and orientation may affect the motion of the AFM cantilever. We imaged unfixed cilia using AFM, using a 'peel-off' method whereby a poly-L-lysine-coated coverslip, placed on top of a layer of cells in a Petri dish, is removed in a single movement, and then imaged *in situ*. The nanoscale surface structure of the immobilised cilia can then be imaged with the AFM. From an analysis of AFM data, we have estimated cilium flexibility, and have probed cilium elasticity using AFM in a nanomechanical mode. By correlating AFM images with single-point AFM force curves, we observe a clear difference in stiffness between the cilium and the cell surface. Using a combined AFM/confocal microscope we have correlated the structures imaged by AFM, to fluorescent markers for tubulin and actin. The staining observed by confocal microscopy confirms that the structures protruding from the cells in the AFM images are the primary cilia. In a further development, we will report results from a combination of AFM imaging and patch-clamp electrophysiology to probe how cilium bending is coupled to ion transport.

1782-Plat Atomic Force Microscopy Studies of Leukocyte Transmigration

Ewa P. Wojcikiewicz[1], Hashem Azad[1], Julia Minkiewicz[1], Robert R. Koenen[2], Vincent T. Moy[1], Christian Weber[2]

[1] *UM Miller School of Medicine, Miami, FL, USA,*
[2] *Rheinisch-Westfalische Technische Hochschule, Achen, Germany.*

Leukocyte trafficking involves the homing of leukocytes to sites of injury during inflammation. It culminates with their migration across the endothelial cell junction, the integrity of which is maintained by adhesion molecules including the junctional adhesion molecule-A (JAM-A). JAM-A is able to form interactions with itself as well as with the integrin LFA-1 (leukocyte function-associated antigen-1). The interaction of LFA-1 expressed on the leukocyte surface with JAM-A as well as the disruption of the homophilic interaction of the JAM-A receptors *in trans* are a critical part of this process. In this study, we used atomic force microscopy (AFM) to characterize the dynamic strength of these interactions in order to determine which (JAM-A/JAM-A v. LFA-1/JAM-A) prevails under pulling forces. In addition we investigated the possible impact that the LFA-1/JAM-A interaction has on the homophilic JAM-A interaction *in trans.* Measurements of unitary LFA-1/JAM-A or JAM-A/JAM-A unbinding forces were obtained between LFA-1 expressing Jurkat cells or JAM-A on the cantilever tip and the JAM-A substrate. All measurements covered three orders of magnitude in force loading rate (50–60000 pN/s). Two loading rate regimes and higher unbinding forces were observed for the LFA-1/JAM-A interaction as compared to one regime and lower unbinding forces for the JAM-A/JAM-A interaction. Our results indicate that the JAM-A/LFA-1 interaction is better able to resist pulling forces than the JAM-A/JAM-A interaction, and imply that LFA-1 may modulate the mechanical strength of the JAM-A homophilic interaction.

1783-Plat Atomic Force Microscopy Exploration of the Extracellular Matrix

Ruchirej Yongsunthon, David E. Baker, Odessa N. Petzold, Wendy A. Baker, Ronald A. Faris, Jin Liu, Theresa Chang, Robert R. Hancock, Wageesha Senaratne, Randall E. Youngman

Corning Incorporated, Corning, NY, USA.

A recent report describes the use of the extracellular matrix (ECM) secreted by HEPG2 cells as a culture surface to promote the *in vitro* establishment of characteristic membrane polarity of hepatocytes (Herrema et. al, Molecular biology of the Cell, vol. *17* (2006)). The re-establishment of the cell membrane polarity is important for global cell function and is thought to be driven by a compromise between cell-cell and cell-surface interactions. It is believed that the cell-surface interactions are influenced by surface properties such as surface energy, charge, topography, modulus and the presentation of ligands at the surface. In the case of the HEPG2 cell-derived ECM surface, the role of these surface properties in achieving the characteristic hepatocyte cell membrane polarity is unknown. We use Atomic Force Microscopy (AFM) and derivative techniques to explore the extracellular matrix on dimensional scales relevant to individual cells. Standard AFM topography imaging yields ECM surface roughness. Nanoindentation measurements provide insight on mechanical properties across the ECM surface. Single molecule force spectroscopy allows examination of the conformational and binding properties of proteins and peptide ligands exposed on the ECM surface. Functionalization of the AFM tip, e.g. via hydrophobic silane chemistry, enables chemical force microscopy probing of specifically targeted domains on the ECM surface. Once a characterization profile is established for the extracellular matrix, we may explore the influence of the underlying substrate on the cell-derived ECM surface. For example, the ECM produced by cells and adsorbed to a hydrophobically-treated glass will be compared to that produced by cells on hydrophilic-treated glass. Correlation of substrate properties to ECM properties to subsequent cell growth will further fundamental understanding of cell-surface interactions and its influence on cell morphology and function.

Symposium 15: Awards Symposium and IUPAB Arne Engstrom Lecture

1784-Symp Breaking the Nanometer Barrier: Biophysics, One Molecule at a Time

Steven Block

Stanford Univ, Stanford, CA, USA.

Advances have led to a new field of exploration, dubbed *single molecule biophysics*. Prominent among the enabling technologies is the optical trap, also called 'optical tweezers.' When combined with *in vitro* assays for function, optical traps can perform measurements

on individual biomolecules with unprecedented precision. Ultrasensitive systems for measuring force and displacement permit the nanomechanical properties of proteins and nucleic acids to be explored, revealing rich behaviors that were heretofore obscured by more traditional, ensemble-based approaches. This talk will focus on our current work with representative single-molecule systems, including translocation by mechanoenzymes such as kinesin, transcription by RNA polymerase, and structural (folding/unfolding) transitions in nucleic acids. We recently developed high-resolution optical instrumentation that has broken the 'nanometer barrier,' and is thereby able to detect displacements down to the atomic level, all in an aqueous buffer at ambient temperature, i.e., under physiological conditions. Consequently, we can now monitor the real-time motions of single RNA polymerase molecules as these step from base to base along the DNA template. On the practical side, basepair resolution makes it possible to sequence single molecules of DNA in an entirely new way, based on enzyme movements, and points to new directions in biological nanoscience. The improved stability against instrumental drift in nanoscale measurements afforded by our current generation of apparatus has allowed us to reconstruct the energy landscapes for folding transitions in nucleic acid hairpins. We are now turning our attention to the problem of co-transcriptional messenger folding, aptamers, and riboswitches formed in nascent RNAs, and to the DNA- and RNA-based sequence elements that regulate transcriptional elongation and termination. We're also beginning to understand key aspects of the molecular basis for 'gating' (head-head communication) exemplified by two-headed motors that walk hand-overhand, such as kinesin and myosin.

1785-Symp Vital structures essential for life

Ben de Kruijff

Utrecht University, Utrecht, The Netherlands.

Membranes are vital structures essential for life. Therefore, it is not surprising that they form a target for many cell-killing agents of which the antibiotics form a special and important class in the fight against infectious diseases. Antibiotics and antimicrobials in general specifically target microorganisms and interfere with cellular functions specific for these microorganisms.

In recent years it became clear that specific membrane lipids can play crucial roles in the killing of bacteria by members of the lantibiotic family. antibiotics are antibiotics with a structure containing lanthionine rings. They are produced by certain bacterial strains and are commonly used as food preservatives.

Several members of that antibiotic family were shown to bind with high affinity to Lipid II the membrane lipid that shuttles the peptidoglycan building blocks across the bacterial membrane. Depending of the type of lantibiotic they kill by targeted pore formation or by removing lipid II out of its functional location.

A parallel can be drawn between the mode action of the lantibiotics and the polyene antibiotics that kill fungi via different mechanisms using the membrane lipid ergosterol as receptor.

References

• Breukink, E., Wiedemann, I., Van Kraaij, C., Kuipers, O.P., Sahl, H.-G. & De Kruijff, B. (1999) Science *286*, 2361–2364.
• Hsu,S-T.D., Breukink, E., Tischenko, E., Lutters, M.A.G., De Kruijff, B., Kaptein, R., Bonvin, A.M.J.J. & Van Nuland, N.A.J. (2004) Nat. Struct. Mol. Biol. *11*, 963–967.
• Breukink, E. & De Kruijff, B. (2006) Nat. Rev. Drug Discov. *5*, 321–332. Review.
• Hasper, H.E., Kramer, N.E., Smith, J.L., Hillman, J.D., Zachariah, C., Kuipers, O.P., De Kruijff, B. & Breukink, E. (2006) Science *313*, 1636–1637.
• De Kruijff, B. & Demel, R.A. (1974) Biochim. Biophys. Acta *339*, 57–70.

1786-Symp The Energy Landscape Theory of Protein Folding

Peter Wolynes

Univ Calif, San Diego, La Jolla, CA, USA.

Twenty-five years ago, how proteins folded into organized structures on the basis of their sequence was a great mystery. By characterizing the energy landscapes of proteins with tools from the statistical mechanics of disordered systems like spin glasses, a "new view" of the folding process became possible. Energy landscape theory provided an incentive to pursue heroic new experiments and to carry out difficult computer simulations addressing protein folding mechanisms.

Many aspects of folding kinetics revealed by these studies can be quantitatively understood using the simple idea that the topography of the energy landscape is that of a "rugged funnel". Folding kinetics can often be predicted quantitatively on the basis of topology alone because of the funneled nature of evolved protein landscapes.

Energy landscape theory also has provided a quantitative means of characterizing which amino acid sequences can rapidly fold. Algorithms based on energy landscape theory have been used to successfully design novel sequences that fold to a given structure in the laboratory.

Energy landscape ideas have transformed the prediction of protein structure from sequence data from being an art to being a science. The success of energy landscape- based algorithms in predicting protein structure from sequence will be highlighted. While still more work is needed to achieve universally reliable structure prediction, many parts of what used to be called "the protein folding problem" can now be considered solved.

Going beyond protein folding, we are presently learning that the folding and functional landscapes overlap. Allosteric proteins and proteins that are modified post-translationally often exhibit landscapes with evolutionarily constructed degeneracies. Understanding these aspects of molecular biology requires new conceptions in energy landscape theory.

Minisymposium 3: Inactivation and Desensitization Mechanisms in Ion Channels

1787-Minisymp A Structural And Functional View Links Toxin-induced Conformational Changes In A Potassium Channel To C-type Activation

Ulrich Zachariae[1], Robert Schneider[1], Phanindra Velisetty[2], Adam Lange[1], Daniel Seeliger[1], Sören J. Wacker[1], Yasmin Karimi-Nejad[3], Gert Vriend[4], Stefan Becker[1], Olaf Pongs[2], Marc Baldus[1], Bert L. de Groot[1]

[1] *Max Planck Institute for Biophysical Chemistry, Goettingen, Germany,*
[2] *University of Hamburg, Hamburg, Germany,*
[3] *Solvay Pharmaceuticals, Hannover, Germany,*
[4] *Radboud University Nijmegen, Nijmegen, The Netherlands.*

A recent solid-state NMR study [1] revealed that high-affinity scorpion toxin binding induces conformational changes in the selectivity filter of potassium channels. The exact nature of these conformational changes, however, remained elusive. We combined all-atom molecular dynamics simulations with solid-state NMR and electrophysiological measurements to investigate these changes. Our simulations covered the complete pathway of spontaneous toxin approach and binding to the membrane-bound channel. The obtained structural model of the complex revealed conformational changes in the selectivity filter that account for the observations made in solid-state NMR. They also explain tight channel blockade and enhanced toxin affinity. We show that these changes are not only structurally, but also functionally closely related to C-type activation [2]. Our study points to heterogeneity in the binding modes that might serve to stabilize the complexes entropically.

References

[1]. Lange, A., et al. Nature *440*, 959–962 (2006).
[2]. Cordero-Morales, J.F., et al., Nat. Struct. Mol. Biol. *13*, 311–319 (2006).

1788-Minisymp Structural basis of K^+ channel C-type inactivation: Crystal Structure of KcsA in the Open/C-type inactivated Conformation

Luis G. Cuello, Vishwanath Jogini, D. Marien Cortes, Eduardo Perozo

University of Chicago; Institute for Molecular Pediatric Sciences and Biochemistry Department, Chicago, IL, USA.

It is well established that in KcsA, proton dependent activation leads to a widening of the inner helical bundle (IHB). In addition, channel activation triggers C-type inactivation at the selectivity filter (SF), a feature common among all potassium selective channels. By establishing the molecular elements responsible for proton sensitivity in KcsA (Cuello et al., this meeting), we have engineered a constitutively open-channel in which the IHB is fully open at basic pH. Additionally, we have demonstrated that deletion of the KcsA C-terminal domain enhances C-type inactivation. These two findings have given us a unique opportunity to structurally evaluate the conformational changes underlying KcsA proton-dependent activation, C- type inactivation at the SF and the conformational coupling between these two processes. By using Fab-assisted crystallization methods we have solved 17 KcsA structures "trapped" in different stages along the transition pathway, from closed to the fully open state (open-inactivated state), including at least four classes of gating intermediates. Analysis of these structures have revealed:

1. The KcsA IHB can be stabilized with several degrees of gate opening, ranging from about 11.5 Å (Cα-Cα distance at residue 112) to 32 Å;
2. We find no ion occupancy at the channel aqueous cavity in the fully open conformation;
3. Major conformational changes at the SF include flipping of carbonyl groups at V76 and a progressive loss of ions at potassium binding sites S2 and S3. Ion occupancy at these sites is strongly correlated with the degree of opening at the IHB;
4. a small but significant conformational change of the P-loop is seen when the IHB is fully open.

These structures reveal, at unprecedented detail, the molecular basis of K^+ channel C-type inactivation and its coupling to activation gating.

1789-Minisymp Allosteric Ion Binding Sites In Kainate Receptors

Andrew J. Plested, Mark L. Mayer

NICHD/NIH, Bethesda, MD, USA.

Almost all fast excitatory signaling in the brain is due to the neurotransmitter glutamate. Hence, the ion channel receptors for glutamate (iGluRs) are cornerstones of excitatory transmission throughout the brain. This family of receptors, with subtypes named AMPA, kainate and NMDA, underlies synaptic currents that have decay times ranging from milliseconds to seconds. Understanding the regulation of their finely tuned kinetic regimes by endogenous modulators is critically important for unraveling their complex roles at central synapses. The recent discovery of an anion binding site in the intersubunit dimer interface of kainate receptors (Plested and Mayer, *Neuron* 2007) provided a simple explanation of how chloride ions control receptor availability and kinetics. Monovalent cations also modulate kainate (but not AMPA) receptors. However, no bound cation was apparent in either the Cl^-- or Br^--bound structures, despite the similarities between modulation of iGluRs by anions and cations (Bowie, *J. Physiol* 2002). We present data that allow us to estimate the coupling between the cation and anion binding sites. These data suggest that kainate receptors are not fully bound by sodium and chloride at physiological salt. We show that this renders a significant fraction of kainate receptors unavailable for activation by glutamate. Using deletion mutagenesis and non-desensitizing mutant receptors, we locate the allosteric cation binding site and demonstrate clearly that external ions are not coactivators of kainate receptors (Wong *et al. J. Neurosci* 2006). We present structural data that illustrate the cation binding site for the first time, and suggest that a simple allosteric mechanism accounts for the apparent concomitant modulation of kainate receptors by anions and cations.