Electronic Supplementary Information (ESI)

Recognition of quinolone antibiotics by the multidrug efflux transporter MexB of *Pseudomonas aeruginosa*

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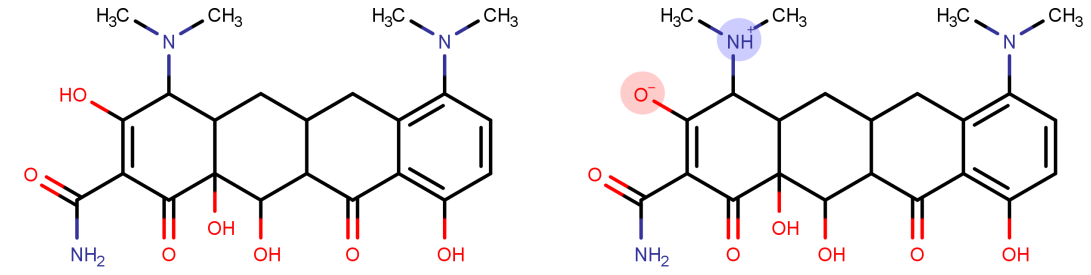
Supplementary Tables and Figures

Table S1 List of 36 FQs simulated in this study – Molecular weights (Da) and net charges are also reported. 11 compounds have been considered in two protonation states.

Quinolones	Molecular weight	Net charge
cinoxacin	261.2	-1
ciprofloxacin	331.4	0 and -1
clinafloxacin	365.8	-1
danofloxacin	357.4	0
delafloxacin	439.8	-1
difloxacin	399.4	0 and -1
dx-619	401.4	0
enoxacin	320.3	0 and -1
enrofloxacin	359.4	0 and -1
fleroxacin	369.3	0 and -1
flumequine	260.2	-1
garenoxacin	426.4	0
gatifloxacin	375.4	0
gemifloxacin	389.4	0
grepafloxacin	359.4	0
levofloxacin	361.4	0 and -1
lomefloxacin	351.4	0 and -1
marbofloxacin	361.4	0
moxifloxacin	401.4	-1
nadifloxacin	359.4	-1
nalidixic acid	231.2	-1
norfloxacin	319.3	0 and -1
ofloxacin	361.4	0 and -1
orbifloxacin	395.4	0
oxolinic acid	260.2	-1
pazufloxacin	318.3	0
pefloxacin	333.4	0 and -1
pipemidic acid	303.3	0
prulifloxacin	460.5	0
rosoxacin	293.3	-1
rufloxacin	363.4	0
sarafloxacin	385.4	0 and -1
sitafloxacin	409.8	0
sparfloxacin	392.4	0
temafloxacin	417.4	0
trovafloxacin	416.4	0



(a) (b)

Figure S1 2D sketches of minocycline protonation states – (a) Un-ionized and (b) zwitterionic.

Table S2 Redocking results – Poses with an RMSD value (Å, in parenthesis) with respect to the corresponding crystal structure below 2.5 Å are highlighted (blue, orange and green for VINA, AD4 and GNINA, respectively). The affinity score (kcal/mol) is reported for VINA and AD4, while the CNN score (adimensional quantity) is given for GNINA. When the programs were not able to produce a docking pose, we inserted the value "NA".

	VINA				AD4				GNINA			
	EX-64	EX-128	EX-512	EX-1024	EX-64	EX-128	EX-512	EX-1024	EX-64	EX-128	EX-512	EX-1024
MexB - ABI-PP												
1	-12.9 (2.2)	-13.3 (2.2)	-13.1 (2.1)	-13.8 (2.1)	-10.6 (4.2)	-10.7 (4.2)	-10.7 (4.1)	-10.7 (4.2)	0.9 (1.1)	0.9 (0.8)	0.9 (0.8)	0.9 (0.9)
2	-11.8 (3.4)	-13.0 (0.9)	-12.8 (0.7)	-13.5 (0.7)	-10.6 (1.0)	-10.6 (1.0)	-10.6 (1.0)	-10.6 (1.0)	0.8 (2.1)	0.8 (2.0)	0.8 (2.0)	0.8 (2.1)
3	-11.4 (4.4)	-12.2 (3.5)	-12.0 (3.5)	-12.7 (3.5)	-10.5 (11.9)	-10.4 (12.0)	-10.4 (12.0)	-10.4 (12.0)	0.6 (6.2)	0.5 (12.9)	0.5 (12.9)	0.5 (12.9)
4	-11.3 (3.2)	-11.8 (4.4)	-11.7 (4.3)	-12.3 (4.3)	-10.5 (12.7)	-9.9 (4.6)	-9.9 (4.6)	-9.9 (4.6)	0.5 (12.9)	0.4 (4.1)	0.4 (4.1)	0.4 (4.1)
5	-11.0 (4.4)	-11.5 (4.5)	-11.4 (4.5)	-12.0 (4.5)	-9.9 (4.6)	-9.6 (1.8)	-9.6 (1.7)	-9.8 (16.4)	0.4 (4.0)	0.4 (12.3)	0.4 (12.3)	0.4 (4.3)
6	-10.9 (2.8)	-11.2 (2.8)	-11.1 (2.8)	-12.0 (3.0)	-9.7 (4.4)	-9.6 (4.3)	-9.5 (4.4)	-9.6 (4.5)	0.4 (4.3)	0.4 (4.5)	0.3 (4.5)	0.4 (3.5)
7	-10.8 (11.9)	-11.1 (11.9)	-11.0 (11.9)	-11.7 (2.8)	-9.1 (2.1)	-8.9 (11.8)	-8.9 (11.8)	-8.4 (4.2)	0.4 (12.3)	0.3 (3.5)	0.3 (3.5)	0.3 (12.6)
8	-10.7 (10.3)	-11.1 (10.3)	-11.0 (10.3)	-11.7 (11.9)	-8.9 (11.8)	-8.4 (4.3)	-8.4 (4.3)	-8.4 (12.4)	0.4 (3.5)	0.3 (12.6)	0.3 (12.6)	0.3 (4.2)
9	-10.6 (15.8)	-10.9 (15.7)	-10.9 (15.7)	-11.6 (10.3)	-8.3 (4.3)	-8.4 (12.4)	-8.3 (12.4)	-8.0 (4.9)	0.3 (12.6)	0.3 (11.2)	0.3 (11.2)	0.3 (11.2)
10	-10.5 (15.7)	-10.8 (15.6)	NA	NA	-8.1 (4.5)	-8.0 (4.9)	-8.2 (4.7)	NA	0.3 (2.6)	0.3 (13.9)	0.3 (13.9)	0.3 (4.3)
AcrB - LFX (0)												
1	-8.5 (1.7)	-8.8 (1.6)	-8.8 (1.6)	-8.6 (1.6)	-7.6 (6.9)	-7.6 (6.9)	-7.6 (6.9)	-7.6 (6.9)	0.9 (0.6)	0.9 (1.6)	0.9 (1.6)	0.9 (1.6)
2	-8.4 (0.6)	-8.7 (3.4)	-8.7 (0.6)	-8.4 (0.6)	-7.5 (1.9)	-7.5 (1.9)	-7.5 (1.9)	-7.5 (1.9)	0.9 (1.6)	0.9 (0.6)	0.9 (0.6)	0.9 (0.6)
3	-8.4 (3.4)	-8.7 (0.6)	-8.7 (3.4)	-8.4 (3.4)	-7.4 (3.6)	-7.4 (3.6)	-7.4 (3.6)	-7.4 (3.6)	0.8 (1.7)	0.8 (1.7)	0.8 (1.7)	0.8 (1.7)
4	-8.1 (6.9)	-8.5 (6.9)	-8.5 (6.9)	-8.3 (6.9)	-7.2 (7.2)	-7.2 (1.6)	-7.3 (7.0)	-7.2 (4.2)	0.7 (2.2)	0.7 (3.7)	0.7 (3.7)	0.7 (3.7)
5	-8.0 (1.7)	-8.3 (1.8)	-8.3 (1.6)	-8.0 (1.7)	-7.2 (1.6)	-7.1 (5.9)	-7.2 (4.2)	-7.2 (1.4)	0.7 (3.4)	0.7 (3.4)	0.7 (3.4)	0.7 (3.4)
6	-8.0 (7.5)	-8.3 (7.5)	-8.3 (7.5)	-8.0 (7.5)	-7.1 (5.9)	-7.1 (7.5)	-7.1 (5.9)	-7.1 (5.9)	0.6 (2.7)	0.6 (2.7)	0.6 (2.7)	0.6 (3.7)
7	-7.9 (8.0)	-8.2 (18.2)	-8.2 (18.2)	-7.9 (18.2)	-7.1 (7.5)	-7.1 (13.2)	-7.1 (7.3)	-7.1 (7.5)	0.5 (3.5)	0.5 (3.5)	0.5 (3.4)	0.6 (2.6)
8	-7.8 (18.2)	-8.1 (8.1)	-8.1 (8.0)	-7.8 (8.0)	-7 (3.9)	-7.1 (4.0)	-7.1 (7.5)	-7.1 (13.4)	0.4 (6.9)	0.5 (3.7)	0.5 (3.7)	0.5 (3.5)
9	-7.4 (3.5)	-8.0 (2.6)	-8 (7.7)	-7.7 (7.7)	-7 (13.3)	-7.0 (3.5)	-7.1 (13.3)	-7.1 (4.0)	0.4 (7.0)	0.5 (3.8)	0.4 (3.8)	0.4 (6.9)
10	-7.2 (18.5)	-7.7 (3.5)	-7.7 (3.5)	-7.7 (2.6)	-7 (5.6)	NA	-7.0 (4.0)	NA	0.4 (4.5)	0.4 (6.9)	0.4 (6.9)	0.4 (7.0)
AcrB - LFX (-1)												
1	-8.9 (1.6)	-8.6 (1.6)	-8.8 (1.6)	-8.9 (1.6)	-7.6 (6.9)	-7.6 (6.9)	-7.6 (6.9)	-7.6 (6.9)	0.9 (1.6)	0.9 (1.6)	0.9 (1.6)	0.9 (1.6)
2	-8.7 (3.4)	-8.4 (0.6)	-8.6 (0.6)	-8.7 (0.6)	-7.5 (1.9)	-7.5 (1.9)	-7.5 (3.6)	-7.5 (3.6)	0.9 (0.6)	0.9 (0.6)	0.9 (0.6)	0.9 (0.6)
3	-8.5 (6.9)	-8.4 (3.4)	-8.6 (3.4)	-8.7 (3.4)	-7.5 (3.6)	-7.5 (3.6)	-7.5 (1.9)	-7.5 (1.9)	0.9 (1.6)	0.9 (1.7)	0.9 (1.8)	0.9 (1.8)
4	-8.3 (1.6)	-8.2 (6.9)	-8.4 (6.9)	-8.5 (6.9)	-7.3 (7.5)	-7.3 (7.2)	-7.3 (7.2)	-7.3 (7.2)	0.7 (3.6)	0.7 (3.7)	0.7 (3.7)	0.7 (3.7)
5	-8.3 (7.5)	-8.1 (1.7)	-8.3 (1.7)	-8.3 (1.7)	-7.3 (7.2)	-7.2 (7.5)	-7.3 (1.6)	-7.2 (7.5)	0.6 (2.5)	0.7 (3.4)	0.7 (3.6)	0.7 (3.4)
6	-8.1 (18.2)	-8.0 (7.5)	-8.2 (7.5)	-8.3 (7.5)	-7.2 (7.5)	-7.1 (5.9)	-7.2 (7.5)	-7.2 (5.9)	0.6 (3.5)	0.6 (2.7)	0.6 (2.5)	0.7 (3.9)
7	-8.1 (8.1)	-7.9 (18.2)	-8.1 (18.2)	-8.1 (18.2)	-7.1 (5.9)	-7.1 (5.4)	-7.2 (5.9)	-7.1 (4.0)	0.5 (7.0)	0.6 (3.5)	0.6 (3.5)	0.6 (3.5)
8	-8.0 (7.7)	-7.8 (8.0)	-8.1 (8.1)	-8.1 (8.1)	-7.1 (4.0)	-7.1 (4.0)	-7.1 (5.6)	-7.1 (5.6)	0.5 (6.9)	0.5 (7.0)	0.5 (7.0)	0.6 (2.5)
9	-7.9 (2.8)	-7.4 (3.5)	-7.9 (7.7)	-8.0 (7.7)	-7.1 (5.6)	-7.0 (3.5)	-7.1 (4.0)	-7.0 (3.4)	0.4 (4.5)	0.5 (6.9)	0.5 (6.9)	0.6 (2.3)
1	-7.7 (3.5)	-7.3 (18.5)	-7.7 (3.5)	-7.7 (3.5)	-7.0 (3.5)	-6.9 (5.2)	-6.9 (5.3)	-6.9 (5.3)	0.3 (7.3)	0.4 (4.5)	0.4 (4.5)	0.6 (3.5)
AcrB - Minocycline												
1	-9.6 (6.2)	-9.6 (6.2)	-9.6 (6.2)	-9.8 (6.2)	-10.1 (14.7)	-10.1 (14.7)	-10.1 (14.7)	-10.1 (14.7)	0.8 (1.1)	0.8 (1.2)	0.8 (3.8)	0.8 (3.7)
2	-9.1 (1.1)	-9.1 (1.1)	-9.1 (1.2)	-9.4 (1.2)	-9.7 (10.4)	-9.7 (10.4)	-9.7 (10.4)	-9.7 (10.4)	0.8 (4.6)	0.8 (19.3)	0.8 (2.5)	0.8 (1.1)
3	-8.9 (5.4)	-8.9 (5.4)	-8.8 (5.4)	-9.1 (5.4)	-9.6 (13.9)	-9.6 (13.9)	-9.6 (13.9)	-9.6 (13.9)	0.8 (2.7)	0.8 (3.7)	0.8 (1.1)	0.8 (3.5)
4	-8.6 (3.5)	-8.6 (3.6)	-8.5 (3.8)	-8.8 (3.6)	-9.4 (11.3)	-9.4 (11.3)	-9.4 (11.3)	-9.4 (11.3)	0.8 (1.6)	0.8 (1.7)	0.8 (1.3)	0.8 (1.3)
5	-8.4 (3.6)	-8.4 (3.7)	-8.4 (4.1)	-8.7 (4.1)	-9.3 (2.0)	-9.4 (2.0)	-9.3 (2.0)	-9.4 (2.1)	0.8 (3.7)	0.8 (1.3)	0.8 (3.8)	0.8 (2.2)
6	-8.4 (4.9)	-8.2 (13.8)	-8.2 (13.8)	-8.4 (13.7)	-9.1 (15.9)	-9.0 (14.5)	-9.0 (14.6)	-9.2 (15.9)	0.6 (6.3)	0.8 (1.7)	0.8 (2.8)	0.7 (4.8)
7	-8.2 (13.7)	-8.1 (10.1)	-8.1 (13.7)	NA	-9.0 (14.5)	-8.9 (13.3)	-9.0 (14.3)	-9.0 (14.6)	0.6 (5.3)	0.7 (3.0)	0.7 (4.9)	0.7 (6.3)
8	-8.2 (10.1)	-8.1 (13.7)	NA	NA	-9.0 (14.3)	-8.9 (14.3)	-8.9 (13.3)	-8.9 (14.3)	0.6 (2.2)	0.7 (5.6)	0.6 (6.3)	0.6 (5.7)
9	-8.1 (13.7)	NA	NA	NA	-8.9 (13.3)	-8.8 (11.1)	-8.8 (15.9)	-8.9 (13.3)	0.5 (11.0)	0.6 (6.3)	0.6 (2.2)	0.6 (5.2)
10	NA	NA	NA	NA	-8.4 (11.7)	-8.4 (11.8)	-8.8 (11.3)	-8.9 (11.2)	0.5 (17.1)	0.6 (5.2)	0.6 (5.3)	0.6 (5.1)

Table S3 Settings for ensemble docking used in this work. A total of 1800 docking poses (600 + 600 + 600) were generated for each FQ.

	VINA 1.2.3	**VINA 1.2.3**	**GNINA**
Scoring function	VINA	AutoDock4	CNN score
Box dimension	30x30x30 Å^3		
Box center	Center of Mass of the DP$_T$		
Exhaustiveness	128		
Poses per run	10	10	10
Number of MexB structures	6 (3 X-ray + 3 MD snapshots [a])		
Number of FQs conformations	10 from MD simulations [b]		
Total poses per FQ	600	600	600

[a] V. K. Ramaswamy et al. Front. Microbiol., 2018, 9, 1144.
[b] S. Gervasoni et al. Sci. Data, 2022, 9, 148.

Table S4 Ligand RMSD (Å) between crystal structures and the input docking conformations – Minocycline is considered in the zwitterionic state. The population of each cluster is reported in parenthesis (%).

cluster	MexB-ABI-PP (3W9J)	AcrB-LFX (-1) (7B8T)	AcrB-LFX (0) (7B8T)	AcrB-Minocycline (4DX5)
0	3.3 (69.6)	0.7 (44.9)	0.6 (72.1)	0.9 (83.5)
1	2.3 (15.4)	0.6 (36.5)	0.8 (12.9)	0.7 (9.0)
2	2.0 (6.9)	0.9 (6.1)	0.5 (9.1)	0.8 (3.6)
3	2.6 (3.3)	0.7 (4.6)	0.5 (2.1)	1.1 (1.2)
4	3.8 (1.5)	1.0 (3.0)	1.0 (1.7)	0.6 (1.1)
5	3.4 (1.3)	1.0 (2.1)	0.7 (0.7)	1.0 (1.0)
6	2.7 (0.7)	0.8 (2.0)	0.6 (0.36)	0.8 (0.2)
7	3.1 (0.5)	0.7 (0.7)	0.7 (0.4)	1.0 (0.2)
8	3.4 (0.4)	1.0 (0.1)	0.9 (0.3)	0.9 (0.2)
9	2.7 (0.3)	0.7 (0.1)	0.9 (0.3)	0.9 (0.1)

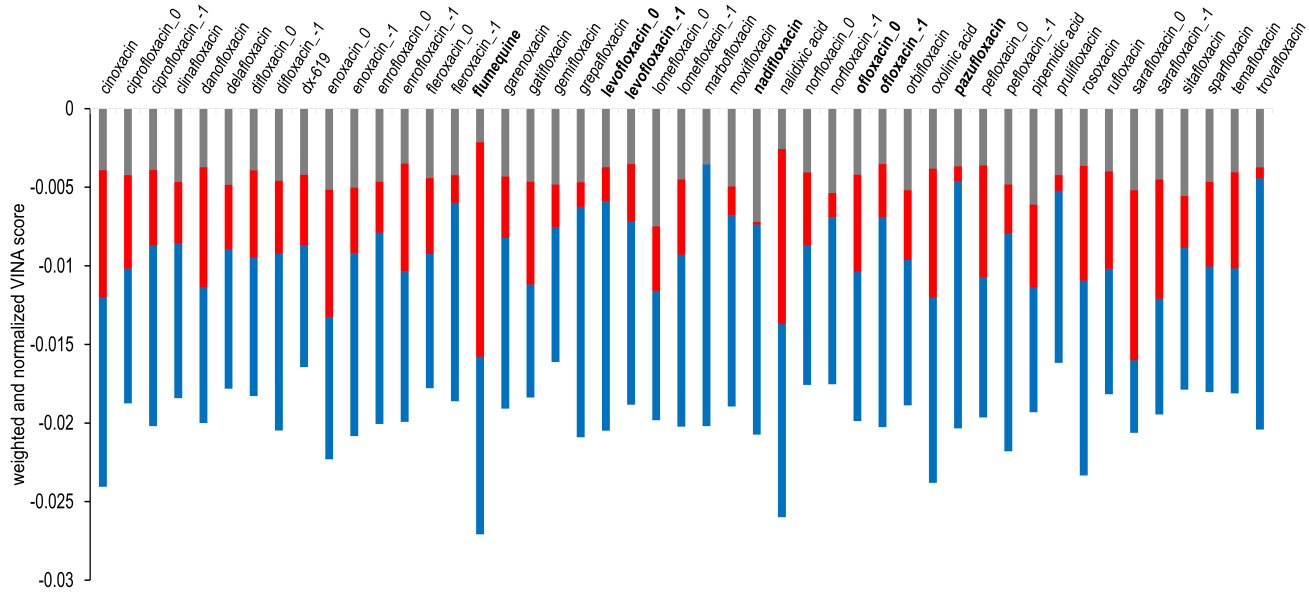


Figure S2 Sub-region distribution of the VINA affinity score, weighted on the cluster population and normalized to the molecular weight – Gray bars refer to the Interface, red to the Cave, blue to the Groove. FQs are in ascending order according to the molecular weight. FQs containing the piperazine group are reported in two net charges (i.e., 0 and -1). LFX and related compounds are highlighted in boldface.

Surface-matching analysis with PLATINUM

In our calculations we selected the Fermi-like function:

$$d(r_{ij}) = \frac{[1+e^{\alpha r_{ij}-c}]^{-1}}{\sum_{k=1}^{N}[1+e^{\alpha r_{ik}-c}]^{-1}} \quad (1)$$

where α = 1 Å^{-1}, and c is a cutoff, usually set to 4 Å. The atomic hydrophobicity constants are derived from octanol-water log P values for different organic compounds and automatically assigned according to the molecular topology. Positive and negative MHP values correspond respectively to hydrophobic and hydrophilic surface regions. The comparison of MHPs on the interfacial surface gives an understanding of the complementarity of the ligand to binding site of the receptor, in terms of hydrophobic and hydrophilic matching. Specifically, the complementarity between MexB and the FQs is estimated by the fraction of matching of total area, projected for both partners onto the Connolly surface of the molecule, and calculated as:

$$SM_{TOT} = \frac{(S_{LL}+S_{HH})}{S_{LL}+S_{LH}+S_{HL}+S_{HH}+S_{LW}+S_{HW}} \quad (2)$$

where S_{LL} and S_{HH} are the area of the match surface between the molecule and receptor hydrophobic and hydrophilic portions, respectively. S_{LH} is the area of surface match between the hydrophobic portion of the molecule and the hydrophilic portion of the receptor, conversely S_{HL} is the area of the match surface between the molecule hydrophilic portion and the hydrophobic portion of the receptor. S_{LW} is the area of surface match between the hydrophobic portion of the molecule and the solvent, and S_{HW} is the area of the match surface between the hydrophilic portion of the molecule and the solvent. The hydrophobic surface matching coefficient (SM_L) was computed as follows:

$$SM_L = \frac{2S_{LL}}{2S_{LL}+S_{HL}+S_{LH}+S_{LW}} \quad (3)$$

SM_{TOT} and SM_L are positive adimensional quantities ranging from 0 (no surface matching between molecule and receptor) and 1 (complete surface matching).