Using force to investigate the stability of proteins and their complexes

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Introduction

Mechanical forces such as those encountered during hydrodynamic flow and cell deformation are ubiquitous in Nature and consequently many proteins are adapted to resist or respond to a mechanical stimulus. The ability to specifically tether and manipulate single biomolecules using techniques such as optical tweezers and the atomic force microscope has allowed experimentalists to measure the effects of force on the stability (or lifetime) of proteins and their complexes, as well as the measurement of the steps size and the effect of load on a wide variety of processive motors.

The aim of our research is to investigate the fundamental effects of force on model proteins and their complexes and apply this knowledge to understand the many seemingly diverse processes *in vivo* where force has been implicated, such as protein translocation and degradation, the remodelling of complexes and signal transduction. Currently research in the group covers three areas: (i) delineation of the mechanical unfolding transition state and rational engineering of the mechanical stability of the small, topologically simple protein L; (ii) measurement of the effects of complexation on the mechanical unfolding energy landscape of proteins and (iii) direct measurement of the unbinding force and pathway of protein:ligand interactions.

Breaking apart high affinity complexes by application of small forces.

As part of their function many proteins form complexes with other proteins or non-proteinaceous ligands. In some cases, the affinity between ligand and receptor is very high to sequester either toxic substances or nutrients vital to other organisms (biotin:streptavidin, for example) or to inhibit the action of a potentially deleterious enzyme. In many cases the high affinity of the complex results from a rapid, diffusion limited on-rate and an off-rate of the order of tens of hours – far longer than the timescales over which most biochemical processes operate.

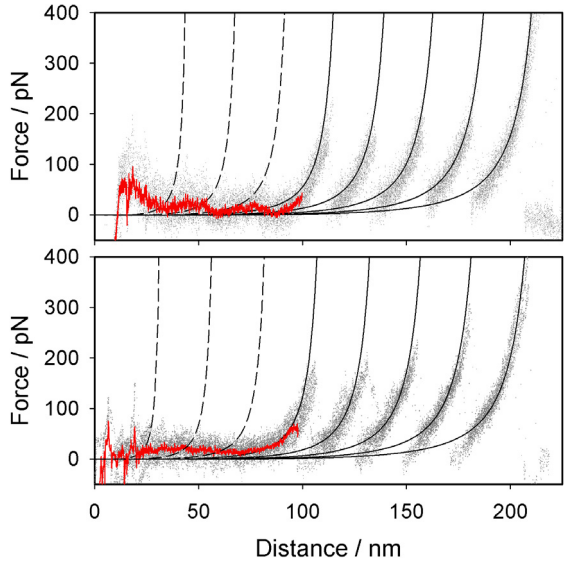

Figure 1: In a heteropolymer comprising of four I27 domains alternating with three Im9 domains, uncomplexed Im9 unfolds at a force below the thermal noise limit of the AFM (bottom). Upon complexation with E9 (top), the mechanical resistance of Im9 increases, but the protein still unfolds at a relatively low force (~35 pN). Multiple force extension profiles (grey dots) are plotted for each experiment. Black continuous and dashed lines show the predicted position of unfolding events for I27 and Im9, respectively. Red lines show a running average of the overlaid plots in the region that Im9 unfolds.

We have been investigating the role that force may play in the remodelling of protein complexes by studying the strong interaction between the colicin nuclease E9 and its ligand, the four helical bacterial immunity protein, Im9. Colicins are bacteriocins produced by some strains of *E.coli* which are toxic to related species, but not the host, owing to the production of an inhibitory immunity protein which binds tightly to the nuclease (K_d ~fM) preventing hydrolysis of the host's DNA. However, once exported out of the host cell, this highly avid interaction must be broken to allow cell invasion and enable catalytic activity. How does this occur on such a

rapid timescale? Many aspects of the colicin import pathway are currently poorly understood but it is thought that force may play an important role *via* either coupling to the proton motive force across the inner membrane or by the action of an energy dependent ATPase protease.

To test this theory the mechanical unfolding strength of Im9 was measured in the absence (Figure 1, bottom) and presence of E9 (Figure 1, top) by constructing a tandem array of alternating Im9 and I27 domains (the latter has previously been shown to display significant mechanical strength and its unfolding profile therefore acts as control to verify the unfolding of Im9). In the absence of E9, Im9 unfolds at a force below the thermal noise limit of the AFM (< 20 pN) in accord with the hypothesis that all α-helical proteins are generally mechanically weak. Surprisingly, complexation with E9 was found to enhance the mechanical strength of Im9 only slightly which still unfolded (and therefore dissociated from E9) under the application of a relatively low level of force (~35 pN). These data suggest that it is possible to disrupt strong complexes *in vivo* by application of a small force to one member of a protein pair. We are now testing this hypothesis by measuring the effects of complexation on the degradation rate of Im9 by a large bacterial protease thought to actively unfold substrate proteins before proteolysis.

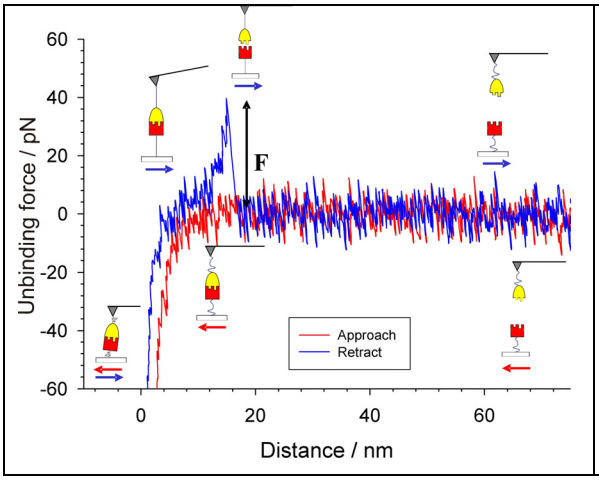

Figure 2: Measurement of the unbinding force (F) for a single E9/Im9 complex by AFM. Upon approach to the surface (red arrows and force-distance trace) a complex is formed between Im9 (yellow) attached to the cantilever tip *via* a polyethylene glycol linker and E9 (red) immobilised to a silicon nitride substrate immobilised in the same manner. Retracting the tip (blue arrows and force-distance trace) increases the force applied across this interaction until it is broken and the force returns to zero.

In addition to measuring the effects of complexation on the mechanical properties of Im9, we have recently developed protocols to specifically immobilise Im9 and E9 to an AFM tip and silicon nitride substrate, respectively (Figure 2). By sequentially approaching and retracting the Im9 labelled tip from an E9 covered surface, it is possible to measure the rupture force of a single protein:protein interaction. Repeating these experiments at different retraction speeds allows the underlying energy landscape of this strong interaction to be mapped, revealing features difficult to detect by standard ensemble methods and allowing estimates of the magnitude of force required to disrupt such complexes *in vivo*.

Collaborators

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Publications

The effect of protein complexation on the mechanical stability of Im9. Hann, E., Kirkpatrick, N., Kleanthous, C., Smith, D., Radford, S. and Brockwell, D. (2007) *Biophys. J.*, **92**:L79-L81.

Probing the mechanical stability of proteins using the atomic force microscope. Brockwell, D. (2007) *Biochem. Soc. Trans*. **35**:1564-1568.

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