downfield relative to 1)); $^1J_{PtP}$ has the surprisingly low value of 505 Hz. We felt that (a) the nonequivalence of P1 and P2, (b) the upfield shift of the resonance of P3 (cf. the similar, though less dramatic trend, for 3[8]), and (c) the weak PtP3 coupling strongly indicated η^2-coordination for **5** in solution.

So that this conclusion could be checked, an X-ray crystal structure determination of **5** was carried out.[11] The structure (Figure 1) shows discrete monomeric units in which the Pt atom has a distorted trigonal coordination. All three P atoms are in one plane with Pt and are σ coordinated to the metal. The Pt-P1 and Pt-P2 bond lengths are in the usual range (228.8 (3) and 229.4 (3) pm, respectively); the Pt-P3 bond is shorter (221.8 pm), as expected for a sp^2 hybridized P atom.[3] Interestingly, the least-squares planes through PtP1P2P3 and PtP3C37C46 form an angle of 67.4° (Figure 2, supplementary material). The near-planarity of PtP3C46C47C53 and the Pt-C46 distance (357 (1) pm) exclude any η^2-type interaction. As in **2**,[3] the ligand **1** is hardly affected structurally by coordination to Pt(0). A noteworthy feature of **1** in **5** is the P=C bond length of 165 (1) pm, which is even shorter than that of CH_2=PH (167.0 pm[12]); there appears to be a trend of P=C bond shortening from **1** (170 pm[2,13]) via **2** (167.9 (4) pm[3]) to **5**. The bond angle at phosphorus (C37-P3-C46, 108.5 (5)°) is smaller than 120° (the angle expected for ideal sp^2 hybridization) but similar to that in free **1** (108.7°) and in **2** (109.8 (2)°).

We are thus left with the somewhat puzzling situation that the ^{31}P NMR and the X-ray data seem to be at variance. Two possible explanations might resolve this apparent discrepancy. First, one might assume that in **5**, **1** is η^1-coordinated and acts as an unusually weak σ donor (as reflected by the small $^1J_{PtP3}$) and an unusually strong π acceptor[14] (as reflected by the strong upfield shift of δ P3). However, at present it is not clear why **1** should behave so differently in **5** and in **2**, where it has been identified as a σ donor and a π acceptor of intermediate strength;[3] moreover, strong π back-donation should result in lengthening of the P=C bond,[14] while the reverse is observed.

A second plausible possibility would be the occurrence of two different structures of **5** in solution and in the crystalline state. In the crystal, η^1-coordination is unambiguously established by the X-ray data. In solution, the bonding situation may have changed to η^2-coordination; the dynamic effects observed in the NMR spectra above −50 °C may be an indication of rapid transformations of this kind. A coordinatively saturated P atom in solution is indicated by the observation that **5** did not react with a second equivalent of **1** or with triphenylphosphine; this behavior is more in line with an η^2 coordination than with an η^1 coordination. On the other hand, in the η^2-coordination mode, **1** would not make use of its HOMO, which is a σ orbital essentially located as a lone pair on phosphorus.[14]

Clearly, further investigations are required before a decision between these interesting alternatives can be made; these investigations are in progress.

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Registry No. 5, 80737-43-5; **6**, 12120-15-9.

Supplementary Material Available: Listings of atomic coordinates, thermal parameters, bond lengths, and bond angles and Figure 2 (7 pages). Ordering information is given ony any current masthead page.

(11) The crystals of **5** were triclinic and crystallized in space group $P\bar{1}$, $Z = 2$, with unit cell constants $a = 12.286$ (2) Å, $b = 13.948$ (2) Å, $c = 14.875$ (3) Å, $\alpha = 100.99$ (2)°, $\beta = 94.88$ (2)°, $\gamma = 101.43$ (2)°. A total of 6698 reflexions with intensity above the 2.5σ level were collected on an Enraf-Nonius CAD-4 diffractometer using graphite monochromatized Mo Kα radiation. No absorption correction was applied. The structure was derived directly from an E^2-Patterson synthesis. Refinement proceeded by anisotropic block-diagonal least-squares calculations. Most of the H atoms were indicated in a difference Fourier synthesis and were introduced and kept fixed at their calculated positions. The final R value was 0.062. A final difference Fourier synthesis showed effects of absorption around Pt and also a large number of peaks of heights of about 0.8 e/Å^3, which could not be interpreted but which are undoubtedly due to some form of disorder. Relevant bond distances (in pm): Pt-P1, 228.8 (3); Pt-P2, 229.4 (3); Pt-P3, 221.8 (3); P3-C46, 165 (1); P3-C37, 182 (1); C46-C47, 156 (2); C46-C53, 151 (2); P1-C1, 187 (1); P1-C7, 183 (1); P1-C13, 185 (1); P2-C19, 182 (1); P2-C25, 182 (1); P2-C31, 180 (1). Relevant bond angles (in degrees): P1-Pt-P3, 120.6 (1); P2-Pt-P1, 121.9 (1); P2-Pt-P3, 117.3 (1); Pt-P3-C46, 133.3 (5); Pt-P3-C37, 118.2 (3); C46-P3-C37, 108.5 (5); C53-C46-P3, 122.6 (8); C53-C46-C47, 133.3 (8); C47-C46-P3, 124.0 (8).

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Interaction of Ions with the Surface Receptor of the Azobenzene-Containing Bilayer Membrane. Discrimination, Transduction, and Amplification of Chemical Signals

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We describe in this article how unique properties of the bilayer membrane are advantageously used for preparing molecular systems in which discrimination, transduction, and amplification of chemical signals are possible.

It has been shown that the bilayer membrane is formed from a variety of single-chain and double-chain amphiphiles which are not directly related to the structure of biolipids.[1] The fundamental physicochemical characteristics of the synthetic bilayer membrane are the same as those of the biolipid bilayer, and therefore, it is expected that some of the molecular machinery of the cell membrane[2] is reproducible by using the synthetic system.

As reported before,[3,4] ammonium amphiphiles with the azobenzene chromophore form stable bilayer assemblies in water. The absorption maximum of this chromophore may undergo hypsochromic shifts in the bilayer matrix relative to that in homogeneous solutions. This spectral shift was used to detect phase separation in the dialkylammonium membrane matrix.[5] The phase-separation phenomenon is useful for the present purpose. The membrane-forming amphiphiles used in the present study are given in Chart I.[6]

In a mixed bilayer membrane of **1** and **4** (molar ratio, 1:10), amphiphile **1** exists as an isolated species at temperatures above T_c (28 °C) of the matrix membrane of **4**. The absorption maximum of the azobenzene chromophore is located at 355 nm in this case, in agreement with that in ethanol. From a drop in temperature, λ_{max} shifts to 320 nm, indicating formation of the clustered species of **1**.

The phase separation depends on the molecular structure of the azobenzene amphiphile. Thus, amphiphile **2**, which possesses a shorter alkyl tail, does not undergo phase separation under the

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(6) Preparations of amphiphiles **1**, **4**, and **5** were described in ref 3, 7, and 8, respectively. Amphiphile **2** is obtained by a procedure similar to that for **1** using *N*,*N*-dimethylaminoethanol. Amphiphile **3** was prepared in refluxing benzene from *p*-hexyloxy-*p'*-(ω-bromodecyloxy)azobenzene and excess ethylenediamine. The product was identified by TLC FID, NMR spectroscopy, and elemental analysis.

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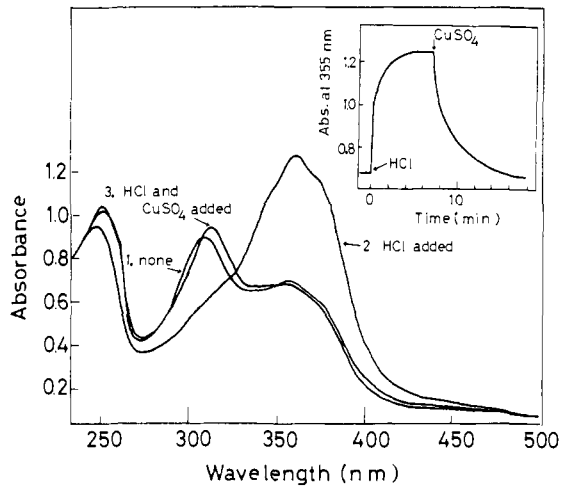
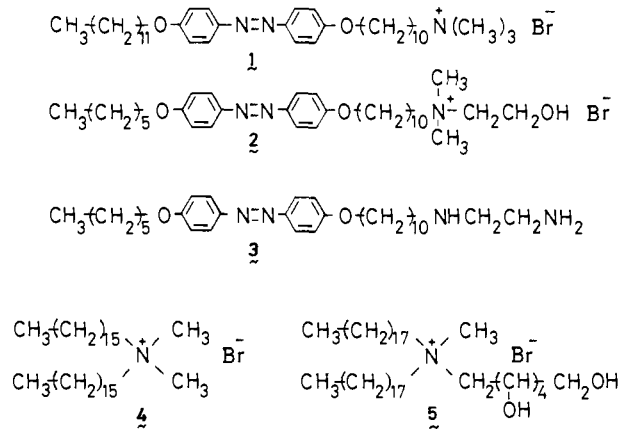


Figure 1. Spectral changes induced by ion addition: (1) [**3**] = 0.5 mM, [**4**] = 5 mM, 35 °C, pH 7; (2) hydrochloric acid added to solution 1, [HCl] = 10 mM; (3) $CuSO_4$ added to solution 2, [$CuSO_4$] = 0.4 mM. The insert indicates the absorbance change at 355 nm upon ion addition.

Chart I



same conditions, λ_{max} being maintained at 355 nm. In contrast, amphiphile **3** undergoes phase separation in spite of the short alkyl tail. In this case, hydrogen bonding among the partially protonated diamine head groups must promote phase separation.[9] This presumption is supported by the fact that amphiphile **3** does not form clusters in the bilayer matrix of **5** even at temperatures below T_c (49 °C). Apparently, the hydrogen-bonding partner of the diamine head group is now replaced by the polyhydroxy head group of **5**, resulting in disappearance of the cluster of **3**.

These results suggest that phase separation of mixed bilayers can be regulated by the interaction of appropriate head groups among themselves or with added ions. In other words, the interaction at the membrane surface can be detected through the physicochemical change (phase separation) of the matrix.

Amphiphile **3** exists as clusters in the fluid bilayer matrix of **4**. When hydrochloric acid is added, λ_{max} shifts from 312 to 355 nm (Figure 1), indicating disintegration of the cluster due to extensive protonation of the diamine head group. Addition of $CuSO_4$ to this solution regenerates the cluster species. The absorbance change at 355 nm (the monomeric species) with these additions is shown in the insert of Figure 1. The regeneration is similarly induced by Na_2SO_4 but not by $CuCl_2$. Addition of NaCl produces no effect. Thus, it is concluded that the phase separation of protonated **3** by $CuSO_4$ is actually induced by SO_4^{2-} ion and not by Cu^{2+} ion.

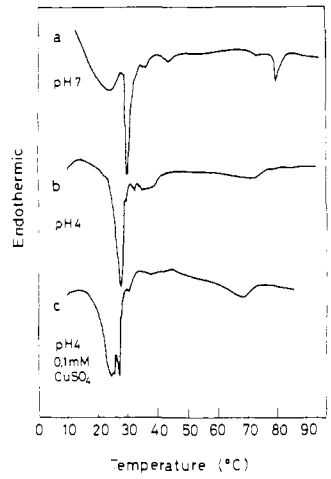


Figure 2. DSC thermograms,[10] heating rate 2.0 °C/min: (a) the sample solution was obtained by sonication of 5 mg of **3** and 20 mg of **4** in 1 mL of water; (b) the pH value of solution a was adjusted to 4 by hydrochloric acid; (c) $CuSO_4$ (0.1 mM) was added to solution b.

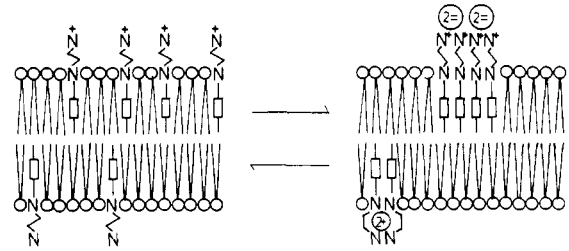


Figure 3. Schematic illustrations of ion-induced phase separation. The phase separation is induced either by the Coulombic interaction of the protonated polyamine head group with divalent anions or by complex formation of the polyamine head group with transition metal ions.

Phase separation by ion addition is supported by differential scanning calorimetry (DSC).[10] As shown in Figure 2a, a mixed membrane of **3** and **4** (weight ratio 1:4) gives three endothermic peaks at 25 (br), 30, and 78 °C at neutral pH. The T_c values of the single-component bilayer of **3** and **4** are 78 and 28 °C, respectively. Therefore, the 78 °C peak of the mixed membrane is attributed to the cluster of **3**. The cluster disappears by lowering of the pH, as indicated by the disappearance of the high-temperature peak in DSC (Figure 2b). Addition of sulfate ion (as $CuSO_4$) to the acidic solution produces a broad peak centered at 69 °C and lower-temperature peaks at 25 and 27 °C (Figure 2c). The latter data clearly indicate regeneration of the heterogeneous domain, although its structure is not necessarily the same as that of the original cluster (Figure 2a).

Different situations are found for the combination of **3** and **5**. As mentioned above, **3** is miscible with the rigid (below T_c) bilayer matrix of **5**. When $CuCl_2$ (0.3 mM) is added to a mixed membrane (5 mM) of **3** and **5** (mole ratio 1:10), a new absorption maximum of the azobenzene cluster appears at 312 nm, with a concomitant decrease in the original monomer peak. Addition of hydrochloric acid (0.01 N) to this solution regenerates the monomer species. Therefore, phase separation is induced by the complex formation between Cu^{2+} ion and the diamine head group of **3**. The protonated head group cannot interact with Cu^{2+} ion.

It is established from these data that phase separation of amphiphile **3** is induced either by the chelate formation of the diamine head group with Cu^{2+} ion or by the electrostatic interaction of the protonated diamine head group with SO_4^{2-} ion (but not with Cl^- ion). These situations are schematically illustrated in Figure 3. The phase separation by Cu^{2+} ion may be compared to the Ca^{2+}-induced phase separation of the biomembrane that contains

(9) The sample solutions were prepared in deionized, distilled water (pH 6–7). Therefore, the diamine head group is partially protonated.

(10) The detailed experimental procedure for DSC in given in: Okahata, Y.; Kunitake, T.; Ando, R. *Ber. Bunsenges. Phys. Chem.* **1981**, *85*, 789–798.

acidic lipids.[11] The latter phenomenon plays important roles in regulation of physiological functions such as membrane fusion and secretion.

The interaction of metal ions with the surface ligand of aqueous micelles and bilayers has been studied by several groups. Le Moigne et al.[12,13] reported enhanced interactions of some metal ions with micellar ligands (crown ether and polyamine) and Grätzel et al.[14,15] described acceleration of photoreduction of Ag^+ in related micellar and bilayer systems. Fromherz and Arden[16] examined pH modulation of energy and electron transfer across the bilayer of docosylamine deposited on the electrode surface.

The characteristics of the present membrane system as a signal receptor are summarized as follows. Chemical signals (H^+, Cu^{2+}, and SO_4^{2-}) interact specifically with the ethylenediamine moiety (receptor) at the membrane surface, and these specific signals are transduced into nonspecific spectral information via the change in the membrane physical state. The signals can be amplified during this process, since the molecular extinction coefficient of the azobenzene chromophore (23 000) is much larger than, for instance, that of the ethylenediamine Cu^{2+} chelate (ca. 50).

The signal recognition can be readily improved by the introduction of other specific ligands at the membrane surface and by the use of more sensitive spectroscopic methods. Efforts directed toward this goal are under way.

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Registry No. 1, 75056-19-8; **2**, 80737-49-1; **3**, 80721-48-8; **4**, 70755-47-4; **5**, 65316-86-1; $CuSO_4$, 18939-61-2.

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Strategy for Nitrogen NMR of Biopolymers

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High-resolution nitrogen NMR spectra of biopolymers are readily obtained by using solid-state ^{15}N NMR techniques on immobile samples, whether crystalline or slowly reorienting solutions, where the molecules have been highly enriched biosynthetically with ^{15}N. Nitrogen NMR spectroscopy is very attractive for biophysical studies because there are relatively few nitrogen atoms in biopolymers (compared to carbon or hydrogen) and they are often located in interesting sites such as functionally active residues of enzymes, the bases of DNA and RNA, or the polypeptide backbone of proteins.

The nuclear properties of the stable isotopes of nitrogen present difficulties for high-resolution NMR spectroscopy. ^{14}N has spin $S = 1$ with a large quadrupole coupling constant in most situations; as a consequence the powder patterns in solids and the resonances in solution are broad. Recent ^{14}N solid-state NMR experiments on single crystals[1] and samples with substantial motional averaging[2,3] show considerable promise for these specialized cases. The other stable isotope of nitrogen is ^{15}N, which has spin $S = {}^1/_2$, but its natural abundance of only 0.3% results in very low sensitivity, and its small, negative gyromagnetic ratio makes even labeled site studies difficult because of long T_1's and negative nuclear Overhauser enhancements. Spin $S = {}^1/_2$ nuclei offer great opportunities for biological NMR spectroscopy because the observation of resonances from individual sites of biopolymers allows measurements that can give structural and dynamical information with atomic resolution. Of the four spin $S = {}^1/_2$ nuclei found in biopolymers (^1H, ^{13}C, ^{15}N, ^{31}P) only ^{15}N has not been widely used in spectroscopic studies. Natural abundance ^{15}N spectra of biopolymers have single-site resolution only when very concentrated large samples are extensively signal averaged in a high-field spectrometer.[4,5]

There are several advantages to solid-state ^{15}N NMR experiments. The sensitivity due to cross polarization of the ^{15}N magnetization from the protons is increased over that from ^{15}N sampling pulses because of the larger amount of ^{15}N magnetization developed per transient and the ability to recycle the experiment according to the generally short ^1H T_1's rather than the long ^{15}N T_1's.[6] The observed nuclear Overhauser enhancement varies with reorientation rates in solution and can completely eliminate signals in spectra of biopolymers, but is avoided in the cross-polarization experiment. Proton decoupling removes the heteronuclear dipolar interactions, making the ^{15}N chemical shift properties available for study.[7,8] Magic-angle sample spinning[9] averages the ^{15}N chemical shift powder patterns to isotropic resonances, with line widths (<0.5 ppm) for polycrystalline amino acids similar to those observed in ^{13}C NMR spectroscopy.[10]

Meselson and Stahl[11] demonstrated that when *E. coli* are grown on a medium with $^{15}NH_4Cl$ as the sole nitrogen source, ^{15}N is incorporated into the newly synthesized biopolymers. From growth of *E. coli* with the appropriate genetic makeup,[12] whether wild type or arranged through mutation, cloning, or viral infection, any gene or gene product of procaryotic or eucaryotic origin can be obtained uniformly labeled with ^{15}N. Very high enrichment with ^{15}N is advantageous from an NMR point of view because of the increased sensitivity due to the large number of spins without the penalty associated with homonuclear couplings, as seen with uniform ^{13}C enrichment, since no nitrogens in biopolymers are directly bonded to other nitrogens. Growth on ^{15}N-containing media has been used to provide several in vivo systems[13-17] and isolated biomolecules,[18-20] including DNA from *E. coli*,[21,22d] for

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