Supporting Information

End-capping of amphiphilic nanotubes with phospholipid vesicles: impact of the phospholipid on the cap formation and vesicle loading under osmotic conditions

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Table of Contents

Materials and Methods

Materials. 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine chloride salt (EPOPC), 1,2-dioleoyl-sn-glycero-3-phospho-L-serine sodium salt (DOPS) were purchased from Avanti Polar lipids and used without further purification. Sodium Chloride (ACS reagent grade) was purchased from Aldrich and used without further purification.

Methods.

Cryo-TEM. Samples for cryo-TEM were prepared by depositing typically 3 μL of nanotube solution on holey carbon coated grids (Quantifoil 3.5/1, Quantifoil Micro Tools, Jena, Germany). After blotting the excess liquid at constant humidity of 100%, the grids were vitrified in liquid ethane (Vitrobot, FEI, Eindhoven, The Netherlands) and transferred to either a Philips CM 12 cryo-electron microscope operating at 120 kV, a Philips CM 120 cryo-electron microscope operating at 120 kV or a FEI Tecnai 20 microscope operating at 200kV. Micrographs were recorded under low-dose conditions with a slow-scan CCD camera.

Standard method for nanotube formation. A stock solution of amphiphile **1** in chloroform was mixed with the desired amount of a stock solution of phospholipid (either DMPC, DOPC, DOPS or EPOPC) in chloroform and subsequently dried under nitrogen gas resulting in the formation a of thin film. After further solvent removal under high vacuum for 15 min, the sample was hydrated with either doubly distilled water and was subjected to three freeze-thaw cycles. The samples with DMPC were either kept at 4 °C or at 30 °C. The samples were kept subsequently in closed 1 ml vials for several days at room temperature which allows for the formation of vesicle caps at the end of the nanotubes. For all samples the concentration of Amphiphile **1** was 1 mg/ml with an additional 1 mg/ml of either DMPC, DOPC, DOPS or EPOPC.

Formation of DOPS vesicles. A thin film of DOPS which was obtained by drying a stock solution of DOPS under nitrogen is dried for 15 min under high vacuum and is subsequently hydrated with doubly distilled water. The samples is then subjected to three freeze/thaw cycles.

Induction of Osmosis by addition of salt.

To a desired sample a NaCl solution of a concentration between 10 mM or 40 mM or 400 mM was added in a 5:1 (v:v) ratio. The samples were then frozen on TEM grids within 20 min after mixing of the solutions.

TEM images nanotubes after 1day in presence of EPOPC

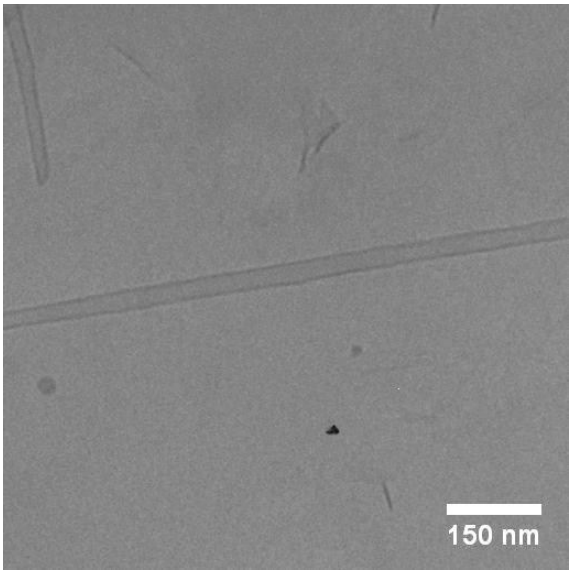

Figure S1 Cryo-TEM image of EPOPC:**1** (1:1, w:w 1 mg/ml each) immediately after sample preparation showing nanotubes without vesicle caps.

TEM images for the inclusion of vesicles during freezing process at 80% humidity

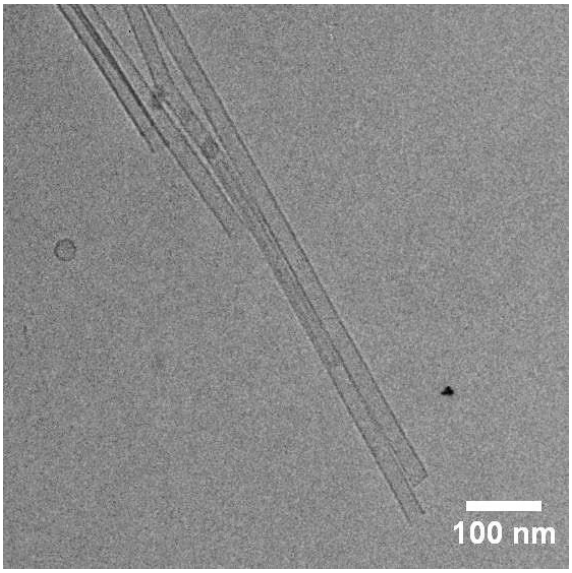

Figure S2 Cryo-TEM image of DOPC:**1** (1:1, w:w 1 mg/ml each) in aqueous 10 mM NaCl solution. In contrast to all other images presented in this publication the humidity was not kept at 100% during the freezing process but at 80%, which results in an evaporation of water of the sample during the freezing process which in turn induces an osmotic gradient which results in the formation of nanotubes with vesicles included into the nanotubes.

TEM images DOPS vesicles under hyperosmotic conditions

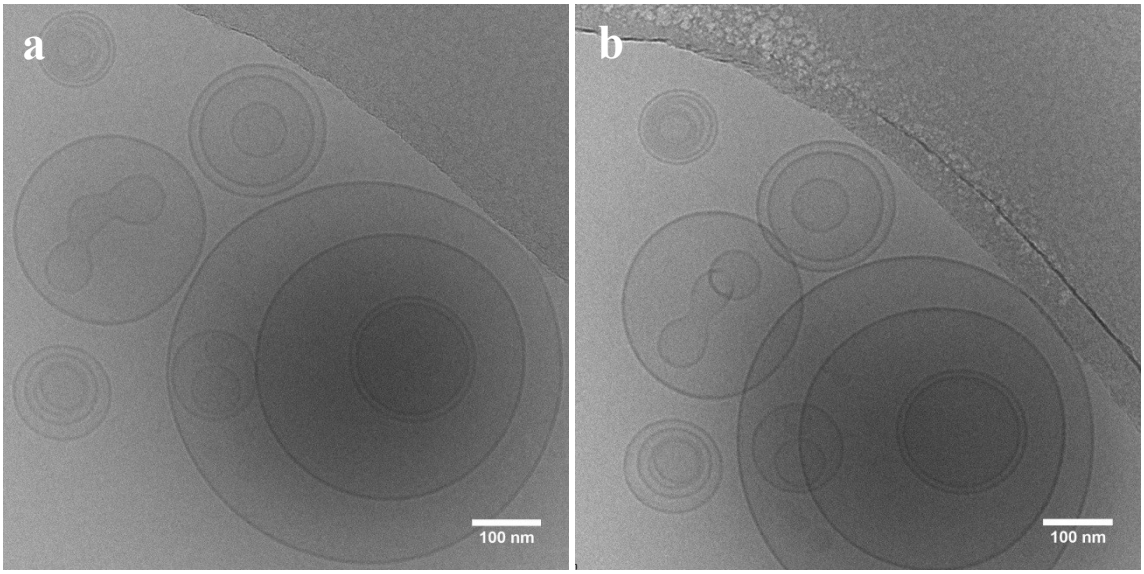

Figure S3 a) Cryo-TEM image of DOPS (1mg/ml) in aqueous solution after the addition of 400 mM aq. NaCl (1:5, v:v) and b) same sample tilted 30 °C to make clear that the inclusions are inside the vesicles.

TEM images of 1 mixed with DMPC below and above the phase transition temperature

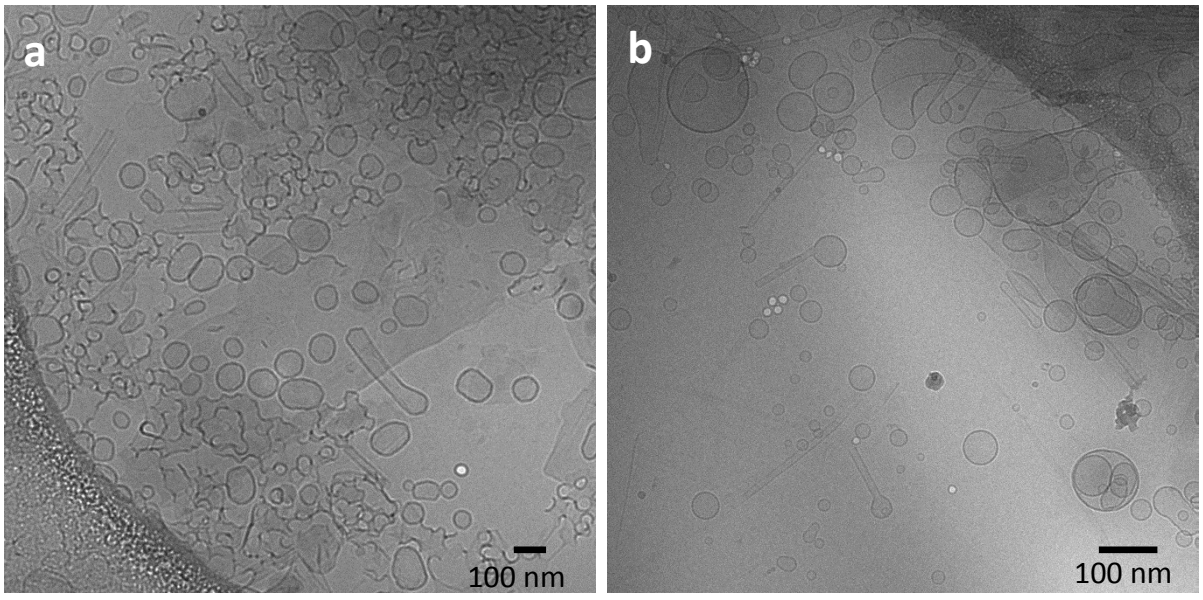

Figure S4 a) Cryo-TEM image of 1:DMPC (1:1 w/w 1 mg/ml each) in aqueous solution prepared at 4 °C. only liposomes and curved bilayers are present. b) same sample but now warmed to 30 °C. end-capped nanotubes are formed.

TEM images of EPOPC vesicles in pure water vitrified at 100% and 80% humidity.

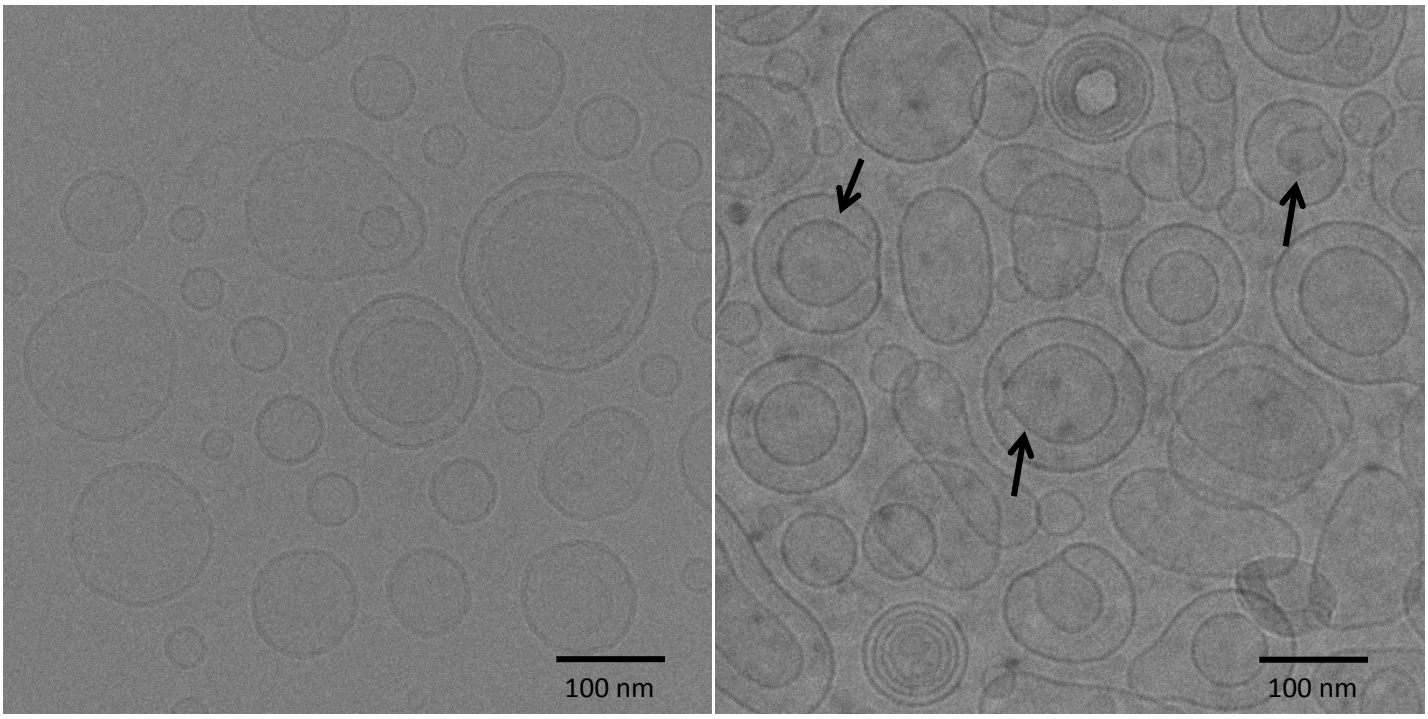

Figure S5; a) Cryo-TEM image of EPOPC liposomes 10 mg/ml in aqueous solution prepared by 200 nm extrusion, vitrified at 100 % humidity. b) Cryo-TEM of the same liposome solution but now vitrified at 80% humidity. due to the evaporation of water outside the vesicles and the presence of counterions an osmotic shock is created which result in the typical stomatocyte shaped vesicles (arrows).