Permeation of β-lactamase inhibitors through the general porins of Gram-negative bacteria

Alessandro Pira,[1] Mariano Andrea Scorciapino,[2] Igor V. Bodrenko,[3] Andrea Bosin,[1] Silvia Acosta-Gutierrez,[4] Matteo Ceccarelli,[1,3,]*

[1]Department of Physics and [2]Department of Chemical and Geological Sciences, University of Cagliari, Cittadella Universitaria di Monserrato, S.P.8 km 0,700 – 09042 Monserrato (CA), Italy; [3]CNR/IOM Sezione di Cagliari, Cittadella Universitaria, S.P. 8 km 0,700 – 09042 Monserrato (CA), Italy; [4]Department of Chemistry, University College London - London, United Kingdom.

* To whom correspondence should be addressed
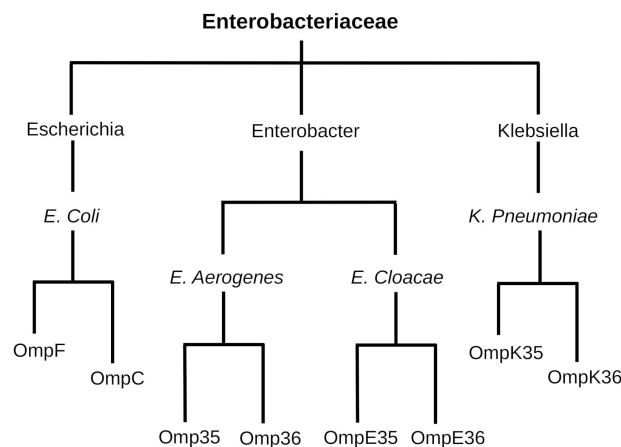
Supplementary Information

Figure S1. The *Enterobacteriaceae* family if divided into about 30 genera, among which *Escherichia*, *Enterobacter* and *Klebsiella*. In this simplified phylogenetic tree, for each of the four selected species, the two main outer membrane porins are reported. OmpF and OmpC orthologs are on the left- and on the right-hand side, respectively.

Figure S2. The OmpF trimer is shown, top-view **(A)** and side.view **(B)**. One monomer is shown in **(C)**, top-view, with the loop L3 highlighted, where negatively charged residues are shown in red. On the opposite side of the channel, the positively charged residues comprising the so-called basic ladder are shown in blue. This charge segregation is responsible for the intense electric field at the constriction region (CR). From **(D)** to **(G)**, one monomer (side-view) of OmpF, Omp35, OmpE35 and OmpK35 is shown, respectively.
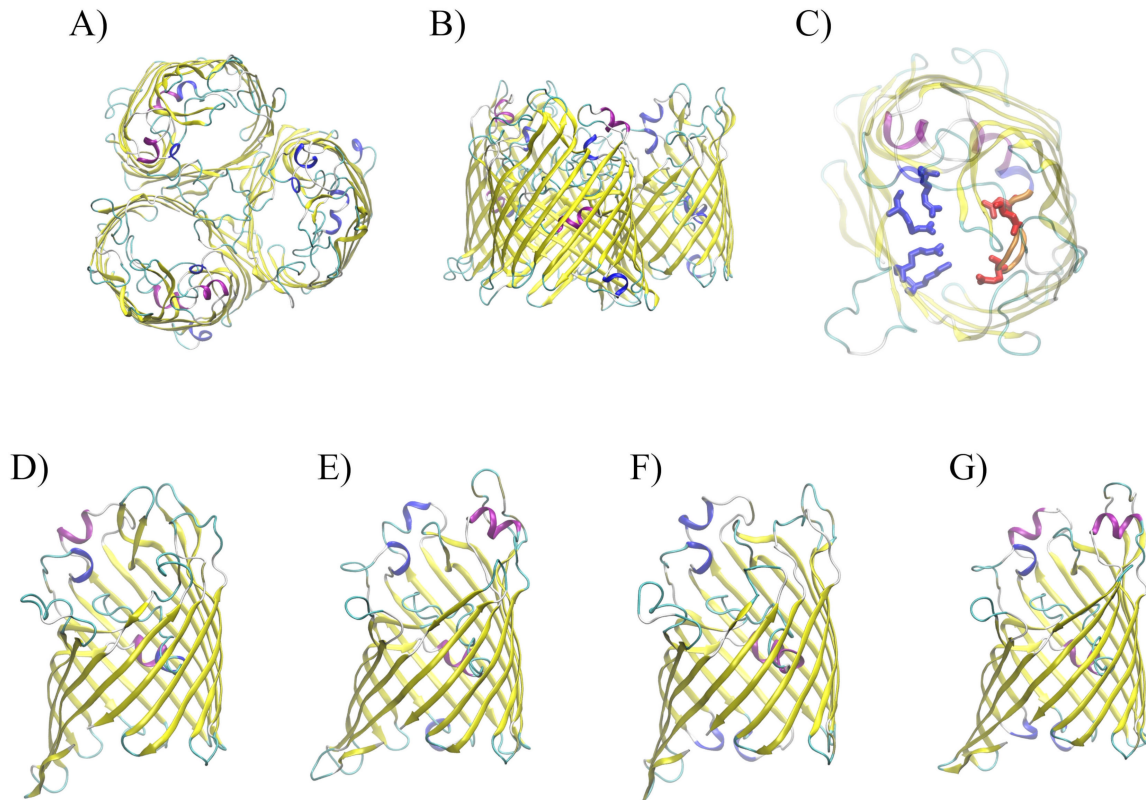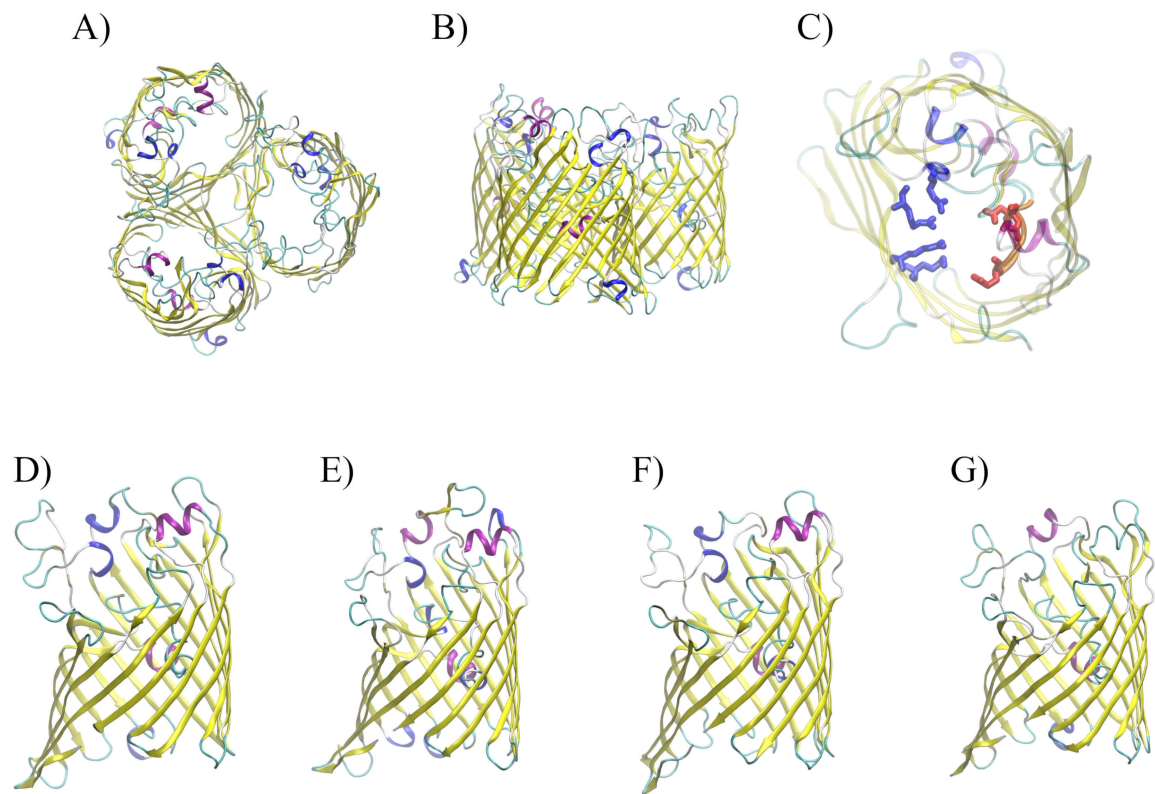
Figure S3. The OmpC trimer is shown, top-view **(A)** and side.view **(B)**. One monomer is shown in **(C)**, top-view, with the loop L3 highlighted, where negatively charged residues are shown in red. On the opposite side of the channel, the positively charged residues comprising the so-called basic ladder are shown in blue. This charge segregation is responsible for the intense electric field at the CR. From **(D)** to **(G)**, one monomer (side-view) of OmpC, Omp36, OmpE36 and OmpK36 is shown, respectively.
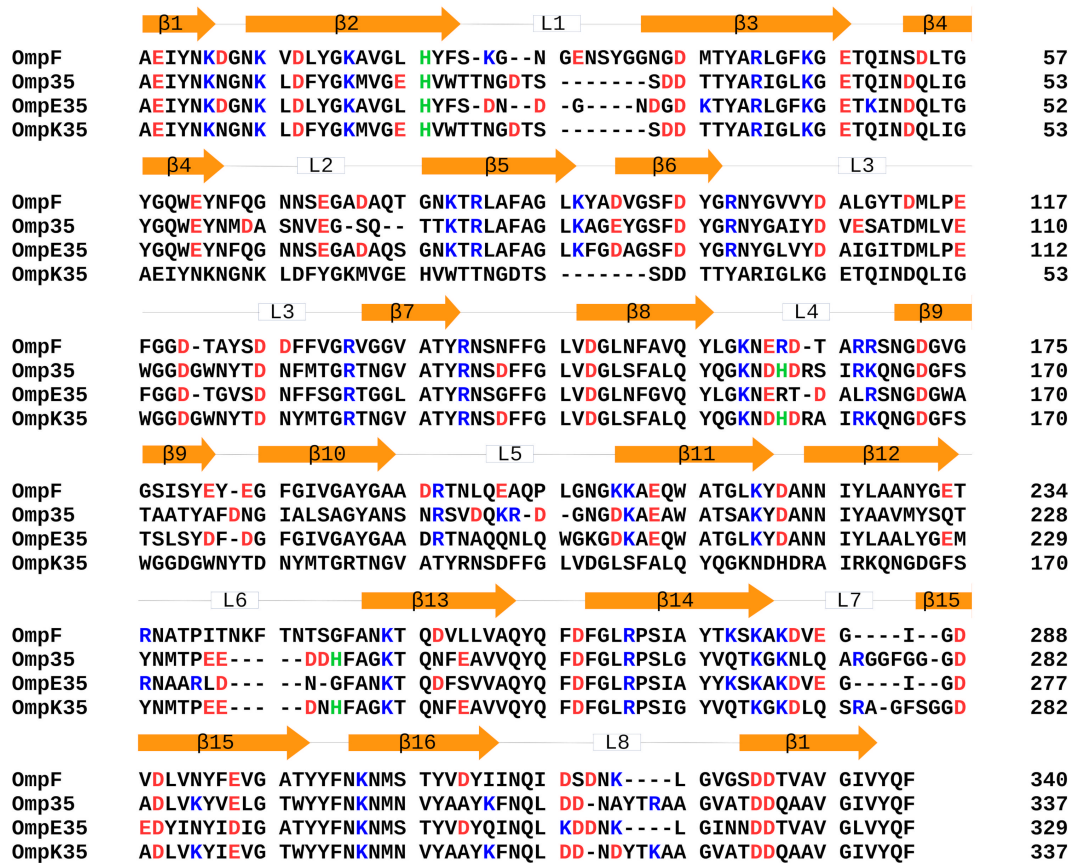
```
              β1           β2                  L1              β3            β4
OmpF    AEIYNKDGNK VDLYGKAVGL HYFS-KG--N GENSYGGNGD MTYARLGFKG ETQINSDLTG    57
Omp35   AEIYNKNGNK LDFYGKMVGE HVWTTNGDTS -------SDD TTYARIGLKG ETQINDQLIG    53
OmpE35  AEIYNKDGNK LDLYGKAVGL HYFS-DN--D -G----NDGD KTYARLGFKG ETKINDQLTG    52
OmpK35  AEIYNKNGNK LDFYGKMVGE HVWTTNGDTS -------SDD TTYARIGLKG ETQINDQLIG    53

             β4          L2             β5            β6              L3
OmpF    YGQWEYNFQG NNSEGADAQT GNKTRLAFAG LKYADVGSFD YGRNYGVVYD ALGYTDMLPE   117
Omp35   YGQWEYNMDA SNVEG-SQ-- TTKTRLAFAG LKAGEYGSFD YGRNYGAIYD VESATDMLVE   110
OmpE35  YGQWEYNFQG NNSEGADAQS GNKTRLAFAG LKFGDAGSFD YGRNYGLVYD AIGITDMLPE   112
OmpK35  AEIYNKNGNK LDFYGKMVGE HVWTTNGDTS -------SDD TTYARIGLKG ETQINDQLIG    53

            L3           β7              β8             L4         β9
OmpF    FGGD-TAYSD DFFVGRVGGV ATYRNSNFFG LVDGLNFAVQ YLGKNERD-T ARRSNGDGVG   175
Omp35   WGGDGWNYTD NFMTGRTNGV ATYRNSDFFG LVDGLSFALQ YQGKNDHDRS IRKQNGDGFS   170
OmpE35  FGGD-TGVSD NFFSGRTGGL ATYRNSGFFG LVDGLNFGVQ YLGKNERT-D ALRSNGDGWA   170
OmpK35  WGGDGWNYTD NYMTGRTNGV ATYRNSDFFG LVDGLSFALQ YQGKNDHDRA IRKQNGDGFS   170

             β9          β10             L5            β11            β12
OmpF    GSISYEY-EG FGIVGAYGAA DRTNLQEAQP LGNGKKAEQW ATGLKYDANN IYLAANYGET   234
Omp35   TAATYAFDNG IALSAGYANS NRSVDQKR-D -GNGDKAEAW ATSAKYDANN IYAAVMYSQT   228
OmpE35  TSLSYDF-DG FGIVGAYGAA DRTNAQQNLQ WGKGDKAEQW ATGLKYDANN IYLAALYGEM   229
OmpK35  WGGDGWNYTD NYMTGRTNGV ATYRNSDFFG LVDGLSFALQ YQGKNDHDRA IRKQNGDGFS   170

            L6          β13            β14             L7      β15
OmpF    RNATPITNKF TNTSGFANKT QDVLLVAQYQ FDFGLRPSIA YTKSKAKDVE G----I--GD   288
Omp35   YNMTPEE--- --DDHFAGKT QNFEAVVQYQ FDFGLRPSLG YVQTKGKNLQ ARGGFGG-GD   282
OmpE35  RNAARLD--- --N-GFANKT QDFSVVAQYQ FDFGLRPSIA YYKSKAKDVE G----I--GD   277
OmpK35  YNMTPEE--- --DNHFAGKT QNFEAVVQYQ FDFGLRPSIG YVQTKGKDLQ SRA-GFSGGD   282

             β15          β16            L8             β1
OmpF    VDLVNYFEVG ATYYFNKNMS TYVDYIINQI DSDNK----L GVGSDDTVAV GIVYQF      340
Omp35   ADLVKYVELG TWYYFNKNMN VYAAYKFNQL DD-NAYTRAA GVATDDQAAV GIVYQF      337
OmpE35  EDYINYIDIG ATYYFNKNMS TYVDYQINQL KDDNK----L GINNDDTVAV GLVYQF      329
OmpK35  ADLVKYIEVG TWYYFNKNMN VYAAYKFNQL DD-NDYTKAA GVATDDQAAV GIVYQF      337
```

Figure S4. Structure alignment of the OmpF orthologs was obtained through the VMD software (https://www.ks.uiuc.edu/Research/vmd/).

```
              β1              β2          L1        β3              β4
OmpC    AEVYNKDGNK LDLYGKVDGL HYFSDNKDVD GDQTYMRLGF KGETQVTDQL TGYGQWEYQI    60
Omp36   AEIYNKDGNK LDLYGKIDGL HYFSSDDSVD GDQTYMRIGV KGETQINDQL TGYGQWEYNV    60
OmpE36  AEIYNKDGNK LDLYGKVDGL HYFSDDDSQD GDQTYMRLGF KGETQVNDQL TGYGQWEYQI    60
OmpK36  AEIYNKDGNK LDLYGKIDGL HYFSDDKSVD GDQTYMRVGV KGETQINDQL TGYGQWEYNV    60

        β4    L2          β5          β6            L3              L3
OmpC    QGNSAENEN- NSWTRVAFAG LKFQDVGSFD YGRNYGVVYD VTSWTDVLPE FGGDTYGSDN    119
Omp36   QANNTESSSD QAWTRLAFAG LKFGDAGSFD YGRNYGVVYD VTSWTDVLPE FGGDTYGSDN    120
OmpE36  QGNSGENEN- NSWTRVAFAG LKFGDAGSFD YGRNYGVVYD VTSWTDVLPE FGGDTYGSDN    119
OmpK36  QANNTESSSD QAWTRLAFAG LKFGDAGSFD YGRNYGVVYD VTSWTDVLPE FGGDTYGSDN    120

        L3    β7          β8            L4              β9
OmpC    FMQQRGNGFA TYRNTDFFGL VDGLNFAVQY QGKNGNPSGE GFTSGVTNNG RDALRQNGDG    179
Omp36   FLQSRANGVA TYRNSDFFGL VDGLNFALQY QGKNGSV--- S-GEDQTNNG RDFQKQNGEG    176
OmpE36  FMQQRGNGFA TYRNSDFFGL VDGLNFAVQY QGKNGSA--- S-GEDQTNNG RTELRQNGDG    175
OmpK36  FLQSRANGVA TYRNSDFFGL VDGLNFALQY QGKNGSV--- S-GEGATNNG RGWSKQNGDG    176

        β9          β10            L5                β11
OmpC    VGGSITYD-Y -EGFGIGGAI SSSKRTDAQN TAA------- -----Y-IGN GDRAETYTGG    224
Omp36   FGTSVTYDIW -DGISAGFAY SSSKRTDEQN N-STFVSKTD GGRYGV-LGE GDHAETYTGG    233
OmpE36  VGGSITYN-L GEGFGIGTAV SSSKRTSSQN --D------- -----LTYGN GDRAETYTGG    220
OmpK36  FGTSLTYDIW -DGISAGFAY SHSKRTDEQN S-V------- -----PALGR GDNAETYTGG    222

        β11         β12        L6        β13             β14
OmpC    LKYDANNIYL AAQYTQTYNA TRVGSLGWAN KAQNFEAVAQ YQFDFGLRPS LAYLQSKGKN    284
Omp36   LKYDANNIYL ATQYTQTYNA TRTGNIGFAN KAQNFEVVAQ YQFDFGLRPS VAYLQSKGKD    293
OmpE36  LKYDANNIYL AAQYTQTYNA TRVGNLGWAN KAQNFEVVAQ YQFDFGLRPS VAYLQSKGKD    280
OmpK36  LKYDANNIYL ASRYTQTYNA TRAGSLGFAN KAQNFEVVAQ YQFDFGLRPS VAYLQSKGKD    282

        L7          β15          β16           L8          β1
OmpC    LGRGYDDEDI LKYVDVGATY YFNKNMSTYV DYKINLLDDN QFTRDAGINT DNIVALGLVY QF    346
Omp36   MG-RYGDQDI LKYVDLGATY YFNKNMSTYV DYKINLLDDN KFTKDASIST DNVVALGLVY QF    354
OmpE36  LENGYGDQDL LKYVDVGATY YFNKNMSTYV DYKINLLDDK EFTRNAGIST DDIVALGLVY QF    340
OmpK36  LERGYGDQDI LKYVDVGATY YFNKNMSTYV DYKINLLDDN SFTRNAGIST DDVVALGLVY QF    344
```

Figure S5. Structure alignment of the OmpC orthologs was obtained through the VMD software (https://www.ks.uiuc.edu/Research/vmd/).
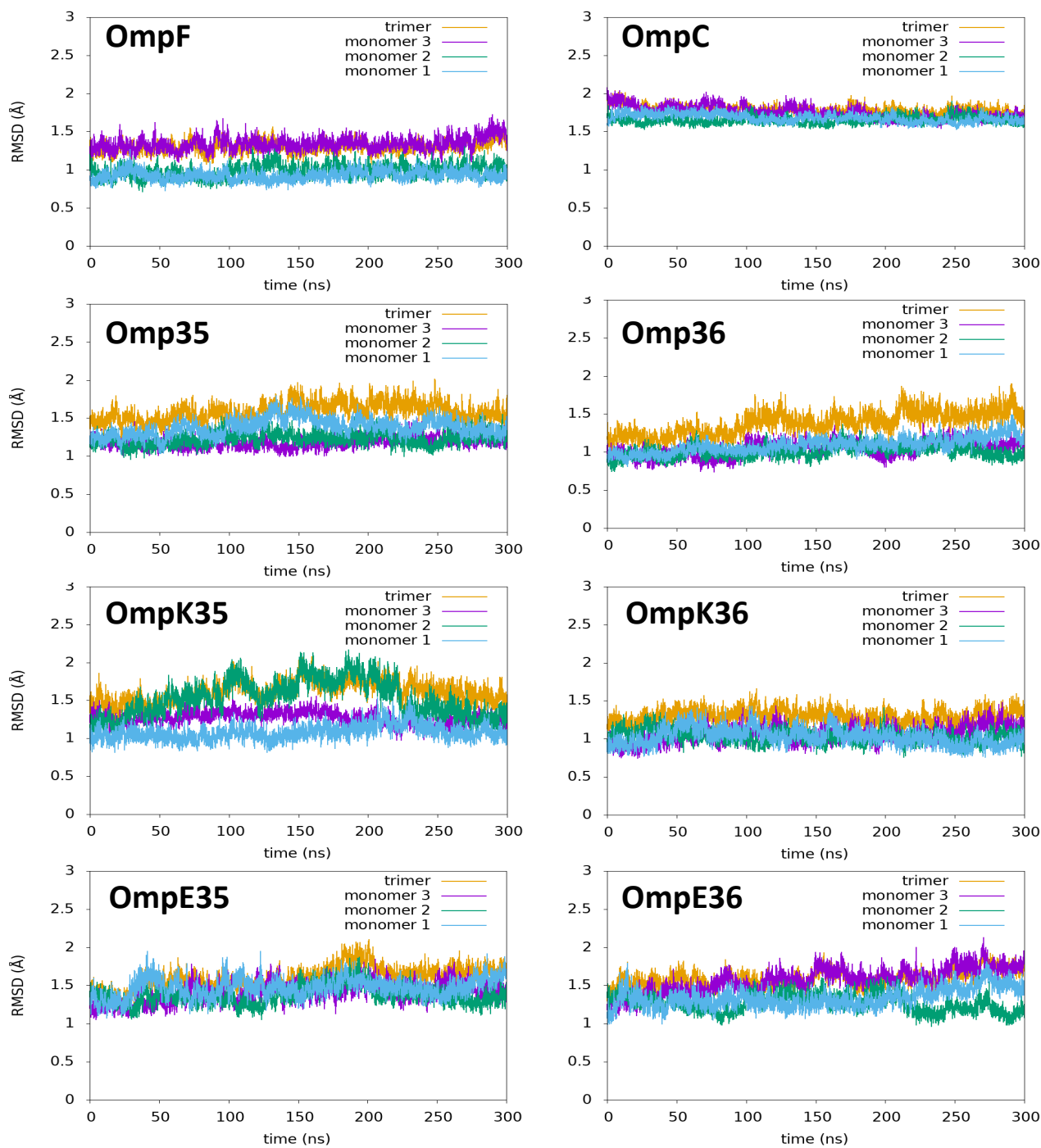
Figure S6 RMSD of the eight porins with respect to the starting X-ray structure, during the last 300 ns of equilibration simulations at 300 K in the NVT ensemble.
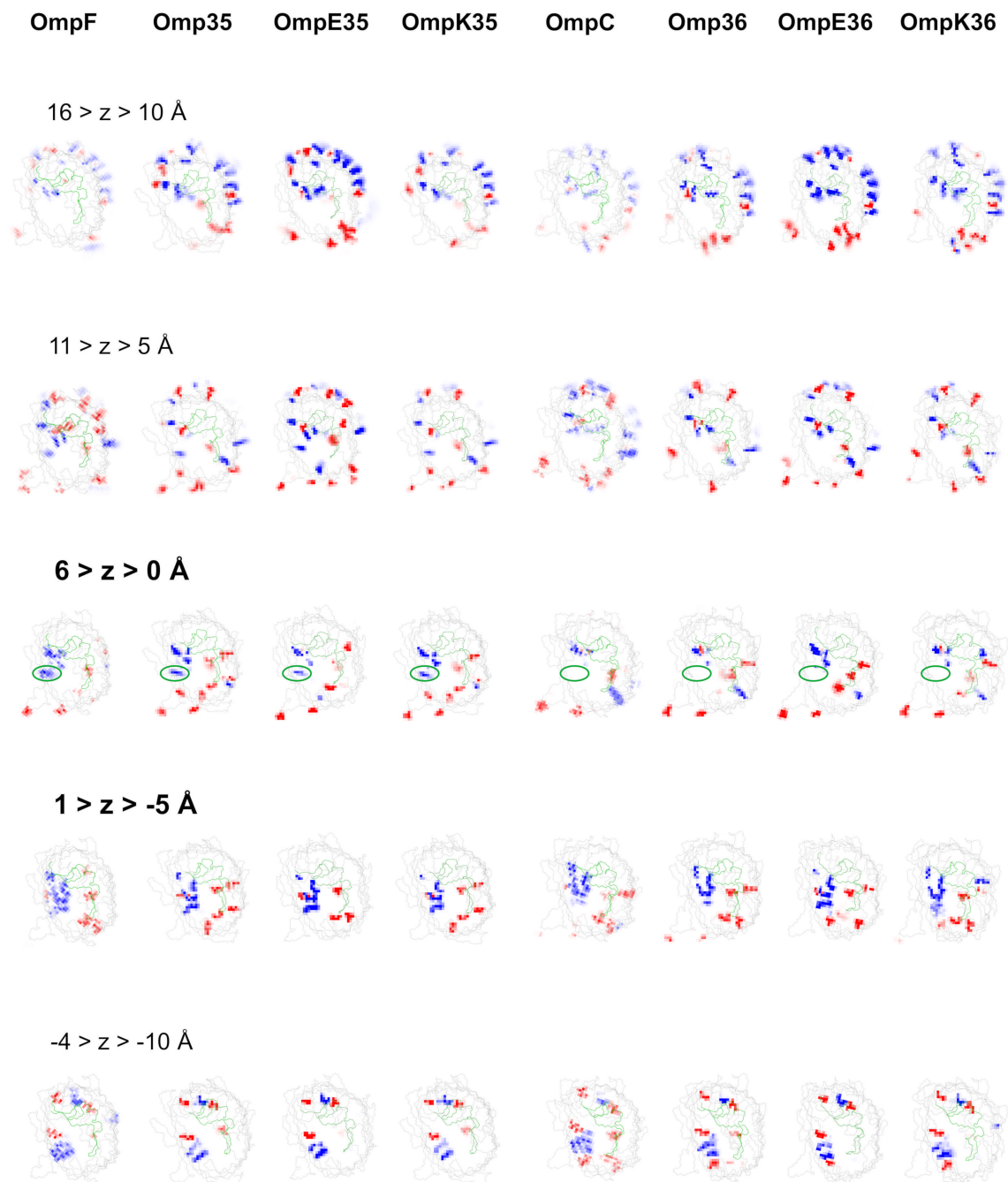
Figure S7. Distribution on the xy-plane of the difference between the density of positively and negatively charged residues, colored in blue and red, respectively. The channel was divided into 5 cross sections along the main axis, namely, +16 Å > z > +10 Å ; +11 Å > z > +5 Å ; +6 Å > z > 0 Å (POR, the pre-orientation region) ; +1 Å > z > -5 Å (CR, the constriction region); -4 Å > z > -10 Å. The circles highlight the major difference found among OmpF orthologs and OmpC orthologs.
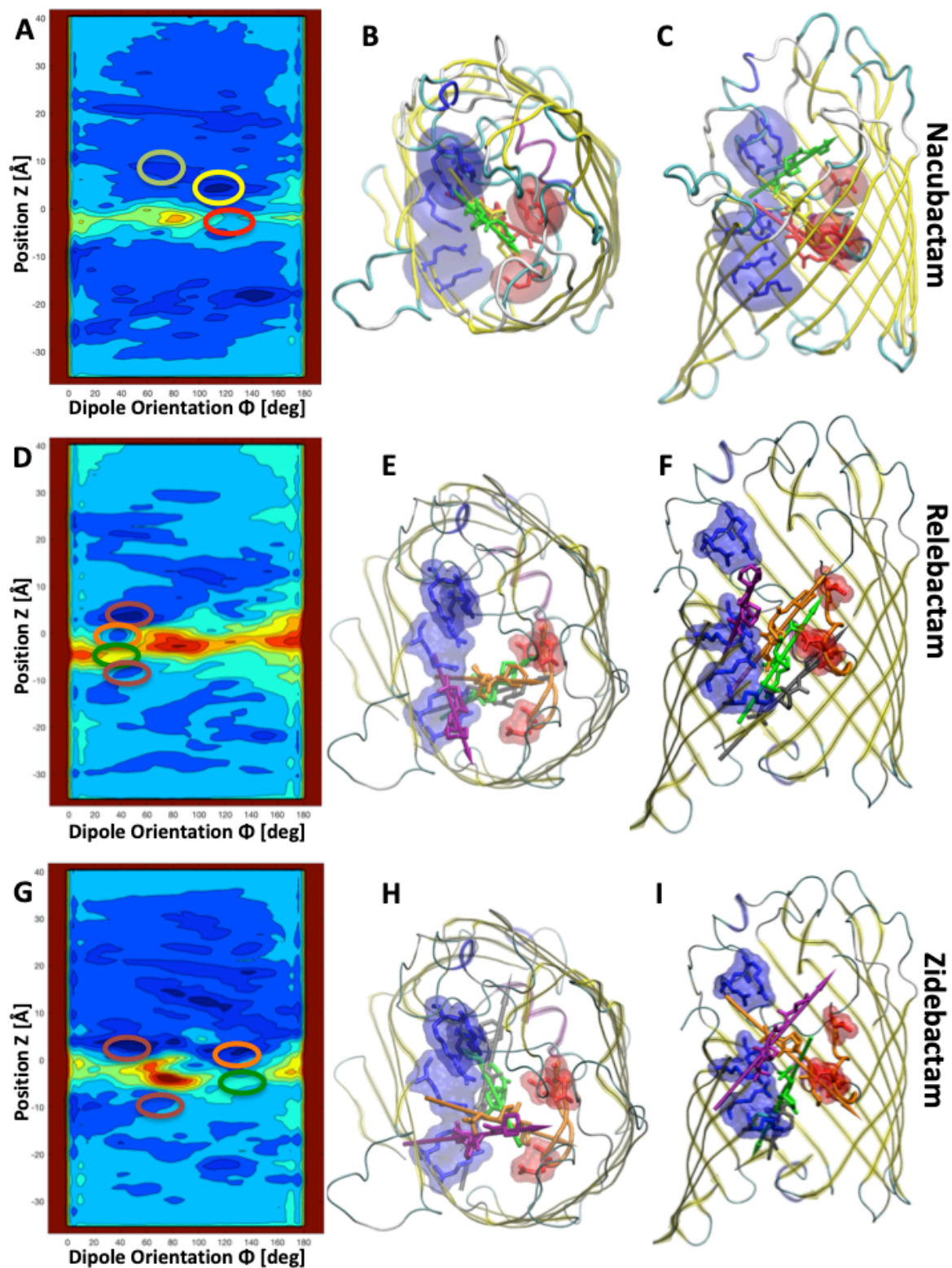
Figure S8. The same FES shown in figure 4 are reported here **(A,D,G)** with differently colored circles to indicate the energy minima and the saddle point analyzed along the minimum energy path in the region 10 Å > z > -10 Å. The same colors are used to show the corresponding representative conformer of the three inhibitors under investigation together with the electric dipole. These structures are shown inside one of the monomers of OmpF from the top view **(B,E,H)** and side view **(C,F,I)**.