Supplementary Information

I. Input data for GROMACS simulation

**Supplementary Table I.** Absolute amount of each representative lipid put into the CHARMM-GUI membrane builder tool per condition per leaflet as well as total amount of solutes. Cardiolipin representative is PMCL2: 1,2-dipalmitoyl-10-palmytoil-20-cis-9,10-methylenehexadecanoyl-cardiolipin; acylphosphatidyl-glycerol representative is DPPGP: 1,2-dipentadecanoyl*-sn-*glycero-3-phospho-(3’-pentadecanoyl)-1’*-sn-*glycerol; phosphatidylcholine representative is DPPC: 2,3-dipalmitoyl-d-glycero-1-phosphatidylcholine; phosphatidylethanolamine representative is DPPE: 2,3-dipalmitoyl-d-glycero-1-phosphatidylethanolamine; menaquinone representative is MEN7: menaquinone-7 (MQ7). Total number of molecules simulated in GROMACS are also given; note that lipid representatives are given per leaflet, and solutes for the whole system.

|  |  |  |  |
| --- | --- | --- | --- |
|  | pH 7.5 | pH 10.5 | pH 11 |
| Cardiolipin | 149 | 79 | 92 |
| Acylphosphatidylglycerol | 7 | 83 | 68 |
| Phosphatidylcholine | 38 | 35 | 32 |
| Phosphatidylethanolamine | 5 | 1 | 0 |
| Menaquinone | 1 | 2 | 8 |
| Sodium | 661 | 526 | 550 |
| Chloride | 51 | 0 | 0 |
| Carbonate | 0 | 44 | 46 |
| Water | 27,925 | 24,734 | 25,137 |
| Total system molecules | 29,037 | 25,704 | 26,133 |

II. Details of identification of acyl-phosphatidylglycerols

Elution of a series of acyl-PGs between the phospholipids and cardiolipins (**Figure 1** of main article, **Supplementary Table II**); assigned from their accurate masses and their fragmentation spectra. Several of the five acyl-PG peaks comprised multiple structural isomers. For example, the first component, with an [M + NH4]+ at *m/z* 964.758, had an assigned elemental composition, based on its accurate mass, of C53H107O11NP. In MS2 it gave rise to a dominant fragment ion at *m/z* 551.504, a commonly observed fragment associated with a diacylglycerol (DAG) lipid comprising 32 acyl carbons (C32:0 DAG), and a less dominant fragment at *m/z* 523.472 (a C30:0 DAG). The neutral loss from the parent ion to produce the C32:0 DAG fragment was C18H40O7NP, after deduction of NH3, this is equivalent to a phosphaditylglycerol moiety bound to a C15:0 fatty acid (IPL analysis does not distinguish between *iso*, *anteiso*, dimethylated or straight chain fatty acids). Indeed the MS2 spectrum also contained fragment ions associated with a C15:0 fatty acid (*m/z* 299.258) and a C17:0 fatty acid (*m/z* 327.290), approximately in the ratio 2:1. It can be deduced therefore, that the C32:0 DAG was made up of a C15:0 and a C17:0 fatty acid, and that there was a C15:0 fatty acid bound via a glycerol to the phospho-moiety. The less dominant isomer, which produced a fragment ion at *m/z* 523.472 (C30:0 DAG), underwent a neutral loss of C22H44O7NP, equivalent to a phosphatidylglycerol moiety bound to a C17:0 fatty acid. The composition of the acyl-PGs is given in **Supplementary Table II**.

**Supplementary Table II.** AEC = Assigned elemental composition; mmu = milli mass unit; Δ mmu = (measured mass – calculated mass) x 1000. \*DAG MS2 fragments listed in order of abundance. PG = phosphatidylglycerol.

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| --- | --- | --- | --- | --- | --- |
| **Observed mass [M+NH4]+** | **AEC** | **Δ mmu** | **Major fragment in MS2\*** | **AEC of associated neutral loss** | **Total fatty acid composition (DAG; PG; additional FA)** |
| 964.758 | C53H107O11NP | 0.8 | 551.504 (C32:0 DAG) | C18H37O7P + NH3 | C15:0,C17:0; PG; C15:0 |
|  |  |  | 523.472 (C30:0 DAG) | C20H41O7P + NH3 | C15:0, C15:0; PG; C17:0 |
| 978.774 | C54H109O11NP | 0.9 | 551.504 (C32:0 DAG) | C19H39O7P + NH3 | C15:0,C17:0; PG; C16:0 |
|  |  |  | 537.488 (C31:0 DAG) | C20H41O7P + NH3 | C15:0,C16:0; PG; C17:0 |
|  |  |  | 565.519 (C33:0 DAG) | C18H37O7P + NH3 | C16:0,C17:0; PG; C15:0 |
| 992.790 | C55H111O11NP | 1.0 | 551.505 (C32:0 DAG) | C20H41O7P + NH3 | C15:0,C17:0; PG; C17:0 |
|  |  |  | 579.535 (C34:0 DAG) | C18H37O7P + NH3 | C17:0,C17:0; PG; C15:0 |
| 1006.806 | C56H113O11NP | 1.2 | 565.520 (C33:0 DAG) | C20H41O7P + NH3 | C16:0,C17:0; PG; C17:0 |
|  |  |  | 579.536 (C34:0 DAG) | C19H39O7P + NH3 | C17:0,C17:0; PG; C16:0 |
| 1020.821 | C57H115O11NP | 0.8 | 579.536 (C34:0 DAG) | C20H41O7P + NH3 | C17:0,C17:0; PG; C17:0 |

III. RMSD and MSD of molecular dynamics simulations

A collage of graphs showing different types of curves

Description automatically generated with medium confidence

**Supplementary Figure I.** RMSD of pH 7.5 (A), pH 10.5 (B) and pH 11 (C); and MSD of pH 7.5 (D), pH 10.5 (E) and pH 11 (F) membranes simulated in GROMACS. Grayscale traces are individual 1 ns (first 15) or 3 ns (subsequent 30) production runs, totalling 105 ns of simulations. The average is given in black, and the standard deviation in both direction is red.

IV. Illustration of pH 10.5 lipid membrane

A blue and red dots

Description automatically generated with medium confidence

**Supplementary Figure II.** Illustration of the membrane of *C. thermarum* TA2.A1 at pH 10.5. Shown in default lipid colours are the acyl-PGs (absolute abundance = 166 out of 400 lipids). Breakdown of default lipid colours: aqua is carbon, white is hydrogen, red is oxygen and yellow is phosphorus. The transparent background matter is the rest of the simulated membrane. The frame used for this illustration is that after 84 ns of simulation.