Electronic Supplementary Material (ESI) for Chemical Communications

Electrostatics and Flexibility Drive Membrane Recognition and Early Penetration by Antimicrobial Peptide Dendrimer bH1

Harish Kumar Ravi[a], Michaela Stach[a], Thereza A. Soares[b], Tamis Darbre[a], Jean-Louis Reymond[a], and Michele Cascella*[a]

[a] Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland, and [b] Departamento de Quimica Fundamental, Universidade de Pernambuco, Av. Jornalista Anibal Fernandes, s/nº Cidade Universitária 50740-560, Recife, Brazil

Supplementary Information

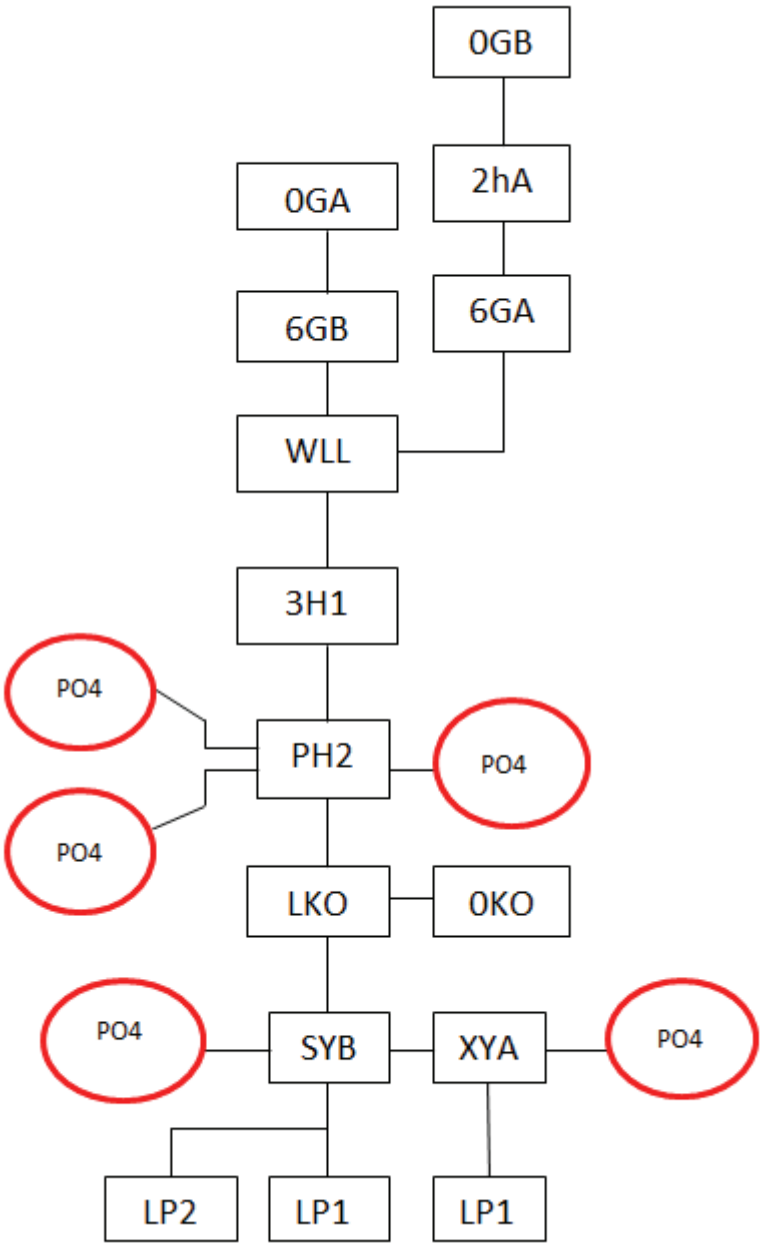
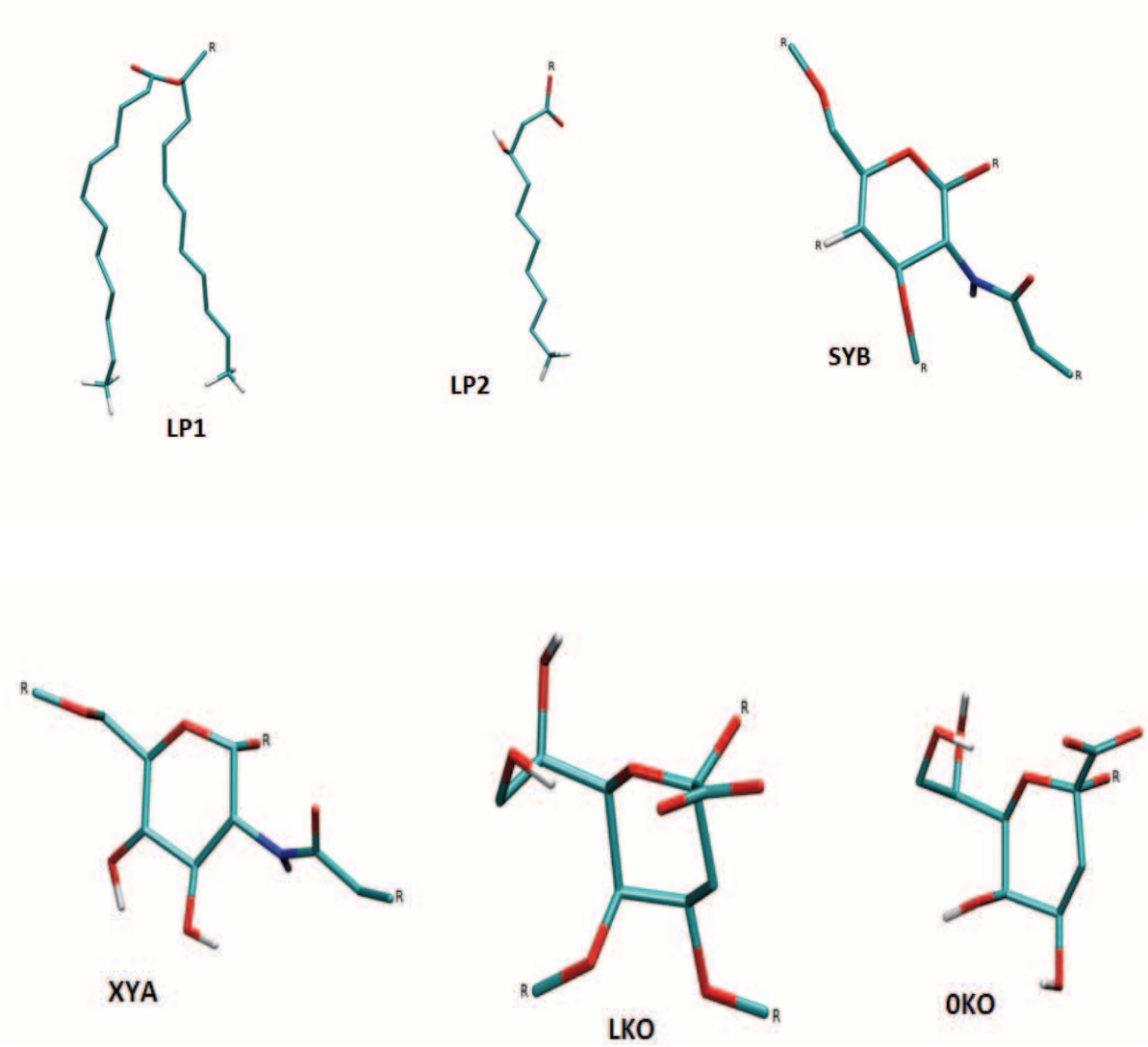


Figure S1: The general architecture of one unit of PA01 LPS chemotype in *Psudomonas aeruginosa*.


LP1
LP2
SYB
XYA
LKO
0KO
PH2
PO4
3H1

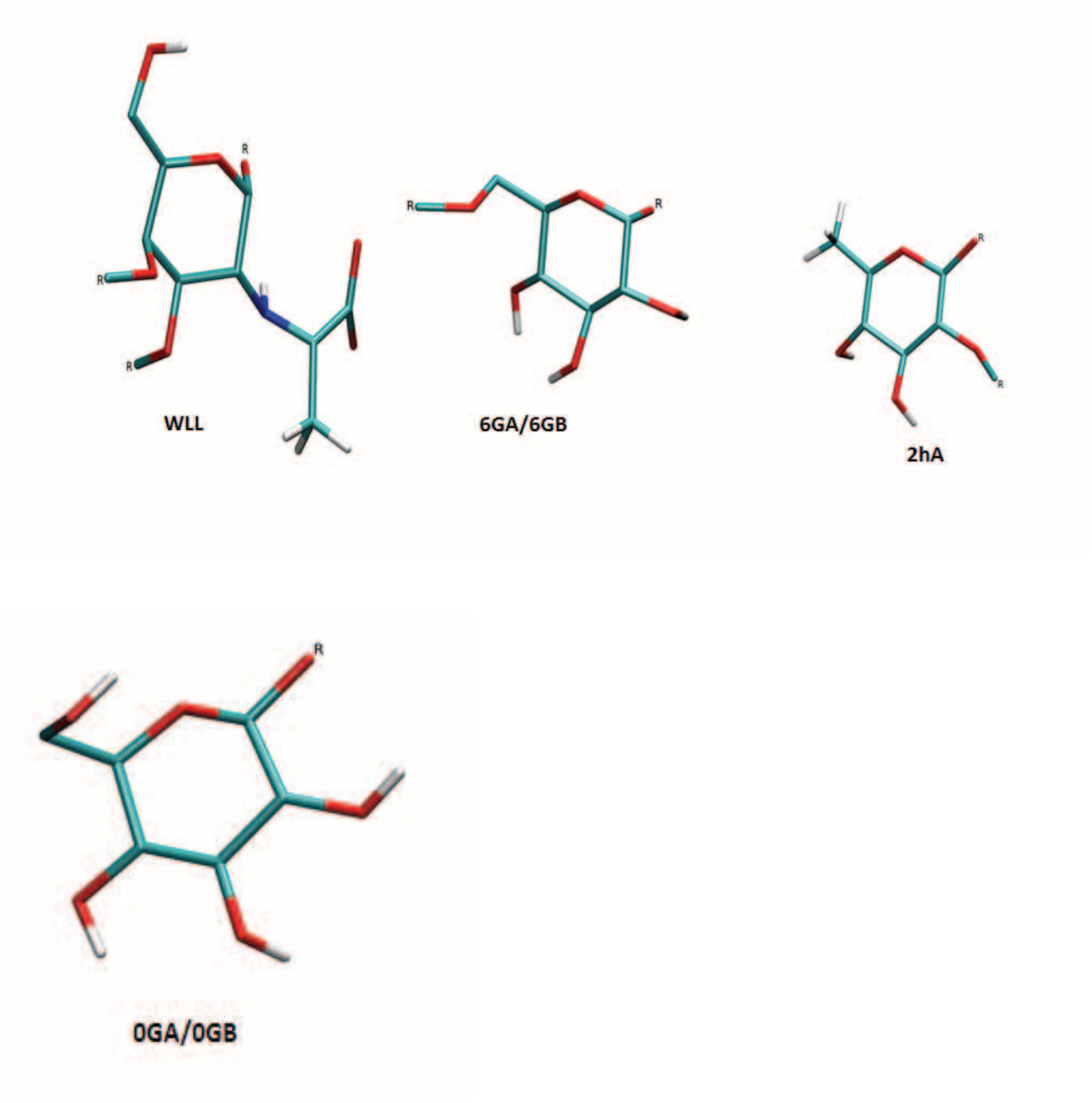
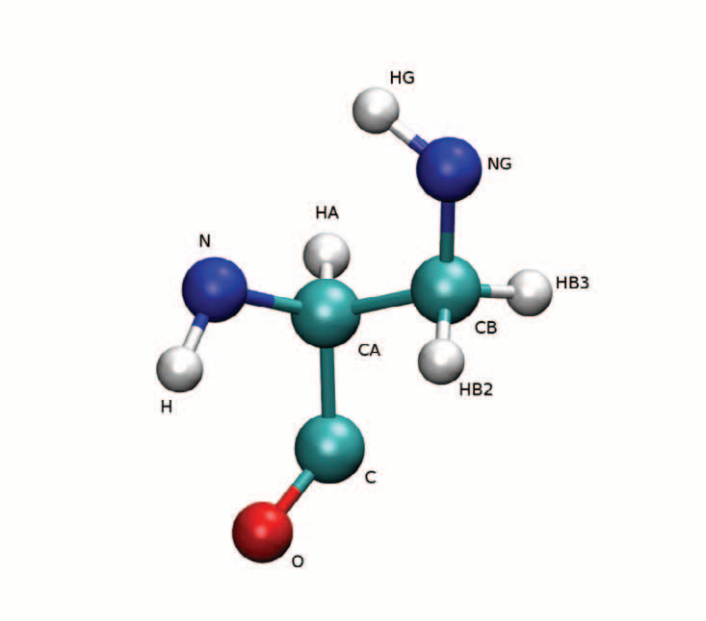


Figure S2: Molecular structure of Individual components that make up the LPS chemotype.



Atom	Charge	Atom	Charge
C	0.597300	N	-0.415700
O	-0.567900	H	0.271900
CA	0.069103	CB	0.018001
HA	0.053650	HB2	0.074392
NG	-0.327105	HB3	0.074392
HG	0.151967		

Figure S3: RESP charges of the branching (*S*)- 2,3-diaminopropionic acid used in the calculation.

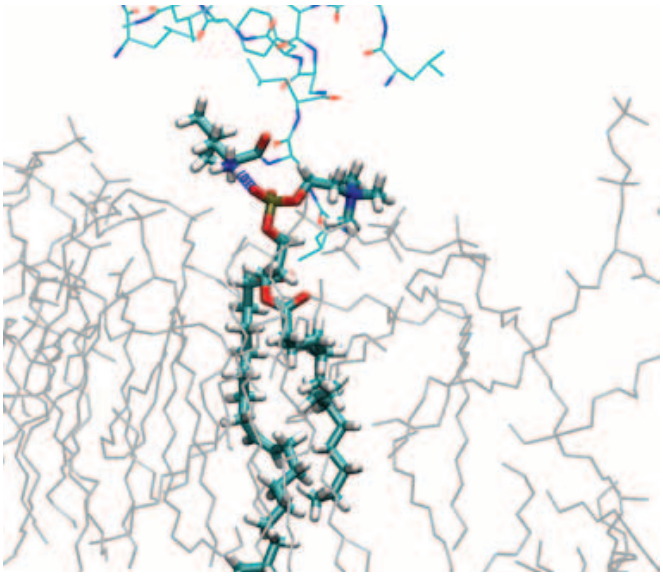


Figure S4: Interaction of bH1 with POPC. The licorice representation was chosen to highlight terminal Leucines and the corresponding POPC lipid they interact with. The other residues in the vicinity are represented as lines.

Material and Methods

Computational Methods

For our simulation we first built the dendrimer bH1 and added the missing hydrogen atoms using the default protonation of the structures by the xleap module in Amber12.[1] The amber force field (FF99SB[2]) was used to describe intra- and inter-molecular interactions. The partial charges for the branching residues were obtained by RESP[3] fitting procedure. The force field parameters for POPC are those from ref. 9a of the main text. A detailed description of the force field parameters for the LPS-DPPE moiety can be found in ref. 9b of the main text. The bH1:POPC system were solvated by 30 222 water molecules and placed in a 120 X 120 X 120 $Å^3$ periodic box. The bH1:LPS-DPPE were solvated by 73 443 water molecules and placed in a 150 X 120 X 120 $Å^3$ periodic box. The TIP3P[4] water model was used. Particle Mesh Ewald routines were used to treat long-range electrostatic interactions.[5,6] A cutoff of 12 Å was used for the van der Waals interactions and the real part of the electrostatic interactions. The simulations were performed in the NpT ensemble with a Langevin chain of thermostats[7]. A total of 9 Cl- ions were added to neutralize both the system. Bonds comprising hydrogen atoms were constrained with the SHAKE constraint algorithm[8] for the protein and the SETTLE

algorithm[9] for the water. Explicit solvent simulations were performed using periodic boundary conditions. All simulations were performed using the NAMD program[10] and the trajectories were analyzed using the Gromacs[11] package. First, energy minimization on the solvent was carried out, while holding the dendrimer and the membrane fixed, using the steepest descent algorithm for the first 300 steps followed by the conjugate gradient algorithm for 7200 steps. Then the same minimization cycles were repeated for the whole system. The energy minimized structures were heated up from 10 to 300 K in 0.3 ns MD, using a time step of 2 fs. Following this production runs of 300 ns and 500 ns were made for the bH1:LPS-DPPE and bH1:POPC membranes respectively.

Material and reagents

All salts, buffers and solvents were of the best grade available from Sigma, Fluka, Acros Organics or Dr. Gogg Chemie AG and used as received. 5(6)-carboxyfluorescein (CF) was from Sigma. Egg phosphatidylcholine (EPC), egg phosphatidylglycerol (EPG) and a Mini-Extruder used for vesicle preparation were from Avanti Polar Lipids. Fluorescence measurements were performed with a Fluorescence spectrophotometer (Cary Eclipse, Varian) equipped with a stirrer and a temperature controller (measurements at 25 °C unless otherwise noted).

Lipid Vesicles Experiments

The experiments were performed following the procedure described in reference 12. A thin lipid film was prepared by evaporating a solution of 25 mg Egg PC or Egg PG in 1 mL MeOH/$CHCl_3$ 1/1 on a rotary evaporator (rt) and then *in vacuo* overnight. The resulting film was hydrated with 1 mL buffer (50 mM CF, 10 mM TIRS, 10 mM NaCl, pH 7.4) for more than 30 min, subjected to freeze-thaw cycles (7x) and extrusion (15x) through a polycarbonate membrane (pore size 100 nm). Extravesicular components were removed by gel filtration (Sephadex G-50) with 10 mM TRIS, 107 mM NaCl, pH 7.4. Final conditions: ~ 2.5 mM Egg PC or Egg PG; inside: 50 mM CF, 10 mM TIRS, 10 mM NaCl, pH 7.4; outside: 10 mM TRIS, 107 mM NaCl, pH 7.4. Egg PC or Egg PG stock solutions (37.5 µL) were diluted with a buffer (10 mM TRIS, 107 mM NaCl, pH 7.4) and placed in a thermostated fluorescence cuvette (25°C) and gently stirred (total volume in the cuvette ~3000 µL; final lipid concentration ~31.25µM). CF efflux was monitored at l_{em} 517 nm (l_{ex} 492 nm) as a function of time after addition of 20 µL of peptide dendrimer in buffer (10 mM TRIS, 107 mM NaCl,

pH 7.4) with final concentrations of 1, 5, 7.5, 10, 15, 20, 25, 30 μg/mL at t = 50 s and 1.2% trition X-100 (30 μL, 0.012% final concentration) at t = 300 s. Fluorescence intensities were normalized to fractional emission intensity *I(t)* using $I(t) = (I_t - I_0) / (I_\infty - I_0)$ where $I_0 = I_t$ at peptide dendrimer addition, $I_\infty = I_t$ at saturation of lysis.

Supporting References

1. D.A. Case, T.A. Darden, T.E. Cheatham, III, C.L. Simmerling, J. Wang, R.E. Duke, R. Luo, R.C. Walker, W. Zhang, K.M. Merz, B. Roberts, S. Hayik, A. Roitberg, G. Seabra, J. Swails, A.W. Goetz, I. Kolossváry, K.F. Wong, F. Paesani, J. Vanicek, R.M. Wolf, J. Liu, X. Wu, S.R. Brozell, T. Steinbrecher, H. Gohlke, Q. Cai, X. Ye, J. Wang, M.-J. Hsieh, G. Cui, D.R. Roe, D.H. Mathews, M.G. Seetin, R. Salomon-Ferrer, C. Sagui, V. Babin, T. Luchko, S. Gusarov, A. Kovalenko, and P.A. Kollman (2012), *AMBER 12*, University of California, San Francisco.
2. V. Hornak, R. Abel, A. Okur, B. Strockbine, A. Roitberg, C. Simmerling, *Proteins: Structure, Function and Genetics*., 2006, **65**, 712-715.
3. F.-Y. Dupradeau, A. Pigache, T. Zaffran, C. Savineau, R. Lelong, N. Grivel, D. Lelong, W. Rosanski, P. Cieplak, *Phys. Chem. Chem. Phys.,* 2010, **12**, 7821-7839
4. W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, M. L. Klein, *J. Chem. Phys.,* 1983, **79**, 926– 935
5. T. Darden, D. York, L. G. Pedersen, *J. Chem. Phys.,* 1993, **98**, 10089– 10092.
6. U. Essmann, L. Perera, M. L. Berkowitz, T. Darden, H. Lee, L. G. Pedersen, *J. Chem. Phys*., 1995, **103**, 8577– 8593.
7. S. A. Adelman, J. D. Doll, *J. Chem. Phys.,* 1976, **64**, 2375– 2388.
8. J-P. Ryckaert, G. Ciccotti, H. J. C. Berendsen, *J. Comput. Phys*., 1977, **23**, 327– 341.
9. S. Miyamoto, P. A. Kollman, *J. Comput. Chem*., 1992, **13**, 952– 962.
10. J. C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kale, K. Schulten, *J. Comput. Chem.,* 2005, **26**, 1781– 1802.
11. B. Hess, C. Kutzner, D. Van der Spoel, E. J. Lindahl, *J. Chem. Theory Comput.,* 2008, **4**, 435– 447.
12. A. Hennig, G. J. Gabriel, G. N. Tew, S. Matile, *J. Am. Chem. Soc.* 2008, **130**, 10338-10344