proportional to the protein's volume, the solvent accessible surface area, the integrated (over the surface area) mean curvature and the integrated (over the surface area) Gaussian curvature. The coefficients proportional to these geometrical measures are geometry independent thermodynamic coefficients, which characterize the interaction between the solvent and the protein. Since the thermodynamic coefficients are independent of the geometry, they can be calculated in a simple test geometry.
The separation of the solvation free energy into four geometrical measures and corresponding geometry-independent thermodynamic coefficients has important consequences: (1) It allows for fast and accurate calculation of the solvation free energy of a protein in a given geometrical configuration, which is important when comparing different structures of a protein. (2) It allows for fast and accurate calculation of the force on the protein in a given configuration mediated by the solvent, which is important in a simulation of the folding process of the protein.

2000-Plat
Dissociation and Unfolding of Insulin Dimers
Ziad Ganim, Kevin C. Jones, Andrei Tokmakoff.
Massachusetts Institute of Technology, Cambridge, MA, USA.
Insulin monomers bind one another through the folding of an interchain β sheet. How does binding mediate protein folding? The energetics and rate of this fast folding process are difficult to translate into the mechanistic details that underlie classical paradigms such as conformational selection and fold-on-contact. We study the conformational dynamics at the dimer interface upon binding and dissociation using two-dimensional infrared spectroscopy (2D IR). 2D IR reveals coupling among α helix and β sheet vibrations for secondary structural sensitivity with picosecond time resolution that can resolve all relevant structural changes. Cross-peak features provide monomer and dimer 2D IR signatures that yield the binding constant and its solvent and temperature dependence. These spectra are interpreted in detail using molecular dynamics simulations of insulin dimers, disordered and compact monomers to quantify the disordered monomer ensemble. Transient dissociation and unfolding are rapidly initiated using a nanosecond temperature-jump. Conformational changes occurring on the fastest resolvable nanosecond timescales are observed for the disordering of the interchain β sheet insulin dimers.

2001-Plat
Evidence For Metastable States Of Lysozyme Revealed By High Pressure FTIR Spectroscopy
Laszlo Smeller.
Semmelweis University, Budapest, Hungary.
Metastable protein conformations play an important role in the folding process because such partially disordered states can be gates for the misfolding pathway, leading sometimes to pathological structures, like fibrous aggregates. High pressure is a very useful tool in the study of metastable states, since application of the pressure is fully reversible, contrary to chemical agents. FTIR spectroscopy allows us to follow simultaneously the secondary structure, the packing (tertiary structure) and the aggregation of the protein using the amide I, amide II and the 1616 cm-1 bands respectively. We performed a systematic study on the temperature-pressure phase diagram of lysozyme and found a two-step unfolding profile both in the pressure and temperature directions. Hydrogen/deuterium exchange results show evidence for the molten globule formation at 57°C @130 MPa and 580 MPa @30°C, which are considerable lower values than those of the complete unfolding (e.g. 75°C@130MPa).
Refolding of the protein after pressure unfolding is a slow process, with a time constant in the range of hours. Partially refolded structures present in this time range have different aggregation propensity. The kinetics of the aggregation has a biexponential character with a time constants of 1060 and 8600 s at 40C ambient pressure. Analyzing the time dependence of the amide I band shape we found that the strengthening of the intermolecular hydrogen bond network was accompanied by decrease of the folded secondary structure content. Moderate pressure of 300 MPa was found to be able to dissociate the aggregates, while the secondary structure is not yet unfolded. This fact together with the small temperature effect on the aggregation kinetics suggests that the rate of the aggregation of the metastable conformations is determined by the high activation volume rather than the high activation energy.

2002-Plat
Single Molecule FRET On Alpha Synuclein Membrane-bound Conformational States
Adam Trexler, Elizabeth Rhoades.
Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA.
Alpha-Synuclein (αS) is the primary component of the Lewy body plaques that are characteristic of Parkinson's disease (PD). Large insoluble αS aggregates compose Lewy bodies, but smaller soluble αS oligomers are implicated as the cytotoxic species in PD. Though αS is natively unstructured in solution, it forms a N-terminal alpha helix upon binding to lipid membranes. Extensive evidence also shows that αS gains structure upon forming oligomeric species. In order to learn more about the transition of monomeric αS to toxic oligomeric species and to identify critical conformational states along this pathway, we use single molecule Förster resonance energy transfer (smFRET) and fluorescence correlation spectroscopy (FCS) to characterize the monomeric conformational states of αS. Our evidence shows that αS populates at least two distinct, monomeric conformational states, as a function of curvature, on lipid membranes or lipid mimetics. This could mean αS forms distinct conformations based on whether it binds synaptic vesicles or other cellular membranes. Perhaps one of these conformations is more susceptible to conversion to toxic species, and so this finding may enhance our understanding of how toxic oligomers are formed in PD.

2003-Plat
Postdoctoral Research Scholar
Harekrushna Sahoo.
University of Massachusetts, Amherst, MA, USA.
Exploring Folding Intermediates of a β-Clam Protein by FRET Analysis
Harekrushna Sahoo[1], Anne Gershenson[2] and Lila M. Gierasch[1,3].
Department of [1]Biochemistry and Molecular Biology and [3]Chemistry, University of Massachusetts Amherst, MA 01003, USA; [2]Department of Chemistry, Brandeis University, Waltham, MA 02454.
A complete understanding of a protein-folding landscape requires detailed characterization of intermediates that are populated during a folding reaction. We are exploring the folding intermediates sampled by a 136-amino acid β-barrel protein, cellular retinoic acid-binding protein I (CRABP I). This model protein is made up of two five-stranded orthogonal β-sheets wrapped around a central ligand-binding cavity. Previous work has shown that the folding of CRABP I involves well-defined stages: An early intermediate forms in ca. 300 μs by hydrophobic collapse, next (~100 ms) an intermediate is populated that has native topology including the ligand-binding cavity, and lastly, in ca. 1 s, interstrand hydrogen bonds form and native packing of side chains develops. The nature of the intermediates is relatively poorly understood, including structural details, compactness, and the size of the intermediate ensembles. To address these questions, we have designed CRABP I mutants with solvent-accessible Cys residues (M1C, S55C, N64C, K106C, and D103C) suitable for attachment of thiol-reactive fluorophores, as well as transglutaminase (TGase) tags at their C-termini for enzymatically mediated labeling with a second fluorophore. The Cys residues have been labeled with HyLite488 or BODIPY-FL (donor), and the TGase tags have been labeled with tetramethylrhodamine (acceptor). As an additional strategy to deduce the nature of the folding intermediates, we are varying solvent conditions using salts that differentially affect species stabilized by hydrophobic, electrostatic, or hydrogen bonding interactions. Taken together, ensemble and single-molecule FRET studies of doubly-labeled variants are providing a increasingly detailed picture of the CRABP I folding landscape. *[Supported by NIH grant OD000945]*

2004-Plat
Measurement of Single Molecule Folding/unfolding Trajectories
Hoi Sung Chung, John M. Louis, William A. Eaton.
NIH/NIDDK, Bethesda, MD, USA.
We have measured folding/unfolding trajectories of single protein G (B1 domain) molecules, a simple two-state folder, by simultaneously measuring the fluorescence intensity, lifetime, and spectrum at various concentrations of denaturant. Protein molecules were labeled by a fluorescence resonance energy transfer (FRET) pair, Alexa Fluor 488 and Alexa Fluor 594 and were immobilized on a glass surface coated with polyethyleneglycol via streptavidin-biotin linkage. The vast majority of molecules (~ 85%) exhibits simple two-state trajectories, with either high or low values of the FRET efficiency, corresponding to the folded and unfolded states, respectively, with unresolvable jumps between them. About 10% of the trajectories show transitions in the unfolded state that can be attributed to a ~20 nm spectral shift of the donor, as revealed by measurements of their emission spectra. The mean FRET efficiency of immobilized molecules matches the value measured in free diffusion experiments. There is a distribution of these values beyond the width expected from shot noise, which can, however, be quantitatively accounted for by the distribution of acceptor lifetimes. In spite of these complications from photophysics, rate coefficients obtained from the exponential distribution of residence times in either the folded or unfolded state yield relaxation times that agree within a factor of 2 with those measured on the dye-labeled protein by stopped flow kinetics. In addition, no correlation is observed between the donor and acceptor intensity in the unfolded state from microseconds to seconds suggesting that structural averaging between unfolded conformations occurs on the nanosecond timescale, as expected from previous measurements by B. Schuler and coworkers (*PNAS*:104,2655,2007). All these results indicate that we have successfully

immobilized the protein without significantly altering its structure, kinetics, or dynamics, and represent a major step forward toward the goal of ''watching'' individual molecules fold.

2005-Plat
Time Resolved Thermodynamics of Fast Protein Folding in Cytochrome c
Randy W. Larsen.
University of South Florida, Tampa, FL, USA.
One of the earliest studies of fast protein folding involved the photolysis of CO bound Cytochorme c in the presence of 4.5 M Gdn-HCl HCl, pH 7.0. These studies revealed fast coordination of non-native His and Met residues resulting in a 'frustrated' folding pathway resulting from non-native coordination. These early events occur on timescales of less than 10 μs. Similar fast folding can be initiated at lower Gdn-HCl at pHs above 9.5. Under these conditions the non-native coordination occurs with time constants of ~ 300 ns and ~ 3 μs at 22 dg C. Previous time resolved CD spectra suggest that 8% of the native like secondary structure forms in $<$ 1 μs (Goldbeck et al., PNAS (1999), 96, 2782). Here, photoacoustic calorimetry (PAC) has been utilized to probe the thermodynamics associated with fast folding and non-native ligand coordination in CO-Cytochrome c ta pH 12.7 and 350 mM Gdn-HCl. At temperatures below 18 ° C the PAC signals indicate multi-phasic kinetics that can not be fit to simple exponential sums suggesting a distribution of conformations in the presence of CO. The integrated thermodynamics (integrated thermodynamics for processes occurring in $<$ ~ 20 μs) give an enthalpy change of 5 ± 2 kcal/mol and a molar volume change of -0.5 ± 0.4 mL/mol. Taking into account the enthalpy and volume changes associated with CO photo-release from the heme, an enthalpy change of - 12 kcal/mol and volume change of -6 mL/mol is obtained for the 8% folding as well as non-native His and Met coordination. These results will be discussed within the context of the protein funnel mechanism for fast folding in cytochorme c.

2006-Plat
Time-resolved Fret Study Shows Sub-populations of A Globular Protein Molecules at The Refolding Transition Zone
Elisha Haas, Dan Amir, Eitan Lerner.
Bar Ilan University, Ramat Gan, Israel.
Cooperative protein folding invokes discrete folded and unfolded ensembles separated by a free-energy barrier. In contrast, downhill folding involves just one ensemble of protein molecules within a single free-energy well. Common method of monitoring the folding transition which yield mean values cannot resolve the two mechanisms. Time-resolved dynamic resonance nonradiative excitation energy transfer (trFRET), which can yield distributions of conformers in ensembles of partially folded protein molecules was applied. *E. Coli* adenylate kinase (AK) was used as a model in a study of the unfolding/refolding transition. Several mutants were prepared which enabled monitoring the folding transition at different parts of the molecule.
The analysis of trFRET monitored chemically induced unfolding/refolding transition yielded a clear evidence for the presence of two distinct sub-populations at the transition zone. One sub-population was native like and the other was unfolded. The proportion of the size of the two sub-populations was varied as function of the concentration of the denaturant.
These experiments yielded solid evidence in support the model of cooperative, barrier crossing, mechanism of folding this protein. At least for this case, the model of downhill mechanism of folding is not applicable.

Platform AP: Membrane Active Peptides

2007-Plat
Effects of Oxidative Stress on Aggregation and Membrane Interaction of alpha-Synuclein Characterized by Single Molecule Fluorescence
Eva Sevcsik, Elizabeth Rhoades.
Yale University, New Haven, CT, USA.
Oxidative stress has been implicated as a major contributing factor to Parkinson's Disease (PD), a neurodegenerative disorder characterized by the deposition of fibrillar amyloid inclusions in the substantia nigra. The primary protein component of these inclusions is alpha-synuclein (aS), an abundant presynaptic protein, whose natural functions have not yet been resolved but presumably involve synaptic vesicle trafficking. Aggregation of amyloid proteins involves sampling of heterogeneous conformational and oligomeric intermediates, and it is actually these species that have been implicated to be responsible for neuronal cell death, possibly by compromising cell membrane integrity. Here, we use single molecule fluorescence techniques (fluorescence correlation spectroscopy and single molecule Förster energy transfer) to investigate the influence of oxidative modifications to both the protein and the lipid matrix on the molecular mechanisms of aS aggregation and membrane interaction. We find that oxidative modification to either protein or lipid leads to a decrease of aS vesicle binding, with the extent of decrease being dependent on the lipid matrix. As aS natural functions most likely involve synaptic vesicle binding, these results might indicate a loss of aS function due to oxidative stress. Further, oxidized aS shows a different aggregation behavior and does not form amyloid fibrils. The systematic characterization of the effects of oxidation on aS aggregation and membrane interaction will help to refine our understanding of the toxic form(s) of aS in order to identify cellular targets for the design of therapeutics to treat or prevent PD.

2008-Plat
Amyloid-β Ion Channels in Artificial Lipid Bilayers and Neuronal Cells. Resolving a Controversy
Ricardo F. Capone[1,2], Felipe Garcia Quiroz[1], Panchika Prangkio[1], Inderjeet Saluja[1], Anna M. Sauer[1], M.R. Bautista[3], R. Scott Turner[1], Jerry Yang[3], Michael Mayer[1].
[1]University of Michigan, Ann Arbor, MI, USA, [2]current address: University of Chicago, Dept. of Medicine, Chicago, IL, USA, [3]University of California, San Diego, CA, USA.
One of the current hypotheses for the pathology of Alzheimer's disease (AD) proposes that amyloid-beta (Aβ) peptides induce uncontrolled, neurotoxic ion flux across cellular membranes. The resulting inability of neurons to regulate their intracellular concentration of ions, in particular calcium ions, has been associated with cell death and may thus contribute to cognitive impairment typical for AD. The exact biophysical mechanism of this ion flux is subject of an ongoing and unresolved controversy. Two mechanisms are currently debated. One proposed mechanism suggests that Aβ assembles into pore-like structures in lipid membranes, leading to stepwise fluctuations of transmembrane current that is typical for ion channels (ion channel hypothesis). The other proposed mechanism postulates a generalized and gradually increasing ion flux as a result of Aβ-induced thinning of membranes.
Here, we resolve this controversy by examining, in detail, the two pivotal protocols for preparing and measuring Aβ induced conductance through planar lipid bilayers and cell membranes. The results clarify that Aβ induces stepwise ion flux across planar lipid bilayers as opposed to a gradual increase in transmembrane current; they show that the previously reported gradual increase in transmembrane current arises from residues of the solvent hexafluoroisopropanol, which is commonly used for the preparation of amyloid samples.
We also examined the effect of Aβ samples on cell membranes. We exposed SH-SY5Y neuroblastoma cells and mouse cortical primary neurons to Aβ at resting potential in the presence and absence of typical ion channel blockers. The results provide additional evidence suggesting that Aβ peptides can form ion channels in cellular membranes that are independent from the postulated ability of Aβ to modulate intrinsic cellular ion channels or transporter proteins.

2009-Plat
Lipid Membrane Penetration Forces from AFM Force Spectroscopy
Elizabeth A. Hager-Barnard, Benjamin D. Almquist, Nicholas A. Melosh.
Stanford University, Stanford, CA, USA.
Understanding how short peptide sequences are able to penetrate cell membranes is important in disease studies and engineering new peptides for drug delivery. While the energetics of membrane penetration has been well studied, the mechanical landscape during contact, translocation, and exit is largely unknown. We used atomic force spectroscopy (AFM) studies on lipid membrane stacks to map the force-distance profile during penetration of short peptides. These force curves reveal the spatial location and magnitudes of penetration barriers that can be related to peptide molecular structure and orientation. We studied the widely used cell penetrating peptide HIV-TAT, a positively charged 9-mer with six arginine groups. The peptides were attached in a single layer at the end of a flat AFM tip giving nanometer spatial resolution relative to the lipid bilayer. Using stacks of lipid membranes rather than individual supported membranes improves data quality by removing substrate effects and providing better statistics.

2010-Plat
Membrane insertion of peptides mimicking E2 domain of Sindbis virus is modulated by cholesterol
Thomas G. Chadwick, Ghada A. Rabah, Brian R. Davies,
Tatyana I. Smirnova.
North Carolina State Univeristy, Raleigh, NC, USA.
In the process of assembly Sindbis enveloped virus uses a host-derived membrane bilayer that is ''sandwiched'' between the concentric protein shells. The transmembrane domains of three glycoproteins penetrate the bilayer and