

Hydrogen-Bonded Supramolecular Assembly of Didodecylmelamine and Thymine at the Air/Water Interface Studied by Heterodyne-Detected Vibrational Sum Frequency Generation

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ABSTRACT

Langmuir monolayers of didodecylmelamine (2C₁₂mela) on thymine aqueous solutions were studied by *in situ* heterodyne-detected vibrational sum frequency generation (HD-VSFG) spectroscopy. The HD-VSFG spectrum of the monolayer on a 10 mM thymine aqueous solution exhibited a positive band at 1665 cm⁻¹, a negative band at 1710 cm⁻¹, and an extremely broad, positive band at the low wavenumber side (2600-2850 cm⁻¹) of the CH, NH, and OH stretching region. The three bands were assigned to the 4-carbonyl, 2-carbonyl, and strongly hydrogen-bonded NH stretching modes of thymine. The signs of the bands indicate that thymine molecules were oriented with the oxygen atoms of the 4-carbonyl pointing downward, the oxygen atoms of the 2-carbonyl pointing upward, and the hydrogen atoms of NH groups of thymine pointing upward along the interface normal, respectively. The spectral data, supported by finite-cluster spectral simulations, favor a polymer-like supramolecular structure over trimeric models. In this model, thymine molecules are bound to neighboring 2C₁₂mela molecules in an approximately 1:1 arrangement, with their methyl group pointing downward along the interface normal and their 4-carbonyl groups sharing a common lateral orientation along the supramolecular chain.

INTRODUCTION

Complementary hydrogen bonds play a key role in a wide range of molecular systems, from biological assemblies to designed artificial supramolecular systems. The binding constants of complementary hydrogen bonds at the air/water interface are reported to be significantly (10⁶-10⁷ times) larger than those in bulk water,¹ making the air/water interface a favorable environment for efficient complementary hydrogen bonding. Several supramolecular structures formed at the interface via complementary hydrogen bonds have been reported, including those between phosphoric acid and guanidinium,¹⁻⁴ guanine and cytosine,⁵⁻⁹ and melamine and barbituric acid (BA)¹⁰⁻¹⁵ headgroups. In particular, didodecylmelamine (2C₁₂mela) and BA form a highly specific 1:1 supramolecular polymer at the air/water interface, as shown in Fig. 1. In the first report of this structure by Koyano and co-workers,¹⁰ however, only Langmuir-Blodgett multilayers on solid substrates were measured, and no *in situ* spectroscopic measurement at the air/water interface was performed.

We previously studied Langmuir monolayers of 2C₁₂mela on a 10 mM BA aqueous solution by heterodyne-detected vibrational sum frequency generation (HD-VSFG) spectroscopy aided

by *ab initio* molecular dynamics simulations.¹¹ This work established the foundation for the present study on the 2C₁₂mela-thymine system. VSFG relies on a second-order nonlinear optical process, and VSFG signals can therefore be generated only from regions lacking centrosymmetry, such as interfaces, under the electric dipole approximation. VSFG spectroscopy enables the selective observation of molecules at the air/water interface without contributions from molecules in the bulk solution, including water and dissolved solutes.^{11, 16} Using the heterodyne-detection method, the SFG signal generated from the sample is interfered with a reference beam used as a local oscillator (LO), allowing both the amplitude and phase of the signal to be measured. Under electronically nonresonant conditions, the phase of the vibrationally resonant response is represented by its sign, which provides information about the absolute orientation of surface species.¹⁷⁻²¹

The HD-VSFG spectrum of Langmuir monolayers of 2C₁₂mela on a 10 mM BA aqueous solution in the SSP polarization combination exhibited two unique bands: a negative band at 1710 cm⁻¹ and a broad positive band at 2500-2950 cm⁻¹, which is at unusually low wavenumbers for an NH stretching band. The former band was assigned to the 2-carbonyl stretching vibration of BA, and the negative sign indicates that BA molecules were oriented with the oxygen atom of the 2-carbonyl group pointing upward. The latter band was assigned to the NH stretching vibration of BA, and its positive sign indicates that the hydrogen atoms of the NH groups pointed upward. From these results, we concluded that 2C₁₂mela and BA form an alternating, one-dimensional hydrogen-bonded supramolecular polymer at the air/water interface (Fig. 1).

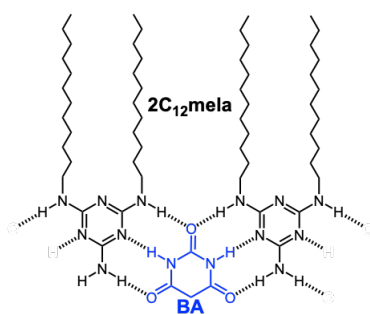


FIG. 1. Schematic representation of the proposed alternating 1:1 hydrogen-bonded supramolecular polymer of 2C₁₂mela and BA at the air/water interface.

Thymine, a nucleobase, has fewer hydrogen-bonding sites than BA, which makes it an interesting comparison to the well-characterized 2C₁₂mela-BA system. Due to this structural difference, multiple possible structures of melamine amphiphiles and thymine can be considered at the air/water interface. Vollhardt and co-workers reported that diundecylmelamine (2C₁₁mela) and thymine form a trimer, as shown in Fig. 2a, based on surface-pressure-area (π -*A*) measurements, Brewster angle microscopy, and grazing incidence X-ray diffraction.²² Xu and co-workers studied Langmuir-Blodgett multilayers of melamine amphiphile molecules with aromatic rings (*N,N'*-bis[4-[(4'-dodecyloxy)phenyl]azo]phenyl]melamine) deposited from the surface of thymine aqueous solution using UV-VIS and IR spectroscopies,²³ and suggested that 1:1 supramolecular polymers, as shown in Fig. 2b, are formed. In these previous studies, however, direct interfacial spectroscopic information on the hydrogen-bonding motifs and functional-group orientations at the air/water interface was not sufficient to unambiguously distinguish between the trimeric and 1:1 polymer-like structures.

In this study, we employed *in situ* HD-VSFG spectroscopy to obtain direct information on the hydrogen-bonding interactions and functional-group orientations of 2C₁₂mela Langmuir monolayers on thymine aqueous solutions at the air/water interface. While 2C₁₂mela and BA form a well-characterized 1:1 supramolecular polymer, thymine has fewer hydrogen-bonding sites than BA, allowing multiple hydrogen-bonding arrangements with melamine amphiphiles to be considered. By utilizing the phase-sensitive capabilities of HD-VSFG spectroscopy, we can determine the absolute orientation of functional groups and thereby elucidate whether 2C₁₂mela and thymine form a 1:1 polymer-like structure similar to the BA system or adopt an alternative arrangement, such as the trimeric structure proposed in previous studies. Such molecular-level understanding provides a basis for understanding how hydrogen-bonding patterns govern supramolecular assembly at the air/water interface.

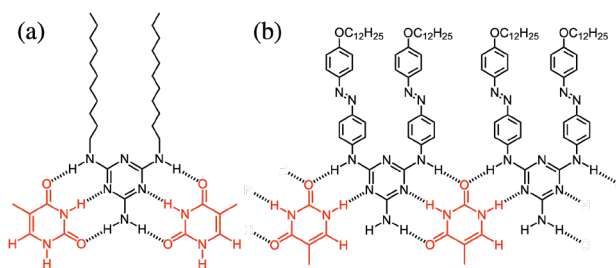


FIG. 2. Schematic representations of previously proposed supramolecular structures of melamine amphiphiles and thymine: (a) a trimeric structure formed by 2C₁₁mela

and thymine, and (b) a 1:1 supramolecular polymer formed by *N,N'*-Bis[4-[[[4'-dodecyloxy]phenyl]azo]phenyl]melamine and thymine.

MATERIALS AND METHODS

Sample preparation

BA and thymine were purchased from TCI, and chloroform was purchased from Nacalai Tesque. These reagents were used as received. 2C₁₂mela was synthesized according to a previously reported method.¹¹ Ultrapure water (resistivity: 18.2 MΩ cm) obtained from an ultrapure water purification system (Sartorius arium 611 UV) was used as the subphase and as the solvent for preparing BA and thymine aqueous solutions.

Surface pressure-area isotherm measurements

The surface pressure-area (π - A) isotherms were measured with a Langmuir-Blodgett trough (KSV Minitrough). The trough consists of a rectangular Teflon vessel measuring 364 × 75 mm², equipped with two hydrophilic Delrin barriers and a Wilhelmy balance. Langmuir monolayers of 2C₁₂mela were prepared by spreading a 0.2 mg·mL⁻¹ chloroform solution onto the surface of pure water or BA/thymine aqueous solutions in the trough. After allowing chloroform to evaporate for 10 min, the monolayer was compressed at a speed of 12 mm·min⁻¹.

HD-VSFG measurements

Details of our HD-VSFG apparatus have been described elsewhere.^{20, 24} The experimental setup is briefly outlined below. The setup was based on a Ti:sapphire regenerative amplifier (Legend Elite, Coherent; average power 3.0 W, repetition rate 1 kHz, pulse width 100 fs, center wavelength 800 nm). The amplified output was divided into two parts to generate a narrowband visible beam (wavelength 600 nm, bandwidth 8 cm⁻¹) and a broadband IR beam (bandwidth 200 cm⁻¹). The IR and visible beams were focused and overlapped in a thin *Y*-cut quartz plate (10-μm thick) to generate sum-frequency light as the local oscillator (LO). The transmitted IR and visible beams, together with the LO, were refocused onto the sample. The LO was passed through a fused silica plate (3 mm) between the *Y*-cut quartz plate and the sample to introduce a time delay of approximately 5 ps. The incident angles of the IR and visible beams were 60 and 73 degrees, respectively. The height of each sample was controlled within 3 μm with a displacement sensor (LT8110, Keyence). The HD-VSFG spectra presented in the main text

were measured with the SSP combination (S-polarized SFG, S-polarized visible, and P-polarized IR). The SPS spectrum in the carbonyl stretching region measured for a 2C₁₂mela monolayer on a 1 mM thymine aqueous solution is shown in the Supporting Material. The 2C₁₂mela Langmuir monolayers for HD-VSFG measurements were prepared on a miniature rectangular 64 × 64 mm² Teflon trough with a fixed surface area. The surface pressure was not actively controlled during the HD-VSFG measurements. The amount of 2C₁₂mela spread onto the surface was adjusted to give a nominal molecular area corresponding to a surface pressure of 15 mN m⁻¹ in the π -A isotherm. For the monolayer on a 10 mM thymine aqueous solution, the corresponding molecular area was 0.473 nm² molecule⁻¹.

Spectral simulations

All quantum chemical calculations used for the spectral simulations were performed using Gaussian 16 Rev. A03.²⁵ Geometry optimizations and analytical vibrational frequency calculations were carried out at the B3LYP/6-31G** level for five supramolecular models (Figs. 3a-e) of *N,N'*-dimethylmelamine (2C₁mela) and thymine. No imaginary frequencies were found for the optimized structures. Thermodynamic quantities, including Gibbs free energies, were obtained using the standard statistical thermochemical treatment implemented in Gaussian 16. The HD-VSFG spectra were then simulated using the calculated Raman tensors and IR transition dipole moments. The five models were gas-phase supramolecular clusters constructed with hydrogen-bonding networks proposed in the studies by Xu et al. and Vollhardt et al. Two of these models were finite 4:4 models consisting of four 2C₁mela and four thymine molecules, in which the 4-carbonyl groups of thymine point in the same direction (identical polymer, i-polymer, in Fig. 3a) or alternate directions (alternate polymer, a-polymer, in Fig. 3b). The other three models were 1:2 trimers in which two thymine molecules are oriented with their methyl group pointing downward and downward (down-down trimer, dd-trimer, Fig. 3c), downward and sideways (down-side trimer, ds-trimer, Fig. 3d), or sideways and sideways (side-side-trimer, ss-trimer, Fig. 3e), respectively.

In these models, 2C₁₂mela was approximated by *N,N'*-dimethylmelamine (2C₁mela), in which the two dodecyl chains of 2C₁₂mela molecules were replaced with methyl groups. This simplified model retains the melamine hydrogen-bonding core while omitting most of the alkyl-chain region. The approximation is appropriate for the present spectral analysis because it focuses on the carbonyl and hydrogen-bonded NH stretching regions rather than on the CH stretching bands of the alkyl chains. However, because the alkyl chains are essential to the amphiphilic character and monolayer formation of 2C₁₂mela, the 2C₁mela models should be

regarded as simplified representations of the hydrogen-bonding core of the supramolecular assemblies. For the water-bound i-polymer model shown in the Supporting Material, a minimal number of water molecules were initially placed at plausible hydrogen-bonding sites around the i-polymer structure, and the resulting structure was then fully optimized at the same level of theory. This model was used to examine the possible effect of hydrogen bonding with interfacial water on the relative intensities of the carbonyl bands, rather than to determine a unique hydrated structure.

Calculated Raman tensors (α) and transition dipole moments (μ) were transformed to correspond to the possible orientations of the supramolecules at the air/water interface. The molecular axes (a, b, c) were defined as shown in Fig. 3. The c -axis was defined as the direction parallel to the sum of the vectors, each of which was drawn from the C atoms at the 4-position (shaded in red in Fig. 3) to the N atoms at the 1-position (shaded in blue in Fig. 3) of each melamine ring. In each supramolecular model, the two terminal thymine molecules each possessed one carbonyl group whose O atom was oriented upward. The bc -plane was defined as the plane containing the O atoms of these upward-oriented carbonyl groups in the two thymine molecules at the edges of the model (shaded in black in Fig. 3). We used Euler angles (ϕ, θ, ψ) depicted in Fig. 4 to specify the orientation of the molecular axes (a, b, c) with respect to the laboratory axes (X, Y, Z). The angle ψ was set to 0° or 180° such that the extension direction of the supramolecule, i.e. the b -axis, was parallel to the air/water interface. The spectra for $\psi = 0^\circ$ and 180° were averaged because there was almost no difference between them (see Supporting Material). Because the spectral shapes were independent of θ (see Supporting Material), θ was set to 0° for the spectra presented in the main text.

If the visible frequency is far from electronic resonance and the IR frequency is near vibrational resonance, the effective second-order nonlinear susceptibility tensors ($\chi_{\text{eff}}^{(2)}$) can be expressed as follows:

$$\chi_{\text{eff}}^{(2)} = \chi_{\text{NR}} + \sum_q \frac{A_q}{\omega - \omega_q + i\Gamma_q} \quad (1)$$

where χ_{NR} arises from the nonresonant background contribution, and A_q , ω_q , and Γ_q denote the amplitude, resonant frequency, and damping coefficient of the vibrational mode q , respectively. We assumed in-plane isotropy of the air/water interface and treated the θ and ψ distributions as δ functions. The simulated spectra were then calculated as follows:

$$\text{Im } \chi_{\text{cal}}^{(2)}(\omega, \theta, \psi) = \sum_q \frac{(-A_q)\Gamma_q}{(\omega - \omega_q)^2 + \Gamma_q^2} = \sum_q \frac{\langle \alpha_q \mu_q \rangle_\phi \Gamma_q}{(\omega - \omega_q)^2 + \Gamma_q^2} \quad (2)$$

where α_q and μ_q represent the Raman tensor and transition dipole moment of the vibrational mode q , respectively. Because the alkyl chains of 2C₁₂mela were replaced with methyl groups in our models, the summation excluded the CH stretching modes. The Γ_q values were set to 100 cm⁻¹ (thymine NH stretching modes), 25 cm⁻¹ (2C₁mela NH and NH₂ stretching modes), and 7 cm⁻¹ (other modes). The calculated vibrational wavenumbers were calibrated with a scaling factor of 0.9627.²⁶

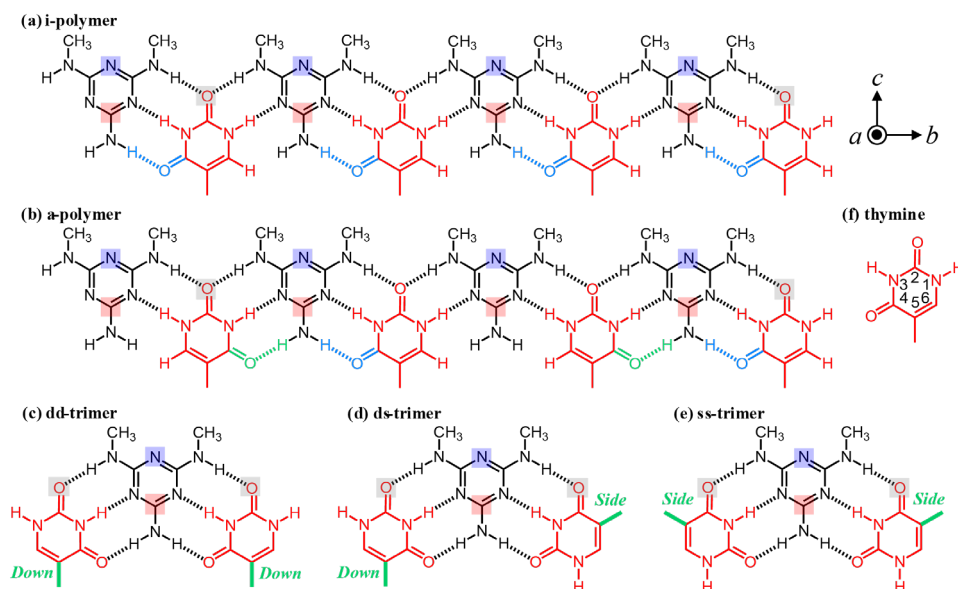


FIG. 3. Schematic representations of the supramolecular models for spectral simulations: (a) i-polymer, (b) a-polymer, (c) dd-trimer, (d) ds-trimer, and (e) ss-trimer. 2C₁mela and thymine are shown in black and red, respectively. The highlighted atoms indicate those used to define the molecular axes (a , b , c), and the labels “Down” and “Side” indicate the methyl-group orientations of thymine in the trimer models. (f) Molecular structure of thymine with ring-atom numbering (1-6).

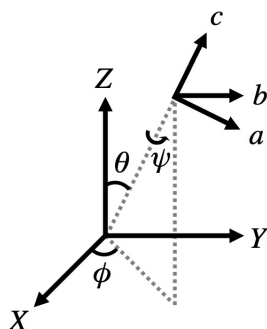


FIG. 4. Definition of the Euler angles (ϕ , θ , ψ) relating the molecular coordinate system (a , b , c) to the laboratory coordinate system (X , Y , Z).

RESULTS AND DISCUSSION

Surface pressure-area (π -A) isotherms

Fig. 5a shows the π -A isotherms of 2C₁₂mela on 10, 1, 0.1, and 0.01 mM thymine aqueous solutions, as well as that on pure water. The isotherm on pure water exhibits a plateau region between 0.56 and 0.68 nm² molecule⁻¹. Because the isotherm on the 0.01 mM solution closely overlaps with that on pure water, the binding of thymine to 2C₁₂mela at the interface is negligible at this thymine concentration. In contrast, the isotherms on the 10 and 1 mM solutions do not exhibit the plateau region observed on pure water. The two isotherms are nearly identical. Moreover, the isotherms on 10 mM thymine and BA solutions (Fig. 5b) display very similar shapes. These results suggest that, on 10 and 1 mM thymine solutions, 2C₁₂mela and thymine form a supramolecular structure that resembles the supramolecular structure of 2C₁₂mela and BA (Fig. 1). To further investigate the structure of 2C₁₂mela and thymine at the air/water interface, we analyzed Langmuir monolayers of 2C₁₂mela on 10 mM thymine solution using HD-VSFG spectroscopy.

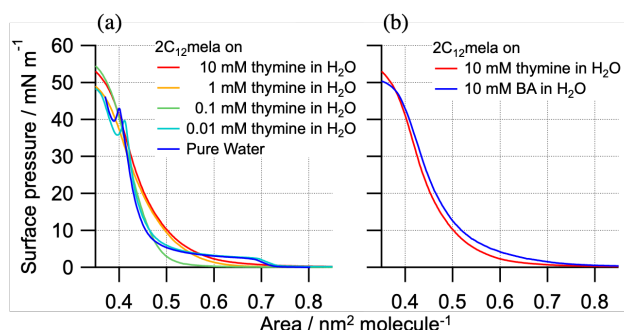


FIG. 5. Surface pressure-area (π -A) isotherms of 2C₁₂mela monolayers. (a) Isotherms measured on pure water and thymine aqueous solutions at concentrations of 0.01, 0.1, 1, 10 mM. (b) Comparison of isotherms measured on 10 mM thymine and 10 mM BA aqueous solutions.

HD-VSFG spectra in the X-H stretching region

Fig. 6 shows the observed HD-VSFG spectra in the X-H (C-H, N-H, and O-H) stretching region of a 2C₁₂mela monolayer on a 10 mM thymine solution, compared with those obtained on a 10 mM BA solution and on pure water. Two negative bands were observed at 2879 and 2940 cm⁻¹ in all three spectra, which are attributed to the symmetric stretching and Fermi resonance bands of terminal methyl groups of alkyl chains.^{20, 27-29} In contrast, no clearly discernible band attributed to methylene stretching modes was observed in the spectra. These spectral features indicate that the alkyl chains of 2C₁₂mela molecules are well-packed on the

three subphases. A negative band around 3350 cm^{-1} is attributed to NH and NH_2 stretching modes of $2\text{C}_{12}\text{mela}$ based on our previous study of $2\text{C}_{12}\text{mela}$ and BA.¹¹ Notably, an extremely broad positive band was observed between 2600 and 2850 cm^{-1} exclusively in the spectra of the monolayers on the thymine and BA solutions. In our previous study of $2\text{C}_{12}\text{mela}$ monolayers on the BA solution, this band was ascribed to the NH stretching mode of strongly hydrogen-bonded BA molecules in a highly inhomogeneous environment within the supramolecular polymer (Fig. 1), with an H-up orientation.¹¹ Taken together, these results suggest that $2\text{C}_{12}\text{mela}$ and thymine form a supramolecular structure similar to that formed by $2\text{C}_{12}\text{mela}$ and BA (Fig. 1).

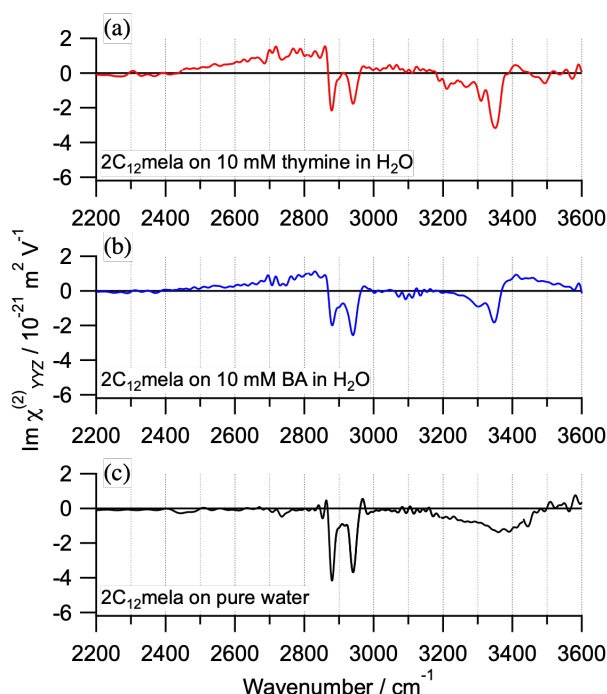


FIG. 6. Observed HD-VSFG spectra in the X-H stretching region of $2\text{C}_{12}\text{mela}$ Langmuir monolayers on (a) a 10 mM thymine solution, (b) a 10 mM BA solution, and (c) pure water. The spectra were measured with the SSP polarization combination.

HD-VSFG spectra in the carbonyl stretching region

Fig. 7 shows the observed HD-VSFG spectrum in the carbonyl stretching region of a $2\text{C}_{12}\text{mela}$ Langmuir monolayer on a 10 mM thymine solution, compared with those obtained on a 10 mM BA solution and on pure water. In contrast to the X-H stretching region, the spectra of the monolayers on the thymine and BA solutions exhibited qualitatively distinct features. The spectrum of the monolayer on the thymine solution exhibits positive and negative bands at 1665 and 1710 cm^{-1} , respectively, whereas that on the BA solution shows only a negative band at 1718 cm^{-1} . The SSP spectra obtained for $2\text{C}_{12}\text{mela}$ monolayers on 1 and 10

mM thymine aqueous solutions were essentially identical in the carbonyl stretching region (Fig. S1). Therefore, the SPS spectrum measured on a 1 mM thymine aqueous solution was used as supplementary polarization information for the supramolecular structure formed on thymine aqueous solutions (Fig. S2). In the next section, we simulate the spectra to assign these vibrational bands and evaluate the supramolecular structure formed by 2C₁₂mela and thymine.

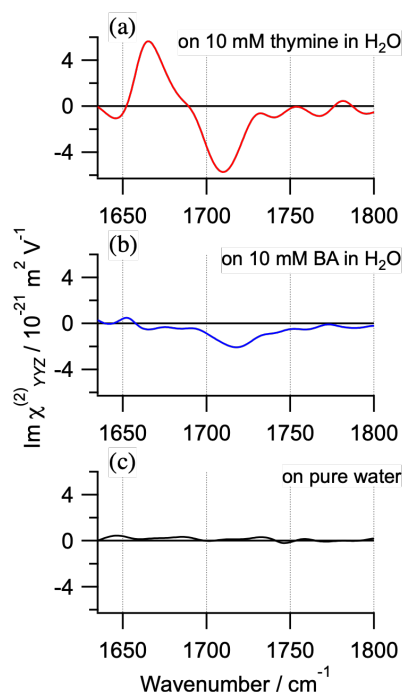


FIG. 7. Observed HD-VSFG spectra in the carbonyl stretching region of 2C₁₂mela Langmuir monolayers on (a) a 10 mM thymine solution, (b) a 10 mM BA solution, and (c) on pure water. The spectra were measured with the SSP polarization combination.

Spectral simulations of the i-polymer and trimer models

To gain further insight into the observed spectral features, we performed spectral simulations using four models: the i-polymer (Fig. 3a), the dd-trimer (Fig. 3c), the ds-trimer (Fig. 3d), and the ss-trimer (Fig. 3e). Fig. 8 presents the simulated spectra for these four models, including both the carbonyl stretching region (left panel) and the X–H stretching region (right panel), allowing a direct comparison among the models. In the X–H stretching region, the simulations focused on the NH and NH₂ stretching modes because the C–H stretching bands were excluded, as described in the MATERIALS AND METHODS section. The simulated spectra reproduced the characteristic bands of NH and NH₂ stretching modes. In contrast, the simulated features in the carbonyl stretching region differ markedly among the four models, as discussed below.

FIG. 8. Simulated HD-VSFG spectra of the i-polymer (Fig. 3a), dd-trimer (Fig. 3c), ds-trimer (Fig. 3d), and ss-trimer (Fig. 3e) models in the carbonyl stretching region (left panel) and X-H stretching region (right panel). The spectra were simulated with the SSP polarization combination.

In the carbonyl stretching region, the spectra of the i-polymer and dd-trimer, in which thymine molecules have their methyl groups oriented downward, exhibit a positive band at the lower-wavenumber side and a negative band at the higher-wavenumber side. In contrast, the ss-trimer model, in which thymine molecules have their methyl groups oriented sideways, shows the opposite sign patterns. Analysis of the calculated normal modes showed that the lower-wavenumber features are associated mainly with normal modes involving 4-carbonyl stretching, whereas the higher-wavenumber features are associated mainly with modes involving 2-carbonyl stretching of thymine. Thus, the sign pattern in the carbonyl stretching region provides information on the orientation of thymine molecules in each model.

The observed spectrum exhibits a positive band at 1665 cm^{-1} and a negative band at 1710 cm^{-1} . The sign pattern is consistent with those of the i-polymer and dd-trimer models, but not with those of the ds- and ss-trimer models, which contain thymine molecules with sideways-oriented methyl groups. These results indicate that thymine molecules at the interface are predominantly oriented with their methyl groups pointing downward. The absence of spectral features attributable to sideways-oriented thymine disfavors the ds- and ss-trimer models as dominant interfacial structures.

The dd-trimer model also gives the same sign pattern as the i-polymer model in the carbonyl stretching region. However, within the limitations of the present finite-cluster calculations, the calculated free energy of the dd-trimer is slightly higher than those of the ds- and ss-trimer models by 0.312 and 0.630 kJ mol^{-1} at 298.15 K , respectively. These small differences do not strongly support the selective population of the dd-trimer among the three trimeric models. If trimeric structures were substantially populated, the ds- and/or ss-trimer would also be expected to contribute to the VSFG spectrum. The observed spectrum, however, does not show features attributable to thymine molecules with sideways-oriented methyl groups. This consideration disfavors the trimeric models as a whole and supports the i-polymer model as the most plausible structure among the finite cluster-models examined here.

In this study, no spectral features attributable to thymine molecules with sideways-oriented methyl groups were detected. Such thymine molecules may be present at the edges of a finite supramolecular polymer. Based on the signal-to-noise ratio of the observed spectra, the population of edge thymine molecules was roughly estimated to be less than one-tenth of that of thymine molecules in the interior of the polymer. This estimate suggests that the supramolecular polymer contains on the order of twenty or more $2\text{C}_{12}\text{mela}$ molecules and thymine molecules, although this value should be regarded only as a rough lower limit. The polymer-like structure proposed here for $2\text{C}_{12}\text{mela}$ and thymine differs from the trimeric structure proposed for $2\text{C}_{11}\text{mela}$ and thymine by Vollhardt and co-workers (Fig. 2a).²² Although this difference might arise from the one-carbon difference in the alkyl chain length, the previous $2\text{C}_{11}\text{mela}$ -thymine model was proposed without direct *in-situ* spectroscopic information on hydrogen-bonding motifs or functional-group orientations at the air/water interface. Our results therefore suggest that the trimer model proposed for the $2\text{C}_{11}\text{mela}$ -thymine system may need to be reconsidered, although direct *in situ* spectroscopic measurements of that system would be required for a definitive conclusion. The present rough estimate of the polymer length for $2\text{C}_{12}\text{mela}$ -thymine system is also consistent with the possibility that $2\text{C}_{12}\text{mela}$ -BA system, in which BA provides more hydrogen-bonding sites than

thymine, forms even longer supramolecular polymers. Information on the polymer length was not reported in the previous studies of 2C₁₂mela and BA.^{10,11}

Spectral simulations of two 4:4 polymer models

Next, we compared the i-polymer and a-polymer models to examine the lateral orientation of the thymine 4-carbonyl groups within the supramolecular polymer. In the i-polymer model (Fig. 3a), all four thymine molecules orient their 4-carbonyl groups in the same lateral direction, whereas in the a-polymer model (Fig. 3b), the 4-carbonyl groups alternate in their lateral orientation. Fig. 9 shows the simulated spectra for these two polymer models. In the X–H stretching region, where the C–H stretching modes were excluded from the simulations, both spectra exhibit positive bands from the strongly hydrogen-bonded NH stretching mode of thymine and negative bands from the hydrogen-bonded NH and NH₂ stretching modes of 2C₁mela. These spectral features are qualitatively consistent with those in the X–H stretching region of the observed spectrum (Fig. 6).

In the carbonyl stretching region, both polymer models exhibit negative features together with positive components at the lower-wavenumber side. However, the origins and intensities of the positive features differ markedly between the two models. In the a-polymer spectrum, the weak positive feature near 1663 cm⁻¹ arises mainly from normal modes characterized by stretching of the exocyclic C–NH₂ bonds and NH₂ scissoring motions of 2C₁mela, rather than by thymine carbonyl stretching. In contrast, the pronounced positive feature near 1686 cm⁻¹ in the i-polymer arises mainly from a normal mode involving 4-carbonyl stretching of thymine, thymine ring motions, and NH rocking motions. Inspection of the displacement pattern shows a particularly pronounced change in the 4-carbonyl bond length, whereas the 2-carbonyl stretching component is much smaller.

The difference between the spectra of the two polymer models cannot be understood solely from the surface-normal orientations of the individual C=O bonds. The SFG contribution of each normal mode depends on both its Raman tensor and IR transition dipole moment. Examination of the individual normal modes and their SSP-SFG contributions showed that the normal mode responsible for the pronounced positive feature of the i-polymer has a large positive SSP-SFG contribution. In the a-polymer, in contrast, normal modes involving 4-carbonyl stretching in the corresponding wavenumber region have only small or negative SSP-SFG contributions. Thus, the different lateral arrangements of thymine molecules alter the normal-mode composition and the associated mode-specific SFG responses, accounting for the pronounced positive carbonyl feature of the i-polymer and the absence of a comparable feature

of the same vibrational origin in the a-polymer. Because the experimentally observed positive band at 1665 cm^{-1} is assigned to the 4-carbonyl stretching vibration of thymine, this difference favors the i-polymer over the a-polymer as a model of the interfacial structure.

The calculated spectrum of the dry i-polymer exhibits a lower relative intensity of the positive band than the experimental spectrum (Fig. 7a). In contrast, when the spectrum was calculated for a water-bound i-polymer model, in which water molecules are hydrogen-bonded to the i-polymer, the positive band intensity increases. As a result, the intensity ratio between the positive and negative bands in the calculated spectrum becomes closer to that of the experimental spectrum (see Supporting Material). Moreover, within the present finite-cluster models, the calculated energy of the i-polymer is much lower than that of the a-polymer ($\Delta G = G(\text{a-polymer}) - G(\text{i-polymer}) = 36.5\text{ kJ mol}^{-1}$ at 298.15 K). Taken together, the spectral comparison and the finite-cluster energy calculations support a structure with a consistent lateral orientation of thymine, as in the i-polymer model, over one with alternating lateral orientations, as in the a-polymer model.

FIG. 9. Simulated HD-VSFG spectra for the i-polymer (Fig. 3a) and the a-polymer (Fig. 3b) models in the carbonyl stretching region (left panel) and X-H stretching region (right panel). The same vertical scale is used for the two polymer models in each spectral region.

CONCLUSION

We investigated the Langmuir monolayers of $2\text{C}_{12}\text{mela}$ on thymine aqueous solutions by π -A and *in-situ* HD-VSFG measurements, and spectral simulations. The π -A isotherms

showed that thymine affects the monolayer properties of 2C₁₂mela at thymine concentrations of 1 and 10 mM, suggesting the formation of a supramolecular structure at the air/water interface. The HD-VSFG spectrum in the carbonyl stretching region exhibited a positive band at 1665 cm⁻¹ and a negative band at 1710 cm⁻¹, which were assigned to the 4-carbonyl and 2-carbonyl stretching modes of thymine, respectively. The signs of these bands indicate that thymine molecules are oriented with their methyl groups pointing downward along the interface normal.

Spectral simulations of finite-cluster models showed that the observed sign pattern is reproduced by models in which thymine molecules adopt this downward methyl orientation. In contrast, trimeric models containing thymine molecules with sideways-oriented methyl groups are inconsistent with the observed spectral signs. Because the calculated free-energy differences among the trimeric models are very small, the selective formation of the dd-trimer alone is not strongly supported. These results disfavor the trimeric models as the dominant interfacial structure and support the formation of a polymer-like supramolecular structure with an approximately 1:1 arrangement of 2C₁₂mela and thymine.

Comparison between the two polymer models further indicated that the i-polymer model better reproduces the experimentally observed positive carbonyl band. The pronounced positive feature of the i-polymer involves the 4-carbonyl stretching of thymine, whereas the weak positive feature of the a-polymer arises mainly from vibrations of 2C₁₂mela. Within the finite-cluster models examined here, the i-polymer was also energetically favored over the a-polymer. Taken together, the present results support a polymer-like 2C₁₂mela–thymine supramolecular structure at the air/water interface, in which thymine molecules are bound to neighboring 2C₁₂mela molecules, their methyl groups point downward, and their 4-carbonyl groups share a common lateral orientation along the supramolecular chain.

SUPPLEMENTARY MATERIAL

The supplementary material contains the comparison of SSP spectra of 2C₁₂mela Langmuir monolayers on 1 and 10 mM thymine aqueous solutions in the carbonyl stretching region, the SPS spectrum of a 2C₁₂mela Langmuir monolayer on a 1 mM thymine aqueous solution, the angular dependence of the simulated spectra, and spectral simulations of a water-bound 4:4 i-polymer model.

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AUTHOR DECLARATIONS

Conflict of Interests

The authors have no conflicts to disclose.

Author Contributions

Shuhei Yamada: Investigation; Formal analysis; Visualization; Writing – original draft; Writing – review & editing. **Katsuhiko Ariga:** Formal analysis; Writing – review & editing. **Taka-aki Ishibashi:** Conceptualization; Methodology; Supervision; Funding acquisition; Formal analysis; Visualization; Writing – original draft; Writing – review & editing.

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TOC Graphic

