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Phospholipids Critical Micellar Concentrations Trigger Different Mechanisms of Intrinsically Disordered Proteins Interaction with Model Membranes

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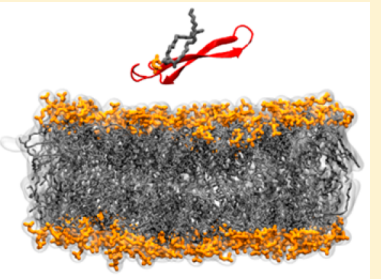
Supporting Information

ABSTRACT: Amyloidogenic proteins are involved in many diseases, including Alzheimer's, Parkinson's, and type II diabetes. These proteins are thought to be toxic for cells because of their abnormal interaction with the cell membrane. Simpler model membranes (LUVs) have been used to study the early steps of membrane-protein interactions and their subsequent evolution. Phospholipid LUVs formed in water solution establish a chemical equilibrium between self-assembled LUVs and a small amount of phospholipids in water solution (CMC). Here, using both experimental and molecular dynamics simulations approach we demonstrate that the insertion of IAPP, an amyloidogenic peptide involved in diabetes, in membranes is driven by free lipids in solution in dynamic equilibrium with the self-assembled lipids of the bilayer. It is suggested that this could be a general mechanism lying at the root of membrane insertion processes of self-assembling peptides.



Lipid membranes are increasingly recognized as key targets of amyloid toxicity. After coming across the host cell, amyloid assemblies are thought to insert into the lipid bilayer thereby disrupting the integrity of the cell membranes. A large number of accounts concerning protein−lipid interactions have been extensively reported.[1−3] However, despite its high relevance to amyloid toxicity, our understanding of the early steps of the spontaneous entry of proteins into lipid membranes is still incomplete. Many biophysical studies have addressed the insertion of peptides in bilayer using simple model membranes.[4−6] Notably, most of the existing reports in the field suggest that lipid influences on the protein/peptide structure are mainly ascribable to a direct binding to membrane-embedded proteins.[6] In other words, peptide/lipid interactions occurring in the lipid phase have attracted major attention, whereas the possibility of peptide−lipid interactions in the pure water phase has been always overlooked. Markedly, most of biophysical studies addressing protein lipid interactions have employed large unilamellar vesicles (LUVs) as the simplest model membrane. In aqueous dispersions of LUVs, however, phospholipids are always in chemical equilibrium between a self-assembled state (the lipid membrane) coexisting with a small amount of monomeric units in water solution. The concentration of aqueous phospholipids in equilibrium with membrane aggregates (i.e., critical micellar concentration, CMC) is a function of the number of hydrophobic carbon atoms contained in the lipid tail.

Within this frame, here we investigate the spontaneous insertion of human islet amyloid polypeptide (IAPP) in zwitterionic lipid bilayers of different thickness.

In a recent paper,[7] we developed a simple nonequilibrium phenomenological model to simulate the transfer kinetics of proteins that may form reversible complexes with the free lipids in equilibrium with the membrane. Accordingly, the insertion of amyloidogenic proteins in membranes can be facilitated by free lipids in solution which are in dynamic equilibrium with the self-assembled lipids of the bilayer (see Scheme 1).

In most biophysical experiments phospholipid LUVs are used. We conjecture that the interaction between protein and free lipids may form protein−lipid complexes of different stoichiometry that could penetrate faster inside the membrane core. To verify our hypothesis, we evaluated the Gibbs free energy of protein transfer from water to the lipid bilayer in the presence of protein-bound free lipids by the umbrella sampling method (11.6 μs of MD simulations, see SI for details). For the sake of comparison, we carried out simulations of the bare protein. In Figure 1, we report the variation of the Gibbs free energy vs the protein distance from the center of a POPC bilayer.

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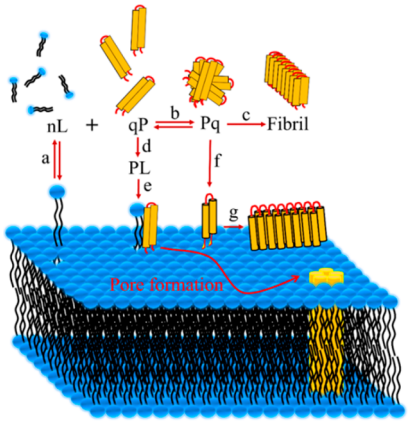
Scheme 1. Pictorial Representation of Different Processes Involved When Amyloidogenic Proteins Dissolved in Water Interact with LUV[a]



[a]Bilayers are in dynamic equilibrium with free lipids (nL) in water (equilibrium a). Monomeric proteins (qP) in absence of free lipids are in equilibrium with unstructured small oligomeric aggregates (Pq) (equilibrium b), and then they self-assemble to form fibrils (irreversible process c, off-pathway in the pore formation). Also, Pqs are adsorbed on the membrane surface forming fibril aggregates (irreversible process f and g) that, eventually, damages the membrane through a detergent-like mechanism. Free lipids (nL) and monomeric proteins (qP) may form lipid-protein complexes (PL) (process d) thermodynamically more stable than the protein−protein ones. The PL complexes can be transported into the lipid bilayer (irreversible process e). Above a concentration threshold, protein molecules within the membrane give rise to pore formation.

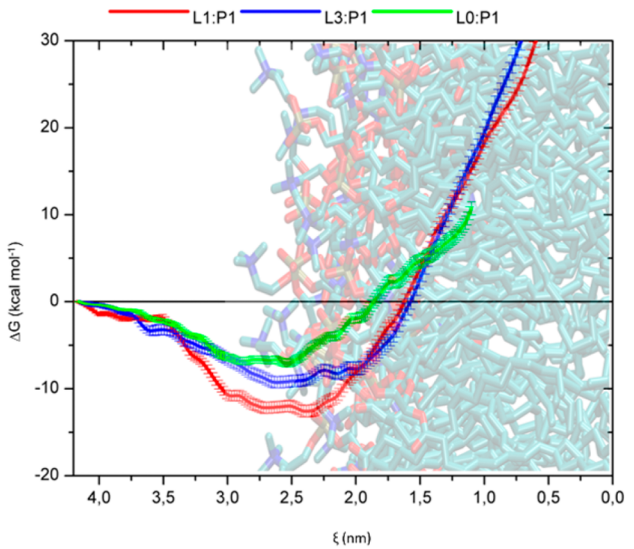


Figure 1. Gibbs free energy variation and statistical indetermination of (n)POPC-hIAPP complex as a function of the distance from the bilayer center, ξ. Green: Bare hIAPP. Red: Complex containing 1 hIAPP and 1 POPC molecules. Blue: Complex containing 1 hIAPP and 3 POPC molecules.

The 1:1 protein−lipids complex shows the more favorable adsorption free energy in comparison to the bare protein (1:0). Interestingly, on increasing the number of bound lipids (1 protein and 3 phospholipid molecules) the protein transfer becomes less favored as suggested by us in ref 7. The lipid-assisted protein transfer mechanism can be ideally portioned into several steps: (a) protein−lipid complexes formation (protein in β-sheet), (b) complexes adsorption on the bilayer surface, (c) bilayer lateral expansion and surface defect formation, (d) protein insertion, (e) β-sheet to coil (or α-helix) protein conformation transition (in the bilayer hIAPP is stable as α-helix), and finally, (f) relaxation of the membrane defect to reach a final stable configuration. All these steps cannot be followed by all atom simulations because of the huge computational time needed. Our all atoms simulations just give the Gibbs free energy and atomistic details of the insertion of lipid−protein complexes into a leaflet of the bilayer (steps a−d). Further details are reported in the SI and ref 7. MD simulations clearly show that free lipids catalyze by a sizable amount the hIAPP transfer from water to membrane. Although this is a reasonable result, the scarce solubility of lipids in water may drastically reduce the efficiency of this mechanism. To confirm the validity of our hypothesis we planned some experimental measurements. Considering that the CMC is strictly related to the number of carbon atoms of lipids, we first investigate the interaction between hIAPP and LUVs formed by diacyl-phosphocolines with different acyl chain length (PC14, PC16, PC18, PC20 and PC22; see SI for legend) as reported in Figure 2. Figure 2A shows typical dye release kinetics from phospholipid LUVs of different chain lengths and different CMCs.

Soon after hIAPP addition at t = 0, LUVs are damaged and start to release the trapped dye. The dye release occurs in two steps, the first is customarily attributed to the formation of pores, the second to the bilayer destruction by a detergent-like mechanism.[8−10] Release data evidence that the bilayer damage in the presence of high concentrations of free lipid (PC14 = high CMC; on SI are reported the CMC measurements) occurs only by pore formation. On the contrary, in the presence of low CMC lipids (PC22) the membrane damage occurs through a detergent-like mechanism. Lipids with halfway CMC show both I and II steps. We also investigated by another fluorescence technique reported in the SI the kinetics of fibrils formation as reported in Figure 3B.

Interestingly, fibrils formation follows an opposite trend as compared to the membrane damage, that is, high concentrations of free lipids inhibit the formation of fibrils, while low concentrations of free lipids promote the formation of fibrils. To further test the role of free lipids, we carried out kinetics experiments on the hIAPP fibrils growth rate in absence of LUVs but at a free phospholipids concentration corresponding to their CMC (Figure 2C)

Data clearly prove that the fibrils growth is markedly influenced by free phospholipids. Indeed, lipids at a concentration of 1×10^{-7} M (the PC14 CMC) totally inhibits the formation of fibrils, while fibrils grown in the presence of a lower free lipids concentrations (at the CMC of PC20, 6.9×10^{-9} M) are more abundant. It is worth noting that the amount of fibrils formed in the presence of membranes (Figure 2B) is higher than that formed in pure water.

Changing the lipids chain length entails also the bilayer thickness other than the CMC. So, we performed focused experiments to exclude the effect of bilayer-protein mismatch on fibril growth or pores formation. Fibrils formation was followed by the classical ThT assay[8] with and without the addition of lipids.

In Figure 3A the magenta curve describes the fibrils formation kinetics in the presence of PC22 LUVs (low CMC). Data show the formation of fibrils. On the contrary, in the presence of PC14 vesicles (high CMC), no fibrils are

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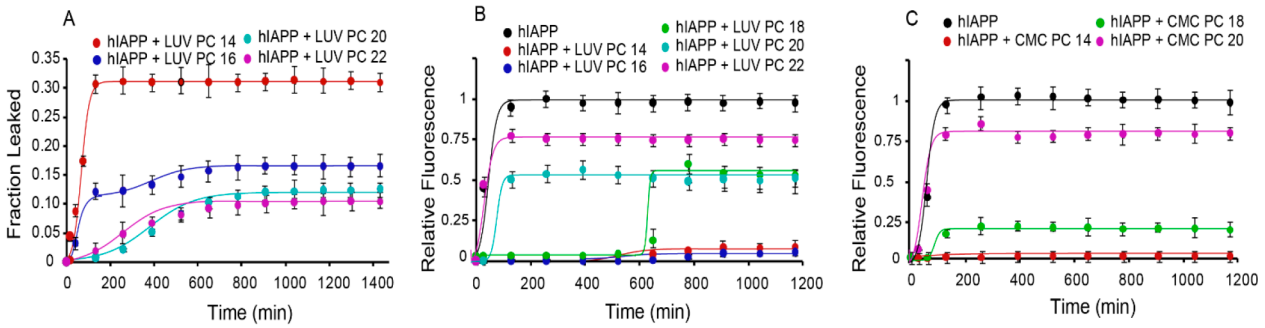

Figure 2. (A) Dye leakage kinetics of LUVs containing diacyl-phosphocholines of different length interacting with hIAPP. Red curve PC14. Blue: PC16. Magenta: PC20. Dark cyan: PC22. (B) Fibril formation kinetics through ThT assay of diacyl-phosphocholine LUVs interacting with hIAPP. Black curve: hIAPP. Dark cyan: PC22. Magenta: PC20. Green: PC18. Blue: PC16. Red: PC14. (C) ThT assay of pure hIAPP (black curve) and in the presence of different diacyl-phosphocholines at their CMC. Red curve: PC14, CMC 1×10^{-7} M. Green curve PC18, CMC 2×10^{-8} M. Magenta curve: PC20, CMC 6.3×10^{-9} M. All experimental conditions are reported in SI.

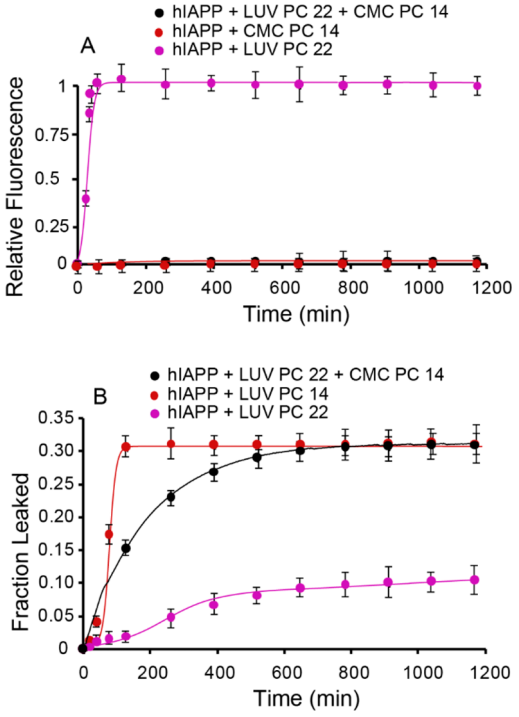

Figure 3. Upper panel: the assay kinetic fluorescence measurements, hIAPP 10 μM in presence of PC22 LUVs 200 μM (magenta curve), free 14PC lipids (at its CMC 1×10^{-7} M) (red curve), PC LUVs 200 μM and 14PC free lipids at its CMC (black curve). Lower panel: dye leakage kinetic measurements of diacyl-phosphatidyl-choline LUVs 200 μM interacting with hIAPP 10 μM. Magenta curve, PC 22 LUVs; red curve PC 14 LUVs; black curve PC22 in presence of 14PC free phospholipid (CMC: 1×10^{-7} M); orange curve LUVs of PC22; blue curve LUVs of PC22 in the presence of free PC14 at its CMC 1×10^{-7}.

formed. Moreover, LUVs formed by PC22 dispersed in a solution of free PC14 phospholipid (at its CMC) do not allow the formation of fibrils. Thus, the presence of either LUVs or the bilayer-protein mismatch does not interfere with fibrils inhibition exerted by free lipids (see Figure 3A). Results suggest that in vesicles-containing systems, free lipids and monomeric proteins are in competitive equilibrium with protein−protein, lipid−lipid (bilayers) and lipid−protein aggregates. In summary, it can be concluded that free lipids in dynamic equilibrium with lipid bilayers might play an essential role in modulating the interaction between amyloidogenic proteins and model membranes, according to ref 7. In the specific case of hIAPP-bilayer, free phospholipids inhibit the formation of fibrils and promote LUVs poration. Previous experimental and MD data had shown that pores are formed by five α-helix peptides, spanning the whole bilayer enabling water and ions permeation.[11,12] Our data support the idea that the transport of amyloidogenic proteins can be mediated by free lipids in solution. Since phospholipids having low values of CMC increase also the bilayer thickness, it is fair to think that thick bilayers do not form pores because of the mismatch of protein-bilayer thickness. To exclude a significant role of bilayer thickness, we performed dye leakage measurements investigating three different systems:

PC14 LUVs (CMC 1×10^{-7} M), PC22 (CMC 8×10^{-10} M), and LUVs of PC22 in the presence of free PC14 at a concentration of 8×10^{-7} M (its CMC). This latter experiment enables us to obtain at the same time a thick membrane and a relatively high concentration of free lipids in water solution.

The results shown in Figure 3 clearly demonstrate our hypothesis: while the interaction between hIAPP and PC14 LUV's induces pore formation, in PC22 LUV's pores are not formed. However, if we perturb the last system by adding free phospholipids (PC14 at its CMC) we observe the formation of pores also on a thicker bilayer (PC22 LUVs).

So, we deduce that the formation of pores does not significantly depend on the mismatch between bilayer and protein lengths, but it is strongly related to the concentration of free lipids as shown in Scheme 1.

Data shown here suggest that free lipids in the aqueous phase in dynamic equilibrium with bilayer lipids show a chaperone-like activity in hIAPP insertion, as hypothesized by us in a previous theoretical paper.[7] Lipids with high CMC form pores, do not exhibit a detergent-like mechanism and repress fibrils formation. On the contrary, lipids with low CMC show a detergent-like mechanism and form fibrils. Moreover, our data suggest that pore formation and detergent-like mechanism are not correlated. Our molecular model was developed using hIAPP as model protein. However, it is likely that this mechanism involves other amyloidogenic proteins. Preliminary data on Aβ(1−40), α-synuclein and rat-IAPP performed in our lab confirm this hypothesis. Our model is also consistent with previous investigations reported by other authors[13] and suggests a new vision of membrane-amyloidogenic protein

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interaction. The evidence that lipid CMC acts as a switch between pore and fibril formation on the membrane can help to shed light on the toxicity of amyloidogenic proteins by solving some apparent controversy in the literature. According to our data, ion channels are formed only if free lipids are present over a threshold concentration. Indeed, one of the first explanations of cell dysfunction and toxicity proposed by Arispe et al. is the so-called channel hypothesis.[14] In vivo evidence for this mechanism have been never reported.

As an example, previous reports have questioned the direct relationship between membrane damage and amyloid toxicity by hIAPP.[15] Those studies addressed these issues by using anionic model membranes and human and rat (rIAPP) islet amyloid polypeptide. rIAPP is not amyloidogenic and is often used as a control model in biophysical studies of amyloid growth. Both hIAPP and rIAPP promote leakage of model membranes but only hIAPP was shown to be toxic to cells. In contrast with these findings, other papers report that, using pure zwitterionic POPC (characterized by a very low CMC 10^{-5} M lower than that anionic lipids[16]), rIAPP does not induce any membrane leakage.[17] This apparent incongruence may be explained in the light of the role played by lipids in water phase. In fact, the absence of free lipids abolished membrane poration of rIAPP leaving the ability of hIAPP to leak the membrane by amyloid growth on the membrane surface (detergent like mechanism).

The aim of this work was to investigate the influence of free lipids in solution on the interaction between proteins and LUVs. It is widely known that the hydrophobic tails length and the chemistry of phospholipids head-groups, influence the CMC. So, do the CMC also affect the membrane-peptide interaction? Mittal and co-workers,[18] for instance, showed that membranes made up of different types of phospholipids lead to various hIAPP morphologies, after adsorption on membrane. The different behavior could be related to the CMC-controlled entry rate of protein in the membrane. For this, we think that future works should focus on the interaction among IDP (Intrisically Desordered Proteins) and phospholipids, paying specific attention to the CMC role and other kinetics and thermodynamic properties (such as multicomponent phases diagram, noncovalent lipid−protein complexes stability) of different systems, including mixed compositions membranes. Another interesting application will concern the role of reactive oxygen species that produce short-chained phospholipids. These low CMC species may act as carriers for IDP contributing to the membrane damage.[19]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.8b02241.

Experimental details; simulations setup; unbiased molecular dynamics simulations details; umbrella sampling simulations details; contact frequency among lipids and protein; membrane thickness of POPC membrane for different systems; area per lipid of POPC membrane for different systems; circular dichroism spectra; fluorescence pyrene spectra; critical micellar concentration; and supplementary references (PDF)

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Author Contributions

[▽]The two first authors contributed equally to the manuscript.

Author Contributions

F.S. and M.F.M.S. performed experiments; C.T. and F.L. performed simulations; F.S., C.T. D.M., A.R., and C.L.R. analyzed the data; D.M., A.R., and C.L.R. designed the research and wrote the paper; C.L.R. directed the project.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Brender, J. R.; Salamekh, S.; Ramamoorthy, A. Membrane Disruption and Early Events in the Aggregation of the Diabetes Related Peptide IAPP from a Molecular Perspective. *Acc. Chem. Res.* **2012**, *45* (3), 454−462.

(2) Caillon, L.; Lequin, O.; Khemtémourian, L. Evaluation of Membrane Models and Their Composition for Islet Amyloid Polypeptide-Membrane Aggregation. *Biochim. Biophys. Acta, Biomembr.* **2013**, *1828* (9), 2091−2098.

(3) Chiti, F.; Dobson, C. M. Protein Misfolding, Functional Amyloid, and Human Disease. *Annu. Rev. Biochem.* **2006**, *75* (1), 333−366.

(4) Hebda, J. A.; Miranker, A. D. The Interplay of Catalysis and Toxicity by Amyloid Intermediates on Lipid Bilayers: Insights from Type II Diabetes. *Annu. Rev. Biophys.* **2009**, *38* (1), 125−152.

(5) Jayasinghe, S. A.; Langen, R. Membrane Interaction of Islet Amyloid Polypeptide. *Biochim. Biophys. Acta, Biomembr.* **2007**, *1768* (8), 2002−2009.

(6) Sciacca, M. F. M.; Tempra, C.; Scollo, F.; Milardi, D.; La Rosa, C. Amyloid Growth and Membrane Damage: Current Themes and Emerging Perspectives from Theory and Experiments on Aβ and HIAPP. *Biochim. Biophys. Acta, Biomembr.* **2018**, *1860* (9), 1625−1638.

(7) La Rosa, C.; Scalisi, S.; Lolicato, F.; Pannuzzo, M.; Raudino, A. Lipid-Assisted Protein Transport: A Diffusion-Reaction Model Supported by Kinetic Experiments and Molecular Dynamics Simulations. *J. Chem. Phys.* **2016**, *144* (18), 184901.

(8) Hsu, J. C. C.; Chen, E. H. L.; Snoeberger, R. C.; Luh, F. Y.; Lim, T. S.; Hsu, C. P.; Chen, R. P. Y. Thioflavin T and Its Photoirradiative Derivatives: Exploring Their Spectroscopic Properties in the Absence and Presence of Amyloid Fibrils. *J. Phys. Chem. B* **2013**, *117* (13), 3459−3468.

(9) Eisert, R.; Felau, L.; Brown, L. R. Methods for Enhancing the Accuracy and Reproducibility of Congo Red and Thioflavin T Assays. *Anal. Biochem.* **2006**, *353* (1), 144−146.

(10) Giehm, L.; Otzen, D. E. Strategies to Increase the Reproducibility of Protein Fibrillization in Plate Reader Assays. *Anal. Biochem.* **2010**, *400* (2), 270−281.

(11) Pannuzzo, M.; Raudino, A.; Milardi, D.; La Rosa, C.; Karttunen, M. α-Helical Structures Drive Early Stages of Self-Assembly of Amyloidogenic Amyloid Polypeptide Aggregate Formation in Membranes. *Sci. Rep.* **2013**, *3*, 2781.

DOI: 10.1021/acs.jpclett.8b02241
J. Phys. Chem. Lett. 2018, 9, 5125−5129

(12) Quist, A.; Doudevski, I.; Lin, H.; Azimova, R.; Ng, D.; Frangione, B.; Kagan, B.; Ghiso, J.; Lal, R. Amyloid Ion Channels: A Common Structural Link for Protein-Misfolding Disease. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (30), 10427−10432.
(13) Martel, A.; Antony, L.; Gerelli, Y.; Porcar, L.; Fluitt, A.; Hoffmann, K.; Kiesel, I.; Vivaudou, M.; Fragneto, G.; De Pablo, J. J. Membrane Permeation versus Amyloidogenicity: A Multitechnique Study of Islet Amyloid Polypeptide Interaction with Model Membranes. *J. Am. Chem. Soc.* **2017**, *139* (1), 137−148.
(14) Arispe, N.; Rojas, E.; Pollard, H. B. Alzheimer Disease Amyloid Beta Protein Forms Calcium Channels in Bilayer Membranes: Blockade by Tromethamine and Aluminum. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90* (2), 567−571.
(15) Cao, P.; Abedini, A.; Wang, H.; Tu, L.-H.; Zhang, X.; Schmidt, A. M.; Raleigh, D. P. Islet Amyloid Polypeptide Toxicity and Membrane Interactions. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (48), 19279−19284.
(16) Marsh, D. *Handbook of Lipid Bilayers*, 2nd ed.; CRC Press, 2013.
(17) Scalisi, S.; Sciacca, M. F. M.; Zhavnerko, G.; Grasso, D. M.; Marletta, G.; La Rosa, C. Self-Assembling Pathway of HiApp Fibrils within Lipid Bilayers. *ChemBioChem* **2010**, *11* (13), 1856−1859.
(18) Dignon, G. L.; Zerze, G. H.; Mittal, J. Interplay between Membrane Composition and Structural Stability of Membrane-Bound hIAPP. *J. Phys. Chem. B* **2017**, *121* (37), 8661−8668.
(19) Korshavn, K. J.; Satriano, C.; Lin, Y.; Zhang, R.; Dulchavsky, M.; Bhunia, A.; Ivanova, M. I.; Lee, Y. H.; La Rosa, C.; Lim, M. H.; et al. Reduced Lipid Bilayer Thickness Regulates the Aggregation and Cytotoxicity of Amyloid-β. *J. Biol. Chem.* **2017**, *292* (11), 4638−4650.

DOI: 10.1021/acs.jpclett.8b02241
J. Phys. Chem. Lett. 2018, 9, 5125−5129