on exchange rate and on uncomplexed nucleoside parameters, and to models utilizing more than one bound conformation. Determination of the exchange rates based on: (1) analysis of the Swift & Connick relations for the observed shift and transverse relaxation rate as a function of the mole fraction of bound nucleoside; (2) CPMG studies of transverse relaxation rates as a function of pulse rate; and (3) rotating frame $T_{1\rho}$ relaxation as a function of the spin lock field provided consistent results for tubercidin exchange kinetics, and were used for analysis of the TRNOE data. Since relaxation matrix simulations indicated that direct and indirect dipolar relaxation pathways cannot always be distinguished via an observable lag of the NOE build-up curves, theoretical and experimental studies utilizing the transferred nuclear Overhauser effect in the rotating frame (TRROE) were carried out in order to determine whether the TRNOE and TRROE data collectively could provide a more useful basis for distinguishing between these possibilities. Preliminary studies support the feasibility of utilizing TRROE measurements in this way. The results support a model in which the predominant conformation of bound tubercidin has the base in a *syn* orientation, and the ribose in a 3'-exo conformation.

Spectral Analysis, Structure Refinement, NMR Techniques

LZ 100 MACROSEARCH: A PROGRAM FOR GENERATING STRUCTURES FROM NMR CONSTRAINTS USING SYSTEMATIC CONFORMATIONAL SEARCH. Denise D. Beusen, Richard D. Head, John D. Clark, E. F. Berkley Shands, Steven F. Karasek, and Richard A. Dammkoehler. Center for Molecular Design, Washington University, Box 1099, One Brookings Drive, St. Louis, MO 63130.

Although distance geometry and molecular dynamics have successfully been used for transforming NMR-derived distance and torsional constraints into three-dimensional structures, *questions remain about* the sampling characteristics of these approaches. In the absence of sufficient constraints, the generated structures may reflect the biases of the computational method rather than a representative sampling of all possible candidates consistent with the data. Because a systematic grid search of torsion angles explicitly considers all conformational possibilities of a molecule, it does not suffer this shortcoming and should be able to address the issue of whether or not a given data set uniquely defines a structure. Historically, the combinatorial nature of systematic search has limited its use to small molecules with their limited degrees of freedom. MACROSEARCH is a new program which combines relaxation matrix calculations with systematic search and makes this tool usable in generating the solution NMR structure of peptides. The program consists of several phases: calculation of distance and torsional constraints from NOEs and coupling constants; systematic search analysis of overlapping molecular fragments with constraints imposed; cluster analysis of the search results for each fragment; construction of the entire molecule by optimizing the overlap of representative fragment conformers; and evaluation of the resulting candidate structures to determine which best fits the experimental data. MACROSEARCH has been evaluated against several simulated datasets for peptides from nine to twenty-nine residues in length, and has been used to determine the structure of the peptaibol antibiotic, emerimicin IV, in DMSO. (Supported in part by the National Institutes of Health.)

LZ 101 RELAXATION MATRIX REFINEMENT OF THE SOLUTION STRUCTURE OF THE ARC REPRESSOR, A.M.J.J. Bonvin, H. Vis, J.N. Breg, R. Boelens and R. Kaptein, Bijvoet Center, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands. Telephone: 31 30 53 2184, telefax: 31 30 54 0980.

The Arc Repressor of *Salmonella* bacteriophage P22 is a dimeric sequence-specific DNA binding protein. The solution structure of Arc has been determined from 2D NMR data using an "ensemble" Iterative Relaxation Matrix Approach (IRMA) followed by direct NOE refinement with DINOSAUR. A set of 51 structures was generated with Distance Geometry and further refined with a combination of restrained Energy Minimization and restrained Molecular Dynamics in a parallel refinement protocol. Distance constraints were obtained from an extensive set of NOE build-ups in H_2O and D_2O via relaxation matrix calculations from the ensemble of structures. Methyl group rotation, aromatic ring flips and internal mobility effects (via order parameters obtained from a free MD run in water) were included in these calculations. The best structures were finally refined with direct NOE constraints following a slow-cooling simulated annealing protocol. In this final refinement stage, theoretical NOE intensities were directly compared to the experimental data and forces were derived using a simple two-spin approximation for the gradient of the NOE function. Dynamic assignment was applied to the peaks involving unassigned diastereotopic groups and to solve the problem of inter-/intramonomer NOEs close to the diad axis of the dimer. The structure is determined to a precision (r.m.s.d. from the average excluding the less defined C- and N-terminal region) of 0.6 and 1.2 Å for backbone- and all atoms, respectively. The final structures have R-factors values around 0.38.

LZ 102 DETERMINATION OF FAST HYDROGEN EXCHANGE RATES BY 2D NMR LINEBROADENING, W. Clay Bracken, and Jean Baum, Department of Chemistry, Rutgers University, Piscataway, NJ 08855

The measurement of hydrogen exchange rates of individual amide protons in proteins and peptides is a widely used NMR method for obtaining information about structure and dynamics. Measuring fast rates is especially important in a number of different areas including, for example: measuring rates under different conditions of pH and temperature, studying the effects of protein binding, and measurement of exchange rates in partially denatured proteins or peptides. Hydrogen exchange rates are usually obtained by acquiring sequential NMR spectra and measuring the change in the observed peak intensity of the amide proton as a function of time.

A method is presented that allows direct measurement of fast hydrogen exchange rates using 2D NMR spectroscopy. The method is based on the fact that the exchange of amide protons with deuterons superimposes an additional exponential decay term in the t1 dimension of a 2D experiment. This results in additional linebroadening upon fourier transformaton of the t1 dimension. This additional linebroadening can be measured directly to give the hydrogen exchange rate from one or more 2D experiments. This method potentially extends the timescale of fast hydrogen exchange rates that can be measured using 2D NMR by roughly a factor of 100.

LZ 103 IMPROVED SPECTRAL RESOLUTION OF 3D AND 4D NMR DATA THROUGH MULTIDIMENSIONAL BAYESIAN ANALYSIS, Roger A. Chylla*, Arthur S. Edison+, William M. Westler*, and John L. Markley*+, Department of Biochemistry* and Graduate Biophysics Program+, University of Wisconsin, Madison, WI 53706

Limited spectral resolution is a drawback of current 3D and 4D NMR data acquisition methods. One approach to overcoming this limitation has been extrapolation of one and two-dimensional time-domain data by linear prediction prior to apozidation and Fourier transformation. Reconstruction of the frequencies contained in *n*-dimensional time-domain data by the maximum entropy method (MEM) has been an alternative and somewhat controversial approach. We discuss here the application of Bayesian methods [1-3] in conjunction with "constant-time" evolution periods to significantly improve the estimation of frequencies in highly-truncated, *n*-dimensional time-domain data. The application of Bayesian probability theory to one-dimensional NMR data analysis has been extensively developed by Bretthorst [1-3]. We have extended this theory to the *n*-dimensional case and have developed a computer program which estimates the number, amplitude, frequency, decay rate, and phase of sinusoids contained in an *n*-dimensional FID. "Constant-time" evolution periods during data acquisition of one or more time axis is advantageous because the sinusoids present in the acquired FID will be stationary. Since decay rates can be removed from the model describing these data, the time required for data processing is significantly decreased. We have applied here Bayesian methods to estimate the number, frequency, and amplitude of sinusoids contained in two-dimensional $^{13}C_{\alpha}$-$^{1}H_{\alpha}$ "constant-time" planes of a 4D $^{1}H^{15}N^{13}C_{\alpha}H_{\alpha}$ experiment [4] performed upon the electron-transport protein, *Anabaena* flavodoxin (MW 20,000).

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LZ 104 PROBING LIGAND-PROTEIN INTERACTIONS IN COMPLEX SYSTEMS: APPLICATION OF CARBON DOUBLE QUANTUM NMR TO HUMAN PLASMA

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One important issue in human biology involves the role of carrier proteins in the transport of sparingly soluble metabolites, hormones and drugs through aqueous compartments, such as the bloodstream. A traditional approach to studying a carrier protein involves its isolation and purification, followed by the characterization of its binding properties by chemical or physical methods. However, in complex biological systems, multiple proteins may simultaneously function in the transport of a single ligand. Therefore, it would be advantageous to devise methods that monitored ligand binding in the intact system, without fractionation of individual components. Here, we describe the novel application of 2-D carbon-carbon double quantum NMR to probe ligand-protein interactions in whole human plasma. This strategy employs selective double-enrichment (>95%) of the ligand and the use of a compensated pulse sequence (Levitt and Ernst, Mol. Phys., 1983, 50, 1109). The double quantum experiment provides a high degree of spectral editing compared with other isotope-directed approaches. It also provides a high resolving power that is necessary to distinguish ligands bound to structurally similar sites. In spite of its relatively low sensitivity, good quality spectra can be obtained in 12-48 hours if a judicious choice of experimental parameters is made. To illustrate this approach, results will be presented for the non-covalent interactions of fatty acids with proteins in human plasma.

LZ 105 COMPUTERIZED ANALYSIS OF 2D-NMR PEPTIDE CORRELATION SPECTRA USING DISCRETE MATRIX REPRESENTATIONS, Edward C. Craig and Irwin D. Kuntz, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446

Methodology is described for generation and analysis of experimental correlation spectra as Discrete Matrix Representations (DMR). DMRs are constructed by equating ω_i and ω_j for each correlation to individual rows and columns in discrete matrices. Experimental connectivity patterns are explicitly preserved by assigning integer labels, equal to the peak labels of experimental correlations, to the individual matrix elements. These labels may be keyed to additional information (e.g. intensity, linewidth or personal comments) pertaining to the individual correlations. Such data reduction is demonstrated with simulated and experimental data obtained for a 27 residue peptide.

Experimental HOHAHA DMRs may be block diagonalized to reveal blocks of correlations belonging to individual peptide residues. Such blocks provide the basis for recognition and resolution of individual spin systems and discrimination between ambiguous and unambiguous correlations. These blocks are subsequently interrogated to obtain lists of frequencies associated with individual peptide residues. These lists are used to analyze complementary correlation spectra (e.g. 2D-COSY or NOESY) for the same sample.

DMRs provide a convenient format for computerized representation, manipulation and analysis of 2D-NMR correlation data. They also permit forms of data abstraction and pattern recognition unavailable with traditional connectivity pathway searches.

LZ 106 MASK-BASED PEAK PATTERN SEARCH IN NMR SPECTRA, D. Croft and H. Oschkinat, NMR Group, EMBL, Meyerhofstr. 1, 6900 Heidelberg, Germany.

Automated assignment of NMR spectra usually starts with the extraction of a peak list from a spectrum. This puts a heavy emphasis on good peak detection. Furthermore, peaks vary in shape in different parts of a spectrum, but peak picking applies the same criterion everywhere. And, once it is done, the original spectrum is lost. In the approach outlined here, two important ideas are employed: i) what we are searching for is *patterns* of peaks, linked to nucleus chemical shift values, as opposed to searching for individual peaks; ii) peaks within a pattern can be detected with maximum accuracy *within the original spectrum*, using masks which have been tuned to select them. For instance, when searching for alanines in a 2D TOCSY spectrum, 3 cross-peaks would be expected above the diagonal and 3 below. Special emphasis should be put on the "fingerprint region" of the spectrum, and diagonal peaks should be excluded. A *mask* is a function that is convolved with the data in the spectrum. If the mask shape has been designed to fit spectrum peaks, then its output will be high when convolved with a peak, negative when convolved with a trough, and zero when convolved with flatness. A mask that is displaced somewhat from a peak will give a weaker response than one sitting right on top of it. A pattern may have many masks associated with it, one for each of the peaks it expects to find in the spectrum. The "response" of a pattern, for a given combination of nucleus chemical shifts, is the sum of all individual mask responses. In order to facilitate pattern specification, a simple pattern description language has been written. The program finds patterns of interest by scanning through all possible chemical shift combinations for the nucleii in the user supplied pattern. The chemical shifts for the most strongly responding patterns are recorded. Multiple spectra can be searched simultaneously. For instance, if the spectra used are TOCSY and NOESY, specifying the patterns for 2 amino acids could be used for locating NOESY cross-peaks between them. Both hetero- and homonuclear spectra can be processed. As an example, when the software was applied to a 2048*2048 TOCSY of a peptide with 8 alanines, 3 of the alanines appeared in the top 10 highest scoring patterns.

LZ 107 UCSF STRIKER: AN INTERACTIVE PROCESSING TOOL FOR MULTI-DIMENSIONAL NMR DATA

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We have developed a graphical front end to a suite of programs for processing high resolution NMR data. A major goal of our efforts has been to supply the spectroscopist with interactive procedures that immediately display the results of calculations as processing parameters are adjusted.

Striker displays one dimensional slices along any axis of two and three dimensional data sets. Dialog boxes are used to provide an interface to processing steps such as apodization, phasing, time domain linear prediction, time domain convolution, and baseline correction. A spectroscopist interactively optimizes settings for these calculations by viewing individual slices, then dispatches a batch mode job to process the entire spectrum. This computational back end can be run on any host on the network.

Striker can simultaneously display multiple views of a spectrum as well as multiple spectra. PostScript output can be sent to a printer, or saved to an Adobe Illustrator compatible file for subsequent annotation.The graphical portions of the program run under OpenWindows 2.0 and 3.0 on Sun SPARCstation hardware. The computational portions have been ported to run on Sun, Hewlett Packard, Silicon Graphics, and IBM equipment

LZ 108 ***Ab Initio*** **Calculations of Backbone Coupling Constants in a Model Peptide**

Arthur S. Edison,[1] William M. Westler,[2] Frank Weinhold,[1,3] and John L. Markley,[1,2] [1]Biophysics Program, [2]Department of Biochemistry, and [3]Department of Chemistry and Theoretical Chemistry Institute, University of Wisconsin, Madison, WI 53706

The number of measured coupling constants in proteins has increased dramatically with the use of multidimensional, multinuclear NMR techniques. All one-, two-, and three-bond homo- and heteronuclear coupling constants in a blocked alanine peptide have been calculated from a fully relaxed HF/3-21G (ϕ,ψ) surface. These calculations have been compared with available experimental results and have been fit to two-dimensional Fourier series. Many of the couplings, including $^3J_{H\alpha,HN}$, show a strong dependence on both torsional angles, ϕ and ψ. Theoretical results at this level can assist the structural interpretation of experimental data.

LZ 109 ACCURATE MEASUREMENTS OF VICINAL $^3J(^{13}C'$-$H\beta)$ COUPLING CONSTANTS FROM ^1J RESOLVED HETERONUCLEAR E.COSY MULTIPLETS.

S. Donald Emerson[a,b] and Gaetano T. Montelione[a]. [a]Center for Advanced Biotechnology and Medicine, Rutgers University, 679 Hoes Lane, Piscataway, NJ 08854. [b]Biophysical Chemistry, Hoffman-La Roche, 340 Kingsland St., Nutley, NJ 07110-1199.

A vicinal coupling constant (^3J) varies in magnitude with changes of the intervening dihedral angle. Coupling constants such as $^3J(^{13}C'$-$H\beta)$, which depend on the dihedral angle χ^1, are particularly useful in generating experimental constraints on amino acid side-chain conformations in proteins and for determining stereospecific methylene H^β resonance assignments. To exploit the structural information content of these vicinal coupling constants, it is important to measure accurately the separation of relevant NMR multiplet components. Here we describe a heteronuclear ^1J resolved E.COSY experiment which allows accurate quantitation of $^3J(^{13}C'$-$H\beta)$ vicinal coupling constants of ^{13}C enriched peptides and proteins. The experiment is based on our previously described HCCH-TOCSY pulse sequences[1,2] for measuring $^3J(H^\alpha$-$H^\beta)$ coupling constants. Combined measurements of $^3J(H^\alpha$-$H^\beta)$ and $^3J(^{13}C'$-$H^\beta)$ provide the information needed to unambiguously determine χ^1 and stereospecific H^β assignments for amino acid residues in proteins.

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LZ 110 Implementation of a relational database for protein NMR results

Elizabeth A. Farr, Beverly R. Seavey, Allison M. Conti, William M. Westler and John L. Markley

A relational database, *BioMagResBank*, [1] has been developed for storage and retrieval of numeric and structural NMR-derived data for proteins and peptides 12 or more amino acids in length published in the primary refereed literature. NMR data such as sequence-specific shift assignments, NOEs, coupling constants, pKas, T_1 values, amide exchange rates and structural elements are indexed by and can be retrieved by pH, temperature, atom, amino acid, protein, organism, redox state of prosthetic group(s), journal article and author. A relational database management system (RDBMS) has been chosen because of the flexibility allowed in design, data retrieval and data entry, and the inherent capabilities for error checking and for ensuring consistency. This model is flexible enough to allow incremental changes in database design without destroying the underlying data, and to allow for the modeling of additional types of data. At present, over 2100 articles (including over 80 reviews and nearly 800 bibliographic entries reporting supporting results) and 600 distinct proteins have been indexed, and shift assignments for over 500 papers are available. The database is in in the early phases of distribution, and is available in three formats: *carrier format*, where the data can be transferred to any RDBMS; via a *network server*; and as *flat files*, where the data will be stored as text in a fixed format with keywords to identify each data type.

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LZ 111 NMR STUDIES ON THE DOMAINE P20 OF E. COLI MANNOSE PERMEASE

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The mannose permease complex is part of the transport system for mannose and other hexoses in E. coli. It consists of two transmembrane subunits and the soluble hydrophilic subunit III^{Man}. This protein forms two domains, the N-terminal domain P13 (with the contact sites for dimerization of III^{Man}) and the C-terminal domain P20 (residues 148-323) of the soluble subunit. P20 contains the contact site for the interaction with the transmembrane subunits and is directly involved in the transfer of a phosphoryl group from N-1 of its residue His175 to the sugar during the transport process in the mannose permease complex.

For structural studies on the soluble subunit III^{Man} of mannose permease, isotopically labeled P20 was employed with uniform ^{15}N and/or ^{13}C enrichment. The results of numerous multidimensional hetero-nuclear and triple-resonance NMR experiments on protein P20 will be presented, as well as modifications of the standard pulse sequences that showed an improved performance in the case of this specific protein.

LZ 112 $^1H^1H$ DQF-COSY, PS-COSY, AND CT-COSY: LOOKING FOR SIGNAL TO NOISE IN A COUPLE OF RIGHT PLACES, Mark E. Girvin and Robert H. Fillingame, Department of Biomolecular Chemistry, University of Wisconsin, Madison, WI 53706.

The double quantum filtered (DQF) COSY is the most commonly used experiment for determining $^1H^1H$ correlations. The phase sensitive (PS) COSY experiment, without the double quantum filter, has recently been revived by using creative data processing to alleviate problems caused by the dispersive nature of the diagonal, and yields a potential factor of two increase in signal to noise over the DQF-COSY experiment. Constant time (CT) experiments, described in 1981 by Bax and Freeman (J. Magn. Reson. **44**, 542.), are now being used as component blocks in 3D and 4D heteronuclear experiments. We report here that the CT sequence is also applicable to the more mundane 2D $^1H^1H$ correlation experiment in biological macromolecules. Increases in signal to noise of a factor of seven over the DQF-COSY, and a factor of four over the PS-COSY are observed. These improvements are due in part to maintaining maximum magnetization transfer over the t_1 period, but they result mainly from the t_1 dimension being decoupled in the CT-COSY experiment, which recovers the intensity lost to mutual cancellation of the antiphase multiplets in the t_1 dimension in the DQF-COSY and PS-COSY experiments. The properties of the diagonal are also much improved in the CT-COSY relative to the PS-COSY. A convenient pulse sequence and data processing procedure are presented. Using this experiment, we have been able to obtain high quality 2D COSY spectra in four hours for protein samples which would require 36 hours to obtain equivalent signal to noise in a DQF-COSY experiment. This sequence is particularly useful for studying time dependent phenomena such as NH exchange. In our laboratory we are using the CT-COSY experiment to titrate the pK_a of an aspartic acid which has been shown to be required for transmembrane H^+ translocation in the F_1F_0 ATP synthase.

LZ 113 A PRACTICAL METHOD FOR UNIFORM ISOTOPIC LABELING OF RECOMBINANT PROTEINS IN MAMMALIAN CELLS, Andrew P. Hansen, Andrew M. Petros, Andrew P. Mazar, Terry M. Pederson, Annemarie Rueter, and Stephen W. Fesik, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064

A method to obtain uniformly isotopically labeled (^{15}N and $^{15}N/^{13}C$) proteins from mammalian cells is presented. The method involves the preparation of isotopically labeled media consisting of amino acids isolated from bacterial and algal extracts supplemented with cysteine and enzymatically synthesized glutamine. The approach is demonstrated by preparing isotopically labeled urokinase from Sp2/0 cells and by successfully growing Chinese hamster ovary (CHO) cells on the labeled media. Using the procedures that will be described uniformly ^{15}N- and $^{15}N/^{13}C$-labeled proteins that have been expressed in mammalian cells can be prepared which will allow their structures to be determined using heteronuclear, multi-dimensional NMR spectroscopy.

LZ 114 **AUTOMATED ASSIGNMENT OF MULTIDIMENSIONAL PROTEIN NOE SPECTRA,** **Timothy S. Harvey, Stefan Bagby and Mitsuhiko Ikura,** Division of Molecular and Structural Biology, Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Canada, M4X 1K9.

Three-dimensional heteronuclear NMR has attained considerable importance in the study of larger (> 15kDa) proteins. This has resulted in part from the spectral simplification achieved by this method, which makes the problem of automated assignment more tractable. An algorithm is presented which, by the use of a reliable peak-picker, and by combining the information contained in ^{15}N-edited 3D HOHAHA and NOE spectra in a simple error function, is able to produce a sequence-specific resonance and NOE assignment of the ^{15}N and amide proton backbone resonances.

The performance of the algorithm will be evaluated using a variety of optimization methods, including Monte Carlo, simulated annealing and conventional minimization techniques. Experimental data from proteins consisting of predominantly α-helix and β-sheet will also be used as examples. The influence of experimental parameters, such as the signal/noise ratio and peak overlap, on the performance of the algorithm will be critically evaluated. Finally, the possibility of extending this method to both conventional ^1H - ^1H as well as 3- and 4- dimensional $^{15}N/^{13}C$-edited NOE spectra will be explored.

LZ 115 COMPARISON OF SIMPLE METHODS FOR ESTIMATING PEAK DISPLACEMENT, Jeffrey C. Hoch*, Robert T. Clubb†, Alan S. Stern*, and Gerhard Wagner†, *Rowland Institute for Science, 100 Cambridge Parkway, Cambridge, Massachusetts 02142, †Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

The task of determining the relative displacement of two peaks is fundamental to NMR spectroscopy, since it serves as the basis for measurement of chemical shifts and coupling constants. The simple method of estimating displacements from peak maxima suffers from well known limitations due to digital resolution and finite line widths, although these can be ameliorated to some extent by zero-filling or model (e.g. lorentzian) fitting. We compare two methods for estimating peak displacements that are not directly limited by digital resolution and that apply to arbitrary lineshapes. The first method uses the difference between the centers of mass of two peaks. The second finds the displacement that optimizes the match of the total lineshapes of two peaks, based on cubic spline interpolation. Factors limiting the precision of these methods are the signal-to-noise ratio, the line widths, and the extent of overlap. In the limit of extremely narrow lines, the precision of these methods is bounded by the digital resolution, but for lines spanning many points and not overlapping, the precision can dramatically exceed the digital resolution when there is sufficient S/N. Both methods are robust, easy to implement (the center of mass is trivial), apply to arbitrary lineshapes (which might result from window functions, unresolved splittings, or maximum entropy reconstruction), and usually obviate the need for extensive zero-filling. We demonstrate using spectra recorded for BPTI that these methods are well suited to the determination of coupling constants from heteronuclear 2D and 3D spectra.

LZ 116 DETERMINATION OF ACCURATE LOCAL CONFORMATION AND 3D-STRUCTURE OF PROTEINS IN SOLUTION USING NOE DATA: APPLICATION OF A PROBABILISTIC APPROACH, **Leela Kar, Simon A. Sherman† and Michael E. Johnson, Department of Medicinal Chemistry & Pharmacognosy, University of Illinois at Chicago, P.O. Box 6998, Chicago, IL 60680**

Application of the probabilistic method of Sherman *et al.* [(1987) *J. Biomol. Str. Dyn.* **4**, 869] to obtain the backbone conformations from NOE data is illustrated for cytochrome *c*, using the published sequential d-connectivity maps of horse cytochrome *c* [Wand *et al. (1989) Biochemistry* **28**, 186; Feng *et al., ibid.* 195]. It is shown that accurate determination of local conformation alone can lead to useful information regarding the structure-functional aspects of proteins in solution. For cytochrome *c*, the angular root mean square deviations for ϕ and ψ are about 17° and 60° between the oxidized and reduced forms of the protein. The largest changes in conformation are seen for residues 27, 28, 32, 42, 47-49, 52, 69, 72, 80, 82, 85 and 86, all of which are within 7 to 14 Å of the heme iron in the high resolution X-ray structures of tuna ferro- and ferricytochrome *c* [Takano & Dickerson (1981) *J. Mol. Biol.* **153**, 79; *ibid.* 95]. The conformational differences between ferro- and ferricytochrome *c* in solution are observed to be much greater than those observed in the same regions for the crystal structures of tuna ferro- and ferricytochrome *c*. Comprehensive statistical analysis shows that the solution structures are significantly different from the crystal structures, particularly in regions close to the heme. Preliminary work is presented for the solution conformation of rat ferricytochrome *c*, enabling the study of structural differences in solution for cytochrome *c* from different species.

An application of the probabilistic method for the determination of the complete three dimensional structure in solution using NMR data is also presented for a 6 kD tryptic fragment of a protein found in human plasma, illustrating the effectiveness of this method with sparse NOE datasets.

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LZ 117 Multidimensional NMR Experiments for Protein Structure Determination, Lewis E. Kay and D. R. Muhandiram, Departments of Medical Genetics, Biochemistry and Chemistry, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

The development of multidimensional, multinuclear NMR spectroscopy has greatly facilitated the structure determination of proteins in the 15-30 kDa range. Recently we have developed a number of new experiments which separate side chain correlations in uniformly $^{15}N,^{13}C$ labeled proteins via the carbonyl chemical shift. One such experiment, the HCACO-COSY provides correlations linking the carbonyl, Hα and Hβ shifts, while the HCACO-TOCSY experiment links the carbonyl shift with all the ^{1}H shifts along a given side chain. Additional experiments for establishing the side chain carbon chemical shifts, with separation achieved by the backbone carbonyl shift have also been developed. The experiments have been demonstrated on a 1.5mM sample of calmodulin (gift of Dr. M. Ikura). The pulse sequences will be presented and an approach for the optimization of their sensitivity will be described.

In collaboration with P. Keifer and T. Saarinen, Varian, a pulse sequence for recording gradient enhanced pure absorption ^{1}H-^{15}N heteronuclear single quantum correlation spectra has been developed which offers significant improvements in sensitivity over existing gradient enhanced pulse schemes for moderately sized proteins (100-150 amino acids). Selection of protons directly bound to ^{15}N and suppression of the intense water resonance is achieved solely through the application of gradient pulses. The method is demonstrated on a 110 amino acid fragment comprising the cellulose binding domain of a cellulase from *Cellulomonas fimi*. Triple resonance gradient enhanced pure absorption pulse schemes will be presented as well.

LZ 118 SYSTEMATIC REFINEMENT OF THE SOLUTION STRUCTURE OF ENDOTHELIN-3 BY RELAXATION MATRIX ANALYSIS Glenn F. King, Kenneth A. Joseph, Séan I. O'Donoghue, and Robyn G. Mills, Department of Biochemistry, University of Sydney, Sydney NSW 2006, Australia.

We have used the 21-residue peptide hormone endothelin-3 (ET-3) to systematically analyse the requirements for optimal structural refinement using relaxation matrix analysis. The starting point for this investigation was the structure of ET-3 published by Mills *et al.* [1]. NOESY assignments were extended to give a total of 209 structurally-relevant interproton distance constraints and 5 χ_1 dihedral angle constraints. The general protocol for structure calculation involved generation of 1000 structures using distance geometry (DG), refinement of the "best" 50 structures (as judged by target function violations) using dynamical simulated annealing (DSA;[2]), then final refinement of the best DSA structures using relaxation matrix analysis (RMA). We used the RMA approach developed by Nilges *et al.* (1991), as implemented in the X-PLOR molecular dynamics program; while this approach is computationally expensive, it is essentially automated and is probably the least subjective approach to RMA. We have systematically examined:

(1) the effect of incorporation of explicit lower interproton distance constraints in both the DG and DSA phases of the calculation;

(2) the effects of r^{-6}-averaging versus centre-averaging in the DSA calculations;

(3) the relative biasing effect of window functions on NOESY crosspeak intensities calculated for the RMA procedure;

(4) the tolerance of the RMA procedure to errors in τ_c.

An optimal approach to structural refinement will be presented, and the final refined structure of ET-3 will be compared to other structures of members of the ET family of peptides which have been presented in the literature.

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LZ 119 UCSF SPARKY: AN NMR DISPLAY, ANNOTATION AND ASSIGNMENT TOOL Donald G. Kneller and Irwin D. Kuntz, Department of Pharmaceutical Chemistry, University of California, San Francisco CA 94143-0446.

We have been developing a graphical tool (Sparky) for the display and annotation of high-resolution NMR spectra. While Sparky meets the usual requirements for displaying multiple NMR spectra and for performing computations such as peak picking and integration, our emphasis has been on designing a tool which aids the NMR spectroscopist in the *assignment* task. This is facilitated in Sparky by a hierarchal data base reflective of the relationships between correlated atoms in the molecule and observed crosspeaks in the spectra.

A spectroscopist, working with one or more 2- or higher-D spectra can search for crosspeaks, annotate them, and define and share assignment information between them. Update of the assignment information at any point is reflected in all crosspeaks that share this assignment. Different experimental conditions can be used and an atom may have different resonant frequencies under the different conditions.

Future directions include an assignment audit trail, incorporation of automatic and computer-assisted assignment algorithms, and porting to machines additional to the currently supported Sun SPARCstations under OpenWindows.

LZ 120 ^{13}C ASSISTED PROTEIN ASSIGNMENTS AND STRUCTURAL CONSTRAINTS UTILIZING ALTERNATING CARBON ENRICHMENT, Diana M. Kushlan and David LeMaster, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208

Uniform ^{13}C enrichment offers numerous benefits for protein structural analysis. Yet it suffers significant limitations as well. At high levels of enrichment, the one bond ^{13}C-^{13}C coupling can give rise to substantial degradation in resolution. Refocusing of these couplings via constant time techniques results in a loss of sensitivity. Using lower levels of uniform enrichment to reduce the effects of the one bond couplings also imply reduced sensitivity. Many of these drawbacks can be overcome with a labeling pattern in which each residue type is enriched in an alternating ^{13}C-^{12}C-^{13}C... pattern from the carbonyl position out along the sidechain. Except for the methine carbons of the branched amino acids, such an alternating enrichment pattern has been obtained by growth of a suitable metabolically blocked E. coli strain on a selectively enriched glycerol or lactate carbon source.

Heteronuclear correlation-TOCSY experiments can be used to obtain both ^{1}H and ^{13}C assignments as well as long range ^{1}H-^{13}C coupling constants. As only ~half of the protons have increased relaxation rates due to directly bonded ^{13}C nuclei, the transfer efficiency is nearer that of natural abundance samples so that ^{13}C separated correlations are observed from H_α to H_δ of the proline rings for a 12 kD protein. The alternating carbon pattern provides a direct determination of the number of transfer steps involved in TOCSY crosspeaks.

The ability to select for or against vicinal interactions is of use in both NOESY and ^{1}H correlation experiments. Editing for ^{13}C (or ^{12}C) in both dimensions of a NOESY experiment strongly selects for interresidue interactions by suppressing the generally less informative vicinal crosspeaks. Conversely, the ability to suppress the diagonal enhances the observation of nearly degenerate vicinal crosspeaks.

LZ 121 ALTERNATING CARBON ENRICHMENT FOR RELAXATION ANALYSIS OF PROTEIN METHINE AND METHYL RESONANCES, David LeMaster and Diana M. Kushlan, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208

^{1}H detected ^{15}N relaxation studies have provided a powerful means of analyzing the mainchain dynamics of proteins. It is clearly desirable to extend such studies to ^{13}C relaxation so that the mainchain and sidechain motion can be monitored simultaneously. All but the smallest of protein systems require ^{13}C enrichment to achieve adequate sensitivity. However, uniform ^{13}C enrichment gives rise to modulation of the apparent relaxation rates by the one bond ^{13}C-^{13}C coupling as determined by the current inverse detection experiments. A number of studies have used selectively labeled individual residue enrichment for relaxation studies, however, this does not provide a practical general solution.

We have developed a suitable metabolically blocked E. coli strain which yields with an appropriate carbon source a labeling pattern in which nearly all residue types are enriched in an alternating ^{13}C-^{12}C-^{13}C... pattern from the carbonyl position out along the sidechain. Growth on [2-^{13}C] glycerol gives rise to C_α enrichment for 75% of the residues (the others have C' & C_β enrichment). This spectral editing combined with elimination of ^{13}C-^{13}C couplings yields a well resolved $^{1}H_\alpha$-$^{13}C_\alpha$ spectra from which T_1, T_2 and heteronuclear NOE measurements have been obtained. Similar determinations have been carried out on the labeled aromatic positions as well as on the enriched threonine C_γ and isoleucine C_δ methyls. Similar experiments are in progress utilizing the opposite labeling pattern so as to extend the dynamics measurements to the othe methine and methyl resonance positions.

LZ 122 **(SOME OF THE) UNSOLVED PROBLEMS IN MULTIDIMENSIONAL NMR DATA PROCESSING,** George C. Levy, G.W. Jeong, Kun Wang, Sophia Wang, and P.N. Borer, *NMR & Data Processing Laboratory*, Syracuse University, Syracuse, NY 13244-4100

Over the past five years, there have been major advances in computer methodology for data reduction and analysis of multi-dimensional NMR spectra. Improved algorithms have joined vastly more capable computers, to efficiently automate or assist in several critical data processing steps, including: FFT or alternative primary data reduction, spectral conditioning and analysis, cross peak assignment and quantitative measurement, and the various steps used to define molecular geometry from NMR (NOESY, COSY) data. Yet, current procedures are not nearly good enough for production-level automated molecular modeling from NMR data. Starting from the primary data, there can be ambiguities arising from peak overlaps and subsequent missed assignments, poorly defined peak integrations, and connectivities that are not seen at all in the automatically prepared spectra. Later computational steps suffer from incompletely sampled or otherwise erroneous conformation determinations. There is little intelligent use of error analysis for the complex cascade of calculations used in NMR molecular modeling. As yet, we simply do not know enough about the interplay of all factors. This paper summarizes some of the outstanding challenges facing the field, and proposes potential solutions for the first type of challenge: optimal *automated* preparation of multidimensional NMR spectra, including accurate automated cross peak quantification that incorporates effective noise suppression, baseplane correction, and separation and accurate assignment (including quantitative partitioning) of overlapped peaks. Our attempt to realize these goals utilizes quantitative 2D or 3D Maximum Likelihood Method (MLM) reconstruction, following automated baseplane recognition and correction methodology that attempts to eliminate baseplane roll and noise ridges without affecting spectral peaks.

LZ 123 AN IMPROVED STRATEGY FOR DETERMINING SEQUENTIAL RESONANCE ASSIGNMENTS OF PROTEINS USING TRIPLE RESONANCE NMR Barbara A. Lyons*, Mitsuru Tashiro, S. Donald Emerson, Keith Newkirk, Lingze Wang, and Gaetano T. Montelione* Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ 08854

We have developed an improved strategy for determining sequence-specific NMR assignments in proteins. In our approach, ^{1}H-^{1}H or ^{13}C-^{1}H planes from a set of 3D ^{15}N- or 4D ^{15}N-,^{13}C- resolved NMR experiments are analyzed to provide both spin system identification and sequential connectivity information. First, multidimensional H_C_CA_N_HN TOCSY[1,2] experiments are used to connect together the resonances of each amino acid spin system and to provide unique identification of many spin system types, including those of asparagine and glutamine. Next, sequential connections between amino acids are established using multidimensional H_C_CO_N_HN TOCSY[3]. The H_C_CA_N_HN TOCSY spectra provide an extensive set of intraresidue connections between proton, carbon, and nitrogen resonances of each amino acid in the sequence, while the complementary H_C_CO_N_HN TOCSY spectra provide sequential connections between aliphatic α, β, γ, and δ proton and carbon resonances of residue i and the amide and nitrogen resonances of residue i+1. Together, these H_C_CA_N_HN and H_C_CO_N_HN TOCSY experiments provide all of the information needed to determine most of the backbone and sidechain resonance assignments in small proteins. These "straight-through" triple resonance experiments work well despite the fact that $^{13}C^\alpha$ transverse relaxation times in proteins are relatively short. The combined experiments have been used to obtain an extensive set of sequence-specific ^{1}H, ^{15}N, and ^{13}C resonance assignments for an isotope-enriched IgG-binding domain of Protein A (Z-domain[4], 8.2 kDa) derived from Staphylococcus aureus. These data were obtained at 2-3 mM protein concentration with a 500 MHz spectrometer. Our progress in determining the three dimensional structure of this Z-domain of Protein A will also be presented.

(1) Montelione & Wagner, **1989** J. Magn. Reson. 87, 183. (2) Montelione & Lyons, submitted for publication. (3) Montelione et al., submitted for publicaton. (4) Nilsson et al., **1987** Protein Engineer. 1, 107.

LZ 124 EVALUATION OF ERRORS OF INTERPROTON DISTANCES AND CORRELATION TIME DETERMINED FROM NMR CROSS-RELAXATION RATES

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We have analyzed propagation of errors for determination of interproton distances and correlation time in a combined use of the two-dimensional nuclear Overhauser effect in the laboratory frame (NOESY) and in the rotating frame (ROESY).[1] In medium-sized rigid molecules this method can be applied in the intermediate motional regime, $0.2 < \omega_o\tau_c < 5$, (τ_c correlation time, ω_o resonance frequency). Error limits are smallest near $\omega_o\tau_c = 1.14$. Generally, the relative error of the calculated correlation time is higher than the error of input cross-relaxation rates. To determine correlation time at the outskirts of the intermediate motional regime, cross-relaxation rates must be measured with high accuracy. With relative error in cross-relaxation rates of 30%, a good estimate of correlation time can be obtained only when $0.2 < \omega_o\tau_c < 5$, and after statistical averaging over a large number of proton pairs. Averaging can be performed over all spin pairs that have the same motional properties (not necessarily the same interproton distances).

The method was tested on six geminal proton pairs in the bicyclic octapeptide (S-deoxo-γ-[R]-OH-Ile[3] amaninamide, Mw = 870) for which at 297°K in DMSO, a correlation time of 1.0 ns, with a standard deviation of 0.12 ns, and an interproton distance of 1.87 Å, with standard deviation of 0.04 Å, are obtained.[2]

1. D.G. Davis, J. Am. Chem. Soc. 109, 3471 (1987).
2. C. Isernia, L. Paolillo, E. Russo, A. Pastore, G. Zanotti and S. Macura, J. Biomol. NMR, in press.

LZ 125 THE USE OF SPIN-LABELS TO MAP PROTEIN SURFACES. Henriette Molinari [1], Gennaro Esposito[2], Monica Pegna[3], Andrea Motta[4], Neri Niccolai[5], Roberto Consonni[6] and Lucia Zetta[6]
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Bound spin labels have been widely used in early seventies as a method of spectral simplification capable to give information on the locations and dimensions of the site where they are bound. As a development of this chemical perturbation approach, we have used soluble free radicals for mapping protein surfaces (assuming that radical-exposed protons are also solvent-exposed). Gramicidine S, Lysozyme and BPTI were employed as model compounds to establish the basis of the method. We report here the results of the studies performed on BPTI using the stable nitroxide TEMPOL as a paramegnetic probe. These studies were aimed i) to clearly assess a model for interaction; ii) to analyze the stability of TEMPOL with time; iii) to determine the proper scaling factor for comparing cross-peak volumes obtained in intrinsically different conditions (with and without the spin label); iv) to study the dependence of the exposed surfaces on the temperature variation. We found that our data can fit a stochastic collision model. The diffusion controlled dipolar relaxations, at 500 MHz, are such that $T_2/T_1<1$, hence the paramagnetic filtering effect is enhanced in TOCSY type experiments where the mixing time acts approximately as a T_2 filter. Our analysis concerns, till now, the backbone protons. For them the dipolar interactions were assumed to be modulated by unique correlation time and a direct comparison between differential effects and nitroxide accessibility have been attempted.

LZ 126 THE ACCURACY OF NOE DATA FROM HETERONUCLEAR 3D AND 4D NOE EXPERIMENTS, David G. Nettesheim, Robert P. Meadows, Edward T. Olejniczak, Stephen W. Fesik, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, Illinois, 60064

With the advent of heteronuclear 3D and 4D NOE experiments, additional NOEs can be resolved, leading to more precise three-dimensional structures and the possibility of determining the structure of larger proteins. However, typical 3D or 4D pulse sequences incorporate time delays that may significantly alter the final NOE intensities of protons undergoing differential relaxation. In addition, spin-diffusion can complicate the interpretation of NOE data. In this presentation, we report on our efforts to better quantitate NOEs from 3D and 4D spectra by taking into account spin diffusion effects and differential relaxation rates that can alter the NOE intensities. By improving the quanitation of the NOE data, our goal is to generate more accurate and precise structures of larger proteins and macromolecules complexes by NMR. We demostrate the approach in our NMR studies on the structure of the FKBP/ascomycin complex in solution.

LZ 127 IS THE FREE R-VALUE USEFUL FOR NMR REFINEMENT?

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Setting the relative weights of experminental and empirical terms in the target function is a non-trivial problem in the refinement of structures with X-ray data or NMR data. If the weight of the experimental term is chosen too small, the model is not fitted to the data, if it is chosen too big, the model is "fitted to the noise" in the experimental data, and the geometry of the model is distorted. The free R-value (Brünger, 1992) has been shown to be a very useful quantity to address this problem in X-ray crystallography. In brief, a randomly chosen, small fraction of the data is not included in the refinement, and the fit of the model to this excluded data set is monitored. In this contribution we investigate if a similar analysis can be carried out for NMR refinement.

The results, obtained with a model system, indicate that an analogous expression to the free R-value can indeed be valuable in NMR refinement. For example, the NMR free R-value displays a minimimum if plotted as a function of the weight on the experimental terms. At this weight, the r.m.s. difference from the ideal structure that was used to calculate the model NOESY spectrum is minimal.

A.T. Brünger, "The Free R Value: A Novel Statistical Quantity for Assessing the Accuracy of Crystal Structures", *Nature*, 355, 472-474 (1992).

LZ 128 HETERONUCLEAR 4D NMR METHODS FOR COMPLETE ASSIGNMENTS OF PROTEINS, Edward T. Olejniczak, Robert X. Xu, Timothy M. Logan, Andrew M. Petros and Stephen W. Fesik, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064

A prerequisite for determining high resolution protein structures by NMR is the resonance assignments. In this presentation new four-dimensional NMR experiments are described for assigning the ^{1}H,^{13}C and ^{15}N resonances of proteins that involve heteronuclear and homonuclear correlations of the backbone and sidechain resonances. The increase in resolution and additional correlations observed in the 4D NMR experiments greatly facilitates the manual and automated assignment of the resonances.The 4D experiments are applied in the assignment of the ^{1}H,^{13}C, and ^{15}N resonances of [U-^{15}N,^{13}C] FKBP binding protein when bound to the immunosuppressant, ascomycin.

LZ 129 THE PRECISION OF NMR PROTEIN STRUCTURES - A MONTE CARLO ANALYSIS, John Shriver and Stephen Edmondson, Medical Biochemistry, Southern Illinois University School of Medicine, Carbondale, IL 62901.

An accurate estimate of the precision with which a protein structure is determined by NMR data is essential for reliable application of the structural information. The present method of basing the precision on an ensemble of structures derived from repeated analyses of the same data set is unreliable due to a lack of consideration of residuals (except for overall R-factors), the influence of arbitrary, user-specified weighting factors on the spread of the ensemble, and the *ad hoc* assignment of effective confidence intervals prior to the data analysis. A straightforward, Monte Carlo procedure is introduced for accurately defining the precision with which the Cartesian coordinates of any macromolecular structure are determined by nuclear Overhauser data. The method utilizes an ensemble of structures obtained from an array of independent simulated data sets derived from the final model structure. Using the noise-free, back-calculated NOE spectrum as the "true" NOE spectrum, 300 simulated data sets are created by superimposing onto the "true" spectrum Gaussian distributed noise with a standard deviation equal to that of the NOE residuals. These data sets represent what would be observed if the NOE experiment were repeated and the model were correct. Full relaxation matrix refinements of the 300 simulated data sets provides confidence probability distributions of the Cartesian coordinates for each atom in the model. The procedure is applied here to the 22 residue peptide hormone motilin, and it is shown that the conventional method of analyzing repeated fits of the same data tends to overestimate the precision (i.e. the root mean square deviations are smaller) when compared to the Monte Carlo method. The inclusion of the noise of the fit enhances the effect of sparse information compared to what is observed with the conventional method.

LZ 130 REFINEMENT OF THE SOLUTION STRUCTURE OF CALBINDIN D_{9k} IN THE APO STATE USING CORMA.
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Members of the calmodulin/troponin C family of Ca^{2+}-binding proteins regulate a wide variety of cellular processes through their ability to "translate" changes in intracellular calcium concentrations, whilst other members are involved in intracellular Ca^{2+} transport and buffering. We have studied a member of this family (calbindin D_{9k} - 75 residues, 8.5 kDa), to determine the exact nature of the structural and dynamical changes associated with Ca^{2+} binding. Analysis of a number of NMR parameters indicate that the differences in structure between the apo and $(Ca^{2+})_2$ states are subtle, and that the highest quality structures would be required to make a meaningful comparison. Structures for the apo form have been calculated using distance geometry, followed by refinement with restrained molecular dynamics calculations. The input for these calculations consisted of over 1000 distance constraints (from NOEs) and over 100 dihedral angle constraints (from scalar coupling constants, and local geometry grid searches). The resulting structures still contained violations of the input constraints which were of an unacceptable size. A simple protocol utilizing CORMA calculations (J. W. Keepers & T. L. James, 1984, *J. Magn. Reson.* 57, 404-426) was used to check the consistency of the input constraints with the final structures. By this method numerous ambiguities and errors in the input constraints were identified. Re-calculation of the structures using updated constraints produced structures that contained significantly lower violations. In addition, several regions of the structures became better defined after the CORMA process, presumably through the removal of conflicting erroneous constraints. Details of the calculations, and the final structures will be presented.

LZ 131 SYMMETRICALLY-SHIFTED PULSES FOR SOLVENT SUPPRESSION,
Steve Smallcombe, Varian Associates, Palo Alto, CA 94063
The use of shaped pulses can be of great benefit in the battle to suppress large solvent resonances such as water. Several topics are discussed herein that are particularly applicable for the observation of protons exchanging with, or buried under the water resonance and are illustrated with a 2 mM DNA sample as well as several protein samples in 90% H_2O.

The first technique illustrates the use of Symmetrically-Shifted pulses, a family of shaped pulses that have been optimized for non-excitation of the water resonance and excitation profile. Symmetrically-Shifted pulses can be seen as having historical precedence in, and many of the properties of, long rectangular pulses and binomial sequences. Further improvements in water suppression are obtained by combining pulse field gradients with symmetrically-shifted pulses.

Linear Prediction software coupled with data shifting is used to eliminate the undesirable frequency dependent phase and baseline problems normally associated with pulses or excitation schemes of this length.

Zero-Frequency Solvent Subtraction is also described.

LZ 132 A METHOD FOR DETERMINING OVERALL PROTEIN FOLD FROM NMR DISTANCE RESTRAINTS, Alan S. Stern and Jeffrey C. Hoch, Rowland Institute for Science, 100 Cambridge Parkway, Cambridge, Massachusetts 02142

We describe a simple method for determining the overall fold of a polypeptide chain from NOE-derived distance restraints. The method uses a reduced representation consisting of two particles per residue and a force field containing pseudo-bond and -angle terms, an electrostatic repulsion term, but no van der Waals or hard shell repulsive terms. The method is fast and robust, the correct mirror image is readily determined, and the results agree well with those obtained using other methods. The method can be implemented using commercially available molecular modeling packages.

LZ 133 Can a Single Structure be Used to Represent an Ensemble of NMR-derived Protein Structures?

Michael J. Sutcliffe, Biological NMR Centre, University of Leicester, PO Box 138, Medical Sciences Building, University Road, Leicester, LE1 9HN, UK

The usefulness of representing an ensemble of NMR-derived protein structures by a single structure has been investigated. Two stereochemical properties have been used to assess this, namely the distribution of $\varphi\psi$ torsion angles and the distribution of χ_1 torsion angles. The results show that the minimised average structure derived from the ensemble does not always correspond well with this ensemble. An alternative method that selects the member of the ensemble which is closest to the "average" of the ensemble has been investigated. Although this method selected a structure which on the whole corresponded more closely to the ensemble than does the minimised average structure, this is still not a totally reliable means of selecting a single structure to represent the ensemble. This suggests that it is advisable to study the ensemble as a whole. A study has also been made of the practice of selecting the "best" rather than the most representative member of the ensemble. This too suggests that the ensemble should be studied as a whole.

LZ 134 BLOCK-DECOUPLED NOESY: DECOMPOSITION OF THE PROTON RELAXATION MATRIX INTO NON-INTERACTING BLOCKS. William M. Westler*, Charles G. Hoogstraten*, Slobodan Macura§, and John L. Markley*, *Department of Biochemistry, University of Wisconsin, Madison, WI 53706; §Department of Biochemistry, Mayo Foundation, Rochester, MN 55905

Block-Decoupled NOESY (BD-NOESY) separates the cross-relaxation matrix into non-interacting blocks by the use of band selective inversion pulses during the NOESY mixing time. The spins outside the inversion region are not affected by the inversion pulses and cross relax among themselves, but no cross-relaxaton occurs between the non-inverted and inverted spins. The spins within the inverted region also cross-relax among themselves. As an example, the aromatic and amide proton region is separated from the aliphatic region by applying band-selective, inversion pulses to the aliphatic region during the mixing time. BD-NOESY eliminates indirect cross-relaxation pathways involving aliphatic resonances that interfere with direct cross relaxation among amide and aromatic protons. The suppression of spin-diffusion contributions to amide-amide NOE cross peaks permits the use of longer NOESY mixing times that yield larger, more accurate peak volumes and derived distances. We report examples of spin-diffusion attenuation within the aromatic-amide region and analyze the behavior with respect to the protein structure.

LZ 135 AUTOMATED APPROACHES TO SEQUENTIAL ASSIGNMENTS OF PROTEIN SPIN-SYSTEMS AND NOE IDENTIFICATION. Michael Wittekind‡, Mark S. Friedrichs‡, Keith L. Constantine‡, William J. Metzler‡, Donna Bassolino§ and Luciano Mueller‡. ‡Macromolecular NMR Department, §Macromolecular Modeling Department, Bristol - Myers Squibb Pharmaceutical Research Institute, P. O. Box 4000, Princeton, New Jersey, 08543-4000

A strategy to assign sequential protein backbone resonances with data from triple resonance NMR experiments is outlined. Sequential $^1H\alpha$, $^{13}C\alpha$ and $^{13}C\beta$ chemical shift information obtained from analysis of 4D-HNCAHA, 4D-HN(CO)CAHA, and 3D-HNCACB experiments were used to establish linkages between spin-systems. The method also makes use of the $^{13}C\alpha$ and $^{13}C\beta$ chemical shift information obtained in the 3D-HNCACB experiment to integrate the backbone and sidechain resonances obtained from HCCH-type experiments and to help align the linked groups with the primary sequence of the protein.

Assignment of proton partners in the NOE spectra are made in an automated fashion using the 3D and 4D NOESY data. When overlap occurs, decisions are made based either on the presence of confirmatory NOEs for other expected close proton pairs or by checking distances against preliminary models.

The assignment algorithms are implemented in a macro language made up of primitive commands that manipulate information about crosspeaks, atoms and structures within a database that has been added to the FELIX 1.0 program (Hare Research, Bothell WA).

LZ 136 HETERONUCLEAR THREE-DIMENSIONAL NMR STUDIES OF WATER MOLECULES BOUND TO THE FK506 BINDING PROTEIN/ASCOMYCIN COMPLEX, Robert X. Xu, Robert Meadows, and Stephen W. Fesik, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, Illinois 60064

Recently, NMR spectroscopy has been shown to be a useful technique for elucidating the location of protein-bound water molecules. However, to date only a limited number of NMR investigations have focussed on the study of protein-bound waters. In this presentation, we report on the location of water molecules buried in the interior of the FKBP/ascomycin complex from ^{15}N-resolved 3D HMQC-ROESY spectra of [U-^{15}N]FKBP/ascomycin and ^{13}C-resolved 3D HMQC-NOESY spectra of [U-^{13}C,^{15}N]FKBP/ascomycin and FKBP/[U-^{13}C]ascomycin. These results were compared to the location of waters observed in the X-ray crystal structure of the FKBP/FK506 complex. In addition to the NOEs involving water molecules, we describe NOEs between rapidly exchanging protons of the protein and ligand. These NOEs help to define a more precise three-dimensional strucure of the FKBP/ascomycin complex.

LZ 137 NMR STUDIES OF THE FK506 BINDING PROTEIN BOUND TO A SPIN-LABELED ASCOMYCIN ANALOG, Liping Yu, Rolf Wagner, Edward T. Olejniczak and Stephen W. Fesik, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064

Solution structures of proteins determined by NMR are based upon sets of short-range (≤ 5 Å) distance and angle constraints. Using only short-range constraints, the relative location of protein segments such as loops and turns may be ill-defined by the NMR data if no NOEs connect these segments to the rest of the protein. One approach to overcome these limitations is to obtain long-range distance constraints (~15 Å) through the use of spin-labels. In this presentation, we describe our NMR studies of the FK506 binding protein when bound to a spin-labeled ascomycin analog. From the relaxation measurements of [U-^{15}N] FKBP when bound to a spin-labeled ascomycin analog, long-range distance constraints between the protein and the spin labeled ligand were measured. The importance of obtaining long-range distance constraints with spin-labeled ligands as a means of defining high resolution structures of protein/ligand complexes will be discussed.

LZ 138 EXTENSION OF DIMENSIONALITY IN 2D NOESY AND 3D HOMONUCLEAR EXPERIMENTS, Erik R.P. Zuiderweg[+*], Hong Wang [+#], Mark Fischer[+], Alex Kurochkin[+], and Gary Glick[#] Biophysics Research Division[+] and Department of Chemistry[#], The University of Michigan, Ann Arbor, MI 48109.

One key problem with the NOESY experiment, zero-quantum coherence transfer correlations, was never fully solved. These correlations interfere destructively with NOE cross peaks between scalar coupled nuclei. This is especially problematic in the NOESY spectra of oligonucleotides. Previous attempts to solve this problem have been efforts to average out these zero-quantum peaks, never assuring complete suppression, or to shift them away in the two-dimensional spectrum thereby obscuring other important cross peaks, or combinations thereof. *We have chosen to place the interfering zero-quantum cross peaks in a third dimension*, thereby removing them rigorously from the two-dimensional NOESY plane. This is achieved by shifting a 180 degree pulse systematically in the NOE mixing time. Upon Fourier transformation in this third time dimension, the interfering zero-quantum peaks are shifted into three-dimensional frequency space while the NOE spectrum remains located in a single (the first) plane of the 3D data set. The experiment is carried out in the same time as a normal NOESY experiment and has the same sensitivity and resolution.

Three-dimensional ^{1}H-^{1}H-^{1}H NOESY-NOESY, NOESY-TOCSY or TOCSY-TOCSY spectra are important analysis tools for the study of deoxy oligonucleotides in solution. The resolution of these experiments is nevertheless insufficient for analysis of the spectra of ribonucleotides, since all ribose protons resonate within 2 ppm. We are investigating the extension of these three dimensional experiments into more dimensions by using ^{13}C shift correlation. Here, we use uniformely ^{13}C labeled AMP to design and carry out *four dimensional* (^{1}H,^{1}H,^{1}H,^{13}C) TOCSY-TOCSY-HMQC, *five dimensional* (^{1}H,^{13}C,^{1}H,^{1}H,^{13}C) HMQC-TOCSY-TOCSY-HMQC and *six-dimensional* (^{1}H,^{13}C,^{1}H,^{13}C,^{1}H,^{13}C) HMQC-TOCSY-HMQC-TOCSY-HMQC. We have recorded all these experiments and are currently processing, analyzing and evaluating this data. The new methods are prototype experiments for the probably more useful TOCSY-NOESY and NOESY-NOESY variants for study of labeled oligo ribonucleotides.

Nucleic Acids, Oligosaccharides, Biomolecules with Special Problems

LZ 200 **TETRAPLEX FORMATION BY dTG_4T IN NaCl SOLUTION,** Fareed Aboul-ela#, Alastair I. H. Murchie*, and David M. J. Lilley*, #Department of Chemistry, University of Colorado, Boulder, CO 80302 *Department of Biochemistry, University of Dundee, DD1 4HN, United Kingdom

We have found that the molecule dTG_4T forms a parallel stranded tetraplex in the presence of 100 mM NaCl. We have assigned all proton and phosphorus peaks with the exception of some 5' and 5" sugar protons and have quantitated corresponding NOEs for a set of mixing times. The assignments have been made using homo and heteronuclear correlations to assign sugar protons and phosphorus resonances without reference to structural models . Aromatic resonances were then assigned using NOEs. We have also observed a pattern of NOEs between exchangeable and aromatic protons which establishes the nature of G-tetrad hydrogen bonding present as well as demonstrating parallel strandedness. Converting NOE data into constraints for model building was complicated by the fourfold symmetry of the system which leads to ambiguities in the interpretation of interstrand versus intrastrand NOEs. We show how these ambiguities can be resolved for the purpose of back calculating NOESY spectra and preliminary model building will be presented.

We would like to thank Dr. Frank van de Ven and Dr. Steven Homans and members of his group for helpful discussions.

LZ 201 A MAGNESIUM-INDUCED CONFORMATIONAL TRANSITION IN A DNA ANALOG OF THE YEAST $TRNA^{Phe}$ ANTICODON IS DEPENDENT UPON CYTOSINE METHYLATION. Paul F. Agris, Richard H. Guenther, Hanna Sierzputowska-Gracz, Vivian Dao and Yan Chen, Department of Biochemistry, North Carolina State University, Raleigh, NC 27695

The $tDNA^{Phe}_{AC}$, d(CCAGACTGAAGAU$_{13}$m^{5}C$_{14}$U$_{15}$GG), with a DNA sequence similar to that of the anticodon stem and loop of yeast $tRNA^{Phe}$, forms a stem and loop structure and has a Mg^{2+}-induced structural transition that was not exhibited by an unmodified $tDNA^{Phe}_{AC}$ d($T_{13}C_{14}T_{15}$) as determined by CD and NMR. Three similar $tDNA^{Phe}_{AC}$ molecules having m^5C_{14} exhibited the Mg^{2+}-induced structural transitions and biphasic thermal transitions, but three other $tDNA^{Phe}_{AC}$, without m^5C_{14} had no Mg^{2+}-induced structural transitions and only monophasic thermal transitions. The $tDNA^{Phe}_{AC}$ d($U_{13}m^5C_{14}U_{15}$) had a single, strong Mg^{2+} binding site [Kd of 1.09×10^{-6} M; $\Delta(\Delta G)$ of -7.75 kcal/mol]. The predominant structure of $tDNA^{Phe}_{AC}$ d(U-m^{5}C-U) in the absence of Mg^{2+} was a hairpin containing a 7 nucleotide loop and a stem composed of 3 stable base pairs. In the Mg^{2+}-stabilized conformation, the loop of $tDNA^{Phe}_{AC}$ d(U-m^{5}C-U) became a two-base turn due to the formation of two additional base pairs across the loop: $C_6 \bullet G_{11}$ and $T_7 \bullet A_{10}$. Adenosine-12 "bulged", but was part of a 3'-stack that includes the anticodon. The Mg^{2+} is in the upper part of the tDNA hairpin loop near the $A_5 \bullet dU_{13}$ base pair. The $tRNA^{Phe}_{AC}$ (U-dm^{5}C$_{14}$-U), in contrast to $tRNA^{Phe}_{AC}$ (U-C$_{14}$-U), also demonstrates a single strong Mg^{2+} binding and conformational transition. The $tRNA^{Phe}_{AC}$ structure [without d(m^{5}C)] in the presence of 5 mM Mg^{2+} had four of the five stem base pairs and no base interactions across the loop. We conclude that m^{5}C facilitates a site-specific Mg^{2+} binding without 2'-OHs and a conformational change in the anticodon loop which for tRNA has important functional implications.

LZ 202 MULTIDIMENSIONAL SOLID-STATE AND SOLUTION NMR STUDIES OF MAGAININ ANTIBIOTIC PEPTIDES IN MEMBRANES, Burkhard Bechinger[1], Jennifer Gesell[1], Michael Zasloff[2], and Stanley J. Opella[1], [1]Department of Chemistry, University of Pennsylvania, Phiadelphia, PA 19104 and [2]Magainin Pharmaceuticals Inc., Plymouth Meeting, PA 19462

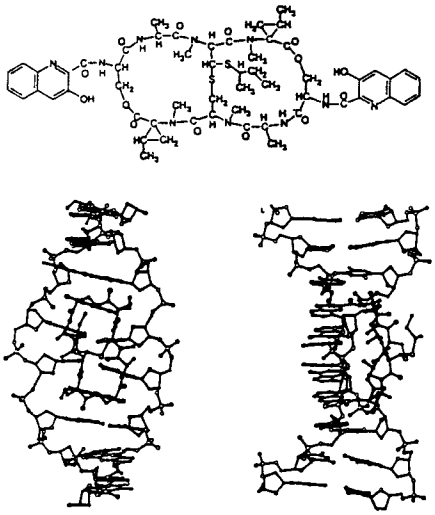
Magainins are a family of broad-spectrum antibiotic peptides originally found in frog skin that decouple the ionic gradient across bacterial, fungal, and tumor cell membranes. Magainin2 (23 residues) and PGLa (21 residues) were chemically synthesized with ^{2}H, ^{13}C, and ^{15}N incorporated into specific residues. Multidimensional solution NMR experiments on these peptides in SDS and DPC micelles showed them to be alpha helical throughout their length. Interestingly, the continuous helix appears to have a break at Gly11 in PGLa, which is the cleavage site of the magainin endopeptidase. Solid-state NMR experiments on these peptides in oriented phospholipid samples also show that they are alpha helical throughout their length and that the axis of these amphipathic helices is parallel to the plane of the bilayers. The peptides are immobilized by their interactions with lipid bilayers, enabling the dynamics of individual lipid and peptide sites to be described by solid-state NMR experiments on unoriented samples.

LZ 203 **INTERACTIONS BETWEEN MAGAININ AND LIPID BILAYERS: A REDOR NMR AND FT-IR STUDY,** Jack Blazyk*, Melody Ferguson*, Jin Hua*, Andrew W. Hing† and Jacob Schaefer†, *Chemistry Department, Molecular and Cellular Biology Program, and College of Osteopathic Medicine, Ohio University, Athens, Ohio 45701, and †Chemistry Department, Washington University, St. Louis, MO 63130.

Magainin 2, a naturally occurring 23-amino acid cationic peptide isolated from the skin of the African clawed frog, is lethal to a wide variety of microorganisms and enveloped viruses. The bactericidal activity of the peptide is believed to be associated with the disruption of the integrity of the membrane system of the target organism. Magainins have the potential to adopt a highly amphiphilic α-helical secondary structure, which is typical for many membrane-active peptides. Unlike other cationic peptides, such as melittin, which are cytotoxic, magainins are nonhemolytic at antimicrobial concentrations. Thus, magainins and related peptides may have therapeutic potential as antibiotics. The molecular nature of the interaction between magainin 2 and the cell surface has yet to be determined. Does magainin act similarly to detergents to disrupt membrane structure, or do oligomeric clusters of magainins form well-defined ion channels through the membrane? Recent evidence suggests that magainins may form cation channels and that the helical axis of magainins lies parallel to the plane of the bilayer. We have applied the new solid-state NMR technique, REDOR (rotational-echo double-resonance), to define the proximity of ^{15}N-labeled magainin and ^{31}P in the polar headgroups of DPPG bilayers. By measuring ^{15}N-^{31}P dipolar coupling in the REDOR experiment, it is possible to determine approximate distances between these nuclei. Lipid and peptide structure also is evaluated by FT-IR spectroscopy to monitor effects on lipid fluidity and peptide secondary structure using C–H stretching and amide I bands, respectively. Future REDOR and TEDOR (transferred-echo double-resonance) experiments to explore oligomer formation and other lipid-peptide interactions will be discussed.

LZ 204 SOLUTION STRUCTURE OF A QUINOMYCIN BISINTERCALATOR-DNA COMPLEX, Huifen Chen[1], Xiucai Liu[1] and Dinshaw J. Patel[1,2], [1]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032 and [2]Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

We have determined the solution structure of the complex between the bisintercalating quinomycin antibiotic UK-63052 and the self-complementary d(A-C-A-C-G-T-G-T) duplex by a combined NMR-molecular dynamics study. The resulting structure provides details about the bisintercalation site where the quinomycin chromophores sandwich the central (C-G)•(C-G) step, the alignment of the bicyclic peptide in the minor groove and the peptide-nucleic acid interactions that stabilise the complex.

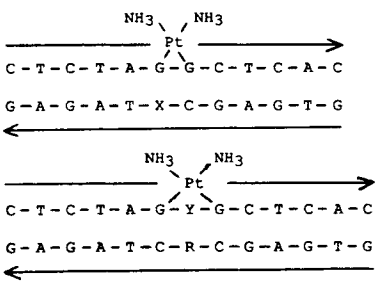


LZ 205 NMR-ENERGY MINIMIZATION STUDIES OF COVALENT STEREOSPECIFIC BENZO[a]PYRENE-DNA ADDUCTS, Monique Cosman[1,2], Carlos de los Santos[1,2], Brian Hingerty[3], Victor Ibanez[4], Nicholas E. Geacintov[4], Suse Broyde[5], and Dinshaw J. Patel[1,2]. [1]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032 [2]Program of Cellular Biochemistry and Biophysics, Sloan Kettering Cancer Center, New York, NY 10021, [3]Oak Ridge National Lab., Oak Ridge TN 37831, and [4]Chemistry and [5]Biology Departments, New York University, New York, NY 10003

The solution conformations of three stereoisomeric covalent adducts derived from the highly tumorigenic (+)-BPDE and the non-tumorigenic (-)-BPDE (*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene) at the C10 position to N2 of guanine in the duplex [d(CCATCG^{BP}CTACC): d(GGTAGCGATGG)] have been studied by 2D-NMR in combination with potential energy minimization techniques. In both the (+)-*trans* and (-)-*trans* adducts, the BPDE moieties are located in a widened minor groove of a minimally perturbed B-DNA helix. In both cases, one face of the aromatic pyrenyl ring system stacks over the sugar residues of the unmodified complimentary strand, while the other face is solvent exposed. An important distinction between these two mirror image stereo-isomers is that the BP ring is oriented towards the 5'-end of the modified strand in the (+)-*trans* adduct, and in the opposite direction (3'-end) in the (-)-*trans*. The conformation of the third stereoisomer studied , the (+)-*cis* adduct, is uniquely different from that of the *trans* adducts. The BP moiety intercalates into the helix forming stacking interactions with the flanking G-C base pairs. The modified guanine and its partner cytosine are denatured and displaced into the minor and major grooves, respectively. The differences in the solution conformations of these three adducts emphasizes the influence of chirality in structure determination. The structure of the adduct, in turn, may predetermine how the BPDE lesion is processed in a living cell.

LZ 206 SOLUTION STRUCTURAL STUDIES OF *cis*-Pt$(NH_3)_2^{2+}$ COORDINATED TO (G-G) AND (G-Y-G) SITES IN DNA Carlos de los Santos,[1,2] Carla van Garderen,[1] Leo van Houte,[1] Lawrence Shapiro,[1] Steve F. Bellon,[3] Stephen J. Lippard[3] and Dinshaw J. Patel[1,2].
[1]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032, [2]Department of Cellular Biochemistry and Molecular Biophysics, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, [3]Department of Chemistry, MIT, Cambridge, MA 02139

We have prepared, purified and structurally characterized by NMR spectroscopy and molecular dynamics calculations two families of *cis*-Pt coordinated DNA oligomer duplexes. The first set included coordination of *cis*-Pt at adjacent guanine N^7 atoms in the 5'(G-G)3' step with either 5'(C-C)3' or 5'(C-A)3' steps positioned opposite the coordination site (see 1 , X=C,A). The second set includes coordination of *cis*-Pt at 5'(G-Y-G) steps with 5'(C-R-C)3' steps positioned opposite the coordination site (see 2, Y•R= T•A and C•G). These studies define the nature and extent of structural distorsions associated with *cis*-Pt coordination on the DNA helix.



1, X = C, A

2
Y•R = T•A, C•G

LZ 207 MULTINUCLEAR MAGNETIC RESONANCE STUDIES OF ARSENIC-GLUTATHIONE COMPLEXES.

Marielle Delnomdedieu, Mufeed M. Basti, James Otvos and David J. Thomas, Center for Environmental Medicine UNC Chapel Hill , HERL US EPA RTP and Dept of Biochemistry NCSU, Raleigh,NC.

Interaction of metals with glutathione (GSH) represents an integral part of the toxic response of many metals. GSH appears to be an early binding target for arsenic. However, little is known about the characteristics of the As/GSH complex(es). ^1H , ^{13}C ,^1H-^{13}C correlation and ^{75}As NMR experiments were carried out to investigate the complexation of As(V), the dominant species in drinking water, with GSH. With As(V), a series of NMR spectral changes are observed up to a GSH/As(V) ratio of 5. When increasing the ratio (R) of [GSH]/[As(V)], two consecutive reactions occur: from R>0 to R=2, As(V) induces the oxidation of GSH to GS-SG, while being reduced to As(III); from R>2 to R=5, the thiol group of GSH reacts with As(III) to form the $(GSH)_3As(III)$ complex. Similar experiments carried out with As(III) did not induce any oxidation of GSH, but demonstrated the same complex formation with GSH, with a ratio 3 to 1. ^{75}As NMR provided new information about the kinetics of the complex formation.

Our findings underlie the critical role of GSH in the reductive metabolism of As(V) to As(III) and the formation of As-GSH complexes, which may play an important role in the subsequent metabolism of As(III) to methylated species.

(This abstract does not necessarily reflect EPA policy)

LZ 208 BINDING MODES OF DISTAMYCIN A WITH VARIOUS I-C CONTAINING DNA OLIGOMERS,

Patricia A. Fagan and David E. Wemmer, Department of Chemistry, University of California, Berkeley, CA 94720

Distamycin A is a cationic peptide antibiotic which binds deep into the minor groove of A-T rich regions of B-form DNA. Distamycin exhibits particular sensitivity to the sequence of the binding site, binding either in a one-drug binding mode, in which one drug molecule is bound in the minor groove, or in a two-drug binding mode, in which two drug molecules are bound antiparallel and *side by side*, in the same region of the minor groove. One drug molecule fits snugly into the narrow minor groove of A-T regions, but the presence of the second drug requires that the groove expand considerably.

We have examined the binding of distamycin to several A-T rich tracts, and various I-C containing analogues. An I-C base pair has minor groove functional groups which are completely equivalent to an A-T base pair, so that any difference in distamycin binding presumably arises from structural rather than functional differences. Distamycin has been shown to bind the site AAATT with intermediate cooperativity; however, it exhibits very high cooperativity (exclusively two-drug binding) toward the minor groove analogue IIICC. We have shown here that the insertion of I-C base pairs in an A-T rich site results in detectable changes in drug binding. The presence of inosine at specific locations within the site may induce a widening of the minor groove, resulting in increased cooperativity of drug binding. More detailed conformational studies are in progress.

LZ 209 NMR INVESTIGATIONS IN TO THE EFFECT OF COMPLEXATION OF A DNA DUPLEX WITH A THIRD STRAND

Fawthrop, Susan A. and Fisher, Julie. School of Chemistry, Leeds University, Leeds, LS2 9JT, England.

The recognition by a double helical DNA molecule of a single stranded DNA molecule, to give a Triplex has become the subject of intense interest over the past few years. Increasingly, evidence is appearing in support of the presence, and hence physiological importance of triplexes *invivo*. There is in consequence an incentive to study DNA, RNA and DNA/RNA hybrid triplexes and to investigate their potential as therapeutic agents (ref 3).

A key to the development of our understanding of the recognition processes which lead to triplex formation is our ability to identify structure / function relationships.

It is now over twenty years since triplexes were observed in x-ray fibre diffraction studies on synthetic homopolymers (ref 1). However, inspite of the increased interest in triplexes, detailed knowledge of their structure is only beginning to emerge (ref 2).

The purpose of this work is to use high resolution nmr to investigate the structure and dynamics of a DNA intermolecular triplex, with the aim to identify the effects of complexation on a "target" duplex and any stabilising interactions. Full characterisation of the "target" duplex using a 500MHz nmr spectrometer must first be carried out if these effects are to be fully quantified. Presented here will be the structural details of the duplex chosen for the complex and the work to date carried out on the triple helix complex.

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LZ 210 STYRENE OXIDE ADDUCTS AT ADENINE N^6 IN AN OLIGONUCLEOTIDE CONTAINING THE RAS CODON 61 SEQUENCE, Binbin Feng, Liang Zhou, Mariella Passarelli, Thomas M. Harris, and Michael P. Stone, Department of Chemistry, Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37235.

Mutations which occur in critical DNA sequences are believed to play an initiating role in molecular carcinogenesis. Chemical modifications which cause single base mutations within codons 12 and 61 in the ras proto-oncogene coding sequence are associated with oncogene activation. We constructed d(CGGACAAGAAG):d(CTTCTTGTCCT), containing n-ras codon 61. NMR analysis of this oligonucleotide reveals it to be in a B-type conformation; a detailed structural refinement is now underway. Styrene is not as toxic as the polycyclic aromatic hydrocarbons, but it is manufactured on a large scale resulting in risk of exposure to workers in the chemical industry. Stereospecific α-styrene oxide adducts have been incorporated at the N^6 positions of A(6) and A(7) in this sequence. These adducts were prepared using a non-biomimetic synthetic strategy whereby the enantiomeric phenylglycinols were reacted with matrix-linked oligomers containing 6-chloropurine deoxyriboside at either position (6) or (7) in the sequence. Subsequent deprotection and purification yielded both R and S stereoisomeric α adducts of styrene oxide at A(6) and A(7). These adducts allow us to probe how stereochemistry of adduction and DNA sequence context influence adduct conformation. Mutagenesis experiments designed to probe for substitution mutations [R.S. Lloyd and coworkers] reveal stereo- and sequence-specific effects in the processing of the these lesions. α-Styrene oxide adduction at adenine N^6 destabilizes the DNA duplex as evidenced by optical measurements of T_m. The greatest destabilization is observed for the S-α-styrene oxide adduct at A(6). The NMR spectra of the R-α-styrene oxide adducts at A(6) and A(7) have been assigned using a combination of NOESY and TOCSY experiments. Structural refinement of these adducts is in progress. Supported by the NIH: ES05355 (M.P.S. & T.M.H.) and RR05805 (NMR Spectrometer).

LZ 211 A TETRAMERIC STRUCTURE OF THE ACID FORM OF 5'-dTCCCCC. Kalle Gehring, Jean-Louis Leroy, Maurice Guéron, Groupe de Biophysique, École Polytechnique & URA CNRS 1254, 91128 Palaiseau, FRANCE.

Using NOESY, COSY and heteronuclear ^1H-^{31}P TOCSY (1), we have completely assigned the resonances of the complex formed by 5'-dTCCCCC, at strand concentrations of 1-10 mM. ¶ There are only six proton spin systems, and five ^{31}P peaks. The imino proton intensity is 0.5 per cytidine. These numbers, and the NOESY signatures of an *anti* glycosidic angle, denote parallel strands forming hemiprotonated base pairs (2,3), as for polycytidine (4). But there are strong NOESY contacts, e.g. between C2 and C5, and C2 and C6, which cannot be explained in a two-stranded structure. ¶ A model which fits the NMR results is a tetramer made of two identical parallel duplexes, (antiparallel to one another) and whose base pairs are *intercalated*. Symbolizing each *base pair* by its number, the tetrad is represented by : 1 6 2 5 3 4 4 3 5 2 6 1. It is a 12 base-pair stack with 4 sugar-phosphate backbones. It has two C2 symmetry axes: one along the 'helix' axis and one perpendicular to it. ¶ The model explains some striking features of the NMR spectra, for instance that the C4 imino proton has NOEs to the H6 protons of only C4 and C3, whereas all other cytidines have such NOEs to *three* H6 protons. A preliminary computed structure also shows agreement with some very unusual inter-residue sugar-sugar NOEs (H1'-H1', H1'-H2", and H1'-H4'), and with the absence of inter-residue aromatic NOEs in D_2O solution. These features could be characteristic of such tetrameric structures. The model may also explain the very long exchange times of the imino protons of C3, C4 and C5 (3). The formation of tetramers has now been confirmed by excluded volume chromatography. ¶An intringing feature of the model is the lack of excluded sites for intercalation. In comparison with recently described G-tetrad structures, the C-tetrads differ by being formed of *base pairs*, but are similar in the long imino proton exchange times (5) and in the presence of a positive charge on the 'helix' axis.

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LZ 212 NMR CHARACTERIZATION OF A HETERO-COMPLEX FORMED BY DISTAMYCIN-A AND ITS ANALOG 2-IMD WITH DNA: IMPLICATIONS FOR THE DESIGN OF SEQUENCE-SPECIFIC MINOR GROOVE BINDING MOLECULES, Bernhard H. Geierstanger, Tammy J. Dwyer and David E. Wemmer, Graduate Group in Biophysics and Department of Chemistry, University of California, Berkeley, CA 94720

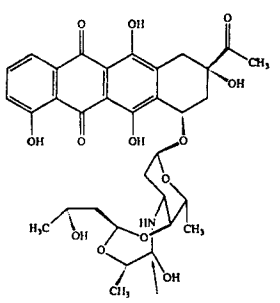
The peptide-linked polypyrrole distamycin-A binds non-covalently to the minor groove of DNA. While distamycin A preferentially targets AT-rich sequences, analogs in which an imidazole ring is substituted for a pyrrole ring can be designed to specifically recognize GC-containing sequences through hydrogen bonds with the guanine amino protons. NMR studies have shown that two ligand molecules bind simultaneously to the minor groove of a five base pair binding site in a head-to-tail orientation. Equimolar amounts of distamycin-A and the pyrrole-imidazole-pyrrole ring system 2-ImD form a specific complex with d(CGCAAGTTGGC):d(GCCAACTTGCG). One 2-ImD and one distamycin-A molecule are staggered similarly to the previously characterized 2:1 homocomplexes. The 2-ImD ligand lies along the AAGTT strand with the imidazole nitrogen of the ligand close to the guanine amino group. NMR titrations confirm that this 2-ImD:distamycin-A heterocomplex has a higher binding affinity for the central AAGTT site than the 2-ImD 2:1 complex as well as the distamycin-A 2:1 complex. This indicates that binding to GC containing sequences can be enhanced when a single hydrogen bond acceptor per guanine amino group is strategically positioned for complexation.

LZ 213 EFFECT OF 5-FU INCORPORATION ON THE STRUCTURE OF DUPLEX RNA, William H. Gmeiner, Simon Sherman, Parag Sahasrabudhe, Eppley Institute For Research in Cancer and Other Allied Diseases, University of Nebraska Medical Center, Omaha, NE 68198-6805

The anti-tumor agent 5-fluorouridine is in widespread clinical use. Although rationally designed to inhibit thymidylate synthase, there is longstanding and growing evidence that 5-FU interferes with RNA mediated processes, including the splicing of pre-mRNA. Nuclear magnetic resonance spectroscopy is used to examine the effects of 5-FU incorporation on the structure of RNA duplexes. The phosphoramidite of 5-FU has been prepared and incorporated into several RNA decanucleotides. 2D NMR studies on these RNA duplexes are performed in order to understand the structural basis for the RNA mediated toxicity of 5-FU. A battery of traditional 2D NMR techniques including COSY, DQCOSY, TOCSY, and NOESY are employed to assign the ^{1}H resonances. Variations of these sequences that incorporate pulsed field gradients to reduce or eliminate phase cycling and therefore the experiment time are being utilized. New methods for determining RNA structure from NMR data that utilize a Bayesian statistics approach to restrict the conformational space searched in the structure determination process are explored. The torsional angles for dinucleotide segments are determined by the evaluation of coupling constants and interproton distances. A comparison of the NMR data and the postulated structure for RNA duplexes with and without 5-FU incorporated is presented.

LZ 214 SOLUTION STRUCTURE OF A COVALENT SN07 ANTHRACYCLINE-DNA ADDUCT, S. Gopalakrishnan and Dinshaw J. Patel, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032 and Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

We have synthesized the N^2-guanine adduct of the SN07 anthracycline chromophore **1**, the active moiety of the SN07 macromolecular antibiotic and solved the solution structure at the DNA oligomer duplex level by a molecular dynamics analysis of the NMR based distance restraints. The SN07 chromophore reacts with the self-complementary d(A-G-C-A-T-G-C-T) duplex to form the symmetrical d(A-[SN]G-C-A-T-G-C-T) duplex. The adduct is anchored at two points through covalent linkage of the SN07 sugar moiety at G2(N^2) and intercalation of the anthracycline ring into the (C3-A4)·(T5-G6) step as depicted in **2**. The long axis of the intercalated chromophore is orthogonal to the long axis of the flanking base pairs. Adduct formation is sequence specific for guanines in 5'-(G-C)-3' steps and this specificity results from intermolecular hydrogen bonds between the SN07 sugar positioned in the minor groove and the DNA.



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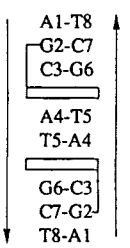

2

LZ 215 STRUCTURAL BASIS FOR ENHANCED AFFINITY OF RNA HAIRPIN LOOP-LOOP INTERACTIONS, Razmic S. Gregorian Jr., John P. Marino, and Donald M Crothers, Department of Chemistry, Yale University, New Haven, CT 06511

Replication of the plasmid Col E1 is regulated by the action of two complementary RNAs, termed RNA I and RNA II, and a plasmid-encoded protein, ROM. The binding of the two RNAs, which leads to inhibition of plasmid replication, begins with the binding of the two RNAs through complementary loop regions. Enzymatic probing has shown that individual RNA hairpin loops can also form a complex and bind ROM. Complexes are formed by a wide variety of sequences and loop sizes. Interestingly, inverting the wild-type loop sequence 5' -to- 3' results in a loop-loop complex which is 1000-fold more stable than wild-type and has markedly slower association and dissociation rates.[1]

The inverted sequence complex forms at one-tenth the magnesium concentration required for wild type and has a lifetime nearly 10,000 times as long. Using UV melting, gel and stopped-flow kinetic methods we have discovered several other mutants which also display such properties and have been able to determine the sequence motif necessary to obtain strong binding and slow kinetics. Mutations in the center of the loops result in stabilities which can be explained in terms of Watson-Crick pairing stabilization energies. However, mutations at the 5' or 3' ends of the loops result in stabilities, association and dissociation rates which cannot be explained by nearest-neighbor interactions alone. Model RNA systems indicate that coaxial stacking of the three helices resulting in the complex is important to achieve enhanced affinity, but can only account for a portion of the increase.

Formation of the RNA-RNA complexes by several sequences has also been demonstrated using one-dimensional exchangeable NMR experiments. Gradient-enhanced two-dimensional H_2O NOESY experiments have enabled assignment of exchangeable resonances to determine base-pairing and stacking schemes. Analysis of NOESY spectra in D_2O and heteronuclear experiments will also be presented. We compare results for wild-type and inverted loop sequences to elucidate principal structural determinants of variations in physical properties, focusing on the stem-loop junction regions.

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LZ 216 FORMATION OF MULTIPLE COMPLEXES BETWEEN ACTINOMYCIN D AND DNA HAIRPINS: SOLUTION STRUCTURES AS DETERMINED BY MULTINUCLEAR NMR
Victor L. Hsu, Michael Kurz, David R. Brown and David R. Kearns, Department of Chemistry, B-042, University of California, San Diego, La Jolla, CA 92093-0342

In an effort to understand the manner in which actinomycin D (ActD) binds unusual DNA structures, we investigated the influence of the binding of the drug on the structure of DNA hairpins. Hairpin loops are important secondary structural elements in nucleic acids which serve as recognition sites for proteins. One of the best characterized families of hairpins contains four thymine nucleotides in the loop region and we chose to study d(TCGCGTTTTCGCGA). Two distinct ActD-d(TCGCGTTTTCGCGA) complexes are formed, which differ by the orientation with which the asymmetric chromophore intercalates at the d(GC) step. We have made the complete assignments of the proton chemical shifts of the free DNA hairpin, and for both ActD:DNA complexes. ^{13}C HMQC and HMBC experiments were used to unequivocally identify each of the two sequence-identical peptide rings attached to the phenoxazone ring of ActD. Assignment of the phosphorus chemical shifts in the free DNA and in the complexes allowed us to monitor conformational changes in the DNA backbone upon intercalation and also assisted in confirming the sequential assignments for the oligonucleotide. Approximately 75 intermolecular NOE crosspeaks were identified for each of the two complexes and were used as distance constraints for restrained molecular dynamics and energy minimization calculations for the structure determination of the complexes. Effects of drug binding on the loop structure for this and a related hairpin will be discussed.

LZ 217 RNA STRUCTURAL MOTIFS - A STUDY ON THE CUUG RNA HAIRPIN BY HETERONUCLEAR MULTIDIMENSIONAL NMR. Fiona Jucker & Arthur Pardi, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309.
RNA is among the oldest known biomolecules and even today this class of molecules is involved in key processes throughout the cell. Knowledge of the structure of RNA folding motifs, the building blocks of larger RNAs, is crucial for understanding both RNA-RNA and RNA-protein interactions, however presently very few 3D structures of RNA have been determined. RNA hairpins (stem-loop structures) represent a group of ubiquitous RNA folding motifs. We have synthesized an RNA hairpin containing the CUUG loop, a tetraloop sequence frequently occuring in ribosomal RNA. To determine the high resolution structure of the CUUG RNA hairpin we applied heteronuclear multidimensional NMR experiments similar to those developed for proteins. A ^{13}C and ^{15}N doubly labeled RNA hairpin was synthesized by *in vitro* transcription. Labeled rNTPs were generated by enzymatic conversion from labeled ribosomal RNA, which was isolated from *E.coli* cells grown on ^{13}C-glucose and ^{15}N-ammonium sulfate. 2D, 3D and 4D heteronuclear multidimensional NMR experiments have been used to obtain complete assignments of the non-exchangeable protons and proton-bound carbons, and to extract noe distance constraints and torsion angle constraints. Structures consistent with the constraints were generated using the programsDSPACE and AMBER. The refined structure of the CUUG RNA hairpin loop will be compared with the published structures of the UUCG and GAAA RNA hairpin loops and the implications for RNA-RNA and RNA-protein interactions will be discussed.

LZ 218 EVIDENCE FOR MOBILITY AT THE TpA JUNCTION IN T_nA_n-CONTAINING DNA OLIGOMERS
Michael A. Kennedy*,‡,§, Sirkku T. Nuutero§, Jeffery T. Davis§, Gary P. Drobny‡,§ and Brian R. Reid*,§,‖ ‡Battelle Pacific Northwest Laboratories, Richland, WA 99352 §Department of Chemistry, University of Washington, Seattle, WA 98195 ‖Department of Biochemistry, University of Washington, Seattle, WA 98195

In 500 MHz ^1H NMR experiments, the H2 and H8 protons of adenine, which reside in the minor and major grooves respectively, are observed to be unusually broadened for the adenine at the TpA junction in the T_nA_n-containing DNA oligonucleotides $[d(CGAGGTTTAAACCTCG)]_2$, $[d(GCTCC-TTTAAAGGAGC)]_2$, and $[d(GCCGTTAACGGC)]_2$. In 16-mers that contain C_2A_3, G_2A_3, and A_3T_3 tracts, all adenine aromatic proton resonances are narrow, indicating that the adenine broadening occurs only at TpA steps in T_nA_n tracts. The usual mechanisms that lead to differential linewidths for aromatic protons in DNA, e.g. unresolved J coupling or strong fluctuating dipolar interactions, do not account for the increased linewidth of the adenine proton resonances at TpA junctions. Other mechanisms that could cause line-broadening, such as paramagnetic impurities, imino proton exchange, and hairpin-duplex equilibria, were systematically eliminated. A classic line-broadening mechanism in solution-state NMR is chemical exchange. Assuming chemical exchange in the form of conformational dynamics, e.g. oscillation of the purine base about the glycosidic torsion angle, χ, 500 MHz ^1H $T_{1\rho}$ and 2D-NOESY data were used to constrain the correlation time of the internal motion of the adenine base to a range between the T_1 and $T_{1\rho}$ minima, which translates into a τ_c of 1.0×10^{-10} sec rad^{-1} to 1.0×10^{-5} sec rad^{-1} for a spin-locking field of ca. 13.8 kHz. Calculated lineshapes for a two-site exchange process using correlation times near the $T_{1\rho}$ minimum indicate that motion with a τ_c of ca. 1.0×10^{-5} sec rad^{-1} to 1.6×10^{-4} sec rad^{-1} is the probable cause of the experimentally observed line-broadening, via ring-current effects.

LZ 219 THE ROLE OF α-HELIX STABILITY IN A PARTLY FOLDED APOMYOGLOBIN INTERMEDIATE, Thomas Kiefhaber and Robert L. Baldwin, Department of Biochemistry, Beckman Center, Stanford University Medical Center, Stanford, CA 94305.
Due to the high level of co-operativity of protein stability transitions, folding intermediates are usually not detectable under equilibrium conditions. One example for the presence of a stable folding intermediate is the low pH-induced unfolding transition of apomyoglobin. Native apo-myoglobin at neutral pH is a compact molecule with an α-helix content of ~55 percent. Upon lowering the pH native apo-myoglobin unfolds in two distinct stages. The first transition leads to a compact intermediate with a helix content of ~35 percent. An expanded state with little helix content is formed in the second transition. Hydrogen exchange studies in combination with two-dimensional ^1H-NMR experiments revealed that in the intermediate state protons in the A, G, and H helices are protected from exchange, whereas protons in the B and D helices exchange freely. This lead to a structural model for the folding intermediate in which a subdomain consisting of the A, G and H helices remains folded while the rest of the molecule is essentially unfolded.

To test the hypothesis that the the structure of the intermediate is determined by the stability of the individual α-helices of apomyoglobin we introduced helix stabilizing mutations at specific sites in the B-helix. In peptides corresponding to the isolated B-helix these amino acid replacements increase the α-helical content from ~5 percent in the wild type sequence to ~25 percent in the variant peptides as determined by circular dichroism. In the intact protein the mutations do not change the stability and helix content of native apo-myoglobin at neutral pH but they influence the structure of the folding intermediate as judged by its CD and fluorescence properties. Whether the B-helix of apo-myoglobin is present in the folding intermediate of the mutant protein will be determined by hydrogen exchange experiments in connection with two-dimensional ^1H-NMR studies.

LZ 220 STRUCTURAL STUDIES OF RNA ENZYMES BY HETERONUCLEAR MULTI-DIMENSIONAL NMR, Pascale Legault and Arthur Pardi, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO, 80309-215

^{13}C and ^{15}N isotopic labelling of RNA in combination with multi-dimensional heteronuclear NMR techniques are becoming invaluable tools for high-resolution structural studies of RNA. They allow for an enormous increase in spectral resolution which is required for complete resonance assignment of larger RNAs (> 15-mer). Specific labelling of certain residues or of a part of the molecule can also simplifies the assignment procedure. The application of these NMR techniques to two RNA enzymes will be presented:

1) a 30-mer lead-dependent ribozyme
2) a 47-mer "hammerhead" ribozyme complex.

Enzymatic activity has previously been used as a biochemical probe to study the secondary structure and some of the sequence requirement of these two ribozymes. The three-dimensional structure of these molecules is being determined in order to understand the structural features that are responsible for RNA cleavage. Relevant structural information derived from our NMR study will be presented.

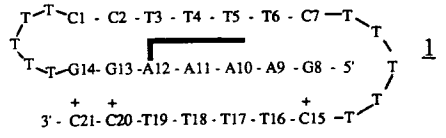
LZ 221 SOLUTION STRUCTURE OF BRANCHED DNA THREE-ARM JUNCTIONS HAVING UNPAIRED NUCLEOTIDES, Neocles B. Leontis, Department of Chemistry, Bowling Green State University, Bowling Green, Ohio 43403. Michael Hills and David Gorenstein, Purdue University, West Lafayette, Indiana 47907. Arun Malhotra, University of Alabama, Birmingham, Alabama 35294. Martial Piotto, Bruker Spectrospin, Wissembourg, France.

We have shown that unpaired nucleotides stabilize the formation of branched, three-arm or three-way helical DNA junctions (1). Two or more unpaired nucleotides located in the junction region enable oligomers ten to fifteen nucleotides long to assemble, forming conformationally homogeneous complexes, as judged by native gel electrophoresis. We have applied two-dimensional and three-dimensional, homonuclear NOESY and TOCSY spectroscopies to assign all the ^1H resonances in junctions containing two unpaired pyrimidines. NMR confirms that these samples form conformationally homogeneous complexes. The pattern of intra- and inter-nucleotide NOESY connectivities observed between sugar protons and base aromatic protons is consistent with the existence of three B-form helices in these complexes. Moreover, NOE connectivities involving imino and amino protons, observed in H_2O solvent, show that two of the three helices are stacked. Correlation of the two linear networks of NOEs via the cytosine H5 and thymidine methyl resonances indicates continous stacking along one strand and cross-strand stacking involving bases flanking the junction in the other two strands. Tertiary contacts are observed involving the methyl groups of unpaired thymidines and sugar protons in the minor groove of one of the helical arms. Strutural models refined using NOE distance constraints will be presented.

1. Leontis, N.B., Kwok, W., and Newman, J. (1991) "Stability and Structure of Three-Way DNA Junctions Containing Unpaired Nucleotides." **Nucleic Acids Research** 19, 759-766.

LZ 222 SITE-SPECIFIC COVALENT DUOCARMYCIN A-INTRAMOLECULAR DNA TRIPLEX ADDUCT Chin H. Lin[a] and Dinshaw J. Patel[a,b], [a]Department of Biochemistry and Molecular Biophysics, Columbia University, [b]Program in Cellular Biochemistry and Biophysics, Memorial Sloan-Kettering Cancer Center, New York.

We have generated and purified the first site-specific covalent antitumor drug-intramolecular pyrimidine•purine•pyrimidine DNA triplex adduct (1), and have characterized the structural properties of the resultant adduct by NMR studies of the exchangeable proton spectra in H_2O solution. Our NMR studies establish that duocarmycin A covalently bonds to the N3 of a specific adenine (2) in the minor groove without disruption of the pyrimidine third strand positioned in the major groove. However, the pKa of the triplex-duplex equilibrium is lowered by approximately two pH units on duocarmycin A adduct formation. These structural results establish cross-talk between the covalently bound duocarmycin A positioned in the minor groove and the pyrimidine third strand positioned in the major groove.



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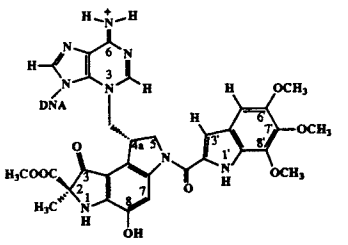


2

LZ 223 ^{19}F NMR STUDIES OF TRANSPORT PROTEINS: INSIGHTS INTO SPECIFICITY, SELECTIVITY AND HYDROGEN BONDING Luck, L.A. and Withers, S.G. Department of Biochemistry, Medical College, University of Vermont, Burlington, VT 05405 and Department of Chemistry, University of British Columbia, Vancouver, BC, Canada V6T 1Y6

^{19}F NMR provides a valuable tool to carry out studies on a variety of proteins too large or insoluble for conventional multidimensional NMR techniques. The nucleus with high sensitivity and low background resonances can be incorporated as a label on the substrate or on the protein. Here we describe both techniques in ^{19}F NMR studies of two periplasmic proteins of the *E.coli* transport system: the D-galactose and D-glucose receptor protein (GGR), and the leucine specific binding protein (LS).

The crystal structures of GGR and LS are known to high resolution (Quiocho et al.), each with two domains having a substrate binding cleft between the domains. In the crystal structure, GGR is shown with glucose in the binding cleft, LS on the other hand, is unliganded. Activation of these receptors by substrate binding induces conformational changes in the proteins. One such change is the closing of the domains engulfing the ligand. We are investigating interactions causing cleft closure and the importance of hydrogen bonding in carbohydrate/protein interactions by introducing a series of fluorinated sugars into the binding site of GGR and probing these by ^{19}F NMR.

Our approach of labeling the protein with fluorine has provided information concerning the binding site of LS. Biosynthetic incorporation of fluorine into TRP-18 in the putative binding cleft of LS has revealed that this residue is affected by leucine binding. Variable temperature ^{19}F NMR shows two unique structural conformations of this protein in the bound form. Trifluoroleucine, a substrate for this protein, has also been used to probe the active site of this protein.

LZ 224 HETERONUCLEAR NMR STUDIES OF THE TRANSMEMBRANE DOMAIN OF GLYCOPHORIN A IN SDS,
Kevin R. MacKenzie*, R. Blake Hill#, James H. Prestegard# and Donald M. Engelman*, Department of Molecular Biophysics and Biochemistry* and Department of Chemistry#, Yale University, New Haven, CT 06511.

Glycophorin A is an integral membrane protein that dimerizes in SDS. The α-helical transmembrane domain has been shown to mediate dimerization: the protein homodimer is specifically disrupted by addition of isolated transmembrane peptide, with the concomitant formation of protein-peptide heterodimers.

We present solution NMR studies of the glycophorin A transmembrane peptide solubilized in aqueous SDS detergent micelles. The large size of the peptide/detergent complex (perhaps 25 kDa) and the poor amide proton chemical shift dispersion (0.8 ppm) make homonuclear ^{1}H NMR methods impractical. Work in this system has been made feasible by the production of uniformly [^{15}N]- and [^{15}N,^{13}C]-labeled peptides by proteolysis of a fusion protein that has been overexpressed in *E. coli*. Our ongoing studies are aimed at complete sequential assignment of the dimer of 35 residue peptides and at the determination of a structure for the dimer using distance constraints derived from ^{13}C-resolved NOESY experiments.

The fact that this peptide forms a parallel dimer introduces a difficulty in interpreting NOE-derived distance constraints: whether a given NOE is due to intra- or inter-monomer effects (or both!) will obviously affect the calculation of compatible structures. While techniques exist that address this problem computationally, we plan to obtain direct experimental evidence for inter-monomer NOEs using ^{13}C half-filtered experiments and a sample consisting of labeled peptide mixed with an excess of unlabeled peptide.

The available structural information will be discussed in the context of the extensive database of disruptive mutants of Lemmon et al (Biochemistry, submitted) and will be used to evaluate the structure predicted by Treutlein et al (Biochemistry, submitted).

LZ 225 NMR STRUCTURAL STUDIES OF COMPLEXES FORMED BY COMPLEMENTARY RNA STEM-LOOPS AND THE COL E1 ROM PROTEIN,
John P. Marino, Razmic S. Gregorian Jr., James H. Prestegard and Donald M. Crothers, Department of Chemistry, Yale University, New Haven, CT 06511

Regulation of replication of the plasmid Col E1 involves the interaction of two plasmid-specific RNA transcripts, RNA I and RNA II, and a plasmid encoded protein, ROM. RNA II acts to prime DNA replication, but is repressed by RNA I which is fully complementary to its 5' end. The complementary regions of RNA I and RNA II form several stem-loop structures and the binding of the RNAs begins by interaction at the loop regions. The initial RNA-RNA complex (or so-called "kissing" complex) is stabilized with respect to dissociation by the ROM protein. Gel studies have shown that individual complementary stem-loops derived from the plasmid RNAs will mimic this interaction and are also stabilized with respect to dissociation by ROM. The dissociation rate of the stem-loop complexes has been shown to be markedly dependent on loop sequence and size. In contrast, the ROM protein appears to bind stem-loop complexes with a specificity for global structural elements of the loop-loop interaction rather than for a specific nucleotide sequence.[1]

The wild type complementary RNA stem-loop sequences have been chosen for initial NMR studies. Formation of the binary RNA-RNA and the ternary RNA-RNA-protein complexes has been demonstrated by using one-dimensional exchangeable NMR experiments. The ^{1}H assignment strategy driven by the assignment of exchangeable resonances using gradient enhanced two-dimensional H_2O NOESY experiments will be presented. In addition, the use of isotopically labeled RNA molecules and ROM protein to study the "kiss" (12 kdal) and "kiss"-ROM (26 kdal) complexes will also be presented.

[1] Eguchi, Y. and Tomizawa, J. (1991) J. Mol. Biol., **220**, 831-842.

LZ 226 MULTIDIMENSIONAL SOLID-STATE AND SOLUTION NMR STUDIES OF FILAMENTOUS BACTERIOPHAGE COAT PROTEINS IN MEMBRANES,
Patricia McDonnell, Yongae Kim, and Stanley J. Opella, Department of Chemistry, University of Pennsylvania, Phiadelphia, PA 19104

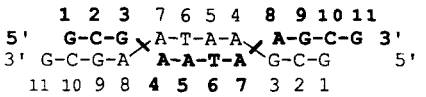
The membrane bound form of fd coat protein and a variety of variants of the protein, including those with additional peptide sequences inserted near the N-terminus are being investigated with multidimensional solid-state NMR experiments on oriented and unoriented lipid bilayer samples and solution NMR experiments on SDS and DPC micelle samples. Proteins selectively labeled with ^{15}N and uniformly labeled with ^{15}N, ^{15}N and ^{2}H, and ^{15}N and ^{13}C are used in these experiments. fd coat protein is a monomer in micelles. It has a turn that is mobile on slow (10^{-3} sec) timescales between the short amphipathic helix near the N- terminus, which has its axis parallel to the plane of the bilayers, and the long transmembrane hydrophobic helix, as determined by solid-state NMR experiments on oriented bilayer samples. The N- and C- terminal residues are mobile on both slow and fast (10^{-9} sec) timescales. Comparisons between the orientations of residues in the membrane bound and viral forms of the coat protein determined by solid-state NMR spectroscopy give a direct view of the assembly mechanism of the viral particles.

LZ 227 CHARACTERIZATON OF A FRAMESHIFT-PRONE SEQUENCE FROM HISD 3052 ADDUCTED WITH 1,N^2-PROPANO-2'-DEOXYGUANOSINE,
James G. Moe, Udai S. Singh, G. Ramachandra Reddy, Lawrence J. Marnett, and Michael P. Stone, Departments of Chemistry and Biochemistry, Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37235.

Malondialdehyde (MDA), a product of polyunsaturated fatty acid oxidation arising from the breakdown of bicyclic peroxide intermediates formed during prostaglandin biosynthesis, induces frameshift mutations in Salmonella typhimurium tester strains. The prevalent lesion in DNA is a 1:1 MDA:dG adduct formed via attack by N^1 and N^2 of dG on the carbonyls and subsequent loss of two molecules of water resulting in the formation of an extra aromatic ring adjoining the pyrimidine ring of dG. Thus, this lesion results in loss of Watson-Crick base pairing capability. This adduct is unstable under the basic conditions required for the deprotection of synthetic oligonucleotides. Therefore 1,N^2-propano-2'-deoxyguanosine was used as a model compound in these studies. The modified base (X) was inserted into the oligonucleotide 5'-CGCXCGGCATG-3' using methodology of Marinelli and coworkers. Modification results in an ~20 °C decrease in T_m of the duplex. NMR experiments reveal that the structure of the adduct within this sequence is dependent upon pH. At pH 5.8, the predominant conformation has the propano-modified guanosine rotated about the glycosyl bond from the anti to the syn conrformation. This structure appears similar to a previous structure reported for 1,N^2-propano-2'-deoxyguanosine paired opposite adenine under acidic conditions [D. Patel and coworkers]. The observed frameshift mutations in this modified oligonucleotide are predominantly two base deletions [L.J. Marnett and coworkers]. These are consistent with the Streisinger hypothesis whereby the modified guanine and adjacent cytosine could loop out of the helix and the lesion could be bypassed by utilizing the subsequent CpG repeat unit as a template. We designed modified oligonucleotides which could form a two nucleotide bulge at the adduct site, to explore the structural consequences of this model. The modified oligonucleotides containing the putative bulge structures have T_m values which are intermediate between the unmodified duplex and the modified fully complementary duplex, suggesting that bulge formation may be thermodynamically favored. NMR structural studies on these oligomers are in progress. Supported by the NIH: CA55678 (M.P.S.), CA47479 (L.J.M.), RR05805 (NMR spectrometer).

LZ 228 NMR STUDIES OF DNA DUPLEXES CONTAINING MISMATCHED BASE PAIRS AND INTERSTRAND STACKING, Kathleen M. Morden and Karol Maskos, Department of Biochemistry, Louisiana State University, Baton Rouge, LA 70803

The solution structure of the oligonucleotide $d(GCGAATAAGCG)_2$ has been studied as a function of temperature and pH using NMR spectroscopy. NOESY and COSY experiments were used to make resonance assignments and the structure has been refined using distance geometry (DSPACE) and simulated annealing. At low pH we are able to observe the amino resonances from several of the adenosines and guanosines. NOEs from these amino resonances confirm the existence of adjacent purine•purine mismatches with unusual hydrogen bonding. The G•A mismatch contains two hydrogen bonds: A(N6) amino---G(N3) and G(N2) amino---A(N7). The A•A mismatch contains only one hydrogen bond, A(N6) amino---A(N3), but is also stabilized by excellent purine-purine stacking with the adjacent base pairs. The adjacent mismatches are incorporated into the DNA helix with interstrand stacking as shown in the schematic below,



The structure of the molecule above will also be compared to a related duplex, $d(GCGAACAAGCG)_2$, that contains six adjacent mismatches and many similar structural properties. These unusual mismatch structures and the unique way of incorporating them into the helix suggest new motifs that may be relevent to DNA recognition and repair, as well as providing information on factors that stabilize unusual DNA structures.
Supported by NIH Grant GM38137.

LZ 229 A HIGHLY CONSERVED SEQUENCE IN HIV-1 GENOME: SOLUTION STRUCTURE BY NMR AND RESTRAINED MOLECULAR DYNAMICS, Anwer Mujeeb, Sean M. Kerwin, William Egan, George L. Kenyon and Thomas L. James, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446.

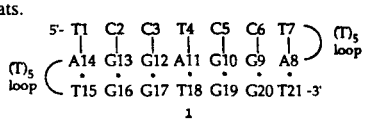
The R region of the long terminal repeat (LTR) in HIV-1 genome has a 21-base sequence, which is found to be consistantly conserved in 147 unique HIV-1 sequences, out of 148 examined. The sequence could serve as a potential target for drug design, to inhibit both RNA transcription from the integrated provirus and the reverse transcription process which possibly uses a DNA template. A 13 base pair duplex, [d(AGCTTGCCTTGAG)·d(CTCAAGGCAAGCT)] from this conserved region has been selected, synthesized and investigated using 2D NMR techniques. A set of structural constraints is obtained: for interproton distances from analysis of 2D NOE peak intensities via the MARDIGRAS algorithm and torsion angle constraints for sugar rings from simulations of 2QF-COSY cross-peak patterns. These structural constraints have been used in restrained molecular dynamics (rMD) calculations to achieve a refined three dimensional structure for the 13-mer DNA sequence.

LZ 230 APPLICATIONS OF HETERONUCLEAR NMR METHODS FOR THE STUDY OF RNAs, Edward P. Nikonowicz and Arthur Pardi, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO. 80309-0215

Isotope edited and heteronuclear multidimensional NMR methods have considerably expanded the size and variety of proteins and protein complexes amenable to structural studies. This has been accomplished largely by reducing resonance overlap of the ^1H spectrum encountered in systems >10 kDa. Similar methods should prove to be invaluable for the study of nucleic acid molecules of comparable size. Recent progress in RNA synthetic methods has enabled the routine production of isotopically (^{13}C and/or ^{15}N) enriched RNA oligomers of defined sequence in quantities sufficient for study by NMR. The preparation of a $^{13}C/^{15}N$ double labelled duplex and two ^{15}N labelled tRNAs is briefly described. We demonstrate here novel methods for the sequence specific sequential assignment of the ^1H, ^{13}C and ^{15}N spectra of RNAs using heteronuclear multidimensional NMR techniques. These methods largely rely on the application of scalar experiments for the assignment procedure. In addition, we demonstrate the simplification of the exchangeable and non-exchangeable regions of the standard NOESY spectrum of RNAs using heteronuclear methods. These methods allow the extraction NOE correlations critical to the structure determination process.

LZ 231 NMR STRUCTURAL STUDIES ON A DNA TRIPLEX BELONGING TO THE PURINE MOTIF, Ishwar Radhakrishnan and Dinshaw J. Patel, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032 and Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

The recently discovered Purine motif is a novel structural motif for oligonucleotide-mediated recognition of duplex DNA sequences. In this motif, a third purine-rich sequence binds to a purine strand in an antiparallel orientation forming reversed-Hoogsteen hydrogen bonds. In contrast to the Pyrimidine motif, very little is known about the structural aspects of this family of triplexes. To address this issue, we have undertaken studies using NMR techniques on a DNA oligomer **1**, designed to form an intra-molecular triplex of this kind. Nearly complete resonance assignments for all the protons in the molecule were obtained by conventional 2D NMR methods. A qualitative analysis of NMR data indicates that the third strand adopts an unusual structure. Especially in the dTpdG steps, the sequential NOE connectivities observed between base and sugar protons are almost absent while in the dGpdG and dGpdT steps these connectivities are weaker than usually observed for canonical B-form DNA. Anomalous chemical shifts in the third strand also reflect this unusual structure. The two strands in the duplex segment by contrast, adopt regular, right-handed helical structures. The sequence **1** at the triplex site can be viewed as an overlapping tetranucleotide repeat **2**, repeated in tandem in all three strands. Particularly interesting is the conservation in the observed patterns of NOE and chemical shifts in both repeats.



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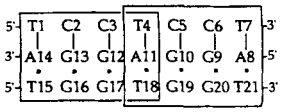


2

LZ 232 5'-CGA SEQUENCE IS A STRONG MOTIF FOR HOMO BASE-PAIRED PARALLEL-STRANDED DNA DUPLEX

Howard Robinson and Andrew H.-J. Wang, Biophysics Division and Department of Cell & Structural Biology, University of Illinois at Urbana-Champaign, IL 61801

Our recent NMR work on d(CGATCG) revealed an unusual homo base-paired parallel-stranded double helix (termed Π-DNA) at low pH (Robinson et al., *Biochemistry* **31**, 1992, in press). In this Π-DNA helix, the 5'-CGA trinucleotide was predicted to be the structural motif that accounts for the stability, with the C^+:C hemi-protonated base pair providing for the nucleation site and the unusual *inter*-strand G-A base stack in the GpA step furnishing the additional stabilizing forces. To unambiguously prove this prediction, the structure of the non-self complementary DNA heptamer d(CGACGAC) at low pH was determined by a quantitative NMR refinement procedure, SPEDREF (Robinson & Wang, *Biochemistry* **31**, 3524, 1992). Acid-base titration of the molecule indicated a prominent n=2 pK_a near 6.8. In the pH range up to 6.0, the heptamer forms a very stable double helix which was conclusively shown to be Π-DNA. We conclude that the 5'-CGA or other related sequences (e.g., 5'-CCGA, or 5'-CGAG) are powerful motifs in promoting the Π-DNA or Π-RNA conformations which may play certain yet to be determined biological functions.

This work was supported by NIH (GM-41612 and CA-52506).

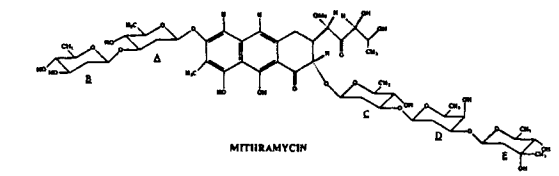
LZ 233 SOLUTION STRUCTURE OF AN HIV-1 OKAZAKI FRAGMENT

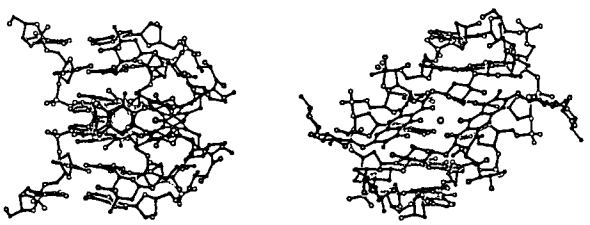
Miguel Salazar*, Seong-Gi Kim†, and Brian R. Reid*
*Departments of Chemistry and Biochemistry, University of Washington, Seattle, WA 98195
†Present Address: Center for Magnetic Resonance, University of Minnesota, Minnneapolis, MN 55455

The three-dimensional solution structure of the nonpalindromic hybrid-chimeric duplex (gccaCTGC):(GCAGTGGC) has been determined by two-dimensional NMR, distance geometry (DG) and NOE back-calculations methods. This sequence consists of a chimeric RNA-DNA strand and its complementary DNA strand that result from the priming of (-)-strand DNA synthesis by $tRNA^{Lys}$ and subsequent (+)-strand DNA synthesis by reverse transcriptase prior to HIV-1 retrovirus integration. Fourteen structures were generated from the automatically refined experimental distance bounds, seven from a classical B-form geometry and seven from a classical A-form geometry. All structures converge to the same family of structures with small RMSD values (typically 0.5 Å to 1.0 Å) Except for the sugar residues which have mixed conformations (the RNA sugars retain a C3'-endo conformation and the DNA sugars have intermediate conformations), the final structures have a geometry closer to B-form DNA than A-form DNA (RMSDs between classical B-form DNA and final structures *ca.* 1.8 Å *vs.* RMSDs of *ca.* 4.0 Å between classical A-form and final structures) contrary to observations in the crystal state for sequences of this type (Egli *et al.*, 1992).

LZ 234 SOLUTION STRUCTURE OF THE MAGNESIUM COORDINATED MITHRAMYCIN DIMER-DNA COMPLEX, M. Sastry[1] and Dinshaw J. Patel[1,2], [1] Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032 and [2]Memorial Sloan-Kettering Cancer Center, New York, NY10021.

Mithramycin is a G·C specific antitumor antibiotic that binds as a Mg(II) coordinated dimer in the minor groove of DNA. We have determined the solution structrure of the Mg(II) coordinated mithramycin-d(T-C-G-C-G-A) complex by a combined NMR-Relaxation matrix refinement study from two different starting structures. The resultant structures (RMSD, 1.06 Å) provide details about the conformational changes in the nucleic acid, the alignment of the Mg(II) coordinated mithramycin dimer in the minor groove and the mithramycin-nucleic acid interactions that stabilse the complex.





LZ 235 NMR STRUCTURAL STUDIES OF *merP* AND *merT* PROTEINS OF THE BACTERIAL MERCURY DETOXIFICATION SYSTEM, Ruth Steele[1], Kathleen Fonda[1], Jennifer Gesell[1], Danielle Brabazon[1], Nancy Hamlett[2], and Stanley J. Opella[1], [1]Department of Chemistry, University of Pennsylvania, Phiadelphia, PA 19104 and [2]Department of Biology, Harvey Mudd College, Claremont, CA 91711

Bacteria possessing the mercury detoxification system on a plasmid have the remarkable ability to transport Hg(II) across the cell membrane into the cell where it is reduced to Hg(0) which is volatile and passively eliminated. The net effect is to allow the bacteria to survive in environments containing toxic Hg(II) compounds. Two key proteins (from the Tn*21* transposon) involved in this process are being investigated by NMR spectroscopy. *merP* protein (periplasm) has 72 residues and binds Hg(II). *merT* protein (transport) has 116 residues and transfers Hg(II) across the membrane. Both of these proteins are being expressed and uniformly labeled with stable isotopes for NMR studies. Multidimensional NMR spectra of *merP* protein in solution are suitable for structure determination. The polypeptide encoded by *merT* appears to be a membrane protein with several transmembrane hydrophobic helices, therefore it must be studied with a combination of solid-state NMR experiments on lipid bilayer samples and multidimensional solution NMR experiments on micelle samples.

LZ 236 THE THREE-DIMENSIONAL STRUCTURES AND DYNAMICS OF CARBOHYDRATES AS STUDIED BY NMR SPECTROSCOPY

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Determining the three-dimensional structure of carbohydrates in solution is critical to understanding their biological function. Although most carbohydrates are highly flexible and adopt multiple conformations when free in solution, the conformations and dynamics of free oligosaccharides in aqueous solution can be probed by *(i)* measurement of scalar $^3J_{HH}$ couplings, *(ii)* measurement of inter-glycosidic scalar $^3J_{CH}$ couplings, and *(iii)* detection of inter-glycosidic $\{^1H,^1H\}$ NOEs, at various magnetic field strengths and sample temperatures [1]. Appropriate experimental conditions (solvent system, sample temperature) enable us to use OH and NH protons in the NOE measurements [1,2]. Since the number of cross-relaxation rates that can be measured for flexible molecules is limited, accuracy of the data obtained is of the utmost importance. We will illustrate various aspects of analyzing the conformation and internal dynamics of sialyl-α(2→6)-lactose [2] and sialyl-α(2→3)-lactose, both in free state and bound to lectins (SNA and MAL, respectively), of an octasaccharide from avian salivary mucus [1,3], and of ganglioside G_{D1a} in a model membrane system.

[Supported by NIH grant P41-RR-05351]

[1] H. van Halbeek and L. Poppe (1992) *Magn. Reson. Chem.* **30**: in press.
[2] L. Poppe, R. Stuike-Prill, B. Meyer and H. van Halbeek (1992) *J. Biomol. NMR* **2**: 109-136.
[3] H. van Halbeek and L. Poppe (1992) in: "*Oligosaccharide and Polysaccharide Structural Determination by Modern Mass Spectrometry and NMR Spectroscopy*" (D.A. Cumming and V.N. Reinhold, eds.) (in press).

LZ 237 SOLUTION STRUCTURE OF THE OCTAMER MOTIF IN IMMUNOGLOBULIN GENES VIA RESTRAINED MOLECULAR DYNAMICS CALCULATIONS, Klaus Weisz, Richard H. Shafer, William Egan and Thomas L. James, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446.

The octamer motif in immunoglobulin genes is believed to play an important role in the B cell type-specific expression of Ig genes. Since it constitutes a binding site for specific protein transcription factors, sequence-dependent structural characteristics may play an important role for its biological activity. Therefore, the DNA duplex d(CATTTGCATC)·d(GATGCAAATG) containing the octamer motif has been studied in detail by 2D NMR spectroscopic techniques coupled with restrained molecular dynamics calculations. 2QF-COSY crosspeaks were simulated to yield torsion angles of the various deoxyribose rings. In addition, 2D NOE crosspeak intensities were analyzed by use of the complete relaxation matrix algorithm MARDIGRAS to give a large number of interproton distances. Both types of experimental data were subsequently used as restraints in molecular dynamics refinements. The refined structure is analyzed in terms of torsion angles and helical parameters taking into account atomic fluctuations for the converged structures during the rMD simulations.

LZ 238 REFINED SOLUTION STRUCTURE OF AN 11 BASE-PAIR OLIGONUCLEOTIDE CONTAINING THE CODON 12 SEQUENCE OF THE N-RAS PROTOONCOGENE, Irene S. Zegar, Jason P. Weisenseel, and Michael P. Stone, Department of Chemistry, Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37235

The solution structure of the oligonucleotide, d(GGCTGGTGGTG)·d(CACCACCAGCC), was investigated using ^1H NMR spectroscopy. This sequence was derived from the n-ras proto-oncogene at codon 12 (underlined). Chemical modification at codon 12 has been demonstrated to cause single base substitutions which lead to oncogene activation [reviewed by Barbacid, M. (1987) *Ann. Rev. Biochem.* **56**, 779-827]. The non-exchangeable protons of this oligonucleotide were characterized by NOESY and TOCSY experiments in D_2O buffer. The standard NOESY pulse program was modified to eliminate artifacts arising from zero-quantum coherence and zz terms observed at short mixing times used in NOE build-up measurements. A systematically shifted composite 180° pulse was implemented within the mixing period and composite 90° pulses were used in place of the second and third 90° pulses in the standard pulse sequence [Bodenhausen et al. (1984) *J. Mag. Res.* **59**, 542-550]. A series of NOESY spectra were acquired as a function of mixing time incremented over the range of 30-150 msec. The two-spin approximation was used to obtain a total of 142 inter-proton distance restraints. These restraints were incorporated into molecular dynamics calculations, as effective potentials in the total energy equation. Classical B-form DNA was energy minimized and used as a starting structure for subsequent restrained molecular dynamics calculations. The experimental NOE distance restraints were refined by comparing observed NOE intensities to those calculated from the model structures using full relaxation matrix analysis. The final structure was determined by energy minimization of the average molecular dynamics structure. A future goal of this research program is to examine the effect of modification by polycyclic aromatic hydrocarbons at guanine N^2 on the solution structure of this oligonucleotide. Supported by the NIH: ES05355 (M.P.S.) and RR05805 (NMR Spectrometer).

Peptides, Protein Folding, Protein Structures

LZ 300 TRANSFORMING GROWTH FACTOR-β1: STRUCTURAL STUDIES USING HETERONUCLEAR NMR SPECTROSCOPY, Sharon J. Archer and Dennis A. Torchia, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892

Transforming growth factor-β1 (TGF-β1) is an important regulator of numerous physiological processes including normal tissue growth and wound repair. We have recently reported the secondary structure of TGF-β1 in solution (Archer et al., *submitted for publication*). Comparison of the solution structure of TGF-β1 with the crystal structures of the highly homologous protein TGF-β2 indicates that, overall, the TGF-β1 and TGF-β2 structures are very similar. However, there are regions in the protein where the solution structure of TGF-β1 differs in conformation and/or mobility from the crystal structure of TGF-β2. These regions may be important in the binding of the TGF-β's to their receptors as well as in distinguishing between the activities of the two isoforms. We will present the structural and dynamic properties of these regions in detail and discuss them in terms of a model for the interaction of TGF-β1 with a TGF-β receptor.

We would like to acknowledge M. B. Sporn and A. B. Roberts (NCI, Bethesda, MD), J. Weatherbee and his colleagues (R&D Systems, Minneapolis, MN), and Y. Ogawa (Celtrix Pharmaceuticals, Santa Clara, CA) for providing protein samples.

LZ 301 SOLUTION STRUCTURE OF THE POU-SPECIFIC SUBDOMAIN FROM THE OCT-1 POU DOMAIN Nuria Assa-Munt[a], Russell Mortishire-Smith[a], Rajeev Aurora[b,c], Winship Herr[c] and Peter Wright[a]
[b]Washington University Medical School, St. Louis, Mo., [c]Cold Spring Harbor Laboratories, and [a]The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037

The POU domain belongs to a sub-class of homeodomain regulatory proteins. The POU domain has a bipartite conservation pattern, consisting of a C-terminal homeodomain and an N-terminal POU-specific region, separated by a non-conserved linker. UV-cross linking studies indicate that when the POU domain is bound to DNA, both segments contact the DNA. Whereas the homeodomain can bind DNA weakly on its own, the role of the POU-specific region in the absence of the homeodomain is not well defined to date. Elucidation of the solution structure of the POU-specific region may provide insight into the function of this segment within the whole POU domain. To this end, we have investigated the solution structure of the POU-specific region, expressed in E. coli and doubly labeled with ^{13}C and ^{15}N isotopes. These studies by NMR are being performed under conditions where the whole POU domain is known to bind DNA. Sequential resonance assignments have been completed, secondary structural elements have been identified and preliminary characteristics of this subdomain's global fold are under study at present.

LZ 302 STRUCTURES OF ANTIFUNGAL THIONIN PROTEINS: ^1H NMR OF SIα1 FROM SORGHUM SEEDS

Carlos Bloch Jr*, Sunil U.Patel†, Peter J. Sadler†, Marketa Zvelebil+ and Janet M. Thornton+, *Dept. Biologia Celular, Universidade de Brasilia, Brasilia-DF 70910, Brazil,†Dept. of Chemistry, Birkbeck College, University of London, London WC1H OPP, UK,+Dept. Biochemistry and Molecular Biology, University College London, London, WC1E 6BT, UK

SIα1 is a basic 5 kDa (47 residue) protein isolated from *Sorghum bicolor* seeds [1]. It is rich in Arg and Lys, has 4 disulphide bridges, and is very stable even in highly acidic media. The protein shows strong sequence similarities to the γ-thionins isolated from wheat, barley, maize and raddish seeds [2]. γ-Thionins are toxic plant proteins which modify membrane permeability, and inhibit *in vitro* protein synthesis in cell-free systems. Some are very potent fungicides, e.g. Rs-AFP1 and Rs-AsPs2 from radish [3]. In spite of this biological interest in thionins, no structural studies on this family of proteins has been reported.

We have investigated the structure of SIα1 in aqueous solution at pH 3 using CD and ^1H NMR spectroscopies. DQF-COSY, TOCSY and NOESY spectra are well-resolved, and preliminary sequential assignments indicate the presence of at least one antiparallel sheet in the overall structure. Several NH protons are inert to H-D exchange. The results of the NMR structure determination will be reported and will be used for constructing models of related thionin proteins.

We thank the MRC, SERC and the Brazilian government (CNPq) for support.

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[3]Garcia-Olmedo,F., Salcedo,G., Sanchez-Monge,R., Gomez,L., Royo,J. and Carbonero, P. (1987) *Oxford Survey of Plant Molecular and Cell Biology* (Miflin, B.J., ed.) OUP, Oxford, Vol 4, pp 275-334.

LZ 303 A STRUCTURAL COMPARISON OF PROTEINS AND SELECTED HOMOLOGOUS PEPTIDES BY NMR METHODS. Kimberly A. Bolin, Daniel P. Raleigh, Maureen Pitkeathly, and Christopher M. Dobson. Inorganic Chemistry Laboratory and Oxford Centre for Molecular Sciences, University of Oxford, Oxford, England, OX1 3QR.

Many techniques have been used to probe the nature of protein folding. These range from direct observation of proteins under a variety of conditions by nuclear magnetic resonance, circular dichroism, and other methods, to studies of specific protein structural subunits. One such approach is the characterisation of synthetic peptides homologous to portions of native proteins. Our studies concentrated on peptides from the sequence of hen egg white lysozyme and α-lactalbumin, specifically, on the regions of helical secondary structure. Comparison of the peptides with NMR data from the native state proteins and folding intermediates (S.Radford et al. *Nature*, 1992) serves as a means of investigating the folding behavior of these closely related proteins, the most obvious difference between them being the molten globule intermediate in α-lactalbumin (J.Baum et al, *Biochemistry*, 1989). A variety of NMR methods were used to characterise the peptides in both aqueous and trifluoroethanol solutions. Comparisons were made with HEWL and α-lactalbumin as well as with characteristic values for NMR observables.

LZ 304 STRUCTURAL STUDIES ON THE SH3 DOMAIN OF PHOSPHATIDYLINOSITOL-3-OH KINASE

Grant W. Booker, Ivan Gout§, Jonathan Boyd, Michael D. Waterfield§ & Iain D. Campbell. *Biochemisty Department, University of Oxord, Oxford OX1 3QU.* §*Ludwig Institute for Cancer Research, London W1P 8BT.*

Phosphatidylinositol-3-OH kinase (PI3K) is a heterodimeric protein involved in cellular signal transduction. The 85,000 MW regulatory subunit (p85) is a modular protein consisting of two src homology (SH) 2 domains, one SH3 domain and a domain with sequence similarity to the breakpoint cluster region (Bcr) gene (1).

While the biological role of the phosphotyrosine-recognising SH2 domain has been well studied, the target of SH3 binding is less clear. A recent study (2) indicates that the SH3 domain from the oncogene *abl* binds to a protein with sequence similarity to Bcr and the guanosine triphosphatase-activating protein (GAP)-rho, and that the SH3 domain may be a negative regulator of transformation

The solution structure of the 104 residue N-terminal SH2 domain of the α form of p85 has been reported (3) and we are currently studying the structure of an 86 residue recombinant protein representing the SH3 domain of p85α using heteronuclear multi-dimensional NMR. Protein samples were prepared from a glutathione-S-transferase fusion protein expressed in *E. coli* allowing uniformly-^{15}N-labelled samples to be produced by bacterial culture in minimal media containing $^{15}NH_4Cl$ (4).

1. Otsu, M. *et al.* (1991) *Cell* 65 91-104.
2. Cichetti, P *et al.* (1992) *Science* 257 803-806.
3. Booker, G.W. *et al.* (1992) *Nature* 358 684-687.
4. Driscoll, P.C. *et al.* (1991) *Nature* 353 762-765.

LZ 305 THE SOLUTION STRUCTURE OF TYROSINE PHOSPHORYLATED PEPTIDES CONTAINING THE PHOSPHATIDYLINOSITOL 3-KINASE BINDING MOTIF.

Bramham, J., Carter, A.N., Downes, C.P. and Homans, S. Dept. Biochemistry, University of Dundee, Dundee DD1 4HN, U.K.

Phosphatidylinositol 3-kinase is one of a number of proteins containing SH2 domains, a protein domain that allows the binding of SH2 containing proteins to tyrosine phosphorylated sequences within other proteins. A concensus sequence for the interaction between PI 3-kinase and its target proteins has been proposed to be **Y(P) x x M** . We have synthesised a series of 18 and 26 amino acid peptides containing this sequence modelled on the kinase insert region of the PDGFβ receptor around Y^{751}, for 18 residue and Y^{740} plus Y^{751} for 26 residue peptides, the known binding site for PI 3-kinase. The peptides have been phosphorylated on tyrosine enzymatically, and their solution structures defined by NMR using J-correlated COSY, NOESY and homonuclear Hartmann-Hahn spectroscopy. The structures of these peptides will be reported and the effects of single amino acid changes on the conformations discussed in the context of the relative abilities of different peptides to bind PI 3-kinase *in vitro* .

LZ 306 THE THREE-DIMENSIONAL STRUCTURE OF CALBINDIN D_{9k} IN THE APO AND CALCIUM-BOUND STATES, Walter J. Chazin, Nicholas J. Skelton, and Johan Kordel, Dept. of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

Structural research on the EF-hand family of calcium-binding proteins is designed to determine the how the wide diversity in the functions of these proteins is achieved within the context of considerable structural homology. To fully understand the structural and dynamical consequences of calcium-binding, the proteins must be examined in both the inactive (apo or magnesium) and activated (calcium) states. Structural information on metal-bound states are available from X-ray crystal structures of six members of the family, but it has not been possible to crystallize any of these proteins with different levels of calcium occupancy. Consequently, three-dimensional structures of EF-hand calcium-binding proteins are being determined *in solution* by nuclear magnetic resonance (NMR) spectroscopy. The first subject of study is calbindin D_{9k}, a 75 residue protein believed to be involved in calcium buffering/transport. The protein consists of a pair of EF-hands joined by a short linker segment, i.e. the basic structural unit for the EF-hand family proteins. Structures of both the apo and calcium-loaded states have been determined by distance geometry and restrained molecular dynamics calculations. More than 1,000 constraints (>15/residue overall, >30/residue in the protein core) were identified for both states of the protein, and very high quality NMR structures have been obtained. The final families of ~30 structures have no distance constraints violations greater than 0.3 Å, are well-defined (e.g. 0.5 Å RMSD for the backbone atoms of the four helices), and have good local geometries as reflected by low total energies (<-900 kcal/mol in the AMBER 4.0 force field). The structure of the calcium-loaded state in solution is very similar to that in the crystalline state, with a 0.88 Å average RMSD for the backbone atoms of the helices. Preliminary comparisons of the apo and calcium-loaded states reveal only small differences in conformation in the N-terminal pseudo EF-hand, but more substantial changes in the C-terminal EF-hand. These structural changes give rise to differences in the van der Waals surface of the protein, although these are clearly less dramatic than the effect on the electrostatic surface associated with the binding of the two cations. The results have implications regarding the nature of the binding process and the cooperativity in the binding of calcium ions by calbindin D_{9k}.

LZ 307 SOLUTION STRUCTURE OF THE *Bacillus subtilis* GLUCOSE PERMEASE IIA DOMAIN AND ITS INTERACTION WITH ANOTHER PHOSPHOCARRIER PROTEIN HPr, Yuan Chen, Wayne J. Fairbrother, Garry P. Gippert, David A. Case, John Cavanagh, Jonathan Reizer, Milton H. Saier, Jr., and Peter E. Wright. Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037 and Department of Biology, University of California at San Diego, La Jolla, CA 92093.

The glucose permease IIA^{glc} domain is a central regulatory and phosphocarrier protein in the bacterial phosphoenolpyruvate-sugar phosphotransferase system. IIA^{glc} of *Bacillus subtilis* is monomeric and consists of 162 amino acid residues. Inter-proton distances of the *Bacillus subtilis* IIA^{glc} have been derived from a combination of 2D, 3D and 4D heteronuclear edited NOESY spectra. Solution structures of the *Bacillus subtilis* IIA^{glc} have been generated using the distance geometry program DISGEO and refined using restrained molecular dynamics calculations using the AMBER force field. The NMR structures obtained satisfy NOE constraints derived from heteronuclear edited NOESY spectra and torsional constraints derived from vicinal coupling constant measurements.

Interaction between the two proteins HPr and IIA^{glc} is necessary for phosphate transfer from HPr to IIA^{glc} during phosphoryl transfer from phosphoenolpyruvate to glucose. HPr consists of a single chain of 88 amino acid residues. The interaction between *Bacillus subtilis* HPr and IIA^{glc} is being characterized by heteronuclear edited and filtered NMR experiments using ^{15}N-labeled or ^{13}C, ^{15}N-doubly-labeled IIA^{glc} and non-labeled HPr. The binding interfaces of the two proteins involve predominantly hydrophobic surfaces near the active site His-15 of HPr and phosphoryl acceptor His-83 of IIA^{glc}.

LZ 308 UREA-UNFOLDING STUDIES ON THE MOLTEN GLOBULE STATE OF GUINEA-PIG α-LACTALBUMIN BY NMR AND CD Chia-Lin Chyan, Seho Kim & Jean Baum, Department of Chemistry, Rutgers University, Piscataway, NJ 08855-0939.

A partially folded state of guinea-pig α-lactalbumin (the A-state), obtained by denaturation at low pH, has been studied using hydrogen exchange methods. We have shown that the most persistent residual structure in the A-state of guinea-pig α-lactalbumin is located in two segments, corresponding to the B- and C- helices of the native structure.[1,2] Urea unfolding of the A-state of guinea-pig α-lactalbumin has been studied by hydrogen exchange methods and CD in order to determine whether there is a hydrophobic core in the A state. CD studies and proton occupancy measurements obtained from 1D bulk hydrogen exchange experiments, show a broad but cooperative unfolding from the A-state to the unfolded state. 2D NMR urea-dilution pH-jump experiments allow us to monitor the percent proton occupancy as a function of urea concentration at the individual residue level. From these experiments, we have found that most of the residues corresponding to the C-helix of the native state are less stable than the residues corresponding the B-helix of the native state, however, some of the residues of the B and C helices appear to be equally stable. These CD and NMR results suggest that the most highly protected regions may be stabilized through hydrophobic interactions.

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2. Chyan, C.-L., Wormald, C., Dobson, C. M., Evans, P. A., Baum, J. *(1992) Biochemistry*, submitted.

LZ 309 SEQUENCE SPECIFIC PROTON ASSIGNMENTS AND PRELIMINARY STRUCTURAL CHARACTERIZATION OF LSIII. Peter J. Connolly, Jeffrey C. Hoch, The Rowland Institute for Science, 100 Cambridge Parkway Cambridge MA 02142 U.S.A.

LSIII is a 66 amino acid toxin from the venom of the blue-green sea snake Laticauda semifasciata. It is a member of the long neurotoxin class of peptides found in the venom of a variety of cobras, mambas, and sea snakes, characterized by five conserved didulfide bonds and a high sequence homology. These toxins act by binding to the acetylcholine receptor and inhibiting neuromuscular response.

We present sequential ^{1}H NMR assignments obtained using two- and three-dimensional homonuclear techniques. In addition, preliminary characterization of the three-dimensional structure and overall fold on the basis of interresidue NOEs will be described using a new method for determining overall fold from distance restraints. We also compare the overall fold of LSIII with known structures of other long neurotoxins.

LZ 310 α- HELIX FORMATION IN A PEPTIDE CORRESPONDING TO THE N-TERMINAL 13 RESIDUES OF T4 LYSOZYME David J Craik, Michael J McLeish and Katherine J Nielsen
School of Pharmaceutical Chemistry, Victorian College of Pharmacy, 381 Royal Pde, Parkville 3052, Victoria, Australia

While there is still considerable debate as to how proteins acquire their final 3-dimensional structure, most models of protein folding require the initial formation of local regions of secondary structure. These regions may subsequently be stabilized by packing against other elements of local folded structure. Recently, 2-dimensional NMR techniques, combined with quench-flow detection of hydrogen-deuterium exchange of amide protons have been used to detect and characterise kinetic intermediates observed during protein folding (Dobson, 1991). When this method was applied to T4 lysozyme (Lu and Dahlquist, 1992) the results showed that helix E (corresponding to residues 93-105) and helix A (residues 3-8) are most likely to be formed first during refolding and that these helices may form the framework for the refolding of the rest of the protein. It is of interest to determine if peptides based on these helices will have a propensity to adopt a helical conformation in solution.

We have used solid phase methods to synthesize a peptide corresponding to T4 lysozyme residues 1-13, and 2-D ^{1}H NMR techniques to investigate its solution structure. Two samples of 2 mM peptide, one in 200 mM perdeuterated SDS micelles, and the other in 50% TFE (v/v) were examined at 500 and 400 MHz respectively. The identification of numerous medium-range NOESY crosspeaks, low $^3J_{NH\text{-}C\alpha H}$ coupling constants and several slowly exchanging NH protons indicated the presence of an α-helical structure (Dyson and Wright, 1991). This was confirmed by simulated annealing calculations.

Dobson, C.M., (1991) *Curr. Opin. Struct. Biol.*, **1**, 22-27.
Dyson, H.J. and Wright, P.E. (1991) *Ann. Rev. Biophys. Chem.*, **20**, 5119-538.
Lu, J. and Dahlquist, F.W. (1992) *Biochemistry*, **31**, 4749-4756

LZ 311 NUCLEAR MAGNETIC RESONANCE STUDIES OF DOMAIN 1 OF RAT CD2. D. Arthur Crawford, Paul C. Driscoll, Bruno Kieffer, Peter Howe and Iain D. Campbell, Department of Biochemistry, Oxford University, Oxford, OX1 3QU, UK.

CD2 is a trans-membrane T cell protein involved in the interaction with antigen presenting cells. The first cytoplasmic domain of CD2 from rats has been expressed in *E.coli* and a low resolution three dimensional structure has been derived with information obtained from two and three-dimensional homonuclear and heteronuclear NMR experiments [1]. The resolution of the NMR structure has now been improved by use of directed, minimal ^{13}C labelling techniques which allow the stereospecific assignment of leucine and valine methyl groups [2]. This alleviates the requirement for large pseudo-atom corrections in the NOE constraints, thus improving the quality of the structure. As a result there has been a marked increase in the definition of many side chains. Increased confidence in the tertiary structure has enabled modelling of human CD2 which has aided recent site-directed mutagenesis studies of the LFA-3(CD58) binding surface. We are currently studying the dynamic properties of rat CD2 using 100% ^{15}N and 10% ^{13}C labelled samples. Relaxation parameters T1, T2 and the heteronuclear NOE have been measured for the backbone amide groups in the fully ^{15}N labelled sample. Side chain dynamics are also being investigated by analysing the relaxation of isolated ^{13}C labelled methyl groups produced by the minimal carbon labelling method. The motional information these experiments yield will be used with reference to the recently obtained crystal structure of two-domain rat CD2 in order to build up a picture of molecular flexibility and its relevance to inter-domain contacts and ligand binding. Recently, CD48(OX45) has been identified as a CD2 ligand in rats and mice and experiments are planned to probe this interaction directly.

1. Driscoll *et al.*, Nature (1991) **353**, pp. 762-765.
2. Senn *et al.*, FEBS Lett. (1989) **249**, No 1, pp. 113-118.

LZ 312 THE SOLUTION STRUCTURE OF THE DNA-BINDING DOMAIN OF A HEAT SHOCK FACTOR BY 2D AND 3D NMR: A NOVEL DNA-BINDING MOTIF, Fred F. Damberger, Celia J. Harrison, Hillary C. M. Nelson, David E. Wemmer, Graduate Group in Biophysics , Department of Molecular Biology, Department of Chemistry, University of California, Berkeley CA, 94720

Heat Shock Transcription Factor appears to recognize DNA using a novel motif. It exists in solution as a trimer and recognizes a DNA sequence which contains an array of three alternately oriented GAA boxes. The DNA binding domain shows no recognizable homology to any known DNA recognition motifs. We are interested in understanding how this novel motif recognizes DNA in terms of structure of the protein and its orientation with respect to the DNA binding site. We have undertaken to determine the three dimensional structure of the DNA binding domain of a Heat Shock Transcription Factor. The protein has been expressed in E. Coli and labeled with both ^{15}N and ^{13}C. NMR experiments are being used to make assignments and determine the secondary structure. The current status and structure analysis will be given.

LZ 313 COMPARISON OF NMR AND X-RAY CRYSTALLOGRAPHICALLY DERIVED STRUCTURES OF THE SH2 MODULE AND MODELLING OF SUBSTRATE BINDING SPECIFICITY, [1]A. Kristina Downing & [2]Marketa J. Zvelebil, [1]Oxford University, Department of Biochemistry, South Parks Road, Oxford OX1 3QU ENGLAND and [2]Biomolecular Structure and Modelling Unit, Department of Biochemistry and Molecular Biology, University College, London WC1E 6BT ENGLAND

Phosphorylation of tyrosine residues by protein-tyrosine kinases is a control mechanism in cellular signal transduction. SH2 (src homology-2) domains recognize phosphorylated tyrosines and are critical to cell signalling. Furthermore differential binding specificity of SH2 domains to phosphotyrosine-containing sequence motifs has been reported (Panayotou, G., *et al.*, EMBO J., *in press.*). The solution structure of the N-terminal SH2 domain of the p85α subunit of phosphatidylinositol-3-OH kinase has been recently determined using multidimensional nuclear magnetic resonance spectroscopy (Booker, G.W. *et al.*, Nature, 358, 684-687.). This structure was compared to the SH2 domain of v-*src* complexed with a phosphotyrosine peptide determined by x-ray crystallography (Waksman, G. *et al.*, Nature, 358, 646-653.) in order to assess the extent of conformational change associated with substrate binding. The structures of the C-terminal SH2 domain of the p85α subunit of phosphatidylinositol-3-OH kinase and the N-terminal SH2 domain of GAP have been homology-modelled based on these two structures to provide a structural rationale for observed binding specifities.

LZ 314 ENZYMATIC THIOL-DISULFIDE EXCHANGE STUDIED BY NMR, H. Jane Dyson, Meifen Jeng, Ivan Slaby and Arne Holmgren, Department of Molecular Biology, The Scripps Research Institute, La Jolla CA 92037 and Karolinska Institute, Stockholm, Sweden.

Thiol-disulfide exchange is an oxidative process which involves the transfer of protons as well as electrons. Such a process should be strongly pH dependent. Thioredoxin from *Escherichia coli* is a small protein (Mr 11200) which mediates thiol-disulfide exchange in the cell. In addition, reduced*E. coli* thioredoxin also functions as a component of DNA polymerase complexes in several phage systems. (Russell & Model, 1986). Oxidized thioredoxin is not active, but the effect is not solely due to the presence of thiol groups, as mutants in which the active-site cysteine residues have been replaced are also functional. Structural (Dyson et al., 1990) and dynamic (Stone et al., 1992) differences between oxidized and reduced thioredoxin are apparently extremely small. The pH dependence of the structure of thioredoxin has been studied using proton NMR spectroscopy to determine titrating groups in the active site region (Dyson et al., 1991). As well as the thiol groups, which titrate in reduced thioredoxin, both forms of the protein show an additional titration of a buried aspartate side chain, with pKa 7-7.5, which affects the entire active site region. A series of mutant thioredoxins has been studied in the same way. The double mutant in which both cysteine residues are replaced by serines shows very similar NMR spectrum and titration behavior to that of reduced thioredoxin, consistent with its behavior in the filamentous phage system. A mutant in which the buried aspartate is replaced by alanine shows marked differences in titration behavior as well as in biological function. Further studies on these and other active-site mutants relevant to the concerted proton-electron transfer mechanism of thioredoxin will be presented.

Dyson et al., Biochemistry *29*, 4129 (1990).
Dyson et al., Biochemistry *30*, 4262 (1991).
Russel & Model, J. Biol. Chem. *261*, 14997 (1986).
Stone et al., Biochemistry (submitted).

LZ 315 ^1H ASSIGNMENT AND SECONDARY STRUCTURE DETERMINATION OF MGSA BY NMR SPECTROSCOPY.
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The solution structure of the 73 residue protein, melanoma growth-stimulating activity (MGSA), a potent inflammatory cytokine, has been investigated using 2D proton NMR spectroscopy. Sequential resonance assignment has been carried out, and elements of secondary structure have been identified on the basis of NOE, coupling constant and amide proton exchange data. Long-range NOEs have established that MGSA is a dimer in solution. The secondary structure and dimer interface of MGSA appear to be very similar to those previously found for the homologous cytokine, interleukin-8 (1). The MGSA monomer contains a three stranded anti-parallel β-sheet arranged in a 'Greek key' conformation, and a C-terminal α-helix (residues 58-69). Unlike IL-8, the last four residues do not appear to be part of the helix, but rather are more flexible than the bulk of the protein. The N-terminal seven residues also appear to be flexible in solution. The dimer interface is formed between the β-sheets of each monomer, resulting in a contiguous six-stranded anti-parallel β-sheet.

(1) Clore, G.M., Appella, E., Yamada, M., Matsushima, K., & Gronenborn, A.M. (1990) *Biochemistry 29*, 1689-1696.

LZ 316 NMR SOLUTION STRUCTURE OF ω-CONOPEPTIDE M-VII-C

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The ω-conopeptide SNX-230(M-VII-C) from *Conus magus*, a fish hunting sea snail, has been shown to bind to neuronal voltage-sensitive calcium channels(1,2). We have synthesized this 26 residue peptide which has 3 disulfides in order to obtain a large amount for NMR structural studies. The ^{1}H NMR spectrum of this peptide was completely *assigned*, with stereo specific assignments made for 12 of the β protons. A correlation time of 1.1(+/- 0.5) ns was calculated from ^{13}C T_1 and T_2 using inverse detection spectra of the natural abundance protein. Noesy spectra were acquired in H_2O using the NEWS water suppression sequence(3) and peaks were integrated with either two dimensional Gaussian line fitting or box integration within the NMR analysis program "Sparky"(4). MARDIGRAS (5) was used to obtain initial distances for input into distance geometry which subsequently provided 15 starting structures for restrained molecular dynamics using the AMBER force field with time averaged distance constraints to account for motional averaging. In addition to the NOE constraints, torsion angle constraints based on ^{1}H-^{1}H coupling constants were included. A total of 265 distance constraints and 20 torsional restraints were used.The final set of structures had an rms deviation of ~1Å.(1) Hillyard, D. *et al.*, **Neuron** (1992) 9:69-77(2) Miljanich, G. *et al.*, submitted.(3) Pucek, L *et al.* **J. Magn. Reson.** (1991)91:120-127 (4) Kneller, D.G. "Sparky, The Users' Manual", (1992) UCSF (5) Keepers,J. and James, T.L. **J. Magn. Reson.** (1988)79:493-512.

LZ 317 SOLUTION STRUCTURE OF RP 71955 , A NEW 21 AMINO ACIDS CYCLIC PEPTIDE ACTIVE AGAINST HIV VIRUS .

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RP 71955 is a secondary metabolite isolated from a strain of actinomycete and showing interesting antiviral activity, in particular as an inhibitor of the cytopathogenic effect of HIV and of the production of reverse transcriptase in cell cultures . It proved to be a peptide of 21 amino acids in length and showing unusual features : it had no terminal NH2 , contained 4 cysteines linked by disulfide bridges , could not be hydrolysed enzymatically and only in a limited and unspecific way chemically . Moreover , it was only sparingly soluble. Therefore , its sequence could not be determined by classical means. From the analysis of the results of HOHAHA and NOESY experiments , the identification of secondary structure motifs and of sequential Hα - HN Overhauser effects , it was shown to be: CLGIGSCNDFAGCGYAVVCFW . An internal amide bond between the HN of C1 and the γ CO of D9 was identified thanks to NOE data . The cysteine pairing pattern could only be determined after generation of families of 3D structures using distance geometry methods (DIANA) and restrained molecular dynamics (INSIGTH DISCOVER) , starting from a set of 212 positive distance constraints (determined using MARDIGRAS), 1870 negative distance constraints and 19 bond angle constraints. The structure was further refined using disulfide bridge constraints . The validity of the structure was confirmed through back calculation of the NOESY profile (CORMA and GIFA), checking of bond angles , chiralities and Ramachandran plots , checking the compatibility of the exposure of amide protons to solvent with experimental results (chemical shift vs temperature) and determination of the R factor. Further insight into the mobility of the molecule was gained thanks to the structures generated from NMR experiments performed at 2 different temperatures .

LZ 318 CONFORMATIONAL STUDIES OF AN EIGHT MEMBERED RING DISULFIDE-CONTAINING PEPTIDE

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Disulfide bridges have been used to restrict the conformational space of highly flexible peptides by covalently cross-linking sites that otherwise are apart in the linear sequence. The conformational bias of the disulfide bond is due to a preferred dihedral angle around ± 90° with a barrier of 7-14 Kcal mol^{-1}, which is intermediate between typical carbon-carbon bond and amide bond barriers. These structural features impose strain in small cyclic disulfide-containing peptides. In 1968 Chandrasekaran and Balasubramanian showed that ring closure by a disulfide bond in an eight membered ring is possible only when the peptide amide bond adopts a non-planar cis conformation. The L-Cys⇔L-Cys dyad has been studied in solution and observed to be an equilibrating mixture of conformers, which has been interpreted in terms of amide bond conformers or disulfide bond rotamers. The ambiguious assignment of the isomerization of this structural motif led us to consider the synthesis and study in solution of two model peptides.

H-Phe-Lys-Cys-Cys-Val-NH_2 (S—S) Boc-Cys-Cys-OBzl (S—S)

We shall report the conformational preferences of these two peptides as determined by nuclear magnetic resonance spectroscopy, circular dichroism, fourier transform infrared spectroscopy, and molecular mechanics methods.

LZ 319 TOWARDS THE STRUCTURE OF THE CATALYTIC DOMAIN OF HUMAN STROMELYSIN-1, P. R. Gooley, B. A. Johnson, A. I. Marcy, S. P. Salowe, G. C. Cuca. Dept. of Biophysical Chemistry, Merck & Co., Rahway, N.J. 07065.

The metalloendoproteinase, Stromelysin-1, is believed to play a central role in the degradation of the connective tissue extacellular matrix and is implicated in disease states such as osteoarthritis. To understand the molecular inhibition of this protein and thus to aid drug design we have undertaken the structural determination of stromelysin by mutidimensional heteronuclear NMR. Resonance assignment employed the two 4D experiments HCaNNH and HCA(CO)NNH and 3D HCCH COSY and TOCSY. NOEs have been assigned in 3D and 4D NOESY experiments and structures calculated by distance geometry or variable target functions with X-PLOR or PEGASUS, respectively. Overall the protein shows 35% regular secondary structure consisting of two helices and a 4 strand β-sheet: 3 strands of parallel and one antiparallel. This protein is unusual in that two zincs are each complexed by three conserved histidines. One zinc is believed to be central in catalysis while the other plays a structural role. The structure of the ligands will be described.

LZ 320 DETERMINATION AND REFINEMENT OF THE STRUCTURE OF THE SH2 DOMAIN OF THE p85α SUBUNIT OF PHOSPHATIDYLINOSITOL 3-OH KINASE BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY Meike Hensmann & A. Kristina Downing, Oxford University, Department of Biochemistry, South Parks Road, Oxford OX1 3QU ENGLAND

SH2 (src homology-2) domains are protein modules of approximately 100 amino acid residues length. They have been identified in proteins involved in the ligand-induced signalling pathway, such as the non-receptor tyrosine kinases of the v-src family, phospholipase C-γ, the p85 regulatory subunit from the phosphatidylinositol 3-OH kinase and GTPase-activating protein. They bind to sequences containing phosphorylated tyrosine residues with high specificity, implying a possible critical role in the assembly of multienzyme signalling complexes.

We have used multidimensional nuclear magnetic resonance spectroscopy to determine the solution structure of an isolated SH2 domain from the p85 regulatory subunit of phosphatidylinositol 3-OH kinase (Booker, G.W. *et al.*, Nature, 358, 684-687.).

Analysis of high resolution x-ray crystal structures has shown a direct correlation between stereochemistry and the quality of protein structure (Morris, A.L. *et al*, PROTEINS, 12, 345-364). Therefore, in order to improve the solution structure of the SH2 domain from p85, we have refined this structure using ϕ, ψ and $\chi 1$ torsion angle constraints, experimentally derived from stereospecific assignments of side-chain protons, as well as additional NOE constraints.

LZ 321 **ISOTOPE-DIRECTED MULTI-DIMENSIONAL NMR STUDIES OF INTESTINAL LIPID-BINDING PROTEINS** Michael E. Hodsdon, James J. Toner, and David P. Cistola, Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO, 63110

In order to compare the molecular properties of three members of a class of 15 kDa lipid-binding proteins from intestine, we have begun a long-term project to completely establish their sequential ^{1}H, ^{13}C, and ^{15}N resonance assignments. Starting with one member of this class, intestinal fatty acid binding protein (FABP), uniformly ^{13}C- and doubly $^{13}C/^{15}N$-enriched proteins have been biosynthesized, purified, and analyzed using isotope-directed 2-D and 3-D NMR methods. The experimental strategy to be presented employed the use of 2-D carbon-carbon double quantum NMR to assign isolated ^{13}C intra-residue spin systems. ^{1}H-^{13}C heteronuclear multiple quantum coherence (HMQC), ^{1}H-^{1}H total correlation spectroscopy, and triple-resonance HNCA NMR were used to identify intra-residue ^{1}H backbone resonances. Triple-resonance HNCO NMR was used to establish inter-residue correlations across the peptide bond. ^{15}N backbone resonances were identified with 3-D triple-resonance HNCO and ^{1}H-^{15}N HMQC NMR experiments. Along with our current resonance assignments for intestinal FABP, preliminary dynamic and structural information will be presented.

LZ 322 DETERMINATION OF THE CONFORMATION OF NEUROPEPTIDE K BY HIGH RESONANCE NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY, James Horne, Maruse Sadek* and David J. CraikSchool of Pharmaceutical Chemistry, Victorian College of Pharmacy, Monash University, 381 Royal Parade Parkville, Victoria, 3052, Australia

*Centre for Protein and Enzyme Technology, La Trobe University, Bundoora, Victoria, 3083, Australia

^{1}H-NMR chemical shift assignments for neuropeptide-K (NPK) have been determined at 600 MHz, in 28% trifluoroethanol/water solution. Two-dimensional NMR techniques were used to assign proton resonances for this 36 amino-acid neuropeptide. Inter-proton distances were estimated from the observed nuclear Overhauser effects. These distances were used as constraints in a simulated annealing protocol within the program XPLOR to generate structures consistent with experimental data. NPK forms a loose, helical structure from Asp 3 to Ala 15. Leu 16, Tyr 17, and Gly 18, are mildly resistant to deuteron exchange, and may be involved in a turn-like structure at the end of the helix. Ala 11, and Thr 29 also resists deuteron exchange, the former helping to stabilise the helix. The remainder of the molecule displays only sequential nOe's, with some i - i+2 contacts, but little evidence of defined secondary conformation. Comparing the tail region of NPK to the related peptide homologue, neurokinin A, in the same solvent system, indicates that both show increasing order when trifluoroethanol is titrated into water solution, with the appearance of sequential nOe's between backbone amide protons.

LZ 323 SOLUTION STRUCTURE OF THE CARDIOTOXIN CTX I FROM *NAJA* *NAJA ATRA*, Wolfgang Jahnke and Horst Kessler, Organic Chemistry Department, Technical University of Munich, 8046 Garching, Germany

The cardiotoxin CTX I from the cobra *Naja Naja Atra* has been investigated by two- and threedimensional NMR techniques. Proton NMR assignments have been obtained and the secondary structure was determined from the patterns of nuclear Overhauser enhancements, from identification of slowly exchanging amide protons, from $^{3}J_{NH\text{-}C\alpha H}$ coupling constants and from $C_{\alpha}H$ chemical shifts. The structure consists of three main loops, rich in β structure, out of which one loop is very flexible. Distance geometry calculations and constrained molecular dynamics have been performed and the obtained structure is compared to the structure of other snake venom proteins.

LZ 324 NMR STRUCTURAL STUDIES OF FRAGMENTS OF THE THYROID HORMONE TRANSPORT PROTEIN TRANSTHYRETIN INVOLVED IN AMYLOID FORMATION

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Transthyretin (TTR) is a 55 kDa plasma protein composed of four identical sub-units, each 127 amino acid residues long. TTR is mainly synthesised in the liver and is a transport protein for thyroid hormones and retinol. Recently, TTR has been implicated in several forms of amyloidosis, with the intact protein and polypeptide fragments of it having been shown to form amyloid-like fibrils *in vitro*[1]. In the currents study the three dimensional solution structures of several fragments of TTR have been investigated using two-dimensional nmr techniques. The peptide corresponding to region 71-93 of TTR shows an intrinsic tendency to form a helical structure between residues 74-82 in solution. This suggests that the helical region, also existing in native TTR, is an important folding nucleation site. Three cases of amyloidogenic TTR have been reported with mutations in this region. It is proposed that these mutations result in improper folding leading to amyloid fibril formation. Other fragments studied include the peptides corresponding to residues 10 -20 and 105-115, known to be amyloidogenic. These correspond to β-strands and some β-turns in the intact protein. Structural studies on these fragments are described. The study of TTR fragments provides an insight into aggregation and amyloidosis and has broader implications in the fields of protein folding and sub-unit assembly.

[1] Gustavsson, A., Engstrom, U., and Westermark, P. (1991) *Biochem Biophys Res Comm.*, 175, 1159-1164.

LZ 325 COMPARISON OF THE FOLDING PATHWAYS OF TWO DISTANTLY RELATED GLOBINS USING HYDROGEN-DEUTERIUM EXCHANGE AND 2D NMR

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The globin family forms an ideal paradigm for investigation of the mechanism of protein folding. The simple all-helical architecture lends itself to detailed biophysical studies such as NMR, CD and fluorescence spectroscopies. In addition, a great deal of structural information is available on these proteins. Pulse labeling and hydrogen - deuterium exchange techniques in combination with homonuclear 2D NMR methods have been used to examine the folding pathway of apomyoglobin. Previous work on the folding of apomyoglobin led to the characterization of a molten globule intermediate at low pH under equilibrium conditions [Hughson, *et al.*, Science (1990) 249, 1544-1548] and kinetic studies have demonstrated that this intermediate is formed early on the folding pathway [Jennings and Wright, submitted]. Similar equilibrium and kinetic studies are in progress on a distantly related plant protein, apoleghemoglobin, to address the question of whether there is a conservation of the "folding code". A comparison of the data from a variety of techniques including CD and NMR will be presented for apomyoglobin and apoleghemoglobin.

LZ 326 HIGH RESOLUTION STRUCTURE OF A MINI PROTEIN.

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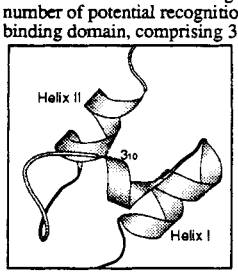
¶ Laboratory of Cell Biology, Building 37, National Cancer Institute, National Institutes of Health, Bethesda, MD 20890.

The three-dimensional structure of an active, synthetic peptide encompassing the peripheral subunit binding domain of dihydrolipoamide acetyltransferase from the pyruvate dehydrogenase multienzyme complex of *Bacillus stearothermophilus* has been determined by means of simulated annealing using restraints derived from ^{1}H nuclear magnetic resonance spectroscopy. A total of 442 experimentally derived restraints including 13 dihedral angle (ϕ,χ^1) restraints were used. A final set of 35 structures was calculated with a root-mean-square deviation from the mean coordinates of 0.36Å for the backbone atoms and 0.96Å when side chain heavy atoms were included for the well-defined region comprising residues 7 to 39. The absence of long range NOEs for the N-terminal six and C-terminal four residues suggests that the terminal regions are largely unstructured. The protein fold is a three-helix bundle with a loop made up of overlapping β-turns whose structure is defined by hydrogen bonds to the side chain of Asp34. We propose that a peripheral subunit-binding site may be located in the loop region which contains a series of highly conserved residues and provides a number of potential recognition sites. The structured region of the binding domain, comprising 33 residues, represents an exceptionally short amino acid sequence with defined tertiary structure that has no disulphide bonds, ligands or cofactors to stabilise the fold. It may be close to the lower size limit for a three-dimensional structure possessing features characteristic of larger structures, including a close-packed, non-polar interior. The organisation of the side chains in the hydrophobic core may have implications for *de novo* protein design.



LZ 327 NMR STUDIES OF DYNORPHIN A(1-17) BOUND TO A LIPID MICELLE,

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The primary function of this peptide *in vivo* is believed to be its action at the κ-opioid receptor to elicit a biological response which produces analgesia. Since little is known about the structure of the opioid receptors, little is known about the biologically active conformations of this peptide, although many active analogs are known. Although both morphine and dynorphin induce analgesia, morphine, which acts at the μ-opioid receptor, is addictive, while dynorphin A is expected to induce analgesia without addictive properties. It is therefore believed that knowledge of the active conformation of dynorphin A will lead to the development of powerful, nonaddictive analgesics.

Many studies to date, both pharmacological and structural, have focused on the deletion analog, dynorphin A(1-13), as it has been shown that it has similar activity to the 17-mer. Thus the structure and properties of the 17-mer are largely unexplored. It has been shown, however, that the 17-mer is the natural ligand for the receptor, and that it has increased resistance to proteolytic degradation in vivo.

There is evidence that the cell membrane catalyzes the conformational conversion of some peptides to their active conformation. A perdeuterated lipid micelle is thus used as a model system to study what stable conformations of the peptide are formed in this environment. The solution conformation of this peptide as it is bound to a micelle is shown using results from ^{1}H NMR studies. The amide hydrogen temperature coefficients and pattern and intensity of NOEs from NOESY spectra indicate a peptide which is (a) in a substantially different conformation than in methanol and (b) mostly bound to the lipid micelle. The secondary structural elements of the lipid-bound peptide derived from the 2D NMR data will be shown.

LZ 328 HETERONUCLEAR NMR STUDIES OF BACTERIALLY EXPRESSED PEPTIDES, Afshin Karimi, Masazumi Matsumara, David P. Millar, Yoshikazu Mikawa, Hiroko Maruyama, Ichiro Maruyama, Peter E. Wright, Department of Cell Biology , Immunology, and Molecular Biology. The Scripps Research Institute. La Jolla, Ca. 92037.

In an effort to better characterize the structure and dynamics of peptides by NMR, it appears helpful to produce isotopically labeled peptides. To this end, we have cloned, expressed (*E. coli*) and purified two different fragments from bacterial coat proteins of *Staphylococcus aureus* (protein A) and *Streptococcus pyogenes* (24M protein).

The gene corresponding to a 27 residue fragment of the coiled coil region of 24M protein has been synthesized and fused to a glutathione S-transferase expression vector. The desired peptide is obtained by thrombin cleavage on glutathione affinity beads followed by cyanogen bromide cleavage.

The gene corresponding to a single B domain of protein A (FB) has been obtained from PCR and fused to a T_4 lysozyme expression vector. Complete ^1H and ^{15}N assignments for the recombinant FB have been obtained. Further NMR analysis of ^{15}N and ^{13}C labeled FB should provide insights into the folding and dynamics of this small globular protein domain.

Both expression systems have high expression levels and yield the desired peptide without any cloning artifacts. The economy and ease of producing recombinantly labeled peptides make molecular biology an attractive alternative to conventional schemes for the synthesis of isotope labeled peptides.

LZ 329 HIGH RESOLUTION PROTEIN STRUCTURE DETERMINATION BY SOLID STATE NMR, Randal R. Ketchem, Weidong Hu and Timothy A. Cross, Institute of Molecular Biophysics and Department of Chemistry, Florida State University, Tallahassee, FL 32306

A technique for determining three dimensional structure has been developed in recent years based on observed nuclear spin interactions in uniformly aligned samples. The angular dependence of the spin interactionns can be used to generate orientational constraints. These local constraints are very high resolution constraints and yield structural detail rarely achieved for proteins even from the best of protein crystals.

Here oriented samples of the monovalent cation selective channel formed by a dimers of gramicidin A in hydrated lipid bilayers have been prepared. Samples have been labeled with ^{13}C, ^{15}N and ^2H for a variety of studies involving the observation of chemical shifts, dipolar and quadrupolar couplings. The Φ and Ψ backbone torsion angles have been solved from solid state NMR data alone. To refine this structure the ω torsion angle that describes the degree of non-planarity of the peptide linkages has been determined for selected sites along the backbone. It is thought that significant distorsions in the peptide plane may occur as the backbone carbonyl oxygens solvate cations in their passage through the channel. The orientation of the four indole rings of the gramicidin monomer have been determined with respect to the bilayer normal. The orientations are very similar and the N-H groups are all directed toward the bilayer surface rather than the bilayer center. Consequently, these groups could be hydrogen bonded to the water of the aqueous interface or to the lipid backbone carbonyl oxygens. These interactions appear to be responsible for the stability of the single stranded gramicidin helix in a lipid bilayer versus the double stranded conformations that exist in organic solvents. Furthermore, the dipole moments of the indole rings are predominately aligned with the channel axis and oriented in such a way as to reduce the potential energy barrier at the bilayer center.

LZ 330 SEQUENTIAL ^1H AND ^{15}N NMR ASSIGNMENTS AND SECONDARY STRUCTURE OF *BRUCELLA ABORTUS* COPPER-ZINC SUPEROXIDE DISMUTASE. Agustin Kintanar and Yen-Liang Chen, Department of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011

The Cu-Zn superoxide dismutase (SOD) of *Brucella abortus* has been cloned and overexpressed in *Escherichia coli*. The overproducing strain was grown in minimal medium containing $^{15}NH_4Cl$ as the nitrogen source, and the uniformly ^{15}N-labelled SOD was purified and remetallated. The enzyme is homodimeric in solution. Each subunit contains 154 amino acid residues and has a molecular weight of ~16,100 daltons. Partial sequence-specific assignments of the backbone ^1H and ^{15}N resonances have been obtained primarily from two double-resonance heteronuclear three-dimensional (3D) NMR spectra (3D NOESY-HMQC and 3D TOCSY-HMQC). The 3D data were supplemented with homonuclear and heteronuclear 2D spectra. The rate of amide proton solvent exchange has been determined from a series of ^1H-^{15}N 2D HMQC spectra recorded after the protein was dissolved in D_2O. Patterns of short-, medium- and long-range nuclear Overhauser effects observed in the 3D NOESY-HMQC spectra, together with the amide proton exchange data revealed the major elements of secondary structure of *B. abortus* Cu-Zn superoxide dismutase. The main structural feature is an eight-stranded β-barrel. The metal-binding sites are formed by several loops. The observed secondary structure is largely similar to that observed crystallographically for the human and bovine Cu-Zn SODs.

LZ 331 CHARACTERIZATION OF A SLOW FOLDING INTERMEDIATE OF APOPLASTOCYANIN BY HYDROGEN EXCHANGE AND 2D-NMR, Shohei Koide and Peter E. Wright, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

We have characterized the equilibrium and kinetic folding of French bean apoplastocyanin (apo-Pc), a small β-sandwich protein. Its major refolding reaction is very slow (relaxation time ≈ 1000 s at 25°C) and limited by the *cis-trans* isomerization of two proline residues. The presence of a kinetic intermediate in this reaction was clarified by means of fluorescence spectroscopy and size-exclusion chromatography. The intermediate is as compact as the native apo-Pc, although it has an unfolded-like peptide CD spectrum (Koide &Wright, manuscript in preparation).

In order to characterize the secondary structure formation of the intermediate in detail, we performed competition experiments between folding and exchange of backbone amide protons for solvent deuterons. The protein was first unfolded in guanidine hydrochloride and the refolding was initiated by a dilution to the native conditions. After a short period which allows completion of the faster refolding reactions, the solution was further diluted into a D_2O buffer and the refolding was completed. Then the amide proton occupancies were measured by 2D-NMR. A preliminary experiment showed several sites which had significantly lower proton occupancies than those in the control sample, indicating that they are less protected than those in native apo-Pc under the labeling conditions used. The results imply that the formation of stable secondary structure may not be an early event in the folding of apo-Pc.

LZ 332 SOLUTION STRUCTURE OF THE RETINOID X RECEPTOR DNA-BINDING DOMAIN, Min S. Lee[¶], Dan Sem,[¶] Steven A. Kliewer,[§] Joan Provencal,[§] Ronald M. Evans,[§] and Peter E. Wright,[¶] [¶]Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, [§]Howard Hughes Medical Institute, The Salk Institute for Biological Studies, La Jolla, CA 92037.

The retinoid X receptor (RXR) is a member of a family of ligand-activated transcription factors that includes receptors for steroid hormones, thyroid hormone, and retinoic acid. RXR binds and activates gene expression in response to its ligand, 9-cis retinoic acid (RA), through hormone response elements (HREs) consisting of direct repeats of the sequence AGGTCA spaced by a single nucleotide (DR-1). Here we describe the three-dimensional solution structure of the DNA binding domain (DBD) of RXR as determined by nuclear magnetic resonance (NMR) spectroscopy. The two zinc fingers of RXR fold to form a single structural domain consisting of two helices perpendicular to each other which resembles the corresponding regions of the glucocorticoid (GR) and estrogen (ER) receptors. However, different from the previously reported structures of GR and ER, RXR contains a third helix downstream of the second helix. Mutations that disrupt this third helix or alter basic amino acids at either end of the helix interfere with binding of the RXR DBD to a retinoid X response element (RXRE). This third helix is absolutely required for cooperative, homodimeric binding of the RXR DBD to its cognate HRE.

LZ 333 NMR STUDIES OF TRIPLE-HELICAL PEPTIDE IN SOLUTION, Ming-Hua Li[1], Chuan Wang[1], Barbara Brodsky[2], and Jean Baum[1], Chemistry Dept., Rutgers University[1] and Biochemistry Dept., UMDNJ-Robert Wood Johnson Medical School[2], Piscataway, New Jersey 08855.

Triple-helical peptides are good model peptides for collagen. Solution studies of triple-helical structures will help understand the interactions that stabilize triple helices as well as provide information, at a molecular level, about the basis of collagen diseases. A design strategy for collagen model peptides was developed whereby real collagen sequences are placed in between $(Pro\text{-}Hyp\text{-}Gly)_n$ extremities. This allows us to study real collagen sequences while maintaining high stability. The sequence $(Pro\text{-}Hyp\text{-}Gly)_3$-Ile-The-Gly-Ala-Arg-Gly-Leu-Ala-Gly-$(Pro\text{-}Hyp\text{-}Gly)_4$ was chosen from human collagen type III (α1). At low temperature, the resonance assignments of three labeled tripeptide units ^{1}Gly-^{1}Leu-^{1}Ala, ^{2}Gly-^{2}Leu-^{2}Ala, and ^{3}Gly-^{3}Leu-^{3}Ala from chains 1, 2, and 3 of the trimer were identified by ^1H-^{15}N heteronuclear experiments. Structural information was obtained from long range NOE's, and torsional constraints measured from ^{15}N HMQC experiments. A computer model of the designed peptide was generated by energy minimization based on the triple-helical structure of $(Pro\text{-}Hyp\text{-}Gly)_{10}$ (Fraser, R. D. B., MacRae, T. P., & Suzuki, E. (1979), *J. Mol. Biol.*, **129**, 463) obtained from X-ray diffraction data. The NMR data was compared with the computer model and the backbone conformation in solution was found to be reasonably consistent with the X- ray diffraction data.

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LZ 334 Glutathione-S-Transferase (α-human liver): Interactions with Cofactor and Substrates

Lu-Yun Lian, Ji-Chun Yang, Gordon C.K.Roberts, Ramin Badii and Andrew Prescott, NMR Centre and Centre for Mechanisms of Human Toxicity, University of Leicester, Leicester LE1 9HN, U.K.

Biotransformation is a process whereby foreign chemicals or xenobiotics are converted, in a 2 phase process, into hydrophilic compounds, the latter being therefore more readily excretable. Glutathione-S-transferases (GST) are a family of enzymes that are involved in the second phase of biotransformation; the enzyme catalyses the nucleophilic attack of the sulphur atom of the cofactor, glutathione, on electrophilic groups on the substrate which are frequently aromatic compounds. There are 4 main classes of this enzyme (π, α, μ and θ) with no more than 30% sequence homology between the different classes; the crystal structure of the three protein complexes from two different classes (π and μ) are known and they have similar topology. The protein is basically made up of two domains; first domain contains a four-stranded anti-parallel β sheet and three α-helices and the larger second domain contains 5-helices. The cofactor-, but not the subtrate-, binding site is known from the crystal structures available
In our laboratory, NMR, crystallographic and kinetics studies are carried out in parallel on the recombinant α-class human liver protein in order to elucidate the mechanistic and structural aspects of the enzymic reaction. The NMR results have so far shown that the protein has a very high helical content with some β-sheet structure, suggesting that the 3D structure of this class of protein is similar to the other two (π and μ) for which the crytal structures are known.

LZ 335 NMR STUDY OF FIRST THREE ZINC FINGERS OF TFIIIA

Xiubei Liao, Karen R. Clemens, John Canaganh, Joel M. Gottesfeld and Peter E. Wright, Department of Molecular Biology, Scripps Research Institute, La Jolla, CA 92037

The first three fingers (ZF123) of TFIIIA from Xenopus has been shown to contribute majority of binding energy to intact TFIIIA DNA interaction. This three finger polypeptide has been isotopically labeled in E.coli and purified to more than 98% homogeneity. Nearly complete assignments of backbone ^1H, ^{15}N, aliphatic ^1H, ^{13}C and aromatic ^1H of ZF123 have been achieved using a combination of single, double and triple resonance multi-dimensional experiments. DG structure and backbone dynamics of ZF123 have been calculated from the data. The calculation of MD structure is currently underway.

LZ 336 FOLDING STUDIES OF A PREDOMINANTLY β-SHEET PROTEIN: CELLULAR RETINOIC ACID BINDING PROTEIN. Zhi-Ping Liu, Josep Rizo, and Lila M. Gierasch, Department of Pharmacology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9041.

Cellular retinoic acid binding protein (CRABP) is a member of a family of β-barrel proteins that bind hydrophobic ligands. In order to study the folding mechanisms of β-sheet proteins, we are analyzing the conformational behavior of CRABP in a variety of conditions, using nuclear magnetic resonance (NMR), circular dichroism (CD) and fluorescence spectroscopies. Unfolding of CRABP by urea is reversible and highly cooperative, and the urea-denatured state contains mostly random structure. However, acid denaturation leads to a state containing substantial amounts of α-helix (A-state), indicating that some regions of CRABP have the tendency to adopt non-native α-helical conformation in the absence of native tertiary interactions. Addition of millimolar concentrations of Na_2SO_4 to acid-denatured CRABP causes a transition to a state containing mostly β-sheet that has a high tendency to aggregate (S-state). One-dimensional NMR data suggest that the S-state shares some similarities with the native state, while the A-state is closely related to the urea unfolded state. The distribution of cross-peaks in the ^1H-^{15}N single quantum coherence spectrum of uniformly ^{15}N-labeled CRABP in the A-state is similar to that observed for the urea denatured state. However, the amide protons of the A-state are in general shifted upfield, which is consistent with protection from the solvent and with the formation of some helical structure in a good part of the sequence. Based on these observations, it is tempting to speculate that non-native α-helices are involved in the early stages of the folding mechanism of CRABP, and that the S-state is a collapsed form that could be related to later folding events. We are currently using heteronuclear three-dimensional NMR techniques to determine the native structure of CRABP, and to characterize in detail its partially folded states.

LZ 337 HETERONUCLEAR MULTIDIMENSIONAL NMR STUDIES OF THE UREA UNFOLDED FORM OF FK506 BINDING PROTEIN, Timothy M. Logan, Heng Liang, Edward T. Olejniczak, and Stephen W. Fesik, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064

The reference state for protein folding and stability studies is the unfolded form of the protein typically obtained in high concentrations of chemical denaturants, such as urea. However, the exact nature of this reference state is unclear due to a lack of detailed structural data. In this presentation, we characterize the equilibrium unfolded form of FK506 binding protein (FKBP) in 6 M urea. A series of 2D $^1H/^{15}N$-HSQC spectra of [U-^{15}N]-FKBP in various concentrations of urea are presented which show the urea-induced unfolding of FKBP to be a reversible, highly cooperative two-state process with a denaturation midpoint of 4 M. The ^1H, ^{15}N and ^{13}C assignments of [U-^{15}N,^{13}C]-FKBP in 6 M urea are also presented. These assignments were obtained using a combination of standard and newly developed heteronuclear 3D NMR methods for resolving the severe spectral overlap generally observed in NMR spectra of unfolded proteins. Finally, the results of the structural characterization of this unfolded protein are presented.

LZ 338 STRUCTURAL STUDIES OF THE CONSERVED DOMAIN FROM THE ACTIN-SEVERING PROTEIN VILLIN, Michelle A. Markus*+, Tomoko Nakayama#, K. Chandrasekhar*, Paul T. Matsudaira# and Gerhard Wagner*+, *Department of Biochemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115, +Department of Biophysics, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138, #Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Nine Cambridge Center, Cambridge, MA 02142

Villin is a member of a family of proteins of so far unknown structure that regulate the organization of actin by severing, capping, and nucleating actin filaments. Severing activity is enhanced by millimolar Ca^{++}, yet the severing proteins lack any known structural motif for binding Ca^{++}. Based on limited proteolytic cleavage and sequence analysis, the smallest unit known to retain severing activity is comprised of one domain of approximately 130 amino acids repeated three times. Each repeat apparently has different specificity for binding Ca^{++}, phosphatidyl 4,5-inositol phosphate, and actin. To provide a framework for understanding its functional properties, we are studying the structure of a typical severing domain by NMR. The first domain (126 amino acids) of villin from chicken intestinal epithelial cells has been overexpressed in *E. coli* and produced with and without uniform ^{13}C and ^{15}N enrichment. Sequential backbone assignments have been made based on triple resonance experiments and confirmed with ^{15}N-resolved noesy. Side chain assignments have been facilitated with ^{15}N-resolved tocsy and heteronuclear crosspolarization experiments. The secondary structure of the protein is mostly β-sheet with a short α-helix around residues 79-92. Initial structural characterization is in progress and will be presented.

LZ 339 THREE-DIMENSIONAL STRUCTURE OF THE FKBP/ASCOMYCIN COMPLEX IN SOLUTION BY HETERONUCLEAR 3D AND 4D NMR, Robert P. Meadows, David Nettesheim, Robert X. Xu, Edward T. Olejniczak, Andrew M. Petros, Thomas F. Holzman, Jean Severin, Earl Gubbins, Harriet Smith, and Stephen W. Fesik, Pharmaceutical Discovery Division Abbott Laboratories Abbott Park, Illinois 60064

We present the high resolution three-dimensional structure of the FKBP/ascomycin complex in solution using heteronuclear multi-dimensional NMR and a distance geometry/simulated annealing protocol. A total of 43 structures of the complex, including 3 tightly bound water molecules, were calculated from 1724 NOE derived distances obtained from 3D and 4D NOESY spectra, 66 $\chi 1$, 46 ϕ and 122 hydrogen bond restraints. The root mean square (RMS) deviations between the 43 FKBP/ascomycin solution structures and the mean atomic coordinates was 0.43 +/- 0.08 Å for the backbone heavy atoms and 0.80 +/- 0.08 Å for all non-hydrogen atoms. Angular order parameters for ϕ, ψ and $\chi 1$ angles exhibited mean values of 0.98, 0.97 and 0.95 respectively, while the mean of the $\chi 2$ order parameter was 0.63. Comparisons were made between the FKBP/ascomycin complex and two NMR-derived solution structures of unbound FKBP and the X-ray crystal structure of an FKBP/FK506 complex. A method for automating the NOE assignments is also discussed.

LZ 340 TOWARDS THE STRUCTURE OF HUMAN PROFILIN

William J. Metzler, Thomas B. Lavoie, Aneka Jan Bell, Eileen G. Ernst and Luciano Mueller, Departments of Macromolecular NMR and Macromolecular Biochemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-4000.

Profilin is a small actin-binding protein found in all eukaryotes examined to date. Profilin has also been shown to bind to phosphoinositol phosphates and to poly-L-proline. The low sequence homology between human and yeast profilins (~30%) suggests that profilin may serve as a potential target for therapeutic intervention. As part of an ongoing effort to characterize the structure-function relationships of this molecule, we have initiated heteronuclear NMR experiments aimed toward the elucidation of the three-dimensional structure of profilin.

Human profilin has been overexpressed in E. *coli* and uniformly enriched with ^{15}N and ^{13}C. Backbone sequential resonance assignments have been obtained through analysis of three and four dimensional, triple-resonance experiments: 4D-HNCAHA[1], 4D-HN(CO)CAHA[1], 3D-HNCACB[2] and 3D-CACB(CO)HN[3]. Progress toward the three-dimensional structure of profilin and analysis of the interaction of profilin with poly-L-proline will be presented.

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LZ 341 STRUCTURAL STUDIES OF HUMAN INTERLEUKIN 2 BY 2D AND 3D NMR.

Helen R. Mott, Paul C. Driscoll, Jonathan Boyd, Robert M. Cooke§, Malcolm P. Weir§ and Iain D. Campbell, *Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, U.K., and §Glaxo Group Reasearch Ltd., Greenford Road, Greenford, Middlesex, UB6 0HE, U.K.*

Interleukin-2 (IL-2) is a cytokine secreted by T cells in response to their stimulation by antigen. It is a growth factor for T cells, natural killer cells and B cells and is one of the major cytokines involved in the initial responses of the immune system. IL-2 has been the target of cancer research, and in its application in clinincal trials it has been found to have a profound effect on tumours. Recombinant IL-2 has been produced in *Escherichia Coli* as inclusion bodies, solubilized, and studied by 2D and 3D NMR using uniformly ^{15}N-labelled protein. The backbone resonances and most of the side-chain resonances have been assigned. The secondary stucture of the protein has been found to consist of four α-helices in an up-up-down-down conformation, and a short section of antiparallel β-sheet. At present, we are attempting to analyse the long-range NOEs so that a full tertiary structure can be calculated. Also, we have studied the structure of a mutant form of IL-2, where residue Phe-42 is replaced by Ala, a mutation which abolishes receptor binding. This mutant form has a structure similar to that of wild-type IL-2. The overall fold of IL-2 suggests that it is a member of a newly emerging family of four helix bundles that includes interleukin 4 (1) and granulocyte-macrophage colony-stimulating factor (2). The NMR structure of IL-2 described is significantly different from a structure of IL-2 previously obtained from low-resolution diffraction data (3), which has since been retracted (4).

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2. Diederichs *et al.* (1991) *Science* **254** 1779-1782.
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LZ 342 INVESTIGATION OF RIBONUCLEASE T1 FOLDING INTERMEDIATES BY HYDROGEN DEUTERIUM AMIDE EXCHANGE - 2D NMR SPECTROSCOPY, Leisha S. Mullins, C. Nick Pace and Frank M. Raushel, Departments of Chemistry and Medical Biochemistry & Genetics and the Center for Macromolecular Design, Texas A & M University, College Station, Texas 77843

The rate of hydrogen bond formation at individual amino acid residues in ribonuclease T_1 (RNase T_1) has been investigated by the hydrogen deuterium exchange - 2D NMR (HDEx-2DNMR) technique to gain insight into the mechanism of protein folding. The HDEx-2DNMR technique combines rapid mixing and 2D NMR techniques to follow the protection of backbone amide deuterons from exchange with solvent protons as a function of folding time. The technique depends on the differences in the exchange rates of hydrogen bonded and non-hydrogen bonded amide residues so that as the protein folds, the amide residues involved in hydrogen bonding are protected from exchange giving structural information about early folding events. The time course for deuterium protection was followed for 20 backbone amide residues that form stable hydrogen bonds in RNase T_1. The time courses are biphasic with 60 - 80 % of the protein molecules showing rapid hydrogen bond formation (11 - 88 s^{-1}) in the α-helix and the β-sheet. The remaining 20 - 40 % of the molecules are protected in a slow phase with a rate constant that has a lower limit of 0.01 s^{-1}. The first phase is complete within the first 100 ms of folding. If the rate constants in this first phase are arbitrarily sub-divided into 2 classes, fast (> 25 s^{-1}) and intermediate (< 25 s^{-1}), then the amide residues that are found in the hydrophobic core are in the fast class while those in the periphery of the three dimensional structure are in the intermediate class. The HDEx-2DNMR results indicate that in the early stages of folding, RNase T_1 folds on at least two parallel pathways each having at least one intermediate. We propose these two intermediates resemble the "structured" and "nonstructured" forms of molten globules.

LZ 343 SOLUTION STRUCTURE OF A FRAGMENT OF THE *E. COLI* ADA PROTEIN CONTAINING THE METAL BINDING AND PHOSPHOTRIESTER REPAIR ACTIVITY, Lawrence C. Myers, Gregory L. Verdine and Gerhard Wagner, Program for Higher Degrees in Biophysics, Harvard University, Cambridge, MA 02138.

The *E. coli* Ada protein repairs the highly mutagenic O^6-methylguanine lesions and methyl phosphotriesters in DNA by the irreversible transfer of the methyl group to a cysteine residue located in its C- and N-terminal domain, respectively. Methyl transfer to the N-terminal domain causes it to acquire a sequence-specific DNA binding activity, which directs binding to the regulatory region of several methylation-resistance genes, including its own. A 10 kD fragment from the N-terminal domain of Ada has been isolated which contains a high-affinity binding site for a single zinc atom and retains phosphotriester repair activity, but does not acquire sequence-specific DNA binding activity upon methylation. A double-labeled, ^{13}C and ^{15}N, sample has been used to obtain sequence specific assignments and the distance constraints necessary to generate a preliminary three-dimensional structure. This data constitutes the first structural information on any of the known forms of the Ada protein. ^{113}Cd NMR has been used to characterize the metal binding site as a four cysteine tetrahedral arrangement. The preliminary structure suggests that the design of the Zn^{2+}-binding domain of Ada is unique among the known structures of (Cys_4) zinc fingers. One of the putative ligand residues, namely Cys_{69}, also serves as the acceptor site for a phosphotriester-derived methyl group. This raises the possibility that methylation-dependent ligand reorganization about the metal plays a role in the conformational switching mechanism that converts Ada from a non-sequence-specific to a sequence-specific DNA-binding protein and that zinc plays a important role in the activation of the thiol for attack on the methylphosphotriester.

LZ 344 ASSIGNMENT, STRUCTURE AND DYNAMICS OF THE 21.5 kD PROTEIN, HUMAN DIHYDROFOLATE REDUCTASE : N. R. Nirmala, Johan Kördel and Gerhard Wagner, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115.

Human Dihydrofolate Reductase (DHFR) catalyzes the NADPH dependent reduction of dihydrofolate to tetrahydrofolate, pools of which are maintained in cells for cell division. As a result, inhibition of the function of DHFR has been a target in the chemotherapeutic treatment of cancer. Methotrexate (MTX) is a potent inhibitor of DHFR and binds very strongly to DHFR. The present study involves the 1:1 binary complex of DHFR with MTX. DHFR has 186 amino acid residues and the complex has a combined molecular weight of ~21.5 kD. Considering the size of the complex, the NMR studies were all done on protein isotopically enriched with ^{15}N, ^{13}C or both. Backbone proton and ^{15}N assignments have been published previously [1]. Triple resonance experiments like the HNCA, HNCOCA, HNCO etc. were used to assign carbon chemical shifts of the backbone. Sidechain proton and carbon assignments were made with the use of the HCCH-TOCSY and HCCH-COSY spectra . The distance geometry program DGII was used to calculate the three-dimensional conformation for the complex in solution. NOEs were semi-qualitatively obtained using upper bounds characterized by weak, medium and strong classifications. The overall structure obtained was similar to that obtained using X-Ray crystallography [2] with some local differences. Relaxation times were measured for the backbone ^{15}N nuclei of this protein. The ratio of spin-lattice relaxation time to the spin-spin relaxation time is very high for the ^{15}N nuclei of this protein. The correlation time calculated from the T1's and T2's indicates slower tumbling than might be expected based on relaxation data from other proteins of various sizes. This poster will present the highlights of the assignment procedure, the structure calculations and dynamics studies.

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LZ 345 STRUCTURAL STUDIES OF THE CALCIUM CHANNEL BLOCKER ω - CONOTOXIN AND A PARTIALLY ACTIVE DISULFIDE ISOMER. Raymond S. Norton[1], Paul K. Pallaghy[1], Brendan M. Duggan[1], & Michael W. Pennington[2] [1] *NMR Laboratory, Biomolecular Research Institute, 381 Royal Parade, Parkville 3052 AUSTRALIA* , [2] *Bachem Bioscience Inc., Philadelphia*

The 27-amino acid residue polypeptide ω- conotoxin G VIA is one of the presynaptic toxins from venom of the cone shell *Conus geographus*, which uses its venom to kill fish. This toxin blocks the voltage-gated calcium channels of nerve and muscle by binding to a novel high-affinity binding site, and is therefore of interest as a new ligand with which to probe the structure and activity of the calcium channel. The focus of the present study is the three-dimensional structure in aqueous solution of the native molecule and the effects on that structure of altering the disulfide pairings.

The structure of native ω-conotoxin (prepared by peptide synthesis) has been determined from high-resolution ^1H NMR spectral data recorded at 600 MHz. Structural constraints consisting of interproton distances inferred from NOEs, dihedral angles from spin-spin coupling constants and hydrogen bonds from the locations of slowly exchanging amide protons were used as input for distance geometry and simulated annealing calculations with the program DSPACE.

The family of structures obtained in this way is well defined by the NMR data. The molecule adopts a compact structure incorporating a number of reverse turns but little regular secondary structure. All three tyrosine residues are located on the molecular surface. There are no negatively charged groups in conotoxin, while the five positively charged groups are distributed in three separate patches on the surface. The implications of the structure in terms of how conotoxin binds to its receptor site on the voltage-gated calcium channel will be discussed.

An analogue of conotoxin with the same amino acid sequence but different disulfide pairings has also been investigated. Its structure is altered profoundly from the native structure and shows heterogeneity, presumably as a result of *cis-trans* isomerism preceding proline residues.

LZ 346 SOLUTION STRUCTURE OF THE BIOLOGICAL ACTIVE DES-(B25-Phe) MUTANT OF HUMAN INSULIN, Helle B. Olsen*, Jens J. Led* and Per Balschmidt†, *Department of Chemistry, University of Copenhagen, The H.C. Ørsted Institute, Universitetsparken 5, DK-2100 Copenhagen Ø, Denmark; †Diabetes Research, Novo Nordisk A/S, Novo Allé, DK-2880 Bagsværd, Denmark.

The solution structure of the des-(B25-Phe) mutant of human insulin at pH 3.0 has been obtained using ^1H-^1H NOE constraints, Distance Geometry and Restrained Molecular Dynamics. The structure of the central parts of the molecule is similar to that of a number of other insulin mutants in solution as obtained by NMR (1-4). However, a less tight network of NOE's in the helix-areas suggests a looser structure of the des-(B25-Phe) mutant in these areas. Moreover, the end of the shortened C-terminus of the B-chain is in close contact with the N-terminus A-chain helix, giving this mutant a more globular shape as compared with the other mutants (1-4). Also, the studies reveal no sign of dimerisation, reflecting the change in one of the major dimer binding sites.

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LZ 347 THREE DIMENSIONAL SOLUTION STRUCTURE OF THE SRC HOMOLOGY 2 DOMAIN OF C-ABL, Michael Overduin, Carlos B. Rios, Bruce Mayer, David Baltimore and David Cowburn. The Rockefeller University, New York, NY 10021

Src Homology 2 (SH2) regions are recognition motifs found in many intracellular signal transducing proteins that bind tyrosyl-phosphorylated protein sequences. The solution structure of the Abl SH2 product, a protein of 109 residues and 12.1 kDa, has been determined by multidimensional nuclear magnetic resonance methods. A pair of antiparallel β sheets and a C-terminal α helix enclose a hydrophobic core. A putative ligand binding groove is formed on an exposed portion of one β sheet between an N-terminal α helix and a five residue loop. Three arginine residues lie within this groove, their sidechains capable of ligating a phospho-tyrosyl group. Sequence homology comparisons to other members of the SH2 domain family show higher conservation in the hydrophobic core and binding area, suggesting a conserved global fold and mode of ligand binding.

LZ 348 ^{1}H-NMR STUDIES OF PROTEIN FRAGMENTS IN SOLUTION

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Recently, we developed a procedure to identify short protein segments that are likely to adopt a well defined conformation in absence of interactions with the rest of the chain[1,2]. Such locally structured segments could be part of early folding intermediates and/or yield *peptides* that adopt well defined structures in solution. Applying our method to 69 known protein structures a repertoire of structured segments has been obtained. Here, we report a study in which four of these segments corresponding to α-helical regions, respectively delimited by residues 63-73 and 97-112 from cytochrome c_2 (Rhodospirillum) and residues 24-36 and 45-55 from Calcium Binding Protein, have been synthesized by solid phase methods and their solution conformations determined by NMR. The peptides were dissolved in ^1H_2O/trifluoroethanol-d_3 (TFE) (60/40; v/v) to give 1-2 mM peptide solutions, pH = 3.8-5.0 and DQF-COSY, TOCSY, ROESY and NOESY were recorded at 25°C. All four peptides show, (i) an extended pattern of sequential $d_{NN}(i,i+1)$ NOE connectivities, the intensities of which are comparable with, or greater than the corresponding $d_{\alpha N}(i,i+1)$ NOEs; (ii) several $d_{\alpha N}(i,i+3)$, $d_{\alpha N}(i,i+4)$, $d_{\alpha\beta}(i,i+3)$ and $d_{NN}(i,i+2)$ NOE connectivities, whose number and intensities vary among the peptides. We conclude that the examined peptides have a detectable population of α-helical conformers in water/TFE (60/40; v/v) at room temperature, and are currently investigating their helical propensities at lower TFE concentrations and in water.

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LZ 349 EXPERIMENTAL AND MOLECULAR MODELING STUDIES OF PEPTIDE AGGREGATION,

Miquel Pons, Imma Fernández, Monika Fuxreiter, Josep Ubach, Ernest Giralt and David Andreu, Departament de Química Orgànica. Universitat de Barcelona.SPAIN

The conformational characteristics of peptides are often strongly affected by intermolecular interactions caused by aggregation. Although this is sometimes a desirable feature built in the actual design of the peptide, more often is an unwanted effect and, if not properly accounted for, may lead to false conclusions and jeopardise the utility of the model system.This is specially important in the case of NMR because, for sensitivity reasons, the concentrations used tend to be higher than with other techniques like Circular Dichroism.

From the NMR point of view the main pitfalls are: (i) Aggregation induced conformational changes, (ii) False NOE assignments arising from the misinterpretation of intermolecular NOE as intramolecular (iii) Anomalous H-D exchange rates and (iv) Improper correction for spin diffusion effects even using full relaxation matrix methods. In our communication we shall present recent examples arising from our work with hybrid peptides containing fragments from cecropin and melittin.

Aggregation can also invalidate the results of modeling studies. To take them into account we have incorporated mutually interacting symmetry replicates in the molecular dynamics calculations of the above peptides. A number of aggregation models have been considered and the results of the simulations will be compared with the experimental results.

LZ 350 **THE HIGH RESOLUTION THREE-DIMENSIONAL SOLUTION STRUCTURE OF HUMAN INTERLEUKIN-4 BY MULTI-DIMENSIONAL HETERONUCLEAR MAGNETIC RESONANCE SPECTROSCOPY,**

Robert Powers, Daniel S. Garrett, Carl J. March, Eric A. Frieden, Angela M. Gronenborn, and G. Marius Clore., NIDDK, Laboratory of Chemical Physics, Building 2 National Institutes of Health, Bethesda, MD 20892

The three-dimensional solution structure of recombinant human interleukin-4, a protein of 133 residues and 15.4 KDa which plays a key role in the immune and inflammatory systems has been solved to a backbone resolution of 0.45 Å and an all atom resolution of 0.9 Å(1). A combination of 3D and 4D heteronuclear and long-range ^{13}C-^{13}C NMR experiments inconjunction with stereosearch and simulated annealing protocols were employed to refine the structure. The NMR structure determination was principally based on ~2300 approximate interproton distance restraints and supplemented with torsional angle restraints and backbone NH-CO hydrogen bond restraints. The structure is dominated by a left-handed four helix bundle with an unusual topology comprising two overhand connections. The linker elements between the helices are formed by either long loops, small helical turns or short strands. The overall topology is remarkably similar to that of growth hormone and granulocyte-macrophage colony stimulating factor, despite the absence of any sequence homology, and substantial differences in the relative lengths of the helices, the length and nature of the various connecting elements, and the pattern of disulfide bridges. These three proteins, however, bind to cell surface receptors belonging to the same hematopoietic superfamily, which suggests that interleukin-4 may interact with its receptor in an analogous manner to that observed in the crystral structure of the growth hormone-extracellular receptor complex.

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LZ 351 A DE NOVO DESIGNED PROTEIN SHOWS A THERMALLY INDUCED TRANSITION FROM A NATIVE TO A MOLTEN GLOBULE LIKE STATE,

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The synthesis and characterization of a de novo designed protein that dimerizes to form a four helix bundle is described. CD indicates that the protein is α-helical and size exclusion chromatography shows that it is compact and dimeric. NMR spectroscopy, Trp fluorescence, and circular dichroism in the near UV indicate that the peptide undergoes a cooperative transition from a native like to a molten globule like state, with a midpoint of 292°K.

LZ 352 THE DISULFIDE BONDING PATTERN AND THREE DIMENSIONAL STRUCTURE OF ω-AGATOXIN IVB DETERMINED NMR ANALYSIS, Michael D. Reily, Venkataraman Thanabal and Michael E. Adamst, Department of Chemistry, Parke-Davis Pharmaceutical Research Division, Warner Lambert Company, Ann Arbor, MI 48105; †Departments of Entomology and Neuroscience, University of California Riverside, Riverside, CA 92521.

The two homologous 48 amino acid peptides, ω-agatoxin IVA[1] and ω-agatoxin IVB, are recently discovered cystine-rich toxins isolated from the venom of the funnel web spider (*Agelenopsis aperta*). These peptides are potent and selective antagonists of p-type calcium channels in mammalian brain. We report verification of the primary structure and determination of the disulfide bonding pattern and tertiary structure of ω-agatoxin IVB based on homonuclear two-dimensional NMR experiments. The unknown location of the peptide's four disulfide bonds provided a rare opportunity to utilize NOE data alone to assess the disulfide bonding pattern in this molecule. The NOE's were used as restraints in distance geometry/simulated annealing calculations, revealing a unique set of disulfides consistent with the experimental data within acceptable limits. This peptide is the first of the agatoxins to have its three dimensional structure identified.

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LZ 353 CONFORMATIONAL STUDIES OF A CYCLIC RGD-CONTAINING PEPTIDE ANALOGUE

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The tripeptide sequence arg-gly-asp (RGD) of fibrinogen is a mediator of the interaction between fibrinogen and the platelet glycoprotein IIb/IIIa which leads to platelet aggregation. The solution conformation of a cyclic RGD-containing compound : cyclo (S,S)-2-mercaptobenzoic acid-RGD-2-mercaptoaniline (the N-demethylated form of SK&F 107260), an inhibitor of this interaction, has been determined by molecular dynamics calculations based upon NMR-derived distance constraints.

The compound was shown to undergo a slow ring opening in solution in DMSO through cleavage at the mercaptobenzoic acid-arginine amide linkage. The 600MHz ^{1}H NMR spectrum was assigned via COSY, TOCSY and NOESY experiments and distance constraints were derived from the intensity of off-diagonal cross peaks in a 100ms spin-lock ROESY spectrum. The mercaptoaniline amide proton had a very small chemical shift temperature dependence indicative of its being involved in a hydrogen-bond. Circular dichroism studies indicated that this compound had a rigid solution conformation with a negative torsion angle about the disulphide bond.

Initial optimisation of molecular conformation was achieved via a simple genetic algorithm in which, iteratively, individual rotatable bonds in a ring-opened structure were sequentially energy minimised before ring closure, to give the best fit to the constraints with minimum atom-atom overlap. Two low energy conformers with minimum distances between the mercaptoaniline amide proton and carbonyl groups were generated by molecular dynamics simulations (using SYBYL) with no initial constraints. These were further energy minimised by SYBYL to obtain the conformation which best fitted the NOE-derived distances.

LZ 354 HETERONUCLEAR THREE-DIMENSIONAL NMR SPECTROSCOPY OF A PARTIALLY DENATURED PROTEIN: THE A-STATE OF HUMAN UBIQUITIN

Terrence A. Scahill, Annica Euvrard and, Brian J. Stockman Physical and Analytical Chemistry Research, Upjohn Laboratories, Kalamazoo, MI 49001

Human ubiquitin is a 76-residue protein that serves as a degradation signal when conjugated to another protein. Ubiquitin has been shown to exist in at least three states: native (N-state), unfolded (U-state), and, when dissolved in 60% methanol:40% water at pH 2.0, partially folded (A-state). Structures for the N-state have been determined by both X-ray crystallography and NMR spectroscopy by others. Comparison of the N-state and the A-state structures may lead to an understanding of the folding pathway of ubiquitin. To this end we have determined the secondary structure of the A-state using three-dimensional NMR spectroscopy of uniformly labelled ^{15}N-enriched ubiquitin. Sequence-specific ^{1}H and ^{15}N resonance assignments have been completed for more than 90% of the residues in the A-state. The assignments were made by concerted analysis of three-dimensional ^{1}H-^{15}N NOESY-HMQC AND TOCSY-HMQC data sets. Because of the degenerate nature of the chemical shifts for many residues, the increased resolution provided by the ^{15}N dimension was critical. Analysis of short- and long-range NOE's indicates that only the first two strands of β-sheet, comprising residues 2-17, remain in the A-state, compared to five strands in the N-state. NOE's indicative of an α-helix comprising residues 25-33 were also identified. These residues are also helical in the N-state. In the N-state, residues in this helix are in contact with residues from the first two strands of β-sheet. However, no NOE's have been definitively identified that indicate the existence of this association in the A-state. It is likely, however, that residues 1-34 comprise a folded domain the A-state of ubiquitin. Based on ^{1}Hα chemical shifts and weak short-range NOE's, residues 34-76 do not adopt rigid secondary structure but do favor a helical conformation. This observation may be related to the helix-inducing effects of the methanol present.

LZ 355 RESIDUE HELICITY IN MODEL PEPTIDES OBSERVED BY 13C NMR, William Shalongo, Laxmichand Dugad and Earle Stellwagen, Department of Biochemistry, University of Iowa, Iowa City, IA 55242

We have explored the potential of one-dimensional 13C NMR measurements for the determination of the residue helical content in two model peptides, acetylW(EAAAR)$_3$Aamide (peptide I) and acetyl(AAQAA)$_3$amide (peptide II). Circular dichroic measurements suggest that about 90% of the molecules of peptide I and about 50% of the molecules of peptide II are in an alpha-helical conformation in aqueous solutions of low ionic strength at zero degrees and neutral pH. Analogs of each peptide were synthesized containing a single alanine or glutamine residue whose carbonyl carbon was 20% enriched in 13C. The thermal dependence of the chemical shift of each enriched carbonyl resonance was measured and fit with a two-state helix/coil transition and the fractional helical content was then calculated at two degrees. The exchange rate of the amide hydrogen adjacent to each enriched carbonyl was measured in an equimolar mixture of H_2O/D_2O at the same temperature by analysis of the lineshape of each enriched carbonyl resonance. The fractional helical content was calculated from these amide exchange rate measurements by comparison with the rates predicted for unstructured model peptides in the same solvent. Both chemical shift and amide exchange measurements of peptide I describe a common symmetrical distribution of residue helical content on sequence position which can be fit using the Lifson-Roig statistical model and global sigma and s values of 0.003 and 1.74, respectively. By contrast, chemical shift and amide exchange measurements of peptide II describe asymmetrical distributions of residue helical content on sequence position. The helical content of the N-terminal residues are enhanced in chemical shift measurements and the helical content of the C-terminal residues are enhanced in amide exchange measurements. We suggest that these asymmetrical distributions result from the partial cha es generated in the frayed end of the helix. The asymmetric distributions observed for peptide II can be fit with an extended Lifson-Roig model using global sigma and s values of 0.003 and 1.32, respectively.

LZ 356 THE METAL ION BINDING PROPERTIES OF DOG OSTEOCALCIN. Judith G. Shelling, Donna T. Isbell, Shan Du, Alan G. Schroering, and Giovanna Colombo, Department of Biochemistry, The University of Kentucky, 800 Rose Street, Lexington, KY 40536.

1D ^{1}H NMR was employed to study how Ca^{2+} and Lu^{3+} bind to dog bone Gla protein (BGP, osteocalcin). Dog BGP is highly homologous to BGPs from other species that bind 1 to 3 mole equivalents of Ca^{2+} with K_ds ranging from 0.14 to 3 mM. In 20 mM NaCl, 2 mole equivalents of Ca^{2+} bound sequentially to apo dog BGP in the NMR slow exchange limit. The same spectral perturbations were observed up to a $[Ca^{2+}]_o/[BGP]_o$ ratio of ~2.3 in 150 mM NaCl. Just 1 Lu^{3+} equivalent produced the same spectral perturbations induced by 2 Ca^{2+}. The addition of 2 Lu^{3+} to Ca^{2+}-saturated BGP had little effect on the spectrum, but the protein aggregated at $[Lu^{3+}]_o/[BGP]_o$ ratios greater than 2 in either the presence or absence of Ca^{2+}. The spectrum of Ca^{2+}-saturated BGP was invariant up to ~60°C, after which all the resonances shifted towards random coil frequencies; in 150 mM NaCl this was observed above ~55°C . At low pH, Ca^{2+} saturation stabilized the protein against the conformational changes observed for apo BGP, but the BGP:Ca^{2+} complex was unstable above pH 9. These results show that dog BGP has a much higher affinity for Ca^{2+} than BGPs from other species, its Ca^{2+} affinity is only slightly reduced at physiological ionic strength, only 1 equivalent of Lu^{3+} is required to mimic the Ca^{2+}-saturated state of the protein, and the BGP:Ca^{2+} complex is unstable at elevated pH or temperature. Multidimensional NMR studies of the BGP:Ca^{2+} complex are currently in progress.

LZ 357 METHOD FOR DETECTING THE EARLIEST PATHWAY SIGNALS OF PROTEIN FOLDING. Simon Sherman, William Gmeiner and Raymond Ruddon, Eppley Institute, University of Nebraska Medical Center, Omaha, NE 68198-6805

The proposed method is based on several assumptions: (i) Proteins fold by a thermodynamically controlled stage mechanism that is programmed by their amino acid sequence. (ii) The folding mechanism is initiated by the earliest pathway signals that are coded in nucleation sites. (iii) In a given protein sequence there are several short peptides that adopt local structure very early in the folding pathway and act as nucleation sites. (iv) The specific conformation of these peptides, corresponds to an equilibrium state that is self stable and cannot be changed significantly during the whole folding process. The conformation of an nucleation site is both an "embryo" of folded structure and one of the earliest pathway signals.

To determine nucleation sites and their specific local conformations on the basis of combined use of NMR data and methods of molecular mechanics and dynamics, it is necessary to: (i) perform conformational analysis of all possible short peptides (up to, say, pentapeptides) from the protein amino acid sequence; (ii) choose the oligopeptides that are most likely to be stabilized by local interactions; (iii) transform available NMR data into conformational characteristics of amino acid residues (see, Sherman & Johnson, *Prog. Biophys. molec. Biol.*, 1992); (iv) determine both the nucleation sites around candidates selected in step (ii) and their specific local conformations determined in step (iii). The candidates with conformations that are statistically indistinguishable from those obtained by NMR data are nucleation sites, and their conformations can be assumed as the earliest pathway signals.

The results of applying the proposed method to study conformational folding pathway signals in the BPTI molecule and in the β-subunit of the human chorionic gonadotropin are discussed.

LZ 358 AMIDE PROTON EXCHANGE IN ONE- AND TWO-DISULFIDE ANALOGS OF BPTI Brenda A. Schulman and Peter S. Kim, Howard Hughes Medical Institute, Whitehead Institute for Biomedical Research, Department of Biology, M.I.T., Cambridge, MA 02142

BPTI is stabilized by three disulfide bonds, between cysteines 30-51, 5-55, and 14-38. We are interested in characterizing the role of these disulfide bonds in determining the structure and dynamics of the protein. Two variants of BPTI have been examined: [5-55], containing only a single disulfide bond between residues 5 and 55, and [30-51; 14-38], containing disulfide bonds between residues 30-51 and 14-38. Both proteins are known to fold into structures essentially identical to native BPTI, making them ideal candidates for comparing the effects of disulfide bonds on protein structure and dynamics. We have assigned the ^{1}H and ^{15}N resonances and have measured the amide proton exchange rates for recombinant models of each variant, in which the cysteines not involved in disulfide bonds have been replaced by alanine or valine. Despite the fact that these proteins contain different disulfide bonds, the chemical shifts and overall patterns of protection from exchange in both analogs are similar to previous measurements made on native BPTI. Nonetheless, there are striking differences among the proteins in the relative degrees of protection of specific residues, indicating that the location of disulfide bonds in proteins affects local dynamics in molecules with similar folds.

LZ 359 MULTIDIMENSIONAL NMR STUDIES OF SKELETAL TROPONIN C. Carolyn M. Slupsky, and Brian D. Sykes. Department of Biochemistry. MRC Group in Protein, Structure and Function. University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

The thin filament protein troponin C (TnC) is a key protein involved in muscle contraction. Avian skeletal TnC has 162 amino acid residues, two homologous domains and is largely helical. Each domain contains two helix-loop-helix calcium binding sites, the C-domain with two high affinity sites and the N-domain with two lower affinity sites. The binding of calcium to the two low affinity sites triggers muscle contraction. Central to the understanding of muscle contraction is the understanding of the structure of TnC in its various stages of calcium binding. We are studying the solution structure of TnC in its calcium saturated form using multidimensional heteronuclear NMR techniques. While TnC is similar in structure to calmodulin (CaM) which has been assigned, there are differences between TnC and CaM which makes the assignment of TnC more difficult. First, TnC contains one SH group which was carboxamidomethylated to prevent oxidation. Second, TnC has larger linewidths than CaM. These are not due to aggregation at concentrations ≤ 1 mM since dilution experiments down to 17 uM do not change the linewidth of TnC. Over 85% of TnC has been assigned using conventional 3D NMR techniques (HNCA, HN(CO)CA, HNCO, HCACO, H(CA)NH, etc.). Comparison of the C-domain assignments of TnC with the SCIII/SCIV synthetic peptide heterodimer assignments reveals a very close correlation. The C-domain assignments of TnC are also homologous to the C-domain assignments of CaM (*Biochemistry* **29**, 4659-4667 (1990)). The N-domain of TnC presents yet another problem for assignment and structure determination since the NH resonances appear to be much weaker in this domain than the C-domain and the homology in assignment to isolated N-domain and/or CaM is not as clear as for the C-domain. We present here the partial assignment of skeletal TnC as well as some of the preliminary secondary structural data.

LZ 360 ESSENTIALLY COMPLETE ^{13}C, ^{15}N AND ^{1}H RESONANCE ASSIGNMENTS FOR THE METHOTREXATE COMPLEX OF *L.CASEI* DIHYDROFOLATE REDUCTASE, BASED ON ISOTOPIC LABELLING OF THE PROTEIN (^{2}H, ^{13}C AND ^{15}N) AND MULTIDIMENSIONAL NMR SPECTROSCOPY, Alice Soteriou, Mark D. Carr, Tom A. Frenkiel, Christopher J. Bauer, John E. McCormick, Berry Birdsall and James Feeney, National Institute for Medical Research, Mill Hill, London NW7 1AA, England.

Three dimensional double and triple resonance NMR spectroscopy, in conjunction with uniform ^{13}C and ^{15}N labelling of *L.casei* dihydrofolate reductase (162 residues) has been used to make essentially complete backbone and sidechain resonance assignments for the methotrexate (MTX) complex of the enzyme. The assignments obtained show complete agreement with a partial set of sequence specific assignments determined previously, by correlating NOE and crystal structure data. This indicates that the overall structure of the protein is similar in the solid and crystal states.

The complete assignments obtained recently will now form the basis of the determination of the 3D structure of the DHFR-MTX complex in solution using data from 3D and 4D NOE experiments. The precision of these calculated structures will be improved by the availability of stereospecific assignments for all leucine methyl groups based on 2D ^{1}H spectra acquired from DHFR in which the leucines have been stereospecifically deuterated.

LZ 361 TOWARD THE SOLUTION STRUCTURE OF INTERLEUKIN-1 RECEPTOR ANTAGONIST PROTEIN DETERMINED BY HETERONUCLEAR THREE-DIMENSIONAL NMR SPECTROSCOPY, Brian J. Stockman, Terrence A. Scahill, Annica Euvrard, Nancy A. Strakalaitis, David P. Brunner, Anthony W. Yem, and Martin R. Deibel, Jr., Upjohn Laboratories and Chemical Division, The Upjohn Company, 301 Henrietta St., Kalamazoo, MI 49001

Interleukin-1 proteins, such as IL-1β, play a key role in the immune and inflammatory responses. Interleukin-1 receptor antagonist protein (IRAP) is a 153-residue, naturally occurring inhibitor of the interleukin-1 receptor. In contrast to IL-1β, IRAP induces no physiological effects upon binding to the interleukin-1 receptor. We are using multidimensional, heteronuclear NMR spectroscopy to determine the antagonist's solution structure. Using a combination of 3D ^{1}H-^{15}N NOESY-HMQC and TOCSY-HMQC and 3D ^{1}H-^{15}N-^{13}C HNCA and HN(CO)CA experiments on uniformly ^{15}N- or doubly ^{13}C/^{15}N-enriched IRAP, we have made resonance assignments for more than 90% of the main-chain atoms. Extensive side-chain assignments have been made using a combination of ^{1}H-^{13}C-^{13}C-^{1}H COSY and TOCSY data sets. Over 1,200 distance constraints have been extracted from 3D ^{1}H-^{15}N NOESY-HMQC and ^{1}H-^{13}C NOESY HMQC spectra. Backbone torsion angle constraints have been determined as well. Structures calculated from these constraints indicate that IRAP is predominantly β-sheet, with the same overall topology as IL-1β. Analysis of the structure, as well as comparisons of $^{1}H^{\alpha}$ and $^{13}C^{\alpha}$ chemical shifts, indicate differences and similarities in the regions of the primary sequences that comprise the β-sheet framework. The 14% sequence identity between IL-1β and IRAP increases to 25% when differences in the locations of secondary structure elements in the primary sequences are taken into account. Still, numerous differences in side chains, which ultimately play a major role in receptor interaction, exist. Comparison with the structure of IL-1β, in conjunction with mutagenesis data, suggests structural similarities and differences that account for the drastically different physiological effects of these two proteins.

LZ 362 THREE-DIMENSIONAL SOLUTION STRUCTURE OF THE CYCLOSPORIN A/CYCLOPHILIN COMPLEX BY NMR. Yves Theriault, Timothy M. Logan, Robert P. Meadows, Liping Yu, Edward T. Olejniczak, Thomas F. Holzman, Robert L. Simmer, and Stephen W. Fesik Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064

Cyclosporin A, a cyclic undecapeptide, is a potent immunosuppressant that binds to a peptidyl-prolyl cis-trans isomerase of 165 amino acids, cyclophilin. The cyclosporin A/cyclophilin complex inhibits the calcium- and calmodulin-dependent phosphatase, calcineurin, resulting in a failure to activate genes encoding interleukin-2 and other lymphokines. In this presentation, we report on the ^{1}H, ^{13}C, and ^{15}N assignments and three-dimensional structure of the complex in solution using heteronuclear multi-dimensional NMR spectroscopy. The structure, one of the largest determined by NMR, is stabilized by several hydrophobic interactions and hydrogen bonds. The complex is analysed in term of the structure/activity relationships for cyclosporin A analogs and is compared to previously determined X-ray structures of free cyclophilin and cyclophilin bound to a tetrapeptide substrate.

LZ 363 THE GCN4 ACIDIC ACTIVATION DOMAIN FORMS A β–SHEET STRUCTURE: AN INVESTIGATION OF ITS ROLE IN TRANSCRIPTIONAL ACTIVATION. Michael N. Van Hoy and Thomas Kodadek, Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712

Transcription is the major pathway governing gene regulation in eucaryotes. In yeast, transcription of class II genes is carried out by RNA polymerase II. In recent years many researchers have identified a host of other factors required to establish pol II at a promoter. This process is known as initiation and first requires TFIID to bind at a TATA element in the promoter followed by TFIIA, TFIIB and finally by pol II. This combination of factors allows "basal transcription" to occur, but other factors known as transcriptional activators are required to give stimulated transcription. These activators have been shown to interact with several components of the basal transcription machinery. The interactions presumably overcome a rate limiting step in initiation, although this has not been demonstrated. Most transcriptional activators have been shown to have two separable domains required for potency. These are a sequence specific, DNA binding domain and an activation domain. One of the most studied classes of activation domains is characterized by its abundance of acidic residues. These "acidic activators" have been speculated to form amphipathic α-helices or to exist as structureless "acid blobs". We have undertaken a structural investigation of the GCN4 transcriptional activation domain and shown it is not an amphipathic α-helix rather a β-sheet. In addition we have investigated the role of the interaction of the activation domain with components of the basal transcription machinery. Results of these experiments will be discussed.

LZ 364 INTERACTION OF CYTOPLASMIC LOOP PEPTIDES OF G PROTEIN-COUPLED RECEPTORS WITH G PROTEINS AND WITH PHOSPHOLIPID MEMBRANES

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Trimeric GTP-binding regulatory proteins (G proteins) on cytoplasmic membranes serve as signal transducers from cell surface receptors to intracellular effectors, such as β-adrenergic receptor/Gs/adenylyl cyclase. G protein-coupled receptors have in common seven transmembrane spans with the N-terminus oriented extracellularly. The second and the third cytoplasmic loop as well as C-terminal tail of β-adrenergic receptor are considered to be involved in the interaction with G proteins. However, it has not been analyzed yet how the specificity between receptor and G protein is ensured, how receptor molecules activate G proteins on molecular level, or how membranes affect their interactions.

We synthesized peptide fragments corresponding to the segments of cytoplasmic loops of β-adrenergic receptor, and studied their interaction with G proteins and with phospholipid membranes to address these questions: (1)what conformation do the loops take upon the interaction with G proteins? (2)which residues of the loops are directly involved in the interaction with G proteins? and (3)how the loops interact with phospholipid membranes in the absence of G proteins?

We found that the peptides corresponding to the N-terminal part of the third intracellular loop of β-adrenergic receptor can directly activate Gs effectively, and Gi less effectively. By transferred NOE (TRNOE) analyses combined with distance geometry calculations, the peptides were found to take an α-helical conformation upon the interaction with both Gsα and Gi1α proteins. Side-chain orientation and/or mobility of bound peptide seems to be different depending on the species of G proteins used. These peptides have strong affinity to anionic phospholipid membranes and take an α-helical conformation also when bound to the membranes. These results suggest that the N-terminal part of the third cytoplasmic loop of β-adrenergic receptor takes α-helical structure to activate G proteins. These studies will help to analyze the detailed mechanism of the activation of G proteins by receptor.

LZ 365 STRUCTURE OF A CYCLIC ESTERASE MIMIC; NMR SPECTROSCOPY, COMPUTER SIMULATION AND HYDROLYTIC ACTIVITY, Björn Walse[1], Magnus Ullner[1], Christer Lindbladh[2], Leif Bülow[2], Olle Teleman[3] and Torbjörn Drakenberg[1,4]. Departments of [1]Physical Chemistry 2, [2]Pure and Applied Biochemistry, Chemical Center, University of Lund, POB 124, S-221 00 Lund, Sweden. [3]Biotechnical Laboratory, VTT, POB 204, [4]Chemical Laboratory, VTT, POB 202, SF-02151 Espoo, Finland.

A small cyclic octapeptide (acetate-ACSPGHCE) was designed to mimic the catalytic triad (Ser, His, Asp) found in serine proteases. To get maximum probability for a β-turn (type II) the amino acids Pro and Gly were inserted at positions 4 and 5 and a disulfide bridge was placed between positions 2 and 7 in the peptide. Glutamate was chosen instead of aspartate in this serine protease mimic since model building showed that this amino acid was more likely to adopt a suitable conformation with His and Ser. The predicted structure of the peptide was subjected to a molecular dynamics (MD) simulation in water. During the simulation, which lasted 173 ps, the peptide stayed in a conformation close to the predicted which indicated that the potential hydrogen bonds could exist as required for the charge relay network. The NMR measurements included ^{1}H-^{1}H NOEs, $^3J_{HN_\alpha}$ and $^3J_{\alpha\beta}$ coupling constants. These measurements were used as input in simulated folding which is restrained molecular dynamics simulation with a simplified description of nonbonded interactions. Each member of the resulting family of accepted structures contained a type II β-turn. None of the hydrogen bonds required for the charge relay network were found in the experimental structure. However in activity measurements, the peptide hydrolysed p-nitrophenyl acetate about 9 times faster than free histidine. This suggests a conformation where the charge relay network is mediated over water.

LZ 366 STRUCTURE DETERMINATION OF HUMAN ELAFIN,

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Excessive leakage of the proteinase elastase from leukocytes and/or reduced in vivo levels of elastase inhibitors results in the extensive tissue damage associated with diseases emphysema, cystic fibrosis and rheumatoid arthritis. Elafin is a natural, low molecular weight (57 residues), highly specific elastase inhibitor isolated from the skin of patients with psoriasis. As such, this protein has proven to be of considerable use as a base compound for anti-emphysema drug development and may itself be of therapeutic value. In the course of the structure determination of elafin, we have assigned over 98% of the proton resonances of the recombinant protein in aqueous solution using a series of 2D and 3D NMR experiments. Elafin is a highly stable protein in terms of both pH and temperature, permitting us to characterise it under numerous conditions. This has led to the characterisation of an intermediate exchange phenomenon occurring in the N-terminal region of the protein.

Furthermore, we have currently identified over 500 distance constraints and torsional constraints, and have calculated preliminary structures using both metric matrix distance geometry and restrained simulated annealing methods, within the programs X-PLOR and NMRchitect. These studies reveal an unusually low regular secondary structure content for the protein - the structure being dominated by four disulphide bonds. In addition, we have characterised the complex formed between elafin and elastase and are in the course of studying isotopically labelled elafin bound to the proteinase. We will present our assignment strategy and 3-D solution structures of elafin, and preliminary studies of the protein-inhibitor complex.

LZ 367 THE SOLUTION STRUCTURE & DYNAMICS OF A PROTEIN MOLTEN GLOBULE: APOCYTOCHROME B562

A. Joshua Wand, Yiqing Feng and Stephen G. Sligar, Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61810

The solution structure and dynamics of apocytochrome b562 are being studied by high resolution NMR spectroscopy. In the absence of the heme prosthetic group, the protein displays many of the characteristics ascribed to the molten globule state. The structure of protein has been determined to intermediate resolution by distance geometry and related computational methods. The structure determination has been complicated by extensive degeneracy of the 1H NMR spectrum. Nonetheless, it can be unequivocally shown that the secondary and tertiary structure of the protein is very similar to the holoprotein. All four helices are present, though some disorder of the C-terminal helix is evident. Superposition of the mean structure of apoprotein on the crystal structure of the holoprotein indicates that one side of the heme binding pocket is preserved and is exposed to solvent. This leads to the speculation that the molten globule state represents a penultimate step in the folding of the protein. We have also characterized the internal dynamics of the protein by hydrogen exchange and NMR relaxation techniques. The NMR relaxation results indicate that the amplitude of motion of amide N-H vectors is not much different from that found in proteins in the native state. In contrast, the effective correlation times are found to vary over a much wider range than is normally seen in proteins in the native state. In sum, these results provide the first comprehensive structural view of a protein in the molten globule state.

LZ 368 NMR STRUCTURE DETERMINATION OF A DICYCLIC LACTAM-BRIDGED HUMAN γ-INTERFERON HELIX F SYNTHETIC PEPTIDE

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The three-dimensional solution structure of a dicyclic lactam-bridged human γ-interferon (hulFN-γ) helix F peptide was studied by proton NMR spectroscopy. The 19 amino acid peptide was designed and synthesized to simulate the observed crystal structure of helix F in hulFN-γ(1). Two lactam bridges were used to stabilize the helical conformation. NMR observations, including sequential and medium-range NOEs, coupling constants, and chemical shift values revealed two α-helical regions around the lactam bridges. The helix content from NMR was consistent with CD observation. A kink centered at histidine 9 separated the two helical regions. The cause for such a bent structure is discussed. The overall conformation was very similar to that of the native helix F in hulFN-γ. The linear analog of the peptide without lactam bridges showed no evidence of helical conformation in aqueous solution. This study indicates that the lactam constrained peptide successfully mimicked the native conformation of helix F in hulFN-γ.

(1) S. E. Ealick, W. J. Cook, S. Vijay-Kumar, M. Carson, T. L. Nagabhushan, P. P. Trotta and C. E. Bugg, *Science*, **252**, 698-252 (1991).

LZ 369 3-D NMR STUDIES OF SHORT RAGWEED ALLERGEN 5 (*Amb. a.* V).

Gregory L. Warren*#, Christopher J. Turner#, Lawrence Goodfriend+, Gregory A. Petsko% and Leo J. Neuringer#, *Dept. of Chemistry and #Francis Bitter Nat'l. Magnet Lab., MIT, Cambridge, MA, 02139, +Royal Victoria Hospital, McGill University, Montreal, Canada, H3A 1A1, %Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02254

The immune system has the task of recognizing and identifying all foreign substances, which enter the body, as harmless or deleterious. There are a few cases were the immune system misidentifies a harmless substance as deleterious, thus invoking a hypersensitive response known as allergy. *Amb. a.* V, a small 45 residue protein with 4 disulfide bonds, elicits such an allergic response in a small human population. We have undertaken 3-D homonuclear and heteronuclear NMR studies of this small protein. Heteronuclear 3-D studies were used to identify a degenerate glycine residue and to unambiguously identify the disulfide bonding pattern. Homonuclear 3-D NMR was used to measure protein hydration effects.

LZ 370 SECONDARY STRUCTURE OF EQUIVOCAL PEPTIDE SEQUENCES DEPENDS ON SOLVENT ENVIRONMENT,

D. Vincent Waterhous and W. Curtis Johnson, Jr., Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331-7305.

Three peptide sequences predicted to be ß-sheet by Chou-Fasman rules, but found to be α-helices in their respective proteins, are studied by circular dichroism in several solvent environments. It is found that these sequences are α-helix in some solvents, ß-strand in other solvents, and random coil in buffer. This study is an extension of a previously published work from this research group (1) where peptides predicted to be α-helix, but found to be ß-sheet in their native proteins, exhibit similar dependencies on local solvent environment. These results are proof of a major limitation of secondary structure prediction algorithms.

(1) Lingxiu Zhong and W. Curtis Johnson, Jr. (1992) PNAS, USA, 4462-4465.

LZ 371 LOW RESOLUTION SOLUTION STRUCTURE OF THE IMMUNOADHESION DOMAIN OF THE T CELL RECEPTOR CD2,

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CD2, a transmembrane glycoprotein expressed on the surface of T lymphocytes facilitates the adhesion of T-Cells to target and antigen presenting cells. This adhesion plays a direct role in the specific recognition of the T-cell receptor and the antigen-major histocompatibility complex and therefore effects the initiation of the cell mediated immune response. CD2, one of several invariant accessory molecules on the T-cell surface binds specifically to the leukocyte function-associated antigen-3 (LFA-3).

The functional extracellular portion of CD2 consists of an adhesion domain, a constant domain and three N-linked oligosaccharides. A low resolution structure of the 105 residue immunoadhesion domain of human CD2 has been determined predominantly by 2D homonuclear methods. Characteristic immunoglobulin folding patterns have been retained which consist of two β sheets each containing four antiparallel β strands. These sheets pack closely together with hydrophobic side chains oriented between the β sheets. The immunoadhesion domain of human CD2 contains one N-linked glycosylation site at Asn 65 which is necessary for stability and adhesion function. Based upon current structural studies, the oligosaccharide appears to be oriented toward the core of the protein. Structural information related to the interaction of the protein with the oligosaccharide will be presented.

In this model, the majority of binding sites of LFA-3 to CD2 as proposed by mutational analysis (Peterson & Seed, Nature, 329, 842-846) have their charged or hydrophobic side chains oriented at the back of the β sheet. This information is consistent with the proposed binding interaction of LFA-3 and CD2 at the surface of the β sheet. Several significant structural differences such as the absence of the C" strand, between human CD2 and the rat form as determined by Driscoll *et. al.*, (Nature, 353, 762-765) have been observed. A structural comparison of these forms will be discussed.

LZ 372 CD AND NMR STUDIES OF THE CONFORMATION OF A 20-AMINO ACID PIP2-BINDING DOMAIN OF GELSOLIN IN MIXED SOLVENT, Wujing Xian, William H. Braunlin, Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE 68588-0304. Paul A. Janmey, Experimental Medicine Division, Brigham and Women's Hospital and Harvard Medical School, 221 Longwood Avenue, Boston, MA 02115

The interaction of the actin binding protein gelsolin with phosphotidylinositol 4, 5-bisphosphate (PIP_2) is a crucial step in biological signal transduction that leads to modification of the cytoskeleton. A 20 amino acid PIP_2 and actin-binding domain is found in gelsolin, and a peptide with the same sequence has been synthesized. CD studies of this peptide in mixed solvent solutions show that it adopts a random coil conformation in H_2O, but a predominantly α-helical conformation in PIP_2 micelle solution and in >20% Trifluoroethanol (TFE). In 25% TFE, 2D NMR DQF-COSY and HOHAHA/TOCSY experiments are performed for spin system assignments, and NOESY is used for sequential assignments. The results suggest that a relatively hydrophobic central region of the peptide is helical. The NOE data provide constraints that are used in molecular modelling studies. The interaction of the peptide with PIP_2 will also be discussed.

LZ 373 SIDE CHAIN EFFECTS ON THE STABILITY OF REVERSE TURNS STUDIED BY NMR, Jian Yao, Jane H. Dyson and Peter E. Wright, Department of Molecular Biology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037

A series of hexapeptides, obtained by substituting either of the two tyrosine residues with other amino acid residues in SYPYDV, was measured by NMR for their ability to form reverse turns. A linear correlation was found between the cis/trans ratio and the coupling constant $^3J_{NH}$ for the second residue, indicating that the only nonrandom structure is a turn. Hydrogen bonding and electrostatic interactions seem to be unimportant in stabilizing the turn. One of the peptides, SYPFDV, was found to have a highly populated side chain rotameric orientation as well as a stable backbone fold of a reverse turn. Calculated structures show that there is a stacking interaction among the side chains of Tyr, Pro and Phe, which probably plays an important role in stabilization of the turn.

LZ 374 SOLUTION STRUCTURE OF SRC-SH3 DOMAIN AND IDENTIFICATION OF ITS PEPTIDE-BINDING SITE
H. Yu, M. K. Rosen, T. B. Shin, C. Seidel-Dugan, J. S. Brugge and S. L. Schreiber, Department of Chemistry, Harvard University, Cambridge, MA 02138

The Src homology 3 (SH3) region is a protein domain of 55-75 amino acids found in many cytoplasmic proteins involved in signal transduction pathways. Mutational analysis suggests that the SH3 domain of Src may act as a regulatory element with dual functions – to modulate catalytic activity and to facilitate binding to other cellular proteins. Recently, Cichetti et al. [*Science*, **1992**, 257, 803] reported the identification of a protein (3BP-1) that binds to Abl and Src SH3 domains *in vitro*. The 3BP-1 finding and other evidence suggest that certain SH3 domains are used in signal transduction pathways involving both tyrosine kinases and small G-proteins.

We have recently reported the structure determination of the Src-SH3 domain and identification of its peptide-binding site (submitted to *Science*). The structure was of medium resolution, based on a total of 668 X-PLOR restraints. The molecule is composed of two short three-stranded anti-parallel β-sheets packed together at approximately right angles. The ligand-binding site was inferred from the identification of residues in the ^{15}N-labelled SH3 domain that underwent a change in their ^{15}N or ^{1}HN chemical shifts following the addition of the peptide ligand derived from the SH3-binding domain of 3BP-1. These studies have revealed a hydrophobic binding site on the surface of the protein that is lined with the sidechains of conserved aromatic amino acids, thus provide explanations for the mutagenesis studies on the Src-SH3 domain.

Protein Structures, Dynamics, Molecular Complexes

LZ 400 EFFECTS OF ION BINDING ON THE INTERNAL DYNAMICS OF CALBINDIN D_{9k}, Mikael Akke[1,2], Nicholas J. Skelton[1], Johan Kördel[1,2], Arthur G. Palmer[1] III and Walter J. Chazin[1], [1]Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, and [2]Department of Physical Chemistry 2, University of Lund, S-221 00 Lund, Sweden.

Calbindin D_{9k}, a 75-residue EF-hand calcium-binding protein structurally homologous to the globular domains of calmodulin and troponin C, binds two calcium ions with positive cooperativity. The molecular basis for this effect is being addressed by the characterization of the structure and dynamics of calbindin D_{9k} in its different metal bound states [apo, $(Cd^{2+})_1$ and $(Ca^{2+})_2$] using NMR spectroscopy.

The three-dimensional solution structures of calbindin D_{9k} show that the structural differences between the three states are rather small. In contrast, a large number of NMR parameters sensitive to the dynamics of the system indicate a marked reduction in flexibility upon binding of ions.

Here we report on two complementary studies of the internal dynamics of calbindin D_{9k}. Uniform labelling of the protein with ^{15}N has allowed quantitative measurement of the ^{15}N relaxation parameters and the rates of amide proton exchange with solvent for all three states of the protein. Together, these parameters are sensitive to molecular processes on a wide range of time scales. The implications of the results in terms of entropic contributions to the cooperativity in calcium binding are discussed.

LZ 401 HELIX DYNAMICS AND PROTEIN LOOP MOTIONS, Niels H. Andersen, Scott M. Harris, Chinpan Chen and Bolong Cao, Department of Chemistry, University of Washington, Seattle, WA 98195.

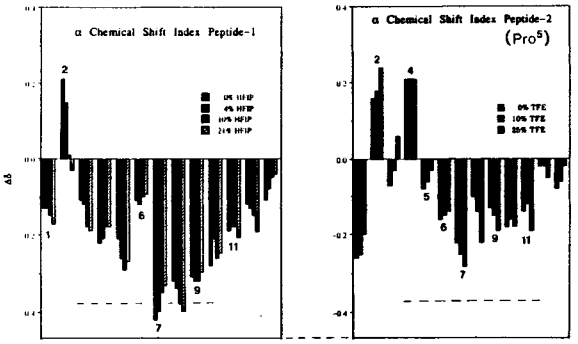
The combined analysis of chemical shifts (their solvent-titration and temperature gradients and deviations from helical or β strand norms), simulated annealing to tightly-defined nOe distances, and additional relaxation data are required to address systems that are conformational mixtures. In the case of linear peptides that are partially helical, fluoroalcohol titration difference CDs provide an additional input to the analysis.

We will attempt to answer the question "how precisely can (should) a protein structure be defined by nOe constraints?" drawing on our studies of hevein, a 43-residue tetradisulfide allergen that is now defined to better than a 0.6Å backbone rmsd by >100 longe-range constraints. Studies of C-peptide analogs —

1, AcA E T A K(Ac) A K F Q R A H A(NH_2)

2, A E T A Pro A K Y Q R A H A —

have served to define the nature of helix unfolding. From that study, we show the deviations from Wishart's Hα shift index values in the presence of varying amounts of added fluoroalcohol.



LZ 402 SOLUTION STRUCTURE OF PROTEIN S S. Bagby[‡], T. S. Harvey[‡], M. Ikura[‡], L. E. Kay[§], S. Eagle[†] and S. Inouye[†]. [‡]Department of Molecular and Structural Biology, Ontario Cancer Institute, Toronto; [§]Department of Medical Genetics, University of Toronto; [†]UMDNJ, Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Protein S is a 173-residue development-specific Ca^{2+}-binding protein from the gram-negative soil bacterium *Myxococcus xanthus*. During the later stages of the unique developmental cycle of this bacterium, when some cells undergo differentiation to produce myxospores, protein S assembles on the spore surface in a Ca^{2+}-dependent manner. In common with the extracellular Ca^{2+}-binding domains of eukaryotic cell adhesion molecules, protein S has been shown to consist largely of β-structure. This contrasts with the intracellular Ca^{2+}-receptors, such as calmodulin and troponin C, which are α-helical. Further, protein S has striking homology to the β- and γ-crystallins (vertebrate eye lens proteins).

We will present the 3D solution structure of protein S determined using data from heteronuclear multidimensional NMR experiments. Comparison will be made with the known structures of calmodulin and γ- and β-crystallins. The implications of the protein S structure for the mechanism of its assembly on the myxospore surface, and for the mode of action of eukaryotic cell adhesion molecules, will be described.

LZ 403 DYNAMICAL STRUCTURE OF PROTEINS IN SOLUTION: COMPARISON OF THEORETICAL AND EXPERIMENTAL NMR PARAMETERS OF BPTI Suganthi Balasubramanian, Nirmala R., David L. Beveridge and Philip H. Bolton, Department of Chemistry, Wesleyan University, Middletown, CT 06459

The backbone dynamics of bovine pancreatic trypsin inhibitor (BPTI) has been studied by comparison of experimental and predicted ^{13}C spin lattice relaxation times (T_1). T_1 values of all the alpha carbons of BPTI were measured by two-dimensional heteronuclear inverse detected ^{13}C-^1H experiments. The T_1 values have also been calculated from a 1 ns molecular dynamics simulation (MD) of BPTI in water. The correlation functions describing the motion of the C-H vectors have been evaluated from the trajectory. The form of the spectral densities, J(ω), is dependent on the dynamics of the C-H bond vector and have been calculated from the the correlation functions. T_1 values have been calculated from the spectral densities. It is seen that the values obtained from experiment and the MD simulation are in reasonable agreement, within the accuracy of the experimental T_1 values. The three-bond vicinal spin-spin coupling constant between the amide proton and the Cα proton ($^3J_{NHC\alpha}$) have been evaluated from the trajectory as an average of structures at every 0.5 ps using the parametrized Karplus curve for BPTI. The experimental and the predicted coupling constants of all residues, except Cys 14 and Ala 40 agree well. The effects of motional averaging on $^3J_{NHC\alpha}$ is negligible as seen from the good correlation between the time averaged coupling constants calculated from the trajectory and the experimental values.

LZ 404 Structural Studies of α-Bungarotoxin-Acetylcholine Receptor Interaction by NMR. V. J. BASUS and D. Hackos (University of California at San Francisco, Department of Pharmaceutical Chemistry), and E. Hawrot (Brown University, Section of Molecular & Biochemical Pharmacology)

Structural studies of ion channels by X-ray diffraction have been limited due to difficulties in obtaining good quality crystals of large membrane-bound proteins. The study of such proteins by NMR are limited by extremely large linewidths and the complexity of the NMR spectra. The best method to date for obtaining structures of the acetylcholine receptor (AChR) has been electron microscopy (1,2). The available resolution (18-22 Å), however, is clearly insufficient to obtain details of the interaction with neurotoxins at the atomic level. Fragments of the α-subunit of AChR have been shown to bind BGTX (3) with high affinity. This offers a novel method for studying the detailed interaction between BGTX and AChR, by obtaining the structure of the complex of BGTX with these fragments of AChR. We will present the structure of the complex involving BGTX and a 12-residue fragment (residues 185-196 of the α-subunit of the *Torpedo* AChR) based on 2-dimensional NMR spectroscopy. This small fragment, nevertheless, binds strongly to BGTX (K_d 1.4 μM), and thus offers an opportunity to investigate the nature of the binding interaction. More than 26 close contacts were identified between these two proteins, thus allowing a detailed view of the contact zone between BGTX and AChR.

(1) Mitra, A.K., McCarthy, M.P., Stroud, R.M., *J. Cell Biol.* **109**:755 (1989).
(2) Toyoshima, C., and Unwin, N., *J. Cell Biol.* **111**:2623 (1990).
(3) Wilson, P.T, Hawrot, E., Lentz, T.L., *Mol. Pharmacol.* **34**:643 (1988).

LZ 405 HETERONUCLEAR NMR STUDIES OF A DOMAIN OF Gs, Dennis R. Benjamin, David W. Markby, Henry R. Bourne, and Irwin D. Kuntz, Depts. of Pharmaceutical Chemistry and Medicine, University of California at San Francisco, San Francisco CA 94143

Much insight into the structure of the alpha chains of G proteins has been gained from the crystallographic studies of the p21-Ras protein. However, many members of the G protein family include a relatively large (~150 amino acid) domain which is not found in Ras. Gs alpha chain mutants missing this domain are deficient in their ability to stimulate adenylyl cyclase.The domain appears to have a persistent structure in solution, as judged by its ability to complement such mutants in biological assays. The ADP-ribosylation site of cholera toxin has been mapped to this site, and it has been postulated that the domain plays a role in G protein function analogous to that played by GAP in Ras function. Additionally, the absence of any structural information about this region has precluded the development of a complete model of G protein structures from the Ras coordinates. In order to obtain such structural information, we have undertaken a heteronuclear NMR study of this domain. The domain has been subcloned and expressed to high levels in E.Coli, allowing the incorporation of 15N and 13C. Standard multidimensional NMR techniques (e.g HMQC-NOESY, HMQC-HOHAHA, HCaCO) are being used on singly and doubly labeled proteins for obtaining assignments and nOe measurements , and a complete relaxation matrix analysis coupled with restrained molecular dynamics will be used to determine the solution structure. This structure will provide a basis for the construction of a model of the structure of the entire Gs alpha chain.

LZ 406 STRUCTURAL ANALYSIS OF TRANSCRIPTION FACTORS OF THE HELIX-LOOP-HELIX CLASS: NMR CHARACTERIZATION OF E47, Stephen L. Brenner, Rita K. Beran-Steed, Tracy M. Handel, William F. DeGrado, Peter J. Domaille, Robert Fairman, Biotechnology Department, The Du Pont Merck Pharmaceutical Company, Experimental Station, Wilmington, DE 19880-0328.

A group of transcriptional activators and inhibitors contain a common sequence motif predicted to form a structural domain composed of a helix, a loop, and a second helix (HLH). The folded helix-loop-helix domain is the interaction interface for the formation of homo- and hetero-oligomers of HLH proteins. Dimers composed of members of this class are site-specific DNA binding proteins. Higher order oligomers can be formed which sequester the proteins and thereby inhibit transcription. We have studied a 71-residue peptide fragment of the immunoglobulin-enhancer binding protein E47 and have shown it to form stable dimers in solution. The peptide was overexpressed in *E. coli* and uniformly labeled with either N15 or N15 and C13, purified, and studied using multi-dimensional homo- and heteronuclear NMR. The spectra have been largely assigned, and analysis shows that the helices are highly dynamic in solution. Distance constraints derived from the NMR experiments allow us to predict the three-dimensional fold and orientation of the helices relative to one another in the native dimer. Preliminary results have been obtained comparing the protein fragment free in solution and bound to DNA containing an E47 binding sequence.

LZ 407 INTERLEUKIN-4: NMR STUDIES OF STRUCTURE DYNAMICS AND LIGAND INTERACTIONS.
Rui M. M. Brito, Christina Redfield, Lorna J. Smith, Gary Christie*, Steve D. Holmes*, Beverley Weston*, Richard A. G. Smith* and Christopher M. Dobson. Inorganic Chemistry Laboratory, University of Oxford, Oxford OX1 3QR, UK & *SmithKline Beecham Pharmaceuticals, Epsom, Surrey KT18 5XQ, UK.

Interleukin-4 (IL-4) is a pleiotropic cytokine which plays a key role in B-cell proliferation, differentiation of T-helper cells and immunoglobulin isotype switching. Modulation of IL-4 activity may prove useful in the control of allergic disease. We have recently described the solution structure of recombinant human IL-4 based on 2-D and 3-D NMR experiments on ^{15}N single labelled and ^{15}N-^{13}C double labelled protein (Redfield et al., (1991) *Biochemistry* 30, 11029; Smith et al., (1992) *J. Mol. Biol.* 224, 899). These studies have shown that IL-4 is a four-helix bundle protein with up-up-down-down connectivity and a short but structurally essential region of beta sheet. This topology has also been found in granulocyte-macrophage colony stimulating factor (GMCSF) and may also apply to Interleukin-2. ^{15}N NOE, T_1 and T_2 measurements showed that the helical core of IL-4 has limited conformational flexibility while other regions of the protein experience substantial fluctuations in the conformation of the main chain. As part of a program to explore structure/function relationships in IL-4, we have examined the effects of ligands on the NMR spectral properties of the protein and these results will be reported.

LZ 408 DYNAMICAL DESCRIPTION OF THE MONOMERIC AND AGGREGATED FORMS OF MELITTIN IN SOLUTION USING C^{13}- AND N^{15}-NMR RELAXATION PARAMETERS AND FLUORESCENCE ANISOTROPY.

Paul Buckley, Marvin D. Kemple*, Franklyn G. Prendergast, Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN, 55905 and *Department of Physics, IUPUI, Indianapolis, IN 46205-2610. Melittin, the principal component of *Apis Melifera* bee venom, forms an amphiphilic helix in methanol, a poorly structured coil in water at low pH, and a highly symmetric tetrameric aggregate of helices in water at high concentration and high pH (Lauterwein, J. et al. *Biochim. Biophys. Acta*, 1979, 1980. 556:244-264, 662:219-230). Longitudinal relaxation times and nuclear Overhauser enhancements were measured at two spectrometer frequencies for C^{13} labeled α-carbon atoms in residues 1, 3, 4, 6, 9, 12, and 15 and in the indole-4 position of the single W19 sidechain. Data were also collected for N^{15} and C^{13} labels in the near-terminal sidechain positions of lysines 7, 21, and 23. The NMR descriptors for the Trp sidechain were combined with the average fluorescence lifetime and the anisotropy values using the Lipari and Szabo model-free formalism (*J. Am. Chem. Soc.*, 1981. 104:4546-4559), as extended by Weaver et al. (*Biochemistry*, 1989. 28:8624-8639), to calculate the molecular rotational correlation time . Order parameters and correlation times were then generated for the local motion of the ^{13}C-H vectors in the framework of the single molecular correlation time. Comparison of the motional parameters between the aggregate and monomeric forms shows a reduction in the chain flexibility with aggregation.

Supported in part by NIH GM 34847 and NSF DMB-9105885.

LZ 409 ^{1}H, ^{13}C, AND^{15}N NMR ASSIGNMENTS AND SECONDARY STRUCTURE OF CHICKEN MUSCLE ADENYLATE KINASE COMPLEXED TO $MgAP_5A$, In-Ja L. Byeon, Arthur S. Edison, Honggao Yan, Ed Mooberry, Fritz Abildgaard, John L. Markley, and Ming-Daw Tsai, Department of Chemistry, Ohio State University, Columbus, OH 43210 and Department of Biochemistry, University of Wisconsin, Madison, WI 53706

Adenylate kinase (AK) is an enzyme (21.7 kDa) which catalyses the interconversion reaction of MgATP + AMP ⇌ MgADP + ADP. Backbone ^{1}H, ^{13}C, and ^{15}N NMR assignments of chicken AK complexed to a bisubstrate analog $MgAP_5A$ have been determined using multiple-resonance 2D and 3D NMR techniques. Unambiguous sequential assignments can be made by a combined use of two standard sequential assignment methods: (i) NOE-directed assignments using the ^{15}N 2D HMQC-NOESY and 3D NOESY-HMQC experiments and (ii) recently developed ^{1}J coupling-directed assignments using the 3D HNCO, HNCA, HN(CO)CA, and HN(CA)H triple-resonance NMR techniques. 2D ^{15}N-HMQC (or HSMQC) experiments on the following specifically labeled samples provided several starting points for the assignments: AK labeled with (i) ^{13}C'-Val and ^{15}N-Leu, (ii) ^{13}C'-Leu and ^{15}N-Val, (iii) ^{13}C'-Leu and ^{15}N-Lys, and (iv) ^{13}C'-Val, ^{15}N-Gly and ^{15}N-Ser. HCCH-TOCSY and HCCH-COSY data as well as $^{13}C^{\alpha}$ chemical shifts were helpful for confirming the assignments. Secondary structure elements in the AK+$MgAP_5A$ complex were determined by extensive analysis of ^{15}N 2D HMQC-NOESY and 3D NOESY-HMQC spectra. The secondary structure of the complex in solution was essentially the same as that of substrate-free porcine analog in crystal: a large proportion of α-helix (~ 60 %) and five-stranded parallel β-sheets (~ 10 %). In addition, several intermolecular NOEs between AK and $MgAP_5A$ were identified.

LZ 410 SEQUENCE-SPECIFIC CARBON-13 RESONANCE ASSIGNMENTS FOR MURINE EPIDERMAL GROWTH FACTOR AT NATURAL ISOTOPE ABUNDANCE: CORRELATION BETWEEN SECONDARY STRUCTURE AND Cα CHEMICAL SHIFTS. Bernardo Celda[1], Maria J. Arnau[1] and Gaetano T. Montelione[2], [1]Depto. Química Física, F. Químicas, U. de Valencia,46100-Burjassot(Valencia), Spain, [2]Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ 08854-5638.

Carbon-13 NMR is a well established method for structural and dynamic characterization of proteins and nucleic acids. Carbon resonance frequencies and coupling constants are very sensitive to hybridization and conformation. Carbon-13 nuclear relaxation rates also can provide information about internal motions in biopolymers. Furthermore, carbon-13 chemical shifts of Cα nuclei have been correlated with regular secondary structures of proteins. In order to carry out studies of internal dynamics, a large part of the carbon-13 resonances for murine epidermal growth factor (mEGF) at pH 3.1 and a temperature of 301 K at natural abundance have been assigned using 2D heteronuclear experiments (HSQC and HSQC-TOCSY). A large number of Hα-Cβ, Hβ-Cα connectivities were identified, allowing the assignment of crowded α-proton regions. A good correlation was found between experimental and theoretical secondary carbon-13 chemical shifts for the regular backbone structures in mEGF.

LZ 411 MULTINUCLEAR, MULTIDIMENSIONAL NMR INVESTIGATIONS OF *ANABAENA 7120* HETEROCYST FERREDOXIN, Young Kee Chae and John L. Markley, Department of Biochemistry, University of Wisconsin - Madison, Madison, WI 53706

Heterocyst ferredoxin is involved in the nitrogen fixation process; it transfers one electron to the nitrogenase component II, which is known as the Fe protein. The gene encoding the heterocyst ferredoxin, cloned by Dr. H.Böhme, was successfully overexpressed in the *E.coli* system by using the T7 promoter. The yield of purified ferredoxin is typically about 20mg (1.8μmole) per liter culture (rich or minimal medium). The heterocyst ferredoxin was labeled with ^{15}N by feeding $(^{15}NH_4)_2SO_4$. The labeled protein was studied by two 3D NMR experiments: ^{15}N-NOESY-HMQC and ^{15}N-TOCSY-HMQC. To facilitate the assignment procedure, several selectively labeled samples were made by incorporating ^{15}N-containing amino acids, for example, Ala, Val, Gly, Leu, etc., and 2D HMQC data were collected with these samples. By combining all these data, the backbone assignments have been completed. A ^{13}C, ^{15}N doubly labeled sample has been purified, and it will be studied by 3D triple resonance experiments in order to check the backbone assignments and extend them to the side chains.

(Supported by USDA grant 92-37306-7699)

LZ 412 THE SOLUTION STRUCTURE OF THE OLIGOSACCHARIDE ANTIGEN FROM GANGLIOSIDE GD2: IMPLICATIONS FOR ITS INTERACTION WITH MAB 3F8, *Dale Cumming, Tom McDonagh, Hsiang-Ai Yu*, Martin Karplus*, and Lynette Fouser* Genetics Institute, 87 CambridgePark Drive, Cambridge, MA 01240, and *Dept. of Chemistry, Harvard University, 12 Oxford Street, Cambridge, MA 02138

Protein/oligosaccharide interactions constitute a molecular recognition mechanism in numerous biological processes. We have embarked upon a detailed study of a particular antibody/antigen system in order to extend our understanding of such interactions . The antibody is murine 3F8, an $IgG_3\kappa$ mAb that exhibits high affinity binding to cells of neuroectodermal origin, including melanoma, neuroblastoma, and small cell lung carcinoma. The tumor associated antigen for this mAb is ganglioside GD_2. 3F8 exhibits tight antigen specificity, binding to GD_2 but not to other gangliosides including GM_2, GD_{1b}, or GD_3, as evidenced by immuno-TLC staining. In addition, the MAb appears to recognize only the glycan portion of the glycolipid antigen. Large quantities of the glycan moiety of GD_2 can be prepared by ceramide glycanase digestion and β-galactosidase digestion of GD_{1b}. The 3D solution structure of the glycan moiety of the antigen has now been determined using quantitative dipole-dipole NMR relaxation data (bulk and selective T_1s, nOe's) and molecular mechanics calculations. In addition, molecular modeling studies of the mAb/Ag complex, using X-ray structures of homologous Ab's as templates, and microcalorimetric studies have been performed. Taken together, these studies suggest the basis for the apparent high affinity binding and specificity exhibited by 3F8.

LZ 413 THE STRUCTURE OF THE MEMBRANE INTERFACE BETWEEN RHODOPSIN AND G PROTEIN USING CONTACT PEPTIDES AND TR-NOESY, Edward Dratz*, Julie Furstenau*, Christophe Lambert*, Theresa Schepers#, and Heidi Hamm#, *Dept. of Chem. and Biochem., Montana State Univ., Bozeman, MT 59717 and #Dept. of Physiol. and Biophys., Univ. of IL Med. School, Chicago, IL 60612

Rhodopsin is an archetype for a large family of membrane receptors thought to contain 7 transmembrane helices and to function by coupling to GTP binding protein (G protein) amplifiers. Rhodopsin is the light receptor in the retinal rod cell. The interface between light excited rhodopsin (MII) and the G protein, transducin, consists of at least three regions on the Gα subunit (residues 340-350, 311-329 and 1-23)(Hamm, et al., Science, 241, 832, 1988) and three regions on rhodopsin (Konig, et al., PNAS 86, 6878, 1989). We studied the bound conformations of the Gα contact peptides binding to rhodopsin and MII in the native disk membrane using Tr-NOESY.

Gα 340-350 and 311-329 bind to MII and shift the MI ⇌ MII equilibrium from the inactive MI to the active MII state in a parallel manner to the full Gαβγ protein but with lower affinity. Much of our structural work has focussed on the Gα 340-350 peptide (I-K-E-N-L-K-D-C-G-L-F) or mutant forms. The specificity of the interaction is high because changing $C_{347} \rightarrow S$ or amidinating the peptide C-terminus (which corresponds to the C-terminus of the intact protein) abolishes all activity. The compact MII bound form of Gα 340-350 was determined based on ca. 125 NOESY distance constraints. The NMR experiments also provided evidence for binding of Gα 340-350 to rhodopsin in the dark (a "precoupled" state) in a conformation substantially different than the MII bound state. The free peptide in solution shows evidence for a preferred bend around Gly_{348} which is much more well defined and compact in the bound forms. We are working on refining the bound structure using backcalculation. The approach appears to be quite general and we are actively applying it to study protein-protein interactions in several other membrane receptor systems. Support: NSF RII8921978 & DMB8804861 and NIH EY06062 & EY06913.

LZ 414 BACKBONE DYNAMICS STUDIES OF TRIPLE HELICAL PEPTIDES BY 2D HETERONUCLEAR NMR, Pei Fan[1], Ming-Hua Li[1], Barbara Brodsky[2] and Jean Baum[1], [1]Department of Chemistry, Rutgers University, and [2]Department of Biochemistry, Robert Wood Johnson Medical School, Piscataway, NJ 08855

The triple helical conformation is the major structural motif in all collagen as well as in a number of other proteins. In triple helices, the repeating amino acid sequence $(X\text{-}Y\text{-}Gly)_n$ is needed, where X and Y can be any amino acid but are often imino acids such as proline and hydroxyproline. The presence of glycine as every third residue is a very stringent requirement in collagen as evidenced by findings which indicate that glycine substitution by other amino acids can result in serious disease. In order to determine how the structure and dynamics of tripeptide units vary with amino acid composition and how these relate to disease, we have studied and compared the dynamics of two triple helical peptides by 2D heteronuclear ^{15}N-^1H NMR spectroscopy. The first, $(Pro\text{-}Hyp\text{-}Gly)_{10}$, is a model peptide containing only imino acids in the X and Y position and the second, a design peptide $(Pro\text{-}Hyp\text{-}Gly)_3$Ile-Thr-Gly-Ala-Arg-Gly-Leu-Ala-$Gly(Pro\text{-}Hyp\text{-}Gly)_4$, contains 9 residues including a glycine interruption site from human type III collagen. The nitrogen T_1, T_2 and heteronuclear NOE were measured, and the data analyzed using the model free formalism (Lipari, G. & Szabo, A. J. Am. Chem. Soc., 1982, 112, 4989). An anisotropic motional model was used because of the rod shape of the triple helix. Both $(Pro\text{-}Hyp\text{-}Gly)_{10}$ and the design peptide exhibit fast internal motions. The order parameters, which reflect the amplitude of internal motion, are very similar for the two peptides and are on the order of 0.8. In addition, the order parameters for the X and Y position on the design peptide are very similar to the order parameter of the glycine residue. These results suggest that the backbone dynamics of the triple helix are quite uniform, and that the types of amino acids in the X and Y position of triple helix sequence do not influence the internal motion.

LZ 415 NMR STUDIES OF CYCLOSPORIN A BOUND TO A MUTANT E.COLI F112W CYCLOPHILIN, Jasna Fejzo, Felicia A. Etzkorn, Robert T. Clubb, Yian Shi, Christopher T. Walsh and Gerhard Wagner, Department of Bilogical Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

Cyclosporin A (CsA) is a clinically used immunosuppressant that binds the human protein cyclophilin (CyP). The CyP/CsA complex inhibits the calcium- and calmodulin-dependent phosphatase, calcineurin. The conformation of CsA when bound to human CyP has been determined by NMR [1,2] and the model of human CyP/CsA complex was generated from the combination of NMR and X-ray data [3].

It has recently been shown that the Trp indole ring in the CsA binding site of cyclophilin proteins is critical for calcineurin recognition [4]. The wild type E. coli cyclophilin, which lacks the Trp indole ring, has peptidyl-prolyl cis-trans isomerase activity but does not bind CsA. The mutant F112W, which has a tryptophan incorporated at the position analogous to Trp 121 in human CyP A, has been found to gain cyclosporin A binding activity as well as inhibition of calcineurin. In order to investigate the three-dimensional structure of the CsA/F112W CyP complex, we have applied a variety of multidimensional NMR methods in the study of uniformly ^{13}C-labeled CsA bound to F112W CyP. The ^1H and ^{13}C NMR resonances of cyclosporin A in the bound state have been assigned, and the bound conformation of CsA has been determined. Three-dimensional structures of CsA, calculated from the NOE data using a distance geometry/simulated annealing protocol, were compared to previously determined solution conformations of CsA complexed to human CyP.

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LZ 416 CALCIUM-INDUCED ACTIVATION OF CALMODULIN EXAMINED BY ^{1}H AND ^{15}N NMR.

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The effect of metal binding on the structure and function of proteins is of fundamental importance to protein biochemistry. One class in particular, the EF-hand family of calcium binding proteins, has been the subject of much interest due to their many regulatory roles in cell metabolism. In order to understand the structural changes induced by the binding of calcium to calmodulin, which possesses four helix-loop-helix EF-hands, we have undertaken a study of the structure of the domain containing the two carboxy-terminal EF-hands of calmodulin. Using ^{1}H and ^{15}N multidimensional NMR, we are examining the structural changes accompanying calcium binding. In contrast to rather subtle effects of the binding of calcium to calbindin D_{9k}, a small member of this family composed of two EF-hand calcium-binding domains, calcium binding to calmodulin induces substantial changes in its structure to render it in an active form. Details of these changes will be discussed.

LZ 417 A SPECIFIC COMPLEX OF PLCγ1 SH2 WITH A PHOSPHOPEPTIDE FROM THE PDGFR Y1021 SITE,

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One of most important recent discoveries of research into the molecular basis of cancer is of the involvement of protein tyrosine kinases in the regulation of cellular proliferation and differentiation. Attachment of a phosphate group on certain tyrosine residues of target proteins creates specific binding sites for other proteins containing regions called SH2 domains, leading to the formation of protein complexes which transduce signals from extracellular growth factors. The 100-residue modular SH2 domains couple growth factor receptor stimulation to the activation of enzymes specific for a number of different biochemical pathways. The structure of a specific complex of the C-terminal SH2 domain from phospholipase-Cγ1 (PLCγ1) with a 12 residue peptide from the Y1021 site of the platelet derived growth factor receptor (PDGFR) has been studied by multidimensional, multinuclear NMR techniques. The affinity of the PLCγ1 C-terminal domain for this particular phosphopeptide has been shown by other groups to be extremely high. ^{31}P NMR has been used to confirm the formation of a tight complex. Nuclear (^{15}N and ^{13}C) editing and filtering methods were helpful in isolating intermolecular peptide-protein NOEs from the intramolecular peptide and protein interactions. Comparison of this structure with other SH2 domains in complex with their target phosphorylated peptides will shed light onto the specificity of SH2 binding and recognition and the general mechanism of protein-protein interactions.

LZ 418 STUDY OF THE N-DOMAIN OF TROPONIN-C BY HETERONUCLEAR MULTIDIMENSIONAL NMR.

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In the thin filament of skeletal muscle, conformational changes are induced in the protein troponin-C by the binding of two calcium ions to the N-domain of the protein; this is the first step in a process that ultimately leads to muscle contraction. This study has been done on a fragment (1-90) containing the intact N-domain of chicken skeletal troponin-C (N-TnC), cloned and expressed in E.coli to allow uniform ^{15}N and ^{13}C labelling.

The ^{1}H, ^{15}N, ^{13}CO and ^{13}C resonances of N-TnC in the apo and calcium forms have been assigned based on a series of conventional 3D double- and triple-resonance NMR experiments. The three-dimensional solution structure of the calcium saturated form of N-TnC has been determined using 3D ^{15}N- and ^{13}C-edited NOESY experiments. Structural information on the apo form has also been obtained using 3D double-resonance experiments on the ^{15}N-labeled protein. The specific effects of the binding of Ca^{2+} were followed by recording ^{15}N- and ^{13}C-HMQC spectra as a function of calcium concentration. Those data allow us to characterize the conformational changes that occur when calcium is bound by the N-domain of troponin-C.

LZ 419 USING 2D-NMR TO PROBE THE CONTACT ZONE BETWEEN α-BUNGAROTOXIN AND PORTIONS OF THE α- SUBUNIT OF THE NICOTINIC ACETYLCHOLINE RECEPTOR,

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Although the nAChR is the best characterized of the ligand-gated ion channels, details of its molecular structure and function remain largely unknown. Binding studies suggest that the agonist and antagonist binding sites reside at least in major part on the N-terminal extracellular portion of the α-subunit prior to the first transmembrane spanning region. The competitive antagonist α-BGTX shows significant binding to regions containing residues 181-200 of the α-subunit.

We have determined the residues in α-BGTX which are significantly perturbed upon binding of an 18mer (181-198) derived from the α-subunit ($Kd=6.5X10^{-8}M$). A ROESY spectrum of the 2:1 complex of α-BGTX:18mer provides exchange cross peaks between bound and free α-BGTX which, when compared with the spectrum of unbound α-BGTX (previously assigned), provides a framework upon which to assign the sequential and nonsequential HOHAHA and NOESY cross peaks of α-BGTX in the 1:1 complex.

Binding-induced chemical shift perturbations show that the N-terminal and middle toxic loop of α-BGTX are both important in the molecular recognition of the nAChR.

LZ 420 **NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY TO STUDY THE MOLECULAR BASIS OF ANTIBODY CROSSREACTIVITY**

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Initial studies of the interaction between the anti-peptide antibody, Gloop1 and the loop peptide of hen egg lysozyme (residues 57 to 84) using COSY NMR experiments have shown that in the bound state a few peptide residues are tightly bound whereas other are highly mobile[1]. The bound residues form a distinct cluster when viewed on the *crystal structure* of HEL. However, any similarity between the conformation of bound peptide and that of free crystalline HEL is uncertain. We have taken advantage of molecular biology techniques and peptide synthesis technology to produce ^{15}N enriched loop peptide for use in the isotope edited NOESY experiment to monitor the conformation of loop peptide bound to Gloop1 Fab. We present the experimental details and preliminary results from these experiments and propose a refined binding model for the complex.

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LZ 421 NMR STUDIES OF PROTEIN-CARBOHYDRATE INTERACTIONS AT A MEMBRANE SURFACE

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The development in our laboratory of model membranes which orient in a magnetic field provides a new method for using NMR to study protein-carbohydrate interactions at a membrane surface. The interactions of the plant lectin wheat germ agglutinin with synthetic glycolipid analogs containing a ^{13}C-labeled N-acetyl-glucosamine headgroup separated from the lipid chain by a variable-length hydrophilic spacer were studied in the oriented membranes. It was found that the saccharide headgroup must be a significant distance from the membrane to interact with the protein. Although the ^{13}C-labeled resonances broadened when the protein bound, the dipolar couplings and chemical shifts remained the same, suggesting that the motion of the headgroup was slowed by protein binding, but that there was no change in overall order. The dissociation constants of the protein-glycolipid complexes were calculated from an analysis of the line width of the labeled glycolipid resonance in competition studies between the glycolipid and free sugar. The results are compared to equilibrium dialysis measurements of the binding constants between the glycolipid analogs incorporated in phospholipid vesicles and wheat germ agglutinin.

LZ 422 STRUCTURAL STUDIES ON A HIGHLY CONSERVED DOMAIN OF DNAJ

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DnaJ is a 41 kDa heat-shock protein from *E. coli* known to participate in numerous cellular reactions including initiation of bacteriophage λ and *E. coli ori-C* dependent DNA replication, regulation of cell division, membrane translocation, and chaperone-mediated protein folding (at least in vitro). Four regions of dnaJ show extensive homology to proteins throughout the animal kingdom that are involved in chaperone-mediated interaction. One of the highly conserved domains (77 residues) has been cloned for structural studies by NMR and further biochemical characterization.

Homonuclear, ^{15}N-edited heteronuclear, and gradient-enhanced experiments were performed. The dnaJ subunit is well structured with about thirty percent α-helix. We use these data to illustrate a new semi-automated assignment strategy based on the use of a relational database and neural networks.

LZ 423 ^{1}H AND ^{15}N ASSIGNMENTS OF TGFα AND PRELIMINARY STUDIES OF THE TGFα/EGFRx COMPLEX USING HETERONUCLEAR 2-D AND 3-D NMR

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Human transforming growth factor α (TGFα) is a 50 residue mitogenic polypeptide that is part of the epidermal growth factor (EGF) family (1). Secreted from epithelial carcinomas, TGFα has been implicated as a factor in maintaining growth of some human cancers (2). Human EGF and TGFα share 40% sequence identity and bind the EGF receptor (EGFR) with about equal affinity (1). However, TGFα and EGF show qualitative differences in their biological response and may bind differently to their receptor. High resolution ^{1}H 2-D NMR studies have yielded the detailed tertiary structures of TGFα and EGF in solution (3). Here we report the ^{15}N and ^{1}H assignments for the backbone and sidechain amides of uniformly ^{15}N-enriched TGFα. The assigments were made using data collected from 2-D and 3-D NMR experiments. These assignments are requisite for the employment of ^{15}N-edited NMR methods for the study of the large TGFα/EGFRx complex (90 kDal). The preliminary NMR data for interaction of ^{15}N-labelled TGFα with the unlabelled extracellular domain of EGFR (EGFRx) will be discussed.

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LZ 424 NMR STRUCTURAL STUDIES OF Cu(I) RUSTICYANIN.

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Rusticyanin is a 155-residue blue copper protein produced by the acidophilic iron-oxidizing bacterium *Thiobacillus ferrooxidans*. Rusticyanin participates in the electron transport system of *T. ferrooxidans*, and there is limited homology in the C-terminal portion of its sequence with other copper-containing electron transport proteins such as plastocyanin. Rusticyanin differs from related proteins, however, in having an unusually high reduction potential (680 mV) and great stability under acidic conditions (pH optimum 1.5).

NMR studies on Cu(I) rusticyanin at pH 3.4 are being pursued in order to learn the structural basis for the unusual properties of the protein in solution. Sequential resonance assignments, secondary structural elements, and a comparison of the global fold to that of plastocyanin will be presented.

LZ 425 ^{1}H NMR OF THE ACTIVE CENTER OF SERINE PROTEASES COMPLEXED TO SLOW- AND RAPID-BINDING INHIBITORS AND TO THE PRO-PEPTIDE OF SUBTILISIN E, A PROTEIN-PROTEASE INHIBITOR, Frank Jordan, Sheng Zhong, Zhixiang Hu, Khadijah Haghjoo, Department of Chemistry, Rutgers University, Newark, New Jersey 07102. Charles Kettner, Du Pont Merck Pharmaceutical Co. Experimental Station, Wilmington, DE 19880-0328.

Recently, by the use of a number of boronic acid inhibitors, including peptide boronic acids varying in K_i from micromolar to sub-nanomolar vis-a-vis chymotrypsin, it was demonstrated that ^{11}B NMR is eminently applicable to the determination of the hybridization at boron in the active center of this and other serine proteases inhibited by such putative "transition-state" inhibitors (Zhong, S. et al. *JACS*, 1991). We here report complementary proton NMR studies in which the His NHs are being observed in the type of experiment first reported by Robillard and Shulman (*J.Mol.Biol.* 1972,74). On repeating the experiment on the native enzymes, the existence of two His environments is deduced near neutral pH, and according to their chemical shifts, these correspond to slow interconversion (on the NMR time-scale) of the His57 protonated, and His57 neutral forms of the enzymes. At the pH extrema the low pH resonance integrated to ca. 1.8x that of the high pH resonance, indicating that under these conditions at acidic pH the two His NHs were in fast exchange (400 MHz). In the inhibited enzymes for both weaker and stronger inhibitor, both His NH resonances were discernible at intermediate pHs: their chemical shifts were independent of the strength of inhibition, however, their linewidths were distinctly characteristic. It could be deduced that the slow-binding inhibitor manifested its effect in a dynamic sense, i.e. differential exchange rates with solvent of the two NH resonances. At both lower and higher pHs more distinct spectra resulted for the enzyme inhibited by the two different inhibitors. Especially the potent, slow-binding inhibitor clearly showed that the active center His experienced an increase in basicity with a pK> 10, in accord with our previous suggestion, based on ^{31}P experiments on the monoisopropylphosphoryl enzymes. As shown before, the pro-peptide of subtilisins is not only required to assure proper folding of this enzyme, but it is also a potent inhibitor of the native enzyme (Ohta, Y. et al. *Mol. Microbiol.* 1991). Preliminary results will be presented on the effect of the pro-peptide on the active center of subtilisins and other serine proteases. Supported by the Rutgers Busch Fund and Ciba-Geigy.

LZ 426 THE SOLUTION STRUCTURE OF THE LEUCINE ZIPPER MOTIF OF THE JUN ONCOPROTEIN HOMODIMER, F. Keith Junius, Séan I. O'Donoghue, Anthony S. Weiss and Glenn F. King, Department of Biochemistry, University of Sydney, Sydney, NSW 2006, Australia.

Proton nuclear magnetic resonance studies have been performed on a 9.8 kDa synthetic fragment comprising the homodimeric leucine zipper domain of the human oncoprotein Jun to ascertain its conformation in aqueous solution. Analysis of two-dimensional scalar- and dipolar-coupling experiments have revealed that the Jun leucine zipper forms a completely symmetric dimer in solution, consistent with the formation of a coiled-coil arrangement of parallel α-helical strands. This symmetry makes it impossible *a priori* to distinguish between *intra-* and *inter*monomer dipolar connectivities. To overcome this problem we have developed a new approach for analysing the NMR spectra of symmetric coiled-coil proteins. This analysis is based on calculations of intra- and intermonomer interproton distances in the recently determined crystal structure of the GCN4 leucine zipper [O'Shea, E. K., Klemm, J. D., Kim, P. S. and Alber, T. (1991) *Science*, **254**, 539-543] and in symmetric coiled-coil models of the leucine zippers of GCN4 and the human oncoprotein Jun which we constructed using a dynamical simulated annealing (DSA) approach. This analysis has enabled the formulation of a set of rules for interpreting the NMR spectra of symmetric coiled-coil proteins. Applying these rules to our spectra, a subset of 209 unambiguous distance constraints was compiled which were used in combination with experimentally-determined hydrogen bond and dihedral angle constraints to compute a set of monomer solution structures using a distance geometry approach. These structures were then used as starting structures for refinement by DSA. Calculation of the interactions at the dimer interface was made by creating a rotationally symmetrical copy of the monomer to form a dimer. The remaining ambiguous distance constraints were added to the calculation and their assignments were allowed to float between being intra- and intermonomer. Both relaxation matrix refinement and r^6 distance averaging was utilized to attempt to resolve the contributions from each of the two possible assignments to the observed NOE intensity. The relative success of these approaches to the calculation of symmetric dimer structures will be discussed and the computed structures at their current stage of refinement will be compared to the structures of the leucine zipper domain of the yeast transcriptional activator GCN4 determined by NMR and X-ray diffraction methods.

LZ 427 DYNAMICS OF ^{13}C LABELED PEPTIDES BOUND TO CALMODULIN, Marvin D. Kemple[§], Peng Yuan[§], Franklyn G. Prendergast[†], and Marie Chabbert[*], [§]Department of Physics, Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46205-2810, [†]Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN 55905, [*]Consorzio Bio-Richerce, 20126 Milano Bicocca, Italy

Side-chain and backbone dynamics of two peptides bound to calmodulin have been investigated by ^{13}C NMR relaxation measurements (primarly T_1 and NOE), at 75.4 and 125.7 MHz. The two peptides, a 26-residue cytolytic peptide (melittin) with sequence

GIGAVLKVLTTGLPALISWIKRKRQQ

and a 19-residue peptide derived from the calmodulin binding region of chicken smooth muscle myosin light chain kinase (ARS-19) with sequence

ARRKWQKTGHAVRAIGRLS,

were synthesized with ^{13}C-labeled amino acid residues at positions 1, 4, 12, and 19 for melittin and 1, 5, 9, 11, and 16 for ARS-19. The NMR results were analyzed along with tryptophan fluorescene anisotropy and lifetime measurements in terms of an order parameter and an effective correlation time for the internal motion, and a correlation time for overall rotational motion of the complex. Compared with melittin in random coil form, melittin bound to calmodulin shows a reduction in the amplitude of internal motion at the various labeled positions. In the complex among the labeled residues, the amino terminus glycine has the largest amplitude of motion. Similarly ARS-19 bound to calmodulin shows restricted internal motion with the degree of restriction varying along the sequence. The results will be considered in light of information concerning the structure of the peptide-calmodulin complexes. The work was supported in part by NSF DMB-9105885 (MDK) and NIH GM34847 (FGP).

LZ 428 STRUCTURAL ASPECTS OF PROTEIN/DNA RECOGNITION: A MULTIDIMENSIONAL NMR STUDY OF THE THREE ZINC-FINGERS OF THE TRANSCRIPTION FACTOR SP1, Richard W. Kriwacki and John P. Caradonna, Biophysics Section, Department of Chemistry, Yale University, New Haven, CT 06511

The amino acid sequence of the transcription factor Sp1 contains three repeats of the Cis_2His_2 zinc-finger motif, with these zinc-binding domains responsible for sequence-specific recognition of DNA with the nucleotide sequence (G/T)GGGCGG(G/A)(G/A)(C/T) (termed GC-box). An interesting aspect of the DNA binding properties of Sp1 is its ability to bind with high affinity to a broad array of sequences embodied by the consensus sequence given above. Biophysical studies with a series of peptides containing the three Sp1 zinc-fingers (1) have shown that a 92 amino acid peptide containing only the three zinc-finger domains binds (i) with high affinity (K_d ~ 10 nM), (ii) in a zinc-dependent manner, and (iii) with Sp1-like sequence specificity to oligonucleotides containing several different GC-box sites. Determination of the limits of the thermodynamic binding site revealed that each of the three Sp1 fingers does not bind to its target DNA subsite with equal affinity and suggests that the three fingers play different roles with regard to sequence recognition. The molecular details of DNA recognition by Sp1 can be modeled on the basis of the 3-dimensional structure of the Zif-DNA complex determined by Pavlitich and Pabo (2). This comparative analysis provides an explanation for the Sp1 binding affinity asymmetry based on the amino acid sequence within the helical portions of the three zinc-finger domains. We present here the results high-resolution, multi-nuclear NMR studies aimed at determining the 3-dimensional structure of both the zinc-bound Zn92 peptide as well as its complex with oligonucleotide DNA. Our ultimate goal is to provide a detailed explanation for Sp1's unique DNA binding properties, as discussed above.

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(2) Pavlitich, N.P., & Pabo, C.O. (1991) *Sci.* **252**, 809-817..

LZ 429 TRANSFER-NOE STUDY OF THE PEPTIDE HORMONE OXYTOCIN BOUND TO ITS CARRIER PROTEIN NEUROPHYSIN, G. Lippens[1], K. C. Russell[2], V. J. Hruby[2] and K. Hallenga[3], [3]CORVAS at [1]UCMB, ULB Brussels, Belgium; [2]University of Arizona, Tucson, Arizona, USA

The potential of transfer-NOE experiments for studying the interaction between small ligands and larger macromolecules has been explored systematically using the peptide hormone oxytocin as a ligand and bovine Neurophysin I as target. In addition to data about the structure of the bound ligand, we will show that the transfer-NOE experiments can also yield information about the dynamics of the bound ligand, and about the interaction between the two molecules.

1) Structural data : due to the limited number of NOE contacts for the small peptide molecule, it is essential to use accurate distance constraints to obtain meaningful distance geometry structures. A single conformation for the oxytocin ring formed by the residues Cys^1-Tyr-Ile-Gln-Asn-Cys^6 was obtained.
2) Dynamics : NOE experiments at different resonance frequences (400 and 600 MHz) showed that residual flexibility exists for some oxytocin residues in the complex, notably in the C-terminal Gly residue and in the Ile^3 side chain.
3) Interaction between oxytocin and NP I : at a peptide protein ratio of of 3:1 in D2O some specific peptide protein NOE contacts were found. Due to previous assignments of the aromatic resonances in NP I, we could attribute a contact to the aromatic groups of Phe^{22} on the protein and Tyr^2 on the peptide.

As the solution conformation of oxytocin is well studied by NMR and other biophysical methods, and an X-ray structure of deamino-oxytocin has been derived, a detailed comparison of the different conformations has been made. This comparison demonstrates the general principle that the structure of a ligand can indeed change upon interaction with its target molecule, which has important consequences for the design of peptide ligands.

LZ 430 SOLUTION STRUCTURE OF A MONOMERIC INSULIN MUTANT.

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Structure studies of insulin and insulin mutants by NMR have been the object of intense research over the last years. Despite the small size of the molecule these studies have been complicated by aggregation, dimerization and unusual fast amide proton exchange rates. Application of partly organic solvent, solutions in the low pH range and/or design of mutants that are partly monomeric have been the standard solution. We have embarked on a structure study of a human insulin mutant B16-His that are monomeric in water at pH 2.5 using NMR and simulated annealing (X-PLOR). The calculated structures are based on more than 800 nuclear Overhauser effects, 73 dihedral angle restraints (44 ϕ-, 23 χ^1-, and 6 χ^2-angles) and include as well a large number of stereospecific assignments. The calculated structures converge well and the atomic RMS difference between the individual structures and the geometrical average structure is 0.99 Å for all backbone atoms, 1.48 Å for all heavy atoms, and 0.63 Å for non-terminal backbone atoms. The last three residues of the B-chain are disordered. No violations of distance restraints exceed 0.25 Å and no dihedral angle restraints are violated by more than 1° in any of the structures.

LZ 431 MULTINUCLEAR NMR STUDIES OF A CALMODULIN: INHIBITOR COMPLEX, Lesley K MacLachlan, Andrew J Edwards and David G Reid, Analytical Sciences Department, SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Herts. AL6 9AR, Great Britain.

An E. coli expression system has been used to produce substantial amounts of cloned calmodulin (CaM) from Trypanosoma brucei, which shows 22 amino acid substitutions relative to the much-studied human protein. One sample with all Phe residues replaced by ring-deuteriated phenylalanine (Phe-d_5) is being used to study the complex of the highly aromatic, potent calmodulin inhibitor calmidazolium with CaM. The absence of phenylalanine aromatic signals from the protein means that we may use standard ^1H experiments to study the bound inhibitor conformation. A second sample consisting of [U-^{13}C,^{15}N]-labelled CaM:calmidazolium complex is being used to study protein:inhibitor interactions, using advanced multinuclear nD experiments.

LZ 432 HETERO-NUCLEAR, MULTI-DIMENSIONAL NMR STUDIES OF AN 186RESIDUE PROTEIN COMPOSING TWO FIBRONECTIN TYPE III DOMAINS.

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The 9-10type III pair from fibronectin has been synthesised, by heterologous expression, in E. coli, using the pGEX2T expression system. An isotopically labelled sample has been obtained and the structure function and the structure function and dynamics of the protein studied. The 10th type III module contains an RGD tripeptide and is known to be involved in cell adhesion. The preceding module increases the binding affinity of the RGD tripeptide. The results below indicate the nature of the synergy observed.

LZ 433 STUDIES OF BACKBONE DYNAMICS OF CARBONMONOXY LEGHEMOGLOBIN USING TWO-DIMENSIONAL ^{1}H-NMR: AMIDE PROTON EXCHANGE WITH SOLVENT, Dimitrios Morikis and Peter E. Wright, Department of Molecular Biology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037.

The hydrogen-deuterium exchange rates for the backbone amide groups of carbonmonoxy leghemoglobin have been measured at pH 7 and 308 K using ^{1}H-NMR spectroscopy. Fast 2D-NOESY experiments, with 5.2 min data collection time for each spectrum, have been performed which make possible the measurement of NOE cross peaks of the fast exchanging amide protons at early time points. Protection factors and plots of the experimental rate constants *vs* the intrinsic rate constants provide a qualitative insight into some of the breathing motions of the protein. Helices A, C and F and the CD loop contain rapidly exchanging NHs suggesting flexibility and solvent exposure. Helices B, E, G and H include extensive regions of medium and slow exchanging NHs suggesting more rigid structure and motional damping. Comparisons with the carbonmonoxy myoglobin exchange rates[1] will be discussed.

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LZ 434 THE ESTABLISHMENT OF TWO DOMAINS IN *CALLINECTIUS SAPIDUS* METALLOTHIONEIN THROUGH HETERONUCLEAR AND HOMONUCLEAR CORRELATION NMR EXPERIMENTS, Surinder S. Narula, Marius Brouwer‡ and Ian M. Armitage, Dept. of Pharmacology, Yale University School of Medicine, 333 Cedar Street, P.O. Box 3333, New Haven, CT 06510 and ‡Duke University Marine Laboratory, Beaufort, NC 28516

Metallothionein is a cysteine-rich metal-binding protein whose biosynthesis is closely regulated by the level of the exposure of an organism to zinc, copper, cadmium, etc. metal salts. The metallothionein from *Callinectus sapidus* is known to bind six distinct ^{113}Cd resonances spanning a chemical shift range of 620-666 ppm, indicating the presence of six distinct tetrahedrally coordinated divalent metal ions binding sites. Heteronuclear ^{113}Cd-^{1}H correlation experiments have revealed all the metal-to-cysteines connectivities present in this protein. Complete sequential ^{1}H NMR assignments have been obtained which reveal that there are two separate domains in this metallothionein. Each of the two domains contain three metal ions and nine coordinating cysteines of which three are bridging. The three dimensional solution structure of this metallothionein calculated using the NOE distances and dihedral angle constraints along with the metal-to-cysteine connectivities obtained from NMR experiments will be presented (NIH DK18778).

LZ 435 USE OF CARBON SECONDARY SHIFTS TO CHARACTERIZE THE SECONDARY STRUCTURE OF h-EGF[1-48], Diana Omecinsky, Michael Reily and Venketaraman Thanabal, Chemistry Department, Parke-Davis Pharmaceutical Research Division, Warner Lambert Company, Ann Arbor, Michigan 48105

We have used a method based on carbon chemical shifts[1,2] to characterize the secondary structure of human epidermal growth factor, h-EGF [1-48]. This method relies on the deviations of the carbon chemical shifts from random coil values, secondary shifts, observed for different types of secondary structure. Helix is characterized by a positive deviation (downfield shifts) of the α-carbon chemical shifts and a negative deviation (upfield shifts) of the ß-carbon chemical shifts; whereas, the opposite is true for ß-sheets. The plot of α-carbon secondary shifts vs residue type shows predominantly the presence of ß-sheet, which agrees with the data obtained using NOE-based NMR methods[3]. The histogram of ß-carbon secondary shifts vs residue type confirms the presence of ß-sheet secondary structure and identifies the residues involved. The magnitude of deviation from random coil values is less pronounced for residues in ß-sheets than for α-helix; however, these deviations are still characteristic of the secondary structure and this information can be used to facilitate the sequential assignment of proteins. Also, the complementary information obtained from both α and ß-carbon secondary shifts may provide a reliable method for the characterization of protein secondary structure.

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LZ 436 SOLUTION STRUCTURE AND IgG BINDING SITE OF THE STREPTOCOCCAL PROTEIN G B2 DOMAIN, John Orban[1], Patrick Alexander[1], Philip Bryan[1], and Diane Hancock[2], [1]University of Maryland, Maryland Biotechnology Institute, [2]National Institute of Standards and Technology, Center for Advanced Research in Biotechnology, 9600 Gudelsky Drive, Rockville, MD 20850

Protein G is a multidomain streptococcal cell wall protein consisting of 2 or 3 55 amino acid repeats (domains B1, B2, and B3), each of which binds to the Fc portion of immunoglobulin G (IgG). The B1 and B2 domains, which differ in their primary structure by only 6 amino acids, differ significantly in their denaturation temperature (T_m 87.5 °C and 79.4 °C, respectively at pH 5.4) and in IgG binding [Alexander *et al.*, *Biochemistry*, **31**, 3597-3603 (1992)]. The solution structure of the B1 domain has been reported [Gronenborn *et al.*, *Science*, **253**, 657-661 (1991)]. We present here the solution structure of the B2 domain and its IgG binding site.

Proton resonance assignments and coupling constant measurements were made using DQF COSY, NOESY, and E. COSY experiments and interproton distances were obtained from NOESY experiments. Stereospecific assignments were made using coupling constants and relative NOE distances. The interproton distance constraints and torsional angle restraints have been used to calculate a series of structures using a hybrid distance geometry-simulated annealing method.

We have measured main chain amide exchange rates of the B2 domain in the free (k_{free}) and in the IgG bound ($k_{complex}$) forms. Residues directly contacting IgG have high protection factors ($k_{free}/k_{complex}$) and a low resolution map of the contact site will be presented.

LZ 437 THE RECEPTOR BOUND CONFORMATION OF THE METASTASIS INHIBITOR, PEPTIDE 11. Gerard J. Ostheimer[1], Jean R. Starkey[2], Terry Landowski[2], and Edward A. Dratz[1], Departments of Chemistry[1] and Microbiology[2], Montana State University, Bozeman, MT 59717.

Peptide 11, CDPGYIGSR-NH_2, is a synthetic peptide, derived from the sequence of the ß1 chain of laminin. It has been shown to block tumor metastasis by competing with laminin for the 67 kDa high affinity laminin receptor. We have previously demonstrated that substituting l-Ala for Gly 7 reduces anti-metastatic activity by >50%; while, substituting d-Ala for Gly 7 yields a peptide with activity equal to that of the native peptide. NMR and molecular dynamics provided evidence that peptide 11 and the active d-Ala 7 analog possess preferred solution conformations that exhibit a similar YIXSR bend, that is not observed in the l-Ala 7 analog.

Our finding that the 67 kDa laminin receptor partitioned into chloroform/methanol allowed us to isolate receptor from EHS tumor where it is found tightly associated with matrix laminin. Sufficient quantities of receptor were isolated in order to permit the performance of Tr-NOESY experiments to determine the structure of the peptides when bound to the 67 kDa laminin receptor. The Tr-NOESY data indicated that the bound peptides exhibit YIXSR conformations that resemble the solution structures of the peptides. In addition, binding of the receptor causes the CDPG region of all three peptides to adopt a conformation that resembles a type II' ß turn. The residual activity of the l-Ala 7 analog is likely to be due to its ability to adopt a CDPG conformation similar to the native peptide. This theory would predict that the peptide, YI(lA)SR, would be completely inactive, as was indeed found to be the case. The receptor bound conformation is preliminary, but after refinement, it may have direct application in structure based drug design. Patent pending, supported by CTR grant #2405.

LZ 438 PROTON NMR DETECTION OF ALBUMIN IN INTACT HUMAN BLOOD PLASMA

Sunil U. Patel, Peter J. Sadler, Alan Tucker and John H. Viles, Department of Chemistry, Birkbeck College, University of London, 29 Gordon Square, London WC1H 0PP, UK.

Blood plasma is a heterogeneous fluid consisting of lipoprotein particles, proteins, small molecules and ions. The major protein is albumin (ca. 0.65 mM), a single chain of 585 amino acids folded into 3 structurally-similar domains with a high helical content [1]. Albumin is involved in the binding, delivery and transport of fatty acids, hormones, metal ions and drugs. Structural flexibility may play a key role in its properties. In our previous ^{1}H NMR studies of plasma [2], we assigned peaks for the various classes of lipoproteins and for small molecules, and used these to study conditions of clinical interest. We show here that albumin is readily detectable in single-pulse spectra of blood plasma if resolution enhancement techniques are used [3]. Remarkably this allows identification of the strong Ni^{2+} and Cu^{2+} binding site on albumin. Differences between isolated albumin and albumin in plasma will be discussed. It should now be possible to use NMR methods to study a range of clinically important problems relating to albumin, e.g. abnormalities, interactions with drugs, anaesthetics, xenobiotics etc.

We thank the MRC, Wellcome Trust, SERC and ULIRS for support.

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LZ 439 ISOTOPE-FILTERED NMR STUDIES OF PROTEIN-BOUND LIGANDS: APPLICATION TO FKBP/IMMUNOSUPPRESSANT COMPLEXES,

Andrew M. Petros, Edward T. Olejniczak and Stephen W. Fesik, *Pharmaceutical Discovery Division, Abbott Laboratories*, Abbott Park, IL 60064

Isotope-edited NMR experiments have proved very useful for obtaining structural information on ^{15}N and ^{13}C-labeled ligands bound to proteins. However, in many cases it may not be possible to obtain the isotopically enriched ligands necessary for such studies. The same type of structural information can be obtained using isotope-filtering techniques in which the proton signals from an unenriched ligand are selectively observed against a background of signals from a uniformly, isotopically enriched protein. In this presentation we apply recently developed isotope-filtering techniques to determine the bound conformation of several analogs of the immunosuppressant ascomycin when bound to uniformly ^{13}C and ^{15}N-labeled FKBP. The bound conformation of these analogs are compared to that of ascomycin and interpreted in terms of the immunosuppressive activities displayed by these analogs.

LZ 440 TRANSFERRED NOE'S TO PROBE PHOSPHOLIPID SUBSTRATE BINDING TO PHOSPHOLIPASE A_2 Leigh A. Plesniak, Scott Boegeman, and Edward A. Dennis, Department of Chemistry, UCSD, La Jolla, CA 92093-0601

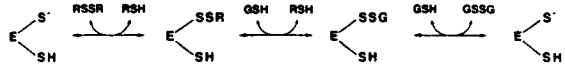
Transferred NOE experiments have been carried out on cobra venom (naja naja naja) phospholipase A_2 (PLA_2) with two types of substrate analogues which serve as inhibitors, 1-hexylthio-2-hexanoylamino-1,2-dideoxy-sn-glycerol-3-phosphorylethanolamine (PE) and the corresponding phosphorylcholine analogue (PC). PLA_2 is an enzyme that catalyzes the hydrolysis of the *sn-2* fatty acid of a phospholipid. The PE and PC inhibitors are short chain substrate analogues which have IC_{50}'s of 4 μM and 55 μM, respectively. Because they are small compounds and monomeric in solution, NOE's develop inefficiently in the absence of enzyme. Thus, the PLA_2-inihibitor system is ideal for analyzing transferred NOE's. The data is the first to determine inhibitor conformation in the catalytic site of cobra PLA_2 in solution. The experiments are carried out under conditions that are optimal for catalysis (pH 7.5). The effect of the thioether in the *sn-1* chain on the chemical shift dispersion of the methylene protons allowed for detailed conformational analysis. Both inhibitors adopt a conformation in which the end of the *sn-2* chain is within 5 Å of the α-methylene of the *sn-1* chain. In addition, intermolecular NOE's show contact points of the inhibitor with the enzyme.

LZ 441 3D STRUCTURE OF PROTEIN INHIBITORS IN THE BOUND STATE BY EXCHANGE-TRANSFERRED NOESY, **Carol B. Post, Michael L. Schneider and Jie Zheng, Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, IN 47907-1333**

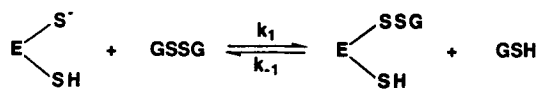
Defining the 3-dimensional structure of small molecule ligands when they are bound to their receptor targets is extremely useful for rational drug design. Exchange-transferred NOESY experiments are being used to determine the 3-dimensional structure of peptide inhibitors and cofactors bound to high molecular weight proteins. Qualitative structural information is readily obtained from simple analysis of the data. We are working toward developing more quantitative approaches to obtain as accurate a bound-state structure as realistic. These procedures will be described with applications to a 15-residue peptide inhibitor of aldolase and NADH bound to lactate dehydrogenase. From our attempts to achieve accurate ligand-ligand interproton distances we have found that relaxation contributions from the protein can be significant. In such cases, distances estimated from exchange-transferred NOESY intensities by ignoring the protein effects would be in error. This feature and others will be described with NMR simulation results and experimental results based on multiple spin-pair (matrix) analyses.

LZ 442 NUCLEAR MAGNETIC RESONANCE STUDY OF THE THIOLTRANSFERASE-CATALYZED GLUTATHIONE/GLUTATHIONE DISULFIDE INTERCHANGE REACTION, Dallas L. Rabenstein and Kevin K. Millis, Department of Chemistry, University of California, Riverside, CA 92521.

Thioltransferase is a low-molecular-weight cystosolic enzyme that catalyzes the formation and reduction of disulfide bonds by thiol-disulfide interchange in a variety of protein and nonprotein substrates. This property enables thioltransferase to affect the sulfhydryl status of metabolic enzymes,which has created interest in its ability to act as a regulatory protein. Thioltransferase has 2 cysteine pairs with sequences of -Cys-Pro-Phe-Cys- and -Cys-Ile-Gly-Gly-Cys-. The first pair (Cys^{22} and Cys^{25}) is located at the active site of the enzyme. The mechanism proposed for thioltransferase-catalyzed thiol-disulfide interchange is:



where RSH represents a small molecule or protein thiol and GSH is glutathione. We have characterized by ^1H NMR the kinetics of the thioltransferase-catalyzed symmetrical GSH/GSSG interchange reaction over a range of solution pH, temperature and GSH, GSSG and thioltransferase concentrations. Interchange rate constants were determined by analysis of exchange-broadened multiplet patterns and by the inversion-magnetization transfer method using concentrations of GSH, GSSG and thioltransferase similar to intracellular concentrations. A rate constant of $k_1=6.4(\pm1.9) \times 10^5\ M^{-1}\ s^{-1}$ was obtained for the reaction of GSSG with thioltransferase:



This reaction is the first step in the thioltransferase-catalyzed formation of protein-GSH mixed disulfides, i.e. the first step in the proposed modulation of enzyme activity by thiol-disulfide exchange. The rate constant is 4-5 orders of magnitude larger than rate constants for analogous reactions of thiolate groups of small molecules with GSSG. Results on other systems suggest that the γ-L-glutamyl-L-cysteinyl moiety of GSSG is critical to its interaction with thioltransferase.

LZ 443 NMR STUDIES OF *Escherichia coli* HPr-ENZYME IIA COMPLEX, Ponni Rajagopal and Rachel E. Klevit, Department of Biochemistry, University of Washington, Seattle, Wa-98195.

HPr and enzyme IIA are two components of the bacterial PTS (phosphpoenolpyruvate: sugar phosphotransferase) system and are involved in the phosphorylation and the concomitant translocation of sugars across the membrane. These PTS protein complexes are also found to regulate sugar transport. HPr is phosphorylated at the N-1 position of His-15 by enzyme I and PEP and transfers the phosphoryl group to the N-3 postion of His-90 in enzyme IIA. A study of phospho-histidine HPr-enzyme IIA complex will enable us to understand the nature and specificity of these protein-protein interactions. However, such a complex will be only transiently stable. Studies on phospho-histidine HPr performed in this lab have shown that HPr undergoes no major structural changes on phosphorylation (to be published). Hence, HPr will serve as a reasonably good model for phospho-histidine HPr in complex with enzyme IIA. *E.coli* HPr and *E.coli* enzyme IIA form a complex with a molecular weight of about 26 kilodaltons. In order to reduce the complexity of the system, various heteronuclear 2D NMR experiments were performed on uniformly ^{15}N labeled HPr complexed with unlabeled enzyme IIA. The binding site of HPr in complex with enzyme IIA was mapped by monitoring chemical shift changes, differential chemical exchange rates, and intermolecular NOEs. The aim of these studies is to probe the structural changes induced in *E.coli* HPr on complexation and provide a key to the fundamental aspects of protein-protein recognition.

LZ 444 CONFORMATION OF Mg ATP AND Mg ADP BOUND AT THE ACTIVE SITE OF ATP UTILIZING ENZYMES BY TWO DIMENSIONAL TRANSFERRED NUCLEAR OVERHAUSER SPECTROSCOPY, B. D. Nageswara Rao, N. Murali, Gotam K. Jarori and Steven B. Landy, Department of Physics, IUPUI, 1125 E 38th Street, Indianapolis, IN 46205

Two dimensional transferred nuclear Overhauser spectroscopy (TRNOESY) experiments were performed to determine the inter-proton distances in MgATP and MgADP when bound at the active site of several ATP utilizing enzymes such as creatine Kinase, 3-phospho glycerate kinase and arginine kinase. Experiments were performed at 500 MHz and at 10^0 C on D_2O solution containing four to ten-fold excess of ligand over enzyme. By performing measurements as a function of ligand concentration, it was possible to determine the extent of contribution to the observed nOe arising from the ligand-protein interactions that are not specific to the active site. The site specific build-up curves, obtained for mixing times between 25 msec. to 300 msec. were analyzed using the relaxation matrix method to obtain the inter proton distances in the adenosine moiety. The distances obtained from such an analysis are used as constraints in the program CHARMM to obtain the conformaton of the nucleotides bound at the active site of the enzymes.

LZ 445 UNUSUAL REACTIVE-SITE DYNAMICS OF A82-CYTOCHROME C PEROXIDASE STUDIED BY 1D AND 2D PROTON NMR SPECTROSCOPY, James D. Satterlee, Steve Alam, James E. Erman[1], J. Matt Mauro[2], Thomas L. Poulos[3] Departments of Chemistry, Washington State University, Pullman, WA 99164-4630, [1]Northern Illinois University, DeKalb, IL 60115; [2]Center for Advanced Research in Biotechnology, 9600 Gudelsky, Drive, Rockville, MD 20850; and [3]Department of Molecular Biology/ Biochemistry, University of California, Irvine, CA 92717

Yeast cytochrome *c* peroxidase is a 34 kDa ferriheme enzyme that has become a paradigm for studying redox-related protein-protein association and long-range electron transfer. An approach that we are pursuing in attempting to dissect the mechanism of this enzyme's function is through use of site specific mutants designed to probe "second sphere" interactions. The mutations made are for testing potentially significant structure/function relationships, but ones that are removed by one molecular layer from the enzyme's heme active-site. One locus for mutations is primary sequence position 82 (N in wild-type enzyme) where the peptide carbonyl acts as a hydrogen bond acceptor from the functionally critical "distal histidine". Two mutants have been studied so far, D82N and A82N. The latter produces proton NMR spectra at 500 MHz that is characteristic of two enzyme forms. These are detected by splitting of proton hyperfine-shifted resonances. The two enzyme forms are interconvertible by temperature so that the equilibrium and kinetics exchange dynamics for the interconversion have been quantitated by both 1D and 2D proton NMR spectroscopy.

LZ 446 APPLICATIONS OF MAXIMUM ENTROPY RECONSTRUCTION, Peter Schmieder, Jeffrey C. Hoch, Alan S. Stern, Gerhard Wagner, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, 02115; Rowland Institute for Science, Cambridge, MA, 02142.

In recent years many procedures for spectral reconstruction have been described as alternatives to the discrete Fourier transform (DFT)[1]. Among them, the Maximum Entropy Reconstruction (MEM)[2] is a particularly general and robust method; however, it bears a considerably heavier computational price than the DFT. This problem has been alleviated by the dramatic development of the available computer resources and many useful applications can be expected in the near future. We want to show two applications where the properties of MEM can be used in an important way. The first application is to constant-time-HSQC experiments of fully carbon labeled molecules. Because of the unfavorable relaxation properties of the carbon nuclei, it can be advantageous to keep the constant time delay as short as possible. For a given sampling interval the maximum number of t_1-values is then restricted to a rather small number, and with the DFT an weighting function has to be applied to avoid truncation artifacts, reducing the resolution and sensitivity. The ability of MEM to produce high resolution spectra from short data records is advantageous in this case. The second application is COSY-type experiments. Here the coupling constants used for the magnetization transfer are usually small. Thus in case of the DFT the most intense FIDs can only be collected after collecting a vast number of low intensity FIDs. Since MEM is capable of processing data that are incompletely sampled[3], the sampling can be weighted toward the most intense FIDs, resulting in increased resolution and signal-to-noise. The usefulness of both applications is shown and the results are compared with other methods such as DFT and linear prediction.

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LZ 447 NMR STUDIES OF YEAST PHOSPHOGLYCERATE KINASES THAT ARE PERDEUTERATED AND PERDEUTERATED BUT HISTIDINE RESIDUES, Engin H.Serpersu, Celeste G. Shibata and Kameshvari M. Pappu, Department of Biochemistry, University of Tennessee, Walters Life Sciences Building, Knoxville, TN 37996-0840

Phosphoglycerate kinase was isolated from yeast cells grown in 99.8% ^2H_2O. Kinetic and NMR studies of perdeuterated phosphoglycerate kinase (^{2}HPGK) revealed that this enzyme is active and behaves similarly to undeuterated enzyme (^{1}HPGK). One dimensional NMR studies indicated that some of the substrate resonances (H2′, H2″, H3′, H4′, H5′ and H5″ of dATP) were obscured by the protein resonances in the ^{1}HPGK•MgdATP complex. On the other hand, in ^{1}H NMR spectrum of matched ^{2}HPGK•MgdATP complex, all of the substrate resonances were observable without any overlap of a few remaining protein ^{1}H resonances. 2D NOESY spectra with bound substrate MgdATP also showed that with ^{2}HPGK none of the intermolecular and protein cross peaks, which obscured some substrate cross peaks in the matched sample with ^{1}HPGK, were visible. Additionally, longer mixing times could be used, which improves the signal/noise ratio, with ^{2}HPGK•MgdATP complex because of the lack of spin diffusion. Another advantage observed with ^{2}HPGK was that lower substrate concentrations were easily observable, which made higher enzyme to substrate ratios obtainable for NOE experiments.

^{2}HPGK was purified from yeast cells grown in deuterated medium with the addition of excess ^{1}H Histidine. ^{1}H NMR spectrum of this enzyme indicated that H2 and H4 resonances of all of the 8 histidine residues were visible and resolved. pH titration of the enzyme confermed that these resonances were histidine H2 and H4 protons.

LZ 448 MULTI-DIMENSIONAL NMR STUDIES OF DNA BINDING PROTEINS, Masahiro Shirakawa, Kouichi Uegaki, Eugin H. Morita and Yoshimasa Kyogoku, Institute for Portein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565, Japan

DNA binding domain of two transcriptional factors, POU-homeo domain of mouse Oct-3 and the DNA binding domain of Interferon Regulatory Factor-2 (IRF2), were studied by means of multi-dimensional heteronuclear NMR spectroscopy.

The 62 residues fragment of mouse Oct3 protein, which corresponds to the POU-homeo domain of Oct3, was produced in *E. coli* in a large amount and extensively doubly-labeled with ^{15}N and ^{13}C. The DNA binding domain of IRF2 was elucidated by limited proteolysis and expression of truncated proteins in *E.coli* [1]. The DNA binding domain of IRF2 which comprises 112 amino acid residues was also doubly-labeled with ^{15}N and ^{13}C.

At first, the backbone ^{1}H, ^{15}N and ^{13}C nuclei of the two proteins were assigned by mainly heteronuclear three-dimensional NMR and triple-resonance multi-dimensional NMR, including four-dimensional triple-resonance experiments. Then the assignments were extended to the side chain ^{1}H and ^{13}C resonances by means of three-dimensional HCCH experiments.

The assignments and the medium range NOEs obtained in three-dimensional ^{1}H-^{15}N NOESY-HSQC and ^{1}H-^{13}C NOESY-HSQC have shown that the POU-homeo domain has three helices, which resembles with other homeo domains.

The structure calculation of the POU-homeo domain and the structure study of the DNA binding domain of IRF2 are now under the way, and will be presented.

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LZ 449 APPLICATION OF ^{1}H COSY AND NOESY TO DELINEATE THE SPIN DENSITY DISTRIBUTION AT THE β-PYRROLE POSITIONS OF PARAMAGNETIC MODEL HEME COMPLEXES, Ursula Simonis, F. Ann Walker, Qing Lin, Department of Chemistry and Biochemistry, San Francisco State University, San Francisco, CA 94132, and Department of Chemistry, University of Arizona, Tucson, AZ 85721

The pattern of unpaired electron spin density delocalization in low-spin iron(III) porphyrins and heme proteins has been the subject of numerous investigations. In the case of heme proteins it has often been possible to assign resonances of the heme substituents by one- and two-dimensional nuclear Overhauser effect (NOE) experiments. However, the combined effect of the low symmetry of the substitution pattern of naturally occurring hemes, the large effect of histidine or methionine π lone pair orientation, and the less quantifiable effect of protein side chains pressing against certain portions of the heme ring, have made it difficult to use these assignments to develop a simple description of the pattern of π-electron density delocalization within the heme ring. We have shown recently that model hemes offer a much simpler system for investigation of the pattern of unpaired electron spin distribution within the porphyrin macrocycle.

Paramagnetic N-methylimidazole complexes of mono-ortho substituted tetraphenylporphyrinatoiron(III), $[TPPFe(N\text{-}MeIm)_2]^+$, have been investigated by two-dimensional proton NMR spectroscopy in order to delineate the inductive or conjugative effect of the phenyl ring, which bears the unsymmetrical substituent, on the pattern of spin delocalization in model hemes. COSY and NOESY spectra of these biologically relevant low-spin model heme complexes reveal cross correlations among the four resonances of the eight pyrrole protons, which are consistent with those theoretically predicted for the electron density distribution in the porphyrin $3e(\pi)$ orbitals, as modified by the unique substituent. This will be discussed in detail, as well as experimental considerations for obtaining COSY and NOESY maps of these low-spin model hemes with short relaxation times.

LZ 450 SECONDARY STRUCTURE ASSIGNMENT OF THE FINGER AND EGF PAIR OF DOMAINS FROM HUMAN TISSUE-TYPE PLASMINOGEN ACTIVATOR BY MULTIDIMENSIONAL NMR, Brian O. Smith & A. Kristina Downing, Oxford University, Department of Biochemistry, South Parks Road, Oxford OX1 3QU ENGLAND

The serine protease tissue-type plasminogen activator (t-PA) catalyzes the conversion of the zymogen Glu-plasminogen to its active form, plasmin, which is the enzyme responsible for degrading the fibrin network of a blood clot. t-PA is composed of five discrete structural domains: a finger domain (F1), a region homologous to the precursor to epidermal growth factor (G), two kringle structures, and a catalytic serine protease region. The solution structure of the finger domain of t-PA has been previously described and compared to the structure of the seventh type 1 repeat from human fibronectin (Downing *et al.*, J. Mol. Biol., 225, 821-833.). One significant difference between the two molecules is that hydrophobic residues cover the exposed surface of the principal β-sheet region in the t-PA finger domain.

Assignment of secondary structure of the F1-G pair of domains from t-PA based on high resolution multidimensional n.m.r. data indicates that the structure of the EGF-like module of t-PA will conform to the consensus EGF structure thereby forming another hydrophobic surface on the second domain of t-PA. It is unclear whether or not the F1 and G domains interact with each other to shield their hydrophobic surfaces. However recent research has indicated a structural cooperativity between the first two domains of t-PA and its serine protease region (Novokhatny, V.V., *et al.*, J. Biol. Chem., 266, 12994-13002; Bennett, W. F., *et al.*, J. Biol. Chem., 266, 5191-5201.). The n.m.r. structure of this pair of modules will shed light on the nature of their interaction with each other and with the serine protease region.

LZ 451 TOWARDS UNDERSTANDING KINETIC CONTROL IN THE FOLDING OF α-LYTIC PROTEASE

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UCSF Dept. of Biochemistry and Biophysics
San Francisco, CA 94143-0448

α-Lytic protease is synthesized as a precursor containing a 166 amino acid pro region which is required both *in vivo* and *in vitro* for proper folding of the enzyme. If one unfolds α-lytic protease and then removes the denaturant, no refolding is seen for periods greater than one month. However if one adds the pro region to this state at any point during the incubation, proteolytic activity is rapidly regenerated. These observations indicate the presence of a stable folding-competent state which in the absence of the pro region has a barrier to folding of >27kcal/mol. Experiments have shown that this intermediate state is greatly expanded in radius over the native state yet retains some native-like secondary structure. In order to further explore the nature of the kinetic block to folding and the mechanism by which the pro region circumvents it, amide exchange NMR experiments are being pursued as well as further characterization of the folding energetics.

LZ 452 TYPE III ANTIFREEZE PROTEINS HAVE A β-SANDWICH STRUCTURE, [1]Frank D. Sönnichsen, [1]Brian D. Sykes, [2]Heman Chao, and [2]Peter L. Davies. [1]Protein Engineering Centre of Centres of Excellence, University of Alberta, Edmonton, Alberta, Canada T6G 2S2, [2]Department of Biochemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Antifreeze proteins have been classified into three biochemically distinct types. Until recently a 3D-structure had been known only for Type I AFP, which consists of an amphipathic helix. In order to understand the structure/function relationship for all AFP's and to determine a structural motif required for ice binding we have used 2D-NMR spectroscopy to obtain detailed structural information on the 65 amino acid Type III AFP. Based on our earlier sequential and secondary structure assignments as well as recent studies of several mutations of the native Type III sequence, we have obtained a 3D-structure for Type III AFP through NOE-based structure calculations. The protein folds into a single, densely packed domain, which primarily consists of a two layer β-sandwich. Several short strands form the triple-stranded layers, which are packed orthogonally. The layers are connected by irregular loops at the close corners and by a two-stranded antiparallel sheet at the splayed corner. Unique features of this novel fold and of the protein will be discussed.

LZ 453 HETERONUCLEAR EDITED STUDIES OF THE *E. coli* METHIONINE REPRESSOR SYSTEM, Leonard D. Spicer,* David Hyre,* Ronald Venters,* and B.T. Farmer II+, *Department of Biochemistry, Duke University Medical Center, Durham, NC 27710 and +Research and Development Department, NMR Instruments, Varian Associates, Palo Alto, CA 94304.

The methionine repressor protein, MetJ, regulates transcription of the genes coding for the methionine biosynthetic enzymes by recognizing and cooperatively binding to contiguous repeats of an 8 base-pair consensus sequence in the operator regions of these genes. This binding is modulated by S-adenosylmethionine, the end-product of this biosynthetic pathway and the main methyl donor in the cell. In the process of characterizing the interactions between the protein, the allosteric cofactor, and the cognate DNA, we are assigning the main chain of the repressor protein. MetJ is composed of a homodimer of two 12 kDa monomers which largely precludes direct assignment by homonuclear 2D methods due to resonance overlap, significant dimer linewidth, and the proximity of the two monomer units.

We are using heteronuclear 3D NMR methods to aid in the assignment of the main chain of the MetJ repressor. These methods are, in general, not compromised by the presence of the intertwined dimer chains since they rely on scalar couplings to directly bonded atoms. Resonance overlap is reduced by the additional dimensionality created by the correlation with the associated hetero-atoms, and these additional correlations themselves are used to connect the individual heteronuclear spin systems into a sequential assignment. We have isotopically labelled the MetJ protein with ^{15}N and ^{13}C using an efficient, cost-effective method developed in our laboratory and have written a coordinated group of cross-correlated, amide-proton-directed, through-bond heteronuclear 3D NMR experiments that are currently being used to assign the main chain of MetJ.

LZ 454 EXAMINATION OF THE COMPLEX FORMED BY THE bHLH DOMAINS OF MyoD AND E47

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The myogenic determination factor, MyoD, and the non-tissue specific enhancer binding protein, E47, are each members of the "basic-helix-loop-helix" (bHLH) family of DNA-binding proteins. Truncated forms of MyoD (68 residues) and E47 (96 residues) that span their respective bHLH regions are necessary and sufficient for dimerization and DNA-binding *in vitro*. Sedimentation equilibrium ultracentrifugation indicates that although the purified proteins exist as tetramers (MyoD) or dimers (E47) at millimolar protein concentrations, mixtures of MyoD and E47 exist predominantly in the form of a MyoD/E47 heterodimer. In DNA-binding assays, the MyoD/E47 heterodimer has a higher apparent DNA-binding affinity than that of either protein alone. We have examined the complex formed by the bHLH domains of MyoD and E47 using $^{1}H/^{15}N$ heteronuclear NMR spectroscopy. Spectra have been obtained on samples containing either uniformly or selectively ^{15}N-labeled MyoD in the presence of unlabeled E47 to examine the secondary structure of MyoD in the heterodimer. Previously, the secondary structure of MyoD in the context of a disulfide-bonded homodimer was examined using a similar approach. Our results with the MyoD/E47 heterodimer will be presented and compared with those obtained for the oxidized MyoD homodimer.

LZ 455 COMPARISON OF BACKBONE AND TRYPTOPHAN SIDECHAIN DYNAMICS OF REDUCED AND OXIDIZED E. COLI THIOREDOXIN USING ^{15}N NMR RELAXATION MEASUREMENTS, Martin J. Stone‡, Kasibhatla Chandrasekhar‡, Arne Holmgren‖, Peter E. Wright‡, and H. Jane Dyson‡, ‡Department of Molecular Biology, The Scripps Research Institute, La Jolla, California 92037, and ‖Department of Biochemistry, The Medical Nobel Institute, Karolinska Institutet, Box 60400, S-10401 Stockholm, Sweden.

The backbone and tryptophan sidechain dynamics of both the reduced and oxidised forms of uniformly ^{15}N-labelled *E. coli* thioredoxin have been characterized using inverse detected two-dimensional ^{1}H-^{15}N NMR spectroscopy. Longitudinal (T_1) and transverse (T_2) ^{15}N relaxation time constants and steady state $\{^{1}H\}$-^{15}N NOEs were measured, at a spectrometer proton frequency of 500 MHz, for more than 90% of the protonated backbone nitrogen atoms and for the protonated indole nitrogen atoms of the two tryptophan residues. These data were analyzed by using the Lipari-Szabo model free dynamics formalism to determine the generalized order parameter (S^2), the effective correlation time for internal motions (τ_e), and ^{15}N exchange broadening contributions (R_{ex}) for each residue, as well as the overall molecular rotational correlation time (τ_m). The reduced and oxidised forms exhibit almost identical dynamic behaviour on the picosecond to nanosecond time scale. The W31 sidechain is significantly more mobile than the W28 sidechain consistent with the positions of W31 on the protein surface and W28 buried in the hydrophobic core. Backbone regions which are significantly more mobile than the average include the N-terminus, which is constrained in the crystal structure of oxidized thioredoxin by specific contacts with a Cu^{2+} ion, the C-terminus, residues 20-22, which constitute a linker region between the first α-helix and the second β-strand, and residues 73-75 and 93-94, which are located adjacent to the active site. In contrast, on the microsecond to millisecond time scale, reduced thioredoxin exhibits considerable dynamic mobility in the residue 73-75 region, while oxidized thioredoxin exhibits no significant mobility in this region.

LZ 456 pH-INDUCED STRUCTURAL TRANSITIONS IN SERUM ALBUMIN AND TRANSFERRIN

Alan Tucker, Gina Kubal and Peter J. Sadler, Department of Chemistry, Birkbeck College, University of London, 29 Gordon Square, London WC1H 0PP, UK.

Albumin (66 kDa) and transferrin (80 kDa) are important blood serum proteins. Albumin has 3 structurally-similar domains each composed of two subdomains [1], and transferrin has two structurally-similar lobes each composed of two domains [2]. Both proteins are involved in the uptake and targeted delivery of small molecules and ions,processes which probably involve interdomain hinge bending and other flexible regions of the proteins. The control of anion (CO_3^{2-}) and metal ion (Fe^{3+}) transport by transferrin is known to be pH-mediated and pH control may also be important to albumin.

We have characterised pH-induced structural changes in both proteins (pH 2.5-11) using ^1H NMR spectroscopy [3,4]. One such transiton for albumin involves a region close to the N-terminus. His titration curves have been constructed for 10 His residues of albumin and 14 in transferrin. Several His residues in each protein have unusually low pK_a values (close to endosomal pH, 5.6). The significance of these results to the structural control of serum proteins will be discussed.

We thank the Wellcome Trust, SERC, MRC and ULIRS for support.

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LZ 457 STRUCTURAL ANALYSIS OF sCD4-183 BY HETERONUCLEAR 3D NMR SPECTROSCOPY

Eldon L. Ulrich[1], Paul E. Fagerness[1], Melinda Roy[1], Kathleen A. Farley[1], Richard J. Kirschner[2], John A. Ceglarek[2], Joe P. Martin[2], and John T. Herberg[2], [1]Control and [2]Fine Chemical Divisions, The Upjohn Company, Kalamazoo, MI 49001

sCD4-183 is a water soluble fragment of the human helper T-cell surface protein CD4. The 183 residue protein contains domains I and II of the full protein and includes the binding site for the coat protein gp120 of the AIDS virus. HMQC-NOESY, HMQC-TOCSY, HNCA, and HN(CO)CA three-dimensional NMR spectra and several two-dimensional spectra are being used to assign the protein backbone resonances. The chemical shift dispersion in the H^α and HN regions of the spectra, HN-HN NOE connectivities, and HN exchange rates are all consistent with the extensive β-sheet structure of the protein determined by x-ray crystallography [Wang et al., **Nature 348,** 411 (1990) and Ryu et al., **Nature 348,** 411 (1990)].

LZ 458 DETERMINATION OF THE SOLUTION STRUCTURE OF STAPHYLOCOCCAL NUCLEASE H124L

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The spatial structure of staphylococcal nuclease H124L (SNase, 17 kDa) has been determined from a network of NMR-derived distance constraints. Sequence-specific backbone and side chain assignment were determined from 2D and 3D homo- and heteronuclear NMR data. Short-, medium-, and long-range interresidue NOE's were identified in spectra of the SNase-pdTp-Ca^{2+} ternary complex. Peak volumes and/or contour levels from 2D and 3D NOE spectra were measured and converted into distances, and these were used to generate a master list of distance constraints. Dihedral angle constraints were developed from spin-spin coupling constants measured from ^1H E.COSY,[1] ^1H-^{15}N HMQC,[2] and ^1H-^{15}N HMQC-NOESY[3] data. The coupling and NOE data led to assignments of a number of prochiral methylene proton signals. Complete assignments of prochiral Leu δ-CH_3 and Val γ-CH_3 groups were determined by incorporating chiral ^{13}C labeled amino acids. The 3D structure of SNase-pdTp-Ca^{2+} ternary complex has been determined by a metric matrix distance geometry/simulated annealing approach (DSPACE).

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LZ 459 STRUCTURAL INVESTIGATION OF A PUTATIVE MINOR GROOVE BINDING PROTEIN: *E. coli.* INTEGRATION HOST FACTOR BOUND TO A 30bp BINDING SITE

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E. coli. Integration Host Factor (IHF) is a moderately sized protein (M_r=21kD) which is involved in λ phage integration into and excision from the *E. coli.* genome. It has been characterized biochemically as primarily a DNA-bending protein which recognizes specific binding sites via the minor groove. In recent years, a variety of eukaryotic transcription factors have also been characterized as interacting with DNA via minor groove interactions and pronounced DNA bending (notably the HMG-1 DNA binding domain and its relatives). We have initiated an extended study of IHF bound to the H' binding site of the λ attachment site P (*attP*) using ^{15}N and ^{13}C double and triple resonance spectroscopy. As the minimum binding site for IHF is 30bp in length, the 40kD complex requires a variety of different approaches to understand the nature of the DNA-protein interface. We describe our approach to this problem and report on our progress in studying such a large and complex system by multidimensional NMR.

LZ 460NMR STUDIES OF AN IMMUNOGLOBULIN LIGHT CHAIN-BINDING PROTEIN L DOMAIN

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Several immunoglobulin (Ig)-binding microbial proteins have been identified and isolated, the two most well known being protein A of *Staphylococcus aureus*, and protein G of group C and G streptococci. Among these various proteins, only one binds Ig through interaction with Ig light chains. This bacterial surface protein is expressed by some strains of the anaerobic bacterial species *Peptostreptococcus magnus*. The protein, named protein L, is an elongated, fibrous molecule, showing affinity for framework structures in the variable domain of κ light chains. The Ig light chain binding activity is located in five homologous domains (B1-B5), each comprising 72-76 amino acid residues. In the present study, ^{1}H and ^{15}N 2D-NMR were employed to obtain information about the secondary structure and global fold for the N-terminal B1 domain.

LZ 461 SOLUTION STRUCTURE OF A PAIR OF TYPE1 MODULES FROM FIBRONECTIN.

Michael J. Williams, Isabelle Phan, Martin Baron, Timothy S. Harvey, Paul C. Driscoll and Iain D. Campbell.
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The explosive growth in protein sequence data has indicated that many diverse so-called mosaic proteins are constructed from small discrete units of conserved sequence. These modules are often encoded on single exons and are detected by consensus sequences, typically of 40-110 amino acids with conserved disulphides and hydrophobic residues. Although direct structural determination of intact mosaic proteins by X-ray crystallography has been hindered by the difficulties of crystallization, the combination of recombinant peptide expression and NMR has proved a powerful tool for studying individual module folds. The structure of one module can provide much useful information about the structures of other members of that module class. However to gain an insight into the structure of larger domains and ultimately whole mosaic proteins, it is necessary to study the nature of inter-module interactions and to confirm that the module does in fact fold as a unique unit. Fibronectin is a large mosaic protein consisting of repeats of three types of module, designated types1, 2 and 3. The solution structures of two single type1 modules have previously been described (Baron M., *et al* 1990, Nature 345, 642-646 & Downing A.K., *et al*, 1992, J Mol Biol 225, 821-833). The consensus structure is constrained by two disulphide bonds and is made up of two regions of anti-parallel B-sheet, both of which have a right handed twist and are stacked on top of each other to enclose a hydrophobic core of conserved residues. As part of a continuing strategy we have determined the tertiary structure of a 93 amino acid pair of type1 modules (^{4}F1.^{5}F1) from fibronectin, by 2D NMR. Both modules of the pair maintain this fold, but appear to be more stable due to the increased number of slow exchanging amides. The two modules connect end-to-end with a well defined interface and limited inter-module flexibility. The interface is dominated by a non-conserved tryptophan from the third turn of the first module which interacts with the first B-sheet of the second module. This structure provides a basis for the further investigation of dynamic aspects of type1 module pairs by ^{15}N labelling, ^{15}N relaxation measurements and molecular dynamics.

LZ 462 TRITIUM NMR SPECTROSCOPY IN KINETICS AND DYNAMICS.

Philip G. Williams and David E. Wemmer*, National Tritium Labeling Facility, Lawrence Berkeley Laboratory 75-123, One Cyclotron Road, Berkeley, CA 94720.

Tritium (^{3}H) is the most sensitive NMR nucleus with the highest resolution in the Periodic Table, and has spin 1/2. It has an extremely low natural abundance ($<10^{-16}$% of H isotopes), which means that it can be used as a true tracer for ^{1}H and ^{2}H. Tritium is radioactive and decays by low energy (18keV) beta particle emission, and straightforward safety measures have allowed tritium NMR spectroscopy to be routinely employed as an analytical tool for more than 20 years. While this aspect of tritium NMR spectroscopy has proven invaluable, there are many dynamic and kinetic situations in both chemistry and biochemistry where the ability to monitor specific hydrogen atoms is essential. Several examples from those described below will be presented.

In chemical research there are a number of mechanistic studies by ^{3}H NMR spectroscopy, but only recently have *in situ* or kinetic measurements of processes such as hydrogen isotope exchange or conformational preference been made. These studies have laid the groundwork for more complex and demanding investigations of proton transfer mechanisms in chemical reactions.

In biological systems there have been several elegant analyses of the ultimate fate of tritium atoms after biological transformations, and a recent example is the oxidation of enantiomerically pure chiral ethane by methane monooxygenase. We have also demonstrated the use of ^{3}H NMR spectroscopy as a monitor of dynamic processes such as glycolysis in canine erythrocytes, sugar binding to a 40kDa periplasmic protein, the solution behaviour of inhibited α-chymotrypsin, and the binding and conversion of ATP to ADP by myosin subfragment-1.

The range of applications of tritium NMR spectroscopy is as broad as the number of substrates in which hydrogen is a substituent. The technique is simple, safe and highly informative.

* This work has involved a large number of collaborators: H. Morimoto, M. Saljoughian (NTLF); R.D. Newmark, S. Un, P.J. Carson, M.P. Klein, D.K. Jaiswal (LBL); K.B. Gehring, M. Kubenic, H. Nikaido, J.G. Pelton, A. Streitwieser, R.E. Dixon (UCB); J.T. Gerig, T.M. O'Connell (UCSB), F.A.L. Anet, D.J. O'Leary (UCLA); A.J. Kresge, Y. Chiang (U. Toronto); M.A Long (UNSW); P.E. Eaton (U. Chicago); H.G. Floss, N.D. Priestley (U. Washington); W.A. Froland, J.D. Lipscomb (U. Minnesota); S. Highsmith (UOP).

LZ 463 NMR Study OF HIV-1 PROTEASE,

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The human immunodeficiency virus (HIV) is the cause agent of the acquired immunodeficiency syndrome (AIDS). The viral protease is responsible for processing polyproteins produced during viral replication and is essential for viral maturation. It is a dimer with molecular weight of 22 kDa and belongs to the aspartic protease family as shown by site-directed mutagenesis, enzymatic analysis, and X-ray crystallography. We have undertaken an NMR study of the recombinant HIV-1 protease complexed with acetyl pepstatin. Homonuclear two-dimensional COSY, TOCSY and NOESY spectra have been collected. The protease has been labeled uniformly and selectively with nitrogen-15. ^{1}H-^{15}N HSMQC and NOESY-HMQC data have been acquired. Analysis of these NMR data will be reported.

* Supported by HFSP fellowship.

LZ 464 ^{1}H-NMR STUDIES OF PROTEIN-PROTEIN ASSOCIATION AND FERRICYTOCHROME *c* EXCHANGE DYNAMICS IN COMPLEXES BETWEEN CYTOCHROME *c* PEROXIDASE AND VARIOUS FERRICYTOCHROMES *c*, Qiang Yi, Yihong Ge, James D. Satterlee and James E. Erman Departments of Chemistry, Washington State University, Pullman, WA 99164-4630 and Northern Illinois University, DeKalb, IL 60115

Cytochrome *c* peroxidase(CcP)/Ferricytochrome *c* (ferriCyt.*c*) complexes have been used as models for studying electron transfer in biology. Knowing the structure, stoichiometry and dynamics of protein-protein interactions is essential to understanding this type of long-range electron transfer. ^{1}H-NMR spectroscopy has been used to examine the stoichiometry and exchange dynamics of CcP/ferriCyt.*c* complexes and CcP-CN/ferriCyt.*c* complexes. Changes in ferriCyt. *c* ^{1}H hyperfine shifts when titrated by either CcP or CcPCN indicate the formation of binary complexes between these two heme proteins, and extensive experiments reveal the stoichiometry of the complexes as 1:1. The simul- taneous detection of both free and bound forms of yeast-iso1 ferriCyt.*c* further reveals a slow exchange process on the NMR time scale. This has enabled us to use dynamic NMR techniques, such as inversion transfer, to study the exchange process in this system. Numerical analysis of the data from inversion transfer experiments, analyzed using the McConnell-modified Bloch Equations, has given estimates of 1--4ms for the life-time of the complex under various conditions of temperature and concentration (ranging from ~1 mM to ~200 μM in total protein).

Late Abstracts

MULTINUCLEAR AND MULTIDIMENSIONAL NMR STUDIES OF GRANULOCYTE COLONY-STIMULATING FACTOR

Alexander L. Breeze[#] and Iain D. Campbell*, *[#]ICI Pharmaceuticals, Protein Structure Group, Mereside, Alderley Park, Cheshire SK10 4TG, U.K. and *Department of Biochemistry, University of Oxford, Oxford OX1 3QU, U.K.*

Protein growth factors of the colony-stimulating factor class are responsible for regulating the proliferation and differentiation of haemopoietic stem cells of the bone marrow into mature white cells of the granulocyte or macrophage lineages, and hence play a central role in host defence. Despite their often complementary actions on their target cells, they are a structurally diverse group sharing little sequence similarity. Granulocyte colony-stimulating factor (G-CSF) is a 174-residue, 19kDa protein with a high predicted α-helical content. The small chemical shift dispersion and very high content of leucine residues (33) of this protein cause severe spectral overlap in many regions and present a significant challenge to current NMR methodologies. We have been studying G-CSF by means of a combination of [^{1}H-^{15}N], [^{1}H-^{13}C] and [^{1}H-^{15}N-^{13}C] multidimensional experiments using both uniformly and selectively heteronuclear-labelled samples. Some results of these studies will be presented here.

SOLUTION STRUCTURE BY 2D ^{1}H–NMR OF A CHIMERIC PEPTIDE RECOGNIZED BY GALANIN AND NPY RECEPTORS, Anders Ehrenberg, Klas Arvidsson, Tiit Land, Ülo Langel and Tamas Bartfai, Depts. of Biophysics and Neurochemistry & neurotoxicology, Arrhenius Laboratories, Stockholm University, S–106 91 Stockholm, Sweden.

The solution structure of the chimeric bireceptor recognizing 25 aa long peptide M32, galanin(1–13)–neuropeptide Y(25–36) amide, was examined in 30% (v/v) of 1,1,1,3,3,3–hexafluoro–2–propanol by ^{1}H–NMR spectroscopy and by CD. Proton resonance assignments were made and distance and angular constraints were deduced from NOESY cross peaks and J–couplings, respectively. Solution structures were calculated using distance geometry and refined by restrained energy minimization and molecular dynamics. The obtained structures contain an α–helical part in the NPY–portion of the peptide including residues 13–20 which in some structures continues to the C terminal Tyr25. Among the ten NMR structures with lowest energy there are some reminding of the horseshoe shape of aPP, a close relative of NPY, studied by X–ray crystallography. The more flexible N terminal portion of the peptide has a freedom to approach this C terminal α–helix, via a reverse turn or a nascent α–helix, which permits the N terminus with Trp2 to come close in space to the C terminus Tyr25. It appears that the strong α–helical character of the NPY(25–36)amide fragment of M32 helps to stabilize structural features in the galanin derived part of the peptide. The peptide is recognized by galanin receptors with unusually high affinity: IC(50%) = 0.1 nM. In addition the peptide appears to act as a galanin receptor antagonist. The chimeric peptide M32 is also recognized by NPY receptors with submicromolar affinity, IC(50%) = 0.25 μM. The structure and dynamics of an analog to M32, galanin(1–12)–Ala–NPY(25–36)amide has also been studied. This peptide is still a high affinity ligand to galanin receptors but not to NPY receptors. The availability of solution structures for these peptides, recognized differently by two peptide receptors, both members of the family of G–protein coupled receptors, may be useful in understanding peptide receptor–ligand interactions.

AUTOMATED AND INTERACTIVE TOOLS FOR ASSIGNING 3D AND 4D NMR DATA: APPLICATION TO INTERLEUKIN-4 AND hnRNP A1

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In order to assist in the analysis of 3D and 4D NMR spectra a suite of automatic and graphically based interactive tools are being developed. The program CAPP (Contour Approach to Peak Picking) automatically and efficiently (>75%) peak picks the 3D triple resonance experiments which are used in assigning protein backbone resonances. The X11 based graphics program PIPP (Primitive Interactive Peak Picker) provides several useful assignment tools which include: peak pick table editing, 3D symmetry locator, ^{15}N, NH strip manipulation tools, and 3D/4D NOESY assignment tools. The strip manipulation tools allow strips from ^{15}N edited experiments to be interactively reordered and compared. The 3D/4D NOESY assignment tools resolve degenerate assignments using 3D/4D symmetry and inter-proton distances obtained from different structural models. The program STAPP (Shift Table Assignment Peak Picker) automatically assigns 3D and 4D spectra making use of symmetry and structural models. STAPP is most useful in assigning 4D NOE experiments which typically have ≥1024 slices. Results from using these tools in analyzing Interleukin-4 and hnRNP A1 NMR data will be shown.

CONFORMATIONAL ANALYSIS OF THE SIDE-CHAIN OF THE VALINE RESIDUES IN RIBONUCLEASE T_1 BASED ON COUPLING CONSTANTS AND NOE'S

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New methods have been developed and existing methods have been applied for the precise measurement of χ_1 for the eight valine residues in $^{13}C/^{15}N$ labeled ribonuclease T_1. They allow to measure seven out of nine possible coupling constants about this dihedral angle: $^3J(H_\alpha,H_\beta)$ (1,2,3), $^3J(C',H_\beta)$ (3), $^3J(N,H_\beta)$ (4), $^3J(C',C_\gamma)$ (5), and $^3J(H_\alpha,C_\gamma)$ (4).

An analysis of these coupling constants together with the NOE's will be given in terms of a dynamic model of the valine sidechains. A comparison with the X-ray structure will be presented.

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STRUCTURE INFORMATION DERIVED FROM CHARGE INDUCED CHEMICAL SHIFT CHANGES.

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Calculations based on the ab initio / LORG method[1] of ^{13}C, ^{15}N and ^{17}O chemical shifts of the model peptides, acetamide and N-methyl acetamide, as a function of charge position (positive and negative) are analysed to investigate the geometrical dependence. The effects of the charge can be divided into contributions from the individual bonds involving the nucleus under observation, with a cosine angular- and a coulombic distance dependence.
By analysing all ^{13}C, ^{15}N and ^{17}O chemical shift changes, the position of charge can be estimated or reversely by knowing the position of charges the position of the nuclei can be found. Examples are given to show how such studies can be used to interprete titration data of e.g. proteins.
Furthermore, the analysis is extended to include the effects of dipoles with the potential to yield conformational information and molecular packing from solid state NMR data. Examples are given to illustrate the use of these principles in the characterization of the chemical shifts in general.

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INTERFERENCE BETWEEN J-COUPLINGS AND CROSS-RELAXATION IN SOLUTION NMR: CONSEQUENCES FOR MACROMOLECULAR STRUCTURE DETERMINATION

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In a NMR spin system in which normal and antiphase coherences have different rates of relaxation, as is usual in macromolecules in solution, the imaginary part of the eigenvalues of the evolution superoperator — which are the observed NMR frequencies — contain contributions from both the coherent interactions and the differential relaxation rates. For this reason, within a J-coupled system undergoing cross-relaxation, the J couplings cannot be obtained from the observed spectral frequencies alone: rather, the entire evolution matrix must be explicitly diagonalized. Furthermore, under these circumstances, individual components of J multiplets acquire a significant dispersive phase, which also affects the apparent splittings. Calculations on simple model spin systems, using internuclear distances, rotational correlation times and J-couplings in the range typically encountered in biological macromolecules, suggest that failure to consider this effect may cause errors of 25% or more in the determination of coupling constants, which would seriously compromise conclusions about structure and dynamics relying on the interpretation of J-couplings. Calculations on deoxyribose ring spin systems suggest that inclusion of this effect raises the estimated population of 2'-*endo* conformers to nearly 100% in solution NMR studies of DNA. Correction of published J-couplings for BPTI for cross-relation effects also significantly change and improve the fit to a parameterized Karplus equation.

A PRACTICAL APPROACH TO CALCULATIONS OF BIOMOLECULAR STRUCTURES FROM HOMONUCLEAR THREE-DIMENSIONAL NOE-NOE SPECTRA, T. A. Holak, J. Habazettl, R. Bernstein, A. Ross and C. Cieslar, *Max Planck Institute for Biochemistry, D-8033 Martinsried, Germany*

The NMR structure determination of proteins using structural information derived from homonuclear 3D NOE-NOE spectroscopy is described. The 3D NOE intensities are used directly in structure calculations without transforming them into distance constraints. A new two-dimensional potential function representing the 3D NOE-NOE intensity has been incorporated into the X-PLOR program and used in the simulated annealing protocol (*1*). The protocol relies also on the simulation of the 3D NOE-NOE spectra with the relaxation matrix method. A 3D NOE-NOE spectrum is calculated based on the available assignments and on a molecular model of the protein. The artificial spectrum is then compared with the experimental spectrum and so on, until all experimental connectivities match the connectivities of the simulated spectrum. The method is illustrated with the determination of the 3D structure of hisactophilin (*2*), an actin binding protein of 118 amino acids which acts as an intracellular pH-sensor.

(*1*) J. Habazettl, M. Schleicher, J. Otlewski, and T. A. Holak (1992) *J. Mol. Biol. 228*, 466-479.

(*2*) J. Habazettl, D. Gondol, R. Wiltscheck, J. Otlewski, M.Schleicher and T. A. Holak (1992) *Nature 359*, 855-858.

LOCAL STRUCTURE DUE TO AN AROMATIC-AMIDE INTERACTION OBSERVED BY ^{1}H-NMR IN PEPTIDES RELATED TO THE N-TERMINUS OF BOVINE PANCREATIC TRYPSIN INHIBITOR, Johan Kemmink and Thomas E. Creighton, European Molecular Biology Laboratory, Meyerhofstr. 1, D-6900 Heidelberg, Germany

A synthetic peptide corresponding to the 15 N-terminal residues of Bovine Pancreatic Trypsin Inhibitor, with serine replacing the two cysteine residues, has been characterized by ^{1}H-NMR. This peptide has a very disordered conformation that is essentially the same when it is part of the analogue of the (30-51) one-disulphide intermediate in folding. This confirms the conclusions of van Mierlo et al. (J. Mol. Biol., 1992, in press) that the (30-51) intermediate is partially folded, with the N-terminal segment disordered.

Local elements of non-random conformation were observed in this peptide. Especially prominent was an apparently electrostatic interaction between the face of the aromatic ring of Tyr 10 and the amide proton of Gly 12, which caused the latter to have a very anomalous chemical shift (δNH = 6.70 ppm). A similar interaction was observed in shorter peptides, especially in tetrapeptides with the sequences Tyr/Phe/Trp-X-Gly-Y. The local nature of this interaction indicates that it should be a general feature in peptides and in unfolded proteins with such a sequence. Other studies indicate that residue X should not be a proline. Sequences like X-Gly-Y-Tyr/Trp do not show anomalous shifts of the Gly amide proton.

^{1}H, ^{13}C and ^{15}N SEQUENTIAL BACKBONE ASSIGNMENTS AND SECONDARY STRUCTURE OF A CONSTRUCT OF CALBINDIN D28k.

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The vitamin D-dependent calcium binding protein calbindin D28 (MW 28kD) contains six «EF-hand» domains. By mutations in evolution, two of these putative EF-hands, domains II and VI, have lost their calcium binding ability[1]. In order to elucidate the unknown function and conformation of evolutionarily mutated domain II in Calbindin D28 a construct (I+II) consisting of domains I and II (Met-1 to Arg-93) of rat Calbindin D28 was generated and expressed at high yield in E.coli. As observed by CD and NMR spectroscopy, the engineered protein proves to reversibly fold into an ordered structure in aqueous solution. ^{15}N-edited 3D TOCSY and 3D NOESY spectra were recorded with a ^{15}N labeled sample, and 3D triple resonance HNCA, HNCO, HN(CO)CA, and HCACO constant time experiments and CBCANH and CBCA(CO)BH spectra[2] with a ^{13}C/^{15}N labeled sample. Cross peaks were correlated as follows to yield partial spin systems and sequential assignments: «Fragments» of the type C'_{i-1}-N_iH_i as obtained from the HNCO experiment were extended towards the N-terminus to C^{α}_{i-1} C'_{i-1}-N_iH_i by use of the HN(CO)CA and towards the C-terminus to $C^{\alpha}_{i-1}C'_{i-1}$-$N_iH_iC^{\alpha}_i$ by the use of the NHCA spectra. H^{α}'s were attached by the use of 3D-TOCSY and the HCACO spectra. Finally the fragments were extended to the C^{β}'s by use of the CBCANH and CBCA(CO)NH spectra. The resulting fragments could then be sequentially ordered on the basis of the combined use of the C^{β} and C^{α} or H^{α} and C^{α} chemical shifts. The NMR-derived preliminary secondary structure of construct I+II will be presented.

[1] M.D.Gross,R.Kumar,W.Hunziker (1988) J.Biol.Chem. 268, 14426-14432.

[2] S.Grzesiek,A.Bax (1992) J.Magn.Reson. 99, 201-207.

OBSERVATIONS OF THE C-TERMINUS OF THE SMALL SUBUNIT OF HERPES SIMPLEX VIRUS RIBONUCLEOTIDE REDUCTASE BY H1 NMR

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Herpes ribonucleotide reductase requires the association of its large and small subunits to be catalytically active. It is known that the C-terminus of the small subunit is involved in this critical association. Thus far there is no reported information from X-ray or NMR of either subunit. The H1 NMR spectrum of the small subunit (76 kDa) shows a minute number of relatively sharp resonance's which arise from the last 14 amino acids of its C-terminus. The assignments were based on comparisons of TOCSY and NOESY spectra of the protein with spectra of analogous peptides and a C-terminus truncated protein.

These results show that the C-terminus of the small subunit exhibits little conformational preference and is more mobile that the rest of the protein. Thus, the C-terminus is probably found at the outer surface of the protein which probably facilitates recognition of two subunits. It is possible that this mechanism of recognition may be general to other ribonucleotide reductases.

STRUCTURAL STUDIES ON THE RNA-BINDING DOMAIN OF THE hnRNP A1

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In the nucleus, RNA is almost always involved in a protein complex, but the function of many RNA-binding proteins is still unknown. hnRNPs are a class of proteins, designated A through U, that bind to RNA. Evidence suggests that they play a role in the metabolism and possibly the splicing of pre-mRNA. These proteins contain a 90 amino acid RNA-binding domain that usually encompasses two well-conserved sequences, called RNP1 and RNP2. The hnRNP A1 is a 320 amino acid protein that has been purified and shown to bind single-stranded nucleic acids. The 92 residue N-terminal fragment of A1 is known to contain both RNA binding sequences, and is able to form an RNA complex. This truncated protein is small enough to allow detailed structural studies in solution by NMR. The knowledge of the structure of hnRNPs will not only aid in developing an understanding of how RNA is processed, but will provide details on a molecular level of how proteins may interact with nucleic acids. We have used 2-and 3-dimensional NMR techniques to determine the ^{15}N, ^{13}C, and ^{1}H NMR assignments of the first 92 amino acids of the hnRNP A1. We will present the secondary structural assignments and the folding pattern of this protein. In addition, we have preliminary structural data of the 92-amino-acid protein in a complex with RNA.

STRUCTURAL AND BIOCHEMICAL STUDIES OF CHE-Y

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The CheY protein of *E. Coli* is essential for bacterial chemotaxis. The phosphorylation state of CheY plays an intimate role in transducing the net signal from membrane receptors into a change in the sense of rotation of the flagellar motors. We have biosynthetically incorporated ^{13}C and ^{15}N into CheY and nearly complete assignments were made using 3-D and 4-D NMR spectroscopy. Solution structure refinement on CheY is in progress. We are recording a time series of 15-minute 2-D HSMQC spectra on several different specifically ^{15}N-labeled CheY samples in order to locate the changes in protein structure when the protein is phosphorylated. In addition, analysis of mutant forms of CheY which appear to be locked in signalling state will be presented.

A COMPARATIVE NMR STUDY OF NEOCARZINOSTATIN BINDING WITH ITS CHROMOPHORE VS. ETHIDIUM BROMIDE.

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Neocarzinostatin (NCS) is a small acidic holo-protein isolated from the culture broth of Streptomyces Carzinostaticus. This protein anti-tumor drug has a protein component (apo-NCS) of M.W. 11,100 and a tightly bound chromophore of M.W. 659. The heat and light sensitive chromophore, which is stabilized by the apoprotein, is responsible for the biological activities of NCS such as DNA strand scission and for the cytotoxic properties of NCS against tumor and bacterial cells. Other streptomyces derived proteins such as Actinoxanthin and Auromomycin have 40-50% sequence homology to NCS and also consists of a protein component (apo-protein) and a tightly bound chromophore. Although NCS strongly binds a number of drugs including ethidium bromide and daunomycin, it does not bind the chromophore of either Auromomycin or Actinoxanthin, nor do these proteins bind to the chromophore of NCS.
To obtain a better understanding of the binding region and drug-protein interactions, NMR investigations of NCS with its chromophore and also with ethidium bromide have been carried out under similar conditions. Ethidium bromide is a stable drug and a known DNA intercalator. The results of this study especially the amino acid residues involved in the binding interactions of both drugs will be discussed.

DNA BINDING AND NMR STUDIES WITH A SINGLE-FINGER PEPTIDE FROM THE ERYTHROID FACTOR GATA-1.

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The erythroid transcription factor GATA-1 was the first identified member of a novel family of 'finger' motif DNA binding proteins, which now includes regulatory proteins expressed in other cell lineages (GATA-2,3,4). Chicken GATA-1 binds as a 35kD monomer to an asymetric DNA target sequence (A/T)GATA(A/G). The protein has two related but non-identical 'finger' elements of the form cys-x-x-cys-$(x)_{17}$-cys-x-x-cys. Unlike other such proteins, multiple fingers are not essential to binding; the binding properties of GATA-1 are only weakly affected by deletion or mutation of the amino-terminal finger. Our studies with several synthetic peptides have shown that a 66-residue peptide containing the carboxy-terminal finger domain binds tightly (1.2×10^8) and specifically to the GATA target sequence. The DNA binding activity requires stoichemtric quatities of $Zinc^{++}$, but other heavy metals can be substituted. The 66-amino-acid fragment has been over expressed in an E.coli expression system, purified to homogeneity and labeled with $^{15}N/^{13}C$ for NMR studies. We will report on the DNA binding studies of the peptide and several mutants as well as on the NMR studies of the labeled fragment both free and bound to the target DNA sequence.

TAUTOMERIC STATES OF THE HISTIDINES OF PHOSPHORYLATED AND UNPHOSPHORYLATED III^{GLC} FROM *E. COLI* USING 2D HETERONUCLEAR NMR TECHNIQUES, J. G. Pelton[1], D. A. Torchia[1], N. D. Meadow[2], & S. Roseman[2]. [1]Bone Research Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892. [2]Department of Biology and McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland 21218.

III^{Glc} is an 18.1 kDa signal-transducing phosphocarrier protein of the phosphoenolpyruvate:glycose phosphotransferase system (PTS) from *E. coli*. The ^1H, ^{15}N, and ^{13}C histidine ring NMR signals of both the phosphorylated and unphosphorylated forms of III^{Glc} have been assigned using two-dimensional ^1H-^{15}N and ^1H-^{13}C HMQC experiments, ^1H-^{15}N HMBC experiments, and a two-dimensional ^1H-^{13}C CCH-COSY experiment. The ^{15}N and ^{13}C chemical shifts were used to determine that His-75 exists predominantly in the $N^{\varepsilon 2}$-H tautomeric state in both III^{Glc} and P-III^{Glc}, and that His-90 exists primarily in the $N^{\delta 1}$-H state in the unphosphorylated protein. Upon phosphorylation of the $N^{\varepsilon 2}$ nitrogen of His-90, the $N^{\delta 1}$ nitrogen remains protonated, resulting in the formation of a charged phospho-His-90 moiety. Results of a pH titration revealed that the pK_a values for both His-75 and His-90 in III^{Glc} and His-75 in phospho-III^{Glc} are less than 5.0, and that the pK_a value for phospho-His-90 is greater than 10. Implications for proposed mechanisms of phosphoryl transfer are discussed.

THREE-DIMENSIONAL STRUCTURE OF THE COMPLEX BETWEEN ACYL-COENZYME A BINDING PROTEIN AND PALMITOYL-COENZYME A, Flemming. M. Poulsen Birhte B. Kragelund, Kim V. Andersen, Jens C. Madsen, Jens Knudsen[1], Carlsberg Laboratorium, Kemisk Afdeling, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark.

Multi-dimensional ^1H, ^{13}C, and ^{15}N nuclear magnetic resonance spectroscopy has been used to study the complex between palmitoyl-coenzyme A and acyl-coenzyme A binding protein. The ^1H and the ^{15}N spectra of the *holo*-protein have been almost completely assigned and so has most of the ^1H spectrum of the coenzyme A part of the protein-bound ligand. The palmitoyl part of the ligand has been uniformly labelled with ^{13}C and the nuclear magnetic resonance signals of the carbon atoms and their protons have been assigned at the two ends of the hydrocarbon chain. A total of 1251 distance restraints from nuclear Overhauser effects and 131 dihedral angle restraints from three-bond coupling constants provided the basis for the structure calculation. A comparison of 20 structures calculated from these data to the average structure showed that they could be aligned with an atomic root-mean-square deviation of 1.3 ± 0.3 Ångström for all C, N, O, P and S atoms in protein and ligand. The *apo*-protein has been shown to be a four helix protein[2] and this structure is maintained in the *holo*-protein. The four α-helices are A_c1 of residues 4 to 15, A_c2 from residue 20 to 36, A_c3 from 51 to 62, and A_c4 from 65 to 84. The binding site for the palmitoyl stretch between the N-terminal end of A_c2 where the carboxyl part binds, to the N-terminal of A_c3 where the ω-end of the palmitoyl part binds. The adenosine-3'-phosphate is bound near residues of each of the four helices in an arrangement where it can form salt bridges and/or hydrogen bonds to either backbone or side chain atoms of Ala9, Tyr28, Lys32, Lys54, and Tyr73.

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[2]Andersen, K. V. & Poulsen, F. M. *J. Mol. Biol.* **226,** 1131-1141.

PROTON DETECTED METHODS FOR THE MEASUREMENT OF $^3J(H,P)$ AND $^3J(C,P)$ COUPLING CONSTANTS IN OLIGONUCLEOTIDES H. Schwalbe†, W. Samstag†, J.W. Engels†, W. Bermel‡, C. Griesinger†, † Institut für Organische Chemie, Universität Frankfurt, Niederurseler Hang, D-W-6000 Frankfurt, FRG, ‡ Bruker Analytische Meßtechnik, Silberstreifen, D-W-7512 Rheinstetten, FRG

A new method for the measurement of $^3J(C,P)$ and $^3J(H,P)$ coupling constants in RNA and DNA molecules will be proposed. These coupling constants provide valuable information concerning backbone conformation of nucleotides. The method relies on recording a spectrum that contains the desired coupling J either in ω_1 or ω_2 and a second spectrum that is decoupled with respect to the desired coupling J. Comparison of the coupled spectrum with the decoupled spectrum that was folded previously with a dublet with a trial coupling J_{trial} shows a minimum in the deviation for $J_{trial} = J$. An evaluation on the precision of the method that needs to fit only one parameter in contrast to other methods (1,2,3) will be presented.

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(2) J.J. Titman, D. Neuhaus, and J. Keeler, J. Magn. Reson. **85,** 111 (1989)

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THE STRUCTURE OF HUMAN PARATHYROID HORMONE (1-34) AS INDUCED BY SOLVENTS AND MICELLES, Lou Anne Strickland, Richard P. Bozzato and K. Anne Kronis, Allelix Biopharmaceuticals Inc., 6850 Goreway Drive, Mississauga, Ontario, Canada, L4V 1P1

The structure of the N-terminal, 34 residue, fragment of human parathyroid hormone was determined in 40 % trifluoroethanol employing two-dimensional ^1H NMR. The proton chemical shifts were assigned from phase sensitive COSY, relayed COSY and NOESY spectra. Distance constraints, estimated from NOESY spectra, were used to create a set of structures by distance geometry (DGEOM) which were subsequently refined by restrained energy minimization and restrained molecular dynamics (CHARMm). The resulting structures contained two helices spanning residues 3 to 12 and residues 17 to 26. The NOE constraints for residues 13 to 16 did not provide a unique structural solution however they were not disordered. The structures prepared by DGEOM and CHARMm contained either irregular turns or bends or were fairly helical at residues 13 to 16. The secondary structure of human parathyroid hormone (1-34) was also assessed by circular dichroism in the presence of methanol, trifluoroethanol and dodecylphosphocholine micelles. Under all three conditions the peptide formed structures containing various amounts of helical content. The formation of helical secondary structure in the presence of micelles supported the proposal that the TFE-induced structure of human parathyroid (1-34) was not an artifact of its environment but was an indication of the conformation that the molecule adopts when in close proximity to the membrane surface and possibly when bound to the parathyroid receptor. These results will be discussed in light of the less ordered structure of human parthyroid hormone (1-34) previously reported which was determined in 10.7 % TFE, by NMR.

ENHANCING COHERENCE TRANSFER PATHWAYS USING ZERO FIELD NUCLEAR MAGNETIC RESONANCE

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Zero field nuclear magnetic resonance (ZF-NMR) is a way to enhance coherence transfer pathways normally not possible with conventional NMR techniques. Coherence transfer pathways rely on the J coupling which transfers phase information back and forth between nuclei. In the presence of strong magnetic fields, the chemical shift interaction is linearly dependent on the applied magnetic field. Therefore it is possible to have two nuclei which are strongly shifted from one another, such as a carbonyl and a methylene carbon in a protein. If the chemical shift is larger than the J coupling, the result is that the two nuclei are weakly coupled. Coherence transfer is weakly allowed among weakly coupled nuclei. Under conditions which eliminate all chemical shifts from the Hamiltonian while retaining the mutual spin-spin couplings, the coherences would be able to migrate through the entire system. In zero field there is no chemical shift or Zeeman terms, so the effect is that all homonuclei which were all chemically shifted from one another, and all heteronuclei which had different resonance frequencies are now strongly coupled. The result is that coherence transfer pathways will be maximized. Both theoretical and practical aspects of the experiment will be presented.

STRUCTURAL STUDIES OF A TRANSLATIONAL ACTIVATING PROTEIN.

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COM (control of MOM) protein is a translational activator from Bacteriophage Mu. This low molecular weight (62 aa) RNA binding protein requires zinc to fold into a "non-classical" zinc finger motif. A report on the purification, metal specificity, and proposed zinc coordination site will be presented. Structural information derived from two-dimensional nuclear magnetic resonance studies will also be discussed.