

The Physics of Soft and Biological Matter

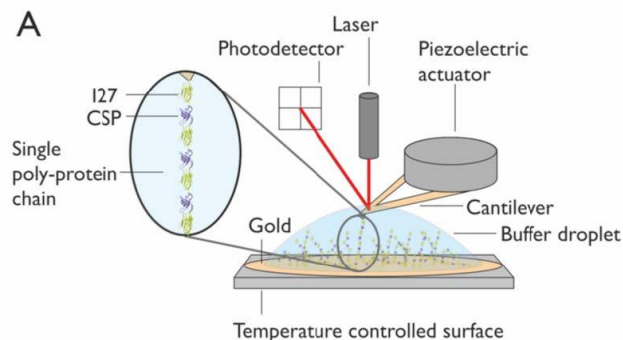
P.07 Variable temperature single molecule force spectroscopy of an extremophilic protein

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Extremophiles (organisms which survive and thrive in the most extreme chemical and physical conditions on Earth) exhibit a range of fascinating cellular- and molecular-level adaptations [1]. The ‘flexibility’ of extremophilic proteins is one of the key determinants of their ability to function at the extremes of environmental temperatures [2].

We use single molecule force spectroscopy (SMFS) by atomic force microscopy (AFM) to measure the effect of temperature on the mechanical stability and flexibility of a protein derived from a hyperthermophilic organism, *Thermotoga maritima* [3]. Our chosen model protein is cold shock protein (CSP) B, a 66-residue nucleotide-binding protein with a known structure and with well-characterised thermodynamic and kinetic properties [4 - 6]. We construct a poly-protein chain consisting of CSP and I27, a marker protein, which provides a fingerprint for our studies [5].



Schematic illustrating the AFM SMFS variable temperature experimental setup – taken from [2]

The study was performed using an AFM SMFS instrument with variable temperature capabilities. In this experimental technique, a constant stretching force or a constant stretching velocity is applied along the end-to-end length of the protein, driving the protein into a fully extended unfolded state. We study temperature-dependent changes in the unfolding energy landscape of this protein by measuring changes in the unfolding force with temperature in combination with Monte Carlo simulations. We find that the position of the transition state to unfolding shifts away from the native state with increased temperature, reflecting a reduction in the spring constant of the protein and an increase in structural flexibility [2]. The mechanical robustness and malleability of this protein provides an insight into the dynamical properties of hyperthermophilic proteins.

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