ATOMIC FORCE MICROSCOPY OF LANGMUIR-BLODGETT MONOLAYERS AND BILAYERS

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Phospholipid monolayers and bilayers prepared by the Langmuir-Blodgett technique are simplified model systems of biological membranes. The molecular organization of these monolayers and bilayers has been intensely studied as they are excellent tools for understanding biological processes such as adhesion, fusion, protein structure and organization, etc. Recent structural studies have included electron diffraction of monolayers deposited on substrates and X-ray diffraction of monolayers at the air-water interface. Both diffraction techniques suggest that in the gel phase, the lipid hydrocarbon chains possess long range orientational order, but relatively short range positional order. As a result, L-B films in the gel state closely resemble the "hexatic" phases better known in thermotropic liquid crystals. However, both short-range and long-range order have not yet been studied in a single Langmuir-Blodgett film.

The Atomic Force Microscope (AFM) should be ideal for visualizing monolayers and bilayers at high resolution. The AFM records interatomic forces between the apex of a cantilevered spring tip and the surface of the sample. Fig. 1 shows a top view of a cadmium arachidate monolayer in air which contains 12 holes, 30 to 140 nm in size. The surface of the films could be imaged repeatedly without damaging the sample. Dark areas correspond to the lower areas in the image, with about 3 nm difference between the bottom of the holes and the top of the monolayer. However, by increasing the applied force on the AFM tip, we could pierce the monolayer to image the 0.5 nm hexagonal lattice of the mica substrate. In this way, we could convince ourselves that we were indeed imaging the cadmium arachidate surface.

Fig. 2 shows a grey scale AFM image of the polar region of a bilayer of DMPE deposited on mica. These images were taken under water at ambient temperature and pressure. The dominant features of the images are long, uniformly spaced rows roughly .7 - .9 nm in spacing. A modulation also can be seen along the rows, with rounded bright spots roughly every .5 nm. We believe that the individual bright spots along the rows correspond to the individual headgroups of the DMPE molecule. The lattice spacing is that measured by X-ray diffraction at the air-water interface. The area per molecule in the AFM image, about .4 nm$^2$, is also in agreement with the .4 nm$^2$ area per molecule measured before deposition. These images also show that the DMPE packing is imperfect, that there are a number of defects and vacancies in the lattice, although the rows of molecules extend for several nanometers. This is suggestive of hexatic ordering. The lipid headgroups are not at a uniform height; the bilayer is rough at the nanometer scale.

The resolution and reproducibility of our images suggest that the localized pressures most likely do not reach the level that we may have expected. AFM tips are rounded, with at least a 50 nm radius, with at least a few atomic-scale projections. When the tip is pushed into the LB film covered substrate, the surface deforms smoothly over some fraction of the tip. This smooth deformation will absorb much of the applied force of the AFM cantilever and distribute the force over a relatively large area of several tens or hundreds of square nanometers. The smoothly varying force over the bulk of the tip should not change much as the AFM tip is rastered across the LB film. However, the local interactions probed by the atomic scale projections do change as the tip is rastered across the specimen surface, and it is these changes that likely dominate the AFM image.

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Fig. 1. AFM image of cadmium arachidate monolayer deposited on mica at a surface pressure of 35±3 mN/m. The image size is 500 x 500 nm. Low regions appear dark while higher regions are light. Many irregularly shaped, 3 nm deep holes can be seen in the monolayer ranging in size from about 30 - 140 nm. The smaller holes are more than twice as small as those ever resolved previously.

Fig. 2. Grey scale AFM images of the polar region of a bilayer of DMPE. The images were taken underwater at ambient temperature and pressure. The uniform rows are .7 - .9 nm in spacing. The modulation along the rows, with rounded spots every .5 nm, correspond to the headgroups of the DMPE molecule. The lattice spacing is identical to that measured by X-ray diffraction at the air-water interface.