**REVIEW**

**Langmuir Monolayer Miscibility of Perfluorocarboxylic Acids with Biomembrane Constituents at the Air-Water Interface**

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Abstract: Over the past two or three decades, Langmuir monolayers comprising various fluorinated amphiphiles such as perfluorinated, partially fluorinated, and fluorinated-hydrogenated hybrid molecules at the air-water interface, have been investigated to deepen the understanding of their characteristic phenomena and to explore the mutual interaction of these amphiphiles with biomembrane constituents. Langmuir monolayers of these fluorinated amphiphiles are potentially applicable in the fields of materials, biological and clinical science, where they may be utilized in two-dimensional protein crystallization, microelectronics, and for surfactant replacement in the lung. This review article focuses on the miscibility between perfluorinated long-chain carboxylic acids (CF$_3$(CF$_2$)$_{n-1}$COOH, or FnCOOH) and phospholipids or fatty acids in biomembranes upon lateral compression, and describes how the chain-length mismatch between the fluorinated and the hydrogenated tails relates to the interfacial phase behavior from the thermodynamic and the morphological aspects at the micrometer and nanometer levels.

**Key words:** Langmuir monolayer, fluorinated amphiphiles, DPPC, DMPE, DPPG

1 INTRODUCTION

Highly fluorinated amphiphiles have multiple applications in materials science as well as in the biomedical field\textsuperscript{1–3}. These applications often involve colloidal systems stabilized by monomolecular films of fluorinated amphiphiles. In fact, fluorinated amphiphiles are potentially applicable as blood substitutes\textsuperscript{4–7} and lung surfactant replacements (as an effective additive in particular)\textsuperscript{8–11}. Therefore, comprehension and judicious control of the structures and properties of these films are essential. Perfluorinated chains (F-chains) differ markedly from hydrogenated chains (H-chains) in terms of bulkiness (cross sections of c.a. 0.28 vs. 0.18 nm$^2$, respectively), helical conformation (all-trans, rather than planar "zigzag" structure) and stiffness\textsuperscript{12}. F-chains are considerably more hydrophobic than H-chains while also possessing lipophobic character\textsuperscript{13}; the lower polarizability of fluorine (c.a. 0.557) in F-chains generates very weak inter-chain van der Waals interactions\textsuperscript{14}; the fluorine atoms in F-chains generally do not engage in hydrogen bonding\textsuperscript{15}, and F-chains are also chemically and thermally more stable than H-chains. These properties of F-chains generate lower surface tension and higher vapor pressure than the corresponding H-chains. Although a number of techniques have been utilized to investigate the properties of various films incorporated with fluorinated amphiphiles, the monolayer at the air-water interface represents a considerably simple and optimal model for mimicry of biological actions of biomembrane components, and has thus been adopted as an experimental paradigm of biomembranes. Consequently, elucidation of the interfacial behavior of surfactants and the mechanism of interactions between compounds such as phospholipids, neutral lipids, and proteins using these monolayers has been widely researched\textsuperscript{16–23}. Moreover, study of the monolayers offers the advantage of visualization of phase variations on the micro and nano scales using Brewster angle microscopy (BAM)\textsuperscript{24,25}, fluorescence microscopy (FM)\textsuperscript{26–28}, and atomic force microscopy (AFM)\textsuperscript{29}.

Thus far, a diversity of partially fluorinated (or perfluorinated) compounds has been synthesized and the effects of...
the fluorination ratio on the properties of these systems have been intensively explored\(^1\). Partially fluorinated alkanes (\(\text{CF}_3(\text{CF}_2)_{n-1}(\text{CH}_2)_{m-1} \text{CH}_3\)) abbreviated \(\text{FnHM}\)) are among the earliest studied of these systems, for the purposes of designing advanced functional polymers and in the quest for novel liquid crystals, due to their structural simplicity. In direct contrast to hydrogenated alkanes, \(\text{FnHM}\) can form a stable Langmuir monolayer at the air-water interface depending on the extent of fluorination and the total carbon number\(^12\). Dynarowicz-Latka and co-workers systematically investigated the monolayer behavior of \(\text{FnHM}\) with various degrees of fluorination\(^3\text{1-37}\). Recently, other types of fluorinated compounds such as long-chain alcohols (\(\text{CF}_3(\text{CF}_2)_{n-1}(\text{CH}_2)_n\text{OH}, \text{FnHmOH}\))\(^1\text{6-41}\), carboxylic acids (\(\text{CF}_3(\text{CF}_2)_{n-1}(\text{CH}_2)_n\text{COOH}, \text{FnHmCOOH}\))\(^3\text{5-56}\), perfluorinated carboxylic acids (\(\text{CF}_3(\text{CF}_2)_{n-1}\text{COOH}, \text{FnCOOH}\))\(^4\text{7-56}\), catanionic surfactants synthesized by equimolar mixing of perfluorinated and hydrogenated compounds\(^5\text{1-56}\), and hybrid-type surfactants covalently combining \(F\)-chains with \(H\)-moieties\(^5\text{7, 58}\) have been reported. Although the compounds containing fluorinated and hydrogenated moieties have a range of potential applications, as mentioned previously, their interactions with biomembranes and their inter-component interactions are still poorly documented. Because perfluorinated alkanes exhibit no film-forming capacity and synthesis of perfluorinated alcohols is difficult, perfluorinated surfactants, \(\text{FnCOOH}\), are the most amenable compounds for understanding the specific properties of partially fluorinated amphiphiles.

This article reviews recent efforts devoted to understanding the interaction and miscibility of two-component monolayers of \(\text{FnCOOH}\) and biomembrane components such as phospholipids and fatty acids. Attention is primarily focused on Langmuir monolayers spread at the air-water interface and Langmuir-Blodgett (LB) monolayers transferred onto solid substrates. Recent results have shown that the miscibility between \(\text{FnCOOH}\) and hydrogenated lipids depends strongly on the chain-length mismatch between \(\text{FnCOOH}\) and hydrogenated amphiphiles, and that the two components have a relatively better affinity at the interface than in the bulk.

The binary miscibility in the monolayer state is examined herein on the basis of thermodynamics and morphology (on micro- and nano-meter scales) by highlighting the following aspects: phase transitions and collapse pressures that are observed in surface pressure (\(\pi\)) - molecular area (\(A\)) isotherms, interaction parameters (or energies), and topologies in \textit{in situ} and \textit{ex situ} microscopic images.

### 2 THERMAL PROPERTIES OF \(\text{FnCOOH}\)

Fluorination of all hydrogen atoms in a \(\text{HmCOOH}\) tail group modifies its fundamental properties such as melting point and vapor pressure. The relationship between the melting points of \(\text{FnCOOH}\), highly purified by repeated recrystallizations (purity\(>99.5\%\)) see Table 1, and carbon numbers \((7 \leq n \leq 17)\) is linear with a gradient \(\partial T/\partial n\) of 13.5 K (Fig. 1)\(^5\text{9}\). Reagent companies generally supply \(\text{FnCOOH}\) at purities of 95%-97% (for \(n \geq 11\)) which is relatively low. The melting points of \(\text{FnCOOH}\) are considerably increased after purification, highlighting the importance of the use of highly purified \(\text{FnCOOH}\) during physicochemical measurements, as discussed below. The melting points of \(\text{HmCOOH}\) \((7 \leq m \leq 17)\) also increase with increasing carbon numbers, with a slope of \(\partial T/\partial m = 5.18\text{ K}\)\(^5\text{9}\). The slope of the melting point \textit{versus} carbon number plot for \(\text{FnCOOH}\) is more than twice as large as that for \(\text{HmCOOH}\). Common to both systems, the variation in melting point is dependent on intermolecular interactions such as hydrogen bonding, dipole-dipole interaction, and van der Waals interaction, which allows us to estimate the difference in the intermolecular interactions of the two systems. The melting point is also influenced by molecular weight (\(M.W.\)) which contributes to the van der Waals interaction in particular. Normalization of the melting point with respect to molecular weights reduces the slope of the \(\text{FnCOOH}\) plot (\(\partial T/\partial M. W. = 0.27\text{ K}\))\(^6\), making it smaller than that for \(\text{HmCOOH}\) (\(\partial T/\partial M. W. = 5.18\text{ K}\))

<table>
<thead>
<tr>
<th>(n)</th>
<th>purity (%)</th>
<th>melting point (K)</th>
<th>purity (%)(^a)</th>
<th>melting point (K)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>99.5</td>
<td>314.2</td>
<td>95 – 99</td>
<td>326.2 – 333.2</td>
</tr>
<tr>
<td>9</td>
<td>99.5</td>
<td>349.2</td>
<td>97 – 98</td>
<td>345.2 – 358.2</td>
</tr>
<tr>
<td>11</td>
<td>99.5</td>
<td>380.2</td>
<td>95 – 97</td>
<td>380.2 – 385.2</td>
</tr>
<tr>
<td>13</td>
<td>99.5</td>
<td>403.2</td>
<td>96 – 97</td>
<td>403.2 – 408.2</td>
</tr>
<tr>
<td>15</td>
<td>99.5</td>
<td>434.2</td>
<td>95</td>
<td>426.2 – 429.2</td>
</tr>
<tr>
<td>17</td>
<td>99.5</td>
<td>448.2</td>
<td>95 – 97</td>
<td>435.2 – 437.2</td>
</tr>
</tbody>
</table>

\(^a\) Data from the followings: Acros Organics (Geel, Belgium), Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), Fluorochem Ltd. (Derbyshire, UK), Sigma-Aldrich (MO, USA), Alfa Aesar (MA, USA), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Apollo Scientific Ltd. (Cheshire, UK).
3 INTERFACIAL PROPERTIES OF FnCOOH MONOLAYERS

3.1 Isothermal behavior

 FnCOOH (pK_α = 1.3 - 3.8) and HmCOOH (pK_α = 4.8) are generally stronger acids than FnCOOH and HmCOOH, respectively (curve 1). Due to the electronegativity of the F-chains, the degree of protonation (α) can be determined from the following equation:

\[ \frac{\alpha}{1-\alpha} = \frac{K_a}{[\text{H}^+]_w} \]  

where [\text{H}^+]_w is the proton concentration in the bulk, \( K_a \) is the acid dissociation constant, and \( \alpha \) is the degree of protonation. The potential difference of the ionic layer can be calculated using the Boltzmann equation:

\[ \phi_0 = \frac{2kT}{e} \sinh^{-1} \left( \frac{1.37\alpha}{A/zc} \right) \]  

where \( e \) is the elementary electric charge, \( k \) is the Boltzmann constant, \( T \) is the Kelvin temperature, and \( A \) is the molecular area, where the monolayer is in a disordered state.

Assuming that the respective pK_α values of FnCOOH and HmCOOH are nearly equal to the above mentioned pK_α values irrespective of the chain lengths, the carboxylate groups of each species will be dissociated under the conditions of a 0.15 M NaCl subphase and close-packed ordering (at 0.3 and 0.2 nm², respectively) by 1.2% - 18.8% for FnCOOH and 0.1% for HmCOOH. Over the large molecular area (1.0 nm²), the ionization degrees of FnCOOH and HmCOOH are 1.4% - 36.7% and 0.2%, respectively; i.e., FnCOOH monolayers are partially in the anionic form over the range of isothermal measurements, whereas HmCOOH monolayers are almost completely protonated. Nevertheless, FnCOOH (n ≥ 11) can form a stable Langmuir monolayer at the air-water interface under certain conditions where it exists on an acidic subphase (pH 2.0).

Fig. 1 Change in melting point of FnCOOH and HmCOOH against carbon numbers (n and m).

\( M.W. = 0.37 \text{ K} \), consistent with the weaker van der Waals interactions between the F-chains.

Fig. 2 The π-A and ΔV-A isotherms of FnCOOH (n = 11 (curve 1), 13 (curve 2), 15 (curve 3), 17 (curve 4)) monolayers on 0.15 M NaCl (pH 2.0) at 298.2 K.
Table 2  Isothermal parameters of the $Fn$COOH monolayers on 0.15 M NaCl (pH 2.0) at 298.2 K.

<table>
<thead>
<tr>
<th>n</th>
<th>$\pi^i$ (mN m$^{-1}$)</th>
<th>$\pi^e$ (mN m$^{-1}$)</th>
<th>$A_0$ (nm$^2$)</th>
<th>$\Delta V_{\text{min}}$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>8.2</td>
<td>59.2</td>
<td>0.29</td>
<td>$\Delta V_{\text{min}}$</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>44.9</td>
<td>0.31</td>
<td>-1052</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>34.8</td>
<td>0.31</td>
<td>-1055</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>35.2</td>
<td>0.29</td>
<td>-1054</td>
</tr>
</tbody>
</table>

$A_0$ denotes the limiting molecular area (ordered monolayers) or the extrapolated molecular area (disordered monolayers). $\Delta V_{\text{min}}$ means the minimum value on monolayer compression.

is thought to induce the reduction of collapse pressures. The limiting (or extrapolated) molecular area ($A_0$) for the $Fn$COOH monolayers converges on $\pi^i \approx 0.3$ nm$^2$, which is almost coincident with the cross-section of a perfluorocarbon chain ($\pi^i = 0.28$ nm$^2$). The $A_\Delta$ isotherms of $Fn$COOH monolayers indicate changes in molecular orientation upon compression; similarly, the $A_\Delta$ value of hydrogenated lipid monolayers indicates positive variation in molecular orientation upon compression. However, the isotherms of the $F$-chains have an sign opposite to those of hydrogenated lipid monolayers (or exhibit negative variation) due to the electronegativity as the molecular areas decrease, which has been thoroughly discussed$^{16,32,34,35,37,40,60}$. Recently, it has been reported that the monolayers of ethers having a long-chain hydrocarbon and a fluorinated headgroup exhibit large negative $A_\Delta$ values.$^{76-78}$ That is, fluorination is found to reverse the molecular dipole moment of these monolayers in the opposite direction relative to unfluorinated analogs. In this case, the $A_\Delta$ value decreases with decreasing molecular area and finally approaches a minimum $A_\Delta$ value ($A_{\Delta \text{min}}$) immediately prior to monolayer collapse. The $A_{\Delta \text{min}}$ values were almost the same ($\approx -1050$ mV) independent of the $F$-chain length (see Table 2).

When unpurified $Fn$COOH was used in the isothermal measurements, various $A_0$ values were generated: $A_0 = 0.32 - 0.35$ nm$^2$ for $n = 11^{47,73}$, $A_0 = 0.22 - 0.24$ nm$^2$ for $n = 13^{47,48,50,74}$, $A_0 = 0.35$ nm$^2$ for $n = 15^{50}$, and $A_0 = 0.28 - 0.37$ nm$^2$ for $n = 17^{47,49,50,75}$. In the case of unpurified $F13COOH$ in particular, the $A_0$ value was unreasonable in terms of the estimated cross-sectional area of the perfluorocarbon chain, which implies that unpurified $F13COOH$ possibly contains a small amount of longer-chain $F_{n}$COOH ($n > 13$) as an impurity. In addition, the $A_{\Delta \text{min}}$ value for unpurified $Fn$COOH monolayers was larger by $\approx 50$ mV than that for purified $Fn$COOH ($n > 13$)$^{50}$. Thus, use of highly purified $Fn$COOH is essential to measure the interfacial properties and to facilitate data interpretation for proper understanding of the interactions.

### 3.2 Thermodynamic behavior

The temperature dependence of $\pi^i$ provides information on the thermodynamic characteristics of the phase transition from disordered to ordered phases of $F$-chains. $F$-chains exhibit exceptional thermal stability (or thermal resistance) in comparison to $H$-chains.$^{12}$ The thermal stability precludes phase transition of the monolayer state upon compression. In general, a positive linear temperature dependence of $\pi^i$ is observed for film-forming materials due to the activation of molecular motions. In the case of $HmCOOH$ monolayers ($m = 12, 13, 14$), the $\pi^i$ value decreases as the carbon number increases (Fig. 3). This is attributed to an increase in cohesion due to the elongation of hydrocarbon chains. On the other hand, the weak van der Waals interaction between $F$-chains generates a similar $\pi^i$
value for both \(F11\text{COOH}\) and \(F13\text{COOH}\) monolayers in spite of the 2 carbon difference of the chain length. In addition, the slope of the temperature dependence of \(\pi^a\) plot \((\partial \pi^a / \partial T)\) for \(Fn\text{COOH}\) is c.a. 1/4 times as small as that for \(Hm\text{COOH}\) (see Table 3), which reflects the thermal stability of the \(F\)-chains.

Based on the slope of \(\partial \pi^a / \partial T\), the phase transition can be evaluated in terms of the apparent molar quantity change. The thermodynamic parameters relating to the transition from the disordered to the ordered phase can be derived from the treatment developed by Motomura et al.\(^{70}\). This equation takes account of subphase contribution in the monolayer. First, the apparent molar entropy change \((\Delta s^a)\) is evaluated from the following relation:

\[
\Delta s^a = \langle A_e - A_c \rangle \left[ \left( \frac{\partial \pi^a}{\partial T} \right)_p - \left( \frac{\partial \pi^b}{\partial T} \right)_p \right] \tag{5}
\]

which corresponds to Eq. (29) of Ref. 76. The terms \(A_e\) and \(A_c\) in this equation represent the molecular areas of the expanded and the condensed phase, respectively. \(A_e\) is the area at the onset of the phase transition at the surface pressure \(\pi^a\). \(A_c\) is the extrapolated area of the ordered phase region of the \(\pi-A\) isotherm to \(\pi^a\). \(\gamma^b\) denotes the surface tension of the subphase. The right side of Eq. (5) can be calculated numerically from the results given in Fig. 3. Moreover, the apparent molar enthalpy change \(\Delta h^a\) accompanying the phase transition is calculated from the following relation:

\[
\Delta h^a = T \Delta s^a \tag{6}
\]

Negative \(\Delta s^a\) and \(\Delta h^a\) values were obtained for all monolayers listed in Table 3, which respectively signify improved ordering and packing of the monolayers, and an exothermic nature of the phase transition induced by compression. The thermodynamic parameters of the phase transition were slightly larger in the case of \(Fn\text{COOH}\) than those of \(Hm\text{COOH}\). The critical temperature, \(T^e\), above which the monolayer cannot be transformed into the ordered phase, can be obtained by extrapolation of a plot of \(\Delta s^a\) against temperature to \(\Delta s^a = 0\) (see Table 3). Larger \(T^e\) values obtained for the \(Fn\text{COOH}\) monolayers relative to \(Hm\text{COOH}\) monolayers confirm the thermal resistance of the \(F\)-chains to molecular reorientation in the monolayer state.

### 4 TWO-COMPONENT MISCIBILITY OF \(Fn\text{COOH}\) WITH PHOSPHOLIPIDS

#### 4.1 The \(\pi-A\) and \(\Delta V-A\) isotherms

The miscibility of binary monolayers can be assessed, as a first approach, from the \(\pi-A\) and \(\Delta V-A\) isotherms measured by varying the compositions (or molar fraction \(X\)) of

<table>
<thead>
<tr>
<th>compounds</th>
<th>(\partial \pi^a / \partial T) (mN m(^{-1}) K(^{-1}))</th>
<th>(\Delta s^a) (J K(^{-1}) mol(^{-1}))</th>
<th>(\Delta h^a) (kJ mol(^{-1}))</th>
<th>(T^e) (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F11\text{COOH})(^a)</td>
<td>0.2</td>
<td>-80</td>
<td>-24</td>
<td>317.6</td>
</tr>
<tr>
<td>(F13\text{COOH})(^b)</td>
<td>0.3</td>
<td>-111</td>
<td>-33</td>
<td>321.4</td>
</tr>
<tr>
<td>(H12\text{COOH})(^c)</td>
<td>1.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(H13\text{COOH})(^d)</td>
<td>1.2</td>
<td>-49</td>
<td>-15</td>
<td>-</td>
</tr>
<tr>
<td>(H14\text{COOH})(^e)</td>
<td>1.0</td>
<td>-72</td>
<td>-21</td>
<td>304.1</td>
</tr>
</tbody>
</table>

\(^a\) On 0.01 M NaCl (pH 2) from ref. 88. \(^b\) On 0.15 M NaCl from ref. 83. 
\(^c\) From ref. 89. \(^d\) From ref. 90.
the monolayer constituents. In general, the isotherms of binary monolayers shift between the original isotherms of each constituent monolayer in compositional order. In the case of binary monolayers comprising a hydrogenated amphiphile and a fluorinated amphiphile, the variation in the $\Delta V$ vs. $A$ isotherms of both amphiphiles. The criteria for miscibility of binary monolayers are the same as those of two-component monolayers of hydrocarbon surfactants and require that there is a variation in $\pi_{eq}$ as well as $\pi_c$ with composition. Determination of the pressures $\pi_{eq}$ and $\pi_c$ can be precisely performed when the inflection point in the corresponding $\Delta V$ vs. $A$ isotherm appears. Although a change in the $\pi_{eq}$ values versus composition suggests miscibility of the two components, the slope $\partial \pi_{eq} / \partial X_{m,T}$ may be either positive or negative. The former and the latter are respectively indicative of fluidization and solidification of one component by the other. Binary phospholipid/FnCOOH monolayers exhibit either a fluidizing or solidifying effect depending on several factors such as mismatches of the hydrophobic chain lengths of the two components and the species comprising the phospholipid headgroups. The fluidizing effect is observed for DMPE/FnCOOH and DPPG/FnCOOH systems (see Fig. 4A). However, in the case of DPPC/FnCOOH systems, the effects vary with $F$-chain lengths; fluidization is observed for $n'$ = 11 and solidification for $n'$ = 13 and 15. Given that no solidifying effect is observed in the DMPE system, the solidifying effect observed in the DPCC/FnCOOH system for $n$ = 13 and 15 is attributed to the increased bulkiness of the DPCC headgroup. In contrast, the longer-chain FnCOOH of the DPPG/FnCOOH system is immiscible with the phospholipids, which is evidenced by the constant $\pi_{eq}$ value, irrespective of variation of the molar fractions (see Fig. 4B). The second criterion, which is a varying $\pi_{eq}$ value for the binary monolayers, can be elucidated by a similar treatment, which is expounded in greater detail below.

4.2 Two-dimensional (2-D) phase diagram

Further details of the phase variation can be derived from two-dimensional phase diagrams, which are constructed by plotting the $\pi_{eq}$ and $\pi_c$ values for the binary monolayers against compositions at a fixed temperature (see Fig. 5). The coexistence phase boundary between the monolayer phase (2-D) and bulk phase (3-D) of the molecules spread on a surface can be theoretically simulated using the Joos equation under the assumption of a regular surface mixture:

$$1 = x_1 \exp \left[ (\pi_{m1} - \pi_{m2}) / kT \right] \exp [\xi (x_1^2)] + x_2 \exp \left[ (\pi_{m1} - \pi_{m2}) / kT \right] \exp [\xi (x_2^2)]$$

where $x_1$ and $x_2$ denote the respective molar fractions of...
2D Miscibility between FnCOOH and Phospholipids

Fig. 5 Two-dimensional phase diagrams based on the variation of the transition pressure (πeq: open circle) and collapse pressure (πc: solid circle), on 0.15 M NaCl (pH 2.0) at 298.2 K, as a function of xFnCOOH: (A) DPPC/, DMPE/, DPPG/F11COOH and (B) DPPC/, DMPE/, DPPG/F17COOH. The dashed lines were calculated according to Eq. (7) for ξ = 0. The solid line at high surface pressures was obtained by curve fitting of experimental collapse pressures to Eq. (7). “M” indicates a mixed monolayer formed by the lipids and F11COOH species, whereas “Bulk” denotes a solid phase of the lipids and F11COOH (“bulk phase” may be called “solid phase”).

components 1 and 2 in the two-component monolayers; πc1 and πc2 are the respective collapse pressures of components 1 and 2; πc is the collapse pressure of the two-component monolayer at a given composition of x1 (or x2); α1 and α2 are the corresponding molecular areas at the collapse; ξ is the interaction parameter. The solid curve at higher surface pressures was drawn by adjusting ξ in Eq. (7) to obtain the best fit for the experimental values of collapse pressures. The profile of the phase diagrams varies for miscible and immiscible monolayers as seen in Fig. 5A and 5B, respectively. In the case of miscible monolayers, the experimental πc1 and πc values vary with composition. The profiles of the two-dimensional phase diagrams of the phospholipid/F11COOH systems presented in Fig. 5A are similar in the collapse pressure region; these systems are of the positive azetotropic type, where the maximum in the phase diagram corresponds to the 2-D positive azetotropic point. On the other hand, the phase diagrams indicate no change in the πc value irrespective of compositional variations, showing one or two πc values at each composition, which correspond to the native values of the respective components of the monolayer. The interaction energy can be calculated as

$$\Delta \varepsilon = \xi RT / \varepsilon$$

where ε is the number of nearest neighbors, equal to 6, in a close-packed monolayer, and the interaction energy is \( \Delta \varepsilon = \xi (\varepsilon_{ij} + \varepsilon_{ji}) / 2 \), \( \varepsilon_{ij} \) denotes the potential energy of interaction between components i and j. The calculated ξ and \( \Delta \varepsilon \) values for the binary phospholipid/FnCOOH66, 78, 79 and phospholipid/HmCOOH81, 82 systems are summarized in Table 4. Comparison of the calculated ξ and \( \Delta \varepsilon \) values of the two phospholipid/FnCOOH systems (n = 11 and 13) indicates that the phospholipids tend to interact more strongly with F13COOH than with F11COOH. DPPG and DPPC have the same hydrocarbon chain length; however, the DPPG/FnCOOH systems exhibit smaller ξ (or \( \Delta \varepsilon \)) values than the DPPC/FnCOOH systems. This may be attributed to the difference in spatial size (or bulkiness) of the polar headgroups (PC > PG) and the difference in the repulsive interaction between the anionic headgroups of DPPG and FnCOOH. In comparison to the HmCOOH systems, the absolute values of the FnCOOH systems are smaller, and only the longer FnCOOH groups are miscible with the phospholipids. That is, FnCOOH exhibits less affinity for the phospholipids in the monolayer state than HmCOOH.

4.3 Fluorescence microscopy

The phase behavior of monolayers as a function of composition and surface pressure can be evaluated via fluorescence microscopy (FM) using fluorescent probes. Contrast in the fluorescence microscopic image is generated by the difference in solubility of fluorescent probes in the disordered (or LE) and ordered (or LC) monolayers. Generally, the probe possesses a spatially bulky moiety in its structure, and forms disordered monolayers at the interface, due to strong affinity for the disordered phase of the monolayers. The fluorescent micrographs of various binary phospholipid/FnCOOH monolayers (see Fig. 6) show the presence of different phase morphologies in the disor-
The balance between the two factors varies with thermodynamic stimuli, e.g., surface pressure and temperature in monolayers. In the DPPC systems, the balance between the line tension and the dipole interaction remains almost constant in spite of an increase in \( \pi \). It is noticed that the ordered domains fuse with each other in DPPC/\( FnCOOH \) monolayers (\( n = 15 \) and 17, immiscibility). The ordered domains for the DMPE systems are transformed to a roughly circular shape with an increase in \( n \), which suggests that the line tension at the phase boundary becomes higher. However, the DPPG systems show dissimilar phase variations between miscible (\( n = 11 \) and 13) and immiscible (\( n = 15 \) and 17) monolayers. In the case of the miscible systems, the ordered domain becomes more branched, which indicates that the repulsive dipole interaction is enhanced.

### Table 4

<table>
<thead>
<tr>
<th>systems</th>
<th>( \xi (\Delta \varepsilon, \text{J mol}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( F11COOH )</td>
<td>0.58/ (−240)</td>
</tr>
<tr>
<td>( F13COOH )</td>
<td>0.91/ (−376)</td>
</tr>
<tr>
<td>( F15COOH )</td>
<td>0.28/ (−116)*</td>
</tr>
<tr>
<td>( H15COOH^a )</td>
<td>0.02/ (−8)†</td>
</tr>
<tr>
<td>( H17COOH^a )</td>
<td>1.71/ (−695)</td>
</tr>
</tbody>
</table>

\( ^a \) on water at 303.2 K. The value is calculated from the data in ref. 81.

\( ^b \) on water at 293.2 K. The value is calculated from the data in ref. 82.

\( ^* \) 0 < \( \chi_{15\text{COOH}} \) ≤ 0.3.

\( ^† \) 0 < \( \chi_{15\text{COOH}} \) ≤ 0.67.

\( ^\dagger \) 0.67 ≤ \( \chi_{15\text{COOH}} \) < 1.

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**Fig. 6** Fluorescent micrographs of the binary DPPC/, DMPE/, and DPPG/\( FnCOOH \) monolayers for \( \chi_{FnCOOH} = 0, 0.1 \) (enclosed by a black frame rectangle), and 1 on 0.15 M NaCl (pH 2.0) at 298.2 K. The number with an open circle denotes the surface pressure value (mN m\(^{-1}\)). Percentages (%) refer to the ratio of ordered domains in the micrograph. The monolayers contain 1 mol\% of fluorescent probe (R18). The scale bar in the lower right represents 100 \( \mu \)m.

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**Ref.** J. Oleo Sci. 61, (4) 197-210 (2012)
hanced due to repulsion among negative charges of the DPPG and FnCOOH headgroups. Likewise, the ordered-phase domains exhibit a dipole density or an excess charge, which leads to long-range repulsion between domains. This long-range dipolar repulsion gives rise to an increase in the energy density with increasing domain size. If the domains are elongated, the electrostatic energy is reduced for a given domain area. Accordingly, in this case, the line energy at the domain boundary also increases. Therefore, elongation and narrowing in ordered domains are caused by an interplay between these two energies. That is, the repulsive dipolar interaction of molecules within a domain favors destabilized interfaces or elongated domains. On the other hand, the ordered domains in the immiscible systems are dispersed into noncircular segments. However, the segments fuse with each other to undergo a transition to nearly circular phases similar to those observed in DPPC and DMPE systems. Judging from these phase variations, a change in domain shape and retention (no fusion) of the dispersed ordered domains are important criteria for elucidating the binary miscibility in FM measurements.

4.4 Atomic force microscopy

The morphological behavior of monolayers at the air-water interface can be visualized at the nano-meter scale by transfer of the monolayers onto a solid mica substrate using the Langmuir-Blodgett (LB) technique. However, potential change of the original monolayer structures by the electric charge between the samples and substrates, and the physical factors during the deposition procedure is a recognized possibility with this technique. AFM images may therefore not provide completely correct information on the phase behavior at the air-water interface but nevertheless allow us to understand phase morphologies that cannot be caught with in situ microscopic techniques (BAM and FM) due to the limited optical resolution and magnification of the microscopes. The AFM topographic images for the miscible monolayers shown in Fig. 7A-C are apparently different from those of the immiscible systems (Fig. 7D). In the miscible systems, the ordered domains (higher molar fraction of F11COOH comprising the phospholipids are dispersed by F11COOH (lower molar fraction) so that worm-like (Fig. 7A) and striped (Fig. 7B, 7C) patterns appear in the images, although a phase separation of the two domains is apparently observed in the images. On the other hand, the AFM image of the immiscible system also exhibits a phase separation of circular domains (F17COOH) and a surrounding dark network (DMPE) (see Fig. 7D). This pattern is indicative of a completely immiscible morphology characterized by a circular-domain dispersion, which is dominantly induced by a driving force of

![AFM images](image-url)

**Fig. 7** Typical AFM topographic images of the binary DPPC/, DMPE/, and DPPG/FnCOOH systems. The scale bar in the lower right represents 500 nm. (A) DPPC/F11COOH for \( X_{F11COOH} = 0.3 \) at 20 mN m\(^{-1}\), (B) DMPE/F11COOH for \( X_{F11COOH} = 0.5 \) at 30 mN m\(^{-1}\), (C) DPPG/F11COOH for \( X_{F11COOH} = 0.7 \) at 30 mN m\(^{-1}\), and (D) DMPE/F17COOH for \( X_{F17COOH} = 0.5 \) at 15 mN m\(^{-1}\).
the line tension at the boundary between DMPE and F17COOH. A similar type of phase separation for the binary fatty acid/FnCOOH monolayers has been reported by Qaqish et al.\textsuperscript{75, 84, 85}.

4.5 Differences in the miscibility of FnCOOH and Hm-COOH

Generally, a fluorinated and a hydrogenated liquid (or solvent) do not mix with each other in the bulk solution due to the weak van der Waals force of the F-chains. The solid states of the F-chains and H-chains also undergo a separation in aqueous solutions. In contrast, F-chains and H-chains in monolayers interact at the air-exposed surface, but not in the aqueous medium. Thus, interplay between the F-chains and H-chains arises without hydrophobic interaction. The binary miscibility of FnCOOH with the phospholipids within a monolayer becomes quite low above n = 15 (see Table 5), which suggests that FnCOOH can be reasonably miscible with phospholipids having relatively short H-chain lengths. In the two-component FnCOOH/HmCOOH monolayers, mutual miscibility is highly limited when n = 11. This difference means that FnCOOH has a higher affinity for phospholipids in monolayers than HmCOOH. Furthermore, the only index affecting monolayer miscibility that is not found to be critical is the difference in chain length between FnCOOH and phospholipids or HmCOOH. Intramolecular attraction between H-chains arises in phospholipids containing two H-chains in their molecular structure, as opposed to typical single chain lipids. This attraction exerts unexpected influence on the monolayer properties such as ordering, packing, and phase transition, which complicate the interfacial behavior between FnCOOH and phospholipids or HmCOOH. In this regard, it appears that the double H-chains produce compatibility with F-chains. In comparison, HmCOOH shows a widespread range of miscibility with both the phospholipids and the other HmCOOH species (see Table 6). Consequently, although the miscibility between FnCOOH and phospholipids is inferior to that between HmCOOH and phospholipids, FnCOOH has a limited and interesting affin-

<table>
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<tr>
<th>Table 5</th>
<th>Monolayer miscibility of FnCOOH with phospholipids or Hm-COOH.</th>
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<tbody>
<tr>
<td>n = 11</td>
<td>n = 13</td>
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<tr>
<td>DMPE</td>
<td>M</td>
</tr>
<tr>
<td>DPPC</td>
<td>M</td>
</tr>
<tr>
<td>DPPG</td>
<td>M</td>
</tr>
<tr>
<td>m=11</td>
<td>M</td>
</tr>
<tr>
<td>m=13</td>
<td>M</td>
</tr>
<tr>
<td>m=15</td>
<td>−</td>
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<tr>
<td>m=17</td>
<td>M</td>
</tr>
<tr>
<td>m=19</td>
<td>−</td>
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</tbody>
</table>
M, P, and I represent miscible, partially miscible, and immiscible monolayers, respectively.

<table>
<thead>
<tr>
<th>Table 6</th>
<th>Monolayer miscibility of HmCOOH with phospholipids.</th>
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<tbody>
<tr>
<td>m = 13</td>
<td>m = 15</td>
</tr>
<tr>
<td>DPPC</td>
<td>M</td>
</tr>
<tr>
<td>POPC</td>
<td>−</td>
</tr>
<tr>
<td>DSPC</td>
<td>M</td>
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<tr>
<td>m = 12</td>
<td>M</td>
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<td>m = 13</td>
<td>M</td>
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<td>m = 14</td>
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<td>m = 15</td>
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<td>m = 16</td>
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<td>m = 18</td>
<td>M</td>
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</tbody>
</table>
M, P, and I represent miscible, partially miscible, and immiscible monolayers, respectively.
ity for phospholipid monolayers, which can be applied to selective recognition of membrane sites.

5 CONCLUSIONS AND PERSPECTIVES

The miscibility of perfluorinated amphiphiles such as \( \text{FnCOOH} \) with the lipids in biomembranes is significantly different from the corresponding \( \text{HmCOOH} \) species due to the non-ideal and weak interaction between \( F \)-chains and \( H \)-chains. Moreover, the miscibility is strongly affected by the media to which the tail moieties in the molecular structure of \( \text{FnCOOH} \) are exposed. In binary fluorinated and hydrogenated surfactant solutions, two kinds of micelles, which are dominated by fluorinated and by hydrogenated surfactants, are formed above the critical micelle concentration. Binary monolayers of phospholipid/\( \text{FnCOOH} \) as well as \( \text{HmCOOH}/\text{FnCOOH} \) amphiphiles exhibit miscibility at the air-water interface over a limited composition range. Notably, \( \text{FnCOOH} \) interacts favorably with phospholipids rather than \( \text{HmCOOH} \) within a monolayer.

The residual effect of \( \text{FnCOOH} \) (particularly, \( n = 7 \)) on the human body and environment has recently been recognized as a serious issue. The environmental fate of \( \text{FnCOOH} \) has been linked to their anionic species. Anionic \( \text{FnCOOH} \) does not partition into the air at all, and its sorption by soils and sediments, which usually carry a net negative charge, is expected to be quite small compared to the undissociated \( \text{FnCOOH} \). The distribution of \( \text{FnCOOH} \) in the global environment is a topic of investigation. Nevertheless, \( \text{FnCOOH} \) is potentially applicable in the medical and pharmaceutical fields as well as in industrial and materials science. Partial fluorination of \( H \)-chains in typical compounds (in a strict sense, perfluoroctyl(F8)) moiety as the maximum) reduces the accumulation period and the toxicity to the human body (or blood). Therefore, partially or hybrid-type fluorinated compounds have been newly synthesized and investigated with the aim of biological application in the clinical field. However, the exact effect of fluorinated chemicals on the human body and the tissue of animals is still unknown. Further investigations of the interaction of perfluorinated compounds with biomembrane components such as phospholipids, neutral lipids, and glycosphingolipids, which are the subject of intensive research on lipid rafts, is thus warranted.

ABBREVIATIONS: DMPE; dimyristoylphosphatidylethanolamine, DPPC; dipalmitoylphosphatidylcholine, DPPG; dipalmitoylphosphatidylglycerol, DSPC; distearoylphosphatidylcholine, POPC; palmitoyloleoylphosphatidylcholine, R18; 3,6-bis(diethylamino)-9-(2-octadecyloxy)phenyl chloride

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