

Early and late stages in protein deposition diseases



A number of prevalent diseases, such as Alzheimer's and Parkinson's disease, involve large conformational transitions in proteins and the formation of amyloid fibrils. In some of these diseases, intrinsically disordered polypeptides form amyloid fibrils in the brains of patients while in others the triggering factor is the partial unfolding of an otherwise globular protein to form aggregation-prone species. Most of these diseases remain incurable as a result of a lack of understanding of the biophysical principles that determine the conformation, interactions and toxicity of partially folded proteins and of the biochemical processes in place for the regulation of their concentration. The Laboratory of Molecular Biophysics seeks to elucidate, at atomic resolution, the mechanism of the key pathogenic processes in protein deposition diseases with the aim to provide opportunities for therapeutic intervention. Key goals of the laboratory include the following: (i) the identification of the residues of the intrinsically disordered amyloid beta ($A\beta$), which are responsible for the establishment of the intermolecular interactions that lead to oligomer formation in Alzheimer's disease; (ii) the rationalisation of the effect of mutations on the stability of human lysozyme in non-neuropathic systemic amyloidosis; and (iii) the identification of the biophysical properties that underlie oligomer and amyloid fibril cytotoxicity in a number of protein deposition diseases.

Beyond single structures: The simultaneous determination of the structure and the dynamics of proteins

Study of the conformation of the species involved in the transitions associated with protein deposition diseases (partially folded proteins, intrinsically disordered proteins and oligomers) is challenging because these species are structurally heterogeneous, *ie*, they cannot be described by a single, average structure and must instead be described by a native ensemble of structures, which, on average, has properties that are compatible with experimental data.

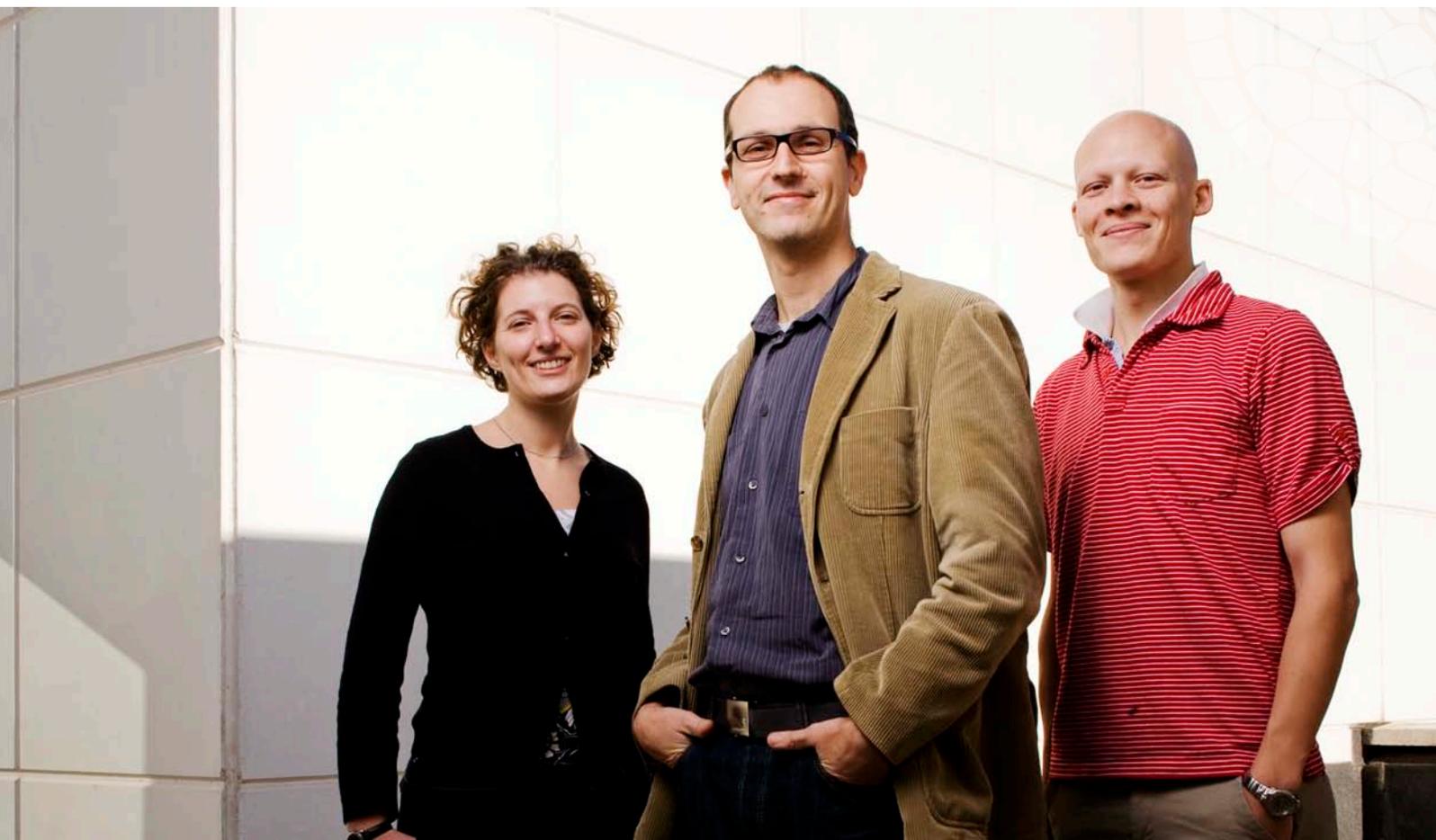
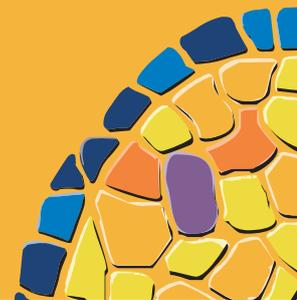
These ensembles can in principle be obtained by using ensemble or time-averaged molecular simulations with constraints derived from Nuclear Magnetic Resonance (NMR) experiments. However, in the first implementations of these approaches it was realised that the NMR data that were then available as restraints, mostly Nuclear Overhauser Effects (NOEs), suffered intrinsic shortcomings that rendered the methods prone to over-fitting (or under-restraining), *ie*, that the geometrical information contained in NOEs was insufficient to allow for the simultaneous determination of the structure of all members of the native ensemble.

Residual Dipolar Couplings (RDCs), which can be measured between pairs of nuclei in proteins that present rotational anisotropy, provide angular information not encoded in conventional NMR restraints and are sensitive to the amplitude of protein motions in the sub-ms time-scale. The availability of such observable NMR makes it possible, for the first time, to determine native ensembles without over-fitting.

During 2008 the Laboratory of Molecular Biophysics has been very active in the development and optimisation of computational methods for the simultaneous determination of the structure and the dynamics of proteins in terms of native ensembles. In collaboration with Griesinger's group (Max Planck Institute for Biophysical Chemistry), which kindly provided us with a very large set of RDCs measured for the protein ubiquitin, we have determined a native ensemble that is in unprecedented agreement with all experimental data available for this protein.

We determine the ensembles using restrained ensemble molecular dynamics simulations, where we simultaneously simulate several (typically 32 or 64) copies of the protein molecule and ensure that at each time-step the simulated ensemble is consistent with the RDCs measured for this protein. We have carried out a thorough validation of this approach by assessing its capac-

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ity to reproduce the distribution of inter-nuclear distances in a reference ensemble produced using unrestrained simulations. The results show that the agreement is highly satisfactory, *ie*, that the method is successful at capturing the fluctuations and that the resulting ensemble is a relatively accurate sample of the Boltzmann ensemble of the protein (De Simone *et al*, in press, 2008).

Understanding binding allostery and folding cooperativity: Correlated motions in the β -sheet of ubiquitin

The recent availability of native ensembles allows the analysis of dynamic properties that could until now be studied only using theoretical methods. We have used the high-resolution native ensemble that we have determined for ubiquitin to carry out a detailed analysis of the presence of correlated motions in the backbone of this protein. By studying the statistical independ-

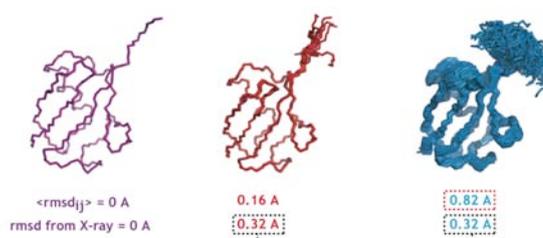


Figure 1. Comparison of the native ensemble determined for the protein ubiquitin (blue) with the X-ray structure (purple - pdb code 1ubq) and with the conventional NMR structure (red - pdb code 1d3z). The average structure of the native ensemble is in as good agreement with the X-ray structure as the conventional NMR structure.

ence of the φ and ψ backbone torsions of all possible pairs of residues, we have identified the crankshaft motion that anticorrelates the $\psi(i)$ and $\varphi(i+1)$ of consecutive residues as a result of the partial double bond character of the amide bond, and a very significant correlation between residues that are far in sequence but connected by hydrogen bonds in the β -sheet.

We have found that the degree of correlation is related to the strength of the hydrogen bonds, as assessed by trans-hydrogen

bond scalar couplings measured by NMR, to the stability of the different elements of secondary structure in the partially folded state (the A state) that ubiquitin forms in aqueous methanol, and to the structure of the transition state for folding, as determined by protein engineering methods and expressed in φ -values.

The results that we have obtained with ubiquitin are relevant because they provide a pathway for the relay of conformational change across the structure of the protein; such a relay is a necessary condition for binding allostery and folding cooperativity but had been very challenging to characterise experimentally until now. We anticipate that the determination of native ensembles and their analysis in terms of correlated motions will provide key insights into these important biological phenomena. Most importantly, we consider this approach a promising avenue for the study of the break-down of folding cooperativity that underlies a number of protein deposition diseases.

Influence of frustration on amyloid formation: Implications for cytotoxicity

Protein sequences have evolved to efficiently fold into the well-packed and uniquely defined structures that characterise native states. Although dynamic, native structures are remarkably resistant to changes of sequence, solution conditions, temperature and concentration. This robustness, summarised in Anfinsen's dogma that the native structure of the protein is determined uniquely by its sequence, is an important property that contributes, as do other relevant biological processes such as proteostasis, to the capacity of organisms to withstand significant changes in environment.

In contrast to native structures, the oligomeric species involved in protein deposition diseases form in a process that can involve the population of a significant large number of long-lived intermediate states and leads to the formation of amyloid fibrils, which are, contrary to native states, not uniquely defined. Indeed, it is possible for the same polypeptide sequence to lead to the formation of stable structurally distinct amyloid fibrils with very different biophysical and biomedical properties. In the Laboratory of Molecular Biophysics we are carrying out a detailed analysis of such polymorphism in the fibrils formed by the human lysozyme protein.

Human lysozyme forms amyloid fibrils in the disease lysozyme non-neuropathic systemic amyloidosis, in which the fibrils accumulate in the spleen and liver of patients that present destabilising mutations in the β domain of this protein. We have found that solution conditions can have a dramatic impact on the properties of the amyloid fibrils. Fibrils formed at low pH and room temperature appear very structured under the microscope, are rich in β secondary structure and show high stability to de-polymerization by chaotropic agents such as guanidinium salts; fibrils formed under physiological conditions and high temperature are much less structured, poor in secondary structure and much less stable to de-polymerisation. We have evidence that this polymorphism is not due to differences in the quaternary structure of the protein in the fibrils but, rather, that it is associated with a significantly different secondary and tertiary structure at the monomer level.

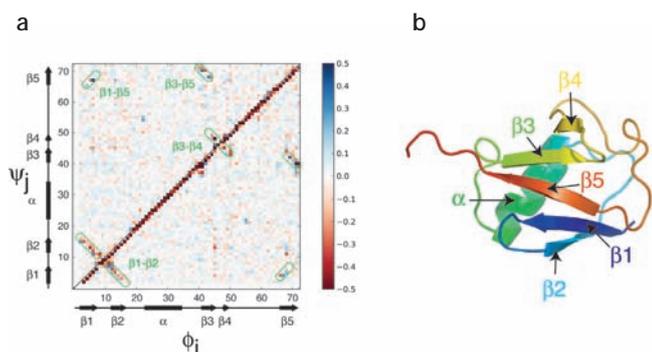


Figure 2. (a) Plot of the degree of correlation between the fluctuations of the ϕ and ψ torsion angles of the residues of ubiquitin in the sub-ms timescale. Zones in the correlation plot that correspond to pairings of β -strands have been highlighted in green to facilitate comparison with the average solution structure of this protein, presented in (b).

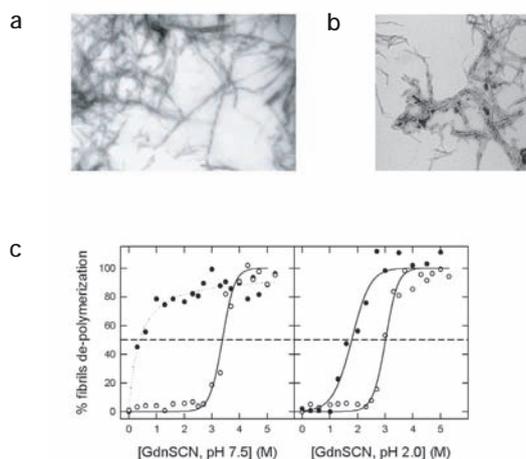


Figure 3. (a) TEM image of fibrils formed at pH 2, where the structure of lysozyme is unfolded. (b) TEM image of fibrils formed under physiological pH, where the protein is only partially unfolded. (c) Comparison of the stability of the two morphologies: under all conditions, the fibrils formed by physiological, partially folded lysozyme are metastable.

Most importantly, we have found that the fibrils formed at low pH are more stable than those formed under physiological conditions even when conditions are exchanged, thereby providing direct evidence that amyloid formation under physiological conditions is under kinetic control, *ie*, that the amyloid fibrils that form are in a kinetic trap. In addition, we have also carried out a study to determine whether these two morphologies can be made to grow in conditions that differ to those used for their formation. In this regard, we have found that, contrary to what is often observed in intrinsically disordered proteins such as A β , there is no propagation of the conformation of the seed.

Our results are compatible with an oligomerisation mechanism that is largely determined by the degree of structure present in the precursor state. In conditions in which the protein is completely unfolded, it can form the amyloid fibrils of highest sta-

bility. In contrast, in conditions in which it is partially folded, only a fraction of the sequence is available for the formation of the intermolecular interactions that lead to oligomerisation, thereby yielding fibrils with a fraction of the amyloid structure and a fraction of the stability of the fully formed fibrils.

Conclusion

The approach that we use in the Laboratory of Molecular Biophysics involves the development of computational methods for the analysis of NMR data to provide high-resolution information about the correlated dynamics of proteins and the use of biophysical methods to study the conformational variability in amyloid fibrils. This combination provides a very powerful tool with which to study key conformational transitions in protein diseases.

SCIENTIFIC OUTPUT

Publications

Calloni G, Lendel S, Campioni S, Gianinni S, Gliozzi A, Relini A, Vendruscolo M, Dobson CM, Salvatella X and Chiti F. The structure and dynamics of a partially folded protein are decoupled from its mechanism of aggregation. *J Am Chem Soc*, 130(39), 13040-50 (2008)

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Salvatella X, Richter B and Vendruscolo M. Influence of the fluctuations of the alignment tensor on the analysis of the structure and dynamics of proteins using residual dipolar couplings. *J Biomol NMR*, 40(1), 71-81 (2008)

Research networks and grants

Amyloid hot spots

European Commission, FP7-PEOPLE-IEF-2008-2367 (2008-2010)

Principal investigator: Xavier Salvatella

Collaborations

Determination of native ensembles for A β and in Alzheimer's disease and identification of the earliest stages of oligomerisation

Christopher Dobson, Department of Chemistry, University of Cambridge (Cambridge, UK)

Methods for the determination of the structure and dynamics of proteins using chemical shifts

Michele Vendruscolo, Department of Chemistry, University of Cambridge (Cambridge, UK)

Methods for the simultaneous determination of the structure and dynamics of native proteins using Residual Dipolar Couplings.

Identification of correlated motions between residues far in sequence in the β -sheet of the protein ubiquitin

Christian Griesinger, Max Planck Institute for Biophysical Chemistry (Göttingen, Germany)

Study of the structural determinants of the cytotoxicity of the oligomers formed by HypF-N, a model protein, in amyloidogenic conditions

Fabrizio Chiti, Department of Biochemical Sciences, University of Florence (Florence, Italy)