SUPPORTING DATA

Anionic lipids modulate the activity of the aquaglyeroporin GlpF

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SUPPORTING DISCUSSION

The activity of GlpF is not affected by eventually remaining trace amounts of OG

To determine, whether varying amounts of OG remaining after detergent dialysis could affect the GlpF activity and tetramer stability, we have analyzed the influence of different OG concentrations on the GlpF activity in proteoliposomes and also on the background flux in GlpF-free diC18:1-PC liposomes.

To do so, GlpF was reconstituted into diC18:1-PC liposomes. After reconstitution, the liposomes were incubated for 1 h at room temperature with OG concentrations varying between 0-7.5 mM. The liposomes were further incubated with 50 mM OG to test, if OG indeed incorporates into the liposomes (Fig. S3A, orange curve). As no signal characteristic for liposomes was detected at 50 mM OG, the liposomes were lyzed by OG, showing that OG incorporates into the diC18:1-PC liposomes. Whereas no influence on the GlpF activity was detected (Fig. S3A), we found a significant impact of increasing OG concentrations on the background ribitol flux (assessed in liposomes without GlpF) (Fig. S3B). Already after incubation of the liposomes with 0.2 mM OG an effect on the light scattering curve can be seen, and at OG concentrations of 0.5 mM (dark blue curve in Fig. S3B) and higher a light scattering signal similar to a signal obtained with a functional GlpF channel (compare purple curve in Fig. S3B) was observed.

However, in all our measurements with GlpF-free liposomes, we never observed a signal indicating functional GlpF, regardless of the respective lipid compositions. Thus, it is very unlikely that any potentially remaining amounts of OG affect the determined GlpF activities. In Fig. S3C light scattering curves of proteoliposomes and the corresponding GlpF-free liposomes are shown, as an example. Neither in the negative control, corresponding to proteoliposomes with highly active GlpF (pure diC18:1-PC liposomes), nor in the negative control, corresponding to proteoliposomes with inactive GlpF (pure diC18:1-PG liposomes), a signal indicating GlpF activity was observed, which could be caused by high OG contents. The light scattering signal of the GlpF-free liposomes stayed nearly constant. We can therefore exclude an influence of different amounts of remaining OG concentrations on the activity measurements.

To study, if varying amounts of remaining OG affect the stability of the GlpF tetramer, a semi-native SDS-PAGE analysis of proteoliposomes incubated with increasing OG concentrations was performed (Fig. S4). This SDS-PAGE analysis did not reveal an influence of OG on the tetramer stability upon an OG concentration of 0.5 mM. However, the remaining OG concentration in the liposomes is lower as concluded from the absence of a fast decay of the light scattering signal of the GlpF free liposomes.

Next, we quantified the remaining amount of OG in diC18:1-PC liposomes at various time points during formation of proteoliposomes (Fig. S5). After detergent dialysis, remaining OG concentrations of 9.0 x 10^{-3} and 1.8 x 10^{-2} mM were determined for the diC18:1-PC liposomes and proteoliposomes, respectively (Fig. S5B). As expected from the measurements with GlpF-free liposomes, those concentrations below 0.2 mM OG, as no drastic influence on the light scattering signal was observed.

SUPPORTING FIGURES

Figure S1

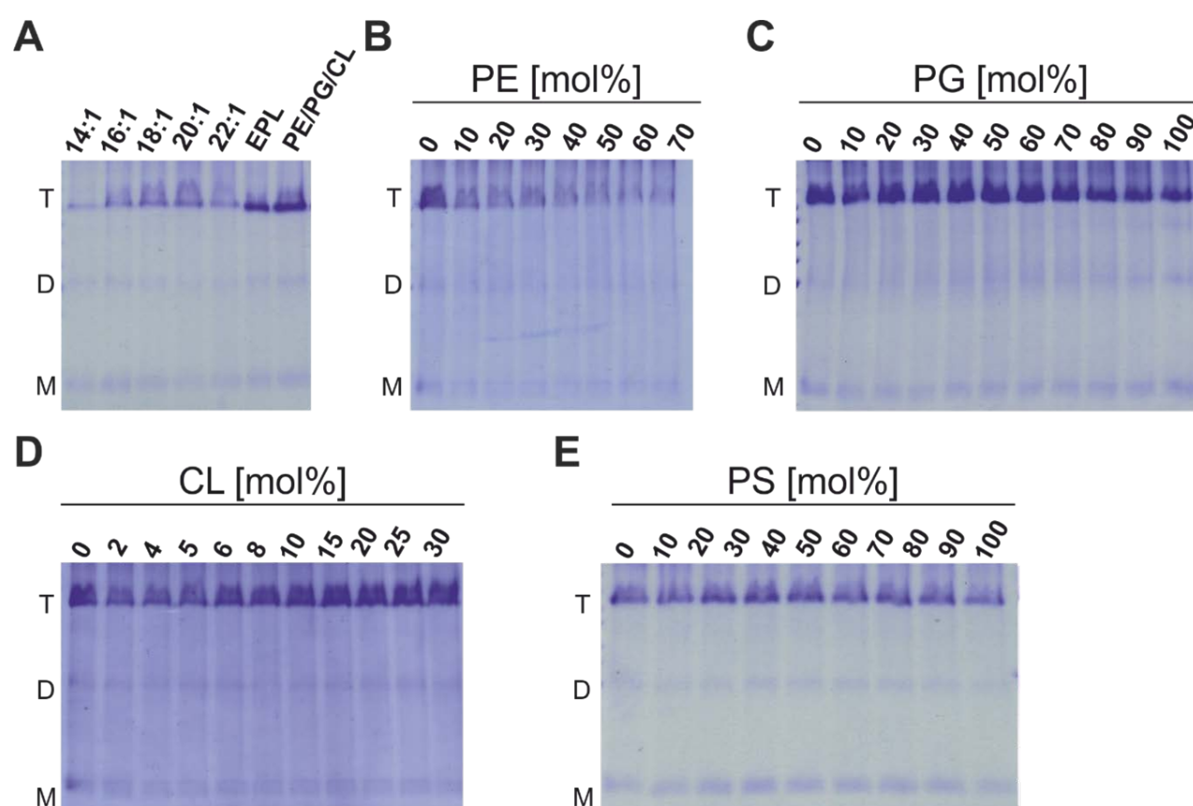

Figure S1: Stability of the GlpF tetramers against SDS-induced unfolding determined in different bilayer environments. GlpF incorporation as well as GlpF oligomeric state in the different lipid composition was monitored via semi-native SDS-PAGE analysis. In an SDS-PAGE analysis, where no SDS is present in the sample buffer, the native tetrameric state of GlpF is preserved. Tetrameric (T), dimeric (D) and monomeric (M) GlpF are indicated. Representative Coomassie Blue-stained SDS-PAGE gels of GlpF incorporated into liposomes. (A) GlpF incorporation into liposomes having increasing PC chain lengths (diC14:1-PC – diC22:1-PC), as well as into liposomes prepared from EPL extract and the ternary PE/PG/CL lipid mixture (70 mol% diC18:1-PE, 20 mol% diC18:1-PG and 10 mol% tetrC18:1-CL). (B-E) GlpF incorporation into diC18:1-PC liposomes with increasing mole fractions of (B) diC18:1-PE, (C) diC18:1-PG, (D) diC18:1-CL, or (E) diC18:1-PS.

Figure S2

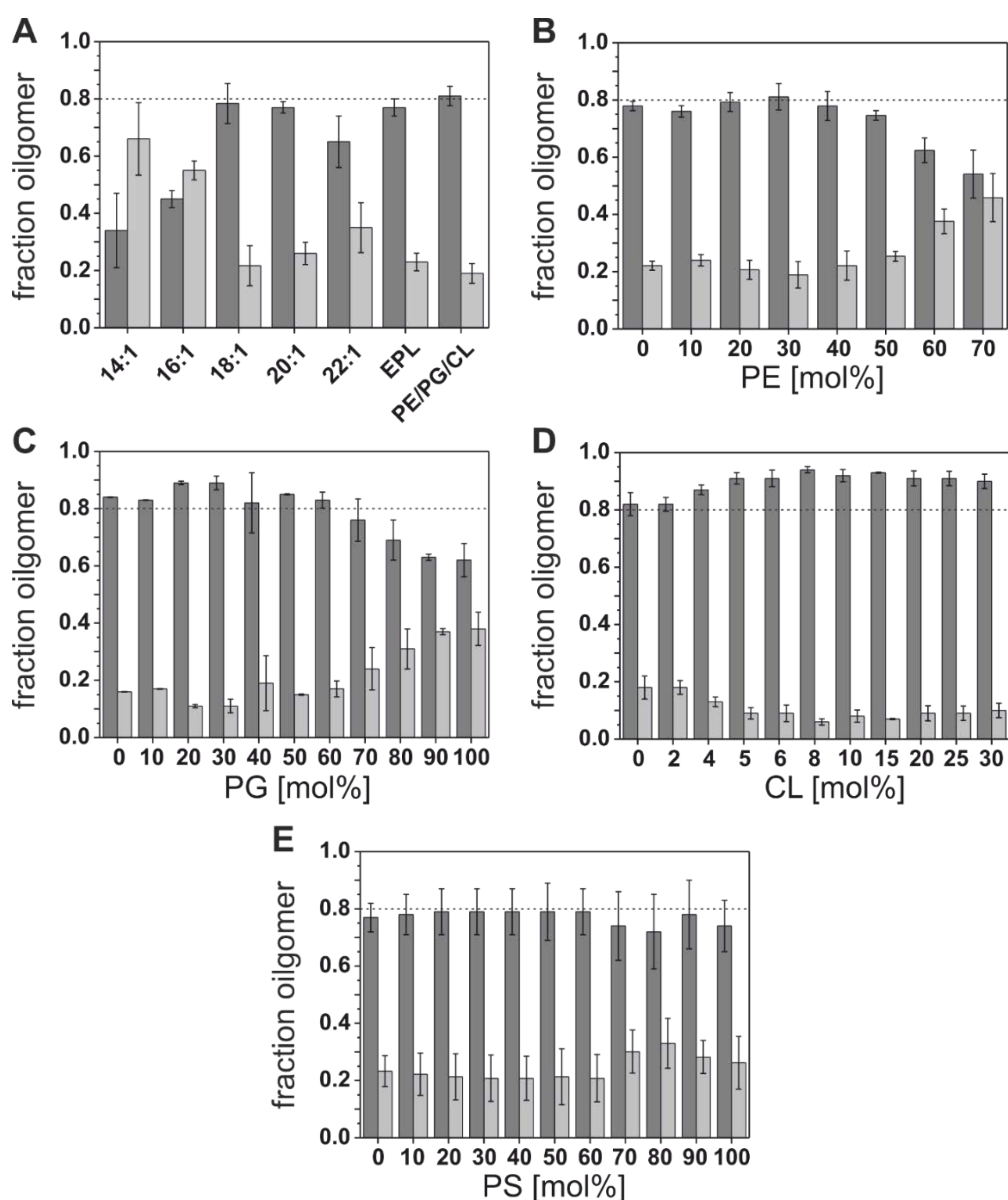

Figure S2: Relative fraction of tetrameric vs. monomeric/dimeric GlpF incorporated in liposomes. The amount of tetrameric GlpF is depicted in dark gray and the amount of incorporated proteins as monomer/dimer is illustrated in light grey. For comparison, the horizontal dashed line (at 0.8) visualizes the fraction of tetrameric GlpF incorporated into liposomes formed using the ternary PE/PG/CL lipid mixture. (A) Relative fraction of tetrameric vs. monomeric/dimeric GlpF in liposomes having increasing PC chain lengths (diC14:1-PC – diC22:1-PC), as well as in liposomes prepared from EPL extract and the ternary PE/PG/CL lipid mixture (70 mol% diC18:1-PE, 20 mol% diC18:1-PG and 10 mol% tetrC18:1-CL). (B-E) Relative fraction of tetrameric and monomeric/dimeric GlpF in liposomes with a diC18:1-PC *background* and increasing mole fractions of (B) diC18:1-PE, (C) diC18:1-PG, (D) diC18:1-CL and (E) diC18:1-PS.

Figure S3

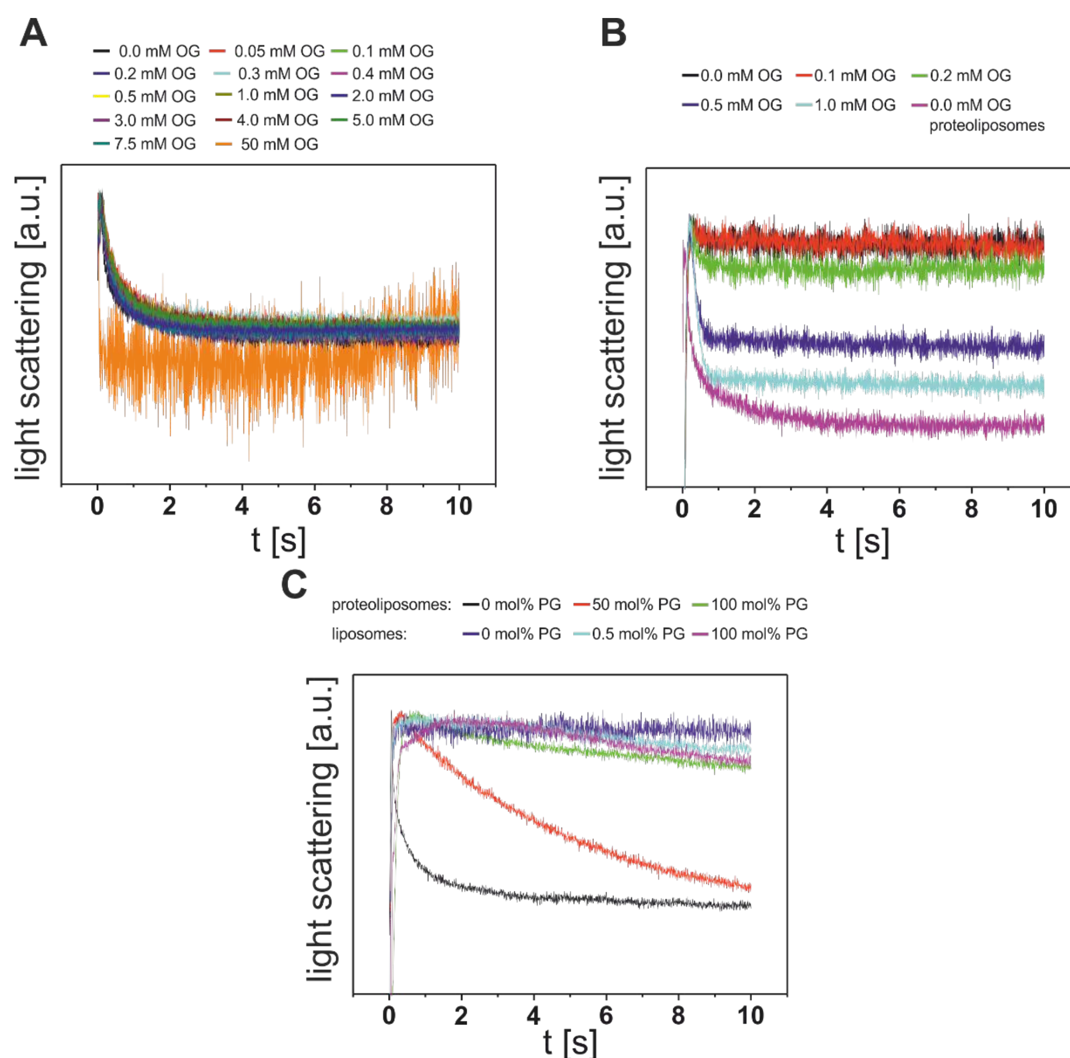

Figure S3: Influence of varying OG amounts on the ribitol conductance of GlpF. (A) To assess the influence of various OG concentrations on the GlpF activity, diC18:1-PC proteoliposomes were incubated with OG concentrations between 0 and 7.5 mM OG as well as with 50 mM OG, and the GlpF activity was determined as described. (B) Influence of varying OG concentrations on the ribitol conductance of GlpF-free diC18:1-PC liposomes (0 mM OG (back), 0.1 mM OG (red), 0.2 mM OG (green), 0.5 mM OG (dark blue), 1.0 mM OG (light blue). After incubation of 2.5 mM diC18:1-PC liposomes with OG concentrations of ≥0.5 mM (dark blue curve), the observed liposom shrinking and reswelling kinetics look very similar to a GlpF-characteristic ribitol flux (purple curve). (C) Representative light scattering curves monitored at 0 (black), 50 (red) and 100 (green) mol% diC18:1-PG. The corresponding negative controls, *i.e.* GlpF-free liposomes, are also depicted at 0 (dark blue), 50 (light blue) and 100 (purple) mol% diC18:1-PG. These data indicate that any potentially remaining trace amounts of OG do not affect the measured GlpF channel activity.

Figure S4

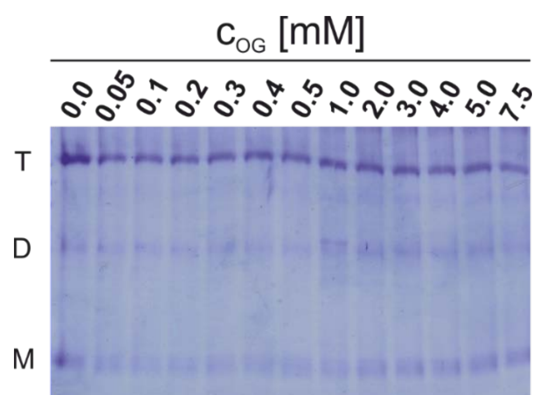

Figure S4: Influence of increasing OG concentrations on the stability of the GlpF tetramer against SDS-induced unfolding. As deduced from the SDS-gel, OG does not influence the tetramer stability up to OG concentrations of 0.5 mM.

Figure S5

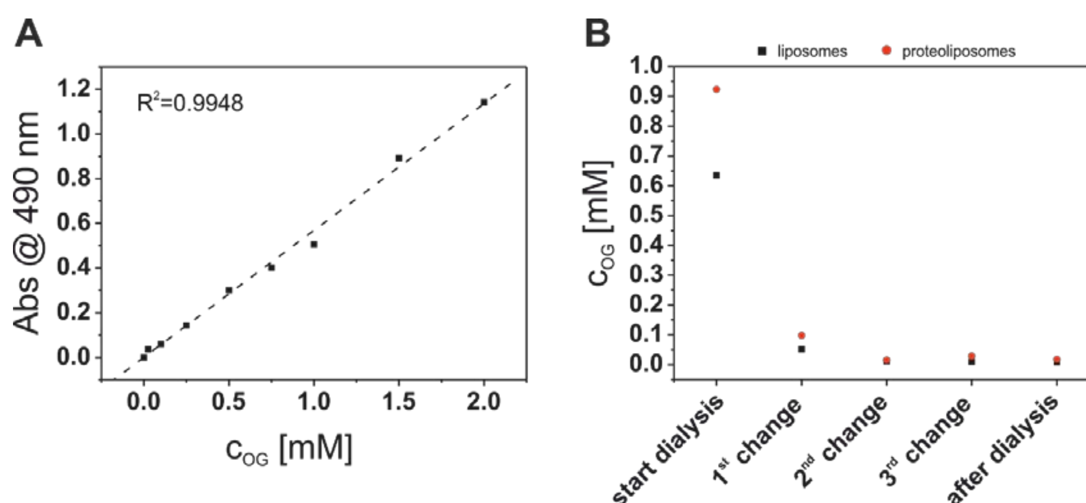

Figure S5: Determination of potentially in proteoliposomes remaining OG traces.
(A) Calibration curve for the determination of the remaining OG concentration in diC18:1-PC liposomes. The calibration curve was recorded with OG concentrations ranging between 0 and 2 mM OG. (B) Quantification of OG concentrations remaining in the liposomes after reconstitution as well as after the individual buffer exchanges during sample dialysis. For quantification of the OG concentrations, a colorimetric based method for glyosidic detergents was employed. For the colorimetric determination 150 µL of the liposome suspension were centrifuged for 40 min at 140,000 g and 4 °C and the supernatant was discarded. The liposomes were then resuspended in 50 µL dialysis buffer containing 1% TritonX-100 (Sigma-Aldrich, München, Germany). Afterwards the OG concentration was determined via a colorimetric based method by addition of 250 µL 5% phenol (Roth, Karlsruhe, Germany) and 600 µL concentrated sulphuric acid (Roth, Karlsruhe, Germany) (1). Via this reaction a p-semiquinonoid chromogen is formed with an absorbance maximum at 490 nm (1). After the samples were allowed to cool to room temperature, they were centrifuged for 10 min at 20,420 g and the absorbance was measured at 490 nm.

Figure S6

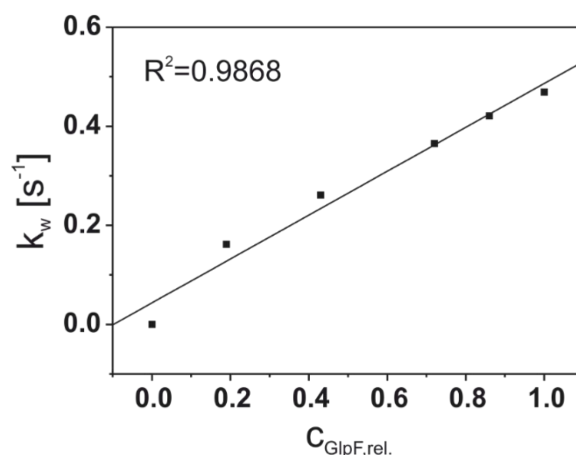

Figure S6: Linear correlation between the amount of liposomal GlpF and the GlpF activity. Increasing GlpF concentrations (3-15 µM) were incorporated into liposomes prepared from EPL extract, while the lipid concentration was kept constant at 5 mM. From SDS-PAGE analyses the amount of incorporated GlpF was determined ($c_{GlpF, rel}$) and plotted versus the rate constants (k_w [s^{-1}]).

SUPPORTING TABLE

Table S1: Hydrophobic thickness of PC bilayers

	d_{P-P} [Å]*	$d_{C=O-C=O}$ [Å] †
diC14:1-PC	29.6	20.0
diC16:1-PC	32.1	23.5
diC18:1-PC	36.8	27.0
diC20:1-PC	38.9	30.5
diC22:1-PC	45.5	34

* Values were obtained from (2)
† Values were obtained from (3)

SUPPORTING REFERENCES

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3. Anbazhagan, V., and D. Schneider. 2010. The membrane environment modulates self-association of the human GpA TM domain--implications for membrane protein folding and transmembrane signaling. Biochim Biophys Acta 1798:1899-1907.