Controlled Vesicle Self-Assembly in Microfluidic Channels with Hydrodynamic Focusing

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Supporting Information[1]

Lipid and Solution Preparation

Dimyristoylphosphatidylcholine (DMPC) and cholesterol (Sigma-Aldrich, St. Louis, MO) in a molar ratio of 1:1 were diluted in chloroform with 1 wt % of 1,1'-dioctadecyl-3,3,3',3' tetramethilindodicarbocyanine perchlorate ($DiIC_{18}$) added for fluorescent imaging. The chloroform solvent was evaporated under a stream of nitrogen at room temperature to form a lipid film on the bottom of a test tube. The test tube was then placed in a vacuum desiccator for at least 24 h to ensure dryness. The dried lipid mixture was resolubilized with 500 µL dry isopropanol yielding a 10 mM concentration of lipid solution. Phosphate buffered saline solution (10 mM phosphate, 27 mM potassium chloride, 137 mM sodium chloride, pH = 7.4) was used as the hydration buffer.

[1] Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Microfluidic Device Fabrication

A microchannel network (200-μm channel width) was anisotropically etched on the surface of a silicon wafer (<100> orientation, 75 mm diameter, 0.3 mm thickness, Virginia Semiconductor, Fredericksburg, VA) to a depth of 40 μm through a photolithographically patterned thermal SiO_2 (100 nm) hard mask using tetramethylammonium hydroxide (TMAH, Alfa Aesar, Ward Hill, MA) (1:2 dilution 25 % w/w aqueous TMAH solution, 80 ºC). The same procedure was followed to etch fluid through-holes on the back-side of the wafer that were aligned to the microchannel network in the front side. Following this, all surface oxide was removed (8 % buffered hydrofluoric acid etch) and then reoxidized to form a 100 nm thick SiO_2 film encapsulating the silicon substrate. Finally, a glass cover wafer (75-mm diameter, 0.1-mm thickness, Corning 7740) was anodically bonded to the front surface of the silicon wafer using a 580 V applied potential and heated to 400 ºC to seal the microchannel network.

Reagent transfers were accomplished using gastight glass syringes interfaced to the microchannel network through capillary tubing (PEEK Tubing, Upchurch Scientific, Oak Harbor, WA) and capillary connectors (Nanoports, Upchurch Scientific, Oak Harbor, WA) that were bonded to the fluid through-holes etched in the silicon wafer. Programmable syringe pumps (Harvard Apparatus, Holliston, MA) were used to control the fluid flow rates by computer using a LabVIEW software interface.

Liposome Characterization

The liposome formulations (100-µL sample size) were collected at each flow condition into polycarbonate cuvets. After collection, 1 mL of phosphate buffered saline solution (10 mM) was added to each formulation and sealed for further characterization.

Liposome size and size distribution was characterized by laser light scattering was performed using a Coulter N4 MD submicron particle analyzer collecting scattered light at 90˚. Confocal fluorescence imaging was performed with a Pascal confocal imaging system (Carl Zeiss, Thornwood, NY).

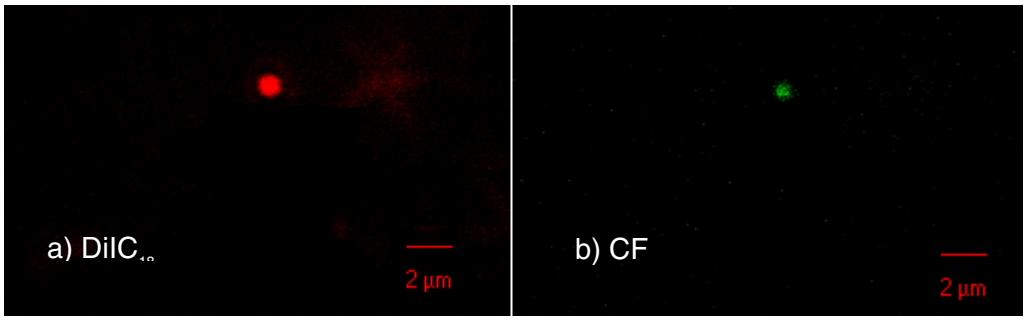

Figure S1. Two-color confocal fluorescence imaging of a single liposome formed in flow conditions of 0.5 µL/min flow rate of the isopropanol stream and 20 µL/min flow rate of the aqueous buffer streams. The flow conditions in this example produced a liposome mean diameter of 133 nm and standard deviation of 26 nm determined by light scattering measurements. (a) Fluorescence image for $DiIC_{18}$ that exhibits intercolation of the dye into the lipid bilayer; and (b) fluorescence image for carboxyfluorescein (CF) that has been encapsulated in the liposome's aqueous interior. *N.B.* The apparent size of the liposome in these images does not give an accurate representation of the actual liposome size due to artifacts of image processing and limitations of the imaging optics. All liposomes in our samples are below the optical resolution accessible by confocal imaging.