

Biointerfacing Luminescent Nanotubes

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Experimental

Materials. Alumina membranes with a pore diameter of 200 nm and a membrane thickness of 60 μm were obtained from Whatman. Poly(ethylenimine) (PEI, M_w 5,000~10,000) was obtained from ICN Co. 3,4,9,10-perylenetetracarboxylic-dianhydride (PTCDA) and egg L- α -phosphatidylcholine (egg PC) was purchased from Sigma–Aldrich, which have been used without further treatment. Texas-Red dihexadecanoylphosphatidyl ethanolamine (TR-DHPE) was purchased from Molecular Probes. All other chemicals used in this work were obtained from Sigma–Aldrich and without further purification.

Preparation of Large, Unilamellar Vesicle by Extrusion. Egg PC was first mixed with 1 % (by molar ratio) TR-DHPE and dissolved in chloroform/methanol (2:1, v/v). The mixed solvent was removed by a rotary evaporator in vacuum to yield a dry lipid film on the inner wall of a glass round-bottom flask. Then the lipid film was hydrated in a PBS buffer solution containing 150 mM NaCl at pH 7.2 at 50 $^{\circ}\text{C}$ (total lipid concentration was 2.5 mg/mL). The lipid suspension was extruded (Avanti POLAR LIPIDS, Alabaster, AL) 20 times through a 0.2 μm polycarbonate filter (Whatman). The size distribution of the extruded vesicles was measured by a Zetasizer Nano ZS

instrument (ZEN 3500, Malvern Instruments Ltd) and was typically 150-180 nm. Vesicle solution was diluted to 0.8 mg/mL in the same buffer solution before use.

Formation of lipid bilayer-modified (PEI/PTCDA)₆ Nanotube. As we previously reported,¹ the (PEI/PTCDA)₆ nanotube is prepared by the combination of layer-by-layer assembly with template removal. Briefly, the PEI and PTCDA were 0.05 wt % dissolved in isopropyl alcohol and *o*-phenol solution, respectively. The alumina membranes were first coated with PEI by filtering the PEI solution through the pores of the membrane. This aids subsequent multilayer buildup. Then the PTCDA solution was deposited by the same procedure through covalent bonding. After several cycles, multilayer films were formed on the inner pore walls of the alumina membrane. The deposited film on the top and bottom surface of the membrane can be removed by mechanical polishing. After the alumina membrane was dissolved by 1 M NaOH solution and the nanotubes were liberated into the solution, the nanotubes were completely washed three times in deionized water, followed by re-dispersing in water. Finally, the (PEI/PTCDA)₆ nanotube solution was incubated with egg PC vesicles for 30 min and the nanotube solution was then washed three times in buffer solution.

Characterization. Scanning electron microscopy (SEM) analysis was conducted with a Gemini Leo 1550 instrument at an operating voltage of 3 keV. A drop of the sample solution was placed onto a glass wafer, dried at room temperature, and sputtered with gold. Similarly for transmission electron microscopy (TEM), a drop of the sample was dried onto a carbon-coated copper grid and analyzed using a Zeiss Omega EM 912 at an operating voltage of 120 kV. Confocal Laser Scanning Microscopy (CLSM) images of lipid-modified nanotubes in water were obtained using a Leica TCS SP confocal scanning system (Leica, Germany) equipped with 100×oil-immersion objective with a numerical aperture of 1.4. In order to carry out fluorescence recovery

after photobleaching (FRAP) measurements, an Ar ion Laser from the CLSM emitting at $\lambda = 488$ nm was used to irradiate the tube about 1 min. Recovery was considered complete when the intensity of the photobleached region had reached a constant value. For quantitative analysis, the fluorescence intensity was integrated by tracing a closed area in the interior, which gave an intensity value for each time. CLSM and FRAP experiments were performed at about 22 °C.

Reference

1. Y. Tian, Q. He, C. Tao, and J. B. Li, *Langmuir* 2006, **22**, 360.