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1 **Higher-Order Mesoscopic Self-Assembly of Fluorinated Surfactants on Water Surfaces**

2 Running title: Hierarchical Self-Assembly of Fluorocarbons

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12

13 **Abstract**

14 Surfactants containing fluorocarbon chains have been drawing increasing attentions, because they
15 self-assemble into a variety of microscopic and mesoscopic domains. They tend to form highly
16 ordered patterns at the air/water interface, which are clearly different from those formed by their
17 hydrocarbon analogues. Focusing on fluorinated surfactants possessing unique physical
18 characteristics, this review aims to give an overview how the interplay of line tension and dipole
19 interaction, the comprehensive principle, governs the pattern formation of two-dimensional self-
20 assemblies. This review also covers several key experimental and analytical techniques that are
21 useful for the characterization of the shape, size, correlation, and viscoelasticity of hierarchical
22 self-assemblies on water surfaces. Finally, several biomedical applications, including biomimetic
23 surface coating, multi-modal contrast agents in medical diagnostics, and controlled delivery of

24 gases (O₂ and NO) for oxygenation and antimicrobial effect, are introduced to highlight how the
25 unique physicochemical properties of fluorinated self-assemblies can be applied in materials
26 science.

27

28 **Higher-order pattern formation by molecular self-assemblies is a universal phenomena.**

29 A wide range of organic molecules (including surfactants, liquid crystals, and diblock copolymers)
30 have various hierarchical two- and three-dimensional patterns and textures.^{1, 2} For example,
31 circular domains, stripes, and chiral crystal structures have been found in Langmuir monolayers
32 of lipids and surfactants at the air/water interface.^{3, 4} Three-dimensional lamellae, inversed
33 hexagonal micelles, and bicontinuous cubic phase assemblies of lipids,^{5, 6} liquid crystals,⁷ and
34 diblock copolymers^{8, 9, 10, 11} have been found. Intriguingly, a small set of structural parameters
35 leads to very similar patterns, independent from the detailed molecular structures. For example,
36 Israelachvili, Mitchell, and Ninham theoretically accounted the shape of self-assembled surfactant
37 aggregates using a geometric packing constraint, $p = v/Al$, where v is the volume of hydrophobic
38 core, A is the surface area (area occupied by a head group), and l is the axial length of the surfactant
39 molecule.¹² This simple geometric constraint can be applied to predict the morphology of various
40 supramolecular architectures formed by a wide variety of molecules with different size and
41 structures, including phospholipids, liquid crystals, and block copolymers. On the other hand, the
42 characteristic length scale and periodicity of patterns can range over several orders of
43 magnitude, from tens of nanometers (e.g. periodicity of phospholipid “ripples”)¹³ to hundreds of
44 micrometers (e.g. stationary patterns driven by chemical reactions).¹⁴ As comprehensively
45 summarized by Seul and Andelman from the theoretical viewpoint,² these patterns are stabilized
46 through interplay between competing intermolecular interactions characterized by the spatial

47 variation of order parameters. In two-dimensional assemblies on water surfaces dealt in this
48 review, the competition of line tension and dipole interaction play dominant roles, as described
49 more explicitly in the following sections.

50

51 **Fluorinated surfactants: what make them unique compared with hydrocarbon analogues?**

52 This review aims to provide a comprehensive overview on the physical principles governing the
53 formation of highly ordered patterns by self-assemblies. As an example, we take fluorinated
54 surfactants that have been shown to form a rich variety of patterns that are distinct from the
55 commonly studied hydrocarbon analogues. Fluorocarbon chains have different structures and
56 physical properties to their hydrocarbon analogues.^{15, 16, 17} A fluorine atom is bulkier than a
57 hydrogen atom, so ordered fluorocarbon chains have rigid 15/7 helical conformations rather than
58 all-*trans* conformations as in the case of hydrocarbon chains. The cross-sectional areas of
59 fluorocarbon chains, 27–30 Å²,¹⁸ are therefore larger than the cross-sectional areas of hydrocarbon
60 chains with all-*trans* conformations, 18–21 Å². The cohesion energy between fluorocarbon chains
61 is lower than the cohesion energy between hydrocarbon chains because fluorocarbon chains are
62 less polarizable than hydrocarbon chains. Moreover, the surface potentials of surfactant
63 fluorocarbon chains at the air/water interface are negative with respect to water,^{19,20} indicating that
64 the molecular dipoles of fluorocarbon chains point in the opposite direction to the molecular
65 dipoles of hydrocarbon chains. Importantly, hydrocarbons and fluorocarbons are both hydrophobic
66 but are totally immiscible, fluorocarbon chains being lipophobic but hydrocarbon chains being
67 fluorophobic.

68 The clear differences in physico-chemical properties between fluorocarbons and hydrocarbons and
69 their mutual immiscibility suggest that the self-assemblies of compounds containing fluorocarbon

70 chains should be distinct from those of their hydrocarbon analogues. Kunitake, Ringsdorf, and
71 their co-workers synthesized phospholipids containing fluorocarbon chains and found that these
72 lipids were hardly miscible with lipids containing hydrocarbon chains.^{21, 22, 23} Riess et al.
73 synthesized various modular single- and double-chain fluorinated surfactants with polar heads
74 derived from phospholipids, amino-acids, sugars, polyols and other biomaterials and characterized
75 their physicochemical properties.^{24, 25} Intriguingly, the compounds containing both fluorocarbon
76 and hydrocarbon chains tend to self-assemble into micelles in either fluorocarbon or hydrocarbon
77 solvents.^{26, 27, 28}

78

79 **“Microstripes” of fluorinated surfactants in the diluted phase: a dipolar monolayer**

80 How will fluorinated surfactants self-assemble when deposited on a water surface? What are the
81 main differences from self-assembly of hydrocarbon analogues? To address these questions,
82 Schneider et al. deposited fully hydrogenated dihexadecylglycerol (diHC16) and its half-
83 fluorinated analogue (diFC8-8) on a water surface.²⁹ They observed the self-assemblies of diHC16
84 and diFC8-8 using a Langmuir film balance coupled to an upright fluorescence microscope while
85 determining the pressure–area isotherms.³⁰ The pressure–area isotherms of diHC16 and diFC8-8
86 are shown in Figure 1a and 1b, respectively. It can be seen that the area per molecule is higher for
87 diFC8-8 than diHC16 at all surface pressures. At $\pi = 25$ mN/m, the area of one diFC8-8 molecule
88 is 57 \AA^2 but the area of one diHC16 molecule is 38 \AA^2 . This is caused by the difference between
89 the cross-sectional areas of a fluorocarbon chain (30 \AA^2) and a hydrocarbon chain (19 \AA^2) and the
90 diFC8-8 and diHC16 molecules each possessing two chains. Near when the pressure starts to
91 increase, diHC16 has circular “bubble-like” domains at $\pi \approx 65 \text{ \AA}^2$ (Figure 1a) similar to the ones
92 found for other lipids with hydrocarbon chains.⁴ In contrast, diFC8-8 forms micrometer-thick,

93 “stripe-like” domains at $A \approx 95 \text{ \AA}^2$ (Figure 1b). Both types of microdomain are thermodynamically
94 stable and found in the coexistence of gas and liquid phases. Further compression of either system
95 causes the film to give homogeneous fluorescence signals.

96 Like for other condensation transitions, the formation of stripe-like domains of diFC8-8 molecules
97 near the first-order phase transition between the gas and liquid phases occurs when attractive
98 interactions between molecules becomes dominant against the mixing entropy. The shapes and
99 thicknesses of stripe-like domains are determined by the competitive interplay between line
100 tension and dipole interactions, as theoretically described by McConnell and Andelman.^{31, 32} Free
101 energy of line tension F_{line} , represented by the energy contribution by line tension γ to create phase
102 boundary is linearly proportional to the peripheral length L of the phase boundary, $F_{\text{line}} = \gamma L$.
103 Namely, the line tension favors circular shapes to minimize the free energy, as found for diHC16
104 and other hydrocarbon-based surfactants. However, the molecular dipole moment tends to stretch
105 isolated domains because of electrostatic interactions. In this scenario, the contribution of the line
106 tension to the domain energy is, $F_{\text{line/stripe}} = \frac{2\gamma}{D}$, where periodicity D is given as the sum of the
107 thicknesses of gas (D_G) and liquid (D_L) phases, $D = D_G + D_L$.

108 Previously, Andelman et al. theoretically predicted that the stripe domains are stabilized through
109 density modulation caused by dipole contributions from the head groups.^{32, 33} However, this cannot
110 explain the formation of bubble-like domains by diFC8-8 and microstripes by diHC16, because
111 they have identical $-\text{OH}$ head groups. Thus, these data suggest that the dipole moments of the
112 chain termini ($-\text{CH}_3$ and $-\text{CF}_3$ groups) as well as those of $-\text{CH}_2-\text{CF}_3-$ junctions play dominant
113 roles in determining the shape and size of domains. The surface potentials of diFC8-8 and diHC16,
114 determined using the Helmholtz equation $V = \frac{\mu}{\epsilon A}$ ³⁴, have opposite signs, indicating that the dipole

115 moment of diFC8-8 points away from the monolayer toward the air,³⁰ as reported previously.^{20, 35}

116 In the Helmholtz equation, μ is the effective dipole moment and A is the area per molecule.

117 Assuming that the infinitely long stripe domains of the gas and liquid phases with thicknesses D_G

118 and D_L , respectively, are separated by sharp boundaries, the free energy of the stripe geometry can

119 be calculated using the following Eq. 1,

$$120 \quad F = \frac{k_B T b^3}{\pi \sqrt{A}} [x_L \phi_L^2 + x_G \phi_G^2] - \frac{b^3}{\pi D} (\phi_L - \phi_G)^2 \log\left(\frac{D \sin \pi x}{\pi \sqrt{A}}\right), \quad \text{Eq. 1}$$

121 where x and ϕ are the fraction and surface concentration (mol/m²) of each phase, $b^3 = \frac{\mu^2 \varepsilon}{k_B T \varepsilon_0 (\varepsilon + \varepsilon_0)}$,

122 and ε and ε_0 are the dielectric constant near the dipoles and the dielectric constant of water,

123 respectively. The first two terms give the mean contribution of the electrostatic energy and are

124 therefore independent of D , but the third term is the sum of the intra- and inter-stripe dipolar

125 interactions.³⁶ The difference between the total free energies of the stripe phase and a reference

126 gas–liquid coexistence is therefore

$$127 \quad \Delta F = \frac{k_B T b^3}{\pi D} (\phi_L - \phi_G)^2 \log\left(\frac{D \sin \pi x}{\pi \sqrt{A}}\right) + \frac{2\gamma}{D}. \quad \text{Eq. 2}$$

128 Minimizing ΔF with respect to the periodicity D gives the equilibrium stripe width:

$$129 \quad D_{eq} = \frac{\pi \sqrt{A}}{\sin \pi x} \exp\left[\frac{2\pi\gamma}{k_B T b^3 (\phi_L - \phi_G)^2} + 1\right]. \quad \text{Eq. 3}$$

130 Using the previously reported line tension $\gamma = 1.6 \times 10^{-12}$ N,³⁷ and the experimentally determined

131 surface area and potential values, the equilibrium thickness $D_{eq} \approx 1$ μm can be calculated using Eq.

132 3. This equilibrium thickness agrees well with the experimentally determined equilibrium

133 thicknesses $D_{exp} = 2\text{--}8$ μm . Decreasing γ by adding a substitutional impurity like cholesterol

134 resulted in a significant decrease in D_{exp} , which gives supporting evidence that the competition

135 between dipole interactions and line tension determine the periodicity.

136

137 **Experimental determination of shape, size, and long-range order of self-assembled domains**

138 Krafft et al. synthesized various semi-fluorinated alkanes $C_nF_{2n+1}C_mH_{2m+1}$ (*F_nH_m* diblocks; Figure
139 2a)) based on covalent connections between fluorocarbon (*F_n*) and hydrocarbon (*H_m*) segments.^{38,}

140 ³⁹ These molecules contain no hydrophilic moieties but form stable Langmuir monolayers at the
141 air/water interface.^{28,40} It is well established that hydrocarbon-based surfactants tend to form a flat
142 monolayer, which can be treated as a two-dimensional continuum. In contrast, the atomic force
143 microscopy (AFM) images of *F_nH_m* monolayers transferred onto solid substrates indicated that a
144 *F_nH_m* monolayer consists of monodisperse, disk-like domains with diameters of 20–40 nm.⁴¹ The
145 orientation of molecules was initially debated because both moieties are hydrophobic. Abed et al.
146 claimed that antiparallel stacking of diblocks occurs,⁴² but X-ray/neutron reflectivity and surface
147 potential measurements indicated that the fluorocarbon chains are in contact with air and the
148 hydrocarbon chains are in contact with water.^{43, 44, 45, 46, 47}

149 A theoretical study⁴⁸ demonstrated that the micelles are mesoscopic domains of a high density
150 phase of the *F_nH_m* molecules taking vertical orientations (the fluorinated segments being extended
151 toward air and the hydrocarbon segments toward water) and the continuous phase is of lower
152 density and the *F_nH_m* diblocks are nearly parallel to the water surface. As described in the previous
153 section, the shape and size of mesoscopic domains are determined by the competitive interplay
154 between line tension and dipole interactions. Here, the line tension favors coalescence of circular
155 domains to minimize the tension free energy of line tension, $F_{\text{line}} = \gamma L$. On the other hand, the
156 repulsive electrostatic interactions between domains contribute to avoid the coalescence of
157 domains and keep their size distribution monodisperse.

158 Notably, the self-assembled mesoscopic domains act like colloidal particles and assemble into
159 hexagonal arrays. Topographic profiles acquired by AFM demonstrated that the mesoscopic
160 domains of *F_nH_m* diblocks have long-range order beyond their own sizes. However, once a
161 monolayer has been transferred onto a solid substrate, the domains lose translational and
162 rotational degrees of freedom. Therefore, it is desired to determine the hierarchical structures of
163 self-assembled semi-fluorinated alkanes on a “free” water surface. Among various surface
164 sensitive techniques, grazing incidence small-angle X-ray scattering (GISAXS) is a powerful tool
165 for determining the long-range correlation between self-assembled mesoscopic objects on the
166 water surface that cannot be visualized by optical microscopy.^{49, 50} Fontaine et al. performed
167 GISAXS measurements of *F8H16* monolayers at the air/water interface. At the surface pressure
168 of $\pi = 5$ mN, they identified several diffraction peaks that were assigned to a hexagonal lattice
169 with a unit length of 33 nm.⁴⁷ Bardin et al. found that the lattice parameter increases monotonically
170 as the hydrocarbon chain length increases, suggesting that subtle changes in molecular structure
171 can be used to change the lattice parameter.⁵¹ On the other hand, it is noteworthy that the lattice
172 parameter does not directly correspond to the domain size, because the discrete domains are
173 separated by the diluted phase. Therefore, quantitative calculations of GISAXS signals are
174 necessary to determine the domain size more precisely.

175 Figure 2b shows the experimental setup and the scattering geometry for GISAXS measurements at
176 the air/water interface. A monolayer of diblocks *F_nH_m* or tetrablocks *di(F_nH_m)* is deposited on the
177 Langmuir film balance and compressed to surface pressure π . A monochromatic synchrotron beam
178 impinges the interface at incident angle α_i (slightly lower than the critical angle of total reflection),
179 e.g., $\alpha_i = 0.12^\circ$ for 8 keV and $\alpha_i = 0.2^\circ$ for 10 keV, and the scattering signals are detected using a
180 two-dimensional pixel detector. The GISAXS signal for a *F10H16* monolayer is shown in Figure 2c.

181 The black rectangle near $q_y = 0$ is the direct beam masked by a beam stop. The maximum scattering
 182 intensity is $q_z \approx 0.28 \text{ nm}^{-1}$, called the Yoneda peak. The maximum scattering intensity decreases
 183 rapidly as q_z increases. To gain sufficient counting statistics, the signal intensity was integrated
 184 between $q_z = 0.7$ and 0.9 nm^{-1} (indicated by white dashed lines), which is sufficiently far from the
 185 Yoneda peak and the beam stop. The integrated GISAXS signal is shown in Figure 2d.

186 The GISAXS signal intensity $I(q_y)$ can generally be determined from the structure factor $S(q_y)$ and
 187 form factor $F(q_y)$,

$$188 \quad I(q_y) = A |F(q_y)|^2 \cdot S(q_y). \quad \text{Eq. 4}$$

189 where A is a scaling factor. Form factor $F(q_y)$ reflects the shapes and sizes of the objects, whereas
 190 structure factor $S(q_y)$ reflects the lateral organization of the objects. The model used to determine
 191 the form factor was selected based on AFM observations. For example, $F(q_y)$ for an oblate
 192 hemispheroid with diameter Φ and height H (Figure 2d inset) was used for the *FnHm* domains,⁵²
 193 using Eq. 5

$$194 \quad F(q_x, q_y, q_z, \Phi, H) = 2\pi \int_0^H \left(\frac{\Phi}{2}\right)^2 \left(1 - \left(\frac{z}{H}\right)^2\right) J_1 \left(\frac{\Phi \sqrt{(q_x^2 + q_y^2) \left(1 - \left(\frac{z}{H}\right)^2\right)}}{2} \right) \exp(-iq_z z) dz. \quad \text{Eq. 5}$$

195 J_1 is the first-order Bessel function of the first kind. The calculated $F(q_y)$ is shown as a gray line
 196 in Figure 2d. However, $F(q_y)$ of an oblate hemiellipsoid was used to give the best fit for di(*FnHm*)
 197 tetrablocks.⁵³ It is notable that form factor should be calculated within the framework of the
 198 distorted wave Born approximation (DWBA) that takes the multiple scattering events caused by
 199 the beam larger than the objects into account.^{54, 55}

200 The structure factor $S(q_y)$ is modeled in the framework using paracrystal theory. The $S(q_y)$ for
 201 *FnHm* is modeled using a two-dimensional hexagonal lattice (the blue line in Figure 2d),⁵² as
 202 shown in Eqs. 6 and 7,

203
$$\frac{(1-\varphi^2)^2}{(1+\varphi^2-2\varphi \cos(q_y L))(1+\varphi^2-2\varphi \cos(\frac{q_y L}{2} + \frac{\sqrt{3}q_x L}{2}))},$$
 Eq. 6

204 where

205
$$\varphi = \exp\left(\frac{-(q_x^2 + q_y^2)\sigma^2}{2}\right) \exp\left(\frac{-L}{\xi}\right).$$
 Eq. 7

206 This gives not only the lattice parameter L and its standard deviation σ but also the correlation
 207 length ξ , which cannot be determined from the peak positions. For example, the size and
 208 correlation length of *F10H16* domains calculated from the fit are $\Phi = 30$ nm and $\xi = 505$ nm,
 209 respectively, indicating that the correlation between hemispherical *F10H16* domains can reach
 210 over the distance that is more than 15 times larger than the domain diameter. As shown in the green
 211 line in Figure 2d, the combination of $F(q_y)$ and $S(q_y)$ shows excellent agreement with the
 212 experimental data. On the other hand, $S(q_y)$ for di(*FnHm*) was modeled using an orthorhombic
 213 (distorted hexagonal) paracrystal lattice.⁵³ The best model for each system was selected by
 214 checking for agreement between the global shape of the GISAXS signals and peak positions using
 215 several candidate models. Yet, the use of GISAXS technique to mesoscopic self-assemblies of
 216 small organic molecules is still technically nontrivial for several reasons. The signals can be
 217 smeared if the domain size distribution is broad. Moreover, one should take care about the trade-
 218 off between the signal intensity (counting statistics) vs. radiation damage. Nevertheless, GISAXS
 219 is an emerging technique that opened a large potential for identifying the shapes, sizes, and long-
 220 range orders of mesoscale self-assemblies of organic molecules that form on water surfaces that
 221 cannot be identified otherwise.

222

223 **Mechanical interactions between self-assembled domains: formation of physical gels**

224 Another remarkable characteristic of fluorocarbon surfactants and semi-fluorinated alkanes at the
 225 air/water interface is that a monolayer is very poorly compressible. Maaloum et al. found that the

226 domain structures in films transferred onto solid substrates at $\pi \geq 0.5$ mN/m suggested that *FnHm*
227 molecules self-assemble into mesoscopic domains even without compression.⁴¹ In fact, the onset
228 of surface pressure increase is immediately followed by a steep increase with very small decrease
229 in area. The isothermal compressibility χ of a *FnHm* monolayer at $\pi = 5$ mN/m, $\chi = -\frac{1}{A} \left(\frac{\partial A}{\partial \pi} \right) \approx 10$
230 m/N, is as low as the isothermal compressibility of a fatty acid in a highly condensed state.⁵⁶ In
231 fact, the domain size Φ and the Φ/L ratio of a *FnHm* determined by GISAXS are remarkably
232 independent of π .⁵²

233 These findings suggest that the self-assembled, mesoscopic domains of *FnHm* establish long-range
234 correlation at a very low π . To quantify the significance of mechanical interactions between the
235 domains, several research groups have measured the interfacial viscoelasticity of *FnHm*
236 monolayers. Klein et al. used an interface stress rheometer based on a gliding magnetic needle
237 under oscillating magnetic fields⁵⁷ and reported that *F12H12* and *F12H20* monolayers are
238 predominantly elastic.⁵⁸ Veschgini et al. used another type of interfacial stress rheometer based on
239 a rotating ring coupled to a Langmuir film balance (Figure 3a).⁵⁹ In this system, an oscillatory
240 shear stress is applied to the monolayer by rotating the ring at frequency ω and driving amplitude
241 γ . The amplitude and phase shift of the ring are used to determine the dynamic surface modulus
242 $G^*(\omega) = G'(\omega) + iG''(\omega)$ as a complex function of ω . The real part, the shear storage modulus G'
243 is a measure of the elastic properties, while the imaginary part the shear loss modulus G'' is a
244 measure of the viscous properties.

245 The storage (G') and loss (G'') moduli of a *F8H16* monolayer are plotted against the area per
246 molecule A in Figure 3b. G' and G'' start increasing at $A \approx 50 \text{ \AA}^2$ but the surface pressure remains
247 $\pi \approx 0$ mN/m (inset). G' is always larger than G'' , indicating that the *F8H16* monolayer is
248 predominantly elastic. This behavior, the formation of two-dimensional gels even in a highly

249 diluted phase, is distinct from two-dimensional gelation of other surfactant monolayers that occurs
250 only at a high surface pressure for the formation of hydrogen bonds or through cross-linking via
251 entanglement for denatured proteins.^{59, 60, 61} The unique viscoelastic properties of *F_nH_m* and
252 di(*F_nH_m*) can be attributed to strong dipole repulsion between CF₃ chain termini and CF₂–CH₂
253 junctions preventing the coalescence of surface domains. The systematic variation of the lengths
254 of *F_n* and *H_m* blocks, indicate that the elongation of the *F_n* block from 8 to 12 units increased *G'*
255 by almost an order of magnitude but elongating the *H_m* block did not change *G'* as much.⁵⁶ Genzer
256 et al.⁶² found that increasing ordering in the “bulkier” fluorocarbon chain by elongating the chain
257 from *n* = 10 to 12 does not disturb the lateral packing of hydrocarbon chains. The marked increase
258 in *G'* caused by elongating the *F_n* block can be attributed to the increase in domain size due to a
259 higher alignment of the molecular dipoles.^{30, 52} Figure 3c shows the frequency dispersion of *G'* and
260 *G''* of *F8H18* monolayers measured under a slight variation of area per molecule; 50 Å² ($\pi = 0$
261 mN/m), 40 Å² ($\pi = 0$ mN/m), 33 Å² ($\pi = 1$ mN/m), and 31 Å² ($\pi = 8$ mN/m). At a surface pressure
262 of zero ($A = 50$ and 40 Å²), the power law exponents in the double logarithmic plots for both *G'*
263 and *G''* are ≈ 1 , which is a characteristic of a soft glassy material.^{63, 64} Intriguingly, the exponents
264 of *G'* become zero as soon as the surface pressure becomes positive but the *G''* exponents remain
265 constant at ≈ 1 . The observed frequency dispersions at $\pi > 0$, $G' \propto f^0$ and $G'' \propto f^1$, are typical for
266 a classical Kelvin–Voigt solid.⁶⁵ When one plots *G''* versus *G'* (Figure 3d), the film behaves
267 predominantly elastic over the whole frequency window at $\pi = 0$ mN/m ($G' < G''$) and the phase
268 shift δ remains at ≈ 0.6 (inset). In contrast, once the film was slightly compressed to $\pi > 0$ mN/m
269 (open symbols), δ shows an increase with increasing frequency *f* to the region where the film
270 becomes predominantly viscous ($\delta > \pi/2$, $G'' > G'$) beyond a critical frequency. The formation of
271 two-dimensional physical gels through mesoscopic domains of *F_nH_m* diblocks even at $\pi = 0$ mN/m

272 shares common features with the formation of gels from “hard” particles but has not previously
273 been found for self-assembled films of small organic molecules. These rheological properties can
274 be attributed to strong dipole repulsion between *FnHm* domains.

275

276 **Higher-order self-assembly in lipid membranes**

277 Sphingolipids, cholesterol, and phospholipids in biological cell membranes form laterally
278 organized functional domains called “rafts”.⁶⁶ Ample evidence has shown that
279 mesoscopic/microscopic domains play key roles in complex cellular functions because spatial
280 confinement of functional molecules allows fine-tuning of cooperative polyvalent interactions
281 between biomolecules.⁶⁷ Many studies showed the formation of raft-like domains by the self-
282 assembly of “raft-like” lipid mixtures⁶⁸, but most of them are either polydisperse in size or
283 coalescing into large domains due to line tension. As described above, using lipids containing
284 fluorocarbon chains is a promising strategy to avoid the tension-driven coalescence of domains.

285 The total free energy F_{total} of a membrane containing domains with diameter D can be described
286 as the sum of the electrostatic energy F_{el} and tension energy F_{λ} ³¹ using Eqs. 8–10,

$$287 \quad F_{\text{total}} = F_{\text{el}} + F_{\lambda}, \quad \text{Eq. 8}$$

288 where

$$289 \quad F_{\text{el}} = \pi D (\Delta m)^2 \ln \left(\frac{e^2 \delta}{4R} \right), \quad \text{Eq. 9}$$

290

291 and

$$292 \quad F_{\lambda} = \pi D \lambda. \quad \text{Eq. 10}$$

293 Δm is the difference between the molecular dipole densities of the lipids in the domains and the
294 matrix lipids, δ is the dipole–dipole distance at the phase boundary, and e is Euler’s constant. The

295 line tension λ contains the mismatch between the surface tension $\Delta\gamma$ and the molecular length Δl ,
 296 $\lambda = \Delta\gamma \cdot l + \gamma \cdot \Delta l$. Minimizing the yields therefore gives the equilibrium domain diameter D_{eq} ,

$$297 \quad D_{\text{eq}} = \left(\frac{e^3\delta}{8}\right) \exp\left(\frac{\lambda}{\Delta m^2}\right). \quad \text{Eq. 11}$$

298 D_{eq} can be calculated because all of the parameters required for the calculation can be determined
 299 experimentally. This suggests that the domain size can be changed by a subtle change in the lengths
 300 and densities of the fluorocarbon chains.

301 Pasc et al. synthesized lipids with perfluorinated anchors (FLn; Figure 4a) and mixed them with
 302 phospholipids with hydrocarbon chains such as dioleoylphosphatidylcholine (DOPC).^{69, 70}
 303 Fluorescence images of DOPC monolayers incorporating FL10, FL13, and FL17 domains at molar
 304 fractions χ of 0.10, 0.20, and 0.33 are shown in Figure 4b. The domains are round and uniform,
 305 showing no sign of coalescence even at a high χ value. The calculated D_{eq} values (T) and
 306 experimentally determined domain size D (E) are shown in Figure 4c. It can be seen that the results
 307 agree well as the fluorocarbon chain is elongated, so increasing n leads to a marked decrease in
 308 the domain size.⁷⁰ The size of the FL17 domains was close to the optical microscope resolution (D
 309 $\approx 0.3 \mu\text{m}$), which was also precisely determined by grazing incidence X-ray diffraction using a Si
 310 (111) analyzer crystal.⁶⁹ Like other colloidal self-assemblies in different length scales,^{71, 72} the
 311 long-range correlation between FLn domains can be assessed by calculating the potential of mean
 312 force $V(r)$ within the framework of inverse work function theorem using Eq. 12

$$313 \quad V(r) \sim -k_B T \ln g(r), \quad \text{Eq. 12}$$

314 where $g(r)$ is the radial distribution function

$$315 \quad g(r) = \frac{dN(r+dr)}{d(A+dA)} \cdot \frac{N}{A}. \quad \text{Eq. 13}$$

316 Figure 4d shows $-\frac{V(r)}{k_B T}$ plotted as a function of r for FL10, FL13, and FL17 at $\chi = 0.33$. The
317 position of the first minimum in each curve coincides with the mean center-to-center distance
318 between the nearest neighbors and monotonically decreases as n increases. The potential near the
319 first minimum can be approximated well as a harmonic potential (red line), and the significance of
320 inter-domain correlation can be evaluated by the second derivative of the potential that is nothing
321 but a spring constant for the potential confinement. The spring constant increases by an order of
322 magnitude as the fluorocarbon chain length increases from $n = 10$ to 17, indicating that the smaller
323 FLn domains are confined in steeper potential wells than the larger FLn domains. Intriguingly, the
324 FL17 domains have distinct minima up to the third order, indicating that the relationships between
325 domains can reach more than an order of magnitude further than the size of a single domain. As
326 expected, the spring constant decreases as χ decreases and uncorrelated interactions following the
327 Yukawa potential⁷³ occur at $\chi < 0.05$.⁷⁰ More recently, Nakahata et al. demonstrated that the
328 tetrablock di(F10H16) incorporated into a dipalmitoylphosphatidylcholine (DPPC) monolayer
329 affects the lateral organization of DPPC, resulting in large, complex flower-like patterns using
330 fluorescence and Brewster angle microscopy.⁷⁴

331

332 **Higher-order self-assembly for biomimetic coating materials**

333 From the biomaterial science viewpoint, adding functionalities to highly ordered meso-/micro-
334 domains of fluorinated lipids embedded in lipid membranes could allow the lipid membranes to
335 act as surrogate surface coatings containing ligand molecules confined in defined, discrete domains.
336 This offers advantages over commonly used surrogate surfaces for studying the adhesive functions
337 of cells. For example, confluent cell monolayers such as monolayers of epithelial cells cannot
338 distinguish between the roles of different ligand-receptor pairs because cells contain complex

339 mixtures of ligand molecules. A surrogate surface based on a supported membrane will allow the
340 surface to be functionalized at precisely controlled inter-ligand densities, but the distribution of
341 functional moieties on the surface is uniform unless a phase-separated membrane is used. However,
342 as described above, the domains of lipid mixtures containing only hydrocarbon chains tend to
343 coalesce due to line tension, resulting in domains with sizes comparable to or even larger than a
344 cell.

345 Kaindl et al. chemically attached simple mannose to the head group of FL10 (FL10Man; Figure
346 5a) and investigated how discrete ligand confinement affects adhesion of macrophage-expressing
347 mannose receptor, which is a 180 kDa C-type lectin containing multiple mannose binding units.⁷⁵
348 Macrophages use multivalent mannose receptor interactions to capture and internalize various
349 mannose-containing proteins.^{75, 76} A fluorescence image and the potential of mean force for
350 FL10Man at $\chi = 0.33$ are shown in Figure 5b. It can be seen that FL10Man forms uniform and
351 highly ordered domains like FL10 (Figure 4b), showing no sign of coalescence. This provides
352 clear proof that dipole repulsion between fluorocarbon chains but not between head groups plays
353 a dominant role in preventing coalescence of self-assembled lipid domains. Confocal fluorescence
354 images and microinterferometry images of a murine macrophage adhered to glass substrates coated
355 with three types of lipid membranes are shown in Figure 5c. Most of the cells in contact with pure
356 phospholipid (DOPC) membranes could be removed even by a gentle medium exchange. The
357 three-dimensional reconstructed confocal image of a cell shown in the upper left panel of Figure
358 5c suggests that the cell is hardly deformed and retains its original ellipsoid shape. The
359 microinterferometry image (lower left of Figure 5c) indicates that very little cell–substrate contact
360 occurred (the relevant area is indicated with a yellow line for clarity), indicating almost negligible
361 non-specific cell adhesion to a pure phospholipid membrane. In contrast, when the same

362 macrophages are in contact with the substrate coated with a membrane only consisting of
363 FL10Man ($\chi = 1.0$), the cells were markedly deformed and spread all over the substrate (upper
364 middle part of Figure 5c). The cell–surface contact area shown as a dark area in the
365 microinterferometry image (lower middle part of Figure 5c) is uniform, indicating that the bottom
366 surfaces of the cells adhere uniformly to the membrane-coated substrate. As shown in the upper
367 right image in Figure 5c, macrophages adhere to and spread on the membrane-displaying domains
368 of FL10Man ($\chi = 0.33$) and extend thin lamellipodia to the surrounding areas. Intriguingly, the
369 adhesion zones visualized by microinterferometry contained uniform dark spots (lower right part
370 of Figure 5c) with comparable sizes to the FL10Mon domains (Figure 5b). During the initial
371 spreading process, the spreading front repeatedly stick and advance rather than continually
372 propagated, indicating that the cells find discrete contact points when they are in contact with a
373 new environment. A membrane containing discrete functionalized fluorinated lipid domains could
374 also be used as a coating for a coverless microfluidic channel on which the medium is driven by a
375 surface acoustic wave⁷⁷ but not by a pump to investigate dynamic adhesion of bacteria onto
376 mannose-functionalized domains (Figure 5d).⁷⁸ As these meso-/micro-domains are formed via
377 self-assembly in the aqueous phase, the domains expressing more complex oligosaccharide
378 moieties can easily be reconstituted in cell-sized, giant lipid vesicles without printing or pre-
379 patterning (Figure 5f).²⁹

380

381 **Higher order self-assembly for medical applications**

382 Hierarchically organized self-assemblies of fluorinated molecules have been drawing increasing
383 attentions for medical applications. Various types of supramolecular formulations including gels,
384 vesicles, emulsions, microbubbles, and phase-shift emulsions have been designed as contrast

385 agents for multimodal diagnosis, delivery of energy, delivery of oxygen, nitric oxide and other
386 gases, drugs and biomarkers.^{16, 79, 80} The few recent examples discussed below illustrate the role
387 of such self-assemblies in diagnosis and therapy.

388 Commercial microbubbles currently used for ultrasound diagnostics are coated with shells made
389 of lipids or albumin, but increasing the control over microbubble size and stability characteristics
390 is a sought after challenge. Among various lipids, DPPC is commonly chosen because they are in
391 mechanically stable liquid condensed phase at 37 °C.⁴¹ Microbubbles consisting of phospholipids
392 and self-assembled fluorinated compounds have been applied for the medical imaging and
393 therapeutic treatment, because the addition of fluorinated compounds increased the life time of
394 microbubbles.⁷⁹ It is notable that pure *FnHm* monolayers tend to collapse under a slight decrease
395 in the surface area due to a poor compressibility originating from the strong dipole repulsions.⁵⁶

396 As the quantitative measurements of structure and viscoelasticity of *FnHm* domains in
397 phospholipid microbubbles are technically not possible, Mielke et al. measured the viscoelasticity
398 of the mixed monolayers of DPPC and diblocks *FnHm* using dilational rheometer.⁸¹ The use of a
399 monolayer at the liquid/gas interface can be considered as a well-defined mechanical model of
400 microbubbles, because the film thickness (≈ 3 nm) is much smaller than the size of microbubbles
401 (~ 1 μm). Although individual *FnHm* domains cannot be visualized by optical microscopy, they
402 accumulate at the boundary of liquid expanded and liquid condensed phases of DPPC.⁴¹ The
403 *FnHm*-DPPC monolayers exhibited viscoelastic properties comparable to those of phospholipid
404 monolayers and persisted under a larger strain under which a pure *F8H16* monolayer collapsed.

405 Therefore, the mixing of *FnHm* with phospholipid matrix is a promising strategy to improve the
406 bubble stability.

407 More advanced formulations involve the incorporation of iron oxide nanoparticles in phospholipid
408 microbubbles for the bimodal ultrasound/MRI contrast agents. This can be realized by coating
409 nanoparticles with amphiphilic bisphosphonate dendrons bearing three oligo(ethylene glycol)
410 chains terminated with $-C_2F_5$ or $-C_4F_9$ groups (Figure 6a). The presence of perfluoroalkyl terminal
411 groups was essential to achieve a high stability, because the nanoparticles coated with fluorinated
412 dendrons are located inside the interfacial film, while those bearing hydrocarbon dendrons remain
413 on the surface.⁸²

414 An increasing number of studies have shown that the presence of a fluorocarbon in the gas phase
415 modulates the molecular self-assembly at the gas/liquid interface and enhances the stability of
416 microbubbles significantly. For example, perfluorohexane (*F*-hexane) leads to an increase in the
417 compressibility of pure *FnHm* monolayers by adsorbing to the gas/liquid interface.⁸³ Moreover,
418 *F*-hexane-enriched gas phase facilitated the displacement of albumin by DPPC at the gas/water
419 interface under periodic deformations mimicking respiration,⁸⁴ suggesting that the presence of
420 fluorocarbon gas inside microbubbles is a promising strategy for the formulation of lung surfactant
421 substitutes. As shown in Figure 6b, the hypoxia biomarker EF5 that is connected to a $-C_2F_5$ group
422 is readily incorporated from the aqueous phase into DPPC microbubbles, whose interior is
423 enriched with *F*-hexane.⁸⁵ In contrast, EF5 remained in the aqueous phase when the bubble is filled
424 with air. This effective and stable recruitment of EF5- C_2F_5 in the presence of *F*-hexane can be
425 explained by the attractive interactions between the C_2F_5 moiety and *F*-hexane. Interestingly, the
426 fluorine-functionalized molecules were shown to partly remain trapped in the DPPC monolayer
427 even after the removal of fluorocarbon gas.⁸⁶

428 As shown in Figure 6c, the self-assembly of oleophobic fluoroalkyl moieties can further be used
429 to design even more complex composite nanomaterials by coating graphene oxide (GO) or

430 oxidized single-walled carbon “nanohorn” with the layers of (i) fluorinated dendrimer (*F*-DEN)
431 and (ii) fluorinated poly(ethylene glycol) (*F*-PEG).⁸⁷ The various components/building blocks
432 contribute additively to O₂ adsorption, and the highest O₂-loading capacity was attained by the
433 combination of nanohorns coated with *F*-DEN and *F*-PEG. O₂ first adsorbs on the outer *F*-PEG
434 shell, penetrates into the middle *F*-DEN region, and eventually reaches the nanohorn core. The
435 irradiation with a light-emitting diode produced singlet oxygen, ¹O₂, which can be used for
436 oxygen-dependent (e.g. photodynamic) therapy of cancer as well as for fighting bacteria and
437 viruses through ¹O₂ generation. A similar formulation built from *F*-DEN, *F*-PEG and nitrogen-
438 doped carbon nanohorns were devised for efficient nitric oxide delivery utilizing the concentric
439 organization.⁸⁸

440

441 **Summary and perspectives**

442 The general physical principle stabilizing various self-assembled patterns of domains is the
443 interplay between competing intermolecular interactions. On two-dimensional water surfaces, the
444 competition of line tension and dipole interaction plays key roles. This review puts a special focus
445 on fluorinated surfactants that form unique patterns of self-assembled domains at the air/water
446 interface. These patterns, ranging from periodic micrometer-wide stripes to highly correlated
447 mesoscopic hemi-ellipsoids, are clearly different from those formed by their hydrocarbon
448 analogues. In contrast to the domains of hydrocarbon surfactants, the fluorocarbon domains do not
449 coalesce by compression, indicating the dipole repulsion dominates the line tension in the free
450 energy minimization. As demonstrated by the quantitative GISAXS analysis, the shape, size, and
451 lateral correlation between mesoscopic domains are modulated by subtle changes in molecular
452 structures, such as the lengths of fluorocarbon- and hydrocarbon chains. Notably, the correlation

453 between mesoscopic domains can reach over the distance that is one order of magnitude larger
454 than the domain diameter. Such repulsive, long-range interactions between domains also affect the
455 mechanical properties of fluorinated surfactant layers significantly. Interfacial shear rheology
456 experiments revealed that the monolayers of semifluorinated diblocks form two-dimensional
457 physical gels even at zero surface pressure. This is clearly different from surfactants with
458 hydrocarbon chains that become predominantly elastic only at high surface pressures.

459 The unique physical principle of self-assembly and pattern formation by fluorinated surfactants
460 open a wide range of biomedical applications. For example, the addition of biofunctional units
461 enables to confine ligand molecules in discrete areas, which can readily be transferred to the
462 surface of solid substrates or micro-fluidic channels. The substrates displaying highly ordered
463 functional mesostructures are ideally suited to study multivalent, cooperative interactions in
464 biological systems, such as cell adhesion caused by multivalent carbohydrate-protein interactions.

465 As discussed in the last section, a wide variety of supramolecular self-assemblies including gels,
466 vesicles, emulsions, and microbubbles have been formulated for numerous medical applications,
467 including ultrasound/MRI diagnosis, delivery of medical gases such as O₂ and NO, as well as the
468 delivery of drugs and biomarkers. The use of self-assembly in the aqueous phase allows for the
469 integration of mesoscopic domains not only into flat substrates but also into three-dimensional
470 micells and cell-sized vesicles, which cannot be achieved by covalent coupling, printing, or
471 patterning techniques.

472

473

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492

493 **Conflict of Interests**

494 The authors declare that there is no conflict of financial interests in relation to the works described
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817

818 **Figure Captions**

819

820 **Figure 1. Self-assembly of lipids with fully hydrogenated and fluorinated chains.**

821 (a) Fully hydrogenated diHC16 forms circular, bubble-like domains. (b) In contrast, partly
822 fluorinated diFC8-8 forms stripe-like domains soon after the surface pressure increases because of
823 corresponding to the coexistence of gas and liquid-expanded phases. The liquid-expanded phase
824 was labeled with fluorescent Texas Red dihexadecyl phosphatidylethanol amine. Partially adapted
825 from Ref. 30.

826

827 **Figure 2. Shape, size and lateral correlation between mesoscopic domains determined by**
828 **quantitative grazing incidence small-angle X-ray scattering (GISAXS) analysis at the**
829 **air/water interface.**

830 (a) Chemical structures of F_nH_m diblocks and $di(F_nH_m)$ tetrablocks. (b) Experimental setup and
831 scattering geometry. (c) GISAXS signal for the $F10H16$ monolayer collected using a two-
832 dimensional detector. The black rectangle is the direct beam masked by a beam stop. The signals
833 in the area surrounded by the white broken line were integrated along q_z . (d) Integrated signal
834 intensity plotted against q_y (circles). The form factor $F(q_y)$ (gray) and structure factor $S(q_y)$ (blue)
835 agreed very well with the experimental data. Partially adapted from Refs. 52 and 53.

836

837 **Figure 3. Mesoscopic domains of F_nH_m forming a two-dimensional network (gel) even at**
838 **zero pressure.**

839 (a) Interfacial stress rheometry experimental setup. (b) Typical storage modulus G' and loss
840 modulus G'' is predominantly elastic ($G' > G''$) of the F_nH_m monolayer ($n = 8, m = 16$). The
841 corresponding isotherm shown in the inset indicates that the monolayer under all conditions. (c)
842 Frequency dispersion of the $F8H16$ monolayer showed that $G' \propto f^1$ and $G'' \propto f^1$ at $\pi = 0$ mN/m,
843 which is a characteristic of soft glassy matter. At $\pi > 0$, the power law exponents change to those
844 of Kelvin–Voigt solid, $G' \propto f^0$ and $G'' \propto f^1$. (d) Plot of G'' against G' for the $F8H16$ monolayer
845 acquired at different f values. The inset is the phase angle δ plotted against the frequency,
846 indicating that the monolayer is predominantly elastic over the whole frequency window at $\pi = 0$
847 mN/m ($G'' < G'$) and the phase shift δ remains constant at ≈ 0.6 . Adapted from Ref. 56.

848

849 **Figure 4. Perfluorinated lipid (FLn) achieving long-range order in a phospholipid membrane.**

850 (a) Chemical structure of FLn lipids ($n = 10, 13, 17$). (b) Fluorescence microscopy images of FL10,
851 FL13, and FL17 measured at $\pi = 20$ mN/m at molar fractions χ of 0.10, 0.20, and 0.33. The bright
852 regions coincide with DOPC (matrix) lipids, while the dark regions are FLn domains. (c)
853 Equilibrium domain sizes D_{eq} calculated using the effective dipole model (T) and experimental
854 data (E) for $\chi = 0.33$. The size distributions (mean \pm standard deviation) and the errors of
855 parameters used for the calculation are presented by the error bars. (d) Normalized potential of
856 mean force $-V(r)/k_B T$ plotted against distance from the center of domain r . Adapted from Refs. 69
857 and 70.

858

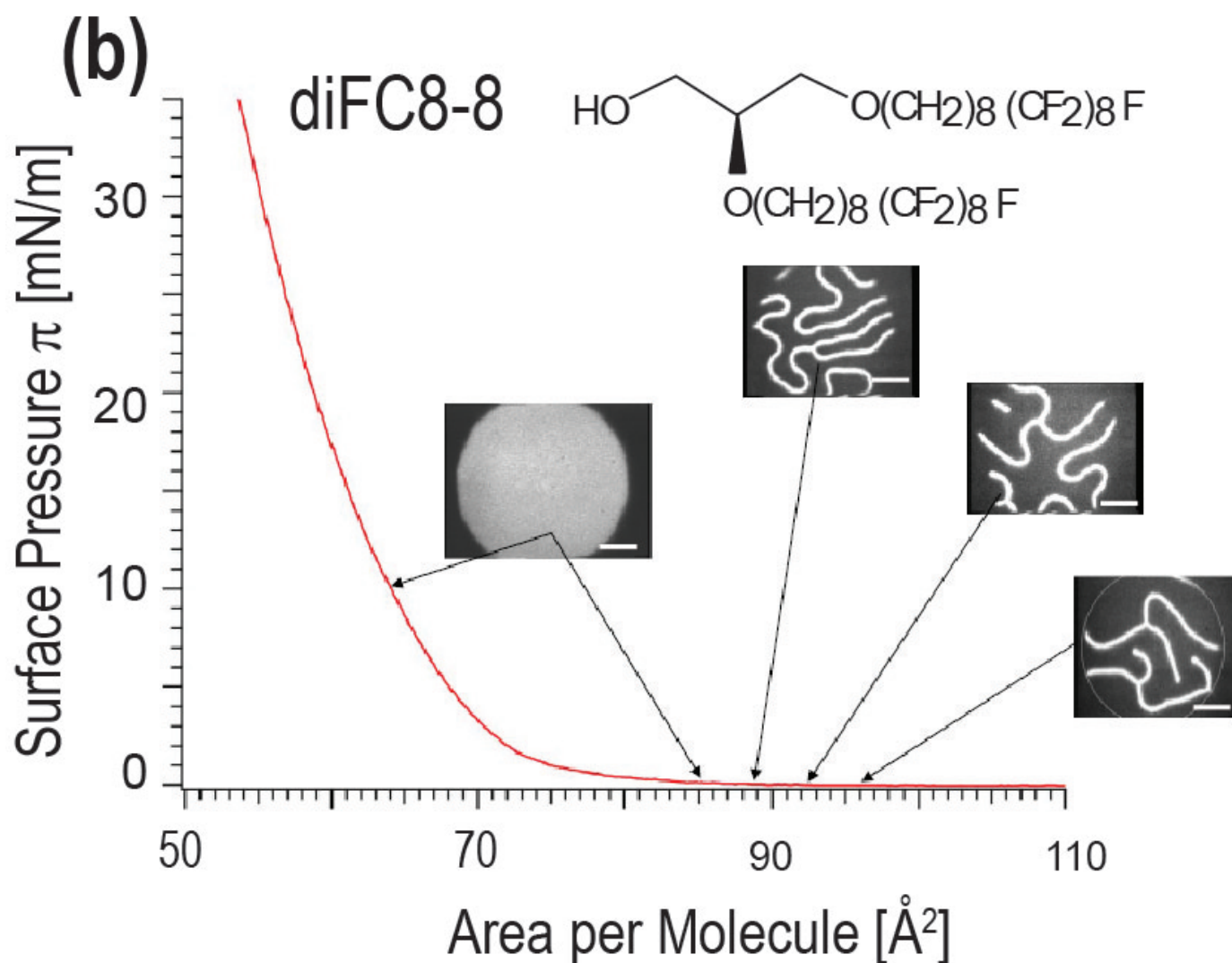
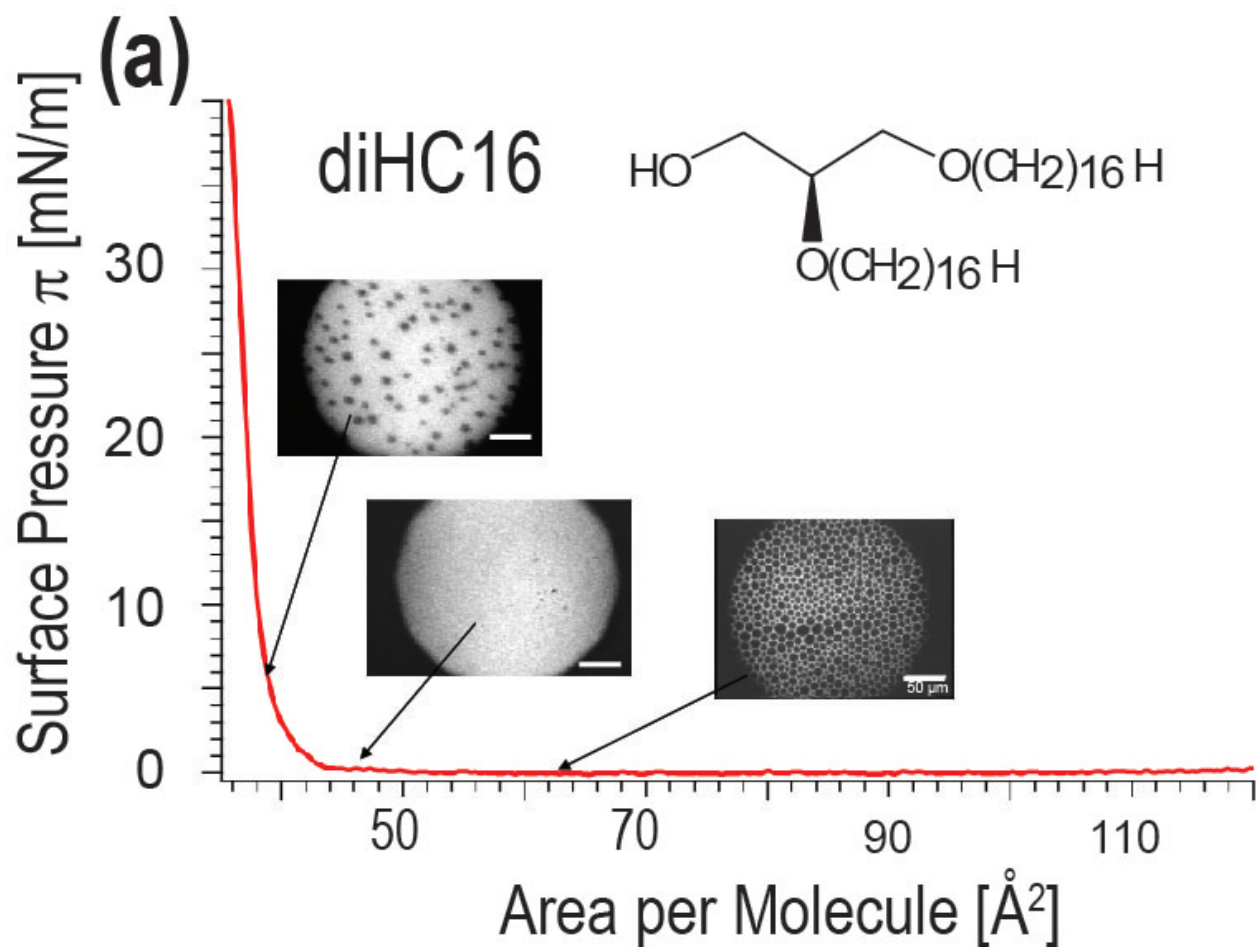
859 **Figure 5. Highly ordered biofunctional domains used as biomimetic coating materials.**

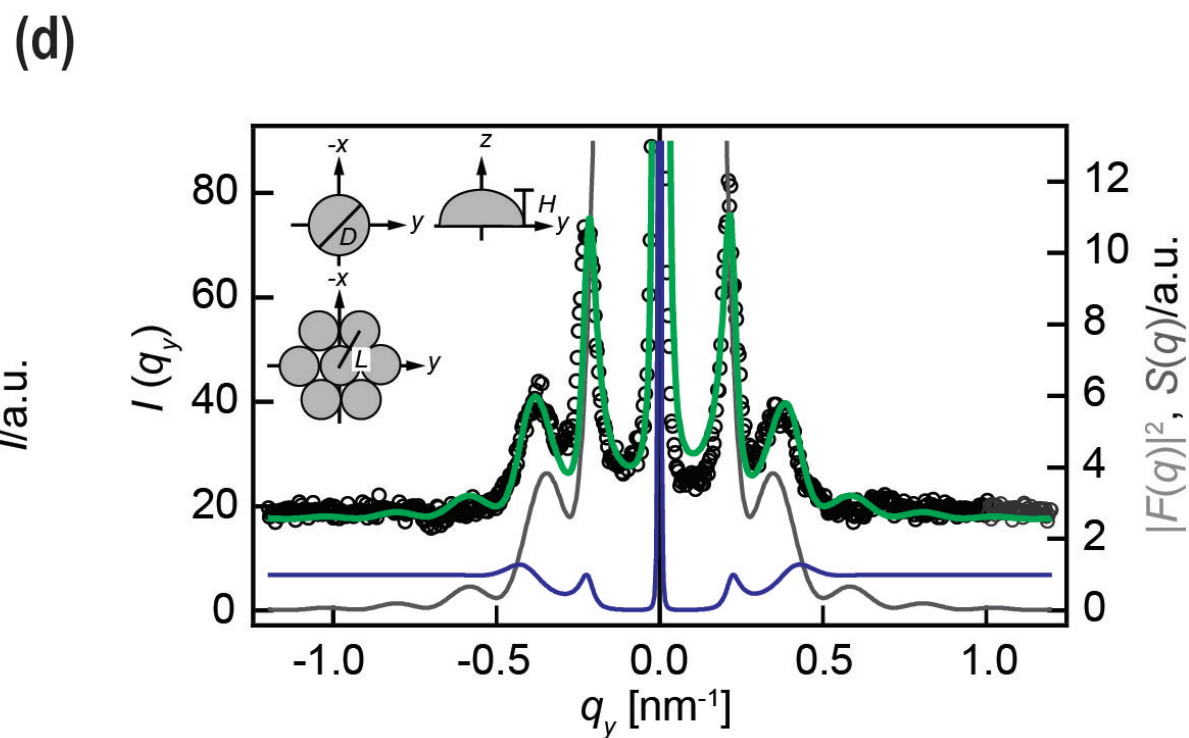
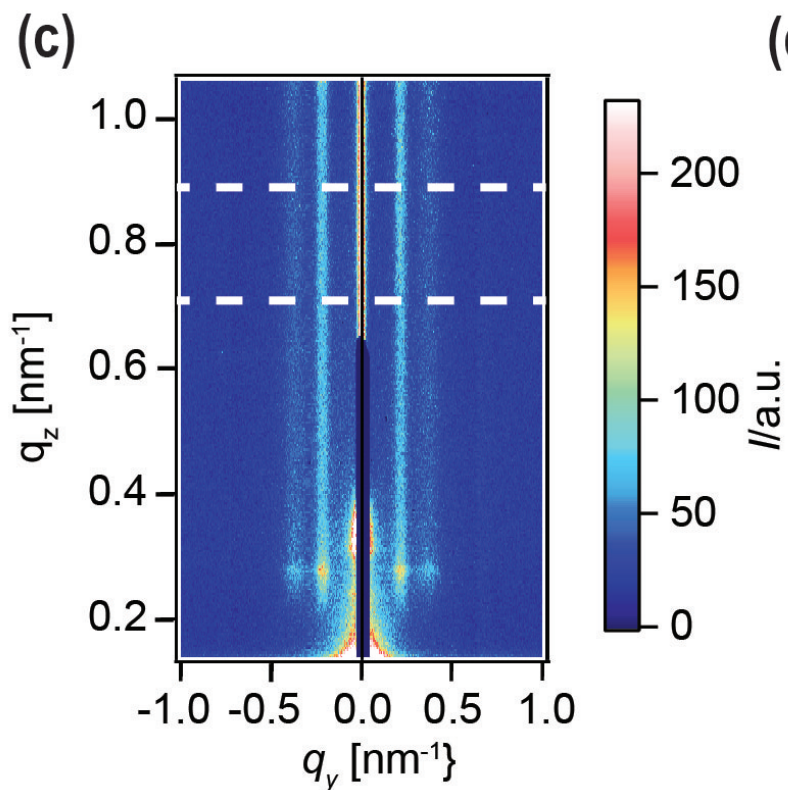
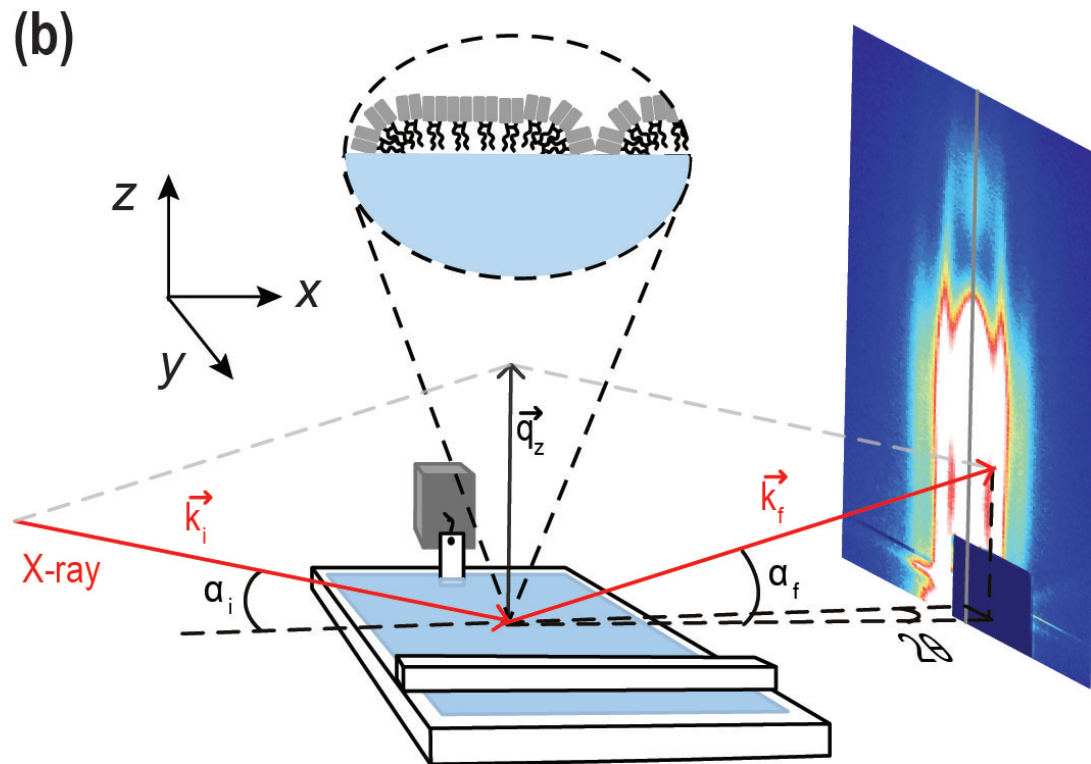
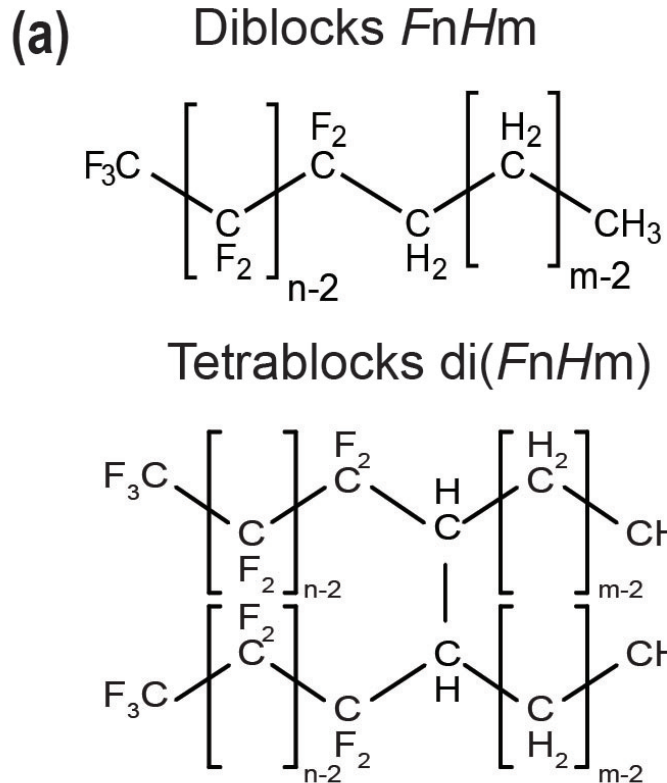
860 (a) Chemical structure of FL10 coupled to mannose (FL10Man). (b) Normalized potential of mean
861 force and fluorescence image of FL10Man indicating the dominant role of dipole repulsion in the
862 formation of highly ordered domains. (c) Membrane with FL10Man domains used as a biomimetic
863 coating material for cells. Adhesion of a macrophage cell on a pure phospholipid membrane (left),
864 pure FL10Man membrane (middle), and membrane incorporating FL10Man at $\chi = 0.33$ (right).
865 The cells form discrete contact points on the FL10Man domains. (d) Integration of FL10Man-
866 incorporating membranes into coverless microfluidics driven by a surface acoustic wave (SAW)
867 to investigate cell adhesion under high shear stress. (e) Cell-sized leukocyte model coated with
868 sialyl-LewisX antigen microdomains. Adapted from Refs. 29, 70, and 78.

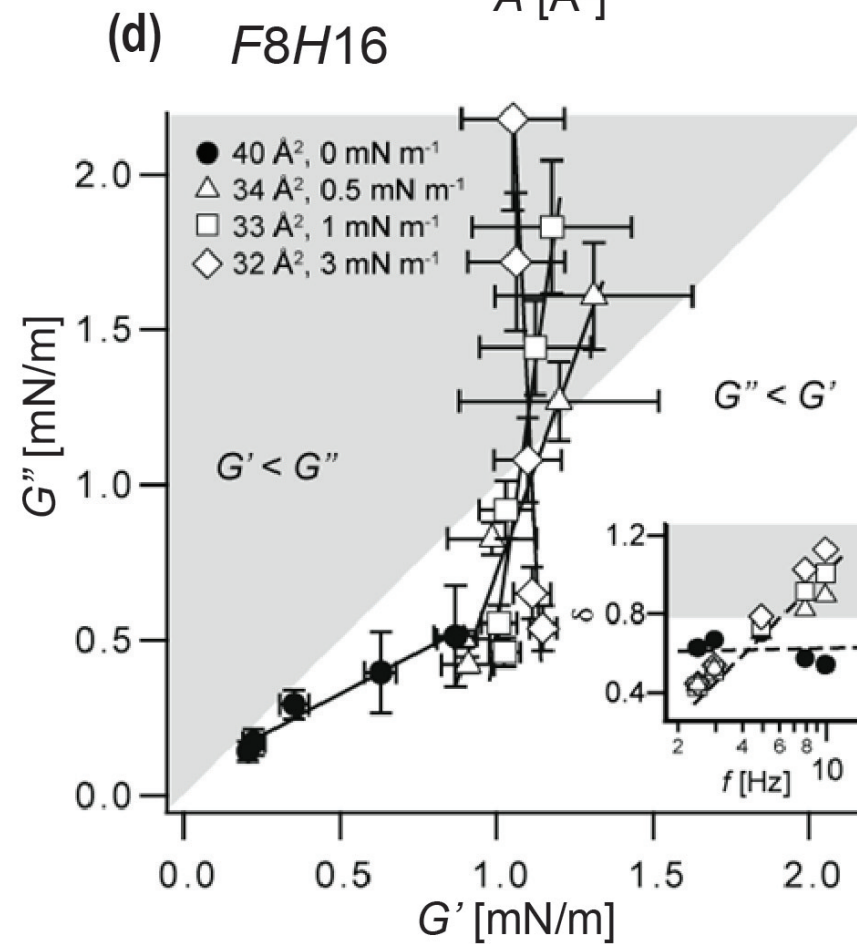
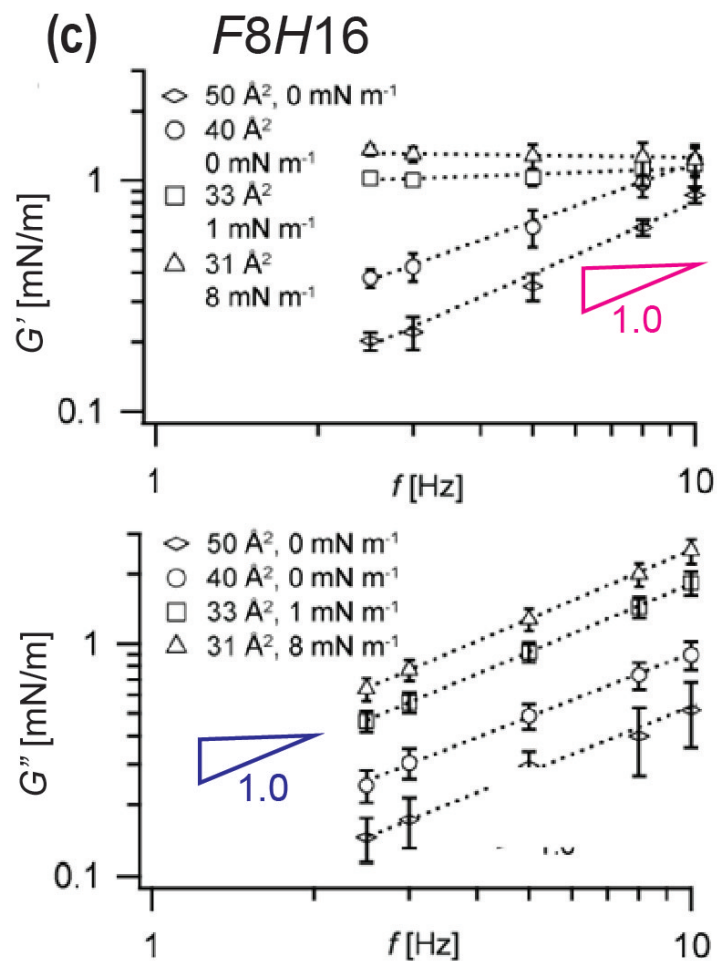
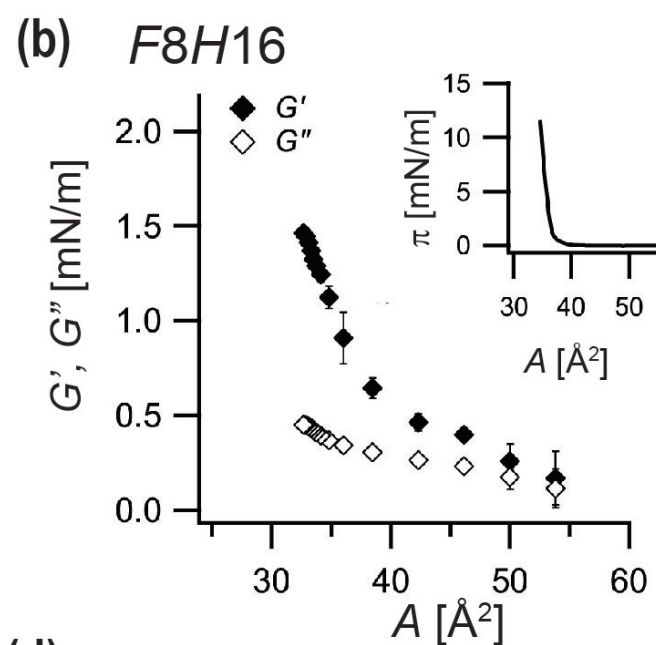
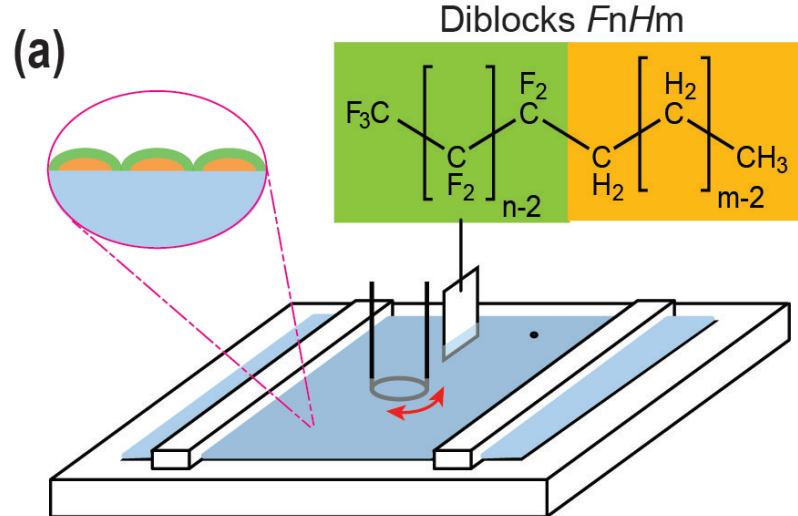
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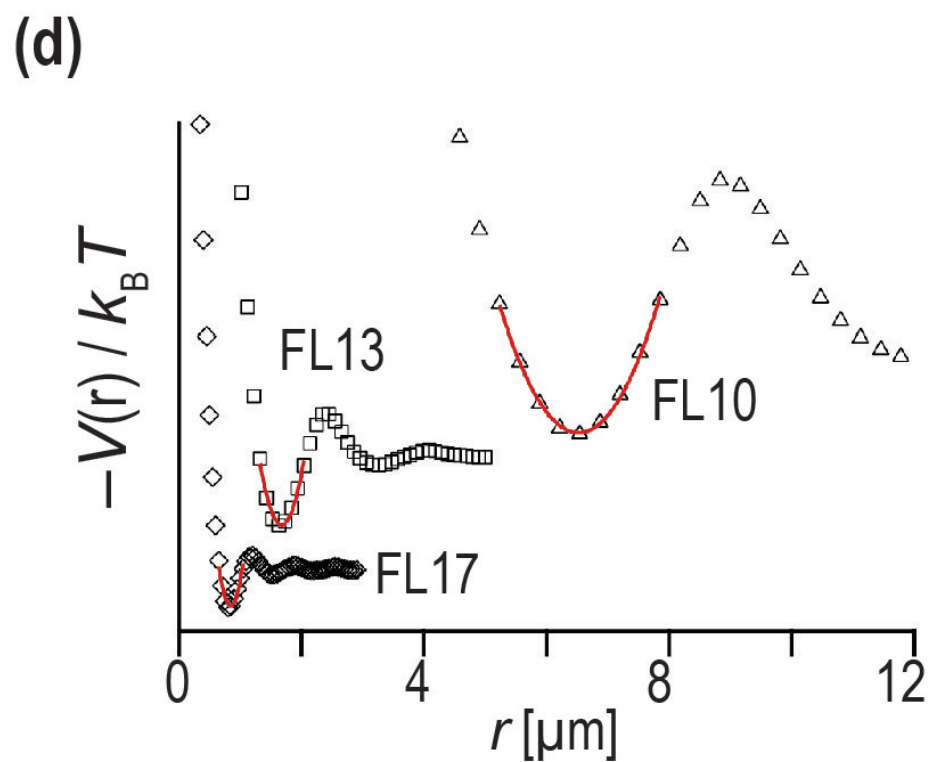
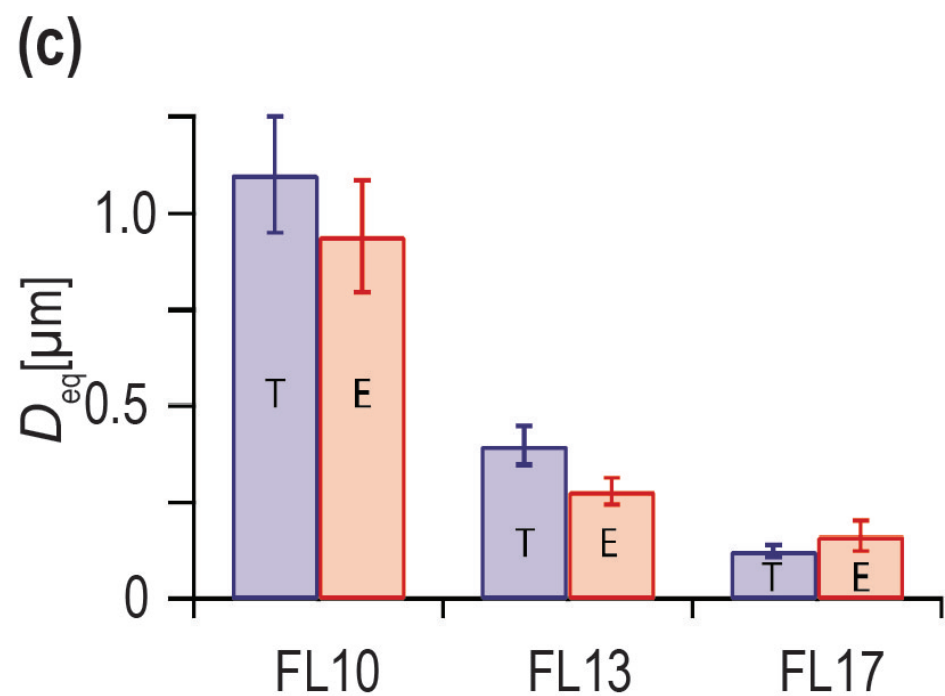
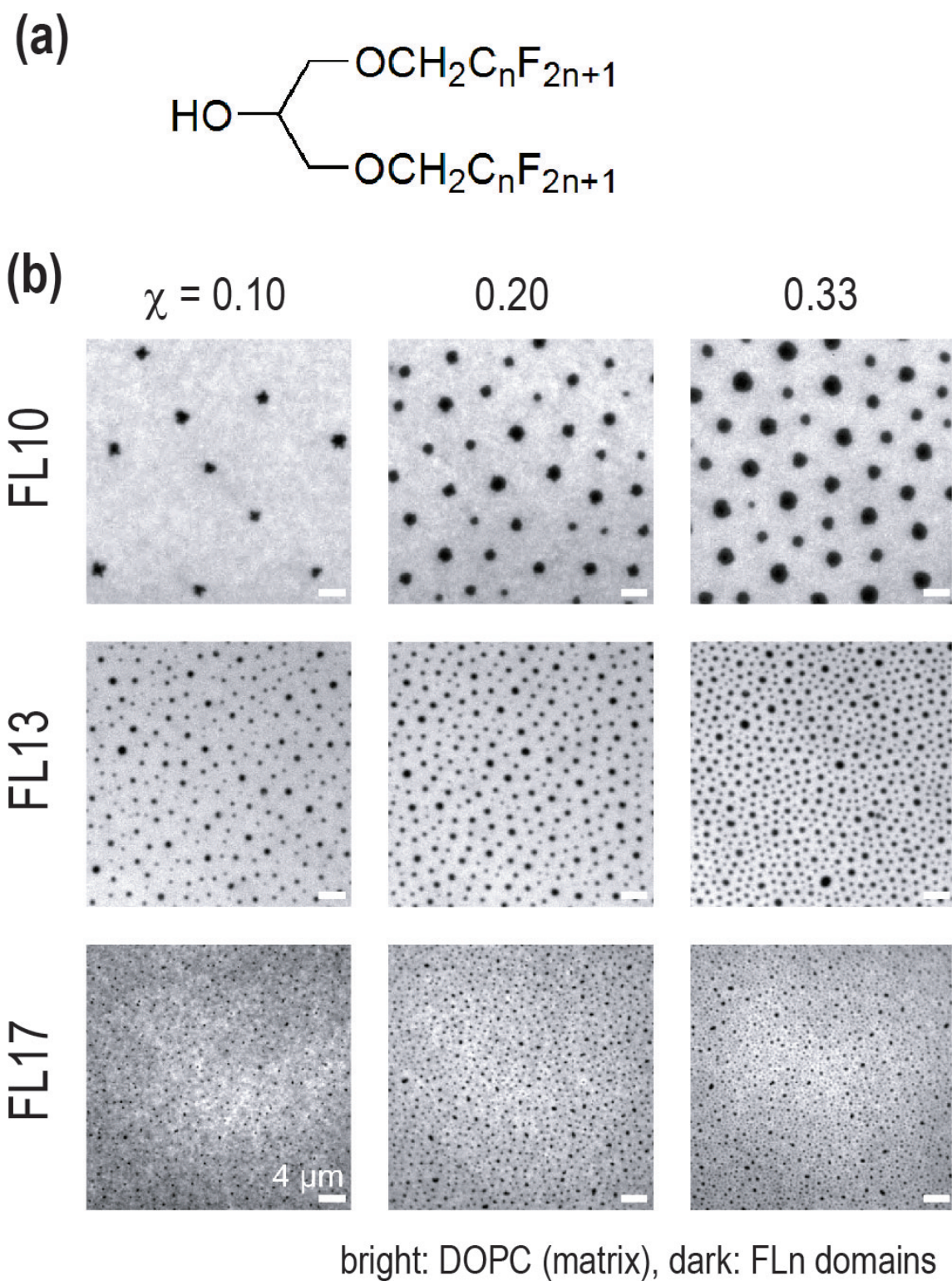
870 **Figure 6. Hierarchically organized fluorinated self-assemblies for biomedical applications.**

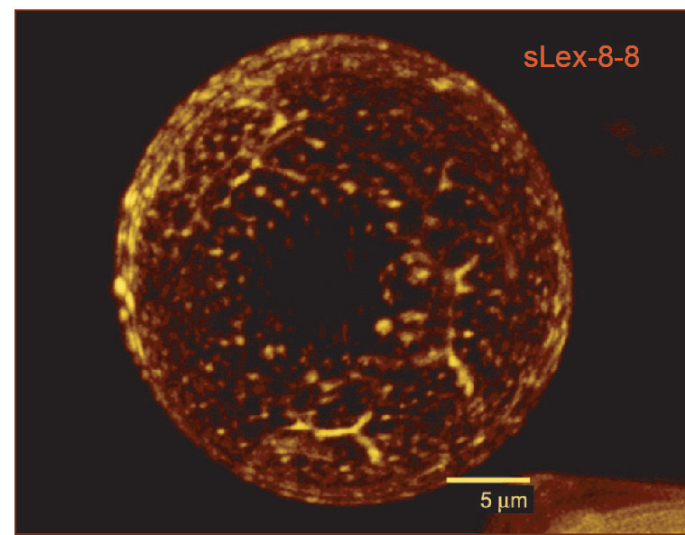
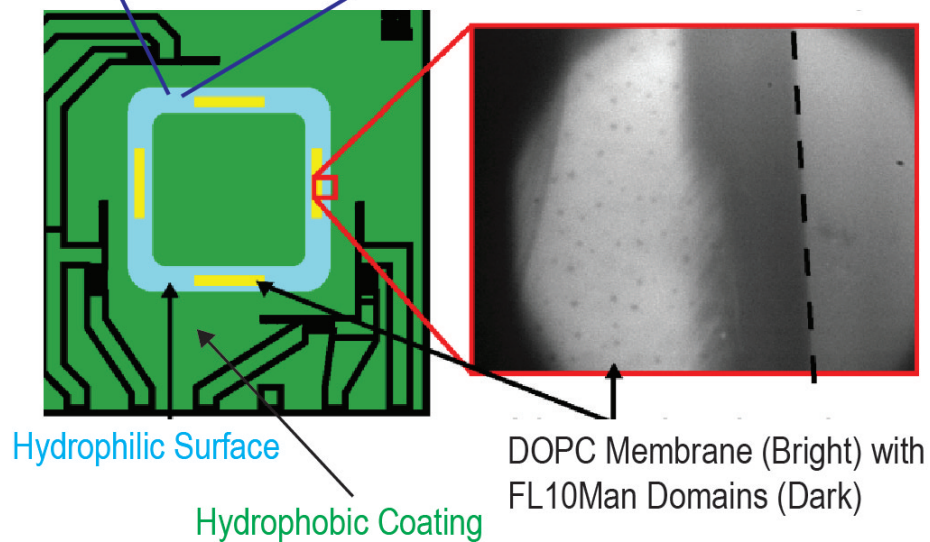
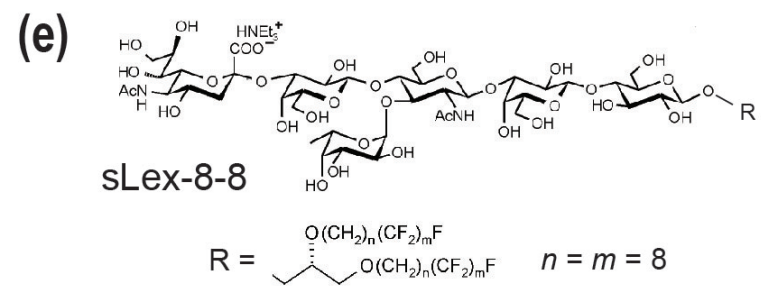
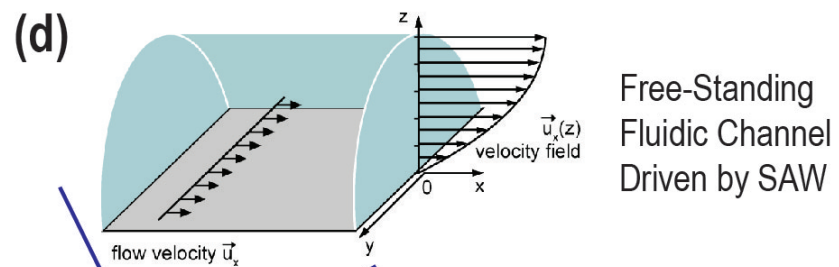
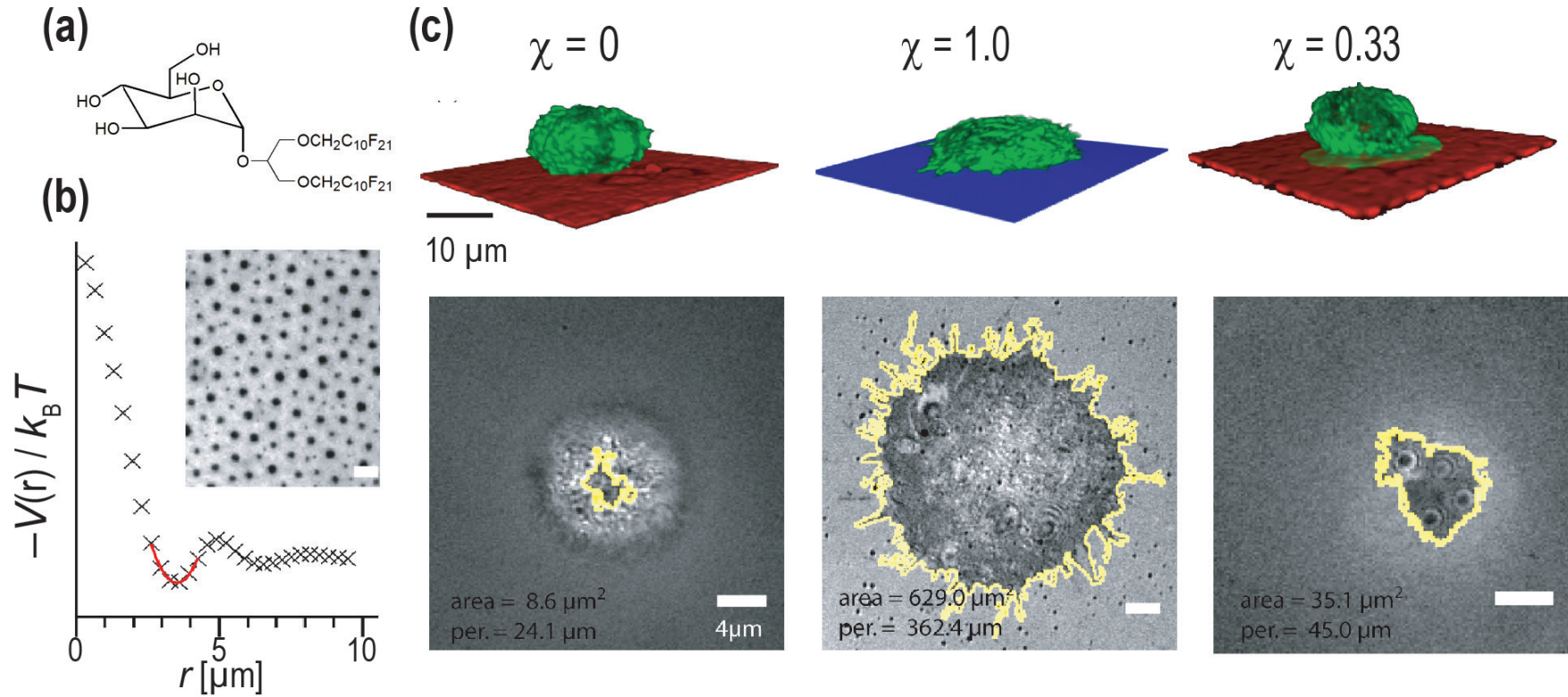
871 (a) Fluorocarbon-stabilized microbubbles with a coating of phospholipids and dendronized iron
872 oxide nanoparticles for bimodal ultrasound/MRI imaging diagnostics. Adapted from Ref. 73. (b)
873 Left: the fluorocarbon saturating the inner gas content of the microbubble allows adsorption of the
874 fluorinated hypoxia biomarker EF5 in a monolayer of phospholipids. Right: Optical microscopy
875 image of an aqueous dispersion of EF5-containing microbubbles. Adapted from Ref. 76. (c)
876 Schematic representation of the functionalization of a graphene oxide (GO) sheet by a layer of
877 fluorinated dendrimers (F -DEN) on which a layer of fluorinated PEG (F -PEG) is adsorbed. These
878 nanocomposites have proved efficacy as O_2 reservoirs for reactive oxygen species production to
879 kill bacteria and perform photodynamic therapy. Adapted from Ref. 87.



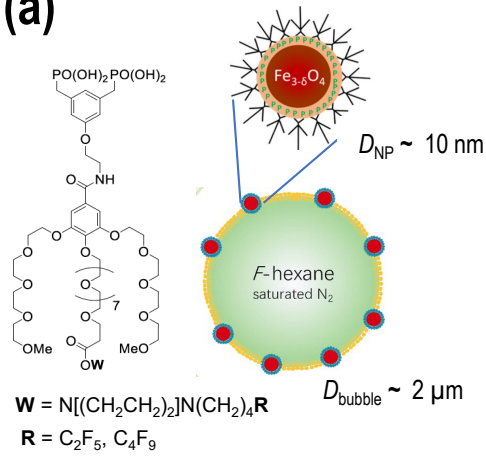




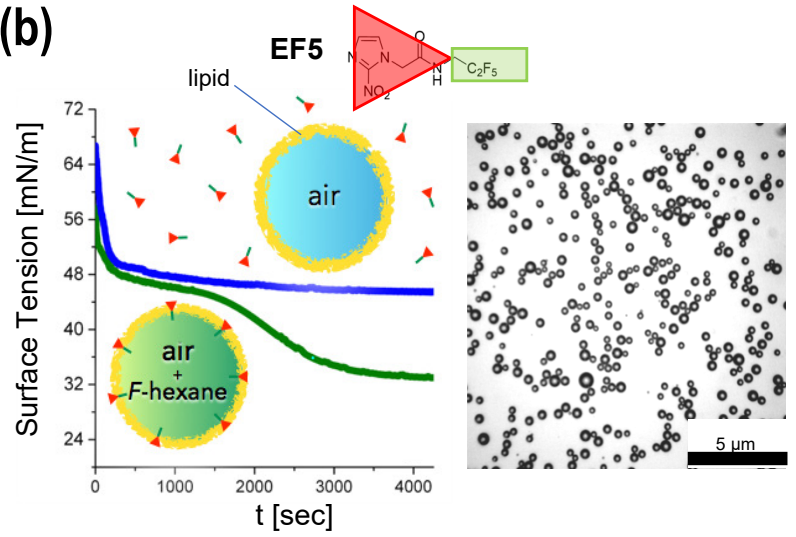




(a)



(b)



(c)

