Mechanisms of Cell Envelope Defense Against Antibiotics in Gram-positive

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1/18/2023

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Disclosures

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- Research support: Merck (ceftolozane-tazobactam), Entasis (sulbactam-durlobactam)

- Other: UpToDate (Enterococcal infections)

Overview

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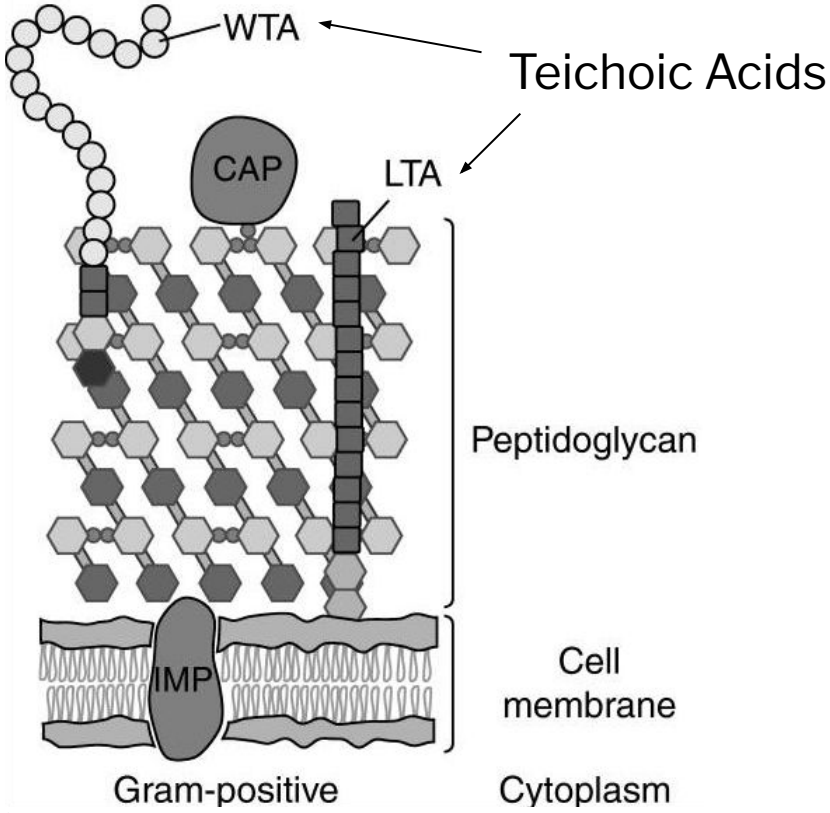
- Brief review of cell envelope synthesis
- Pathways of bacterial cell envelope defense in Gram-positive organisms
- Adaptation to cell envelope stress, a case study in *Enterococcus faecalis*

Gram-positive cell envelope



Silhavy T, CSH Perspect 2010

Cell envelope synthesis

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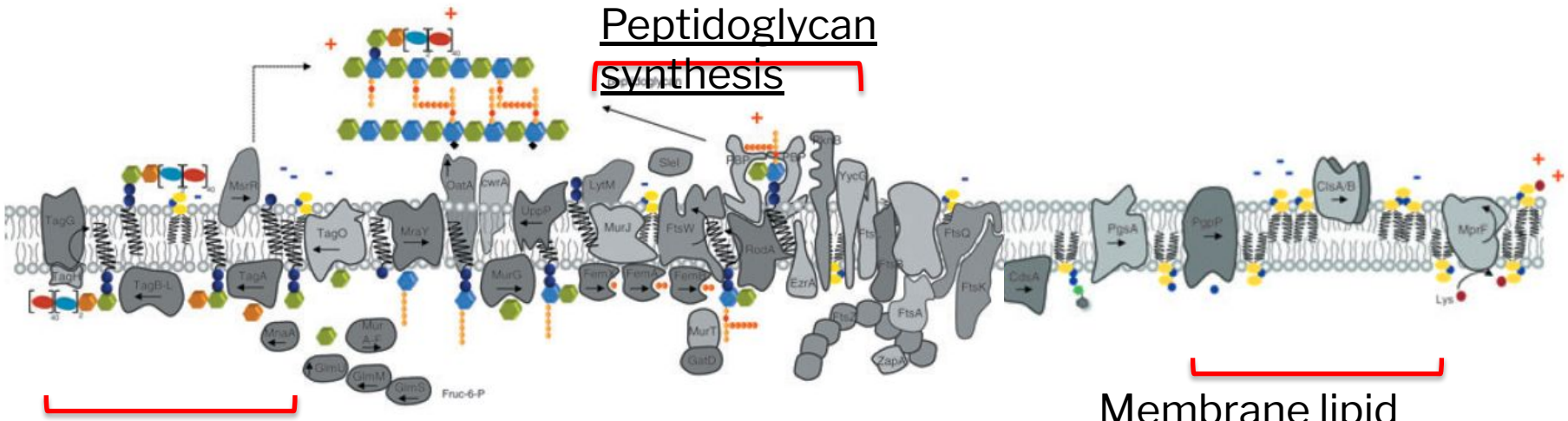
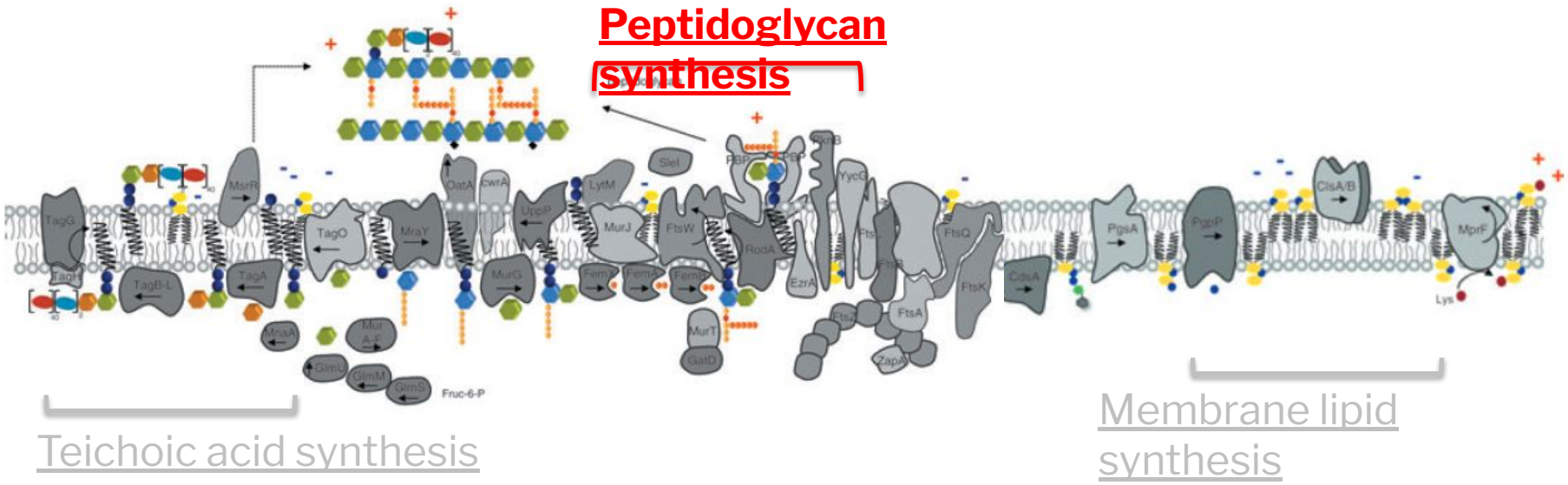


Bayer AS, Ann NY Acad Sci 2013
1277:120-50

Cell envelope synthesis



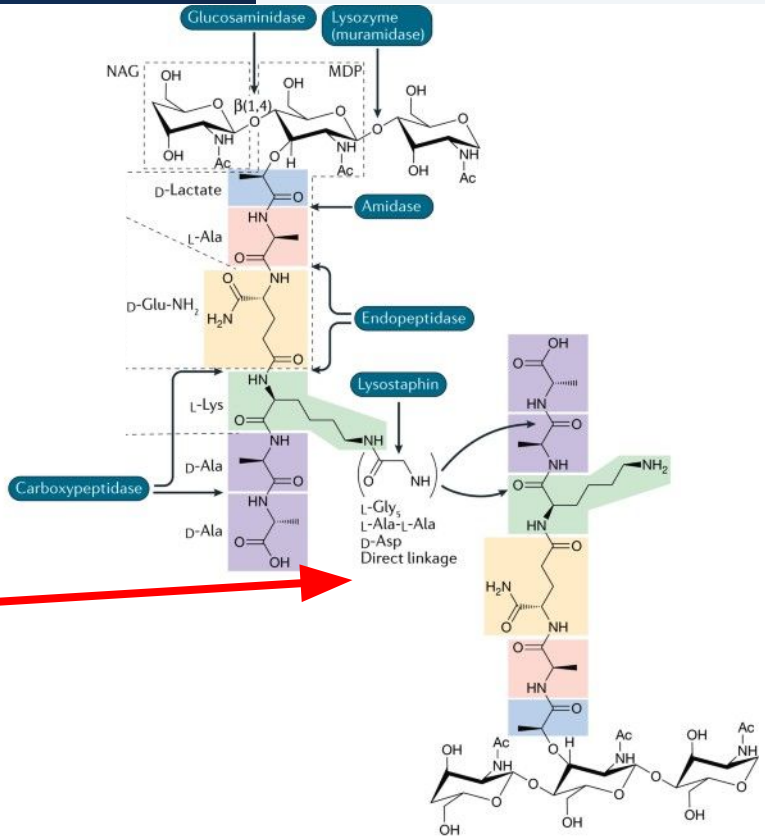
Bayer AS, Ann NY Acad Sci 2013
1277:139-50

Peptidoglycan

- Polymer of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), with an oligopeptide chain attached to NAM

- Peptide chain serves as substrate for cross-linking

<u>Crosslink:</u>
S. aureus – L-Gly_5
E. faecalis – L-Ala-L-Ala
E. faecium – D-Asp



Nat Rev Immunol. 2018 Apr;18(4):243-254.
Biochemistry. 2008 Aug 12; 47(32): 8378–8385.
Biochim Biophys Acta Biomembr. 2017

Peptidoglycan synthesis

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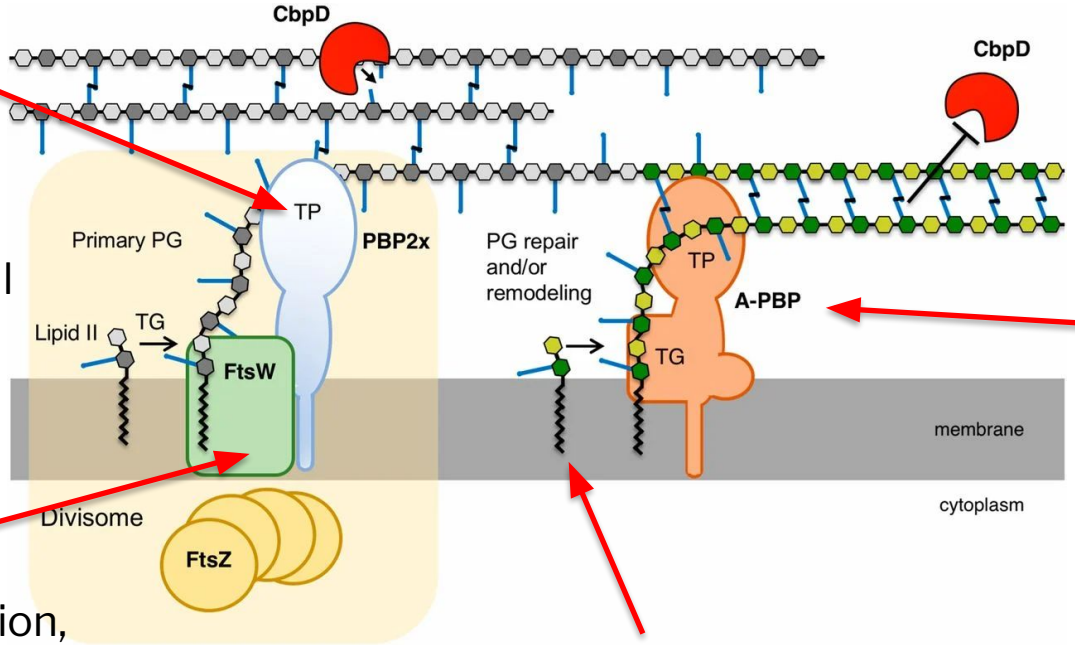
Class B PBP

- Low molecular weight
- Monofunctional transpeptidase activity



Class A PBP

- High molecular weight
- Bifunctional enzyme – transglycosylase and transpeptidase activity

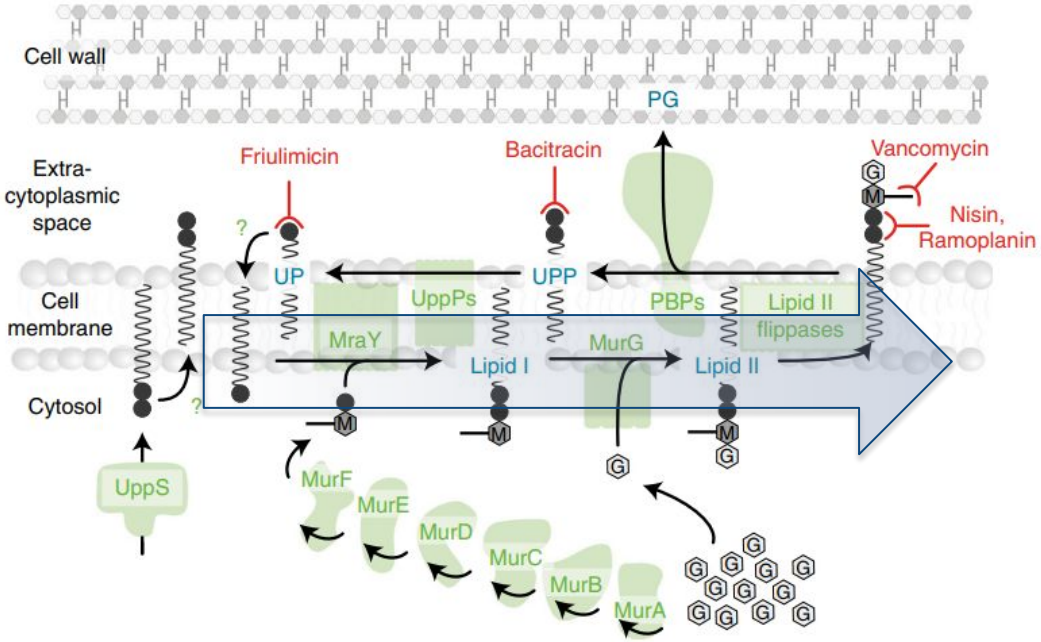
SEDS

- shape, elongation, division and sporulation
- Partner transglycosylase

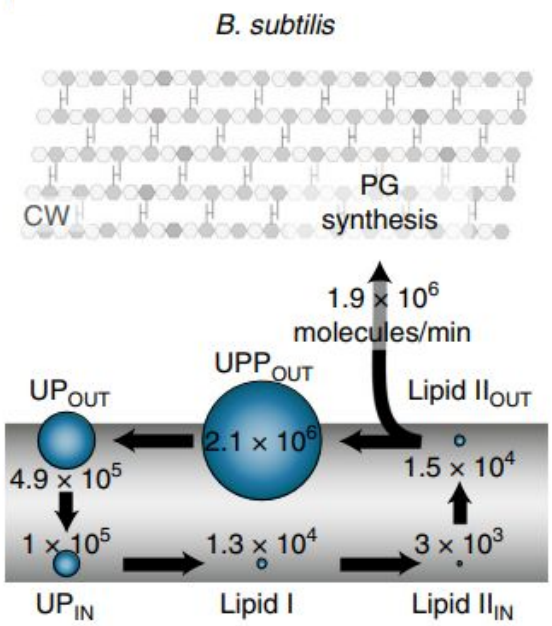
Lipid II (and its intermediates) are critical to cell wall synthesis

Nature. 2016 Sep 29; 537(7622): 634–638.
Proc Natl Acad Sci U S A. 2020 Mar 17;117(11): 6100–6108.

The cell wall supply chain



Passage of lipid intermediates is driven by a substrate affinity gradient



Peptidoglycan synthesis rate is coupled to Lipid II flux

Nat Commun. 2019 Jun 21;10(1):2733.
Nat Commun. 2022 Apr 27;13(1):2278.

Antimicrobials targeting Lipid II flux

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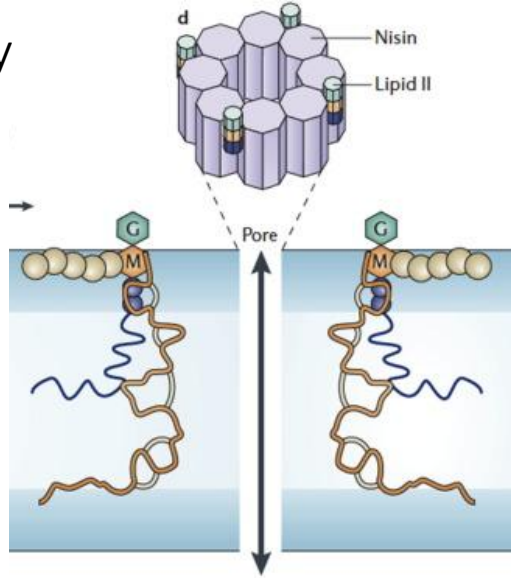
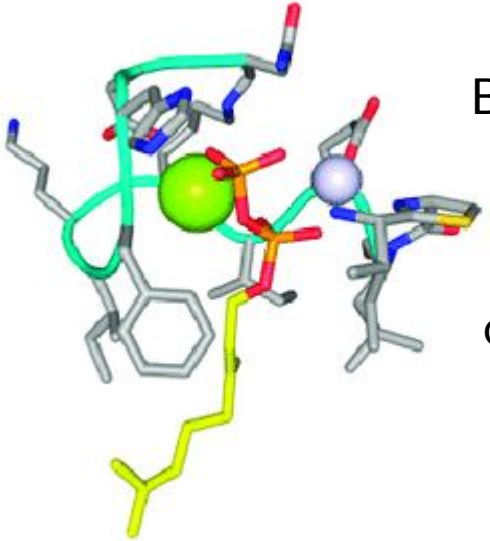
Nisin

Binding affinity
K_D – 0.015 μM

Effective concentration
~ 1 μM



Bacitracin



Binding affinity
K_D – 1 μM

Effective concentration
~ 1.8 μM

What accounts for the difference?

Biochim Biophys Acta. 2016 May;1858(5):947-57; Proc Natl Acad Sci U S A. 2013 Aug 27;110(35):14207-12.

Lipid II cycling explains differential activity of antibiotics

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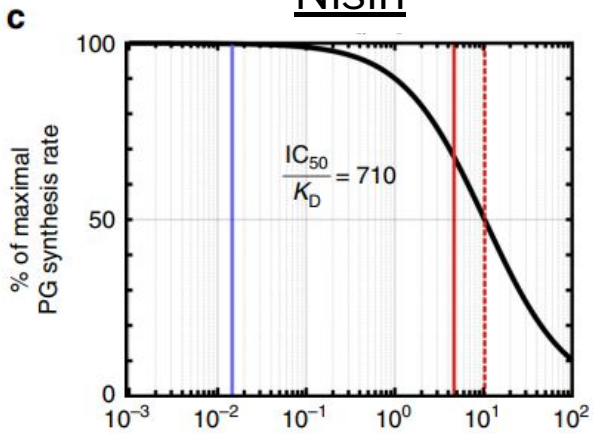
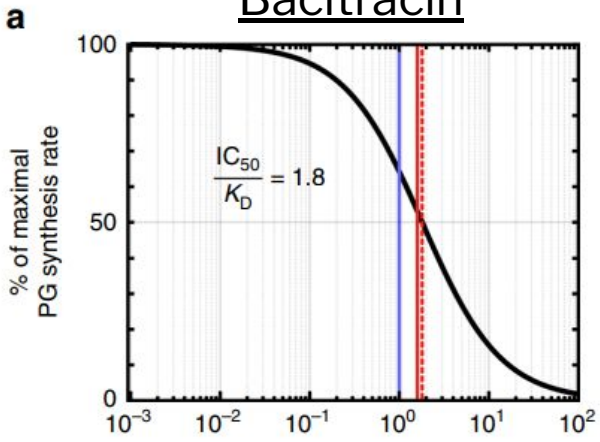
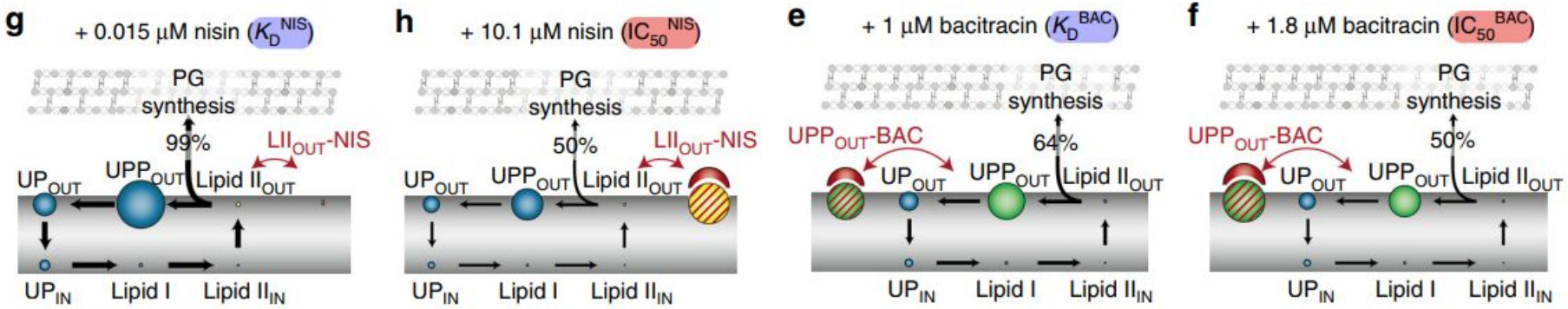
Nisin

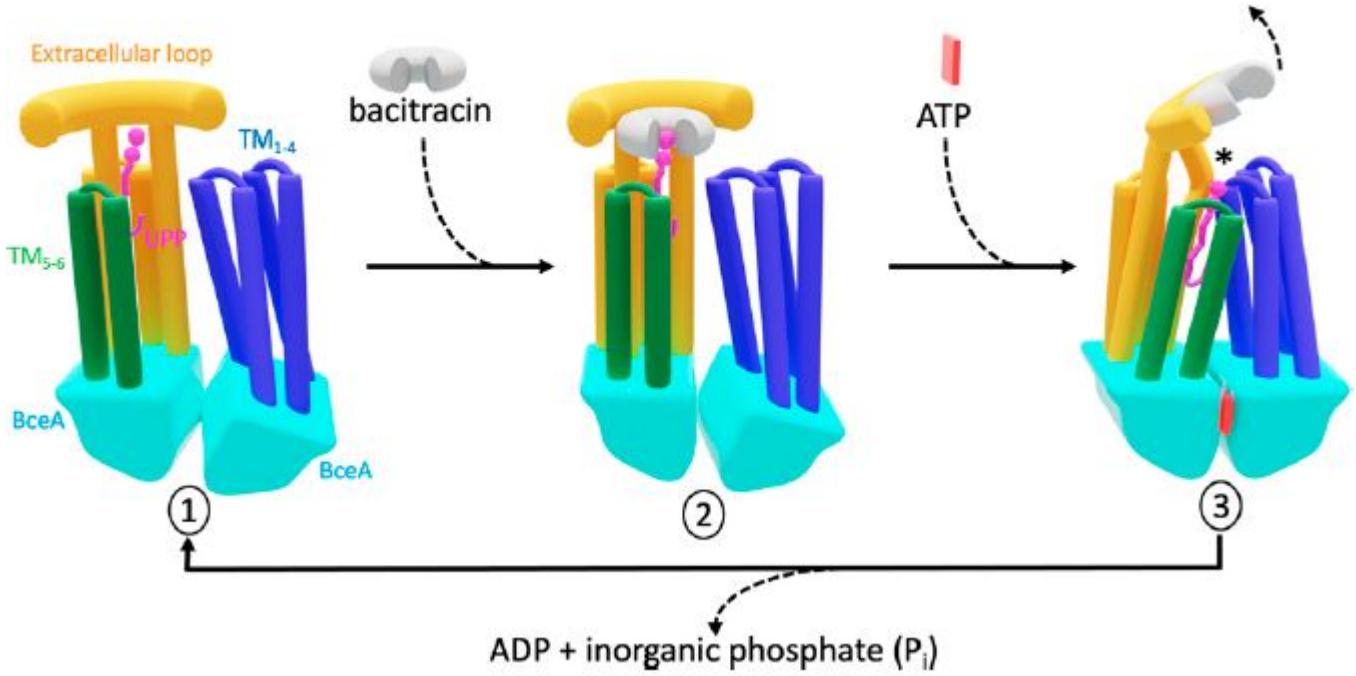


Bacitracin



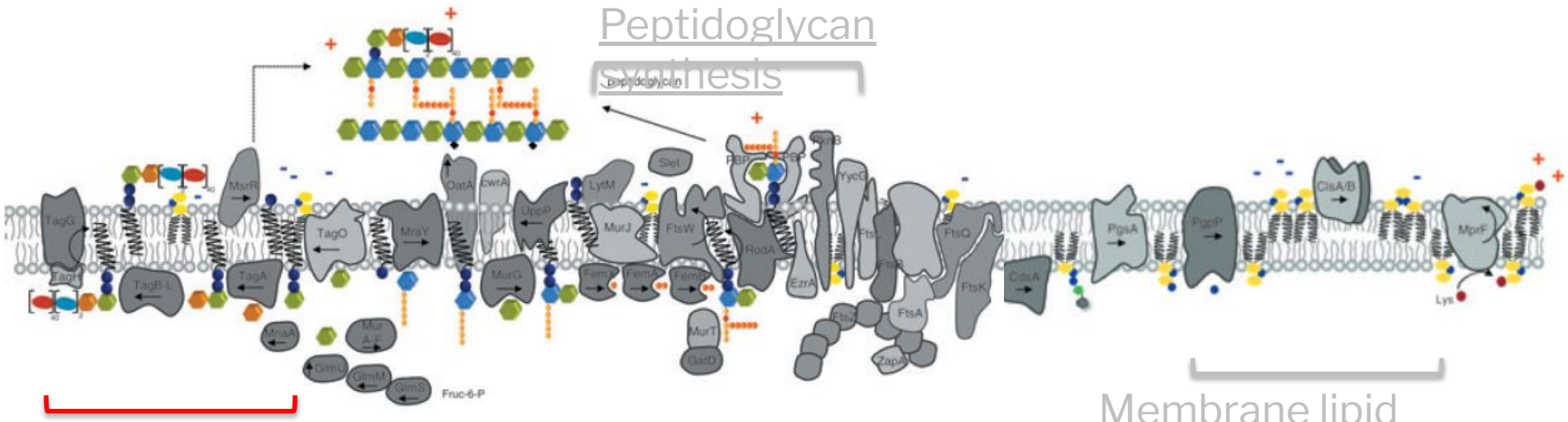


Resistance to Bacitracin mediated by target protection



Proc Natl Acad Sci U S A. 2022 Apr 5;119(14):e2123268119.

Cell envelope synthesis



Teichoic acid synthesis

Membrane lipid synthesis

Bayer AS, Ann NY Acad Sci 2013
1277:139-50

Teichoic Acids (TA)

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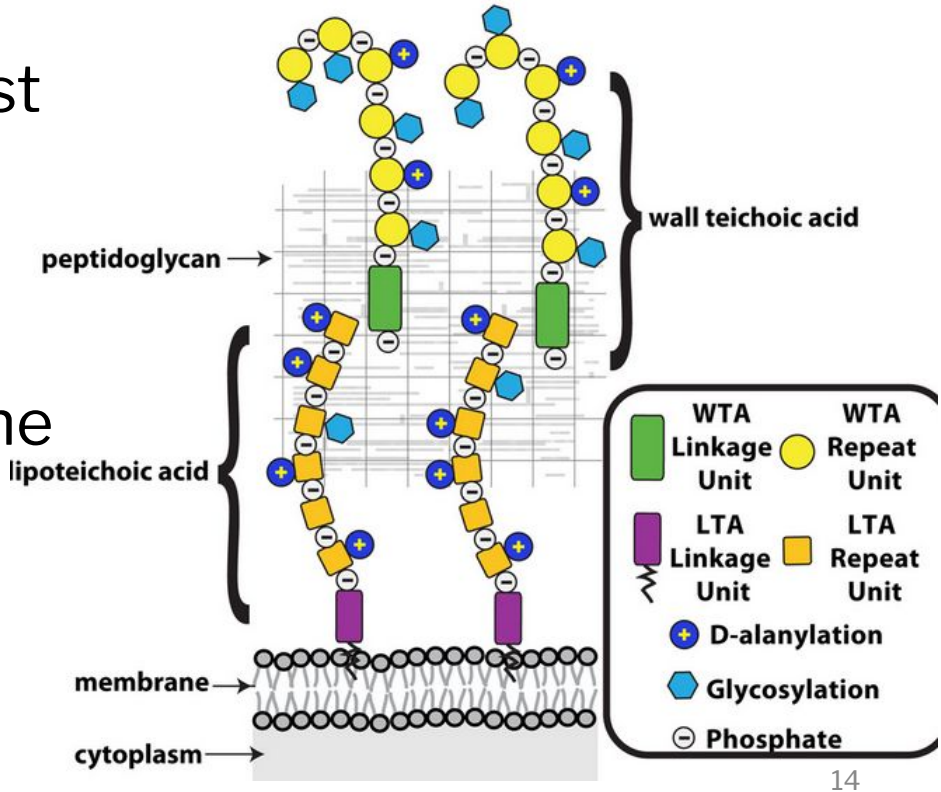
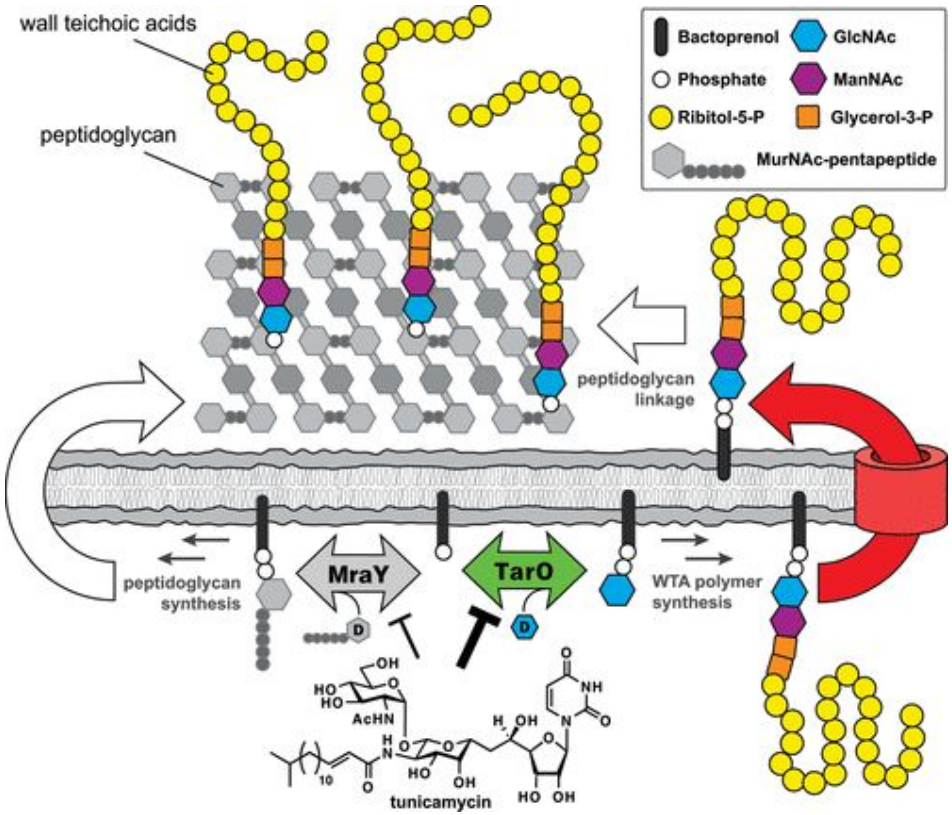
- Teichoic acids are glycopolymers present in most Gram-positive cell envelopes.
- Lipoteichoic acids (LTA) are anchored to the cell membrane
- Wall teichoic acids (WTA) are bound to the peptidoglycan matrix



Annu Rev Microbiol.

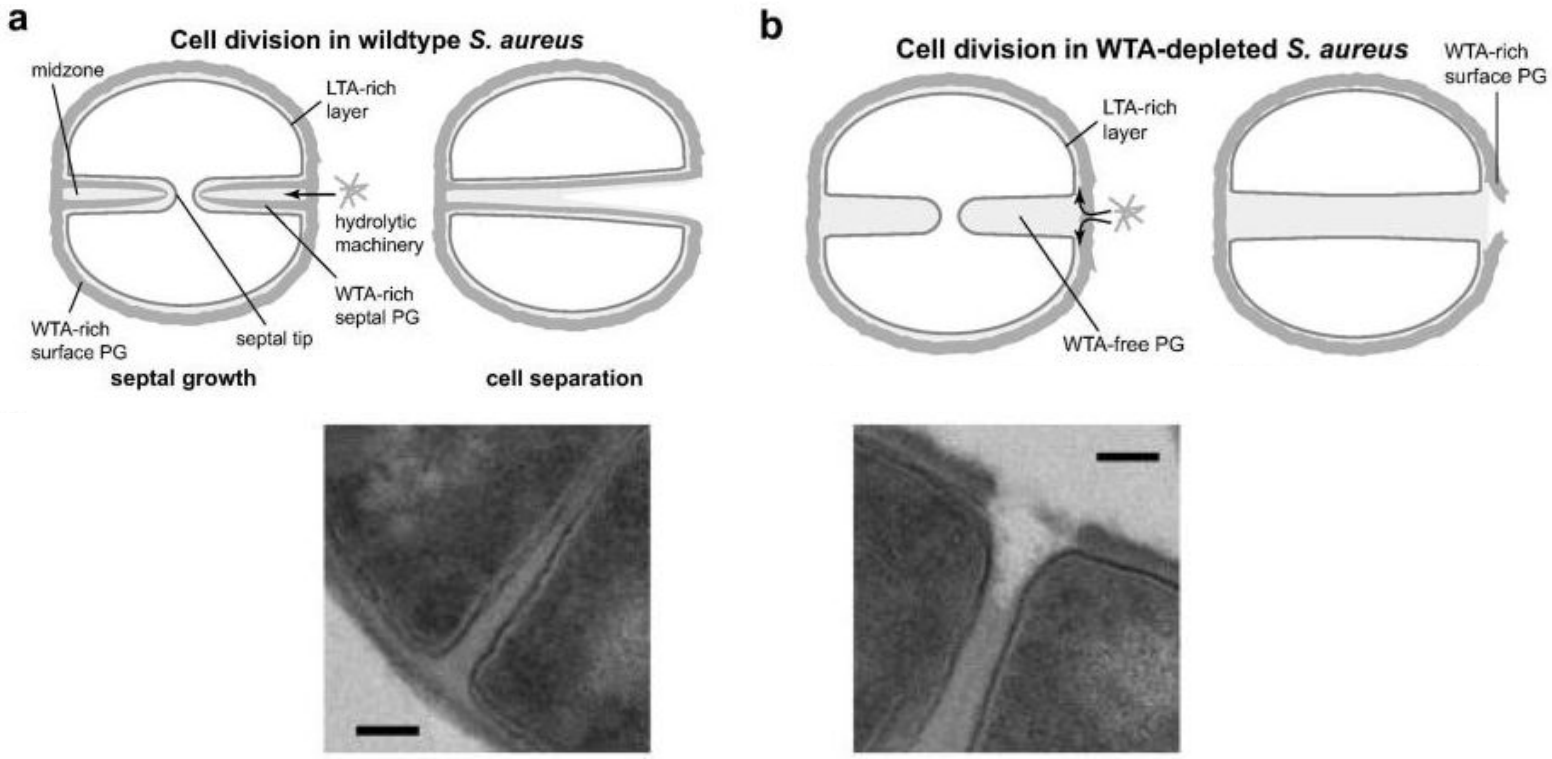
Wall teichoic acid synthesis



Annu Rev Microbiol. 2013;67:313-336; ACS Chem Biol. 2011 Jan 21; 6(1): 106–116

- TA synthesis shares the undecaprenyl pool with PG biosynthesis
- In *S. aureus*, WTA has been implicated in the cooperative action of PBP2 and PBP4
- Disrupting WTA synthesis leads to increased sensitivity to β-lactams which target PBP2

WTA regulate PG autolysis



ACS Chem Biol. 2011 Jan 21; 6(1):
106–116

Teichoic Acids as reservoirs of positive charge

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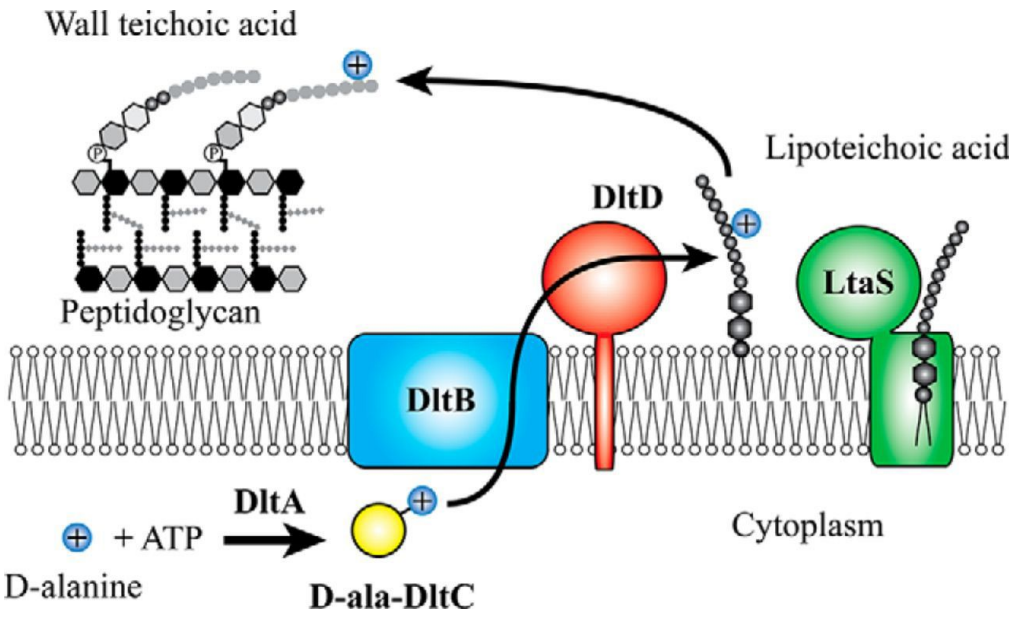
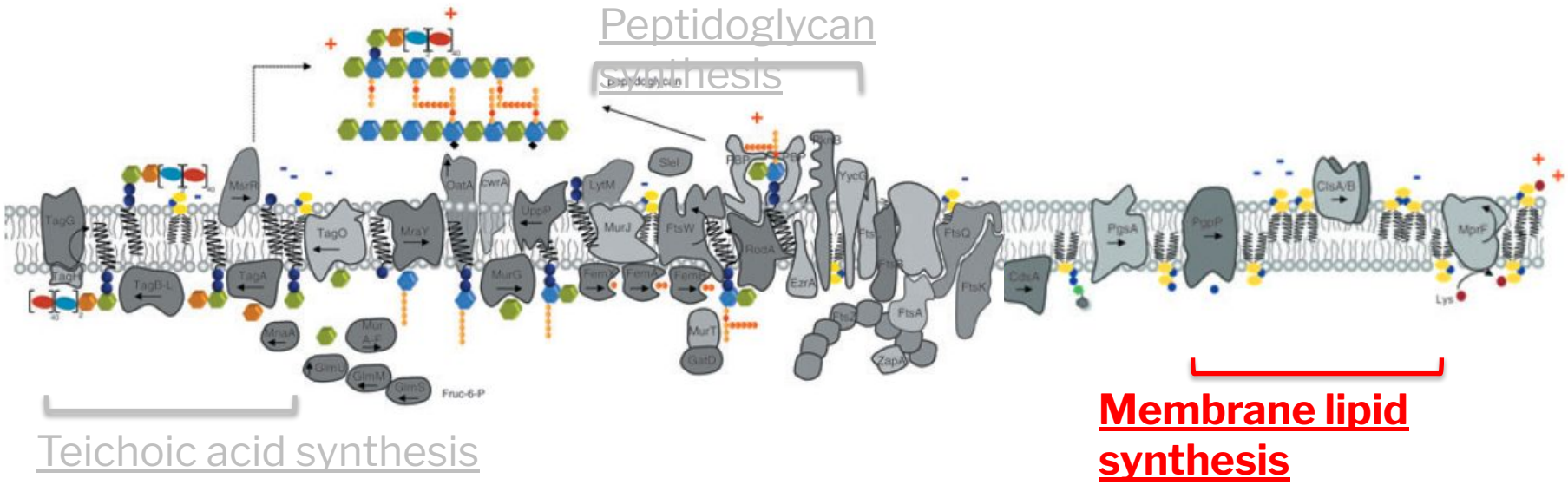


- D-alanylation of LTA occurs via pathway encoded by *dlt* operon
- This modulates the activity of PG hydrolases/amidases in the cell wall.
- WTA also functions in the pathway to resistance antimicrobial peptides and antibiotics active at the cell envelope.

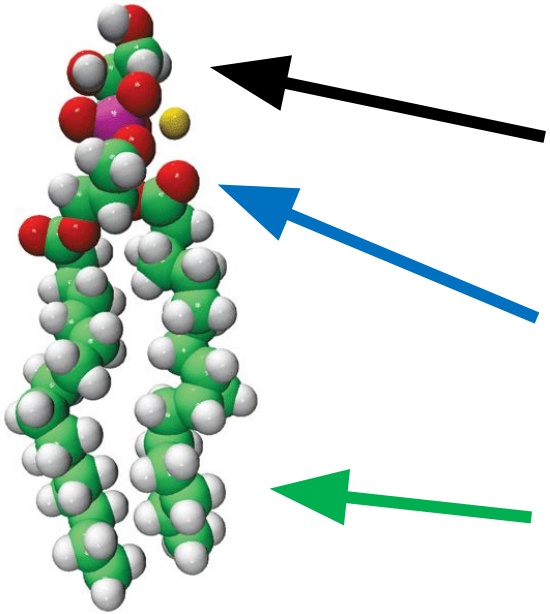
J Biol Chem. 2018 Nov 16 293(46) 17895-17906

Cell envelope synthesis



Bayer AS, Ann NY Acad Sci 2013
1277:120-50

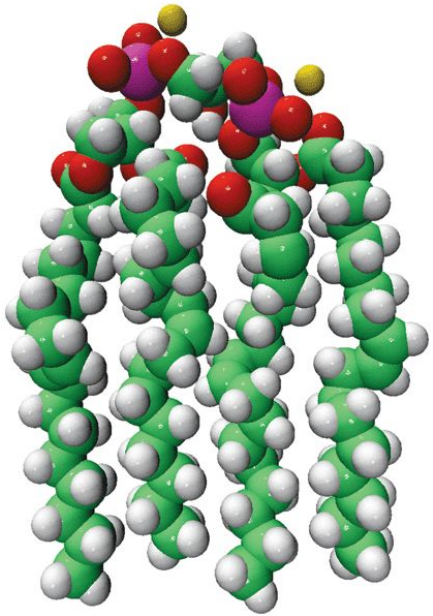
Membrane lipids



Polar head group

Glycerol

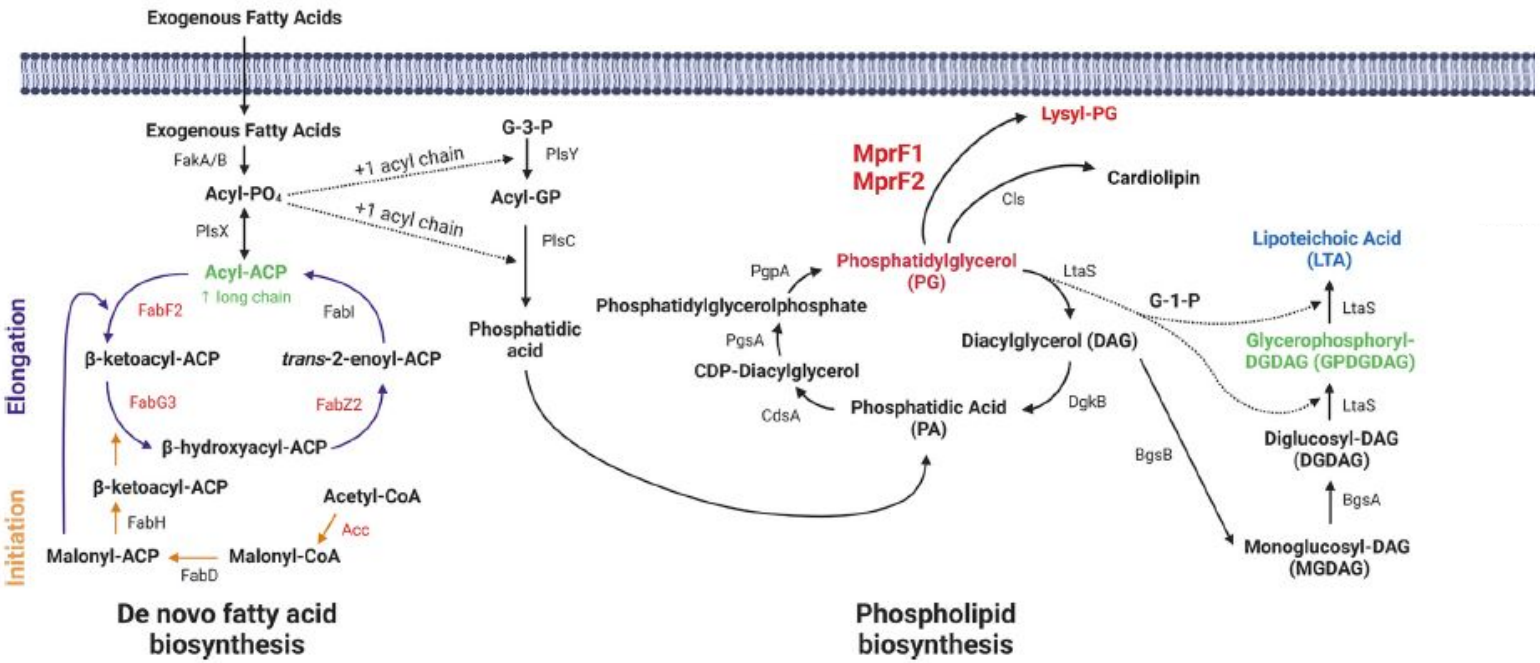
Fatty acid tail



Phosphatidylglycerol (PG)

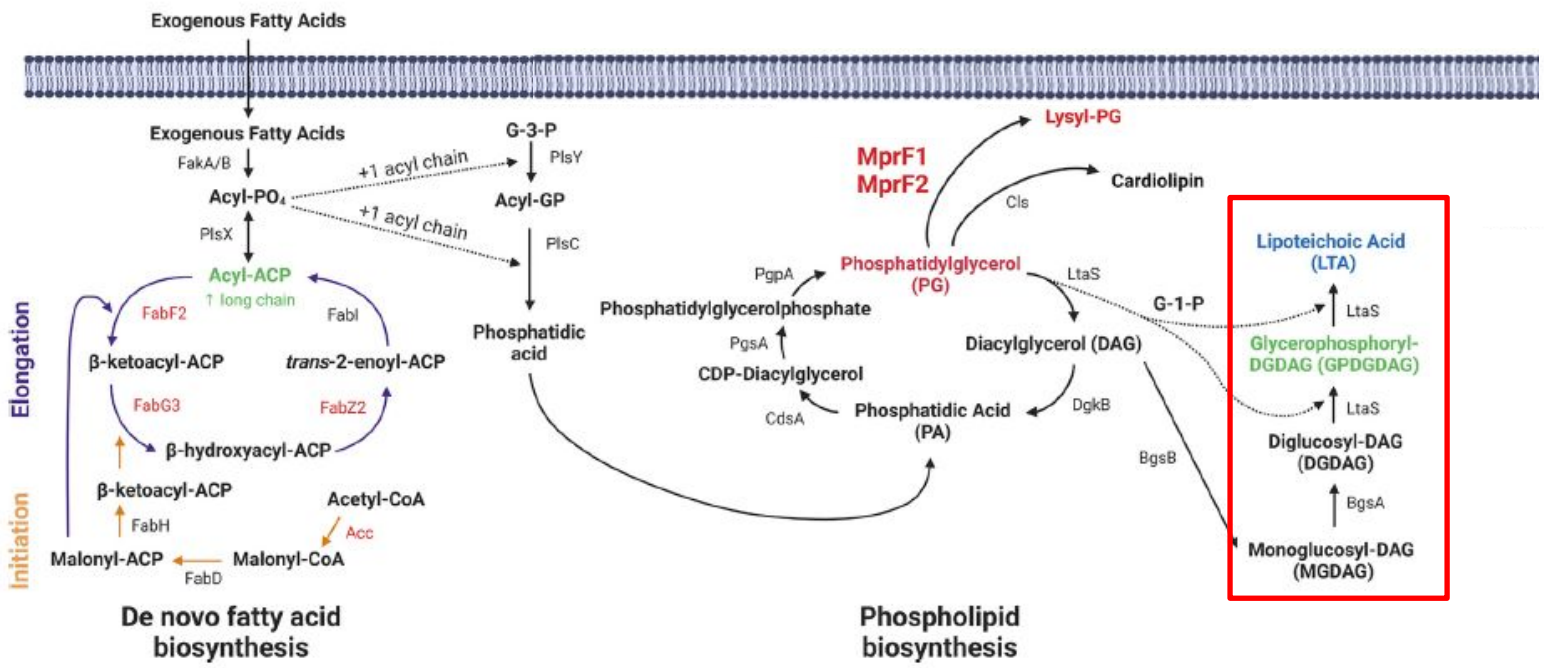
Cardiolipin (CL)

Phospholipid synthesis



mBio. 2023 Jan 11;e0307322.

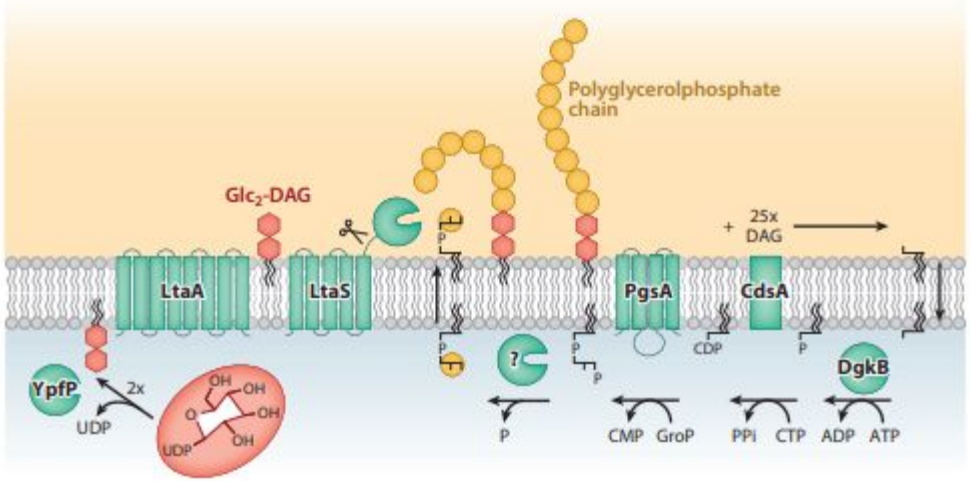
Phospholipid synthesis



mBio. 2023 Jan 11;e0307322.

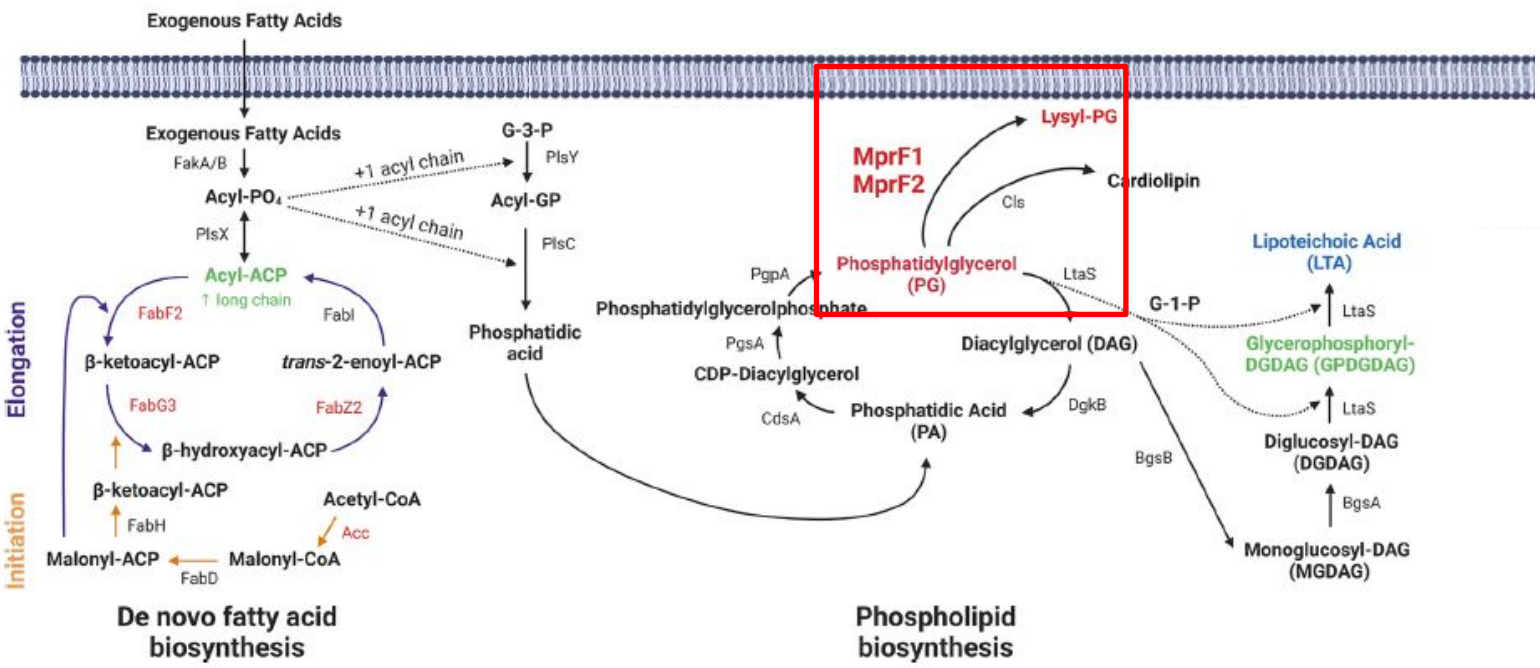
Lipoteichoic acid synthesis

- Closely linked to lipid biosynthesis
- Each elongation with glycerolphosphate consumes one phosphatidylglycerol(PG) molecule
- Estimated that *S. aureus* will turn over the entire PG pool twice to support LTA synthesis each generation



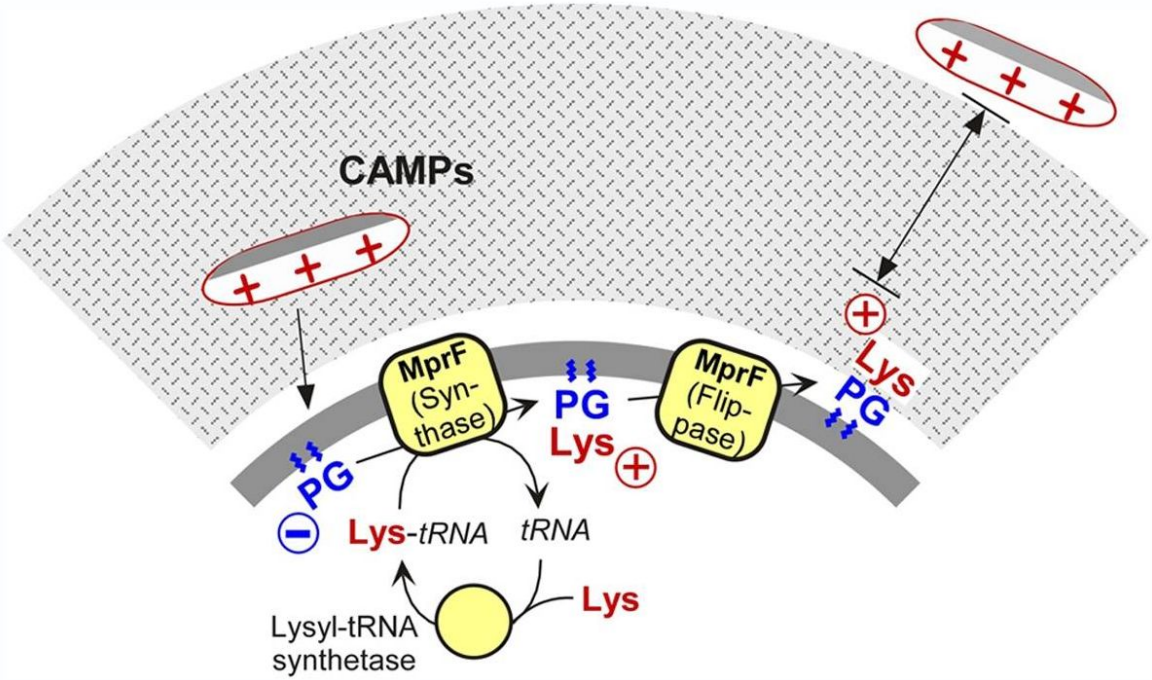
Annu Rev Microbiol. 2014;68:81-100.

Phospholipid synthesis



mBio. 2023 Jan 11;e0307322.

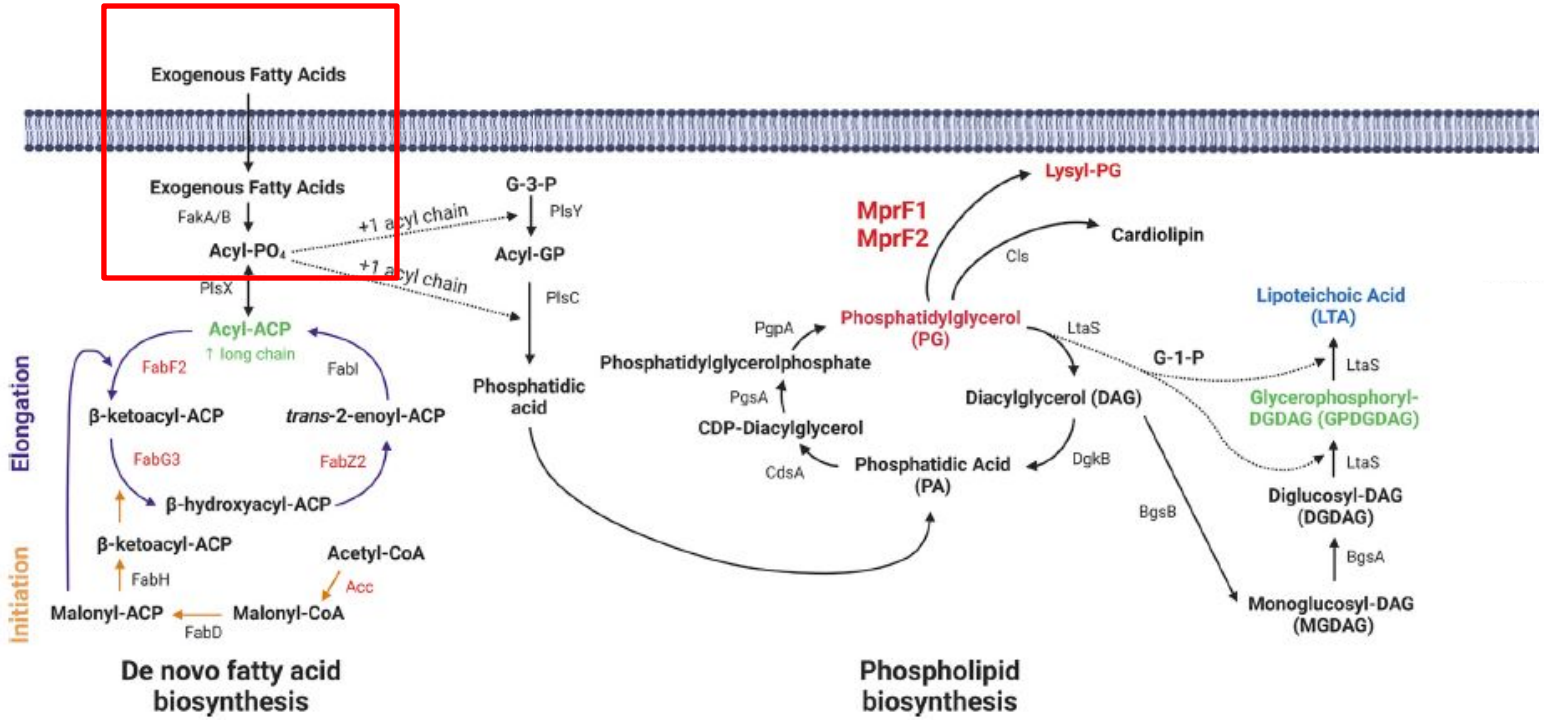
MprF and the repulsion hypothesis



- MprF – Multiple peptide resistance factor
- Catalyzes transfer of lysine from Lys-tRNA to phosphotidylglycerol
- Alters cell surface charge and masks anionic phosphotidylglycerol
- Can change membrane composition and fluidity

PLoS Pathog. 2009

Phospholipid synthesis



mBio. 2023 Jan 11;e0307322.

Exogenous fatty acids protect against membrane stress

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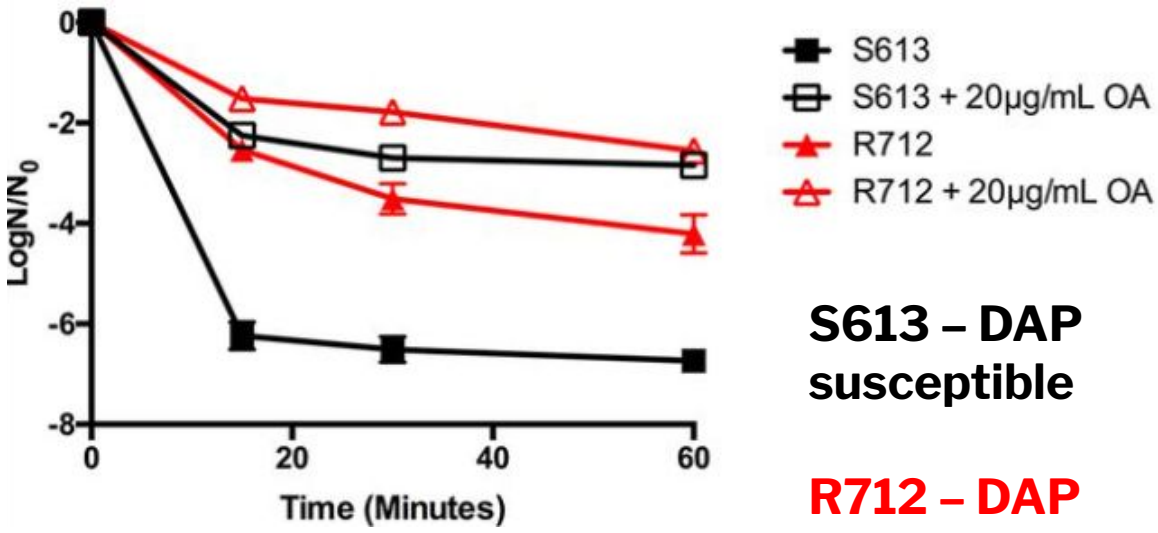

S613 – DAP susceptible

R712 – DAP resistant

- Decreased killing with daptomycin seen with oleic acid ($C_{18:1\ cis\text{-}9}$) and linoleic acid ($C_{18:2\ cis\text{-}9,12}$)
- These lipids are derived from eukaryotic cells
- Supplementation with exogenous fatty acids altered membrane lipid acyl chains

Appl Environ Microbiol. 2016 Jun 30;82(14):4410-4420.
Appl Environ Microbiol. 2018 Jan 1; 84(1): e01633-17.

Case Study

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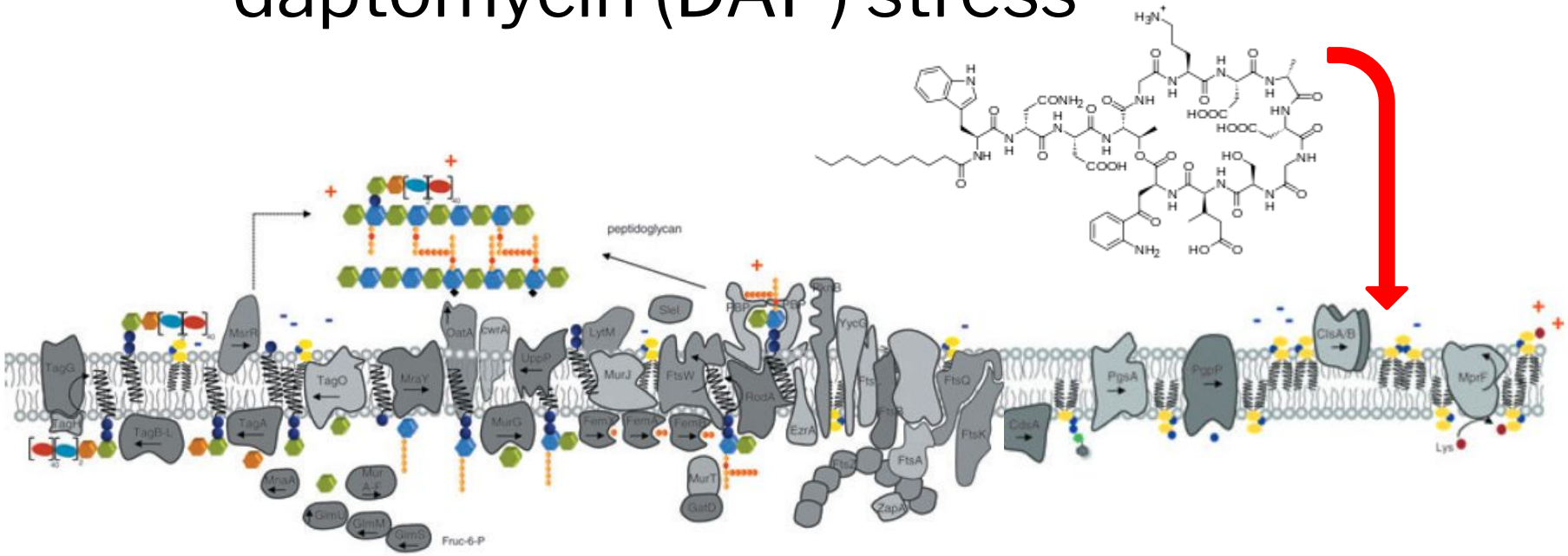
E. faecalis Cell envelope adaptation to daptomycin (DAP) stress



Bayer AS, Ann NY Acad Sci 2013

Daptomycin – mechanism of action

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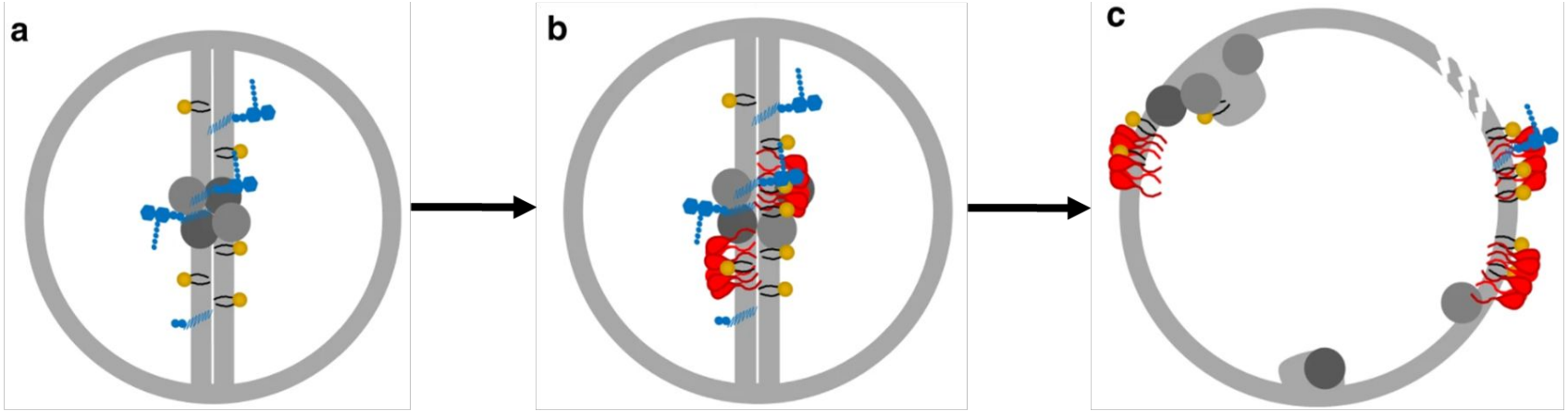

Septal synthesis

DAP binding at septum

Enzyme delocalization and loss of cell envelope integrity

Nat Commun. 2020 Mar 19;11(1):1455.

Pathways linked to DAP-R in enterococci

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Two Component Signaling Systems

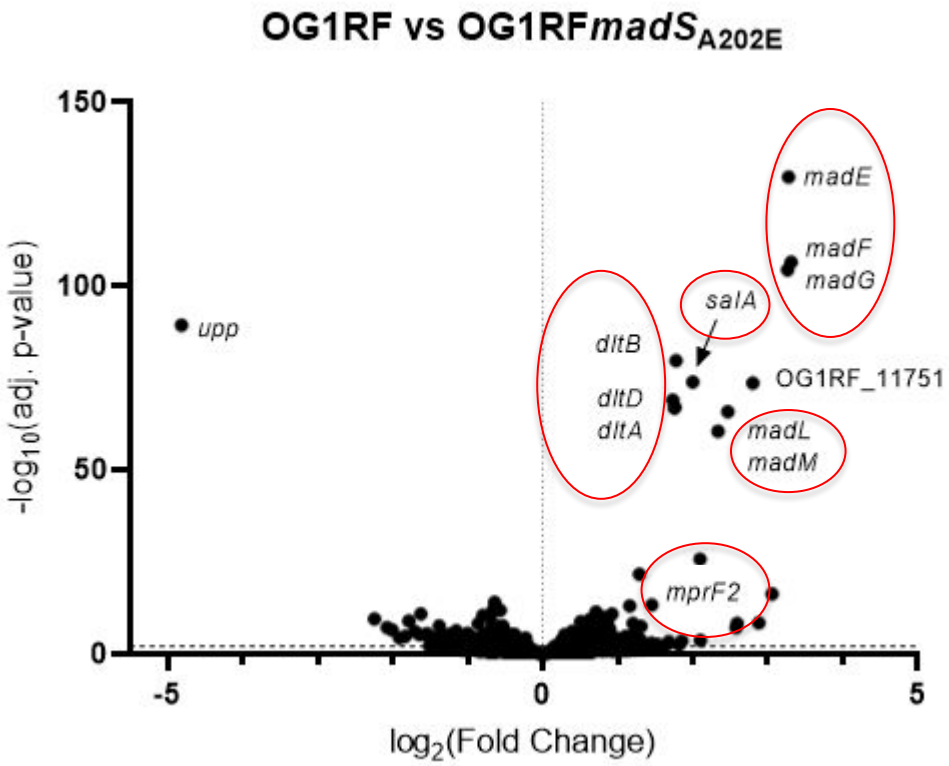
- LiaFSR
- YycFG (WalKR)
- YxdJK/YxdLM/YvcRS (MadRS)

Lipid metabolism

- Cardiolipin Synthase (Cls)
- GdpD (glycerophosphoryldiester phosphodiesterase)
- Cfa (cyclopropane fatty acid synthase)
- Dak (fatty acid kinase)

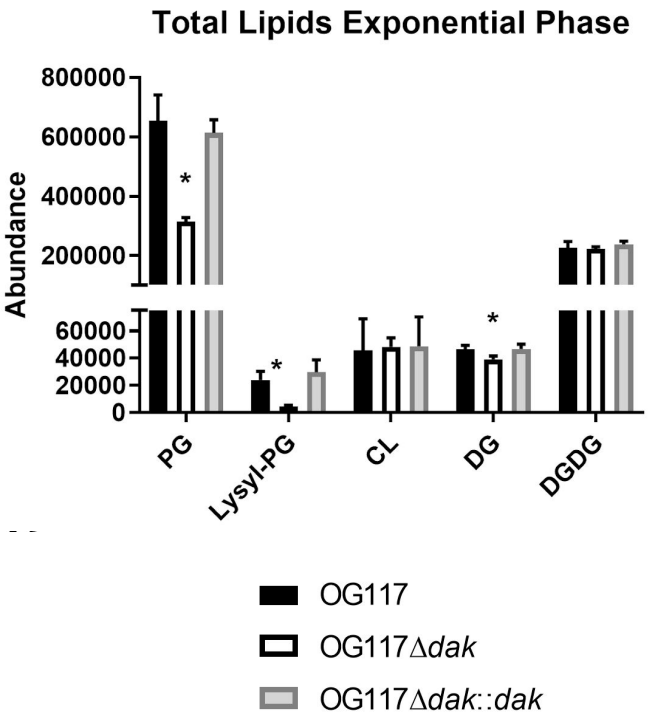
N Engl J Med. 2011 Sep 8;365(10):892-900.
Antimicrob Agents Chemother. 2013 Jan;57(1):261-8.

Activation of MadRS regulon



- Ala->Glu at position 202 leads to activation of MadS histidine kinase
- Upregulation of genes:
  - Target protection UPP
  - Target protection against antimicrobial peptides
  - D-ala of LTA
  - Lys-PG MprF
  - Peptidoglycan hydrolase

Loss of Dak is associated with alterations in membrane lipids



- Decreased levels of phosphatidylglycerol during exponential growth
- Decreased growth rate
- Increased membrane rigidity

Fatty acid profile in Dak mutant

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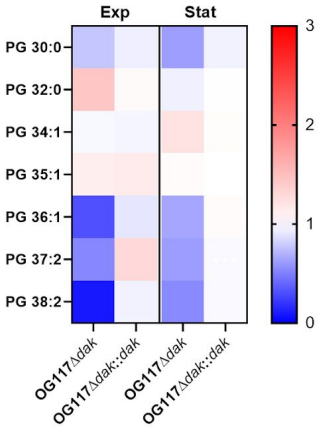
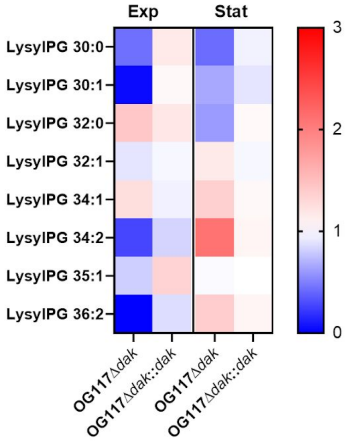
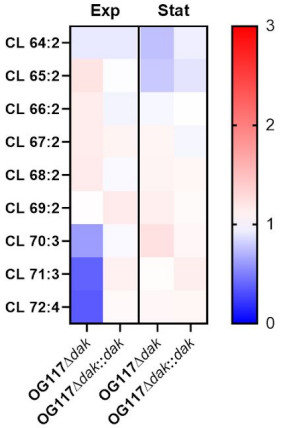
PG



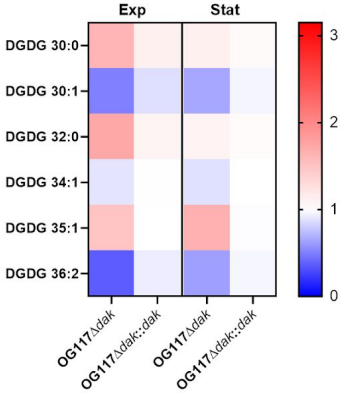
LPG



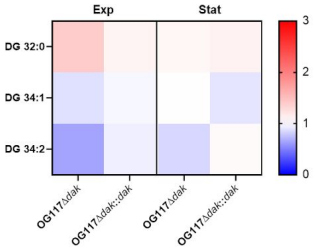
CL



DGDG



DG



Loss of *dak* is associated with decrease in unsaturated long chain fatty acids

MadRS System

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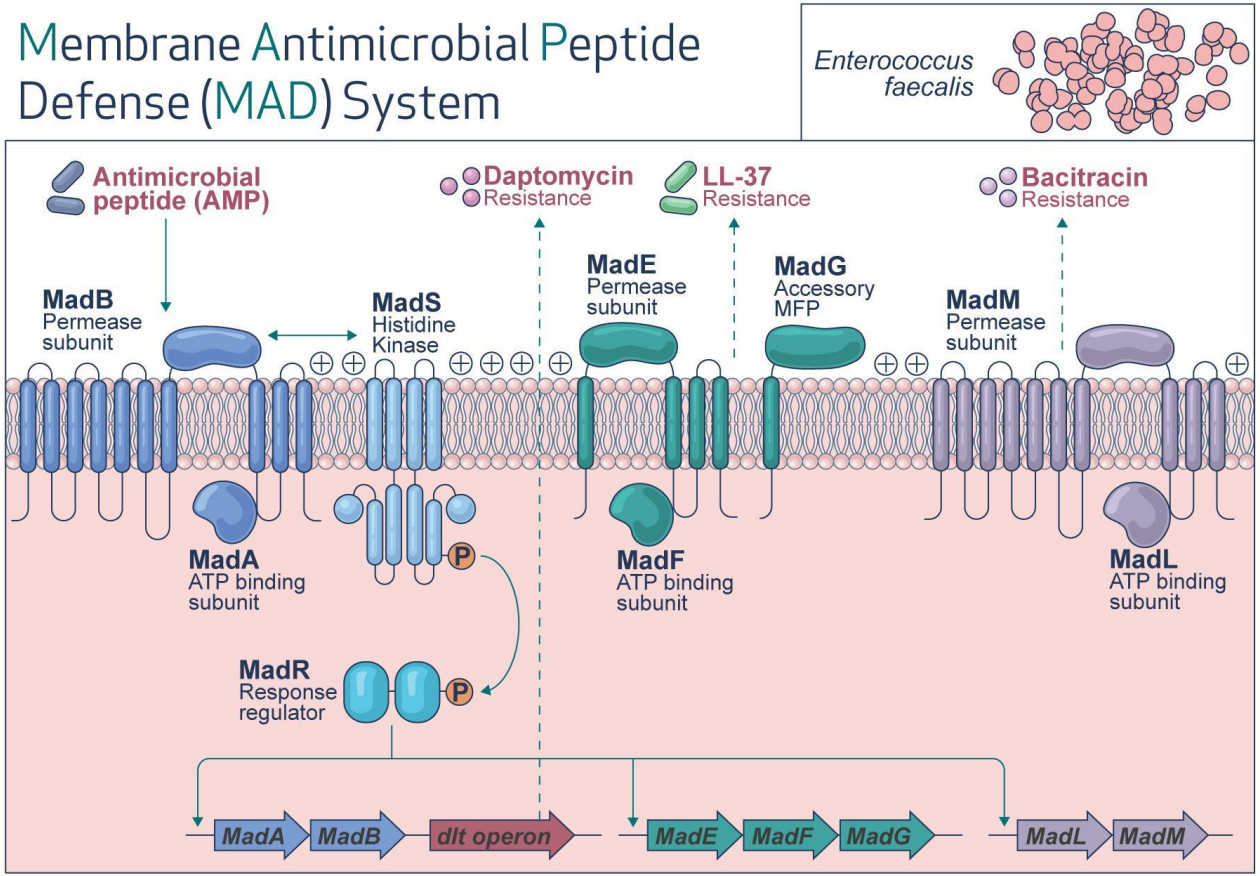

Summary

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- The Gram-positive bacterial cell envelope is a complex, interwoven machine
- Bacteria possess a variety of stress response systems to respond to antibiotics and environmental stressors
- A potent resistance phenotype can emerge via activation of one or more of these pathways

Acknowledgements

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Jinnethe Reyes

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Questions?

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