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Selective ion–permeable membranes by insertion of biopores into polymersomes

Supporting Information

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Size, morphology and stability of $A_6B_{44}A_6$ polymersomes - TEM and LS characterization

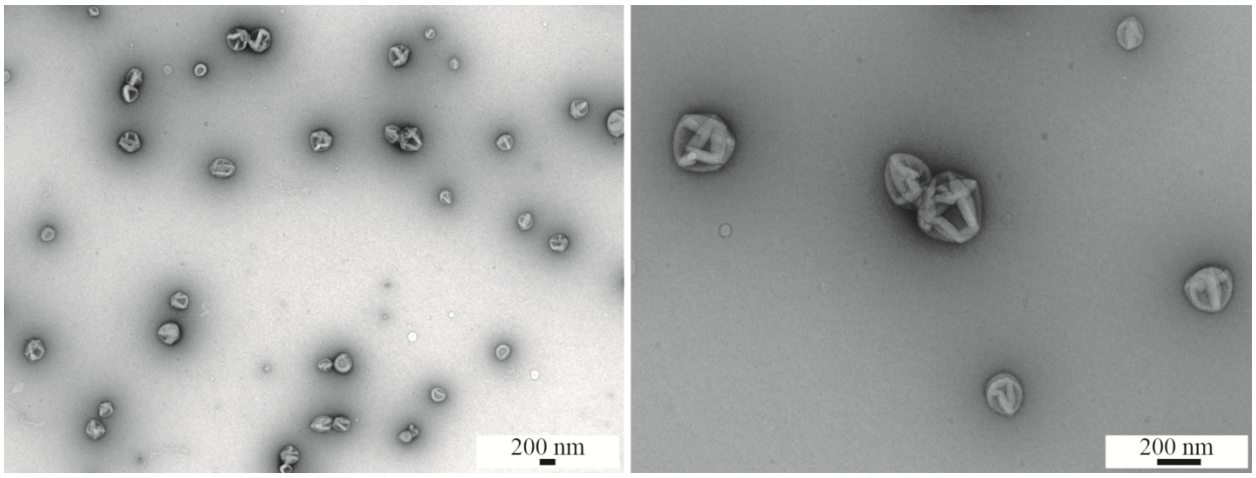

Fig. S1 TEM micrograph of ACG loaded polymersomes of $A_6B_{44}A_6$ in the presence of 3.74 μM ionomycin: sample overview (left) and zoom in micrograph (right).

Table S1 Molecular parameters of 3-D assemblies of $PMOXA_6$-$PDMS_{44}$-$PMOXA_6$ without and with ionomycin, and in the presence of 830 μM $CaCl_2$.

Size and stability of calcium sensing nanodevices	DLS/SLS*			
	$M_{w,agg}x10^{-8}$ [g/mol] **	$N_{agg}x10^{-4}$**	A_2x10^8 [mol·dm^3/g^2] **	PDI**
$A_6B_{44}A_6$	1.82	4.04	- 9.44	0.09
$A_6B_{44}A_6$ + ionomycin	5.75	12.77	- 0.14	0.12
$A_6B_{44}A_6$ + ionomycin + $CaCl_2$	4.43	9.84	- 0.06	0.09

*Dynamic – and static light scattering experiments in dilute polymersome solutions;

** $M_{w,agg}$ – apparent molecular weight, N_{agg} – the average aggregation number, A_2 – second virial coefficient, and PDI – polydispersity index for polymersomes.

Table S2 Surface (ζ) Potential

Parameters	ACG*	Empty $A_6B_{44}A_6$ polymersomes	$A_6B_{44}A_6$ polymersomes with entrapped ACG	$A_6B_{44}A_6$ polymersomes with entrapped ACG + 3.74 µM Ionomycin	$A_6B_{44}A_6$ polymersomes with entrapped ACG + 3.74 µM Ionomycin + $CaCl_2$
ζ [mV]	- 7.54	5.43	4.56	2.04	2.31
±SD, n=3	1.67	0.30	0.43	0.24	0.33

*Asante Calcium Green;

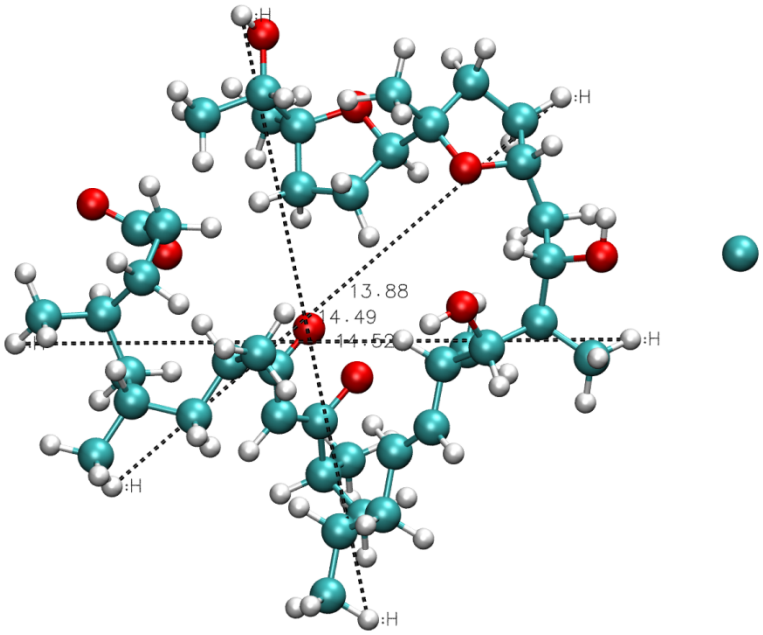

Fig. S2 Size estimation of ionomycin molecule, taking into account the molecule diameter as the largest distance from one end to another.

Behaviour of fluorescence intensity of free ACG

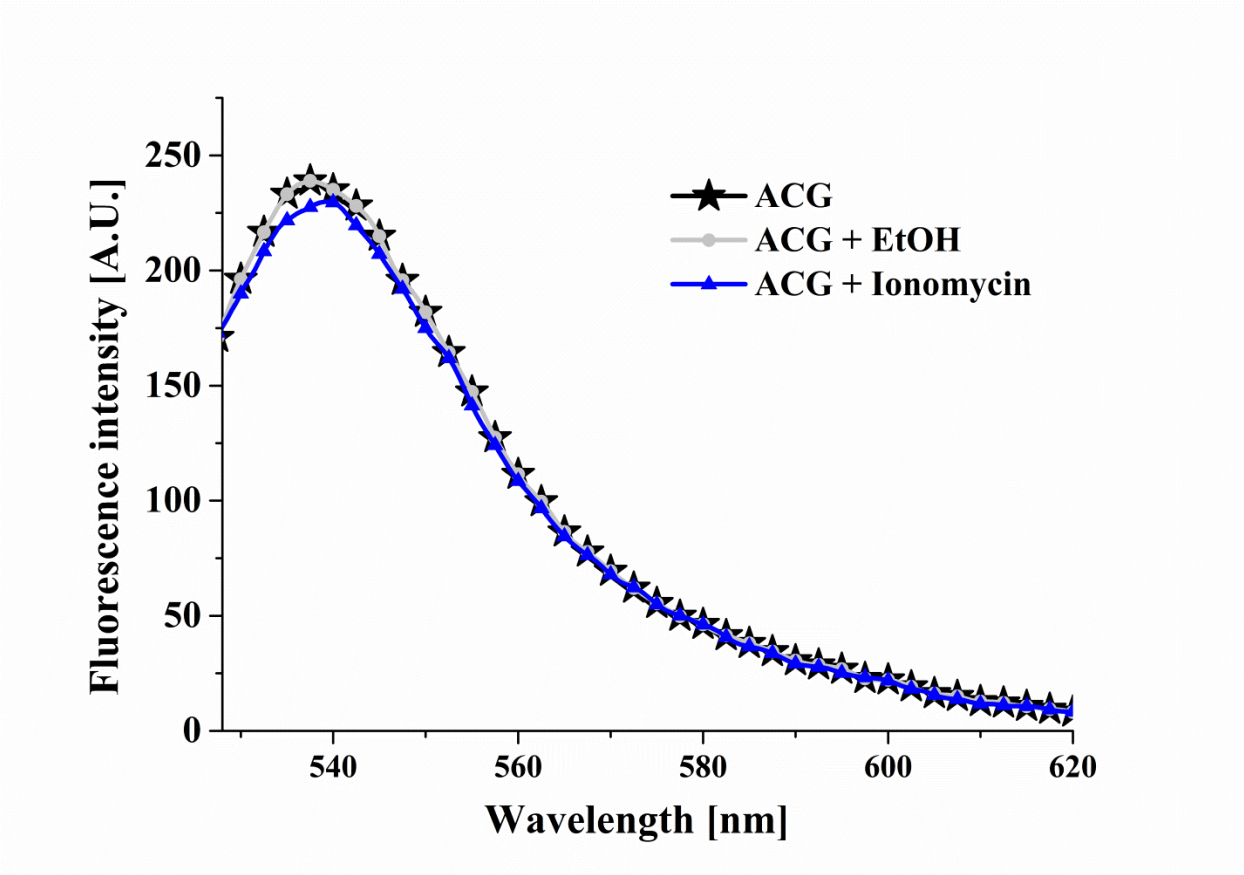

Fig. S3 Influence of EtOH and ionomycin upon fluorescence intensity increase of the free Asante Calcium Green (ACG) solution in the presence of 830 μM $CaCl_2$.

Polymersomes with inserted and functional ionomycin

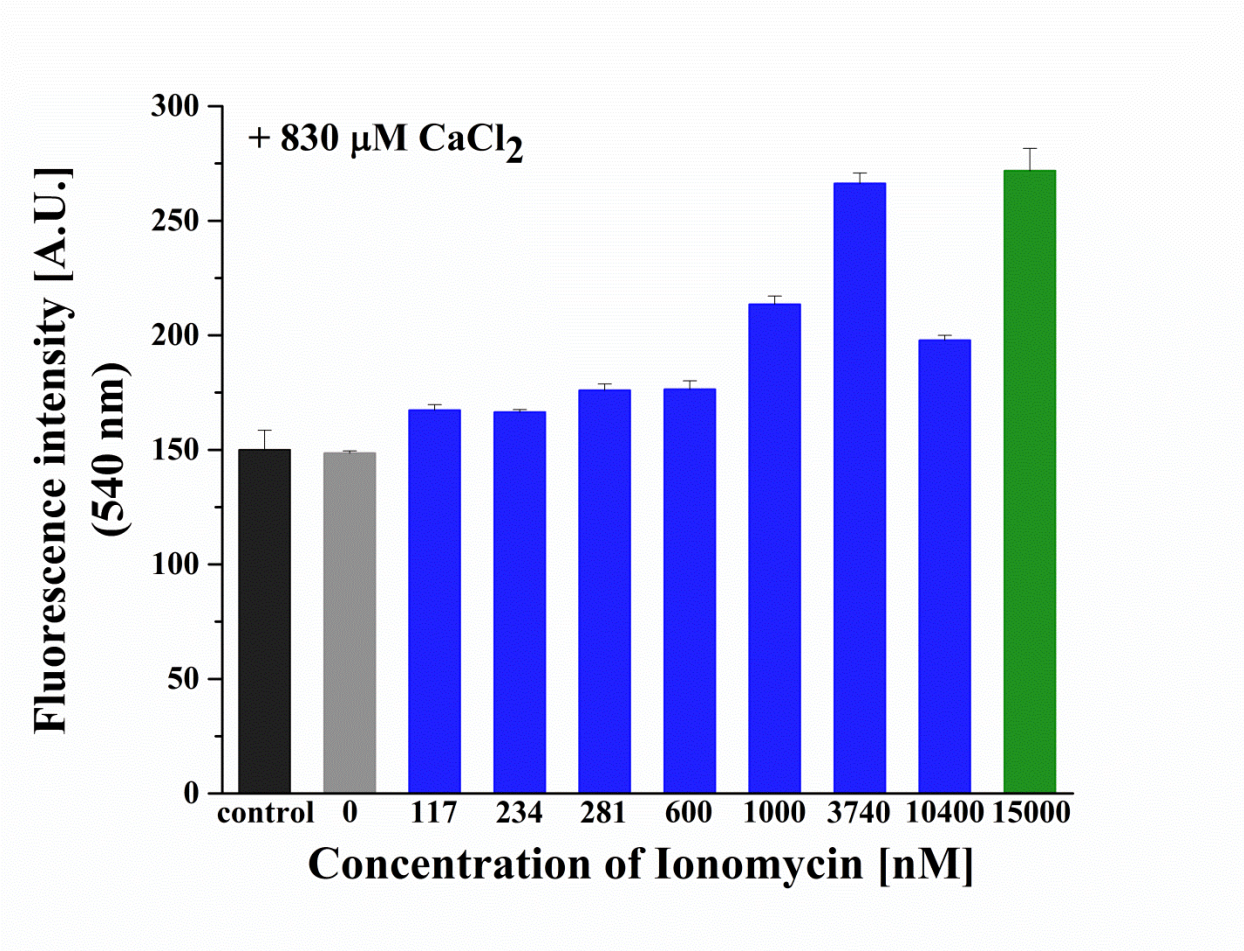

Fig. S4 Influence of ionomycin concentration on membrane permeabilization of $A_6B_{44}A_6$ polymersomes. The concentration of $CaCl_2$ was maintained constant at 830 µM for ACG loaded $A_6B_{44}A_6$ polymersomes (black bar), in the presence of ethanol (grey bar) and increasing concentrations of ionomycin (blue and green bars). Error bars are shown as standard deviations of three individual measurements.

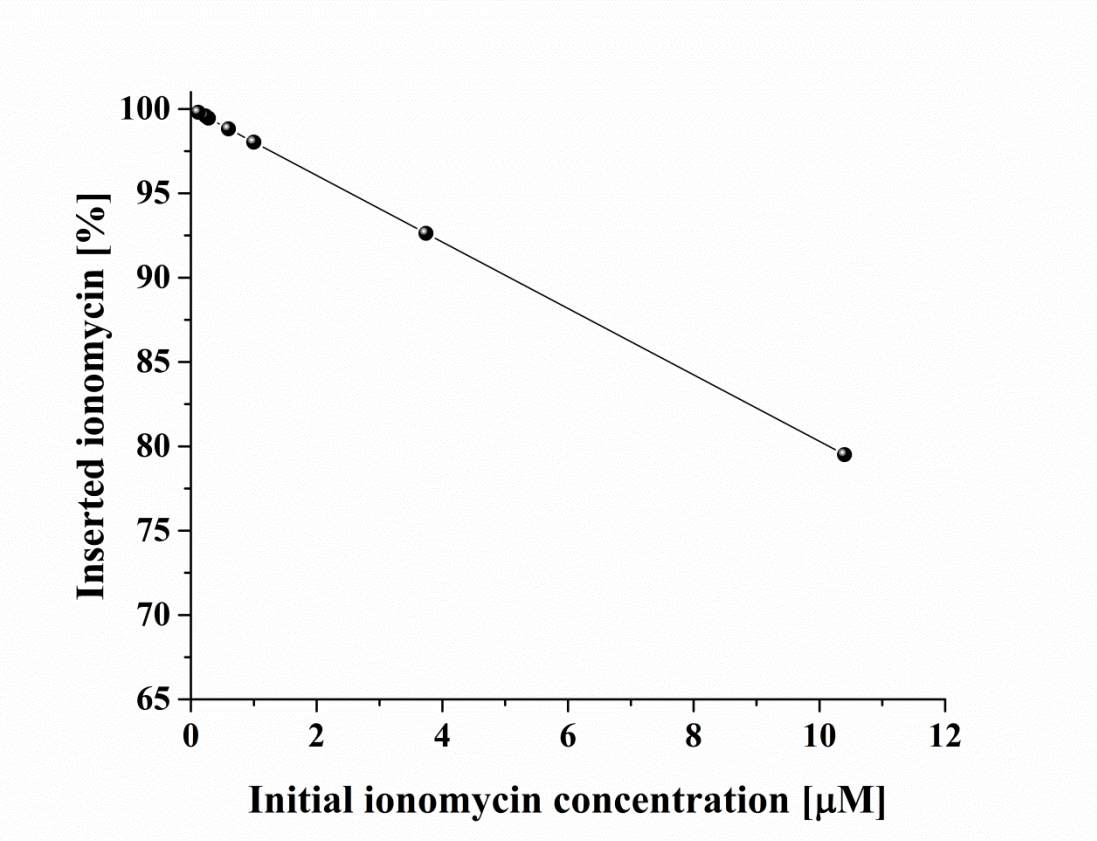

Fig. S5 Ionomycin insertion in polymersome membrane, given as percentage.

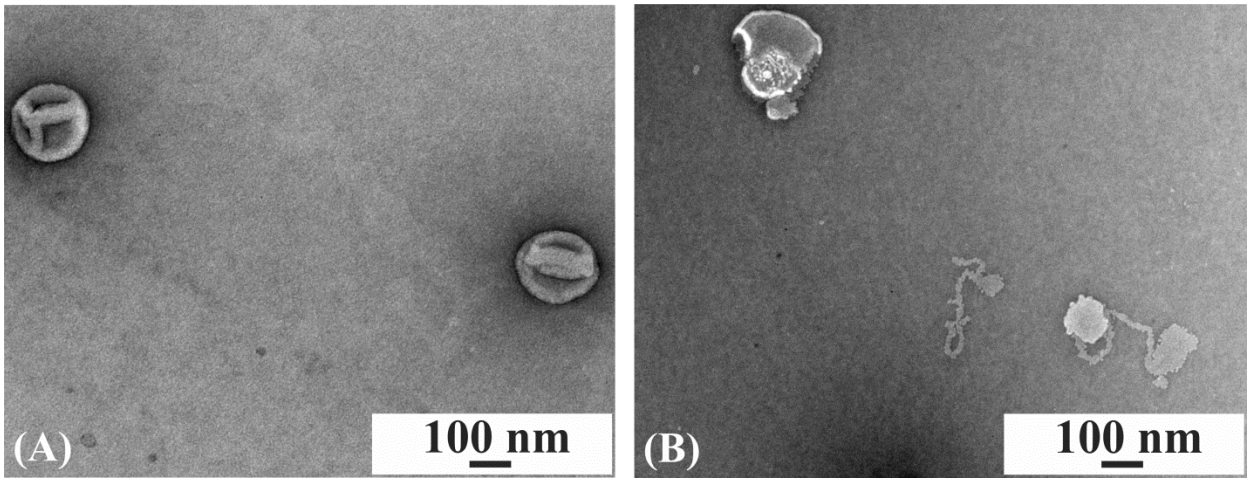

Fig. S6 TEM micrograph of ACG loaded polymersomes of $A_6B_{44}A_6$ in the presence of 10.4 μM (A) and 15 μM ionomycin (B).

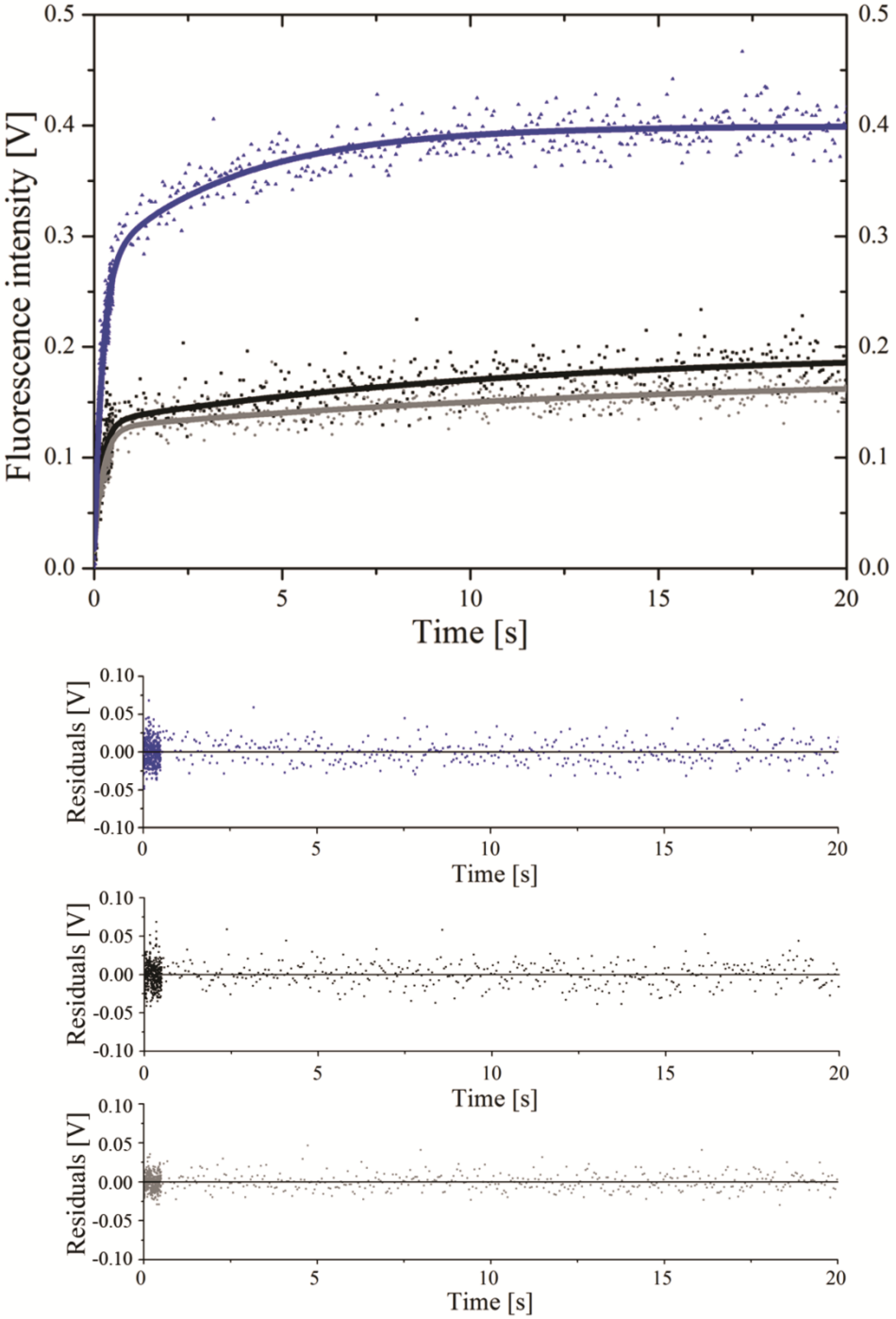

Fig. S7 Fluorescence intensity increase of ACG-loaded $A_6B_{44}A_6$ polymersomes observed by stopped-flow spectroscopy - before (black and grey curves), and after insertion of ionomycin (blue curve). Residuals for fits to a double exponential equation are shown in the lower panel.

Table S3 Diffusion coefficients of different fluorescent species within polymer and lipid membranes.

Membrane type	Measured species	$M_{w,species}$ [g/mol]	D [µm²/s] (20 °C)
$A_7B_{49}A_7$	SRB-$A_7B_{49}A_7$	5100	1.4 ± 0.1[1]
	Bodipy 630/650	660	4.7 ± 0.5
POPC	Rhod-PE	1000	12.5 ± 0.5[1]

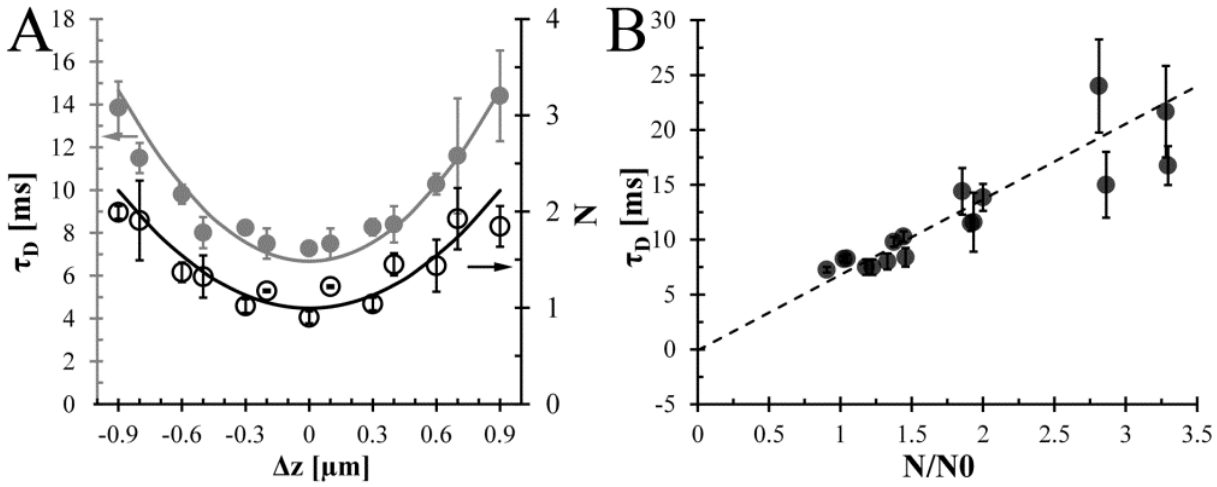

Fig. S8 Diffusion measurements of Bodipy 630/650 within polymersome membrane. A) Z-scan FCS data of Bodipy diffusion within $A_7B_{49}A_7$ membrane. B) Z-scan FCS law showing the free-diffusion character of small hydrophobic molecules.

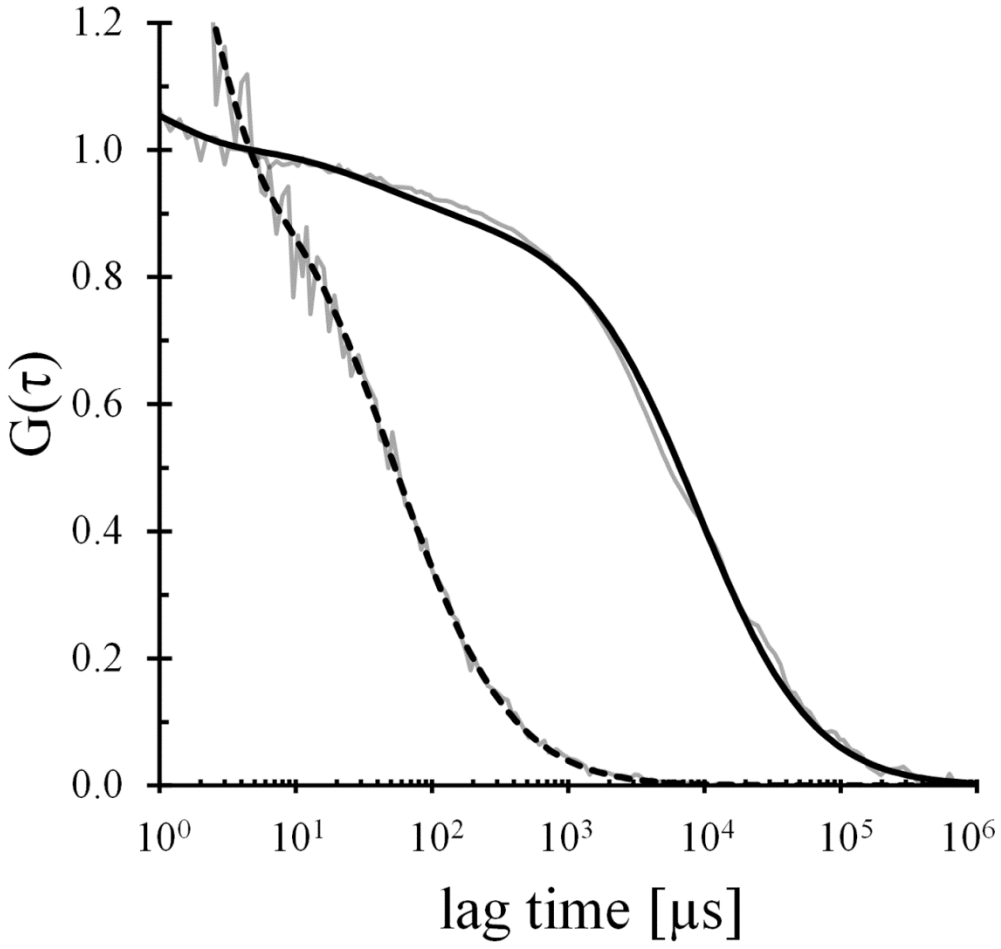

Fig. S9 Fluorescence correlation spectroscopy (FCS) measurements of ACG interaction with polymersomes. Polymersomes were prepared in absence of ACG. Then ACG was added (20 µM) outside to the polymersomes. After overnight incubation, the diffusion time showed a strong shift to larger particles (polymersome fraction). 90 % of ACG was interacting with the polymersome membrane (high diffusion time – slow diffusing fraction – solid line), while only 10 % of the ACG remained in the solution (fast diffusion time – fast diffusing fraction – dotted line).

GUV membrane interaction in the presence of ACG - visualization by CLSM

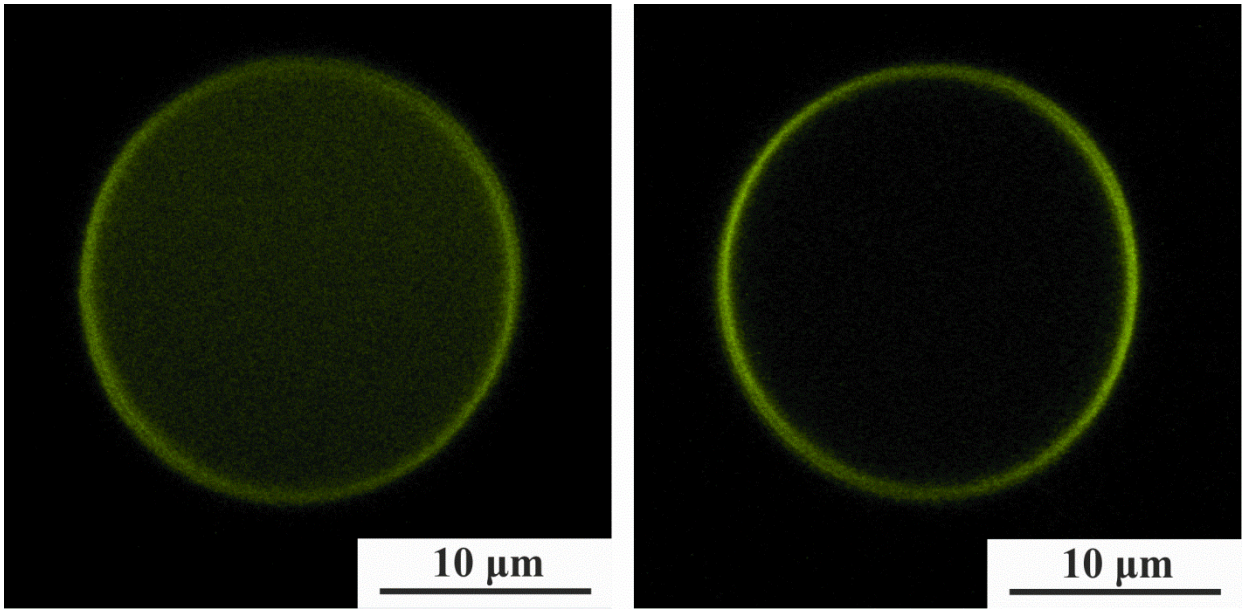

Fig. S10 Confocal laser scanning microscopy (CLSM) micrographs showing ACG interaction with $A_6B_{44}A_6$ GUVs membrane. Left: before bleaching. ACG shows fluorescence inside the vesicle, while outside of the vesicle the ACG concentration is reduced due to dilution of the GUVs into the buffer. Right: After bleaching. The polymeric membrane stays fluorescent, while the free ACG encapsulated in the GUV is bleached. This shows that ACG interacts strongly with the membrane having therefore a strong fluorescence signal emitted from the membrane.

Table S4 Stopped-flow data analysis. Rate constants (k_1 and k_2) and amplitude changes (ΔA_1 and ΔA_2) were determined from fitting the stopped-flow signal with a double exponential function (equation 3).

Stopped-flow kinetic parameters	$A_6B_{44}A_6$ polymersomes with entrapped ACG + $CaCl_2$	$A_6B_{44}A_6$ polymersomes with entrapped ACG + EtOH + $CaCl_2$	$A_6B_{44}A_6$ polymersomes with entrapped ACG + 3.74 μM Ionomycin + $CaCl_2$
k_1 [s^{-1}]	4.70	4.35	4.30
k_2 [s^{-1}]	0.09	0.07	0.27
ΔA_1	0.11	0.11	0.26
ΔA_2	0.06	0.05	0.125

References

1. F. Itel, M. Chami, A. Najer, S. Lörcher, D. Wu, I. A. Dinu and W. Meier, *Macromolecules*, 2014, **47**, 7588-7596.