Oriented Self-Assembly of Cyclic Peptide Nanotubes in Lipid Membranes

Hui Sun Kim, Jeffrey D. Hartgerink, and M. Reza Ghadiri*

Contribution from the Departments of Chemistry and Molecular Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California 92037

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Abstract: Polarized attenuated total reflectance (ATR), grazing angle reflection−absorption, and transmission Fourier transform infrared (FT-IR) spectroscopy methods have been used to investigate the orientation of peptide nanotubes in the functionally relevant environment of ordered phospholipid multibilayers. Eight-residue cyclic peptides of alternating D- and L-amino acids which self-assemble to form open-ended, tubular structures have been shown previously to function as transmembrane channels for ion transport. Although the tubular structure of the peptide assembly has been established in the pure solid state, this study presents the first detailed biophysical investigation of the peptide nanotubes within the context of the lipid film to afford a quantitative estimate of their angle of orientation relative to the lipid bilayer. We find by ATR IR that in accordance with the previous structure−function model hypothesis for a transport-competent channel, the central axis of nanotubes composed of *cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] is aligned parallel to the dimyristoyl phosphatidylcholine (DMPC) hydrocarbon chains, at approximately 7° from the axis normal to the bilayer plane. This upright orientation for the DMPC/peptide film is also qualitatively supported by grazing angle and transmission FT-IR data. By contrast, FT-IR studies of two- and three-dimensionally ordered peptides, Langmuir−Blodgett films and microcrystals, respectively, show that the peptide tubes lie parallel to the substrate surface in the absence of a hydrophobic supportive environment. These studies support a transport-competent, membrane-spanning orientation of the peptide nanotubes in the lipid bilayers. Furthermore, they indicate that the orientation of the tubes can be controlled by a supporting environment, implicating biological and nanotechnological applications.

Introduction

The importance of channels in sustaining biological systems and their potential utility in nanotechnology and biomedicine have kept the structure−function relationship of channels an active area of investigation.[1] Previously, we reported an artificial channel system composed of a tubular ensemble of stacked cyclic peptides which was shown by vesicular assays and single channel conductance measurements to function as a transmembrane channel for ion and biomolecule transport.[2b,c] Briefly, the design for this tubular structure is based on the postulate that cyclic peptides of an even number of alternating D- and L-amino acids can adopt a low-energy, flat-ring conformation in which the hydrogen-bonding backbone moieties stand perpendicular to the plane of the ring and the sides chains point radially away from the ring. Such a conformation poises the cyclic peptide units to self-assemble into an antiparallel β-sheetlike cyclindrical structure,[2,3] and the appropriate decoration of this structure with hydrophobic side chains is hypothesized to allow its partition into the lipid environment to function as membrane-spanning channels (Figure 1).

Unlike natural peptide and protein channel systems in which secondary folding, tertiary interactions, and/or quaternary assembly as well as conformational dynamics must often be considered, the structure−function analysis of the nanotube system is simplified by the highly restricted conformational and assembly options inherent in its design. To function as a transmembrane channel with a discrete, bilayer-spanning pore as in the hypothesized model, the cyclic peptides must meet two structural criteria in the context of the lipid environment: (1) they must self-assemble to form tubes in the membrane, and (2) the central axis of the membrane-incorporated tubes should be oriented approximately perpendicular to the plane of the lipid membrane to provide a solute-accessible through-path. The formation of the tightly hydrogen-bonded antiparallel β-sheet tubular structure has been well established in the solid state of the pure peptide by cryoelectron microscopy, electron diffraction, FT-IR, molecular modeling, and X-ray crystallography,[2a,d] and the incorporation of peptides into the membrane has been supported by FT-IR studies.[2b]

To determine the formation of the tubular structure and its orientation in the lipid membrane, FT-IR studies of the cyclic

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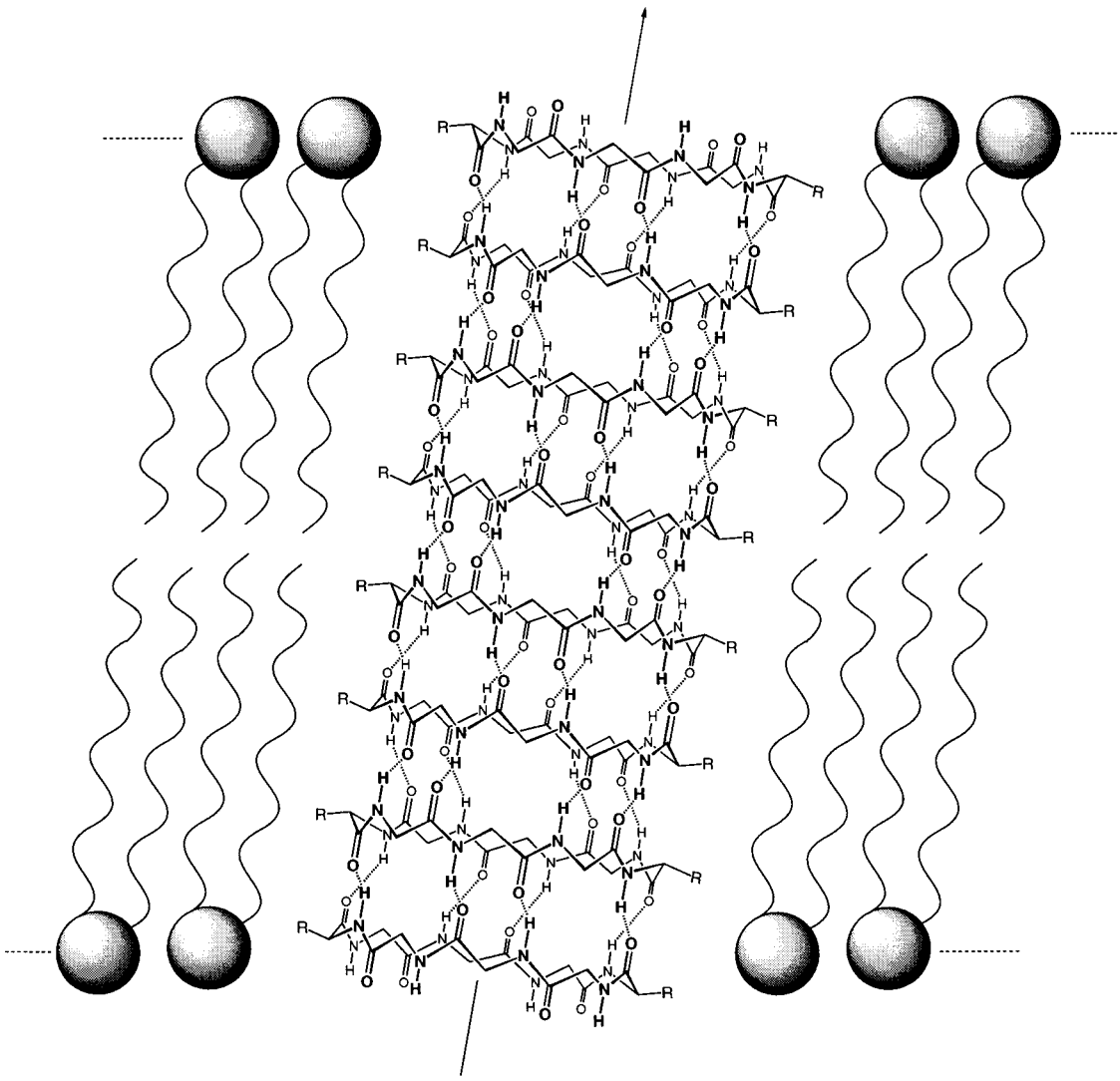

Figure 1. Schematic representation of the cyclic peptide channel assembly in the lipid bilayer. The picture emphasizes the antiparallel β-sheetlike cylindrical structure formed by the cyclic peptide subunits and its tilt imposed by the lipid bilayer. Most of the side chains have been omitted for clarity. The central axis of the peptide nanotube is defined as the longitudinal axis of the cylindrical structure.

peptide in dried, oriented, phospholipid multibilayers were carried out. Polarized FT-IR has been used extensively to determine the secondary structure and orientation of a number of membrane proteins and peptides by using such oriented lipid bilayers as model membranes.[4,5] As our structural model for the "stackable", flat-ring cyclic peptides assumes a conformationally restricted backbone amide in which the hydrogen-bonding carbonyl and NH moieties are aligned with the central axis of the nanotube, the IR-active amide modes can serve as references for the orientation of the entire tubular assembly. In fact, the peptide nanotube assembly affords an ideal model for a highly ordered, conformationally homogeneous antiparallel β-sheet system to study by the current method. Here we report a detailed study of the orientation of the peptide nanotubes in the functionally relevant, ordered dimyristoyl phosphatidylcholine (DMPC) multibilayers by polarized attenuated total reflectance (ATR), grazing angle reflection−absorption, and transmission FT-IR techniques to afford both quantitative and qualitative orientational analysis. Furthermore, these observations are then compared to the orientation of two-dimensionally ordered films and three-dimensionally ordered microcrystals of the peptide in the absence of a supportive lipid matrix.

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Results and Discussion

FT-IR Studies of the Cyclic Peptide in DMPC Multibilayers. The IR spectra of *cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] in the oriented lipid membrane display amide bands which correlate well with those expected for a tightly hydrogen-bonded antiparallel β-sheet structure (Figure 2, Table 1). The amide I peak frequencies of 1635 (perpendicular component, strong, hereafter designated as amide I) and 1688 cm^{-1} (parallel component, weak, hereafter designated as amide I$_{||}$), with a splitting of 53 cm^{-1}, and the amide II band at 1538 cm^{-1} are

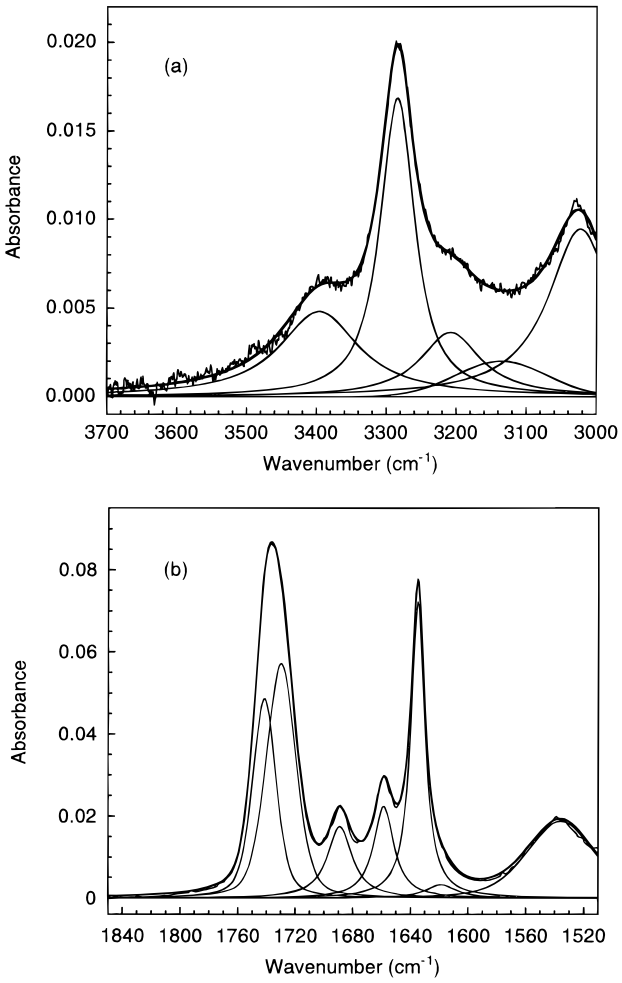

Figure 2. Deconvoluted ATR IR spectra with use of parallel polarization of (a) the amide A region and (b) the amide I and II region of the peptide nanotube *cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] in the DMPC multilayer. (The peak at 1738 cm^{-1} is a lipid band.)

Table 1. Assignment of Relevant IR Peaks in a Film of *cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] in Air-Dried DMPC Multibilayers

freq (cm^{-1})	assignment	
3281	amide A	N−H stretch of peptide amide
2850	ν_s (CH_2)	symmetric stretch of lipid hydrocarbon methylene
2919−2918	ν_{as} (CH_2)	antisymmetric stretch of lipid hydrocarbon methylene
1738	ν (C═O)	C═O stretch of lipid fatty acid ester
1688 (w)	amide $I_{\|\|}$	primarily C═O stretch of peptide amide, parallel component
1635 (s)	amide I	primarily C═O stretch of peptide amide, perpendicular component
1537	amide II	primarily NH in-plane bend and CN stretch of peptide amide
1257	ν_{as} (PO_2^-)	antisymmetric stretch of lipid PO_2^- double bond

highly characteristic of the antiparallel β-sheet structure.[6] The observed amide A (NH stretch) frequency at 3281 cm^{-1} supports a tight ring-to-ring network of backbone hydrogen bonding.[2a] The band at 1659 cm^{-1} correlates with the reported frequency of ∼1650 cm^{-1} for the glutamine C═O stretch in peptides.[7] These results, which agree well with those from previous IR studies of the pure microcrystalline nanotube peptides,[2a,d] indicate that the cyclic peptides in the lipid membrane environment form an assembly that is very similar to the tubular, antiparallel β-sheet structure formed in the solid state.

Polarized ATR allows a quantitative evaluation of the orientation of both the peptide amide and the lipid components. It selectively measures the *x* and *z* components of the transition dipole moment with the parallel electric field component of the incident light (p-polarized light) and the *y* component with the perpendicular electric field component (s-polarized light). Figure 3a depicts the experimental coordinates and polarization vector conventions used in this study. We have chosen to measure the dichroic ratios of the strong amide I band (perpendicular component), which consists primarily of the backbone C═O stretch, to quantitatively measure the orientation of the peptide tube. The more complex amide II band, an out-of-phase combination of largely NH in-plane bend and CN stretch whose transition moment is approximately perpendicular to that of amide I,[6] and the amide A band, a localized backbone NH stretch that is approximately parallel to amide I, were used in comparison with the amide I to qualitatively assess the peptide tube orientation. To evaluate the lipid chain orientation, the symmetric and antisymmetric CH_2 stretches of the DMPC lipid, which are perpendicular to the all-trans hydrocarbon chain axis,[8,9b] were used. The lipid headgroup components, the fatty acid ester C═O stretch and the antisymmetric stretching of the PO_2^- group, were also used to assess lipid orientation and order. Quantitative calculations of the angles of orientation were carried out according to Fraser's equations as detailed in the Experimental Section.

To assess the orientation of the peptide relative to the lipid layer, it is imperative to establish the orientation and order of the physisorbed lipids relative to the substrate surface. Without the peptide, the hydrocarbon chains of the lipid DMPC show an average tilt of 28° relative to the surface normal (Table 2, Figure 4a). This value correlates well with previous studies of phospholipid multibilayer systems, which report tilts in the approximate range of ∼20−30°.[4,9] The values of the angle of transition moment orientation for the lipid head group C═O and PO_2^- relative to the surface normal, 62° and 61°, respectively, are also in agreement with previously reported values of 64° for C═O and 63° for PO_2^- in similar phosphatidylcholine systems.[9a,4a] Insertion of the peptides into the lipid membrane shows negligible change in the polar region of the lipid, but the hydrocarbon chain shows a slight increase in tilt to approximately 32°. The minimal tilt change upon peptide incorporation, coupled with the minor frequency shift (+1 cm^{-1} for CH_2 antisymmetric stretch, Table 2) and bandwidth broadening (∼5% of bandwidth at half-maximal height) in the lipid hydrocarbon bands, suggests that the perturbation in the lipid bilayers caused by the incorporated nanotube peptides is minimal and localized. A similar conclusion was drawn in a comparable ATR FT-IR study of gramicidin/lipid multibilayer systems.[4b]

The ATR IR spectra of ordered DMPC/*cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] film show a dramatic decrease in amide I and A intensities upon changing the polarization of the incident light from parallel to perpendicular (Figure 4b). This result, which

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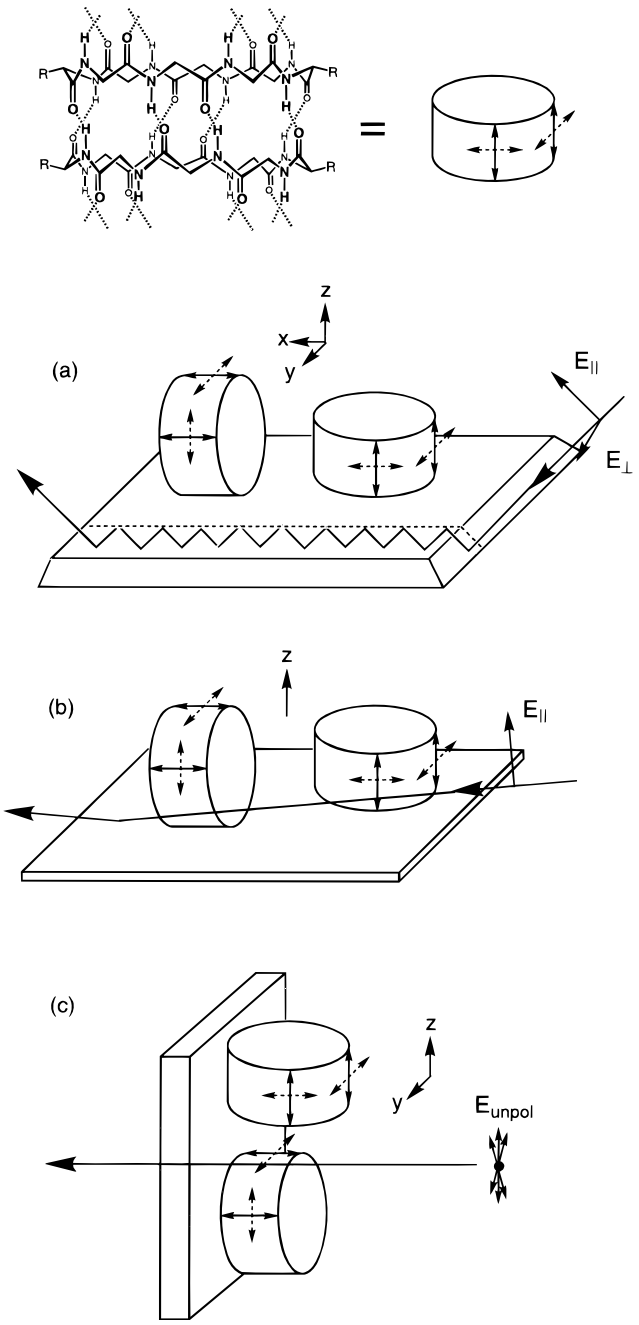

Figure 3. Experimental setup for (a) ATR, (b) grazing angle, and (c) transmission IR. The cylindrical structure represents a dimer of self-assembled cyclic peptides. The solid double arrows on the representation of the dimer indicate the general direction of the amide I transition moment; the dashed double arrows indicate the general direction of the amide II transition moment. $E_{\perp}$, $E_{||}$, and E_{unpol} are the polarization components of the incident light; *x*, *y*, and *z* are the experimental coordinates which are detectable by the indicated method.

indicates that the amide carbonyl and NH bonds are predominantly aligned along the *z* coordinate, is qualitatively consistent with peptide tubes whose central axis is aligned perpendicular to the substrate surface (Figure 3). Quantitatively, the nanotube tilt angle as determined by the amide I transition was measured to be 39° relative to the surface normal, which is approximately 7° relative to the average membrane plane normal of 32° (Table 2). The nanotube tilt angle of 7° is comparable to findings from the IR studies of the β-helical gramicidin A in dried DMPC multibilayers, which report a helix tilt angle of <15° relative to the bilayer normal.[4a]

Table 2. ATR IR Orientation Parameters of DMPC with and without *cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu]

	DMPC[a]			DMPC/peptide[a]		
assignment	freq (cm^{-1})	$[A_{||}/A_{\perp}]^b$	angle (deg)	freq (cm^{-1})	$[A_{||}/A_{\perp}]^b$	angle (deg)
υ (C═O)$_{\text{peptide}}$				1635	4.34 ± 0.14	39[c]
υ_{as} (CH$_2$)$_{\text{lipid}}$	2918	1.15 ± 0.02	29[c]	2919	1.26 ± 0.01	33[c]
υ_s (CH$_2$)$_{\text{lipid}}$	2850	1.10 ± 0.02	26[c]	2850	1.20 ± 0.03	31[c]
υ (C═O)$_{\text{lipid}}$	1738	1.51 ± 0.03	62[d]	1738	1.51 ± 0.02	62[d]
υ_{as} (PO_2^-)$_{\text{lipid}}$	1257	1.56 ± 0.06	61[d]	1257	1.60 ± 0.08	60[d]

[a] Data are an average of 3−4 samples. [b] $[A_{||}/A_{\perp}]$ indicates the dichroic ratio of the band intensity with parallel polarized incident light to the band intensity with perpendicular polarized light. [c] Values are angles of the molecular axis relative to the substrate surface normal, as determined from the dichroic ratio of the indicated transition moment. Error is ≤±1°. [d] Values are angles of the indicated transition moment relative to the substrate surface normal. Error is ≤±1°.

The tilt angles were calculated assuming that the lipid bilayers were oriented perfectly parallel to the IR substrate surface. Previous X-ray and neutron diffraction studies have reported values of <15° to 30° for the mosaic spread of various lipid multibilayer samples on solid surfaces.[10] Taking into consideration any contribution to disorder arising from an imperfect parallelism between the lipid multibilayers and the substrate surface effectively reduces the average angle of orientation of both the lipid hydrocarbon chains and the peptide nanotubes, making them even more closely aligned to the surface normal.[4a,5a−c,e] If an even distribution of the peptide in the lipid sample is assumed, the effect of membrane mosaicity on the current difference value between the orientations of the lipid chains and the peptide nanotubes would be minor.

Grazing angle and transmission IR also qualitatively support the observation of nanotubes which are oriented parallel to the lipid acyl chains and perpendicular to the bilayer plane. Metal-surface IR selection rules for grazing angle impose that only those transition dipole moments which are normal to the surface will absorb[11] (Figure 3b). Because amides I and II are approximately perpendicular to each other, the ratio of these two band intensities gives a way to qualitatively assess the orientation of the tube. The DMPC/*cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] system in grazing angle IR shows a spectrum with a significantly large amide I/amide II band ratio (Figure 5a). Along with the strong amide A intensity, the observed preferential absorption of the amide I band is expected for nanotubes which are aligned perpendicular to the substrate surface. For unpolarized transmission IR, every transition moment absorbs except the one aligned with the direction of light propagation when the sample surface is placed at the conventional 90° to the incident light (Figure 3c). Hence, the transition moments which are aligned with the central axis of the up-right nanotube are preferentially nonabsorbing in transmission IR, while they are preferentially absorbing in grazing angle IR. The transmission spectrum shows a dramatic decrease in the amide I peak intensity in comparison to that of the grazing angle spectrum, again qualitatively confirming the perpendicular orientation of the nanotubes relative to the lipid membrane plane (Figure 5b, Table 3). The amide I/amide II dichroic ratio is not reduced further than observed, however, due to the bilayer-imposed tilt of the peptide tubes in the membrane which allows partial amide

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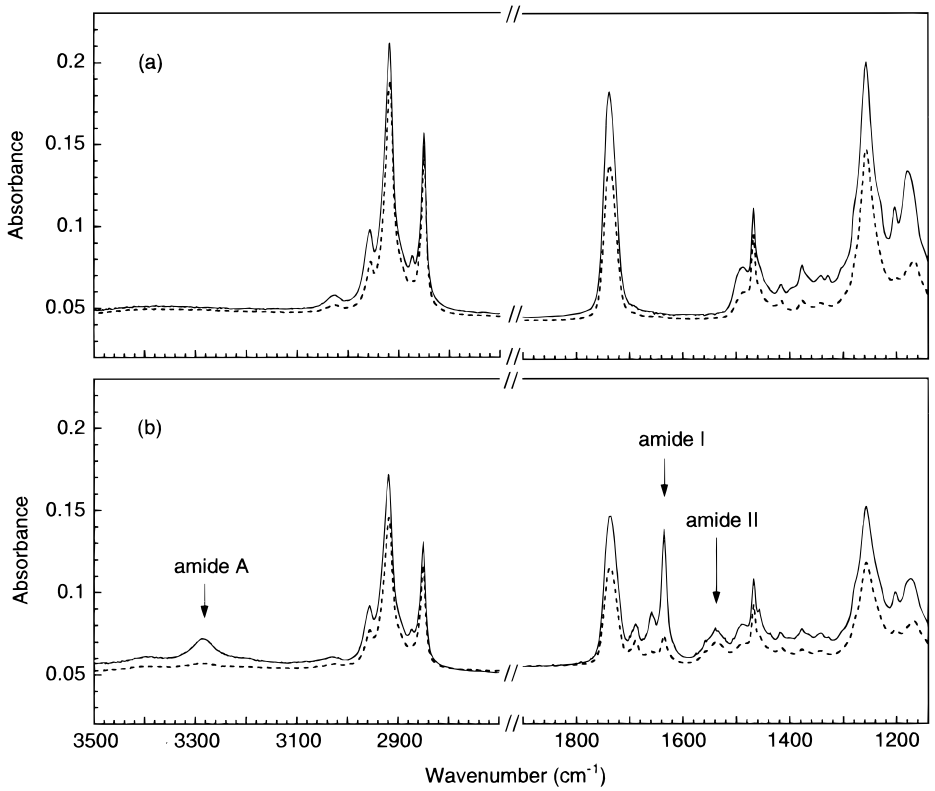

Figure 4. ATR IR spectra of (a) a DMPC multilayer and (b) a DMPC/*cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] multilayer. The solid trace indicates absorbance with parallel polarized light; the dashed trace indicates absorbance with perpendicular polarized light.

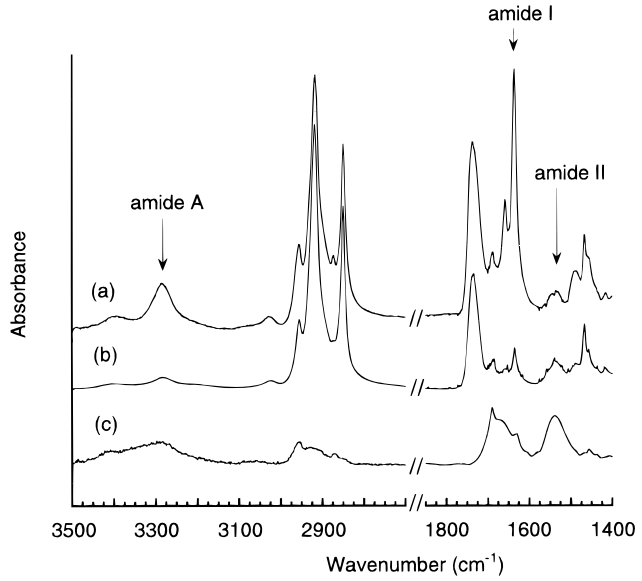

Figure 5. (a) Grazing angle IR spectrum of a DMPC/*cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] multilayer on a gold surface. (b) Transmission IR spectrum of a DMPC/*cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] multilayer on a CaF_2 plate. (c) Grazing angle IR spectrum of a Langmuir−Blodgett film of *cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] on a gold surface. The spectra have been scaled to facilitate visual comparison.

I absorption, and the higher relative absorption coefficient of the amide I over that of amide II. The two IR methods of grazing angle and transmission give results which correlate well with those obtained by ATR IR, in that there is a qualitative similarity between the grazing angle and the parallel polarized ATR spectra, and between the transmission and perpendicular polarized ATR spectra (compare spectra a and b in Figure 5 to spectrum b in Figure 4).

In a further support for the proposed tube orientation in an ordered hydrocarbon supportive environment, recent studies of the related cyclic peptide *cyclo*[(L-Trp-D-Leu)$_4$] incorporated into self-assembled monolayers (SAMs) of dodecanethiols on a gold surface[12] show an amide I/II dichroic ratio very similar to that of the DMPC multilayer/peptide system in comparable grazing angle IR studies. SAMs of chemisorbed alkanethiols on gold surfaces have been studied extensively in the past, and the alkyl chain orientation has been measured to be in approximately the same tilt range as that for oriented phospholipid membranes.[13]

The above IR results of the nanotube peptides in ordered hydrophobic films strongly suggest that in the functionally relevant lipid membrane environment, the peptide tubes are oriented in a membrane-spanning, transport-competent direction perpendicular to the bilayer plane.

Study of Ordered Peptide Nanotubes in the Absence of the Lipid Matrix. Two types of ordered nanotube assemblies were used to assess the peptide tube orientation in the absence of the lipid matrix: the two-dimensionally ordered Langmuir−Blodgett films and the three-dimensionally ordered microcrystals.

Langmuir−Blodgettry was used to transfer compressed films of *cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] from the air−water interface onto a gold surface for IR analysis. In direct contrast to the grazing angle IR spectrum of the lipid-supported peptides which has a large amide I/amide II dichroic ratio, the grazing angle spectrum of the compressed Langmuir−Blodgett films of *cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] shows a significantly larger amide II band intensity in comparison to the amide I intensity (Figure 5c, Table 3). The display of a significant dichroism indicates first that a high degree of order exists in

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Table 3. Summary of IR Dichroisms for Various Peptide Systems

environment	system	IR method	dichroism[a]
with hydrophobic matrix	DMPC:*cyclo*[(L-Trp-D-Leu)$_3$−L-Gln−*D*−Leu] oriented multilayer	ATR	$A_{\|\|}$(amide I) > $A_\perp$(amide I)
	DMPC:*cyclo*[(L-Trp-D-Leu)$_3$−L-Gln−*D*−Leu] oriented multilayer	grazing angle	*A* (amide I) > *A*(amide II)
	DMPC:*cyclo*[(L-Trp-D-Leu)$_3$−L-Gln−*D*−Leu] oriented multilayer	transmission	*A*(amide I) ∼ *A*(amide II)
	Alkanethiol:*cyclo*[(L-Trp-D-Leu)$_4$] monolayer on gold	grazing angle	*A*(amide I) > *A*(amide II)
without hydrophobic matrix	*cyclo*[(L-Trp-D-Leu)$_3$−L-Gln−*D*−Leu] Langmuir−Blodgett film	grazing angle	*A*(amide I) < *A*(amide II)
	cyclo[(L-Gln-D-Leu)$_4$] microcrystals	grazing angle	*A*(amide I) < *A*(amide II)
	cyclo[(L-Gln-D-Leu)$_4$] microcrystals	transmission	*A*(amide I) > *A*(amide II)

[a] "*A*" indicates absorption intensity, "$A_{||}$" indicates intensity with parallel polarized incident light, and "$A_\perp$" indicates intensity with perpendicular polarized light, of the IR bands enclosed in the parantheses.

the transferred peptide film. Second, the dichroism in which amide II is more intense than amide I shows that in the absence of a lipid matrix, the central axis of the ordered nanotubes is primarily parallel to the gold surface. The decrease in the intensity of the amide I perpendicular component is also accompanied by an increase in the intensity of its parallel component, amide $I_{||}$, at 1688 cm^{-1}. The opposite grazing angle IR dichroisms of the peptide nanotubes in and out of the lipid matrix suggest that the average molecular orientations in the two systems are orthogonal to each other (compare spectra a and c in Figure 5).

Crystalline nanotubes assembled from the cyclic peptide *cyclo*[(L-Glu-D-Leu)$_4$] which were prepared as previously described have been characterized by light and electron microscopy to be rod-shaped (approximately 10−30 μm long and 100−500 nm wide).[2a,d] The grazing angle IR spectrum of the peptide microcrystals applied onto the gold surface exhibits an amide II/amide I dichroism that is similar to, but more pronounced than, that of the Langmuir−Blodgett film of the peptide *cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] (compare Figures 6b and 5c, see Table 3). This dichroism, in which the amide II band is much more intense than the amide I band, shows that the peptide microcrystals are lying parallel to the surface in the absence of the lipid matrix. The more exaggerated dichroism exhibited by the crystals in comparison to that of the Langmuir−Blodgett films reflects the higher order of crystalline assembly and alignment on the surface. As expected for the parallel tube orientation and in contrast to the peptide in the lipid matrix, the transmission IR spectrum of the peptide crystals displays a much higher intensity for the amide I than for the amide II band (Figure 6c, Table 3). In fact, the transmission spectrum of the peptide crystals looks very similar to the grazing angle IR spectrum of the peptide in the lipid film (Figure 5a), reflecting the orthogonal nature of the two IR techniques. The orientation of the peptide crystals suggested by these IR methods is verified by scanning probe microscopy (SPM) images, which depict the crystals lying exclusively parallel to the surface (Figure 7). In contrast to the ordered techniques described above, the transmission IR spectrum of the ground nanotube crystals in a KBr pellet, in which the crystals are expected to be randomly oriented, displays intense amide I and amide II bands (Figure 6a). Together with the observations from the peptides in lipid multilayers, these results suggest that the elongated nature of the self-assembled peptide nanotubes predisposes them to lie with their central axis parallel to the surface, unless they are supported in an upright orientation by an appropriate supportive matrix such as lipid bilayers (Figure 8).

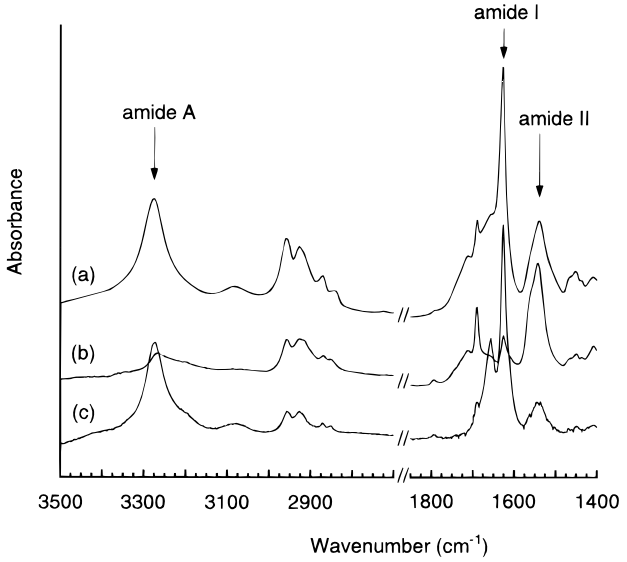

Figure 6. IR spectra of microcrystals of *cyclo*[(L-Gln-D-Leu)$_4$] by (a) transmission IR spectroscopy in a KBr pellet, (b) grazing angle IR spectroscopy on a gold surface, and (c) transmission IR spectroscopy on a CaF_2 plate. The spectra have been scaled to facilitate visual comparison.

Conclusion

Polarized ATR IR has given a quantitative estimate of the orientation of the peptide nanotube relative to the lipid membrane, and has shown that the central axis of the nanotube is approximately 7° from the bilayer normal. Qualitative analysis of the lipid/peptide system by grazing angle and transmission IR modes, and comparison to 2-D and 3-D ordered peptide systems in the absence of the lipid matrix, support the observation that the central axes of the tubes are approximately perpendicular to the bilayer normal in the functionally relevant lipid environment. In the absence of the supportive lipid matrix, the tubes were observed to lie parallel to the surface. This study provides direct structural evidence for the functional model hypothesis of the artificial nanotubular channels for which transmembrane channeling activity has been demonstrated. The study also demonstrates that the orientation of the nanotubes can be manipulated by the presence of lipids, giving a powerful parameter to use in nanotechnical applications, especially toward the design of novel biosensors.

Experimental Section

Peptide Synthesis. All reagents and solvents were purchased as previously reported,[2d] except tetra-*N*-butylammonium fluoride hydrate (TBAF, Aldrich), (*O*-(7-azabenzotriazol-1-yl)-1,1,3)tetramethyluronium hexafluorophosphate (HATU, PerSeptive Biosystems), and amino acids and 4-(hydroxymethyl)phenylacetamidomethyl (Pam) resin (Calbiochem-Novabiochem). *cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] was synthesized by solid-phase Boc chemistry on Pam resin following the protocols of Kent,[14] cleaved by TBAF,[15] and cyclized in solution with HATU. It was purified by reverse phase HPLC in a methanol/H_2O/trifluoroacetic

(14) Schnolzer, M.; Alewood, P.; Jones, A.; Alewood, D.; Kent, S. B. H. *Int. J. Pept. Protein Res.* **1992**, *40*, 180−193.

(15) Ueki, M.; Kai, K.; Amemiya, M.; Horino, H.; Oyamada, H. *J. Chem. Soc, Chem. Commun.* **1988**, 414−415.

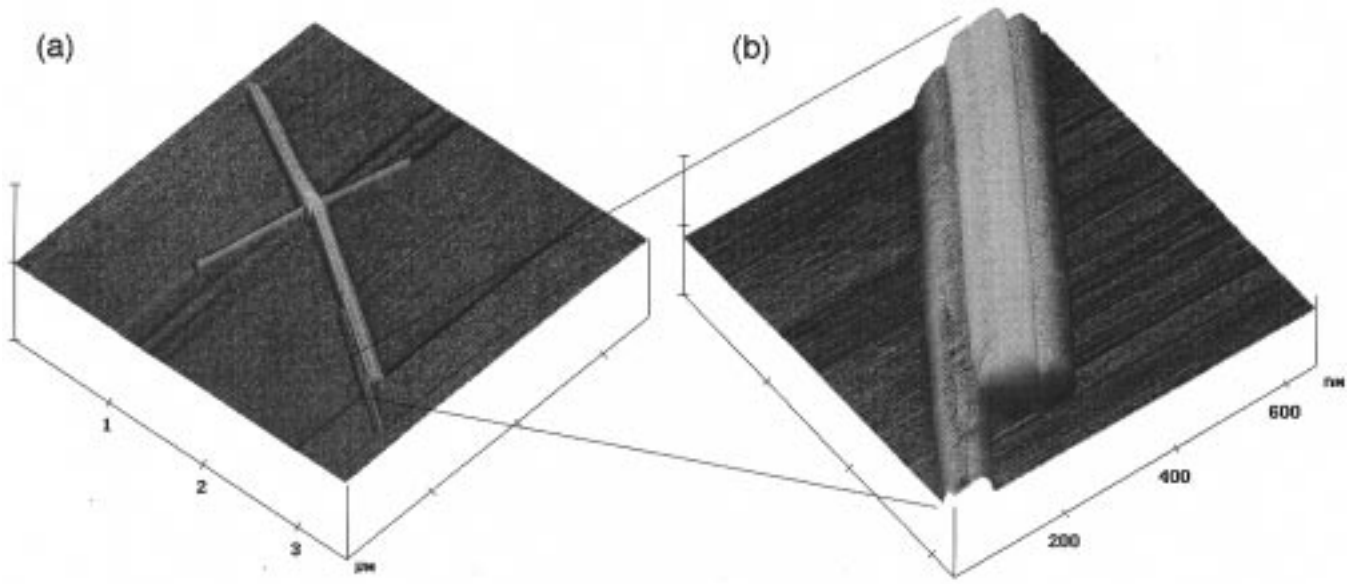

Figure 7. Scanning probe microscope (SPM) images of nanotube microcrystals on a glass surface obtained in the tapping mode. Part b is an expansion of the indicated portion of part a.

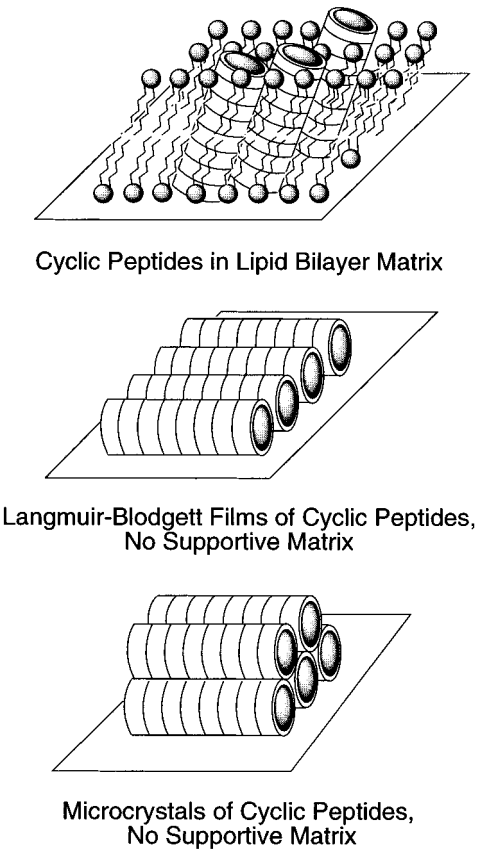

Figure 8. Pictorial representation of the effect of the lipid bilayer matrix on the orientation of the cyclic peptide nanotubes.

acid solvent system, and characterized as previously reported.[2d] *cyclo*[(L-Gln-D-Leu)$_4$] was synthesized, purified, and characterized as previously reported.[2d]

Peptide/Lipid Multibilayer Sample Preparation for IR. Vesicles were prepared from 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC, T_c = 24 °C, Avanti Polar Lipids, Inc.) by the reverse-phase evaporation method of Papahadjopoulos.[16] Briefly, to a solution of 20 mg of DMPC in 1:1 volume of chloroform/ether, 2 mL of ddH_2O was added. The suspension was sonicated to yield a milky white solution, then the organic solvents were rotovapped off to yield the aqueous vesicle suspension. The *cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu] peptide solution (10 mM in DMSO) was added to the vesicle suspension diluted 1:1 by volume with ddH_2O, to yield a final peptide:lipid molar ratio of 1:9 and DMSO content of less than 8% in the final volume. The mixture was vortexed vigorously, allowed to incubate at room temperature for 15−20 min, then gel-filtered through a fine-grade Sephadex G-25 (Pharmacia) column to remove the nonincorporated peptide. The filtrate was applied onto the appropriate FT-IR substrate (germanium crystal for ATR, gold-coated silicon wafer prepared as previously reported[12] for grazing angle, and CaF_2 plate for transmission IR). The lipid/peptide film was allowed to orient by air-drying at room temperature without disturbance.

Pure Peptide Sample Preparation for IR and SPM. To prepare the Langmuir−Blodgett film of *cyclo*[(L-Trp-D-Leu)$_3$-L-Gln-D-Leu], a Nima 611M/win Langmuir film balance trough was used. The peptide solution (0.1 mg/mL in ∼5% TFA/chloroform) was spread onto the ddH_2O subphase at room temperature and compressed to a pressure of 28 mN/m. An eight-layer film was transfrerred onto a gold-coated silicon wafer by dipping at the constant pressure of 28 nN/m and then allowed to dry at room temperature before the grazing angle IR analysis.

The peptide *cyclo*[(L-Gln-D-Leu)$_4$] was crystallized as previously reported.[2d] Suspensions of the peptide microcrystals in ddH_2O were applied onto a gold-coated silicon wafer for grazing angle IR, a CaF_2 plate for transmission IR, or a glass surface for SPM, then dried under a nitrogen stream prior to analysis. For the preparation of the KBr pellet for transmission IR, the peptide microcrystals were dried under vacuum and then ground with KBr powder.

SPM images were obtained with a multimode nanoscope IIIA scanning probe microscope (Digital Instruments) with use of tapping mode and Nanoprobe TESP (*T*apping Mode *E*tched *S*ilicon *P*robes, Digital Instruments) SPM tips at a scan rate of 2 Hz.

FT-IR Spectroscopy. Spectra were obtained on a Nicolet 550 Magna Series II FT-IR instrument equipped with a liquid nitrogen-cooled mercury−cadmium−telluride detector at a 2 cm^{-1} resolution and under nitrogen atmosphere. A total of 800 scans were averaged for each spectrum. For ATR IR, the instrument was equipped with a baseline horizontal ATR optical bench with a 45° germanium plate and a ZnSe polarizer (Spectra-Tech). For grazing angle IR, a grazing angle accessory with a fixed incidence angle of 80° was used (Spectra-Tech), with a ZnSe polarizer set to allow selective transmission of p-polarized light. For transmission IR, CaF_2 plates positioned at a 90° angle of incidence were used with unpolarized light.

(16) Szoka, F., Jr.; Papahadjopoulos, D. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4194−4198.

The spectral data were analyzed by Omnic 3.0 software (Nicolet). For the calculation of the dichroic ratios for the peptide and the lipid hydrocarbon chain, the spectral data in the peptide amide I and the lipid CH_2 stretch regions were Fourier deconvoluted and further analyzed by the deconvoluting software Lab Calc Search Arithmetic s2.22 (Galactic Industries Corp). Deconvolution was performed by using a single-component, mixed Lorentzian and Gaussian function with an iterative, linear least-squares algorithm.

Calculations of Orientation. The orientational analysis approximated for ordered thin films by ATR IR was carried out according to the method reviewed by Fringelli and Günthard,[8] using equations derived by Fraser.[17] This method has been used to determine the molecular orientation for a number of membrane-active peptide systems in ordered lipid multibilayer films.[4d−f,h,i] According to the convention used by Fringelli and Günthard and as depicted in Figure 3, parallel polarization is defined as the incident light components which are in the plane containing the incident and reflected radiation (plane containing the surface normal), and corresponds to the electric field components in the *x* and *z* coordinates. The perpendicular polarization corresponds to the electric field component in the *y* coordinate. The dichroic ratio *R* is defined as the ratio of the band intensity with parallel polarized incident light to the band intensity with perpendicular polarized light, and is related to the electric field amplitudes and the molecular orientation in the following way:

$$R = A_{||}/A_{\perp} = (E_x^2/E_y^2) + (E_z^2/E_y^2)[(f\cos^2\alpha + (1/3)(1-f))/((1/2)f\sin^2\alpha + (1/3)(1-f))]$$

E_x, E_y, and E_z are the electric field amplitudes for ATR which have been calculated by Harrick[18] to be:

$$E_y = [2\cos\phi]/[(1-n_{21}^2)^{1/2}]$$

$$E_x = [2\cos\phi\,(\sin^2\phi - n_{21}^2)^{1/2}]/[(1-n_{21}^2)^{1/2}((1+n_{21}^2)\sin^2\phi - n_{21}^2)^{1/2}]$$

$$E_z = [2\cos\phi(\sin\phi)]/[(1-n_{21}^2)^{1/2}((1+n_{21}^2)\sin^2\phi - n_{21}^2)^{1/2}]$$

The notations n_2 and n_1 are the refractive indices of the lipid film (1.50) and the germanium (4.03), respectively, and n_{21} is the ratio n_2/n_1. For our experimental setup, the angle of incidence ϕ is 45°, giving the following electric field component amplitudes: $E_x = 1.40$, $E_y = 1.52$, and $E_z = 1.64$. The parameter α is the angle between the transition moment and the molecular axis. The parameter *f* is the orientational order parameter, and is equal to $(3\cos^2\theta - 1)/2$, where θ is the average angle between the molecular axis and the surface normal.

The equation for the orientational order as derived by Fraser[17b] represents the effect of imperfect ordering by supposing that all the molecules in the sample are displaced by the same average angle from the surface normal, and is valid for the analysis of a well-ordered specimen. In this model, a symmetric, conical, uniaxial distribution of the measured axis around a central reference axis is assumed, in which the molecular axis is inclined at an average angle of θ from the surface normal, and transition moment being measured is oriented at an angle of α to the molecular axis. For the nanotube peptides, the molecular axis is the central, longitudinal axis of the cylindrical peptide tube, and the transition moment being measured is the amide I (perpendicular component). For the phospholipids, the molecular axis is the axis running through the hydrocarbon chain in the fully extended, all-trans conformation, and the transition moments being measured for the purposes of orientation calculations are the symmetric and antisymmetric CH_2 stretches. The average of the angles calculated from the two CH_2 stretches was used to determine the lipid chain orientation.

To fulfill the hydrogen-bonding requirements for the formation of the tubular ensemble and as determined structurally,[2a,d,e] the cyclic peptides in the nanotube assume a conformation in which the backbone carbonyl (and the NH) bonds are aligned parallel to the longitudinal axis of the nanotube. In an antiparallel β-sheet structure, such as that of the peptide nanotubes, the directionalities of the backbone carbonyl and the amide I (perpendicular component) coincide;[6] thus the angle α between the nanotube axis and the amide I (perpendicular component) transition moment is 0°. The transition moments of the lipid hydrocarbon CH_2 symmetric and antisymmetric stretches lie perpendicular to the hydrocarbon chain axis assuming that the chain axis is perpendicular to the plane of the CH_2 group,[8] so the angle α between the CH_2 transition moments and the hydrocarbon chain is 90°. The C═O stretch of the lipid head group is approximately parallel to the C═O double bond, and the antisymmetric PO_2^- double bond stretch is perpendicular to the bisector of the O−P−O angle.[8] Since these absorption bands were used to measure only the orientation of the indicated transition moment itself, not the orientation of the lipid molecule, relative to the surface normal, α is 0 for these groups.

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Supporting Information Available: Plots showing the dependence of the angle of molecular orientation (θ) on the dichroic ratio and on α (1 page print/PDF). See any current masthead page for ordering information and Web access instructions.

JA9735315

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(18) Harrick, N. J. *Internal Reflection Spectroscopy*; Interscience Publishers: New York, 1967.