Soluble Surfactant and Nanoparticle Effects on Lipid Monolayer Assembly and Stability

Dissertation

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By

Matthew D. Nilsen, B.S., M.Sc.

Graduate Program in Chemical Engineering

The Ohio State University

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Dissertation Committee:

Dr. James F. Rathman, Advisor
Dr. L. James Lee
Dr. Isamu Kusaka
Abstract

The study of self-assembly dynamics that lead to ultra-thin films at an interface remains a very active research topic because of the ability to generate interesting and useful structures that can perform specific tasks such as aiding in magnetic separations, acting as two-dimensional biosensors and the modification of surfaces without changing their morphology. In some cases this is critical to surface functionality. This area is also important because it may ultimately yield simple screening techniques for novel surfactants and nanoparticle species before they are introduced onto the market, as is already happening. In the work presented here we studied the assembly dynamics of the insoluble lipid DPPC, common to most mammalian cell lines, in the presence of soluble surfactant and nanoparticle species. Preliminarily, we investigated the anomalous behavior of soluble surfactants at the air/water interface as an extension to work done previously and then used these results in an attempt to explain the behavior of the combined surfactant/lipid monolayer under dynamic and static conditions. Separately, we performed nanoparticle dynamics studies for a commercially available product and for particles created in-house before performing similar statics/dynamics work on the combined nanoparticle/lipid monolayer. Additionally, we constructed a novel molecular dynamics (MD) simulation code in an effort to understand this type of system at the micro-scale as our data represent averaging at the macroscopic level only.
What we found is that these types of systems are significantly more complex than they would appear otherwise. Soluble surfactant diffusion kinetics toward and away from the interface differ by at least an order of magnitude. What is even more unusual is that nanoparticle diffusion kinetics, although the particles differ in weight and in chemical nature from the surfactants, take the same form of and, in some cases, even the same magnitude of the soluble species. While merely coincidence, it is nonetheless an interesting finding. The combined soluble/lipid and nanoparticle/lipid monolayers exhibited unusual self-assembly dynamics than for either species by itself and both systems showed temporal instability upon compression to moderate or elevated pressures, indicating that there is rearrangement and exclusion from the monolayer of the non-dominant species. The computer simulation work showed that generating extended lipid structures such as stripes and islands requires much larger systems with higher lipid densities than it is possible to handle using our limited resources. However, it is still possible even with a small system containing a limited number of lipid molecules to gain useful insights into the micro-scale behavior of the system. In this case it is clear that having long-range repulsive forces and short range dipole interactions is critical to structure formation. We also determined that, due to the nanoparticles having a mass two orders of magnitude greater than the lipid, the amount of simulation time required to see nanoparticle agglomeration is far beyond our ability to study without the use of a massively parallel computing system. Nonetheless, we are able to draw conclusions by studying lipid diffusion into the particles and compare the structures that form there rather than in free solution.
For Heather.

My friend and companion, without whom I could not have completed this work.

You have my love and my gratitude.
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Vita


1999 - 2000  ......................... Engineering Intern, Marathon Ashland Petroleum LLC, Findlay Ohio

2002  ................................. B.S. - Chemical Engineering, The Ohio State University

2004  ................................. M.S. - Chemical Engineering, The Ohio State University

2004 - 2005  ......................... Researcher, Battelle Memorial Institute, Columbus Ohio

2008 - 2010  ......................... Chair, Department Chemical Hygiene Committee

2005 - 2010  ......................... Graduate Researcher, The Ohio State University

Publications

Research Publications

Brodkey, Zhao, Nilsen et. al, “Particle Tracking Velocimetry Applied to an Opposed-Jet Mixer Configuration,” APS Meeting Abstracts, November 2001


Nilsen, Rathman and Vogel, “Experimental Study of Lipid/Nanoparticle Langmuir Films,” In preparation
Nilsen and Rathman, “Molecular Dynamics Simulation of Lipid/Nanoparticle Composite Monolayers,” *In preparation*

**Fields of Study**

Major Field: Chemical Engineering

Studies in:

- Turbulence and mixing
  - Prof. Robert S. Brodkey
- Numerical modeling
  - Battelle Memorial Institute
- Self-assembly at interfaces
  - Prof. James F. Rathman
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Chapter 1: Introduction

Molecular self-assembly remains an area of active interest across multiple disciplines and is useful in producing ultra-thin films of pure or mixed components that have direct applications in layer stability testing, advanced materials, surface treatment and the construction of artificial cell membranes. It is possible to generate, for example, a monolayer of lipids (biomolecules that comprise the majority of a cell membrane) in order to study the dynamic assembly process and to perturb that process by introducing outside factors like a soluble/insoluble species or active molecules like enzymes or nanoparticles. In doing so, we gain an understanding of the forces at work in such a system and can determine the effect that the secondary species have on the assembly process. The resulting thin film will have potentially interesting properties and functionality that would not otherwise be present, leading to novel applications in a range of areas that include magnetic cell sorting technology, two-dimensional enzymatic surfaces for biomolecular reactions, toxicology studies and many others. That we are able to generate these kinds of films at very low cost and in a non-cleanroom environment is encouraging from a practical standpoint. Because the technology required has been available and in-use for decades is also encouraging
as it helps to alleviate concerns about reproducibility and homogeneity that sometimes arise. In this case it is the volume of experience and practical knowledge that makes this approach attractive.

While experimental work may yield information that lends a greater understanding of self-assembly, it is becoming increasingly common to try and explain the observed behavior through computer modeling. With a computer, unlike an experiment that is dependent on many variables that we cannot control or easily quantify in some cases, we have access to every variable and understand exactly what is being done while the model is running. If the governing equations are valid and solved appropriately along with the use of reasonable initial and boundary conditions, it should be possible to begin approximating the system of interest. This is true even for self-assembly where the system size is truly vast in terms of atomic length scales. However, in order to do this it is necessary to make certain assumptions and simplifications that must be corrected within the model. In this way, computer simulation is perhaps not so different from physical experimentation in that preparation and proper execution are both critical for achieving success. But even so, it is left to the researcher to demonstrate that the output is descriptive of the intended system of interest.

The remainder of this chapter will describe the Langmuir-Blodgett (LB) film assembly and transfer process in a detailed manner followed by a brief discussion of monolayers in biological systems and some applications that are mentioned in the literature. Following this is a review of the more recent papers concerning nanoparticle interactions with self-assembled and naturally assembled (that is, derived from living cells) mono- and bi-layer structures. We will not review the literature related to molecular dynamics simulation (MD) in this chapter as it seems more appropriate
to deal with the topic in its own section. Chapter 2 will cover some of our earlier work involving anomalous soluble surfactant behavior which is important when discussing monolayer assembly in the presence of such compounds. This will be covered at the end of the chapter. In Chapter 3 we will discuss the interactions between nanoparticles and lipids beginning with a short literature review and then moving into the experimental work. Chapter 4 is concerned exclusively with the MD simulation work and will be arranged in the same manner as Chapter 3. Finally, I will present some conclusions drawn from the work and give recommendations for those interested in pursuing these topics further. The various appendices will include data and observations not included in the body of this document as well as the simulation code along with anything else that may be of interest.

1.1 The Langmuir-Blodgett Technique

The Langmuir-Blodgett (LB) technique has the ability to not only study the dynamics of transient monolayer formation for essentially any material of interest that is sufficiently insoluble in the subphase (usually water) but also to transfer an assembled structure onto a solid substrate for further analysis. An example of a two-barrier LB trough is shown in figure 1.1. Once transferred, analysis of the film may include essentially any imaging technology since the layer can tolerate being in an evacuated chamber which is necessary for AFM or electron microscopy. Physical measurements are acquired through the Wilhelmy Plate Method, in which a small paper plate is held at the air/water interface by a highly sensitive scale. Differences in the force on this scale yield surface tension measurements, although the usual convention is to quote surface pressure instead. Surface pressure is a deviation from the initial surface
tension (ultra pure water) and is expressed very simply by Equation 1.1.

\[ \pi = \gamma_0 - \gamma \]  

Here, \( \gamma_0 \) is the initial surface tension (usually expressed in units of mN/m) minus the current value as measured by the Wilhelmy plate. Values obtained in this way represent a truly large population sample and as such are an appropriate measure of the average effect of the surface active molecules on the air/water interface. Figure 1.2 shows an example of a typical isotherm obtained using a common lipid species. Each change in slope represents a two-dimensional phase change in which the molecules either change orientation or move into a more packed structure (note: it is important not to confuse pressure here with the three-dimensional analog). Commonly the various phases are gas (diffuse), liquid expanded, liquid condensed and solid (crystalline) each with a corresponding increase in slope until the solid phase forms at which time the film breaks. Phase change is dominated by the thermodynamics of the system (hence temperature is a key factor in layer stability) and by molecular orientation...
which is due to different factors depending on the phase under investigation. In the gas phase the lipids do not interact frequently and van der Waals forces cause the hydrophobic tail group to lay parallel with the interface. In the liquid region physical packing of molecules is the dominant orienting force. Once the monolayer reaches the solid phase breakage is immediate and unavoidable because the layer acts essentially as a crystalline phase at maximum density - deviations beyond this point cause the layer to pass under and over itself forming a multi-layer structure and therefore no longer a monolayer. It is for this reason that we perform film transfer in the liquid-condensed phase and sufficiently removed from the known breaking point. The transfer process works by depositing a solution of the candidate molecules onto the air/water interface, waiting for pseudo-steady equilibrium and then steadily decreasing the area of the interface using a movable barrier (see Figure 1.3).
compressed, the molecules behave essentially as a liquid crystal so when an appropriate substrate is passed through the interface the molecules should transfer to the surface with no disruption in any features that were present in the formerly floating film. To account for loss of material in the monolayer as it being removed, the LB trough uses a feedback controller to maintain the appropriate surface pressure. Multi-layered structures are also possible by repeating the deposition process multiple times. These deposited films have many applications in microelectronics such as resistors of electron tunneling thickness\(^1\) or as a basis of patterned electronics\(^2\), sensing\(^3,4\) and as surface patterning and modification agents\(^5\). This approach has also made it possible to study the effects of including bio-active molecules such as proteins and enzymes or inorganics like silicates into the membrane-like structure either through penetration from the subphase or deposition onto the layer from above\(^6\). It is important to note however, that this self assembly process, which occurs in a non-cleanroom environment under ambient conditions, is highly sensitive to perturbations beyond the usual concerns about contamination or impurities. For this reason reproducibility may represent the primary concern when considering the commercial application
of this technology although it does not necessarily disqualify the concept if proper techniques are being employed.

1.2 Lipid Monolayers and Biological Systems

In cell membranes the most commonly occurring lipids are phosphatidylcholines (PC) and phosphatidylethanolamines (PE). These zwitterionic species self-assemble into a highly organized bilayer which separates the internal cellular environment from the continuous phase existing outside. Monolayers, or a single closely-packed layer of molecules, which may contain a mixture of different PC’s and/or PE’s containing saturated or unsaturated acyl-chains with varying length typically between 12 and 22 carbon atoms (Figure 1.4 gives examples of these structures) exhibit interesting two-dimensional patterns when treated appropriately. For instance, Dufrene\(^7\) studied the separation of phases between distearoylphosphatidylenthanolamine (DSPE) and dioleoylphosphatidylethanolamine (DOPE), finding that DSPE micro-domains embed in the continuous DPPE phase and that these small domains were significantly more stable under mechanical shear. Work by Shahgaldian\(^8\) also studied phase separation in mixed monolayers using non-biologically derived lipid-like species while Maxloff\(^9\) used a phosphorescently dyed lipid species to directly observe phase formation using Brewster Angle Microscopy (BAM). Gyorvaary\(^10\) and Solletti\(^11\) also studied domain formation, the former studying the effect of ‘linker’ lipids on domain stability and the latter interested in spontaneous bi-layer and tri-layer formation. Alexandre investigated the degree of order present in DPPE monolayers\(^12\) and later the formation of fibrillar and circular domain formation\(^13\) in the presence of enzymes. The structures form due to differences in headgroup charges and in the degree of saturation of the
Figure 1.4: DPPC (straight chain) and POPC (one unsaturated bond) lipid structures

hydrophobic tail groups between different lipid species and are highly enriched in one or other similar lipids. These condensed structures, commonly referred to as ‘rafts’, also occur in living cells and provide a particularly stable environment on the cell surface. It is because of this increased stability that functional molecules such as signaling groups, characteristic proteins and other active structures tend to aggregate there\textsuperscript{14,15}. The lipid bilayer is highly sensitive to small molecule perturbations from the outside environment, such as the addition of a soluble surfactant species, which may be present to prevent cell damage from mechanical mixing forces but which may also have negative properties that can cause cell damage. This complex structure is also temperature dependent such that even a deviation of a few degrees in the cell culture may cause a phase transition in the bilayer resulting in either homogenization of the lipids (almost certainly resulting in cell death), or stronger separation of the lipid phases and consequent contraction of the membrane itself\textsuperscript{16,17}. Anchorage-dependent cells, when exposed to a candidate surface, can provide important information for
understanding both cell-cell communication and cell-biomolecule interactions such as phospholipids and extracellular matrix (ECM) proteins in vivo. Elbert\textsuperscript{18} and Yam\textsuperscript{19} showed that, depending on their thickness, thin layers of polyelectrolytes could either promote or discourage cell adhesion and growth with possible applications for biomedical implants. Garcia\textsuperscript{20} investigated osteoblast adhesion on fibronectin-treated glass, showing robust behavior under applied shear. Overall this indicates that a properly engineered surface that consists of regions promoting or inhibiting cell adhesion either by virtue of the chemical nature of the region or due to nanostructured surface morphology can localize cells in a well-defined manner, leading to specific cell array and pattern formation. Spatial organization of cells with controlled shape, function\textsuperscript{21} and location can be realized on a pre-patterned surface which has essential applications in cell-based biosensors\textsuperscript{22}, medical implants and diagnostic tests (Toxicity sensing using hepatocyte protein cultured on surfaces treated with collagen\textsuperscript{23}, self-assembled monolayers and micro-patterning for selective adhesion\textsuperscript{24}, ability of bone cells to adhere to surfaces\textsuperscript{25}, protein-driven domain formation in cells\textsuperscript{26} and a good review of surface patterning techniques\textsuperscript{27}).

1.3 Nanoparticle Interactions with lipid Monolayers

The addition of functional nanoparticles that may have inherent surface activity or of particles that can be perturbed via an externally applied magnetic field may have other interesting effects on the types and nature of raft formation. As nanotechnology advances in the development of functionalized particles for use in these applications (such as magnetic particles to increase a cell’s magnetophoretic mobility\textsuperscript{28}), it is important that we develop tools to quantify the effects of these particles.
using model membrane systems and molecular-dynamics (MD) simulations to determine their effect on viability and predicted success in performing their anticipated function. In order to begin understanding these effects we must study the dynamics of particle/lipid interactions at interfaces, biological or otherwise. The effects of inorganic nanoparticles in organic or man made biomimetic systems is dependent on their size, chemical nature and on particle alignment. There have been previous attempts to understand these effects by studying how fabricated particles arrange in one or two-dimensional geometries\textsuperscript{29,30,31}. Specifically, with respect to the assembly and dynamics of magnetic nanoparticles, there are a few interesting studies that should be mentioned. Lee \textit{et. al} showed that magnetic nanoparticles could be arranged using the LB technique to generate a ‘vast’ monolayer whose properties were superior to similar systems generated using the spin-coating technique\textsuperscript{32}. Researchers under Clemente-Leon\textsuperscript{33} created magnetic monolayers of cesium/nickel/chromate nanoparticles coordinated with dioctadecyldimethylammonium bromide (DODAB) and studied both the dynamic surface effects using BAM and static effects by transferring the layer to a solid surface for AFM. They determined that the morphology of the generated layers was highly dependent on the history of construction and the ratio between surfactant and nanoparticle concentrations. The magnetic properties of the layer indicated highly localized regions of particles rather than homogenization - which was the desired result. While working with researchers in Chalmers’ laboratory\textsuperscript{34} we observed that micron-sized magnetite particles arrange into ‘string-of-pearl’ structures when an externally applied magnetic field is present. Although this finding has not yet been published it was observed independently by other researchers in our group.
Applied magnetic fields represent a non-mechanical method for spontaneously self-arranging particles into coherent structures that may be useful in other applications such as cell-scaffolding materials. Their paramagnetic properties have the potential to expand the usefulness of these structures beyond their physical arrangements.

The ability of biological structures such as lipid mono- or bilayers to interact with nanoparticles introduces the potential to control their two-dimensional organization\textsuperscript{35}. This is different from the studies mentioned because the 2-D continuous phase component is now biological in nature and the system dynamics change dramatically as a result. One method used to incorporate nanoparticles into patterned LB films is by fabricating the particles at the interface with a partially or fully-developed monolayer template\textsuperscript{36}. The nucleation and growth of nanoparticles is initiated by introducing the appropriate reagents directly at the surface or by adsorption from the subphase. This has the advantage of limiting the particle size distribution. Or, there is the simpler method of simply introducing a pre-fabricated nanoparticle suspension via the subphase under a Langmuir film, deposition from above, or by immersing a transferred film into the nanoparticle solution (although the last method does not provide any information about the rate or degree of incorporation into the template). Stuart\textsuperscript{37} investigated the interaction of DPPC monolayers with gelatin based nanoparticles adsorbed from the subphase and found that the particles tend to penetrate rapidly into the monolayer at moderate pressures, indicating that these particles may have an application in pulmonary drug delivery. Terheiden\textsuperscript{38} deposited multilayers of common lipids such as DPPC and DOPC onto a substrate and investigated the rate of magnetic FePt particle penetration from the gas phase, a novel approach. Results indicate that particle incorporation is highly dependent on the lipid head groups to
form a vesicle around the particles. If the temperature is sufficiently high, these vesicles become mobile and can penetrate into the multilayer structure.
Chapter 2: Anomalous Surfactant Behavior and Interactions with Lipid Monolayers

The ability to form a stable monolayer at an interface is primarily dependent on the nature and magnitude of the forces between the monolayer material and the surface under investigation. In the case of the LB technique the determining factors are the balance between the hydrophilicity and hydrophobicity of the assembling molecules and their tendency to phase separate. It is no surprise therefore that molecules possessing both types of regions, amphiphiles, are good candidates for monolayer formation. Important also is the degree of solubility in the subphase (usually water) and the ability to undergo compression at the interface. In other words, can we control the density of this species by confining them to ever decreasing surface areas. For the case of a soluble amphiphilic species the conventional wisdom says this should not be possible. That it was recently demonstrated that soluble surfactants can, under certain conditions, be compressed to form pseudo-stable Gibbs monolayers represents an interesting deviation from the conventional wisdom. Furthermore, the effect of these species on a lipid monolayer as it is condensed or on a layer that has already been compressed, is not fully understood although a good compilation of the current theories of how soluble surfactants may disrupt monolayers by diffusion from the subphase was recently assembled by Vollhardt and Fainerman and were
applied to previous research efforts within our group\textsuperscript{42}. Correlations between surfactant penetration into the monolayer and the effects observed in Chinese hamster ovary (CHO) cell cultures showed that there was a strong effect between the species chosen (Pluronic F-68, sodium dodecyl sulfate or ‘SDS’) and concentration on cell viability\textsuperscript{43}. The conclusion drawn was that the soluble surfactants were potentially having a disruptive effect on the cell lipid bilayers. Seeing that a disruptive effect was observed in similar structures in the Langmuir Trough in the presence of the same surfactants suggest that this process may play a role in cell death, at least in the case of a non-block copolymer species (like F-68).

2.1 Experimental Approach

Unlike the dodecyldimethylamine N-oxide (DDAO) used in the previous study we chose to use sodium dodecyl sulfate (SDS or sometimes sodium lauryl sulfate) because it is a relatively well-understood surfactant that is employed for multiple reasons in many laboratory and industrial settings, even as a food additive. For instance it is commonly used in biological studies to lyse cells. The structure for SDS is shown in Figure 2.1. The experimental methodology was to generate a range of SDS bulk concentrations and then study what effect was observed under compression in the Langmuir trough. We wanted to answer several questions:

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{structure_sodium_dodecyl_sulfate.png}
\caption{Structure of sodium dodecyl sulfate.}
\end{figure}
• First, do we see increases in surface pressure for this simple surfactant, as was observed previously for the more complicated amine-oxide species?

• Secondly, if this behavior is apparent, can we isolate the effect of compression speed on the ultimate surface pressure value?

• Thirdly, after compression, what is the time value associated with dynamic decompression and does it follow a logical pattern?

• Finally, what is the effect of the presence of a soluble species that tends to concentrate at the surface on the compression of an insoluble lipid species?

To answer these questions a series of bulk solutions of SDS were prepared at even intervals between 0 and 20 mmol/L and placed into the Langmuir Trough. After waiting for equilibrium (approximately 30 minutes - as determined by observing pressure changes from a freshly generated surface over the subphase SDS solution) the experiment began with compression runs at ever increasing speeds from 25 cm$^2$/min to 200 cm$^2$/min. Between runs the system was allowed to re-equilibrate over the customary waiting period. In some of the runs, rather than opening the barrier immediately after reaching the fully closed position (fully closed simply indicates that the barrier has traveled to the minimum allowed area, which is not necessarily zero.), data acquisition continued so that we could observe the Gibbs layer as it relaxed. This was important because it answers questions about the relative rate of diffusion of the surfactant away from the surface as compared with the reverse process. After the initial series of experiments were complete and noting some unusual behavior we decided to perform the same runs on concentrations that would increase resolution in the range where we saw these effects.
The fact that a soluble surfactant would tend to aggregate at the air/water interface is a frequently noted effect. What makes this process interesting is that the surfactant molecules would remain at the interface under compression, indicating that there is an energy barrier to overcome before a given molecule can diffuse into the bulk. Thermodynamically, for a simple ionic surfactant like SDS, we would expect that under compression the molecules would rapidly diffuse into the subphase as the apparent surface concentration deviated from equilibrium conditions. Indeed, this is a common assumption made throughout the literature relating to soluble surfactants. To quantify the surface excess of surfactant molecules (as opposed to what would be expected had the bulk concentration prevailed to the interface) we performed an analysis using titration curves and the Gibbs Isotherm (Equation 2.2) for both SDS and DDAO.

### 2.2 Results for the Free Surfactants

What became readily apparent was that there is compression at the interface, reaching significant pressure values, and that there is a strong relationship between subphase concentration and the ultimate surface pressure achieved in pseudo-stable Gibbs monolayers. As was mentioned, unlike the previous studies this experimental data extended to subphase concentrations of SDS that encompassed values well beyond the critical micellar concentration (CMC) of 8 mmol/L. Figure 2.2 shows a titration curve (via our SensaDyne bubble tensiometer) for SDS and DDAO for comparison with the dashed vertical lines indicating the CMC for each species. The difference between the titration curves indicates that the repulsive forces between SDS molecules, being ionic, is greater than the force between DDAO molecules. As a
result the CMC for SDS is higher by nearly an order of magnitude and is characterized by a much more rapid decline in surface tension. The points marked (a), (b) and (c) on the SDS curve correspond to the compression isotherms shown in Figures 2.3, 2.4 and 2.5, respectively. Initially the curves are flat because of a relative lack of available surfactant which causes the process of diffusion from the bulk to the interface to be significantly lower than for higher bulk concentrations. After the CMC the derivative is again zero because we have fully populated the interface with surfactant and the act of adding additional material will only affect the bulk. These experiments demonstrate that there are marked differences in behavior when the solution strength is varied. We hypothesize that the ability of these soluble molecules to remain confined at the 2-D interface is related to the formation of dimers or trimers which are
Figure 2.3: Isotherm for 0.25 mmol/L SDS.

Figure 2.4: Isotherm for 2 mmol/L SDS.
more stable than the monomeric surfactant alone. We have investigated the time stability of these compressed Gibbs layers to determine kinetic rate coefficients using an equation suggested by Maget-Dana\textsuperscript{44} (analogous to a first order reaction) resulting in these polymer structures.

\[ \ln \frac{\pi(t) - \pi_0}{\pi_f - \pi_0} = -kt \quad (2.1) \]

In Equation 2.1 \( \pi_f \) is the maximum surface pressure experienced by the system, \( \pi(t) \) is the surface pressure at some intermediate time, \( t \), \( \pi_0 \) is the initial pressure and \( k \) is the kinetics parameter. Note that \( k \) can describe both adsorption to the surface as well as desorption from the surface depending on our definitions of the various \( \pi \)'s. The desorption kinetics are quite modest compared to the reverse process (diffusing from bulk to surface) which seems to occur very quickly. Analysis of the dynamic data (see
Figure 2.6) revealed that Equation 2.1 is not sufficient to describe the phenomenon as the first order reaction assumption is apparently not appropriate. Initially, the model over predicts the surface pressure while in the long term (approaching several minutes) the opposite is true. Table 2.1 gives the regressed values for the reaction constant for first and second order reactions over the first 30 seconds (region of fast relaxation) and again for the region ending at five minutes. The values are of the same order of magnitude in each region, but differ by a factor of two. There are clearly different forces dominating the system behavior depending on how recently compression took place. One possible explanation is that upon compression we begin forming two-dimensional analogs of the sorts of structures we would expect to see at high surfactant concentrations in the bulk. That is, we have pseudo two-dimensional micelles, tubular structures and branched formations. At high compression it is possible that these structures may spontaneously disengage from the Gibbs monolayer and pass into the subphase, forming into three dimensional forms in the process. This would have the effect of removing large amounts of surfactant from the monolayer and thereby rapidly reduce the surface pressure. Once sufficiently relaxed, the slower process of dissolving these structures into individual monomers which then diffuse into the bulk would begin to dominate. Therefore, it makes sense that we see strong variations in the apparent rate of relaxation with the initial stage occurring much faster. The concept is shown in Figure 2.7 in which a cross section of the subphase shows diffuse surfactant migrating to the interface where it tends to concentrate and incorporate into pseudo two-dimensional micellar structures.

Using the definition of the chemical potential, with a few assumptions and rearrangements, it is possible to derive an expression for the number of additional
Figure 2.6: Maget-Dana model as applied to the experimental data for SDS Gibbs layer relaxation.

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Table 2.1: Rate constants based on reaction order
molecules at the surface relative to what would be expected had the bulk concentration prevailed all the way to the surface, the ‘surface excess’. This expression, Equation 2.2, is known as the Gibbs Isotherm.

\[
\Gamma = -\frac{1}{RT} \cdot \left( \frac{\partial \gamma}{\partial \ln(c)} \right)_{T,P} = -\frac{c}{RT} \cdot \left( \frac{\partial \gamma}{\partial c} \right)_{T,P}
\] (2.2)

In this equation \( \Gamma \) is the surface excess in [mol/area], \( \gamma \) is the surface tension [N/m], \( R \) and \( T \) have the usual meanings and the concentration \( (c) \) is given in [mol/L]. This expression is applicable only to dilute binary solutions of solute but is valid wherever the derivative of \( \gamma \) vs. \( \ln(c) \) is available. It is also applicable for stronger solutions by replacing the concentration with the activity. In order to use this equation we must have data that show the relationship between surface tension and bulk concentration, which was already available for SDS and DDAO (Figure 2.2) and then...
apply Equation 2.2 to both systems. The difference between these two surfactants is immediately apparent but not unexpected. Figures 2.8 and 2.9 are regressions of the surface tension data; the slopes show that the surface excess for SDS (6.290x10^{-6} mol/m^2) and for DDAO (4.585x10^{-6} mol/m^2) are of the same order of magnitude but that the ionic surfactant tends to aggregate at the air/water interface in greater numbers than would be expected with the non-ionic amine oxide. Note that for the selected figures we are using only the titration data from the negative slope linear region although this analysis applies to every point on the curve; this is merely the convention.

Figure 2.8: Derivative calculation for use in Gibbs Equation, SDS.
Experimental results also showed that the maximum surface pressure value experienced as a function of subphase concentration (rate of compression and initial areas being the same) is non-monotonic in that it begins at zero, passes through a maximum value and then decreases slowly back to zero (see Figure 2.10. The likely explanation is that at low concentrations ($\leq 2$mmol/L) there simply is not enough surfactant at the surface to generate a large pressure, at intermediate values ($2 - 5$ mmol/L) we go through a local maximum and at higher values (approaching CMC) the presence of structures such as micelles and extended branching network formations allow the surfactant to escape from the surface quickly by incorporating into these structures. These curves unfortunately do not give us any information on what surface structures
are present or how they evolve in time. However, they indicate conclusively that surface patterns with a soluble component should be stable over long enough periods to have marked effects on any structures formed from an insoluble second phase.

The issue of compression speed is a problem as it can be argued that as the compression is taking place surfactant will be diffusing away from the air/water interface which means that we cannot assume the layer is static. To determine the effect of dynamics on our compression data, a series of experiments were performed with the only variable being compression speed. This was done in parallel with the series that varied the bulk SDS concentration. Results showed that there is indeed an effect due to compression speed but that it becomes significant only in the range of concentrations in which we observe elevated pressures (the peaked region in Figure 2.10).
Figure 2.11 shows a comparison of the effects of compression speed for 0.5 mmol/L SDS versus 2 mmol/L run at 50 cm²/min and 200 cm² respectively. Although the maximum pressure for the 0.5 mmol/L run is significant, the difference in speed only changes this value by 3 mN/m. By contrast, the 2 mmol/L sample shows a change of 9 mN/m which is a significant deviation underlining the need to compress at the maximum possible speed when operating within this concentration range. The logical extension is to do likewise when working with any surfactant possessing this unusual surface behavior. For the sake of completeness, Figure 2.12 shows the effect as the

Figure 2.11: Isotherms of SDS illustrating effect of concentration and compression speed.
concentration was increased up to, and beyond, the CMC. For direct comparison the vertical axes for both figures have the same limiting values.

Figure 2.12: Isotherms of SDS at the CMC and beyond with compression speed effects.

2.3 Lipid Monolayers and Soluble Surfactants

The fact that surfactant molecules at an interface exist in pseudo-stable equilibrium with subphase is understood conceptually but attempts to quantify and understand why some species concentrate at the interface (while others do not) and to what degree they concentrate is an ongoing research challenge. The adsorption of small molecules such as ions or other ionic compounds (surfactants as an example) into monolayers of polymers, lipids or the membranes of living cells.
adds an even greater degree of complexity as this process involves multiple complicating factors that dictate both the rate and degree of penetration of these molecules. Our group investigated this effect previously in an attempt to find potential replacement molecules for the commercially available Pluronic F-68, a block co-polymer used widely in industrial bio-reactors. Our experimental techniques allowed for the study of the interaction of surfactants with lipid monolayers using different vectors. As an example, consider a system in which the soluble component is initially equilibrated at the air/water interface before introduction of the lipid monomers. After addition of the insoluble component compression proceeds in the normal fashion but is different from a system in which the surfactants diffuse into an already assembled layer in that the monolayer forces the surfactant away from the interface. In this kind of system we are well above equilibrium behavior which should give rise to even more unusual structures which could prove useful in future studies. It is important to note however that this scenario will result in rapidly evolving structures at the surface such that transferring the monolayer to a substrate will result in a space and time dependent arrangement of structures. If we are careful about the pressure we compress to and the rate at which we make the transfer, it may be possible to study this time behavior simply by analyzing different regions of the coated sample. This should reduce the number of experiments necessary to understand the transient behavior of the system which is attractive for obvious reasons so long as the film dynamic decompression time vs. substrate position information is correct.

The combination of an insoluble lipid species with a soluble component complicates matters significantly because it changes both the statics and the dynamics of the system. Figure 2.13 is a cartoon showing the penetration of a soluble component

28
(small head group, single hydrocarbon chain) into a partially assembled monolayer of lipids (double hydrocarbon chain) in equilibrium with the subphase. We also studied the effect these surfactants have on the dynamic compression and decompression of the monolayer.

2.4 Results for the Binary System

Consider Figure 2.14 which shows the dynamic decompression of SDS alone versus SDS in the presence of the insoluble lipid DPPC. Both systems were compressed at the same speed and to the same absolute area at which point the barrier was held stationary while recording the value of $\pi$ vs. time. From this graph it is clear that in the presence of an insoluble monolayer the dynamic decompression proceeds in the same manner as with the surfactant alone but that the initial pressure from which we decompress is higher due to less available area per molecule. The important feature
to note is that the kinetic rates are approximately equal, indicating that the process preventing the soluble component from escaping into the subphase was accelerated by the presence of the DPPC. This indicates that lipid-surfactant interactions are not negligible in magnitude for this system. What was also clear is that the presence of these ‘mobile’ species which can escape into the subphase has a pronounced effect on the compression curve of the lipid under consideration when compared to the lipid alone. Table 2.2 shows the regressed rate constants for the data in Figure 2.14. As expected, the reaction rate dropped sharply as time progressed showing that simple first or second order reaction kinetics are not sufficient to describe this system behavior. Furthermore, when compared to Table 2.1 the visual observation that the desorption rates were higher in the binary system is confirmed.
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Table 2.2: Rate constants based on reaction order for 2 mmol/L SDS and a DPPC monolayer.

The overall isotherm curves for the mixed system are given in Figure 2.15 which shows SDS and DPPC alone (a,b) versus the binary combination (c). Certain lipids, rather than having generally positive changes in $\pi$-Area slopes as the area is incrementally decreased, possess plateau regions which are due to rearrangement of the hydrocarbon chains. It is not a coincidence therefore that sterically hindered lipids such as DOPC and POPC are the types of molecules that possess these unusual isotherms although straight alkyl chain lipids like DPPC also show this behavior under certain circumstances. In this case the plateau is more likely to result from tail groups moving into a surface normal position rather than laying horizontally along the surface. Upon addition of SDS the plateau regions disappeared almost completely and the $\pi$-Area slope, rather than having distinct transition regions which mark a phase change, increase in a steady fashion before reaching the solid phase at which time they collapse. This indicates that the SDS is determining phase behavior as the layer self-assembles but that the DPPC, because it occupies a certain non-trivial fraction of the interface, causes much higher pressures than would be expected with either component by itself. However, if we make the assumption that the effect from each of the two species is additive, we would not expect to see the binary isotherm increasing so slowly at the onset of compression. In fact, the values do not exceed that of the lipid-only isotherm until the area is substantially reduced. This may indicate,
Figure 2.15: Example isotherms of (a) DPPC, (b) 2 mmol/L SDS, (c) DPPC over 2 mmol/L SDS, dashed lines show solid phase monolayer collapse point for lipid alone and for binary system.

for this particular system, the presence of attractive forces that cause an effective contraction of the monolayer resulting in a lower pressure.
Chapter 3: Interactions Between Lipids and Nanoparticles

Understanding how nanoparticles interact with biological systems is critical to their safe application and therefore it makes sense to study their influence on the building blocks of living cells. In this case, we are interested in how typical magnetite particles affect the self-assembly process of a monolayer of DPPC, a commonly occurring lipid discussed in Chapter 1 that is a major component of most cell membranes. By quantifying these interactions we make use of a potential screening methodology that may find use in preliminary health and safety studies. There are several interesting studies available that detail the penetration of nanoparticles into an assembled monolayer using different vectors which are discussed in Chapter 1.

3.1 Experimental Approach

As with the Gibbs and mixed lipid/soluble surfactant work described in previous chapters, a Langmuir-Blodgett trough was used to measure surface pressure changes in a mixed monolayer of DPPC and magnetite nanoparticles. Two types of particles were studied: A polydisperse aqueous suspension of untreated particles generated in-house and a commercially available monodisperse suspension in chloroform of particles treated with oleic acid (to maximize hydrophobicity). In all cases the lipid
species was deposited first from a chloroform solution at 1 mg/mL lipid concentration. After waiting approximately 10 minutes the nanoparticles were introduced from a solution that varied from 1.000 mg/mL to 0.950 mg/mL (the total sample volume was maintained at 1 mL which resulted in a downward concentration drift - this was necessary for continued accuracy). Runs were performed at 15 cm$^2$/min, unless stated otherwise, to reduce the likelihood of monolayer breakage and other transient effects that occur at higher speeds. The data were then compared with results from the lipid-only and nanoparticle-only runs to determine if the binary system behavior was additive or if nanoparticle/lipid forces were non-zero.

### 3.2 Results

When studying nanoparticles that have treated surfaces (in this case a strongly hydrophobic compound) it is important to understand effects due to surface interactions versus effects due to the physical size of the particle. This is especially true when considering that self-assembly in a monolayer is driven by both. The question remained as to the relative magnitude of these competing effects. In this initial case the particles used were created in-house, treated with oleic acid and were polydisperse. A series of experiments was conducted in which the isotherm for oleic acid was obtained and then compared to a monolayer compression of the acid in the presence of the treated nanoparticles. Figure 3.1 shows compression cycles for the two systems and indicates very good reproducibility. The slight shift on consecutive cycles is normal and not considered an issue. In addition, by performing cycle analysis it is possible to determine the correct operating range in surface pressure that indicates a stable monolayer and at what pressure the isotherm is likely to break. In this case
we estimated 30 - 35 mN/m. Figure 3.2 shows the full isotherm for both systems and indicates that layer assembly is dominated by the chemical nature of the particle surfaces rather than their physical size. The question now was how the presence of these particles would affect the monolayer assembly of a lipid species. In these tests it apparently made no difference if the particles were deposited at the surface first or if it were the lipids. For consistency however, it was determined that the lipids would be first. The experiments determined, as shown in Figure 3.3, that nanoparticles may act as ‘spring-like’ structures that pad lipid molecules during compression. In this figure the curves (a), (b) and (c) indicate DPPC, magnetite nanoparticle and dual DPPC/nanoparticle isotherms, respectively. The lack of a coherent solid phase

Figure 3.1: Compression cycles for oleic acid and for oleic acid treated polydisperse magnetite nanoparticles.
transition (dashed box) on the particle and mixed isotherms is believed to occur because of particle ‘hopping’ in which the nanoparticles begin to arrange into multilayer structures by moving out of the air/water interface much like ping-pong balls in a swimming pool if forced into a small enough area. This is only apparent with the polydisperse nanoparticles. Also, it is likely that smaller particles were forced out of the monolayer first and that larger particles remain because it is more difficult to disperse them in the aqueous subphase.

For the next phase we decided to perform similar experiments using a commercially available particle suspension that would be identical to those generated in-house but with the advantage of being nearly monodisperse and of a known concentration. This made it possible to talk in terms of area per particle rather than total surface
Figure 3.3: Isotherms of DPPC (a), 5nm magnetite nanoparticles (b) and the binary system (c).
area. The purchased particles, shown in Figure 3.4, had an average size of 5 nm and were treated with oleic acid to make them hydrophobic. As was done previously, we performed compression cycle analysis to determine the best experimental conditions for future runs and to estimate the monolayer breakage pressure. Figure 3.5 shows the results which indicate good reproducibility over the three cycles but, interestingly, show changes in cycle onset over time. The delay, in cycles two and three, of the onset of non-zero surface pressure and the sharper curves indicate possibly that there was some rearrangement of the particles on the initial cycle and that a more compact structure was retained through expansion. Another possibility is that the particles were not monodisperse enough which would cause smaller particles to be forced out of the monolayer in the same manner observed with the in-house particles. Indeed, a

Figure 3.4: TEM of the 5 nm commercially available particles (image courtesy of Ocean Nanoworks, with permission).
check with the MSDS revealed the listed particle size as $5 \pm 2.5$ nm which is a fairly wide range. However, by comparison, the in-house particles had a maximum size of $10 \, \mu\text{m}$ and a minimum under 5 nm which made them much more disperse. Or it may simply be the case that no matter how monodisperse a nanoparticle monolayer is, at any significant pressure there will be some small degree of particle dispersion into the subphase.

The differences between the SOR commercial particles and those produced in-house was equally apparent when we attempted to generate the full particle isotherm. Regardless of the amount of material deposited there did not appear to be any transition region in which particle movement out of the monolayer was apparent. In all cases the barrier moved into the minimum area position without the data showing a collapse point, even at the maximum barrier speed or with high surface concentration.
Figure 3.6 shows a sampling of the various runs in which we changed the concentration and barrier speed. Clearly the particles are unable to form a two-dimensional crystalline phase which would break upon sufficient compression as we observe frequently in lipids; this was the case despite flooding the surface with particles and compressing at high rates. The observed behavior, while unexpected, was not particularly troubling as we ultimately were interested in particle effects on lipid monolayers rather than particle dynamics. One explanation is that the particles generate a high enough surface pressure before compression to escape into the subphase as we believe they do under mechanical compression. Having fewer particles available when the isotherm begins would then naturally result in lower realized pressures. That this happens at
such low pressures would seem to indicate that sufficiently small particles, despite their degree of hydrophobicity, experience an equally small thermodynamic energy barrier at the air/water interface and, as a result, move easily into the bulk liquid. The rate of particle loss was possible to measure using pressure versus time data which we analyzed to obtain kinetic constants assuming two different order reactions. Figure 3.7 shows the data which appear quite similar to the data obtained on the soluble surfactant SDS in a Gibbs monolayer. The resulting rate constants were regressed and are given in Table 3.1. As with SDS the rates decreased over time and were of the same order of magnitude despite the markedly different nature of the two species.

![Dynamic Decompression of SOR Nanoparticles](image)

Figure 3.7: Dynamic relaxation of SOR nanoparticles showing Gibbs monolayer type behavior.
To proceed we performed an experiment, identical in setup as the one done previously for the in-house particles, using the commercial product. Figure 3.8 shows isotherms for two different particle/lipid deposition amounts at a constant volume ratio. The data show that curve behavior is identical until significant pressures develop. Because the nanoparticle/lipid mass ratio was the same for both experiments this is expected. However it is apparent that in the lower mass run there simply was not enough material on the surface to reach a collapse point. The second curve, however, clearly shows a collapse near 60 mN/m which is consistent with DPPC alone (refer to Figure 1.2). Figure 3.9 shows a comparison of the mixed monolayer with DPPC alone.

The two isotherms track well initially, but begin to deviate significantly at moderate to high pressures. This indicates that when the monolayer is diffuse and particle/lipid interactions are infrequent that there is very little effect on layer assembly. However, as the pressure increases to values expected in the liquid phase, particle/lipid interactions become much more frequent and cause the apparent surface pressure to be artificially lower than what would be expected with the lipid species alone. This indicates relatively strong attractive interactions between the hydrophobic tail groups and the oleic acid treated nanoparticle surfaces. It is not possible to infer whether or not this is an indication of how these particles would interact with a living cell bilayer structure but does show that they may have a tendency to accumulate at

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Table 3.1: SOR nanoparticle rate constants during dynamic decompression.
Figure 3.8: Isotherms for 10/10 mL and 15/15 mL DPPC/SOR nanoparticles.

Figure 3.9: Comparison between DPPC monolayer and the binary SOR particle isotherms.
the cell surface. Potentially, they could penetrate into the hydrophobic interlamellar region and cause a disruption in normal cell functions. Whether this represents an attractive or an undesirable situation depends completely on their intended use.

We also were interested in the time-dependent stability of the binary monolayer as it was assumed that even if there were some apparent relaxation that it would be relatively modest when compared to earlier studies involving soluble surfactants. What we discovered was completely unexpected: not only does a mixed monolayer of lipid molecules and treated nanoparticles relax from a compressed state, it does so at a rapid pace (see Figure 3.10). It seemed appropriate to do numerical analysis of the data in a manner consistent with what was described in Chapter 2. We attempted to determine rate parameters as though this process were a chemical reaction of first or second order. The results showed that the parameters vary significantly over time, which was the same behavior observed with the soluble species. The analog of Table 2.1 is presented here as Table 3.2. For the first order approximation the rate constants are nearly identical between the two systems for the period 0 - 30 seconds with the second order being an order of magnitude smaller than observed for SDS. The period 30 - 300 seconds showed a first-order kinetic parameter that was smaller by a power of ten while the change in the second order approximation was modest. It is important to note at this point that these two systems are not analogous; because of this fact, any apparent congruency in the data is merely coincidence. What is important to note is that the relaxation data both follow similar trends and that the rate constants are of a similar magnitude. This illustrates that the ability for a species, whether soluble surfactant or nanoparticle, to move away from the air/water interface and disperse into the bulk subphase is dominated by the same force.
Figure 3.10: Mixed monolayer relaxation from a compressed state for DPPC and SOR nanoparticles.

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Table 3.2: Rate constants for the binary system based on reaction order
By performing an L-B transfer to a cleaved mica substrate (Ted Pella Inc.) it was possible to analyze the equilibrium (although we use the word loosely here) surface features of the mixed monolayer under AFM. The nanoparticles, encountering the somewhat hydrophobic mica surface, should tend to attach and remain in positions that may be similar to where they were at the interface. We believed, however, that the effect of a soluble/insoluble species being present must have an effect on structure formation. Figure 3.11 shows a cartoon of how the LB transfer process is thought to occur with a soluble/insoluble surfactant acting as the ‘chaperone’ phase.

In this case there is a soluble entity that is washed away in the final step to leave the nanoparticle patterns which may be useful in applications where surface heterogeneity may be important such as cell attachment and separation via magnetic influence. In our case we decided to study the assembly and transfer of the nanoparticles in the presence of DPPC as we understood both systems very well from our body of previous work. A control sample of nanoparticles was transferred to a mica substrate under moderately high pressures (to assure a monolayer) and this was followed by transfers for the mixed lipid/particle layer at reasonably low (17.7 mN/m) and high (39.7 mN/m) pressures. We analyzed the samples using the bio-AFM device at Nanotech West and the resulting images are shown below. As is apparent in Figure 3.12, the sample obviously was not displaying the characteristics of a true monolayer as there are large regions in which particles were absent. Either the sample originally did have these properties and then rearranged on drying, or surface particle dynamics may be much more complicated than we had anticipated. For instance, what may have happened is that, upon compression to high pressures, the particles agglomerate into larger scale structures in a non-reversible manner. The drying process then
Figure 3.11: Cartoon showing soluble surfactant-mediated patterning and transfer of nanoparticles (use of an insoluble species is also possible).
would only cement these structures into more or less solid magnetite stripes. Closer

inspection, shown in Figure 3.13, shows that the larger structures were themselves
an agglomeration of meso-scale constructs on the order of approximately 0.1 microns;
this was still orders of magnitude larger than the stated free particle size of 5 microns.

Proceeding to the limit of meaningful resolution in the sample plane (in the case of this
instrument, X and Y resolution is limited to 1 nanometer while vertical, Z, resolution
is on the order of an angstrom) Figure 3.14 shows the meso-scale agglomerations were
composed of yet smaller particle constructs. This was intuitive when considering
particle dynamics but it was reassuring to see that the governing concepts of such

Figure 3.12: AFM image of SOR nanoparticles on mica; 20x20 microns.
Figure 3.13: AFM image of SOR nanoparticles on mica; 5x5 microns.
processes were readily apparent. It is important to note that the height scale in this image indicates that the highest structures, greater than 6 nm, and lowest, -6 nm, represent a total layer depth of about 12 nm. As the particles themselves are on the order of 5 nm this indicates nearly a perfect bilayer in thickness, which makes sense considering that this is precisely what should have happened during sample acquisition. Therefore we may conclude that although there was discontinuity on the mica plane that a reasonable monolayer of hydrophobic nanoparticles was present and that any subsequent changes in structure, while occurring either at the air/water interface or upon drying, did not cause any unusual thickening of the deposited sample - this is a critical consideration when talking about reproducibility. The binary system

![Figure 3.14: AFM image of SOR nanoparticles on mica; 1x1 microns.](image)

50
LB transfers yielded equally interesting results. Figure 3.15 shows that most of sample was homogeneous (from this scale) except for several striped regions and a few circular structures that formed in the continuous phase. The striped regions have jagged edges and a depth of approximately 2 nm and may be due to either a discontinuity in the mica substrate or to monolayer contraction during transfer and drying. We became immediately interested in looking more closely at these two distinct types of structures and increased magnification accordingly. Figures 3.16 and 3.17 are 5x5 and 1x1 micron images, respectively. Figure 3.16 showed that the nanoparticles in the continuous phase (small white dots) were clearly the highest structures on the

Figure 3.15: AFM image of SOR nanoparticles and the lipid DPPC on mica; 20x20 microns.
film - evidence that we had a true monolayer of a thickness smaller than the diameter of the particles. Also apparent was that these particles were not agglomerating to the extent seen in the control sample and that they were well-dispersed in the lipid phase. Proceeding to maximum resolution we selected one of the circular structures to investigate this unexpected behavior. Figure 3.17 shows that the structure was actually a ring-like formation having a thickness of about 2 nm (consistent with a monolayer of lipids) and that this structure was quite free of the nanoparticles seen in the continuous phase. The larger agglomerated particles near the region at the center

Figure 3.16: AFM image of SOR nanoparticles and the lipid DPPC on mica; 5x5 microns.
Figure 3.17: AFM image of SOR nanoparticles and the lipid DPPC on mica; 1x1 microns.
of the formation were quite large compared to the disperse particles. It is entirely possible that as this formation was undergoing self-assembly that several disperse particles were forced into an ever decreasing central area and experienced a higher pressure than those outside. In this way, the formation of the structure may itself be responsible for causing the particle agglomeration. Alternately, it may be that many particles had agglomerated in the monolayer as it was compressed but that, as we have observed previously, these larger particles were forced out of the interface such that only diffuse particles remained stable there. In this case, the formation of this lipid structure may have preserved some of these larger particles when otherwise they would have been lost. This does however run counter to our previous assertion that smaller particles should have a greater tendency to escape into the subphase than larger particles. In the presence of an insoluble continuous phase it may well be that exactly the opposite is true. Unfortunately the only way to truly understand what happens in a dynamic process like this would be to observe it in real time. Currently we are not aware of how this might be done, but are hopeful that AFM technology may advance to the point where a modified system could be incorporated into the LB trough system.

Clearly, systems of nanoparticles and binary systems that incorporate both particles and other species are complex and difficult to study without the benefit of having dynamic feedback during the entire process of monolayer generation. Nonetheless it is still possible to hypothesize about the assembly method of the observed structures and why we observe such distinct regions rather than a completely homogeneous system. For instance, we had assumed that the particles would remain at the interface and form their own particle-rich domains while the lipids would pack around and in
between them. Instead, it is the particles that tend to disperse or escape and the lipids themselves that form structured domains. The forces driving this behavior are not adequately understood and it is for this reason that the study of nanoparticle/biomolecule dynamics must continue for the foreseeable future.
Chapter 4: Molecular Dynamics Simulation

The simulation of Langmuir films is not a novel concept although as technology has evolved the level of detail and scope of the simulation environment have both increased dramatically. Atomistic simulations, being of the highest degree of complexity, have been able to predict pattern formation in Langmuir films\(^5\), but because of computational cost it proved difficult to elucidate the structure of larger scale lipid ‘rafts’ which are usually observed at intermediate pressures. Larger scale simulations, which treat the lipid molecules essentially as spheres have been performed by Singer’s Group (The Ohio State University) to address the question of why certain patterns form and under what conditions they are stable. For instance, the melting of ‘stripes’\(^5\) is closely related to an increase in the relative repulsion energy while a decrease causes the size of these structures to increase exponentially. Later work by Singer involved the testing of his model scaling theory which gave an indication as to what forces cause coherent structure breakdown and how this decomposition process may occur\(^5\). The reproduction of full \(\pi\)-Area isotherms have also been attempted\(^5,5\) with a remarkable degree of success. In the cited cases the authors were able to model the full isotherm for their candidate lipids (DPPC and DMPA, respectively) at the air/water interface from the gas (diffuse) phase through the liquid phases and ultimately to layer collapse upon reaching the solid phase. Coarse-grain
models have also had some success at predicting the method of collapse in these layers - one accepted method being a novel bridge formation between zwitterionic lipid head groups\textsuperscript{57} that allows them to escape into the aqueous subphase. Singer’s group used a modified Lennard-Jones potential that included a long-range repulsive term that decreases as $r^{-3}$.

4.1 Simulation Approach

Our methodology, as with the work of Singer’s group, was to use a modified Lennard-Jones potential with an added repulsion term to account for long-range forces. There are several methods used to account for long range repulsions as experienced in systems with a dispersion of screened charges or with dipole moments. The first is direct summation in which each dipole or charge experiences forces from every other dipole and screened charge in the system and its corresponding mirror images with the minimum image convention usually observed. The second is the Ewald summation in which contributions from mirror images are summed in a converging sequence. This amounts to solving the Poisson equation on a grid and tends to be quite computationally expensive. The third is the hybrid techniques in which a compromise is reached between direct summation and an approximation for interactions that extend beyond the region of direct sum. Rather than using an Ewald summation we decided to use a direct summation only within the cut-off region (which the code was already taking into account) and to account for the long-range interactions through the third term, $\mu^2/r^6$, in the L-J equation. Equation 4.1 was the form used in our simulation. Figures 4.1 and 4.2 show the modified potential and resulting intermolecular force curves in their reduced form. The force is computed by taking
the negative gradient of the potential, Equation 4.2. In this equation $f_{LJM}$ is simply the total force between two particles; these forces are summed to find the net acceleration for predicting future positions and velocities. Note that the $\mu$ used in the Lennard-Jones potential is not the same as that used for the lipid dipoles. In this case our intent was to approximate long-range forces using a simple expression while short-range forces use a direct and more complex summation.

$$u_{LJM} = 4 \epsilon \left[ \left( \frac{r}{\sigma} \right)^{12} - \left( \frac{r}{\sigma} \right)^{6} \right] + \frac{\mu^2}{r^3} \quad (4.1)$$

$$f_{LJM} = -\frac{\partial \vartheta_{LJM}}{\partial r} = 48 \frac{1}{r^2} \left[ \left( \frac{1}{r} \right)^{12} - 0.5 \left( \frac{1}{r} \right)^{6} \right] + \frac{3\mu^2}{r^4} \quad (4.2)$$

In addition to the modified LJ potential it was necessary to consider the contribution of dipole-dipole interactions over short ranges for which the additional term in the
intermolecular force was not intended to account. The simplistic Reaction Field Method described by Laudan and Binder\textsuperscript{58} was considered initially but ultimately was too coarse for our purposes. We decided instead to use a direct summation method as described by Weis\textsuperscript{59} for the potential energy, the resulting inter-lipid force and the torque on the interacting molecules. The total potential for the dipole-dipole field is then given by Equation 4.3 in which $r_{ij}$ is the intermolecular distance, $\mu_i$ and $\mu_j$ are the dipole vectors for the $i^{th}$ and $j^{th}$ particles respectively, and $p$ is the dimensionality of the system (2-D in our case). The $\vartheta_{LJM}$ is simply the contribution from the modified potential given by Equation 4.1.

\[
\vartheta(r_{ij}, \mu_i, \mu_j) = \vartheta_{LJM}(r_{ij}) + \frac{1}{r_{ij}^p} \left[ \mu_i \cdot \mu_j - \frac{p(\mu_i \cdot r_{ij})(\mu_j \cdot r_{ij})}{r_{ij}^2} \right] \tag{4.3}
\]
The force is found by taking the gradient of Equation 4.3 and the torque is given by Equation 4.4 in which $I$ is the moment of inertia and $\omega$ is the angular velocity.

$$\vec{\tau}_i = I \left( \frac{\partial \omega}{\partial t} \right)_i = \sum_{i \neq j} \frac{\vec{\mu}_i \times \vec{\mu}_j}{r_{ij}^3}$$  \hspace{1cm} (4.4)$$

Once calculated, the force was added to the contribution from the Lennard-Jones equation and the Velocity-Verlet scheme was applied to predict the future positions of the lipids and nanoparticles. A similar formulation was used to find the orientational change for the dipoles. The Velocity-Verlet formulation is especially powerful when compared to other techniques because it eliminates higher order terms like $\frac{\partial a(t)}{\partial t}$ and $\frac{\partial^2 a(t)}{\partial t^2}$. The governing mathematics (Equations 4.5, and 4.6) use the present values of position and velocity combined with the local accelerations, computed using Equation 4.7, to compute values at the future time.

$$r(t + \delta t) = r(t) + v(t)\delta t + \frac{1}{2}a(t)\delta t^2 \hspace{1cm} (4.5)$$

$$v(t + \delta t) = v(t) + \frac{\delta t}{2} [a(t) + a(t + \delta t)] \hspace{1cm} (4.6)$$

$$-\nabla v_{LJ} \quad \frac{m}{a}$$ \hspace{1cm} (4.7)$$

Initial values for the various parameters were selected by varying the potential and visually evaluating system behavior. Scenarios that seemed physically unreasonable (meaning that they displayed instabilities or caused the program to frequently correct predicted future positions, indicating that the time step size was too large, or in which the lipids were clearly behaving as a gas) were discarded. The model, as mentioned, employed the robust and relatively fast Velocity-Verlet formulation to predict future positions and velocities of the lipid and nanoparticles with a time step of between 10 and 40 femtoseconds ($1 \text{ fs} = 1 \times 10^{15} \text{ seconds}$). The time step size was selected
based on trial and error although methods do exist to predict this value based on
the average system energy distribution. Verlet lists, which allow the simulation to
loop through only the particles within a cutoff radius ($r_c$) is an especially useful
technique when dealing with large number of entities. The technique is attractive
because it scales as $N \ln(N)$ rather than $N^2$ if we select the cutoff radius to $L/2$, its
maximum allowable value, and loop over all particles in the system without a list.
In this case we initially selected a cutoff radius of $10\sigma$ with a buffer region of $12\sigma$
in which particle interaction was weighted to allow for a smooth introduction to the
interaction region. This is necessary because MD simulations require potentials that
do not cause abrupt changes as particles pass through the cutoff region; the resulting
discontinuity in potential energy and the force can cause instabilities. This concept is
illustrated in Figure 4.3 which shows the inner circle with full weight and the annular
buffer zone with varying weight. We believed the value for the cutoff radius was
appropriate because interactions at that range are a small percentage of their value
when $\sigma$ is less than 5. We discovered, however, that for good stability a much larger
cutoff region was needed and scaled the values to a direct region of $20\sigma$ and weighted
zone extending to $30\sigma$. The stability of later simulations proved that the changes
were appropriate. The weighting function is given by Equation 4.8 which shows that
it is linear in the buffer region, as is desired. An illustrative plot for generic values of
the cutoff and buffer region is shown in Figure 4.4.

$$
\omega_{ij} = \begin{cases} 
1 & \text{if } r_{ij} \leq r_c \\
\frac{1}{r_v} \cdot \left( r - \frac{r_c}{r_v} \right) + 1 & \text{if } r_c < r_{ij} \leq r_v \\
0 & \text{if } r_{ij} > r_v 
\end{cases} 
$$

(4.8)

While the modified LJ potential was appropriate for the lipids it does not apply to
nanoparticle/nanoparticle or lipid/nanoparticle interactions. Therefore we chose a
Figure 4.3: Cutoff zone and weighted buffer region for Verlet listing.

Figure 4.4: Plot of the weighting function for the buffer zone, arbitrary units.
smoothed hard-sphere potential for the nanoparticles and for the lipid/nanoparticle interactions with no long-range repulsive interactions.

A generalized flowchart of the simulation code is shown in Figure 4.5. The simulation initializes by reading in values for changeable variables from a list of cases in a file. These variables can include the number of time steps to run, $\epsilon$ (changing the temperature) and any other variable to be changed. Essentially any variable used in the simulation can be read in from the input case file, although only a few key variables were selected for simplicity. It is important to note that all variables in the code are in reduced form not only for ease of calculation and enhanced accuracy but, equally as important, to check for the equivalence of states. For example, the reduced temperature (Equation 4.10) may be increased either by raising the real temperature or by lowering $\epsilon$. So it is possible to arrive at two equivalent states by adjusting different variables - reduced units provide a method to avoid this issue. Following this, the code assigned initial positions for all entities based on a simple square lattice. The reasoning is that, if the simulation is behaving properly, the choice of initial
position should have little effect on positions far into the future. The particles and lipids were segregated into two different areas of the available space (100 nm X 100 nm) because this is simply easier to do than having the two species mixed initially. It also allowed the lipids to begin forming any short or extended domains prior to direct interaction with the nanoparticles. See Figure 4.6 for an example with 250 lipids and 25 nanoparticles \((l/p = 10)\). Initial velocities came from the selection of a random number on the interval \([-0.5,0.5]\) which is then scaled up to reflect the reduced temperature. Speaking theoretically, the initial values for velocity should not matter as in free particle systems they should tend toward a Maxwell-Boltzmann
Distribution over time (Equation 4.9).

\[ f(v) = \sqrt{\frac{2}{\pi}} \left( \frac{m}{kT} \right)^{3/2} v^3 \exp\left( -\frac{mv^2}{2kT} \right) \]  

(4.9)

In our case, however, we are dealing with a system in which dipolar attractions and repulsions are a significant factor in driving the formation of local condensed domains. Therefore it was not possible to apply the Maxwellian Distribution as a measure of convergence to equilibrium. Instead we must speak in terms of domain formation and stability over time with a small sub-population of free lipid or particle species moving between the domains. Because force and energy are conserved quantities, it was not necessary to correct the system center of mass beyond the correction to the initial velocity assignments. Because the Velocity-Verlet technique requires the positions from the previous time step, the code simply back calculated the positions from the initially assigned velocities. The program then looped over all species, computing the relative distance between the \( i^{th} \) and \( j^{th} \) particles and enforcing the minimum image convention. This was necessary because we were attempting to simulate a system that was essentially infinite from the perspective of the interacting particles but we do not possess the computing power to do this. Instead we must approximate an infinite system by assuming exact copies of the particles in boxes surrounding the simulation space. The mirror images of the particles and particles participate in force calculations through the minimum image convention in which a lipid or particle whose cut-off region extends beyond the system boundaries may interact with particles on the other side of the system through a simple translation vector of magnitude \( L \), the system length on a side. Figure 4.7 illustrates the concept. Regardless of the value for \( \sigma \) or \( \epsilon \) the potential function is shifted in order to make the value at the cutoff radius equal to zero (note: this does not affect the force calculation as the gradient is not zero at
the cutoff radius). Following this the intermolecular forces were computed by looping over each particle in the system and summing the result from all of a given particles’ neighbors (Equation 4.2) and then the dipole-dipole contribution was computed from the gradient of Equation 4.3. This was then used to predict the future position using the equations mentioned previously for the Velocity-Verlet scheme and then each particles potential and kinetic energy were added to an accumulating total. There was a check to ensure that any particles leaving the simulation box were recycled back by simply subtracting the box length from their predicted future positions outside the simulation space and making the same correction for their previous position because not doing so would result in the calculation of an artificially high velocity. At that point we had the future positions and velocities, which replaced the current values, we calculated the total potential and kinetic energy along with the current value of
the temperature (Equation 4.10).

\[ T_{\text{inst}}^* = \sum_i \frac{v_i^2}{pN_i} \quad (4.10) \]

In this expression \( p \) is the dimensionality of the system and \( N_t \) is the total number of lipids or nanoparticles (the instantaneous temperature will be different for both) - essentially, the total number of degrees of freedom. All variables of interest were written to data files at regular intervals (usually every 0.1 ns) and JPEG images were created for use in making animations to show dynamic behavior. Once a particular case was completed, the program referred back to ‘Cases.dat’ to find the next set of parameters to run and the process repeated. Because we chose to run the simulation on a desktop PC, we limited the number of lipid species to 1250 and nanoparticles to 25 as we believed that this represented a good compromise between density and computing resources. The total simulation time varied from run to run, but it seemed clear that a minimum of 10 ns is required for equilibrium. Sampling at 0.1 ns increments throughout each run also seemed sufficient to obtain a reasonable average for system potential and kinetic energy.

4.2 Results

The first step in evaluating program output was to vary the number of lipid molecules in the simulation space and monitor the system as it evolved in time. This was done to determine stability as particles were added and also to find an appropriate value for the time step size. Figure 4.8 shows the total potential energy as a function of the number of lipid particles without the presence of nanoparticles. The non-linear decrease is consistent with what would be expected as the average particle spacing decreases. A simple second-order polynomial fit yielded a very good \( R^2 \) value
and indicates that the potential energy is a smoothly varying function whose absolute value will continue to increase along with the total number of particles. After adding dipole moments on the lipids ($\mu^* = 0.22$ as a starting point from the literature) and adding the nanoparticles in their separate region we began seeing results like those shown in Figure 4.9. The figure shows how the lipid molecules have dispersed between the particles and that the particles have not moved significantly over the course of the simulation (9.9 nanoseconds). This makes sense because the particle mass was two orders of magnitude greater than the lipids which affects not only their velocity but also their simulated time step size. What was also apparent was that the lipid-nanoparticle potential was not strong enough to prevent some particle/lipid overlap.
Figure 4.9: Graphical output for $l/p = 40$, $T^* = 0.411$, $\rho^* = 0.02$ and time = 9.9 ns.

This was not a significant problem as we could simply increase the value of our parameters to compensate. Quantitative simulation output included the potential and total kinetic energy and the instantaneous temperature as shown in Figures 4.10 and 4.11, respectively. Clearly there is a period of adjustment over the first 30% of the simulation time with a steady-unsteady state evolving later. One check for free particle system stability, as mentioned in the introduction, is to compare the steady velocity distribution with the Maxwell-Boltzmann distribution. Despite the fact that our system has restraints on the particles through the dipolar attractive and repulsive forces that we should not see a deviation from the theory. As expected, Figure 4.12 shows that there was a good agreement between the particle energy distribution (through the velocities) and the M-B distribution. As the simulations proceeded it became
Figure 4.10: Numerical output for potential energy $l/p = 40, T^* = 0.411, \rho^* = 0.02$ and time = 9.9 ns.

Figure 4.11: Numerical output of total kinetic energy and instantaneous temperature for $l/p = 40, T^* = 0.411, \rho^* = 0.02$ and time = 9.9 ns.
apparent that we needed to find a balance between total simulation time (in terms of the number of time steps) and the actual time step size. To begin with we selected 10 fs but found that approximately $3 \times 10^6$ steps were necessary before observing equilibrium in the potential and kinetic energies. Obviously this posed a problem as the total run time for such a case was on the order of 100 days on our computers. Upon increasing the step size to 50 fs we determined that stability was still present and that the total number of time steps could be reduced accordingly. A full listing of the simulation runs performed is given by Table 4.1. Note that in this table the value of $\epsilon_p$ was multiplied by $1 \times 10^{22}$, $\epsilon_l$ by a value of $1 \times 10^{20}$ and $\delta t$ by a value of $1 \times 10^{14}$. In each case the number of nanoparticles was constant at 25 as were the values for $\sigma$ for the particles and lipids and the long range repulsion strength with a value of 0.1.
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</tbody>
</table>

Table 4.1: Listing of MD simulation runs.
This was done because it was possible to vary the lipid to particle ratio simply by varying the number of lipids and the long range repulsion, while having an effect on the system, was not the primary variable of interest to us and has been investigated by others. Instead we were interested in determining a stable operating envelope and appropriate range of values for the potential function and the dipolar strength as well as appropriate cutoff and buffer region dimensions. In many cases the simulation experienced an initial period of stability of between 5 and 10 ns before an instability caused one or more groups of particles to suddenly accelerate. This effect then propagated through the system causing a dramatic increase in the instantaneous temperature and velocity distribution. Ultimately, the lipids moved to unrealistically large distances which caused the program to cease calculation. These runs showed that the instability was caused by unusually large force calculations with the primary contribution being the magnitude of the dipole moments on the lipids. We determined that this value should be decreased for subsequent runs and that a force correction algorithm was necessary. Run 13 was the first such run and showed vastly increased stability so all subsequent simulations used this new correction scheme. That we observed stability for some earlier scenarios was most likely due to the relatively sparse nature of the system, having only 100 to 200 lipid molecules. This would result in larger average distances between particles leading to much smaller force values; this made the calculation inherently more stable. As mentioned previously, we determined that the relatively modest cutoff radius was still insufficient to enable smooth transitions so we enlarged both the full and partial weight regions to $20\sigma$ and $30\sigma$, respectively. Run 17 was the first to use this approach and showed stable output in energy. Figures 4.13, 4.14, 4.15 and 4.16 show the instantaneous temperature, potential energy,
kinetic energy and final computed positions for the lipids and nanoparticles, respectively. There is some instability in the computed potential energy but the kinetic energy and instantaneous temperature indicate that we have a stable system under when operating under these settings. As expected, the nanoparticles themselves have not moved appreciably and the lipids have diffused into the spaces between them. Also apparent is the phenomenon, observed previously, of the lipids tending to accumulate around the nanoparticles, despite there being no attractive force between them. Again, we believe this is due to the particles representing lipid-free zones that do not exert the usual long-range and dipolar forces that are present elsewhere. So in this case the lipids push themselves into the regions bounding the particles rather than the particles attracting them. If we were to add an attractive term this effect would only be increased.
Figure 4.13: Instantaneous temperature for Run 17 versus simulated time.

Figure 4.14: Potential energy for Run 17 versus simulated time.
Figure 4.15: Kinetic energy for Run 17 versus simulated time.

Figure 4.16: Final positions for the lipids (blue) and nanoparticles (brown) for Run 17 at 10 ns.
Chapter 5: Conclusions and Recommendations

This work was concerned with surfactant and nanoparticle effects at an interface between air and water and the dynamics of lipid monolayer assembly in their presence. Understanding the effect of soluble surfactants in such a system is directly related to industrial cell culture maintenance and viability as numerous studies have shown that some surfactants are helpful in reducing shear stresses that can destroy cells, while others are linked to an increased rate of cell death due to chemical interactions. Similarly, a knowledge of how nanoparticles interact with cell-like structures is the first step in determining what effects will be present in cell cultures. Therefore, the techniques used in this study provide a general screening tool with significant potential. In addition, there exists the ability to pattern, at the micro and nano scale, nearly any substrate to enhance performance, functionalize, or to reduce unwanted effects. Of particular importance is this occurs in a non-cleanroom environment and uses vanishingly small quantities of material. The molecular dynamics portion of the work has laid a foundation for future students who can improve and continue to study mixed nanoparticle/lipid systems. However, the initial study using the code has been informative and allowed us to understand the dynamics of this type of system in a way that our instruments cannot.
With respect to the Gibbs monolayer studies on sodium dodecyl sulfate we conclude the following:

1. Even relatively simple surfactants like SDS are compressible due to equilibrium and dynamic effects at the interface. There are surface effects that inhibit the molecules from escaping this region either due to a thermodynamic energy barrier or because monomeric surfactant cannot diffuse into the subphase until it has been removed from any coherent structures in which it participates or until the structure itself diffuses away. Because of this barrier effect, it is therefore possible to generate large surface pressures which decay slowly over time.

2. Diffusion to the surface is at least an order of magnitude faster than diffusion from the surface into the bulk.

3. Isotherm behavior and maximum observed surface pressure are a strong function of concentration. When dilute, the pressure will be lower due to the scarcity of surfactant. At intermediate values (1 - 4 mmol/L) we see a maximum and, at higher values (CMC and above), we see almost no compression. This is due to the presence of micellar, tubular branch and other structures in the subphase into which the monomer may incorporate and escape. This would indicate that the energy of incorporation is much lower than the energy barrier across the air/water interface.

4. The time-dependent nature of the Gibbs monolayers we studied showed that a simple reaction model (Maget-Dana) is not sufficient to describe the behavior. Depending on the amount of time the layer has been allowed to relax the rate
constants, whatever the order of reaction, change significantly and show that a more complex model is necessary.

For future studies we recommend that this work be expanded to include a wide range of surfactants: ionic, non-ionic, amphoteric and a block co-polymer like F-68. For the ionic species we recommend ammonium lauryl sulfate (similar to SDS), dioctyl sodium sulfosuccinate and dimethyldioctadecylammonium chloride as these are pH-sensitive which would add a dimension of complexity to the work. Also, these are relatively inexpensive and reasonably safe for use in our laboratory. For the zwitterionic species we recommend any primary, secondary or ternary amine species with a sulfate or carboxylate group. For the non-ionic species we recommend a simple compound such as cetyl or stearyl alcohol. As there are entire product lines at multiple chemical suppliers concerned with the generation of novel block co-polymeric species, the choice of compound is left to the reader. Simple compression studies followed by lipid monolayer effects should be conducted followed by adjustment of pH on a few selected experiments. If possible, it would be interesting to attempt to image the monolayer by AFM upon transfer to a mica substrate and determine how normal structures (usually stripes) are affected.

Regarding the soluble/insoluble surfactant composite layers we find:

1. Much of the characteristics of the lipid-only monolayer are preserved during compression but the lack of a plateau region indicates that there is a pre-arrangement of the lipid tail groups before monolayer assembly. Because of van der Waals forces the lipids should have a tail orientation parallel to the interface under normal circumstances; the reorientation to the normal position is what causes the observed flat region in the isotherm. That we do not see
this region in the presence of SDS shows that the tail groups are aligned in the normal position at equilibrium prior to compression.

2. Compression speed is less of a factor in the mixed monolayer than for the Gibbs layer excluding those speeds which may cause premature breakage of any lipid-rich regions.

3. Desorption of the soluble component in the mixed layer is an order of magnitude lower than for the soluble species alone. This indicates a stabilizing force due to the presence of the lipid, possibly due to an attraction between the hydrophobic tail groups or an ionic/dipolar interaction between the hydrophilic head groups.

Concerning the nanoparticle dynamics and lipid/nanoparticle interaction work we conclude the following:

1. The ability for properly treated nanoparticles to act as surface active agents is clearly demonstrated in this work. That they have an unusual and transient nature upon forming a monolayer is also evident in the data with the degree of size variation being a critical factor. The more polydisperse the size distribution the more notable is the effect as smaller particles are forced out of the monolayer and are thus unable to fully participate in future compression cycles.

2. When a suitable particle suspension is used and is treated under the proper conditions, highly reproducible experiments are possible. This gives a range of conditions over which particle exclusion from the monolayer is kept to a minimum and can be virtually eliminated as a confounding variable.
3. Compression isotherms for the mixed nanoparticle/lipid system showed that at low to moderate compression the lipid species dominates the behavior. At higher compression it is clear that particle/lipid interactions have a significant effect by artificially lowering the pressure, showing that the effect of the particles encompasses more than simply occupying space. This indicates that interactions between the two components is attractive. It is important to note, unfortunately, that quantifying the force magnitude is not directly possible and would require more assumptions than would be appropriate.

4. As with the soluble surfactant work, it was apparent that mixed monolayers of nanoparticles and lipids also experience a relaxation following their assembly. The pressure decay curves closely resembled those of the lipid/SDS monolayers and rate constants, regressed from the data, showed the same magnitude and degree of variation depending on the relaxation time. It was noted in the text that similar values for these constants is purely coincidental.

5. Transfer of the mixed monolayer to a cleaved mica substrate and subsequent analysis by AFM showed particles and lipids mixed in a fairly uniform fashion. This behavior was expected as we hypothesized that the presence of this species would disrupt normal lipid domain formation.

In the future we believe that these studies should be expanded to include a greater variety of nanoparticles. For instance, a study using SOR particles in the 20 - 50 nm size range would be interesting as the company claims $\pm 2.5$ nm for the entire product line. This will result in a tighter distribution and would us to test our hypothesis that particles will be more stable as polydispersion decreases in a more thorough manner.
It would also be informative to vary the nature of the particle surface modification to determine the effect. That the particles were magnetite was not important in this study - they could have just as easily been pure iron or cobalt. What would be interesting is to take advantage of the particles paramagnetic nature and study their dynamics in the presence of an externally applied magnetic field. This would require some specialized planning as much of the langmuir trough contains materials that are effected by such fields but certainly it is not impossible. Finally, varying the type of lipid used would be helpful as it would answer questions about the effect of head-group charge and size on the lipid/particle effects. If there is no effect then we may say conclusively that the attractive forces are due to tail-oleic acid interactions.

In reviewing our results from the molecular dynamics work we conclude:

1. As the number of particles or lipids is increased the net system potential energy increases. This is due to the spacing between particles being reduced on average and thereby forcing the system into a more negative region on the potential function.

2. After studying system dynamics as a function of time step size, we conclude that for such systems a time step size of 50 fs ($10^{-14}$ seconds) represents a practical ceiling above which the simulation should not run. Larger time step sizes caused instability and resulted in particle overlap and unreasonable behavior.

3. A hard sphere potential for inter-particle and particle/lipid interactions is reasonable.

4. The effect of the ratio of the number of lipids to the number of particles appears not to have a significant effect on overall system behavior beyond the
physical exclusion zones that the particles represent. The apparent affinity of
the lipids to group around the particles is due to the repulsive forces between
the lipids themselves, making the region surrounding the nanoparticles attrac-
tive because of the absence of this force. Including an attractive term in the
lipid/nanoparticle potential would only increase this affinity. Clearly, this be-
havior is one of the underlying causes of the net attractive force observed during
the monolayer experiments.

5. Variation of the depth of the potential well caused the requisite scaling in veloci-
ties and temperature which caused the system to behave more like an expanded
liquid when the depth was increased versus a gas-like phase when decreased.
This effect was anticipated and is reasonable for molecular dynamics simula-
tions.

6. The effect of the long range repulsion due to the additional term in the Lennard-
Jones potential was not immediately apparent. What we determined was that
dipole-dipole interactions are far more dominant in this system, although exclu-
sion of the long range term would have been unrealistic and so it seems unwise
to neglect its contribution.

7. As with the time step size, the selection of cutoff range and weighting region
was in dispute. We determined that our initial estimate of $5 - 10\sigma$ for the
fully weighted region and an additional $2 - 5\sigma$ for the weighted region were
too conservative for the purposes of stability. Upon increasing these values to
$20\sigma$ and $10\sigma$, respectively, we observed greatly increased stability and the code
outputs seemed quite reasonable at all simulated times. Future work will utilize these new values.

8. The magnetite nanoparticles, being two orders of magnitude heavier than the lipids, move proportionally much slower than the lipids do over the course of any given simulation. We believe that for the purposes of speeding convergence, especially when particle/particle interactions are of interest, the mass of the nanoparticles should be artificially decreased for an appropriate length of simulated time to facilitate their penetration into the lipid-rich regions. At that time the we would reset the mass to its true value and proceed normally.

9. When speaking about validation, especially with atomistic codes, it is difficult to make direct comparisons on the micro scale to macro scale observations. As the simulation showed, the physical presence of the nanoparticles which did not have any attractive force on the lipids, nevertheless showed lipid grouping due to the absence of local repulsions. This is likely to be one of the reasons for the observed monolayer contraction seen in the laboratory and underlines the fact that any additional attractive force due to the chemical nature of the particles will only strengthen this effect. That there has been no previous mention of positive lipid interaction by virtue of exclusion of material due to the presence of a nanoparticle species makes this work a first step in attempting to explain what may happen when these particles interact with living cells.

Expansion of this work should include the ‘softening’ of the inter-particle and particle/lipid potentials to further test the validity of our hard sphere assumptions. This will be especially important if we wish to consider surface-treated particles whose
chemical nature will play an important role in interactions. It would also be interesting to fully explore our simulation space by evaluating the effect of all appropriate variables including the depth of the potential well, lipid and particle masses, system size, temperature, etc. Transition from single to parallel computing would represent a significant advantage in time savings. However, it may not be possible to do so using the current programming language. Therefore we recommend that the code be rewritten in C##, Fortran or any other suitable format and then run in parallel using machines at the OSC (Ohio Supercomputing Center).

In general terms, we recommend obtaining funds for a replacement laser for the Brewster Angle Microscope (BAM) and imaging CCD. The technology has advanced appreciably enough to render the current setup nearly obsolete. We believe also that having an AFM in the laboratory would remove a severe bottleneck in sample processing and would save time that would otherwise be wasted in transit to laboratories that have this capability. And a replacement motor on the water ultra-purification system and acquisition of all necessary supplies for cartridge installation, including liquid sterilizer, would be desirable.
Appendix A: List of Equipment and Supplies

The following is a listing of the equipment, supplies and software used in the previous work. Commonly used items such as gloves, pipettes, kimwipes, etc. are excluded.

- Sensadyne Bubble Tensiometer, Model# PC500-LV, Serial# 470
- Langmuir-Blodgett Trough, NIMA Technology, Model# 611, Serial# 087
- NIMA TR516 DAQ Software
- PowerSpec PC, Microcenter Inc., Windows Vista, Serial# V201020820338
- MatLAB R2009a Math Environment Software, 32-bit
- Mega-Pure System, Barnstead, Version# MP-1, Model# A440267, Serial# 674930897328
- Asylum Bio-AFM, Asylum Systems Inc.
- Confocal Microscope, Leica
- Mica Sheets - 1 x 4 cm - Fine Condition, Ted Pella Inc.
- Various Lipid Species, Avanti Polar Lipids Inc.
- Cholesterol, Avanti Polar Lipids Inc.
- Sodium Dodecyl Sulfate, Aldrich
- Dual-Frequency UV Lamp, Fisher Scientific
- dodecyldimethyl-N-amine oxide, Fluka
- SOR 5nm Magnetite Particles with Oleic Acid, Ocean Nanoworks
- \LaTeX\ document preparation and typesetting program
Appendix B: Study of Rheological Modification of HEMA and Surface Effects with Included Nanoparticles

Part of the very first work we were interested in was surface modification using ultrathin film technologies. One promising material for use in cell-attachment cultures was 3-hydroxyethyl-methacrylate (HEMA) because the abundance of hydroxy groups for hydrophilicity combined with an unsaturated bond that would participate in polymerization. The structure for HEMA and for the polymerization initiator used in this study are shown in Figure B.1. We decided to investigate the effect of a thickening agent, poly(ethylene glycol), on HEMA to determine how changing the weight percent of PEG and the molecular weight of PEG on the surface tension and viscosity as these are a critical factor for any coating material. The measured effects are shown in Figures B.2 and B.3 where it is clear that both the surface tension and viscosity are strongly affected by the addition of PEG8000. We also were interested in the effect of adding magnetite nanoparticles to the HEMA to potentially create a functionalized magnetic surface coating. Figure B.4 shows a thin layer of HEMA on a polycarbonate substrate where cracking is evident. This indicates that the layer was not cured quickly enough. Figure B.5 is the same system but with nanoparticles mixed into the HEMA/initiator liquid. Particle movement during the curing process is evident due to the lack of particles near surface defects and the tendency to cluster.
toward the center of each domain. Figure B.6 is a closeup showing extensive particle agglomeration such that these are essentially microparticles rather than nano. These images were obtained using confocal microscopy. Ultimately we decided to move the research in a different direction due to our group specializing in self-assembly of ultra-thin films at an interface rather than the ‘bulk’ approach in this study.

(a) HEMA.

(b) Irgacure 819.

Figure B.1: Atomic structures for monomer and initiator.
Figure B.2: Surface tension as a function of weight % PEG8000.

Figure B.3: Viscosity as a function of weight % PEG8000.
Figure B.4: Acrylic surface with thin layer of polymerized HEMA.

Figure B.5: Acrylic surface with thin layer of polymerized HEMA and magnetite nanoparticles.
Figure B.6: Closer view of the HEMA/particle surface.
Appendix C: Additional Bulk SDS Isotherms

The following data are included here, rather than in the body of the text, because they only further underline our conclusions. Namely, that the ability to form a Gibb’s monolayer that is stable under compression is a strong function of bulk subphase concentration. At lower concentrations we observe stable layer formation, near the CMC and above no compression is observed. As noted in the text, this is due to the formation of micellar structures as the CMC is approached and the surfactant at the interface can easily escape by incorporating itself into these structures. The energy required to do so is much lower than diffusing over the energy barrier and into the bulk. Note that for Figures C.11 and C.12 the maximum values on the vertical axes are intentionally kept large to emphasize the lack of change in surface pressure in comparison to lower concentrations.
Figure C.1: Isotherm comparison between 2 mmol/L and 4 mmol/L SDS at 50 cm²/min.

Figure C.2: Isotherm comparison between 2 mmol/L and 4 mmol/L SDS at 200 cm²/min.
Figure C.3: Isotherm for 2 mmol/L SDS at 100 cm$^2$/min.

Figure C.4: Isotherm for 2 mmol/L SDS at 200 cm$^2$/min.
Figure C.5: Isotherm for 4 mmol/L SDS at 100 cm$^2$/min.

Figure C.6: Isotherm for 4 mmol/L SDS at 200 cm$^2$/min.
Figure C.7: Isotherms for 0.25 mmol/L SDS at various speeds showing both compression and expansion curves.

Figure C.8: Isotherms for 1 mmol/L SDS at various speeds showing both compression and expansion curves.
Figure C.9: Isotherms for 2 mmol/L SDS at various speeds showing both compression and expansion curves.

Figure C.10: Isotherms for 4 mmol/L SDS at various speeds showing both compression and expansion curves.
Figure C.11: Isotherms for 12 mmol/L SDS at various speeds showing both compression and expansion curves.

Figure C.12: Isotherms for 20 mmol/L SDS at various speeds showing both compression and expansion curves.
Appendix D: Molecular Dynamics Simulation Code

1 % md5.m
2 % This is the fifth and final code for the MD simulation
3 % of lipids and nanoparticles using standard coding approach
4 % with a modified LJ potential.
5
6 mpl = 1.219e-21; % lipid molecular mass, gm
7 mpp = 2.40e-19; % nanoparticle mass, gm
8 kb = 1.3806503e-23; % boltzmann constant, m2*kg/s2*K
9 sigmat = 1e-9; % unit of length from LJ equation
10 sigmap = 5e-9; % unit of length from LJ equation
11 etat = 1e-19; % unit of energy from LJ equation, lipid
12 etap = 1e-22; % unit of energy from LJ equation, particle
13 trt = sqrt(etat/mpl/sigmat^2); % Reduced time
14 trp = sqrt(etap/mpp/sigmap^2); % Reduced time
15 tstep = 2e-14; % Real time step size, relative to reduced
16 % step size
17 tsteprt = trt*tstep; % Reduced time step size
18 tsteprp = trp*tstep; % Reduced time step size
19 T = 298; % real temperature
20 Trt = kb*T/etat; % reduced temperature
21 Trp = kb*T/etap;
22 %P = 101325; % real pressure, Pa
23 %p = P*sigmat^3/etat; % reduced pressure
24 L = 1e-7/sigmat; % system length, on a side
25 rc = 20; % Cutoff radius for LJ potential, multiple of
26 % sigmat
27 rv = rc + 10; % Defines border region for partial weight
28 rc2 = rc^2; % Cutoff squared, to save time
29 rv2 = rv^2; % Border cutoff squared
30 lwt = 1/(rc-rv); % Slope of the weighting factor
\texttt{\texttt{inwt = 1 - rc/(rc-rv); % Intercept of the weighting factor}}
\texttt{\texttt{mu = 0.1; % Long range repulsion}}
\texttt{\texttt{mu_dp = 0.1; % Dipole moment for the lipids}}
\texttt{\texttt{dr = 0.1; % Increment used for computing gradient of}}
\texttt{\texttt{%dipole potential}}
\texttt{\texttt{ecut = 4*(1/rc^12 - 1/rc^6) + mu/rc^3; % Value of LJ}}
\texttt{\texttt{%potential at cutoff}}
\texttt{\texttt{\%fid4 = fopen('Cases.dat','r'); % Read case settings file}}
\texttt{\texttt{\%caseSettings = fscanf(fid4,'%g %g %g',[3,inf]);}}
\texttt{\texttt{\%fclose(fid4);}}
\texttt{\texttt{\%caseSettings = caseSettings'; % Import and invert matrix}}
\texttt{\texttt{\%[row,col] = size(caseSettings); % Get the size of the matrix}}
\texttt{\texttt{tic; % Start internal stopwatch (gives total run time)}}
\texttt{\texttt{\%for gcount = 1:row}}
\texttt{\texttt{Ntp = 250; % number of lipid particles}}
\texttt{\texttt{Npp = 25; % number of nanoparticles}}
\texttt{\texttt{tts = 2e5; % Total Time Steps}}
\texttt{\texttt{fid = fopen(sprintf('%gNtp_%gNpp_%gtts.dat',Ntp,Npp,tts),...}}
\texttt{\texttt{'wt')}}
\texttt{\texttt{fid2 = fopen(sprintf('%gNtp_%gNpp_%gtts_xy_0.dat',Ntp,Npp,...}}
\texttt{\texttt{tts),\texttt{'wt')}}
\texttt{\texttt{fid3 = fopen(sprintf('%gNtp_%gNpp_%gtts_uv_0.dat',Ntp,Npp,...}}
\texttt{\texttt{tts),\texttt{'wt')}}
\texttt{\texttt{Ntt = Ntp+Npp;}}
\texttt{\texttt{\% Create settings file for reference}}
\texttt{\texttt{fid7 = fopen(sprintf('Settings_%gNtp_%gNpp_%gtts.dat',Ntp,...}}
\texttt{\texttt{Npp,tts),\texttt{'wt')}}
\texttt{\texttt{fprintf(fid7,'Ntp = %g\n',Ntp);}}
\texttt{\texttt{fprintf(fid7,'Npp = %g\n',Npp);}}
\texttt{\texttt{fprintf(fid7,'Sigmat = %g\n',sigmat);}}
\texttt{\texttt{fprintf(fid7,'Sigmap = %g\n',sigmap);}}
\texttt{\texttt{fprintf(fid7,'Etat = %g\n',etat);}}
\texttt{\texttt{fprintf(fid7,'Etap = %g\n',etap);}}
\texttt{\texttt{fprintf(fid7,'T = %g\n',T);}}
\texttt{\texttt{fprintf(fid7,'Trt = %g\n',Trt);}}
\texttt{\texttt{fprintf(fid7,'Trp = %g\n',Trp);}}
\texttt{\texttt{fprintf(fid7,'ttrt = %g\n',ttrt);}}
\texttt{\texttt{fprintf(fid7,'ttrp = %g\n',ttrp);}}
\texttt{\texttt{fprintf(fid7,'tstep = %g\n',tstep);}}
\texttt{\texttt{fprintf(fid7,'tsteprt = %g\n',tsteprt);}}
fprintf(fid7,'tsteprp = %g
',tsteprp);
fprintf(fid7,'tts = %g
',tts);
fprintf(fid7,'mu = %g
',mu);
fprintf(fid7,'mu_dp = %g
',mu_dp);
fprintf(fid7,'rc = %g
',rc);
fprintf(fid7,'rv = %g
',rv);
close(fid7);

% Generate evenly spaced particles on a lattice with random velocity vectors
sumvt = zeros(1,2); % Velocity sum
sumvt2 = zeros(1,2); % Mean square velocity sum
sumvp = zeros(1,2); % Velocity sum
sumvp2 = zeros(1,2); % Mean square velocity sum
x = zeros(Ntt,4); % Create matrices for position and velocity
v = zeros(Ntt,2);
lp = sqrt(L^2/Ntp)*0.94; % Particle length scale for lattice, corrected
% The correction factor (default 0.98) is arbitrary.
% Plot the initial lattice to ensure that no particles are outside the boundaries. If so, then scale back.
%b = 0; % Lattice generation counters
%c = 0;
tempdipole = 0;
lacount = 1;
for i = 4:4:100
    for j = 5:5:50
        x(lacount,1) = i-2+L;
        x(lacount,2) = j-2.5+L;
        x(lacount,3) = 0;
        x(lacount,4) = rand*2*pi; % Dipole vector angle
        lacount = lacount + 1;
    end
end
for i = 20:20:100
    for j = 10:10:50
        x(lacount,1) = i-10+L;
        x(lacount,2) = j-5+50+L;
        x(lacount,3) = 1;
        x(lacount,4) = 0; % No dipole on the particles
        lacount = lacount + 1;
    end
end
```matlab
for i = 1:Ntp
    v(i,1) = rand - 0.5;  % Random particle velocities
    v(i,2) = rand - 0.5;
    sumvt(1,1) = sumvt(1,1)+v(i,1);  % Add to velocity
    sumvt(1,2) = sumvt(1,2)+v(i,2);
    sumvt2(1,1) = sumvt2(1,1)+v(i,1)^2;  % Add to v^2
    sumvt2(1,2) = sumvt2(1,2)+v(i,2)^2;
end
for i = Ntp+1:Ntt
    v(i,1) = rand - 0.5;  % Random particle velocities
    v(i,2) = rand - 0.5;
    sumvp(1,1) = sumvp(1,1)+v(i,1);  % Add to velocity
    sumvp(1,2) = sumvp(1,2)+v(i,2);
    sumvp2(1,1) = sumvp2(1,1)+v(i,1)^2;  % Add to v^2
    sumvp2(1,2) = sumvp2(1,2)+v(i,2)^2;
end
% Create graphic of initial positions
xpos = sprintf('%gNpt_%gNnt_%gtts_X_%d',Ntp,Ntt,tts,0);
figure(1)
plot(x(1:Ntp,1),x(1:Ntp,2),'o','MarkerSize',6,...
     'MarkerEdgeColor','b','MarkerFaceColor','b')
hold on
plot(x(Ntp+1:Ntt,1),x(Ntp+1:Ntt,2),'o','MarkerSize',20...'
     'MarkerEdgeColor',[0.36,0.2,0.09],...
     'MarkerFaceColor',[0.36,0.2,0.09])
xlabel('X')
ylabel('Y')
title('t = 0')
print(1,xpos,'-djpeg')
close(1)

% Correct the center of mass
sumvt = sumvt/Ntp;  % Average particle v and v^2
sumvt2 = sumvt2/Ntp;
sumvp = sumvp/Npp;
sumvp2 = sumvp2/Npp;
xm = zeros(Ntt,4);
% Scale factor of the velocities
fst = sqrt(2*Trt/(sumvt2(1,1)+sumvt2(1,2)));
fsp = sqrt(2*Trp/(sumvp2(1,1)+sumvp2(1,2)));
% Scale factor increases velocities to be consistent
```
% with the temperature. Factor of 2 since we have
% 2*Ntp degrees of freedom. For 3-D system it
% would be 3*Ntp, and so on.

% Correcting center of mass, generate velocity scale factors
vtzero = zeros(1,2);
vpzero = zeros(1,2);
for i = 1:Ntp
    v(i,1) = (v(i,1)-sumvt(1,1))*fst; % V correction
    v(i,2) = (v(i,2)-sumvt(1,2))*fst;
    xm(i,1) = x(i,1)-v(i,1)*tsteprt; % X,Y last time step
    xm(i,2) = x(i,2)-v(i,2)*tsteprt; % 'Bootstrap' approach
    xm(i,3:4) = x(i,3:4);
    vtzero(1,1) = vtzero(1,1) + v(i,1)^2;
    vtzero(1,2) = vtzero(1,2) + v(i,2)^2;
end
for i = Ntp+1:Ntt
    v(i,1) = (v(i,1)-sumvp(1,1))*fsp; % V correction
    v(i,2) = (v(i,2)-sumvp(1,2))*fsp;
    xm(i,1) = x(i,1)-v(i,1)*tsteprp; % X,Y last time step
    xm(i,2) = x(i,2)-v(i,2)*tsteprp; % 'Bootstrap' approach
    xm(i,3:4) = x(i,3:4);
    vpzero(1,1) = vpzero(1,1) + v(i,1)^2;
    vpzero(1,2) = vpzero(1,2) + v(i,2)^2;
end
vtzero(1,1) = sqrt(vtzero(1,1))/Ntp;
vtzero(1,2) = sqrt(vtzero(1,2))/Ntp;
vpzero(1,1) = sqrt(vpzero(1,1))/Npp;
vpzero(1,2) = sqrt(vpzero(1,2))/Npp;

% Output initial position and velocities to file
for i = 1:Ntt
    fprintf(fid2,’%-3.4f	 %-3.4f	 %-1.0f
’,x(i,1),...
x(i,2),x(i,3));
    fprintf(fid3,’%-2.4f	 %-2.4f
’,v(i,1),v(i,2));
end
fclose(fid2);
fclose(fid3);

% Create initial verlet list
verll = zeros(1,8); % This is the verlet matrix
countll = 0; % Counting variable
for i = 1:Ntt-1 % Loop over all particles
for j=i+1:Ntt

    xr = (x(j,1)-x(i,1))^2 + (x(j,2)-x(i,2))^2;
    xr1 = (x(j,1)-L-x(i,1))^2 + (x(j,2)-L-x(i,2))^2;
    xr2 = (x(j,1)-x(i,1))^2 + (x(j,2)-L-x(i,2))^2;
    xr3 = (x(j,1)+L-x(i,1))^2 + (x(j,2)-L-x(i,2))^2;
    xr4 = (x(j,1)-L-x(i,1))^2 + (x(j,2)-x(i,2))^2;
    xr5 = (x(j,1)+L-x(i,1))^2 + (x(j,2)-x(i,2))^2;
    xr6 = (x(j,1)-L-x(i,1))^2 + (x(j,2)+L-x(i,2))^2;
    xr7 = (x(j,1)-x(i,1))^2 + (x(j,2)+L-x(i,2))^2;
    xr8 = (x(j,1)+L-x(i,1))^2 + (x(j,2)+L-x(i,2))^2;

    if xr<rv2  % This is the central box
        countll = countll + 1;
        verll(countll,1) = i;
        verll(countll,2) = j;
        verll(countll,3) = 0;
        verll(countll,4) = 0;
        verll(countll,5) = x(j,3) + x(i,3);
    end

    if xr1<rv2  % Box lower left
        countll = countll + 1;
        verll(countll,1) = i;
        verll(countll,2) = j;
        verll(countll,3) = -1;
        verll(countll,4) = -1;
        verll(countll,5) = x(j,3) + x(i,3);
    end

    if xr2<rv2  % Box below central box
        countll = countll + 1;
        verll(countll,1) = i;
        verll(countll,2) = j;
        verll(countll,3) = 0;
        verll(countll,4) = -1;
        verll(countll,5) = x(j,3) + x(i,3);
    end

    if xr3<rv2  % Box lower right
        countll = countll + 1;
        verll(countll,1) = i;
        verll(countll,2) = j;
        verll(countll,3) = 1;
        verll(countll,4) = -1;
        verll(countll,5) = x(j,3) + x(i,3);
    end

    if xr4<rv2  % Box to left of central box

countll = countll + 1;
verll(countll,1) = i;
verll(countll,2) = j;
verll(countll,3) = -1;
verll(countll,4) = 0;
verll(countll,5) = x(j,3) + x(i,3);

end

if xr5<rv2 % Box to right of central box
    countll = countll + 1;
    verll(countll,1) = i;
    verll(countll,2) = j;
    verll(countll,3) = 1;
    verll(countll,4) = 0;
    verll(countll,5) = x(j,3) + x(i,3);
end

if xr6<rv2 % Box upper left
    countll = countll + 1;
    verll(countll,1) = i;
    verll(countll,2) = j;
    verll(countll,3) = -1;
    verll(countll,4) = 1;
    verll(countll,5) = x(j,3) + x(i,3);
end

if xr7<rv2 % Box above central box
    countll = countll + 1;
    verll(countll,1) = i;
    verll(countll,2) = j;
    verll(countll,3) = 0;
    verll(countll,4) = 1;
    verll(countll,5) = x(j,3) + x(i,3);
end

if xr8<rv2 % Box upper right
    countll = countll + 1;
    verll(countll,1) = i;
    verll(countll,2) = j;
    verll(countll,3) = 1;
    verll(countll,4) = 1;
    verll(countll,5) = x(j,3) + x(i,3);
end
end

eoutcount = 1; % Counter for output
out = zeros(1,4);  % Output matrix
for k = 1:tts;  % Loop over time steps
    % Compute forces
    en = 0;  % Potential energy to zero
    f = zeros(Ntt,2);  % Set forces to zero
    tq = zeros(Ntt,1);  % Set torques to zero
    [m,n] = size(verll);  % Verlet list size
    for i = 1:m
        xr=zeros(1,2);
        ff = 0;
        fdp = 0;
        dpi = zeros(1,2);
        dpj = zeros(1,2);
        tdp = 0;
        rdm2 = 0;
        rdp2 = 0;
        % X,Y distance betw. particles
        xr(1,1) = x(verll(i,1),1)-x(verll(i,2),1)+verll(i,3)*L;
        xr(1,2) = x(verll(i,1),2)-x(verll(i,2),2)+verll(i,4)*L;
        r2 = xr(1,1)^2 + xr(1,2)^2;  % Square of the distance
        sr2 = sqrt(r2);
        if verll(i,5) == 0  % If two lipids
            if sr2 > rc && sr2 < rv
                w = lwt*rc + inwt;
            else
                w = 1;
            end
            r2i = 1/r2;  % Find quantities to use in JL expression
            r6i = r2i^3;
            rdm2 = 1/((xr(1,1)-dr)^2 + (xr(1,2)-dr)^2);
            rdp2 = 1/((xr(1,1)+dr)^2 + (xr(1,2)+dr)^2);
            % Dipole vectors on ith and jth
            dpi = [sin(x(verll(i,1),4))*mu_dp,cos(x(verll(i,1),4))*mu_dp];
            dpj = [sin(x(verll(i,2),4))*mu_dp,cos(x(verll(i,2),4))*mu_dp];
            % Dipole force
            fdp = (rdm2*(dot(dpi,dpj)-2*dot(dpi,xr)*dot(dpj,xr)+

*rdm2)-rdp2*(dot(dp1,dpj)-2*dot(dp1,xr)*dot(dpj,xr)...
*rdp2))/2;

% Dipole torque
tdp = dpi(1,1)*dpj(1,2)-dpi(1,2)*dpj(1,1);
% Compute force (-dU/dr)
ff = 48*r2i*(r6i*r6i - 0.5*r6i + 3/48*mu/sr2^4)*w+fdp*w;
verll(i,6) = ff*xr(1,1)/sr2;
verll(i,7) = ff*xr(1,2)/sr2;
verll(i,8) = tdp/sr2^3;
en = en + 4*r6i*(r6i - 1) + mu/sr2^3 - ...
dot(dpi,dpj) - ecut; % We don't use x and y here,
%ecut is defined in preamble

end

if verll(i,5) == 1 % If a lipid and a particle
    if sr2 <= sigmat + sigmap/2
        ff = 40;
        verll(i,6) = ff*xr(1,1)/sr2;
        verll(i,7) = ff*xr(1,2)/sr2;
    end
end
if verll(i,5) == 2 % If two particles
    if sr2 <= sigmap
        ff = 50;
        verll(i,6) = ff*xr(1,1)/sr2;
        verll(i,7) = ff*xr(1,2)/sr2;
    end
end
for i = 1:m
    f(verll(i,1),1) = f(verll(i,1),1) + verll(i,6);
    f(verll(i,1),2) = f(verll(i,1),2) + verll(i,7);
    f(verll(i,2),1) = f(verll(i,2),1) - verll(i,6);
    f(verll(i,2),2) = f(verll(i,2),2) - verll(i,7);
    tq(verll(i,1),1) = tq(verll(i,1),1) + verll(i,8);
    tq(verll(i,2),1) = tq(verll(i,2),1) - verll(i,8);
end
fresid = sum(f);

% Verlet Algorithm
sumvt=zeros(1,2); % velocity sum
sumvt2=zeros(1,2); % mean square velocity sum
108
sumvp=zeros(1,2); % velocity sum
sumvp2=zeros(1,2); % mean square velocity sum
xx = zeros(Ntt,4);
factort = zeros(1,2);
factorp = zeros(1,2);

for i = 1:Ntp
    % Kinetic energy, X, Y
    sumvt2(1,1) = sumvt2(1,1) + v(i,1)^2;
    sumvt2(1,2) = sumvt2(1,2) + v(i,2)^2;
end
for i = Ntp+1:Ntt
    sumvp2(1,1) = sumvp2(1,1) + v(i,1)^2;
    sumvp2(1,2) = sumvp2(1,2) + v(i,2)^2;
end
% Calculate force correction from velocity correction
factort(1,1) = vtzero(1,1)/sqrt(sumvt2(1,1))/Ntp;
factort(1,2) = vtzero(1,2)/sqrt(sumvt2(1,2))/Ntp;
factorp(1,1) = vpzero(1,1)/sqrt(sumvp2(1,1))/Npp;
factorp(1,2) = vpzero(1,2)/sqrt(sumvp2(1,2))/Npp;

% Clear sums
sumvt=zeros(1,2); % velocity sum
sumvt2=zeros(1,2); % mean square velocity sum
sumvp=zeros(1,2); % velocity sum
sumvp2=zeros(1,2); % mean square velocity sum

for i = 1:Ntp % Verlet algorithm for lipids
    xx(i,1) = 2*x(i,1) - xm(i,1) + tsteprt^2*f(i,1)*factort(1,1); % Compute future x
    xx(i,2) = 2*x(i,2) - xm(i,2) + tsteprt^2*f(i,2)*factort(1,2); % Compute future y
    xx(i,3) = x(i,3);
    % New dipole orientation
    xx(i,4) = 2*x(i,4) - xm(i,4) + tsteprt^2*tq(i,1);
    % x/y velocities
    v(i,1) = (xx(i,1) - x(i,1))/(2*tsteprt);
    v(i,2) = (xx(i,2) - x(i,2))/(2*tsteprt);
    % Velocity center of mass
    sumvt(1,1) = sumvt(1,1) + v(i,1);
    sumvt(1,2) = sumvt(1,2) + v(i,2);
    sumvt2(1,1) = sumvt2(1,1) + v(i,1)^2;
    sumvt2(1,2) = sumvt2(1,2) + v(i,2)^2;
418 if xx(i,4) > 2*pi % If dipole swings past 2*pi
419     xx(i,4) = xx(i,4) - 2*pi;
420 end
421 if xx(i,4) < 0 % If dipole swings under zero
422     xx(i,4) = xx(i,4) + 2*pi;
423 end
424 % Return particles that leave box
425 if xx(i,1)>2*L
426     xx(i,1) = xx(i,1)-L;
427     x(i,1) = x(i,1)-L;
428 end
429 if xx(i,2)>2*L
430     xx(i,2) = xx(i,2)-L;
431     x(i,2) = x(i,2)-L;
432 end
433 if xx(i,1)<L
434     xx(i,1) = xx(i,1)+L;
435     x(i,1) = x(i,1)+L;
436 end
437 if xx(i,2)<L
438     xx(i,2) = xx(i,2)+L;
439     x(i,2) = x(i,2)+L;
440 end
441 % Current positions become previous
442 % Predicted positions become current
443 xm(i,:) = x(i,:);
444 x(i,:) = xx(i,:);
445 end
446 for i = Ntp+1:Ntt % Verlet algorithm for particles
447     xx(i,1) = 2*x(i,1) - xm(i,1) + tsteprp^2*f(i,1)...
448         *factorp(1,1); % Compute future x
449     xx(i,2) = 2*x(i,2) - xm(i,2) + tsteprp^2*f(i,2)...
450         *factorp(1,2); % Compute future y
451     xx(i,3) = x(i,3);
452     xx(i,4) = x(i,4);
453 % x/y velocities
454     v(i,1) = (xx(i,1) - xm(i,1))/(2*tsteprp);
455     v(i,2) = (xx(i,2) - xm(i,2))/(2*tsteprp);
456 % Velocity center of mass
457     sumvp(1,1) = sumvp(1,1) + v(i,1);
458     sumvp(1,2) = sumvp(1,2) + v(i,2);
459     sumvp(1,1) = sumvp(1,1) + v(i,1)^2;
460     sumvp(1,2) = sumvp(1,2) + v(i,2)^2;

110
% Return particles that leave box
if xx(i,1)>2*L
    xx(i,1) = xx(i,1)-L;
    x(i,1) = x(i,1)-L;
end
if xx(i,2)>2*L
    xx(i,2) = xx(i,2)-L;
    x(i,2) = x(i,2)-L;
end
if xx(i,1)<L
    xx(i,1) = xx(i,1)+L;
    x(i,1) = x(i,1)+L;
end
if xx(i,2)<L
    xx(i,2) = xx(i,2)+L;
    x(i,2) = x(i,2)+L;
end
% Current positions become previous
% Predicted positions become current
xm(i,:) = x(i,:);
x(i,:) = xx(i,:);

% Instantaneous temperature
% Total energy per particle
Tinst = (sumvt2(1,1)+sumvt2(1,2))/(2*Ntp);
etot = (en + sumvt2(1,1) + sumvt2(1,2))/(2*Ntp);
%(2 is for 1/2mv^2)

% Update verlet list
% See list initialization for comments
if mod(k,50) == 0 % Every X time steps
    verll = zeros(1,8);
countll = 0; % Counting variable
for i = 1:Ntt-1 % Loop over all entities
    for j=i+1:Ntt
        xr = (x(j,1)-x(i,1))^2 + (x(j,2)-x(i,2))^2;
xr1 = (x(j,1)-L-x(i,1))^2 + (x(j,2)-L-x(i,2))^2;
xr2 = (x(j,1)-x(i,1))^2 + (x(j,2)-L-x(i,2))^2;
xr3 = (x(j,1)+L-x(i,1))^2 + (x(j,2)-L-x(i,2))^2;
xr4 = (x(j,1)-x(i,1))^2 + (x(j,2)-x(i,2))^2;
xr5 = (x(j,1)+L-x(i,1))^2 + (x(j,2)-x(i,2))^2;
xr6 = (x(j,1)-L-x(i,1))^2 + (x(j,2)+L-x(i,2))^2;
xr7 = (x(j,1)-x(i,1))^2 + (x(j,2)+L-x(i,2))^2;
    end
end
xr8 = (x(j,1)+L-x(i,1))^2 + (x(j,2)+L-x(i,2))^2;
if xr<rv2 % This is the central box
    countll = countll + 1;
    verll(countll,1) = i;
    verll(countll,2) = j;
    verll(countll,3) = 0;
    verll(countll,4) = 0;
    verll(countll,5) = x(j,3) + x(i,3);
end
if xr1<rv2 % Box lower left
    countll = countll + 1;
    verll(countll,1) = i;
    verll(countll,2) = j;
    verll(countll,3) = -1;
    verll(countll,4) = -1;
    verll(countll,5) = x(j,3) + x(i,3);
end
if xr2<rv2 % Box below central box
    countll = countll + 1;
    verll(countll,1) = i;
    verll(countll,2) = j;
    verll(countll,3) = 0;
    verll(countll,4) = -1;
    verll(countll,5) = x(j,3) + x(i,3);
end
if xr3<rv2 % Box lower right
    countll = countll + 1;
    verll(countll,1) = i;
    verll(countll,2) = j;
    verll(countll,3) = 1;
    verll(countll,4) = -1;
    verll(countll,5) = x(j,3) + x(i,3);
end
if xr4<rv2 % Box to the left of central box
    countll = countll + 1;
    verll(countll,1) = i;
    verll(countll,2) = j;
    verll(countll,3) = -1;
    verll(countll,4) = 0;
    verll(countll,5) = x(j,3) + x(i,3);
end
if xr5<rv2 % Box to the right of central box
    countll = countll + 1;
verll(countll,1) = i;
verll(countll,2) = j;
verll(countll,3) = 1;
verll(countll,4) = 0;
verll(countll,5) = x(j,3) + x(i,3);
end
if xr6<rv2 % Box upper left
  countll = countll + 1;
  verll(countll,1) = i;
  verll(countll,2) = j;
  verll(countll,3) = -1;
  verll(countll,4) = 1;
  verll(countll,5) = x(j,3) + x(i,3);
end
if xr7<rv2 % Box above central box
  countll = countll + 1;
  verll(countll,1) = i;
  verll(countll,2) = j;
  verll(countll,3) = 0;
  verll(countll,4) = 1;
  verll(countll,5) = x(j,3) + x(i,3);
end
if xr8<rv2 % Box upper right
  countll = countll + 1;
  verll(countll,1) = i;
  verll(countll,2) = j;
  verll(countll,3) = 1;
  verll(countll,4) = 1;
  verll(countll,5) = x(j,3) + x(i,3);
end
end
end
end
if mod(k,10000) == 0 % For every X time steps record data
  s = outcount;
  out(s,1) = tstep*k;
  out(s,2) = en;
  out(s,3) = etot;
  out(s,4) = Tinst;
  outcount = outcount + 1;
  % Print current time, pot. en., kinetic en.
  % and instantaneous temperature to file
fprintf(fid, '%3.3E %3.3E %3.3E %3.3E\n', out(s,1),...\n, out(s,2), out(s,3), out(s,4));

fid2 = fopen(sprintf('%gNtp_%gNpp_%gtts_xy_%g.dat',...\nNtp,Npp,tts,out(s,1)),'wt');

fid3 = fopen(sprintf('%gNtp_%gNpp_%gtts_uv_%g.dat',...\nNtp,Npp,tts,out(s,1)),'wt');

% Print current positions, velocities to file
for i = 1:Ntt
fprintf(fid2,'%-3.4f	 %-3.4f	 %-1.0f\n',x(i,1),...\nx(i,2),x(i,3));
 fprintf(fid3,'%-2.4f	 %-2.4f\n',v(i,1),v(i,2));
end
fclose(fid2);
fclose(fid3);

% Screen output of current progress
sprintf('The case is %g percent complete.',k/tts*100)
sprintf('Instantaneous temperature is %g with a...\nforce residual of %g and %g.',out(s,4),fresid)

% Create graphic showing current positions
xpos = sprintf('%gNtp_%gNpp_%gtts_X_%d.jpg',Ntp,...\n,Npp,tts,out(s,1));
figure(1)
plot(x(1:Ntp,1),x(1:Ntp,2),'o','MarkerSize',6,...\n'MarkerEdgeColor','b','MarkerFaceColor','b')
hold on
plot(x(Ntp+1:Ntt,1),x(Ntp+1:Ntt,2),'o','MarkerSize',20,...\n'MarkerEdgeColor',[0.36,0.2,0.09],'MarkerFaceColor',...\n[0.36,0.2,0.09])
xlabel('X')
ylabel('Y')
title(sprintf('t = %g',out(s,1)))
print(1,xpos,'-djpeg')
close(1)
end
end
% Simulation is over
% Output history of energies and temperatures
enfig = sprintf('%gNtp_%gNpp_%gtts_en_%g',Ntp,Npp,tts,k);
tenfig = sprintf('%gNtp_%gNpp_%gtts_ten_%g',Ntp,Npp,tts,k);
tempfig = sprintf('%gNtp_%gNpp_%gtts_T_%g',Ntp,Npp,tts,k);
figure(1) % Plot output data
plot(out(:,1),out(:,2))
print(1,enfig,'-djpeg')
close(1)
figure(1)
plot(out(:,1),out(:,3))
print(1,tenfig,'-djpeg')
close(1)
figure(1)
plot(out(:,1),out(:,4))
print(1,tempfig,'-djpeg')
close(1)
fclose('all');
close('all');
toc % Running time in seconds
Bibliography


