Movement of Arginine through OprD: The Energetics of Permeation and the Role of Lipopolysaccharide in Directing Arginine to the Protein

Firdaus Samsudin¹ and Syma Khalid¹*
¹School of Chemistry, University of Southampton, Southampton SO17 1BJ, UK
*Corresponding author: s.khalid@soton.ac.uk

Supporting Information
Figure S1. Equilibrium simulations of OprD in a model outer membrane. a, A snapshot of OprD embedded in a model outer membrane with key loop regions labelled. b, All-atom root mean square deviation (RMSD) progression along two independent simulations of the key loop regions (same colour scheme as a) and the rest of the protein (black). c, Root means square fluctuation of each residue of OprD averaged over the two simulations. d, Secondary structure preservation of OprD during one of the simulations.
Figure S2. OprD pore profile. a, The shape of the channel connecting the two solvent exposed sides of OprD. Dotted red lines indicate parts of the protein that are exposed to bulk water in the extracellular and periplasmic sides. Enlarged images show the smallest constriction of the pore encircled by residues S130, D295, S296 and R391. b, (Top) Minimum distance between the side chain of R391 and that of S130, D295 and S296 during two independent simulations. (Bottom) The number of water molecules permeating through OprD during these simulations.
**Figure S3. Steered MD simulation of arginine across OprD.** (Left) Snapshots of arginine substrate from the steered MD simulations taken every 100 ps with the eyelet residues highlighted. (Right) The same snapshots with the basic ladder residues labelled.
Figure S4. Evaluation of sampling and convergence. Histogram overlap from umbrella sampling simulations for arginine translocation through a, OprD and b, outer membrane. PMF value at two points along the reaction coordinate (indicated by the red asterisk in inset) generated from increasing amount of simulation data for arginine translocation through c, OprD and d, outer membrane.
Figure S5. Average number of hydrogen bonds made between the permeating arginine molecules with OprD (blue) and LPS (red) in all umbrella sampling windows.
**Figure S6. Diffusion of arginine in bulk outer membrane.** a, Correlation between the direction of motion of arginine and lipids (LPS in blue and phospholipids in red) found within 1 nm in all umbrella sampling windows. Error bars indicate standard deviations. LPS is coloured dark grey in the left snapshot and the approximate position is indicated by the dotted lines. b, Centre of mass trajectories of arginine along the plane of the membrane in bulk solution (grey), LPS headgroups (blue) and phospholipid headgroups (red). c, Comparison of centre of mass trajectories of arginine (blue) and an adjacent LPS molecule (green). d, Snapshots of arginine taken every 10 ns from the last 100 ns of the simulation corresponding to c, with four surrounding LPS molecules (orange, pink, green, purple).
Figure S7. Water molecules around arginine and LPS headgroups. A side and top view of water molecules found within 3 Å of arginine as it embedded into the LPS sugar moieties. Three nearby LPS molecules are shown in pink, green and orange.