via localized atomic deformation functions;[13] these can, in favorable cases, be handled to give a conventional orbital description. For the phthalocyanine complexes, the Fourier series indicates a total delocalization of spin density onto the ligand of >20%, and we shall discuss these results in detail elsewhere.[13]

For the binuclear copper(II) complex, the copper atoms interact ferromagnetically with an exchange integral of 24 cm^{-1};[14] the observed density distribution shows no significant spin density *between* the copper atoms (Figure 2), whereas spin density is present on the bridging hydroxyl ligands, and this indicates that the mechanism of magnetic coupling is essentially one of superexchange.

Note Added in Proof. Further analysis of the data for Cs_3CoCl_5 to include refinement of the cobalt scattering curve shape and consideration of the bias introduced into the Fourier maps by an incomplete data set has led to d-orbital populations on the cobalt closer to the expected t_2^3.

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Photochemical Probes for Model Membrane Structures

Sir:

We have described the selective attack of benzophenone probes on rigid steroids[1] and the more random attack on flexible chains. The latter, useful chiefly as evidence on conformations of the chain, was examined in homogeneous solution[2] and in micelles.[3] We concluded that a probe such as benzophenone-4-carboxylate (**2**) incorporates into a micelle such as that from sodium dodecyl sulfate (SDS) so that the probe orients largely perpendicular to the micelle surface. However, on photoexcitation it attacks a range of the CH_2's of SDS because of the disorder of the SDS chains.

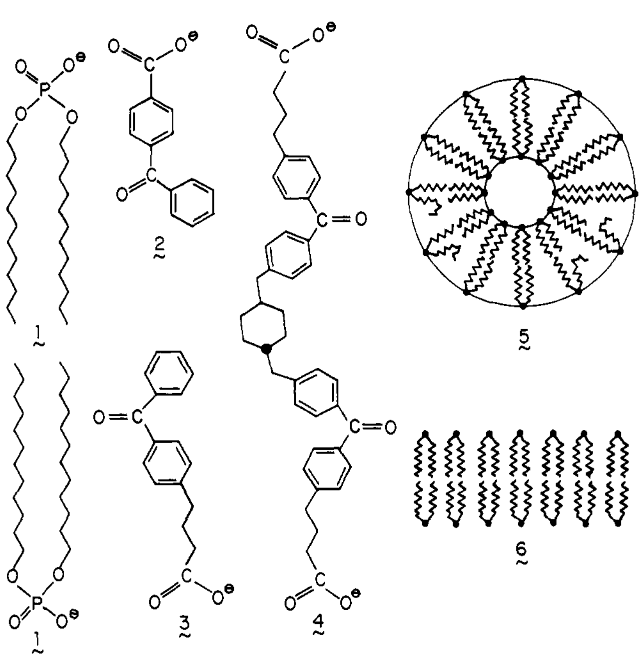

Figure 1. Probes and bilayers.

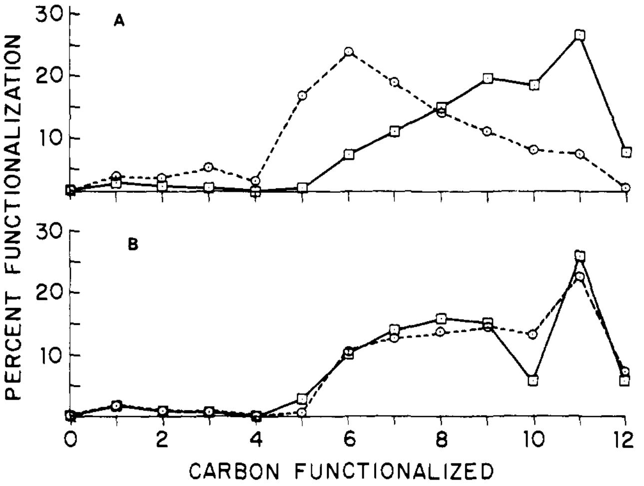

Figure 2. Distribution of functionalized positions. A: probe **2** with bilayer **1** as a vesicle (⊡—) and as a multilamellar system (⊙- - -). B: vesicles of **1** with probe **3** (⊡—) and probe **4** (⊙- - -).

Amphiphiles with two chains per charged head group, such as didodecyldimethylammonium cation,[4] dicetylphosphate anion,[5] and the lecithin of biomembranes[6] tend to form bilayers, not micelles, in which the chains are presumably more ordered than those in simple micelles. Such bilayers can exist as flat sheets, as in multilamellar structures, or instead as the curved surface of a spherical vesicle.[6] NMR evidence has been interpreted[7] to indicate that the bilayers of small spherical vesicles are more disordered than are the bilayers of multilamellar structures, although this interpretation has been disputed.[8] Thus it was of interest to apply our benzophenone probe method to the study of bilayers.[9] With this technique we have been able to explore the amount of disorder in such structures, and to demonstrate a striking difference between the disorder in spherical vesicles (**5**) and that in flat multilamellar systems (**6**) (Figure 1).

Our bilayers were constructed from didodecyl phosphate (**1**).[10] When this was sonicated[11] at 16 mM with 8 mM **2** in 50 mM Tris buffer at pH 8.0, a clear stable solution was formed

Table I. Randomness Ratios (R_r) for Reactions of Benzophenone Probes with Surfactant Micelles and Bilayers

probe, concn (mM)	surfactant, concn (mM)	phase	R_r (positions compared)
2, 5.0	SDS, 10	micelle/premicelle	3.73[a] (11/6)
2, 50	SDS, 100	micelle	1.00[a] (11/6)
2[b]	SDS/dodecanol[b]	multilamellar	0.35[a,b] (11/6)
2, 8.0	**1**, 16	vesicle	4.29[c] (11/6)
2, 8.0	**1**, 16	multilamellar	0.27[d] (11/6)
3, 2.6	**1**, 16	vesicle	1.72[c] (11/9)
4, 1.3	**1**, 16	vesicle	1.55[c] (11/9)

[a] Taken from ref 3. [b] Ratio of SDS/dodecanol/H_2O/**2**, 6:6:15:2. [c] Vesicles prepared in 50 mM Tris HCl, pH 8.0. [d] Multilamellar bilayers prepared in 100 mM sodium borate, pH 8.9.

indicating the production of vesicles.[11] The solution was photolyzed, degraded,[12] and analyzed by mass spectrometry as we have described previously.[2,3] The distribution of functionalized positions in the dodecyl chain is shown in Figure 2. We have also described[3] a parameter R_r, the randomness ratio, which can be used to quantitate the disorder revealed in such distributions: R_r = attack at penultimate carbon/attack for perfect orientation. The R_r from this curve, the ratio of attack at C-11 resulting from disorder to the attack at C-6 expected, from models, if the head groups of **1** and **2** are aligned and the chains of **1** are fully extended, is 4.29. This value is listed in Table I.

The curve and the R_r reveal considerable disorder in this bilayer. For comparison, our data[3] for a micelle of SDS with probe **2** had an R_r of only 1.0 at a 2:1 SDS to probe ratio.[13] At ~30 °C in this system, **1** is certainly above its transition temperature to a more fluid state,[14,15] as are biomembranes. As we found with micelles,[3] the absence of significant attack on carbons 1–5 indicates that the probe is indeed correctly oriented largely perpendicular to the vesicle surface. The possibility that we are seeing significant misalignment of the head groups of **1** and **3** is also made unlikely by this fact, and by the data on other systems described below.

When **1** and **2** were sonicated in a sodium borate buffer, the result was an almost opaque dispersion characteristic of multilamellar systems.[16] The ability of Na^+ to promote the formation of such structures has been noted previously.[5] In this system, the functionalization profile (Figure 2) is now grossly different from that with the vesicles, and the R_r (Table I) has the remarkably low value of 0.27, the lowest value we have ever observed. The ordering observed is even greater than that we have seen[3] in a dodecanol/SDS system (Table I) which also is known[17] to form bilayer structures. Thus it seems that such multilamellar bilayers are indeed much more ordered than are curved vesicles.

Two other probes were examined in the study of spherical vesicles. With probe **3**, the longer chain moves the probe deeper into the bilayer, leading to the profile in Figure 2 and an R_r (Table I) of 1.72. Probe **4** was synthesized[18] since models suggest that its length is correct to *span* the bilayer and anchor its orientation with two charged groups at the bilayer surface.[19] The similar profile of **3** and **4** (Figure 2) and the similar R_r values (Table I) support the idea that the principal contributor to these profiles is disorder of the bilayer, rather than disorder of probe orientation.

Of course, all such chemical probe techniques carry the hazard that the observed disorder is to some extent *induced* by the probe, or that the probe is selectively taken up into disordered regions. Thus such data should be used in conjunction with that from other physical methods to give a complete picture of the unperturbed structure. However, even with such caveats it seems clear from our data that vesicles derived from **1** show disorder comparable with that in simple micelles, but that multilamellar bilayers derived from **1** are much more ordered in structure.

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(9) The idea of using a photochemically generated species to probe biomembrane structures is not unprecedented. Several laboratories have proposed carbenes or nitrenes, although no structural data have yet been reported. See for example, the following. (a) Klip, A.; Gitler, C. *Biochem. Biophys. Res. Commun.* **1974**, *60* 1155–1162. (b) Klip, A.; Darszon, A.; Montal, M. *ibid.* **1976**, *72*, 1350–1358. (c) Chakrabarti, P.; Khorana, H. G. *Biochemistry* **1975** *14*, 5021–5033, (d) Bayley, H.; Knowles, J. R. *ibid.* **1978**, *17*, 2414–2419. (e) Bayley, H.; Knowles, J. R. *Ibid.* **1978**, *17*, 2420–2423.

(10) Didodecyl phosphate was conveniently prepared from reaction of dodecanol and *p*-nitrophenyl phosphorodichloridate, followed by saponification of the triester with KOH to give, after acidification, *p*-nitrophenol and didodecyl phosphate. The didodecyl phosphate was purified by fractional crystallization (2–3×) from 0.01 M HCl in ethanol, mp 58–59 °C.

(11) Sonication was performed using a Branson Sonifier Model 350, equipped for processing large volumes, with a $^3/_4$-in. high gain disrupter horn. Typical conditions were power level 6–7 for 30–45 min. Comparable conditions produced vesicles in ref 4 and 5.

(12) We used a modified version of our previously described degradation, ref 2 and 3. After photolysis, the mixture was acidified and extracted with $CHCl_3$. The dry organic material was reduced with $LAlH_4$ according to the method of Karrer, P.; Jucker, E. *Helv. Chim Acta* **1952**, *35*, 1586. Subsequently the procedure paralleled our other degradations with acetylation by Ac_2O/pyridine, elimination of the tertiary carbinol with $SOCl_2$/pyridine, oxidation with $RuO_2/NaIO_4$, and finally thioketalization with ethanedithiol/BF_3Et_2O. Final chromatographic purification of the thioketal acetates was done on μ Porasil (4 mm × 25 cm) using 1% ether in hexane.

(13) However, in the bilayer the formal ratio of hydrocarbon chains to probe is 4:1; furthermore we do not know what percent of the total probe is actually incorporated in the bilayer. Thus the concentration comparisons are not exact.

(14) Although no systematic studies on transition temperatures in these simple membrane models have been published, preliminary measurements on dimethyldioctadecylammonium bromide[4c] indicate a T_c of 30–50 °C. If, by analogy to diacylphosphatidyl cholines,[6a] our 12-carbon analogue is 35 °C lower than the 18-carbon species, we would predict a T_c of −5 to 15 °C.

(15) So far several attempts to incorporate and react **2** in dihexadecyl phosphate vesicles have not been successful. This may be related to a higher transition temperature for the 16-carbon case.

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(18) Probe **4** was prepared by saponification of its dimethyl ester. The diester was the product of the bis Friedel–Crafts acylation on *trans*-1,4-dibenzylcyclohexane with the acid chloride of methyl 4-(*p*-carboxyphenyl)butyrate. The dibenzylcyclohexane was the product of catalytic hydrogenation of *trans*-1,4-dibenzoylcyclohexane which was obtained from the acylation product of 2 equiv of benzene and 1 equiv of the diacid chloride of *trans*-1,4-cyclohexanedicarboxylic acid. The butyrate was obtained by the acylation of methyl 4-phenylbutyrate by oxalyl chloride.

(19) The profile in Figure 2, with no functionalization at C(1)–C(5), suggests that both carboxyls are *not* oriented at the *same* surface.

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