Optimization of efflux avoidance and outer membrane permeation for antibiotic development

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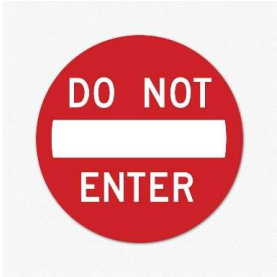
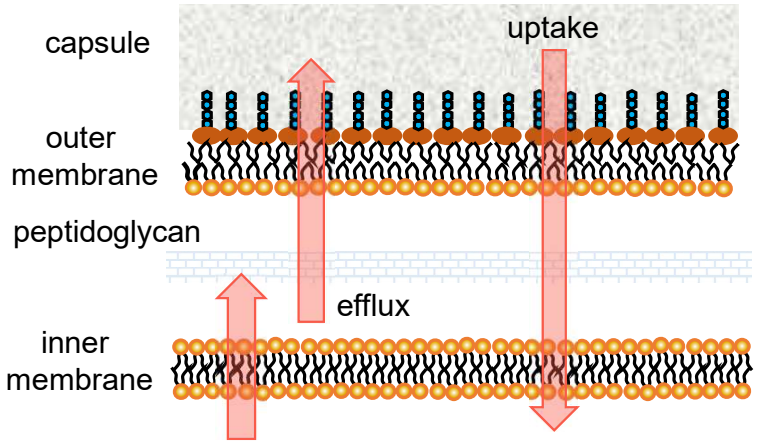
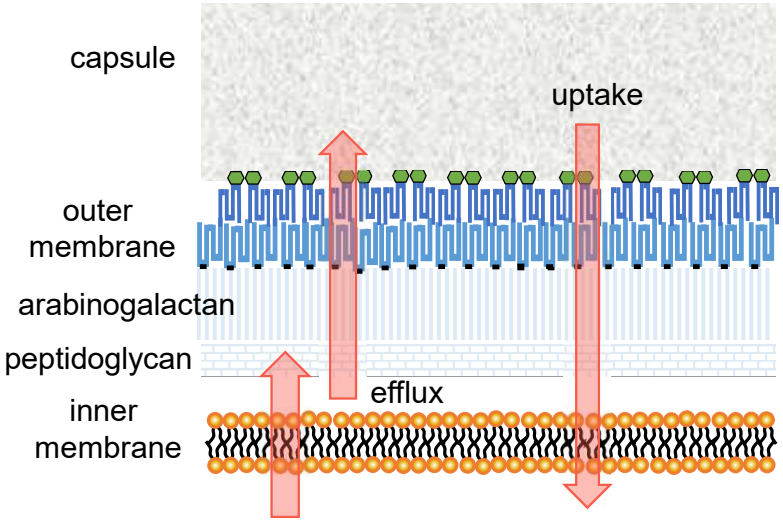
Bacterial cell envelopes are diverse, but rules are the same:



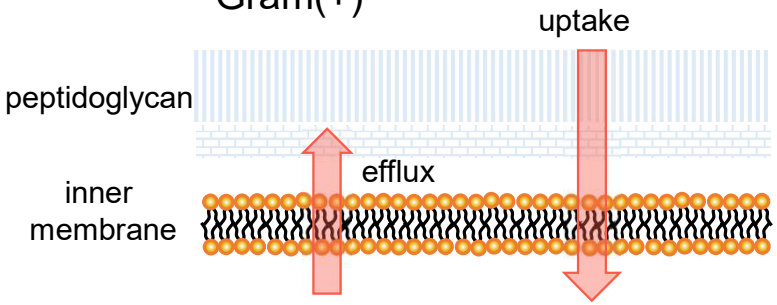
Gram(-) cell envelope



Mycobacteriales

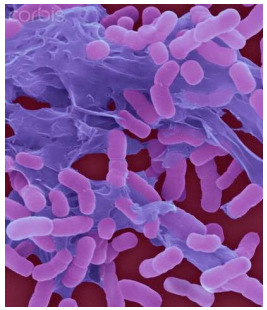


Gram(+)



Multidrug resistant Gram-negative pathogens

Acinetobacter baumannii



% MULTIDRUG RESISTANT IN 2020

39.3% Resistant

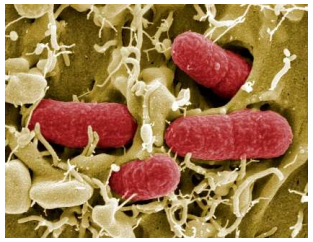
(205 resistant/521 tested)

% MULTIDRUG RESISTANT BY PATIENT AGE IN 2020

Pediatric: 2.3%

Adult: 42.8%

Escherichia coli



% MULTIDRUG RESISTANT IN 2020

10.3% Resistant

(1541 resistant/15,000 tested)

% MULTIDRUG RESISTANT BY PATIENT AGE IN 2020

Pediatric: 9.9%

Adult: 10.3%

Pseudomonas aeruginosa

% MULTIDRUG RESISTANT IN 2020

7.7% Resistant

(578 resistant/ 7,484 tested)

% MULTIDRUG RESISTANT BY PATIENT AGE IN 2020

Pediatric: 5.5%

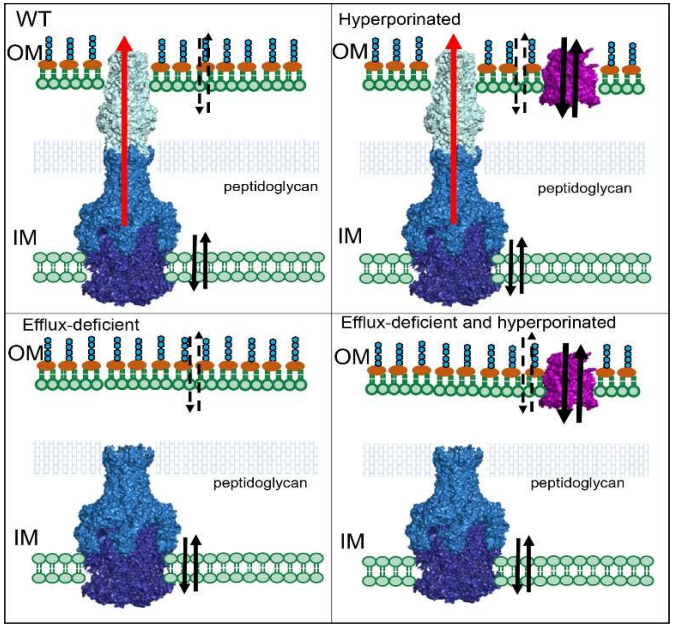
Adult: 7.8%

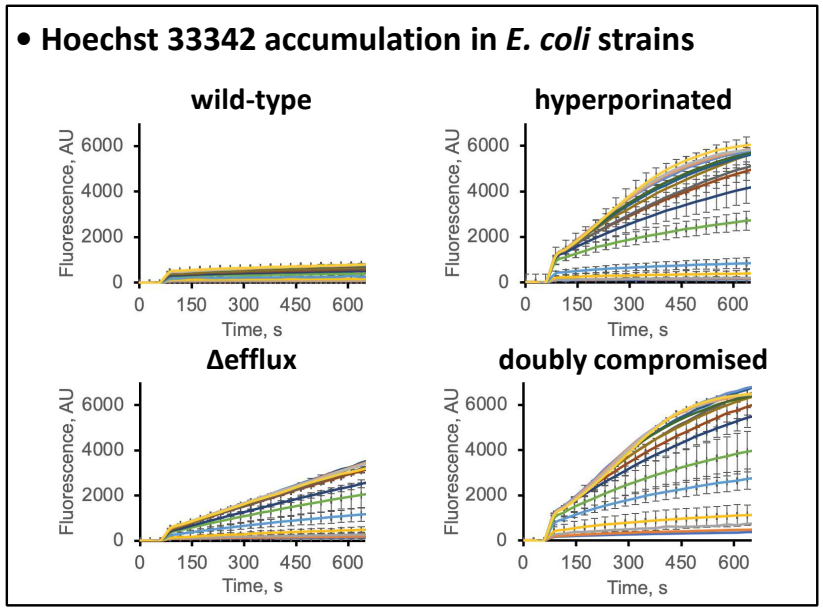
CDC

Active efflux is synergistic with the limitations on transmembrane diffusion

4-Strain Sets	OM	efflux
1) wild-type	+	+
2) hyperporinated	–	+
3) efflux knockout	+	–
4) doubly compromised	–	–

	Fold change in MICs			
Species	AZI	LEVO	RIF	NOVO
Ecoli/Ec-Δ9-Pore	64	8	16	1000
Abau/Ab-Δ3-Pore	16	4	8	1024
Pae/Pae-Δ6-Pore	8000	64	32	4000

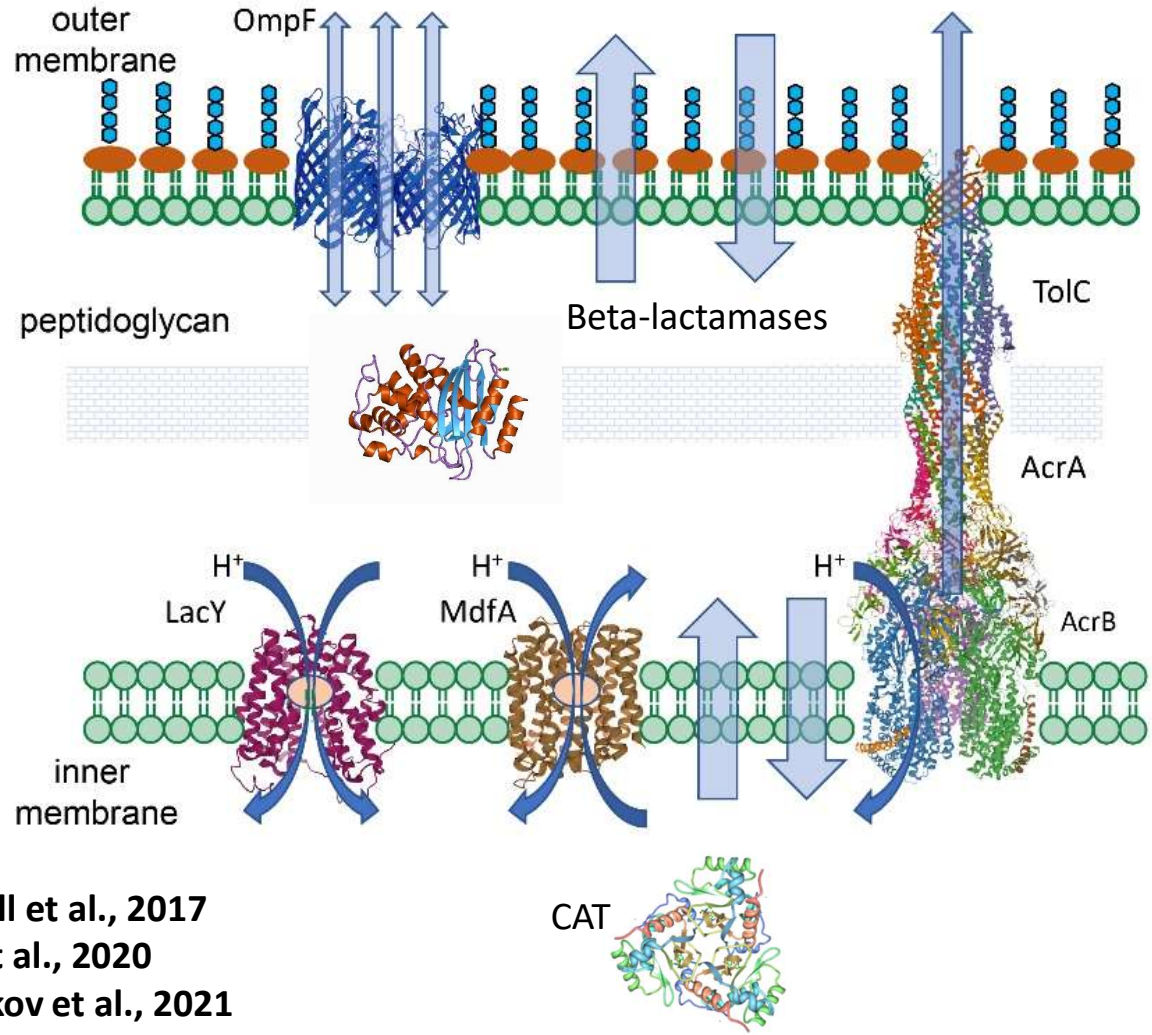




Krishnamoorthy et al., mBio, 2017

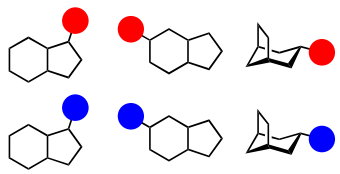
An integrated antibiotic permeation and resistance model

- Transporters are synergistic with transporters in the other membrane and additive with transporters acting across the same membrane.
- Enzymatic inactivation of an antibiotic could be either additive or synergistic with efflux and transmembrane diffusion
- When active influx is more efficient than efflux, the compound will be accumulated inside the cell.

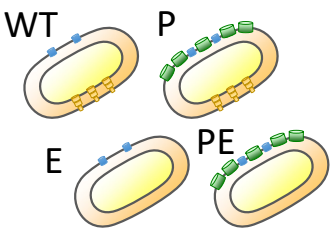


Westfall et al., 2017
Saha et al., 2020
Rybenkov et al., 2021

Toward Predictive Models for Bacterial Penetration

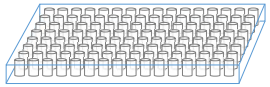


diverse scaffolds
shared substituent sets
cheminformatic analysis

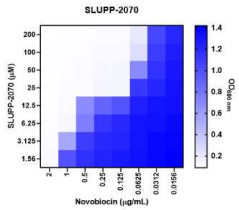


species-specific
subcellular localization
OM vs IM vs efflux

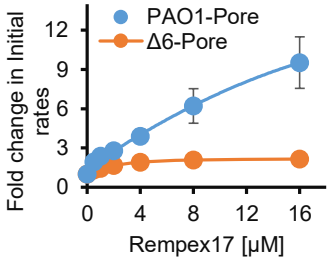
MIC/IC$_{50}$

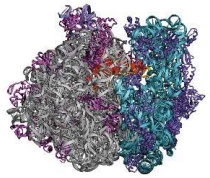


MPC



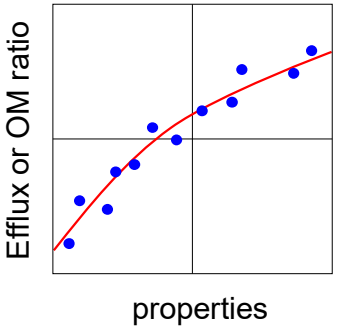
target inhibition





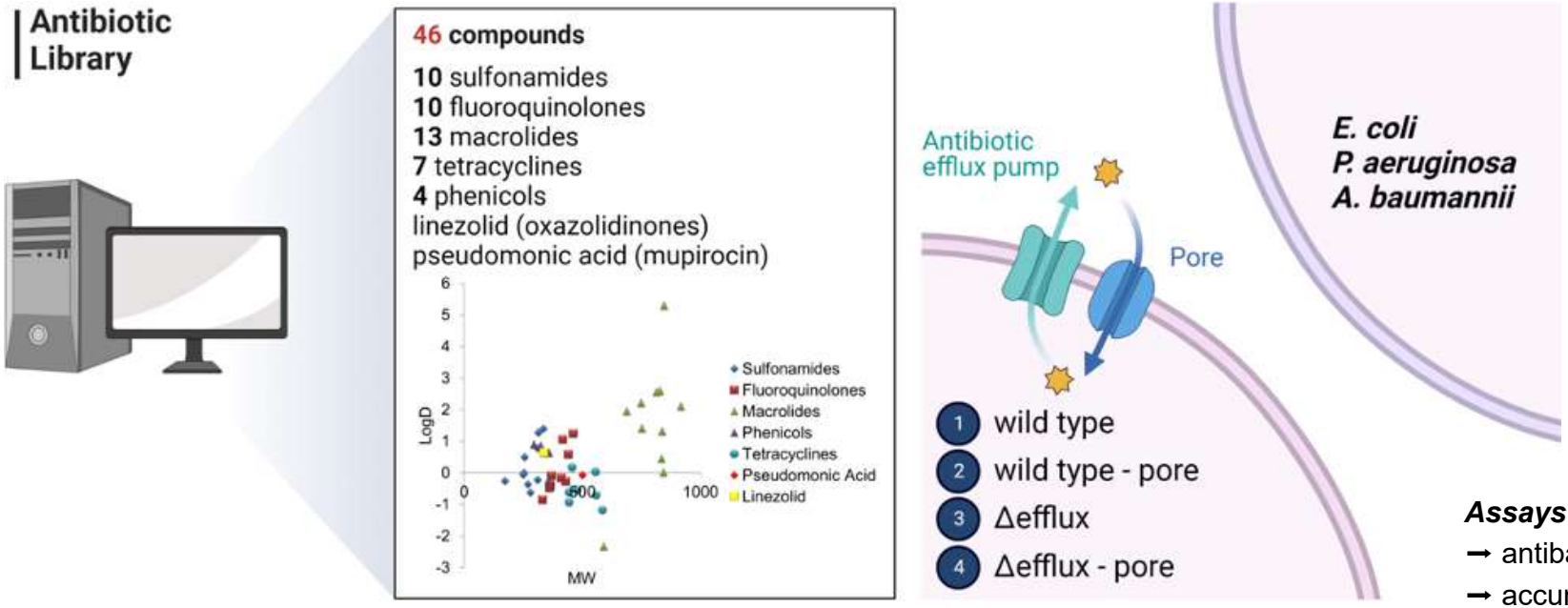
higher-throughput
MS and fluorescence
accumulation assays

WT/PE- total barrier ratio
P/PE- efflux impact ratio
E/PE- OM impact ratio



Machine Learning (ML)
modeling

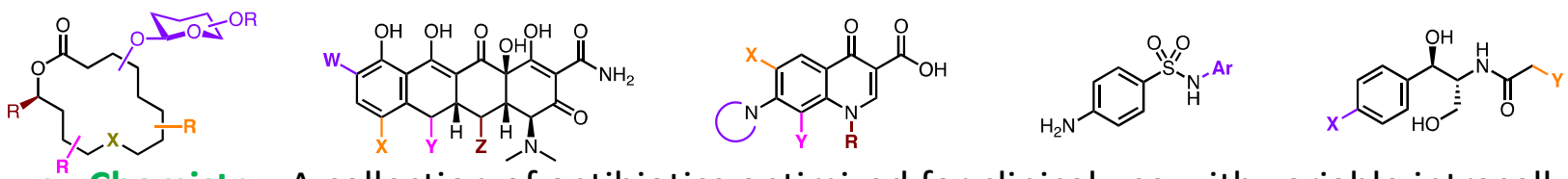
Prevalence of OM barrier and compound-specific uptake pathways



Assays

→ antibacterial (MIC, IC_{50})

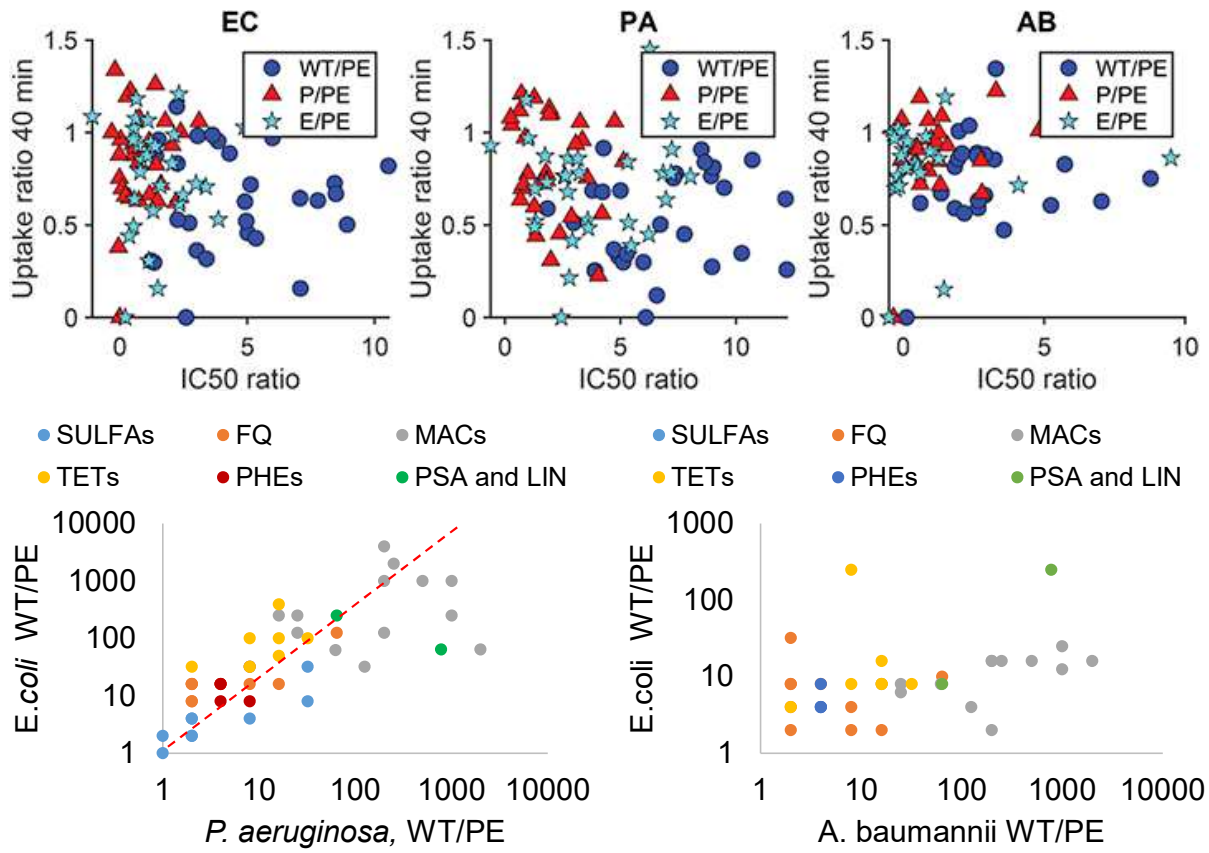
→ accumulation (MS)



- **Chemistry:** A collection of antibiotics optimized for clinical use with variable intracellular permeation properties
- **Goal:** Establish bacterial species-specific similarities and differences of permeability barriers and uptake pathways

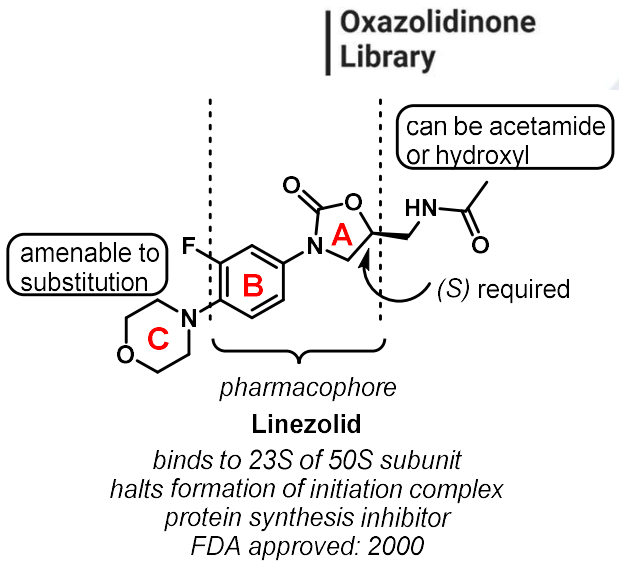
Prevalence of OM barrier and compound-specific uptake pathways

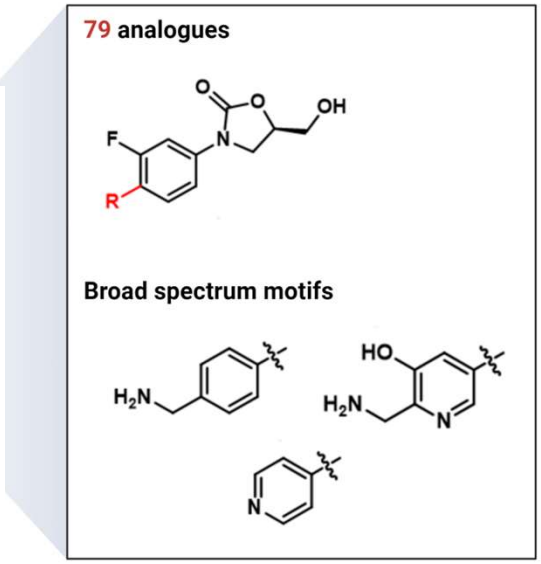
- **Findings:**
  - The OM is the major barrier in *E. coli* and *P. aeruginosa,* efflux for *A. baumannii.*
  - No quantitative correlations between MIC/IC50 and accumulation
  - A common, porin-independent OM penetration pathway in *E. coli* and *P. aeruginosa*.
  - Even compounds of the same chemotype could be using different uptake pathways to gain intracellular accumulation.

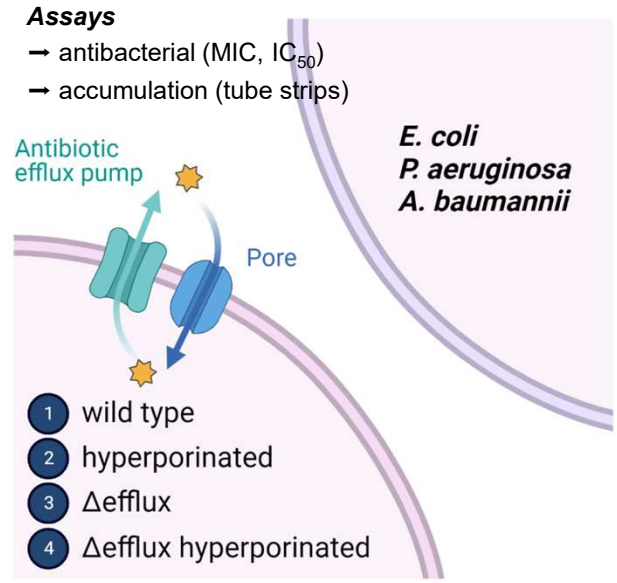


Leus et al., AAC, 2023

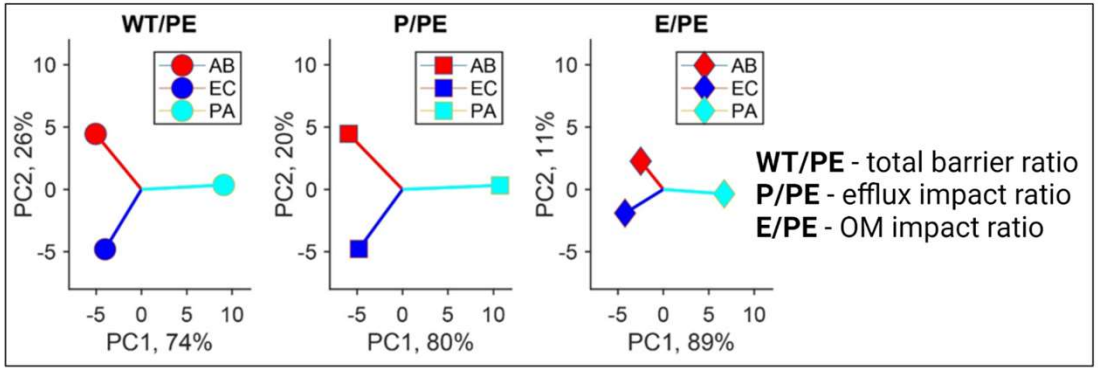
New structural motifs that correlate with penetration







- **Chemistry:** A pharmacophore-based series incorporating alkynes, aryl boronic acids/esters, and aryl bromides
- **Goal:** identify specific chemical groups facilitating and impending permeation

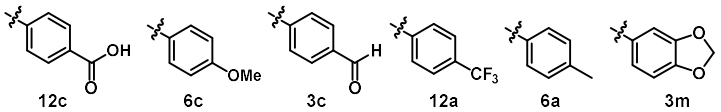


Hu, Z et al., J. Med. Chem. 2022

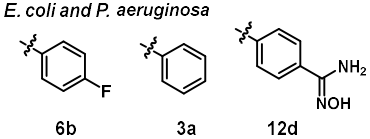
New structural motifs that correlate with penetration

- **Findings:**
  - PA > AB > EC barriers
  - Orientation, location, and composition of functional groups are the determinants on chemotype accumulation.
  - SAR analysis identified substituents detrimental to OM penetration & efflux susceptibility, most of which are species-specific
  - 3 analogues identified with antibacterial activity against all three wt species

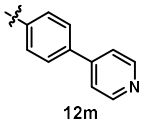
E. coli, A. baumannii, and *P. aeruginosa*



E. coli and P. aeruginosa

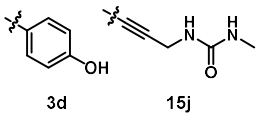


A. baumannii and P. aeruginosa



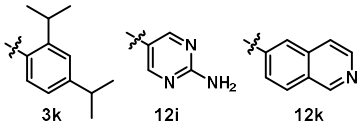
compound	EC	P/PE AB	P/PE PA
12c	>39	5	>11
6c	14	>15	>82
3c	12	14	>254
12a	10	11	>72
6a	10	>62	>26
3m	8	>67	>309
6b	10	-	40
3a	7	-	9
12d	7	-	36
12m	-	>16	>104

E. coli specific



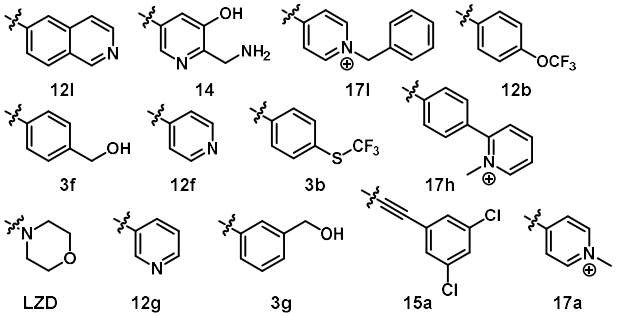
compound	P/PE
3d	10
15j	>9

A. baumannii specific



compound	P/PE
3k	10
12k	10
12i	8

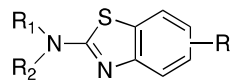
P. aeruginosa specific



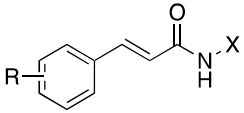
compound	P/PE
12l	>1127
14	259
17l	38
12b	>33
3f	>18
12f	16
3b	>14
17h	13
LZD	12
12g	12
3g	>8
15a	>6
17a	6

Chemical library of antibacterials and efflux pump inhibitors (EPIs)

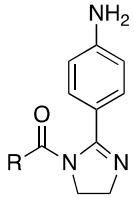
Structural class, source	#
Rempex EPIs	255
Basilea EPIs	8
Known Antibiotics (46 from Merck)	137
Oxazolidinones (Duerfeldt, UM)	84
Gyrase inhibitors (Merck)	126
Dihydroimidazoline EPIs (Walker, SLU)	176
Benzoylimidazolines EPIs	14
2,7-diaminoquinoline EPIs	69
2-aminobenzothiazoles antibacterial	79
Condensin inhibitors antibacterial	108
Cinnamoyl derivatives EPI/antibacterial	73
Thienylpiperidines EPI	8
Diamino pyrimidines EPI	50
Miscellaneous	171
Enamine Screening compounds	160
Total	**1493**



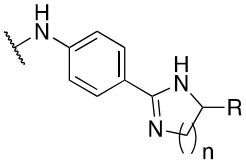
2-aminobenzothiazoles



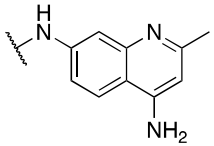
cinnamoyl derivatives



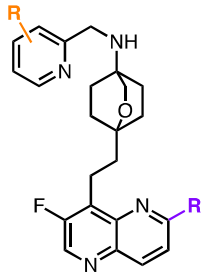
benzoylimidiazolines



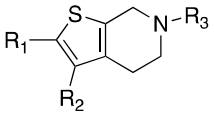
dihydroimidazoline



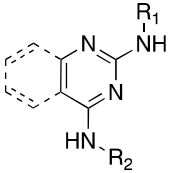
2,7-diaminoquinoline

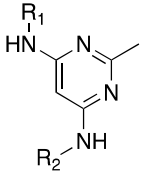


Merck gyrase library

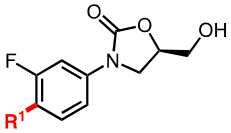


thienylpyridines

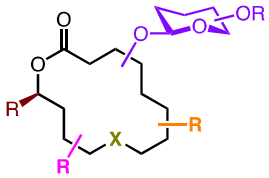


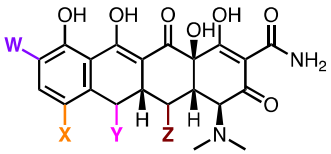


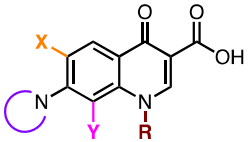
diaminopyrimidines

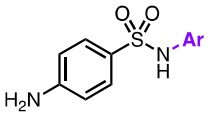


oxazolidinones

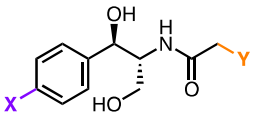


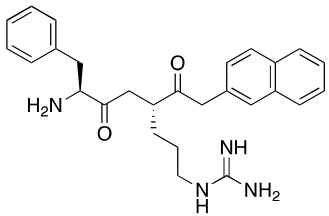






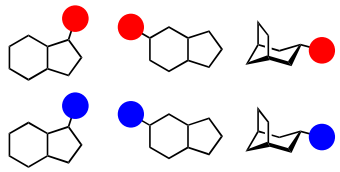
antibiotics





Rempex peptidomimetics library

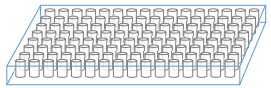
Predictive models for efflux avoidance, inhibition and bacterial penetration

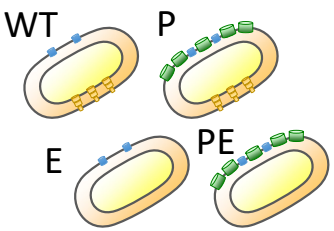


diverse scaffolds
shared substituent sets
cheminformatic analysis

MIC/IC$_{50}$

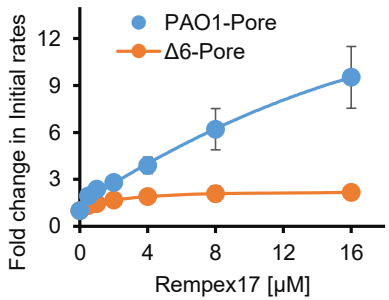
WT/PE- total barrier ratio
P/PE- efflux impact ratio
E/PE- OM impact ratio



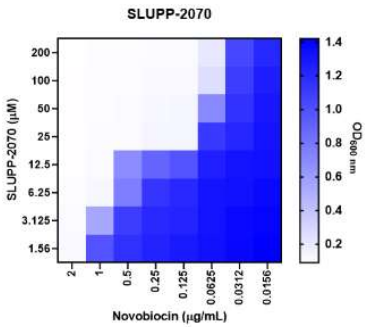


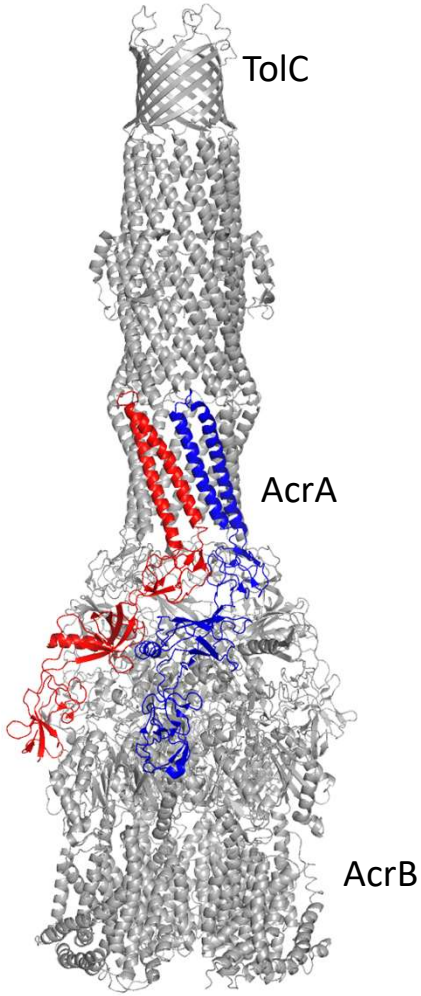
species-specific
subcellular localization
OM vs IM vs efflux

Efflux inhibition

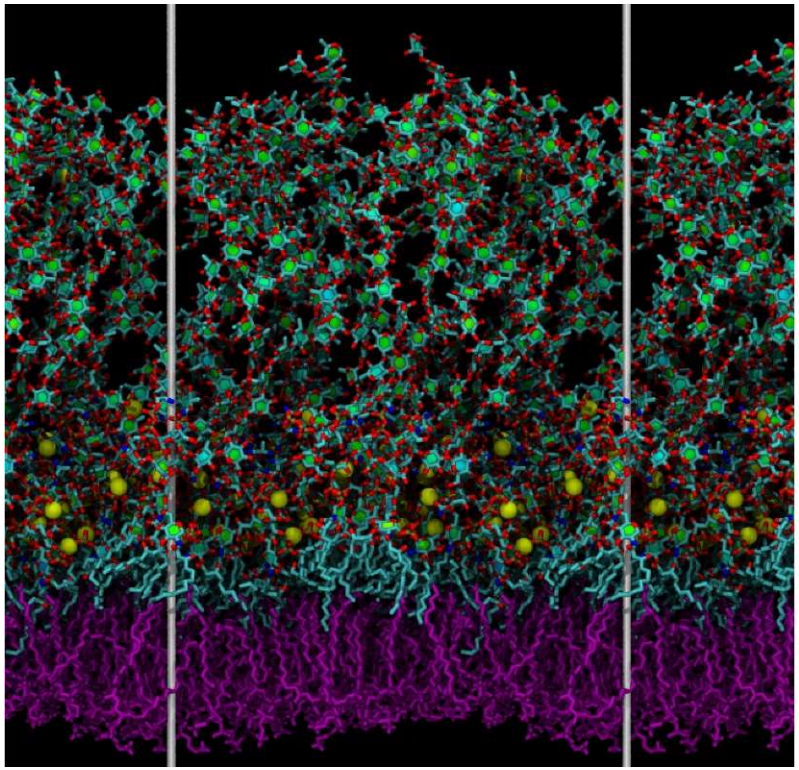


MPC



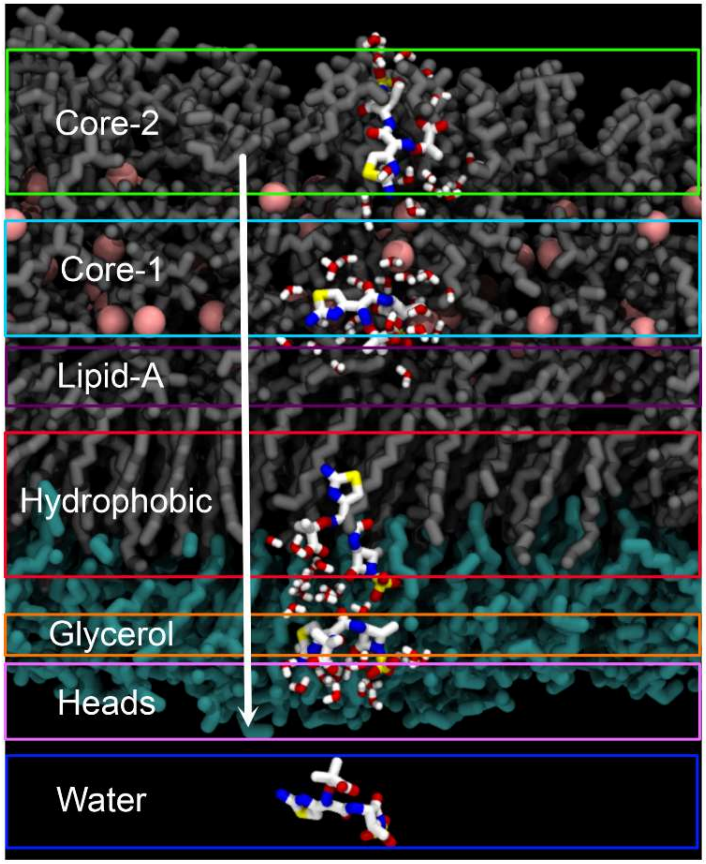


A model of *P. aeruginosa* outer membrane and numerical descriptors of permeation



Mansbach et al., 2020
Lopez et al., 2019
Manrique et al., submitted

OM environment and permeation descriptors



HB-CORE-2
HB-WATER-CORE-2
Δ_{xy}-CORE-2 ($cm^2\ s^{-1}$)
Δ_h-CORE-2 ($kJ\ mol^{-1}$)
Δ_s-CORE-2 ($J\ mol^{-1}\ K^{-1}$)

HB-CORE-1
HB-WATER-CORE-1
Δ_{xy}-CORE-1 ($cm^2\ s^{-1}$)
Δ_h-CORE-1 ($kJ\ mol^{-1}$)
Δ_s-CORE-1 ($J\ mol^{-1}\ K^{-1}$)

HB-LIPID-A
HB-WATER-LIPID-A
Δ_{xy}-LIPID-A ($cm^2\ s^{-1}$)
Δ_h-LIPID-A ($kJ\ mol^{-1}$)
Δ_s-LIPID-A ($J\ mol^{-1}\ K^{-1}$)

HB-TAILS
HB-WATER-TAILS
Δ_{xy}-TAILS ($cm^2\ s^{-1}$)
Δ_h-TAILS ($kJ\ mol^{-1}$)
Δ_s-TAILS ($J\ mol^{-1}\ K^{-1}$)

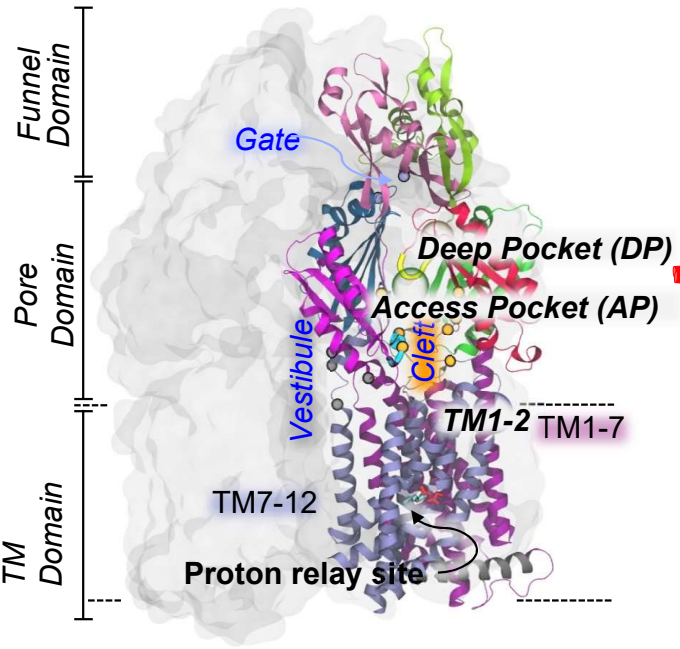
HB-IL-GLY
HB-WATER-IL-GLY
Δ_{xy}-IL-GLY ($cm^2\ s^{-1}$)
Δ_h-IL-GLY ($kJ\ mol^{-1}$)
Δ_s-IL-GLY ($J\ mol^{-1}\ K^{-1}$)

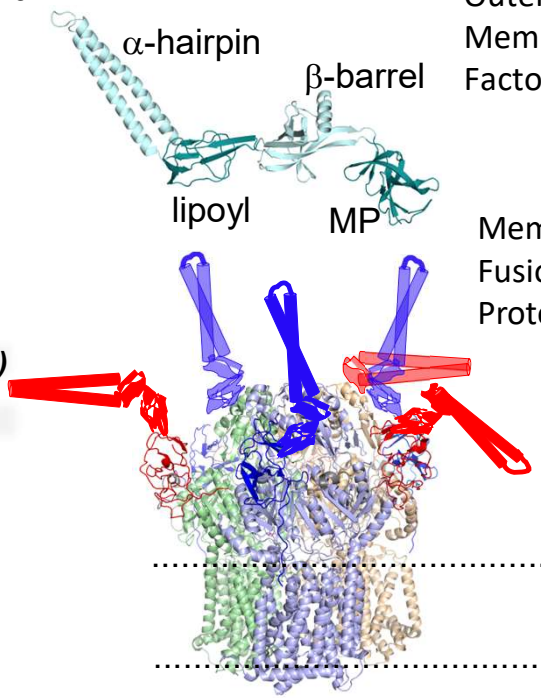
HB-IL-HEAD
HB-WATER-IL-HEAD
Δ_{xy}-IL-HEAD ($cm^2\ s^{-1}$)
Δ_h-IL-HEAD ($kJ\ mol^{-1}$)
Δ_s-IL-HEAD ($J\ mol^{-1}\ K^{-1}$)

HB-MEM-INTER
HB-WATER
Δ_{xy}-SOL ($cm^2\ s^{-1}$)
Δ_h-SOL ($kJ\ mol^{-1}$)
Δ_s-SOL ($J\ mol^{-1}\ K^{-1}$)

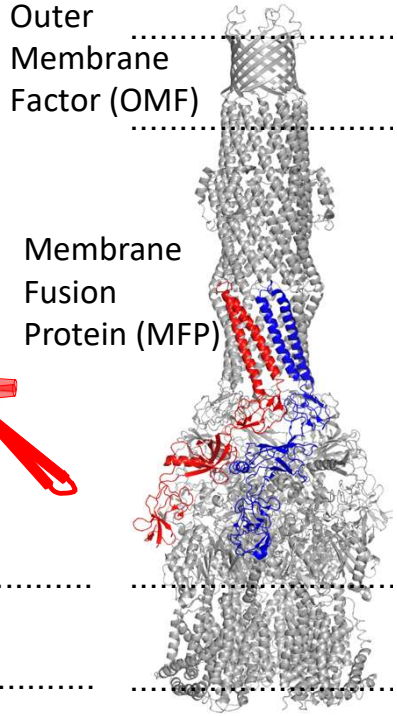
Resistance-nodulation-division (RND) transporters: efflux across the outer membrane

Structural studies by Martin Pos, Akihito Yamaguchi, Satoshi Murakami, Edward Yu, and other groups

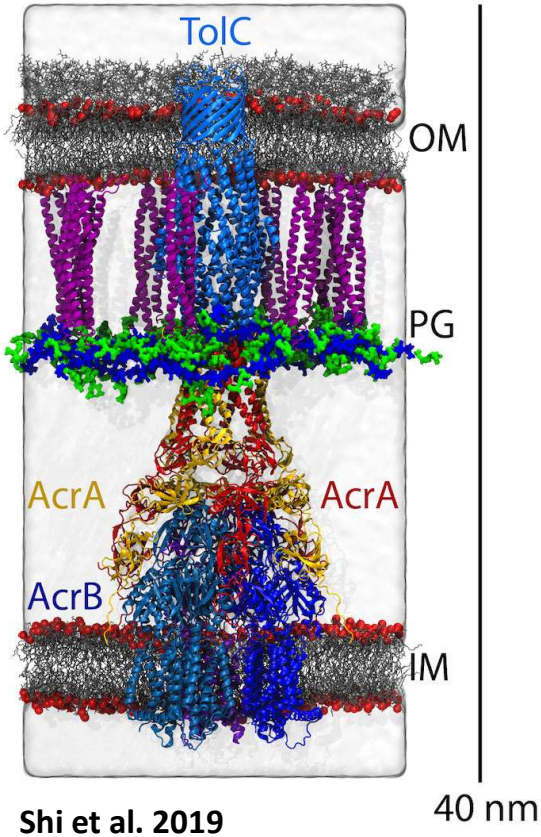




Fabre et al. Structure, 2021



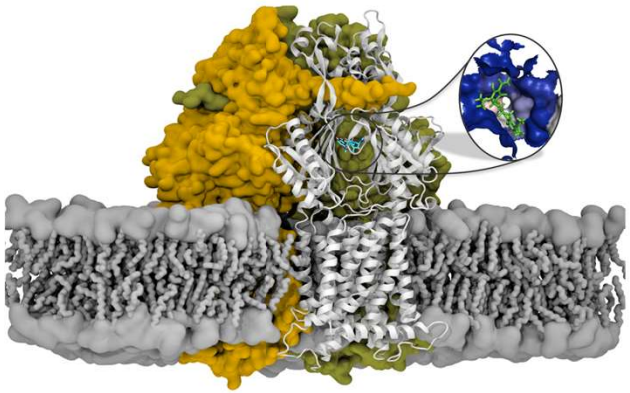
Du et al. Nature, 2014



Shi et al. 2019
Gumbart et al. 2021

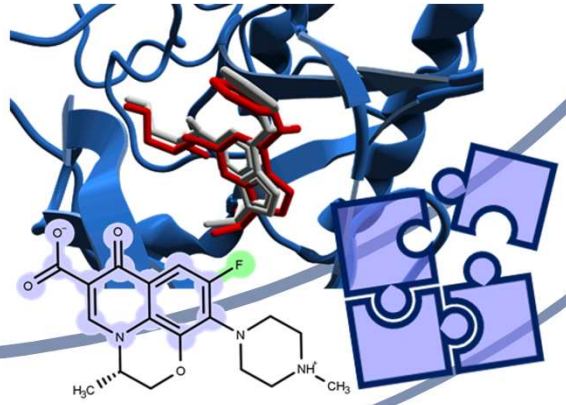
Models of *P. aeruginosa* RND transporters MexB, MexY and MexF and numerical descriptors of ligand-transporter interactions

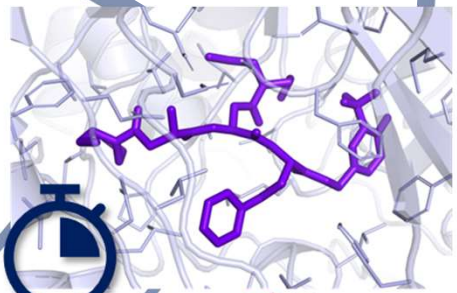
Paolo Ruggerone's group at University of Cagliari, Italy



DYNAMIC
STRUCTURE-BASED
Dynamics

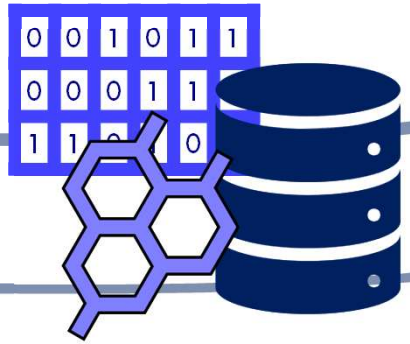
"STATIC"
STRUCTURE-BASED
Interactions





REMPEX Pharmaceuticals

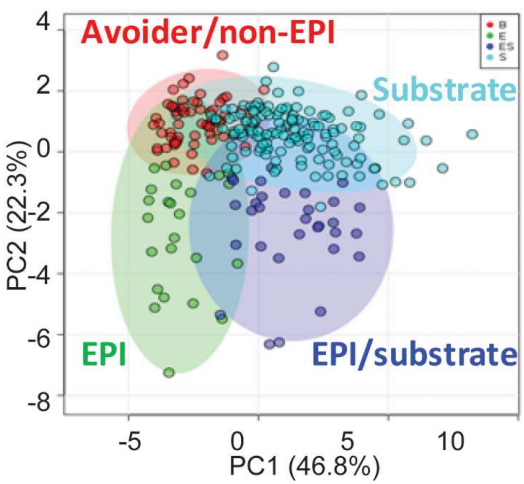
LIGAND-BASED
Descriptors



Gervasoni et al., PCCP, 2022
Gervasoni et al., Sci Data, 2022

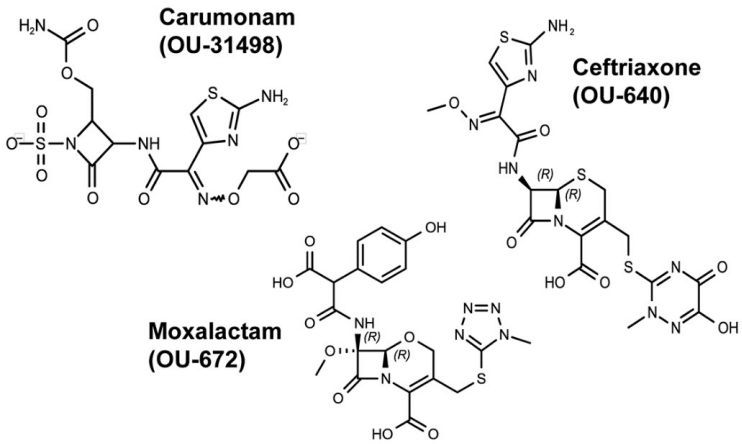
Predictive model of efflux avoiders, inhibitors and substrates

Calculated descriptors for 674 compounds with unknown antibacterial properties, known efflux inhibitors, and traditional antibiotics



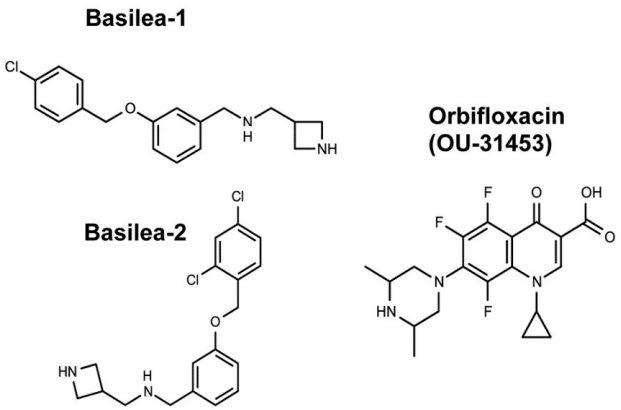
Avoiders

	OU-31498	OU-672	OU-640
Probability	0.85	0.83	0.81
Efflux ratio	ND	1	4
Acylindricity	1.81	1.58	1.34
PRO668_A	0	0	0
LEU674_A	7	3	1
Aromatic_rings	1	2	1
Acceptors	12	11	10
logD	-8.1	-7.3	-4.3
Ani_pol	149	217	178
Aff_DPB	-9.6	-10.5	-9.8



Inhibitors

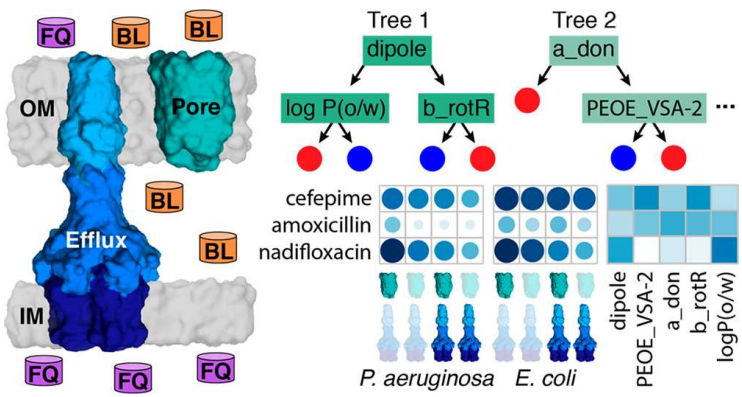
	Basilea-1	Basilea-2	Orbifloxacin
Probability	0.85	0.84	0.80
SS_{conc} ratio	4.5	7.2	ND
Acylindricity	1.42	1.43	1.35
PRO668_A	16	11	0
LEU674_A	4	3	10
Aromatic_rings	2	2	2
Acceptors	1	1	4
logD	0.2	0.8	-1.1
Ani_pol	134	133	206
Aff_DPB	-8.5	-8.5	-9.4



Mehla et al., mBio, 2021

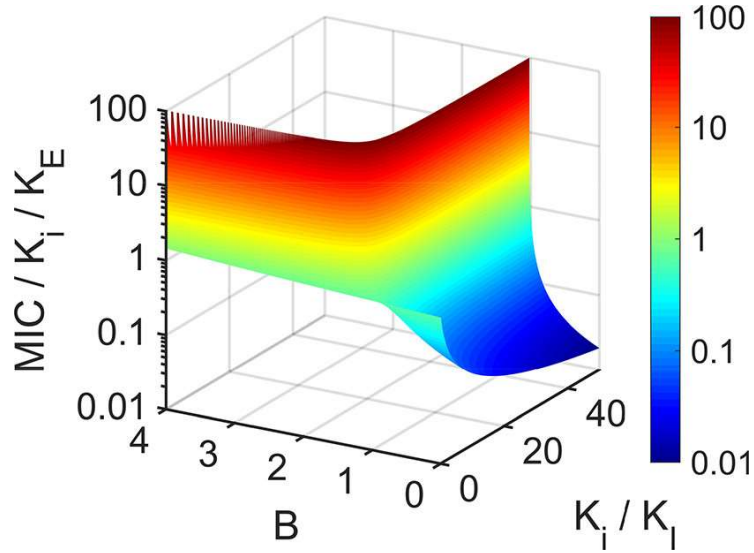
What we learned: 1. MICs vs. intracellular accumulation

Both biological activities and accumulation data are usable to derive permeation rules when concentrations are measured in cells with variable permeability barriers



What we learned : 2. Various resistance mechanisms

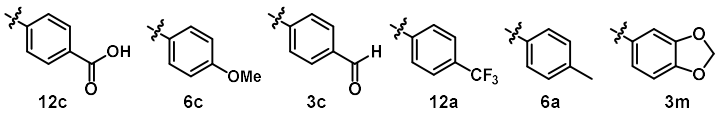
Diffusion barriers and enzymatic inactivation in combination with efflux pumps can generate either additive or multiplicative changes in intracellular concentrations of antibiotics and hence, in their antibacterial activities



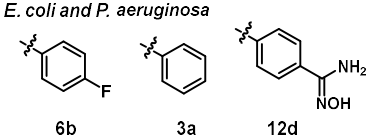
What we learned : 3. Property descriptors vs. chemical groups

Certain chemical groups are associated with good and bad permeation into *P. aeruginosa, E. coli* and *A. baumannii*

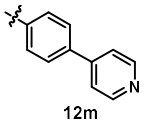
E. coli, A. baumannii, and *P. aeruginosa*



E. coli and P. aeruginosa

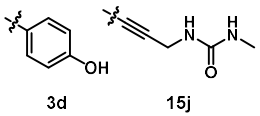


A. baumannii and P. aeruginosa



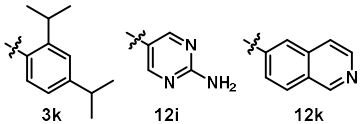
compound	EC	P/PE AB	P/PE PA
12c	>39	5	>11
6c	14	>15	>82
3c	12	14	>254
12a	10	11	>72
6a	10	>62	>26
3m	8	>67	>309
6b	10	-	40
3a	7	-	9
12d	7	-	36
12m	-	>16	>104

E. coli specific



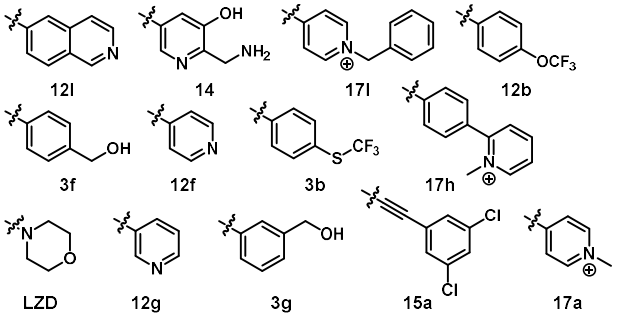
compound	P/PE
3d	10
15j	>9

A. baumannii specific



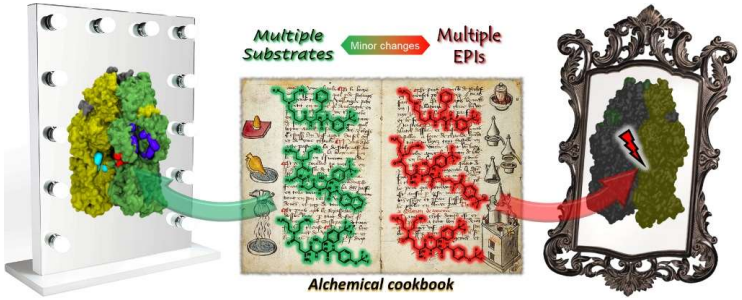
compound	P/PE
3k	10
12k	10
12i	8

P. aeruginosa specific

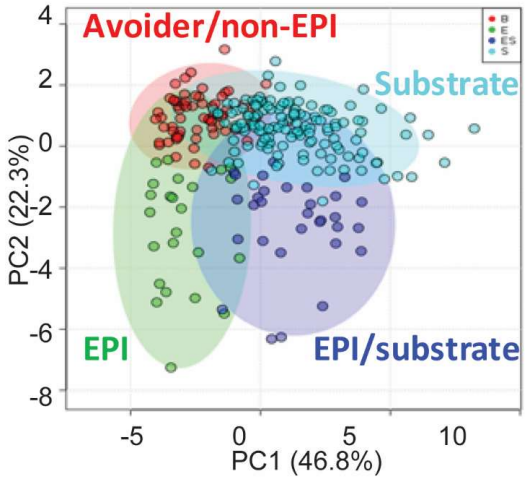


compound	P/PE
12l	>1127
14	259
17l	38
12b	>33
3f	>18
12f	16
3b	>14
17h	13
LZD	12
12g	12
3g	>8
15a	>6
17a	6

What we learned : 4. Efflux avoiders vs. substrates and inhibitors

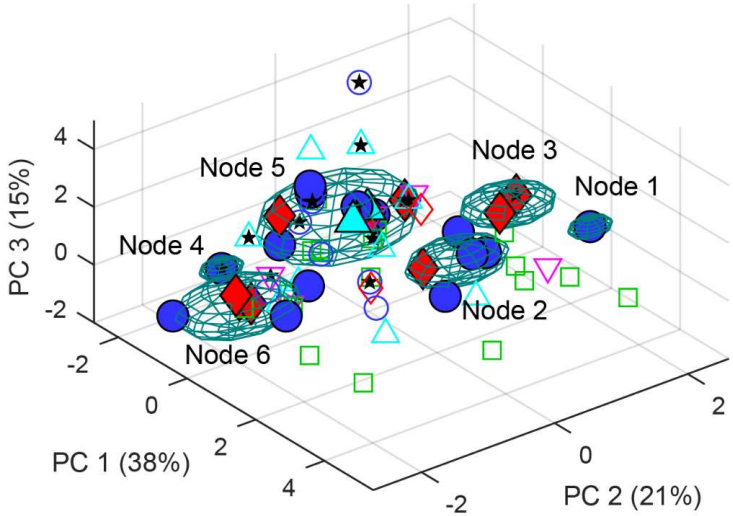


- Structurally diverse compounds possess properties of efflux inhibitors and substrates, often in the same chemical scaffold
- Efflux pump ligands (EPIs and substrates) have physico-chemical and molecular level properties that are distinct from efflux avoiders
- Efflux avoidance and inhibition models are predictive of such properties among unrelated compounds and the two models select different chemical classes of compounds



What we learned: 5. Universal vs scaffold specific rules

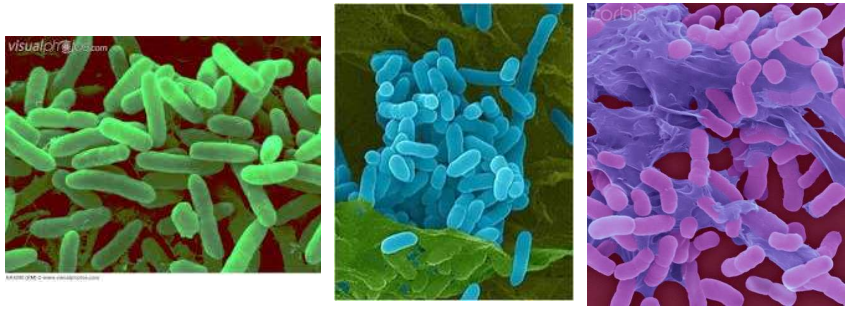
Good permeators can be found among compounds occupying different property areas (many scaffolds can be optimized)



What did not work

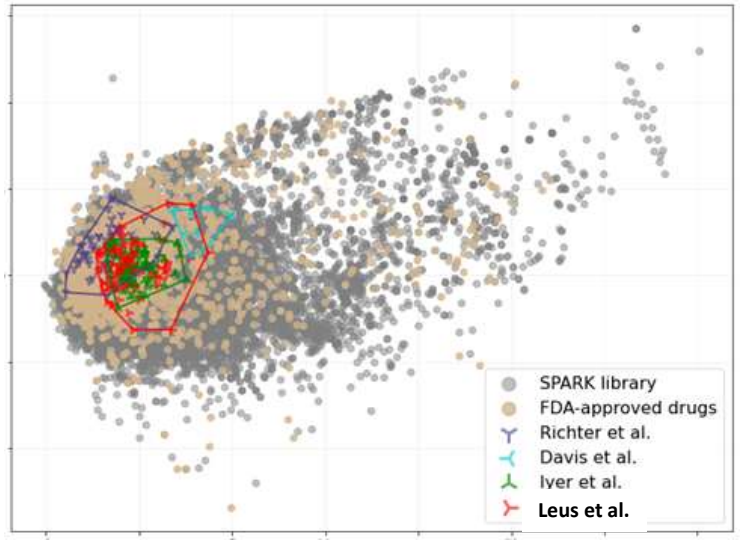
- No quantitative correlations between bacterial growth-dependent and independent assays (for both antibiotics and EPIs)
- SPE-MS is laborious and as other MS approaches, reports the total compound concentration bound to cells
- No universal descriptors or values of descriptors

What is next



CDC

- Species- specific differences are significant and important: need similar studies for *challenging bacteria*; different efflux transporters
- Mapped and modeled relatively small property space: need more data and for diverse chemical libraries (NIAID CC4CARB)
- Searchable databases and user-friendly websites to analyze compounds (AB-DB, SPARK…)



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