Supporting Information

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SI Text

Surface Potential Created upon Binding of +8 Charge TAT Peptides to 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphocholine Liposomes and Its Relation to $E_{2\omega}$

Starting with the Poisson–Boltmann equation (1),

$$\frac{d^2\Phi}{dz^2} = -\frac{\rho(z)}{\varepsilon}, \qquad \textbf{[S1]}$$

we consider a system where C is the concentration of bound TAT, C_0 is the total concentration of TAT, and $C_0 - C$ is the concentration of TAT in the bulk solution. The counter ions in the solution are OH^-, at a concentration $C_{OH^-} = 8C_0$. The Boltzmann distribution expresses the population of ions at a charged surface,

$$\begin{aligned} C_+(z) &= (C_0 - C)e^{-(8e\Phi(z)/kT)} \\ C_-(z) &= C_{OH^-}e^{e\Phi(z)/kT}. \end{aligned} \qquad \textbf{[S2]}$$

$C_+(z)$ and $C_-(z)$ are the concentrations of the cations and of the anions at position z. The charge density ρ at position z is thus given by

$$\rho(z) = 8e(C_0 - C)e^{-(8e\Phi(z)/kT)} - 8eC_0e^{e\Phi(z)/kT}. \qquad \textbf{[S3]}$$

Substituting Eq. **S3** into [**S1**] yields

$$\frac{d^2\Phi}{dz^2} = -\frac{8e(C_0 - C)}{\varepsilon}e^{-(8e\Phi/kT)} + \frac{8eC_0}{\varepsilon}e^{e\Phi/kT}. \qquad \textbf{[S4]}$$

Multiplying by $2(d\Phi/dz)$ yields

$$\begin{aligned} 2\frac{d\Phi}{dz}\frac{d^2\Phi}{dz^2} &= \left[-\frac{8e(C_0 - C)}{\varepsilon}e^{-(8e\Phi/kT)} + \frac{8eC_0}{\varepsilon}e^{e\Phi/kT}\right]2\frac{d\Phi}{dz} \\ \frac{d}{dz}\left(\frac{d\Phi}{dz}\right)^2 &= \left[-\frac{8e(C_0 - C)}{\varepsilon}e^{-(8e\Phi/kT)} + \frac{8eC_0}{\varepsilon}e^{e\Phi/kT}\right]2\frac{d\Phi}{dz}. \end{aligned} \qquad \textbf{[S5]}$$

Integrating from 0 to ∞, the left side of Eq. **S5** from 0 to ∞ results in

$$\int_0^\infty \frac{d}{dz}\left(\frac{d\Phi}{dz}\right)^2 dz = \left(\left(\frac{d\Phi}{dz}\right)^2\right)_\infty - \left(\left(\frac{d\Phi}{dz}\right)^2\right)_0 = -\left(\left(\frac{d\Phi}{dz}\right)^2\right)_0. \qquad \textbf{[S6]}$$

The right side in Eq. **S5** becomes

$$\begin{aligned} &\int_0^\infty \left[-\frac{8e(C_0 - C)}{\varepsilon}e^{-(8e\Phi/kT)}2\frac{d\Phi}{dz} + \frac{8eC_0}{\varepsilon}e^{e\Phi/kT}\right]2\frac{d\Phi}{dz}dz \\ &= -\frac{16e(C_0 - C)}{\varepsilon}\left(\frac{-kT}{8e}\right)\left(1 - e^{-(8e\Phi(0)/kT)}\right) \\ &\quad + \frac{16eC_0}{\varepsilon}\left(\frac{kT}{e}\right)\left(1 - e^{-(e\Phi(0)/kT)}\right). \end{aligned} \qquad \textbf{[S7]}$$

We then obtain

$$\sigma = -\int_0^\infty \rho dz = \int_0^\infty \varepsilon\frac{d^2\Phi}{dz^2}dz = -\varepsilon\left(\frac{d\Phi}{dz}\right)_{z=0}. \qquad \textbf{[S8]}$$

Combining Eqs. **S6–S8**, we get

$$\frac{\sigma^2}{\varepsilon^2} = \frac{16e(C_0 - C)}{\varepsilon}\frac{kT}{8e}\left(e^{-(8e\Phi/kT)} - 1\right) - \frac{16eC_0}{\varepsilon}\frac{kT}{e}\left(1 - e^{-(e\Phi/kT)}\right). \qquad \textbf{[S9]}$$

For $\Phi \geq 5$ mV, $e^{-(8e\Phi/kT)}$ is at least a factor of 5 smaller than 1 and is neglected. This yields Eq. **S10**,

$$\frac{\sigma^2}{\varepsilon^2} = -\frac{16e(C_0 - C)}{\varepsilon}\frac{kT}{8e} - \frac{16eC_0}{\varepsilon}\frac{kT}{e}\left(1 - e^{-(e\Phi/kT)}\right), \qquad \textbf{[S10]}$$

which yields

$$\Phi = \frac{kT}{e}\ln\left(\frac{\sigma^2}{16\varepsilon C_0 kT} + \frac{C_0 - C}{8C_0} + 1\right). \qquad \textbf{[S11]}$$

When $C << C_0$, we obtain

$$\Phi = \frac{kT}{e}\ln\left(\frac{\sigma^2}{16\varepsilon C_0 kT} + \frac{9}{8}\right). \qquad \textbf{[S12]}$$

We assume a modified Langmuir binding for TAT onto POPC liposomes. The surface charge density σ is given by (2–4)

$$\sigma = \frac{8eN}{4\pi R^2}, \qquad \textbf{[S13]}$$

where N is the number of TATs bound per unit volume.

The reaction of TAT with empty sites (ES) yields filled sites (FS),

$$\underset{C_0 - C}{\mathrm{TAT}} + \underset{C_{\max} - C}{\mathrm{ES}} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{C}{\mathrm{FS}}.$$

C_{max} is the maximum bound concentration and C is the concentration of bound TAT at equilibrium, where $C = d \times N$, $C_{max} = d \times N_{max}$, and d is the liposome number per unit volume.

The reaction kinetics are described by

$$\frac{dC}{dt} = k_1\frac{C_0 - C}{55.5}(C_{\max} - C) - k_{-1}C. \qquad \textbf{[S14]}$$

At equilibrium,

$$\frac{dC}{dt} = 0,$$

which leads to

$$C = \frac{(C_{\max} + C_0 + 55.5K_d) - \sqrt{(C_{\max} + C_0 + 55.5K_d)^2 - 4C_{\max}C_0}}{2}$$

$$N=\frac{(N_{max}+C_0/d+55.5K_d/d)-\sqrt{(N_{max}+C_0/d+55.5K_d/d)^2-4N_{max}C_0/d}}{2}. \quad \textbf{[S15]}$$

By substituting Eq. **S13** into Eq. **S12**, we obtain the following expression for the surface potential,

$$\Phi=\frac{kT}{e}\ln\left(\frac{\sigma^2}{16\varepsilon C_0 kT}+\frac{9}{8}\right)=\frac{kT}{e}\ln\left(\frac{(8eN/4\pi R^2)^2}{16\varepsilon C_0 kT}+\frac{9}{8}\right). \quad \textbf{[S16]}$$

The total second harmonic electric field, $E_{2\omega}$, originating from a charged interface in contact with water can be expressed as

$$E_{2\omega}\propto\sum_i\chi^{(2)}_{c,i}E_\omega E_\omega+\sum_j\chi^{(2)}_{\mathrm{inc},j}E_\omega E_\omega+\chi^{(3)}_{\mathrm{H_2O}}E_\omega E_\omega\Phi, \quad \textbf{[S17]}$$

where $\chi^{(2)}_{c,i}$ represents the coherent contribution of the second-order susceptibility of the species i at the interface, $\chi^{(2)}_{\mathrm{inc},j}$ represents the incoherent contribution of the second-order susceptibility of the species j present in solution, referred to as the hyper-Rayleigh scattering, $\chi^{(3)}_{\mathrm{H_2O}}$ is the third-order susceptibility of water molecules, and Φ is the surface potential. The second-order susceptibility, $\chi^{(2)}_{c,i}$, can be written as the product of the number of molecules, N, at the surface and $\alpha_i^{(2)}$ is the hyperpolarizability of surface species i, yielding $\chi^{(2)}_{c,i}=N\langle\alpha_i^{(2)}\rangle$ (5). The bracket, $\langle\rangle$, indicates an orientational average over the interfacial molecules.

By substituting Eq. **S16** into Eq. **S17**, we obtain the following expression for $E_{2\omega}$, where A and B are defined in the text,

$$E_{2\omega}\propto A+B\ln\left(\frac{(8eN/4\pi R^2)^2}{16\varepsilon C_0 kT}+\frac{9}{8}\right). \quad \textbf{[S18]}$$

Surface Potential of TAT Binding to the 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phospho-1′-*rac*-Glycerol Liposomes and Its Relation to $\mathbf{E_{2\omega}}$. The concentration of Na^+ is determined by the number of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-1′-*rac*-glycerol (POPG) lipids; i.e., one Na^+ is released by each POPG lipid that composes the POPG liposome. The spatial distribution of ions is given by

$$C_+(z)=(C_0-C)e^{-(8e\Phi(z)/kT)}+C_{\mathrm{Na^+}}e^{-(e\Phi(z)/kT)}$$
$$C_-(z)=C_{\mathrm{OH^-}}e^{e\Phi(z)/kT}. \quad \textbf{[S19]}$$

Thus,

$$\rho(z)=8e(C_0-C)e^{-(8e\Phi(z)/kT)}+C_{\mathrm{Na^+}}e^{-(e\Phi(z)/kT)}-8eC_0e^{e\Phi(z)/kT}. \quad \textbf{[S20]}$$

Substituting Eq. **S20** into Eq. **S1** yields

$$\frac{d^2\Phi}{dz^2}=-\frac{8e(C_0-C)}{\varepsilon}e^{-(8e\Phi/kT)}-\frac{eC_{\mathrm{Na^+}}}{\varepsilon}e^{-(e\Phi(z)/kT)}+\frac{8eC_0}{\varepsilon}e^{e\Phi/kT}. \quad \textbf{[S21]}$$

Multiplying by $2(d\Phi/dz)$ yields

$$2\frac{d\Phi}{dz}\frac{d^2\Phi}{dz^2}=\left[-\frac{8e(C_0-C)}{\varepsilon}e^{-(8e\Phi/kT)}-\frac{eC_{\mathrm{Na^+}}}{\varepsilon}e^{-(e\Phi(z)/kT)}+\frac{8eC_0}{\varepsilon}e^{e\Phi/kT}\right]2\frac{d\Phi}{dz}$$
$$\frac{d}{dz}\left(\frac{d\Phi}{dz}\right)^2=\left[-\frac{8e(C_0-C)}{\varepsilon}e^{-(8e\Phi/kT)}-\frac{eC_{\mathrm{Na^+}}}{\varepsilon}e^{-(e\Phi(z)/kT)}+\frac{8eC_0}{\varepsilon}e^{e\Phi/kT}\right]2\frac{d\Phi}{dz}. \quad \textbf{[S22]}$$

Integrating from 0 to ∞, the left side of Eq. **S22** becomes

$$\int_0^\infty\frac{d}{dz}\left(\frac{d\Phi}{dz}\right)^2dz=\left(\left(\frac{d\Phi}{dz}\right)^2\right)_\infty-\left(\left(\frac{d\Phi}{dz}\right)^2\right)_0=-\left(\left(\frac{d\Phi}{dz}\right)^2\right)_0. \quad \textbf{[S23]}$$

The surface charge density is given by

$$\sigma=-\int_0^\infty\rho dz=\int_0^\infty\varepsilon\frac{d^2\Phi}{dz^2}dz=-\varepsilon\left(\frac{d\Phi}{dz}\right)_{z=0}. \quad \textbf{[S24]}$$

Integrating from 0 to ∞, the right side of Eq. **S22** yields

$$2\int_0^\infty\left[-\frac{8e(C_0-C)}{\varepsilon}e^{-(8e\Phi/kT)}-\frac{eC_{\mathrm{Na^+}}}{\varepsilon}e^{-(e\Phi(z)/kT)}+\frac{8eC_0}{\varepsilon}e^{e\Phi/kT}\right]\frac{d\Phi}{dz}dz$$
$$=-\frac{2}{\varepsilon}\left[-(C_0-C)kT\left(1-e^{-(8e\Phi(0)/kT)}\right)-C_{\mathrm{Na^+}}kT\left(1-e^{-(e\Phi(0)/kT)}\right)-8C_0kT\left(1-e^{-(e\Phi(0)/kT)}\right)\right]. \quad \textbf{[S25]}$$

Combining Eqs. **S24** and **S25**, we obtain

$$-\frac{\sigma^2}{\varepsilon^2}=-\frac{2kT}{\varepsilon}\left[-(C_0-C)\left(1-e^{-(8e\Phi(0)/kT)}\right)-C_{\mathrm{Na^+}}\left(1-e^{-(e\Phi(0)/kT)}\right)-8C_0\left(1-e^{-(e\Phi(0)/kT)}\right)\right]$$
$$\frac{\sigma^2}{2kT\varepsilon}=-9C_0-C_{\mathrm{Na^+}}+C+(C_0-C)e^{-(8e\Phi(0)/kT)}+C_{\mathrm{Na^+}}e^{-(e\Phi(0)/kT)}+8C_0e^{-(e\Phi(0)/kT)}. \quad \textbf{[S26]}$$

For the experiments using POPG,

$$\frac{\sigma^2}{2kT\varepsilon}=-9C_0-C_{\mathrm{Na^+}}+(C_0-C)e^{-(8e\Phi(0)/kT)}.$$

We thereby obtain an expression for the surface potential given by Eq. **S27**:

$$\Phi=-\frac{kT}{8e}\ln\left(\frac{\sigma^2}{2\varepsilon C_0kT}+\frac{C_{\mathrm{Na^+}}}{C_0}+9\right). \quad \textbf{[S27]}$$

We used a modified Langmuir model, Eq. **S15**, for binding of TAT to POPG liposomes. The surface charge density σ is given by

$$\sigma = \sigma_0 - \sigma_{\mathrm{ads}} = \frac{8eN_{\max}}{4\pi R^2} - \frac{8eN'}{4\pi R^2}. \qquad \textbf{[S28]}$$

By substituting Eq. **S28** into Eq. **S27**, we are able to write the expression

$$\Phi = -\frac{kT}{8e}\ln\left(\frac{\sigma^2}{2\varepsilon C_0 kT} + \frac{C_{\mathrm{Na}^+}}{C_0} + 9\right)$$

$$= -\frac{kT}{8e}\ln\left(\frac{\left(8eN_{\max}/4\pi R^2 - 8eN'/4\pi R^2\right)^2}{2\varepsilon C_0 kT} + \frac{C_{\mathrm{Na}^+}}{C_0} + 9\right). \qquad \textbf{[S29]}$$

By substituting Eq. **S29** into Eq. **S17**, we obtain the expression

$$E_{2\omega} \propto A' + B'\ln\left(\frac{\left(8eN_{\max}/4\pi R^2 - 8eN'/4\pi R^2\right)^2}{2\varepsilon C_0 kT} + \frac{C_{\mathrm{Na}^+}}{C_0} + 9\right). \qquad \textbf{[S30]}$$

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