Supplemental Materials

Additional details of lipid A-SL synthesis

3-Carboxy-proxyl (0.27 mmol) from Molecular Probes was dissolved in 0.75 mL benzene [S1] in a brown glass vial with a teflon-coated cap. This was followed by the addition of approximately one equivalent (1 drop) of pyridine (Aldrich) and 1.4 equivalents of thionyl chloride (0.37 mmol). This mixture was stirred at room temperature for 1 h with a needle inserted into the Teflon stopper to open the reaction to air. The solvents were evaporated under a gentle stream of nitrogen, and the remaining solute was dissolved in 1 mL $CHCl_3$ (Aldrich). This produced the proxyl-acid chloride solution, which was then used immediately to covalently spin-label lipid A.

One mL of $CHCl_3$ was added to 1 mg of diphosphoryl lipid A from *E. coli* K12 lacking LPS (List Biological Laboratories). For the lipid A spin labeling reaction, 0.2 μL of triethylamine and 3 μL (1.3 equivalents, assuming 100% conversion in the above reaction) of the proxyl-acid chloride solution described above were added to the lipid A solution in chloroform. This mixture was stirred at 4°C in the original lipid A vial for 19 h. At room temperature, the reactivity in esterification reactions of primary alcohols is greater than for secondary alcohols [7], and the nearly 1:1 ratio of reagents help to select for the spin label to react with the primary alcohol of lipid A. The overnight reaction time at the lowered temperature was chosen to ensure as complete an esterification reaction as possible and to minimize side reactions [S6]. The excess proxyl-acid chloride is quenched when exposed to air and becomes proxyl-acid. The lipid A-SL was either dried down under nitrogen or stored in chloroform aliquots at -20°C.

As a note, the purchased lipid A was estimated at 1600 g/mol, whereas characterization by mass spectrometry indicates that this lipid A stock was largely 6-acyl and not 4-acyl, therefore putting the average molecular mass of the lipid A used probably closer to 1796 g/mol.

Additional details of lipid A-SL characterization

Lipid A-SL was added at 1 mol% into 20:40:40 mol% DOPE:DOPG:DOPC lipid bilayers by drying the appropriate mixture of lipids solubilized in chloroform under a stream of nitrogen. The lipids were then resuspending in 20 mM MOPS, pH 7 buffer, followed by addition of 0.01% dodecylmaltoside (DM) detergent, sonication, and additional DM buffer to make a 0.1% final concentration, as done previously for producing proteolipids [S2]. Detergent is removed using Biobeads (Biorad). To remove the excess unreacted proxyl-acid, the liposomes were washed after ultracentrifugation by the addition of 20 mM MOPS, pH 7 and pelleted once again.

Power saturation experiments described in the text and shown in Figure 2 were carried out on an X-band Varian E-109 spectrometer using a loop-gap resonator. Liposome samples in 50 mM $NaPO_4$, pH 7 were contained in a gas-permeable TPX capillary and placed under a stream of air for oxygen studies or under nitrogen in the presence of 200 mM NiEDDA or 5 mM CROX, as previously carried out (e.g., [11, S2). Known depths from the phosphate groups for TEMPO-PC and 5-doxyl-PC [12] were used with our experimental data to calculate an approximate depth calibration equation: depth (Å) = $-5.56\Phi + 2.8$ where $\Phi = \ln[\Pi(\text{air})/\Pi(\text{Ni})]$ and Π is the accessibility parameter [S3]. Depths calculated using this method are linear within the membrane (S3) and we have extended this slightly to include the position of the headgroup spin label TEMPO-PC, though other labs have calculated distances even beyond the phosphate headgroups (e.g., [S4;S5]).

Purified MsbA protein (5 μM) in DM in the presence of a 250 molar excess of inner membrane lipids (65:25:10 POPE:POPG:CL), 25x lipid A or 25x lipid A-SL was assayed for its ATPase activity in the presence of 3 mM ATP containing [γ-^{32}P]ATP (Perkin Elmer) over 2 minutes. Rates of hydrolysis were determined by plotting nmol of P_i released, as quantitated by a scintillation counter, per mg of protein against time (Figure 3).

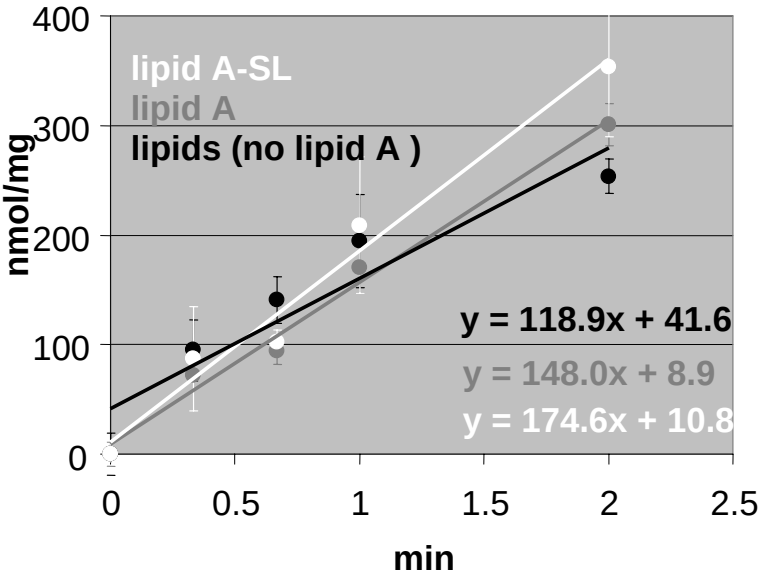

Figure 3. Stimulation of the ATPase activity of purified MsbA in the presence of lipid A-SL, lipid A, and lipids without lipid A.

Supplemental Materials Reference List

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