Direct Demonstration of Lipid Phosphorylation in the Lipid Bilayer of the

Biomimetic Bicontinuous Cubic Phase Using the Confined Enzyme Lipid A

Phosphoethanolamine Transferase

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Figure S1.1. RP-HPLC and ESI-TOF MS data of a sample and fractions with pure MO + DOPE + DOG, similar to Figure 3(A) in 70% ethanol (60 μg loaded on the column). (A) RP-HPLC result: DAD signal 203 nm. (B) MS data sample, (C) MS data 22 – 23 minutes: MO, (D) MS data 38 – 39 min: DOPE, (E) MS data 40 – 41 min: DOG.



Figure S1.2. RP-HPLC and ESI-TOF MS data of the sample and fractions with MO + DOPE + *Nm*EptA, 24 hours (Figure 3(D)). (A) RP-HPLC result: DAD signal 203 nm. (B) MS data sample, (C) MS data 17 – 18 minutes: MOPE, (D) MS data 19 – 20 min: MO, (E) MS data 36 – 37 min: DOPE, (F) MS data 37 – 38 min: DOG.



Figure S2.1. Reproducibility SAXS data Figure 5(B,C), results in Figure 5(A) is data with 0% DOPE in Figure 6(A) and the reproducibility of this data is shown in Figure S2.2(A). (A) MO with different concentrations of DOPE and DOG. D stands for the $Q_{II}{}^{D}$ phase, H stands for the H_{II} phase. (B) MO with different concentrations of DMPE and DMG. At concentrations of 6% DMPE and higher a coexisting L_a phase with a lattice parameter of 51 Å, typical for pure DMPE, was observed. At concentrations of 3% DMG and higher a coexisting L_a phase with a lattice parameter of 42 Å, typical for pure DMG, was observed.



Figure S2.2. Reproducibility SAXS data Figure 6. (A) MO with different concentrations of DOPE and *Nm*EptA after 12 hours, all from the Q_{II}^{D} phase. (B) MO with different concentrations of DOPE and *Nm*EptA after 20 hours, all from the Q_{II}^{D} phase.



Figure S2.3. Reproducibility SAXS data Figure 7. (A) MO with different concentrations of DMPE and *Nm*EptA after 12 hours, all from the Q_{II}^{D} phase. (B) MO with different concentrations of DMPE and *Nm*EptA after 20 hours, all from the Q_{II}^{D} phase. At concentrations of 6% DMPE and higher a coexisting L_a phase with a lattice parameter of 51 Å, typical for pure DMPE, was observed.

Supplementary Information 3. High-throughput data with DMPE, presented in individual graphs for every % w/w DMPE.



Figure S3.1. High-throughput SAXS results for 0 to 10 % w/w DMPE and the respective linear fits for (A) 0% w/w DMPE, (B) 2% w/w DMPE, (C) 4% w/w DMPE, (D) 6% w/w DMPE, (E) 8% w/w DMPE, (F) 10% w/w DMPE.