Investigating non-native protein assemblies

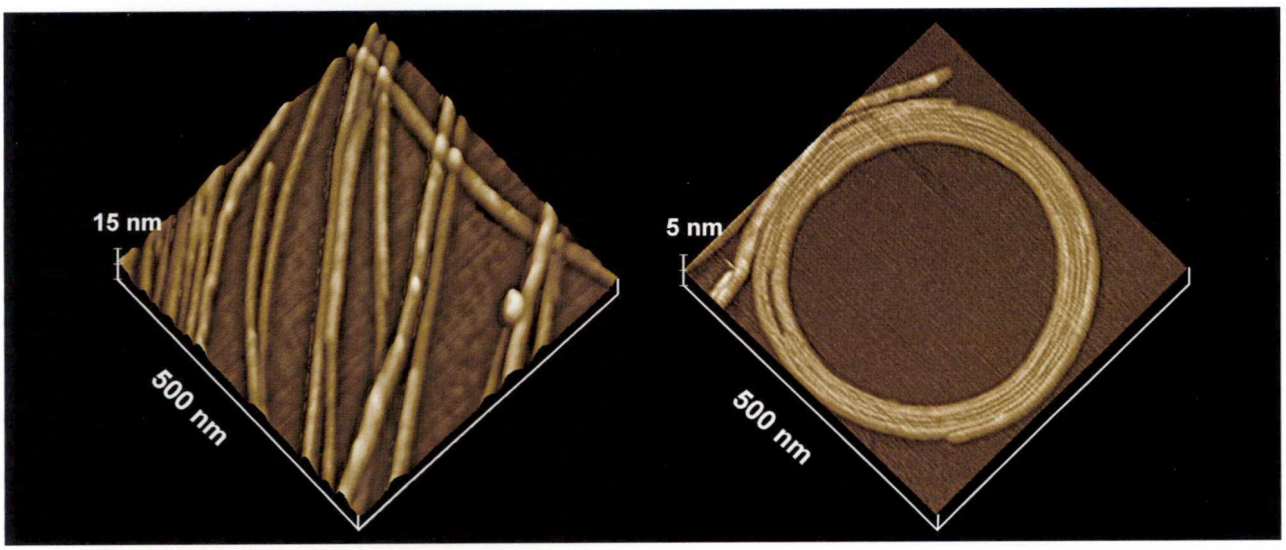
Alternative States of Protein Matter

WOJCIECH DZWOLAK, SYLWESTER POROWSKI
High Pressure Research Center, Warszawa
Polish Academy of Sciences
wdzwolak@unipress.waw.pl, sylvek@unipress.waw.pl

High-pressure physics in quest of the mechanisms of protein non-native self-assembly: from hard-boiled eggs to prions

One of the fundamental tenets of molecular biology holds that under physiological conditions, only one 3D structure may be formed out of a single amino acid sequence of a biologically functional protein. This elegant concept has received plentiful experimental support, ultimately becoming the pillar of protein folding theories equating the global energy minimum with the protein's native state. In other words, nascent, as-yet unstructured protein chains naturally tend to minimize energy (as any physical object does), which automatically leads them to acquire their native, "biological" structure. This implies, however, that protein denaturation (e.g. by heating) should only persist as long as externally-sustained high energy prevents protein from reverting to its native structure.

Nevertheless, the perplexing case of hard-boiled eggs, which apparently refuse to liquefy even upon prolonged cooling, is perhaps the most obvious contradiction to this scenario. Denaturation - a rather reversible event *per se* - is in fact often followed by a spontaneous aggregation of protein molecules, which warrants an irreversible loss of biological function. Aggregation is a common fate of destabilized protein molecules, which subsequently tend to cluster and form ß-sheet-rich precipitates. The delicious white ovalbumin of hard-boiled hen eggs serves as a prominent example here. Protein ß-pleated aggregates are either amorphous or fibrillar (the so-called amyloids) entities, which are biologically dysfunctional and resistant to temperature, proteases, and chemical denaturants. This phenomenon, which despite its commonness was long trivialized and identified with spoilt *in vitro* protein samples, has only recently entered mainstream bioscience. This is a consequence of the realization that protein aggregation underlies certain degenerative, mostly neurological conditions such as Alzheimer's, Huntington's, Parkinson's, and Creutzfeldt-Jakob (the prion-associated illness) diseases. On the other hand, spontaneous aggregation upon over-expression of recombinant proteins (e. g. human recombinant insulin) decreases the efficiency and quality of pharmaceutical products.

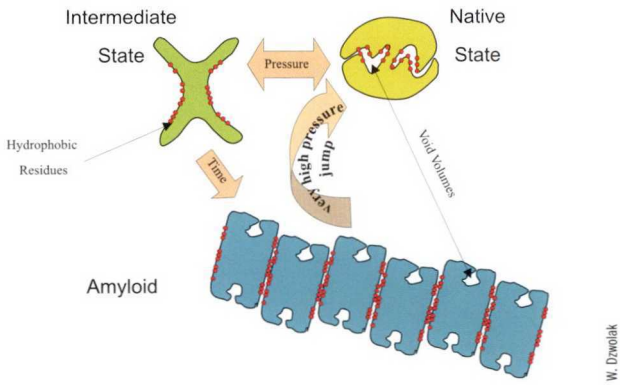


W. Dzwolak

Comparative surface Atomic Force Microscopy (AFM) images reveal fundamental morphological differences between "regular" straight insulin amyloid grown at atmospheric pressure (left), and the new circular form obtained via 1500-atmosphere incubation (right)

Since amyloidogenic stacking of protein molecules involves non-covalent interactions (hydrophobic, electrostatic and hydrogen bonding), hydration of the protein is expected to have a large influence. Indeed, the cellular membrane (or membrane vicinity) - meaning the highly hydrophobic habitat of many proteins implicated in pathological aggregation (e. g.: Alzheimer's peptide or the prion precursor protein PrP^{C}) - suggests that dramatic changes in water-protein interactions play a role in triggering the disease. This has inspired our own studies, where protein hydration is tuned by means of high hydrostatic and osmotic pressure. The paradox of the pressure effect on protein aggregation consists, on the one hand, in the induction of aggregation-prone intermediate states of proteins, and on the other, in the ability of high pressure to prevent aggregation or even dissociate once-formed aggregates. While aggregation-susceptible conformations may be induced under a variety of destabilizing conditions, such as high temperature, extreme pH, or the presence of chemical denaturants, high pressure seems to be uniquely suited to bring an aggregating protein to quasi-reversible conditions. Our study on insulin, which forms amyloid *in vitro* with notorious ease, has shown that since aggregation must proceed through a bulky intermediate state, it can be prevented under high-pressure conditions. This suggests that amyloid-free and non-allergenic insulin can be obtained under high pressure. Even more exciting prospects arise from our recent study on high-pressure tuned amyloidogenesis of insulin, wherein a unique and previously unknown circular form of amyloid was obtained - Figure 2. Unlike the regular straight, unbranched fibrils obtained under ambient conditions (shown in the left panel in Figure 2), at moderately high pressure, ring-shaped forms roughly 400 nm in diameter prevail (the right panel). This provides a rare insight into the amyloids' intrafibrillar architecture, suggesting an anisotropic distribution of void volumes within the ambient fiber (under high pressure, the growing fiber bends steadily in one direction to minimize the volume expansion). More importantly, it shows how quaternary folds and morphology of an amyloid may be finely tuned under high pressure, which translates into a means of synthesizing protein-based nanostructures.

The subtle yet tangible effect of hydration on protein aggregation pathways may be tuned in a more "physiological" way than high pressure does. Namely, instead of pressing the water molecules into the protein interiors (which accounts for the pressure-driven protein destabilization and subsequent aggregation) or in between the aggregated proteins (which explains the pressure-induced dissociation of amyloids), protein molecules may be forced into preferential interactions with water in the presence of naturally occurring protein stabilizers - the so-called osmolytes, including sugars, alcohols and certain amino



High hydrostatic pressure may evoke aggregation-prone intermediate states in proteins. Increasing pressure favors the collapse of void volumes in the native conformation and the exposure of hydrophobic residues. High surface hydrophobicity of the intermediate state is thermodynamically unfavorable and enhances the „recombination" of the non-polar residues in the form of amyloid. Even higher hydrostatic pressure is likely to dissociate the amyloid, which may be advantageous in getting rid of pathological amyloids, such as prions

acids. These osmolytes increase the thermodynamic cost of denaturation and, when acting on an aggregating protein, appear to promote different amyloidal structures and morphologies. We have just reported that structurally diverse types of amyloid may be induced in a single aggregating amino acid sequence through transient environmental fluctuations. Therefore, the aforementioned "one sequence - one structure" dogma may not necessarily be the case for all amyloids. While this seems to closely parallel the puzzling phenomenon of "prion strains," i.e. the observation that a single amino acid sequence of a prion propagating in genetically identical hosts may yet evoke distinct phenotypes of the disease, it also suggests multiple modes of self-assembly of peptides and proteins that could be explored *in vitro* for the sake of custom-structured protein-based nanomaterials. A structural polymorphism is inherent to amyloids, and may be controlled by physical parameters affecting the protein hydration, promising many fascinating bio-and nanotechnological applications. ■

Further reading:

W. Dzwolak, R. Ravindra, C. Nicolini, R. Jansen, R. Winter (2004). The Diastereomeric Assembly of Polylysine Is the Low-Volume Pathway for Preferential Formation of ß-Sheet Aggregates. *J. Am. Chem. Soc. 126*, 3762-3768.

W. Dzwolak, R. Ravindra, J. Lendermann, R. Winter (2003). Aggregation of Bovine Insulin Probed by DSC/PPC Calorimetry and FTIR Spectroscopy. *Biochemistry 42*, 11347-11355.

R. Jansen, S. Grudzielanek, W. Dzwolak, R. Winter (2004). High Pressure Promotes Circularly Shaped Insulin Amyloid. *J. Mol. Biol. 338*, 203-206

W. Dzwolak, V. Smirnovas, R. Jansen, R. Winter (2004). Insulin Forms Amyloid in a Strain-Dependent Manner: an FT-IR Spectroscopic Study. *Protein Sci.* 13, 1927-1932.