138-Symp
Folding and Dynamics in an Allosteric Response
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Allosteric mechanisms that incorporate protein folding and/or changes in dynamics have been reported for many systems. However, the connection between local events and global changes in dynamics remains unclear. The *Escherichia coli* biotin repressor, BirA, provides a model for investigating the relationship of these two phenomena in an allosteric process. Corepressor binding to BirA results in a −4.0 kcal/mole enhancement of the Gibbs free energy of dimerization, thereby enabling assembly of a transcriptional regulatory complex on DNA. Thermodynamic measurements on single amino acid variants of BirA reveal that corepressor-induced folding of a flexible loop that is distal to the dimerization interface is required for the full allosteric response. Hydrogen-deuterium exchange measurements indicate a correlation between the allosteric activation and a decrease in the backbone hydrogen exchange throughout the protein. This decreased exchange is consistent with an allosteric mechanism that involves suppression of protein dynamics in the entire protein. The connection between the local ligand-induced folding and the global dynamic changes was investigated using hydrogen-deuterium exchange studies of BirA variants in which loop folding and the allosteric response are compromised.
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139-Symp
Membrane Induced Conformational Transitions in Alpha-Synuclein Aggregation and Function
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Alpha-synuclein is a small (140 residue) pre-synaptic protein that is genetically and histopathologically linked to the etiology of Parkinson'S disease. While intrinsically disordered when isolated in solution, the protein transitions into a number of helical conformations upon interactions with membranes or membrane-mimetics. Using NMR, ESR and CD spectroscopy, we delineate the structural features of these conformations and show that the associated transitions can favor the formation of small aggregates that are similar to those linked with the protein'S toxicity to cells, and that the three Parkinson'S-linked mutations, A30P, A53T and E46K, can influence these transitions in ways that may influence both the normal and pathological activities of the protein. Our results highlight the importance of membrane interactions in alpha-synuclein function and pathology.

PLATFORM K: Protein Folding & Stability I

140-Plat
Monitoring Proteostasis in Populations of Living E. coli Cells at the Single-Cell Level
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Here we describe an *in vivo* system to quantitatively monitor proteostasisin populations of living *Escherichia coli* cells. We use this system to estimate, for the first time in any organism, the spontaneous error rate that leads to the collapse of proteostasis.
The system is conceptually founded on the theory of linked equilibria and it utilizes carefully selected temperature sensitive mutant proteins as sentinels of cellular proteostasis. A subpopulation of cells carrying the sentinel *ts* mutations displays the mutant phenotype even at permissive temperature. The sentinel proteins were selected for easy microscopic and cytometric identification of this subpopulation. Overexpression of chaperones suppresses this partial penetrance at the permissive temperature arguing that the *ts* mutant allele generates a mutant phenotype at the permissive temperature in cells where chaperone capacity is limiting.
The deficiency in chaperone capacity in this subpopulation could be due to 1) lower chaperone concentration as a result of molecular noise; or 2) the accretion of errors leading to failures of proteostasis and the consequent titration of chaperones by unfolded or partially folded proteins. Using fluorescent reporters we measure the concentration of chaperones in individual cells and find no deviations from the mean in the subpopulation displaying the mutant phenotype. The failure of the function of the sentinel protein is therefore due to errors in proteostasis that engender the titration of chaperones by unfolded or partially folded proteins in the proteome. This is further supported by the fact that this proteostatic deficiency is inherited epigenetically in lineages of cells and the fact that these cells die at a higher rate when faced with proteotoxic stresses.
We use this system to calculate the spontaneous error rate at which proteostasis fails in *Escherichia coli* under standard growth conditions.

141-Plat
How Pulling Forces Tune Redox Potentials of Disulfide Bonds
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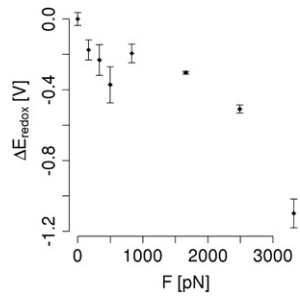

Mechanical pulling forces have been shown to significantly alter the rate of redox reactions in various thiol/disulfide exchange systems [1]. However, it has been questioned if force can directly affect the electron affinity of such redox systems [2]. We here investigated the impact of mechanical force on disulfide bonds. We used hybrid quantum/molecular mechanical simulations of cystine in water and extensive conformational sampling to estimate redox potentials under pulling forces in the range of 0 to 3300 pN. We found that forces as small as a few 100 pN alter the redox potential by several 100 mV, which is significant regarding that similar variations are found among simple reducing agents or upon mutations in thioredoxin. Thus, mechanical stress as proteins in living organisms often experience can tune biochemical reactivity not only by protein unfolding and exposure of reaction sites but also by directly tuning the electronic energy of a bond.
[1] Wiita et al. 2007. Nature. 450:124-127; Jiminez et al. 2009. Nat. Struct. Mol. Biol. 16: 890-896.
[2] Kucharski et al. 2009. Angew. Chem. Int. Ed. 48:7040-7043.

142-Plat
Structure, Function and Folding of Phosphoglycerate Kinase are Strongly Perturbed by Macromolecular Crowding
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We combine experiment and computer simulation to show how macromolecular crowding dramatically affects the structure, function and folding landscape of phosphoglycerate kinase (PGK). Fluorescence labeling shows that compact states of yeast PGK are populated as the amount of crowding agents (Ficoll 70) increases. Coarse-grained molecular simulations reveal three compact ensembles: C (crystal structure), CC (collapsed crystal) and Sph (spherical compact). With an adjustment for viscosity, crowded wild type PGK and fluorescent PGK are about 15 times or more active in 200 mg/ml Ficoll than in aqueous solution. Our results suggest a new solution to the classic problem of how the ADP and diphosphoglycerate binding sites of PGK come together to make ATP: rather than undergoing a hinge motion, the ADP and substrate sites are already located in proximity under crowded conditions that mimic the in vivo conditions under which the enzyme actually operates. We also examine T-jump unfolding of PGK as a function of crowding experimentally. We uncover a non-monotonic folding relaxation time vs. Ficoll concentration. Theory and modeling explain why an optimum concentration exists for fastest folding. Below the optimum, folding slows down because the unfolded state is stabilized relative to the transition state. Above the optimum, folding slows down because of increased viscosity.

143-Plat
Nanomechanics of Membrane Proteins Probed by Atomic Force Microscopy
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Understanding of how membrane proteins fold in their native lipid bilayers is lacking - a major reason being that membrane proteins aggregate outside the lipid bilayer. Therefore, detergents and lipid vesicles are used to study these important proteins. However, the folding and function of a membrane protein in detergents or in lipid vesicles may not mimic its behavior inside the native lipid bilayer. It is, therefore, crucial that new techniques be employed to study membrane protein (un)folding inside their native lipid bilayers.
Atomic force microscope based single-molecule force spectroscopy (SMFS) experiments have provided us with insight into the (un)folding of proteins hitherto inaccessible by conventional methods which probe the bulk behavior of