



# PCCP

## Pathways of amyloid-beta absorption and aggregation in membranous environments

Journal:	<i>Physical Chemistry Chemical Physics</i>
Manuscript ID	CP-ART-01-2019-000040.R2
Article Type:	Paper
Date Submitted by the Author:	26-Mar-2019
Complete List of Authors:	Sahoo, Abhilash; University of Maryland, Fischell Department of Bioengineering Xu, Hongcheng; University of Maryland, College Park, Fischell Department of Bioengineering Matysiak, Silvina; University of Maryland, Fischell Department of Bioengineering

SCHOLARONE™  
Manuscripts

Cite this: DOI: 10.1039/xxxxxxxxxx

# Pathways of amyloid-beta absorption and aggregation in membranous environment<sup>†</sup>

Abhilash Sahoo,<sup>a</sup> Hongcheng Xu,<sup>b</sup> and Silvina Matysiak<sup>\*ab</sup>Received Date  
Accepted Date

DOI: 10.1039/xxxxxxxxxx

www.rsc.org/journalname

Aggregation of misfolded oligomeric Amyloid-beta ( $A\beta$ ) peptides on lipid membranes has been identified as a primary event in Alzheimer's pathogenesis. However, the structural and dynamical features of this membrane assisted  $A\beta$  aggregation has not been well characterized. The microscopic characterization of dynamic molecular-level interactions in peptide aggregation pathways has been challenging both computationally and experimentally. In this work, we explore differential patterns of membrane-induced  $A\beta$  16-22 (K-L-V-F-F-A-E) aggregation from the microscopic perspective of molecular interactions. Physics-based coarse-grained molecular dynamics (CG-MD) simulations were employed to investigate the effect of lipid headgroup charge — zwitterionic (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine: POPC) and anionic (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine: POPS) on  $A\beta$  16-22 peptide aggregation. Our analyses present an extensive overview of multiple pathways for peptide absorption and biomechanical forces governing peptide folding and aggregation. In agreement with experimental observations, anionic POPS molecules promote extended configurations in  $A\beta$  peptides that contribute towards faster emergence of ordered  $\beta$ -sheet-rich peptide assemblies compared to POPC, suggesting faster fibrillation. In addition, lower cumulative rates of peptide aggregation in POPS due to higher peptide-lipid interactions and slower lipid diffusion, results in multiple distinct ordered peptide aggregates that can serve as nucleation seeds for subsequent  $A\beta$  aggregation. This study provides an in-silico assessment of experimentally observed aggregation patterns, presents new morphological insights and highlights the importance of lipid headgroup chemistry in modulating peptide absorption and aggregation process.

## 1 Introduction

Aberrant aggregation of peptides and proteins on cellular membranes has been associated with the pathogenesis of a number of neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD) and Huntington's (HD) disease<sup>1-3</sup>. Alzheimer's disease, characterized by extra-cellular amyloid plaques<sup>4-6</sup> and intracellular neurofibrillary tangles<sup>7,8</sup>, is a significant social challenge which has affected 5.7 million people in the United States<sup>9</sup>. The amyloid cascade hypothesis for AD presents aggregation of a 39-43 residue long intrinsically disordered peptide-amyloid beta ( $A\beta$ ) as the trigger for a cascade of events culminating in neuronal deaths and dementia<sup>10,11</sup>. Multiple alloforms of  $A\beta$  peptides with an intrinsic tendency to form fibrillar aggregates are created by successive excisions of Amyloid precursor proteins

(APP) by beta secretase in the endosomal pathway and gamma secretase in the plasma membrane<sup>12-14</sup>. Recent evidences have implicated soluble, low molecular weight  $A\beta$  oligomers as the primary cytotoxic agents, correlating strongly with cognitive defects<sup>15-18</sup>. The structural diversity of polymorphic  $A\beta$  oligomers contributes towards multiple pathways for  $A\beta$ -induced neuronal toxicity<sup>19</sup>.

A broad range of proteins and peptides, regardless of large variations in the amino acid sequence, have been shown to form amyloid fibril at high concentrations<sup>20-22</sup>. Experimental characterization of peptide aggregates through X-ray diffraction has revealed common structural features such as the cross beta sheet architecture<sup>23,24</sup>. Structural studies of  $A\beta$  peptides through solid state NMR<sup>25</sup>, hydrogen-deuterium exchange<sup>26</sup> and electron microscopy<sup>27-29</sup> have also shown the presence of similar cross beta sheet patterns. The central hydrophobic core (CHC), residues 17-21 (L-V-F-F-A) of the complete  $A\beta$  is crucial for fibrillation<sup>30-34</sup>. In addition, solid state NMR studies have confirmed that  $A\beta$  16-22 (K-L-V-F-F-A-E) is one of the the smallest peptide sequence capable of forming highly ordered, stable beta sheet rich fibrils at neutral

<sup>a</sup> Biophysics Program, Institute of Physical Science and Technology, University of Maryland, College Park, MD

<sup>b</sup> Fischell Department of Bioengineering, University of Maryland, College Park, MD

<sup>†</sup> Electronic Supplementary Information (ESI) available: Alterations to the coarse grained model and other supporting evidences. Movie presenting membrane assisted emergence of secondary structures on POPS. See DOI: 10.1039/CXCP00000x/

pH<sup>35</sup>. Therefore studies of the structural and kinetic properties of a simpler tailorable model peptide, A $\beta$  16-22, can provide a better understanding of the molecular forces responsible for fibril formation/elongation<sup>36–38</sup>.

The production of A $\beta$  peptides occurs in a membranous environment, exposing the peptides to a number of lipid-peptide interactions<sup>39</sup>. Also, the perturbation of cellular membranes, followed by ion-dysregulation due to oligomeric forms of A $\beta$  peptides is hypothesized to be a central part of A $\beta$  assisted AD pathology<sup>39–41</sup>. Therefore, a mechanistic understanding of bio-mechanical interactions of A $\beta$  peptides with cellular membranes is necessary to gain insights on the A $\beta$  cascade pathway. A $\beta$  peptides have been shown to exhibit varying aggregation patterns on lipid bilayers depending on their structure and composition<sup>42–49</sup>. CD and Thioflavin T assay studies on unilamellar vesicles have revealed an accelerated aggregation of A $\beta$  16-28 peptides into ordered beta sheets on anionic bilayer (DPPG) as compared to zwitterionic bilayer (DPPC)<sup>45</sup>. In addition, an AFM experiment using supported bilayers, has shown that disruption of zwitterionic membranes (DOPC) is higher than anionic membranes (DOPG) in presence of A $\beta$  peptides<sup>43</sup>. Recent evidences from imaging studies using TEM, AFM and total internal reflection fluorescence microscopy have suggested that small unilamellar vesicle (SUVs) with a larger curvature promotes amyloid fibril formation when compared to large unilamellar vesicles<sup>50,51</sup>.

A morphological characterization of A $\beta$  oligomers is difficult due to its transient and soluble nature<sup>52–54</sup>. On the other hand, computational studies, particularly molecular dynamics (MD) can be an ideal alternative to access this small time scale, transient behaviour<sup>55</sup>. Atomistic simulations, often coupled with advanced sampling techniques have been extensively implemented to study small-scale peptide oligomerization in solution<sup>56–63</sup>. Some recent atomistic studies on peptide-lipid interactions have investigated pre-formed membrane-inserted oligomers and the very initial phases of oligomer-lipid interaction<sup>64–66</sup>. Due to high computational costs and sampling issues, atomistic simulations have not been used to study peptide aggregation on lipid bilayers starting from a solvated monomeric configuration. Coarse grained molecular dynamics (CG-MD), which provides a reduced resolution description of a system and significantly improved sampling of protein conformational landscape, is an effective tool to study complex systems with extended spatio-temporal scales<sup>67</sup>, specifically peptide aggregation starting from monomeric peptides. Many novel coarse grained force-fields (In-lattice and Off-lattice) have successfully characterized ordered amyloid aggregation<sup>68–73</sup>. PRIME 20, an intermediate resolution unbiased peptide coarse graining scheme has been implemented with discontinuous molecular dynamics (DMD) on many amyloidogenic sequences including A $\beta$  peptides to study fibril formation in solution<sup>74</sup>. Zheng, et. al. explored the aggregation free energy landscape of A $\beta$  peptides using AWSEM-MD — a predictive coarse grained force-field<sup>75</sup>. A minimalistic, phenomenological model (Clafisch model) was implemented with a variable dihedral term to generate peptide aggregation on model vesicles<sup>69</sup>. Recently, another phenomenological coarse grained three bead-per-residue, amyloidogenic peptide model (Shea model) was em-

ployed with implicit solvent to demonstrate spontaneous peptide aggregation into beta sheets on model lipid membranes<sup>70</sup>. These coarse graining techniques, either have been designed only for peptides (PRIME20-Hall model) or do not provide peptide sequence/lipid type specificity (Clafisch and Shea models).

Here, we present a CG-MD study of partitioning, folding and aggregation dynamics of A $\beta$  16-22 peptides (K-L-V-F-F-A-E), in presence of model lipid bilayers composed with zwitterionic-POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) and anionic-POPS (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine) starting from their solvated monomeric state. We designed the peptide using a modified version of prior-developed coarse grained model - Water-Explicit Polarizable PROtein Model (WEPPROM)<sup>76,77</sup>, which generated secondary structures of small peptides from primary amino acid sequences without any built-in bias. Lipids were modeled by another slightly altered variant of recently created Water-Explicit Polarizable MEMbrane (WEPMEM) model that could accurately reproduce dielectric properties at the lipid-headgroup (interface) region<sup>77,78</sup>. Physically, peptide aggregation involves an interplay between hydrophobic and hydrophilic effects, indicating the importance of precise modeling of electrostatic interactions<sup>79,80</sup>. The novelty of these models is the introduction of structural polarization at the peptide-backbone and lipid-headgroup which is necessary for studying peptide-lipid interaction and membrane induced peptide folding<sup>77</sup>. Both these models have been parameterized to use Yesylevskyy's polarizable water model<sup>81</sup>.

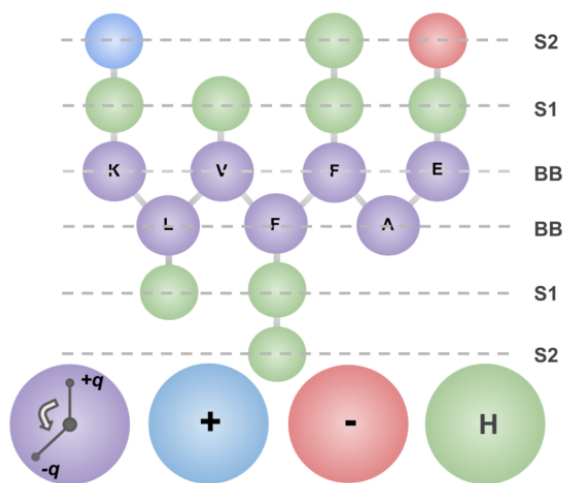
Outer neuronal cell membranes are extremely diverse with a wide variety of glycerophospholipids and ceramides<sup>82</sup>. A $\beta$  peptides show an enhanced propensity to aggregate into ordered beta sheets on negatively charged membranes composed with anionic lipids<sup>45,79,83–86</sup>. Also, many experimental studies to understand the effect of lipid type on peptide aggregation have used POPS as one of the anionic components<sup>87–89</sup>. This prompts our choice for using POPS as the model anionic lipids to study the oligomerization of A $\beta$  peptides in membranous environments. In this paper we explore the differences in the morphology and kinetics of membrane-assisted beta sheet formation by A $\beta$  16-22 on model lipid membranes using coarse grained MD simulations. The paper also presents a mechanistic explanation to experimentally observed kinetic behaviour. To our knowledge, this is the first coarse grained simulation study that captures the complex process of peptide aggregation in membranous environment, from peptides solvated in their monomeric state to formation of ordered secondary structures, while maintaining peptide sequence and lipid type specificity.

## 2 Methods

### 2.1 Peptide Model

The A $\beta$  peptide model is primarily derived from WEPPROM — which has been successfully applied to study interfacial folding and aggregation of small peptides without any externally added bias<sup>76,77</sup>, with certain modifications (Table S1). In short, the CG peptide consists of three types of beads - charged (+/-), hydrophobic (H) and polar (P) mapped from an atomistic amino

acid sequence (Figure 1). Each amino acid's peptide backbone has been mapped into a single polarizable backbone bead (BB) with structural polarization added through dummy positive/negative charges (BBp/BBm). These dummy charges interact with the central backbone bead through a harmonic potential, with each other through a cosine squared angle potential and with its environment through electrostatic forces. This results in an induced-dipole effect, adding directionality to peptide backbone-backbone interactions, generating secondary and super-secondary structures<sup>76</sup>. Charged residues (K and E) are represented as two side chain beads (S1/S2) - one hydrophobic another charged. The side-chains in the CHC region (L-V-F-F) just have hydrophobic beads with sidechains of Leucine and Valine represented as single bead (S1) and Phenylalanine with two beads (S1 and S2).



**Fig. 1** A schematic description of peptide coarse-grained model

Further details about forcefield parameters, details of parametrization and model validation is provided in Ganesan *et al.*<sup>76</sup> and modifications are listed in supplementary (Table S1).

The peptide model was validated using experimental and computational evidences of A $\beta$  16-22 aggregation into ordered cross beta structures in aqueous solution<sup>23,24,36,90</sup>. Simulations with 12 A $\beta$  monomers, solvated in water (0.14 M) aggregated into stable beta sheet rich oligomers (Figure S1).

## 2.2 Lipid Model

Similar to the original MARTINI forcefield<sup>91</sup>, lipids - POPC and POPS in our lipid model — adapted from WEPMEM<sup>77</sup>, are modelled by 13 CG beads each through a 4:1 mapping scheme. Some alterations made to the WEPMEM forcefield have been described in Table S2. At the lipid-headgroup, phosphate (PO4) and choline (NC3) are mapped to a charged coarse grained bead, whereas glycerol-esters (GL1 and GL2) and serine (CNO) are represented by polarizable beads with structural polarization generated through two dummy charges similar to the peptide model. Lipid oleoyl tails are designed with five hydrophobic beads, whereas the palmitoyl tails with four. Please refer to Ganesan *et al.* for further description of the coarse grained model<sup>77</sup>. Both the lipid and peptide models are compatible with each other

and use polarizable MARTINI water model<sup>81</sup> for solvation.

## 2.3 Simulation Protocol

The model POPC bilayer, composed with 240 lipids and POPS bilayer with 242 lipids were solvated with CG water (lipid to water ratio fixed approximately at 20). The simulations were performed on GROMACS 4.5.4<sup>92</sup>. After the preliminary energy minimization, the bilayers and counter-ions (in case of POPS bilayer) were equilibrated for 10 ns (time step, dt = 10 fs) using NPT ensemble. Temperature was maintained at 300K through a Noose-Hoover thermostat<sup>93,94</sup> with a time constant of 1 ps. Parinello-Rahman barostat<sup>95</sup> with a time constant of 1 ps and compressibility of  $3 \times 10^{-5}$ /bar was used alongside with semi-isotropic pressure coupling to maintain a pressure of 1 bar. Particle mesh Ewald (PME)<sup>96,97</sup> with a relative dielectric constant of 2.5 and cutoff distance of 1.6 nm was used to compute long range electrostatics. The Lennard-Jones interactions were modified starting from 0.9 nm to 0 at 1.2 nm by the GROMACS shift scheme.

After creation of an equilibrated bilayer, 48 peptides (peptide to lipid ratio of about 0.2) are randomly added into the solution. The peptide to lipid molar ratio of 1:5 has been previously investigated in small (A $\beta$  25-35) peptide-membrane experiments<sup>98</sup>. The composite system is then energy minimized and re-equilibrated for 50 ns with position restraints placed on 4th residue backbone (on F-19 BB in x, y and z directions) in peptides and phosphate (on PO4 in z direction) in lipids. The bilayers were simulated with a fixed surface tension (Berendsen barostat) to mimic an enhanced area per lipid due to the outer membrane curvature of SUVs. The area per lipid of both POPC and POPS bilayers were fixed at  $95 \text{ \AA}^2$  to simulate outer membrane of a SUV with 13.4 nm diameter. These values were obtained through a WEPMEM simulation of a small unilamellar vesicle system composed of 877 lipids and 61113 CG-water molecules and also verified against other coarse grained molecular dynamics simulations with comparable vesicle sizes<sup>99,100</sup>. All other simulation parameters were kept similar to the first equilibration step. This second step allows us to equilibrate the monomeric peptides in the solution in presence of a lipid bilayer.

Finally, position restraints were removed and a production run of 1.5  $\mu\text{s}$  was carried out using the remaining simulation parameters from the second equilibration step. To verify the statistics presented in this paper, we also simulated a replica of bilayer-peptide systems with different initial states.

## 2.4 Analysis

Built-in functions of GROMACS, analysis modules of Visual Molecular Dynamics<sup>101</sup> (VMD) and in-house developed scripts were used to analyze molecular simulation trajectory.

### 2.4.1 Peptide absorption

The relative position of two F-S2 beads from the lipid bilayer surface, described by locally close (six nearest neighbors from center of mass) phosphate (PO4) beads has been used as metrics to differentiate between absorbed and unabsorbed peptides. To accommodate for the local curvature of lipid bilayers, the height of bi-

layer surface ( $h$ ) is determined by the average heights of six nearest PO4 beads to each individual peptide. The peptides are then categorized into three classes - *completely absorbed* (CA), *partially absorbed* (PA) and *unabsorbed* (UA) based on positions of Phenylalanine S2 (F-19/F-20 S2) on a single peptide chain. If the position of both F-S2 along bilayer normal ( $z$ ) is less or equal to  $h$ , the peptide is classified as completely absorbed (CA) whereas if  $z > h$ , the peptides are considered unabsorbed (UA). Figure S2 provides a schematic representation of this algorithm and the three classes of peptides differentiated on the basis of absorption onto bilayer surface.

#### 2.4.2 Peptide-aggregate Clusters

Peptide aggregation in the simulated systems was quantified by clustering connected peptides. In this analysis, the peptides are described by main beads - BB, S1 and S2. This connectedness is established using distance cutoffs determined from the first peak of radial distribution functions ( $g(r)$ ) between these beads (Figure S3). Two peptides are connected if they have at-least one pair of beads within their respective cutoff distance. These cutoffs are detailed in the Table 1.

**Table 1** An example of a caption to accompany a table

Interaction	Cutoff (nm)
BB - BB	0.45
BB - S1	0.40
BB - S2	0.61
S1 - S1	0.65
S1 - S2	0.42
S2 - S2	0.70

The number of peptide clusters, representing peptide aggregates at a particular time is computed by determining connected components. In addition, the peptide aggregates is also categorized into three groups based on absorption classes of component peptides - *completely absorbed aggregates*, *partially absorbed aggregates* and *unabsorbed aggregates*. A peptide-aggregate has been listed as a completely absorbed aggregate, if *all* of its component peptides can be classified as either completely absorbed or partially absorbed. On the other hand, an aggregate is designated as a partially absorbed aggregate, if *at-least* one of its component peptide can be classified as partially absorbed and *at-least* one another as unabsorbed. Finally, if *all* of the component peptides are marked as unabsorbed, then the peptide-aggregate is categorized as unabsorbed aggregate.

#### 2.4.3 Beta Sheet Content

A backbone contact between two peptides is defined by alignment of back-bond dipoles (BBm–BBp), characterized using a distance cut off of 2.5 nm between two oppositely charged BB-dummies. This distance was determined from the interaction peak of radial distribution function,  $g(r)$ , between opposite charged dummy particles. We considered two peptides as beta sheets, if they have at least five (71.5 %) such backbone-backbone contacts and end-to-end distance greater than 1.2 nm, similar to beta sheet determination technique used by Lu et.al.<sup>90</sup>. The end-to-end length is the distance between the back-bone (BB) beads of flanking amino

acids - K and E. The fraction of peptides in mutual beta sheets constitute the total beta sheet content.

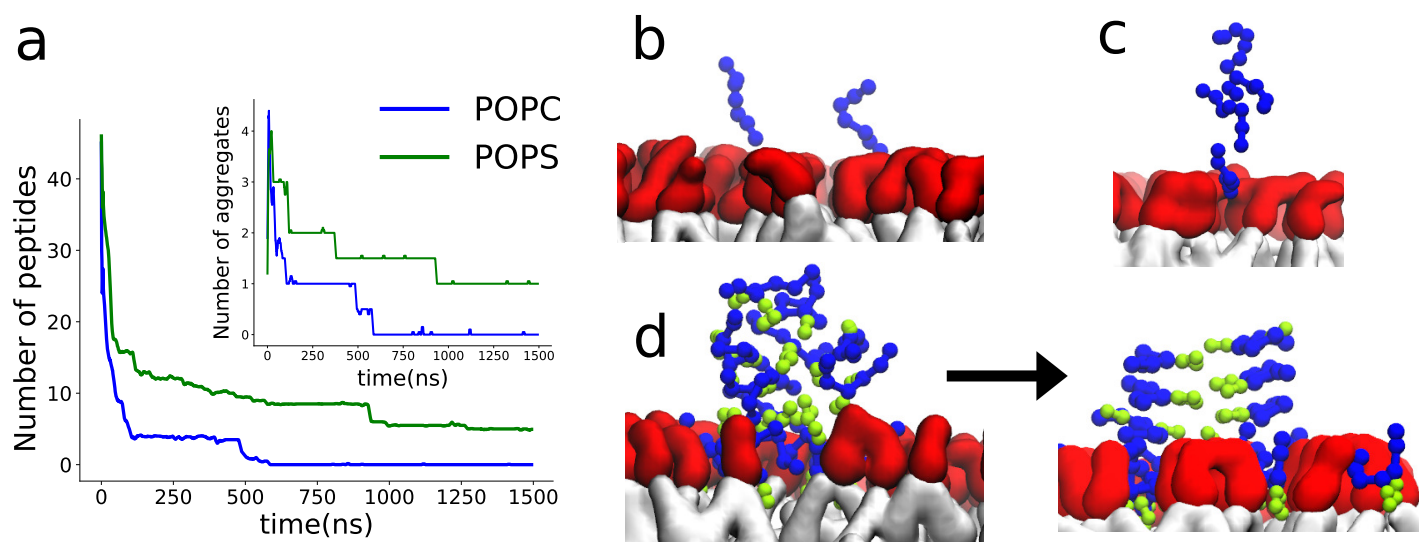
### 3 Results and discussion

Figure 2a shows the number of unabsorbed peptides over time whereas Figure 2a-inset presents the variation in the number of partially absorbed aggregates with time. The peptides can partition into lipid membranes as individual monomers (Figure 2b), as small oligomeric aggregates (Figure 2c) or by slow dissociation of larger peptide aggregations (Figure 2d) bound to the lipid membrane. Previous AFM studies with full length A $\beta$  peptide have also reported some of these pathways — absorption as monomers and oligomers into supported bilayers<sup>43</sup>. There was a sudden significant decrease in the number of unabsorbed peptides within about first 15 ns for both anionic-PS and zwitterionic-PC simulations (Figure 2a). Following that, the absorption slowed down due to formation of a number of partially absorbed aggregates. These aggregates arranged themselves to protect the hydrophobic cores of their component peptides (Figure 2d), which afforded some stability and reduced absorption rates. Over time, these partially absorbed aggregates continued to slowly lose peptides to the membrane and decrease in total number and size. Particularly, in contrast to POPC where all inter-facial aggregates were completely absorbed, one such aggregate on POPS rearranged and conformed into a stable layered beta sheet structure (Figure 2d) attached to the membrane, similar to structures observed in membrane-free systems (Figure S1).

The backbones of the absorbed peptides had variable residue-wise insertion into a lipid membrane modulated by their side chain hydrophobicity and relative position along the peptide chain (Figure S4). Throughout the 1.5  $\mu$ s simulation, the absorbed peptides remained close to the bilayer headgroup region (Figure 2d, S6). Once absorbed into the membrane, the peptides (monomers and aggregates) start diffusing laterally on bilayer surface as shown in the supplementary movie. In addition, as the peptides are pre-dominantly hydrophobic (71.43 %), the aggregation on the membrane occurs primarily by pushing away polar/charged lipid heads thereby exposing the hydrophobic tail region as is evident in Figure 3a-d, where peptides have pre-dominantly aggregated on top of the hydrophobic alkyl tails (white region).

#### 3.1 Rate of peptide aggregation

The variation in cumulative number of peptide clusters (ordered + disordered) over time is presented in Figure 3e. Over time, due to continued aggregation, the number of peptide clusters on POPC steadily reduced in number (Figure 3e) to about three clusters and increased in size — number of peptides (Figure S5). In contrast, on POPS membrane, the number of peptide aggregates/clusters continued to be relatively high, featuring significant variations in aggregate sizes, pointing to a comparatively slower aggregation rate. This disparity in aggregation patterns can be explained through difference in diffusion rates of PC and PS. Lateral diffusion of lipids is relatively slower in POPS with a lateral coarse grained diffusion constant of  $0.0369 \times 10^{-5} \text{ cm}^2/\text{s}$



**Fig. 2** a) Variation of number of “unabsorbed peptides” with time, averaged over two replica-simulations. The variation of the number of partially absorbed aggregates has been provided as *inset*. b,c,d) Different pathways for peptide absorption into lipid bilayer. b) Single (monomeric) peptide absorption. c) Peptide absorption as oligomeric aggregates. d) Peptide aggregation through dissociation and rearrangement of partially absorbed aggregates. Coloring scheme: Light green beads - Sidechains of Phenylalanines (F); Blue beads - Peptide backbones; Red region - Polar/charged lipid headgroup; White region - Hydrophobic alkyl tails (Lipids).

as compared to  $0.0899 \times 10^{-5} \text{cm}^2/\text{s}$  in POPC. While coarse grained diffusion constant is not directly comparable to experimental and atomistic results, it can capture relative trends. Similar qualitative trend has also been observed in other reported values for lateral diffusion constants<sup>102</sup>. The rigid headgroup of POPS due to intra-molecular pseudo-hydrogen bonds captured by CNO dipole-dipole interactions in this coarse grained scheme, prevents faster diffusion for lipids. This restricts the effective exclusion of lipid molecules<sup>77</sup>, preventing peptide aggregation. In addition, increased interaction between lipid head group (CNO/PO4) and peptides in POPS can further result in better mixing of peptides and lipids, compared to POPC (Figure 3f).

### 3.2 Beta Sheet Content

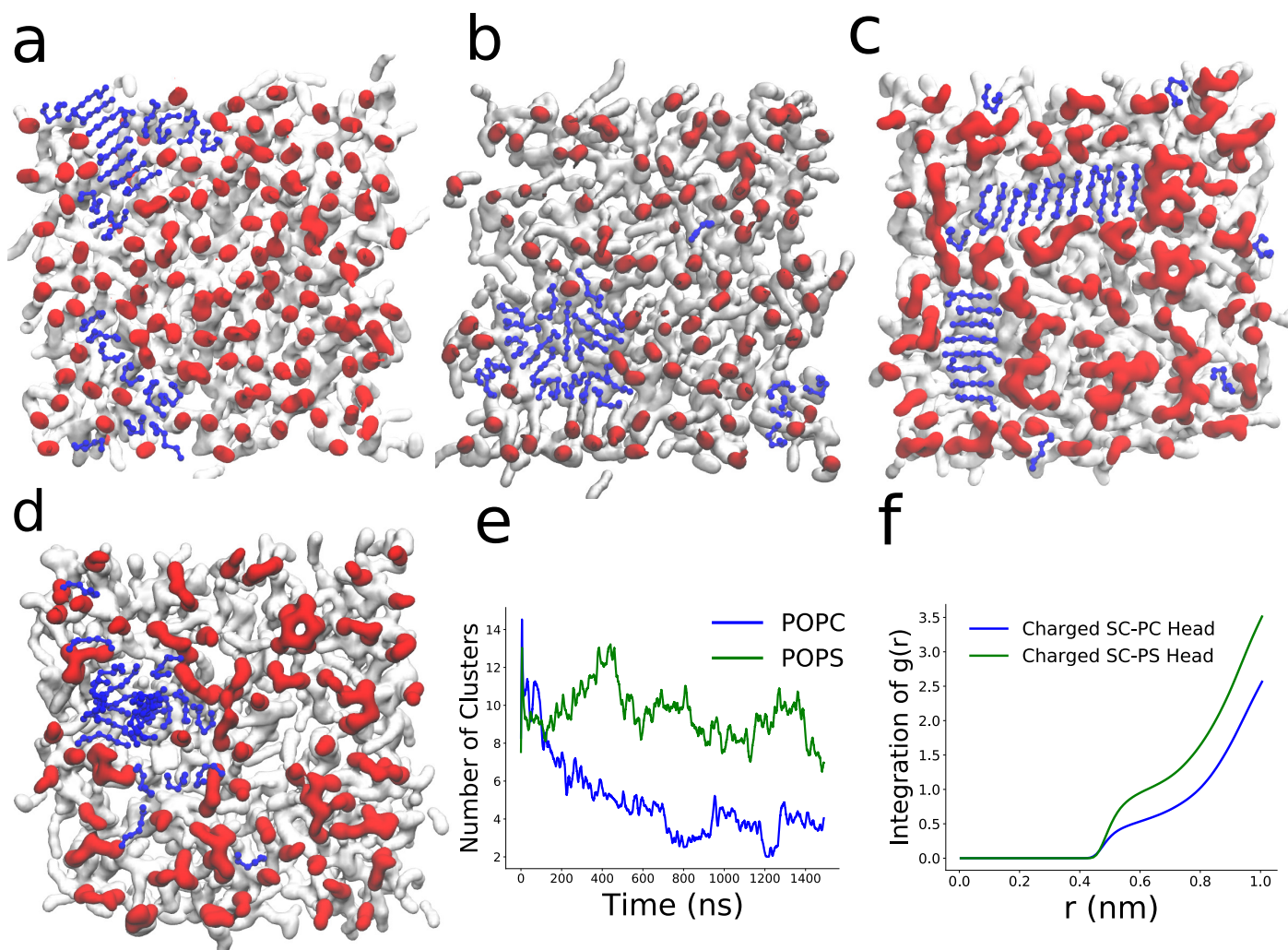
Over time, peptides rearranged into aggregates with high beta sheet content on both bilayers. Figure 4a presents time evolution of beta sheet content over time. Similar to reported CD and Thioflavin T assay studies<sup>45</sup> with A $\beta$  16-28, the beta sheet content in peptide aggregates was significantly larger (approximately double) for POPS as compared to POPC. Even with larger sized aggregates, peptides on PC bilayer were more unstructured. The higher beta sheet content on PS bilayer can be explained by the distribution of end-to-end distance of peptides. Peptides on PS are in general more elongated (Figure 4b) which exposes their backbone to more peptide backbone-backbone interactions, thereby increasing the overall beta sheet content. In agreement with our coarse grained simulations, atomistic molecular dynamics simulations of single peptide-membrane systems also reproduce a trend towards more elongated peptides in PS than PC (Figure S6). This lower end-to-end distance of peptides in PC can be reasoned in terms of membrane compressibility and peptide-lipid insertion. The higher compressibility of POPC membranes as compared to

POPS, that has been previously reported<sup>103</sup> and also captured by our CG model<sup>77</sup>, increases the relative penetration of peptides into POPC membranes. Figure 4c presents the distribution of F-S2, the “highest inserted” side chain bead into the membrane. This increased insertion of F-S2 on POPC membranes, coupled with charged residues at the ends — K and E which prefer to stay close to bilayer surface, distorts the shape of the peptide into a sharper “U/O - like” shape (Figure 4b) thereby decreasing the average end-to-end distances and backbone-bone contacts. The higher membrane disruption for some zwitterionic lipids like DOPC compared to anionic DOPG has also been recorded in AFM experiments by Hanes *et. al.*<sup>43</sup>. Moreover, similar to FTIR experiments on A $\beta$  peptides in membranous environment by Yu *et. al.*<sup>104</sup>, a relatively higher insertion of F-19 as compared to F-20 is observed. In addition, as apparent from Figure 4d the sidechain of Glutamate (E-S2) in POPS is positioned slightly away from the bilayer-headgroup as compared to POPC because of the interaction between positively charged choline (NC3) and negatively charged E-S2 which is absent in POPC. This further increases possibility of intra-peptide K-E interactions which results in a less expanded peptide conformation.

In addition, due to higher number of total inter-peptide interactions in large amorphous peptide aggregations on POPC, it is difficult for peptides to effectively rearrange into ordered beta sheets necessary for efficient fibrillation. On the other hand, peptides on PS form numerous slow diffusing smaller oligomeric aggregates which can reorganize comparatively easily into beta sheet rich structures before coalescing into larger aggregates.

Oligomeric deposits on membranes can act as nucleation-seeds for subsequent peptide aggregation from solution. To test this hypothesis, after the initial production run of  $1.5 \mu \text{s}$ , we added 48 more peptides into both bilayer systems (with pre-existing pep-





**Fig. 3** a,b) Last frame snapshot of peptide aggregates on two opposing leaflets of POPC lipid membrane in simulation 1. c,d) Last frame snapshot of peptide aggregates on two opposing leaflets of POPS lipid membrane in simulation 1. The red part in this representation corresponds to polar headgroup and the white part corresponds to hydrophobic tails. The blue connected beads represent peptide backbone. e) Variation in the number of A $\beta$  16-22 aggregates over time, averaged over both replica-simulations. Even connected components of size one (monomers) have been designated as a single cluster. f) Integration of radial distribution function between charged peptide sidechains (E/K-S2) and lipid headgroup (POPC:NC3/PO4, POPS:CNO/PO4), averaged over both replica-simulations.

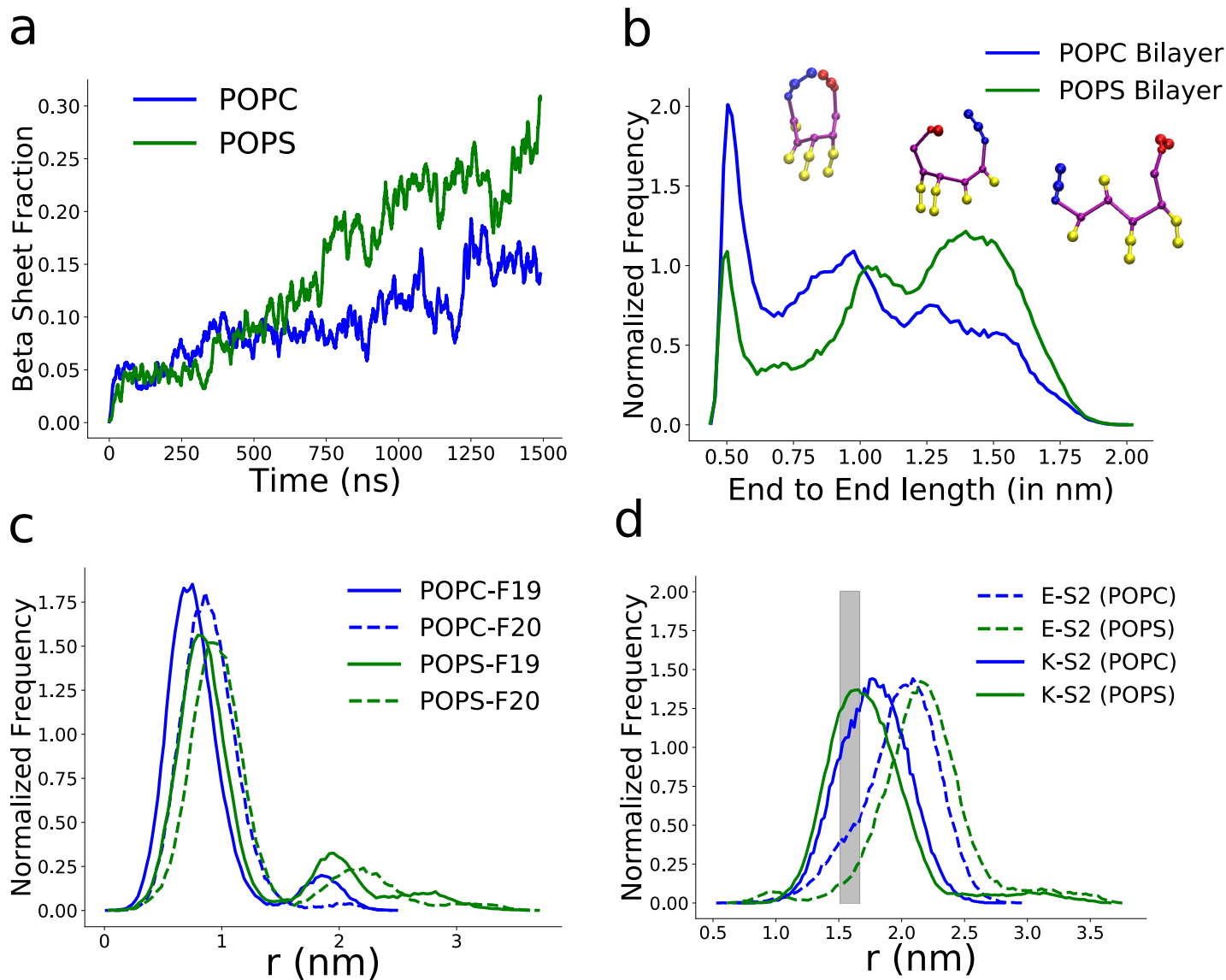
tide aggregates) and recorded their dynamics for 500 ns. Similar to previous experimental observations<sup>105</sup>, initial pre-existing oligomers acted as nucleating sites, recruiting either individual monomers or smaller oligomers to create large fibrillar aggregates (Figure S7). This presents a possibility of multiple ordered aggregates on POPS (larger than POPC) to act as nucleation sites that can progressively increase subsequent aggregation rates. These fibrils are attached to the membranes primarily through inter-peptide interactions with embedded peptides and extend into solution. The aggregate sizes of fibrils in solution, attached to the bilayers is on average larger than oligomers lying flat on membrane surfaces.

Studies with central hydrophobic core (CHC) provides insights about how structures develop in peptide aggregates<sup>106–108</sup>. Faster development of ordered beta sheet rich structures in A $\beta$  16-22 can be corroborated with faster ordered fibrillation and resulting toxicity of full length A $\beta$  peptides in POPS. Similar to our

observations, Lindberg et. al.<sup>109</sup> also found about two fold increase in fibrillation of A $\beta$  1-42 in an anionic membrane (DOPS). This effect of lipid headgroup chemistry has also been corroborated in previous work with surface plasmon resonance and magnetic bead assay on A $\beta$  1-40<sup>110</sup>.

## 4 Conclusion

Our coarse grained molecular dynamics simulations reproduce and provide a mechanistic explanation to a broad spectrum of experimental results<sup>43,45,104,109,110</sup>. We characterized multiple pathways for peptide absorption into membranes composed of POPC and POPS. Both lipid molecules have distinct effects on aggregation patterns of absorbed peptides. While rapid cumulative aggregation (ordered + disordered) was observed in zwitterionic PC bilayer, the emergence of ordered beta sheets and by extension, fibrillation was faster in presence of anionic POPS lipids. The results are in agreement with previous experimental stud-



**Fig. 4** a) Time evolution of beta sheet fraction. b) Distribution of end-to-end length of peptides over the last 200 ns. The gray line shows the end-to-end distance criteria used to determine beta sheets. (*inset*)-single peptide representative snapshots describing end-to-end lengths of peptides. Peptide backbone of A $\beta$  17-21 (LVFFA) is presented in magenta, whereas residues K and E are represented by blue and red respectively. The connected blue beads represent hydrophobic sidechains. c) Density distribution of E-S2/K-S2 (POPC/POPS) along bilayer normal from bilayer center over the last 200 ns of simulation time. The gray region describes the average location of bilayer headgroup (PO4). d) Density distribution of F19-S2/F20-S2 (POPC/POPS) beads along bilayer normal from bilayer center over last 200 ns of simulation time. All the results presented here have been averaged over all replica-simulations.

ies<sup>45,79,83–86,109,110</sup> that had observed faster growth of amyloid fibrils in presence of anionic lipids. The discrepancy in the cumulative aggregation rates is a consequence of faster lateral diffusion of POPC lipid molecules compared to POPS. On the other hand, increased beta sheet content in POPS membranes is due to the differences in membrane compressibility. Higher membrane compressibility of POPC membrane compared to POPS, results in a relatively higher peptide insertion into the bilayer. This distorts the geometry of individual peptide molecules which hinders their participation in beta sheet formation. Some of the morphological aspects of membrane assisted A $\beta$  aggregation reported in this study such as relatively higher membrane insertion of F19 compared to F20, have been supported by previous experimental ev-

idences<sup>104</sup>. We also revealed the propensity of initial oligomeric deposits to act as nucleation seeds to enhance further fibrillation. Considering the presence of multiple, ordered aggregates in POPS due to slow cumulative aggregation and peptide aggregates operating as nucleation seeds, POPS membranes can have an increased progressive peptide aggregation rates. This work unravels how lipid headgroup driven biochemical interactions in homogeneous model membranes shape peptide absorption and aggregation.

## Acknowledgement

This research was supported by the National Science Foundation under the Grant CHE-1454948 and through XSEDE resources pro-



vided by the Texas Advanced Computing Center (TACC) under the grant number TG-MCB120045.

## References

- 1 D. R. Howlett, K. H. Jennings, D. C. Lee, M. S. Clark, F. Brown, R. Wetzels, S. J. Wood, P. Camilleri and G. W. Roberts, *Neurodegeneration*, 1995, **4**, 23–32.
- 2 G. B. Irvine, O. M. El-Agnaf, G. M. Shankar and D. M. Walsh, *Mol. Med.*, 2008, **14**, 451–64.
- 3 M. Arrasate and S. Finkbeiner, *Exp Neurol.*, 2012, **238**, 1–11.
- 4 G. G. Glenner and C. W. Wong, *Biochem. Biophys. Res. Commun.*, 1984, **122**, 1131–1135.
- 5 G. G. Glenner and C. W. Wong, *Biochem. Biophys. Res. Commun.*, 1984, **120**, 885–890.
- 6 C. L. Masters, G. Simms, N. A. Weinman, G. Multhaup, B. L. McDonald and K. Beyreuther, *Proc. Natl. Acad. Sci. U.S.A.*, 1985, **82**, 4245–9.
- 7 C. Ballatore, V. M.-Y. Lee and J. Q. Trojanowski, *Nat. Rev. Neurosci.*, 2007, **8**, 663–672.
- 8 H. Braak and E. Braak, *E. Braak*, Springer, Vienna, 1998, pp. 127–140.
- 9 *Alzheimers Dement*, 2018, **14**, 367–429.
- 10 B. Yankner, L. Dawes, S. Fisher, L. Villa-Komaroff, M. Oster-Granite and R. Neve, *Science*, 1989, **245**, 417–420.
- 11 D. J. Selkoe, *Neuron*, 1991, **6**, 487–498.
- 12 M. Goedert and M. G. Spillantini, *Science*, 2006, **314**, 777–781.
- 13 D. J. Selkoe, *Physiol. Rev.*, 2001, **81**, 741–766.
- 14 R. Tanzi, J. Gusella, P. Watkins, G. Bruns, P. St George-Hyslop, M. Van Keuren, D. Patterson, S. Pagan, D. Kurnit, R. Neve and al. Et, *Science*, 1987, **235**, 880–884.
- 15 D. M. Walsh and D. J. Selkoe, *J. Neurochem.*, 2007, **101**, 1172–1184.
- 16 D. M. Walsh and D. J. Selkoe, *Neuron*, 2004, **44**, 181–193.
- 17 W. L. Klein, G. A. Krafft and C. E. Finch, *Trends Neurosci*, 2001, **24**, 219–224.
- 18 C. C. Glabe, *Amyloid Accumulation and Pathogenesis of Alzheimer's Disease: Significance of Monomeric, Oligomeric and Fibrillar A $\beta$* , Springer US, 2005, pp. 167–177.
- 19 J. Meinhardt, C. Sachse, P. Hortschansky, N. Grigorieff and M. Fändrich, *J. Mol. Biol.*, 2009, **386**, 869–877.
- 20 J. D. Gillmore, P. N. Hawkins and M. B. Pepys, *Br. J. Haematol.*, 1997, **99**, 245–256.
- 21 E. H. Koo, P. T. Lansbury and J. W. Kelly, *Proc. Natl. Acad. Sci. U.S.A.*, 1999, **96**, 9989–9990.
- 22 R. N. Rambaran and L. C. Serpell, *Prion*, 2008, **2**, 112–117.
- 23 K. L. Morris and L. C. Serpell, *Methods in molecular biology (Clifton, N.J.)*, 2012, vol. 849, pp. 121–135.
- 24 M. Sunde, L. C. Serpell, M. Bartlam, P. E. Fraser, M. B. Pepys and C. C. Blake, *J. Mol. Biol.*, 1997, **273**, 729–739.
- 25 H. A. Scheidt, I. Morgado, S. Rothmund and D. Huster, *J. Biol. Chem.*, 2012, **287**, 2017–2021.
- 26 A. Olofsson, M. Lindhagen-Persson, M. Vestling, A. E. Sauer-Eriksson and A. Öhman, *FEBS J.*, 2009, **276**, 4051–4060.
- 27 V. L. Anderson and W. W. Webb, *BMC Biotechnol.*, 2011, **11**, 125.
- 28 C. Sachse, M. Fändrich and N. Grigorieff, *Proc. Natl. Acad. Sci. U.S.A.*, 2008, **105**, 7462–7466.
- 29 L. Gremer, D. Schölzel, C. Schenk, E. Reinartz, J. Labahn, R. B. G. Ravelli, M. Tusche, C. Lopez-Iglesias, W. Hoyer, H. Heise, D. Willbold and G. F. Schröder, *Science*, 2017, **358**, 116–119.
- 30 C. Hilbich, B. Kisters-Woike, J. Reed, C. L. Masters and K. Beyreuther, *J. Mol. Biol.*, 1992, **228**, 460–473.
- 31 H. Inouye, P. E. Fraser and D. A. Kirschner, *Biophys. J.*, 1993, **64**, 502–519.
- 32 D. A. Kirschner, H. Inouye, L. K. Duffy, A. Sinclair, M. Lind and D. J. Selkoe, *Proc. Natl. Acad. Sci. U.S.A.*, 1987, **84**, 6953–6957.
- 33 S. J. Wood, R. Wetzels, J. D. Martin and M. R. Hurle, *Biochemistry*, 1995, **34**, 724–730.
- 34 K. A. Ball, A. H. Phillips, P. S. Nerenberg, N. L. Fawzi, D. E. Wemmer and T. Head-Gordon, *Biochemistry*, 2011, **50**, 7612–7628.
- 35 J. J. Balbach, Y. Ishii, O. N. Antzutkin, R. D. Leapman, N. W. Rizzo, F. Dyda, J. Reed and R. Tycko, *Biochemistry*, 2000, **39**, 13748–13759.
- 36 A. K. Mehta, K. Lu, W. S. Childers, Y. Liang, S. N. Dublin, J. Dong, J. P. Snyder, S. V. Pingali, P. Thiagarajan and D. G. Lynn, *J. Am. Chem. Soc.*, 2008, **130**, 9829–9835.
- 37 K. Lu, J. Jacob, P. Thiagarajan, V. P. Conticello and D. G. Lynn, *J. Am. Chem. Soc.*, 2003.
- 38 K. Tao, J. Wang, P. Zhou, C. Wang, H. Xu, X. Zhao and J. R. Lu, *Langmuir*, 2011, **27**, 2723–2730.
- 39 M. P. Murphy and H. I. LeVine, *J. Alzheimers Dis.*, 2010, **19**, 311–323.
- 40 A. Itkin, V. Dupres, Y. F. Dufrêne, B. Bechinger, J.-M. Ruyschaert and V. Raussens, *PLoS ONE*, 2011, **6**, e18250.
- 41 A. Demuro, I. Parker and G. E. Stutzmann, *J. Biol. Chem.*, 2010, **285**, 12463–12468.
- 42 E. Drolle, A. Negoda, K. Hammond, E. Pavlov and Z. Leonenko, *PloS ONE*, 2017, **12**, e0182194.
- 43 F. Hane, E. Drolle, R. Gaikwad, E. Faught and Z. Leonenko, *J. Alzheimers Dis.*, 2011, **26**, 485–494.
- 44 M. Vestergaard, T. Hamada and M. Takagi, *Biotechnol. Bioeng.*, 2008, **99**, 753–763.
- 45 N. Sureshbabu, R. Kirubakaran, H. Thangarajah, E. J. P. Malar and R. Jayakumar, *J. Mol. Neurosci.*, 2010, **41**, 368–382.
- 46 J. A. Lemkul and D. R. Bevan, *Protein Sci.*, 2011, **20**, 1530–1545.
- 47 I. Morgado and M. Garvey, *M. Garvey*, Springer, Cham, 2015, pp. 67–94.
- 48 L. Dominguez, L. Foster, J. E. Straub and D. Thirumalai, *Proc. Natl. Acad. Sci. U.S.A.*, 2016, **113**, E5281–E5287.
- 49 K. Matsuzaki, *Biochim. Biophys. Acta, Biomembr.*, 2007, **1768**, 1935–1942.
- 50 M. S. Terakawa, Y. Lin, M. Kinoshita, S. Kanemura, D. Itoh,

- T. Sugiki, M. Okumura and Y.-H. Lee, *Biochim. Biophys. Acta, Biomembr.*, 2018, **1860**, 1741–1764.
- 51 Y. Sugiura, K. Ikeda and M. Nakano, *Langmuir*, 2015, **31**, 11549–11557.
- 52 W. Yu, K. Zou, J. S. Gong, M. Ko, K. Yanagisawa and M. Michikawa, *J. Neurosci. Res.*, 2005, **80**, 114–119.
- 53 D. M. Walsh, A. Lomakin, G. B. Benedek, M. M. Condrón and D. B. Teplow, *J. Biol. Chem.*, 1997, **272**, 22364–22372.
- 54 R. Kaye, E. Head, J. L. Thompson, T. M. McIntire, S. C. Milton, C. W. Cotman and C. G. Glabe, *Science*, 2003, **300**, 486–489.
- 55 P. Das, S. Matysiak and J. Mittal, *ACS Cent Sci*, 2018, **4**, 534–542.
- 56 R. Wu, J. Liu, X. Qiu and M. Deng, *Mol Simul*, 2017, **43**, 1227–1239.
- 57 B. Urbanc, L. Cruz, F. Ding, D. Sammond, S. Khare, S. Buldyrev, H. Stanley and N. Dokholyan, *Biophys. J.*, 2004, **87**, 2310–2321.
- 58 M. Biancalana and S. Koide, *Biochim. Biophys. Acta, Proteins Proteomics*, 2010, **1804**, 1405–1412.
- 59 N. G. Sgourakis, M. Merced-Serrano, C. Boutsidis, P. Drineas, Z. Du, C. Wang and A. E. Garcia, *J. Mol. Biol.*, 2011, **405**, 570–583.
- 60 N. G. Sgourakis, Y. Yan, S. A. McCallum, C. Wang and A. E. Garcia, *J. Mol. Biol.*, 2007, **368**, 1448–1457.
- 61 N. Nishikawa, P. H. Nguyen, P. Derreumaux and Y. Okamoto, *Mol Simul*, 2015, **41**, 1041–1044.
- 62 D. K. Klimov and D. Thirumalai, *Structure*, 2003, **11**, 295–307.
- 63 S. Santini, N. Mousseau and P. Derreumaux, *J. Am. Chem. Soc.*, 2004, **126**, 11509–11516.
- 64 C. H. Davis and M. L. Berkowitz, *Biophys. J.*, 2009, **96**, 785–797.
- 65 L. N. Zhao, S.-W. Chiu, J. Benoit, L. Y. Chew and Y. Mu, *J. Phys. Chem. B*, 2011, **115**, 12247–12256.
- 66 A. Brown and D. Bevan, *Biophys. J.*, 2016, **111**, 937–949.
- 67 S. Kmiecik, D. Gront, M. Kolinski, L. Wieteska, A. E. Dawid and A. Kolinski, *Chem. Rev.*, 2016, **116**, 7898–7936.
- 68 S. Abeln, M. Vendruscolo, C. M. Dobson and D. Frenkel, *PLoS ONE*, 2014, **9**, e85185.
- 69 R. Friedman, R. Pellarin and A. Caflisch, *J. Mol. Biol.*, 2009, **387**, 407–415.
- 70 A. Morriss-Andrews, F. L. H. Brown and J.-E. Shea, *J. Phys. Chem. B*, 2014, **118**, 8420–8432.
- 71 M. Chiricotto, T. T. Tran, P. H. Nguyen, S. Melchionna, F. Sterpone and P. Derreumaux, *Isr. J. Chem.*, 2017, **57**, 564–573.
- 72 Y. Chebaro, S. Pasquali and P. Derreumaux, *J. Phys. Chem. B*, 2012, **116**, 8741–8752.
- 73 M. Cheon, I. Chang and C. K. Hall, *Proteins*, 2010, **78**, 2950–2960.
- 74 M. Cheon, M. Kang and I. Chang, *Sci. Rep.*, 2016, **6**, 38196.
- 75 W. Zheng, M.-Y. Tsai, M. Chen and P. G. Wolynes, *Proc. Natl. Acad. Sci. U.S.A.*, 2016, **113**, 11835–11840.
- 76 S. J. Ganesan and S. Matysiak, *J. Chem. Theory Comput.*, 2014, **10**, 2569–2576.
- 77 S. J. Ganesan, H. Xu and S. Matysiak, *Phys. Chem. Chem. Phys.*, 2016, **18**, 17836–17850.
- 78 S. J. Ganesan, H. Xu and S. Matysiak, *J. Phys. Chem. B*, 2017, **121**, 787–799.
- 79 C. Ege, J. Majewski, G. Wu, K. Kjaer and K. Y. C. Lee, *ChemPhysChem*, 2005, **6**, 226–229.
- 80 S. J. Ganesan and S. Matysiak, *Phys. Chem. Chem. Phys.*, 2016, **18**, 2449–2458.
- 81 S. O. Yesylevskyy, L. V. Schäfer, D. Sengupta and S. J. Marrink, *PLOS Comput. Biol.*, 2010, **6**, e1000810.
- 82 R. O. Calderon, B. Attema and G. H. DeVries, *J. Neurochem.*, 2002, **64**, 424–429.
- 83 X. Yu, Q. Wang, Q. Pan, F. Zhou and J. Zheng, *Phys. Chem. Chem. Phys.*, 2013, **15**, 8878.
- 84 J. McLaurin and A. Chakrabarty, *Eur. J. Biochem.*, 1997, **245**, 355–363.
- 85 E. Terzi, G. Hoelzemann and J. Seelig, *Biochemistry*, 1994, **33**, 7434–7441.
- 86 E. Terzi, G. H. Hoelzemann and J. Seelig, *Biochemistry*, 1997.
- 87 C. Canale, S. Seghezze, S. Vilasi, R. Carrotta, D. Bulone, A. Diaspro, P. L. San Biagio and S. Dante, *Biophys. Chem.*, 2013, **182**, 23–29.
- 88 M.-A. Sani, J. D. Gehman and F. Separovic, *FEBS Lett.*, 2011, **585**, 749–754.
- 89 T.-L. Lau, J. D. Gehman, J. D. Wade, K. Perez, C. L. Masters, K. J. Barnham and F. Separovic, *Biochim Biophys Acta Biomembr*, 2007, **1768**, 2400–2408.
- 90 Y. Lu, P. Derreumaux, Z. Guo, N. Mousseau and G. Wei, *Proteins*, 2009, **75**, 954–963.
- 91 S. J. Marrink, H. J. Risselada, S. Yefimov, D. P. Tieleman and A. H. de Vries, *J. Phys. Chem. B*, 2007.
- 92 S. Pronk, S. Páll, R. Schulz, P. Larsson, P. Bjelkmar, R. Apostolov, M. R. Shirts, J. C. Smith, P. M. Kasson, D. van der Spoel, B. Hess and E. Lindahl, *Bioinformatics*, 2013.
- 93 S. Nosé, *Mol. Phys.*, 1984, **52**, 255–268.
- 94 Hoover, *Phys. Rev. A*, 1985, **31**, 1695–1697.
- 95 M. Parrinello and A. Rahman, *J. Appl. Phys.*, 1981, **52**, 7182–7190.
- 96 T. Darden, D. York and L. Pedersen, *J. Chem. Phys.*, 1993, **98**, 10089–10092.
- 97 U. Essmann, L. Perera, M. L. Berkowitz, T. Darden, H. Lee and L. G. Pedersen, *J. Chem. Phys.*, 1995, **103**, 8577–8593.
- 98 N. Kandel, T. Zheng, Q. Huo and S. A. Tatulian, *J. Phys. Chem. B*, 2017, **121**, 10293–10305.
- 99 S. J. Marrink and A. E. Mark, *J. Chem. Phys.*, 2003.
- 100 C.-M. Lin, C.-S. Li, Y.-J. Sheng, D. T. Wu and H.-K. Tsao, *Langmuir*, 2012, **28**, 689–700.
- 101 W. Humphrey, A. Dalke and K. Schulten, *J Mol Graph*, 1996, **14**, 33–38.
- 102 F. E. Herrera and S. Pantano, *J. Chem. Phys.*, 2012, **136**, 015103.

- 103 J. Pan, X. Cheng, L. Monticelli, F. A. Heberle, N. Kučerka, D. P. Tieleman and J. Katsaras, *Soft Matter*, 2014, **10**, 3716.
- 104 Y. Hu, P. Kienlen-Campard, T.-C. Tang, F. Perrin, R. Opsomer, M. Decock, X. Pan, J.-N. Octave, S. N. Constantinescu and S. O. Smith, *Sci. Rep.*, 2017, **7**, 17159.
- 105 N. R. Anthony, A. K. Mehta, D. G. Lynn and K. M. Berland, *Soft Matter*, 2014, **10**, 4162–4172.
- 106 S. Chimon, M. A. Shaibat, C. R. Jones, D. C. Calero, B. Aizezi and Y. Ishii, *Nat. Struct. Mol. Biol.*, 2007, **14**, 1157–1164.
- 107 N.-V. Buchete and G. Hummer, *Biophys. J.*, 2007, **92**, 3032–3039.
- 108 J. Li, R. Liu, K. S. Lam, L.-W. Jin and Y. Duan, *Biophys. J.*, 2011, **100**, 1076–1082.
- 109 D. J. Lindberg, E. Wesén, J. Björkeröth, S. Rocha and E. K. Esbjörner, *Biochim Biophys Acta Biomembr*, 2017, **1859**, 1921–1929.
- 110 J. J. Kremer and R. M. Murphy, *J. Biochem. Biophys. Methods*, 2003, **57**, 159–169.