Molecular-level Mixing Behavior, Structure and Interactions of Mixed Hydrogenated-Fluorinated Surfactant Langmuir Monolayers

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By

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ABSTRACT

This thesis constitutes findings from several projects designed with an ultimate goal of understanding the mixing behavior, molecular structures and interactions that play out in mixtures of perfluorotetradecanoic acid with hydrogenated surfactants at the micro and molecular-level. In particular, this thesis explores the importance of chemical structure of surfactants in controlling properties of surfactant mixtures. Notably, the difference in surfactant chain length and head group are key chemical identities which control mixing behavior.

Therefore, mixtures of perfluorotetradecanoic acid (PF; CF$_3$(CF$_2$)$_{12}$COOH) with nonadecanoic acid (ND; CH$_3$(CH$_2$)$_{17}$COOH) were explored in the context of closely related mixed surfactant systems to understand how the difference in chain length controls miscibility, and to validate the miscibility rule proposed for these types of mixed systems. In addition, the impact of surfactant head group was explored by switching the carboxylic functional group on the nonadecanoic acid to an alcohol functional group (nonadecanol; NDOH; CH$_3$(CH$_2$)$_{18}$OH). Both the fatty acid and fatty alcohol were immiscible with PF in monolayers, and formed phase-separated domains. These studies were all carried out using Langmuir trough compression isotherms, Brewster angle microscopy imaging and Atomic force microscopy imaging to characterize the monolayer films both at air-water and solid-air interfaces. However, during the course of these studies, it was noted that these characterization techniques do not provide direct information about film structure at the molecular length scale.

In recognition of this shortcoming, a molecular-level study of several mixed surfactant systems at the air-water interface was performed using the synchrotron-based liquid surface X-ray scattering techniques Grazing incidence X-ray diffraction (GIXD), specular X-ray reflectivity (XR) and X-ray fluorescence near total reflection (XFNTR). A benchmark mixed film system comprised of arachidic acid (AA, C$_{19}$H$_{39}$COOH) and PF was adopted for this study. In all cases, the two components in the mixed film behaved entirely independently of film composition, which is exactly the expected result for a fully phase-separated, immiscible system. The mixed film systems explored here were monomeric fatty acids and were chosen to understand how the molecular-level interaction of the films contribute to phase-separation. However, with the emerging interest in dimeric compounds (gemini surfactants), the mixing behavior of anionic gemini surfactants (Ace(12)-2-Ace(12) and Ace(18)-2-Ace(18)) with PF was explored
comparison to the monomeric hydrogenated-PF mixed film systems. The gemini surfactant Ace(12)-2-Ace(12) was chosen to understand the effect and role dimeric hydrogenated surfactants play in miscibility with fluorinated surfactants (PF). The Ace(12)-2-Ace(12)-PF mixed film system was found to be miscible at the molecular-level with Ace(12)-2-Ace(12) showing an amorphous state while PF was highly crystalline. The mixing behavior of the longer chain variant of the gemini surfactant Ace(18)-2-Ace(18) with PF was also explored to understand the chain length effect in such systems. Similar miscibility trends in the short chain gemini surfactant with PF were observed. These studies were carried out at the molecular-level using GIXD and XR. Finally, the potential of the gemini surfactant as a chelating agent was explored by investigating its association with Fe$^{3+}$ at the air-water interface using XFNTR.
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DEDICATION

This thesis is dedicated to my Lord and Savior Jesus Christ. I also dedicate this thesis to the Spirit of the Living God.
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LIST OF ABBREVIATIONS

AA: Arachidic acid, C₁₉H₃₉COOH

ACS: American Chemical Society

AFM: Atomic Force Microscope

APS: Advance Photon Source

BAM: Brewster Angle Microscope

BXDS: Brockhouse X-ray Diffraction and Scattering Sector

CCD: Charge-coupled device

cf.: Compare

CLS: Canadian Light Source

CMCs: Critical Micelle Concentrations

DFT: Density Functional Theory

DMPE: Dimyristoylphosphatidylethanolamine

DPPC: Dipalmitoylphosphatidylcholine

DPPG: Dipalmitoylphosphatidylglycerol

EDP: Electron density profile

EDTA: Ethylenediaminetetraacetic acid

FWHM: Full width at half maximum

GIXD: Grazing incidence X-ray diffraction

LB: Langmuir Blodgett

Lₙₓᵧ: Coherence length

MMA: Mean molecular area

MMFF: Merk molecular force field
ND: Nonadecanoic acid

NDOH: Nonadecanol

NN: nearest neighbor

NNN: next-nearest-neighbor

PA: Perfluorotetradecanoic acid

PCDA: Polydiacetylene

PF: Perfluorotetradecanoic acid

XFNTR: X-ray fluorescence near total reflection

XR: Specular X-ray reflectivity
CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Overview of physical chemistry of surfactant Langmuir monolayers

Surfactant Langmuir monolayers are formed when insoluble amphiphilic molecules such as lipids organize at the air-water interface by adsorbing their hydrophilic part (polar head group) directly in contact with the air-water interface, and orienting their hydrophobic part (non-polar tail group) away from the interface. Molecular organization of insoluble surfactant Langmuir monolayers at the air-water interface can be explored using a Langmuir trough in which the surface tension ($\gamma$) as a function of the mean molecular area (MMA) occupied by the surfactant molecules can be measured. The surface tension of the film-covered surface ($\gamma$) is often described using the concept of surface pressure ($\pi$) according to the mathematical expression:

$$\pi = \gamma_0 - \gamma$$  (1.1)

where $\gamma_0$ is the surface tension of the bare water surface. Typical isotherm measurements, $\pi$ vs. MMA, for surfactant monolayers reveal different phases (gaseous, liquid or solid) depending on factors such as temperature, surface pressure, surfactant structure and packing density. The uncompressed monolayer, where molecules are far apart on the water surface, has been described as a two-dimensional gas phase. Compressing the monolayer so that the area of the molecules is reduced transitions the monolayer into the liquid expanded phase. Here, the hydrocarbon chain of the molecules begin to interact forming a random film with an irregular molecular orientation at the air-water interface. Further compression of the monolayer results in a transition from the liquid expanded phase to a liquid condensed phase, and then to a solid condensed phase until its collapse state. These transitions are often observed on the isotherm as kinks, depicting a change in structural orientation of the molecules. It should be noted, however, that gaseous, liquid expanded and liquid condensed are historical names that do not fully capture modern understanding of phase structures.
Early studies on phase transition of surfactant monolayers could not fully-explain these observations, nor provide a molecular-level description of the differences between liquid condensed and solid condensed phases until the introduction of X-ray diffraction studies. From X-ray diffraction, the orientation of the molecular chains in the liquid condensed phase were seen to be tilted with respect to the water surface, and perpendicular for the solid condensed phase. Hence, the terminology liquid condensed and solid condensed phases which are descriptors for phase behavior of fatty acids were replaced in the literature with tilted condensed and untilted condensed phase, respectively. From here, important characteristic behaviors of the monolayer such as compressibility, viscosity and electrical effects (surface potentials and dipole moments) could be assessed in the context of crystallographic properties of the film to provide some insight to the phase changes. Furthermore, the phase rule and phase diagrams ($\pi$ vs. MMA at several temperatures) could be fully assessed to understand the role that factors such as temperature and number of surfactant components play in the phase behavior of surfactant monolayers. \(^2\text{-}^5\)
1.2 Thermodynamics of mixing in Langmuir monolayers

Mixed surfactant Langmuir monolayers consist of two or more surfactants at the air-water interface. Mixed surfactant Langmuir monolayers are known to behave differently from single component surfactant monolayers and provide a tractable model for complex systems such as biological membranes. Consider a binary mixed surfactant monolayer film system made up of surfactant 1 and surfactant 2. If the two components are immiscible, then the mixed monolayer film effectively consists of two separate monolayers. In this case, the area occupied by the mixed film is the sum of the individual component film areas, which can be expressed according to the additivity equation below:

\[ A_{12} = \chi_1 A_1 + \chi_2 A_2 \]  

(1.2)

where \( A_{12} \) is the average molecular area occupied by the mixed film, \( A_1 \) and \( A_2 \) the molecular areas of the respective surfactant film components, and their corresponding mole fractions \( \chi_1 \) and \( \chi_2 \) at a specific surface pressure. In an ideal mixed film behavior, a straight line should be observed when \( A_{12} \) is plot against \( \chi_1 \). Deviations above the straight line are an indication of positive (repulsive) interactions, while deviations below the straight line indicates negative (attractive) interactions within the films. The thermodynamic stability of the films can be assessed from the excess Gibbs energy of mixing (\( \Delta G_{ex} \)), which is given according to the equation:

\[ \Delta G_{ex} = G_{12} - \chi_1 G_1 - \chi_2 G_2 \]  

(1.3)

where \( G_{12} \) is Gibbs free energy in the two-component film system, \( G_1 \) and \( G_2 \) the Gibbs free energy for the respective surfactant film components, \( \chi_1 \) and \( \chi_2 \) the corresponding mole fractions at specific surface pressures. Therefore, the excess Gibbs free energy is the difference between the energy of mixing of the mixed monolayer and that of the individual surfactant film components. Gibbs free energy as a function of the surface pressure at constant temperature, pressure and chemical composition can be expressed as:

\[ \left( \frac{dG}{d\pi} \right)_{T P N} = - A \]  

(1.4)

The excess Gibbs free energy can be measured experimentally by integrating the \( \pi \) versus MMA compression isotherm (with respect to surface pressure) of the mixed monolayer film and the individual films to a specified surface pressure, and then subtracting those of the individual films.
at their respective mole fractions from the mixed monolayer film. Therefore, equation (1.4) above can be re-written in terms of excess Gibbs free energy as:

\[
\Delta G_{ex} = \int_0^\pi [A_{12} - (\chi_1 A_1 + \chi_1 A_2)] d\pi \quad (1.5)
\]

A negative \(\Delta G_{ex}\) value indicates that the mixed films experience some kind of internal attraction and are more thermodynamically stable than the individual films, whereas a positive \(\Delta G_{ex}\) will mean less stability.

1.3 Morphology of mixed Langmuir monolayers containing perfluorinated surfactants

Perfluorinated surfactants are surface active compounds that can form stable monolayers at the air-water interface under appropriate conditions. They exhibit excellent properties such as high surface activity, strong mechanical rigidity and good spreading qualities in comparison to their hydrogenated counterparts. Hence, they have found several applications in the pharmaceutical, medical and industrial fields. Mixed Langmuir monolayers containing perfluorinated surfactants provide a model platform to understand their properties and applications. Of particular interest are mixtures of hydrogenated and perfluorinated fatty acids, as these mixed surfactants are known to phase-separate into distinct morphological domains under appropriate conditions. Factors such as chemical composition, surface pressure and temperature are known to control phase-separation in these mixed film systems. For example, Overney et al. in an investigation of mixed films of arachidic acid and a partially fluorinated surfactant, identified a phase-separated system of circular domains which were assigned to the perfluorinated surfactant on the basis of difference in chain length. Also, Matsumoto et al. identified a phase-separated system in mixtures of arachidic acid and perfluorotetradecanoic acid revealing highly-structured regular polygonal domains which were chemically assigned to arachidic acid on the basis of chain length difference. Furthermore, Imae et al. in a study of mixtures of arachidic acid and perfluorinated fatty acids such as perfluorotetradecanoic acid and perfluoroctadecanoic acid, observed a phase-separated system of irregularly shaped domains for perfluoroctadecanoic acid but no discernible domains for perfluorotetradecanoic acid.

Domain formation in surfactant systems is generally controlled by two competing forces, line tension between different phases and dipole-dipole interactions between surfactant headgroups. Line tension is defined as the energy per unit length applied on the interface between two co-
existing phases. These competing forces control the shape and size of monolayer domains. A large line tension favors the formation of circular domains (domain contraction), while a large dipole-dipole headgroup repulsion favors elongated domains (domain expansion).\textsuperscript{20} Morphology of the monolayer domains can be observed directly at the air-water interface or at the solid-air interface when deposited onto a solid substrate. However, the deposition process may slightly alter the morphology of the films at the solid-air interface.\textsuperscript{19} The Paige research group has contributed significantly to this field of research by establishing an empirical relationship between domain morphology and chemical composition in mixed hydrogenated-perfluorinated fatty acid mixed films.\textsuperscript{21} Key to this relationship is a simple parameter, $\Delta_{H-F}$, which is defined by the difference in methylene groups in the hydrogenated surfactant to the number of difluoro-methylene groups in the perfluorinated surfactant as shown below.\textsuperscript{21}

$$\Delta_{H-F} = \# \text{CH}_2 \text{ groups in hydrocarbon chain} - \# \text{CF}_2 \text{ groups in perfluorocarbon chain} \quad (1.6)$$

For mixed Langmuir monolayers containing perfluorinated surfactants where the $\Delta_{H-F} > 5$, the films phase-separate into well-defined polygonal domains enriched by the hydrocarbon. The perfluorinated surfactant surrounds the polygonal domains as a continuous matrix. Dipole-dipole repulsions of the headgroup is dominant in the film mixture, while line tension is minimal. Film mixtures with $\Delta_{H-F} < 5$ phase-separated into different morphologies such as stripes. Here, line tension is large in such film mixtures with minimal dipole-dipole headgroup repulsions. Much work on mixed Langmuir monolayers containing perfluorinated surfactants have centered on mixtures of hydrogenated and perfluorinated fatty acids, however, there have been reports on other mixtures. For example, mixtures of dipalmitoylphosphatidylcholine (DPPC) and perfluorinated fatty acids or alcohols, mixtures of dipalmitoylphosphatidylglycerol (DPPG) and perfluorinated fatty acids, and mixtures of dimyristoylphosphatidylethanolamine (DMPE) with perfluorinated fatty acids have been highlighted in literature. Investigations by Shibata et al.\textsuperscript{22} on mixtures of DPPC with perfluorinated fatty acids revealed either a partial dissolution of the phospholipid domains or a phase-separated system depending on the chain length of the perfluorinated fatty acid. In addition, studies of DPPC mixed with perfluorinated alcohols in our research group revealed the formation of lobes and circular domain morphologies of the phospholipids. Here, the perfluorinated alcohols promoted either fluidity or solidity of the mixed monolayer films. However, the lobes and circular morphologies were explained on the basis of long range dipole-
dipole repulsions which gave rise to lobes and the domination of line tension giving rise to circular domains. Finally, mixtures of polydiacetylenes (PCDA’s) with perfluorinated fatty acids have also been reported, revealing a phase-separated system of irregularly shaped domains enriched by the PCDA. Furthermore, our research group has studied mixed films of gemini surfactants with perfluorinated fatty acids.

1.4 Morphology of mixed Langmuir monolayers containing gemini surfactants

Gemini surfactants are dimeric surface active agents comprised of two surfactant molecules that are linked by an organic spacer group. They are able to form Langmuir monolayers and have found applications in many technological fields. Mixed Langmuir monolayers containing gemini surfactants are of great interest in the scientific literature. Much of the investigations stem from the rich physico-chemical properties gemini surfactants possess and their relevance in drug-delivery and biomedical applications. Gemini surfactants in mixed monolayers help lower surface tension greatly, promote a higher surface activity and form complex solution-phase aggregate structures. Therefore, mixing behavior and morphology of gemini surfactant in mixed Langmuir monolayers remains a relevant area of study to help understand their applications. To this end, Tsubone studied the interaction of an anionic gemini surfactant (having N,N-dialkylamide and a carboxylate group) with conventional anionic surfactants such as sodium dodecylsulfate (SDS) and sodium N-dodecanoylglutamate (AGS). The mixtures showed stronger interactions synergistically resulting in effective surface tension reduction, and also exhibited expansions in packing. Li et al. also investigated the interaction of a series of cationic gemini surfactants with decyl-β-maltoside, decyl-β-glucoside, and dodecyl-β-maltoside. These mixed films containing the cationic gemini surfactant showed stronger interactions than conventional cationic surfactants. In addition, the mixed films exhibited contracted or expanded packing depending on the hydrophobicity or hydrophilicity of the gemini spacer. However, the morphology of gemini surfactants in mixed Langmuir monolayers have not been substantively explored in the literature and remains an active area of research.

1.5 Langmuir trough compression and Langmuir-Blodgett

The Langmuir trough is an instrument which is used to measure surface pressure-area properties of monolayers films at the air-water interface. The trough, which is typically made of hydrophobic polymers like Teflon or Delrin, is equipped with movable barriers, which are used to
compress the monolayer films at the air-water interface and generate $\pi$-A compression isotherms. A photograph of a typical trough is shown in Figure 1-2. Surface pressure changes are typically monitored using an electro-balance together with a Wilhemy plate (typically made of either platinum or paper) mounted above the trough.30

Figure 1-2: Langmuir trough and its accessories (this figure has been modified from reference [31]).

The movement of the barrier during compression results in a change in the MMA of the surfactant films. Furthermore, a dipping device (dipper) is often attached to the trough and is used for depositing monolayer films onto solid substrates. Here, the substrate is first immersed into the subphase, then the surfactant solution is spread and then finally the monolayer film is compressed to a targeted surface pressure or MMA. The monolayer films are deposited onto the substrate by lifting the substrate through the air-water interface while maintaining the target surface pressure or MMA. The quality of the deposition can be inferred from the transfer ratio (TR) which is defined by the equation:32

$$\text{Transfer Ratio} = \frac{\text{Decrease in the area occupied by monolayer on water surface}}{\text{Coated area of the solid substrate}}$$

(1.7)

For the surfactant films to be ideally transferred as a monolayer onto the substrate, the TR should be exactly or close to 1. A hydrophilic substrate is typically preferred as it allows the hydrophilic headgroups of the surfactant film to adsorb to the substrate. Glass and mica are typical solid
substrates used for depositing surfactant monolayer films. This method of depositing monolayer films is known as Langmuir-Blodgett deposition. The Langmuir trough can be further integrated with other characterization techniques like Brewster angle microscopy, fluorescence microscopy and synchrotron-based liquid surface X-ray scattering.\textsuperscript{33}

1.5.1 Brewster angle microscopy imaging

Brewster angle microscopy is an imaging technique that uses the reflection of polarized light to visualize structures and morphology of surfactant monolayer films at the air-water interface.\textsuperscript{34} First developed commercially by Dirk Honig and introduced in 1991, the technique has become a worldwide standard for characterizing micro-structures at the air-water interface.\textsuperscript{35} When a ray of light is directed through a boundary between two media of different refractive index, a part of the light ray is reflected and the rest refracted at the interface, as shown in Figure 1-3.

\textbf{Figure 1-3:} A schematic representation of the Brewster angle microscopy principle (this figure has been redrawn from reference \textsuperscript{[35]}).

The angle of incidence and angle of refraction with respect to the surface normal can be described by Snell’s law. In Snell’s law, the sine of the angles of incidence and refraction of light wave are constant when it passes through two media. Here, the relationship between the angle of incidence and refraction is measured with respect to the surface normal. However, when the angle of incidence is adjusted so that light ray of a particular polarization state cannot be reflected, then the Brewster angle ($\theta_B$) has been attained.\textsuperscript{36} The $\theta_B$ relates to the refractive indices of the mediums according to the equation:\textsuperscript{35}
\[ \tan \theta_B = \frac{n_2}{n_1} \quad (1.8) \]

where, \( n_1 \) and \( n_2 \) are the refractive indices of the first and second medium. If we consider a glass medium which has a refractive index \( (n_2 \approx 1.5) \) placed in ambient air \( (n_1 \approx 1) \); the Brewster angle is found to be \( 56^\circ \). For an air-water interface, where the refractive index of water \( (n_2 \approx 1.33) \), the Brewster angle is found to be \( 53^\circ \). Therefore, at this Brewster angle of the air-water interface, light can be reflected by the monolayer film and be captured by a CCD camera. However, this only occurs if the refractive index of the monolayer films is different from water. So here, the bare water surface looks dark (no reflected light) but when there is some surfactant film which has a different refractive index you get reflected light. Therefore, the morphology and structure of the monolayer films can be visualized.

**1.5.2 Atomic force microscopy imaging**

Atomic force microscopy falls under the family of scanning probe microscopies where a sharp probe is scanned across a sample surface. The technique was first developed in 1986 by Binning, Quate, and Gerber, after the Nobel Prize in Physics won by Binning and Rohrer for developing scanning tunneling microscopy in 1982. The atomic force microscope is designed to have a scanner made from a piezoelectric material that responds by contractions and expansions when a voltage is applied. A probe generally consisting of either silicon (Si) or silicon nitride \( (\text{Si}_3\text{N}_4) \) cantilever is mounted on a tip holder attached to the scanning head of the instrument. When a laser light is focused onto the back of the cantilever, the laser light is reflected from the back of the cantilever onto a position-sensitive photodetector, as shown in Figure 1-4.
Figure 1-4: A schematic diagram of the Atomic Force Microscopy principle (this figure has been modified from reference [46]).

Movement of the tip across the sample surface results in a corresponding change in position of the reflected light onto the photodetector. Therefore, topographical properties of the sample can be captured easily from the tip interaction with the sample as it moves across the surface. The AFM can resolve to the atomic-level with a tip resolution limit of 0.1 nm. Topographical images captured by the AFM can be collected using different scanning modes, including contact mode, non-contact mode, and tapping mode.\textsuperscript{45,47} In contact mode imaging, the tip interacts with surface through the capillary layer of water formed on the sample surface. As the tip is scanned across the sample surface, a constant tip deflection is maintained by a feedback loop. Maintaining this constant cantilever deflection ensures that the force between the tip and sample remains unchanged. Non-contact mode imaging is performed by oscillating the cantilever at or slightly above its resonant frequency.\textsuperscript{45,48} Here, the tip has no contact with the sample surface but oscillates above the capillary layer of water formed on the sample surface. The oscillations decrease when the cantilever approaches the sample surface due to decrease in the cantilever resonance frequency by van der Waals forces and long range forces above the sample surface. The piezo is then adjusted by a feedback loop to maintain a constant oscillating frequency, which aids in capturing images of the sample surface.\textsuperscript{48} Tapping mode is also a kind of an oscillating cantilever AFM imaging mode where the tip slightly taps on the sample surface during scanning. Here, the cantilever is oscillated at or slightly below the cantilevers resonance frequency while maintaining a constant oscillation.
amplitude through the feedback loop. Images captured by the AFM can be used to measure nanometer-scale surface structures.45-48

1.6 Overview of Synchrotron-based Liquid Surface X-ray Scattering Techniques on Surfactant Monolayers

For over a century, X-ray scattering and diffraction have been essential techniques to characterize the structure of materials. Interactions of X-rays with materials reveals their internal structure as either crystalline or non-crystalline. While the intrinsic structure of crystalline materials can be described by discrete and periodic scattering functions, non-crystalline materials give rise to a continuous scattering function. Non-crystalline materials often exhibit weak X-ray scattering because of their low electron density.49 Crystalline materials show strong X-ray scattering, however weak X-ray scattering may also be observed for very thin (e.g. monolayer) materials. Weak scattering is typically seen with soft-matter materials, and to precisely probe their structure, the use of brilliant X-ray sources of high photon flux are preferred over conventional X-ray sources. Synchrotrons provide highly collimated, brilliant X-rays capable of probing the structure of soft-matter materials such as surfactant Langmuir monolayers. Therefore, synchrotron-based liquid surface X-ray scattering techniques are frequently used to obtain molecular information about Langmuir monolayers directly at the air-water interface. A liquid surface diffractometer, illustrated in Figure 1-5 is required for these X-ray scattering techniques.28
In brief, a synchrotron X-ray beam of wave vector $K_{\text{syn}}$ from the upstream beam-line optics is directed towards a steering crystal typically made of germanium or silicon which operates as a monochromator. The crystal Bragg planes reflect the X-ray beam to select a specific energy of the incoming X-ray beam and then steers it through slit S1. The size of the X-ray beam is reduced as it passes through slit S1, then through the beam monitor or in some cases a Kapton film to the sample surface for scattering at specified incident angles. However, prior to scattering, thin metal foils are often placed in the path of the beam to reduce the intensity on the sample and the detector. The sample stage consists of a Langmuir trough built into a gas tight box (canister) mounted on an anti-vibration stage. X-rays scattered from the sample surface are directed out of the canister through slits 2 and 3 to a detector mounted on the output arm.\textsuperscript{28} For liquid monolayers, one usually measures X-rays that are either diffracted at low angles or are specularly reflected. These approaches will be discussed in the following sections in more detail.

1.6.1 Grazing incidence X-ray diffraction (GIXD)

Grazing incidence X-ray diffraction (GIXD) is a synchrotron-based liquid surface X-ray scattering technique which is sensitive to the in-plane structure of monolayers and is primarily used to study their molecular packing directly at the air-water interface.\textsuperscript{5,50} In GIXD, an incident X-ray beam is steered by the monochromator crystal onto the sample surface at an incident angle ($\alpha_i$) below the critical angle ($\alpha_c$) of the air-water interface. The X-rays are diffracted by the monolayer. The angles and intensity of the diffracted beam can be used to extract information about the 2D organization of the film. If the monolayer is oriented with lattice planes so that it meets the Bragg condition ($\lambda = 2d_{hk} \sin \theta_{hk}$), then a Bragg diffraction peak can be observed.\textsuperscript{2,51} The scattering geometry for GIXD measurement is shown in Figure 1-6 below.
Figure 1-6: Illustration of the grazing incidence X-ray diffraction principle (this figure has been modified from reference [52]).

An incident X-ray beam of wave vector $K_{in}$ is directed at the sample surface at very shallow incident angle, typically about or slightly less than 0.1°. The X-ray beam is diffracted by the monolayer over the in-plane angle $2\theta$, along the three dimensional wave vector $q$ as defined by the equation:

$$ q = (q_{xy}, q_z) \quad (1.9) $$

With the components $q_{xy}$ and $q_z$ defined by the equations:

$$ q_{xy} = \left(2\pi/\lambda\right) \left[ \cos^2 (\alpha_i) + \cos^2 (\alpha_f) - 2 \cos (\alpha_i) \cos (\alpha_f) \cos (2\theta_{xy}) \right]^{1/2} \approx (4\pi/\lambda) \sin (2\theta_{xy}/2) \quad (1.10) $$

$$ q_z = \left(2\pi/\lambda\right) \left[ \sin (\alpha_i) + \sin (\alpha_f) \right] \approx (2\pi/\lambda) \sin (\alpha_f) \quad (1.11) $$

The diffracted intensity is collected as function of the vertical ($q_z$) and horizontal ($q_{xy}$) momentum transfer. The diffraction patterns observed for surfactant monolayers are shown in Figure 1-7 below.
In the simplest model, six diffraction maxima (six first-order wave vectors) of the monolayer at the air-water interface are observed when the reciprocal disk intersects with the first-order Bragg rods. When the reciprocal disk lies in the plane of the monolayer, then the molecules do not tilt and hence all the peaks also lie in the plane of the monolayer and are degenerate. Movement of the peaks out of the plane of the monolayer indicates molecular tilts and results in a break of the peak degeneracy. Molecular tilts may occur toward the nearest neighbor (NN) direction where four peaks move out of the plane: two above and two below, with the peaks above showing equal wave vector components. Tilts toward the next-nearest-neighbor (NNN) shows an unequal wave vector.

**Figure 1-7:** X-ray diffraction patterns of surfactant Langmuir monolayers. (this figure has been modified from reference [5]).
components for the peaks above the plane. Crystallographic assignments of the peaks are generally
given as either a hexagonal or centered rectangular (distorted hexagonal) unit cell.\(^5\)

### 1.6.2 Specular X-ray reflectivity (XR)

Specular X-ray reflectivity (XR) is a type of synchrotron-based liquid surface X-ray scattering
technique that measures the electron density of the monolayer film at the air-water interface.\(^5\) In XR, an incident X-ray beam of wave vector \(K_{\text{in}}\) is directed onto the monolayer film at the air-water interface as shown in Figure 1-7 so that the condition \((\alpha_i = \alpha_f = \alpha)\) is established. That is, the incident angle \((\alpha_i)\) equals the exit angle \((\alpha_f)\) and \(2\theta_{xy} = 0.\(^4\)\(^9\)\(^5\)\(^3\) The incident X-ray beam is reflected
along the wave vector \(K_{\text{out}}\) to the detector. The detector collects the reflected X-ray signal
intensity as a function of \(q_z\). In XR theory, if we consider a planar surface where a plane wave reflects and
refracts, then the index of refraction may change due to dispersion and absorption processes.\(^5\)\(^1\) The X-ray refractive index of a medium \(j\) may be generally given by the expression:\(^4\)\(^9\)

\[
n_j = 1 - \delta_j + i\beta_j \tag{1.12}
\]

Where \(n\) is the refractive index, \(\delta\) and \(\beta\) are the dispersion and absorption contribution respectively. 
The dispersion contribution \((\delta)\) is given by the expression:\(^4\)\(^9\)

\[
\delta_j (q, \lambda) = [(e^2 \lambda^2)p_j/(8\pi^2 m_e c^2 \varepsilon_\infty)]\sum \left[f_k^0 (q, \lambda) + f_k^1 (\lambda)\right]/\sum M_k \tag{1.13}
\]

and the absorption contribution given as:\(^4\)\(^9\)

\[
\beta_j (\lambda) = [(e^2 \lambda^2)p_j/(8\pi^2 m_e c^2 \varepsilon_\infty)]\sum \left[f_k^0 (\lambda)\right]/\sum M_k \tag{1.14}
\]

Where \(c\) is the speed of light, \(e\) the elementary charge, \(m_e\) the electron rest mass, \(\varepsilon_\infty\) the permittivity
constant, \(p_j\) the mass density, \(\lambda\) the wavelength, \(M_k\) the atomic weight, \(f_k^0\), \(f_k^1\) and \(f_k^\prime\) are the
dispersion corrections.

The reflectivity \(R(q_z)\) across the interface of the medium can be calculated using the distorted wave
Born approximation (DWBA) according to the expression:\(^5\)\(^3\)

\[
R(q_z) = R_F(q_z) \left| (\rho_{\text{sub}})^{-1} \int (d\rho(z)/dz) \exp(i q_z z) \ dz \right|^2 \tag{1.15}
\]

Where \(R_F(q_z)\) is the Fresnel reflectivity for a flawless sharp interface, \(\rho (z)\) the electron density
across the interface, \(\rho_{\text{sub}}\) the electron density of the subphase \((\rho_{\text{water}} = 0.334 \text{ e/Å}^3)\).
The profile depth of the monolayer film can be obtained by inverting the normalized reflectivity, $R(q_z)/R_F(q_z)$ to yield an averaged $p(z)$ of the film as a function of the vertical direction ($z$ coordinate). However, since the molecular structure of the surfactant that makes up the monolayer is typically known, an electron density model can be constructed from a stack of homogeneous slabs. Here, the head and tail group thickness of the monolayer film can be modelled and compared to the known length of the molecule.

1.6.3 X-ray fluorescence near total reflection (XFNTR)

X-ray fluorescence near total reflection (XFNTR) is a synchrotron-based X-ray technique that is sensitive to elemental (most commonly metal) ions associating with monolayer films at the air-water interface. The technique was first introduced by Bloch et al., and has since found extensive use in the investigation of elemental ions distributed across layered structures. In XFNTR, an incident X-ray beam is directed onto the monolayer-metal ion interface below the critical angle such that metal ions at the interface are excited and emit fluorescence signals to the detector, as shown in Figure 1-8.

![Figure 1-8: A schematic representation of X-ray fluorescence near total reflection (XFNTR) (this figure has been modified from reference [54]).](image)

The incident X-ray beam is usually brought in below the critical angle to ensure signals are from the interface and not the bulk phase. In addition, the detector is positioned perpendicular (90°)
to the sample surface to reduce background scattering. The fluorescence spectra that are captured are often taken as a function of small angles of \( q_z \) near total reflection. The fluorescence intensity is estimated by integrating the X-ray intensity over the footprint region where the metal ion coverage overlaps with the incident beam at the interface. The total fluorescence intensity \( I \) is the sum of the interfacial \( (I_{\text{int}}) \), bulk \( (I_{\text{bulk}}) \), and background \( (I_{\text{bg}}) \) contributions assuming a uniform metal ion concentration in the sub-phase except at the interface.\(^{55}\) This is given by the expression:\(^{55}\)

\[
\frac{I}{I_o} = \frac{I_{\text{int}} + I_{\text{bulk}} + I_{\text{bg}}}{I_o} \quad (1.16)
\]

Intensity of the fluorescence signal from the bare surface of the bulk metal ion concentration \( (n_b) \), \( I_{\text{bulk}} \), is given as:\(^{56}\)

\[
I_{\text{bulk}}(\alpha_i) = C |t(\alpha_i)|^2 D(\alpha_i)g(\alpha_i)A_o n_b \quad (1.17)
\]

whereas the intensity of the fluorescence signal from the monolayer covered region on the metal ion sub-phase, \( I_{\text{int}} \), has two contributions. One contribution is from the bound ions at the interface \( (n_s) \) and the other contribution from the bulk ions, which is given by the expression:\(^{56}\)

\[
I_{\text{int}}(\alpha_i) = C |t(\alpha_i)|^2 g(\alpha_i)A_o n_s \exp[-\left|z_{\text{ion}}\right|/D(\alpha_i)] \quad (1.18)
\]

Where \( C \) is the scale factor that accounts for the effect of scattering geometry, beam intensity and detector efficiency. \( A_o \) is the mean molecular area of the monolayer film, \( t(\alpha_i) \) the transmission function, \( g(\alpha_i) \) the penetration depth perpendicular to the interface, \( D(\alpha_i) \) a geometrical correction and \( z_{\text{ion}} \) the position of the metal ions at the interface.\(^{55,56}\) Analysis of the \( I_{\text{int}} \) provides an estimate of the number of bound metal ions per headgroup of the surfactant molecule at the air-water interface.\(^{56}\)

1.7 Motivation and research objectives

The objective of this PhD thesis research is to gain an understanding of the molecular-level interactions that give rise to phase-separation in simple mixed hydrocarbon-perfluorocarbon monolayer surfactant films. In addition, an understanding of the mixing behavior and molecular-level film structures of the mixed monolayer surfactant films were sought. The Paige research group has explored several factors that contribute to phase-separation in mixed surfactant monolayer films such as simple mixtures of hydrogenated and perfluorinated fatty acids, photopolymerizable surfactants mixed with perfluorocarbons, phospholipids mixed with peptides
and perfluorocarbons, and developed rules that allow prediction of phase-separation. However, these rules are empirical and limited in scope. Therefore, with the view of understanding at the molecular-level why these empirical rules work, this project was undertaken to gather a molecular-level understanding of factors that cause phase-separation in mixed hydrogenated-fluorinated surfactant monolayer systems at the air-water and solid-air interfaces. In detail, a simple mixed hydrogenated-fluorinated surfactant monolayer system that comprises of nonadecanoic-perfluorotetradecanoic acid monolayers was first explored to understand the effect chain tail length differences have on phase-separation and structural morphology, and to validate a predictive model. The formation of phase-separated highly crystalline elongated hexagonal domains in this mixed films was hypothesized and tested. Further, nonadecanol-perfluorotetradecanoic acid monolayers were explored to understand how the difference in headgroup of the surfactant influences phase-separation and structural morphology.

A molecular-level GIXD and XR study of a benchmark system comprising of arachidic-perfluorotetradecanoic acid monolayer films was done to assess the structure of the mixed, phase-separated films that are not fully captured and understood at the micron-scale. Next, the mixing behavior of an anionic gemini Ace(12)-2-Ace(12) mixed with perfluorotetradecanoic acid was studied to further refine current understanding of how molecular structure affects phase-separation in mixed hydrogenated-fluorinated surfactant monolayer films. The anionic gemini surfactant Ace(12)-2-Ace(12) was selected based on its structural resemblance with hydrogenated fatty acids, which are studied in the earlier thesis chapters and elsewhere in the literature. A longer tail chain variant, Ace(18)-2-Ace(18) was also explored in mixed films with perfluorotetradecanoic acid to understand the effect change in tail length has in these mixtures. This study was undertaken based on existing knowledge of monomeric systems showing phase-separation with regular polygonal structures when the tail length of the hydrogenated surfactant is greatly increased keeping the tail length of the fluorinated surfactant constant.
1.8 References


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CHAPTER 2

THE INFLUENCE OF SURFACTANT HEADGROUP ON MISCIBILITY IN MIXED NONADECANOIC-PERFLUOROTETRADECANOIC ACID AND NONADECANOL-PERFLUOROTETRADECANOIC ACID MONOLAYERS

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In this study, the effect of chemical structure (surfactant headgroup) on miscibility in mixed nonadecanoic-perfluorotetradecanoic acid and nonadecanol-perfluorotetradecanoic acid monolayers was explored at the air-water and solid-air interfaces using surface pressure-area isotherms, Brewster angle microscopy and atomic force microscopy. This study was performed to understand the molecular factors that control miscibility in hydrogenated-perfluorinated monolayer films from the perspective of thermodynamics and micron-scale morphology. The isotherm measurements provided information on the thermodynamic mixing behavior of the mixed films while Brewster angle microscopy was used to characterize micron scale morphologies of the mixed films directly at the air-water interface. Finally, atomic force microscopy was used to access the domains and morphologies of the mixed films at the solid-air interface.

The formation of phase-separated highly crystalline elongated hexagonal domains in mixed films of nonadecanoic acid-perfluorotetradecanoic acid was hypothesized based on differences of surfactant tail length and the empirical model developed in our research group. This hypothesis was confirmed using the characterization techniques mentioned above. Also, the headgroup effect on the thermodynamics of mixing and morphology was explored. The headgroup greatly influenced the film morphologies. Circular morphological domains were observed in mixed films of nonadecanol-perfluorotetradecanoic acid monolayers. Findings from this project were placed in context to other works and is disclosed in this chapter in detail.

The author’s contribution to this article includes: sample preparation, performing all experiments, analyzing and interpreting all results, and finally preparing and writing the manuscript. My supervisor, Professor Matthew F. Paige further polished the manuscript by writing extensively and interpreting the results.
The influence of surfactant head group on miscibility in mixed hydrocarbon-perfluorocarbon monolayers

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2.1 Abstract

Simple binary mixtures of hydrogenated and perfluorinated surfactants are typically immiscible in monolayers, with mixtures forming highly-structured films at the air-water and solid-air interfaces. While dispersion interactions between tail groups of the surfactants play a key role in dictating the micron-scale morphology of such mixed films, the influence of the head group on film morphology has been minimally explored. In this work, surfactant head group effects are investigated by comparing thermodynamic miscibility in combination with film morphology in binary mixed films comprised of nonadecanoic acid (ND; CH$_3$(CH$_2$)$_{17}$COOH) with perfluorotetradecanoic acid (PF; CF$_3$(CF$_2$)$_{12}$COOH), as well as the corresponding fatty alcohol nonadecanol (NDOH; CH$_3$(CH$_2$)$_{18}$OH) with PF. Both the fatty acid and fatty alcohol were immiscible with PF in monolayers, with the systems exhibiting small, negative deviations from ideal mixing and forming phase-separated domains under a variety of preparation conditions. Micron and nanometer-scale film morphologies for the two sets of films were significantly different, however, with fatty acid mixtures tending to form polygonal, faceted domains enriched in hydrocarbon whereas the fatty alcohol mixtures formed significantly less structured domains, both at the solid- and liquid-air interfaces. Differences in film morphologies are discussed in terms of head and tail group interactions, and placed in context of existing literature on closely-related monolayer systems.
2.2 Introduction

Fluorinated surfactants typically exhibit excellent surface tension lowering abilities, chemical stability and rapid spreading at the air-water interface, and often outperform their hydrogenated counter-parts in a variety of technological applications.¹ Fluorinated surfactants are frequently mixed with conventional hydrogenated surfactants to improve overall surface activity in various surfactant applications; for example, surfactant mixtures containing a fluorinated component are frequently used in fire-fighting foams, microemulsions and mixed micelles. Despite the varied and important uses of fluorocarbon-hydrocarbon surfactant mixtures, the mixing behaviour of this broad class of molecules is often nonideal and quite complex because of the very different chemical properties of fluorocarbons and hydrocarbons. Simple fluorocarbon molecules are typically non-polarizable, mechanically inflexible and highly immiscible with water, whereas hydrocarbons are more polarizable, flexible but also immiscible with water. The two classes of compounds tend to be mutually immiscible, though for surfactants in aqueous systems, the presence of a polar head group adds further chemical complexities to their miscibility.

There have been significant recent research efforts aimed at understanding molecular-level factors which control miscibility in mixed surfactant systems, with an emphasis on using mixtures of hydrogenated and perfluorinated fatty acids in monolayers as simple, tractable model systems; various aspects of immiscibility and resulting film morphology in mixed surfactant systems have been reviewed, and readers are referred to these works for an overview of the field.²-⁴ Mixed Langmuir or Langmuir Blodgett (LB) monolayer films comprised of simple hydrogenated and perfluorinated fatty acids are typically immiscible, with components undergoing phase-separation at solid and liquid-air interfaces. Immiscibility can broadly be attributed to a combination of the comparatively strong homomolecular dispersion interactions between hydrogenated surfactants and correspondingly weak homo- and heteromolecular dispersion interactions with the poorly-polarizable perfluorocarbons. However, subtleties in terms of the degree of immiscibility and the overall morphology of the resulting phase-separated film are highly-dependent upon the composition of the underlying liquid sub-phase (e.g. pH, ionic strength), the degree of film compression and more importantly, the precise chemical identity of the film components, including surfactants’ tail group lengths and the identity of the head groups.
Much of our research group’s work in this area has centred on correlating mixing thermodynamics and micron-scale film morphology with the surfactant tail length for compressed films (fatty acids in the untilted condensed phase). By keeping the headgroups fixed (carboxyl groups) and systematically varying the length of the alkyl fatty acid tail length, correlations between mixing properties and molecular structure can be made. These mixed systems are generally phase-separated, with repulsive, non-ideal interactions between film components, though variations in this exist. Our combined studies suggest a basic empirical relationship for phase-separation in these systems; in terms of film morphology, mixed systems in which the difference in tail length between the hydrogenated fatty acid and the perfluorinated fatty acid ($\Delta_{H-F} = \# CH_2 \text{ groups in hydrocarbon tail chain} - \# CF_2 \text{ groups in perfluorocarbon tail chain}$) are greater than six form micron-sized, phase-separated polygonal domains enriched in the hydrogenated component.

Decreasing $\Delta_{H-F}$ favours formation of extended, line-like domains. These results can be broadly rationalized using McConnell’s thermodynamic model developed for single-component monolayers, which invokes a competition between dipole-dipole repulsion between negatively-charged headgroups and line-tension between different phases; in the mixed system, altering the length of the alkyl tail under close-packing conditions enables tailoring of the line tension interaction and therefore the overall film morphology. Matsumoto et al. have built upon this framework, through their investigations of phase-separated diblock fatty acids, and refined a model to describe phase-separation in binary hydrocarbon-fluorocarbon systems which accounts for molecular-level interactions between the different functional groups on the surfactant tails.

We note that while these models are simple and provide an empirical, predictive ability for when systems will phase-separate and what the resulting gross film morphology will be, numerous subtle but important effects are lost. For example, domain growth kinetics and solubility in the subphase are known to affect phase-separation and morphology. Further, an important factor that has been minimally addressed in the literature is the chemical nature of the surfactant headgroup; the majority of studies to date have investigated fatty acids and the role played by the surfactant head group remains largely unexplored.

In this work, we have characterized the mixing thermodynamics and micron-scale domain structure of mixed monolayer films comprised of nonadecanoic acid (ND; CH$_3$(CH$_2$)$_{17}$COOH) with perfluorotetradecanoic acid (PF; CF$_3$(CF$_2$)$_{12}$COOH), as well as the corresponding fatty
alcohol nonadecanol (NDOH; CH₃(CH₂)₁₈OH) with PF. Based on the empirical predictive understanding described above, we hypothesized that the mixed ND:PF system (ΔH_f = +5) should form phase-separated, elongated polygonal domains comprised of ND dispersed in a PF matrix. For the subphase conditions typically used in our other works described above, the carboxyl head groups will be negatively charged giving a high density of negative charge at the air-water interface. In this work, replacing the carboxyl group with a simple, uncharged hydroxyl by using a fatty alcohol surfactant can reasonably be anticipated to influence dipole density and thus overall film morphology. This effect is explored in detail using a combination of thermodynamic mixing and film morphology measurements.

2.3 Materials and methods

2.3.1 Chemicals and substrates

Nonadecanol, nonadecanoic acid and perfluorotetradecanoic acid were purchased from Sigma-Aldrich and used as received. The solvents n-hexane and THF (ACS grade) were purchased from Fisher Chemicals and Sigma-Aldrich respectively, and used as received. Stock surfactant solutions were prepared by dissolving the solid surfactant in a 9:1 n-hexane:THF volume ratio to a desired molarity of 2.0 × 10⁻³ mol/L. Mixed surfactant solutions were prepared by mixing appropriate volumes of the surfactant solutions to obtain the desired mole fraction. The nomenclature XX:YY is used in the text to specify the mole fraction ratio of either NDOH:PF or ND:PF mixtures. Microscope slide cover glass (22 mm × 22 mm, VWR International) was used for LB deposition experiments. The cover glass substrates were sequentially rinsed with isopropanol, absolute ethanol and ultrapure water, dried with nitrogen, and finally treated with a plasma cleaner (Harrick Plasma) prior to film deposition.

2.3.2 Compression isotherms and Langmuir Blodgett (LB) film preparation

A commercial Langmuir trough system (KSV Instruments) with ultrapure water (18.2 MΩ·cm, Millipore) as a subphase, was used in symmetric compression mode to collect surface pressure-area compression isotherms at a temperature of 22 ± 1 °C. For compression isotherm measurements, aliquots of the surfactant solution of interest was spotted onto a clean subphase surface using a Hamilton syringe. A period of at least ten minutes was allowed to ensure the evaporation of the solvent before compression. Surface pressures were measured with a platinum
Wilhemy plate and isotherms were collected using a compression rate of 7.0 Å$^2$ molecule$^{-1}$ min$^{-1}$. Isotherms were measured in triplicate to ensure reproducibility, and uncertainties in measured mean molecular areas were < 0.1 % as determined by standard deviations in replicate measurements. LB deposition of surfactant films were carried out using the same apparatus and conditions as described above, but with a compression rate of 3.5 Å$^2$ molecule$^{-1}$ min$^{-1}$. Films were deposited onto cleaned glass substrates using a single upwards stroke with a deposition rate of 5 mm-min$^{-1}$. Deposited films were allowed to dry in a clean environment before additional characterization.

2.3.3 Atomic force and Brewster angle microscopy imaging

Atomic force microscope (AFM) images were collected using a Dimension Hybrid Nanoscope system (Veeco Metrology Group) operating in Tapping Mode, using silicon probes with spring constant in the range of 12–103 N·m$^{-1}$, and resonance frequency in the range of 307–332 kHz. Imaging was carried out in air, using a scan rate of 0.50 Hz, a resolution of 512 pixels per line and with scan sizes typically 10 µm × 10 µm or 15 µm × 15 µm.

An UltraBAM system (KSV NIMA), equipped with a 658 nm imaging laser was used for Brewster angle microscope (BAM) imaging. Images were collected at an image acquisition rate of 20 frames·s$^{-1}$ and the spatial resolution of the microscope was limited by diffraction to ~ 2 µm.

2.4 Results and discussion

2.4.1 Compression isotherms

Compression isotherms for monolayers of NDOH, ND, PF, and various mixed films were collected at the air-water interface at 22 ± 1 °C, and are shown in Figure 2-1. Isotherms of pure ND and NDOH were similar to those reported elsewhere in the literature, and exhibited three phases before the film collapse pressure, including a liquid expanded (LE) phase, a tilted condensed (TC) phase, and an untilted condensed (UC) phase, as is typical for these films. To highlight phase transitions in the monolayers, compressibility modulus plots ($C^{-1} = -A(d\pi/dA)_{\pi}$ vs. $\pi$) for pure films of NDOH, ND, PF, and a representative (1:1) mixture of NDOH:PF and ND:PF are shown in Figure 2-2. The lower-pressure limiting areas ($A_0$) of ND and NDOH were estimated to be ~ 23.0 Å$^2$·molecule$^{-1}$, and ~ 21.8 Å$^2$·molecule$^{-1}$ respectively, as determined by extrapolating a straight line from the TC phase region of the isotherm to zero surface pressure. Higher-pressure
limiting areas were ~ 19 Å²·molecule⁻¹ for ND and ~ 21 Å²·molecule⁻¹ for NDOH, again in good agreement with previous reports for closely-affiliated systems. The TC phase of ND spanned surface pressures of ~ 1 mN·m⁻¹ – 28 mN·m⁻¹, whereas that for NDOH spanned ~ 1 mN·m⁻¹ – 15 mN·m⁻¹. Isotherms for pure PF consisted of a single, smoothly rising curve, typically assigned to a liquid expanded (LE) phase,³ with no evidence of other phases before film collapse. The A₀ of pure PF was estimated to be ~ 26 Å²·molecule⁻¹, agreeing well with previously reported values and consistent with the expected diameter of the perfluorocarbon molecules. Collapse pressures (π_c) of pure ND and pure NDOH films were comparable to one another (ca. 49 mN·m⁻¹ and 48 mN·m⁻¹ respectively), whereas that of pure PF was larger, occurring at ~ 65 mN·m⁻¹.

Isotherms for the mixed films of NDOH:PF and ND:PF fell between the isotherms for the pure individual components. For all mixtures, isotherms shifted to higher mean molecular areas as the fraction of PF in the mixture increased, as expected for increasing amounts of the bulkier component. The phase transition between TC-UC phases became increasingly difficult to resolve with increasing fraction of PF, as a result of relative dilution of the hydrogenated surfactant’s contribution to the overall isotherm. However, phase transitions could still be detected in compressibility modulus plots (summarized for all mixtures in the Supplementary Information). A phase transition occurs at a higher surface pressure of ~ 24 mN·m⁻¹ for the 1:1 ND:PF mixed film compared to the 1:1 NDOH:PF mixed film, which occurs at a lower surface pressure of ~ 12 mN·m⁻¹.
Figure 2-1: Surface pressure-area isotherms for pure NDOH (A) or pure ND (B), pure PF and their mixed films on ultrapure water at 22 ± 1 °C. (B) Surface pressure-area isotherms for pure ND, pure PF and their mixed films on ultrapure water at 22 ± 1 °C. Ratios noted in the figure.
legend are mole fraction ratios of hydrocarbon to perfluorocarbon in the mixed films. Uncertainties in mean molecular areas were less than 0.1 % as noted in the preceding section.

Figure 2-2: (A) to (E): Compressibility modulus plots for isotherms of pure NDOH (A), pure ND (B), mixed film NDOH:PF (1:1) (C), mixed film ND:PF (1:1) (D), and pure PF (E). Ratios noted in the figure legend are mole fraction ratios of hydrocarbon to perfluorocarbon in the mixed films.
To explore the extent of miscibility in the mixed films, the validity of the additivity relationship (2.1) was assessed:

\[ A_{12} = A_1 \chi_1 + A_2 \chi_2 \]  (2.1)

where \( A_{12} \) is the mean molecular area of the mixed monolayer film, \( A_1 \) and \( A_2 \) are the mean molecular areas of the individual film components, and \( \chi_n \) is the mole fraction of each component.\textsuperscript{16} From the additivity relationship, positive deviations (experimental points falling above the ideal line) indicates a repulsive interaction between film components, leading to expanded films, whereas a negative deviation (experimental points falling below the ideal line) is indicative of attractive interaction between film components, leading to contracted films. Mixed systems which follow equation (2.1) precisely are either fully miscible or fully immiscible. Plots showing mean molecular area as a function of mole fraction of NDOH and ND for three different surface pressures are shown in Figure 2-3A and 2-3B respectively, along with the additivity plot calculated from equation (2.1).
Figure 2-3: Mean molecular area of NDOH:PF (A) or ND:PF (B) mixed films as a function of the mole fraction of NDOH or ND, respectively. Solid dots are experimental points and the dotted
lines are the expected behaviour from the additivity rule described in equation (2.1). Uncertainties in mean molecular areas were less than 0.1% as noted in the preceding section.

The NDOH:PF and ND:PF mixed systems showed only minor deviations from ideal mixing, with both systems showing small positive and negative deviations from ideality over the range of compositions measured. There was a slight tendency towards negative deviations in both mixed systems, with the greatest deviations from ideality being observed for the ND:PF system occurring at the highest mole fractions of ND. Thus, the mixed films tended to be marginally more compacted than films of the pure individual components, though the greatest film compaction ($\chi_{ND} = 0.8$ at $\pi = 30 \text{ mN}\cdot\text{m}^{-1}$) was less than 3.5% of the area of the ideal mixture. We compare these results with those reported for the $\Delta_{HF} = +8$ and +4 systems, which showed positive and negligible deviations from ideal mixing, respectively.\textsuperscript{7,17} The $\Delta_{HF} = +8$ mixed films are the most expanded of the mixed fatty acid systems described in the literature, and have the greatest degree of immiscibility between perfluorocarbon and hydrocarbon; comparatively large dispersion forces within the hydrocarbon domains result in rigorous exclusion of the perfluorocarbons and resulting compacted domains. For the $\Delta_{HF} = +4$ system, deviations from additivity are effectively negligible, and indicating that shortening the tail group significantly weakens dispersion forces within the hydrocarbon regions. The current $\Delta_{HF} = +5$ system shows significantly smaller deviations than the $\Delta_{HF} = +8$ system, which is the expected result for the weaker dispersion forces with the shorter hydrocarbon. It is currently unclear why there appears to be a small tendency towards film compaction in this system in comparison with the nearly ideally mixed $\Delta_{HF} = +4$, though given the small size of these deviations, minor experimental factors such as spreading solvent might play a role.

The minimal difference in deviations between the NDOH:PF and ND:PF mixed systems at all surface pressures suggests that any difference in the extent of head group interactions (OH-COOH vs. COOH-COOH) are negligible in comparison with the dispersion interactions between tail chains, even at relatively low film compressions. While this may seem initially surprising, it is worth considering with more care. A crude estimate of the difference in interaction energies between fatty alcohols and fatty acids can be made through a fixed dipole interaction potential calculation (e.g. $w(r) = -u_1u_2K/r^3$ where $u_n$ is the dipole moment of molecule of interest, $K$ is a constant and $r$ is spatial separation between dipoles\textsuperscript{18}). Substituting typical gas phase dipole moments for linear alcohols and carboxylic acids from the literature\textsuperscript{19} into this relationship gives
fatty acid – fatty alcohol interaction potentials which are comparable (< 1% smaller) to the actual fatty acid – fatty acid interaction potentials. Thus, the minimal effect of headgroup on the bulk mixing energetics is actually entirely reasonable. Of course, ionization of the carboxylic acid plays a role, as will the precise value of the dipole moment for the surfactants. Under the subphase conditions used here, ND is expected to be > 95% ionized (calculated using a rudimentary Gouy-Chapman approach; cf.20) and this will affect the overall dipole moment of the headgroup. But nonetheless the relative impact of dipole density on the bulk thermodynamics of mixing in these systems is clearly minimal.

To more fully quantify the thermodynamics of mixing in these systems, the excess Gibbs free energy of mixing \( \Delta G_{ex} \), was determined according to equation (2.2):

\[
\Delta G_{ex} = \int_0^\pi [\sigma_{12} - (\chi_1 \sigma_1 + \chi_2 \sigma_2)] d\pi
\]

where \( \sigma_{12} \) is the molar area of the mixed monolayer film, \( \sigma_1 \) and \( \sigma_2 \) are the molar areas of the pure films, and \( \chi_n \) is the mole fraction of the “n-th” film component.16 Plots of \( \Delta G_{ex} \) as a function of mole fraction at select surface pressures were calculated for NDOH:PF and ND:PF mixed films, and are reported in Figure 2-4A and 2-4B, respectively. As expected from the deviations from ideal mixing, \( \Delta G_{ex} \) values for the NDOH:PF and ND:PF mixed films showed both positive and negative values which varied as a function of composition. Values were small in comparison with thermal energy (kT) and ranged from +72 to -92 J·mol\(^{-1}\) for the NDOH:PF mixtures and +98 to -137 J·mol\(^{-1}\) for ND:PF mixed films, respectively. Excess energies were small in comparison with the affiliated \( \Delta H_F = +8 \) system described above.7 While the combined additivity and Gibbs excess mixing energies described above indicate that there are minimal interactions between film components and that there are only small energetic differences between the NDOH:PF and ND:PF mixed systems, there are significant and nuanced structural differences between the resulting mixed monolayer film structures. Structures of the mixed films for these systems have been investigated using micron and nanometer-scale imaging methods and are described in the following sections.
Figure 2-4: Excess Gibbs free energy of mixing of NDOH:PF (A) or ND:PF (B) mixed films as a function of the mole fraction of NDOH or ND, respectively. Uncertainties in energies are <0.1% as determined by appropriate error propagation calculations using data from Figure 2-3.
2.4.2 Brewster angle microscopy

The micron-scale morphology of pure and mixed monolayer films was measured at the air-water interface using BAM imaging. Imaging in these systems is limited to a low surface pressure regime (< 2 mN·m⁻¹) because at higher pressures, domain structures are compressed to sizes and separations that are smaller than the diffraction-limited spatial resolution of the instrument (~2 μm). BAM images for the pure films and mixed films were collected, with representative images for the pure films and mixtures with compositions of 4:1 NDOH:PF and 4:1 ND:PF shown in Figure 2-5. Other compositions for which isotherms were measured are reported as Supplementary Information.

BAM images of pure PF consisted of a dark background with no reflective contrast, consistent with fluorinated films with a refractive index comparable to that of the water subphase.¹ Reflective features observed in the pure or mixed films could be ascribed to NDOH or ND and the dark background to either the underlying water subphase or PF (in the case of the mixed films). For pure NDOH, the film morphology consisted of amorphous patches (typically tens to hundreds of microns in size) dispersed over the low-reflectivity subphase, whereas the ND film tended to form patches with sharp edges and facets, suggesting a higher degree of crystallinity in the fatty acid monolayers in comparison with the fatty alcohol. The precise morphology and patch size of NDOH and ND varied as a function of surface pressure as shown in the Supplementary Information (data distributed across Figures (2-S3)-(2-S7)).

The principal result from these measurements is that structured films were observed for all the mixed monolayers. The 4:1 NDOH:PF mixed films consisted of reflective, amorphous domains (assigned to NDOH) interspersed with occasional low reflectivity regions (assigned to either subphase water or PF). The films were significantly different in structure from the pure NDOH alone, but many of the domains appeared to be close in size to the instrument resolution or were likely so closely packed that they could not be resolved, and thus could not be examined in detail. For the 4:1 ND:PF mixed film, the reflective ND domains were significantly larger (length scales on the order of tens of microns) and at higher film compressions had a faceted, polygonal structure. There was significant domain-to-domain variations in reflectivity, with domains exhibiting various shades of white and grey within the same BAM image. Both the domain structures and differences in reflectivity are entirely consistent with that we have reported previously for the Δ_HF = +6 mixed
system, which formed phase-separated, crystalline domains of hydrocarbon (the polygonal domains) in a perfluorocarbon matrix. The same overall film morphology is the same as observed for the ND:PF mixtures. The variation in reflectivity between domains has been attributed to hydrocarbon molecules that have different azimuthal tilts in their aliphatic tail groups; this gives films the same thickness but different effective refractive indices and hence reflectivity.

The greater tendency of the fatty acid mixed system to form faceted, polygonal structures in the mixed films in comparison with the fatty alcohol mixed system is consistent with the behavior observed for the pure films shown in Figure 2-5, but the precise molecular-level explanation for this difference presently remains unclear to us. It is well-known from glancing incidence X-ray diffraction experiments that monolayer films of both fatty acids and alcohols are crystalline and diffract even at near zero surface pressures. There are subtle crystallinity differences between the two types of films which become particularly important at low surface pressures where the interaction of head groups on molecular packing dominate over tail interactions. Fatty alcohols tend to form significantly smaller tilt angles with respect to the subphase normal in comparison with fatty acids, and the alcohols exhibit one fewer crystalline phases (the so-called D phase, using nomenclature used by Dutta et al.). However, it is unlikely that these small differences in crystal structure would result in such large morphological differences between domains in the two systems. It would appear that the very small difference in head group dipole moment between the fatty alcohol and acid contributes to this effect, with the slightly larger dipole for the fatty acid promoting the formation of faceted domains at the micron length scale. At present, we can simply report the tendency of the phase-separated ND to form polygonal domains in comparison with NDOH forming circular domains. However, the structures and behavior persist at the solid-air interface for more compressed films as well, as detailed below.
Figure 2-5: BAM images of pure and mixed monolayer films. (A) Pure NDOH, (B) Pure ND, (C) NDOH:PF 4:1, (D) ND:PF 4:1, and (E) Pure PF, taken between $\pi = 1$ to $2 \text{ mN} \cdot \text{m}^{-1}$ and $T = 22 \pm 1 \, ^\circ\text{C}$.
2.4.3 Atomic force microscopy

To assess nanometer-scale film structure of the mixed films at the solid-air interface, deposited monolayers (LB films) were measured using AFM imaging. Pure and mixed films were deposited onto glass substrates at $\pi = 30 \text{ mN} \cdot \text{m}^{-1}$, a surface pressure selected to allow for comparison of film structures with those reported previously in the literature (systems described in the Introduction). Films deposited with transfer ratios of ~1, indicating stable transfer of single monolayers had been achieved. AFM height images of the pure control films and mixed films with representative compositions of 4:1 NDOH:PF and 4:1 ND:PF are shown in Figure 2-6, and films with a range of other compositions are shown in Figure 2-7 (for ND:PF) or are reported as Supplementary Information. Images of the pure films showed no discernible topographical features, as expected for the pure components deposited at high surface pressures. AFM images for the 4:1 NDOH:PF mixed system consisted of discrete, elliptical domains that were heterogeneous in size (though typically on the order of 200 nm diameter) and dispersed in a continuous matrix. Cross-sectional and frequency histogram analysis of 4:1 NDOH:PF mixed films (Figures 2-S9 and 2-S10 in Supplementary Information) show an average height difference of $0.7 \pm 0.2 \text{ nm}$ between the elevated elliptical domains and the continuous matrix. Molecular lengths of ND, NDOH and PF were calculated to be ~2.5 nm, ~2.5 nm and ~1.8 nm (values calculated using DFT in vacuum using Spartan ’14, V.1.1.4), respectively and thus the height difference between domains and the underlying matrix measured in the AFM is consistent with the difference in length between the hydrocarbon and perfluorocarbon which are adsorbed normal to the substrate. The film morphology measured in the AFM was consistent with that observed for the BAM images at lower pressures, indicating that the deposition process itself does not significantly affect domain structure, as reported previously for the $\Delta HF = +6$ system.$^{22}$
Figure 2-6: AFM height images (15 µm × 15 µm) of pure and mixed monolayer films. (A) Pure NDOH, (B) Pure ND, (C) NDOH:PF 4:1, (D) ND:PF 4:1, and (E) Pure PF, deposited at $\pi = 30$ mN·m$^{-1}$ and $T = 22 \pm 1$ °C.
AFM height images for the 4:1 ND:PF mixed film show that this system also undergoes phase-separation but the overall film morphology differed considerably from that of the fatty alcohol, similar as to observations from the BAM measurements. Morphology consisted of elevated polygonal domains, typically several microns in diameter, dispersed on a continuous matrix. The height difference between the polygonal domains and the underlying matrix was 1.0 nm ± 0.2 nm (cross-sectional and histogram analysis reported in Supplementary Information), consistent with the difference in molecular length of fully-extended ND and PF. This is the expected result for polygonal domains comprised of ND and a surrounding matrix of PF, as found in the affiliated mixed systems.

Some final additional questions of interest are how the nanoscale film morphology of the mixed films vary as a function of film composition (hydrocarbon to perfluorocarbon ratio) and whether the morphology of the fatty acid system follows the general trend of favouring extended domains as a function of shorter alkyl tail length reported in the literature. Compositionally-dependent AFM images are shown for the NDOH:PF and ND:PF systems in the Supplementary Information and in Figure 2-7, respectively. There was a significant degree of heterogeneity in terms of domain shape observed for both systems, both within the same film and between different films of different compositions. However, some general results can be extracted. There was no significant change in molecular orientation (vertically adsorbed surfactants) as a function of composition, as determined through monitoring domain height differences. For both systems, as the relative mole fraction of the PF increased the total area of the continuous matrix increased relative to that of the dispersed domains. This is the expected result, though precise quantitation was problematic because of film heterogeneity. There were only small morphological variations as a function of composition seen in the NDOH:PF system (Figure 2-S8), with the greatest deviations from simple elliptical domains being seen at the highest mole fractions of PF. Because this system showed minimal changes, we will focus the remaining discussion on the ND:PF system.
Figure 2-7: AFM height images (15 µm × 15 µm) and (10 µm × 10 µm) of ND:PF mixed monolayer films, of different compositions. (A) 4:1, (B) 3:1, (C) 2:1, (D) 1:1 (E) 1:2, (F) 1:3, and (G) 1:4, deposited at π = 30 mN·m⁻¹ and T = 22 ± 1 °C.

As noted in the Introduction, general trends in gross domain shape for the mixed fatty acid systems are regulated by a combination of line tension and dipole repulsion, and for the ND system, we predicted that the domains will be more spatially extended than those for the behenic acid – perfluorotetradecanoic acid (Δ_H-F = +8) system. While rigorous quantification of this effect is challenging because of the sample heterogeneity described above, we have carried out a semi-quantitative analysis of the image data by calculating aspect ratios (ARs; major domain diameter / minor domain diameter) for the hydrocarbon domains shown in Figure 2-7 and comparing those with samples prepared under comparable conditions for the Δ_H-F = +8 system reported previously. Aspect ratios were calculated using the ImageJ (v1.49) particle analysis function from appropriately thresholded AFM images. Domain aspect ratios for the ND (Δ_H-F = +5) system were approximately 1.4-fold greater than that of the Δ_H-F = +8 system, with differences determined to be significant at the 95% confidence level. While we treat the precise numerical value itself as semi-quantitative, the general trend of shorter fatty acid chain length leading to more spatially elongated domains holds well for this system, and provides further general support for the empirical rules which can usefully describe morphology in these systems.

2.5 Conclusions

Miscibility in the ND:PF mixed monolayer system is consistent with predictions based on our previously established empirical models, and there are only minimal differences in terms of bulk thermodynamic mixing properties between the fatty acid and fatty alcohol mixed systems. Despite these, there were significant morphology differences between the two systems at the micron and nanometer length scale. The NDOH:PF system tended towards formation of phase-separated, elliptical domains of fatty alcohol dispersed in a matrix of perfluorocarbon, while the ND system formed polygonal, crystal-like domains in the PF matrix. The underlying explanation of why the two different head groups give rise to morphological differences in these systems, despite minimal differences in thermodynamic mixing properties in the two systems, remains unclear. In terms of closely-related affiliated mixed fatty acid systems, the overall domain morphology of the ND:PF system agrees well with empirical predictions, forming domains with shapes that were elongated
in comparison with fatty acid systems with longer tail chains, but less so than systems with shorter tail chains. This result follows the general expectation of domain morphology which is dominated by a combination of dipole-dipole repulsion in combination with line tension that is dominated by tail chain-tail chain dispersion interactions.

2.6 Acknowledgements

Financial support for this work has been provided by the Natural Sciences and Engineering Research Council of Canada, the Canadian Foundation for Innovation and the University of Saskatchewan.

2.7 Appendix A. Supplementary data

Figure 2-S1: Compressibility modulus plots for isotherms of mixed films NDOH:PF (4:1) (A), (3:1) (B), (2:1) (C), (1:2) (D), (1:3) (E), and (1:4) (F).
Figure 2-S2: Compressibility modulus plots for isotherms of mixed films ND:PF (4:1) (A), (3:1) (B), (2:1) (C), (1:2) (D), (1:3) (E), and (1:4) (F).
**Figure 2-S3:** BAM images of pure and mixed monolayer films. (A) Pure ND, (B) 4:1, (C) 3:1, (D) 2:1, (E) 1:1 (F) 1:2, (G) 1:3, (H) 1:4 and (I) Pure PF, taken at $\pi = 0 \text{ mN} \cdot \text{m}^{-1}$ and $T = 22 \pm 1 ^\circ \text{C}$. 
**Figure 2-S4:** BAM images of pure and mixed monolayer films. (A) Pure ND, (B) 4:1, (C) 3:1, (D) 2:1, (E) 1:1 (F) 1:2, (G) 1:3, (H) 1:4 and (I) Pure PF, taken between \( \pi = 0 \) to 1 mN·m\(^{-1} \) and \( T = 22 \pm 1 \) °C.
Figure 2-S5: BAM images of pure and mixed monolayer films. (A) Pure ND, (B) 4:1, (C) 3:1, (D) 2:1, (E) 1:1 (F) 1:2, (G) 1:3, (H) 1:4 and (I) Pure PF, taken between $\pi = 1$ to 2 mN·m$^{-1}$ and $T = 22 \pm 1$ °C.
Figure 2-S6: BAM images of pure and mixed monolayer films. (A) Pure NDOH, (B) 4:1, (C) 3:1, (D) 2:1, (E) 1:1 (F) 1:2, (G) 1:3, (H) 1:4 and (I) Pure PF, taken between $\pi = 0$ to 1 mN·m$^{-1}$ and T = 22 ± 1 °C.
Figure 2-S7: BAM images of pure and mixed monolayer films. (A) Pure NDOH, (B) 4:1, (C) 3:1, (D) 2:1, (E) 1:1 (F) 1:2, (G) 1:3, (H) 1:4 and (I) Pure PF, taken between $\pi = 1$ to $2 \text{ mN} \cdot \text{m}^{-1}$ and $T = 22 \pm 1 \, ^\circ\text{C}$. 
Figure 2-S8: AFM height images (15 µm × 15 µm) of NDOH:PF mixed monolayer films, of different compositions. (A) Pure NDOH, (B) 4:1, (C) 3:1, (D) 2:1, (E) 1:1 (F) 1:2, (G) 1:3, (H) 1:4 and (I) Pure PF, deposited at $\pi = 30 \text{ mN} \cdot \text{m}^{-1}$ and $T = 22 \pm 1 ^\circ\text{C}$. 
**Figure 2-S9:** AFM image cross-sectional analysis of NDOH:PF mixed monolayer films, of different compositions. (A) 4:1, (B) 3:1, (C) 2:1, (D) 1:1 (E) 1:2, (F) 1:3, and (G) 1:4

**Figure 2-S10:** (A): AFM height image (15 µm × 15 µm) of 4:1 NDOH:PF mixed monolayer film, with corresponding histogram analysis (percent frequency of image pixels versus pixel height). (B): AFM height image (15 µm × 15 µm) of 4:1 ND:PF mixed monolayer film, with corresponding histogram analysis (percent frequency of image pixels versus pixel height).
2.8 References


CHAPTER 3

MOLECULAR-LEVEL STRUCTURE AND PACKING IN PHASE-SEPARATED ARACHIDIC ACID-PERFLUOROTETRADECANOIC ACID MONOLAYER FILMS

This chapter is a modified version of a paper published in Langmuir in August 2018. [Reprinted with permission from Langmuir 2018, 34(36) 10673-10683. Copyright (2018) American Chemical Society.]

In this study, the molecular-level structure of arachidic acid-perfluorotetradecanoic acid mixed monolayers was investigated directly at the air-water interface using the synchrotron-based liquid surface X-ray scattering techniques grazing incidence X-ray diffraction and X-ray reflectivity. The characterization of mixed hydrogenated-perfluorinated monolayer films at the micron scale does not give direct information about the film structure at molecular length. Therefore, this study was undertaken to gather information about the molecular-level film structure which reveals new insights and deepens the knowledge and understanding of the factors that control miscibility in these mixed films.

Grazing incidence X-ray diffraction measurements provided information on the crystallographic structure and packing of these mixed films. X-ray reflectivity was used to measure the film thickness and electron density distribution at the air-water interface. From GIXD studies, it was found that all of the mixed films were crystalline, manifesting diffraction peaks at distinct specific in-plane positions which clearly demonstrates phase-separation at the molecular-level. GIXD and XR studies both revealed the fluorinated surfactant, whether in pure or mixed film state, formed a hexagonal lattice with a vertical order perpendicular to the air-water interface. The hydrogenated surfactant was seen to exhibit significant tilts with respect to the surface normal. These findings at the molecular-level solidifies the understanding of phase-separation and miscibility in hydrogenated-perfluorinated monolayer films.

The author’s contribution to this article includes: sample preparation, performing all experiments, processing, analyzing and interpreting all results, and finally preparing and writing the manuscript. My supervisor, Professor Matthew F. Paige also assisted in the measurements at the synchrotron facility and further polished the manuscript by writing extensively. Kyle M. Fransishyn and Chelsea Cayabab also assisted in the experimental measurement. Ivan Kuzmenko and Michael
Martynowycz are beamline scientists who provided training on the instrument and gave insight to processing and interpreting the results.

**Molecular-level Structure and Packing in Phase-Separated Arachidic Acid-Perfluorotetradecanoic Acid Monolayer Films**

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### 3.1 Abstract

Synchrotron-based X-ray scattering measurements of phase-separated surfactant monolayers at the air-water interface provide molecular-level structural information about the packing and ordering of film components. In this work, grazing incidence X-ray diffraction (GIXD) and X-ray reflectivity (XR) measurements were used to collect crystallographic structural information for binary mixed monolayers of arachidic acid (AA, C₁₉H₃₉COOH) with perfluorotetradecanoic acid (PA, C₁₃F₂₇COOH), a system that has previously been investigated using a variety of thermodynamic and micron-scale structural characterization methods. GIXD measurements at surface pressures of π = 5, 15 and 30 mN/m indicated that AA in pure and mixed films forms a rectangular lattice at π = 5 and 15 mN/m but a hexagonal lattice at π = 30 mN/m. PA formed hexagonal lattices under all conditions, with films being highly-ordered and crystalline (as determined by Bragg peak width) at even the lowest surface pressures investigated. Phase-separation occurred for all mixed monolayer film compositions and surface pressures, manifesting as diffraction peaks characteristic of the individual components appearing at different in-plane scattering vector qₓᵧ. For both pure and mixed films, the molecular tilt angle of the AA hydrocarbon chain towards the nearest-neighbor was substantial at low pressures but decreased with increasing pressure. The PA fluorocarbon chain showed negligible molecular tilt under all conditions, and was oriented normal to the subphase surface regardless of surface pressure or the presence of AA in the films. In all cases, the two components in the mixed film behaved entirely independently of film composition, which is exactly the expected result for a fully phase-separated, immiscible system. XR measurements of film thickness at the air-water interface supported these
results; overall film thickness approached the calculated ideal surfactant tail lengths with increasing surface pressure, indicating nearly-normal oriented surfactants. The overall surfactant packing and crystallographic features of the mixed monolayers are discussed in terms of the lipophobic nature of the perfluorinated surfactant, as well as in context of thermodynamic miscibility and domain structure formation reported elsewhere in the literature for these mixed monolayer systems.

3.2 Introduction

The patterning and self-assembly of surfactants at interfaces has long been an area of both fundamental and applied interest in the physical sciences. While a variety of approaches for the patterning of surface active molecules exist, an approach that offers the benefits of simplicity and controllability is the phase-separation of mutually immiscible surfactants. Numerous immiscible, phase-separating surfactant systems can be found in the literature, but a particularly promising and tractable system of recent interest is simple mixtures of fatty acids with long-chain, perfluorinated fatty acids. Perfluorinated surfactants are a well-studied class of molecules which have a demonstrated variety of useful and important technological properties.\(^1\) A key feature of this class of molecules is their strong lipophobicity, a consequence of the very low polarizability of perfluorocarbons, which can be exploited in mixtures of hydrogenated surfactants for phase-separation and patterning purposes.

Our group and others have demonstrated that a wide variety of binary mixed fatty acid-perfluorinated fatty acid systems undergo phase-separation at both solid-air and liquid-air interfaces to yield highly-structured, patterned surfaces with diverse morphology over various length scales.\(^2\)\(^-\)\(^5\) Factors such as the chemical nature of the constituent surfactants, the composition of the mixed film, surface pressure and others affect the extent of phase-separation, surfactant miscibility and hence overall film structure; for a recent overview see the review by Paige et al.\(^4\) A benchmark phase-separated monolayer system that has been investigated a number of times in the literature are mixtures of arachidic acid (AA; \(C_{19}H_{39}COOH\)) and perfluorotetradecanoic acid (PA; \(C_{13}F_{27}COOH\)).\(^2\)\(^,\)\(^6\)\(^-\)\(^8\) When mixed monolayers of this system are prepared on pure water subphases, the system undergoes phase-separation to form distinct, micron-sized polygonal domains surrounded by a continuous matrix, as verified by Brewster Angle Microscope (BAM) imaging (Figure 3-1).\(^2\) Monolayers could be successfully transferred onto various types of solid-
substrates with negligible alteration to domain morphology, as measured using Atomic Force 
Microscope (AFM) imaging.\textsuperscript{6,7} These investigations also revealed that the polygonal domains are 
composed of AA, whereas the surrounding matrix is comprised exclusively of PA. We have 
subsequently explored a range of affiliated phase-separated systems, and demonstrated that 
domain morphology is broadly affected by a combination of line tension – dipole repulsion effects 
first described by McConnell for single-component phospholipid monolayers,\textsuperscript{9} as well as kinetics 
of domain growth, fluid drainage (in the case of deposited Langmuir Blodgett (LB) films) and 
other factors.

While micron-scale film morphology and domain shape in the mixed AA:PA monolayer system 
are well-studied, the molecular-level structure of the mixed, phase-separated films remains largely 
unexplored. Whereas the underlying reason for forming polygonal structures of AA in the presence 
of PA at the air-water interface is unclear, the various domain textures observed in BAM imaging 
experiments (appearing as different shades of gray in Figure 3-1) indicates the presence of highly-
crystalline domains of AA with an assortment of azimuthal tilt angles with respect to the subphase 
surface.\textsuperscript{2} The domain structure and texturing does not occur with AA alone, suggesting that the 
PA component induces domain formation and structuring. To explore these effects, along with the 
detailed crystallographic structures of the phase-separated films, we have made use of synchrotron-
based, surface sensitive X-ray scattering measurements, namely grazing incidence X-ray 
diffraction (GIXD) and X-ray reflectivity (XR).

![Figure 3-1: (A) BAM image of a phase-separated AA-PA mixed monolayer at the air-water interface ($\pi = 10$ mN/m), with AA forming the polygons and PA forming the black phase in](image_url)
between. The arrow indicates an aggregate of polygonal domains, and the different shades of gray (reflectivity) results from variations in azimuthal tilt angles of constituent AA molecules. Reproduced and modified with permission from Eftaiha et al.\textsuperscript{2}. \textbf{(B)} AFM image (20 $\mu$m $\times$ 20 $\mu$m) of a phase-separated AA-PA mixed monolayer deposited at onto a solid mica substrate, with AA forming the polygons and PA forming the black phase in between. Reprinted (adapted) and with permission from Qaqish et al.\textsuperscript{6} Copyright 2007 American Chemical Society.

The application of GIXD and XR methods to Langmuir monolayer film characterization is well-known and has been reviewed in detail elsewhere (see Als-Nielsen et al.\textsuperscript{10}, for example). Both fatty acids and fluorinated fatty acid monolayers have been characterized using these approaches, the former more extensively. Fatty acid monolayer ordered structures and phases are a strong function of tail chain length, surface pressure and subphase temperature, with features such as molecular tilt angles, lattice spacing and backbone order varying considerably as a function of these parameters.\textsuperscript{10} At low surface pressures, fatty acid molecules typically pack into a distorted hexagonal unit cell, with a considerable molecular tilt towards their nearest neighbours (NN).\textsuperscript{11} The unit cell distortion and the molecular tilt decrease at higher film compressions, with molecules aligned perpendicular to the subphase at highest compressions. Partially fluorinated and perfluorinated monolayer systems have also been measured.\textsuperscript{12-14} In a seminal work in this field, Barton et al.\textsuperscript{15} noted the tendency of the fluorinated fatty acid C$_{10}$F$_{21}$CH$_2$CO$_2$H to spontaneously form crystalline aggregates at very low concentrations, with molecules packing into a hexagonal lattice. Further, the highly-inflexible fluorocarbon chains tended to align with a very small tilt angle (2° $\pm$ 3) to the subphase surface. Jacquemain et al.\textsuperscript{13} expanded on this work, suggesting that the potential for conformational disorder in fluorocarbon chains is more limited than in corresponding hydrocarbon systems because of high backbone rigidity and stronger chain-chain van der Waals attraction. Additional results that support these models can be found in closely affiliated systems, including PA\textsuperscript{16}, CF$_3$(CF$_2$)$_{10}$COOH\textsuperscript{17}, and semifluorinated alkanes\textsuperscript{18}.

While thermodynamic and micron-scale structural investigations of phase-separated fatty acid–fluorocarbon mixed monolayers are plentiful in the literature, there appears to be very few reports of the use of X-ray scattering methods for structural analysis on these or affiliated systems. Takiue\textsuperscript{19} have characterized phase-separated mixed monolayer films of 1-tetradecanol (CH$_3$(CH$_2$)$_{13}$OH) with 1,1,2,2-tetrahydrohenicosafluorododecanol (CF$_3$(CF$_2$)$_6$(CH$_2$)$_2$OH) using
GIXD, in combination with BAM imaging and affiliated thermodynamic miscibility studies. In-plane diffraction patterns for the mixtures consisted of two distinct Bragg peaks, with each peak corresponding to the diffraction pattern for the pure individual component. Both components were assigned as adopting a hexagonal packing arrangement, and the intensity of the diffraction peak associated with the hydrogenated alcohol increased as a function of surface pressure. The authors attributed these results to both components de-mixing and forming condensed states. Research from Dupres et al.\textsuperscript{20} has demonstrated complete phase-separation in a mixture of stearic acid with a fluorinated amphiphile through a combination of GIXD measurement with optical and thermodynamic studies. A key observation was that the tilt angle of the stearic acid molecules in both the pure stearic acid film and mixed film decreased with time, with the tilt angle decreasing from 25° to a non-measurable value at $\pi = 5$ mN/m.

In this work, GIXD and XR measurements have been used for structural characterization of binary mixed monolayer films of AA and PA over a range of film compositions and surface pressures, with a view towards determining the crystallographic structures of these films as well as achieving a molecular-level understanding of the domain structures observed at micron length-scales. Results from these measurements are discussed in context of the comparator systems described above, as well as the previously measured BAM and AFM data.

3.3 Materials and methods

3.3.1 Materials

Perfluorotetradecanoic acid, arachidic acid, THF and n-hexane were purchased from Sigma-Aldrich. The PA was further purified by repeated recrystallization from 2\% (v/v) of acetone in hexane/acetone mixture with small amount of trifluoroacetic acid. All other reagents were used as received. Ultrapure water (MilliQ, 18 MΩ-cm) was used as the subphase. The solid surfactants were dissolved in a 9:1 n-hexane:THF solvent and the stock solutions were then mixed in appropriate volumes to make mixed surfactant solutions in various mole ratios. Mixtures are described using the notation XX:YY AA:PA, where XX and YY are the mole fractions of AA and PA in the mixture, respectively.
3.3.2 Liquid Surface X-ray Scattering (GIXD and XR)

GIXD and XR measurements were performed on the liquid surface spectrometer on beamline 9-ID-B at the Advanced Photon Source (Argonne National Laboratory). Measurements were carried out using a Langmuir trough with a single, movable barrier, encased in a gastight chamber mounted on an anti-vibration system. The trough was filled with ultrapure water as the subphase and an aliquot of the AA:PA solution of interest was spread drop by drop over the subphase with a Hamilton syringe. The solvent was allowed to evaporate before beginning measurements. The gastight chamber containing the trough was continuously purged with helium to reduce oxidative degradation of the monolayer and air scattering. The monolayer was compressed to the desired surface pressure ($\pi = 5, 15$ and $30$ mN/m) to observe microscopic structural behavior at different phases (ranging from liquid to condensed phases), and was held constant during the measurements. The experiments were all conducted at room temperature and a Ge (111) steering crystal was used to re-direct monochromatic X-ray beam of wavelength $0.920 \text{ Å}$ onto the liquid surface at different incidence angles. The scattered X-ray beam was collected with a Dectris “Mythen” linear detector. The intensity of diffracted beam in the GIXD scan was collected as a function of the horizontal momentum transfer component ($q_{xy}$) and vertical momentum transfer component ($q_z$). The intensity of the reflected beam in the XR scan was collected as a function of $q_z$. The trough position was translated horizontally in the direction perpendicular to the beam following each GIXD or XR scan so as to minimize potential sample damage.

3.4 Results and Discussion

3.4.1 Grazing incidence X-ray diffraction

GIXD measurements were used to determine the crystallographic structures of monolayer films of PA, AA and their mixtures at the air-water interface. Fig. 3-2 shows GIXD patterns for pure PA and pure AA at surface pressures of $\pi = 5, 15$ and $30$ mN/m.

For pure PA at all surface pressures, diffraction patterns consisted of a single, sharp Bragg peak appearing at $q_{xy} = 1.26 \pm 0.01 \text{ Å}^{-1}$ and with an out-of-plane diffuse scattering maximum at $q_z = 0 \text{ Å}^{-1}$. The PA peak position is in good agreement with reports by Barton et al.\textsuperscript{15} and Fukuto et al.\textsuperscript{16} for fluorinated amphiphiles. The PA diffraction peak was indexed as triple degenerate $\{1,0\}$, $\{0,\bar{1}\}$, $\{1,1\}$ reflection. It corresponds to a hexagonal lattice, with $a = b = 5.77 \text{ Å}$, with a unit cell
area per chain of 28.79 Å² at π = 5 mN/m. The Bragg rod, with its maximum q_z = 0 Å⁻¹, and its characteristic width correspond to the molecular long axis perpendicular to the water surface, similar to observations by Acero et al. on fluorinated amphiphiles. Comparable results were obtained for pure PA films measured at π = 15 and 30 mN/m; GIXD patterns were also indexed as a hexagonal lattice, a = b = 5.78 Å with a unit cell area per chain of 28.94 Å² and a = b = 5.76 Å with a unit cell area per chain of 28.69 Å² respectively. The Bragg peak broadening in q_{xy} for the triple degenerate reflection is given by the average 2D crystallite size, i.e. coherence length, L_{xy}. For pure PA films, it was ~ 437 Å according to Scherrer’s relation at all pressures. In total, the measurements indicate that PA was highly crystalline for all surface pressures explored in these measurements, which is in good agreement with observations by Barton et al. and Acero et al. on fluorinated amphiphiles. It is worth briefly considering the underlying chemical factors that promote the vertical alignment of the PA monolayers, particularly given the very low surface pressures at which the films are prepared. Perfluorocarbons adhere weakly to the air-water interface, due in part to their extremely low polarizability, and the long fluoroalkyl chains are mechanically rigid. It appears that minimization of fluoroalkyl chain interactions with the underlying aqueous subphase can be achieved by vertically orienting the chain, leaving only the carboxylate headgroup to associate with the subphase. Since there is no conformational flexibility in the fluoroalkyl chains, the entire molecule is forced into a vertical alignment at even the most modest of surface pressures.

GIXD patterns for pure AA at π = 5 mN/m consisted of two Bragg peaks at q_{xy} = 1.40 ± 0.02 Å⁻¹ and 1.46 ± 0.01 Å⁻¹, indicating two low-order reflections with a distorted hexagonal lattice (centered rectangular lattice), with cell parameters a = 5.3 Å and b = 8.5 Å corresponding to a unit cell area of 45.09 Å², which contains two hydrocarbon chains. This observation stems from the long axis tilt towards nearest neighbors as the Bragg rods of the degenerate reflections ([1,1]r, [1,1]̅r) center at q_z ≥ 0 Å⁻¹, while that of the non-degenerate reflection ([0,2]r) at q_z = 0 Å⁻¹. Thus, AA molecules tilt toward the NN in the L₂ phase, as was established earlier. The molecular tilt from the vertical, calculated based on q_z/q_{xy} positions of the diffraction peaks, gives 30.8° at π = 5 mN/m. The AA film is less crystalline than PA, with a coherence length L_{11} of ~ 142 Å and L_{02} of ~ 224 Å consistent with studies by Peterson et al. on eicosanoic acid. Similar results were obtained for pure AA monolayers at π = 15 mN/m but with a less distorted lattice, lower molecular
tilt angle and weaker degenerate reflections. At $\pi = 30$ mN/m (S phase), the peak shifts to higher scattering vector ($q_{xy} = 1.53 \pm 0.01$ Å$^{-1}$) and the two reflections merge to a single reflection, depicting a more compact organized film, consistent with studies by Kjaer et al.$^{12}$ on AA.

For the mixed monolayers, GIXD measurements for films with a higher mole fraction of PA at $\pi = 5$ mN/m are first described, followed by the same compositions at $\pi = 15$ and 30 mN/m. Subsequently, films with higher mole fraction of AA are described.

GIXD patterns for mixed AA:PA monolayers as a function of increasing PA mole fraction at $\pi = 5$ mN/m are shown in Fig 3-3A. Distinct Bragg peaks characteristic of both PA and AA appearing at different in-plane $q_{xy}$ were observed, similar to GIXD studies by Dupres et al.$^{20}$ on stearic acid mixed with partially fluorinated amphiphilic molecules. This is precisely the result that is expected for a phase-separated, mutually immiscible monolayer system, and agrees with our thermodynamic and micron scale measurements carried out previously on this system.$^{2,5-7}$ The crystallographic structure of the PA component in the mixed films was essentially unchanged from that measured in the pure PA films. The Bragg peak for PA was at $q_{xy} = 1.26 \pm 0.01$ Å$^{-1}$ and was very sharp, with an out-of-plane scattering vector $q_z = 0$ Å$^{-1}$. For all mixed composition films, the PA component was indexed as a hexagonal lattice $a = b = 5.8$ Å (for details see Table 3-1), corresponding to a unit cell area per chain of 28.9 Å$^2$ at $\pi = 5$ mN/m. An average $L_{xy}$ of $\sim 365$ Å PA crystallite domain

Figure 3-2: (A) GIXD patterns for pure PA at surface pressures $\pi = 5$, 15 and 30 mN/m. (B) GIXD patterns for pure AA at surface pressures of $\pi = 5$, 15 and 30 mN/m.
size was measured for all mixed compositions. For the AA component in all of the mixed films, two Bragg peaks at $q_{xy} = 1.40 \pm 0.01 \text{ Å}^{-1}$ and $1.47 \pm 0.01 \text{ Å}^{-1}$, correspond to a centered rectangular lattice, $a = 5.3 \text{ Å}$ and $b = 8.5 \text{ Å}$, with a unit cell area per two hydrocarbon chain of 45.1 Å$^2$ (for details see Table 3-1). AA molecules in the mixed composition films tend to be tilted toward the NN in the L$_2$ phase with an average $L_{11}$ of $\sim 81 \text{ Å}$ and $L_{02}$ of $\sim 193 \text{ Å}$.

Further insight into the crystallographic structure of these films comes from contour plots of the intensity along the $q_{xy}$ versus intensity along the $q_z$ axis (Figure 3-3(B-E)). As expected, the relative intensities of the two sets of peaks for PA and AA scales as a function of the relative amounts of the two components. Plotting the percentage of the total diffraction signal contributed by PA as a function of its mole fraction yielded a straight line with slope $= 0.91 \pm 0.7$ (see Figure 3-S5 in Supplemental Information). A fully immiscible system is expected to follow a simple thermodynamic additivity relationship, and the near unity slope is thus in good agreement with this expectation.

At higher pressures, the diffraction signal associated with PA remained effectively unchanged, whereas that associated with AA changed to reflect an increasingly vertical orientation of the fatty acid molecules. For pure PA, results are indicative of an ordered, crystalline hexagonal unit cell.$^{11,25}$ Contour maps for the mixed films indicates a hexagonal unit cell for the PA and a centered rectangular unit cell with NN tilt in the L$_2$ phase for the AA.$^{11,23}$ Therefore, the PA packs hexagonally but the AA packs in a centered rectangular cell with tilts in NN. As noted in the Introduction, we have previously reported that different polygonal domains had different reflectivity values in BAM measurements taken at low surface pressures (Figure 3-1A), and attributed this to domains that had different azimuthal tilts for the AA molecules that comprise the domains. The GIXD measurements for the mixed films indeed confirm that at the surface pressures used for the BAM measurements, the AA will be tilted with respect to the surface normal, and the micron-scale BAM measurements are in excellent agreement with the GIXD results.
Figure 3-3: (A) GIXD patterns for mixed monolayers of mole fraction of PA ($\pi = 5$ mN/m). (B) to (E) Contour plot showing intensity along $q_z$ versus intensity along $q_{xy}$ for pure PA, 1:1, 1:2 and 1:4 AA:PA mixed films.
Table 3-1. GIXD data for mixed monolayer films at $\pi = 5$ mN/m.

<table>
<thead>
<tr>
<th>Composition</th>
<th>$\pi$ (mN/m)</th>
<th>In-plane Bragg Peaks</th>
<th>Out-of-plane Bragg Rods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\pi$</td>
<td>Lattice Parameters</td>
<td>Unit Cell Area / Chain (Å$^2$)</td>
</tr>
<tr>
<td>Pure PA</td>
<td>5</td>
<td>a (Å) (± 0.01)</td>
<td>b (Å) (± 0.01)</td>
</tr>
<tr>
<td>AA:PA 1:1</td>
<td>5</td>
<td>5.77</td>
<td>5.77</td>
</tr>
<tr>
<td>AA:PA 1:2</td>
<td>5</td>
<td>5.77</td>
<td>5.77</td>
</tr>
<tr>
<td>AA:PA 1:4</td>
<td>5</td>
<td>5.76</td>
<td>5.76</td>
</tr>
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</tr>
<tr>
<td>AA:PA 1:2</td>
<td>5</td>
<td>5.25</td>
<td>8.53</td>
</tr>
<tr>
<td>AA:PA 1:4</td>
<td>5</td>
<td>5.26</td>
<td>8.53</td>
</tr>
</tbody>
</table>

GIXD measurements for films with a higher mole fraction of PA at $\pi = 15$ and 30 mN/m are now described. Fig. 3-4 shows GIXD patterns for mixed monolayer films as a function of increasing PA content at surface pressures of $\pi = 15$ and $\pi = 30$ mN/m. The peak related to the PA component showed no significant difference from the values measured at lower surface pressure. However, the central peak position and width for the AA component at $\pi = 15$ mN/m in all compositions appeared at $q_{xy} = 1.45 \pm 0.02$ Å$^{-1}$ and $1.48 \pm 0.02$ Å$^{-1}$ but shifted to $1.53 \pm 0.01$ Å$^{-1}$ at 30 mN/m.
We interpret this as a change in the NN-tilted phase to an untilted phase (S phase) due to films being compressed (see Figure 3-S1 and 3-S2).\textsuperscript{11} The NNN-tilted phase was not observed at the surface pressures measured here, but can reasonably be expected to exist somewhere between $\pi = 15$ mN/m - 30 mN/m, consistent with studies by Peterson et al. on eicosanoic acid.\textsuperscript{23} Again, the large fraction of PA had no significant effect on the orientation of AA at these surface pressures, indicating that neither component appreciably affects the orientation of the other. GIXD parameters for these measurements are reported in detail in the Supporting Information.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3-4.png}
\caption{(A) GIXD patterns for mixed monolayers of mole fraction of PA ($\pi = 15$ mN/m). (B) GIXD patterns for mixed monolayers of mole fraction of PA ($\pi = 30$ mN/m).}
\end{figure}

GIXD measurements for films with a higher mole fraction of AA at $\pi = 5$ mN/m are now described. As seen previously, films of all compositions measured at $\pi = 5$ mN/m exhibited phase-separation, as shown in Fig 3-5. Distinct Bragg peaks characteristic of PA and AA appearing at different in-plane $q_{xy}$ were observed. Bragg peaks for PA appeared at $q_{xy} = 1.25 \pm 0.01$ Å$^{-1}$, with $q_z$ peaking at 0 Å$^{-1}$. A hexagonal lattice $a = b = 5.8$ Å, corresponding to a unit cell area per chain of 29 Å$^2$ was indexed for the PA with an average $L_{xy}$ of ~ 211 Å for all mixed compositions (for details see Table 3-2). Also, two Bragg peaks at $q_{xy} = 1.40 \pm 0.01$ Å$^{-1}$ and 1.47 \pm 0.01 Å$^{-1}$ indicating two low-order reflections with a centered rectangular lattice, $a = 5.3$ Å and $b = 8.5$ Å corresponding to a unit cell area per two hydrocarbon chains of 45.1 Å$^2$. This was typical for the AA component within all mixed composition films, with an average $L_{11}$ of ~ 111 Å and $L_{02}$ of ~ 206 Å (summarized in Table 3-2). Thus, AA molecules tilt toward the nearest-neighbor in the L$_2$ phase.
with tilt angle of ~ 30° in all mixed composition films (see Table 3-2 and Figure 3-5B). GIXD parameters for PA and AA in the mixed sytem as composition of AA was increased at $\pi = 5 \text{ mN/m}$ are summarized in Table 3-2.

The contour plot of the intensity along $q_{xy}$ versus intensity along the $q_z$ (Fig. 3-5B) for pure AA is indicative of a centered rectangular unit cell. The AA monolayer packs in a centered rectangular cell with tilts in NN. Contour maps for the mixed films (Fig. 3-5C, 3-5D & 3-5E) indicates a hexagonal unit cell for the PA and a centered rectangular unit cell with NN tilt in the L2 phase for the AA. Thus, the PA packs hexagonally but the AA packs in a centered rectangular cell with tilts in NN. The centered rectangular cell for the AA further stretches towards NN due to molecular tilts and molecular backbone plane ordering being less rigid compared to PA.
Figure 3-5: (A) GIXD patterns for mixed monolayers of mole fraction of AA ($\pi = 5 \text{ mN/m}$). (B) to (E) Contour plot showing intensity along $q_z$ versus intensity along $q_{xy}$ for pure AA, 1:1, 2:1 and 4:1 AA:PA mixed films.
Table 3-2. GIXD data for mixed monolayer films at $\pi = 5$ mN/m.

<table>
<thead>
<tr>
<th>Composition</th>
<th>$\pi$ (mN/m)</th>
<th>In-plane Bragg Peaks</th>
<th>Out-of-plane Bragg Rods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lattice Parameters</td>
<td>Unit Cell Area / Chain (Å$^2$)</td>
<td>Lxy (Å)</td>
</tr>
<tr>
<td>Pure AA</td>
<td>5</td>
<td>5.28 8.54 90 19.74 142 224 0.71 30.8</td>
<td></td>
</tr>
<tr>
<td>AA:PA 1:1</td>
<td>5</td>
<td>5.78 5.78 120 28.94 306 - 0 0</td>
<td></td>
</tr>
<tr>
<td>AA:PA 2:1</td>
<td>5</td>
<td>5.82 5.82 120 29.32 128 - 0 0</td>
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</tr>
<tr>
<td>AA:PA 4:1</td>
<td>5</td>
<td>5.79 5.79 120 29.03 200 - 0 0</td>
<td></td>
</tr>
<tr>
<td>AA:PA 1:1</td>
<td>5</td>
<td>5.26 8.54 90 19.38 98 207 0.70 30.3</td>
<td></td>
</tr>
<tr>
<td>AA:PA 2:1</td>
<td>5</td>
<td>5.19 8.51 90 19.46 138 191 0.65 28.2</td>
<td></td>
</tr>
<tr>
<td>AA:PA 4:1</td>
<td>5</td>
<td>5.26 8.53 90 19.60 96 219 0.67 29.2</td>
<td></td>
</tr>
</tbody>
</table>

Finally, GIXD measurements for films with a higher mole fraction of AA at $\pi = 15$ and 30 mN/m are described. Fig. 3-6 shows GIXD results for mixed films of high AA content for $\pi = 15$ and 30 mN/m. Films with higher mole fractions of AA at $\pi = 15$ and 30 mN/m showed no appreciable difference in diffraction patterns from those reported above; data is summarized in Supporting Information. Again, the peak related to the PA component showed no significant difference from the values measured at lower pressure. Also, the AA peak at 15 mN/m in all compositions appeared
at $q_{xy} = 1.46 \pm 0.01$ Å$^{-1}$ and $1.49 \pm 0.01$ Å$^{-1}$ but shifted to $1.53 \pm 0.01$ Å$^{-1}$ at 30 mN/m. This again shows change in the NN-tilted phase to an untilted phase (S phase) due to films being compressed (see Figure 3-S3 and 3-S4). The NNN-tilted phase was again not observed but as noted above, can reasonably be expected to lie somewhere between $\pi = 15$ mN/m - 30 mN/m. The effect of increasing surface pressure on the ordering of AA molecules is observed here. When the surface pressure reaches $\pi = 30$ mN/m there was no longer a significant degree of molecular tilt to either AA or the PA components. Furthermore, higher compositions show no discernible tilt in AA at $\pi = 30$ mN/m since films become compact and organized, consistent with studies by Kjaer et al. GIXD parameters for PA and AA in the mixed system as composition of AA for $\pi = 15$ and 30 mN/m are reported as Supporting Information.

![GIXD patterns](image)

**Figure 3-6:** (A) GIXD patterns for mixed monolayers of mole fraction of AA ($\pi = 15$ mN/m). (B) GIXD patterns for mixed monolayers of mole fraction of AA ($\pi = 30$ mN/m).

### 3.4.2 X-ray reflectivity

X-ray reflectivity measurements were carried out as a complement to GIXD measurements in elucidating microscopic structural details for the mixed films at the air-water interface. Fig. 3-7 shows normalized electron density profiles of pure PA and AA extracted from X-ray reflectivity data over the surface pressures described in the preceding section. The electron density profile (EDP) was obtained from a model constructed from a stack of two homogeneous slabs describing the head group and the tail chains of the lipid molecule, with details shown in Supporting Information. More complex (three slab) models did not show significant improvements in
goodness-of-fit parameters, and so a simple two slab model was deemed sufficient to model these systems.

**Figure 3-7:** (A) Normalized electron density profiles of pure PA at $\pi = 5$, 15 and 30 mN/m. (B) Normalized electron density profiles of pure AA at $\pi = 5$, 15 and 30 mN/m.

The difference in EDP of PA and AA indicates that AA and PA molecules orient differently at the air-water interface. The tail layer thickness for pure PA was $16.1 \pm 0.1$ Å at $\pi = 5$ mN/m, $16.1 \pm 0.2$ Å at $\pi = 15$ mN/m and $17.1 \pm 0.4$ Å at $\pi = 30$ mN/m, consistent with the value reported by Fukuto et al.\(^{16}\) These values are in excellent agreement with the untilted orientation observed in GIXD. DFT calculations of PA tail length (in vacuum using Spartan ‘14, V.1.1.4) gave values of 16.8 Å, which supports the proposal that PA molecules are vertically oriented, fully-extended and highly ordered at the air-water interface. For pure AA monolayers, experimentally measured tail layer thicknesses were $19.3 \pm 0.1$, $19.7 \pm 0.1$ and $21.4 \pm 0.1$ Å at $\pi = 5$, 15 and 30 mN/m respectively, while calculated tail lengths (calculated by DFT in vacuum using Spartan ‘14, V.1.1.4) were of 24.2 Å. Comparison of these values indicates that AA adopts significant tilts at lower surface pressures, consistent with the GIXD measurements for pure AA films. The molecular tilt angle ($t$) was estimated from the relationship: $\cos (t) = L_T/L_{CH}$ where $L_T$ is the tail layer thickness and $L_{CH}$ the maximal length of a saturated CH chain. The latter terms were calculated using the expression: $L_{CH} = (n + 9/8)\cdot 1.265$ Å, where $n$ is the number of CH\(_2\) groups and the CH\(_3\) terminal group contributing the 9/8 value.\(^{14}\) Using this approach, tilt angles of 37.1°, 35.5° and 27.8° from the surface normal were measured at $\pi = 5$, 15 and 30 mN/m respectively.
Figure 3-8: Normalized X-ray reflectivity for mixed monolayers of mole fraction of PA at $\pi = 5$, 15 and 30 mN/m. Corresponding normalized electron density profiles are shown on the right.
Figures 3-8 and 3-9 show the normalized X-ray reflectivity (R/R_F; reflectivity normalized to the Fresnel reflectivity) as a function of the vertical scattering vector q_z for the mixed film systems at all surface pressures as a function of composition, along with the corresponding EDPs. Reflectivity plots reveal the presence of Kiessig fringes typical of monolayer systems and generally displays an increase in R/R_F as the fraction of PA in the monolayers was increased. We will not attempt to analyze all of the various changes in film thickness that can be seen in the data (see Supporting Information for tabulated results), but rather will describe and summarize the key observations from these measurements. At the lowest surface pressures, tail layer thickness increased from 3.8 ± 0.1 Å to 16.9 ± 0.3 Å as the fraction of PA was increased (see Supporting Information). The increase in tail layer thickness can reasonably be interpreted as a simple concentration effect; the average film thickness will increase as the relative contribution to the reflectivity signal from the tilted AA molecules is dwarfed by the contribution from the vertically-oriented PA. The effect is lessened at higher surface pressures, as the degree of tilt in the AA component is significantly reduced.

Intriguingly, as the relative proportion of PA decreases and the AA becomes the predominant component in the mixed films, the relative thickness of the head group increases. For example, for π = 15 mN/m, the head group thickness changes from 3.9 ± 0.1 Å to 16.1 ± 0.2 Å as the PA:AA ratio shifts from 4PA:1AA to 1PA:4AA; similar effects can be observed at other surface pressures. We interpret this result as meaning that at these surface pressures, the highly-tilted AA molecules effectively have a higher electron density closer to the subphase surface, thus effectively increasing the head group thickness during the fitting routines, and this effect makes an increasingly greater contribution to the effective overall film thickness as the relative fraction of AA in the monolayer film increases. More sophisticated models for reflectivity that account for phase-separation and the relative fraction of the two phase-separated components might be useful to more fully clarify the influence of the relative weight of the two discrete components here, but modelling of this type is beyond the scope of the present work and will need to be rigorously explored elsewhere.
Figure 3-9: Normalized X-ray reflectivity for mixed monolayers of mole fraction of AA at $\pi = 5$, 15 and 30 mN/m. Corresponding normalized electron density profiles are shown on the right.
It is worth considering the interpretation of these cumulative GIXD and XR data in the context of the micron-scale film morphology reported previously\(^2\)\(^-\)\(^8\) and shown in Figure 3-1. The PA component, either alone or in mixtures, forms a rigid, highly-crystalline matrix with a hexagonal lattice under all film conditions explored. The AA component of the film is tilted at all but the highest surface pressures. For mixed films that are subjected to compression, the hexagonal PA crystallites at the air-water interface will be forced together; there is no change in the crystal packing of the PA component with compression and thus compression forces crystallites into closer proximity, squeezing the phase-separated deposits of AA into vacant space between PA crystallites. We can reasonably speculate that the polygonal domains observed at the micron scale, along with their different reflectivity in the BAM images are a direct consequence of this; as the rigid, hexagonal lattice PA crystallites pack increasingly closely together, the edges and interfaces between them and the phase-separated AA takes on sharp edges and asperities that are consistent with the hexagonal lattice of the PA. The micron scale polygonal structures are an entirely reasonable outcome of the highly-crystalline, hexagonal symmetry films on the air-water interface. Finally, the AA component retains some of its tilt relative to the subphase until the highest compression pressures, hence the variations in reflectivity observed in the BAM images.

3.5 Conclusions

We demonstrate the crystallographic structure of binary mixed monolayers comprised of the hydrogenated fatty acid, AA, and the perfluorinated fatty acid, PA, at the air-water interface using X-ray-based scattering methods. In agreement with previously-reported micron-scale imaging methods, the X-ray scattering measurements indicate that phase-separation of the two surfactants occurs over a wide range of surface pressures and film compositions, with the lattice structures of each individual component remaining unaffected by the other. The perfluorinated component aligned vertically to the air-water interface under all surface pressures and mixed film compositions, likely driven by the combination of perfluorocarbon chain rigidity and the low work of cohesion with the water subphase. The PA packed into a hexagonal lattice, whose lattice parameters were invariant with film conditions. The hydrogenated component showed a more conventional change in molecular tilt with surface pressure, with the AA tilting considerably from vertical alignment at lower pressures and approaching normal with increased compression. These results with internally consistent between GIXD and XR measurements. One the basis of these
measurements and the micron-scale imaging measurements reported in previous studies, we can rationalize the existence of the polygonal, micron-sized domains comprised of AA as resulting from the hydrogenated component being embedded in a crystalline, hexagonal symmetric matrix of the PA component, with the AA maintaining significant tilt to the subphase under modest surface pressures.

3.6 Acknowledgements

Financial support for this work has been provided by the Natural Sciences and Engineering Research Council, the Canadian Foundation for Innovation and the University of Saskatchewan. This research used resources of the Advance Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357.

3.7 Supporting information

GIXD Fitting Parameters

GIXD fitting parameters were obtained from fitting a Lorentzian function to the in-plane $q_{xy}$ diffraction peaks. Extracting the maximum $q_{xy}$ from the fit, d-spacing was obtained according to the equation $d = (2\pi)/q_{xy}$ while $q_{xy}$ was determined using equation (3.51):

$$q_{xy} = \frac{(2\pi/\lambda)}{\left[\cos^2(\alpha_i) + \cos^2(\alpha_f) - 2\cos(\alpha_i)\cos(\alpha_f)\cos(2\Theta_{xy})\right]^{1/2} \approx \frac{(4\pi/\lambda)}{\sin(2\Theta_{xy}/2)} \quad (3.51)$$

Thus, lattice parameters (a, b, $\gamma$) were obtained using the d-spacing of the diffraction peaks. Peaks for two low-order reflections were indexed as a distorted hexagonal lattice (centered rectangular lattice) having degenerate reflections ([1,1]$_r$, [1,1]$_r$) and a non-degenerate reflection ([0,2]$_r$). Single diffracted peaks were indexed as a hexagonal lattice having degenerate reflections {1,0}, {0,1}, and {1,1}. The unit cell area per chain was then calculated from the lattice parameters and the coherence length ($L_{xy}$) from the Scherrer relation given by equation (3.52):

$$L_{xy} = \frac{(0.9 \times 2\pi)}{\left(FWHM_{\text{measured}}^2 - FWHM_{\text{res}}^2\right)^{1/2}} \quad (3.52)$$

Where ‘FWHM$_{\text{measured}}$’ is the full width at half maximum measured for the fitted peak and ‘FWHM$_{\text{res}}$’ is the slit-limited instrumental resolution, with a value of $9.58 \times 10^{-3}$ Å$^{-1}$. Bragg rods were analysed from the diffracted X-ray beam intensity along the vertical momentum transfer component ($q_z$) given by equation (3.53):
\[ q_z = (2\pi/\lambda) \left[ \sin (\alpha_i) + \sin (\alpha_f) \right] \approx (2\pi/\lambda) \sin (\alpha_f) \quad (3.33) \]

The molecular chain tilt angle in the NN direction was calculated according to equation (3.34):

\[ q_z^{hk} = q_{xy}^{hk} \cos (\Psi^{hk}) \tan (t) \quad (3.34) \]

where \( t \) is the molecular chain tilt angle, \( q_{xy} \) and \( q_z \) are the in-plane and out-of-plane coordinates of the peak in the \( q_{xy}-q_z \) contour plot and \( \Psi \) is the tilt azimuth between the tilt direction projected onto the \( xy \)-plane and the corresponding reciprocal lattice vector. Uncertainties in \( q_{xy} \) were estimated from uncertainties determined in the peak fitting and these were used in conjunction with uncertainty propagation calculations to estimate uncertainties in lattice parameters and unit cell area per chain.
Table 3-S1. GIXD data for PA and AA in the mixed systems as PA fraction is increased relative to AA at 15 mN/m.

<table>
<thead>
<tr>
<th>Composition</th>
<th>$\pi$ (mN/m)</th>
<th>In-plane Bragg Peaks</th>
<th>Out-of-plane Bragg Rods</th>
</tr>
</thead>
<tbody>
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<td>Lattice Parameters</td>
<td>Unit Cell Area / Chain (Å²)</td>
<td>$L_{qz}$ (Å⁻¹)</td>
</tr>
<tr>
<td></td>
<td>a (Å) (± 0.01)</td>
<td>b (Å) (± 0.01)</td>
<td>γ (°)</td>
</tr>
<tr>
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<td>5.78</td>
</tr>
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<td>5.78</td>
</tr>
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<td>5.76</td>
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<td>8.49</td>
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Table 3-S2. GIXD data for PA and AA in the mixed systems as PA fraction is increased relative to AA at 30 mN/m.

<table>
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<th>Composition</th>
<th>$\pi$ (mN/m)</th>
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<th>Out-of-plane Bragg Rods</th>
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</thead>
<tbody>
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<td>$\pi$ (mN/m)</td>
<td>Lattice Parameters</td>
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<td>19.78</td>
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Table 3-S3. GIXD data for PA and AA in the mixed systems as AA fraction is increased relative to PA at 15 mN/m.

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<th>Unit Cell Area / Chain (Å$^2$) (± 0.02)</th>
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<td></td>
<td>Lattice Parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>a (Å) (± 0.01)</td>
<td>b (Å) (± 0.01)</td>
<td>$\gamma$ (°)</td>
</tr>
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<td>8.45</td>
<td>90</td>
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<tr>
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<td>5.78</td>
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<td>120</td>
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<td>5.00</td>
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<td>90</td>
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</table>
Table 3-S4. GIXD data for PA and AA in the mixed systems as AA fraction is increased relative to PA at 30 mN/m.

<table>
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<th>Composition</th>
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<th>In-plane Bragg Peaks</th>
<th>Out-of-plane Bragg Rods</th>
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</thead>
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<td></td>
<td>Lattice Parameters</td>
<td>Unit Cell Area / Chain (Å²)</td>
<td>$L_{xy}$ (Å)</td>
</tr>
<tr>
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<td>0 0</td>
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<td>30 AA:PA 2:1</td>
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<td>30 AA:PA 4:1</td>
<td>4.76 4.76 120 19.60 101</td>
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Figure 3-S1: Contour plot showing intensity along $q_z$ versus intensity along $q_{xy}$ for pure PA, 1:1, 1:2 and 1:4 AA:PA mixed films at 15 mN/m.
Figure 3-S2: Contour plot showing intensity along $q_z$ versus intensity along $q_{xy}$ for pure PA, 1:1, 1:2 and 1:4 AA:PA mixed films at 30 mN/m.
Figure 3-S3: Contour plot showing intensity along $q_z$ versus intensity along $q_{xy}$ for pure PA, 1:1, 2:1 and 4:1 AA:PA mixed films at 15 mN/m.
**Figure 3-S4:** Contour plot showing intensity along $q_z$ versus intensity along $q_{xy}$ for pure PA, 1:1, 2:1 and 4:1 AA:PA mixed films at 30 mN/m.
Figure 3-S5: Plot showing % of total diffraction signal contributed from PA as a function of mole fraction of PA extracted from GIXD contour plots. The % Signal was determined by integrating the peak associated with the PA component alone and dividing by the sum of the integrated signal from PA with the integrated signal from AA. The dotted line is the predicted behavior for pure additive behavior (eg. % Signal from PA directly proportional to $\chi_{PA}$).

**XR Fitting Parameters**

Specular X-Ray reflectivity (XR) is possible when the incident and reflected beam angles are the same ($\alpha_i = \alpha_r = \alpha$) and $2\theta_{xy} = 0$. Thus, vertical momentum transfer component ($q_z$) is given by equation (3.S5):\(^{14}\)

$$q_z = \left(\frac{2\pi}{\lambda}\right) \sin (\alpha) \quad (3.55)$$

The X-ray reflectivity, $R(q_z)$, according to the Born approximation is given by (3.S6):\(^{14}\)

$$R(q_z) = R_F(q_z) \left| \left( \rho_{\text{sub}} \right)^{-1} \int (dp(z)/dz) \exp(i q_z z) \, dz \right|^2 \quad (3.56)$$

Where the Fresnel reflectivity for a flawless sharp interface is represented by $R_F(q_z)$, $\rho(z)$ is the electron density and $\rho_{\text{sub}}$ is the electron density of the subphase.\(^{14}\) However, $R(q_z)$ was obtained
from the ratio of the specularly scattered intensity to the incident X-ray beam intensity and the reflectivity data plotted as $R/R_F$ as a function of $q_z$. The XR fitting software (StochFit 1.7.0) and procedure described by Danauskas et al.\textsuperscript{27} was used to generate stochastic model-independent and model-dependent fits to the XR data. In stochfit, both the model-independent and model-dependent fits depend on a calculated reflectivity based on Parratt’s recursive method.\textsuperscript{27} Here, the measured reflectivity is calculated from the Parratt’s method based on the optical theory that, for an exact wave equation for an s-polarized plane wave at an ideal surface, wavevector ($q$) values are greater than the critical wavevector ($q_c$, see equation 3.S7) for total internal reflection.

$$q_c = 4 \left[ \pi (SLD_{\text{sub}} - SLD_{\text{sup}}) \right]^{1/2} \quad (3.S7)$$

$SLD_{\text{sub}}$ and $SLD_{\text{sup}}$ are the scattering length density for the subphase and superphase respectively. The scattering length density may be obtained according to equation (3.S8):

$$SLD = \frac{r_e N_A \rho}{MW} \sum_{i=1}^{N} f_{1i} \approx \sum_{i=1}^{N} \frac{Z_i r_e}{V_m} \quad (3.S8)$$

where $N$ is the number of atoms, $N_A$ the Avogadro’s number, $Z_i$ the number of electrons of each atom $i$, $r_e$ the classical radius of the electron, $V_m$ the molecular volume, $\rho$ the bulk density, and $f_{1i}$ the real anomalous scattering factor. If we consider the normal component of the wavevector at the interface for a given $q$, the superphase refractive index, Fresnel coefficient and phase factor, the amplitude reflectivity for the $i$th layer may calculated according to equation (3.S9):

$$R_i = a_i(R_{i+1} + f_i)/(R_{i+1}f_i + 1) \quad (3.S9)$$

Assuming a superphase boundary condition of no reflected wave and an infinite thickness in the superphase and subphase layers. The total measurable reflectivity ($R$) after recursion may be now obtained by the complex modulus of the amplitude reflectivity at the interface of the first layer and superphase according to equation (3.S10):

$$R = R_0^2 \quad (3.S10)$$

From here, the model independent fit can now be used to generate an electron density profile (EDP) on the basis of the estimated sample thickness and average SLD of the film sample. The generation of the EDP is done by taking a number of boxes of fixed thickness ($\sim 0.5$ Å per box) and a
smoothing parameter ($\sigma$), dispersion parameter ($\delta$), together with the individual box contributions smeared by a Gaussian error function (erf) given by equation (3.511):

$$\delta(z) = \delta_0 + \sum_{k=0}^{NB} \left( \frac{\delta_{k+1} - \delta_k}{2} \right) \left[ 1 + \text{erf} \left( \frac{z - kB_t}{2\sigma} \right) \right] \quad (3.511)$$

where, NB is the total number of boxes, $B_t$ the box thickness in angstroms, and $z$ the spatial parameter oriented perpendicular the surface. The reflectivity is then calculated by iterating through each of the EDP points treated as a layer, considering the absorption of the films. Now, a model dependent fit is applied to the model independent EDP to extract physical meaning. The base of model dependent EDP is fitted using a Levenberg-Marquardt nonlinear least-square fit. In Brief, XR parameters such as layer thickness (d), electron density ($\rho/\rho_{H2O}$), interfacial roughness ($\sigma$) and distinct inter-slab roughness ($\sigma_i$) of the lipid molecule are varied to find the best overall fit for the data.26,27 The model parameters that provided the best fit were ultimately selected based upon chi-squared values. Fitting parameters are provided in the tables below.
**Table 3-S5.** XR fitting results for pure PA at 5 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{sub}$</th>
<th>d [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>Head</td>
<td>1.687 ± 0.131</td>
<td>4.0 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>Tail</td>
<td>1.910 ± 0.102</td>
<td>16.1 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>3.6 ± 0.1</td>
</tr>
</tbody>
</table>

**Table 3-S6.** XR fitting results for pure PA at 15 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{sub}$</th>
<th>d [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>Head</td>
<td>1.759 ± 0.152</td>
<td>3.9 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>Tail</td>
<td>1.937 ± 0.127</td>
<td>16.1 ± 0.2</td>
<td>2.9 ± 0.1</td>
<td>3.6 ± 0.1</td>
</tr>
</tbody>
</table>

**Table 3-S7.** XR fitting results for pure PA at 30 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{sub}$</th>
<th>d [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
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<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>Head</td>
<td>1.621 ± 0.135</td>
<td>3.4 ± 0.4</td>
<td>3.0 ± 0.2</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>Tail</td>
<td>1.924 ± 0.118</td>
<td>17.1 ± 0.4</td>
<td>3.0 ± 0.1</td>
<td>3.6 ± 0.2</td>
</tr>
</tbody>
</table>
Table 3-S8. XR fitting results for pure AA at 5 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{\text{sub}}$</th>
<th>$d$ [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Head</td>
<td>1.164 ± 0.183</td>
<td>5.9 ± 0.1</td>
<td>2.3 ± 0.2</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Tail</td>
<td>0.962 ± 0.160</td>
<td>19.3 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>2.6 ± 0.2</td>
</tr>
</tbody>
</table>

Table 3-S9. XR fitting results for pure AA at 15 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{\text{sub}}$</th>
<th>$d$ [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Head</td>
<td>1.131 ± 0.112</td>
<td>7.1 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Tail</td>
<td>0.973 ± 0.107</td>
<td>19.7 ± 0.1</td>
<td>3.0 ± 0.1</td>
<td>2.2 ± 0.2</td>
</tr>
</tbody>
</table>

Table 3-S10. XR fitting results for pure AA at 30 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{\text{sub}}$</th>
<th>$d$ [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.6 ± 0.1</td>
</tr>
<tr>
<td>Head</td>
<td>1.296 ± 0.121</td>
<td>3.9 ± 0.1</td>
<td>4.0 ± 0.1</td>
<td>4.6 ± 0.1</td>
</tr>
<tr>
<td>Tail</td>
<td>0.950 ± 0.112</td>
<td>21.4 ± 0.1</td>
<td>3.2 ± 0.1</td>
<td>4.6 ± 0.1</td>
</tr>
</tbody>
</table>
Table 3-S11. XR fitting results for PA:AA 1:1 composition at 5 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{\text{sub}}$</th>
<th>$d$ [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>Head</td>
<td>1.483 ± 0.132</td>
<td>17.0 ± 0.1</td>
<td>3.7 ± 0.1</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>Tail</td>
<td>0.890 ± 0.101</td>
<td>3.8 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>3.8 ± 0.1</td>
</tr>
</tbody>
</table>

Table 3-S12. XR fitting results for PA:AA 2:1 composition at 5 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{\text{sub}}$</th>
<th>$d$ [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>Head</td>
<td>1.423 ± 0.186</td>
<td>3.3 ± 1.0</td>
<td>2.2 ± 0.2</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>Tail</td>
<td>1.631 ± 0.126</td>
<td>16.9 ± 0.3</td>
<td>2.9 ± 0.1</td>
<td>3.0 ± 0.6</td>
</tr>
</tbody>
</table>

Table 3-S13. XR fitting results for PA:AA 4:1 composition at 5 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{\text{sub}}$</th>
<th>$d$ [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Head</td>
<td>1.423 ± 0.962</td>
<td>4.3 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Tail</td>
<td>1.688 ± 0.201</td>
<td>16.1 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
</tbody>
</table>
Table 3-S14. XR fitting results for PA:AA 1:1 composition at 15 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>ρ / ρ_sub</th>
<th>d [Å]</th>
<th>σ_i [Å]</th>
<th>σ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.5 ± 0.1</td>
</tr>
<tr>
<td>Head</td>
<td>1.688 ± 0.102</td>
<td>10.5 ± 0.1</td>
<td>4.3 ± 0.2</td>
<td>5.5 ± 0.1</td>
</tr>
<tr>
<td>Tail</td>
<td>1.110 ± 0.123</td>
<td>9.3 ± 0.1</td>
<td>2.9 ± 0.1</td>
<td>5.5 ± 0.1</td>
</tr>
</tbody>
</table>

Table 3-S15. XR fitting results for PA:AA 2:1 composition at 15 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>ρ / ρ_sub</th>
<th>d [Å]</th>
<th>σ_i [Å]</th>
<th>σ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td>Head</td>
<td>2.037 ± 0.119</td>
<td>4.9 ± 0.9</td>
<td>1.4 ± 0.4</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td>Tail</td>
<td>1.716 ± 0.137</td>
<td>12.1 ± 0.1</td>
<td>3.5 ± 0.1</td>
<td>5.4 ± 0.3</td>
</tr>
</tbody>
</table>

Table 3-S16. XR fitting results for PA:AA 4:1 composition at 15 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>ρ / ρ_sub</th>
<th>d [Å]</th>
<th>σ_i [Å]</th>
<th>σ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>Head</td>
<td>1.487 ± 0.293</td>
<td>3.9 ± 0.7</td>
<td>1.6 ± 0.5</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>Tail</td>
<td>1.795 ± 0.115</td>
<td>16.6 ± 0.5</td>
<td>2.9 ± 0.1</td>
<td>3.1 ± 0.4</td>
</tr>
</tbody>
</table>
Table 3-S17. XR fitting results for PA:AA 1:1 composition at 30 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{\text{sub}}$</th>
<th>d [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.9 ± 0.1</td>
</tr>
<tr>
<td>Head</td>
<td>2.204 ± 0.112</td>
<td>4.1 ± 0.1</td>
<td>3.8 ± 0.2</td>
<td>8.9 ± 0.1</td>
</tr>
<tr>
<td>Tail</td>
<td>1.291 ± 0.104</td>
<td>9.8 ± 0.1</td>
<td>3.1 ± 0.1</td>
<td>8.9 ± 0.1</td>
</tr>
</tbody>
</table>

Table 3-S18. XR fitting results for PA:AA 2:1 composition at 30 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{\text{sub}}$</th>
<th>d [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>Head</td>
<td>2.169 ± 0.187</td>
<td>3.6 ± 1.1</td>
<td>2.1 ± 0.3</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>Tail</td>
<td>1.703 ± 0.061</td>
<td>12.1 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>5.6 ± 0.2</td>
</tr>
</tbody>
</table>

Table 3-S19. XR fitting results for PA:AA 4:1 composition at 30 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{\text{sub}}$</th>
<th>d [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
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<td>-</td>
<td>-</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Head</td>
<td>1.439 ± 0.109</td>
<td>4.7 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Tail</td>
<td>1.774 ± 0.105</td>
<td>16.9 ± 0.1</td>
<td>3.2 ± 0.1</td>
<td>3.5 ± 0.1</td>
</tr>
</tbody>
</table>
**Table 3-S20.** XR fitting results for PA:AA 1:2 composition at 5 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>ρ / ρ_{sub}</th>
<th>d [Å]</th>
<th>σ_i [Å]</th>
<th>σ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>Head</td>
<td>1.142 ± 0.001</td>
<td>7.1 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>Tail</td>
<td>1.303 ± 0.001</td>
<td>16.1 ± 0.1</td>
<td>2.8 ± 0.3</td>
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</tbody>
</table>

**Table 3-S21.** XR fitting results for PA:AA 1:4 composition at 5 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>ρ / ρ_{sub}</th>
<th>d [Å]</th>
<th>σ_i [Å]</th>
<th>σ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Head</td>
<td>1.102 ± 0.111</td>
<td>11.9 ± 0.2</td>
<td>2.9 ± 0.1</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Tail</td>
<td>1.178 ± 0.115</td>
<td>13.3 ± 0.2</td>
<td>1.8 ± 0.3</td>
<td>1.2 ± 0.3</td>
</tr>
</tbody>
</table>

**Table 3-S22.** XR fitting results for PA:AA 1:2 composition at 15 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>ρ / ρ_{sub}</th>
<th>d [Å]</th>
<th>σ_i [Å]</th>
<th>σ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.3 ± 1.7</td>
</tr>
<tr>
<td>Head</td>
<td>1.507 ± 0.164</td>
<td>10.9 ± 3.2</td>
<td>4.3 ± 1.2</td>
<td>7.3 ± 1.7</td>
</tr>
<tr>
<td>Tail</td>
<td>1.218 ± 0.138</td>
<td>9.9 ± 0.8</td>
<td>2.9 ± 0.1</td>
<td>7.3 ± 1.7</td>
</tr>
<tr>
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<td>$\sigma_i$ [Å]</td>
<td>$\sigma$ [Å]</td>
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<td>----------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Sub-phase</td>
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<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>Head</td>
<td>1.187 ± 0.115</td>
<td>16.1 ± 0.2</td>
<td>1.8 ± 0.5</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>Tail</td>
<td>1.136 ± 0.123</td>
<td>9.9 ± 0.2</td>
<td>3.1 ± 0.1</td>
<td>2.7 ± 0.1</td>
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</table>

Table 3-S24. XR fitting results for PA:AA 1:2 composition at 30 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{\text{sub}}$</th>
<th>$d$ [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.6 ± 0.2</td>
</tr>
<tr>
<td>Head</td>
<td>1.452 ± 0.161</td>
<td>12.5 ± 1.5</td>
<td>1.8 ± 0.1</td>
<td>10.6 ± 0.2</td>
</tr>
<tr>
<td>Tail</td>
<td>1.195 ± 0.141</td>
<td>11.3 ± 0.2</td>
<td>3.6 ± 0.1</td>
<td>10.6 ± 0.2</td>
</tr>
</tbody>
</table>

Table 3-S25. XR fitting results for PA:AA 1:4 composition at 30 mN/m.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\rho / \rho_{\text{sub}}$</th>
<th>$d$ [Å]</th>
<th>$\sigma_i$ [Å]</th>
<th>$\sigma$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>Head</td>
<td>1.188 ± 0.102</td>
<td>18.4 ± 0.1</td>
<td>3.6 ± 0.1</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>Tail</td>
<td>0.958 ± 0.113</td>
<td>11.1 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.6 ± 0.1</td>
</tr>
</tbody>
</table>
3.8 References


8. Qaqish, S. E.; Paige, M. F., *J. Colloid Interface Sci.* **2008**, 325, 290-293.


CHAPTER 4

MIXING BEHAVIOR IN BINARY ANIONIC GEMINI SURFACTANT-
PERFLUORINATED FATTY ACID LANGMUIR MONOLAYERS


In this work, molecular-level information about anionic gemini surfactant-perfluorotetradecanoic acid mixed monolayers was studied directly at the air-water interface using synchrotron-based grazing incidence X-ray diffraction and X-ray reflectivity. The anionic gemini surfactant, based on the N, N, N’, N’-diacyl-N,N’-diacetate ethylenediamine structure, is shown in Figure 4-1. The surfactant, referred to as Ace(12)-2-Ace(12), has a 12 carbon long alkyl chain tail group. Its mixing behavior in monolayer films with the fluorinated surfactant, PF, was explored because of the structural similarities with monomeric hydrogenated fatty acids. Here, the short tail length of Ace(12)-2-Ace(12) is expected to promote mixing with PF as has been seen in mixed films of short tail length monomeric hydrogenated surfactants with PF.

Ace(12)-2-Ace(12) monolayers did not diffract, indicating an amorphous state. Also when mixed with fluorinated surfactant, there was significant destabilization of the crystalline fluorinated surfactant component. In XR studies, the orientation of Ace(12)-2-Ace(12)-PF mixed films become more ordered when the PF component is increased. Details of the findings are shown in this chapter and correlated to other works performed at the micron scale using other characterization techniques.

In this article, the thesis author shares equal first authorship with Jeveria Rehman. The thesis author’s contribution in this work includes: Sample preparation for all synchrotron measurements, performing all synchrotron experiments, analyzing and interpreting all synchrotron results, and writing the synchrotron part of the manuscript. Measurements other than the synchrotron work were performed, analyzed, interpreted and written by Jeveria Rehman. Prof. Matthew F. Paige also assisted in the synchrotron measurement and further polished the manuscript by writing extensively. Dr. Amy L. Stevens also assisted in the synchrotron measurement. Dr. Wei Bu is the
beamline scientist who trained us on the instrument and gave insight on some of the interpretation of the results.

**Mixing behavior in binary anionic gemini surfactant – perfluorinated fatty acid Langmuir monolayers.**

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**4.1 Abstract**

The miscibility and film structure of mixed Langmuir monolayer films comprised of an anionic gemini N, N, N’, N’dialkyl-N,N’-diacetate ethylenediamine surfactant (Ace(12)-2-Ace(12)) with perfluorotetradecanoic acid (C₁₃F₂₇COOH; PF) has been investigated using a variety of thermodynamic and structural characterization methods. The two film components were found to be miscible in monolayers at the air-water interface over a range of compositions and at all but the lowest surface pressures, with attractive interactions occurring between the two components. While pure PF monolayers formed crystalline lattices with hexagonal symmetry, and with the surfactant tails oriented normal to the underlying water subphase, the pure gemini surfactant formed amorphous films with little tendency to orient at the subphase. In mixed films with mole ratios of PF:Ace(12)-2-Ace(12) < 2.5, the miscibility of the two components resulted in a nearly complete loss of crystallinity of the PF, though films at higher mole fractions of PF showed some residual crystallinity, albeit with lattice structures that were significantly different from that of pure PF. Miscibility and film structure in this mixed system is discussed in comparison with other mixed gemini surfactant systems in the literature, as well as binary mixtures of phospholipids or monomeric fatty acids with PF.
4.2 Introduction

Gemini surfactants, surface active agents comprised of two “monomer” surfactant molecules connected through their headgroups by a short organic linker, are compounds of great academic interest and hold considerable potential for industrial and biomedical applications.\(^1\)-\(^4\) Much of the interest in gemini surfactants is based on their improved technological performance over monomeric surfactants, including superior surface tension lowering abilities, extremely low critical micelle concentrations and their ability to form a variety of complex solution-phase aggregate structures. The latter are of particular interest in drug-delivery formulations and other in vitro applications where there is demand for a high degree of control over aggregate properties while ensuring minimal concentrations of reagents are introduced into biosystems.\(^2\) Significant progress in regulating and understanding structure-function relationships in gemini surfactants as well as diverse applications of these molecules have been made in recent years and have been reviewed by a number of authors.\(^5\)-\(^10\)

To date, the majority of surfactants that have been investigated in depth are cationic surfactants, typically variants of the alkanediyl-\(\alpha,\omega\)-bis(alkyldimethylammonium bromide) family.\(^11\)-\(^13\) However, there are several examples of anionic gemini surfactants to be found in the literature. Of particular recent interest is a class of anionic gemini surfactants based on the N, N, N’, N’- dialkyl-N,N’-diacetate ethylenediamine structure; these compounds, first introduced by Wattebled et al.\(^14\) and further developed by Lv et al.\(^15\)-\(^17\), can be prepared in high-purity and yield through a facile and flexible synthesis. The chemical structure of one member of this class of compounds, referred to as Ace(12)-2-Ace(12) using Lv et al.’s nomenclature, is shown in Figure 4-1. This compound was demonstrated by Wattebled et al.\(^14\) to form very small (~3 nm) micellar aggregates over a wide range of pHs, and required notably high concentrations of aqueous Ca\(^{2+}\) before undergoing precipitation. Further, this compound, as well as other variants of its kind, exhibit a reversible, pH induced aggregation, which has been used for controlled vesicle and micelle formation, as well as to toggle a dodecane-in-water emulsion between O/W to W/O forms. The aggregation properties of the molecules could be further refined by introducing asymmetries in the surfactant tail lengths.\(^15\) While the very few studies of this class of compound have been carried out in bulk aqueous solution, our group has recently reported a surfactant monolayer (Langmuir and Langmuir Blodgett film) investigation of Ace(12)-2-Ace(12), and observed that
the compound forms stable monolayers at the air-water interface, and that binding of subphase Na\(^+\) and Ca\(^{2+}\) results in substantive monolayer film expansion, accompanied by the formation of multi-molecular aggregates.\(^{18}\)

\[ \text{Figure 4-1: Chemical structure of Ace(12)-2-Ace(12).} \]

While the solution-phase and monolayer properties of the pure Ace(n)-m-Ace(n) compounds are important, there is growing appreciation for the importance of the physical chemical properties of mixed surfactant systems, particularly those containing a gemini surfactant. For example, surfactant mixtures containing both a cationic and anionic surfactant frequently exhibit so-called synergism, with the mixed systems often having superior surface tension lowering properties, lower CMCs and a greater propensity to form desirable complex structures in comparison with the individual pure components (see Khan et al.\(^{19}\) for a recent review). In many systems, the basis of these synergistic effects are related to miscibility or phase-separation of the various components in micelles or films. Investigations of aggregate structures and thermodynamics of miscibility in mixed gemini surfactant systems are not particularly abundant in the literature, though there are a number of key observations in this area. The majority of work to date has been directed towards cationic-anionic mixtures and to the best of our knowledge, there are no published reports investigating binary mixed gemini monolayers involving two anionic species. Wang and Marques\(^{20}\), as part of a larger investigation of the thermal properties of phase transitions in mixed alkanediyl-\(\alpha,\omega\)-bis(alkyldimethylammonium bromide) – sodium dodecyl sulphate monolayers, measured compression isotherms for various geminis and reported the formation of stable monolayers for the mixtures over a range of temperatures. The authors also proposed that the strong electrostatic interactions between cationic and anionic surfactant molecules led to significant layer compaction. In another investigation of cationic-anionic mixed monolayers, Li
et al.\textsuperscript{21} explored the influence of gemini spacer length \(s\) in the \(\text{C}_{18}\text{H}_{37}(\text{CH}_3)_2\text{N}^+-(\text{CH}_2)_s-\text{N}^+(\text{CH}_3)_2\text{C}_{18}\text{H}_{37}\) system in monolayers comprised of the gemini mixed with anionic stearic acid, and observed either complete miscibility \((s \leq 8)\) or partial miscibility \((s > 8)\) for the mixed films. For the longest linkers, the degree of miscibility was a strong function of the degree of ionization of the stearic acid headgroup, with higher miscibility observed when the stearic acid underwent complete ionization. For a closely-related set of geminis, Li et al.\textsuperscript{22} have investigated miscibility and aggregate structure in mixed gemini - alkylglucoside (or maltoside) surfactant films. The authors reported that the mixed monolayers adsorbed more strongly to the air-water interface than mixtures containing simple monomeric surfactants, and that the interactions between film components was stronger with the alkylglucoside than with the maltoside-based surfactant.

Our research group has a long-standing interest in understanding and controlling phase-separation in monolayer surfactant films comprised of mixtures of hydrogenated and perfluorinated surfactants.\textsuperscript{23-29} For simple binary monolayer mixtures of hydrogenated and perfluorinated fatty acids, film components are typically immiscible and undergo phase-separation to yield highly-structured films. Micron-scale film morphology of the phase-separated monolayers is broadly-controlled by an interplay between dipole-dipole repulsion of headgroups and line tension effects at phase-boundaries, a general principle first elucidated by McConnell in single-component phospholipid monolayers.\textsuperscript{30} By keeping the headgroup repulsion fixed through controlled subphase pH and varying the line tension through selection of surfactants with different tail lengths, the micron-scale morphology of the films can be modulated from compact, polygonal domains (line tension dominant; longer hydrocarbon tail groups) to extended, linear domains (dipole repulsion dominant; shorter hydrocarbon tail groups).\textsuperscript{26,28-29,31-32} Thermodynamic measurements at the air-water interface have also allowed for careful determination of Gibbs excess energies of mixing and other key miscibility properties of the mixtures.

Gemini surfactants provide an intriguing chemical system to further explore miscibility effects in mixed hydrocarbon-perfluorocarbon systems. Because of the similarity of their chemical structures to those of simple fatty acids, we can reasonably anticipate that the anionic Ace\(n\)-m-Ace\(n\) surfactants will either be miscible or undergo phase-separation in mixtures containing perfluorinated fatty acids, based on the choice of an appropriate surfactant tail length. There appear to be very few reports of mixed monolayer gemini systems containing a fluorinated component,
though there is some precedence for this in the literature. Nishdia et al.\textsuperscript{33} have studied mixing behavior of a novel fluorinated cationic surfactant with a hydrogenated cationic surfactant, both variants of the alkanediyl-\(\alpha,\omega\)-bis(alkyldimethylammonium bromide) surfactants, and observed phase-separation of the two components at the air-water interface if the two components were deposited separately onto the water surface, but the components were miscible if first mixed in solution then deposited onto the substrate. An aqueous solution study of a partially fluorinated cationic gemini surfactant mixed with a hydrogenated anionic surfactant has been reported by Sakai et al.\textsuperscript{34}, with the mixtures having poor miscibility and exhibiting only limited synergistic effects.

In this work, we have investigated the miscibility and film structure of binary mixed monolayers comprised of Ace(12)-2-Ace(12) and perfluorotetradecanoic acid (C\textsubscript{13}F\textsubscript{27}COOH; PF) using a suite of Langmuir monolayer characterization approaches. PF was selected as a representative perfluorinated fatty acid as it has been systematically studied in a variety of mixed monolayer films and can be readily purified. Further, based on the relative size of the tail groups of the two film components and the existing literature on phase-separated fatty acid systems monolayer systems (citations described above), we can reasonably predict this system to be miscible at the air-water interface. While subphase pH and ionic strength can reasonably be expected to affect mixing behavior in this system, we have selected a minimal model subphase of ultrapure water alone for this work, as it will allow comparison of this system with the majority of those described above. Results from the liquid surface X-ray scattering measurements (glancing incidence X-ray diffraction; GIXD and X-ray reflectivity; XR) are described in the following sections, along with discussion of the results in terms of miscibility of affiliated systems discussed in the literature.
4.3 Materials and methods

4.3.1 Materials

The synthesis, purification and characterization of the surfactant Ace(12)-2-Ace(12) has been described elsewhere\textsuperscript{14,18}, and the yields and purities for the surfactant synthesized for this work were consistent with previous reports. Solvents (typically ACS grade) were purchased from Fisher Chemicals or Sigma-Aldrich, and used as received. PF was also purchased from Sigma-Aldrich, but was additionally purified by repeated recrystallization from an acetone / hexane mixture with traces of added trifluoroacetic acid.

4.3.2 Liquid surface X-ray scattering (GIXD and XR)

GIXD and XR measurements were carried out on beamline 15-ID-C (ChemMatCARS) at the Advanced Photon Source (Argonne National Labs). Monolayers were prepared in a Langmuir trough equipped with a single barrier, and the trough was mounted on a dynamic vibration isolation pad and enclosed in a sealed sample chamber. After spreading the surfactant solution on a clean water subphase and allowing the solvent to evaporate as described above, the sample chamber was purged with helium to minimize air scattering. A Ge (111) steering crystal was used to direct monochromatic X-rays of energy 10.0 keV ($\lambda = 1.24 \text{ Å}$) onto the air-water interface of the trough at appropriate incident angles. Diffracted or reflected X-rays were collected on a Pilatus 100K X-ray detector (Dectris). Diffraction intensity was measured as a function of both the in-plane ($q_{xy}$) and out-of-plane ($q_z$) scattering vectors, while reflected intensity was measured as a function of $q_z$. The incident X-ray flux was adjusted to ensure that no beam-induced damage was occurring during the time-frame of the experiments; this was verified by monitoring GIXD peak intensity and width as a function of illumination time, and under the conditions used, these did not change significantly during the course of an experiment.
4.4 Results and Discussion

4.4.1 Liquid surface X-ray scattering

To assess crystallographic structure and molecular packing of the surfactants in the films at the air-water interface, monolayers with a range of compositions and surface pressures were measured using GIXD and XR, which can provide complementary information on crystal packing and film thickness, respectively. GIXD contour plot ($q_z$ versus $q_{xy}$) measurements for the pure and mixed monolayers, all at $\pi = 30.0$ mN·m$^{-1}$, are shown in Figure 4-2. Pure PF monolayers give a single, strong diffraction peak centered at $q_{xy} = 1.257$ Å$^{-1}$, with an out-of-plane scattering vector at $q_z = 0$ Å$^{-1}$. The peak was indexed as a hexagonal lattice with lattice parameters $a = b = 5.77$ Å, $\gamma = 120^\circ$, and a unit cell area per chain of 28.84 Å$^2$. The Bragg rods indicated zero tilt angle in the nearest neighbor direction, corresponding to PF molecules that were normal to the subphase surface. These results are in good agreement with literature values for closely-related systems$^{35-36}$, and in general, it is well-known that perfluorinated fatty acids pack to form crystalline, highly-ordered monolayers at the air-water interface. Average 2D crystallite sizes were calculated using Scherrer’s relation and the Bragg peak broadening (for an overview of this analysis see references $^{37-38}$), and gave a coherence length $L_{xy} = 640$ Å. There was no substantial change in the diffraction pattern or crystallographic properties for PF monolayers over a wide range of surface pressures. Monolayers of pure Ace(12)-2-Ace(12) did not give a detectable diffraction signal at any scattering angle within our measurable range, nor was diffraction detected at even the highest surface pressures (below $\pi_c$). The likely cause of this is that the bulky headgroup of the gemini surfactant prevents close packing of the surfactants, resulting in a film that is effectively amorphous.
Figure 4-2: GIXD contour plots for pure Ace(12)-2-Ace(12), mixed monolayer films of PF: Ace(12)-2-Ace(12) 1:1, 2:1, 2.5:1, 3:1, 4:1, and for pure PF at \( \pi = 30.0 \text{ mN} \cdot \text{m}^{-1} \).

GIXD contour plots for mixed monolayers at the highest mole fraction of PF exhibited a single diffraction peak (\( q_{xy} = 1.282 \text{ Å}^{-1} \) for \( \pi = 30.0 \text{ mN} \cdot \text{m}^{-1} \)), which corresponded to diffraction from the PF component. Intriguingly, the intensity, width and position of the PF peak changed as a function of composition, with the intensity decreasing, width increasing and peak position shifting to a higher in-plane scattering vector as the mole fraction of PF decreased in the films. Lattice parameters assigned to the PF peak are summarized in Table 4-1 as a function of film composition. In general, there was a decrease in unit cell area per chain, as well as a decrease in the average crystallite size as the fraction of PF in the film decreased. For monolayer films with a PF: Ace(12)-2-Ace(12) ratio that was < 2.5:1, the diffraction peak associated with PF was too weak and broad to be meaningfully distinguished from background for any surface pressure (Figure 4-2).

Table 4-1. GIXD parameters for monolayer films at \( \pi = 30.0 \text{ mN} \cdot \text{m}^{-1} \).

<table>
<thead>
<tr>
<th>Monolayer Composition</th>
<th>( q_{xy} ) maxima ((\text{Å}^{-1})) (\pm 0.0001)</th>
<th>d-spacing ((\text{Å})) (\pm 0.0003)</th>
<th>Lattice Parameters</th>
<th>Unit cell area ((\text{Å}^2)) (\pm 0.002)</th>
<th>Coherence Length, (L_{xy} \text{ (Å)}) (\pm 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure PF</td>
<td>1.257 (\pm 0.0001)</td>
<td>5.00 (\pm 0.0003)</td>
<td>5.77 (\pm 0.0002)</td>
<td>5.77 (\pm 0.0002)</td>
<td>120 (\pm 15)</td>
</tr>
<tr>
<td>4:1</td>
<td>1.282 (\pm 0.0001)</td>
<td>4.90 (\pm 0.0003)</td>
<td>5.66 (\pm 0.0002)</td>
<td>5.66 (\pm 0.0002)</td>
<td>120 (\pm 15)</td>
</tr>
<tr>
<td>3:1</td>
<td>1.283 (\pm 0.0001)</td>
<td>4.90 (\pm 0.0003)</td>
<td>5.66 (\pm 0.0002)</td>
<td>5.66 (\pm 0.0002)</td>
<td>120 (\pm 15)</td>
</tr>
<tr>
<td>2.5:1</td>
<td>1.293 (\pm 0.0001)</td>
<td>4.86 (\pm 0.0003)</td>
<td>5.61 (\pm 0.0002)</td>
<td>5.61 (\pm 0.0002)</td>
<td>120 (\pm 15)</td>
</tr>
</tbody>
</table>

The GIXD data collected here supports the previously described model of miscibility in the mixed films. In fully immiscible, binary phase-separated films described in the literature, GIXD peaks behave entirely independently of film composition (see Takiue et al.\textsuperscript{39} for example), which is clearly not the case here. For systems which have mole ratios < 2.5, the films are essentially non-crystalline, indicating that the attractive interactions between PF and Ace(12)-2-Ace(12), in combination with the large quantity of gemini surfactant, was sufficient to entirely dissolve any crystallites of PF. This result is actually quite remarkable, given the very high-degree of
crystallinity and structural rigidity of fluorocarbon monolayers. There are several reports highlighting the tendency of fluorocarbons (including perfluorinated fatty acids and semifluorinated alkanes) to spontaneously form highly-crystalline films at very low surface pressures\textsuperscript{35-36,40}, and impeding this requires a significant thermodynamic stabilization of the resulting mixed film. The significantly weakened, broadened and shifted diffraction peak for PF at the highest compositions of perfluorocarbon also reflects this miscibility. Clearly, in films containing large amounts of PF, there are still occasional PF crystallites forming. However, the data in Table 1 indicates that these crystallites are markedly smaller than those formed in the pure films, with a slightly smaller unit cell area per chain and that the presence of Ace(12)-2-Ace(12) in the mixture is strongly perturbing the crystal structure. It is not immediately apparent why the PF unit cell area should be slightly reduced as the relative amount of Ace(12)-2-Ace(12) increases; intuition would suggest that miscibility would, if anything, expand the lattice. At present we are unable to account for this expansion, though the effect is reasonably small.

We note some parallels in terms of structural effects seen in the mixed PF-gemini system and those seen in mixtures of perfluorinated fatty acids with phospholipids (see \textsuperscript{41-43}, for example). Nakahara et al.\textsuperscript{44} have investigated miscibility between a series of perfluorinated fatty acids with dipalmitoyl phosphatidylcholine (DPPC; monomeric headgroup surfactant, with two tails) on high salinity subphases, and reported that several of these systems aremiscible and that the perfluorinated fatty acid can significantly soften or even dissolve condensed phases of the phospholipid. Similar reports on affiliated systems can be found elsewhere. A similar solubilization effect appears to be taking place in the mixed gemini system here, though of course, there are striking differences in terms of headgroup bulk and charge density between Ace(12)-2-Ace(12) and DPPC, as well as differences in chain length, so the analogy should not be taken too far.

As a final complement to the GIXD measurements, XR measurements were performed to assess monolayer film thickness. All films gave measurable reflectivity, with curves exhibiting typical Kiessig fringes as expected. Normalized XR curves ($R/R_f$ vs. $q_z$; $R$ is reflectivity, $R_f$ the Fresnel reflectivity) for both the pure and mixed films measured at $\pi = 30.0$ mN·m$^{-1}$ are shown in Figure 4-3, along with representative electron density profile plots and fits from a conventional two-slab model for the films. Results from the fitting calculations are shown in Table 4-2, with additional
details of the fitting calculation routines and fitting parameters provided in Supporting Information.

A)

B)
Figure 4-3: (A) Normalized X-ray reflectivity plots for pure Ace(12)-2-Ace(12), PF and mixed monolayer films of 4:1, 3:1, 2:1, 2.5:1, 1:1 at \( \pi = 30.0 \text{ mN} \cdot \text{m}^{-1} \). The points are experimental values while the solid curve is the fit. Plots have been vertically offset to aid clarity of presentation. (B) Corresponding normalized electron density profiles for data in (A).

Table 4-2. XR layer length parameter for monolayer films at \( \pi = 30.0 \text{ mN} \cdot \text{m}^{-1} \).

<table>
<thead>
<tr>
<th>Monolayer composition</th>
<th>Head layer length (Å)</th>
<th>Tail layer length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Ace(12)-2-Ace(12)</td>
<td>9.0 ± 0.1</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td>1:1</td>
<td>11.6 ± 0.1</td>
<td>9.8 ± 0.1</td>
</tr>
<tr>
<td>2:1</td>
<td>10.9 ± 0.3</td>
<td>10.2 ± 0.3</td>
</tr>
<tr>
<td>2.5:1</td>
<td>9.9 ± 1.2</td>
<td>10.8 ± 1.2</td>
</tr>
<tr>
<td>3:1</td>
<td>4.0 ± 0.7</td>
<td>16.8 ± 0.9</td>
</tr>
<tr>
<td>4:1</td>
<td>3.9 ± 0.1</td>
<td>17.7 ± 0.3</td>
</tr>
<tr>
<td>Pure PF</td>
<td>4.6 ± 0.1</td>
<td>17.1 ± 0.1</td>
</tr>
</tbody>
</table>
XR measurements of pure PF monolayers gave a tail thickness that was comparable to the expected length of a PF molecule tail chain (17.1 ± 0.1 Å) measured vs. ~17 Å (as calculated by DFT in vacuum), and a relatively compact headgroup. This indicates that PF molecules in the pure monolayers are oriented normal to the surface, in good agreement with the GIXD measurements described above. For the pure Ace(12)-2-Ace(12) monolayers, the thickness of the tail groups was markedly smaller than for PF (8.2 ± 0.3 Å) and significantly smaller than the fully-extended tail length of the molecule (~18 Å as calculated by MMFF in vacuum). The headgroup thickness was also substantially larger than PF (9.0 ± 0.1 Å), as can reasonably be expected because of the significantly larger headgroup of the gemini surfactant in comparison with the PF. A measured film thickness that is smaller than the extended tail length is entirely consistent with a poorly-organized (amorphous) film in which, presumably, there is a wide range of molecular tilts and orientations with respect to the surface normal. This result is consistent with the lack of a diffraction peak associated with the gemini component in any of the monolayer films. As shown in Table 4-2, the overall tail thickness for the mixed films increased as a function of the PF content of the film, and the headgroup thickness correspondingly decreased. Again, this is the expected result for a miscible system in which the relative fraction of the longer, fully-extended component increases. We also note that a significant change in the overall film layer structure takes place between the 2.5:1 and the 3:1 mixtures, which agrees with the composition at which a diffraction peak from PF becomes detectable in the GIXD measurements. At higher fractions of PF, the apparent tail layer length approaches that of the pure PF, consistent with the formation of crystalline deposits of normally oriented perfluorocarbon. At lower fractions, the head and tail layer lengths are comparable, which is consistent with the fully mixed, amorphous film.

A key question which arises from these results is why is this anionic gemini surfactant system miscible with PF, whereas the previously studied simple fatty acid mixed systems, which also have simple carboxylic acid headgroups and alkane tails, are immiscible. It is likely that several structural factors are at play here. The alkane tails for the Ace(12)-2-Ace(12) surfactant are significantly shorter than those for the phase-separating fatty acids, with the shortest reported fatty acid we have found to undergo phase-separation being palmitic acid (C15H31COOH). Kimura et al. have developed a simple thermodynamic model based on dispersion interactions to justify the influence of tail chain length on controlling phase-separated film structure in mixed fatty acid-hybrid (CmF2m+1CnH2nCOOH) fatty acid films, and argued that decreasing “n” for these mixed
systems increases miscibility through a decrease in line tension between phases; it is likely that this is an important contributing factor for the gemini system here, with line tension differences between a potential gemini phase and PF phase being so small that de-mixing is unfavorable. However, we also believe the steric bulk of the Ace(12)-2-Ace(12) headgroup and the molecule’s poor packing ability must also play a contributing role here. Dispersion interactions can significantly stabilize the hydrocarbon phase in phase-separated fatty acid mixtures precisely because the tail chains can align, pack and effectively form a crystalline monolayer; dispersion interactions in the perfluorocarbons are much less important because of their negligible polarizabilities. In the gemini system, the bulky headgroups impede packing, and thus the already short tail groups pack poorly and the ability to stabilize a phase-separated film is minimal. Some additional control might be gained through adjustment of subphase pH to allow for manipulation of headgroup dipole-dipole repulsion interactions, and a rigorous investigation of film structure as a function of subphase conditions might be useful here. Nonetheless, one might reasonably expect to observe phase-separation occurring in the mixed Ace(n)-m-Ace(n) systems only when dealing with very long tail chains, or variants of the system with much less steric bulk in the headgroup. Whether a molecule with these properties can be prepared and dissolved in a solvent that is sufficiently nonpolar for useful applications remains to be explored.

**4.5 Conclusions**

We report, for the first time, on the miscibility of binary monolayer films comprised of the new class of anionic Ace(n)-m-Ace(n) surfactant mixed with perfluorotetradecanoic acid. While simple anionic fatty acid-perfluorinated fatty acid monolayer systems described in the literature are typically immiscible and fully phase-separated, the system here is miscible over a wide range of mixing ratios and at all but the lowest surface pressures. The miscibility observed in these systems is more typical of that commonly observed in mixed phospholipid-perfluorinated fatty acid mixtures, in which the perfluorcarbon can effectively solubilize liquid-condensed domains. At a molecular level, the gemini surfactant packs poorly, forming what are essentially amorphous films, likely because of the steric bulk of the headgroup, and this behavior is observed in all of the mixed films. The association of the two components effectively results in dissolution of PF crystallites in mixed films for all but the highest PF content films. The miscibility in this system can reasonably be attributed to a combination of the relatively short alkane tail groups in the gemini, which
precludes phase-separation into discrete domains, as well as the bulky gemini headgroups which impedes close-packing of the alkane tails.

4.6 Acknowledgements

Financial support for this work has been provided by the Natural Sciences and Engineering Research Council, the Canadian Foundation for Innovation and the University of Saskatchewan. ChemMatCARS Sector 15 is principally supported by the Divisions of Chemistry (CHE) and Materials Research (DMR), National Science Foundation, under grant number NSF/CHE-1346572. Use of the Advanced Photon Source, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science by Argonne National Laboratory, was supported by the U.S. DOE under Contract No. DE-AC02-06CH11357.

4.7 Supporting information

Specular X-ray Reflectivity (XR)

XR provides the electron density distribution of the monolayer film across the interface. The electron density profile was extracted from a homogeneous two-slab model describing the head group and the tail chains of the lipid molecule. The StochFit 1.7.0 software was used in performing the fit, with parameters such as layer length, electron density (ρ/ρH2O), interfacial roughness, and subphase roughness obtained by χ² minimization.
Table 4-S1. XR parameters for head layer of monolayer films at $\pi = 30.0 \text{ mN} \cdot \text{m}^{-1}$.

<table>
<thead>
<tr>
<th>Monolayer composition</th>
<th>Electron density ($\rho/\rho_{\text{H}_2\text{O}}$)</th>
<th>Interfacial roughness (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Ace(12)-2-Ace(12)</td>
<td>1.18 ± 0.01</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>1:1</td>
<td>1.382 ± 0.001</td>
<td>3.112 ± 0.001</td>
</tr>
<tr>
<td>2:1</td>
<td>1.423 ± 0.007</td>
<td>3.162 ± 0.008</td>
</tr>
<tr>
<td>2.5:1</td>
<td>1.42 ± 0.02</td>
<td>3.12 ± 0.02</td>
</tr>
<tr>
<td>3:1</td>
<td>1.43 ± 0.03</td>
<td>2.93 ± 0.01</td>
</tr>
<tr>
<td>4:1</td>
<td>1.29 ± 0.03</td>
<td>3.02 ± 0.01</td>
</tr>
<tr>
<td>Pure PF</td>
<td>1.379 ± 0.001</td>
<td>2.041 ± 0.001</td>
</tr>
</tbody>
</table>
Table 4-S2. XR parameters for tail layer of monolayer films at $\pi = 30.0 \text{ mN} \cdot \text{m}^{-1}$.

<table>
<thead>
<tr>
<th>Monolayer composition</th>
<th>Electron density ($\rho/\rho_{\text{H2O}}$)</th>
<th>Interfacial roughness (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Ace(12)-2-Ace(12)</td>
<td>0.89 ± 0.06</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>1:1</td>
<td>1.250 ± 0.001</td>
<td>3.112 ± 0.001</td>
</tr>
<tr>
<td>2:1</td>
<td>1.363 ± 0.008</td>
<td>3.162 ± 0.008</td>
</tr>
<tr>
<td>2.5:1</td>
<td>1.38 ± 0.03</td>
<td>3.12 ± 0.02</td>
</tr>
<tr>
<td>3:1</td>
<td>1.52 ± 0.02</td>
<td>2.93 ± 0.01</td>
</tr>
<tr>
<td>4:1</td>
<td>1.64 ± 0.01</td>
<td>3.02 ± 0.01</td>
</tr>
<tr>
<td>Pure PF</td>
<td>1.927 ± 0.001</td>
<td>2.997 ± 0.001</td>
</tr>
</tbody>
</table>

The average subphase roughness in all monolayer compositions was $2.88 \pm 0.04 \text{ Å}$.
Grazing Incidence X-ray Diffraction (GIXD)

GIXD provides information about crystallographic structure of the monolayer directly at the air-water interface. Bragg peak positions were used to obtain d-spacing of the diffracting planes according to the relation \( d = (2\pi)/q_{xy} \). A Lorentzian function was used in the fitting of the Bragg peaks, in order to obtain fitting parameters. The coherence length (\( L_{xy} \)) was obtained according to the Scherrer relation, \( L_{xy} = (0.9 \times 2\pi)/W \) where ‘W’ is the peak’s full width at half maximum (FWHM) corrected for instrumental resolution \( W = (\text{FWHM}_{\text{measured}}^2 - \text{FWHM}_{\text{res}}^2)^{1/2} \), with \( \text{FWHM}_{\text{res}} \) being the slit-limited instrumental resolution, with a value of \( 6 \times 10^{-3} \text{ Å}^{-1} \) for the system used here). Bragg rods were analysed from the diffracted X-ray beam intensity along the vertical momentum transfer component (\( q_z \)) according to the relation \( q_z = (2\pi/\lambda) [\sin (\alpha_i) + \sin (\alpha_f)] \approx (2\pi/\lambda) \sin (\alpha_f) \).

\[ \text{Figure 4-S1: GIXD patterns for pure PF and pure Ace(12)-2-Ace(12), with corresponding plots of intensity along } q_z \text{ at } \pi = 30.0 \text{ mN.m}^{-1}. \]
Figure 4-S2: GIXD patterns for mixed monolayer films of PF:Ace(12)-2-Ace(12) at 1:1, 1:2, 1:2.5, 1:3, 1:4, with corresponding plots of intensity along $q_x$ at $\pi = 30.0$ mN·m$^{-1}$. 
4.8 References

CHAPTER 5
IMMISCIBLE ANIONIC GEMINI SURFACTANT-PERFLUORINATED FATTY ACID
LANGMUIR MONOLAYERS


In this work, molecular-level structures of anionic gemini surfactant-perfluorotetradecanoic acid mixed monolayers were studied directly at the air-water interface using grazing incidence X-ray diffraction and X-ray reflectivity. The anionic gemini surfactant (abbreviated Ace(18)-2-Ace(18)) is based on the N, N, N’, N’-dialkyl-N,N’-diacetate ethylenediamine structure as shown in Figure 5-1B, and has a longer tail chain than the compound investigated in Chapter 4. This version of the anionic gemini surfactant was studied to understand how chain length affects the mixing behavior and molecular-level film structure in monolayers with the perfluorinated surfactant.

GIXD studies on pure Ace(18)-2-Ace(18) monolayers showed no diffraction, revealing an amorphous state as was the case for the shorter-chain gemini variant. Also when mixed with fluorinated surfactant, there was significant destabilization of the crystalline fluorinated surfactant. Further, XR studies revealed less well-oriented Ace(18)-2-Ace(18) films, which is improved when the gemini surfactant is mixed with the fluorinated surfactant. The thermodynamics of mixing in Ace(18)-2-Ace(18)-perfluorotetradecanoic acid films showed an immiscible behavior. However, the destabilization of perfluorinated component of the monolayer by Ace(18)-2-Ace(18) as was the case of Ace(12)-2-Ace(12) in chapter 4 is an indication of mixing at the molecular-level. Details of the finding are disclosed and correlated to other works carried at the micron scale using other characterization techniques in this chapter.

In this article, the thesis author shares equal first authorship with Jeveria Rehman. The thesis author’s contribution in this work includes: Sample preparation for all synchrotron measurements, performing all synchrotron experiments, analyzing and interpreting all synchrotron results, and writing the synchrotron part of the manuscript. Measurements other than the synchrotron work were performed, analyzed, interpreted and written by Jeveria Rehman. Prof. Matthew F. Paige also assisted in the synchrotron measurement and further polished the manuscript by writing
extensively. Dr. Amy L. Stevens also assisted in the synchrotron measurement. Dr. Wei Bu is the beamline scientist who trained us on the instrument and gave insight on some of the interpretation of the results.

**Immiscible anionic gemini surfactant-perfluorinated fatty acid Langmuir monolayer films.**

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5.1 Abstract

A new member of the N, N, N’, N’-dialkyl-N, N’-diacate ethylenediamine family of anionic gemini surfactants has been synthesized and its miscibility with the model perfluorocarbon, perfluorotetradecanoic acid, has been investigated in monolayer films at the air-water interface. Thermodynamics of mixing and the accompanying changes in mixed film structure have been probed using a combination of compression isotherm measurements supported by Brewster angle microscope imaging and X-ray scattering measurements, and results have been compared with those collected for a previously studied, shorter tail-chain variant of the surfactant. Thermodynamic measurements showed that the gemini surfactant and perfluorotetradecanoic acid were immiscible, with weak repulsive interactions, manifesting as small positive deviations from ideal mixing, observed between the two film components. Films were highly-textured, with micron-scale, phase-separated domains readily detectable. Grazing Incidence X-ray Diffraction measurements showed that the gemini surfactant was disordered in the monolayers, whereas the perfluorocarbon formed discrete crystallites in the disordered matrix. Despite the small deviations from ideal mixing detected in the thermodynamic measurements, the X-ray measurements indicated that the presence of the gemini perturbs the PF crystal lattice from that of pure PF. Finally, X-ray reflectivity measurements showed that the addition of equimolar PF to the gemini monolayer induces a significant increase in the nominal head group thickness of the film, suggesting that interactions between the two surfactants can lead to structural re-arrangements of the gemini’s head group near to the water surface.
5.2 Introduction

Dimeric “gemini” surfactants, surface active molecules comprised of two polar head groups and two non-polar tail groups, continue to attract considerable research interest because of their excellent technological properties, their potential applications in drug-delivery systems and their unique self-assembly behavior in solution.\(^1\) Much of the pioneering research in gemini surfactants has been directed towards characterizing physicochemical properties of cationic surfactants in solution, with the most thoroughly investigated systems being the so-called \(m-s-m\) (alkanediyl-\(\alpha,\omega\)-bis(alkyldimethylammonium bromide) compounds originally described by Alami et al.\(^6\) Anionic gemini surfactants, while also synthetically tractable, have been minimally investigated and their chemical properties remain an active subject of investigation; a small number of these compounds have been reported in the literature and some aspects of their synthesis and chemical properties have been recently surveyed.\(^5\)

A promising new class of anionic gemini surfactants based on \(N, N', N'-\text{dialkyl-}N, N'-\text{diacetate ethylenediamine}\) has been described in the literature by Wattebled and Laschewsky.\(^8\) The first of these surfactants (Figure 1A), dubbed \(\text{Ace}(12)-2\text{-Ace}(12)\) after the diacetate head group, linker and surfactant tail length, exhibits a very low critical micelle concentration (< \(10^{-4}\) mM), excellent metal ion chelating capabilities and forms a variety of different aggregate structures in bulk solution, depending on pH. Since Wattebled and Laschewsky’s pioneering work, additional investigations of this class of compound have been carried out by Lv et al.\(^9\)–\(^11\) who further explored molecular structure effects on aggregation in bulk solution by introducing asymmetries in surfactant tail lengths and explored emulsification behavior in binary oil-water mixtures. Our group has also reported on fundamental surfactant monolayer properties of the compound in Figure 1A, including \(\text{Na}^+\) and \(\text{Ca}^{2+}\) ion-binding capacity\(^12\), and most recently, miscibility in mixtures with a perfluorinated fatty acid.\(^13\)

In this work, we report on the miscibility of a newly-synthesized, symmetric tail chain member of this class of compounds, \(\text{Ace}(18)-2\text{-Ace}(18)\) (Figure 1B), with the model perfluorocarbon surfactant perfluorotetradecanoic acid (\(\text{C}_{13}\text{F}_{27}\text{COOH}\); PF) noted above. Our interest in mixtures of the \(\text{Ace}(n)-m\text{-Ace}(n)\) compounds with perfluorocarbon stems from previous research aimed at understanding monolayer film structures and miscibility relationships in mixtures of monomeric fatty acids with perfluorinated surfactants (for a recent review see\(^14\)). The miscibility of
hydrogenated surfactants with perfluorinated surfactants continues to be a topic of considerable interest in the literature, as the lower-dimensionality of monolayer systems allows for complex intermolecular interactions between components to be unraveled in a tractable way. Fluorocarbons and hydrocarbons tend to be immiscible in fluid media, generally because dispersion interactions between the hydrogenated components dominates over those between the poorly-polarizable perfluorocarbons. However, in mixed fatty acid monolayer film systems this effect is often balanced by subtle details of the intermolecular interactions between film components which depend on factors such as surfactant tail chain length and head group dipole, as well as other factors including molecular packing density and mixing kinetics. For sufficiently long fatty acid tail groups, mixtures of fatty acids with PF undergo phase-separation at the air-water interface and exhibit rich and complex film morphologies at the micron and nanometer length scale. For example, mixtures of arachidic acid (C_{19}H_{39}COOH) and PF phase-separate at the air-water interface to form crystalline, polygonal domains of the hydrocarbon surrounded by a crystalline matrix of PF. We view the Ace(n)-m-Ace(n) compounds as simple dimeric mimics of fatty acid systems, and thus provide a novel new system to further explore intermolecular interaction effects and their role in surfactant miscibility.

![Figure 5-1: Chemical structures of (A) Ace(12)-2-Ace(12), (B) Ace(18)-2-Ace(18), (C) PF.](image)

While mixing behavior in gemini surfactant systems has been reported for several different types of binary films, most commonly combinations of cationic hydrogenated gemini surfactants with monomeric cationic surfactants, there is only one report to date of mixing in anionic Ace(n)-m-Ace(n)–perfluorocarbon monolayers. In this previous work, the mixing of Ace(12)-2-Ace(12) with PF on a pure water subphase was explored. The two film components showed attractive
interactions, manifest as negative deviations from ideal mixing under all conditions, and the surface phase rule suggested that the film components were miscible (at the equilibrium spreading pressure). However, at lower film compressions, immiscibility was observed, with micron-scale, phase-separated domains observed in Brewster Angle Microscope (BAM) imaging experiments. Synchrotron-based Grazing Incidence X-ray Diffraction (GIXD) measurements, supplemented by X-ray reflectivity (XR) thickness mapping indicated that the Ace(12)-2-Ace(12) films were amorphous and disordered, likely because of the very poor ability of the bulky gemini head groups to pack into an ordered lattice, and that in mixed films, the gemini surfactant impeded the crystallization of PF, which typically forms strongly-diffracting crystallites at even the lowest surface pressures.

The current work expands on the previous study by investigating mixing in the PF: Ace(18)-2-Ace(18) system, a monolayer system which has never before been described in the literature. While care must be taken in extrapolating results from monomeric surfactant systems to the gemini systems, empirical trends gleaned from the mixed fatty acid - PF systems described in the literature (see studies of arachidic acid with PF^{16,20}, nonadecanoic acid with PF^{21} and behenic acid with PF^{23}, for example) suggest that the longer tail chain of the Ace(18)-2-Ace(18) should lead to a fully immiscible, phase-separated system under all compositions and surface pressures. We have also previously postulated that a longer tail chain will provide additional stability to the gemini surfactant monolayer over and above that of its shorter-chain counterpart, which would offset the steric bulk of the head group and thus allow for the formation of crystalline monolayers. These hypotheses are tested in this current work through structural analysis of the monolayer films at the air-water interface using GIXD and XR measurements.

5.3 Materials and methods

5.3.1 Materials

The surfactant Ace(18)-2-Ace(18) was synthesized by coupling N-methyloctadecylamine with ethylenediamine tetraacetic acid anhydride, with the synthesis and purification following the same procedure used for the shorter-chain Ace(12)-2-Ace(12) variant described previously.\textsuperscript{8,12} A synthetic scheme, along with characterization data, including \textsuperscript{1}H-NMR and HR-MS is reported as Supporting Information. All solvents were purchased from Sigma-Aldrich and were ACS grade or better. PF was also purchased from the same source and was further purified by recrystallization.
from a mixed acetone - hexane solvent containing a trace amount of trifluoroacetic acid. Stock surfactant solutions were prepared in a 1:1 v/v mixture of tetrahydrofuran: chloroform and were combined in appropriate volumes to prepare mixtures with the desired mole ratio. In the text, monolayer film compositions are described using the notation X:Y to indicate the relative mole fractions of PF:Ace(18)-n-Ace(18) in the mixtures.

5.3.2 Liquid surface X-ray scattering (GIXD and XR)

GIXD and XR measurements were carried out on the liquid surface scattering spectrometer of sector 15-ID-C (NSF’s ChemMatCARS) at the Advanced Photon Source (Argonne National Laboratories). Films were prepared on a Langmuir trough equipped with a single movable barrier, and the trough was encased in a sealed chamber mounted on a vibration isolation pad. The liquid footprint inside the trough had dimensions of 42.0 cm (length) × 8.9 cm (width) × 0.7 cm (depth). Surfactant solution was deposited onto a clean water surface in the trough, as described in the preceding section, and the chamber was purged with He to minimize X-ray beam scattering by air and to minimize potential damage to the film caused by the incident beam. A monochromatic beam of X-rays with energy 10.0 keV and approximate size of 0.1 mm × 2.0 mm was directed onto the subphase surface at an appropriately selected incident angle (~ 0.1 ° for GIXD; a range of angles for XR) and diffracted (or reflected) X-rays were collected on a Pilatus 100K X-ray detector (Dectris). The X-ray beam intensity was adjusted to ensure that no damage was being caused to the film during the time course of the experiments. X-ray diffraction intensity was measured as a function of in-plane (q_{xy}) and out-of-plane (q_{z}) scattering vectors, and reflected intensity was measured as a function of out-of-plane scattering (q_{z}).

5.4 Results and discussion

5.4.1 GIXD and XR measurements

Crystal structure and thickness of the various films was measured at the air-water interface using GIXD and XR. GIXD contour plots (q_{z} versus q_{xy}) for the pure and mixed monolayer films, all measured at \(\pi = 30.0 \text{ mN·m}^{-1}\), are shown in Figure 5-2. There was no detectable diffraction signal at any scattering angle for pure monolayer films of Ace(18)-2-Ace(18), indicating that the films were disordered. We had hypothesized that the longer tail-chain of this gemini surfactant in comparison with the 12-carbon long variant would promote formation of an ordered monolayer
through enhanced chain-chain dispersion interactions, but clearly this is not the case; it appears that the steric bulk of the head group plays a dominant role in hindering crystal packing in comparison with tail length and extending the alkyl chain length by six additional alkyl groups cannot offset this effect. Thus it is reasonable to propose that, for all practical purposes, this approach will not lead to crystalline gemini surfactant monolayers in the Ace(n)-m-Ace(n) systems. In the case of pure PF monolayers, a single, strong diffraction peak centered at $q_{xy} = 1.262 \text{ Å}^{-1}$ was observed, with out-of-plane scattering vector at $q_z = 0 \text{ Å}^{-1}$. The zero tilt angle for the Bragg rods in the nearest neighbor direction indicates a normal orientation of PF molecules to the water subphase. The pure PF monolayers were indexed as a hexagonal lattice with lattice parameters $a = b = 5.75 \text{ Å}$, $\gamma = 120^\circ$, and a unit cell area per chain of $28.62 \text{ Å}^2$. A coherence length $L_{xy} = 566 \text{ Å}$ (average 2D crystallite sizes) was calculated using Scherrer’s relation (see 24-25 for an overview of GIXD analysis approaches; specific data processing details for our system are described in Supporting Information). In short, the pure PF films were highly-ordered crystalline monolayers at the air-water interface, with crystallographic characteristics consistent with values reported in the literature for similar systems.26-27
1PF : 1Ace(18)-2-Ace(18)

2PF : 1Ace(18)-2-Ace(18)

2.5PF : 1Ace(18)-2-Ace(18)

3PF : 1Ace(18)-2-Ace(18)

4PF : 1Ace(18)-2-Ace(18)

Pure PF

Intensity (a.u.)

Intensity (a.u.)

Intensity (a.u.)

Intensity (a.u.)

Intensity (a.u.)
Figure 5-2: GIXD contour plots for pure Ace(18)-2-Ace(18), mixed monolayer films of PF:Ace(18)-2-Ace(18) 1:1, 2:1, 2.5:1, 3:1, 4:1, and for pure PF at π = 30.0 mN·m⁻¹.

GIXD contour plots for all mixed films consisted of a single peak corresponding to diffraction from the PF component (hexagonal lattice), suggesting films consisted of PF crystallites dispersed in an amorphous matrix of gemini surfactant. We contrast this result with that for mixed PF-arachidic acid monolayers, which might be viewed as “monomer” versions of the films; the arachidic acid systems formed phase-separated films in which both components were crystalline and both gave distinct, well-separated diffraction peaks. Crystallographic data for the films is tabulated in Table 5-1 and for comparison, corresponding measurements from the mixed PF-Ace(12)-2-Ace(12) system are reported in Supporting Information. The intensity of the PF diffraction peak was dependent upon film composition (vide infra); signals were strongest at the largest PF mole fraction and correspondingly decreased as the fraction of PF decreased. Films with mixing ratios of < 2.5:1 gave diffraction signals which were comparable to background levels and thus we report only on films with this or greater PF content. Crystallographic features for the PF component showed a significant dependence on film composition, manifesting as changes in qₓᵧ peak position and width with composition. As the relative fraction of PF decreased, the d-spacing and accompanying lattice parameters decreased, with unit cell areas for the 2.5:1 films (the smallest PF content) decreasing to ~90% of that of the pure PF. Coherence lengths (crystallite sizes) also decreased markedly, with the smallest PF content films having values which were ~ 8% of the pure films.
The diffraction data indicates that the interactions between the two different components are significant enough to induce crystallographic changes in the PF lattice. This is atypical, as the expected result for a fully-immiscible system is that the behavior of one component should be entirely independent of the other indicating that the GIXD measurements are registering subtle molecular-level changes that are blurred over in the classical thermodynamic measurements. For the Ace(12)-2-Ace(12) system, similar effects were observed in pressure regimes where the components were immiscible. We previously attempted to rationalize the results, particularly the decrease in coherence length of crystallites as a function of increasing gemini content, in terms of dissolution of the crystallites. A variety of groups, including ours, have reported on the fluidization and dispersion of rigid, solid-phase phospholipid films by fluorinated surfactants, and it has been proposed, for monolayers comprised of simple fluorinated fatty alcohols and DPPC, also a two-tailed surfactant, that the crucial factor dictating the extent of this fluidization are the extent of interactions between surfactant head groups. However, careful analysis of the GIXD data suggests that PF crystallites are not being dissolved by addition of the gemini in the Ace(18)-2-Ace(18) mixed system. Integration of the PF diffraction peak and normalizing this value against total PF content for the mixed films indicates that, within error, there was no significant change in diffraction intensity as a function of gemini content. Complete dissolution of crystallites would lead to a net decrease in diffraction signal (i.e. less diffracting material in probe volume) and thus the results indicate that the gemini promotes formation of smaller PF crystallites rather than substantially dissolving them. Note, we have applied the same analysis on our previously reported

Table 5-1. GIXD parameters for PF: Ace(18)-2-Ace(18) monolayer films at $\pi = 30.0$ mN·m$^{-1}$.

<table>
<thead>
<tr>
<th>Monolayer composition</th>
<th>q$_{xy}$ maxima ($\AA^{-1}$) (± 0.001)</th>
<th>d-spacing ($\AA$) (± 0.01)</th>
<th>Lattice Parameters</th>
<th>Unit cell area ($\AA^2$ ± 0.01)</th>
<th>Coherence Length, L$_{xy}$ (Å ± 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure PF</td>
<td>1.262</td>
<td>4.98</td>
<td>5.75</td>
<td>5.75</td>
<td>120</td>
</tr>
<tr>
<td>4:1</td>
<td>1.299</td>
<td>4.84</td>
<td>5.59</td>
<td>5.59</td>
<td>120</td>
</tr>
<tr>
<td>3:1</td>
<td>1.312</td>
<td>4.79</td>
<td>5.53</td>
<td>5.53</td>
<td>120</td>
</tr>
<tr>
<td>2.5:1</td>
<td>1.313</td>
<td>4.79</td>
<td>5.53</td>
<td>5.53</td>
<td>120</td>
</tr>
</tbody>
</table>
data for the Ace(12)-2-Ace(12) mixed system, but the relative uncertainties in the values made results ambiguous and we cannot make definitive statements about the shorter tail-system based on current data. The accompanying small decrease in lattice parameters (< 4%) is more difficult to rationalize, and at present we can only provide some reasoned speculation as to its source. Our current hypothesis is that the changes in lattice parameters reflect crystallographic microstrain; as PF crystallites are reduced in size, the average strain on crystallites is likely to increase, particularly at asperities, resulting in a systematic change in lattice structure. Traditional approaches to quantify microstrain using crystallographic data (e.g. Williamson-Hall plots) generally requires analysis of multiple diffraction peaks, which cannot be carried out here because the PF diffraction pattern consists of a single peak only, and thus we simply note the possibility of this effect rather than attempt to quantify it. Nonetheless, this rationalization remains purely speculative and will require further investigation to clarify.

To assess monolayer film thickness directly at the air-water interface, XR measurements were carried out. Normalized XR curves (R/R\text{f} vs. q); R is reflectivity, R\text{f} is the Fresnel reflectivity) are shown in Figure 5-3, along with corresponding electron density profile plots for both pure and mixed films at \( \pi = 30.0 \text{ mN\cdot m}^{-1} \). XR curves from all monolayer films showed Kiessig fringes and gave measurable reflectivity as expected. Electron density profile plots for the monolayer films were generated from XR curves using a conventional two-slab fitting model, with one slab corresponding to the monolayer head group and the other, the tail group.\textsuperscript{35} Fitting parameters for the mixed PF: Ace(18)-2-Ace(18) films are shown in Table 5-2, with comparable results from the corresponding Ace(12)-2-Ace(12) system reported as Supporting Information. Details of the fitting routine followed and additional fitting parameters such as electron density together with roughness values are shown in Supporting Information.
Figure 5-3: (A) Normalized X-ray reflectivity plots for pure Ace(18)-2-Ace(18), PF and mixed monolayer films of 4:1, 3:1, 2:1, 2.5:1, 1:1 at \( \pi = 30.0 \text{ mN} \cdot \text{m}^{-1} \). (B) Normalized X-ray reflectivity plots for pure Ace(12)-2-Ace(12), PF and mixed monolayer films of 4:1, 3:1, 2:1, 2.5:1, 1:1 at \( \pi = 30.0 \text{ mN} \cdot \text{m}^{-1} \). The points are experimental values while the solid curve is the fit. Plots have been vertically offset to aid clarity of presentation. (C) & (D) Corresponding normalized electron density profiles for data in (A) & (B) respectively.
Table 5-2. XR layer length parameters for PF: Ace(18)-2-Ace(18) films at $\pi = 30.0 \text{ mN} \cdot \text{m}^{-1}$.

<table>
<thead>
<tr>
<th>Monolayer composition</th>
<th>Head layer length (Å)</th>
<th>Tail layer length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Ace(18)-2-Ace(18)</td>
<td>8.5 ± 0.4</td>
<td>15.8 ± 0.3</td>
</tr>
<tr>
<td>1:1</td>
<td>12.1 ± 0.1</td>
<td>13.8 ± 0.1</td>
</tr>
<tr>
<td>2:1</td>
<td>8.1 ± 0.1</td>
<td>15.9 ± 0.1</td>
</tr>
<tr>
<td>2.5:1</td>
<td>8.2 ± 0.3</td>
<td>16.0 ± 0.4</td>
</tr>
<tr>
<td>3:1</td>
<td>6.5 ± 0.1</td>
<td>16.8 ± 0.1</td>
</tr>
<tr>
<td>4:1</td>
<td>5.4 ± 0.1</td>
<td>17.0 ± 0.1</td>
</tr>
<tr>
<td>Pure PF</td>
<td>4.5 ± 0.1</td>
<td>16.9 ± 0.1</td>
</tr>
</tbody>
</table>
XR measurements and accompanying data fitting for pure Ace(18)-2-Ace(18) monolayers yielded a head group thickness of \(8.5 \pm 0.4\ \text{Å}\) and a tail group thickness of \(15.8 \pm 0.3\ \text{Å}\). The calculated head group thickness is comparable with the \(9.0 \pm 0.1\ \text{Å}\) reported previously for Ace(12)-2-Ace(12), while the tail group thickness is larger \((8.2 \pm 0.3\ \text{Å})\).\(^{13}\) The larger value for the tail in comparison with the Ace(12)-2-Ace(12) is qualitatively consistent with the six additional alkyl groups. However, the measured value is slightly shorter than that calculated for a fully-extended alkane (~18 Å as calculated by MMFF in vacuum), providing further support for the existence of disorder in the tail-region of the film. Thickness values for the pure PF film were comparable with previous measurements (head group \(4.5 \pm 0.1\ \text{Å}\), tail group \(16.9 \pm 0.1\ \text{Å}\)) and consistent with the fully-extended molecule oriented normal to the subphase, again as expected from the GIXD measurements.

For the mixed films, the calculated tail thickness generally increased as a function of the PF content of the film, while the head group thickness correspondingly decreased. While the general trend in thickness values as a function of mole fraction of PF was not convincingly linear, as might be reasonably from a simple additivity relationship noted earlier, the basic effects observed here can reasonably be attributed to a simple statistical increase in the fraction of PF present in the film. Disappointingly, there is no clear correlation between structure of the headgroup region and the change in structure of the PF crystallites that was detected in the GIXD measurements as a function of gemini content. However, a potentially important observation is that there was a significant difference in the nominal headgroup region of the film for the pure gemini surfactant and that for the 1:1 mixtures. While we are cautious about over-interpretation of the calculated thicknesses, the data set for the 1:1 mixture is particularly intriguing. The thickness assigned to the head group slab is the largest for this mixture (lowest measured electron density), and in fact is greater than that for the pure gemini surfactant alone. A similar observation was made in the shorter-chain system, suggesting a systematic effect. We postulate that there is significant structural re-arrangement of the gemini surfactant head group upon mixing with the PF at this mole fraction, possibly involving the lifting of some of the functional groups away from the subphase surface. Alami et al.\(^6\) have described a variation of this in the cationic \(m-s-m\) system, in which alkyl ammonium gemini surfactants with large, flexible spacer units \((s = 10-12)\) adopt a “wicket-like” conformation in which the spacer group is forced off the water surface. Of course, the ethyl spacer unit for the Ace(18)-2-Ace(18) molecule will have significantly less conformational flexibility than an \(s = 10-\)
12 alkyl linker, but the data do suggest a structural change adjacent to the subphase is occurring. In the absence of the PF, the gemini headgroup “flattens” on the water subphase, but when mixed with PF, interactions between the polarizable headgroup of the gemini and PF push the head group away from the subphase. This effect would likely be indistinguishable in the bulk thermodynamic mixing data, which effectively convolves all head group and tail group interactions. We also note that there are concurrent effects on the tail length thickness for the 1:1 mixture, but cannot easily rationalize this based on the current data. While the head group effect is intriguing, it is certainly small, and further measurements are required to probe this more carefully. High-sensitivity, surface-based vibrational spectroscopies (IR-sum frequency generation, for example) may provide insightful here, and this will be explored in future works.

5.5 Conclusions

We have reported, for the first time, on the miscibility of the newly-synthesized Ace(18)-2-Ace(18) anionic gemini surfactant with the benchmark perfluorinated carboxylic acid, PF. Unlike the shorter tail-chain variant investigated previously, this mixed system was phase-separated and immiscible under all conditions explored, as predicted. However, the Ace(18)-2-Ace(18) failed to pack into crystalline, diffracting films, regardless of whether in pure monolayers or in mixtures with PF. These results suggest, more broadly, that this class of surfactants will not form crystalline monolayers under normal monolayer film preparation conditions, likely because the steric bulk of the head group impedes close-packing. While thermodynamic measurements indicated that the mixed films were fully immiscible and that there were only very weak repulsive interactions between Ace(18)-2-Ace(18) and PF, crystallographic measurements indicated that there were subtle effects of mixing on film structure. The main effect of mixing was to promote formation of smaller PF crystallites in comparison with pure films. There also appeared to be a minor but detectable thickening in the head group region of the gemini in 1:1 mole fraction mixtures with PF, which was tentatively attributed to a structural rearrangement induced by head group interactions.

5.6 Acknowledgements

Financial support for this work has been provided by the Natural Sciences and Engineering Research Council, the Canadian Foundation for Innovation and the University of Saskatchewan.
Partial support for ALS was provided by the College of Arts and Science, University of Saskatchewan. Prof. Ron Steer is gratefully acknowledged for partial funding support for ALS and for helpful discussions. NSF's ChemMatCARS Sector 15 is principally supported by the Divisions of Chemistry (CHE) and Materials Research (DMR), National Science Foundation, under grant number NSF/CHE-1834750. Use of the Advanced Photon Source, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science by Argonne National Laboratory, was supported by the U.S. DOE under Contract No. DE-AC02-06CH11357.
5.7 Supporting information

![Chemical structure](attachment:image.png)

**Figure 5-S1:** Synthetic scheme for Ace(18)-2-Ace(18).

![NMR spectrum](attachment:image.png)

**Figure 5-S2:** $^1$H NMR of purified Ace(18)-2-Ace(18) in CDCl$_3$ and proton labeling scheme.

Spectrum was measured using a 500 MHz, 5 mm SEI NMR spectrometer (Bruker Biospin).
**Figure 5-S3:** Mass spectrum for purified Ace(18)-2-Ace(18). Predicted mass based on formula was 821.27 Da. The TOF–mass spectra were collected using QSTAR XL System (PE Sciex), with samples infused at a rate of 5 mL/min in positive-ion mode using an ESI source.
Grazing Incidence X-ray Diffraction (GIXD)

GIXD fitting parameters were extracted from analyzing Bragg peaks obtained from the intensity of the diffracted X-ray beam as a function of the horizontal momentum transfer component \( q_{xy} \). Diffraction intensity along the vertical momentum transfer component \( q_z \) was used to analyze the resulting Bragg rods. Bragg peaks were fitted with a Lorentzian function to obtain peak position and full width at half maximum (FWHM). Next, d-spacings of the diffracting planes were deduced using the relation \( d = (2\pi)/q_{xy} \). Lattice parameters \( (a, b, \gamma) \) were then determined from the d-spacings and used to estimate the unit cell area. From Scherrer’s relation, \( L_{xy} = (0.9 \times 2\pi)/W \), the coherence length \( (L_{xy}) \) was determined. Here, ‘W’ is the peak’s full width at half maximum corrected for instrumental resolution \( W = (\text{FWHM}_{\text{measured}}^2 - \text{FWHM}_{\text{res}}^2)^{1/2} \), with \( \text{FWHM}_{\text{res}} \) being the slit-limited instrumental resolution, with a value of 6 ×10\(^{-3}\) Å\(^{-1}\) for the system used here).

Table 5-S1. GIXD parameters for PF: Ace(12)-2-Ace(12) monolayer films at \( \pi = 30.0\) mN·m\(^{-1}\).

<table>
<thead>
<tr>
<th>Monolayer Composition</th>
<th>( q_{xy} ) maxima ( (\text{Å}^{-1}) ) (± 0.001)</th>
<th>d-spacing ( (\text{Å}) ) (± 0.01)</th>
<th>Lattice Parameters</th>
<th>Unit cell area ( (\text{Å}^2 \pm 0.01) )</th>
<th>Coherence Length, ( L_{xy} ) (Å ± 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure PF</td>
<td>1.257 (± 0.001)</td>
<td>5.00 (± 0.01)</td>
<td>5.77</td>
<td>5.77</td>
<td>120</td>
</tr>
<tr>
<td>4:1</td>
<td>1.282 (± 0.001)</td>
<td>4.90 (± 0.01)</td>
<td>5.66</td>
<td>5.66</td>
<td>120</td>
</tr>
<tr>
<td>3:1</td>
<td>1.283 (± 0.001)</td>
<td>4.90 (± 0.01)</td>
<td>5.66</td>
<td>5.66</td>
<td>120</td>
</tr>
<tr>
<td>2.5:1</td>
<td>1.293 (± 0.001)</td>
<td>4.86 (± 0.01)</td>
<td>5.61</td>
<td>5.61</td>
<td>120</td>
</tr>
</tbody>
</table>
Specular X-ray Reflectivity (XR)

XR data was analyzed using StochFit 1.7.0. XR fitting software. The software calculates electron density profile and fitting parameters (layer length, electron density ($\rho/\rho_{H2O}$), interfacial roughness, and subphase roughness) through a simple homogeneous two-slab model describing the head and tail group of the lipid molecule. Fitting parameters were obtained by minimizing $\chi^2$ values. Fitting parameters for the various films are summarized in the following tables.

Table 5-S2. XR layer length parameters for PF: Ace(12)-2-Ace(12) films at $\pi = 30.0 \text{ mN}\cdot\text{m}^{-1}$.

<table>
<thead>
<tr>
<th>Monolayer composition</th>
<th>Head layer length (Å)</th>
<th>Tail layer length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Ace(12)-2-Ace(12)</td>
<td>9.0 ± 0.1</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td>1:1</td>
<td>11.6 ± 0.1</td>
<td>9.8 ± 0.1</td>
</tr>
<tr>
<td>2:1</td>
<td>10.9 ± 0.3</td>
<td>10.2 ± 0.3</td>
</tr>
<tr>
<td>2.5:1</td>
<td>9.9 ± 1.2</td>
<td>10.8 ± 1.2</td>
</tr>
<tr>
<td>3:1</td>
<td>4.0 ± 0.7</td>
<td>16.8 ± 0.9</td>
</tr>
<tr>
<td>4:1</td>
<td>3.9 ± 0.1</td>
<td>17.7 ± 0.3</td>
</tr>
<tr>
<td>Pure PF</td>
<td>4.6 ± 0.1</td>
<td>17.1 ± 0.1</td>
</tr>
</tbody>
</table>
Table 5-S3. XR parameters for head layer of PF: Ace(18)-2-Ace(18) films at $\pi = 30.0$ mN·m$^{-1}$.

<table>
<thead>
<tr>
<th>Monolayer composition</th>
<th>Electron density ($\rho/\rho_{H2O}$)</th>
<th>Interfacial roughness (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Ace(18)-2-Ace(18)</td>
<td>1.23 ± 0.02</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>1:1</td>
<td>1.407 ± 0.001</td>
<td>0.515 ± 0.001</td>
</tr>
<tr>
<td>2:1</td>
<td>1.472 ± 0.008</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>2.5:1</td>
<td>1.44 ± 0.03</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>3:1</td>
<td>1.544 ± 0.001</td>
<td>0.927 ± 0.001</td>
</tr>
<tr>
<td>4:1</td>
<td>1.556 ± 0.001</td>
<td>1.496 ± 0.001</td>
</tr>
<tr>
<td>Pure PF</td>
<td>1.448 ± 0.001</td>
<td>2.073 ± 0.001</td>
</tr>
</tbody>
</table>
Table 5-S4. XR parameters for tail layer of PF: Ace(18)-2-Ace(18) films at $\pi = 30.0 \text{ mN} \cdot \text{m}^{-1}$.

<table>
<thead>
<tr>
<th>Monolayer composition</th>
<th>Electron density ($\rho/\rho_{\text{H}_2\text{O}}$)</th>
<th>Interfacial roughness (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Ace(18)-2-Ace(18)</td>
<td>1.03 ± 0.02</td>
<td>3.92 ± 0.06</td>
</tr>
<tr>
<td>1:1</td>
<td>1.425 ± 0.001</td>
<td>3.434 ± 0.001</td>
</tr>
<tr>
<td>2:1</td>
<td>1.554 ± 0.006</td>
<td>3.348 ± 0.009</td>
</tr>
<tr>
<td>2.5:1</td>
<td>1.57 ± 0.01</td>
<td>3.27 ± 0.01</td>
</tr>
<tr>
<td>3:1</td>
<td>1.604 ± 0.001</td>
<td>3.292 ± 0.001</td>
</tr>
<tr>
<td>4:1</td>
<td>1.670 ± 0.001</td>
<td>3.235 ± 0.001</td>
</tr>
<tr>
<td>Pure PF</td>
<td>2.039 ± 0.001</td>
<td>2.966 ± 0.001</td>
</tr>
</tbody>
</table>
5.8 References

CHAPTER 6. SUMMATIVE DISCUSSION, CONCLUSIONS AND FUTURE WORK

6.1 Summative discussion and conclusions

In this thesis, mixed Langmuir monolayer films of hydrogenated-fluorinated surfactants were explored, as these films are known to undergo phase-separation under appropriate conditions and can be used for interfacial patterning.\textsuperscript{1-3} However, the underlying factors that drive phase-separation in these mixed films are shrouded. In addition, the crystalline behavior and lattice structure of the films directly at the air-water interface are not well-known. A combination of GIXD and XR studies have now revealed the lattice structure and crystalline behavior of the films, where the micron scale polygonal domains of the hydrogenated surfactant are as a result of being embedded in a hexagonal lattice structural packing of the PF. This thesis presents major advances in these important areas of research.

The first project, discussed in Chapter 2 of the thesis, was undertaken to further clarify how molecular structure influences phase-separation in these mixed monolayer systems. The nature of the headgroup and tail length of the surfactant are determinants of phase-separation within these mixed films. The majority of research on these mixed films have shown results of polygonal domain formation and the effect of tail length in controlling morphology.\textsuperscript{4-10} However, for the first time, the effect of the surfactant headgroup in the mixing behavior and morphology of these mixed films was explored. Also, the tail length effect and how it fits with previously recognized trends was explored.

This first project hypothesized the formation of phase-separated highly crystalline elongated hexagonal domains in the mixed films of nonadecanoic acid-perfluorotetradecanoic acid based on empirical trends (tail length effect) and a predictive model. Also, the mixing behavior and predicted morphology was hypothesized to change based on the headgroup. These hypothesis were tested using surface pressure-area compression isotherms, Brewster angle microscopy (BAM) and atomic force microscopy (AFM). Phase-separation predicted on the basis of tail length in these mixed films was demonstrated to fit within the previously recognized trends in hydrogenated-fluorinated mixed films. However, it was also noted that phase-separation and interfacial patterning behavior of these mixed films were significantly affected by the change in headgroup. Furthermore, the morphologies observed by BAM and AFM were dependent on the composition of the surfactant mixtures in the films. Highly crystalline elongated hexagonal domains were
observed for higher compositions of nonadecanoic acid in the mixed film. In the BAM measurement, assorted grey shades of crystalline polygonal domains were observed for the hydrogenated surfactant, in good agreement with previous trends. However, the underlying cause of the hexagonal domains was unclear.

One major finding in this project was that an alcohol functional group on the hydrogenated surfactant (nonadecanol) in mixed monolayer films with a fluorinated surfactant bearing a carboxylic functional group (perfluorotetradecanoic acid), resulted in the formation of circular morphological domains of nonadecanol surrounded by a continuous matrix of perfluorotetradecanoic acid. These circular domains were observed for the first time in these mixtures. They were observed for higher compositions of nonadecanol in the mixed film and changed marginally when the mixing concentration compositions were changed. However, morphology in the mixed films of nonadecanoic acid and perfluorotetradecanoic acid were polygonal in shape for higher compositions of nonadecanoic acid. The mechanism of domain formation rationalized by McConnel stems on a competition between dipole-dipole repulsion between negatively charged headgroups and line tension between different phases. This model was developed on the premise that increasing the tail length of the surfactant favors line tension and controls the overall morphology. However, no model exist to explain the morphological differences that result from changing the headgroup of the surfactant. Therefore, the underlying explanation as to why the two different headgroups result in different morphologies despite minimal thermodynamic differences remains unclear and should be investigated more closely. A combination of sum frequency generation (SFG) and infrared reflection-absorption spectroscopy (IRAS) could be possible techniques to probe the surfactant headgroup (functional group) effect on morphology in these mixed films and help understand the cause of change in morphology. Nonetheless, this current study and results shown does contribute to the scientific body of knowledge, as it reveals an important piece in the mixing behavior of these films that needs to be carefully considered when applying in interfacial patterning.

To gain an in-depth understanding of the mixing behavior, phase-separation and molecular-level structure in these mixed films, a molecular-level study was performed using synchrotron-based liquid surface X-ray scattering techniques GIXD and XR. These types of experiments had never been done in our research group and to the best of our knowledge has never been used to investigate
phase-separation, miscibility, and molecular-level structure in mixed hydrogenated-fluorinated monolayer films at the molecular-level. In the first project as explained above, the underlying cause of different shades of highly reflective grey polygonal domains observed in the BAM measurements were unclear. To this end, molecular-level mixing behavior of a simple benchmark mixed film system comprising of arachidic acid-perfluorotetradecanoic acid monolayer films were explored using GIXD and XR. One unique result was the demonstration of phase-separation in these mixed films at the molecular-level, confirmed by the manifestation of independent characteristic in-plane diffraction peaks of the respective surfactants within the mixed film. In addition, micron scale measurements in previous reports could only suggest crystallinity of the hydrogenated surfactant films as polygonal structures through BAM and AFM. However, in this project the two surfactant films, whether in their pure form or in a mixed state, showed diffraction peaks which indicated crystallinity. Therefore, one could rationalize the polygonal structures of the hydrogenated surfactant as being an embedment component in a crystalline hexagonal matrix of the fluorinated surfactant. This result clearly demonstrates that while phase-separation within these mixed films is thermodynamically driven, molecular-level structural interactions are important in regulating overall film morphology.

Another major finding was the formation of crystalline, vertically ordered perfluorotetradecanoic acid (PF) films at very low surface pressures. This behavior of PF films was observed in the pure and mixed films. The vertical ordering of PF can be explained on the basis of its weak adhesion to the air-water interface as a result of low polarizability and rigid helical conformation of fluorine atoms along the molecular tail portion. In the hydrogenated surfactant, hydrogen atoms along the molecular tail adopt a zig-zag conformation which is less rigid compared to PF, hence significant tilts are observed.\(^{14}\) Also, this is why for a higher composition of PF in the mixed films, vertical ordering of the films were favored, as the overall film thickness increased. Clarification of the influence of PF on the overall film thickness is beyond the scope of the two slab model used in modelling the reflectivity. Therefore, a more sophisticated model that takes into account phase-separation and the relative amounts of the two surfactant components mixed films could be developed and used in the future to de-convolute the influence of PF on the overall mixed film thickness.
Because of the structural similarities of gemini surfactants (dimeric hydrogenated surfactants) to monomeric hydrogenated surfactants, the mixing behavior of an anionic gemini surfactant Ace(12)-2-Ace(12) with PF was explored. The molecular-level mixing behavior and structure of the films were investigated using GIXD and XR. One major finding was that the anionic gemini surfactant Ace(12)-2-Ace(12) which is a 12 carbon tail length did not diffract in GIXD, thus, indicating an amorphous film. The amorphous state of Ace(12)-2-Ace(12) could again be explained from XR as the head and tail group thicknesses calculated revealed a film that comprised of a bulky headgroup with tilted tails that cannot effectively pack into crystals. Furthermore, when Ace(12)-2-Ace(12) was mixed with PF in low compositions the crystalline behavior of PF was severely perturbed. The films at both micron scale and molecular-level seemed to be miscible at high surface pressures. This kind of miscibility is more commonly seen in phospholipid-perfluorinated fatty acid films, where the perfluorinated surfactant component solubilizes the liquid condensed domains.\textsuperscript{15} The molecular-level results collated in this third project were unique and novel and complements the thermodynamics of mixing observed in the films at the micron scale.

In view of tail length effect on phase-separation, mixed films containing the anionic gemini surfactant Ace(18)-2-Ace(18) were investigated. Ace(18)-2-Ace(18) was mixed with PF and the films investigated at the molecular-level using GIXD and XR. While the isotherm measurements indicated immiscibility, the molecular-level behavior was similar to that observed in mixed films of Ace(12)-2-Ace(12) with PF. GIXD and XR measurements of Ace(18)-2-Ace(18) revealed an amorphous behavior of a bulky headgroup compound that lack a well-organized structure. Therefore, this class of gemini surfactant is amorphous in nature, irrespective of the tail length. Furthermore, the crystalline behavior of PF was heavily perturbed by the presence of Ace(18)-2-Ace(18) in low compositions of PF in the mixed films. Hence, the molecular-level measurements revealed subtle effects that are shrouded at the micron scale, as crystal solubilization which is an indication of mixing at the molecular-level was observed.
6.2 Future work

Surfactants play a vital role in many of the products we use in our day to day life and activities. Therefore, understanding their fundamental behavior and activity is essential to human growth and development. On this premise, the research described in this thesis has sought to understand the mixing behavior in mixed hydrogenated-fluorinated surfactant monolayer films at the air-water and solid-air interface. In all the projects undertaken, it was understood that the development of gemini surfactant has witnessed tremendous growth in applications and continues to pave way for research studies. The class of gemini surfactants studied in this thesis have an EDTA based structure with a headgroup that has potential to associate to metal ions and can be applied as a chelating agent. This class of gemini surfactant is useful for administering certain essential elements (e.g. iron) that are deficient in agricultural crops for better growth. Therefore, as a new direction, our research group has begun to investigate the association of the gemini surfactants to metal ions at the air-water interface. Synchrotron-based X-ray fluorescence near total reflection (XFNTR), a surface sensitive technique that can measure the ion-monolayer association directly at the air-water interface has been used to carry out some of the seminal work. The fluorescence spectra of the gemini surfactants associating with Fe$^{3+}$ ions is shown in Figure 6-1 below:

**Figure 6-1:** Fluorescence spectra of the gemini surfactants association with Fe$^{3+}$ ions.

The Fe K$_\alpha$ emission lines were strong in intensity confirming an association with the gemini surfactant. The presence of Cu and Zn were as a result of trace contaminants in the subphase. Further work will have to be done in this area to eliminate the presence of trace contaminants and
to numerically quantify the association of Fe$^{3+}$ ions with the gemini surfactants. However, the question of whether a gemini surfactant structure can be modified to optimize binding remains unanswered. Designing gemini surfactants with greater structural balance and having headgroups whose pKa’s will permit easy association with the metal ions of interest will be a great step forward.

In the latter part of this thesis, Ace(12)-2-Ace(12) and Ace(18)-2-Ace(18) in mixed monolayer films with PF were explored at the molecular-level. This class of gemini surfactants were found to be amorphous, most likely because of its bulky headgroup nature, promoting mixing and solubilization of the PF crystals. Therefore, the structure of the anionic gemini surfactant and its ability to organize well at the air-water interface does affect the mixing behavior and film properties. The question of whether a gemini surfactant of less bulky headgroup that has potential to structurally organize into crystalline films at the air-water interface can be synthesized remains.

To answer this question, a new synthetic pathway where the molecular structure of the starting reagent required to form the headgroup bears less bulky groups will be used. Synthetic work in this direction is already underway in our research group as shown in Figure 6-2 below:

![Figure 6-2: Scheme 1 of the synthetic step to making the new gemini surfactant.](image)

The first step of this synthetic scheme was carried out by mixing stoichiometric amounts of cis-1,2,3,6-tetrahydrophthalic anhydride 1 and 1-octadecanol in the presence of p-toluenesulfonic acid monohydrate as an acid catalyst under a solvent free environment and heating in an oil bath at 110 °C for 15 minutes to form an alkylcyclohex-4-ene-1,2-dicarboxylate 2$^{16}$.

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Figure 6-3: Scheme 2 of the synthetic step to making the new gemini surfactant.

In the second step, the alkylcyclohex-4-ene-1,2-dicarboxylate 2 formed is then stirred in the presence of water an KMnO₄ to promote oxidative cleavage of the double bond of the diester to form the gemini surfactant 3. Lastly, the molecular-level structure of the newly synthesized gemini surfactant will be characterized using GIXD and XR.

Finally, the development of a synchrotron-based liquid surface X-ray scattering beamline at the Canadian Light Source (CLS) should be pursued. This new direction is a challenging one to be established but developmental work to this project is already underway as shown in Figure 6-4.

Figure 6-4: Image of CLS’s BXDS beamline for the installation of a liquid surface diffractometer.
Indeed, the insights gained from this thesis has solidified the underlying fundamentals in Langmuir monolayer films of hydrogenated-fluorinated surfactants. However, there remains more work to be done and to advance this area of research. Therefore, the future work outlined here is anticipated to provide useful guidelines to help achieve this goal.

6.3 References


