

## Supporting Movies

**Movie S1: Nanotube fluctuation at the ATPS interface.** The movie captures the fluctuation of two nanotubes at the interface after the 2<sup>nd</sup> deflation step ( $r = 1.4$ ) using confocal microscopy. The nanotubes are constantly moving along the two-phase interface. The imaging area is  $4 \times 2 \mu\text{m}$ , pixel size was set to 100 nm to allow fast video capture.

**Movie S2: Nanotube movement used to illustrate the tube persistence length.** Nanotubes in a vesicle at  $r = 1.6$  are undulating with persistence length around  $5.9 \mu\text{m}$  (S.D. of  $3.9 \mu\text{m}$ ) as captured with STED, suggesting cylindrical structure of the tubes. With time, some of the nanotubes leave the imaging area resulting from the optical tweezer effect of the STED laser.

**Movie S3: Membrane neck of an ATPS budding GUV.** The thermal undulation of the membrane neck was captured with 2D STED. The real curvature of the membrane neck can be revealed (with higher intensities on the membrane showing the two buds are in sharp focus) but with time the equators of the two buds are getting out of focus due to membrane undulations; this adds some uncertainty when analyzing the membrane neck radius.