**3243-Plat**
**A Biological Friendly High-Resolution Optical Tweezers for Single Molecule Studies**
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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**
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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**
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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 ($\Delta G$) 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 ($\Delta G$) to lipid vesicles with formation of trapped interfacial intermediate state is about $-8$ kcal/mole, and this $\Delta G$ value does not change with pH, while the $\Delta G$ difference between transmembrane state and the interfacial state ranges from $-1.5 \div -4$ 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 $\Delta G$ stabilizing the structure of constitutive membrane proteins. Supported by NIH GM069783(-04S1)

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