REFOLDING OF A HIGH MOLECULAR WEIGHT PROTEIN: SALT EFFECT ON COLLAPSE

D. Lairez[1], J. Pelta[2]

[1]Laboratoire Léon Brillouin (CEA-CNRS), CEA-Saclay, 91191 Gif-sur-Yvette cedex
[2]ERRMECe, Université de Cergy-Pontoise, BP 222, 95302 Cergy Pontoise cedex

The understanding of protein folding is a challenge for biologists. Proteins function are determined by their three dimensional structure and many diseases are linked to protein misfolding. Small globular proteins, generally refold spontaneously to their correct functional conformation after removal of the denaturing agent. Based on folding landscapes, in which the protein conformation moves on a minimum free energy pathway from unfolded conformations to a unique refolded and globular state, theoretical models have been proposed and computer simulations performed. For large or multidomain proteins (>10^5g/mol), the number of possible conformations is beyond the scope of such approaches. Even so, this apparent complexity would allow to benefit from progresses in polyampholytes scaling theories. To a certain extent, the behaviour of a high molecular weight protein should be simpler than expected.

We have studied the refolding of fibronectin (a multidomain protein of the extracellular matrix, M=5.3 10^5 g/mol) once unfolded with 8M-urea and urea slowly removed by dialysis at pH=7.4 and at two different ionic strengths. In dilute (C=8 10^{-3} g/cm^3) and salt containing solutions, measurement leads to the protein form factor *P(q)* as a function of the scattering vector *q* (see Fig.1).

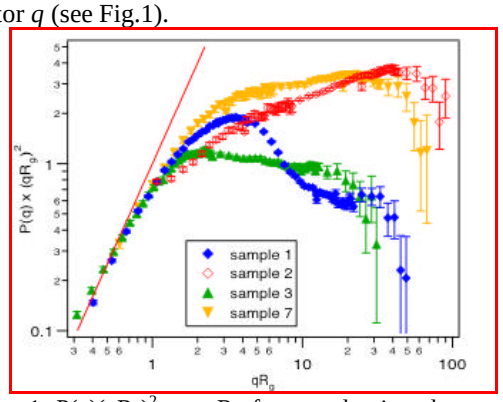

Figure 1. P(q)(qR$_g$)^2 vs. qR$_g$ for samples in salt-containing solution. 1: "native" fibronectin; 2: unfolded fibronectin; 3: "refolded" in salt-containing solution; 7: "refolded" in salt-free solution and added salt after refolding. Radius of gyration R$_g$ are equals to 153±2, 300±10, 88.5±3.5, 220±5 Å for samples 1, 2, 3 and 7, respectively. The largest values of R$_g$ are measured by static light scattering.

Native fibronectin was known to be made of 56 domains which have been mainly identified as resistant to proteolysis. We have shown [1] that native fibronectin adopts the statistical conformation of a flexible string of 56 globules of 25 Å radius each. In 8M-urea solution the protein is unfolded and swells as a linear polymer in good solvent. Data are nicely fitted using the form factor of a swollen chain of 1250 statistical segments of 13.2 Å each (see Fig.2).

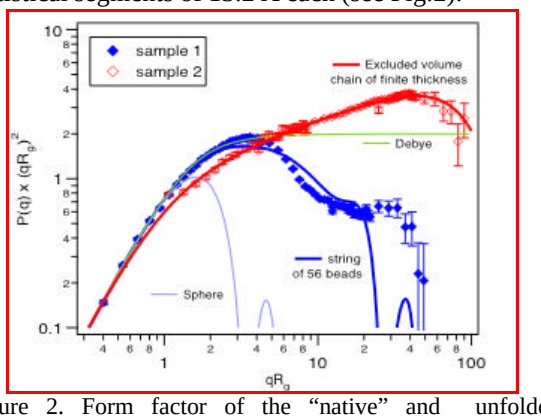

Figure 2. Form factor of the "native" and unfolded fibronectin. The full lines correspond to the theoretical expectation for a sphere, a string of 56 beads, a gaussian chain with infinitely small monomer (Debye function), and a swollen chain with a finite thickness.

As urea is removed, fibronectin collapses as proved by the deacreasing radius of gyration. From polyampholytes theory [2], in the case of neutral polyampholytes, electrostatic interactions reduce to the attractive term between charges of opposite sign, that can be included within an effective two-body volume interaction term. The chain is expected to collapse in a globule with an internal concentration resulting from a balance between attractive two-body and repulsive three-body interactions. The former need a minimum volume (or distance) to be significant, meaning that small chain segments remain gaussian, whereas the overall chain collapses at large length scale. The globule density is governed by the added salt concentration and scales as the screening length, κ_o^{-1}, of electrostatic interactions. Without added salt, the globule density is governed by the polyampholyte charge concentration. In the case of non neutral or asymmetric polyampholytes in salt-free solution, polyelectrolyte like repulsions distort the shape of the collapsed globule at large length scales. A cigar-shape rather than a spherical conformation minimizes the free energy [3]. However, the cigar-shape is unstable and splits into smaller spherical globules linked by narrow strings [4] leading to a "necklace" conformation. Depending on the net charge of the chain (long range repulsions), on the solvent quality and on charge asymmetry (short range attractions), cascade of transitions is expected between necklace conformations of various numbers of beads [5]. Our SANS data interpretations are guided by these expectations that qualitatively explain our results.

Our key observation [6] is the following: as urea is slowly removed from the solution, fibronectin does not recover its native conformation and two different collapsed conformations have been clearly identified depending on the added salt concentration.
For collapse driven at physiological ionic strength, data are accounted for using Monte-Carlo simulations to compute the form factor of a chain made of 1250 statistical segments confined in a sphere of radius R_c (Fig.3). Our results prove unambiguously that the chain in salt-containing solution collapses at large length scales ($r>x$) but remains gaussian at small length scales ($r<x$). The globule embodies a large quantity of solvent compared to the compact situation. The volume fraction of protein inside the globule is found to be f^*=0.3±0.1. The "blob" size x, below which the chain remains gaussian is found of the order of 33 Å.

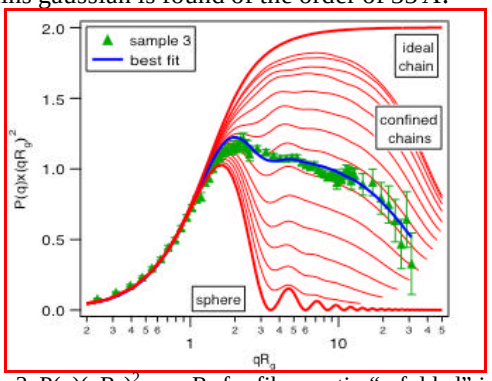

Figure 3. $P(q)(qR_g)^2$ vs. qR_g for fibronectin "refolded" in salt-containing solution (points) compared to confined chains (lines). From the top to the bottom, the lines correspond to: Debye function; confined chains with radius of confinement, R_c from 260 to 35 Å; compact sphere. The line fitting the data corresponds to R_c =139±15 Å.

For collapse driven in salt-free solution (sample 6), in view of the globule density measured in salted solution, one expects a fully collapsed and compact globule split into a "necklace" conformation. However, in order to access the form factor, salt was added after the collapse stage. Then the form factor shows that the badly refolded protein is not globular but displays both a coil-like and open conformation at large length scales and a local high density area, i.e. exactly the reverse situation as in salted solution. Data are accounted for using either a model of a star polymer with a dense core (the best fit gives 2 arms) or a model of a Gaussian chain scattered with small globules (the best fit gives 1 globule). In both models the local high density area is found to extend over 20 Å. Although the conformation of the collapsed protein in salt-free solution (sample 6) was not unambiguously determined, the analysis of the high q behaviour and of the interaction peak pleads in favour of a necklace conformation (partly dissolved by salt addition) that may be reminiscent of the native structure.

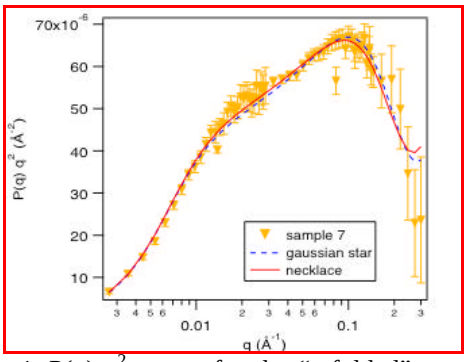

Figure 4. P(q) q^2 vs. q for the "refolded" protein in salt-free solution and salt added after refolding (sample 7). The solid and dashed lines correspond to a gaussian chain scattered with small globules (best fit gives 1 globule) and a gaussian star with core (best fit gives 2 arms), respectively.

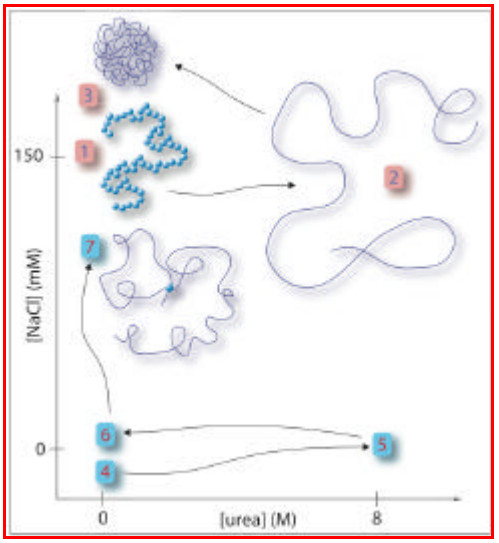

Figure 5. Conformation of fibronectin and sample history.

Although samples 1, 3 and 7 are at the same pH and ionic strength, fibronectin displays three different conformations. Two of these samples are not at the equilibrium. As a high local density may favour quenched and metastable states, it can reasonably be assumed that samples 1 and 7 are not at equilibrium. For the native conformation, this is quite puzzling and opens biological questions, mainly concerning *in vivo* 1) the possible sequential refolding of the newly translated and nascent fibronectin; 2) the assistance of molecular chaperones.

References

[1] J. Pelta, H. Berry, G.C. Fadda, E. Pauthe, D. Lairez, *Biochemistry*, **39** (2000) 5146.
[2] P.G. Higgs, J.-F. Joanny, *J. Chem. Phys.*, **94** (1991) 1543.
[3] A. Dobrynin, M. Rubinstein, *J. Phys. II France*, **5** (1995) 677.
[4] Y. Kantor, M. Kardar, *Phys. Rev. E*, **51** (1995) 1299.
[5] A. Dobrynin, M. Rubinstein, S. Obukhov, *Macromolecules*, **29** (1996) 2974.
[6] D. Lairez, E. Pauthe, J. Pelta, *Biophysical J.*, **84** (2003) 3904