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Self-assembly of Amyloid-beta (A β) Peptides from Solution to near in Vivo Conditions

Phuong. H. Nguyen,¹ Fabio Sterpone,¹ Philippe Derreumaux^{1,2*}

1 CNRS, Université Paris Cité, UPR 9080, Laboratoire de Biochimie Théorique, Institut de Biologie Physico-Chimique, Fondation Edmond de Rothschild, 13 rue Pierre et Marie Curie, 75005 Paris, France.

2 Institut Universitaire de France (IUF), 75005, Paris, France.

Abstract: Understanding the atomistic resolution changes during the self-assembly of amyloid peptides or proteins is important to develop compounds or conditions to alter the aggregation pathways and suppress the toxicity, and potentially aid in the development of drugs. However, the complexity of protein aggregation and the transient order/disorder of oligomers along pathways to fibril are very challenging. In this perspective, we discuss computational studies of amyloid polypeptides carried out under various conditions, including conditions closely mimicking *in vivo*, and point out the challenges in obtaining physiologically-relevant results, focusing mainly on the amyloid-beta A β peptides.

1. Introduction

Amyloid fibril with a cross β -structure and intermolecular H-bonds parallel to the long fibril axis is a hallmark of many sequences differing in amino acid length and composition involved in neurodegenerative diseases. These involve A β and tau proteins in Alzheimer's disease (AD), α -synuclein in Parkinson disease, superoxide dismutase 1 (SOD1) protein in amyotrophic lateral sclerosis, transthyretin protein in cardiac and systemic amyloidosis, prion protein in prion disease, and human islet amyloid polypeptide (hIAPP) in type 2 diabetes, among others. Fragments of these proteins form fibrils as well, which represent ideal systems for computational studies.¹ In this perspective, we mainly focus on the A β peptides.

2. Results and Discussion

2.1. Protein self-assembly under quiescent solution conditions

The aggregation kinetics of amyloid proteins in aqueous solution, as measured by Thioflavin T (ThT) fluorescence assays, displays a sigmoidal curve with a lag-phase where monomers undergo conformational conversion and self-assemble into oligomers until the growth phase where the fibril elongates. This is followed by the saturation phase where the system is in equilibrium between fibrils and a low concentration of monomers. Aggregation is described by microscopic events involving primary nucleation, elongation and dissociation of a fibril by one monomer, and secondary (fibril fragmentation and fibril surface-catalyzed) nucleation. The rates of the different microscopic processes are obtained by fitting the experimental aggregation kinetic curves using multiple initial monomer concentrations. It was shown the aggregation kinetics is sensitive to variations in temperature, pH, protein concentration, ionic strength, and the presence of cofactors and preformed aggregates.²

General questions probing amyloid fibril formation of short peptides adopting straight β -strands in the bulk solution were addressed by on-lattice models and Monte Carlo simulations. It was demonstrated that the energy difference between the monomeric native state and the fibril-prone (N^*) state, and therefore the population of the ensemble of N^* structures in the full spectrum of conformations, are the major determinants of the time for fibril formation.^{3,4} Based on a cubic lattice that considers the formation of H-bonds and pairwise side chain interactions,⁵ and replica exchange Monte Carlo simulations with the OPEP force field,⁶ the critical nucleus size of A β 16-22 was estimated to be 10 monomers,⁷ and the fibril state of A β 37-42 with parallel β -strands was populated 4% for 20-mers.⁸ Figure 1A shows

the free energy landscape of 10-mers A β 37-42 as function of the nematic order parameter P2 and the number of intermolecular H-bonds at room temperature.⁷ Up to 91% of states are disordered (P2 < 0.3 and N-HB < 15). The two ordered S7 and S8 states consisting of two β -sheets with populations of 2% and 1% match the experimental microcrystal structures of A β 16-22 and A β 37-42 fibrils,⁹ indicating that the parallel and antiparallel orientations of the β -strands are encoded in the C-terminus amino acid sequence of A β 42.

Cafilisch and Shea proposed two off-lattice coarse-grained models in aqueous solution by tuning the probability of the N* monomer adopting a β -strand. Using Langevin dynamics, Cafilisch et al. showed that at a concentration of 8.5 mM, the size of primary nucleus varies from 4 to 50 monomers as the function of the population of the N* state, and amyloid polymorphism is under kinetic control.¹⁰ Using Langevin dynamics, Shea et al. demonstrated that aggregation into fibrils at a concentration of 80 mM occurs via three main mechanisms: (i) directly from the assembly of early ordered oligomers (one-step primary nucleation, 1SN), (ii) through on-pathway, nonfibrillar aggregates rich in β -sheet content, and (iii) formation of amorphous aggregates followed by reorganization to β -sheet aggregates and to fibrils (two-step primary nucleation, 2SN).¹¹

Incorporating more degrees of freedom, the aggregation of A β 16-22 peptides was followed by discrete molecular dynamics (DMD) simulations using the PRIME four-bead coarse-grained protein model with implicit solvent. Assuming 1SN mechanism, which was confirmed by electron spray ionization ion-mobility spectrometry mass spectrometry,¹² the thermodynamic phase diagram of A β 16-22 fitted well TEM (transmission electron microscopy) fibrillation experiments at peptide concentrations varying from 10 to 200 μ M at 277–330 K.¹³ It is to be noted that the N- and C-terminal unprotected A β 16-22 peptides at concentration of 30 μ M and above show rapid kinetics fibrillation within seconds to minutes consistent with the nucleation-elongation 1SN and suggest a nucleus size of 6-7 peptides displaying two perpendicular β -sheets (see Figure 1B).¹⁴ Coarse-grained OPEP Lattice-Boltzmann molecular dynamics (LBMD) simulations of 1000 A β 16-22 peptides protected by acetyl and NH₂ with hydrodynamics also reported critical nuclei of 6-10 mers.¹⁵ The PRIME model coupled to DMD investigated the co-aggregation of A β 40 by A β 16-22 and found that A β 16-22 increases the aggregation rate of A β 40 by a surface-catalyzed secondary nucleation mechanism.¹⁶

A challenging problem is the simulation of fibril formation using atomistic protein force field and explicit solvent. Bias-exchanged (BE) metadynamics with several collective variables

(CV) describing various ordered β -sheets and side chain packings revealed two distinct primary nucleation events at a concentration of 120 mM: (i) transition from mixed parallel/antiparallel to parallel β -sheets for 18 Val₈ peptides,¹⁷ and (ii) formation of specific interdigitation of the side chains for 18 A β ₃₅₋₄₀ peptides.¹⁸

Multi-*eGo*, a recent atomistic, hybrid-structure-based model that takes advantage on the knowledge of monomers conformational ensemble in explicit solvent and of the fibril structure was developed to follow in time and at atomic resolution the primary and secondary nucleation processes of a system consisting of 4000 transthyretin 105-115 peptides. The concentration dependence of the kinetics was found consistent with ThT fluorescence assays at concentrations of 7, 10 and 13 mM. Molecular dynamics (MD) simulations in explicit solvent revealed multiple primary nuclei consisting of 10 monomers organized in two opposed β -sheets (Figure 1C) populated for less than 0.1% in the lag phase time, and secondary nucleation was the main effect contributing to the exponential growth of the fibril mass.¹⁹

A few studies also combined all-atom representation and coarse-grained water model. The PACE-ASM force field with an atomistic representation of the peptide and the MARTINI water model representing four water molecules by a single site was used to study the self-assembly of 18 A β ₁₆₋₂₂ and tau PHF6 peptides in a cubic box of 3.0 nm size. At the end of 3 and 10 μ s of conventional MD at 330 K, the majority (>70%) of the A β ₁₆₋₂₂ and PHF6 peptides formed a double-layered filament, which each layer composed of in-register antiparallel (A β ₁₆₋₂₂) and parallel (PHF6) β -strands, consistent with the experimental fibrils.²⁰ It is unclear why the PACE-ASM-generated free energy landscape of both peptides is funnelled towards fibrillar states, as MD is sufficient to explore very rapidly fibrillar states, and peptides should spend most of their times in misregistered β -sheets.^{21,22} Using the PACE force field, BE-metadynamics with multiple CVs, and cluster statistical mechanics, Han et al. showed that A β ₁₆₋₂₁ self-assembly follows a 2SN mechanism at 10 mM or above. At the critical oligomer concentration of 360 μ M, aggregation follows a mechanism reminiscent of 1SN mechanism, but with atypical pathways characterized by growing oligomers with structured cores wrapped by disordered surfaces (Figure 1D).²³

Much less is known on the self-assembly of full-length A β peptides at atomic resolution and understanding transient disorder along pathways to amyloid is a real challenge experimentally,^{24,25} and computationally. It was demonstrated that the difference between A β ₄₂ and A β ₄₀ aggregation kinetics is due to a shift of more than one order of magnitude in

the relative importance of primary nucleation versus fibril-catalyzed secondary nucleation.²⁶ It is not clear whether our current repertoire of fibrillar U-shaped (strand-loop-strand) topology and S-shaped (three strands separated by loops) topology is complete, as cryo-electron microscopy of A β 42 fibrils from human brains is revealing new filament types for individuals with familial Alzheimer's disease and other pathological conditions.²⁷ Thermodynamics of the formation of all types of A β amyloid fibrils from non-equilibrium experiments of growth and dissociation would be also very useful.²⁸ Coarse-grained (SOP-IDP) simulations determined that the N* state of A β 2 monomer coding for the U-shaped conformation should form earlier than the N* state coding for the S-shaped conformation, and both N* states have very low populations in solution.²⁹ Using one particle per amino acid and lattice simulations, the critical nucleus size of A β 40 was estimated to be 11 monomers for the U-shape conformation at a concentration of 290 μ M.³⁰

Saric et al. developed a simple stick-like hard spherocylinder model with attractive patches and three interaction parameters: ϵ -bb for two monomers interacting in the parallel β -form, ϵ -bs for one amyloid-competent β monomer interacting with a soluble monomer, and ϵ -ss for two interacting soluble monomers. Using Monte Carlo simulations at concentrations varying between 60 μ M and 4mM, primary A β 42 nucleation in aqueous solution takes place through a 2SN mechanism involving a rare oligomer size rather than the most prevalent oligomers with a primary nucleus of 7-8 peptides.³¹ Using the same model, they found that secondary nucleation of A β 40 dominates primary nucleation in a very narrow regime of inter-protein interactions.³²

Structural characterization of A β 40/42 oligomers in the bulk solution is still very scarce despite extensive experimental and computational studies. Visualizing and trapping transient oligomers is a real challenge.³³ This is particularly important as amyloid plaques formed by these peptides in the extracellular space have, in fact, been observed in humans not affected by Alzheimer's disease,³⁴ and soluble A β oligomers were shown to directly inhibit ubiquitin proteasome system³⁵ and to be the most toxic species.³⁶ It should be emphasized that impaired cellular processing by proteasome and autophagy was also suggested to contribute to the accumulation of toxic hIAPP.³⁷

There is experimental discrepancy in the β -sheet content of oligomers, varying between 13% and 39%, as the function of the sample preparation.^{38,39} Atomistic simulations on A β 40 monomer⁴⁰ and A β 42 dimer⁴¹ demonstrated that the conformational ensemble

varies from one force field to another, a feature observed for many intrinsically disordered proteins and oligomers.⁴²⁻⁴⁴ Simulations starting from disordered states and nuclear magnetic resonance (NMR) studies suggest small oligomers with transient antiparallel β -sheets.⁴⁵⁻⁵⁰ For example, simulations on A β 40 dimer using the PACE-MARTINI force field showed that the antiparallel β -sheet structure forms with a time scale of 200 μ s and a probability of 1.3% and the U-shaped fibril conformation forms with a time scale of 25.8 ms and a probability of 0.02%.⁴⁸ Simulations in explicit solvent revealed β -barrel motifs in A β 42 trimer⁴⁹ and tetramer.⁵⁰

Recently, the AlphaFold2 method, which revolutionized structure prediction of single domain folded proteins,⁵¹ predicted parallel α -helix bundles spanning the C-terminal residues in equilibrium with β -hairpin assemblies spanning the central hydrophobic core and part of the C-terminus in A β 42 aggregates < 6 peptides.⁵² A short α -helix spanning residues 17-23 and a 3-10 helix spanning residues 13-23 were reported for A β 40 monomer by a predictive coarse-grained force field in solution free of salt,⁵³ and a NMR study at pH 7.3 with 50 mM NaCl,⁵⁴ respectively. Interestingly, circular dichroism (CD) experiments on A β 42 peptides in pure buffer give 19% of α -helix structure after 4 days of incubation.³⁸ Addition of trifluoroethanol (TFE) concentrations on A β fibril assembly suggested partially helical intermediates,⁵⁵ and partial helical structures of A β 40 induced by low solvent polarity accelerated amyloid fibrillation.⁵⁶ Furthermore, ThT assay and FTIR (Fourier Transform Infrared) spectroscopy on polyQ-A β ₃₀₋₄₂ peptides at μ M concentration suggested an aggregation mechanism initiated by a rapid formation of α -helical oligomers mediated by the C-terminal residues, in agreement with AlphaFold2 results.⁵⁷ Detection of helical intermediates during amyloid formation by intrinsically disordered peptides and proteins was also reported.⁵⁸ All these results may indicate very low population of metastable α -helix topologies that may not be detectable within the current experimental precision. Another intermediate discussed by Daggett et al. is the formation of oligomers with the nonstandard α -sheet secondary structure during the lag phase of A β aggregation.⁵⁹

2.2. Protein self-assembly near in vivo conditions

The brain is composed of neural cells (neurons, astrocytes, oligodendrocytes, and microglia), the vascular system including arteries, arterioles, capillaries, venules and veins, and the interstitial system (ISS). The brain ISS, which is circumscribed by the plasma membrane of

neurons or glia, and the wall of the blood vessels, is composed of the interstitial fluid (ISF) and the extracellular matrix (ECM), and occupies 15-20% of the total brain volume. The brain ISS is an irregular, tortuous and narrow space among neural cells and capillaries. ISF includes waters, ions, gaseous molecules, and organic molecules, such as proteins, peptides, enzyme, dopamine, osmolytes, glucose, extracellular vesicles and the floating chains of glycoproteins attached to the ECM. The ECM consists of collagens, glycosaminoglycans, elastin, proteoglycans and glycoproteins (reelin, laminin, fibronectin and tenascin).⁶⁰ A β peptides accumulate in the crowded extracellular space and removal of A β products can occur through ISF, and the cerebrospinal fluid (CSF) which fills the cerebral ventricles and the subarachnoid space.⁶¹ In what follows we describe what experimental and computational studies tell us about A β -membrane interactions, and the aggregation of A β peptides in crowded environment and under fluid flows.

Amyloid-membrane interactions. The interaction between amyloidogenic proteins and the lipid cell membrane catalyzes *in vitro* amyloid nucleation.⁶² AFM images of α -synuclein and A β 42 revealed that self-assembly on 1-(3-aminopropyl) silatrane mica surfaces occurs at nanomolar concentrations, a concentration found for A β 42 in human brains.⁶³ Cholesterol was found to enhance the primary nucleation rate of A β 42 aggregation in the presence of lipid bilayers by up to 20-fold *in vitro*.⁶⁴ The binding of A β to ganglioside-containing membranes also plays an important role in the pathogenesis of AD.⁶⁵ Interactions of A β species and other amyloid proteins with membrane cell are very transient and oligomer-size dependent.^{62,66-68} Helical intermediates appear to be particularly important in membrane-catalyzed amyloid formation.⁶⁹

Extending their cubic lattice to interactions of A β 42 with hydrophilic and hydrophobic surfaces, Li et al. showed that, consistent with experimental studies, an increase in roughness slows down fibril formation, and this process becomes inhibited at a very highly level of roughness.⁷⁰ Introducing a three-bead model for lipids to their protein stick model, Saric et al. identified two membrane-assisted nucleation mechanisms: (i) formation of A β 42 oligomers on the membrane surface and subsequent nucleation into pure fibrils, and (2) stabilization of the β -prone protein conformation through hydrophobic contacts with lipids and the associated mixing of membrane lipids with fibril-forming proteins enhanced by membrane fluidity.⁷¹

A β adsorbs and interacts with lipid membranes through the carpeting effect and amyloid-pore mechanisms as assessed by TEM, EM (electron microscopy), conductance and NMR experiments.^{62,72-74} Ramamoorthy et al. proposed a two-step mechanism of membrane disruption by A β through membrane fragmentation and pore formation.⁷⁵ Based on a variety of biophysical methods and MD simulations, La Rosa et al. proposed a lipid-chaperone hypothesis as a mechanism of protein-membrane poration.⁷⁶ Like tau oligomers, A β oligomers have a detergent-like activity by removing lipids from the bilayer and creating amyloid-lipid complexes.^{71,77-79} Senile plaques of AD patients accumulate phospholipids, promoting the destabilization of plaques into toxic oligomers by a lipid-promoted fibril fragmentation.⁸⁰ Accumulation of cholesterol was also assessed in microdissected AD senile plaques by mass spectrometry.⁸¹ Overall, the accumulation of free phospholipid, cholesterol and ganglioside in the ISS is most likely to modify the free energy surface of amyloid oligomers.⁸²⁻⁸⁵

Change in lipid composition of the membrane due to AD aging conditions, i.e., the amount of sphingomyelin,⁸⁶ ganglioside GM1, glycerophospholipids, and omega-3 and omega-6 polyunsaturated fatty acids,⁸⁷ may also trigger amyloid toxicity in AD as assessed by *in vitro* experiments⁸⁸ and simulations.⁸⁹⁻⁹¹ Finally, the enhance of lipid peroxidation in brain of AD patients compared to matched-age controls⁹² also changes membrane fluidity and permeability on an atomic scale by computational means.⁹³

Amyloid-crowding interactions. *In vitro*, low and high percentages of dextran70 and Ficoll400 promote and inhibit A β 40 fibrilization, respectively.⁹⁴ Primary nucleation, fiber elongation and secondary nucleation steps of α -synuclein are accelerated by the presence of 140 and 280 mg/mL Ficoll70, while sucrose, a small-molecule osmolyte and building block of Ficoll70, slows down α -synuclein amyloid formation.⁹⁵ Ficoll70 and dextran70 also enhance tau244-372 fibrillization.⁹⁶ As a result of molecular crowding, trimethylamine-N-oxide (TMAO), a gut microbiota metabolite derived from dietary choline and carnitine, enhances A β aggregation kinetics.⁹⁷

At computational level, the addition of the osmolyte TMAO shifts the atomistic conformations of tau273-286 monomer, but does not generate new conformations,⁹⁸ and favors the formation of β -strands in dimer, and tetramer of hIAPP23-29.⁹⁹ Using single-particle hard sphere crowders, a coarse-grained DMD simulation suggested that crowding increases the rate of oligomer formation and fibril growth of A β 16-22 peptide, and this effect is crowder-size dependent at a volume crowder fraction of 0.10.¹⁰⁰ Using all-atom protein model for the

transthyretin fragment 105-115, implicit solvent MD simulations showed that hard sphere crowding destabilizes in-register dimeric β -sheets, but stabilizes β -sheets made of trimers and tetramers, and emphasized the influence of nanoparticle shape on protein aggregation.¹⁰¹ Finally, crowding can have specific interactions with amyloid proteins favoring misfolding and competing with the aggregation. For example, a semi-unfolded intermediate SOD1 monomeric state was identified by a multi-scale simulation in a crowded BSA (bovine serum albumin) solution.¹⁰²

Amyloid-fluid flow interactions. Using *in vivo* two-photon and *ex vivo* fluorescence imaging, Nedergard et al. showed that cerebral arterial pulsation resulting from the pulsative blood flow drives paravascular CSF-ISF exchange in the murine brain. There is a ten-fold increase in CSF tracer influx and a doubling of A β clearance from wake to sleep, during which the ISS volume increases by about 60% due to the fluid expelled from astrocytes.¹⁰³ Recent reports revealed that reduced sleep quality occurs with brain aging suggesting a causal relationship between sleep disturbance and progression of the neurodegenerative diseases,¹⁰⁴ and the sleep-wake cycle also regulates ISF tau in mice and CSF tau in humans.¹⁰⁵

Both bulk flow and hindered diffusion are mechanisms of substance transport in the ISS.¹⁰⁶ Tortuosity (λ) and volume fraction (α) are two parameters to describe the geometry of the brain ISS. λ is the square root ratio between the diffusion coefficient in a free medium and that measured in the brain ISS. α is defined as the ratio of ISS volume to total brain volume. Increase of these two parameters was reported in the cortex in a mouse AD model.¹⁰⁷ Local restriction in the interstitial channels of the extracellular space, as the width of the ECS varies from 38 to 64 nm in adult rat neocortex (Figure 2A),^{108,109} and interactions with cell membrane and ECM network have a significant influence on substrate transport in the ISS¹⁰⁶ and should generate locally different flow patterns. By analogy with computational modelling of ISF in brain tumours, accumulation of A β should change the properties of the flow.¹¹⁰

It is known that extensional flows caused by a gradient in velocity in the direction of travel induce protein aggregation of BSA in microfluidic devices (Figure 2B) by inducing partial unfolding.¹¹¹ Aggregation kinetics of A β 40 in a Couette cell (Figure 2C), that is subject to a shear flow generated by a gradient in velocity perpendicular to the direction of travel, showed that increased shear rates decrease the lag phase time, increase the growth rate and the final concentration of fibrils (Figure 2D). It was shown that the addition of a depolymerization event

(leading to the formation of a monomer and a (i-1) fibril)) to primary and secondary nucleation and elongation steps suffices to reproduce the time evolution of fibril concentrations at different shear rates.¹¹² Similar kinetics behaviour was reported for A β 42 under fluid flows.¹¹³

While shear-induced monomeric protein dynamics were explored by experimental^{114,115} and computational¹¹⁶⁻¹²⁰ studies, and lipid systems were also investigated under shear flows,¹²¹⁻¹²⁴ we still lack an atomic description of A β aggregates under fluid flows in the bulk solution and at the surface of membranes. Yet it is known that flowing acting on the membrane surface showed desorption, diffusion and collision of A β -lipid aggregates forming larger plaque-like aggregates.¹²⁵ Overall, hydrodynamic flow fields in ISS are likely to change the conformational ensemble states assessed by small A β oligomers and may promote very rare excited oligomers.¹²⁶

3. Conclusions

In summary, we are gaining a better atomistic description of the aggregation of short amyloid peptides in the bulk solution. Whether all relevant oligomeric microstates with α -helix and β -barrel¹²⁷⁻¹³⁰ contents or associated with multiple strains¹³¹ are sampled correctly by current simulations remains to be determined.

It is a real challenge to characterize the relevant physical and pathological conformations of A β 40/42 oligomers in the bulk solution or at the membrane cells as they are likely to be marginally populated. Results are likely to depend on the force field and the membrane composition. Metadynamics simulations in the bulk solution of small A β 40/42 oligomers with CVs featuring α -helix, α -sheet,⁵⁹ misregistered β -sheets, α -helix bundles⁵² and β -barrels¹³² could identify population and structures of rare aggregates. Path sampling simulations may also reveal unexpected excited states.¹³³ These simulations could be followed by the development of a Markov state model to characterize the kinetics between on-pathway and off-pathway oligomers and the role of water mobility in the early steps of aggregation.¹³⁴ The concentration-dependence of aggregation pathways and oligomers at nM concentrations or lower as it is observed for A β 40/42 peptides in human brains has also to be considered,¹³⁵ and liquid-liquid phase separations may help in this context.

There is still a very large gap between simulations and *in vivo* conditions. Constant progress regarding the interactions of amyloid oligomers with membranes is noted and experimental data may guide simulations to further explore the conformational ensemble.¹³⁶

Simulations under crowding conditions are far behind, and going from *in vitro* to *in vivo* observations is not an easy task.¹³⁷ Simulations based on implicit solvent models without hydrodynamics effects cannot capture, however, the size fluctuations of the oligomers.^{138,139} Furthermore, the role of crowding is far from being evident as human CSF, which contains antibodies, albumin, fibrinogen among others, delays A β 40/42 aggregation under quiescent conditions.^{140,141}

Finally, coarse-grained simulations, taking into account the non-regular and tortuous geometry and the alterations of the ISS space, crowding including prion protein and NMDA receptor, and fluid flow variations during sleep-wake cycle could unveil unexpected phenomena and aggregates. More elaborated microfluidic experiments could also approach *in vivo* conditions.^{120,125,142} These combined studies could help design more efficient drugs targeting A β monomer and oligomers.^{135,143}

AUTHOR INFORMATION

Corresponding Author

E-mail: philippe.derreumaux@ibpc.fr

ORCID:

Derreumaux: 0000-0001-9110-5585, philippe.derreumaux@ibpc

Sterpone: 0000-0003-0894-8069, fabio.sterpone@ibpc.fr

Nguyen: 0000-0003-1284-967X, phuong.nguyen@ibpc.fr

Biographies

Phuong H. Nguyen is a CNRS Researcher at the Laboratoire de Biochimie Théorique at the Institut de Biologie Physico-Chimique (IBPC) in Paris. He received his M.Sc. from ICTP, Italy, and his Ph.D. from the University of Bielefeld, Germany, in Theoretical Condensed Matter Physics. He was then a Postdoctoral Fellow at the University of Frankfurt. His current research focuses on the development and application of theoretical methods for studying equilibrium and nonequilibrium structure, dynamics, and thermodynamics of single proteins, amyloids, and membranes at all-atom and coarse-grained levels.

Fabio Sterpone is a Research Director at the CNRS, and works at the Laboratoire de Biochimie Théorique at IBPC in Paris. He graduated from the University of Paris UPMC (biophysics). He has a broad experience in the application and development of computational methods to soft-matter systems. Presently, he is mainly interested in the study of protein stability and aggregation by applying and developing multiscale simulation methodologies.

Philippe Derreumaux is Professor at Université Paris Cité and Senior Member of the Institut Universitaire de France. He has been working at IBPC on the development of coarse-grained

protein model, peptide structure prediction, and the self-assembly of amyloid proteins in various environments by computer simulations.

Notes

The authors declare no competing financial interest.

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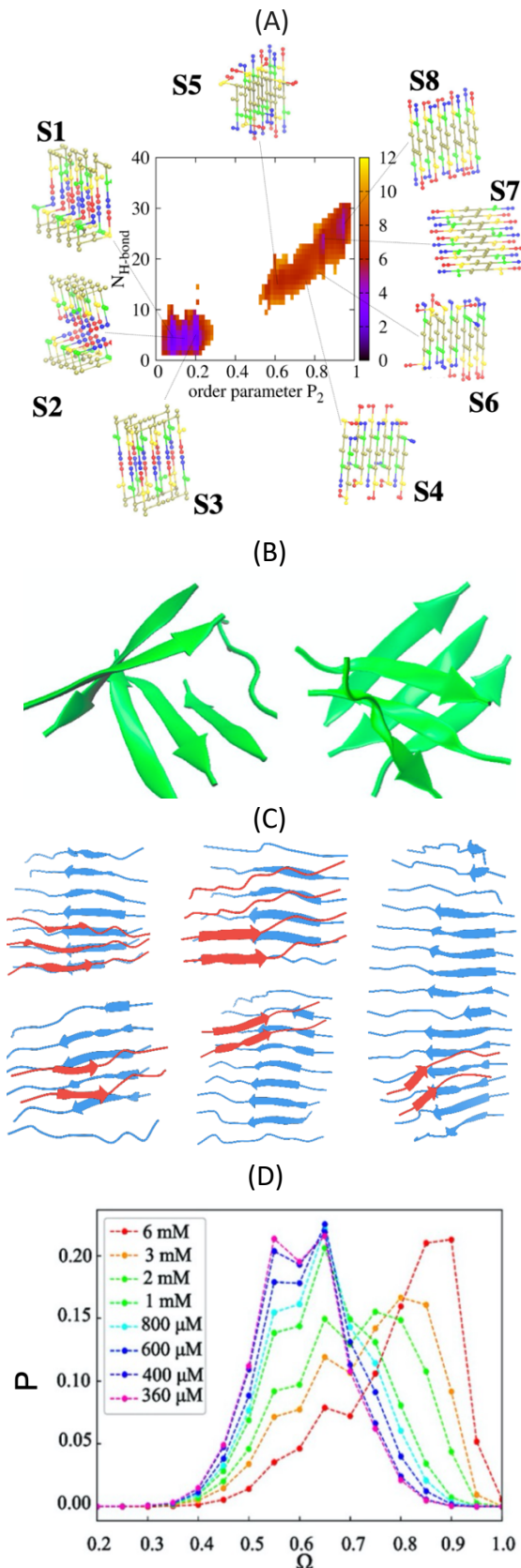


Figure 1. Free energy landscape of 10-mers A β 37-42 as a function of P_2 and N-HB (A).⁷ Representative structures of A β 16-22 aggregation nuclei (B).¹⁴ Representative structures of transthyretin fragment primary nuclei (C).¹⁹ Ω probability distributions of A β 16-21 pathways at various concentrations. Perfect 1SN or 2SN pathway has $\Omega = 0$ or 1, respectively (D).²³

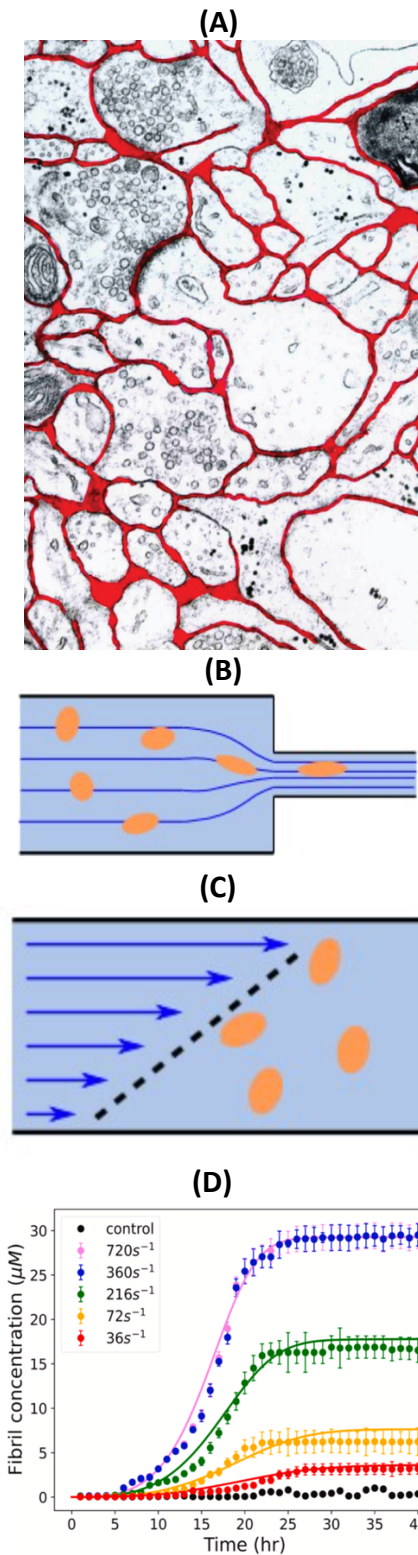


Figure 2. Electron micrograph showing a small area of cortical neuropil from the brain of a rat. The interstitial extracellular space around neurons is in red (A).¹⁰⁸ Schematic representation of the experimental microfluidic set up used to study BSA aggregation with blue arrows showing representing the velocity profile (B).¹¹¹ Schematic depiction of shear flows in a Couette experiment (C). Time evolution of A β 40 fibril concentrations at different shear rates using Couette cell (D).¹¹²

TOC Graphic

