Published in final edited form as:

Soft Matter. 2011 May 9; 7(11): 5267–5273. doi:10.1039/C1SM05162H.

Polymorphism of amyloid β peptide in different environments: implications for membrane insertion and pore formation[†]

Fernando Terán Arce^{1,a}, Hyunbum Jang^{1,b}, Srinivasan Ramachandran^a, Preston B. Landon^a, Ruth Nussinov^{*,b,c}, and Ratnesh Lal^{*,a}

^aDepartment of Bioengineering and Department of Mechanical and Aerospace Engineering, University of California, San Diego, La Jolla, CA 92093, U.S.A.

^bCenter for Cancer Research Nanobiology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, Maryland 21702, U.S.A.

^cSackler Inst. of Molecular Medicine, Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Abstract

Amyloid- β (A β) peptides are thought to be involved in neurodegenerative diseases such as Alzheimer's disease and Down's syndrome. They form a large number of polymorphic structures, including heterogeneous ionic pores in membranes as well as different types of fibrillar and globular structures on surfaces and in solution. Understanding the origin of these structures and the factors that influence their occurrence is of great biomedical interest because of the possible relationship between structure and pathogenicity. Here, we use atomic force microscopy (AFM) and molecular dynamics (MD) simulations to demonstrate that at room temperature a truncated A β peptide which is generated in vivo and shown to be toxic in vitro forms fibrillar structures on hydrophobic graphite surfaces, but not on hydrophilic mica or lipid bilayers. Our results suggest that the toxic pores and fibrillar polymorphic organizations can be explained in terms of the U-shaped β -strand-turn- β -strand structural motif observed for full length A β and other amyloids, as well as the physicochemical properties at the interfaces. The interactions of the hydrophobic, truncated A β with its environment illustrate that the universal amyloid motif can provide a link between the pore and fibrillar structures and indicate that surfaces with different physicochemical properties can shift the polymorphic landscape toward other conformational states.

1. Introduction

The N-terminal truncated β -amyloid A β_{17-42} peptide (known as p3, with the sequence LVFFAEDVGSNKGAIIGLMVGGVVIA) is found in brain tissues of patients with Alzheimer's disease (AD) and Down's Syndrome (DS).¹⁻⁴ The p3 peptide is produced from its amyloid precursor protein (APP) via enzymatic cleavage by two enzymes, α and γ secretases.^{1, 2} Most of the amino acids (81%) comprising p3 are hydrophobic with an average hydropathy index of 1.45,^{5, 6} compared to 0.2 for the full length A β_{1-42} or 0.75 for the smaller A β_{26-35} fragment. Full length A β peptides (A β_{1-42} and A β_{1-40}) as well as the truncated p3 possess hydrophilic extracellular and hydrophobic transmembrane domains,⁷⁻⁹ allowing them to form polymorphic structures: globular, annular and fibrillar (filaments,

[†]Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here].

© The Royal Society of Chemistry

* rlal@ucsd.edu . * ruthnu@helix.nih.gov .

¹These authors contributed equally

protofibrils, fibrils and mature fibers).^{9–18} Polymorphism is a fundamental property of amyloid peptides, and aggregates can acquire different preferred fibril architectures depending on sequence or environmental factors.^{19, 20} However, the mechanism and extent of structural polymorphism of amyloid peptides in different environments is not yet fully understood.

Recently, X-ray microcrystallography and solid state nuclear magnetic resonance (NMR) provided detailed three-dimensional (3D) structural information of A β fibrillar structures.^{21–23} The 3D structure of the small A β _{1–42} protofibril is based on two-dimensional NMR in combination with solid state NMR (ssNMR), mutational data and electron microscopy (EM).²² Because the N-terminal is disordered, only A β _{17–42} coordinates were defined. These illustrated that the A β peptide adopted a U-shaped, β -strand-turn- β -strand motif as predicted by modeling of A β _{16–35}.²⁴ Similarly, A β _{9–40} peptide coordinates derived from the ssNMR-based A β _{9–40} oligomer also exhibit the U-shaped structure.²³ The U-shaped conformation is a general motif in amyloid organization in A β and in other amyloids.^{25, 26} However, amyloid fibrils exhibit different fibril morphologies when grown from preformed seeds, validating amyloid fibril polymorphism.²⁷

Using molecular dynamics (MD) simulations, atomic force microscopy (AFM) and other complementary experimental techniques, we recently demonstrated that p3 peptides insert into lipid bilayers and form annular structures, which self-organize into heteromeric, mobile and loosely-connected oligomeric subunits.^{4, 28–32} Those results showed that the toxic pores can be explained in terms of the U-shaped β -strand-turn- β -strand structural motif observed for full length A β ^{22, 23} and other amyloids.^{25, 26} Here we address the question whether this motif is retained at interfaces with different physicochemical properties. Our current results suggest that this universal amyloid motif can provide a link between the pore and fibrillar structures and indicate that surfaces with different physicochemical properties can shift the polymorphic landscape towards different preferred conformational states and polymorphic organizations.^{20, 33–35}

2. Materials and Methods

2.1 AFM imaging

A Multimode AFM equipped with a Nanoscope IVa controller (Veeco, Santa Barbara, CA) was used. Oxide sharpened cantilevers with spring constants of 0.12 N/m or 0.58 N/m were employed. Since experiments were performed in liquid, a fluid cell (Veeco) was utilized. In most experiments, the fluid cell was cleaned in detergent (for ~20 min) and rinsed vigorously in a continuous flow of ultrapure water (Millipore, Billerica, MA). To test the possible influence of surfactant components of the detergent in the fibrillar structures observed experimentally, the fluid cell was also sonicated for ~15 min in ultrapure water and ethanol in a detergent-free environment. Similar fibrillar structures were observed using both cleaning protocols. Time sequential AFM images were typically acquired in tapping mode at oscillation frequencies of ~9 kHz and drive amplitudes between 10 – 20 mV. Although filaments were observed primarily using tapping mode, they could also be observed in contact mode by minimizing the applied contact force. The filaments width was measured as the full width at half maximum (FWHM) in the height profile. Thickness measurements were performed in contact mode, whereby a portion of the film was removed by scanning at a higher force and subsequently the same region was imaged at a lower force. The force was minimized by adjusting the setpoint to the lowest possible value before the tip came out of contact with the surface. All experiments were performed at room temperature.

2.2 Sample preparation

Muscovite mica disks and Highly Oriented Pyrolytic Graphite (grade SPI-1) were purchased from SPI supplies (West Chester, PA). p3 peptides (purchased from Anaspec, Santa Ana, Ca) were aliquoted in 1% NH_4OH (Sigma-Aldrich, St. Louis, MO) aqueous solutions and further diluted (1:10 by vol) in PBS without nominal Ca^{2+} or Mg^{2+} content (Fisher Scientific, Pittsburgh, PA). For time sequential imaging at low concentrations (1.5 μM), peptides were diluted in PBS solutions without NH_4OH and sonicated for 20 min in an ice water bath at temperatures of $\sim 4^\circ\text{C}$. A similar procedure was followed at higher concentrations (7.5 μM), except that the 1% NH_4OH solution was used. For all AFM imaging on graphite and mica surfaces, samples were freshly cleaved before each experiment and a region of the sample was imaged for ~ 1 hr as reference before injecting p3 peptides into the AFM fluid cell.

The contact angles of water on these surfaces have been reported to be $\sim 86^\circ$ for graphite and $\sim 23^\circ$ for mica,^{36–41} although these values decreased to $\sim 46^\circ$ for freshly cleaved, highly oriented pyrolytic graphite,^{37, 38} and $\sim 10^\circ$ or not possible to measure due to static charge for cleaned or freshly cleaved mica samples.^{36, 39} The latter results correlate better with the conditions of our experiments, as the larger contact angles values were measured in graphite samples with low degrees of crystal orientation,³⁸ or mica samples stored for several days.³⁹ DOPC (Dioleoyl, 1,2-dioleoyl-sn-glycero-3-phosphocholine, purchased from Avanti Polar Lipids, Alabaster, Al) supported lipid bilayers were prepared by rupture and fusion of liposomes. These were deposited on mica at lipid concentrations of 0.1 – 1 mg/mL and allowed to adsorb for periods of time of ~ 1 min. Samples were rinsed with PBS buffer. Peptide reconstitution in supported lipid bilayers and imaging of pore structures was carried out as described elsewhere.^{4, 11, 13} Sonication of the liposome-peptide mixture was performed in an ice water bath at $\sim 4^\circ\text{C}$ temperature, as measured before sonication. We observed pore structures only when liposomes were sonicated, but not when peptides were added to supported lipid bilayers, suggesting that water pore formation under sonoporation, in addition to local heating due to sonication, might provide a favorable mechanism for peptide insertion into the bilayer and subsequent pore formation.⁴²

2.3 Explicit molecular dynamics (MD) simulations

The initial p3 ($\text{A}\beta_{17-42}$) monomer coordinates were taken from the three-dimensional structure of the Alzheimer's $\text{A}\beta_{1-42}$ fibrils (PDB code: 2BEG; deposited as pentamers; Residues 1–16 are missing due to disorder).²² The p3 monomer conformation was used to construct 16-mer p3 filament with a linear shape. The 16-mer p3 filament was minimized with a rigid body motion for the peptides in order to enhance the formation of backbone hydrogen bonds (H-bonds) within a β -sheet, and then deposited on the surfaces of graphite and DOPC bilayers. In the graphite simulations, three layers of graphite in a hexagonal phase were created to mimic highly ordered pyrolytic graphite (HOPG). The lateral dimensions of the HOPG substrate were chosen to be $123.8 \times 125.0 \text{ \AA}^2$. The distance between graphite layers was set to $3.354 \text{ \AA}$. The graphite sheets were located in the middle of a unit cell within the periodic boundary, and TIP3P water molecules were added at both sides. To neutralize the system, 16 counterions (Na^+) were inserted. In addition to the counter ions, the system contains NaCl to satisfy a total ions concentration near 100 mM. For the graphite, we adopted the potential parameter for the atom type “CA”. In the bilayer simulations, 400 DOPCs (200 DOPCs each side) constitute the lateral cell dimension $119.3 \times 119.3 \text{ \AA}^2$. In the bilayer construction, our method closely follows previous β -sheet channel simulations.^{4, 28–30, 32}

The CHARMM program⁴³ was used to construct the set of starting points and to relax the systems to a production-ready stage. In the pre-equilibrium stages, the initial configurations

were gradually relaxed, with the peptides held rigid. A series of dynamic cycles were performed with the harmonically restrained peptides in the filaments, and then the harmonic restraints were gradually diminished with the full Ewald electrostatics calculation and constant temperature (Nose'-Hoover) thermostat/barostat at 300 K. For production runs to 50 ns, the NAMD code⁴⁴ on a Biowulf cluster at the National Institutes of Health, Bethesda, MD (<http://biowulf.nih.gov>) was used for the starting point.

Averages were taken after 10 ns discarding initial transient conformations. Since AFM images of filament growth were acquired on timescales of minutes, MD simulations are not able to provide information on the growth of filament structures.

2.4 Thioflavin staining

Amyloid fibrillogenesis was monitored using Thioflavin-T (ThT) fluorescence measurements. Briefly, 1 mg of p3 peptide (Anaspec, Fremont, CA) was dissolved in 100 μ l of 1% ammonium hydroxide (Sigma-Aldrich) and diluted to 1mg/ml in phosphate buffered saline (Thermo Scientific) and aliquoted into 50 μ l and stored at -20°C . A vial was thawed and diluted to 1.5 and 7.5 μM concentrations to which ThT (Anaspec) at a final concentration of 20 μM was mixed. 100 μ l of this mixture is deposited in 8 wells (replicates) in an opaque black walled 96 well plate (Nunc) and incubated at RT. Fluorescence was recorded (Ex/Em: 450/482 nm) on a spectrofluorometer (Spectra Max XS, Molecular Devices) at 0.5, 4, 6, 12 and 24 hrs. $\text{A}\beta_{1-42}$ at 10 μM and 100 μM concentrations were used as a positive control and ThT mixed with PBS alone was used as a blank. Blank fluorescence was subtracted from all treatments and plotted against time.

3. Results and discussion

3.1 p3 fibril morphology on graphite

Linear fibrillar structures with heights of ~ 1 nm, widths ~ 5 nm and varying lengths were observed on highly ordered pyrolytic graphite (HOPG) surfaces exposed to aqueous solutions of p3 peptides (Figure 1a). Based on their dimensions, geometrical shape and existing literature,^{45, 46} these structures are identified as filaments, the simplest form found for amyloid peptides. MD simulations suggest that the irregular cylindrical morphology in Figure 1a correlates well with stable filaments formed from rows of p3 peptides aligned in parallel. For the simulations, the 16-mer p3 filament was initially deposited on the HOPG surface with the hydrophobic C-terminal β -strands in contact with the surface. The filament was constructed from U-shaped p3 peptides containing two parallel β -sheets, which are ~ 1 nm apart. The filament length along its long axis is ~ 8 nm, and the width ~ 4.5 nm. The resulting height and width of MD simulations agree closely with the values measured with AFM in Figure 1a.

Two independent simulations for the preassembled p3 filament with different orientations were performed (Figure 1b–e). The p3 filaments with its fibril axis were initially aligned 45° (Trajectory 1) and parallel (Trajectory 2) to the reference axis. During the simulations, the p3 filaments appeared very stable on the HOPG surface, with less than 1.5 $\text{\AA}$ root-mean-square deviation (RMSD) with respect to the starting point, suggesting a favorable interaction between the filament hydrophobic face and the graphite surface (Figure 1c,e). The p3 filaments tend to orient their long axis parallel to the reference axis. Peaks around $\theta = 0^{\circ}$ indicate high probability of the filament orientational angle with respect to the reference axis on the hexagonal lattice (Figure 1f). An additional simulation for the p3 filament with the polar/hydrophilic N-terminal facing the graphite surface led to a disrupted filament structure which drifted from the graphite surface, suggesting highly unfavorable interactions (see Figure S1 in ESI).

3.2 Kinetics of p3 filament growth on graphite

Figure 2 shows a time series of AFM images illustrating the morphology and growth pattern of p3 filaments on a graphite surface. The same atomically flat terraces separated by steps with heights of 1 – 2 atomic layers ($\sim 0.33 - 0.66$ nm) are observed in all images. Filament formation on the graphite terraces starts after ~ 100 min of p3 ($C \approx 1.5 \mu\text{M}$) contact with the surface (Figure 2b and Figure 3a). The filaments are ordered, arranged in parallel, and with an order parameter, $S = (1/N) \sum [(3 \cos^2 \theta_i - 1)/2]$, with the sum extended over all terrace filaments. In Figure 2, $S = 1$ in each terrace, independent of time.

After a slow initial growth phase at low concentrations, with no observed filaments, the filaments surface density increased at a rate of ~ 1.6 filaments/ $(\mu\text{m}^2 \cdot \text{min})$ (solid squares in Figure 3a). Their lengths increased at rates of ~ 2.5 nm/min (Figure 3b), compatible with a mechanism of sudden growth followed by long pauses, similar to $A\beta_{25-35}$ and $A\beta_{1-42}$.⁴⁷ In addition to this mechanism, where lengths increase by monomeric or oligomeric peptides joining the ends (marked by black, red, and green arrows in Figure 2), filaments in close proximity can merge together (marked by white arrow in Figure 2) and this mechanism can predominate at high surface densities.

At higher peptide concentrations ($C \approx 7.5 \mu\text{M}$), filaments still grow predominantly in parallel, although occasionally forming criss-cross patterns (Figure 4a) with $S \sim 0.5$ and $\sim 60^\circ$ angles between filaments. At these concentrations, the lag phase is only 10 min and the surface density subsequently increases at a rate of ~ 11 filaments/ $(\mu\text{m}^2 \cdot \text{min})$ (open squares in Figure 3a). In contrast, lengths increase at rates similar to those at lower concentrations. Therefore, merging of pre-existing filaments becomes the predominant filament growth mechanism. After extended periods of time, a film with ~ 1.1 nm thickness with globular structures and fairly amorphous appearance is formed (Figure 4b,c). While the parallel alignment of filaments observed with PBS appears to be lost, higher resolution images (Figure 4c,d), show it is still subtly preserved at the nm scale.

The MD results shown in Figure 1f and the $\sim 60^\circ$ angles between filaments observed on different as well as the same graphite terraces (Figures 2 and 4a) suggest a strong interaction of filaments with the graphite surface, forcing the filaments to follow the graphite's hexagonal lattice symmetry. These results, along with the globular substructure of the filaments (Figure 1a), suggest an aggregation mechanism driven by hydrophobic oligomer-oligomer (or oligomer-monomer) interactions whose orientation is largely influenced by hydrophobic p3-graphite interactions. This surface-dependent mechanism was also suggested by staining experiments in which thioflavin, known to become fluorescent upon binding to amyloid fibrils (Figure 3c),⁴⁸ was used to stain possible filaments present in solution. Since no significant fluorescence change for p3 compared to $A\beta_{1-42}$ was detected even after 24 hrs, we concluded that the p3 filaments observed on the graphite surface were not formed in solution under these experimental conditions. Higher fluorescence for $A\beta_{1-42}$ at 0.5 hrs suggests the presence of pre-existing seeds and significant fiber formation.

3.3 Unfavorable p3 adsorption on mica

Following the same protocol as on graphite, no adsorption of p3 peptides was observed on mica surfaces. Considering their high hydropathy index,⁵ p3 peptides do not adsorb, or physisorb too weakly, to withstand the shear forces exerted by the AFM tip during imaging. Such a behavior contrasts the structures formed by $A\beta$ peptides with lower hydropathy index (e.g. $A\beta_{26-35}$ or $A\beta_{1-42}$) on the mica surface, which adsorb in globular form or produce fibrillar structures similar to their native structure in solution.^{9, 13, 49} The average hydropathy index of the $A\beta_{1-16}$ segment in full-length $A\beta$ is -1.8 , conferring hydrophilic

properties on A β ₁₋₄₂/A β ₁₋₄₀ peptides. These results correlate well with a recent study that showed the importance of hydrophobic interfaces in amyloid fibrilization.⁵⁰

3.4 Absence of p3 filaments on a lipid bilayer

Following our results on (hydrophobic) graphite and (hydrophilic) mica surfaces, we investigated the possible formation of p3 filamentous structures on lipid bilayers. In the MD simulations the filament was placed on the DOPC bilayer surface with the hydrophobic C-terminal β -strands in contact with the surface. Our results indicated the filaments' instability, as significant loss of contact between the initial filaments and the bilayer surface is observed after 50 ns (Figure 5a). Consequently, there is a significant difference between the initial and final filament structures on DOPC bilayers (Figure 5b). These results are in stark contrast with the stability of p3 filaments on graphite, where the initial and final structures almost coincided (Figures 1c, e). An additional simulation also indicates the instability of the p3 filament when its polar/hydrophilic N-terminal face interacts with the bilayer surface, obtaining a disrupted filament structure (see Figure S2 in ESI). In both cases, whether the hydrophobic or the polar/hydrophilic β -strands face the DOPC bilayer surface, the interactions between the pre-assembled 16-mer p3 filaments and the bilayer surface are unfavorable. The destabilized hydrophobic β -strands lead to the disruption of the p3 filaments, either because they interact with the polar bilayer surface (former case) or because they are exposed to solution (latter case). Unlike the highly ordered fibrillar structures on graphite, the bilayer surface does not provide the hydrophobic-surface-induced p3 aggregation. In contrast, recent studies demonstrated that A β ₁₋₄₀ and A β ₂₆₋₃₅ peptides can form filaments on supported lipid bilayers,^{7, 51} which correlates with their lower hydrophobicity indices.

In good agreement with the MD results described above, p3 filaments were not observed on DOPC bilayers by AFM, although on few occasions the AFM images suggested the presence of globular oligomers adsorbed on the bilayer surface (see Figure S3 in ESI). The combined AFM and MD, along with the presence of filaments on graphite surfaces and the absence of thioflavin staining in aqueous solutions, strongly suggest a surface-induced oligomerization mechanism that allows p3 monomers to associate into stable filament structures on graphite surfaces. These promote p3 aggregation by providing peptide-peptide and peptide-graphite hydrophobic interactions.

3.5 p3 pore formation in the lipid bilayer

The absence of p3 aggregation into large fibrillar structures on DOPC bilayer surfaces, combined with our observations of pore structures embedded in lipid bilayers (Figure 5c,d),⁴ suggest the pathway that p3 follows in channel formation. Larger p3 aggregates are not able to insert into the bilayer because of the high energy penalty; however, individual or small p3 oligomers can insert with subsequent associations of the small mobile subunits in the fluidic bilayer, leading to the observed pores.^{4, 28-32}

During the proteoliposome sample preparation, water pores created via sonoporation in lipid bilayers⁵² or local heating during sonication might provide possible gaps in the bilayer for the peptides to insert, thus presenting a possible favorable mechanism. As p3 monomers/oligomers are weakly adsorbed on the liposome surface and form unstable structures at the lipid bilayer/solution interface, individual peptides find a more energetically favorable environment inside the lipid bilayer, where they can associate and dissociate into dynamic toxic pore structures with varying number of subunits.^{4, 28-32, 53}

In agreement with the simulations, pore-like structures with outer (inner) diameters of ~6 – 10 nm (~1 – 2 nm) and variable number of subunits are observed in AFM images (Figure

5d). Significantly, the same structural motif adopted by filaments on graphite also appears to be adopted by p3 peptide pores in lipid bilayers,⁴ thus suggesting that p3 can assemble into pores in the hydrophobic bilayer environment or organize in parallel filaments at the graphite/aqueous solution interface. On graphite, the hydrophobic C-terminus, with a hydrophathy index of 2.5, interacts with the surface via hydrophobic interactions, while the N-terminus (hydrophathy index of 1.4) is exposed to the aqueous environment. Inside the bilayer the C-terminus interacts with the hydrocarbon chains of the lipid molecules, while the N-terminus lines to form a solvated pore. In both cases, the presence of a hydrophobic surface/aqueous solution interface allows the p3 peptides to adopt organized polymorphic pores or fibrillar structures, while these are not observed at the interface of a hydrophilic surface and an aqueous solution.

Thus, our results are compatible with a common structural motif for fibrillar and pore organizations of the N-terminal truncated A β peptides in hydrophobic environments or surfaces. Associations of these peptides when adopting the U-shaped motif can lead to filament and pore morphologies. This however is not the case when placed on the hydrophilic mica or zwitterionic bilayer surfaces, where weakly adsorbed alternate polymorphic states present higher populations; thus no filament formation. The absence of the A β hydrophilic/charged N-terminal shifts the polymorphic free energy landscape. Therefore, the physicochemical properties of the A β sequence lead to different preferred amyloid organizations in different environments. Polymorphic states at the protofilament level were also observed for mature A β ₁₋₄₀ fibrils.²⁷ Further, different β -arches (such as the U-shaped peptides discussed here) can stack into polymorphic β -arcade fibrillar organizations with parallel in-register β -sheets.⁵⁴ These observations combine to illustrate the highly polymorphic range of A β conformations, and the shift in the prevailing conformational states induced by the sequence properties and the environments and thus the interactions. Within this framework, our results indicate that the universal U-shaped motif-containing states can populate the landscape for toxic pore and fibrillar organizations, and as such, substantiate its importance.

4. Conclusions

In conclusion, we have investigated the structural polymorphism of the p3 peptide. We observed that the preferred structural organizations depend on the physicochemical properties of the peptide and its environment: N-terminal truncated A β (p3) is able to form fibrillar structures on a hydrophobic graphite surface and heterogeneous pores inside a lipid bilayer; however, no filaments or protofibrils form on the bilayer or on mica at room temperature. The differential hydrophobicity of the peptide termini, with a hydrophobic C-terminus and hydrophilic N-terminus, determines the final structures based on peptide-peptide, peptide-surface and peptide-solvent interactions. Importantly, these results suggest why *in vivo* deposition of such β -strand-turn- β -strand motifs on the lipid bilayers favors the formation of toxic pores, which is currently believed to be the key to their pathology rather than a fibrillar organization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the National Institutes of Health (National Institute on Aging) extramural program (RL). This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under contract number HHSN261200800001E. This research was supported (in part) by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. All

simulations had been performed using the high-performance computational facilities of the Biowulf PC/Linux cluster at the National Institutes of Health, Bethesda, MD (<http://biowulf.nih.gov>).

References

1. Selkoe DJ. *Nat Cell Biol.* 2004; 6:1054–1061. [PubMed: 15516999]
2. Thinakaran G, Koo EH. *J Biol Chem.* 2008; 283:29615–29619. [PubMed: 18650430]
3. Lalowski M, Golabek A, Lemere CA, Selkoe DJ, Wisniewski HM, Beavis RC, Frangione B, Wisniewski T. *J Biol Chem.* 1996; 271:33623–33631. [PubMed: 8969231]
4. Jang H, Arce FT, Ramachandran S, Capone R, R. A, B.L. K, Nussinov R, Lal R. *Proc. Natl. Acad. Sci. USA.* 2010; 107:6538–6543. [PubMed: 20308552]
5. Voet, D.; Voet, JG. *Biochemistry.* 3rd edn.. Wiley; Hoboken, NJ: 2004.
6. Kyte J, Doolittle RF. *J. Mol. Biol.* 1982; 157:105–132. [PubMed: 7108955]
7. Zhang L, Zhong J, Huang LX, Wang LJ, Hong YK, Sha YL. *J. Phys. Chem. B.* 2008; 112:8950–8954. [PubMed: 18593149]
8. Zheng J, Jang H, Ma B, Tsai CJ, Nussinov R. *Biophys J.* 2007
9. Kowalewski T, Holtzman DM. *Proc. Natl Acad Sci USA.* 1999; 96:3688–3693. [PubMed: 10097098]
10. Dobson CM. *Nature.* 2003; 426:884–890. [PubMed: 14685248]
11. Lin H, Bhatia R, Lal R. *Faseb J.* 2001; 15:2433–2444. [PubMed: 11689468]
12. Querfurth HW, LaFerla FM. *New Engl. J. Med.* 2010; 362:329–344. [PubMed: 20107219]
13. Quist A, Doudevski I, Lin H, Azimova R, Ng D, Frangione B, Kagan B, Ghiso J, Lal R. *Proc Natl Acad Sci U S A.* 2005; 102:10427–10432. [PubMed: 16020533]
14. Kaye R, Pensalfini A, Margol L, Sokolov Y, Sarsoza F, Head E, Hall J, Glabe C. *J Biol Chem.* 2009; 284:4230–4237. [PubMed: 19098006]
15. Lashuel HA, Hartley D, Petre BM, Walz T, Lansbury PT Jr. *Nature.* 2002; 418:291. [PubMed: 12124613]
16. Glabe CG. *J. Biol. Chem.* 2008; 283:29639–29643. [PubMed: 18723507]
17. Schmechel A, Zentgraf H, Scheuermann S, Fritz G, Pipkorn R, Reed J, Beyreuther K, Bayer TA, Multhaup G. *J Biol Chem.* 2003; 278:35317–35324. [PubMed: 12840025]
18. Butterfield SM, Lashuel HA. *Angew. Chem. Int. Ed.* 2010; 49:5628–5654.
19. Marshall KE, Serpell LC. *Soft Matter.* 2010; 6:2110–2114.
20. Miller Y, Ma B, Nussinov R. *Chem Rev.* 2010; 110:4820–4838. [PubMed: 20402519]
21. Nelson R, Sawaya MR, Balbirnie M, Madsen AO, Riekel C, Grothe R, Eisenberg D. *Nature.* 2005; 435:773–778. [PubMed: 15944695]
22. Luhrs T, Ritter C, Adrian M, Riek-Loher D, Bohrmann B, Dobeli H, Schubert D, Riek R. *Proc Natl Acad Sci U S A.* 2005; 102:17342–17347. [PubMed: 16293696]
23. Petkova AT, Yau WM, Tycko R. *Biochemistry.* 2006; 45:498–512. [PubMed: 16401079]
24. Ma B, Nussinov R. *Proc Natl Acad Sci U S A.* 2002; 99:14126–14131. [PubMed: 12391326]
25. Ferguson N, Becker J, Tidow H, Tremmel S, Sharpe TD, Krause G, Flinders J, Petrovich M, Berriman J, Oschkinat H, Fersht AR. *Proc Natl Acad Sci U S A.* 2006; 103:16248–16253. [PubMed: 17060612]
26. Iwata K, Fujiwara T, Matsuki Y, Akutsu H, Takahashi S, Naiki H, Goto Y. *Proc Natl Acad Sci U S A.* 2006; 103:18119–18124. [PubMed: 17108084]
27. Petkova AT, Leapman RD, Guo Z, Yau W-M, Mattson MP, Tycko R. *Science.* 2005; 307:262–265. [PubMed: 15653506]
28. Jang H, Arce FT, Ramachandran S, Capone R, Lal R, Nussinov R. *J Phys Chem B.* 2010; 114:9445–9451. [PubMed: 20608696]
29. Jang H, Arce FT, Capone R, Ramachandran S, Lal R, Nussinov R. *Biophys J.* 2009; 97:3029–3037. [PubMed: 19948133]
30. Jang H, Zheng J, Nussinov R. *Biophys J.* 2007; 93:1938–1949. [PubMed: 17526580]

31. Jang H, Arce FT, Ramachandran S, Capone R, Lal R, Nussinov R. *J Mol Biol.* 2010; 404:917–934. [PubMed: 20970427]
32. Jang H, Zheng J, Lal R, Nussinov R. *Trends Biochem Sci.* 2008; 33:91–100. [PubMed: 18182298]
33. Miller Y, Ma BY, Nussinov R. *Biophys. J.* 2009; 97:1168–1177. [PubMed: 19686665]
34. Miller Y, Ma BY, Nussinov R. *Proc. Natl. Acad. Sci. USA.* 2010; 107:9490–9495. [PubMed: 20448202]
35. Miller Y, Ma BY, Tsai CJ, Nussinov R. *Proc. Natl. Acad. Sci. USA.* 2010; 107:14128–14133. [PubMed: 20660780]
36. Bryant EM, Bowman RS, Buckley JS. *J. Petrol. Sci. Eng.* 2006; 52:244–252.
37. Helmy AK, Ferreiro EA, de Bussetti SG. *Appl. Surf. Sci.* 2007; 253:4966–4969.
38. Schrader ME. *J. Phys. Chem.* 1980; 84:2774–2779.
39. Spagnoli C, Loos K, Ulman A, Cowman MK. *J. Am. Chem. Soc.* 2003; 125:7124–7128. [PubMed: 12783566]
40. Werder T, Walther JH, Jaffe RL, Halicioglu T, Koumoutsakos P. *J. Phys. Chem. B.* 2003; 107:1345–1352.
41. Adamson, AA. *Physical Chemistry of Surfaces.* Fifth edn.. Wiley; New York: 1990.
42. Koshiyama K, Yano T, Kodama T. *Phys. Rev. Lett.* 2010; 105
43. Brooks BR, Brucoleri RE, Olafson BD, States DJ, Swaminathan S, Karplus M. *J. Comp. Chem.* 1983; 4:187–217.
44. Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L, Schulten K. *J. Comp. Chem.* 2005; 26:1781–1802. [PubMed: 16222654]
45. Harper JD, Lieber CM, Lansbury PT. *Chem Biol.* 1997; 4:951–959. [PubMed: 9427660]
46. Ionescu-Zanetti C, Khurana R, Gillespie JR, Petrick JS, Trabachino LC, Minert LJ, Carter SA, Fink AL. *Proc. Natl. Acad. Sci. USA.* 1999; 96:13175–13179. [PubMed: 10557293]
47. Kellermayer MSZ, Karsai A, Benke M, Soos K, Penke B. *Proc Natl Acad Sci USA.* 2008; 105:141–144. [PubMed: 18162558]
48. Ban T, Hamada D, Hasegawa K, Naiki H, Goto Y. *J. Biol. Chem.* 2003; 278:16462–16465. [PubMed: 12646572]
49. Arimon M, Diez-Perez I, Kogan MJ, Durany N, Giralt E, Sanz F, Fernandez-Busquets X, Faseb J. 2005; 19:1344–+. [PubMed: 15919759]
50. Pronchik J, He XL, Giurleo JT, Talaga DS. *J. Am. Chem. Soc.* 2010; 132:9797–9803. [PubMed: 20578692]
51. Yip CM, McLaurin J. *Biophys. J.* 2001; 80:1359–1371. [PubMed: 11222297]
52. Koshiyama K, Yano T, Kodama T. *Phys. Rev. Lett.* 2010; 105
53. Jang H, Arce FT, Ramachandran S, Capone R, Lal R, Nussinov R. *J Phys Chem B.* 2010; 114:9445–9451. [PubMed: 20608696]
54. Kajava AV, Baxa U, Steven AC. *FASEB J.* 2010; 24:1311–1319. [PubMed: 20032312]

Fig. 1.

(a) AFM height image of p3 fibrils adsorbed on a graphite surface and corresponding cross sections along 1 and across 2 fibrils (dotted white lines). A globular substructure is visible in 1. The fibrils width was measured as the full width at half maximum (FWHM) in the height profile. A 7.5 μM p3 peptide concentration in PBS was used. (b), (d) MD snapshots of the 16-mer p3 fibril on the surface of HOPG at the end of the 50 ns simulations for two independent trajectories. In the peptides, hydrophobic residues are shown in white, polar and Gly residues in green, negatively charged residues in red, and positively charged residues in blue. The orientation of a monomer on the graphite surface is indicated as inset. (c), (e) Superpositions of the p3 fibril conformations on the surface of HOPG from the starting points (blue) and the 50 ns simulations (yellow) for two independent trajectories. (f) The probability distributions of the angle θ between the p3 fibril axis (arrow in the right panel) and the reference axis (horizontal line in the right panel) obtained from two independent simulations.

Fig. 2.

a)–c) Time sequential AFM images of p3 adsorption at (a) $t = 0$, (b) $t = 110$, (c) $t = 178$, (d) $t = 207$, (e) $t = 249$, and (f) $t = 303$ min on graphite surface in PBS buffer at $1.5 \mu\text{M}$ peptide concentration. Times in images (b)–(d) refer to time points after the peptide solution was inserted into the liquid cell. Image in (a) was acquired before peptide insertion. Height scale is 5 nm for all images. Fibril growth occurs following well-defined directions of graphite. The fibrils marked by black, red, and green arrows in (c), (d) and (e) correspond to fibrils growing by aggregation of oligomers/monomers to a preexisting fibril. The fibril marked by a white arrow is an example of growth as two fibrils merge together. A Si_3N_4 cantilever with a nominal 0.12 N/m spring constant was used for all images.

Fig. 3.

a) Plot of fibrils surface density, ρ , as a function of time for a $1.5 \mu\text{M}$ p3 concentration (solid squares) and $7.5 \mu\text{M}$ (open squares). (b) Length of fibrils, L , as a function of time for the fibrils marked with arrows in Figure 2. c) Time series measurements of ThT fluorescence intensity, I , specific to amyloid fibrillogenesis for p3 at the concentrations of $1.5 \mu\text{M}$ (black) and $7.5 \mu\text{M}$ (red), and for A β_{1-42} , at a concentration of $10 \mu\text{M}$ (blue).

Fig. 4.

a) p3 fibrils on graphite surface in PBS buffer at $7.5 \mu\text{M}$ peptide concentration after 221 min adsorption time. Height scale is 3 nm. AFM image was acquired in tapping mode using a Si_3N_4 cantilever of nominal 0.58 N/m. b) Thickness measurement of layer formed on graphite after ~ 450 min of adsorption time. Film in the central square of image was removed by applying a larger force during scanning. Image was acquired in a different spot of the same sample used in (a). c) and d) Higher resolution images of the continuous, amorphous film in (b) still display a subtle sub-jacent fibrillar structure (d). Height information is shown in (c) (height scale is 2 nm) and amplitude data of the region marked with the dashed square is shown in (d). Contact mode using a Si_3N_4 cantilever of nominal 0.12 N/m was employed for b)–d).

Fig. 5.

a) Snapshot of 16-mer p3 fibril on the surface of DOPC bilayer taken from the 50 ns simulation. Approximately half of the initial fibril loses contact with the bilayer during the simulation. In the peptides, hydrophobic residues are shown in white, polar and Gly residues are shown in green, negatively charged residues in red, and positively charged residues in blue. In the lipids, nitrogen atoms are shown in red, carbon chains in white threads, and phosphate atoms in red spheres. Red spheres represent phosphate atoms. b) Superposition of the p3 fibril conformations on the surface of DOPC bilayer from the starting point (blue) and the 50 ns simulation (yellow). c) Channel structure with subunits of p3 peptides in DOPC bilayer. The different subunits are highlighted in different color. The orientation of a p3 monomer on the DOPC bilayer and inside the DOPC lipid bilayer is indicated as insets in a) and c). d) AFM images of individual pore structures of p3 peptides reconstituted in DOPC lipid bilayer. Possible subunits are marked in the images. Notice that a variable number of subunits are possible. Additional information about these pore structures can be found in our previous work^{4, 28, 29, 31, 32}