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
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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
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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
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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
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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
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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
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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