

## LETTER TO THE EDITOR

### *Perturbation of Membrane Structure by Uranyl Acetate Labeling*

Dear Sir:

Stamatoff et al. (1) have recently elaborated a method of Furuya et al. (2) for labeling membranes with uranyl acetate to measure membrane dimensions directly with x-ray scattering. The authors recognized that "Direct interpretation in terms of phosphate separation for unstained isolated bilayers is not possible due to our inability to rule out changes in the bilayer imparted by  $\text{UO}_2^{++}$  binding" (1). In their demonstration study they found no change in the apparent 51-Å thickness of dipalmitoylphosphatidylcholine (DPPC) bilayers for  $\text{UO}_2^{++}$ :DPPC ratios from 1:1 down to 1:20. The expectation then was that no perturbation was likely for lower  $\text{UO}_2^{++}$ :DPPC ratios and that the label method was nondisruptive. We now find that uranyl labeling causes the hydrocarbon chains to become perpendicular to the plane of the bilayer.

While using Stamatoff et al.'s (1) measurement for our studies with DPPC multilayers in  $\text{CaCl}_2$  solutions, we compared the wide angle reflections of these bilayers in both  $\text{CaCl}_2$  and  $\text{UO}_2^{++}$  acetate solutions. Tardieu et al. (3), Rand et al. (4), and, more recently, McIntosh (5,6) have shown how the shape of this scattering, near  $(4.2 \text{ \AA})^{-1}$ , may be used to infer the tilt of the lipid hydrocarbon chains with respect to the plane of the bilayer. The tilting has been observed more directly by Levine (7-9) and Stamatoff et al. (10) on oriented multilayers. DPPC in 30 mM  $\text{CaCl}_2$  solutions (Fig. 1) always shows the same shouldered distribution seen for DPPC in pure water, a pattern indicative of chains tilted with respect to the bilayer (2, 3). (This distribution is reportedly altered at 0.5 M  $\text{CaCl}_2$  [11].) Bilayers in the  $\text{UO}_2^{++}$  acetate solutions at the ion concentrations used by Stamatoff et al. (1) always show the sharp symmetric peak characteristic of chains perpendicular to the bilayer (Fig. 1). We can rule out any suggestion that the effects seen result from close bilayer apposition and resultant interactions. This is because the wide angle patterns, for both  $\text{CaCl}_2$  and uranyl acetate, are unvarying over bilayer separations from 20 to 90 Å in multilayers, corresponding to 70 to 33% lipid, respectively, and then down to dilutions of 5% lipid/95% water where scattering from single bilayers is observed.

Change in tilt as a function of  $\text{UO}_2^{++}$  concentration appears to be sudden, as would be associated with a cooperative transition. Such cooperativity cannot be certified without investigating all  $\text{UO}_2^{++}$  concentrations between zero and one  $\text{UO}_2^{++}$  per 10 DPPC molecules.

From known lipid volume fraction and lattice repeat spacing in the multilayer, we have measured a thickness of 44 Å for the unperturbed bilayer (12) (assuming a bilayer region that contains all the phospholipid and no water). This thickness, determined for a multilayer in equilibrium with excess water, is consistent with the hydrocarbon chain tilt of 30° found by Tardieu et al. (3). Then, for a bilayer whose chains have been rotated to be perpendicular to the bilayer plane, we infer a thickness of  $44 \text{ \AA} / \cos 30^\circ = 50.8 \text{ \AA}$ . This is in apparent agreement with Stamatoff et al.'s (1) 50.3-Å separation between  $\text{UO}_2^{++}$  ion layers on the bilayer surface.

Therefore,  $\text{UO}_2^{++}$  labeling does appear to perturb the DPPC bilayer by removing the tilt angle, even when only one  $\text{UO}_2^{++}$  is bound per 10 DPPC molecules. It has recently been reported that trivalent lanthanide at much higher concentrations, one ion per three DPPC molecules, will also remove chain tilt; there is some indication (11) that  $\text{Ca}^{++}$  at or above 500 mM will also affect hydrocarbon chain arrangement. Neither of these ions seems to be as powerful a perturbant as  $\text{UO}_2^{++}$ .

Uranyl acetate might now be expected similarly to affect the structure of related materials. Even with the differences in resolution and preparation for x-ray diffraction and electron microscopy, we believe it is fair to question the common assumption that uranyl acetate is a nonperturbing stain.

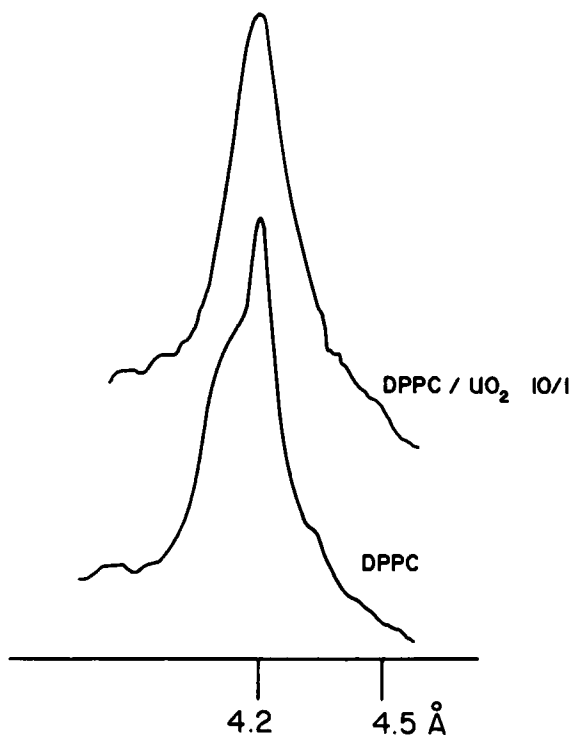


FIGURE 1 Photodensitometer tracing of x-ray scattering by DPPC bilayers at 25° in the vicinity of  $(4.2 \text{ \AA})^{-1}$ . The shouldered distribution is seen for DPPC in pure water or in  $\text{CaCl}_2$  solution. The symmetric curve is seen for DPPC exposed to uranyl acetate.

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