

INVITED LECTURE T1

Force-clamp spectroscopy of single proteins

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We have developed single molecule AFM techniques to study how mechanical forces affect the dynamics and chemistry of proteins. Using molecular biological techniques, we engineer tandem modular proteins that are made of identical repeats of a protein of interest. These polyproteins act as handles for atomic force microscopes, without the need for linkers or special attachment chemistry. When such polyproteins are extended by an AFM, their force properties are unique mechanical fingerprints that unambiguously distinguish them from the more frequent non-specific events that plague single molecule studies. We combine polyprotein engineering together with active force-clamp AFM techniques. With this approach, the length of an extending polyprotein is measured while the pulling force is actively kept constant by negative feedback control. The force-clamp technique combined with polyprotein engineering has become a powerful approach to study proteins. We have investigated the force-dependency of protein folding, unfolding and of chemical reactions. From the force-dependence, we extract features of the transition state of these reactions that reveal underlying molecular mechanisms. Our data will help guide the development of new theories on areas such as the statistical dynamics of a folding polymer and *ab-initio* studies of a chemical reaction while placed under a stretching force.

References:

1. Fernandez., J.M *et al.*(2004), *Science*, 303: 1674-1678
2. Wiita *et al.* (2007), *Nature*, 450:124-7.