Amyloidosis.11 Formed by misfolded proteins have been associated with devoted to protein assemblies because i.e., Lewy bodies in some cases of hereditary Parkinson disease.13 This protein that forms a native membrane protein and one of the important questions how the membrane proximity influences this peptide self-assembly process.12 Another example is the α-synuclein that is found concentrated in abnormal deposition of cellular material, i.e., Lewy bodies in some cases of hereditary Parkinson’s disease.13 This protein that forms fibers is also known to interact with membranes and to play a role in the preservation of the pool of synaptic vesicles within the neuron.14

**INTRODUCTION**

Self-assembled architectures are omnipresent in nature. Membranes, microtubules, microfilaments, chromatin, etc., are all dynamic and functional architectures playing a crucial role in the compartmentation of cellular functions. These functional and versatile materials have been selected by evolution, and the chemical and physicochemical rules governing their formation are yet to be understood in detail. This is of great importance not only in biology but also for material science, as the understanding of the strategies selected among which electrostatic plays an important role. In the present work we examined the interaction between a small dicationic peptide, that possesses self-assembly properties, and lipid model membranes. The peptide, lanreotide, spontaneously forms nanotubes in water that have a strictly uniform diameter. In the current work, we show that the interaction between the cationic peptide and negatively charged bilayers of lipids induces the formation of myelin sheath-like structures that we call nanoscrolls. By deciphering the different steps of formation and the molecular structure of the self-assembly, we show how electrostatics modify the spontaneous peptide and lipid way of packing.

On the other hand, in material science, biological molecules and molecular self-assemblies are promising templates to organize well-defined inorganic nanostructures. For example, DNA–cationic membrane complexes, maintained by strong electrostatic interactions, allowed the alignment of the CdS (002) polar planes parallel to the negatively charged sugar–phosphate DNA backbone, suggesting that molecular details of the DNA molecule have been replicated onto the inorganic crystal structure.15,16 Hierarchical self-assembly of quantum dots has been realized by using a self-assembled three-dimensional crystal template of helical actin protein filaments and lipid bilayers.17 More recently, multilamellar nanocomposite membranes composed of phospholipid multilayers and silicon nanoparticles sandwiched between each adjacent lipid layer were fabricated. In these ordered composite materials, the silicon nanoparticles achieved different photoluminescence properties compared to the nanoparticles in suspension.18 Therefore, understanding the influence of membranes on protein self-assembly is important not only for biology and medicine but also for material science.

**Supporting Information**

Supporting Information is available for this article. To view it, go to the journal online at pubs.acs.org/doi/10.1021/acs.langmuir.9b01542. It can be found, with any supporting data, in the Supporting Information section of the Supporting Information.

**ABSTRACT:** An important aspect of cells is their shape flexibility that gives them motion but also a high adaptation versatility to their environment. This shape versatility is mediated by different types of protein–membrane interactions among which electrostatic plays an important role. In the present work we examined the interaction between a small dicationic peptide, that possesses self-assembly properties, and lipid model membranes. The peptide, lanreotide, spontaneously forms nanotubes in water that have a strictly uniform diameter. In the current work, we show that the interaction between the cationic peptide and negatively charged bilayers of lipids induces the formation of myelin sheath-like structures that we call nanoscrolls. By deciphering the different steps of formation and the molecular structure of the self-assembly, we show how electrostatics modify the spontaneous peptide and lipid way of packing.

**Organic Nanoscrolls from Electrostatic Interactions between Peptides and Lipids: Assembly Steps and Structure**

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In the present work, we studied the interaction between lanreotide and lipid membranes. Lanreotide, an oligopeptide of eight amino acids, is a therapeutic analogue of somatostatin hormone (Figure 1D). Lanreotide retained from the natural peptide hormone the self-assembly properties.19,20 However, if somatostatin forms reversible polydispersed amyloid structures, lanreotide forms very well-defined nanotubes (Figure 1A–C) with a strictly uniform diameter that is essentially controlled by the close contacts between molecules within the crystalline wall of the nanotubes (Figure 1D–F). This crystalline nanotube wall is formed by a bilayer of peptide. This bilayer presents two hydrophilic surfaces that protects hydrophobic residues such as D-Nal and Tyr from water. This unique capacity of lanreotide to enable very well organized self-assembly allowed very fundamental studies for which we deciphered not only the structure2,21,22 but also the mechanism of formation of the nanotubes 23 and the role of the counterions in this mechanism.24,25 Lanreotide is therefore a

Figure 1. Morphology and structure of the nanotubes formed by the self-assembly of lanreotide in water. Freeze-fracture micrographs of nanotubes that have been transversally and longitudinally cut (A), zoomed-in longitudinally (B) and transversally (C) cut nanotubes. (D) Detail of the unit cell of the peptide crystal that forms the wall of the nanotube. The two cationic charges of the peptide are indicated in red and are brought by the N-ter and the lysine residue. The wall is formed by a peptide bilayer, and the two leaflets are not equivalent, hence the spontaneous wall curvature. (E) The nanotube structure and (F) detail on the internal structure of the wall; the wall presents two external hydrophilic surfaces, and the hydrophobic residues (yellow) are concentrated within the wall at the interface between the internal and external leaflets (in gray and green, respectively).

Figure 2. Ultrafiltration experiments of solutions containing lanreotide (A, blue points) and solutions containing lanreotide and liposomes (A, orange points, B and C). For all experiments in the presence of lipids, we kept constant the total lipid concentration and increased the lanreotide concentration. (A) Evolution of the free lanreotide concentration measured in the filtrate with the initial lanreotide concentration in the solution (before ultrafiltration) for lanreotide solutions (blue points) and for lanreotide–ePC liposomes (orange points) and \[\text{lip}] = 10 \text{ mM}. (B and C) Evolution of the lanreotide concentration in the filtrate with the charge ratio \(R_q\) (see text for definition). (B) Ultrafiltration of solutions containing lanreotide and liposomes (\[\text{lip}] = 10 \text{ mM}) formed by either 90% of ePC and 10% of ePA (violet points) or 30% of ePC and 70% of ePA (red points). (C) Ultrafiltration of solutions containing lanreotide and liposomes (\[\text{lip}] = 26 \text{ mM}) formed by 90% of DMPC and 10% of DMPA (violet points), 50% of DMPC and 50% of DMPA (green points), or 30% of ePC and 70% of ePA (red points). In all the samples containing lipids, the absence of lipid in the filtrate was controlled thanks to the fluorescent probe laurdan (\(\lambda_{\text{em}} = 360 \text{ nm}; 360 < \lambda_{\text{em}} < 640 \text{ nm}\) incorporated within the initial liposomes at 0.1% (M/M). The lipid concentration within the filtrate never exceeds 0.01% of the initial lipid concentration.
very simple model for deciphering the molecular and physicochemical determinants driving the self-assembly processes.

In this study, we ask the question "How do lipid membranes influence the self-assembly process of lanreotide?". Indeed, in a physiological context, self-assembly processes are influenced by many different environmental parameters and in particular by the presence of membranes. In some cases, the environment can drive the assembly to another pathway resulting in the stabilization of an architecture that is less stable than the one formed in the absence of these new parameters. In the present work, we studied the interaction between lanreotide and lipid membranes containing increasing proportions of negatively charged lipids.

**RESULTS AND DISCUSSION**

To determine the conditions of interaction between lanreotide and lipids, we performed ultrafiltration experiments with the idea that the large self-assemblies will remain in the retentate, while the nonassembled peptide will cross the filter. Thus, after centrifugation, quantification of the peptide in the filtrate by UV−visible spectroscopy (ε_m^280 nm =12000 cm−1 M−1) will give access to the concentration of the nonassembled peptide in equilibrium with the assemblies.

The capacity of such experiment to give access to the nonassembled peptide was tested on solutions of increasing lanreotide concentration. In Figure 2A, we show the results obtained after ultrafiltration on Amicon Ultra with a membrane in regenerated cellulose and a 50 KD cut off. Other ultrafiltration units were tested but showed strong peptide absorption (see Material and Methods for details and Supporting Information, Figure SI-1.). The evolution of the peptide concentration in the filtrate describes a curve showing two distinct domains (Figure 2 A, blue points): from 0 to 17 mM, the points describe a straight line with a slope of 0.98 followed at higher concentrations by a plateau. In the first part of the curve, the slope, close to 1, indicates that (i) the absorption of the peptide onto the filter is negligible and (ii) all the peptide is free and not assembled. The break between this straight line and the plateau determines a critical assembly concentration (CAC) of lanreotide of 17 mM. This concentration is in very good agreement with previous determination.21,23

Ultrafiltration of solutions that contain lanreotide together with unilamellar liposomes composed of neutral lipids shows an evolution of lanreotide concentration in the filtrate with the initial lanreotide concentration similar in shape to the one obtained for lanreotide alone (Figure 2 A, orange points). However, the initial straight line has a lower slope (0.69 compared to 0.98), and the break between the straight line and the plateau is also lower than for the peptide alone (12.6 mM compared to 16.6 mM). This means two things: (i) at low concentrations part of the peptide partitions between water and lipid membrane and (ii) the critical assembly concentration of lanreotide is lowered by this interaction. Electron micrographs of the ePC−lanreotide retentate above this new critical assembly concentration show the coexistence of liposomes and lanreotide nanotubes (Supporting Information, Figure SI-2) indicating that upon the "mixed critical assembly concentration", lanreotide self-assembles into classical nanotubes. Therefore, the interaction between neutral lipids and lanreotide indicates that lanreotide can probably cross the lipid membrane by passive diffusion and that upon a critical concentration, the peptide forms regular nanotubes.

The same ultrafiltration experiments have been performed on samples containing lanreotide together with negatively charged membranes (Figure 2, panels B and C). For these experiments, we plot the evolution of the concentration of lanreotide determined in the filtrates after spinning versus the charge ratio R_q defined by the ratio between the positive charges of the peptide and the negative charges of the lipids:

\[
    R_q = 2 \times \frac{[\text{lanreotide}]}{[\text{anionic lipids}]}
\]

This representation allows the direct comparison of the experiments performed with different lipid compositions and concentrations. Contrary to neutral lipid membranes, as soon as negatively charged lipids (ePA or DMPA) are added to the membranes, the peptide interacts strongly with the lipids, as no peptide is measured in the filtrates until an R_q of 0.5, i.e., the electroneutrality (Figure 2B,C). Moreover, independently of the proportion of negatively charged lipids in the membrane (from 10% to 70% (M/M) or the nature of negatively charged lipids (ePA or DMPA), the R_q^sat for saturation is close to 1 (Table 1). A slight difference can be seen when ePA/ePC or DMPA/DMPC mixtures of lipids are used, the R_q^sat for natural lipids being slightly higher than for synthetic lipids (Table 1). Above this ratio, nonassembled lanreotide is detected.

Electron microscopy of DMPA/DMPC (30/70% M/M)−lanreotide mixture for R_q values of 0.5, 1, and 2 has been performed either after negative staining (Figure 3A–C) or after freeze-fragmentation (Figure 3D,E). For negative staining, we had to dilute the samples to get observable grids. This dilution can break nanotubes of pure lanreotide, as they are sensitive to concentration, but the mixed lanreotide/lipid architectures were not affected by dilution, as no free peptide is in equilibrium. As soon as the peptide was added to a suspension of unilamellar liposomes, we detected the presence of different assemblies and the disappearance of liposomes. For R_q = 0.5, liposomes, planar lamellae, curved lamellae, and “myelin sheath-type structures” that we called “nanoscrolls” coexist. For R_q = 1, curved lamellae and a majority of nanoscrolls coexist and for R_q = 2, only nanoscrolls are detected. The photos taken after freeze-fracture show the internal structure of these nanoscrolls that are formed by the winding of multilamellar structures (Figure 3 D). The density profiles determined using ImageJ, on different parts of a multilamellar nanoscroll (Figure 3, D lower panel), give an interlamellar distance of 60 ± 10 Å. The micrographs in Figure 3E show the internal structure of different nanoscrolls that can be composed of the winding of one, two, three, or four stacked lamellae. At

<table>
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<th>[lip]_sat</th>
<th>R_q^sat</th>
<th>[DMPC]</th>
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Table 1. Saturation Charge Ratio R_q^sat for Membranes Composed of Different Lipids (ePC, ePA, DMPC, and DMPA) and of Different Negatively Charged Lipid Proportions

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The spontaneous curvature of the multilamellae suggests an asymmetric structure of the assembly. To solve the question of the molecular structure of these nanoscrolls, we performed both X-ray scattering experiments and ATR-FTIR measurements (Figure 5). The amide I ATR-FTIR spectrum of lanreotide gives access to the conformation of the peptide within the assemblies. A typical ATR-FTIR amide I spectrum of nanotubes of lanreotide shows five components that were previously assigned, in agreement with the literature, to turn secondary structure (1664 cm$^{-1}$) and two different antiparallel $\beta$-sheet organizations (1620 and 1682 cm$^{-1}$, and 1640 and 1692 cm$^{-1}$) (Figure 5A). These vibrations were shown to correspond to a $\beta$-hairpin peptide backbone conformation developing an inner- and interantiparallel $\beta$-sheet network. The ATR-FTIR spectra of the DMPC/DMPA (30/70% M/M) unilamellar vesicles are shown on Figure 5C. The decomposition of the peak between 1700 and 1770 cm$^{-1}$ gives two bands that correspond to H-bonded ester (1725 cm$^{-1}$) and unbounded ester (1742 cm$^{-1}$) of the glycerol backbone$^{28}$ leading to a proportion of 61% of unbounded ester and 39% of H-bonded ones. In this spectra we can also see a large band between 1600 and 1700 cm$^{-1}$. This remaining band probably comes from water, as we could only remove it after drying the sample on the ATR crystal using nitrogen flux. This large band that always remains after bulk water subtraction could be due to either encapsulated water within the liposomes or water in strong interaction with lipids that should both have a slightly different spectrum than that of bulk water. Figure 5B represents the spectra recorded for a solution that contains DMPC/DMPA and lanreotide for an $R_q$ of 1. The decomposition of both amide I and ester bands shows exactly the same peak positions as for peptide and lipids separately. The amide I decomposition gives the same proportions of the different structural elements as for lanreotide nanotubes and indicates that in the presence of negatively charge membranes (i) the peptide is self-assembled, (ii) the peptide is in a $\beta$-hairpin peptide backbone conformation, and (iii) it develops an inner- and an interantiparallel $\beta$-sheet network. The ATR-FTIR spectra of the DMPC/DMPA–lanreotide mixtures with $R_q$ = 1, 1.6, and 2. For reference, we also plot the patterns obtained for lipid unilamellar vesicles (Figure 5, lower black trace) and peptide nanotubes (Figure 5, upper black trace). In the small angle range (Figure 5D), the X-ray patterns obtained for lipid–peptide demonstrate the absence of peptide nanotubes and of unilamellar vesicles. The intense peaks at about 0.1 and 0.2 Å$^{-1}$ indicate that the interaction between the dicationic peptide and the anionic membranes induces a lamellar stacking with a typical interdistance of 66 ± 1 Å. This interdistance is in very good agreement with the interdistance determined from the freeze-fracture replica of multilamellar nanoscrolls (Figure 3).
Interestingly, from $R_q$ 0.5 to 1.6, the interlamellar distance is independent of the concentration of peptide. Other peaks are also present on the traces and will be discussed and assigned later.

In the intermediate $q$ range (Figure 5E), the series of peaks centered at 0.35 Å$^{-1}$ is typical of the $\beta$-sheet network of the lanreotide packed in the nanotubes. These patterns show that lanreotide, even at low concentration, well below its CAC, self-assembles as soon as it interacts with the negatively charged membranes.

Finally in the wide angle region (Figure 5F), the peak detected in all the patterns reveals the organization of the lipid aliphatic chain. As the value of $R_q$ increases, the wide angle peak (i) decreases in intensity, indicating that the order of the aliphatic chains decreases from interaction of increasing amounts of peptide, and (ii) changes in shape, indicating that the aliphatic chains change orientation within the bilayer. The analysis of the peak shape at $R_q = 1$ gives a maximum angle of 40° (Supporting Information, Figure SI-5).

For samples containing higher amounts of lanreotide ($R_q > 2$), distinct macroscopic phases coexist that contain preferentially either nanoscrolls or peptide nanotubes (Supporting Information, Figure SI-6). The X-ray scattering patterns have been recorded as a function of temperature between 23.5 °C and 55 °C for different values of $R_q$ (0 < $R_q$ < 3.6). In Figure 6A–C we plot the evolution of the X-ray pattern for DMPC/DMPA (30/70% M/M) and $R_q = 1.2$ with temperature (from 23.5 °C to 55 °C). Membranes formed by mixtures of DMPC and DMPA present gel to fluid transition that depends on the proportion of each lipid. For membranes composed of DMPC/DMPA (30/70% M/M), we detected the transition between gel and fluid phases to be 40–45 °C in agreement with the literature. At small angles (Figure 6A), the pattern of DMPC/DMPA/lanreotide ($R_q = 1.2$) at 23.5 °C already evidences a coexistence of gel and fluid phases of lipids as seen by the higher angle shoulders of the two major Bragg peaks. With the temperature increase, the major Bragg peaks disappear in favor of a wider angle Bragg peak at the position of the previous shoulders. Together with the temperature-induced disappearance of the wide angle peak at about 1.5 Å$^{-1}$ (Figure 6C), this evidences a gel-to-fluid lipid phase transition within the nanoscrolls. In Figure 6B, the X-ray patterns show higher order Bragg peaks that shift with temperature but also some peaks that remain unchanged with temperature: 0.35 Å$^{-1}$, 0.62 Å$^{-1}$, and 0.89 Å$^{-1}$ are the most visible. These peaks are related to peptide packing within the nanoscrolls.

In Figure 6D (and in Supporting Information, Table SI-1) we depicted the different phases that we observed in the X-ray patterns: NSβ′ for nanoscrolls with lipids in gel phase (β′ indicates that the aliphatic chains are tilted), NStr for nanoscrolls with lipids in fluid phase, and NT for nanotubes. The interaction of lanreotide with DMPC/DMPA membranes induces a drastic decrease of the phase transition temperature (of about 20 °C) until the saturation of membranes by the peptide, i.e., for $R_q$ about 1.

Altogether, these results indicate that (i) the peptide is self-assembled at the surface of lipid bilayers, (ii) the peptide packing in this self-assembly is very similar to the peptide packing within the nanotube walls, (iii) this interaction decreases drastically the temperature of the gel to fluid phase transition of the lipids, and (iv) the mixed lipid–peptide lamellae spontaneously stack to become multimellar and roll up on themselves.

The indexation of the X-ray pattern of DMPC/DMPA–lanreotide (30/70%; [lip] = 26 mM and lanreotide $R_q$ 1.2) at 23.5 °C and at 48.5 °C was performed by trial and error approach using a homemade program to find a unique set of unit cell parameters and an indexation for all the observed peaks. Lamellar peaks (00l) were first indexed. The 2D lattice (hk0) was then indexed with an unit cell close to the lanreotide...
nanotubes. Additional peaks require the formation of a $2 \times 2$ superlattice, doubling the in-plane parameters. The cell parameters were then optimized to reduce the mean squared error. As a result, the X-ray patterns obtained at $23.5^\circ C$ and $48.5^\circ C$ have been indexed in a 3-D triclinic and a monoclinic unit cell, respectively. The parameters of these two 3-D cells are reported in Table 2 (the indexations of the patterns at $23.5^\circ C$ and $48.5^\circ C$ are detailed in Supporting Information, Table SI-2).

At $23.5^\circ C$, the $c$ parameter (67.2 Å) corresponds well to the lamellae interdistance measured by electron microscopy (Figure 3, 60 ± 10 Å). This distance (67.2 Å) corresponds to a stacking of a lipid bilayer and of a peptide bilayer.

The cell parameters $a$ (46.7 Å) and $b$ (40.2 Å) (Table 2) represent the unit cell for the peptide packing. The surface delimited by $a$ and $b$ (1820 Å$^2$) is about 4.5 times higher than the 2-D unit cell of the peptide in the nanotubes$^2$ (Figure 1D, $a = 20.7$ Å and $b = 20.8$ Å, $\gamma = 119^\circ$ and $S = 400$ Å$^2$), indicating that instead of two molecules of peptide, eight peptides can fit within the surface delimited by $a$ and $b$ parameters.

If we consider that these objects are formed for electroneutrality conditions, 25 molecules of lipids should fit within this surface, allowing the presence of 16 molecules of DMPA required for electroneutrality. This amount of lipids gave an average surface/lipid of 79 Å$^2$. The area we deduced from the analysis of the wide-angle X-ray scattering pattern was on the

**Figure 5.** ATR-FTIR spectra (A–C) and X-ray scattering patterns (D–F). For ATR-FTIR spectra, we focused on the amide I band of the peptide backbone and the ester bond of the lipids (between 1580 and 1780 cm$^{-1}$). (A) ATR-FTIR spectrum of the amide I band of nanotubes of peptide ([Lan] = 55.2 mM). (B) ATR-FTIR spectrum of unilamellar vesicles of DMPC/DMPA (30/70% m/M-[lip] = 158 mM) and lanreotide ([Lan] = 55.2 mM). (C) ATRFTIR spectrum of the unilamellar vesicles of DMPC/DMPA (30/70% m/M and [lip] = 158 mM). For X-ray scattering patterns, we focused on three different regions of the patterns: (D) $0 < q < 0.3$ Å$^{-1}$ (small angles), (E) $0.25 < q < 0.55$ Å$^{-1}$ (intermediate angles), and (F) $1.2 < q < 1.7$ Å$^{-1}$ (wide angles). In each panel the same patterns are presented. Black patterns: unilamellar vesicles of DMPC/DMPA (30/70% M/M and [lip] = 26 mM) (black lower traces) and peptide nanotubes ([Lan] = 70 mM) (back upper traces). The dotted black upper trace represents the fit of the peptide nanotubes by a Bessel function of zero order leading to a diameter of 238.5 Å. The other patterns were recorded for DMPC/DMPA (30–70% M/M and [lip] = 26 mM) containing increasing concentration of lanreotide to reach $R_q = 0.5$ (green pattern), $R_q = 1$ (orange pattern); $R_q = 1.6$ (blue pattern), and $R_q = 2$ (brown pattern).
same order of magnitude, i.e., 76 Å² (Supporting Information, Figure S1-4). These area values are significantly higher than, for example, 60 Å² determined for fully hydrated DMPC. Therefore, the assembly of the peptide at the membrane surface induces strong constraints on the lipid organization that increases the average surface occupied by the lipid. This observation very well explains the decrease of the transition temperature as well as the 40° angle of the aliphatic chain when lipids are in the gel phase. Using this structural information, we built a molecular model (Figure 7). We show that the interaction between lanreotide and anionic membrane induces the formation of multilamellar structures, i.e., nanoscrolls, the curvature radius of the lamellae continuously varying within the same object. The resulting nanoscrolls are formed by alternate peptide and lipid bilayers in strong electrostatic interaction. The structural and spectroscopic data also indicate that the molecular packing of lanreotide within the nanotubes and within the nanoscrolls are very similar. The most important change is the surface of the unit cell that is four times higher than in the nanotubes. For the pure lanreotide architectures, i.e., nanotubes, we have previously shown that the curvature radius of the nanotube is due to the difference of the peptide packing on the two layers forming the nanotube wall. We have also shown that the strictly uniform diameter of the nanotubes is controlled by close contacts between the lateral chains within

Table 2. Unit Cell Parameters at 23.5 °C and 48.5 °C of DMPC/DMPA-Lanreotide \( R_q = 1.2 \)

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<th>( a ) (Å)</th>
<th>( b ) (Å)</th>
<th>( c ) (Å)</th>
<th>( \alpha ) (deg)</th>
<th>( \beta ) (deg)</th>
<th>( \gamma ) (deg)</th>
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<tr>
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<td>56.9</td>
<td>90</td>
<td>90</td>
<td>94.3</td>
</tr>
</tbody>
</table>

Figure 6. (A–C) Evolution of the X-ray patterns with temperature from 23.5 °C to 55 °C by increments of 3.5 °C (from the bottom to the top) for DMPC/DMPA (30/70% M/M and \([lip]\) = 26 mM)—lanreotide \( R_q = 1.2 \). (D) Phase diagram. Blue points: lamellar stacking with lipids in \( L_\beta \) phase, orange points: lamellar stacking with lipids in \( L_\alpha \) phase, and green points: peptide nanotubes.
the peptide crystalline self-assembly induces a reorganization of the lipid packing.

Another analogy between the mixed peptide–membrane studied in this work can be made with the myelin sheath. The intracytoplasmic proteins that are directly involved in the tight membrane packing are positively charged, and the driving force for the membrane packing within the myelin is essentially electrostatic. In particular, stacking of lipid membranes can be induced in vitro by the interaction of P2 basic proteins, one of the two major proteins involved in the myelin sheath structure. More recently, septin, an ubiquitous protein regarded as one of the cytoskeleton proteins, was shown not only to interact with clusters of negatively charged lipids but also to self-assemble into filaments when interacting with membranes either in vivo or in vitro. Lanreotide—negatively charged lipid membrane assemblies mimic well natural protein–membrane assemblies that have structural functions for cells.

**MATERIAL AND METHODS**

**Materials.** Lanreotide was provided by IPSEN. The lipids (ePC, ePA, DMPC, DMPA) were purchased from Avanti Polar Lipids and used without any further purification. The ultrafiltration units (Amicon centrifugal filter units) of 0.5 mL were used.

**Methods. Small Unilamellar Liposomes.** The lipids are dissolved in chloroform:methanol (90:10 v/v) and then evaporated using a Büchi rotavapor R-200 for obtaining a thin and homogeneous film. The film is then hydrated with a precise volume of pure water, and the lipid solution is vigorously vortexed. This solution is dispatched in different small vials that have been previously weighed. The samples are then frozen and lyophilized overnight. After lyophilization, each vial containing lipids are weighed to estimate the quantity of lipids.

Liposomes—Lanreotide Mixtures. The unilamellar vesicles are mixed with the appropriate lanreotide solution and rigorously vortexed. For synthetic lipids such as DMPC and DMPA, the lipid suspensions are kept at room temperature. When liposomes are prepared for ultrafiltration experiments, 0.1% (M/M) laurdan, a lipid fluorescence probe, is added to the lipids, allowing the quantification of lipids.

**Ultrafiltration Experiments.** To find the most convenient ultrafiltration unit for our experiments, we tested different cutoffs (between 3KD and 100 KD) and two different filtration membranes (poly(ether sulfone) and cellulose). Lanreotide forms nanotubes when the total peptide concentration exceeds 17 mM, i.e., its critical self-assembly concentration (CAC). After spinning a solution of 41 mM using the different ultrafiltration units, only the Amicon Ultra, 0.5 mL (cellulose membrane and 50KD cutoff), gave a satisfactory result (Supporting Information, Figure SI-5), i.e., a concentration of lanreotide in the filtrate corresponding to its critical assembly concentration.

The experiments were performed as follows: 0.4 mL of each sample is pipetted into the filter unit and is subjected to either 14000 rpm during 10 min or 5000 rpm during 0.5 h. The UV spectra of the solutions in the resulting filtrate are recorded on a UV–visible spectrophotometer (GE Healthcare, Ultraspec7000). The concentration of lanreotide in the filtrate is calculated from the optical density at 280 nm using the molar absorption coefficient determined previously at 12000 M⁻¹ cm⁻¹ and compared to the initial lanreotide concentration in the samples. For the measurement of lanreotide within the samples containing lipids and to avoid errors due to light scattering, the initial solutions are in DMSO.
**Sample Preparation for Electron Microscopy.** Electron microscopy is performed on the samples either after negative staining or after freeze-fracture for visualizing the object formed by lanreotide and lipids. For negative staining, we used 1% uranyl acetate as previously described. For freeze-fracture, we used a Balzers (BAF 600) apparatus. Different techniques have been used depending on the sample and the information we wanted to obtain: simple Pt (30 Å) carbon replica after surface fracturing, Pt (30 Å) carbon replica obtained after surface fracturing and etching, or rotative Pt pulverization and carbon replica after fracturing and etching.

**Small Angle X-ray Scattering.** SAXS was performed on the high brilliance SWING beamline (12 keV) at the Soleil Synchrotron Facility using sample–detector distances of 0.5 m. The diffraction patterns were recorded for reciprocal spacing q (Å⁻¹) from 0.02 to 1.8 Å⁻¹. The X-ray patterns were detected and recorded via a chip charge-coupled device camera detector, AVIEX. The samples were prepared in 1.1 to 1.5 mm glass capillaries (Gläser Technik and Konstruktion, Schönewalde, Germany) and introduced into a homemade capillary holder accommodating 20 capillaries at controlled temperature. For each capillary, 34 patterns (exposure time 50 ms) were recorded from the top to the bottom to test homogeneity of the sample and to avoid degradation during measurements. All samples exhibited powder diffraction and scattering intensities as a function of the radial wave vector q (q = 4π sin(θ)/λ), which was determined by circular integration. The diffraction spacing was calibrated using the lamellar peaks of silver behenate (d = 53.380 Å).

For temperature scanning experiments, the samples were heated directly in a homemade capillary holder connected to a programmable thermostatic bath. The heating rate was fixed at 0.16 °C/min, leading to an average difference of 3 °C between each recorded X-ray pattern of the same sample.

**ATR-FTIR Spectroscopy.** Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) spectra were measured at 4 cm⁻¹ resolution with a Bruker IFS 66 spectrophotometer equipped with a 45° n ZnSe ATR attachment. The spectra resulted from the average of 50 scans. Spectra were corrected for the linear dependence on the wavelength of the absorption measured by ATR. The water signal was removed by subtraction of a pure water spectrum recorded the day of the experiment. Analysis of the lanreotide conformation was performed by decomposition of the absorption spectra using GRAMS software, as a sum of Gaussian–Lorentzian (10%) components.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.9b01542.

Additional material includes complementary ultrafiltration experiments, electron micrographs of ePC–lanreotide solution after ultrafiltration, fit of the inside structure of nanoscrolls with Archimedean spiral, X-ray patterns of DMPC/DMPA (30/70% m/M)–lanreotide Rg > 1.2, wide-angle X-ray analysis, and indexation tables of the patterns at 23.5 °C and 48.5 °C of DMPC/DMPA (30/70% m/M)–lanreotide Rg = 1.2 (PDF)

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**Notes**

The authors declare no competing financial interest.

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**ABBREVIATIONS**

CAC, critical self-assembly concentration; ePC, egg-phosphatidylcholine; ePA, egg-phosphatidic acid; DMPC, dimyristoyl-phosphatidylcholine; DMPA, dimyristoylphosphatidylcholine; ATR-FTIR, attenuated total reflectance Fourier transformed infrared spectroscopy; Rg, charge ratio between the cationic charges and the anionic charges; NS, nanoscrolls; NT, nanotubes.

**REFERENCES**


