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Interaction of Alzheimer's β-Amyloid Peptides with Cholesterol: Mechanistic Insights into Amyloid Pore Formation

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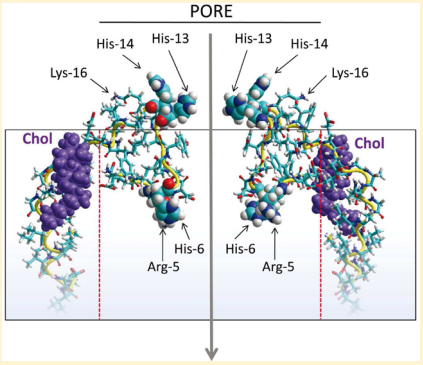
ABSTRACT: Brain cholesterol plays a critical role in Alzheimer's disease and other neurodegenerative diseases. The molecular mechanisms linking cholesterol to neurotoxicity have remained elusive for a long time, but recent data have allowed the identification of functional cholesterol-binding domains in several amyloidogenic proteins involved in neurodegenerative diseases, including Alzheimer's disease. In this review, we analyze the cholesterol binding properties of β-amyloid (Aβ) peptides and the impact of these interactions on amyloid pore formation. We show that although the cholesterol-binding domains of Aβ peptides and of transmembrane precursor C99 are partially overlapping, they involve distinct amino acid residues, so that cholesterol has a greater affinity for Aβ than for C99. Synthetic 22−35 and 25−35 fragments of Aβ retained the ability of the full-length peptide 1−42 to bind cholesterol and to form zinc-sensitive, calcium-permeable amyloid pores in cultured neural cells. Studies with mutant peptides allowed the identification of key residues involved in cholesterol binding and channel formation. Cholesterol promoted the insertion of Aβ in the plasma membrane, induced α-helical structuration, and forced the peptide to adopt a tilted topology that favored the oligomerization process. Bexarotene, an amphipathic drug currently considered as a potential candidate medication for the treatment of neurodegenerative diseases, competed with cholesterol for binding to Aβ and prevented oligomeric channel formation. These studies indicate that it is possible to prevent the generation of neurotoxic oligomers by targeting the cholesterol-binding domain of Aβ peptides. This original strategy could be used for the treatment of Alzheimer's and other neurodegenerative diseases that involve cholesterol-dependent toxic oligomers.



CHOLESTEROL AND ALZHEIMER'S DISEASE

Cholesterol is usually considered a causative factor in Alzheimer's disease (AD), but the mechanistic link between cholesterol and AD remains to be formally established.[1] Several studies have suggested that high serum cholesterol, especially after midlife, is a potential risk for AD,[2,3] but this conclusion has been recently challenged.[1] On the other hand, the effectiveness of cholesterol-lowering drugs (statins) for the prevention and treatment of AD has been questioned.[4,5] Clinical studies in humans markedly contrast with the data obtained in animal and cell culture studies,[6] which showed that increasing cholesterol levels, especially in the plasma membrane of neural cells, potentiates both the production and neurotoxicity of Aβ peptides,[7] whereas decreasing membrane cholesterol levels have rather neuroprotective effects.[1] How can we reconcile these apparently conflicting results obtained in humans, animals, and cell cultures? First, we have to consider that cholesterol is not randomly distributed in the plasma membrane but concentrated in specific microdomains termed lipid rafts.[8] Thus, estimating total cholesterol levels in brain may not reflect subtle changes in the cholesterol content of lipid rafts. Second, in addition to their high cholesterol levels, lipid rafts are also enriched in specific lipids (especially sphingolipids) that may also play a role in AD symptoms.[9−11] Third, with regard to statins, one should take into account the fact that most of them do not cross the blood−brain barrier, so that they may have little, if any, effect on the homeostasis of brain cholesterol.[12] Therefore, if statins exert any protective or curative effect in AD patients, this is probably unrelated to brain cholesterol levels.[13,14] Instead, it has been suggested that by limiting atherosclerosis, statins may improve blood circulation and thus ameliorate brain oxygenation.[12] An alternative explanation to link cholesterol and AD is to consider that this is not cholesterol that has an impact on AD but AD that affects cholesterol homeostasis,[1,12] yet why in animal and cell culture studies cholesterol has such a stimulatory effect on Aβ production and toxicity remains to be determined. In other words, what could be the simplest explanation for linking cholesterol and AD? A rational approach could be to determine, among the proteins that have been involved in AD, which have the highest probability of physically interacting with cholesterol. With this in mind, we can then proceed through a series of simple questions and clear-cut answers. Which proteins are involved in AD? They are chiefly Tau, Aβ peptides, and the amyloid precursor protein (APP). Where is cholesterol? It is in the plasma membrane, especially in lipid rafts. Where are Tau, Aβ peptides, and APP? Tau is intracellular; APP is a transmembrane protein, and Aβ peptides are both extracellular and associated with the plasma membrane. Therefore, whatever the mechanistic link between cholesterol and AD is, the most likely hypothesis is that cholesterol physically interacts with APP and/or Aβ peptides and

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that these molecular interactions play a role in AD symptoms.[15] The scope of this review is to describe the interactions between cholesterol and APP or Aβ at the molecular level and to assess the impact of these interactions on Aβ production and on the formation of toxic oligomers of Aβ peptides in the context of AD. In-depth reviews of the literature on related but distinct topics such as the interaction of Aβ peptides with gangliosides, the structure of Aβ fibrils, or the role of apolipoprotein E can be found elsewhere.[16−20]

HOW MEMBRANE PROTEINS INTERACT WITH CHOLESTEROL

The long-standing interest in elucidating the molecular mechanisms controlling the binding of cholesterol to proteins[1,21] has allowed the definition of two main consensus linear binding domains termed CRAC [(L/V)-X_{1-5}-(Y)-X_{1-5}-(K/R)][22] and CARC [(K/R)-X_{1-5}-(Y/F)-X_{1-5}-(L/V)],[23] yet some proteins that do not fulfill all the consensus criteria can also bind cholesterol with high affinity.[24] Cholesterol has a large apolar domain consisting of a flexible terminal iso-octyl chain linked to a semirigid sterane backbone (Figure 1). In contrast, its polar part

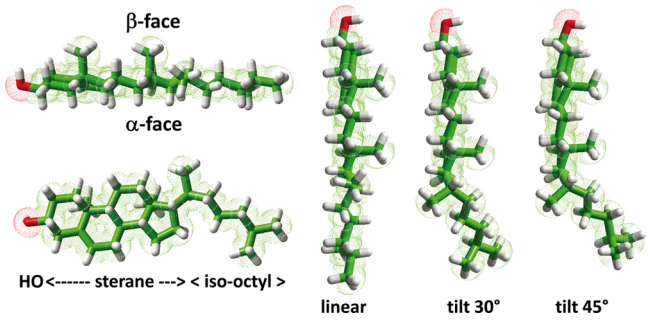

Figure 1. Structural features of cholesterol. In the left panel, the chemical structure of cholesterol consists of a sterane tetracyclic backbone linked to a small polar group (hydroxyl) and an iso-octyl chain. The methyl groups linked to sterane form two spikes, rendering the β-face of cholesterol "rougher" than the opposite "smooth" α-face. The right panel shows three possible conformers of cholesterol differing by the orientation of the iso-octyl chain with respect to sterane. The conformer with a 45° tilt is involved in the interaction of cholesterol with both Aβ and α-synuclein (see Figure 5).

is restricted to a single hydroxyl group. An important feature of cholesterol structure is that the methyl groups linked to the sterane backbone render the surface of one cholesterol face rough (β-face), compared with the smooth opposite face devoid of spiking groups (α-face).[25] By analyzing the interaction of cholesterol with various unrelated cellular and pathogenic proteins, Fantini and Barrantes[24] observed that cholesterol binding involved a common subset of amino acid residues that appeared to be particularly suited for interacting with the α or β face of cholesterol. This explains why in addition to *bona fide* CRAC or CARC motifs, other specific amino acid sequences can form functional cholesterol-binding sites.[24,26,27] A typical cholesterol-binding domain consists of (i) a polar area for accommodating the hydroxyl group of cholesterol, which contains both a donor and an acceptor hydrogen bond atom (the hydrogen and oxygen atoms, respectively, of the OH group) and (ii) a succession of apolar residues capable of interacting with the sterane rings of cholesterol (CH-π stacking with aromatic side chains)[28] or intercalating between the methyl and iso-octyl groups of cholesterol (van der Waals interactions with branched amino acid residues such as Val, Leu, or Ile).[25] Thus, both the smooth α face and the rough β face of cholesterol can optimally interact with selected transmembrane domains of proteins, involving in each case an adequate combination of amino acid residues. Moreover, the flexibility of the iso-octyl chain, which can be either linear or tilted (Figure 1), can generate numerous cholesterol conformers that can interact with distinct transmembrane domains, as explained in the comprehensive analysis of cholesterol-binding sites published by Fantini and Barrantes.[24]

HOW MEMBRANE CHOLESTEROL INTERACTS WITH THE AMYLOID PRECURSOR PROTEIN APP

Because APP is a large membrane protein (Figure 2), it is difficult to study its interaction with cholesterol in reconstituted membranes. An elegant study published by Lahdo et al.[29] described the insertion of nanomolar concentrations of APP into cholesterol-containing monolayers. This study is based on the Langmuir system (Figure 3). In these experiments, a lipid monolayer was prepared at the air−water interface at an initial surface pressure of 10 mN m^{-1}. Then APP (purified from porcine brain) was added in the aqueous subphase and its insertion into the monolayer followed by real-time measurements of the surface pressure, as explained in Figure 3 (left panel). When incubated with a cholesterol monolayer, APP immediately increased the surface pressure. After incubation for 10 min, the surface pressure reached 25 mN m^{-1}. The maximal surface pressure increase was obtained after incubation for 160 min. At that time, the surface pressure was 40 mN m^{-1}. The maximal surface pressure increase ($\Delta\pi_{max}$) was calculated as the difference between maximal pressure π_{max} (40 mN m^{-1}) and initial surface pressure π_i of the cholesterol monolayer (10 mN m^{-1}); i.e., $\Delta\pi_{max}$ = 30 mN m^{-1} in this specific case. This formally demonstrated that APP interacts with cholesterol in a reconstituted model membrane. In contrast, when APP was incubated with monolayers of other membrane lipids (phosphatidylcholine, phosphatidylserine, or sphingomyelin), $\Delta\pi_{max}$ did not exceed 5−10 mN m^{-1}, indicating that APP has a marked preference for cholesterol versus membrane phospholipids. Because the mean surface pressure of a natural plasma membrane has been estimated to be 30 mN m^{-1}, it was important to check that APP could still interact with cholesterol monolayers at such high pressures. This can be done by measuring the insertion of the protein with a series of lipid monolayers prepared at various π_i values (specificity studies). It is expected that the insertion of the protein into the lipid monolayer becomes more difficult as π_i increases. This is because the strength of lipid−lipid interactions is obviously higher in densely packed monolayers (high π_i) than in loose monolayers (low π_i). Thus, if the interaction is specific, the value of $\Delta\pi_{max}$ should gradually decrease as π_i increases (Figure 3, right panel). The extrapolated value of π_i at $\Delta\pi_{max}$ = 0 is termed the "critical pressure of insertion" (π_c). When π_c is >30 mN m^{-1} (the mean value for a representative plasma membrane), the interaction is considered biologically relevant.[30] In the case of APP, the value of π_c for cholesterol monolayers has been estimated to be 32 mN m^{-1},[29] whereas in the same study, the value of π_c for phosphatidylserine was as low as 11 mN m^{-1}. Overall, these results demonstrate that APP is indeed a cholesterol-binding protein. The Langmuir system has several advantages over other methods for studying lipid−protein interactions.[31] Because it uses a half-membrane, one can easily control the actual molar ratio of lipids in perfectly defined mixed monolayers.[32] Monolayers with two, three, or more lipid species, in various amounts, can be prepared and probed with the protein

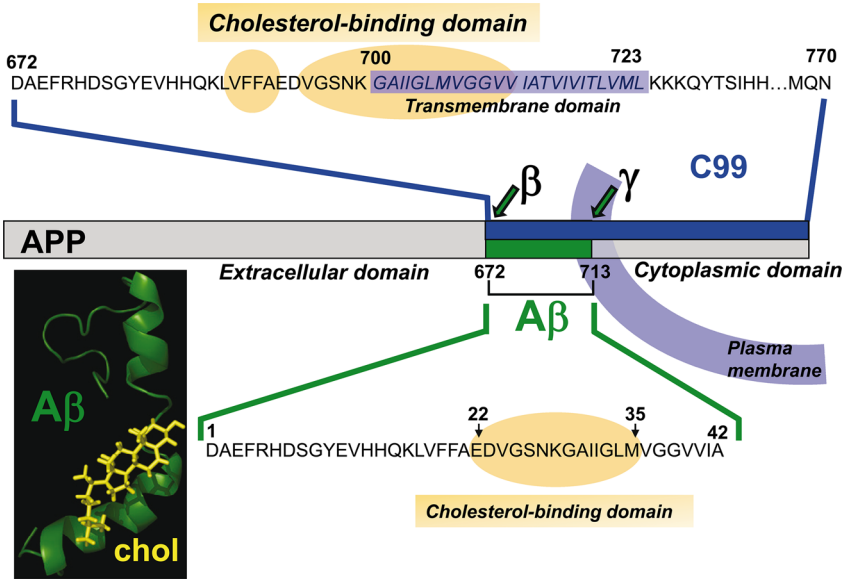

Figure 2. Location of cholesterol-binding domains in C99 and Aβ_{1-42}. Amino acid residues involved in the binding of cholesterol to C99 (top sequence) and Aβ_{1-42} (bottom sequence) are colored orange. The membrane topology of APP (N-terminal domain extracellular and C-terminal domain intracellular) is shown in the middle. The α-helical structure of Aβ_{1-42} (in complex with cholesterol, colored yellow) is visible in the cartoon (bottom left).

of interest (Figure 3). This is a very important point because in most widely used bilayer systems such as liposomes or black lipid membranes, the lipid distribution in each monolayer is not warranted and often not even known by the researcher. Moreover, the Langmuir system works finely with biologically compatible concentrations of proteins (e.g., 4.5 nM in the APP experiments described above). Finally, the molecular mechanisms involved in the lipid−protein interaction can be studied with chemically modified lipids and mutant proteins, either recombinant, purified, or even chemically synthesized in the case of peptides such as β-amyloid peptides (see below). Correspondingly, surface pressure measurements of lipid monolayers have been successfully used to decipher the interactions between membrane lipids (cholesterol, phospholipids, or glycolipids) and a wide range of cellular,[33−39] microbial,[40−46] and amyloidogenic proteins.[32,45,47−54]

Nuclear magnetic resonance (NMR) spectroscopic studies have also been used to identify the amino acid residues physically involved in cholesterol binding. The group of Sanders has reported a series of data obtained with C99,[55−57] a fragment (residues 672−770) lacking most of the extracellular domain of APP (Figure 2). In neural cells, C99 is generated by proteolytic cleavage of APP with β-secretase and is the substrate of γ-secretase that liberates Aβ peptides.[58] This process is regulated by membrane lipids, especially cholesterol.[59] In a first series of experiments, the C99 protein has been incorporated in mixed lysomyristoylphosphatidylglycerol (LMPG) micelles containing a water-soluble cholesterol analogue.[55] In a subsequent study by the same group, C99 was prepared in dimyristoylphosphatidylcholine (DMPC)/cholesterol micelles.[56] The data showed that the transmembrane domain of C99 has such a high flexibility that it can accommodate several sterol conformers. This may explain why divergent data have been obtained according to the nature of the micellar system in which C99 is incorporated. Indeed, NMR studies of LMPG micelles indicated that Gly-696 and Lys-699 were critical for the binding of the water-soluble cholesterol analogue.[55] However, when C99 was prepared in DMPC micelles, cholesterol strongly interacted with Gly-700 and Gly-704 but only weakly with Gly-696 and Lys-699.[56] In this case, the critical role of Gly-700 and Gly-704 was further confirmed by NMR studies with mutants of C99.[56] In summary, these elegant studies indicate that C99 interacts with membrane cholesterol through a domain encompassing the quasi-totality of amino acid residues 689−711, corresponding to amino acid residues 18−40 in Aβ_{1-42} (Figure 2). Gly-700 and Gly-704 residues, which form a typical Gly-XXX-Gly motif,[60] seem particularly important for the flexibility of the membrane-spanning region of C99 and/or for direct binding to cholesterol. Whether this Gly motif is also involved in the interaction between cholesterol and full-length APP has not been determined yet. In any case, one should keep in mind that the avidity of APP for cholesterol needs to be finely tuned so that Aβ peptides can be liberated from the membrane and not trapped by membrane cholesterol. In this respect, the involvement of Gly residues is highly significant because (i) Gly residues confer unique flexibility properties that are probably required for optimal interaction of the transmembrane domain of C99 with γ-secretase and (ii) the side chain of Gly residues (restricted to a hydrogen atom) offers a very small surface for cholesterol interaction, so that cholesterol can easily detach from C99 just before, during, or shortly after the action of γ-secretase.

HOW MEMBRANE CHOLESTEROL INTERACTS WITH β-AMYLOID PEPTIDES

The finding of a cholesterol-binding domain in C99 or APP is consistent with the location of these precursor proteins in lipid raft microdomains, and with the critical role played by cholesterol in the generation of Aβ peptides.[58,61,62] However, it has also been shown that cholesterol has a direct impact on the neurotoxicity of Aβ peptides.[63] Therefore, it was important to determine whether Aβ peptides could interact with cholesterol and how this interaction could be related to the neurotoxicity of Aβ peptides in the context of AD. Indeed, a seminal study by Avdulov et al.[64] demonstrated that Aβ_{1-40} had a marked preference for cholesterol versus phosphatidylcholine. Transmission electron

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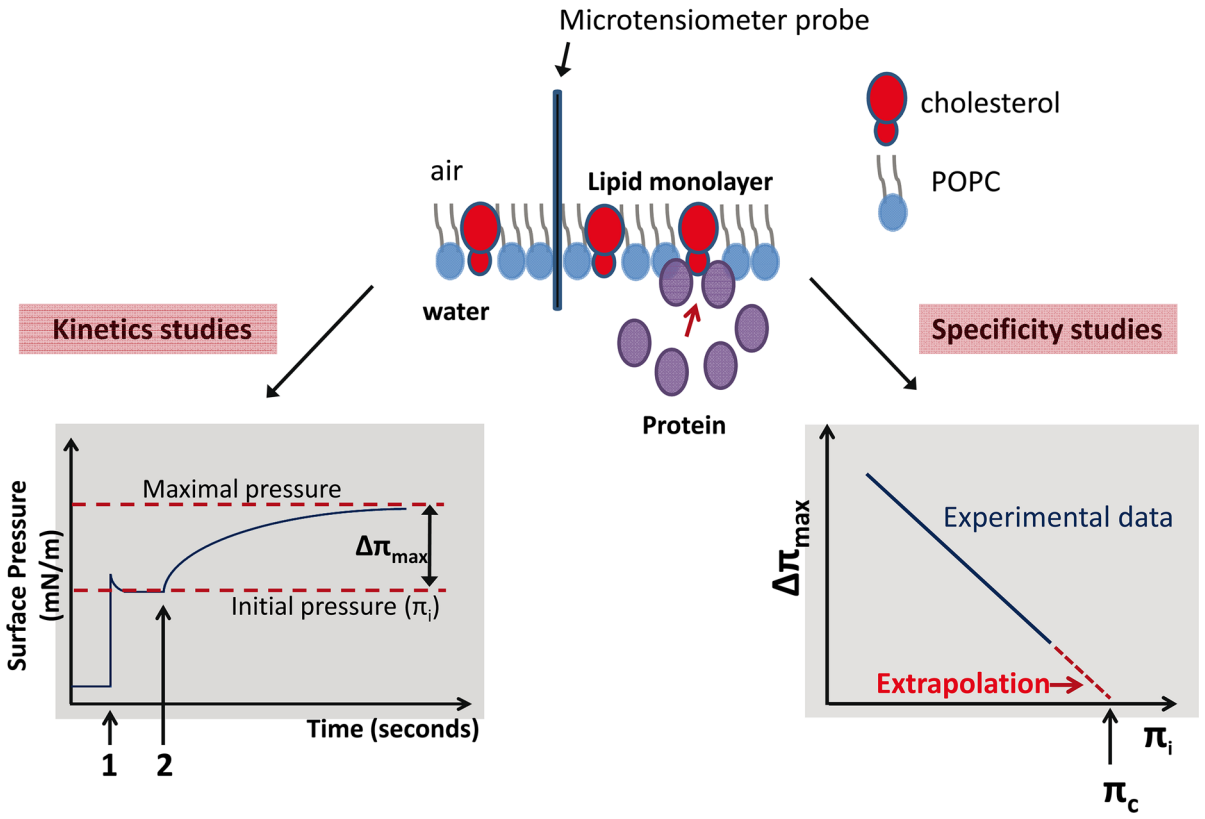

Figure 3. Microtensiometry at the air−water interface for quantitative studies of lipid−protein interactions. A lipid monolayer is prepared at the air−water interface by deposing a drop of lipid [either a pure lipid preparation of a lipid mixture, e.g., cholesterol with palmitoyloleoylphosphatidylcholine (POPC)] at the surface of a water droplet. This induces an immediate increase in surface pressure followed by a rapid stabilization (left panel, step 1). The lipid is usually dissolved in a chloroform/methanol mixture [1:1 or 2:1 (v:v)] or in a hexane/ethanol/chloroform mixture [11:5:4 (v:v:v)]. After evaporation is allowed for 2−5 min, the protein is injected in the aqueous phase (either pure water or a buffer) and the kinetics of protein insertion within the monolayer are followed by real-time measurements of surface pressure. The pressure at which the protein is injected is noted π_i (initial surface pressure). If the protein interacts with the lipid monolayer in such a way that the polar heads of the lipids are spaced by the protein (i.e., if protein insertion occurs), the lateral packing of the molecules in the monolayer (lipids and proteins) increases, as does the surface pressure. At equilibrium, a maximal surface pressure is reached. The difference between this maximum and π_i is termed $\Delta\pi_{max}$. Specificity studies consist of determining $\Delta\pi_{max}$ at different values of π_i (right). Experimental data are determined with a series of monolayers prepared at different values of π_i and probed with the protein at a unique concentration (usually in the nanomolar to micromolar range). The extrapolated value of π_i for $\Delta\pi_{max} = 0$ is termed the critical pressure of insertion, π_c. This parameter reflects the avidity of the protein for the monolayer and should ideally be >30 mN m^{-1}, which is the value measured for a natural plasma membrane.

microscopy studies showed that soluble cholesterol micelles bind to $A\beta_{1-42}$ protofibrils.[15,65] Molecular dynamics simulations suggested that cholesterol promotes the insertion of Aβ into membrane bilayers.[66,67] With the aim of understanding how Aβ peptides recognize membrane cholesterol, our group has studied the interaction of a panel of short Aβ peptides with cholesterol monolayers prepared at the air−water interface.[68−70] This allowed us to delineate the sequence of residues 20−35 of Aβ as a functional cholesterol-binding domain.[68] Of all the peptide fragments tested in these physicochemical experiments, only those containing the region of residues 25−35 ($A\beta_{17-40}$, $A\beta_{22-35}$, and $A\beta_{25-35}$) could penetrate into the cholesterol monolayer. The critical pressure of insertion, π_c, of $A\beta_{22-35}$ for cholesterol monolayers has been estimated to be 41 mN m^{-1}, which is significantly higher than the value published[29] for APP (i.e., 32 mN m^{-1}). Their results strongly suggest that membrane cholesterol has a higher avidity for Aβ peptides than for APP. This is not totally unexpected because, as stated above, the cholesterol−APP complex should be displaced to allow the extraction of the cleaved Aβ peptides from the cholesterol-containing membrane. In contrast, extracellular Aβ peptides are attracted in cholesterol-containing plasma membrane domains where they can organize into neurotoxic amyloid pores. Not only the affinity but also the molecular mechanisms involved in the binding of cholesterol to APP and Aβ were significantly different. In the case of APP and C99, Gly residues appeared to play a critical role in cholesterol binding.[55,56] However, binding of Aβ to cholesterol involved both Lys-28 and a series of branched amino acid residues, including Ala-21, Val-24, and Ile-32.[68] Replacing any of these critical residues with a Gly in synthetic mutant peptides significantly decreased the level of cholesterol binding. Moreover, when the Gly residues involved in C99−cholesterol interactions were mutated in $A\beta_{22-35}$ (Gly → Ala substitutions at positions 29 and 33, i.e., corresponding to Gly-700 and Gly-704, respectively, in C99), these changes had very little effect on cholesterol binding. These data further demonstrated that C99 and Aβ peptides do not interact with membrane cholesterol through the same molecular mechanisms. The choice of branched amino acids (Ala, Val, and Ile) for Aβ instead of the less bulky Gly for C99 significantly improved cholesterol binding, in full agreement with Langmuir data. An interesting aspect of Aβ−cholesterol interactions is that although the cholesterol-binding domain of $A\beta_{1-40}$ can be delineated to amino acid residues 20−35,[68] shorter peptides fragments (i.e.,

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Aβ_{22-35} and Aβ_{25-35}) could still bind cholesterol, yet logically with lower affinity (Figure 4). Specifically, the energies of

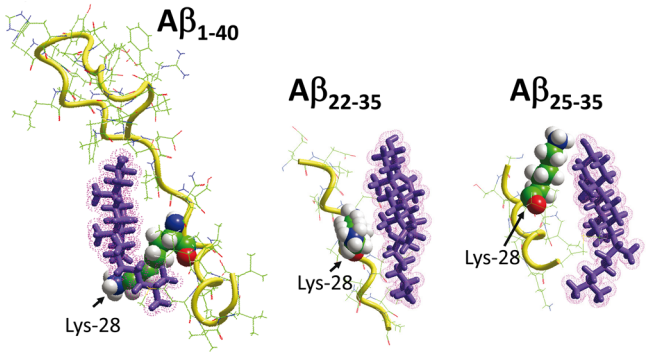

Figure 4. Comparison of cholesterol-binding sites in Aβ_{1-40}, Aβ_{22-35}, and Aβ_{25-35} polypeptides. Note that in all models the main axis of the β-amyloid peptide is tilted with respect to membrane cholesterol. Cholesterol is colored purple. The side chain of Lys-28 (space filled atoms with carbon colored green, nitrogen blue, oxygen red, and hydrogen white) can adopt three distinct orientations, allowing in each case an optimal adaptation to cholesterol. It is only in the complex with Aβ_{25-35} that the ε-NH_3^+ group of Lys-28 forms a hydrogen bond with the OH group of cholesterol (snorkeling topology). In the case of both Aβ_{1-40} and Aβ_{22-35}, the side chain of Lys-28 is totally immersed in the apolar phase of the membrane. This insertion is favored by the deprotonation of the ε-amino group.

interaction between these peptides and cholesterol were estimated to be −76.9, −67.8, and −37.1 kJ mol^{-1} for Aβ_{1-40}, Aβ_{22-35}, and Aβ_{25-35}, respectively.[68−70]

Modeling studies suggested that each Aβ peptide has its own way of interacting with cholesterol. For instance, Aβ_{1-40} selected the α face of the sterol, whereas Aβ_{22-35} and Aβ_{25-35} interacted with the β face (Figure 4). Overall, this indicates that even overlapping protein fragments (C99, Aβ_{1-40}, Aβ_{22-35}, and Aβ_{25-35} in this case) may greatly differ in the way they interact with membrane cholesterol. This is fully consistent with the identification of various distinct cholesterol-binding motifs, including CRAC, CARC, and tilted domains in a series of membrane and amyloidogenic proteins.[24] The key residues forming the cholesterol-binding site were Ala-21, Val-24, Lys-28, and Ile-31 (Aβ_{1-40}), Glu-22, Val-24, Asn-27, Lys-28, and Met-35 (Aβ_{22-35}), and Lys-28, Ile-31, and Met-35 (Aβ_{25-35}).[68−70] In fact, only Lys-28 appeared to be common to the cholesterol-binding site of these peptides. Nevertheless, it is important to note that the side chain of Lys-28 interacts with the apolar part of cholesterol in the case of Aβ_{1-40} (iso-octyl chain) and Aβ_{22-35} (sterane) but forms a hydrogen bond with the OH group of cholesterol in the case of Aβ_{25-35}. By adjusting its orientation in the lipid phase during the membrane insertion process,[71] the flexible side chain of Lys-28 plays a key role in the progressive adaptation of the shape of the peptide to cholesterol, ensuring in each case an optimal interaction with the sterol.

A molecular model of cholesterol bound to Aβ_{1-40} in membrane-consistent conditions is shown in Figure 5. One can see that the orientation of cholesterol with respect to the membrane forces the peptide to adopt a typical tilted topology. As discussed below, this orientation is critical for the oligomerization of Aβ into calcium-permeable amyloid pores. A similar tilted topology is induced by cholesterol on α-synuclein[32] (Figure 5). It is meaningful that in both cases (Aβ and α-synuclein), the iso-octyl chain of cholesterol is tilted by 45°

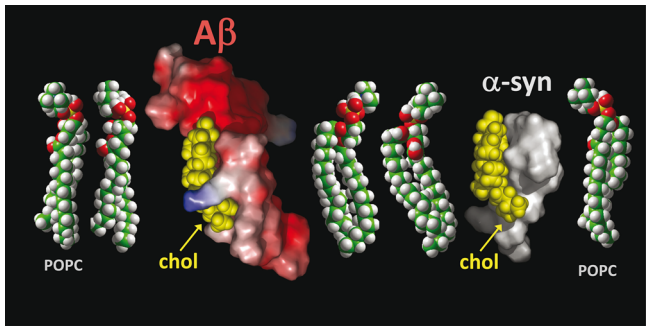

Figure 5. Membrane topology of Aβ_{1-40} and α-synuclein in a cholesterol-rich domain. Cholesterol is colored yellow and POPC atom colors (green for carbon, red for oxygen, yellow for phosphorus, and white for hydrogen). The molecular model of the Aβ−cholesterol complex is adapted from ref 68, and that of the α-synuclein−cholesterol complex (domain 67−78 of α-synuclein) is from ref 32 (Copyright 2011 Elsevier). Note that in both cases the amyloid protein interacts with a tilted conformer of cholesterol and the transmembrane part of the protein is also tilted with respect to the main axis of cholesterol. Thus, cholesterol and the membrane-embedded α-helix form a tight complex in which each component wraps around the other one in a topology reminiscent of a "double-helix".

from the sterane ring system (compare the conformation of cholesterol in Figure 5 with the 45°-tilted conformer of Figure 1). It is interesting to observe how the membrane-dipped α-helix of both Aβ and α-synuclein offer a complementary surface for the 45°-tilted cholesterol conformer, in a topology that resembles a part of a double helix (this is particularly obvious for the α-synuclein−cholesterol complex), yet one should realize that both Aβ and α-synuclein are intrinsically disordered proteins whose three-dimensional (3D) structure is controlled by the water or lipid environment.[72,73] Indeed, the cholesterol-binding domain of Aβ consists of amino acid residues that have a higher propensity to form a β-structure than a α-helix.[68,74] Thus, it is only because the Aβ peptide strongly interacts with membrane cholesterol that it adopts a α-helical fold, as demonstrated by Ji et al.,[75] who combined Langmuir and circular dichroism studies. It is likely that during this membrane-assisted folding, the conformation of cholesterol is also affected by the peptide, so that at the end of the process, the membrane part of Aβ is an α-helix and the iso-octyl chain of cholesterol is tilted (Figure 5). The consequence of this mutual conformational influence is that Aβ is forced to adopt a tilted orientation that favors the oligomerization into annular pores. These oligomeric structures, which can induce marked alterations in calcium homeostasis in brain cells, are probably involved in the neurotoxicity of Aβ peptides.[76−79] For this reason, amyloid pores are a target of choice for developing new anti-Alzheimer's drugs.[80] We will now examine the role of cholesterol in amyloid pore formation and determine whether inhibiting binding of cholesterol to Aβ peptides can prevent amyloid pore formation.

MEMBRANE CHOLESTEROL AND AMYLOID PORE FORMATION

Molecular dynamics simulations combined with calcium flux studies have shed some light on the role of cholesterol in amyloid pore formation. First, we observed that the isolated cholesterol-binding domain of Aβ, i.e., Aβ_{22-35}, was able to induce the entry of a massive amount of calcium into human neuroblastoma SH-SY5Y cells.[69] These minimal calcium channels were functionally similar to those formed by the full-length Aβ_{1-42} peptide.[81]

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Indeed, both Aβ_{22-35} and Aβ_{1-42} channels were blocked by zinc, a classical inhibitor of amyloid channels.[82] In both cases, the calcium flux induced by these peptides involved extracellular calcium, as demonstrated by the lack of activity when cells were bathed in calcium-free medium.[69] Depletion of membrane cholesterol with methyl-β-cyclodextrin abrogated Aβ-induced calcium fluxes, showing that the formation of calcium channels by both full-length and minimal Aβ peptides is a cholesterol-dependent phenomenon.[69,70] Molecular dynamics simulations of cholesterol−Aβ_{22-35} complexes suggested a molecular mechanism for the oligomerization process (Figure 6). The typical

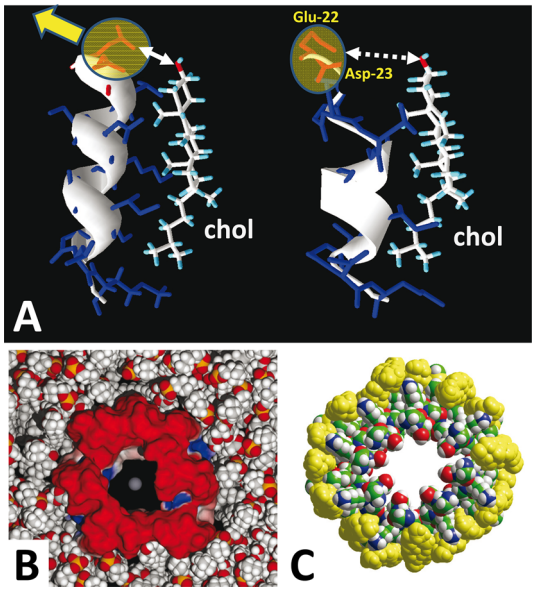

Figure 6. Molecular modeling of annular Aβ channels in the presence of cholesterol. (A) Rearrangements of the cholesterol−Aβ_{22-35} complex in the oligomeric channel. Note the conformational adjustments of Glu-22 and Asp-23 that are close to the OH group of cholesterol in the monomeric Aβ_{22-35}−cholesterol complex (model on the left) and are rejected on the opposite side in each Aβ_{22-35}−cholesterol subunit (model on the right) in the oligomeric channel. This explains why the pore mouth of the Aβ_{22-35} channel forms a negatively charged crown that can efficiently attract Ca^{2+} ions (B). Aβ_{25-35} can also form an annular channel in the presence of cholesterol, as shown in panel C. The channel in panel B is reproduced and adapted from ref 69. The channel in panel C is reproduced and adapted from ref 70.

tilted orientation of Aβ_{22-35} bound to cholesterol favored the establishment of a hydrogen bond network involving the side chains of Asn-27 and Lys-28 of two distinct peptide monomers.[69] Correspondingly, mutating these residues abrogated amyloid pore formation. The involvement of Lys-28 in the assembly of the amyloid pore is particularly interesting from a structural point of view. Because it has an amphipathic structure, the side chain of a Lys residue that belongs to a transmembrane domain has to conciliate the opposite properties of its apolar methylene chain, -$(CH_2)_4$, with its highly polar cationic ε-NH_3^+ group. Usually, the methylene chain is dipped in the apolar lipid phase, whereas the terminal ε-NH_3^+ group emerges from the membrane, where it can interact with water molecules and/or with lipid polar headgroups. This phenomenon is metaphorically termed "snorkeling".[83] This is indeed the case in the complex between cholesterol and Aβ_{25-35}, but not for Aβ_{1-40} and Aβ_{22-35} (Figure 4). In these latter cases, the side chain of Lys-28 is completely immersed in the apolar lipid phase. To avoid a clash between the cationic ε-NH_3^+ group and the apolar environment of the membrane, the side chain of Lys-28 has to find an electrostatically compatible group, i.e., a negative charge or a hydrogen bond acceptor group. The topology of Aβ peptides in a cholesterol-rich membrane domain is consistent with the establishment of a hydrogen bond network involving the ε-NH_3^+ group of Lys-28 on one peptide and the oxygen atoms of both the peptidic bond and the side chain of Asn-27 on another peptide unit (Figure 7A). Because upon the insertion process the ε-NH_3^+ group of

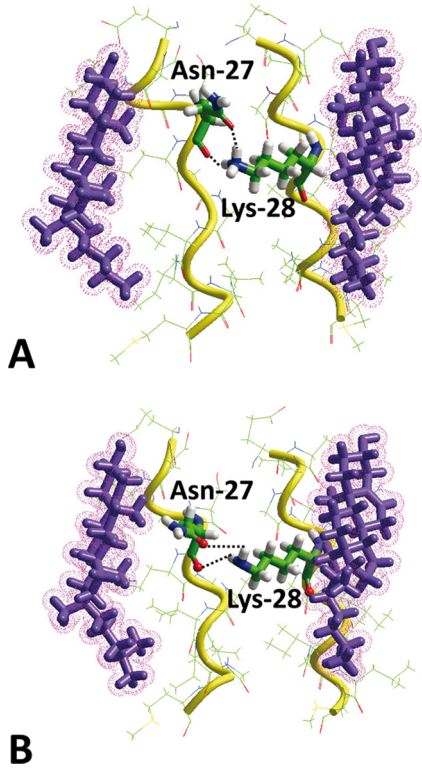

Figure 7. Molecular mechanisms of cholesterol-driven oligomerization of Aβ_{22-35} peptides. The tilted geometry of Aβ_{22-35} peptides induced by cholesterol favors the formation of a hydrogen bond network linking Asn-27 on one peptide and Lys-28 on another. This hydrogen bond can be established with the protonated ε-NH_3^+ group of Lys-28 (A) or with the deprotonated -NH_2 group that is likely to occur during the insertion of the peptide into the cholesterol-rich plasma membrane domain (B).

Lys-28 is probably deprotonated,[84] it is likely that the hydrogen bond is preferentially formed by the neutral ε-NH_2 group of Lys-28 (instead of the cationic ε-NH_3^+ group), as shown in Figure 7B. This may lead to further conformational adjustment[84] of the peptide−cholesterol complex, either before or during the oligomerization process. Molecular dynamics simulations in a membrane environment indicated that the progressive organization of eight Aβ_{22-35} peptides, each interacting with one cholesterol molecule, eventually formed an annular channel (Figure 6B). A key feature of these channels is the recruitment of negatively charged residues (Glu-22 and Asp-23) that eventually form the pore mouth (Figure 6A,B). During the oligomerization process, subtle conformational rearrangements of each cholesterol−Aβ_{22-35} complex allow a reorientation of these acidic side chains that are rejected toward the channel side, leaving cholesterol on the outer wall of the channel, facing membrane lipids (Figure 6A). Calcium ions are attracted by this crown of negative residues whose carboxylic acid groups can also bind

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Zn^{2+} ions, explaining the inhibition of Ca^{2+} fluxes in the presence of $ZnCl_2$.[69] Both the pore size diameter of the channel (~1.5 nm, hence slightly larger than the diameter of a hydrated Ca^{2+} ion) and the fact that the central pore is formed by electronegative residues are consistent with a selectivity for calcium.[85] We have also shown that cholesterol interacts with a shorter fragment of Aβ, i.e., Aβ_{25-35}, a neurotoxic peptide that lacks both acidic residues Glu-22 and Asp-23,[70] yet we demonstrated that Aβ_{25-35} can also form Ca^{2+}-permeable channels via a cholesterol-dependent mechanism. In this case, the pore mouth is formed by the recruitment of Ser-26 residues that form an attractive crater for Ca^{2+} ions (Figure 6C). Because the hydroxyl group of the side chain of Ser residues can bind Zn^{2+} ions, $ZnCl_2$ could efficiently inhibit the Ca^{2+} fluxes induced by the oligomerization of Aβ_{25-35}−cholesterol subunits (with a specific stoichiometry of 8 peptides for 16 cholesterol). In contrast with that of Aβ_{22-35}, this oligomerization is not driven by the formation of a hydrogen bond network but through multiple van der Waals interactions involving Gly-25, Asn-27, and Ile-31 on one peptide subunit and Ser-26, Gly-29, and Gly-33 on its partner.[70] Finally, it is intriguing that the octameric channel formed by Aβ_{25-35} in the presence of cholesterol has a crystalline-like structure that recalls the channel formed by hexameric Aβ_{1-42} β-barrels[85] (compare Figure 6B in this review with Figures 5B and 6B of ref 85). This further illustrates the conformational plasticity of Aβ peptides that can probably use different oligomerization mechanisms to form functional amyloid pores.

Does cholesterol play a similar role for full-length Aβ_{1-42} channels? Although several models have been proposed for the structure of Aβ ion channels,[86−91] none of them have taken into account the interaction of the peptide with cholesterol. This is an important omission, because it is well-established that cholesterol both controls the insertion of Aβ_{1-40} and Aβ_{1-42} peptides within the plasma membrane[67,92] and stimulates channel formation.[93,94] Whatever the molecular mechanisms of amyloid channel formation,[95] one important functional characteristic of Aβ channels is that Zn^{2+} preferentially binds and blocks only one side of the channel.[96] This suggests that His residues of Aβ_{1-42}, and acidic residues (Glu/Asp) in the case of Aβ_{22-35}, should be located at the entry of the channel. Accordingly, plausible models of the Aβ_{1-40} ion channel have been previously constructed with a ring of His residues around the entrance of the pore.[86,96] In these models, the lining of the pore is also formed by a cluster of acidic residues that includes Glu-3, Asp-7, and Glu-11, as we also suggested for the Aβ_{22-35} channel model (Glu-22 and Asp-23 in this case, as shown in Figure 6). On our side, we found that the structure of Aβ_{1-42} bound to cholesterol[68] is compatible with the topology of a tetrameric channel that shares some interesting features with previous models,[71,86] especially the crucial role of His residues (Figure 8). Our modeling strategy was to position first the cholesterol molecules in a realistic fashion with respect to the lipid organization of the membrane, i.e., the OH group at the polar−apolar interface and the apolar part dipped into the membrane. Thus, the position of cholesterol determined the membrane topology of the whole Aβ_{1-42} peptide, including the typical tilt. If we introduce another Aβ−cholesterol complex, in a face-to-face geometry, one can see that His-13 and His-14 are located just above the membrane, forming a functional mouth for the Aβ ion channel (Figure 8). With this principle in mind, we constructed a tetrameric channel (7.8 nm width and 1.5 nm diameter of the central pore) immersed in a phosphatidylcholine membrane (Figure 9). Aβ assemblies with a similar size of 7 nm, which are consistent with our model, have been evidenced by

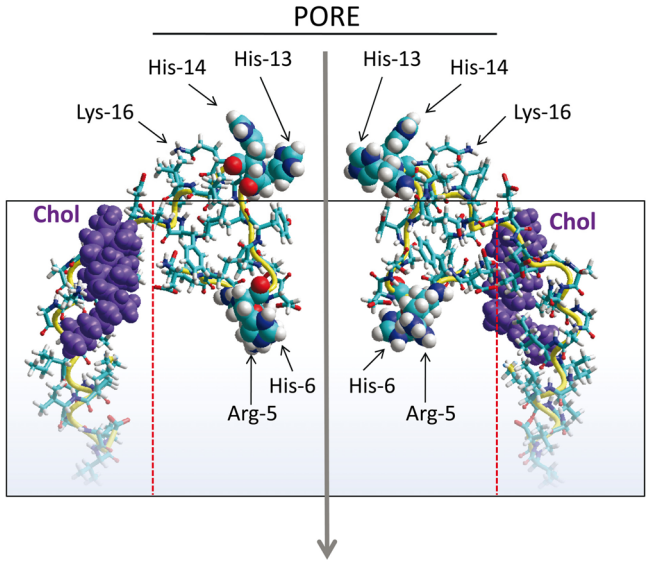

Figure 8. Possible topology of a membrane-bound Aβ_{1-42} ion channel interacting with cholesterol. This topology of a membrane-bound Aβ ion channel is inspired by previous studies.[86,96] Two cholesterol−Aβ subunits have been placed in a two-dimensional array to illustrate the formation of a hydrophilic pore. Note that the position of cholesterol ensures that His-13 and His-14 form the mouth of the pore, as hypothesized in the previous models. This is consistent with the key role of these His residues in the activity of Aβ ion channels.

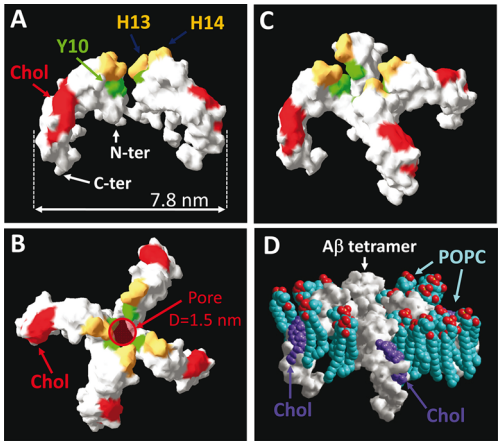

Figure 9. Modeling of an oligomeric channel with four cholesterol−Aβ units embedded in a POPC matrix. Two Aβ_{1-42} chains interacting with cholesterol[68] have been placed face to face (A) as described in Figure 8. Then two other Aβ−cholesterol subunits have been added to form a tetramer with the cholesterol molecules located at the periphery of the supramolecular structure. Phosphatidylcholine molecules (POPC) were randomly introduced into the system, surrounding the tetramer to create a membranelike environment. After further geometry optimization, molecular dynamics simulations were conducted for 10 ns *in vacuo*. Under these conditions, the oligomer formed a channel-like structure delineating a central pore, with a diameter of 1.5 nm. This channel is represented without (B and C) or with (D) POPC molecules. The positions of Tyr-10 (Y10), His-13 (H13), and His-14 (H14) are indicated.

freeze-fracture electron microscopy studies in phospholipid bilayers.[85] Moreover, structures of 13−14 nm were also detected by this ultrastructural approach, which may suggest that the 7 nm species could correspond to transient structures that can coalesce into larger channels.[85] In line with this notion, an interesting

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feature of our model is that it consists of an annular oligomer with four tails. Such multitailed structures have been previously observed by atomic force microscopy studies of amyloid oligomers formed by immunoglobulin light chains.[97]

Other types of channel structures have been described on the basis of molecular dynamics simulations of truncated Aβ peptides.[89] The authors have used the 3D structure of amyloid fibrils formed in water by $Aβ_{1-42}$ peptides.[98] In this structure, residues 18−42 form a typical β-strand−turn−β-strand fold that constitutes an aggregation-prone motif theoretically able to form either amyloid fibrils or amyloid pores. The annular structures constructed with this motif fit remarkably well with the atomic force microscopy images of amyloid pores obtained by the same authors,[95,99] which render their models particularly attractive. It will be interesting to assess whether these β-rich pores can bind cholesterol and/or need cholesterol to penetrate the membrane. In this respect, one cannot exclude the possibility that oligomers of Aβ could be formed outside the membrane, without the help of cholesterol. Such β-rich oligomers could bind to the cell surface and then span the membrane for the formation of active channels.[85] A realistic scenario describing the formation of amyloid channels through the oligomerization of $Aβ_{1-42}$ β-barrels has been published by Shafrir et al.[85] The impact of cholesterol on the membrane insertion and/or oligomerization of such β-structured Aβ peptides remains to be determined. Molecular modeling studies have suggested that sphingomyelin/cholesterol micelles could favor β-sheet structuration of Aβ and fibrillogenesis through a mechanism involving the establishment of a stacking interaction between the aromatic ring of Phe-19 and cholesterol.[100] This is consistent with the recent observation that sphingomyelin-containing membranes can induce Aβ aggregation.[101] However, other data obtained *in silico* and/or by circular dichroism spectroscopy have suggested that cholesterol not only greatly facilitates the insertion of Aβ peptides into the lipid bilayer but also constrains the peptide to adopt an α-helical conformation instead of a β structure.[67,75] Similarly, an α-helix-rich structure of Aβ has been experimentally characterized by NMR spectroscopy in aqueous micelles mimicking a water−membrane medium.[102,103] The balance between α-helical and β secondary structures of Aβ is also dependent upon peptide:lipid ratios.[104] When the peptide is diluted, the lipids win and force Aβ to fold into α structures. At higher peptide concentrations, the influence of lipids is weakened and the peptides are free to adopt the structure they prefer in water, i.e., β-strands that self-aggregate into higher-order structures.[104] Hence, these data support the notion that cholesterol plays a critical role in the insertion, α-helical folding, and oligomerization of Aβ monomers into functional amyloid pore channels. In this respect, it is interesting to note that a minimal synthetic calcium channel can be formed by a tetrameric α-helical peptide that shares some structural homology with our models of Aβ pores.[105] On the other hand, the possibility that the membrane insertion of large amyloid oligomers, preformed in the aqueous extracellular milieu, could proceed through a "perforating" mechanism, independent of membrane lipids, cannot be ruled out.[85]

INDIRECT EFFECTS OF CHOLESTEROL

Apart from direct binding to Aβ peptides, cholesterol could nonspecifically affect the physical properties of biological membranes, such as curvature, fluidity, and permeability.[106−109] A large excess of cholesterol is expected to rigidify the plasma membrane in such a way that it could "mechanically" prevent the insertion of extracellular proteins, including Aβ peptides. Indeed, several groups have reported that increases in the level of membrane cholesterol can inhibit the insertion of amyloidogenic proteins and impede their cytotoxic properties.[110−112] In contrast, other have reported that a high level of membrane cholesterol in both neural cells and lipid vesicles increases the extent of Aβ insertion and Ca^{2+} permeability.[7] Thus, there is an apparently paradoxical situation with respect to the role of membrane cholesterol on Aβ toxicity. Nevertheless, it is important to mention that such apparently opposite effects of cholesterol are in fact a hallmark of the regulatory effects that cholesterol exerts on membrane proteins. The Na^+,K^+-ATPase, for instance, does not work in lipid vesicles lacking cholesterol.[113] When cholesterol is incorporated into these vesicles, the ATPase activity gradually increases until reaching a maximum at a molar ratio of cholesterol versus other lipids of 30%, yet the activity of the pump decreases when the amount of cholesterol is further increased. Similarly, it has been observed that above a molar ratio of 20%, cholesterol inhibited the insertion of Aβ peptides into reconstituted membrane bilayers.[114] Thus, it appears that there is a balance between the positive and negative effects of cholesterol, which, in the case of Aβ peptides, could represent the frontier between specific effects (cholesterol binding to Aβ, which promotes amyloid pore formation) and nonspecific physical alterations of the plasma membrane (which, at high cholesterol ratios, may become too rigid to allow Aβ insertion). Accordingly, an increasing cholesterol content above a threshold level could indeed prevent Aβ insertion and toxicity, in agreement with several reports.[115−117]

Cholesterol can also indirectly control the insertion of Aβ peptides through a conformational effect on gangliosides.[51,118] It has been shown that Aβ specifically interacts with GM1,[16] a ganglioside abundantly expressed in neuronal plasma membranes,[119] and that GM1 efficiently promotes Aβ aggregation and neurotoxicity.[17] Moreover, gangliosides and cholesterol not only segregate into lipid raft domains but strongly interact to form stable cholesterol−ganglioside complexes.[120,121] We have shown that cholesterol exerts a specific conformational effect on the sugar headgroup of GM1 (a chaperone-like effect), so that it accelerates the binding of Aβ to the ganglioside.[118] Thus, cholesterol could first help GM1 to attract Aβ peptides on the cell surface and second stimulate Aβ insertion through a direct Aβ−cholesterol interaction.[73] Such functional cooperation between cholesterol and gangliosides has also been reported for α-synuclein,[32,53] the protein associated with Parkinson's disease that forms α-helical ion channels in membrane bilayers.[122,123] Overall, these data are in line with the notion that Aβ binding and insertion are distinct processes that involve different membrane properties.[104]

THERAPEUTIC STRATEGIES: BEXAROTENE AND BEYOND

There is a growing body of evidence that oligomers, but not fibrillar aggregates, are the main neurotoxic forms of Aβ peptides in the brain of AD patients.[124,125] Among these oligomers, amyloid pore channels than can perturb calcium homeostasis in neural cells probably play a critical role in AD, especially in the early stages of the disease.[73,126] By linking membrane cholesterol to amyloid pore formation,[69] our group has provided a molecular mechanism accounting for the potentiating effects of cholesterol on the toxicity of Aβ peptides. Indeed, one can consider that it is because cholesterol stimulates both the insertion and oligomerization process of Aβ in target cell membranes that any changes in the cholesterol content of these membranes can affect the

neurotoxic potential of Aβ peptides. Moreover, we have shown that it is possible to inhibit the formation and/or function of amyloid pores by preventing the interaction of Aβ peptides with cholesterol.[70] More generally, we assumed that any molecule that can occupy the cholesterol-binding site of Aβ peptides could potentially interfere with amyloid pore formation. We identified several candidate compounds and selected bexarotene, a potential anti-Alzheimer's drug whose mechanism of action is under debate,[127−134] as the first molecule to be tested to validate our hypothesis. The rationale for this choice is that bexarotene and cholesterol share several structural features, including a similar amphipatic organization of a cyclic apolar domain with a small polar part, and a similar conic topology in an apolar milieu such as the plasma membrane. Beyond a real chemical homology, cholesterol and bexarotene can be considered as two compounds of approximately the same size sharing an interesting structural analogy (Figure 10). In agreement with these observations,

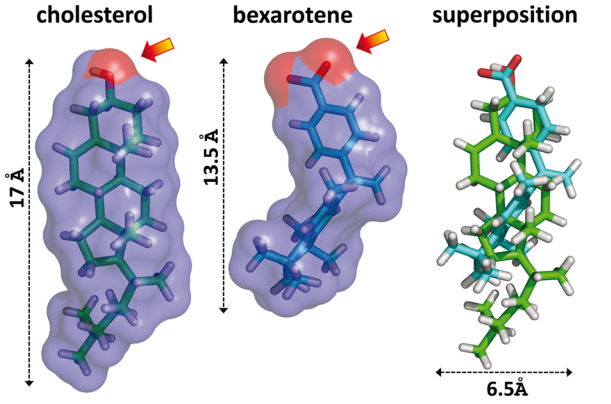

Figure 10. Structural and functional similarities between cholesterol and bexarotene. Both molecules are amphipathic compounds with an apolar polycyclic hydrocarbon domain and a small polar head consisting of a single hydroxyl group for cholesterol and a carboxylic acid for bexarotene (red arrows).

docking of bexarotene on various Aβ peptides (Aβ_{1-42}, Aβ_{17-40}, and Aβ_{25-35}) confirmed that this compound could in each case occupy the cholesterol-binding domain.[70] This is clearly visible for Aβ_{25-35} in the models shown in Figure 11A. In the case of Aβ_{1-42}, bexarotene not only competed with cholesterol for binding to Aβ but also induced an important conformational rearrangement of the peptide so that the cholesterol-binding domain was significantly distorted (Figure 11B,C). Treatment of the cells with nanomolar concentrations of bexarotene efficiently prevented amyloid pore formation by Aβ_{1-42} and Aβ_{25-35}, as shown by the lack of Aβ-induced Ca^{2+} fluxes compared with those under bexarotene-free conditions.[70] It is important to note that in these experiments, the concentration of Ca^{2+} exceeded by more than 5600-fold the concentration of bexarotene (bexarotene is used at 220 nM, whereas calcium is used at 1.25 mM). Thus, we can totally rule out the possibility that bexarotene, because of its carboxylic acid group, could exert its effects by binding to calcium rather than to Aβ peptides.

Taken together, these data demonstrate that bexarotene can bind to Aβ peptides extracellularly and inhibit the cholesterol-driven insertion of these peptides into the plasma membrane, thereby preventing amyloid pore formation. Moreover, the amphipathic structure of bexarotene is consistent with its own insertion within the plasma membrane, so that it could also interact with Aβ peptides in the membrane environment, in

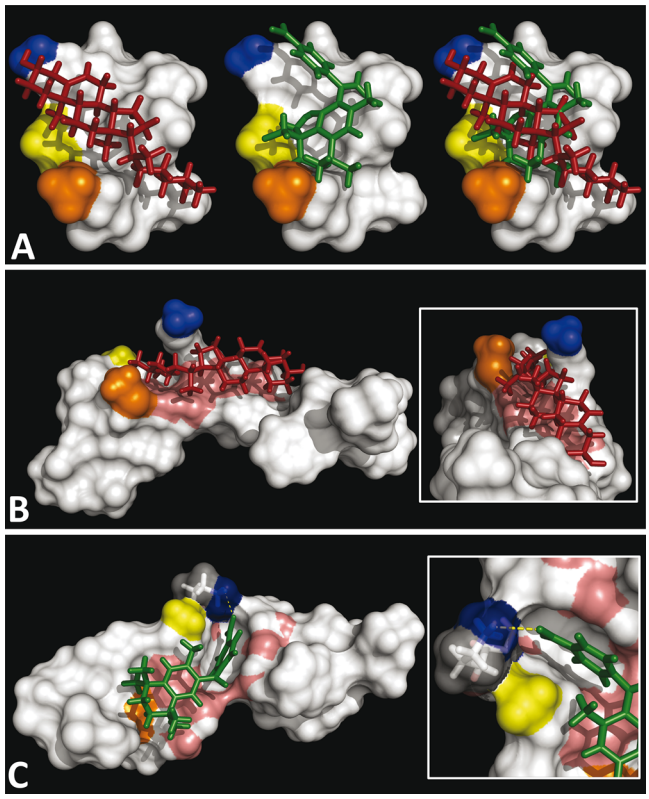

Figure 11. Bexarotene and cholesterol share the same binding site on Aβ. (A) Docking of cholesterol (left) and bexarotene (middle) on Aβ_{25-35}. The superposition of cholesterol and bexarotene on Aβ_{25-35} (right) shows that these compounds may compete for the same binding site. (B and C) Docking of cholesterol and bexarotene, respectively, on Aβ_{1-42}. Cholesterol is colored red, bexarotene green, Lys-28 blue, Ile-31 yellow, and Met-35 orange. An electrostatic bond between Lys-28 and bexarotene (yellow) stabilizes the complex. Bexarotene is predicted to induce a major conformational rearrangement of the cholesterol-binding domain of Aβ_{1-42} (salmon fingerprint). Reproduced from ref 70. Copyright 2014 American Chemical Society.

agreement with monolayer experiments. Drugs that can interfere with the oligomerization of Aβ peptides into annular channels have *de facto* an interesting anti-Alzheimer's potential and could represent an alternative therapeutic strategy for AD.[135,136] The discovery of the neuroprotective activity of bexarotene does not exclude other mechanisms of action such as apoE-mediated Aβ clearance.[127] Nevertheless, its functional resemblance with cholesterol suggests that this drug could prevent the cholesterol-dependent formation of amyloid pores by other protein oligomers, such as the α-synuclein associated with Parkinson's disease.[137]

■ FUTURE STUDIES AND PERSPECTIVES

We are at the onset of developing new therapeutic strategies for the treatment of neurodegenerative diseases. Targeting and/or preventing amyloidogenesis through vaccinal or medication-based therapies has not had the expected success.[135] It is now admitted that curing these disease requires an arsenal of new drugs that can reach the sites of formation of neurotoxic oligomers, especially amyloid channels.[136,138] Understanding how cholesterol interacts with Aβ precursor proteins (APP and C99) and with Aβ peptides has been an important step. Elucidating the role of membrane cholesterol in the oligomerization of Aβ peptides into annular Ca^{2+} channels has been a second

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breakthrough. It is interesting to note that the calcium hypothesis of amyloid toxicity was proposed more than 20 years ago,[139,140] at a time when amyloid fibrils were considered as the main toxic aggregates of Aβ peptides. The time has come to identify, design, and test potential inhibitors of cholesterol binding and/or cholesterol-assisted channel formation that can limit the neurotoxicity of Aβ and other amyloidogenic proteins involved in neurodegenerative diseases. Bexarotene has been the first, but several other candidates can be considered, e.g., bexarotene analogues, amphipathic drugs, cyclic compounds, or aromatic molecules. Computer-assisted drug design[141−143] will be particularly helpful for identifying or designing leading compounds that can occupy cholesterol-binding sites and prevent the oligomerization of various amyloidogenic proteins, including Aβ. In this respect, one should mention 22(R)-hydroxycholesterol (a steroid derivative of cholesterol in the biosynthetic pathway of pregnenolone formation), which has been shown to exhibit neuroprotective properties against Aβ-induced cell death.[144] A radio-assay has demonstrated that 22(R)-hydroxycholesterol binds to Aβ_{1-42} and Aβ_{17-40} peptides. Moreover, docking studies have suggested that the steroid binds to a linear domain encompassing amino acid residues 19−36, which is almost identical to the cholesterol-binding site we have characterized.[68] Accordingly, one could reasonably hypothesize that the protective effects of 22(R)-hydroxycholesterol against Aβ_{1-42} toxicity are, at least in part, inherent to its capacity to lock the cholesterol-binding domain of Aβ.

Now several issues still need to be clarified. Our modeling studies suggest that monomeric Aβ peptides first penetrate the plasma membrane, so that oligomerization occurs in the membrane under the control of key lipids, among which cholesterol plays a critical role. This is likely to happen at the early stages of AD, when the low Aβ:lipid ratio favors the membrane insertion and α-helical folding of Aβ peptides, yet another mechanism could be considered, i.e., the insertion of a preformed β-rich oligomer into the membrane. This could take place later during the course of the disease, when the concentration of Aβ has sufficiently increased to reverse the protein:lipid ratio in favor of the amyloid peptides. Few studies have analyzed the possible involvement of cholesterol in the incorporation of these preformed oligomers into model membranes,[145] so this possibility remains to be assessed. It has also been suggested that Aβ peptides could regulate plasma membrane channels and thus indirectly perturb calcium homeostasis.[146] For instance, the type 1 voltage-dependent anion channel (VDAC) located in the plasma membrane could be the prototype of a family of "amyloid-regulated channels" that could perturb Ca^{2+} homeostasis.[147] This interesting distinction between "amyloid-made" and "amyloid-regulated" channels warrants further consideration.

Another important question that remains to be answered concerns the physiological role of secreted Aβ peptides. The strong propensity of Aβ peptides to form annular pores in the plasma membranes of neural cells suggests that this may be related to a normal physiological function, e.g., an antimicrobial activity.[148−150] Our data, which strongly suggest that cholesterol plays a key role in the formation of these pores, provide a common molecular mechanism of pore formation that takes into account membrane lipids. Our model also predicts that any changes in lipid:cholesterol and/or cholesterol:Aβ ratios could transform a regulated antimicrobial function into a highly cytotoxic process. Thus, it will be interesting to evaluate the impact of a gradual increase in cholesterol levels on amyloid pore formation. Finally, it is known that Aβ_{1-42} peptides strongly interact with ganglioside GM1.[17,151−153] This explains why these peptides are concentrated in lipid raft microdomains of the plasma membrane of neural cells.[154,155] The GM1-binding domain of Aβ has been delineated to a linear fragment encompassing amino acid residues 5−16.[54,118] Thus, this motif contains a pair of contiguous histidine residues, His-13 and His-14, that have been identified as being critical for GM1 binding,[118] yet in the amyloid channel, these residues line the pore mouth, explaining the inhibitory effect of Zn^{2+} cations on channel function.[96] Therefore, it is likely that the oligomerization of Aβ requires a conformational change allowing His-13 and His-14 to leave GM1, so that they can be recruited to form the pore mouth. In this respect, a realistic scenario describing the formation an oligomeric channel by full-length Aβ_{1-42} peptides should include in the beginning both GM1 and cholesterol and explain how these raft lipids cooperate to make possible the formation of a functional channel in a cholesterol- and GM1-rich domain of the plasma membrane. This might not be an easy task, because cholesterol binds not only to Aβ but also to GM1 and influences the conformation of the ganglioside to allow an optimal interaction with Aβ.[51,118] Deciphering this complex molecular ballet involving several distinct lipid species in lipid raft domains[156,157] will probably allow the development of even more therapeutic strategies for Alzheimer's disease and other neurodegenerative diseases.

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Notes

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REFERENCES

(1) Wood, W. G., Li, L., Muller, W. E., and Eckert, G. P. (2014) Cholesterol as a causative factor in Alzheimer's disease: A debatable hypothesis. *J. Neurochem. 129*, 559−572.

(2) Kivipelto, M., Helkala, E. L., Laakso, M. P., Hanninen, T., Hallikainen, M., Alhainen, K., Soininen, H., Tuomilehto, J., and Nissinen, A. (2001) Midlife vascular risk factors and Alzheimer's disease in later life: Longitudinal, population based study. *BMJ [Br. Med. J.] 322*, 1447−1451.

(3) Pappolla, M. A., Bryant-Thomas, T. K., Herbert, D., Pacheco, J., Fabra Garcia, M., Manjon, M., Girones, X., Henry, T. L., Matsubara, E., Zambon, D., Wolozin, B., Sano, M., Cruz-Sanchez, F. F., Thal, L. J., Petanceska, S. S., and Refolo, L. M. (2003) Mild hypercholesterolemia is an early risk factor for the development of Alzheimer amyloid pathology. *Neurology 61*, 199−205.

(4) McGuinness, B., O'Hare, J., Craig, D., Bullock, R., Malouf, R., and Passmore, P. (2013) Cochrane review on 'Statins for the treatment of dementia'. *International Journal of Geriatric Psychiatry 28*, 119−126.

(5) Silva, T., Teixeira, J., Remiao, F., and Borges, F. (2013) Alzheimer's disease, cholesterol, and statins: The junctions of important metabolic pathways. *Angew. Chem., Int. Ed. 52*, 1110−1121.

(6) Simons, M., Keller, P., Dichgans, J., and Schulz, J. B. (2001) Cholesterol and Alzheimer's disease: Is there a link? *Neurology 57*, 1089−1093.

(7) Abramov, A. Y., Ionov, M., Pavlov, E., and Duchen, M. R. (2011) Membrane cholesterol content plays a key role in the neurotoxicity of β-amyloid: Implications for Alzheimer's disease. *Aging Cell*, 595−603.

(8) Fantini, J., Garmy, N., Mahfoud, R., and Yahi, N. (2002) Lipid rafts: Structure, function and role in HIV, Alzheimer's and prion diseases. *Expert Rev. Mol. Med. 4*, 1−22.
(9) Posse de Chaves, E., and Sipione, S. (2010) Sphingolipids and gangliosides of the nervous system in membrane function and dysfunction. *FEBS Lett. 584*, 1748−1759.
(10) van Echten-Deckert, G., and Walter, J. (2012) Sphingolipids: Critical players in Alzheimer's disease. *Prog. Lipid Res. 51*, 378−393.
(11) Hartmann, T., Kuchenbecker, J., and Grimm, M. O. (2007) Alzheimer's disease: The lipid connection. *J. Neurochem. 103* (Suppl. 1), 159−170.
(12) Martins, I. J., Berger, T., Sharman, M. J., Verdile, G., Fuller, S. J., and Martins, R. N. (2009) Cholesterol metabolism and transport in the pathogenesis of Alzheimer's disease. *J. Neurochem. 111*, 1275−1308.
(13) Laufs, U., Gertz, K., Dirnagl, U., Bohm, M., Nickenig, G., and Endres, M. (2002) Rosuvastatin, a new HMG-CoA reductase inhibitor, upregulates endothelial nitric oxide synthase and protects from ischemic stroke in mice. *Brain Res. 942*, 23−30.
(14) Mohmmad Abdul, H., Wenk, G. L., Gramling, M., Hauss-Wegrzyniak, B., and Butterfield, D. A. (2004) APP and PS-1 mutations induce brain oxidative stress independent of dietary cholesterol: Implications for Alzheimer's disease. *Neurosci. Lett. 368*, 148−150.
(15) Harris, J. R., and Milton, N. G. (2010) Cholesterol in Alzheimer's disease and other amyloidogenic disorders. *Subcell. Biochem. 51*, 47−75.
(16) Yanagisawa, K. (2007) Role of gangliosides in Alzheimer's disease. *Biochim. Biophys. Acta 1768*, 1943−1951.
(17) Yanagisawa, K. (2011) Pathological significance of ganglioside clusters in Alzheimer's disease. *J. Neurochem. 116*, 806−812.
(18) Greenwald, J., and Riek, R. (2010) Biology of amyloid: Structure, function, and regulation. *Structure 18*, 1244−1260.
(19) Dasilva, K. A., Shaw, J. E., and McLaurin, J. (2010) Amyloid-β fibrillogenesis: Structural insight and therapeutic intervention. *Exp. Neurol. 223*, 311−321.
(20) Kanekiyo, T., Xu, H., and Bu, G. (2014) ApoE and Aβ in Alzheimer's disease: Accidental encounters or partners? *Neuron 81*, 740−754.
(21) Yanagisawa, K. (2005) Cholesterol and amyloid β fibrillogenesis. *Subcell. Biochem. 38*, 179−202.
(22) Li, H., and Papadopoulos, V. (1998) Peripheral-type benzodiazepine receptor function in cholesterol transport. Identification of a putative cholesterol recognition/interaction amino acid sequence and consensus pattern. *Endocrinology 139*, 4991−4997.
(23) Baier, C. J., Fantini, J., and Barrantes, F. J. (2011) Disclosure of cholesterol recognition motifs in transmembrane domains of the human nicotinic acetylcholine receptor. *Sci. Rep. 1*, 69.
(24) Fantini, J., and Barrantes, F. J. (2013) How cholesterol interacts with membrane proteins: An exploration of cholesterol-binding sites including CRAC, CARC, and tilted domains. *Front. Physiol. 4*, 31.
(25) Fantini, J., and Barrantes, F. J. (2009) Sphingolipid/cholesterol regulation of neurotransmitter receptor conformation and function. *Biochim. Biophys. Acta 1788*, 2345−2361.
(26) Rosenhouse-Dantsker, A., Noskov, S., Durdagi, S., Logothetis, D. E., and Levitan, I. (2013) Identification of novel cholesterol-binding regions in Kir2 channels. *J. Biol. Chem. 288*, 31154−31164.
(27) Levitan, I., Singh, D. K., and Rosenhouse-Dantsker, A. (2014) Cholesterol binding to ion channels. *Front. Physiol. 5*, 65.
(28) Nishio, M., Umezawa, Y., Fantini, J., Weiss, M. S., and Chakrabarti, P. (2014) CH/π hydrogen bonds in biological macromolecules. *Phys. Chem. Chem. Phys. 16*, 12648−12683.
(29) Lahdo, R., Coillet-Matillon, S., Chauvet, J. P., and de La Fourniere-Bessueille, L. (2002) The amyloid precursor protein interacts with neutral lipids. *Eur. J. Biochem. 269*, 2238−2246.
(30) Seelig, A. (1987) Local anesthetics and pressure: A comparison of dibucaine binding to lipid monolayers and bilayers. *Biochim. Biophys. Acta 899*, 196−204.
(31) Thakur, G., Pao, C., Micic, M., Johnson, S., and Leblanc, R. M. (2011) Surface chemistry of lipid raft and amyloid Abeta (1−40) Langmuir monolayer. *Colloids Surf., B 87*, 369−377.
(32) Fantini, J., Carlus, D., and Yahi, N. (2011) The fusogenic tilted peptide (67−78) of α-synuclein is a cholesterol binding domain. *Biochim. Biophys. Acta 1808*, 2343−2351.
(33) Hammache, D., Yahi, N., Pieroni, G., Ariasi, F., Tamalet, C., and Fantini, J. (1998) Sequential interaction of CD4 and HIV-1 gp120 with a reconstituted membrane patch of ganglioside GM3: Implications for the role of glycolipids as potential HIV-1 fusion cofactors. *Biochem. Biophys. Res. Commun. 246*, 117−122.
(34) Aubert-Jousset, E., Garmy, N., Sbarra, V., Fantini, J., Sadoulet, M. O., and Lombardo, D. (2004) The combinatorial extension method reveals a sphingolipid binding domain on pancreatic bile salt-dependent lipase: Role in secretion. *Structure 12*, 1437−1447.
(35) Chakrabandhu, K., Huault, S., Garmy, N., Fantini, J., Stebe, E., Mailfert, S., Marguet, D., and Hueber, A. O. (2008) The extracellular glycosphingolipid-binding motif of Fas defines its internalization route, mode and outcome of signals upon activation by ligand. *Cell Death Differ. 15*, 1824−1837.
(36) Taieb, N., Maresca, M., Guo, X. J., Garmy, N., Fantini, J., and Yahi, N. (2009) The first extracellular domain of the tumour stem cell marker CD133 contains an antigenic ganglioside-binding motif. *Cancer Lett. 278*, 164−173.
(37) Hamel, S., Fantini, J., and Schweisguth, F. (2010) Notch ligand activity is modulated by glycosphingolipid membrane composition in *Drosophila melanogaster*. *J. Cell Biol. 188*, 581−594.
(38) Chattopadhyay, A., Paila, Y. D., Shrivastava, S., Tiwari, S., Singh, P., and Fantini, J. (2012) Sphingolipid-binding domain in the serotonin(1A) receptor. *Adv. Exp. Med. Biol. 749*, 279−293.
(39) Heuss, S. F., Tarantino, N., Fantini, J., Ndiaye-Lobry, D., Moretti, J., Israel, A., and Logeat, F. (2013) A glycosphingolipid binding domain controls trafficking and activity of the mammalian notch ligand delta-like 1. *PLoS One 8*, e74392.
(40) Hammache, D., Pieroni, G., Yahi, N., Delezay, O., Koch, N., Lafont, H., Tamalet, C., and Fantini, J. (1998) Specific interaction of HIV-1 and HIV-2 surface envelope glycoproteins with monolayers of galactosylceramide and ganglioside GM3. *J. Biol. Chem. 273*, 7967−7971.
(41) Fantini, J., Hammache, D., Pieroni, G., and Yahi, N. (2000) Role of glycosphingolipid microdomains in CD4-dependent HIV-1 fusion. *Glycoconjugate J. 17*, 199−204.
(42) Fantini, J., Maresca, M., Hammache, D., Yahi, N., and Delezay, O. (2000) Glycosphingolipid (GSL) microdomains as attachment platforms for host pathogens and their toxins on intestinal epithelial cells: Activation of signal transduction pathways and perturbations of intestinal absorption and secretion. *Glycoconjugate J. 17*, 173−179.
(43) Hammache, D., Pieroni, G., Maresca, M., Ivaldi, S., Yahi, N., and Fantini, J. (2000) Reconstitution of sphingolipid-cholesterol plasma membrane microdomains for studies of virus-glycolipid interactions. *Methods Enzymol. 312*, 495−506.
(44) Mahfoud, R., Mylvaganam, M., Lingwood, C. A., and Fantini, J. (2002) A novel soluble analog of the HIV-1 fusion cofactor, globotriaosylceramide (Gb(3)), eliminates the cholesterol requirement for high affinity gp120/Gb(3) interaction. *J. Lipid Res. 43*, 1670−1679.
(45) Fantini, J. (2003) How sphingolipids bind and shape proteins: Molecular basis of lipid-protein interactions in lipid shells, rafts and related biomembrane domains. *Cell. Mol. Life Sci. 60*, 1027−1032.
(46) Fantini, J., Garmy, N., and Yahi, N. (2006) Prediction of glycolipid-binding domains from the amino acid sequence of lipid raft-associated proteins: Application to HpaA, a protein involved in the adhesion of *Helicobacter pylori* to gastrointestinal cells. *Biochemistry 45*, 10957−10962.
(47) Mahfoud, R., Garmy, N., Maresca, M., Yahi, N., Puigserver, A., and Fantini, J. (2002) Identification of a common sphingolipid-binding domain in Alzheimer, prion, and HIV-1 proteins. *J. Biol. Chem. 277*, 11292−11296.
(48) Taieb, N., Yahi, N., and Fantini, J. (2004) Rafts and related glycosphingolipid-enriched microdomains in the intestinal epithelium: Bacterial targets linked to nutrient absorption. *Adv. Drug Delivery Rev. 56*, 779−794.

(49) Levy, M., Garmy, N., Gazit, E., and Fantini, J. (2006) The minimal amyloid-forming fragment of the islet amyloid polypeptide is a glycolipid-binding domain. *FEBS J. 273*, 5724−5735.
(50) Fantini, J. (2007) Interaction of proteins with lipid rafts through glycolipid-binding domains: Biochemical background and potential therapeutic applications. *Curr. Med. Chem. 14*, 2911−2917.
(51) Yahi, N., Aulas, A., and Fantini, J. (2010) How cholesterol constrains glycolipid conformation for optimal recognition of Alzheimer's β amyloid peptide (Aβ1−40). *PLoS One 5*, e9079.
(52) Mazargui, H., Leveque, C., Bartnik, D., Fantini, J., Gouget, T., Melone, M. A., Funke, S. A., Willbold, D., and Perrone, L. (2012) A synthetic amino acid substitution of Tyr10 in Aβ peptide sequence yields a dominant negative variant in amyloidogenesis. *Aging Cell 11*, 530−541.
(53) Fantini, J., and Yahi, N. (2013) The driving force of α-synuclein insertion and amyloid channel formation in the plasma membrane of neural cells: Key role of ganglioside- and cholesterol-binding domains. *Adv. Exp. Med. Biol. 991*, 15−26.
(54) Fantini, J., and Yahi, N. (2011) Molecular basis for the glycosphingolipid-binding specificity of α-synuclein: Key role of tyrosine 39 in membrane insertion. *J. Mol. Biol. 408*, 654−669.
(55) Beel, A. J., Sakakura, M., Barrett, P. J., and Sanders, C. R. (2010) Direct binding of cholesterol to the amyloid precursor protein: An important interaction in lipid-Alzheimer's disease relationships? *Biochim. Biophys. Acta 1801*, 975−982.
(56) Barrett, P. J., Song, Y., Van Horn, W. D., Hustedt, E. J., Schafer, J. M., Hadziselimovic, A., Beel, A. J., and Sanders, C. R. (2012) The amyloid precursor protein has a flexible transmembrane domain and binds cholesterol. *Science 336*, 1168−1171.
(57) Beel, A. J., Mobley, C. K., Kim, H. J., Tian, F., Hadziselimovic, A., Jap, B., Prestegard, J. H., and Sanders, C. R. (2008) Structural studies of the transmembrane C-terminal domain of the amyloid precursor protein (APP): Does APP function as a cholesterol sensor? *Biochemistry 47*, 9428−9446.
(58) Wolozin, B. (2001) A fluid connection: Cholesterol and Aβ. *Proc. Natl. Acad. Sci. U.S.A. 98*, 5371−5373.
(59) Chauhan, N. B. (2003) Membrane dynamics, cholesterol homeostasis, and Alzheimer's disease. *J. Lipid Res. 44*, 2019−2029.
(60) Johnson, R. M., Rath, A., Melnyk, R. A., and Deber, C. M. (2006) Lipid solvation effects contribute to the affinity of Gly-xxx-Gly motif-mediated helix-helix interactions. *Biochemistry 45*, 8507−8515.
(61) Fassbender, K., Simons, M., Bergmann, C., Stroick, M., Lutjohann, D., Keller, P., Runz, H., Kuhl, S., Bertsch, T., von Bergmann, K., Hennerici, M., Beyreuther, K., and Hartmann, T. (2001) Simvastatin strongly reduces levels of Alzheimer's disease β-amyloid peptides Aβ42 and Aβ40 in vitro and in vivo. *Proc. Natl. Acad. Sci. U.S.A. 98*, 5856−5861.
(62) Kojro, E., Gimpl, G., Lammich, S., Marz, W., and Fahrenholz, F. (2001) Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the α-secretase ADAM 10. *Proc. Natl. Acad. Sci. U.S.A. 98*, 5815−5820.
(63) Arispe, N., and Doh, M. (2002) Plasma membrane cholesterol controls the cytotoxicity of Alzheimer's disease AβP(1−40) and (1−42) peptides. *FASEB J. 16*, 1526−1536.
(64) Avdulov, N. A., Chochina, S. V., Igbavboa, U., Warden, C. S., Vassiliev, A. V., and Wood, W. G. (1997) Lipid binding to amyloid β-peptide aggregates: Preferential binding of cholesterol as compared with phosphatidylcholine and fatty acids. *J. Neurochem. 69*, 1746−1752.
(65) Harris, J. R. (2008) Cholesterol binding to amyloid-β fibrils: A TEM study. *Micron 39*, 1192−1196.
(66) Qiu, L., Buie, C., Reay, A., Vaughn, M. W., and Cheng, K. H. (2011) Molecular dynamics simulations reveal the protective role of cholesterol in β-amyloid protein-induced membrane disruptions in neuronal membrane mimics. *J. Phys. Chem. B 115*, 9795−9812.
(67) Yu, X., and Zheng, J. (2012) Cholesterol promotes the interaction of Alzheimer β-amyloid monomer with lipid bilayer. *J. Mol. Biol. 421*, 561−571.
(68) Di Scala, C., Yahi, N., Lelievre, C., Garmy, N., Chahinian, H., and Fantini, J. (2013) Biochemical identification of a linear cholesterol-binding domain within Alzheimer's β amyloid peptide. *ACS Chem. Neurosci. 4*, 509−517.
(69) Di Scala, C., Troadec, J. D., Lelievre, C., Garmy, N., Fantini, J., and Chahinian, H. (2014) Mechanism of cholesterol-assisted oligomeric channel formation by a short Alzheimer β-amyloid peptide. *J. Neurochem. 128*, 186−195.
(70) Fantini, J., Di Scala, C., Yahi, N., Troadec, J. D., Sadelli, K., Chahinian, H., and Garmy, N. (2014) Bexarotene blocks calcium-permeable ion channels formed by neurotoxic Alzheimer's β-amyloid peptides. *ACS Chem. Neurosci. 5*, 216−224.
(71) Mobley, D. L., Cox, D. L., Singh, R. R., Maddox, M. W., and Longo, M. L. (2004) Modeling amyloid β-peptide insertion into lipid bilayers. *Biophys. J. 86*, 3585−3597.
(72) Uversky, V. N. (2009) Intrinsic disorder in proteins associated with neurodegenerative diseases. *Front. Biosci., Landmark Ed. 14*, 5188−5238.
(73) Fantini, J., and Yahi, N. (2010) Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: Common mechanisms in neurodegenerative diseases. *Expert Rev. Mol. Med. 12*, e27.
(74) Kallberg, Y., Gustafsson, M., Persson, B., Thyberg, J., and Johansson, J. (2001) Prediction of amyloid fibril-forming proteins. *J. Biol. Chem. 276*, 12945−12950.
(75) Ji, S. R., Wu, Y., and Sui, S. F. (2002) Cholesterol is an important factor affecting the membrane insertion of β-amyloid peptide (Aβ1−40), which may potentially inhibit the fibril formation. *J. Biol. Chem. 277*, 6273−6279.
(76) Cecchi, C., and Stefani, M. (2013) The amyloid-cell membrane system. The interplay between the biophysical features of oligomers/fibrils and cell membrane defines amyloid toxicity. *Biophys. Chem. 182*, 30−43.
(77) Lashuel, H. A., and Lansbury, P. T., Jr. (2006) Are amyloid diseases caused by protein aggregates that mimic bacterial pore-forming toxins? *Q. Rev. Biophys. 39*, 167−201.
(78) Zhao, L. N., Long, H., Mu, Y., and Chew, L. Y. (2012) The Toxicity of Amyloid β Oligomers. *Int. J. Mol. Sci. 13*, 7303−7327.
(79) Kagan, B. L., Azimov, R., and Azimova, R. (2004) Amyloid peptide channels. *J. Membr. Biol. 202*, 1−10.
(80) Ono, K., and Yamada, M. (2011) Low-n oligomers as therapeutic targets of Alzheimer's disease. *J. Neurochem. 117*, 19−28.
(81) Lin, H., Bhatia, R., and Lal, R. (2001) Amyloid β protein forms ion channels: Implications for Alzheimer's disease pathophysiology. *FASEB J. 15*, 2433−2444.
(82) Arispe, N., Pollard, H. B., and Rojas, E. (1996) Zn^{2+} interaction with Alzheimer amyloid β protein calcium channels. *Proc. Natl. Acad. Sci. U.S.A. 93*, 1710−1715.
(83) Strandberg, E., and Killian, J. A. (2003) Snorkeling of lysine side chains in transmembrane helices: How easy can it get? *FEBS Lett. 544*, 69−73.
(84) Gleason, N. J., Vostrikov, V. V., Greathouse, D. V., and Koeppe, R. E., II (2013) Buried lysine, but not arginine, titrates and alters transmembrane helix tilt. *Proc. Natl. Acad. Sci. U.S.A. 110*, 1692−1695.
(85) Shafrir, Y., Durell, S., Arispe, N., and Guy, H. R. (2010) Models of membrane-bound Alzheimer's Aβ peptide assemblies. *Proteins 78*, 3473−3487.
(86) Durell, S. R., Guy, H. R., Arispe, N., Rojas, E., and Pollard, H. B. (1994) Theoretical models of the ion channel structure of amyloid β-protein. *Biophys. J. 67*, 2137−2145.
(87) Jang, H., Zheng, J., and Nussinov, R. (2007) Models of β-amyloid ion channels in the membrane suggest that channel formation in the bilayer is a dynamic process. *Biophys. J. 93*, 1938−1949.
(88) Jang, H., Arce, F. T., Capone, R., Ramachandran, S., Lal, R., and Nussinov, R. (2009) Misfolded amyloid ion channels present mobile β-sheet subunits in contrast to conventional ion channels. *Biophys. J. 97*, 3029−3037.
(89) Jang, H., Arce, F. T., Ramachandran, S., Capone, R., Azimova, R., Kagan, B. L., Nussinov, R., and Lal, R. (2010) Truncated β-amyloid peptide channels provide an alternative mechanism for Alzheimer's

disease and Down syndrome. *Proc. Natl. Acad. Sci. U.S.A. 107*, 6538−6543.
(90) Arispe, N., Diaz, J., Durell, S. R., Shafrir, Y., and Guy, H. R. (2010) Polyhistidine peptide inhibitor of the Aβ calcium channel potently blocks the Aβ-induced calcium response in cells. Theoretical modeling suggests a cooperative binding process. *Biochemistry 49*, 7847−7853.
(91) Strodel, B., Lee, J. W., Whittleston, C. S., and Wales, D. J. (2010) Transmembrane structures for Alzheimer's Aβ(1−42) oligomers. *J. Am. Chem. Soc. 132*, 13300−13312.
(92) Qiu, L., Lewis, A., Como, J., Vaughn, M. W., Huang, J., Somerharju, P., Virtanen, J., and Cheng, K. H. (2009) Cholesterol modulates the interaction of β-amyloid peptide with lipid bilayers. *Biophys. J. 96*, 4299−4307.
(93) Micelli, S., Meleleo, D., Picciarelli, V., and Gallucci, E. (2004) Effect of sterols on β-amyloid peptide (AβP 1−40) channel formation and their properties in planar lipid membranes. *Biophys. J. 86*, 2231−2237.
(94) Meleleo D., Notarachille G., Gallucci, E., and Micelli, S. (2009) Cholesterol modulates amyloid beta peptide 1−42 channel formation in planar lipid membranes. In *New Trends in Alzheimer and Parkinson Related Disorders: ADPD 2009, Medimond International Proceedings*, Fisher, A., Hanin, I., Eds.; MEDIMOND S.r.l.: Bologna, Italy, pp. 167−171.
(95) Lal, R., Lin, H., and Quist, A. P. (2007) Amyloid β ion channel: 3D structure and relevance to amyloid channel paradigm. *Biochim. Biophys. Acta 1768*, 1966−1975.
(96) Diaz, J. C., Linnehan, J., Pollard, H., and Arispe, N. (2006) Histidines 13 and 14 in the Aβ sequence are targets for inhibition of Alzheimer's disease Aβ ion channel and cytotoxicity. *Biol. Res. 39*, 447−460.
(97) Zhu, M., Han, S., Zhou, F., Carter, S. A., and Fink, A. L. (2004) Annular oligomeric amyloid intermediates observed by in situ atomic force microscopy. *J. Biol. Chem. 279*, 24452−24459.
(98) Luhrs, T., Ritter, C., Adrian, M., Riek-Loher, D., Bohrmann, B., Dobeli, H., Schubert, D., and Riek, R. (2005) 3D structure of Alzheimer's amyloid-β(1−42) fibrils. *Proc. Natl. Acad. Sci. U.S.A. 102*, 17342−17347.
(99) Jang, H., Zheng, J., Lal, R., and Nussinov, R. (2008) New structures help the modeling of toxic amyloid β ion channels. *Trends Biochem. Sci. 33*, 91−100.
(100) Zhou, X., and Xu, J. (2012) Free cholesterol induces higher β-sheet content in Aβ peptide oligomers by aromatic interaction with Phe19. *PLoS One 7*, e46245.
(101) Yuyama, K., and Yanagisawa, K. (2010) Sphingomyelin accumulation provides a favorable milieu for GM1 ganglioside-induced assembly of amyloid β-protein. *Neurosci. Lett. 481*, 168−172.
(102) Coles, M., Bicknell, W., Watson, A. A., Fairlie, D. P., and Craik, D. J. (1998) Solution structure of amyloid β-peptide(1−40) in a water-micelle environment. Is the membrane-spanning domain where we think it is? *Biochemistry 37*, 11064−11077.
(103) Shao, H., Jao, S., Ma, K., and Zagorski, M. G. (1999) Solution structures of micelle-bound amyloid β-(1−40) and β-(1−42) peptides of Alzheimer's disease. *J. Mol. Biol. 285*, 755−773.
(104) Wong, P. T., Schauerte, J. A., Wisser, K. C., Ding, H., Lee, E. L., Steel, D. G., and Gafni, A. (2009) Amyloid-β membrane binding and permeabilization are distinct processes influenced separately by membrane charge and fluidity. *J. Mol. Biol. 386*, 81−96.
(105) Zhorov, B. S., and Ananthanarayanan, V. S. (1996) Structural model of a synthetic Ca^{2+} channel with bound Ca^{2+} ions and dihydropyridine ligand. *Biophys. J. 70*, 22−37.
(106) Gondre-Lewis, M. C., Petrache, H. I., Wassif, C. A., Harries, D., Parsegian, A., Porter, F. D., and Loh, Y. P. (2006) Abnormal sterols in cholesterol-deficiency diseases cause secretory granule malformation and decreased membrane curvature. *J. Cell Sci. 119*, 1876−1885.
(107) Ivankin, A., Kuzmenko, I., and Gidalevitz, D. (2012) Cholesterol mediates membrane curvature during fusion events. *Phys. Rev. Lett. 108*, 238103.
(108) Yesylevskyy, S. O., Demchenko, A. P., Kraszewski, S., and Ramseyer, C. (2013) Cholesterol induces uneven curvature of asymmetric lipid bilayers. *Sci. World J. 2013*, 965230.
(109) Barenholz, Y. (2002) Cholesterol and other membrane active sterols: From membrane evolution to "rafts". *Prog. Lipid Res. 41*, 1−5.
(110) Kawahara, M., and Kuroda, Y. (2000) Molecular mechanism of neurodegeneration induced by Alzheimer's β-amyloid protein: Channel formation and disruption of calcium homeostasis. *Brain Res. Bull. 53*, 389−397.
(111) Kawahara, M., and Kuroda, Y. (2001) Intracellular calcium changes in neuronal cells induced by Alzheimer's β-amyloid protein are blocked by estradiol and cholesterol. *Cell. Mol. Neurobiol. 21*, 1−13.
(112) Mirzabekov, T. A., Lin, M. C., and Kagan, B. L. (1996) Pore formation by the cytotoxic islet amyloid peptide amylin. *J. Biol. Chem. 271*, 1988−1992.
(113) Yeagle, P. L. (1991) Modulation of membrane function by cholesterol. *Biochimie 73*, 1303−1310.
(114) Curtain, C. C., Ali, F. E., Smith, D. G., Bush, A. I., Masters, C. L., and Barnham, K. J. (2003) Metal ions, pH, and cholesterol regulate the interactions of Alzheimer's disease amyloid-β peptide with membrane lipid. *J. Biol. Chem. 278*, 2977−2982.
(115) Zhou, Y., and Richardson, J. S. (1996) Cholesterol protects PC12 cells from β-amyloid induced calcium disordering and cytotoxicity. *NeuroReport 7*, 2487−2490.
(116) Yip, C. M., Elton, E. A., Darabie, A. A., Morrison, M. R., and McLaurin, J. (2001) Cholesterol, a modulator of membrane-associated Aβ-fibrillogenesis and neurotoxicity. *J. Mol. Biol. 311*, 723−734.
(117) Lin, M. C., and Kagan, B. L. (2002) Electrophysiologic properties of channels induced by Aβ25−35 in planar lipid bilayers. *Peptides 23*, 1215−1228.
(118) Fantini, J., Yahi, N., and Garmy, N. (2013) Cholesterol accelerates the binding of Alzheimer's β-amyloid peptide to ganglioside GM1 through a universal hydrogen-bond-dependent sterol tuning of glycolipid conformation. *Front. Physiol. 4*, 120.
(119) Schnaar, R. L., Gerardy-Schahn, R., and Hildebrandt, H. (2014) Sialic acids in the brain: Gangliosides and polysialic acid in nervous system development, stability, disease, and regeneration. *Physiol. Rev. 94*, 461−518.
(120) Radhakrishnan, A., Anderson, T. G., and McConnell, H. M. (2000) Condensed complexes, rafts, and the chemical activity of cholesterol in membranes. *Proc. Natl. Acad. Sci. U.S.A. 97*, 12422−12427.
(121) Rondelli, V., Fragneto, G., Motta, S., Del Favero, E., Brocca, P., Sonnino, S., and Cantu, L. (2012) Ganglioside GM1 forces the redistribution of cholesterol in a biomimetic membrane. *Biochim. Biophys. Acta 1818*, 2860−2867.
(122) Zakharov, S. D., Hulleman, J. D., Dutseva, E. A., Antonenko, Y. N., Rochet, J. C., and Cramer, W. A. (2007) Helical α-synuclein forms highly conductive ion channels. *Biochemistry 46*, 14369−14379.
(123) Di Pasquale, E., Fantini, J., Chahinian, H., Maresca, M., Taieb, N., and Yahi, N. (2010) Altered ion channel formation by the Parkinson's-disease-linked E46K mutant of α-synuclein is corrected by GM3 but not by GM1 gangliosides. *J. Mol. Biol. 397*, 202−218.
(124) Glabe, C. G. (2008) Structural classification of toxic amyloid oligomers. *J. Biol. Chem. 283*, 29639−29643.
(125) Esparza, T. J., Zhao, H., Cirrito, J. R., Cairns, N. J., Bateman, R. J., Holtzman, D. M., and Brody, D. L. (2013) Amyloid-β oligomerization in Alzheimer dementia versus high-pathology controls. *Ann. Neurol. 73*, 104−119.
(126) Shirwany, N. A., Payette, D., Xie, J., and Guo, Q. (2007) The amyloid β ion channel hypothesis of Alzheimer's disease. *Neuropsychiatr. Dis. Treat. 3*, 597−612.
(127) Cramer, P. E., Cirrito, J. R., Wesson, D. W., Lee, C. Y., Karlo, J. C., Zinn, A. E., Casali, B. T., Restivo, J. L., Goebel, W. D., James, M. J., Brunden, K. R., Wilson, D. A., and Landreth, G. E. (2012) ApoE-directed therapeutics rapidly clear β-amyloid and reverse deficits in AD mouse models. *Science 335*, 1503−1506.
(128) Price, A. R., Xu, G., Siemienski, Z. B., Smithson, L. A., Borchelt, D. R., Golde, T. E., and Felsenstein, K. M. (2013) Comment on "ApoE-

directed therapeutics rapidly clear β-amyloid and reverse deficits in AD mouse models". *Science 340*, 924-e.
(129) LaFerla, F. M. (2012) Preclinical success against Alzheimer's disease with an old drug. *N. Engl. J. Med. 367*, 570−572.
(130) Fitz, N. F., Cronican, A. A., Lefterov, I., and Koldamova, R. (2013) Comment on "ApoE-directed therapeutics rapidly clear β-amyloid and reverse deficits in AD mouse models". *Science 340*, 924-c.
(131) Landreth, G. E., Cramer, P. E., Lakner, M. M., Cirrito, J. R., Wesson, D. W., Brunden, K. R., and Wilson, D. A. (2013) Response to comments on "ApoE-directed therapeutics rapidly clear β-amyloid and reverse deficits in AD mouse models". *Science 340*, 924-g.
(132) Tesseur, I., Lo, A. C., Roberfroid, A., Dietvorst, S., Van Broeck, B., Borgers, M., Gijsen, H., Moechars, D., Mercken, M., Kemp, J., D'Hooge, R., and De Strooper, B. (2013) Comment on "ApoE-directed therapeutics rapidly clear β-amyloid and reverse deficits in AD mouse models". *Science 340*, 924-e.
(133) Veeraraghavalu, K., Zhang, C., Miller, S., Hefendehl, J. K., Rajapaksha, T. W., Ulrich, J., Jucker, M., Holtzman, D. M., Tanzi, R. E., Vassar, R., and Sisodia, S. S. (2013) Comment on "ApoE-directed therapeutics rapidly clear β-amyloid and reverse deficits in AD mouse models". *Science 340*, 924-f.
(134) LaClair, K. D., Manaye, K. F., Lee, D. L., Allard, J. S., Savonenko, A. V., Troncoso, J. C., and Wong, P. C. (2013) Treatment with bexarotene, a compound that increases apolipoprotein-E, provides no cognitive benefit in mutant APP/PS1 mice. *Mol. Neurodegener. 8*, 18.
(135) Gandy, S., and DeKosky, S. T. (2013) Toward the treatment and prevention of Alzheimer's disease: Rational strategies and recent progress. *Annu. Rev. Med. 64*, 367−383.
(136) Jang, H., Connelly, L., Arce, F. T., Ramachandran, S., Lal, R., Kagan, B. L., and Nussinov, R. (2013) Alzheimer's disease: Which type of amyloid-preventing drug agents to employ? *Phys. Chem. Chem. Phys. 15*, 8868−8877.
(137) McFarland, K., Spalding, T. A., Hubbard, D., Ma, J. N., Olsson, R., and Burstein, E. S. (2013) Low dose bexarotene treatment rescues dopamine neurons and restores behavioral function in models of Parkinson's disease. *ACS Chem. Neurosci. 4*, 1430−1438.
(138) Malchiodi-Albedi, F., Paradisi, S., Matteucci, A., Frank, C., and Diociaiuti, M. (2011) Amyloid oligomer neurotoxicity, calcium dysregulation, and lipid rafts. *Int. J. Alzheimer's Dis. 2011*, 906964.
(139) Pollard, H. B., Rojas, E., and Arispe, N. (1993) A new hypothesis for the mechanism of amyloid toxicity, based on the calcium channel activity of amyloid β protein (AβP) in phospholipid bilayer membranes. *Ann. N.Y. Acad. Sci. 695*, 165−168.
(140) Arispe, N., Pollard, H. B., and Rojas, E. (1994) β-Amyloid Ca^{2+}-channel hypothesis for neuronal death in Alzheimer disease. *Mol. Cell. Biochem. 140*, 119−125.
(141) Shaikh, S. A., Li, J., Enkavi, G., Wen, P. C., Huang, Z., and Tajkhorshid, E. (2013) Visualizing functional motions of membrane transporters with molecular dynamics simulations. *Biochemistry 52*, 569−587.
(142) Brooks, B. R., Brooks, C. L., III, Mackerell, A. D., Jr., Nilsson, L., Petrella, R. J., Roux, B., Won, Y., Archontis, G., Bartels, C., Boresch, S., Caflisch, A., Caves, L., Cui, Q., Dinner, A. R., Feig, M., Fischer, S., Gao, J., Hodoscek, M., Im, W., Kuczera, K., Lazaridis, T., Ma, J., Ovchinnikov, V., Paci, E., Pastor, R. W., Post, C. B., Pu, J. Z., Schaefer, M., Tidor, B., Venable, R. M., Woodcock, H. L., Wu, X., Yang, W., York, D. M., and Karplus, M. (2009) CHARMM: The biomolecular simulation program. *J. Comput. Chem. 30*, 1545−1614.
(143) Singh, R. P., Brooks, B. R., and Klauda, J. B. (2009) Binding and release of cholesterol in the Osh4 protein of yeast. *Proteins 75*, 468−477.
(144) Yao, Z. X., Brown, R. C., Teper, G., Greeson, J., and Papadopoulos, V. (2002) 22R-Hydroxycholesterol protects neuronal cells from β-amyloid-induced cytotoxicity by binding to β-amyloid peptide. *J. Neurochem. 83*, 1110−1119.
(145) Ashley, R. H., Harroun, T. A., Hauss, T., Breen, K. C., and Bradshaw, J. P. (2006) Autoinsertion of soluble oligomers of Alzheimer's Aβ(1−42) peptide into cholesterol-containing membranes is accompanied by relocation of the sterol towards the bilayer surface. *BMC Struct. Biol. 6*, 21.
(146) Thinnes, F. P. (2014) Concerning a Clue on the Enigma of "Amyloid Made" versus "Amyloid Regulated" Channels in Cell Membranes: A Comment on M. A. Mukhamedyarov et al., Neuroscience and Behavioral Physiology, Vol. 43, No. 4, pp 479−484 (2013). *Neuroscience and Behavioral Physiology 44*, 125−126.
(147) Thinnes, F. P. (2013) On a solution to the riddle of "amyloid made" versus "amyloid regulated" > channels in cell membranes. *Letter to the Editor of JAD*, September 1, 2013.
(148) Soscia, S. J., Kirby, J. E., Washicosky, K. J., Tucker, S. M., Ingelsson, M., Hyman, B., Burton, M. A., Goldstein, L. E., Duong, S., Tanzi, R. E., and Moir, R. D. (2010) The Alzheimer's disease-associated amyloid β-protein is an antimicrobial peptide. *PLoS One 5*, e9505.
(149) Last, N. B., and Miranker, A. D. (2013) Common mechanism unites membrane poration by amyloid and antimicrobial peptides. *Proc. Natl. Acad. Sci. U.S.A. 110*, 6382−6387.
(150) Kagan, B. L., Jang, H., Capone, R., Teran Arce, F., Ramachandran, S., Lal, R., and Nussinov, R. (2012) Antimicrobial properties of amyloid peptides. *Mol. Pharmaceutics 9*, 708−717.
(151) Yanagisawa, K., Fantini, J., Chakrabartty, A., and Eckert, A. (2011) Aβ behavior on neuronal membranes: Aggregation and toxicities. *Int. J. Alzheimer's Dis. 2011*, 286536.
(152) Kakio, A., Nishimoto, S. I., Yanagisawa, K., Kozutsumi, Y., and Matsuzaki, K. (2001) Cholesterol-dependent formation of GM1 ganglioside-bound amyloid β-protein, an endogenous seed for Alzheimer amyloid. *J. Biol. Chem. 276*, 24985−24990.
(153) Matsubara, T., Iijima, K., Yamamoto, N., Yanagisawa, K., and Sato, T. (2013) Density of GM1 in nanoclusters is a critical factor in the formation of a spherical assembly of amyloid β-protein on synaptic plasma membranes. *Langmuir 29*, 2258−2264.
(154) Kakio, A., Nishimoto, S., Kozutsumi, Y., and Matsuzaki, K. (2003) Formation of a membrane-active form of amyloid β-protein in raft-like model membranes. *Biochem. Biophys. Res. Commun. 303*, 514−518.
(155) Hicks, D. A., Nalivaeva, N. N., and Turner, A. J. (2012) Lipid rafts and Alzheimer's disease: Protein-lipid interactions and perturbation of signaling. *Front. Physiol. 3*, 189.
(156) Malchiodi-Albedi, F., Contrusciere, V., Raggi, C., Fecchi, K., Rainaldi, G., Paradisi, S., Matteucci, A., Santini, M. T., Sargiacomo, M., Frank, C., Gaudiano, M. C., and Diociaiuti, M. (2010) Lipid raft disruption protects mature neurons against amyloid oligomer toxicity. *Biochim. Biophys. Acta 1802*, 406−415.
(157) Barenholz, Y. (2004) Sphingomyelin and cholesterol: From membrane biophysics and rafts to potential medical applications. *Subcell. Biochem. 37*, 167−215.