

SUPPLEMENTARY INFORMATION

Title: *Membrane permeabilization by the Bordetella Adenylate Cyclase Toxin involves growing-size pores of toroidal characteristics*

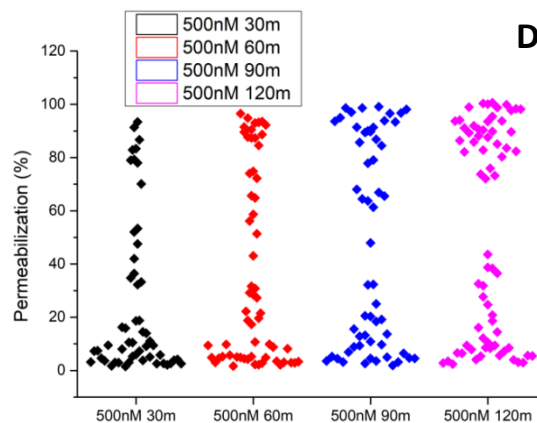
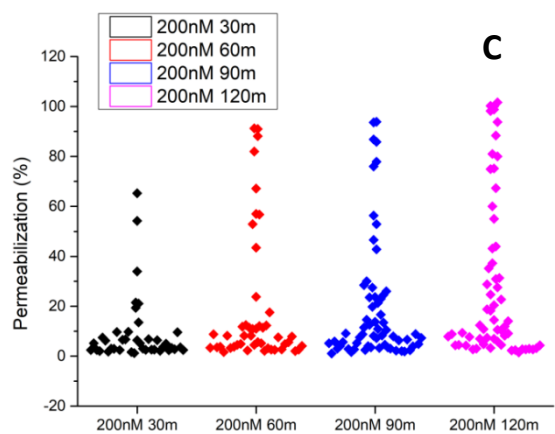
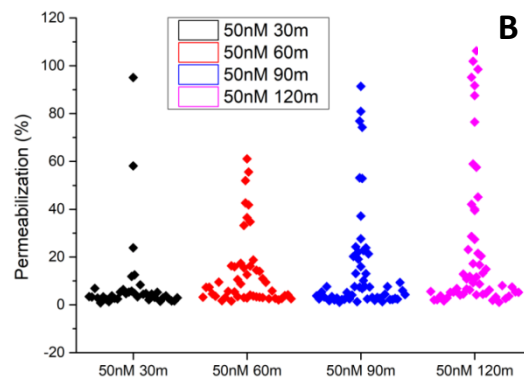
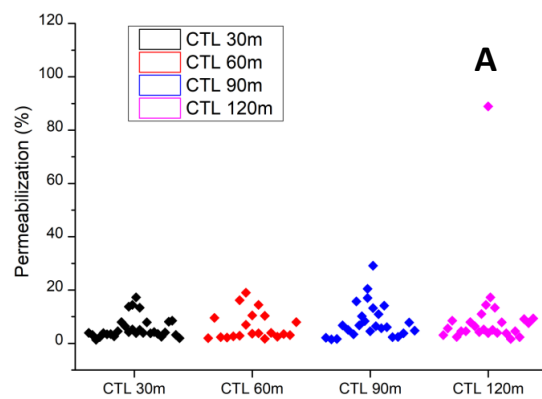
Authors: *David González-Bullón, Kepa B. Uribe, Eneko Largo, Garazi Genbelzu, Aritz B. García Arribas, César Martín and Helena Ostolaza*

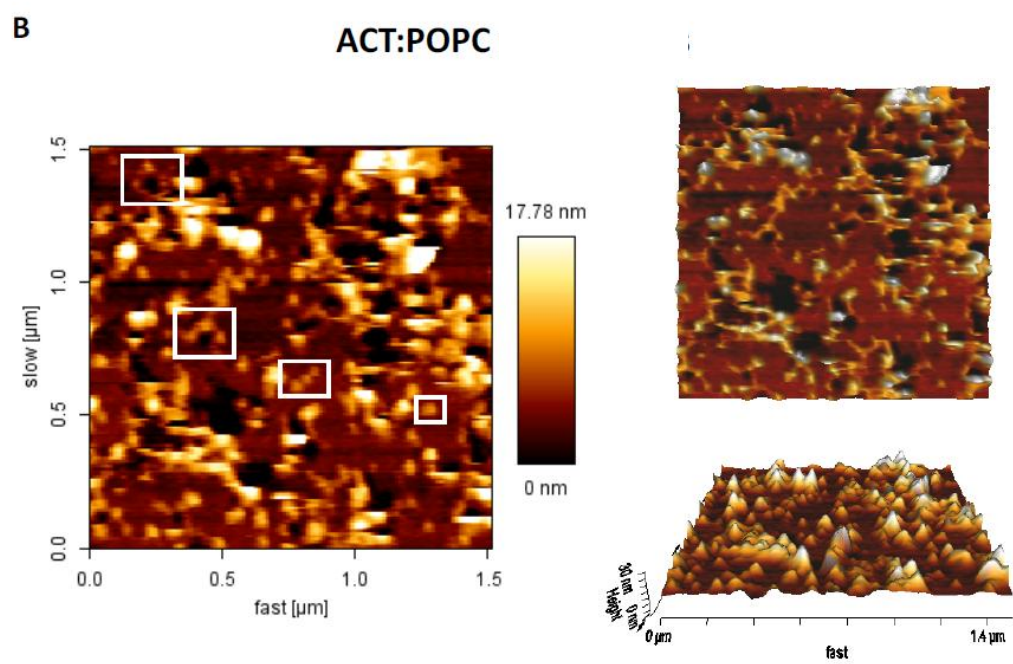
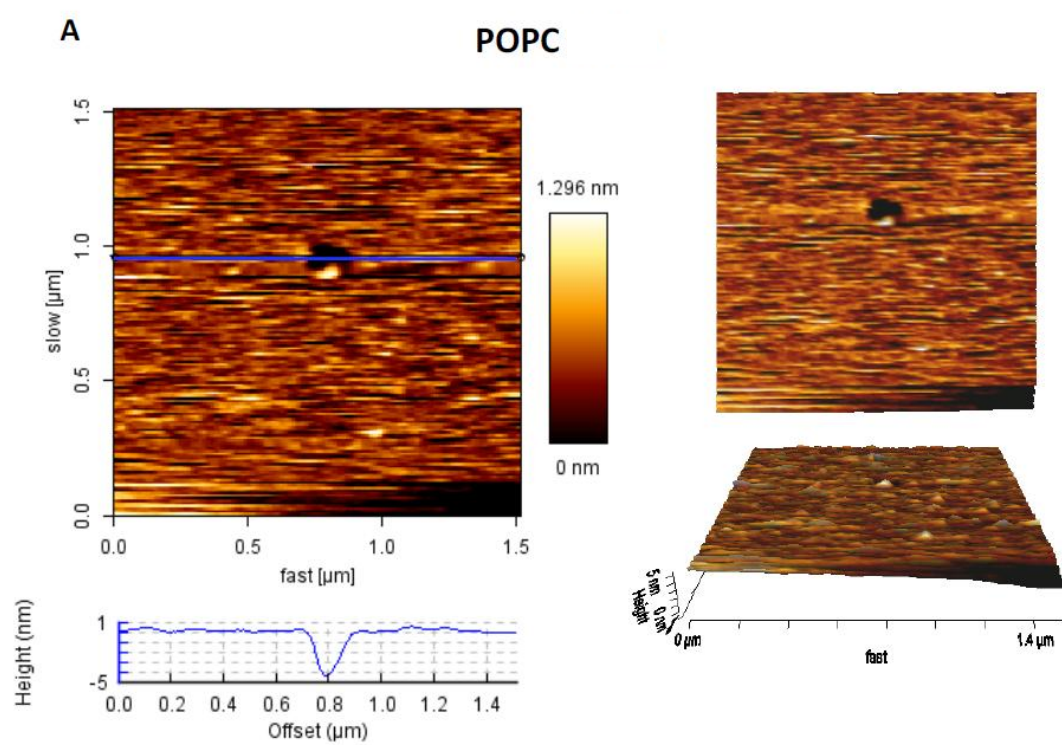
Supplementary Figure S1. Permeabilization of GUVs for the 10kDa-Dextran-FITC determined at different incubation times and toxin concentrations. DOPC giant unilamellar liposomes (GUVs) at RT were exposed to control buffer (CTL) or to ACT toxin at three different concentrations (50, 200 and 500 nM) and for different times (30 to 120 min). The external medium contained the fluorescently labelled 10 kDa-Dextran-FITC in buffer with CaCl_2 10 mM. Vesicle permeabilization (influx of the fluorescent solute) was visualized by confocal microscopy. The filling degree of the vesicles, represented in the figure as percentage of permeabilization, was calculated, for each incubation time and each toxin concentration, from the fluorescence values obtained from ≈ 200 individual vesicles, as detailed in the Experimental Procedures section.

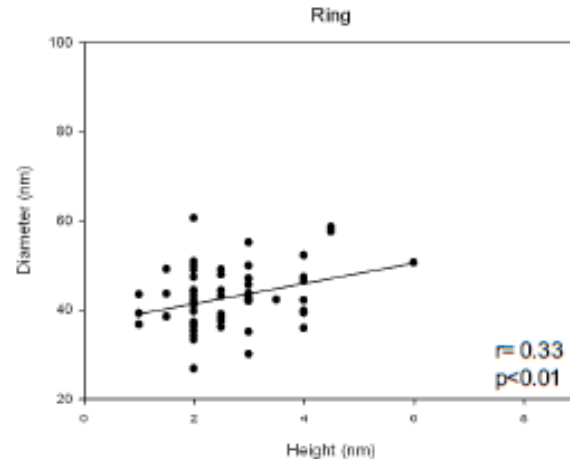
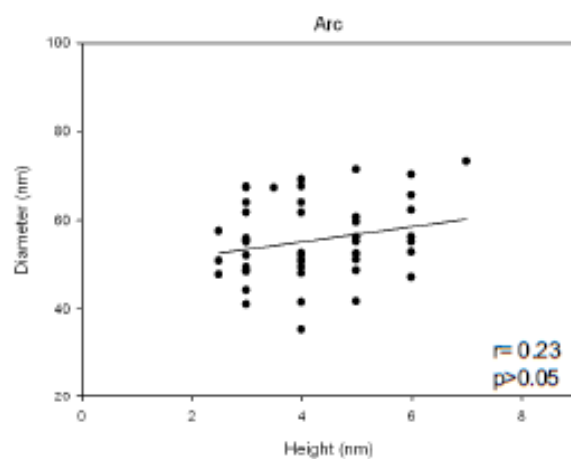
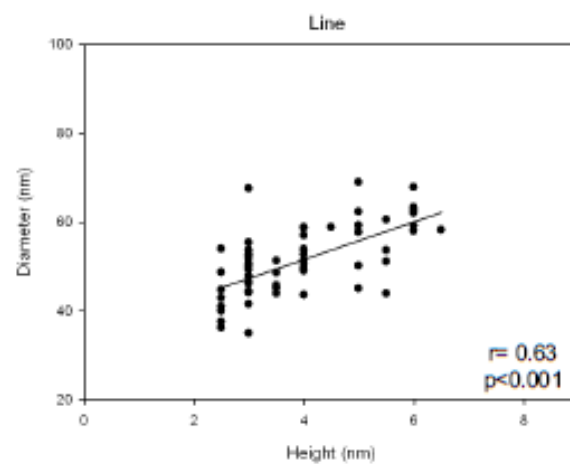
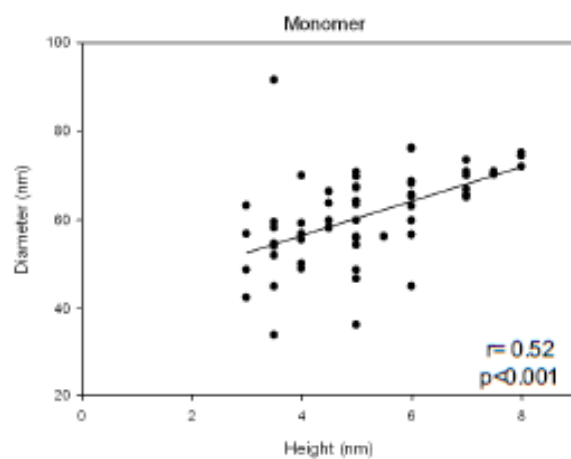
Supplementary Figure S2. . Analysis by AFM of control POPC and ACT:POPC supported bilayers. AFM image of a supported lipid bilayer extended from a control sample of POPC liposomes, or from ACT:POPC proteoliposomes, and analyzed with AFM (room temperature). The topographical analysis of the bilayer is shown below each one of the images. On the right side of each panel a lateral view is shown.

Supplementary Figure S3. Correlation between the monomeric particle diameter and particle height for the different ACT assemblies visualized by atomic force microscopy. Graphical representations of the correlation between the monomeric particle diameter (in nm) and the monomeric particle height (in nm) for the different ACT assemblies (monomer, lines, arcs and full rings) visualized by atomic force microscopy.

Supplementary Figure S4. Comparative analysis by BN-PAGE of CR3 (+) and CR3(-) cells exposed to ACT. CR3(+) J774A.1 cells (1×10^6 cells/ml) or CR3(-) cells (1×10^6 cells/ml) were incubated with ACT (30 nM) at 37°C and then the separated membranes were electrophoresed by BN-PAGE, blotted into a nitrocellulose membrane and stained with anti-ACT MAb 9D4. Several protein bands of apparent high molecular masses (≈ 800 and 1000 kDa) were resolved in both cell membranes, corresponding most likely to ACT oligomers of variable stoichiometry.







J774A.1

1236 kDa —

1048 kDa —

720 kDa —

480 kDa —



J774A.1

CR3⁻

1236 kDa —

1048 kDa —

720 kDa —

480 kDa —

