New Insights into Peptidoglycan Biosynthesis

Louis B. Rice
Warren Alpert Medical School of Brown University
Providence, RI

Outline of Presentation

- Review role of penicillin-binding proteins in cell wall synthesis, antibiotic susceptibility and resistance
- Discuss the role of auxiliary proteins in cell wall synthesis
- Point out where simple models and ideas, though appealing, break down
- Discuss the interplay between cell wall synthesis proteins and the bacterial membrane
- Review new targets and some compounds in development

Cell Wall Synthesis

- Bacterial peptidoglycan synthesis and remodeling are accomplished through the activities of transpeptidases, transglycosylases and carboxypeptidases referred to as penicillin-binding proteins (PBPs)
- Cell wall maintenance during stationary phase also involves PBPs and cell wall hydrolases

Proc. Nat. Acad. Sci. USA
Vol. 69, No. 12, pp. 3751–3755, December 1972

Isolation by Covalent Affinity Chromatography of the Penicillin-Binding Components from Membranes of *Bacillus subtilis*

(D-alanine carboxypeptidase/transpeptidase/detergent solubilization)

PETER M. BLUMBERG AND JACK L. STROMINGER

Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Contributed by Jack L. Strominger, October 13, 1972

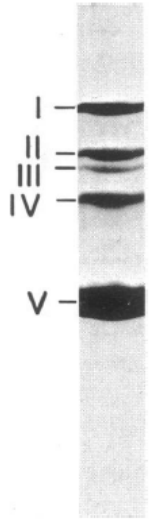


FIG. 1. SDS gel of the isolated penicillin-binding components stained with Coomassie brilliant blue. The components are identified by Roman numerals.

Penicillin-Binding Protein Classes

- Class A - an N-terminal transglycosylase domain and a C-terminal transpeptidase domain
- Class B - a C-terminal transpeptidase domain and an N-terminal morphogenic domain that does not possess transglycosylase function
- Carboxypeptidases

Penicillin-Binding Proteins

- Recognized early on that some (*E. coli* 1-3) were essential while others(4-6) were not
- Differential binding correlated with activity and with effect (filaments vs. round cells)
- Antibiotic development sought to inhibit a broader range of Pbps (*Pseudomonas*)
- In most, but not all, cases, inhibition was associated with bacterial cell death

Enterococcal Tolerance

- Characteristic whereby the antibiotic concentration required to kill the bacterium is much greater than that required to inhibit it
- Important in treatment of endocarditis
- Synergistic bactericidal activity achievable by combining cell wall active agent with an aminoglycoside

Synergism vs. Enterococci

Moellering and Weinberg J. Clin. Invest. 1971 50: 2580-4

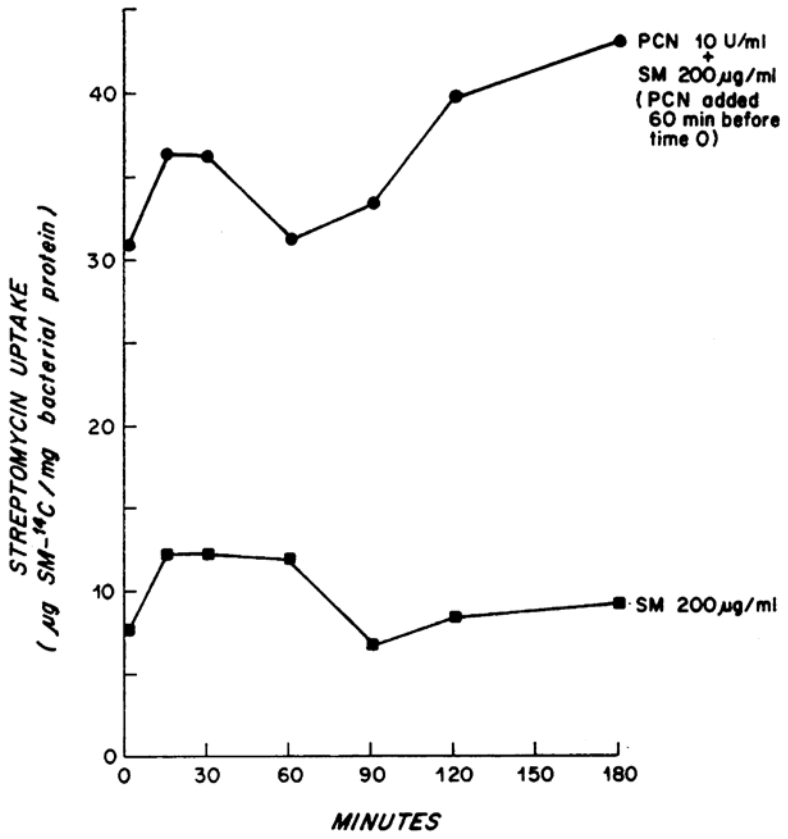


FIGURE 2 Effect of pretreatment with penicillin (PCN) on uptake of streptomycin-^{14}C (SM) by *S. faecalis* strain EI.

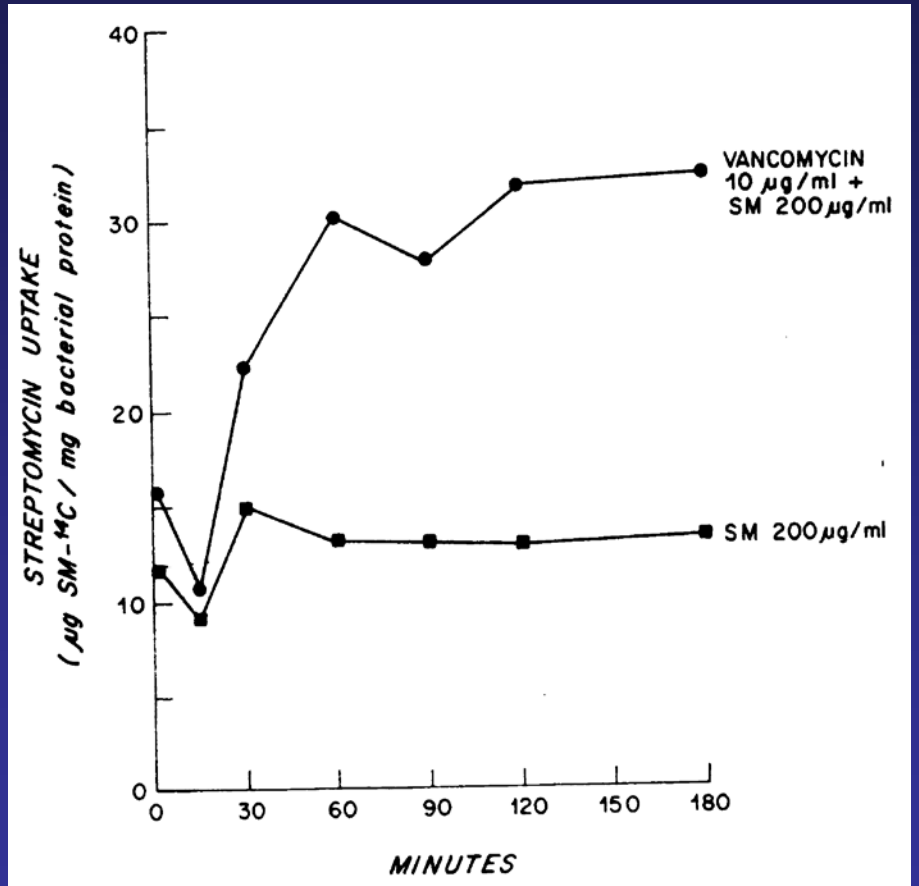
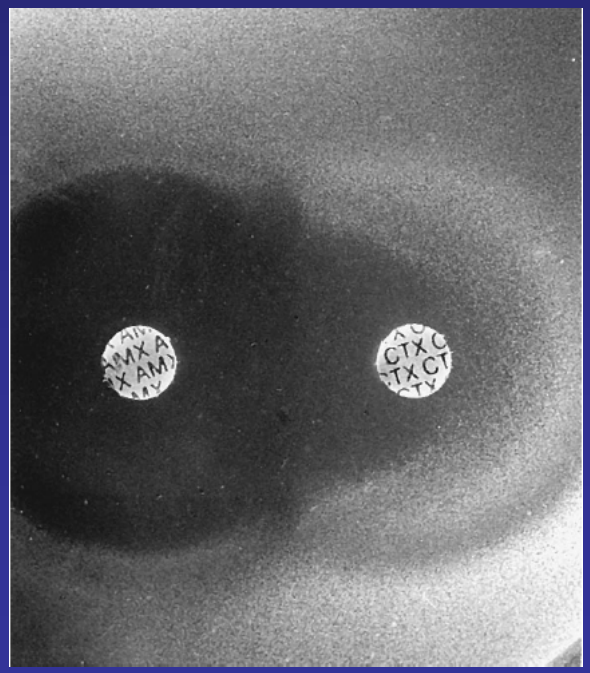


FIGURE 5 Effect of vancomycin on uptake of streptomycin-^{14}C (SM) by *S. faecalis* strain EI.

Ampicillin Plus Ceftriaxone Is as Effective as Ampicillin Plus Gentamicin for Treating *Enterococcus faecalis* Infective Endocarditis

Nuria Fernández-Hidalgo,[1] Benito Almirante,[1] Joan Gavaldà,[1] Mercè Gurgui,[2] Carmen Peña,[3] Arístides de Alarcón,[4] Josefa Ruiz,[5] Isidre Vilacosta,[6] Miguel Montejo,[7] Nuria Vallejo,[8] Francisco López-Medrano,[9] Antonio Plata,[10] Javier López,[11] Carmen Hidalgo-Tenorio,[12] Juan Gálvez,[13] Carmen Sáez,[14] José Manuel Lomas,[15] Marco Falcone,[18] Javier de la Torre,[16] Xavier Martínez-Lacasa,[17] and Albert Pahissa[1]



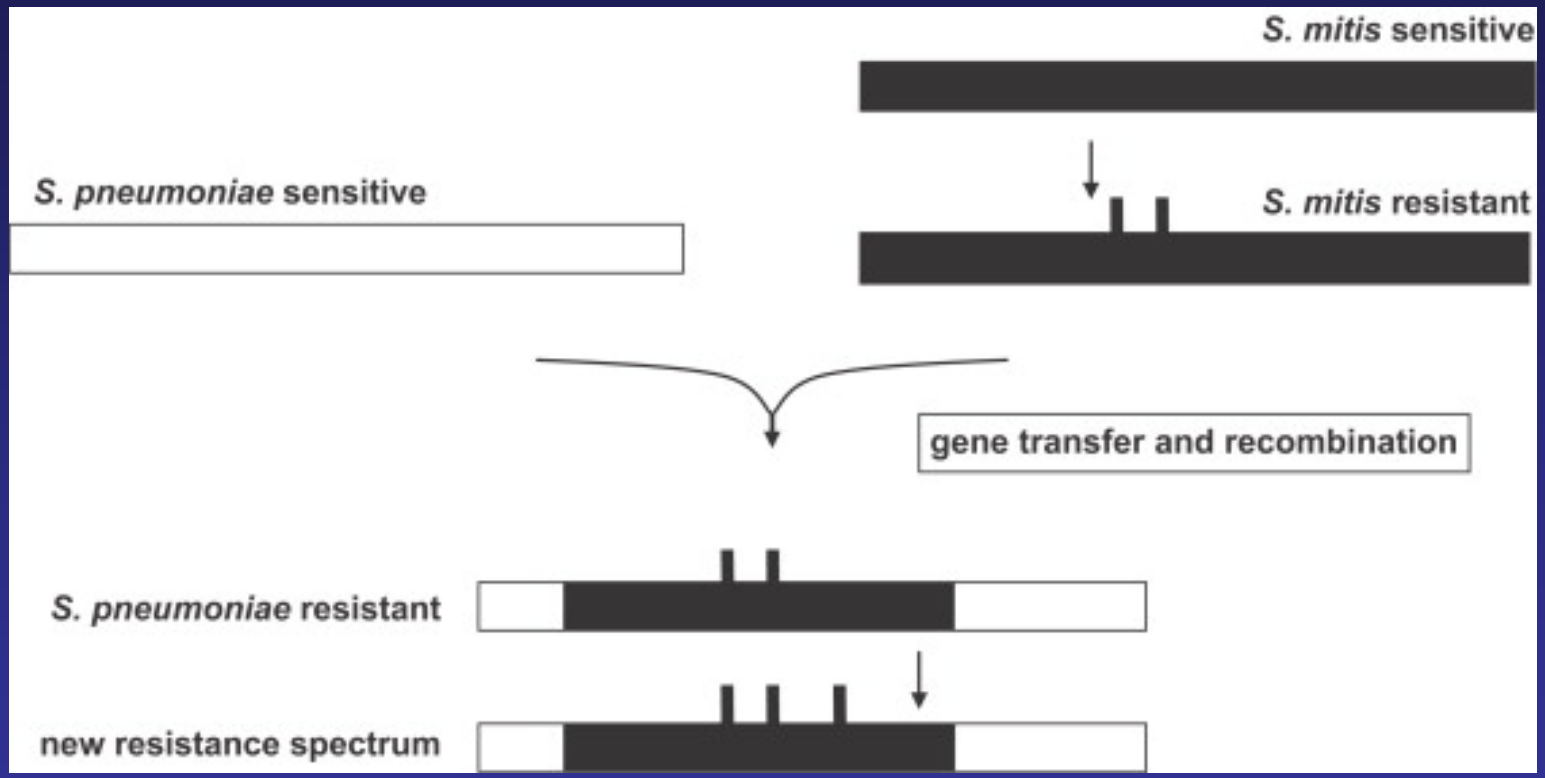
Clinical Infectious Diseases (2013) 56: 1261

Mainardi, et al (1995) *AAC* 39: 1984

Clinical use of antibiotics was associated with the emergence of strains resistant because of changes in Pbps

(Almost exclusively a Gram-positive phenomenon)

Mosaic Pbps



Chi, et al (2007) Int. J. Med. Microbiol. 297: 503

E. faecium PBP5 Mutations and Resistance

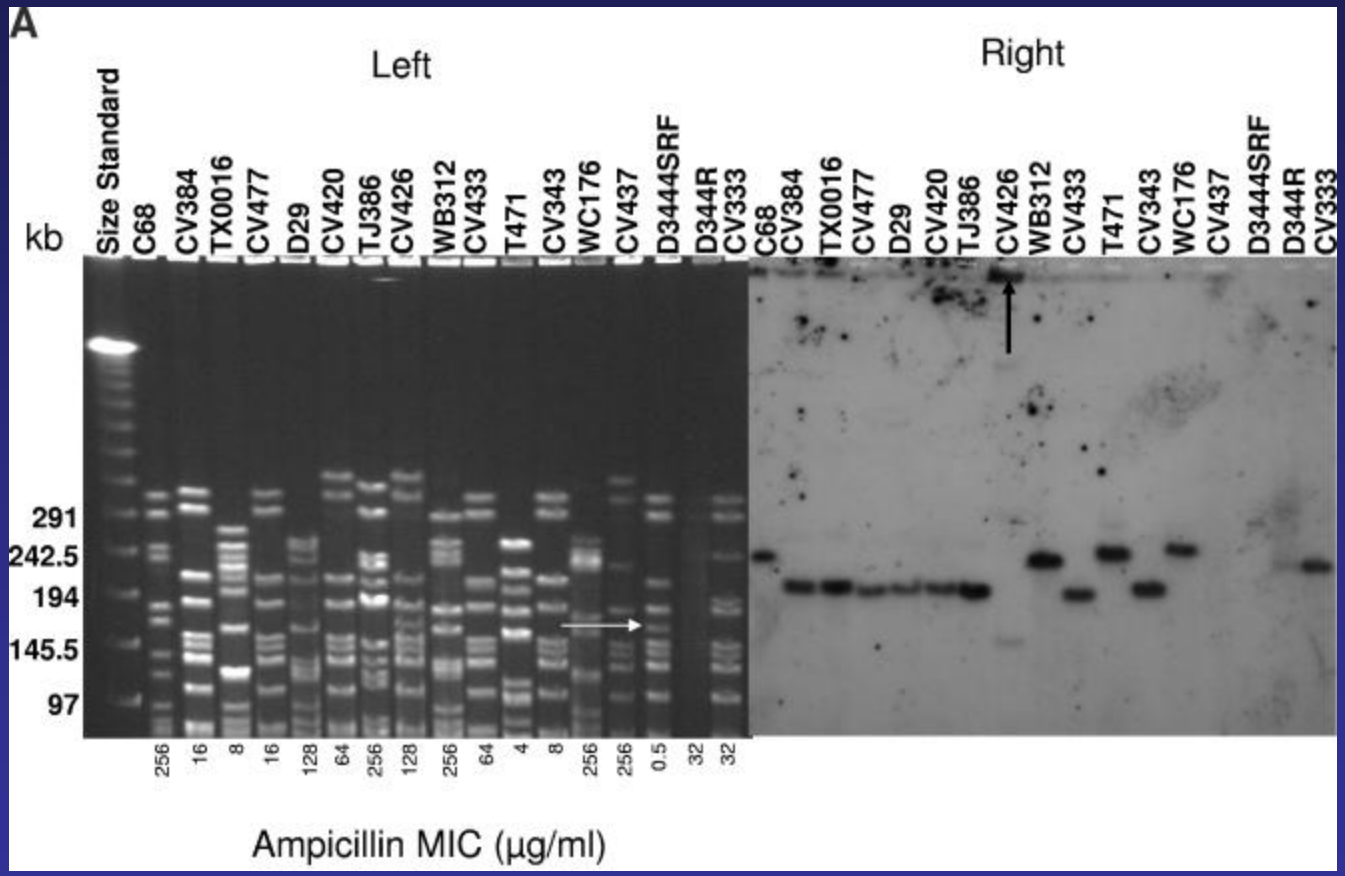
Plasmid	Amino acid at position within PBP5				Penicillin (µg/ml)								S_{50} (µgml)
	485	499	629	Ser 466'	256	128	64	32	16	8	4	2	
pCWR624	M	I	E	-									8
pCWR633		T		-									12
pCWR634			V	-									11
pCWR662			V	+									16
pCWR661		T		+									16
pCWR651				+									16
pCWR635	T			-									40
pCWR697	A			-									64
pCWR663	T			+									64
pCWR698	A			+									100
pCWR666	A	T	V	+									128

What about *S. pyogenes*?

Rice, et al (2004) *AAC*

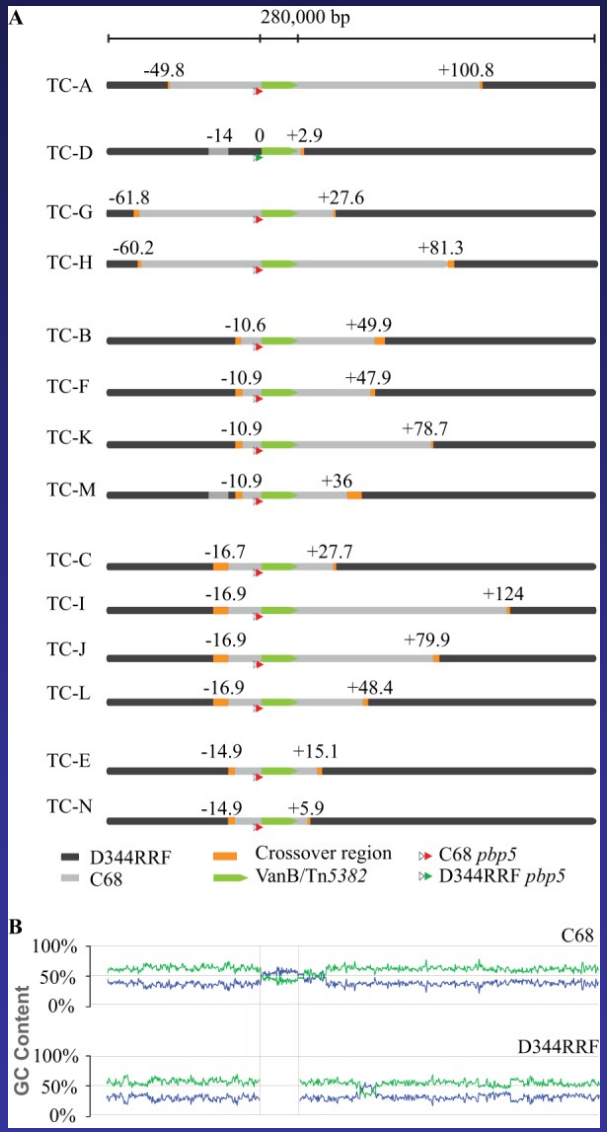
Some shared the wealth

Transferability of *pbp5*



Rice, et al (2005) *AAC*: 5007-12

Transferable Genomic DNA – *E. faecium*

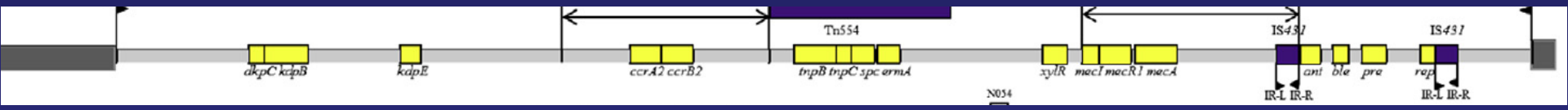


Garcia-Solache (2016) AAC

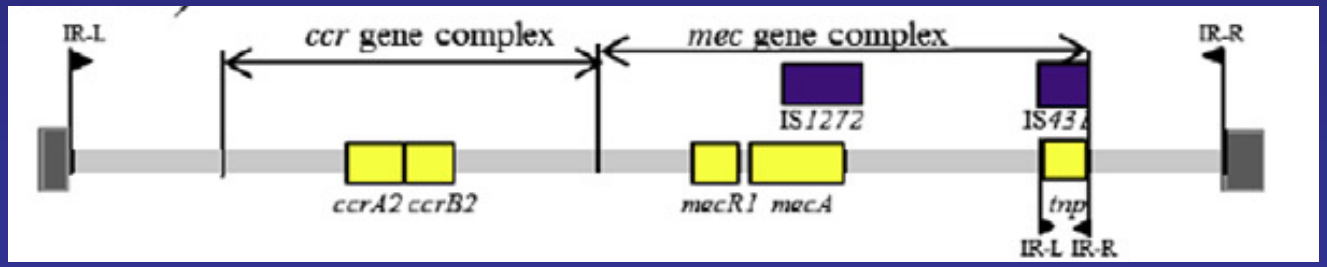
Others acquired necessary genes from other species

Acquired Pbps (Pbp2a)

SCCmec Type II

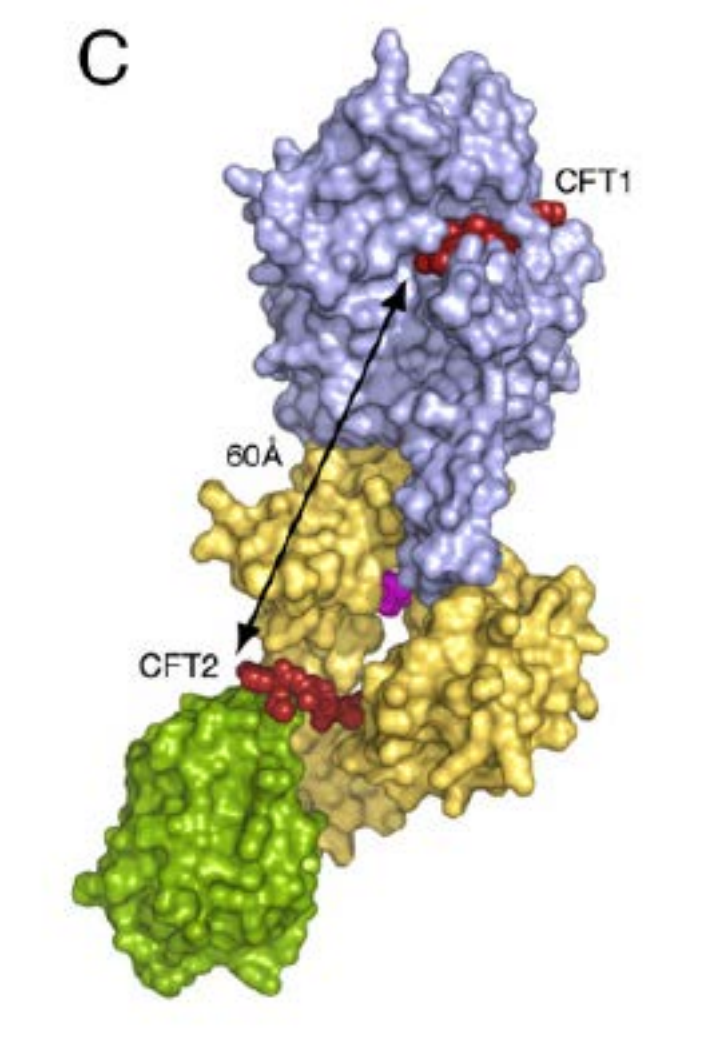


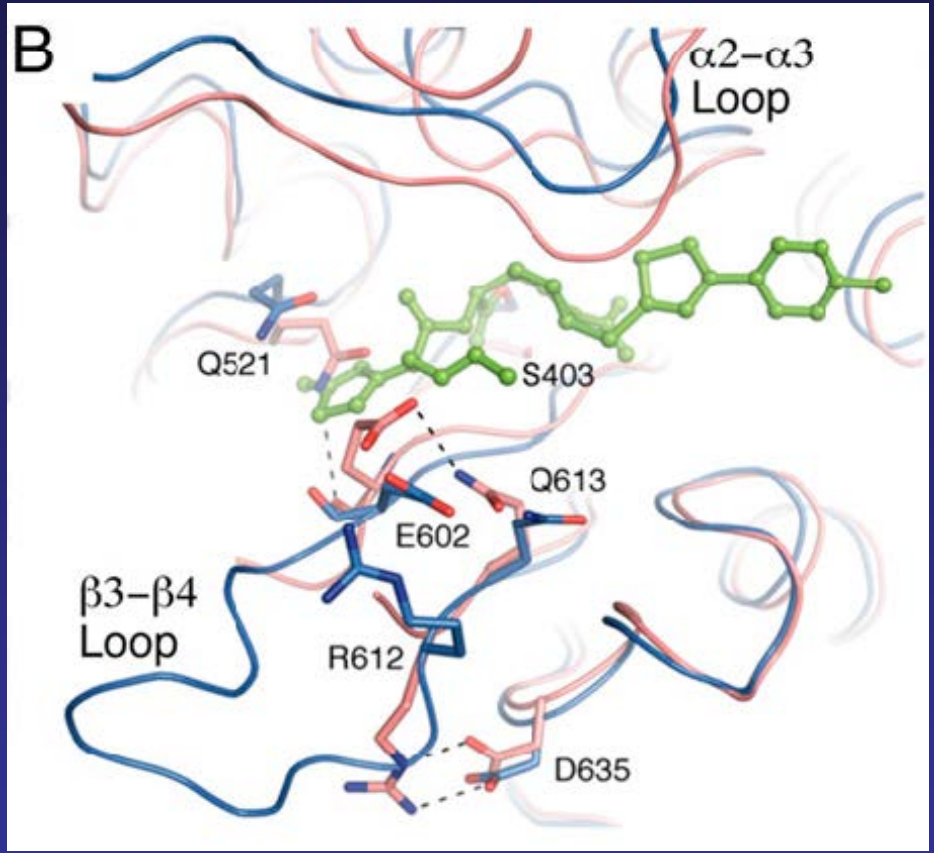
SCCmec Type IVb



Liu, et al (2016) Microb Pathogen 101:56

Allostery and Ceftaroline Activity

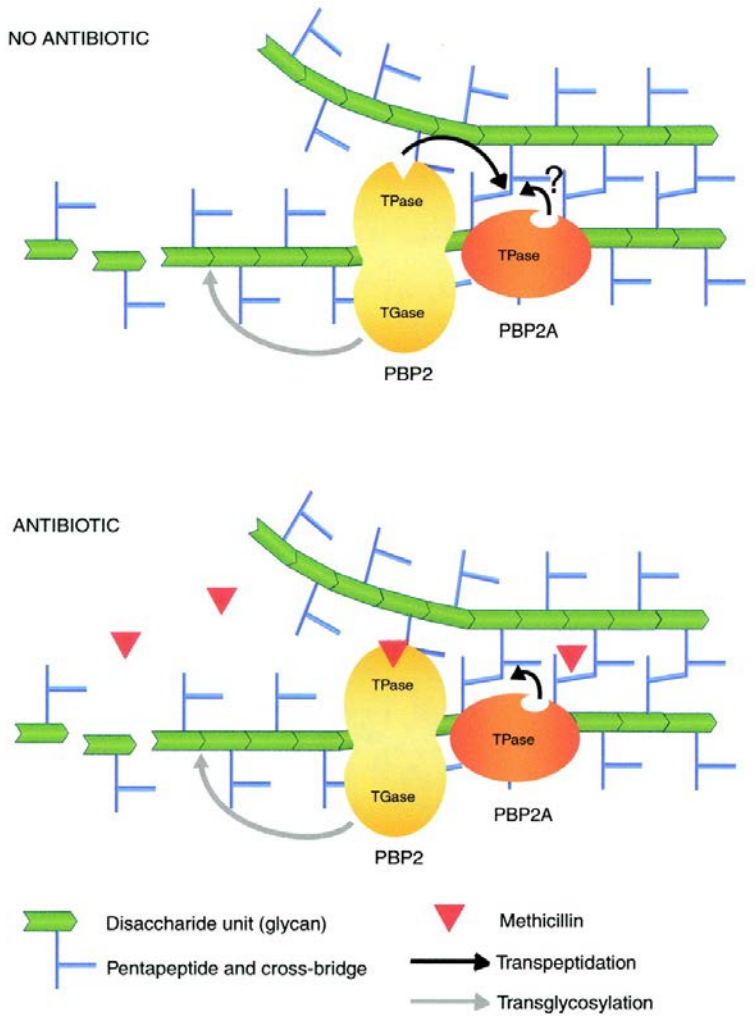




Otero, et al (2013)
PNAS 110: 16808

Pbp2a , as it turned out, needs a lot of help. It seemed almost petulant in its fussiness!

Model for the cooperative functioning of the TGase domain of PBP2 and the TPase activity of PBP2A in methicillin-resistant S. aureus



Pinho M. G. et.al. PNAS 2001;98:10886-10891

fem Factors

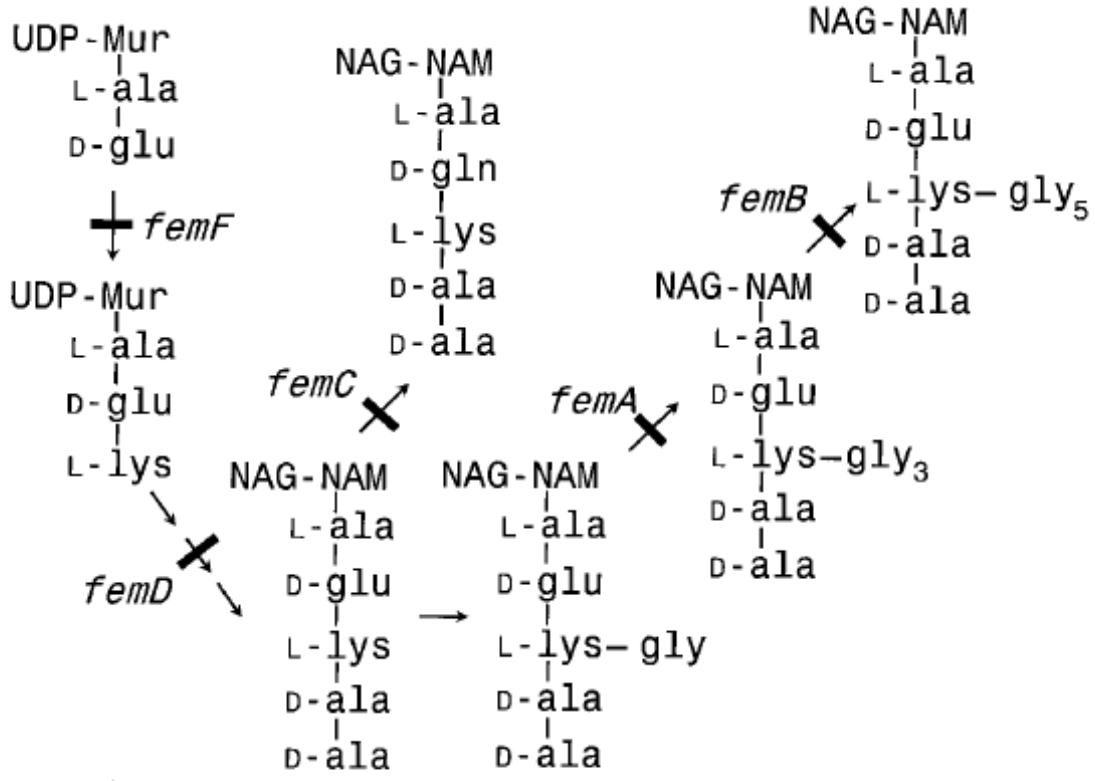


FIG. 3. Sites of peptidoglycan precursor synthesis at which blocks occur in *fem* mutants. UDP-Mur indicates uridine diphosphomuramyl peptide precursor; NAG-NAM, *N*-acetylglucosamine-*N*-acetylmuramic acid disaccharide.

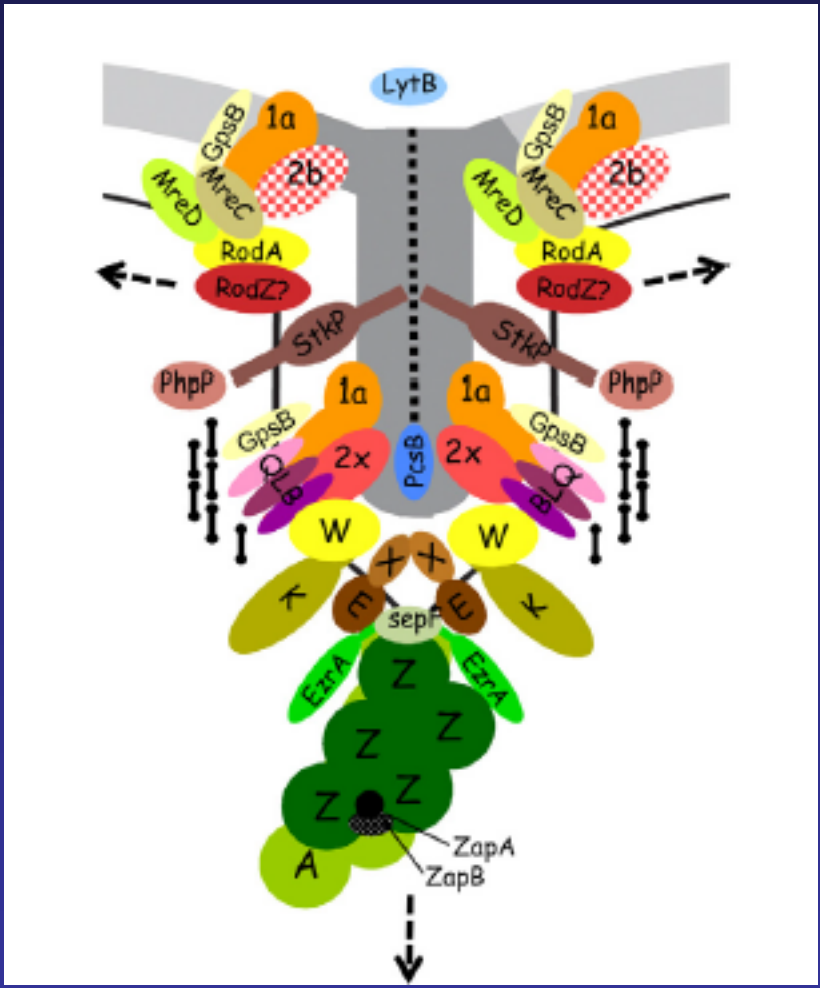
Chambers, HF CMR (1997) 10: 781

Pbp2a is not finicky outside of *S. aureus*

- The *mecA* gene of *S. aureus* conferred resistance to ceftriaxone in *E. faecalis* JH2-2Δ*pbp5* and *E. faecium* D344S … Because the D,D-transpeptidases are the essential target of β-lactams, PBP2a acted as a surrogate of the host D,D-transpeptidases and therefore catalyzed peptidoglycan cross-linking. This implies a low substrate specificity of PBP2a, because the amino group of the acceptor participating in the transpeptidation reaction was located on side chains consisting of five Gly, L-Ala-L-Ala, and D-Asx in *S. aureus*, *E. faecalis*, and *E. faecium*, respectively. These observations establish for the first time that *mecA* of *S. aureus* can confer β-lactam resistance in distantly related hosts belonging to the genus *Enterococcus*, despite substantial diversity in the structure of peptidoglycan precursors.

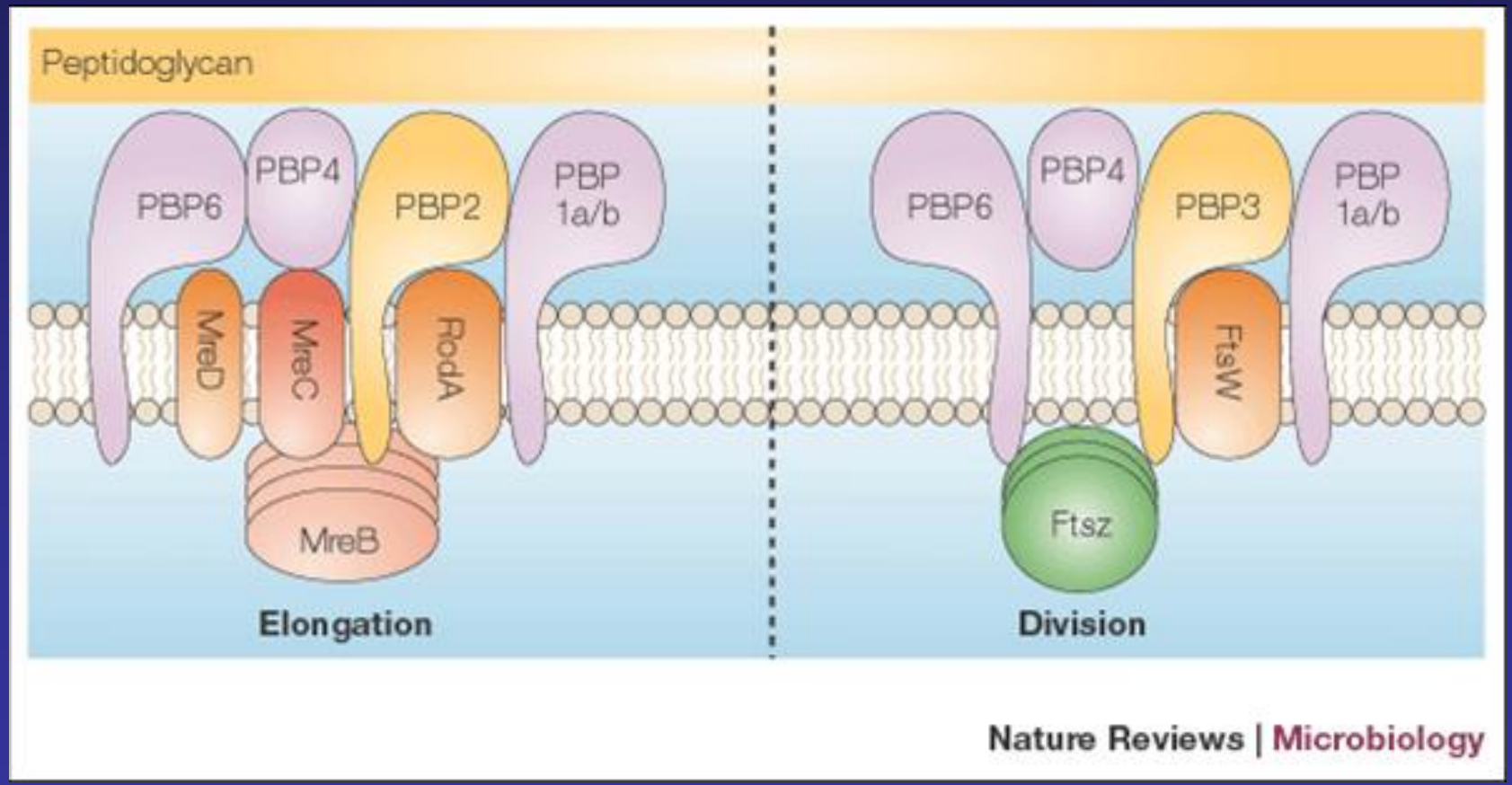
Arbeloa, et al (2004) J. Biol. Chem. 279: 41546

S. pneumo PG synthesis machinery



Massidda, et al (2013) Env. Microbiol. 15: 3133

Peptidoglycan synthesis is a coordinated process involving several enzymes integrated into bacterial cytoplasmic membrane



Daptomycin-Ceftaroline synergism vs. daptomycin non-susceptible MRSA and *E. faecium*

Rose, et al (2012) *AAC* 56: 5296
Sakoulas, et al (2014) *AAC* 58: 1494

Dapto^{r} and membrane fluidity

Author	Species			
		Membrane fluidity		PL Content
		s	r	
Mishra	*S. aureus*	Dec	Inc	2/3 sets L-PG>PG
Kang	*S. aureus (Clinical strains)*	Same	Same	3/3 L-PG> in R
Mishra	*E. faecalis* and *E. faecium*	Inc	Dec	No diff. in faecalis; dec U FA in R in Faecium
Jones	*S. aureus*	Dec	Inc	Dec neg chg PG in R strains

Mishra, et al (2014) PLOS One; Kang, et al (2017) J Microbiol 55:153
Mishra, et al (2012) PLOS One; Jones, et al (2008) *AAC* 52: 269

E. faecium LiaFSR.

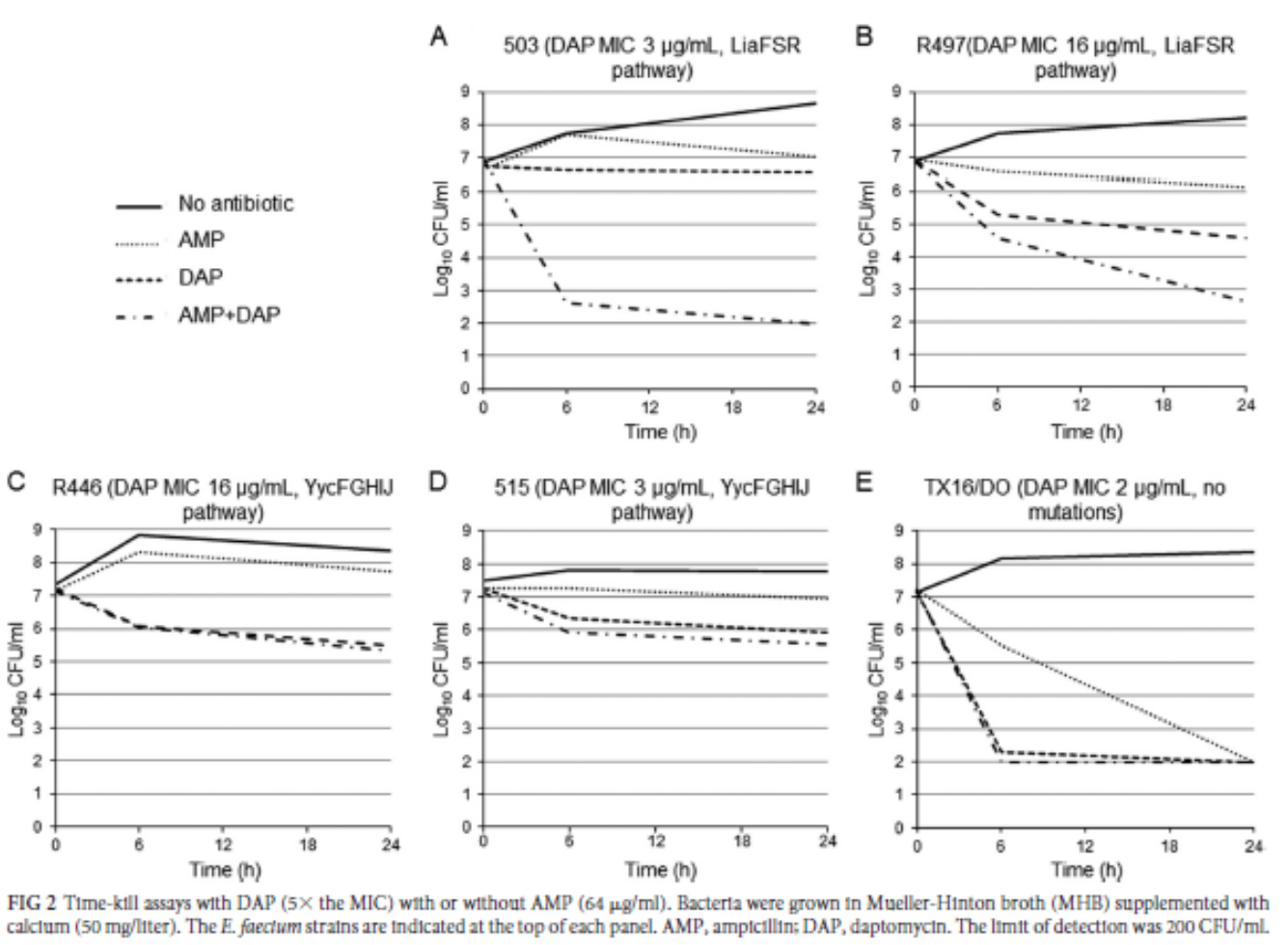


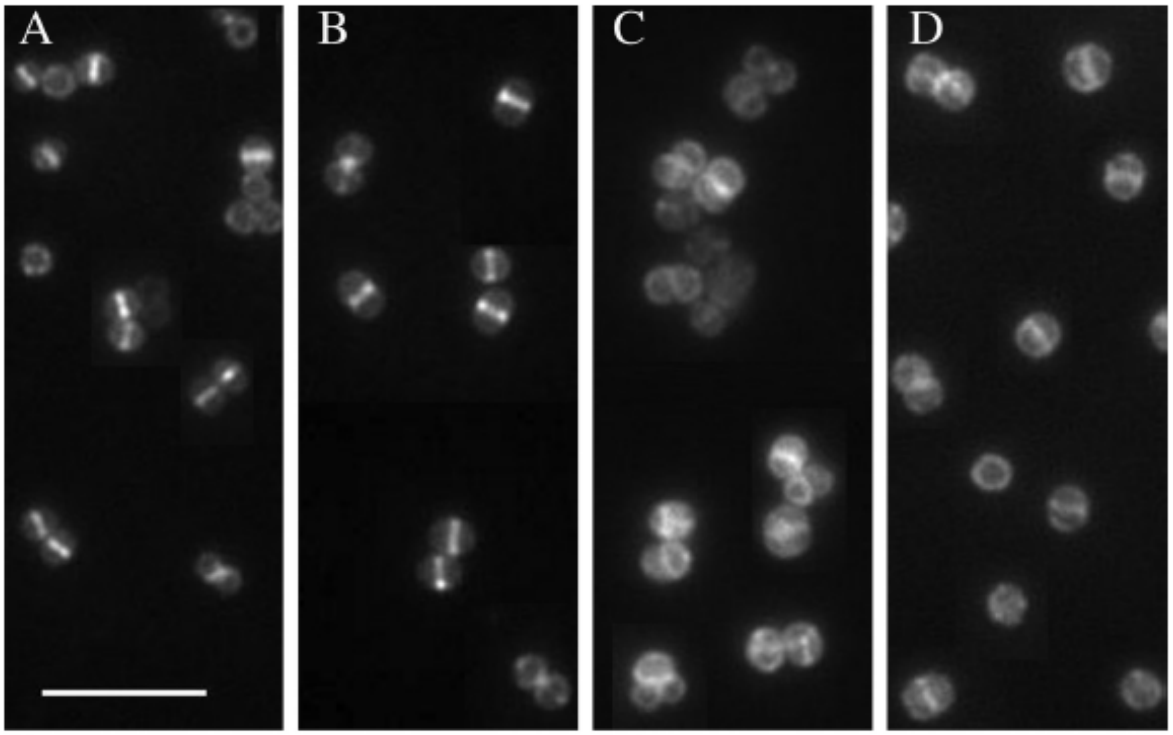
FIG 2 Time-kill assays with DAP (5× the MIC) with or without AMP (64 µg/ml). Bacteria were grown in Mueller-Hinton broth (MHB) supplemented with calcium (50 mg/liter). The *E. faecium* strains are indicated at the top of each panel. AMP, ampicillin; DAP, daptomycin. The limit of detection was 200 CFU/ml.

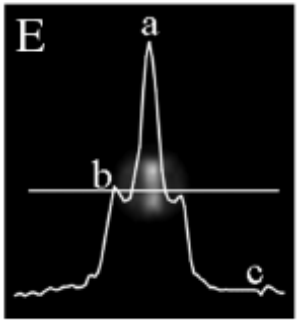
LiaFSR is a 3-component regulatory pathway involved in cell membrane stress response.

- MICs>3 µg/ml associated with decreased daptomycin membrane binding
- Amp/Dapto synergism seen only in strains with LiaFSR mutations

Diaz, et al (2014) *AAC* 58: 4527

Epicatechin Gallate induced changes in membrane fluidity and Pbp2 positioning

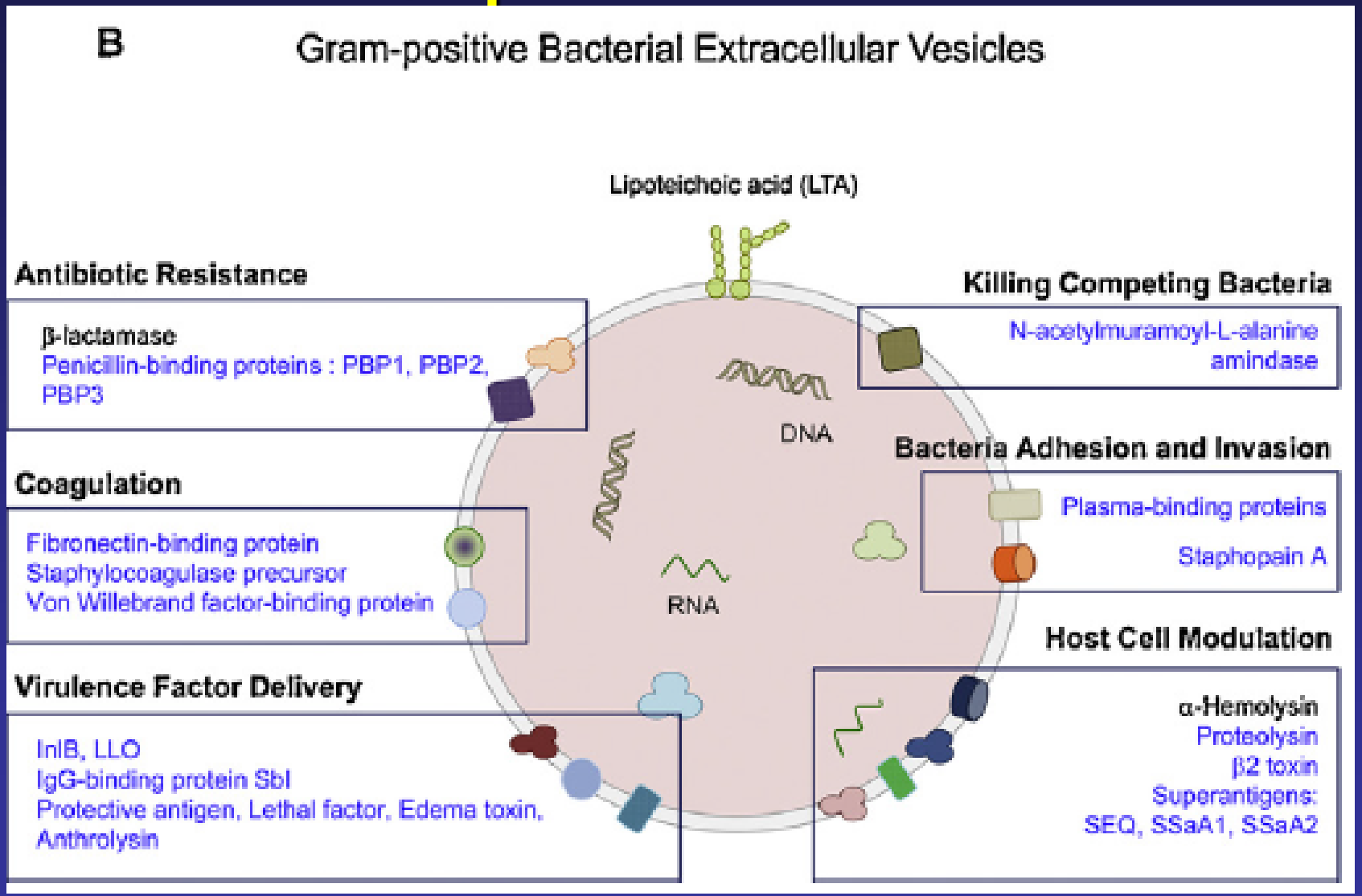




Compound	Ratio	Delocalization of PBP2
Ø	2.91±0.06	No
Oxa	2.77±0.04	No
ECg	1.84±0.02	Yes
Oxa + ECg	1.54±0.02	Yes

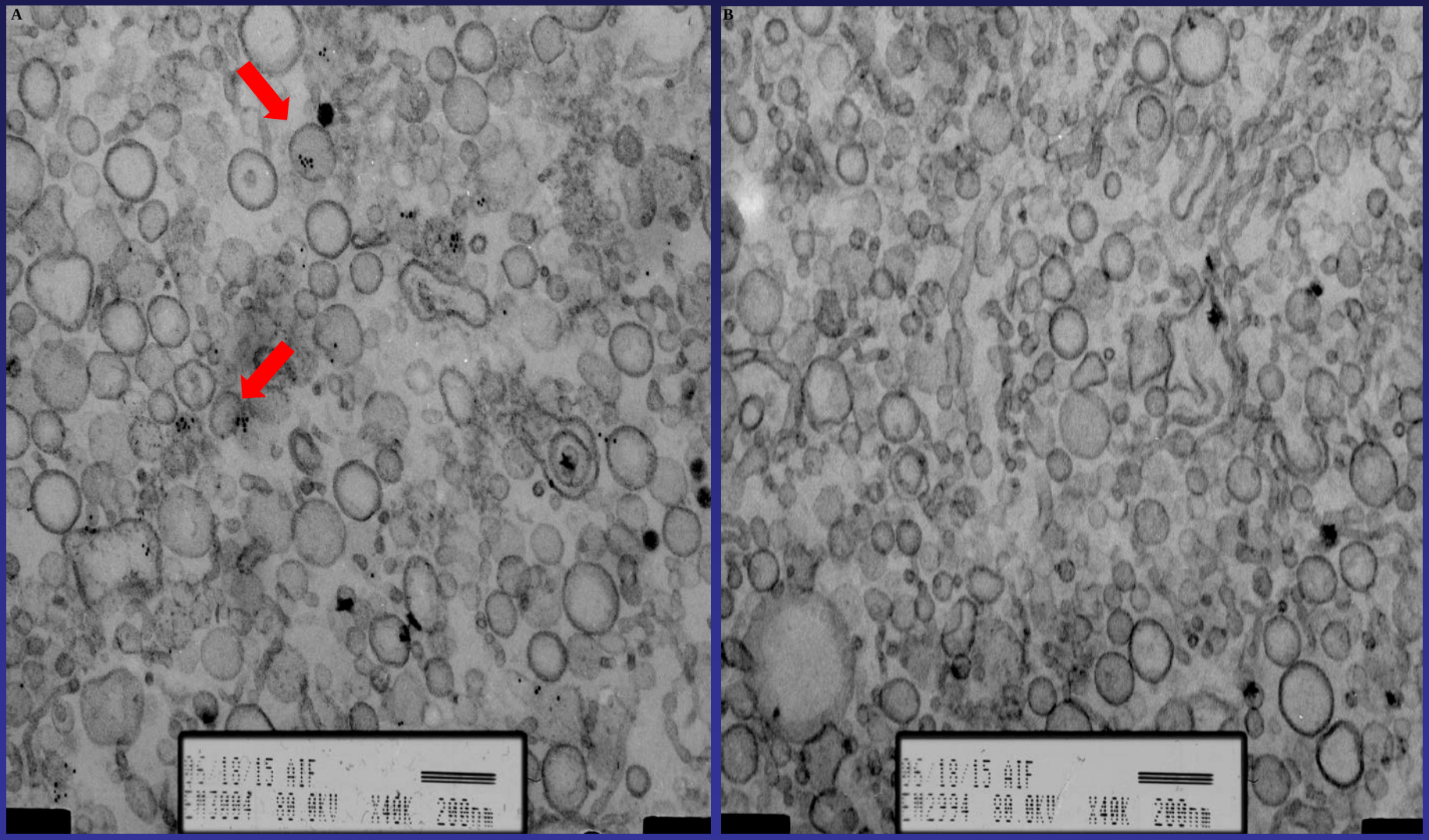
Bernal, et al JBC 285: 24055

Gram-positive vesicles



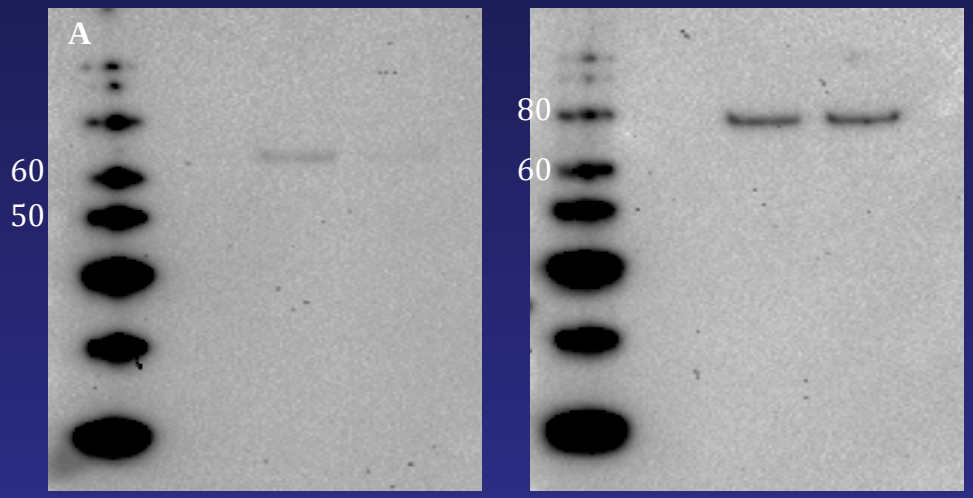
Kim, et al (2015) Se, Cell Dev Biol 10: 97

Microvesicles and Pbp5



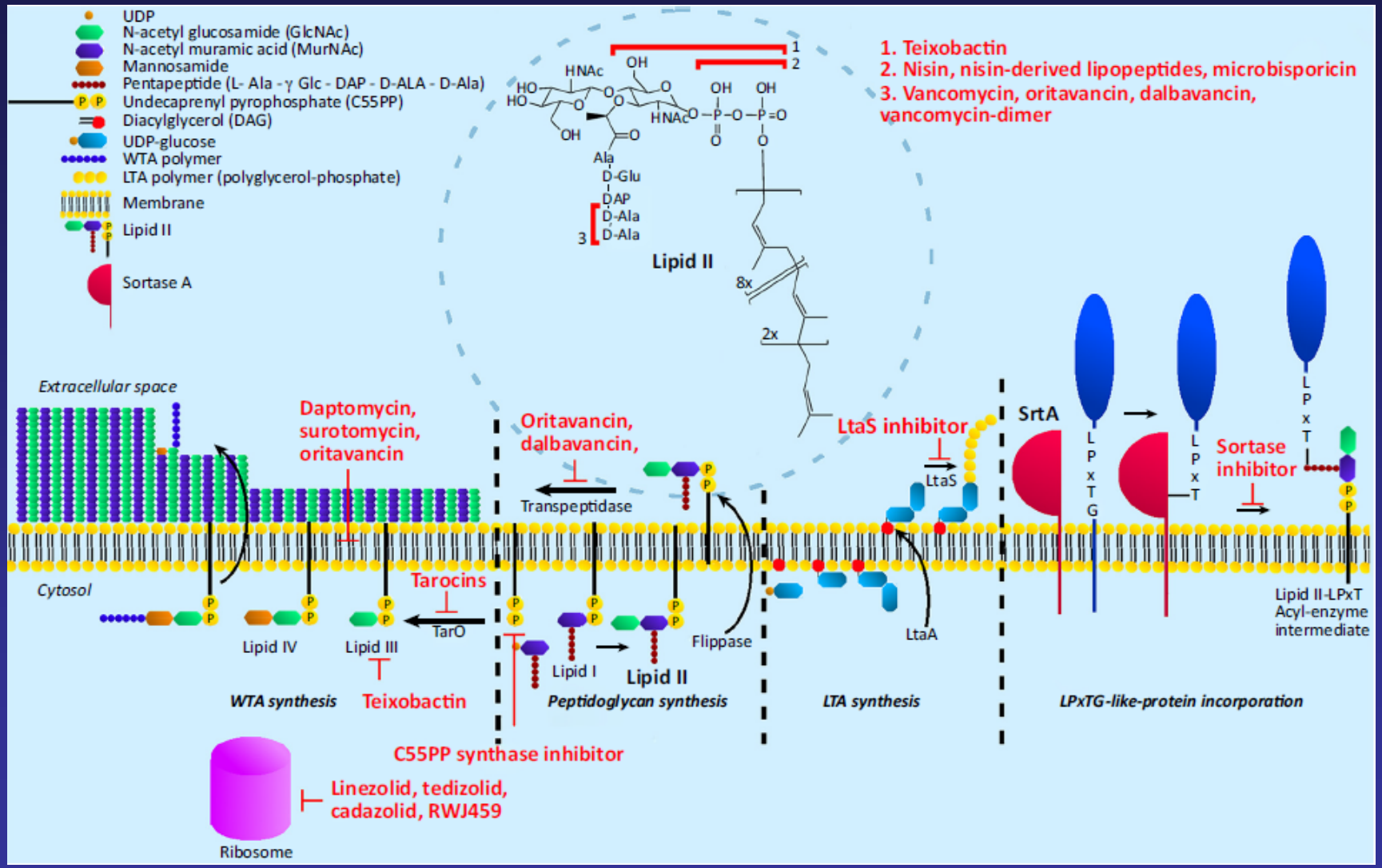
Immuno EM. C68-derived vesicles. A) Rabbit anti-Pbp5. B) Rabbit anti isotype. Red arrows show stain.

E. faecium Microvesicle Content



Western blot of vesicles isolated from clinical *E. faecium* strain C68. Lane 1: Size standard (KDa); lane 2: empty, lane 3: C68 MV, lane 4: C68 + PenG EMB. A) Rabbit anti-P_5AP. B) Rabbit anti-Pbp5

Newer Enterococcal Targets



Van Harten and colleagues (2017) Trends Microbiol 25: 467

Conclusions

- Bacterial cell wall synthesis occurs through the coordinated actions of several different proteins
- PBPs are the most prominent and the ones for whom we have lethal inhibitors
- Understanding the interactions of the different proteins with each other and with the membrane to which many of them are attached will give us a deeper understanding of microbial physiology and may identify new lethal targets