close to the holo-form and α3 undergoes extensive conformational changes in response to different TCAs (Fig. 1). The specificity binding loop in NCBD adopts intermediates that enables the bending and twisting of α1 and α2 into its final orientation with the target TCA. We hope this quantitative view of NCBD:TCA landscape can aid the design of novel cancer therapeutics.

54-Plat
Inferring Aggregation Mechanisms of Polyglutamine Through Quantitative Studies of Phase Behavior
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Many proteins associated with neurodegenerative diseases are intrinsically disordered, i.e. they lack a stable, folded structure under physiological conditions. It is currently believed that the aggregation of these proteins plays a causative role in neurodegenerative disease pathogenesis. Therefore, understanding how and why these proteins aggregate is crucial to understanding and possibly suppressing disease.
Proteins are polymers and protein aggregation is akin to phase separation. We employ techniques and theories borrowed from polymer physics to help understand the driving forces and mechanisms of protein aggregation. Our system of interest is polyglutamine, as expansions of polyglutamine are thought to be causally linked to the development of at least nine different neurodegenerative diseases. In this work, we measure the saturation concentrations of aqueous polyglutamine solutions containing 30 and 40 glutamines and either 2 or 4 lysines. We use classical polymer physics theory to construct the entire (soluble-insoluble) phase diagram from the measured saturation concentrations. The low-concentration arm of the phase diagram provides a thermodynamic basis for assessing aggregation propensity. For a given chain length of polyglutamine, we find that aggregation propensity increases with fewer lysines, and, for a fixed number of lysines, the aggregation propensity increases with increasing chain length. Although the phase diagrams are thermodynamic in nature, they still provide insights regarding the kinetic mechanisms of phase separation. For the concentrations used in most in vitro experiments, the phase diagrams predict that intrinsically disordered monomers first form disordered, higher-order oligomers or clusters which then undergo a nucleated structural conversion into an ordered, insoluble form. These predictions are supported by detailed atomic force microscopy studies. This work highlights the prominence of intrinsic disorder even in multimolecular complexes and its role in facilitating conformational conversion to ordered supramolecular aggregates.

55-Plat
Comparison of α-Synuclein and Amyloid Beta Membrane Interactions
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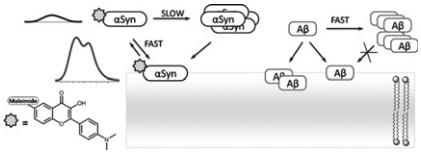
α-synuclein (αSyn) and amyloid beta (1-42) peptide (Aβ) are the main constituents of pathological deposits in the midbrain of individuals affected by Parkinson's disease and Alzheimer's disease respectively. Defining the interactions of αSyn and Aβ with membranes is essential for understanding the neurotoxicity caused by mutations and/or overexpression.



We performed a systematic *in vitro* study of the effects of membrane charge, phase, curvature, defects and lipid unsaturation on αSyn and Aβ binding using model vesicles and proteins labeled with a new solvatochromic fluorescent probe. The probe's emission spectrum strongly depends on the membrane properties, allowing clear discrimination of the protein bound to vesicles of different composition that enables measurements of the kinetics of αSyn migration between membranes of different compositions [1]. The interaction of αSyn with vesicular membranes is fast and reversible while the membrane binding of Aβ is mainly kinetically controlled and competes with aggregation. By introducing the probe at different positions of αSyn and Aβ we were able to estimate the relative immersion of different protein domains into membrane, and its changes depending on membrane composition and lipid-to-protein ratio.
[1] Shvadchak, et al., *J. Biol. Chem.*, (2011).

56-Plat
Protein Disorder and Degradation: Is Ubiquitin the Missing Link?
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The ubiquitin-proteasome system is responsible for degradation of numerous proteins. How the proteasome successfully degrades such a large variety of proteins is not well understood. It is thought that in order to be degraded, proteins must have a disordered region (an unstructured stretch of residues of a certain length) that can be used by the proteasome to initiate degradation. We have assembled a dataset of proteins that undergo ubiquitination and degradation, and characterized their structural properties (**Hagai et al., JMB, 2011**). Surprisingly, we observed that ~25% of the proteins that are successfully degraded lack the needed disordered region. Therefore, an additional mechanism should somehow provide a disordered region to enable degradation. Since the majority of proteins are ubiquitinated prior to their degradation, it is possible that the ubiquitin chain itself can influence the structure and thermal stability of the protein.
Using molecular dynamics simulations we showed that ubiquitination can significantly alter the protein's structure and stability (**Hagai and Levy, PNAS, 2010**). Ubiquitination of the substrate Ubc7 at the residues that are modified in vivo prior to degradation uniquely results in significant thermal destabilization and a local distortion near the modification site. These effects are specific to these sites, while other lysine residues which are not used in vivo, display diverse behavior upon ubiquitination. This indicates that ubiquitination can facilitate the unfolding process and create disordered regions, and therefore can assist degradation.
Our findings suggest that in addition to its signaling role, ubiquitination may alter protein biophysics to support degradation.
References:
Intrinsic disorder in ubiquitination substrates.Hagai et al. ***JMB***, 2011.
Ubiquitin not only serves as a tag but also assists degradation by inducing protein unfolding. Hagai and Levy. ***PNAS***, 2010.

Platform: Molecular Mechanics & Force Spectroscopy

57-Plat
Highly Covalent Ferric-Thiolate Bonds in Rubredoxin Exhibit Surprisingly Low Mechanical Stability
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Depending on their nature, different chemical bonds show vastly different stability with covalent bonds being the most stable ones that rupture at forces above nanoNewton. Studies revealed that Fe-thiolate bonds in metalloprotein are highly covalent and are conceived to be of high mechanical stability. Here we used single molecule force spectroscopy techniques to directly determine the mechanical strength of ferric-thiolate bonds in rubredoxin. We observed that the ferric-thiolate bond ruptures at surprisingly low forces of ~200 pN, one order of magnitude lower than that of typical covalent bonds, such as C-Si, S-S and Au-thiolate bonds. And the mechanical strength of Fe-thiolate bonds is observed to correlate with the covalency of the bonds in different protein systems. Our results shed new lights on the nature of Fe-thiolate bonds and suggest that highly covalent Fe-thiolate bonds are mechanically labile and clearly distinguish themselves from typical covalent bonds.

58-Plat
Towards a General Platform to Study Single-Bond Chemistry Under Force
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The application of force to reagents that participate in a chemical reaction can probe the transition state of the reaction with sub-Angstrom resolution. Using single-molecule force-clamp spectroscopy, this approach has been extensively applied to the cleavage of disulfide bonds in proteins. However, to date there is no methodology that can expand this class of experiments to bonds not naturally present in proteins. Here, we introduce an experimental platform with the potential to fulfill the requirements to perform single-bond rupture determinations on any covalent bond. We engineered polyproteins based on the I27 domain of titin including two cysteine residues. Bifunctional crosslinking molecules specific for thiol groups were then used to generate intradomain covalent bridges between the engineered cysteines, much similar to the manner disulfides link polypeptide chains. Different bismaleimide reagents containing cleavable covalent bonds were tested for their ability to crosslink the I27 domain. We used single-molecule force- spectroscopy to pull from the modified polyproteins. Successfully crosslinked domains gave rise to lower increments in contour length after mechanical unfolding, consistent with the number of amino acids protected in the protein loop formed by the covalent bridge. Using our new strategy, virtually any covalent bond can be used to generate intradomain crosslinks in proteins. Our method can be adapted to produce hybrids