Structural Characterization of the Amyloid Precursor Protein Transmembrane Domain and Its γ-Cleavage Site

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ABSTRACT: Alzheimer’s disease is the most common form of dementia that affects about 50 million of sufferers worldwide. A major role for the initiation and progression of Alzheimer’s disease has been associated with the amyloid β-peptide (Aβ), which is a protease cleavage product of the amyloid precursor protein. The amyloid precursor protein is an integral membrane protein with a single transmembrane domain. Here, we assessed the structural integrity of the transmembrane domain within oriented phosphatidylcholines lipid bilayers and determined the tilt angle distribution and dynamics of various subdomains using solid-state NMR and attenuated total reflectance Fourier transform infrared spectroscopies. Although the overall secondary structure of the transmembrane domain is α-helical, pronounced conformational and topological heterogeneities were observed for the γ- and, to a lesser extent, the ζ-cleavage site, with pronounced implications for the production of Aβ and related peptides, the development of the disease, and pharmaceutical innovation.

INTRODUCTION

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder and is the most common form of dementia. The statistics for 2015 states that nearly 47 million people worldwide have AD or a related dementia. The disease is characterized by brain cell destruction, memory loss, and deterioration of cognitive and behavioral processes, severe enough to affect work, lifelong hobbies, and social life. Symptoms worsen over time, and the disease is always fatal. Small soluble or protofibrillar assemblies of amyloid β peptide (Aβ) are believed to be involved in the onset and progression of AD.1-4 The length of Aβ peptides varies from 38 to 43 residues, with Aβ40 and Aβ42 being the major species.5 All Aβ peptides are products of a proteolytic cleavage of the amyloid precursor protein (APP), an integral membrane protein with a single transmembrane domain (TMD), expressed in many tissues and concentrated in the synapses of neurons.6-9 Its primary function is not known, although cell-associated APP has been shown to be involved in some aspects of neuronal development, signalling, intracellular transport, and neuronal hemostasis.10 Nevertheless, it is most commonly studied in connection with Aβ peptides’ production.6-8,11

Notably, the resulting cleavage products and their oligomeric fiber intermediates have been suggested to be involved in memory loss via their cell cytotoxicity. Because membranes assist in the formation of such toxic species, the bilayer interactions are considered of utmost importance for the development of AD and need further investigation.12,13

Amyloidogenic processing of APP begins with proteolytic cleavage of the bulk ectodomain by membrane-bound β-secretase, leading to a secreted fragment sAPPβ and a membrane-bound β-C-terminal fragment (β-CTF), also known as C99.8,14 Following transmembrane proteolysis of β-CTF by γ-secretase at the ε- and γ-sites (Figure 1) results in Aβ production and release of the APP intracellular domain.8 Accumulated evidence suggests that Aβ peptides are released via a sequential proteolytic cleavage mechanism, where the first cut occurs at residue 720 or 719 of β-CTF, that is, at the ε-site located at the cytoplasmic edge of the TMD (Figure 1).15 Products Aβ49/Aβ48 remain membrane-bound and are further processed in a sequential mode through the ζ-site (residue 717 of APP) to yield Aβ46.16 Aβ46 is the precursor of Aβ40 and Aβ42, processed via γ-cleavage.17 The Aβ production in the β-secretory pathway is promoted by APP homodimer formation,18 and further cleavage that generates e.g., Aβ38 and Aβ37.
will occur only if not inhibited by strong dimerization between two TMD helices.20

Multidimensional solution NMR spectroscopy in micellar environments indicates that the TMD of APP is a curved helix with its apex close to G708 and G709, that is, in the center of the hydrophobic region, which results in a 17 ± 7° change in helix alignment.21,22 However, MD calculations show that this kinked structure is specific for micellar but not bilayer environments.23 At its amide terminus, the TMD is connected by a loop to another less-stable helical domain in the juxtamembrane region oriented along the lysomyristoylphosphatidylglycerol (LMPG) micellar surface.22 When investigated at peptide-to-dodecylphosphocholine (DPC) ratios of 1/50, a mixture of monomer and dimer occurs with closely related structures.22,23 The dimer interaction site involves the extended heptad repeat I702—L723 encompassing the γ-secretase cleavage sites.22

In view of the dynamic nature of the APP TMD and the influence of membrane curvature on structural details, biophysical measurements in lipid bilayers are sought after.23 Indeed, magic angle spinning NMR chemical shift measurements indicate that in lipid bilayers helical and nonhelical conformations of individual residues within the TMD are in slow exchange,24 conformational equilibria that are sensitive to lipid composition and temperature. Whereas helical structures are favored by cryotemperatures and/or PG lipids, the amount of nonhelical conformations at residues around the γ-cleavage site increases in membranes mimicking more closely the brain lipid composition and at room temperature.24,25 The helical TMD ends around residues L720/V721, which positions the ε cleavage in a less-structured region.25

In lipid bilayers, solid-state NMR intermolecular cross-peaks suggest that the APP TMD tends to assemble into a right-handed coiled-coil dimer,26 in agreement with recent MD calculations.20 The interaction surface of the homodimer includes G700, G704, and G708, but not G709 and A713.22,24 Indeed, such GxxG motifs are known to mediate transmembrane helix dimerization,22 and mutational studies have confirmed the importance of these glycines for APP processing.28–30 Furthermore, accumulated structural and biochemical data suggest that the conformational variability of the TMD segment plays an important role in APP enzymatic cleavage and therefore in Aβ production.24,26,28,31,32 Thereby, intramembraneous proteolysis by γ-secretase probably requires local unraveling of the helical secondary structure of the TMD to expose the backbone carbonyl carbons for nucleophilic attack by polarized water in the enzyme active site.

Importantly, whereas a number of different conformers are observed during MD calculations, the type of conformer is heavily dependent on the macroscopic shape and curvature of the membrane model system,21 where the right-handed coiled-coil dimer is dominant in 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) bilayers26 but a left-handed parallel dimer predominates in dodecylphosphocholine micelles.22 Important structural details such as the helical outlines, the overall molecular shape, or the topology of the dimer differ between micelle and lipid bilayer environments.21–25 Therefore, these previous investigations underline the importance of structural investigations in liquid crystalline bilayers. Here, we used solid-state NMR spectroscopy of supported planar lipid bilayers to study conformational details of the TM homodimer, where the same peptide used in a previous NMR investigation was studied by highly complementary approaches.24 The investigations are all performed in liquid crystalline bilayers, which imposes additional challenges due to the fluidity of the lipids and the dynamics of the embedded peptides. Solid-state NMR spectroscopy is unique in providing accurate structural information under such near-physiological conditions. This is particularly important when a lipid bilayer environment is required to reveal precise structural details of APP but also because its cleavage products aggregate in a lipid-dependent manner.21,22 Notably, these are the first solid-state NMR investigations of the APP TMD reconstituted in supported lipid bilayers that are oriented relative to the magnetic field of the NMR spectrometer. In particular, the data reveal a structuration into subdomains, dynamic features of individual sites and the resulting membrane topologies, and orientational distribution and conformational heterogeneity of the TMD.

RESULTS

Attenuated Total Reflection Fourier Transform Infra-red Spectroscopy (ATR-FTIR) Spectroscopy. To investigate the global secondary structure of the APP_TMD4K peptides reconstituted into POPC bilayers and the alignment of lipids and peptides in the sample and to assess the overall peptide orientation (i.e., transmembrane or in-plane), ATR-FTIR spectroscopy was used. When recorded at ambient temperatures, the amide I region of the spectrum (1700–1600 cm⁻¹) exhibited a symmetric peak with a maximum at 1653 cm⁻¹, characteristic of predominantly helical conformations (Figure 2). Fourier self-deconvolution revealed several components within the amide I region, which were quantified by an iterative least-squares curve-fitting procedure.33 The calculated secondary structure content was found to be about 74% for the helical structure and 18% for random coil and turn conformations. The remaining 8% are within the experimental error.

The amide I region of the spectra was also used to determine the orientation of the α-helical APP_TMD4K peptide relative to the POPC hydrocarbon chains of the bilayer. A dichroic spectrum of the amide I band is strongly 90° polarized, as seen from the positive deviation in the helical region (Figure 2, black

Figure 1. Partial sequence of APP, numbered following the full-length protein sequence. The TMD region in LMPG micelles from amino acid 700 to 723 is underlined;21 in bilayers, the helix ends at L720/V721.25 The shaded sequence marks APP_TM4K, the region synthesized for this study. Underlined with red are isotopically labeled amino acids that were incorporated for solid-state NMR studies. Positions of the glycines in three consecutive GxxxG sequences are shown in bold and numbered according to the APP sequence (i.e., 696, 700, 704, 708). Cleavage sites of α-, β-, and γ-secretases are indicated by triangles. γ-secretase cleavage sites correspond among others to amino acids 709, 710, 711, and 713 and upon cleavage by secretases produce Aβ38, Aβ39, Aβ40, and Aβ42, respectively.
This indicates that the α-helix orientation is perpendicular to the germanium plate and therefore parallel to the acyl chains of the lipids. A quantitative estimation of the helix orientation was obtained by computing an isotropic dichroic ratio of 1.16 using the lipids‘ ν(C=O) band at 1738 cm\(^{-1}\) as a reference.\(^3\) With this calibration, the dichroic ratio of the amide I band was 1.74. When only the helical component of the amide I band was analyzed, the tilt angle relative to the normal of the germanium plate was found to be ≤20°. The orientation of the lipid acyl chains was also assessed. The lipid dichroic ratio for the band at 2872 cm\(^{-1}\) (νs (CH\(_3\))) was found to be 3.71 (data not shown), and accordingly, the tilt angle between acyl chains and the normal of the germanium crystal was ≤25°. This result indicates that APP_TM4K reconstituted into POPC bilayers forms a predominantly transmembrane α-helix.

\(^{15}\)N and \(^2\)H Solid-State NMR Spectroscopy: APP_TM4K Orientation and Conformation. Local conformational details of the membrane-inserted APP_TM4K helix were revealed by preparing peptides selectively labeled with stable \(^{15}\)N and \(^2\)H isotopes, followed by reconstitution into uniaxially oriented POPC bilayers and solid-state NMR investigations at 296 K (Figure 3A–E). In the presence of POPC, dynamic nonhelical conformers are favored at some positions within the overall helical transmembrane region, similar to the observations made in brain lipid extracts and membrane mimetics.\(^2\) These are thought to be of particular importance for cleavage by secretases.\(^1\)\(^9\),\(^2\)\(^4\),\(^2\)\(^5\) Peptides with three different labeling

![](image)
schemes were used: 15N-Gly708/2H-Ala713, 15N-Ala713/2H-Ala701, and 15N-Val717/2H-Ala701.

On the one hand, the proton-decoupled 15N solid-state NMR spectra of the 15N-labeled peptide amide bonds provide a direct indicator of the approximate tilt angle of the 15N-H vector and thereby also of helical domains.35 Whereas chemical shifts in the 200 ppm region are associated with transmembrane helices, on the opposite, values <100 ppm are indicative of in-planar orientations. On the other hand, the 2H quadrupolar splitting of methyl-deuterated alanine provides highly complementary information on the orientation of the Cα−Cβ−Hβ vector, which can be used to refine the helical tilt and pitch angles.26,34 Finally, proton-decoupled 31P solid-state NMR spectra were recorded for each sample to assess the quality and the alignment of the lipids. The POPC lipids predominantly resonate at 30 ppm, typical of phosphatidylcholine molecules in liquid-disordered bilayers that are oriented with their normal parallel to the magnetic field direction and thereby the bilayer normal.36,37 In the presence of polypeptides that disrupt the membrane packing, the 31P solid-state NMR spectra often extend up to −15 ppm that arise from different lipid alignments.38,39 A representative spectrum is shown in Figure 3F and spectra from other samples in the Supporting Information (Figure S1).

Figure 3A shows the 15N-Gly708 spectrum with a chemical shift value at 191 ppm, which indicates that Gly708 is in a transmembrane orientation.35 The line width at half-height is <12 ppm, demonstrating that the peptide is well-oriented with respect to the magnetic field direction. The 2H spectrum of Ala713 recorded on the same sample did not exhibit one clearly distinguishable quadrupolar splitting, but rather a distribution of Cα−Cβ−Hβ alignments that add up to the shape seen in Figure 3E. The width of the spectrum is ~20 kHz, which can be simulated by Cα−Cβ−Hβ alignments relative to the membrane normal (magnetic field direction) at an average angle of 52° and a Gaussian distribution with 10° standard deviation (Figure 3E).

When this same site was labeled with 13N, the corresponding 13N chemical shift solid-state NMR spectrum of Ala713 exhibited a complex spectral line shape, where the 13N chemical shift values were spread over 160 ppm (Figure 3B), that is, covering almost the full chemical shift anisotropy, indicating that the 1H−13N vector of the Ala713 site is characterized by a wide range of orientations relative to the membrane normal, due to structural and/or orientational heterogeneity of the Ala713 site. Notably, the 2H solid-state NMR spectrum recorded from the 2H1-Ala701 label of the same sample did not resemble a powder pattern line shape (Figure 3D), as would have been expected if the peptide or the sample as a whole was misaligned. In addition, the corresponding proton-decoupled 31P solid-state NMR spectrum exhibited a line shape typical of well-aligned POPC lipids (Figure S1C).

Consequently, the combination of 13N and 2H solid-state NMR spectra of Ala713 (Figure 3B,E) shows that this site adopts a range of different orientations, which exchange slowly on the NMR time scale (kHz range).

When the peptide carrying the 13N-Val717 site is reconstituted into uniaxially oriented membranes, 13N chemical shift intensities are observed at 209 ± 28 ppm (Figure 3C). Although a broad spectral resonance with a correspondingly higher noise level is observed, the chemical shift values undoubtedly indicate a transmembrane orientation of the Val717 segment.35 The width of the spectrum points toward a distribution of orientations around a main value of the Val717 15N−H vector relative to the magnetic field direction. Whereas the mean value of 209 ppm is indicative of a helical tilt angle around 15° (5−25° depending on the pitch angle), a rather large range of possible helix/15N−H vector alignments from 0 to 50° results from taking into account the full spectral width (Figure 3C).

The same peptide carrying the 15N-Val717 label was also labeled at 2H-Ala701, and the corresponding 2H spectrum exhibits a maximal quadrupolar splitting of 26 kHz (Figure 3D). The width of the peptide spectrum and its shape point toward a heterogeneous distribution of alignments also for Ala701.

### DISCUSSION

Multidimensional solution-,21,22 MAS-,24,25 and oriented solid-state NMR spectroscopies, as well as MD simulations,35 all agree that in membrane environments the TMD of APP exhibits predominantly helical conformations. The helix extends from residues G700–L723 in LMPG micelles and from K699–K724 in the presence of DPC and ends around residues L720/V721 in lipid bilayers, that is, the position of the γ-cleavage site.25 ATR FTIR spectra indicate a global tilt angle of the helical part of approximately 20° (Figure 2). Furthermore, for several amino acid positions along the TMD, equilibria between helical and nonhelical conformers were detected by MAS solid-state NMR spectroscopy.24,25 Whereas helical conformations are favored by cryo-temperatures and/or the presence of PG lipids,23 the amount of nonhelical conformations at residues close to the amino- and carboxy-termini of the helix and around the γ-cleavage site increases in POPC, brain lipid extracts, or model membranes mimicking the brain lipid composition and at higher temperatures. Here, such previous investigations of conformational equilibria were considerably extended and refined not only by identifying nonhelical residues but also by investigating the structure, dynamics, and topological distribution of the resulting trans-membrane subdomains. The supported POPC lipid bilayers used here were selected to obtain new and more detailed structural insight into physiological temperatures and under conditions emphasizing the nonhelical structures also observed in brain lipids.

Using solid-state NMR on planar supported lipid bilayers, orientational restraints have been obtained from various sites along the TMD of APP labeled selectively with 2H3-alanine and/or 15N at the corresponding peptide bond. In contrast to MAS solid-state NMR, the technique exploits the full range of the chemical shift anisotropy and thereby provides information, at atomic resolution if a site is part of a well-defined secondary structure, on motions that cause dynamic averaging as well as structural information in terms of angles and their distribution. Importantly, in the experiments designed here both the amide 15N and the 2H3-alanine labels reveal details about the alignment and the alignment distribution of the peptide backbone relative to the magnetic field of the NMR spectrometer (B0), which is collinear with the sample normal. The 31P solid-state NMR spectra indicate well-oriented samples, where all of them exhibit closely related 31P NMR line shapes (Figures 3F and S1). The pronounced differences of 2H and 15N spectra from various labeled sites of the peptides (Figure 3A−E) thus represent conformational and topological differences of the polypeptide, rather than artifacts of individual preparations.
The $^{15}$N solid-state NMR spectra of Gly708 and Val717 are indicative of structured helical domains with transmembrane topology (Figure 4), thereby extending previous NMR and MD structural data. Notably, the Gly708 site represents the apex of the bent helical conformation, which is observed only in micellar environments. Whereas the simulations in Figure 3 compare the spectra that result from a specific alignment to those from the experimental result, Figure 4 indicates all combinations of tilt and pitch angles that agree with a $^{15}$N spectrum. On the other hand, the $^2$H spectra are a direct indicator of $C_\alpha$-$C_\beta$ orientations relative to magnetic field direction $B_0$ (coincident with the membrane normal) and highly sensitive to its mosaicity.

Furthermore, the effects of wobbling and rocking motions of the helices on the tilt and rotational pitch angles are estimated in Figures S2 and S3. These spectral simulations indicate that taking into consideration typical motions of transmembrane sequences shifts the fits to a more transmembrane orientation and larger fluctuations of the $C_\alpha$-$C_\beta$ bonds. Both change by about $5-10^\circ$, thereby also providing an estimate of error bars that arise from uncertainties about the exact motional regime.

In an oriented POPC lipid bilayer and at ambient temperatures, the A713 $^{15}$N solid-state spectrum shows a broad resonance with a maximum at 85 ppm extending to about 200 ppm (Figure 3B). The spectral line shape is indicative of a preference for angles close to perpendicular to the membrane normal albeit with additional alignments of the $N$-$H$ vector covering almost all possible directions. A spectral simulation with a helix tilt angle of $70^\circ$ and a Gaussian distribution of $35^\circ$ reproduces the experimental spectrum reasonably well (Figure 3B). Therefore, the broad, nonaveraged $^{15}$N spectral line shape (Figure 3B) refines previous MAS NMR measurements, which had been rationalized by exchange effects between helical and nonhelical conformations that is slow on the millisecond time scale. Despite the broad distribution of the $^{10}$N-$^1$H vector, the $^1$H-solid-state NMR spectrum from the deuterated methyl group of the same amino acid agrees with a comparatively well-defined tilt angle of the $C_\alpha$-$C_\beta$ bond of $52 \pm 10^\circ$ (Figure 3E). The combination of both the $^{15}$N and $^2$H measurements indicates that the relative orientation of the $C_\alpha$-$C_\beta$ bond undergoes only modest variation relative to the sample normal, when at the same time, it suggests that rotation around the $C_\alpha$-$C_\beta$ bond scans a wide variety of $N$-$H$ alignments (Figure 5). Additional variability of the NH bond topology is obtained through motions involving the Ramachandran angles $\Phi, \Psi$ (not shown).

Figure 4. Contour plots illustrating the helical topologies of the APP_TM4K peptide, which agree with the experimental measurements of the $^{15}$N chemical shift of (A) (192 ± 7) ppm of Gly708 and (B) (209 ± 28) ppm of Val717. The x-axis shows the rotational pitch angle around the helix long axis, whereas the y-axis shows the tilt angle, relative to the membrane normal. The gray area represents the possible pitch/tilt angular pairs that agree with the most intense chemical shift value (bold line) and the chemical shifts corresponding to the intensities at half-height, where the intervals of the contours are in steps of 7 ppm.

Figure 5. Model of the Ala713 site. (A) Oriented $^2$H solid-state NMR spectra (Figure 3E) are indicative of an alignment of the $C_\alpha$-$C_\beta$ vector (black line and cone) at $52 \pm 10^\circ$ relative to the magnetic field direction $B_0$ (arrows pointing upward). The predominant $^{15}$N chemical shift intensity agrees with a $^{15}$N-$H$ vector alignment of ca. 70 ± 35° (or because of symmetry 110 ± 35°, blue line). (B) The broad line shape of the $^{15}$N spectrum with smaller intensities reaching into the 200 ppm region indicates that NH alignments parallel to $B_0$ also occur (Figure 3B). Indeed, rotation around the $C_\alpha$-$C_\beta$ bond results in a range of NH orientations from nearly parallel to $B_0$ to 110°. The carbons are black, nitrogen is blue, oxygen is red, and hydrogen is white. Additional degrees of freedom occur around two Ramachandran angles (not shown).
The V717 site represents the most C-terminal residue investigated in this article (Figure 3C). The spectrum is indicative of a transmembrane helical arrangement albeit with considerable topological and/or conformational dispersion. Interestingly, this site is part of the V717/I718 ζ-cleavage site (Figure 1).

Finally, the ²H solid-state NMR spectrum of A701 (Figure 3D) agrees with a Cα−Cβ bond orientation of 49 ± 10°. However, additional contributions are needed to fully account for the spectral features. The predominant ²H quadrupolar splitting of ~27 kHz indicates that motions are slow or intermediate on the corresponding time scale (Figure 3D,E). Indeed, in the same lipid bilayer used here, two sets of Cα, Cβ and CO chemical shifts were observed in ¹³C MAS solid-state NMR spectra, indicative of helical and nonhelical conformations in slow exchange.24 In micellar environments, position 701 is within the first helical turn of the TMD.21,22 Notably, the residue is also within a domain that has been identified to be part of the cholesterol binding site,23 a lipid important for γ-secretase activity and raft formation.42,43 The dynamic nature of the APP TMD contrasts with that of other membrane-associated polypeptide sequences for which it was possible to determine a unique topology/tilt angle for the helical domain.41,44,45

Previous investigations show that the same transmembrane sequence investigated here adopts a right-handed coiled-coil dimer arrangement in POPC bilayers,25 in excellent agreement with studies in the presence of detergent micelles at similar peptide-to-amphiphile ratios.22,46 Familial Alzheimer mutants of APP have been found to increase the population of monomers46 when at the same time increased dimerization results in decreased Aβ/ production.29,47 Notably, only small differences in chemical shift were observed in previous investigations when the mono- and dimer were compared with each other in micellar environments, suggesting only small conformational changes upon dimer formation.22

A model summarizing our structural data is shown in Figure 6. Whereas residue 701 is relatively mobile being close to the amino-terminal end of the transmembrane helix, residue 708 is well-structured and exhibits a unique and well-defined topology in POPC planar lipid bilayers. Notably, 708 is also part of the helical domain involved in homodimer formation in lipid bilayer where the interaction surface involves G700, G704, and G708.25 Residue 708 is also involved in dimerization when the APP TMD is studied in DPC micellar environments,27 albeit the handedness of the dimer arrangement depends on the membrane curvature,23 being left-handed in micelles22 and right-handed in bilayer environments.25 On the other hand, the bilayer topologies measured here (Figure 4) agree with coiled-coil helical crossing angles ≤60° observed in DPC micelles22 or previously established models that were used to analyze measurements in phospholipid bilayers.23 On the other hand, in our study, both the amino- and carboxy-terminal helices adopt transmembrane alignments, whereas a pronounced kink between these helices and consecutively a more in-planar alignment of the N-terminal domain have been suggested for the monomeric protein in detergent micelles.21

Indeed, not only the conformation of the cleavage site itself but also the overall shape of the transmembrane domain has been suggested to be important for interactions with the secretases.21 The oriented solid-state NMR data presented here (Figures 3 and 5) show a conformational equilibrium, which interrupts the helix around position 713 and provides flexibility for increased topological heterogeneity of its carboxy-terminal domain including residue 717. The latter is characterized by a ¹⁵N–H vector being aligned in a transmembrane fashion, albeit covering a considerable angular distribution. In addition to the A713 residue (Figure 3B,E), the G708/G709 site has also been identified as a site with high flexibility.19,23,22 Such hinge regions within the helical domain probably help the TMD to fit into the secretase binding site. At the same time, pronounced conformational and orientational heterogeneity of the γ- and, to a smaller extent, the ζ-cleavage site (Figure 3B,C) may have important implications for the cleavage mechanism of APP and hence for Aβ production and can serve as sites of enzymatic activity.

This view of a rather heterogeneous structural arrangement, enriched by detailed information on the topology of ¹⁵N–H and Cα=CβH directors, much refines MAS solid-state NMR investigations, which showed the coexistence of nonhelical and helical conformations25 as well as MD simulations, which exhibit a dynamic exchange of conformers where the conformational equilibria are shifted by single-residue mutagenesis24 or lipid composition.26 Additional structural and topological details could be obtained from two-dimensional solid-state NMR spectra of uniformly ¹³N-labelled APP reconstituted into oriented phospholipid bilayers48 although the dynamic nature of some of the sites will probably make their investigation, together with many other residues, more difficult. The dimerization and structural transitions, which are regulated by the lipid composition24 and membrane curvature,37 may be coupled to each other and important for regulation of availability and access of the substrate to the γ-secretase enzymatic site. Notably, in recent electron microscopy structural investigations, single transmembrane domains of γ-secretase also appear quite dynamic49 and such regulatory mechanisms may be enhanced by the enzyme activity itself, being dependent on the membrane lipid composition.32,45,47

## EXPERIMENTAL SECTION

### APP_TM4K Peptide Synthesis.

The 30 amino-acid-long polypeptides, which correspond to the transmembrane part of full-length APP (amino acids 699–726, KGAIIILMVGGV-VIATVIVTLVMLKKK), were synthesized by solid-phase peptide synthesis using Fmoc chemistry40 on a Millipore 9050 automated peptide synthesizer (Millipore, Darmstadt,
Germany) using a TentaGel R RAM resin (polyethylene glycol polystyrene-graft copolymer support) with a degree of substitution of 0.190 meq/g (Rapp Polymere GmbH, Tübingen, Germany). The synthesis was carried out at 40 °C, with double couplings for Val715 and Ile716 and acetylation of nonreacted peptides. Side-chain protecting groups and peptide cleavage from the resin was carried out at room temperature for 4 h in 94% aqueous trifluoroacetic acid (TFA) with the addition of 2.5% ethanedithiol, 1% of the silane derivative trisopropylsilan and 2.5% water as scavenger cocktail. The yield of the crude peptide varied from 60 to 90%.

Peptides were purified by high-performance liquid chromatography (HPLC) using a reversed-phase Prontosol 300-5-C4 (particles size of 5 mm with 300 Å pores) column (Bishoff, Leonberg, Germany). The mobile phase consisted of a gradient of isopropanol with 0.1% TFA (buffer B) mixed with water and 0.1% TFA (buffer A). The content of buffer B increased from 10 to 60% in 23 min at a flow rate of 10 mL/min. All of the solvents were of highest purity (HPLC grade, Sigma-Aldrich Chimie S.a.r.l., Lyon, France). The quality of the purified peptides was assessed by matrix-assisted laser desorption/ionization (MALDI) with a time-of-flight (TOF) mass spectrometer (Autoflex from Bruker Daltonics, Bremen, Germany). The yield of the pure peptide varied between 10 and 30%. Purified APP_TM4K peptides were lyophilized 3 times from 10 mM HCl to remove the TFA counterions. In the presence of TFA, FTIR spectra show a sharp and intense band at 1738 cm−1 was used to characterize the isotropic dichroic ratio.

**Solid-State NMR Spectroscopy.** The following isotopic labeling schemes were introduced into the APP_TM4K peptides during solid-phase synthesis: 15N-Gly708/2H-Ala713, 15N-Ala713/2H-Ala701, and 15N-Val717/2H-Ala701. The oriented samples were prepared as described in detail in ref 54. A peptide-to-lipid ratio of 1:50 was obtained by mixing 3.5 mg of dry peptide dissolved in HFIP (Sigma-Aldrich Chimie S.a.r.l., Lyon, France) with 46.4 mg of POPC dissolved in chloroform. Before NMR measurements, the samples were equilibrated at 93% relative humidity at room temperature. For 2H experiments, the saturated solution was prepared with deuterium-depleted water with a residual degree of deuteration of 10−6. During the solid-state NMR experiments, the samples were cooled by a stream of air at 296 K.

Proton-decoupled 31P solid-state NMR spectra were recorded at 202.41 MHz on a Bruker Avance wide-bore NMR spectrometer operating at 11.7 T (Bruker, Rheinstetten, Germany). A Hahn echo pulse sequence55 was used with the typical parameters: 1 H B1 field 33 kHz, 90° pulse length 10 μs, echo delay 100 μs, spectral width 100 kHz, 2048 data points, typically 512 scans, and repetition time 3 s. The spectra were referenced relative to 85% phosphoric acid (0 ppm). An exponential apodization function corresponding to a line broadening of 100 Hz was applied before Fourier transformation.

Proton-decoupled 15N cross-polarization (CP) spectra of static aligned samples were acquired at 50.67 MHz on a Bruker Avance wide-bore NMR spectrometer operating at 11.7 T (Bruker, Rheinstetten, Germany). A commercial E-free triple-resonance solid-state NMR probe equipped with a flat coil was used (Bruker, Rheinstetten, Germany). The adiabatic passage through the Hartman-Hahn CP condition (APHH-CP) pulse sequence57 was used with the typical parameters: spectral width 35.7 kHz, acquisition time 14.4 ms, and CP contact time 600 μs. The 1H B1 field was swept from 40 to 60% following a tangent amplitude-modulated slope, and the average value corresponded to 47.6 kHz. About 60 000 scans were accumulated with 1024 points. An exponential apodization function corresponding to a line broadening of 300 Hz was applied before Fourier transformation. Spectra were externally referenced to 15NH4Cl at 41 ppm.58

2H solid-state NMR spectra were recorded using a quadrupolar echo pulse sequence59 with the typical parameters:
Calculation of Orientational Constrains Obtained from ssNMR Data. To evaluate APP_TM4K orientations that agree with the experimental results, the static $^{15}$N chemical shift tensor of the polypeptide structure using an Euler coordinate system of the molecular framework was oriented with respect to the molecular coordinate system of the polypeptide structure using an Euler shift tensor. To evaluate the peptide orientations that agree with the experimental results of the $^{2}$H quadrupole interactions of the alanine residue, a maximal quadrupolar coupling constant of 74 ppm was used for the alanine $^{2}$H$_{13}$C$_{2}$-group.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b00619.

Additional $^{31}$P solid-state NMR spectra, simulations of spectral line shapes in the presence of motions and resulting topological analysis, and a more detailed analyses of the $^{2}$H and $^{15}$N spectral line shapes of Ala713 (PDF)

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**Notes**

The authors declare no competing financial interest.

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**ABBREVIATIONS**

AD Alzheimer’s disease
APP amyloid precursor protein
ATR FTIR attenuated total reflection Fourier transform infrared
$\beta$-CTF $\beta$-C-terminal fragment

**REFERENCES**

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