**Phospholipid Monolayers**

**Purpose**
You will determine the heat and entropy change for the liquid-condensed to liquid-expanded phase transition of a phospholipid monolayer. You will also directly observe this phase transition with a Brewster Angle Microscope (BAM), and use reflectivity data to calculate the thickness of the two phases.

**Introduction**
The occurrence of molecules that have both hydrophobic and hydrophilic moieties in chemical combination, that is, amphiphiles, gives rise to an extensive range of phenomena and structural patterns, the importance of which can hardly be overestimated in both the physical and life sciences. Amphiphilic molecules are employed as soaps and detergents, in the processing of beverages, as stabilizers in paints, and in secondary recovery of oil from porous rock beds. Further, it is almost inconceivable to consider the existence of life without amphiphilic molecules. In general, amphiphilic molecules consist of one or more nonpolar or hydrophobic tail groups (usually an alkyl, acyl, or aromatic groups) and one or more polar or hydrophilic head groups. Amphiphilic molecules that are insoluble in aqueous solutions can form monomolecular layers at air–water interfaces. Benjamin Franklin was one of the pioneers in the field of fatty acid monolayers and was known to conduct experiments where he would spread a spoonful of oil on a large pond to demonstrate the effects of monomolecular films.¹

Phospholipids are a special group of amphiphilic molecules that self-assemble to form bilayer structures (Figure 1), which provide the framework for cell membranes. The factors that affect the structure of phospholipid bilayers have long been studied by exploring the effects in

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**Figure 1:** Phospholipids can form monolayers at the air-water interface and bilayers in bulk water.
(often chloroform) that will spread the amphiphile over the surface. Once the solvent has evaporated a monomolecular film of the amphiphile remains, which can then be compressed and expanded with the barriers. As the monolayer is compressed, the surface tension of the water decreases. The decreased surface tension can be understood the difference between the surface tension of pure water, which tends to contract the surface, and the surface pressure, $\pi$, of the monolayer, which tends to expand the surface.

$$\gamma_{\text{water with monolayer}} = \gamma_{\text{water}} - \pi$$  \[1\]

Thus, by monitoring the surface tension, we can determine the surface pressure.

The relatively high surface tension of water is a function of the relatively strong intermolecular forces present in liquid water. Liquids with strong intermolecular forces have large surface tensions. As an example of the role intermolecular forces play in the surface tension we can look at three liquids at 20°C: mercury (metallic bonding and dispersion) has a surface tension of 485 mN/m, water (hydrogen bonding, dipole, and dispersion) has a surface tension of 73 mN/m, and heptane (dispersion only) has a surface tension of 20 mN/m. The high surface tension of water, in comparison to other common liquids, makes it surprisingly difficult for water to wet many surfaces.

In this experiment you will be exploring the structure and phase transitions of phospholipid monolayers using a Langmuir trough. A phospholipid monolayer provides a convenient model for understanding bilayer structures. Phospholipid bilayers can exist in two different states, a highly ordered gel state and a more fluid liquid crystalline state. The transition between these two phases occurs as the bilayer is heated (Figure 3).

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Figure 3: The gel to liquid crystal phase transition occurs in bilayers.
As can be seen in Figure 3, the phospholipid molecules are more expanded in the liquid crystalline phase than in the gel phase. This expansion is a result of the increased thermal energy, which produces trans-gauche conformational changes about the C-C bonds along the alkyl chain. The mean molecular area, \text{mmA}, of a phospholipid molecule in a bilayer (that is, the area occupied by a phospholipid molecule) is thus dependent on the temperature. A similar transition occurs in phospholipid monolayers between liquid expanded (LE) and liquid condensed (LC) phases. Since monolayers can be studied with a Langmuir trough, where both the temperature and the mean molecular area can be controlled, we are able to extensively probe the thermodynamics of the LE-LC phase transition. Much work has been done in this area and the results have been critical to the understanding of phospholipid bilayer structure.

In this experiment, we will be studying phosphatidylcholines (Figure 4), which consist

![Figure 4](image)

Figure 4: All phosphatidylcholines have a phosphocholine headgroup and a glycerol backbone.

of a two-chain nonpolar or tail group \((R1\) and \(R2\)) and a zwitterionic head group (with a positive and negative charge from the amine and the phosphate groups respectively). We will be depositing phosphatidylcholines from a chloroform solution onto the water surface of a Langmuir trough and compressing the resulting monomolecular film at various temperatures. The constant temperature compressions that we will be obtaining are called \(\pi-A\) isotherms; typical \(\pi-A\) isotherms for a common phosphatidylcholine are shown in Figure 5. These \(\pi-A\) isotherms are analogous to \(P-V\) diagram for 3-D systems. The liquid expanded and liquid condensed states are separated by a transition region where the surface pressure remains approximately constant as the film is compressed.

The mean molecular area of the LC phase is obtained by drawing a tangent to the steepest part of the isotherm in that region and extrapolating it to zero surface pressure. For the isotherm in Figure 5, that area is \(\sim 52 \text{ Å}^2/\text{molecule}\). The mean molecular area for the LE phase is taken at \(\pi_T\). Under ideal circumstances, the isotherm would be horizontal at \(\pi_T\), which would simplify our analysis. Unfortunately, tiny amounts of impurities make the isotherm slightly curved in the transition region, making it somewhat difficult to determine \(\pi_T\). To determine \(\pi_T\) more precisely,
plot the isothermal compressibility\(^1\) \(\left(\frac{-1}{A} \left(\frac{\partial A}{\partial \pi}\right)_T\right)\) as a function of surface pressure from 1.0 mN/m to 20 mN/m. The compressibility will go through a maximum at \(\pi_T\).

**Figure 5:** Typical \(\pi\)-A isotherms for a phosphatidylcholine monolayer are shown in this figure. For the isotherm with \(\pi_T = 5\) mN/m, \(A_{LC}\) would be 52 \(\text{Å}^2\) and \(A_{LE}\) would be about 83 \(\text{Å}^2\).

From Figure 5 we can see that at higher temperatures a larger surface pressure is required to compress the liquid expanded phase to the liquid condensed phase (again, this is analogous to 3-D compression of a gas into a liquid). For a 3-D system in which two phases are in equilibrium one can show that

\[
\frac{dp}{dT} = \frac{\Delta S_m}{\Delta V_m} \quad [2]
\]

where \(\Delta S_m\) is the molar entropy change and \(\Delta V_m\) is the molar volume change for the phase transition. Equation 2 is an important result called the Clapeyron equation and is valid for all first-order phase transitions. Since we are at phase equilibrium, \(Q_m = T\Delta S_m\). Substituting this into Equation 2 one obtains

\[
\frac{dp}{dT} = \frac{Q_m}{T\Delta V_m} \quad [3]
\]

\(Q_m\) is the molar latent heat of transition and thus at constant pressure equates to \(\Delta H_m\) and we get the more familiar form of the Clapeyron equation. In an analogous manner one can obtain an expression for the phase transition of a monolayer.

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\(^1\) You know this from your class as \(\kappa_T\), but the trough software uses the symbol \(\beta\).
\[
\frac{d\pi_T}{dT} = \frac{Q_m}{T \Delta A_m}
\]  \[4\]

where \(\pi_T\) is the transition surface pressure (shown in Figure 5) and \(\Delta A_m\) is the change in the molar area \((A_{LE} - A_{LC})\) for the transition. (Since \(\Delta\) is defined as final–initial, all the thermodynamic quantities we calculate will be for the LC to LE phase transition, not the reverse.) If the change in the molar area is independent of temperature and the transition occurs at constant pressure, then we can replace \(Q_m\) with \(\Delta H_m\); however, this is rarely the case for phospholipid monolayers. Since the LC to LE transition does not occur at constant pressure and \(\Delta A_m\) is not temperature-independent we must determine \(\Delta A_m\) at each temperature and then determine \(d\pi_T/dT\) in order to calculate \(Q_m\) at each temperature.

\[
Q_m = T \Delta A_m \left( \frac{d\pi_T}{dT} \right)
\]  \[5\]

Temperature is not the only factor that affects the LC to LE phase transition. The number of carbons and degree of saturation in the two-alkyl chains on the nonpolar group also has a large effect on the phase behavior of a monolayer. Shorter chains (fewer than 10-14 carbons) exist only in the LE state whereas longer chains (more than 18-22 carbons) exist only in the LC state. Further, phospholipids with unsaturated alkyl chains often exist only in the LE state due to the increased fluidity afforded by the unsaturation.

**Direct observation of phase transitions**

Microscopy techniques allow for the evaluation of morphological properties of monolayer films. Fluorescence microscopy and Brewster Angle Microscopy (BAM) are the two leading microscopy techniques for direct visualization of monolayers. Fluorescence microscopy was the first of these two techniques to be developed, and was a key mode for proving the two-phase coexistence seen in the first order phase transition of DPPC.8 With the development of the Brewster Angle Microscope in 1991, clever use of Brewster’s Law (explained below) allowed for a new way to image monolayers without introducing the external perturbation of fluorescent markers. While both techniques serve as important tools for the evaluation of monolayers, when afforded, BAM serves as a primary technique for characterizing the film structure and dynamics of phospholipids.9

**How BAM works**

At the Brewster Angle \((\theta_B)\) for a medium, all incident \(p\)-polarized light is refracted while only \(s\)-polarized light is reflected. The relationship between this angle and the refractive indices, also known as Brewster’s Law, is shown in Equation 6 where \(n_1\) is the refractive index of the initial medium and \(n_2\) is the refractive index of the second medium. Because BAM is conventionally used for studying systems at an air-water interface, \(n_1\) is typically the refractive index of air while \(n_2\) is the refractive index of water.

\[
\tan(\theta_B) = \frac{n_2}{n_1}
\]  \[6\]
Given the dependence of Brewster’s Law on the refractive indices of two media at an interface, if a third component film is deposited onto the surface of the water, regions the film occupies will possess a different refractive index, and therefore will reflect light (Figure 6). Hence, the reflected light observed and used to produce surface images is representative of the surface films on water, allowing for observation of the morphological properties of these films and qualitative determination of certain characteristics including surface density or molecular tilt.\textsuperscript{10}

![Figure 6](image1.png)

**Figure 6.** When a light source projected at the Brewster Angle is incident on water, $p$-polarized light is only refracted. Under the same conditions, when light is incident on a region in the presence of another media, like phospholipid domain shown above, $p$-polarized light is reflected.

![Figure 7](image2.png)

**Figure 7.** This schematic shows the BAM set-up for evaluating film properties.

The BAM you will be using today uses a 75 mW, 532 nm green laser as the light source. It is mounted on the Langmuir trough with two motorized arms that allow for the adjustment of the incident and camera angle. A polarizer that allows the passage of $p$-polarized light has been placed in front of the laser and CCD (camera) to ensure that all reflected light resulting from the film is $p$-polarized.
Calculations
You will be using the BAM to determine the surface density of LC and LE domains formed during the coexistence phase of DPPC. By choosing representative reflectivity values for the liquid condensed and liquid expanded phases, you will be able to monitor the change in each phase over time. This reflectivity will then be used to calculate the film thickness and surface density of the two coexisting phases.

An adaption of the Fresnel equations, which model the reflection and transmission of light incident on an interface between two different mediums, gives the relationship between this reflectivity \( R \), the refractive indices \( n_{\text{film}} \) and film thickness \( d \) of DPPC, and the refractive index of water \( n_w \) (Equation 7).\(^{11-14}\) It is important to note that this relationship is only valid when the wavelength of the incident light \( \lambda \) is significantly larger than the film thickness \( d \).

\[
R = \left( \pi \frac{d}{\lambda} \right)^2 \frac{\left(n_{\text{film}}^2 - n_w^2 - 1 + \frac{n_w^2}{n_{\text{film}}^2}\right)^2}{1 + n_w^2} \tag{7}
\]

Using this relationship, you will determine the film thickness of the two phases from your collected reflectivity values. Prior work has determined that refractive index of water is 1.333, while that of DPPC is 1.453.\(^{12}\) After determining the film thickness, you will use the difference in refractive indices of the film and water along with a known value for the refractive index increment (dn/dC) of DPPC to determine the surface density (\( \Gamma \)) of the selected regions. This can be done using the relationship between surface density and the refractive indices for two media at an interface given by Equation 8; note that dn/dC for DPPC is 1.167x10^{-28} m^3/molecule.\(^{12}\)

\[
\Gamma = \frac{(n_{\text{film}} - n_w)d}{dn/dC} \tag{8}
\]

After determining these values, you will discuss the differences in surface density both between the LE and LC phases as well as any variability (or lack thereof) seen within each phase. You will also take advantage of your collected images to discuss any notable morphological/nucleation patterns.
Hazards

- You will be working with an open flame for a portion of the experiment. Before lighting the flame, remove all solvents and other combustible materials that are within three meters of the torch. Tie back long hair and remove any gloves. Roll up your sleeves, which could carry flames from the torch to your torso. Just before lighting the torch, say, “Open flame!” loudly enough to be heard by everyone in the room, so that they do not approach your working area with a combustible material.

- Chloroform is a suspected carcinogen, so you must clean and load syringes in the hood. When working in the hood, wear an apron. Chloroform can penetrate the skin, so wear two sets of nitrile gloves.

Procedure for compression isotherms

You will obtain a series of π-A isotherms for a L-α-dipalmitoylphosphatidylcholine monolayer at several different temperatures (start with the temperature bath at 17°C and increase in 1.7°C increments). NOTE: the temperature measured in the trough will always differ from the temperature set on the bath, the settings above refer to the bath! You will use your data to determine $Q_m$ and $\Delta S_m$ for the LC to LE transition of L-α-dipalmitoylphosphatidylcholine at each temperature and to make comparisons between the behaviors of the phospholipids as a function of chain length.

Using the Langmuir Trough

Your instructor will show you how to use the Langmuir trough and associated software.

Film Preparation

You will be using chloroform as the spreading solvent and the phosphatidylcholines will be dissolved at concentrations of approximately 1 mg/mL. Be sure to record the exact concentration in your notebook and in the software.

1. Set the circulating bath to the desired temperature.

2. You will first have to fill the trough with nanopure water so that the water surface is elevated approximately 2 mm above the trough edges.

3. Next you will clean the Wilhelmy plate (use care, as it is fragile) by heating it until it glows orange in a flame from a propane torch.

4. Use the positive pressure forceps to suspend the Wilhelmy plate just above the tensiometer hook, and then drop the plate onto the hook, so that the forceps do not pull down on the tensiometer (it, too, is quite fragile). The plate should be suspended approximately half in and half out of the water.

5. Open the manual controls, and click on the tensiometer (called the balance in control software) tab. Zero the tensiometer, and close the barriers while monitoring the surface pressure. If the surface pressure changes by less than 0.2 mN/m then the surface can be considered clean, if not then you must clean the surface. Close the barriers all the way to concentrate the contamination. Clean the glass tip of the aspirator with a chemwipe and
use the aspirator to remove the surface contamination, using the following technique: with the aspirator tip at right angles to the water surface, peck at the surface for about 15 seconds. Add water outside the barriers until the water returns to the original level.

6. Repeat step 5 until the surface pressure changes by no more than 0.2 mN/m during the compression. If the surface pressure was initially several mN/m, you may have to re-flame the plate.

7. Once you have determined that the surface is clean, open the barriers all the way and zero both the barriers and the tensiometer.

8. In the fume hood: Clean out the microsyringe by drawing up pure chloroform, dispensing it in the designated waste, and wiping the syringe with a clean chemwipe. Repeat this four more times. Then draw up 15 μL of the designated DPPC solution. Return the DPPC solution the refrigerator.

9. Deposit the monolayer by depressing the syringe enough to make a drop that hangs from the tip of the syringe. Touch the bottom of the drop to the surface of the water in the trough, allowing it to break from the syringe tip. Do not let the DPPC bead drop into the trough solution before contact and be careful not to let the syringe touch the water. Repeat until you have expelled all 15 μL, then wait 5 minutes for the solvent to evaporate.

π-A Isotherms
1. Obtain an isotherm at bath setting of 17 °C (remember the trough temperature will be slightly different).
   a. Make sure the barriers are completely open, and then re-zero the film balance. You will do this before every run.
   b. Click the LB command button , and then click on ISO.
   c. Enter the required information in the experimental set-up form: user name (CHEM 354), your group’s name, trough name (mini), substance, and the amount and concentration of the spreading solution.
   d. Click on start. A second form will appear. Verify that it is set to “constant rate compression”, “10 mm/min”, “go to target”, the target is set to 30 mN/m, and then click on “go”.

2. When the isotherm is complete, open the barriers immediately, and then re-zero the tensiometer. Increase the temperature of the circulating bath by 1.7 °C. The water bath is not controlled by the computer; use the control pad on the water bath itself: press set, adjust the temperature with the arrow pads, and then press set again. Once the bath has stabilized allow another 5 minutes for the water in the trough to equilibrate with the bath and then collect another isotherm. Repeat this process collecting a total of 5 isotherms. Make sure to record both the bath temperature and the trough temperature.
3. Return the Wilhelmy plate to its case using reverse forceps. Remove the monolayer from the trough with the aspirator.

4. Examine your results. Click on the Browse Experiments icon on the KSV Research Lab Window, and double-click on the experiment you wish to examine.
   a. Right-click on the experiment and choose “graph” to see your results.
   b. To export your data in a usable form, right click and choose “copy to clipboard”.
      Paste the data into Excel. The headers will be missing, so enter the headers for temperature, surface pressure, and area above the appropriate columns.

5. Using your data construct a plot of the molar heat of transition ($Q_m$) as a function of temperature in Kelvin. Explain why the heat of transition varies with temperature. Determine the temperature at which $Q_m = 0$. What is the significance of this temperature?

**Procedure for BAM**

*Setting up the BAM (Your instructor may do some of this for you)*

1. Turn on the computer. This computer has four programs that are used to control the BAM angle, analyze the camera signal, do histogram analysis, and control the Langmuir trough.
2. Turn on the interface unit for the trough (the grey tower to the left of the trough) and open the LB software (SG server). The manual control window should open automatically and give you readings for all the Langmuir devices.
3. Turn on the laser using the power strip to the left of the trough. Do not look at the laser spot in the trough.
4. Add nanopure water to the trough, outside the barriers, until the water is about 3 mm below the microscope objective.
5. Make sure the black polymer square is not floating on the water. It should be positioned underwater centered on the laser spot. If you need to reposition it, do it with a clean pair of forceps.
6. In the manual control window, click on the “Balance(s)” tab and zero the tensiometer balance.
7. Click on the “Barrier 1” tab, and press “close” to begin compressing the barriers. Make sure the surface pressure does not rise above 0.20 mN/m during this initial compression. If it is under this value, re-expand the barriers using the “open” button. If it does rise slightly over 0.20 mN/m, try to aspirate a small amount of water off until the surface pressure drops. Make sure to wipe off the tip of the aspirator with a kimwipe before using it in the trough. If the pressure rises above 0.20 mN/m, repeat the cleaning step. If the pressure rises above 2.0 mN/m, empty the trough and refile with fresh nanopure water before repeating steps 3-5.
8. Turn on the LABVIEW interface (center button on box labeled NXT inside the trough cabinet).
9. On the Desktop open up both “Camera Acquisition Controls” and “Motor Controls”.
10. Near the top left corner of the LabVIEW window in Camera Acquisition press the Run icon (an arrow at upper left) and the camera feed should appear on screen.
11. Add more nanopure water to the trough until the laser spot is in the correct position (~200 mL). The laser spot is a bright white circle that moves if the trough is moved. The laser spot should be seen in the middle of the video screen. If the bright spot is at the bottom of the screen, the water level is too high; if it is at the top of the screen, it’s too low. If you need to add water, close the barriers part way and add water outside the barriers.

12. If you are at the Brewster angle the laser spot should become very dim (and may disappear completely) as the water rises to the correct level. The steps below fine tune the angle. Tapping the table very gently will bounce the surface of the water, and allow you to see the light spot. In the following steps we will now fine-tune the Brewster angle.

13. Open up “Motor Controls”.

14. Near the top left corner of the LabVIEW window in Motor Controls press the Run icon (an arrow at upper left).

15. Set the power to -8 to move arms up and 8 to move arms down. Pressing “ok”, should make the arms move (you should hear it). Keep changing the angle until the greyscale (go back to the Camera Acquisition press is below 4. There is a time lag, so wait one second before pressing the button each time. You can decrease the power to make finer adjustments.

16. Open the barriers and re-zero the balance one final time.

**Depositing the monolayer**

1. **In the fume hood**: Clean out the microsyringe by drawing up pure chloroform, and dispensing it in the designated waste, and wiping the syringe with a clean chemwipe. Repeat this four more times. Then draw up 30 μL of the designated DPPC solution. Return the DPPC solution the refrigerator.

2. Deposit the monolayer by depress the syringe enough to make a drop that hangs from the tip of the syringe. Touch the bottom of the drop to the surface of the water in the trough, allowing it to break from the syringe tip. Do not let the DPPC bead drop into the trough solution before contact and be careful not to let the syringe touch the water. Repeat until you have expelled all 30 μL, then wait 5 minutes for the solvent to evaporate.

3. Go back to the Camera Acquisition software, and see if the data collection area (the green ellipse in the image) is centered on the laser spot. If not, drag it there with the mouse.

**Running the isotherm**

1. Make sure the barriers are completely open, and then re-zero the film balance.

2. While you are waiting, set up your isotherm experiment parameters by clicking on the LB Control Software icon found on the KSV Research Lab window. Click on the “Iso” button in the top left corner of the LB Control Software window. Continue on to the next two steps while you wait.

3. Enter in your group name in the Experimental Setup window, and select CHEM 354 in the user dropdown menu. Make sure the following parameters are properly listed:
   - Probe for balance 1: Paper Wilhelmy Plate
   - Trough: Mini
   - Subphase: Water
• Substance 1: DPPC (enter in correct solution concentration, MW, and volume if not entered already).

4. When all experimental parameters are set press the Start button at the bottom of this menu. After doing this your Trough Controls menu should appear. Make sure the following trough controls are set, but do not press Go.
- Compression method: Constant rate compression
- Recording options: From go to target
- Target options: Surface pressure
- Compression Parameters: Target (mN/m) = 35 and Rate (mm/min) = 5

5. If your 5-minute waiting period has elapsed at this point, click on the stop icon in the Camera Acquisition window.

6. In the Camera Acquisition window, click on the folder icon next to the Histogram Path textbox (next to the Log Histogram button). Navigate to the CHEM 354 folder, and then make a new folder labeled with your group’s name and the word histogram. Click on the folder icon next to the Image Path textbox (next to the Log Images button). Navigate to CHEM 354 folder, and then make a new folder labeled with your group’s name and the word images.

7. Click on the stop icon in the camera acquisition window. Activate the log histogram and log images switches; they will turn bright green when on.

8. Press Go in the trough software the same time as the run icon in the Camera Acquisition window.

9. While the experiment is running, make note of different reflectivity values and greyscale values that seem to be associated with the LE and LC phases (the peaks on the histogram in the upper right of the camera acquisition window will help with this).

10. When the surface pressure reaches 35 mN/m, end your image and reflectivity value collection by pressing the stop icon in the LabView program. If the LB software doesn’t stop automatically at 35mN/m, stop it as well, and be sure to press the keep data button.

Accessing your data

1. Click on the Browse Experiments icon on the KSV Research Lab Window and find your group name.

2. Double click on your experiment to display the data. Right-click on your experiment and copy the data, and then paste it, without a header row, to an Excel spreadsheet. Then save the spreadsheet as a text (.txt) file; use the path to the CHEM 354 folder, and then make a new folder labeled with your group’s name and the word isotherm.

3. Open the program Histogram Analyzer. There are three text boxes for the data paths for your images, your trough data (from the .txt file), and your reflectivity histogram data. Fill in these boxes. Put the greyscale (coexistence) cut-off at a level that is between the values for the LC and LE phases; this will allow the software to plot the fraction of each phase during the isotherm. Toggle the show video switch to on (it will turn bright green), and press the run icon (the arrow at upper left). The computer will display a time-lapse version of your experiment over the next minute or two. Observe it to see if there is anything you missed during the experiment. When finished, click on the raw data tab to retrieve your results. Right-click on each of the graphs, export the data to the clipboard, and then paste it into a spreadsheet. Choose three representative images of the monolayer: one before, one after, and one during the phase transition. Email the Excel
sheet with the results and the three images to all members of your lab group. Make sure that you include representative images for the LE, LC, and LE/LC coexistence portions of the isotherm in the *Results* section of your reports.

**Clean up**

1. Remove the monolayer from the trough with an aspirator, and then empty the water from the trough with the aspirator.

**Writing Guidelines**

See the Author Guidelines document and review the rubrics for general guidance. Among other appropriate discussion, the following items should be included in your manuscript.

**Results, Discussion and Conclusion**

- $Q_m$ and $\Delta S_m$ for the LC $\rightarrow$ LE transition.
- Isotherms for different temperatures; $\pi$ vs $T$ plot.
- Film thickness and surface density of LE phase and LC phase.
- A graph displaying the change in reflectivity counts of LC and LE phases over time for coexistence of DPPC.
- At least three representative BAM images of monolayer behavior during the isothermal compression.
- Discussion of how and why the transition pressure varies with temperature.
- Discussion of the balance between enthalpy and entropy via intermolecular forces (dispersion) and Thermal energy.
- Discussion of how morphological changes in the film correlate to physical and chemical thermodynamic properties.
- Uncertainties for each measured and calculated value (not required for BAM data).
- Comparison of results to relevant and properly cited existing literature.
- Discussion of the limitations of the results/conclusions.
- Discussion of possible future work to extend the value of the line of study. This should go beyond “collect more data.”
References