Extreme biophysics: a single molecule approach to explore proteins from extreme environments

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Introduction

Life has adapted to a vast range of environmental conditions and it is now difficult to find any place on Earth devoid of life. Some conditions are extreme in the sense of being unfavourable to most eukaryotic life forms. The adaption of proteins played a key role in enabling extremophilic organisms to colonise such ecological niches (Figure 1). Understanding the physical properties of proteins from extremophilic organisms and their remarkable preservation capability is not only of fundamental interest, but also pivotal to our ability to rationally engineer biological materials for exploitation.

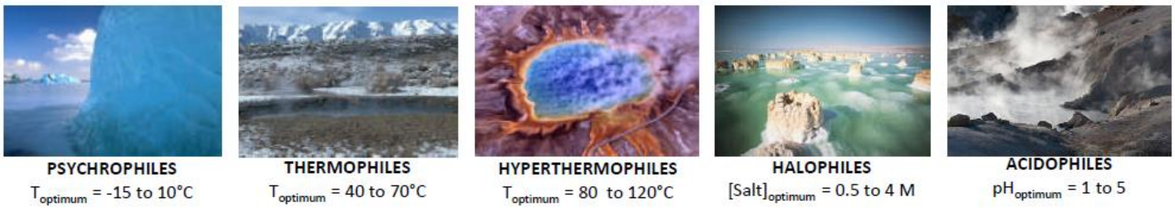

Figure 1: survival in the most adverse of environmental conditions requires extensive adaptations to enable life at the extremes of temperature, pressure or salinity. Extremophilic (extreme-loving) organisms have evolved unique features to enable them to function in these conditions (T_{opt}=optimum organism temperature).

Developing new tools to explore extremophile proteins

We are developing quantitative biophysical approaches to characterise the physical mechanisms of protein folding and stability in extreme environments. We have built a force spectroscopy instrument which we use to examine the conformational dynamics of single extremophilic proteins. This technique is used to apply a constant stretching force along a well-defined reaction coordinate, the end-to-end length of the protein, driving proteins to a fully extended unfolded state (Figure 2). By examining single molecules one at a time, the individual dynamics of protein subpopulations can be measured, revealing information which may be crucial for understanding and designing 'artificial' extremophilic proteins.

New insight gained from this approach

A single molecule approach provides a new perspective and reveals novel insight into the mechanisms of protein folding particularly with regard to how peptide chains fold in interplay with various environmental parameters. Moreover, this approach could aid in the design of extremophilic proteins which possess specific thermal or mechanical stability, binding specificity and conformational flexibility for exploitation in biotechnology and medicine. Our study will not only help to better understand life on Earth but also its prospects elsewhere.

Harnessing the power of extremophile organisms

Proteins with high stability are a prime target for biotechnology, for their prolonged life in storage and application and for their utility under extreme conditions. In addition to withstanding extreme temperatures, such proteins are resilient to organic solvents and proteolytic attack. While specific extremophilic proteins with particular capabilities have been sought, engineering stability into mesophilic proteins is an attractive alternative. The increased availability of complete genomes, coupled with the improved resolution of protein structures, has provided the potential to obtain stable proteins designed for individual

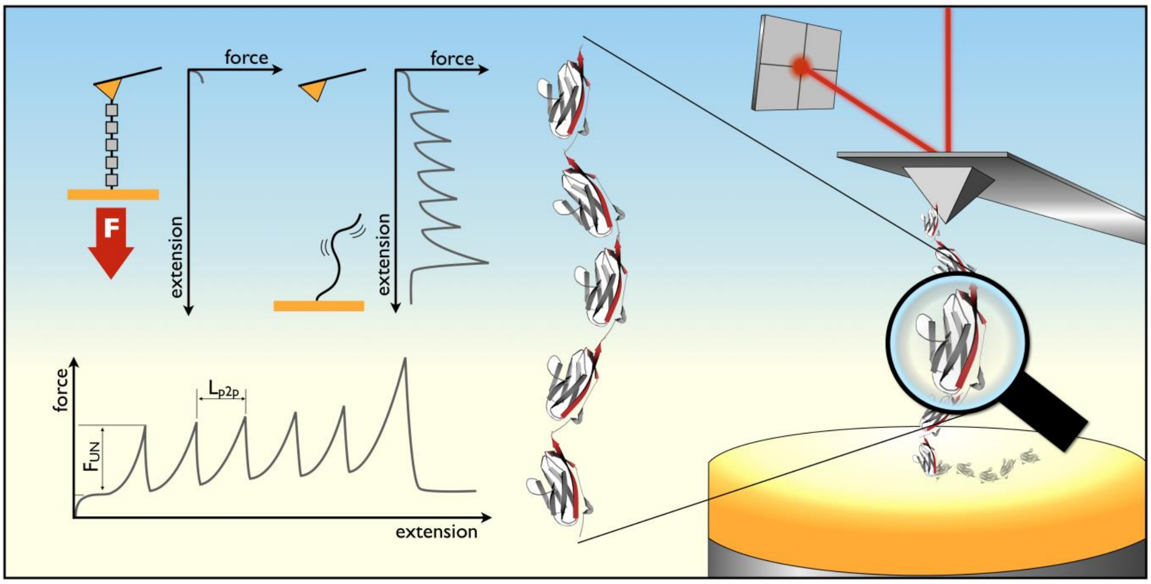

Figure 2: in single molecule force spectroscopy, the polyprotein chain is tethered between a substrate (e.g. gold) and the tip of a silicon nitride cantilever. Cysteine residues can be used to anchor one end of the protein by thiol-gold interactions while the opposite end of the polyprotein chain attaches non-specifically to the cantilever tip. The force acting on the protein displaces the cantilever with a known spring constant. That displacement is detected by the change of deflection of a laser focused on to the back of the cantilever tip.

potential applications. However, the advancement of extremophilic research relies on the continual development of enabling technology to examine and characterise the stability, flexibility and function of these proteins.

Publications

Hoffmann, T. & Dougan, L. (2012) Single molecule force spectroscopy using polyproteins. *Chem. Soc. Rev.* **41:** 4781-4796.

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Collaborators

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