Electronic Supplementary Material (ESI) for Nanoscale.

Supporting information for:

Unveiling Growth and Dynamics of Liposomes by Graphene Liquid Cell-Transmission Electron Microscopy

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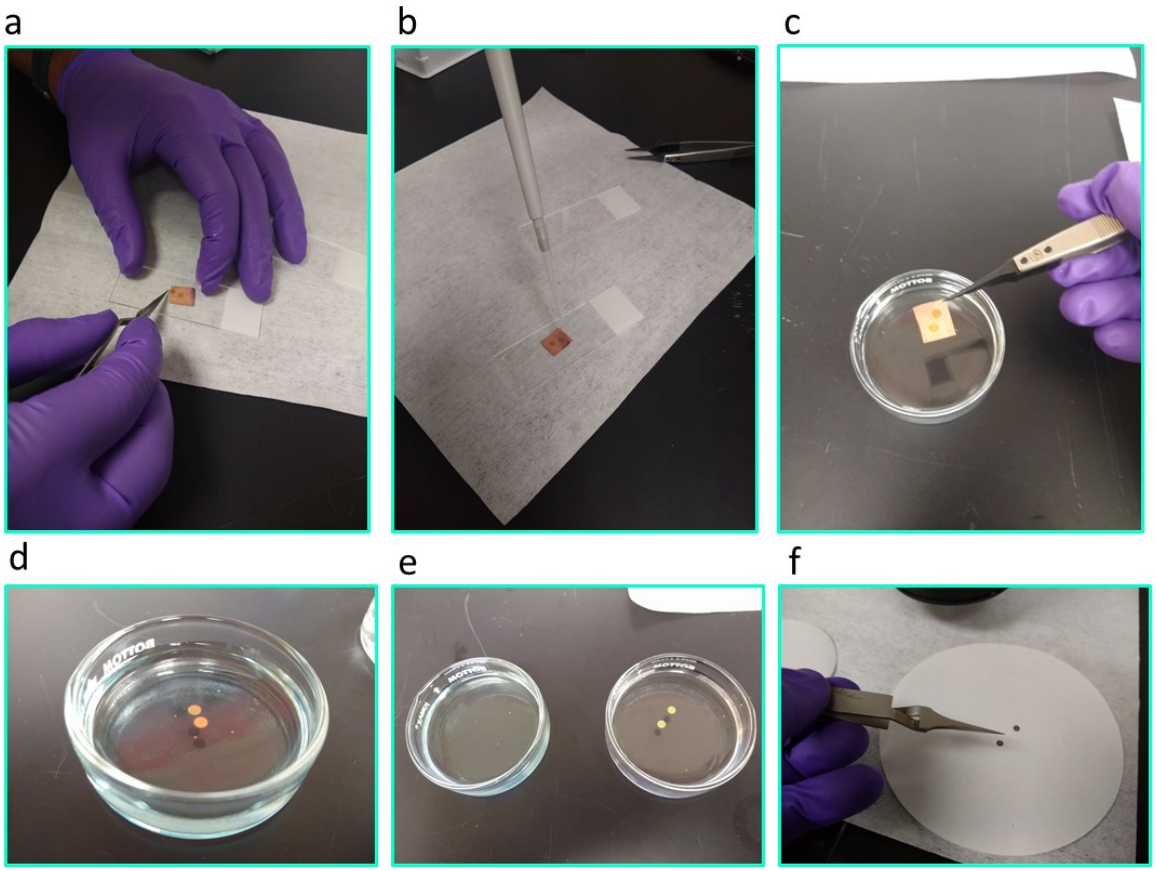

Figure S1. Protocol for making graphene-coated TEM grids.

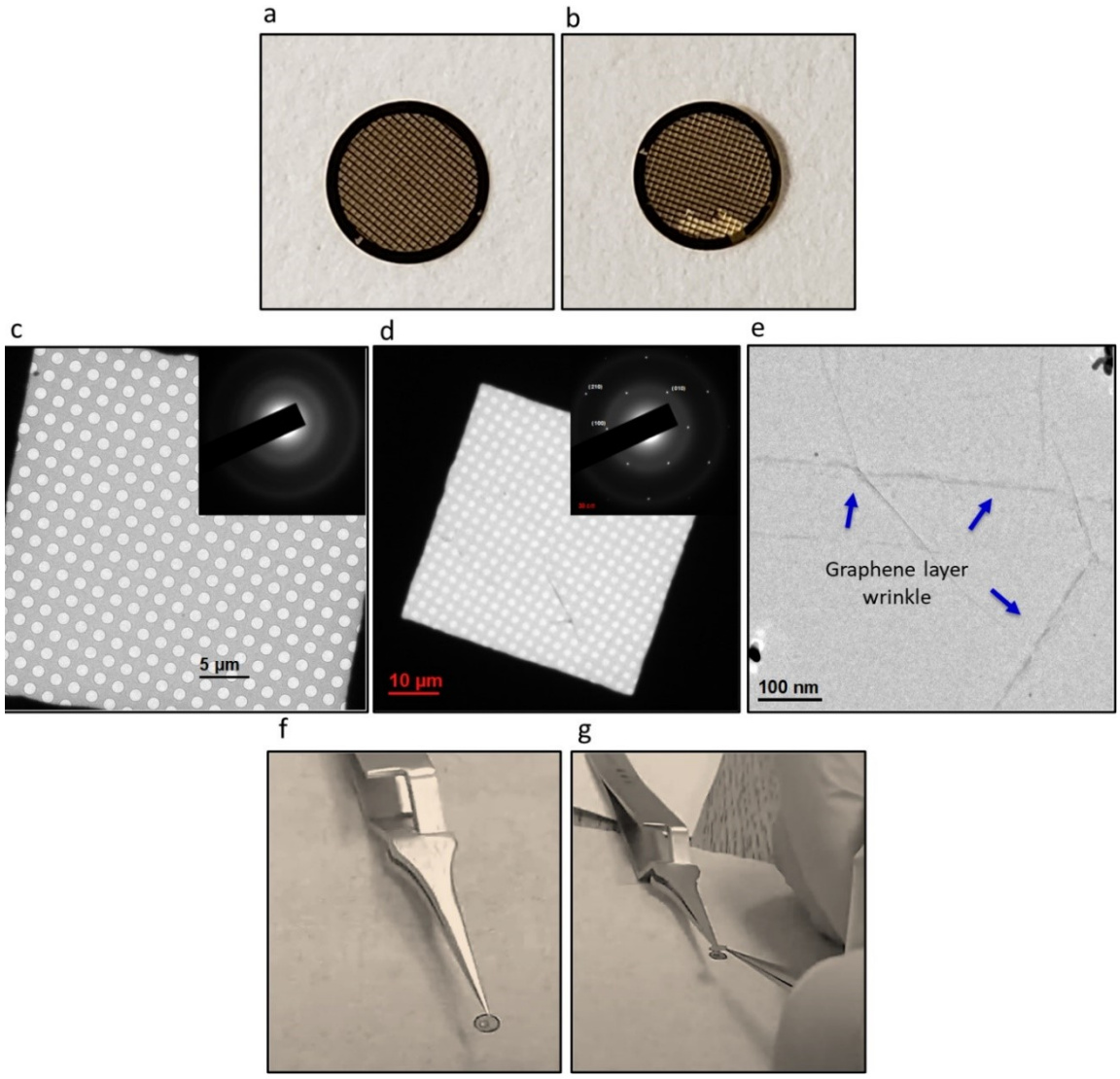

Figure S2. Protocol for assembly of the GLC. A carbon-supported Au grid with undefective uniform graphene coating (a) and with defective graphene coating (b). Low magnification TEM image of the amorphous carbon-coated Au grid before (c) and after graphene coating (d) and (e). Insets show selected area diffraction (SAED) pattern of the Au grids with and without graphene layer coating. (f) A small droplet (~0.1-0.2 µl) of the lipid solution placed on the graphene-coated TEM grid. (g) Gently sliding the top grid on top of the bottom grid to form a liquid pocket.

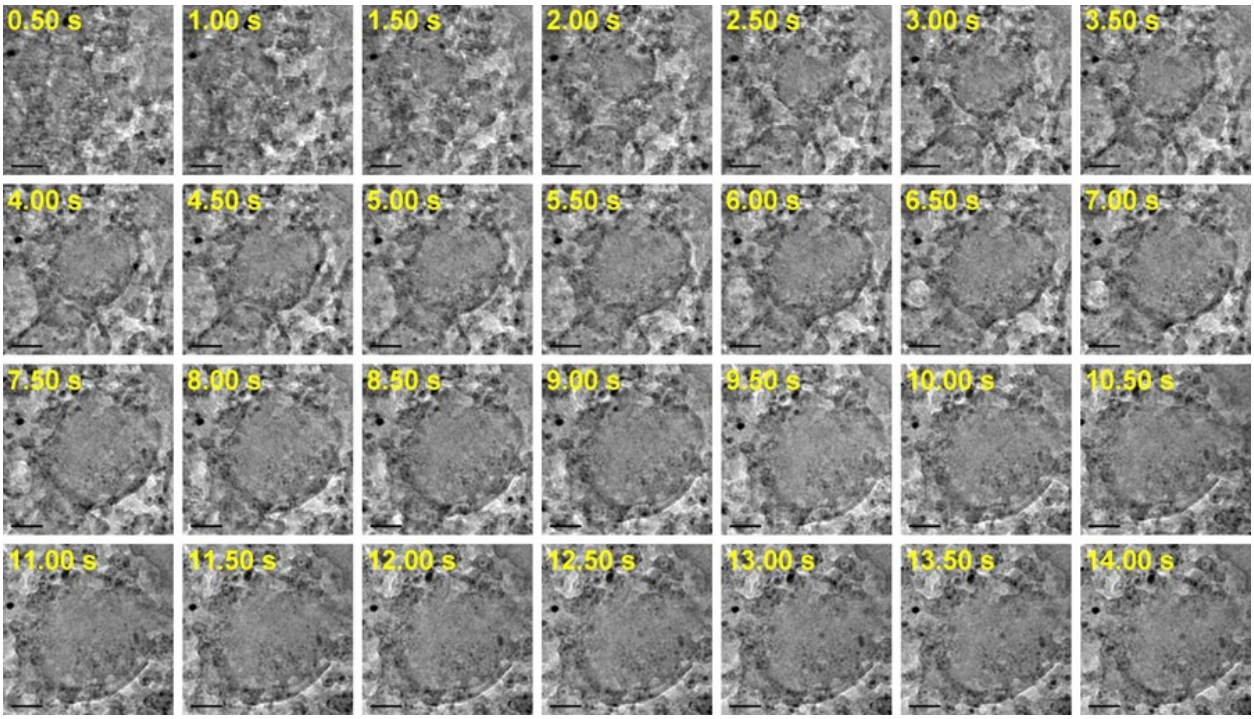

Figure S3. The original GLC-TEM images for the Figure 2a. The scale bar is 20 nm. TEM images are taken from Video S1.

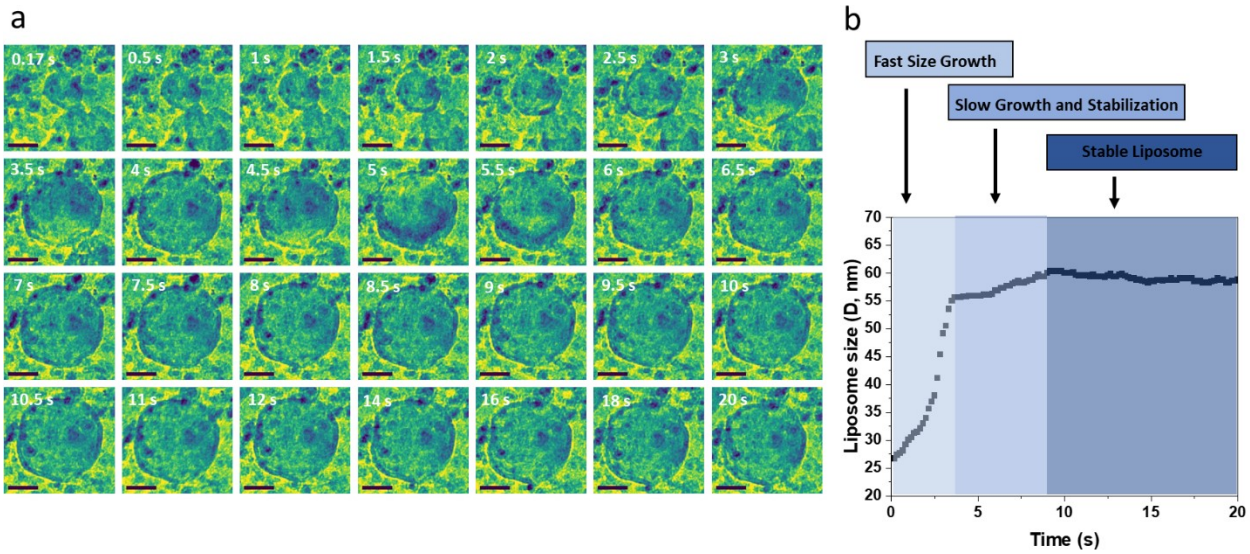

Figure S4. Formation of liposomes visualized in real-time by GLC-TEM. Time-lapse colored TEM images (a) and the corresponding size growth plot (b) for the formation and growth of a liposome from an aqueous solution of phosphatidylcholine lipids. The scale bar is 20 nm. TEM images are taken from Video S2.

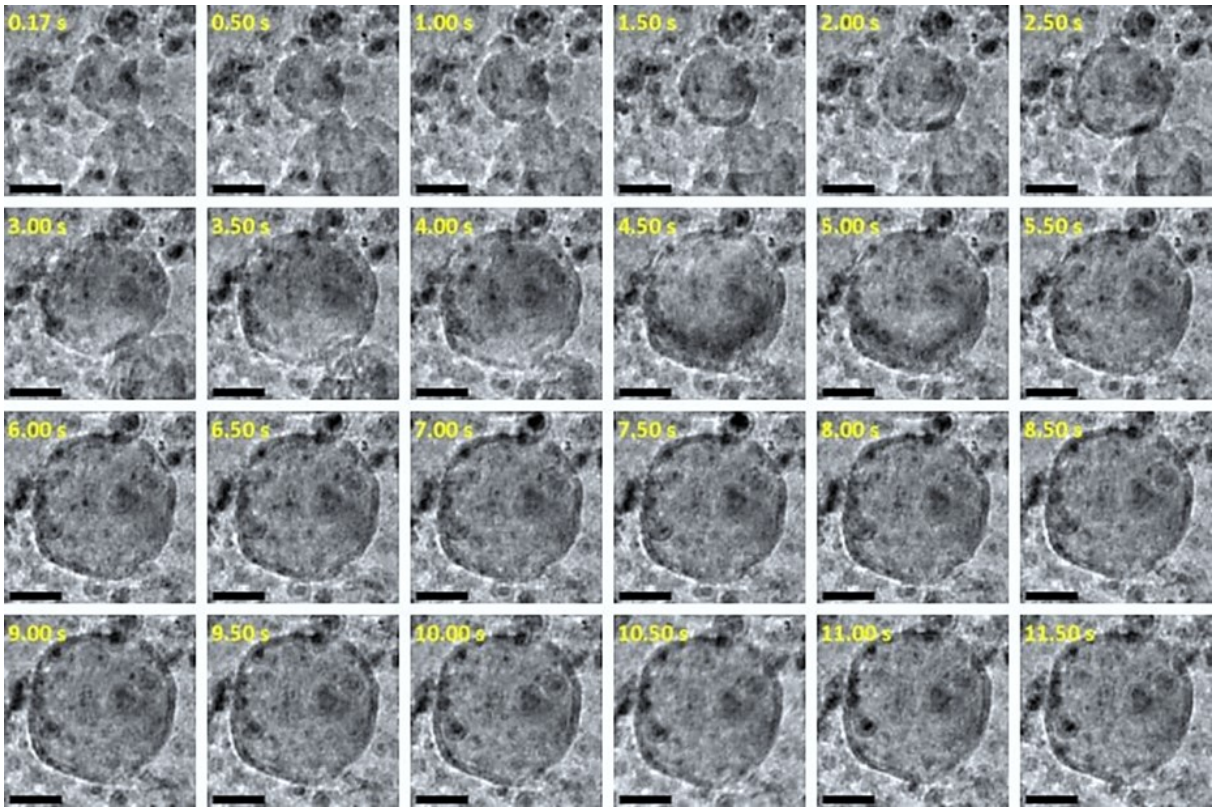

Figure S5. The original GLC-TEM images for the Figure S4. The scale bar is 20 nm. TEM images are taken from Video S2.

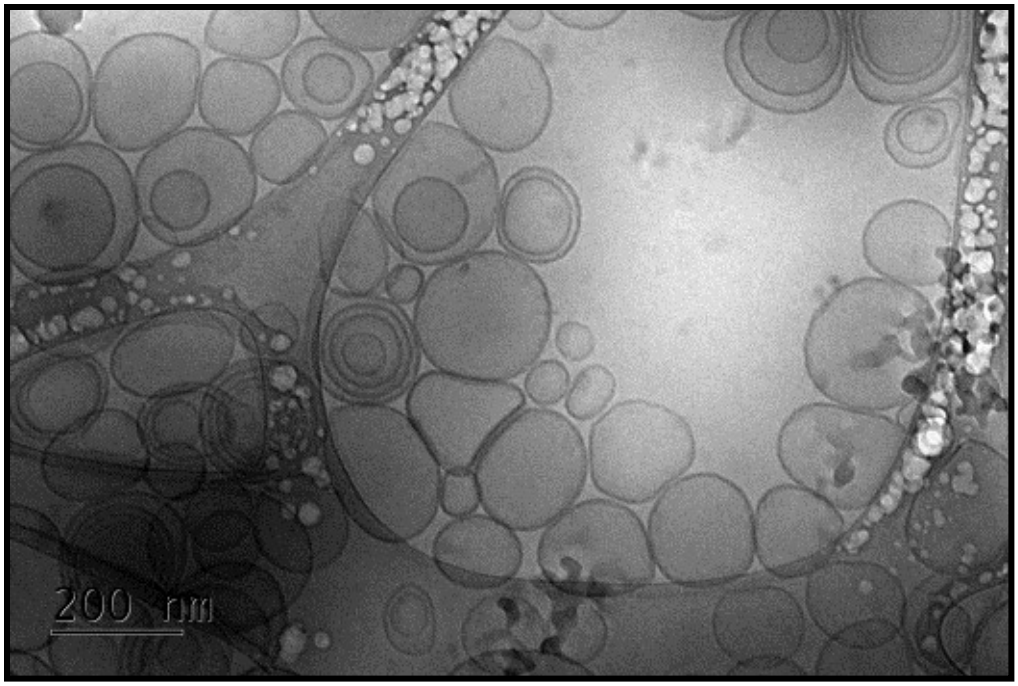

Figure S6. Cryo-TEM image of the synthesized liposomes.

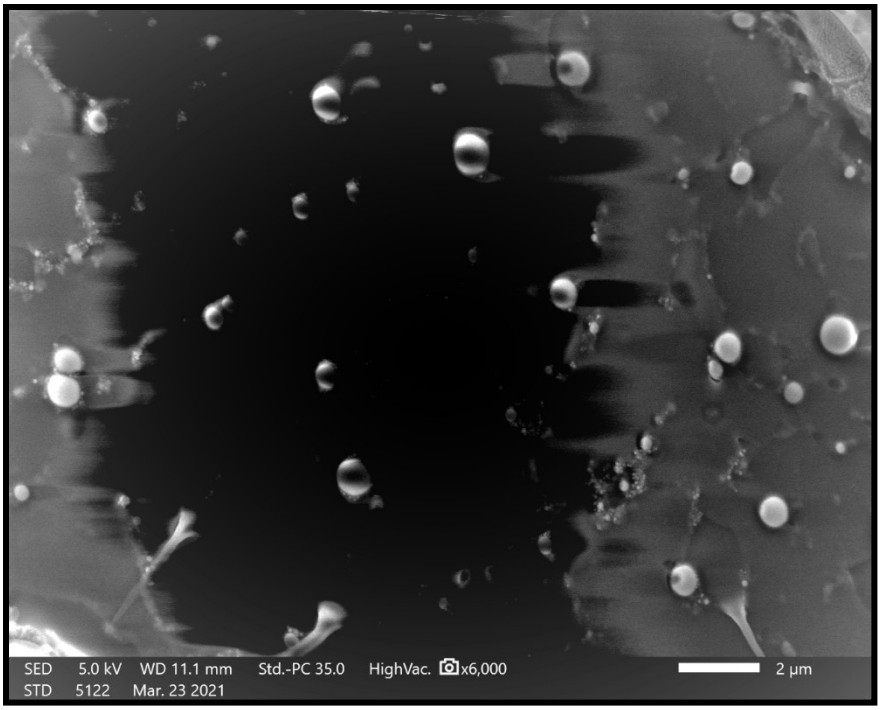

Figure S7. SEM image of the synthesized liposomes.

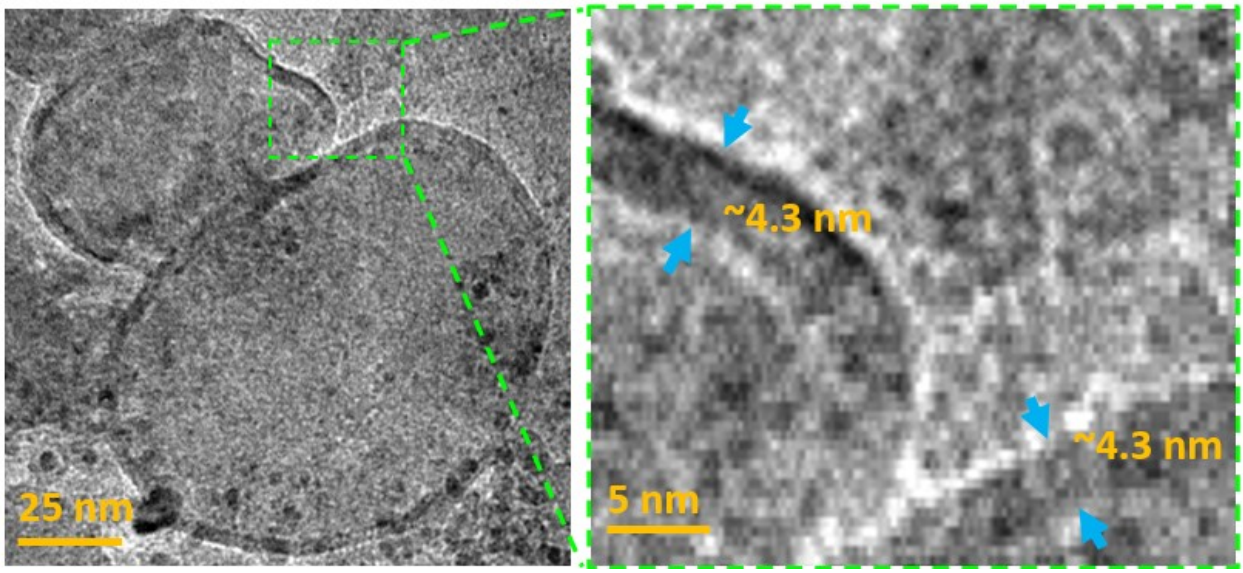

Figure S8. Membrane (lipid bilayer) thickness of liposomes.

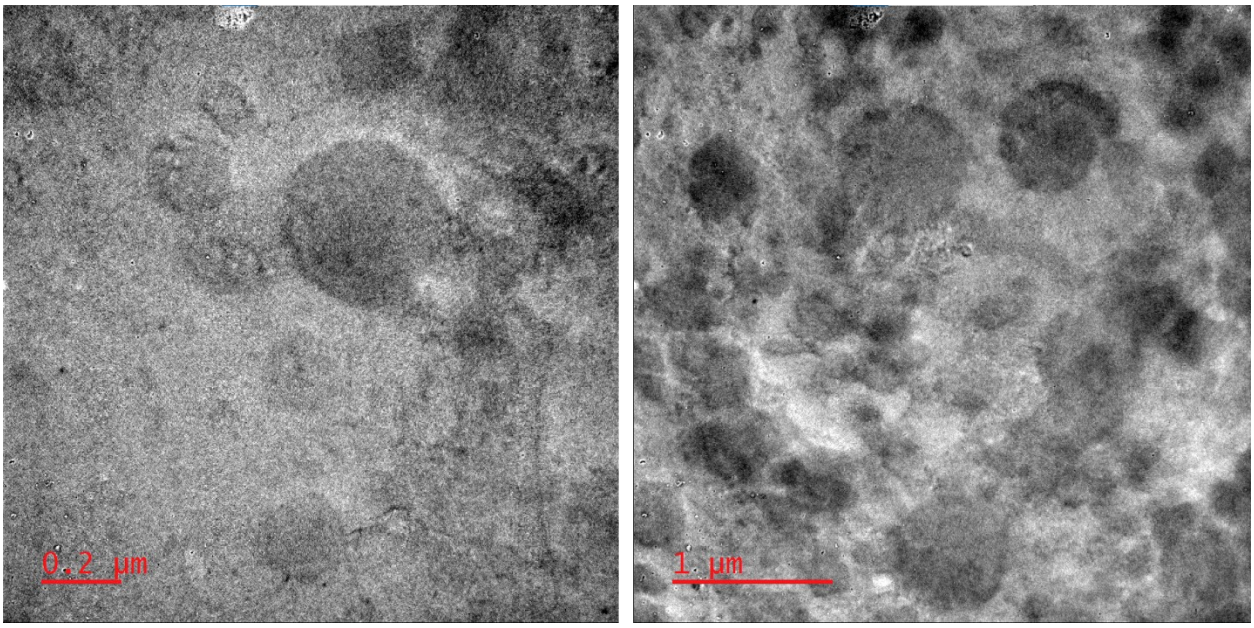

Figure S9. The GLC-TEM images for the PC liposomes with cholesterol in ultrapure water (no salt).

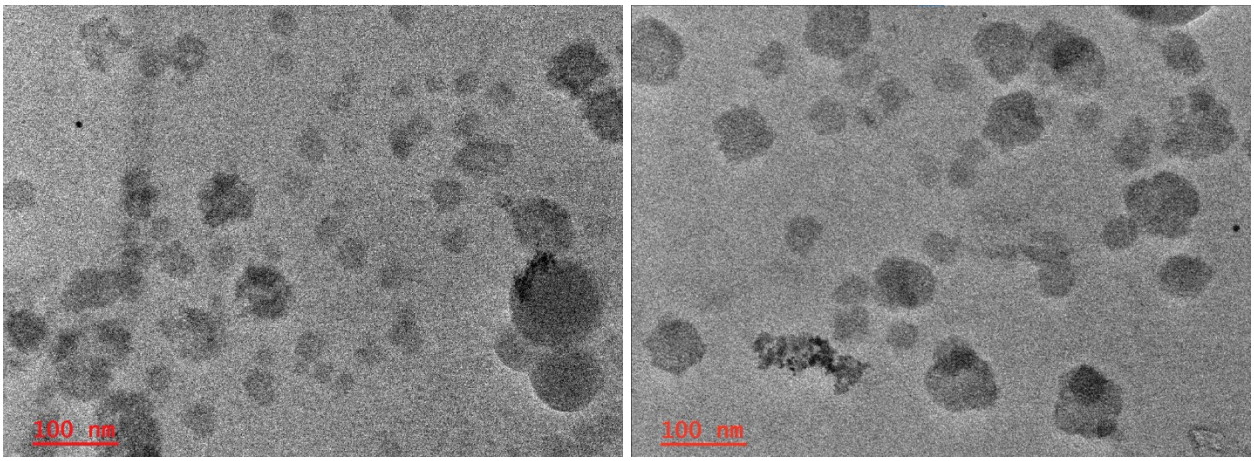

Figure S10. The GLC-TEM images for the PC liposomes with no cholesterol in ultrapure water (no salt).

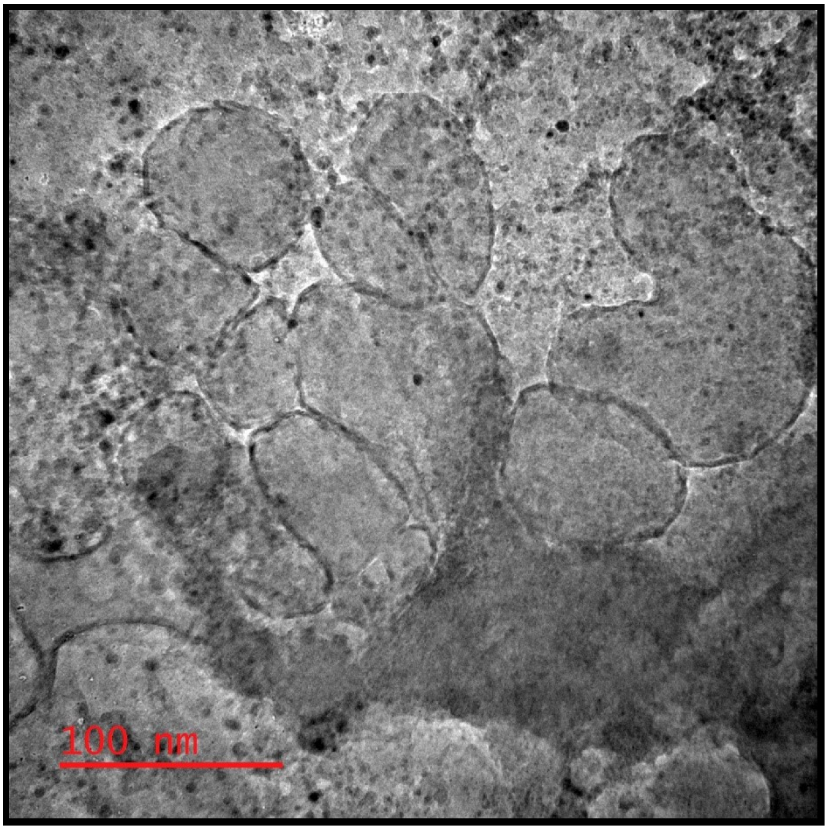

Figure S11. TEM image of a large liposomes with irregular shape formed by fusion of small liposomes, taken during in-situ GLC experiment.

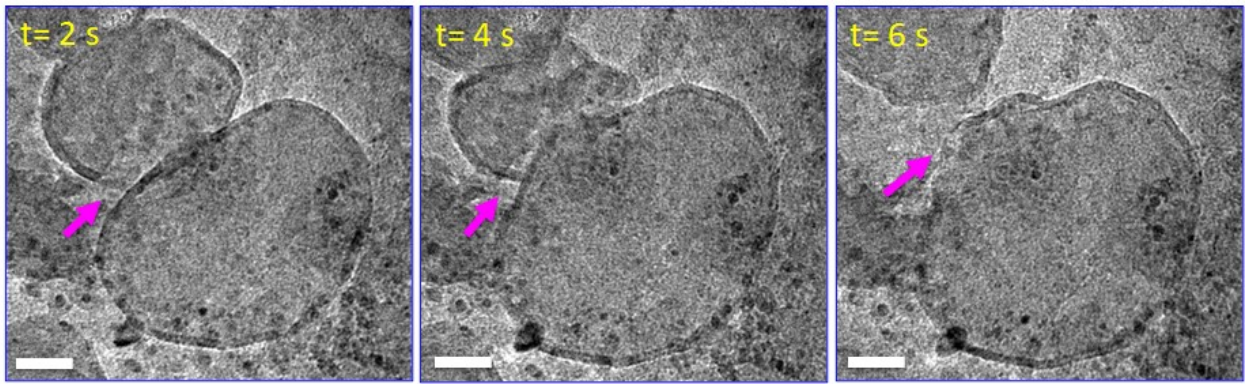

Figure S12. Denaturation of a small liposome during fusion with a larger liposome visualized in real-time by GLC-TEM. The constituting lipids of the small liposome integrate into the large liposome upon the membrane breakdown. White arrows point out the evolving interface between the two liposomes. The scale bar is 20 nm.

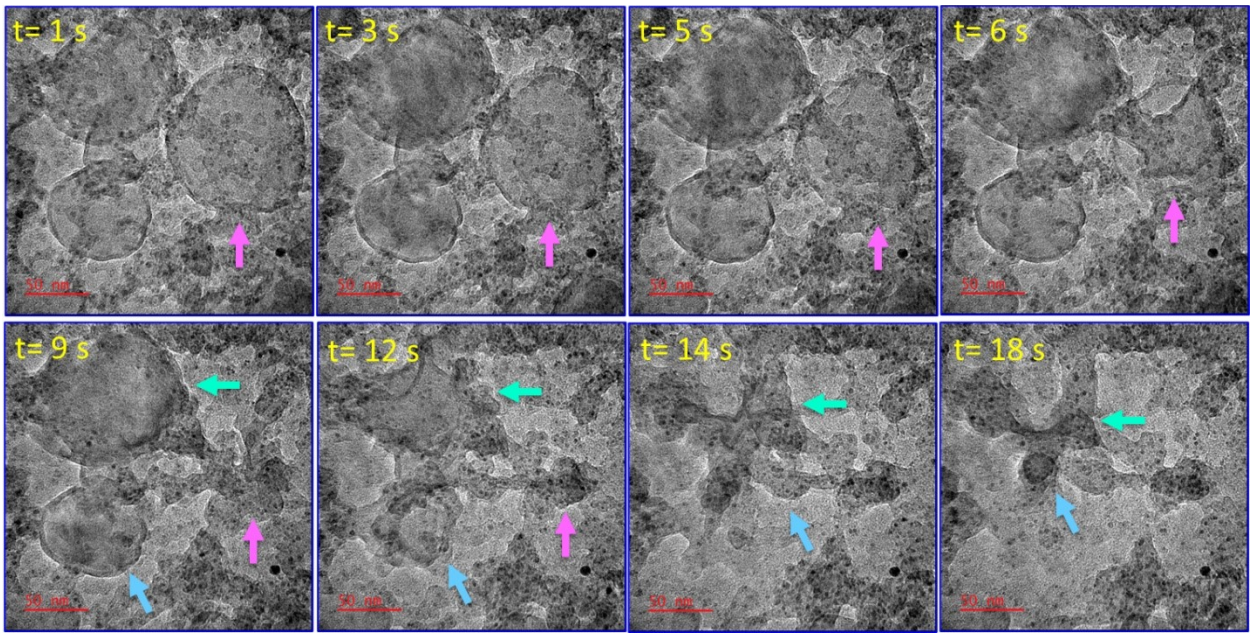

Figure S13. Time-lapsed TEM images of denaturation (breakdown) of liposomes visualized in real-time by GLC-TEM (a-e). Green, blue, and pink arrows point out different denaturing liposomes.

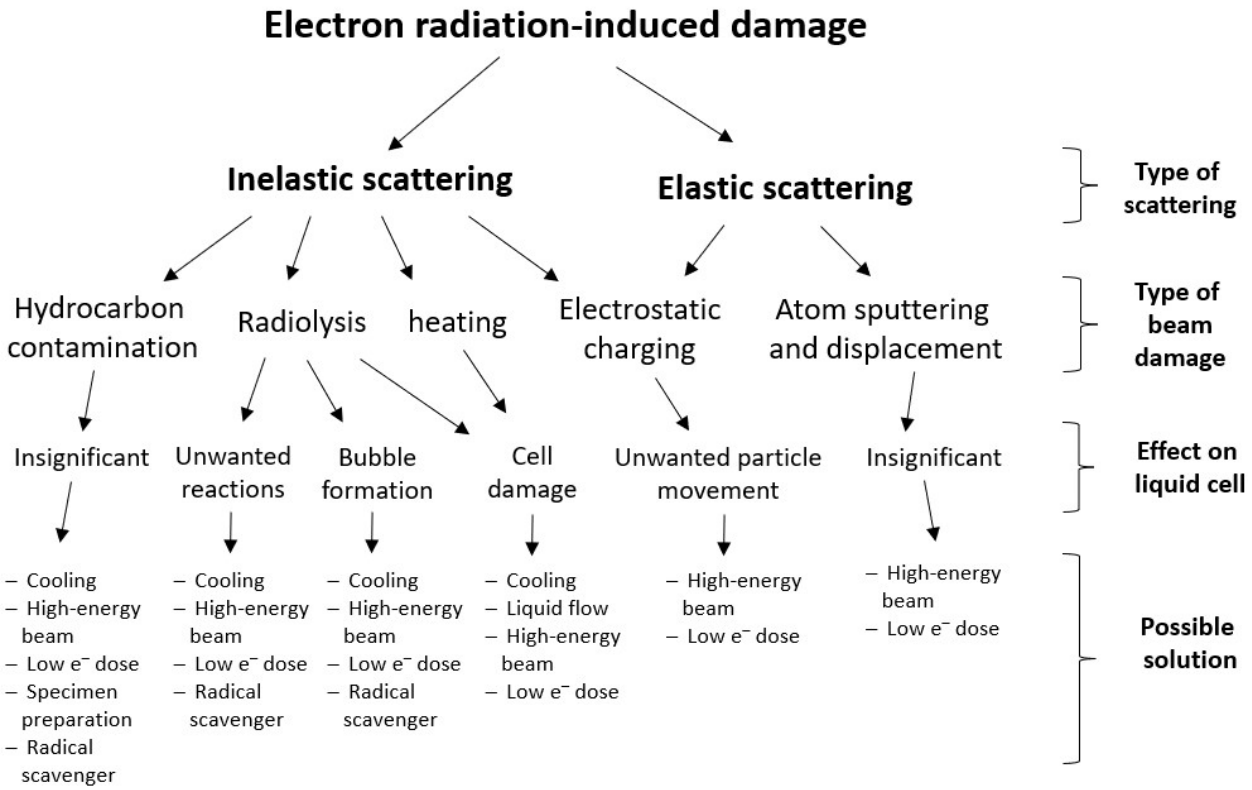

Figure S14. Different types of electron beam-induced damages and their corresponding effects on liquid cell-TEM.

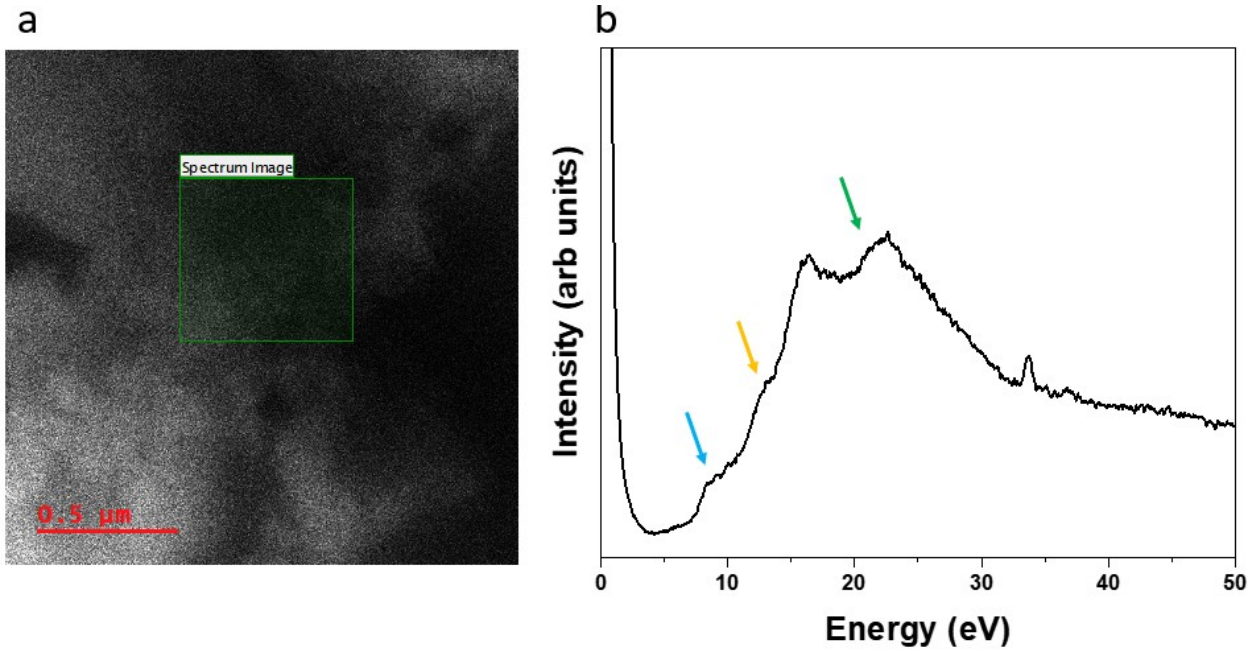

Figure S15. (a) HAADF STEM image of liposomes in GLC. The white contrast in the image represents the liquid (water). (b) Low-loss EELS representing the peaks at ~9 eV related to water exciton, ~14 eV related to graphene σ+π bond, and ~ 24 eV related to the plasma maxima of water shown with blue, orange, and green arrows, respectively.

Supporting Videos:

Video S1. Formation of liposomes of by molecular self-assembly of phosphatidylcholine lipid in aqueous solution, visualized *in situ* by GLC-TEM. Time-lapse TEM images shown in Figure 2 are taken from this video. The video is in real-time.

Video S2. Formation of liposomes of by molecular self-assembly of phosphatidylcholine lipid in aqueous solution, visualized *in situ* by GLC-TEM. Time-lapse TEM images shown in Figure S4 are taken from this video. The video is in real-time.

Video S3. MD simulations of phosphatidylcholine self-assembly. Simulation snapshots of 512 PC molecules in aqueous solution in a 19 nm periodic box. Red = zwitterionic PC fragments; Grey = hydrophobic PC fragments.

Video S4. MD simulations of phosphatidylcholine self-assembly. Analogous PC system in the presence of 128 cholesterol molecules. These images are taken from Video S4. Red = zwitterionic PC fragments; Grey = hydrophobic PC fragments; Green = cholesterol molecules

Video S5. Fusion of liposomes visualized *in situ* by GLC-TEM. Time-lapse TEM images shown in Figure 3 are taken from this video. The video is in real-time.

Video S6. Fusion of liposomes visualized *in situ* by GLC-TEM. The video is in real-time.

Video S7. Liposome denaturation visualized *in situ* by GLC-TEM. Time-lapse TEM images shown in Figure 4 are taken from this video. The video is in real-time.

Video S8. Simultaneously occurring fusion-denaturation of liposomes visualized *in situ* by GLC-TEM. Time-lapse TEM images shown in Figure 5 are taken from this video. The video is in real-time.

Video S9. A liquid cell desiccation under electron beam irradiation. As can be seen, a wet liquid cell (with a dark contrast and mobile small micelle-like structures) transform into a dry liquid cell (with a colorless or white contrast and still micelle-like structures).

Video S10. Bubble formation in GLC during TEM imaging. The video is in real-time.