Supporting information

Ring-shaped nanoparticle assembly and cross-linking on lipid vesicle scaffolds

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1. Materials and methods

Preparation of lipid vesicle suspension: Lipid vesicles were formed by the gentle hydration method described by Karlsson *et al*.[1]: 50 % w/w *E.coli* polar lipid extract, 49 % w/w soybean polar lipid extract and 1 % w/w 16:0 Liss Rhod PE (all from Avanti Polar Lipids, USA) were mixed in chloroform (Sigma Aldrich) reaching to a total concentration of 10 mg/ml. To remove the solvent, 300 µl of this chloroform solution was placed in a 10 ml round bottom flask in a rotary evaporator at reduced pressure (20 kPa) for 6 hours. The dry lipid film was rehydrated with 3 ml of PBS buffer containing 5 mM Trizma Base, 30 mM KH_2PO_4, 3 mM $MgSO_4*7H_2O$, 0.5 mM Na_2EDTA (pH of the PBS buffer was adjusted with KOH to 7.4) and 30 µl glycerol. The rehydrated lipid film was kept at +4 °C overnight, and was sonicated the next day for 10 seconds at room temperature to form the multilamellar lipid vesicle suspension. The deionised water was taken from a Milli-Q system (Millipore) for all buffers used in this work.

Surface fabrication: 84 nm thick SiO_2 films were deposited on glass cover slips #1 (Menzel Gläser) by reactive sputtering using a MS 150 Sputter system (FHR Anlagenbau GmbH) in the clean room facility MC2, at Chalmers University of Technology, Sweden.

Preparation of vesicle-nanotube networks: 4 µl of the lipid suspension described above was dehydrated on a glass cover slip in an evacuated desiccator for 20 min. The dry lipid film was rehydrated with 0.5 ml of HEPES buffer containing 10 mM HEPES and 100 mM NaCl (pH= 7.8, adjusted with NaOH) for 5 min, and transferred to the sample chamber via an automatic pipette. The observation chamber consisted of a SiO_2 surface submerged in ~1 ml of HEPES buffer containing 10 mM HEPES, 100 mM NaCl and 4 mM $CaCl_2$ (pH= 7.8, adjusted with NaOH). The vesicle networks developed autonomously in these conditions as described in detail in earlier work[2-4].

Preparation of nanoparticle suspensions: Carboxylate-modified fluorescent polystyrene nanoparticles (2% solids, Thermo Fisher Scientific, 0.1 µm, yellow-green fluorescence) were diluted 1:10 000 with the same Ca^{2+}-HEPES buffer in which the vesicle networks were developed. The particle suspensions were added to the vesicle networks via an automatic pipette. For the experiment shown in **SI Fig. 2** polystyrene particles with a diameter of 20 nm (2% solids, Thermo Fisher Scientific, 0.02 µm) were used with the same fluorescent label.

Carbodiimide chemistry-based cross-linking of nanoparticles: 0.1 gram of crystalline N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, Sigma-Aldrich) was dissolved in 1 mL of HEPES buffer resulting in a concentration of 0.1 g/mL (0.5 M). With an automatic pipette, 250 µl of this

EDC solution was added to the surface-adhered vesicle networks containing the particle assemblies. The sample chamber already contained ~1000 µL of HEPES buffer, therefore the final concentration of EDC therein reached 0.02 g/mL. 1 hour following the EDC addition, ethylenediamine (EDA, Sigma-Aldrich) solution was added to the sample. The EDA solution was prepared by adding 300 µl HEPES buffer to 0.07 mL of EDA (0.07 g/ml). 1 hour upon EDA addition, 10% detergent Triton X-100 (Sigma-Aldrich) was added to the sample chamber to dissolve and remove the lipid fraction. Subsequently, the whole liquid volume in the chamber was removed using a Pasteur pipette and replaced with deionized water (Milli-Q, Millipore, USA). The last step was repeated a few times until all excess lipid or particle residues are removed.

Microscopy imaging. An inverted confocal laser scanning microscopy system (Leica SP8, Germany), with an HC PL APO CS2 40x oil objective (NA: 1.30) was used for acquisition of the confocal images. The excitation/emission wavelengths used for the imaging were as follows: for yellow-green fluorospheres, λ_{ex}: 505 nm, λ_{ex}: 515 nm, for lipid-conjugated Rhodamine λ_{ex}: 560 nm; λ_{em}: 583 nm. The grayscale images for fluorescence emission channels of the particles were arbitrarily false colored in green in the Leica microscope system's original acquisition software.

Image analyses: Leica Application Suite X Software (Leica Microsystems, Germany) was used to perform the fluorescence intensity based image analyses on the confocal micrographs and the graphs were plotted in Matlab. Schematic drawings and figures were created with Adobe Illustrator (Adobe Systems, USA).

2. Extended Figure 1

In extended **SI Fig. 1** we provide multiple micrographs corresponding to **Fig. 1** in the main script in xy plane. Panel A shows the cross section of the equator of the vesicles in a lipid patch, where panel B shows the cross section at the base of the vesicles. Panel C: particle assemblies around the vesicle bases. The 3D confocal image in Panel D is identical to **Fig. 1I**, where the region framed in dashed lines corresponds to the region in the frames shown in **SI Fig. 1A-C**.

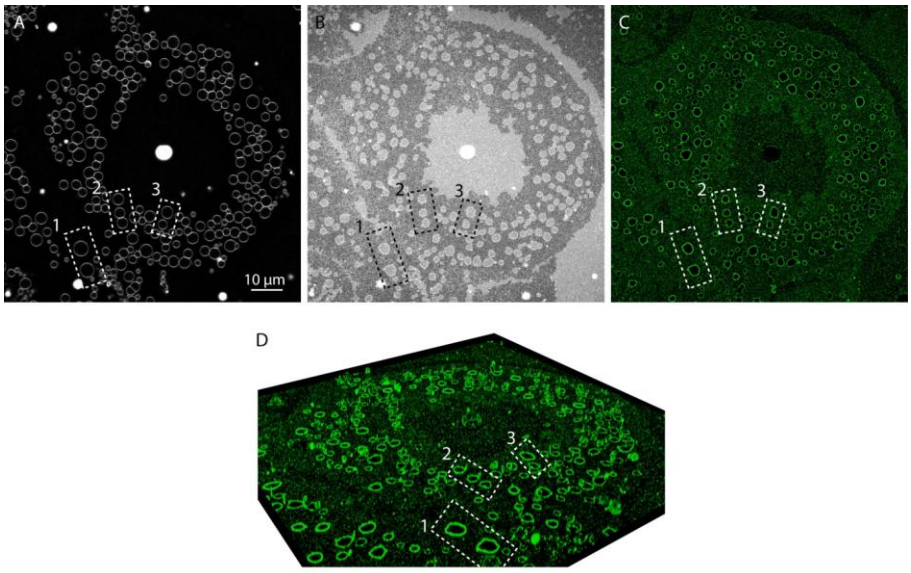

Figure S1 Extended Panel (I) of Figure 1 of the main article. (A-C) Confocal micrographs showing, in the xy plane, the cross section of the (A): equator of the surface-adhered vesicles, (B): base of the surface-adhered vesicles, (C): nanoparticle assemblies. (D) 3D confocal micrograph corresponding to (C), the panel is identical to the panel (I) of Figure 1 of the main article.

3. Size-analyses of self-assembled rings from particles with a diameter of 20 nm

SI Figure 2 shows size analyses of the ring-like structures assembled from polystyrene particles of 20 nm diameter. The results are in agreement with the analyses shown in **Fig. 1J** which indicates that the nanoparticle assemblies are localizing along the base of the vesicles. Here, the ratio of the vesicle diameter over the diameter of the vesicle base is higher, therefore the offset of the purple line over the black dashed line is larger. This can be due to the denser pinning between the vesicles and the underlying bilayer caused by Ca^{2+} present in the aqueous ambient solution[2].

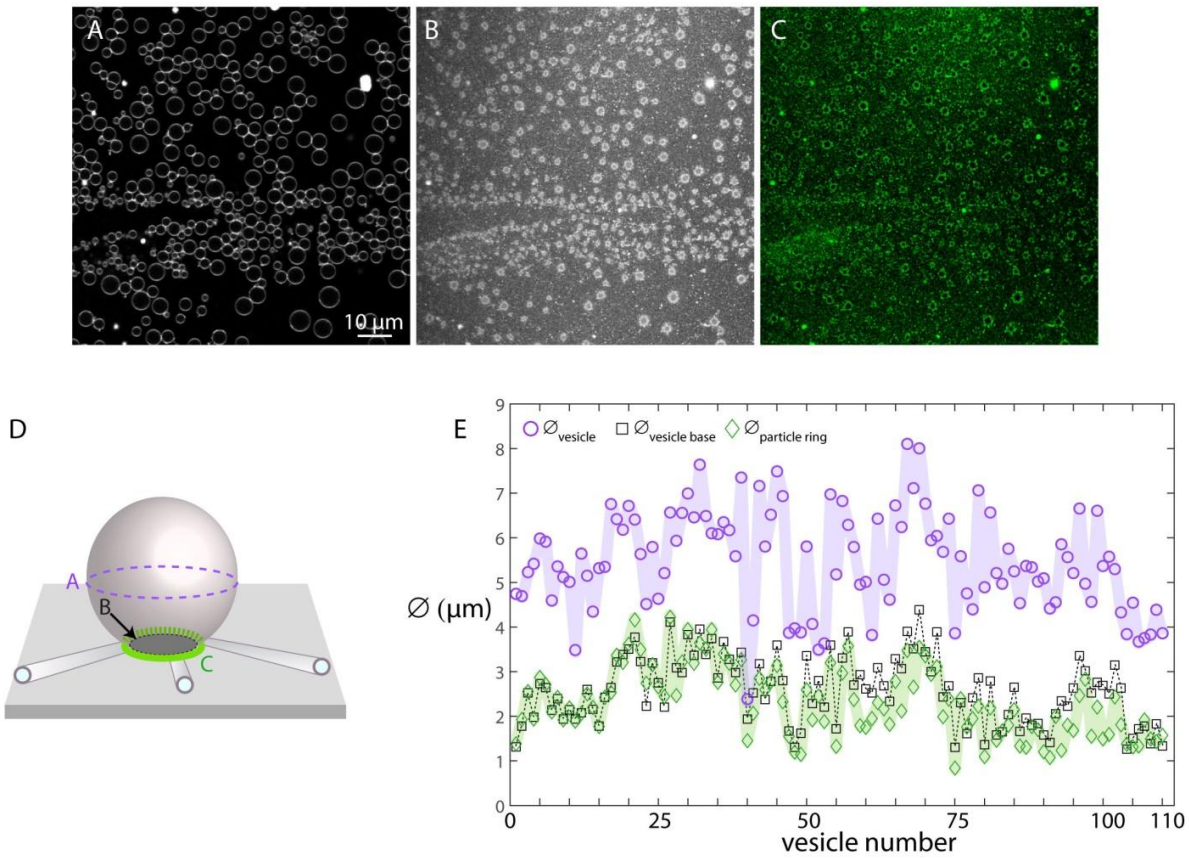

Figure S2 Size analyses of ring-like assemblies from particles of 20 nm diameter. (A-C) 2D confocal micrographs showing the cross section of (A): the equator of the surface-adhered vesicles, (B): the base of the surface-adhered vesicles, (C) :nanoparticle assemblies from particles with a diameter of 20 nm. (D) Schematic drawing showing the circumference of the three different diameters analyzed from panels (A-C). The diameter of the cross section of the equator of the vesicle is indicated with a purple-colored dashed line in panel D, where the diameter of the vesicle base is indicated with a black-colored dashed line, and the diameter of the particle assemblies is indicted with a green line. (E) Size analyses corresponding to (A-C). The graph shows the diameter of 110 vesicles from (A-C), and of the corresponding vesicle bases and particle assemblies. The diameter of the particle rings is shown with green diamond-shaped data markers, the diameter across the equator of the vesicles in the network (indicated by the purple dashed lines in panel D) is shown in purple round data markers, and the diameter of the base of the vesicles in the network is shown with black squares (indicated by the black dashed lines in panel D). The lines connecting the data points in each data set are to facilitate the comparison between the data sets.

4. Examples of confocal microscopy images after key cross-linking steps and detergent exposure

Confocal micrographs of the vesicle-nanoparticle assembly system upon EDC, EDA and Triton X addition are shown in **SI Figure 3**. EDC features a dimethyl ammonium ion moiety, which may interact with negative headgroup charges, which would explain membrane deformations as seen in **SI Fig. 3A**. This

appears to be a relatively mild interaction which is well tolerated as the vesicle integrity remains. EDA is commonly employed to solubilize proteins[5] and was previously reported to act as a binding agent within a phospholipid monolayer or between bilayers[6]. The EDA addition in our system leads to the agglomeration of lipid vesicles (orange arrows in **SI Fig. 3D**).

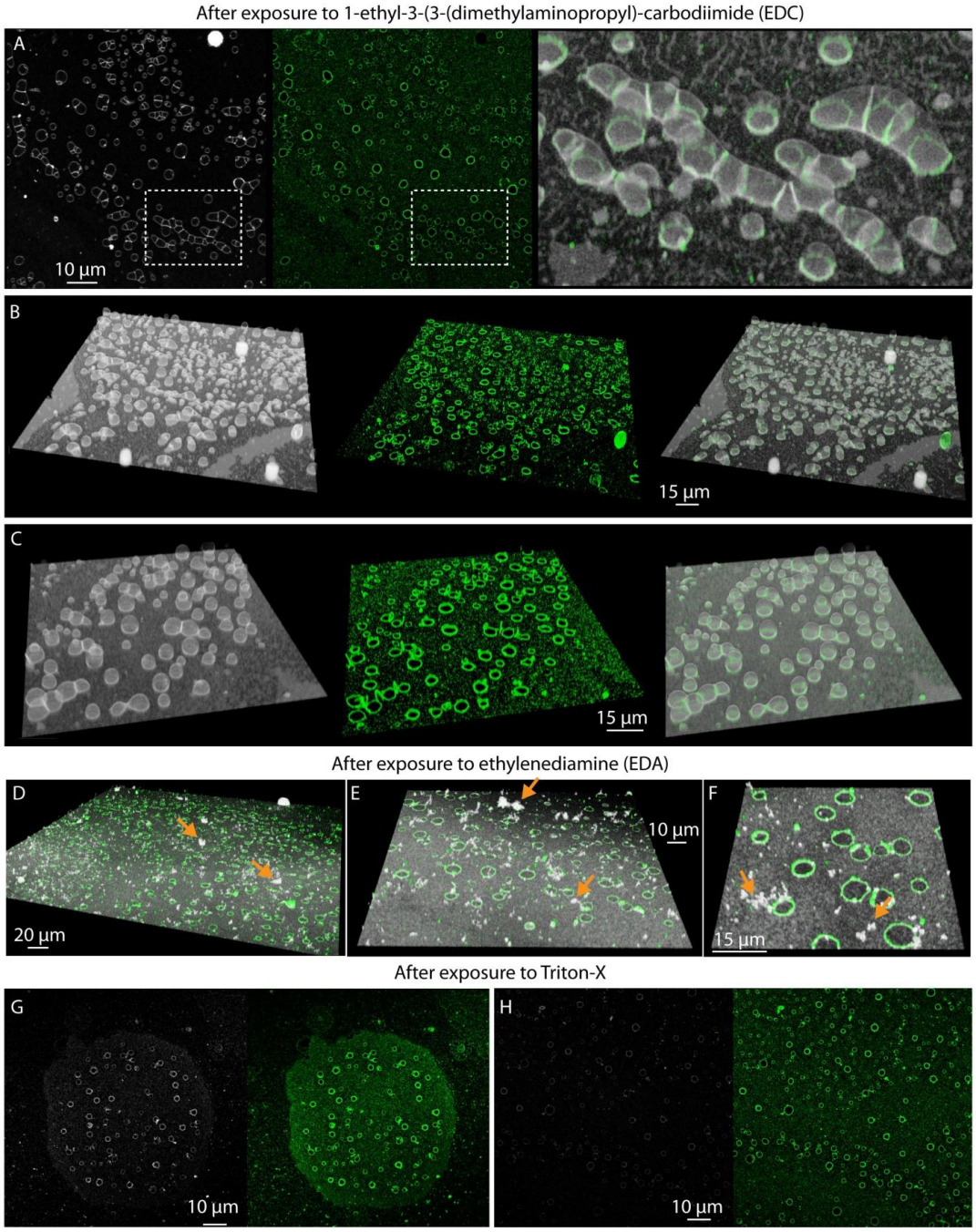

Figure S3 Confocal microscopy images after key cross-linking steps and detergent exposure. (A-C) Confocal micrographs showing three different sample regions after EDC exposure: lipid vesicles (left panel), nanoparticle assemblies (middle panel), lipid vesicles and particle assemblies overlaid (right panel). (D) Confocal micrographs showing three different sample regions after EDA exposure. Arrows

indicate lipid agglomerates.(G-H) Two different sample regions after detergent exposure upon particle cross-linking. The lipid fluorescence emission channel is on the left, and the particle fluorescence emission channel is on the right of each panel.

5. Inter-vesicle nanoparticles assemblies

SI Figure 4 contains the confocal micrographs showing the inter-vesicular formation of nanoparticle assemblies. In this case, the rings align between two adjacent vesicles, vertically with respect to the planar surface underneath. Panel A and B shows two examples of inter-vesicular rings between a large and relatively smaller lipid vesicle. In Panel C there are three rings assembled between 4 vesicles and in panel D there are two rings aligned among 3 vesicles.

Upon cross-linking of nanoparticles and removal of lipids, some of these inter-vesicular rings remained on the surface, however they collapse as the lipid vesicles they were adhering to were dissolved (panel E). This indicates that the lipid compartments were mechanically supporting the inter-vesicular rings.

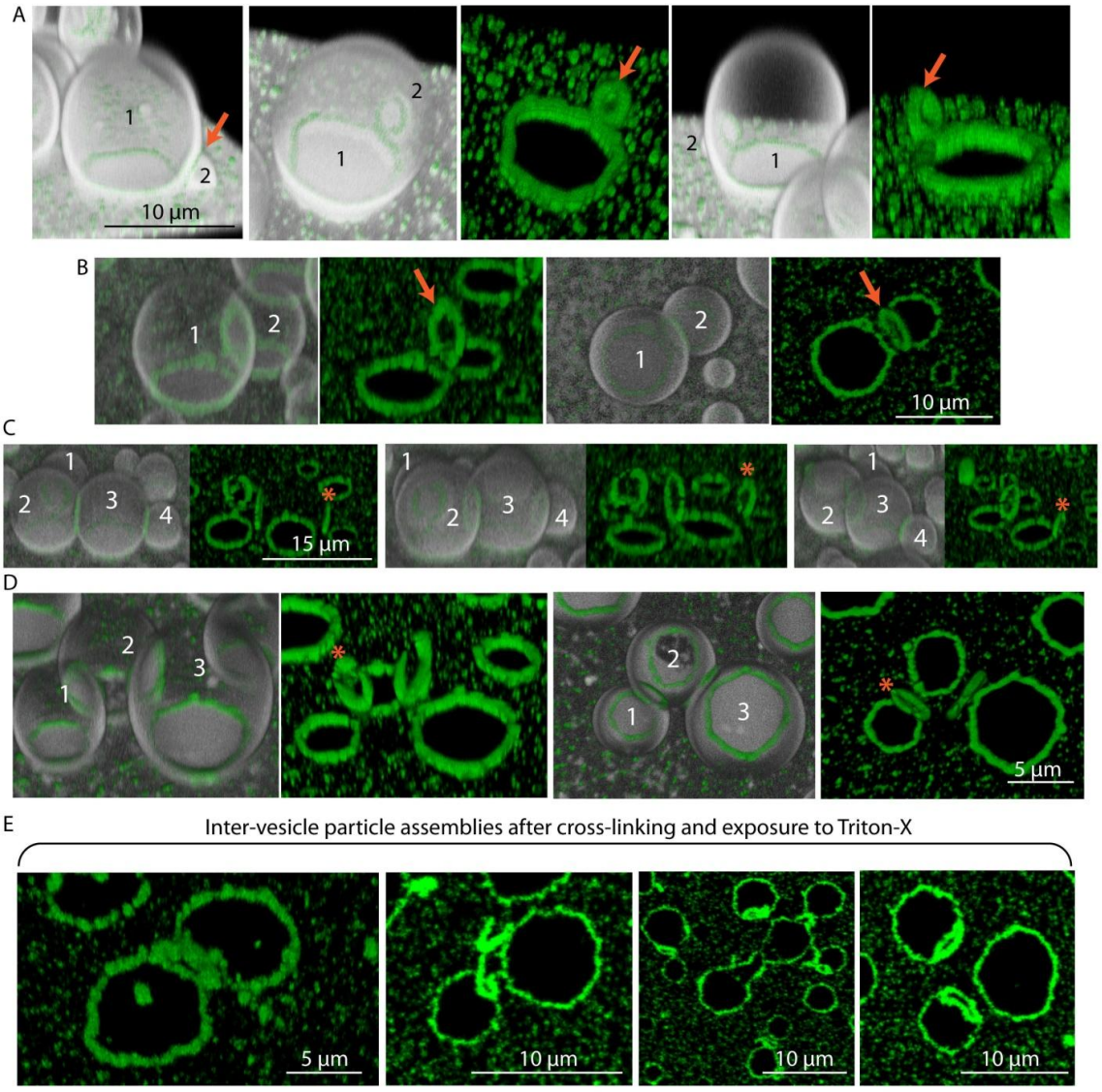

Figure S4 Inter-vesicle nanoparticle assemblies. Confocal microscopy images showing the nanoparticle assemblies forming at the interface between adjacent vesicles. (A-D) each panel shows the same vesicle/vesicle group and their associated particle assemblies from different angles. Same vesicles in different micrographs of each panel are labeled with the same number. (E) Four different sample regions showing the inter-vesicle particle assemblies after cross-linking and detergent exposure.

References

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