Teixobactin-
Entdeckung und Wirkungsmechanismus

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NOVOBIOTIC PHARMACEUTICALS

DZIF

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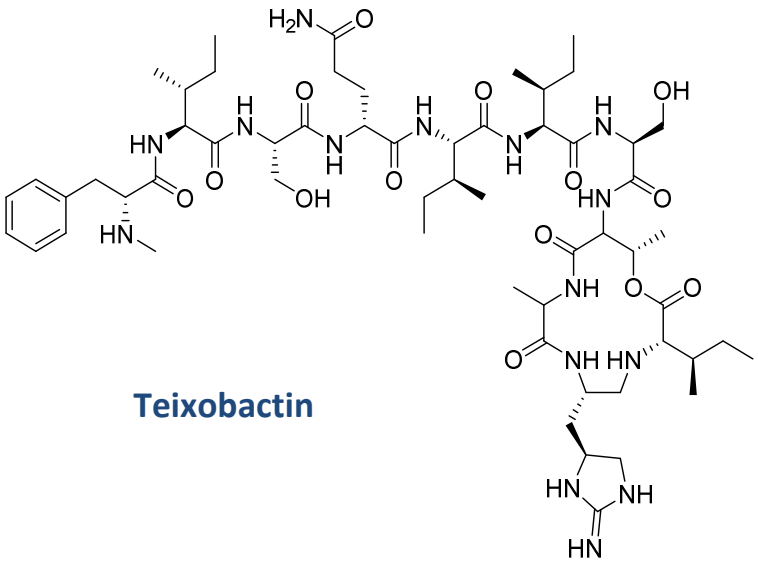
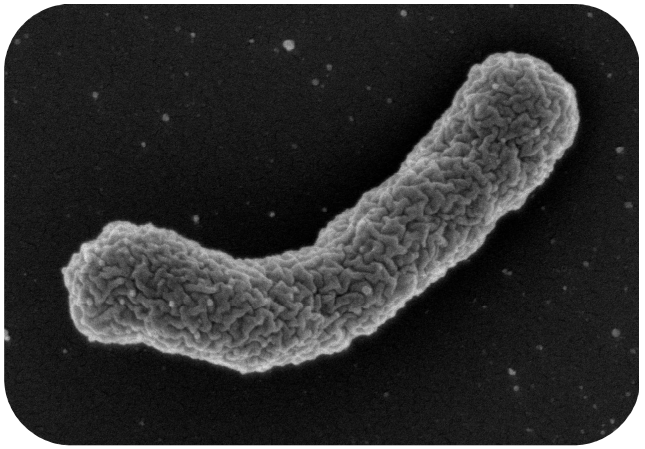
Teixobactin

- 11 amino acid depsipeptide
- enduracididine, methylphenylalanine, D-amino acids
- MW: 1.242 Da



Teixobactin

- new β-proteobacterium: *Eleftheria terrae*
- Gram-negative, related to Aquabacteria
- isolated from a grassy field in Maine



Eleftheria terrae

(William Fowley, Norteastern University)

DZIF Northeastern University NOVOBIOTIC PHARMACEUTICALS Ling & Schneider *et al.*, Nature, 2015 bonn

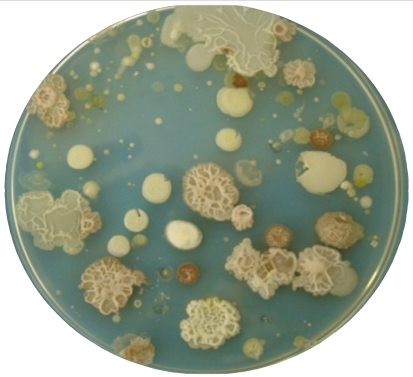
Growing the „uncultured“ – *„Great plate count anomaly“*

- most antibiotics are produced by environmental mircoorganisms

  → only 1% of all microbial species grow on nutrient media

  ⟶ ~ 99% constitute so far „uncultured bacteria“
  ⟶ „dark matter“: promising source for new antibiotics

(Stewart, 2012; Hongoh & Toyoda, 2001, Lewis *et al.*, 2010)

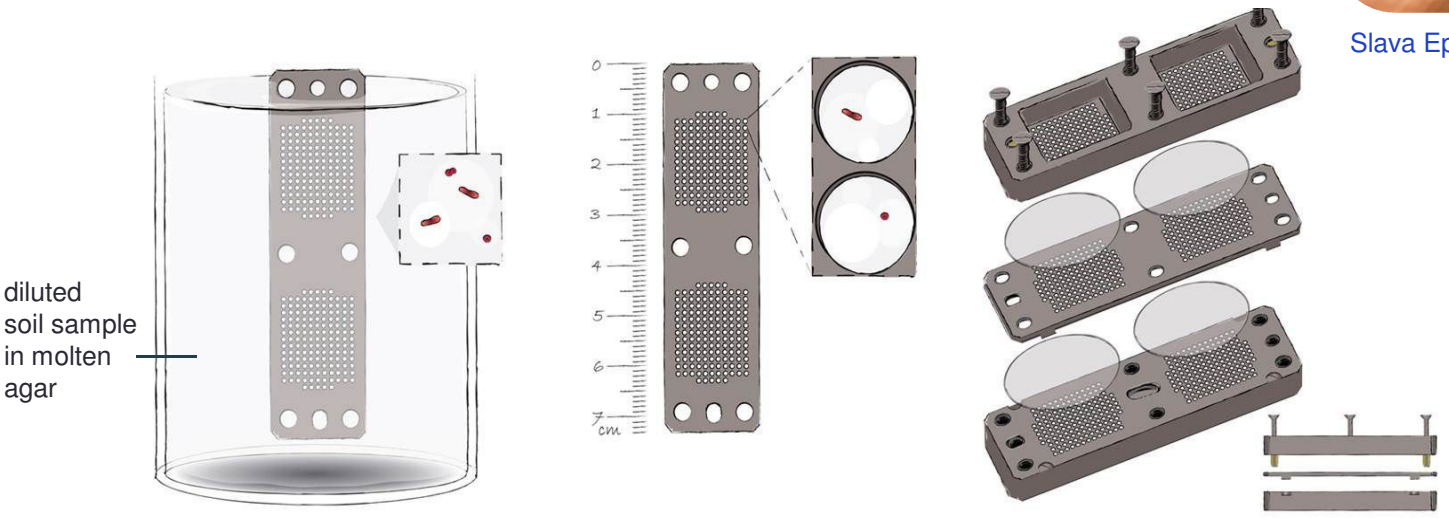


iChip

- grow and isolate „uncultured“ bacteria in one step in their natural environment

- high-throughput microbial cultivation

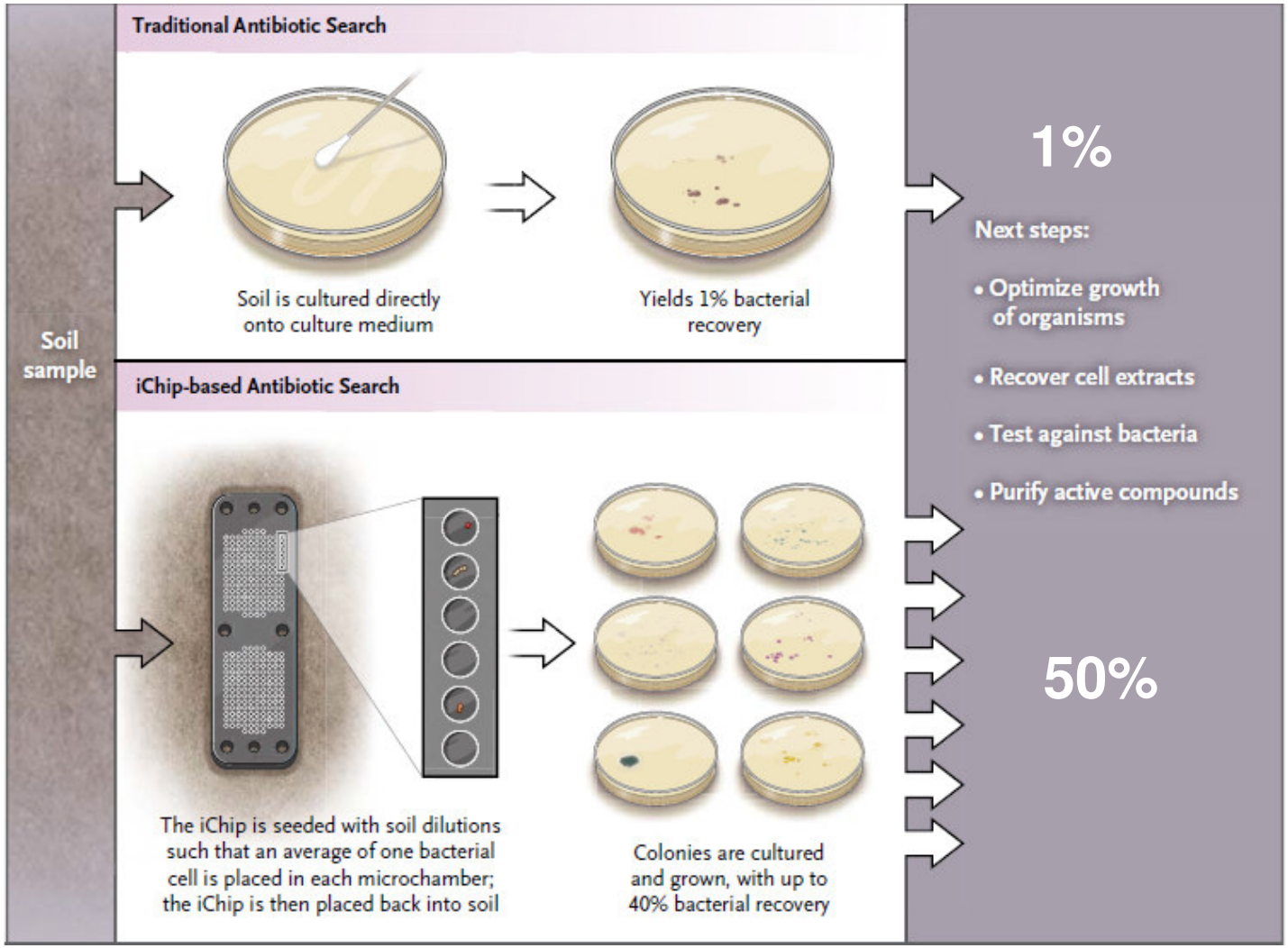
Slava Epstein, Northeastern University



(Nichols *et al.*, AEM, 2010)

Traditional vs iChip based search

Growth recovery → iChip: 50% *vs*. petridish : 1 %

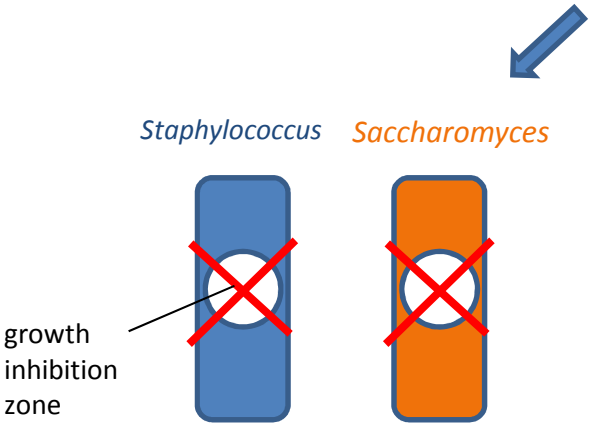


Arias *et al.*, NEJM, 2015
Nichols *et al.*, AEM, 2010

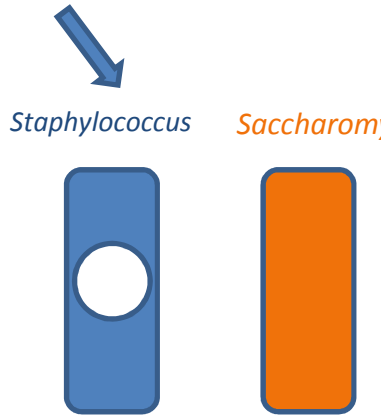
Growing the „unculturable“ - iChip (isolation chip)

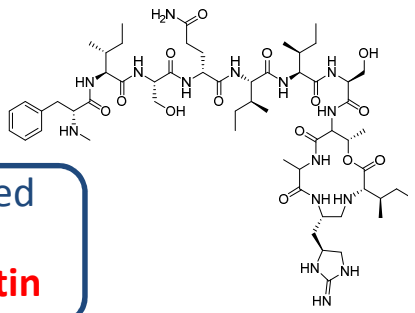
- ~50.000 previously „uncultered“ bacteria were isolated from soil samples
- extracts of 10.000 isolates have been screened for antimicorbial activity
- 25 new compounds identified

10.000 extracts



discarded to eliminate toxic compounds





25 new compounds identified

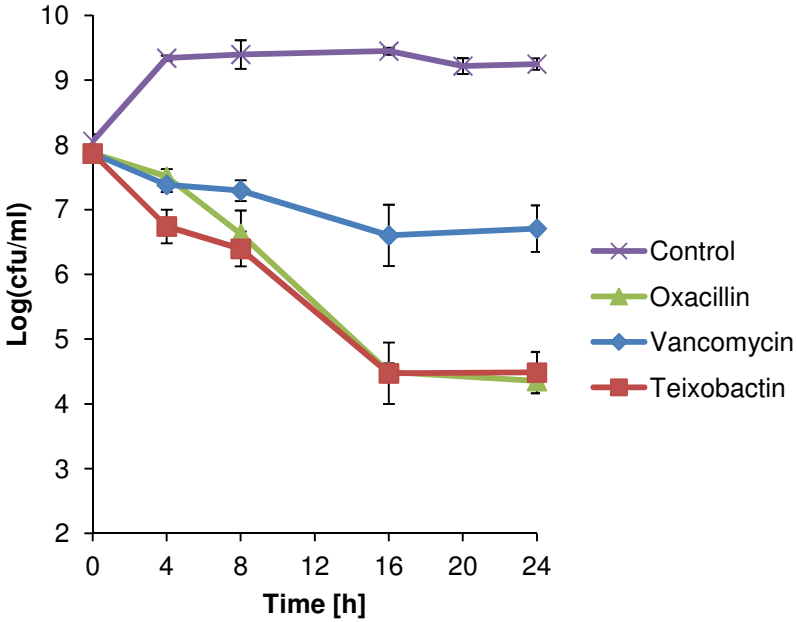
→ most promising : **Teixobactin**

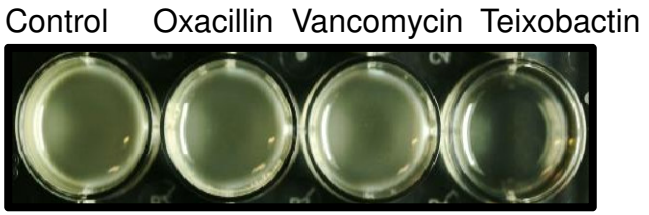
Activity of teixobactin against pathogenic microorganisms

- excellent activity against Gram-postive pathogens

pathogen	MIC (µg/ml)
Staphylococcus	
S. aureus ATCC 33591 (MRSA)	0.16
S. aureus ATCC 700699 (GISA)	0.32
S. aureus (DAP-R)	0.12
S. aureus (LIN-R)	0.12
S. aureus (VISA)	0.12
Enterococcus	
E. faecium (VRE)	0.31
E. faecalis (VRE)	0.31-0.63
Mycobacterium	
M. tuberculosis H37Rv	0.125
M. tuberculosis (clin. isolate 70)	0.125-0.25
Streptococcus	
S. pneumoniae ATCC 6303	0.02
S. pyogenes	0.31
Bacillus anthracis	<0.06
Clostridium difficile CD 196	0.005

Time dependent killing of *S. aureus*



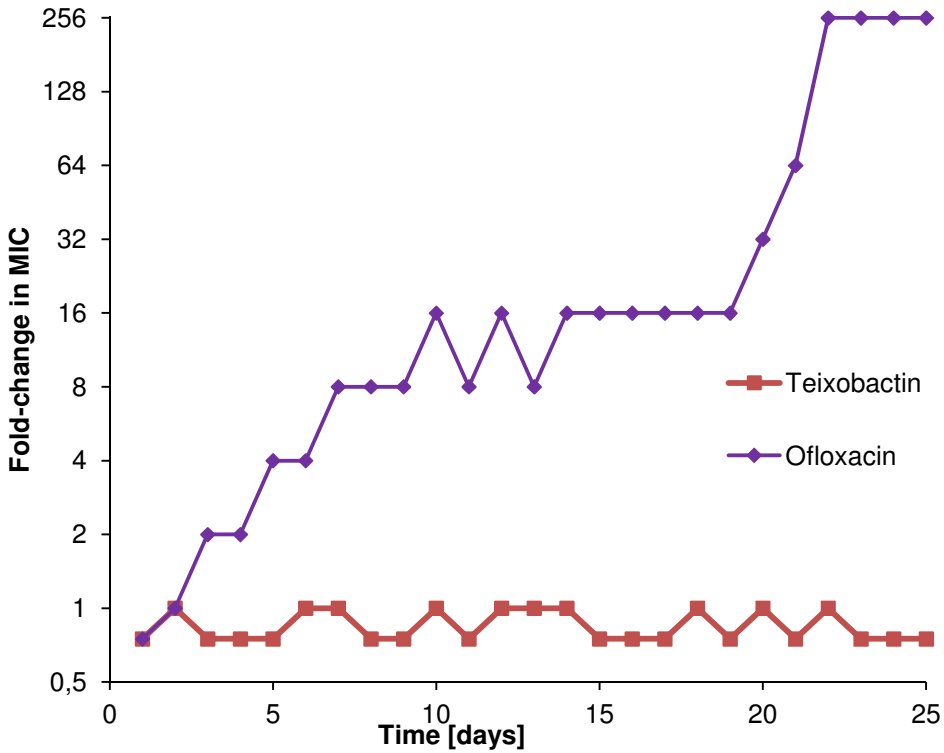


- superior to vancomycin in killing *S. aureus*
- excellent bactericidal activity

No resistance acquisition detected during serial passaging

- no selection of resistant mutants of *S. aureus* oder *M. tuberculosis,* even at low doses

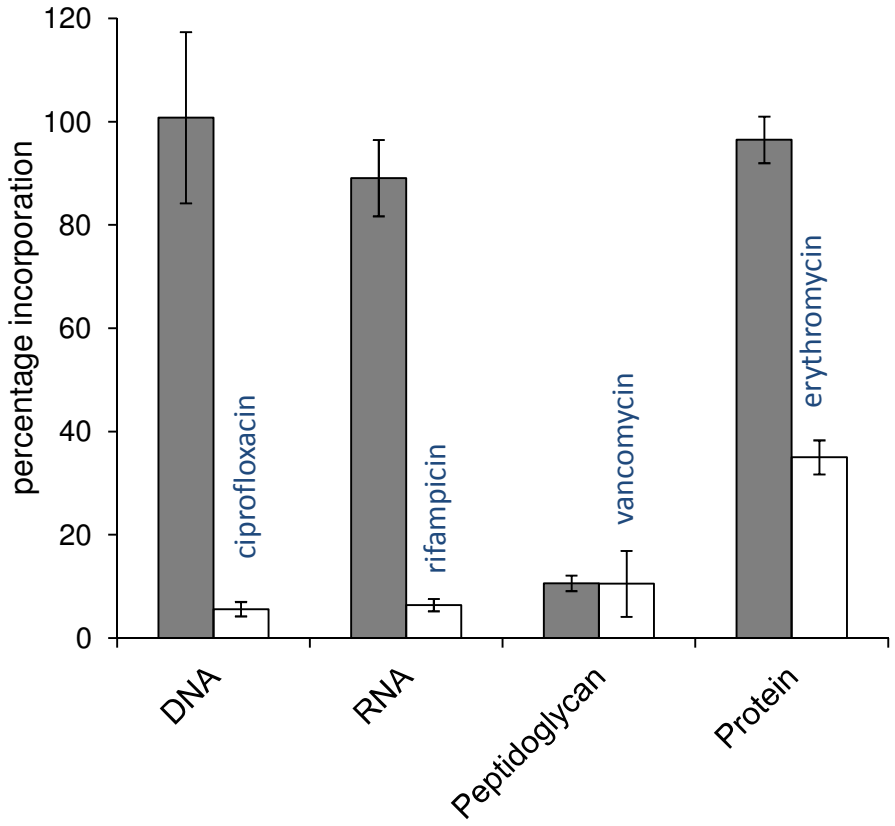
- serial passaging of *S. aureus* on sub-MIC levels of teixobactin
  → failed to produce resistant mutants



→ no toxicty against mammilian cells at 100 µg/ml (highest dose tested)

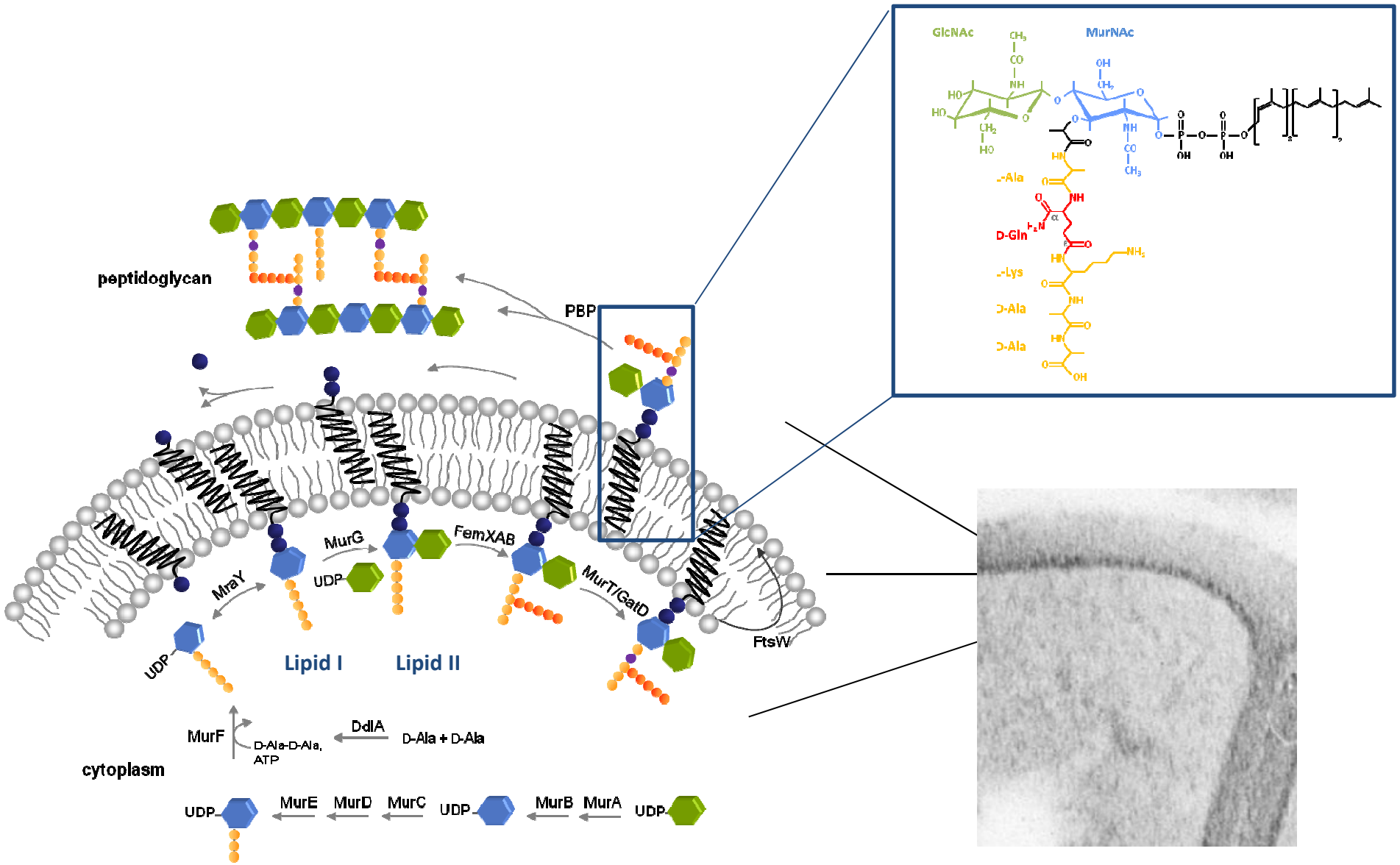
Mode of action analysis

Impact of teixobactin on macromolecular biosyntheses

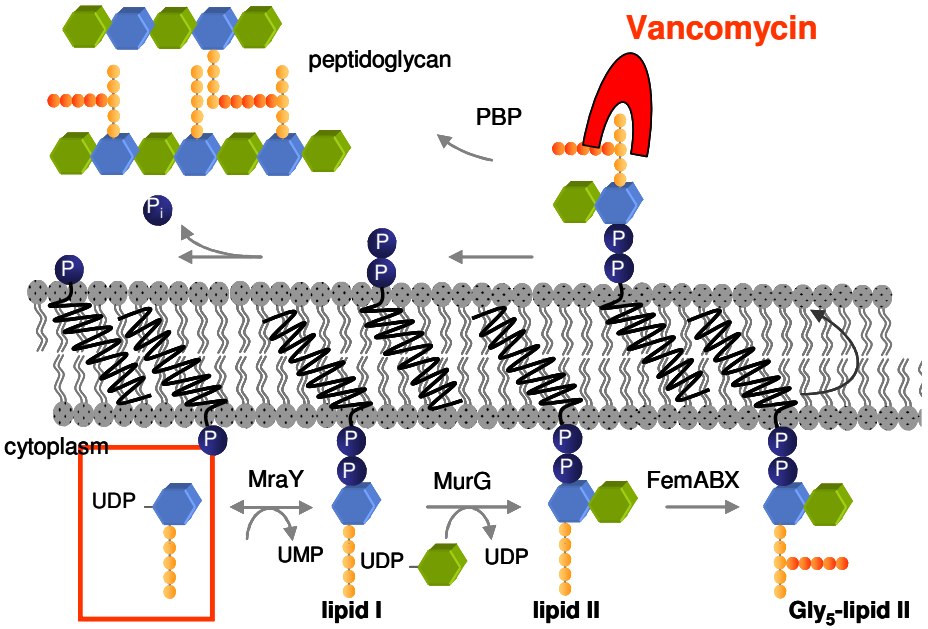


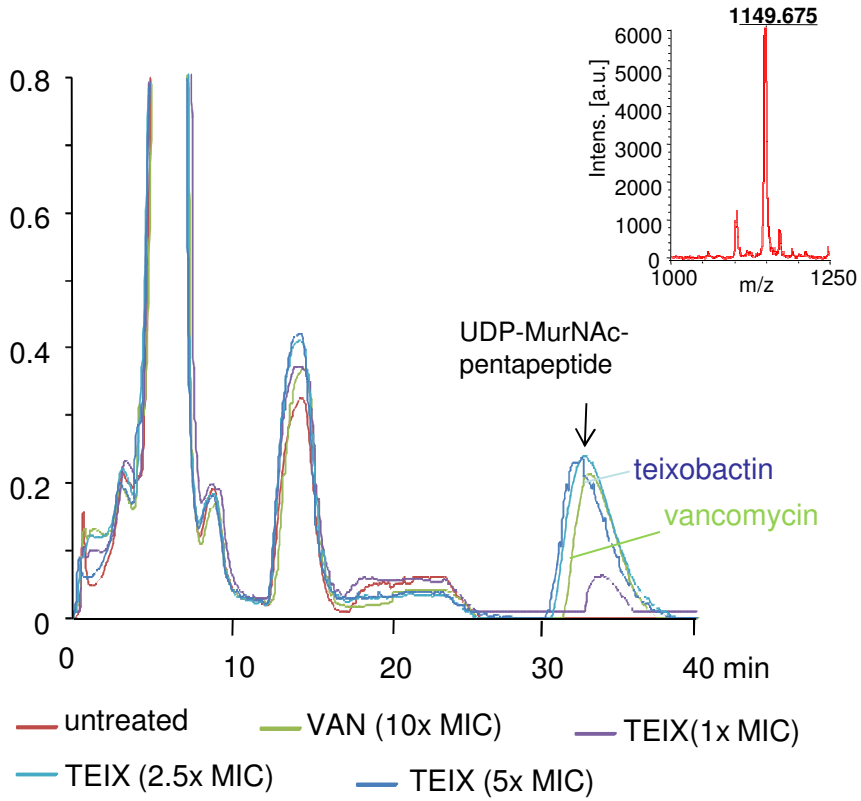
- incorporation of radiolabeled leucin (protein), thymidine (DNA) and uridine (RNA) was unaffected
- glucosamine was no longer incorporated – pointing towards cell wall biosynthesis as a target

Peptidoglycan biosynthesis in *S. aureus*



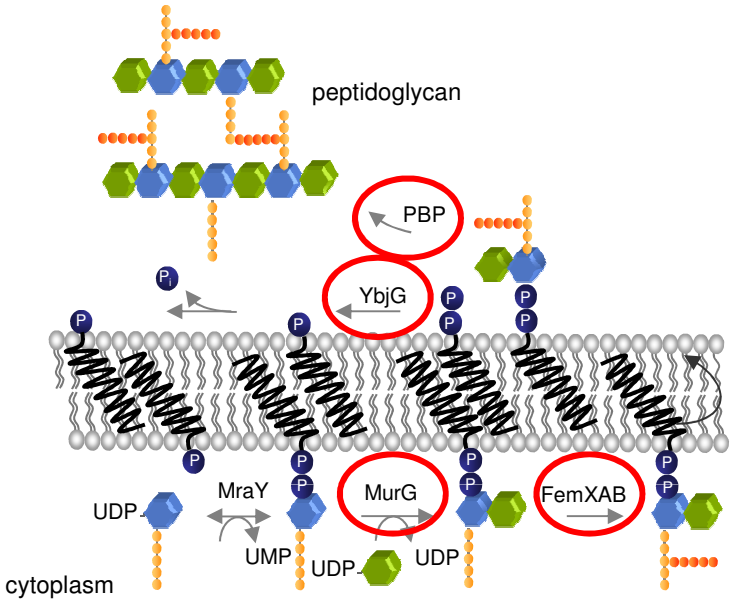
Teixobactin causes the accumulation of UDP-MurNAc-pentapeptide



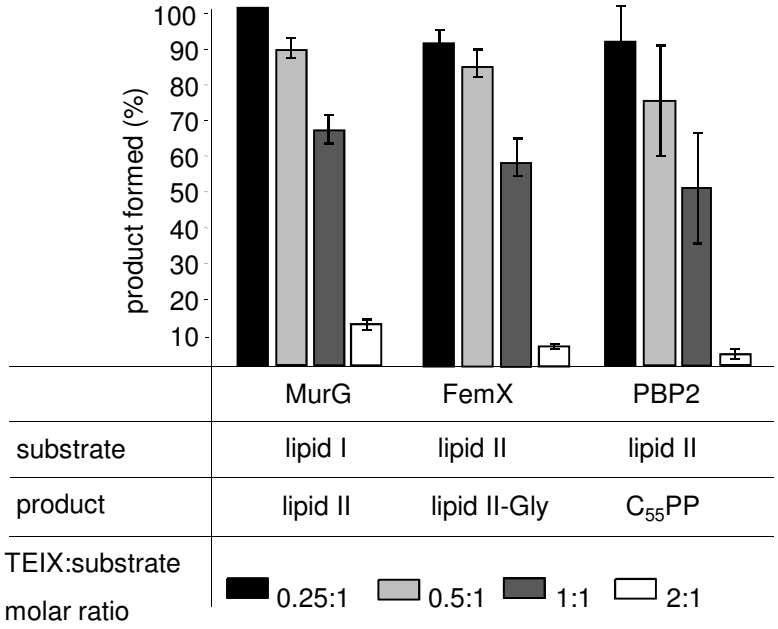


- teixobactin-treated cells accumulate the ultimate soluble cell wall precursor UDP-MurNAc-pp, indicating that a later, membrane-associated step of cell wall biosynthesis is inhibited

Inhibition of membrane associated cell wall biosynthesis steps



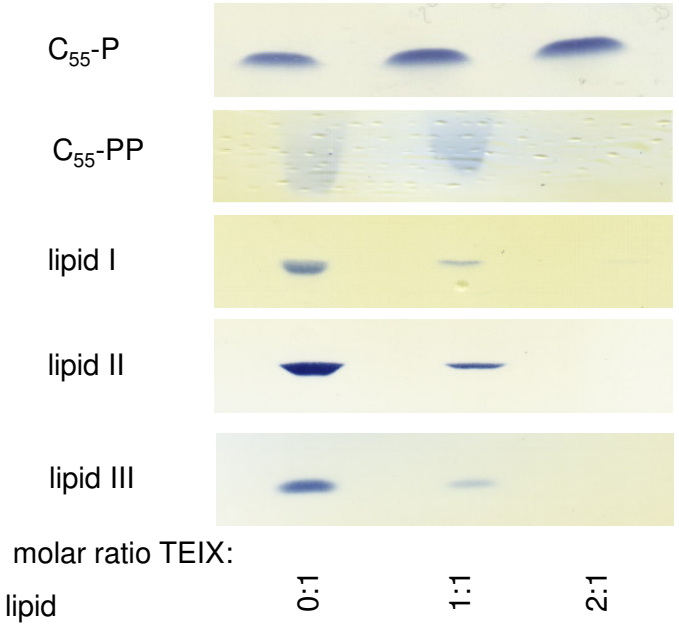
Lipid I **Lipid II**



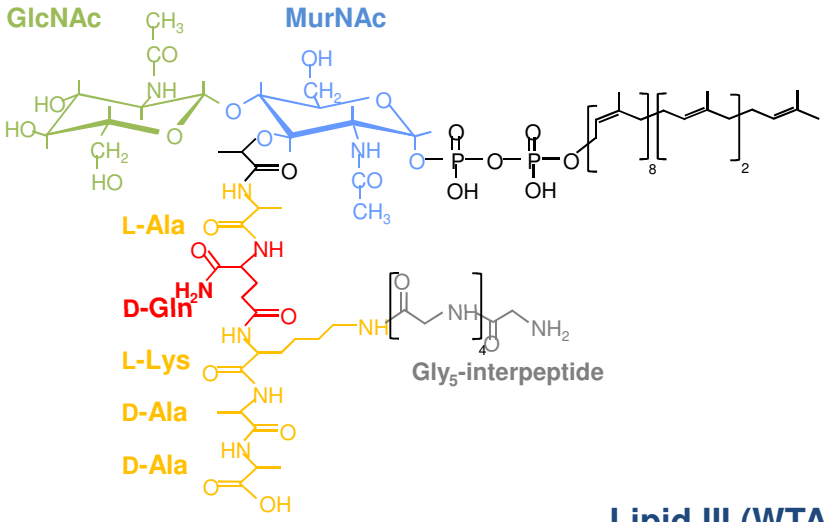
- full inhibition is achieved at 2:1 molar ratio (Teixobactin:lipid)
- teixobactin forms a stoichiometric complex with the substrates lipid I and lipid II, rather than inhibiting the enzyme itself
- analogous WTA & capsule biosynthesis reactions are similarly inhibited
- secondary target: universial lipid carrier (un)decaprenyl(pyro)phosphate

Complex formation with cell envelope precursors

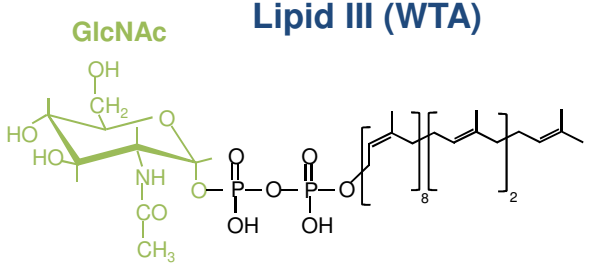
- evaluate the minimal motif required for high affinity binding of teixobactin



Lipid II (peptidoglycan)



Lipid III (WTA)



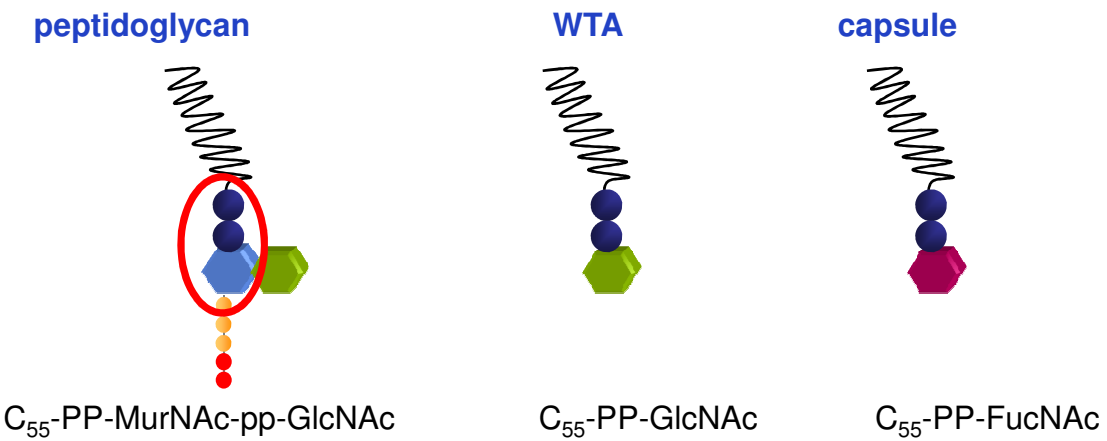
BuOH extraction

- pyrophosphate moiety is crucial for TEIX binding

Cell envelope precursors antagonize teixobactin antimicrobial activity

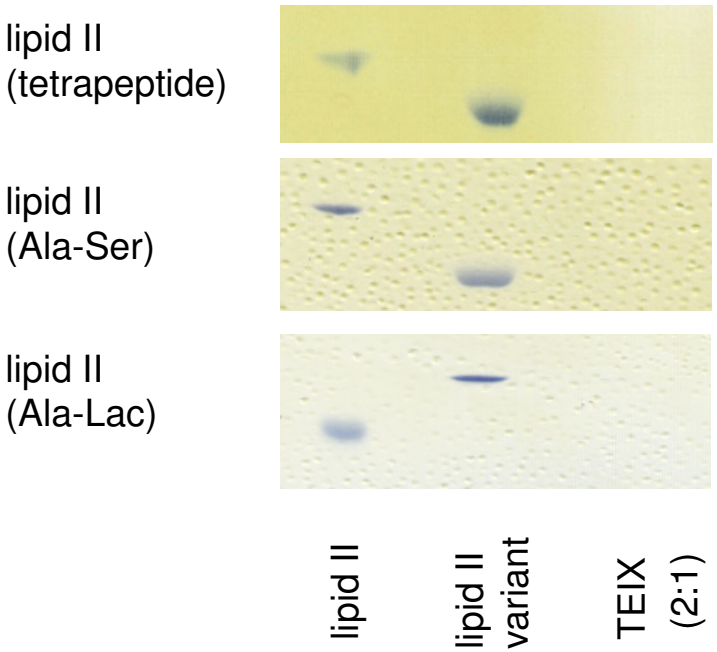
lipid intermediate	molar ratio of precursor to teixobactin						
	0 x	0.5 x	1 x	2.5 x	5 x	7.5 x	10 x
lipid II	-	+	+	+	+	+	+
C_{55}-PP	-	-	-	-	+	+	+

- high affinity binding relies on the interaction with the PP-sugar moiety
- the nature of the first sugar is less important



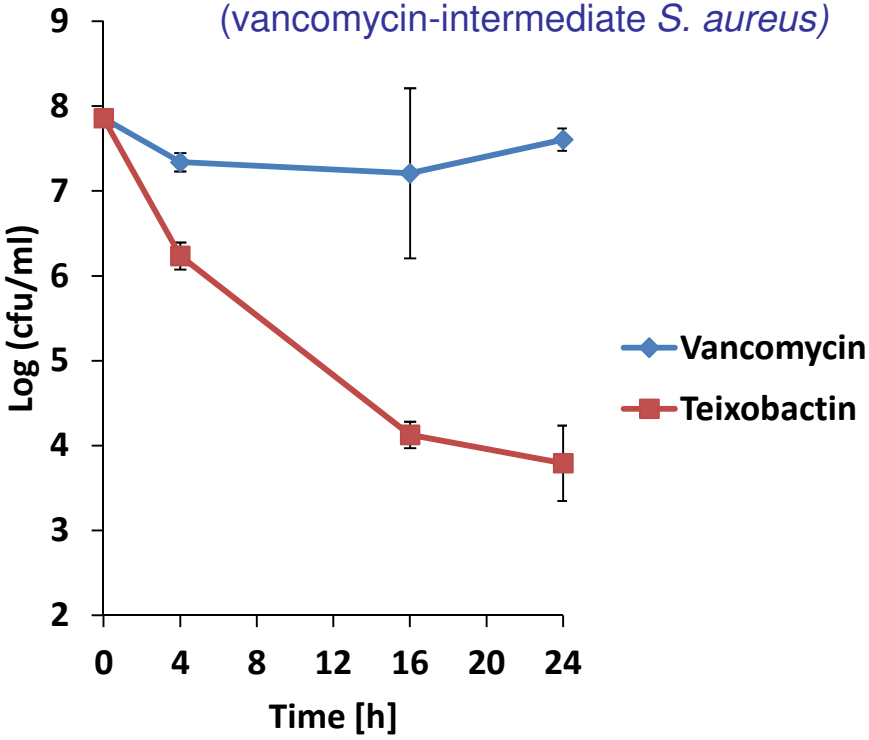
Teixobactin is active against Vancomycin resistant *S. aureus*

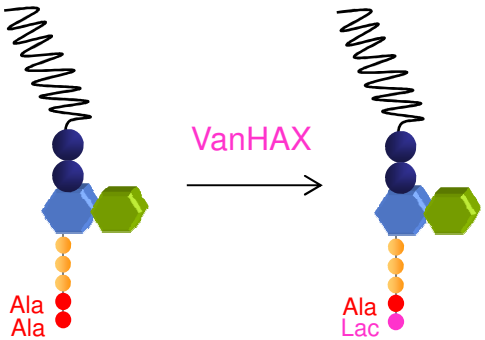
VRSA

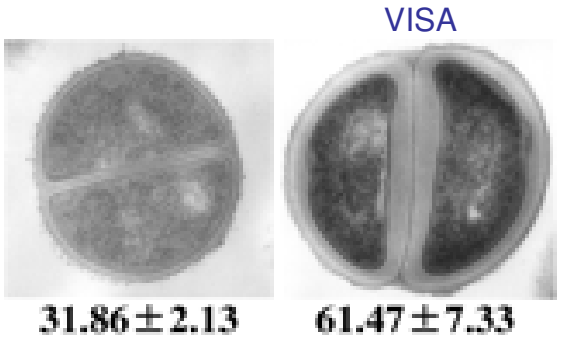


Killing of VISA

(vancomycin-intermediate *S. aureus*)





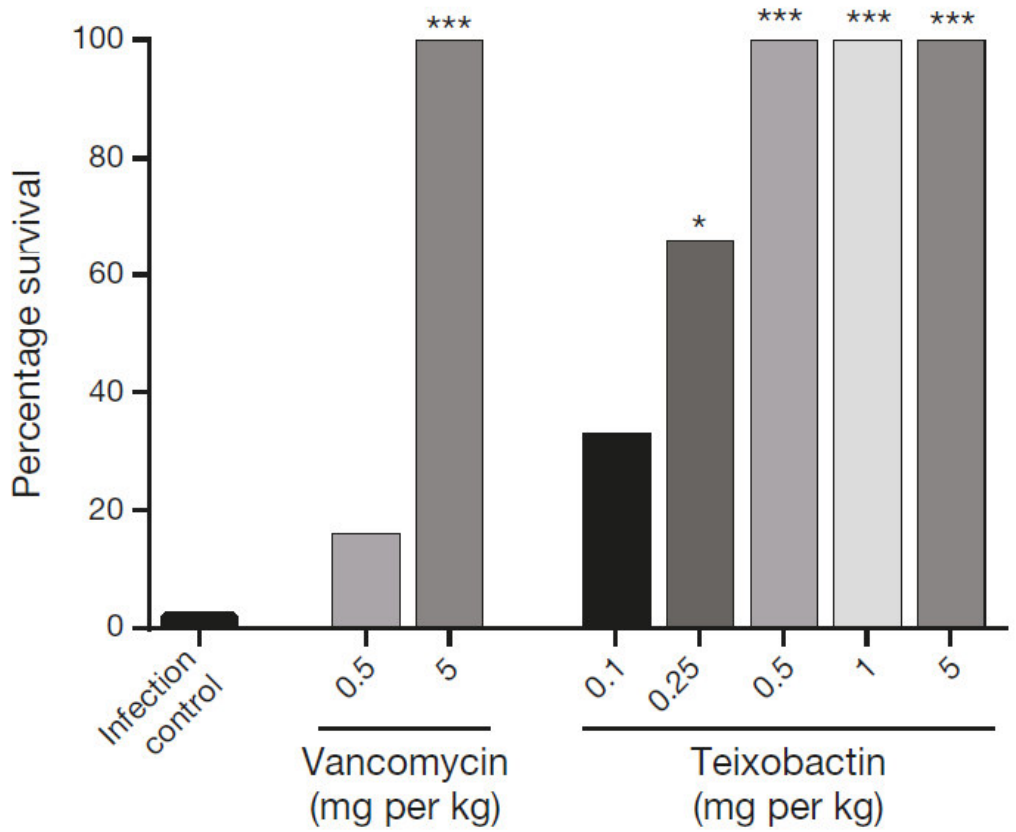


(Cui *et al.*, AAC, 2006)

Teixobactin is efficacious in mouse models of infection

- effective in 3 mouse models
- 100% survival of mice at 0.5 mg/kg

Mouse sepsis model

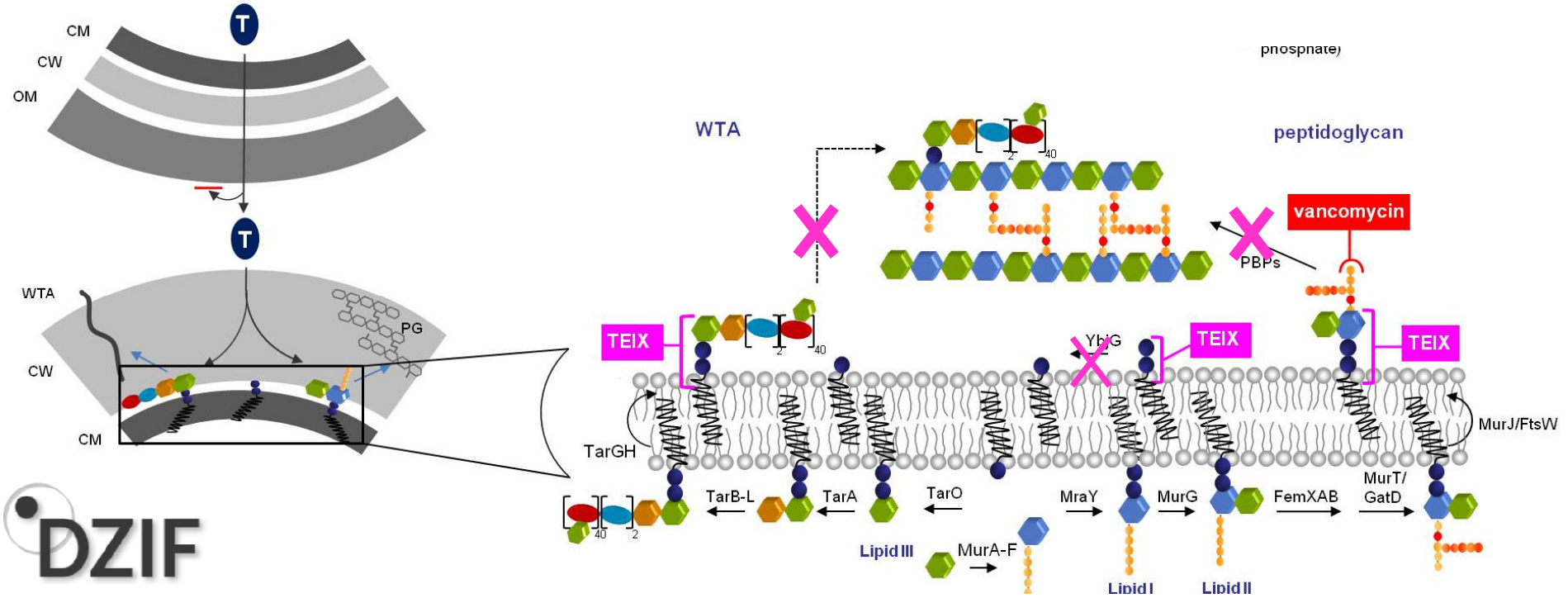


Mode of action model

- binds to multiple targets > polyprenol-coupled precursors of different cell envelope pathways
- highly conserved *non-protein* target structures
- binding sites are extremly hard to modify

→ strongly limits resistance development

Teixobactin



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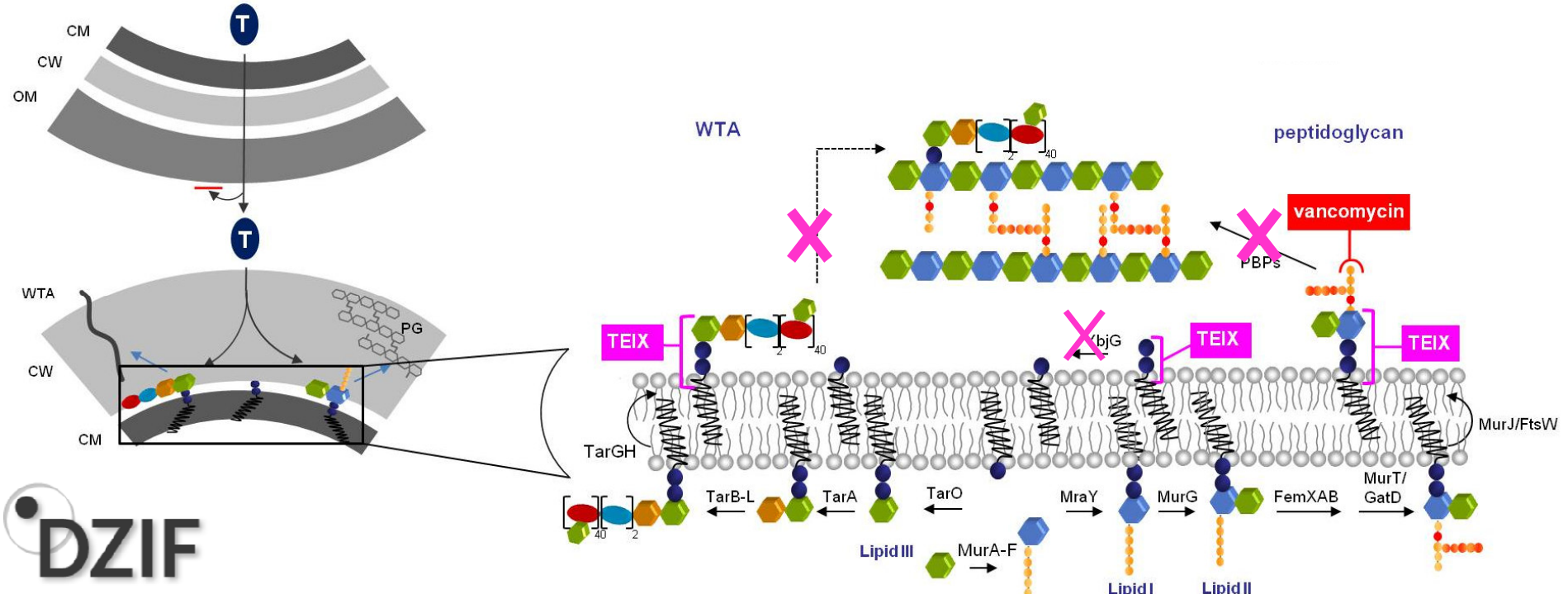
Mode of action model

- the producer is a gram-negative bacterium and the outer membrane protects it from re-entry of teixobactin → there appear to be no additional resistance mechanisms that other bacteria could borrow
- no cross-resistance to strains resistant to other „cell wall-active“ antibiotics, including VISA or DAP^{R}

BUT:

- antibiotic modifying or degrading enzymes might be produced by other microbes



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Brian Conlon, Northeastern University