Supplementary Information for

Real-Time Monitoring the Staged Interactions between Cationic Surfactants and a Phospholipid Bilayer Membrane

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Photo-Voltage Transient Experiment

Setup of the system. The key part of the photo-voltage test system is the photoelectrode (i.e., working electrode), which consists of a supported phospholipid bilayer (SPB) deposited on a silicon wafer surface.

The setup of the photo-voltage test system includes three steps, i.e., substrate cleaning before SPB preparation, sample installation, and the subsequent photo-voltage tests.

The silicon wafer was sonicated with acetone and ethanol respectively for 30 min, and washed completely with ultrapure water. It was then boiled in a mixture of sulfuric acid (98%) and hydrogen peroxide (30%) with a ratio of 7:3 for 45 minutes, washed again with ultrapure water, blown dry with nitrogen, and treated in a plasma cleaner for 5 minutes (air; 18 W) to ensure its good hydrophilicity. Then the back and sides of the wafer were coated with a layer of insulating glue (with a blank area of 15 × 10 mm^2 left for SPB deposition) to prevent the photogenerated electrons from escaping from these regions.

The wafer surface was coated with DOPC bilayer following the traditional vesicle fusion method. Briefly, DOPC lipids were dissolved in chloroform at 4.0 mg mL^{-1} for absolute dissolution, and 100 mL solution was added to a glass bottle, blown dry with nitrogen, and dried in vacuum overnight. The dried membrane was rehydrated in ultrapure water (to 0.4 mg mL^{-1}), and sonicated for 45 minutes to form vesicles. Using an extruder, the vesicle dispersion was extruded through a polycarbonate membrane (with 100 nm pores; Avanti Polar Lipids) for 21 times, and small unilamellar vesicles (SUVs) with a size of 110±10 nm (determined by DLS) were obtained.

The newly cleaned silicon wafer was set up into a home-made incubation chamber. 15 μL SUV dispersion was injected to cover the bare wafer surface, and incubated at 37°C for 3 hours. The dispersion was then replaced with ultrapure water using a slow water flow, so as to remove the excessive vesicles. After that, a high-quality SPB was formed.[1, 2]

The wafer (with SPB) was quickly transferred to the reaction cell (containing deionized water) of the photo-voltage system. Note that in the test system, the wafer surface immersed in water was either coated with SPB membrane (i.e., the working electrode, with an area of 15 × 10 mm^2) or insulating glue. The whole setup was placed in a closed electromagnetic shielding box.

The SPB was incubated in deionized water to equilibrate for no less than 10 min. After that, voltage pulses of electrodes under illumination were recorded as baselines for subsequent in-situ tests. At t = 0 min, an amount of high-concentrated surfactant solution was added to the chamber (to a final solution volume of 1.5 mL and a surfactant concentration ranging from 0.5 μM to 10.0 mM, as described in the main text) for surfactant-membrane interaction. A xenon lamp, equipped with a monochromator, is used as the light source. A square-wave modulated illumination, e.g., 100 ms ON/300 ms OFF, or 250 ms ON/500 ms OFF, is applied in the experiments. Therefore, a time resolution of <1 s is acquired.

Analysis of τ. For each photo-voltage pulse, the charge relaxation time constant, τ, is obtained through fitting the descending edge with $y=C_1e^{-\frac{t}{\tau}}+C_2e^{-\frac{t}{\tau'}}+C_3$, in which C_1, C_2, C_3 are constants. Here, τ represents the diffusion of electrons into the solution (i.e., the relaxation time) and τ' represents the diffusion of electrons on the surface of the silicon wafer which is about 50 μs.[3] For clarity, the initial 70% of the descending edge is used for each pulse in this work.

Situation of a bare silicon substrate or a SPB in water

A bare silicon wafer in deionized water was firstly tested as a control. Figure S1(a) shows a representative photo-voltage pulse. The descending edge (marked in red) is well fitted as shown in Figure S1(b), with a τ value of 3.34 ms (τ=2~4 ms in parallel tests) and a fitting degree as high as 0.954. In contrast, for a SPB in deionized water, a τ value of 20~80 ms, which is much larger than that of a bare silicon, is generally obtained (Figure S1c, d). This confirms the formation of a continuous SPB membrane on the silicon surface (for the following SPB-surfactant interactions). For comparation, the τ value of a SPB in water is normalized as 40 ms throughout this paper.

Supplementary images

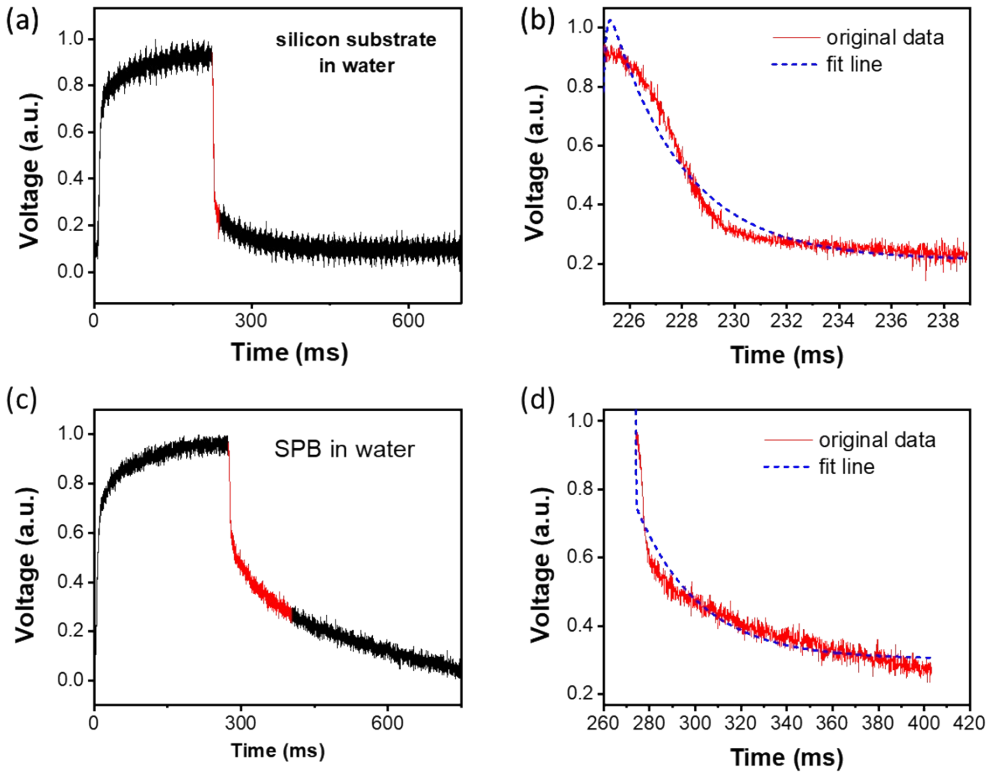

Figure S1. Fitting and acquirement of τ. (a-b) Typical photo-voltage pulse of a bare silicon wafer in water. (c-d) Typical photo-voltage pulse of a SPB in water. (b) and (d) show fittings of the descending edges, with a fitting goodness of 0.96 and 0.89, respectively.

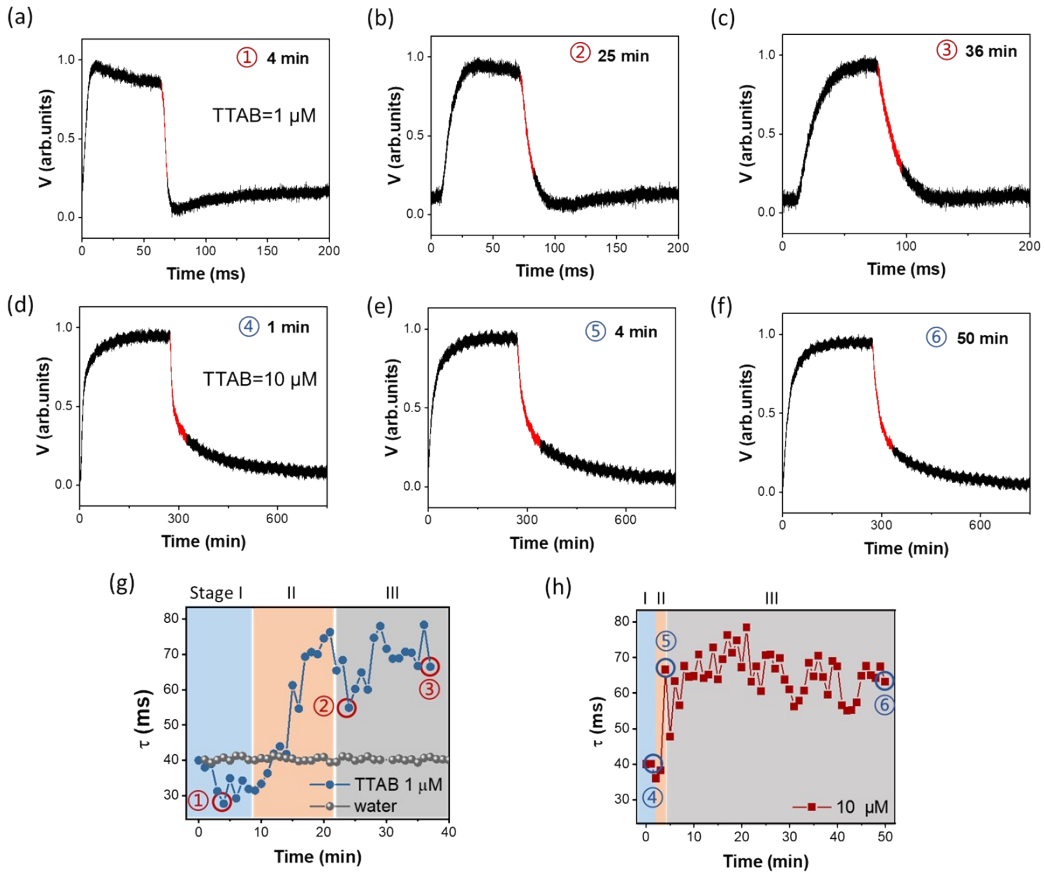

Figure S2. Membrane actions of TTAB at low concentrations (≤10 μM) under photo-voltage transient monitoring. (a-f) Typical voltage pulses captured at different times after TTAB addition. (g, h) Time evolution of τ during the interactions. The TTAB concentration is 1 (a-c, g) or 10 μM (d-f, h). The pulses (①-⑥) are marked correspondingly in the profiles with circles. The red region in (a-f) is the stage used for τ determination. The profiles are representative results.

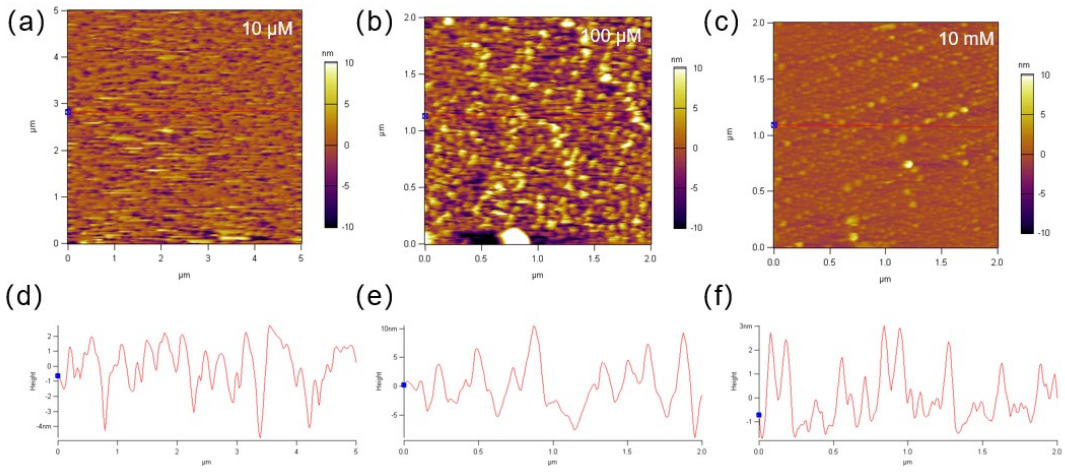

Figure S3. AFM images of SLB after treatment of TTAB at different concentrations,

i.e., 10 μM (a), 100 μM (b) and 10 mM (c). (d-f) height diagrams of the corresponding red lines. The SLBs were incubated with TTAB for 30 min, after which, the peptide solution was replaced gently with deionized water right before AFM observation. The AFM images were taken using an Asylum Research MFP-3D-SA atomic force microscope setup in a tapping mode in the liquid phase.

Corresponding note

At a relatively low concentration of 10 μM (Figure S3-a), surface binding of surfactants was observed on the membrane surface. Moreover, defects (e.g., transmembrane pores with a depth approaching 4 nm) were distinguished. At a higher concentration of 100 μM (Figure S3-b), a much rougher surface was observed. At a high concentration of 10 mM (Figure S3-c), the SLB was completely disrupted, with some molecular aggregates left on the substrate surface.

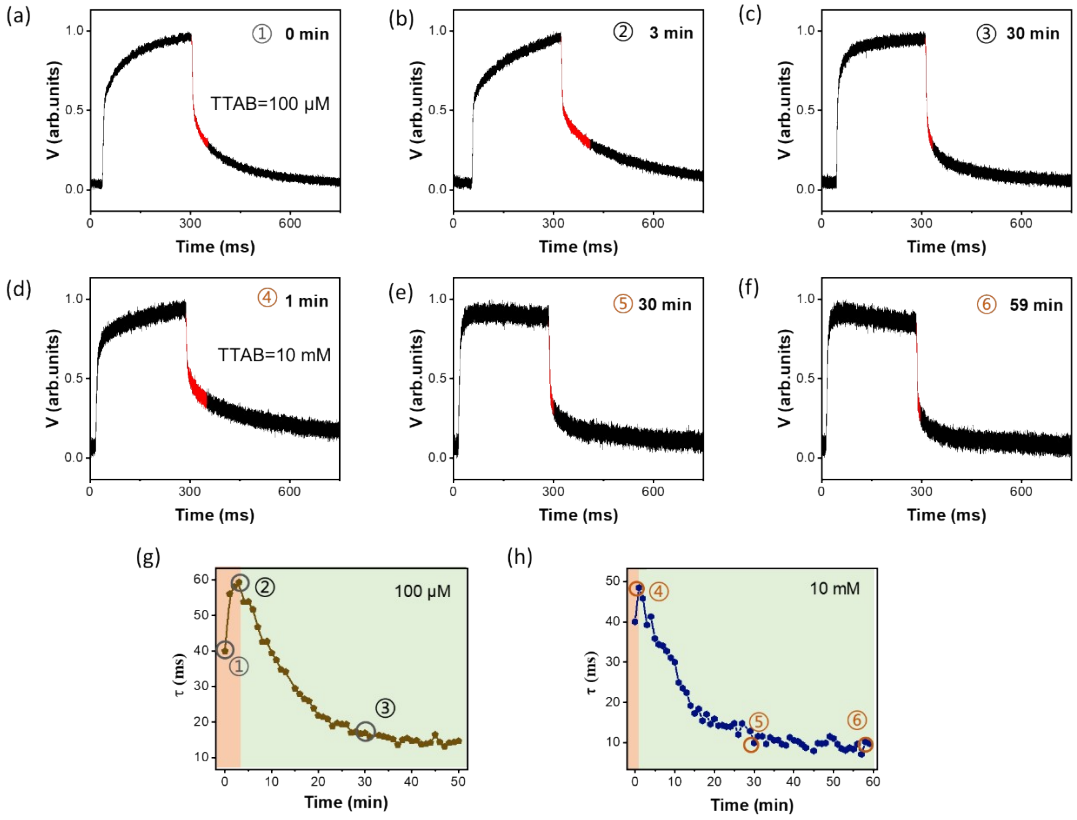

Figure S4. Membrane actions of TTAB at higher concentrations (100 μM~10 mM) under photo-voltage transient monitoring. (a-f) Typical voltage pulses captured at different times after TTAB addition. (g, h) Time evolution of τ during the interactions. The TTAB concentration is 100 μM (a-c, g) or 10 mM (d-f, h). The pulses (①-⑥) are

marked correspondingly in the profiles with circles. The red region in (a-f) is the stage used for τ determination. The profiles are representative results.

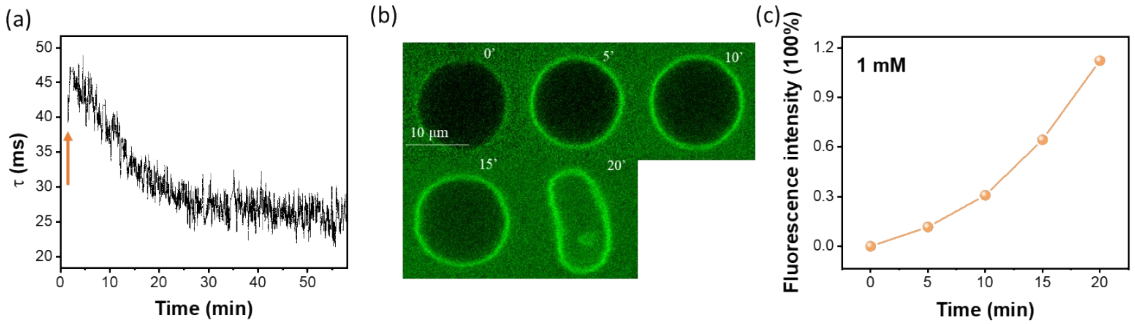

Figure S5. Membrane actions of 1 mM TTAB under photo-voltage and GUV leakage tests. (a) Typical time-evolution of τ upon TTAB addition under photo-voltage test. (b-c) Representative TTAB-induced transmembrane diffusion of calcein in GUV leakage assays, including the fluorescent images (b) and corresponding time evolution of the fluorescence intensity of the interior of the GUV (after normalization, c).

Corresponding note

Upon TTAB actions at 1 mM, the relaxation time constant, τ, increases first and then decreases with time until reaching equilibrium. Such a trend is similar as that with 100 μM or 10 mM. In the GUV leakage assay, transmembrane diffusion of calcein occurs quickly after TTAB addition. Obvious membrane deformation occurs at 15 min and vesicle rupture occurs after 20 min.

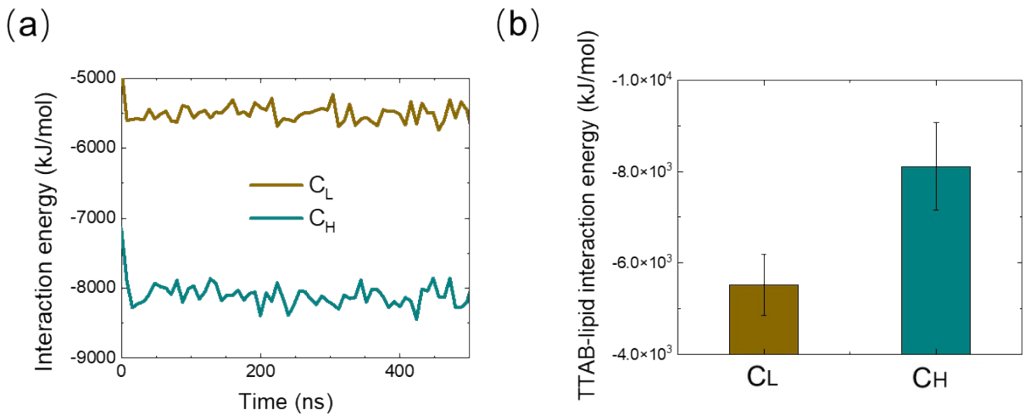

Figure S6. Fluctuations in interaction energy between TTAB and a bilayer membrane with different surfactant concentrations.

Reference

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3 Q. Wang and B. C. Li, Electronic transport characterization of silicon wafers by spatially resolved steady-state photocarrier radiometric imaging, *J. Appl. Phys.*, 2015, **118**, 125705.