Protein unfolding *in vitro* vs. *in cellulo* – from dilute solutions into the cell

Module time 4-5 hours

When 10 a.m.
Where NCDF 03/30
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Abstract The cellular solvent better known as the cytosol is a complex solution of various salts, metabolites and macromolecules where the macromolecular concentration can be as high as 400 g/l. These peptides and proteins can influence each other, affecting folding, mobility or association properties. Furthermore this high concentration of macromolecules increases the viscosity of the solution, slowing down diffusion controlled processes.
Specific and unspecific interactions between biomolecules can take place resulting in the modulation of activity, folding or aggregation. For example chaperones as part of the protein quality control network can prevent / reverse aggregation or misfolding of proteins.

To investigate the influence of the cellular environment on the unfolding / refolding behavior of proteins we have created different fluorescent folding probes with fluorescent proteins, AcGFP1 and mCherry which can interact with each other forming a FRET[1] pair (Figure 1). In the folded state the two fluorophores are in close proximity to each other leading to a high FRET signal. If the folding probe unfolds the polypeptide chain extends leading to a reduction in the FRET state.

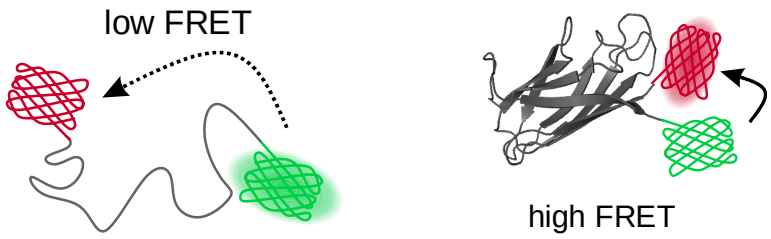

Figure 1 Schematic representation of the folding sensor (e.g. SOD1)

The used folding probe will be a variant of either SOD1 or PAPSS2b which are well understood model system of protein folding. Both model systems unfold in a 2-state manner[2] and their folding transition is highly reversible which makes it an ideal candidate for studying protein folding *in vitro* or *in cellulo*.

As part of an ongoing project we have generated different thermo-sensitive mutants of those proteins to study the free energy landscape inside cells. To investigate the unfolding process of the protein *in vitro* as well as in living cells we apply FRel (Fast Relaxation Imaging)[3]. This method utilizes high speed fluorescence imaging in combination with tailored infrared laser pulses to heat the cells until the protein unfolds (Figure 2). The unfolding can be detected by analyzing the recorded FRET signal of the fused fluorophores (Figure 1).

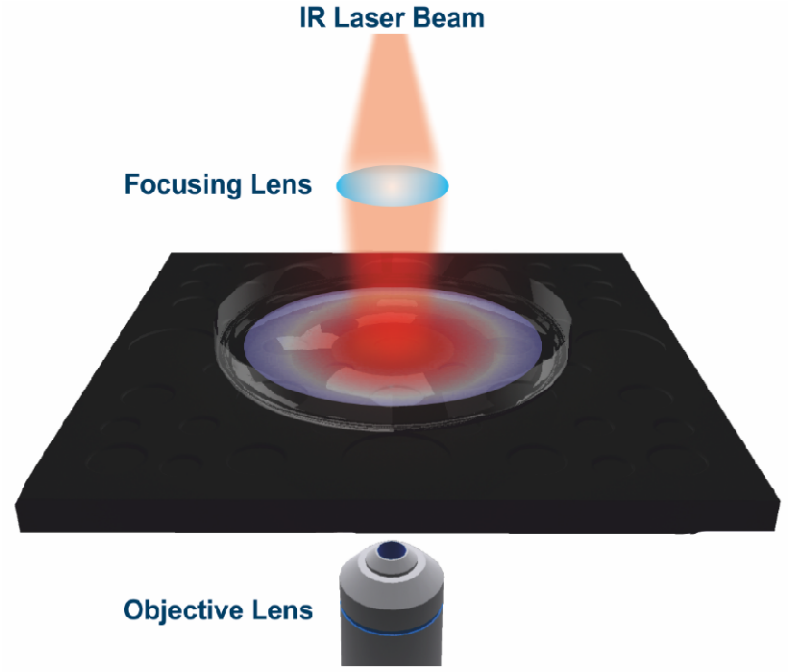

Figure 2 Schematic of the FReI setup

As part of the advanced module participants will have the opportunity to apply FReI to one folding model either *in cellulo* or *in vitro*. Furthermore, an introduction into subsequent analysis of the recorded data is given.

Suggested reading

1. J. R. Lakowicz, *Principles of fluorescence spectroscopy*, 3rd edn., Springer, New York, 2006.
2. P. L. Privalov, *J. Mol. Biol.*, 1996, 258, 707-725.
3. S. Ebbinghaus, A. Dhar, J. D. McDonald and M. Gruebele, *Nature methods*, 2010, 7, 319-323.