Interfacial Properties of Binary Systems Composed of DPPC and Perfluorinated Double Long-Chain Salts with Divalent Counterions of Separate Electric Charge

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Abstract: The interfacial behavior of binary monolayers of 1,1’-(1,ω-alkanediyl)-bispyridinium perfluorotetradecanecarboxylate (CnBP(FC14), n = 2, 6, or 10) and dipalmitoylphosphatidylcholine (DPPC) was studied using surface pressure (π)-area (A) and surface potential (ΔV–A) isotherm measurements and morphological observations. In a previous study, we showed that the spacer moiety of C2BP(FC14), and C6BP(FC14), are completely dissociated after spreading on 0.15 M NaCl, whereas the C10BP(FC14), spacer moieties do not dissociate in the monolayer state. However, in the present study, the C10BP moiety partially dissociates in the presence of DPPC monolayers. The excess Gibbs free energy of mixing and two-dimensional phase diagrams suggest that CnBP(FC14), is miscible with DPPC monolayers and also has a fluidizing effect on DPPC monolayers. The phase behavior of the binary monolayers was observed with Brewster angle microscopy (BAM), fluorescence microscopy (FM), and atomic force microscopy (AFM). The dispersion mode of DPPC-rich domains by C10BP(FC14), is significantly different from those of the other CnBP(FC14), monolayers. These results suggest that the aliphatic chains in phospholipids control the dissociation of divalent spacer bound to fluorinated surfactants or amphiphiles.

Key words: fluorinated amphiphiles, DPPC, surface pressure, surface potential, BAM, FM, AFM

1 Introduction

It is well known that fluorocarbons have unique features such as simultaneous hydrophobicity and lipophobicity. Compared with hydrogenated amphiphiles, fluorinated amphiphiles display different and superior behavior in both the bulk phase and at the air-water interface. Fluorinated surfactants can generally be classified into two types: perfluorinated surfactants and partially-fluorinated surfactants including gemini compounds. Substituting fluorine for hydrogen drastically changes the properties of the surfactant. Both the extent of fluorination and the position of the fluorine atoms in a molecule affect the characteristics of a surfactant, such as its solution properties and interfacial behavior. In comparison to hydrogenated surfactants, fluorinated surfactants have a higher surface activity and can lower the surface tension of water to less than 20 mN m⁻¹. In addition, the C-F bond is very stable toward acids, alkalis, oxidation, reduction, and changes in temperature. Therefore, fluorinated surfactants are receiving significant attention in various fields such as industry and biomedical research. Fluorinated amphiphiles are useful for various applications including nucleic acid transfer; the aggregates formed using fluorinated compounds exhibit high transfection efficiency due to the inertness of the fluorinated compounds against biocompounds. However, perfluorinated compounds have a residual effect on the human body and the environment. Therefore, the interaction between biomembrane components and perfluorocarboxylic acids has been systematically investigated to better understand the effects of perfluorinated compounds on biomembranes. The chain length and polar-head group species were found to affect the interaction and miscibility in these studies.

Using partially fluorinated amphiphiles results in their lower accumulation as the specific properties are remained. In fact, it has been reported that partially fluorinated amphiphiles exhibit high biodegradability compared with perfluorinated amphiphiles. With a view towards biomedical...
applications, we previously studied the interaction of partially-fluorinated amphiphiles with biomembrane components using the Langmuir monolayer method\textsuperscript{21}. We found that the solidification or fluidization of partially-fluorinated amphiphiles on lipid monolayers depends on their hydrophobic-chain length and degree of fluorination. Therefore, we expect fluorinated salts and partially-fluorinated amphiphiles to display advantages over perfluorinated amphiphiles, such as reduced bioaccumulation.

The double long-chain salts (gemini type) used in the present study consist of two hydrophobic chains and a hydrophilic section consisting of two electric charges separated by a spacer chain. Both of the chains are connected electrostatically to the hydrophilic section. It has been reported that the spacer controls the solution properties of these salts\textsuperscript{22, 23}. In particular, folding the long spacer chain (longer than C8) results in a high surface activity and micelle-forming ability. That is, these properties are induced by even low concentrations of salts with long spacers. The monolayer behavior of typical double long-chain salts is considerably different from the corresponding single-chain salts; this results from changes in the surface pressure ($\pi$)-molecular area ($\alpha$) isotherms and variation of the phase-transition pressure\textsuperscript{24}. Furthermore, increasing the salt spacer length leads to a loose-packed monolayer. However, the spacers in salts with spacers longer than C8 orientate toward the air, which results in folding and a wicket-like conformation\textsuperscript{25, 26}. The interfacial behavior of fluorinated salts (CnBP ($\text{FC14}$)$_z$, $n = 2, 6,$ or 10) at the air-water interface has been studied previously\textsuperscript{27}. The spacer moieties of C2BP (FC14)$_z$ and C6BP (FC14)$_z$ dissociate to the bulk just after spreading, whereas the C10BP (FC14)$_z$ headgroups remain bonded to the hydrophobic chains when spread on a 0.15 M NaCl solution. However, the interactions between CnBP (FC14)$_z$ and a lipid monolayer have not been reported yet.

Herein, the objective is to elucidate the effect of the spacer length in CnBP (FC14)$_z$ on its interaction with dipalmitoylphosphatidylcholine (DPPC) in a monolayer state. DPPC is a major component in the outer leaflet of cell membranes\textsuperscript{27} and pulmonary surfactants\textsuperscript{28}. The $\pi$-$\alpha$ and surface potential ($\Delta V$)-$\alpha$ isotherms were measured for binary DPPC/CnBP (FC14)$_z$ systems. The excess Gibbs free energy of mixing and two-dimensional phase diagrams were used to analyze the mutual interactions. The phase behavior of the binary monolayers was also observed with Brewster angle microscopy (BAM), fluorescence microscopy (FM), and atomic force microscopy (AFM).

2 Experimental

2.1 Materials

Perfluorotetradecanoic acid (FC14) was purchased from Fluorochem (Derbyshire, United Kingdom) and purified by thrice recrystallization from n-hexane/acetone mixed solvent. 1,1'-((1,ω-alkanediyi)-bispyridinium perfluorotetradecanecarboxylate (CnBP; $n = 2, 6,$ or 10) were kind gifts from the former Kawanura laboratory of Gunma University\textsuperscript{23, 24, 26}. 1,1'-((1,ω-alkanediyi)-bispyridinium perfluorotetradecanecarboxylate (CnBP (FC14)$_z$, $n = 2, 6,$ or 10) were synthesized and purified as reported previously\textsuperscript{25, 26}. L-α-dipalmitylophosphatidylcholine (DPPC; >99%) and the fluorescent probe 1-palmitoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yi) amino] hexanoyl]-sn-glycero-3-phosphocholine (NBD-PC; >99%) were obtained from Avanti Polar Lipids (Alabaster, AL) and used without further purification. Methanol (99.8%) and ethanol (99.5%) were obtained from Nacalai Tesque (Kyoto, Japan). n-Hexane (99.0%), chloroform (99.7%), and 2,2,2-trifluoroethanol (99.0%) were obtained from Merck (Uvasol, Darmstadt, Germany), Kanto Chemical (Tokyo, Japan), and Wako Chemical (Osaka, Japan), respectively.

Fig. 1 Chemical structure of CnBP (FC14)$_z$. 
roethanol (5/3/1 v/v for CnBP(FC14)2 where n = 2) or n-hexane/ethanol/methanol (4/1/1 v/v for CnBP(FC14)2 where n = 6 or 10). Sodium chloride (Nacalai Tesque) was roasted at 1023 K for 24 h to remove any surface-active organic impurities. A 0.15 M NaCl subphase solution (pH = ~6) was prepared using thirice-distilled water (surface tension = 72.0 mN m⁻¹ at 298.2 K; electrical resistivity = 18 MΩ cm).

2.2 Methods

2.2.1 Surface Pressure-Area Isotherms

The surface pressure of the monolayers was measured using an automated homemade Wilhelmy film balance. The surface-pressure balance (Mettler Toledo, AG-245) has a resolution of 0.01 mN m⁻¹. The surface measuring system was equipped with filter paper (Whatman 541, periphery = 4 cm). The trough was made from Teflon-coated brass (area = 750 cm²), and Teflon-made barriers (both hydrophobic and lipophobic) were used in this study. The π-A isotherms were recorded at 298.2 K ± 0.1 K. The solvents used to spread DPPC (0.5 mM) and CnBP(FC14)2 (0.5 mM) were allowed to evaporate for 15 min prior to compression. The monolayers were then compressed at a speed of ~0.23 nm² molecule⁻¹ min⁻¹. The standard deviations (SD) for molecular surface area and surface pressure were 0.01 nm² and 0.1 mN m⁻¹, respectively.

2.2.2 Surface Potential-Area Isotherms

The surface potential was measured simultaneously with the surface pressure while the monolayer was compressed. An ionizing ³²⁴Am electrode located 1.2 mm above the interface was used to monitor the surface potential, with a reference electrode dipped in the subphase. The SD of the surface potential measurement was 5 mV.

2.2.3 Brewster Angle Microscopy (BAM)

The monolayer was directly visualized using a Brewster angle microscope (KSV Optrel BAM 300, KSV Instruments Ltd., Finland) coupled to a commercially-available film balance system (KSV Minitrough, KSV Instruments Ltd.). A 20 mW He-Ne laser emitting p-polarized light of 632.8 nm wavelength and a 10 × objective lens were used; the lateral resolution was 2 μm. The angle of the incident beam to the interface was fixed to the Brewster angle (53.1°) at 298.2 K. The reflected beam was recorded with a high-grade CCD camera (EHDKamPro02, EHD Imaging GmbH, Germany), while the BAM images were digitally saved to a computer hard disk.

2.2.4 Fluorescence Microscopy (FM)

An Olympus microscope BX51WI (Tokyo, Japan) coupled to the film balance system (KSV Minitrough) was used to observe the monolayer. The Olympus microscope was equipped with a 100 W mercury lamp (USH-1030L), a 50 × objective lens (SLMPlan50 ×, working distance = 15 mm), and a 3CCD camera with a camera control unit (IKTU51CU, Toshiba, Japan). The z-directional focus was adjusted precisely using an automation controller (MAC 5000, Ludl Electronic Products Ltd., NY). Spreading solutions of the surfactants were prepared and doped with 1 mol% of the fluorescence probe (NBD-PC). The image processing and analysis were carried out using ImageJ software (Wayne Rasband, Bethesda, MD).

2.2.5 Atomic Force Microscopy (AFM)

Langmuir-Blodgett (LB) films were made using the KSV Minitrough. The film deposition (vertical dipping method) was performed using a freshly-cleaved mica supporting substrate (Okenshio Co., Tokyo, Japan). At the selected surface pressures, a 5 nm min⁻¹ transfer velocity was used for monolayer deposition on 0.15 M NaCl at 298.2 K. The transfer occurs such that the hydrophilic part of the monolayer is juxtaposed to the mica while the hydrophobic part is exposed to the air. AFM experiments were performed using an SPA 400 instrument (Seiko Instruments Co., Chiba, Japan) in tapping mode and both topographical and phase-contrast micrographs were recorded. The transferred samples were observed in air and at room temperature. The atomic force micrographs were collected at a 1 Hz scan rate and using a silicon cantilever (SI-DP3, Seiko Instruments Co.) with a nominal spring constant of 1.7 N m⁻¹ under normal atmosphere and at room temperature.

3 Result and Discussion

3.1 π-A and ΔV-A Isotherms

The π-A and ΔV-A isotherms of binary DPPC/CnBP(FC14)2 systems on 0.15 M NaCl at 298.2 K are shown in Fig. 2. The π-A isotherm of DPPC (curve 1) exhibits a change from a liquid-expanded (LE) to a liquid-condensed (LC) phase at a phase transition pressure (πᶜ) of ~11 mN m⁻¹ and a collapse pressure (πᵥ) of ~55 mN m⁻¹. The CnBP(FC14)2 monolayers exhibit a phase transition from a disordered state to an ordered state at πᶜ = ~6 mN m⁻¹ (for n = 2 or 6) and ~22 mN m⁻¹ (for n = 10). The π-A isotherm of C2BP(FC14)2 is almost the same as that of C6BP(FC14)2 because of the CnBP moieties dissociating to the subphase. On the other hand, the difficulty of CnBP dissociation results in the π-A isotherm of C10BP(FC14)2 shifting to large molecular areas and changes in the πᶜ and πᵥ values compared to those when n = 2 or 6. Considering the similar electronic environments in the vicinity of the carbonyl and CnBP moieties among CnBP(FC14)2, the association of hydrophobic and spacer moieties in a C10BP(FC14)2 molecule on water is attributed to a hydrophobic condensing force between the FC14 chains and the C10BP spacer. Detailed discussion of pure CnBP(FC14)2 monolayers was presented in a previous paper²⁰. Almost all of the π-A isotherms for binary DPPC/CnBP(FC14)2 monolayers are located between those for the pure monolayers. In addition, the πᶜ and πᵥ values vary with mole fraction of CnBP
In contrast, the minimum Δ\(\pi\)-value for C10BP \(\cdot\) 2 monolayers shifts to more negative values up to 0.7. However, the Δ\(\pi\)-value for the monolayer when \(X_{\text{C10BP}(FC14)}\) is 0.9, which corresponds to condensation or expansion of the monolayers. In the case of (2), the surrounding DPPC molecules are considered to disturb the intramolecular binding of C10BP/(FC14)\(_2\) due to hydrophobic condensing forces. This is deeply related to the fact that the hydrophobic C10BP spacer interacts more strongly with the DPPC hydrocarbon chain than the fluorocarbon chain. Consequently, we believe that the second explanation is more reasonable.

### 3.2 Excess Gibbs Free Energy Analysis

The two-component interaction in the binary systems was evaluated using the excess Gibbs free energy of mixing (\(\Delta G_{\text{mix}}^{\text{ex}}\)). \(\Delta G_{\text{mix}}^{\text{ex}}\) values were calculated using Eq. \(1^{30}\):

\[
\Delta G_{\text{mix}}^{\text{ex}} = \int_{0}^{1} (A_{i2} - X_{i}A_{1} - X_{2}A_{2}) \, dx,
\]

where \(A_{i}\) and \(X_{i}\) are the area per molecule and mole fraction, respectively, of component \(i\) and \(A_{12}\) is the mean area per molecule in binary monolayers of components 1 and 2.

There are two possible explanations for this negative contribution: (1) an improvement in the FC14 chain orientation when \(n = 10\) or a reduced orientation of the DPPC hydrocarbon chain and (2) dissociation of some CnBP moieties from C10BP/(FC14)\(_2\). Comparing the isotherms does not support the first interpretation because there is no significant shift in the isotherm when \(X_{\text{C10BP}(FC14)}\) = 0.9, which is discussed in more detail later. The Δ\(\pi\)-A isotherm of the monolayers provides more information on the monolayer orientation in the direction of the close-packed state, which is similar to that for a pure FC14 monolayer. In contrast, the minimum Δ\(\pi\)-value for C10BP/(FC14)\(_2\) monolayers is nearly \(-650\) mV. We attribute this difference in and negative variations, respectively, as the molecular areas decrease. The negative Δ\(\pi\)-value for C10BP/(FC14)\(_2\) is caused by the strong electronegativity of the fluorine atoms\(^{26, 29, 30}\). The C2BP/(FC14)\(_2\) and C6BP/(FC14)\(_2\) monolayers have a minimum Δ\(\pi\)-value of \(-1000\) mV in the close-packed state, which is similar to that for a pure FC14 monolayer. In contrast, the minimum Δ\(\pi\)-value for C10BP/(FC14)\(_2\) is nearly \(-650\) mV. We attribute this difference in the minimum Δ\(\pi\)-value to the binding of the hydrocarbon spacer to C10BP/(FC14)\(_2\). The positive contribution of the binding to the Δ\(\pi\)-value results from a loose packing of the fluorocarbon chains and orientation of the hydrocarbon spacer chains toward the air. Considering the Δ\(\pi\)-A isotherm (Fig. 2) of the C10BP/(FC14)\(_2\) monolayers in the close-packed state, the orientation of the spacer chains towards the air contributes more to the less negative Δ\(\pi\) than loose packing of the fluorocarbon chains. The Δ\(\pi\)-A isotherm for the binary system when \(n = 2\) or 6 shifts successively from positive to negative Δ\(\pi\) values with increasing \(X_{\text{C10BP}(FC14)}\). In addition, the isotherms lie within those for the pure components. In contrast, the Δ\(\pi\)-A isotherm for the DPPC/ C10BP/(FC14)\(_2\) monolayers shifts to more negative values with increasing \(X_{\text{DPPC}(FC14)}\), up to 0.7. However, the minimum Δ\(\pi\)-value when \(X_{\text{C10BP}(FC14)} = 0.8\) (data not shown here) or 0.9 is smaller than that for pure C10BP/(FC14)\(_2\). This indicates that there is a negative contribution to the Δ\(\pi\)-value for the monolayer when \(X_{\text{C10BP}(FC14)}\) = 0.9 (or 0.8).

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\(\Delta G_{\text{mix}}^{\text{ex}}\) was also probed as a function of \(X_{\text{FC14}}\) for the systems where \(n = 2\) and 6; this is because the...
spacers in C2BP(FC14)2 and C6BP(FC14)2 are completely dissociated and dissolved in the bulk phase (Fig. S1). The variation of ΔG_{mix} when n = 2 was similar to that when n = 6 and, for both systems, ΔG_{mix} increases with increasing surface pressure. The change in ΔG_{mix} is particularly clear when n = 6 (Fig. 3B). The minimum ΔG_{mix} values at 5 mN m⁻¹ are −2800 J mol⁻¹ (X_{C2BP(FC14)2} = 0.4, Fig. 3A) and −2600 J mol⁻¹ (X_{C6BP(FC14)2} = 0.3, Fig. 3B). That is, lateral compression reduces the attractive interaction between the two components. On the other hand, the behavior when n = 10 was completely different from n = 2 or 6. ΔG_{mix} when n = 10 decreases with increasing surface pressure from 5 to 25 mN m⁻¹. Above 25 mN m⁻¹, ΔG_{mix} is constant up to 45 mN m⁻¹. The minimum ΔG_{mix} value (−2300 J mol⁻¹) is also smaller than the other systems (n = 2 or 6). In contrast to C2BP(FC14)2 and C6BP(FC14)2, C10BP(FC14)2 can interact more attractively with DPPC at high surface pressures. This is attributed to the van der Waals interaction between the hydrocarbons of DPPC and the C10BP moiety. Considering the dissociation of the spacer moieties when n = 2 or 6 (see Fig. S1), the minimum ΔG_{mix} value increases to more than −2500 J mol⁻¹ in Fig. S1, which is comparable to ΔG_{mix} for the C10BP(FC14)2 system. However, the miscibility among these systems is thermodynamically different at different surface pressures. Consequently, these results support existence of the spacer effect of C10BP(FC14)2 on their interaction with DPPC.

3.3 Two-Dimensional Phase Diagrams

Two-dimensional phase diagrams were constructed by plotting the disordered/ordered phase-transition pressure and the monolayer collapse pressure against X_{CnBP(FC14)2} (Fig. 4). For all systems, the π^{eq} values (open circles) increase as X_{CnBP(FC14)2} increases from 0 to 0.3. This reflects a fluidization of the DPPC monolayer by CnBP(FC14)2. Conversely, at high X_{CnBP(FC14)2}, values π^{eq} does not change with X_{CnBP(FC14)2}. This indicates a weak interaction between the two components, which may be due to the weak condensing force of fluorocarbons. In addition, π^{eq} (solid circles) varies with X_{CnBP(FC14)2}. All of the phase diagrams are classified as positive azeotropic and suggest miscibility between the DPPC and CnBP(FC14)2 monolayers.

The coexistence phase boundary between the ordered monolayer phase and the bulk phase can be theoretically simulated using the Joos equation⁶; this assumes a regular surface mixture with a hexagonal lattice in the DPPC/CnBP(FC14)2 systems.

Fig. 3 Excess Gibbs free energy of mixing (ΔG_{mix}) for the binary (A) DPPC/C2BP(FC14)2, (B) DPPC/C6BP(FC14)2, and (C) DPPC/C10BP(FC14)2 monolayers as a function of X_{CnBP(FC14)2} at selected surface pressures on 0.15 M NaCl at 298.2 K.

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and 2, respectively, in the two-component monolayer; using the solid curve at high surface pressures was obtained for the experimental components 1 and 2, respectively; and is the product of the Boltzmann constant and Kelvin temperature. The dotted curve was obtained by curve fitting of experimental $\pi'$ to Eq. 2. M. indicates a mixed monolayer formed by the DPPC and C$\text{BP}$(FC14)$_2$ species, whereas Bulk denotes a solid phase of DPPC and C$\text{BP}$(FC14)$_2$ ("bulk phase" may be called "solid phase").

$$1 = x_1 \exp \left[ (\pi_m^1 - \pi_c^1) / kT \right] \exp \left[ \frac{\lambda_1 (x_1^2)^2}{\omega_1} \right] + x_2 \exp \left[ (\pi_m^2 - \pi_c^2) / kT \right] \exp \left[ \frac{\lambda_2 (x_2^2)^2}{\omega_2} \right], \tag{2}$$

where $x_1^1$ and $x_2^2$ denote the mole fractions of components 1 and 2, respectively, in the two-component monolayer; $\pi^1$ and $\pi^2$ are the collapse pressure of components 1 and 2, respectively; $\pi_m^1$ is the collapse pressure of the binary monolayers at a given composition of $x_1^1$ and $x_2^2$; $\omega_1$ and $\omega_2$ are the corresponding extrapolated areas at the collapse points of components 1 and 2, respectively; $\xi$ is interaction parameter; and $kT$ is the product of the Boltzmann constant and the Kelvin temperature. The dotted curve was obtained by curve fitting the experimental $\pi'$ values when $\xi = 0$ in Eq. 2. The solid curve at high surface pressures was obtained using $\xi$, which is calculated from Eq. 2 and is the best fit for the experimental $\pi'$ values. The DPPC/C$\text{BP}$(FC14)$_2$ systems have two negative interaction parameters (Fig. 4). The negative $\xi$ values indicate that the interaction energy between different molecules is greater in magnitude than that among like molecules$^{17,18}$.

When $0 \leq X_{\text{C$\text{BP}$(FC14)}_2} \leq 0.5$, the $\xi$ values are very similar regardless of the spacer chain length in C$\text{BP}$(FC14)$_2$. By contrast, when $0.5 \leq X_{\text{C$\text{BP}$(FC14)}_2} \leq 1$, the $\xi$ values when $n = 2$ or 6 are smaller than that when $n = 10$. The phase diagram was reconstructed for the DPPC/C$\text{BP}$(FC14)$_2$ and DPPC/C$\text{BP}$(FC14)$_2$ systems and plotted against $X_{\text{FC14}}$ in Fig. S2. The $\xi$ values for both of these systems become larger than that of the DPPC/C$\text{BP}$(FC14)$_2$ system. That is, C$\text{BP}$(FC14)$_2$ can interact more strongly with DPPC in the close-packed state. These results suggest that the association of the C10BP spacer prevents the interaction with DPPC at high $X_{\text{C$\text{BP}$(FC14)}_2}$.  

3.4 FM Measurements

Figure 5A shows a fluorescence micrograph of the DPPC monolayers in an LE/LC coexistence state. The dark-contrast domains of the LC phase have three anticlockwise arms, which is characteristic of l-DPPC monolayers$^{34-36}$. The domain shapes change significantly upon addition of a small amount of C$\text{BP}$(FC14)$_2$ to the DPPC monolayers, as seen in Fig. 5C-E. It is well-known that domain formation is controlled by the balance of line tension at the boundary between the disordered and ordered domains and long-range dipole-dipole interactions between the ordered domains$^{37,38}$. The strong dipole repulsion of the CF$_3$ groups in C$\text{BP}$(FC14)$_2$ dominate the domain shape of the binary monolayers. Consequently, the thick arms of the DPPC monolayers change to spiral arms with thicknesses of a few μ. This kind of shape in ordered domains has been observed for binary DPPC/Cholesterol$^{39,40}$ and DPPC/cyanopentylibiphenyl$^{15}$ systems. Closer inspection reveals that the domains having both a left-handed and a right-handed arm (indicated by white circles) at the same time coexists in the fluorescence micrographs for the $n = 2$ or 6 systems.
Miscibility of CnBP(FC14)2 and DPPC

The formation of such domains has been well probed by energy minimization modeling\(^\text{41}\). Although these two-armed domains also appear in the micrograph of the FC14 system (Fig. 5B), the C10BP(FC14)\(_2\) system does not show such domains. In the present conditions (pH = \(~6\)), both dissociated and undissociated FC14 carbonyl groups exist in the surrounding DPPC monolayers\(^\text{19, 31, 42}\). The domain is expected to consist of DPPC and dissociated FC14 molecules because of its shape. Nevertheless, the presence of two-armed domains implies strong binding between the FC14 moieties and the C10BP spacers even in the sub-phase.

Fluorescence micrographs of the binary systems with large mole fractions ($X_{\text{FC14}}$ and $X_{\text{C10BP}}$) = 0.7) are shown in Fig. 6. Ordered domains, derived mainly from the fluorinated molecules, emerge just above the respective transition pressures (see the left column). Identification of this domain is supported by the fluorescence micrographs of single FC14 and CnBP(FC14)\(_2\) monolayers\(^\text{20}\). The size of the ordered domains shrinks with increasing surface pressure, as seen in in Fig. 6A-C. During this process, the domain edge visually changes from black to gray and the black domain nearly disappears above 15 mN m\(^{-1}\); the ordered domains cannot be practically observed with BAM and FM under these conditions. The micrographs in Fig. 8A-C show the coexistence state of DPPC (bright domains) and FC14 or CnBP (FC14)\(_2\) (dark domains) disordered phases. The domain classification was confirmed by varying the mole fraction in the binary systems (Fig. S3). Apart from the inclination of monolayer orientation, the height difference of 0.6-0.7 nm between the two domains in these micrographs results primarily from the hydrophobic chain lengths between DPPC and FC14. The appearance of the DPPC domains is very similar among the micrographs in Fig. 8A-C due to the complete dissociation of the spacer from C2BP(FC14)\(_2\) and C6BP(FC14)\(_2\). Conversely, the C10BP(FC14)\(_2\) system appears to display a different morphology from the C2BP (FC14)\(_2\) and C6BP(FC14)\(_2\) systems (Fig. 8D). Given that the 15 mN m\(^{-1}\) surface pressure is less than the transition pressure (Fig. 4), the monolayer state is in a disordered phase. Nevertheless, the image consists roughly of two regions. The first region (left-hand side in Fig. 8D) appears...
similar to the micrographs in Fig. 8A-C. Although the resolution of this region is somewhat more unclear, the height difference (∼0.5 nm) between the bright and dark domains is almost the same as that for the systems of FC14, n = 2 and 6. The second region (right-hand side in Fig. 8D) is an island-like region, with a height difference of ∼0.1 nm between the first and second regions. The island structure may correspond to an ordered domain of undissociated C10BP(FC14)₂ and DPPC monolayers as such island domains are not present in the micrographs of the other systems. This interpretation is also supported by the aforementioned behavior of the ΔV-A isotherms for the DPPC/C10BP(FC14)₂ system (Fig. 2C). Consequently, we propose that the hydrocarbon spacer (C10) in C10BP(FC14)₂ is partially dissociated due to interaction with the DPPC monolayer.

4 Conclusion
We have elucidated the monolayer behavior of CnBP (FC14)₂ with different hydrocarbon spacers that are connected electrostatically with two FC14 chains. Two-component monolayers of DPPC with CnBP(FC14)₂ (n = 2 or 6) exhibited almost the same isotherms and morphology as DPPC/FC14 monolayers due to the dissociation of the Cn spacers from CnBP(FC14)₂. Analysis of the excess Gibbs free energy of mixing and two-dimensional phase diagrams suggests that the two components are miscible. Furthermore, studying the morphology with BAM and FM indicat-
ed that the ordered domain consisting of the two components transitions into a disordered phase with increasing surface pressure. Conversely, a cohesive force or van der Waals attractive force between the DPPC hydrophobic chains and the C10 moieties in C10BP\textsuperscript{FC14}\textsubscript{2} induces a partial dissociation of these moieties. The undissociated C10BP\textsuperscript{FC14}\textsubscript{2} and DPPC monolayers are also miscible with each other. The interaction parameter indicates that DPPC interacts more favorably with dissociated molecules or FC14 because of the limited molecular motion (or orientation) of the FC14 chains in undissociated C10BP\textsuperscript{FC14}\textsubscript{2}. These new findings provide useful information for molecular designing of gemini-type compounds in various fields such as industry and biomedical research.

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**Supporting Information is available**

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