# Supporting Information

## Getting drugs into Gram-negative bacteria: Rational rules for permeation through general porins

Silvia Acosta-Gutiérrez[1], Luana Ferrara[2], Monisha Pathania[3], Muriel Masi[4], Jiajun Wang[5], Igor Bodrenko[1], Michael Zahn[3], Mathias Winterhalter[5], Robert A. Stavenger[6], Jean-Marie Pagès[4], James H. Naismith[7], Bert van den Berg[3], Malcolm G. P. Page[5] & Matteo Ceccarelli[1*]

**Affiliations**

[1]Department of Physics, University of Cagliari, Cittadella Universitaria di Monserrato, SP Monserrato-Sestu Km 0.8, 09042 Monserrato (CA).

[2]Biomedical Sciences Research Complex, University of St Andrews, St Andrews, KY16 9RH, United Kingdom

[3]Institute for Cell and Molecular Biosciences, The Medical School, Newcastle University, Newcastle upon Tyne NE2 4HH, United Kingdom.

[4]UMR_MD1 Inserm U1261, Membranes et Cibles Thérapeutiques, 27 Bd Jean Moulin, Aix-Marseille Université, Facultés de Pharmacie et de Médecine, 13005 Marseille, France

[5]Department of Life Sciences and Chemistry, Jacobs University Bremen, 28719 Bremen, Germany.

[6]Antibacterial DPU, GlaxoSmithKline, 1250 Collegeville Rd., Collegeville, Pennsylvania, PA 19426, USA

[7]Division of Structural Biology, Nuffield Department of Medicine, Roosevelt Drive, Oxford, OX3 7BN, United Kingdom

Research Complex at Harwell, Rutherford Laboratory, Didcot, OX11 0FA, United Kingdom

Corresponding author: Matteo Ceccarelli, matteo.ceccarelli@dsf.unica.it

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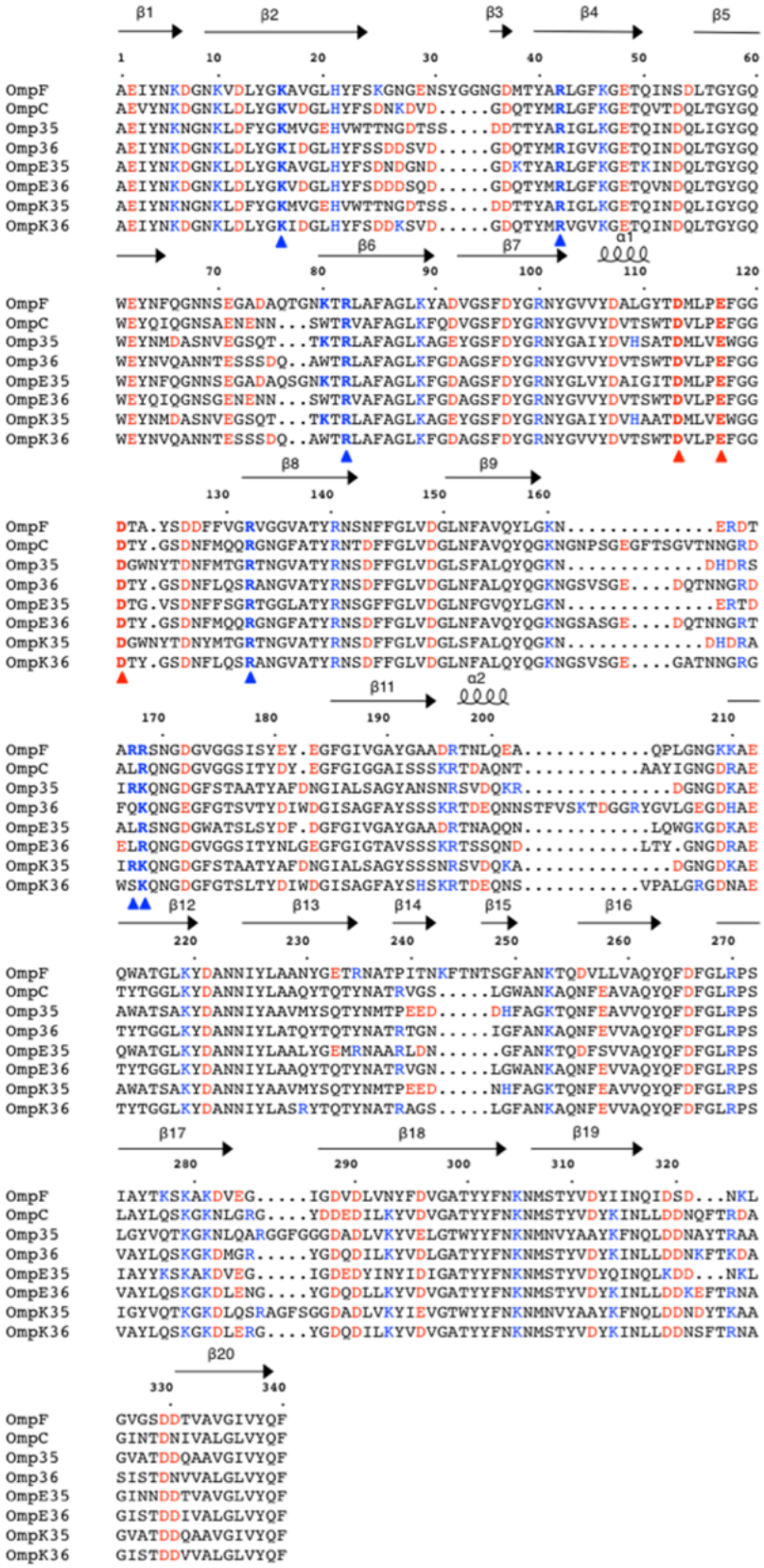
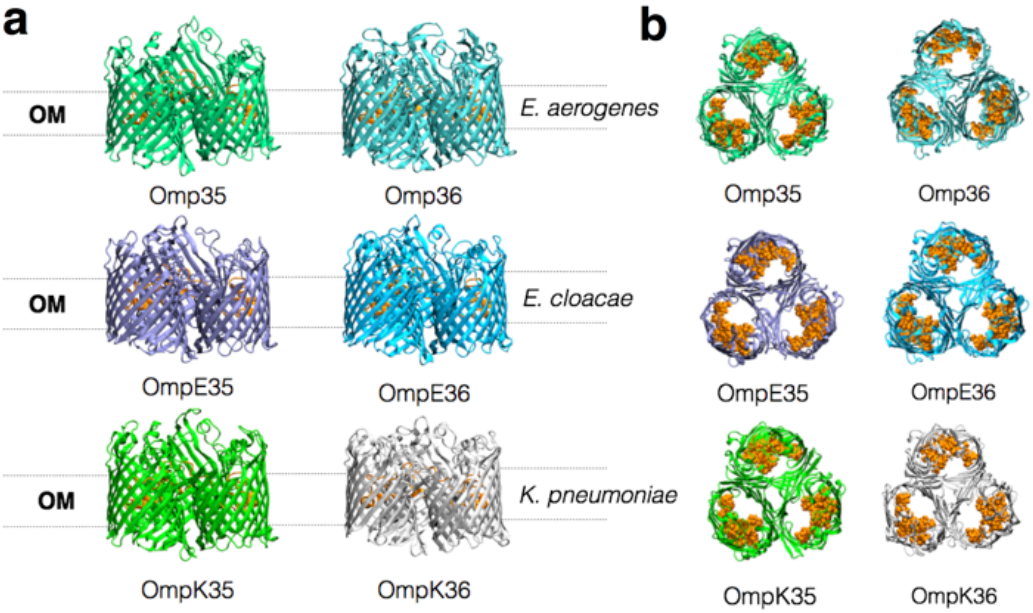
# Supplementary Figures

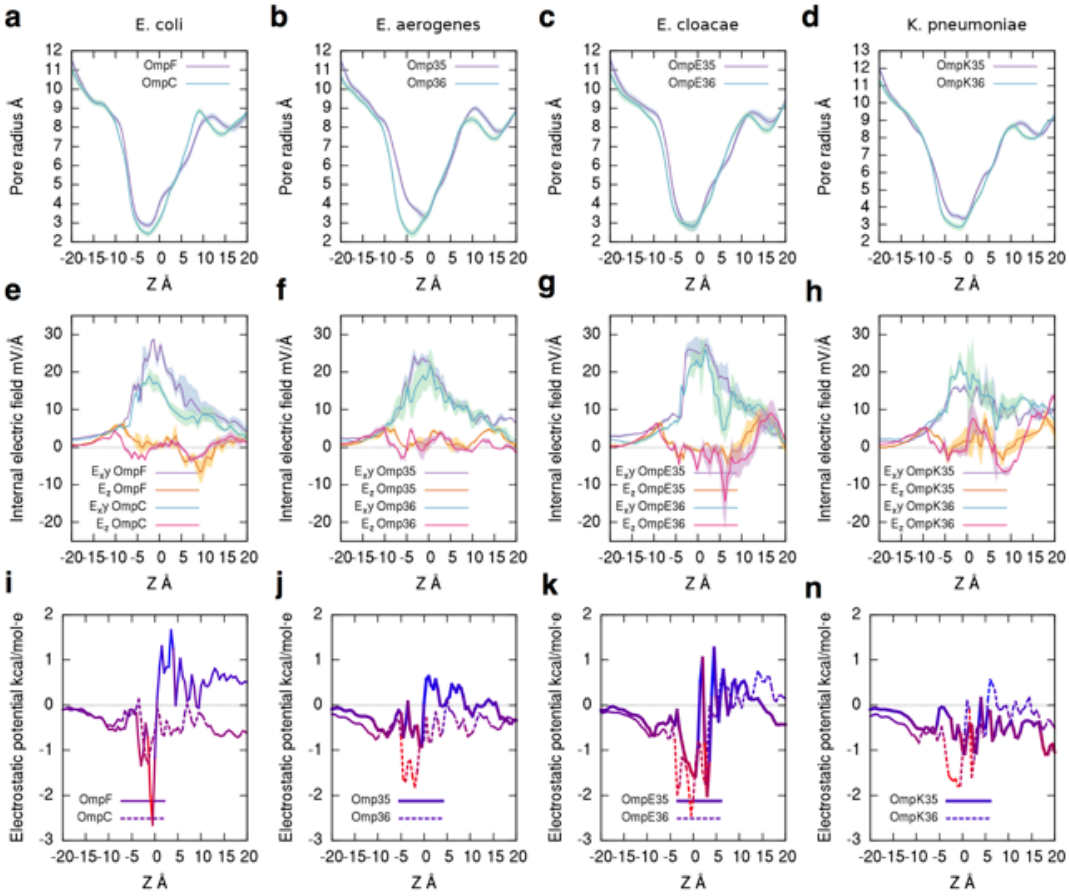



**Supplementary Figure 1 | OmpF/C orthologues sequence alignment.** Structural alignment of the seven porins investigated (OmpC, Omp35, Omp36, OmpE35, OmpE36, OmpK35, OmpK36) to OmpF (*E.coli*). All charged residues are coloured

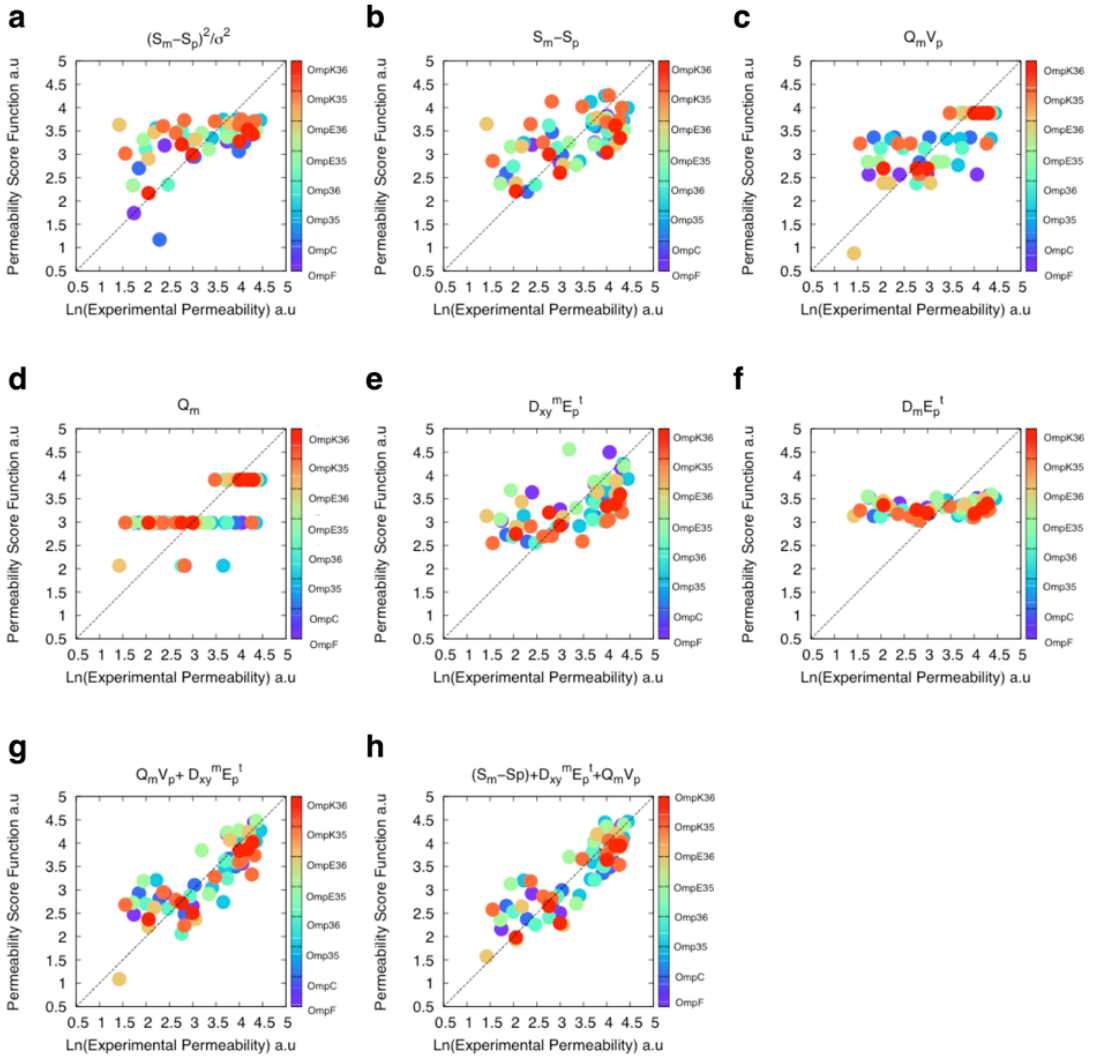
according to their net charge. Key residues from the OmpF electrostatic filter are highlighted in bold and mark with a triangle (K16, R42, K80, R82, D113, E117, D121, R132, R167, R168).



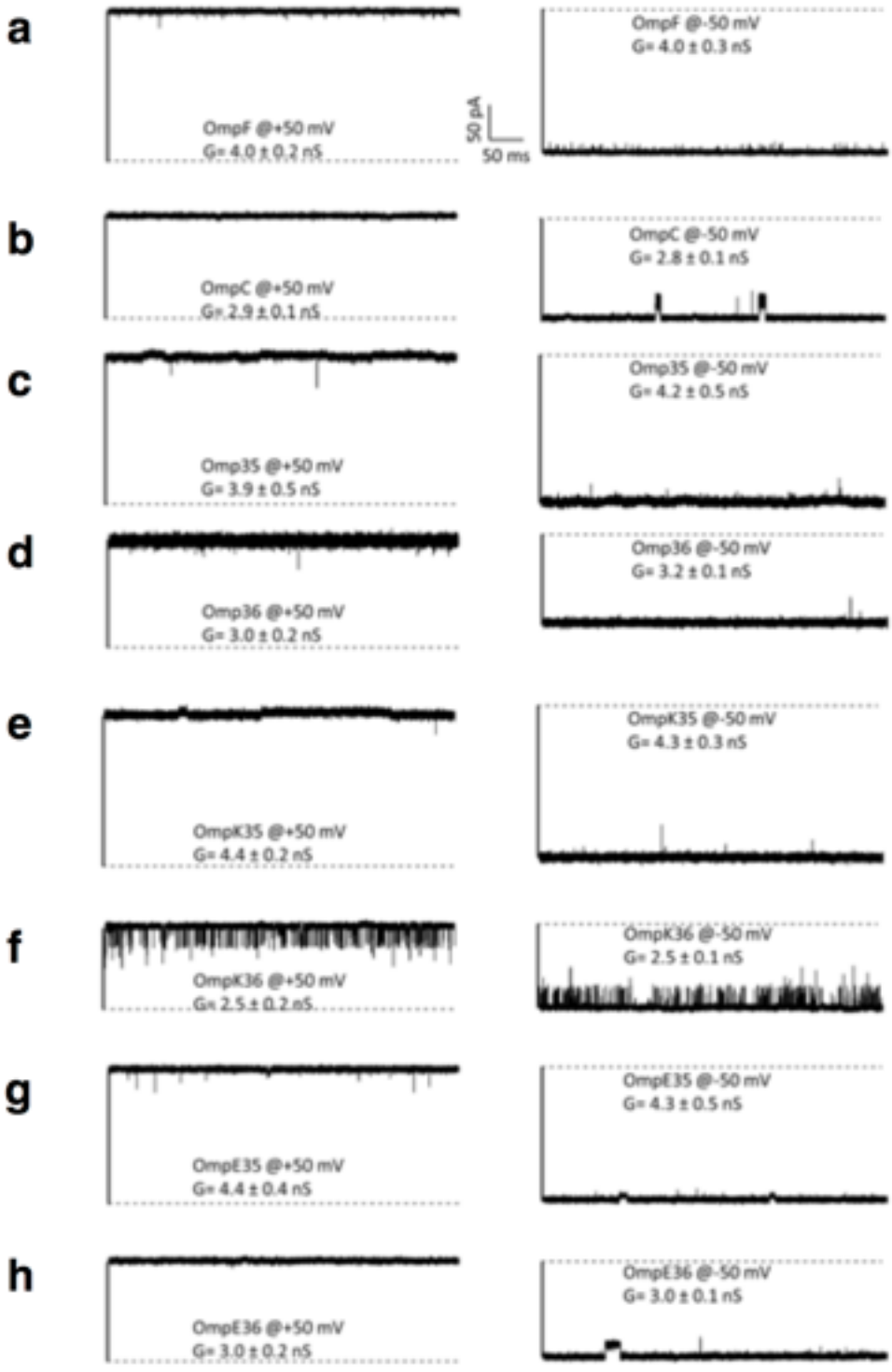

**Supplementary Figure 2 | OmpF/C orthologues architecture.** OmpF/C orthologues are depicted as cartoon for each Enterobacteriaceae family. **a,** Longitudinal view of the trimeric arrangement in the OM of each porin. **b**, Top view of the eyelet region for each porin. Constricting loop (L3) is depicted in van der waals representation and highlighted in orange.



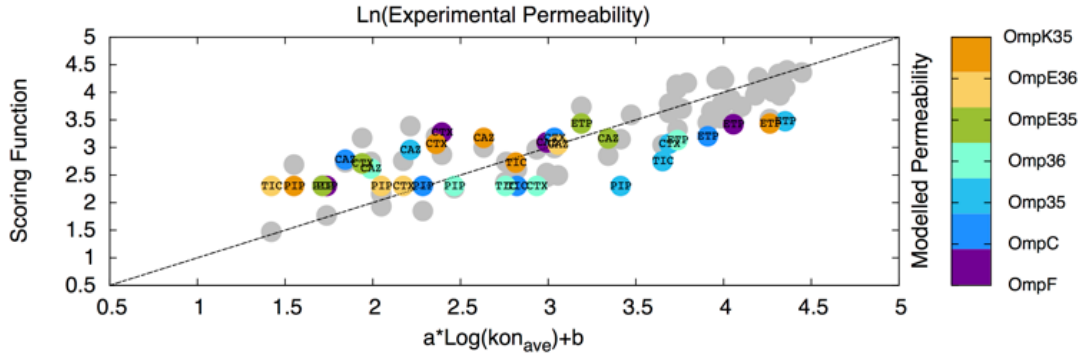

**Supplementary Figure 3 | Porins size and electrostatic properties. a-d**, Average pore radius for each porin; **e-h**, average internal electric field decomposed in its longitudinal ($E_z$) and transversal ($E_{xy}$) components with respect to the diffusion axis of each porin; **i-n**, average internal electrostatic potential along the axis of diffusion of each porin.



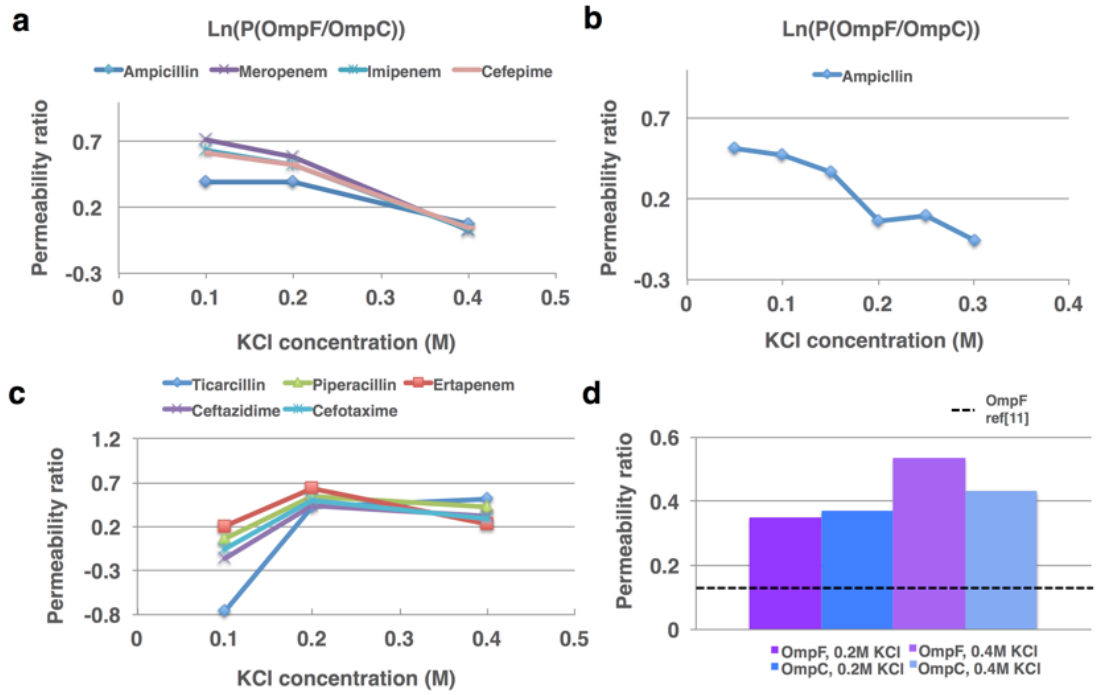

**Supplementary Figure 4 | Evaluation of the scoring function.** Measured vs. predicted permeability using different terms from the scoring function (Eq.4) and different analytical forms for some terms: **a**, $U_{steric}$, **b**, modified $U_{steric}$, **c**, $U_{charge}$, **d**, modified $U_{charge}$, **e**, $U_{dipole}$, **f**, modified $U_{dipole}$, **g**, $U_{charge}+U_{dipole}$ and **h**, full scoring function using modified $U_{steric}$.



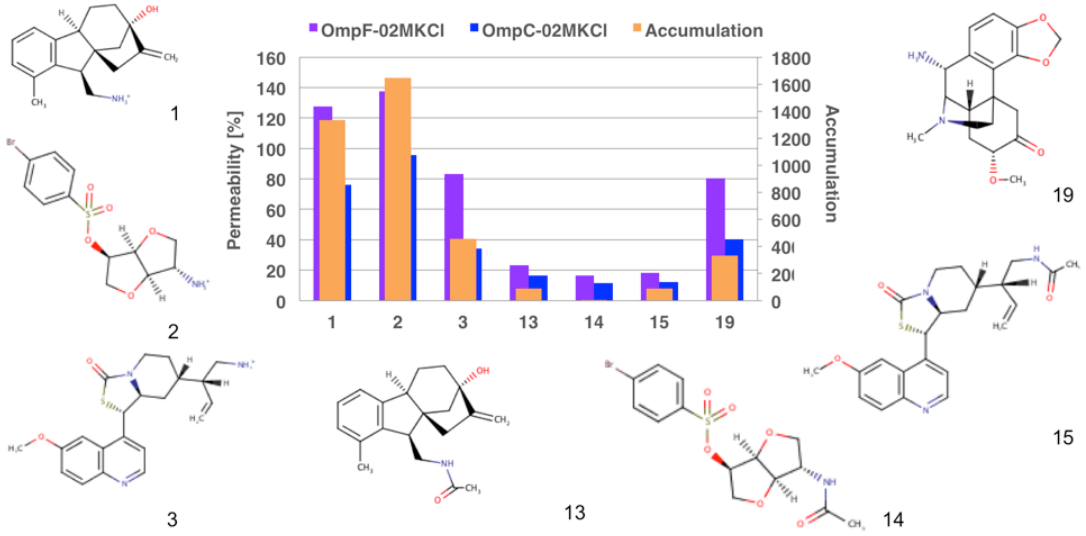

**Supplementary Figure 5 | Intrinsic current recordings of single channel reconstituted in the artificial bilayer. a**, OmpF, **b**, OmpC, **c**, Omp35, **d**, Omp36, **e**, OmpK35, **f**, OmpK36, **g**, OmpE35, **h**, OmpE36. 1 M KCl, 20 mM MES at pH 6.0 was used.




**Supplementary Figure 6 | Linear correlation of electrophysiology data for charged molecules with the scoring function.** Linear regression of the ln($k_{on\_ave}$), where $k_{on\_ave}$ is the average of $k_{on}$ at +50mV and -50mV, onto the LSA data for charged antibiotics. Linear regression parameters fitted to scoring function values. Gray circles represent the modelled permeability versus relative permeability from LSA experiment.




**Supplementary Figure 7 | Permeability prediction vs published data. a**, The scoring function predicted permeability through OmpF and OmpC ratio is shown for different zwitterionic antibiotics. **b**, Experimental permeability ratio (OmpF/OmpC) of ampicillin from [15], **c**, The scoring function predicted permeability through OmpF and OmpC ratio is shown for different charged antibiotics. **d**, Predicted permeability ratio (benzylpenicillin/ampicillin) at different ion concentration through OmpF and OmpC is compared with the experimental permeability ratio through OmpF from [14] (black dashed line).




**Supplementary Figure 8 | Permeability vs. accumulation.** Permeability prediction for a subset of molecules from ref. [10] through OmpF and OmpC at 200mM KCl. Only molecules which properties indicate porin-mediated uptake have been selected. Accumulation results from ref.[10] are overlaid in orange.

# Supplementary Tables

| | **OmpF** | **OmpE35** | **OmpK35** | **Omp35** | **OmpC** | **OmpE36** | **OmpK36** | **Omp36** |
|---|---|---|---|---|---|---|---|---|
| **OmpF** | | 0.62(317) | 1.0(312) | 1.0(313) | 0.93(324) | 0.93(322) | 1.0(324) | 1.0(288) |
| **OmpE35** | 80 | | 0.92(317) | 1.0(317) | 0.81(325) | 0.79(325) | 0.85(325) | 0.89(288) |
| **OmpK35** | 58 | 58 | | 0.58(328) | 0.90(324) | 0.93(325) | 0.90(322) | 0.90(317) |
| **Omp35** | 56 | 56 | 95 | | 0.96(318) | 0.96(317) | 0.94(314) | 0.94(315) |
| **OmpC** | 64 | 66 | 56 | 57 | | 0.51(340) | 0.61(339) | 0.68(334) |
| **OmpE36** | 66 | 67 | 58 | 57 | 88 | | 0.60(340) | 0.56(335) |
| **OmpK36** | 64 | 66 | 62 | 62 | 80 | 83 | | 0.47(335) |
| **Omp36** | 60 | 66 | 61 | 60 | 78 | 81 | 91 | |

**Supplementary Table 1 | Root mean square deviation (upper triangle) and sequence identity (lower triangle) of OmpC and OmpF orthologues.**

| Name | Charge | M.Weight (Da) | E.Dipole (Debye) | Min. Proj. Area (Å$^2$) |
|---|---|---|---|---|
| **Substrates** | | | | |
| **Glycine** | (+,-) | 75 | 14 | 18 |
| **Penicillins** | | | | |
| **Ampicillin** | (+,-) | 349 | 35 | 50 |
| **Ticarcillin** | (-,-) | 382 | 9 | 48 |
| **Piperacillin** | (-) | 517 | 31 | 77 |
| **Carbapenems** | | | | |
| **Ertapenem** | (+,-,-) | 475 | 27 | 57 |
| **Imipenem** | (+,-) | 299 | 14 | 45 |
| **Meropenem** | (+,-) | 383 | 31 | 51 |
| **Cephalosporins** | | | | |
| **Cefotaxime** | (-) | 454 | 20 | 59 |
| **Cefepime** | (+,-) | 481 | 17 | 58 |
| **Ceftazidime** | (+,-,-) | 546 | 26 | 68 |

**Supplementary Table 2 | Key molecular descriptors for permeability trough porins.** Molecular descriptors: charge, molecular weight, total electrical dipole moment and minimal projection area for the nine clinically relevant antibiotics considered in the study. The last two descriptors were obtained averaging them over standard MD trajectories.

| **log($k_{on}$)** | ***E. coli*** | | ***E,aerogenes*** | | ***K. pneumoniae*** | | ***E. cloacae*** | |
|---|---|---|---|---|---|---|---|---|
| Drug Name | **OmpF** | | **Omp35** | | **OmpK35** | | **OmpE35** | |
| | **+50mV** | **-50mV** | **+50mV** | **-50mV** | **+50mV** | **-50mV** | **+50mV** | **-50mV** |
| Ertapenem | 4.15 | 4.11 | 4.48 | 4.11 | 4.26 | 4.00 | 4.30 | 4.00 |
| Cefotaxime | 3.48 | 3.60 | 2.78 | 2.78 | 3.30 | 2.30 | 3.00 | <2 |
| Ceftazidime | 2.60 | 3.18 | 2.48 | 2.30 | 2.90 | 3.48 | 3.00 | 3.30 |
| Piperacillin | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 |
| Ticarcillin | 3.00 | <2 | 3.30 | <2 | 3.15 | <2 | 3.00 | <2 |
| | **OmpC** | | **Omp36** | | **OmpK36*** | | **OmpE36** | |
| | **+50mV** | **-50mV** | **+50mV** | **-50mV** | **+50mV** | **-50mV** | **+50mV** | **-50mV** |
| Ertapenem | 3.48 | 3.11 | 3.18 | 2.90 | | | 3.70 | 3.48 |
| Cefotaxime | 3.08 | 3.26 | <2 | <2 | | | <2 | <2 |
| Ceftazidime | 3.48 | <2 | 2.30 | <2 | | | 3.04 | 2.48 |
| Piperacillin | <2 | <2 | <2 | <2 | | | <2 | <2 |
| Ticarcillin | <2 | <2 | <2 | <2 | | | <2 | <2 |

**Kinetics cannot be determined due to the frequent gating observed from the single channel recordings. Supplementary Figure 5F.*

**Supplementary Table 3 | Electrophysiology data.** Logarithm of association rate ($k_{on}$) from interaction between antibiotics and porin orthologues. 1 M KCl, 20 mM MES, pH 6.0 has been used as bath solution at room temperature. The calculation of $k_{on}$ has been introduced in selectivity and conductance measurements method's section. The logarithm values of the association rate <2 correspond to rare interaction spike been observed.

| Name | Charge | $D_{xy}$(Debye) | Min. Proj. Area (Å$^2$) | $\sigma_{\text{Min. Proj. Area}}$ (Å$^2$) |
|---|---|---|---|---|
| **1** | +1 | 13 | 48 | 0.8 |
| **2** | +1 | 11.5 | 44 | 4.4 |
| **3** | +1 | 19.3 | 69 | 1.6 |
| **13** | 0 | 4.4 | 51.4 | 1.1 |
| **14** | 0 | 5.2 | 72 | 4 |
| **15** | 0 | 7 | 71 | 4 |
| **19** | +1 | 14 | 62 | 1 |

**Supplementary Table 4 | Key molecular descriptors for permeability through porins.** Molecular descriptors: net charge, transversal dipole moment ($D_{xy}$) with respect to the main axis of inertia, minimal projection area and fluctuations, from [10], obtained averaging them over standard MD trajectories.

| | **Crystallisation condition** |
|---|---|
| **OmpE35** | 0.2 M $(NH_4)_2SO_4$, 0.05 M ADA, 13% w/v PEG 4000, pH 6.5 |
| **OmpE36** | 0.4 M $(NH_4)_2SO_4$, 0.1 M MES, 10% w/v PEG 3350, pH 6.5 |
| **OmpK35** | 13% PEG 2000 MME, 0.1 M $CaCl_2$, 0.1 M Tris pH 6.5 |
| **OmpK36** | 0.08 M Magnesium acetate, 0.1 M sodium citrate, 14% w/v PEG 5000 MME, pH 6.0 |
| **Omp35** | 0.2 M LiCl, 0.05 M $MgSO_4$, 8% (w/v) PEG 8000 |
| **Omp36** | 0.1 M Tris-HCl pH 8.5, 8% (w/v) PEG 8000 |

**Supplementary Table 5 | Data collection and refinement statistics for all crystallised proteins.**

| | | OmpK35 | OmpK36 | OmpE35 | Omp35 | Omp36 |
|---|---|---|---|---|---|---|
| **Data collection** | | | | | | |
| **Beamline** | | DLS i04-1 | DLS i04 | DLS i04-1 | DLS iO2 | DLS i04 |
| **Wavelength**(Å) | | 0.9282 | 0.9793 | 0.9282 | 0.9795 | 0.9174 |
| **Space group** | | $P6_3$ | C2 | $C222_1$ | I222 | P3 |
| **Cell dimensions** | **(a,b,c)** | 77.3,77.3,114.0 | 232.3,74.5,90.8 | 137.6,187.3,123.0 | 111.57,115.94,216.66 | 125.09,125.09,126.54 |
| | **(α, β, γ)** | 90,90,120 | 90.00,112.17,90.00 | 90,90,90 | 90, 90, 90 | 90,90,120 |
| **Molecules/AU** | | 1 | 3 | 3 | 3 | 3 |
| **Solvent content (%)** | | 56 | 63 | 65 | 57 | 47 |
| **Resolution** (Å) | | 66.95-1.50 | 107.57-1.65 | 46.81-2.30 | 108.56-2.85 | 56.07-2.46 |
| **Completeness** | | 99.8 (100)[#] | 98.5 (97.3) | 100 (99.9) | 92.9 (85.5) | 94.0(96.1) |
| **Redundancy** | | 5.1 (4.7) | 3.8 (3.9) | 7.6 (7.5) | 2.5 (2.5) | 3.1 (3.1) |
| **I/sI** | | 14.3 (2.0) | 11.6 (1.6) | 16 (1.4) | 6.3 (1.9) | 9.6 (3.8) |
| **$R_{pim}$(%)** | | 4.4 (52.1) | 3.9 (60) | 3 (56) | 5.6 (18.5) | 7.9 (36.8) |
| **$CC_{1/2}$** | | 0.99 (0.72) | 0.99 (0.68) | 0.99 (0.63) | 0.99 (0.68) | 0.99 (0.60) |
| **Refinement** | | | | | | |
| **Resolution (Å)** | | 66.95-1.50 | 107.57-1.65 | 45.8-2.3 | 108.56-2.85 | 56.07-2.46 |
| **Unique reflections (n)** | | 61689 | 161366 | 70510 | 33222 | 74780 |
| **$R_{work}/R_{free}$ (%)** | | 15.7/18.5 | 14.6/18.9 | 20.6/24.6 | 21.33/26.0 | 19.5/23.9 |
| **Atoms (n)** | **protein/solvent** | 2657/284 | 8032/635 | 7736/188 | 7776/1 | 10924/98 |
| | **ligand/detergent** | 16/23 | -/54 | 35/79 | -/- | -/71 |
| **B factors ($Å^2$)** | **protein/solvent** | 20/27 | 29/39 | 53/46 | 57/37 | 26/32 |
| | **ligand/detergent** | 19/38 | -/50 | 67/61 | -/- | -/64 |
| **R.m.s.d.** | **Bond lengths (Å)** | 0.020 | 0.014 | 0.015 | 0.010 | 0.007 |
| | **Bond angles** | 2.087 | 1.588 | 1.405 | 1.47 | 0.97 |
| **Ramachandran plot (%) most favoured/outliers**[1] | | 93.6/0.9 | 96.7/0.6 | 95.2/0.1 | 95/0.3 | 93.5/0.4 |
| **Molprobity clashcore** | | 6.0 | 1.0 | 3.6 | 3.1 | 6.3 |
| **PDB ID** | | 5O77 | 5O79 | 6ENE | 5O78 | 5O9C |

[#] Values in parentheses are for the highest resolution shell
[1] As determined by Molprobity

## Supplementary Table 6 | Data collection and refinement statistics for porins.