Amyloid-β Peptide Triggers Membrane Remodeling in Supported Lipid Bilayers Depending on Their Hydrophobic Thickness

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5 Supporting Information

ABSTRACT: Amyloid-β (Aβ) peptide has been implicated in Alzheimer’s disease, which is a leading cause of death worldwide. The interaction of Aβ peptides with the lipid bilayers of neuronal cells is a critical step in disease pathogenesis. Recent evidence indicates that lipid bilayer thickness influences Aβ membrane-associated aggregation, while understanding how Aβ interacts with lipid bilayers remains elusive. To address this question, we employed supported lipid bilayer (SLB) platforms composed of different-length phosphatidylcholine (PC) lipids (C12:0 DLPC, C18:1 DOPC, C18:1-C16:0 POPC), and characterized the resulting interactions with soluble Aβ monomers. Quartz crystal microbalance-dissipation (QCM-D) experiments identified concentration-dependent Aβ peptide adsorption onto all tested SLBs, which was corroborated by fluorescence recovery after photobleaching (FRAP) experiments indicating that higher Aβ concentrations led to decreased membrane fluidity. These commonalities pointed to strong Aβ peptide–membrane interactions in all cases. Notably, time-lapsed fluorescence microscopy revealed major differences in Aβ-induced membrane morphological responses depending on SLB hydrophobic thickness. For thicker DOPC and POPC SLBs, membrane remodeling involved the formation of elongated tubule and globular structures as a passive means to regulate membrane stress depending on Aβ concentration. In marked contrast, thin DLPC SLBs were not able to accommodate extensive membrane remodeling. Taken together, our findings reveal that membrane thickness influences the membrane morphological response triggered upon Aβ adsorption.

INTRODUCTION

Amyloid-β (Aβ) peptide is typically associated with Alzheimer’s disease, which is a neurodegenerative condition that led to over 1.9 million deaths worldwide in 2015.1,4 Aβ is the intramembrane domain of the amyloid precursor protein (APP) and is produced by proteolytic cleavage of APP.5 Upon its accumulation in the brain, Aβ aggregates into fibrils that form senile plaque, which is a hallmark of Alzheimer’s disease.1,3 The pathological role of Aβ peptide in disease pathogenesis is not fully understood,1,5,6 yet it is closely related to its aggregation state: While Aβ fibrils are considered to be nontoxic, smaller Aβ oligomers have demonstrated pathogenic character.7–9 Notably, the lipid membrane plays a meaningful role in these processes.10–13 Membrane properties such as lipid bilayer thickness regulate the generation and surface-induced aggregation of Aβ peptides.14–16 As alterations in lipid bilayer properties are associated with aging and Alzheimer’s disease, they may affect Aβ biological functionality.12,17,18 Recently, it has been shown that Aβ interacts differently with model membranes mimicking healthy and diseased cellular states.18 Therefore, understanding membrane–Aβ peptide interactions and their dependence on membrane characteristics is essential.12,17

The cellular membrane influences Aβ biological functionality by modulating its generation and self-assembly. In model systems, it has been shown that lipid membranes catalyze or inhibit Aβ fibrillation depending on the lipid composition, and lipid–peptide ratio.10,14,16,19–25 Among membrane characteristics, reduced fluidity was reported to increase Aβ aggregation; in turn, Aβ decreased membrane fluidity upon its adsorption.16,24,25 Additionally, the presence of cholesterol, GM1 (monosialotetrahexosylganglioside), and negatively charged lipids has been observed to increase the effect of Aβ on lipid membranes.13,16,23,26 Moreover, recent studies indicate that neutral membrane composed of phosphatidylcholine (PC) lipids also interacts with Aβ and influences its aggregation properties.14,21,22,25 Recently, the curvature of the lipid bilayer was reported to dictate the interaction of Aβ with the bilayer and its aggregation properties as well.21,22 Packing

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defects, which have higher density in smaller, higher curvature vesicles, expose hydrophobic clefts on the vesicle surface. These hydrophobic clefts were found to be nucleation sites of Aβ that modulated its aggregation depending on the lipid/peptide ratio.21,22 This observation correlates with additional studies that demonstrated Aβ affinity to hydrophobic regions.14,15 The thickness of model membranes, which consisted of zwitterionic PC lipids in solution-phase vesicles, regulated the proteolytic cleavage of APP to produce Aβ. Particularly, thinner model membranes inhibited the generation of Aβ, in comparison to thicker model membranes such as 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC).15,20 Moreover, model PC membranes with the thin hydrophobic domain, i.e., 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) vesicles, prevented fibril formation. Instead, they promoted the formation of toxic Aβ oligomers.38 Vesicles with thicker hydrophobic regions, such as DOPC and POPC, catalyzed the Aβ fibrillation. This illustrates the importance of hydrophobic thickness in Aβ–membrane interactions.

Upon interacting with the membrane, Aβ disrupts the lipid bilayer integrity and, in turn, may initiate neurotoxicity.3,4 The peptide induced pores, as well as membrane fragmentation, and morphological changes in the lipid bilayers.5,6–12 The proposed mechanism involves α-helix insertion into the hydrophobic domain of the membrane followed by conformational changes of the peptide and its aggregation in the membrane.13,23,25,26,28,29,33-38 Aβ insertion into PC zwitterionic membranes was more pronounced than into negatively charged membranes.25 In addition, molecular dynamics investigations supported Aβ aggregation in zwitterionic membranes and that fibrils formed remained in the membrane.39,40 Such membrane-associated aggregation was accompanied by morphological changes in the lipid bilayer and reduced mobility of the lipids.38,28,35,36,41–43 This mode of action resembles other amyloidogenic proteins and pore-forming antimicrobial peptides such as MSI-78 and polymyxins, all of which induce membrane remodeling following by membrane fragmentation.13,25,46,47

Aβ–membrane interactions, and the resulting membrane remodeling, were mostly explored by employing unilamellar vesicles in solution or typically by atomic force microscopy (AFM). The former led to numerous valuable mechanistic insights but lacked the direct visualization of morphological changes in the lipid bilayer. The latter, AFM characterization, revealed informative morphological changes, yet was focused on surface-adsorbed peptide aggregates; distinguishing lipids clusters from peptide aggregation and capturing rapid changes in the lipid bilayers were more evasive. Hence, early stages of the membrane-associated aggregation and its accompanied membrane disruption remain elusive,44 including the occurrence and growth of such morphological changes, exploration of their nature, and their dependence on membrane properties such as hydrophobic thickness.

Supported lipid bilayers (SLBs) are a convenient platform to study morphological changes, as they enable the localized visualization of the membrane as well as the measurement of the average membrane response to interactions with active agents.48–51 Although SLBs have reduced flexibility compared with giant unilamellar vesicles (GUVs),51 and may limit the analysis of curvature and stoichiometric peptide/lipid ratio aspects, their particular advantages make them a fitted platform to gain insights on Aβ–membrane interactions. Herein these interactions were investigated using zwitterionic SLBs of varying hydrophobic thickness by employment of different-length PC lipids, C12:0 DLPC, C18:1 DOPC, and C18:1-C16:0 POPC. The model membranes were carefully selected for their ability to either catalyze Aβ fibril formation, DOPC and POPC, or to completely inhibit fibrillation, DLPC, while presenting the same headgroup and therefore similar surface characteristics.14,20,38 The interactions were investigated using quartz crystal microbalance with dissipation (QCM-D)52 to evaluate the overall effect and the binding of the peptide to the model membranes upon their immediate interaction; fluorescence recovery after photobleaching (FRAP)53 was employed to study the changes in lipid mobility following the interactions with Aβ, and time-lapsed fluorescence visualization of the model membranes was used to identify rapid morphological changes, within seconds, upon Aβ administration. This work presents that Aβ peptide adsorbed strongly to PC SLBs and in turn impaired their fluidity irreversibly. In addition, Aβ peptide induced morphological changes, which were identified as membrane remodeling upon peptide addition. Markedly, membrane remodeling was observed solely for thick lipid bilayers, i.e., DOPC and POPC, hence associating the Aβ ability to induce membrane remodeling with the hydrophobic thickness of the membrane.

## RESULTS AND DISCUSSION

### Fabrication of Supported Lipid Bilayers with Varying Membrane Thickness

The disruption of the membrane integrity by Aβ has been evaluated on model membranes of varying thickness. We fabricated three SLBs of varying thickness by the spontaneous rupture of large unilamellar vesicles (LUVs) of 80 nm size, on the silica surface (Figure 1). To modulate the lipid bilayer thickness, zwitterionic PC lipids with different hydrocarbon chain lengths were employed: C18:1 DOPC, C18:1-C16:0 POPC, and the short C12:0 DLPC (Figure 1a). The rupture of the LUVs to form a complete SLB was monitored using QCM-D (Figure 1b).54,55 The resonance frequency (Δf) and the energy dissipation (ΔD) shifts were recorded as a function of time; decrease in Δf reflected mass increase on the surface, and the ΔD increase indicated greater viscoelasticity of the adlayer. Vesicles in Tris buffer 10 mM, 150 mM NaCl, pH 7.5, of either DOPC, POPC, or DLPC, were injected following 5 min of stabilization with buffer. Upon injection, the Δf decreased and thus indicated a greater mass on the surface due to vesicles adsorption. Vesicles were adsorbed up to a critical concentration, following which they were ruptured to form a complete lipid bilayer on the surface as was reflected by the typical increase in Δf and a decrease in ΔD values associated with this process.54,55 Final Δf and ΔD values agreed with previously reported values and confirmed the formation of a lipid bilayer on the surface:54,55 DOPC Δf = −25.8 ± 0.7 Hz, ΔD = 0.3 ± 0.2 (10⁶); POPC Δf = −26.6 ± 0.6 Hz, ΔD = 0.3 ± 0.2 (10⁶); DLPC Δf = −23.5 ± 0.4 Hz, ΔD = 0.3 ± 0.2 (10⁶). The DLPC lipid bilayer exhibited a lower Δf shift due to DLPC shorter alkyl chain, i.e., lower molecular mass. The SLB thickness was calculated on the basis of QCM-D data using the Sauerbrey model (Figure 1c, Table 1).54 DLPC with the shorter alkyl chain demonstrated the lowest thickness value,
Aβ Adsorption on Zwitterionic Supported Lipid Bilayers. To study the interactions of Aβ1–40 monomers with SLBs, Aβ1–40 monomers were first disaggregated. Different disaggregation protocols produce different polymeric fibrils in the presence of model membranes; therefore, to produce comparable data with previous studies on hydrophobic thickness, the following protocol was selected: lyophilized Aβ was treated with hexafluorosilpropanol (HFIP), dried under nitrogen into thin film, and then treated with a NH2OH solution to achieve monomer disaggregation.

The interactions of Aβ1–40 monomers with the SLBs were evaluated in real time using QCM-D (Figure 2 and Figures S1–3). Arrow 1 marks the lipid vesicles injection, followed by buffer rinse to remove excess lipids. Subsequently, Aβ1–40 peptide was injected for 1 h (arrow 2), and the excess peptide was rinsed for additional 15 min (arrow 3). Changes in Δf (Hz) and ΔD (10−6) were recorded throughout the process. Representative plots, upon administration of 20 μM Aβ, are presented in Figure 2a. Values of Δf and ΔD shifted dramatically upon the injection of the peptide (arrow 2) implying that the peptide immediately interacted with the membrane, without any lag-time (Figure 2a). The net shift in Δf values, which correlates with the mass of the adsorbed Aβ peptide, was calculated by subtracting the Δf value prior to the peptide injection from the final Δf value recorded. The net shift in Δf values for 20 μM Aβ were the following: DOPC −50.4 ± 4.5 Hz, POPC −70.8 ± 7.8 Hz, and DLPC −71.8 ± 2.8 Hz (Figure 2). This implied strong interactions between the peptide and the membrane and a considerable peptide adsorption. ΔD values increased correspondingly upon peptide adsorption: DOPC 6.9 ± 0.5 × 10−6, POPC 8.3 ± 0.8 × 10−6, DLPC 8.1 ± 0.6 × 10−6 (Figure 2a). This demonstrated greater viscoelasticity of the adlayer due to peptide attachment, and hence supported peptide adsorption on the lipid bilayer. Similar trends were recorded for lower Aβ concentrations, 1–10 μM, yet with a moderate response compared with 20 μM Aβ (Figure 2b and Figures S1–3). The net changes in Δf and ΔD decreased depending on the Aβ concentration applied (Figure 2b). Hence, adsorption of Aβ and in turn the peptide–membrane interactions were dependent on the Aβ administered concentration. In addition, a linear correlation was found between the Δf and ΔD shifts (Figure S4), which suggests that the softening of the bilayer, as evinced by the ΔD increase, was directly related to the amount of adsorbed Aβ. This finding represents a possible insertion of Aβ into the hydrophobic core upon its adsorption onto the lipid bilayer, as it was observed for Aβ and other amyloidogenic proteins.

The Aβ adsorption rate on DOPC was moderately slower, with ∼15–20% lower Δf and ΔD shifts, compared with POPC and DLPC. Notably, Aβ favored dense bilayers with low area per lipid molecule, i.e., POPC and DLPC, without dependence on their membrane thickness (Table 1). As dense bilayers provide a more hydrophobic environment in their core, the higher adsorption rates on them agree with the typical affinity of Aβ to hydrophobicity.

The Δf and ΔD values measured with QCM-D were in excellent agreement with previous reports of hydrophobic thickness measured by X-ray scattering.

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Table 1. DOPC, POPC, and DLPC Bilayers: Selected Physicochemical Properties and a Summary of Their Response to Aβ Peptide Interactions

<table>
<thead>
<tr>
<th></th>
<th>DOPC</th>
<th>POPC</th>
<th>DLPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>acyl chain</td>
<td>18:1</td>
<td>18:1,16:0</td>
<td>12:0</td>
</tr>
<tr>
<td>thickness (nm)</td>
<td>4.5 ± 0.1</td>
<td>4.7 ± 0.2</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>area per molecule (Å²)</td>
<td>72.4 ± 27</td>
<td>68.3 ± 70</td>
<td>63.2 ± 26</td>
</tr>
<tr>
<td>bending modulus (N/m)</td>
<td>1.3 ± 0.79</td>
<td>3.9 ± 0.69</td>
<td>9.2 ± 0.59</td>
</tr>
<tr>
<td>fluidity (μm²/s)</td>
<td>2.3 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>Aβ adsorption</td>
<td>medium</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>membrane remodeling</td>
<td>high</td>
<td>medium</td>
<td>low</td>
</tr>
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*Based on the data presented and discussed in this work.*

4.1 ± 0.1 nm, whereas DOPC and POPC exhibited greater membrane thicknesses of 4.5 ± 0.1 and 4.7 ± 0.2 nm, respectively. The difference in the thickness of DOPC and POPC was accounted for the number of unsaturated bonds in the hydrocarbon chain, which resulted in a kink structure and hence a slightly thinner hydrophobic domain. All values agreed with previous reports of hydrophobic thickness measured by X-ray scattering.
terms of trends and in final values measured, throughout the Aβ concentration range (Figure 2 and Figures S1–3).

The Δf and ΔD values were recorded during a 15 min buffer rinse, following the Aβ adsorption. The values were stable during the rinse for all the Aβ concentrations employed and the different SLBs (Figure 2 and Figures S1–3). The relatively stable Δf and ΔD values imply that neither Aβ detachment nor membrane fragmentation occurred under these conditions, which are typically accompanied by shifts in Δf and ΔD values.43 These observations support irreversible adsorption of Aβ and reject a reversible equilibrium-controlled adsorption of the peptide to PC lipid bilayers; this is in contrast to fatty acids or detergents, whose dissociation from the bilayer following buffer rinsing is accompanied by a major shift in Δf and ΔD values.43 Moreover, the trends of both Δf and ΔD shifts were distinct to amyloidogenic species, i.e., similar to additional amyloidogenic proteins, yet differ from other membrane-active peptides.24,43–65 Overall, findings imply an immediate interaction, without any lag-phase, between Aβ and the lipid bilayer, similar to additional amyloidogenic proteins,24,43–65 and an irreversible adsorption of Aβ on the zwitterionic membranes. This observation was similar for all the model membranes, regardless of their hydrophobic thickness.24 Second, QCM-D data deny major fragmentation or solubilization of the membranes and agree with previous observations, which were recorded with lipid vesicles.19,26

Concentration-Dependent Aβ–Bilayer Interaction Hinders Membrane Fluidity. The lipid bilayers were visualized by epifluorescence microscopy using Rhodamine B labeled lipids to study the effect of Aβ on the mobility and morphology of the membranes. Epifluorescence visualization and FRAP measurements confirmed the formation of homogeneous, fluidic SLBs prior to the peptide injection (Figure 3a, Table 1, and Movies S1–3). Lateral diffusion coefficients of the SLBs were calculated on the basis of >15 measurements. The SLBs presented diffusion coefficients within the expected range: DLPC 3.8 ± 0.2, POPC 2.7 ± 0.2, and DOPC 2.3 ± 0.2 μm²/s.66,67 Differences in the fluidity of the SLBs can be contributed either to the size of the lipid molecule and van der Waals interactions between phospholipid molecules, which are both governed by the length of the hydrocarbon chain, i.e., shorter chain leads to greater fluidity, or to the packing density, which is influenced by the saturation degree, i.e., higher saturation degree decreases the fluidity. DLPC SLB, with the shortest hydrocarbon chain and the highest saturation degree, presented the highest fluidity. This suggests that the chain length is the dominant parameter under these experimental conditions.

The mobility of the model membranes was measured following Aβ administration (Figure 3b, Figure S5, and Movies S4–5). FRAP measurements of the lipid bilayer fluidity were conducted twice for each experiment: prior to Aβ injection, i.e., the SLB untreated, and following Aβ administration for 30 min and rinse to remove excess of Aβ. Lateral diffusion coefficients driven from the FRAP experiments following administration of Aβ are presented in Figure 3. With the increase Aβ concentration, the membrane mobility decreased. Upon administration of high Aβ concentrations, 10–20 μM, the fluidity of the lipid bilayers was decreased considerably, yet a moderate degree of mobility remained.24,61 In particular, the peptide–membrane interactions upon exposure to 20 μM Aβ hindered the mobility of the three model membranes similarly: DOPC 1.04 ± 0.03, POPC 1.1 ± 0.1, and DLPC 1.13 ± 0.05 μm²/s. The diffusivity coefficients measured were similar despite the distinct intrinsic fluidity of the model membranes.
Generally, changes in the fluidity can be attributed to structural changes in the bilayer due to the insertion of the peptide, or to a physical restrain of the lipid diffusion by a peptide-adlayer on the membrane. On the basis of previous reports, the peptide interacts with the hydrophobic core, and it is not merely physical restrain of adlayer. The insertion of the peptide, which is governed by hydrophobic interactions, decreases the lipid density in the bilayer. Yet, the observed fluidity decrease is concentration-dependent manner, and not increased as may be expected. This implies that Ap may act as a docking agent upon its interaction with the lipids. Furthermore, the fluidity hindrance corresponds with the QCM-D measurements and the high ΔD shifts measured. The increased ΔD values represent a rupture in lipid–lipid interactions due to the peptide insertion into the hydrophobic core, as was demonstrated for the amyloidogenic protein, human islet amyloid polypeptide (hIAPP). The similar trends in fluidity inhibition and ΔD shifts mark similar peptide–membrane interactions motivating the adsorption of Ap to the PC membranes and support the insertion of Ap into the hydrophobic core and its interference in lipid–lipid interactions.

Ap Induces Membrane Remodeling Depending on the Thickness of the Lipid Bilayer. Morphological changes in the SLBs due to Ap–membrane interactions were studied with epifluorescence imaging and photobleaching (FRAP) measurements to estimate the fluidity of DOPC, POPC, and DLPC SLBs, image size 0.14 × 0.14 mm², with fluorescence intensity recovery (%) dependent on time (s). (a) Fluorescence recovery after photobleaching (FRAP) measurements to estimate the fluidity of DOPC, POPC, and DLPC SLBs, image size 0.14 × 0.14 mm², with fluorescence intensity recovery (%) dependent on time (s). (b) Lateral diffusion coefficients (μm²/s) calculated on the basis of FRAP data following exposure to 1, 3, 10, 20 μM Ap, and without Ap, 0 μM.

![Figure 3](image_url)

Figure 3. Lateral diffusivity coefficients of DOPC, POPC, and DLPC SLBs following 30 min of exposure to Ap depending on the peptide concentration. (a) Fluorescence recovery after photobleaching (FRAP) measurements to estimate the fluidity of DOPC, POPC, and DLPC SLBs, image size 0.14 × 0.14 mm², with fluorescence intensity recovery (%) dependent on time (s). (b) Lateral diffusion coefficients (μm²/s) calculated on the basis of FRAP data following exposure to 1, 3, 10, 20 μM Ap, and without Ap, 0 μM.

To evaluate the intensity of the morphological changes induced by Ap following 30 min, analysis of the number of lipid aggregation events and their size was achieved by ImageJ function, particle analysis (Figure 5). The background was subtracted, followed by thresholding of the image to enable the count of distinct aggregation events and measure their size. A summary of the number of distinct lipid aggregation events and the total size (μm²) of lipid clusters per image captured was plotted (Figure 6). DOPC was the SLB most sensitive to morphological changes (Figures 4 and 6). DOPC demonstrated both tubular and globular clusters as a response to Ap adsorption at all concentrations tested, except for the minimal concentration, 1 μM Ap. POPC lipid bilayer exhibited tubule and bud lipid aggregates upon administration of the maximal peptide concentration tested, 20 μM, whereas for lower concentrations minimal morphological changes were observed (Figures 4 and 6). In marked contrast, DLPC did not present stable morphological changes under these conditions (Figures 4 and 6). Minor morphological changes of tubular shape appeared, yet they rapidly disappeared within a period of seconds. No major fragmentation of the lipid bilayer or its solubilization was observed (Figure S7).

Membrane remodeling by proteins, i.e., membrane bending, is typically dependent on the rigidity of the bilayer, as reflected by its bending modulus. Differences in the acyl chain of DLPC, POPC, and DLPC resulted in a range of SLB rigidities (Table 1), with DLPC being the most inflexible SLB due to its shortest acyl chain. Even though Ap interacted vigorously with DLPC, it failed to produce membrane remodeling. This disagreement is clarified once the high rigidity of DLPC is accounted for. The moderate response of POPC SLBs, which have medium bending moduli, supports this as well. Taken together, this points to the membrane bending abilities as a
critical factor in Aβ-induced membrane remodeling. Fluidity is an additional factor in the membrane remodeling. As fluidity reduces the energetic barrier for tubulation, DLPC, the most fluidic SLB, was expected to exhibit intense membrane changes. This is in contrast to the findings. Therefore, this implies that the membrane fluidity has a minor role in the Aβ-induced membrane remodeling, and that membrane elasticity is more essential to this process.

Additional clarification to the different intensities of membrane remodeling relates the tubulation mechanism. Tubulation by the amyloidogenic protein, α-synuclein, as well as by additional proteins was described via “wedge”

Figure 4. Morphological changes in DOPC, POPC, and DLPC SLBs induced by Aβ peptide, depending on administered Aβ concentration. Images were captured following 30 min of continuous administration of Aβ and buffer rinse.

Figure 5. Evaluation of the number of lipid aggregation events and their size per image captured via ImageJ particle analysis: First, background of the crude image was subtracted, and then, threshold was used and particle analysis function to retrieve number of “particles”, i.e., number of lipid aggregation events, and their size in μm². (a) A representative analysis for buds on DOPC SLB, and (b) a representative analysis for tubules and buds on POPC SLBs, following 30 min of exposure to 20 μM Aβ peptide.
model.70,74–77 Monomers of α-synuclein formed tubules on PC membranes, which were induced by weak interactions. These interactions were efficient for hydrophobic insertion of an amphipathic α-helix that acted as a partition and interfered with lipid–lipid interactions to generate membrane curvature.74 A similar model of peptide insertion was suggested for tubulation on GUVs by Aβ.31,78 Taken together with the aforementioned hindered fluidity of the SLBs due to Aβ insertion into the hydrophobic core, indeed, a “wedge” model can be considered for Aβ. Interestingly, in this model, induced curvature by α-helix insertion is dependent on the penetration depth, and hence on the hydrophobic thickness of the bilayer.77,79 For a given hydrophobic thickness, α-helix insertion induces positive curvature, yet with deeper protein partition, the effect is lost or even reversed into negative curvature. As Aβ insertion is similar in PC SLBs, for thicker SLBs positive curvature is expected, whereas for thinner SLBs, a milder effect should be seen on the basis of this model. This provides a secondary explanation for the lack of membrane remodeling in DLPC and its appearance on the thicker membranes, DOPC and POPC. Taking together, both the rigidity of the SLB and the insertion depth of Aβ contribute to the membrane remodeling, both of which are a derivative of the hydrophobic thickness.

Aβ induces Tubule and Bud Formations in Zwitter-ionic Supported Lipid Bilayers. Aβ affected DOPC morphology the most, compared with morphologies of POPC and DLPC. The morphological changes, i.e., buds or tubules, deepened on the administrated Aβ concentration. For DOPC SLB, at lowest Aβ concentration, 1 μM, no lipid aggregation was observed. For higher concentration, 3 μM, tubules grew over time with a secondary event of bud lipids aggregates. At higher concentration of the peptide, 10–20 μM, only bud aggregates were observed. This may be contributed to prior aggregation of Aβ in the solution at high concentrations. Administration of high Aβ concentration may have resulted in Aβ aggregation in the solution into intermediate oligomers; therefore, Aβ may have adsorbed to the lipid bilayer as small aggregates that presented as buds on the membrane. This is consistent with the limited ability of bigger proteins to form tubules.72 At lower Aβ concentration, the aggregation may occur mainly at the membrane to form tubules. With the flow of additional peptide, the peptide-accumulated membrane may have interacted more slowly with Aβ. The slower peptide–membrane interactions may enable Aβ to aggregate in the solution, before adsorbing into the membrane. Indeed, QCM-D plots imply that the Aβ adsorption rate was decreasing with time (Figure 2a). POPC SLB had less tendency to morphological changes, yet it exhibited related behavior to DOPC. At the lowest Aβ concentration that induced major morphological changes, i.e., 20 μM, tubules with buds as a secondary event were observed; this is in similarity to DOPC at 3 μM Aβ, i.e., the lowest Aβ concentration that induced morphological changes.

Closer examination of the growth of such morphological changes was achieved by counting the number of lipid aggregation events and assessment of the total size of these aggregates over time (Figure 7 and Figure S8). Bud lipid aggregates were initially observed as small seeds on the SLB that grew up to 25 μm² within 30 min (Figure 7a). Their growth was characterized by separate nucleation and growth steps (Figure 7c and Figure S8). A rapid nucleation period of 5 min, with subsequent growth step and minor seeding events of new aggregates, began 5 min later. The first 3 min are a time-gap between the injection of the peptide and its encounter with the SLBs. Tubule growth was more complex, as it was accompanied by bud formation as a later event (Figure 7b). Within 5 min, tubular lipid clusters grew from small seeds to tubules of >140 μm length and <1 μm width. Following an additional 5 min, several tubules collapsed into buds or waned, and the number of tubules over time decreased (Figures 7d and 8, and Figure S8). Tubules growth halted following 5 min, and their size remained stable; this is in contrast to continuous bud growth. With time, bud seeds appeared as a secondary event. The bud number and size increased with time. A similar pattern of growth was observed in all the events of major morphological changes of DOPC (Figure S8).

The growth pattern differed from previously described cases of tubulation due to protein insertion or osmotic pressure.51,72 The tubulation typically follows a separate nucleation phase and a subsequent tubule growth phase. This is due to a higher energy barrier for forming an initial high curvature bud than extension of an existing tubule. The presented analysis demonstrated bud formation following tubulation, i.e., additional nucleation sites after tubule growth. This supports our aforementioned suggestion that the membrane remodeling was also affected by the accumulation of the peptide in the membrane, and perhaps was induced by monomers and intermediate oligomers. An additional explanation points to the decrease in the SLB fluidity with the insertion of the peptide. As tubulation is typically promoted by fluidity,72 such a decrease may impair formation of additional tubules and limit their frequency on the SLBs. These two factors, Aβ aggregation and reduced fluidity, may collaborate to limit tubule growth.

To understand whether the lipid clusters were entwined lipid–peptide aggregates, or an intact lipid bilayer in complex structures, i.e., remodeled, FRAP analysis of the lipid clusters was used (Figure 9). All tubules and buds analyzed were recovered following the photobleaching due to lipid diffusion. Their recovery time was consistent with that of the rest of the bilayer. This implies that the lipid clusters remained in a
reasonably organized two leaflet structure, i.e., monolayer coupling, which enabled lateral diffusion.

The overall observations illustrate a passive stress relief response of the membrane to peptide insertion into the hydrophobic region of the lipid bilayer. The peptide, due to its hydrophobic nature, penetrates the lipid bilayer, and induces the membrane remodeling (Figure 10). This notion is supported by the findings presented here and by previous reports: (1) QCM-D experiments pointed to Aβ insertion, which was favored in dense SLBs composed of saturated lipids. The accompanied inhibited fluidity of the SLBs agreed with Aβ α-helix insertion, and suggested that Aβ was acting as a docking agent to restrain membrane fluidity. (2) Membrane remodeling, i.e., tubule and bud formation, occurred on thicker membranes. This pointed to membrane hydrophobic thickness as an enabling factor, due to its effect on membrane rigidity as well as peptide insertion depth. (3) Lateral diffusion of the lipids in the tubule and bud clusters support that the membrane was intact and structured to enable a possible diffusion of Aβ peptides as well. The dynamics of such structures may act to promote Aβ crowding and conformational changes, and hence its aggregation. This correlates well with the tendency of DOPC and POPC membranes to catalyze Aβ fibril formation whereas DLPC inhibited this process. Notably, with a resemblance to IAPP and additional amyloidogenic proteins, this indicates a relation between the lipid bilayer flexibility to form complex structures without membrane fragmentation, and its ability to catalyze the aggregation of Aβ.

Figure 7. Image snapshots at various time points present the growth of tubule and bud lipid aggregation upon continuous administration of 20 μM Aβ for 30 min: (a) buds on DOPC SLB, (b) tubules on POPC SLB, (c) size and number of bud aggregation events on DOPC dependent on time, (d) size and number of tubule and bud aggregation events on POPC dependent on time. t = 0 represents the Aβ injection time.
We studied the interactions of Aβ monomers with PC SLBs of varying hydrophobic thickness. The employment of SLBs enabled to explore the immediate membrane response to Aβ. Utilization of QCM-D and fluorescent microscopy facilitated the investigation of rapid changes in the membrane upon the earliest peptide−membrane interaction. Indeed, time-lapsed fluorescence microscopy enabled detailed characterization and tracking of membrane morphological responses; all morphological changes described herein were identified as membrane remodeling induced by Aβ.

Aβ interacted greatly with phosphatidylcholine SLBs and impaired membrane fluidity. This implied Aβ insertion into the membrane core. Aβ adsorption and insertion were dependent on the degree of lipid saturation, however, regardless of their hydrophobic thickness. Nevertheless, membrane remodeling occurred in thicker lipid bilayers, composed of DOPC or POPC, and was absent from thinner DLPC bilayers. This finding separates Aβ insertion from Aβ-induced membrane remodeling, as different physicochemical characteristics of the membrane influence these distinct processes.

In particular, bud formation was observed at high Aβ concentrations, and tubules were observed at lower concentrations. Overall, their occurrence and growth were dependent on the applied peptide concentration, and the membrane hydrophobic nature. The lipid aggregates maintained their fluidity; hence, they were identified as part of the intact lipid bilayer. No signs of membrane fragmentation were observed under the experimental conditions applied. Whereas thicker membranes catalyze Aβ fibril formation, thinner membranes inhibited this process. Taking together, these observations offer a linkage between membrane remodeling and membrane-catalyzed Aβ aggregation, suggesting that membrane remodeling may facilitate Aβ crowding and conformational change, as suggested for other amyloidogenic proteins. Significantly,
this is the first report that associates membrane remodeling upon Aβ adsorption to the thickness of the hydrophobic core. These findings shed light on the interplay between Aβ-induced membrane disruption and Aβ aggregation. In perspective with Alzheimer’s disease, which is associated with lipid dysregulation and alterations in membrane properties, such alterations can influence the Aβ aggregation state, and therefore its biological function. Moreover, membrane remodeling induced by Aβ potentially affects additional protein–membrane interactions and in turn may disorder the signaling and trafficking of neuron cells. Insights regarding the mutual interactions between the peptide and the membrane can assist in understanding Aβ homeostasis and its role in health and disease.

### Experimental Details

**Materials.** 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1-palmityl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC), and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (Rhod PE) were purchased from Avanti polar lipids. Lyophilized powder of amyloid β (Aβ40) peptide was obtained from Bio Basic. Additional reagents and solvents were purchased from Sigma-Aldrich. Milli-Q-treated water (18 MΩ cm) (Millipore) was used for all buffer solutions mentioned.

**Preparation of Aβ 40 Peptide.** Lyophilized powder of Aβ40 peptide was dissolved in hexafluoroisopropanol (HFIP), 5 mg/mL, and vortexed gently. The solution was incubated at RT (room temperature) for at least 1 h until the solution was clear. The solvent was removed under a nitrogen stream. The dried peptide was redissolved in HFIP, sonicated for 5 min, and dried under a gentle nitrogen stream to form a thin film of the peptide. The dried film was kept under vacuum overnight to remove residues of HFIP. The dried peptide was dissolved with minimal amount of 1% NH4OH solution, following which solution was diluted with Tris 10 mM buffer, pH 7.5, to give a final concentration of 1 mg/mL. Aliquots of 50 μL were prepared and lyophilized overnight. Aliquots were stored at −80 °C until use. Solution characterization can be found in Supporting Information, Figure S9.

**Preparation of Large Unilamellar Vesicles (LUVs).** Vesicles of composed DOPC, POPC, or DLPC were prepared by the extrusion method.51 Dried lipid films were hydrated in 10 mM Tris, pH 7.5, and 150 mM NaCl buffer solution at a concentration of 5 mg/mL. Then, the sample was a vortex, and vesicles were extruded through track-etched polycarbonate members with 50 nm diameter nominal pore sizes. Size of the vesicles was confirmed by dynamic light scattering (DLS) using a 90 Plus particle size analyzer (Brookhaven Instruments) with a 658.0 nm monochromatic laser. The extruded vesicles were diluted in the appropriate buffer solution shortly before the experiment.

**Quartz Crystal Microbalance with Dissipation (QCM-D).** QCM-D experiments were performed with a QSense E4 instrument (Biolin Scientific, Stockholm, Sweden). Shifts in the resonance frequency (Δf) and energy dissipation (ΔD) of an oscillating, piezoelectric quartz crystal were recorded as a function of time. These shifts relate to the acoustic mass, frequency (Hz), and viscoelastic properties, with energy dissipation (10^−6) of the adsorbed molecules on the surface.52 The sensor chips used were coated with a 50 nm thick layer of silicon dioxide (QSX 303, Biolin Scientific). Data were recorded at n = 3–11 odd overtones and normalized according to the overtone number. Overtone n = 7 was chosen as a representative overtone in all presented QCM-D plots. The thickness of the lipid bilayer was driven using the Sauerbrey model.53 Data processing was performed in the QTools (Biolin Scientific) and OriginPro 2016 (OriginLab) software programs.

Prior to each experiment, the chips were washed multiple times with water and ethanol, dried with nitrogen gas, and treated with oxygen plasma for 1 min using a Plasma Cleaner (Harrick Plasma).

Measurements were performed under continuous flow conditions, with a set flow rate of 50 μL/min. Final frequency and energy dissipation shifts were reported as the mean ± standard deviation of at least 3 repeats. All experiments were conducted with Tris 10 mM buffer, pH 7.5, 150 mM NaCl. General procedure for QCM-D experiments with Aβ peptide: SLB on the silicon dioxide surface was fabricated by vesicles fusion method.54,55 Briefly, vesicles of either DOPC, POPC, or DLPC were diluted, 0.125 mg/mL, with buffer and injected. Following the formation of SLB, as indicated by the frequency and energy dissipation values, the buffer was administered for 10 min to remove vesicle residues. Then, Aβ was introduced for 1 h with continuous flow, following which excess of the peptide was rinsed with buffer for 15 min. During the experiments, the temperature in the measurement chamber was maintained at 24.0 ± 0.5 °C. Each measurement set was repeated at least three times. Net change in frequency and energy dissipation reported (Figure 2b) was calculated as follows: the final frequency/energy dissipation value at the end of the measurement, i.e., following rinse of not adsorbed peptide, minus frequency/energy dissipation value upon SLB formation.

**Fluorescence Microscopy.** Epifluorescence microscopy was conducted to observe morphological changes in SLBs due to adsorption of the Aβ peptide. Eclipse Ti-U inverted optical microscope (Nikon, Japan) with a 60× magnification (NA = 1.49) oil-immersion objective lens (Nikon) was used to capture images, 0.140 × 0.140 mm², with an iXon 512 pixel × 512 pixel EMCCD camera (Andor Technology). A fiber-coupled mercury lamp (Intensilight C-HGFI, Nikon) illuminated the labeled phospholipids with a TRITC filter. Experiments were conducted on glass slides attached to the microfluidic flow-through chamber (sticky slide VI 0.4, Ibidi) similarly to QCM-D experiments, with the flow rate set to 50 μL/min. Time-lapse micrographs were recorded every 5 s for a total duration of 30 min. The time, t = 0 s, was defined upon the injection of the peptide solution to the channel inlets. The fluorescence intensity of each micrograph was normalized using a custom-written script for the Python(x,y) 2.7.5 software program. Image analysis to extract the number of aggregation events and their size was conducted using the “particle analysis” function of ImageJ software.56 Briefly, the protocol was based on the recommended protocol as published by ImageJ. Background of the image was subtracted, following manual thresholding, and particle analysis function. Images following particle analysis are presented in Figure 5.

**Fluorescence Recovery after Photobleaching (FRAP) Measurements.** FRAP measurements were carried out to monitor the lateral diffusivity of SLBs twice: (1) prior to treatment with the Aβ peptide and (2) following the administration of the peptide for 30 min and rinse to remove excess of not adsorbed peptide. A 20 μm diameter circular spot was photobleached for 5 s by using a 532 nm, 100 mW laser (Klaster Laser Technologies), and fluorescence micrographs were taken every 1 s for 180 s in total. Lateral diffusion coefficients were computed on the basis of the Hankel transform method.52 Lateral diffusion coefficients were presented as the mean ± SEM of at least 4 repeats.

### Associated Content

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.8b01196.

QCM-D recording of Aβ peptide interactions, dependence of dissipation energy shifts on frequency shifts following administration of Aβ, lateral diffusivity coefficients of the SLBs before and after introducing Aβ peptide, morphological changes following 24 h, relative fluorescence intensity before and after introducing Aβ peptide, size and number of tubules or buds dependent on time, MS analysis, and descriptions of movie files (PDF)
Movie S1, a representative time-lapse epifluorescence of FRAP experiment of DOPC SLB (ZIP)
Movie S2, a representative time-lapse epifluorescence of FRAP experiment of POPC SLB (ZIP)
Movie S3, a representative time-lapse epifluorescence of FRAP experiment of DLPC SLB (ZIP)
Movie S4, a representative time-lapse epifluorescence of FRAP experiment of DOPC SLB, following the administration of 20 μM Aβ (ZIP)
Movie S5, a representative time-lapse epifluorescence of FRAP experiment of POPC SLB, following the administration of 20 μM Aβ (ZIP)
Movie S6, a representative time-lapse epifluorescence of FRAP experiment of DOPC SLB, following the administration of 20 μM Aβ (ZIP)
Movie S7, a representative time-lapse epifluorescence of bud formation on DOPC SLB, upon administration of 20 μM Aβ (ZIP)
Movie S8, a representative time-lapse epifluorescence of tubulation on POPC SLB, upon administration of 20 μM Aβ (ZIP)
Movie S9, a representative time-lapse epifluorescence of DLPC SLB, upon administration of 20 μM Aβ (ZIP)

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