Novel inhibitors of class C beta-lactamases: from the chemical lab to pre-clinical evaluation

Malcolm G. P. Page

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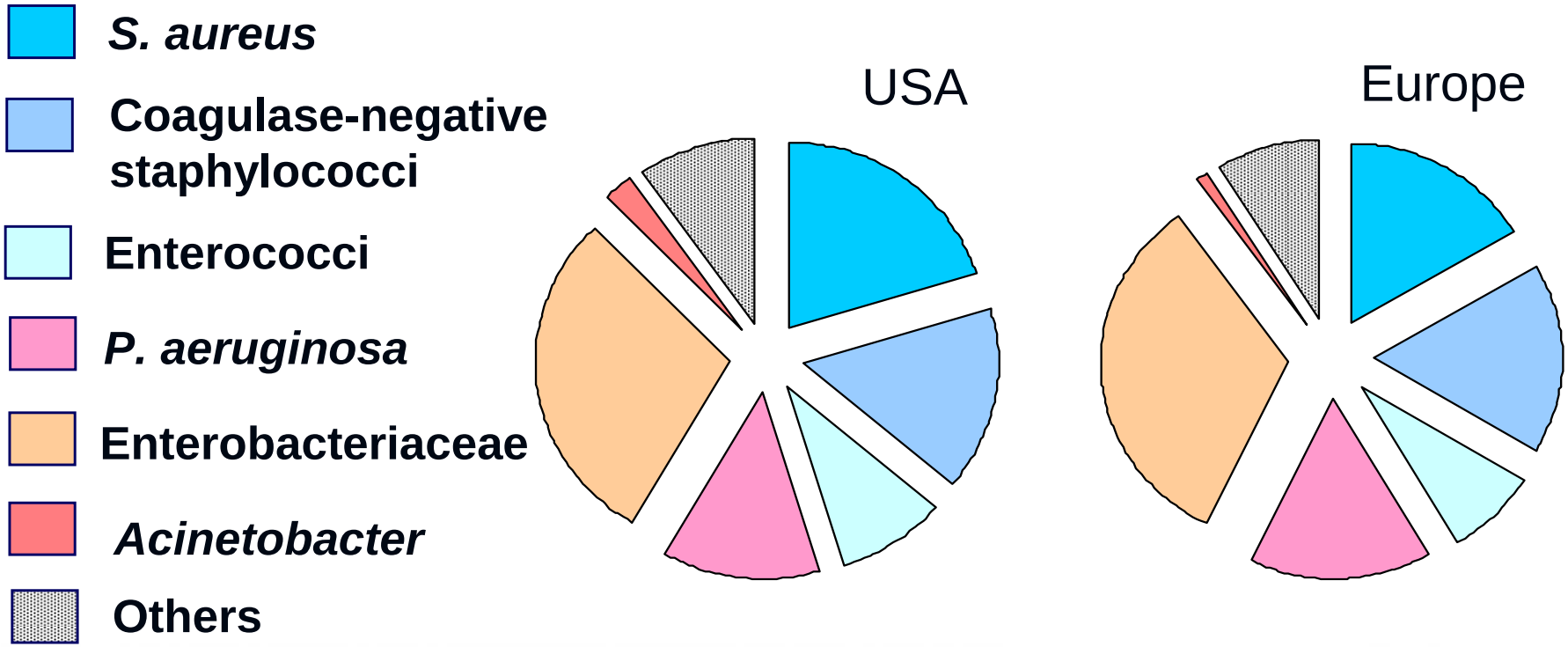
Incidence of Pathogenic Bacteria in Hospital-acquired Infections

Gram-negative bacteria account for approximately half of the severe hospital-acquired infections

P. aeruginosa is the 4th most frequently isolated nosocomial species



M. E. Jones et al., Emerging resistance among bacterial pathogens in the intensive care unit – a European and North American Surveillance study (2000–2002). Annals of Clinical Microbiology and Antimicrobials 2004, 3:14

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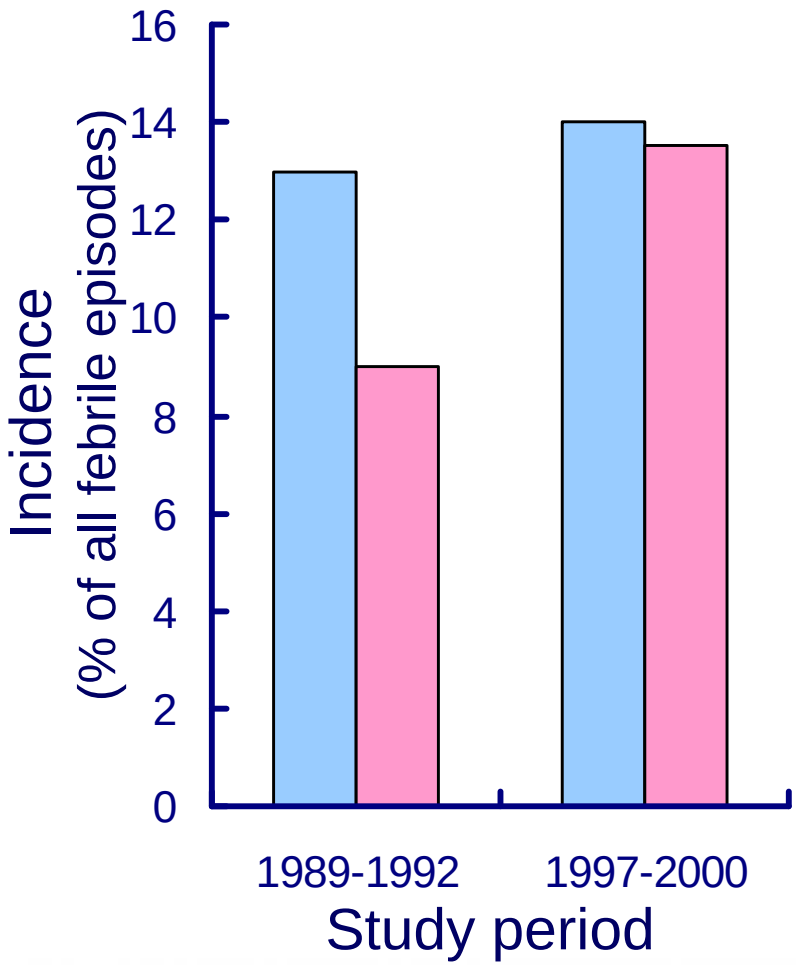
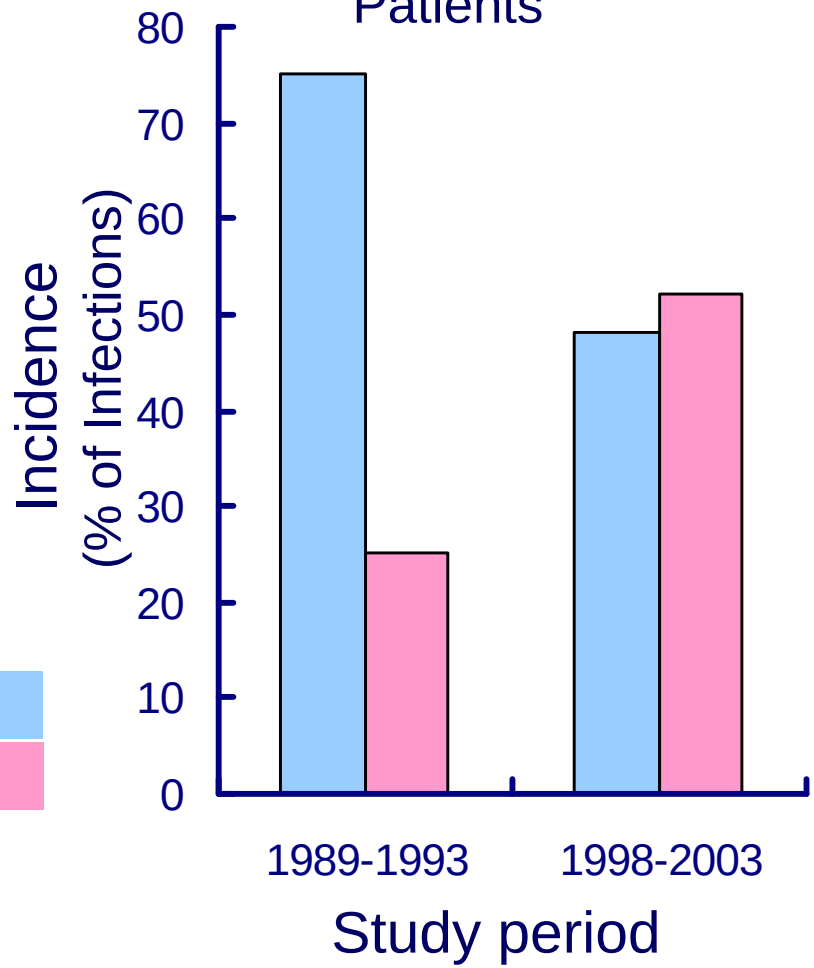
Incidence of Gram-negative Bacilli is Increasing

Occurrence of Monomicrobial infections in Cancer Patients



Occurrence of Bacterial Infections in Liver Transplant Patients



Gram +

Gram -

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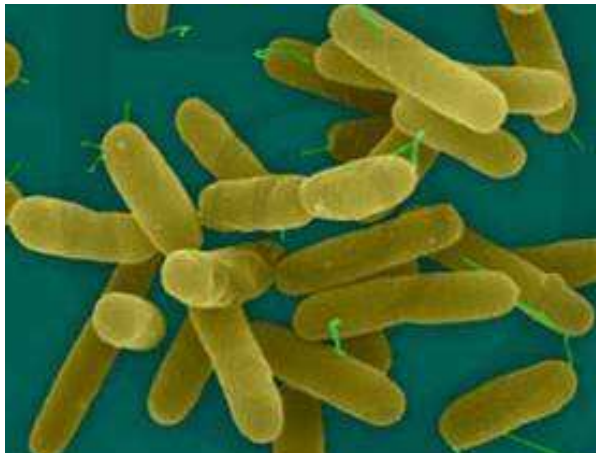
MDR Gram-negative Bacilli: the Rising Tide

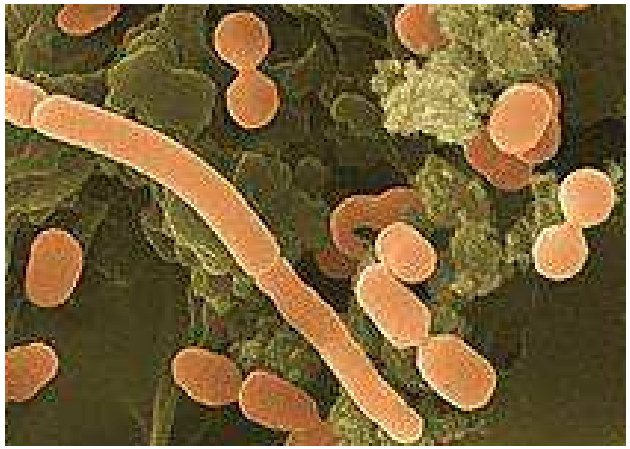
Bad Bugs, No Drugs
As Antibiotic Discovery Stagnates
. . . A Public Health Crisis Brews

Infectious Diseases Society of America

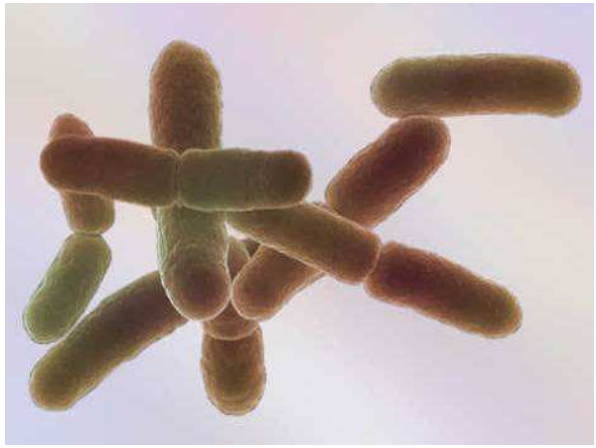
Pseudomonas aeruginosa



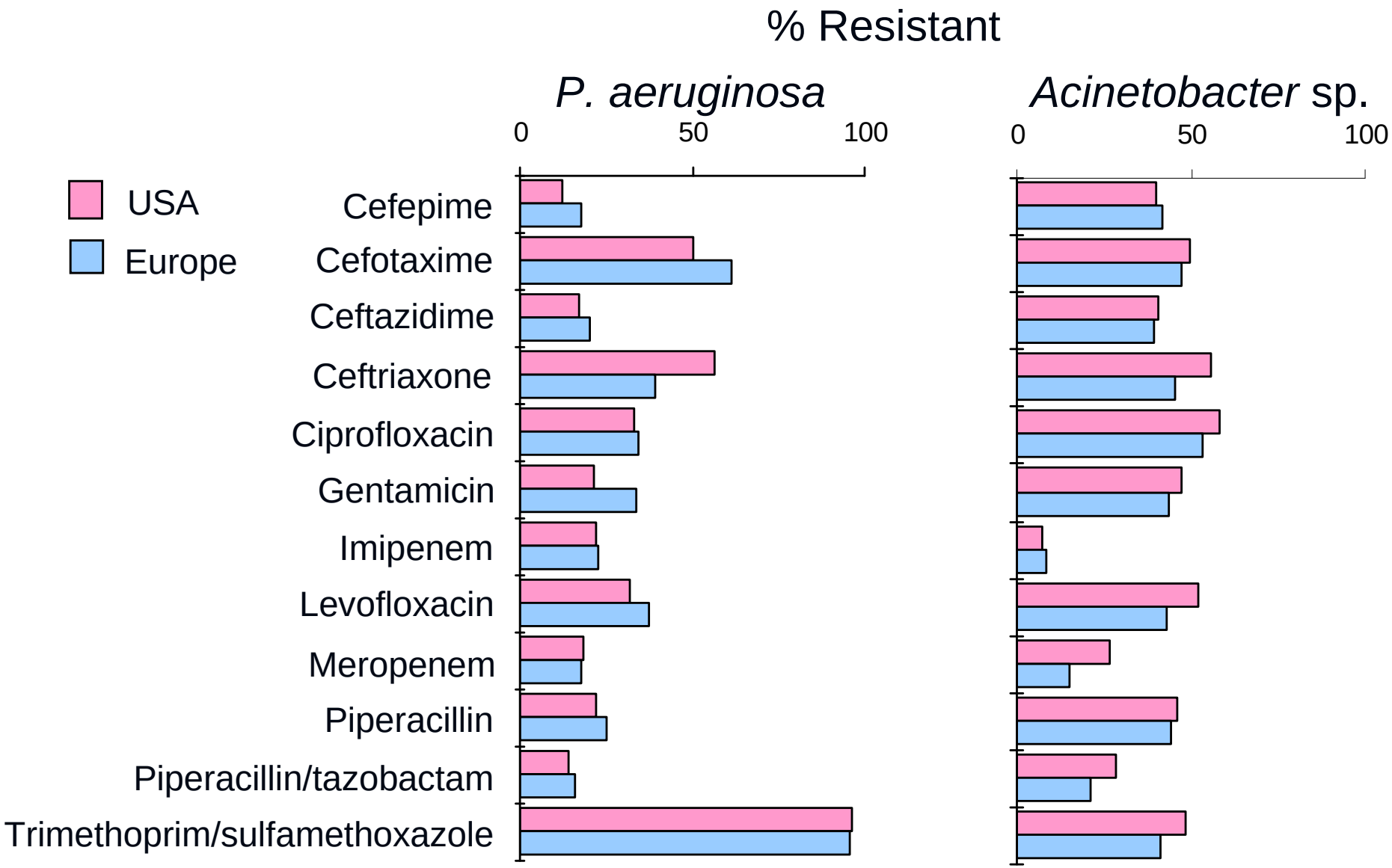
Acinetobacter baumannii



E. coli* and *K. pneumoniae



Resistance Rates are Already High in the Non-Fermentors



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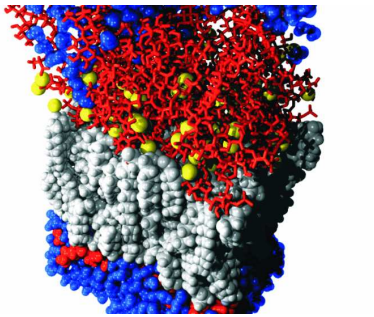
M. E. Jones et al., Emerging resistance among bacterial pathogens in the intensive care unit – a European and North American Surveillance study (2000–2002). Annals of Clinical Microbiology and Antimicrobials 2004, 3:14

Gram-negative resistance mechanisms (1) General

Lack of activity is due to the interplay of several distinct mechanisms

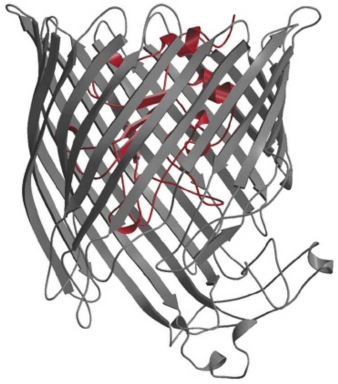
Each is capable of conferring high-level resistance



Lipopolysaccharide

Repels negatively charged compounds

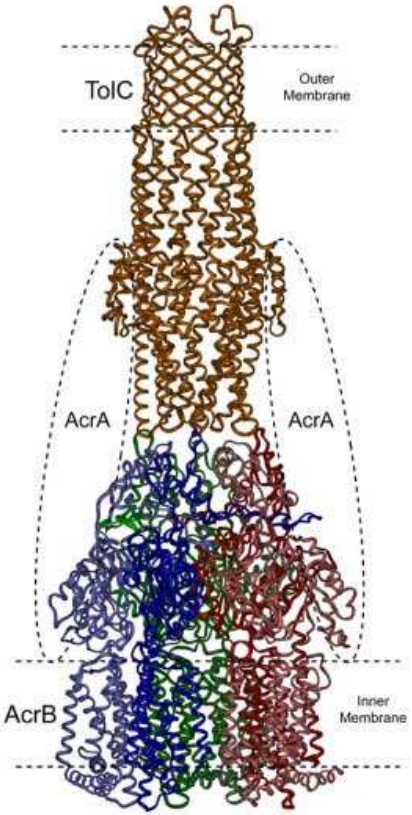
Modification with aminoarabinose confers reistance to cationic peptides



Porins

Filter out large compounds

Can be mutated to increase filtering effect or deleted to decrease influx



Multiple efflux pumps

Pump compounds back outside

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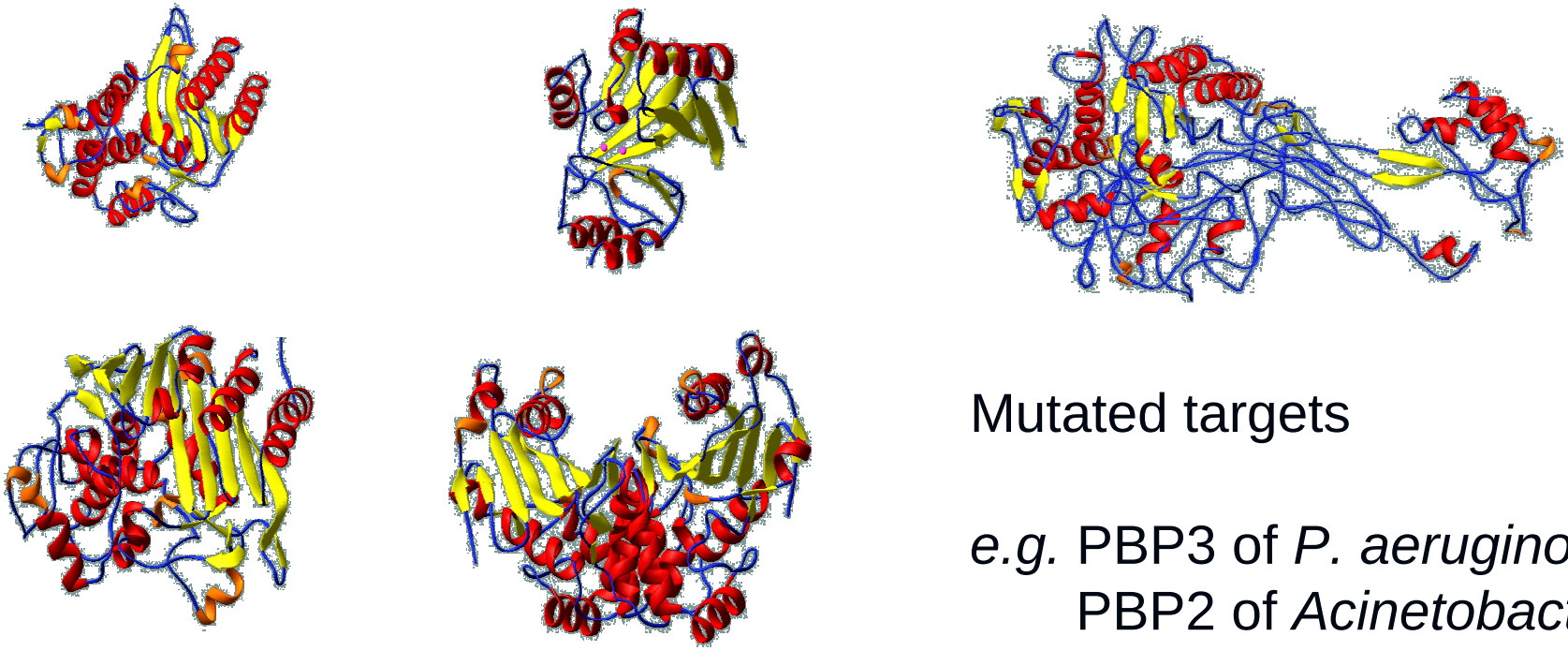
Gram-negative resistance mechanisms (2) Specific



Protective enzymes that destroy or modify antibiotics so that they are no longer active

e.g. aminoglycoside modifying enzymes, multiple β-lactamases

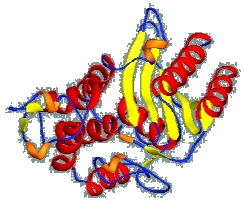
Mutated targets

e.g. PBP3 of *P. aeruginosa*
PBP2 of *Acinetobacter*

Mutations make the penicillin-binding protein (PBP) immune to β-lactams but leave it active for cell wall biosynthesis

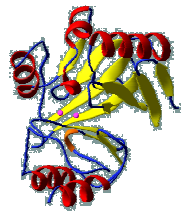
β-Lactamases

Class A



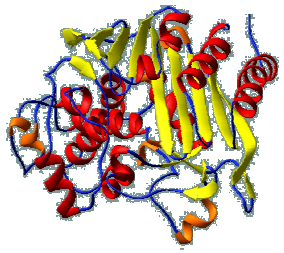
- The most abundant class (>300 known). Rare in *P. aeruginosa* & *Acinetobacter* sp. More common in *S. maltophilia* and in Enterobacteriaceae (*K. pneumoniae)*
- Generally not very active against carbapenems and readily inhibited by clavulanate and penam sulfones
- The KPC carbapenemases are poorly inhibited by the marketed inhibitors

Class B



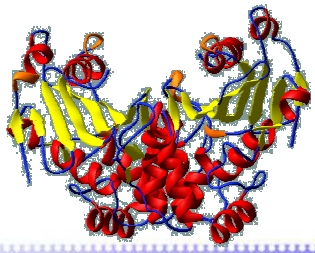
- Relatively rare, but on the increase in Europe, Asia & S. America. Found in *P. aeruginosa, Acinetobacter* & *S. maltophilia* and spreading to Enterobacteriaceae
- Very broad substrate specificity. No useful inhibitor

Class C



- Widely distributed in *P. aeruginosa*, *Acinetobacter* and Enterobacteriaceae
- Broad substrate specificity, can confer resistance to carbapenems. No inhibitor on the market

Class D



- ESBL variants with broad substrate specificity in *P. aeruginosa*
- Emerging carbapenemase variants in *Acinetobacter*
- No useful inhibitor

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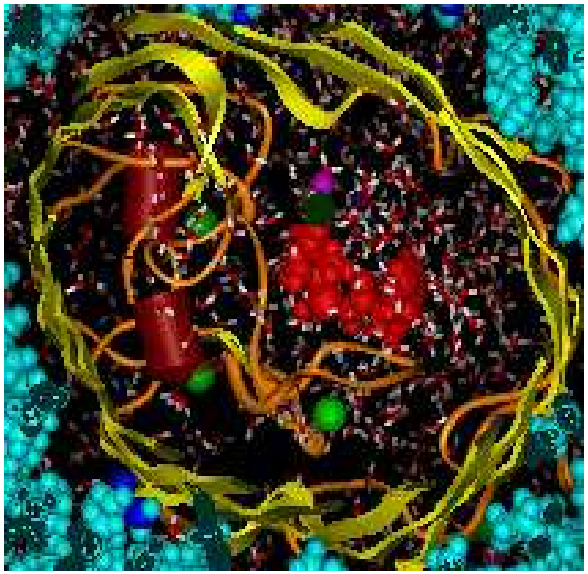
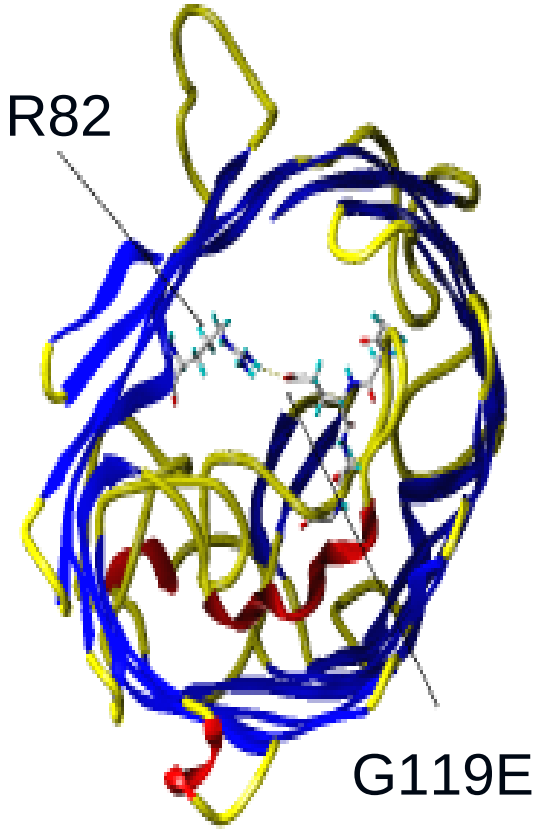
Transport Through Porins

Transport mediated by porins can be restricted by:

- Decreased expression
- Shift to expression of porins with narrower pores
- Mutation: bulkier, charged restrict free diffusion





Substitution of glycine 119 with glutamate increases size and allows formation of a cross-bridge with Arg 82.

Effective diameter of the channel is halved.

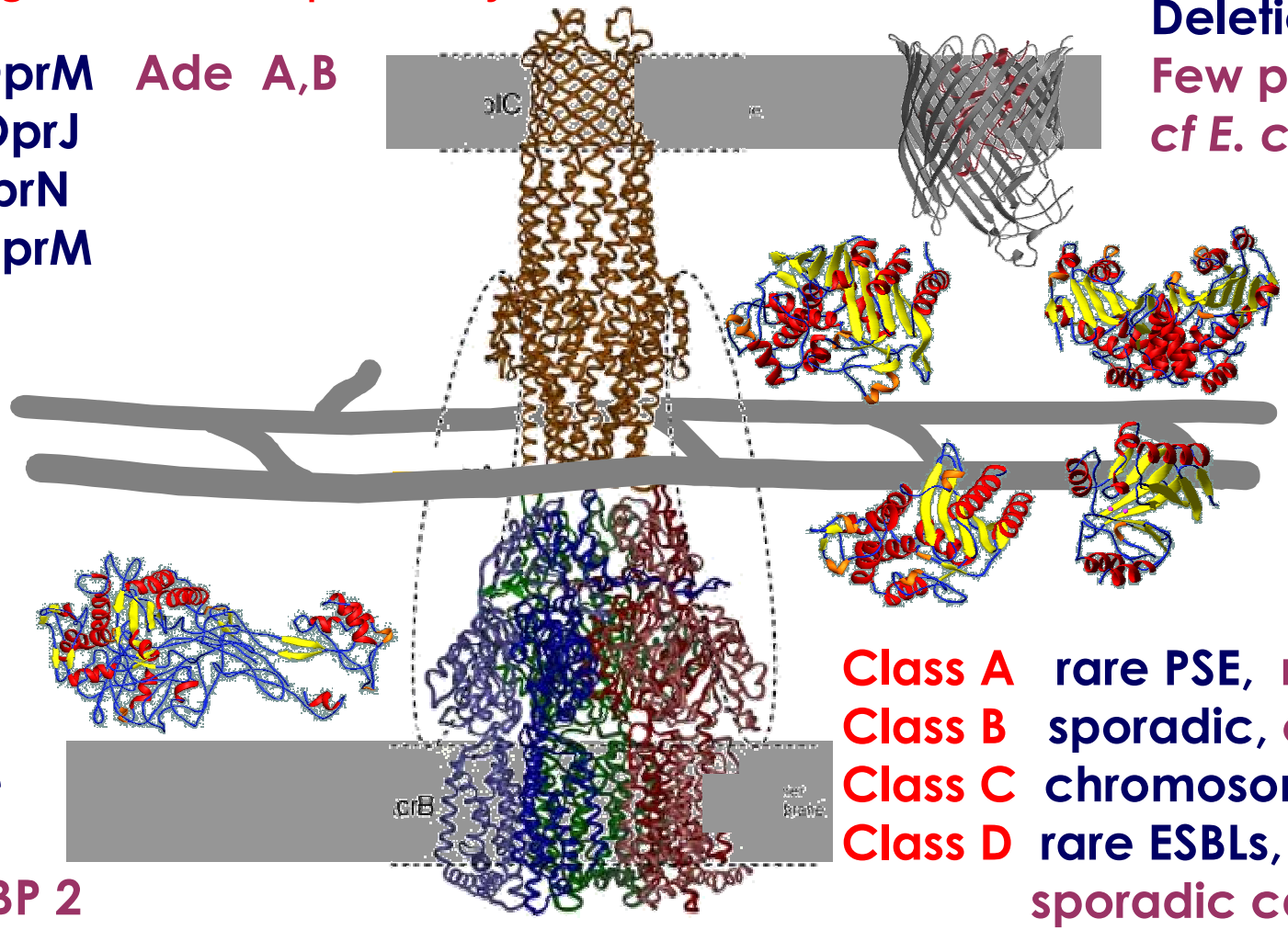
β-Lactam Resistance in *P. aeruginosa* & *Acinetobacter*

Multiple efflux systems with overlapping substrate specificity

Mex A,B-OprM **Ade A,B**
Mex C,D-OprJ
Mex E,F-OprN
Mex X,Y-OprM

Mutation or altered expression of porins

Deletion of D2 porin
Few porins (<5% flux *cf E. coli*)



Multiple β-lactamases

Mutated PBPs

Alternative PBP3*
Mutated PBP 2

Class A rare PSE, rare ESBL
Class B sporadic, common
Class C chromosomal in both
Class D rare ESBLs, sporadic carbapenemases

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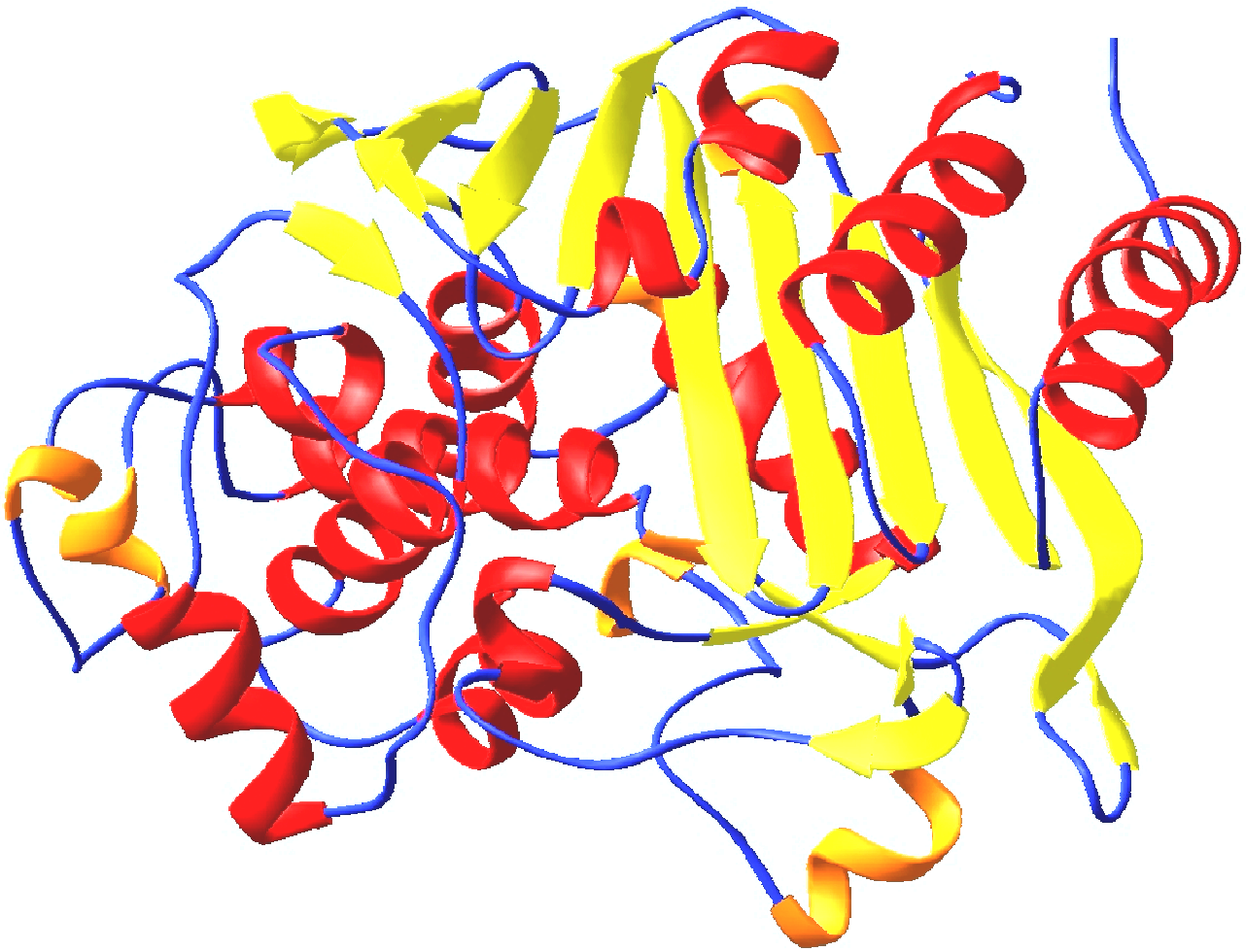
Class C β-Lactamases

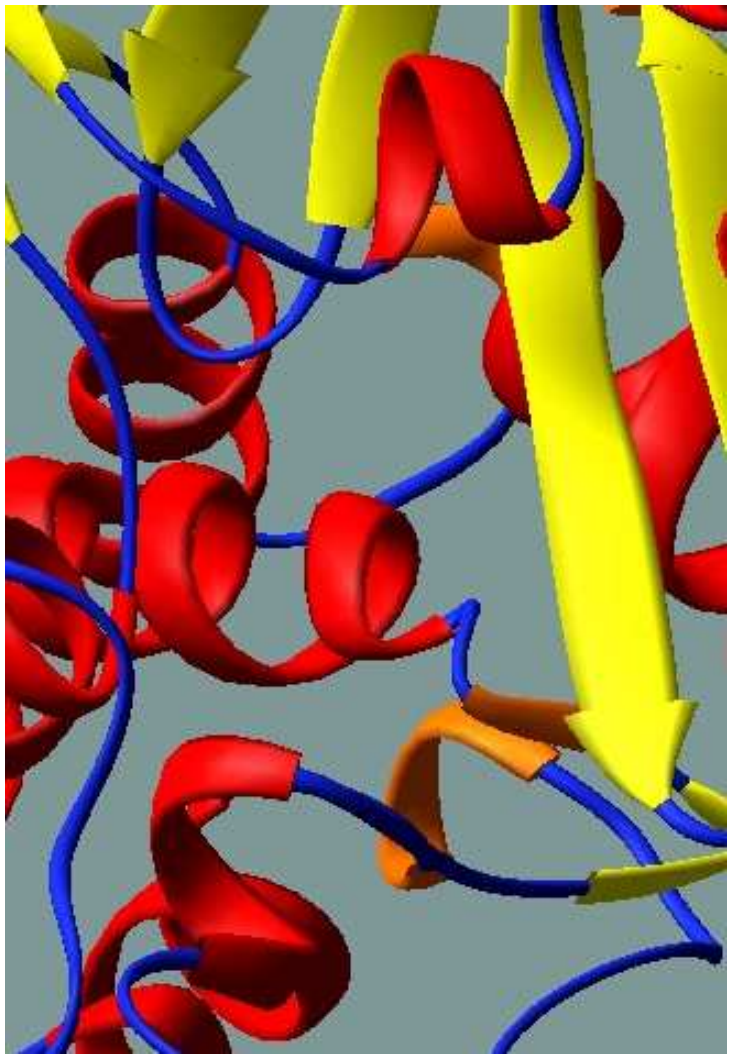


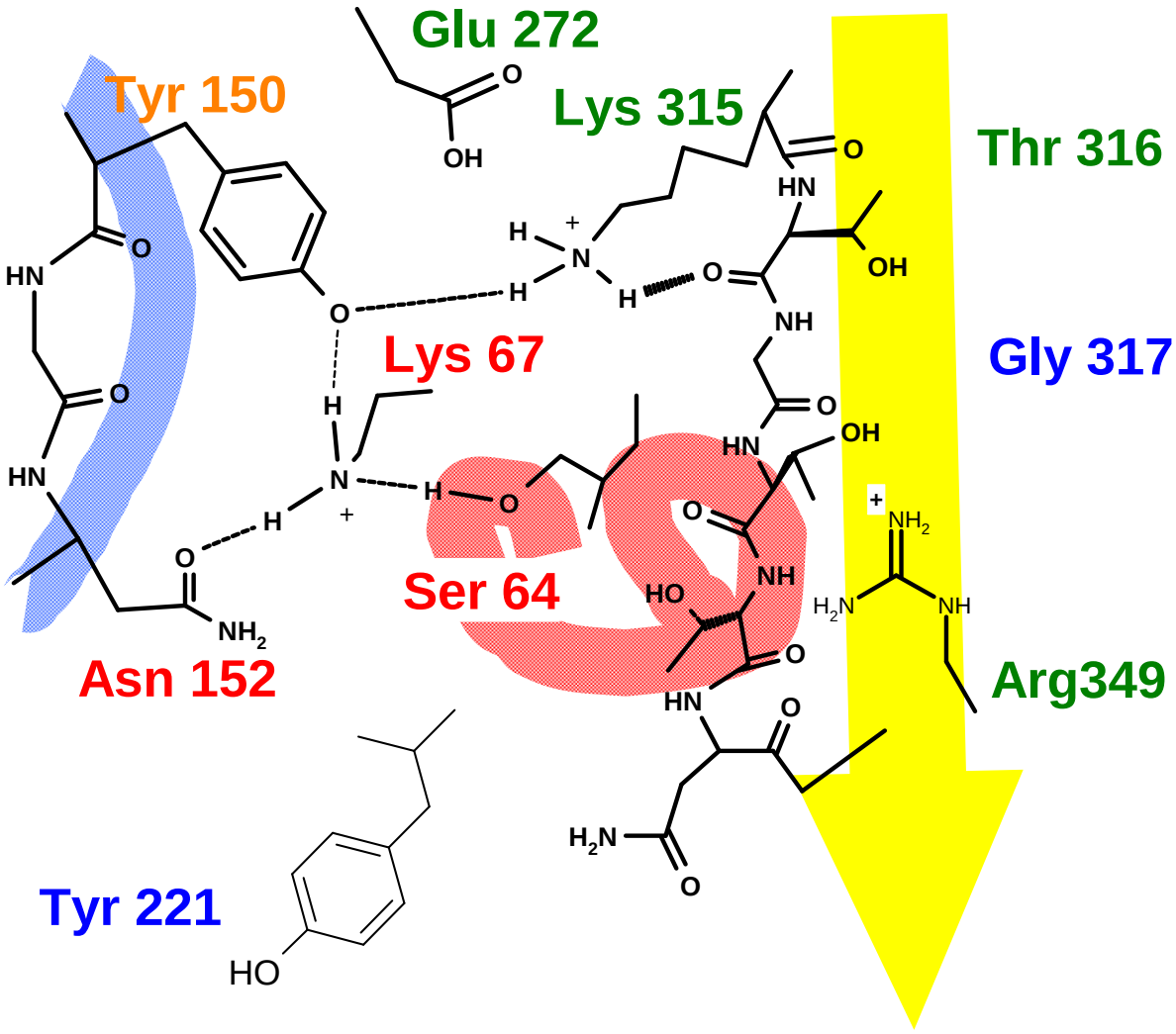
Chromosally encoded in
Citrobacter freundii
Enterobacter cloacae
Morganella morgani
Providencia rettgeri
Serratia marcescens
Pseudomonas aeruginosa
Acinetobacter baumannii
…

Transferrable variants in
Escherichia coli
Klebsiella pneumoniae
Enterobacter aerogenes
…

Can confer resistance to carbapenems, cephamycins, cephalosporins, monobactams, penicillins …

Active Site of Class C β-Lactamases





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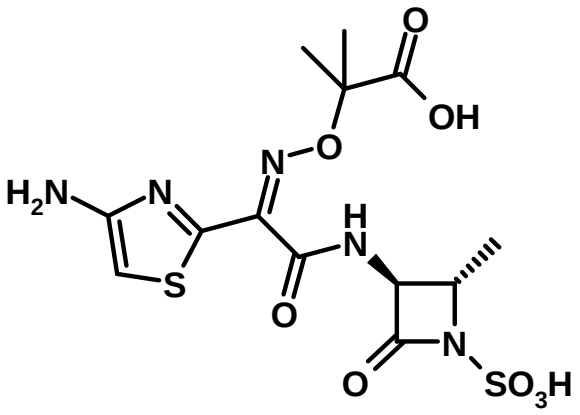
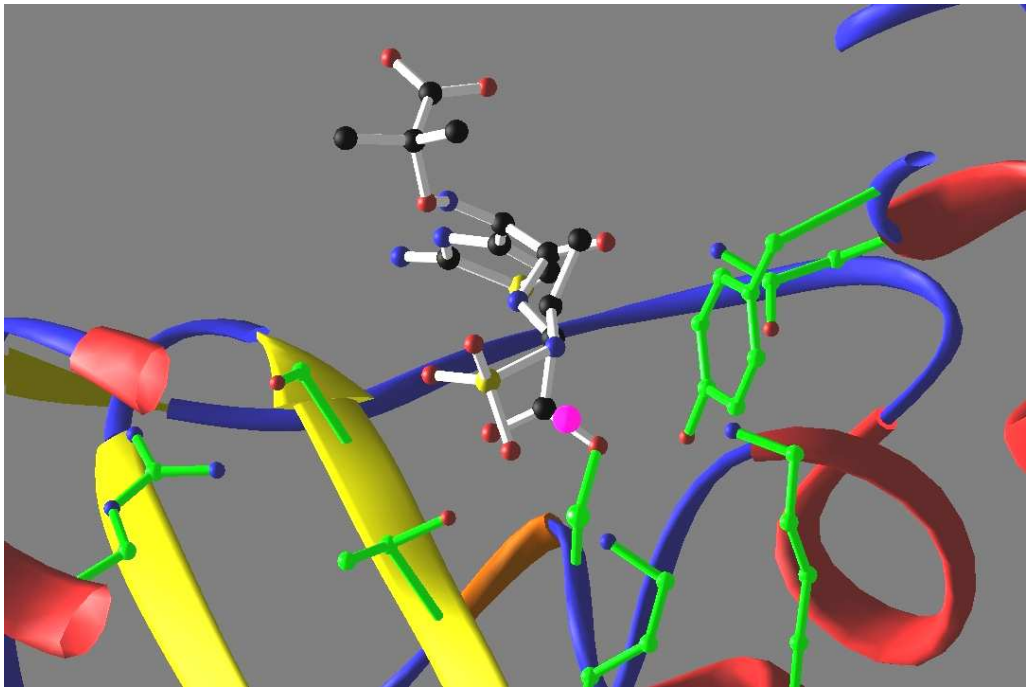
Inhibition of Class C β-Lactamase By Aztreonam





Antibiotic

Potent inhibitor of class C β-lactamase IC_{50} <0.1 μM

Inhibition due to stable acylation of the enzyme
$t_{1/2}$ for deacylation >45 min

Acyl-enzyme can be isolated

X-ray structure of acyl-enzyme obtained by soaking aztreonam into crystals of *C. freundii* class C β-lactamase

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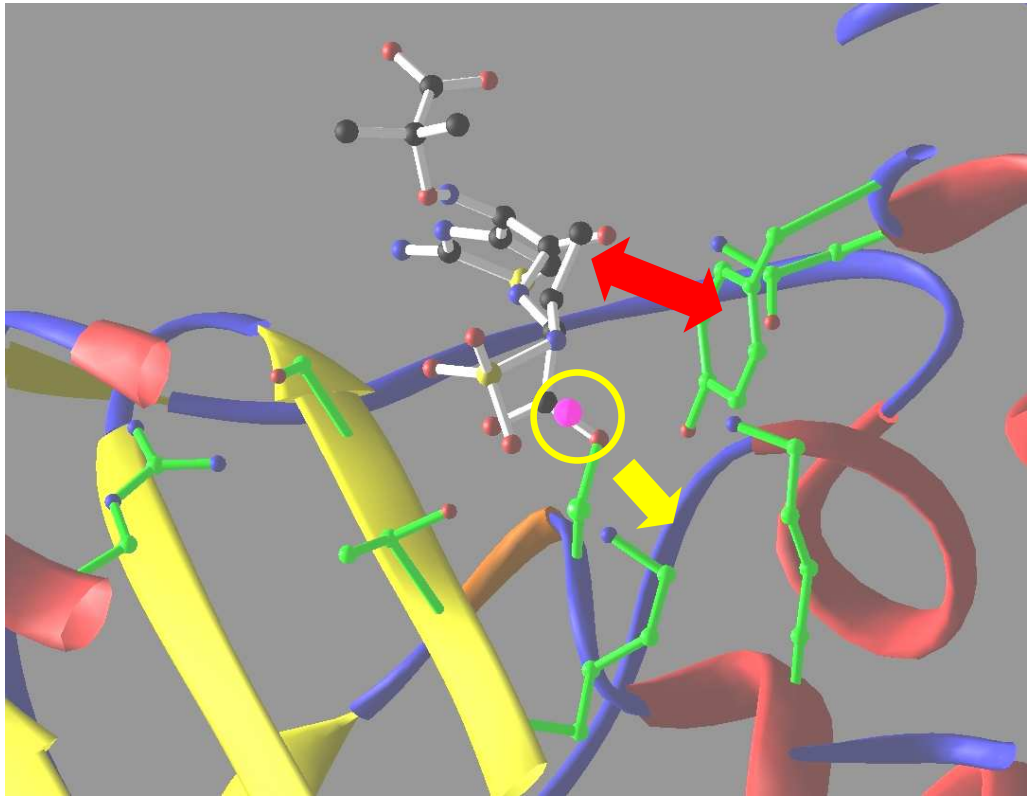
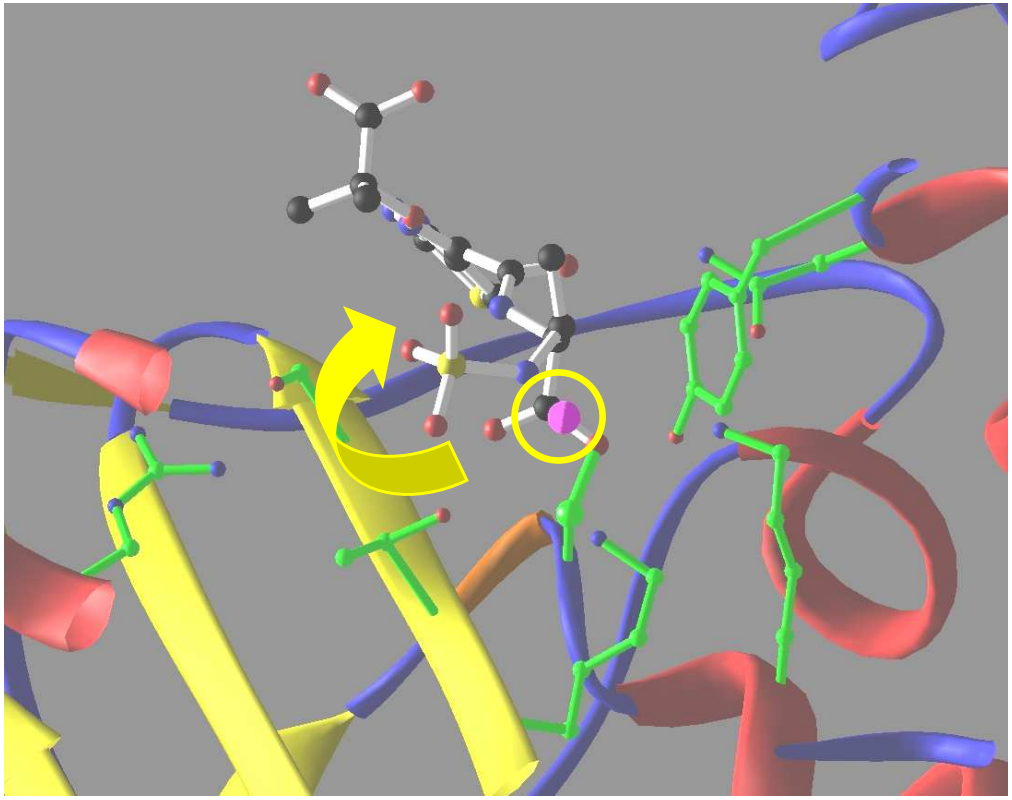
Structural Basis of Inhibition of AmpC by Aztreonam

Crystal structure of *C. freundii* AmpC - aztreonam complex



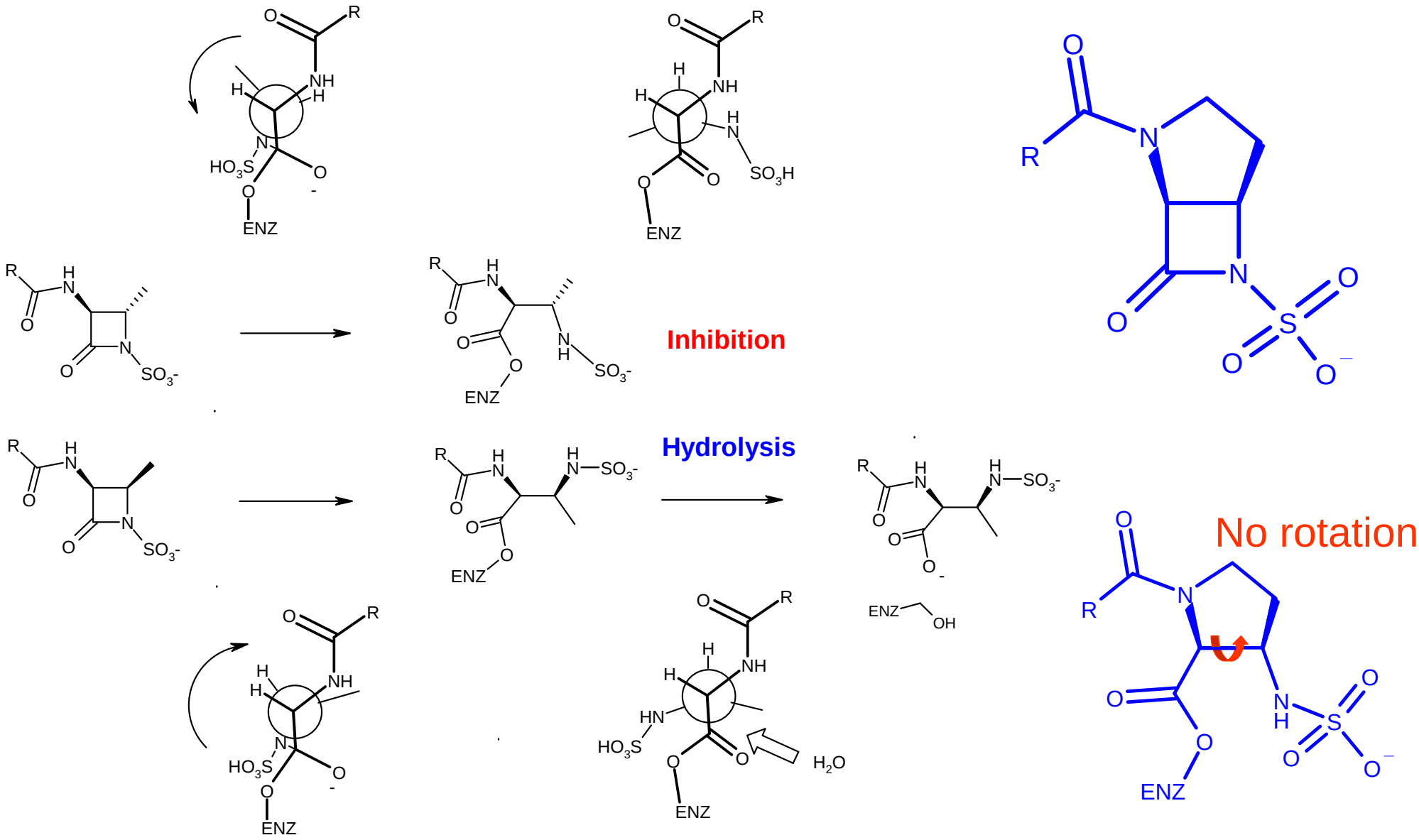
Trans isomer stable because motion restricted by steric conflict between methyl group and tyrosine side chain

Model of *C. freundii* AmpC complex with analogue having a *cis* methyl

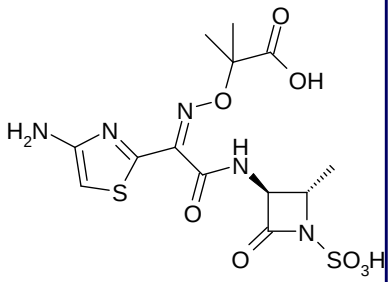
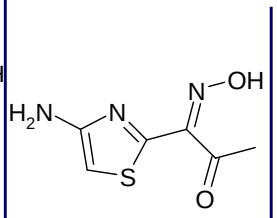
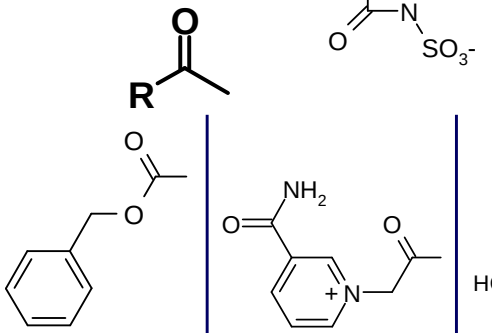
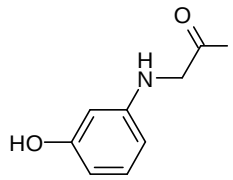


Cis isomer rapidly hydrolysed because motion possible, allowing water to attack ester bond

Going One Step Further – the Bridged Monobactams



Comparative Activity of Bridged Monobactams

Enzyme source	Inhibition Constant (IC_{50}) [nM]				
	Aztreonam 				
C. freundii	170	>10000	75	18	0.1
E. coli	250	>10000	100	23	3
P. aeruginosa MK751	300	>10000	180	12	0.9
P. aeruginosa 18SH	500	>10000	200	27	25

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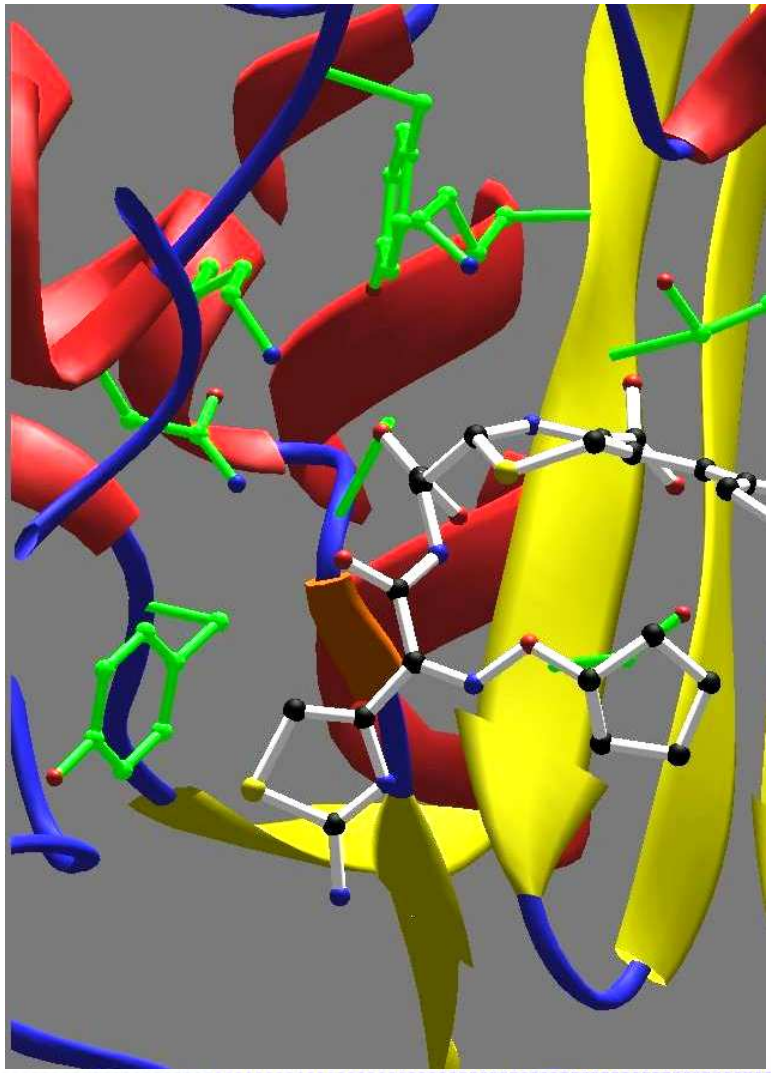
The Role of the Aminothiazolyloxyiminoacetamide Side Chain in Binding to Class C β-Lactamases

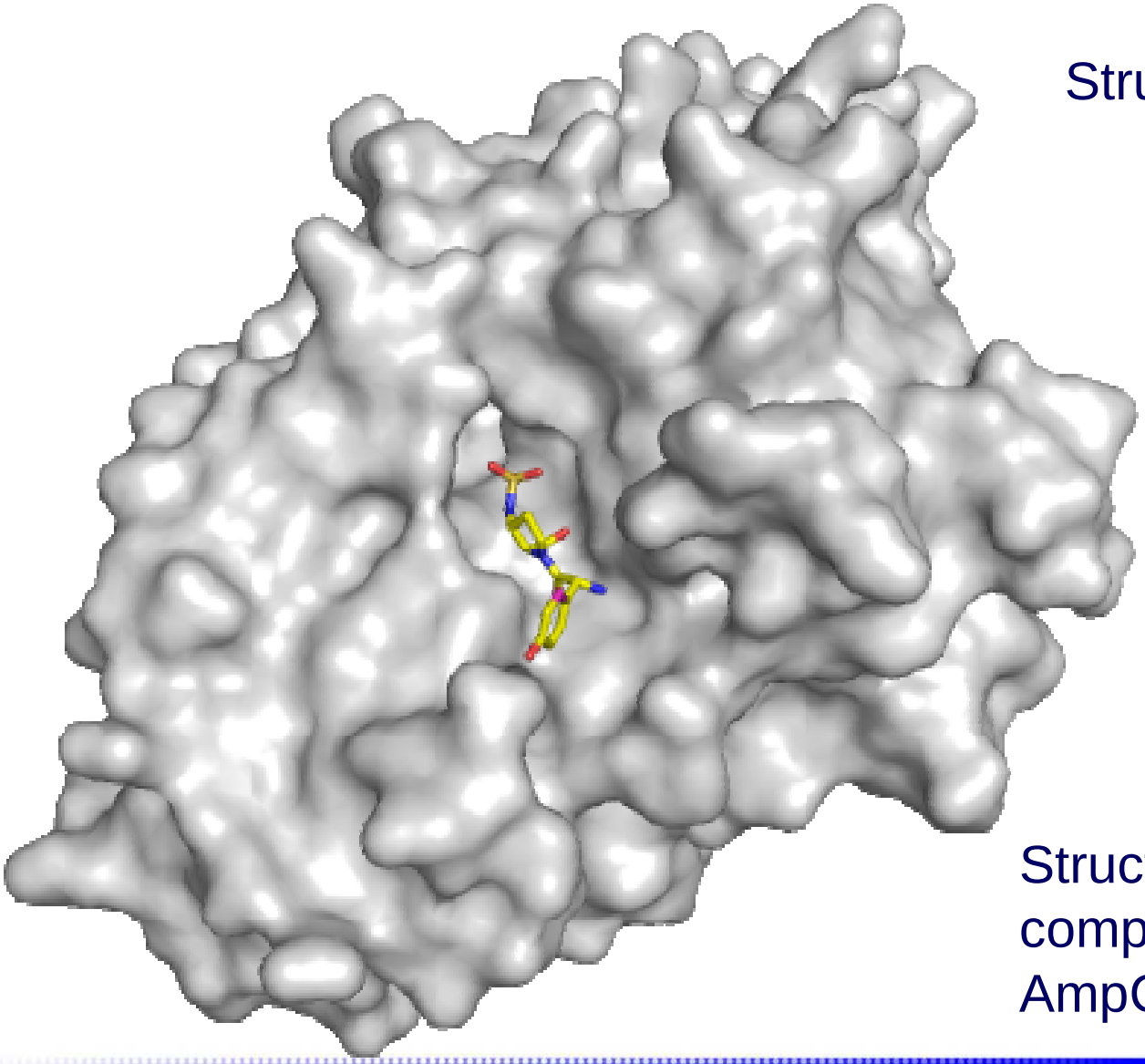


In aztreonam and cephalosporins the aminothiazolylacetamide lines up against the edge of the β-sheet and interacts with residues in it, as well as with Asn152 and Tyr221.

The interactions stabilize the acyl-enzyme forms, contributing to inhibition.

In the bridged monobactams the acetamide has a different vector and steric interference prevents binding.

Structures of Acyl-enzyme Complexes



Structures confirm expectations

- tight fit to pocket
- minimal rearrangement
- interaction with key catalytic residues

Structure of the acyl-enzyme complex of BAL with *C. freundii* AmpC β-lactamase

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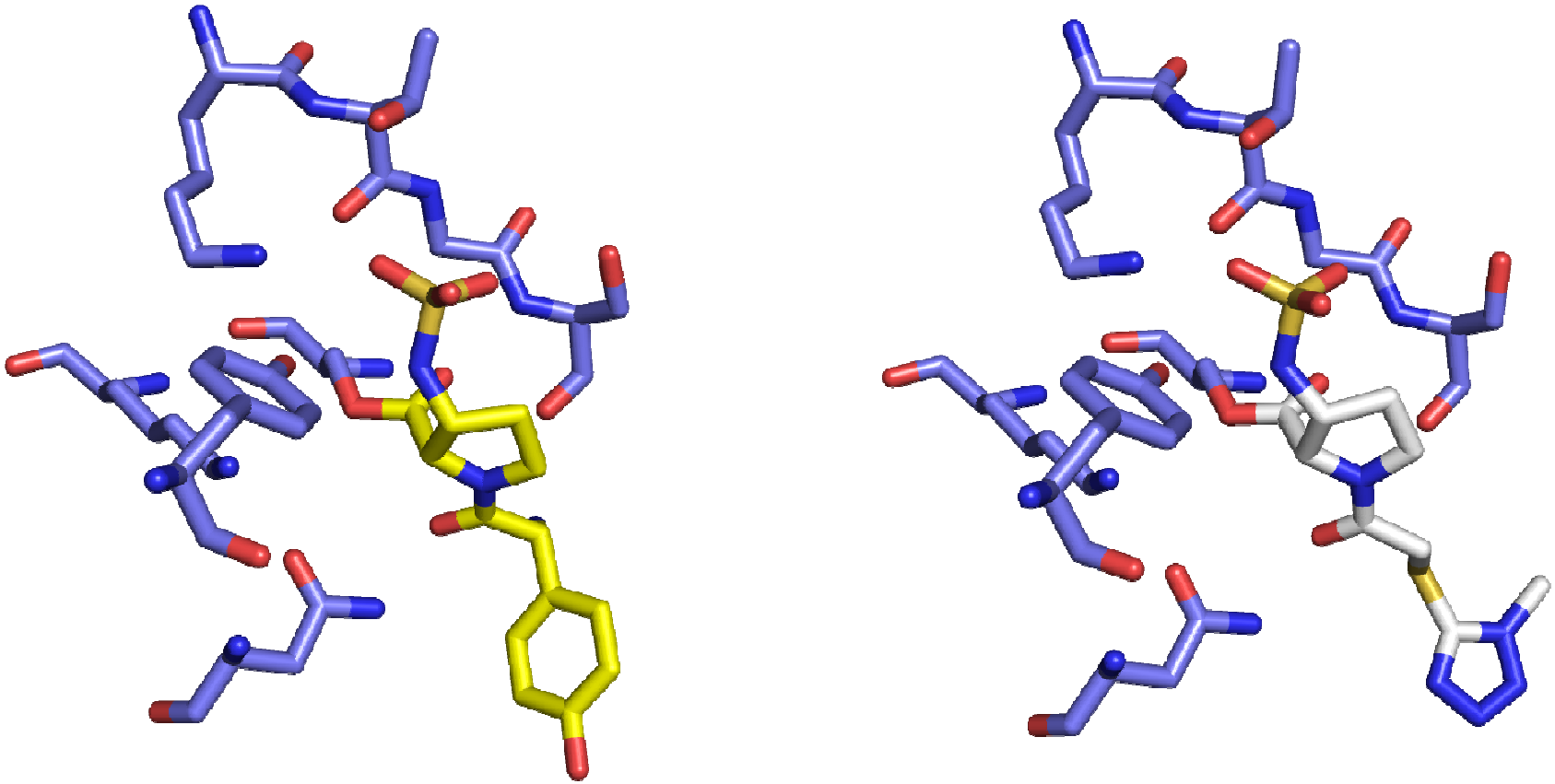
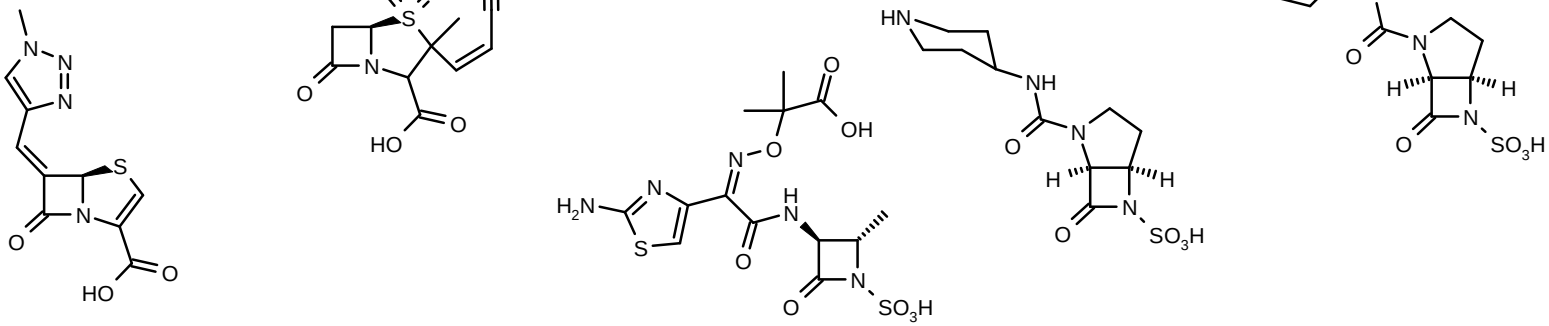
Interactions with Essential Catalytic Residues Ensure Specificity and Low Resistance Development



Permeability of *P. aeruginosa* Outer Membrane



Inhibition of *P. aeruginosa* 18S/H class C β-lactamase in cells (IC_{50}, μM)					
	BRL42715	R0 48-1220	Aztreonam	R0 48-1256	BAL29880
With intact membrane	1.1	12	18	3.9	0.062
Permeable membrane	0.025	0.4	0.28	3.9	0.056
Permeability factor	44	30	64	1	1.1

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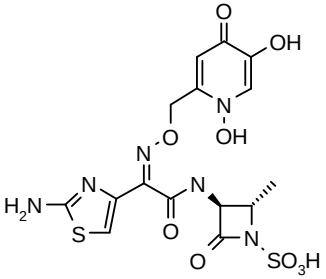
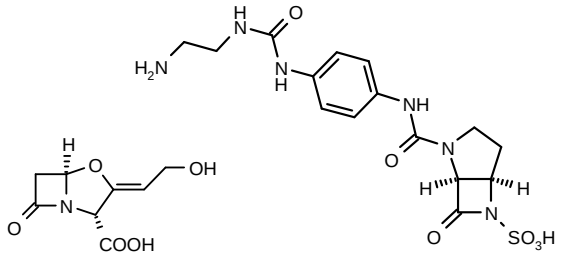
Ability to Afford Protection to a Labile Antibiotic

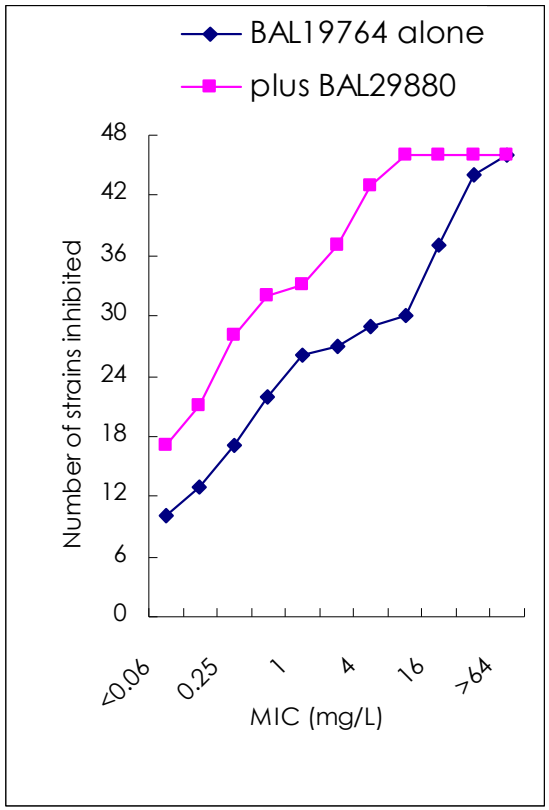
The contribution of BAL29880 to BAL30376

BAL29880 at 4 mg/L reduces the MIC of BAL19764 against strains with class C enzymes from ≥ 64 mg/L to 4 mg/L

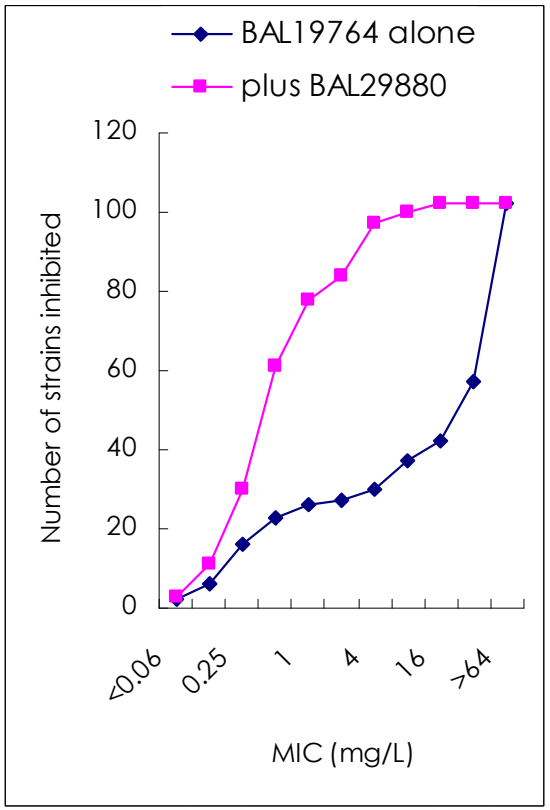




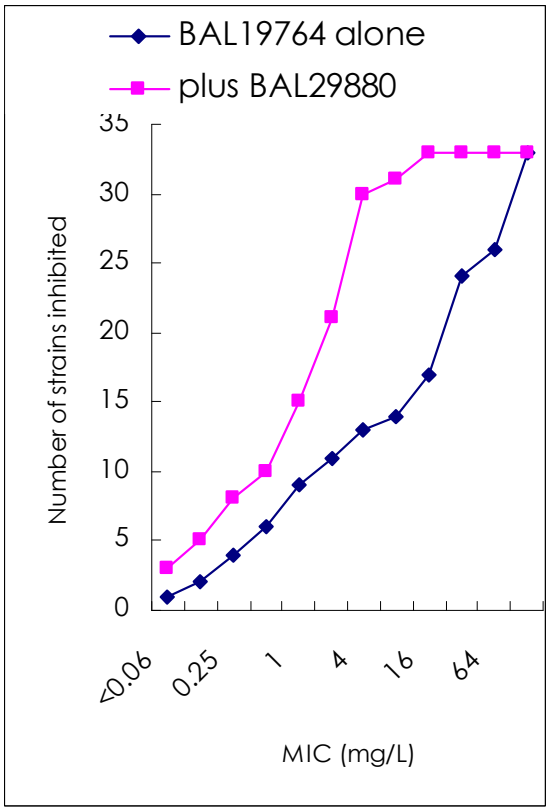
E. coli



E. cloacae



P. aeruginosa



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Activity of BAL30376 Against Organisms with Class C β-Lactamases

Species	ID	MIC (mg/L)							
		BAL	IMP	CAZ	FEP	TZP	CIP	GEN	TIG
Citrobacter freundii	8609	1	1	>32	0.5	32	<0.06	nt	4
Enterobacter aerogenes	1U595	1	0.5	>32	0.5	>32	<0.06	4	8
Enterobacter cloacae	P99	4	2	>32	2	>32	<0.06	4	4
	SMH99	4	2	8	1	16	32	>32	32
	SMH84	4	0.5	>32	1	>32	8	>32	8
Pseudomonas aeruginosa	18 S/H	4	4	>32	32	>32	1	8	nt
	M9788	4	4	>32	32	>32	16	>32	nt
	QR5	2	8	8	16	>32	16	8	nt

Activity of BAL30376 Against Carbapenemase Producers

Species	β-Lactamase	MIC (mg/L)				
		BAL	IMP	MEP	CAZ	FEP
Acinetobacter baumannii	OXA-23	2	32	>32	>32	32
	IMP-1	2	16	>32	>32	>32
Pseudomonas aeruginosa	IMP-13	1	128	128	128	64
	SPM-1	4	256	256	256	256
	GIM-1	2	>128	>128	128	16
	VIM-2	1	16	16	16	16
	VIM-4	4	>64	>16	>32	32
St. maltophilia	L1 + L2	1	>32	>32	32	32
E. cloacae	NmcA	2	128	128	256	256
Serratia marcescens	SME-1	0.25	>32	32	0.5	<0.06
	IMP-4	0.06	64	16	>128	64

Activity of BAL30376 Against "Pan-resistant" *Klebsiella pneumoniae*

Greek strains with multiple chromosomal mutations + VIM-1 + SHV-5
(V. Miriagou *et al*., *J. Antimicrob. Chemother.* 2005**)**

ID	MIC (mg/L)													
	BAL	IMP	MEP	ATM	FOX	FEP	CTX	TZP	LVX	CIP	GEN	SXT	MIN	TIG
54	0.25	>32	16	>32	>32	16	>32	>32	32	16	8	>32	32	16
55	0.13	16	8	>32	>32	16	>32	>32	>32	>32	8	>32	>32	16
848	2	32	32	>32	>32	>32	>32	>32	>32	>32	>32	>32	4	4

Activity of BAL30376 Against "Pan-resistant" Non-fermentors

Species	ID	MIC (mg/L)									
		BAL	SUL	MEP	CAZ	FEP	TZP	CIP	GEN	SXT	TIG
P. aeruginosa	1477	1	nt	>32	>32	>32	>32	>32	>32	>32	nt
	1586	1	nt	>32	>32	>32	>32	>32	>32	>32	nt
	996	0.5	nt	32	32	16	>32	>32	>32	>32	nt
A. baumannii	J11	4	>32	8	>32	>32	>32	>32	>32	nt	>32
S. maltophilia	155	1	nt	>32	32	>32	>32	nt	nt	>32	nt

In-vivo Efficacy

Strain	Inoculum (CFU)	Substance	MIC mg/L	MBC mg/L	MED (mg/kg t.i.d.)
P. aeruginosa 527	10^7	BAL30376	2	4	6.25
P. aeruginosa HPA101-1477	10^7	BAL30376	0.5	32	6.25
		Meropenem	>32	≥ 64	≥ 25
A. baumanni HPA74510	4×10^5	BAL30376	0.25	1	≤ 1.6
A. baumanni J2	10^3	BAL30376	2	16	3.1
E. cloacae R947	10^5	BAL30376	1	2	0.8
E. cloacae S9615	10^7	BAL30376	0.5	4	0.8

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