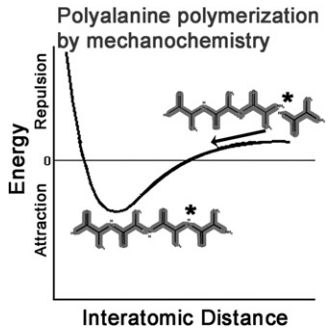
and is proposed to be a remnant of the earliest cell divisions (Leaver, *Nature*09).

Fluid percolated into and out of spaces between mica sheets, providing cycles of wetting and drying that favor the polymerization of amino acids.

The discovery of Intrinsically Disordered Proteins (IDP) turns the protein structure-function dogma upside down, because individual IDPs can assume many transient structures and perform many functions (Dunker, JMolecGraphicsModelling2001).



Prebiotic peptides, crowded at the edges of mica sheets, could have had simple functions.

Platform BH: Protein Structure

3948-Plat

Structure of the Yellow Fever Virus Membrane Fusion Envelope E

Eric Crampon[1,2], Stéphane Duquerroy[1,3], Giovanna Barba-Spaeth[1], Félix A. Rey[1].

[1]Institut Pasteur, Unité de Virologie structurale and CNRS URA 3015, Paris, France, [2]Université Paris 7 Denis Diderot, Paris, France, [3]Université Paris-Sud, Faculté d'Orsay, Orsay Cedex, France.

Enveloped viruses enter into cells by a membrane fusion mechanism which leads to the release of their viral genome into the cytosol of the host . Yellow fever virus (YFV), of a flavivirus of approximately 50 nm in diameter, displays on its surface 180 envelope glycoproteins E arranged in a herringbone pattern , which are responsible for both receptor recognition and membrane fusion. It causes 200,000 illnesses and 30,000 deaths every year. A vaccine is avalaible, which differs from the wild-type by 12 mutations in E out of 400 residues. We were interested in understanding changes induces by these mutations

We produced the recombinant E protein in Drosophila Schneider 2 (S2). We determined the crystal structure of the ectodomain of the YFVE for both the wild-type Asibi and vaccinial 17D strains at 2.75Å and 3.5Å respectively. The overall tertiary structure of the YFV-E is typical of class II membrane fusion proteins observed for other flavivirus E and alphavirus E1 proteins. YFV-E has no N-glycosylation site as other Flavivirus E proteins, but interestingly it presents unexpected O-mannosylation. Furthermore, improvement of crystal resolution has been obtained after urea denaturation and renaturation, which is an unusual approach for improving crystal resolution.

The structure of Asibi WT YFV-E and its comparison to vaccinial 17D strain as well as to other class II fusion proteins will be presented to stress on its salient characteristics.

3949-Plat

Crystal Structures of *P. Aeruginosa* Reveal a Dynamic type IV Pilus Motor Protein

Ana M. Misic, Kenneth A. Satyshur, Katrina T. Forest.

UW-Madison, Madison, WI, USA.

Type IV pili are extracellular bacterial virulence factors that are retracted into cells by the powerful molecular motor, PilT. *Pseudomonas aeruginosa* PilT crystal structures, both AMP-PCP bound and unliganded, have been refined at 2.6 and 3.1 Å resolution, respectively. The structures reveal an interlocking asymmetric hexamer mortared with extensive ionic interactions. The three subunits in each asymmetric unit exhibit differing conformations, implying domain motions during the ATP-coupled mechanism of pilus retraction and disassembly into membrane-localized pilin monomers. The force-generating swing of PilT upon nucleotide binding has a magnitude of ~20° and a direction diagonal to the polar axis. Future work will focus on identifying protein interaction partners of PilT to more fully understand the pilus retraction process.

3950-Plat

Structural, Biochemical, and Functional Studies on the Regulation of the *S. Cerevisiae* AMPK Homolog SNF1

Gabriele A. Amodeo[1,2].

[1]Weill Cornell Medical College, New York, NY, USA, [2]Columbia University, New York, NY, USA.

The 5'AMP-activated protein kinase (AMPK) is a master regulator of energy homeostasis. AMPK is activated by a high AMP:ATP ratio, and functions as a metabolic thermostat. By sensing when energy is low, AMPK upregulates energy-producing pathways (e.g., glycolysis, glucose transport, fatty acid oxidation, food intake) while downregulating energy-consuming pathways (e.g., gluconeogenesis, fatty acid synthesis). Due to its central role in controlling these processes, AMPK represents a key drug target for both diabetes and obesity. In *S. cerevisiae*, the AMPK homolog Sucrose Non-Fermenting 1 (SNF1) controls many of the same pathways as AMPK and, like AMPK, is a heterotrimeric protein comprised of a catalytic alpha subunit and regulatory beta and gamma subunits. We present here structures of the heterotrimer core of SNF1 and the catalytic protein kinase domain/auto-inhibitory domain (KD-AID) of the alpha subunit. Our studies elucidate important differences between SNF1 and higher eukaryotic AMPKs, especially with regards to AMP activation. In addition, we provide the first structural insight into the Regulatory Sequence (RS) of the alpha subunit, a region that interacts with the gamma subunit of SNF1. GST pull-down experiments demonstrate strong, direct interactions between the RS and the heterotrimer core. These interactions can be greatly reduced *in vitro* by the introduction of single-site mutations, although no effect is observed *in vivo*. We also probed the role of an AID N-terminal to the RS through crystallographic studies of a KD-AID protein. Interestingly, the AID in this structure is disordered, but the KD reveals a novel DFG-out conformation blocking ATP binding to the active site. Together, these data indicate that the RS is constitutively bound to the SNF1 gamma subunit, and the AID may be required to regulate SNF1 activity.

3951-Plat

Investigation of Protonation Effects on ATP Binding in ABC Transporters

Jussi Aittoniemi, Frances M. Ashcroft, Mark S.P. Sansom.

University of Oxford, Oxford, United Kingdom.

ATP binding cassette (ABC) transporters consist of two characteristic nucleotide binding domains (NBD) and two transmembrane-spanning domains (TMD). Binding and hydrolysis of ATP at the NBDs controls substrate transport or, in rare cases, other physiological functions.

Previous studies have identified residue motifs of functional importance, but our understanding of the hydrolysis process in ABC NBDs remains incomplete. An acidic residue of the Walker-B motif has been suggested to act as a general base that abstracts a proton from the hydrolytic water. Other work has suggested a greater role of a histidine (in the switch motif) in activating the hydrolytic water. Also the role of the Mg^{2+} ion in orienting hydrolytic residues and waters is poorly understood.

One limitation in interpreting existing ABC NBD structures for their hydrolytic function is the assignment of protonation states to the relevant residues and the exact orientation of water molecules in the NBDs. The highly charged nature of the NBS renders protonation assignment particularly challenging.

In this study, we vary protonation states at the NBDs of the multidrug ABC exporter Sav1866 and simulate the ATP-bound NBD dimers by molecular dynamics. We consider combinations of protonation of the Walker-B glutamate, the switch histidine and the ATP itself with and without Mg^{2+}. The resulting 24 systems are simulated to at least 50 ns duration.

We show that the Mg^{2+} and residue protonation affect protein dynamics. Crucially. we show that the local geometries of ATP-binding residues in many available ABC NBD crystal structures can potentially be rationalized by different protonation states. Conformational changes of the Glu and His upon protonation support the idea of the Glu acting as a general base. Furthermore, we discuss the coordination and dynamics of putative hydrolytic waters.

3952-Plat

Nano-Mechanical and -Electromechanical Heterogeneity in Single Collagen Fibrils

Majid Minary-Jolandan, Min-Feng Yu.

University of Illinois at Urbana-Champaign, Urbana, IL, USA.

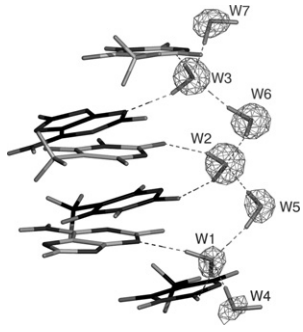
Type I collagen, as the most abundant protein in mammals, is the main organic component of bone, tendon, dentine, and cornea. Functioning in such diverse tissues shows the multifunctional capability of collagen fibrils. The gap and overlap regions in axial direction of a fibril with a characteristic period of ~67 nm is believed to be an important factor in microstructure of the fibrils enabling its multifunctionality. For example, in bone mineral nano-crystals are deposited specifically in the gap region. In this study, we focus on studying mechanical and electromechanical properties at different scale levels, ranging from subfibrillar microstructure of single collagen fibrils ~100 nm in diameter up to bone samples.

In terms of mechanical (elastic and viscoelastic) properties, implementing near-surface static and dynamic nanoindentation technique with AFM, we show that the gap and overlap regions in single collagen fibril have significantly different elastic and energy dissipation properties, correlating the significantly different molecular structures in these two regions. We further show that such subfibrillar heterogeneity holds in collagen fibrils inside bone and might be related to the excellent energy dissipation performance of bone.

It is known that bone and tendon are piezoelectric, and it is believed that the piezoelectric charges produced under mechanical deformation in these tissues have a potential role in mechanoelectric transduction leading to their growth and remodeling. With high resolution PFM we probe piezoelectric properties in bone and show that single collagen fibrils are responsible for piezoelectric behavior of bone and behave predominantly as shear piezoelectric materials. Furthermore, we show that there is an intrinsic electromechanical heterogeneity in axial direction of individual fibrils that holds even for the collagen fibrils embedded in bone matrix. Such heterogeneity may have implications in regulating the ionic environment in bone responsible for bone remodeling.

3953-Plat
SAXS: Structure Verification of an S-Layer Protein using a Fractal Mean Force Potential
Christine Horejs[1], Dietmar Pum[1], Uwe B. Sleytr[1], Rupert Tscheliessnig[2].
[1]Center for Nanobiotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Austria, [2]Institute for Applied Microbiology, University of Natural Resources and Applied Life Sciences, Vienna, Austria.
Using a combination of SAXS and molecular dynamics simulations we get an integrated picture of the structure of S-layer proteins, for which no crystallized structure is currently available. S-layers are the most commonly observed cell surface structure of prokaryotic organisms and they are made up of identical protein subunits. One of the most striking properties of S-layers is that they are able to self-assemble into crystalline lattices in suspension and on various solid substrates. The resulting ordered molecular layer provides a matrix for the binding of various biomolecules and nanoparticles. Due to the complex biochemical properties of these proteins, classical techniques such as NMR or X-ray crystallography have not been able to provide an atomistic structural model for S-layer proteins. Motivated by the results obtained through the use of a fractal concept for the analysis of SAXS data in cluster physics, here we employ such fractal concept for the investigation of the structure of S-layer proteins. We fit the SAXS intensity as a function of the scattering angle using both a fractal form factor and a fractal structure factor. We compute the form factor by a Fourier transform of an average fractal delta function of characteristic units, thereby allowing us to investigate the presence of local, rather than global, electron densities in the structure of S-layer protein monomers. The structure factor is calculated by a Fourier transform of a fractal potential of mean force. Using this fitting function, we calculate the shape of the monomers, which is in good agreement with the shape obtained using molecular dynamics simulations. Our approach reveals itself as a novel means to get a detailed insight into the structure of proteins that adopt a fractal self-assembly and that lack of crystallized structure.

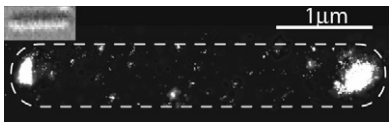
3954-Plat
Is Theory Leading Neutron Diffraction in Macromolecular Solvent Networks?
Timothy Fenn, Michael Schnieders, Vijay Pande, Axel Brunger.
Stanford University, Stanford, CA, USA.
Standard crystallographic practice models electron scattering as a spherically symmetric phenomenon about atoms in the absence of any external effects. We have recently developed a polarizable atomic multipole refinement method for macromolecular crystallography that presents a significant improvement to the resultant information contained in an atomic model. We apply this method to high resolution lysozyme and trypsin data sets and validate its utility for precisely describing biomolecular electron density as indicated by a decrease in 5-6% in the R and Rfree values relative to the deposited values. The resultant models also illustrate the ability of force field electrostatics to orient water networks and catalytically relevant hydrogens that can be used to make predictions regarding active site function and activity. Finally, a DNA model generates the zig-zag spine pattern of hydrogen bonding in the minor groove without manual intervention. Comparison of the solvent networks with macromolecular neutron models suggest the hydrogen bonding patterns and distances generated by our protocol are more consistent with condensed phase measures and more likely to yield energetically favorable hydrogen bonds. The refinement technique proposed should be useful in applications to enzymology, drug design, and protein folding.



3955-Plat
Identification of Functional Surfaces of Proteins from Sequences
Jieling Zhao, Sema Kachalo, Zheng Ouyang, Jie Liang.
Department of Bioengineering, University of Illinois at Chicago, Chicago, IL, USA.
The identification of protein functional surfaces is important for understanding enzyme mechanism, protein function prediction, compound-protein docking, and drug design. As the speed of rapid accumulation of protein sequence information far exceeds that of structures, it is important to construct accurate models of protein functional surfaces and identify key residues on these surfaces. A promising approach is to build comparative models from sequences using known structural templates. We assess how well this approach works by building three-dimensional comparative models of proteins using standard tools and determine how well functional surfaces can be accurately reproduced. We use the pocket algorithm based on alpha shapes computed for the modeled protein structures and characterize potential binding surfaces on these structures. Based on a large scale study, we give general criteria on when such comparative models can give accurate information on functional surfaces. We also provide assessment on the applicability of this approach to the universe of currently known protein sequences. We further point out methods for improved models of protein functional surfaces.

Platform BI: Imaging & Optical Microscopy II

3956-Plat
Self-Organization of the Escherichia Coli Chemotaxis Network Imaged with Super-Resolution Light Microscopy
Ann McEvoy[1], Derek Greenfield[1], Hari Shroff[2], Gavin E. Crooks[3], Ned S. Wingreen[4], Eric Betzig[2], Jan Liphardt[1].
[1]UC Berkeley, Berkeley, CA, USA, [2]Janelia Farm HHMI research campus, Asburn, VA, USA, [3]Lawrence Berkeley National Labs, Berkeley, CA, USA, [4]Princeton University, Princeton, NJ, USA.
The Escherichia coli chemotaxis network is a model system for signal transduction and processing. Chemotaxis receptors assemble into large clusters containing tens of thousands of proteins which have been observed at cell poles and future division sites. Despite extensive study, it remains unclear how chemotaxis clusters form, what controls cluster size and density, and how the cellular location of clusters is robustly maintained in growing and dividing cells. Here, we use a super-resolution optical technique called photoactivated localization microscopy (PALM) to map the cellular locations of three proteins central to bacterial chemotaxis (the Tar receptor, CheY, and CheW) with a precision of 15 nm. We find that cluster sizes are approximately exponentially distributed, with no characteristic cluster size. One-third of receptors are part of smaller lateral clusters that have not been previously observed. Analysis of the relative cellular locations of 1.1 million individual proteins (from 326 cells) suggests that clusters form via stochastic self-assembly. The super-resolution PALM maps of E. coli receptors support a growing collection of evidence that stochastic self-assembly can create and maintain periodic structures in biological membranes, without direct cytoskeletal involvement or active transport.



3957-Plat
K-Space Image Correlation Spectroscopy of Quantum dot Labeled T Cell Receptors Characterizes their Nanoscale Clustering in Living Cells
Paul W. Wiseman[1], David L. Kolin[1], Sarah Boyle[2], Joan G. Bieler[2], Jonathan P. Schneck[2], Michael Edidin[2].
[1]McGill University, Montreal, QC, Canada, [2]Johns Hopkins University, Baltimore, MD, USA.
Changes in distribution of membrane receptor organization are used by cells to modulate the dynamic range of their responses to environmental cues. Consequently, it is important to develop experimental methods that can accurately measure receptor transport and aggregation. We used quantum dot (QD) labeling of T cell receptors (TCR) and a recently developed technique, k-space image correlation spectroscopy (kICS) to characterize TCR state as a function of cell differentiation. We developed kICS to measure transport coefficients of fluorescently labeled membrane proteins while taking into account nanoparticle emission blinking. We use kICS to measure T cell receptor (TCR) aggregation in live cells by characterizing quantum dot (QD) blinking and distribution on the cell surface. 2C TCR transgenic cells in culture were observed from the naïve state to 12 days after activation by antigen. Cells were labeled