Supporting Information: Exploring the Structure and Phase Behavior of Plasma Membrane Vesicles under Extreme Environmental Conditions

Janine Seeliger, Nelli Erwin, Christopher Rosin, Marie Kahse, Katrin Weise, and Roland Winter*

Physical Chemistry I – Biophysical Chemistry, Department of Chemistry and Chemical Biology, TU Dortmund University, Otto-Hahn-Str. 6, 44227 Dortmund, Germany

Additional Results

Establishment of a cell growth and GPMV vesiculation protocol that generates GPMVs with lipid phase coexistence at room temperature.

Table S1. Cell growth and GPMV blebbing conditions including 25 mM formaldehyde and 2-5 mM DTT.

medium			growth	*c*(DTT)	GPMVs with phase coexistence	
MEM	RPMI	Gln			~5 °C	RT
60%	30%	2 mM	++	5 mM	0	0
70%	20%	2 mM	+	5 mM	<1%	0
90%	0%	2 mM	~	5 mM	2%	<1%
80%	10%	0.2 mM	+	2 mM	10%	2%
90%	0%	0.2 mM	~	2 mM	30%	10%
90%	0%	0	~	5 mM	100%	100%
90%	0%	0	~	3 mM	100%	100%
90%	0%	0	~	2 mM	100%	60%

The growth medium for RBL-2H3 cells recommended by the supplier consists of 60% MEM (+ 2 mM L-Gln), 30% RPMI 1640 (+ 2 mM L-Gln), 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. The observed cell growth was very good but even use of 25 mM formaldehyde and 5 mM DTT did not lead to GPMVs with lipid phase coexistence at room temperature (Table S1, row 1). To reach that aim, conditions were modified until almost all GPMVs showed lipid phase coexistence at RT. Therefore, RBL-2H3 cells were cultured at 37 °C under 5% CO_2 in complete medium composed of 90% MEM (+ 2 mM L-glutamine) supplemented with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. Five days before GPMV isolation, cells were passaged by gentle trypsination and seeded at a density of 1.3×10⁴ cells/cm² into fresh 75-cm² cell culture plates containing 25 mL GPMV medium composed of 90% MEM (without L-glutamine) supplemented with 10% FCS, 100 U/mL penicillin and 100 µg/mL streptomycin. If chemically induced blebbing was carried out with 25 mM formaldehyde and 3 mM DTT, almost all GPMVs showed lipid phase coexistence at RT (Table S1, row marked in green). In general, higher T_{misc} was observed with increasing DTT concentrations (as reported previously [Levental, I., Grzybek, M., and Simons, K. (2011) *Proc. Natl. Acad. Sci. U.S.A. 108*, 11411–11416]) and poorer cell growth.

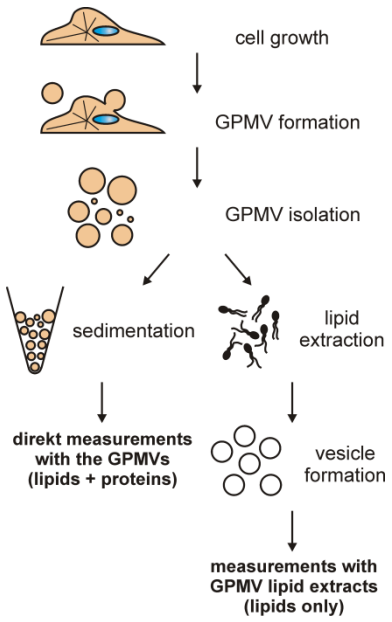

Figure S1. Schematic representation of GPMV isolation and lipid extraction.

Quantification of lipid and protein content.

Table S2. Lipid and protein contents.

	***n*(lipid) / nmol**	***m*(lipid) / µg[a]**	***m*(protein) / µg[b]**	**protein content / %**
GPMVs	46.5 ± 19.0	34.8 ± 14.3	23.2 ± 9.5	~40
GPMV lipid extracts	19.1 ± 2.5	14.3 ± 1.9	n.d.[c]	n.d.[c]

[a] Assumption M(lipid) = 750 g mol^{-1}

[b] Lipid/protein content per confluent grown 75 cm² cell culture plate

[c] Not detectable (using the Roti®-Nanoquant protein quantification assay)

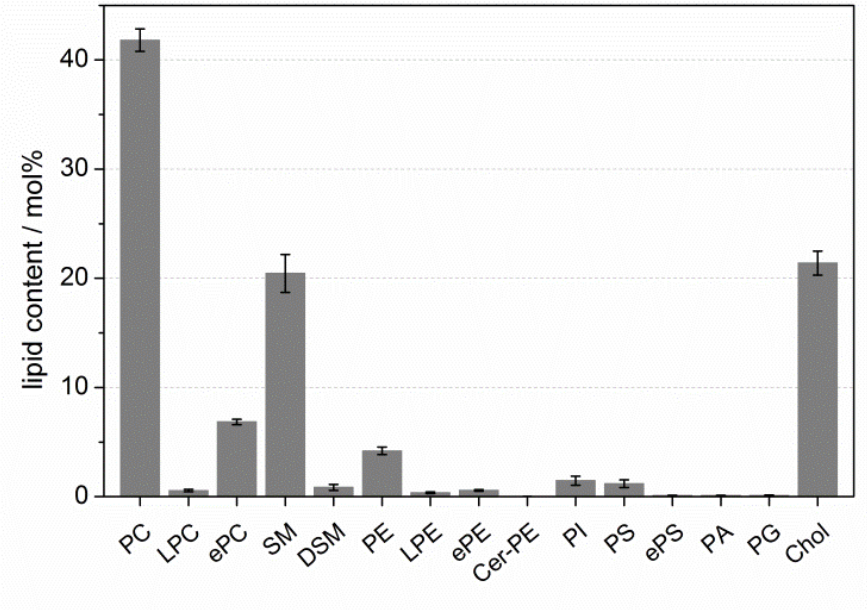

Figure S2. Content of cholesterol as well as phospholipid headgroups of GPMV lipid extracts as analyzed by a non-enzymatic cholesterol assay and mass spectrometry analysis. PC: phosphatidylcholine, LPC: lysoPC, ePC: ether linked PC, SM: sphingomyeline, DSM: dihydroSM, PE: phosphatidylethanolamine, LPE: lysoPE, ePE: ether linked PE, Cer-PE: ceramide-PE, PI: phosphatidylinositol, PS: phosphatidylserine, ePS: ether linked PS, PA: phosphatidic acid, PG: phosphatidylglycerol.

The major phospholipid headgroup is the zwitterionic phosphatidylcholine (PC, LPC, ePC, SM, and DSM) with a fraction of ~71 mol%. The remaining part consists of phosphatidylethanolamine (PE, LPE, ePE, Cer-PE, ~5 mol%) and ~3 mol% anionic lipid headgroups (PI, PS, ePS, PA, PG). With regard to the lipid backbone, glycerophospholipids (~58 mol%) are dominant over phosphosphingolipids (~21 mol%), whereupon 1 mol% of the glycerophospholipid fraction exists as lysolipids exhibiting only one lipid chain, and 8 mol% are linked via an ether instead of an ester group to their fatty acid chain. The phospholipid fatty acid chains themselves were analyzed to consist of mostly 34 to 36 C-atoms and up to nine double bonds for both lipid chains combined, corresponding to an average single chain length of 16 to 18 C-atoms (Fig. S3).

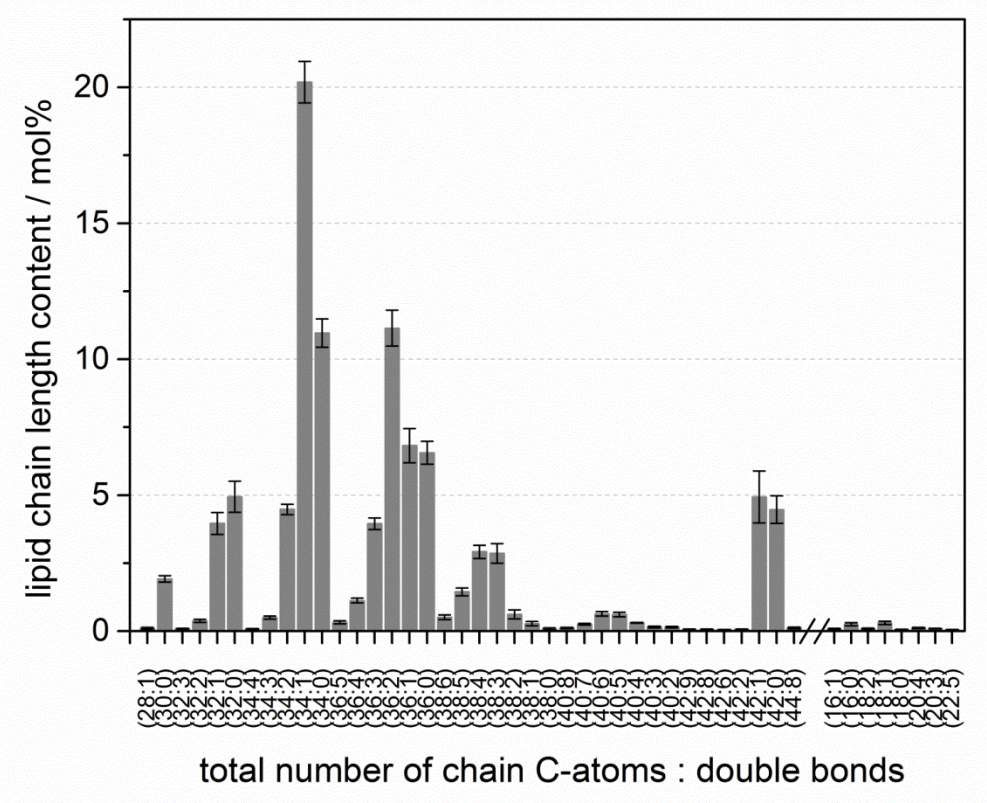

Figure S3. Content of phospholipid total chain lengths and double bonds of GPMV lipid extracts as analyzed by mass spectrometry analysis. On the left hand side the analysis of lipids carrying two chains is displayed, whereas the right hand side shows results for lysolipids exhibiting only one lipid chain.

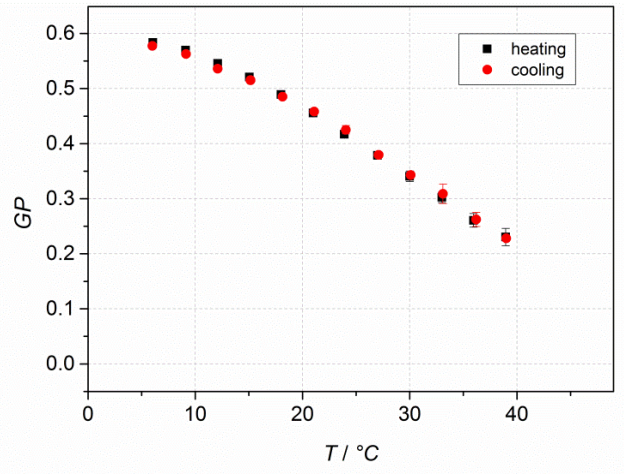

Figure S4. Temperature-dependent Laurdan *GP*-values of GPMVs in the range of 5 to 40 °C, where membrane proteins remain in their folded state.

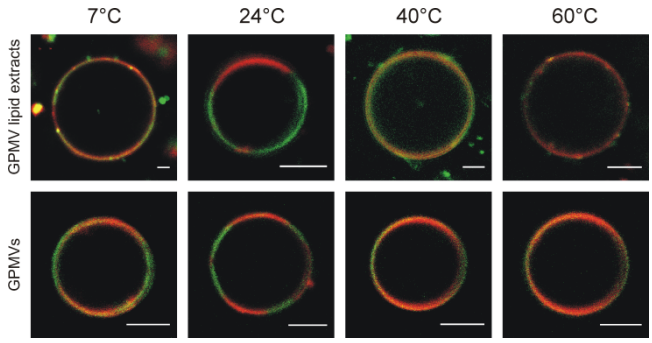

Figure S5. Temperature-dependent fluorescence microscopy images of GUVs of GPMV lipid extracts and GPMVs. Vesicles are labeled with the fluorescence dyes NBD-DHPE (preferentially partitioning into l_o phases; green) and N-Rh-DHPE (favors partitioning into l_d phases; red). The scale bar represents 5 µm.