

3243-Plat**A Biological Friendly High-Resolution Optical Tweezers for Single Molecule Studies**

Wei Cheng, Ximiao Hou.

University of Michigan, Ann Arbor, MI, USA.

Exciting progress has been made recently in biophysical techniques that allow optical manipulation and measurement for single molecules at subnanometer scale as they undergo conformational transitions in real time. However, a serious problem that could limit the future application of this technique is the photo damage to biological molecules brought by intense laser irradiation. The state-of-the-art high resolution optical tweezers instrument utilizes lasers that operate at 1064 nm, which coincides with the absorption of molecular oxygen in water. As a consequence, reactive oxygen species are generated during experiments that irreversibly modify the chemical structures of molecules under study. To solve this problem, we have constructed an optical tweezers instrument using a new generation high power diode laser that operates at 830 nm. Molecular oxygen has no absorption at this wavelength. We show that the choice of this laser not only eliminated photo damage associated with reactive oxygen species, the instrument also gained a faster frequency response, which stems from the overlap between trapping laser wavelength and the peak absorption of silicon photodetectors. Moreover, the sample temperature during experiments is much better controlled due to negligible absorption of water at 830 nm. All these advantages could significantly benefit future application of this single molecule technique in biological studies. We present our results from this instrument, and the status of spatial resolution for single molecule manipulation.

3244-Plat**Stretching Single DNA Molecules and using High-Speed Camera Power Spectral Analysis to Demonstrate High Force Capabilities of Holographic Optical Tweezers**Astrid van der Horst¹, Arnau Farré², Nancy R. Forde¹.¹Simon Fraser University, Dept. of Physics, Burnaby, BC, Canada,²Universitat de Barcelona, Dept. Física Aplicada i Òptica, Barcelona, Spain.

Holographic optical tweezers (HOTs), in which a Spatial Light Modulator is used to change the phase pattern of the laser light, enable the manipulation in three dimensions of many particles simultaneously. This can be used for the probing of extended structures such as cells and extended protein networks. To allow for quantitative force measurements, the HOT traps need to be calibrated. However, nanometer-scale position modulations are introduced by the Spatial Light Modulator. In power spectral analysis, modulations at specific frequencies and drift can be readily identified in the spectrum and omitted before analysis, making this the preferred method of calibration for our HOTs. We use high-speed camera imaging for position detection of multiple trapped particles simultaneously, from which we obtain power spectra with 1.25 kHz bandwidth. For stiff traps, however, blur due to image integration time affects the detected particle positions significantly. Taking the effects of blur, aliasing and position detection error into account, as put forward by Wong and Halvorsen [Opt. Express 14, 12517, 2006], we are able to obtain the corner frequency f_c of the power spectrum for stiff traps with f_c up to 3.5 kHz. We demonstrate the utility of our calibration approach by measuring the force-extension curve for 4-micrometer-long DNA.

Platform BB: Membrane Protein Structure II**3245-Plat****Thermodynamics of Interfacial Membrane Binding and Transmembrane Insertion of Diphtheria Toxin T-Domain: Fluorescence Correlation Spectroscopy Study**Yevgen O. Posokhov¹, Mykola Rodnin¹, Alexander Kyrychenko¹, Christine Contino², Bernard Pucci², Alexey S. Ladokhin¹.¹KUMC, Kansas City, KS, USA, ²Université d'Avignon et des Pays du Vaucluse, Avignon, France.

Recent studies of kinetic behavior of binding and insertion of diphtheria toxin translocation domain (DTT) into lipid membranes [Kyrychenko et al. Biochemistry 2009, 48:7584] revealed the presence of several interfacial intermediates on the insertion pathway leading from soluble W-state to transmembrane T-state. It has been found that an intermediate interfacial I-state can be trapped in membranes with low content of anionic lipids (10%), while in membranes of greater anionic lipid content, another pH-dependent transition results in the formation of the insertion-competent state and subsequent transmembrane insertion. In this work we applied fluorescence correlation spectroscopy (FCS) to determine the free energy (ΔG) stabilizing final transmembrane and interfacial intermediate states. To avoid aggregation of DTT and to chaperone its membrane insertion, the FCS measurements were performed in the presence of fluorinated surfactants FTAC-C6. Our results indicate that the free energy of binding (ΔG) to lipid vesicles with formation of trapped interfacial intermediate state is about -8 kcal/mole, and this ΔG value does not change with pH, while the ΔG difference between transmembrane state and the interfacial state ranges from -1.5 to -4 kcal/mole depending on membrane lipid composition and pH of media. Our results confirm the interface-directed model of spontaneous insertion of non-constitutive membrane proteins and provide an important benchmark for future measurements of ΔG stabilizing the structure of constitutive membrane proteins. Supported by NIH GM069783(-04S1)

copy (FCS) to determine the free energy (ΔG) stabilizing final transmembrane and interfacial intermediate states. To avoid aggregation of DTT and to chaperone its membrane insertion, the FCS measurements were performed in the presence of fluorinated surfactants FTAC-C6. Our results indicate that the free energy of binding (ΔG) to lipid vesicles with formation of trapped interfacial intermediate state is about -8 kcal/mole, and this ΔG value does not change with pH, while the ΔG difference between transmembrane state and the interfacial state ranges from -1.5 to -4 kcal/mole depending on membrane lipid composition and pH of media. Our results confirm the interface-directed model of spontaneous insertion of non-constitutive membrane proteins and provide an important benchmark for future measurements of ΔG stabilizing the structure of constitutive membrane proteins. Supported by NIH GM069783(-04S1)

3246-Plat**The Spontaneous Refolding of Opacity-Associated Proteins into Lipid Membranes**

Alison H. Dewald, Jacqueline C. Hodges, Linda Columbus.

University of Virginia, Charlottesville, VA, USA.

The spontaneous folding of outer membrane proteins (OMPs) into lipid vesicles provides a means to study the determinants, kinetics, and thermodynamics of membrane protein folding in manipulatable systems modeling the native lipid environment. This information increases our ability to understand and utilize membrane proteins, but it remains sparse, with direct lipid refolding reported for fewer than a dozen unique OMPs. We present the spontaneous refolding of recombinant Opa proteins into lipid vesicles, with a systematic investigation of the impact protein and lipid bilayer variables have on the folding. Opa proteins are eight-stranded β -barreled monomeric integral outer membrane proteins found in the bacterial pathogens *N. gonorrhoeae* and *N. meningitidis*. There are at least 26 characterized Opa proteins, nearly identical in sequence, but varying in three extracellular loops. *In vivo*, these proteins interact with specific human host cell receptors to breach the plasma membrane and gain entry to targeted human cells. The basis for host-receptor specificity is not well understood but is determined by the variable extracellular loops. These loops also play a role in folding. The β -sheets of Opa variants OpaI and OpaA are nearly identical in sequence, but OpaI refolds in DMPC vesicles while OpaA does not. These variants therefore provide a natural system to probe protein folding determinants. The effects of lipid composition (in particular both head group and chain length), buffer pH, ionic strength, and temperature on refolding have been characterized for these Opa variants. Ultimately, reconstitution into lipid bilayers required the matching of hydrophobic thicknesses as well as optimization of parameters mediating electrostatic interactions between the protein and lipids used. The reconstituted systems provide a new model in the study of membrane protein folding, with an exploration of refolding parameters that may be applicable to additional OMP-lipid systems.

3247-Plat**Structure of the Mycobacterium Tuberculosis Virulence Factor Rv0899 (ompATb)**Peter Teriete¹, Yong Yao¹, Adrian Kolodzik¹, Leigh A. Plesniak²,Michael Niederweis³, Francesca M. Marassi¹.¹The Burnham Institute, La Jolla, CA, USA, ²University of San Diego, San Diego, CA, USA, ³University of Alabama, Birmingham, Birmingham, AL, USA.

Rv0899 (OmpATb) is a 326-residue membrane-associated virulence factor of *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis. It is essential for the adaptation of *Mtb* to acidic environments and has been identified as an outer membrane protein. The M domain (residues 1-72) contains a 20-residue hydrophobic sequence that may form a membrane-anchoring helix. The B domain (residues 73-195) has been described as pore-forming. It shares sequence homology with the "BON" domain of Bacterial Osmotic-shock-resistance, Nodulation-specificity and lipid-binding proteins. The C domain (residue 196-326) shares significance homology with other bacterial peptidoglycan-binding domains, including the C-terminus of the *E. coli* outer membrane protein OmpA, after which Rv0899 was originally named. Using NMR spectroscopy, we show that residues 73-326, spanning the joint B and C domains, adopt a well-defined three-dimensional structure in water solution, however, the individual B and C domains fold independently and interact with each other to a minimal extent. The B domain adopts a rigid and stable structure, that forms a six-stranded β -sheet protected on one side by three α -helices. No comparable arrangement of secondary structure elements could be found in the databases, suggesting that is a new fold. The structure of the B domain is

suggestive of a lipid binding or structural function, and provides insights to the role of Rv0899 in *Mtb* virulence.

(This research was supported by the National Institutes of Health (NIH), and utilized the Burnham Institute NMR Facility supported by the NIH National Cancer Institute).

3248-Plat

Structural Investigations of an Amphipathic Region of the Twin-Arginine Translocase TatA Subunit

Catherine S. Chan^{1,2}, Evan F. Haney², D. Peter Tieleman², Hans J. Vogel², Raymond J. Turner².

¹University of British Columbia, Vancouver, BC, Canada, ²University of Calgary, Calgary, AB, Canada.

The twin-arginine translocase (Tat) system is used by many bacteria and plants to move folded proteins across the cytoplasmic membrane. Tat substrates contain a signature S/TRR \times FLK twin-arginine motif in their N-terminal sequence. In most bacteria, the translocon consists of the TatABC subunits where TatA is the postulated pore subunit through homo-oligomerization with other TatA protomers, whereas TatBC is the substrate-receptor complex. The predicted structure of TatA includes a transmembrane helix, an amphipathic helix and a potentially unstructured C-terminal region. Biochemical and structural investigations were targeted at a peptide which represents the amphipathic region consisting of residues 22 to 44 of TatA (TatAH2). The dual topology of the region corresponding to TatAH2 in TatA was previously shown to be dependent on the membrane potential (Chan *et al.* 2007 *Biochemistry* 46: 7396-404), and thus warranted further investigations on its role for protein translocation. NMR and CD spectroscopy of TatAH2 show that it adopts helical structure in a membrane mimetic environment, in comparison to the random coil structure in aqueous solution. Microcalorimetry studies also show that it interacts with DPPG lipid vesicles to affect the phase transition temperatures. The solution NMR structure of TatAH2 shows conformation flexibility of the peptide around the acidic Asp31 at the center of the helix, a residue potentially important for the function of the TatA pore. The C-terminal half (residue 32 onwards) is α -helical, whereas the N-terminal half (23 to 30) has helical-like structure, suggesting that TatAH2 does not form a 'typical' α -helix.

3249-Plat

Investigating the Structural and Dynamic Properties of Membrane Proteins with Solid-State NMR, CW-EPR, ESEEM, and DEER Spectroscopic Techniques

Gary A. Lorigan.

Miami University, Oxford, OH, USA.

Currently, we have very limited structural and dynamic information on membrane proteins and peptides. New spectroscopic methods are needed to probe these systems in a lipid bilayer. In order to address these issues, the Lorigan lab developing unique hybrid solid-state NMR and spin-label EPR spectroscopic techniques. Magnetic resonance spectroscopic data of ¹⁵N-, g²H-labeled and/or spin-labeled membrane proteins incorporated into vesicles and bicelles will be presented. State-of-the-art solid-state NMR and pulsed EPR techniques such as Magic Angle Spinning (MAS) NMR, Electron Spin Echo Envelope Modulation (ESEEM) spectroscopy, and Double Electron-Electron Resonance (DEER) spectroscopy will be used. The ESEEM technique can determine short to medium range distances (out to about 8 Å) between a site-specific nitroxide spin label (MTSL) and a nearby NMR-active isotopic labeled residue for a variety of different peptides and proteins which ultimately can be used to determine the difference between an α -helical and β -sheet secondary structure. DEER can be used to measure distances between 2 spin labels out to about 70 Å. The advantages and disadvantages of applying solid-state NMR and EPR spectroscopy to probe the structural and dynamic properties of membrane proteins will be discussed.

3250-Plat

Secondary-Structure Analysis of Human Peripheral Cannabinoid Receptor CB2 Based on Solid-State ¹³C-, ¹⁵N-MAS NMR and Molecular Dynamics Simulations

Tomohiro Kimura¹, Krishna Vukoti¹, Diane L. Lynch², Dow P. Hurst², Alan Grossfield³, Michael C. Pitman⁴, Patricia H. Reggio², Alexei A. Yeliseev¹, Klaus Gawrisch¹.

¹NIH/NIAAA, Bethesda, MD, USA, ²University of North Carolina Greensboro, Greensboro, NC, USA, ³University of Rochester Medical Center, Rochester, NY, USA, ⁴IBM Thomas J. Watson Research Center, Yorktown Heights, NY, USA.

The secondary structure of the human peripheral cannabinoid receptor CB2 was investigated according to chemical shift data obtained on lipid-reconsti-

tuted, functional CB2. The CB2 receptor, uniformly labeled with ¹³C and ¹⁵N, was produced by bacterial expression, purified in milligram quantities, and reconstituted in an agonist-bound form into unilamellar liposomes. One- and two-dimensional ¹³C and ¹⁵N spectra were recorded by solid-state magic-angle spinning (MAS) NMR. Homogeneity of the reconstitution was examined by measurement of the ³¹P chemical-shift anisotropy of the membrane phospholipids and functional integrity monitored by a G-protein activation assay. The acquired one- and two-dimensional ¹³C-, and ¹⁵N-MAS NMR spectra of CB2 were compared with C α , C β , C=O, and N-H chemical shifts predicted from a computer model of CB2, obtained in micro-second time scale, all atom molecular dynamics simulations. Although experimental spectra suffered from heavy superposition of resonances, the comparison of theoretical and experimental spectra gave insights into secondary structure of protein domains. There is sufficient spectral resolution to partially assign the resonances of specifically labeled CB2. The prospects for spectroscopic targeting of structural changes that take place upon CB2 activation by a combination of NMR experiments and computer modeling will be discussed.

3251-Plat

Spectroscopic Investigation of Phosphorylated Pentameric Phospholamban Bound to the Sarcoplasmic Calcium ATPase

Raffaello Verardi, Nathaniel J. Traaseth, Lei Shi, Gianluigi Veglia.

University of Minnesota, Minneapolis, MN, USA.

The sarco(endo)plasmic reticulum Ca-ATPase (SERCA) is the main enzyme responsible for the reuptake of Ca²⁺ into the sarcoplasmic reticulum (SR) during muscle relaxation. Phospholamban (PLN), a homopentameric membrane protein expressed in the SR, binds to and decreases the rate of Ca²⁺ transport of SERCA. This inhibitory action is relieved when PLN is phosphorylated at residues Ser-16 or Thr-17 in response to different signal pathways. Although a great deal of knowledge has been accumulated on the functional consequences of PLN phosphorylation, very little is known about the molecular details leading to relief of inhibition. Previous work suggested a crucial role of protein topology in the complex between SERCA and PLN, with the latter showing different tilt angles in the presence and absence of SERCA. Our hypothesis is that SERCA activity is regulated by changes in topology in PLN transmembrane helices upon phosphorylation. In order to probe for the orientation of protein domains, solid-state NMR in oriented lipid bilayers has established itself as the method of choice in many studies. Here we present data on the topological changes taking place in phospholamban pentamer in the presence of SERCA upon phosphorylation at Ser-16 or Thr-17. SERCA and PLN are reconstituted in lipid bilayers and uniaxially aligned either mechanically (on glass plates) or magnetically (upon formation of bicelles). The two proteins are fully functional under these conditions as demonstrated by the ability of SERCA to transport Ca²⁺ in functional assays as well as PLN dependent decrease in apparent affinity for Ca²⁺.

3252-Plat

Protein Folding at the Membrane Interface: The Structure of Nogo-66

Melanie J. Cocco, Sheeja Vasudevan, Rosemarie Vithayathil, Jessica Schulz, Chunyi Zhou, Sudipta Majumdar, Gregory Weiss.

University of California, Irvine, CA, USA.

Compelling evidence indicates that repair of damage to the central nervous system (CNS) is inhibited by the presence of protein factors within myelin that prevent axonal regrowth. Myelin growth inhibitors and their common receptor have been identified as targets in the treatment of damage to the CNS.

We have recently determined the NMR structure of one of the myelin growth inhibitors, the neurite outgrowth inhibitor (Nogo). We studied the structure of this protein alone and in the presence of dodecylphosphocholine micelles to mimic the natural cell membrane environment. Using several paramagnetic probes, we have defined portions of the growth inhibitor that are accessible to solvent (and consequently the Nogo receptor). Mutagenesis probed through phage-display confirms that the positions predicted to be extra-cellular are sensitive to receptor binding. Using computational docking methods and the mutagenesis results, we calculated the optimal protein-protein interface between our structure of Nogo and the Nogo receptor. The structure of Nogo and the predicted Nogo/receptor inhibitory complex structure will be presented.

Funding was provided by: NIH 1R01GM078528-01 and Cal Roman Reed Res Fund RR03-072, +114, +155