MOLECULAR DYNAMICS CONTROLLING THE ROBUSTNESS OF CU(II)-COMPLEXED THIN FILMS AND THE EXCITED-STATE BEHAVIOR OF PHOTOACTIVE REAGENTS

By

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ABSTRACT

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Understanding the molecular interactions that control chemical processes is essential for developing novel advancements in any chemical industry, especially for the fields of surface modifications and photoactive chemical reagents. Monolayers and thin films are essential to a wide range of surface chemistry and materials science applications, such as chemical separations, heterogeneous catalysis, and tribology. However, adlayers currently utilized have fixed properties upon deposition onto substrates. Alternatively, photoactive reagents, specifically super-photobases, are pivotal for controlling the temporal and spatial extents of photochemical reactions used in industries such as precision chemistry and high-speed chemical sensing. Nonetheless, few known super-photobases currently exist, and little is understood about the molecular dynamics that give rise to such reactive properties upon photoexcitation. As such, this two-part work focused on the characterization of metal ion-complexed films as candidates for the creation of films with reversible properties, as well as the investigation of the excited-state behavior and spectral dynamics of super-photobase precursor molecules.

The first part of this work focused on the study of the molecular properties that lead to the robustness of Cu^{2+} -complexed amphiphilic Langmuir-Blodgett films. Incorporation of Cu^{2+} ions into the film system resulted in the unique formation of a highly ordered, rigid film system resistant to collapse. Film orientation and mobility were determined to be controlled by Cu^{2+} amphiphile interactions and a shift in dominating intermolecular forces as subphase pH increased. The unique presence of a rigid film at high surface pressures and pH conditions was attributed to the extrusion of Cu^{2+} -amphiphile moieties into the aqueous subphase rather than traditional amphiphile buckling, which occurs at low pH conditions.

In the second part of this work, the excited-state behavior of FR0, a substituted fluorene precursor to the recently developed super-photobase FR0-SB, was studied to determine the statedependent solvent-solute interactions that give rise to the unique reactivity of the photobase molecule upon photoexcitation ($\Delta p K_b \sim 15$). Using time-resolved fluorescence spectroscopy and quantum chemical calculations, the electronic structure and relaxation dynamics of FR0 were examined in protic and aprotic solvents. Hydrogen-bonding interactions in protic solvents, particularly those in the S₂ excited state, were determined to mediate relaxation dynamics. Solvent hydroxyl functionality concentration was also determined to play a role in such dynamics. Examination of the behaviors of structurally modified versions of FR0 demonstrated that the spectral dynamics observed for FR0 are structurally invariant, but that the nature of hydrogen bonding changes depending on the placement and type of modification.

Overall, this work has led to a fundamental characterization of unique classes of surface modifications and photoactivated chemical reagents. First, the dynamics leading to unusually robust thin films were determined, the understanding of which can lead to the creation of selective interfaces using such films. Second, the intermolecular interactions lending themselves to unique spectral properties of super-photobase precursor molecules were identified, which can serve as a vantage point for the future synthesis and development of additional photobase compounds. In both aspects of this work, such advancements contribute to the furthering of each field by providing important fundamental frameworks for future applications in surface chemistry and photoactive reaction chemistry.

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CHAPTER I: INTRODUCTION

1.1 Motivation for Work

The development of unique surface modifications and photoactive chemical reagents requires a comprehensive understanding of the intrinsic molecular interactions characteristic to each class of chemical system. Surface modifications are pivotal for a variety of applications (*e.g.*, chemical separations, lubrication, chemical sensing, surface protection).¹⁻⁵ As such, the behavior of adlayers and their corresponding molecular properties must be fully characterized prior to implementation in any industry. Despite the many advancements to film organization, stability, and structure that have been reported in recent years, monolayers and thin films maintain fixed properties upon deposition onto solid supports, regardless of the formation method employed. Thus, creating films with reversible properties after deposition would enable molecular-level control over an existing film system and expand the range of applications for which such films can be utilized.

The formation of metal ion-complexed Langmuir-Blodgett (LB) films has been reported on for nearly a century, with the incorporated metal ions serving as a means to improve film stability and fluidity.⁶⁻¹⁰ One avenue for achieving reversible control of film systems is through modification of the charge state of the metal ion incorporated into an LB film. Copper (II) serves as a viable candidate for such a task given its reduction potential window in the context of the other monolayer constituents. However, use of Cu^{2+} in LB films has been limited, and a complete characterization of the effects of Cu^{2+} ions on LB films must first be achieved. The first part of this work aims at addressing this gap in knowledge and fully understanding the effects of Cu^{2+} ions on film structure, organization, and mobility as the fundamental step towards gaining reversible control over monolayer properties.

Separately, but equally important, the ability to control the reactivity of photoactive reagents enables highly sensitive chemical reactions to be performed, such as those in the fields

of precision chemistry and high-speed chemical sensing. While these molecules are central to photochemical reactions, a full understanding of the excited-state properties and behavior must be known prior to their use. Super photobase compounds have been of recent interest due to their ability to achieve changes in K_b as great as 15 orders of magnitude upon photoexcitation. The properties of a specific super photobase, FR0-SB, has been studied in detail;¹¹⁻¹³ however, very little is understood about the excited-state behavior of FR0, the precursor molecule used for the synthesis of FR0-SB. As such, the second part of this work focuses on the investigation of these properties in the context of the surrounding solvent environment and the induced solvent-solute interactions occurring in the molecule's excited states. Understanding such properties can lead to the synthesis of additional super photobases for expanded use in photoactive chemical reactions. As described, the work presented in this dissertation is divided into two separate segments and is presented as such.

1.2 Metal-Ion Complexed Langmuir-Blodgett (LB) Films

Monolayers and thin films formed *via* the LB technique are widely versatile in their applications. In a typical LB experiment, films are formed by compressing molecules together at the air-water interface until the highest order of molecular packing is achieved and a cohesive film is formed.¹⁴ Opposed to other thin film formation techniques, such as self-assembly and vapor deposition,¹⁵⁻¹⁹ the LB technique inherently ensures that molecular orientation and packing is consistent for each system; thus, monolayer formation is highly reproducible (Figure 1.1).



Figure 1.1 Schematic of a Langmuir-Blodgett (LB) film formed by compressing barriers to form a cohesive layer of amphiphiles on a water surface.

Films are typically formed using amphiphilic constituents that contain both hydrophilic and hydrophobic components, such that the hydrophilic portion (headgroup) orients closest to the water surface. Common amphiphiles consist of carboxylic and phosphonic acids but can also extend to more complicated systems such as lipids, polymers, and dendrimers.¹⁴ When incorporating additional additives into the film system (*e.g.*, chromophores, metal ions) studying a simple carboxylic acid film that is well characterized allows for direct observation of the additive's effects on the system. For this work, we use arachidic acid, a 20-carbon carboxylic acid (Figure 1.2), as the amphiphile, as its use in LB technology is well established.²⁰⁻²¹



Figure 1.2 Structure of arachidic acid (AA) molecule.

Film adhesion strength and stability can be modified by incorporating metal ions into the aqueous subphase. Characterization of metal ion-complexed films has demonstrated that the charged ion species complex to amphiphile headgroups mainly through electrostatic interactions, such that deposition of metal ion-complexed films on a variety of substrates can be reproducibly achieved.^{9-10, 22} Additionally, monolayer morphology and formation behavior at the water surface has been proven to be dependent on not only metal ion identity but ion charge magnitude. A vast number of characterization studies have been conducted using various metal ion identities and charge states; therefore, we highlight here the ones most relevant to this dissertation work.

One of the most studied and characterized metal ion-complexed carboxylic acid film systems is the cadmium-arachidate (CdA) LB film. Kurnaz and Schwartz reported on the pH dependence of CdA films and characterized the morphology of deposited films using atomic force microscopy (AFM).⁸ Noticeable changes in film structure as a function of subphase pH were reported prior to deposition in the corresponding surface-pressure area (Π-A) isotherm. As pH increased, the presence of the liquid phase in the corresponding isotherms diminished until completely absent at the highest pH condition. This change was attributed to the formation of the cadmium-arachidate soap, with the mole fraction of soap moieties directly proportional to the extent of deprotonation among arachidic acid molecules.⁸ Morphology of multilayer films was studied using AFM; however, as morphology is highly dependent on the number of layers deposited, such characterization is outside the scope of this work as we focus on single layer systems.

Kundu *et al.* reported in 2008 the study of stearic acid (C_{18}) monolayer collapses as a function of metal ion presence and identity as well as subphase pH.⁷ For the five divalent cations studied (Mg²⁺, Ca²⁺, Ba²⁺, Zn²⁺, and Cd²⁺), a transition in collapse behaviors was observed and attributed to both cation presence and subphase pH. At the lowest pH condition for each ion subphase system, the resulting monolayer exhibited collapses similar to those formed on a pure water subphase, in which the surface pressure rapidly decreased following the solid phase region (termed "constant area" collapse).⁷ As pH increased, the collapse transitioned from typical "constant area" collapses to "constant pressure" collapses, in which the surface pressure remained consistent as molecular area decreased. Kundu *et al.* attributed the "constant area" collapse to incomplete dissociation among the stearic acid molecules. At the collapse point, the amphiphiles buckled and formed multilayers on top of the initial layer, creating gaps in the film system and therefore a sharp decrease in surface pressure. The "constant pressure" collapses occurred at higher pH systems, which the authors attributed to cation-cation or cation-headgroup

interactions.⁷ However, a more detailed depiction of molecular movement at the air-water interface was not determined for this collapse condition. As such, the cause for the "constant pressure" collapse remains unknown.

More recently, less conventional methods of characterizing metal ion-complexed film behavior have been employed, focusing on molecular motion and diffusion to assess film adhesion and fluidity. Using fluorescence recovery after photobleaching (FRAP), Baumler *et al.* studied the translational diffusion of phospholipid LB films in the presence of alkaline earth and transition metals.¹⁰ Irrespective of metal ion identity, the diffusion of the metal-lipid film systems was significantly lower than that of films formed on a pure water subphase (no metal ions). Relating the calculated translational diffusion constants for each metal-lipid film system and the corresponding metal-ligand formation constant, complexation was determined to occur in a 1:1 metal:ligand fashion. Additionally, it was determined that the organization of the lipid film system was more affected by transition metals than alkaline earth metals.¹⁰ While this work provides a basis for the use of time-resolved fluorescence spectroscopy for studying film fluidity, a tethered chromophore was used to measure the diffusion of the phospholipid film system, which does not directly translate to the carboxylic acid system used in this work.

The effects of divalent metal ions on the mobility of carboxylic acid LB films was studied by Kijewska *et al.*, using FRAP to measure translational diffusion and film mobility.⁹ Perylene, a nontethered chromophore, was incorporated into a stearic acid (C_{18}) LB film system complexed with various metal ions (*i.e.*, Ca^{2+} , Cd^{2+} , or Ba^{2+}), and deposited films were subject to FRAP analysis. Incorporation of each metal ion induced a morphological change in the resulting layers. Translational diffusion constants were determined to be statistically different for each metal ion; however, the trend was nonlinear in nature.⁹ For a nontethered chromophore, diffusion

is studied as movement between the amphiphile tail groups, which also serves as a gauge of film uniformity. Diffusion constants were higher for metal ion-complexed films compared to stearic acid films formed on pure water; the authors attributed this increase in mobility to the film nonuniformity induced by inconsistent binding between the metal ions and amphiphiles.⁹ While this work reported on characterizing the mobility of a metal ion-carboxylic acid film, there still exists a need to characterize a film system capable of possessing reversible properties.

As a means to reversibly modify film properties, we focus on the charge state of the metal ion. Changing this state through electrochemical means (*e.g.*, voltammetry) would target the charged species while leaving the remainder of film constituents unaffected. While various metals have been studied and used for complexation purposes in LB films, copper (II) is one of the only metal ions with a reduction potential window (for $Cu^{2+} \rightarrow Cu^+$) that does not overlap with that of other film components, thereby isolating the electrochemical reaction solely to this species. Despite this inherent advantage of Cu^{2+} , formation of LB films with this metal ion has not been widely studied, and the resulting effects on monolayer organization, orientation, and stability have not been determined. Therefore, prior to achieving reversible film control using Cu^{2+} ions, a thorough characterization is necessary. The first part of this work addresses such characterization, with the aim of investigating film behavior uniquely induced by Cu^{2+} ions.

1.3 Super-Photobase Compounds and Precursor Molecules

The development of super-photobase compounds address a need for reagents that enable temporal or spatial control in a chemical reaction. Super-photobase compounds and their counterparts, super-photoacids, experience extreme increases in basicity and acidity, respectively, upon photoexcitation (> 10 order-of-magnitude increase).¹¹ The reactivity of these compounds can be controlled using light activation, and when in an excited state, such reagents

can be utilized in applications that require spatial and temporal control during photochemical reactions (*e.g.*, nanolithography, localized catalysis). While super-photoacids have been studied in detail and utilized in applications such as proton-transfer lasers and dye-sensitized ion exchange membranes,²³⁻²⁴ the properties of their super-photobase counterpart have not been investigated to the same extent. Thus, there exists a need for exploration of these useful photoreagent compounds.

Despite the existence of only a few photoactive reagents, their use enables control over photoactivated acid-base chemistry, in which excited state proton transfer reactions can be studied. However, in order to utilize such reagents in photochemical reactions, their specific excited-state properties and reactivities must first be understood. Such characterization has been achieved for FR0-SB, one of the few existing super-photobases. In its excited state, FR0-SB exhibits unique behaviors, one being the ability to induce proton abstraction when in protic solvent media. Such behavior has been studied by Lahiri et al. using time-correlated single photon counting (TCSPC) to probe the various excited-state lifetimes of FR0-SB.^{12-13, 25} Through experimental lifetime data and theoretical computations, it was determined that the rotational diffusion dynamics of FRO-SB depend on solvent viscosity, whereas the proton transfer dynamics between the solvent and solute depend on solvent -OH concentration and solvent dielectric response. While the reactivity of FR0-SB has been studied in detail, its precursor molecule, FR0, has yet to be examined in its excited state. Initial fluorescence lifetime data suggest different excited-state behavior of the FR0 precursor than its super-photobase counterpart. Characterization of this unique behavior and a more thorough understanding of the driving forces behind the observed solvent-dependent phenomena is imperative to the creation of additional super-photobase molecules using the FR0 precursor and sets a precedent for the characterization of additional photoreagent precursor molecules.

1.4 Research Objectives

As the contents of this dissertation work are separated into two major components, the objectives for such work are twofold. The first part of this work focuses on the characterization of Cu²⁺-complexed arachidic acid LB films. In an effort to create a monolayer with reversible properties, film behavior, morphology, and mobility must be thoroughly studied. Characterization is first conducted using surface pressure-area (Π -A) isotherms and Brewster angle microscopy (BAM) photomicrographs to monitor film formation and collapse as a function of Cu²⁺ ion presence, Cu²⁺ concentration, and subphase pH. Monolayer orientation is characterized using various spectroscopic techniques. Infrared reflection-absorption spectroscopy (IRRAS) is employed to determine aliphatic chain orientation through calculations of average aliphatic chain tilt angle with respect to the surface normal for Cu²⁺-complexed films formed at different pH conditions. Fluorescence recovery after photobleaching (FRAP) is subsequently used to quantitatively determine film fluidity through measurements of translational diffusion of a nontethered chromophore throughout the LB film system. Such measurements are conducted as a function of Cu²⁺ presence and subphase pH. The complete characterization of these films, which has yet to be achieved, will advance the understanding of LB carboxylic acid monolayers formed in the presence of Cu²⁺ ions and provide support for use of such ions to mediate film formation and cohesion. Such work serves as a fundamental building block towards creating films with controllable and reversible properties.

The second part of this work focuses on examining the unique excited-state behavior of FR0, the precursor compound to the super-photobase FR0-SB. Using time-correlated single

photon counting (TCSPC), the fluorescence lifetimes and anisotropy decay functions of FR0 in various solvents is investigated. Protic solvent (*i.e.*, aliphatic alcohols) and aprotic solvent media are utilized to assess the likelihood of proton abstraction and/or hydrogen bonding interactions between solvent-solute moieties. Based on the fluorescence lifetime data, the presence of multiple excited-state species is investigated, and the relaxation dynamics of the different excited states is predicted using kinetic modeling. Theoretical computations provide insight as to the driving forces behind the observed experimental phenomena, including excitation energies, Mulliken charges, and permanent dipole moments for several of the lowest singlet excited states of FR0. The comprehensive understanding of excited-state behavior achieved through this work provides imperative knowledge of the reactivity of this precursor molecule, in turn enabling the creation of additional super-photobase molecules for use in a wide range of photoactivated chemical reactions and precision chemistry applications.

REFERENCES

REFERENCES

- 1. Tieke, B. Langmuir-Blodgett Membranes for Separation and Sensing. *Adv. Mater.* **1991**, *3*, 532-541.
- 2. Novotny, V.; Swalen, J. D. Tribology of Langmuir-Blodgett Layers. *Langmuir* **1989**, *5* (2), 485-489.
- 3. Meyer, E.; Overney, R.; Brodbeck, D.; Howald, L.; Luthi, R.; Frommer, J.; Guntherodt, H. J. Friction and Wear of Langmuir-Blodgett Films Observed by Friction Force Microscopy. *Phys. Rev. Lett.* **1992**, *69* (12), 1777-1780.
- 4. Swalen, J. D., et al. Molecular Monolayers and Films. Langmuir 1987, 3, 932-950.
- 5. Petty, M. C. Possible applications for Langmuir-Blodgett films. *Thin Solid Films* **1992**, *210/211*, 417-426.
- 6. Blodgett, K. B.; Langmuir, I. Built-Up Films of Barium Stearate and Their Optical Properties. *Phys. Rev.* **1937**, *51* (11), 964-982.
- 7. Kundu, S.; Langevin, D. Fatty Acid Monolayer Dissociation and Collapse: Effect of pH and Cations. *Colloids Surf. A.* **2008**, *325* (1-2), 81-85.
- Kurnaz, M. L.; Schwartz, D. K. Morphology of Microphase Separation in Arachidic Acid-Cadmium Arachidate Langmuir-Blodgett Multilayers. J. Phys. Chem. 1996, 100, 11113-11119.
- 9. Kijewska, K.; Blanchard, G. J. Using Diffusion To Characterize Interfacial Heterogeneity. *Langmuir* **2017**, *33* (5), 1155-1161.
- 10. Baumler, S. M.; Blanchard, G. J. The Influence of Metal Ions on the Dynamics of Supported Phospholipid Langmuir Films. *Langmuir* **2017**, *33* (12), 2986-2992.
- 11. Sheng, W., *et al.* Ultrafast Dynamics of a "Super" Photobase. *Angew. Chem. Int. Ed.* **2018**, *57* (45), 14742-14746.
- 12. Lahiri, J., *et al.* Proton Abstraction Mediates Interactions between the Super Photobase FR0-SB and Surrounding Alcohol Solvent. *J. Phys. Chem. B* **2019**, *123* (40), 8448-8456.
- 13. Lahiri, J., *et al.* Steric effects in light-induced solvent proton abstraction. *Phys. Chem. Chem. Phys.* **2020**, *22* (35), 19613-19622.
- 14. Petty, M. C. Langmuir-Blodgett Films; Cambridge University Press: NY, 1996.

- 15. Seshan, K. *Handbook of Thin Film Deposition Processes and Techniques*. 2nd ed.; William Andrew: Norwich, NY, 2001.
- 16. Abegunde, O. O.; Akinlabi, E. T.; Oladijo, O. P.; Akinlabi, S.; Ude, A. U. Overview of thin film deposition techniques. *AIMS Mater. Sci.* **2019**, *6* (2), 174-199.
- 17. Bain, C. D.; Troughton, E. B.; Tao, Y.; Evall, J.; Whitesides, G. M.; Nuzzo, R. G. Formation of Monolayer Films by the Spontaneous Assembly of Organic Thiols from Solution onto Gold. J. Am. Chem. Soc. **1989**, 111, 321-335.
- 18. Gun, J.; Sagiv, J. On the Formation and Structure of Self-Assembling Monolayers. III. Time of Formation, Solvent Retention, and Release. J. Colloid Interface Sci. **1986**, *112*, 457-472.
- 19. Oberts, B. P.; Blanchard, G. J. Headgroup-dependent lipid self-assembly on zirconium phosphate-terminated interfaces. *Langmuir* **2009**, *25* (24), 13918-13925.
- 20. Chen, C. W.; Liu, T. J. Maximum withdrawal speed for Langmuir-Blodgett film deposition of arachidic acid. *J. Colloid Interface Sci.* **2006**, *298* (1), 298-305.
- 21. Roy, D.; Das, N. M.; Ganesan, V.; Gupta, P. S. Growth and Structural Study of Cadmium Arachidate Multilayers Deposited by LB Technique. *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry* **2015**, *45* (4), 560-566.
- Peltonen, J.; Linden, M.; Fagerhoim, H.; Györvary, E.; Eriksson, F. The influence of multivalent salts on the processability of a stearic acid monolayer: a stability, electron spectroscopy for chemical analysis and atomic force microscopy study. *Thin Solid Films* **1994**, 242, 88-91.
- 23. Kwon, J. E.; Park, S. Y. Advanced organic optoelectronic materials: harnessing excited-state intramolecular proton transfer (ESIPT) process. *Adv. Mater.* **2011**, *23* (32), 3615-3642.
- White, W.; Sanborn, C. D.; Fabian, D. M.; Ardo, S. Conversion of Visible Light into Ionic Power Using Photoacid-Dye-Sensitized Bipolar Ion-Exchange Membranes. *Joule* 2018, 2 (1), 94-109.
- 25. Lahiri, J., *et al.* Isoenergetic two-photon excitation enhances solvent-to-solute excited-state proton transfer. *J. Chem. Phys.* **2020**, *153* (22), 224301.

CHAPTER 2: EXPERIMENTAL TECHNIQUES

2.1 Thin Film Formation and Deposition: Langmuir-Blodgett Technique

Formation and deposition of thin films, particularly monolayers, are processes that require great precision and control over film properties. Among the various techniques available (*e.g.*, self-assembly, vapor deposition, dip coating, chemical bonding),¹⁻³ the Langmuir-Blodgett (LB) technique enables extreme precision and control over molecular-level film properties. Formation of highly-ordered amphiphilic films dates back to the study of oil films on water reported by Irving Langmuir in 1919.⁴ Together with Katherine Blodgett, the LB method of reproducibly forming thin films on water surfaces was developed in 1935.⁵⁻⁶ The early reports of this method detailed the ability to form such films by compressing amphiphilic molecules together to create a uniform layer on the water surface. Subsequent deposition was performed by lifting a pre-submerged glass substrate out of the water. Still utilized today, this technique allows for not only a high degree of precision when forming films, but the ability to control properties such as amphiphile identity, metal ion presence and identity, film orientation, and film thickness.

Modern LB instruments consist of a polytetrafluoroethylene (PFTE) trough as the well to hold the aqueous subphase on which the monolayer is formed. If metal ions (*e.g.*, calcium, barium, copper) are being incorporated into the film, the metallic salt will be dissolved in this subphase (Figure 2.1A).⁶⁻⁷ In addition to metal ions, the pH of the subphase has also been studied as another variable to control amphiphile protonation and induce the formation of metal ion-amphiphile moieties.⁸ Moving barriers sit on the outside of the trough above the subphase.



Figure 2.1 Schematic of the (A) Langmuir-Blodgett (LB) technique for film formation with metal ions (M^+) present in the subphase, (B) vertical dipping method for thin film deposition, and (C) representative surface pressure-area (π -A) isotherm for an arachidic acid LB film.

Amphiphiles are transferred onto the surface of the subphase *via* syringe. Due to their amphiphilic nature, the molecules are inherently arranged with the hydrophilic portions closest to the water surface and hydrophobic portions oriented away from the subphase (Figure 2.1A). Amphiphile identities can range from long, single-chain fatty acids such as stearic acid (C_{18}) or arachidic acid (C_{20}) (as in this work), or more complex molecules such as phospholipids.⁹⁻¹⁰ The barriers subsequently compress to initiate monolayer formation, and deposition is performed by withdrawing the substrate from the subphase at surface pressures indicative of solid-phase films (*ca.* 30 mN/m) (Figure 2.1B). Surface pressure is measured *via* the Wilhelmy plate method,¹¹⁻¹² which measures the force acting on a platinum plate when wetted by a liquid.

Monolayer formation is monitored in real time using surface pressure area (Π -A) isotherms, which depict the surface pressure measured by the Wilhelmy plate versus the area of the amphiphile system (Figure 2.1C). Isotherms are read from right to left and can be divided into three distinct phases indicating the extent of monolayer formation as the barriers compress. The first of which is the initial phase upon transfer of the amphiphile solution to the top of the water subphase and is termed the "gas phase". In this phase, amphiphiles are highly disordered and sporadically arranged at the air-water interface. As the barriers compress, the space between amphiphiles decreases and prompts an increase in surface pressure, known as the "liquid phase," depicted by the linear increase in pressure starting at *ca*. 22.5 Å²/molecule in Figure 2.1C. During this phase, amphiphile orientation becomes more uniform until the monolayer reaches the "solid phase" region, indicative of maximum cohesion and uniformity among amphiphiles. In the isotherm, this third region is indicated by a sharp increase in surface pressure (Figure 2.1C). Monolayer deposition is typically performed at this stage. Extrapolation of this region to 0 mN/m should result in an x-axis intersection at a mean molecular area consistent with the area of one amphiphile molecule, which is useful in determining the absence of any contaminants within the film.

As the barriers continue to compress and surface pressure increases, the monolayer reaches the point of collapse, in which amphiphiles begin to buckle and are extruded above the layer. Such a phenomenon creates gaps in the layer at a rate faster than the barriers can compress to maintain the solid phase, and a sharp decrease in surface pressure is observed in the isotherm (Figure 2.1C). Differences in monolayer formation induced by the incorporation of metal ions or changes in subphase pH can be observed by monitoring the corresponding Π -A isotherm and any deviations from the typical isotherm shape shown in Figure 2.1C.

For deposition *via* the vertical dipping method,^{5, 7} a substrate (*e.g.*, glass, gold, silicon) is suspended in the subphase prior to spreading the amphiphile solution and initiating barrier compression. Upon reaching the solid phase, the film is transferred onto the solid support by raising the substrate out of the water. The resulting deposited layer consists of similarly oriented amphiphiles situated on the substrate. Metal ions, if incorporated in the subphase, are positioned between the amphiphiles and substrate (Figure 2.2).



Figure 2.2 Langmuir-Blodgett (LB) carboxylic acid film deposited onto solid substrates, with metal ions situate between the substate and amphiphiles.

2.2 Characterization of Film Morphology and Dynamics

2.2.1 Brewster Angle Microscopy

Theory and instrumentation for Brewster angle microscopy (BAM). Characterizing thin film morphology is commonly performed through various microscopy techniques; however, many techniques are intended for use following monolayer deposition, rather than *in situ* (during layer formation). Assessing the morphology of LB films provides useful information of amphiphile behavior and film cohesion as a function of surface pressure. As such, Brewster angle microscopy (BAM) was developed as a technique to measure film morphology in real time without requiring the use of dyes or additives, which can alter film structure and orientation. For two media with different optical characteristics, reflection and transmission of polarized light at the interface of such media are governed by the angle of the incident light and refractive indices of the two media (Figure 2.3).



Figure 2.3 Reflection and refraction (transmission) of incident light at the interface of two optically different media, with n_1 and n_2 being the refractive indices of the two media.

Polarized light directed at a surface is both reflected and refracted (transmitted), the extent of each phenomenon dependent on the law of reflection (Equation 2.1), in which the angle of incidence (θ_i) is equal to the angle of reflection (θ_r), and Snell's law (Equation 2.2). From these fundamental definitions, the angles of incidence (θ_i) and transmittance (θ_i) can be related to the refractive indices of two isotropic media through which light passes (n_1 and n_2).¹³

$$\theta_i = \theta_r \tag{2.1}$$

$$n_1 \sin \theta_i = n_2 \sin \theta_t \tag{2.2}$$

Given that the sum of the angles of reflection (and thus incidence) and transmittance must equal 90°, this identity ($\theta_t = 90 - \theta_i$) can be substituted for θ_t into Equation 2.2. Rearranging to solve for the angle of incidence at Brewster's angle (θ_B) results in Equation 2.3, Brewster's law,¹⁴ which describes the angle of incidence of maximum polarization, for which $n_2 > n_1$.^{13, 15-16}

$$\theta_B = \tan^{-1} \left(\frac{n_2}{n_1} \right) \tag{2.3}$$

For *p*-polarized light, in which the electric field is polarized parallel to the plane of incidence and the magnetic field polarized perpendicular to plane of incidence, there exists an incident angle at which zero reflection occurs, as described by the Fresnel equation for the transverse magnetic mode (Figure 2.4).¹³



Figure 2.4 The reflectance (%) of light for the transverse electric (TE) and transverse magnetic (TM) modes of electromagnetic radiation for the air-water interface, showing Brewster's angle (θ_B) (point of zero reflectance) for TM mode.

This angle, also known as Brewster's angle, is dependent on the refractive indices of the media through which the incident light passes. For an air-water system ($n_{air} \approx 1.33$; $n_{water} \approx 1.00$), such as that for LB experiments, Brewster's angle equals approximately 53°. The BAM light source is set to this angle, and no reflection is observed (Figure 2.5). An organic monolayer forming at the air-water interface has a different refractive index ($n \approx 1.5$) than either medium, thereby changing the ratio of $n_2:n_1$ in Equation 2.3. Thus, reflection of light occurs (Figure 2.5), enabling visualization of the film forming on the water surface.¹⁵⁻¹⁶



Figure 2.5 Incident light directed at the air-water interface at Brewster's angle (53.2°), in which (A) no reflection is observed and (B) reflection is observed with the formation of an organic monolayer at the air-water interface.

The instrumentation used for BAM imaging is shown in Figure 2.6. The light source is a single wavelength diode laser, which emits light at 658 nm with an incident angle range of $52 - 57^{\circ}$ with respect to the surface normal (resolution = 0.001°). The emitted light passes through a polarizer (Glan-Thompson prism) with a resolution of 0.001°. Upon reaching the sample surface, the reflected light passes through a 10x microscope objective (Olympus, LMPLANF1), with a field of view of 720 x 440 μ m and a resolution of 1.6 μ m. The light is then directed through an analyzer (resolution = 0.001°) to correct for any ellipticity in the beam upon reflection. The beam ultimately reaches the CCD camera, which allows for visualization of the surface (image frame = 1360 x 1024 pixels).



Figure 2.6 Schematic of Brewster angle microscope (BAM) instrument.
2.2.2 Infrared Reflection-Absorption Spectroscopy

Assessing the orientation of aliphatic chains in amphiphilic thin films can be achieved by measuring the associated vibrational frequencies using infrared reflection-absorption spectroscopy (IRRAS). The technique measures the vibrational modes of individual chemical bonds present in a single layer. For analysis, such layers are deposited on a reflective metallic surface, such as gold (Au), which enables increased sensitivity of the method ($< 10^{-4}$).¹⁷ The magnitude of sensitivity stems from the polarization of the incident infrared beam, which is comprised of s and p components, with polarizations oriented perpendicular and parallel to the incident plane, respectively (Figure 2.7). Upon reflection of the incident light on a metal surface, the direction of the s-component polarization undergoes an approximate 180° phase reversal, thereby canceling out the associated electric vector.¹⁷⁻¹⁹ Conversely, the *p*-component intensifies upon reflection due to its polarization being in the incident plane, resulting in increased measurement sensitivity. Additionally, the absorption intensity increases as the angle of incidence (θ) approaches 90°, given that the infrared light encounters more molecules at higher incident angles.¹⁸⁻¹⁹ As such, the sensitivity of the measurement can be further increased by using incident angles closer to 90°.





For IRRAS experiments on monolayers or thin films, the formed layer deposited onto a metal substrate is placed on a sample holder suitable for such measurements (*e.g.*, Au attenuated total reflectance attachment), and the infrared light is directed at the sample. Due to the small thickness scale of the adsorbed layer, it is imperative that the system is purged with N_2 gas prior to collecting any spectra. To increase the instrument sensitivity further, infrared spectrometers used for IRRAS measurements are usually equipped with a mercury cadmium telluride (MCT) detector.¹⁷ In addition to a sample spectrum, a reference spectrum is also obtained using a clean substrate (absent of any adsorbed layer), and the sample spectrum is divided by the reference spectrum to produce the final absorbance spectrum. Because many monolayers and films contain hydrocarbon regions (*e.g.*, aliphatic chains), the vibrational frequencies of the C-C and C-H bonds can be used to determine the orientation of the aliphatic chains with respect to the surface normal as described by Allara and Nuzzo.²⁰

2.3 Measurement of Inter- and Intramolecular Motion through Time-Resolved Fluorescence Spectroscopy

2.3.1 Fluorescence Recovery After Photobleaching

First developed to measure the fluidity of cells and model membranes,²¹⁻²² fluorescence recovery after photobleaching (FRAP) has more recently been applied to assessing diffusion and

mobility of thin films deposited onto solid substrates. The technique utilizes the concepts of confocal laser scanning microscopy, and multiple regions of interest (ROIs) within one sample can be analyzed. For measuring mobility of a film system, a fluorescent probe molecule is incorporated into the sample. In LB film systems, the probe (fluorophore) is added to the amphiphile solution prior to spreading of the solution *via* syringe at the air-water interface. For the purposes of this work, the probe molecule will be discussed in the context of a purely diffusion limited regime, in which there are no binding events that occur between the fluorophore and amphiphiles. As such, FRAP measurements assess the degree of mobility of the probe, in our case perylene, a nontethered probe (Figure 2.8A), among the aliphatic chains (Figure 2.8B).



Figure 2.8 (A) Structure of perylene, and (B) diffusion of perylene among aliphatic chains in a fatty acid monolayer.

Fluorescence recovery experiments can be divided into three main regions. The initial fluorescence intensity of an ROI within a sample is first recorded by directing a laser beam at low intensity at the chosen region. Second, the probe molecules in the ROI are photochemically bleached by increasing the laser power to maximum intensity for a short period of time (several seconds). Lastly, following the photobleaching event, the laser returns to the low intensity state,

and surrounding unbleached probe molecules exchange with the bleached molecules and diffuse into the ROI. The fluorescence intensity of the ROI is monitored over time until the system reaches an equilibrium state; this process is also known as the fluorescence recovery of the system (Figure 2.9).²³ This diffusion process is directly affected by molecular interactions within the film and can be an indication of the film system's mobility. The corresponding diffusion constant for a given FRAP experiment can be calculated as a quantitative measure of film mobility.



Figure 2.9 Example fluorescence recovery after photobleaching (FRAP) curve for an arachidic acid monolayer with incorporated Cu²⁺ ions, showing the three main regions (pre-bleach, bleach, post-bleach) of a FRAP experiment.

Background fluorescence of a separate region (ideally furthest from the ROI) is also monitored over time and should be relatively constant; however, acquisition bleaching is inevitable even with a low laser intensity during prebleach and recovery periods of an experiment. To account for any acquisition bleaching, the resulting FRAP curve is normalized according to Equation 2.4,¹⁰

$$I_{norm}(t) = \frac{I_{frap}(t) - I_{bkg}}{I_{frap-pre}}$$
(2.4)

where I_{frap} is the FRAP intensity signal at a given time *t*, I_{bkg} is the background fluorescence intensity signal, and I_{pre} is the fluorescence intensity of the initial, pre-bleach signal. Curves are then normalized between 0 and 1. The translational diffusion constant (D_T) for a fluorescent probe in a given system can be calculated using Equation 2.5,

$$\tau_D = \frac{\omega^2}{D_T} \tag{2.5}$$

where τ_D is the diffusion time, or the time at which the fluorescence intensity recovers to half of the maximum intensity, in seconds, and ω is the bleaching radius, or spot size, in μ m. Fitting τ_D in Equation 2.5 is performed using curve fitting software (Origin) according to Equation 2.6, determined by Soumpasis in 1983,²⁴ where I_0 and I_1 are modified Bessel functions.

$$I(t) = e^{\left(\frac{-2\tau_D}{t}\right)} \left[I_0\left(\frac{-2\tau_D}{t}\right) + I_1\left(\frac{-2\tau_D}{t}\right) \right]$$
(2.6)

For this work, a Nikon C2+ inverted confocal laser scanning microscope (CLSM) was utilized, which is equipped with a diode laser source (Nikon LU-N4) with four wavelength options for bleaching (405, 488, 561, and 640 nm) (Figure 2.10). A dichroic mirror directs light of the chosen wavelength to the sample, which is positioned on a moveable stage. The fluorescence from the sample is directed back towards the dichroic mirror, passes through several bandpass filters and through the confocal pinhole, the opening of which can be changed to modify the number of photons that reach the detector. The light then reaches the detector (3 PMT detector), which detects light between 400 - 700 nm (Figure 2.10).



Figure 2.10 Schematic of fluorescence recovery after photobleaching (FRAP) instrument.

2.3.2 Time-Correlated Single Photon Counting

The behavior of a molecule in its excited state can reveal important information regarding the inter- and intramolecular interactions governing its reactivity and potential use in chemical reactions. One technique used to measure excited-state behavior is time-correlated single photon counting (TCSPC). In this form of time-resolved fluorescence spectroscopy, the molecules in a sample are excited with a pulse of light generated from a laser source, and a photon is emitted. The difference in time that it takes for the excitation pulse and emitted photon to reach the TCSPC electronics is recorded. This difference is plotted as a single count at the corresponding time interval.²⁵⁻²⁶ Using the pulsed-laser light source, the excitation process is continuously repeated, and the number of photons detected at certain time points results in a histogram depicting the decay, or fluorescence lifetime, of the molecule in the excited state, which can be numerically determined by fitting the lifetime curve to an exponential decay function. The

fluorescence transients for the parallel $(I_{\parallel}(t))$ and perpendicular $(I_{\perp}(t))$ components of the excitation polarization are monitored and combined $(I_{\parallel}(t) + 2I_{\perp}(t))$ to produce the anisotropy decay function, R(t) (Equation 2.7).²⁶ The rotational diffusion decay time constant (τ_{OR}) can be extracted and related to molecular reorientation times within the sample.

$$R(t) = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)}$$
(2.7)

The light source for the TCSPC instrument consists of a CW passively mode-locked Nd:YVO₄ diode laser, with a 1064 nm output that is frequency doubled (532 nm) and tripled (355 nm), both at 2.5 W of power. The laser produces 13 ps pulses at a repetition rate of approximately 80 MHz and pumps a cavity dumped dye laser with a tunable wavelength range of 430 – 850 nm. The dye laser has a repetition rate between 80 kHz – 80 MHz, producing approximately 5 ps FWHM pulses (4 MHz repetition rate) that have a power of approximately 0.5 mW. The excitation beam from the dye laser is split, with half going to a reference detector and the other half going to the sample (Figure 2.11). Given the high repetition rate of the laser, a photon may not always be emitted by the sample; thus, the TCSPC electronics are operated in "reverse mode" for optimum performance. The electronics, acting like a stopwatch, are turned "on" upon detecting the pulse from the emitted sample photon, and "off" upon detecting the pulse from the time (in ps) between these two events is recorded and plotted in the resulting memory histogram, and this process is repeated with the pulsed-laser source. Additional experimental details are provided in Chapter 5, Section 5.3.

The TCSPC electronics consist of three main components (Figure 2.11). The first component consists of two constant fraction discriminators (CFDs), one for the reference pulse and one for the sample pulse, which measure the time at which each associated pulse arrives.

Upon arrival, each pulse signal passes through the corresponding CFD to the time-to-amplitude converter (TAC), the second major component of the TCSPC electronics. Operating in reverse mode (as shown below in Figure 2.11) to maintain linearity of the TAC response and a low duty cycle of the TCSPC electronics, the signal from the emitted photon reaches the TAC first. The arrival of the sample photon signal triggers a voltage ramp that increases proportionally with time and stops once the TAC detects the reference signal pulse. The TAC signal then passes through an amplifier and to the analog-to-digital converter (ADC), at which point the voltage is converted to a numerical value and plotted versus the associated time delay in the resulting histogram.²⁵⁻²⁶



Figure 2.11 Schematic of the time-correlated single photon counting (TCSPC) instrument.

REFERENCES

REFERENCES

- 1. Massé, P.; Ravaine, S. The Langmuir–Blodgett technique: A powerful tool to elaborate multilayer colloidal crystals. *Colloids Surf.*, A 2005, 270-271, 148-152.
- 2. Abegunde, O. O.; Akinlabi, E. T.; Oladijo, O. P.; Akinlabi, S.; Ude, A. U. Overview of thin film deposition techniques. *AIMS Mater. Sci.* **2019**, *6* (2), 174-199.
- 3. Seshan, K. *Handbook of Thin Film Deposition Processes and Techniques*. 2nd ed.; William Andrew: Norwich, NY, 2001.
- 4. Langmuir, I. The Constitution and Fundamental Properties of Solids and Liquids. II. Liquids. *J. Am. Chem. Soc.* **1917**, *39*, 1848-1906.
- 5. Blodgett, K. B. Films Built by Depositing Successing Monomolecular Layers on a Solid Surface. J. Am. Chem. Soc. **1935**, *57*, 1007-1022.
- 6. Blodgett, K. B.; Langmuir, I. Built-Up Films of Barium Stearate and Their Optical Properties. *Phys. Rev.* **1937**, *51* (11), 964-982.
- 7. Petty, M. C. Langmuir-Blodgett Films; Cambridge University Press: NY, 1996.
- 8. Kundu, S.; Langevin, D. Fatty Acid Monolayer Dissociation and Collapse: Effect of pH and Cations. *Colloids Surf. A.* **2008**, *325* (1-2), 81-85.
- 9. Baumler, S. M.; Blanchard, G. J. The Influence of Metal Ions on the Dynamics of Supported Phospholipid Langmuir Films. *Langmuir* **2017**, *33* (12), 2986-2992.
- Baumler, S. M.; Reidy, T. M.; Blanchard, G. J. Diffusional motion as a gauge of fluidity and interfacial adhesion. Supported alkylphosphonate monolayers. J. Colloid Interface Sci. 2016, 468, 145-155.
- 11. Heertjes, P. M.; de Smet, E. C.; Witvoet, W. C. The Determination of Interfacial Tension with the Wilhelmy Plate Method. *Chem. Eng. Sci.* **1970**, *26*, 1479-1480.
- 12. Wu, N.; Dai, J.; Micale, F. J. Dynamic Surface Tension Measurement with a Dynamic Wilhelmy Plate Technique. J. Colloid Interface Sci. **1999**, 215, 258-269.
- 13. Lvovsky, A. I. Fresnel Equations. In *Encyclopedia of Optical Engineering* Taylor and Francis: New York; 1-6.
- 14. Brewster, D. On the Laws Which Regulate the Polarisation of Light by Reflexion from Transparent Bodies. *Philosophical Transactions of the Royal Society of London* **1815**, *105*, 125-159.

- 15. Hönig, D.; Möbius, D. Direct Visualization of Monolayers at the Air-Water Interface by Brewster Angle Microscopy. J. Phys. Chem. **1991**, *95*, 4590-4592.
- 16. Hönig, D.; Möbius, D. Reflectometry at the Brewster Angle and Brewster Angle Microscopy at the Air-Water Interface. *Thin Solid Films* **1992**, *210-211*, 64-68.
- 17. Hollins, P. Infrared Reflection–Absorption Spectroscopy In *Encyclopedia of Analytical Chemistry* John Wiley & Sons, Ltd.: 1-17.
- Yoshinobu, J. Infrared Reflection–Absorption Spectroscopy. In *Compendium of Surface and Interface Analysis*, Springer Nature Singapore Pte Ltd.: Kashiwa, Chiba, Japan, 2018; pp 295-299.
- Mendelsohn, R.; Brauner, J. W.; Gericke, A. External Infrared Reflection Absorption Spectrometry of Monolayer Films at the Air-Water Interface. *Annu. Rev. Phys. Chem.* 1995, 46, 305-334.
- 20. Allara, D. L.; Nuzzo, R. G. Spontaneously organized molecular assemblies. 2. Quantitative infrared spectroscopic determination of equilibrium structures of solution-adsorbed n-alkanoic acids on an oxidized aluminum surface. *Langmuir* **1985**, *1* (1), 52-66.
- 21. Axelrod, D.; Koppel, D. E.; Schlessinger, J.; Elson, E.; Webb, W. W. Mobility Measurement by Analysis of Fluorescence Photobleaching Recovery Kinetics. *Biophys J.* **1976**, *16*, 1055-1069.
- 22. Sprague, B. L.; Pego, R. L.; Stavreva, D. A.; McNally, J. G. Analysis of Binding Reactions by Fluorescence Recovery after Photobleaching. *Biophys. J.* **2004**, *86* (6), 3473-3495.
- 23. Wachsmuth, M. Molecular Diffusion and Binding Analyzed with FRAP. *Protoplasma* **2014**, *251* (2), 373-382.
- 24. Soumpasis, D. M. Theoretical Analysis of Fluorescence Photobleaching Recovery Experiments. *Biophys. J.* **1983**, *41*, 95-97.
- 25. Becker, W. *The bh TCSPC Handbook;* 3rd ed.; Becker & Hickl GmbH: Berlin, Germany, 2008.
- 26. Wahl, M. Time-Correlated Single Photon Counting. Technical Note, PicoQuant, Berlin, Germany, 2014, 1-14.

CHAPTER 3: EFFECTS OF CU(II) ON THE FORMATION AND ORIENTATION OF AN ARACHIDIC ACID LANGMUIR-BLODGETT FILM

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3.1 Abstract

The influence of the copper (II) ion on the formation, morphology, and organization of an arachidic acid monolayer was investigated using Langmuir-Blodgett (LB) monolayers, Π -A isotherms and Brewster angle microscopy (BAM). Our findings indicate that a Cu²⁺-complexed LB film exhibits order that depends on the subphase pH, analogous to other metal ions.¹⁻² The metal ion facilitates formation of solid-phase films at surface pressures as low as 5 mN/m. The films exhibit a rigid, ordered phase, evidenced by the absence of a collapse point and an increase in surface pressure rather than the typical sharp decrease in surface pressure indicative of film failure. The role of amphiphile ionic charge *vs.* pH (*i.e.*, the extent of arachidic acid protonation) plays a role in the observed absence of collapse and the ability of the films to maintain order and cohesion at high surface pressures (*ca.* 65 mN/m). Additionally, film thickness data suggest that the incorporation of Cu²⁺ ions induces a change in orientation of the aliphatic chains of the amphiphiles, and that amphiphile solubility in the subphase may play a role in the observed film behavior at low surface areas and high pH.

3.2 Introduction

Interfaces are used to mediate many processes, ranging from chemical separations and sensing, through heterogeneous catalysis and tribology.³⁻¹⁴ Gaining molecular-scale understanding and control over interface organization enables the advancement of a broad range of science and technology. The details of monolayer organization and bonding to the support surface determines the potential utility of the resulting film.¹⁵⁻¹⁷ Changing or modifying the properties of these films can alter monolayer organization and dynamics. Gaining reversible control over the organization and dynamics of monolayers and thin films *after* they have been formed is essential to creating a class of more robust and versatile interfaces with expanded

applications. In the work we report here, we take the initial step in this direction, through a study of Cu^{2+} complexation with the monolayer-forming amphiphile arachidic acid (AA).

There are several well-established means of bonding monolayers to surfaces in a robust manner, ranging from thiol-gold interactions through covalent bonding (*e.g.*, modified silica, alkene binding to diamond)¹⁸⁻²⁷ and ionic complexation chemistry (*e.g.*, zirconium-phosphate (ZP) chemistry),^{1, 28-29} in addition to a plethora of physisorption schemes. In all of these cases, the surface-monolayer interactions are determined by the chemical constituents and cannot be altered reversibly once they are formed. In many instances the means of attachment determines the organization of the resulting monolayer. Modifying surfaces chemically allows them to be tailored for specific uses. Especially for covalently- or ionically-bound monolayers, the interactions are established at the time of monolayer deposition, precluding subsequent (reversible) modification of monolayer properties. Interfaces that can be controlled and modified post-deposition are, in principle, more versatile, providing broader utility as chromatographic stationary phases, films with selective and controllable permeability, or surfaces with specific optical properties, for example, ^{5-6, 30}

The Langmuir-Blodgett (LB) technique^{15, 28, 31-32} has proven to be a facile means of amphiphilic film deposition onto solid supports. It has experienced a renaissance due to its facility in controlling interfacial design and organization. The LB technique allows user control over film properties such as amphiphile identity, packing density, and the presence or absence of metal ions interacting with the amphiphile interfacial polar head groups.^{28, 31, 33} The methods used to characterize LB films (Wilhelmy plate measurement of surface tension, Brewster angle microscopy imaging of monolayer morphology)^{31, 34-38} provide both the quantitative and

qualitative information needed to evaluate the formed monolayers prior to transfer from trough to support.

The modification of LB monolayer films by adding metal ions to the (aqueous) subphase is well established.^{1, 28, 39-41} Typically, mono- or divalent cations added to the subphase in an LB experiment coordinate with the amphiphile polar headgroup and, upon monolayer deposition, exist in a single layer between the amphiphiles and support. These metal ions function as binding and potentially as ordering agents due to the inherent charge of the ion and its ability to coordinate polar organic functionalities. The presence of metal ions tends to increase LB monolayer stability.^{1, 28, 39, 42-43} In addition, metal ion complexation can be used to mediate monolayer fluid properties on a solid support,⁴³⁻⁴⁴ depending on metal ion identity and charge. Divalent cations have been investigated most extensively, with Cd²⁺ being the ion studied most thoroughly. With arachidic acid as the amphiphile, cadmium arachidate has become the reference system for transition metal-amphiphile LB monolayers.^{1-2, 42-46}

Monolayer formation with metal ions is known to depend on the subphase pH. Several studies with Cd^{2+} have shown that the metal-arachidate film forms more readily with increasing subphase pH,^{2, 42, 46} owing to the extent of amphiphile deprotonation. Despite the literature on metal ion-containing monolayers, comparatively little attention has been given to Cu^{2+} . We aim to investigate the role of Cu^{2+} on the morphology, organization, and orientation of an arachidic acid monolayer. Our choice of Cu^{2+} is deliberate. Divalent Cu has been shown to exhibit nanoscale structural order under conditions favoring the formation of $Cu(OH)_x$ species,⁴⁷ and we examine the potential role of such structure on the macroscopic properties of LB films. In addition, unlike many other divalent metal ions, Cu^{2+} can be converted electrochemically to Cu^+ . If the oxidation state of Cu can be reversibly changed between these two oxidation states, it

offers the opportunity to modify monolayer organization *in situ* by electrochemical means. This work is the first step in this effort, establishing the monolayer properties for the Cu^{2+} system. We use Cd^{2+} in the LB trough aqueous subphase as a reference system.

3.3 Experimental Methods

Materials. Arachidic acid (AA, \geq 99.0%) was purchased from Sigma-Aldrich and used as received. Hexane (BDH[®], HPLC grade) was used as the solvent for the monolayer spreading solutions, which were prepared at concentrations of 1 mg/mL. Copper (II) chloride (CuCl₂, Jade Scientific, 99.0%) was used as the source of metal ions and dissolved in Milli-Q water (18 M Ω) at concentrations of 1- and 5 mM for the subphases. HCl and NaOH were used to adjust the pH of the subphase solutions.

Monolayer formation. Monolayers were formed using the LB technique.^{15, 28, 31-32} Film formation and deposition was performed with a LB trough (KN 2003, KSV Nima) with a platinum Wilhelmy plate to measure the surface pressure of the films. The subphase surface was cleaned by suction to ensure minimal contamination, and the subphase (temperature controlled, 20.0 ± 0.5 °C) was allowed to equilibrate for *ca.* 1 hr before spreading the monolayer solution. For each monolayer, 100 µL of the arachidic acid solution was spread by syringe underneath the subphase surface, and 20 minutes was allowed for hexane evaporation and AA molecule equilibration at the surface. During spreading, it was ensured that the surface pressure did not exceed 0.5 mN/m such that the solution spread evenly in the trough. Monolayer compression was carried out at a speed of 5 mm/min. For experiments in which only isotherms were obtained, compression of the films continued past the collapse points to obtain full isotherm data. For deposition experiments, films were held at surface pressures corresponding to solid phases (*ca.* 30-35 mN/m) and post-collapse points (*ca.* 60-65 mN/m). Prior to deposition, the solid phasefilms were allowed to sit for approximately 10-15 minutes for monolayer equilibration. Films at both stages were deposited onto solid substrates at a rate of 2 mm/min. Following deposition, the substrates remained in the dipping apparatus to dry for approximately 15-20 minutes.

Silicon wafers (SiO₂-coated, University Wafer) were cut (0.5 mm \times 10 mm \times 20 mm) and used as solid substrates for film deposition. Substrates were cleaned in piranha solution (1H₂O₂:3H₂SO₄; *Caution! Strong oxidizer!*) for no more than 15 minutes and subsequently rinsed with Milli-Q water. Substrates were stored in Milli-Q water to minimize contamination.

Brewster angle microscopy. A Brewster angle microscope (BAM, Accurion UltraBAM) was used to characterize the morphology of films formed at the air/water interface. The instrument employs a 658-nm solid-state laser to visualize the films and an angle of incidence of 53.2° for organic layers on an aqueous medium. Spatial resolution of the microscope is 2 μ m. Visualization of layers is achieved through a CCD camera which communicates to the respective Accurion UltraBAM software.

Ellipsometry. Film thickness measurements were conducted using a spectroscopic ellipsometer (M-44[®], J.A. Woollam Co., Inc.). The instrument uses a 75 W Xenon light source (LPS-3000) and an M-44[®] detector with an EC110 multi-wavelength control module. A clean silicon substrate was used for calibration and calculating the correct optical constants. Measurements were conducted using an angle of incidence of 75°. Various areas of the coated substrates were measured to account for possible irregularities in film thicknesses. Thickness measurements reported are averages of 20 individual measurements.

3.4 Results and Discussion

Effects of Cu^{2+} *on Monolayer Formation and Morphology*. To evaluate the effects of Cu^{2+} on an arachidic acid (AA) LB monolayer, surface pressure-area (Π -A) isotherms and Brewster angle microscopy (BAM) images were acquired for experiments using a water subphase, a 1 mM Cd²⁺ aqueous subphase, and 1 mM Cu²⁺ aqueous subphase (Figure 3.1).



Figure 3.1 Surface pressure-mean molecular area (Π -A) isotherms and corresponding BAM images for an AA monolayer with subphases containing (A) no metal ions (H₂O), (B) 1 mM CdCl₂, and (C) 1 mM CuCl₂. Dashed line represents mean molecular area at which the film in (A) reached the solid phase and was superimposed onto (B) and (C) for comparison. BAM images taken at approx. 0.25 mN/m. Light areas indicate film, dark areas indicate subphase. Scale bars represent 100 μ m.

The isotherms depict monolayer formation in real time. For a fatty acid system on a strictly aqueous subphase (*i.e.*, no metal ions), a typical isotherm consists of three distinct phases (gas, liquid, solid) and a final collapse point at which the amphiphiles buckle and the surface pressure decreases dramatically (Figure 3.1A, left panel). For this system, the mean molecular area at which the solid phase is reached was calculated by extrapolating the linear portion of the solid phase region to the *x*-axis and was determined to be 18.9 ± 0.1 Å² (indicated by the dashed line).

The corresponding BAM image shows the amphiphilic system in the gas phase once compression began. Consistent with a typical gas-phase AA film, the amphiphiles appear to form a loosely compressed film, indicated by the circular regions where no film is present.

Compared to the isotherm and BAM data of the AA monolayer formed on a water subphase, the data for monolayers formed on subphases containing Cd^{2+} (Figure 3.1B) and Cu^{2+} (Figure 3.1C) are markedly different. In both systems, the liquid phase is absent, and the film transitions from the gas phase directly to the solid phase at low surface pressures (below 10 mN/m). This form of the isotherm indicates that the metal ions facilitate monolayer formation, presumably by metal-amphiphile interactions. From the respective BAM images, the Cd²⁺arachidate film appears to form similarly to the AA film on water at low surface pressures, as it has the same regions of loosely compressed film as the AA layer formed on water. The Cu²⁺arachidate film appears to form much more cohesively at low surface pressures than the AA and Cd²⁺-arachidate films, and it resembles a sheet-like layer in the BAM image (Figure 3.1C, right panel). It is useful to note that, even though neither the Cd²⁺- or Cu²⁺-arachidate film isotherms exhibit a liquid phase region, the collapse behavior of the two monolayers differs; the isotherm for the Cd²⁺-arachidate system plateaus and then decreases, and the isotherm for the Cu²⁺arachidate system increases monotonically with decreasing area per molecule. This finding indicates that the Cu²⁺-arachidate monolayer retains some manner of structural integrity even at high surface pressures and low mean molecular areas. We note that when decompressed, the Cu²⁺-arachidate films form island-like structures, providing support for the notion of film structural integrity being associated with metal ion complexation.

The formation constants of Cu^{2+} -acetate and Cd^{2+} -acetate and those for $Cu(OH)_2$ and $Cd(OH)_2$ could help explain the differences observed here. Metal-acetate formation constants are

used because the formation constants for the metal-arachidate systems are not available to the best of our knowledge. The formation constant for $Cu(OAc)_2$ ($K_1 = 140$) is a factor of three larger than that of $Cd(OAc)_2$ ($K_1 = 50$), while the formation constant for $Cu(OH)_2$ ($K_1 \approx 10^6$) is approximately two orders of magnitude greater than that of $Cd(OH)_2$ ($K_1 \approx 10^4$). Since the formation constants for both Cu^{2+} complexes are greater than those for the Cd^{2+} complexes, the respective interactions of the Cu^{2+} ions with the arachidate headgroups and free hydroxides in solution would be more favorable energetically than the respective interactions with Cd^{2+} ions. Thus, the Cu^{2+} -complexed film in the solid phase may have greater physical integrity than a Cd^{2+} -complexed film in this same region, and thus be able to maintain its integrity in the collapse region. The formation of reinforcing metal hydroxide structures could explain, in part, why the Cu^{2+} -complexed film does not collapse with increasing pressure at higher subphase pH values (*vide infra*).

Subphase pH and metal ion concentration dependence. To understand more fully the effects of Cu^{2+} on the AA monolayer system, the pH of the subphase was varied to resolve the pH-dependence of film formation. Because the p K_a of arachidic acid is 5.4,² a pH range of 3.5 – 6.5 was chosen, with experiments conducted at incremental pH values within this range. Figure 3.2 shows the results of these experiments, and it is clear that the formation of the Cu²⁺- arachidate monolayer isotherm depends sensitively on subphase pH.



Figure 3.2 Π-A isotherms of AA monolayers formed on subphases containing (A) no metal ions (H₂O), (B) 1 mM CdCl₂, and (C) 5 mM CuCl₂ as subphase pH is increased. Isotherms shown are at pH conditions at approximately pH 4 (black solid), 4.5 (red dash-dot), 5 (green dash), 5.5 (blue short dash), and 6 (yellow short dash-dot).

Over this pH range, the Π -A isotherms for AA monolayers formed on the water subphase (Figure 3.2A) do not exhibit a change in shape or area per molecule, demonstrating that

amphiphile film formation in the absence of metal ions in the subphase does not depend greatly on subphase pH. In contrast, the 1 mM and 5 mM Cu^{2+} systems (Figures 3.2B and 3.2C, respectively) exhibit a pH dependence. For the 1 mM Cu^{2+} system, the form of the isotherm below pH 5.0 is the same as that of an AA monolayer formed on a water subphase, with clear transitions between the gas, liquid, and solid phases. The effect of the Cu^{2+} ions is apparent at pH 5.0, where the liquid phase is no longer present and the film transitions directly from the gas phase to the solid phase. Above pH 5.0, the transitions between the gas and solid phases become less distinct. Also, at and above pH 5.0, the surface pressure continues to increase for areas smaller than the arachidic acid "footprint". Typically, this region of the isotherm exhibits a decrease in pressure which is interpreted as film buckling or collapse.

The isotherm data reported here, which is unique to copper (II) to the best of our knowledge, indicates that monolayers formed with Cu^{2+} in the subphase have considerably greater structural integrity than monolayers formed on a water subphase. Monolayer stability is maintained even at surface pressures of *ca*. 60 mN/m. This same behavior is observed for the 5 mM Cu²⁺ system, but it is seen at *ca*. 0.5 pH units lower than for 1 mM Cu²⁺. This finding is strongly supportive of the anomalous isotherm behavior being related to complexation of the Cu²⁺ with arachidate as well as the formation of Cu²⁺ hydroxide species. The absence of the liquid phase is attributed to the change in protonation of the arachidic acid molecules as the system pH approaches the p K_a . As the pH increases, an increasing fraction of amphiphiles are deprotonated and can interact more strongly with Cu²⁺. Future work will involve investigation into the interactions at the interface as a function of bulk solution composition at smaller pH increments (*e.g.*, 0.2 pH units) within this pH range. Such information could provide additional

insight into the transitions of the film from typical fatty acid film behavior to the behavior we report here.

As noted above, another potential contributing factor in the apparent rigidity of the films with increasing subphase pH is the interaction between Cu^{2+} and hydroxide ions. Previous studies have shown that at pH ~ 6 Cu^{2+} interacts with hydroxide ions to form nano-strand structures.⁴⁷⁻⁴⁸ If analogous structures are formed in the subphase, their contribution to the properties of the LB film could be manifested as an increase in film lateral integrity, as observed in the Π -A isotherms at higher pH values (Figure 3.2). More information is clearly required to resolve the role of metal hydroxide species in the properties of LB films.

In addition to the pH dependence, it is evident that the morphologies of the films differ at a given pH based on the subphase composition. BAM images of the AA film formed at pH 5 on the water, 1 mM Cu^{2+} , and 5 mM Cu^{2+} subphases at a progression of surface pressures are shown in Figure 3.3. Additional BAM images at each pH across the pH range are presented in Appendix 3A, Figures A.1 – A.3. The formation of the monolayers depends not only on the presence or absence of metal ions in the subphase, but also on the concentration of metal ions. The gas, liquid, and solid phases can all be identified for the monolayer formed on the water subphase.



Figure 3.3 BAM images and corresponding isotherms of AA films formed on water (left column), 1 mM CuCl₂ (middle column), and 5 mM CuCl₂ (right column) subphases, all at pH 5. Images taken at increasing surface pressures to depict film composition at the different film phases. Scale bars represent 100 μm.

For 1 mM Cu^{2+} in the subphase, a cohesive film starts to form at 10 mN/m, and its structural integrity is maintained as the pressure increases (Figures 3.3B-1 – B-4). When the concentration of Cu^{2+} in the subphase is increased to 5 mM, the BAM images show what appears to be a less smooth film as the pressure increases, suggesting that a cohesive film does not form, despite the shape of the corresponding isotherm (Figures 3.3C-1 – C-4). This effect could be due to steric hindrance in the headgroup region associated with more extensive complexation of the arachidate functionalities with Cu^{2+} . In addition to the information available from the isotherms, the BAM data provide complementary information on the morphologies of the monolayers in the presence of Cu^{2+} .

Identity and relative abundance of species at the air-water interface controlled by competitive equilibria. Due to the number of competing reactions in the metal ion-containing solutions, modeling the equilibria is useful as a way to gain insight into the dominant constituents and how they vary with pH. The relevant reactions are the interaction of Cu^{2+} with arachidic acid (Equation 3.1), the dissociation of arachidic acid (Equation 3.2), and the interaction of Cu^{2+} with OH⁻ (Equation 3.3).

$$Cu^{2+} + AA^{-} \underbrace{\xrightarrow{K_{1}}} CuAA^{+} + AA^{-} \underbrace{\xrightarrow{K_{2}}} Cu(AA)_{2}$$
(3.1)

$$HAA = H^{+} + AA^{-}$$
(3.2)

$$Cu^{2+} + OH^{-} \underbrace{\overset{K_{OH_{1}}}{\longleftarrow}} Cu(OH)^{+} + OH^{-} \underbrace{\overset{K_{OH_{2}}}{\longleftarrow}} Cu(OH)_{2}$$
(3.3)

Using these equilibrium expressions, the relevant equilibrium constants, and the concentrations of Cu^{2+} and H^+ , expressions can be derived for [HAA], [AA⁻], [CuAA⁺] and [Cu(AA)₂]. The full derivation is presented in Appendix 3B. The dependence of the concentration of each species on pH and Cu^{2+} are shown in Figure 3.4. From these data, monolayer composition at a given pH can be determined.



Figure 3.4 Plots of the fractional contributions of the indicated species present at the air-water interface with a (A) 1 mM CuCl₂ subphase and (B) 5 mM CuCl₂ subphase. Species present include arachidic acid (HAA, green), arachidate (AA⁻, red), copper monoarachidate (CuAA⁺, blue), and copper diarachidate (CuAA₂, pink). Dotted lines represent pH values at which experiments were conducted. Plots are based on the equilibria shown in Equations 3.1 - 3.3.

Trends in relative concentrations at the surface of the aqueous subphase can be seen to depend on both pH and $[Cu^{2+}]$. The fractional amounts of HAA and AA⁻ behave as expected: in each plot, the protonated form of the acid is the dominant species at pH values below the p K_a (at which point the species reach a 1:1 equilibrium), and the deprotonated form is the dominant species for pH values above the p K_a . At 5 mM Cu²⁺, the fractional amount of HAA decreases more rapidly around pH 4.5, which we attribute to depletion of HAA by the larger amount of

Cu(AA)₂ present. The fractional amount of Cu(AA)₂ increases in proportion to the amount of Cu^{2+} in the subphase, while the amount of CuAA⁺ species remains at a relatively low, constant level across the relevant pH range. The majority of Cu²⁺ binds preferably to two arachidate molecules. This is not a surprising result based on the values of K₁ and K₂ for this reaction, which we estimate from the equilibrium constant data for Cu(OAc)₂.⁴⁹ We recognize that these K values may be off by a small amount, which could affect the specific values for the species shown in Figure 3.4, but the relative magnitudes of K₁ and K₂ are expected to be correct and the qualitative trends in Figure 3.4 will not change. From this model, it is evident that both the pH and [Cu²⁺] in the subphase greatly influence the fractional amount of each monolayer constituent. This model provides predictability of the dominant species present and identifies the role of the competing equilibria in this system.

Film thickness influenced by subphase pH and deposition conditions. To further characterize the Cu²⁺-arachidate monolayers, thickness measurements were performed using optical ellipsometry for monolayers formed on a 1 mM Cu²⁺ subphase and deposited on silicon. A central point in measuring thickness as a function of surface pressure at which the film was deposited is to understand the anomalous post-collapse behavior of these systems. Π -A isotherms of monolayers formed on a 1 mM Cu²⁺ subphase at pH *ca.* 5 (Figure 3.3B) show that the monolayer does not exhibit a post-collapse change in thickness, as is seen for AA monolayers formed on water (Figure 3.3A). Monolayers were formed and deposited at typical solid phase surface pressures (30 – 35 mN/m), and separate films were formed and deposited at surface pressures of 60 – 65 mN/m, beyond the solid phase region. Figure 3.5 shows the thickness measurements for solid phase- and collapsed films across the experimental pH range. Raw ellipsometric data are presented in Appendix 3C, Tables A.1 and A.2.



Figure 3.5 Plot of film thickness vs. pH for films formed on a 1 mM CuCl₂ subphase and deposited at the solid phase (blue squares) and post collapse (red circles). Uncertainties are reported as $\pm 1\sigma$ (n = 20).

The thicknesses of the solid phase monolayers formed at low pH values (pH 3.5 - 4.5) agree with literature values of monolayers formed with fatty acid amphiphiles with chain lengths ranging from 18-20 carbons on water subphases (*ca*. 23 Å),⁵⁰ in which the amphiphiles typically assemble at a ~30° tilt angle with respect to the surface normal.¹⁶ The isotherm and thickness data, taken collectively, are consistent with the Cu²⁺ ions not interacting extensively (*i.e.*, limited extent of Cu(AA)₂ formation) with the arachidic acid molecules at these pH values because the majority of AA molecules remain protonated. At pH values of 5 and above, the thickness increases to roughly 30 Å. We expect that this observed thickness increase is a consequence of several factors. As the pH increases, an increasing fraction of AA molecules become deprotonated, and AA⁻ complexes more efficiently with Cu²⁺ than HAA. This situation leads to an increase in monolayer strength and integrity, and the AA intermolecular distance is expected to become shorter due to complexation with Cu²⁺. Such a condition will lead to an AA chain

orientation closer to the surface normal (*i.e.*, smaller tilt angle). For such films, rather than buckling, the monolayer maintains its thickness and even increases slightly. There are a limited number of possibilities consistent with this finding. As noted above, the interaction of AA⁻ in the monolayer with Cu²⁺ could lead to a change in tilt angle. Based on molecular mechanics calculations, the length of an all-*trans*, fully extended, arachidic acid molecule is ~25 Å. The presence of the Cu²⁺ ion may also play a role; a six-coordinate Cu²⁺ ion layer is *ca*. 5.4 Å in diameter.⁵¹ Combined, the total thickness of these two film components agree with our measurements of \geq 30 Å at high pH. We attribute these thickness values with a change in orientation of the AA amphiphiles, in which they are oriented in the usual tilted manner at low pH conditions when the interaction with copper ions is limited but change to an orientation closer to normal to the substrate surface at higher pH conditions when the interactions with Cu²⁺ play a greater role.

When the films were deposited at surface pressures past the collapse points, a similar trend is observed as the subphase pH increases. These data are shown in Figure 3.5 and there are two important points to be noted. The first point is that with increasing pH the monolayer thickness trends higher, consistent with the argument made above regarding the interactions of Cu^{2+} with the AA⁻ constituents in the film. The second point is that for all pH values the film thickness post-collapse is equal to (pH 4, 4.5) or less than (\geq pH 5) the thickness in the solid phase region. Because the thickness data are acquired using optical ellipsometry, the thickness values reported represent averages over the beam spot on the film. It is thus not possible to get direct molecular-scale information from these data but the nature of the change in thickness is consistent with a limited number of explanations. Specifically, while on the Cu²⁺-containing subphase, if all of the film mass remained on the surface of the trough, compression beyond the

collapse point would give rise to the film forming multi-layer regions where its lateral integrity had been compromised by pressure and the resulting average film thickness would be greater than a single monolayer. Such a process would diminish the ability of the film to maintain integrity at high pressure and the roll-over in the Π -A isotherm would be the expected result. The associated increase in thickness would obtain regardless of the orientation of the AA constituents in the multilayer regions. Film pressure collapse is seen at low pH and the change in thickness seen ellipsometrically for the supported monolayer at higher pH is not seen at low pH.

The fact that the film thickness is seen to decrease in the collapse region and that the pressure remains constant or increases slightly in this region can be accounted for only if the monolayer maintains its structural integrity (i.e., does not exhibit breaks or structural compromises). This prediction is consistent with the BAM data, in which the ordered film formed at solid phase pressures appears to remain intact at high surface pressures (> 50 mN/m) (Appendix 3A, Figure A.2). The thickness of the monolayer in this region can be accounted for if the excess monolayer constituents (presumably CuAA⁺ and Cu(AA)₂) are forced into the aqueous subphase. This phenomenon can occur in one of two ways. The first is if the Cu^{2+} ions are coordinating to the arachidate amphiphiles in such a way to increase the solubility of the amphiphiles in the aqueous subphase. The second is if some arachidate molecules could be dissolved in the subphase given that the deprotonated form of arachidic acid is more soluble in water than the protonated form. Considering that this phenomenon occurs at pH values above the AA pK_a and nearing neutral, it is likely that the second explanation plays a significant role since the majority of amphiphiles should be deprotonated at the higher pHs used in this work. While we are not aware of any solubility data for CuAA⁺ or Cu(AA)₂ in water, the amount of monolayer constituent(s) that would be required to be accommodated into the aqueous phase

(which contains excess Cu^{2+}) would be low, making solubility issues a modest concern. Work is currently underway to evaluate the solubility of AA⁻ in aqueous Cu^{2+} solutions.

It is also useful to consider the role of electrostatic repulsion as a function of subphase pH, which can also influence the organization and thickness of the solid phase and "collapsed" films. Since Cu^{2+} ions arrange in a layer between the substrate and amphiphiles in supported monolayers, the ionic charge density could naturally induce a slight in-plane repulsion of the Cu^{2+} ions as well as the AA⁻ ions, limiting the achievable arachidate molecules density in the supported layer, and accounting for the observed decrease in thickness at high pressures. The effect of electrostatic repulsion is consistent with the average thickness remaining within $\pm 1 - 2$ Å of the solid phase value above pH 5.

3.5 Conclusions

We have investigated the effects of Cu^{2+} on the organization and formation of an arachidic acid LB film. The ion produces some unexpected effects on monolayer formation and produces structures with enhanced structural integrity. While exhibiting a dependence on the subphase pH and metal ion concentration, the films do not exhibit traditional collapse points at high subphase pH conditions, likely due to the complexation of the Cu^{2+} with the arachidic acid/arachidate molecules. Additionally, thickness measurements show that while film integrity is likely maintained at pH conditions near and above the HAA p K_a , the orientation of the amphiphiles likely changes due to the addition of Cu^{2+} to the subphase. These measurements demonstrate that a film system with considerable lateral integrity can be deposited onto a substrate not only in the solid phase, but also at surface pressures typical of normal collapse points. Further investigation into the specific nature of the Cu^{2+} -amphiphile bond(s) and the manner in which this metal ion influences amphiphile-substrate binding directly (*e.g.*, monolayer

mobility) will be necessary to obtain important information about the full influence of copper (II) on creating a film system with reversible properties following deposition.

APPENDICES

APPENDIX 3A:

Brewster Angle Microscopy (BAM) Images from pH Dependence Studies



pH 5.3

A

B

рН 5.0 в

A

рН 5.5 В





Figure A.1 BAM images of AA monolayer formed on plain water subphase at incremental pH conditions across pH range. Each set of images is of the same monolayer at increasing surface pressures. For each set, individual images correspond to (A) 0.25 mN/m, (B) 10 mN/m, (C) 30 mN/m, and (D) 50 mN/m. Scale bars represent 100 μm.


pH 5.3



pH 6.0 B

pH 6.2





Figure A.2 BAM images of AA monolayer formed on 1 mM Cu²⁺ water subphase at incremental pH conditions across pH range. Each set of images is of the same monolayer at increasing surface pressures. For each set, individual images correspond to (A) 0.25 mN/m, (B) 10 mN/m, (C) 30 mN/m, and (D) 50 mN/m. Scale bars represent 100 μ m.







Figure A.3 BAM images of AA monolayer formed on 5 mM Cu²⁺ water subphase at incremental pH conditions across pH range. Each set of images is of the same monolayer at increasing surface pressures. For each set, individual images correspond to (A) 0.25 mN/m, (B) 10 mN/m, (C) 30 mN/m, and (D) 50 mN/m. Scale bars represent 100 μm. Experiments for pH 6.2 and 6.5 subphases not possible due to the precipitation of CuOH₂ as a result of the increased Cu²⁺ concentration.

APPENDIX 3B:

Mathematical Model Workflow

For the mathematical model plots of the fractional amount of species present at the subphase surface at a given pH and Cu^{2+} concentration, the following series of reactions and corresponding equilibria expressions were used:

$$Cu^{2+} + AA^{-} \underbrace{\xrightarrow{K_{1}}} CuAA^{+} + AA^{-} \underbrace{\xrightarrow{K_{2}}} CuAA_{2}$$
(A.1)

$$K_{1} = \frac{[\text{CuAA}^{+}]}{[\text{Cu}^{2+}][\text{AA}^{-}]}$$
(A.2)

$$K_2 = \frac{[\text{CuAA}_2]}{[\text{CuAA}^+][\text{AA}^-]}$$
(A.3)

where K_1 and K_2 represent equilibrium constants for the formation of copper (II) monoacetate $(Cu(OAc)^+)$ and copper (II) diacetate $(Cu(OAc)_2)$ to represent the interactions of Cu^{2+} ions with the carboxylate headgroups of the arachidate molecules. Literature values for K_1 and K_2 were used $(K_1 = 140; K_2 = 11)$,⁵² as well as the literature value for the p K_a of arachidic acid (p $K_a = 5.4$).² Further relationships utilized are as follows:

$$HAA \underbrace{K_a}{} H^+ + AA^-$$
 (A.4)

$$K_a = \frac{[\mathrm{H}^+][\mathrm{AA}^-]}{[\mathrm{HAA}]} \tag{A.5}$$

$$\operatorname{Cu}^{2+} + \operatorname{OH}^{-} \xleftarrow{K_{\operatorname{OH}_{1}}} \operatorname{Cu}(\operatorname{OH})^{+} \xleftarrow{K_{\operatorname{OH}_{2}}} \operatorname{Cu}(\operatorname{OH})_{2}$$
 (A.6)

$$K_{\rm OH_1} = \frac{[{\rm CuOH}^+]}{[{\rm Cu}^{2+}][{\rm OH}^-]}$$
(A.7)

$$K_{\text{OH}_2} = \frac{[\text{Cu(OH)}_2]}{[\text{CuOH}^+][\text{OH}^-]}$$
(A.8)

where the K_{sp} for copper (II) hydroxide $(2 \times 10^{-20})^{52}$ was used as the product of K_{OH_1} and K_{OH_2} . Using this plus the expression for water dissociation, an expression for the concentration of free Cu^{2+} ions in the subphase was derived (Equation A.9):

$$[Cu^{2+}] = \frac{\frac{C_{Cu}K_{sp}}{K_{w}^{2}}[H^{+}]^{2}}{1 + \frac{K_{sp}}{K_{w}^{2}}[H^{+}]^{2}}$$
(A.9)

Among the variables in Equations A.2 – A.9, the independent variables are $[H^+]$ (pH) and $[Cu^{2+}]$ (subphase concentration), and the dependent variables are [HAA], $[AA^-]$, $[CuAA^+]$, and $[CuAA_2]$. Rearranging the above equilibria expressions to solve for each of the dependent variables and assigning each one an arbitrary variable, we get Equations A.10 – A.13:

$$[AA^{-}] = \Phi_1 = \frac{K_a[HAA]}{[H^{+}]}$$
(A.10)

$$[CuAA^{+}] = \Phi_{2} = K_{1}[Cu^{2+}][[AA^{-}]]$$
(A.11)

$$[CuAA_2] = \Phi_3 = K_2[CuAA^+][AA^-]$$
(A.12)

$$[HAA] = 1 - \Phi_1 - \Phi_2 - \Phi_3 \tag{A.13}$$

Substituting Equation A.11 into Equation A.12, we get Equation A.14.

$$[CuAA_{2}] = \Phi_{3} = K_{1}K_{2}[Cu^{2+}][AA^{-}]^{2}$$
(A.14)

Replacing the [HAA] variables with the equivalent variables defined in Equation A.12 and all $[AA^{-}]$ species with Φ_1 , the four dependent variables can be defined by Equations A.15 – A.18.

$$[HAA] = 1 - \Phi_1 - \Phi_2 - \Phi_3 \tag{A.15}$$

$$[AA^{-}] = \Phi_1 = \frac{K_a}{[H^{+}]} (1 - \Phi_1 - \Phi_2 - \Phi_3)$$
(A.16)

$$[CuAA^{+}] = \Phi_{2} = K_{1}[Cu^{2+}]\Phi_{1}$$
(A.17)

$$[CuAA_{2}] = \Phi_{3} = K_{1}K_{2}[Cu^{2+}]\Phi_{1}^{2}$$
(A.18)

Rearranging all unknown variables in terms of Φ_1 , the expression for [AA⁻] becomes:

$$[AA^{-}] = \Phi_{1} = \frac{K_{a}}{[H^{+}]} (1 - \Phi_{1} - K_{1}[Cu^{2+}]\Phi_{1} - K_{1}K_{2}[Cu^{2+}]\Phi_{1}^{2})$$
(A.19)

which can be rearranged to form the following polynomial expression (Equation A.21):

$$0 = \frac{K_a}{[H^+]} - \left(1 + \frac{K_a}{[H^+]} + \frac{K_a K_1}{[H^+]} [Cu^{2+}]\right) \Phi_1 - \frac{K_a K_1 K_2}{[H^+]} [Cu^{2+}] \Phi_1^2$$
(A.20)

Using the quadratic equation, the value of Φ_1 can be solved for and used to solve for the values of [CuAA⁺] and [CuAA₂] in Equations A.17 and A.18, respectively. Microsoft Excel was used to calculate the values of each species from pH 2 – 7.

APPENDIX 3C:

Ellipsometric Film Thickness Measurements

measurements. Errors reported are standard deviations.				
Subphase pH	Thickness (Å)			
3.5	26.1 ± 0.3			
4.0	26.6 ± 0.5			
4.5	27.1 ± 0.4			
5.0	30.4 ± 0.8			
5.3	29.8 ± 1.0			
5.4	29.9 ± 0.4			
6.0	31.8 ± 0.6			
6.2	31.9 ± 0.6			
6.4	334 + 04			

Table A.1 Numerical ellipsometric thickness data for AA films formed on 1 mM Cu²⁺ subphase at each pH and deposited at the solid phase. Thickness values are averages of 20 individual measurements. Errors reported are standard deviations.

Table A.2 Numerical ellipsometric thickness data for AA films formed on 1 mM Cu²⁺ subphase at each pH and deposited at surface pressures indicative of a typical collapsed film. Thickness values are averages of 20 individual measurements. Errors reported are standard deviations.

Subphase pH	Thickness (Å)
3.6	24.2 ± 0.8
4.0	26.2 ± 0.3
4.6	27.4 ± 0.4
5.1	28.7 ± 1.0
5.4	28.4 ± 0.3
5.6	28.4 ± 0.4
6.0	29.0 ± 0.4
6.2	29.3 ± 0.7
6.5	31.3 ± 0.6

REFERENCES

REFERENCES

- 1. Yazdanian, M.; Yu, H.; Zografi, G. Ionic Interactions of Fatty Acid Monolayers at the Air-Water Interface. *Langmuir* **1990**, *6*, 1093-1098.
- Kurnaz, M. L.; Schwartz, D. K. Morphology of Microphase Separation in Arachidic Acid-Cadmium Arachidate Langmuir-Blodgett Multilayers. J. Phys. Chem. 1996, 100, 11113-11119.
- 3. Novotny, V.; Swalen, J. D. Tribology of Langmuir-Blodgett Layers. *Langmuir* **1989**, *5* (2), 485-489.
- 4. Meyer, E.; Overney, R.; Brodbeck, D.; Howald, L.; Luthi, R.; Frommer, J.; Guntherodt, H. J. Friction and Wear of Langmuir-Blodgett Films Observed by Friction Force Microscopy. *Phys. Rev. Lett.* **1992**, *69* (12), 1777-1780.
- 5. Petty, M. C. Possible applications for Langmuir-Blodgett films. *Thin Solid Films* **1992**, *210/211*, 417-426.
- 6. Tieke, B. Langmuir-Blodgett Membranes for Separation and Sensing. *Adv. Mater.* **1991**, *3*, 532-541.
- 7. Swalen, J. D., et al. Molecular Monolayers and Films. Langmuir 1987, 3, 932-950.
- 8. Mirčeski, V.; Quentel, F.; L'Her, M.; Elleouet, C. Homogeneous versus Heterogeneous Catalysis at Electrodes Modified with a Thin Organic Layer: Theoretical and Experimental Study under Conditions of Square-Wave Voltammetry. *J. Phys. Chem. C* **2007**, *111*, 8283-8290.
- 9. Nieuwenhuys, B. E. Applications of Thin Films to Fundamental Studies in the Field of Heterogeneous Catalysis. *Thin Solid Films* **1983**, *100*, 355-358.
- 10. Eloul, S.; Batchelor-McAuley, C.; Compton, R. G. Thin film-modified electrodes: a model for the charge transfer resistance in electrochemical impedance spectroscopy. *J. Solid State Electrochem.* **2014**, *18* (12), 3239-3243.
- 11. Moriizumi, T. Langmuir-Blodgett Films as Chemical Sensors. *Thin Solid Films* **1987**, *160* (1-2), 413-429.
- 12. Çaycı, D.; Stanciu, S. G.; Çapan, İ.; Erdoğan, M.; Güner, B.; Hristu, R.; Stanciu, G. A. The influence of the surface morphologies of Langmuir Blodgett (LB) thin films of porphyrins on their gas sensing properties. *Sens. Actuators, B* **2011**, *158* (1), 62-68.

- 13. Pedraz, P.; Montes, F. J.; Cerro, R. L.; Díaz, M. E. Characterization of Langmuir biofilms built by the biospecific interaction of arachidic acid with bovine serum albumin. *Thin Solid Films* **2012**, *525*, 121-131.
- 14. Pedraz, P., *et al.* Affinity separation by Langmuir–Blodgett deposition of bovine serum albumin using arachidic acid as specific ligand. *Sep. Purif. Technol.* **2015**, *143*, 161-168.
- 15. Langmuir, I. The Adsorption of Gases on Plane Surfaces of Glass, Mica and Platinum. J. Am. Chem. Soc. **1918**, 40 (9), 1361-1403.
- 16. Tao, Y. Structural Comparison of Self-Assembled Monolayers of *n*-Alkanoic Acids on the Surfaces of Silver, Copper, and Aluminum. *J. Am. Chem. Soc.* **1993**, *115*, 4350-4358.
- 17. Tamm, L. K.; McConnell, H. M. Supported Phospholipid Bilayers. *Biophys J.* **1985**, *47*, 105-113.
- 18. Bain, C. D.; Biebuyck, H. A.; Whitesides, G. M. Comparison of Self-Assembled Monolayers on Gold: Coadsorption of Thiols and Disulfides. *Langmuir* **1989**, *5*, 723-727.
- Bain, C. D.; Evall, J.; Whitesides, G. M. Formation of Monolayers by the Coadsorption of Thiols on Gold: Variation in the Head Group, Tail Group, and Solvent. J. Am. Chem. Soc. 1989, 111, 7155-7164.
- 20. Hamers, R. J. Formation and Characterization of Organic Monolayers on Semiconductor Surfaces. *Annu. Rev. Anal. Chem.* **2008**, *1*, 707-736.
- Nuzzo, R. G.; Zegarski, B. R.; Dubois, L. H. Fundamental Studies of the Chemisorption of Organosulfur Compounds on Au(111). Implications for Molecular Self-Assembly on Gold Surfaces. J. Am. Chem. Soc. 1987, (109), 733-740.
- 22. Finklea, H. O.; Avery, S.; Lynch, M. Blocking Oriented Monolayer of Alkyl Mercaptans on Gold Electrodes. *Langmuir* **1987**, *3*, 409-413.
- Porter, M. D.; Bright, T. B.; Allara, D. L.; Chidsey, C. E. D. Spontaneously Organized Molecular Assemblies. 4. Structural Characterization of *n*-Alkyl Thiol Monolayers on Gold by Optical Ellipsometry, Infrared Spectroscopy, and Electrochemistry. *J. Am. Chem. Soc.* 1987, 109, 3559-3568.
- Wasserman, S. R.; Tao, Y.; Whitesides, G. M. Structure and Reactivity of Alkylsiloxane Monolayers Formed by Reaction of Alkyltrichlorosilanes on Silicon Substrates. *Langmuir* 1989, 5, 1074-1087.
- 25. Maoz, R.; Sagiv, J. On the Formation and Structure of Self-Assembling Monolayers. J. *Colloid Interface Sci.* **1984**, *100*, 465-496.

- 26. Gun, J.; Iscovici, R.; Sagiv, J. On the Formation and Structure of Self-Assembling Monolayers. II. J. Colloid Interface Sci. **1984**, 101, 201-213.
- 27. Gun, J.; Sagiv, J. On the Formation and Structure of Self-Assembling Monolayers. III. J. *Colloid Interface Sci.* **1986**, *112*, 457-472.
- 28. Blodgett, K. B.; Langmuir, I. Built-Up Films of Barium Stearate and Their Optical Properties. *Phys. Rev.* **1937**, *51* (11), 964-982.
- 29. Oberts, B. P.; Blanchard, G. J. Headgroup-dependent lipid self-assembly on zirconium phosphate-terminated interfaces. *Langmuir* **2009**, *25* (24), 13918-13925.
- Penner, T. L.; Armstrong, N. J.; Willand, C. S.; Schildkraut, J. S.; Robello, D. R. Langmuir-Blodgett Films for Second-Order Nonlinear Optics. *Prop. Org. Mater. IV* 1991, 1560, 377-386.
- 31. Blodgett, K. B. Films Built by Depositing Successing Monomolecular Layers on a Solid Surface. J. Am. Chem. Soc. **1935**, *57*, 1007-1022.
- 32. Zasadzinski, J. A.; Viswanathan, R.; Madsen, L.; Garnaes, J.; Schwartz, D. K. Langmuir Blodgett Films. *Science* **1994**, *263*, 1726–1733.
- 33. Petty, M. C. Monolayers: Two-Dimensional Phases. In *Langmuir-Blodgett Films*, Cambridge Press: NY, 1996; pp 12-15.
- 34. Heertjes, P. M.; de Smet, E. C.; Witvoet, W. C. The Determination of Interfacial Tension with the Wilhelmy Plate Method. *Chem. Eng. Sci.* **1970**, *26*, 1479-1480.
- 35. Wu, N.; Dai, J.; Micale, F. J. Dynamic Surface Tension Measurement with a Dynamic Wilhelmy Plate Technique. *J. Colloid Interface Sci.* **1999**, *215*, 258-269.
- 36. Hönig, D.; Möbius, D. Direct Visualization of Monolayers at the Air-Water Interface by Brewster Angle Microscopy. J. Phys. Chem. **1991**, 95, 4590-4592.
- 37. Hönig, D.; Möbius, D. Reflectometry at the Brewster Angle and Brewster Angle Microscopy at the Air-Water Interface. *Thin Solid Films* **1992**, *210-211*, 64-68.
- 38. Hénon, S.; Meunier, J. Microscope at the Brewster Angle: Direct Observation of First-Order Phase Transitions in Monolayers. *Rev. of Sci. Instrum.* **1991,** *62* (4), 936-939.
- 39. Baumler, S. M.; Blanchard, G. J. The Influence of Metal Ions on the Dynamics of Supported Phospholipid Langmuir Films. *Langmuir* **2017**, *33* (12), 2986-2992.
- 40. Peltonen, J.; Linden, M.; Fagerhoim, H.; Györvary, E.; Eriksson, F. The influence of multivalent salts on the processability of a stearic acid monolayer: a stability, electron

spectroscopy for chemical analysis and atomic force microscopy study. *Thin Solid Films* **1994,** 242, 88-91.

- 41. Chen, C. W.; Liu, T. J. Maximum withdrawal speed for Langmuir-Blodgett film deposition of arachidic acid. *J. Colloid Interface Sci.* **2006**, *298* (1), 298-305.
- 42. Kundu, S.; Langevin, D. Fatty Acid Monolayer Dissociation and Collapse: Effect of pH and Cations. *Colloids Surf. A.* **2008**, *325* (1-2), 81-85.
- 43. Kijewska, K.; Blanchard, G. J. Using Diffusion To Characterize Interfacial Heterogeneity. *Langmuir* **2017**, *33* (5), 1155-1161.
- 44. Baumler, S. M.; Reidy, T. M.; Blanchard, G. J. Diffusional motion as a gauge of fluidity and interfacial adhesion. Supported alkylphosphonate monolayers. *J. Colloid Interface Sci.* **2016**, *468*, 145-155.
- 45. Roy, D.; Das, N. M.; Ganesan, V.; Gupta, P. S. Growth and Structural Study of Cadmium Arachidate Multilayers Deposited by LB Technique. *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry* **2015**, *45* (4), 560-566.
- 46. Yanklovich, M. A.; Ivanov, N. S.; Sukhodolov, N. G.; Zhukov, A. N. A Study of the Properties and Composition of Stearic Acid Monolayers on an Aqueous Subphase Containing Cadmium Ions. *Colloid J.* 2016, 78 (2), 277-280.
- 47. Luo, Y. H.; Huang, J.; Jin, J.; Peng, X.; Schmitt, W.; Ichinose, I. Formation of Positively Charged Copper Hydroxide Nanostrands and Their Structural Characterization. *Chem. Mater.* 2006, 18, 1795-1802.
- 48. Ichinose, I.; Kurashima, K.; Kunitake, T. Spontaneous Formation of Cadmium Hydroxide Nanostrands in Water. J. Am. Chem. Soc. **2004**, *126*, 7162-7163.
- 49. Skoog, D. A.; West, D. M. *Fundamentals of Analytical Chemistry;* 3 ed.; Holt, Rinehart, and Winston: New York, 1976.
- Takenaka, T.; Nogami, K.; Gotoh, H.; Gotoh, R. Studies on Built-up Films by Means of the Polarized Infrared ATR Spectrum. I. Built-up Films of Stearic Acid. *J. Colloid Interface Sci.* 1971, 35, 395-402.
- 51. Yang, L.; Frauenheim, T.; Ganz, E. Properties of the Free-Standing Two-Dimensional Copper Monolayer. *J. Nanomater.* **2016**, *2016*, 1-6.
- 52. Skoog, D. A.; West, D. M. *Fundamentals of Analytical Chemistry*; 3rd ed. ed.; Holt, Rinehart and Winston: New York, 1963.

CHAPTER 4: SPECTROSCOPIC ANALYSIS OF CU(II)-COMPLEXED THIN FILMS TO CHARACTERIZE MOLECULAR-LEVEL INTERACTIONS AND FILM BEHAVIOR

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Capistran, B. A.; Blanchard, G. J. Spectroscopic Analysis of Cu(II)-Complexed Thin Films to Characterize Molecular-Level Interactions and Film Behavior. *Langmuir* **2021**, *37*, 5089-5097. 4.1 Abstract

We report on the structure and dynamics of a Cu²⁺-complexed arachidic acid (AA) monolayer formed by Langmuir-Blodgett (LB) deposition. Infrared reflection-absorption spectroscopy (IRRAS) was used to characterize aliphatic chain -CH₂ symmetric and asymmetric stretching modes and determine the chain tilt angle and order as a function of subphase pH. Monolayer structure is controlled by metal ion-amphiphile interactions. At low subphase pH (< 5), film buckling at high surface pressure is observed, while for high subphase pH (\geq 5), monolayer buckling is not observed. This finding is correlated with monolayer structural mediation by metal ion-amphiphile interactions. Dynamics and mobility of a fluorophore incorporated into the monolayer were also affected by Cu²⁺-AA interactions, determined by fluorescence recovery after photobleaching (FRAP) measurements. These data are consistent with the formation of a rigid film due to Cu²⁺ coordination to AA headgroups, with the extent of headgroup protonation being determined by the pH of the subphase during monolayer deposition.

4.2 Introduction

The design and characterization of thin interfacial films is central to a host of applications, including chemical separations, chemical sensing, and surface passivation.¹⁻⁹ Monolayers and thin film properties can be controlled by means of monolayer constituent identity and the details of surface-bonding. For amphiphilic films, properties such as film robustness and adhesion strength can be altered by modifying amphiphile chain length, headgroup identity and bonding modality to the surface, including physisorption, covalent bonding and metal ion-complexation.¹⁰⁻¹⁴ Characterizing monolayer constituent structure, orientation and dynamics are required to understanding whether or not modified interfaces are

appropriate for their intended purposes. Typically, monolayer properties are determined at the time of formation based on the (amphiphilic) monolayer constituents used and the means of deposition, and once a monolayer is deposited, modifications involving monolayer morphology or organization are generally not feasible. Modifying monolayer properties *in situ* and reversibly after deposition has not been demonstrated generally but would prove useful in certain applications.

A variety of methods exist to deposit monolayer films, including self-assembly of thiols on coinage metals, vapor deposition, dip coating, and chemical bonding.¹⁵⁻¹⁷ Langmuir-Blodgett (LB) deposition is one of the oldest methods and it provides a facile means of forming and depositing monolayers under conditions of controlled pressure and organization.^{13, 18-19} LB deposition affords control over amphiphile identity, the incorporation of metal ions and/or chromophores, number of layers deposited, and orientation of each adlayer. Metal ioncomplexation, where metal ions reside between the substrate and amphiphilic adlayer, has been shown to enforce monolayer organization and affect the mobility of monolayer constituents.¹⁹⁻²⁷ The effects of monovalent ions (*e.g.*, Li⁺, Na⁺, K⁺) and divalent ions (*e.g.*, Mg²⁺, Ca²⁺, Ba²⁺, Zn²⁺) on film properties have been studied in detail and shown to enhance amphiphile packing and film robustness.^{20-22, 24, 26, 28-29} Such enhancements of film structure can broaden the range of applications for which these interfaces are used.

Our previous work demonstrated the formation of a highly ordered arachidic acid (AA) LB monolayer resulting from the incorporation of Cu^{2+} ions during monolayer formation and deposition.²⁸ The literature on the use of Cu^{2+} ions in LB films is not as extensive as it is for other metal ions, such as Cd^{2+} .^{25-27, 30-31} However, unlike many metal ions, Cu^{2+} can undergo reversible oxidation to Cu^{+} at a potential that does not give rise to redox processes in other

monolayer constituents. We have demonstrated that Cu^{2+} -complexed arachidic acid monolayers exhibit pressure-area (Π -A) isotherms that depend on the pH and Cu^{2+} concentration in the aqueous subphase, analogous to that seen with other metal ions.^{25, 30} However, changes in film formation and integrity unique to Cu^{2+} -complexation were observed. As the subphase pH increased toward the (solution phase) p K_a of the amphiphile and beyond, the formation of solidphase Cu^{2+} -complexed monolayer domains were observed at low surface pressures (*e.g.*, < 5 mN/m), and the monolayers ceased to exhibit a structural collapse point at surface pressures associated with amphiphile layer buckling (*e.g.*, > 50 mN/m). Brewster angle microscopy (BAM) images revealed that the films maintained non-buckled monolayer organization at these high surface pressures. It is thought that under such conditions it is energetically more favorable for excess Cu^{2+} -amphiphile complexes to move into the aqueous subphase than it is to form bilayer regions on top of the monolayer.

The work presented here is aimed at understanding the role that Cu^{2+} complexation plays in mediating the structure and dynamics of arachidic acid films. We report on the aliphatic chain order and dynamics of Cu^{2+} -complexed AA films as a function the pH of the subphase on which the monolayers were formed. Amphiphile chain tilt angle and organization were characterized using -CH₂ symmetric and asymmetric stretching frequencies and band intensity ratios. Film fluidity was measured with fluorescence recovery after photobleaching (FRAP) measurements of a chromophore confined within the aliphatic chain region of the monolayer.^{23, 32-33} We find that Cu^{2+} -amphiphile interactions affect the diffusional behavior of the chromophore, and these interactions depend on the subphase pH. These findings point to the central role of the Cu^{2+} arachidic acid complex in controlling the properties of the monolayer.

4.3 Experimental Methods

Materials. Arachidic acid (AA, Sigma Aldrich, \geq 99.0%) was purchased and used as received. All solutions used for monolayer formation were prepared at a concentration of 1 mg/mL in hexane (BDH[®], HPLC grade). Perylene (3 mol %, Aldrich, > 99%) was dissolved in the AA solution for FRAP measurements. For the growth of Cu²⁺-AA monolayers, copper (II) chloride (CuCl₂, Jade Scientific, 99.0%) was dissolved in the aqueous subphase (1 mM). Filtered water (18 MΩ) from a Milli-Q filtration system was used for the subphase in all experiments. Subphase pH was controlled with hydrochloric acid (HCl, 8.91 mM, Columbus Chemical Industries, Inc., Columbus, WI, USA) and sodium hydroxide (NaOH, 4.66 mM, Mallinckrodt Chemicals, Phillipsburg, NJ, USA).

For infrared spectroscopic measurements, films were deposited on gold-coated silicon wafers (99.999% Au, Sigma-Aldrich, St. Louis, MO, USA) cut to *ca.* 1 cm x 2 cm. For FRAP measurements, films were deposited on glass cover slides (#1, 22 x 22 mm, Alkali Scientific, Inc.). All substrates were cleaned by immersion in piranha solution (1H₂O₂:3H₂SO₄; *Caution! Strong oxidizer!*) for 10 to 15 minutes followed by rinsing with Milli-Q water until the pH returned to *ca.* 7. Substrates were stored in Milli-Q water to minimize contamination by airborne organic compounds.

Langmuir-Blodgett (LB) film formation. All LB monolayers were formed using a LB trough (KN 2003, KSV Nima, Biolin Scientific, Gothenburg, Sweden) equipped with a Brewster angle microscope (BAM) attachment (UltraBAM, Accurion, Göttingen, Germany). A platinum Wilhelmy plate was used for measuring surface pressure. For each film, the aqueous subphase solution was deposited in the trough (temperature controlled, 20.0 ± 0.5 °C) and a vacuum pump was used to clean the subphase surface of any dust or contaminants. Upon cleaning, the substrate (gold or glass) was submerged in the center well of the trough using the dipper attachment.

Approximately 1 hr was allotted for subphase equilibration. After this time, 100 μ L of the arachidic acid (or arachidic acid/perylene) spreading solution was deposited beneath the subphase surface using a syringe, with care taken to ensure the surface pressure did not exceed 0.5 mN/m to minimize the formation of multilayers. A waiting period of approximately 20 minutes was allotted for solvent evaporation. After this time, monolayer formation was initiated, with a barrier compression speed of 5 mm/min.

For films deposited at the tilted condensed phase,³⁴ barrier compression stopped when the surface pressure reached 30 mN/m. For films deposited at the post collapse phase, film formation was monitored in the corresponding isotherm and barrier compression was stopped once the surface pressure reached common collapse pressures (*ca.* 50 - 60 mN/m). For condensed phase and post collapse phase films, the barrier position was held at the respective surface pressures for *ca.* 10 - 15 minutes to allow for monolayer equilibration. Film deposition was performed by the vertical dipping method by removing the substrate from the subphase at a dipper speed of 2 mm/min. Once fully removed from the subphase, the substrate sat for *ca.* 30 minutes to allow for any residual water to dry. All experiments were controlled using the KSV NIMA LB software (Version 2.2, Biolin Scientific). The Brewster angle microscope was controlled with the UltraBAM 1.1.2 software (Accurion) and set at 53.2°. Film formation was visualized using Accurion Image 1.1.3 software (Accurion).

Infrared reflection-absorption spectroscopy (IRRAS). Infrared spectroscopy measurements were performed using a Mattson Research Series Fourier-transform infrared (FTIR) spectrometer equipped with a mercury cadmium telluride (MCT) detector and attenuated total reflectance (ATR) attachment. At each pH condition, solid phase and post collapse phase films were formed and deposited onto separate gold substrates. A clean gold substrate was used

to acquire a background scan prior to film analysis. All reflectance spectra were collected over the scan range 2000 - 3500 cm⁻¹, with 1024 scans and a resolution of 4 cm⁻¹. All spectra were collected and subsequently baseline corrected using the WinFIRSTTM software (Mattson Instruments, Madison, WI).

Fluorescence Recovery after Photobleaching (FRAP). FRAP measurements were obtained using a Nikon C2⁺ confocal laser scanning microscope equipped with a Nikon Eclipse Ti-E inverted microscope and Nikon Ti-S-CON confocal scanning system (Nikon Instruments Inc., Nikon Corporation, Tokyo, Japan). Data was collected using NIS-Elements AR (Version 4.30, Nikon Instruments Inc.) acquisition software. Measurements were taken for solid phase films deposited on glass cover slides. Excitation in all fluorescence measurements was performed at 405 nm, with emission detected using a photomultiplier tube (Nikon C2-DU3). A 20x microscope objective was used for all measurements. Prior to bleaching, a laser power of ca. 4% of the maximum laser intensity was used to maintain sufficient initial fluorescence intensity (I_0) without substantial ambient bleaching. A circular region of interest was used with a spot size of 4 μ m for all measurements. For photobleaching experiments, the fluorescence intensity in the prebleach period was acquired for one minute, followed by bleaching at 100% laser power for 4 seconds. Fluorescence recovery acquisition periods ranged from 5 to 20 minutes depending on the rate and extent of recovery. Following each bleaching measurement, the microscope stage was moved to focus on a different area of the film, and a new bleaching measurement was recorded. A minimum of six replicate bleaches were recorded for each film.

The fluorescence recovery data was exported into Microsoft Excel (Microsoft Office 365, Microsoft Corporation, Redmond, WA) and was background subtracted. Normalization was performed according to Equation 4.1,

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$$I_0 = \frac{I_{frap}(t) - I_{bleach}}{I_0 - I_{bleach}}$$

$$\tag{4.1}$$

where I_0 is the initial fluorescence intensity, $I_{frap}(t)$ is the fluorescence intensity at time (*t*), and I_{bleach} is the minimum fluorescence intensity immediately after bleaching. Given that perylene is a non-tethered chromophore, fluorescence recovery was assumed to follow the pure diffusion model.³⁵⁻³⁶ The normalized recovery curve was manually fit in Origin (OriginPro 9.0, OriginLab Corporation, Northampton, MA) to obtain the diffusion time constant (τ_D), which was used to calculate translational diffusion (Equation 4.2),

$$D_T = \frac{\omega^2}{\tau_D} \tag{4.2}$$

where ω is the spot size (in μ m) and D_T is the translational diffusion constant.

4.4 Results and Discussion

Aliphatic tail group orientation mediated by interactions between Cu^{2+} and amphiphile head group. Analysis of the Cu²⁺-arachidate films by infrared reflection-absorption spectroscopy (IRRAS) across a range of subphase pH values provides information on AA aliphatic chain orientation and organization and the effects of Cu²⁺ ions on the integrity of the monolayers, especially at high surface pressures (> 50 mN/m). Consistent with our earlier report, condensed phase (tilted and untilted)³⁴ and post collapse phase arachidic acid LB films were formed with Cu^{2+} ions for subphase pH values in the range of 3.5 to 6.5, which were chosen based on the solution pK_a of arachidic acid ($pK_a = 5.4$).²⁵ We note that the pK_a of arachidic acid in solution may differ from the pK_a of the same molecule incorporated in an organized monolayer, but we do not have a facile means of characterizing such a difference. In the discussion that follows, the term pK_a refers to the known solution phase pK_a of AA. Monolayers formed at high subphase pH conditions, where monolayer collapse was not observed, were deposited at high surface pressures (> 50 mN/m). Figure 4.1 shows example surface pressure-area (Π -A) isotherms with indicated deposition points for LB films deposited at the condensed phase (Figure 4.1A) and post-collapse phase (Figure 4.1B) at subphase pH values below and above the amphiphile p K_a .



Figure 4.1 Example isotherms for Cu²⁺-complexed arachidic acid (AA) films formed under various pH conditions at the (A) solid phase, and (B) post collapse phase. The corresponding shape at the end of each isotherm marks the surface pressure at which the film was deposited onto substrates for infrared reflection-absorption spectroscopy (IRRAS) and fluorescence recovery after photobleaching (FRAP) measurements.

As can be seen from isotherm data,²⁸ film formation was influenced by the Cu²⁺ ions as subphase pH increased, evidenced by the absence of the tilted condensed phase in the isotherms at pH \geq 5.3. For the monolayer deposited at subphase pH 5.5, the rise in surface pressure began to slow at *ca*. 25 mN/m, and thus deposition was initiated slightly below 30 mN/m to prevent film collapse. For the post collapse film deposited at subphase pH 3.5, deposition was initiated at a surface pressure lower than the others due to immediate film collapse for surface pressures slightly higher than that used to achieve the solid phase.

At pH 5.5, the isotherm for the post collapse film exhibited a surface pressure plateau at ca. 4 mN/m, which was absent in the remaining isotherms (Figure 4.1B). A surface pressure of ca. 4 mN/m is relatively small compared to those of the condensed phase region, at which these films achieve maximum organization (ca. 30 mN/m). As such, any increases in surface pressure at such low pressures are likely due to initial interactions of the amphiphiles and metal ions in the subphase. Using BAM, small "island"-like clusters of monolayer have been observed to form at low surface pressures.²⁸ The pH of this system in particular (pH 5.5) is just above the pK_a of bulk arachidic acid, resulting in an increased number of deprotonated amphiphiles available to interact with Cu^{2+} ions than at the lower pH conditions in Figure 4.1. Thus, we attribute the slight increase in surface pressure beginning at *ca*. 30 Å²/molecule to formation of these "islands" due to initial metal ion-amphiphile interactions upon the start of barrier compression. Once such clusters have formed, the system likely reaches an equilibrium until barrier compression induces larger scale film formation, which begins at *ca*. 25 Å²/molecule. We attribute the plateau at *ca*. 4 mN/m to this equilibrium state, which transitions into the liquid-condensed phase at pressures greater than 5 mN/m.

Based on the isotherms alone, changes in monolayer morphology were observed as a function of subphase pH. For an arachidic acid monolayer formed in the absence of metal ions, the molecular "footprint" is *ca.* 19 Å²/molecule, determined by extrapolation to 0 mN/m along the condensed phase region of the isotherm. With the addition of Cu^{2+} ions, however, the area

per molecule increased by an amount greater than the measurement uncertainty (± 1 Å²/molecule), suggesting a change in molecular packing (Figure 4.2).



Figure 4.2 Mean molecular area of arachidic acid (AA) as a function of subphase pH at deposition. Uncertainties represent measurement error ($\pm 1 \text{ Å}^2$ /molecule).

Similar increases in molecular area as a function of pH have been observed for copper stearate and other metal ion-fatty acid monolayers, which have been attributed to metal ion-fatty acid complexation.³⁷⁻³⁸ Consistent with these findings, we attribute the increase in area per molecule to the changes in film morphology as a function of pH.

Characterizing the amphiphile aliphatic chain tilt angle is determined by the asymmetric $(v_a, ca. 2920 \text{ cm}^{-1})$ and symmetric $(v_s, ca. 2850 \text{ cm}^{-1})$ -CH₂ stretch intensities. Figure 4.3 shows the reflectance spectra for monolayers deposited from the solid phase region of the isotherm at pH values below and above the AA pK_a. We believe that the Cu²⁺ is transferred with the monolayer onto the support during the deposition process, based on literature precedent.^{22, 26, 39}



Figure 4.3 Infrared reflection-absorption spectroscopy (IRRAS) spectra for control arachidic acid (AA) Langmuir-Blodgett (LB) monolayer (no Cu²⁺ ions) (black), and example Cu²⁺- complexed AA LB films deposited at the solid phase at pH 3.5 (blue), 5.3 (red), 5.5 (green), and 6.2 (yellow) across the truncated range 2950 – 2800 cm⁻¹. Dashed lines represent approximate maxima for absorption peaks corresponding to the asymmetric (v_a) and symmetric (v_s) -CH₂ stretching modes associated with amphiphile tail groups.

A reference spectrum for an AA film without metal ions is also shown, with the asymmetric and symmetric -CH₂ stretching bands present at 2918 cm⁻¹ and 2849 cm⁻¹, respectively (Figure 4.3), consistent with literature values.^{11, 40-41} The spectra of Cu²⁺-complexed films deposited at low subphase pH values resemble that for the AA layer without Cu²⁺ ions (Figure 4.3). In the spectra of monolayers deposited at pH values approaching and surpassing the AA p K_a (*i.e.*, pH 5.3, 5.5, and 6.2 in Figure 4.3), band positions were seen to be at slightly lower frequencies; 2915 cm⁻¹ and 2846 cm⁻¹ for asymmetric and symmetric stretches, respectively (Figure 4.3). These band positions depend on aliphatic chain order, with lower frequencies correlating with all-*trans* chains.^{11, 41} For monolayers exhibiting predominantly all-*trans* chains, it is possible to evaluate the average tilt angle, a quantity that is expected to depend on intermolecular spacing.

Calculation of aliphatic chain tilt angle. The absorbances for the peaks corresponding to the asymmetric and symmetric -CH₂ stretches were extrapolated from the IRRAS spectra. To calculate the average tilt angle for the amphiphile tail groups in each LB film, the method described by Allara and Nuzzo¹⁰⁻¹¹ was adapted for this work. Briefly, for a species such as an all-*trans* chain that has a point of attachment on a surface (*e.g.*, amphiphile head group), movement about the surface is defined by two main angles: rotation angle around the molecular axis, ϕ , and the tilt angle, θ (Figure 4.4A). The symmetric stretch dipole moment remains in the plane of the backbone and perpendicular to the aliphatic chain axis, while the asymmetric stretch dipole moment lies in the plane perpendicular to that of the aliphatic chain backbone and perpendicular to the symmetric stretch (Figure 4.4A). Rotation of the aliphatic chain by ϕ and tilt from the surface normal by θ in the reference plane (x_0 , y_0 , z_0) results in individual vector components in the new coordinate plane (x, y, z).





The rotation angle can be related to the angles between the symmetric (χ_{sym}) and asymmetric (χ_{asym}) stretches and the surface normal though Equation 4.3,¹⁰⁻¹¹ followed by the relationship of the tilt angle and the intensities of the absorbance bands (Equation 4.4),¹¹

$$\frac{\cos \chi_{asym}}{\cos \chi_{sym}} = \tan \phi \tag{4.3}$$

$$\cos^2 \theta = \frac{I_{obsd}}{3I_{calcd}} \tag{4.4}$$

where, using Allara and Nuzzo's notation,¹¹ I_{obsd} is the experimentally observed intensity, and I_{calcd} is the corresponding intensity from an isotropic spectrum of the same bands, where intensity

is proportional to absorbance (*A*) in an IR spectrum. Using Equation 4.4 for each stretching mode and simplifying results in the relationship between the modes expressed in Equation 4.3,

$$\frac{\cos^2 \chi_{asym}}{\cos^2 \chi_{sym}} = \frac{A_{asym}}{A_{sym}}$$
(4.5)

where A_{asym} and A_{sym} are the absorbances of the asymmetric and symmetric -CH₂ stretching bands, respectively. Combining Equations 4.3 and 4.5 results in Equation 4.6, the chain rotation angle in the *x*-*y* plane,

$$\tan\phi = \left(\frac{A_{asym}}{A_{sym}}\right)^{1/2}$$
(4.6)

which can then be used to calculate the tilt angle using the relationships between ϕ and θ for the individual vector components indicated in Figure 4.4B and 4.4C.¹⁰⁻¹¹

For the condensed phase and post-collapse phase films formed and deposited across the entire pH range of 3.5 to 6.5, the frequencies of the asymmetric and symmetric stretching bands exhibited a clear pH dependence (Table 4.1, Figure 4.5). Consistent with the shifts seen in the reference spectrum in Figure 4.3, the frequencies of both modes were observed to decrease in a stepwise manner as pH increased (Figure 4.5).

Table 4.1 Frequencies corresponding to the asymmetric (v_a) and symmetric (v_s) -CH₂ stretching modes for arachidic acid (AA) amphiphile tail groups, as well as the corresponding absorbance ratio and calculated tilt angle for aliphatic chains as a function of pH for Cu²⁺-complexed thin films formed at the solid and post collapse phases. All wavenumbers have uncertainties of ± 2 cm⁻¹.

	Condensed Phase			Post Collapse				
рН	-CH ₂ , v _a (cm ⁻¹)	-CH ₂ , v _s (cm ⁻¹)	Abs. Ratio	Tilt Angle (°)	-CH ₂ , v _a (cm ⁻¹)	-CH ₂ , v _s (cm ⁻¹)	Abs. Ratio	Tilt Angle (°)
3.5	2919	2850	0.90	20	2916	2849	3.53	26
4.0	2919	2850	0.67	18	2916	2850	3.71	26
4.5	2916	2850	1.00	21	2916	2850	2.35	25
5.0	2917	2849	1.43	23	2916	2850	3.62	26
5.3	2914	2846	1.00	21	2915	2846	1.00	21
5.5	2915	2846	1.00	21	2916	2847	2.11	24
6.0	2915	2846	1.50	23	2915	2847	2.24	25
6.2	2915	2846	0.99	21	2915	2847	2.15	24
6.5	2915	2846	1.50	23	2915	2847	1.97	24
Average tilt angle: 21 ± 1					Avera	ge tilt an	gle: 25 ± 2	



Figure 4.5 Frequencies for -CH₂ asymmetric (v_a) and symmetric (v_s) stretching modes obtained *via* infrared reflection-absorption spectroscopy (IRRAS) *vs* subphase pH for Langmuir-Blodgett (LB) films formed and deposited at the (A) solid phase and (B) post collapse phase.

The decrease for both modes was most noticeable for condensed phase films, which is reasonable because these films have the highest degree of order and thus the strongest attractive intermolecular interactions; however, decreasing frequencies were still observed for post collapse films (Figure 4.5). For monolayers deposited from condensed phase LB films, the asymmetric -CH₂ stretching band was centered at 2919 cm⁻¹ at pH 3.5 and shifted to 2915 cm⁻¹ at pH 6.5. Similarly, the symmetric band was centered at 2850 cm⁻¹ at pH 3.5 and 2846 cm⁻¹ at pH 6.5. These band shifts to lower frequencies are associated with increased conformational ordering, consistent with an all-*trans* aliphatic chain.⁴⁰⁻⁴³ The shift in band positions is correlated with the pK_a of AA, indicating that Cu²⁺ complexation of the carboxylate functionality plays a role in determining the ordering of the aliphatic chains. Measurement of these band positions for the post collapse films demonstrates the presence of a robust film system where the Cu²⁺ ions enforce a monolayer structure even at high surface pressures. This finding is supported by previous work,²⁸ in which the addition of Cu²⁺ ions was demonstrated to facilitate film formation and enhance film integrity at pH values above the pK_a of the amphiphile.

A comparison of Cu²⁺-arachidate films deposited at condensed phase pressure and post collapse phase pressure shows that the tilt angle of the amphiphiles does not exhibit a subphase pH dependence (Table 4.1). The absorbances and absorbance ratios for the post-collapse films were generally greater than those for the condensed phase films, particularly at pH values ≤ 5 (Table 4.1). The post-collapse films, especially those formed at lower subphase pH levels (pH < 5.3), experience buckling, typical of fatty acid monolayers and thin films, due to the limited extent of metal ion-amphiphile interactions. Buckled amphiphilic films give rise to a thickness that is slightly greater than a planar monolayer formed at condensed phase pressures. Despite the increased absorbances, the tilt angles for the post collapse films remained relatively constant across the pH range (Table 4.1). For both the condensed phase and post collapse films, an average tilt angle was calculated across the range for condensed phase and post collapse phase

experimental uncertainty, with the angle being determined by the intermolecular spacing imposed by the headgroup functionality.

The value of the tilt angle we observe here can provide some insight into the average intermolecular spacing in the monolayer. It is known that the tilt angle observed for alkanethiol monolayers on Au is 30°, and this value results from optimizing the interchain van der Waals interactions for all-*trans* chains given the enforced lattice spacing of 5.0 Å.^{11, 43-45} Indeed, there is abundant literature from investigations of alkanethiol self-assembled monolayers (SAMs) relating aliphatic chain tilt angle to the nearest neighbor distance. In addition to the data for Au(111),⁴⁴⁻⁴⁵ it is known that for Pd(111), with a nearest neighbor distance of ~4.8 Å,⁴⁶ the chain tilt angle is ~16° and for Ag(111) with a nearest neighbor distance of ~4.5 Å, $^{47-48}$ the tilt angle is ~10°.⁴⁹ In addition, the molecular spacing for a tilt angle of 0° was computationally modelled to be ca. 4.24 Å.⁴² Assuming that the arachidic acid molecules in the LB monolayers are arranged in their lowest energy orientation in both deposition phases (condensed and post collapse), comparing the tilt-angle data to that reported for SAMs allows an estimate of the intermolecular spacing, which we estimate to be *ca*. 4.9 Å. This spacing is determined by the dimensions of the carboxylate headgroups and/or coordination of these moieties to Cu²⁺. The effective "footprint" per molecule is *ca*. 24 Å²/molecule.

The molecular interactions occurring at the air-water interface are likely influenced by the change in the extent of protonation as pH increases for the condensed phase and post collapse phase films. At low pH conditions (pH < 5), the arachidic acid headgroups are protonated, thus limiting the extent of ionic interactions between the Cu²⁺ ions and neutral amphiphiles. Isotherms acquired under these conditions are consistent with this interpretation, based on their resemblance to isotherms of AA monolayers in the absence of metal ions in the aqueous subphase, where a characteristic collapse point is observed. For monolayers formed at these subphase pH conditions, the issue is whether it is energetically more favorable to move a monolayer constituent into the aqueous subphase or into the atmosphere (above the monolayer). Experimentally, for low subphase pH values, with rising surface pressure, the monolayer buckles and amphiphiles are seen to be extruded above the monolayer into atmosphere. The monolayer buckling likely induces slight relaxation of the aliphatic chains to tilt angles further from the surface normal, which is observed for the post collapse films at low pH conditions (pH < 5) (Table 4.1).

At higher subphase pH values (pH \geq 5), different behavior is seen. For anionic amphiphiles, it is energetically more favorable to extrude amphiphiles into the aqueous subphase. For subphase pH values above 5, ionic interactions between the (anionic) arachidate functionality and metal ions in the subphase are energetically closer to van der Waals interactions in the aliphatic chain regions of the AA monolayer, and molecules that are extruded from the monolayer with increasing pressure move into the aqueous subphase. Metal ionamphiphile complexes form, resulting in increased solubility of the arachidic acid molecules in the aqueous subphase. Based on the absence of collapse in the isotherms collected at subphase pH \geq 5, it appears that the extrusion into the aqueous subphase is closer to an individual molecular event than is seen for low pH conditions, where monolayer buckling is seen to involve a larger number of molecules in a cooperative extrusion process.

Characterization of amphiphile orientation for condensed phase and post collapse films provides further evidence of the formation of a robust film unique to Cu^{2+} complexation. Pending further investigation, it is likely that the Cu^{2+} ions complex *via* octahedral coordination, with two AA molecules coordinating to one metal ion in a bridging bidentate fashion.^{38, 50} Despite the limited reports on the use of Cu^{2+} in LB films, those that do involve Cu^{2+} -fatty acid complexation report the formation of crystalline-like films and nanostructures with substantial rigidity.^{37-38, 50-51} Our data are consistent with these findings. By coordinating to multiple AA molecules, the divalent metal ions effectively lock the film in place, thereby creating a rigid monolayer that resists collapse at high surface pressures.

Film fluidity mediated by Cu^{2+} -amphiphile interactions as a function of subphase pH. The diffusional motion of chromophores located in the monolayer aliphatic chain region deposited at each pH level revealed changes in monolayer organization that increased with subphase pH and Cu²⁺-amphiphile interactions. A trend is seen that as subphase pH increases, the translational diffusion of the chromophore decreases, where a rigid monolayer is seen for high subphase pH values (Table 4.2).

Table 4.2 Translational diffusion constants (D_T) and corresponding diffusion time (τ_D) for arachidic acid (AA) and Cu²⁺-complexed AA films formed across the pH range 3.5 – 6.5. Uncertainties are reported as $\pm 1\sigma$ (n = 6).

pH	$D_T (\mu \mathrm{m}^2/\mathrm{s})$	$ au_D(\mathbf{s})$
AA (no Cu^{2+})	1.89 ± 0.38	9 ± 2
3.5	1.54 ± 0.46	11 ± 3
4.0	1.09 ± 0.13	15 ± 2
4.5	0.78 ± 0.07	21 ± 2
5.0	0.35 ± 0.16	54 ± 24
5.3	0.26 ± 0.11	69 ± 22
5.5	0.20 ± 0.05	88 ± 26
6.0	0.12 ± 0.02	137 ± 25
6.2	0.16 ± 0.04	113 ± 32
6.5	0.13 ± 0.02	130 ± 24

For most pH values, the addition of Cu^{2+} ions to the monolayer resulted in lower translational diffusion constants (longer diffusion times) compared to the monolayers formed without Cu^{2+} in the subphase (Table 4.2). Comparatively smaller diffusion constants for perylene in the amphiphiles were observed with the addition of Cu^{2+} ions to the subphase. While a slight

decrease in D_T was observed even for subphase pH in the range of 3.5 to 4.5, the fluorescence recovery was similar to that of the AA layer. This effect was especially evident at pH 3.5, for which the fluorescence recovery curve resembles the recovery for the AA layer (Figure 4.6), with almost immediate fluorescence recovery following bleaching. At low pH values, interactions of arachidate amphiphiles with Cu²⁺ ions are limited due to the protonated headgroups, consistent with the similar diffusion constants with and without Cu²⁺ in the subphase.



Figure 4.6 Example fluorescence recovery curves for (A) arachidic acid (AA) monolayer with no Cu²⁺ ions (pH 6), and for Cu²⁺-complexed AA films at (B) pH 3.5, (C) pH 4.5, (D) pH 5.3, (E) pH 5.5, and (F) 6.2. Green squares represent residuals for each fluorescence recovery measurement.

At intermediate pH values, near the AA pK_a value (*i.e.*, subphase pH 5.0 to 5.5), film fluidity continues to decrease, with D_T values approximately half of those measured at lower pH

values (Table 4.2). As the extent of AA deprotonation increases, more ordered monolayers are seen to form, with a concomitant increase in monolayer rigidity. The isotherm shapes seen for subphase pH 5.0 and above, (*i.e.*, an absence of liquid expanded phase during monolayer formation and post collapse phase at high surfaces pressures, Figure 4.1B) correlate with the trends seen in D_T . This decreased fluidity is reflected in the FRAP recovery data for monolayers formed at subphase pH 5.3 and 5.5 (Figure 4.6D and 4.6E, Table 4.2). Translational diffusion (D_T) in the monolayer continues to decrease with increasing subphase pH values above the AA pK_a (*i.e.*, subphase pH 6.0 to 6.5), consistent with greater monolayer rigidity. We reported previously that for high subphase pH values, the isotherms lack clear transitions between the gaseous, liquid expanded and condensed phases (Figure 4.1B). Surface pressure increases almost immediately upon barrier compression, indicating film formation at high mean molecular areas (ca. 35 Å²/molecule). It is worth noting that monolayers formed at these subphase pH values were previously determined to lack uniformity and exhibited a higher defect density relative to those formed at subphase pH values closer to the AA pK_{a} .²⁸ It is thus not surprising that there was a greater spread in residuals for the fluorescence recovery data compared to monolayers formed at lower subphase pH values (Figure 4.6F). As noted above, this trend is likely driven by the presence of Cu²⁺ ions and directly related to the areal density of charged species at the airwater interface.

4.5 Conclusions

A full understanding of the role of Cu^{2+} ions on the organization of an arachidic acid LB monolayer has been achieved using complementary spectroscopic characterization techniques. While typically deposited at pressures in the condensed phase region of the isotherm, films deposited at surface pressures consistent with the post collapse phase (*ca.* \geq 50 mN/m)
maintained integrity as subphase pH increased, attributed to metal ion-amphiphile interactions. The asymmetric and symmetric -CH₂ stretching band positions reveal a shift toward a more ordered film at high subphase pH values. Aliphatic chain orientation for both condensed phase and post collapse phase films was determined by the competition between chain-chain and metal ion-amphiphile interactions. The Cu^{2+} ions were shown to play a central role in determining monolayer organization, effectively creating monolayers with tilt angles independent of subphase pH. Translational diffusion measurements of perylene in the aliphatic chain region of the monolayer were shown to depend on both the presence of Cu^{2+} ions and subphase pH. As subphase pH increases, film fluidity decreases because of increased interactions between arachidate and Cu^{2+} . The information reported here provides a more thorough understanding of the role that Cu^{2+} ions play on amphiphilic monolayer behavior.

REFERENCES

REFERENCES

- 1. Eloul, S.; Batchelor-McAuley, C.; Compton, R. G. Thin film-modified electrodes: a model for the charge transfer resistance in electrochemical impedance spectroscopy. *J. Solid State Electrochem.* **2014**, *18* (12), 3239-3243.
- 2. Meyer, E.; Overney, R.; Brodbeck, D.; Howald, L.; Luthi, R.; Frommer, J.; Guntherodt, H. J. Friction and Wear of Langmuir-Blodgett Films Observed by Friction Force Microscopy. *Phys. Rev. Lett.* **1992**, *69* (12), 1777-1780.
- 3. Moriizumi, T. Langmuir-Blodgett Films as Chemical Sensors. *Thin Solid Films* **1987**, *160* (1-2), 413-429.
- 4. Nieuwenhuys, B. E. Applications of Thin Films to Fundamental Studies in the Field of Heterogeneous Catalysis. *Thin Solid Films* **1983**, *100*, 355-358.
- 5. Petty, M. C. Possible applications for Langmuir-Blodgett films. *Thin Solid Films* **1992**, *210/211*, 417-426.
- 6. Swalen, J. D., et al. Molecular Monolayers and Films. Langmuir 1986, 3, 932-950.
- 7. Swalen, J. D.; Novotny, V. Tribology of Langmuir-Blodgett Layers. *Langmuir* **1989**, *5* (2), 485-489.
- 8. Tieke, B. Langmuir-Blodgett Membranes for Separation and Sensing. *Adv. Mater.* **1991**, *3*, 532-541.
- 9. Wirth, M. J.; Peter Fairbank, R. W.; Fatunmbi, H. O. Mixed Self-Assembled Monolayers in Chemical Separations. *Science* **1997**, *275*.
- 10. Allara, D. L.; Nuzzo, R. G. Spontaneously organized molecular assemblies. 1. Formation, dynamics, and physical properties of n-alkanoic acids adsorbed from solution on an oxidized aluminum surface. *Langmuir* **1985**, *I* (1), 45-52.
- 11. Allara, D. L.; Nuzzo, R. G. Spontaneously organized molecular assemblies. 2. Quantitative infrared spectroscopic determination of equilibrium structures of solution-adsorbed n-alkanoic acids on an oxidized aluminum surface. *Langmuir* **1985**, *1* (1), 52-66.
- Bain, C. D.; Evall, J.; Whitesides, G. M. Formation of Monolayers by the Coadsorption of Thiols on Gold: Variation in the Head Group, Tail Group, and Solvent. J. Am. Chem. Soc. 1989, 111, 7155-7164.
- 13. Petty, M. C. Langmuir-Blodgett Films; Cambridge University Press: NY, 1996.

- 14. Tao, Y. Structural Comparison of Self-Assembled Monolayers of *n*-Alkanoic Acids on the Surfaces of Silver, Copper, and Aluminum. *J. Am. Chem. Soc.* **1993**, *115*, 4350-4358.
- 15. Massé, P.; Ravaine, S. The Langmuir–Blodgett technique: A powerful tool to elaborate multilayer colloidal crystals. *Colloids Surf.*, A 2005, 270-271, 148-152.
- 16. Oluwatosin Abegunde, O.; Titilayo Akinlabi, E.; Philip Oladijo, O.; Akinlabi, S.; Uchenna Ude, A. Overview of thin film deposition techniques. *AIMS Mater. Sci.* **2019**, *6* (2), 174-199.
- 17. Seshan, K. *Handbook of Thin Film Deposition Processes and Techniques*. 2nd ed.; William Andrew: Norwich, NY, 2001.
- 18. Blodgett, K. B. Films built by depositing successive monomolecular layers on a solid surface. J. Am. Chem. Soc. 1935, 57, 1007-1022.
- 19. Blodgett, K. B.; Langmuir, I. Built-Up Films of Barium Stearate and Their Optical Properties. *Phys. Rev.* **1937**, *51* (11), 964-982.
- 20. Avila, L. V. N.; Saraiva, S. M.; Oliveira, J. F. Stability and collapse of monolayers of stearic acid and the effect of electrolytes in the subphase. *Colloids Surf.*, A **1999**, *154*, 209-217.
- 21. Baumler, S. M.; Blanchard, G. J. The Influence of Metal Ions on the Dynamics of Supported Phospholipid Langmuir Films. *Langmuir* **2017**, *33* (12), 2986-2992.
- Baumler, S. M.; McHale, A. M.; Blanchard, G. J. Surface Charge and Overlayer pH Influence the Dynamics of Supported Phospholipid Films. *J. Electroanal. Chem.* 2018, *812*, 159-165.
- 23. Kijewska, K.; Blanchard, G. J. Using Diffusion To Characterize Interfacial Heterogeneity. *Langmuir* **2017**, *33* (5), 1155-1161.
- 24. Kundu, S.; Datta, A.; Hazra, S. Effect of Metal Ions on Monolayer Collapses. *Langmuir* **2005**, *21*, 5894-5900.
- 25. Kurnaz, M. L.; Schwartz, D. K. Morphology of Microphase Separation in Arachidic Acid/Cadmium Arachidate Langmuir-Blodgett Multilayers. *J. Phys. Chem.* **1996**, *100*, 11113-11119.
- 26. Roy, D.; Das, N. M.; Ganesan, V.; Gupta, P. S. Growth and Structural Study of Cadmium Arachidate Multilayers Deposited by LB Technique. *Synth. React. Inorg., Met.-Org., Nano-Met. Chem* 2014, 45 (4), 560-566.
- 27. Yazdanian, M.; Yu, H.; Zografi, G. Ionic interactions of fatty acid monolayers at the air/water interface. *Langmuir* **1990**, *6*, 1093-1098.

- 28. Capistran, B. A.; Blanchard, G. J. Effects of Cu(II) on the Formation and Orientation of an Arachidic Acid Langmuir-Blodgett Film. *Langmuir* **2019**, *35* (9), 3346-3353.
- 29. Liu, M.; Kira, A.; Nakahara, H. Complex Formation between Monolyaers of a Novel Amphiphilic Thiazolylazo Dye and Transition Metal Ions at the Air/Water Interface. *Langmuir* **1997**, *13*, 779-783.
- 30. Kundu, S.; Langevin, D. Fatty acid monolayer dissociation and collapse: Effect of pH and cations. *Colloids Surf.*, A **2008**, *325* (1-2), 81-85.
- 31. Yanklovich, M. A.; Ivanov, N. S.; Sukhodolov, N. G.; Zhukov, A. N. A study of the properties and composition of stearic acid monolayers on an aqueous subphase containing cadmium ions. *Colloid J.* **2016**, *78* (2), 277-280.
- 32. Baumler, S. M.; Reidy, T. M.; Blanchard, G. J. Diffusional motion as a gauge of fluidity and interfacial adhesion. Supported alkylphosphonate monolayers. *J. Colloid Interface Sci.* **2016**, *468*, 145-155.
- 33. Wachsmuth, M. Molecular Diffusion and Binding Analyzed with FRAP. *Protoplasma* **2014**, *251* (2), 373-382.
- 34. Kaganar, V. M.; Mohwald, H.; Dutta, P. Structure and phase transitions in Langmuir monolayers. *Rev. Mod. Phys.* **1999**, *71*, 779-819.
- 35. Baumler, S. M. Diffusional Motion as a Gauge of Interfacial Fluidity and Adhesion of Supported Model Membrane Films. Ph.D. Dissertation, Michigan State University, East Lansing, MI, 2017.
- 36. Soumpasis, D. M. Theoretical Analysis of Fluorescence Photobleaching Recovery Experiments. *Biophys. J.* **1983**, *41*, 95-97.
- 37. Stickland, F. G. W. The Formation of Monomolecular Layers by Spreading a Copper Stearate Solution. *J. Colloid Interface Sci.* **1971**, *40* (2), 142-153.
- 38. Wolstenholme, G. A.; Schulman, J. H. Metal-monolayer interactions in aqueous systems. Part I.-The interaction of monolayers of long-chain polar compounds with metal ions in the underlying solution. *Trans. Faraday Soc.* **1950**, *46*, 475-487.
- Peltonen, J.; Linden, H.; Fagerholm, H.; Gyorvary, E.; Eriksson, F. The influence of multivalient salts on the processability of a stearic acid monolayer: A stability, electron spectroscopy for chemical analysis and atomic force microscopy study. *Thin Solid Films* 1994, 242, 88-91.
- 40. Cheng, S.; Li, S.; Tsona, N. T.; George, C.; Du, L. Insights into the Headgroup and Chain Length Dependence of Surface Characteristics of Organic-Coated Sea Spray Aerosols. *ACS Earth Space Chem.* **2019**, *3* (4), 571-580.

- 41. Simon-Kutscher, J.; Gericke, A.; Hühnerfuss, H. Effects of Bivalent Ba, Cu, Ni, and Zn Cations on the Structure of Octadecanoic Acid Monolayers at the Air-Water Interface As Determined by External Infrared Reflection-Absorption Spectroscopy. *Langmuir* 1996, 12, 1027-1034.
- 42. Mendelsohn, R.; Brauner, J. W.; Gericke, A. External Infrared Reflection Absorption Spectrometry of Monolayer Films at the Air-Water Interface. *Annu. Rev. Phys. Chem.* **1995**, *46*, 305-334.
- 43. Ulman, A. Formation and Structure of Self-Assembled Monolayers. *Chem. Rev.* **1996**, *96*, 1533-1554.
- 44. Camillone, N. I.; Chidsey, C. E. D.; Liu, G.-Y.; Scholes, G. Superlattice structure at the surface of a monolayer of octadecanethiol self-assembled on Au(111). *J. Chem. Phys.* **1993**, 98, 3503 3511.
- Dubois, L. H.; Zegarski, B. R.; Nuzzo, R. G. Molecular ordering of organosulfur compounds on Au(111) and Au(100): Adsorption from solution and in ultrahigh vacuum. J. Chem. Phys. 1993, 98, 678 - 688.
- 46. Speller, S.; Rauch, T.; Bomermann, J.; Borrmann, P.; Heiland, W. Surface structures of S on Pd(111). *Surf. Sci.* **1999**, *441*, 107 116.
- 47. Rovida, G.; Pratesi, F. Sulfur overlayers on the low-index faces of silver. *Surf. Sci.* **1981**, *104*, 609-624.
- Schwaha, K.; Spencer, N. D.; Lambert, R. M. A single crystal study of the initial stages of silver sulphidation: The chemisorption and reactivity of molecular sulphur (S₂) on Ag(111). *Surf. Sci.* 1979, 81, 273-284.
- 49. Wang, Z.; Chen, J.; Oyola-Reynoso, S.; Thuo, M. The Porter-Whitesides discrepancy: Revisiting odd-even effects in wetting properties of n-alkanethiol SAMs. *Coatings* **2015**, *5*, 1034-1055.
- 50. Rardin, R. L.; Tolman, W. B.; Lippard, S. J. Monodentate carboxylate complexes and the carboxylate shift: Implications for polymetalloprotein structure and function. *New J. Chem.* **1991**, *15*, 417-430.
- Khomutov, G. B.; Yakovenko, S. A.; Soldatov, E. S.; Khanin, V. V.; Nedelcheva, M. D.; Yurova, T. V. Interaction of copper ions with stearic acid Langmuir monolayer and formation of cluster structures in monolayers and Langmuir-Blodgett films *Membr Cell Biol.* **1997**, *10* (6), 665-681.

CHAPTER 5: EXCITED STATE DYNAMICS OF A SUBSTITUTED FLUORENE DERIVATIVE. THE CENTRAL ROLE OF HYDROGEN BONDING INTERACTIONS WITH THE SOLVENT

5.1 Abstract

Substituted fluorene structures have demonstrated unusual photochemical properties. Previous reports on the substituted fluorene Schiff Base FR0-SB demonstrated super photobase behavior with $\Delta p K_b \sim 15$ on photoexcitation. In an effort to understand the electronic structural basis for this unusual behavior, we have examined the electronic structure and relaxation dynamics of the structural precursor of FR0-SB, the aldehyde FR0, in protic and aprotic solvents using time-resolved fluorescence spectroscopy and quantum chemical calculations. The calculations show three excited singlet states in relatively close energetic proximity. The spectroscopic data are consistent with relaxation dynamics from these electronic states that depend on the presence and concentration of solvent hydroxyl functionality. These results underscore the central role of solvent hydrogen-bonding to the FR0 aldehyde oxygen in mediating the relaxation dynamics within this molecule.

5.2 Introduction

Chemical reactions are typically performed in the liquid phase or at the interface between phases with reactants in their ground states. Under such conditions there is little opportunity to control the temporal or spatial extent of the reaction. While excitation of one reactant is required to initiate photochemical reactions, the use of these reactions has historically been limited. Gaining broader utility in photochemical reactions affords new opportunities for temporal and spatial control of reaction chemistry, properties that are central to the emerging field of precision chemistry and high-speed chemical sensing. Essentially all chemical reactions are either acidbase (proton transfer) or redox (electron transfer) reactions. The ability to alter the acidity or basicity of certain compounds through photoexcitation has been explored extensively for photoacids (proton donation), but the development of photobases (proton abstraction upon photoexcitation) has been much more limited.

Recently, we have focused on the design and characterization of super photobases, molecules that are capable of exhibiting changes in K_b of 15 orders of magnitude ($\Delta p K_b \sim 15$) upon photoexcitation.¹⁻² While not as well understood as their super photoacid counterparts,³⁻⁴ super photobases become highly reactive when in their excited states, allowing for proton abstraction by the photobase from its surrounding environment. The behavior of one of the few known super photobases, FR0-SB (Figure 5.1A), (FR0-SB*) in protic and aprotic media has been reported previously,¹⁻² providing fundamental insight into the proton abstraction reaction that occurs in protic solvents. Among the issues that requires further investigation is the chemical structural basis that affords such a large change in the basicity of the imine nitrogen upon photoexcitation. In an effort to understand more completely the structural properties that lead to super photobase behavior, we have examined the excited state properties of the aldehyde precursor of FR0-SB, FR0 (Figure 5.1B).



Figure 5.1 (A) Structure of the FR0-SB Schiff base and (B) structure of the FR0 precursor.

A comprehensive understanding of the reactivity and excited state population relaxation dynamics of such precursor molecules is central to the rational design of super photobases with controlled properties for specific applications. In this work, we report on the reactivity and excited-state relaxation dynamics of the FR0 precursor molecule in protic and aprotic solvent media using steady-state and time-resolved spectroscopy in conjunction with quantum chemical computational data.

The spectroscopic properties of the super photobase FR0-SB lend themselves to the study of its photoreactivity. Emission bands for unprotonated and protonated forms of the Schiff base are well resolved, and the time-evolution of those band intensities provides direct insight into solvent proton abstraction by FR0-SB*. The data reported here for FR0 also exhibit spectral dynamics, although resolution of individual bands is not as facile. The emission spectrum of FR0 exhibits time-dependent changes in band shape and position in protic solvents, suggesting a significant role of the FR0 chromophore structure in the unusual photobase behavior of the corresponding Schiff base. We report on the presence of overlapped electronic singlet state manifolds within the FR0 emission band and population exchange between those singlet states in protic solvents. The excited state relaxation dynamics of FR0 depend on the identity of the (protic) solvent. Spectral reconstruction from the time-resolved emission data reveals the details of band evolution and demonstrates that the relaxation dynamics of the FR0 precursor molecule are mediated by hydrogen-bonding interactions between FR0 and the surrounding solvent medium.

5.3 Experimental Methods

Materials. n-Propanol (99.7%, anhydrous), *n*-pentanol (\geq 99%, ACS grade), and dimethyl sulfoxide (DMSO, \geq 99.9%, anhydrous) were all purchased from Sigma-Aldrich and used as received. *n*-Pentanol, which was not available in the anhydrous form, was stored over molecular sieves (Type 3A).

Synthesis of FR0. The synthesis of FR0 followed the procedure reported previously⁵ and is described briefly here. The synthesis commenced with fluorene, which was converted to 2-Bromofluorene by treating with *N*-bromosuccinimide (NBS) and subsequently methylated at the C9 position with iodomethane in the presence of NaOH. Fuming nitric acid was used for nitration to yield 2-bromo-9,9-dimethyl-7-nitro-9*H*-fluorene, which was subsequently reduced to the corresponding amine with iron powder suspended in an aqueous ammonium chloride solution. The resulting product was heated with ethyl iodide and potassium carbonate to introduce the diethyl substituents on the amine. FR0 was obtained upon formylation of the resultant metal/halogen exchanged intermediate which exchange promoted by the addition of *n*butyllithium, as a yellow solid. ¹H-NMR (500 MHz, CDCl₃), δ (ppm) = 9.98 (d, *J* = 0.8 7.90 – 7.85 (m, 1H), 7.78 (dt, *J*1 = 7.8 Hz, *J*2 = 1.1 Hz, 1H), 7.68 – 7.59 (m, 2H), 6.73 – 6.66 (m, 2H), 3.46 (q, *J* = 7.1 Hz, 4H), 1.50 (s, 6H), 1.30 – 1.20 (m, 7H). ¹³C-NMR (125 MHz, CDCl₃): δ (ppm) = 192.05, 157.33, 153.19, 148.99, 146.90, 133.41, 131.27, 125.20, 122.47, 122.35, 118.12, 110.87, 105.06, 46.62, 44.71, 27.24, 12.59.

The compound was stored in ethanol (1 mM). To prepare samples of FR0 in each solvent, aliquots of FR0 were brought to dryness using nitrogen and reconstituted with the solvent system to be studied at a concentration appropriate for the measurement, typically *ca*. $5x10^{-6}$ M.

Steady-State Emission Spectroscopy. Emission spectra for FR0 in each of the solvents were collected using a Hitachi F-4500 fluorescence spectrometer and Spex Fluorolog 3 emission spectrometer. Quartz cuvettes (1 cm) were used for all measurements and spectra were acquired using an excitation wavelength of 440 nm, with excitation and emission spectral resolution of 1 nm.

Time-Resolved Fluorescence Spectroscopy. Time-resolved fluorescence measurements were collected using a time-correlated single photon counting (TCSPC) instrument that has been described in detail elsewhere.⁶⁻⁷ Briefly, the light source is a CW passively mode-locked Nd:YVO₄ laser (Spectra Physics Vanguard), that produces 13 ps pulses at 1064 nm (80 MHz repetition rate). The output of this laser is at the second harmonic (532 nm) and the third harmonic (355 nm), with 2.5 W average power at both wavelengths, with the same pulse duration. For the experiments reported here, the cavity-dumped dye laser (Coherent 702-2) is excited by the third harmonic output of the pump laser. The dye used is Stilbene 420 (Exciton) and the dye laser output at 440 nm is 5 ps FWHM pulses at a repetition rate of 4 MHz. The pulses from the dye laser are divided, with approximately half going to a reference channel diode (Becker and Hickl PHD-400-N), and the remaining light going to the sample. The vertically polarized excitation light was focused in the sample cuvette and a reflecting microscope objective (40X, Ealing) was used to collect emission. The collected emission is passed through a polarization-selective beamsplitter and sent to two identical detection channels. Each detection channel has a subtractive double monochromator (Spectral Products CM-112) and a microchannel plate PMT detector (Hamamatsu R3809U-50). Signals from each detector are sent to TCSPC detection electronics (Becker & Hickl SPC-132). Typical instrument response function for this system is ca. 35 ps FWHM. The TCSPC acquisition electronics, PMT bias and monochromator wavelengths are controlled using a computer program written in-house using LabVIEW[®] (National Instruments) software.

For these experiments, time-resolved fluorescence data were acquired from 470 nm to 620 nm, in 10 nm increments. All raw time-domain data were exported to Microsoft Excel (Microsoft Office 365, Microsoft Corporation, Redmond, WA). Data analysis, including extraction of fluorescence lifetime decay constants and band fitting, was performed using Microcal Origin (OriginPro 9.0, OriginLab Corporation, Northampton, MA).

5.4 Results and Discussion

The super photobase FR0-SB exhibits an anomalous change in its excited state pK_b ,¹⁻² and among the issues that remain to be resolved is the role that the fluorene derivative chromophore structure plays in producing this behavior. In this work, we investigate the precursor to FR0-SB, the aldehyde FR0 (Figure 5.1). This molecule exhibits unusual fluorescence relaxation dynamics in protic solvents. We present the time-resolved spectra for FR0 in *n*-propanol (Figure 5.2A), *n*-pentanol (Figure 5.2B) and DMSO (Figure 5.2C). These spectra were extracted from time-domain experimental data with integrated emission intensity normalized to the steady state fluorescence spectrum (Appendix, Figures A.4 – A.5).



Figure 5.2 Time-resolved spectra for FR0 in (A) *n*-propanol, (B) *n*-pentanol, and (C) DMSO extracted from experimental fluorescence lifetime data.

For the time-resolved spectral evolution seen with FR0-SB, there were well resolved emission bands corresponding to unprotonated and protonated species,¹ but for FR0, there is no

corresponding imine and consequently no analogous spectrally-resolved features. It is thus important to consider first whether the data shown in Figures 5.2A and 5.2B represent spectral shifts of a single electronic state in time or the evolution of populations in multiple spectrally overlapped bands. There are several factors that point to the latter explanation being correct.

There is a substantial literature related to spectral evolution and its relationship to solvation dynamics. The probe molecule Coumarin 153 has been studied extensively, and it has been found to exhibit a time-resolved florescence Stokes-shift⁸⁻¹⁴ that is mediated by solvent relaxation.¹⁵⁻¹⁶ The data for the time-resolved Stokes-shift seen for Coumarin 153 resemble, at least qualitatively, the data shown in Figures 5.2A and 5.2B. For Coumarin 153 the observed spectral relaxation correlates with the solvent Debye relaxation time, and this timescale (*ca.* 50-100 ps) is somewhat shorter than that of the spectral shift we observe, but not necessarily at odds with the relaxation times of normal alcohols.¹⁷ Significantly, the solvent-relaxation model presumes that the spectral dynamics are mediated by relaxation along a featureless reaction coordinate that is determined by the time required for the solvent surrounding the dipolar excited state to accommodate to the change in dipole moment orientation and magnitude resulting from excitation. If other intramolecular processes such as intramolecular vibrational relaxation (IVR) or relaxation between multiple electronic state manifolds can be shown to contribute, then the spectral relaxation process cannot be assigned to solvent relaxation alone.

There are several pieces of information, both experimental and computational, that point to the spectral dynamics we report here being understood in the context of intramolecular relaxation dynamics that are mediated by specific intermolecular interactions. In addition to the analysis provided in the following sections, we have taken the time-resolved emission data at several different wavelengths across the FR0 emission band and have extracted the rotational diffusion time constants from different emission wavelengths. The reorientation time of FR0 in the alcohols exhibits a measurable wavelength-dependence. We will return to a more thorough discussion of this point below, but wavelength-dependent reorientation times requires the existence of multiple excited electronic states, each with a finite radiative lifetime. We assert that the correct analysis of the data involves multiple electronic states rather than the temporal evolution along a solvent-mediated relaxation coordinate for a single electronic state.

Computational chemistry has demonstrated its importance in understanding molecularscale phenomena and spectroscopic dynamics of the super photobase FR0-SB.¹⁻² We have applied the same computational approach to FR0 and the results of those calculations reveal several interesting features that assist in our understanding of the complex spectral dynamics shown in Figure 5.2. The computations performed were done following procedures described previously.^{1, 18} The results of those computations are shown in Tables 5.1 and 5.2, and Figures 5.3 and 5.4. The Mulliken charges in the S₀, S₁, S₂ and S₃ states are shown in Figure 5.3 and the comparison of the magnitude and direction of the permanent dipole moments in each state are shown in Figure 5.4.

Table 5.1 Vertical excitation energies ω_n (in eV) along with the permanent dipole moments μ_n and their cartesian components (all in Debye) of the lowest four singlet excited states, S₁–S₄, characterizing FR0 in the gas phase. The dipole moment of the ground electronic state, S₀, is given for comparison.

State	ωn ^a	$\mu_x^{\mathbf{b}}$	$\mu_y^{\mathbf{b}}$	$\mu_z^{\mathbf{b}}$	μ_n^{b}
S_0		6.1	0.5	-0.5	6.2
S_1	3.56	15.1	-1.3	-1.4	15.2
S_2	3.81	2.4	-0.1	-0.1	2.5
S ₃	3.95	10.0	0.3	-0.8	10.0
S_4	4.27	7.8	0.4	-0.7	7.8

^a Calculated as $\omega_n = \omega_n^{(\text{EOMCCSD/6-31+G*})} + [\omega_n^{(\delta-\text{CR-EOMCC/6-31G})} - \omega_n^{(\text{EOMCCSD/6-31G})}].$

^b Calculated using (EOM)CCSD/6-31+G*.

Target State		Initial State									
		S ₀		S ₁		S2	S ₃				
	TDM	Osc. Str.	TDM	Osc. Str.	TDM	Osc. Str.	TDM	Osc. Str.			
S_1	7.5	0.84									
S_2	0.2	0.00	0.2	0.00							
S ₃	2.9	0.14	3.4	0.02	0.1	0.00					
S 4	0.5	0.00	0.2	0.00	0.0	0.00	0.8	0.00			

Table 5.2 Transition dipole moments (in Debye) and oscillator strengths characterizing the electronic excitation/de-excitation between the ground and the lowest four excited singlet states characterizing the FR0 in the gas phase. Only unique transitions are given in the table.^a

^a Calculated using EOMCCSD/6-31+G*.

There are several interesting points that stem from these results. The first is that there are three excited electronic singlet states in relatively close energetic proximity (Table 5.1). The energetic proximity of these states suggests facile population relaxation from S_3 to S_2 or S_1 and from S_2 to S_1 . A second important result of these calculations is that the S_0 , S_1 and S_3 states all exhibit relatively similar electron densities for the aldehyde moiety, with the carbonyl oxygen carrying a Mulliken charge of *ca.* –0.4 and, not surprisingly, the dipole moments are oriented along similar axes (Table 5.1, Figure 5.3). The S_2 state, however, exhibits a significantly different electron density distribution for the aldehyde group (Figure 5.4), with the carbonyl oxygen exhibiting a Mulliken charge of *ca.* –0.1 and the aldehyde carbon being substantially more negative (Table 5.1, Figure 5.3). These differences in Mulliken charges are reflected in the relatively small permanent dipole moment in S_2 relative to that seen for the other electronic states (Table 5.1, Figure 5.4). These findings are consistent with our experimental data, as discussed below.



Figure 5.3 Mulliken charges for individual atoms in the FR0 molecule in the S₀, S₁, S₂, S₃ states.



Figure 5.4 Magnitude and direction of permanent dipole moments for excitation of FR0 in the ground state to each of the lowest three excited singlet states.

The existence of several excited electronic states in close energetic proximity combined with the unusual spectral dynamics in alcohols suggests that these experimental results are accounted for by population relaxation between the excited electronic states and radiative relaxation from each state, rather than the temporal evolution of a single band. We propose a kinetic model that is consistent with the observed spectroscopic behavior (Figure 5.5), as defined by Equations 5.1 - 5.3.



Figure 5.5 Proposed kinetic model for the excitation and relaxation of FR0 to the lowest three excited singlet states, in which *k* denotes the rate constant associated with each transition. Dashed arrows represent radiative decays and solid arrows represent non-radiative decays.

$$\frac{dS_3}{dt} = -(k_{30} + k_{31} + k_{32})S_3$$
(5.1)

$$\frac{dS_2}{dt} = k_{32}S_3 - (k_{20} + k_{21})S_2$$
(5.2)

$$\frac{dS_1}{dt} = k_{31}S_3 + k_{21}S_2 - k_{10}S_1 \tag{5.1}$$

In this model, the transitions associated with k_{30} , k_{20} and k_{10} are all considered to be radiative and the relaxation phenomena described by k_{32} , k_{31} and k_{21} are non-radiative. Also in this model we consider excitation to be instantaneous and indicated by the quantity $\delta(t)$ (Figure 5.5), which is taken to indicate a short laser pulse. The temporal evolution of the populations of each state is given by Equations 5.4 - 5.6,

$$S_{3}(t) = S_{3}(0) \exp\left(-\left(k_{30} + k_{31} + k_{32}\right)t\right)$$
(5.4)

$$S_{2}(t) = \left(S_{2}(0) + \int_{0}^{t} k_{32} \exp(k_{20} + k_{21}) x \cdot S_{3}(x) dx\right) \exp(-(k_{20} + k_{21})t)$$
(5.5)

$$S_{1}(t) = \left(S_{1}(0) + \int_{0}^{t} \exp(k_{10}x) \cdot \left(k_{21}S_{2}(x) + k_{31}S_{3}(x)dx\right)\right) \exp(-(k_{10})t)$$
(5.6)

With the time-resolved intensities of the three bands determined, it is possible to estimate the rate constants for the model shown in Figure 5.5 and Equations 5.1 – 5.3. Comparing the kinetic model described above to experimental data is not as straightforward as fitting individual time-resolved fluorescence intensity decays at specific wavelengths. As noted above, the emission spectrum of FR0 is broad and relatively featureless, and the presence of multiple radiative decays will be complicated by spectral overlap of (unresolved) bands. We have fitted the steady-state emission spectra of FR0 in the solvents used to three (Gaussian) bands (Figure 5.6). Using these fitted band energies, we have taken time-slices of the normalized time-domain data and have fitted those time-resolved spectra to determine the band intensities of each of the fitted bands as a function of time (Appendix, Figure A.6). In that manner we extract the timeevolution of each of the three spectral features. Based on the transition energies, we assign the highest energy emission band to be $S_3 \rightarrow S_0$ emission, the intermediate band to be $S_2 \rightarrow S_0$ emission and the lowest energy band to be $S_1 \rightarrow S_0$ emission. While not without precedent, it is noteworthy that $S_2 \rightarrow S_0$ emission is dominant.¹⁹⁻²⁰



Figure 5.6 Steady-state (ss) fluorescence emission spectra of FR0 in (A) *n*-propanol (PrOH), (B) *n*-pentanol (PeOH), and (C) DMSO in the frequency domain fit to three Gaussian bands.

In addition to the complexities associated with spectral overlap and limited signal-tonoise ratio, extracting the rate constants from the time-resolved spectral data is inherently underdetermined because we are trying to evaluate six rate constants using the decay profiles of three bands. For this reason, we apply the following constraints to place physically realistic estimates on our estimated rate constants. The first constraint is that all rate constants must be greater than or equal to zero. The second constraint is that the lifetime of the first excited singlet state, S₁, is on the order of several nanoseconds (*i.e.*, $k_{10} \le 10^{\circ}$). With these basic constraints, we have used a Monte Carlo fitting routine (coded in-house) to estimate the rate constants for the temporal evolution of the fitted emission bands (Table 5.5). The fits of Equations 5.1 – 5.3 to the experimental data using the rate constants in Table 5.5 are shown in Figure 5.7. We note that the spectral dynamics we have discussed to this point are seen in protic solvents but not in aprotic solvents, such as DMSO. Based on this information, we assert that the spectral dynamics we observe are associated with hydrogen bonding between solvent molecules and the FRO chromophore.

Table	5.3	Fitted	rate	constants	for	the	relax	ations	associa	ated	with	each	of	the	three	fitted
emissi	on t	oands (spect	ral feature	es) fo	or F	FR0 i	n <i>n-</i> pr	opanol	and	<i>n</i> -pe	ntanol	. U	Jnce	rtaintie	es are
reporte	ed as	$\pm 1\sigma$ (n = 10)).												

Rate Constant and	Solvent					
Associated Transition	<i>n</i> -propanol (× 10 ⁹)	<i>n</i> -pentanol (× 10 ⁹)				
k ₃₂	9.47 ± 1.02	9.02 ± 0.22				
k ₃₁	1.89 ± 1.10	1.70 ± 0.20				
k30	1.86 ± 0.63	4.21 ± 0.50				
k ₂₁	2.73 ± 0.62	0.40 ± 0.15				
k_{20}	3.33 ± 0.81	0.23 ± 0.14				
k ₁₀	0.42 ± 0.05	0.84 ± 0.26				



Figure 5.7 Normalized intensities of the three fitted bands (spectral features) extracted from the normalized time-domain data as a function of time for FR0 in (A) *n*-propanol and (B) *n*-pentanol.

While the ability of the solvent to hydrogen bond with FR0 is related to the observed spectral dynamics, it is important to consider what the dominant factor in that dependence is. Based on the data shown in Table 5.5 it is not possible to determine whether the observed solvent-dependent changes in rate constants are dependent primarily on the hydroxyl

concentration or the viscosity of the solvent. An alternative way to phrase this uncertainty is whether or not large amplitude molecular motion, such as rotation of the diethylamino moiety, mediates the observed spectral relaxation dynamics. We can resolve this issue experimentally.

We have performed a series of measurements in binary solvent systems of DMSO and *n*-propanol in which the amount of each solvent is controlled. The reason for the choice of DMSO and *n*-propanol as the two solvents is twofold: first, the spectral dynamics seen in *n*-propanol are not seen in DMSO, and second, the two solvents have essentially the same bulk viscosity ($\eta_{DMSO} = 1.99$ cP and $\eta_{n-propanol} = 1.96$ cP). Thus, the binary solvent systems used will allow control over hydroxyl group concentration ([OH]) under constant viscosity conditions. We report the fitted rate constants for 0%, 25%, 50% and 75% DMSO in Table 5.6, determined from the time evolutions of each fitted band (full series of data used for such determinations presented in the Appendix, Figures A.7 – A.10).

Table 5.4 Fitted rate constants for the relaxations associated with each of the three fitted emission bands (spectral features) for FR0 in *n*-propanol/DMSO binary solvent systems. Uncertainties are reported as $\pm 1\sigma$ (n = 10).

Rate Constant	Solvent							
and Associated Transition	<i>n</i> -propanol (× 10 ⁹)	75% <i>n</i> -propanol (× 10 ⁹)	50% <i>n</i> -propanol (× 10 ⁹)	25% <i>n</i> -propanol (× 10 ⁹)				
k ₃₂	9.47 ± 1.02	13.67 ± 0.26	14.64 ± 0.79	16.74 ± 1.04				
k ₃₁	1.89 ± 1.10	4.10 ± 0.56	4.91 ± 0.36	5.31 ± 0.50				
k ₃₀	1.86 ± 0.63	1.01 ± 0.34	1.76 ± 1.10	0.30 ± 0.36				
k ₂₁	2.73 ± 0.62	2.80 ± 0.14	2.70 ± 0.16	2.60 ± 0.60				
k ₂₀	3.33 ± 0.81	1.29 ± 0.13	0.80 ± 0.06	0.14 ± 0.13				
k ₁₀	0.42 ± 0.05	0.36 ± 0.02	0.37 ± 0.01	0.32 ± 0.04				

These data are shown graphically in Figure 5.8, and they reveal several interesting features. Specifically, the rate constants k_{32} and k_{31} decrease with increasing [OH], k_{20} increases with [OH] and the rate constants k_{30} , k_{21} and k_{10} are independent of [OH]. One conclusion from these data is that the spectral dynamics we observe are associated with [OH] and not large

amplitude molecular motion. This finding is consistent with the computational results of Mulliken charges (Figure 5.3) showing no significant accumulation of positive charge on the amino nitrogen in any of the excited electronic states, precluding any contribution from charge-separated species.



Figure 5.8 Fitted rate constants for FR0 in *n*-propanol/DMSO binary mixture as a function of percent *n*-propanol present.

The [OH] concentration-dependence of k_{32} and k_{31} indicates that the formation of a hydrogen bond between FR0 and the solvent alcohol mediates intramolecular relaxation from S_3 to both S_2 and S_1 . The formation of a hydrogen bond between FR0 and an alcohol does not, however, influence k_{30} or k_{10} . It is possible that the formation of a hydrogen bond between the alcohol proton and the FR0 carbonyl oxygen serves to reduce the dipole moment of the excited FR0, thereby diminishing the dipolar coupling between the excited FR0 and DMSO. This explanation is based on the observation that k_{3x} is faster than we can measure with our instrumentation in DMSO, implying either facile quenching of S_3 by DMSO or dipolar

enhancement of internal conversion in FR0. The observation that k_{20} increases with [OH] is consistent with the formation of the H-bond between the alcohol proton and FR0 carbonyl oxygen in S₂ increasing the magnitude of the dipole moment relative to the gas phase value, enhancing dipolar DMSO-FR0(S₂) interactions. An implication of these explanations for our experimental observations is that there may be observable state-dependent solvent-interactions with the FR0 chromophore.

We can test this hypothesis by examining the rotational diffusion behavior of FR0 as a function of emission wavelength, and we provide these data in Table 5.7. These data demonstrate state-dependent reorientation dynamics for FR0 in protic solvents and state-independent reorientation dynamics in DMSO. The reorientation time constants for FR0 in *n*-propanol and *n*-pentanol exhibit three regions, with the reorientation time being fastest in S_3 and slowest in S_2 , with S_1 reorientation being intermediate in value. There is no state dependence for FR0 in DMSO outside the experimental uncertainty, consistent with rapid relaxation of higher excited singlet states in this dipolar solvent. While it may be tempting to extract more quantitative information from these data relating to solvent association between FR0 and the alcohols, there are several factors (*e.g.*, local heating, hydrogen bond lifetime in each state) that we cannot determine with sufficient certainty to allow further interpretation. We note that it is unusual for a molecule to exhibit significant radiative lifetimes for each of three singlet electronic states and this anomalous behavior invites further investigation.

λ (nm)	R (0)ргон	тоr-proн (ps)	R (0) _{РеОН}	ток-реон (ps)	R(0)dmso	tor-dmso (ps)
490	0.17 ± 0.05	271 ± 90	0.10 ± 0.03	491 ± 98	0.17 ± 0.02	242 ± 36
500	0.19 ± 0.02	400 ± 58	0.11 ± 0.01	788 ± 53	0.19 ± 0.01	331 ± 27
520	0.17 ± 0.01	610 ± 48	0.15 ± 0.01	941 ± 45	0.18 ± 0.01	309 ± 22
540	0.17 ± 0.01	571 ± 36	0.15 ± 0.01	1250 ± 43	0.19 ± 0.01	277 ± 19
560	0.18 ± 0.01	678 ± 40	0.19 ± 0.01	1489 ± 36	0.22 ± 0.01	319 ± 24
570	0.17 ± 0.01	734 ± 32	0.21 ± 0.01	1235 ± 29	0.21 ± 0.01	351 ± 28
580	0.19 ± 0.01	512 ± 19	0.21 ± 0.01	1057 ± 30	0.26 ± 0.03	319 ± 33
590	0.22 ± 0.01	518 ± 20	0.27 ± 0.01	1057 ± 29	0.36 ± 0.04	252 ± 26

Table 5.5 Fluorescence anisotropy and lifetime components for FR0 in *n*-propanol (PrOH), *n*-pentanol (PeOH), and DMSO. Uncertainties reported are associated with the individual fits.

The spectral dynamics data, taken in conjunction with the wavelength-dependent reorientation data, support the validity of our interpretation of the dynamics data in the context of multiple emissive states relaxing at different rates rather than as a single electronic state exhibiting a spectral shift. The computational results indicate that the S_2 state is characterized by an electron density distribution that differs significantly from the electron density distributions seen in S_1 and S_3 (Figures 5.3 and 5.4). The singlet state S_2 is characterized by a permanent dipole moment that is substantially smaller in magnitude than that for S_1 and S_3 (Figure 5.4) and this difference is localized significantly on the aldehyde carbonyl functionality (Figures 5.3 and 5.4). The rate constant data and its dependence on the concentration of [OH] in solution points to the importance of dipole-dipole interactions in determining coupling between the excited singlet states in FR0. The data are consistent with the hydrogen-bonding interactions between the solvent alcohol proton and the FRO carbonyl oxygen modulating the dipole moment in each excited state (the calculated results do not take solvent H-bonding into account) and thereby controlling the efficiency of solvent-solute dipole-dipole interactions. It is these interactions that appear to facilitate relaxation between the excited singlet states.

5.5 Conclusions

We have examined the excited state relaxation dynamics of the substituted fluorene derivative FR0. The experimental data, in conjunction with the quantum chemical calculations reveal that relaxation between three excited singlet electronic states accounts for the spectral relaxation dynamics we observe in protic solvents. The relaxation dynamics are related to the concentration of hydroxyl functionality in the solvent system, indicating that hydrogen bonding in the S₂ state between the FR0 aldehyde oxygen and the solvent hydroxyl proton diminishes the role of dipolar solvent-solute coupling, reducing the relaxation rates for some of the relaxation pathways. Further experimentation will be required to address in detail the dependence of the electronic state coupling and its dependence on solvent interactions on chemical structure.

APPENDIX



Figure A.4 Time-resolved spectra for FR0 in (A) *n*-propanol, (B) *n*-pentanol, and (C) DMSO at wavelengths characteristic to the blue end (480 nm), intermediate (540 nm), and red end (590 nm) of the wavelength studied.



Figure A.5 Three-dimensional spectra of time-resolved fluorescence as a function of wavelength for FR0 in (A) *n*-propanol, (B) *n*-pentanol, and (C) DMSO.



Figure A.6 Examples of time-slices taken from the normalized time-domain data for FR0 in *n*-propanol at (A) 50 ps, (B) 500 ps, and (C) 5000 ps fitted to the three band positions determined using the corresponding steady-state emission spectrum. The blue band (—) represents the $S_3 \rightarrow S_0$ transition, the green band (—) represents the $S_2 \rightarrow S_0$ transition, and the red band (—)

represents the $S_1 \rightarrow S_0$ transition. The pink band (—) represents the overall fit of the three bands. All time slices were fit to the three band positions; however only the intermediate (green) and

lowest (red) energy bands were present at longer times, such as in (B) and (C).



Figure A.7 Time-resolved spectra for FR0 in the binary mixture of (A) 75% *n*-propanol in DMSO, (B) 50% *n*-propanol in DMSO, and (C) 25% *n*-propanol in DMSO at wavelengths characteristic to the blue end (480 nm), intermediate (540 nm), and red end (590 nm) of the wavelength studied.



Figure A.8 Steady-state (ss) emission spectra of FR0 in the *n*-propanol (PrOH)/DMSO binary solvent mixture of (A) 75% *n*-propanol in DMSO, (B) 50% *n*-propanol in DMSO, and (C) 25% *n*-propanol in DMSO in the frequency domain fit to three Gaussian bands.



Figure A.9 Time-resolved spectra and corresponding three-dimensional spectra of time-resolved fluorescence as a function of wavelength for FR0 in the binary mixture of (A) 75% *n*-propanol in DMSO, (B) 50% *n*-propanol in DMSO, and (C) 25% *n*-propanol in DMSO extracted from experimental fluorescence lifetime data.


Figure A.10 Normalized intensities of the three fitted bands (spectral features) extracted from the normalized time-domain data as a function of time for FR0 in the binary mixture of (A) 75% *n*-propanol in DMSO, (B) 50% *n*-propanol in DMSO, and (C) 25% *n*-propanol in DMSO. Each evolution was fit using the kinetic model relationships described in Chapter 5.

REFERENCES

REFERENCES

- 1. Lahiri, J., *et al.* Proton Abstraction Mediates Interactions between the Super Photobase FR0-SB and Surrounding Alcohol Solvent. *J. Phys. Chem. B* **2019**, *123* (40), 8448-8456.
- 2. Lahiri, J., *et al.* Steric effects in light-induced solvent proton abstraction. *Phys. Chem. Chem. Phys.* **2020**, *22* (35), 19613-19622.
- 3. Finkler, B., *et al.* Highly photostable "super"-photoacids for ultrasensitive fluorescence spectroscopy. *Photochem. Photobiol. Sci.* **2014**, *13* (3), 548-62.
- 4. Malval, J. P.; Diemer, V.; Savary, F. M.; Jacques, P.; Allonas, X.; Chaumeil, H.; Defoin, A.; Carré, C. Excited state proton transfer in a 'super' photoacid based on a phenol–pyridinium biaryl chromophore. *Chem. Phys. Lett.* **2008**, *455* (4-6), 238-241.
- 5. Sheng, W., *et al.* Ultrafast Dynamics of a "Super" Photobase. *Angew. Chem. Int. Ed.* **2018**, *57* (45), 14742-14746.
- 6. Pillman, H. A.; Blanchard, G. J. Effects of Energy Dissipation on Motional Dynamics in Unilamellar Vesicles. J. Phys. Chem. B 2010, 114, 13703-13709.
- Baumler, S. M. Diffusional Motion as a Gauge of Interfacial Fluidity and Adhesion of Supported Model Membrane Films. Ph.D. Dissertation, Michigan State University, East Lansing, MI, 2017.5
- Castner, J., E. W.; Fleming, G. R.; Bagchi, B. Influence of non-Debye relaxation and of molecular shape on the time dependence of the Stokes shift in polar media. *Chem. Phys. Lett.* 1988, 143, 270-276.
- 9. Su, S. G.; Simon, J. D. Solvation dynamics in ethanol. J. Phys. Chem 1987, 91, 2693-2696.
- Castner, J., E. W.; Maroncelli, M.; Fleming, G. R. Subpicosecond resolution studies of solvation dynamics in polar aprotic and alcohol solvents. *J. Chem. Phys.* **1987**, *86*, 1090-1097.
- 11. Simon, J. D.; Su, S. G. Picosecond Stokes shift studies of solvent friction: experimental measurements of time-dependent band shape and integrated intensity. *Chem. Phys.* **1991**, *152*, 143-152.
- 12. Nagarajan, V.; Brearley, A. M.; Kang, T. J.; Barbara, P. F. Time-resolved spectroscopic measurements on microscopic solvation dynamics. J. Chem. Phys. 1987, 86, 3183-3196.
- 13. Bagchi, B.; Oxtoby, D. W.; Fleming, G. R. Theory of the Time Development of the Stokes Shift in Polar Media. *Chem. Phys.* **1984**, *86*, 257-267.

- 14. Maroncelli, M.; Fleming, G. R. Comparison of time-resolved fluorescence Stokes shift measurements to a molecular theory of solvation dynamics. *J. Chem. Phys.* **1988**, *89* (2), 875-881.
- 15. Maroncelli, M.; Fleming, G. R. Picosecond solvation dynamics of coumarin 153: The importance of molecular aspects of solvation. *J. Chem. Phys.* **1987**, *86*, 6221-6239.
- 16. Eom, I.; Joo, T. Polar solvation dynamics of coumarin 153 by ultrafast time-resolved fluorescence. *J. Chem. Phys.* **2009**, *131* (24), 244507.
- Böhmer, R.; Gainaru, C.; Richert, R. Structure and dynamics of monohydroxy alcohols— Milestones towards their microscopic understanding, 100 years after Debye. *Phys. Rep.* 2014, 545 (4), 125-195.
- 18. Yuwono, S. H. Michigan State University, East Lansing, MI. Personal communication, May, 2021.
- 19. Eber, G.; Grüneis, F.; Schneider, S.; Dörr, F. Dual fluorescence emission of azulene derivatives in solution. *Chem. Phys. Lett.* **1974**, *29*, 397-404.
- 20. Huppert, D.; Jortner, J.; Rentzepis, P. M. S2 \rightarrow S1 emission of azulene in solution. *Chem. Phys. Lett.* **1972**, *13*, 225-228.

CHAPTER 6: INTRAMOLECULAR RELAXATION DYNAMICS MEDIATED BY SOLVENT-SOLUTE INTERACTIONS OF SUBSTITUTED FLUORENE DERIVATIVES

6.1 Abstract

Several fluorene derivatives are known to exhibit excited-state reactivity and relaxation dynamics that remain to be understood fully. We report in this work on the spectral relaxation dynamics of structurally modified versions of FRO, the precursor of the FRO-SB superphotobase, to more fully understand the role of the FR0 chromophore and the effect of specific substituents of the photobase behavior of FR0-SB. We have examined the time-resolved spectral relaxation dynamics of two compounds, NCy-FR0 and MK-FR0, in protic and aprotic solvents using time-resolved spectroscopy and computational chemistry. Both compounds exhibit similar spectral relaxation characteristics to those seen in FR0, underscoring the central role of hydrogen bonding interactions with solvent protons in mediating the relaxation pathways available to three excited electronic states. The modification of FR0 to produce the corresponding methyl ketone, MK-FR0, alters the nature of the hydrogen bond between the FR0 carbonyl oxygen and the solvent alcohol proton, resulting in changes in the observed intramolecular relaxation kinetics. These investigations demonstrate that the spectral relaxation dynamics of the FR0 precursor molecule depend to a limited extent on structural variations at the carbonyl carbon and are not affected by the rotational freedom of the terminal amino group. This information is useful to understanding the interplay between hydrogen-bonding and dipole-dipole intermolecular interactions in mediating intramolecular relaxation processes.

6.2 Introduction

Photoactivated chemical reactants are a unique class of compounds with reactivities that can be controlled through light-activation. These molecules display reactive properties only when in an excited electronic state, and this property is central to controlling spatial and temporal aspects of photochemical reactions. Applications such as precision chemistry and high-speed chemical sensing can benefit greatly from the use of these compounds. Prior to utilizing a photoactivated reagent in a chemical reaction, the relevant excited-state properties of the reagent must be understood at a sufficient level to provide insight into the reaction mechanism and, potentially, the reaction coordinate. In principle, photoreagents can be used to perform proton or electron exchange reactions, with proton exchange reactions being studied more extensively to date. The photoreagent for proton exchange reactions can function as a proton donor (photoacid) or as a proton acceptor (photobase). Photoacids are more numerous than photobases, and recent work has demonstrated a family of photobases capable of very large changes in basicity ($\Delta pK_b \sim$ 15).¹⁻²

The excited-state behavior of one of the few known super-photobases, FR0-SB (Figure 6.1A), has recently been characterized in protic and aprotic solvent environments, and the reaction coordinate and excited-state identities have been identified for the solvent proton abstraction reaction.¹⁻² In an effort to understand more fully the photoinduced change in reactivity of FR0-SB from a structural perspective, we have studied the excited-state relaxation dynamics of the aldehyde precursor, FR0 (Figure 6.1B), the results of which we presented in Chapter 5. Using time-resolved spectroscopy in conjunction with quantum chemistry calculations at the *ab initio* level, multiple unresolved bands were found to contribute to the broad steady-state emission spectrum of FR0 in both protic and aprotic solvents. The individual

bands were associated with three excited singlet electronic states in relatively close energetic proximity.

The solvent medium was found to exhibit significant control over the coupling and relaxation rates between the electronic states in FR0. State-dependent relaxation dynamics were observed in protic solvents, whereas the coupling between electronic excited states was markedly different for FR0 in dipolar aprotic solvents. The state-dependent relaxation dynamics of FR0 in protic solvents was found to depend on solvent proton concentration, analogous to the behavior seen for the super photobase FR0-SB. For the FR0 precursor, quantum chemical calculations demonstrated that the S₂ state exhibited an electron density distribution in the vicinity of the aldehyde moiety that is significantly different than the electron density distributions for either the S₁ or S₃ states. This state-dependent variation in electron density distribution at the aldehyde gives rise to state-dependent differences in permanent dipole moment, and we postulated that hydrogen-bonding to FR0 in its S₂ state mediated intramolecular relaxation pathways involving that electronic state. The site for the relevant hydrogen bond is the carbonyl oxygen, with the lone pairs on the oxygen presumably interacting with solvent hydroxyl protons.



Figure 6.1 Structures of (A) FR0-SB Schiff base, (B) FR0 precursor, (C) NCy-FR0 structural precursor, and (D) MK-FR0 structural precursor.

The postulate that solvent hydrogen bonding interactions with the FR0 aldehyde carbonyl moiety is primarily responsible for the observed spectral dynamics can be tested by making systematic structural changes in FRO and relating them to changes in the spectral relaxation dynamics. We report those studies here. Two structurally modified FR0 derivatives were examined, NCy-FR0 (Figure 6.1C) and MK-FR0 (Figure 6.1D). These structural modifications were targeted because of their ability to address two specific issues. The first issue, that of whether or not there are contributions from charge-transfer excited state(s) to the observed dynamics, can be evaluated by restricting the ability of the FRO amino group to undergo rotation. The cyclization of the amino functionality of FR0 and comparison of the spectral relaxation dynamics of the free and cyclized species addresses this issue directly. The second issue is the role of the aldehydic proton on the observed spectral dynamics. Replacement of the aldehydic proton with a methyl group to form the methyl ketone affords a direct examination of the role of the carbonyl moiety. The data we report here demonstrate the absence of contributions from charge-transfer excited states to the observed spectral dynamics. Subtle differences are seen in the spectral dynamics of the aldehyde and ketone. While both compounds undergo hydrogen

bonding with protic solvents, the differences in the electron density distributions of the aldehyde and ketone moieties leads to differences in dipolar contributions to the observed dynamics.

6.3 Experimental Methods

Materials. n-Propanol (99.7%, anhydrous) and dimethyl sulfoxide (DMSO, \geq 99.9%, anhydrous) were purchased from Sigma-Aldrich and used as received.

Synthesis of structural precursors. All chemicals and solvents were purchased from Sigma-Aldrich and used without further purification. The synthesis of NCy-FR0 was performed starting from 9,9 dialkylated monobromo fluorene through nitrating selectively at the 7 position. Reduction of the nitro group was commenced with Fe powder and NH₄Cl in an ethanol-water (1:1) solution of starting material. The resulting amine was alkylated and subsequently cyclized with excess of 1-bromo-2-chloroethane in the presence of sodium carbonate. Finally, the cyclized derivative was obtained as a yellow solid by formylating the starting material with *n*-butyllithium and dimethylformamide. ¹H NMR (500 MHz, CDCl₃) δ : 9.95 (s, 1H), 7.81 (d, *J* = 1.0 Hz, 1H), 7.73 (dd, *J* = 8Hz, 1.5Hz, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.25 (s, 1H), 3.22-3.26 (m, 4H), 3.03 (t, *J* = 6.4 Hz, 2H), 2.84 (t, *J* = 6.5 Hz, 2H), 2.10 – 1.88 (m, 4H), 1.59 (s, 6H).

The synthesis of MK-FR0 started with 9,9-dimethyl-9*H*-fluoren-2-amine, which was amine was protected with boc anhydride (di-*tert*-butyl decarbonate), prior to ethylation with iodoethane, giving the mono-alkylated protected amino-fluorene. The protecting group was cleaved under acidic conditions, and subsequently treatment with acetic anhydride to give the acylated product *N*-(9,9-dimethyl-9*H*-fluoren-2-yl)-*N*-ethylacetamide. Then, to install the carbonyl group, the fluorene derivative underwent a Friedel-crafts acylation with aluminum chloride and acyl bromide to yield the methyl ketone product. The acyl group underwent cleavage under basic conditions, generating the free mono-ethylated amine, followed by

alkylation using iodoethane and potassium carbonate to yield the final methyl ketone derivative of FR0. ¹H-NMR (500 MHz, CDCl₃): 7.98 (d, J = 1.6 Hz, 1H), 7.91 (dd, J = 7.9, 1.7 Hz, 1H), 7.60 (dd, J = 15.7, 8.2 Hz, 2H), 6.70 (m, 2H), 3.46, (q, J = 7.1 Hz, 4H), 2.64, (s, 3H), 1.49 (s, 6H), 1.24 (t, J = 7.1 Hz, 6H).

Following synthesis, each compound was stored in ethanol at a concentration of 1 mM. The compounds were prepared for spectroscopic analysis following a procedure similar to that used for FR0. Briefly, each compound was brought to dryness with $N_2(g)$ and reconstituted in the solvent system to be studied (*n*-propanol, DMSO, binary solvent mixture of *n*-propanol/DMSO). Sample concentrations were *ca*. 5 x 10⁻⁶ M for the spectroscopic measurements.

Steady-state emission and time-resolved fluorescence spectroscopy. Collection of steadystate and time-resolved emission spectra was performed using same instrumentation and parameters for analysis of FR0 (see Chapter 5, Section 5.3). For time-domain TCSPC measurements, emission decays were acquired across the wavelength range 470 – 620 nm, in ten nm increments. The raw lifetime data corresponding to the parallel and perpendicular polarization components were exported to Microsoft Excel (Microsoft Office 365, Microsoft Corporation, Redmond, WA). Microcal Origin (OriginPro 9.0, OriginLab Corporation, Northampton, MA) was used for subsequent data processing and analysis, band fitting, and determination of fluorescence lifetime decay constants.

6.4 Results and Discussion

To investigate the effects of structural changes to the FR0 molecule on spectral dynamics, spectroscopic fluorescence lifetime measurements were performed for NCy-FR0 and MK-FR0 in *n*-propanol and DMSO to represent the same protic and aprotic media used in the analysis of FR0. For both NCy-FR0 and MK-FR0 quantum chemical calculations (see Chapter 5, Section

5.4) were performed and the results used in conjunction with the time-resolved emission data to gain insight into the structure-dependence of the relaxation dynamics.

We have studied the spectral dynamics of NCy-FR0 to evaluate the role of large amplitude motion. For NCy-FR0, the diethyl amino group on FR0 is tethered to the fluorene ring system (Figure 6.1C), preventing rotation about the ring carbon to amino nitrogen bond. This structural modification prevents the amino nitrogen from participating in an excited state charge-transfer structure.

Quantum chemical calculations of NCy-FR0 show three excited singlet states in close energetic proximity (Table 6.1), similar to FR0. The energetic proximity of these singlet states is consistent with facile relaxation between the S_3 , S_2 , and S_1 states. The permanent dipole moment for NCy-FR0 in the S_2 singlet excited state is noticeably smaller than that in the other excited states (Table 6.1). Analogous to FR0, solvent-solute interactions with the S_2 state appear to play a substantial role in mediating intramolecular relaxation dynamics for NCy-FR0. The transition dipole moments and oscillator strengths for each excited state are similar to those for FR0 and are presented in Appendix 6A, Table A.3.

Table 6.1 The vertical excitation energies ω_n (in eV) along with the permanent dipole moments μ_n and their cartesian components (all in Debye) of the lowest four singlet excited states, $S_1 - S_4$, characterizing NCy-FR0 in the gas phase. The dipole moment of the ground electronic state, S_0 , is given for comparison.

State	$\omega_n^{\rm a}$	$\mu_x^{\mathbf{b}}$	$\mu_y^{\mathbf{b}}$	$\mu_z^{\mathbf{b}}$	$\mu_n^{\mathbf{b}}$
S_0		5.9	0.6	-0.6	6.0
S_1	3.46	14.3	-0.8	-1.7	14.4
S_2	3.82	2.2	0.0	0.0	2.2
S ₃	3.80	8.8	-0.1	-1.2	8.9
S_4	4.15	2.6	3.2	-1.3	4.3

^a Calculated as $\omega_n = \omega_n^{(\text{EOMCCSD/6-31+G*})} + [\omega_n^{(\delta-\text{CR-EOMCC}(2,3)/6-31G)} - \omega_n^{(\text{EOMCCSD/6-31G})}].$

^b Calculated using (EOM)CCSD/6-31+G*.

The Mulliken charges for the aldehyde functionality, specifically the carbonyl group, in the excited form of NCy-FR0 in the S₂ state (Figure 6.2) are almost identical to those of FR0 in the S₂ excited state. The carbonyl oxygen exhibits a more positive charge in the S₂ state, with an effective charge of *ca*. -0.12, than in the S₁ and S₃ states, where the carbonyl oxygen carries *ca*. -0.4 effective charge (Figure 6.2). The state-dependent changes in Mulliken charges in the aldehyde moiety are manifested as state-dependent changes in permanent dipole moments for each state (Table 6.1), shown visually along with the corresponding electron density distributions in Figure 6.3. The Mulliken charge on the amino nitrogen atom is close to zero in the ground state and the excited states (Figure 6.2). There is no evidence from the quantum chemical calculations of any contribution from a charge-transfer species to the excited electronic states. The interactions between solvent protons and the amino nitrogen are expected to be modest and no state-dependence is expected. Not surprisingly, the quantum chemical calculations for NCy-FR0 are very similar to those for FR0. Large-amplitude rotational motion of the amino group is thus shown not to contribute to the spectral dynamics seen for the FR0 chromophore.



Figure 6.2 Mulliken charges for individual atoms in the NCy-FR0 molecule in the S₀, S₁, S₂, S₃ states.



Figure 6.3 Magnitude and direction of permanent dipole moments for excitation of NCy-FR0 in the ground state to each of the lowest three excited singlet states. Red indicates increasing electron density (more negative upon excitation) and blue indicates decreasing electron density (more positive upon excitation).

The excited-state relaxation dynamics of NCy-FR0 are consistent with the quantum chemical calculations. Time-resolved fluorescence lifetime decays in *n*-propanol and DMSO were collected over a wavelength range (470 - 620 nm) and normalized to the steady-state fluorescence emission spectra collected for each solvent medium. The time-resolved spectra were generated from the corresponding experimental lifetime decays (Appendix 6B, Figure

A.11) at various time points across the collection period as a function of emission wavelength (Figure 6.4). The results display a spectral shift (wavelength-dependence) in protic media (Figure 6.4A), whereas the dynamics remain constant in aprotic media (Figure 6.4B).



Figure 6.4 Time-resolved spectra for NCy-FR0 in (A) *n*-propanol and (B) DMSO extracted from experimental fluorescence lifetime data.

To further investigate the observed spectral dynamics of NCy-FR0, the steady-state emission spectrum in each solvent was treated in the same way described for those of FR0 to identify the three Gaussian bands within the broad spectra (Appendix 6B, Figure A.12). The corresponding intensities of each fitted band within the extracted time-resolved spectra (Figure 6.4) were determined to generate the time evolution of each band, which corresponds to the solvent-solute complex in each excited state. Consistent with the analysis of FR0, the highest energy emission band was assigned to the $S_3 \rightarrow S_0$ emission, the intermediate band assigned to the $S_2 \rightarrow S_0$, and the lowest energy band assigned to the $S_1 \rightarrow S_0$ emission. Taking the resulting time-dependent band intensities, we applied the kinetic model developed in Chapter 5 to determine the fitted rate constants associated with the relaxations in each state, which are presented in Table 6.2 and Figure 6.5. We remind the reader that the determination of best-fit rate constants does not determine the rate constants uniquely because this is an inherently underdetermined problem. With that caveat in mind, we present the fitted rate constant values in Table

6.2.

Table 6.2 Fitted rate constants for the relaxations associated with each of the three fitted emission bands (spectral features) for NCy-FR0 in *n*-propanol and DMSO. Uncertainties are reported as $\pm 1\sigma$ (n = 10).

Data Constant	Solvent				
Rate Constant	<i>n</i> -propanol (× 10 ⁹)	DMSO (× 10 ⁹)			
k ₃₂	9.21 ± 0.87	0.08 ± 0.03			
k ₃₁	4.93 ± 1.14	0.08 ± 0.07			
k ₃₀	8.26 ± 1.22	0.21 ± 0.08			
k ₂₁	0.52 ± 0.30	0.05 ± 0.02			
k ₂₀	1.46 ± 0.41	0.24 ± 0.02			
k ₁₀	0.65 ± 0.25	0.80 ± 0.14			



Figure 6.5 Normalized intensities of the three fitted bands (spectral features) extracted from the normalized time-domain data as a function of time for NCy-FR0 in (A) *n*-propanol and (B) DMSO.

Several trends emerge from these fitted results. The relaxation dynamics in *n*-propanol appear to be qualitatively different than those in DSMO (Table 6.2). The fitted rate constants for NCy-FR0 in *n*-propanol are consistent with those determined for FR0 in *n*-propanol, indicating that hydrogen bonding with solvent protons plays the same role in both systems. For NCy-FR0 in DMSO, three rate constants are observed with magnitudes larger than the associated error; these are all associated with direct transitions from the excited electronic state to the ground state (S₃ \rightarrow S₀, S₂ \rightarrow S₀, S₁ \rightarrow S₀) (Table 6.2). The magnitudes of the rate constants for relaxation between excited states (S₃ \rightarrow S₂, S₃ \rightarrow S₁, S₂ \rightarrow S₁) are an order of magnitude smaller and in all cases barely larger than their uncertainties. We attribute this finding for DMSO to an absence of excited state-to-excited state relaxation in aprotic media. For NCy-FR0 in *n*-propanol, hydrogenbonding with the solvent mediates access to relaxation channels between excited states. Most importantly, however, we see similar dynamics in protic media for NCy-FR0 and FR0, reinforcing the assertion that solvent-solute hydrogen-bonding interactions proceed predominantly at the chromophore carbonyl oxygen, independent of the amino nitrogen.

We next investigated the excited-state behavior of MK-FR0 to determine the effects of substituting the aldehyde proton with a methyl group on FR0. To investigate the effects of this substitution on the corresponding excited-state behavior, time-resolved lifetime data were collected for MK-FR0 in *n*-propanol and DMSO (Appendix 6B, Figure A.13). Consistent with the spectral dynamics of FR0 and NCy-FR0, prominent time-domain spectral dynamics were seen in the experimental data for MK-FR0 in *n*-propanol (Figure 6.6A), and an absence of spectral dynamics was observed in DMSO (Figure 6.6B). Alternate presentations of these time spectra are presented in Appendix 6B, Figure A.14. It is worth noting that the shift in λ_{max} observed for MK-FR0 in DMSO (Figure 6.6B) compared to NCy-FR0 in DMSO (Figure 6.4B) is

due to the addition of the methyl group, which alters the electron density distribution of the excited state(s) as well as the magnitudes of the permanent dipole moments.



Figure 6.6 Time-resolved spectra for MK-FR0 in (A) *n*-propanol and (B) DMSO extracted from experimental fluorescence lifetime data.

Quantum chemical calculations revealed similar excited state energetics for MK-FR0 and

FR0. The vertical excitation energies for the first three excited states are within close energetic

proximity, and the permanent dipole moment of the S₂ state is significantly smaller than that for

the S_0 , S_1 and S_3 states (Table 6.3), analogous to the computed results for FR0.

Table 6.3 The vertical excitation energies ω_n (in eV) along with the permanent dipole moments μ_n and their cartesian components (all in Debye) of the lowest four singlet excited states, $S_1 - S_4$, characterizing MK-FR0 in the gas phase. The dipole moment of the ground electronic state, S_0 , is given for comparison.

State	<i>Wn</i> ^a	μ_x^{b}	$\mu_y^{\mathbf{b}}$	$\mu_z^{\mathbf{b}}$	μ_n^{b}
S_0		5.1	1.1	-0.4	5.2
S_1	3.64	13.2	-0.6	-1.2	13.3
S_2	3.89	1.6	0.2	0.0	1.6
S ₃	3.95	9.2	0.9	-0.6	9.3
S_4	4.28	-0.7	1.7	4.0	4.4

^a Calculated as $\omega_n = \omega_n^{(\text{EOMCCSD/6-31+G*})} + [\omega_n^{(\delta-\text{CR-EOMCC(2,3)/6-31G})} - \omega_n^{(\text{EOMCCSD/6-31G})}].$

^b Calculated using (EOM)CCSD/6-31+G*.

Computational results for spectroscopic properties (transition dipole moment, oscillator strengths) are also similar for FR0 and MK-FR0 and are presented in Appendix 6A, Table A.4.

Despite the similarity of the results for MK-FR0 and FR0, slight differences in Mulliken charges and corresponding electron density distributions in the vicinity of the carbonyl moiety have important significant consequences, as seen in the experimental time-resolved data. The Mulliken charges and the magnitudes and directions of permanent dipole moments for MK-FR0 are presented in Figures 6.7 and 6.8, respectively.

While the experimental time-resolved spectra indicate the same qualitative role of solvent-solute hydrogen-bonding interactions for FR0 and MK-FR0 (Figure 6.6), the Mulliken charges reveal different charge distributions around the carbonyl moiety for FR0 and MK-FR0. The S₂ state plays a solvent-dependent mediating role in intramolecular relaxation dynamics for both chromophores. However, comparison of the Mulliken charges on the aldehyde group in NCy-FR0 (Figure 6.2), which are very similar to those for the FR0 aldehyde group, the Mulliken charges on the MK-FR0 ketone carbon, oxygen, and methyl carbon (Figure 6.7) all differ from those seen for FR0. We believe these differences account for subtle differences in the solvent-dependent trends in the fitted rate constants seen for the two molecules.

The carbonyl carbon in the S₀, S₁, and S₃ states is much more positive in MK-FR0 (Figure 6.7) than that in NCy-FR0 (Figure 6.2), with an effective charge of *ca*. 0.4. The effective charge decreases to 0.16 in the S₂ state. Consistent with NCy-FR0, the charge of the carbonyl oxygen is sufficiently negative in the S₀, S₁, and S₃ states (*ca*. -0.5) and decreases to -0.17 in the S₂ state. The methyl carbon in MK-FR0, however, accumulates substantial negative charge (*ca*. -0.8) in all states, which affects the local dipole moment of the methyl ketone moiety. Given the effective charges for these atoms in the S₀, S₁, and S₃ states, the net dipole moment would fall along the axis of the bond between the carbonyl carbon and ring carbon. However, in the S₂ state, there is a comparatively smaller dipole moment between the carbonyl carbon and oxygen,

contrasted by a much larger local dipole moment between the carbonyl carbon and methyl carbon. Thus, the net dipole moment would lie along the carbonyl carbon-methyl carbon bond in the direction of the methyl carbon. Compared to the aldehyde in NCy-FR0 (and FR0), the carbonyl carbon is sufficiently more negative in the S₂ state (*ca.* -0.3 effective charge; Figure 6.2), giving rise to a net local dipole moment in the opposite direction than that in MK-FR0, which would in turn affect the nature of hydrogen bonding. The effect of the methyl group on electron density distribution are also apparent in Figure 6.8, where the presence of the methyl group appears to modify the electron densities in the terminal groups of the MK-FR0 molecule.



Figure 6.7 Mulliken charges for individual atoms in the FR0ktn molecule in the S₀, S₁, S₂, S₃ states, highlighting the atomic charges on the aldehyde group.



Figure 6.8 Magnitude and direction of permanent dipole moments for excitation of MK-FR0 in the ground state to each of the lowest three excited singlet states. Red indicates increasing electron density (more negative upon excitation) and blue indicates decreasing electron density (more positive upon excitation).

To assess the effects of hydroxyl group concentration ([OH]) on the relaxation dynamics of MK-FR0, time-resolved spectra were acquired for MK-FR0 in binary *n*-propanol and DMSO solvent systems, as was performed for FR0 in Chapter 5. The individual time-resolved spectra and corresponding time-domain lifetime data from which the spectra were extracted are presented in Appendix 6B, Figures A.15 – A.16. The positions of the unresolved bands within the broad steady-state spectrum of MK-FR0 in each solvent system were fitted (Appendix 6B, Figures A.17 and A.18), and the corresponding intensities were extracted to generate the time evolution of each band (Figures 6.9 and 6.10). The rate constants corresponding to each band decay were fitted using the previously developed kinetic model, and the fitted rate constants are reported in Table 6.4.



Figure 6.9 Normalized intensities of the three fitted bands (spectral features) extracted from the normalized time-domain data as a function of time for MK-FR0 in (A) *n*-propanol and (B) DMSO.



Figure 6.10 Normalized intensities of the three fitted bands (spectral features) extracted from the normalized time-domain data as a function of time for MK-FR0 in the binary mixture of (A) 75% *n*-propanol in DMSO, (B) 50% *n*-propanol in DMSO, and (C) 25% *n*-propanol in DMSO.

Table 6.4 Fitted rate constants for the relaxations associated with each of the three fitted emission bands (spectral features) for MK-FR0 in *n*-propanol, DMSO, and binary solvent systems of increasing *n*-propanol concentration in DMSO. Uncertainties are reported as $\pm 1\sigma$ (n = 10).

,	Solvent							
Rate Constant	n-propanol (× 10 ⁹)	75% n-propanol (× 10 ⁹)	50% n-propanol (× 10 ⁹)	25% n-propanol (× 10 ⁹)	DMSO (× 10 ⁹)			
k ₃₂	14.76 ± 0.42	11.78 ± 3.16	9.69 ± 0.65	6.91 ± 0.34	0.24 ± 0.25			
k ₃₁	8.94 ± 0.34	7.54 ± 1.22	3.44 ± 0.59	5.19 ± 0.59	14.58 ± 0.85			
k30	2.26 ± 0.47	2.14 ± 0.38	1.25 ± 0.63	1.16 ± 0.48	9.92 ± 0.58			
k ₂₁	0.39 ± 0.09	0.49 ± 0.14	0.27 ± 0.12	0.28 ± 0.07	0.28 ± 0.11			
k ₂₀	0.11 ± 0.07	0.51 ± 0.14	0.22 ± 0.14	0.14 ± 0.07	0.26 ± 0.10			
k ₁₀	0.66 ± 0.11	0.64 ± 0.16	0.65 ± 0.27	0.53 ± 0.10	0.46 ± 0.10			

As seen for FR0, there is a [OH] dependence for several of the rate constants for MK-FR0, particularly for the transitions from the S_3 state (Table 6.4). The solvent-dependent trends for MK-FR0 are opposite to those reported for FR0 in the binary solvent system, as k_{32} , k_{31} , and k_{30} increase with increasing [OH]. This is likely an indication of facile interstate relaxation to the S_2 state, at which point the kinetics are slowed. This is supported by a sufficient decrease in rate constants observed for the transitions associated with the S_2 state (k_{21} and k_{20}), demonstrating the modulating effect of this state on the relaxation dynamics of MK-FR0.

Based on these results, it appears that hydrogen bonding interactions of the chromophore carbonyl oxygen with solvent hydroxyl protons still plays a central role in determining the spectral dynamics of MK-FR0, but the details of the relevant solvent-solute interactions are likely different than those for NCy-FR0 and FR0, which are both aldehydes. The interactions between the carbonyl oxygen and solvent proton create an associated local dipole moment, which will interact with the local dipole moments of the chromophore carbonyl functionalities. As discussed above, the local dipole moments for the ketone and aldehyde for MK-FR0 and FR0, respectively, are oriented differently. While hydrogen bonding interactions still occur for MK-FR0 at the carbonyl oxygen, the nature of this interaction will be altered by the difference in local dipole moment of the MK-FR0 methyl group compared to the FR0 aldehyde proton. As such, the R-O-H…O=C solvent-solute interaction may align in a spatially different orientation compared to that for FR0. This hypothesis will be the focus of future work currently underway as part of this collaborative effort. Based on the data we have presented in this work, hydrogenbonding interactions in the S_2 state remain the mediating factor in the excited-state behavior of FR0 structural precursors, indicating that the spectral dynamics are structurally invariant. However, as demonstrated with MK-FR0, while hydrogen bonding mediates relaxation, the nature of the hydrogen bonds is directly related to the functionalities surrounding the FR0 carbonyl group.

6.5 Conclusions

We have reported on the excited-state behavior and spectral dynamics of two structural precursors to the FR0 molecule, NCy-FR0 and MK-FR0. Through the use of quantum chemical calculations in concert with experimental data, we have determined that solvent-solute hydrogen bonding interactions still occur upon structural modification to either pendent functionality on the FR0 core structure. The effect of amino group rotation on the spectral relaxation dynamics was determined to be negligible, as the spectral dynamics, Mulliken charges, and permanent dipole moments for NCy-FR0 agreed well with those for FR0. The ketone analog, MK-FR0, exhibited similar spectral relaxation dynamics to FR0 in protic solvents; however, the subtle differences are seen for the aldehyde and the ketone, and these differences are thought to result from differences in the local charge density distributions seen for the aldehyde and ketone functionalities. These differences give rise to changes in the details of the optimum solvent-solute hydrogen bonding configuration. Despite this difference, both structural precursors were

determined to exhibit spectral dynamics similar to FR0, demonstrating the effect of solventsolute interactions, particularly those in the S_2 excited state, as the mediators in molecular relaxation dynamics. The implications of these findings on the structural factors that are important to the super photobase properties of FR0-SB remain to be understood fully. APPENDICES

APPENDIX 6A:

Results of Computational Calculations for Structural Precursors

Table A.3 Transition dipole moments (in Debye) characterizing the electronic excitation/deexcitation between the ground and the lowest four excited singlet states characterizing the NCy-FR0 in the gas phase. Only unique transitions are given in the table.^a

	Initial state							
Target	So		S_1		S_2		S 3	
State	TDM	Osc. Str.	TDM	Osc. Str.	TDM	Osc. Str.	TDM	Osc. Str.
S ₁	6.9	0.70						
S_2	0.1	0.00	0.1	0.00				
S ₃	2.4	0.10	2.7	0.01	0.0	0.00		
S_4	1.7	0.05	5.2	0.07	0.1	0.00	3.1	0.01

^a Calculated using EOMCCSD/6-31+G*.

Table A.4 Transition dipole moments (in Debye) and oscillator strengths characterizing the electronic excitation/de-excitation between the ground and the lowest four excited singlet states characterizing the MK-FR0 in the gas phase. Only unique transitions are given in the table.^a

	Initial state							
Target	So		\overline{S}_1		S_2		S 3	
State	TDM	Osc. Str.	TDM	Osc. Str.	TDM	Osc. Str.	TDM	Osc. Str.
S_1	7.2	0.80						
S_2	0.3	0.00	0.5	0.00				
S ₃	3.1	0.16	3.6	0.01	0.1	0.00		
S_4	0.8	0.01	2.9	0.02	0.1	0.00	1.8	0.00

^a Calculated using EOMCCSD/6-31+G*.

APPENDIX 6B:

Steady-State and Time-Resolved Spectral Data for Structural Precursors



Figure A.11 Normalized time-resolved lifetime decay curves for NCy-FR0 in (A) *n*-propanol and (B) DMSO at emission wavelengths representing the blue end (480 nm), intermediate (540 nm), and red end (590 nm) of the wavelength range studied.



Figure A.12 Steady-state (ss) emission spectra of NCy-FR0 in (A) *n*-propanol (PrOH) and (B) DMSO in the frequency domain fit to three Gaussian bands.



Figure A.13 Normalized time-resolved lifetime decay curves for MK-FR0 in (A) *n*-propanol and (B) DMSO at emission wavelengths representing the blue end (480 nm), intermediate (540 nm), and red end (590 nm) of the wavelength range studied.



Figure A.14 Three-dimensional spectra of time-resolved fluorescence as a function of wavelength for MK-FR0 in (A) *n*-propanol and (B) *n*-pentanol.


Figure A.15 Normalized time-resolved lifetime decay curves for MK-FR0 in (A) 25% *n*-propanol in DMSO, (B) 50% *n*-propanol in DMSO, and (C) 75% *n*-propanol in DMSO at emission wavelengths representing the blue end (480 nm), intermediate (540 nm), and red end (590 nm) of the wavelength range studied.



Figure A.16 Time-resolved spectra and corresponding three-dimensional spectra of time-resolved fluorescence as a function of wavelength for MK-FR0 in the binary mixture of (A) 75% *n*-propanol in DMSO, (B) 50% *n*-propanol in DMSO, and (C) 25% *n*-propanol in DMSO extracted from experimental fluorescence lifetime data, as well as corresponding three-dimensional time-resolved spectra of MK-FR0 in (D) 75% *n*-propanol in DMSO, (E) 50% *n*-propanol in DMSO, and (F) 25% *n*-propanol in DMSO.



Figure A.17 Steady-state (ss) emission spectra of MK-FR0 in (A) *n*-propanol (PrOH) and (B) DMSO in the frequency domain fit to three and two Gaussian bands, respectively.



Figure A.18 Steady-state (ss) emission spectra of MK-FR0 in the *n*-propanol (PrOH)/DMSO binary solvent mixture of (A) 25% *n*-propanol in DMSO, (B) 50% *n*-propanol in DMSO, and (C) 75% *n*-propanol in DMSO in the frequency domain fit to three Gaussian bands.

- 1. Lahiri, J., *et al.* Proton Abstraction Mediates Interactions between the Super Photobase FR0-SB and Surrounding Alcohol Solvent. *J. Phys. Chem. B* **2019**, *123* (40), 8448-8456.
- 2. Lahiri, J., *et al.* Steric effects in light-induced solvent proton abstraction. *Phys. Chem. Chem. Phys.* **2020**, *22* (35), 19613-19622.

CHAPTER 7: CONCLUSIONS AND FUTURE WORK

7.1 Conclusions and Future Work

7.1.1 Cu²⁺-Complexed Langmuir-Blodgett (LB) Films

Monolayers and thin films are used to enhance many chemical processes and applications $(e.g., \text{chemical sensing, surface protection, and optical enhancements});^{1-4}$ however, such adlayers have fixed properties (e.g., adhesion strength, mobility) upon deposition onto solid supports. Gaining control over molecular-level interactions after deposition has yet to be achieved; such ability would enable customization of film systems *in situ* for specific applications. The first part of this work was motivated by the creation of interfaces with selective properties through reversible control over the oxidation state of the metal ion in Cu²⁺-complexed Langmuir-Blodgett (LB) films. Prior to achieving this goal, however, the dynamics influencing the formation and morphology of these understudied films must be characterized.

The first step in understanding the influence of Cu^{2+} ions on monolayer organization and dynamics involved monitoring film formation as a function of Cu^{2+} presence and pH conditions.⁵ Incorporation of Cu^{2+} ions in arachidic acid LB films resulted in a highly rigid film system. Formation of the amphiphilic films was facilitated by Cu^{2+} ions as a function of subphase pH. As pH increased, approaching and exceeding the p K_a of AA, film formation began at low surface pressures commonly indicative of system disorder (*ca.* < 5 mN/m). Brewster angle microscopy monitoring of film formation confirmed this phenomenon, as "islands" of monolayer were observed at these low surface pressures and high pH conditions. Out of the several effects of Cu^{2+} ions on film structure, most notable was the absence of collapse for films formed at higher pH conditions (pH \geq 5), which indicated the presence of a structurally rigid film even at high surface pressures (*ca.* > 55 mN/m). Film thickness measurements demonstrated such film integrity, attributed directly to Cu^{2+} -amphiphile interactions. To accompany experimental data, mathematical modeling was performed to determine the dominant species present in the film

system as a function of pH and Cu^{2+} concentration using the equilibrium expression for each species. Such calculations enabled understanding of the evolution of the species across the pH range and the dominant metal ion-amphiphile interactions as a function of acid protonation.

The factors influencing the Cu²⁺-AA film system were then analyzed using spectroscopic techniques designed to assess amphiphile orientation and film mobility.⁶ Infrared reflectionabsorption spectroscopy (IRRAS) was used to assess AA aliphatic chain tilt angle in solid and post collapse phase films by measuring the -CH₂ stretching modes as a function of pH. Frequencies of the stretching modes were pH-dependent for both phases of film deposition and indicated increasing conformational ordering among amphiphiles, which was directly related to Cu²⁺-amphiphile complexation. Film orientation was determined to be independent of pH. In general, the average tilt angle for solid phase films was smaller than that for post collapse phase films due to tighter intermolecular spacing at the solid phase. For post collapse phase films, the tilt angle was determined to directly depend on the extent of amphiphile protonation and Cu²⁺-AA interactions (*i.e.*, induced buckling at low pH conditions and extrusion of metal ionamphiphile moieties into the subphase at high pH conditions). For a complementary assessment of Cu²⁺-AA interactions, film mobility was characterized using fluorescence recovery after photobleaching, which measured the translational diffusion of a non-tethered chromophore among the AA aliphatic chains. At low pH conditions (pH < 4), diffusion resembled that of an AA layer sans metal ions. Film fluidity decreased with increasing pH, indicating a rigid film system at high pH conditions (pH \geq 6) due to increased Cu²⁺-amphiphile interactions and further confirming the influence of Cu^{2+} on the rigidity of the monolayer system.

The factors affecting the formation and integrity of a Cu^{2+} -AA LB film were characterized to provide a comprehensive understanding of this unique system. The work presented here not only provides characterization of an understudied metal ion-film system but provides the foundation for creating tunable interfaces using Cu^{2+} ions as the mediating factor. The microscopic and spectroscopic analysis techniques used throughout this work enabled the determination of the specific molecular-level interactions causing the anomalous behavior observed upon Cu^{2+} incorporation. Such an understanding is central to the future use of these and related adlayers.

Future directions for this work involve the investigation of creating selective interfaces by changing the oxidation state of the Cu^{2+} ions. While the use of electrochemistry to induce charge transfer in LB films has been previously reported,⁷⁻⁸ focus has been on charge-transfer salts and polymeric surfactants with conductive properties.⁹ Electrochemistry of LB films containing transition metal ions has been performed for phthalocyanines and related coordination complexes,^{7-8, 10} while very few have focused on metal ion-fatty acid films such as those in this work. Future work to achieve redox control over Cu²⁺-AA film systems would involve a technique such as potential sweep voltammetry to reduce Cu²⁺ to Cu⁺. Initial investigations to optimize experimental parameters would need to be accomplished. An optimum subphase pH would first need to be determined to ensure that Cu^{2+} ions are available for redox chemistry (e.g., pH with some degree of acid protonation). Second, optimization of electrochemical parameters such as electrolyte solution identity and scan rate would enhance electron flow to the metal ions. Upon reducing Cu²⁺ to Cu⁺, film characterization using the techniques described in this work would be helpful to understand the effects that monovalent copper ions have on the film system compared to divalent ions. Overall, the creation of selective films using such means shows promise for generating a novel class of interfaces whose properties can be altered in a facile manner and utilized in wide range of chemical applications.

7.1.2 Excited-State Dynamics of Super-Photobase Precursors

Super-photobases have recently been studied as a class of photoactivated molecules with unique reactivities (*i.e.*, proton abstraction) in their excited states. While very few super-photobases have been identified, it is necessary to study the excited-state dynamics of precursor molecules used to synthesize such photoactive compounds. This understanding can lead to the development of additional photobases for applications in which temporal and spatial control of reactions is necessary. The excited-state behavior of one of the few known super-photobases, FR0-SB, has been studied previously¹¹⁻¹² and was found to participate in proton abstraction in protic solvents. Well-resolved bands corresponding to the unprotonated and protonated forms of this molecule in the excited state were identified.

The second part of this dissertation work focused on the investigation of the excited-state dynamics of the FR0 precursor chromophore to better understand its role in the behavior of the super-photobase FR0-SB. In contrast to FR0-SB, the spectral relaxation dynamics of FR0 are not as easy to examine, owing to the absence of spectral resolution of distinct species. Time-correlated single photon counting was used to measure the excited-state lifetimes of FR0 in protic and aprotic media. Spectral shifting was observed in protic solvents and determined to be a result of hydrogen-bonding interactions between the FR0 carbonyl oxygen and solvent protons. Quantum chemical calculations support these experimental findings, revealing multiple excited electronic singlet states within close energetic proximity for FR0. Calculations of Mulliken charges and permanent dipole moment directions and magnitudes revealed properties for the S₂ excited state that differed from those of the adjacent S₃ and S₁ states, suggesting solvent interactions with this state to be the mediating factor in the observed emission spectral relaxation dynamics. Spectral reconstruction of the normalized time-resolved experimental data revealed three spectrally overlapped bands within the broad and comparatively featureless steady-state

emission spectrum of FR0 in each solvent system examined. Examination of the time-evolution of each fitted band and modeling of the rate constants associated with relaxation between the three excited electronic states and the ground state indicated that the S_2 state played a central role in controlling the relaxation dynamics of FR0. Hydrogen-bonding interactions between FR0 and protic solvents altered the rates of relaxation to and from the S_2 state relative to the relaxation dynamics observed in the polar aprotic solvent DMSO.

Analysis of structural modifications to the FR0 molecule demonstrated that the spectral dynamics observed for the precursor molecule are structurally invariant. Upon analysis of two structurally modified versions of FR0, NCy-FR0 and MK-FR0, similar spectral dynamics and computational results were observed. Such results indicated that interactions between the carbonyl oxygen and solvent protons, most notably in the S₂ excited state, dominate the excited-state behavior and relaxation dynamics of FR0 in protic solvents. Concurrently, it was determined that replacement of the aldehyde proton in FR0 with a methyl group in MK-FR0 changed the nature of solvent-solute hydrogen-bonding interactions due to differences in local dipole moments. Using time-resolved spectroscopy and computational chemistry, the molecular dynamics owing to the unique excited-state behavior of the FR0 precursor were determined, and the mediating factor in controlling the dynamics was identified. Such determinations lead to a better understanding of the characteristics of FR0 that lead to the super-photobase behavior of the FR0-SB Schiff base.

As this research is part of a rapidly developing field, many avenues exist for future expansion on the results presented in this work. One such future direction involves the analysis of additional compounds related to the FR0 precursor to determine why the structure gives rise to super-photobase properties in the corresponding Schiff base. Pertaining to the MK-FR0

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structural precursor, the methyl ketone group could be ring locked, effectively attaching the methyl group to the fluorene ring *via* a six-membered ring. This modification would alter the electron density distribution of the molecule, which may affect spectral dynamics. Additional future work involves the synthesis of different photobase compounds related to the Schiff base structure, which is currently underway. Studying other related super-photobase compounds would prove valuable in the context of the findings in this work to determine exactly how the excited-state dynamics of the FR0 precursor affect the dynamics of photobases. Ultimately, understanding the spectral dynamics of such molecules will enable the creation of additional novel photoactive reactants to further the field of photochemistry and its related applications.

- 1. Moriizumi, T. Langmuir-Blodgett Films as Chemical Sensors. *Thin Solid Films* **1987**, *160* (1-2), 413-429.
- 2. Tieke, B. Langmuir-Blodgett Membranes for Separation and Sensing. *Adv. Mater.* **1991**, *3*, 532-541.
- Penner, T. L.; Armstrong, N. J.; Willand, C. S.; Schildkraut, J. S.; Robello, D. R. Langmuir-Blodgett Films for Second-Order Nonlinear Optics. *Prop. Org. Mater. IV* 1991, 1560, 377-386.
- 4. Swalen, J. D.; Novotny, V. Tribology of Langmuir-Blodgett Layers. *Langmuir* **1989**, *5* (2), 485-489.
- 5. Capistran, B. A.; Blanchard, G. J. Effects of Cu(II) on the Formation and Orientation of an Arachidic Acid Langmuir-Blodgett Film. *Langmuir* **2019**, *35* (9), 3346-3353.
- 6. Capistran, B. A.; Blanchard, G. J. Spectroscopic Analysis of Cu(II)-Complexed Thin Films to Characterize Molecular-Level Interactions and Film Behavior. *Langmuir* **2021**, *37* (16), 5089-5097.
- 7. Goldenberg, L. M. Use of electrochemical techniques to study the Langmuir-Blodgett films of redox active materials. *Russ. Chem. Rev.* **1997**, *66* (12), 1033-1052.
- 8. Goldenberg, L. M. Electrochemical properties of Langmuir-Blodgett films. *J. Electroanal. Chem.* **1994**, *379*, 3-19.
- 9. Bryce, M. R.; Petty, M. C. Electrically conductive Langmuir-Blodgett films of charge-transfer materials. *Nature* **1995**, *374*, 771-776.
- Petty, M.; Lovett, D. R. Electrochromism in ytterbium bisphthalocyanine-(stearic acid or cadmium stearate) films deposited by the Langmuir-Bodgett technique. *Thin Solid Films* **1989**, *179*, 387-395.
- 11. Lahiri, J., *et al.* Steric effects in light-induced solvent proton abstraction. *Phys. Chem. Chem. Phys.* **2020**, *22* (35), 19613-19622.
- 12. Lahiri, J., *et al.* Proton Abstraction Mediates Interactions between the Super Photobase FR0-SB and Surrounding Alcohol Solvent. *J. Phys. Chem. B* **2019**, *123* (40), 8448-8456.