Gel-assisted formation of giant unilamellar vesicles

Andreas Weinberger[1], Feng-Ching Tsai[2], Gijsje H. Koenderink[2], Thais F. Schmidt[3], Rosângela Itri[4], Wolfgang Meier[5], Tatiana Schmatko[1], André Schröder[1] and Carlos Marques[1]

[1]Institut Charles Sadron (UPR22-CNRS), Université de Strasbourg, Strasbourg, France
[2]Biological Soft Matter Group, FOM Institute AMOLF, Amsterdam, The Netherlands
[3]Universidade Federal do ABC, Santo André, SP, Brazil
[4]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, SP, Brazil
[5]Department of Chemistry, University of Basel, Basel, Switzerland

SUPPORTING MATERIAL

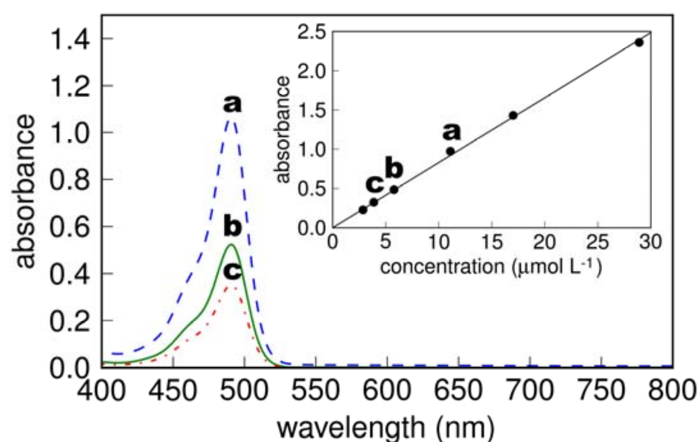

Fig. S1: Absorbance spectra of DTAF standard solutions and standard curve for DTAF solutions in water (inset). For the inset the absorbance at 495 nm, the absorption maximum of DTAF, was used to draw the standard curve. Points a, b, c on the standard curve correspond to the spectra marked as a, b, c.

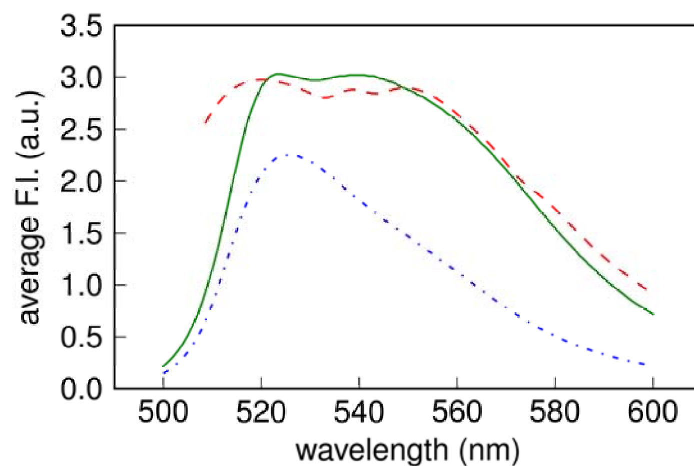

Fig. S2: Fluorescence emission spectra of DTAF free in PBS solution (dash-dotted) and in the presence of PVA (solid line) obtained by excitation at 488 nm with a fluorescence spectrophotometer. Spectra of the DTAF labeled PVA obtained by CLSM at 488 nm after swelling with PBS (dashed line).

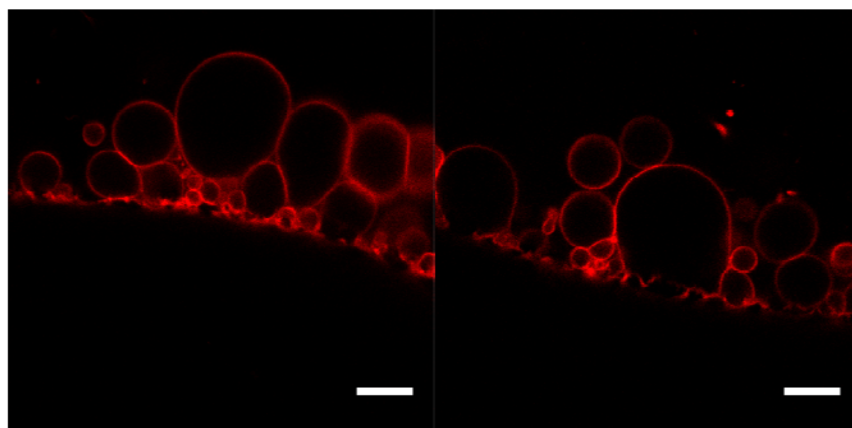

Fig. S3: CLSM images (XZ plane) of DOPC-GUVs labeled with 0.5 mol% RhodB-PE, grown on unlabeled PVA film. Scale bars 20 µm.

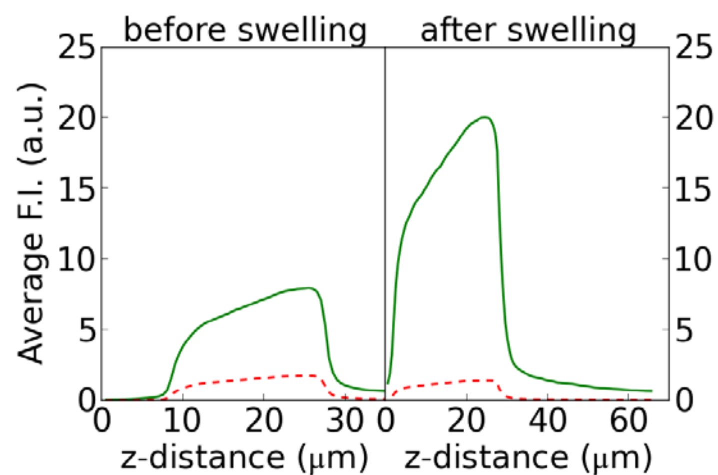

Fig. S4: CLSM z-profiles of a DTAF-labeled PVA film. Emission bleeding in the red channel can be observed. A z-distance of zero microns signifies the bottom of the sample.

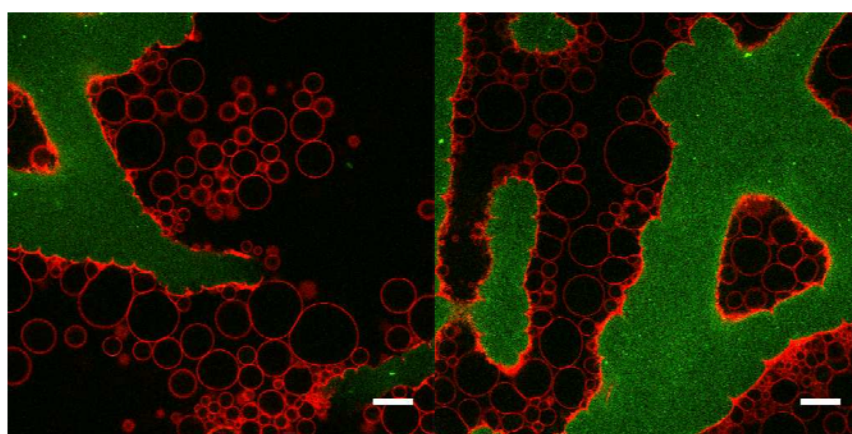

Fig. S5: Confocal laser scanning microscopy (CLSM) image of DOPC-GUVs labeled with 0.5 mol% RhodB-PE, grown on a fluorescently (DTAF) labeled PVA film. Scale bars 20 µm.

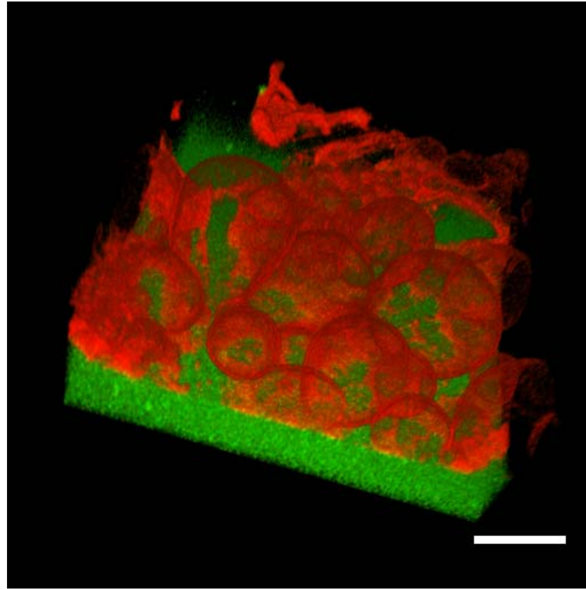

Fig. S6: 3D reconstruction of a z-stack recorded by CLSM of RhodB-labeled DOPC vesicles grown on a DTAF-labeled PVA gel. Scale bar 20 µm.

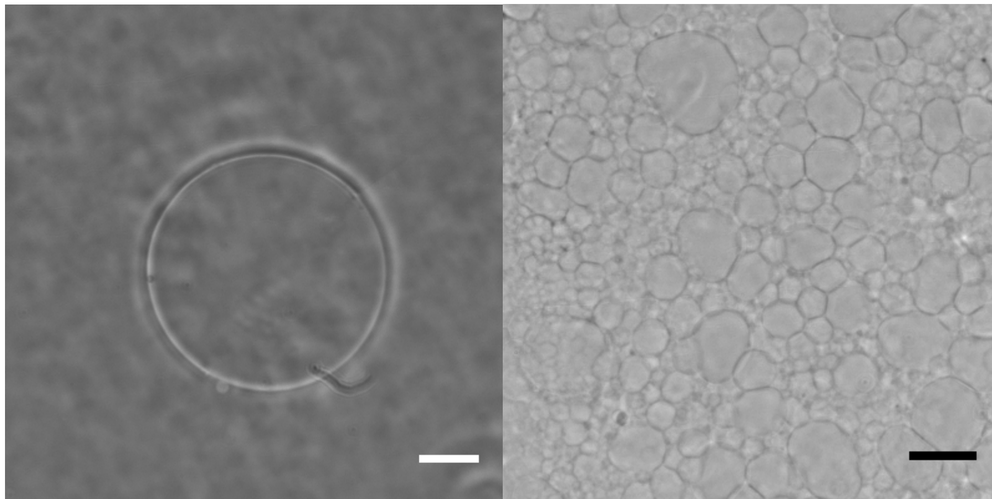

Fig. S7: Left image: free floating DOTAP-GUV. Right image: DOTAP vesicles in the growing chamber. Both were formed by PVA swelling at room temperature in PBS. Scale bar 20 µm.

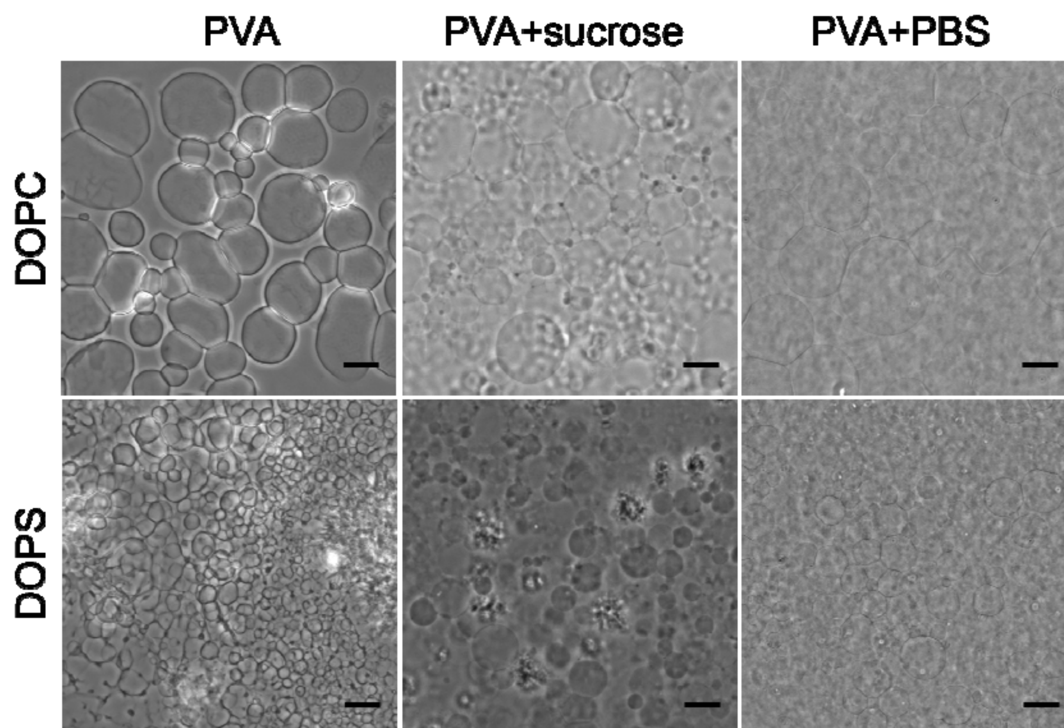

Fig. S8: Phase contrast images of GUVs formed from DOPC and DOPS by PVA swelling. Left column: growth on pure PVA gel. Middle column: growth on PVA containing sucrose. Right column: growth on PVA containing PBS. Swelling solution: PBS. Scale bars: 20 µm.

Table S1: Summary of grown lipid compositions and experimental conditions. Note that the table lists only GUVs that could successfully be grown from one single species of a lipid. If the single lipid mixture was successful, mixtures of 2 different lipids could also be grown by the mentioned method.

Method	**Temperature**	**Lipid Composition**	**Inside buffer**	**Success**
EF	RT	DOPC	Sucrose	+
EF	50°C	DPPC	Sucrose	+
EF	RT	DOTAP or DOPS or DOPG	PBS	Detachment problems
EF	RT	Cardiolipin	Sucrose	Vesicle defects
PVA	RT	DOPC	Sucrose	+
PVA	RT	DOPC	PBS	+
PVA	RT	DPPC	Sucrose	No, gel state
PVA	50°C	DPPC	Sucrose	+
PVA	RT	DOTAP	PBS	+
PVA	RT	DOPG	PBS	+
PVA	RT	DOPS	PBS	+
PVA	RT	PMOXA-PDMS-PMOXA	PBS	See Fig. 4
PVA	RT	Cardiolipin	Sucrose	+
PVA	RT	60% Sphingomyelin/ 35% Cholesterol/ 5% PEG-PE	Sucrose	+
PVA	4°C	Encapsulation of proteins requires low temperature		

In the table above the different lipids used in this paper are summarized. Success is generally obtained for mixtures composed of 2 pure lipids with at least 50% of a lipid that can be successfully grown by this method. The PVA method in this table does not distinguish between PVA, PVA+sucrose in the gel and PVA+PBS in the gel. However, generally it was observed that efficiency of GUV formation is ranked as followed: PVA < PVA+PBS < PVA+sucrose. Substitution of a pure lipid by fluorescently-labeled and biotinylated lipid in the range of 5% does not reduce the yield. However, as mentioned in the main text, adding a small amount of pegylated lipids can further increase the yield.

We have not precisely quantified the yield of vesicles swollen on the gel. In both methods i.e. with the electroformation method and with the gel swelling method, a high number of vesicles could be obtained for neutral lipids. For charged lipids the number and average size of the vesicles is higher in the gel swelling method than in the electroformation method. In a few cases e.g. for cationic lipids we had some difficulties in extracting them by simple pipetting. Putting the sample for 1-2 seconds in an ultrasound bath (35 kHz, HF-power 80 W) detaches a significant number of GUVs, enough for obtaining diluted vesicle samples of typically a couple of hundred GUVs.