

Joint Physical/Chemical Biology

Tuesday,
September 21, 2010

Seminar
11:00 a.m.

Room 1315
Chemistry Building

Protein Yoga: Stretching and Relaxing Proteins One Molecule at a Time



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In order to fold and function, proteins must explore structural fluctuations away from their unique native conformations. Such fluctuations result in a distribution of conformations of differing stabilities that, together with the barriers separating them, constitute a protein's energy landscape. In spite of their general importance, unfolding events occur very rarely under native conditions, and the populations of unfolded conformations are very small, rendering experimental characterization of these non-native regions of the landscape extremely challenging. I will present our studies using both ensemble and single molecule methods to probe these high-energy fluctuations. In particular we have developed a novel method to explore the mechanical stability of single protein molecules in the low force regime of the laser tweezers. Individual protein molecules are tethered between two polystyrene beads using two dsDNA molecules, which are in turn attached to the protein through unique engineered cysteine residues. One bead is held by suction on the tip of a pipette while the other is held in the optical trap. The force and the distance between the beads are controlled and monitored by moving one of the beads relative to the other. The DNA handles can be attached to any exposed cysteine residues, allowing a variety of pulling geometries to be used to investigate the anisotropy of the protein energy landscape. Recently we uncovered a novel unfolding intermediate for the model protein RNase H identifying a force-dependent change in the transition state for unfolding which suggests that the region of a protein that resists unfolding by molecular machines will depend on the applied force. I will also present our recent developments of thiol exchange methods that allow partitioning of fluctuations and partially unfolded conformations to either side of the rate limiting step for unfolding.

Refreshments will be available prior to the seminar at 10:45 a.m. outside room 1315

Graduate Students may meet with the speaker at 1:15 p.m. in Room 8335