within alanine peptides linked to short proline arms.(1) Two peptides were employed in our studies,, OO-T*-PPPA*PPPA*-OO and OO-T*-PPPAAAA*-OO, where O is Ornithine, T* is Toac (2,2,6,6-Tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid), A* is 15N labelled alanine. Mesostate based Monte-Carlo sampling calculations (2) were carried out to interpret the relaxation data. The conclusion of the study is that over 90% occupation of extended conformations, i.e. PII and β mesostates, is required to reproduce the experimentally observed distance averaging. Compact structures including αR, αL and turns are clearly present but are not dominant in the conformational ensemble. Analysis of the conformation of other side chains will be presented.

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23-Plat

A Solution NMR and Crystallographic Study of the Role of the Quaternary Shift in the Allosteric Regulation of Phosphofructokinase from B. stearothermophilus

Rockann Mosser, Manchi Reddy, James C. Sacchettini, Tatyana Igumenova, Gregory D. Reinhart.
Texas A&M Univ, College Station, TX, USA.

The 136 kDa Bacillus stearothermophilus phosphofructokinase (BsPFK) is a homotetramer that is allosterically inhibited by phospho(enol)pyruvate (PEP), which binds along one dimer-dimer interface. Fru-6-P binds along the other dimer-dimer interface. The substrate bound and the inhibitor bound structures of wild-type BsPFK exhibit a 7° rotation about the substrate binding site interface, termed the quaternary shift. The binding of substrate and inhibitor to BsPFK have been studied using methyl TROSY NMR. By selectively labeling all 30 Ile residues in the BsPFK monomer with 13CH3 in an otherwise fully deuterated enzyme, only the Ile are detected on a 2D-1H-13C correlation spectrum. Several distinct Ile cross-peaks change position when PEP is added to wild-type apo BsPFK. To distinguish between changes associated with the quaternary shift and those associated with intra-subunit tertiary changes, the variant D12A BsPFK is currently being studied using kinetics, x-ray crystallography, and methyl TROSY NMR. When compared to wild-type, D12A BsPFK shows a 100-fold increase in the binding affinity for PEP, a 50-fold decrease in the binding affinity for F6P, and an allosteric coupling comparable to wild-type. Crystal structures of apo and PEP bound forms of D12A BsPFK both indicate a shifted structure similar to the inhibitor bound structure of wild-type. Since PEP still inhibits D12A BsPFK substantially despite the fact that it has already adopted the inhibited quaternary structure, the inhibition likely involves further tertiary changes to the enzyme structure. NMR of deuterated, 13CH3 labeled D12A is being performed in an effort to identify residues involved in these tertiary changes. Supported by grant GM33216 from NIH and grant A1543 from the Welch Foundation.

24-Plat

From Data or Dogma? The Myth of the Ideal Helix

Daniel J. Kuster, Sergio Urahata, Jay W. Ponder, Garland R. Marshall.
Washington University in St. Louis, Saint Louis, MO, USA.

In the course of building detailed surface representations for peptidomimetics, we were motivated to analyze the detailed surfaces of helices in proteins. Unexpectedly, we observed few ideal helical forms in high-resolution protein structures. Instead of a bimodal distribution matching the well-known alpha and 3(10) helical forms, we observed a smooth, single-peaked population, characterized by intermediate helices with shared hydrogen bonds. Bifurcated, or three-center hydrogen bonds, have been well-documented in small molecules and peptides, but they're rarely highlighted in the context of folded proteins. The data suggests shared hydrogen bonds are a major component of helices in proteins. Contrary to the Pauling-Corey-Branson models, we did not restrict our analysis to single hydrogen bonds–shared three-center hydrogen bonds were included. High resolution (<2.0 Å), electron density data is sharp enough that a helical structure should be unambiguous and accurately modeled. These helices are centered on an intermediate helical form. At poorer resolutions (2.0-5.0 Å), electron density is ambiguous; refinement fitting methods are employed to model ideal structures into the data. Here there is an enriched population of ideal structures. A structurally representative subset of proteins reveals the same trends as the whole PDB. The data support the observation that ideal helical parameters do not accurately describe the distribution of real helices in proteins. Hydrogen bonds are a polar (and polarizable) moiety and an accurate model must account for this. We present data comparing molecular dynamics simulations using popular monopole force fields (OPLS-AA and CHARMM) with a next generation force field (AMOEBA) implementing polarizability and multipole electrostatics. AMOEBA simulations are shown to quantitatively reproduce the experimentally observed trends in helical populations. These results emphasize the importance of using appropriate force field potential models when simulating hydrogen bonded structures in proteins.

25-Plat

Conformation Coupling Between The I-like Domain Alpha7 Helix And The Hybrid Domain Of Beta3 Integrins

Jizhong Lou, Wei Chen, Mei-Yin Chou, Cheng Zhu.
Georgia Institute of Technology, Atlanta, GA, USA.

Integrins are αβ heterodimers that mediate cell adhesion and transduce signals bidirectionally across the cell membrane. Integrins often exist in low affinity (or inactive) states for ligand binding on the cell curface, but change conformations to high affinity (or activated) states when induced by stimuli from inside or outside the cell. Crystallographic and electron microscopic studies have obtained evidence that the low affinity states correspond to bent conformations where the integrin headpiece interacts with the legs, whereas the high affinity states correspond to more extended conformations where integrins stand up, suggesting a switchblade-like unbending model for integrin activation. In this model, the key conformational changes on integrin headpiece include the swing out of the hybrid domain and the downward movement of I-like domain α_7 helix. These conformational changes are suggested by crystal structures of β_3 integrin headpieces. The unliganded $\alpha_v\beta_3$ headpiece displays a closed hybrid domain with the I-like α_7 helix in an upper position and the pseudo-liganded $\alpha_{IIb}\beta_3$ headpiece shows an open hybrid domain with the I-like α_7 helix in a lower position. Using molecular dynamics simulations we studied the stability of the two conformations of the I-like domain α_7 helix and the hybrid domain, the transition between the two conformations in each structure, and the coupling between the conformational changes in the two structures. We observed that the β-propeller domain of the α subunit helped stabilize the hybrid domain at the close conformation. The down/upward movements of the I-like α_7 helix correlated with the opening/closing of the hybrid domain. Our simulations identify key residues to the α_7 helix movement and suggest the existence of intermediate conformations of the hybrid domain between the open and the close conformations.

26-Plat

Spin-label EPR of alpha-Synuclein on Vesicles Reveals Antiparallel Arrangement and Differences in the Membrane Binding Affinity of the two Helices

Martina Huber[1], Malte Drescher[1], Frans Godschalk[1], Sergey Milikisyants[1], Gertjan Veldhuis[2], Bart van Rooijen[2], Vinod Subramaniam[2].
[1]Leiden University, Leiden, Netherlands, [2]TwenteUniversity, Enschede, Netherlands.

The Parkinson's disease-related protein α-Synuclein (αS) is a 140 residue protein that is natively unfolded in solution. Its membrane-binding properties are implied in its physiological or pathologic activity. αS was investigated by spin-label EPR. By Electron-Electron Double Resonance (DEER) the distance between the spinlabels in four double mutants was determined in the vesicle-bound and free form of αS, revealing antiparallel arrangement of the helices. Thus, even in the vesicle-bound form αS has the horseshoe conformation, revealing that this conformation is intrinsic to the protein, rather than induced by the small size of micelles investigated previously. Mobility analysis of five single spin-labeled mutants showed that the membrane affinity of helix 2, comprising residues 45 - 90, decreases with decreasing negative charge of the membrane surface, suggesting differential binding of αS to membranes. The findings reveal molecular details of the membrane-bound conformation of αS not previously obtained.

27-Plat

A Coarsened Network Model Reveals Allosteric Machinery

Ali Rana Atilgan, Canan Atilgan.
Sabanci University, Istanbul, Turkey.

The past decade has witnessed the development and success of coarse-grained network models of proteins for predicting many equilibrium properties related to collective modes of motion(1,2). We first use a systematic approach on a large set of globular proteins to find a basis for why the network models work well to define certain properties of the system. The analysis is based on the radial distribution function and the spectral dimensions(3) of proteins as well as newly defined quantities, the angular distribution function, and the contact order per mean path length of individual residues. We prove that the network construction is free of the cut-off distance problem if one is interested in the collective motions of the residues.

We next undertake a linear response analysis. If the collection of forces applied on a specific residue is independent and large in number, they will appear in a spherically symmetric set of directions. With the aid of responses deviating from such a spherically symmetric distribution, we rigorously determine the residues involved in the remote control of the ligand. These are usually charged surface loop residues, providing binding locations for ions which are known to influence ligand release kinetics(4). We prove that by perturbing any one of these residues, the tip of the cap that opens the exit of the ligand is made to operate coherently, irrespective of the direction of the perturbation. We thereby

provide a mechanism on how proteins remotely modulate bound ligands to create preferred kinetic pathways(5,6).
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Platform B: Biotechnology & Bioengineering

28-Plat
Sequencing Paired Reads using True Single Molecule Sequencing (tSMS)™ Technology
Jeff G. Reifenberger, Doron Lipson, Jennifer Colonell, Tal Raz,
Bill Pierceall, John Thompson, Patrice Milos.
Helicos BioSciences Corp., Cambridge, MA, USA.
Single molecule DNA sequencing provides novel methods for interrogating DNA molecules. For example, genomic rearrangements such as insertions, deletions, and inversions that are often associated with cancers or variations within the transcriptome of specific genes can be difficult to detect with conventional sequencing strategies. Paired reads sequencing, where a spacer is inserted between two single molecule sequencing reads, offers a more viable method for detecting genomic rearrangements. We have developed a paired reads strategy using True Single Molecule Sequencing (tSMS)™ in which a large number of individual templates of DNA were analyzed using a proprietary form of sequencing-by-synthesis. To create paired reads DNA strands are attached to a surface and sequenced-by-synthesis for a known number of cycles. A spacer was then added to the DNA strands in a controlled manner and then sequencing by synthesis continued for the same number of cycles. Data on test oligonucleotides of known length and sequence demonstrate the viability of the technique and our ability to control the length of the spacer between the two reads on an individual strand. We have now extended our Paired Reads technique to biological samples, initially with a 12kb PCR product encompassing the CETP gene to demonstrate our ability to sequence the whole gene product and identify mutations which have been inserted into the CETP reference. Finally we have utilized this novel method to examine a human placental transcriptome cDNA library to demonstrate the ability to span exon boundaries.

29-Plat
Self-assembly via Active Transport By Biomolecular Motors
Parag Katira, Isaac Luria, Shruti Seshadri, Henry Hess.
University of Florida, Gainesville, FL, USA.
Modified kinesin gliding motility assays display striking self-assembly phenomena,[1] in particular the formation of non-equilibrium structures. Biotinylated microtubules partially coated with streptavidin form ‘‘wires’’ and ‘‘spools’’ while gliding on kinesins adhered to the surface.[2] The spool formation process was investigated in detail, and we found that the assembled spools exhibit a narrow distribution of spool diameters, and that the average diameter is an order of magnitude smaller than what would be obtained from a thermally driven assembly process. We also observe that pinning of the microtubule leading tip on the surface initiates the spool formation in 80% of the cases. By modeling the mechanism of microtubule tip buckling and the resultant microtubule spool diameter as a function of the microtubule persistence length and the number of motors attached to the microtubule, we can predict the average spool diameter and the observed spool diameter distribution. The model suggests a strong dependence of spool diameters and size distribution on the surface kinesin density. The goal of our research is to obtain a better understanding of the dynamics of this multi-agent process and its implications for self-assembly in general.
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30-Plat
Single Cell Detection and Analysis with Asynchronous Rotation of Driven Magnetic Microspheres
Brandon H. McNaughton, Paivo Kinnunen, Rodney R. Agayan,
Ron G. Smith, Alan J. Hunt, Raoul Kopelman, Roy Clarke.
University of Michigan, Ann Arbor, MI, USA.
The nonlinear rotational response of a magnetic micropshere, suspended in a viscous fluid, occurs when a driving magnetic field, used to rotate the magnetic particle, exceeds a critical frequency. Above this critical frequency, the particle is asynchronous with the external field. Shifts in this nonlinear rotational frequency of the magnetic microsphere offer a dynamic approach for the detection (see Appl. Phys. Lett. 2007 **91**, 224105) and analysis of bacterial cells (i.e. growth and response to chemical agents).

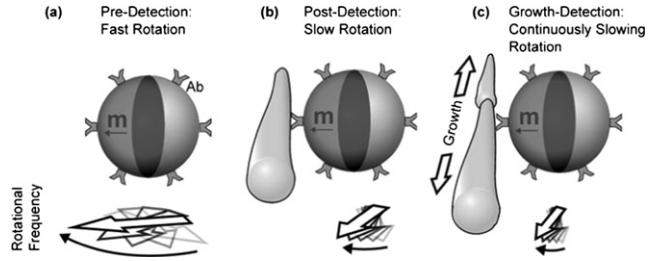

Figure: Schematic of the asynchronous (nonlinear) rotation rates of an antibody-coated magnetic microsphere with (a) no bacteria, (b) a single bacterium and (c) growth of the attached bacterium.

31-Plat
Remote Steering of C. Elegans Using Nanoparticle Heating
Heng Huang, Savas Delikanli, Hao Zeng, Arnd Pralle.
SUNY Buffalo, Amherst, NY, USA.
Remote and parallel stimulation of a subpopulation of neurons will aid the understanding of signal processing in the complex neuronal networks tremendously. We present a method capable of stimulating neurons deep inside the body. It relies on manganese iron oxide nanoparticles targeted to the neuron’s plasma membrane to convert energy from an alternating magnetic field to local heat which opens the temperature sensitive Calcium channel TRPV1. Expressing this channel in neurons will allow us to remotely stimulate and control the neurons. We apply the method to trigger the ASH and ADL neurons in C. elegans which control the chemical avoidance reaction.
In the course of this study, we have characterized nanoscale heating and heat propagation inside cells quantifying heat conduction over nanometer distances, important knowledge to optimize the efficacy of hypothermia treatment for cancer.

32-Plat
Probing Conformational Changes In Rhodopsin With Site-specific Azido Labels
Shixin Ye[1], Thomas Huber[1], Reiner Vogel[2], Thomas P. Sakmar[1].
[1]Rockefeller University, New York, NY, USA, [2]Albert-Ludwigs-University Freiburg, Freiburg, Germany.
Transmembrane signaling via heptahelical G protein-coupled receptors (GPCRs) is essential for a cell’s communication with its environment and represents a major target for drug development. Although recent advances have provided high-resolution crystal structures of several GPCRs, understanding the conformational dynamics of receptor activation in bilayers remains paramount. Fourier-transform infrared (FTIR) difference spectroscopy has proven to be a powerful biophysical technique for structure/function relationships in the prototypical GPCR, rhodopsin. Here we report a new conceptual advance in FTIR difference spectroscopic analysis of heterologously expressed eukaryotic proteins. We demonstrate the site-directed incorporation of an IR-active unnatural amino acid, *p*-azido-L-phenylalanine (azidoF), into rhodopsin using amber condon suppression technology. The intense antisymmetric stretch vibration of the azido group absorbs at around 2100 cm^{-1} in a clear spectral window devoid of other protein bands and is exceptionally sensitive to the polarity of its surroundings. Using FTIR difference spectroscopy on azidoF rhodopsin mutants, we report the changes in the electrostatic environments of selected side chains on both the cytoplasmic and extracellular receptor surfaces during the conformational transition associated with receptor activation.

33-Plat
Towards Mapping Domain Boundaries of Proteins
Shambaditya Saha[1], Thomas D. Pollard[1,2].
[1]Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA, [2]Dept. of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, USA.
GFP fluorescence from bacterial colonies expressing a polypeptide upstream of GFP depends on the solubility of the polypeptide. Inspired by this idea, we developed a method to test for the folding of any polypeptide longer than about 66 amino acids. This method bypasses the need for purification of the polypeptide to test for folding. We employed this technique to identify the independently folded domains of Mid1, a fission yeast protein of 920