Interfacial Ordering of Thermotropic Liquid Crystals Triggered by

the Secondary Structure of Oligopeptides

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Electronic Supplementary Information (ESI)

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Materials

Glass slides and hydrogen peroxide were purchased from Fisher Scientific (Pittsburgh, PA). The mesogen 4-cyano-4'-pentylbiphenyl (5CB), E7 (a mixture of three alkylcyanobiphenyls and one alkylcyanoterphenyl: 51 wt% 5CB, 25 wt% 4-cyano-4'-n-heptyl-biphenyl, 16 wt% 4-cyano-4'-noxyoctyl-biphenyl, and 8 wt% 4-cyano-4'-n-pentyl-p-terphenyl) and the reactive mesogenic monomer 4-(3-acryloyloxypropyloxy) benzoic acid 2-methyl-1,4-phenylene ester (RM257) were purchased from EM Sciences (New York, NY). N,N-dimethyl-N-octadecyl-3-aminopropyltrimethoxysilyl chloride (DMOAP; 42% in methanol), photo-initiator 2-dimethoxy-2-phenyl acetophenone (DMPAP), dodecyltrimethylammonium (DTAB), sulfuric acid (95%), and oligopeptide Alamethicin (with a minimum purity of 90%) were obtained from Sigma-Aldrich (St. Louis, MO). Texas red-labeled 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethyl-ammonium salt (DHPE) was purchased from Molecular Probes (Eugene, OR). 4'-octyl-4-biphenyl-carbonitrile (8CB) was purchased from Alfa Aesar (Ward Hill, MA). Oligopeptides Cecropin P1, Tachyplesin I, Cecropin A (1-8) - Melittin (1-18) hybrid peptide, Protegrin I and Lactoferricin B were purchased from Peptide2.0 (Chantilly, VA). Oligopeptide MSI-78 and MSI-594 were provided by the Marsh group and Ramamoorthy group at the University of Michigan, respectively. Phosphate buffer saline (PBS, pH 7.4) was obtained from Hyglos GmbH (Regensburg, Germany). L-dilauroylphosphatidylcholine (DLPC) was purchased from Avanti Polar Lipids, Inc. 20 µm-thick gold-coated specimen grids were purchased from Electron Microscopy Sciences (Hatfield, PA). Calcium fluoride prisms for SFG characterization were purchased from Altos (Bozeman, MT). Deionized water (resistivity of 18.2 MQ cm) was obtained using a Milli-Q system (Millipore, Bedford, MA).

Methods

Piranha cleaning of glass slides

Glass slides were Piranha-cleaned according to previously published methods.¹ Briefly, glass slides were immersed in piranha solution (70 v% v sulfuric acid and 30 v% v hydrogen peroxide) at $\sim 80^{\circ}$ C for 30 min. (Warning: Piranha solution reacts strongly with organic compounds and should be handled with extreme caution). The slides were subsequently rinsed with deionized water, and ethanol, and dried under nitrogen.

Preparation of DMOAP-coated glass slides

We functionalized the surfaces of the cleaned glass slides with DMOAP to induce a perpendicular alignment of LC at the LC—glass interface. First, we incubated the glass slides in aqueous DMOAP (1 v %/v) for 10 min. Next, we rinsed the glass slides with water followed by ethanol. Finally, we dried the glass slides with a stream of gaseous nitrogen.

Preparation of lipid-laden aqueous—LC interfaces

The lipid-laden aqueous—LC interfaces were prepared according to previously published procedures.² Briefly, a thin film of nematic 5CB was prepared by filling the pores of a 20 μ m-thick gold-coated specimen grid supported on a DMOAP- functionalized glass slide. Next, we incubated the supported LC-filled grid under PBS containing DLPC and DTAB (the concentration of DLPC and DTAB were 8 μ M and 2.5 mM, respectively) for 20 min to generate a lipid-laden LC film. Subsequently, we rinsed the interface of the LC with copious amounts of PBS to remove the DTAB from the interface of the LC.

Optical microscopy characterization of adsorption of oligopeptides at LC interfaces

A small volume of oligopeptide in PBS was added to an aqueous phase incubated against a lipid-laden LC film. For consistency, the final concentration of oligopeptides in the aqueous solution was held at 2 μ M, although exploratory experiments revealed the optical response of the LC to be independent on the oligopeptide concentration for concentrations between 2 μ M and 8 μ M. The optical appearance of the LC during adsorption of the oligopeptides at the aqueous— LC interface was recorded using an Olympus BX60 microscope (Center Valley, PA) equipped with crossed polarizers. Images were captured using a digital camera (Olympus C2020 Zoom) on the microscope. In addition, we determined the nematic—isotropic phase transition temperature of LC films by using polarized light microscopy. The transition temperatures of lipid-free and lipid-decorated 5CB films under water were measured to be 35.0 ± 0.1 °C, which is same as that of pure 5CB.³ This measurement indicates that any changes in the composition of the 5CB during our experiments do not impact the clearing temperature. After oligopeptides were adsorbed at the LC interface, no measurable difference in the nematic—isotropic transition temperature was measured. We also comment here that the thicknesses of 5CB films used in our study are 20 µm, which is substantially thicker than the LC films used in past studies of pretransitional wetting of 5CB.⁴



Fig. S1 Optical micrographs (crossed polarizers) of DLPC-laden aqueous—LC interfaces in contact with aqueous PBS. Inset in (B) is a conoscopic image confirming homeotropic alignment. Scale bars: 100 μm.

Fluorescence microscopy of optical response of LCs to adsorption of oligopeptides

First, we incubated the LC-filled grid under aqueous PBS containing Texas red-labeled DHPE and DTAB (the concentration of Texas red-labeled DHPE and DTAB were 8 μ M and 2.5 mM, respectively) for 25 min. Next, we exchanged the Texas red-labeled DHPE—DTAB aqueous solution with PBS buffer solution. Subsequently, PBS containing Cecropin P1 was incubated against the lipid-laden aqueous—LC interface. The final concentration of Cecropin P1 in PBS was 2 μ M. Finally, the sample was imaged using an Olympus IX71 inverted fluorescence microscope equipped with a 100 W Mercury lamp. A fluorescence filter cube with an excitation of Texas red-labeled DHPE. The images were collected with a Hamamatsu 1394 ORCA-ER CCD camera (Brightwater, NJ) connected to a PC and controlled via SimplePCI imaging software (Compix, Inc., Cranbury Twp, NJ).

Fractal dimension analysis

A "box-counting" method was used to calculate the linear-related fractal dimension *D* from the shape of the optical domains observed in the optical micrographs.⁵ Briefly, we cropped a ~ 350 * 300 pixels image from the original micrograph. Next, we assembled square boxes over the border of each optical domain using boxes with different size (2, 3, 4, 6, 8, 12, 16, 32, 64 pixels). The number of boxes required to cover the border is dependent on the size of the box. The number of boxes of each size required to cover the border (log scale) was plotted against the size of each box (log scale). A straight line with negative slope S is obtained. *D* is calculated as D =1 - S. The "box-counting" method was performed by using ImageJ. Fig. S2 showed an example of calculation of *D* in the case of Cecropin P1 or Cecropin A-Melittin hybrid peptide.



Fig. S2 PL micrograph of an ellipsoidal domain formed by (A) Cecropin P1 or (D) Cecropin A-Melittin hybrid peptide at L-DLPC-laden aqueous—LC interfaces. (B and E) Selection of the border of the ellipsoidal domain. (C and F) Fractal dimension *D* of the border calculated by the plot of number of boxes (in log) against size of boxes (in log).

Preparation of polyRM257-stabilized LC films

The experimental system described above (LC hosted within the pores of a TEM grid) could not be used for the SFG measurements because the scattering of light from the specimen grid interfered with the SFG signal. To form LC films that could be immersed under PBS without dewetting, we formed a LC polymer network within LC films. We mixed toluene with 20 wt/wt % LC mixture, which consisted of 70 wt % 8CB and 30 wt % RM257. The photo-initiator DMPAP was added at 5 wt/wt% based on the mass of RM257. Next, 50 µL of the LC toluene solution was applied to the prism, followed by a spin coating process at 3,000 rpm for 1 min. Finally, the film was exposed to UV light for 10 min. Photo-polymerization was performed using a UV lamp (365 nm) that delivered 2.5 mW/cm². The polyRM257-stabilized LC films were observed to be stable under an aqueous phase for at least 4 h, as shown in Fig. S3. DLPC-laden polyRM257stabilized LC films were prepared by incubation of the films under PBS containing 20 µM L-DLPC for 30 min, followed by rinsing with deionized water to remove excess L-DLPC. We note that both 5CB and 8CB films share the same chemical functionality and present predominantly either nitrile or aliphatic groups to aqueous solutions or lipid monolayers, respectively. We also comment that the molecular area and two-dimensional elasticity of monolayers of 5CB and 8CB formed at the aqueous surface are similar.⁶



Fig. S3 Polarized light micrographs of (A) pure 5CB film or (B) polyRM257-stabilized 8CB film on calcium fluoride prism after immersing into PBS buffer solution for (A) 60 s or (B) 4 h. Scale bars: 100 µm.

Sum frequency generation (SFG) vibrational spectroscopy

SFG is uniquely well-suited to characterize macromolecules at interfaces.⁷ Two pulsed laser beams, one with a fixed frequency in the visible frequency range (ω_{Vis}), and the other with a tunable frequency in the infrared frequency range (ω_{IR}), are overlapped on the aqueous—LC interface (Fig. S4).⁸ Briefly, sum frequency generation is a process in which two input beams

 $(\omega_{Vis} \text{ and } \omega_{IR})$ mix in a medium and generate an output beam at the sum frequency $\omega_{sum} = \omega_{Vis} + \omega_{IR}$.⁹ This is a second-order non-linear optical process, and the selection rules result in SFG signals only being generated from a medium with no inversion symmetry under the electric dipole approximation.¹⁰ Most bulk media possess this inversion symmetry (due to the random orientation of molecules), and thus contribute no SFG signal.¹¹ On the other hand, molecules at interfaces are located in an asymmetric environment, which leads to the generation of a SFG signal.¹²⁻¹⁴ SFG is therefore a surface (interface)-sensitive non-linear optical vibrational spectroscopic technique, and it has been successfully applied previously to examine the conformation and orientation of peptides at the aqueous—lipid bilayer and aqueous—polymer interfaces.⁸⁻¹³ The peptide secondary structure can be characterized by analyzing the SFG signal in the amide I frequency region (1500 cm⁻¹ to 1800 cm⁻¹).⁸⁻¹³ By collecting SFG spectra using different combinations of the polarization of the input/output beams and by fitting the collected spectra, it is also possible to infer the apparent orientation of peptides/proteins relative to the surface.

The design of our SFG spectrometer has been described elsewhere, and detailed descriptions of the principles underlying SFG can be found in prior publications.⁷⁻¹⁴ Both the DLPC-decorated LC films as well as DLPC-free LC films (stabilized by polymerization, see above) were used as substrates in the SFG experiments. The LC interfaces were contacted with a reservoir filled with 2 mL of ultrapure water. A specified volume of aqueous solution containing oligopeptide was injected into the subphase water, and allowed to diffuse to the LC surface over 30 min. The final peptide concentrations used in our SFG experiments are shown in Table S1. SFG spectra from oligopeptides adsorbed at the aqueous—LC interface were collected at room temperature (24 °C) using a near total internal reflection geometry with ssp (s-polarized output SFG signal, s-polarized input visible beam, and p-polarized input IR beam) and ppp polarization combinations. We chose such polarization combinations because the ratio of ppp and ssp intensity is related to the peptide orientation. Additional details can be found in prior publications.⁷⁻¹⁴



Fig. S4 Schematic illustration of the SFG experimental geometry.

Oligopeptide	At lipid-laden aqueous—LC interface	At bare aqueous—LC interface					
Cecropin P1	2 µM	36 µM					
MSI-594	8 µM	8 µM					
MSI-78	20 µM	20 µM					
Alamethicin	2 µM	30 µM					
Tachyplesin I	40 µM	40 µM					
Cecropin A-Melittin	2 µM	88 µM					
Protegrin I	40 µM	40 µM					
Lactoferricin B	40 µM	40 µM					

Table S1. Concentration of oligopeptides used in SFG measurement

Results

Quantification of coverage of lipid monolayer at aqueous—LC interfaces.

The lipid monolayer coverage at aqueous-LC interfaces was quantified according to previously published methods.¹⁵ Briefly, lipid monolayers comprised of pure DLPC or DLPC— DTAB mixture were formed at the aqueous—5CB interface according to the procedures described in the Experimental section. In both samples, Texas red-labeled DHPE (2% mol / mol based on DLPC) was added as fluorescent probe. When amphiphilic molecules adsorbed at the LC interfaces, the amphiphiles organized to present their hydrophobic aliphatic tails towards the LC phase, leading to interdigitation of LC mesogens and aliphatic tails with the subsequent homeotropic anchoring of LCs.^{2,15-17} After formation of lipid monolayers, we rinsed the LC interfaces to remove amphiphiles in the supernatant and DTAB from the interfaces. We comment here that DTAB can be further removed from the interfaces by repeat rinsing because it forms Gibbs monolayer, whereas DLPC forms Langmuir monomer that makes it stable against rinsing.¹⁸ Therefore, after rinsing, the preparation process involving pure DLPC gave rise to a full coverage monolayer of DLPC, whereas the procedure with DLPC-DTAB resulted in a partial monolayer of DLPC. Fluorescence imaging was performed using a 20x objective and an exposure time of 0.1 s. The fluorescence intensities (gray scales) from the samples prepared by pure DLPC and DLPC-DTAB mixture were measured to be ~ 203 and ~ 169, respectively. Based on these values, we calculate the lipid monolayer coverage to be 169 / 203 = -83 %. We also comment here that the elasticity of LCs and the mobility of phospholipids permit reorganization of adsorbed peptides, which can be detected by using crossed-polarized microscope.

Fluorescence microscopy of optical response of LCs to adsorption of oligopeptides

As described in main text, we interpret the planar anchoring regions (bright optical domains in Fig. 1 and 2) to be oligopeptide-rich, whereas the matrix of a dark optical appearance of the LC surrounding the domains is rich in phospholipids. Here we use fluorescence microscopy to support our statement. A monolayer of Texas red[®]-labeled DHPE, instead of DLPC, was prepared at aqueous—LC interfaces. We characterized the optical appearance of the LC interface with adsorption of Cecropin P1. Inspection of Fig. S5 reveals that bright ellipsoidal domains and dark matrix observed in PL image (Fig. S5A) coincide with the regions exhibiting low and high

intensity in fluorescence signal in fluorescence micrograph (Fig. S5B), respectively. The above observation supports our statement that the planar anchoring regions in PL micrographs were oligopeptide-rich.



Fig. S5 (A) Polarized light and (B) fluorescence micrographs of optical appearance of Texas red[®]-labeled DHPE-laden aqueous—LC interface to adsorption of Cecropin P1. The concentration of Cecropin P1 was 2 μ M. The images were captured at 1 hr after addition of Cecropin P1. Scale bars: 100 μ m.

Influence of LC elasticity on formation of optical domains by oligopeptides at LC interfaces

LC films recapitulate some of the physics of biological membranes such as the elasticity of biological membranes. In our experiments, we see conceptual connections between the influence of the elasticity of the LC on the behavior of adsorbates and the influence of the elasticity of biological membranes on positioning of constituents (e.g., oligopeptide and lipid constituents). Here, we performed one additional experiment to provide insight into the role of the elasticity of the LC on the formation of optical domains by adsorbed peptides at aqueous—LC interfaces. First, DLPC-decorated films of 5CB were prepared as described in the main text. Next, the 5CB films were heated to form an isotropic phase, and a small amount of Cecropin P1 or Cecropin A-Melittin hybrid peptide was added to the aqueous phase above the isotropic 5CB film. After 30 min, we cooled the film in the nematic phase to characterize the dynamic response of LC using PL microscope.

The results are shown in Fig. S6. When the 5CB was heated into the isotropic phase (38°C), the PL micrographs appeared dark (crossed-polars), as shown in Fig. S6A. Upon cooling the sample to T_{NI} , the sample appeared (Fig. S6C) bright between crossed polars consistent with formation of the nematic phase. As shown in Fig. S6E, the initial optical texture of the LC was consistent with tilting of the LC at the interface rather than homeotropic anchoring. We make two comments on the origin of bright optical texture at the interface. First, the tilting of LC is

consistent with adsorption of Cecropin P1 at the lipid-laden aqueous—LC interface and disruption of homeotropic anchoring of the LC. Second, the absence of domains, as shown in Fig. 1 of the main text, suggests that the distribution of peptides over the interface is initially uniform following the quench into the nematic phase. Over the course of the next 3-4 min (Fig. S6G, I, K and M), we observed the formation and coalescence of domains across the sample, resulting in elliptical domains with sizes in the range of tens of micrometers, which are same as those observed in Fig. 1 and 2 in the main text. In contrast, Cecropin A-Melittin hybrid peptide gave rise to dendritic domains within the course of 3-4 min after cooling back to nematic phase (Fig. S6H, J, L and N). We repeated the experiments with E7 instead of 5CB at 38 °C (the nematic—isotropic phase transition temperature of E7 is 59 °C), and we observed the formation of oligopeptide-rich domains at lipid-decorated aqueous—E7 interfaces. These results suggest that the domains rich in oligopeptide only form in the presence of the nematic phase.

In addition, we used fluorescence microscope to confirm that domains of oligopeptides and lipids did not form when 5CB was in an isotropic phase, and that domain formation was induced by the presence of nematic order in the 5CB. Specifically, a monolayer of Texas red[®]-labeled DHPE was deposited at the aqueous-LC interfaces, as described above. Next, the 5CB film was heated to 38 °C to form an isotropic phase and a small amount of Cecropin P1 was added to the aqueous phase above the isotropic 5CB film. After 30 mins, the film was cooled to nematic phase. During this process, we imaged the LC interface using fluorescence microscopy. When the 5CB was heated into the isotropic phase, the fluorescence micrograph appeared uniformly bright, as shown in Fig. S7A and B, indicating the absence of domains at the interface. However, after the sample was cooled to the nematic phase, we observed the formation of ellipsoidal domains with low fluorescence intensities, as shown in Fig. S7C and D, corresponding to the oligopeptide-rich domains (see previous section). Previous studies have reported the presence of long-range interactions between adsorbates (molecules and colloids) suspended in a nematic solvent or adsorbed at a nematic interface. These interactions can lead to formation of assemblies.^{2, 15-17, 19-31} The interactions are mediated by distortions of the LCs (elasticity) in the vicinity of the adsorbates. These long-range interactions are not present when adsorbates are suspended in an isotropic medium or adsorbed at an isotropic interface. Guided by these previous studies, our experimental observations suggest that formation of domains of oligopeptides at LC interfaces is driven by nematic order-mediated long-range interactions. This result leads us to

conclude that the shapes of the interfacial oligopeptide domains depend on the secondary structure of oligopeptides, the elasticity and the lateral mobility of LC film.



Fig. S6 PL micrographs of the dynamic response of the LC to the adsorption of (A, C, E, F, G, I, K, M) Cecropin P1 or (B, D, F, H, J, L, N) Cecropin A-Mellitin hybrid peptide at a DLPC-laden aqueous—5CB interface. The times at which the micrographs were taken are indicated after injection of Cecropin P1 into the aqueous phase. The 5CB was incubated at isotropic phase

(38°C) in the first 30 mins, and then cooled down to nematic phase. The final concentration of peptides is 2 μ M. Scale bars: 50 μ m.



Fig. S7 (A and C) Polarized light and (B and D) fluorescence micrographs of the optical appearance of Texas red[®]-labeled DHPE-laden aqueous—LC interfaces following adsorption of Cecropin P1 at (A and B) the isotropic or (C and D) nematic 5CB interface. The concentration of Cecropin P1 was 2 μ M. Scale bars: 100 μ m.

In addition, we calculate the time required for domain formation by diffusion (in the absence of elasticity of LCs) as

$$t=x^2/4d$$

in which x is the distance of diffusion (300 μ m; the size of the pores of the grid), d is the lateral diffusivity of the lipids (1 x 10⁻¹² m²/s).¹⁵ We calculate t ~ 3 hours, which is long compared to the time over which the domains form in Fig. S6. This result suggests that the elasticity of the LC also likely influences the dynamics of the transport processes underlying the segregation of the lipid on the interface of the LC.

Fractal dimensions analysis

We used a linear-related fractal dimension (D)⁵ to quantify the shape of the optical domains of LCs induced by adsorption of oligopeptides. Briefly, the value of *D* for a spherical domain is close to 1, whereas the value of *D* for a dendritic domain is close to 2. We employed the "boxcounting" method to calculate *D* of the optical domains in micrographs shown in the main text. As shown in Fig. 4 (main text), three oligopeptides rich in α -helix (Cecropin P1, MSI-594 and MSI-78) generate ellipsoidal domains with *D* between 1.05-1.20, whereas three oligopeptides rich in β -sheet and β -turn (Tachyplesin I, Lactoferricin B and Protegrin 1) generate elongated domains with *D* between 1.47-1.62. In addition, two more peptides (Alamethicin and Cecropin A-Melittin hybrid peptide) that adopted neither α -helix nor β -sheet/ β -turn, generate *D* falling out of the range of α -helix (1.05-1.20) and β -sheet/ β -turn (1.47-1.62). These results clearly show that *D* is able to determine α -helix and β -sheet/ β -turn structures.

SFG spectra collected from polymer-stabilized 8CB films

SFG spectra collected from polymer-stabilized 8CB films (no adsorbed DLPC) are shown in Fig. S8. Both in air and in PBS buffer solution, a peak at ~1610 cm⁻¹ is observed, which coincides with vibration of $-CH=CH_2$ (vinyl group) in previous IR measurement.³² We note that we observe this peak in a subset of the SFG spectra (Fig. 3C and F).



Fig. S8 SFG spectra collected from bare polymer-stabilized 8CB film in (A) air or (B) water. (C) Molecular structure of RM257.

SFG signals from oligopeptides at the aqueous—LC interface

Both ppp and ssp SFG signals were collected from peptides at the aqueous—LC interface. The SFG spectra were fitted using the following equation:⁹

$$\chi_{eff}^{(2)} = \chi_{nr} + \sum_{q} \frac{A_q}{\omega_2 - \omega_q + i\Gamma_q}$$
 Equation

where ω_2 and ω_q represent the frequencies of the infrared beam and the qth vibrational peak center, respectively. Γ_q is the damping coefficient (or peak width), χ_{nr} is the nonresonant background, and A_q is the signal strength.⁹ The peak center of the SFG signal is related to the secondary structure of the peptide. After fitting the spectra using the above equation, the peak centers were determined for various SFG spectra. We used Equation 1 to fit all the SFG spectra presented in this Communication, and the fitting parameters are shown in Table S2.

Table S2. Fitting parameters used in SFG measurement																				
Cecropin P1 Fig. 3A					χnr			A ₁					ω_1 (cm ⁻¹)				$\Gamma_1 (\text{cm}^{-1})$			
PP	P		+	0.19			44.43					1658				12				
SS	P		+	0.39				36 32						1658			13			
																I				
MSI-594 Fig. 3B					χnr			A ₁					(ω_1 (cm ⁻¹	¹)		Γ_1 (cm ⁻¹)			
PP	P D				-0.06			96.84						1658						
SS	P		-		0.00			65.17				1658					14			
										-						I				
MSI-78 Fig. 3C		χ	χnr		A	1	0	ω_1 (cm ⁻¹)		$\Gamma_1 (\text{cm}^{-1})$		n ⁻¹)	A ₂			$\omega_2 (\text{cm}^{-1})$		$\Gamma_2 (\text{cm}^{-1})$		
PPP		2.	42		31.	07		1610			8		155 85			1658		18		
SSP		0.	96		28.	26		161	0	8			1	127.51		1658		18		
									•					127.01			-			
Lac. B Fig. 3D	χn	ır	А	-1	ω_1		[(c)	Γ_1 A		-2	$2 \qquad \qquad$		Γ_2			A ₃	۵ (cm	3 -1)	Γ_3	
ppp	-0.2	29	8	8 1600		$\frac{1}{00}$	(0	7 7		7	1635			16		18	166	53	$\frac{(\cos \beta)}{23}$	
SSP	_0 ($\frac{2}{17}$	3	,	16	00		$\frac{7}{7}$		1	1635			$\frac{10}{16}$ $\frac{10}{22}$		23	1663		23	
551	0.0	57		,	1000			/		<u>r 1055</u>		055	10			25	100		25	
Alamethicin χ Fig. 3E		χ_{nr}		A ₁			ω_1 (cr		Γ	Γ_1 (cm ⁻¹)			A ₂		$\omega_2 (\text{cm}^{-1})$		$\Gamma_2 (\text{cm}^{-1})$			
PPP	PPP -0.3		0.87	7	29.90			16	42		13			138.21		16	58		21	
SSP		-	0.04	ŀ	22.84			1642		13		3	123.36		1658		58 21		21	
	•																			
Hybrid peptide Fig. 3F	χr	ır	A	A ₁ (c		(ω_1) (cm^{-1})		$[\Gamma_1] \qquad A$ cm ⁻¹)		A ₂	(c	(cm^{-1})		$\begin{array}{c c} \Gamma_2 \\ (cm^{-1}) \end{array}$		A ₃	ω ₃ (cm ⁻¹)		Γ_3 (cm ⁻¹)	
PPP	2.7	77	29	.18	16	510		6	62	.87	1	1655		14		13.38 10		53	6	
SSP	2.5	51	9.	81	16	510		10	109	9.90	1	655		18	19.34		1663		8	
Tachyplesin 7 Fig. S9A		χ	nr	A ₁		ω (cm	1 -1)	Γ_1	-1)	A ₂		(cm^{-1})		Γ_2 (cm ⁻¹)	A ₃		(cr) ₃ n ⁻¹)	Γ_3 (cm ⁻¹)	
SSP	SSP 3		02	29	9.06 160)5	10		47.8	6	1635		5 20		45.19	16	63	20	
				A	A 4	ω (cm	4 -1)	Γ ₄ (cm	-1)	A ₅		ω ₅ (cm ⁻¹)	Γ_5 (cm ⁻¹)						
SSP			16	6.23 168		33	20)	4.78	3	1710		15							

Our group has performed extensive SFG research to study peptides at either aqueous—lipid bilayer or aqueous—polymer interfaces; these past studies provide reference behaviors with which we can compare measurements obtained at the aqueous—LC interface.⁸⁻¹³ In our previous work, we found that the SFG amide I peak centers from melittin, magainin 2, and MSI-78 associated with lipid bilayers are at 1655, 1657, and 1650 cm⁻¹, respectively;^{8,9,11} the SFG peak center from Cecropin P1 on polymer surface was around 1650 cm⁻¹.¹⁰ These amide I signals were assigned to an α -helical structure on the basis of comparisons to other analytical techniques. Accordingly, we assign the SFG signals at 1658 cm⁻¹ for Cecropin P1, MSI-78 and MSI-594 at the aqueous—LC interfaces (see Fig. 3 and Table 1 in the main text) to the α -helical structure. The slight difference for this peak center is likely due to the limited resolution of the SFG spectrometer (~ 5 cm⁻¹).

In our previous work, we also collected SFG signals from Tachyplesin I at the aqueous lipid bilayer interface: the 1635 cm⁻¹ peak was assigned to the B2 mode of the β -sheet structure, and the 1665 cm⁻¹ peak was attributed to the β -turn structure.¹³ In this work, we used five peaks to fit the ssp SFG signal detected from β -sheet oligopeptides (Tachyplesin I, Lactoferricin B and Protegrin 1) at the bare aqueous—8CB interface: The 1605 cm⁻¹ peak comes from the LC film (-CH=CH₂ of polyRM257); the 1635 cm⁻¹ peak is contributed by the B2 mode of the β -sheet structure; the 1663 cm⁻¹ peak comes from the β -turn structure; the 1683 cm⁻¹ peak comes from the B1 mode of the β -sheet structure; and the 1710 cm⁻¹ peak arises from the B3 mode of the β sheet structure. The SFG signal of β -sheet oligopeptides is dominated by contributions from the B2 mode of the β -sheet structure (1635 cm⁻¹) and the β -turn structure (1663 cm⁻¹). For Lactoferricin B, we observed two amide I peaks at ~ 1635 cm⁻¹ and ~ 1663 cm⁻¹, corresponding to β -sheet/ β -turn content, respectively. For Protegrin 1, no SFG signal was obtained from 10 independent measurements, as shown in Fig. S9B. For Tachyplesin I, a weak ssp signal was measured but no signal for ppp, as shown in Fig. S9A. Interestingly, although the Tachyplesin I signal was noisy, by using parameters obtained for the fit of the Lactoferricin B, we were able to obtain a reasonable description of the data consistent with β -sheet/ β -turn content (see Table S2 for fitting parameters). Overall, these results provide evidence that oligopeptides that generate elongated LC domains contain β -sheet/ β -turn content. In addition, our results indicate that either the oligopeptides are adsorbed with a distribution of orientations that give rise to a weak signal or the hyperpolarizability of the β -sheet structures is low. We comment here that ssp and ppp

probe different components of the second order nonlinear optical susceptibility, and thus they can have different behaviors.

Our previous research found that the SFG signal detected from Alamethicin at the aqueous lipid bilayer interface comes from two different secondary structures (α -helical and 3₁₀-helical segments).¹² The SFG peak at 1638 cm⁻¹ was attributed to the 3₁₀-helical structure.¹² Accordingly, the SFG peak detected at 1642 cm⁻¹ from Alamethicin at the aqueous—LC interface (see Fig. 3 and Table 1 in the main text) is assigned to a 3₁₀-helical structure, while the SFG peak at 1658 cm⁻¹ is associated with the presence of α -helical structure.

For Cecropin A-Melittin hybrid peptide, three peaks were used to fit the SFG signal: 1610 cm⁻¹ (from –CH=CH₂ of polyRM257), 1655 cm⁻¹ (from α -helical structure) and 1663 cm⁻¹ (from β -turn structure). Therefore, we conclude that Cecropin A-Melittin hybrid peptide adopted α -helical and β -turn structures at LC interfaces.



Fig. S9 SFG spectra collected from aqueous—LC interfaces with adsorbed (A) Tachyplesin I or (B) Protegrin 1.

SFG spectra collected from DLPC-decorated aqueous—8CB interfaces with adsorbed oligopeptides

The SFG spectra of oligopeptides adsorbed at LC interfaces decorated with DLPC are shown in Fig. S10. Measurements were performed on both DLPC-free (shown in Fig. 3 in the main text) and DLPC-decorated LC interfaces to explore the effect of interfacial environment on the conformational states of the oligopeptides, and in particular, the extent to which the LC provides an interfacial environment that is similar to a lipid monolayer.

The SFG spectra of oligopeptides adsorbed on DLPC-decorated aqueous—LC interfaces are similar to those measured on DLPC-free interfaces (Fig. 3 in the main text). For example, for

Cecropin P1, we measured an amide I peak at around 1658 cm⁻¹ on bare LC films (Fig. S10A), which is consistent with an α -helical secondary structure. A similar signal was observed for MSI-594 and MSI-78 (Fig. S10B and C), which reveals the secondary structures of these LC-adsorbed oligopeptides to be similar to that found in a lipid bilayer. For Alamethicin (Fig. S10G), we observed amide I peaks at ~1642 cm⁻¹ and ~1658 cm⁻¹, corresponding to 3₁₀-helical and α -helical secondary structure, respectively. Finally, for the Cecropin A-Melittin hybrid peptide, amide I peaks at ~1655 cm⁻¹ and 1663 cm⁻¹ were observed, which correspond to α -helical and β -turn structures, respectively, as shown in Fig. S10H.



Fig. S10 SFG spectra collected from DLPC-laden aqueous—8CB interfaces with adsorbed oligopeptides: (A) Cecropin P1; (B) MSI-594; (C) MSI-78; (D) Lactoferricin B; (E) Tachyplesin I; (F) Protegrin I; (G) Alamethicin; (H) Cecropin A-Melittin hybrid peptide.

Here we comment that experiments performed with Lactoferricin B, Tachyplesin I or Protegrin 1 at DLPC-laden aqueous—8CB interfaces (Fig. S10D-E) did not result in a measureable SFG signal. We did, however, observe LCs to reorganize upon adsorption of the above oligopeptides at lipid-laden aqueous—5CB interfaces (see Fig. 1 and 2 in the main text), indicating that these oligopeptides were adsorbed to these interfaces. We speculate that the absence of SFG signal indicates that either the oligopeptides are adsorbed with a distribution of orientations that give rise to a weak signal or the hyperpolarizability of the β -sheet structures is low.

Overall, these results suggest that the interfacial environment defined by DLPC-free and DLPC-decorated LC interfaces has a similar effect on the conformational states of the oligopeptides (with exception of Tachyplesin I and Lactoferricin B), and that the interfacial environment of both LC interfaces (DLPC-free and DLPC-decorated) is similar to a lipid bilayer.

SFG spectra collected from oligopeptide-adsorbed air—LC interfaces

The DLPC-laden aqueous—8CB films were removed from the peptide solutions and exposed to air. After the films were dry, ppp and ssp SFG spectra were collected (Fig. S11). The SFG signal at 1610 cm⁻¹ comes from the LC film (–CH=CH₂ of polyRM257) and is not related to the secondary structure of the peptides. Comparing Fig. S11 to Fig. 3 in the main text, it can be seen that the SFG amide I signals detected from peptides disappear. We believe that this is due to the random orientation of the peptides on LC in air.



Fig. S11 SFG spectra of (A) Cecropin P1, (B) MSI-78, (C) MSI-594, (D) Alamethicin, (E) Cecropin A-Melittin hybrid peptide, (F) Lactoferricin B, (G) Tachyplesin I and (H) Protegrin 1 at the air—DLPC-laden 8CB interfaces.

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