Supplementary Materials

SERS Studies on the Dynamic Response of Membrane Interface Lipids to Proteins: Concentration Effect and Redox State Conversion

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1. Experimental Details

1.1. Preparation of Silver Nanoparticles (AgNPs)

Silver nanoparticles were prepared via reduction of $AgNO_3$ by sodium citrate using the modified method of Lee and Meisel [1]. 18 mg of $AgNO_3$ was dissolved in 100 mL of deionized water in a three-necked flask. The solution was heated to near-boiling under magnetic stirring at 600 rpm by the rapid addition of 2 mL of 1 wt% sodium citrate solution. The solution color changed from colorless to light yellow, then to dark yellow, and finally to grayish green. Upon color stabilization, the mixture was cooled to 90 °C and held at 90 °C for 40 min, then allowed to cool naturally to room temperature.

1.2. Preparation of DOPC Vesicles

The DOPC solid was dissolved in chloroform, dried under a nitrogen stream, and then vacuum-dried for 8–12 h. The dried lipid film was subsequently rehydrated with deionized water to prepare an aqueous solution at a concentration of 5 mg/mL. Finally, DOPC vesicles were formed by sonicating the solution for 5 min.

1.3. Preparation of Ag@RCM

To prepare Ag@RCM, a 4 mL suspension of 4% mouse red blood cells was first mixed with 10 volumes of 0.25× PBS to induce osmotic lysis during a 1 h incubation in an ice bath. The lysate was then centrifuged at 12,000 g (4 °C, 30 min) to pellet the membrane fragments, followed by two washes with 1× PBS to remove residual hemoglobin and obtain purified erythrocyte membranes. The membrane precipitate was resuspended in deionized water to a concentration of 2.5 mg/mL and extruded 9 times through a 400 nm polycarbonate membrane to form vesicles. Subsequently, the vesicle suspension was mixed with Ag NPs at a 1:1 volume ratio and sequentially extruded 11 times through 200-nm and 100-nm polycarbonate membranes to obtain uniform Ag@RCM nanoparticles, thereby ensuring efficient encapsulation and enhanced biocompatibility.

1.4. Instruments

Transmission electron microscope (TEM, JEM-2100 F, Tokyo, Japan), ultraviolet-visible spectrophotometer (UV-vis, USB4000, Ocean Optics, Dunedin, FL, USA), dynamic light scattering instrument (DLS, Nano ZS, Malvern Zetasizer, Malvern, UK), Vacuum-type Fourier Transform Infrared Spectrometer (VERTEX 8OV-ATR, BRUKER OPTIK GMBH, Ettlingen, Germany), Raster-type Raman spectrometer (T64000, HORIBA JOBIN YVON, Villeneuve d'Ascq, France).

When preparing the Ag@DOPC sample for TEM image, chromic acid diuranate was used for staining. 1 mL of the prepared Ag@DOPC was centrifuged at 5300 rpm for 7 min to remove the supernatant. We resuspended it in deionized water to prepare a 1.0 mL solution. 5 μL of the solution was dropped onto a copper mesh and allowed

to dry in air. Then, 2% chromic acid diuranate was dropped above for 2 min of staining. The mesh was washed twice with deionized water and dried before taking TEM images.

1.5. Figures

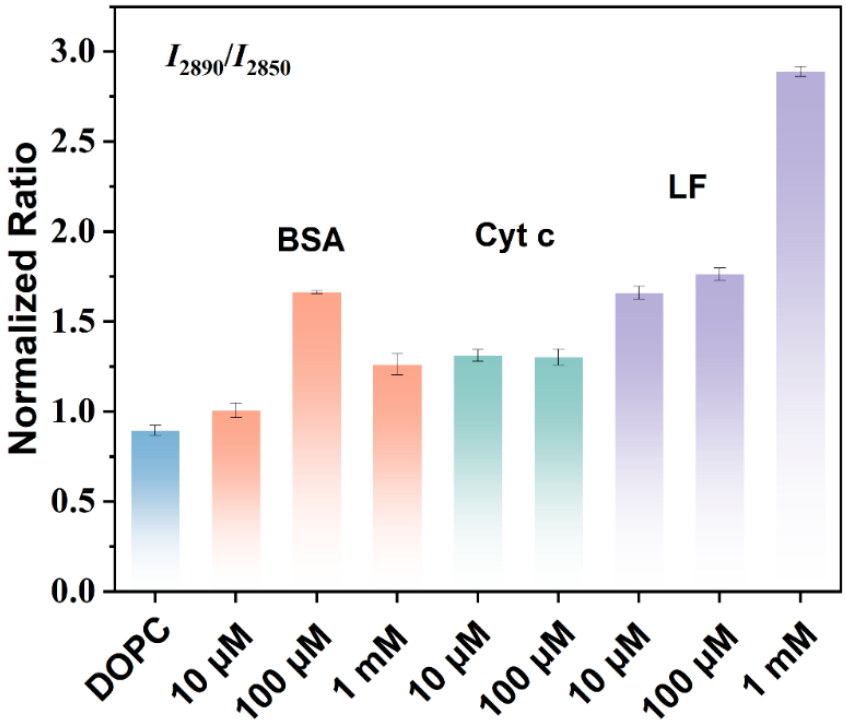

Figure S1. Normalized I_{2890}/I_{2850} ratio of DOPC@Ag treated with BSA, Cyt c, and LF at varying concentrations. Data are normalized to pure DOPC@Ag and presented as mean ± SD (n = 3). The ratio refers to the Raman peak intensity ratio I_{2890}/I_{2850}.

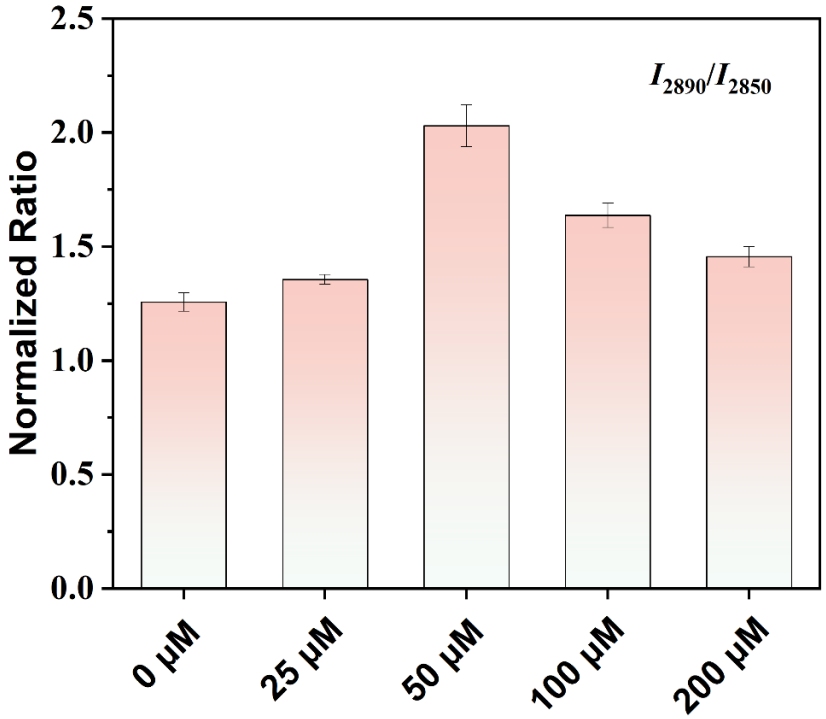

Figure S2. Normalized I_{2890}/I_{2850} lipid ratio of DOPC@Ag during the reduction of adsorbed Cyt c. Data are normalized to pure DOPC@Ag and presented as mean ± SD (n = 3). The ratio refers to the Raman peak intensity ratio of lipid acyl chains at 2890 cm^{-1} and 2850 cm^{-1}.

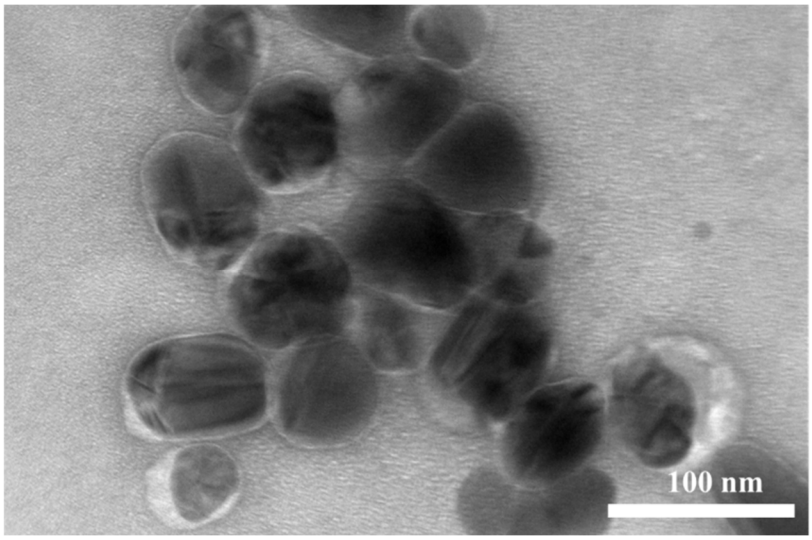

Figure S3. TEM images of RCM@AgNPs.

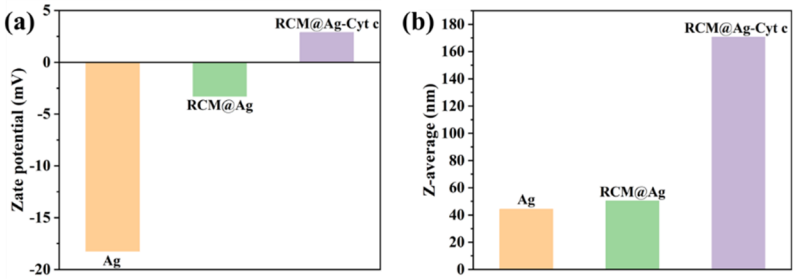

Figure S4. (**a**) Zeta potential of Cyt c assembled RCM@AgNPs; (**b**) Size distribution of Cyt c assembled RCM@AgNPs.

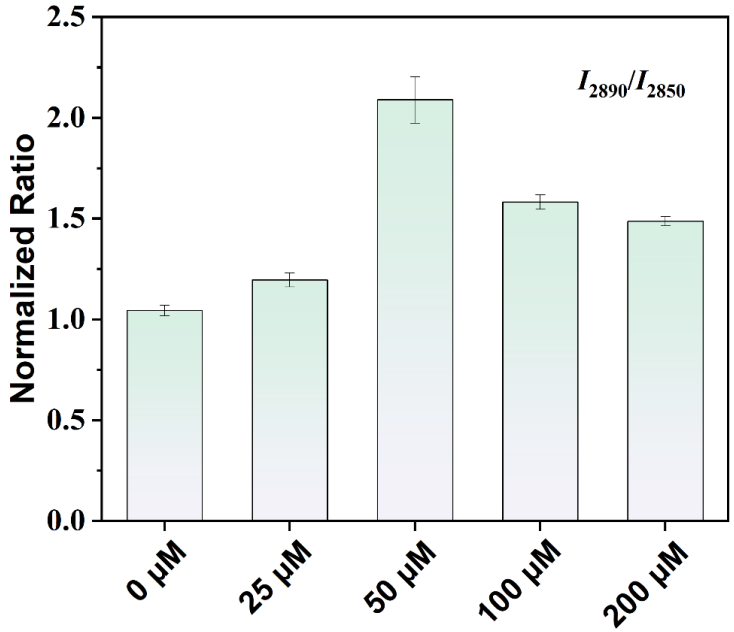

Figure S5. Normalized I_{2890}/I_{2850} lipid ratio of RCM@Ag during the reduction of adsorbed Cyt c. Data are normalized to pure RCM@Ag and presented as mean ± SD (n = 3). The ratio refers to the Raman peak intensity ratio of lipid acyl chains at 2890 cm^{-1} and 2850 cm^{-1}.

Reference

1. Lee, P.C.; Meisel, D. Adsorption and surface-enhanced Raman of dyes on silver and gold sols. *J. Phys. Chem.* **1982**, *86*, 3391–3395.