Electronic Supplementary Information

Phase Transfer-driven Rapid and Complete Ligand Exchange for Molecular Assembly of Phospholipid Bilayer on Aqueous Gold Nanocrystals

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1. Experimental details

Chemicals: Silver nitrate ($AgNO_3$, ≥99.0%), L-ascorbic acid ($C_6H_8O_6$, ≥99.0%), gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, ≥99.9%), sodium borohydride ($NaBH_4$, 98%), Sodium citrate tribasic dihydrate ($HOC(COONa)(CH_2COONa)_2 \cdot 2H_2O$, 99.0%), phosphate buffered saline (PBS, pH 7.4), poly(ethylene glycol) methyl ether thiol (PEG-SH, M_n 6,000), chloroform (CH_3Cl), sodium chloride (NaCl) and were all obtained from Sigma-Aldrich (USA). Hydrogen peroxide (H_2O_2, 30 wt.% in H_2O), Sulfuric acid (H_2SO_4, 95% wt.% in H_2O) and hydrochloric acid (HCl, 37 wt.% in H_2O) and acetone (CH_3COCH_3, ≥99.5%) were all obtained from Samchun Chemicals (Korea). 1,2-dipalmitoyl-sn-glycero-3-phosphothioethanol (DPPTE), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-dioleoyl-sn-glycero-3-phosphoglycerol (DOPG) were all obtained from Avanti Polar Lipids, Inc (USA). Cetyltrimethylammonium bromide (CTAB, ≥99.0%) was obtained from Acros Organics (USA). Ethyl alcohol (CH_3CH_2OH, 99.9%) was obtained from J. T. Baker (USA). Human neuroblastoma cell (SH-SY5Y) was obtained from ATCC (USA). DMEM/F-12 from Welgene (Korea), penicillin from Gibco (USA) and fetal bovine serum (FBS) from Young In Frontier (Korea) were utilized to prepare the media. Cell viability assay kit was acquired from Young In Frontier (Korea) to investigate the cell viability after particle injection. 96 well plate was purchased from Corning Incorporated (USA). Deionized (DI) water with a resistivity of 18.2 MΩ · cm at room temperature was used throughout the experiments.

Preparation of gold nanorods (GNR): Before synthesis, all glassware was treated with piranha solution (H_2SO_4:H_2O_2 = 7:3 *v*/*v*, warning: this solution was highly acidic and a strong oxidant) for 30 min, and then rinsed with copious amounts of DI water. GNRs were synthesized by the seed-mediated growth method with modification.[1] Seed solution was prepared by mixing 10 mL of 0.2 M CTAB and 25 μL of 0.1 M $HAuCl_4$ followed by addition of 0.6 mL of ice-cold 0.01 M $NaBH_4$. Growth solution was prepared by mixing 9.5 mL of 0.1 M CTAB, 85 μL of 0.01 M $AgNO_3$, and 0.5 mL of 0.01 M $HAuCl_4$ followed by addition of 55 μL of 0.1 M ascorbic acid with mild stirring. After 3 h, 36 μL of the seed solution was added to the growth solution and was left without disturbance overnight. The solution was centrifuged twice at 10,000 rpm for 15 min. The supernatant was removed, and the rest of the solution was re-dispersed in DI water.

Preparation of gold nanospheres (GNS): To synthesize 20 nm GNS, 100 mL of 0.1 mg mL^{-1} $HAuCl_4$ solution was poured in round bottom flask and was heated in oil bath at reflux with vigorous stirring for 10 min. After mixing, 3 mL of sodium citrate aqueous solution (1.0 %) were added with stirring continuously for 20 min before cooling down to room temperature. 50 nm GNS were prepared by a standard sodium citrate reduction method, where the GNS with a diameter of 20 nm was used as a seed.[2] Briefly, 100 mL of 0.1 mg mL^{-1} $HAuCl_4$ solution was poured in round bottom flask and was heated in an oil bath at reflux with vigorous stirring for 10 min. After mixing, 4 mL of seeds (20 nm) and 0.4 mL of sodium citrate aqueous solution (1.0 %) were added with stirring continuously for 20 min more before cooling down to room temperature.

Synthesis of phospholipid bilayer-coated GNR and GNS: 2 mL of aqueous gold nanoparticle (GNP, *i.e.,* GNR and GNS) solution with 250 nM PEG-SH and 2 mL chloroform with 1 mM DPPTE were mixed to form two phases. Then 10 μL of HCl was added to the aqueous phase and the mixture was vortexed for 2 min and shaken for 20 min for phase transfer to chloroform. To facilitate precipitation of the nanoparticles, 0.2 mL of ethanol was added to 1mL of GNP solution in chloroform.[3] Then, the mixture was centrifuged for 20 min (GNR: 7,000 rcf, 20 nm GNS: 6,900 rcf, 50 nm GNS: 1,100 rcf) and re-dispersed in 0.2 mL acetone after removing supernatant. The acetone solution containing GNP was then added dropwise to 65 °C 4 wt. % ethanol/water mixture where 1 mM lipid molecules (*i.e.*, DSPC and DOPG) were dissolved. After evaporation of acetone and ethanol by stirring at room temperature for 2 h, the resulting GNR solution was washed 2 times with DI water by centrifugation for 20 min (GNR:7,000 rcf, 20 nm-GNS:6,900 rcf, 50 nm-GNS:1,100 rcf).

Characterization methods: TEM images were taken on a LIBRA 120 electron microscope (Carl Zeiss) operating at an acceleration voltage of 120 kV. The UV-visible absorbance spectra were taken by V-530 UV spectrometer (JASCO). Zeta-potential measurements to observe surface ligands changes and dynamic light scattering for GNP size were determined using a nano partica SZ-100 (HORIBA, Ltd.). To measure Raman spectra of GNR in each process for encapsulation with the phospholipid bilayer, all GNR solutions were centrifuged and dried on a silicon wafer. Micro-Raman system combining a spectrometer SR-303i (Andor Technology), 785 nm laser module IO785SR0100B (IPS), and Olympus BX-53M TRF microscope (Olympus) with an MPlanFL N 20 × (NA = 0.45) objective lens used for analysis and the integration time was 3 s. The concentration of CTAB-GNR and DSPC-GNR for MTT cell viability assay was estimated by using the molar extinction coefficient and absorbance from UV-vis spectra.[4]

Analysis of TEM images for interparticle distance measurement: CTAB-GNR and resulting phospholipid bilayer-coated GNR solutions (10 μL) were dropped onto TEM grid to analyze using TEM. To quantitatively analyze the side-to-side distance between GNR, the average and standard deviation of interparticle distance was obtained by measuring the distance between neighboring GNR (≥ 100 particles) in a TEM image.

Analysis of colloidal stability under physiological conditions: To confirm colloidal stability, CTAB-GNR, 20 nm citrate-GNS, 50 nm citrate-GNS, DSPC-GNR, 20 nm DSPC-GNS and 50 nm DSPC-GNS were added to DI water, 100 mM NaCl, PBS buffer solution (PBS 1× (pH 7.4)) respectively. After a certain period of time (GNR: 1 day, GNS: 1 h), the relative change in the SPR peak for each sample is measured using a V-530 UV spectrometer (JASCO).

Cell viability test based on MTT assay: To verify the nontoxicity of the synthesized particles, cell viability assay kit was introduced. First of all, 100 μL of SH-SY5Y (Concentration: 5×10^5 numbers of cells mL^{-1}) was seeded in the 96 well plates and incubated for 24 h. After incubation, media was extracted and the synthesized particle solution (ca. 5.1×10^{11} particles/ml for CTAB-GNR and ca. 6.6×10^{11} particles/ml for DSPC-GNR) prepared in media (Media:particle solution = 9:1 *v*/*v*) was added in the 96 well plates for 6 h. Then, after washing by PBS, cell viability test solution in cell viability assay kit was added in the well plates for 1 h. As cell viability test solution, water-soluble tetrazolium salt (WST) was used for absorbance detection by living cells. After 1 h from incubation with WST, prepared 96 well plates were investigated by EL800 microplate reader (BioTek Ins.). To

verify the nontoxicity of the synthesized particles, 96 well plates with only SH-SY5Y and with CTAB-GNR were prepared and investigated as control samples for comparison.

2. Supplementary figures

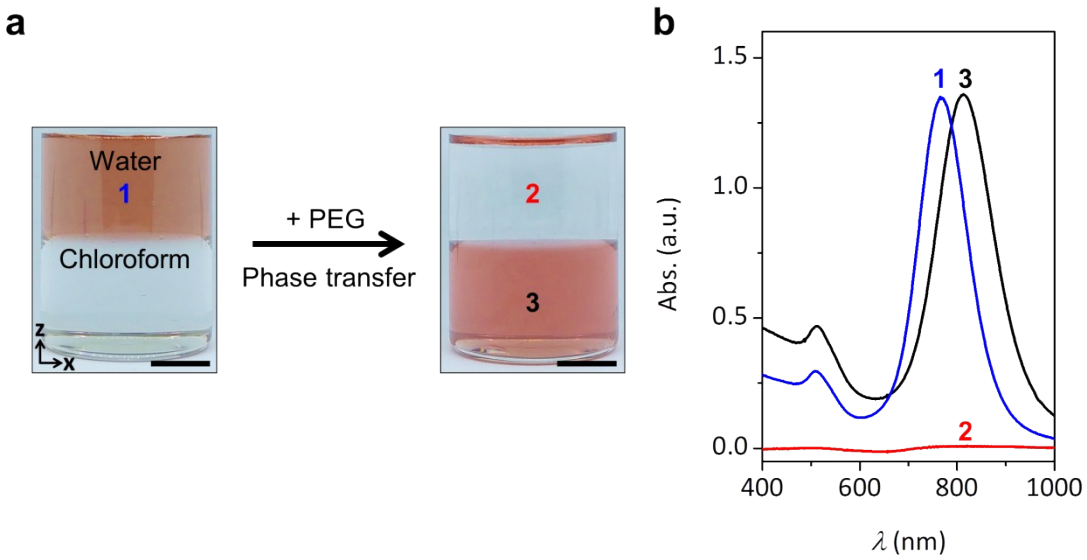

Fig. S1 (a) Real photographs of aqueous and chloroform phase before and after phase transfer. Scale bars, 1 cm. (b) Representative absorbance spectra of aqueous phase before (blue line) and after (red line) phase transfer, chloroform phase after phase transfer (black line).

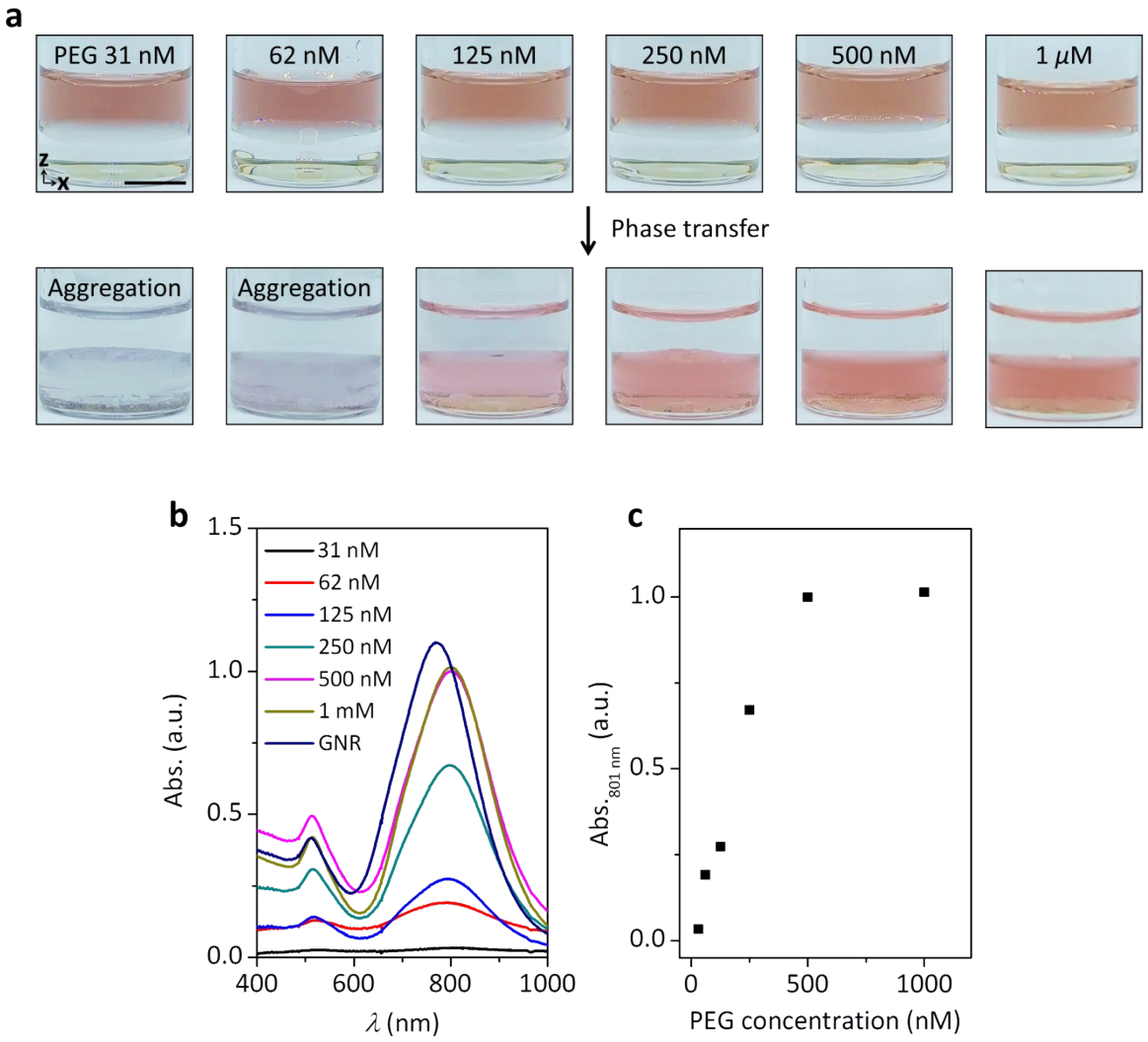

Fig. S2 a) Real photographs of water and chloroform phase at various concentrations of PEG-SH. The color changed after phase transfer at 31 nM and 62 nM PEG-SH because of GNR aggregation. Scale bar, 1 cm. b) Representative absorbance spectra of GNR at various concentrations of PEG-SH. c) The change of SPR bands as the increasing concentration of PEG-SH.

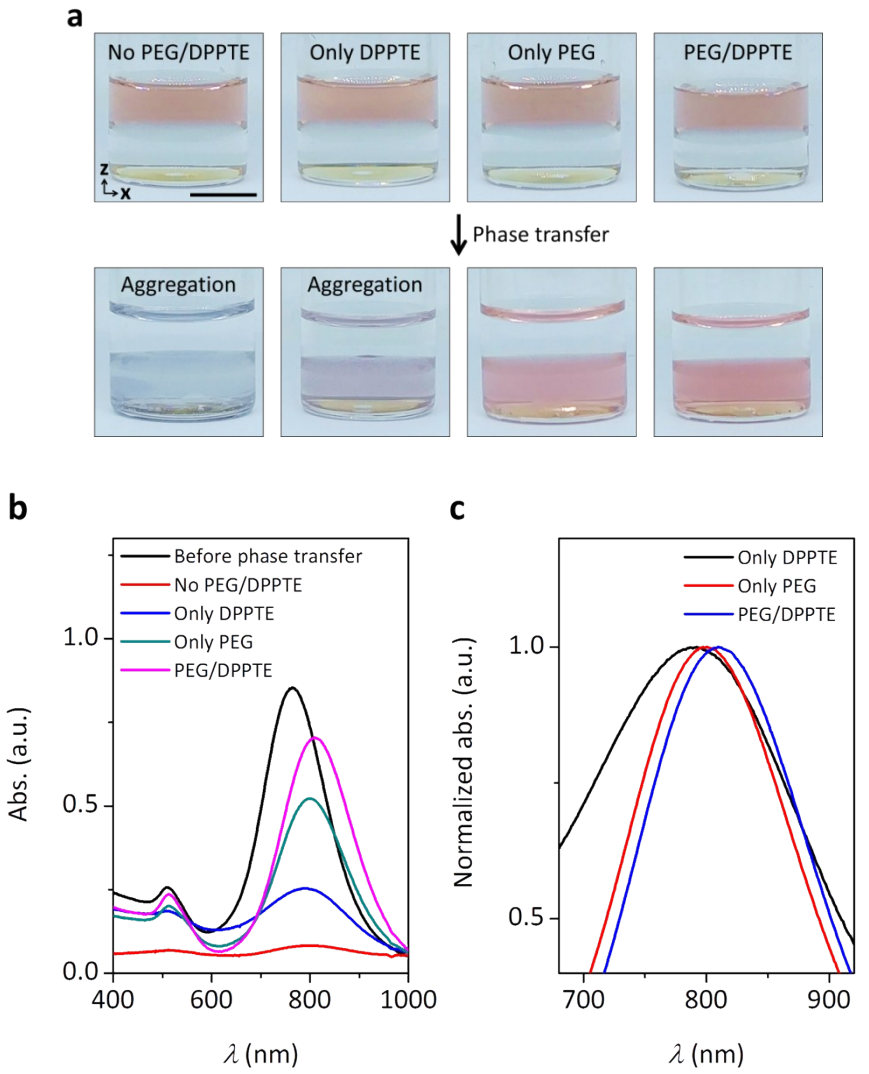

Fig. S3 a) Real photographs and b) Representative absorbance spectra with/without PEG-SH and DPPTE molecules. Scale bar, 1 cm. c) Normalized absorbance spectra with/without PEG and DPPTE molecules.

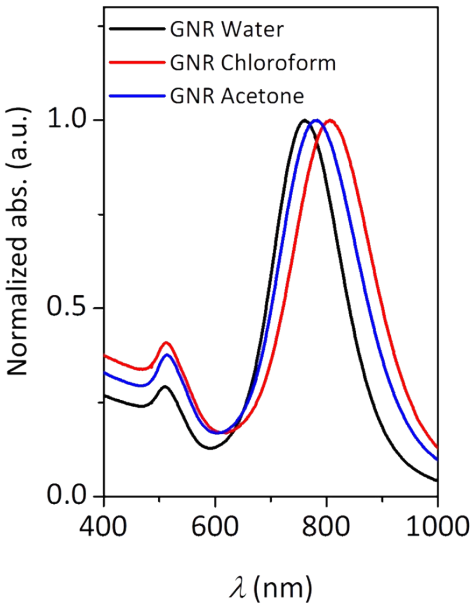

Fig. S4. Normalized absorbance spectra of GNR in water, chloroform and acetone.

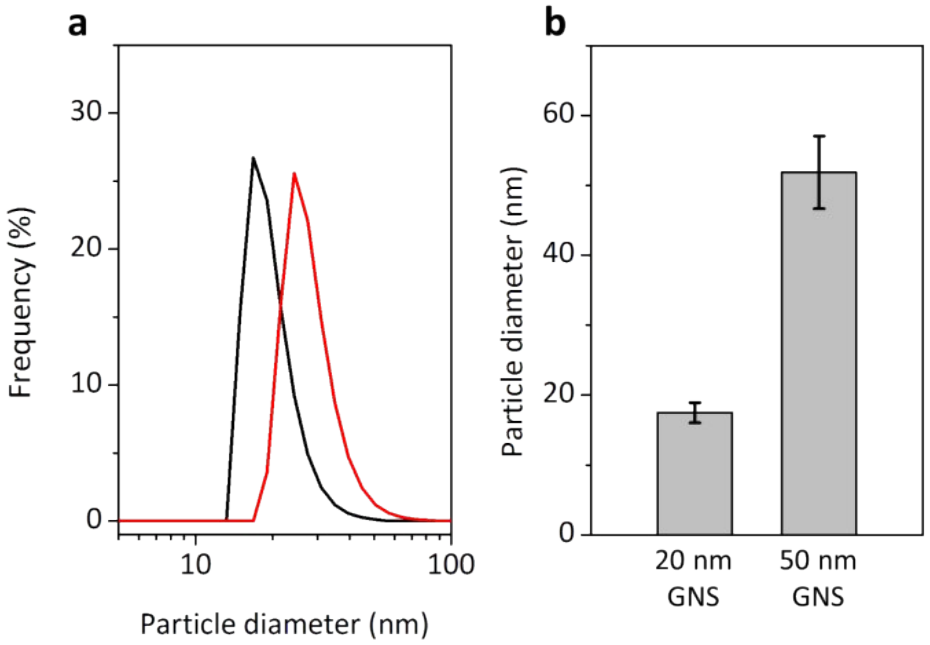

Fig. S5 a) Dynamic light scattering spectra for 20 and 50 nm GNS. b) Mean particle diameter measured in TEM images (≥ 50 particles).

3. Reference

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4 C. J. Orendorff and C. J. Murphy, *J. Phys. Chem. B*, 2006, **110**, 3990-3994.