## 4B08   pH at the Lipid/Water Interfaces Revealed by Heterodyne-Detected Electronic Sum Frequency Generation

(Mol. Spectrosc. Lab., RIKEN) <u>KUNDU, Achintya</u>; YAMAGUCHI, Shoichi; TAHARA, Tahei

The biological cell membrane is a complex mixture of lipids, proteins, carbohydrates, and cholesterols. The pH and polarity at the cell membrane are very important for many biological processes, such as proton transfer, ion transfer, etc. Despite their importance, however there is no systematic study of the pH and polarity at the cell membrane. Because the complexity of the real cell membrane makes it difficult to probe, we use a lipid monolayer on a water surface (air/lipid/water interface) as a model of a cell membrane. We report that heterodyne-detected electronic sum frequency generation (HD-ESFG) with a surface-active pH indicator dye allows us to estimate the pH and polarity at the model cell membrane.
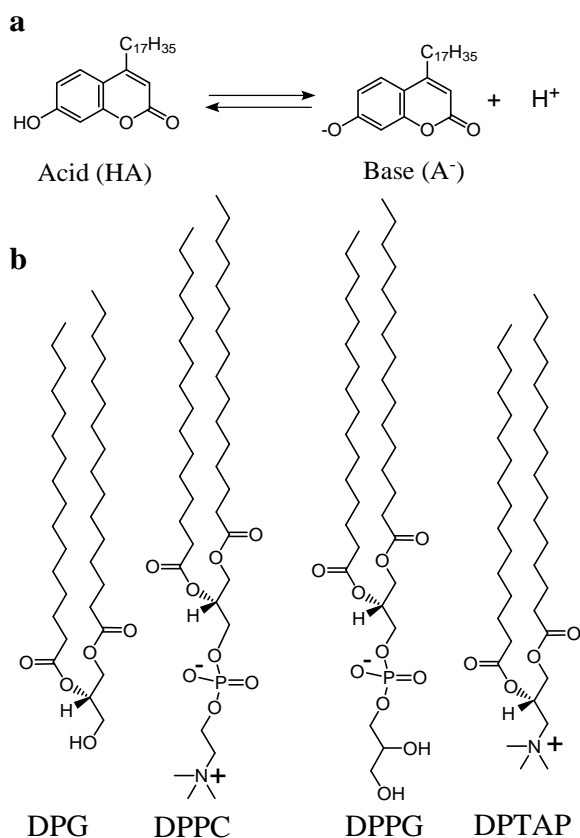
Figure 1. **a,** Acid-base equilibrium of the surface-active pH indicator, HHC. **b,** Chemical structure of the four model lipids: DPG (nonionic), DPPC (zwitterionic), DPPG (anionic) and DPTAP (cationic).
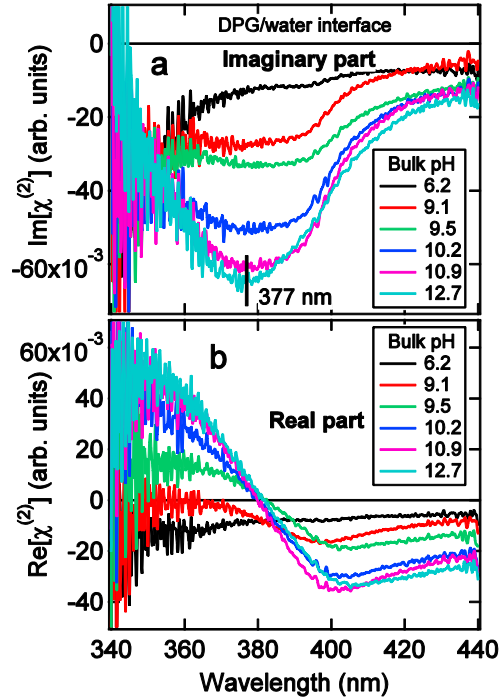
The most reliable method of determining bulk pH is UV-visible spectrometry with a pH indicator dye. It is also possible to apply spectrometry to the lipid/water interface by using HD-ESFG, which can provide the second-order nonlinear optical susceptibility ($\chi^{(2)}$) of the interfacial molecules. Using this technique, we systematically determine the pH at lipid/water interfaces. Figure 1a shows the equilibrium the acid (HA) and base (A$^-$) of a pH indicator, 4-heptadecyl-7-hydroxycoumarin (HHC). Studies were performed at the water surface with each of four model lipids, shown in Figure 1b: 1,2-dipalmitoyl-*sn*-glycerol (DPG, nonionic), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, zwitterionic), 1,2-dipalmitoyl-*sn*- glycero-3-phospho -(1'-*rac*-glycerol) (DPPG, anionic), 1,2-dipalmitoyl-3-(trimethyl-ammonium) propane (DPTAP, cationic).

Figures 2a and 2b show the surface-selective $\mathrm{Im}[\chi^{(2)}]$ and $\mathrm{Re}[\chi^{(2)}]$ spectra of the indicator at the DPG/water interface, with the bulk pH regulated by sodium hydroxide. By analyzing the bulk-pH dependence of the $\chi^{(2)}$ spectra, the bulk pH at which [HA] = [A$^-$] is determined as 9.6. Generally, the pH is equal to the pK_a (negative logarithm of the acid dissociation constant) when [HA] = [A$^-$]. However, this bulk pH at which [HA] = [A$^-$] is not the pK_a of the indicator at the DPG/water interface, because the pH is measured in the bulk. If we analyze the $\chi^{(2)}$ spectra against the interfacial pH, we can obtain the pK_a. Assuming interfacial pH = bulk pH + Δ, pK_a is given as 9.6 + Δ.

The pK_a is equal to the standard reaction Gibbs energy of HA$\rightarrow$ H$^+$ + A$^-$ divided by $RT\ln10$. The standard reaction Gibbs energy is given as a function of the effective relative dielectric constant of the surrounding medium which can be estimated by the

Figure 2. a, b, Surface-selective electronic spectra of the pH indicator at the nonionic lipid (DPG)/water interface. The imaginary and real parts of $\chi^{(2)}$ at different pH are shown in **a** and **b**, respectively.

solvatochromism of A$^-$. The peak wavelength of the $\mathrm{Im}[\chi^{(2)}]$ spectrum of A$^-$ in Figure 2a allows us to estimate the effective relative dielectric constant of the DPG/water interface as 12. From this effective relative dielectric constant, the pK_a of HHC at the DPG/water interface is evaluated as 11.6. Because this pK_a should be equal to 9.6 + Δ, Δ is obtained as +2.0. In the same manner, the values of Δ for the other lipid/water interfaces are obtained as shown in Table 1. This work clearly shows that the interfacial pH is substantially deviated from the bulk pH and that is changes largely depending on the head group of the lipids.

Table 1. Peak position of the base (A$^-$), effective relative dielectric constant, and pH difference Δ.

Interface	λ_{peak} / nm	Effective relative dielectric constant	Δ Interfacial pH - bulk pH
DPG/water	377	12	+2.0
DPPC/water	375	22	-1.1
DPPG/water	365	41	-2.6
DPTAP/water	376	20	+4.4