Supplemental Information

Antimicrobial peptide mechanism studied by scattering-guided molecular dynamics simulation

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KDO2 Simulations

General description. The simulated 72-lipid KDO2 bilayer was built based on previous simulations of an LPS-containing membrane[1]. A monomer of WLBU2 was added initially at a distance of 10 Å from the membrane surface and then simulated for 400 ns, resulting in the interaction shown in Fig. S1. The KDO2:WLBU2 molar ratio was 72:1.

KDO2 MD methods. KDO2 simulations with and without WLBU2 were performed using NAMD 2.13[2]. The CHARMM36 lipid force field and CHARMM36m protein force field were used for lipids and peptide, respectively[3-4]. All covalent hydrogen bonds were kept rigid, which allowed for a 2-fs time step. Van der Waals interactions were cut off at 12 Å, with a force-based smoothing function applied between 10 and 12 Å to ensure a smooth decay to zero. The particle mesh Ewald summation method was used for long-range electrostatic interactions[5], which were calculated every two time steps. Temperature was kept constant at 328 K using Langevin dynamics. Pressure was maintained at 1 atm using the Langevin piston method[6], with the pressure in the plane of the membrane held separately from that in the orthogonal direction.

A bilayer containing 72 lipid A molecules (36 per leaflet), each having two attached KDO sugars, was constructed based on previous simulations of an LPS-containing membrane[1]. The membrane was solvated with 6549 TIP3P water on each side and 432 Na+ ions were added to neutralize the -6e charge on each KDO2 molecule (-4 *e* on lipid A and -1 *e* on each KDO residue). Over the course of a 200 ns simulation, the area/KDO2 for this membrane equilibrated to 160.7 +/- 0.8 Å^2 (averaged over the last 50 ns), in excellent agreement with experiment[7]. For systems with WLBU2, the peptide was added to the equilibrated membrane system. The peptide was placed oriented horizontally (helical axis in the membrane plane) 10 Å away from the

membrane surface, with overlapping water molecules removed. The whole new system was minimized and water and ions were equilibrated for 1ns while membrane and peptide atoms were restrained using a harmonic force constant of 2 kcal mol^{-1} $Å^{-2}$. Next, all restraints were removed and the system was simulated for an additional 400 ns to observe WLBU2 approach and insert into the headgroup region of the membrane. Simulated form factors and electron-density profiles were produced using the SimtoExp software[8]. Code is freely available on the WEB.

KDO2 results.

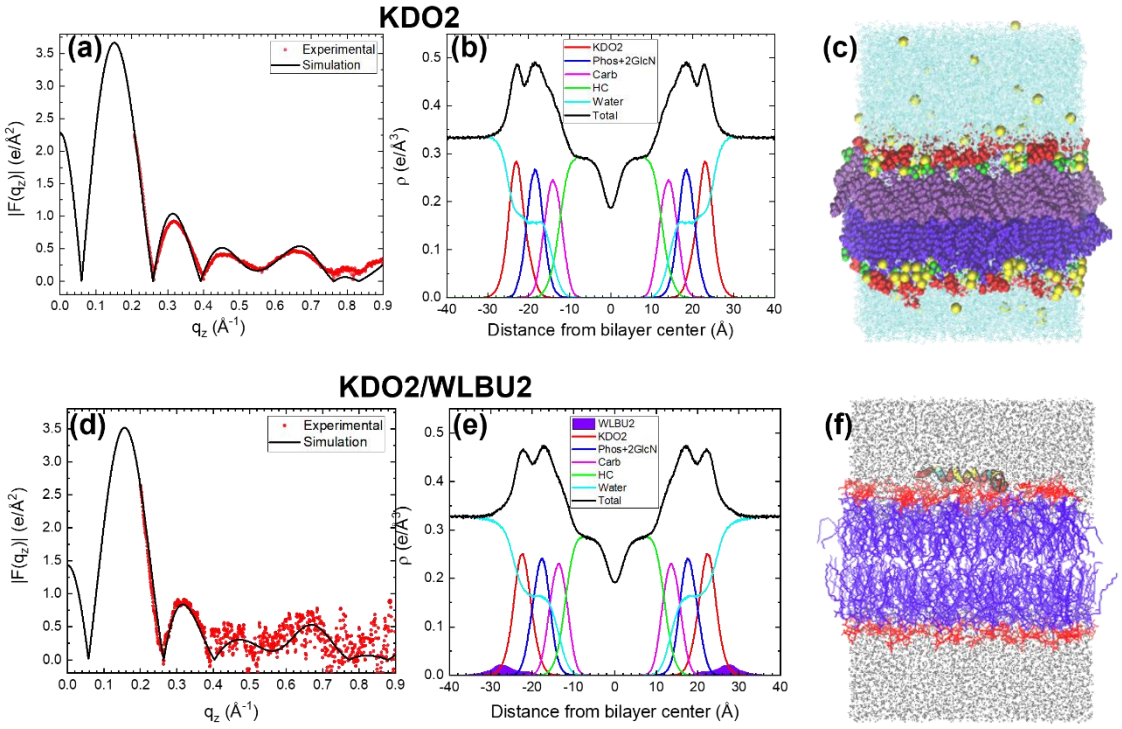

Figure S1. MD simulation and experimental results. (a) KDO2 control simulated form factors (black trace) and experimental (red circles). (b) KDO2 control simulated EDP. Colors: total, black; octulosonic acids, orange; phosphate groups + mannoses, blue; carbonyls, magenta; CH2 region, green; WLBU2, filled purple. (c) VMD visualization. Colors: lipid chains, lavender and purple; phosphate groups, green; octulosonic acids, red; Na^+ ions, yellow; water, cyan. (d) KDO2/WLBU2 (75:1), colors as in (a). (e) KDO2/WLBU2 (75:1) EDP, colors as in (b), with WLBU2, dark filled blue. (f) VMD visualization. Colors: lipid chains, purple; octulosonic acids, red; water, grey; WLBU2 with amino acids: R, red, V, yellow, W, blue. Figure reproduced with permission from Ref.[7].

Additional details of Robetta modeling

A shortcoming of Robetta is that its *de novo* modeling stems from the assumption that proteins typically form a soluble domain with a hydrophobic core, whereas short sequences such as the 24-mer WLBU2 often do not follow this trend. As a result, the Robetta server has a 28-residue minimum input length, so two valine residues were added to the C-terminus and N-terminus each of WLBU2. Valine was chosen as the additive to reduce steric and electrostatic effects. After Robetta outputted the potential initial structure, the four added valine residues were spliced out to recover the original 24-residues WLBU2.

Stability tests of inserted WLBU2 in G(-) IM simulation

In Figs. S2 and S3 are plotted the physical positions in the Z dimension across the membrane of different labeled amino acids in the inserted WLBU2 over time. These fluctuations are normal when dealing with the stochastic nature of the molecular dynamics simulations and show that WLBU2 is staying inserted in the membrane after being placed there and that there is no slow drift in any one direction. It is unlikely that the peptide would just suddenly start coming out of the membrane later after running it longer. It is more likely that it would either immediately come out as it did in the beginning over 20-40 ns without any surface tension, or alternatively just come out even more slowly with the surface tension. So, these stability tests show that for WLBU2 in G(-) IM LMM, there is no indication that there is a drift; this indicates that there is a low chance that the peptide would come out of the membrane if run longer.

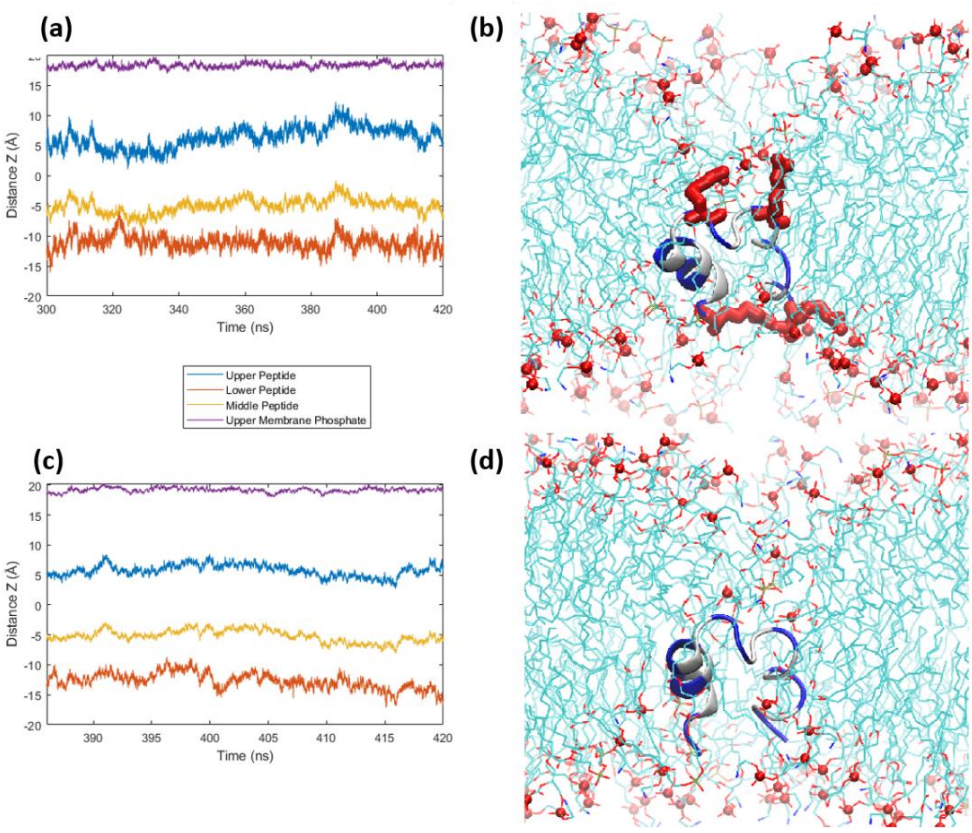

Figure S2. (a) and (b) correspond to one simulation and (c) and (d) correspond to another simulation. The (a) and (c) trials were used to fit the X-ray crossing points and were equilibrated for 320 ns before the data collection began. The (b) and (d) figures show a copy of the system that was set to 0 dyne/cm to test the extreme cases of stability. The residues that were tracked to generate the position vs. time graph were the hydrogens on the guanidinium groups of R12 and R16 to generate the upper peptide (blue). Amino acids R1 and R24 (the termini) were used for the bottom peptide (orange). The choice of which hydrogen was the same for all trials (HH12). Additionally, all of the residues that were used were always arginine for the long chain that extends to the furthest edges of the space occupied by the peptide. The middle peptide was tracked by using the center of mass of the entire peptide. The simulation snapshot (b) depicts the residues that were selected (red tubular shapes) and the phosphates (small red spheres) that are shown in both visualizations.

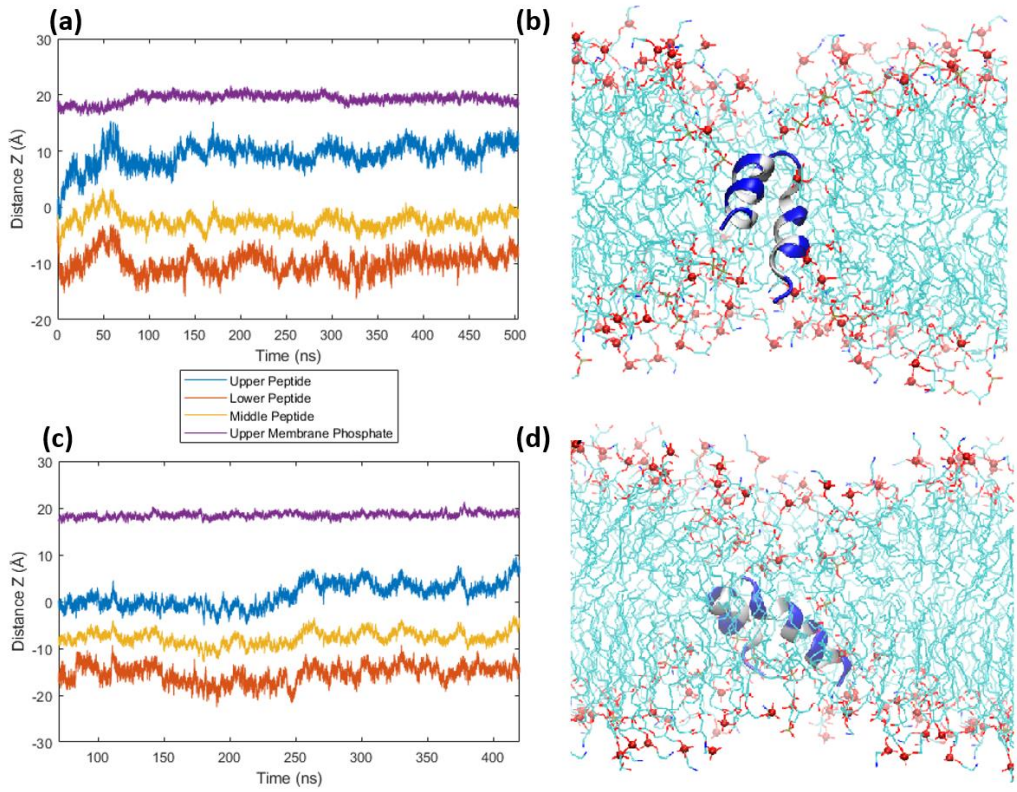

Figure S3. (a) and (b) correspond to one simulation and (c) and (d) correspond to another simulation. The (a) and (b) simulation was the longest simulation that was set at or below the target surface tension to ascertain if it the peptide was staying inserted at 15 dyne/cm. It was changed to 9 dyne/cm when it was stable. The residues that were used to test stability were R12 and R16 for the upper peptide (blue), R1 and R24 for the lower peptide (orange) and the entire peptide for the middle peptide (yellow). The (c) and (d) figures show the longest simulations that were continuously set to 15 dyne/cm. This simulation used R8 and R12 for the upper peptide to monitor its trajectory.

Stability test of inserted WLBU2 in G(+) LMM

In Fig. S4 is plotted the physical position in the Z dimension across the membrane of inserted WLBU2 over time. At 0 ns the peptide had been restrained near the bilayer center for 200 ns and then released. As shown, there are normal fluctuations in the position of the peptide, and very slight movement away from the bilayer center towards 6 Å from the bilayer center.

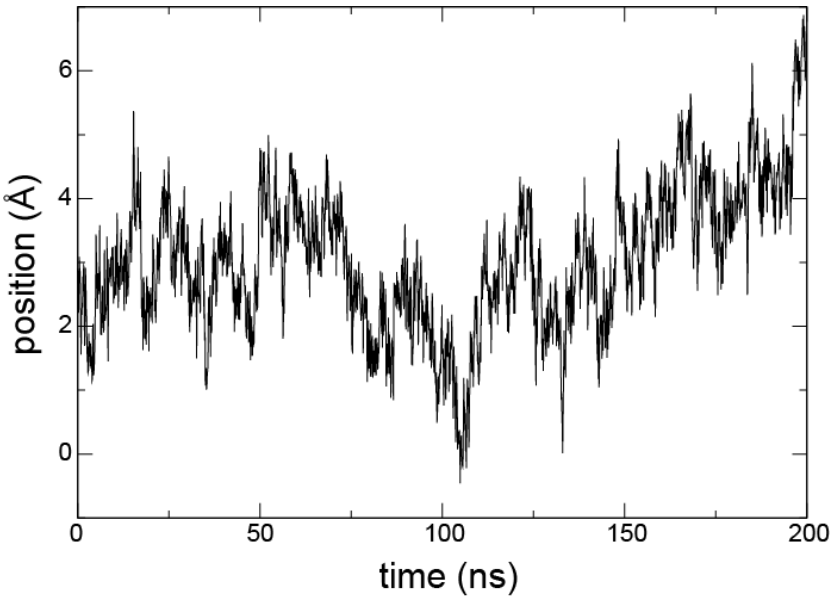

Figure S4. Distance between center of mass of WLBU2 and center of the Gram-positive membrane vs. time during a 200-ns unrestrained simulation with surface tension of 15 dyne/cm.

Neutron reflectivity (NR)

NR general description. Lipid:peptide mixtures are cosolubilized in organic solvent, dried under vacuum and hydrated via bath sonication. A single membrane bilayer was deposited onto a lipid-tethered gold-covered 3" silicon wafer using the vesicle fusion method[7]. NR data were collected at the NGD-MAGIK reflectometer[9] at the NIST Center for Neutron Research (Gaithersburg, MD) over a momentum transfer range 0-0.25 $Å^{-1}$. 6-hour scans were collected in either H_2O or D_2O at 37 °C for all models except KDO2 model, which was collected at 55 °C. Data were analyzed at NIST; 1D-structural profiles are parameterized using a continuous distribution model[10] using Refl1D software packages[11].

NR materials. Lipids and peptides were as for x-ray experiments. LPS model consisted of LPS/DLPG 1:3 and KDO2 model consisted of KDO2/DLPG 1:11. DLPG was added to both OM membrane models in order to achieve complete bilayer coverage on the tethered silicon wafer.

NR methods. While XDS uses a stack of membranes, NR probes a single bilayer attached to a gold substrate. 3'' diameter, 5 mm thick n-type Si:P [100] wafers (El-Cat Inc., Ridgefield Park, NJ) were cleaned with the SC-1 step of a RCA clean[12]. The substrates were coated with Cr (~20 Å) and Au (~150 Å) by magnetron sputtering (ATC Orion; AJA International, Scituate, MA). Coated substrates were soaked in a 7:3 (mol/mol) ethanol solution of HC18 20 and ß-mercaptoethanol at a total concentration of 0.2 mM to form a self-assembled monolayer (SAM). Substrates were assembled in NCNR fluids cells[11] and 5 mg/mL solutions of vesicles in 2 M NaCl of the desired lipid composition were allowed to incubate the dry SAM for ~2 h. Afterwards, the system was flushed with pure water to complete stBLM formation.

NR data collection. NR measurements were performed at the CGD-Magik reflectometer[9] at the NIST Center for Neutron Research (NCNR). Reflectivity curves were recorded for momentum transfer values $0.01 \leq qz \leq 0.25$ Å−1. For each measurement, adequate counting statistics were obtained after 5–7 h. The NCNR fluids cell[11] allows for in situ buffer exchange; therefore, subsequent measurements were performed on the same sample area. The entire flow cell was maintained at room temperature. After in situ completion of the stBLM, NR data were sequentially collected with D_2O and H_2O in the measurement cell. Buffer exchange was accomplished by flushing ~10 ml of buffer through the cell (volume ~1.3 ml) using a syringe. Temperature during the scan was 37 °C for all of the lipid models except 55 °C for KDO2 model.

NR data analysis. 1D-structural profiles of the substrate and the lipid bilayer along the lipid bilayer normal were parameterized using a continuous distribution model as described earlier[10]. The component volume occupancy (CVO) profile of the protein was defined by a Hermite spline with control points on average 15 Å apart. The spatial extension of the protein along the bilayer normal determined the number of control points which were iteratively refined during model optimization. Optimization of model parameters was performed using the ga refl and Refl1D software packages developed at the NCNR[11]. All reflectivity curves of one data set were fit simultaneously to the same model, sharing fit parameters, for example, for the solid substrate. A Monte Carlo Markov Chain-based global optimizer[11] was used to determine fit parameter confidence limits.

NR results.

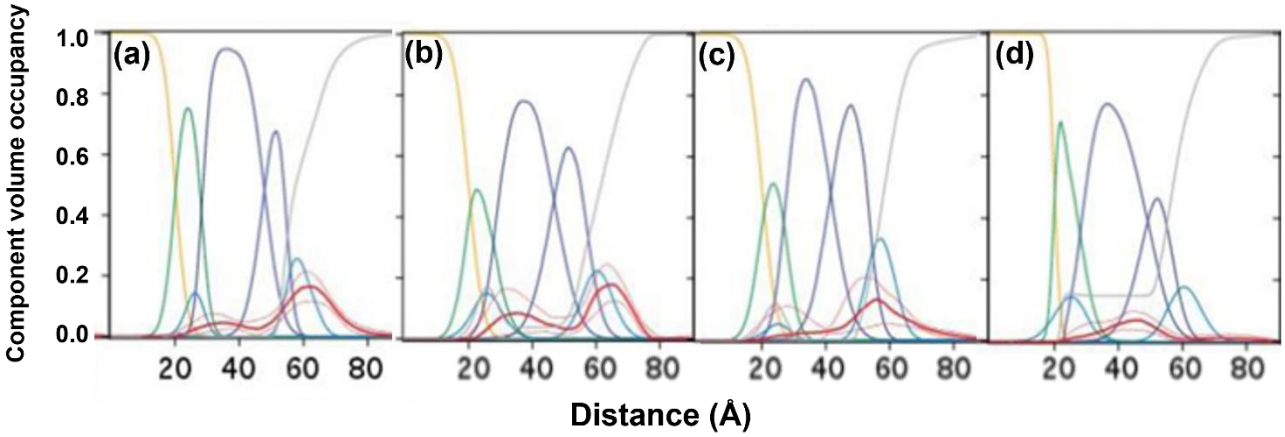

Figure S5. Component volume occupancy results from NR for WLBU2 in the following LMMs: (a) KDO2, (b) G(-) inner membrane, (c) G(+), (d) LPS. Colors: Gold-covered silicon wafer, gold; lipid tether, green; headgroups, light blue; hydrocarbons, dark blue; water, gray; WLBU2, red; 68% confidence limit on WLBU2's envelope, pink. Distance is measured from the underlying silicon wafer. These data were reproduced with permission from Ref.[7].

X-ray diffuse scattering (XDS)

XDS general description. Lipid model membranes (LMMs) were prepared using the Rock and Roll procedure[13] which mixes lipids and peptides in organic solvent, plates them onto chromic acid-cleaned silicon wafers or curved, cut glass rods and then dries them under vacuum. Samples were fully hydrated through the vapor in a hydration chamber[14]. When oriented samples of lipids are fully hydrated, they fluctuate and produce lobes of diffuse x-ray data (Fig. 3 in the main paper). X-ray diffuse scattering data were collected at the Cornell High Energy Synchrotron Source (CHESS), Ithaca, NY, on three trips using x-ray wavelengths ~1.1 or ~0.8 Å with the Flicam CCD detector or the Eiger 4M hybrid detector, and at the home source using a Rigaku (The Woodlands, TX) RUH3R rotating anode generator with x-ray wavelength 1.5418 Å and Mercury CCD detector. Membranes were measured at 37 °C, except for KDO2 which was measured at 55 °C. The XDS data are analyzed using liquid crystal theory[15-16] with methods described in detail in the SI to Ref.[17]. Full hydration causes the membrane stacks to fluctuate, producing lobes of diffuse data, which provide the intensity data that is the basis for the form factors.

XDS materials. The 24-mer peptide WLBU2 was synthesized by Genscript (Piscataway, NJ). Mass spectroscopy showed the purity to be ~98%. The synthetic lyophilized lipids 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(10-rac-glycerol) sodium salt (POPG), 10,30-bis-[1,2-dioleoyl-sn-glycero-3-phospho]-*sn*-glycerol sodium salt (TOCL, i.e., cardiolipin), 1,2-dioleoyl-3-trimethylammonium-propane chloride salt (DOTAP) were purchased from Avanti Polar Lipids (Alabaster, AL) and used as received. HPLC organic solvents were purchased from Sigma/Aldrich (St. Louis, MO).

XDS methods. Lipid model membranes were prepared by dissolving lyophilized lipids in chloroform. These lipid stock solutions were combined to create lipid mixtures in molar ratios mimicking the G(-) inner membrane (IM), POPE/POPG/TOCL (7:2:1 molar ratio), and G(+) cell membrane, POPG/DOTAP/POPE/TOCL (6:1.5:1.5:1). Lipid compositions were based on Ref.[18]. WLBU2 stock solution was prepared by mixing the lyophilized peptide powder with hexafluoroisopropanol (HIP). Multilamellar stacked samples for x-ray scattering were prepared by mixing 4 mg of the G(-) lipid mixture plus WLBU2 in a lipid:peptide ratio of 75:1. Solvents were removed by evaporation under vacuum and samples were redissolved in 200 µl chloroform:TFE (1:1, v:v) for plating onto highly polished silicon wafers (1 x 15 x 30 mm^3) via the Rock-and-Roll[13] method to produce stacks of ~1800 well-aligned bilayers, where the silicon wafer is rocked continuously during solvent evaporation. Plating was carried out in the fume hood due to the toxicity of HIP. Once immobile, the thin film was placed under vacuum for at least 2 h. The sample was trimmed to a central 5 mm wide strip parallel to the long edge of the wafer and hydrated through the vapor in a thick-walled x-ray hydration chamber[14]. Condensation of water into the sample was enhanced by a Peltier cooler under the silicon wafer. Alternatively, hydration was expedited by adhering the silicon wafer onto a rectangular stone block using Dow Corning heat sink compound, then pre-cooling the wafer and block in a

refrigerator held at ~5°C. The wafer on the block was then quickly transferred to the hydration chamber held at 37 °C where full hydration through the vapor occurred within 5-10 minutes. Samples on cut glass rods were similarly hydrated by rapid transfer from cold storage to the warm chamber. When the oriented lipid/peptide samples are fully hydrated they fluctuate, producing lobes of diffuse data, in addition to 2 lamellar orders of Bragg diffraction (shown in Fig. 3 as green dots through the white diffuse lobes). Form factors were obtained as previously described[15-16] by collecting the diffuse intensity (I_q) under the blue band shown in Fig. 3 in the main paper and then applying this equation:

$$|F(q_z)| = \sqrt{q_z \cdot I(\boldsymbol{q})/S(\boldsymbol{q})}$$

where q_z is the Lorentz polarization correction and $S(\mathbf{q})$ is the structure factor, which is obtained by application of liquid crystal elasticity theory as described previously[15-17].

XDS results.

The second key data besides NR for simulations to compare to is the XDS form factor, which leads to the area per lipid and membrane thickness. Form factors for all four mimics were previously published[7]. As shown in Fig. S6(a-d), form factors for mimics containing 75:1 lipid:WLBU2 (red) move to slightly higher q_z values compared to their controls (black), indicating membrane thinning upon the addition of WLBU2.

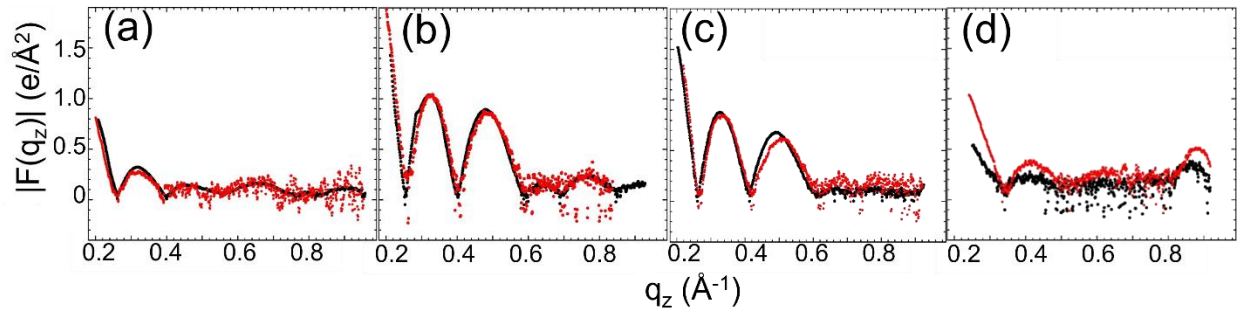

Figure S6. Form factor data obtained from XDS data collected at 37 °C for the following LMMs: (a) KDO2, (b) G(-) IM, (c) G(+), (d) LPS. Colors: Control data (black solid circles), lipid:WLBU2 75:1 (red solid circles). These data were reproduced with permission from Ref.[7].

NR peak decomposition.

For G(-) IM, WLBU2 components in the Component Volume Occupancy graph shown in Fig. 5(b) were fit to Gaussian distributions, as shown in Fig. 7(a). The percentage of the total peptide volume was determined by integrating the area under the individual peaks and dividing by the total. For G(+), the midpoint of the hydrocarbon error function was first located at 54.5 Ångstroms from the substrate. Subsequently, the area under each side of the peak was integrated and converted to percentage as shown in Fig. 7(b).

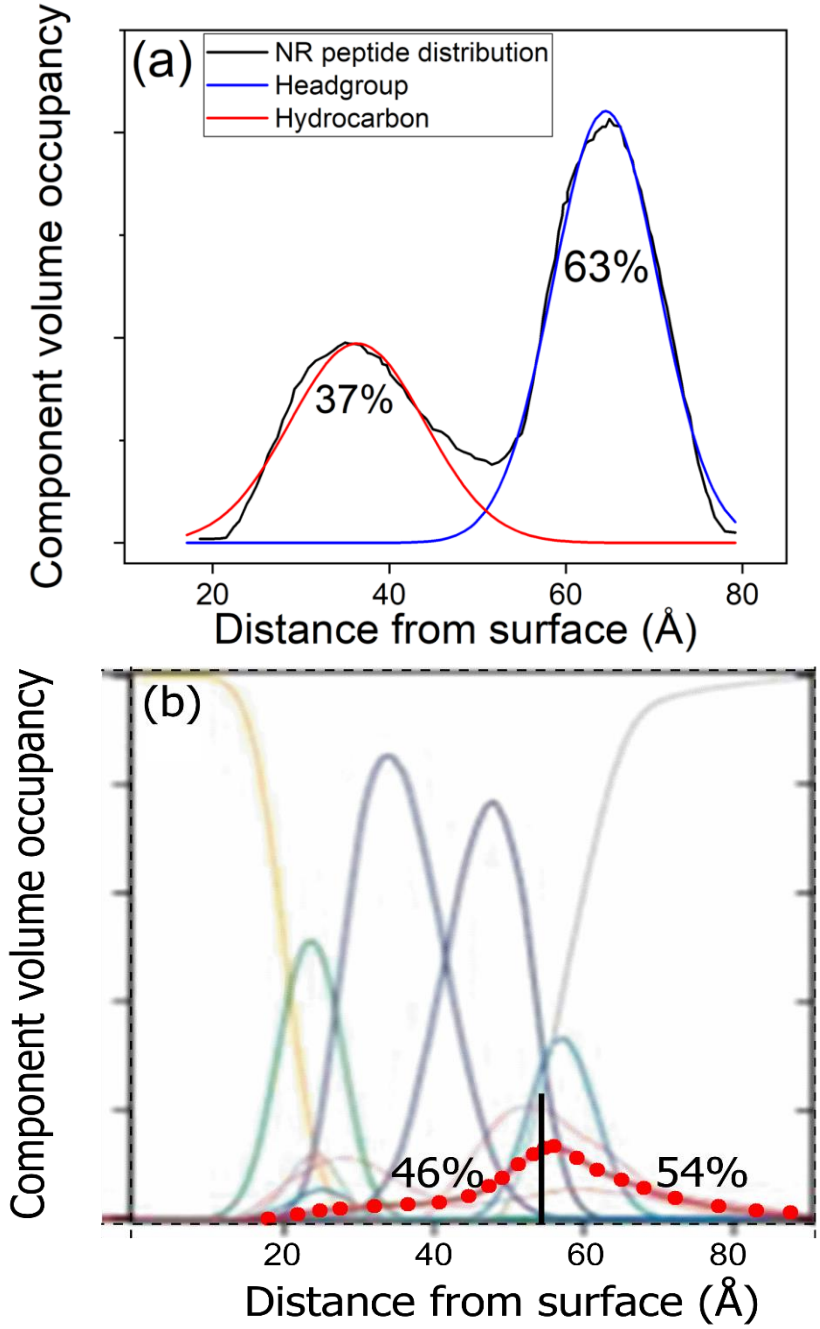

Fig. S7. Method of determining WLBU2 partitioning in (a) G(-) IM, and (b) G(+) lipid model membranes with NR peptide distribution shown as red solid circles. Components as in Fig. S5.

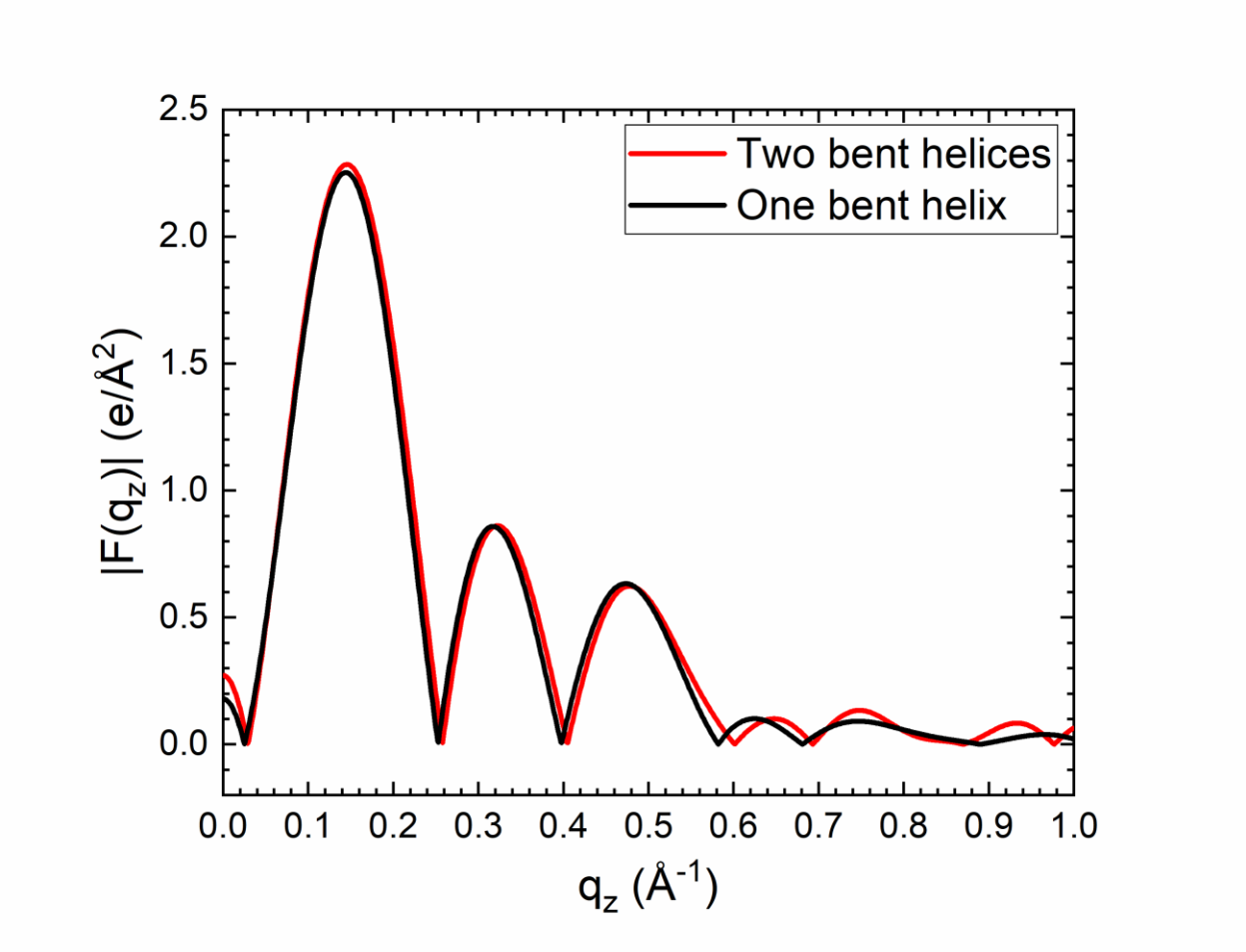

Figure S8. Simulated form factors of WLBU2 in the surface state where two bent helices were added to G(-) LMM, one on each side (red line), or only one bent helix was added to one side (black line).

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