FRONTIERS OF NMR IN MOLECULAR BIOLOGY - IV

Organizers: Stephen W. Fesik, Thomas L. James and Gerhard Wagner

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Poster Sessions

Protein Structure

D2-001 **STRUCTURE OF SIGNALLING PROTEINS** Iain D. Campbell
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Certain structures are used repeatedly in signalling proteins[1]. Cytokines, for example, are found in a relatively small number of structural types, including the "four helix bundle" clas that contains numerous interleukins, colony stimulating factors and interferons. The extracellular parts of the various cytokine receptors also show the same kind of structural economy and are usually made up from various combinations of domains, such as the fibronectin type III (Fn3) module[2]. Modularity also extends to intracellular domains of signalling proteins; well known examples being SH2, SH3 and plextrin homology (PH) domains. There have been numerous recent structural studies of cytokines, as well as intra- and extracellular modules using both NMR and X-ray crystallography. Examples of recent NMR work, done in this laboratory, on the structure and ligand binding of some of these proteins and dissected structural units will be presented. These will include: some members of the four-helix bundle cytokine family - interleukin-2[3], granulocyte colony stimulating factor[4] and an intriguing homologue of interferon γ[5] found in the HIV matrix; the structure and assembly of extracellular modules of the kind that form cytokine receptors[6,7,8] and intracellular SH2[9,10] and SH3[11] domains. Major goals in this work are to define functional patches in protein-protein interactions[8] and to understand the assembly and control of modular proteins[6,7].

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D2-002 PROTEIN STRUCTURE AND DYNAMICS, Gerhard Wagner, Harvard Medical School, Boston.

The function of most proteins is governed by a particular way they interact with other proteins, nucleic acids membranes or other ligands. These interactions are determined by the conformation of particular surface regions. Both the 3D structure such surface patches and their mobility may be important for these recognition processes. Properties of protein surfaces can also be important as recognition sites for viruses, tumor-causing proteins, proteases or other degrading agents. Consequently, precise knowledge about surface properties is desirable for rational drug design.

The surface conformation is particularly important for cell surface glycoprotein receptors. Here, the solution structure of the glycosylated adhesion domain of the human T-cell glycoprotein receptor CD2 will be presented. Methods for assignment of the glycan in the glycoprotein, as well as the determination of the carbohydrate structure will be discussed. This will be related to structural data known from isolated carbohydrates or X-ray structures of glycoproteins where carbohydrate structures are defined. Aspects of the structure relevant for recognition of the counterreceptor or antibodies will be discussed. Other results on T-cell surface proteins will also be presented. The lecture will also focus on recent results on nucleic acid binding proteins and other interesting systems.

D2-003 PROTEIN SURFACE AND PROTEIN HYDRATION IN SOLUTION, Kurt Wüthrich, Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule-Hönggerberg, CH-8093 Zürich, Switzerland.

The area of structural biology, in particular atomic resolution studies of the three-dimensional structure of biological macromolecules and their intermolecular interactions, has never before had as central a role in biological and biomedical research and enjoyed as much popularity as today. Experimental approaches in this field are dominated by X-ray diffraction with single crystals and by NMR in solution, and the addition of NMR spectroscopy to the arsenal of techniques applied in structural biology has greatly added to the current excitement. The two methods provide intrinsically complementary data, and the introduction of the NMR method for protein structure determination enabled for the first time direct comparison at atomic resolution between corresponding protein structures in crystals and in solution. Such comparative studies promise to lead to the identification of specific areas where the two techniques can provide unique information. For globular proteins one finds on the one hand, close similarity between the molecular architectures in the different environments, and on the other hand significant differences near the surface in the protein–solvent interface. The contrasting situation of finding in the same molecule both nearly identical global architectures and characteristically different surface properties in single crystals and in solution can be demonstrated in a particularly direct fashion from studies of hydration. The hydration of peptides, proteins and DNA has by now been studied in numerous instances by ^1H–^1H nuclear Overhauser effects (NOE) between the resonances of the individually assigned protons of the biomacromolecules and the water protons. NOE measurements in the laboratory frame (NOESY) and the rotating frame of reference (ROESY) recorded without pre-saturation of the water signal reveal two different time scales for solvent–solute interactions: (i) The NOE interaction between the macromolecule and the water is characterized by NOESY cross-peaks which have the same sign as the diagonal peaks. This has so far been observed for water molecules located in the interior of proteins, bound in the minor groove of B-DNA duplexes, or located in the intermolecular interface of protein–DNA complexes. (ii) Surface hydration waters show NOESY cross-peaks with opposite sign relative to the diagonal peaks, which can be rationalized by the spectral density functions calculated for free translational diffusion of spheres of different sizes representing the macromolecule and the water, respectively. In today's presentation, recent results on hydration in solution will be reviewed in the context of other, independent observations on the surface structure of proteins and nucleic acids.

Protein Folding

D2-004 PROTEIN FOLDING PATHWAYS AS REVEALED BY AMIDE PROTON EXCHANGE MEASUREMENTS, Heinrich Roder[1,2], J. Michael Sauder[1,2], Sepideh Khorasanizadeh[1,3], Paul B. Laub[1,2], and Iain D. Peters[1,4], [1]Fox Chase Cancer Center, Philadelphia, PA 19111, Depts. of [2]Biochemistry & Biophysics, and [3]Chemistry, Univ. of Pennsylvania, Philadelphia, PA 19104. [4]Present address: Hawaii Biotechnology Group, Aiea, HI 96701.

NMR observation of amide proton exchange rates has become one of the principal approaches for the structural characterization of protein folding intermediates (reviewed in ref.1) and protein-ligand complexes (2). Both applications rely on the ability to measure the protection against solvent exchange for individual amide protons involved in stable intra- or intermolecular hydrogen bonds. Pulsed H-D exchange experiments performed on a quenched-flow apparatus make it possible to measure the time course of amide protection during protein folding. The method is illustrated by our work on ubiquitin, a 76-residue protein with simple topology (an α-helix packed against a 5-strand β-sheet surrounding a single hydrophobic core). Most amide probes involved in regular secondary structure and those bridging the helix-sheet interface were found to be protected in a highly concerted early (8 ms) folding event (3). Other amide protons, including several on the C-terminal strand of the β-sheet involved in H-bonds with the parallel N-terminal strand, are protected at a ~3-fold slower rate. After the initial folding phase, the two parallel β-strands appear to be misaligned, leaving the amides on the C-terminal strand without H-bond acceptors; the native H-bond pattern is formed in a subsequent rearrangement step. Hydrogen exchange measurements under native conditions showed that the slowly protected amide protons on the C-terminal strand exchange more rapidly than other H-bonded amide groups in the β-sheet, suggesting that equilibrium fluctuations of the folded state can lead to a similar structural defect. Complementary information on the formation of the hydrophobic core during folding was obtained by fluorescence measurements of the folding kinetics for a F45W mutant of ubiquitin (4). Functional, thermodynamic and solution structure analysis of this variant showed that replacement of the largely buried Phe 45 by a tryptophan results in a fully active ubiquitin with slightly reduced stability and limited structural perturbations. Kinetic folding studies on a series of mutants with aliphatic substitutions at position 26 on the inside face of the α-helix highlight the importance of helix-sheet packing interactions during the initial stages of folding.

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D2-005 ACCESSING THE ENERGETICS & DYNAMICS OF PROTEINS. A. J. Wand, Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA 61801

The dramatic increase in the power, flexibility and efficiency of modern multidimensional and multinuclear NMR spectroscopy has provided the structural biologist with a previously unavailable avenue to high resolution structural information in solution. As the determination of the solution structure becomes increasingly facile, interest is growing in the use of NMR spectroscopy to characterize the dynamics around equilibrium structures and the associated energetics of these processes. For example, though the structure of the final, compact complex is now known, little is known about how calmodulin initially recognizes the domain, how it collapses from an initial encounter complex to the final compact state, or the energetics of these events. Experiments using high pressure to populate metastable states of the complex and the use of hydrogen exchange to probe the manifold of states accessible to the peptide while bound to calcium saturated calmodulin will be described. The results provide insight into the energetics of collapse from the encounter complex to the final compact state. The relationship between perturbation of local stabilities in proteins and fast internal dynamics is also being explored by hydrogen exchange and 15N and 13C NMR relaxation techniques in the protein ubiquitin. 13C relaxation studies have utilized randomly fractionally labeled protein using low pass filtered relaxation experiments which will be described. Results of the localized destabilization brought about by small molecule denaturants will be presented. Finally, the structural features of a protein molten globule - apocytochrome b562 - will be discussed in terms of its observed thermodynamic behavior. Though of relatively modest precision, the determined structure appears to explain many of the classic features of the protein molten globule such as a small change in heat capacity upon unfolding and low cooperativity for unfolding. Hydrogen exchange and NMR relaxation studies indicate the presence of unusual dynamics in the protein which are consistent with a "frustrated" spin-glass like packing.

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Nucleic Acid Structure

D2-006 HIGH-RESOLUTION STRUCTURES OF DOUBLE-HELICAL NUCLEIC ACIDS, Thomas L. James, He Liu, Anwer Mujeeb, Uli Schmitz, Marco Tonelli, Nicolai Ulyanov, and Yate-Ching Yuan, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446.

Sequence-dependent structural variations in nucleic acids are important for protein recognition. There are indications that the three-dimensional arrangement of hydrogen-bonding sites, bases, and helix geometry may play an important role in protein recognition, with specific promoter sequences exhibiting 10^3-10^4 times stronger affinity for the transcription factors than random sequences which appear to interact with the proteins electrostatically. The short range interactions which influence measurable NMR parameters are quite sensitive to the local molecular environment. Crystal structures of protein/DNA complexes indicate that bound DNA can be modified significantly from canonical B-DNA, but it is not known whether the particular DNA sequence had some tendency for these structural deformations in the absence of protein. This lack of knowledge is chiefly due to the lack of high-resolution structures for DNA sequences which are specifically recognized by proteins. Within the past couple years, the quality of DNA structure determination via NMR has improved sufficiently to provide an accurate, high-precision structure of nearly any DNA double helix of length less than 15 base pairs if sufficient care and effort are expended. Although the number of well-determined structures is not high, we are able to draw a few tentative generalizations regarding the sequence-dependent structural features of DNA duplexes in solution. While nucleic acid duplexes do assume a "structure" in solution, internal inconsistencies in some of the NOE and scalar coupling data do suggest that there is some conformational flexibility. For conformationally flexible molecules in solution, NMR-derived distance and torsion angle restraints are time-averaged. The present challenge is to provide an adequate description of the conformational ensemble.

Nucleic Acid Complexes

D2-007 STRUCTURE AND DYNAMICS IN PROTEIN-DNA INTERACTION, Robert Kaptein, Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Specific DNA recognition by proteins lies at the heart of several cellular processes such as gene expression, replication and DNA repair. DNA-binding proteins often have a modular architecture. For instance, many eukaryotic transcription factors have separately folded domains for DNA-binding, transactivation and possibly ligand-binding. Because the DNA-binding domains are often relatively small, NMR spectroscopy has been quite successful in solving the structure of these domains. In particular for several sub-families of zinc-finger proteins the structural information came first from NMR and was later confirmed by X-ray crystallography. Some recent contributions from our group in this area will be discussed. We have been studying these classes of DNA-binding proteins such as prokaryotic repressors (lac and lexA repressors) and eukaryotic transcription factors including the nuclear hormone receptors (glucocorticoid and retinoic acid receptors) and POU-domain proteins. The use of stable isotope labeling has been extremely fruitful. Thus, in the case of the Arc and Mnt repressors ^{13}C and ^{15}N labeling has been used in conventional NOE editing, but also in the discrimination of intra- versus intersubunit NOE's in these dimeric proteins. The complex of lac repressor heapiece with an 11 bp lac operator was solved by a restrained Molecular Dynamics method using constraints from experimentally observed NOE's. The structure of the comlex and the various specific interactions observed are in full agreement with a large body of genetic and biochemical data. Changes in protein flexibility upon complex formation have been studied via ^{15}N relaxation measurements. When a full structural study of protein-DNA complex is not possible one can obtain useful information using docking procedures, when the structure of the protein is known. We have developed the program MONTY for this purpose, which uses a Monte Carlo sampling procedure. A simplified force field is used that represents H-bond and van der Waals interactions. In addition, biochemical data such as from footprinting, contact point analysis and mutagenesis can also be taken into account. The program will be illustrated with the lexA repressor, which interacts with a variety of SOS operators in E. coli.

D2-008 MOLECULAR BASIS FOR NUCLEIC ACID RECOGNITION BY ZINC FINGER PROTEINS. P. E. Wright[1], R. Brüschweiler, K. Clemens, M. Foster, J. Gottesfeld, W. Jahnke, X. Liao, D. Millar, D. Wuttke, [1]Department of Molecular Biology - MB2, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037.

The zinc finger is a key DNA binding motif found in a large number of eukaryotic transcription factors. *Xenopus* transcription factor IIIA is the prototypical zinc finger protein, containing nine Cys_2His_2 zinc fingers, which regulates 5S RNA gene transcription. In addition to binding to the internal control region of the 5S RNA gene, TFIIIA binds to the 5S RNA transcript. We have expressed a truncated form of TFIIIA containing only the three N-terminal zinc fingers (termed zf1-3) that binds DNA with full specificity and high affinity. The protein has been uniformly labeled with ^{13}C and ^{15}N for multidimensional NMR studies. Solution structures have been determined for free zf1-3 using conventional NOE-based methods, and ^{15}N relaxation measurements have been used to investigate the internal backbone dynamics and the overall molecular tumbling. Tumbling is found to be anisotropic with long correlation times, giving clear evidence for motional correlation between the zinc finger domains. Linker mutations that influence the molecular reorientation are deleterious to DNA binding. Progress with and methods for NMR studies of the structure of a complex of zf1-3 with a 15 base pair oligonucleotide containing the cognate binding site will be reviewed.

New NMR Methods

D2-009 FUNDAMENTALS OF AND NEW IDEAS IN NMR APPLIED TO ISOTOPICALLY LABELED RNA. Bennett T. Farmer II, Arthur Pardi*, and Luciano Mueller. Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton NJ 08543-4000 USA; *Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-0215 USA.

A variety of $^{13}C/^{15}N$ and $^{13}C/^{31}P$ triple-resonance correlation experiments have been developed to facilitate both the intra-residue sugar-to-base correlation [*1-3*] and the sequential sugar-to-sugar correlation [*4*] in $^{13}C/^{15}N$ isotopically labeled RNAs, respectively. We have previously noted, however, that the simplest and most sensitive sugar-to-base correlation experiment, $H_sC_sN_b$ [2], did not work well for one purine base in the CUUG duplex [5] and failed out-right on a 47bp RNA hammerhead ribozyme. The basis for this failure is examined; and improved NMR experiments are presented. In addition, the heteronuclear and homonuclear coupling network within the nitrogenous base and between the base and ribose sugar is described in detail. Finally, we discuss both novel ^{15}N-edited NOESY experiments, which may yield additional ^1H-^1H distance constraints in RNAs, and the measurement of rapid amino and imino H_N exchange rates in RNAs using pulsed field gradients, as previously described for proteins [6].

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D2-010 NEW EXPERIMENTS DIRECTED TO THE ASSIGNMENT AND MEASUREMENT OF COUPLING CONSTANTS IN PROTEINS AND OLIGONUCLEOTIDES

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Assignment oriented methods for $^{13}C/^{15}N$ labeled oligonucleotides (1) will be presented that correlate the sugar moieties via the ^{31}P resonances of the backbone of RNA in HCP and HCP-CCH-TOCSY (2) experiments. In addition, correlations of the sugar resonances with those of the nucleobases will be shown.

Methods for the determination of coupling constants of the sugar ring conformation based on HCCH-E.COSY methods (3,4) and the determination of phosphodiester backbone conformation based on the P-FIDS-HSQC (4,5) will be presented on oligonucleotides. Approaches for the reduction of spectral complexity will be discussed for the severely overlapping spectra of oligoribonucleotides.

The FIDS-HSQC and a method based on the evolution of double and zero quantum coherence will also be applied to the determination of heteronuclear coupling constants in proteins such as $^{3}J(H^{N},C')$ couplings. The method will be demonstrated on the protein rhodniin.

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D2-011 Pulsed Field Gradient Triple Resonance Experiments for the Study of the Structure and Dynamics of Proteins, T. Yamazaki[1], S. M. Pascal[1,2], D. R. Muhandiram[1], W. Lee[3], C. H. Arrowsmith[3], N. A. Farrow[1], O. Zhang[1,2], J. D. Forman-Kay[2] and L. E. Kay[1], [1]Departments of Medical Genetics, Biochemistry and Chemistry, Medical Sciences Bldg., University of Toronto, Toronto, Ontario, M5S 1A8, [2]Biochemistry Research Division, Hospital for Sick Children, 555 University Ave, Toronto, Ontario M5G 1X8 and [3]Division of Molecular and Structural Biology, OCI, 500 Sherbourne St. Toronto, Ontario, M4X 1K9.

The past several years have witnessed a significant growth in the use of NMR to study aspects of protein structure and function. Multi-dimensional, multi-nuclear methods exist for the facile assignment of $^{15}N,^{13}C$ labeled proteins with molecular weights on the order of 25 kDa or less. A suite of pulse schemes is presented for the assignment of arginine guanidino ^{15}N and ^{1}H chemical shifts based on magnetization transfer employing scalar connectivities exclusively. The unambiguous assignment of these resonances is of considerable importance in the study of many complexes, such as protein-DNA complexes or complexes of Src homology 2 (SH2) domains bound to phosphotyrosine peptides where the arginine sidechains participate in stabilizing interactions between the protein and the ligand. The methods are demonstrated on a complex of the C-terminal SH2 domain from phospholipase-Cγ1 bound to a 12 residue phosphotyrosine containing peptide comprising the high affinity binding site of the platelet derived growth factor receptor.

Recently it has been demonstrated that fractional substitution of deuterons for protons bound to ^{13}C nuclei can increase the sensitivity and/or resolution of spectra that record carbon chemical shifts or in which carbon spins are involved in the relay of magnetization in a complex pulse sequence. A suite of triple resonance experiments for the assignment of $^{15}N,HN,^{13}C^{\alpha}$ and $^{13}C^{\beta}$ spins in 100% $^{15}N,^{13}C$, fractionally ^{2}H labeled proteins will presented. The methods are applied to a 37 kDa ternary complex of a $^{15}N,^{13}C,$~70% ^{2}H labeled sample of trp-repressor, fully protonated 5 methyl-tryptophan and a fully protonated 20 basepair trp-operator DNA fragment.

Membrane-Associated Proteins

D2-012 NMR STUDIES OF STRUCTURAL AND MEMBRANE PROTEINS, Stanley J. Opella, Chemistry Department, University of Pennsylvania, Philadelphia, PA 19104.

Recent progress in the development of methods for determining the structures and describing the dynamics of structural and membrane proteins will be described. A combination of multidimensional solution and solid-state NMR methods are being applied to these important, but difficult to study, classes of proteins. The experiments rely on the ^{1}H chemical shift, ^{1}H-^{15}N dipolar, and ^{15}N chemical shift interactions at ^{15}N labeled sites in peptides and proteins. Peptides and proteins associated with membranes are studied in micelles with solution NMR methods and in oriented and unoriented bilayers with solid-state NMR methods. The major emphasis in solid-state NMR studies of both membrane and structural proteins is to take advantage of the properties of oriented molecular systems.

D2-013 APPLICATIONS OF REDOR NMR SPECTROSCOPY, Lynda M. McDowell, Christopher A. Klug, Daniel R. Studelska, Kenzabu Tasaki, Denise D. Beusen, Robert A. McKay, and Jacob Schaefer, Department of Chemistry, Washington University, St. Louis, MO 63130.
We are performing new solid-state NMR experiments to determine the structure and dynamics of protein complexes which are unsuited for diffraction studies and too big for solution-state NMR. The versatile rotational-echo, double-resonance (REDOR) and trasferred-echo, double-resonance (TEDOR) analytical methods that we have developed in the last five years for the accurate determination of internuclear distances (and hence geometry and structure), and new combined TEDOR-REDOR and TEDOR-TEDOR experiments, are heteronuclear NMR techniques that are directly applicable to the characterization of protein complexes. These complexes can be examined either as polycrystalline materials in contact with mother liquor, or as freeze-quenched lyophilized solids embedded in cryoprotectant buffer glasses. About 1 μmole of complex is needed with our current instrumentation. The heteronuclear techniques are supplemented by controlled-excitation for dephasing rotational amplitudes (CEDRA) and XY8-dipolar restoration at the magic angle (XY8-DRAMA), new REDOR-like homonuclear dephasing experiments which we have introduced recently for accurate distance measurements. These pulse sequences are used to select two or three of a cluster of stable-isotope labels to generate multiple, long-range distance measurements of binding-site geometry. We report results of REDOR/TEDOR/CEDRA/DRAMA solid-state experiments and computer modeling studies to characterize: (1) domain closure of enolpyruvylshikimate-3-phosphate synthase on substrate binding; (2) the indole-tunnel reorganization of tryptophan synthase on serine binding; (3) the hinge motion of glutamine binding protein on substrate binding; and (4) the geometry of peptide drugs bound to thermolysin.

Pharmaceutical Applications

D2-014 PROTEIN STRUCTURES OF POTENTIAL DRUG TARGETS. Stephen Fesik, Edward Olejniczak, Andrew Petros, Liping Yu, David Nettesheim, Robert Meadows, Ho Sup Yoon, Philip Hajduk, Heng Liang, Ming-Ming Zhou, Andrew Hansen, and John Harlan. Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064.

Due to the development of heteronuclear multi-dimensional NMR methods, the availability of isotopically labeled proteins, and computational tools for analyzing NMR data, three-dimensional structures of biomacromolecules and molecular complexes can be rapidly determined. The structural information gained from the NMR experiments could aid in the design of molecules that bind and block the function of proteins that are important for critical cell processes. These proteins may be involved in signal transduction, binding to DNA, or carrying out key enzymatic reactions. In this presentation, the solution structures of proteins and protein/ligand complexes will be described. Each of these proteins are potential drug targets and of pharmaceutical interest.

D2-015 THE THREE-DIMENSIONAL STRUCTURE OF THE INHIBITED CATALYTIC DOMAIN OF STROMELYSIN-1. Paul R. Gooley, John F. O'Connell, Bruce A. Johnson, Alice I. Marcy, Scott P. Salowe and Greg C. Cuca. Deptartment of Biophysical Chemistry, Merck Research Laboratories, Rahway, N.J. 07065.

Stromelysin-1 is a member of the matrix metalloendoprotease (MMP) family which includes the collagenases and gelatinases. These enzymes collectively degrade the extracellular matrix and basement membrane and have been implicated in a variety of diseases such as arthritis, corneal ulceration, periodontal disease and cancer metastasis. Using multidimensional heteronuclear NMR techniques we have determined the structure of the 19 KDa catalytic domain of $^{13}C,^{15}N$ labelled stromelysin-1 inhibited with several unlabelled small peptide-like inhibitors. Isotope filtered experiments were used to assign the resonances of the inhibitor and to assign NOEs between the protein and the inhibitor. Over 1800 NOE constraints have been derived with approximately 40% long range and intermolecular. The structures were calculated with DIANA and refined with FANTOM. The protein is globular in shape with a five-stranded β-sheet, three helices and two zincs: where one zinc is catalytically important and the other structural. Inspection of the family of structures shows that the inhibitors bind in an extended manner running antiparallel to the outer strand of the β-sheet and parallel to a non-regular loop region. The primary specificity subsite S1' is deep and capable of accommodating large hydrophobic groups. Comparison of the bound conformation of the inhibitors used in this study shows that several residues of the S1' subsite can move to fit different P1' groups suggesting that this subsite is not only large but flexible. These similarities and differences form the molecular basis for further inhibitor design.

D2-016 STRUCTURES OF THE HIV-1 NUCLEOCAPSID AND MATRIX PROTEINS: IMPLICATIONS FOR THE TREATMENT OF AIDS,

Michael F. Summers[1], Wesley I. Sundquist, III[2], Michael M. Massiah[1], Chiana M. Paschall[1], Mary R. Starich[1], Allyson M. Christensen[2], Terri L. South[1], Brian Lee[1], and Paul R. Blake[1], [1]Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, Maryland 21228, and [2]Department of Biochemistry, University of Utah, Salt Lake City, Utah 84132.

Three dimensional structures of the HIV-1 nucleocapsid (NC) and matrix (MA) proteins have been determined by NMR methods. The nucleocapsid protein contains two copies of a conserved CCHC zinc finger sequence (CCHC = Cys-X_2-Cys-X_4-His-X_4-Cys; X = variable amino acid) that bind zinc, forming what may be the smallest known mini-globular protein domains. A total of five NH--S and six NH--O hydrogen bonds are present in the N-terminal CCHC zinc finger, and the covalent nature of the NH--S hydrogen bonds (determined from proton-metal scalar interactions in a rubredoxin with similar metal binding structure) may serve as major determinants of stability in zinc fingers. A synthetic peptide with sequence of the N-terminal HIV-1 NC zinc finger, Zn(HIV1-F1), exhibits zinc-, sequence- and conformation-dependent binding to nucleic acids. Ejection of zinc by novel antiviral agents leads to loss of nucleotide binding, providing insights into the mechanism of action of these potential chemotherapeutic agents. The structure of recombinant HIV-1 matrix protein was determined using more than 600 NOE-derived distance restraints derived from ^{15}N-double resonance and $^{15}N,^{13}C$-triple resonance NMR data. The protein is composed of five α-helices, a short 3_{10} helical stretch, and a three-stranded mixed β-sheet. Helices I-III and the 3_{10} helix pack about a central helix (IV) to form a compact globular domain that is capped by the β-sheet. The C-terminal helix (V) projects away from the β-sheet to expose carboxyl-terminal residues essential for early steps in the HIV-1 infectious cycle. Basic residues implicated in membrane binding and nuclear localization functions cluster about an extruded cationic loop that connects β-strands 1 and 2. The structure suggests that both membrane binding and nuclear localization may be mediated by complex tertiary structures rather than simple linear determinants.

D2-017 STRUCTURE, DYNAMICS AND INTERACTIONS OF HIV-1 PROTEASE-INHIBITOR COMPLEXES,

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Although numerous crystal structures of HIV-1 protease/inhibitor complexes have been solved, relatively little detailed information about protease/inhibitor structure, dynamics and interactions is available in solution. Recently, we have obtained signal assignments of the protease complexed with two strong C2 symmetric inhibitors (a) DMP323, a novel, highly specific, cyclic urea based inhibitor and (b) P9941 a linear diol based inhibitor. I will discuss NMR experiments that elucidate interactions between water molecules and the protease/inhibitor complexes, and then present an analysis of 15N relaxation measurements that identifies regions of the inhibited protease molecules that undergo significant internal motions. I will also report the pH dependence of chemical shifts of all Asp and Glu terminal methylene and carboxyl carbons, obtained from proton detected 3D spectra. The pK_a values of the active site Asp residues have been derived from this data, from H-D isotope shifts, and from information about the environments of the carboxyl groups in the crystal structure. Finally, I will discuss the 3D solution structure of the protease/DMP323 complex derived from NOE and J-coupling restraints.

Carbohydrates and Peptides

D2-018 THE USE OF COUPLING CONSTANTS IN STRUCTURE REFINEMENT OF PEPTIDES AND PROTEINS

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A lack of sufficient NOE data in peptides and proteins may result from different sources: small size (peptides) or location in areas with few neighbours (surface); internal dynamics or saturation transfer from exchange with water. Hence, more experimental data are required to get reliable information about structure and dynamics. Examples will be shown in which the inclusion of J coupling yields unequivocal answers in those cases.

Techniques to obtain homo- and heteronuclear J coupling constants in small peptides as well as in large proteins (30 kDa) will be presented. For cases where exchange with water occurs techniques measuring exchange rates or suppressing exchange will be presented. Several different procedures will be discussed to achieve a flip-back of the water resonance before acquisition. These include the design of experiments with gradients in a proper way as well as the use of radiation damping to orient water back to the z-axis. Application to triple resonance experiments of the II^A domaine of the mannose transporter (31 kDa) demonstrate that a significantly improved signal-to-noise ratio is achieved for signals where exchange is involved.

The use of J couplings in Distance Geometry, Distance Driven Dynamics and Molecular Dynamics calculations will be shown in addition with time dependent distance restraints.

D2-019 STRUCTURES OF CARBOHYDRATES ON THE MEMBRANE SURFACE, Kathleen P. Howard, Brian A. Salvatore, and James H. Prestegard, Chemistry Department, Yale University, New Haven, CT06520-8107.

Carbohydrates serve as receptors for a variety of physiologically and pathologically important agents that invade cells or alter cell function. In most cases where carbohydrates serve in this capacity they are anchored to cell membranes as parts of glycolipids or glycoproteins. The conformational properties of the carbohydrate portions of these molecules are of obvious importance, and have been the target of solution NMR studies for many years. Questions remain, however, as to the degree to which conformations allowed in solution are represented when molecules are anchored to membrane-like surfaces, or bound to protein as a part of a protein-receptor complex. We are able to answer some of these questions for simple glycolipids attached to field orientatble arrays of membrane-like fragments. The answers come from NMR measurements of dipole-dipole splitings and anisotropic chemical shift offsets that fail to average to zero in oriented media. Examples will be presented using synthetically and biosynthetically prepared ^{13}C and ^{15}N labeled glycolipids that show selection of certain conformations in membrane anchored environments. Possibilities of extending studies to complexes with proteins that use these molecules as receptors will be discussed.

D2-020 NMR STUDIES OF THE STRUCTURE AND DYNAMICS OF CARBOHYDRATES, Herman van Halbeek, Complex Carbohydrate Research Center and Department of Biochemistry, University of Georgia, Athens, GA 30602-4712

A detailed NMR study of the conformation of an oligosaccharide in aqueous solution typically yields a number of constraints that are not all compatible with the existence of a single rigid structure, implying that the oligosaccharide is, to a greater or lesser extent, dynamic with respect to torsional vibrations around each glycosidic bond. The past few years have witnessed a vast increase in the number of efforts aimed at the measurement of NMR parameters directly related to the flexibility of carbohydrates, including ^{1}H and ^{13}C relaxation rates, homo- and heteronuclear cross-relaxation rates, and global and local correlation times. Notable recent developments in NMR methods used to study carbohydrate structure include the application of tailored homo- and heteronuclear (multi-)selective two- and three-dimensional techniques, improved measurements of long-range heteronuclear scalar couplings, and the observation of hydroxyl proton signals used for the detection of intramolecular hydrogen bonds in aqueous solution. In addition, technological advances in ^{13}C isotopic labeling of carbohydrates are creating new ways of measuring NMR conformational parameters. We will review the application of the aforementioned NMR methods to the investigation of the structures of oligosaccharides free in solution, carbohydrates in complexes with proteins (antibodies, lectins), and the orientation and restrained dynamics of carbohydrates covalently attached to model membranes [1-3].

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Late Breaking Developments

D2-021 STRUCTURES AND DYNAMICS OF CYTOSKELETAL PROTEINS AND THE Fv FRAGMENT OF AN ANTIBODY, Tad A. Holak, Christian Freund, Andreas Plückthun, Michael Schleicher, Arndt Schnuchel, Alfred Ross, Ronald Wiltscheck, Max-Planck-Institute for Biochemistry, D-82152 Martinsried, F.R.G.

We describe here structures of two F-actin binding proteins and compare two approaches used for their structure determination. In the first approach, the structure of **hisactophilin**, an actin binding protein of 118 amino acids, was derived from the homonuclear 3D NOE-NOE spectroscopy [1]. 2500 3D NOE intensities that could be extracted from a single **3D NOE-NOE** spectrum of the protein in water were used directly in structure calculations without transforming them into distance constraints. The most unusual feature of hisactophilin is the presence of 31 histidines which are responsible for intracellular pH-sensoring properties of hisactophilin. The second protein studied was severin. The NMR structure determination of domain 2 of **severin** (111 residues) was carried out with the heteronuclear ^{1}H-^{15}N-^{13}C double and triple-resonance spectra. Together with biochemical experiments, the structures of both proteins explain how they interact with actin.

F_v fragments are the smallest functional antibody units. The structural and dynamic properties of the F_v fragment and corresponding single-chain F_v fragment (scF$_V$: V$_H$-linker-V$_L$; **252 amino acids**) of the phosphoryl-choline-binding antibody McPC603 in the presence of hapten have been studied by heteronuclear multidimensional NMR spectroscopy [2]. Both the ^{15}N TOCSY-HMQC and triple resonance experiments (HNCA, HN(CA)H, with ^{15}N-^{13}C labeled protein) gave poor spectra, due to short T_2 relaxation times for most of the backbone ^{1}H, ^{15}N, and $^{13}C^{\alpha}$ atoms. The assignment procedure therefore relied upon the combination of amino acid and **domain** (V$_L$-) specifically labeled spectra and the 3D NOESY-HMQC spectrum of the uniformly ^{15}N labeled F_v and scF$_V$ fragments. Strategies for the preparation of the ^{15}N-V$_L$ domain labeled F_v fragment will be discussed. Approximately 80% of the ^{15}N and ^{1}H backbone and 60% of the ^{1}H side-chain resonances have been assigned. Short and long range NOEs were used to determine the extent of β-sheet structure. The ^{1}H-^{15}N NOE data indicate that the scF$_v$ backbone has a well-defined structure of limited conformational flexibility. However, the linker of the scF$_V$ fragment exhibits substantial fast internal motion compared with the overall rotational correlation time of the whole molecule which is of the order of 13 ns.

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D2-022 PEPTIDE BINDING TO THE SH3 DOMAINS OF GRB2. Michael Wittekind, Claudio Mapelli, Bennett T. Farmer II, Valentina Goldfarb, Ving Lee, Barbara Leiting, Kelly Pryor, Robert Bruccoleri, Chester A. Meyers, Luciano Mueller. Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-4000.

The triple-domain Grb2 protein, comprised of a single SH2 domain flanked by two SH3 domains, has been shown to mediate signal-transduction between activated cell surface receptors and the RAS oncogenic protein. The SH2 domain binds to the tyrosine-phosphorylated intracellular domains of activated tyrosine kinase receptors whereas the two SH3 domains bind to proline-rich polypeptide sequences in the C-terminal domain of the SOS protein, a RAS-activating guanine-nucleotide releasing factor. Grb2 therefore appears to acts as an "adaptor" protein that, when cells are stimulated via the cell-surface receptor, recruits the SOS protein to the intracellular cell surface leading to activation of RAS [1,2,3].

We have used multi-dimensional NMR spectroscopy and isothermal titration calorimetry (ITC) to study the binding of synthetic peptides derived from the C-terminal region of murine SOS proteins, and of a number of analogs, to the two isolated SH3 domains of murine Grb2 [4,5]. Half-reverse filtered NOESY experiments allowed the identification of the intermolecular interactions between the [^{13}C-^{15}N] labeled N-terminal Grb2 SH3 (N-SH3) domain and unlabeled peptide ligands. In other isotope-edited experiments, [^{13}C-^{15}N] labeled or perdueterated amino-acid residues were incorporated into peptides in order to obtain unambiguous assignments. The conserved aromatic residues of the N-SH3 domain (Tyr_7, Phe_9, Trp_{36}, and Tyr_{52}) define two hydrophobic ligand-binding sub-sites with an adjacent negatively-charged sub-site binding to essential basic residues at the C-termini of the SOS peptide ligands. The orientation of the peptide backbone of these peptide ligands in the binding-site of the N-SH3 domain is opposite to those reported for the complex of a combinatorial-library derived peptide with the P13K SH3 domain[6] and the 3BP2 peptide:Fyn SH3 and 3BP1 peptide:Abl SH3 complexes[7]. Details of the structures of the SH3:peptide complexes will be presented.

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Spectral Analysis and Structure Determination

D2-023 AUTOMATED ANALYSIS OF RESONANCE ASSIGNMENTS FOR PROTEINS USING TRIPLE RESONANCE NMR AND METHODS FROM ARTIFICIAL INTELLIGENCE, Gaetano T. Montelione[a], Diane Zimmerman[a,b], Casimir Kulikowski[b], Carlos Rios[a], Mitsuru Tashiro[a], Wenqing Feng[a], Chen-ya Chien[a], Zhigang Shang[a], and Clelia Biamonti[a].
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We have developed an automated strategy for determining sequence-specific NMR assignments in small proteins[1-3]. In our approach, ^1H-^1H and ^{13}C-^1H planes from a set of 3D NMR experiments are analyzed to provide both spin system identification and sequential connectivity information. First, PFG ^{15}N-HSQC, HNCO, DEPT-DEPT, and H-C-CA-N-H TOCSY[4] experiments are used to connect together the resonances of each amino acid spin system and to provide unique identification of many spin system types. Next, sequential connections between amino acids are established using multidimensional PFG H-C-CO-N-H TOCSY[1]. The PFG H-C-CA-N-H 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 PFG H-C-CO-N-H TOCSY spectra provide sequential connections between aliphatic α, β, γ, δ, and ϵ proton and carbon resonances of residue i and the amide and nitrogen resonances of residue i+1. Variant versions of the H-C-CO-N-H TOCSY experiment also allow unique identification of amino acid spin systems with aromatic, amide, or carboxyl sidechain functional groups. Together, these experiments provide sufficient constraints to assign most proton, carbon, and nitrogen resonances of small proteins. Constraint propagation methods from artificial intelligence[3] progressively narrow the set of possible assignments of amino acid spin systems to sequence-specific positions in the process of NMR data analysis. The constraint satisfaction paradigm provides a framework in which the necessary constraint-based reasoning can be expressed, while an object-oriented representation structures and facilitates the extensive list processing and indexing involved in matching. A prototype expert system, AUTOASSIGN, uses a "best-first" algorithm to generate unique, error-free, and nearly complete resonance assignments with real and simulated 3D NMR data. The remaining spin systems are then assigned to sequence-specific positions using a "branch-and-bound" search algorithm. The combined experiments have been used to obtain sequence-specific ^1H, ^{15}N, and ^{13}C resonance assignments for an IgG-binding domain of Protein A[2] (Z-domain, 8 kDa) derived from *S. aureus* and for the major RNA-binding cold-shock protein[5] (CspA, 8 kDa) from *E. coli*.

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D2-024 STRUCTURE REFINEMENT FROM NMR DATA, Michael Nilges, Séan I. O'Donoghue and Karina Abagian, European Molecular Biology Laboratory, Meyerhofstr. 1, D-69117 Heidelberg, Federal Republic of Germany.

Distance geometry algorithms usually require that the distance restraints extracted from NOE experiments are unambiguously assigned to pairs of atoms. Recently, we have shown that the methodology can be extended to ambiguous distance data, using simulated annealing [1,2]. The method is equally suited for dealing with ambiguous NOEs in symmetric multimers, ambiguities due to overlap, and unassigned disulfide or hydrogen bond connectivities. In the first part of the present paper, experiences with several experimental data sets will be described. The emphasis will thereby be on ways of extending the radius of convergence of the method. Ambiguities in symmetric multimers are more difficult to deal with than ambiguities in NOE data due to overlap. However, we were able to apply the method successfully to the calculation of several symmetric multimers.

The method is a step towards automating the interpretation of the NOE spectra, once the chemical shifts are assigned. Complete automation gives rise to additional problems. Potential difficulties are for example incomplete or erroneous assignments of the chemical shifts. Another source of error is the automatic peak picking, which may yield wrong peak positions for some NOE peaks, and include artefacts as restraints. In the second part of the paper, possible ways of adressing these problems during a structure calculation will be discussed.

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D2-025 COMPUTER AIDED ANALYSIS OF NMR DATA FOR STUDIES OF PROTEIN STRUCTURE, FUNCTION, FOLDING AND DYNAMICS. Flemming M. Poulsen and Mogens Kjær. Carlsberg Laboratory, Department of Chemistry, Gamle Carlsberg vej 10, DK-2500 Valby, Copenhagen, Denmark.

The NMR software package PRONTO[1] has been developed over the last 10 years and extended as the demands for a comprehensive and easy to use computer program have been growing. The latest developments of the computer program will be presented and discussed. These will include new examples of the application of the program performing spin system and NOE assignment of heteronuclear multidimensional NMR data of folded, partly folded and unfolded proteins; the application to studies of protein-ligand interactions; the application to studies of heteronuclear relaxation data as well as amide hydrogen exchange kinetics. A recently developed and highly integrated molecular graphics facility for displaying the molecular structures in the NMR investigation along with the spectra will also be presented.

The program has schemes for automated and semi-automated resonance assignments and NOE assignments, however, the basic presumption in PRONTO is that the expertise of the user will always be crucial for a correct analysis of complex NMR data. PRONTO is therefore made to ensure that the analyst always has easy access to all data and all assignment decisions made throughout the course of analysis.

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Protein Structure

D2-100 SOLUTION STRUCTURE OF THE TETRAMERIC MINIMUM TRANSFORMING DOMAIN OF P53, Cheryl H. Arrowsmith[1], Weon Tae Lee[1], Timothy S. Harvey[1], Ya Yin[1], Paul Morin[1], and David Litchfield[2], [1] Division of Molecular and Structural Biology, Ontario Cancer Institute and Depart-ment of Medical Biophysics, University of Toronto, 500 Sherbourne St., Toronto, Ontario M5X 1K9 and [2] Manitoba Institute of Cell Biology, 100 Olivia Street Winnipeg, Manitoba R3E 0V9

Mutations in the gene of the tumor suppressor, p53, are found in over half of all human cancers. The oligomerization of p53 is the origin of the transdominant phenotype of mutant p53. Residues 302-360 of murine p53 have been termed the "minimum transforming domain" because they can transform as efficiently as full length mutant p53 in the *ras* cotransformation assay. The efficiency of transformation was coupled with the ability to oligomerize with WT p53 (Shaulian et al. 1992, *Mol. Cell. Biol.* **12**, 5581). We have determined the solution structure of residues 303-366 of human p53 (p53tet) which form a 28 kDa symmetric tetramer in solution. This domain contains a number of important functions associated with p53 activity including oligomerization, nuclear localization and a phosphorylation site for $p34^{cdc2}$ kinase. We show that residues 325-355 form a highly stable dimer-of-dimers. Each dimer consists of an antiparallel β-sheet packed against a pair of antiparallel α-helices. The two dimers pack together in a symmetric manner with parallel packing of the helices across the dimer-dimer interface, in contrast to a previously reported structure (Clore et al, 1994, *Science* **265** 389). Residues 303-324 and 356-366 are largely unstructured in solution including the nuclear localization signal and phosphorylation site. Phosphorylation of Ser315 has only minor effects on adjacent residues and does not effect the globular tetramerization domain. The procedure for identifying intersubunit NOEs in a symmetric tetramer will be presented along with the method of calculation used. Results of characterization by NMR of the stability of the tetramer and rates of subunit exchange are also described. Modelling based on the p53tet structure suggests possible modes of interaction between adjacent domains in full-length p53 as well as modes of interaction with DNA.

D2-101 Multidimensional ^{13}C, ^{15}N heteronuclear NMR spectroscopy of the h-Interleukin 6.

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Cells can interact in immune, hemopoietic and neuronal systems through soluble mediators called cytokines or interleukins. Interleukin 6, which belongs to this family of proteins, plays a role in inflammation, viral infection, autoimmunity, and cancer. Receptor studies have shown that many cytokine receptors consist of two polypeptide chains, a ligand binding receptor, and a non binding signal trasducer. In order to characterize in great detail this path we started the characterization of the molecular structure of the human Interleukin 6.

The recombinant fully labeled ^{13}C and ^{15}N protein is currently being analysed by means of multidimensional heteronuclear NMR techniques. The assignments and structure characterization is here presented.

D2-102 COMPLETE ^{1}H, ^{13}C and ^{15}N NMR ASSIGNMENTS AND SECONDARY STRUCTURE OF THE 269 RESIDUE SERINE PROTEASE PB92 FROM BACILLUS ALCALOPHILUS, Rolf Boelens[1], Dick Schipper[2], Rasmus Fogh[1], Matteo Mariani[1] and Robert Kaptein[1]; [1]Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, 3584CH Utrecht, The Netherlands; [2]Gist-Brocades BV, CT&S/ARS, PO Box 1, 2600MA Delft, The Netherlands

The constant improvement of NMR techniques has permitted the assignment of several large proteins (see for a review Bax and Grzesiek [1]). The use of $^{13}C/^{15}N$ labeled proteins in combination with heteronuclear 3D and 4D experiments alleviates the problem of spectral overlap. We have applied these methods to the serine protease PB92 (maxacal[tm]), an enzyme of the subtilase family which is used commercially as a protein degrading component of washing powders. The 269 residue protease PB92 (molecular weight 27 kD) is one of the largest monomeric proteins studied by NMR in detail. The assignment of the backbone ^{1}H, ^{13}C and ^{15}N resonances has been described previously [2]. Recently, we have obtained almost complete assignments for this protein using 3D HCCH COSY and TOCSY measurements [3]. The set of assignments encompasses all backbone carbonyl and CH_n carbons, all amide (NH and NH_2) nitrogens and 99% of the amide and CH_n protons. The secondary structure and general topology appears to be identical to the one found in the crystal structure of PB92 [4], as judged by chemical shift deviations from random coil values, NH exchange data, and analysis of NOEs between backbone NH groups. The obtained assignments form the basis for further structural work on PB92. Molecules of this size (27 kD) thus seem to be well within the limits of current NMR technology, at least for reasonable well-behaved proteins under suitable measuring conditions.

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D2-103 SOLUTION STRUCTURE OF CAPSICEIN BY 3D TRIPLE RESONANCE NMR SPECTROSCOPY

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Elicitins, 10 kD holoproteins, are elicitor molecules having toxic and signalling properties that are secreted by Phytophthora fungi. They are responsible for the incompatible hypersensitive reaction of diverse plant species leading to resistance against fungal or bacterial plant pathogens. The protein was uniformly labelled with ^{15}N and ^{13}C to overcome spectral overlap of the proton resonances. The ^{1}H, ^{15}N and ^{13}C resonance assignments, solution secondary and tertiary structure determination of capsicein are presented. A Combination of 3D HOHAHA-HMQC, NOESY-HMQC, HMQC-NOESY-HMQC, HNCA, HN(CO)CA and HNCO experiments allowed the identification of spin systems with through-bond correlations, which were in turn correlated by through-space connections. The sequential assignment was obtained for main and side chain resonances and led to the identification of secondary structures. Measurement of the vicinal coupling constants $^{3}J_{HN\alpha}$ was carried out and 53 ϕ torsion angles were determined. 964 NOE cross-peaks, identified on the NOESY spectra, were converted into three distance classes depending on their intensity. The DIANA program was used for the structure calculation from the NMR data, and the generated conformers with the lowest final target function values were subjected to a restrained energy minimisation using the X-PLOR program in four stages: energy minimisation without constraints using standard X-PLOR parameters, increase of the temperature to 2000 K introducing gradually the constraints, cooling of the system from 2000 K progressively to 0 K and a minimisation of the obtained structures by a molecular dynamics minimisation at 0 K. The resulting structures are in good agreement with the NMR-derived constraints, with only a few NOE constraint violations and no angle constraint violations greater than 5 degrees.

D2-104 TOWARDS A STRUCTURE FOR THE IL-2/IL-2 RECEPTOR COMPLEX

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Understanding the structural basis of protein–protein interaction is an important goal in biology. Circumvention of the upper size limitations of n.m.r. spectroscopy may be achieved practically as follows: (i) solve the structure of the components of a protein–protein complex to high definition, (ii) probe the nature of the interactions of the components both by direct observation using n.m.r. techniques, and by solving structures of mutant proteins with altered affinities for other components in the complex; (iii) use computational techniques to build models of the intact complex, making use of data obtained in (i) and (ii) above.

We have initiated this approach in the study of the four-helix bundle cytokine, interleukin-2 (IL-2) and the extracellular domains (ECDs) of its receptor chains. The ECDs transduce binding of cognate messenger cytokines into cytoplasmic signals that trigger developmental processes in the cell (a recent X-ray structure of the human growth hormone – receptor complex provides direct evidence in support of the idea that ligand-induced receptor oligomerization is important for signal transduction). IL-2 belongs to a protein superfamily including which includes human growth hormone. We present progress towards our goal of building up a picture of the entire IL-2/IL-2R complex, focussing on the dynamic properties of functionally important surface side-chains of the components, and on rationalizing and predicting differences in main-chain conformation between mutant and wild-type proteins.

D2-105 NMR STRUCTURAL STUDIES OF A NOVEL ONCOGENIC RAS VARIANT AND A RAS EXCHANGE FACTOR MUTANT

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The Ras proteins are members of a large superfamily of guanine nucleotide-binding proteins. These proteins cycle between active GTP- and inactive GDP-bound states. Ras proteins are positively regulated by guanine nucleotide exchange factors (GEFs) that promote formation of the active GTP-bound state and negatively regulated by GTPase activating proteins (GAPs) that stimulate formation of the inactive GDP-complexed protein. Structural mutations that activate Ras oncogenic potential either impair the GAP-stimulated GTPase activity or promote enhanced intrinsic nucleotide exchange. The net result of either biochemical effect to favor elevated levels of Ras-GTP *in vivo*.

Two residues in the helix (5) of Ras are strictly conserved in all Ras-related proteins. These residues make several hydrophobic contacts that have been speculated to be involved in the formation and/or maintenance of the Ras three dimensional structure. Thus, the strict conservation of these residues is believed to reflect their essential role in maintaining the structural integrity and function of Ras superfamily proteins rather than direct association with the guanine nucleotide. We have introduced a F156L mutation into wild type Ras to evaluate the role of this residue in Ras structure and function. We observed that this mutation activated Ras transforming potential and greatly accelerated guanine nucleotide turnover. We will present the structural perturbations resulting from mutation of this strictly conserved residue, and relate the structural changes with its biological and biochemical properties.

We have also studied a Ras mutant containing a D69N substitution in helix (2) which disrupts guanine nucleotide exchange factor (GEF) binding. NMR structural studies indicate that the mutation causes localized structural perturbations surrounding the mutation site. Consistent with our structural analyses, the biochemical properties of the protein are unaffected by the mutation. These studies provide the first direct evidence that residues in helix (2) are important determinants of exchange factor binding to Ras proteins.

D2-106 THREE DIMENSIONAL STRUCTURE OF THE HIV-1 MATRIX PROTEIN

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The HIV-1 matrix protein forms an icosahedral shell associated with the inner membrane of the mature virus. Genetic analyses have indicated that the protein is important for many important steps throughout the viral life cycle, including stabilizing the envelope structure, viral penetration, nuclear localization, and cell membrane localization. In order to understand the structural bases for the diverse functions of this protein, we have determined the three dimensional solution structure of recombinant matrix protein to high resolution using multidimensional heteronuclear NMR spectroscopy.

NMR signal assignments required recently developed triple-resonance (^{1}H, ^{13}C, ^{15}N) NMR methodologies owing to significant degeneracies in the α and backbone amide proton regions of the spectra. A total of 636 NOE-derived distance restraints were employed for distance geometry-based structure calculations. An ensemble of 25 refined distance geometry structures with penalties of 0.32 $Å^2$ or less were generated. Best-fit superposition of ordered backbone heavy atoms relative to mean atom positions afforded rms deviations of 0.50±0.08 Å.

The folded HIV-1 matrix protein structure is composed of five α–helices, a short 3_{10} helix, and a three-stranded mixed ß-sheet. Helices I to III and the 3_{10} helix pack about a central helix (IV) to form a compact globular domain that is capped at one end by the ß-sheet. The C-terminal helix (V) projects away from the ß-sheet to expose residues essential for early steps in the HIV-1 infectious cycle. Basic residues implicated in membrane binding and nuclear localization functions cluster about an extruded cationic loop that connects ß-strands 1 and 2. The structure suggests that these two functions may be mediated by complex tertiary structures rather than simple linear determinants.

Massiah, et. al, *Journal of Molecular Biology*, **244**, 198-223 (1994)

D2-107 STRUCTURAL BIOLOGY OF PROTEIN DOMAINS INVOLVED IN INTRACELLULAR SIGNAL TRANSDUCTION, David Cowburn, The Rockefeller University, New York, NY, 10021.

Many of the proteins involved in intracellular signal transduction use similar motifs, which are apparently defined domains. Src homology (SH) domains are common sequence motifs within protein tyrosyl kinases, and other proteins involved in intracellular signal transduction. SH2 domains bind to phosphotyrosyl containing peptides and proteins in a very large range on pathways linking the activation of cell surface receptors to events in the cytoplasm and the nucleus. SH3 domains appear to recognize prolyl-rich sequences. Many proteins binding to SH3's contain the Pleckstrin Homology domain (PH), of currently unknown function. Three aspects of these domains under study are (A) the protein structure of individual domains, (B) recognition of ligands by these motifs, and (C) the interactions among the motifs.

The previously reported solution structure of Abl SH2, has been complemented by structural studies of the Abl SH3 and SH3+2 constructs. Although in the full Abl gene product the SH2 is required for transforming potential, and the SH3 must be critically placed for kinase down regulation, on the basis of shift, nOe, ligand affinity, and hydrogen exchange comparisons, there appears to be virtually no interactions between the SH2 and SH3 domains in the SH3+2 product, and no evidence for dimerisation..

The Pleckstrin Homology (PH) domain from dynamin has been expressed and its structure derived by us and others. The solution structure is monomeric, compared to the intimate dimer of the crystal form. Phospatidylinositol-3,4-bisphosphate, and many other lipids bind to dynamin PH, and the basis of specificty is being investigated.

D2-108 THE STRUCTURE OF *DESULPHOVIBRIO AFRICANUS* FERREDOXIN I BY ^{1}H NMR, S.L. Davy[1], M. J. Osborne[1], J. Breton[1], A.J. Thomson[1], C. Luchinat[2], I. Bertini[3],G.R. Moore[1]. [1]Centre for Metalloprotein Spectroscopy and Biology, School of Chemical Sciences, University of East Anglia, Norwich, England, [2]University of Bologna, Institute of Agricultural Chemistry, Bologna, Italy, [3]University of Florence, Department of Chemistry, Florence, Italy.

Desulphovibrio africanus ferredoxin I (Fd I) is a 64 amino acid protein containing one [4Fe-4S] cluster which is coordinated to the protein backbone by the thiolate groups of four cysteine residues positioned at 11, 14, 17 and 54 in the primary structure. All the ^{1}H NMR data has been recorded on the oxidised protein with a formal cluster charge of 2+, and a diamagnetic ground state of S=0. However our studies have shown the protein exhibits some paramagnetic nature, even at ambient temperatures, with large, characteristic, hyperfine shifts, and fast relaxing nuclei detected in the NMR data. The hyperfine shifts are contact in origin and thus are attributed to the βCH_2 protons and αH protons of the cluster coordinating cysteine residues.

Using conventional methods employed for structure determination of diamagnetic proteins preliminary 2D data recorded on oxidised *D. africanus* Fd I allowed the identification of 48 NH-αCH connectivites from which 32 of the spin systems identified from the scalar connectivities were then sequence-specifically assigned by utilisation of NOESY spectra (100-200ms). With correlations between the fast relaxing nuclei undetected in these preliminary 2D data sets, 1D nOe experiments were successfully employed to afford the pairwise assignment of the cysteinyl βCH_2 protons which in turn permitted their sequence-specific assignment to Cys11, 14, 17 or 54. These assignments were validated by "paramagnetic" 2D experiments, both TOCSY and NOESY, with the experimental parameters tailored to optimise detection of specific correlations.

At the present time 48 residues (75%) have been sequence-specifically assigned, with a total of 62 out of 64 detected. Three secondary structures, two β–sheets and an α-helix have been identified comprising of a total of 22 spin systems.

Thus with these secondary structure elements, and the constraints imposed on the folding of the protein backbone by the cluster itself, we believe that we have enough NMR constraints to generate 3D structures of *D. africanus* Fd I in the solution state. This computational analysis is currently underway.

D2-109 PROTEIN-LIGAND INTERACTIONS IN THE THYROID SYSTEM, Brendan M. Duggan, David J. Craik and Sharon L.A. Munro, School of Pharmaceutical Chemistry, Victorian College of Pharmacy, Monash University, Parkville VIC 3052 Australia

Thyroid hormones are synthesized in the thyroid gland and secreted into the bloodstream. The lipophilic molecules bind to serum transport proteins which deliver the hormones to receptors within the cell nuclei. The proteins involved in the thyroid system are too large (~60kDa) for structure determination by NMR, but the interaction of the proteins with the thyroid hormones (and their analogues) can be investigated by a variety of NMR techniques.

Experiments have been performed on complexes of the thyroid hormones with two of the serum transport proteins, transthyretin and human serum albumin. Line broadening experiments have been used to identify systems in slow and fast exchange, and subsequently calculate dissociation rates for the complexes. Systems that fall into the fast exchange regime were investigated further using transferred NOE experiments to identify the bound conformation and determine dissociation constants.

D2-110 FUNCTIONAL IMPLICATIONS OF THE STRUCTURE OF THE BACTERIAL RESPONSE REGULATOR, Spo0F Victoria A. Feher[1], James Zapf[2], James A. Hoch[2], John M. Whiteley[2], John Cavanagh[2], and Frederick W. Dahlquist[1] [1] Intitute of Molecular Biology, University of Oregon, Eugene, OR 97403 [2] The Scripps Research Institute, La Jolla, CA 92037

Bacillus subtilis responds to nutrient deprivation and high cell density by expressing a series of gene products which change the cell morphology and transform the bacteria from a vegetative form to a dormant form, a dehydrated endospore. Commitment to grow or begin sporulation is dependent on the action of a four component phosphorelay system. The first two steps of the system involve autophosphorylation of a kinase, Kinase A, and transfer of the phosphate to a response regulator, Spo0F. A phosphotransferase, Spo0B, then catalyzes the transfer of the phosphate from the Spo0F protein to the transcriptional activator, Spo0A. We are utilizing high resolution NMR for structural studies of one of these phophorelay proteins, Spo0F. Study of the structural responses to magnesium binding and phosphorylation will aid in our understanding of how Spo0F functions in this phosphorelay. In addition, comparison of similarities and differences of structural responses of Spo0F and those of other response regulators will help in our understanding of responses that are unique to each protein and those that are general to this family of proteins.

Currently we have determined the backbone and side chain resonance assignments for Spo0F and are using these assignments to determine the tertiary solution structure. Analysis of the secondary structure shows Spo0F is an α/β protein containing five alpha helices and 5 parallel β-strands, as was predicted by sequence homology to other response regulators. Comparison of the secondary structure to the structure determined for the chemotactic response regulator, CheY, reveals several differences located at the C-terminal end. Studies of Spo0F in the magnesium bound and phosphorylated forms have been carried out, the structural changes observed and comparisons to CheY will be discussed.

D2-111 NMR STRUCTURAL STUDIES OF pp60$^{c\text{-}src}$ SH3-2 DOMAINS, Robert Gampe, Robert Xu, Donald Davis, Michael Word, Martin Rink and Byron Ellis, Department of Bioanalytical and Structural Chemistry, Division of Molecular Sciences,Glaxo Research Institute, Five Moore Drive, Research Triangle Park, NC 27709

Cytosolic *src* is a nonreceptor kinase that participates in intracellular signal transduction and has been implicated in the development of malignant tumors in the human colon and breast. *Src* is comprised of conserved domains that engage in various functions during intracellular signal transduction and in self regulatory processes. One of the domains, the *src*-homology 2 or SH2 domain, mediates with high selectivity the signal transduction by binding phosphorylated tyrosine motifs located on specific receptors. Another domain, SH3, which is situated on the N-terminal side of the SH2 domain in *src*, is beleived to interact with unidentified proteins containing proline rich sequences. Mutations in these domains can lead to cellular transformation. In order to further our understanding of complexes of these proteins our effort and interests have been directed towards examining the structural similarities or differences between complexes of native *src* SH32 domains and the solution structure of the native *src* SH2 domain bound with a phosphorylated tyrosine pentapeptide (see poster R.Xu et al.). We have employed well established heteronuclear, multi-dimensional/-frequency NMR methodologies to assign the H, C and N resonances of the isotopically enriched *src* SH3-2 protein. Isotope-edited and -filtered NOESY data have been used to elucidate the intra-/inter-molecular interactions of the protein and the bound ligand(s). Our progress towards the analysis and understanding of the native *src* SH32 domain complexes compared to the *src* SH2 complex are presented.

D2-112 **Investigation of the 19kDa Peptide-Binding Domain of DnaK by Multi-dimensional NMR.** Michael J. Goger[1], Roger McMacken[2], Lila M. Gierasch[1], Department of Chemistry[1], University of Massachusetts, Amherst, MA 01003, Department of Biochemistry[2], Johns Hopkins University, Baltimore, MD 21205.

DnaK is a member of the stress related protein 70 (HSP70) family and is a prototypical molecular chaperone. This family of proteins is essential for normal cellular function and is involved in binding unfolded proteins in a number of important processes. These 70kDa proteins are composed of an N-terminal ATPase domain and a C-terminal peptide binding domain. The crystal structure of the ATPase domain has been solved, but, there is no direct structural information available for the peptide binding domain. We are therefore determining the 3D-solution structure of a 19kDa fragment of DnaK that encompasses the peptide binding domain. The fragment, obtained from overexpession in *E. coli* cultured in minimal medium, is stable, protease resistant, and interacts with substrate peptide. The far UV-CD spectrum of the peptide binding domain demonstrates that the fragment is stabely folded with an approximate secondary structure of 30% α-helix and 33% β-sheet. In 1D- and 2D-NMR experiments the resonances of the fragment are narrow and well dispersed with many downfield shifted Cα proton resonances, likely to arise from β-sheet stucture. These data are being used, along with other multi-dimensional heteronuclear NMR experiments, in the resonance assignment process. Thus far, the CD and initial NMR results are all consistent with a model of the DnaK peptide binding domain predicted to be structurally homologous to the major histocompatibility complex.

D2-113 PLECKSTRIN HOMOLOGY DOMAINS: STRUCTURE AND FUNCTION, Philip J. Hajduk, John E. Harlan, Ho Sup Yoon, Andrew M. Petros, Edward T. Olejniczak, Robert P. Meadows and Stephen W. Fesik, Pharmaceutical Discovery Division, AP10, Abbott Laboratories, Abbott Park, IL 60064.

The pleckstrin homology (PH) domain is a new protein module of approximately 100 amino acids that is found in several proteins involved in signal transduction. Although the specific function of the PH domain is not known, it has been postulated that it may be involved in membrane association. In this presentation we describe: 1) the solution structure of the N-terminal domain of pleckstrin as determined from heteronuclear three-dimensional NMR spectroscopy, 2) evidence that many PH domains bind with high affinity to phosphatidylinositol-4,5-bisphosphate (PIP_2), and 3) a characterization of the PH domain's interaction with PIP_2 using site-directed mutagenesis and binding studies of PIP_2 analogs. The results presented here demonstrate that the PH domains bind to PIP_2 primarily through charge-charge interactions involving three lysines (K13, K14, and K22). The implications of these findings for the role of the PH domain in membrane association are discussed.

D2-114 SOLUTION STRUCTURE OF THE STREPTOCOCCAL PROTEIN G B2 DOMAIN AT pH 5.4, Diane K. Hancock[1], Patrick Alexander[2], and Philip Bryan[2], John Orban[2], [1]National Institute of Standards and Technology, [2]University of Maryland, Maryland Biotechnology Institute, Center for Advanced Research in Biotechnology, 9600 Gudelsky Drive, Rockville, MD 20850

Protein G, a multidomain streptococcal cell wall protein, consists of either two or three 55 amino acid repeats (domains B1, B2, and B3), each of which binds to the Fc portion of human immunoglobulin G (IgG). The primary structures of these domains differ by six amino acids. Solution structures of B1 and a B2 analog have been determined using homonuclear techniques [Gronenborn *et al.*, *Science*, **253**, 657-661 (1991); Lian *et al.*, *J.Mol.Biol.*, **228**, 1219-1234 (1992)]. These structures differ in the helix and loop 4. We present here the solution structure of the B2 domain. Comparison with the above solution structures and X-ray structures of B1 and B2 will be described.

Proton resonance assignments and coupling constant measurements were made using DQF COSY, NOESY, and E. COSY experiments. In addition ^{13}C and ^{15}N assignments were obtained from HMQC-COSY, HMQC-NOESY, and HMQC-TOCSY spectra. Interproton distances were acquired from NOESY experiments at 50, 75, 100 and 150 msec mixing times. Stereospecific assignments were made using coupling constants and relative NOE distances. A hybrid distance geometry-simulated annealing method was used to calculate a series of structures from the interproton distance and torsion angle constraints.

D2-115 ORGANIZATION OF THE COLD SHOCK DOMAIN: STRUCTURE OF THE MAJOR COLD SHOCK PROTEIN FROM *BACILLUS SUBTILIS* IN SOLUTION, T. A. Holak[1], A. Schnuchel [1], R. Wiltscheck[1], M. Czisch [1], M. Herrler [2], G. Willimsky [2], P. Graumann [2], and M. A. Marahiel [2], [1]Max-Planck-Institut für Biochemie, D-82152 Martinsried bei München, Germany, and [2]Department of Biochemistry, Philipps University of Marburg, D-3550 Marburg, Germany

The cold shock domain (CSD) was first discovered among many eukaryotic transcriptional factors and was shown to be responsible for the specific DNA-binding to a cis-element called the Y-box [1]. The same domain was also found within the sequence of the *Xenopus* proteins FRG Y_1 and FRG Y_2, which have been later identified as RNA-binding proteins [1]. We present here the three-dimensional structure of the major cold shock protein of *B. subtilis* (CspB) determined by 2D NMR spectroscopy [2]. The 67-residue protein consists of an antiparallel five-stranded β-barrel with strands connected by turns and loops. The structure bears resemblance to that of staphylococcal nuclease and the gene 5 single stranded DNA binding protein [3] A three-stranded β-sheet, that contains the conserved RNA binding motif RNP1 as well as a motif similar to RNP2 in two neighboring antiparallel β-strands, has basic and aromatic residues at its surface that could serve as a binding site for single-stranded DNA. In gel retardation experiments, binding of CspB to single-stranded DNA is observed.

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D2-116 SOLUTION STRUCTURE OF N-TERMINAL SH3 DOMAIN OF GRB2 COMPLEXED WITH PROLINE-RICH PEPTIDE FROM THE GUANINE NUCLEOTIDE RELEASING FACTOR SOS, F. Inagaki, Department of Molecular Physiology, The Tokyo Metropolitan Institute of Medical Science, Tokyo 113, Japan.

We reported the three dimensional structures of the SH3 of PLCγ (1) and C-terminal SH3 of GRB2 (2). Unfortunately, due to weak binding of the proline-rich peptides to these SH3 domains, we could not determine the structure of the complex. Recently, we succeeded to determine the solution structure of the complex between GRB2 N-terminal SH3 (N-SH3) and the proline-rich peptide (VPPPVPPRRR) (3). The NMR data show that the peptide adopts the conformation of a left-handed polyproline type II helix (PPII) and interacts with the three hydrophobic sites on the SH3 domains. Pro2' and Pro3' of the peptide bind to the first site formed by Tyr7 and Tyr52. Val5' and Pro6' binds to the second site which is surrounded by Tyr52, Pro49, Trp36 and Phe9. Finally, the side chain of Arg8' interacts with the third site formed by the aromatic rings of Trp36 and Phe47. These hydrophobic residues are conserved in the family of SH3 domains and form a characteristic patch on the protein surface. The aromatic rings of conserved Trp36 and Tyr52 are parallel to each other and separated by a distance of 9.0 Å. This is similar to the interplanar distance between Pro3' and Pro6' in the PPII helix. In this arrangement, Pro3' interacts with Tyr52 and Pro6' interacts with Trp36. The third site is also characterized by acidic groups. Glu16 and Asp33, located on the RT-src and n-src loops, which possibly bind Arg8' through ionic interaction. Thus, the SH3 domain is a structural motif that accommodates the PPII helix on the hydrophobic patch formed by the conserved aromatic and proline residues.

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D2-117 SOLUTION STRUCTURE OF THE EGF-HOMOLOGY DOMAIN OF HEREGULIN, Neil E. Jacobsen, Mark X. Sliwkowski, Daniel Yansura, Nasrin Abadi, Dorothea Reilly, Nicholas J. Skelton and Wayne J. Fairbrother, Genentech, Inc., South San Francisco, CA 94080

Heregulin is a 45-kDa glycoprotein which activates the receptor tyrosine kinase p185^{HER2}. A construct corresponding to residues 177-239 of heregulin α has been expressed in *E. Coli* and shown to activate p185^{HER2} (Holmes *et al.*, Science **1992**, *256*, 1205-1210). This construct has 26% identity with human EGF. Complete backbone and side-chain ^{1}H and ^{15}N assignments have been obtained at 20°C and 30°C, pH 4.5. The NOE, amide proton-deuterium exchange and coupling constant data are consistent with a three stranded antiparallel β-sheet (residues 3-6, 17-24, and 31-37), a two stranded antiparallel β-sheet (39-41 and 47-49), a short helix-like region (7-13), and a solvent-exposed and conformationally undefined C-terminus (51-63). The three residue Ser-Asn-Pro insertion (27-29) in HRG$_{177-239}$ relative to EGF is accomodated in a loop without changing the β-strand alignment or extending the β-sheet. Residues 3-6 of HRG$_{177-239}$ have slowly-exchanging backbone amide protons and strong cross-strand NOE interactions which indicate that Cys-6 is aligned with its disulfide-bonded partner Cys-20. The equivalent region of human EGF is disordered (Hommel *et al.*, J. Mol. Biol. **1992**, *227*, 271-282), and in mouse EGF it is aligned with the β-sheet with Gly-5 across from Cys-20 (Montelione *et al.*, Biochem. **1992**, *31*, 236-249). The solution structure has been calculated, and a comparison with the structure of a similar construct (Nagata *et al.*, EMBO J. **1994**, *13*, 3517-3523) will be presented.

D2-118 STRUCTURE AND DYNAMICS DIFFERENCES BETWEEN OXIDIZED AND REDUCED *ESCHERICHIA COLI* THIOREDOXIN

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The high-resolution three-dimensional solution structures of oxidized and reduced forms of *Escherichia coli* thioredoxin, a protein of molecular weight ~11.7 kDa, which catalyzes a number of redox reactions, including both oxidative and reductive thiol-disulfide exchange of proteins, have been determined using three-dimensional heteronuclear NMR spectroscopy. 30 structures of both forms were generated from distance geometry program DIANA, and refined by molecular dynamics (AMBER). The atomic rms. distribution about the mean coordinate positions for residue 4-107 is 0.35 Å (oxidized) and 0.29 Å (reduced) for the backbone atoms, 0.72 Å (oxidized) and 0.76 Å (reduced) for heavy atoms. The N- and C-terminal residues (1-3, 108, respectively) appear to be disordered. Detailed comparison of NMR solution structures of the oxidized and reduced forms will be presented and observed differences with the x-ray crystal structure of the oxidized form will be discussed.

In addition to ^{15}N relaxation studies of the backbone dynamics of thioredoxin (Stone et al., 1993)[a] we have recently measured the backbone mobility of the two forms by amide proton exchange detected by ^{15}N-HSQC NMR spectra. For slowly-exchanging protons the exchange rates were obtained by hydrogen-deuterium exchange. For those amide protons with rates too fast to measure by this method, the measurements were made by saturation transfer. The exchange rates of most protons are very similar in the two forms. Significant differences are observed in the active site sequence and in the regions of the protein that are close to this sequence in the 3-dimensional structure, including protons of the β-strand and α-helical sequences immediately flanking the active site. In general, the amide proton exchange rates are consistently lower for the oxidized form of thioredoxin. This result provides further evidence that the functional differences observed between the two forms of thioredoxin can most likely be ascribed to the greater flexibility of the reduced form of the protein.

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D2-119 ISOTOPE LABELLING TECHNIQUES FOR STRUCTURE DETERMINATION OF LARGE PROTEINS

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The introduction of uniform isotope labelling with ^{13}C and ^{15}N in conjunction with multidimensional heteronuclear experiments has allowed determination of the structures of proteins with molecular weights of up to 18 kDa and the complete assignment of even larger proteins. A major limitation for the application of multidimensional heteronuclear experiments to the study of large proteins is the rapid transverse relaxation rates in these molecules. Deuteration has been proposed as one method to improve the relaxation properties of ^{13}C-, ^{15}N-labelled proteins and reduce losses due to relaxation during magnetization transfer. Initial studies have demonstrated a reduction in line widths for $^{13}C_{\alpha}$ nuclei in proteins enriched in ^{2}H (1,2).

Samples of two protein domains (8 kDa and 19 kDa respectively), with very different relaxtion properties, uniformly enriched with ^{13}C (100%) and ^{15}N (100%), and with levels of random fractional deuteration between 0-75% have been prepared. Data from several triple-resonance experiments performed with these samples is presented and the implications for structural studies of large proteins are discussed.

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D2-120 LOW-RESOLUTION STRUCTURE OF THE A-DOMAIN OF ESCHERICHIA COLI ENZYMEII^{mtl}, Gerard J. A. Kroon, Klaas Dijkstra, Ruud M. Scheek and George T. Robillard, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands.

The EnzymeII mannitol (EII^{mtl}) of *Escherichia coli* is part of the phospho-*enol*pyruvate dependent phosphotransferase system (PTS). The system regulates the uptake and phosphorylation of specific carbohydrate substrates. The carboxy-terminal domain of EII^{mtl} (EIIA^{mtl}, 148 residues, 16.4 kDa) has been cloned and overexpressed in *E. coli*. The backbone resonances were assigned using a three-dimensional ^{1}H-^{1}H NOE {^{1}H-^{15}N} NOESY-HSQC and a three-dimensional ^{1}H-^{1}H total correlation {^{1}H-^{15}N} TOCSY-HSQC in combination with several triple-resonance experiments. Determination of the secondary structure of EIIA^{mtl} is based on characteristic NOE patterns from the ^{15}N NOESY-HSQC and on the ^{13}Cα, ^{13}CO and ^{1}Hα secondary chemical shifts. The resulting secondary structure is considerably different from that reported for EnzymeIIA glucose (EIIA^{glc}) of *E. coli* and for the EnzymeIIA glucose domain of *Bacillus subtilis* determined by NMR and X-ray. Neither proteins are homologous to EIIA^{mtl} although they have a comparable function in the PTS: all three proteins are phosphorylated at a histidine by HPr (Histidine containing Protein) and transfer the phosphoryl group to the B domain of the sugar specific EnzymeII. Assignment of the sidechain resonances was carried out using three-dimensional {^{1}H-^{1}H-^{13}C} correlation spectroscopy (HCCH-COSY), three-dimensional {^{1}H-^{1}H-^{13}C} total correlation spectroscopy (HCCH-TOCSY) and a new three-dimensional {^{13}CO-^{13}Cα-^{1}H} total correlation spectroscopy experiment (COCCH-TOCSY). The COCCH-TOCSY correlates sidechain proton resonances to the ^{13}Cα and ^{13}CO resonances of the backbone. This was helpful in cases of overlapping ^{1}Hα and ^{13}Cα resonances. In order to determine the low-resolution structure of IIA^{mtl}, NOEs from the {^{1}H-^{15}N} NOESY-HSQC and a three-dimensional ^{1}H-^{1}H NOE {^{1}H-^{13}C} NOESY-HSQC are analyzed.

D2-121 THE THREE-DIMENSIONAL SOLUTION STRUCTURE OF REDUCED PLASTOCYANIN FROM *Anabaene Variablis*. Jens J. Led,[1] Ulla Balsberg,[1] Jan M. Hammerstad-Pedersen[2] and Jens Ulstrup,[2] [1]Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark. [2]Chemistry Department A, The Technical University, DK-2800 Lyngby, Denmark.

Plastocyanins are small copper proteins which are essential components of the electron transfer process in the photosynthesis in higher plants and algae. The size of the plastocyanins (approximately 100 amino acids depending on the source) and the high content of ß-sheet make them suitable for NMR investigations.

Reactions of plastocyanin have indicated the presence of two potential areas for interaction with physiological redox partners. These have been termed the north site (or hydrophobic patch) and the east site (or the acidic patch). In all but two cases plastocyanins are acidic with a charge on the reduced protein of -9 ± 1 at pH = 7. One of the two exceptions is *A. variabilis* plastocyanin. In this plastocyanin the negative charge at the east site is not retained resulting in a charge of +1 at pH 7. Further deviations from other plastocyanins are a lower reduction potential, a much greater self-exchange rate constant, and different rate constants in the reaction with the redox partners. Moreover, the sequence homology of *A. variabilis* plastocyanin with other plastocyanins is low, and its peptides chain is slightly longer.

We have determined the three-dimensional NMR solution structure of *A. variabilis* using standard NMR techniques, and simulated annealing and restrained energy minimization calculations. The obtained structure will be presented and compared with the structure of other plastocyanins, and the structural basis for the differences from other plastocyanins mentioned above will be discussed.

D2-122 NMR STRUCTURE AND PHOSPHOTYROSINE BINDING STUDIES OF THE MURINE BLKSH2 DOMAIN, Barbara Leiting, William J. Metzler, KellyAnn D. Pryor, Joseph B. Bolen*, Hann-Guang Chao, Gary R. Matsueda, Luciano Mueller and Bennett T. Farmer II. Department of Macromolecular Structure, * Department of Molecular Biology, Bristol-Myers Squibb, Princeton, NJ 08543-4000.

Blk, the smallest known B-cell specific protein tyrosine kinase (PTK) in the Src family, plays an important role in both the positive and negative signaling pathways induced by the B-cell antigen receptor complex (mIgM). Blk contains one catalytic domain and two regulatory domains: an SH3 and an SH2 domain. The SH2 domain is an approximately 100-amino-acid fragment found in a variety of proteins implicated in growth control. We have determined the three-dimensional structure of murine BlkSH2 domain (residues 107-218 of the native protein, MW ~ 12,700 daltons) by Nuclear Magnetic Resonance (NMR).

The regulation of the PTK's activity is often associated with the binding of SH2 to tyrosine phosphorylated polypeptides. In order to understand the Blk enzyme activation induced by the B cell receptor, we have characterized the binding of the BlkSH2 domain to chemically synthesized peptides and recombinant protein domains containing sequences of the mIg-α and mIg-β cytoplasmic domains. In this poster, we will present the three-dimensional structure of the BlkSH2 domain and the results of our binding studies.

D2-123 THE THREE DIMENSIONAL STRUCTURE OF THE CYTOKINE hMIP-1β AND COMPARISON TO THE RELATED CYTOKINE IL-8, Patricia J. Lodi, D.S. Garrett, J. Kuszewski, M. L.-S. Tsang, J. A. Weatherbee, A.M. Gronenborn, and G.M. Clore, Laboratory of Chemical Physics, National Institutes of Heath, Bethesda, MD 20892-0505

Multidimensional NMR has been used to determine the high resolution structure of the cytokine human macrophage inflammatory protein (hMIP-1β). This protein is a member of the β subfamily of "chemokines" which are chemoattractants for monocytes. hMIP-1β shares about 20% sequence identity with interleukin-8 (IL-8), which belongs to the α subfamily of chemokines and which is a chemoattractant of neutrophils but not monocytes. Both hMIP-1β and IL-8 form homodimers of approximate overall mass 16 kDa, and while the tertiary structure of the monomeric unit for each protein is quite similar, the quaternary structure is entirely distinct. The dimer interface is formed by a different set of residues for the two proteins, and while hMIP-1β is elongated and cylindrical, IL-8 is compact and globular. ^{15}N relaxation measurements are used to compare the backbone dynamics of the two proteins.

D2-124 STRUCTURE AND BACKBONE DYNAMICS OF A BIOLOGICALLY POTENT HUMAN INSULIN MUTANT,

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The solution structure of insulin is not easily accessible from NMR studies due to dimerization, higher order aggregation, or fibrillation. The need of having purely monomeric insulin has been a major obstacle, but within the last few years this has been more or less successfully achieved either by the use of organic solvents or by genetically engineered mutants of insulin. A general result of these studies has been structures that are generally poorly defined. We have probed several mutations of human insulin with the aim to achieve molecules that are monomers still at high concentrations. One of the most promising candidates is the one where B16 Tyr has been substituted with Histidine, B16 His. This site of mutation is in the monomer-monomer interface, thus preventing dimerization, and monomeric molecules in water are then accessible for NMR at pH 2.4. B16 His has maintained 43 % activity in receptor binding compared to native human insulin. The structure studies of His B16 human insulin (and a ^{15}N enriched mutant) were performed using two- or three-dimensional NMR. Intensities of 910 NOESY cross peaks were used for structure calculation along with 44 ϕ-angle restraints based on $^{3}J_{H^{N}H^{\alpha}}$ coupling constant measurements, 23 χ^{1}-, 6 χ^{2}-angle restraints. Structure calculations were performed with the program X-PLOR version 3.0 using the combined distance geometry / simulated annealing protocol. Twenty converged structures were calculated that fulfilled the restraints very well. The atomic RMSD of the twenty structures when compared to their average is 0.46 Å for backbone atoms (A2-A19, B4-B28) and 0.89 Å for all backbone atoms. The solution structure shows great resemblance to molecule 1 of the 2Zn pig insulin crystal structure. Backbone dynamics of uniformly ^{15}N-labelled B16His insulin were investigated using two-dimensional inverse detected heteronuclear ^{15}N-^{1}H NMR spectroscopy. ^{15}N T_1, T_2, and NOE data were measured and interpreted according to the model-free approach. Backbone dynamics of insulin provide important information for an understanding of the structure function relationship.

D2-125 BACKBONE DYNAMICS OF *ESCHERICHIA COLI* RIBONUCLEASE H1: CORRELATIONS WITH STRUCTURE AND FUNCTION IN AN ACTIVE ENYZME,

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Ribonuclease H hydrolyzes the RNA moiety of RNA-DNA duplex molecules. *Escherichia coli* ribonuclease H is involved in DNA replication; retroviral ribonuclease H is essential for reverse transcription of the viral genome. Spin-lattice relaxation rate constants, spin-spin relaxation rate constants and the steady state heteronuclear nuclear Overhauser effects for the ^{15}N nuclear spins of *E. coli* RNase H have been determined by using proton-detected heteronuclear NMR spectroscopy. The relaxation data were analyzed by using a series of dynamical models in conjunction with a statistical model selection protocol to characterize the intramolecular dynamical properties of the protein. Ribonuclease H exhibits a complex array of dynamical features, most notably in the parallel β-strands of the principal five stranded β-sheet, the coiled-coil helical interface, the active site and in the loop regions surrounding the active site. The dynamical properties are correlated with the local structural environments of the ^{15}N spins and suggest possible relationships to the functional properties of ribonuclease H. Results for *E. coli* ribonuclease H are compared to previously-reported results for the human immunodeficiency virus type 1 ribonuclease H domain of reverse transcriptase.

D2-126 NMR structure determination of cytochromes c_{553} of *Desulfovibrio vulgaris* Hildenborough and c_{551} of *Ectothiorhodospira halophila*

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Cytochromes *c* are small soluble heme proteins with a great diversity of sequences [80 → 120 aa] implicated in electron transfer processes in both eukaryotic and prokaryotic organisms. We here report the solution structure of two small ferrocytochromes *c* of low redox potential, as determined by NMR and restrained molecular dynamics. A three stage protocol was developed, involving a 60ps stage to determine the global fold, a 20ps exploratory stage with a simplified potential and a 26 ps refinement stage with the full force field.

① The cytochrome c_{553} of *Desulfovibrio vulgaris*, a strict anaerobic sulfate-reducing bacterium, exhibits a low potential of +20 mV. Its structure was determined using about 900 NMR distance constraints and two conformations of the turn motif at the solvent/heme cleft interface are identified, with equally viable energetic caracteristics.

② The cytochrome c_{551} of *Ectothiorhodospira halophila*, a phototrophic purple bacterium, has a potential of +58 mV. Its ^{1}H and ^{13}C resonances were assigned and more than 1200 constraints were used for the molecular refinement.

In these cytochromes, the conserved structural elements (α-helices) and the solvent exposure of the heme are compared and discussed with regard to their low redox potential.

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D2-127 STRUCTURE AND FUNCTION OF RETRO-VIRAL MATRIX PROTEINS

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Retroviral matrix proteins (MA's) form the outer shell of the core of the virus and therefore line the inner surface of the viral membrane. This protein has several key functions, which vary according to the type of retrovirus. In C-type retroviruses it orchestrates viral assembly via targeting signals that direct the gag precursor polyprotein, to the host cell membrane and it interacts with the transmembrane protein to retain the env-encoded proteins in the virus. In addition, it contains a nuclear localisation signal that directs the pre-integration complex to the nucleus of infected cells. In D-type viruses the assembly is initiated within the cytoplasm. We report the nuclear magnetic resonance (NMR) solution structures of two matrix proteins. The first is an example from a C-type lentivirus (Human Immunodeficiency Virus, HIV) and the other is from the unrelated Human T-cell and Bovine Leukaemia Virus (HTLV/BLV) family. The MA from HIV-1 consists of four helices connected by short loops and an irregular, mixed β sheet that provides a positively charged surface for interaction with the inner layer of the membrane. The helical topology is unusual; the Brookhaven protein database contains only one similar structure, that of the immune modulator interferon-γ. MA from the BLV is also largely helical but lacks several key features of the HIV MA structure. These can be extensively interpreted in terms of subtle differences in function. The comparison of the two structures also enables us to predict how these molecules interact to form the retroviral matrix.

D2-128 STRUCTURE DETERMINATION OF RHODNIIN WITH TRIPLE-RESONANCE EXPERIMENTS

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The protein Rhodniin that can be purified from the blood-sucking insect *Rhodnius prolixus* is a thrombin-specific inhibitor (1) with a molecular mass of 11 kDa. The amino acid sequence (103 residues) is homologous to Kazal-type inhibitors from egg white (Ovomucoid) or the saliva of predatory animals.
Sequential assignment of the $^{13}C/^{15}N$ labeled protein was obtained from a combination of HNCO, HN(CA)CO, HNCA and H(N)COCA as well as CBCACO(N)H and CBCANH experiments. All of these triple-resonance experiments rely on magnetisation transfer via heteronuclear one-bond J couplings. Essential information of the amino acid type is included from the sidechain proton and carbon resonances obtained from (H)CCCO(N)H and H(CC)CO(N)H spectra. Complete sidechain assignment was done by a HCCH-TOCSY. NOE's are measured with simultanous edited $^{13}C,^{1}H/^{15}N,^{1}H$- HSQC-NOESY (2) and 4D-HSQC-NOESY-HSQC experiments. The measurement of coupling constants is done with C'-FIDS-HSQC and DQ/ZQ-methods (3). In order to achieve excellent water suppression and optimum sensitivity all experiment employ heteronuclear gradient echoes in combination with Coherence Order Selective Coherence Transfer (COS-INEPT), i.e. using Double INEPT and/or planar TOCSY for coherence transfer (4).

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D2-129 **STRUCTURE OF LEUKAEMIA INHIBITORY FACTOR.**

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Leukaemia Inhibitory Factor (LIF) is a pleiotropic cytokine that acts on a wide range of target cells, including megakaryocytes, osteoblasts, hepatocytes, adipocytes, neurons, embryonic stem cells and primordial germ cells. Many of its activities are shared with other cytokines, particularly interleukin-6, oncostatin-M, ciliary neurotrophic factor and granulocyte colony-stimulating factor (G-CSF). Although secreted *in vivo* as a glycoprotein, non-glycosylated recombinant protein expressed in *E. coli* is fully active and has been used in our nuclear magnetic resonance (NMR) studies of the three-dimensional structure and structure-function relationships of LIF in aqueous solution.

With 180 amino acids and a molecular mass of *ca* 20 kDa, LIF is too large for direct structure determination by ^{1}H NMR methods alone. It has therefore been necessary to label the protein with the stable isotopes ^{15}N and ^{13}C and employ heteronuclear 3D NMR in order to resolve and interpret the spectral information required for 3D structure determination. This has been undertaken with both human LIF and a mouse-human chimaera, MH35, which binds to the human LIF receptor with the same affinity as the human protein and yet expresses in *E. coli* at much higher levels. Sequence-specific resonance assignments and secondary structure elements for the doubly-labelled chimaera will be presented and its 3D structure described.

This is a contribution from the Cooperative Research Centre for Cellular Growth Factors

D2-130 STRUCTURAL STUDIES OF HUMAN CD5 DOMAIN-1 USING MULTIDIMENSIONAL NMR,

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CD5 is a type-1 transmembrane glycoprotein expressed on T-cells and a subset of B-lymphoctyes. Functional and biochemical evidence suggest a co-stimulatory role for CD5 in T-cell activation, and a study of CD5 ligands is being investigated using a range of recombinant fusion proteins. The extracellular portion of CD5 contains three domains which belong to the rapidly emerging scavenger receptor superfamily (ScR). Electron microscopy suggests that each of these domains is independently folded, although there is no structural information for a single domain. Domain-1 was chosen for NMR studies since it is thought to be involved in ligand interactions and there are unlikely to be inter-domain contacts between domains-1 and 2 as they are separated by a long, O-glycosylated, proline-rich linker. Recombinant, glycosylated CD5 domain-1 has been expressed at high levels in mammalian cells and the structure of the carbohydrate has been determined. CD5d1 has also been expressed at high levels in yeast allowing simple enzymatic manipulation of carbohydrate size and isotopic labelling for multidimensional NMR. The length of the CD5d1 construct and its carbohydrate content have been optimised for homogeneity and performance in NMR experiments. The protein has been characterised by mass-spectrometry and the disulphide arrangement has been determined. NMR analysis suggests that the structure is composed mainly of β-sheet and the spectra were of sufficiently high quality to allow the determination of a three-dimensional structure. This structure will provide insights into the structure and function of CD5 and other members of the ScR superfamily.

D2-131 THE STRUCTURE AND ENZYMATIC MECHANISM OF *BACILLUS CIRCULANS* XYLANASE

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Bacillus circulans xylanase (BCX) is an endo-β-(1,4) glycosidase that cleaves xylan. This insoluble sugar is a major component of plant cell walls, and thus there is considerable biotechnological interest in the use of xylanases for bio-bleaching in paper manufacture and for the saccharification of biomass for food production. The goals of our research are to understand the catalytic mechanisms of xylanase and to apply protein engineering towards the design of these enzyme for industrial applications.

We have measured the pKa's of the catalytic glutamic acid residues using ^{13}C-NMR to monitor the pH titration of BCX specifically labelled with δ-^{13}C-Glu. The pKa of the nucleophilic E78 sidechain is 4.6, whereas that of the general acid catalyst E172 is 6.7. In the presence of a mechanism-based fluorinated xylobiose inhibitor that forms a stable covalent linkage to E78, the pKa of E172 is *lowered* to 4.2. Therefore the pKa of this catalytic Glu residue "cycles" over the course of the hydrolysis reaction to match its function as a general acid and then base. We are investigating the structural and electrostatic factors that establish the pKa's of the active site Glu residues, as well as those of the seven additional aspartic acids in BCX.

We have also assigned the ^{1}H, ^{13}C, and ^{15}N resonances of this 20.4 kDa protein and are characterizing its solution structure by NOE measurements and dynamics by heteronuclear relaxation studies. We have characterized a conserved buried histidine with a pKa < 2.5 in the native protein.

D2-132 SYNTHESIS AND PRELIMINARY STRUCTURE OF THE FOURTH EGF-LIKE DOMAIN OF THROMBOMODULIN, David P. Meininger, Michael J. Hunter, and Elizabeth A. Komives, Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA 92037

Thrombomodulin is an endothelial cell surface protein which binds to thrombin and changes thrombin's catalytic activity from procoagulant to anticoagulant by switching thrombin's substrate specificity away from fibrinogen and towards protein C. Throbomodulin contains six epidermal growth factor (EGF)-like domains. The fourth and fifth EGF domains are required for the activation of protein C by the thrombin-thrombomodulin complex. A 44 amino acid peptide corresponding to the fourth EGF domain in thrombomodulin has been synthesized and oxidized under redox conditions to form the three disulphide bonds. The major oxidation product has been shown to have the same disulphide bonding pattern as that found in EGF. This domain appears well structured in solution, and contains regions of B-sheet structure commonly found in EGF-like domains. The 3-dimensional structure of the fourth EGF domain has been determined by two-dimensional NMR spectroscopy at pH 5.2 from over 500 observed NOEs.

D2-133 REFINED SOLUTION STRUCTURE OF HUMAN PROFILIN: COMPARISON WITH THE X-RAY STRUCTURE FOR BOVINE PROFILIN William J. Metzler, Keith L. Constantine, Bennett T. Farmer II, Thomas Lavoie and Luciano Mueller, Department of Macromolecular Structure, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-4000 USA.

Profilin is a ubiquitous eukaryotic protein that binds to both cytosolic actin and the fatty acid phosphatidyl-inositol-4,5-bisphosphate (PIP_2). These dual competitive binding capabilities of profilin suggest that profilin serves as a link between the phosphatidyl inositol cycle and actin polymerization, and thus profilin may be an essential component in the signaling pathway leading to cytoskeletal rearrangement. The refined three dimensional solution structure of human profilin I has been determined using multi-dimensional heteronuclear NMR spectroscopy. Twenty structures were selected to represent the solution conformational ensemble. This ensemble of structures has root-mean-square distance deviations from the mean structure of 0.58Å for the backbone atoms and 0.98 Å for all non-hydrogen atoms. Comparison of the solution structure of human profilin to the crystal structure of bovine profilin reveals that although profilin adopts essentially identical conformations in both states, the solution structure is more compact than the crystal structure. Interestingly, the regions that show the most structural diversity are located at or near the actin binding site of profilin. We suggest that the structural differences are reflective of dynamical properties of profilin that facilitate favorable interactions with actin.

D2-134 STRATEGIC NMR STUDIES OF A PARAMAGNET-CONTAINING ENZYME : SUPEROXIDE DISMUTASE, Anne-Frances Miller, Y. M. Kang, D. L. Sorkin, C. K. Vance, Chemistry and Biophysics Depts., The Johns Hopkins University, Baltimore MD 21218.

We are exploiting NMR as a probe of function as well as structure to better understand fundamental aspects of enzyme catalysis. The enzyme superoxide dismutase (SOD) protects against toxic by-products of O_2 respiration by catalyzing the disproportionation of $O_2^{\cdot-}$. SOD's unique ability to alternately reduce and oxidize the same substrate rests on its ability to provide at least one proton to the reaction. Thus, the pK_A of the proton donor is a key determinant of the reaction energetics. We are exploiting the ability of NMR to directly observe protons and monitor the titrations of individual amino acids, in order to identify the proton donor in SOD and measure its pK_A. In the state of SOD that donates a proton to substrate, the active site contains a paramagnetic Fe^{2+} ion. Although even protons of ligands to Fe are visible, the spectrum of the active site residues is dispersed over 100 ppm, and active site resonances can be semiselectively observed. We observe distinctive shifts in several proton resonances in the pH range of 7.5 to 9.7 and are using specific labeling to identify these with specific amino acids and measure pK_As. We are also determining whether the hydrogen bonding network that supports the active site could conduct protons to it, or propagate conformational effects associated with proton transfer.

D2-135 MECHANISM OF ACTION OF THE CYTOTOXIC DOMAIN OF COLICIN E9 AND ITS INHIBITION BY ITS COGNATE IMMUNITY PROTEIN. G.R. Moore[1], M.J. Osborne[1], S.B.M. Whittaker[1], H. Videler[2], L-Y. Lian[2], A. Pommer[3], R. Wallis[3], R. James[3] and C. Kleanthous[3]. [1]. School of Chemical Sciences, UEA, Norwich NR4 7TJ, U.K. [2]. Biological NMR Centre, University of Leicester, Leicester, U.K. [3]. School of Biological Sciences, UEA, Norwich NR4 7TJ, UK.

Colicins are plasmid-encoded bacterial proteins which have antibiotic activity and are secreted as part of the stress response system of the producing organism. Colicin E9 is a member of the DNase group of E-colicins. These proteins have a mass of 61.5 kDa,and consist of at least three domains. The C-terminal domain, with a mass of 14.5 kDa, carries the cytotoxic DNase activity. We have over-expressed this domain in *E. coli* together with the corresponding 86 amino acid inhibitor protein, which confers immunity against the colicin onto the host cell, and are using NMR to determine the structures of the colicin domain, the inhibitor protein, and their bimolecular complex, and to investigate the mechanism of action of the DNase. In this poster we report the progress we have made on the latter two subjects.

A variety of E colicins and corresponding immunity protein (Im) exist, all exhibiting a high degree of specificity. Thus although Im8 binds to the DNase domain of colicin E9 Im8 does not confer biological immunity against colicin E9. To discover why this is so we have studied ^{15}N labelled Im8 and Im9 interacting with unlabelled E9 DNase by a variety of methods including ^{15}N-^{1}H HSQC NMR. The results of these studies indicate that Im8 binds less tightly to E9 than does Im9 and that, at least in the case of Im9, complex formation is accompanied by a conformational change.

Histidine residues are implicated in the DNA hydrolysis mechanism of the E9 DNase by mutagenesis experiments in which inactive DNase is produced by single site substitutions of two histidines. Investigation of the pK_a values of these histidines, and the effect that Im9 binding and nucleotide binding has on the histidines leads to a mechanistic model that will be presented.

D2-136 THE STRUCTURE AND PEPTIDE BINDING OF FYN-SH3 - AN NMR STUDY, Craig J. Morton, David J.R. Pugh, Emma L.J.Brown, Iain D. Campbell, Oxford Centre for Molecular Sciences, University of Oxford, South Parks Rd, Oxford, OX2 3QU, U.K..

The src family of tyrosine kinases are identified by the presence of three regions of sequence similarity; the kinase domain, the SH2 domain and the SH3 domain. The SH2 and SH3 domains are responsible for regulating the activity of the molecule and controlling its interactions with other proteins. While the activity of the SH2 domain is fairly well understood, the mechanism by which the SH3 domain acts is less clear.

As part of our on-going investigation into the structure and function of SH2 and SH3 domains, we present here the 3-dimensional solution structure of the SH3 domain from the tyrosine kinase fyn and data describing the interaction of fynSH3 with a peptide derived from the p85 subunit of phosphatidylinositol 3' kinase. In agreement with the X-ray and NMR structures previously determined for SH3 domains, fynSH3 forms a compact β structure consisting of two orthogonal β-sheets, each of three strands. Chemical shift analysis of a peptide-SH3 complex identified a region on the molecule opposite the N and C-termini responsible for peptide binding, while the kinetics of the interaction indicate a fast exchange binding mechanism.

D2-137 STRUCTURAL AND DYNAMIC STUDIES OF HUMAN INTERLEUKIN 2.

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Interleukin-2 (IL-2), a 15kDa cytokine, is secreted by T cells and is one of the major determinants in the initial response of the immune system. Recombinant IL-2 has been produced in *E. Coli* as inclusion bodies, solubilized and purified. The secondary structure of wild-type human IL-2 determined by NMR suggested that it is a member of the four-helix bundle family of cytokines [1], in agreement with a corrected crystal structure [2]. It has been found that the NMR spectra of wild-type IL-2 are of relatively poor quality, precluding the determination of a three-dimensional structure. However, it has been possible to study the backbone dynamics of the wild-type protein by ^{15}N NMR methods, and these results will be presented.

Two mutants of IL-2 have been analysed whose spectra are of sufficiently high quality to allow further structural analysis of this important cytokine. The first of these, mutant Phe-42 to Ala (F42A), is unable to bind the α chain of the IL-2 receptor [3]. The solution structure of this mutant has been determined and is compared to the structure of wild-type IL-2 determined by X-ray crystallography. The second mutant IL-2, Leu-94 to Lys, is fully functional and refolds from inclusion bodies 100% more efficiently than wild-type IL-2 [4]: the improved yield allowed the protein to be uniformly ^{15}N and ^{13}C labelled. The 3D structure of this mutant is presented and compared to both the crystal structure of wild-type IL-2 and the structure of the F42A mutant IL-2.

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D2-138 NMR-INVESTIGATIONS OF THE HORMONE/RECEPTOR COMPLEX OF INTERLEUKIN-4

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Interleukin-4 (IL-4) is a typical cytokine, which acts as a pleiotropic growth factor on B- and T-cells. Its biological activities concern proliferation and differentiation of B-cells, immunoglobulin class switching of activated B-cells to IgE and IgG1 and expression of the low affinity receptor of IgE. Therefore IL-4 is of particular interest for medical applications in cancer therapy and allergic diseases.

Due to the potential interest in its mechanism of acting as a hormone, several structural investigations by NMR and X-ray were performed. The combination of mutagenesis studies and NMR investigations identified two distinct functional sites on IL-4 impaired in either receptor binding or receptor activation. The location of these sites on the hormone and the mechanism of binding and activation reveal high similarity to the mechanism obtained from the 3D-model of the human growth hormone/growth hormone receptor. Further insights in the mechanism of the recognition of IL-4 by its high affinity IL-4 receptor α-subunit can be obtained from structural investigations of the hormone/receptor complex. Heteronuclear NMR-experiments on N15-labeled interleukin-4 bound to the extracellular domain of the interleukin-4 receptor α-subunit exhibit details on the binding epitope of the hormone.

D2-139 **THREE DIMENSIONAL SOLUTION STRUCTURE OF CARBOXY-TERMINAL SH2 DOMAIN OF HUMAN SYK,** **Surinder S. Narula,Ruth W. Yuan, Susan E. Adams, Thomas B. Phillips, Jeremy Green, Oluyinka M. Green, Ellen R. Laird, Jennifer Karas,David C. Dalgarno, ARIAD Pharmaceuticals, 26 Landsdowne St., Cambridge, MA 02139.**

Src homology 2 (SH2) domains provide specificity in intracellular signaling processes by binding to specific phosphotyrosine containing peptide sequences. The interaction between SH2 domain proteins and phosphorylated receptor tails is critical for the formation of signal transduction complexes. In the activated mast cell the tyrosine kinase Syk associates with the FcεRI receptor tail. Human Syk is known to have two SH2 domains. The three dimensional solution structure of the carboxy-terminal SH2 domain of human Syk (112 residues) has been investigated using multidimensional heteronuclear nmr methods. The structure is characterized by a central region of β–sheet flanked on either side by one α-helix. The helix close to the C-terminus is less well characterized than the N-terminal helix which may reflect its transient nature. However, many backbone amide protons of the central β–sheet region show slow hydrogen - deuterium exchange rates indicating their restricted accessibility and greater stability of the β–sheet region.

D2-140 **ASSIGNMENTS AND SECONDARY STRUCTURE OF HUMAN APOLIPOPROTEIN(A) KRINGLE IV37.** **N. R. Nirmala*, Kayhan G. Akyel*, John. S. Gounarides*, Philip V. LoGrasso* # and Brian Boettcher*, *Departments of Central Technologies & Atherosclerosis and Vascular Biology, Preclinical Research, Sandoz Research Institute, Sandoz Pharmaceuticals Corporation, 59 Route 10, East Hanover, NJ 07936; # current address: Merck Research Laboratories, P.O. Box 2000, R50A300, Rahway, NJ 07065.**

Kringles are lysine-binding domains composed of roughly 85 amino acids with three intramolecular disulfide bridges.These domains are found in many proteins associated with fibrinolysis including apolipoprotein(a), plasminogen,tissue plasminogen activator and urokinase. A number of studies have associated high levels of lipoprotein (a) (Lp(a)) with coronary artery disease, cerebrovascular disease and peripheral vascular disease. There also exists evidence to suggest that the Kringle IV37 (KIV37) domain of apolipoprotein(a) plays a major role in the binding of Lp(a) to fibrin and cellular surfaces through lysine-dependent interactions.

A portion of KIV37 was cloned and expressed in *E.coli* (LoGrasso et al., J. Biol. Chem. 269 21820-21827 (1994)). The purified protein was then subjected to NMR Spectroscopy. Assignments were made based on two-dimensional homonuclear spectra and three-dimensional heteronuclear spectra. The assignments and secondary structure of KIV37 will be presented.

D2-141 SOLUTION STRUCTURE OF THE N-TERMINAL RECEIVER DOMAIN OF NTRC AND INITIAL STUDIES ON THE MAGNESIUM BOUND AND PHOSPHORYLATED FORMS. Michael J. Nohaile, Brian F. Volkman, Sydney Kustu, and David. E. Wemmer. Department of Chemistry, University of California, Berkeley, CA 94720.

Two-component regulatory systems have been widely studied in bacteria and recently identified in yeast. A kinase domain and the receiver domain, which is phosphorylated, are the conserved features of all two-component systems. NTRC is a transcriptional enhancer-binding protein involved in nitrogen regulation. Phosphorylation of the receiver domain of NTRC turns on an ATPase in the central domain of NTRC, which activates transcription of genes controlling nitrogen metabolism. Using 3- and 4-dimensional NMR spectroscopy, we have completed the ^{1}H, ^{15}N , and ^{13}C resonance assignments and used distance geometry methods to determine the solution structure of the N-terminal receiver domain of the NTRC protein. The receiver domain is comprised of the N-terminal 124 amino acids of NTRC, and contains five α-helices and a five-stranded parallel β-sheet in a $(\beta\text{-}\alpha)_5$ topology. Studies on the magnesium bound and phosphorylated forms of the protein will be presented. Also, implications for the further characterization of the signal transduction by receiver domains will be discussed.

D2-142 THE NMR-DERIVED SOLUTION STRUCTURE OF THE IMMUNITY PROTEIN OF COLICIN E9. M.J. Osborne[1], L-Y. Lian[2], A. Breeze[3], A. Reilly[4], R. Wallis[4], R. James[4],C. Kleanthous[4] and G.R. Moore[1]. [1] School of Chemical Sciences, UEA, Norwich NR4 7TJ, UK. [2] Biological NMR Centre, University of Leicester, Leicester, UK. [3] Zeneca Pharmaceuticals, Alderley Park, Macclesfield Cheshire SK10 4TG, UK. [4] School of Biologial Sciences, UEA, Norwich NR4 7TJ, U.K.

E group colicins are plasmid encoded bacterial toxins which recognise and bind the btuB receptor of *E. coli* cells, which is formally required for vitamin B12 transport. These toxins are released from the host bacterial cell in response to DNA damage (*via* an *SOS* lexA/recA dependent system) whereupon they bind to the appropriate receptor and translocate into the foreign bacterial cell by a little understood process involving the tolA pathway. Several cytotoxic classes of E-colicins exist; we are interested in the non-specific DNases (RNases and pore forming activities are also known) E2, E7, E8 and E9.

To protect the producing cell against the cytotoxic activity of its colicin, the E-colicin producing cell co-synthesises a small inhibitor protein (known as an Immunity Protein) which binds to the enzymic C-terminal domain of the E-colicin and neutralises its cytotoxic activity.

We are studying the mechanism of the interaction between the DNase-E-colicin and its immunity proteins and the specificity of these interactions. This specificity is highlighted by the fact that an immunity protein will only protect *E. coli* cells against its cognate E-colicin, even though the four DNase E-colicins and their immunity proteins exhibit a high degree of sequence homology (~80% and 60%, respectively).

To understand fully the mechanism and specificity of these interactions a high resolution structure is required. Since crystallization of the DNase colicins and immunity proteins have proved unsuccessful we have employed 2D and 3D multinuclear NMR experiments on an isotopically enriched immunity protein of colicin E9 (Im9 ≈ 95 kDa) with the aim of determining its 3D solution structure. Complete assignment of ^{13}C, ^{15}N and ^{1}H NMR signals have been made and a solution structure compiled which comprises three helices, spanning residues 12-24, 33-45 and 66-77. Biochemical and mutagenesis studies have established that residues comprising the central helix mediates specificity and a model for the uniqueness of the interaction of the immunity proteins and the DNase E-colicins is presented.

D2-143 REFINED STRUCTURE AND SIDECHAIN DYNAMICS OF AN SH2 DOMAIN FROM PLC-γ1 COMPLEXED WITH A pY1021 PEPTIDE FROM PDGFR

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Binding of an extra-cellular messenger to a receptor tyrosine kinase causes auto-phosphorylation of several tyrosine residues in the cytoplasmic domain of the receptor protein. These phosphotyrosine residues are then specifically recognized and bound by SH2 domains of intra-cellular proteins which function to couple the external signal with intra-cellular activation of enzymes specific for a number of different biochemical pathways, often leading to cell growth.

The structure of a specific complex of the C-terminal SH2 domain from the γ1 isoform of phospholipase C (PLC-γ1) with a 12 residue peptide from the Tyr-1021 site of platelet derived growth factor receptor (PDGFR) has been determined to high precision by 2D, 3D and 4D NMR. Backbone and sidechain positions of the peptide-SH2 hydrophobic interface region have been determined to an rmsd of 0.55 Å, while regions of the backbone including the central beta sheet and the interface have been determined to an rmsd of 0.3 Å. This degree of refinement allows detailed analysis of the mechanism of specific SH2-phosphopeptide recognition. Also, dynamic and exchange properties of SH2 arginine sidechain groups involved in hydrogen bonds with the peptide phosphate group have been studied and used to further elucidate the nature of the interaction.

D2-144 STRUCTURAL STUDIES OF THE REPEAT MOTIF OF α-SPECTRIN BY NMR SPECTROSCOPY. *Jaime Pascual, Matti Saraste, Annalisa Pastore and Chris Sander* European Molecular Biology Laboratory (EMBL). Meyerhofstrasse 1, Heidelberg, D-69012 Germany.

The cell's shape is determinated by an integrated system of molecules called cytoskeleton. Spectrin is a major component and interacts with actin, ankyrin, band 3 and others[1]. It is an elongated flexible molecule whose primary structure is dominated by tandem homologous 106-residues motifs. Because the repeated segments account for most of the lenght of the protein, the structure of these repeats must in large part determine the flexibility and mechanochemical properties of the meshworks they form.

Sequence analysis suggests that the repeat folds into a triple stranded alpha helical coiled coil bundle[2]. Up to now, there is only a crystal structure of a homodimer of the 14th repeat of Drosophila melanogaster alpha spectrin[3].

The 16th repeat of chicken brain alpha spectrin[4] was cloned and expressed in E. coli. ^{15}N and ^{13}C uniformly labeled samples have been used to acquire a variety of three dimensional doble and triple resonance experiments. Sequencial assingment and structure calculations are under progress.

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D2-145 NMR STUDIES ON THE C-TERMINAL DOMAIN OF THE HUMAN GUANINE NUCLEOTIDE EXCHANGE FACTOR EF-1ß, Janice M.J. Pérez[1], Arnout P. Kalverda[2], Jan Dijk[1], Gerard W. Canters[2], Wim Möller[1], [1] Department of Medical Biochemistry, Sylvius Laboratories, Leiden University, Wassenaarseweg 72, 2333 AL [2] Leiden institute of chemistry, Gorlaeus Laboratories, Leiden University

The protein elongation factor EF-1α in the GTP form delivers aminoacyl-tRNA to the empty A site of the ribosome. During this process GTP is hydrolysed to GDP and EF-1α becomes inactive. At this stage EF-1ß binds to EF-1α and induces the dissociation of GDP and the re-uptake of GTP. This nucleotide exchange reaction does not only occur during protein synthesis but also in signal transduction pathways. Knowledge of the 3D structure of EF-1ß will give a more detailed understanding of these processes. Previous structural studies carried out in our lab on EF-1ß from *Artemia* showed the existence of two domains. The C-terminal region (amino acids 106-206) of EF-1ß from *Artemia* still possesses the full nucleotide exchange activity of the intact protein. Based on this finding we decided to obtain the two domains of the human factor by PCR amplification of the recently cloned EF-1ß cDNA. These clones were ligated in a pET-11a vector and brought to overexpression in E.coli. The recombinant protein of the C-terminal domain proved to be active in a heterologous assay with EF-1α from *Artemia*. The NMR data acquired on a 600MHz NMR spectrometer were of good quality and showed that rEF-1ß adopts a well-defined structure in solution. Structural elements such as α-helices and ß-sheets could be recognized in the NOESY spectra. Several spinsystems could already be assigned. Sequential assignment was made possible by enrichment of the protein with ^{15}N isotope.

D2-146 DYNAMIC STUDIES OF A FIBRONECTIN TYPE I MODULE PAIR BY NMR SPECTROSCOPY: AN ANISOTROPIC MODEL, Isabelle Q.H. Phan, Jonathan Boyd, Michael J. Williams[§], Bruno M. Kieffer[§§], and Iain D. Campbell, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, U.K., [§]The Scripps Research Institute, La Jolla CA, [§§]Institut de Biologie Moléculaire et Cellulaire du CNRS, Rue Descartes, Strasbourg, France

We have determined the fold of the fibronectin $^4F1^5F1$ module pair, which contains 93 residues, by ^1H-NMR. The five residue linker sequence between $^4F1$ and $^5F1$ forms a tight turn-like structure, and four loops are involved in intermodule interactions. The relative orientation of the two modules in the pair was estimated by calculating the tilt and twist angles between $^4F1$ and $^5F1$. The restricted range of values obtained, 4.9° to 39.0° for the tilt and -0.1° to 49.4° for the twist, seems to indicate that there is a preferred orientation of one module with respect to the other. To assess the degree of rigidity of the module pair, we studied the backbone motion of a $^4F1^5F1$ sample uniformly labelled with ^{15}N. For this purpose, T1 and T2 relaxation rates and the ^1H-^{15}N nuclear Overhauser effect (NOE) were experimentally determined for 93% of the backbone amides. No decrease in the ^1H-^{15}N NOE was observed for the 3 residues in the linker region for which NOE data was available, in marked contrast to the sharp 'dip' in the NOE value observed for the flexible helical hinge of calmodulin. Furthermore, T1 and T2 relaxation times determined for $^4F1^5F1$ appear to cluster around one set of T1 and T2 value, around 600 and 90 msec respectively, without distinction between the two modules. Theoretical T1 and T2 were calculated using the isotropic approximation, neglecting the effect of rapid internal motion. Under these assumptions, an apparent rotational correlation time of 7.9 ns was obtained. However, it is apparent from the structure that the $^4F1^5F1$ molecule is not spherical, but rod-like, and that therefore the isotropic model is not appropriate for the interpretation of the ^{15}N relaxation data. It is known that for an axially symmetric molecule, the spectral density function at each ^{15}N amide site is dependent on the angle α between the backbone N-H bond and the main axis of symmetry, and also on the two components of the rotational diffusion tensor. We determined these using a hydrated beads model, and from the α angles, we performed a back-calculation of T1/T2 for each residue. We found that at 600MHz, fluctuations of up to 30% in the T1/T2 ratio could be explained by the anisotropic effect alone.

D2-147 MULTIDIMENSIONAL ^1H-^{13}C-^{15}N NMR SPECTROSCOPY OF THE MAC-1 β2 INTEGRIN α-SUBUNIT I DOMAIN: PROGRESS TOWARDS DETERMINING THE SOLUTION STRUCTURE, John R. Pollock, Terrence A. Scahill, Michael B. Fairbanks, Robert L. Heinrikson and Brian J. Stockman, Upjohn Laboratories, The Upjohn Company, Kalamazoo, MI 49001

Integrins are a family of cell surface proteins that mediate cell-cell and cell-extracellular matrix adhesion. In addition to their roles in fundamental cellular processes such as anchorage, migration, growth and differentiation, integrins are involved in platelet aggregation, immune functions, tissue repair and tumor migration. The I domain of the Mac-1 β_2 integrin α subunit has been shown to be the major recognition site for several adhesion ligands, including iC3b, fibrinogen and ICAM-1. Understanding the molecular basis for I domain/ligand recognition is of critical importance to the design of small molecules that may alter these interactions. Since detailed structural studies of the entire Mac-1 α (160 kDa) subunit would represent a significant challenge, we have chosen to investigate the structure of the I domain portion alone. Two-dimensional ^1H NMR spectroscopy indicates that the I domain adopts a predominantly folded structure in solution, indicating that the I domain is a legitimate choice for functional and structural investigation. We are currently in the process of elucidating the detailed solution structure of the I domain. Resonance assignments have been obtained for one-third of the residues by analysis of heteronuclear three-dimensional NMR spectra recorded on [^{15}N]I domain and [^{13}C,^{15}N]I domain. Characteristic NOE patterns and $^{13}C^{\alpha}$ chemical shift values for these residues have so far delineated helical elements in the I domain. The location of the helices in the primary sequence is in good agreement with predicted locations of helical structure [S. J. Perkins et al., *J. Mol. Biol. 238*, 104-119 (1994)]. Additional resonances have chemical shifts characteristic of β-sheet structure.

D2-148 SOLUTION STRUCTURE OF CALCYCLIN DIMER, A CELL-CYCLE DEPENDENT CA^{2+}-BINDING PROTEIN, Barbara C. Potts[1], Goran Carlstrom[1], Mikael Akke[1], K. Okazaki[2], H. Hidaka[2], and W.J. Chazin[1], [1]The Scripps Research Institute, La Jolla, CA 92037, [2]Nagoya University School of Medicine, Nagoya, Japan

A primary strategy for studying growth regulation involves the search for genes or gene products that undergo cell-cycle dependent changes of expression. One such example is calcyclin, a product of the 2A9 gene which is preferentially expressed in G_1 phase. A growing body of evidence points to a specific regulatory function for this protein which involves modulation of the activity of a membrane-associated annexin, CAP-50. Calcyclin is a member of the S-100 branch of the calmodulin superfamily of Ca^{2+}-binding proteins which is characterized by the EF-hand binding motif. Distinctive features of the S-100 subfamily include an affinity for both calcium and zinc ions, as well as the propensity to form disulfide-linked dimers; these features are apparent in calcyclin isolated from Ehrlich ascites tumor cells. Interestingly, calcyclin from rabbit contains no free cysteine residues, precluding disulfide-linked dimer formation. However, our NMR data and other evidence indicate that rabbit calcyclin also forms homodimers in solution.

Calcyclin from rabbit (90 amino acids/monomer) was studied in the apo state. All resonance assignments and input constraints for structure calculations were obtained from homonuclear ^1H NMR data acquired from a single 8 mg sample of native protein. Despite linewidths exceeding 10 Hz, >95% of all proton resonances were assigned, allowing identification of the elements of secondary structure and determination of the global folding pattern. Four distinct helices were identified along with an antiparallel β-type interaction between the two putative Ca^{2+}-binding loops, consistent with two EF-hand motifs for the monomer structure.

Over 550 proton-proton distance constraints have been identified and used as input for distance geometry (DG) and restrained molecular dynamics (rMD) calculations. Initially, only those NOEs consistent with a monomer were included; this strategy generated a family of monomer structures that are highly homologous to the S-100 protein calbindin D_{9k}. The results of structure calculations for the symmetric homodimer will be presented, generated by a combination of DG and rMD strategies.

D2-149 HIGH RESOLUTION NMR STUDIES OF THE AMINO TERMINAL REGION OF FIBRONECTIN, Jennifer R. Potts, Isabelle Q. Phan, ¶Moshe M. Werber, Andrew R. Pickford and Iain D. Campbell, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, U.K., ¶BioTechnology General (Israel) Ltd., Rehovot, Israel

Fibronectin is a ubiquitous extracellular glycoprotein which plays a major role in many important physiological processes such as embryogenesis and wound healing. Fibronectin is a mosaic protein composed of three types of protein module (Fn1, Fn2 and Fn3). NMR studies of Fn1 modules from fibronectin and t-PA have revealed a consensus fold characterised by a short double-stranded β-sheet (strands A and B) followed by a triple-stranded β-sheet (strands C, D, and E). The structure is stabilised by two disulphide bonds, and by hydrophobic interactions. It has become increasingly clear that the way the Fn modules fit together is important for function. The amino terminal region of fibronectin contains five Fn1 modules joined by linker regions of between five and nine residues. ^{4}Fn1 and ^{5}Fn1 are joined by a five residue linker and NMR structural studies of this module pair carried out previously in this laboratory revealed that each module forms a stable compact structure having the consensus Fn1 fold and that the ^{4}Fn1 docks onto the ^{5}Fn1 module *via* a hydrophobic interface involving residues at the N-terminal end of strand D of ^{4}Fn1 and strands A and B of ^{5}Fn1. There is a second inter-module interface involving the linker region and the turns between strands B and C and between strands D and E of ^{5}Fn1[1]. There is evidence that the intact N-terminal region of fibronectin is required for binding to *Staphylococcus aureus* and for assembly into a fibrillar matrix, thus we are interested if the intermodule interfaces evident in the ^{4}Fn1^{5}Fn1 module pair are also present between a pair of Fn1 modules joined by the longer nine residue linker. In the present work we have determined the solution structure of the ^{1}Fn1^{2}Fn1 module pair obtained as a refolded *E. coli*-expressed recombinant 12kD protein [2] and of the ^{1}Fn1 single module expressed in using a yeast α-factor expression system. We compare the structure of ^{1}Fn1 in isolation with its structure in the module pair and the structures of the ^{1}Fn1^{2}Fn1 and ^{4}Fn1^{5}Fn1 module pairs to assess the effect of the longer linker on the intermodule interface.

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D2-150 TOWARDS THE SOLUTION STRUCTURE OF ONCOSTATIN M, A 23 kDa α-HELICAL PROTEIN. Robert Powers, Franklin J. Moy#, Ross C. Hoffman*,Virginia Price*, Julie King*, Eric A. Frieden*, Beverly J. Castner*, Mike Comeau*, and Carl J. March*, Department of Protein Chemistry and #Spectroscopy Support and Protein Analysis American Cyanamid, Medical Research Division, Pearl River, NY and *Immunex Corp. 51 University St. Seattle, WA 98101

Oncostatin M (196 residues, 23 kDa) is a member of the cytokine family that includes interleukin-6 (IL-6), leukemia inhibitory factor (LIF), and granulocyte-colony stimulating factor (G-CSF); lymphoid cell-derived cytokines that share a number of functional and structural properties. Functionally, they induce differentiation of murine myeloid leukemia cells to macrophages and induce acute-phase protein synthesis in hepatocytes. It has been shown that these shared functions are mediated by the signal transducing membrane glycoprotein, gp130, that interacts with the extracellular domains of these cytokine receptors. In addition to its familial attributes, Onco M inhibits the proliferation of several tumor cell lines, e.g. human A375 melanoma cells. Structurally the members of this family are believed to be related to growth hormone, a four helix-bundle with characteristic up-up-down-down helix topology.

In order to better understand the structure/function relationship in Onco M specifically and this cytokine family in general, a structural study of Onco M is underway. A mutant version of human Onco M in which two N-linked glycosylation sites and an unpaired cysteine have been mutated to alanine (N76A/C81A/N193A) has been expressed and shown to be as active as wt Onco M. The triple mutant has been doubly isotope-labeled with 13C and 15N in order to utilize heteronuclear multi-dimensional NMR techniques for structure determination. Approximately 90% of the backbone resonances have been assigned by a combination of triple resonance experiments: HNCA, HNCO, CBCACONH, HBHACONH, HNHA and HCACO; intra and sequential NOEs observed in the 3D ^{15}N-NOESY-HMQC and ^{13}C-NOESY-HMQC experiments and side-chain information obtained from the CCONH and HCCONH experiments. Preliminary analysis of the ^{15}N-NOESY-HMQC spectra's NOE pattern and the $^{13}C\alpha$ and $^{13}C\beta$ secondary chemical shifts predict a secondary structure for Onco M consisting of four α–helices with two intervening helical turns, consistent with the four helix-bundle motif predicted for this cytokine family.

D2-151 NMR ASSIGNMENTS OF MURINE LEUKAEMIA INHIBITORY FACTOR

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Leukaemia inhibitory factor (LIF) is a cytokine involved in the cellular control and development of many types of mammalian cells. Originally named after its effects on a cancerous cell line, it also regulates haematopoietic, neural, and liver cells, and is necessary for the implantation of mouse blastocysts [1]. It shares some activities with the related cytokines oncostatin-M and ciliary neurotrophic factor. It is a member of the four α-helix bundle structural family of cytokines, which include growth hormone and granulocyte colony stimulating factor. With a molecular weight of 20 kDa and extensive α-helical content, homonuclear spectra of LIF are too complex to analyse. We have therefore isotopically labelled recombinant murine LIF with ^{15}N and ^{13}C and are using heteronuclear multi-dimensional NMR methods to gain structural information. We report here the sequential assignment of the backbone resonances of murine LIF and progress towards the determination of a three-dimensional solution structure. Comparing a solution structure with the recently reported crystal structure of LIF [2] will lead to better understanding of the structure and dynamics of this cytokine.

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D2-152 The Three-dimensional Solution Structure of Human Thioredoxin Complexed with Its Target Binding Site from NFκB

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Thioredoxin has been recently found to be involved in the redox regulation of the DNA binding activities of several transcriptional factors such as NFκB, AP-1 etc. To characterize the detailed molecular mechanism of this important biological process, we have determined the high-resolution three-dimensional solution structure of human thioredoxin mutant (Cys35-Ala35) complexed with its target binding peptide from NFκB using multi-dimensional heteronuclear NMR spectroscopy. A total of 60 structures were calculated by means of hybrid distance geometry-simulated annealing, and the atomic rms distribution about the mean coordinate positions is ~0.2 Å for the backbone atoms, ~0.5 Å for all atoms. The binding peptide was found to be located in a cleft of the thioredoxin by forming extensive contacts including hydrophobic interactions, hydrogen bonding, and salt bridges. The results reveal that the redox reaction between thioredoxin and FNκB may be facilitated through the specific interactions between the two proteins, and thioredoxin therefore serves as a molecular redox sensor for regulating the DNA binding activity of the NFκB.

D2-153 CORRELATION OF PROTEIN STRUCTURE AND FUNCTION---AN INVESTIGATION BY NMR,

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With a view toward correlating protein structure and function, the mechanism of histidine phosphorylation in the *Escherichia coli* phosphoenolpyruvate:sugar phosphotransferase system (PTS) was investigated using NMR. A series of phospho-protein intermediates are involved in the phosphorylation and the concomitant translocation of sugars across the bacterial membrane. Two of the intermediates, HPr and EIIA^{glc} form a complex with a molecular weight of 27 kD, thus being fairly amenable to NMR studies. HPr serves as the phosphoryl group donor to EIIA^{glc}, with transfer occuring between histidine side chains on each protein. EIIA^{glc} has two histidine residues, H90 and H75. H90 is important for phosphoacceptor activity and H75 is crucial for phosphotransfer activity. A complex of P-His-HPr and EIIA^{glc} will be difficult to study due to (1) rapid transfer of the phosphoryl group from HPr to EIIA and (2) rapid phosphohydrolysis of the N1-P bond in HPr. Hence, a mutant, H90Q EIIA^{glc}, which abolishes phosphoacceptor activity was chosen for the study. X-ray studies have shown that H90Q EIIA^{glc} is structurally similar to EIIA^{glc}. A constant level of P-His-HPr was maintained using an *in-situ* enzymatic regeneration system. A complex of P-His-HPr and H90Q EIIA^{glc}, thus presents a novel system for study by NMR.

Using labeled HPr and unlabeled H90Q EIIA^{glc}, a variety of $^{13}C/^{15}N$-edited experiments are performed to investigated the changes undergone by HPr, the smaller of the two components in the complex. Assignments are done by standard 3D heteronuclear methods. ^{15}N T_{1s} and T_{2s} and amide proton exchange rates are measured for both the free and complexed forms of HPr. The tautomeric state of the histidine ring in HPr is determined. Intermolecular NOEs are also investigated in order to understand the role of conserved residues in HPr.

D2-154 THREE-DIMENSIONAL STRUCTURE OF A BIOTINYL DOMAIN FROM THE BIOTIN CARBOXYL CARRIER PROTEIN OF *ESCHERICHIA COLI* ACETYL-COA CARBOXYLASE AND ITS INTERACTIONS WITH THE BIOTINYLATING ENZYME, BIOTINYL LIGASE.

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The high definition structure of the biotinylated domain from the biotin carboxyl carrier protein (BCCP) of *Escherichia coli* acetyl-CoA carboxylase (1) has been determined by means of 2D and 3D nuclear magnetic resonance (NMR) spectroscopy. Nuclear Overhauser effect distance constraints, dihedral angle restraints and hydrogen bond restraints were employed as the input for the structure calculations, which were performed using the program X-PLOR. The overall structure of the biotinyl domain (comprising the C-terminal 87 residues of the *E. coli* BCCP sequence), is that of a flattened eight-stranded β-barrel folded around a core of well-defined hydrophobic residues. In the biosynthesis of BCCP a biotin molecule is attached through its carboxyl group, via an amide linkage, to the biotinyl domain. The biotinylation site, lysine 50, is located in the middle of a protruding β-turn, and the N- and C-terminal residues of the domain are close together in adjacent β-strands at the opposite end of the molecule. The protein backbone exhibits a 2-fold axis of quasi-symmetry and the structure closely resembles that of the lipoyl domain of 2-oxo acid dehydrogenase complexes (2). The interaction of the biotinyl domain with the enzyme biotinyl ligase (3) which catalyses the post-translational modification is being studied by NMR spectroscopy to identify residues involved in this interaction.

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D2-155 BACKBONE NMR ASSIGNMENTS, SECONDARY STRUCTURE AND Ca^{2+} BINDING PROPERTIES OF THE FIRST C2 DOMAIN OF SYNAPTOTAGMIN.

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Synaptotagmin is a synaptic vesicle protein that has been proposed to be the Ca^{2+} receptor that triggers exocytosis, due to its Ca^{2+}-dependent phospholipid binding properties [Brose et al (1992) *Science 256*, 1021]. These properties can be ascribed to the presence in the cytoplasmic region of synaptotagmin of two repeats homologous to the C2 regulatory region of protein kinase C. We are analyzing the structure and Ca^{2+}-binding properties of the first C2 domain of synaptotagmin using a combination of spectroscopic techniques. We have assigned all the backbone ^{1}H and ^{15}N resonances and most of the side chains of the first C2 domain, in the presence and absence of Ca^{2+}, using multidimensional NMR methods. The secondary structure of both forms of the C2 domain consists of two four-stranded β-sheets. Substantial changes in chemical shifts upon addition of Ca^{2+} show that the C2 domain binds Ca^{2+} even in the absence of phospholipids, and suggest that Ca^{2+} induces a conformational change. These conclusions are consistent with the observation of a slight change in the circular dichroism spectrum of the C2 domain upon Ca^{2+} titration, and of a Ca^{2+}-induced increase of 15 °C in the temperature of denaturation (T_m). Analysis of the Ca^{2+} affinity of the C2 domain and of Ca^{2+}-induced conformational changes, together with the implications of these results for the function of synaptotagmin, will be presented.

D2-156 **SOLUTION STRUCTURE OF RANTES, A C-C CHEMOKINE**, Nicholas J. Skelton, Fernando Aspiras & Thomas J. Schall, Department of Protein Engineering, Genentech Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080, USA.

^{1}H NMR has been used to investigate the structural properties of RANTES, a protein from the C-C branch of the chemotactic cytokine family that has a strong chemoattractive effect on monocytes, lymphocytes and eosinophils. RANTES is extensively aggregated in solution above pH 4.0, hence resonance assignments were obtained at pH 3.7 where the protein is dimeric (K_d = 35 μM for dissociation to monomer). Structures of the monomer units within the dimer have been determined using 13.9 NMR-derived restraints per residue as input to distance geometry and restrained molecular dynamics calculations. The structure is well defined for residues 9 to 31 and 37 to 66 (backbone RMSD 0.52±0.08 Å), and consists of a C-terminal α-helix packing against a three stranded antiparallel β-sheet. The observed intermolecular NOEs indicate that the quaternary structure is very different to that of the C-X-C chemokines IL-8 and MGSA, but similar to that found for the C-C chemokine MIP-1β. Over 70 intermolecular NOEs have been identified, allowing determination of a structure for the RANTES dimer. The quaternary structure is subtly different from that of MIP-1β, possibly because of differences in the primary sequence at the interface. The biological implications of the structures and the pH dependent aggregation of RANTES are discussed with regard to the distinct biological functions of C-C and C-X-C chemokines. At the monomer level, some differences are noted between RANTES and other chemokine molecules suggesting a structural basis for differences in receptor interactions.

D2-157 NMR EVIDENCE FOR TWO EXCHANGING CONFORMATIONS OF THE HUMAN α3-CHAIN TYPE VI COLLAGEN C-TERMINAL KUNITZ DOMAIN, Morten Dahl Sørensen[1], Jens J. Led[1], Søren Bjørn[2], Ole W. Sørensen[2] and Kjeld Norris[2], [1]Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen Ø, Denmark, [2]Novo Nordisk A/S, DK-2880 Bagsværd, Denmark.

The Kunitz domain, which primarily consists of a central two strand β-sheet and a C-terminal α-helix, is a widespread globular fold of small protein proteinase inhibitors found in living organisms. Recently Wüthrich and coworkers[1] found that the well-known Kunitz type inhibitor BPTI (bovine pancreatic trypsin inhibitor) exists in two exchanging conformations, that differ from one another by the chirality of the Cys14-Cys38 disulfide bond.

Here we present the results of a NMR study of the C-terminal Kunitz domain from human collagen (α3-VI), a 58 residue protein with 33% homology to BPTI including the six cysteines. The results include the sequential assignment, the secondary structure and evidence for two exchanging conformations of the protein.

The sequential assignment was obtained using the standard methods outlined by Wüthrich (1986). The secondary structure determined from characteristic NOE connectivities is strikingly similar to that of BPTI. The observation of TOCSY correlations at 303 K between backbone amide protons shows that the protein exists in two exchanging conformations. The preliminary results suggest that in the case of α3-VI the two conformations may have different chirality of the Cys30-Cys51 disulfide bond which link the secondary structure elements together.

1. G. Otting, E. Liepinsh & K. Wüthrich (1993) Biochemistry *32*, 3571-3582.

2. K. Wüthrich (1986) *NMR of Proteins and Nucleic Acids*, Wiley, New York

D2-158 **THE E-DOMAIN OF *STAPHYLOCOCCAL* PROTEIN A: SOLUTION STRUCTURE AND FV BINDING SURFACE** Melissa A. Starovasnik, Mark P. O'Connell, Dorothea Reilly, Robert F. Kelley, Nicholas J. Skelton, & Wayne J. Fairbrother, Departments of Protein Engineering and Cell Culture, Genentech, Inc., South San Francisco, CA 94080

The extracellular portion of *staphylococcal* protein A contains five homologous domains that each have the ability to bind the Fc portion of IgG. Starting from the N-terminus the domains are designated E, D, A, B, and C, and are 65-90% identical to one another. In addition to Fc binding, the isolated E-domain (56 residues) can bind to Fv fragments having a type III V_H domain with micromolar affinity. Multidimensional NMR spectroscopy is being used to study the structure of E-domain both free in solution and in complex with an Fv. Complete ^{1}H and ^{15}N resonance assignments have been obtained and structures have been calculated for the free domain. The solution structure contains three α-helices that pack together to form a compact helical bundle. The secondary structure of the protein is consistent with that determined previously for the free B-domain by NMR [Gouda *et al.* (1992) *Biochemistry* **31**, 9665-9672]. However, the specific alignment of helix-1 and helix-2 is more similar to that observed in the B-domain/Fc complex determined by X-ray crystallography [Deisenhofer (1981) *Biochemistry* **20**, 2361-2370]. The Fv under study is a noncovalent heterodimer of V_H and V_L domains, composed of 120 and 109 residues, respectively. Titration experiments reveal that E-domain/Fv complex formation exhibits slow-exchange behavior. In contrast to Fc-binding which takes place through residues located within the first and second α-helices, chemical shift mapping suggests that the Fv-binding surface is contained within the second and third α-helices of E-domain. Thus, it appears that Fc and Fv bind to distinct sites on E-domain, consistent with the lack of competition observed between Fc and Fv for E-domain binding.

D2-159 MULTIDIMENSIONAL NMR STUDIES OF A TRANSPORT PROTEIN, MERP, FROM THE BACTERIAL MERCURY DETOXIFICATION SYSTEM. Ruth Steele, Nancy Hamlett, and Stanley J. Opella. Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104 and Department of Biology, Harvey Mudd College, Claremont, CA 91711.

Bacteria with plasmids encoding the mercury detoxification system have the ability to transport Hg(II) across the cell membrane into the cell, where it is reduced to Hg(0) and passively eliminated. The net effect is to allow the bacteria to thrive in environments contaminated with toxic Hg(II) compounds. We are investigating a key protein (from the Tn21 transposon) involved in this process by NMR spectroscopy. MerP (periplasmic) has 72 residues and binds Hg(II). The *merP* gene has been subcloned as a maltose binding protein fusion (MBP). MBP is expressed with a Factor Xa cleavage site and MerP fused to its C-terminal end. MerP has been isolated by affinity chromatography using the well behaved properties of MBP and uniformly labeled with stable isotopes. This results in a substantial improvement of protein yield over previous methods which utilized a heat inducible promoter inversion so that only merP was expressed. The structure of three forms of merP (oxidized, reduced and Hg^{2+} bound) are being determined using triple resonance multidimensional solution NMR. Initial sequential and secondary structure assignments have come from the complementary 3D ^{15}N TOCSY-HMQC, NOESY-HMQC experiments, which give through-bond and through-space correlations. Additional unambiguous sequential assignments were obtained from the 3D ^{15}N/^{13}C HNCA experiment which correlates the intraresidue amide ^{1}H and ^{15}N shifts with its C_a chemical shift and that of the preceding residue. J-coupling constraints were determined using the 3D ^{15}N HNHA experiment. The structure of the protein as determined by NMR will be presented.

D2-160 STRUCTURAL CHANGES CAUSED BY SITE-DIRECTED MUTAGENESIS OF TYROSINE-98 IN *DESULFOVIBRIO VULGARIS* FLAVODOXIN DELINEATED BY ^{1}H AND ^{15}N NMR SPECTROSCOPY: IMPLICATIONS FOR REDOX POTENTIAL MODULATION, Brian J. Stockman[1], Thomas E. Richardson[1] and Richard P. Swenson[2], [1]Upjohn Laboratories, The Upjohn Company, Kalamazoo, MI 49001 and [2]Department of Biochemistry, Ohio State University, Columbus, OH 43210

Flavodoxins mediate electron transfer at low redox potential between the prosthetic groups of other proteins. Interactions between the protein and the flavin mononucleotide cofactor shift both the ox/sq and sq/hq redox potentials significantly from their free-in-solution values. In order to investigate the possible role that the tyrosine at position 98 plays in this process, we have used heteronuclear three-dimensional NMR spectroscopy to determine the solution conformation of wild-type and four position-98 mutants, Y98W, Y98H, Y98A and Y98R, of *Desulfovibrio vulgaris* flavodoxin. Assigned ^{1}H and ^{15}N chemical shifts indicate that the secondary structure and topology of the proteins are identical. However, residues that undergo substantial mutation-induced changes in chemical shift are spread throughout the flavin cofactor binding site. Distance and dihedral angle constraints were used to generate solution structures for the wild-type and mutant proteins. Collectively, the mutant proteins have no gross conformational changes in the flavin binding site. The changes that do occur are minor, and result from the different packing interactions required to accommodate the new side chain at position-98. The solvent accessibility and electrostatic nature of the flavin binding site in the mutant proteins is compared to the wild-type structure. The structural data support the hypothesis that the very low midpoint of the sq/hq couple in the wild-type protein is modulated to a large extent by the energetically unfavorable formation of the flavin hydroquinone anion in the apolar environment of the flavin binding site.

D2-161 **Heteronuclear and Triple Resonance NMR Studies of a 25 kDa Protein: The Extracellular Domain of Human Tissue Factor.**

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Tissue factor (TF) is the cell surface transmembrane receptor that initiates both the extrinsic and intrinsic blood coagulation cascades. TF is implicated in myocardial infarction, stroke, septic shock, and tumor metastasis. The abilities of TF to associate with factor VIIa and factor X in a ternary complex and to enable proteolytic activation of factor X by factor VIIa reside in the 25 kDa extracellular domain of TF. This domain was produced in both *Saccharomyces cerevisiae* and *E. coli* expression systems and a novel renaturation protocol was developed. Protein from these systems had comparable biochemical and physical properties. Protein was isotopically enriched with ^{15}N and ^{13}C, and 3D heteronuclear (TOCSY-HSQC and NOESY-HSQC) and triple resonance (HNCA, HN(CO)CA, HNCO, CBCA(CO)NH and HNCACB) spectra were recorded. Progress towards complete resonance assignments will be described. In addition a comparison of the sensitivities of the experiments will be presented and strategies for improving the spectral quality for large proteins will be discussed.

D2-162 SOLUTION STRUCTURE OF A NOVEL HIRUDIN VARIANT HM2, ITS FRAGMENT 1-47 AND THE MUTANT(N64→V+G65): A NMR AND MD STUDY, Marco Tatò, Luca Baumer, Giuseppe Nicastro and Giorgio Bolis, Analytical Chemistry and Structural Biochemistry, PHARMACIA, via Giovanni XXIII n°23, 20014 Nerviano (MI), Italy

A novel variant of Hirudin, defined as HM2, exhibiting antithrombin activity, has been isolated in Pharmacia-Farmitalia from the leech *H.manillensis*. The 64-residue polypeptide was also efficiently produced in recombinant E.coli (Scacheri et al. 1993, Eur.J.Biochem. *214*, 295). The NMR data of HM2, HM2(1-47) and HM2(N64→V+G65) were obtained at 600 MHz, in water at temperature 301°K and pH=3.3, using 2D and 3D homonuclear NMR techniques. Stereospecific assignments were determined for 28 β-methylene groups and 19 slowly-exchanging NHs, likely H-bonded, were identified. The Hα chemical shifts of the three polypeptides were compared with those of Hirudin-HV1 and its Lys47 Glu mutant (Folkers et al. 1989, Biochem.*28*, 2601). We used the method of Wishart and Sykes (Biochemistry *31*,1647, 1992) to verify the assumption: "Same Structure + Different Sequence = Similar Secondary Chemical Shifts (Δδ)", and to check the secondary structures derived from NOEs against those derived from Δδ (^{1}H and ^{13}C). The 3D solution-structure of the three polypeptides were determined with a Simulated Annealing procedure, using the software Discover/Insight. The structure calculations were based on the NOE restraints togheter with the backbone torsion angle restraints, derived from ^{3}J(NH-Hα), and the stereospecific assignment of 28 βCH_2. A total of some 30 good structures were computed for each of the three polypeptides. The fold of the residues 5-30 and 35-45, which form the core of the N-terminal domain (held togheter by three disulfide linkages), is well determined with an average r.m.s. difference of 0.41, 0.53 and 0.85 Å for the Cα, backbone atoms and all heavy atoms respectively. The C-terminal domain is very flexible, but it tends to fold back on the rest of the protein.

D2-163 SOLUTION STRUCTURE OF SRC HOMOLOGY-2 DOMAIN OF GROWTH FACTOR RECEPTOR BINDING PROTEIN (Grb2). Kevin Thornton and Venkataraman Thanabal, Department of Chemistry, Parke-Davis Pharmaceutical Research, Division of Warner Lambert Company, Ann Arbor, MI 48105.

Src homology-2 (SH2) domains have been identified in proteins that play an important role in the transmission of growth factor initiated signals to the downstream components of cellular signaling pathways. SH2 domains bind to the phosphorylated tyrosine sites on the activated growth factor receptor. Growth factor receptor binding protein (Grb2) is involved in recruiting a Ras activator to the receptor. The structure of the SH2 domain of Grb2 has been studied by multinuclear, multidimensional NMR techniques. Constraints obtained from NOE, coupling constants and amide proton exchange were used to calculate the three dimensional structure. Amide proton exchange rates were used to identify the hydrogen bonding network present in the molecule. Structural elements include the core three stranded anti-parallel β-sheet and two α-helical segments.

D2-164 OVEREXPRESSION AND NMR STUDIES OF THE BLUE COPPER PROTEIN RUSTICYANIN, Anna Toy-Palmer, Danilo Casimiro, Robert C. Blake II†, and H. Jane Dyson, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, †College of Pharmacy, Xavier University, New Orleans, LA.

Rusticyanin, a blue copper protein of the acidophilic bacterium *Thiobacillus ferrooxidans*, acts as a principal component in the iron oxidation pathway. Unusual properties of the 16.5 kD protein include optimal activity and structural stability under highly acidic conditions (pH = 2) and a high redox potential for a single copper site protein (+680 mV vs. NHE). Studies by NMR are underway to determine the source of rusticyanin's unusual characteristics. The ^{15}N-labelled protein, used to complete the NMR sequential assignments, was obtained by growing *T. ferrooxidans* in the presence of $(^{15}NH_4)_2SO_4$. However, this bacterium would not grow on $NaH^{13}CO_3$.

Therefore, to obtain ^{13}C, ^{15}N-doubly labelled rusticyanin, an artificial gene for rusticyanin using codons optimized for *E. coli* was designed and then synthesized utilizing the polymerase chain reaction (PCR) methodology. Insertion into the pET24a plasmid and transformation into the BL21 strain of *E. coli* provided a system in which rusticyanin expression is induced using IPTG. Optimization of growing conditions in minimal media results in the production of ^{13}C, ^{15}N-doubly labelled rusticyanin in yields of >50 mgs/L.

Triple resonance NMR experiments including CBCA(CO)NH and C(CO)NH-TOCSY aided in the assignment of the main chain and side chain carbons of almost all residues, including 13 of 14 prolines that are present in rusticyanin. The Pro Cα and Cβ carbon chemical shifts also revealed the *cis/trans* conformation of each proline. NOEs from the ^{13}C-HSQC-NOESY and the ^{15}N-NOESY-HSQC allow refinement to the preliminary structures obtained through distance geometry and simulated annealing. The structures of rusticyanin are compared to those of related blue copper proteins in order to define which properties contribute to the structural stability and high redox potential of rusticyanin.

D2-165 STRUCTURE OF CALMODULIN/PHOSPHORYLASE KINASE COMPLEXES, APO-CALMODULIN, AND THE APO-CALMODULIN/NEUROMODULIN COMPLEX Jeffrey L. Urbauer, James H. Short, Linda K. Dow, and A. Joshua Wand, Department of Biochemistry and the Program in Biophysics, University of Illinois, Urbana, IL 61801

The structures of the complexes between calmodulin and two different calmodulin binding domains of phosphorylase kinase, as well as the structures of apo-calmodulin and the complex between apo-calmodulin and the calmodulin binding domain of neuromodulin are being examined. Calmodulin binds many target protein molecules in response to the cellular Ca^{2+} concentration. In almost every case, calmodulin and its target peptide totally dissociate from one another when Ca^{2+} is absent. High resolution models of such complexes (i.e. calmodulin/skeletal muscle myosin light chain kinase, Ca^{2+} loaded) have been determined previously. Calmodulin may, however, simply change the manner of its interaction with a target protein in response to Ca^{2+} concentration without dissociation, as in the case of the phosphorylase kinase multisubunit enzyme, of which calmodulin is an integral, non-dissociable subunit. That the interaction between calmodulin and at least one of the calmodulin binding domains of phosphorylase kinase is fundamentally different than interactions between calmodulin and other peptides, complexes for which high resolution structural models are already available, is indicated from results of x-ray and neutron scattering studies. Binding of calmodulin to a target protein may also occur in the absence of Ca^{2+}, with dissociation occuring in the presence of Ca^{2+}, as is the case with the neuromodulin/calmodulin complex. ^{15}N,^{13}C labeled apo-calmodulin, the complex between ^{15}N,^{13}C labeled apo-calmodulin the neuromodulin peptide, and the complexes between ^{15}N,^{13}C labeled calmodulin and two peptides from phosphorylase kinase (Ca^{2+} loaded) have been prepared. The resonances have been assigned using 2D-4D heteronuclear techniques. Secondary structural elements have been determined from standard chemical shift and NOE data. Structural models are being determined from NOE and J coupling based restraint methodologies.

D2-166 THE THREE DIMENSIONAL STRUCTURE OF THE α-SPECTRIN MODULE DETERMINED BY TRIPLE RESONANCE NMR METHODS. K.G. Valentine, T.M. DeSilva, D.W. Speicher and S.J. Opella, Chemistry Department, University of Pennsylvania, Philadelphia, PA 19104 and Wistar Institute for Anatomy and Biology, Philadelphia, PA 19104.

Spectrin is one of a network of proteins in the erythrocyte cells which imparts architecture and pliability to the red cell membrane. Spectrin is composed of a series of tandem motifs forming an elongated flexible molecule. It is constructed as a tetramer containing 2 alpha and 2 beta subunits. Two antiparallel hetero-dimers are linked in a head to tail fashion. A 112 residue segment of a representitive single compact folding motif of α spectrin was expressed in E.coli as a glutathione S-transferase fusion protein. The ^{15}N / ^{13}C labeled protein was studied with multi-nuclear NMR techniques.

^1H , ^{13}C and ^{15}N resonance assignments were analyzed from the 3D ^{15}N,^1H NOESY -HMQC , TOCSY-HMQC and the ^{13}C, ^1H NOESY -HMQC experiments. Sequential assignments were traced from the HNCA and HN(CO)CA and CBCA(CO)NH experiments.Site specific labeling was used to aid in the assignment task.The HNHA experiment was used to measure three bond $J(H^NH\alpha)$ coupling constants. NOE and dihedral angle constraints were used in determining the three dimensional structure. The protocol of simulated annealing and restrained molecular dynamics then energy minimization was used for the structure determination.

D2-167 TWO DIMENSIONAL NMR AND BIOCHEMICAL STUDIES OF FLUORO-Tyr AND FLUORO-Phe CALMODULIN, Hans J. Vogel, Alexis David and Deane D. McIntyre, Department of Biological Sciences, The University of Calgary, Calgary, Canada, T2N 1N4.

Calmodulin (CaM) is a ubiquitous eukaryotic calcium binding regulatory protein that regulates the activity of ≈ 30 different target proteins and enzymes. It comprises 148 amino acids, including 2 Tyr and 8 Phe residues. We have biosynthetically incorporated 30%-90% m-fluoro-Tyr using an *E. coli* expression system, harbouring a synthetic CaM gene. The labelled proteins bound to phenyl-Sepharose, in a calcium-dependent manner, moreover they showed the characteristic calcium-dependent band shift during SDS electrophoresis. As expected, UV titrations showed that the two f-Tyr residues in CaM had a lower pKa than Tyr. F-Tyr-CaM activated Myosin Light Chain Kinase in the usual fashion, but full activation of calcineurin required a two-fold increased CaM concentration. Starting from the known assignments for the proton Tyr resonances, it was possible to assign the ß aliphatic and ring H5 and H6 resonances, from DQF-COSY and NOESY spectra. Interestingly, the ß protons were not markedly affected by the fluoro substitution. The ring H2 proton, as well as the F19 resonances could be assigned with the aid of ^1H, ^{19}F HMQC spectra, starting from the ring H5 protons. The ^{19}F chemical shifts of fTyr99 and fTyr138 are both sensitive to the binding of calcium ions. Surface exposure of these two residues was measured by the solvent perturbation technique using varying ratios of H_20 and D_20; both Tyr are solvent exposed in apo- as well as Ca^{2+}-CaM. Our results confirm that fTyr-CaM does not have a structure that is significantly different from native CaM, making it a suitable probe for future ^{19}F NMR studies of their interactions between CaM and its high molecular weight target proteins; such studies are presently in progress. In addition, similar NMR and biochemical studies with o, m, p fluoro Phe labelled CaM have also been performed (supported by MRC Canada).

D2-168 THE REFINED SOLUTION STRUCTURE OF HUMAN STEFIN A, Jonathan P. Waltho, John R. Martin, C. Jeremy Craven, Roman Jerala+, Eva Žerovnik+ and Vito Turk+, Krebs Institute, University of Sheffield, S10 2UH, U. K. and +Department of Biochemistry and Molecular Biology, Jozef Stefan Institute, 6100 Ljubljana, Slovenia.

Stefin A is a member of the cystatin superfamily of proteins which are tight and reversibly binding inhibitors of the papain-like cysteine proteinases. These inhibitors are thought to protect the cells from inappropriate endogenous or external proteolysis and are implicated in the mechanism responsible for intracellular or extracellular protein breakdown. Stefin A is a single-chain protein with a molecular weight of *ca* 11kD, which lacks disulphide bonds and carbohydrates. It is found at high concentrations in various types of epithelial cells and in polymorphonuclear leucocytes which has led to the proposal of its involvement in the first line of defence against invasive organisms.

The proton and nitrogen resonances of ^{15}N-enriched human stefin A have been completely assigned using multi-dimensional homo- and heteronuclear NMR methods, and the solution structure of the protein has been determined. Although stefin A exhibits the same global fold as the homologous protein stefin B in its complex with papain, there are some significant differences in regions which are central to the binding event. The principal difference is the presence of two disordered regions in stefin A which form two of the components of the "tripartite wedge" which docks into the active site of the target proteinase. Specifically, these regions are the 5 N-terminal residues and the second binding loop. The dynamic properties of these regions of stefin A in solution have been investigated using ^{15}N relaxation experiments. In addition, rapid back-calculation procedures have been used to refine the solution structure.

D2-169 STRUCTURE-FUNCTION RELATIONSHIPS OF CELLULAR RETINOIC ACID BINDING PROTEINS: SOLUTION NMR STUDIES OF THE WILD-TYPE AND SITE-DIRECTED MUTANTS. Lincong Wang and Honggao Yan, Department of Biochemistry, Michigan State University, East Lansing, MI 48824

Retinoic acid, an active metabolite of vitamin A, has profound effects on cell growth, differentiation, and morphogenesis. Two types of proteins have been found to interact with retinoic acid: nuclear retinoic acid receptors (RARs and RXRs) and cellular retinoic acid binding proteins (CRABPs). RARs and RXRs are retinoic acid-activated transcriptional factors that regulate expression of target genes. Although the physiological roles of CRABPs have not been well defined yet, they are believed to be involved in cellular transport and metabolism of retinoic acid, and modulating cellular retinoic acid concentrations. Although great advances have been made in our understanding of the roles of retinoic acid in recent years, little is known about how retinoic acid interacts with CRABPs, RARs, and RXRs. We are interested in studying the structure-function relationships of CRABPs by a combination of site-directed mutagenesis, thermodynamics, and NMR. CRABPs are monomeric proteins with a molecular weight of 15.7 kDa. Sequential resonance assignment of CRABP II and a site-directed mutant (R112M) has been undertaken by multidimensional multinuclear NMR spectroscopy. The results of sequential resonance assignment will be presented and discussed in relation to the ligand binding properties of the proteins.

D2-170 SOLUTION NMR STUDIES OF HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR. **Jörn M. Werner, Alexander L. Breeze§, and Iain D. Campbell,** Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K. *and* §Protein Structure Laboratory, Zeneca Pharmaceuticals, Mereside, Alderley Park, Cheshire SK10 4TG, U.K.

Human granulocyte colony-stimulating factor (hG-CSF) is a cytokine which is involved in the proliferation, differentiation and maturation of bone marrow haemopoietic stem cells. Structural studies by NMR (1,2) and X-ray crystallography have shown that, like many cytokines, hG-CSF is a 4-helix bundle with an overhand loop topology (3,4).

We are studying the solution-state structure and backbone dynamics of two highly active analogues of hG-CSF (19 kDa; 175 residues) by NMR. The small chemical shift dispersion and very high content of leucine residues (33) of this protein cause severe spectral overlap and have necessitated the application of a range of multidimensional and multiple resonance techniques.

Resonance assignment has been accomplished by means of 2D and 3D ^{15}N-separated experiments on uniformly ^{15}N- and residue type-specific labelled hG-CSF samples, together with ^{1}H-^{15}N-^{13}C triple resonance experiments on ^{15}N-^{13}C double-labelled samples.

The backbone dynamics of hG-CSF in solution have been studied by heteronuclear (^{1}H-^{15}N) relaxation measurements at different field strengths. G-CSF exhibits motion on a wide range of time scales. A detailed analysis of the relaxation data will be presented.

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D2-171 GLYCOPROTEIN STRUCTURE OF THE ADHESION DOMAIN OF THE HUMAN T-CELL RECEPTOR CD2

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CD2, a T-cell specific surface glycoprotein, is critically important for the adherence of T cells to antigen-presenting cells or target cells. In humans, CD2 binds specifically to CD58 expressed on the cell to which the T cell binds. The extracellular amino-terminal 105 residue domain of human CD2 (hu-sCD2$_{105}$) is responsible for cell adhesion and requires a single N-linked carbohydrate to maintain its native conformation and ability to bind CD58.

To better understand the structural aspects that regulate human CD2 adhesion functions we previously determined the solution structure of the protein part of hu-sCD2$_{105}$ using predominantly homonuclear NMR methods and DG calculations [J. M. Withka, D. F. Wyss et al. *Structure* **1**, 69-81 (1993)]. We recently achieved complete ^{1}H and ^{13}C resonance assignments of the heterogeneous high mannose N-glycan in hu-sCD2$_{105}$ on the intact glycoprotein at natural abundance. Protein-glycan contacts as well as intra-carbohydrate NOEs were identified and used together with conformational restraints for the polypeptide to determine the complete structure of the glycoprotein. To our knowledge, this is the first time that the structure has been obtained by NMR for both the protein and glycan components of an intact glycoprotein of this size. Based on the glycoprotein structure a series of mutations was made to probe the requirement of specific interactions observed between the N-glycan and the polypeptide for the ability of CD2 to bind CD58. We have also obtained information about the dynamics of the glycoprotein and further refined the structure of hu-sCD2$_{105}$ using a ^{15}N-labeled sample.

D2-172 **MULTI-NUCLEAR NMR STUDIES OF HYPERFINE-SHIFTED RESONANCES OF *CLOSTRIDIUM PASTEURIANUM* RUBREDOXIN, Bin Xia, Hong Cheng, Jean-Marc Moulis,[#] and John L. Markley, Graduate Biophysics Program and Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706; and [#]DBMS-Métalloprotéines, CENG 85X, 38041 Grenoble, France**

Rubredoxins are non-heme, iron-sulfur proteins. Their active sites consist of an iron atom tetrahedrally coordinated to four cysteine sulfur atoms. Rubredoxin serves as a potential model for NMR studies of hyperfine-shifted resonances in iron-sulfur proteins. The rubredoxin from *Clostridium pateurianum* has been over-expressed in *E. coli* with a protein yield of ~ 30 to 40 mg per liter culture. This protein has been labeled uniformly with ^{15}N and ^{13}C, and labeled selectively with $^{2}H^{\alpha}$-cysteine, $^{2}H^{\beta}$-cysteine, ^{15}N-cysteine, and $^{13}C^{\beta}$-cysteine. The labeled rubredoxin samples have been studied by NMR spectroscopy. Preliminary results from multinuclear NMR studies will be presented along with assignments of hyperfine-shifted resonances from the selective labeling experiments. (Supported by NSF grant MCB-9215142.)

D2-173 SOLUTION STRUCTURE OF THE PH DOMAIN FROM *DROSOPHILA* β–SPECTRIN, Peili Zhang*,†, Sekhar Talluri†, Haiyan Deng§, Daniel Branton§ and Gerhard Wagner*,†, *Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115; †Committee on Higher Degrees in Biophysics, Harvard University, Cambridge, MA 02138; §Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

The solution structure of the PH domain from *drosophila* β-spectrin has been solved by 2D and 3D NMR spectroscopy. The overall structure is similar to previously published structures of other PH domains, consisting of a seven-stranded antiparallel β-sheet with two helices. However, compared to the pleckstrin N-terminal PH domain (1), this domain from *Drosophila* β-spectrin contains an insertion of eight residues at the loop connecting the first and the second β-strand. It undergoes conformational exchange and is ill defined. This is similar to the structure of the PH domain from β-spectrin of mouse brain (2). It may play a role in the localization of the *Drosophila* cytoskeleton. An additional insertion is between the third and fourth β-strand. This insertion, although shorter, is found also in mouse β-spectrin. It adapts a helical conformation.

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D2-174 SOLUTION STRUCTURE OF THE SHC SH2 DOMAIN COMPLEXED WITH A TYROSINE-PHOSPHORYLATED PEPTIDE, Ming-Ming Zhou, Timothy M. Logan, Robert P. Meadows, Ho Sup Yoon, Kodimangalam S. Raichandran*, Steven J. Burakoff*, Stephen, W. Fesik, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, Illinois 60064, *Division of Pediatric Oncology, Dana-Farber Cancer Institute, and Department of Pediatrics, Harvard Medical School, Boston, MA 02115

The *shc* oncogene product couples T-cell activation to the Ras signaling pathway by linking together the tyrosine-phosphorylated ζ chain of the T cell receptor (TCR) with Grb2. Here, we report the solution structure of the SHC SH2 domain complexed to a phosphotyrosine-containing peptide corresponding to the ζ chain of TCR. The structure is compared to previously determined structures of other SH2/phosphopeptide complexes. We also report on the binding specificity of the SHC SH2 domain for different phosphopeptides as determined by NMR.

Peptides; Protein Folding

D2-200 TOWARD SOLVING THE FOLDING PATHWAY OF BARNASE: THE BACKBONE ^{13}C, ^{15}N AND ^{1}H NMR ASSIGNMENTS OF ITS pH- AND UREA-DENATURED STATES. Vickery L. Arcus, Stephane Vuilleumier, Stefan M. V. Freund, Mark Bycroft and Alan R. Fersht,
Medical Research Council Unit for Protein Function and Design, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW United Kingdom.

The structures of the major folding intermediate, the transition state for folding, and the folded state of barnase have been previously characterized. We now add a further step towards a complete picture of the folding of barnase by reporting the backbone ^{15}N, ^{13}C and ^{1}H NMR assignments for the protein unfolded at pH 1.8 and 30°C. We also report NMR assignments for barnase unfolded in 5.5M urea at 30°C. These assignments, which were obtained from a combination of heteronuclear magnetization transfer and backbone triple resonance NMR experiments, constitute the first stage in the structural characterization of these denatured states by NMR. Inter-residue NOE contacts and deviations from ^{1}H random coil chemical shifts provide evidence for stable residual structure in the pH-denatured state. The structured regions span residues in the native protein that contain its major α-helix and central strands of the ß-sheet. Earlier experiments have shown that these regions are predominantly intact in the major folding intermediate and that their docking is partly rate determining in folding.

D2-201 **CONFORMATIONS AND DYNAMICS OF A PENTAPEPTIDE FRAGMENT OF THE SUBUNIT OF THE NICOTINIC-ACETYLCHOLINE RECEPTOR STUDIED BY NMR**
Daina Avizonis, Shauna Farr-Jones, Phyllis Ann Kosen and Vladimir J. Basus, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143.

The nicotinic-Acetylcholine Receptor (nAChR), a ligand-gated sodium channel, exists in 4 states. In the resting state, with no acetylcholine (ACh) bound, the channel is closed. Upon ACh binding, the receptor undergoes an allosteric rearrangement and, the channel opens, allowing cation flow through the membrane for a few milliseconds. Next, the channel enters a fast desensitized or intermediate state where the flow of cations is decreased by a factor of 250. Finally, the receptors fully desensitized and no ion flux is detected across the membrane. The binding of ACh is 10^4 times tighter when the receptor is fully desensitized than the active state[1]. Fluorescence and circular dichroism spectroscopy indicate that the resting and desensitized states of AChR are conformationally dissimilar[2,3]. The conformational transitions that are triggered by binding ACh may involve a change in the conformational state of a rare structural element: an eight membered ring formed by joining cysteines 192 and 193 of the AChR α-subunit. The two cysteines and an adjacent proline 194 are essential for the proper function of all known acetylcholine receptors[4]. We synthesized the pentapeptide, TCCPD, to examine the conformational heterogeneity of this 8-membered ring. ^{1}H-NMR shows that this peptide exists both the *cis*- and *trans*-conformations of the amide bond between the two cysteines. Four different conformations have been assigned at low temperatures. Conformational exchange rate constants were measured using a modified HSQC pulse sequence. From the NMR data, we calculated the thermodynamics and activation energies for the four conformations. The relationship of these data to the function of a molecular switch in the receptor shall be discussed.

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D2-202 SOLUTION STRUCTURE OF SPECTRIN SH3 DOMAIN AND NMR STUDIES ON THREE CIRCULARLY PERMUTATED SEQUENCES. *Francisco J. Blanco, Ana Rosa Viguera and Luis Serrano.* European Molecular Biology Laboratory (EMBL). Meyerhofstrasse 1, Heidelberg, D-69012 Germany.

The Src-homology region 3 domain of chicken spectrin is a β-sheet barrel containing 62 residues[1]. The kinetic and thermodynamic analysis of this protein shows that the folding and unfolding reactions can be described by a two state model, with no accumulating intermediate and with a concomitant recovery of secondary and tertiary structure during refolding[2]. This simple folding mechanism makes this protein a good model to study protein folding.

In order to be able to apply the different NMR based approaches to characterize in detail the folding of this protein the first step is the obtention of the NMR spectrum assignment. We present here the complete ^{1}H and ^{15}N resonances assignment of this domain and the calculated structure based on NOE data in comparison with that in the crystal (the two structures are very similar).

The proximity of the chain termini and the existence of three loops or turns connecting three pairs of antiparallel β-strands offered the possibility of exploring the stability and folding kinetics of all the possible circularly permutated sequences of this protein[3]. The mutant proteins are less stable and refold with different rates than the wild type. Aromatic and methyl protons, appearing in a well resolved region of the one and two-dimensional spectra, indicated that the overall structures of the three chimeric proteins are very similar to that of the wild type. The complete backbone proton assignment obtained for one them shows that the native and mutant protein structures are nearly identical. tese results show that the order of the secondary structure elements does not determine the structure but affects to the folding kinetics.

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D2-203 A STRUCTURAL COMPARISON OF TWO PEPTIDES CORRESPONDING TO HUMAN AND BABOON APOLIPOPROTEIN C-I RESIDUES 1-38. *Garry W. Buchko, Annett Rozek, Patrick Kanda[1] and Robert J. Cushley,* Institute of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada, [1]Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas, U.S.A.

Human apolipoprotein C-I (apoC-I) is a low molecular weight protein (57 a.a.) associated with the lipid of the high density lipoproteins (HDL), very low density lipoproteins (VLDL) and chylomicron subfractions of blood plasma. A family of baboons (*papio* sp.) has been selectively bred which accumulate HDL_1 when fed a diet high in cholesterol and fat. The latter feature is of interest since high concentrations of HDL are negatively correlated with the development of atherosclerosis in both humans and baboons. Associated with the baboon HDL of this family is a protein, corresponding to baboon apoC-I residues 1-38, which may be responsible for the accumulation of HDL by inhibiting the activity of cholesteryl ester tranfer protein (CETP). On the other hand, a synthetic peptide corresponding to residues 1-38 of human apoC-I is only 60% as effective in inhibiting baboon CETP activity. Baboon and human apoC-I residues 1-38 differ in 7 amino acid residues, 6 located towards the C-terminal. To assess the structural features responsible for the different biological activities, the two peptides were studied in deuterated sodium dodecyl sulfate (SDS) solutions using two-dimensional NMR spectroscopy. The proton resonances for both peptides were assigned from TOCSY, NOESY and DQF-COSY data. Preliminary analysis of the H^{α}-secondary shifts indicate both peptides adopt helical structures in the presence of SDS. Detailed structures for both SDS-associated peptides, obtained from distance geometry calculations (DGII), will be presented.

D2-204 STRUCTURE OF BRAZZEIN, A NOVEL SWEET-TASTING PROTEIN, Jane Caldwell,[a] Frits Abildgaard,[b] John L. Markley,[a,b] Ding Ming,[c] Goran Hellekant,[c] [a]Department of Biochemistry, [b]National Magnetic Resonance Facility at Madison, and [c]Department of Veterinary Science, University of Wisconsin-Madison, Madison, WI 53706

The fruit of an African tropical plant, *Pentadiplandra brazzeana* Baillon, contains a small (6 kDa), potently sweet-tasting protein named brazzein. The homonuclear proton 2D NMR resonances of brazzein spin systems have been completely assigned sequentially. A preliminary structure has been calculated using distance geometry with simulated annealing refinement, from constraints derived from ^{1}H-^{1}H NOE cross peak intensities and $^3J_{HNH\alpha}$ coupling constants. The structure reveals that brazzein contains two short alpha helices, which are cradled by a three-stranded beta sheet. This topology is supported by chemical shift calculations, hydrogen exchange data, and coupling constant information.

(Supported by NIH grants GM35976 and RR02301. J.C. is a NSF Predoctoral Fellow.)

D2-205 SECONDARY STRUCTURE CHANGE of HIV-1 gp120 PEPTIDE (413-456) INDUCED BY SOLVENT AND INTERACTION WITH A PEPTIDE FROM CD4 Domain 1: IMPLICATION ON THE MECHANISM OF FUSION BETWEEN HIV-1 AND ITS TRAGET CELLS
Dind-Kwo Chang*, Shu-Fang Cheng, Wei-Jyun Chien and Cheng-Chao Liang, Institute of Chemistry, Academia Sinica, Taipei, Taiwan, Republic of China, 11529

Interaction of the envelope glycoprotein gp120 of HIV-1 with CD4 of T-cell and the ensuing membrane fusion is the first step of HIV-1 replication cycle. We seeked to use spectroscopic technique to understand the structure and toinfer the mechanism.

A peptide of 44 amino acids containing a critical CD4 binding site on C5 of HIV-1 envelope glycoprotein gp120 was systhsized and its secondary structure in aqueous and TFE solutions was determined by NMR and circular dichroism spectroscopies. It was found the peptide exists in aqueous solution primarily in βstrand and turn. α helix content is significantly increased following addition of TFE (trifluoroethanol) by NMR study. The finding is supported by CD study which also shows similar change by complexing the peptide with a peptide of CD4 (39-57) containing an HIV-1 gp120 binding site. Since this domain of HIV-1 gp120 is near a domain involved in association with gp41, its conformational switch may be essential to the fusion of HIV-1 with the membrane of its target cell.

D2-206 2D ^{1}H NMR AND CD STUDIES OF MEP-22, A SYNTHETIC ZINC-BINDING PEPTIDE FROM HUMAN METALLOENDOPEPTIDASE, Woei-Jer Chuang, Ja-Shan Yang , and Yuh-Dar Chang, Department of Biochemistry, National Cheng Kung University Medical College, Tainan, Taiwan 70101 ROC

MEP-22, a peptide based on residues 89-110 of the human intestinal brush border metalloendopeptidase containing a HEXXHXXGXXH consensus sequence for zinc binding site, binds zinc tightly (K_D = 1.5 μM) with a 1:1 stoichiometry as found by CD titration at pH 7.0. CD spectra of MEP-22 in 20 mM HEPES at pH 7.0 show 6.7% helix, 38.6% β-structure, and 54.7% coil. Zinc-binding induces the folding of MEP-22, changing the CD to 20.4% helix, 51.2% β-structure, and 28.4% coil. CD titrations show that MEP-22 also binds Cu^{2+}, Co^{2+}, and Ni^{2+} but not Mg^{2+} divalent metal ions. Both 1D NMR and CD spectra indicate that MEP-22 binds Zn^{2+} in a 1:1 complex. The resonances associated with the δ and ε C-H protons of three His residues of MEP-22 are very close in the absence of Zn^{2+}, but shift into downfield after addition of a equivalent of Zn^{2+}. The 600 MHz proton NMR spectra of MEP-22 with equivalent Zn^{2+} in 20 mM Tris-HCl at pH 7.0 have been 60% assigned by 2D NMR method (DQF-COSY, TOCSY, and NOESY with gradient water suppression). Based on our preliminary results, the structure of MEP-22 is similar to that of the catalytic domain of astacin, stromelysin, and collagenase.

D2-207 CHARGE INTERACTIONS IN THE STRUCTURE DETERMINATION OF SOME ENDOTHELIN ANALOGUES, Murray Coles, Sharon L. A. Munro and David J. Craik., Dept. of Pharmaceutical Chemistry, Victorian College of Pharmacy, Monash University, Victoria Australia.

The Endothelins are a family of endogenous peptide hormones which have important roles in the maintainence of vascular homeostasis. Abnormal levels of the Endothelins have been associated with the pathology of many disease states, including cardiovascular disease, asthma, renal failure, diabetes and cancer. Each member of the Endothelin family consists of 21 residues and is bicyclic via two disulphide bridges. We have used several monocyclic and linear analogues of Endothelin-1, ET-1, (Fig. 1a) as conformational probes. These analogues are formed by replacement of the cysteine residues by α-aminobutyric acid, Aba (X in Fig.1. b-d). Such analogues have been shown to have activity and binding profiles at the two Endothelin receptors very different from the native form.

We present here some of the experimental and computational techniques used to derive the solution conformations of these peptides. In particular a Monte-Carlo based structural refinement protocol for the program XPLOR has been used to include sidechain to backbone charge interactions which are often not included in *in vacuo* simulations.

a) ET-1 CSCSSLMDKECVYFCHLDIIW

b) [1,15 Aba]-ET-1 XSCSSLMDKECVYFXHLDIIW

c) [3,11 Aba]-ET-1 CSXSSLMDKEXVYFCHLDIIW

d) 4ABA-ET1 XSXSSLMDKEXVYFXHLDIIW

D2-208 SOLUTION STRUCTURE OF BMY-29303 (SIAMYCIN II), A PEPTIDE THAT INHIBITS HIV, Keith L. Constantine[1], Mark S. Friedrichs[1], David Detlefsen[2], Maki Nishio[2], Mitsuaki Tsunakawa[2], Hiroaki Ohkuma[2], Toshikazu Oki[2], Susan Hill[2], Robert E. Bruccoleri[1], Pin-Fang Lin[2], Tamotsu Furumai[2] and Luciano Mueller[1], Bristol-Myers Squibb Pharmaceutical Research Institute, [1]Princeton, NJ 08543 and [2]Wallingford, CT 06492.

The 21 amino acid peptides BMY-29303 (siamycin II) and BMY-29304 (simycin I), derived from *Streptomyces* strains AA3891 and AA6532, respectively, have been found to inhibit HIV-1 induced synticia formation. The BMY-29303 and BMY-29304 amino acid sequences were determined by amino acid composition analysis and NMR spectroscopy. The primary sequence of BMY-29303 is CLGIGSCNDFAGCGYAIVCFW (one letter amino acid code). BMY-29304 differs by only one amino acid; it has a Val residue at position 4. In both peptides, disulfide bonds link Cys1 with Cys13 and Cys7 with Cys19, and the sidechain of Asp9 forms an amide bond with the N-terminus. A BMY-29303 sample yielded 335 NOE distance constraints and 13 dihedral angle constraints. An ensemble of 30 BMY-29303 structures was generated; the structures have average backbone atom and all heavy atom RMSDs to the mean coordinates of 0.24 Å and 0.52 Å, respectively. The peptide displays an unusual wedge-shaped structure, with one face being predominantly hydrophobic and the other being predominantly hydrophilic. Chemical shift and NOE data show that the BMY-29304 structure is essentially identical to BMY-29303. These peptides may act by preventing oligomerization of the HIV transmembrane glycoprotein gp41, or by interfering with interactions between gp41 and the envelope glycoprotein gp120, the cell membrane or membrane-bound proteins. The amphipathic nature of the peptides suggests that a polar (or apolar) site on the target protein may be masked by the apolar (or polar) face of the peptide upon peptide/protein complexation.

D2-209 SYNTHESIS AND NMR STUDIES ON THE CONFORMATIONS OF THE THREE DISULFIDE BOND ISOMERS OF CONOTOXIN GI, David J. Craik, John Gehrmann, Paul F. Alewood and Diane Alewood, Centre for Drug Design and Development, University of Queensland, St. Lucia, Queensland, Australia 4072.

The alpha conotoxin GI, isolated from *Conus geographus*, is a highly toxic peptide that targets the acetylcholine receptor at the neuromuscular junction. The four cysteine residues in this peptide can form three different disulfide bond combinations: the native Cys 2-7 and 3-13 (globular), and two non-native Cys 2-3 and 7-13 (string) and Cys 2-13 and 3-7 (ribbon)[1]. The structures of these non-native isomers are of interest due to their biological activity (approximately 1/10th of the native activity in mice[2]). Solid phase methods have been used to synthesise the individual isomers. Structures of the major conformations of the two non-native isomers determined by gradient enhanced multi-dimensional NMR and simulated annealing calculations are presented. The "string" isomer has more than one distinct conformation, whereas the "ribbon" isomer is well defined in a single conformation. Structural similarities with the native GI are identified.

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D2-210 **NMR AND SAXS STUDIES OF THE FOLDING OF SPERM WHALE APOMYOGLOBIN.**

David Eliezer, Patricia A. Jennings, and Peter E. Wright. Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

We are investigating the nature of the information which can be obtained about folding intermediates of recombinant sperm whale apomyoglobin using a combination of multidimensional heteronuclear nuclear magnetic resonance techniques and static and time-resolved synchrotron small angle x-ray scattering techniques. Initial progress has addressed the compactness of the kinetic and equilibrium molten globule intermediates as well as the spectral chemical shift dispersion of isotopically labeled protein at different stages of equilibrium unfolding.

D2-211 A COMPARATIVE STUDY OF THE BACKBONE DYNAMICS OF THE FOLDED AND UNFOLDED FORMS OF AN SH3 DOMAIN, Neil A. Farrow, Ouwen Zhang, Ranjith Muhandiram, Julie D. Forman-Kay and Lewis E. Kay, Departments of Medical Genetics, Biochemistry and Chemistry, University of Toronto, Toronto, Ontario M5S 1A8 Canada.

Two-dimensional NMR ^{15}N relaxation studies have been used to characterize the backbone dynamics of the N-terminal SH3 domain of the protein drk (drkN SH3). In aqueous buffer and near neutral pH the isolated drkN SH3 domain exists in equilibrium between folded and unfolded states. For 12 of the 59 residues in the protein the backbone dynamics of both the folded and the unfolded states have been determined, and the rates of the folding/unfolding transition estimated. The mean value of the rate of the folding transition, determined from the rate of exchange between the two states was 0.9 s^{-1}, similar to previous measurements for the rate of formation of beta structure. A more extensive characterization of the dynamics of the two states has been performed by studying the folded and unfolded forms independently. The backbone dynamics of the unfolded form of drkN SH3 have been determined in the presence of 2 M guanidine hydrochloride, while the dynamics of the fully folded form were studied in the presence of 0.4 M sodium sulphate. In all cases the motional properties of the protein are described in terms of the values of the spectral density function of the backbone amide bond vector determined at a number of frequencies. Relatively little dynamical heterogeneity was observed throughout the folded form of drkN SH3. In both the equilibrium mixture and in the presence of denaturant, the unfolded form of the domain has considerably greater dynamic behaviour than that associated with the folded form. However, the motional properties of the unfolded state are consistent with the adoption of a reasonably compact form in solution.

D2-212 **SOLUTION STRUCTURE OF THE RECOMBINANT IRON-SULFUR PROTEIN ISO I (HiPIP I) FROM *E. halophila*, IN BOTH OXIDATION STATES,** **Isabella Felli, Lucia Banci, Ivano Bertini, Lindsay D. Eltis, Dieter H. W. Kastrau, Claudio Luchinat, Mario Piccioli, Roberta Pierattelli and Michael Smith, Department of Chemistry, University of Florence**

The recombinant high potential iron sulfur protein (HiPIP) I from *Ectothiorhodospira halophila* expressed in *E. coli*. contains 73 aminoacids and a 4Fe4S cluster as prostetic group. It represents a suitable system to investigate wether structural or dynamic changes occur between the two oxidation states through which electron transfer is achieved.

In the reduced form, the $[Fe_4S_4]^{2+}$ cluster has no unpaired electrons in the ground state but the contribution of the excited states at room temperature yields a paramagnetism of about 0.8 μ_B per iron ion. In the oxidized state, the $[Fe_4S_4]^{3+}$ cluster has S=1/2 that yields a paramagnetism of about 1.8μ_B per iron atom. In both oxidation states, the paramagnetic effects on nuclear relaxation are so strong as to yield T_1 values of few milliseconds and linewidths of hundreds Hz for the nuclei closest to the paramagnetic center.

Despite these features a high resolution solution structure of the protein in the reduced form has been obtained. 1246 NOESY intensities involving 71 assigned residues were used in distance geometry calculations (DIANA), and this preliminary structure was further refined through restrained energy minimization and restrained molecular dynamics simulations (Amber).

We are now refining the solution structure of the same protein in the oxidized form. Surprisingly the effect of the paramagnetic center seems to be more dramatic in the reduced form as far as relaxation is concerned. 73 out of 73 residues were assigned and the overall folding is essentially the same. Slight differnces can be found examining carefully the intensities of the dipolar connectivities.

In parallel also point mutants of this protein are under investigation.

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D2-213 KALIOTOXIN SHOWS STRUCTURAL DIFFERENCES WITH RELATED POTASSIUM CHANNEL BLOCKERS, I.Fernández#, R.Romi†, S.Szendeffy#, M.F. Martin-Eauclaire†, H.Rochat†, J.Van Rietschtoten†, M.Pons# and E.Giralt#,#Department of Organic Chemistry, University of Barcelona, Martí i Franqués 1-11, Barcelona 08028, SPAIN. †Laboratoire d'Ingénierie des Protéines, URA 1455, Centre National de la Recherche Scientifique, Faculté de Médecine. Marseille, France.

The three dimensional structure of Kaliotoxin(KTX), a toxin from the scorpion *Androctonus mauretanicus mauretanicus* that blocks calcium dependent potassium channels, and its analog KTX(1-37) has been determined by NMR. This toxin is homologous with other scorpion toxins such as Charybdotoxin (ChTX) or Iberiotoxin (IbTX) for which the structures are already known but the presence of prolines in the expected α-helical region suggested that there may be some major difference in the structure of KTX that could be related to its different selectivity. Proline residues are also found in the homologous region of other scorpion toxins such as Noxiustoxin or Margatoxin.

Our results indicate that KTX and its analog contains the same sequence of secondary structure elements as ChTX but that the helical region is shorter and distorted due to the presence of two prolines. The distortion consists of a bending in the α-helix and in the presence of a 3_{10} helix turn in the last three residues. Furthermore the increased length of the extended structure preceding the helix favours a different packing of this part of the molecule with respect to the secondary structure elements. This change in folding modifies the accessibility of the conserved ^{27}Lys which is known, from mutation studies, to be involved in channel blocking by ChTX.

In this communication we shall present a refined structure of KTX using a full relaxation matrix approach with special attention paid to dynamic aspects of this molecule.

D2-214 IDENTIFICATION OF NUCLEATION SITES FOR FOLDING OF hCG-β FROM ANALYSIS OF PEPTIDES BY MOLECULAR DYNAMICS, NMR, AND CD SPECTROSCOPY, William H. Gmeiner, Parag Saharabudhe, Fulvio Perini, and Raymond W. Ruddon, Eppley Institute and Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE 68198-6805

General rules for protein folding remain to be elucidated. Early steps in folding include formation of nucleation sites, short regions of ordered structure that form independently of sequence context. Common nucleation site structures are α-helix and β-hairpin. The glycoprotein hormone hCG-β has important biological functions in reproduction physiology and cancer detection. The subunit contains six disulfide bonds and the kinetic order of disulfide bond formation has been studied intracellularly. We have investigated the secondary structures of peptides that correspond to those regions of hCG-β that kinetic analyses suggest promote formation of disulfide bonds 34-88 and 38-57. Molecular dynamics calculations have been performed on substructures from the X-ray analysis of hCG-β. Potential nucleation sites are identified as regions that are conserved structurally throughout the dynamics trajectory. CD and NMR spectroscopy are used to analyze peptides that contain these putative nucleation sites. The 38-60 long loop was found to adopt a novel nucleation site consisting of residues 43-51. The structure contains a turn comprising residues 44-47 that is stabilized by hydrogen bonding and hydrophobic contacts between the side chain of R43 and the side chains and main chain of residues 48-51. The nucleation site is analogous to a β-hairpin with the side chain of R43 replacing one arm of the hairpin to form a pseudo hairpin structure.

D2-215 POLARITY DEPENDENT STRUCTURAL REFOLDING -: AN NMR STUDY OF A 15 RESIDUE PEPTIDE, Andreas Graf v. Stosch, Jennifer Reed and Volker Kinzel, Department of Pathochemistry, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

A close association between the HIV surface protein gp 120 and its CD4 receptor initiates the viral multiplication cycle. A 15 amino acid peptide (LAV) within the CD4 binding domain of gp 120 has been shown to retain binding ability. We have studied the structural behaviour of the LAV peptide by CD and NMR methods in aqueous solution and upon addition of trifluoroethanol (TFE) to emulate the relatively apolar conditions at the membrane bound receptor. The LAV peptide folds into a ß-pleated structure in more polar buffer/TFE mixtures, while a concerted structural change can be observed at a concentration of 60% TFE (v/v). This abrupt, cooperative refolding from a regular ß-sheet to a helical secondary structure is known as "switch" behaviour. At 70% TFE the refolding process is complete, further addition of TFE not leading to any increase in helicity. CD experiments with LAV sequence variants indicated that four amino acids at the N-terminus are indispensable for the "switch". The N-terminal LPCR-tetrad has a strong ß-turn forming potential. It was assumed that the tetrad can act as a nucleation site governing the cooperative transition of the helical structure. In order to gain further insight into this folding mechanism, the structure of the LAV peptide in pure TFE has been elucidated by NMR methods. Distance geometry and MD simulations give clear evidence for a 3_{10} helix. This result supports the hypothesis that the refolding starts at the N-terminal tetrad, possibly adopting a type III ß-turn and promotes the formation of a similar bend in the next overlapping tetrad until the sequence is restructured into a 3_{10} helix at a critical polarity favouring intrachain hydrogen bonds. The cooperative structural transition of the LAV peptide is interesting not only as a prerequisite for the gp120/CD4 interaction, as previously shown. The results suggest that a folding motif was identified which governs a complex cooperative restructuring of secondary structure elements.

D2-216 **NMR STUDIES OF ROBUSTOXIN, A POLYPEPTIDE NEUROTOXIN FROM THE VENOM OF THE SYDNEY FUNNEL WEB SPIDER, *ATRAX ROBUSTUS***

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Robustoxin and versutoxin are highly potent 42-residue polypeptide neurotoxins from the Sydney funnel web spider (*Atrax robustus*) and Blue Mountains funnel web spider (*Hadronyche versutus*), respectively. These neurotoxins have four disulphide bonds and display 83% sequence identity[1]. Human fatalities have been attributed to the bite of the male *A. robustus* spider. Robustoxin and versutoxin are responsible for the major symptoms of envenomation from these spiders.

We describe the complete sequence-specific assignment of resonances in the ^{1}H-NMR spectrum of robustoxin. Amide exchange experiments and chemical shift analyses have determined those residues that lie within the β-sheet secondary structure. Comparison at the chemical shift level between robustoxin and ω-Agatoxin-IVB, a four disulphide bonded 48-residue neurotoxin from the venom of the American funnel web spider, *Agelenopsis aperta*, whose three dimensional structure has recently been determined[2], demonstrates that the structures are not identical.

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D2-217 SYNTHESIS AND PRELIMINARY STRUCTURE OF THE FIFTH EGF-LIKE DOMAIN OF THROMBOMODULIN, Michael J. Hunter and Elizabeth A. Komives, Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA 92037

Thrombomodulin is an endothelial cell membrane bound protein which binds thrombin. The formation of the thrombin-thrombomodulin complex on the surface of the endothelium is responsible for the thrombin catalyzed activation of the anticoagulant serine protease zymogen, protein C, and the initiation of the anticoagulant pathway. The fourth and fifth EGF-like domains of thrombomodulin are required for protein C activation and the fifth EGF-like domain contains the major thrombin-binding determinants. The fifth epidermal growth factor (EGF)-like domain of thrombomodulin has been synthesized, purified, and refolded. This single EGF-like domain binds to thrombin and is a potent inhibitor of thrombin induced fibrin clot formation as well as of the thrombin-thrombomodulin interaction that leads to the activation of protein C. Preliminary 2-D NMR data suggests that this molecule is flexible in solution with the majority of the structural information coming from the C-terminal 18 residues. These results correspond well with recent transferred NOE data which indicates that the loop peptide comprised of these 18 residues adopts a more rigid structure upon binding to thrombin. The flexibility of the N-terminal half of this molecule is believed to be necessary for the proper positioning of the fourth EGF-like domain (in the context of the native protein) when the C-terminal half is bound to thrombin.

D2-218 STRUCTURAL STUDIES OF THE N1 CELLULOSE BINDING DOMAIN FROM *Cellulomonas fimi* ENDOGLUCANSE C,

Philip E. Johnson, Peter Tomme, and Lawrence P. McIntosh, Departments of Chemistry, Biochemistry, and Microbiology, and the Protein Engineering Network of Centres of Excellence, University of British Columbia, Vancouver, BC, Canada.

The bacterium *Cellulomonas fimi* produces a plethora of cellulases, each having a modular design. Binding to the substrate, cellulose, is mediated by the cellulose binding domain (CBD). The cellulase CenC is unique with two CBDs (N1 and N2) in tandem at its N terminus. The N1 CBD is interesting in that it has poor sequence similarity to other CBDs from *C. fimi*, and has an unusual binding specificity for amorphous but not crystalline cellulose.

The goal of this research is to elucidate the mechanism of the interaction between the N1 CBD and cellulose by determining the three dimensional structure of this protein using NMR. CBD samples selectively and uniformly enriched in ^{13}C and ^{15}N have been prepared and we are currently assigning the NMR spectra. The CD, FTIR, and NMR spectra of this CBD demonstrate that this protein is composed mostly of β sheets.

The N1 CBD reversibly unfolds with thermal denaturation. The thermodynamics of folding are also being studied using CD and NMR spectroscopy.

D2-219 **STRUCTURAL AND DYNAMICAL PROPERTIES OF THE F30→A CAVITY MUTANT OF THE Z DOMAIN OF PROTEIN A**

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The 8 kD Z domain of the Staphylococcal protein A binds to human IgG. The binding region is located on the surface of Z and as a consequence the F30 A cavity mutation does not change the IgG_1 binding properties and CD spectral analysis indicate that very minor, if any, changes in the secondary structure are introduced. This mutation does however destabilizes the protein towards Gdn-HCl unfolding by as much as 3.5 kcal/mol[1]. To shed more light on the changes in structural and dynamical properties leading to the decreased thermodynamical stability in this mutant protein we have turned to NMR studies. To this end Z-F30A has been uniformly labelled with ^{15}N and ^{13}C and sequence specific assignments have been arrived at using conventional triple resonance methodology. Relaxation parameters (^{15}N T_1, T_2 and NOE) have been measured for the backbone amide groups. Motional information derived from these measurements will be used in reference to the structural and dynamical information available from the native Z domain.

1. Cedergren *et al.*, (1993) *Prot. Eng.* **6**, 441-448.

D2-220 **ANALYSIS OF DYNAMIC MODELS OF ONE- AND TWO-HELICAL SUBUNITS OF BACTERIOOPSIN.**

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To investigate the influence of the internal dynamics of two polypeptides comprising transmembrane α-helix A or couple of α-helixes A and B of bacterioopsin on the experimentally accessible ^{15}N NMR relaxation rates the molecular dynamics (MD) simulations in vacio combined with more simple mechanic considerations where used . For these models "model-free" order parameters and correlation times of internal motions (Lipari and Szabo, (1982) *J. Am. Chem. Soc.*, **104**, 4546-4559) were calculated. It was found that both peptides exhibit two types of internal motions of the amide bonds in the pico- and nanosecond time-scales affecting ^{15}N NMR relaxation. The fast motions characterized by the order parameters S_f^2 of 0.75-0.90 and effective correlation times τ_f of 0.1-0.4 ps. These motions are scarcely correlated with the secondary structure of peptides and display oscillation character. In contrast, the motions in the nanosecond time-scale is strongly dependent on the secondary structure. These motions have the order parameters S_s^2 of 0.70 - 0.90 in α-helixes and 0.20-0.60 in unstructured parts of the peptides. The slow internal motions are relevant to the concerted collective vibrations of a large number of atoms and could be represented as bending oscillation of α-helixes, witch has underdamped character in vacio MD simulation but strongly overdamped by the ambient solvent. A few other molecular mechanisms of slow internal motion were found. It was shown that the bending motion of α-helixes is in good correspondence with the simple mechanic model, where α-helix is considered as a rod-shaped elastic body in viscous environment stochastically vibrating under a random force.The results are compared with the ^{15}N NMR relaxation data measured for the (1-36)bacterioopsin and (1-71)bacterioopsin polypeptides in chloroform-methanol (1:1) and SDS micelles (Orekhov et al.,(1994a) *Eur. J. Biochem.*,**219**, 887-896; Orekhov et al., (1994b),paper in preparation).

D2-221 **AUTONOMOUS FOLDING ABILITY OF ISOLATED HELICES. Studies of fragments from acyl-coenzyme A binding protein, ACBP.**

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Bovine acyl-coenzyme A binding protein, ACBP is an 86 residue protein folded into a four-helix bundle motif[A]. The amide hydrogen in the four helices are retained from exchange by factors in excess of 10^4 to 10^7 and during a highly cooperative folding process of ACBP, bulk protection of amides from exchange follows an apparent two-state behaviour[B]. To determine if any of the four helices in ACBP exhibit propensities to form structured conformers in isolation, and to see whether any combination of two, three or all four helices is able to stabilize and induce secondary structure, four peptides corresponding to regions of helical secondary structure in bovine ACBP, have been synthesized and structurally characterized both individually and in different combinations using circular dichroism and NMR. Extensive NMR characterizations, including sequential assignment, NOE analysis, amide hydrogen exchange rate determination and structure calculations have been initiated for those helices showing a clear preference for adopting significant structure. When studied in isolation either in water or in various concentrations of additive, i. e. trifluoroethanol, the four peptides show different tendencies to form nascent or stable helical structure. The most stable helix has been shown to be the *C*-terminal 19-residue helix A4, Ser65-Tyr84, whereas the least stable is helix A3, Gly51-Lys62, containing 11 residues. The structural content of the four helices at the different conditions and the results obtained on residue specific amide hydrogen exchange rates will be presented and compared to similar data on the intact protein.

[A]Andersen, K. V. & Poulsen, F. M. *J. Biomol. NMR.* **3**, 271-284. (1993).
[B]Kragelund *et al.*, *J. molec. Biol.* submitted (1994); Kragelund *et al.*, *Nature Struc. Biol.* submitted (1994).

D2-222 NMR STUDIES OF G PROTEIN-BOUND RECEPTOR PEPTIDE FRAGMENTS

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G proteins transduce information from liganded receptor such as β-adrenergic receptor (β-AR) to effectors such as adenylyl cyclase. Though β-AR molecule is too large to physicochemically analyse the receptor-G protein-interactions, some peptide fragments of β-AR are known to activate Gs protein, hence are promising low molecular weight models of β-AR.

The analysis of the interaction between the peptide fragments and a G protein can be facilitated by TRNOE measurements. However, this method often suffers from poor proton signal dispersion of peptides, because short linear peptides are usually in a 'random-coil state' in a free state.

Such a drawback of the TRNOE methods can be overcome by expanding the peptide signals by heteronuclear chemical shifts into two or three dimensions, hence we developed an expression system to produce isotope-labeled peptides in *E. coli* cells. The resultant expression system enabled us to produce a peptide at several milligram quantity within a week.

We first prepared a $u[^{15}N]$ β-AR peptide. Though the amide proton signals of the peptide in 1D NMR spectra were severely overlapped, amide signals were completely resolved in a $[^1H,^{15}N]$ HSQC spectra. The ^{15}N-edited TRNOE-HSQC spectrum of the $u[^{15}N]$ β-AR peptide in the presence of Gs showed well-resolved TRNOE cross peaks involving amide protons, hence is very useful to extract structural information of the interaction.

We have also prepared a $u[^{13}C,^{15}N]$ β-AR peptide to resolve both NH and CH signals. The application of the $u[^{13}C,^{15}N]$ β-AR peptide will also be disscussed.

D2-223 **Structure of FK506 Bound to Triple Mutant FKBP13**

Christopher Lepre*, Olga Futer, David Livingston, and Jonathan Moore *Vertex Pharmaceuticals, Inc., Cambridge, MA 02139*

FK506 is a potent immunosuppressant that binds to a family of homologous 'FK506 binding proteins' (FKBP's) ranging in size from 12 to 52 kDa. Only the FK506 complex of FKBP12, however, inhibits calcineurin (CN), the putative cellular target of FK506. FKBP13 has a 123-fold lower FK506 binding affinity than FKBP12, and its FK506 complex has a >2200-fold lower CN affinity. However, mutation of three FKBP13 surface residues (N50, A95, and K98) to the corresponding FKBP12 residues (R42, H87, and I90) confers FKBP12-like FK506 and CN binding activity to FKBP13 [O. Futer, M. DeCenzo, S. Park, B. Jarrett, R. Aldape, and D. Livingston, unpublished].

This work reports the solution structure of 13C-labeled FK506 bound to triple mutant FKBP13. Assignments and NOE measurements were made from inverse-detected 1H-13C experiments. Structures were calculated by distance geometry (DGII) and refined using conventional MD (Discover). The NMR structures of the bound ligand are well defined by the NOE restraints. An independent set of time averaged MD calculations (Amber) was carried out in order to allow for motional averaging of the NOE restraints. The resulting structures are compared to the X-ray structures of FK506 bound to FKBP12 and FKBP13.

D2-224 DIFFERENT CONFORMATIONS FOR THE TRANS-CIS ISOMERS OF A CONSTRAINED PEPTIDE CONTAINING THE PRO-X-X-PRO SEQUENCE, Guy Lippens, C. Francart, S. Petitfrère and A. Tartar, SCBM, Institut Pasteur de Lille, 59000 Lille, France

It has been observed that the peptide

H-Cys-Leu-Pro-Arg-Glu-Pro-Gly-Leu-Cys-OH

S----------S

is eluted as a broad peak at room temperature whereas it splits into two separate peaks at low temperature [1]. This splitting was attributed to the different isomers of the prolines, with an unusually slow equilibrium between the trans and cis form. Furthermore, it has been observed in our laboratory that changing the extremities of the peptide (Ac or H, NH_2 or OH) has a distinct influence on the HPLC pattern.

We have used different NMR techniques to 1) elucidate the conformations of the trans- and cis-isomers, 2) to characterize the dynamics of the processus and 3) to explain the structural role of the extremities. To this end, we have used gradient-enhanced NMR techniques on the different isomers isolated chemically by HPLC, as well as on samples where the the different isomers are in thermal equilibrium.

Our results indicate that the Pro3 is present in both cis- and trans form, whereas the trans form is dominant for Pro6. Moreover, comparison of the peptides with modified extremities has shown that the difference in HPLC behaviour is not due to changes in the equilibrium populations of the isomers, but to lowering of the energetic barrier and thus of the interconversion rates.

The importance of Pro cis-trans isomerisation and interconversion rates will be discussed in the perspective of protein folding, where this isomerisation step is often the rate-limiting step.

1) Gesquière et al, (1989) J. Chromatog. 478, 121

D2-225 STRUCTURE AND STABILITY OF A SECOND MOLTEN GLOBULE APOMYOGLOBIN FOLDING INTERMEDIATE. Stewart N. Loh and Robert L. Baldwin, Department of Biochemistry, Stanford University, Stanford, CA 94305.

Apomyoglobin folding is known to proceed through a molten globule intermediate (I_1) that is observed in both kinetic (pH 6) and equilibrium (pH 4) folding experiments. Of the eight helices in myoglobin, three (A, G, H) are structured in I_1 while the rest are unfolded. Here we characterize a second intermediate (I_2) which is induced either from the acid-unfolded protein (U) or from I_1 by ≥ 5 mM sodium trichloroacetate (see Goto et al., *Biochemistry 29*, 3480; 1990). Circular dichroism measurements monitoring urea- and acid-induced unfolding indicate that, compared to I_1, I_2 is more highly structured as well as more stable relative to U. Although I_2 exhibits properties closer to those of the native protein, 1D NMR spectra show that it maintains the lack of fixed tertiary structure that is the hallmark of a molten globule. Amide proton exchange and ^1H-^{15}N 2D NMR experiments are used to identify the source of the extra helicity observed in I_2. The results reveal that the existing A, G and H helices present in I_1 have become more stable in I_2, and that a fourth helix—the B helix—has been incorporated into the molten globule. The implications of this finding towards understanding the folding pathway of apomyoglobin are discussed.

D2-226 **PROBING HYDROGEN BOND INTERACTIONS IN CARBOHYDRATE-PROTEIN SYSTEMS: APPLICATION OF ^{19}F NMR TO FLUORO-DEOXY SUGARS AND RECEPTOR PROTEINS** Linda A. Luck, Clarkson University, Potsdam, N.Y.13699

Protein-ligand interactions and molecular recognition are frequently defined by hydrogen-bonding. These bonds are critical for conferring specificity, ensuring the proper fit of substrates, inducing conformational changes and enhancing stability. Carbohydrate-protein interactions present a unique system to study the role of hydrogen bonds due to the large number of hydroxyls present. The *E. coli* Glucose and Galactose Receptor (GGR) has been shown to be an ideal system to gain insight into the importance of specific hydrogen bonding interactions. GGR serves as the initial receptor for chemotaxis and transport. This protein sequesters both sugars in a tight affinity binding cleft between two similar domains of this bilobal 33 kDa protein. Binding of the sugar produces a large conformational change in the protein which is necessary for recognition of the liganded protein by membrane components for transport and signalling.

Measurement of the binding capacity of a series of fluoro-deoxy analogues of glucose and galactose to GGR by ^{19}F NMR and titrating calorimetry has provided information about the role of each of the hydroxyls in the binding event. One and two dimensional NMR studies have been key in obtaining structural details of these interactions. The studies have revealed that the 2-OH on both glucose and galactose is critical for binding and the protein has an overwhelming preference for the beta anomer. The data demonstrate the utility of ^{19}F NMR for characterizing carbohydrate-protein interactions as well as providing information about exchange rates, anomeric preferences and kinetics.

D2-227 SOLUTION CONFORMATION OF THE NMDA ANTAGONIST CONANTOKIN T

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Conantokin T is a 21 residue polypeptide derived from the venoms of fish-hunting Conus. It exhibits the N-methyl-D-aspartate (NMDA) antagonist property that inhibits calcium entry into neurons. Unlike the other toxins purified from Conus venoms, conantokins contain no disulfide bonds but have a significant content of a post-translationally modified residue, γ-carboxyglutamate (Gla). Conantokin T has been shown to adapt an α-helical conformation in solution in a calcium-dependent way. To address the importance of the γ-carboxyglutamate residue in folding, we have chemically synthesized an conantokin T analog by replacing all the Gla residues into Glu as to compare their ability in chelating calcium ion in an α-helical conformation. Two-dimensional NMR spectroscopy has been used to characterize the solution conformation of both conantokin T and its analog and detailed comparisons of two conformations will be presented.

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D2-228 SPATIAL STRUCTURE OF TRANSMEMBRANE SEGMENT M2 OF α-SUBUNIT OF NICOTINIC ACETYLCHOLINE RECEPTOR, Innokenty V.Maslennikov, Ivan A.Sorokin and Alexander S. Arseniev, *Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Science, Miklukho-Maklaya 16/10, Moscow, 117871 Russia.*

The nicotinic acetylcholine receptor (AchR) is a ligand-gated cation channel composed of four homologous transmembrane subunits in a stoichiometry of $\alpha 2\beta\gamma\delta$. AchR carries the cholinergic binding sites, contains the ion channel and mediates the conformational transitions responsible for the regulation of ion translocation. Electron microscopic image analysis of the AchR indicate that the subunits are arranged pseudosymmetrically around the central axis with all five subunits contributing structurally to form the ion channel. Each subunit contains four hydrophobic segments of about 30 amino acids in length, reffered to as M1 - M4, that are proposed to be membrane-spanning α-helices and might be organized as a four-helical bundle, with one segment from each subunit associating at the central axis to form the ion channel. Affinity label studies as well as mutagenesis provide strong evidence that segments M2 from each subunit are α-helical and associate around the central pore.

The solution structure of the synthetic peptide corresponding residues 253-285 (membrane spanning segment M2) of α-subunit of nicotinic AchR from *Torpedo californica* has been determined. Peptide was solubilized in membrane mimetic solution (methanol-chloroform, 1:1), the ^{1}H-NMR spectra was completely assigned and spatial structure determined from NMR data. The reconstruction of M2 spatial structure includes the following steps: (1) analysis of the local structure; (2) structure calculation with the distance geometry program DIANA; (3) the systematic search of allowed side chain rotamers by using the program CONFORNMR; (4) energy refinement of 20 best DIANA structures by using the program CHARMM.

The data unequivocally define the peptide conformation as the right-handed α-helix, extremely rigid in central region from Leu-245 to Val-261 and flexible in the N- and C-terminal parts. Our results are compared with the limited information available about M2 secondary structure.

D2-229 NMR STRUCTURE OF A HIGHLY THERMOSTABLE SUBDOMAIN WITHIN VILLIN HEADPIECE

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We have developed a 35 residue thermostable subdomain to use as a model system for investigating the limits on the amino acid sequence required for native protein folding. This model system was derived from the 76 residue f-actin binding headpiece domain of villin. Heteronuclear NMR spectroscopy of this domain indicated that the N-terminal half of this domain was unfolded and/or sampling multiple conformation while the C-terminal region formed a helical subdomain. A combination of proteolysis and NMR was used to minimize the folded subdomain to only 35 residues. This subdomain is highly thermostable with a thermal melt midpoint of 70 °C at pH 7.0. Unlike other small folded domains the headpiece subdomain contains no cysteine residues so the folded structure can not be stabilized by disulfide bond formation. Thermal melts in the presence of EDTA rule out stabilization by metal binding. Furthermore, analytical ultracentrifugation indicates that the headpiece subdomain is monomeric and therefore not stabilized by self association. NMR analysis of the subdomain indicates that it is composed of three short helices each of which contribute hydrophobic residues to a central hydrophobic core.

D2-230 SOLUTION CONFORMATION OF FRAGMENTS OF HUMAN BIG-ENDOTHELIN-1 BY 2D NMR SPECTROSCOPY. Sharon L.A. Munro, Murray Coles and David Craik. School of Medicinal Chemistry, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Vic., AUSTRALIA, 3052.

Endothelins are a newly discovered hormone family which play a major role in the development and progression of many disease states, including asthma, cancer, hormonal imbalances in women, cardiovascular disorders (for example hypertension) and diabetes. Understanding the structure and functionality of the precursors of these hormones, typified by big-endothelin-1 (big-ET-1), will allow insight into the manner in which these hormones are produced. Additionally, it will provide a basis for rational drug design of agents which may play a role in the treatment of diseases associated with these hormones.

Four fragments encompassing two enzyme cleavage sites of big-endothelin-1, namely (1) that between I20 and W21, where endogenous endothelin-1 (ET-1) is metabolized to a des-Trp derivative and (2) between W21 and V22, where big-ET-1 is cleaved to produce ET-1 have been studied using NMR spectroscopy. The results of these studies will be compared to the conformations of other analogues investigated in our laboratory.

D2-231 **STRUCTURE OF A POLYPEPTIDE CONTAINING ALANINE HEPTAD REPEAT UNITS FROM A MEROZOITE SURFACE ANTIGEN OF *PLASMODIUM FALCIPARUM*.** Raymond S. Norton[1], Terrence D. Mulhern[1], Gavin E. Reid[2], Richard J. Simpson[2] & Robin F. Anders[3]. [1] *NMR Laboratory, Biomolecular Research Institute, 381 Royal Parade, Parkville 3052 AUSTRALIA*, [2] *Joint Protein Structure Laboratory, Ludwig Institute for Cancer Research, Parkville 3050 AUSTRALIA*, [3] *Walter & Eliza Hall Institute for Medical Research, Parkville 3050 AUSTRALIA.*

The *Plasmodium falciparum* antigen SPAM (secreted polymorphic antigen associated with merozoites) contains an unusual set of heptad repeat units with alanine at the **a** and **d** positions. Twelve heptads with the consensus sequence AXXAXXX occur in three blocks of four, linked by short non-repetitive sequences. A 38-residue polypeptide comprising the first block of four heptad units and five flanking residues at either end, SPAM-H1, has been synthesized and its structure in aqueous solution determined from ^{1}H NMR data.

The polypeptide contains an α-helix extending from Ser10 (position **e** of the first heptad) to at least Lys32 (position **f** of the fourth heptad) and possibly as far as Val35. The helix is bent, partly as result of a kink around residues 19-20. The conformations of the nine N-terminal residues and the six C-terminal residues are not well defined by the NMR data. The RMS deviation from the average of the 20 best structures over the well-defined region (residues 11-31, which have backbone angular order parameters > 0.8) was 1.56 Å for backbone heavy atoms (N, C^α and C) and 2.12 Å for all heavy atoms. Possible interactions of the H1 sequence with the other two heptad repeat units in the intact merozoite antigen are discussed.

^2H_2O exchange experiments identified slowly exchanging amide protons near the C-terminus and the last two turns of the helix. The unusual stability of the C-terminus reflects the presence of a novel C-capping motif. The essential features of this C-capping motif have been probed with a series of synthetic peptide analogues.

D2-232 THERMODYNAMIC PARAMETERS FROM HYDROGEN-DEUTERIUM EXCHANGE IN THE PROTEIN G B1 AND B2 DOMAINS: COMPARISON WITH CALORIMETRIC DATA, John Orban, Patrick Alexander, Philip Bryan, and Devesh Khare, University of Maryland Biotechnology Institute, Center for Advanced Research in Biotechnology, 9600 Gudelsky Drive, Rockville, MD 20850.

Hydrogen-deuterium exchange experiments have been used to measure backbone amide proton (NH) exchange rates in the B1 and B2 IgG-binding domains of protein G. Exchange rates were determined from ^{1}H-^{15}N HSQC spectra acquired without phase cycling [Marion et al., J. Mag. Res. 85, 393-399 (1989)]. Using this method, as well as ^{15}N-edited 1D spectra, exchange rates up to 300 h^{-1} were measured.

In B2, exchange rates for H-bonded NHs are more clustered than in B1, and the distinction between global and local unfolding mechanisms is not obvious for some NHs. In B1, exchange is approximately five-fold slower than in B2 for NHs which exchange through global unfolding - local and global unfolding modes can be readily differentiated. This difference in exchange rates corresponds to $\Delta\Delta G_{op}$(B1-B2) ~ 1 kcal/mol which is in agreement with the calorimetric $\Delta\Delta G_u$. Comparison of NH exchange in the two domains therefore allows us to differentiate between NHs which exchange through global versus local unfolding. Exchange rates were also determined as a function of temperature (298-343K) and apparent activation enthalpies for NHs were obtained. Comparison of ΔG_{op} (HX) and ΔG_u (calorimetry) versus temperature will be presented.

The poster will highlight the utility of H-D exchange in obtaining thermodynamic parameters for individual amide protons in G_{B1} and G_{B2} and illustrate how this data can be used to interpret exchange mechanisms.

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D2-233 **BACKBONE DYNAMICS OF (1-71) AND (1-36) BACTERIOOPSIN STUDIED BY TWO DIMENSIONAL NMR SPECTRSCOPY**, Vladislav Orekhov, Konstantin V. Pervushin, Dmitry M. Korzhnev and Alexander S. Arseniev. *Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, RAS, Miklukho-Maklaya 16/10, Moscow, 117871, Russia.*

The backbone dynamics of uniformly 15N-labelled fragments (residues 1-71 and 1-36) of bacterioopsin, solubilized in two media (methanol- chloroform (1:1) and SDS micelles) have been investigated using 2D proton-detected heteronuclear 1H-15N NMR spectroscopy at two spectrometer frequencies 600 and 400 MHz. Contributions of the conformational exchange to transverse relaxation rates of individual nitrogens are elucidated using a set of different rates of the CPMG spin-lock pulse train. We found that most of the backbone amide groups of (1-71)bacterioopsin in SDS micelles are involved in the exchange process over a rate range of 10^{3}-10^{4} s^{-1}. It is supposed that this conformational exchange is induced by the interaction between two α-helices of (1-71)bacterioopsin, since the hydrolysis of peptide bond Asn36-Pro37 leads to the disappearance of the exchange line broadening. Contribution of the conformational exchange to the measured transverse relaxation was essentially suppressed by the high frequency CPMG spin-lock. 15N relaxation rates and NOE values were interpreted using the model-free approach of Lipari and Szabo (1982, *J. Am. Chem. Soc.*, 104, 4546-4559). In addition to overall rotation of the molecule, the backbone N-H vectors of the studied peptides are involved in two types of internal motions - fast, on a time scale of <20 ps, and intermediate, close to 1 ns. Distinctly mobile regions are identified by a large decrease in order parameter of nanosecond range motions, S_S^2 and correspond to N and C termini, and loop connecting α-helices of (1-71)bacterioopsin. A decrease in the order parameter S_S^2 was also observed for residues next to Pro50, indicating an anisotropy of the overall rotational diffusion of the molecule. Thus, backbone dynamic parameters of (1-71)bacterioopsin are in good correspondence with its spatial structure (Pervushin et al., (1994) *Eur. J. Biochem.*, 219, 571-583) The observed conformational exchange in the millisecond time scale and collective nanosecond motions of α-helices should be taken to account in the development of the dynamic model of bacteriorhodopsin functioning.

D2-234 NMR CONFORMATIONAL STUDIES OF IMMUNOGENIC PEPTIDE FRAGMENTS DERIVED FROM HIV COAT PROTEIN gp120, L. D. Spicer, H. Vu, R. de Lorimier, M.A. Moody, B.F. Haynes, Departments of Biochemistry, Radiology, and Medicine, Duke University Medical Center, Durham NC 27710.

A series of four 39 amino acid peptide fragments derived from binding sites in the C4 and V3 regions of the HIV gp120 coat protein are characterized by NMR with respect to their conformational preferences. Each peptide sequence has an invariant domain and a variable domain and contains immunogenic T-helper cell epitopes and B-cell determinants. Correlation of NMR-observed conformer domains with biological activity and with stable secondary structures observed in functionally active proteins with homologous sequences has led to new rationally designed mutations which have been constructed and studied both by NMR and functional assays. Initial results with a peptide designed to achieve a more extended conformation in the C-4 domain which would be more representative of immunoglobulin binding sites has shown NMR evidence for successful design and has demonstrated increased immunogenicity in preliminary tests. In particular, the nascent helical propensity in this region of the peptide was overcome by point substitutions based on electrostatic considerations. The resultant peptide molecule is thus constructed on the basis of a combination of structure and sequence homology. Other designs to increase conformer exposure in apolar binding regions are also being constructed to better understand reactivity differences in the parent peptides. Of particular interest is the sequence of turns in the variable region near the principal neutralizing determinant of the V3 domain which show distinctive conformer properties in each of the peptides studied. Models based on NMR-derived conformer preferences show considerable variation in both the specific exposure of known binding residues and in the overall effective molecular size of the synthetic peptides.

D2-235 **STRUCTURAL STUDIES ON THE BACTERIAL ENTEROTOXIN, STABLE TOXIN B BY MULTIDIMENSIONAL NMR AND CIRCULAR DICHROISM**, Muppalla Sukumar[1], Josep Rizo[2], Mark Wall[2], Lawrence A. Dreyfus[3], Yankel M. Kupersztoch[2], Lila M. Gierasch[1]; [1]University of Massachusetts, Amherst, MA 01003, [2]UT Southwestern Medical Center, Dalls, TX 75235, [3]University of Missouri, Kansas City, MO.

Stable Toxin B (STB) is secreted by enterotoxigenic E. coli that cause secretory diarrhea in humans and domestic animals. It is a 48 amino acid peptide and contains two disulfide bridges, between residues 10-48 and 21-36, which are crucial for its biological activity. Here, we report the solution structure of STB determined by three dimensional NMR methods. The effect of reducing the disulfides on the structure of STB and the geometry of disulfide bridges were investigated by circular dichroism (CD). A set of 390 approximate interproton distances derived from nuclear Overhauser effect (nOe) data were used to construct structures of STB using distance-geometry analysis. Results indicate that STB is largely helical between residues 5-22 and 36-48. Specific interactions have been identified between residues Ile16 and Ala17 of the N-terminal helix and Ala39 of the C-terminal helix. The structure of the loop region between residues 21-36 is poorly defined and we are exploring the possibility that this is due to true conformational mobilty. If so, this mobility may be of functional significance because several residues in this region have been shown to be critical for activity. CD studies indicate that reduction of disulfide bridges results in a dramatic loss of structure which correlates with loss of function. Reduced STB is predominantly a random coil. Chromatographic measurement of concentrations of native, fully reduced and single disulfide species in equilibrium mixtures of STB in redox buffers indicate that the formation of the two disulfide bonds is only moderately cooperative. Similar measurements in the presence of 8M urea suggest that the native secondary structure significantly stabilizes the disulfide bonds.

D2-236 STUDIES OF A GP-120 PEPTIDE AND ITS ANTI-PEPTIDE ANTIBODY COMPLEXES, Pearl Tsang *, Gang Wu* and Paul J. Durda+, *Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221 and +E.I.DuPont de Nemours & Co., Inc., 331 Treble Cove Rd., N. Billerica, MA 01862

The fifteen residue peptide RIQRGPGRAFVTIGK (derived from the V3 loop of the gp-120 protein of HIV-1 virus (strain IIIB[1]) has been studied using a variety of NMR techniques to obtain physical information regarding its structure and interactions with two monoclonal anti-peptide antibodies, 5023 and 5025A[1]. Both antibodies specifically recognize and bind this peptide with affinities of $10^8 M^{-1}$ or higher. While 5023 and 5025A also neutralize HIV-1, their neutralization capabilities differ and are virus strain-dependent[2]. NMR studies of these Fab-peptide complexes are being conducted to obtain information regarding physical differences which exist between them (vis à vis the bound peptide) and which may account for observed antigen specificity differences between the antibodies. Studies of the unbound peptide involved use of homonuclear NMR experiments while heteronuclear techniques were applied to study isotopically labeled peptide-antibody complexes. NMR differences which have been observed between the free and antibody-bound forms of the peptide are consistent with formation of specific interactions between the peptide and each Fab. The peptide in these complexes undergoes slow chemical exchange on the chemical shift timescale so isotopically labeled peptides were used to form the complexes which were then studied using isotope-edited NMR methods similar to those employed in a previous study of a different peptide-Fab complex[3]. Comparative NMR studies of the 5023 and 5025A Fab-peptide complexes indicate that binding effects upon the peptide differ and depend upon which Fab the peptide is bound to. NOE studies of the two Fab complexes indicate that interactions between the peptide and other residues (due to the peptide or Fab) differ and are antibody-dependent as well.

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D2-237 NMR STUDIES ON THE STRUCTURE OF PEPTIDE FRAGMENTS OF SPECTRIN SH3 DOMAIN IN WATER SOLUTION. *Ana Rosa Viguera, María Angeles Jímenez,[1] Manuel Rico[1] and Luis Serrano.* European Molecular Biology Laboratory (EMBL). Meyerhofstrasse 1, Heidelberg, D-69012 Germany and [1]Instituto de Estructura de la Materia. CSIC 28006 Madrid. Spain

We have study the structure of the synthetic peptides that comprises the whole structure of spectrin SH3 domain. They all are ß-hairpins in the protein and range between 17 and 20 aminoacids. The stability of the wild type and the influence of the order of secondary elements were previously investigated[2,3]. The structure of the original domain contains no helical component, only a 3^{10} helix is also present[1]. Previous studies on clycic permutations over the domain indicated that the disrruption of each ß-hairpin had different influence in the refolding rate, some increased and others decreased it. In order to discover wether this difference in rate has to see with the early stages of folding we have study by NMR the structure of all the fragments that contain a complete secondary strucural element. It is surprising the result that none of them have a significant population of regular structure although they do not tend to form helical structure even in the presence of the helical promoter TFE and in spite of the presence of a known helix cap in one of them. This suggest that the intricisic tendency of this sequence is mainly ß but tertiary interaction should play an important role in the attainment of the final structure. One important coclussion is that the folding of this little all ß-structure domain is a cooperative process with no clear local nucleation region. This is in agreement with the observation that fluorescence and far-CD signal are recovered simultaneously during refolding.

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D2-238 FOLDING STUDIES OF H124L STAPHYLOCOCCAL NUCLEASE BY PULSED HYDROGEN EXCHANGE AND RAPID MIXING METHODS, William F. Walkenhorst, John L. Markley†, and Heinrich Roder, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111 and †Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706

The refolding of H124L SNase from acid pH has been examined by stopped-flow fluorescence and by pulse labeling hydrogen exchange methods. Three kinetic phases have been identified in the refolding of H124L SNase by tryptophan fluorescence. The major phase (66%) has a time constant of 180 ms at 15 °C, and two minor phases have time constants of 1 second (24%) and 13.5 seconds (10%), respectively. NH exchange rates under the unfolding conditions (pH 3.0, low salt) showed no evidence for protection both in the presence and absence of 2.5 M GuHCl, indicating that the protein is fully unfolded by acid pH alone. Pulsed hydrogen exchange methods were applied to H124L using uniformly ^{15}N labeled protein. ^1H-^{15}N HSMQC spectra were acquired for folding times between 10 ms and 160 s. Analysis of the data reveals that for more than half of the observed amide sites the initial proton occupancy (A_o) at 10 ms is significantly lower ($A_o < 0.7$) than the value expected for a fully labeled amide site in the unfolded state ($A_o = 1$). Thus, an early folding intermediate accumulates during the deadtime of the quenched-flow experiment in which many amide sites are already protected against exchange. The protected sites in the intermediate include all of the observed protons in the β-barrel, and several in adjacent regions of secondary structure. Most of the sites in the three α-helices, however, show no protection in the intermediate. The time course of protection shows two phases (100-200 ms and 5-10 s, respectively) and is similar for all probes. These results are in agreement with those of Jacobs and Fox (PNAS, **91**, 449-453, 1994), who reported similar observations for 39 amide protons of another variant (P117G) of SNase. In our studies, we were able to follow more than 60 amide probes and have evidence for the involvement of regions outside the β-sheet domain.

D2-239 **A ^1H-NMR SPECTROSCOPIC INVESTIGATION OF THE MOLECULAR BASIS OF PILUS SUBUNIT BINDING TO THE PAPD CHAPERONE**

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Gram negative bacteria which cause severe urinary tract infection assemble supra-molecular structures on their surfaces that mediate binding to galabiose [α-D-Gal-(1-4)-β-D-Gal] containing glycolipids on epithelial cells. These rod-like surface organelles called Pap-pili (encoded by the pyelonephritis-associated pilus operon) are composite fibers consisting of repetitive subunits. The tip fibrillum contains the bacterial adhesin PapG that mediates binding to the galabiose receptor. Pap-pili are assembled by a periplasmic chaperone PapD [1] which is not itself incorporated into the pilus. PapD binds to each of the pilus subunit types as they are translocated across the cytoplasmic membrane and escorts them to their assembly site. PapD binds to the highly conserved [2] COOH-terminal end of the different subunits. Synthetic peptides corresponding to this conserved region also bind to the chaperone PapD [3]. To investigate the molecular basis of this chaperone-subunit interaction PapD-binding peptides were analysed with both CD spectroscopy and ^1H-NMR spectroscopy. To determine the conformation of the peptides when bound to PapD transfer-NOE experiments were performed.

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D2-240 STRUCTURAL STUDIES OF PEPTIDE INHIBITORS OF VOLTAGE-GATED K^+ CHANNELS (Kv1.3) ON HUMAN T LYMPHOCYTES, J.M. Withka*, J.P. Rizzi*, K.A. Borzilleri*, D.H. Singleton*, G.C. Andrews*, W. Lin*, J.G. Boyd*, J. G. Stroh*, K.J. Rosnack*, D.C. Hanson*, R.J. Mather*, G.A. Gutman+, J. Aiyar+, and K.G. Chandy+, *Pfizer Inc., Central Research Division, Eastern Point Road, Groton, CT 06340,+Dept. of Physiology and Biophysics, UCI, Irvine, CA 92717

Blockage of the voltage-gated Kv1.3 K^+channel on T lymphocytes modulates the immune response. Scorpion toxins that are potent inhibitors of these channels effectively inhibit T cell activation. Three families of these toxins have been identified based upon sequence homology and have been found to block Kv1.3 channels with different potencies. Solution NMR structures of synthetic samples of kaliotoxin, margatoxin, noxiustoxin and agitoxin-2 will be presented. All toxins (37-39 residues) consist of an antiparallel β-sheet and an α-helix with three conserved disulfide bonds. Interactions of the toxins with the channels are being predicted by correlating structural changes with differences in activity. Additionally, complementary studies involving toxin and Kv1.3 channel mutations have demonstrated kaliotoxin-Kv1.3 interactions between R24-D386, F25-H404, and L15 and R31 with G380. These data together with structural information allows an estimation of the dimensions for the channel vestibule as well as a prediction of the orientation for the toxin in the channel.

D2-241 DETERMINATION AND IDENTIFICATION OF THE INTERACTION OF GRAMICIDIN A WITH SOLVENT IN AN ETHANOL/BENZENE SOLVENT SYSTEM USING 2D NMR SPECTROSCOPY, *F. Xu, * J.B. Vaughn, Jr., and T.A. Cross, Institute of Molecular Biophysics and Department of Chemistry, Florida State University, Tallahassee, FL 32306

The mechanistic details of how solvent modifies conformation and function remain unclear. Furthermore, despite a great deal of effort the process for inserting polypeptides and proteins into a membrane environment is not well characterized. In an effort to better understand these processes we have investigated the nature of catalytic solvent (solvent that can promote hydrogen bond exchange) interactions with a well defined polypeptide system, Gramicidin A (Pascal and Cross, J. Biomol. NMR 3:495-513, 1993). The conformation of this polypeptide is highly dependent upon the solvent environment and therefore, an immediate question lies in whether the solvent influences structure through specific or non-specific interactions. In this investigation we have used a minimal amount of ethanol in benzene as a mixed solvent system for the polypeptide. Under these conditions the residence time of the ethanol on gramicidin increases, as does the intensity of the rotating frame Overhauser enhancements (ROEs). These results reveal that there are specific backbone amide and Trp indole protons which are in chemical exchange with the solvent hydroxyl proton. Additionally, there are several other exchangeable amide and indole protons which exhibit ROEs with the solvent. It is significant that there are also several ROE peaks at the catalytic solvent hydroxyl frequency which are attributable to non-labile Trp aromatic, backbone Hα, and side chain protons. These observations document the presence of specific solvent/protein interactions. Such interactions may play a pivotal role in defining the three dimensional conformation of this polypeptide. As a membrane bound polypeptide, the conformation of gramicidin A is very different and the transformation is certainly dependent upon the influence of the heterogeneous lipid environment. The interactions observed here provide some clues as to how this process might occur.

D2-242 **SOLUTION STRUCTURE DETERMINATION OF HUMAN INTERLEUKIN 6 BY HETERONUCLEAR MULTIDIMENTIONAL NMR**, *Guang-Yi Xu, Mark Stahl, Jin Hong, Thomas McDonagh, Jasbir Seehra, and Dale Cumming*, Small Molecule Drug Discovery, Genetics Institute Inc., Cambridge, Ma. 02140

Interleukin-6 is a multifunctional cytokine that exhibits a multitude of stimulatory effects on hematopoetic cells and the cells of the immune system. Emerging evidence suggests that pathological overproduction of IL-6 may be causal in multiple myeloma, autoimmune disease, and postmenopausal osteoporosis.

Based on the primary amino acid sequence homology to G-CSF, a cytokine of known tertiary structure, a computer modeled structure of IL-6 has been proposed by others which exhibits the 4-helix bundle topology common to many cytokines. To test this hypothetical structure and to further expand our understanding of cytokine structure and function, we have initiated efforts to determine the solution structure of recombinant human IL-6.

Uniformly labeled ^{15}N and $^{15}N/^{13}C$ hIL-6 was produced in an E. coli expression system. A series of multi-dimensional heteronuclear pulse field gradient NMR experiments have been utilized to determine backbone and sequential assignments. Determination of the secondary and tertiary structures of rIL-6 using ^{15}N- and ^{13}C-edited NOESY spectroscopy is ongoing.

D2-243 SOLUTION STRUCTURE OF THE HUMAN pp60c-src SH2 DOMAIN COMPLEXED WITH A PHOSPHORYLATED TYROSINE PENTAPEPTIDE, Robert X. Xu*, J. Michael Word, Donald G. Davis, Martin J. Rink, Derril H. Willard, Jr. and Robert T. Gampe, Jr., Glaxo Research Institute, Five Moore Drive, Research Triangle Park, N.C. 27709

Human $pp60^{c\text{-}src}$ is a cellular nonreceptor tyrosine kinase that participates in cytosolic signal transduction and has been implicated in the development of malignant tumors in the human breast and colon. Signal transduction is mediated by highly specific interactions between the SH2 domain and receptor phosphorylated tyrosine binding motifs. To elucidate the molecular conformation and interactions in solution, a family of highly resolved nuclear magnetic resonance (NMR) structures were determined for the *src* SH2 domain complexed with a high affinity phosphorylated pentapeptide, Acetyl-pYEEIE-OH. The 23 structures, generated with a DG/SA procedure, satisfied 2072 experimental restraints derived from a variety of multidimensional and isotope filtered NMR data. Superimposition of residues 143-245 upon the mean coordinate set yielded an atomic rmsd of 0.58±0.09 Å for the N,Cα,C' atoms and 1.04±0.08 for all the nonhydrogen atoms. Residues in the ordered secondary structure regions superimpose to 0.29±0.04 Å for the N,Cα,C' and 0.73±0.08 Å for all the nonhydrogen atoms. The angular order parameter for the φ, ψ angles was > 0.9 for 81 of the 106 protein residues. The observed intermolecular nuclear Overhauser effects (nOe) from the pY, +1E and +3I residues positioned the ligand in an extended conformation across the SH2 domain surface with the pY and +3I sidechains inserted into the protein binding pockets. In general the protein conformation is consistent with previously reported structures of different SH2 domain complexes determined by X-ray crystallography. However, the guanidinium sidechains from the solvated RαA2 or the buried RβB5 did not report any intermolecular nOes at pH=5.5 or 7.0. A more detailed comparison between the crystal structure and the NMR derived solution structures of the same *src* SH2 domain complex is presented. Small conformational differences for ligand residues exposed to observed crystal contacts and at the +3I binding region are discussed.

D2-244 NMR STUDIES OF LOCAL STRUCTURAL ELEMENTS OF PROTEINS, Jian Yao, H. Jane Dyson, Peter E. Wright, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

Structural elements found in short peptides and unfolded proteins are due to local interactions. Understanding their structure and stability is important for understanding the initiation of protein folding and protein stability. Certain short peptides are found to be able to form reverse turns in high populations. Sequence dependence studies have shown that peptides containing two aromatic resudues that flank a proline residue exhibit the greatest population of type VI turn conformation. In order to understand this propensity, structural calculations were carried out for the folded form of the *cis* isomer of a representitive peptide, SYPFDV. The structure will be presented. Judicious use of NMR parameters, including NOE distance restraints and ϕ and χ_1 dihedral angle restraints is emphasized, to account for the flexible nature of peptides. The structure is highly specific and is consistent with all available NMR information including upfield chemical shifts for the proline protons, differential hydration, and proline ring pucker.

D2-245 STRUCTURAL STUDIES OF PEPTIDE FRAGMENTS OF THE PRION PROTEIN, Hong Zhang, Kiyotoshi Kaneko, Jack T. Nguyen, Tatiana L. Livshits, Michael A. Baldwin, Fred E. Cohen, Thomas L. James, and S. B. Prusiner, University of California San Francisco, California 94143-0518

Prions are composed largely, if not entirely, of the scrapie isoform of the prion protein (PrP^{Sc}). Conversion of the cellular isoform (PrP^{C}) to PrP^{Sc} is accompanied by a diminution in the α-helical content and an increase in the β-structure (Pan et al. (1993)*Proc. Natl. Acad. Sci. USA* **90**:10962-10966). Attempts to identify a post-translational chemical modification which triggers this conformational change have been unsuccessful. To investigate the structural basis of this transition, peptide fragments corresponding to Syrian hamster PrP residues 90-145 and 109-141, which are the most conserved across species, were studied using infrared spectroscopy (IR) and circular dichroism (CD). The peptides could be converted to α-helices in aqueous solutions in the presence of organic solvents, such as trifluoroisopropanol (TFE) and hexafluoroisopropanol (HFIP), or detergents, such as sodium dodecyl sulfate (SDS) or dodecyl phosphocholine (DPC). Acetonitrile or NaCl at physiological concentration converted the peptides into polymers rich in β-structures. The intermolecular nature of the β-structures was evident in the formation of rod-shaped amyloids as detected by electron microscopy. Resistance to hydrolysis by proteinase K and epitope mapping suggests that the β-structures were formed by the interaction of residues lying between 109-141. By nuclear magnetic resonance (NMR), the same peptide was shown to be capable of forming α-helices under appropriate conditions. The α-helical structures seem to require hydrophobic support from either intermolecular interactions or detergents, which is in agreement with the predicted hydrophobic nature of the packing surface among the four helices of PrP^{C} (Huang et al. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 7139-7143). The secondary structures of the peptides as determined by the NMR are consistent with the PrP^{C} model. Our data indicate that PrP peptides can form either α-helical or β-sheet structures analogous to the two PrP isoforms. Perturbation of the packing environment of the highly conserved residues is therefore suggested as a possible mechanism for triggering the structural conversion of PrP^{C} into PrP^{Sc} where α-helices appear to be converted into β-structure.

D2-246 STRUCTURAL CHARACTERIZATION OF A FOLDED AND AN UNFOLDED STATE OF AN SH3 DOMAIN IN AQUEOUS BUFFER AND SOLUTION STRUCTURE OF THIS DOMAIN BY NMR Ouwen Zhang[a,b], Lewis E. Kay[a] and Julie D. Forman-Kay[b]

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The isolated N-terminal SH3 domain of the drosophila signalling adaptor protein, drk, was found to exist in equilibrium between a folded state and an unfolded state under normal aqueous conditions. By adding either 400 mM Na2SO4 or 2 M guanidium chloride, we obtained only a folded state or an unfolded state, respectively. Backbone assignments of both states for this protein in equilibrium were made simultaneously by 3D gradient sensitivity-enhanced HSQC-TOCSY and HSQC-NOESY with minimum water saturation techniques. The uniqueness of this system allowed structural characterization of folded and unfolded states in the same solution without added denaturant. NOE patterns, backbone NH, Hα and 15N chemical shifts, 3JHαNH coupling constants and amide-water exchange rates show that the folded states in equilibrium and in high salt are identical, while there are significant differences between the unfolded states in equilibrium and in denaturant solution. The structure of the folded state in equilibrium strongly resembles other known SH3 structures. SH3 domains mediate specific protein-protein interactions by binding to poly-proline regions in their biological targets. A titration study of this SH3 domain with peptides derived from its biological target, Sos, demonstrated that the folded state in equilibrium is biologically active. We also present the three dimensional structure of the folded state of this protein in 400 mM Na2SO4, which stabilizes the folded structure. Structural data coupled with thermodynamic data have been used to understand the stability of this SH3 domain. Comparisions with other SH3 domains will also be presented.

Nucleic Acid Structure; Nucleic Acid Complexes

D2-300 HETERONUCLEAR NMR STUDIES OF THE STRUCTURE OF THE MutT ENZYME AND ITS INTERACTION WITH SUBSTRATE ANALOGS, C. Abeygunawardana,[1] D.J. Weber,[1] A.G.Gittis,[1] D.N. Frick,[2] A. F. Miller,[2] M.J. Bessman,[2] and A.S. Mildvan,[1] [1] Johns Hopkins School of Medicine, Baltimore, MD 21205 and [2]University, Baltimore, MD 21218

The MutT enzyme (129 residues) catalyzes the hydrolysis of normal and mutagenic nucleoside triphosphates, such as 8-oxo-dGTP, by substitution at the rarely attacked β-P, to yield NMP and pyrophosphate. Previous NMR studies of MutT have assigned the backbone Hα, Cα, NH, and N resonances and have shown the secondary structure to consist of a 5-stranded mixed β-sheet connected by the loop I-α helix I-loop II motif, two tight turns, and loop III, and terminated by loop IV-α helix II (*Biochem.* 32, 13071; 13081). Complete side chain assignments of ^{1}H and ^{13}C resonances have now been made by 3D CC(TOCSY)(CO)NNH and HCCH TOCSY experiments. Interproton proximities, obtained by 3D ^{15}N NOESY HMQC and 3D ^{13}C NOESY HSQC spectra, including >200 remote NOEs were used to determine the tertiary structure of MutT by distance geometry, simulated annealing, and energy minimization with the program XPLOR. The conformation of the enzyme-bound substrate analog, Mg^{2+}-AMPCPP, determined by 10 transferred intramolecular NOEs, is very high *anti* (χ = 100±16°) with a C1'-endo ribose pucker (δ = 126±2°). Similarly the conformation of the bound product dGMP is very high *anti*, C2'-endo (χ = 90±10°; δ = 145±6°) based on 7 intramolecular NOEs. Such conformations avoid intramolecular clashes in substrates such as 8-oxo-dGTP. Nucleotides bind near the loop I-helix I-loop II motif based on intermolecular NOEs and selective paramagnetic broadenings by Mn^{2+}-AMPCPP in ^{1}H-^{15}N HSQC spectra. Both Mg^{2+} and AMPCPP alter the chemical shifts in ^{1}H-^{15}N HSQC spectra of residues in the loop I-helix I-loop II motif as well as in β-strands A, B, and E, and loop III. The tertiary structure of MutT shows most of these regions to be near each other. The location of the active site determined by NMR is consistent with mutagenesis studies and with sequence homologies among MutT-like pyrophosphohydrolases.

D2-301 ROLES OF MODIFICATIONS AND METAL IONS IN RNA STRUCTURE/FUNCTION DETERMINED BY NMR, Paul F. Agris, Mufeed M. Basti, Stephen C. Brown*, John Stuart, Richard Guenther, Adam Kraszewski+, and Andrzej Malkiewicz#
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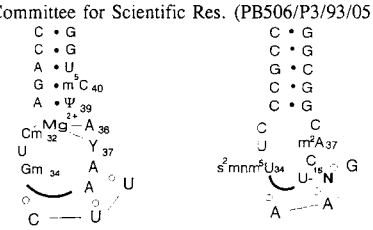
Site-specific introduction of isotopically-labelled and modified nucleosides by chemical synthesis has aided the NMR investigation of tRNA structure and function. Some 70 modified nucleosides are found in tRNAs and, in the absence of high concentrations of Mg^{2+}, unmodified tRNA transcripts neither functionally nor structurally resemble native tRNAs. Using NMR spectroscopy of tRNA domains and their DNA analogs, we have determined that specific modified nucleosides are involved in tight metal ion binding directly or impart structure that provides ligands for binding. A modification-dependent, structural transition to a closed loop is produced by one particular Mg^{2+} binding in the anticodon domain and is counterproductive to tRNA function. Anticodon-codon binding studied by NMR, demonstrates that the evolution of other modifications has corrected this to produce an effective codon binding and negate translational frame shifting, simultaneously. The unique positioning of an $^{15}N_{1,3}$-uridine in the second position of the anticodon demonstrated the specificity of anticodon-codon binding, *in vitro*. Therefore, modified nucleosides contribute functionally important structures that facilitate Mg^{2+} binding and correct codon recognition. [Support by: NIH (GM23037 to P.F.A.), Glaxo Res. Inst.; Polish Committee for Scientific Res. (PB506/P3/93/05 to A.M.)]



D2-302 HIGH RESOLUTION NMR STRUCTURE OF THE BIOLOGICALLY ACTIVE DNA ANALOGUE OF YEAST $tRNA^{Phe}_{AC}$, Mufeed M. Basti, John W. Stuart, Richard Guenther, and Paul F. Agris, Department of Biochemistry, North Carolina State University, Raleigh, NC 27695-7622

A seventeen-residue DNA analogue of yeast $tRNA^{Phe}$ anticodon domain ($tDNA^{Phe}_{AC}$), with d(m^5C) and d(m^1G) at positions corresponding to those of m^5C_{40} and Y_{37} in the tRNA, was chemically synthesized. The hairpin has been shown to have two tight Mg^{+2} binding sites and effectively inhibits the binding of native tRNA to the ribosome. A number of one- and two-dimensional NMR experiments were carried out to determine the three dimensional structure and the metal binding positions of the hairpin. An average of 8 interproton NOEs per residue were observed and their intensities were then used to generate distance constraints using iterative relaxation matrix algorithm (IRMA, Biosym Technologies). Restrained molecular dynamics (rMD) calculations followed to obtain structures consistent with the experimental constraints. The NMR data and the computer-generated models indicate that the stem of the hairpin adopts mainly a 3'-stacked, B-DNA form with few local violations especially around the m^5C_{40}. The broadness of the NMR peaks of loop residues prohibited obtaining the number of NOEs necessary to achieve a well-defined loop structure. Substitution of Mg^{+2} by paramagnetic Mn^{+2} which introduced selective broadening of proton signals helped in determining the metal binding sites in the hairpin. Extending the use of NMR, paramagnetic elements, and molecular dynamics as tools in the study of tRNA hairpin structure and function will be discussed.

D2-303 HETERODIMERIZATION OF THE YEAST HOMEODOMAIN TRANSCRIPTIONAL REGULATORS a1 AND α2: EFFECTS OF DIMERIZATION ON BACKBONE DYNAMICS STUDIED BY ^{15}N-NMR SPECTROSCOPY, Susan M. Baxter and Frederick W. Dahlquist, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

The yeast proteins, a1 and α2, act cooperatively to regulate cell type specific genes in yeast. The basis of this cooperativity is the formation of a heterodimer that binds DNA tightly and specifically. Fragments containing the DNA-binding regions of the a1 and α2 proteins have been studied by NMR spectroscopy to define secondary structural elements and localize changes upon heterodimerization.[1,2,3] Both proteins contain the three helices, two turns and an unstructured n-terminal tail characteristic of the homeodomain protein family. Heterodimer formation does not seem to affect the conformation of the DNA recognition helices. To characterize the dynamics of the individual proteins and the complex they form, ^{15}N spin-lattice (T_1) and spin-spin (T_2) relaxation rate constants and $\{^{1}H\}^{15}N$ nuclear Overhauser effect (NOE) enhancements have been measured for the backbone amides by two-dimensional, proton-detected heteronuclear NMR experiments. Relaxation parameters were analyzed using a model-free formalism that characterizes the dynamics of the N-H bond vectors in terms of generalized order parameters (S^2), effective correlation times (τ_e), ^{15}N exchange broadening contributions (R_{ex}) and the overall rotational correlation time (τ_m). These results provide insight into the molecular details of heterodimer formation and evidence that heterodimerization may "prepay" the entropic cost associated with subsequent DNA binding.

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D2-304 STRUCTURE OF A 24-NUCLEOTIDE RNA HAIRPIN, Philip N. Borer, Yong Lin, Deborah Kerwood, & Istvân Pelczer, *Chemistry Department, Syracuse University, Syracuse, NY 13244-4100.*

The three-dimensional conformation of a 24-nucleotide variant of the RNA binding sequence for the coat protein of bacteriophage R17 has been analyzed using NMR and molecular mechanics. The imino proton spectrum is consistent with base pairing requirements for coat protein binding known from biochemical studies [Witherell, G. W., Gott, J. M. & Uhlenbeck, O. C. (1991) *Prog. Nucl. Acid Res. 40,* 185.] All 185 of the non-exchangeable protons were assigned using a variety of homonuclear 2D and 3D-NMR methods. Measurements of nuclear Overhauser enhancements and two-quantum correlations were made at 500 MHz. New procedures were developed to characterize as many resonances as possible, including deconvolution and path analysis methods. An average of 21 distance constraints per residue were used in molecular mechanics calculations to get folded structures for residues 3 - 21. The unpaired A8-residue is stacked in the stem, and the entire region from G7 - C15 in the upper stem and loop appears to be flexible. Several of these residues have a large fraction of S-puckered ribose rings, rather than the N-forms characteristic of RNA duplexes. There are at least two low-energy loop conformations that satisfy the distance constraints at a preliminary level of refinement. The Shine-Dalgarno ribosome binding site is exposed and only two apparently weak base pairs would have to break for the 16S ribosomal RNA to bind and the ribosome to initiate translation of the replicase gene. Although the loop forms must be regarded as tentative, the known interaction sites with the coat protein are easily accessible from the major groove side of the loop. Progress of further refinement will be reported at the meeting.

D2-305 COMPLETE ASSIGNMENT AND PRELIMINARY 3-DIMENSIONAL STRUCTURE OF THE ZINC CLUSTER PROTEIN, ALCR.

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The transcription factor ALCR of the ethanol utilisation pathway in *Aspergillus nidulans* contains a motif : Cys-X2-Cys-X6-Cys-X16-Cys-X2-Cys-X6-Cys (1). The amino acid sequence (1-65) of this zinc cluster is very similar to the DNA-binding domains of ascomycete transcription factors, which have six cysteine residues. However, several features of the ALCR zinc-binding motif are interesting. An unusual extended loop of 16 amino acids is present between the third and fourth cysteine whereas 6 residues are usually found in the sequence of zinc motif, as exemplified by GAL4. Unlike many transcription factors of this class, whose recognition sequences are frequently inverted repeats, the ALCR zinc-binding motif is able to bind to sites that occur in two configurations, a direct repeat and an inverted repeat, with the same consensus core 5'-CCGCA-3' (2). Structural studies of ALCR were performed using two- and three-dimensional NMR at pH = 5.9 and T = 293K. Sequential assignment was obtained for main and side chain resonances and led to the identification of all secondary structures. The region between the first and second cysteine contains an α-helix.

Replacement of the two zinc atoms with ^{113}Cd allowed us to obtain more details about the zinc cluster complex geometry.

A preliminary tertiary structure and a comparison with GAL4 (3,4) will be discussed. The structural features of ALCR may give information about alternative DNA binding modes.

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D2-306 NMR STUDIES OF THE 23 MER DNA OLIGONUCLEOTIDES CONTAINING THE ENHANCER OF THE HUMAN PROENKEPHALIN GENE SEQUENCE, Hong Cheng, Naned Juranic, Slobodan Macura, Cynthia T. McMurray, Mayo Foundation, 200 First Street, SW, Rochester, MN 55905 USA

Receptor-mediated induction of the human proenkephalin gene has been mapped to short semi-palindrome of 23 base pairs between -104 and -86, upstream of the transcription start site. Several lines of evidence suggest that the synthetic 23-base-pair oligonucleotide duplex containing the enhancer of the human proenkephalin gene sequence undergoes a reversible conformation change from duplex to a hairpin state of the enhancer. Two hairpins can be formed from each of the two strands. Each hairpin contains two mismatched base pairs and one three-base loop. We have used NMR spectroscopy to study the structure of two synthesized 23 mer oligonucleotides:
GT hairpin: d(GCTGGCGTAGGGCCTGCGTCAGC)
and
AC hairpin: d(GCTGACGCAGGCCCTACGCCAGC)
whose sequence is identical to the top and bottom strands of the native enhancer, respectively. NMR spectroscopy has been used to determine the structures of the two oligonucleotides. The NMR data show that each oligonucleotide strand exists primarily as a hairpin structure over a wide range of oligonucleotide concentration and over a wide range of temperature (0-45 °C). By using 2D NMR methods, NOESY, COSY, and TOCSY, we have assigned the most proton resonances from the bases in the stem of GT hairpin. For those from the bases in the loop of GT hairpin, however, their sequential connections were unable to be identified from the normal homonuclear 2D proton NMR spectra.

D2-307 SOLUTION STRUCTURE OF THE LEUCINE ZIPPER MOTIF OF HIV TYPE 1 INTEGRASE

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Integration of virus DNA into the infected host cell DNA is an essential step in the life cycle of human immunodeficiency virus 1 (HIV-1). This integration step is only performed by the viral protein - HIV-1 integrase (IN). It has been shown that IN contains at lease four functional domains, namely an N-terminal HHCC box zinc finger domain, a central catalytic domain, a leucine zipper motif and a C-terminal non-specific DNA binding domain. The leucine zipper motif was suspected to be the multimerization region of IN. Secondary structure of the leucine zipper motif of IN was predicted to be an α-helix based on structure prediction methods and molecular dynamics simulations. We now present the experimental evidences that the solution structure of the leucine zipper motif of IN adopts an α-helical conformation based on two-dimensional ^1H-NMR and CD studies.

D2-308 **Investigations of the DNA binding Domain of Transposase: Backbone Dynamics and Mutagenesis Studies.**

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Transposons are mobile genetic elements capable of translocation from one site on the DNA to another. Transposition, the recombination reaction utilized to accomplish this task, is also the mechanism employed to integrate cDNA copies of retroviral and retrotransposon RNA into the chromosomal DNA of their host. The largest and most efficient transposon known is the bacteriophage Mu genome, which utilizes the phage encoded transposase (MuA protein) to pair the ends of the phage DNA, cleave the termini, and promote strand transfer. Mu transposase is a monomeric 75kD protein in solution but functions as a tetramer during transposition. The transposase can be divided into four structurally and functionally distinct domains. We have recently determined the three-dimensional solution structure of the internal DNA binding domain of transposase (residues 1-76 of MuA, Mu^{76}) which facilitates the assemble the transposase:DNA complex. The fold of Mu^{76} is a novel single "winged", winged helix-turn-helix DNA binding domain. Here we present the results of rationally designed site-specific mutations of the Mu^{76} domain and their effects on the transposition reaction. We also present the results of ^{1}H-^{15}N heteronuclear NMR relaxation measurements of the Mu^{76} domain. An interpretation of this data utilizing the model-free formalism has provided information about backbone mobility of the HTH unit and wing utilized in DNA recognition. The results of both biochemical and biophysical studies provides a detailed view of the function of the Mu^{76} domain in the transposition of the phage genome.

D2-309 STABILIZATION OF RNA STACKING BY PSEUDOURIDINE. Darrell R. Davis, Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT 84112.

Pseudouridine is the most common modified nucleoside in RNA, occurring ubiquitously in tRNA and with high frequency in ribosomal RNAs and small nuclear RNAs. Pseudouridine is unique compared to uridine in that there are two NH imino protons available to serve as hydrogen bond donors[1]. The effect of the modified nucleoside pseudouridine on RNA stacking was compared to uridine. Base stacking was measured by ^{1}H NMR spectroscopy using model single-stranded oligoribonucleotides composed of a single uridine or pseudouridine residue within a sequence of adenosines. The extent of stacking for each residue was determined from the percent 3′-endo sugar conformation as indicated by the H1′-H2′ scalar coupling. NMR measurements as a function of temperature indicate that pseudouridine forms a more stable base stacking arrangement than uridine, an effect that is propagated throughout the helix to stabilize stacking of neighboring purine nucleosides. Stabilization of pseudouridine nucleoside conformation is accomplished by forming a stable intramolecular hydrogen bond involving a water molecule bridged between the N1 proton and the phosphate backbone. The presence of the water bridge was determined by 2D NOESY experiments with a selective NODE-1 read pulse. The three-dimensional structure of the pseudouridine containing model single-stranded tetramer, AAψA, was determined using typical 2D NMR experiments to obtain constraints.

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D2-310 STRUCTURAL INVESTIGATIONS OF RNA AND DNA APTAMERS IN SOLUTION.

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Aptamers are nucleic acid molecules which selectively bind ligands. They can be isolated by their specific properties from large pools of random sequences by *in vitro* selection. RNA aptamers are excellent model systems for the study of molecular recognition processes and the structural basis of RNA function and folding. Furthermore, they may provide insight into the processes which took place about 2.5 billion years ago, when the first self-replicating systems appeared on earth: the so-called RNA-world and the origin of life.

The aptamers currently under investigation in our laboratory include RNA and DNA forms of an ATP binding aptamer as well as Riboflavin binding aptamers. The RNA ATP-binder has been studied by homo- and heteronuclear, multi-dimensional NMR techniques. The molecular interactions upon binding of ATP have been studied using combinations of labeled and unlabeled RNA and ATP. Large changes of proton chemical shifts and a number of intermolecular NOEs show that the adenine part of the ATP strongly interacts with bases and sugars in the RNA.

D2-311 THE ETS-DOMAIN IS A MEMBER OF THE WINGED HELIX-TURN-HELIX SUPERFAMILY OF DNA-BINDING PROTEINS.

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The Ets family of eukaryotic transcription factors and proto-oncoproteins is characterized by a highly conserved sequence, termed the ETS-domain, that mediates specific binding to dsDNA sites containing a GGA core motif. The archetypal member of this family is the *v-ets* oncogene from avian leukosis *retrovirus*. The broad objectives of our research are to characterize the structure, DNA-binding, and intramolecular regulation of the ETS-domain.

Based on the NMR-derived α/β secondary structure of a cloned 12 kDa fragment of murine Ets-1 containing the ETS-domain, we have shown that this transcription factor is a member of the superfamily of "winged helix-turn-helix (wHTH)" DNA-binding proteins. The prototype of this superfamily is *E. coli* catabolite activator protein (CAP). We propose a model in which a helix-turn-helix motif binds DNA in the major groove while β-sheet wings provide flanking contacts.

A combination of NOESY experiments including 4D-[^{1}H,^{13}C,^{15}N,^{1}H] , 4D-[^{1}H,^{13}C,^{13}C,^{1}H] , 3D-[^{1}H,^{15}N,^{1}H], 3D-[^{1}H,^{13}C,^{1}H], and 2D spectra of selectively labelled aromatic residues are currently being analysed to determine the teritiary structure of the 12 kDa fragment and to validate the wHTH model.

D2-312 COUNTERION DEPENDENCE OF STRAND ASSOCIATION AND DISSOCIATION IN A DNA DODECAMER CONTAINING AN A_3T_3 TRACT. Amy S. Duffield, Ewa J. Folta-Stogniew, and Irina M. Russu, Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, CT 06459-0175

Several laboratories have previously reported that strand dissociation in DNA oligonucleotides is either independent or very slightly dependent on counterion concentration. Subsequent work in this laboratory, however, found that the number of sodium ions which are adsorbed and released during the helix-coil transition of $[d(CGCGAATTCGCG)]_2$ is equally distributed between association and dissociation processes. In order to establish the general relevance of this finding, we have used ^1H NMR spectroscopy to investigate the salt dependence of the equilibria and kinetics of the helix-coil transition of the dodecamer $[d(CGCAAATTTGCG)]_2$. This dodecamer was chosen because it exhibits anomalous migration in gel electrophoresis, suggesting that it is bent. Moreover, crystallographic structures of the dodecamer indicate variations in phosphate-phosphate separation along the sequence. These factors are expected to influence the interactions of the DNA with counterions. The helix-coil transition of the dodecamer was monitored using the proton resonance of the methyl group of the thymine in position 7. Chemical shift of this resonance was followed to calculate the equilibrium constant for helix formation (K_a). Line width measurements of the same resonance were used to obtain rate constants for strand association (k_a) and duplex dissociation (k_d). The helix-coil transition was monitored as a function of sodium ion concentration. The salt dependence of K_a, k_a, and k_d was used to calculate the number of counterions released in the helix-coil transition and their distribution between the association and dissociation processes. The number of sodium ions which associate with $[d(CGCAAATTTGCG)]_2$ during duplex formation is found to be comparable to the number of sodium ions released upon helix dissociation. These results support the data previously obtained for $[d(CGCGAATTCGCG)]_2$, namely, both strand dissociation and association are significantly dependent upon counterion concentration. (Supported by the Hughes Program in Life Sciences, Wesleyan University)

D2-313 NMR EXPERIMENTS ON PERDEUTERATED AND $^{13}C/^{15}N$ LABELED HUMAN CARBONIC ANHYDRASE II: ^{13}C SIDECHAIN ASSIGNMENTS AND LONG-RANGE ^1H_N-^1H_N NOES. Bennett T. Farmer II, Ronald A. Venters*, Leonard D. Spicer*, and Luciano Mueller. Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-4000. *Department of Biochemistry, Duke University Medical Center, Durham, NC 27710.

High-level deuteration of aliphatic carbons in large proteins has been previously demonstrated to have a beneficial effect on the $^{13}C_\alpha$ and ^1H_N T_2 relaxation rates. Although high-level deuteration of large proteins has greatly enhanced the ability to obtain sequential assignments based on inter-residue and intra-residue $^{13}C_\alpha/^{13}C_\beta$ correlations to the backbone ^1H_N-^{15}N spin pair, there has been no published attempt to obtain additional ^{13}C sidechain assignments. These assignments are useful for confirming amino-acid types based on spin-system patterns and as a starting point in the analysis of the 4D $^{13}C/^{13}C$-separated NOESY on a fractionally deuterated protein sample. We present here the application of the C(CC)(CO)NH experiment to perdeuterated and $^{13}C/^{15}N$ labeled human carbonic anhydrase II (HCA II) for the assignment of the additional ^{13}C sidechain resonances. The sensitivity of this experiment on the perdeuterated protein is compared both theoretically and experimentally to that of the HC(CC)(CO)NH experiment on the protonated protein.

Cross-strand and short-range ^1H_N-^1H_N NOEs are important in β-sheets and helices/turns, respectively. Perdeuteration of aliphatic carbons should dramatically increase both the overall signal-to-noise of NOEs in a 4D $^{15}N/^{15}N$-separated NOESY and the maximum observable range for this particular NOE interaction. Results from perdeuterated HCA II will be presented.

D2-314 SOLUTION STRUCTURES OF R- and S-α-(N^6-ADENYL)-STYRENE OXIDE ADDUCTS AT EACH OF TWO ADJACENT ADENINES IN AN OLIGONUCLEOTIDE CONTAINING CODON 61 OF THE *N-RAS* PROTOONCOGENE, Binbin Feng, Liang Zhou, Mariella Passarelli, Constance M. Harris, Thomas M. Harris, and Michael P. Stone, Department of Chemistry, Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37235.

The solution structures of d(CGGACAAGAAG):d(CTTCTTGTCCG), containing n-ras codon 61 (underlined), and of the R- and S-diastereomeric α-(N6-adenyl)-styrene oxide adducts incorporated at A^6 and A^7 in this sequence, were investigated by ^1H NMR. The NMR spectra were assigned using a combination of NOESY and TOCSY experiments. Analysis of NOEs between the styrene and DNA protons, and of chemical shift perturbations, revealed that these α-styrene oxide adducts were located in the major groove of the DNA duplex. The solution conformation was dependent upon the stereochemistry of adduction. For the R diastereomers, the styrene ring was oriented towards the 5' direction from the site of adduction. For the S stereoisomers, the styrene ring was oriented towards the 3' direction from the site of adduction. For each adduct, distance restraints were calculated from NOE data using relaxation matrix analysis. These were incorporated as effective potentials into the total energy equation. The solution structures were refined using restrained molecular dynamics calculations which incorporated a simulated annealing protocol. The accuracy of the emergent structures was evaluated by complete relaxation matrix methods. For the two R-α-(N^6-adenyl)-styrene oxide adducts slight DNA sequence effects upon adduct conformation were observed. These sequence effects are discussed in the context of mutagenesis experiments designed to probe for R-α-styrene oxide-induced mutations [R.S. Lloyd and coworkers, University of Texas Medical Branch, Galveston, TX], which revealed stereo- and sequence-specific effects in the processing of these lesions. A similar analysis of DNA sequence effects upon the two S-α-(N^6-adenyl)-styrene oxide adducts is underway. Supported by the NIH: ES-05355 (M.P.S), ES-05509 (T.M.H.), and RR-05805 (NMR spectrometer).

D2-315 STRUCTURAL FEATURES OF THE TRANSCRIPTIONAL ACTIVATION DOMAIN OF MOTA FROM BACTRIOPHAGE T4, Michael S. Finnin, David W. Hoffman and Stephen W. White, Department of Microbiology, Duke University Medical Center, Durham, NC 27710.

The bacteriophage T4 middle-mode transcription factor MotA consists of two domains of approximately equal size. The C-terminal domain (MotCF) has been shown to contain the DNA-binding elements of the molecule, and the N-terminal domain (MotNF) appears to interact with RNA polymerase. Three-dimensional triple-resonance NMR techniques are being used to solve the solution structure of a 96 amino acid construct of MotNF. The backbone resonances for residues 4-96 were identified through gradient-enhanced CBCA(CO)NH and HNCA experiments and many side chain resonances were assigned through a gradient-enhanced HCCH-TOCSY experiment. An NMR secondary structure model of MotNF has been built which contains five α-helical segments and two four-residue antiparallel β-strands. Three-dimensional ^{13}C and ^{15}N-edited NOESY spectra have been collected and are in the process of being interpreted in order to find long-range NOE resonances. In tandem, crystals of MotNF that diffract beyond 2.4Å have been obtained and work is proceeding to solve the structure by X-ray crystallographic methods. The three-dimensional structure of MotNF should provide insight into the mechanism of transcription since little structural information is known about activation domains of transcription factors. Our previous NMR studies on the DNA-binding domain, MotCF, suggests homology to the TATA-binding protein. Therefore, the MotA protein may provide structural and functional insights into eukaryotic transcriptional regulation.

D2-316 SOLUTION STRUCTURE OF THE SINGLE-STRANDED DNA BINDING PROTEIN OF PHAGE PF3.

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The single-stranded DNA binding protein (ssDBP, 78 residues) encoded by the filamentous *Pseudomonas* bacteriophage Pf3 has been studied by multidimensional heteronuclear NMR spectroscopy. The molecule forms a symmetric dimer with a molecular mass of 18 kDa. We designed a 'solubility mutant' [1] (Phe36→His) because the wild-type protein has a large tendency to aggregate at millimolar concentrations. NMR measurements and structure calculations have been performed on this mutant.

The three-dimensional structure of the protein will be presented, which was calculated using restrained molecular dynamics (without the use of distance geometry). Structures are based on 1440 distance and 32 dihedral constraints per monomer.

Notwithstanding the low sequence homology, the folding of Pf3 ssDBP strongly resembles that of the M13 gene 5 protein[2] (GVP). Furthermore, the structure of the Pf3 ssDBP is strikingly similar to the major cold shock protein of *Bacillus subtilis* (CspB)[3]. CspB is a putative single-stranded DNA binding protein which has an apparent sequence homology with the cold-shock domain of Y-box factors. Based upon these striking structural and functional similarities it is postulated that the Pf3 ssDBP fold forms a common motif implicated in nucleic acid binding.

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D2-317 ON THE ENERGETICS OF BASE-PAIR OPENING PROCESSES IN DNA, Ewa J. Folta-Stogniew, James G. Moe* and Irina M. Russu, Molecular Biology and Biochemistry Department, Wesleyan University, Middletown, CT 06459-0175, *GENE-TRAK, 31 New York Ave, Framingham, MA 01701

Previous work from this laboratory has used proton NMR spectroscopy to characterize the kinetics and energetics of base-pair opening in six self-complementary DNA dodecamers. The base sequences in the DNA dodecamers of interest encompassed homopurine·homopyrimidine tracts, (AAA/TTT, GAA/TTC, AGA/TCT, GAG/CTC), an alternating purine·pyrimidines tract, (CACA/GTGT) and a mismatched G·T base pair (GAA/TTT). The opening rates and the equilibrium constants for formation of the open state of each base pair were obtained from the dependence of imino proton exchange rates on the concentration of ammonia catalyst at several temperatures. Depending on base sequence, the opening rates were found to range from 3 to 1000 s^{-1} (at 15°C). A strong sequence-dependence was observed for enthalpy changes: the activation enthalpies for opening ranged from -6 to 22 kcal/mol and the standard enthalpy changes to form the open state of the base pairs varied from 10 to 26 kcal/mol. In the present work, we present a full analysis of these results for 21 base pairs in the dodecamers of interest, each placed in a distinct sequence context. The analysis shows that, for all base pairs investigated, the standard enthalpy changes are linearly related to the standard entropy changes. This enthalpy-entropy compensation exists in the activation step as well as in the equilibrium between open and closed states of the base pairs (corresponding compensation temperatures 316±12 K and 293±34 K, respectively). The relevance of these findings for the mechanisms and energetics of base-pair opening in DNA will be discussed. (Supported by grant 88-17589 from NSF)

D2-318 SOLUTION STUDY OF TFIIIA ZINC FINGERS 1-3 COMPLEXED TO A 15-BASE PAIR DNA DUPLEX.

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The first three zinc fingers of the nine-finger *Xenopus laevis* transcription factor TFIIIA contribute most of the binding energy for recognition of its cognate DNA sequence in the internal control region of the 5S RNA gene. We have undertaken a solution study of a protein construct containing TFIIIA zinc fingers 1-3 complexed to a 15-bp DNA oligonucleotide containing the high affinity (<6nM) binding site.

Our approach has been to use double- and triple-resonance NMR experiments at 500, 600 and 750 MHz to obtain resonance assignments for the isotopically labeled (^{13}C, ^{15}N) protein, and isotope filtered 2D experiments to assign DNA resonances and distinguish protein-protein, protein-DNA and DNA-DNA cross-relaxation. Data on the protein component of the complex will be presented.

D2-319 **UNUSUAL SOLUTION STRUCTURES UNVEILED BY NMR STUDIES OF SINGLE-STRANDED DNA TRIPLET REPEATS.**

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The discovery that trinucleotide repeat expansions condition a number of neurological disorder diseases has attracted great attention. The focus of the research in this area has been on the role that nucleic acid repeat sequences may play in the mutational mechanisms by which certain inherited human disorders are transmitted.[1] 5'-$(CTG)_n$ located within a gene exon, is associated with the onset of a number of neurodegenerative disorders: spinocerebellar ataxia type I, X-linked spinal and bulbar atrophy (SMBA), Huntington's disease, and hereditary dentatorubral-pallidoluysian atrophy (DRPLA). The trinucleotide repeat expansion disease (TRED) myotonic dystrophy also shows anticipation related to the CTG repeats, but these are located in a 3' untranslated region of the gene. A second sequence of unstable trinucleotide repeats, $(CGG)_n$ and $(GCC)_n$, is implicated in fragile X syndromes, FRAXE and FRAXA, respectively.

We are interested in using NMR spectroscopy to characterize the solution conformation of the triplet repeats, which may serve as basic structural motifs in large molecular assemblies. To determine whether short stretches of DNA triplet repeats could form stable secondary structures, we have extensively investigated several DNA triplet repeats in single-stranded form by using high resolution NMR spectroscopy. A wealth of information has been obtained on sequence alignment and base pairing as a function of the number of repeats, temperature, pH and ionic strength. Each of the triplet repeating sequences exhibits spectral features which indicate that these single-stranded sequences tend to form stable structures rather than random coils. Structure refinement using DG/MD has revealed a novel helical structure that differs from any of the known DNA structures.

Acknowledgments: KS is a NASA graduate fellow in Professor George Fox's laboratory.

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D2-320 NMR CHARACTERIZATION OF DIMERIC MINOR GROOVE COMPLEXES OF DISTAMYCIN ANALOGS: DESIGNED "HAIRPIN" DIMERS AND A SIDE-BY-SIDE DIMER WITH AN EXTENDED RECOGNITION SITE,
Bernhard H. Geierstanger, Timothy D. Cole and David E. Wemmer, Department of Chemistry, University of California, Berkeley, CA 94720

NMR, footprinting and affinity cleavage studies have previously shown that the peptide-linked polypyrrole distamycin and its analogs can be combined in head-to-tail dimeric complexes with the minor groove of DNA. The specificity of these complexes can be engineered by replacing pyrrole rings with imidazole rings. Recently the imidazole-pyrrole-pyrrole ligand 2-ImN was covalently linked to the pyrrole-pyrrole-pyrrole system P3 to enhance binding affinity and specificity. NMR titrations and 2D NOESY data indicate that, consistent with the design, ligands with linkers of two, three and four methylene groups preferentially form a "hairpin" structure with the TAACA·TGTTA target site. 2-ImN-Gly-P3 does not form a 'hairpin" complex but forms a dimeric complex with a 13 base pair site consisting of TTTTAGACAAATT·AATTTGTCTAAAA. The central AGACA· TGTCT sequence is occupied by a dimeric motif formed by the two 2-ImN ring systems while the flanking AT sequences are complexed by the P3 parts of the ligands similar to the 1:1 complexes of distamycin. This extended dimeric complex has significant implications for the design of sequence-specific minor groove binders that recognize longer sites.

D2-321 NMR STRUCTURAL STUDIES OF RNA MOLECULES USING $^{13}C/^{15}N$ LABELED RNA

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Structural studies of RNA molecules by NMR have for a long time been hampered by strong resonance overlap, in particular in the sugar proton region. Fortunately this situation has changed, mainly because RNA fragments, uniformly enriched in ^{13}C and/or ^{15}N are now efficiently synthesized on a more or less routine basis [1,2].
Using enriched RNA we devised a number of heteronuclear experiments that make the most out of these labels: X-filter experiments unambiguously determine RNA-RNA, RNA-DNA and DNA-DNA NOEs in a triple helix composed of a DNA hairpin, Hoogsteen base paired to a [$^{15}N/^{13}C$]-uniformly enriched RNA strand (compare the accompanying poster of Van Dongen et al.). Heteronuclear 3D and 2D triple resonance experiments allow for a complete through bond sequential resonance assignment of ^{1}H , ^{13}C and ^{31}P resonances [4,5]. We also devised 2D and 3D heteronuclear experiments that allow for a more accurate determination of J-couplings for deriving backbone torsion angles. These experiments are crucial in high resolution structure determination of RNA molecules by NMR, which will be demonstrated on a RNA hairpin with a tetranucleotide CAAA loop.

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D2-322 NMR INVESTIGATION OF THE C195A RETINOID X RECEPTOR DNA BINDING DOMAIN

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The retinoid X receptor (RXR) is a zinc finger protein and a member of the nuclear hormone receptor superfamily. The receptor regulates gene expression by binding as either a homodimer or heterodimer (formed with other nuclear hormone receptors) to two DNA half-site sequences (AGGTCA) arranged as a direct repeat [AGGTCA$(X)_n$AGGTCA]. Selective DNA recognition of the dimers is achieved, in part, through variable spacing between the two half-sites (n= 1-5). The DNA recognition function of the receptor is localized to an 80 residue DNA binding domain (RXR 130-212). Previous NMR structural studies of the RXR DNA binding domain revealed the presence of a novel third helix, which plays a role in both DNA binding and protein-protein dimerization. Our goal is to refine this NMR structure.

To increase the solubility of the protein, Cys195, which is not a zinc ligand and resides in the hydrophobic core, was mutated to an Ala. The mutant protein was cloned into a pET24a expression vector and expressed at high levels in minimal media, allowing ^{13}C and ^{15}N labeling of the protein. ^{13}C, ^{15}N, and ^{1}H assignments were made using a combination of 3D heteronuclear pulse methods. Analysis of chemical shift and NOESY data show that the mutant has an overall global fold and secondary structure similar to that of the wild type RXR DNA binding domain.

D2-323 SOLUTION STRUCTURE OF RIBOSOMAL PROTEIN S17 BY HIGH RESOLUTION NMR SPECTROSCOPY,
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The solution structure of a primary 16S rRNA binding ribosomal protein, S17 is being investigated by two and three dimensional homonuclear and heteronuclear magnetic resonance spectroscopy. Complete chemical shift assignments for main chain and side chain ^{1}H, ^{15}N and ^{13}C resonances have been obtained. A preliminary structure has been obtained by distance geometry using some of the NOE constraints. Further analysis of the NMR data, to obtain a more rigorous structure is in progress. The preliminary structure of S17 shows that the protein consists of 5 β strands that form a single twisted antiparallel β sheet with Greek key topology which gives it the appearance of an incomplete β barrel. The β strands are connected by extended loops two of which contain residue types that are usually found in RNA binding sites of proteins.

D2-324 A DOUBLE HELIX-TURN-HELIX MOTIF FOR THE DNA BINDING DOMAIN OF THE ONCOPROTEIN C-Myb.

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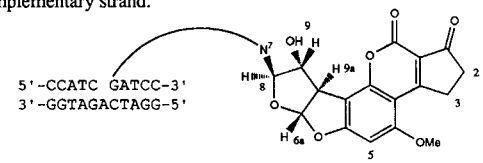
The c-Myb oncoproteins are transcription factors which are involved in the proliferation and differentiation of hematopoietic cells. They are characterized by a DNA binding domain which contains two to three imperfect repeats of about 50 amino acids each. Only two of these repeats (R2 and R3) are necessary and sufficient for sequence specific DNA binding. The polypeptide composed of these two repeats has been studied by heteronuclear magnetic resonance spectroscopy using uniformly 15N-labeled and 13C,15N doubly-labeled samples as well as a sample selectively enriched on all lysine, histidine and leucine residues.
Using a combination of 3D 1H-15N NOESY-HMQC, TOCSY-HMQC, HMQC-NOESY-HMQC as well as triple resonance 1H-15N-13C HNCA and HN(CO)CA experiments, we have made assignments of about 90% of the main chain atoms.
The analysis of sequential and medium range NOE connectivities indicates that the R3 repeat is composed of three helices (residues 62-75, 78-85 and 91-100) while for the R2 repeat only two helices are found (residues 10-23 and 28-34). The remaining C-terminal part of the R2 repeat, predicted to be helical undergoes intermediate conformational exchange processes. Stabilization of this segment might occur upon DNA binding.
The interaction of c-Myb with two DNA fragments of different length (12 and 16 base pairs) and containing the consensus sequence TAACGGTC has been approached by 1H NMR. The titration of the imino protons of the DNA when adding the protein indicates the presence of two complexed forms in slow exchange with the free protein.

D2-325 REFINED SOLUTION STRUCTURE OF 8,9-DIHYDRO-8-(N^7-GUANYL)-9-HYDROXY-AFLATOXIN B_1 OPPOSITE CpA IN THE COMPLEMENTARY STRAND OF AN OLIGODEOXYNUCLEOTIDE DUPLEX AS DETERMINED BY ^{1}H NMR. David S. Johnston and Michael P. Stone. Center in Molecular Toxicology and Department of Chemistry, Vanderbilt University, Nashville, TN 37235.

We constructed the oligodeoxynucleotide duplex d(CCATC^{AFB}GATCC)·d(GGATCAGATGG), in which 8,9-dihydro-8-(N^7-guanyl)-9-hydroxy-aflatoxin B_1 was positioned opposite CpA in the complementary strand of the DNA duplex. The modified guanosine was positioned opposite cytosine, while the covalently-attached aflatoxin moiety was positioned opposite adenosine in the complementary strand.



Using a combination of one- and two-dimensional methods, the ^{1}H NMR spectrum was successfully assigned. Numerous NOEs were observed between the DNA protons and the aflatoxin protons. Molecular dynamics calculations restrained with experimentally-derived distances refined a family of structures characterized by pairwise rms differences of less than 0.89 Å excluding terminal base pairs. Complete relaxation matrix calculations yielded a sixth root rms difference between the refined structures and the 250 ms NOE intensity data of 0.072, which confirmed that the refined structures were in reasonable agreement with the data. In the refined structures, the aflatoxin was intercalated into the helix on the 5'-face of the modified guanine and A^{16} was inserted between the $^{AFB}G^6$:C^{15} base pair and aflatoxin. We have also carried out initial studies with the duplex d(CCATC^{AFB}GATCC)·d(GGATCCGATGG), in which a cytosine was positioned opposite the aflatoxin for comparison to the adenine structure. Supported by NIH grants CA-55678, RR-05805, ES-00267, ES-07028.

D2-326 NMR STUDIES ON AN HDV RIBOZYME MODEL

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Self-cleaving RNA sequences have been found in a number of small pathogenic viruses in both plants and animals. The Hepatitis Delta Virus RNA contains such domains on both the genomic and the anti-genomic strand. Through minor modifications in the primary structure they can both be transformed into trans-acting, and therefore truly enzyme-like components. RNA catalysts have gained considerable interest because of their potential use as designer drugs. Ribozymes modeled after the HDV wild type sequences consist of a minimum of about 50 nucleotides, and are capable of cleaving 8-mer substrates. Cleavage is preceded by binding of the substrate to the complementary sequence on the ribozyme through the formation of Watson-Crick base pairs. However, whereas some parts of the ribozyme appear to be relatively stable, the ribozyme-substrate interaction helix is not. This lability makes analysis of the NMR data extremely difficult.
The quality of the NMR spectra could be significantly improved by constructing an alternative unimolecular model, which consists of the three-way-junction containing the supposed active site in addition to a 6-mer single-stranded region. We propose a model in which two stems form a quasi-continuous helix. Tertiary interactions between the single-stranded region and the central loop are believed to stabilize the three-dimensional structure. Preliminary results will be presented of NMR experiments on this model system. We believe that the elucidation of the three-dimensional structure of the three-way-junction model will lead to a better understanding of the principles underlying RNA cleavage by the HDV ribozyme.

D2-327 NMR ANALYSIS OF THE DNA-BINDING DOMAIN DERIVED FROM THE TRANSCRIPTION FACTOR PHO4.

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The yeast gene PHO5 is regulated by the transcription factors PHO4 and PHO2. In high phosphate buffer (repressing conditions) PHO4 is hyperphosphorylated, and the transcription of PHO5 is not induced. Under phosphate starvation PHO4 is not hyperphosphorylated, and the transcription of PHO5 is activated by PHO4 in conjunction with PHO2.
PHO4 is a member of the basic-helix-loop-helix (bHLH) family. The circular dichroism spectrum of the 49-residue HLH domain indicates a high content of α-helical secondary structure. ^{15}N decoupled ^{1}H 2D NMR and half-filtered ^{15}N edited ^{1}H 2D NMR has been carried out to determine the secondary and tertiary structure. The assignments and structural features will be discussed.

D2-328 SOLUTION STRUCTURE OF THE SECOND RNA-BINDING DOMAIN OF SEX-LETHAL AND INITIAL STUDIES ON THE PROTEIN/RNA COMPLEX. Andrew L. Lee, Roland Kanaar, Donald. C. Rio, and David. E. Wemmer. Department of Chemistry, University of California, Berkeley, CA 94720.

The RNA-binding protein, Sex-lethal (Sxl), is a critical regulator of sexual differentiation and dosage compensation in *Drosophila*. This regulatory activity is a consequence of the ability of Sxl to bind uridine-rich RNA tracts involved in pre-mRNA splicing. Sxl contains two RNP consensus-type RNA-binding domains (RBDs). A structural study of a portion of Sxl (amino acids 199-294) containing the second RNA-binding domain (RBD-2) using multidimensional heteronuclear NMR is presented here. Nearly complete ^{1}H, ^{13}C, and ^{15}N resonance assignments were used to analyze 3D ^{15}N-separated NOESY and $^{13}C/^{13}C$-separated 4D NOESY spectra which produced 494 total and 169 long-range NOE-derived distance restraints. Along with 41 backbone dihedral restraints, these distance restraints were employed to generate an intermediate-resolution family of calculated structures, which exhibits the βαβ-βαβ tertiary fold found in other RBD-containing proteins. A capping box was identified at the N-terminus of the first helix and has been characterized by short and medium-range NOEs. Finally, initial results on protein/RNA complexes are presented.

D2-329 INFLUENCE OF GLYCOSIDIC TORSION ANGLE ON ^{13}C SHIFTS IN NUCLEOTIDES, David Live, Karen Greene, Yong Wang, Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., NY, NY 10021

With methods of indirect detection and isotopic labeling making ^{13}C chemical shift information from RNA and DNA oligonucleotides readily available, the prospect of using the shifts for conformational analysis as well as for dispersing the crowded proton spectrum becomes more attractive. While efforts have been made to understand the impact of sugar pucker on the sugar carbon shifts, the impact of changes in the glycosidic bond angle are less well characterized. This angle is less of an issue in regular duplex DNA structures. However, unusual secondary and tertiary structures found in higher order DNA systems and in RNA may have less predictable glycosidic angles and shift information may prove to be a useful parameter in their characterization. We have used a model G-tetrad system with well defined syn and anti base orientation, but with a very similar C2' endo sugar pucker, to quantify the effects of rotation about the glycosidic bond on the shifts of the protonated base and sugar carbons. Significant effects are seen for C1', C2', C3', and C4' sugar positions and in the C8 of the base. Correlations will be made with other available data.

D2-330 PRELIMINARY NMR INVESTIGATION OF THE HMG DOMAIN FROM THE LYMPHOID ENHANCER BINDING FACTOR

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LEF-1 (Lymphoid Enhancer-binding Factor) is a member of a family of DNA associated proteins that have in common the High Mobility Group DNA binding domain. LEF-1 is an immune system transcription factor expressed in pre-B and T lymphocytes that has been shown to regulate expression of the α-subunit of the T cell receptor. The HMG domain of LEF-1 binds DNA monomerically and selectively to the sequence CCTTTGAA. It has been shown to bend DNA by approximately 130° and to interact with the minor groove of DNA. The HMG domain binds DNA with moderate specificity, high affinity, and with a half life of approximately two hours.

To increase solubility of the HMG domain a C24S mutation was introduced followed by overexpression in a T7 expression system. Biological activity was shown to be preserved as determined by electrophoretic mobility shift assays. Circular dichroic analysis indicated the HMG domain to be highly helical. Initial NMR studies suggested that the free HMG domain may not be highly structured in solution. A dideoxynucleoside-terminated primer extension reaction was combined with gel-shift methods for analysis of the binding strength of various protein-DNA complexes. Size exclusion chromatography was utilized to show that the HMG DNA binding domain successfully formed a complex with a minimal binding site portion of DNA.

Preliminary multidimensional NMR studies on isotopically enriched protein indicate the induction of a more highly ordered protein structure upon formation of a complex with DNA. Changes to the structure of the DNA can be inferred via significant differences between the chemical shifts of DNA in complex with protein as opposed to those from free duplex DNA.

D2-331 ^{13}C NMR MEASUREMENT OF INTERHELICAL ELECTROSTATIC INTERACTIONS IN THE GCN4 LEUCINE ZIPPER

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Transcription factors of the basic-region leucine zipper (bZIP) family bind to DNA as homo- or heterodimers. Dimerization of these proteins is controlled by the leucine zipper, a parallel, two-stranded coiled coil. The leucine zipper thus mediates the DNA-binding specificity of the bZIP transcription factors by determining which bZIP proteins form stable dimers. Accurate prediction of bZIP dimerization specificity will require an understanding of the principles governing leucine zipper dimerization.

It is commonly assumed that electrostatic interactions between oppositely charged residues on different helices of the leucine zipper contribute favorably to dimerization specificity. Crystal structures of the GCN4 leucine zipper contain interhelical salt bridges from Lys 15 to Glu 20' and Glu 22 to Lys 27'. ^{13}C-NMR pK_a measurements in physiological solution indicate that the salt bridge to Glu 22 contributes equally to stability, while the salt bridge to Glu 20 is even less favorable, than the corresponding interactions between the charged Lys and neutral (protonated) Glu residues. Moreover, the E20Q substitution is stabilizing. Thus, salt bridges will not necessarily contribute favorably to bZIP dimerization specificity, and may indeed be unfavorable, relative to alternative neutral/charge interactions.

D2-332 ^{15}N - LABELING AND HETERONUCLEAR NMR STUDIES OF A DNA OLIGONUCLEOTIDE CONTAINING AN A_3T_3 TRACT, Ryszard Michalczyk*, Louis A. Silks III#, and Irina M. Russu*, *Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, CT 06459-0175, and #NIH Stable Isotopes Resource, Los Alamos National Laboratory, Biochemistry and Spectroscopy Section, CST-4, MS C-345, Los Alamos, NM 87545

A_nT_n tracts possess unique properties, such as bending of the helix axis, specific patterns of hydroxyl radical cleavage, and anomalously long base-pair lifetimes. Work in this laboratory has used NMR spectroscopy to study the self-complementary DNA dodecamer $[d(CGCAAATTTGCG)]_2$ in order to probe conformational and dynamic features of A_nT_n tracts in solution. Adenine amino protons are of particular importance in A_nT_n tracts since, in addition to participating in Watson-Crick hydrogen bonds, they have also been postulated to be involved in three-center hydrogen bonds across the major groove. Direct observation of these protons by NMR is impaired by exchange broadening and by severe overlap of their resonances with resonances of aromatic protons. To overcome this limitation, we have labeled the Adenine amino group with ^{15}N. 6-^{15}N labeled deoxyadenosine phosphonate was synthesized starting from 6-chloropurine and ^{15}N-NH_3 via a combination of chemical and enzymatic synthesis, and used for DNA synthesis via phosphonate chemistry on a solid support. ^{15}N chemical shift and relaxation times as well as ^1H-^{15}N nuclear Overhauser effect and ^1H-^{15}N coupling constants were measured as a function of temperature. The same set of data was also obtained for a control dodecamer, $[d(CGCGAGCTCGCG)]_2$, which contains a single labeled Adenine. The results for the two dodecamers will be compared, and their relevance to structural and dynamic properties of A_nT_n tracts in solution will be discussed. (Supported by NSF grant 88-17589 to IMR, and NIH Division of Research Resources grant RR 02231 to LAS)

D2-333 **DNA BINDING AND HETERONUCLEAR NMR STUDIES WITH SINGLE AND DOUBLE ZINC FINGER PEPTIDES FROM GATA-1**

James G. Omichinski[1], Angela M. Gronenborn[1], Cecelia Trainor[2], Stephen Stahl[3], Gary Felsenfeld[2] and G. Marius Clore[1]. Lab of Chemical Physics, NIDDK[1], Lab of Molecular Biology NIDDK[2] , and Protein Expression Lab[3] National Institutes of Health, Bethesda, MD 20892 U.S.A.

The erythroid transcription factor GATA-1 is a member of a family of DNA binding proteins, which now includes regulatory proteins expressed in other cell lineages. GATA-1 binds as a monomer to an asymetric DNA target sequence (A/T)GATA(A/G). The protein has two related but non-identical zinc finger elements of the form cys-x-x-cys-(x17)-cys-x-x-cys. We have shown that a 66-residue peptide containing only the carboxy-terminal zinc finger domain is capable of binding specifically to the AGATAA sequence.The three-dimensional structure of a complex between the 66-residue DNA binding domain of the GATA-1 and a 16-mer fragment of DNA containing an AGATAA site has been determined using multidimensional heteronuclear magnetic resonance spectroscopy (NMR). Interactions between the DNA are observed from the zinc binding domain, from the α-helix located just outside the zinc binding domain and from a highly basic region on the very C-terminus of the peptide. Binding studies with chimaeric proteins of GATA-1 and other GATA family members shows that the differences in DNA recognition between various family members appears to be determined within the zinc binding region of the DNA binding domain. Further experiments with double finger peptides of GATA-1 suggest that in most cases the carboxy-terminal finger is solely responsible for DNA specificity but in a few select cases both fingers are involved in DNA recognition. The results with the chimaeric peptides and the double finger peptides will be presented as well as heteronuclear NMR studies with the double-finger peptide in both the free and bound state.

D2-334 SOLUTION STRUCTURAL STUDIES OF A LEAD-DEPENDENT RIBOYZYME, Arthur Pardi and Pascale Legault, Department of Chemistry and Biochemistry, Campus Box 215, University of Colorado at Boulder, Boulder, CO 80309

We are using state-of-the-art heteronuclear multi-dimensional NMR to determine the 3D structure of a several catalytic RNAs, including a lead-dependent ribozyme, known as the leadzyme. The leadzyme system being studied consists of a 99% $^{13}C/^{15}N$ labeled 30 nucleotide RNA. A variety of 2D and 3D double and triple resonance NMR experiments are used to make resonance assignments in this system and complete proton, carbon and nitrogen resonance assignments have been made for the leadzyme. The active site of the leadzyme consists of an internal loop that displays a variety of interesting structural features, including several *syn* nucleotides and an adenine base with an unusual pK_a of 6.5. The three dimensional structure of this leadzyme will be reported.

D2-335 hmU DNA- STUDIES OF STRUCTURE AND DYNAMICS BY NMR SPECTROSCOPY, Laura B. Pasternack, Janice Bramham, Luciano Mayol, Xin Jia and David R. Kearns, Department of Chemistry, University of California, San Diego, La Jolla, CA 92093

The presence of 5-hydroxymethyl-2'-deoxyuridine (hmU) in place of thymine in DNA occurs due to oxidative attack on the methyl group of thymine or deamination of 5-hydroxylmethyl-cytosine. The production of hmu DNA has been linked to high fat diets. hmU DNA is also suspected to play a role in the aging process. Reduced excision of hmU from DNA has been implicated in diseases associated with early onset of aging such as Werner's syndrome. In addition, the nucleoside is proving useful in anti-leukemic and antiviral therapeutic regimes. hmU DNA occurs in bacteriophages such as SPO1 along with hmU specific proteins such as Transcription factor 1 (TF1). TF1 binds specifically to DNA containing hmU in place of thymine and has been shown to bend DNA through angles of roughly 180^0. DNA footprinting has defined sequence specific binding sites of TF1[1]. A 16 basepair hmU DNA (portion of the footprint region with the insertion of one base pair) has been synthesized along with the thymine containing derivative. The sequence is symetric and contains 8 G-C basepairs and 8 A-T (or A-hmU) base pairs. Comparative studies of the structure and dynamics of these DNAs have been performed in order to consider the role of the hydroxymethyl moiety in the binding of hmU DNA to TF1. Differences can be seen in the thermal stability of these two DNAs by consideration of imino proton 1D resonances and interstrand NOEs at different temperatures. Presented here is ongoing work in the study of the structure and dynamics of hmU DNA alone and bound to TF1.

[1]Schneider, G. T., Sayre, M. H., Geiduschek, E. P. (1991) *J. Mol. Biol.*, **221**, 777-794.

D2-336 **NMR STRUCTURE DETERMINATION OF COMPLEXES BETWEEN HEDAMYCIN AND THE OLIGONUCLEOTIDE DUPLEXES d(CACGTG)$_2$ AND d(CGTACG)$_2$.** Spiro Pavlopoulos[1], Wendy Bicknell[2], David Craik[1], Geoffrey Wickham[3], (1) School of Pharmaceutical Chemistry, Victorian College of Pharmacy(Monash University) 381 Royal Parade, Parkville, Victoria, Australia 3052 (2) Peter McCallum Cancer Institute, 481-Lt Lonsdale Street, Melbourne, Victoria, Australia, 3000 (3) Department of Chemistry, University of Wollongong,Wollongong, NSW, Australia 2500

Hedamycin is an antitumour antibiotic isolated from *Streptomyces greisoruber* c1150. It consists of two sugar rings attached to an anthraquinone chromophore, which also contains a diepoxide side chain. Hedamycin displays two types of binding to DNA, one reversible by dialysis against 1M NaCl and the other irreversible. Reversible binding of hedamycin involves intercalation of the chromophore while the irreversible binding involves alkylation of DNA presumably through the epoxide side chain of the chromophore. Hedamycin has been shown to bind preferentially to CGT sequences, so the two sequences d(CACGTG)$_2$ and d(CGTACG)$_2$ were designed to investigate a covalently-bound hedamycin/oligonucleotide adduct.

A 2D NMR spectroscopic study of a complex between d(CACGTG)$_2$ and hedamycin was undertaken with the aim of proposing a model for binding of the ligand to DNA. The ligand formed a 1:1 complex with the oligonucleotide and initially exhibited slow exchange binding kinetics. Evidence for intercalation and alkylation was observed and a model for the interaction was determined based on intermolecular NOEs. We are currently investigating the interaction of hedamycin with d(CGTACG)$_2$ where there is the potential for a 2:1 complex to be formed based on the results of the previous study.

D2-337 NMR STRUCTURAL STUDIES OF HIV-1 REV-BINDING ELEMENT (RBE) RNA. Robert D. Peterson, and Juli Feigon, Department of Chemistry and Biochemistry and Molecular Biology Institute, University of California, Los Angeles, CA 90024.

The genome of HIV-1 codes for two essential regulatory proteins, Tat and Rev. Rev mediates the transition from early to late gene expression in HIV-1. A portion of the HIV-1 mRNA called the Rev Responsive Element (RRE) is bound by Rev, and the Rev-RRE complex allows the HIV-1 structural proteins, which are made from incompletely spliced mRNA, to be translated.

The RRE contains a high-affinity binding site for Rev. This high-affinity binding site has been incorporated into small RBE RNAs. These have been studied both free and when bound by a Rev peptide that contains the arginine-rich RNA binding domain. The internal loop of the free RNA seems to be conformationally flexible, but to have a predominant conformation which includes two purine-purine base pairs and a bulged U. NMR data indicate that the RNA when bound by Rev peptide contains many of the same structural elements as the free RNA.

D2-338 **NMR STUDIES OF AN ARGININE BINDING DNA HAIRPIN** Stephanie A. Robertson*, Kazuo Harada+, Alan D. Frankel+, and David E.Wemmer*, University of California, Berkeley and Lawerence Berkeley Laboratories, Berkeley, CA 94720*, and University of California, San Francisco, CA+.

Selex methodology was used to screen a library of single stranded DNA sequences for TAR RNA-like arginine binding affinity. A ten base loop hairpin motif, distinct in both sequence and secondary structure from TAR RNA, was selected. Mutational and chemical modification studies indicate the top stem basepair leading into the loop and eight of the ten residues in the loop are necessary for binding of arginine. NMR data of the unbound DNA hairpin compared to the arginamide bound DNA complex indicate the two have dramatically different tertiary structures. The data indicate that the loop in the unbound structure is dynamic and generally not well defined by NMR. However, the arginamide-bound structure has a definable loop including unusual basepairing and backbone conformation.

D2-339 AN INVESTIGATION OF THE INTERACTION OF Mn^{2+} IONS WITH A DNA DODECAMER CONTAINING THE RECOGNITION SITE FOR *EcoRV* ENDONUCLEASE, Daniel L. Rosewater and Irina M. Russu, Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, CT 06459-0175

Proton NMR spectroscopy was used to examine the interaction of Mn^{2+} ions with the DNA dodecamer [d(CGCGATATCGCG)]$_2$, which contains the recognition site for *EcoRV* endonuclease (underlined). Samples of the dodecamer in a 10 mM phosphate buffer containing 100 mM NaCl at pH 7.0 were titrated with a $MnCl_2$ stock solution at 25°C. Addition of Mn^{2+} to a Mn^{2+}/DNA phosphate ratio of 2.0 x 10^{-5} increased the longitudinal relaxation rates and linewidths for three proton resonances. Using 2D NOE spectroscopy (NOESY), the three resonances were assigned to the G4-H8, C3-H5, and C3-H6 protons. The selective effect of Mn^{2+} on these three protons suggests that the ion interacts preferentially at the CG3 and/or GC4 base pairs. To further characterize this site of interaction, we have also measured the linewidths and longitudinal relaxation rates of scalar-coupled protons using 2D NMR. In these experiments, the conventional inversion-recovery pulse sequence is modified by replacing the "observe" pulse with the pulse sequence of a double-quantum filtered correlation (DQF-COSY) experiment. The increased resolution provided by this method allowed the measurement of longitudinal relaxation rates and linewidths for 38 additional sugar protons, including the H1', H2' and H2" of C3 and G4. The relaxation rates were used to calculate distances between the Mn^{2+} and nearby protons within the binding site of the ion. (Supported by the Hughes Program in Life Sciences, Wesleyan University)

D2-340 HIGH RESOLUTION SOLUTION STRUCTURE AND DYNAMICS OF THE MINOR GROOVE BINDING DRUG SN-6999 COMPLEXED WITH d(GGGAAAAACGG)·d(CCGTTTTTCCC), J.M. Rydzewski, J.A. Smith, W. Leupin, and W.J. Chazin, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

A number of antitumor drugs and other ligands that bind reversibly to DNA serve as excellent models for the study of the physical, thermodynamic and kinetic parameters characteristic of recognition of the minor groove of duplex DNA. SN-6999 is a member of the family of bis(quaternary ammonium) heterocycles which have been shown to possess potent antitumor activity. NMR spectroscopy is being used to carry out a detailed study of the interactions between SN-6999 and an A-tract DNA duplex with the sequence designed to produce a narrow minor groove width and maximize the van der Waals complementarity of the drug and DNA. Complete ^{1}H NMR resonance assignments have been made and 31 intermolecular contacts have been unequivocally assigned. The drug binds primarily in the $d(A)_5 \cdot d(T)_5$ region, and in contrast to previous studies, with a preferred orientation along the DNA axis. Doubling of numerous drug and DNA resonances of the 1:1 complex observed at 1 °C indicates slow exchange between a major and minor binding mode with a ratio of ~7:3. An activation energy for the reorientation of the drug in the binding site using DNA and drug resonances is calculated to be ~60 kJ mol^{-1} which is similar to the value observed previously, and is consistent with a "flip-flop" mechanism of reorientation. Lower limits for the rate of exchange at coalescence are determined for selected resonances, providing limiting lifetimes for each binding mode at specific temperatures. High resolution three dimensional solution structures are being determined using NMR-derived distance and dihedral angle constraints in restrained molecular dynamics (rMD) simulations. DNA starting structures are generated using a distance geometry approach followed by independent refinement. Drug starting structures are generated by taking snapshots from rMD simulations. Pairs of drug and DNA conformations are arbitrarily selected, docked, and then rMD refined. The results are being analyzed to identify the elements responsible for the unique binding orientations of SN-6999 in this complex, and the factors which determine the DNA sequence preferences of this class of antitumor agents.

D2-341 NMR STUDIES OF THE IMPORTANCE OF A SINGLE BASE MUTATION IN THE HAIRPIN LOOP OF THE IRON RESPONSIVE ELEMENT (IRE) IN FERRITIN mRNA, Hanna Sierzputowska-Gracz*+, R. Ann McKenzie+, and Elizabeth C. Theil+#, Departments of Chemistry*, Biochemistry+, and Physics#, North Carolina State University, Raleigh, NC 27695

The iron responsive element (IRE) is the best characterized group of eurkaryotic mRNA regulatory elements and is one of the longest conserved RNA sequences known (n=28-30). Noncoding sequences regulate the function of mRNA and DNA. In animal mRNAs, iron responsive elements (IREs) regulate the synthesis of proteins for iron storage, uptake and red cell heme formation. The secondary structure is a hairpin loop with an internal loop or bulge. Folding of the IRE was indicated previously by reactivity with chemical and enzymatic probes. Using DNA templates and T7 polymerase, we were able to synthesize an oligonucleotide (n=30) wild type and a mutant G18A. ^{1}H and ^{31}P NMR spectra now confirm the IRE folding; an atypical ^{31}P spectrum, differential accessibility of imino protons to solvents, multiple long-range NOEs, and heat stable subdomains were observed. Biphasic hyperchromic transitions occurred (52° and 73°). A G-C base pair occurs in the hairpin loop (HL) (base on dimethylsulfate, RNAase T1 previously used and changes in NMR imino proton resonances typical of G-C base pair after G/A substitution). Mutation of the hairpin loop also decreased temperature stability and changed ^{31}P NMR spectrum; regulation and protein (IRP) binding were previously shown to change. Alteration of IRE structure shown by NMR spectroscopy, occurred at temperatures used in studies of IRE function, explaining loss of IRP binding. The effect of the HL mutation on the IRE emphasizes the importance of HL structure in other mRNAs, viral RNAs (e.g. HIV-TAR), and ribozymes.

D2-342 Secondary Structure And Global Fold Of The ERR2 DNA Binding Domain: A Model For Monomer Binding Nuclear Hormone Receptors, Daniel S. Sem[1], Danilo Casimiro[1], Steven A. Kliewer[2], Joan Provencal[3], Ronald M. Evans[3], and Peter E. Wright[1]

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ERR2 is a member of a subfamily of nuclear hormone receptors that bind DNA as monomers, along with FTZ-F1, NGFI-B and SF1. Other, structurally characterized members of the nuclear hormone receptor superfamily (ER, GR, RXR & RAR) bind DNA as homo- and heterodimers, but little is known regarding the structural basis of monomer binding. Several mutagenesis and protein chimera studies have implicated a carboxy terminal region in the determination of monomer versus dimer binding. This region is thought to make key contacts with the DNA at the 5'-end of the recognition site for the monomer binders.

We have determined preliminary resonance assignments, secondary structure, and the global folding pattern for the ERR2 DNA binding domain using multidimensional multinuclear NMR. The core 66 residue zinc finger domain has a global fold similar to the other structurally characterized nuclear hormone receptors, with two helices that originate from the carboxy terminal ends of the two zinc fingers. These helices lie orthogonal to each other and pack together forming a hydrophobic core. Electrospray mass spectral analysis indicates that two zincs are bound by ERR2, and gel shift analysis of a C163A mutant indicates that this ninth cysteine, which resides in the hydrophobic core, is not essential for DNA binding. The carboxy terminal region appears disordered, with the few residues that could be assigned in this region showing near random coil chemical shift values.

It is likely that the carboxy terminal region of ERR2 will become ordered upon binding DNA, since it provides considerable binding energy for high affinity and specificity. In the dimer binders, the complete binding energy is only realized because of cooperative protein-protein interactions, which are accompanied by the entropic cost of bringing together two protein monomers. In the monomer binders, the full binding energy may only be realized after the entropically costly ordering of the carboxy terminal region, which allows for additional specific interactions with the DNA.

D2-343 STRUCTURES OF R-α-(N^2-GUANYL)-STYRENE OXIDE ADDUCTS AT EACH OF TWO ADJACENT GUANINES IN AN OLIGODEOXYNUCLEOTIDE DUPLEX CONTAINING CODON 12 OF THE *N-RAS* PROTOONCOGENE, Feloora R. Setayesh, Irene S. Zegar, Bart DeCorte, Constance M. Harris, Thomas M. Harris, and Michael P. Stone, Department of Chemistry, Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37235.

The solution structures of d(GGCTGGTGGTG)·d(CACCACCAGCC), which contains codon 12 of the *n-ras* protooncogene (underlined), and of two R-α-(N^2-guanyl)-styrene oxide adducts located respectively at G^5 or G^6 in codon 12, were investigated using ^{1}H NMR spectroscopy. The ^{1}H resonance assignments were obtained using NOESY and TOCSY experiments. The pattern of the NOE cross-peaks observed between the styrene protons and the DNA, and the changes in chemical shifts for both adducts indicated that the styrene moiety was situated in minor groove, with phenyl ring oriented toward the 3' end of the DNA molecule. Distance restraints were calculated from NOE data using relaxation matrix analysis. These were incorporated as effective potentials into the total energy equation. The solution structures were refined using restrained molecular dynamics calculations which incorporated a simulated annealing protocol. The accuracy of the emergent structures was evaluated by complete relaxation matrix methods. For these two R-α-(N^2-guanyl)-styrene oxide adducts only slight DNA sequence effects upon adduct conformation were observed. Single base substitutions at the codon 12 site correlate with oncogene activation [reviewed by Barbacid, M. (1987) *Ann. Rev. Biochem.* **56**, 779-827]. Supported by the NIH: ES-05355 (M.P.S.), ES-05509 (T.M.H.), ES-00267, and RR-05805 (NMR spectrometer).

D2-344 PROBING THE ENANTIOSELECTIVE RECOGNITION OF DNA BY RH(EN)$_2$PHI^{3+} VIA ^{1}H NMR.

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Rh(en)$_2$phi^{3+}, (en = ethylene-diamine, phi = phenanthrene-quinone diimine), displays remarkable enantioselectivity in binding and cleaving DNA. Both Δ- and Λ- Rh(en)$_2$phi^{3+} bind to DNA through the intercalation of the aromatic phi ligand, and thus the enantioselectivity observed is the result of the enantiomeric disposition of the small ethylenediamine ligands. Δ-Rh(en)$_2$phi^{3+} binds DNA at 5'-GC steps, due to hydrogen bonding from the axial amines of the ancillary ethylene-diamine ligands to the O6 of guanine. Λ-Rh(en)$_2$phi^{3+} displays lower overall sequence selectivity, since, in addition to hydrogen bonding at 5'-GC steps, it also recognizes 5'-TA and TX (X = any base) steps through van der Waals contacts between the ethylenediamine ligands and the thymine methyl group in the major groove of DNA.

Enantioselective recognition of DNA by Δ- and Λ-Rh(en)$_2$phi^{3+} has been probed through a series of one dimensional (1D-) and two dimensional (2D-) 500 MHz ^{1}H NMR experiments both to extend our understanding of the observed enantioselectivities as well as to gain detailed structural information on the intercalation site. Δ-Rh(en)$_2$phi^{3+} forms a clean 1:1 complex with d(GTGCAC)$_2$ and the metal complex is in slow exchange at 295° K. The large upfield shifts of the phi ligand's aromatic protons (H2,7 = 1.27, H1,8 = 1.12, H4,5 = 0.69, H3,6 = 0.55 ppm) are consistent with intercalation of the phi ligand into the base stack of DNA. 2D-NOESY experiments show that Δ-Rh(en)$_2$phi^{3+} is bound between G3 and C4 bases, and the symmetry of the 1:1 complex indicates that the phi complex is not canted towards one strand. Results from NOESY experiments which place Δ-Rh(en)$_2$phi^{3+} in the major groove will be discussed. Λ-Rh(en)$_2$phi^{3+} displays remarkably different binding behavior with the oligo d(GTGCAC)$_2$. The metal complex is now in intermediate exchange at 295° K, and approaches slow exchange at 285° K. In 2D-NOESY experiments, two sets of resonances are observed for the T2 and A5 bases, consistent with binding at the T2-G3 step.

D2-345 TF1-STUDIES OF STRUCTURE AND DYNAMICS BY NMR SPECTROSCOPY, Maria-Victoria Silva, Xin Jia, E. Peter Geiduschek, and David R. Kearns, Department of Chemistry, Department of Biology and Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093

Transcription Factor 1 (TF1), encoded by the bacteriophage *Bacillus subtilis*, is a dimeric protein that acts to inhibit transcription of native SPO1 DNA by bacterial DNA-dependent RNA polymerase. The core-binding site for TF1 has been previously determined by DNA footprinting. The sequence-specific ^{1}H and ^{15}N NMR assignments have been made for TF1 using six complementary selectively deuterium-labeled TF1 variants and uniformly ^{15}N-labeled TF1 variant[1]. Discrete protein secondary structure domains have been defined for the non-flexible region by 2D and 3D NMR. This structure has been compared to the HU-based model (another type II DNA-binding protein). The solution structure of TF1 indicates that the most flexible structural domain in TF1 is the β-ribbon domain indicative of the concave β-ribbon assembly proposed for type II binding proteins. The secondary structure of this β-ribbon domain is not completely assigned due its flexibility. TF1 binds specifically to DNA containing 5-hydroxymethyl-2'-deoxyuridine (hmU) in place of thymine and has been shown to bend DNA through angles of roughly 180^0. Presented here is ongoing work in the study of the structure and dynamics of TF1 alone and bound to hmU DNA.

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D2-346 STRUCTURAL STUDIES ON TRIPLE HELICES.

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Triple helices are thought to play a important role in various cellular processes *in vivo*, as well as to be a new and potentially useful concept in nucleic acid recognition. We will present the results of structural NMR studies on a DNA intramolecular triple helix and on a DNA/RNA hybrid of a DNA hairpin with a ^{15}N,^{13}C labeled RNA oligonucleotide acting as the third strand. For the intramolecular triple helix we will for example show that the loop connecting the two Watson-Crick bound strands undergoes a conformational change upon binding of the third strand[1]. The DNA/RNA hybrid triple helix has been chosen for NMR structural studies because of the advantage of higher stability[2]. More importantly however it offers the possibility to isotopically label the third strand to reduce spectral overlap, and thereby obtain a much more detailed picture of the structural features. We will for example show the use of X-filtered NMR experiments for this purpose. Furthermore the application of assignment via through-bond coherence transfer techniques will be presented.

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D2-347 DOES DNA BINDING MODULATE THE STRUCTURE OF THE GR DBD ? AN NMR STUDY.

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The steroid hormone receptor superfamily represents the largest known family of transcription factors in eukaryotes. The glucocorticoid receptor (GR) is structurally organized in three domains related to the function of the protein as a ligand dependent transcription factor. The C-domain or DNA binding domain (DBD) is involved in protein-DNA interactions. The structure of the dimeric GR DBD-DNA complex was determined by crystallography[1], and the structure of the monomeric unbound GR DBD by NMR [2,3]. The overall structure of the protein in the complex is similar to that in the free state, but there are differences in the second zinc coordinating domain where an extra (distorted) helix was found. The present structure refinement[2] also revealed a different folding of this specific region then that of another refined structure of GR DBD[3] in which a distorted helix was found. Our results indicate that DNA binding is accompanied by relatively large conformational changes in the second zinc finger region. Furthermore, two point mutants (GR S459A and GR P493R) of GR DBD are now studied by 2D and 3D NMR. The mutants are capable of activating transcription normally, but unlike the wild-type receptor, they interfere with particular transcriptional activators in yeast and mammalian cells, and inhibit growth when overexpressed in yeast. From biochemical data it is suggested that specific DNA acts as an allosteric effector to direct the functional interaction of the receptor with targets of the transcriptional activation, and that the 2 mutants mimic the allosteric effect of specific DNA, allowing the receptor to interact with regulatory targets even in the absence of specific DNA binding.

1 Luisi *et al.*, (1991) Nature 352, 497-505. 2 van Tilborg *et al.*, submitted

3 Baumann *et al.*, (1993) Biochemistry 32, 13463-13471.

D2-348 NMR ANALYSIS OF A DNA HAIRPIN FROM THE *SALMONELLA TYPHIMURIUM hisD3052* GENE CONTAINING 1,N^2-PROPANODEOXYGUANOSINE, Jason P. Weisenseel, G. Ramachandra Reddy, Lawrence J. Marnett, and Michael P. Stone, Center in Molecular Toxicology, Departments of Chemistry and Biochemistry, Vanderbilt University, Nashville, TN 37235.

The oligodeoxynucleotides d(CGCGGTGTCCGCG) and d(CGCGGTXTCCGCG) (where X=1,N^2-propanodeoxyguanosine; PdG), were examined using NMR spectroscopy. The sequence of the oligodeoxynucleotide was derived from the *hisD3052* gene of *Salmonella typhimurium*. Melting data collected as a function of salt concentration and of DNA concentration were consistent with the formation of a hairpin in both the modified and unmodified oligodeoxynucleotides. We were unable to measure the difference in T_m for the modified and unmodified hairpins; both exhibited a T_m of 74 ± 1 °C, as determined by thermal melting analysis monitored by UV spectroscopy. NOESY and TOCSY NMR experiments were used to assign the ^1H resonances of the modified and unmodified oligodeoxynucleotides. NOE-derived distance restraints were calculated by relaxation matrix analysis using the program MARDIGRAS. Restrained molecular dynamics calculations using a simulated annealing protocol to refine the solution structures of these oligodeoxynucleotides are currently in progress. These calculations include the experimental restraints derived from NOE and scalar coupling data. Supported by the NIH: CA-55678 (M.P.S.), CA-47479 (L.J.M.), ES-00267 and RR-05805 (NMR spectrometer).

D2-349 STRUCTURAL STUDIES OF PROTEIN/NUCLEIC ACID COMPLEXES BY ISOTOPE-SELECTED MULTIDIMENSIONAL NMR, Milton H. Werner, Jeff Huth, G. Marius Clore and Angela Gronenborn, Laboratory of Chemical Physics, National Institutes of Health, Bethesda MD 20892

We have determined the structures of two novel DNA-binding domains derived from human transcription factors by a variety of $^{13}C/^{15}N$- and $^{12}C/^{14}N$- selected multi-dimensional NMR techniques.

The structure of the DNA-binding domain of the human testis determining factor SRY has been determined bound to an octanucleotide duplex dervied from the promoter of the human Müllerian Inhibitory Substance gene (16kD complex). The structure represents the first description of a minor-groove binding protein by NMR and the first protein characterized by NMR which dramatically bends the DNA duplex. The DNA bending is the consequence of protein sidechain intercalation involving isoleucine-13 in the N-terminal helix and several hydrophobic sidechains in the N-terminus. More than 30 specific contacts have been identified between protein and DNA which define the interface. We discuss the novel structure of the DNA/protein complex and how these novel conformations are characterized by NMR using different isotope-selected techniques.

The structure of the DNA-binding domain from the human ETS-1 oncogene product has also been characterized by multi-dimensional NMR bound to a 17 nucleotide oligonucleotide duplex derived from *in vitro* selection experiments (25kD complex). The DNA-binding domain appears to be of the helix-turn-helix type, but, recognizes a purine-rich GGA consensus motif in a way distinct from other helix-turn-helix proteins. Despite the long half-life of the complexes (≈1000s), there was significant broadening for amino-acids forming the interface which needed to be characterized by approaches distinct from those utilized for the SRY/DNA complex. We discuss the structure of the complex and the general strategy employed to determine macromolecular complex structures of different sizes by multi-dimensional NMR.

D2-350 NMR STUDIES OF ANTISENSE RNA•DNA HYBRID DUPLEXES CONTAINING 3'-THIOFORMACETAL AND FORMACETAL BACKBONES, Jeffrey S. Rice and Xiaolian Gao, Dept. of Chemistry, University of Houston, Houston, TX 77204-5641

Two major problems with using deoxyoligonucleotides containing normal phosphodiester backbones for antisense therapy are inefficient uptake by target cells and degradation by cellular nucleases. Formacetal and thioformacetal groups in place of the phosphodiester backbone provide a neutral, achiral backbone moiety that should penetrate the lipophilic cell membrane more readily and should be resistant to most cellular nucleases. How a single 3'-thioformacetal or formacetal backbone substitution is accommodated in an RNA•DNA duplex d[CGCGTT-SCH_2O-TTGCGC]•r[GCGCAAAACGCG] (RIII) or d[CGCGTT-OCH_2O-TTGCGC]•r[GCGCAAAACGCG] (RII) and the effect of this substitution on the stability of an RNA•DNA duplex is being investigated.

A set of 1D NMR melting experiments and a set of 2D ^1H NOESY and COSY and ^1H-^{31}P experiments with RIII allow for comparison of Tm's, NOEs, chemical shifts, and J-coupling information between RIII and DIII, a (T6-SCH_2O-T7)-DNA•DNA duplex with the same base sequence. This comparison provides information about the binding of a modified DNA strand to an RNA strand as opposed to a DNA strand. By 1D ^1H NMR analysis of the imino proton regions, RIII was found to have a T_m very close to that of DIII (331-332 K). No premelting was observed at the modification site for either RIII or DIII. The Tm of RII will be reported. The 2D NMR experiments indicate that although the DNA strands of the RIII and DIII duplexes adopt a B-type conformation, the conformation of the former does deviate from that of its counterpart in the DIII duplex, especially in their central T4•A4 regions. The sugar puckers of T5-T8 of RIII have more N-character (C3'-endo like) than the those of T5-T8 of DIII. The NOEs connecting the methylene protons of the 3'-thioformacetal group (HP1 and HP2) with sugar protons of T6 and T7 are significantly different between RIII and DIII. The conformation of the DNA strand of RII will be compared to that of DII, its DNA•DNA counterpart.

The NMR results of RIII allow for comparison between RIII and RI, a hybrid duplex of the same sequence as RIII but without a backbone modification, in order to provide information about the effects of an RNA strand binding to a modified DNA strand as opposed to an unmodified DNA strand. The NOE patterns and proton chemical shifts of the RNA strands of RIII and RI are very similar and show patterns close to A-family RNA. The NOE patterns and proton chemical shifts of RII will be compared to those of RI and RIII.

D2-351 MULTIDIMENSIONAL HETERONUCLEAR NMR STUDIES OF THE DNA COMPONENT OF THE *XENOPUS LAEVIS* TFIIIA ZINC FINGER 1-3 PROTEIN/DNA COMPLEX, Deborah S. Wuttke,* Mark P. Foster and Peter E. Wright, The Scripps Research Institute, 10666 North Torrey Pines Road, MB-2, La Jolla, California 92037

NMR data at proton frequencies of 500, 600 and 750 MHz of have been obtained on the tightly bound (K_d < 6 nM) complex between a ^{15}N- and ^{13}C-labelled protein consisting of the first three zinc fingers of the nucleic acid binding domain of *Xenopus laevis* transcription factor IIIA and a 15 base pair DNA oligonucleotide containing the recognition sequence from the internal control region of the 5S RNA gene. Heteronuclear $^{13}C/^{15}N$ ω1 and ω2 half-filtered experiments have been used to assign the complexed DNA proton resonances. Standard methodologies have been utilized to assign the proton resonances in the free duplex DNA. NOEs between the uniformly ^{13}C- and ^{15}N-labelled protein and the unlabelled DNA oligonucleotide have been directly observed using ^{13}C-selected-$^{13}C/^{15}N$-filtered 2D and 3D experiments. Sites on the DNA interacting with the protein have been identified confirming the positioning of the protein relative to the DNA.

D2-352 SOLUTION STRUCTURE OF THE C-TERMINAL DOMAIN OF *E. COLI* DNA TOPOISOMERASE I, Liping Yu[1], Chang-Xi Zhu[2], Yuk-Ching Tse-Dinh[2] and Stephen W. Fesik[1], [1]Pharmaceutical Discovery Division, D47G, AP10, Abbott Laboratories, Abbott Park, IL 60064, [2]New York Medical College, Dept. of Biochemistry and Molecular Biology, Valhalla, NY 10595.

Topoisomerase I is responsible for removing the negative supercoiling from DNA during transcription. The protein consists of three domains: (1) the N-terminal domain (67 kd) which is responsible for the DNA cleavage, (2) the zinc binding domain (17 kd) which is important for DNA relaxation and efficient formation of the DNA cleavage complex, and (3) the C-terminal domain (14 kd) which binds to single-stranded DNA. In this presentation, we report on the ^{1}H, ^{13}C, and ^{15}N NMR assignments and three-dimensional structure of the C-terminal DNA binding domain of *E. coli* DNA topoisomerase I in solution using heteronuclear multi-dimensional NMR spectroscopy. In addition, we discuss the binding of DNA to this domain of topoisomerase I from NMR studies of the protein/DNA complex.

D2-353 A BENZO[A]PYRENE ADDUCT IN AN 11 BASE-PAIR OLIGONUCLEOTIDE CONTAINING THE CODON 61 SEQUENCE OF THE *N-RAS* PROTOONCOGENE, Irene S. Zegar, Seong J. Kim, Pamela Horton, Constance M. Harris, Thomas M. Harris, and Michael P. Stone, Department of Chemistry, Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37235.

The structure of d(CGGACXAGAAG)•d(CTTCTTGTCCG), for which X is the (-)-7*S*, 8*R*, 9*S*-trihydroxy-10*R*-(N^6-adenyl)-7,8,9,10-tetrahydrobenzo[a]pyrene (10*R*-BP) adduct corresponding to *trans* attack of adenine N^6 on (-)-7*S*,8*R*dihydroxy-*anti*-9*R*,10*S*-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (10*S*-BPDE), was refined using molecular dynamics simulations restrained by NOE-derived distances from ^{1}H NMR spectroscopy. This sequence was derived from the *n-ras* proto-oncogene at the bases encoding for amino acid 61 of the *n-ras* protein (underlined). The assignments of the non-exchangeable and exchangeable proton resonances of this duplex were obtained using NOESY and TOCSY experiments. The sequential NOE cross peaks between the fifth, sixth and seventh bases of the modified strand were missing or very weak. The sequential NOE connectivities were interrupted between the fifth and sixth bases of the complementary strand. This indicated disruption of the DNA conformation at and one base-pair flanking the site of adduction. The chemical shift dispersion of the benzo[a]pyrene aromatic protons indicated that the pyrenyl moiety existed in a less homogenous environment than would be expected if it was situated in the major groove. NOE cross-peaks were observed between the aromatic protons of benzo[a]pyrene and minor groove protons of the DNA adjacent to the site of adduction. These indicated that the pyrenyl moiety was stacked between the fifth and sixth base-pairs of this duplex. Broadening of the imino resonances was observed near the site of adduction. Thermal melting indicated a 15 °C destabilization of this duplex relative to the unmodified duplex. Distance restraints were calculated from the NOE intensities using MARDIGRAS. 400 NOE restraints were incorporated into molecular dynamics calculations, as effective potentials in the total energy equation. The emergent structure confirmed that the BPDE was partially intercalated with two of the benzene rings in the pyrenyl moiety stacked with residues C^5 and T^{17}. Benzo[a]pyrene adduction caused partial unwinding of the duplex adjacent to the site of adduction. Supported by the NIH: ES-05355 (M.P.S. & T.M.H.) and RR-05805 (NMR spectrometer).

D2-354 NMR STUDIES OF THE ZINC FINGER MOTIFS OF URBS1 PROTEIN, Qin Zhao,[1] Zhiqiang An,[2] Sally A. Leong,[2,3] and John L. Markley,[1] Departments of Biochemistry[1] and Plant Pathology,[2] University of Wisconsin-Madison and USDA ARS Plant Disease Resistance Research Unit,[3] Madison, WI 53706

The goal of our research is to elucidate the structure and function of the zinc-finger like motifs of Urbs1 using multidimensional NMR spectroscopy. Urbs1 is a putative transcription repressor of the *sid1* gene which encodes an enzyme involved in ferrichome siderophore biosynthesis in the fungus *Ustilago maydis*. The Urbs1 protein itself has a molecular weight of 100 kD and contains two zinc-finger-like motifs (GATA-1 type). Expression or structural studies of this protein and its motifs have not been reported. An 8 kD polypeptide encompassing the C-terminal finger domain of the Urbs1 protein has been subcloned and overexpressed in *E.coli* using the pET system (Novagen). Preliminary NMR results will be described.

Methods; Membranes; Solid State NMR

D2-400 Isotope Effects on ^{13}C and ^{15}N Chemical Shifts in Proteins From Electric Field Effects.

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One and multiple bond isotope effects on the ^{13}C- and ^{15}N chemical shifts in proteins due to proton-deuterium substitution at exchangeable sites are related to structure in a complex manner and are useful in the study of hydrogen bonding [1].

Deuteriation results in very small geometric rearrangements like the H-X distance which is shortened in the order of 0.01 Å. The hydrogen bond acceptor gives rise to a small electric field perturbation and the isotope effect can be rationalized as electric field effects on the chemical shifts [2]. The effect in non hydrogen bonded situations are likewise analyzed in terms of electric field contributions originating from nearby oxygens and thus depends on the local conformation of the peptide chain.

We have calculated these effects by ab-initio methods on model compounds using the LORG method (Localized Orbital, Local Origin) [3], and present comparison with experimental data.

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D2-401 DESIGN AND APPLICATIONS OF A PROBE WITH SWITCHABLE Q-FACTOR

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A design for a probe with switchable Q-factor is presented together with its applications. The high Q-factor of modern NMR probes leads to strong radiation damping effects in aqueous solutions. The water resonance itself and also NOE correlation peaks between water and the solute are broadened by radiation damping. Lowering the Q-factor during evolution and mixing periods in multidimensional experiments results in significant sharpening of these correlation peaks. Measurements of T_1 relaxation times of bulk water are also presented. The current design of the Q-switch allows switching between high and low Q in less than 2 microseconds.

D2-402 New Pulse Sequences for Chemical Shift Correlation in N-15, C-13 fully labelled proteins. Application to human interleukin-6 (MW=21KD).,

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New pulse sequences are described for the chemical shift correlation in N-15, C-13 fully labelled proteins. Optimization of sensitivity and/or resolution is achieved either through modification of existing pulse schemes or via entirely new design.. Examples are HSQC 2D experiments for the correlation of proton with nitrogen and carbon resonances, and 3D experiments of the HACACO, (HACA)CONH, and HCCH types. The performances of the new experiments are illustrated by the application to a 21 KD MW protein: human interleukin-6.

D2-403 NEW SOFTWARE FOR THE NMR DETERMINATION OF MACROMOLECULAR STRUCTURES AND DYNAMICS

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Two new software packages, XEASY and OPAL, are presented. These complete a line of software developments in our laboratory, which support the different steps during the determination of NMR solution structures of proteins and have fully compatible data formats. XEASY is used for the spectral analysis, and jointly with DIANA and supporting programs for repeated cycles of data collection and structure calculation, and OPAL performs structure refinements and molecular dynamics simulations. XEASY is laid out for peak picking, support of sequence-specific resonance assignments, cross peak assignments, cross peak integration, and rate constant determination for dynamic processes in homonuclear and heteronuclear 2D, 3D and 4D spectra. Due to the use of X-windows and *Motif*, XEASY is portable on a wide range of UNIX workstations. Maximal computer support in the analysis of spectra is combined with complete user control over the final resonance assignments. XEASY has capabilities for proper treatment of spectral folding and efficient transfer of resonance assignments between spectra of different types and different dimensionality. It can use and flexibly display "strips", *i.e.*, two-dimensional spectral regions that contain the relevant parts of 3D or 4D NMR spectra. Automated sorting routines allow to narrow down the selection of strips that need to be interactively considered in a particular assignment step.

Refinement tasks may include a description of hydration water molecules and of dynamic aspects such as fluctuating networks of non-covalent intermolecular interactions in molecular complexes. OPAL is a program for efficient molecular mechanics and molecular dynamics calculations of large systems, reaching an average rate of 1.5 GFlops on a NEC SX3.

An application of OPAL to an *Antp* homeodomain–DNA–water system will be presented yielding a long time simulation of the protein–DNA interface that includes interior water molecules, and thus describes dynamical aspects of the DNA recognition by the protein.

D2-404 **ANTIMICROBIAL PEPTIDES IN LIPID BILAYERS: A SOLID-STATE NMR AND FT-IR STUDY**, Jack Blazyk*, Amy E. Bankert*, Janet Hammer*, Silvana Lesnoff-Caravaglia*, Donald J. Hirsh† and Jacob Schaefer†, *Chemistry Department, College of Osteopathic Medicine, Ohio University, Athens, Ohio 45701, and †Chemistry Department, Washington University, St. Louis, MO 63130.

The molecular mechanism of magainins, a class of amphipathic cationic peptides with broad antimicrobial properties, remains unclear. Since these peptides selectively permeabilize the plasma membrane of target microorganisms at concentrations which have no apparent effect on mammalian plasma membranes, their potential use as clinical antibiotics is real. We are using the complementary techniques of solid-state NMR and FT-IR spectroscopy to determine the secondary structure, orientation and location of these cationic peptides in phosphoglyceride bilayers in the presence or absence of cholesterol. Ala_{19}-magainin 2, in which Glu at position 19 is replaced by Ala, possesses much greater bactericidal potency than naturally-occurring magainins, yet remains nonhemolytic. Using FT-IR, analysis of the amide I' band of the peptide in lipid bilayers reveals substantial, but not complete, α-helical secondary structure when the peptide is associated with lipid bilayers. We used REDOR (rotational-echo double resonance) NMR to probe the structure of the peptide by making a labeled analog containing [1-^{13}C]Ala_{15}, [2-^{13}C]Gly_{18} and [^{15}N]Ala_{19}. The distance between [1-^{13}C]Ala_{15} and [^{15}N]Ala_{19} is determined by the peptide's three-dimensional structure. The experimentally determined distance of 4.3±0.1 Å is consistent with α-helical secondary structure. In addition, we are using polarized attenuated total reflectance (ATR) IR spectroscopy to estimate the orientation of the helical axis with respect to the bilayer. Similar experiments are under way with an even more potent antimicrobial peptide, $(KIAGKIA)_3$-NH_2, with [1-^{13}C]Ala at position 10 and [^{15}N]Gly at position 11.

D2-405 USE OF ENSEMBLE-AVERAGED DISTANCE CONSTRAINTS FOR NMR REFINEMENT: VALIDITY ASSESSMENT USING CROSS-VALIDATION. Bonvin A.M.J.J. and Brünger A.T., Howard Hughes Medical Institute and Department of Molecular Biophysics, Yale University, New Haven CT 06520, USA.

In the past years much work has been done to better take into account the dynamic properties and conformational variability of NMR experimental data into structure refinement. In general, the focus has been on the use of time-averaged restraints. Ensemble-averaging can be used as well with as advantage that it does not require very long simulations. With ensemble-averaged NOE constraints an ensemble of structures rather than one single structure is required to satisfy the constraints at the same time. This implies a multifold increase of the number of parameters. A potential danger of this approach is that the improvement of the fit obtained in this way might only reflect the increased number of parameters and thus not be significant. This would result in overfitting the experimental data. This is particularly true for NMR structure determination where the ratio of the number of experimental constraints to the number of parameters is usually unfavorable. It is therefore important to develop a method that allows one to assess the validity of the use of ensemble-averaged constraints in NMR structure determination. Cross-validation, which was initially introduced for X-ray structure refinement (Brünger, Nature 355, 472 (1992)), seems an appropriate method for assessing the validity of this approach. Cross-validation estimates the quality of the fit between data calculated from a model and the experimental data for a subset of the data (the test set) that has been omitted during the refinement. Cross-validation provides in this way an unbiased measure of the fit. First, we will demonstrate using two test cases that complete cross-validation can be used to assess the validity of multi-conformer refinement with NMR constraints. The method will then be applied to two proteins, interleukin 4 (Dobson *et al.*, J. Mol. Biol. 224, 899 (1992)) and interleukin 8 (Clore *et al.*, Biochemistry 29, 1689 (1990)) for which both X-ray and NMR data are available.

D2-406 STRUCTURAL CHARACTERIZATION OF OLIGO - N - SUBSTITUTED GLYCINE PEPTOIDS Erin K. Bradley, Janice M. Kerr, Lutz S. Richter, Gianine M. Figliozzi, Dane A. Goff, Ronald N. Zuckermann, David C. Spellmeyer, Jeffrey M. Blaney
Chiron Corporation, Emeryville, CA 94608

Synthesis and screening of peptide libraries for pharmaceutical lead discovery has been a topic of great interest in recent years. Oligo-N-substituted glycines (NSGs) have also been used as a source of molecular diversity in chemical libraries.[1,2] Two NSG trimers, CHIR-2279 and CHIR-4531, have recently been identified as nM ligands for two 7-transmembrane G-protein coupled receptors.[3] In view of the pharmaceutical relevance of this new category of compounds, the NMR characterization of NSGs becomes important, both for establishing covalent connectivity and for determining solution and/or receptor bound conformations. A new strategy has been developed for assigning ^{1}H and ^{13}C resonances of NSGs, using both traditional homonuclear assignment techniques and the additional heteronuclear pulse sequences, HMQC and HMBC. Sequential chemical shift assignments were performed, and solution conformational preferences were determined for CHIR-2279 and CHIR-4531. NSGs are expected to be more flexible than peptides due to the tertiary amide, with both *cis* and *trans* amide bond conformations being accessible. Solution NMR studies indicate that although CHIR-2279 and CHIR-4531 have identical backbones and termini, and very similar side chains, they do not display the same solution conformational characteristics.

1 Simon, et al., (1992) *Proc. Natl. Acad. Sci. USA* **89**: 9367-9371.
2 Zuckermann, et al., (1992) *J. Am. Chem. Soc.* **114**: 10646-10647.
3 Zuckermann, et al., (1994) *J. Med. Chem.* **37**: 2678-2685.

D2-407 RIBOSOMAL PROTEIN L9: AN RNA-BINDING PROTEIN ANALYZED USING NMR AND NMR-X-RAY JOINT REFINEMENT, Chris S. Cameron and David W. Hoffman, Department of Chemistry and Biochemistry, University of Texas, Austin, Tx 78712

The structure of L9, a 15.7 kD primary RNA-binding of the prokaryotic ribosome, has been determined using an unusual combination of NMR spectroscopy and x-ray crystallography. The protein consists of two domains connected by a central nine turn alpha helix, giving L9 the appearance of a dumbbell. While the C-terminal domain and connecting alpha helix were well defined by x-ray data alone, information from both NMR and x-ray diffraction were needed to accurately define the structure of the N-terminal domain. The N-terminal domain contains a hydrophobic core and slowly exchanging amide protons, suggesting that its poor definition in the x-ray structure is due to packing disorder within the crystal, rather than structural instability. Conserved aromatic residues on the protein surface form likely sites for binding ribosomal RNA. This analysis provides a model as to how x-ray and NMR data can by practically and logically combined to determine. the structure of a single protein molecule.

D2-408 **PROBING BOUND WATER IN PROTEINS USING TWO-DIMENSIONAL ^{19}F-^{1}H NMR**

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A simple approach for localizing internal water molecules in proteins is described. This approach combines ^{19}F-detected heteronuclear Overhauser and exchange spectroscopy (HOESY) with site- or residue-specific ^{19}F substitution. For intestinal fatty acid binding protein complexed with [2-mono-^{19}F]-palmitate (MW 15.4 kDa), an intense nuclear Overhauser enhancement (NOE) was observed between the fluorine atom(s) on the ligand and an adjacent ordered water molecule, as defined in the 1.98Å crystal structure of the fatty acid-protein complex. Other NOEs were observed between the fluorinated ligand and protein side-chain moieties and were also consistent with the crystal structure. NOE build-up curves for the water cross-peaks revealed anomolous intensities and an unusual oscillatory behavior. We hypothesize that the anomolous build-up curves are related, in part, to chemical exchange of the water molecule between its internal binding site and other sites, possibly bulk solvent. This approach has several distinct advantages over ^{1}H-detected methods. The use of ^{19}F detection circumvents the need for exotic solvent suppression schemes, sequential proton assignments, and a third-dimension. Also, ^{19}F is relatively inexpensive to incorporate into specific amino acids and nucleotides. This method should be particularly useful for macromolecules complexed with drugs that naturally contain ^{19}F atoms.

D2-409 THE BINDING OF A HYDROPHOBIC DRUG TO THE C-TERMINAL DOMAIN OF CALMODULIN, C. Jeremy Craven, Sheena K. Jones, Johan Evenäs+, Torbjörn Drakenberg+, Bryan Finn+, and Jonathan P. Waltho+, Krebs Institute, University of Sheffield, S10 2UH, U. K. and +Department of Physical Chemistry II, University of Lund, Sweden.

The binding of a naphthalene sulphonamide derivative drug (J8) to the C-terminal domain of bovine calmodulin (TR2C) has been studied using NOE and chemical shift analysis, in conjunction with a dynamic method for the assignment of intermolecular NOEs.

The proton and nitrogen resonances of TR2C were assigned in both the free and the drug-bound states by a combination of standard homonuclear and heteronuclear experiments and a ^{15}N-HSQC titration. Analysis of the chemical shifts indicated that the conformational changes of the protein on binding were extremely small, although ring-current effects due to the drug molecule were detectable. Drug-protein NOEs were identified using a ^{15}N-edited NOESY experiment. These NOEs were assigned using a dynamic NOE assignment method in which ambiguities were resolved during a simulated annealing procedure. This procedure yielded two families of binding orientations for the naphthalene ring within the hydrophobic pocket of the protein molecule, neither family being individually able to satisfy all NOEs.

The automatic assignment of the NOEs allowed detailed study of the uniqueness and completeness of the family of binding conformations found. At the same time, the simplicity of the system studied also allowed an analysis of the use of the dynamic assignment method in a real application.

D2-410 **TRANSLATION OF UNCERTAINTIES IN NOESY PEAK VOLUMES INTO UNCERTAINTIES IN THE COORDINATES OF COMPUTED STRUCTURES,** ***Zeljko Dzakula,[1] Nenad Juranic,[2] Zsolt Zolnai,[1] William M. Westler,[1] Slobodan Macura,[2] and John L. Markley,[1]***
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Analytical expressions have been derived for the derivatives of atomic Cartesian coordinates with respect to interatomic distances. These new equations, in conjunction with the full relaxation matrix approach, have been applied in analyzing uncertainties in molecular structures calculated from experimental proton-proton nuclear Overhauser effect (NOE) data. Uncertainties in interatomic distances and in atomic coordinates have been computed from the estimated uncertainties in the measured NOE intensities. These calculations have made it possible to establish a missing link between the quality of the input spectral parameters and the output NMR structure. Applications of this technique will be demonstrated.

D2-411 IMPROVED HCCH-TOCSY EXPERIMENT FOR DETERMINING ^{3}J(H$^{\alpha}$-H$^{\beta}$) COUPLING CONSTANTS FROM ^{1}J RESOLVED HETERONUCLEAR E.COSY MULTIPLETS.

S. Donald Emerson and David C. Fry.
Department of Physical Chemistry, Hoffmann-La Roche, 340 Kingsland St., Nutley, NJ 07110-1199.

Coupling constants such as ^{3}J(H$^{\alpha}$-H$^{\beta}$), which depend on the dihedral angle χ^{1}, are useful in generating experimental constraints on amino acid side-chain conformations in proteins and for determining stereospecific methylene H$^{\beta}$ resonance assignments. Accurate quantitation of ^{3}J(H$^{\alpha}$-H$^{\beta}$) from small-flip-angle HCCH-TOCSY spectra is complicated by distortions in the intensities of sub-multiplet elements of the heteronuclear E.COSY cross-peak. We describe modifications to the previously reported HCCH-TOCSY pulse sequence[1] which improve the quality of data for measuring ^{3}J(H$^{\alpha}$-H$^{\beta}$) coupling constants.

[1]S.D. Emerson and G.T. Montelione. *J. Magn. Reson.* **99**, 413-420 (1992).

D2-412 **Demonstration of Positionally Disordered Water Within a Protein Hydrophobic Cavity By NMR**

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The location of bound water in the solution structure of human interleukin-1β (hIL-1β) has been investigated using water-selective two-dimensional heteronuclear magnetic resonance spectroscopy. We show that, in addition to water at the surface of the protein and ordered internal water molecules involved in bridging hydrogen bonds, positionally disorded water is present within a large naturally occurring hydrophobic cavity located at the center of the molecule. The residency time of these bound water molecules lies in the range of 1-2ns to 100-200μs and can be readily detected by NMR. The NMR experiment is only dependent on the spatial proximity of water protons to protons of the protein and on the lifetime of the bound water, and therefore does not require uniform ordering of water molecules. In contrast, no water inside this cavity was observed in the crystallographically determined structures of hIL-1β. This reflects the inherent positional disorder of these water molecules, resulting in a smearing out of their electron density beyond the limits of detection. Thus hydrophobic cavities in proteins may not truly be empty as has been proposed form analysis of crystal structures but may contain crystallographically invisible, mobile water molecules which are detectable by NMR. The factors involved in stabilizing water within hydrophobic cavities are discussed.

D2-413 A MODEL-FREE ALGORITHM FOR THE REMOVAL OF BASELINE ARTIFACTS, Mark S. Friedrichs, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543. A novel algorithm for removing baseline distortions in NMR spectra is presented. The algorithm approximates the baseline as the median of the noise extrema. Consequently, the method does not require that NMR peaks be discriminated from noise peaks. In addition, the algorithm makes no assumptions regarding the source of the distortion or its functional form. The application of the algorithm to a particularly distorted, two-dimensional NOESY spectrum removed all baseline artifacts, thereby revealing previously obscured peaks. Also the algorithm's behavior is explored for different parameter settings and spectral characteristics (signal-to-noise ratio, NMR peak density, peak linewidths).

D2-414 **AUTOMATED AND INTERACTIVE TOOLS FOR ASSIGNING 3D AND 4D NMR SPECTRA OF PROTEINS: CAPP, STAPP AND PIPP**

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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 program STAPP (Shift Table Assignment Peak Picker) automatically assigns or correlates 3D and 4D spectra making use of the prior information known about each NMR experiment, proposed assignments, and previously determined structural models. STAPP will be shown to correlate peaks from several 3D triple resonance experiments into a flat assignment database file which greatly aids the semi-automatic assignment of proteins. STAPP has been most useful in assigning 4D NOE experiments which typically have ≥1024 slices. The X11 based graphics program PIPP (Primitive Interactive Peak Picker) provides several useful interactive assignment tools which include: peak pick table editing, 3D symmetry locator, and 3D/4D NOESY assignment tools. The 3D/4D NOESY assignment tools resolve degenerate assignments using 3D/4D symmetry and inter-proton distances obtained from different structural models. Results from using these tools in analyzing Interleukin-4, hnRNP A1 and MIP-1β NMR data will be shown.

D2-415 MULTIDIMENSIONAL SOLUTION NMR STUDIES OF MEMBRANE SPANNING SEGMENT 2 OF HUMAN NMDA RECEPTOR IN MEMBRANE ENVIRONMENTS, Jennifer J. Gesell, William Sun, Mauricio Montal, Stanley J. Opella, Department Of Chemistry, University of Pennsylvania, Philadelphia, PA 19104; Department of Biology and Physics, University of California San Diego, La Jolla, CA 92093

Conventional structural studies of major functional channel proteins are severely limited by their size, complexity and difficulty of crystallization. A promising approach to their analysis is to synthesize and study peptide sequences corresponding to functional segments of the protein. We have chemically synthesized and biologically expressed a peptide corresponding to the amino acid sequence of membrane spanning segment 2 (M2) of the NMDA receptor. This segment is the proposed channel lining of the receptor. The peptide has been purified and incorporated into deuterated detergent micelles for solution NMR studies. Two dimensional ^1H /^1H homonuclear pulsed field gradient experiments have been used to determine that the secondary structure of the peptide in detergent micelles at neutral pH is primarily helix, a structural motif that is compatible with the pore-lining function of this segment in the authentic channel protein. In addition, solid state NMR experiments of M2 in oriented planar lipid bilayers indicate that the peptide is embedded in the bilayer in a transmembrane orientation.

D2-416 DETERMINATION OF STRUCTURAL CHANGES IN BPTI MUTANTS USING 13C NMR CHEMICAL SHIFTS.

Poul Erik Hansen, Conni Lauritzen, Lars Chr. Petersen*, Søren Bjørn*, Kjeld Norris* and Ole Hvilsted Olsen*, Department of Life Sciences and ChemistRy, Roskilde University and Novo-Nordisk*, Denmark

Structural changes via ^{13}C chemical shift comparisons are demonstrated using capped BPTI analogues with point mutations. The ^{13}C chemical shift changes can easily be separated into local and global effects. ^{13}C ring currents effects are calculated. The effects are analysed mainly in terms of electric field effects and ring current effects. Removal of the terminal salt bridge in BPTI 3-58 and its mutants is clearly seen in terms of changes in ^{13} chemical shifts and is discussed in relation to the increased moblity of the side-chain of Tyr-23. Comparison of ^{13}C chemical shifts can also be used to identify salt bridges via comparison of mutants lacking one of the partners. Changes in stability as a function of amino acid composition or due to variations in pH are measured by DSC and the results are correlated to NH exchange rates. NH exchange rates show a dramatic increase for the slowly exchanging protons in going from WT to 3-58, but a levelling effect for less stable mutants.

D2-417 CARBON-13 CHEMICAL SHIFT AND RELAXATION DATA IN COMPACT PEPTIDES: APPLICATION TO NEUROTOXINS ISOLATED FROM SPIDER VENOM.

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Many neurotoxins isolated from animal venoms are small proteins rich in cystine residues which represent a large (15-25) percentage of the molecule's amino acids. Common "cystine motifs" appear in neurotoxic peptides from varied sources displaying diverse functional properties. The abundance of cystines in this class of molecules results in compact structures composed of both regular and irregular secondary structure elements. We have measured ^{13}C NMR chemical shifts for 3 peptides isolated from the venom of the funnel web spider and correlated these to independently determined three dimensional structures. A retrospective analysis of the carbon chemical shift data suggests that it will be valuable for monitoring protein structure refinement. Using indirect detection techniques, we measured ^{13}C-^{1}H NOE and $^{13}C\alpha$ relaxation data on ω-Aga-IVB and find that this information correlates well with the rmsds measured from an ensemble of solution structures.

D2-418 CALCULATION OF PROTEIN SOLUTION STRUCTURES USING IMPROVED DISTANCE INPUT FROM NETWORK EDITING EXPERIMENTS,

Charles G. Hoogstraten, William M. Westler, Slobodan Macura*, and John L. Markley, Department of Biochemistry, University of Wisconsin, Madison, WI 53706, and *Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN 55905

We have developed a series of experiments designed to measure interproton distances in proteins without the interfering effects of spin diffusion (*J. Magn. Reson. B*, **102**, 232-235 (1993); Abstract M10, XVIth ICMRBS, Veldhoven, August 14-19, 1994). It remains to be shown that these improvements in cross-relaxation analysis significantly affect the course of protein structure calculations in the presence of a dense NOE network, steric interactions, and other energetic terms. In this work, we conduct side-by-side structure calculations to compare results from "unimproved" NOESY-based datasets interpreted with standard methods to datasets "improved" using network editing experiments. We show structural calculations based both on data simulated from an assumed structure, to allow the assessment of absolute accuracy, and from experimental network editing data. The use of the latter set of calculations in refinement of the previously determined structure of the small protein turkey ovomucoid third domain (OMTKY3) is discussed.

D2-419 CONFORMATIONAL STUDY OF DIACYLGLYCEROL GLYCOLIPIDS BOUND TO MAGNETICALLY-ORIENTED PHOSPHOLIPID BILAYERS.

K. P. Howard and J.H. Prestegard, Department of Chemistry, Yale University, New Haven, CT 06511.

A wide variety of biological processes are mediated by interactions between carbohydrates and proteins at the surface of cells. Diacylglycerol glycolipids occur widely in higher plants, algae and photosynthetic microorganisms. Here we present membrane-bound conformations determined from NMR spectroscopy of diacylglycerol glycolipids isolated from extracts of algae grown on ^{13}C enriched media. The study is conducted using methods which rely on the observation of dipolar interactions between neighboring spin 1/2 nuclei in ^{13}C labeled molecules. Dipolar couplings are measured in a model membrane system consisting of phospholipid bilayers which orient in an applied magnetic field. Molecular modeling using a version of AMBER modified to include a membrane interaction energy term is used to study the energetics of membrane surface interactions and aid in analysis of the experimental observations.

D2-420 DEPT EDITING OF CROSS-RELAXATION NETWORKS IN PROTEINS, Nenad Juranić and Slobodan Macura, Department of Biochemistry and Molecular Biology, Mayo Graduate School Mayo Clinic and Foundation, Rochester, MN 55905

Manipulation of magnetization exchange networks during the mixing time, exchange network editing[1], is very useful for removal of unwanted magnetization exchange pathways and for the accuracy increase in measurements of desired ones. The major limitation in the previously proposed experiments is limited selectivity due to spectral overlap of different classes of spins. Here we demonstrate that on isotopically labeled samples the cross-relaxation network of proteins can be edited independently of chemical shifts overlap, by the inverse DEPT technique. For example, one can remove all CH_2 protons from the cross-relaxation network by inverting them during the mixing time. In another experiment CH and CH_3 protons were inverted, dividing the network into subnetworks of NH, CH_2 and CH, CH_3 protons. The method allows precise probing of the spin-diffusion pathways of peptides. The essence of the approach is demonstrated on uniformly doubly labeled human ubiquitin, a small globular protein, Mw 8565.

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D2-421 USE OF OVERLAPPED NOE VOLUME RESTRAINTS AND LONGER MIXING TIME NOE DATA FOR THE REFINEMENT OF PROTEIN STRUCTURES, M. W. Kalnik, S. Szalma, & P. F Yip, BIOSYM Technologies, Inc., San Diego, CA 92121

With the recent introduction of fast and accurate methods[1] for the direct refinement of protein structures using NOE cross-peak volumes as pseudo-energy restraints within restrained molecular dynamics simulations, we have begun to investigate the effects of including longer mixing time data (> 500 ms) and summed volume restraints for overlapped crosspeaks within NOE-MD protocols.

The 58-residue protein, Zn-Rubredoxin, whose high-resolution structure has been determined by both X-ray crystallography[2] and NMR spectroscopy[3] serves as our model system. We have simulated realistic NOE data from these two structures (including noise and ambiguous resonance assignments) and have used these data to verify the protocols and to quantitate the improved accuracy and precision of the refined structures. The results of these studies using simulated NOE data, along with a comparison to refined structures obtained with experimentally-determined NOE volumes[4] will be presented.

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D2-422 HIGH DEFINITION PROTEIN STRUCTURE DETERMINATION AND REFINEMENT FROM SOLID-STATE NMR DERIVED ORIENTATIONAL CONSTRAINTS, Randal R. Ketchem, Kwun-Chi Lee, Shouqin Huo, Weidong Hu, Fang Tian, Myriam Cotten and Timothy A. Cross, National High Magnetic Field Laboratory, Institute of Molecular Biophysics and Department of Chemistry, Florida State University, Tallahassee, FL 32306-4005

A solid-state NMR technique for determining three dimensional protein structure has been developed. This technique is based on the observation of nuclear spin interactions in uniformly aligned samples. The resulting local orientational constraints provide a level of structural detail rarely achieved in either protein crystallography or solution-state NMR.

Samples of gramicidin A were labeled with ^{13}C, ^{15}N and ^{2}H, and chemical shifts, dipolar and quadrupolar couplings were observed. From these orientational constraints the initial backbone and sidechain torsion angles were characterized. The structure was refined based on the solid-state NMR data using a simulated annealing protocol developed in this lab. This protocol utilizes torsional changes to vary the protein structure and minimizes a penalty based on differences between calculated and observed NMR data.

The refined structure is being used to investigate how this polypeptide facilitates cation transport. The carbonyl oxygens are important for solvating the cations and the dipole moment of the tryptophan indoles reduces the potential energy barrier at the center of the bilayer.

D2-423 SOFTWARE FOR THE ANALYSIS OF BIOMOLECULAR NMR SPECTRA, Mogens Kjær and Flemming M. Poulsen, Carlsberg Laboratory, Dept. of Chemistry, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark

At Carlsberg Laboratory, two major program complexes have been developed during the recent years:

MNMR is a suite of programs used to Fourier transform multi-dimensional spectra. FID's can be read from several types of spectrometers.

Pronto/3D[2] is used for analysis of the transformed spectra. Pronto/3D[2] combines heteronuclear multidimensional NMR spectrum analysis and molecular graphics display facilities. It is now widely applicable to studies of biomolecules, such as proteins, oligosaccharides, and oligonucleotides.

The programs run on the major X11-based UNIX workstations.

A flexible and easy-to-use contour diagram display is the central lightbox in Pronto/3D[2]. All steps of the assignment process can be examined, and the alignment of cross peaks verified. 2D, 3D, or 4D spectra can be displayed simultaneously.

Pronto/3D[2] has tools for semi-automatic build up of spin systems, cross peak assignments, and sequential assignments. Molecular structures calculated can be displayed, as stick models, spheres, or cartoons.

The poster outlines the steps taken during the assignment of a 65-residue protein, using 2D COSY, TOCSY, and NOESY spectra, 3D ^{15}N-HMQC-TOCSY and ^{15}N-HMQC-NOESY, 3D ^{13}C-HCCH, as well as triple resonance HNCO, HN(CO)CA, and HNCA spectra.

D2-424 COMPLETE RELAXATION AND CONFORMATIONAL EXCHANGE MATRIX (CORCEMA) ANALYSIS OF NOESY SPECTRA: APPLICATION TO TRANSFERRED NOESY AND PROTEIN FOLDING STUDIES, N. Rama Krishna, Hunter N.B. Moseley and Ernest V. Curto, Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL 35294.

We have developed a general program called CORCEMA, an extension of earlier work from this laboratory (Biopolymers **19**: 2003 (1980) and J. Mag. Reson., **98**:36 (1992). It incorporates multistate conformational exchange for a single molecule or a set of interacting molecules (e.g., ligand and enzyme). For a given dynamical model, it computes NOESY intensities which can be compared to experimental intensities. We demonstrate its application using simulation of (i) transferred NOESY experiment on a thermolysin-inhibitor complex showing hinge-bending motion, and (ii) a three-state model of folding pathway of penta-L-alanine exhibiting (αhelix-3_{10}helix-βturn transitions). It is shown that slow hinge-bending motions in enzymes can severely restrict the applicability of transferred NOESY technique in deducing the active-site conformation of a ligand. Other pertinent factors that govern the intensities in the NOESY spectra of dynamical systems have also been examined.

D2-425 HYDROGEN BONDING NETWORKS IN INTESTINAL FATTY-ACID BINDING PROTEIN STUDIED BY AMIDE $^1J_{NC}$ COUPLING CONSTANT, Elizabeth Kurian, Nenad Juranic, Franklyn Prendergast, Slobodan Macura, Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN 55905, U.S.A.

We have recently shown that amide group $^1J_{NC}$ coupling is a valuable probe for amide hydrogen bonding in proteins [1]. Analysis of coupling constants in proteins reveals correlations of $^1J_{NC}$ values with secondary structural features which seem to be characteristic of specific hydrogen bond networks for backbone amides in α-helices, β-sheets and reverse turns. We have extended these studies to include the $^1J_{NC}$ coupling constants in intestinal fatty-acid binding protein (IFABP). IFABP (Mr = 15000) has ten antiparallel β-strands comprising a β-barrel. The coupling constants ranged from 13 to 17 Hz depending on the residues' position in the secondary structure. Results obtained were analyzed for evidence of intrastrand hydrogen bonding chains.

[1] N. Juranic, P.J.Ilich and S. Macura, J. Amer. Chem. Soc., 1994, in press.
Key words: Hydrogen bonding, proteins, NMR, scalar coupling.

D2-426 CHEMICAL SHIFT DISPERSION EXPECTED IN A 900 MHZ SPECTROMETER FROM A 14.1 T (600 MHZ) MAGNET

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The study of large molecules using NMR is complicated by the approximately linear increase in the number of correlation peaks with a linear increase in, for example, the number of amino acids in a protein. The dispersion of chemical shifts is, however, constant for proteins with 50, 100 or 200 amino acids. Large proteins not only have more peaks in the same chemical shift range, these peaks are broader due to the inevitable decrease is rotational correlation time. One advantage to having larger magnetic fields is that increasing B_0 from 14.1 to 17.6 Tesla (the difference in using a 600 MHz and a 750 MHz spectrometer) gives an increase of 25% dispersion in each dimension. For larger proteins, 3D and 4D experiments on ^{13}C and ^{15}N labeled samples are necessary to increase the effective dispersion even more so that sequential assignments can be made and NOE cross peaks can be assigned.

We present here a simple method that enhances the chemical shift dispersion in the indirect dimension of any 2D or 3D or 4D experiment. Using a 600 MHz spectrometer, non-degenerate chemical shifts can be dispersed as they would appear in a 900 MHz spectrometer, if one existed. The method involves fusing together two evolution periods for each indirect dimension. The intrinsic linewidth in the indirect dimension of linearly incremented and fused evolution periods is identical so that the 50% greater dispersion in the fused evolution experiment is real.

Fusing together two time periods requires a simple modification that can be made to any indirect constant time or linearly incremented time period. This method should be valuable for the study of molecules that are highly helical (RNA, DNA and helical proteins) and, in general, for larger proteins.

D2-427 **PROBING PROTEIN/GLYCAN INTERACTIONS: THE SOLUTION STRUCTURE OF AN OLIGOSACCHARIDE (GD2) ANTIGEN AND ITS INTERACTIONS WITH MAB 3F8,** *Thomas McDonagh, Dale Cumming, Hsiang-Ai Yu, Martin Karplus*, and Lynette Fouser,* Small Molecule Drug Discovery, Genetics Institute Inc., 85 Bolton Street, Cambridge 02140, 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 IgG3k 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 GD2. 3F8 exhibits tight antigen specificity, binding to GD2 but not to other gangliosides including GM2, GD1b, or GD3, 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 GD2 can be prepared by ceramide glycanase digestion followed by ß-galactosidase treatment of GD1b. The 3D solution structure of the glycan moiety of the antigen has now been determined using dipole-dipole NMR relaxation (bulk and selective T1's and nOe's) and molecular modeling studies of the mAb/Ag complex, using X-ray structures of homologous Ab's as templates, and calorimetric studies have been performed. Taken together, these studies suggest the basis for the apparent high affinity binding and specificity exhibited by 3F8.

D2-428 Solid-state NMR Analysis of the Conformation of an Inhibitor Bound to Thermolysin

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Structural methods, such as X-ray diffraction (for crystals) and solution NMR (for soluble proteins smaller than 20 kDa), are commonly used to determine the receptor-bound conformation of ligands in the process of rational drug design. Rotational-echo double-resonance (REDOR) NMR is a solid-state, magic-angle spinning technique which can be used for structural studies of insoluble, non-crystallizable, and large proteins. Previous studies of helical peptides verified that dipolar couplings measured by REDOR NMR yield accurate and precise internuclear distances. In this study we use double REDOR to measure distances between backbone atoms in a phosphonamidate transition-state inhibitor bound to thermolysin. The ^{31}P-^{13}C', ^{31}P-^{15}N, and ^{31}P-^{13}Cα distances measured in a complex of Cbz-Gly^{P}-[1-^{13}C]Leu-[^{15}N,2-^{13}C]Ala and the enzyme (3.61 ± 0.15, 3.89 ± 0.12, and 5.37 ± 0.13 Å, respectively) are consistent with those observed by X-ray diffraction in four phosphonamidate inhibitor/thermolysin complexes (average values of 3.58 ± 0.04, 3.91 ± 0.13, and 5.17 ± 0.18 Å, respectively).

D2-429 A COMPARISON OF TAILORED BAND-SELECTIVE PULSES IN TRIPLE RESONANCE EXPERIMENTS

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Triple-resonance NMR experiments often involve excitation of a particular region of interest such as the carbonyl carbons using relatively simple band-selective pulses such as sin x/x.
We have examined various tailored excitation schemes in a constant-time version of the HCACO experiment using an ^{15}N, and ^{13}C enriched hexapeptide. The advantages of various window functions for these pulses are demonstrated and discussed.

D2-430 THE DQ/ZQ-METHOD: A NEW APPROACH FOR MEASURING ^{3}J-COUPLING CONSTANTS

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Heteronuclear and homonuclear vicinal coupling constants in proteins provide useful information about local conformations and complement structural information obtained from NOEs. Here we introduce a set of new experiments relying on the DQ/ZQ-principle (1) to measure coupling constants in macromolecules with linewidth larger than the coupling of interest:
A zero quantum coherence between spins A and B evolves the difference of the couplings to a third spin C: Δ=J(A,C)-J(B,C), whereas double quantum coherence evolves the sum Σ=J(A,C)+J(B,C) to C. If e.g. J(A,C) is a large coupling, the interesting coupling J(B,C) including its relative sign with respect to J(A,C) can be determined from J(B,C)=1/2(J_{Σ}-J_{Δ}). The DQ/ZQ-method is robust against effects of differential relaxation (2). Examples will be shown for the determination of $^{3}J(H^{N},H_{\alpha})$ and $^{3}J(N_{i+1},H_{\alpha i})$ coupling constants defining the backbone angles ϕ and ψ from the appropriate triple resonance experiments derived from HNCA and HN(CO)CA respectively.
The DQ/ZQ-method further allows in an DQ/ZQ-{HC}CH-E.COSY experiment to determine $^{n}J(H,H)$ couplings in CH_2-CH_2 fragments defining χ_2 and χ_3 in proteins, which has thus far been difficult to obtain in HCCH-E.COSY experiments (3).

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D2-431 A SIMULTANEOUS ^{15}N,^{1}H- AND ^{13}C,^{1}H-HSQC WITH SENSITIVITY ENHANCEMENT AND A HETERO-NUCLEAR GRADIENT ECHO

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New pulse sequences are introduced and discussed, that allow for simultaneous acquisition (1) of ^{15}N,^{1}H- and ^{13}C,^{1}H-HSQC correlations for fully ^{13}C, ^{15}N labeled biomacromolecules in combination with heteronuclear gradient echoes and sensitivity enhancement (2, 3). The simultaneous COS-INEPT (4) transfer experimentally found to be optimal can be used as a building block especially in time consuming multidimensional NMR experiments. Due to the excellent solvent suppression obtained by employing heteronuclear gradient echoes, which allows detection of resonances under the water resonance, it is possible to record two sensitivity enhanced experiments simultaneously on one sample dissolved in H_2O, e.g. a 3D NOESY-^{15}N,^{1}H/^{13}C,^{1}H-HSQC or a 4D ^{13}C,^{1}H-HSQC-NOESY-^{15}N,^{1}H/^{13}C,^{1}H-HSQC.

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D2-432 MAXIMUM ENTROPY RECONSTRUCTION OF NON-LINEARLY SAMPLED DATA, 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.

Data processing with Maximum Entropy reconstruction (MaxEnt) is a robust alternative to the usual processing with the DFT. It is particularly powerful if combined with non-linear sampling of data points, where the sampling of the data points is tailored to achieve the necessary resolution with adequate signal-to-noise in the shortest time possible. The dramatic development of available computer resources has made routine application of this method feasible[1-5]. To achieve optimal results, however, a careful design of the sampling schedule is necessary. Non-linear sampling is also possible in more than one dimension in multidimensional experiments, further reducing the time necessary to achieve the desired resolution. Examples of the optimal sampling schemes will be shown for different types of spectra and the application of one- and two-dimensional non-linear sampling will be demonstrated.
One major drawback of MaxEnt is the fact that it is a non-linear method. Thus quantification is not straightforward and we would like to propose a solution to this problem using internal calibration. It will be applied to a ^{15}N-relaxation-time series, where non-linear sampling has been used to reduce the measuring time by a factor of two.

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D2-433 **THE HCC-TOCSY-CCH-E.COSY-EXPERIMENT FOR THE DETERMINATION OF J(H,H)-COUPLING CONSTANTS IN RNA**, *H. Schwalbe[#†], J. Marino[‡], S. Glaser[#], G. King[£], D.M. Crothers[‡], C. Griesinger[#]
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In isotopically enriched oligonucleotides (1), the ribose ring conformation can be derived by measuring proton-proton coupling constants using methods based on the E.COSY principle (2). However, the severe overlap for ribose protons prohibits the determination of all ^3J(H,H) coupling constants from an HCCH-E.COSY experiment.
We therefore introduce a novel pulse sequence: 3D HCC-TOCSY-CCH-E.COSY experiment which solves this problem by two features. All magnetization is derived from the H1' plane which is the best resolved in RNA spectra. The C,C-TOCSY transfer can be adjusted such that out of 17 possible (ω_2,ω_3)-cross peaks in ribose only five, namely the C1',H2'; C2',H3'; C3',H4' and C4',H5' are observed in the H1' plane. This allows to disentangle the cross peaks in the H2',C2'/H3',C3' region that are otherwise severely overlapping in RNA. Applications of the technique to GMP and a 19mer RNA-hairpin are shown in order to derive the dynamical conformation of the stem and the loop-region of this hairpin RNA.

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D2-434 HETERO-TOCSY-BASED EXPERIMENTS FOR MEASURING HETERONUCLEAR RELAXATION IN NUCLEIC ACIDS AND PROTEINS. Barry I. Schweitzer, Kevin H. Gardner, and Gregory Tucker-Kellogg. Walt Disney Memorial Cancer Institute, Florida Hospital, Orlando, FL. 32826.
While both ^{31}P and ^{113}Cd are present at locations of interest in many different macromolecular systems, heteronuclear-detected relaxation measurements on these nuclei have been restrained by limitations in either resolution or signal to noise. In the present study, we have developed heteroTOCSY-based methods to overcome both of these problems and thus to facilitate the study of heteronuclear relaxation rates on systems containing either of these nuclei. 2-D versions of these experiments were utilized to measure ^{31}P T_1 and T_2 values in DNA oligonucleotides; the additional resolution offered by a second dimension allowed determination of these values for most of the ^{31}P resonances in a DNA dodecamer. We used this data to ascertain that the terminal portions of the helix have higher mobility than internal portions, in agreement with previous studies using other methods. ^{31}P relaxation measurements also suggested that incorporation of the nucleoside analog cytosine arabinoside into a DNA dodecamer causes a reduction in backbone mobility. One-dimensional, frequency-selective versions of these experiments were also developed for use on systems containing a smaller number of heteronuclear spins. These methods were applied to investigate the heteronuclear relaxation properties of ^{113}Cd in ^{113}Cd(2)LAC9(61), a Cys(6)Zn(2) DNA binding domain. Data from these experiments confirms biochemical evidence that there are more significant differences in the metal-protein interactions between the two metal-binding sites than has been previously identified for proteins containing this motif. These demonstrations of the utility of heteroTOCSY based relaxation measurements suggest that these methods may be more generally applicable to other systems.

D2-435 Two-dimensional triple-resonance NMR experiments: application to the conformational study of labelled electron-transfer proteins.
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Institut de Biologie Structurale Jean-Pierre Ebel, C.N.R.S. - C.E.A. - 38027 Grenoble Cedex - France

The resonance assignment of doubly labelled proteins is usually based on a set of three-dimensional triple-resonance experiments, as originally proposed by Kay *et al* (1). In this case, assignment ambiguities are reduced, because 3 nuclei are correlated in a single experiment. Two different techniques can be devised to simultaneously label two spins in the same indirect detection dimension: ① a two-spin coherence is created during a single evolution period or ② a single-quantum coherence is generated for each nucleus during two evolution periods which are incremented in concert.

Szyperski *et al* have used these methods for designing 3D experiments of reduced-dimensionality [4D→3D] (2,3) and we have recently proposed a complete set of 2D schemes (4,5), as an alternative to established 3D sequences. For intermediate size proteins (< 120 aa), the complete backbone assignment can be obtained on the sole basis of such 2D experiments, where the increased digital resolution balances the reduced dimensionality. Indeed, this resolution makes possible the use of a computerized assignment procedure (6), based on a simulated annealing algorithm. We have extended the use of these 2D methods for measuring J-couplings and relaxation parameters of the cytochrome c_2 from *Rhodobacter capsulatus*.

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D2-436 BIOMAGRESBANK

Eldon L. Ulrich, Miron Livny, Yannis Ioannids, Clara Mortezai-Zanjani, Amy Klimowicz, and John L. Markley, Departments of Biochemistry and Computer Science, Univ. of Wisconsin-Madison, Madison, WI 53706

The scope of *BioMagResBank (BMRB)* is being expanded to include dynamic, structure, kinetic, and thermodynamic data on biological macromolecules derived by NMR spectroscopy. Specific data will involve order parameters, T1, T1rho, and T2 values, local and global rotational correlation times, H-exchange rates, chemical shifts, coupling constants, secondary structure predictions, structure coordinate data, and pKa values. In addition to the primary archived data, sufficient experimental detail will be captured to allow users of the database to judge the quality and measure the accuracy of the information they retrieve. Software will be developed and distributed to authors for use in formatting and submitting their data to the database directly. Data validation routines will be embedded in the software distributed to authors to increase the quality of data submitted to BMRB. Further validation software will be utilized at BMRB to check submissions for proper format, nomenclature, completeness, and that data values are within expected ranges, before they are entered in the distribution form of the database. Value added information such as semantic links to data in related databases (SWISS-PROT, Molecular Structural Database, GenBank, and Protein Information Resource) will be added to the files. These procedures will allow for rapid data acquisition, processing, and distribution and will place the burden of accuracy on authors, who are most familiar with the data. The data will be available in a format compatible with the Crystallographic Information File being developed by the crystallographic community and in an Abstract Syntax Notation One format. Access to the database is provided on the World Wide Web (WWW) and by anonymous ftp. Other means of accessing and querying the database will be pursued and brought on-line as they are developed.

D2-437 NMR STUDIES OF $^{2}H/^{13}C/^{15}N$ LABELED HCA II, Ronald A. Venters[a,*], Chih-Chin Huang[a], Bennett T. Farmer II[c], Ronald Trolard[d], Leonard D. Spicer[a,b], and Carol A. Fierke[a]; [a]Department of Biochemistry and [b]Radiology, Duke University Medical Center, Durham, North Carolina 27710; [c]Macromolecular NMR, Pharmaceutical Research Institute, Bristol-Myers Squibb, P.O. Box 4000, Princeton, New Jersey 08543-4000; [d]Cambridge Isotope Laboratories, 50 Frontage Road, Andover, Massachusetts 01810.

The protein human carbonic anhydrase II (HCA II) has been isotopically labeled with ^{2}H, ^{13}C, and ^{15}N for high-resolution NMR assignment studies and pulse sequence development. ^{2}H has been incorporated into HCA II in order to decrease the rates of ^{13}C and $^{1}H_N$ T_2 relaxation. NMR quantities of protein with essentially complete aliphatic ^{2}H incorporation have been obtained by growth of *E. coli* in defined media containing D_2O, [1,2-$^{13}C_2$, 99%] sodium acetate, and [^{15}N, 99%] ammonium chloride. Complete aliphatic deuterium enrichment is optimum for ^{13}C and ^{15}N backbone NMR assignment studies since the ^{13}C and $^{1}H_N$ T_2 relaxation times and, therefore, sensitivity are maximized. In addition, complete aliphatic deuteration removes the line-broadening effects of the differential ^{2}H isotopic shift in partially deuterated CH_nD_m moieties.

Replacement of ^{1}H with ^{2}H on non-Gly Cα nuclei can be calculated to increase the ^{13}C T_2 relaxation time approximately 12.5-fold and the $^{1}H_N$ T_2 relaxation time approximately 1.7-fold for rotational correlation times consistent with the size and packing of HCA II. These increases in ^{13}C and $^{1}H_N$ transverse relaxation times allows many otherwise marginal heteronuclear 3D and 4D pulse sequences, which are important in current assignment strategies, to be executed successfully on proteins with rotational correlation times larger than HCA II. However, resonance assignment of deuterated proteins necessitates the development of new sequences and new assignment strategies due to the absence of all aliphatic protons and the introduction of the ^{2}H isotopic shift on the ^{13}C chemical shifts.

D2-438 ^{1}H NMR STUDIES OF SMALL RAGWEED ALLERGEN 5 (*Amb. a.* V), Gregory L. Warren*, Christopher J. Turner#, Gregory A. Petsko% and Axel T. Brünger*, * Howard Hughes Medical Institute and Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, 06511, #Francis Bitter Nat'l. Magnet Lab., MIT, Cambridge, MA, 02139, %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 homonuclear NMR studies of this small protein to determine its three dimensional structure in an effort to understand what part structure may play in the allergic response. Structure calculations have been made using three methods: Distance Geometry/Simulated Annealing (DG/SA), quasi-relaxation matrix, and relaxation matrix refinement using an extended version of the program X-PLOR. The quality of the structures derived from and the computational efficiency of these methods were compared.

D2-439 THE MEASUREMENT OF PROTON AUTO-RELAXATION RATES BY NETWORK EDITING: MODIFIED QUENCHER SEQUENCES. William M. Westler, John L Markley, and Slobodan Macura.* Department of Biochemistry, University of Wisconsin, Madison WI 53706, and *Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN 55905

We have demonstrated an experiment to extract proton T_1 and T_2 relaxation times in the absence of cross relaxation. The pulse train Quencher cancels cross relaxation between protons by compensating NOE cross relaxation with an appropriate amount of ROE cross relaxation, which is of opposite sign from NOE in macromolecules. Under Quencher conditions, each spin relaxes with a single exponential rate since the relaxation is not dependent on the deviation from equilibrium of other spins. Both T_1 and T_2 can be obtained from the final steady state value and the relaxation rate under the conditions of the pulse train.

A disadvantage of the relaxation measurement described above is that to extract both T_1 and T_2 the steady state value must be measured. This requires measuring the magnetization after a long ($>T_1$) Quencher sequence. Long irradiation times causes heating of the sample. It is therefore of interest to modify the experimental protocol to eliminate the need for obtaining the steady state value.

We are investigating alternatives to the original Quencher sequence in order to reduce the need for long irradiation times. For example, the flip angle for the Quencher sequence can be reduced to 65.282° leading to a windowless sequence. This is possible since for this a flip angle the NOE buildup during the counter-rotating pulses exactly cancels the ROE buildup. Reintroducing delays into the windowless sequence generates an infinite number of possible sequences that will cancel cross relaxation.

D2-440 TOWARDS THE STRUCTURE OF THE MEMBRANE PROTEIN, RHODOPSIN, Philip L. Yeagle, James L. Alderfer and Arlene D. Albert, Department of Biochemistry, University at Buffalo, Buffalo, NY 14214; Department of Biophysics, Roswell Park Cancer Institute, Buffalo, NY 14263

The structure of rhodopsin is essential to an understanding of visual transduction. Furthermore, rhodopsin is a member of the family of G-protein receptors. Considerable progress is being made on the structure of the transmembrane portion of rhodopsin. However, the region that is responsible for interaction of the receptor with the G protein in signal transduction is extramembraneous. We have approached the question of the structure of the extramembraneous portions of rhodopsin by determining the structure of those regions as if they were protein domains. The data accumulated to date support that hypothesis. Initially the carboxyl terminal segment (the last 33 amino acids on the carboxyl terminus of rhodopsin) was studied. The circular dichroism spectrum indicates that this carboxyl-terminal peptide has some β-like structure. This peptide inhibited the cGMP cascade, indicating that the peptide had structure with functional relevance. Therefore, high field multidimensional NMR studies were undertaken to determine the 3-dimensional structure of this peptide. The resulting peptide is globular. Serines and threonines that can be phosphorylated are clustered. The structure opens a new discussion on the interaction between G-proteins and their receptors and may offer a new paradigm for the determination of the structures of membrane proteins. (NEI EY03328)

D2-441 **LOOP CONFORMATIONS BY A MONTE CARLO METHOD,**
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We introduce a novel methodology to help the determination of loop conformations. Typically, loops are the least defined parts of a protein, due usually to the lack of restraints, and/or structural and dynamical disorders. To gain insight into the possible multi-conformers, it is desirable to have a technique which samples the allowable conformations efficiently and effectively. Our method uses a Monte Carlo scheme in internal torsional space of the loop. The method searches through the conformations of the loop without affecting the rest of the molecule. We will present the basic methodology as well as an application using simulated data.

Late Abstracts

ASSOCIATION OF BIOMOLECULAR SYSTEMS *VIA* PFG-NMR DIFFUSION MEASUREMENTS Amanda S. Altieri, Denise P. Hinton† and R. Andrew Byrd, Macromolecular NMR Section, ABL-Basic Research Program, NCI-FCRDC, Frederick, MD 21702 and †Department of Chemistry, University of Virginia, Charlottesville, VA 22908

In one of the earliest applications of pulsed-field gradients (PFG) in NMR , Stejskal and Tanner showed that the translational self-diffusion (D_S) coefficient of molecules can be measured using a PFG spin echo experiment[1]. These measurements are indicative of molecular size and shape, but for large molecules such as protein systems, D_S measurements using spin echoes are difficult because of short protein transverse relaxation times, J modulation and the requirement for large gradients. A later development of Gibbs and Johnson[2] reduces T_2 and J effects and allows measurement of D_S for larger molecules. Clearly, D_S can be valuable in determining the aggregation state of biomolecular systems that are undergoing structural analysis by high-resolution, high-field, multidimensional NMR. Applications of this method include determining the monomeric or multimeric state of the system, finding optimal conditions for structure determination of a system that undergoes non-specific aggregation and for binding studies of protein-protein or protein-nucleic acid complexes. We have modified the LED[2] experiment to include water suppression[3,4]. Using standard PFG triple resonance high-resolution hardware (35 G/cm PFG capability at 600 MHz) and the modified LED experiment, we have measured D_S values for model monomer-dimer protein systems and shown that these experiments can be used to determine the dimeric state of an unknown protein system up to sizes of at least 38 kDa.

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EFFECT OF CYTOKINE DIMERIZATION ON RECEPTOR BINDING, **Donna M. Baldisseri*, Amanda S. Altieri*, Monica Tsang§, Jim Weatherbee§, Li Zhou§, Hua Chen§, Denise P. Hinton# ,Lewis Pannell¶ and R. Andrew Byrd*, *Macromolecular NMR Section, ABL-Basic Research Program, NCI-FCRDC, Frederick, MD 21702, §R&D Systems, Minneapolis, MN 55413, #Deptartment of Chemistry, University of Virginia, Charlottesville, VA 22908, ¶National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892**

Interleukin-10, IL-10, is a pleiotropic cytokine that is a powerful immunosuppressant of macrophage functions. It is a member of the class of α-helical cytokines which bind to a superfamily of hematopoeitic receptors (1,2). A point mutant, Y154C IL-10, has been isolated which exhibits low affinity for the IL-10 receptor. In the context of structural studies, a biophysical characterization has been performed for both wild-type and mutant IL-10 using mass spectrometry, gel filtration, a novel NMR diffusion experiment (see poster by A. Altieri) and circular dichroism. The mass spectrum of the mutant indicates that the protein is a Cys-glutathione adduct. Results of the gel filtration and the NMR diffusion experiment indicate that while the wild-type protein is a noncovalent homodimer in solution, the mutant is primarily monomeric. These results concur with ^1H-^{15}N HSQC data for both wild type and mutant protein. Since the CD spectrum of the mutant does not exhibit a large change in secondary structure relative to that of the wild-type, the decrease in receptor binding ability is most probably attributable to the dimer to monomer transition which results from the Tyr to Cys conversion at position 154.

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STRUCTURE AND DYNAMICS OF THE HUMAN MACROPHAGE CHEMOTACTIC PROTEIN (MCP-1) HOMODIMER USING HETERONUCLEAR TRIPLE RESONANCE NMR. Tracy M. Handel* and Peter J. Domaille%, Department of Molecular and Cell Biology, University of California, Berkeley*, CA 94720; The DuPont Merck Pharmaceutical Company%, P.O. Box 80328, Wilmington, Delaware 19880-0328.

Recent work has demonstrated the importance of proteins from the β-chemokine (chemoattractant cytokine) family because of their involvement in immune regulation and their demonstrated role in several chronic diseases (e.g., atherosclerosis, rheumatoid arthritis). Until recently, no 3-dimensional structures had been reported for the β-chemokines (C-C) although there are structures available for the α-family members (C-X-C) interleukin-8, bovine PF-4, and very recently MGSA. We report here the NMR assignments (^{1}H, ^{13}C, ^{15}N) and structure determination of the β-chemokine MCP-1 (or MCAF) on the basis of heteronuclear triple resonance (^{13}C, ^{15}N) experiments. The protein forms a homodimer under the conditions studied, so inter-monomer and intra-monomer NOE's are unambiguously distinguished on the basis of a mixed isotope experiment to complement data obtained with uniform isotope incorporation. We describe the quaternary and monomer structure, dynamics of the protein backbone, comparisons with the recently determined structure of MIP-1β, and the implications for the function of this important chemokine.

MOLECULAR DYNAMICS OF SEMISYNTHETIC CYTOCHROME C: IMPLICATIONS FOR ELECTRON TRANSFER, Sheri A. Hunt, R. Scott Prosser, Regitze R. Vold and David N. Hendrickson, Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093-0359

Of current interest in molecular biophysics is the connection between the electron transfer process and dynamical features of an electron transfer protein. Typically, electron transfer reactions have been studied by measuring the dependence of the rate of electron transfer on the distance between the donor and the acceptor. This study, however, focuses on dynamic features of two amino acid residues (met80, phe82) implicated in modulating the electron transfer potential, in both oxidation states of the iron center. Two semisynthetic horse heart cytochrome c's were prepared, each with a singly substituted deuterated amino acid, the methionine methyl, in one and the five ring positions of phenylalanine in the other. Both isotopically- and deuterium exchange-labeled cytochrome c were incorporated into a magnetically orientable bilayer mimetic system.[1] Deuterium multipulse NMR experiments (quadrupole echo, inversion recovery, and broadband Jeener-Broekaert) were employed on oriented cytochrome c to investigate local and global dynamical and structural features.

[1]C.R. Sanders II and G.C. Landis (1994) J. Am. Chem. Soc. *116*, 6470.

DNA Complexes with the Fluorescent Bis-intercalator TOTO.

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We have used one and two dimensional ^{1}H NMR spectroscopy to characterize the binding of a homodimeric thiazole orange dye, 1,1'-(4,4,8,8-tetramethyl-4,8-diaza-undecamethylene)-bis-4-(3-methyl-2,3-dihydro-(benzo-1,3-thiazole)-2-methylidene)-quinolinium tetraiodide (TOTO), to various double stranded DNA oligonucleotides. TOTO binds strongly to all the oligonucleotides used, but with CTAG:CTAG as the preferred binding site. We have determined the solution structure of the DNA oligonucleotide d(5'-CGCTAGCG-3')$_2$ complexed with TOTO. The determination of the structure was based on total relaxation matrix analysis of the NOESY cross peak intensities. The polypropyleneamine linker chain is located in the minor groove of dsDNA. The benzothiazole ring system is twisted relative to the quinoline in the free TOTO molecule. The site selectivity of TOTO for the CTAG:CTAG site is explained by its ability to adapt to the base pair propeller twist of dsDNA to optimize stacking.

EXTENDED RESIDENCE TIMES OF HYDRATION WATER MOLECULES IN THE ACTIVE SITE CLEFT OF HEN EGG-WHITE LYSOZYME, Edvards Liepinsh, Gottfried Otting, Department of Medical Biochemistry and Biophysics, Karolinska Institute, S-171 77 Stockholm, Sweden

The hydration of hen egg-white lysozyme was investigated by NMR spectroscopy, using intermolecular water-protein NOEs. Analysis of 3D NOESY-TOCSY, 3D ROESY-TOCSY, 2D radiation damping excited NOE-NOE and NOE-ROE experiments of lysozyme recorded at 36 °C and 20 °C enabled the identification of about 250 NOEs between lysozyme and the water resonance. Comparison with X-ray structures [1] enabled to identify about 24 hydration water molecules which are characterized by a negative sign of the water-protein NOE in NOESY and a positive sign in ROESY, i. e. by residence times longer than ~500 ps [2]. 4 of them are interior water molecules with 0% solvent accessibility and 6 of them are almost completely buried with solvent accessibilities below 5%. The rest of the molecules with long residence times were located on the enzyme surface, mostly in surface grooves and in the active site cleft (solvent accessibility 10 -30%). Almost all of the active site waters observed in the single crystal could be detected in the NMR experiments and seem to have long residence times. Complexation with 2-N-acetylglucosamine in the binding groove at site B did not change the residence times of the interior waters or of the active site waters located in the binding groove at site D. The presence of immobilized hydration water in the active site cleft is likely to be significant for the substrate recognition and catalysis.

1. Brookhaven Data Bank, entries 2lym, 3lym, 4lym, 1lzt, 2lzt, 1hel, 5lyt, 6lyt, 6lyz, 1hew.
2. G.Otting, E.Liepinsh, K.Wüthrich, Science, 254, 974-980 (1991).

NMR STRUCTURAL STUDIES OF TRANSCRIPTION ELONGATION FACTOR TFIIS, Paul E. Morin, Weon Tae Lee, Don Awrey*, Al Edwards*, and Cheryl Arrowsmith, Ontario Cancer Institute and Dept. of Biophysics, University of Toronto, Princess Margaret Hospital, Toronto, ON and *Dept. of Biochemisty, McMaster University, Hamilton, ON, Canada

In vivo transcription of DNA by RNA polymerase II is known to be arrested at particular DNA sequences called pause sites. Further advance of the polymerase requires interaction of a transcription elongation factor with the polymerase/RNA complex. In yeast, the protein TFIIS is known to act as such a transcription factor. Upon binding TFIIS, two bases are cleaved off the 3' end of the nascent RNA chain by an unknown mechanism. The polymerase then reads through the pause site extending the RNA chain and re- transcribing the two cleaved bases along the way. Similar functions are carried out in *E. coli* by the proteins GreA and GreB, however, up to nine bases are cleaved form the nascent chain in the presence of GreB instead of two. For TFIIS, the tertiary structure of the amino terminal zinc ribbon domain has already been determined using NMR methods (Quian X., Weiss, M.A. and co-workers, Nature 365:277, 1993). The present work centers upon the remaining COOH terminal domain which shows a high alpha helix content similar to GreA and GreB. Progress of the structure determination will be reported as well as comparisons among GreA/B and larger domains of TFIIS which retain functional activity. Supported by HFSP grant #LT688.

NEW NMR EXPERIMENTS WITH ENHANCED RESOLUTION AND SENSITIVITY FOR THE STUDY OF BIOMOLECULES, Gottfried Otting, Edvards Liepinsh and Anna Hammarström, Department of Medical Biochemistry and Biophysics, Karolinska Institute, S-171 77 Stockholm

Experimental schemes are devised which enable to reduce the dimensionality of water-protein NOE experiments by selectively exciting the water resonance. The schemes involve the selective excitation of the water signal either by radiation damping or by the use of a special probehead where the Q-factor can be switched low during free precession intervals of a DANTE-type excitation pulse. The experiments enable the recording of the water-protein NOEs in short one(two)-dimensional experiments, where two(three)-dimensional experiments were required earlier.

A universally applicable scheme for resolution enhancement is presented by the development of semi-selective acquisition modulated (SESAM) decoupling [1]. Spectral regions of finite width are irradiated during data acquisition which can be used to reduce the multiplet fine-structure of the amide protons to singlets and to decouple the αH-βH couplings during detection.

Decoupling of the couplings between α and β-protons during the evolution period of COSY experiments leads to αH-NH cross-peaks with minimum multiplet finestructure. As a result of the reduced cancellation of the different multiplet components in the antiphase cross-peaks, considerably enhanced signal-to-noise ratios are observed for the cross-peaks.

[1] A. Hammarström & G. Otting, J. Am. Chem. Soc. 116, 8847-8848 (1994).

SEQUENCE-SPECIFIC ASSIGNMENTS OF THE BACKBONE ^{1}H, ^{13}C AND ^{15}N RESONANCES AND SECONDARY STRUCTURE OF *FUSARIUM SOLANI PISI* CUTINASE BY HETERONUCLEAR MULTIDIMENSIONAL NMR.

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Essentially complete sequence-specific assignments were made for the backbone ^{1}H, ^{13}C and ^{15}N resonances of *Fusarium solani pisi* Cutinase, produced as a 214-residue heterologous protein in *E. coli*. Three-dimensional spectra of a doubly uniformly labeled sample in H_2O correlated the peptide ^{1}H-^{15}N with backbone nuclei of the residue itself and the preceding residue, namely with $^{13}C'$ $^{13}C_\alpha$ and $^{1}H_\alpha$. Concerted analysis of all these spectra using interactive graphics allowed to transverse the protein backbone in both directions, yielding continuous stretches of assigned residues terminating at Pro residues and at residues with undetectable or ambiguous resonances. Some ambiguities were solved by the checking whether all previously identified sets of $^{13}C'$, $^{13}C_\alpha$ and $^{1}H_\alpha$ signals do indeed come from the same residue in a H_2O version of the COCAH experiment. Stretches of residues were positioned in the sequence by identification of the amino acid types, using the combination of C_α, C_β and H_β chemical shifts. The latter were obtained from 3D ^{1}H-^{15}N-$^{13}C_\beta$ and ^{1}H-^{15}N-$^{1}H_\beta$ correlation experiments respectively. The backbone assignment was performed using a single sample containing 2mM Cutinase in H_2O.

Secondary structure elements were identified by the typical secondary chemical shifts and NOE's as observed in a ^{15}N-edited NOESY. This allows a first comparison of the structure in solution with the crystal structure from X-ray diffraction.

RATIONALLY DESIGNED N,N'-BIS[N-*p*-GUANIDINOBENZYL-N-METHYL)AMINOCARBONYL]1,3-DIAMINOBENZENE, "BIGBEN," BINDS TO THE MINOR GROOVE OF d(CGCGAATTCGCG)$_2$ AS DETERMINED BY TWO-DIMENSIONAL NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY, Charles R. Watts,[†] Sean M. Kerwin,[‡*] George L. Kenyon,[§] Irwin D. Kuntz,[§] and Deborah A. Kallick[†*], [†]Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN 55455, [‡]Division of Medicinal Chemistry, University of Texas at Austin, Austin, TX 78712, and [§]Department of Pharmaceutical Chemistry, University of California at San Francisco, San Francisco, CA 94728

We have used homonuclear NMR techniques to investigate the interactions between the *de novo* designed minor groove ligand, BIGBEN, and the receptor for which it was designed, the d(CGCGAATTCGCG)$_2$ dodecamer. Our NMR results show unequivocally the minor groove interaction of the non-exchangeable and exchangeable protons of BIGBEN with the dodecamer. These interactions were observed with the use of 1D NMR titrations to establish the fast-chemical exchange regime of the ligand with the DNA, and homonuclear NOESY experiments to establish the connectivities between the ligand and the DNA. This represents the first complete iteration of our design cycle applied to the minor groove of DNA. The cycle begins with the selection of a receptor for which there is high-resolution structural data. A structural database is then searched for putative ligands which may have shape complementarity to the desired binding site on the receptor. The ligand or a derivative is synthesized and its ability to bind to the desired receptor is tested [Kerwin et al., 1991]. The cycle culminates with the high resolution structural determination of the interactions in the complex, elucidated here for BIGBEN and the dodecamer d(CGCGAATTCGCG)$_2$.

RECENT ADVANCES IN COMPUTER AUTOMATED AND ASSISTED ROUTINES, Paul L. Weber, Tripos Inc., 1699 South Hanley Road, St. Louis MO 63144 USA

Determination of protein structures from NMR data can be enhanced in several ways through the development and use of computer software. Data reduction (e.g., peak picking), resonance assignments, NOE peak identification, constraint generation, structure calculations, structure refinement and error analysis are tasks that are repetitive but based on relatively simple rules. Such tasks can be handled well by computer software routines, resulting in a faster and more efficient structure determination. I will show our most recent advances in automating these tasks and in the related tasks of molecular structure analysis.