Synthesis of Ptsome: A platinum-based liposome-like nanostructure

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Supporting Information

Experimental Sections

Materials. Hydrogenated L-α-phosphatidylcholine (EggPC), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*N*-lissamine rhodamine B sulfonyl (DMPE-RhB), N-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-phytosphingosine (C_6NBD), and cholesterol were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Stearic hydrazide was purchased from Tokyo Kasei Kogyo Co. Ltd. and used directly. Potassium tetrachloroplatinate (II) and all other chemicals used herein were purchased from Sigma-Aldrich Co. and used without further purification.

Synthesis and characterization of* cis-*Dichloridobis(2-stearoylhydrazide)platinum(II) (DCSP). DCSP was synthesized in biphasic solvent at room temperature. In a typical experiment, 10 mg (0.024 mmol) of potassium tetrachloroplatinate (II) was dissolved in 2 mL of 0.05 M HCl and was reacted with 14.38 mg (0.048 mmol) of stearic hydrazide dissolved in 2mL of chloroform. After three days of reaction under vigorous stirring, the red color of the aqueous layer disappeared as the organic phase turned to yellow. Then the organic phase was collected and precipitated in ether. Finally, the product was purified by column chromatography (5% methanol in chloroform) and thin layer chromatography (3% methanol in chloroform) with product Rf = 0.44. ^{195}Pt NMR spectra were recorded in $CDCl_3$ using a Varian Mercury 400 MHz

spectrometer. For ^{195}Pt NMR measurement, the shift in DCSP resonance was measured with respect to the standard saturated solution of potassium tetrachloroplatinate (II) in 0.05 M HCl containing 10% of D_2O for sample lock. Samples were measured at spectral width of 21.62 KHz with spectral frequency of 107.22 MHz within 200 ppm offset. ^{195}Pt NMR δ ppm; -1578.0 (standard, saturated solution in 0.05 M HCl in D_2O), -1377.2 (DCSP product, saturated solution in $CDCl_3$). Electrospray ionization mass spectrometry (ESI-MS, Thermo LCQdeca spectrometer) was used to determine the mass of the compound. ESI-MS (negative): m/z: 861.08 $[M-H]^-$, 896.94 $[M+Cl]^-$, 825.33 $[M-HCl-H]^-$, which corresponds to the theoretical value for $[M-H]^-$ 861.49.

Preparation and characterization of Ptsome. Ptsomes were prepared using an extrusion method that has been widely used to prepare phospholipid liposomes.[1, 2] Briefly, 100 μg of DCSP were dissolved in 1 mL of chloroform. The solvent was evaporated with argon gas for 15 min. Then, the dried DCSP films were hydrated with 3 mL of deionized water, followed by vortexing for 1 min and sonicating for 3 min in a bath sonicator (Fisher Scientific FS30D). A Ti probe (Branson 450 sonifier) was then used to sonicate the solution for 1~2 min at 20 W. Lastly, the solution was extruded through a 100 nm pore-sized polycarbonate membrane for 11 times. DSPE-PEG stabilized Ptsomes were prepared by adding 20 mol% of DSPE-PEG to DCSP prior to the preparation of Ptsomes. The hydrodynamic size and surface zeta potential of the prepared Ptsomes were assessed by using the Malvern Zetasizer ZS (Malvern Instruments, UK). The mean diameter and zeta potential of Ptsomes were determined through dynamic light scattering (DLS) and electrophoretic mobility measurements, respectively. All characterization measurements were repeated three times at 25 °C. The morphology and structure of Ptsomes were characterized using scanning electron microscopy (SEM). Samples for SEM characterization were prepared by dispersing a diluted solution of Ptsomes onto the surface of a silicon wafer and dried at room temperature. Prior to SEM measurement the dried film was coated with chromium for 30 sec.

Ptsome fusion study. Ptsome fusion study was conducted following a previously described protocol based on fluorescence resonance energy transfer (FRET) technique.[2] Specifically, a fluorescence donor (0.1 mol% C_6NBD, excitation/emission = 470/520 nm) and a fluorescence acceptor (0.5 mol% DMPE-RhB, excitation/emission = 550/590 nm) were incorporated into the Ptsome membranes. At this composition, the fluorescence emission from the donor was maximally quenched by the acceptor. Then the FRET pair-loaded Ptsomes were

mixed with unlabeled Ptsomes at a molar ratio of 1:7 for 30 min. The fluorescence emission spectra at the range of 500 to 700 nm were obtained with an excitation at 470 nm. As a negative control, the FRET pair-loaded Ptsomes containing 20 mol% DSPE-PEG were tested in parallel to examine their fusion activity with unlabeled Ptsomes. All measurements were carried out at 25 °C using a fluorescent spectrophotometer (Infinite M200, TECAN, Switzerland).

References:

[1] L. D. Mayer, M. J. Hope, P. R. Cullis, *Biochim. Biophys. Acta* **1986**, *858*, 161-168.

[2] D. Pornpattananangkul, S. Olson, S. Aryal, M. Sartor, C. M. Huang, K. Vecchio, L. Zhang, *ACS Nano* **2010**, *4*, 1935-1942.