Editorial

Special Issue on Protein Folding and Aggregation

The theme of protein folding is increasingly becoming a hot topic for the attention of not only biochemists, biophysicists, biotechnologists, cell and molecular biologists but also of researchers in the fields of molecular evolution and molecular medicine. Actually, protein folding has progressively revealed multi-faceted aspects linking it to two other, strictly related aspects, protein misfolding and aggregation that are being shown to be at the basis of many physiological and pathological processes.

In the past 15-20 years, all these themes have undergone profound changes of paradigms. The energy landscape theory of protein folding has provided a solid theoretical basis to interpret old experimental data and to design new experimental approaches also taking benefit of newly introduced spectroscopic and fluorescence methods. It has also exploited the single-mutant approach first introduced by Alan Fesht to assess the contribution of each single residue in the overall folding process. Presently, we can consider with confidence the possibility that in a near future we will be able to decrypt the folding code encrypted in the amino acid sequence of each polypeptide chain enabling us to propose with good approximation a three-dimensional structure from any given one-dimensional string of amino acid residues under specific environmental conditions.

Protein misfolding is increasingly seen as much more than a mere defect of protein folding. Rather, presently it is considered the other side of the coin of protein folding. The protein conformational states available to a polypeptide chain go well beyond the natively folded, biologically active, form. Aberrantly folded, or misfolded, states in dynamic equilibrium with the correctly folded conformation appear continuously in the population of a protein's molecules. Accordingly, a protein solution can be considered a collection of different conformational states undergoing very rapid interchange where the native state is the most highly populated, which occupies a minimal energy state. This is the theoretical basis to understand the effects of structural (amino acid substitutions) or environmental (pH, temperature, chemical modification, presence of surfaces or stabilising ligands, protein over-expression) perturbations affecting the folded-misfolded equilibrium with the resulting quantitative modification of the different structures of the polypeptide chain populated at the equilibrium. The review by Paavo Kinnunen strengthens the importance of surfaces in affecting the behaviour of polypeptide chains making them more or less susceptible to misfolding/unfolding. This is a very important point, considering that the intracellular milieu is dramatically crowded by macromolecules and membranes and hence of surfaces with different physicochemical features affecting in various ways the folding/misfolding processes of peptides and proteins.

Protein aggregation has been since a long time matter of study of protein biochemists or biophysicists on one side and of pathologists on the other. The latter was due to the presence of proteinaceous aggregates in tissues from a number of pathologies mainly of degenerative type. However, only since about fifteen years *in vitro* studies have provided a theoretical link between protein folding and misfolding and the presence of protein aggregates in specific pathologies. First of all, it has been possible to distinguish between different types of protein aggregates; i.e. disordered or ordered aggregates. Ordered aggregates, either of amyloid or of non-amyloid type, are found deposited in many degenerative diseases (for instance amyloidoses or serpinopathies, respectively) inside or outside the cells, depending on the case; present knowledge depicts them as the main culprits of cell/tissue impairment underlying the clinical signs of the disease. Accordingly, investigating the structure, molecular origin and biological effects of those aggregates has became a key issue even in molecular medicine. The review by Louise Serpell highlights some important aspects of protein aggregation. It describes our present knowledge on the structural features of ordered amyloid aggregates, particularly their stable fibrillar end products, a very important issue that can help to understand the molecular process of their formation to gain clues enabling us to hinder it.

The importance of protein aggregation in protein biology has gained even more importance since when, in 1998, it was first reported that two proteins not associated with any amyloid disease were able to aggregate into fibrils of amyloid type similarly to the well known disease-associated peptides and proteins. The subsequent confirmation of these results with a large number of disease-unrelated peptides and proteins, including synthetic very short peptides, led to the currently accepted view that, under suitable environmental conditions, in principle most, but possibly all, polypeptide chains can undergo aggregation into

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amyloid fibrils. This has also led to re-think of natural proteins and peptides as a very tiny subset of polypeptide chains selected by evolution at the best in order to maximise their folding efficiencies while minimising their intrinsic tendencies to enter the aggregation pathway. Actually, several bio-informatics studies carried out by scanning the whole human, and other organism, proteomes led to highlight specific structural adaptations in natural proteins specifically aimed at reducing their intrinsic aggregation potential. Co-evolution of structural features in natural proteins and their chaperones helping the latter to recognise aberrantly folded proteins has also been highlighted. The review by Joost Schymkowitz holds this point providing strong support to the above hypothesis.

A further step forward to a better knowledge of the protein folding/misfolding/aggregation world was the assessment that amyloid aggregates can be highly toxic to cultured cells mainly in their unstable, immature forms appearing before their organisation into stable highly ordered fibrils. Such a change of paradigm is very important, re-directing the efforts to develop anti-amyloid substances in order to design molecules able to hinder the appearance of misfolded forms, and hence their oligomeric, toxic entities, rather than the growth of relatively harmless mature fibrils. This has also stimulated many efforts to study the structural features of such unstable, highly dynamic oligomeric pre-fibrillar aggregates, even though such a study is made very difficult by their intrinsic instability.

The importance of oligomer cytotoxicity has grown even more since 2002, when it was intriguingly reported that amyloid oligomers were intrinsically cytotoxic independently of the protein/peptide they were made of. In other words, this means that, in principle, any natural polypeptide chain can undergo aggregation generating at the onset of the process oligomeric species that are intrinsically toxic to the cells. Such an idea describes the protein universe as a two-face world. Actually, proteins can display the well known benign face as fundamental molecules for life or a dark face featuring them as possible rogues able to kill cells, further underscoring the importance of the evolutionary adaptations making natural proteins and peptides as less as possible aggregation-prone. The review by Charlie Glabe underlies the importance of these ideas providing us the state-of-the-art of the shared structural features of protein/peptide oligomers and the molecular basis of their cytotoxicity.

Finally, increasing attention is paid to the process of misfolding and aggregation inside the cell. Actually, proteins and peptides can aggregate *in vivo* either inside or outside the cells depending on the site where they are generated and perform their function(s). Due to the peculiar physicochemical and biological features of the intracellular environment, studying protein aggregation inside the cell is of the outmost importance to gain knowledge on the molecular and cell determinants of the process and of aggregate cytotoxicity. The reviews by Anna von Mickecz and Giorgio Casari tell us about protein misfolding and aggregation into the nucleus and into the mitochondria, respectively, providing us valuable information on such key events in the amyloid-disease relation. In particular, the latter is best underlined in the review by Vittorio Bellotti and Mark Pepys, where the authors provide a comprehensive overview of the protein aggregation diseases of amyloid type and their therapeutic approaches.

This special issue of the Open Biology Journal on protein folding, misfolding and aggregation is aimed at providing the reader an updated state-of-the-art of this increasingly important field of protein biology and molecular medicine.

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