ADVANCED FUNCTIONAL MATERIALS

Supporting Information

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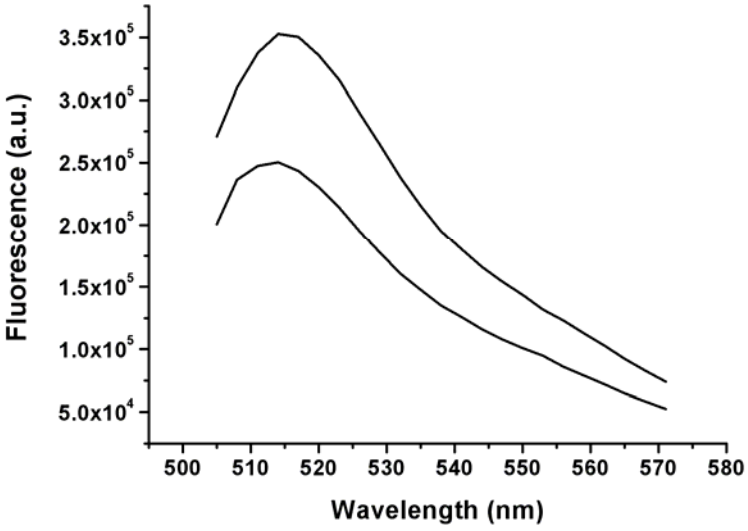
Giant chromatic lipid/polydiacetylene vesicles for detection and visualization of membrane interactions

Supporting Information

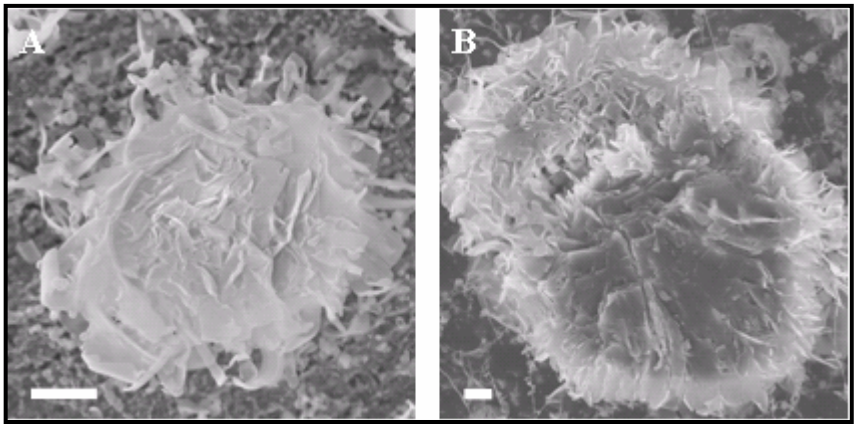
1. Fluorescein-encapsulation experiment.



Bottom curve: fluorescence emission (excitation at 490 nm) recorded for as-prepared giant DMPC/PDA vesicles (1:1.5 mole ratio) encapsulating fluorescein. **Top curve**: fluorescence recorded after destroying the vesicles through addition of ethanol. The increase in fluorescence indicates that the prepared vesicles had an enclosed volume containing fluorescein.

Experimental details: The vesicle constituents (lipids and PDA) were separately dissolved in chloroform/ethanol (1:1 volume ratio) and then added to a 250 ml round-bottom flask containing 1 ml of chloroform. 5 mL of 0.4 mM fluorescein in water pH 7.4 was then carefully added along the flask walls. The organic solvent was removed in a rotary evaporator under reduced pressure (final pressure 40 mmHg) at 65°C and 40 rpm. After evaporation for 2 min the resultant volume of about 2 ml was cooled to room temperature and kept at 4°C overnight. The solution was then irradiated at 254 nm for 30-40 s. To remove non-trapped fluorescein, the giant vesicles were sedimented for several days. The supernatant was exchanged by the same volume of 0.5 mM sucrose solution. Fluorescein leakage from the giant vesicles was induced by addition of ethanol. Fluorescence was measured at room temperature on an Edinburgh FL920 spectrofluorimeter.

2. Cryo-SEM experiment

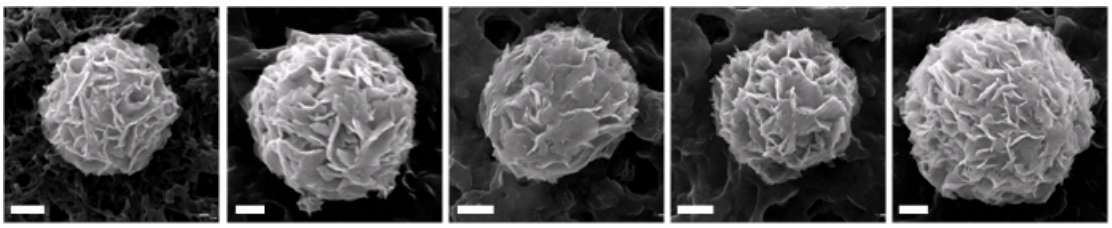


A: PDA/DMPC (1:0.67 mole ratio); **B**. PDA/DMPE/DMPG (1:0.33:0.33 mole ratio). Scale bar is 1 μm. The cryo-SEM images display a "cut-through" plane through the vesicles, thus exposing the vesicle interior. These images reveal that, in contrast to conventional giant phospholipid vesicles in which the interior of the vesicle is essentially "empty" (i.e. contains the aqueous solution), the PDA/lipid giant vesicles exhibit sheet layers inside, most likely corresponding to the PDA assemblies.

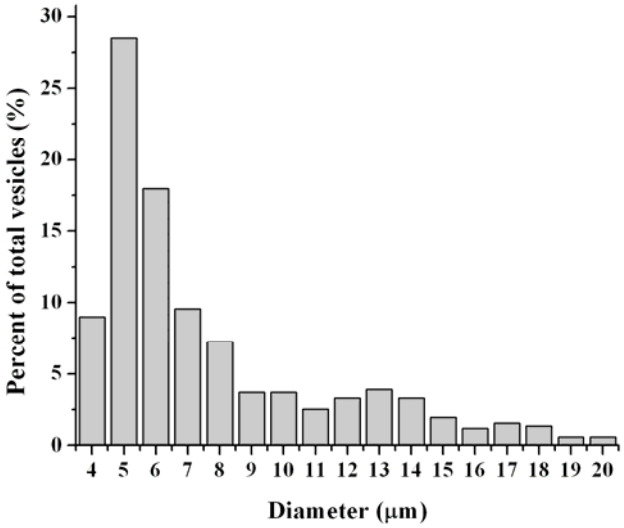
Experimental details: 3 μL of the vesicle sample were deposited on the copper grid. The grid was then sandwiched between two copper carriers and frozen in liquid ethane using a custom-built spring loaded plunger. The frozen sample was subsequently put into a BAF 60 freeze fracture devise using Vacuum Cryo Transfer system - VCT100 (Bal-Tec, Balzers, Liechtenstein). The sample was fractured and etched at -105°C for 30 min. and double-layer coated by platinum-carbon rotary shadowing at angle of 45° (thickness 2.5 nm) followed by carbon coating (thickness 5 nm) at 90°. Following coating the sample was cryo transferred and imaged on a Zeiss Ultra 55 SEM (Germany) equipped with cryo stage (Bal-Tec, Balzers, Liechtenstein). We are grateful to Dr. Eyal Shimoni, Microscopy Unit, Weizmann Institute of Science, for help with the cryo-SEM experiments.

3. Size distribution of DMPE/DMPG/PDA vesicles

A. vesicles recorded in the SEM experiment (scale is 2μm)



B. statistical distribution determined by image analysis



Experimental details: Giant DMPC/PDA vesicles sedimented for several days at 4°C were transferred onto a microscopy glass cover-slip. Light microscopy images were recorded on an Olympus IX-70 microscope equipped with a cooled CCD camera (MicroMax, Princeton, NJ) using a UPLanFLN 20x/0.50 objective. To determine the statistical distribution of vesicle diameters, the microscopy images were analyzed by the ImageJ software (http://rsb.info.nih.gov/ij/). Approximately 500 vesicles were used in the database.