## 54-Plat Biofunctional Micropatterned Surfaces to Study Individual LFA-1&ICAM-1 Interactions in Living Cells

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Lymphocyte function-associated antigen-1 (LFA1;αLβ2) is a leukocyte specific integrin transmembrane protein that mediates migration across the endothelium and within tissues. It also takes part of the immunological synapse by binding with high affinity to its ligand ICAM-1. We have recently shown that integrin mediated adhesion depends not only on receptor occupancy but also on its nano-cluster organization on the cell membrane. In order to get deeper insight on the mechanisms that control and regulate LFA-1 clustering we have fabricated large areas of biofunctional micropatterned surfaces containing ICAM-1 ligands using soft-lithography. Homogenous ICAM-1 regions (from 10μm-1μm) have been achieved over 1cm$^2$ areas, with variations in ICAM-1 density below 9%. THP-1 cells (monocytic cell line) expressing LFA-1 have been stretched over the patterned surfaces and the diffusion of labeled LFA-1 in the ICAM-1 regions has been followed in time using single molecule sensitive TIRF microscopy. Single molecule fluorescence trajectories show an increase of intensity on individual fluorescent spots as well as reduction of its mobility consistent with selective recruitment of individual LFA-1 clusters on the ICAM-1 patterned areas. Fluorescent spots on the non-patterned areas (BSA coated) show on the other hand random diffusion. Surprisingly, *on-off* blinking and large intensity fluctuations were observed on the LFA-1&ICAM-1 bound (immobile) clusters compared to the randomly diffusing LFA-1. We are currently performing statistical analysis on the *on-off* times to correlate the binding kinetics of LFA-1&ICAM-1 with receptor-ligand density. These studies will shed light on the role of affinity *vs.* avidity of LFA-1 to its ligand ICAM-1.

### Symposium 3: Membrane Protein Structure: Freed from the Lattice

## 55-Symp Solid-state NMR

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Solid-state NMR (ssNMR) offers structural insight into the formation of molecular complexes for a wide range of molecular sizes and binding affinities. In our contribution we will discuss recent methodological progress in probing structure, topology and complex formation in membranes. In addition, we will show how to follow protein activation/inactivation in membrane-embedded ion channels and seven-helix receptors by multidimensional ssNMR.

## 56-Symp Insights Into Disulfide Bond Formation In The Periplasm

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Insights into Disulfide Bond Formation in the Periplasm.

We have used solution NMR methods to study the structure and function of the integral membrane enzyme DsbB. The proteins DsbA and DsbB work in concert to mediate the formation of disulfide bonds in the periplasm of E. coli. DsbA is a soluble protein that functions as the primary oxidant for proteins in the periplasm whereas DsbB is an integral membrane protein that reoxidizes DsbA and is itself reoxidized by various quinones. Mutations of DsbA in pathogenic bacteria are avirulent, suggesting this pathway may be a useful target for the development of novel antibiotics.

In order to quench extensive conformational exchange seen in the spectra of wildtype DsbB, we have employed an inter-loop disulfide bonded species, referred to as DsbB(CSSC), which is a reaction intermediate in the mechanism of DsbB. Using a combination of backbone dihedral angle restraints, a limited number of NOE constraints, pramagnetic relaxation effect (PRE) restraints, and an extensive set of dipolar couplings, we have determined the backbone structure of DsbB(CSSC) in DPC micelles to high-resolution. To our knowledge, this represents the first solution structure of a bona fide α-helical membrane protein with more than 2 TM helices. The structure shows the predicted 4 TM helices as well as an additional N-terminal helix. Examination of the two periplasmic loops, which are critical for enzymatic function, reveals that the region of the protein which binds to DsbA has increased mobility, likely mimicing the typically unfolded substrates of DsbA. NMR chemical shifts for the inter-loop disulfide bond suggest a highly polarized disulfide bond primed for disulfide exchange. We have also been able to determine the binding site for ubiquinone on DsbB and shown the importance of residues lining this binding site for function.

## 57-Symp Potassium channels conduct and regulate potassium flux across the cell membrane

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Potassium channels conduct and regulate potassium flux across the cell membrane. Several crystal structures and other biophysical studies of tetrameric ion channels have revealed many of the structural details of ion selectivity and gating. A narrow pore lined with four arrays of carbonyls is responsible for ion selectivity, whereas a conformational change of the four inner transmembrane helices is involved in gating of the channel. Here, we studied full-length KcsA, a prototypical K+ channel, by solution-state

Nuclear Magnetic Resonance Spectroscopy (NMR) at open, closed and intermediate states. These studies elucidate that at least two conformational states occur both in the selectivity filter and near the C-terminal end of the inner transmembrane helice 2 (TM2). In

the ion-conducting open state, rapid structural exchange between two conformations, presumably K+ high- and low-affinity, of the filter is observed. These millisecond dynamics provide the basis for simultaneous ion selection and gating of the selectivity filter at a timescale distinguishable from that of the gating action by the TM2.

## 58-Symp The human voltage-dependent anion channel

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The human voltage-dependent anion channel (VDAC) is a 283-residue integral membrane protein located in the outer mitochondrial membrane. It forms an aqueous pore through which metabolites and other small molecules pass between the cytosol and the inter-membrane space. The essential life-sustaining function of VDAC in metabolite trafficking is regulated by proteins of the Bcl-2 family, and previously pro- and anti-apoptotic Bcl-2 proteins have been shown to interact with VDAC.

We have expressed, purified, and refolded VDAC1 into LDAO detergent micelles. We have shown that this preparation is amenable to NMR studies exhibiting excellent multidimensional spectra. The recombinant protein is functional as it is capable of interacting with substrates and inhibitors. A large fraction of the backbone and side-chain resonances were assigned and secondary structure elements were identified through analysis of chemical shifts and inter-strand Nuclear Overhauser effects (NOEs). Initial models of the polypeptide fold were compared with predicted models of the channel. Determination of the tertiary structure is in progress. Various biophysical experiments indicate that micelle-bound VDAC is in intermediate exchange between monomer and trimer. Using NMR spectroscopy, gel filtration, and chemical cross-linking we obtained direct evidence for binding of Bcl-xL to VDAC in a detergent micelle system. The VDAC-interacting region of Bcl-xL was characterized by NMR with chemical shift perturbation and transferred cross saturation. The interaction region was mapped to a putative helical hairpin motif of Bcl-xL that was found to insert into detergent micelles. Our results suggest that Bcl-xL can bind to 1 or 2 VDAC molecules forming heterodimers and heterotrimers. Our characterization of the VDAC/Bcl-xL complex offers initial structural insight into the role of anti-apoptotic Bcl-xL in regulating apoptotic events in the mitochondrial outer membrane.

### Symposium 4: Putting the Move on Myosin

## 59-Symp Molecular Movements Associated with Force Generation

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The myosin actin-binding cleft located between the upper and lower 50kD domains is thought to undergo changes as a function of nucleotide state and binding to actin. Cleft closure is needed to allow tight binding to actin and might be coupled to the opening of active site. To detect cleft closure we measured the distances between the upper- and lower-domains with various nucleotides and in the presence of actin using conventional and pulsed dipolar EPR (DEER). Double cysteine mutants were engineered in upper domain and in lower domain that were labeled with spin probes/fluorophores. We found two major populations of distances and shifting in the populations as a function of nucleotide state and actin binding. The average distances and the trends in distance distribution were in excellent agreement between FRET and EPR. In presence of actin, the short distance population increased significantly and broad distance distribution was found, which suggest that the cleft is closed upon actin binding, however, multiple conformations exist. In smooth muscle, force generation is regulated by phosphorylation of regulatory light chain (RLC). The molecular mechanism is uknown, although it is clear that it involves the interaction between the myosin heads. To determine the relative positions of the heads we have measured distances between the selected cysteine mutants of RLC exchanged into smooth muscle myosin. In the unphosporylated SMM monomers, the measured distances were 11.5 Å for C38, 28 Å for C59 and over 40 Å for C108 and C84. Upon phosphorylation of SMM all the distances were beyond the sensitivity range of EPR. Using novel distance geometry algorithm, we have a constructed a model for the 6S monomer. Our model at this stage is not unique but the family of current solutions includes model of Wendt & Taylor.

## 60-Symp Structural Studies Of Myosin VI Provide Important Insights For The Motor Mechanism Of Myosin Motors

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Force is produced by myosin motors upon the actin-driven conformational changes of the motor that leads to sequential release of the hydrolysis products of ATP. Structural information on the multiple states the motor adopt along its motor cycle is essential for understanding how chemical energy is converted into force production. Myosin VI is the most enigmatic of myosins. First, it produces its force and traffics toward the minus-end of actin filament in the opposite direction to other myosins. Second, this motor uses a number of unique mechanisms that are not well understood to take multiple steps on an actin filament without detachment. Surprisingly, these steps are similar in size to those of myosin V, even though the lever arm of myosin VI contains only one IQ motif, whereas that of myosin V contains six. We have recently revealed the structure of the myosin VI motor at the end of its powerstroke (in the rigor conformation). This structure reveals that a specific insert wraps around the converter and binds a calmodulin that interacts with the converter. The result is a ~120° repositioning of the myosin VI lever arm, which explains its reverse directionality. However, to account for the large powerstroke of myosin VI, this study clearly predicted that the pre-powerstroke state of myosin VI must differ from that of plus-end directed myosins. We have recently solved this structure. It reveals that unexpected rearrangements in the converter are critical to position the lever arm ideally to produce a very large stroke.