Supplementary figures

Figure S1

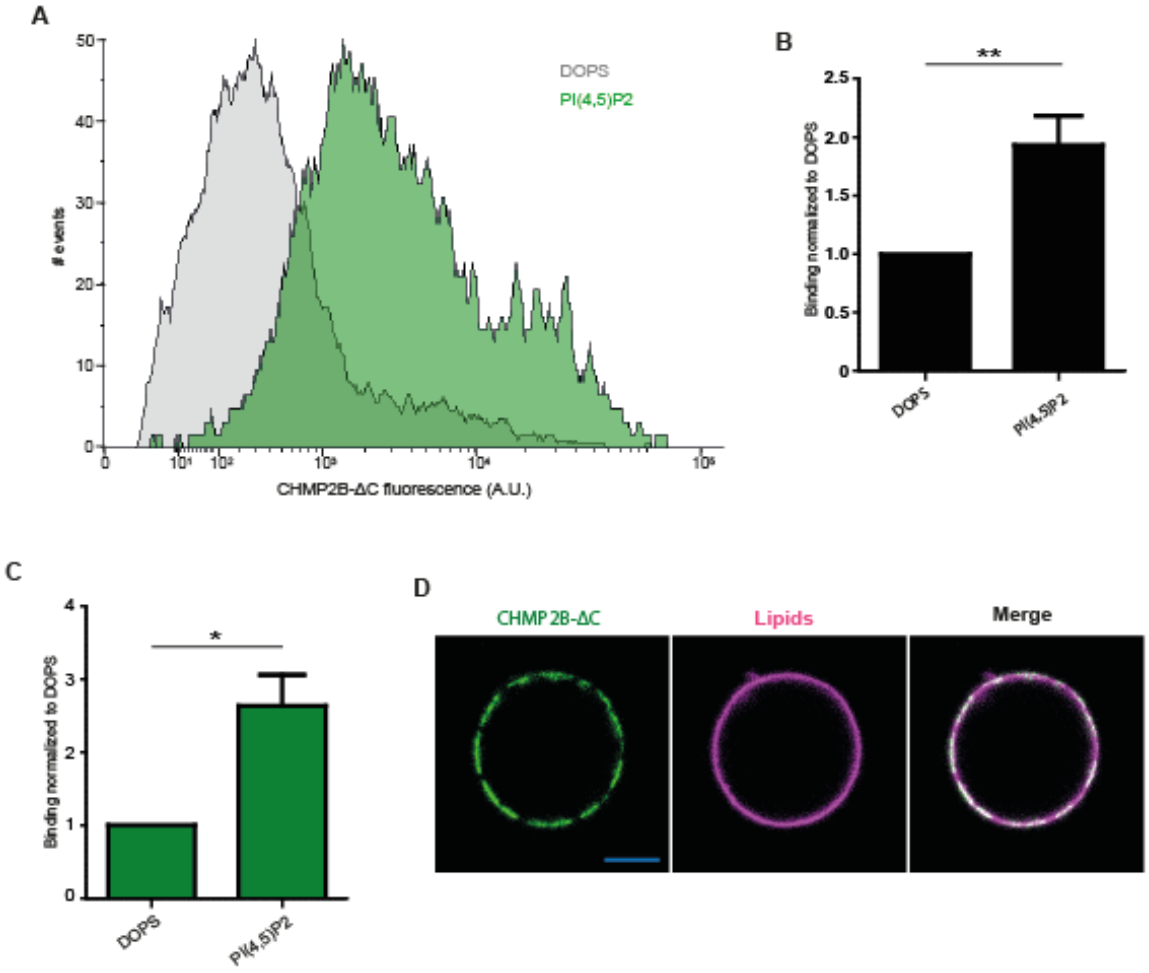


Figure S1: membrane binding specificity of CHMP2B

- **S1A:** histograms obtained by flow-cytometry of CHMP2B-ΔC binding to GUVs containing either DOPS or PI(4,5)P2. CHMP2B fluorescence is represented on a logarithmic scale. Each event represent a single GUV.
- **S1B:** Quantification of CHMP2B-FL binding to GUVs containing DOPS and PI(4,5)P2 by flow cytometry. Equimolar amount of DOPS and PI(4,5)P2 (2% of total lipids) have been used. **=p-value<0.01 (Student's t-test); n=6.
- **S1C:** Quantification of CHMP2B-ΔC binding to GUVs containing DOPS and PI(4,5)P2 by flow cytometry. Equal net charge has been achieved by using 6% and 2%

of DOPS and PI(4,5)P2, respectively, according to (Visco, Hoege et al. 2016). *=p-value<0.01 (Student's t-test); n=5.

- **S1D:** Transient protein patches formed by CHMP2B-ΔC in PI(4,5)P2-containing GUVs. Scale bar: 10µm

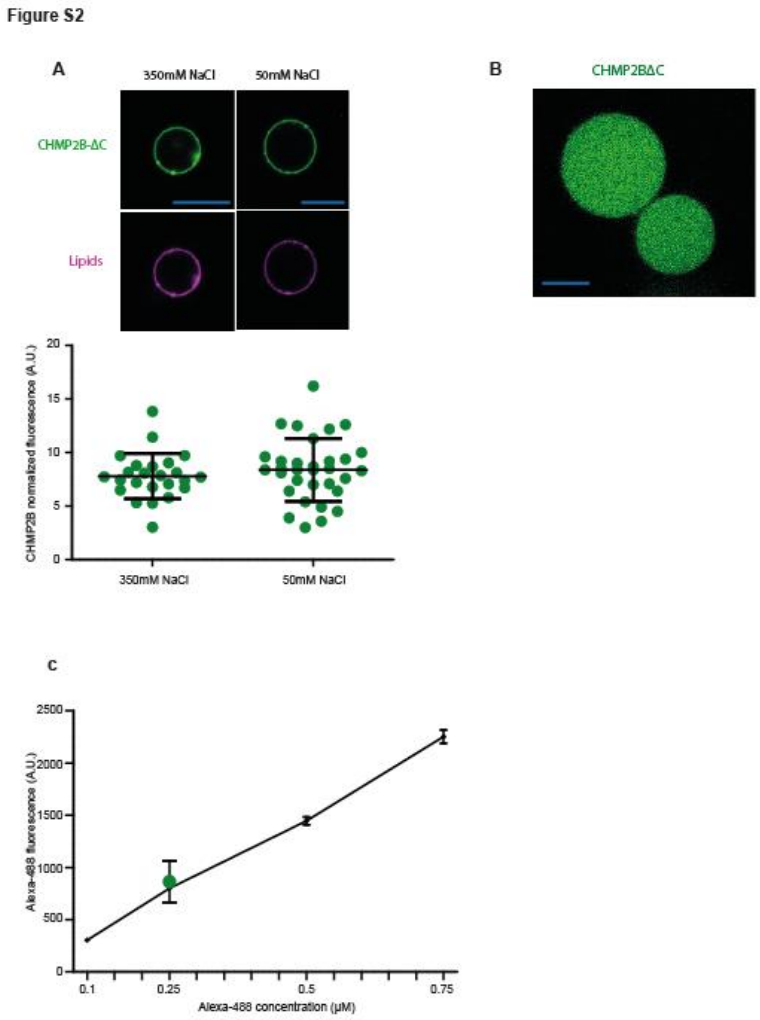


Figure S2: encapsulation of CHMP2B in GUVs

- **S2A**: no detachment of CHMP2B-ΔC from a PI(4,5)P2-containing GUV upon incubation in high salt buffer. Scale bar: 10µm
- **S2B**: encapsulation of CHMP2B-ΔC in GUVs devoid of PI(4,5)P2. Scale bar: 10µm.
- **S2C**: level of fluorescence of CHMP2B-ΔC-Alexa488 encapsulated inside GUVs (green dot) as compared with a titration curve of Alexa-488 (black line). The labelling efficiency for CHMP2B-ΔC is 95%, hence the protein concentration inside GUVs is ≈250nM. See methods for more details.

Figure S3

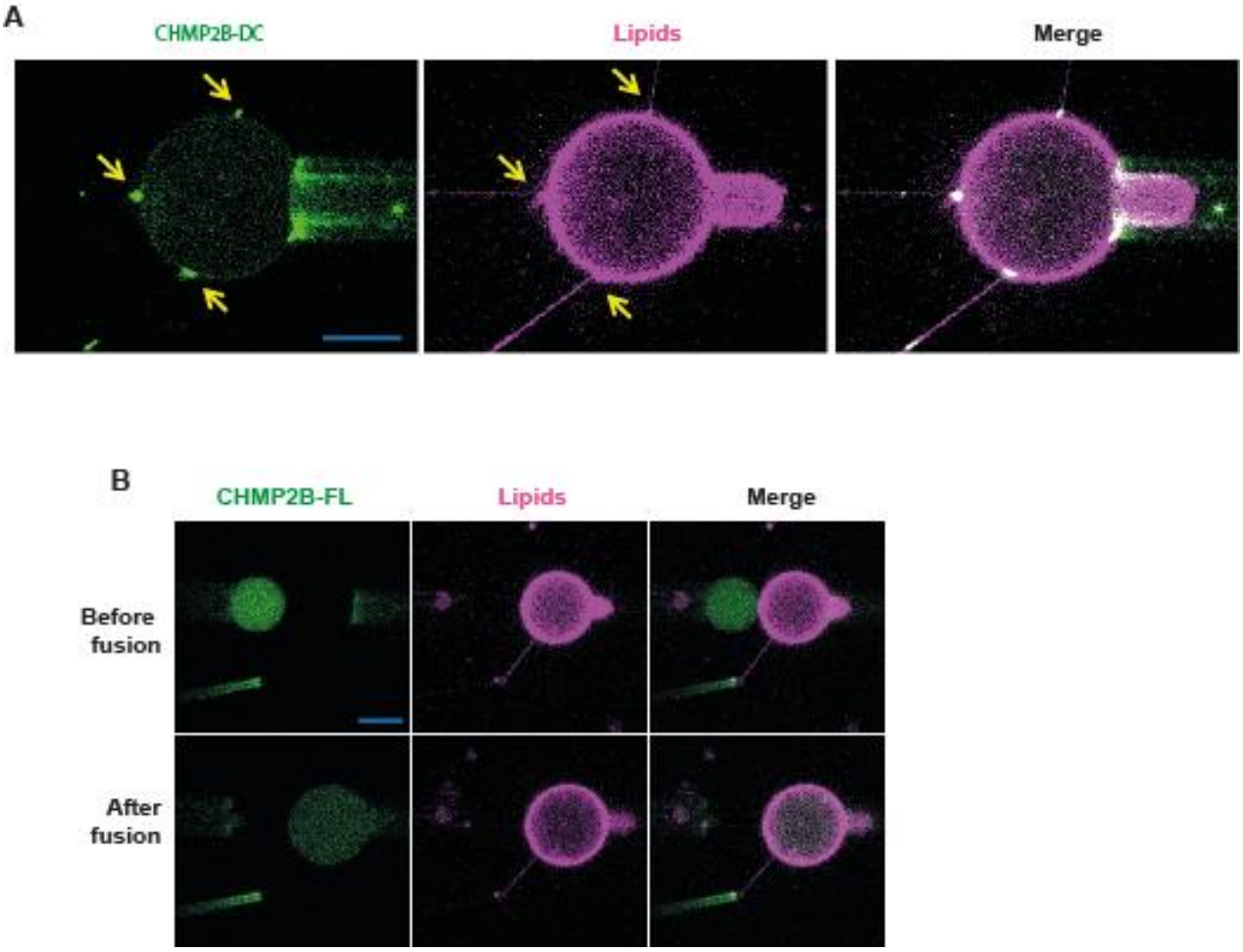


Figure S3: CHMP2B clusters localization at the base of membrane nanotubes.

- **S3A**: CHMP2B-ΔC clusters localizing at the base of membrane nanotubes after fusion. In this particular example, three nanotubes emanate from the GUV resulting from fusion. The lipid signal has been increased in order to visualize the position of the nanotubes, which exhibit a weak signal due to their small diameter. Arrows indicate the position of the necks. Scale bar: 10µm
- **S3B**: Fusion of GUVs containing CHMP2B-FL and PI(4,5)P2. Scale bar: 10µm.

Figure S4

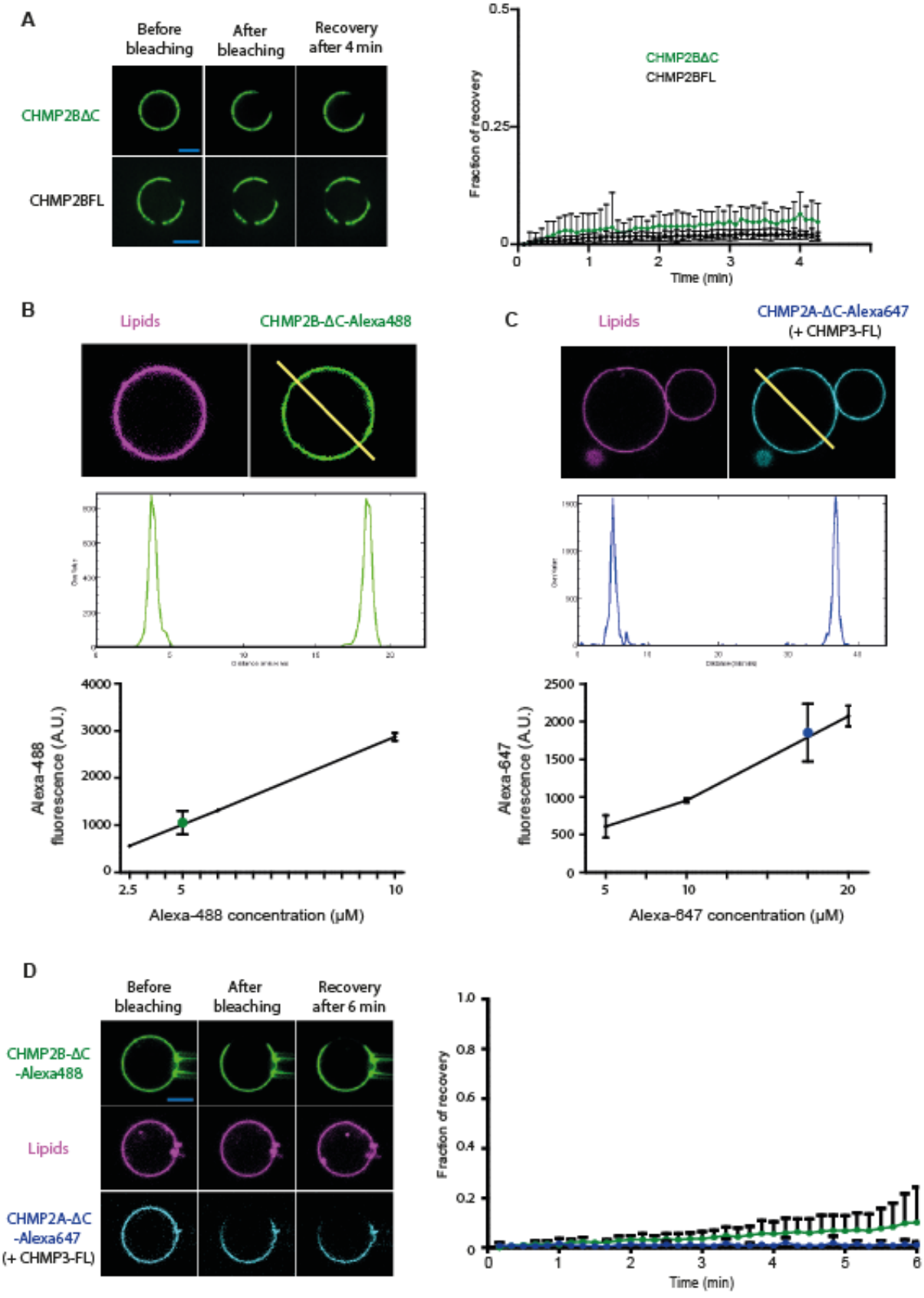


Figure S4: comparison between diffusion properties of CHMP2B-ΔC and CHMP2B-FL polymers.

- **S4A**: FRAP experiment on CHMP2B-ΔC and CHMP2B-FL bound to the external leaflet of GUVs containing PI(4,5)P2. Soluble proteins in bulk have been removed by dilution. Protein polymers were bleached on the side of the GUV and protein recovery measured over time. One confocal plane is shown. Scale bar: 10µm.
- **S4B:** measurement of CHMP2B-ΔC density at GUV surface. The peak of Alexa-488 dye intensity, corresponding to the GUV outer surface, was measured by line scan and averaged (13 GUVs). This value was compared to a standard curve of soluble Alexa-488. A degree of labeling of CHMP2B-ΔC of 95% results in a protein density at GUV surface corresponding to a bulk concentration ≈4µM.
- **S4C:** measurement of CHMP2A-ΔC density at GUV surface. The peak of Alexa-647 dye intensity, corresponding to the GUV outer surface, was measured by line scan and averaged (24 GUVs). This value was compared to a standard curve of soluble Alexa-647. A degree of labeling of CHMP2A-ΔC of 78% results in a protein density at GUV surface corresponding to a bulk concentration ≈23µM.
- **S4D**: FRAP experiment on a co-polymer of CHMP2B-ΔC-Alexa488/CHMP2A-ΔC-Alexa647/CHMP3-FL(unlabelled) bound to the external leaflet of GUVs containing PI(4,5)P2. Soluble proteins in bulk have been removed by dilution. Protein polymers were bleached and protein recovery in both 488 and 647 channels was measured over time. One confocal plane is shown. Scale bar: 10µm.

Figure S5

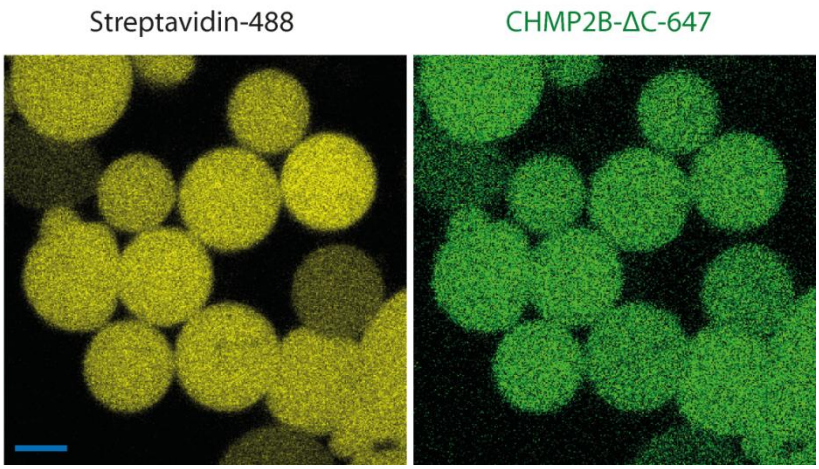
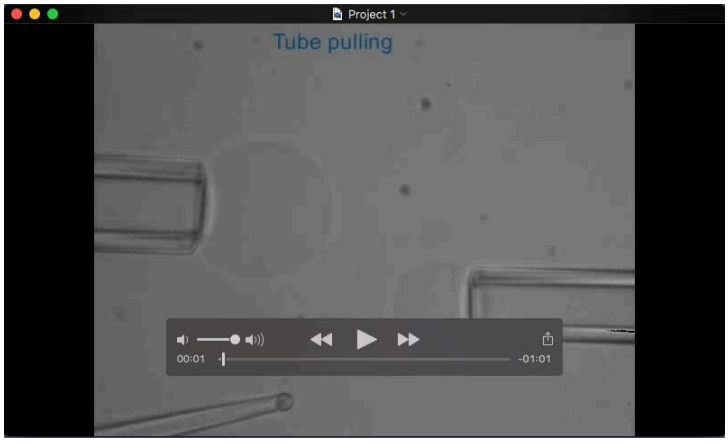


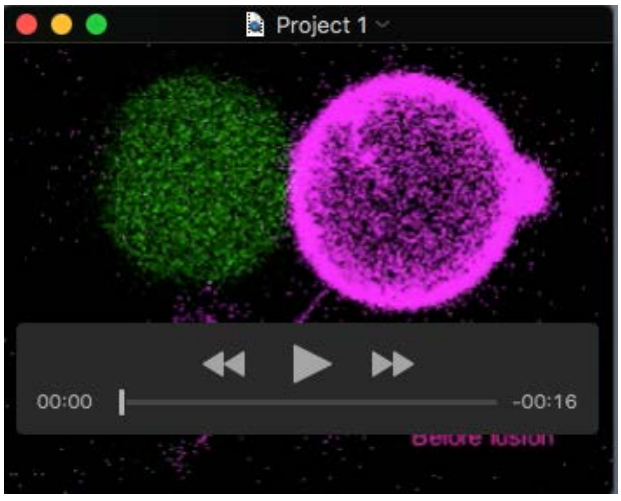
Figure S5: streptavidin and CHMP2B co-encapsulation.

Representative image of co-encapsulation of Alexa-647 labelled CHMP2B-ΔC and Alexa-488 labelled streptavidin in EPC vesicles. Scale bar: 10µm.



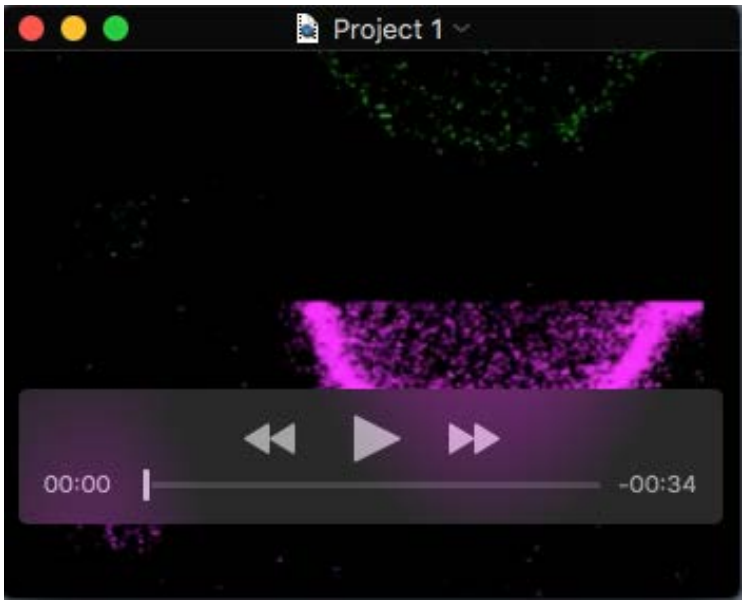
Movie 1: example of GUV fusion.

The movie shows the whole process of GUV fusion. Initially, a nanotube is pulled. Then the two GUVs are brought in contact and their position is then adjusted so that the contact point is aligned with the optical tweezers. For sake of time, this part of the video has been speeded up 4x. Upon activation of the optical tweezers, instantaneous fusion occurs. This part of the video has been slowed down 5x.

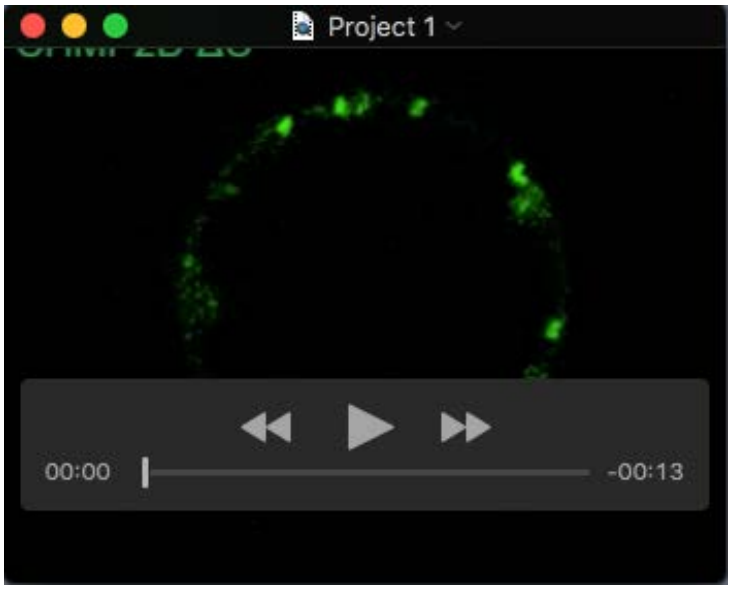


Movie 2: formation of CHMP2B-ΔC clusters upon fusion.

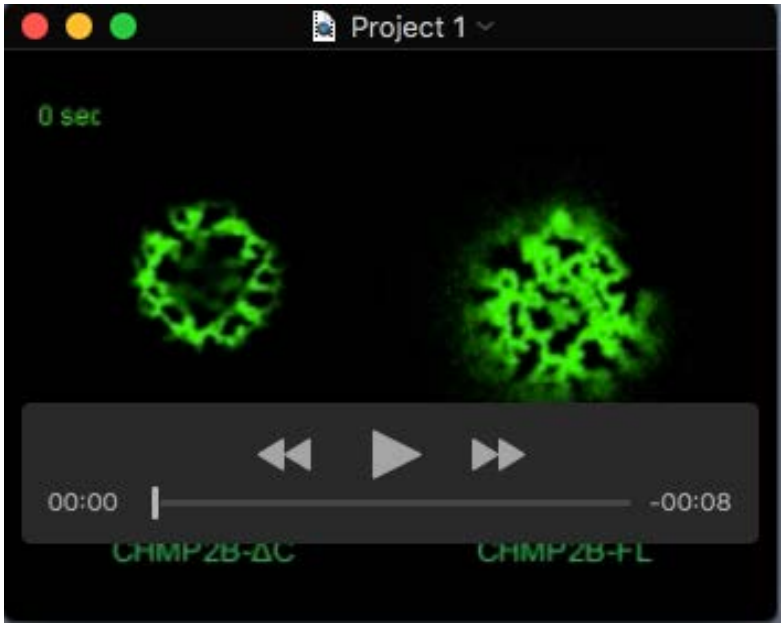
The movie shows a still image of the GUVs before fusion, and subsequently the evolution in time of CHMP2B-ΔC binding and formation of clusters. Only the protein channel has been acquired to allow for maximal time resolution. Time frame: 2sec.



Movie 3: engagement of CHMP2B-ΔC clusters at the neck.
The movie shows that the CHMP2B-ΔC cluster are freely diffusing in the membrane, until they reach the nanotube neck. Time frame: 2sec.

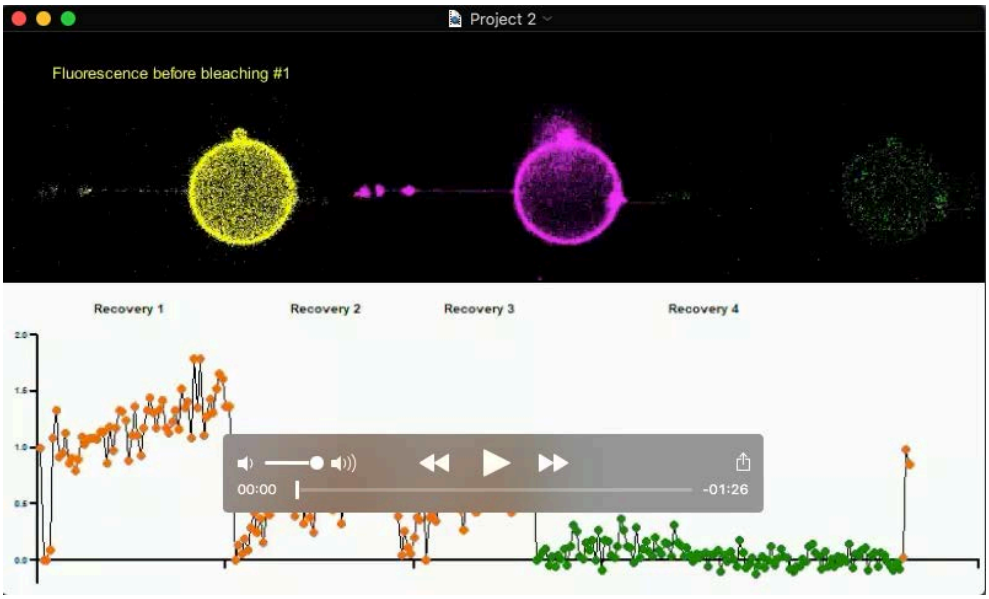


Movie 4: CHMP2B-ΔC clusters formed on the outer leaflet of GUVs are freely diffusing
The movie shows diffusion of CHMP2B-ΔC clusters obtained by short incubation at low protein concentration on the outer leaflet of a GUV. Time frame: 2sec.



Movie 5: FRAP experiment on CHMP2B reticulum.

The movie shows FRAP experiments on GUVs incubated with either CHMP2B-ΔC (left) or CHMP2B-FL (right), in condition of GUV partial coverage. A region corresponding to the upper spherical cap of the GUV is shown.



Movie 6: example of streptavidin recovery in nanotube upon fusion with a GUV containing CHMP2B-ΔC.

The movie shows four sequential bleaching and recovery events on a nanotube with streptavidin bound to the inner leaflet. Streptavidin, lipids and CHMP2B- ΔC are depicted in yellow, magenta and green, respectively. In the first three FRAP events, no CHMP2B cluster is visible at the neck, and streptavidin recovers very quickly. At the end of the third recovery, a CHMP2B cluster happens to localize at the neck region. After a subsequent bleaching, no recovery of streptavidin is observed. However, as soon as the CHMP2B cluster moves away from the neck, quick recovery occurs. Frame rate: 5fps. The movie has been paused before and after each bleaching event to better visualize the fluorescence of the nanotube. The plot in the lower part of the movie displays the quantification of streptavidin fluorescence in the nanotube during the recovery, normalized for the fluorescence before each bleaching event. Green and orange dots indicate frames in which the CHMP2B cluster was localized at the neck or not, respectively.