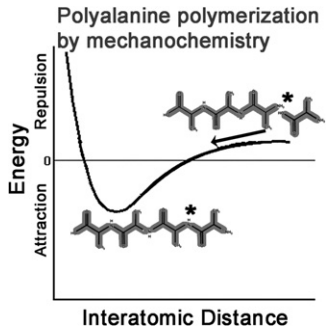
and is proposed to be a remnant of the earliest cell divisions (Leaver, *Nature*09).

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.