H4. PROTEIN REFOLDING AND TRANSLOCATION: BIOLOGY MEETS NANOSCIENCE

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The understanding of protein folding is a central problem in present post-genomic biology. On one hand, protein misfolding is involved in many diseases: Alzheimer's and Parkinson's diseases, bovine spongiform encephalopathy... On the other hand, in many cases recombinant protein synthesis comes up against the formation of inclusion bodies. These inclusion bodies are solubilized using a denaturing agent such as urea or guanidinium chloride. Then *in vitro* refolding difficulties are often encountered, more particularly for high molecular weight and multidomain proteins. Current view of protein folding involves a minimum of free energy pathway through the conformational energy landscape. To progress in the understanding of protein folding, this paradigm needs to be overcome. Actually *in vivo*, the nascent protein folding mechanism, as well as unfolding-translocation-refolding cycles observed in many cases, suggests that sequential refolding is a key feature. Sequential refolding means that one extremity of the peptide chain begins to refold without the knowledge of the remaining peptide chain sequence. This is the key point we try to mimic *in vitro*.

Our approach consists in studying protein refolding by performing *in vitro* translocation (see Fig. 1), developing techniques to measure and control translocation time and developing nanoporous media adapted to this application. To this end, different strategies are investigated.

Protein translocation through single protein nanochannel in lipid bilayer is studied. Here, nanochanel is α-Hemolysin from Staphylococcus Aureus that has been already used for DNA translocation. This single molecule experiment allows patch-clamp technique to be used for measurement of translocation events (frequency of events, duration of a single event, see Fig. 1).

Ref. 1 reports the first experiment concerned with *in vitro* translocation of an unfolded protein, Maltose Binding Protein, that has the ability to be unfolded at low concentration of denaturing agent ([Gdm-HCl ~1 M) leaving intact the proteic nanochannel. This result demonstrates translocation feasibility in the case of unfolded protein and opens up to new means of investigation for unfolding-refolding mechanism.

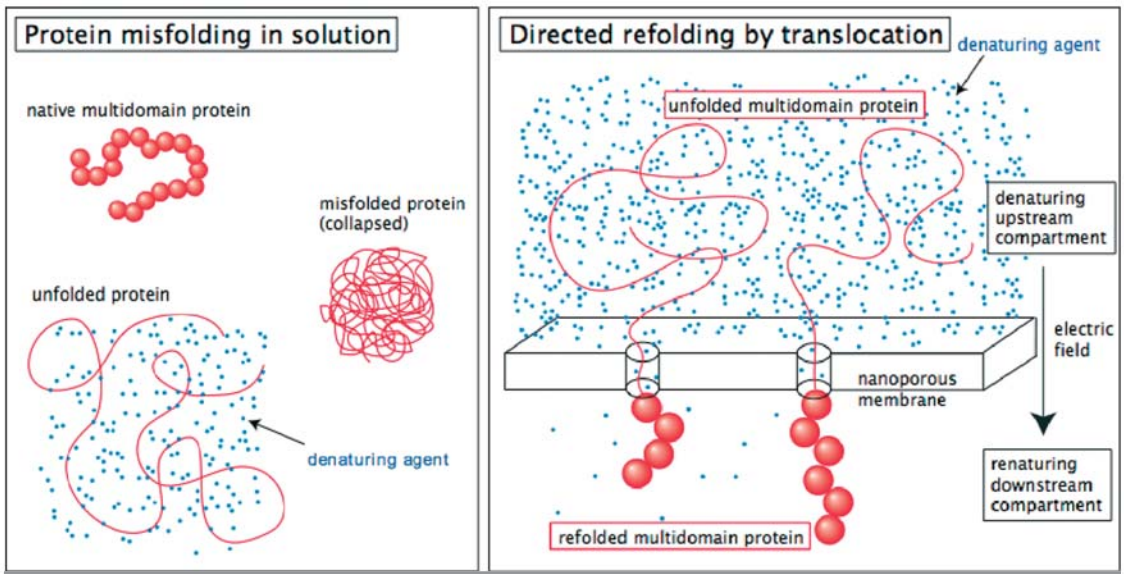

Figure 1. Protein refolding directed by in vitro translocation. An adequate translocation velocity should favour a correct refolding of multidomain proteins.

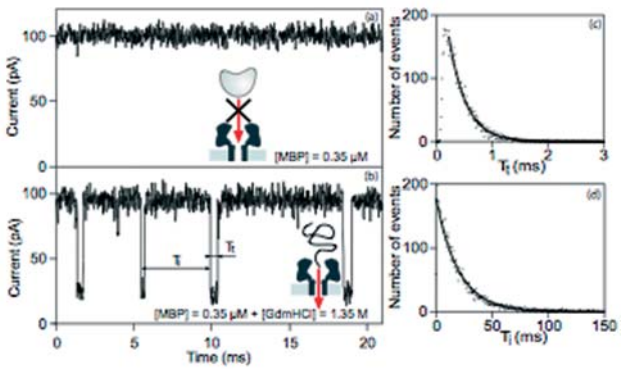

Figure 2 (from Ref.1). Current traces through a-Hemolysin (at100mV) in presence of Maltose Binding Protein (MBP) at 0.35µM. Left: a) with denaturing agent, native protein cannot passes through the nanochannel. Measured current is constant (100 pA). b) with denaturing agent ([GdmHCl=1.35M), MBP is unfolded the current trace decreases down to 20 pA when a molecule is in the pore. Right: c) Distribution of translocation times. d) Distribution of time intervals between two events.

Protein nanochannels technique knows limitations: fragility to osmotic gradient that should be necessary to direct the refolding of translocated proteins; low frequency of translocation events (single pore) leading to production of small quantities... For these reasons, synthetic nanoporous membranes have to be preferred and specially designed for this application. Two ways are currently investigated.

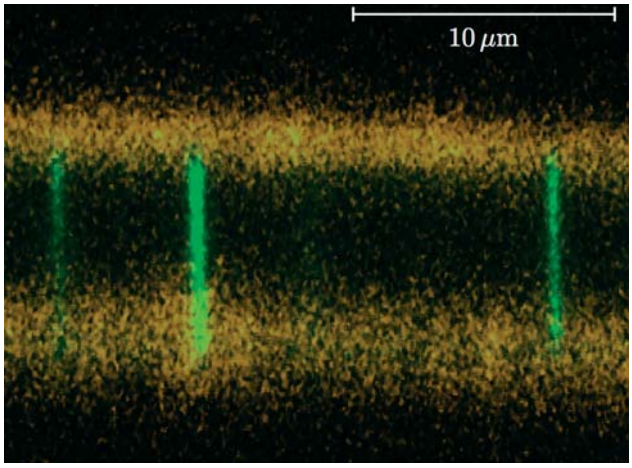

Figure 3 (from Ref.2). PVDF membrane obtained by heavy ion irradiation and ion-track etching. Radicals that persist in nanopores after etching allows us a selective radiografting of poly(acrylic acid). Then a selective pore labelling with fluorophore is possible. The image the *xz*-plan cross-section of the membrane obtained by confocal laser scanning microscopy.

Nanoporous track-etched PVDF membranes are obtained by heavy ion irradiation of 9 µm thick PVDF films and track-etching. Nanopores have a nice straight cylindrical shape that has been evidenced by Small Angle Neutron Scattering. Recently, radiographting of poly(acrylic-acid) has been selectively driven at the surface of pore-walls. This chemical modification allows membrane pores to be selectively labelled with fluorescence molecules and then to be imaged by confocal laser scanning microscopy (see Fig. 3 and ref. 2). With so prepared membranes, measurement of translocation events on single nanopore using fluorescence techniques usually associated with confocal microscopy (such as Fluorescence Resonance Energy Transfer, or Fluorescence Correlation Spectroscopy) are now conceivable.

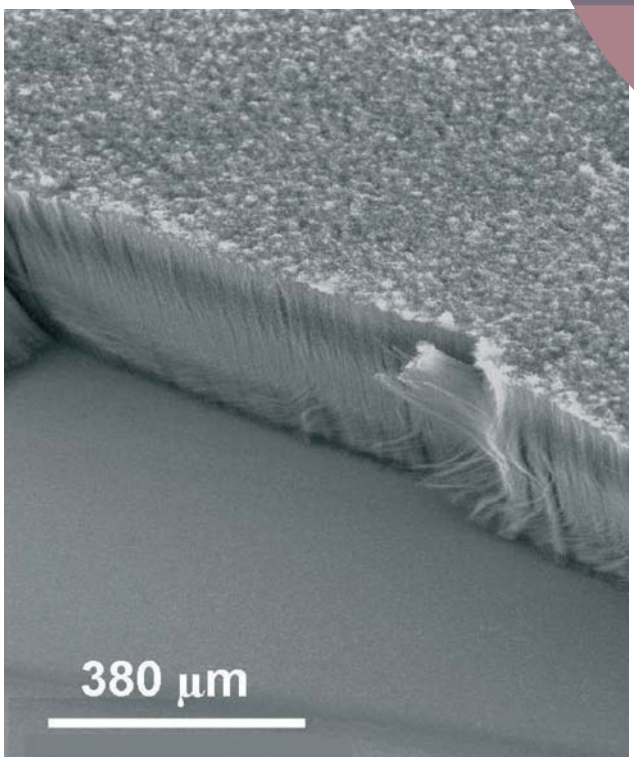

Figure 4 (from Ref.3). Scanning Electron Microscopy image of aligned carbon nanotubes (a-CNT) synthesized on silicon substrates by aerosol-assisted catalytic chemical vapour deposition from toluene/ferrocene aerosol. The a-CNT obtained are multiwalled (] M. Pinault *et al.*, Nano Lett., 5, 12, 2394-2398 (2005).

Aligned carbon nanotubes (nanotubes carpet see Fig. 4) are suitable to elaborate polymer-based composites in which the nanotube fillers exhibit a unidirectional orientation. After impregnation with polystyrene or epoxy-resin, a thinning procedure with a polishing device is performed to adjust the thickness of the composite and to open nanotube ends. Nanoporous membranes of 100-200 µm tihckness are so elaborated with the hollow central channels of nanotubes as nanopores (Ref. 3). With respect to our application, the permeation characteristics of these membranes are very interesting with a monodisperse internal diameter of the order of 7 nm (that can be adjusted) and an optimal pores density of the order of 10^{10} tubes/cm^2.

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