Toward Molecular Mechanoelectric Sensors: Flexoelectric Sensitivity of Lipid Bilayers to Structure, Location, and Orientation of Bound Amphiphilic Ions

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The curvature-induced polarization (*i.e.* flexoelectricity) of lipid bilayers produces ac currents (I_{vib}) on application of vibrations. Binding of aromatic amphiphilic ions to one or both interfaces of a planar bilayer membrane decreases the amplitude of $I_{vib.}$ On asymmetric binding of such ions I_{vib} is further reduced to a minimum at an applied dc voltage (V_{min}), which has a sign driving the ion outward and a magnitude determined by the lipophilic group of the amphiphile. A voltage with reversed sign always increases $I_{vib.}$ The mobile lipophilic ion, tetrakis(*p*-chlorophenyl)borate, can amplify I_{vib} by ∼10-fold by enlarging the polarizibility but causes little shift of V_{min}. The novel voltage dependence of I_{vib} induced by amphiphilic ions is attributed to the influence of their relocalization and orientation on the surface tension of the bilayer. This finding offers a new method for study of molecular interactions at the lipid−water interface and an entry into molecular mechanoelectric sensors.

Introduction

The flexoelectric effect is the curvature-induced electric polarization in structure, which is conceptually analogous to the piezoelectric effect. Flexoelectricity is a well-understood property of liquid crystals.[1] This concept has been extended to lipid bilayer membranes.[2] Pressure wave-induced vibration of the membrane can produce ac currents[3,4] or voltages[5] of the same frequency across the bilayer. This periodic polarization is caused by the curvature-induced unbalanced charge displacement and dipole orientation in the two polar or interfacial regions of the bilayer. Since flexoelectricity converts mechanical energy to electrical energy (and vice versa), this property of the lipid bilayer has been hypothesized as a biological mechanoreceptor[3] and active driving force[2] across cell membranes. The influence of lipid properties, membrane conductivity, applied voltage, vibration frequency, and photoinduced charge transfer on the flexoelectricity of lipid bilayers has been reported.[3−6] Theoretical models describing the flexoelectric effect[2−5] and the effect of electrostatic double layers on the curvature elasticity of membranes[7,8] have been proposed. The converse flexoelectric effect (voltage-induced curvature) has been experimentally demonstrated.[9] These results permit in principle the use of lipid bilayer membranes as micromechanoelectric transducers and sensors.

Compared to other known mechanoelectric devices, the lipid bilayer begins on the molecular scale. Its flexoelectricity is sensitive to molecular interactions. Thus there is a possibility that lipid bilayers can be used as mechanoelectric sensors of lipophilic and amphiphilic molecules. However, little research in this direction has been previously reported. Presented herein is the first observation of flexoelectric sensitivity of the bilayer to structure, location, and orientation of bound amphiphilic ions. In the presence of amphiphilic ions at a single interface of a planar bilayer membrane, applied dc voltages produce dramatic changes in amplitude of the vibration-induced ac current (I_{vib}). A specific voltage which reduces I_{vib} to a minimum amplitude has a close relation to the lipophilic binding energy of the amphiphile. It will be shown that tetrakis(*p*-chlorophenyl)borate and its fluoro analogue, lipophilic anions with high mobility in the lipid bilayer,[10] are efficient flexoelectric enhancers of membranes. Their presence can amplify I_{vib} more than 10-fold depending on the bound amphiphilic ion. These results may stimulate the development of molecular mechanoelectric sensors.

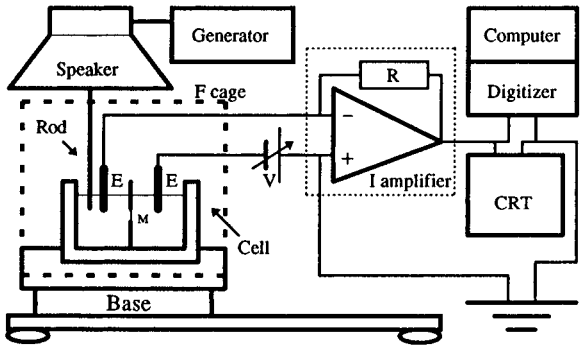

Figure 1. Schematic of the setup for observing flexoelectric sensitivity of lipid bilayers to binding of ions. M represents a planar lipid bilayer membrane formed across a 0.5−2.5 mm diameter hole in a thin Teflon partition and bathed in aqueous electrolyte. Amphiphilic ions can be added to either side of M. V is a voltage source for applying dc voltage. Vibration-induced currents (I_{vib}) of M were measured via two calomel electrodes (E) and the amplifier in negative feedback mode. The system was shielded by a Faraday cage. The vibration of M is induced by that of a loudspeaker and transmitted via an insulated rod.

Materials and Methods

1,2-Diphytanoyl-3-*sn*-phosphatidycholine was obtained from Avanti Polar Lipids. Potassium tetrakis(*p*-chlorophenyl)borate ($TCPB^-$) was obtained from Fluka Chemika. Sodium tetraphenylborate (TPB^-), chloroquine (*N*-(7-chloro-4-quinolinyl)-*N*′,*N*′-diethyl-1,4-pentanediamine) phosphate, and valinomycin were obtained from Sigma Chem. Co.. Sodium tetrakis(*p*-fluorophenyl)borate ($TFPB^-$), sodium anthraquinone-2-sulfonate (AQS^-), and other compounds were obtained from Aldrich Chem. Co..

Figure 1 shows schematically the experimental setup for observing the flexoelectric sensitivity of lipid bilayers to molecular binding. Planar lipid bilayers were formed in a 0.5−2.5 mm diameter hole in a 0.38 mm thick Teflon partition, which symmetrically divides a 4 mL polyethylene cell with glass

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windows containing electrolyte solution. The bilayer-forming solution is 30 mM diphytanoylphosphatidylcholine in *n*-decane. By gently brushing the lipid solution along the lipid-precoated hole edge under a microscope, a membrane was spontaneously formed across the hole. The quality of the lipid bilayer was monitored by measuring its conductance and capacitance and by checking the symmetry of vibration-induced ac currents to the sign of applied dc voltage. The amplitude of I_{vib} was proportional to the square of the membrane diameter. Typically the membrane was formed in a 1.5 mm diameter hole and bathed in 0.1 M NaCl and 0.01 M Hepes with pH 7−8. Their steady state resistance and capacitance were 10^{10} Ω and 6 nF. The capacitance was determined from the square-wave current response to the applied triangle-wave voltage across the bilayer in the $1-10^4$ Hz range below ±20 mV. When the amphiphile in aqueous solution was added to one side of the membrane, the hydrostatic pressure was balanced by adding water to the other side. Since the amphiphilic ions were not permeable and their concentrations were ≤2% of the electrolyte, there was no observable transmembrane osmotic gradient. When used, the lipophilic borate in ethanol−water solvent was added symmetrically to both sides of the membrane. The content of ethanol in the bathing solution is always less than 0.2%, which has no influence on the properties of lipid bilayers.

Currents across the lipid bilayer were measured via two calomel electrodes (Fisher 620-79) and the current amplifier consisting of an operational amplifier (Teledyne 1021) and a homemade feedback circuit. The amplifier was set at a gain of 10^7-10^8 V/A with a time constant of 0.1−1 ms. Dc voltages below ±140 mV were applied across the membrane via the same electrodes, using a variable voltage source in series. Other details of short-circuit current measurements were described earlier.[10] Mechanical vibrations were transmitted from a loudspeaker (Sanyo IJ-23WBF) to the membrane via a polyethylene rod immersed in the bathing solution. The rod had a 3 mm diameter and was hardened by a metal core. The loudspeaker was powered by $10-10^4$ Hz sine-wave voltages below ±2 V from a function generator (Exact 119B). It vibrated with the same frequency as the input but with nonlinearly varying responses to different frequencies. Under these conditions, observable I_{vib} was produced in the 10−600 Hz range. The sensitivity of the available pressure wave detectors (air microphones and piezoelectrodes) varied with frequency in this range. The dependence of I_{vib} on vibrational frequency is too small to measure under these conditions, which has been reported to vary with properties of lipid bilayers.[4] The intensities of the pressure wave and I_{vib} were linearly proportional to the magnitude of the sine-wave voltage. However, resonances and reflections of the vibrations at some frequencies in the system made it difficult to determine the absolute applied pressure on the membrane. When the loudspeaker was not coupled mechanically to the cell system in the Faraday box, no observable ac current was caused by its vibration or by electromagnetic interference. Vibrations transmitted to the membrane via the cell holder by a solid−solid coupling method also produced the amphiphile-sensitive $I_{vib.}$ When the membrane diameter ≥ 1 mm and $TCPB^-$ (or $TFPB^-$) is present, I_{vib} induced by 25−30 Hz vibrations of the laboratory floor was easily observable even though the cell base was supported by viscoelastic polymer. These vibrations were determined by an acoustically isolated microphone (Knowles BL-1785).

To ensure the mechanical symmetry and stability of membranes, very weak vibrations were applied in most experiments. In the voltage dependence measurements of I_{vib} amplitudes, a dc voltage was applied to the membrane for <10 s, then the reversed voltage was always applied for the same time. The amphiphilic and borate ions change I_{vib} only by interacting with the lipid bilayer. They did not affect I_{vib} by altering any property of the electrodes. This was proven by using a system without holes in the partition but with a circuit containing resistance and capacitance similar to a bilayer membrane. Current oscillations in the $1-10^4$ Hz range caused directly by applied voltage oscillations across the bilayer were not affected by amphiphilic binding and dc voltages.

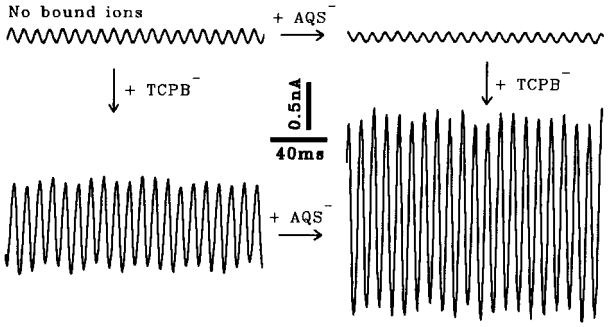

Figure 2. Relative I_{vib} amplitudes of the lipid bilayer clamped at 0 mV on binding of different ions. The ac currents are induced by the 100 Hz vibration. There is no dc current offset (<5 pA) across the membrane. When the applied vibration is absent, only in the presence of $TCPB^-$ is there an observable 25−30 Hz ac current arising from vibrations of the laboratory floor, which is recorded as noise (∼5% of the signal) in the two lower current traces. When used, 4 μM $TCPB^-$ (lipophilic) is added to both sides of the bilayer. 0.2 mM AQS^- (anthraquinone-2-sulfonate) is added to one side. The symmetric addition of AQS^- causes 5−10% smaller I_{vib} than that shown by the two right curves in the absence and presence of $TCPB^-$.

Results

Upon application of mechanical vibrations in the 10−600 Hz range, I_{vib} with the same frequency occurs at zero voltage across the lipid bilayer (Figure 2). The amplitude of I_{vib} increases linearly with the vibrational amplitude. Such a current cannot be caused by the curvature-induced capacitance and resistance changes of the membrane during its vibration, since these changes can produce ac currents only in the presence of voltage and here the membrane is clamped at 0 mV by the feedback amplifier. In the absence and presence of the lipophilic borate anions, the membrane resistances are $\geq 10^{10}$ and $\sim 10^8$ Ω, respectively. A greater than 0.1 V ac voltage is needed to drive a trans-membrane ion flow equivalent to the ac currents (∼0.1 and 1 nA) shown in Figure 2. However, previous studies[5] and the present measurements have shown that bending of lipid bilayers can produce only <0.1 mV ac voltage across the membrane. Thus the measured ac currents are 10^3 larger than the ion leak currents across the bilayer. The addition of valinomycin or gramicidin, which increases the ionic conductance of lipid bilayers by several orders of magnitude,[11] has no significant influence on I_{vib} even with an applied dc voltage across the bilayer. This shows that I_{vib} is independent of transmembrane ion transport and is coupled through the capacitance of the bilayer, as has been reported.[4] Such ac currents can be caused only by the curvature-induced electric polarization in the bilayer structure. This flexoelectric effect arises from unbalanced charge shift and dipole orientation in the two polar or interfacial regions of the bilayer.[4−6] In the absence of lipophilic and amphiphilic ions, the curvature-induced ac currents are mainly carried by displacements of charges and dipoles on the polar head groups of lipid molecules.[5]

Figure 2 shows the changes of I_{vib} caused by binding of amphiphilic AQS^- and lipophilic $TCPB^-$ anions to the lipid

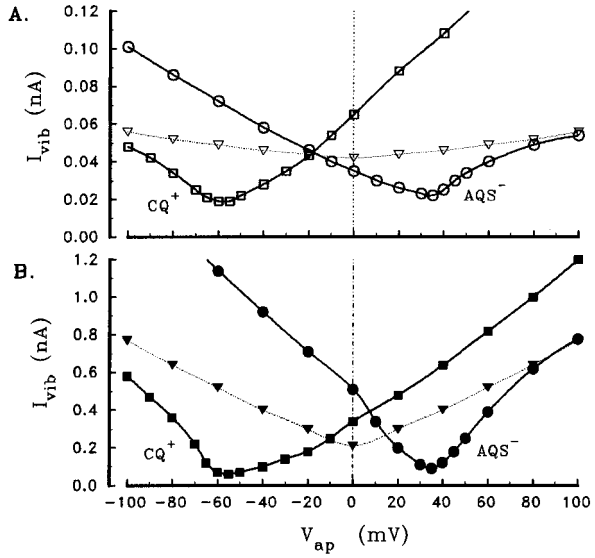

Figure 3. Novel dependence of I_{vib} on applied dc voltage (V_{ap}) induced by amphiphilic ions. The sign of V_{ap} is that on the amphiphile side. The I_{vib} data are the averaged values of peak-to-peak amplitudes. To ensure the symmetry and stability of the bilayer, the weakest vibrations producing observable currents have been applied. The current unit indicates only the smallest observable I_{vib} level. In at least the 20−200 Hz range, the changes of the relative I_{vib} amplitudes with V_{ap} are the same. When present CQ^+ (protonated chloroquine) is 2 mM at pH 7.0 and AQS^- is 0.3 mM. Triangle symbols and symmetric V-shape curves show the amphiphile-free systems. (A) no $TCPB^-$; (B) 4 μM TCPB. Note the 10-fold larger vertical scale.

bilayer. Insertion binding of AQS^- into the lipid bilayer has been known from the anomalously large apparent second-order rate constant of electron transfer from an excited lipophilic porphyrin (10^{11} M^{-1} s^{-1}).[12] The presence of AQS^- at a single interface of the bilayer reduces the amplitude of I_{vib} by 20−30%. A further 5−10% decrease is caused if the same amount of AQS^- is added to the other interface. Similar reduction of I_{vib} occurs when other aromatic amphiphilic ions are bound both asymmetrically and symmetrically to the bilayer. The amplitude of I_{vib} is much more sensitive to binding of $TCPB^-$ anions, but the effect is opposite. In the presence of AQS^- at one or both interfaces, the symmetric addition of $TCPB^-$ increases I_{vib} more than 10-fold. $TCPB^-$ increases I_{vib} by ∼5-fold in the absence of AQS^-. This nonlinear effect indicates that there are interactions between the $TCPB^-$ and AQS^- anions at the interface. These results show that the presence of $TCPB^-$ greatly enhances the flexoelectric effect of the bilayer and changes its sensitivity to interfacial binding of amphiphilic ions. No other anions or cations have been found to exhibit comparable amplification effects besides anionic structural analogues of $TCPB^-$.

For a symmetric bilayer with or without bound lipophilic ions, an applied dc voltage (V_{ap}) of any sign increases the amplitude of I_{vib} (Figure 3). The presence of bound ions alters only the increasing rate of I_{vib} with the magnitude of V_{ap}. Figure 3 shows the novel $I_{vib}-V_{ap}$ dependence induced by binding of amphiphilic ions at a single interface. When V_{ap} has a negative sign on the AQS^- side, it causes a larger increase of I_{vib} than in the AQS^- free system over all the voltage range. However, V_{ap} with a positive sign reduces I_{vib} to a minimum amplitude at 35 mV. Further increase of V_{ap} increases I_{vib} to the current level of the AQS^- free system. This $I_{vib}-V_{ap}$ dependence is strikingly amplified by the presence of $TCPB^-$ in the bilayer (Figure 3B). Note that the I_{vib} amplitudes at V_{min} (+35 mV) in both the absence and presence of $TCPB^-$ are smaller than those of the corresponding AQS^- free system at 0 mV.

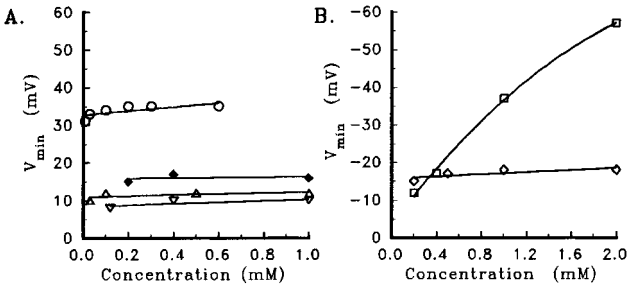

Figure 4. Dependence of V_{min} on concentrations and structures of amphiphilic ions. The data are at the measurable concentrations. (A) Anions, circle, AQS^-; diamond, indole-3-acetate; triangle up, naphthyl-2-acetate; triangle down, naphthyl-2-sulfonate. (B) Cations, diamond, *N*-methyltryptammonium, pH 5.5; square, CQ^+, pH 7.0.

This $I_{vib}-V_{ap}$ dependence does not occur with $AQDS^{2-}$, which has a sulfonate on the lipophilic end of AQS^-. It occurs with other amphiphilic anions, such as indole-3-acetate, naphthyl-2-acetate and naphthyl-2-sulfonate, and with the cations *N*-methyltryptammonium and protonated chloroquine (CQ^{+}). The sign of V_{min} is reversed for cations (Figure 3). $TCPB^-$ (or $TFPB^-$) can similarly produce many-fold amplification of I_{vib} depending on the amphiphile. For octanoate and hexylamonium ions with flexible chain structure, the novel $I_{vib}-V_{ap}$ dependence is observable only in the presence of the borates. Except for CQ^+ cation,[13] V_{min} of all the other ions with a highly hydrophilic charge is basically constant over their measurable concentration ranges (Figure 4), but the relative amplitude of I_{vib} changes with their concentrations. I_{vib} at a V_{ap} with a sign pushing amphiphilic ions deeper into the bilayer increases, but the minimum I_{vib} at V_{min} decreases with increased amphiphile concentration. Earlier study has shown that binding of AQS^- to the bilayer is not saturated in the <0.3 mM range.[12] Thus V_{min} of these ions is independent of the interfacial bound density and determined only by their structures. *N*-methyltryptammonium has the same V_{min} value (∼17 mV) as indole-3-acetate, although of reverse sign. These two amphiphilic ions are of opposite charge but have the same lipophilic end group (an indole). This shows that the magnitude of V_{min} represents the lipophilic binding ability of the inserted structure.

Discussion

The reduction of I_{vib} on asymmetric binding of amphiphilic ions cannot arise from their influence on the electrochemical potentials across the bilayer, since this reduction does not disappear on symmetric binding of the ions. Binding of lipophilic ions, such as tetraphenylborate anion and tetraphenylphosphonium cation, to the bilayer always increases the amplitude of $I_{vib.}$ This suggests that I_{vib} is unlikely to have been reduced by the local fields caused by bound amphiphilic ions. The increased charge density in the electrostatic double layer favors the curvature of the lipid bilayer.[7,8] The amplitude of I_{vib} is usually increased by electrical and mechanical polarizations of the bilayer.[3] The interfacial bound charges themselves cannot directly reduce the flexibility or the polarizibility of the bilayer. Thus amphiphilic ions reduce the flexoelectricity of lipid bilayers most likely by their structural effects on the flexibility of membranes, not by their electrostatic effects. The novel $I_{vib}-V_{ap}$ dependence induced by amphiphilic ions cannot be explained by any effect of V_{ap} on the asymmetric electrochemical potentials of the bilayer. Although the potential caused by the bound amphiphilic ions may be neutralized by the voltage-driven inward movement of aqueous counterions, such an effect may decrease I_{vib} only to the amplitude of the AQS^- free system, not below as is observed.

The above discussion shows that V_{ap} must affect the flexoelectricity of the bilayer by manipulating the amphiphile. The increase of I_{vib} caused by V_{ap} with a sign pushing amphiphilic ions deeper into the bilayer may arise from an enlarged vibration-induced curvature. The insertion of charged groups may make the bilayer more flexible by weakening the hydrophobic attraction of lipid chains, which determines the surface tension of the bilayer.[14] At 0 mV, orientations of amphiphilic ions are limited to a small angular range by the lipophilic and hydrophilic interactions at their two ends. Bending of the bilayer needs to overcome the extra energy required for altering orientations of the rigid amphiphilic ions. This orienting effect may cause some increase of the surface tension by affecting movements of the lipids. Thus the reduced flexibility of the membrane can explain the decrease of I_{vib} on binding of amphiphilic ions at 0 mV. It has been demonstrated that an applied electric field of 10^6 V cm^{-1} can turn a bound molecule with a dipole moment of 14 D by an angle of $\sim 60°$ at a solid−liquid interface.[15] The interfacial electric fields caused by a voltage across the lipid bilayer can be more than 10-fold stronger than those calculated from the voltage value and the bilayer thickness.[16] A 10 mV voltage across the bilayer can cause a interfacial electric field of $>10^5$ V cm^{-1}. In the $0-V_{min}$ range, where I_{vib} is reduced by increased V_{ap}, the applied electric field most likely reduces I_{vib} by further orienting the amphiphile and thus limiting movements of the lipids. When the electrostatic energy of the charge in the outward driving field is less than the binding energy of the amphiphile to the bilayer, its lipophilic end remains partially bound to the lipid molecules. The increased magnitude of V_{ap} may increase the orientation of the amphiphilic structure at the interface. The orientation of enough bound amphiphilic ions may further order the lipids and increase the surface tension of the bilayer. Such an effect on structure is supported by the larger $I_{vib}-V_{ap}$ dependence of the amphiphilic ions with rigid polycyclic aromatic structures than those with only flexible chains. When $V_{ap} > V_{min}$, the increase of I_{vib} to that of the amphiphile-free system (Figure 3) suggests that the lipophilic binding energy is overcome by the increased interfacial field. Once the lipophilic end of the amphiphile becomes relatively free, the ordering effect of the amphiphile on the lipids diminishes. Thus the vibration-induced curvature and I_{vib} of the membrane are increased.

The enhancing effect of $TCPB^-$ and $TFPB^-$ on the amplitude of I_{vib} functions mainly by enlarging the polarizibility of lipid bilayers. Their presence increases the trans-membrane capacitance by $\sim$10-fold at <1 kHz.[10] Amphiphilic ions have only a small influence ($<10\%$) on the capacitance whether the borates are present or not. In the presence of the borates, the addition of AQS^- at 0 mV causes a more than 2-fold increase of I_{vib} (Figure 2), not a decrease as in their absence. This nonlinear effect indicates that other causes, besides changes of the surface tension, must be considered. The dynamic interactions of the borate anions and the interfacial bound ions may be the cause of the further increase of I_{vib}. $TCPB^-$ and $TFPB^-$ anions are more mobile in an interfacial region of the bilayer than tetraphenylborate (TPB^-) and other hydrophobic ions.[10] They may oscillate in the polar regions or across the interfaces during vibration of lipid bilayers and thus increase the electric polarizibility. The presence of borates may increase the curvature by weakening the hydrophobic attraction of lipid chains and by causing space charge.[17] The small increase of I_{vib} caused by TPB^-, which has a hydrogen in place of the halogen on each phenyl ring, shows that these two effects are not the main cause for the large amplification caused by $TCPB^-$ and $TFPB^-$. Compared to the latter, TPB^- has about the same bound density, but much lower mobility, and has only a small effect on the capacitance in the >1 Hz frequency range.[10] Thus the increased electric polarizibility in the polar or interfacial regions caused by $TCPB^-$ and $TFPB^-$ should make the most contribution to the enhanced flexoelectricity.

The value of V_{min} offers a measurable quantity to represent lipophilic binding energy of structures in bilayers. No report on methods that can directly measure this energy has been seen up to now. Structural lipophilicity has often been described quantitatively by using its hydrophobicity,[18] because of lack of data for the former. In many cases, however, the latter cannot actually reflect the former. Indole-3-acetate has a larger V_{min} (15 mV) than naphthyl-2-acetate (10 mV) and phenylacetate (≤ 5 mV), but the indole group has been commonly reported to be less hydrophobic than phenyl and naphthyl groups.[18] This suggests that dipole−dipole interactions in the polar region of lipid bilayers may greatly affect the molecular insertion binding. A deeper and quantitative study of flexoelectricity might lead to knowledge of lipophilic binding energies, configurations, and surface tensions of lipid bilayers.

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(13) The V_{min} of CQ^+ is significantly affected by its concentration (Figure 4B). Its binding ($V_{ap} = 0$ mV) at <1 mM decreases I_{vib} but at ≥ 2 mM increases $I_{vib.}$ These changes may arise from its distributive binding sites to the bilayer with increased concentration. As the positive charge in CQ^+ is surrounded by two ethyl groups and a longer hydrocarbon chain, its charged part can locate deeper into the bilayer at high concentrations. CQ^+ can slowly cross the membrane (Poole, B.; Ohkuma, S. *J. Cell Biol.* **1981**, *90*, 665−669).

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