Exploring the effect of mechanical forces on biopolymers through theory, models and novel experimental techniques

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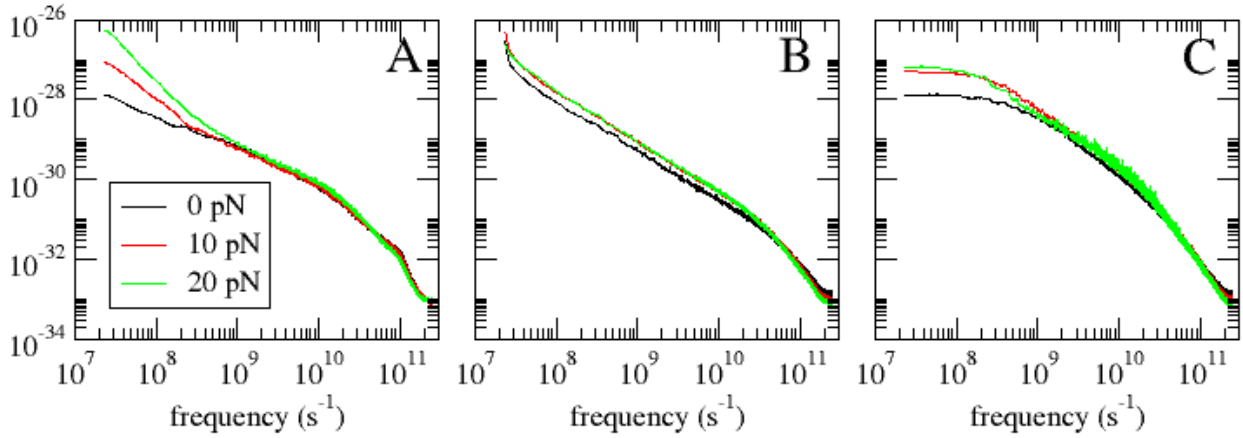
In the past 10 years mechanical experiments on single proteins has not only revealed how proteins unfold when extended, but has also addressed key questions on the initial stages of protein folding. Within the Astbury Centre, mechanical unfolding and mechanical properties of biopolymers in general have been extensively studied using innovative experimental and theoretical approaches.

On the experimental side we have developed a technique where, in addition to the force that is applied to the protein by retracting the AFM cantilever, a small periodical perturbation force is added. We have also perfected our novel low noise AFM force spectroscopy apparatus, with damping of thermal noise on a very soft AFM cantilever down to an effective temperature of 3 Kelvin (in a 300 K buffer solution). Recent results using the system in a force-clamp mode have shown a second unfolding event in the mechanical unfolding of protein L for the first time.

A theoretical framework based on viscoelastic models with internal friction and two- and three-state models has been developed to interpret the fluctuations of the distance between the two ends of a tethered protein. This provides the elasticity, relaxation times and internal frictions and has been tested on polysaccharides (cellulose, dextran, pectin). We are currently using this approach to investigate peptides and proteins. Computer simulation on increasingly complex systems was used to test the theory: starting from a freely jointed chain, we then simulated polysaccharides (dextran and pectin) and we have recently simulated short peptides of different structure (α-helical, β-hairpin and a flexible structureless peptide). Even in these more complicated cases where the free energy landscape has multiple minima, the spectrum of the fluctuations can be interpreted in terms of the simple models mentioned above. These simulations provide a proof of principle of the possibility of extracting more information from single molecule mechanical experiments than the force of unfolding and distance to the transition state (xu) obtainable using force velocity and force clamp experiments.



Power Spectral Density (PSD) of the fluctuations of end-to-end distance for three peptides with different secondary structural propensity: A) α-helical (Ala12), B) β-hairpin and C)3- flexible (Gly10) peptides. The PSD is the Fourier transform of the time correlation function of the end-to-end distance of peptide and thus defines some characteristic time τ (or times τ_i) for system. This function can be measured by single molecule AFM and is related to the energy dissipation spectral density $D(\omega)= \zeta\, \omega PSD(\omega)$ at given frequency ω (where ζ is the friction). In the case of a simple dynamical process with characteristic time τ (or friction coefficient ζ) and elasticity coefficient K it has a plateau at low frequencies and linear decay at higher ones in log-log scale. If

there are more processes each of these corresponds to a plateau and a linear decay in log-log scale. The frequency at which PSD decreases to half of its plateau value is equal to reciprocal characteristic time τ of process. The plateau value of PSD is a function of both elasticity coefficient K and characteristic time τ. Such fitting at different forces F applied to the end of peptide in single molecule AFM have been used to obtain the dependence of $K(F)$ and $\tau(F)$ on force. Thus PSD(ω) provides a dynamical fingerprint of the peptides.

Force clamp and constant velocity experiments have traditionally used a Bell-type model to exctract thermodynamic information about the unfolding (or unbinding) properties of a protein (see Using force to investigate the stability of proteins and their complexes Eleanore Hann, Jim Pullen, David Sadler, Sheena Radford and David Brockwell in these reports). Improved methods to treat the experimental data still have the drawback of involving the projection of the free-energy landscape on a single variable. We have recently analysed the distribution of unfolding times while a constant force is applied to a protein, which are measurable with force-clamp technology. This provides the model-free estimation of parameters which characterise the free energy landscape and the dynamics, but it still assumes a projection onto a one-dimensional variable. Ongoing work to improve this theory includes devising and modelling simple potentials for unfolding, which can be generalized systematically by adding more degrees of freedom. This will provide additional physical mechanisms that can contribute to the effective 1D parameters, and provide guidance for new experiments to probe the directional dependence of mechanical resistance, as well as how the control of noise can, perhaps unexpectedly, be a valuable probe of the shape of the energy landscape.

Publications

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