Cholesterol Molecules Alter the Energy Landscape of Small A β 1-42 Oligomers

Son Tung Ngo, Phuong H Nguyen, Philippe Derreumaux

► To cite this version:

Son Tung Ngo, Phuong H Nguyen, Philippe Derreumaux. Cholesterol Molecules Alter the Energy Landscape of Small A β 1-42 Oligomers. Journal of Physical Chemistry B, 2021. hal-03430931

HAL Id: hal-03430931

<https://hal.science/hal-03430931>

Submitted on 16 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Cholesterol Molecules Alter the Energy Landscape of Small A β 1-42 Oligomers

Son Tung Ngo,¹ Phuong H Nguyen,² and Philippe Derreumaux^{2,3*}

1 Laboratory of Theoretical and Computational Biophysics & Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh, City, Vietnam.

2 CNRS, Université de Paris, UPR 9080, Laboratoire de Biochimie Théorique, 13 rue Pierre et Marie Curie, F-75005 Paris, France ; Institut de Biologie Physico-Chimique, Fondation Edmond de Rothschild, PSL Research University, Paris 75000 France.

3 Laboratory of Theoretical Chemistry, Ton Duc Thang University, Ho Chi Minh City, Vietnam ; Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, Vietnam.

ABSTRACT

Small amyloid-beta (A β) oligomers are believed to be key pathogenic species in Alzheimer's disease (AD). One suggested toxicity mechanism is the detergent model where oligomers remove lipid molecules from the bilayer. Senile plaques of AD patients also accumulate a ratio 1:1 of cholesterol/A β . What are the dominant structures of small A β 42 oligomers with cholesterol molecules in aqueous solution? Here we answer this question by performing atomistic replica exchange molecular dynamics simulations of A β 42 dimers and trimers. Our simulations demonstrate that the interactions with cholesterol molecules change completely the energy landscape of small A β 42 oligomers. This result, which provides a mechanistic explanation for the observed enhanced effect of cholesterol in the aggregation of A β 42, indicates that simulations in bulk solution cannot recapitulate aggregation in the brain extracellular space.

Introduction

Experimental studies have shown that the interactions between amyloid oligomers and membrane may lead to the carpeting model where accumulation of oligomers on the surface imparts unequal stress on bilayer, the detergent model where oligomers have a profound effect to remove lipids from the membrane as assessed by atomic force spectroscopy (AFM) and electron microscopy (EM), and the membrane-pore model where oligomers insert into the membrane and create a pore as assessed by AFM, EM, ion mobility-mass spectrometry and conductance studies.¹⁻⁶

Both experimental and computational studies showed that binding of A β (linked to AD) and α -synuclein (linked to Parkinson's disease) varies with the membrane composition.^{7,8} AFM images of α -synuclein, A β 42 and A β 14-23 revealed that self-assembly on 1-(3-aminopropyl) silatrane mica surfaces occurs at nanomolar concentrations and simulations reported that surface interactions stabilize the dimer assembly and could lead to more stable oligomers that remain intact after unbinding to the membrane.⁹ Phospholipids can also be removed from the membrane, and it has been shown that phospholipid/tau oligomers, tau being linked to AD, are toxic to primary hippocampal cultures.¹⁰ It is known that the cholesterol and ganglioside lipids favor formation of A β and α S pores in the membrane of brain cells,¹¹ and cardiolipin, phospholipid signature of mitochondrial membranes, enhances α S-lipid interaction and the membrane pore-forming activity of α S oligomers.¹² Finally, a recent NMR study provided the first high-resolution structure of tetrameric A β 42 β -barrels,¹³ and computational studies explained why A β 42 and not A β 40 peptides can form tetrameric β -barrels in membrane-mimicking environment.^{14,15}

It is well established that the level of plasma cholesterol is 10% higher in AD patients than in healthy individuals.¹⁶ Using transmission EM, Harris *et al.* demonstrated that cholesterol micelles bind to A β protofibrils and fibrils.¹⁷ Accumulation of cholesterol was also assessed in microdissected AD senile plaques by mass spectrometry with a cholesterol/A β ratio of 1:1,¹⁸ and cholesterol was found to enhance the primary nucleation rate of A β 42 aggregation by up to 20-fold *in vitro*.¹⁹ All together these data indicate that a full understanding of the conformational ensemble of small A β 42 oligomers in the brain interstitial space must integrate cholesterol.

While the equilibrium ensemble of A β 42 monomer and dimer has been studied by experimental and computational means in bulk solution,²⁰⁻³⁰ and the interactions between A β 42 aggregates and membranes with different cholesterol compositions have been investigated,³¹⁻³⁷ the

impact of free cholesterol molecules on small A β 42 oligomers is unknown. In this study, we have determined the impact of cholesterol with a ratio 1:1 on A β 42 dimers, the first amyloid- β species known to be toxic,³⁸ by replica exchange molecular dynamics (REMD) simulations. By using the atomistic AMBER99SB-ildn force field³⁹ and TIP3P water force field,⁴⁰ it is possible to compare to previous REMD simulations in bulk solution.²⁹ We also report on REMD simulations of A β 42 trimer, known to be more stable than A β 42 dimer by AFM force spectroscopy,⁴¹ with cholesterol.

Materials and Methods

The cholesterol (Chol) molecule was geometrically optimized by quantum chemical calculation with the B3LYP functional at 6-31G(d,p) level of basis set, and the molecule was parameterized using general Amber force field (GAFF).⁴² In particular, the atomic charges were allocated via the Restrained Electrostatic Potential (RESP) method⁴² computed with quantum chemical calculations at the B3LYP functional in combination with 6-31G(d,p) basis set.⁴³

The initial structures of the A β 42 dimer and trimer with β -hairpin were taken from an extensive atomistic simulation of a tetrameric A β β -barrel structure, made of two distinct motifs and an asymmetric arrangement of eight antiparallel β -strands, that showed that this barrel exists transiently for A β 42, but not for A β 40 in bulk solution.⁴⁴ This β -hairpin propensity has also already been discussed in many simulations of the monomer.^{8,22,23} The helical structure of the A β 42 dimer was taken from the A β 40 monomer PDB entry 2LFM, which displays a 3-10 helix from residues 13 to 23 in aqueous environment by NMR,²¹ and the addition of the residues 41 and 42 in extended conformation.

The peptides were inserted into a dodecahedron periodic boundary condition water box with a volume ranging from 360 to 400 nm³ (cf. Table 1) and the cholesterol molecules were randomly inserted, as shown in Figure 1. The AMBER99SB-ildn force field³⁹ was employed for the A β 42 peptide, and water was represented by the TIP3P model.⁴⁰ All simulations were performed using GROMACS⁴⁵ and all parameters for the Van der Waals and electrostatic interactions described in Ref. 29. The peptides at pH 7 have NH₃⁺ and CO₂⁻ termini, deprotonated Glu and Asp, protonated Arg and Lys, and neutral His with a protonated N ϵ atom. All A β 42/Chol systems were neutralized by sodium ions.

For analysis, a contact is considered formed between a cholesterol molecule and non-hydrogen atoms of a side chain and backbone residue when the distance is smaller than 0.45 nm. The DSSP

protocol was used to determine the secondary structure of A β peptides.⁴⁶ Using GROMACS tools, the free energy landscape (FEL) were projected on the first two principal components obtained after diagonalization of the positional fluctuation covariance matrix of the backbone peptide atoms. The population of each minimum in the FEL was determined by counting all conformations within 1 kcal mol⁻¹ of each minimum and the representative structure of each minimum was obtained by the Daura clustering method⁴⁷.

Results and Discussion

All simulations performed in this study are summarized in Table 1. The secondary structure compositions of the dimer starting from β -sheet (run R1) and helix (run R2) structures was calculated at 315 K using the time interval 50-250 ns of the REMD simulations. A total of 250 ns per replica is not sufficient to obtain full equilibrium as the two simulations diverge in β -sheet (38% in R1 vs. 15% in R2) and helix (3% in R1 vs. 16% in R2) contents, while displaying similar coil (47% vs. 49%) and turn (12% vs. 20%) values. Though longer simulation times are needed for convergence, these simulations allow, however, to extract important trends. Average of the two REMD simulations with cholesterol gives 26% β -sheet, 48% coil, 16% turn and 10% helix to be compared to the REMD values of 24% β -sheet, 29% coil, 41% turn and 6% helix in bulk solution.²⁹ Cholesterol therefore favors coil over turn, while leaving the β -sheet and helix percentage invariant.

To investigate how the cholesterol molecule impacts the conformational ensemble, Figure 2 reports on the FEL of A β 42 dimer at 315 K projected on the first two principal components using the time interval 50-250 ns of both REMD simulations. The representative structures of the eight minima designated as A-H with decreasing populations are shown in Figure 3. The eight minima totaling 71% of the full conformational space display three topologies.

The first topology with α/β composition, characterized by minimum A with population of 26%, displays a helix at residues 13-16 (chain A) and three helices at residues 7-15, 24-26, and 29-37 in chain B with a small intermolecular parallel β -sheet between the N- and C-termini and β -hairpin ordering of the C-terminus of one chain (Figure 3A). The second topology of β class, consisting of minima B, C and D with populations of 20, 8 and 5% in Figures 3B-D, displays two β -hairpins of different lengths with various interfaces. The minimum B displays a three-stranded β -sheet at residues 5-7, 11-20, 30-37 (chain A) perpendicular to a β -sheet formed by the residues

4-5, 18-22, and 31-35 (chain B). The minimum C shows one β -hairpin in the N-terminus and one β -hairpin spanning residues 15-19 and 29-31 (chain A) anti-parallel to a β -hairpin spanning residues 18-23 and 31-35 (chain B), while minimum D displays two β -hairpins (residues 10-20 – 30-35 and residues 18-24 – 29-34) in between the antiparallel and perpendicular orientation.

The third topology, consisting of minima E to H (Figures 3E-H) with a total population of 12%, is largely disordered with both chains having small amounts of helix and β -sheet at different locations. The minimum E shows one chain with helix at positions 13-17 and one chain with a two-stranded β -sheet at residues 18-20 and 30-32, while the minimum H shows a helix at positions 28-32 in one chain and a three-stranded β -sheet at residues 19-20, 24-27 and 38-40 in the second chain. The minimum F forms a β -hairpin at the N-terminus (residues 4-5 and 10-11) of chain A, and two small 3-10 helices in chain B (residues 17-19 and 30-32). Interestingly, the minimum G forms an intermolecular parallel β -sheet spanning residues 29-30 and 31-32.

Comparison of this conformational ensemble to the simulation results of the A β 42 dimer in bulk solution²⁹ reveals striking differences using the same protein and water force fields. Here the N-terminus spans residues 1-16, the central hydrophobic core (CHC) spans residues 17-21, the loop region spans residues 22-29, and the long hydrophobic core (LHC) spans residues 30-42. First, differences are observed at the single-molecule states, i.e., states of the intramolecular conformation of one chain in the presence of the other chain. Without cholesterol as described in Table S1 of Ref. 29, 34% of the s-m states have only helices in one of the following three regions: residues 24-27 (loop region), residues 9-11 and 15-17 (N-terminus), and residues 28-36 (loop and N-region of the LHC); 30% and 6% of the s-m states have a β -hairpin-like at the N-terminus (residues 4-6 and 10-12), and at residues 21-27 and 30-36, respectively; and 31% of the s-m states show short 3-10 helices at the N-terminus or the residues 31-35 with β -hairpins spanning residues 21-27 and 30-36, or residues 17-20 and 30-32.²⁹ In the presence of cholesterol, none of these single-molecule states are present in the minima A to G. Rather the intramolecular three-stranded β -sheet spanning the N-terminus - CHC - C-terminus is statistically populated (see minimum B), and multiple β -hairpins with various β -strand and loop lengths are identified from residues 11 to 37. Second, differences are also observed for the orientation and packing of each chain where many states are side-by side for the dimer free of cholesterol (see Figure 7 in Ref. 29) vs. entangled and compact as a result of the competition between the A β 42 peptides and the cholesterol molecules (Figure 3).

Figure 4A shows the probability of contacts between the side-chains and backbone atoms of A β 42 dimer and the cholesterol molecules at 315 K. As expected, the high hydrophobic character of cholesterol favors strong interactions with hydrophobic side-chains and notably the residues F4, H14, I31, I32, L34, M35 and the CHC region (binding probability > 40%). In contrast, the hydroxyl group of cholesterol favors interactions with hydrophilic residues and in particular the N-terminus Y10 residue (binding probability of 35%) and to a lesser extent the loop E22 and K28 residues (probability on the order of 20%). The role of the aromatic interaction with F19 was already noted in very short MD simulations of A β 42 and its F19S variant with cholesterol.⁴⁸

Overall, it should be stressed that, like all simulations of A β dimer in bulk solution,^{8,26,27} random orientation of the two chains with transient and very different secondary structures is still preferred over the antiparallel and perpendicular orientations of the chains with β -hairpins, but in the presence of cholesterol the populations of the two latter configurations become statistically significant (minima B, C and D). None of our conformations show high resemblance with the parallel U-shape fibril structure (i.e., with two β -strands at residues 10-23 and 30-38),⁴⁹ though the minimum G displays a short parallel intermolecular β -sheet. Whether this state or other conformations may have characteristics of the U-shape topology at very long time-scales, as reported by extensive simulation in bulk solution,²⁶ is a computational intense task. To understand the lifetime of aggregates in the presence of cholesterol, Figure 5 reports the evolution of the backbone RMSD starting from the configuration with two β -hairpins (shown in Figure 1A) using standard MD simulation at 310 K. Our results indicate that the lifetime of this topology with many distinct microstates is at least of 2 μ s. Finally, besides the U-shape topology, A β 42 fibrils can also form with a S-shape (i.e., with β -strands at residues 12-18, 24-33 and 36-40) *in vitro*.⁴⁹ In the presence of cholesterol molecules, we did not find any conformations displaying this motif.

As the conformational ensemble is sensitive to the force field, we performed a REMD simulation starting from the most populated state (minimum A, Figure 3) using the recently designed CHARMM36m/TIP3P-modified force field for intrinsically disordered proteins.⁵⁰ This force field was recently tested on A β 16-22, A β 40 and A β 42 oligomers.^{44,51,52} Averaged over the full 150 ns trajectory at 315 K, the secondary structure is 14% β -sheet, 40% coil, 24% turn and 22% helix, almost identical to that of the starting conformation. The dimer is highly dynamic, however, as evidenced by the distribution of the backbone RMSD with respect to the starting structure centered at 1.6 nm and the distributions of secondary structures, with both β -sheet and

helices varying between 0 and 40% (Figure 6). Figure 7 reports the first five dominant clusters at 315 K, representing 33% of the full ensemble, using Daura algorithm and a backbone RMSD of 0.3 nm. These clusters, deviating by 1.5-1.7 nm from the minimum A, preserve however the α/β topology with a multitude of distinct conformations sharing a tendency to form a helix at the C-terminus in one chain and a β -hairpin at the C-terminus in the second chain.

Finally, we characterized the FEL of the A β 42 trimer in the presence of cholesterol projected on the first two principal eigenvectors of the covariance matrix at 315 K using the REMD time interval 50-250 ns (Figure 8). Again, we cannot expect full convergence starting from our conformation, but interesting trends can be extracted. We observe that the content of β -sheet structure is enhanced from the dimer (26%) to the trimer (41%). This increase of β -sheet ordering was already noted in previous experiments and simulations of A β 42 in bulk solution.^{53,54} The representative 3D structures and the secondary structures of the eight minima designated as A2 to H2 and representing 65% of the full ensemble are described in Figure 9.

The FEL is dominated by the first two minima (A2 and B2, 40%) characterized by three individual chains forming a β -hairpin covering the CHC region and the C-terminus, and one hairpin laying above the plane formed by the other two hairpins. The F2 and H2 minima share the same topological characteristics, but in all minima, the β -hairpins display high variation from one unique conformation. For instance, the state A2 shows hairpins at residues 11-22 – 26-33, 19-22 – 31-38, and 17-21 – 28-31 in the three chains, whereas the H2 minimum has a hairpin located at residues 17-19 and 34-36 in one chain. The FEL is also characterized by two individual chains with β -hairpin character perpendicular to each other and a third disordered chain stacked and attached with no preferred orientation and interface, see minima C2, D2, E2 and G2.

It is interesting that the A β 42/cholesterol trimer displays such a small number of topologies with a high tendency of the peptides to form β -hairpins, and even three-stranded β -sheets covering the full sequence. The stability of these two motifs is enhanced from dimer to trimer owing to interpeptide and peptide-cholesterol interactions. It is found that the cholesterol molecules have a much higher probability (> 30%) to bind to residues K16, L17 to A21 (CHC region), and residues N27 to M35 (except G33) than the N-terminus and the amino acids 35-42 (Figure 4B). Note that the increased β -structure ordering of trimers in the presence of cholesterol is also observed for trimer simulations using the coarse-grained OPEP^{55,56} and AWSEM⁵⁴ force fields in bulk solution,

and we can anticipate the same β -structural effect on the conformational ensemble of tetramers, hexamers and dodecamers, which may form from the trimer seed,⁴⁹ and are important species during the lag phase of A β 42 aggregation and in toxicity.^{57,58}

Conclusions

In summary, we have shown that the A β 42/cholesterol dimeric states are markedly different from the A β 42 dimeric states in bulk solution. The presence of cholesterol molecules, which induces changes in the population of the intra- and inter-molecular states, enhances the population of multiple β -hairpins spanning the residues 11-37 and decreases the population of amorphous dimers. This tertiary and quaternary ordering is found to be enhanced from dimer and trimer. The role of the hairpin conformation being critical in defining amyloid pathways and in the conversion to fibril-like states,^{8,49,59} our simulations provide a mechanistic explanation for the reduced A β 42 lag phase during primary nucleation upon cholesterol addition, observed experimentally.¹⁹ Of note, it has also been shown that lipids significantly promote the destabilization of amyloid fibrils into toxic oligomers⁶⁰ by a lipid-promoted fibril fragmentation.⁶¹

Our findings have two implications for AD and other neurodegenerative diseases. First, they indicate that the presence of phospholipids will shift equilibrium and therefore the populations of the oligomeric states of any amyloid proteins that are otherwise marginally populated in bulk solution. How these amyloid oligomers/phospholipids complexes will affect the communication with the cellular partners, such as the prion protein, remains to be determined. It is interesting that tau/phospholipid complexes with five molecules of tau per complex share high structural similarities with fibrils, are taken up by neuronal cells, and may play an important role in transporting tau between different neurons.¹⁰ Second, our results also raise interesting questions regarding the impact of lipids on the conformations of amyloid oligomers in the presence of inhibitors and therefore in the design of effective drugs.⁶²⁻⁶⁸

AUTHOR INFORMATION

Corresponding Author

*E-mail: philippe.derreumaux@tdtu.edu.vn, philippe.derreumaux@ibpc.fr

ORCID

Phuong H. Nguyen: [0000-0003-1284-967X](#)

Son Tung Ngo: [0000-0003-1034-1768](#)

Philippe Derreumaux: [0000-0001-9110-5585](#)

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by grants from the French IDRIS and CINES computer centers, and the “Initiative d’Excellence” program from the French State, Grants “DYNAMO”, ANR-11-LABX-0011, and “CACSICE”, ANR-11-EQPX-0008.

References.

1. Lashuel, H.A.; Hartley, D.; Petre, B.M.; Walz, T.; Lansbury, P.T. Jr. Neurodegenerative disease: Amyloid Pores from Pathogenic Mutations. *Nature* **2002**, *418*, 291.
2. 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*, 10427-10432.
3. Jang, H.; Zheng, J.; Nussinov, R. Models of beta-amyloid Ion Channels in the Membrane Suggest that Channel Formation in the Bilayer is a Dynamic Process. *Biophys. J.* **2007**, *93*, 1938-1949.
4. Jang, H.; Connelly, L.; Arce, F. T.; Ramachandran, S.; Kagan, B. L.; Lal, R.; Nussinov, R. Mechanisms for the Insertion of Toxic, Fibril-like beta-Amyloid Oligomers into the Membrane. *J. Chem. Theory Comput.* **2013**, *9*, 822-833.
5. Berthelot, K.; Cullin, C.; Lecomte, S. What Does Make an Amyloid Toxic: Morphology, Structure or Interaction with Membrane? *Biochimie* **2013**, *95*, 12-19.
6. Österlund, N.; Moons, R.; Ilag, L.L.; Sobott, F.; Gräslund, A. Native Ion Mobility-Mass Spectrometry Reveals the Formation of β -Barrel Shaped Amyloid- β Hexamers in a Membrane-Mimicking Environment. *J. Am. Chem. Soc.* **2019**, *141*, 10440-10450.
7. Sahoo, A.; Matysiak, S. Computational Insights into Lipid Assisted Peptide Misfolding and Aggregation in Neurodegeneration. *Phys. Chem. Chem. Phys.* **2019**, *21*, 22679-22694.
8. Nasica-Labouze, J.; Nguyen, P.H.; Sterpone, F.; Berthoumieu, O.; Buchete, N.V.; Côté, S.; De Simone, A.; Doig, A.J.; Faller, P.; Garcia, A. et al. Amyloid β Protein and Alzheimer's Disease: When Computer Simulations Complement Experimental Studies. *Chem. Rev.* **2015**, *115*, 3518-3563.
9. Banerjee, S.; Hashemi, M.; Lv, Z.; Maity, S.; Rochet, J.C.; Lyubchenko, Y.L. A Novel Pathway for Amyloids Self-assembly in Aggregates at Nanomolar Concentration Mediated by the Interaction with Surfaces. *Sci. Rep.* **2017**, *7*, 45592.

10. Ait-Bouziad, N.; Lv, G.; Mahul-Mellier, A.L.; Xiao, S.; Zorludemir, G.; Eliezer, D.; Walz, T.; Lashuel, H.A. Discovery and Characterization of Stable and Toxic Tau/phospholipid Oligomeric Complexes. *Nat. Commun.* **2017**, *8*, 1678.
11. Di Scala, C.; Yah, N.; Boutemour, S.; Flores, A.; Rodriguez, L.; Chahinian, H.; Fantini, J. Common Molecular Mechanism of Amyloid Pore Formation by Alzheimer's β -amyloid Peptide and α -synuclein. *Sci. Rep.* **2016**, *6*, 28781.
12. Ghio, S.; Camilleri, A.; Caruana, M.; Ruf, V.C.; Schmidt, F.; Leonov, A.; Ryazanov, S.; Griesinger, C.; Cauchi, R.J.; Kamp, F. et al. Cardiolipin Promotes Pore-Forming Activity of Alpha-Synuclein Oligomers in Mitochondrial Membranes. *ACS Chem. Neurosci.* **2019**, *10*, 3815-3829.
13. Ciudad, S.; Puig, E.; Botzanowski, T.; Meigooni, M.; Arango, A.S.; Do, J.; Mayzel, M.; Bayoumi, M.; Chaignepain, S.; Maglia, G. et al. $A\beta(1-42)$ Tetramer and Octamer Structures Reveal Edge Conductivity Pores as a Mechanism for Membrane Damage. *Nat. Commun.* **2020**, *11*, 3014.
14. Nguyen, P.H.; Campanera, J.M.; Ngo, S.T.; Loquet, A.; Derreumaux, P. Tetrameric $A\beta 40$ and $A\beta 42$ β -Barrel Structures by Extensive Atomistic Simulations. I. In a Bilayer Mimicking a Neuronal Membrane. *J. Phys. Chem. B.* **2019**, *123*, 3643-3648.
15. Ngo, S.T.; Nguyen, P.H.; Derreumaux, P. Impact of A2T and D23N Mutations on Tetrameric $A\beta 42$ Barrel within a Dipalmitoylphosphatidylcholine Lipid Bilayer Membrane by Replica Exchange Molecular Dynamics. *J. Phys. Chem. B.* **2020**, *124*, 1175-1182.
16. Popp, J.; Meichsner, S.; Kölsch, H.; Lewczuk, P.; Maier, W.; Kornhuber, J.; Jessen, F.; Lütjohann, D. Cerebral and Extracerebral Cholesterol Metabolism and CSF Markers of Alzheimer's Disease. *Biochem. Pharmacol.* **2013**, *86*, 37-42.
17. Harris, J.R.; Milton, N.G. Cholesterol in Alzheimer's Disease and Other Amyloidogenic Disorders. *Subcell. Biochem.* **2010**, *51*, 47-75.
18. Panchal, M.; Loeper, J.; Cossec, J.C.; Perruchini, C.; Lazar, A.; Pompon, D.; Duyckaerts, C. Enrichment of Cholesterol in Microdissected Alzheimer's Disease Senile Plaques as Assessed by Mass Spectrometry. *J. Lipid Res.* **2010**, *51*, 598-605.
19. Habchi, J.; Chia, S.; Galvagnion, C.; Michaels, T.C.T.; Bellaiche, M.M.J.; Ruggeri, F.S.; Sanguanini, M.; Idini, I.; Kumita, J.R.; Sparr, E. et al. Cholesterol Catalyses $A\beta 42$ Aggregation through a Heterogeneous Nucleation Pathway in the Presence of Lipid Membranes. *Nat. Chem.* **2018**, *10*, 673-683.
20. Roche, J.; Shen, Y.; Lee, J.H.; Ying, J.; Bax, A. Monomeric $A\beta(1-40)$ and $A\beta(1-42)$ Peptides in Solution Adopt Very Similar Ramachandran Map Distributions That Closely Resemble Random Coil. *Biochemistry* **2016**, *55*, 762-75.
21. Vivekanandan, S.; Brender, J. R.; Lee, S. Y.; Ramamoorthy, A. A Partially Folded Structure of amyloid-beta(1-40) in an Aqueous Environment. *Biochem. Biophys. Res. Commun.* **2011**, *411*, 312-316.
22. Rosenman, D.J.; Connors, C.R.; Chen, W.; Wang, C.; García, A.E. $A\beta$ Monomers Transiently Sample Oligomer and Fibril-like Configurations: Ensemble Characterization using a Combined MD/NMR Approach. *J. Mol. Biol.* **2013**, *425*, 3338-3359.
23. Rosenman, D.J.; Wang, C.; García, A.E. Characterization of $A\beta$ Monomers through the Convergence of Ensemble Properties among Simulations with Multiple Force Fields. *J. Phys. Chem. B.* **2016**, *120*, 259-277.

24. Liao, Q.; Owen, M.C.; Bali, S.; Barz, B.; Strodel, B. A β under Stress: The Effects of Acidosis, Cu²⁺-Binding, and Oxidation on amyloid β -peptide Dimers. *Chem. Commun (Camb)*. **2018**, *54*, 7766-7769.
25. van der Munnik, N.P.; Sajib, M.S.J.; Moss, M.A.; Wei, T.; Uline, M.J. Determining the Potential of Mean Force for Amyloid- β Dimerization: Combining Self-Consistent Field Theory with Molecular Dynamics Simulation. *J. Chem. Theory Comput.* **2018**, *14*, 2696-2704.
26. Cao, Y.; Jiang, X.; Han, W. Self-Assembly Pathways of β -Sheet-Rich Amyloid- β (1-40) Dimers: Markov State Model Analysis on Millisecond Hybrid-Resolution Simulations. *J. Chem. Theory Comput.* **2017**, *13*, 5731-5744.
27. Das, P.; Chacko, A.R.; Belfort, G. Alzheimer's Protective Cross-Interaction between Wild-Type and A2T Variants Alters A β ₄₂ Dimer Structure. *ACS Chem. Neurosci.* **2017**, *8*, 606-618.
28. Tarus, B.; Tran, T.T.; Nasica-Labouze, J.; Sterpone, F.; Nguyen, P.H.; Derreumaux, P. Structures of the Alzheimer's Wild-Type A β 1-40 Dimer from Atomistic Simulations. *J. Phys. Chem. B*. **2015**, *119*, 10478-87.
29. Man, V.H.; Nguyen, P.H.; Derreumaux, P. High-Resolution Structures of the Amyloid- β 1-42 Dimers from the Comparison of Four Atomistic Force Fields. *J. Phys. Chem. B*. **2017**, *121*, 5977-5987.
30. Nguyen, P.H.; Derreumaux, P. Structures of the Intrinsically Disordered A β , tau and α -synuclein Proteins in Aqueous Solution from Computer Simulations. *Biophys. Chem.* **2020**, *264*, 106421.
31. Dias, C.L.; Jalali, S.; Yang, Y.; Cruz, L. Role of Cholesterol on Binding of Amyloid Fibrils to Lipid Bilayers. *J. Phys. Chem. B*. **2020**, *124*, 3036-3042.
32. Karimi, H.; Heydari Dokoochaki, M.; Zolghadr, A.R.; Ghatee, M.H. The Interactions of an A β Protofibril with a Cholesterol-enriched Membrane and Involvement of Neuroprotective Carbazolium-based Substances. *Phys. Chem. Chem. Phys.* **2019**, *21*, 11066-11078.
33. Owen, M.C.; Kulig, W.; Poojari, C.; Rog, T.; Strodel, B. Physiologically-relevant Levels of Sphingomyelin, but not GM1, Induces a β -sheet-rich Structure in the amyloid- β (1-42) Monomer. *Biochim. Biophys. Acta Biomembr.* **2018**, S0005-2736(18)30106-8.
34. Lockhart, C.; Klimov, D.K. Cholesterol Changes the Mechanisms of A β Peptide Binding to the DMPC Bilayer. *J. Chem. Inf. Model.* **2017**, *57*, 2554-2565.
35. Grouleff, J.; Irudayam, S.J.; Skeby, K.K.; Schiøtt, B. The Influence of Cholesterol on Membrane Protein Structure, Function, and Dynamics Studied by Molecular Dynamics Simulations. *Biochim. Biophys. Acta*. **2015**, 1848, 1783-95.
36. Yu, X.; Zheng, J. Cholesterol Promotes the Interaction of Alzheimer β -amyloid Monomer with Lipid Bilayer. *J. Mol. Biol.* **2012**, *421*, 561-571.
37. Azouz, M.; Cullin, C.; Lecomte, S.; Lafleur, M. Membrane Domain Modulation of A β ₁₋₄₂ Oligomer Interactions with Supported Lipid Bilayers: An Atomic Force Microscopy Investigation. *Nanoscale* **2019**, *11*, 20857-20867.
38. Lesné, S.E.; Sherman, M.A.; Grant, M.; Kuskowski, M.; Schneider, J.A.; Bennett, D.A.; Ashe, K.H. Brain amyloid- β Oligomers in Ageing and Alzheimer's Disease. *Brain* **2013**, *136*, 1383-1398.
39. Shaw, D. E.; Maragakis, P.; Lindorff-Larsen, K.; Piana, S.; Dror, R. O.; Eastwood, M. P.; Bank, J. A.; Jumper, J. M.; Salmon, J. K.; Shan, Y. et al. Atomic-level Characterization of the Structural Dynamics of Proteins. *Science* **2010**, *330*, 341-346.
40. Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L., Comparison of Simple Potential Functions for Simulating Liquid Water. *J. Chem. Phys.* **1983**, *79*, 926-935.

41. Maity, S.; Lyubchenko, Y.L. AFM Probing of Amyloid-Beta 42 Dimers and Trimers. *Front. Mol. Biosci.* **2020**, *7*, 69.
42. Wang, J.; Wang, W.; Kollman, P. A.; Case, D. A., Automatic Atom Type and Bond Type Perception in Molecular Mechanical Calculations. *J. Mol. Graph. Model.* **2006**, *25*, 247-260.
43. Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A., Development and Testing of a General Amber Force Field. *J. Comput. Chem.* **2004**, *25*, 1157-1174.
44. Nguyen, P. H.; Campanera, J. M.; Ngo, S. T.; Loquet, A.; Derreumaux, P., Tetrameric A β 40 and A β 42 β -Barrel Structures by Extensive Atomistic Simulations. II. In Aqueous Solution. *J. Phys. Chem. B* **2019**, *123*, 6750-6756.
45. van der Spoel, D.; Lindahl, E.; Hess, B.; Groenhof, G.; Mark, A. E.; Berendsen, H. J. GROMACS: Fast, Flexible, and Free. *J. Comput. Chem.* **2005**, *26*, 1701-1718.
46. Kabsch, W.; Sander, C. Dictionary of Protein Secondary Structure: Pattern Recognition of Hydrogen-bonded and Geometrical Features. *Biopolymers* **1983**, *22*, 2577-2637.
47. Smith, L.J.; Daura, X.; van Gunsteren, W.F. Assessing Equilibration and Convergence in Biomolecular Simulations. *Proteins* **2002**, *48*, 487-96.
48. Zhou, X.; Xu, J. Free Cholesterol Induces Higher β -sheet Content in A β Peptide Oligomers by Aromatic Interaction with Phe19. *PLoS One* **2012**, *7*, e46245.
49. Kreutzner, A.G.; Nowick, J.S. Elucidating the Structures of Amyloid Oligomers with Macrocyclic β -Hairpin Peptides: Insights into Alzheimer's Disease and Other Amyloid Diseases. *Acc. Chem. Res.* **2018**, *51*, 706-718.
50. Huang, J.; Rauscher, S.; Nawrocki, G.; Ran, T.; Feig, M.; de Groot, B.L.; Grubmüller, H.; MacKerell, A.D. Jr. CHARMM36m: An Improved Force Field for Folded and Intrinsically Disordered Proteins. *Nat. Methods* **2017**, *14*, 71-73.
51. Man, V.H.; He, X.; Derreumaux, P.; Ji, B.; Xie, X.Q.; Nguyen, P.H.; Wang J. Effects of All-Atom Molecular Mechanics Force Fields on Amyloid Peptide Assembly: The Case of A β ₁₆₋₂₂ Dimer. *J. Chem. Theory Comput.* **2019**, *15*, 1440-1452.
52. Samantray, S.; Yin, F.; Kav, B; Strodel, B. Different Force Fields Give Rise to Different Amyloid Formation Pathways in Molecular Dynamics Simulations. *J. Chem. Inf. Model.* **2020** Nov 11. doi: 10.1021/acs.jcim.0c01063. Epub ahead of print.
53. Ono, K.; Condron, M.M.; Teplow, D.B. Structure-neurotoxicity Relationships of Amyloid beta-protein Oligomers. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 14745-14750.
54. Zheng, W.; Tsai, M.Y.; Wolynes, P.G. Comparing the Aggregation Free Energy Landscapes of Amyloid Beta(1-42) and Amyloid Beta(1-40). *J. Am. Chem. Soc.* **2017**, *139*, 16666-16676.
55. Chebaro, Y.; Jiang, P.; Zang, T.; Mu, Y.; Nguyen, P.H.; Mousseau, N.; Derreumaux, P. Structures of A β 17-42 Trimers in Isolation and with Five Small-molecule Drugs using a Hierarchical Computational Procedure. *J. Phys. Chem. B.* **2012**, *116*, 8412-8422.
56. Sterpone, F.; Melchionna, S.; Tuffery, P.; Pasquali, S.; Mousseau, N.; Cragolini, T.; Chebaro, Y.; St-Pierre, J.F.; Kalimeri, M.; Barducci, A. et al. The OPEP Protein Model: From Single Molecules, Amyloid Formation, Crowding and Hydrodynamics to DNA/RNA Systems. *Chem. Soc. Rev.* **2014**, *43*, 4871-4893.
57. Bernstein, S.L.; Dupuis, N.F.; Lazo, N.D.; Wyttenbach, T.; Condron, M.M.; Bitan, G.; Teplow, D.B.; Shea, J.E.; Ruotolo, B.T.; Robinson, C.V. et al. Amyloid- β protein Oligomerization and the Importance of Tetramers and Dodecamers in the Aetiology of Alzheimer's Disease. *Nat. Chem.* **2009**, *1*, 326-31.

58. Lesné, S.E.; Sherman, M.A.; Grant, M.; Kuskowski, M.; Schneider, J.A.; Bennett, D.A.; Ashe, K.H. Brain Amyloid- β Oligomers in Ageing and Alzheimer's Disease. *Brain* **2013**, *136*, 1383-1398.
59. Abelein, A.; Abrahams, J.P.; Danielsson, J.; Gräslund, A.; Jarvet, J.; Luo, J.; Tiiman, A.; Wärmländer, S.K. The Hairpin Conformation of the Amyloid β Peptide is an Important Structural Motif along the Aggregation Pathway. *J. Biol. Inorg. Chem.* **2014**, *19*, 623-634.
60. Widenbrant, M.J.; Rajadas, J.; Sutardja, C.; Fuller, G.G. Lipid-induced Beta-amyloid Peptide Assemblage Fragmentation. *Biophys. J.* **2006**, *91*, 4071-4080.
61. Friedman, R.; Pellarin, R.; Caflisch, A. Amyloid Aggregation on Lipid Bilayers and its Impact on Membrane Permeability. *J. Mol. Biol.* **2009**, *387*, 407-415.
62. Doig, A.J.; Del Castillo-Frias, M.P.; Berthoumieu, O.; Tarus, B.; Nasica-Labouze, J.; Sterpone, F.; Nguyen, P.H.; Hooper, N.M.; Faller, P.; Derreumaux, P. Why Is Research on Amyloid- β Failing to Give New Drugs for Alzheimer's Disease? *ACS Chem. Neurosci.* **2017**, *8*, 1435-1437.
63. Doig, A.J.; Derreumaux, P. Inhibition of Protein Aggregation and Amyloid Formation by Small Molecules. *Curr. Opin. Struct. Biol.* **2015**, *30*, 50-56.
64. Cox, S.J.; Lam, B.; Prasad, A.; Marietta, H.A.; Stander, N.V.; Joel, J.G.; Sahoo, B.R.; Guo, F.; Stoddard, A.K.; Ivanova, M.I. et al. High-Throughput Screening at the Membrane Interface Reveals Inhibitors of Amyloid- β . *Biochemistry* **2020**, *59*, 2249-2258.
65. Zhang, T.; Xu, W.; Mu, Y.; Derreumaux, P. Atomic and Dynamic Insights into the Beneficial Effect of the 1,4-naphthoquinon-2-yl-L-tryptophan Inhibitor on Alzheimer's A β 1-42 Dimer in terms of Aggregation and Toxicity. *ACS Chem. Neurosci.* **2014**, *5*, 148-159.
66. Zhang, T.; Zhang, J.; Derreumaux, P.; Mu, Y. Molecular Mechanism of the Inhibition of EGCG on the Alzheimer A β (1-42) Dimer. *J. Phys. Chem. B.* **2013**, *117*, 3993-4002.
67. Lee, J.C.; Kim, H.Y.; Lee, S.; Shin, J.; Kim, H.V.; Kim, K.; Baek, S.; Lee, D.; Jeon, H.; Kim, D et al. Discovery of Chemicals to Either Clear or Indicate Amyloid Aggregates by Targeting Memory-Impairing Anti-Parallel A β Dimers. *Angew. Chem. Int. Ed. Engl.* **2020**, *59*, 11491-11500.
68. Heller, G.T.; Aprile, F.A.; Michaels, T.C.T.; Limbocker, R.; Perni, M.; Ruggeri, F.S.; Mannini, B.; Löhr, T.; Bonomi, M.; Camilloni, C et al. Small-molecule Sequestration of Amyloid- β as a Drug Discovery Strategy for Alzheimer's Disease. *Sci. Adv.* **2020**, *6*, eabb5924.

Table 1. Simulations summary with a A β /Chol ratio of 1:1.

System	Simulation Method/Force Field	Time (ns)	Replicas	T range (K)	Volume (nm ³)	Water molecules
A β 42 dimer (β -hairpin)	MD AMBER99SB-ildn	2000	--	310	368.57	11567
A β 42 dimer (β -hairpin)	REMD AMBER99SB-ildn	250	96	300-450	368.57	11567
A β 42 dimer (Helix)	REMD AMBER99SB-ildn	250	96	300-450	402.24	12723
A β 42 dimer (Helix/ β - hairpin)	REMD CHARMM36m	150	96	idem	idem	Idem
A β 42 trimer	REMD AMBER99SB-ildn	250	96	300-450	402.24	12723

Figure 1. Initial REMD conformations. (A) Aβ42 dimer in hairpin + 2 Chol form a β-sheet at residues 12-19 and 30-36 of chain A (red) and residues 16-23 and 30-37 of chain B (green). (B) Aβ42 dimer with helix + 2 Chol form helices at residues 13-23 in both chains A (red) and B (green). (C) Aβ42 trimer + 3 Chol form β-sheets at residues 13-21 and 29-35 of chain A, residues 16-22 and 31-37 of chain B, and residues 13-21 and 27-35 of chain C. Red, green, and yellow colors show chains A, B, and C, respectively, and the N-terminus is shown by a blue ball.

Figure 2. FEL of A β 42 dimers + 2 Chol molecules using the interval 50-250 ns of the two REMD simulations at 315 K. The eight minima A, B, C, D, E, F, G, and H are shown.

Figure 3. Representative structures of the eight minima obtained using the time interval 50-250 ns of the two REMD simulations. The blue ball shows the N-terminus. The population of the eight minima A, B, C, D, E, F, G, and H is 26, 20, 8, 5, 3, 3, 3, and 3 %, respectively.

Figure 4. Probability of contacts between the cholesterol molecules and the side-chains and backbone atoms of Aβ42 at 315 K. (A) Aβ42 dimer + 2 Chol, (B) Aβ42 trimer + 3 Chol.

Figure 5. MD simulation of A β 42 dimer in hairpin + 2 Chol at 310 K. (*Left*) C_{α} RMSD, (*right*) snapshot at 2000 ns, where a β -sheet is located at residues 3-4, 13-22 and 26-38 in chain A (red) and residues 19-22 and 31-34 in chain B (green), and a helix forms at residues 11-13 of chain B. The blue ball shows the N-terminus. Over the full trajectory, the averaged secondary structure is 36% β -sheet, 54% coil, 8% turn and 2% helix.

Figure 6. Distributions of 3D and 2D properties sampled by REMD simulation using the CHARMM36m/TIP3P-modified force field at 315 K. (A) RMSD with respect to the initial structure, (B) beta-sheet, (C) helix, (D) turn and (E) coil.

Figure 7. First five dominant clusters of Aβ42 dimer + 2 Chol system using REMD and the CHARMM36m/TIP3P-modified force field. For clarity, the cholesterol molecules are not shown.

Figure 8. FEL of A β 42 trimer + 3 Chol from PCA using the time interval 50-250 ns of REMD simulation at 315 K.

Figure 9. Representative structures of eight A β 42 trimer + 3 Chol minima. The populations of the A2, B2, C2, D2, E2, F2, G2 and H2 minima are 12, 28, 2, 6, 4, 8, 1, 3 and 2%, respectively. Red, green, and yellow colors show chains A, B, and C. Blue ball shows the N-terminus. Minimum A2 forms a β -sheet at residues 2-5, 11-22, and 26-33 of chain A, 19-22 and 31-38 of chain B, and 3-5, 17-21, and 28-31 of chain C. Minimum B2 displays a β -sheet at residues 5-6, 10-11, 13-22 and 26-35 of chain A, residues 16-22 and 31-37 of chain B, and residues 15-22 and 26-33 of chain C. Minimum C2 has a β -sheet at residues 4-5, 14-20, and 28-34 of chain A, residues 4-5, 18-20, and 34-35 of chain B, and residues 16-20, 28-32 and 39-40 of chain C. Minimum D2 forms a β -sheet spanning 2-5, 11-21, 27-34 and 39-40 of chain A, 2-3, 19-23, and 30-34 of chain B, and 13-23, 26-35, 39-40 of chain C. Minimum E2 forms a β -sheet at positions 2-6, 10-20, 30-34 of chain A, 18-19, 34-35, 39-41 of chain B, and 6-7, 12-13, 15-22 of chain C. Minimum F2 forms a β -sheet at residues 13-22, 26-35 of chain A, residues 4-6, 16-24, 29-35 of chain B, and residues 2-6, 9-11, 13-22, 27-35 of chain C. Minimum G2 forms a β -sheet at the residues 5-6, 15-22, 26-33, 38-39 of chain A, and residues 3-6, 9-10, 15-23, 31-38 of chain B. Minimum H2 forms a β -sheet at residues 14-20, 29-34, and 40-41 of chain A; 17-19 and 34-36 of chain B; 13-20, 28-35, and 38-40 of chain C, and a helix at residues 25-27 of chain A.

TOC