Mechanisms of Daptomycin Resistance and the Seesaw Effect in Multi-Drug Resistant Enterococci

NAME OF STUDENT

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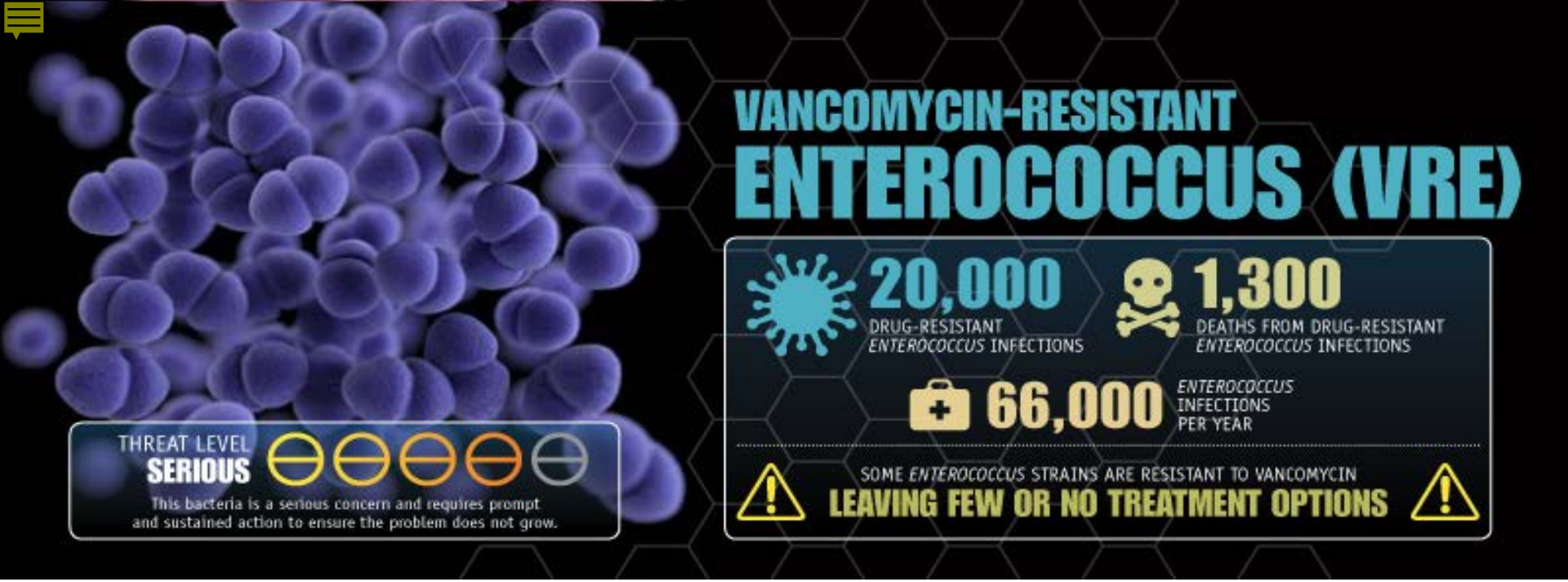
THE GRADUATE SCHOOL OF BIOMEDICAL SCIENCES
GSBS
SYNERGY IN SCIENCE

MMG
Microbiology and Molecular Genetics

UTHealth
The University of Texas Health Science Center at Houston

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

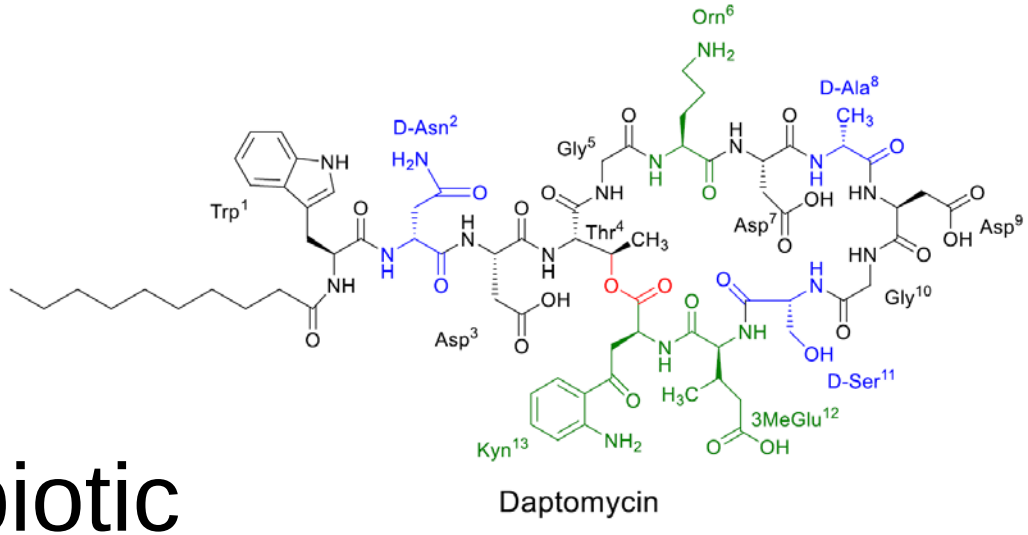
Graduate School of Biomedical Sciences



- Major nosocomial pathogen
- Endocarditis, bacteremia, UTIs, meningitis
- High intrinsic resistance to antibiotics (aminoglycosides, cephalosporins, beta-lactams)
- High genetic plasticity

http://www.cdc.gov/drugresistance/biggest_threats.html

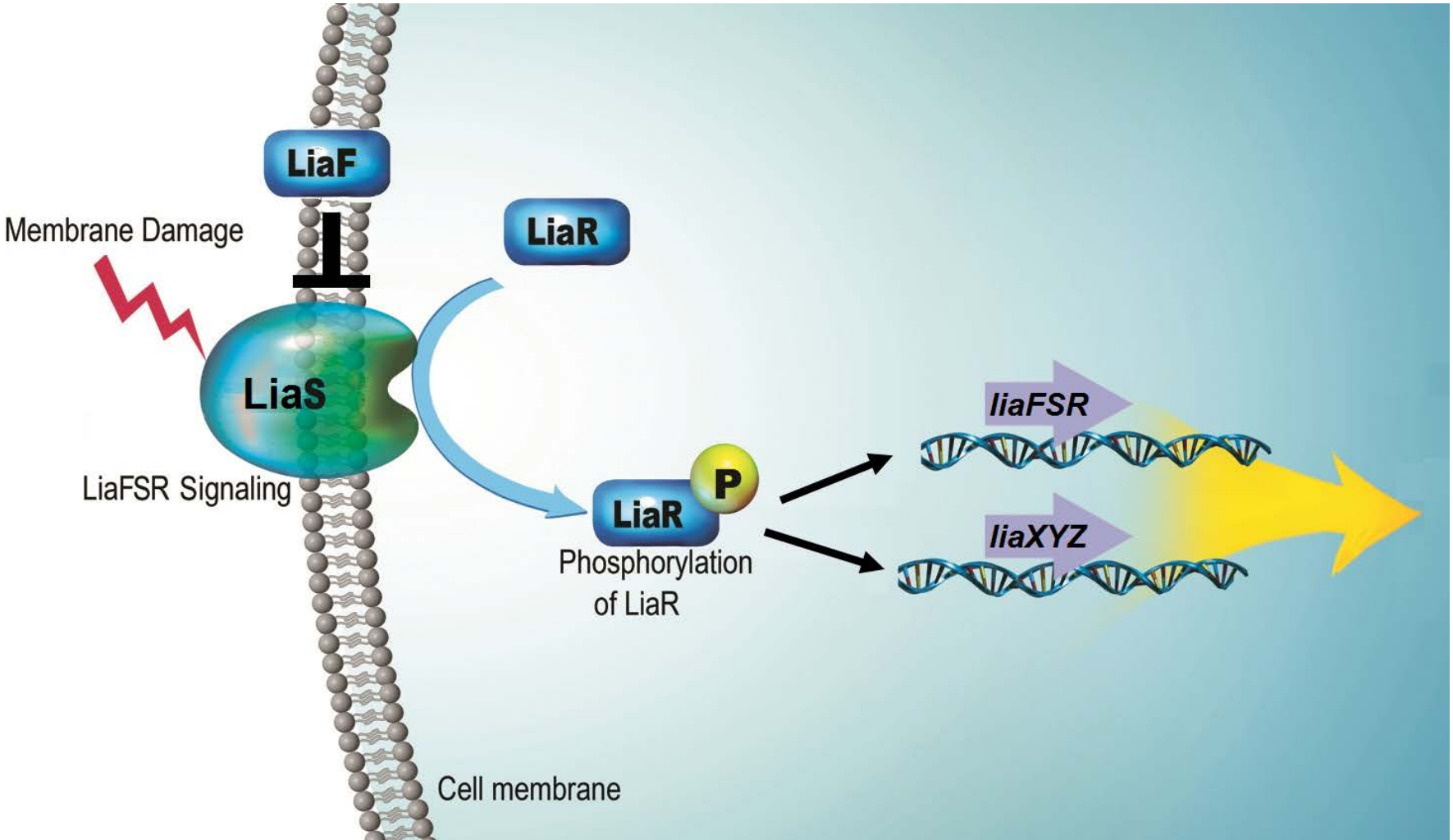
Daptomycin



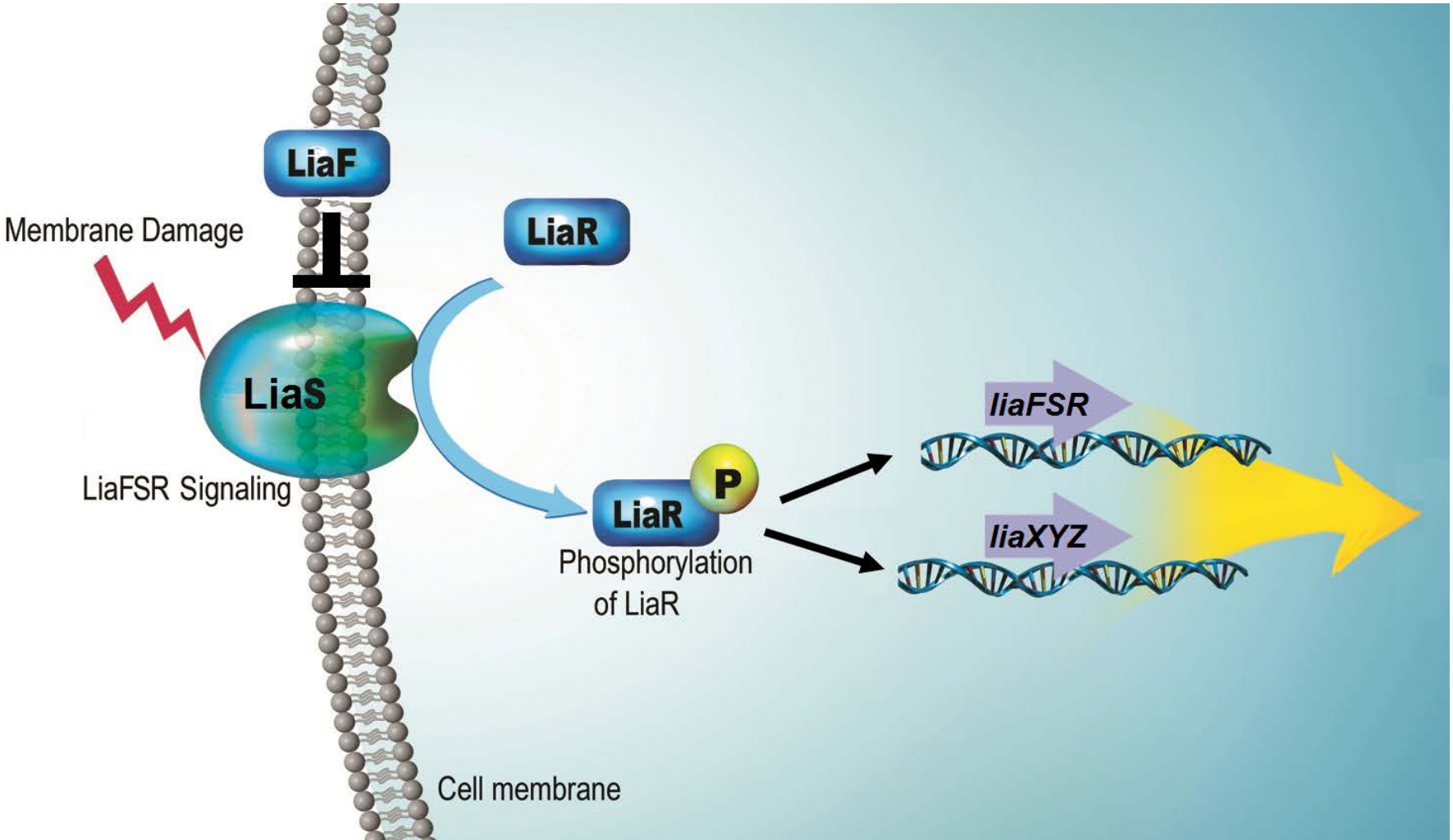
Daptomycin

- Lipopeptide antibiotic
- Used as a ”last resort” for MDR-enterococcal infections (Breakpoint MIC= 4µg/ml)
- Observed clinical resistance in VRE
- Disrupts cell membrane integrity

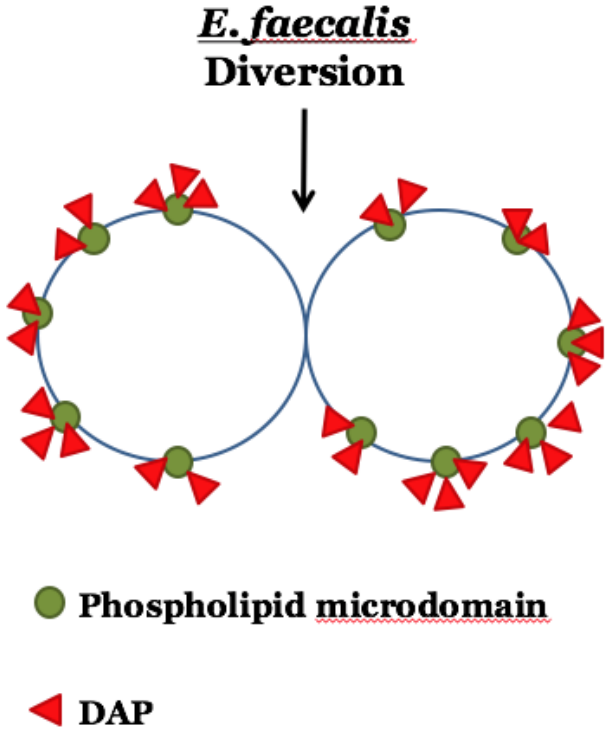
The LiaFSR system regulates DAP-R in enteroccoci



liaXYZ are effectors of the LiaFSR stress response



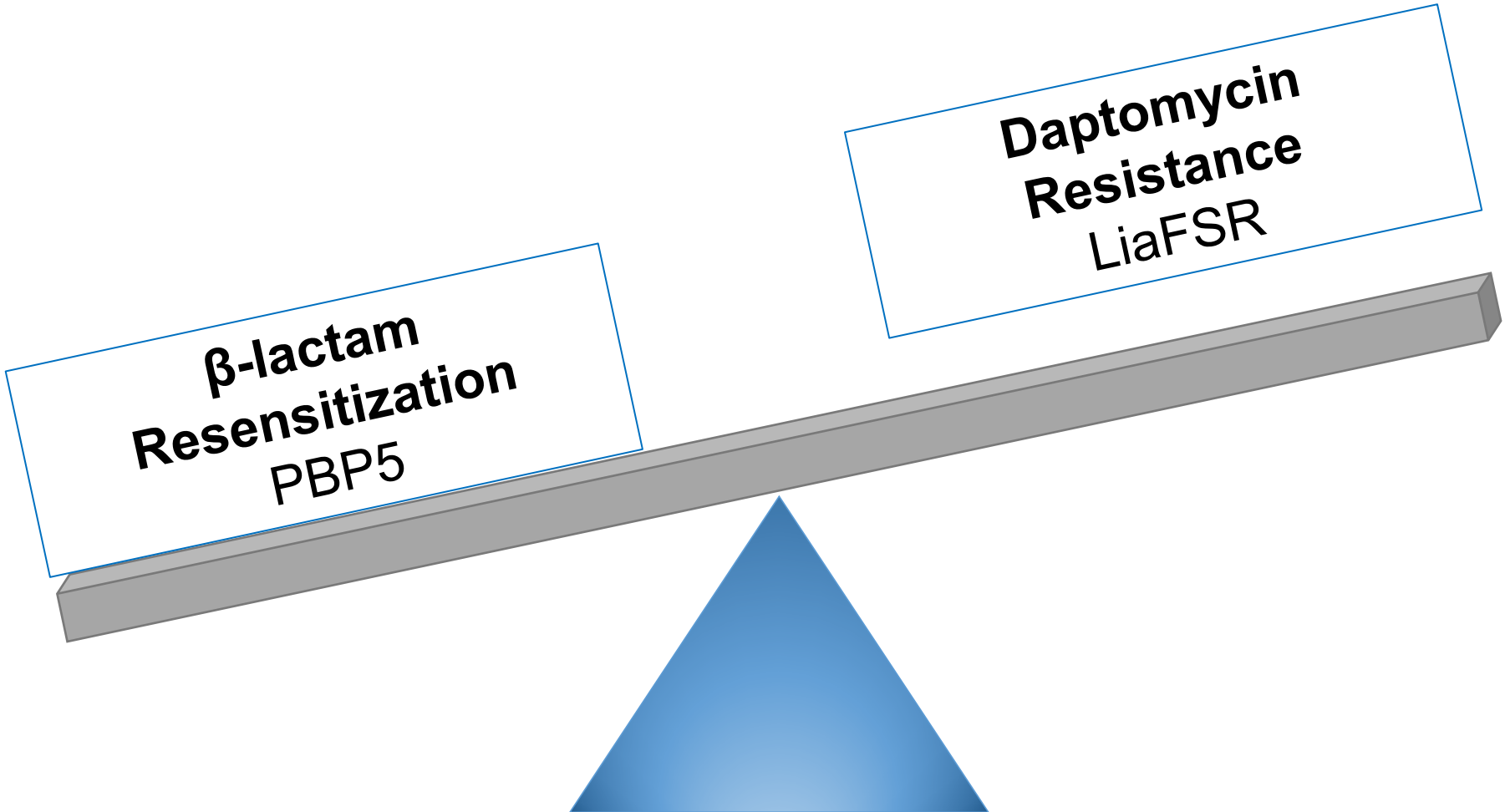
DAP-R leads to redistribution of anionic phospholipids



NAO Staining= Visualization of enriched anionic PL microdomains (Cardiolipin)

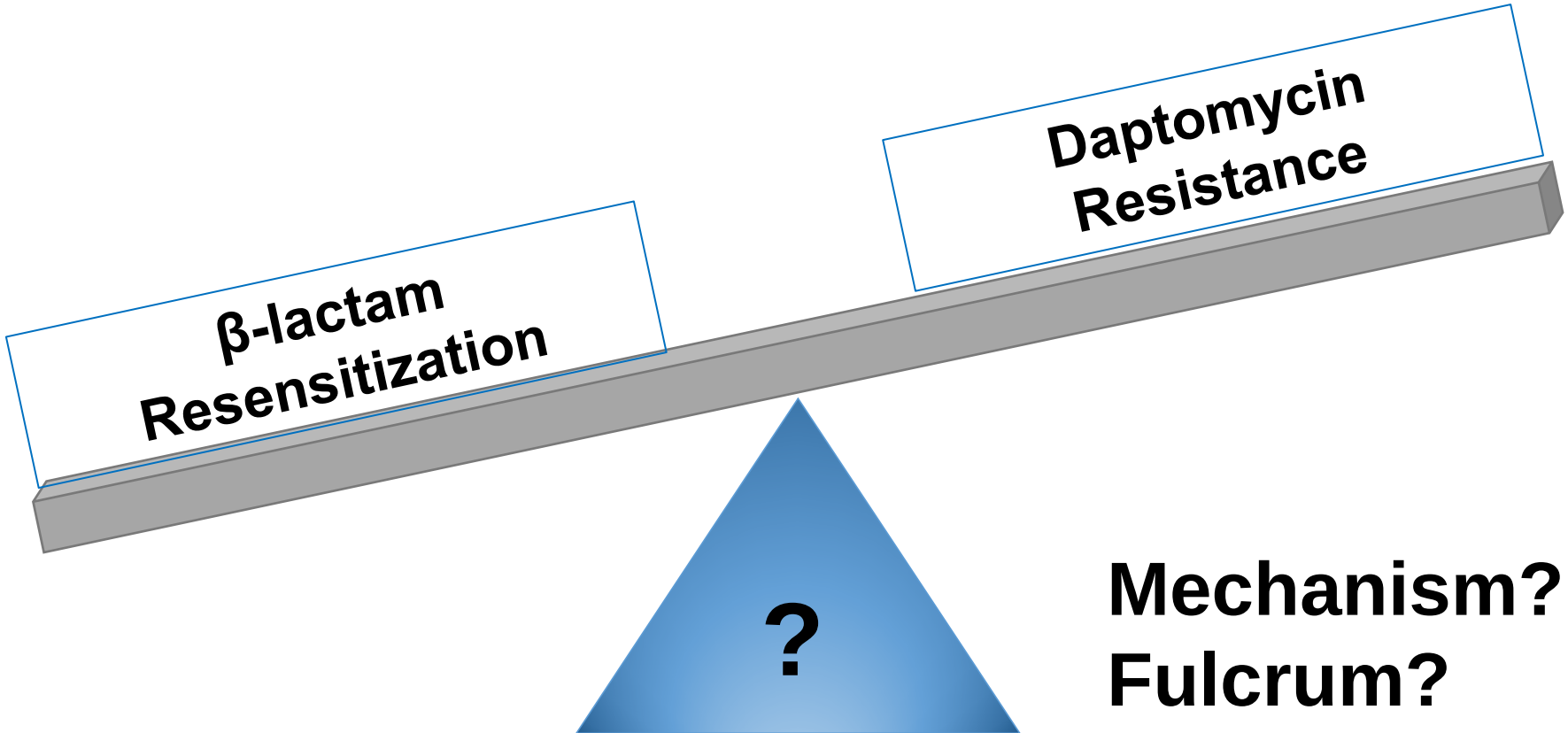
Tran T, et al. MBio. 2013 Jul 23;4(4). pii: e00281-13.

The Seesaw Effect- *Efs*, *Efm*, *MRSA*



The Seesaw Effect- *Efs*, *Efm*, *MRSA*

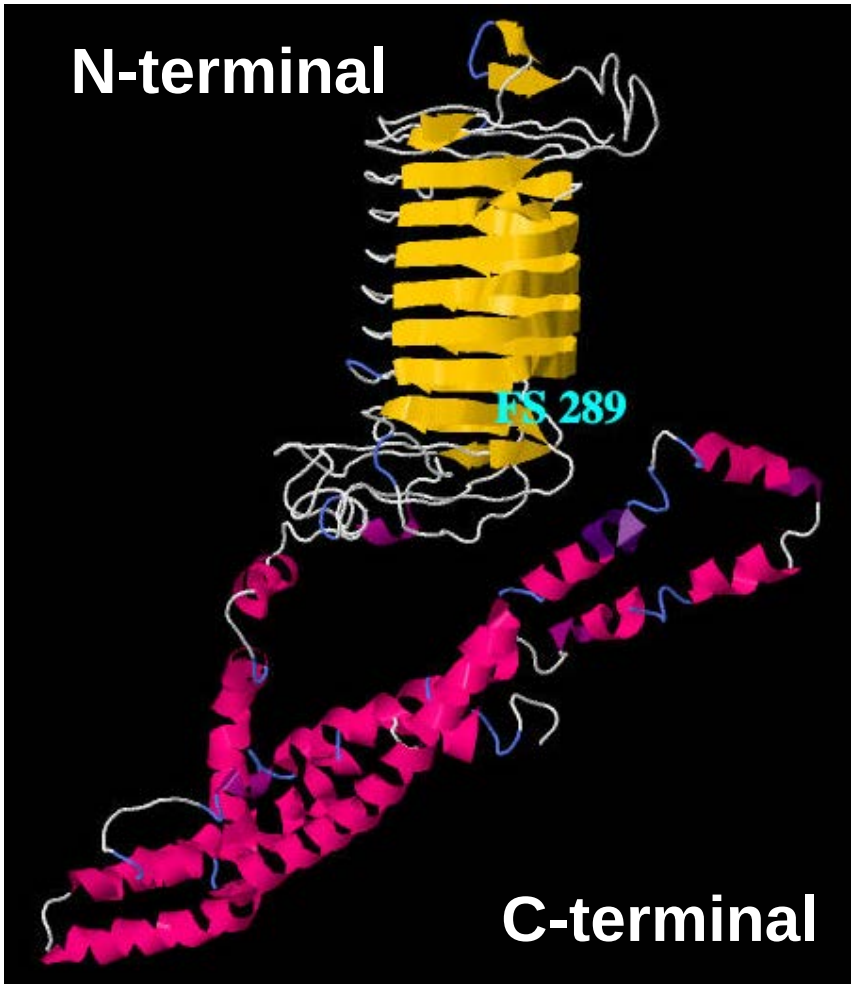
Exploited in combination therapy with DAP + β-lactam for severe MDR infections



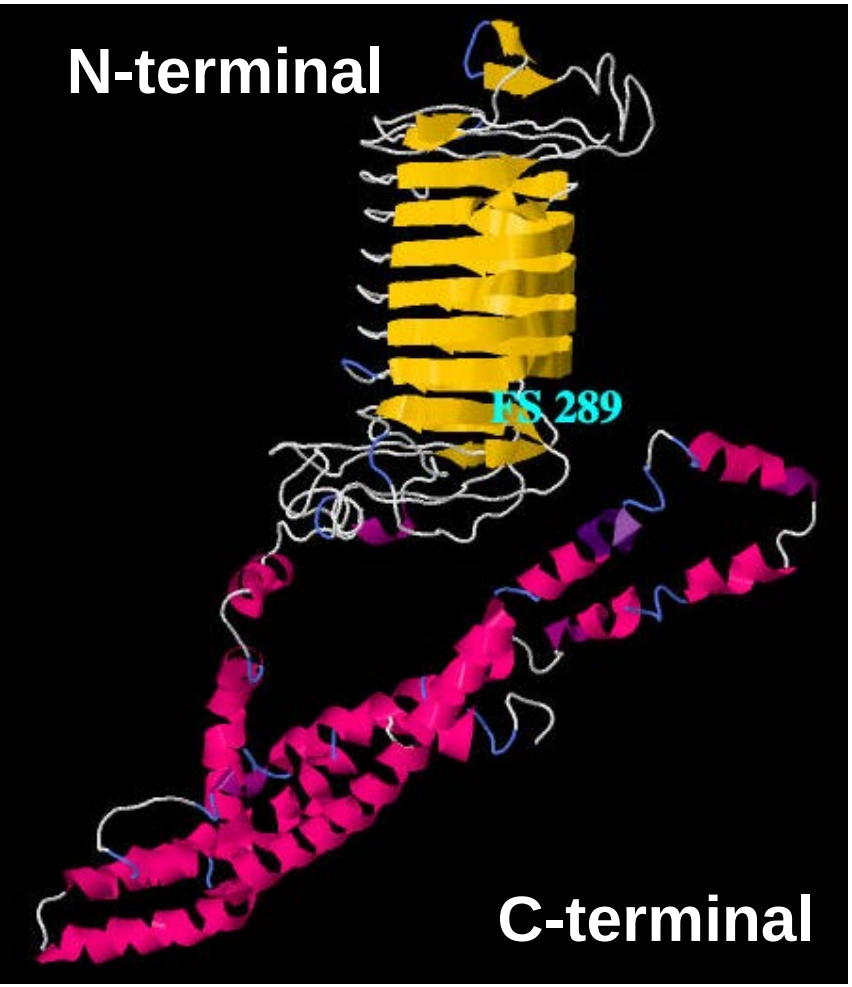
Mechanism?
Fulcrum?

Overall Goal: Characterize LiaX and determine its role in antibiotic resistance

- 533 AA
- Surface exposed
- Mutations present in DAP-R clinical strains
- Evolutionary adaptation of DAP-S clinical strain-– Ct truncation of liaX (fs AA 289) sufficient for high level resistance



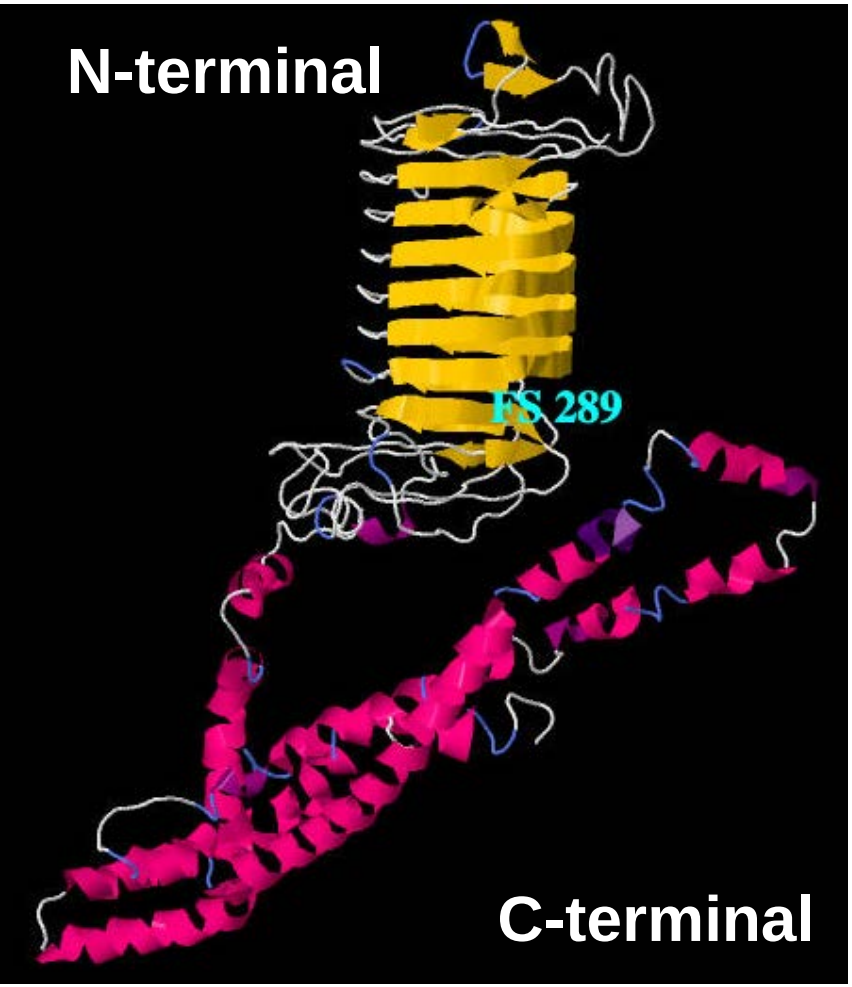
Overall hypothesis



LiaX is a multifunctional protein that

→Regulates daptomycin resistance through negative inhibition of liaYZ

Overall hypothesis



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→Activates the liaFSR system in the presence of extracellular stress

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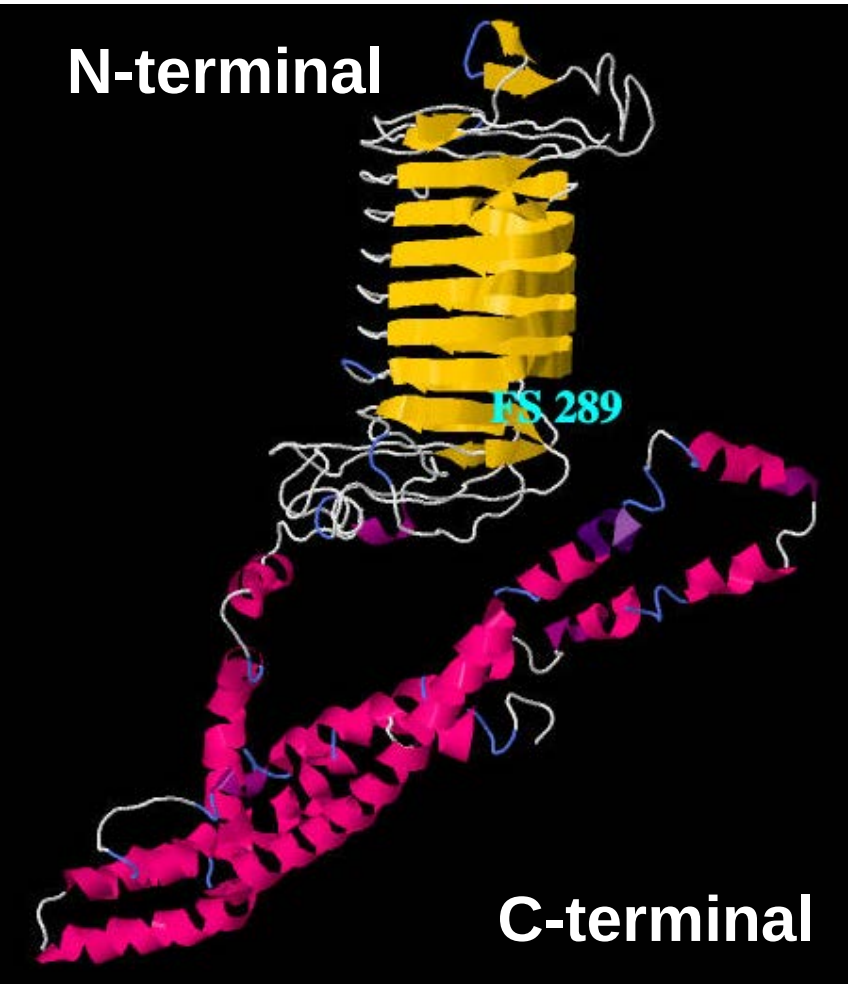

LiaX is a multifunctional protein that

→Regulates daptomycin resistance through negative inhibition of liaYZ

→Activates the liaFSR system in the presence of extracellular stress

→Modulates the seesaw effect through interactions with PBP5

Aim 1: Characterize the localization of LiaX as it pertains to the CE stress response to AMPs

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1. Evaluate LiaX protein levels and localization under DAP stress and upon the development of resistance

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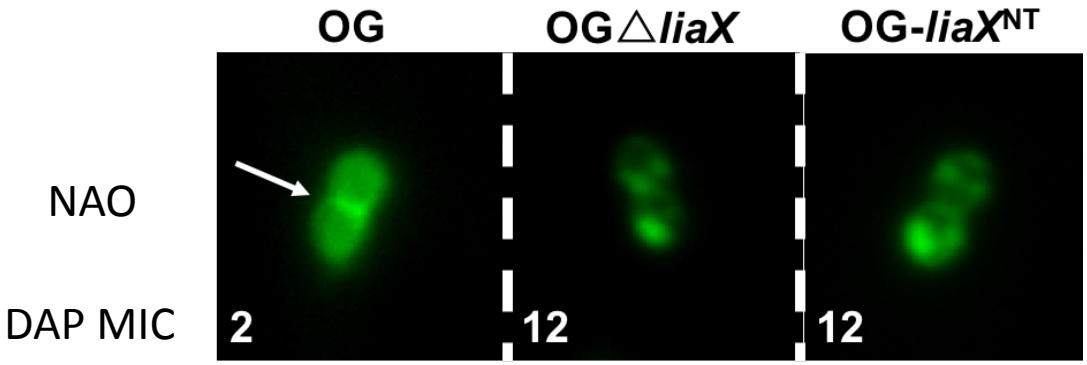
2. Determine the role of LiaX in resistance to AMPs *in vitro* and *in vivo*

Aim 1: Characterize the localization of LiaX as it pertains to the CE stress response to AMPs

1. Evaluate LiaX protein levels and localization under DAP stress and upon the development of resistance
2. Determine the role of LiaX in resistance to AMPs *in vitro* and *in vivo*
3. Assess if extracellular LiaX can protect DAP-S strains from antibiotic attack by activating the liaFSR stress response

Aim 1 Preliminary Data



LiaX (with the Ct alone) negatively regulates DAP-R and CM remodeling

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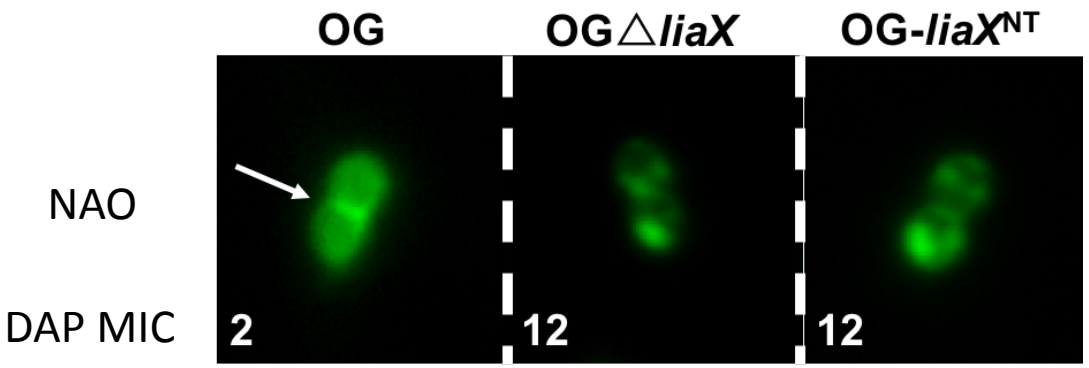
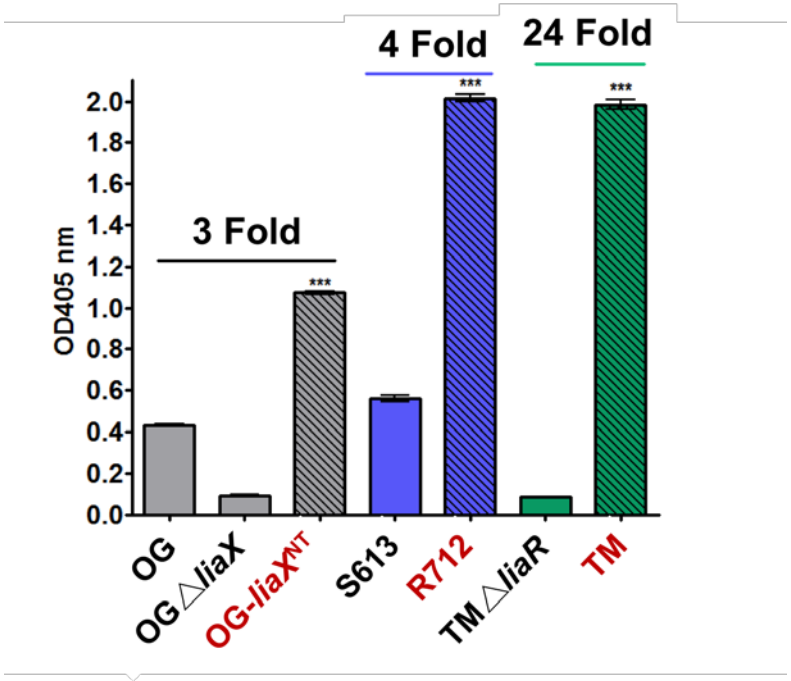
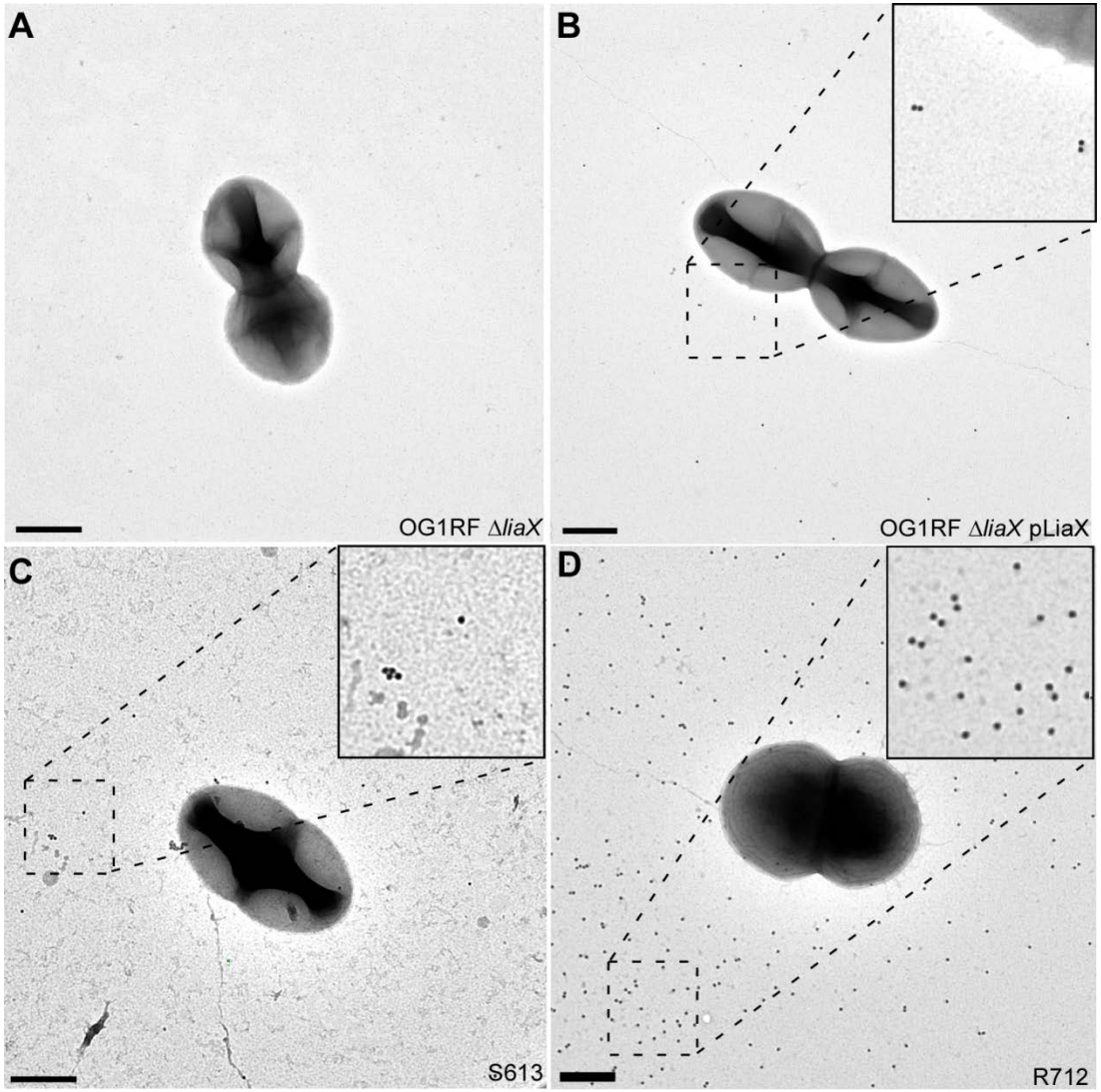


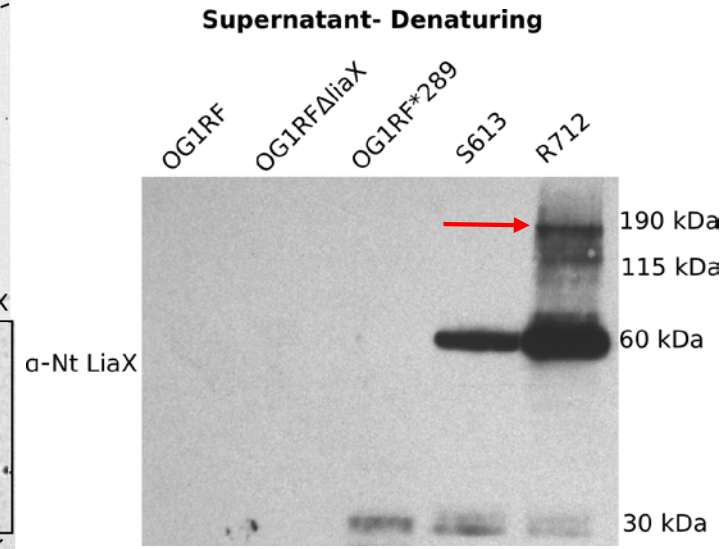
LiaX (with the Ct alone) negatively regulates DAP-R and CM remodeling

Whole-cell ELISA



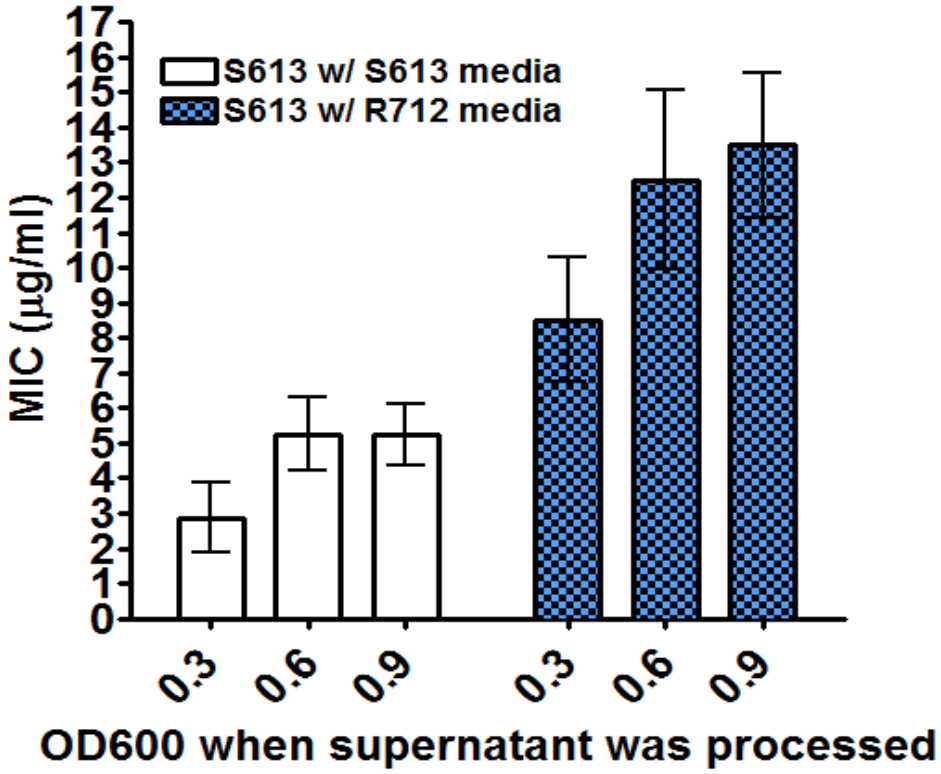
Extracellular LiaX in DAP-R strains





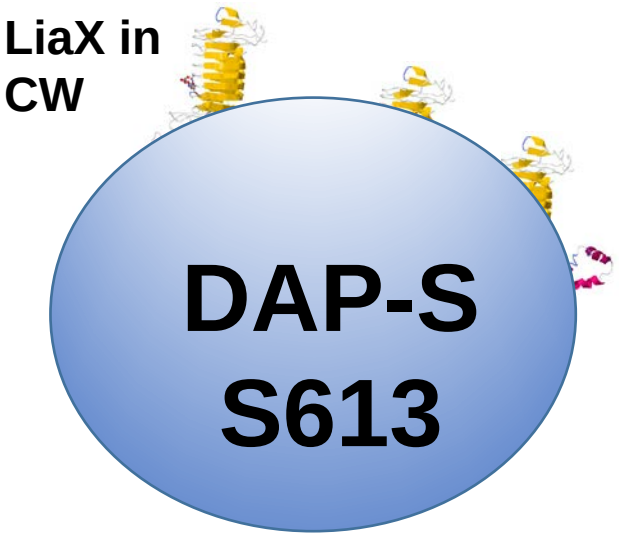
Scale bar 0.5 μM. Secondary antibody conjugated to 18 nM gold particles.

DAP-R spent media protects DAP-S strain



LiaX binds DAP (Kd= 0.05uM)

Localization hypothesis



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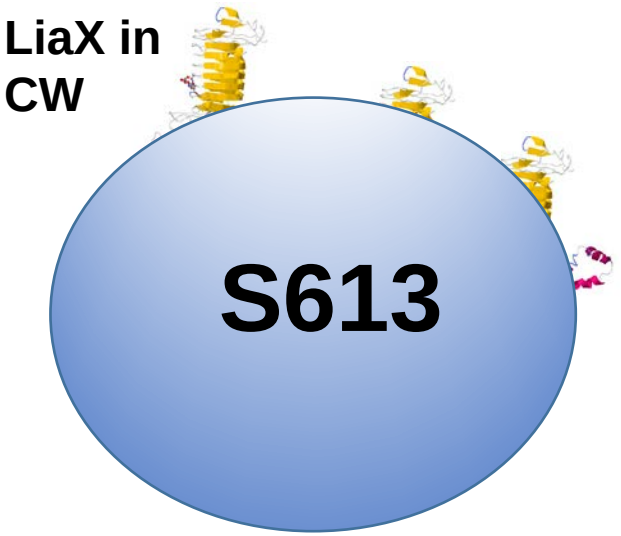
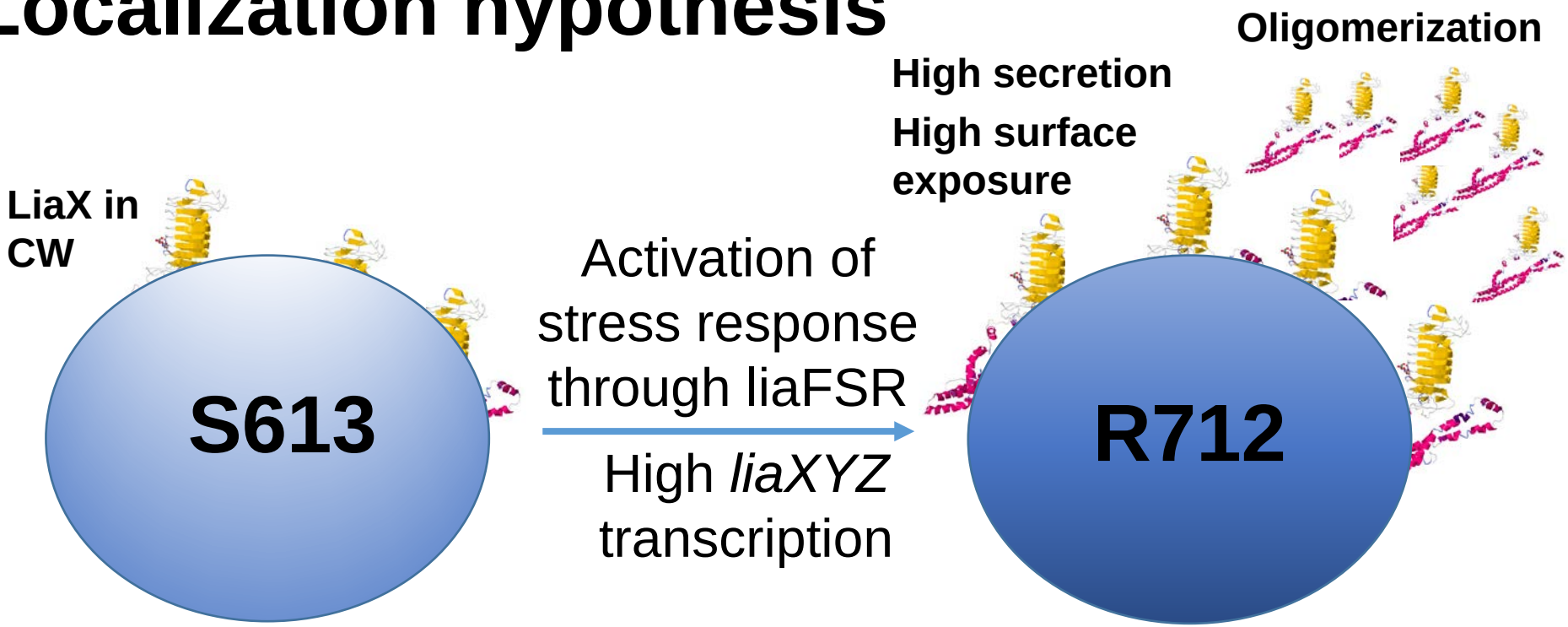


Activation of stress response through liaFSR

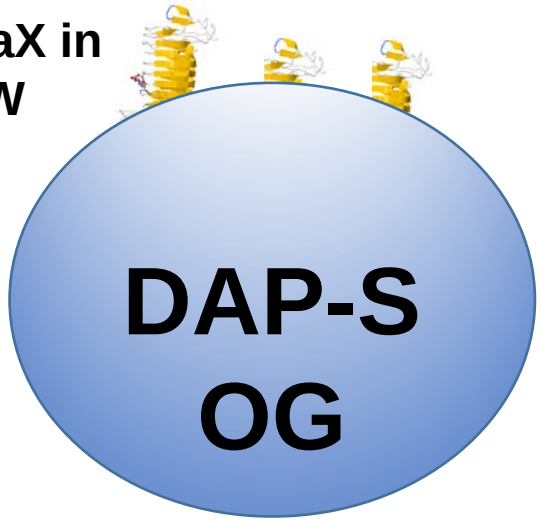
High *liaXYZ* transcription

Localization hypothesis



Localization hypothesis

LiaX in CW



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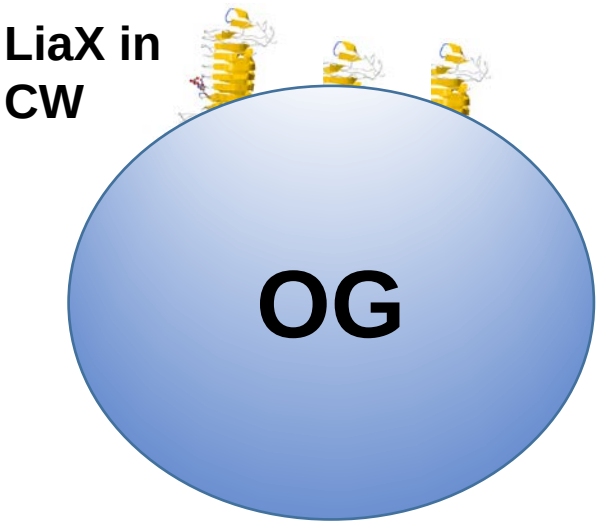
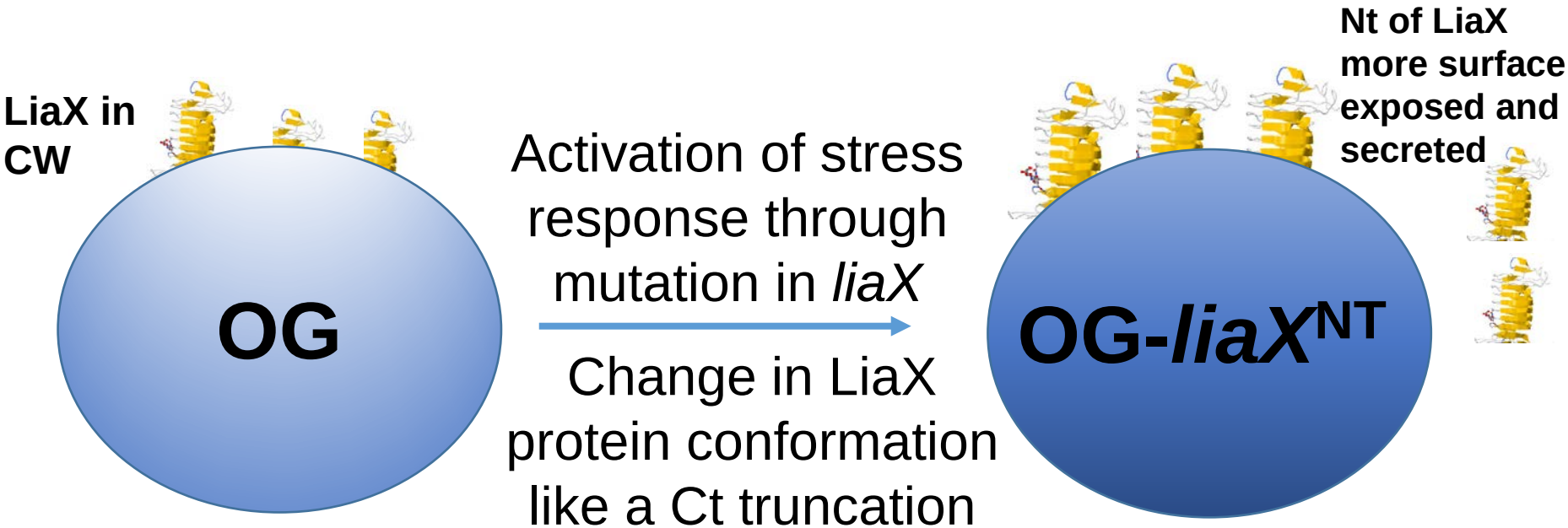


Activation of stress response through mutation in *liaX*

Change in LiaX protein conformation like a Ct truncation

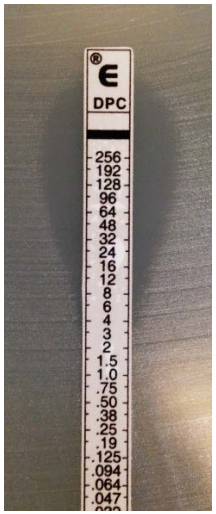
Localization hypothesis



AMP resistance hypothesis

DAP-R

MIC 8



ΔLiaR

Susceptible
DAP
LL37
Nisin
HBD 3
Broad spectrum

Reyes J, et al. *J Infect Dis*, 2015; Panesso D, et al. *Antimicrob Agent Chemother*, 2015

AMP resistance hypothesis

DAP-R

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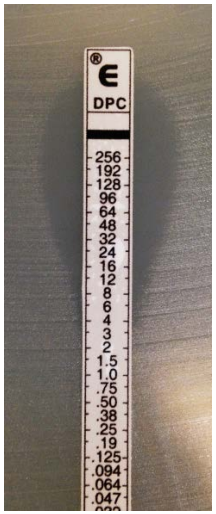

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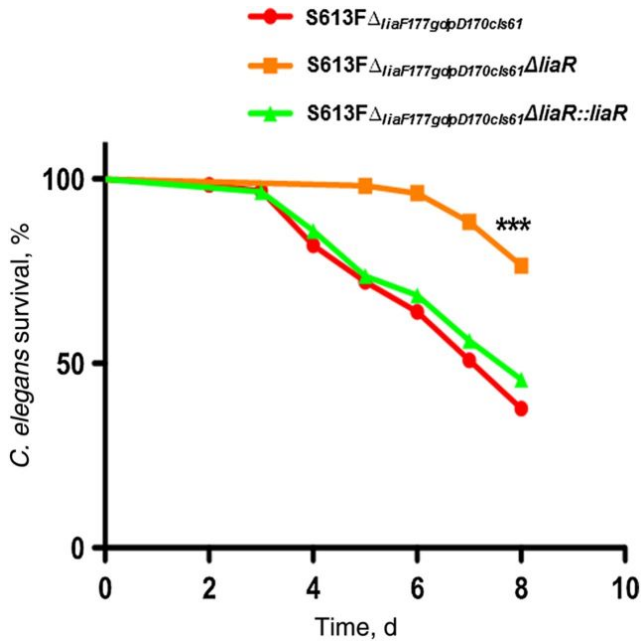

AMP resistance hypothesis

DAP-S

MIC 2

ΔLiaX

ΔLiaX-Ct

Resistant

DAP

LL37

Nisin

HBD3

Broad Spectrum

AMP resistance hypothesis

DAP-S

MIC 2

ΔLiaX

ΔLiaX-Ct

Resistant
DAP
LL37
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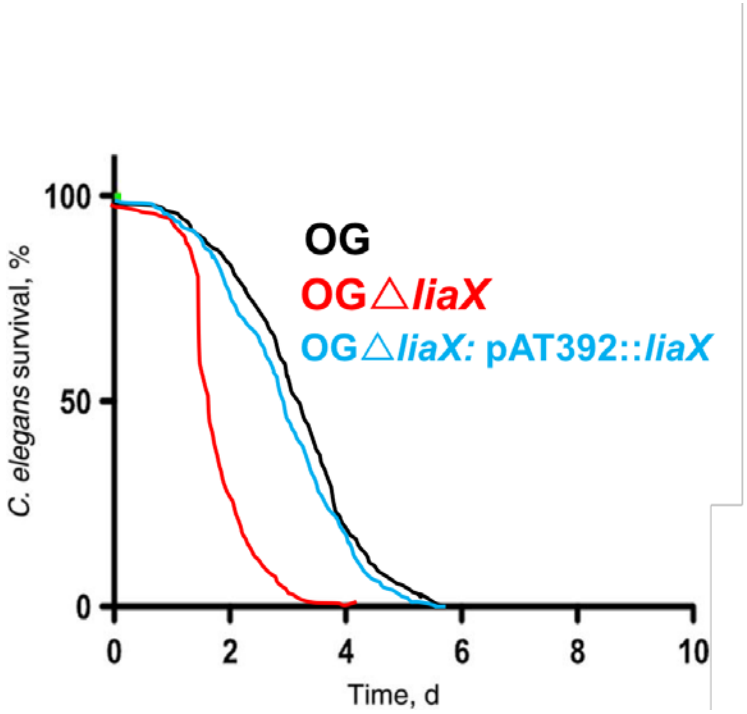
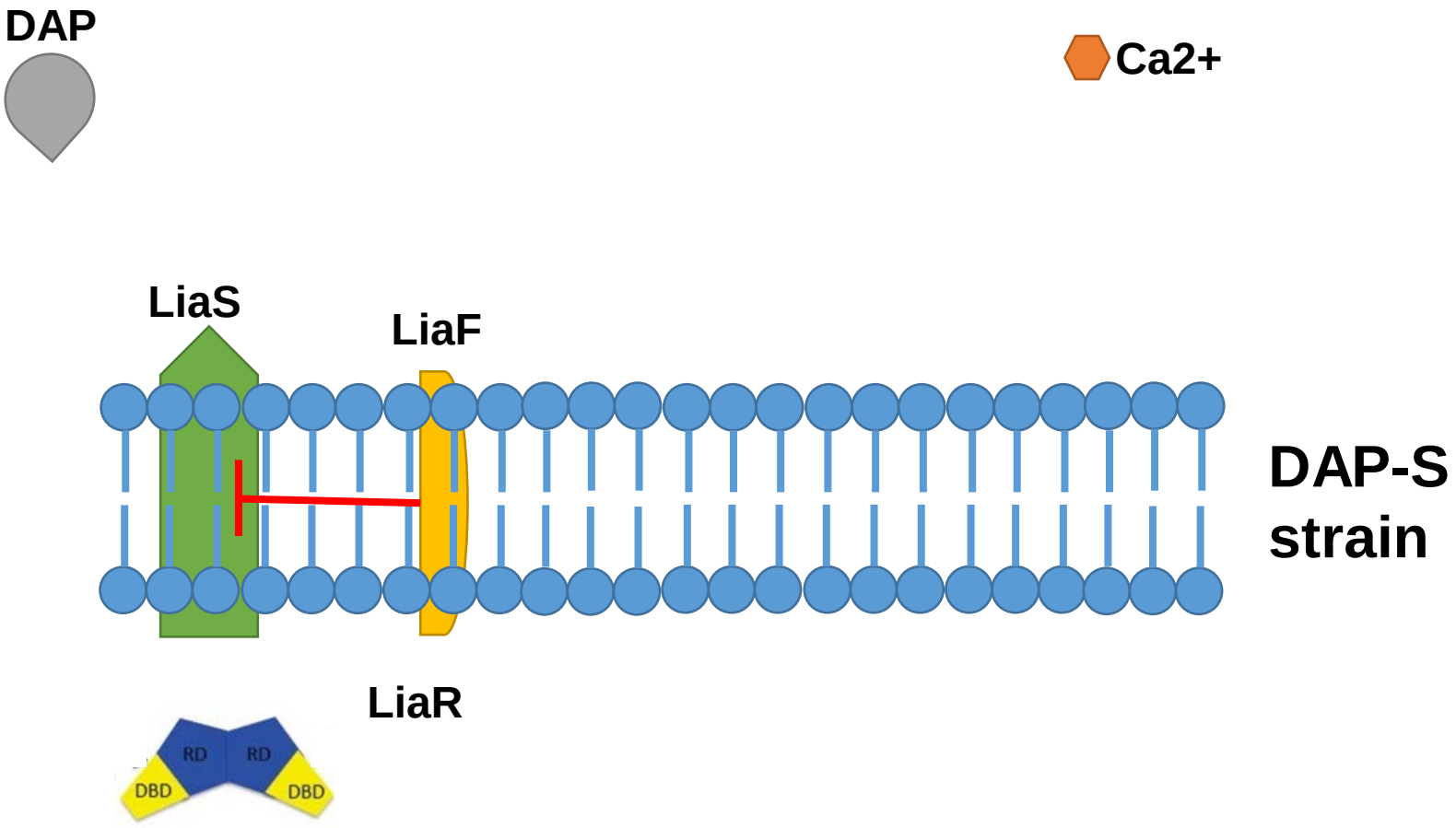
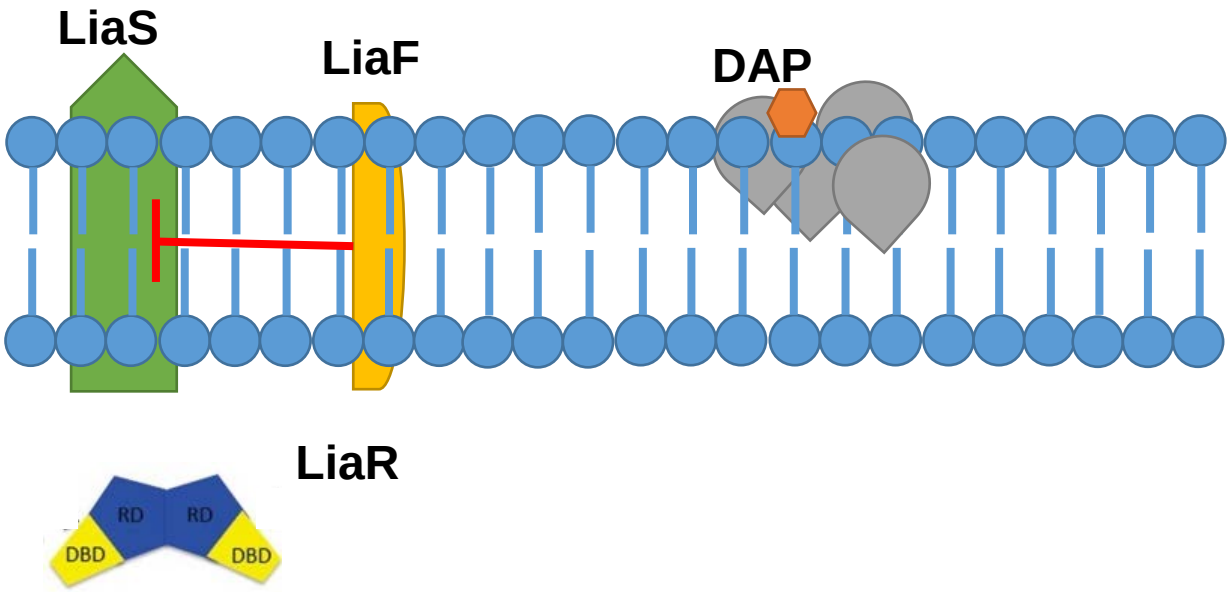
Broad
Spectrum



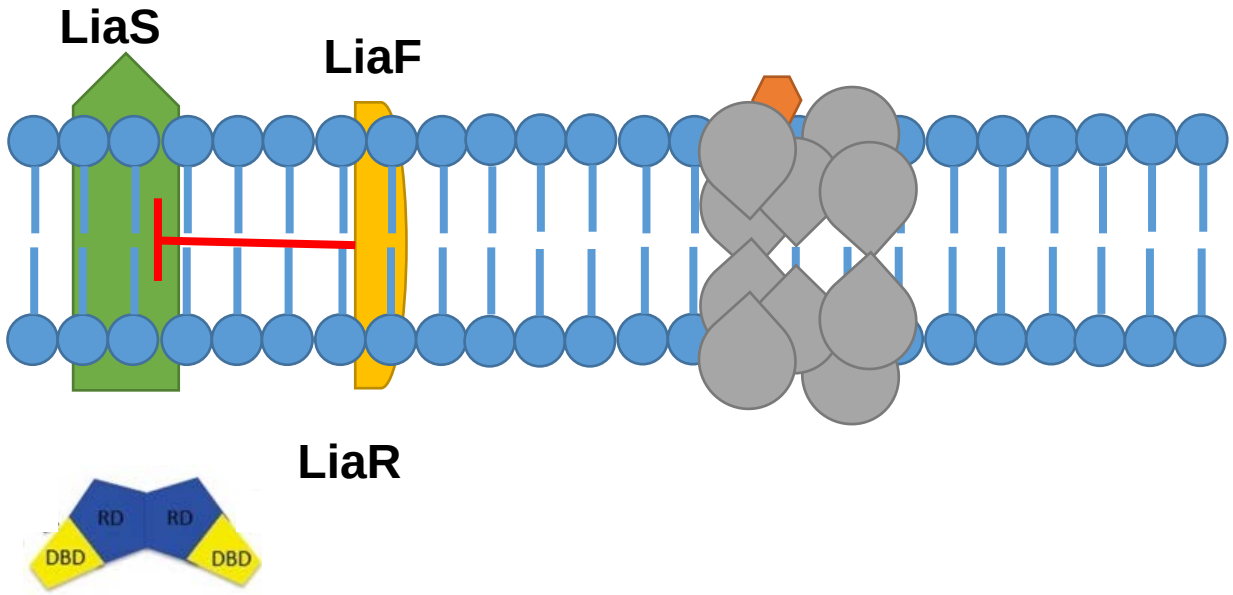
DAP attack on a DAP-S strain



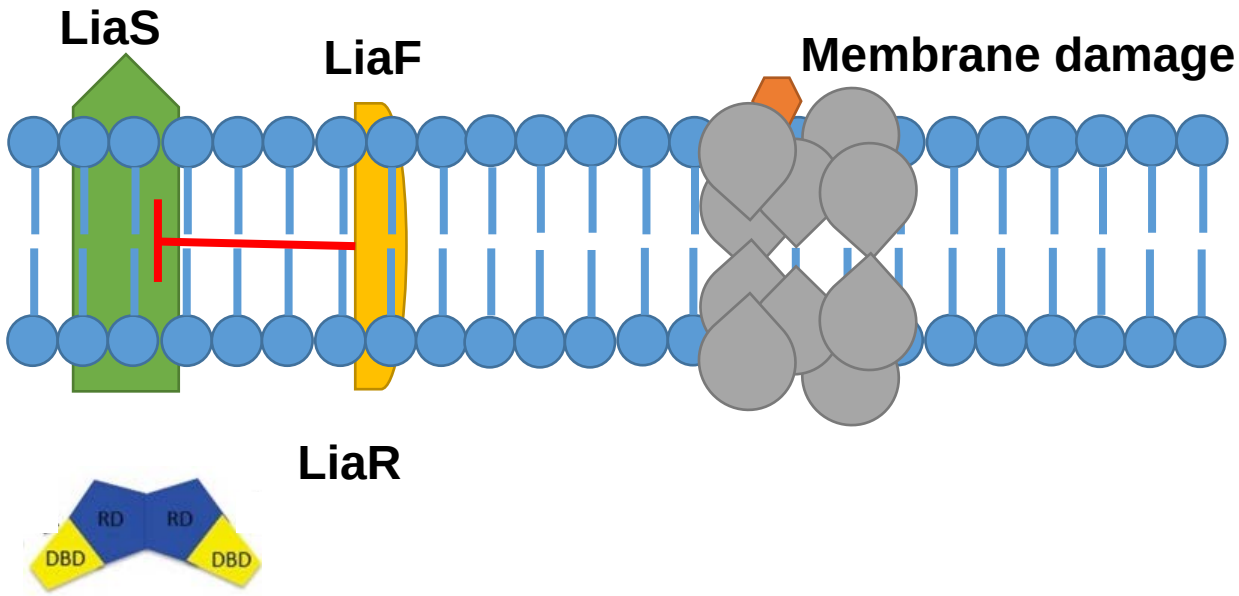
DAP insertion

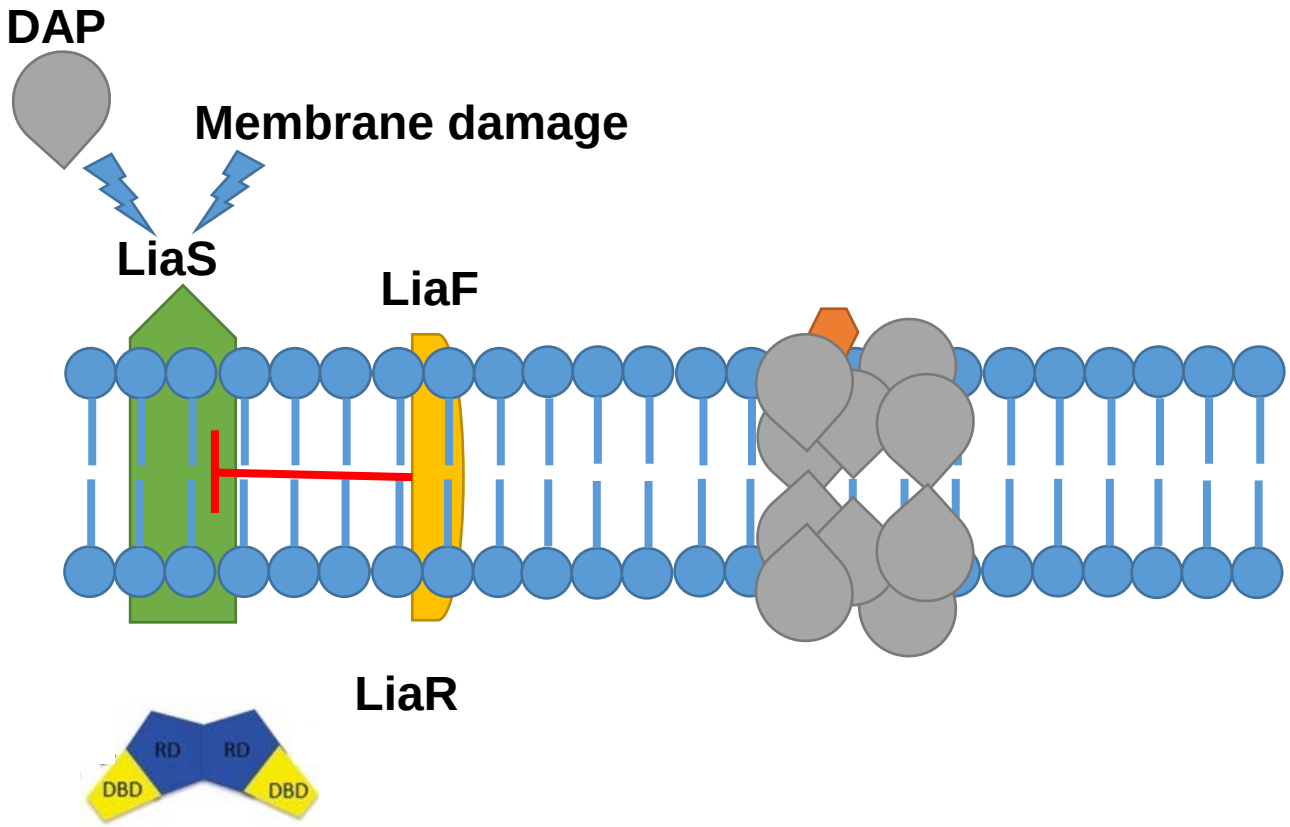


Oligomerization

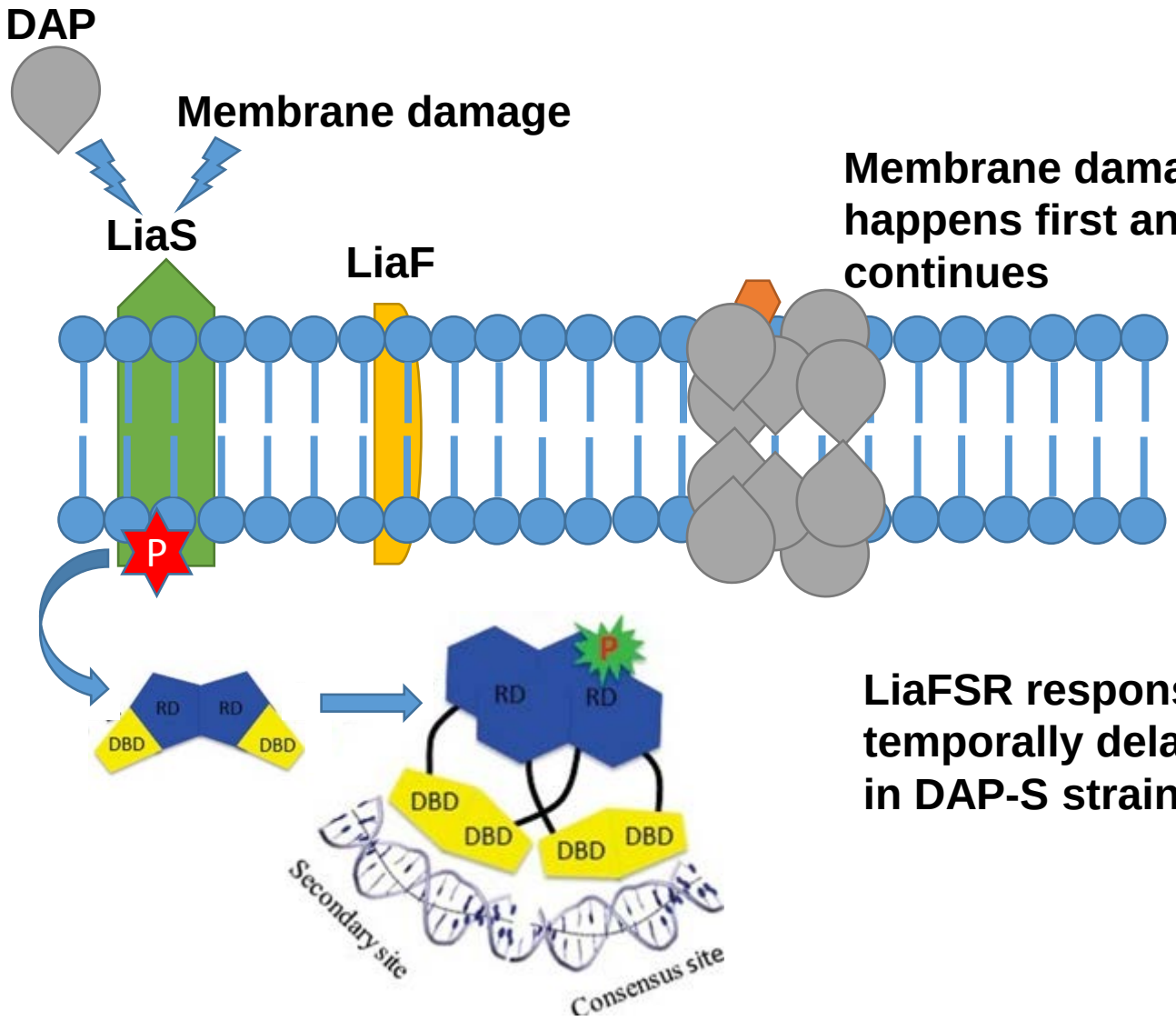


Damage begins




DAP
Membrane damage
LiaS
LiaF
LiaR
RD
RD
DBD
DBD

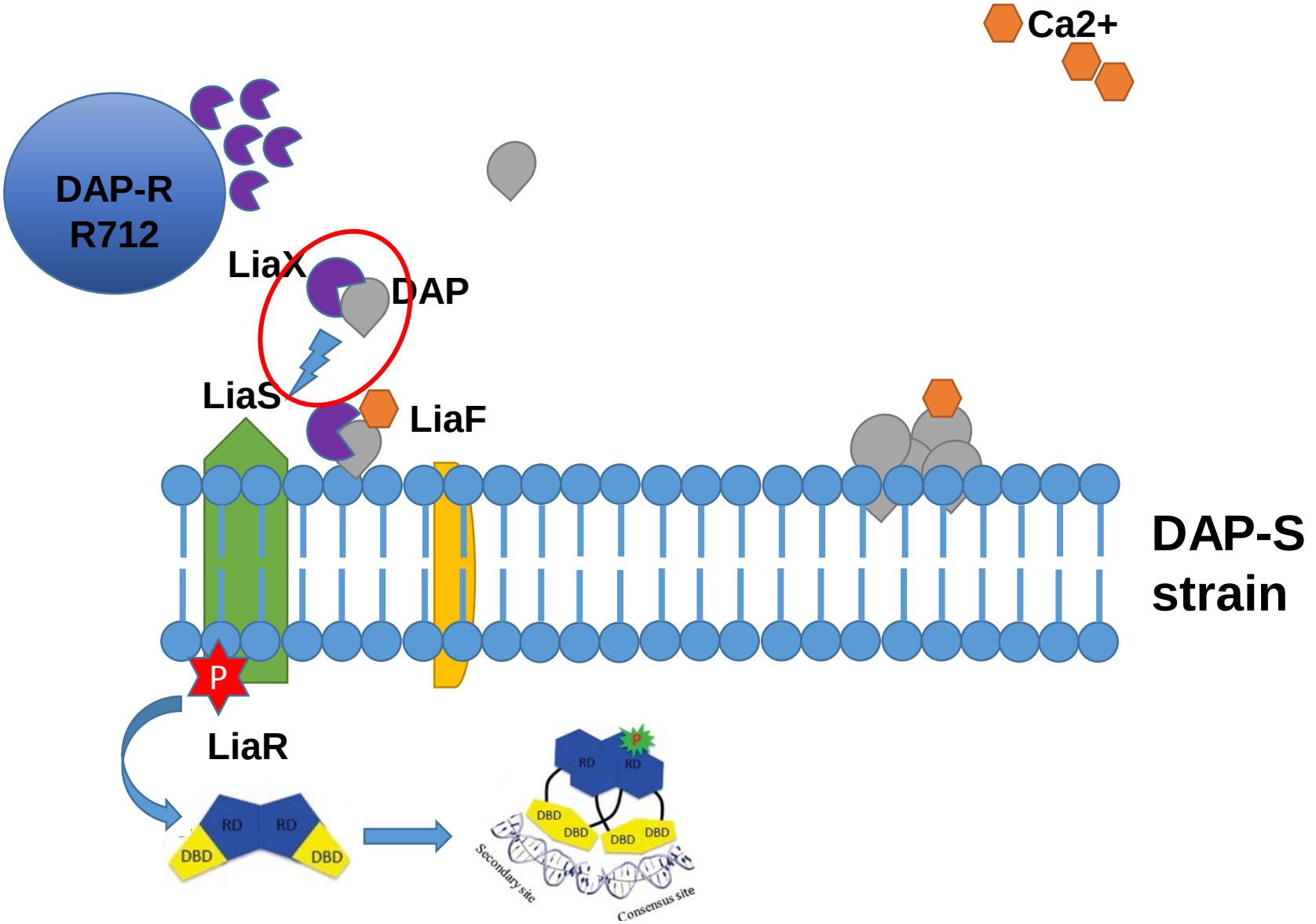
Time to cell death < Time to mount a response



LiaFSR response is temporally delayed in DAP-S strains

Extracellular protection hypothesis

LiaX-DAP complex activates stress response before cell death



Aim 2: Dissect the role of LiaX in regulating DAP-R through protein interactions

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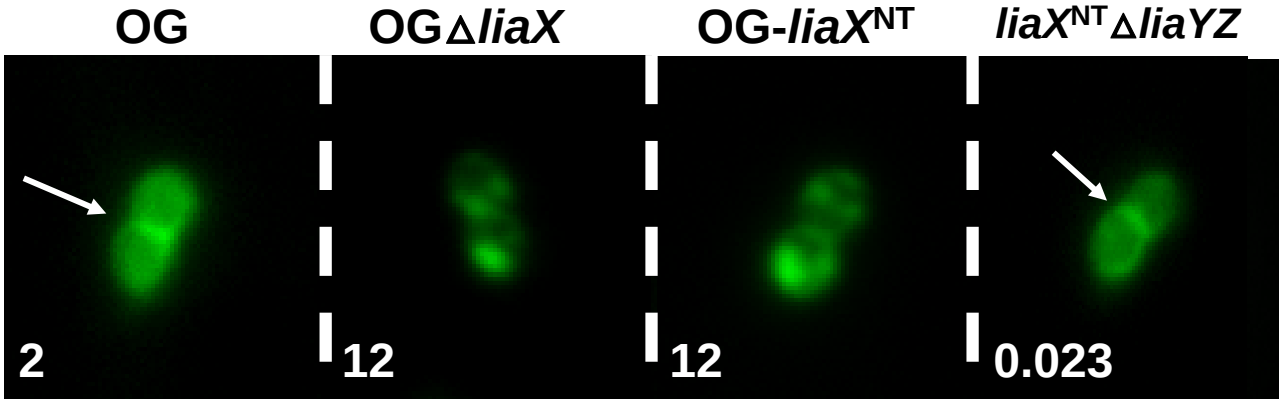
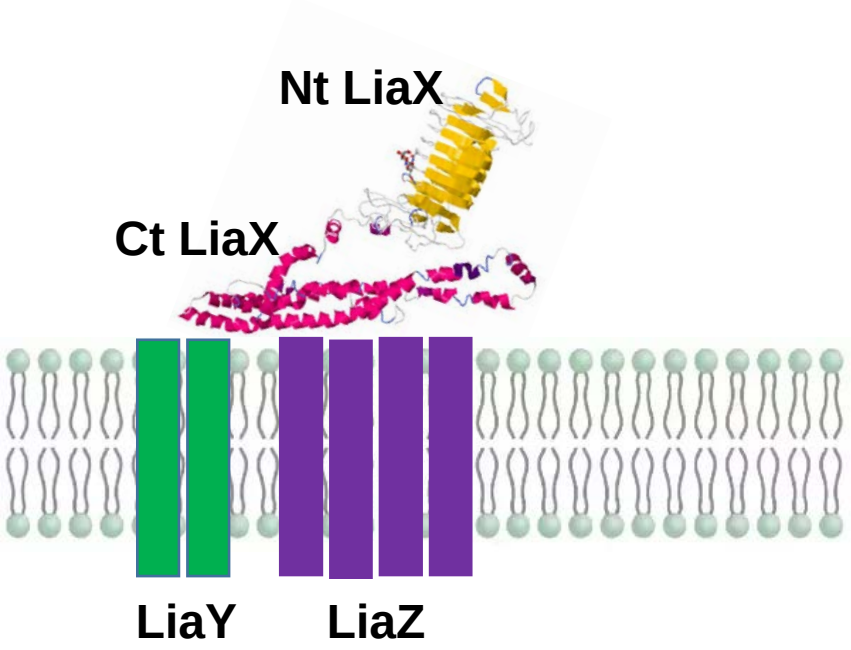
1. Characterize the liaX interactome in DAP-R and DAP-S strains
2. Study the liaX and liaYZ interaction as mechanism of regulation of DAP-R

Aim 2 Preliminary Data



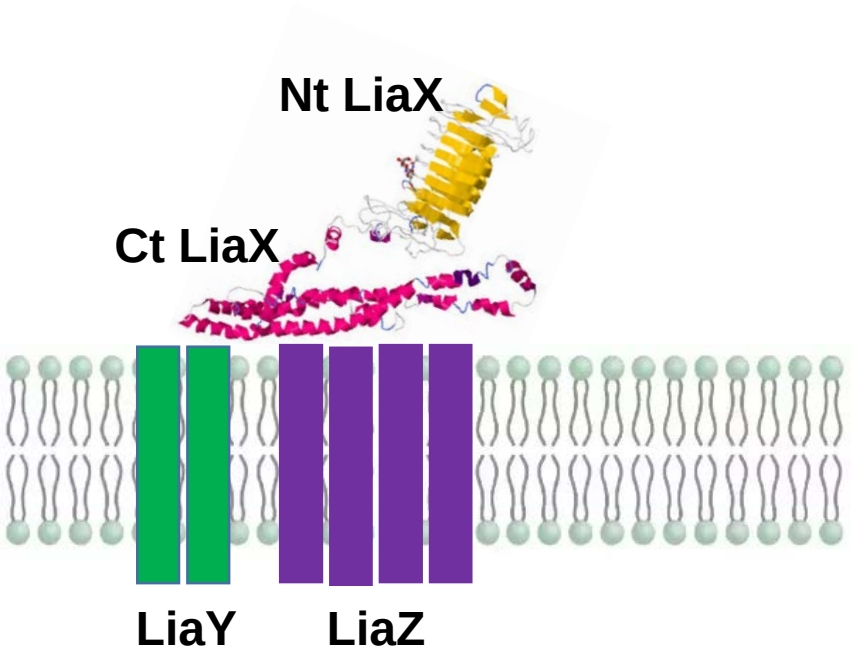
LiaX regulates DAP-R by inhibiting liaYZ

Aim 2 hypothesis- LiaX and LiaYZ interaction



Full length LiaX in DAP-S strains

Aim 2 hypothesis- LiaX and LiaYZ interaction



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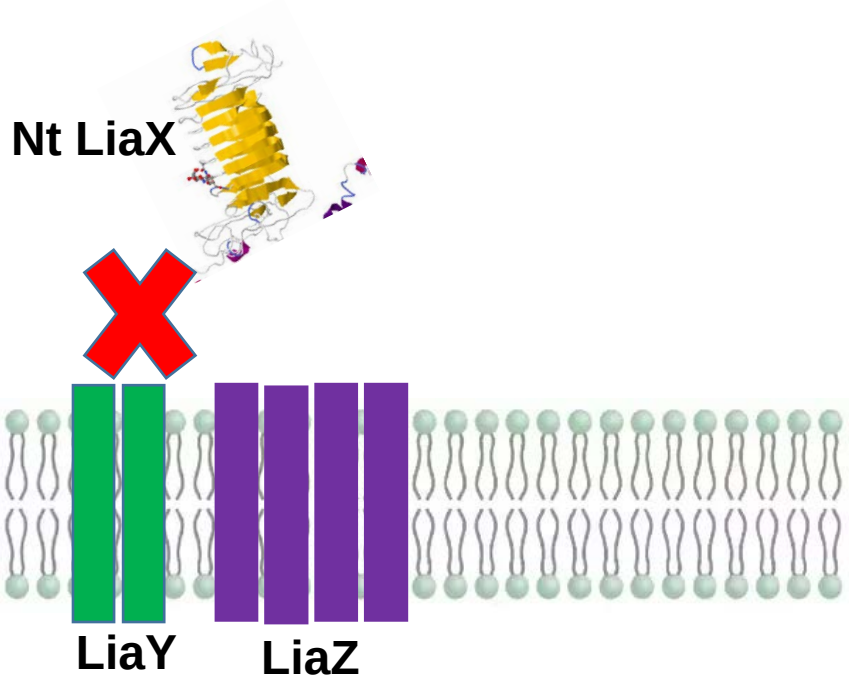
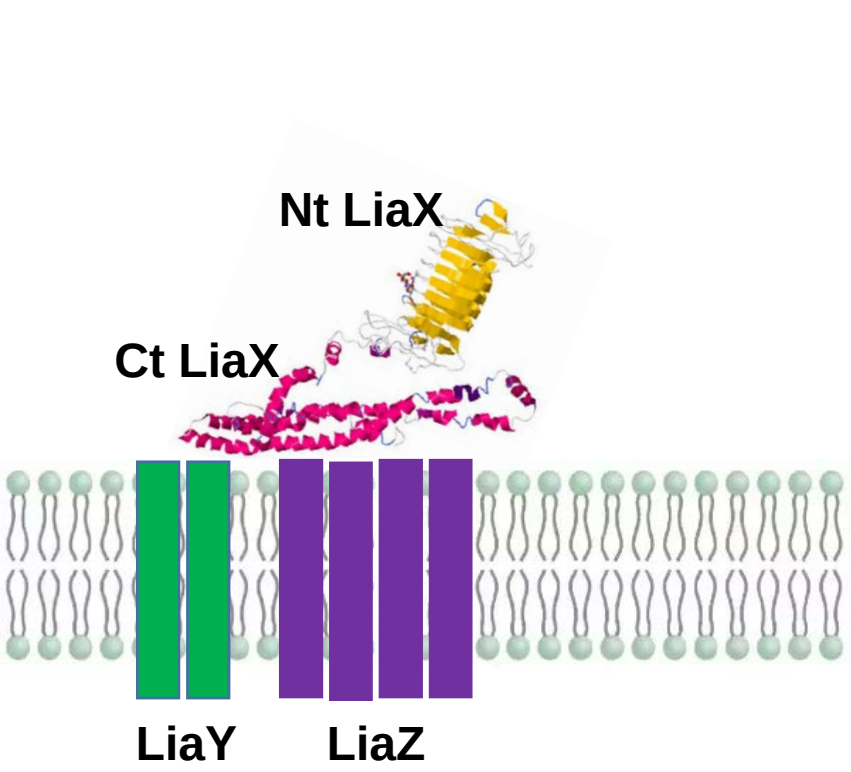


DAP-R strain with Ct truncation of LiaX

Aim 2 hypothesis- LiaX and LiaYZ interaction



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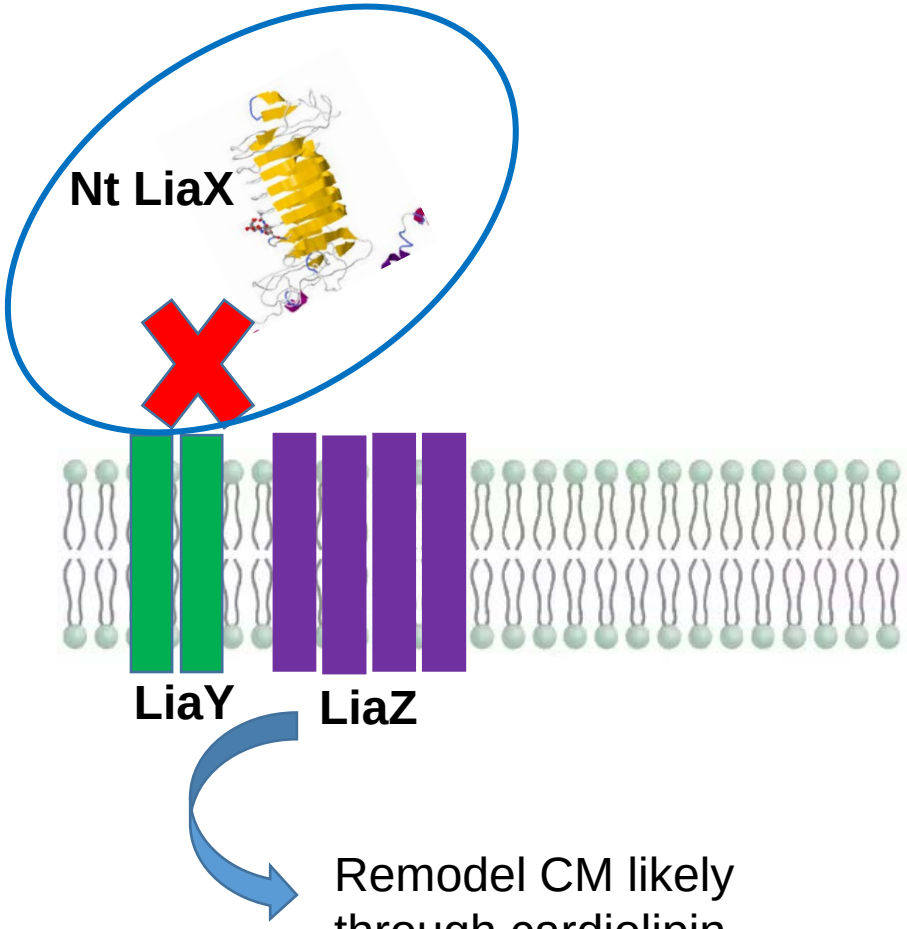

Remodel CM likely through cardiolipin synthase

Aim 3:Elucidate the role of LiaX in mediating the seesaw effect through interaction with PBP5

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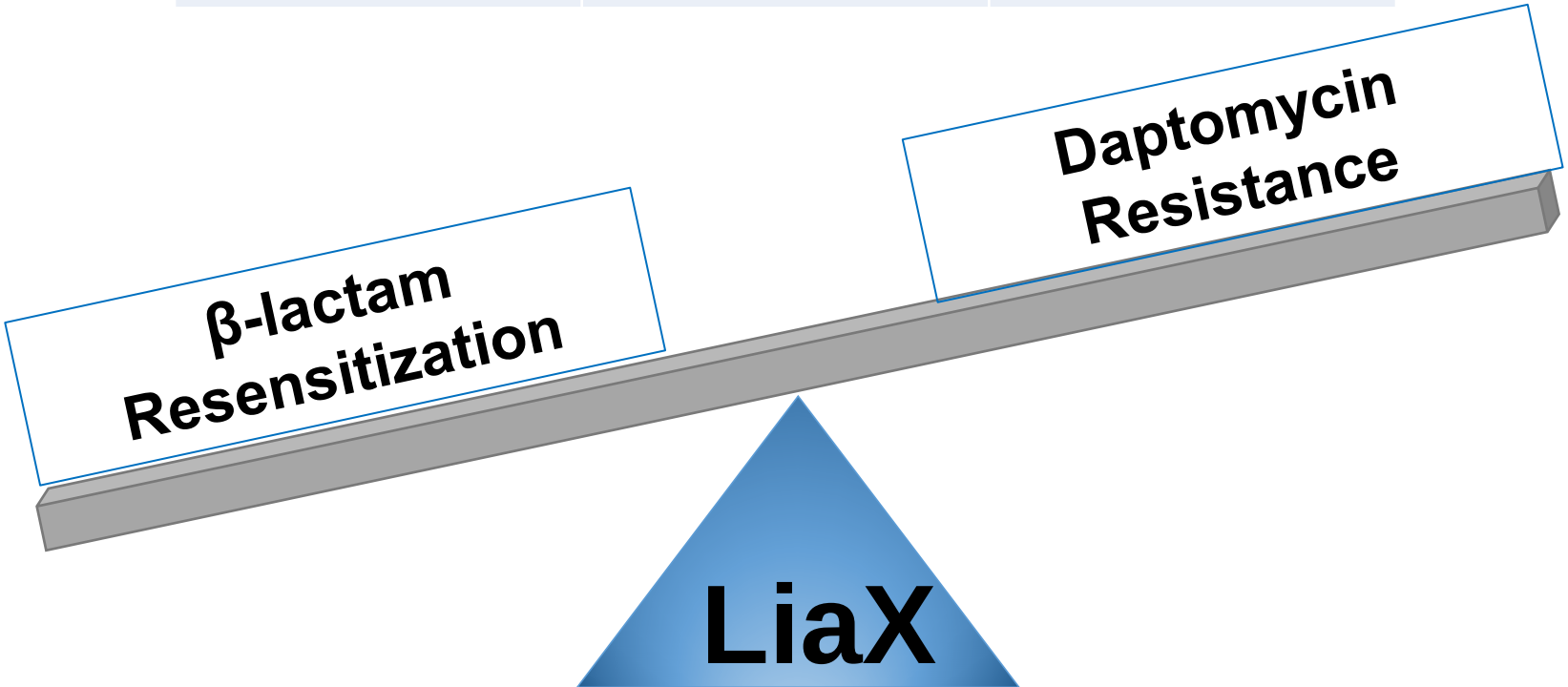
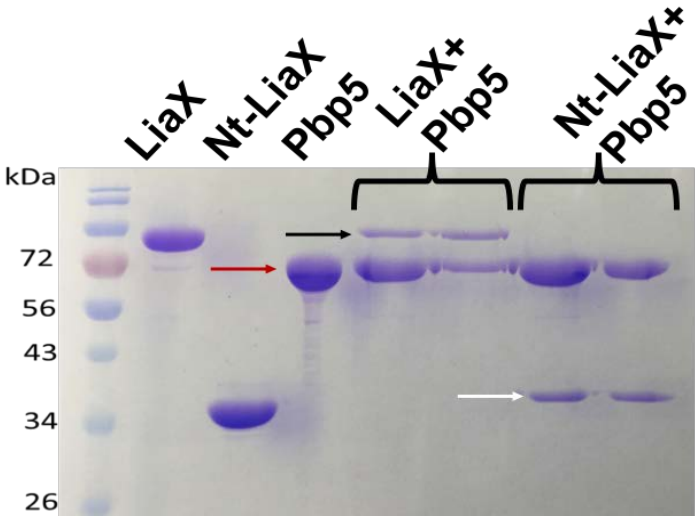
1. Study PBP5-liaX colocalization in DAP-S strains and PBP5 mislocalization in DAP-R strains
2. Assess PBP5 protein levels and β-lactam binding to PBPs in DAP-R strains

Aim 3 Preliminary Data

Strain	DAP MIC (ug/ml)	Ceftriaxone MIC
OG	2	32
OGΔ*liaX*	12	6
OG-*liaX*^{NT}	12	6
Complements	4	32



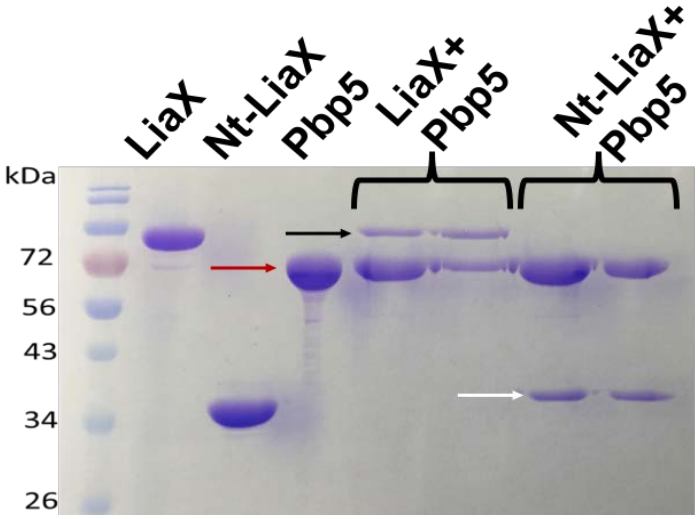
LiaX-Pbp5 pull down

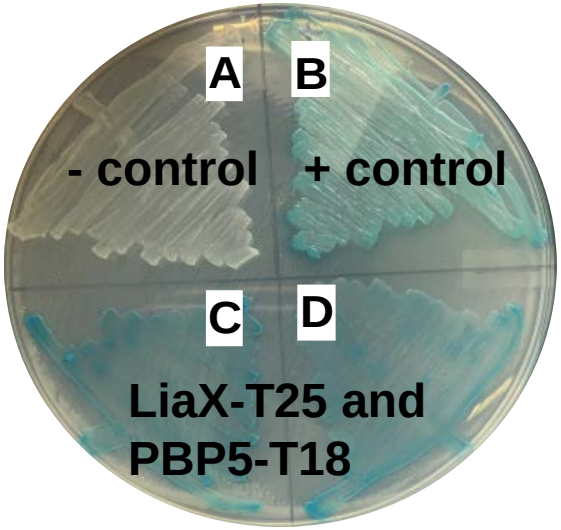


Used LiaX or Nt-LiaX as bait and PBP5 as prey

Controls: no bait, GFP used as bait/ prey

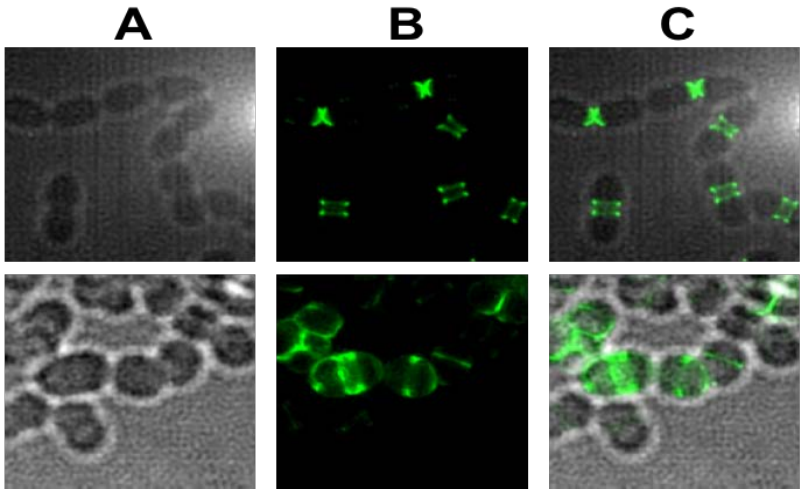
Pull-down and Bacterial 2hybrid show interaction





Bacterial 2 hybrid system
Tags are on the Ct end of both LiaX and PBP5

PG synthesis mislocalized



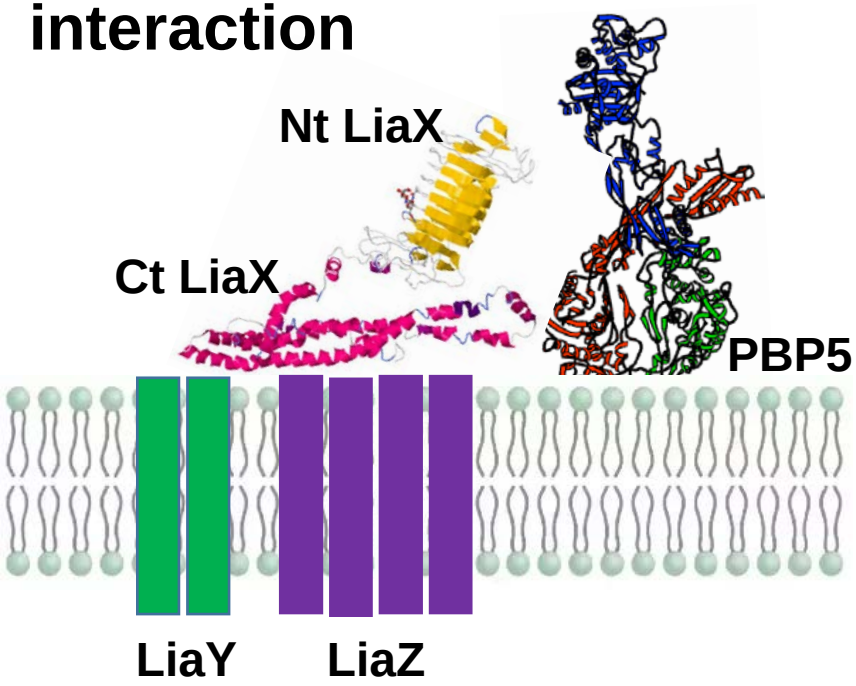
S613 Equatorial rings

R712 Aberrant staining and side wall synthesis

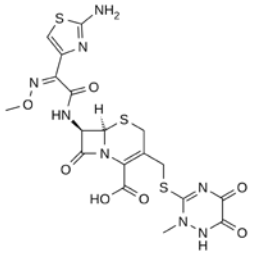
NADA Staining of nascent PG synthesis

Aim 3 hypothesis

LiaX- PBP5 interaction

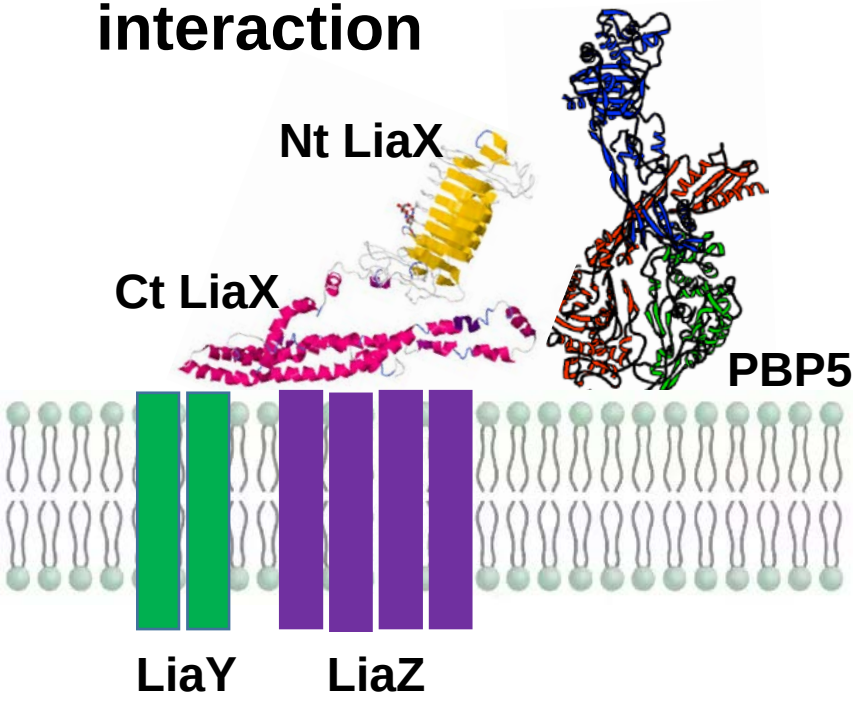


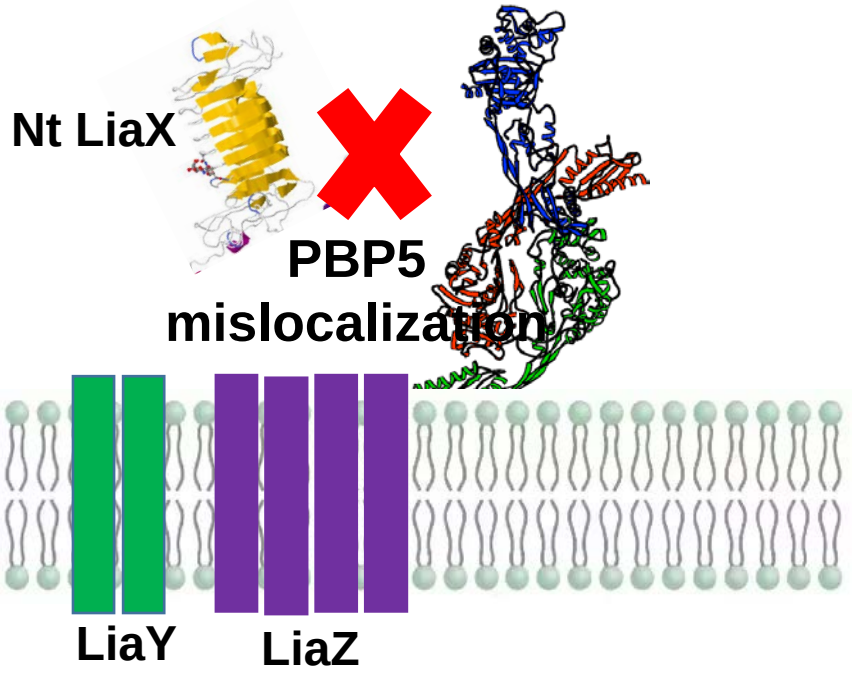
Full length LiaX in DAP-S strains



Increased β-lactam access

LiaX- PBP5 interaction



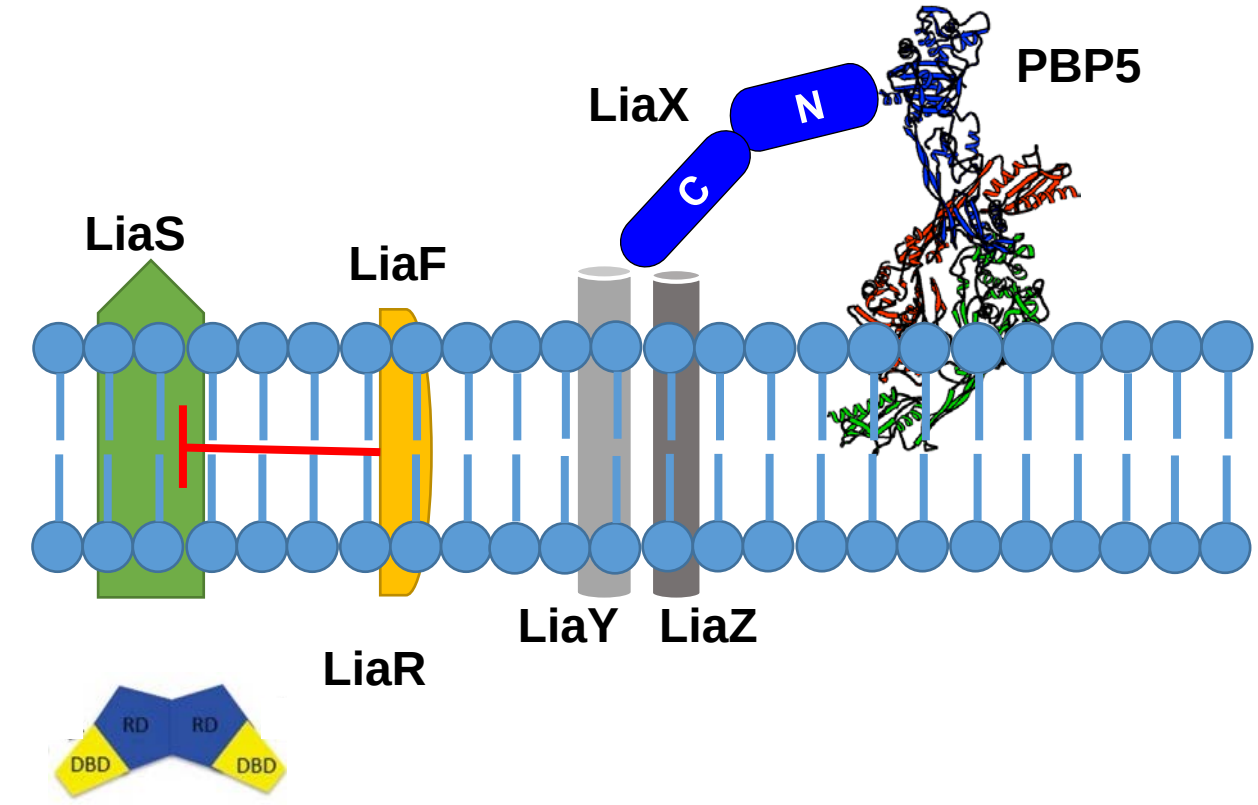


Full length LiaX in DAP-S strains

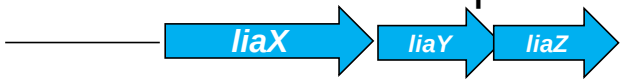
Ct truncation in DAP-R strains

Model of the LiaFSR and LiaX mediated stress response

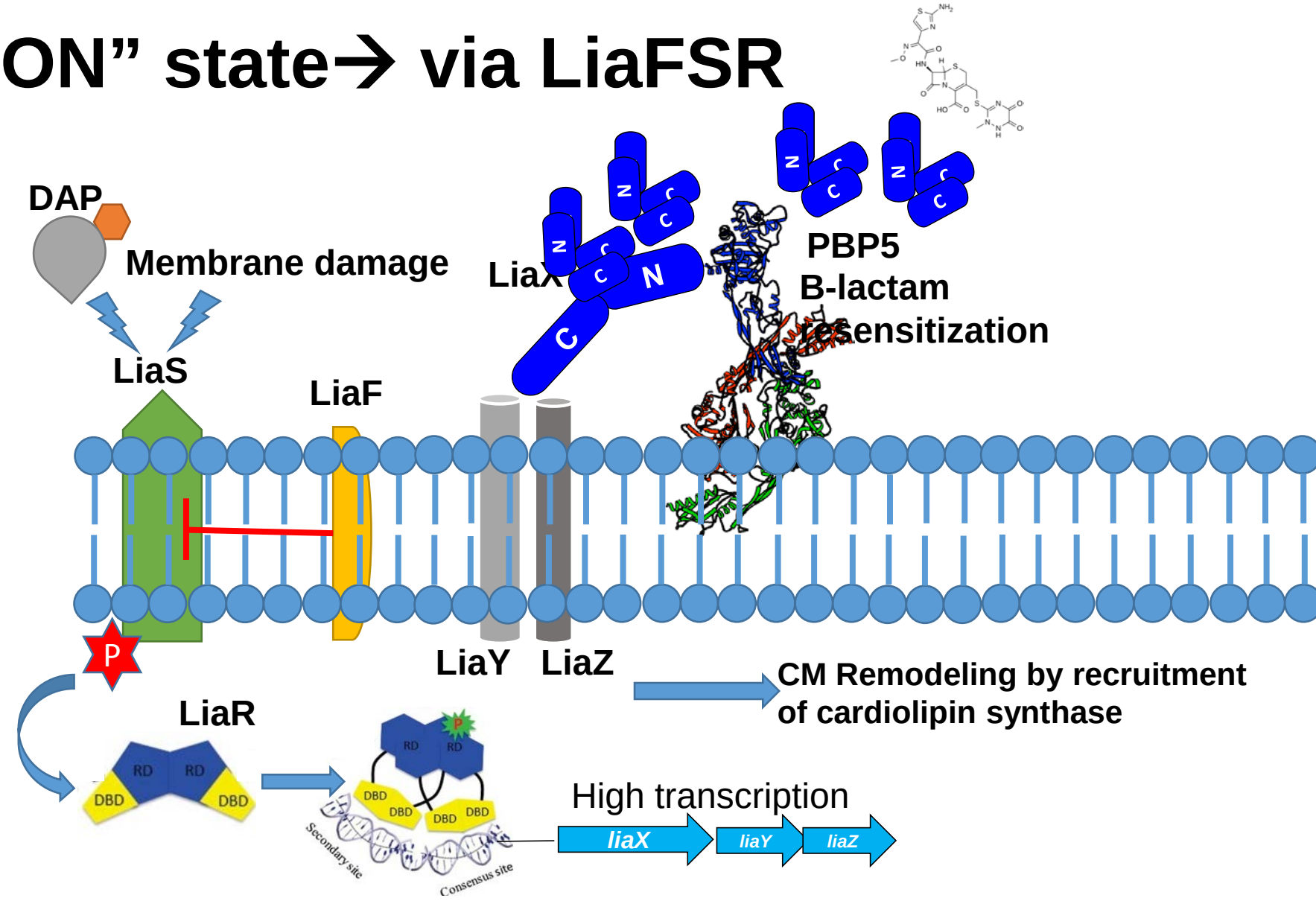
Absence of stress (“OFF”)



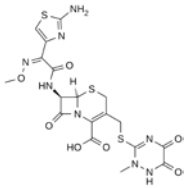
Basal transcription



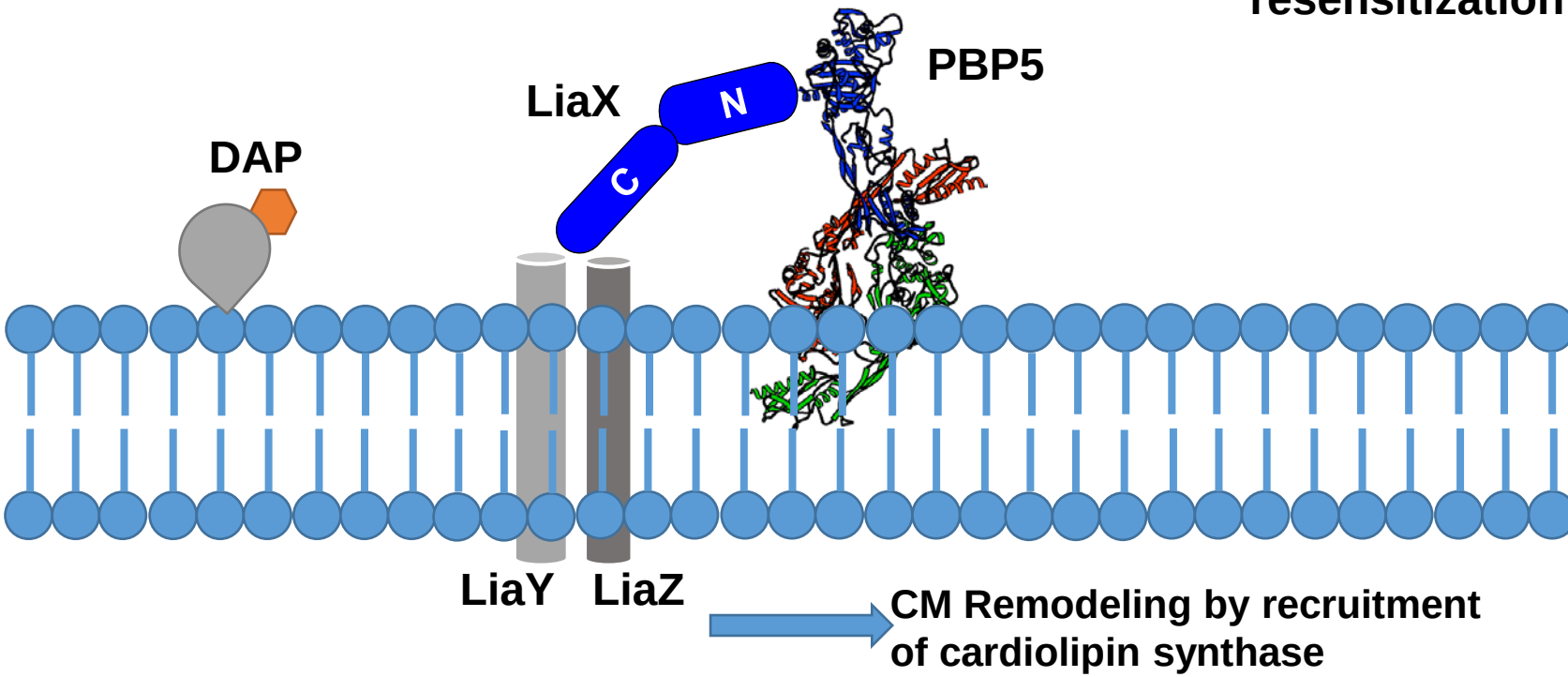
“ON” state→ via LiaFSR



“ON” state→ via LiaX

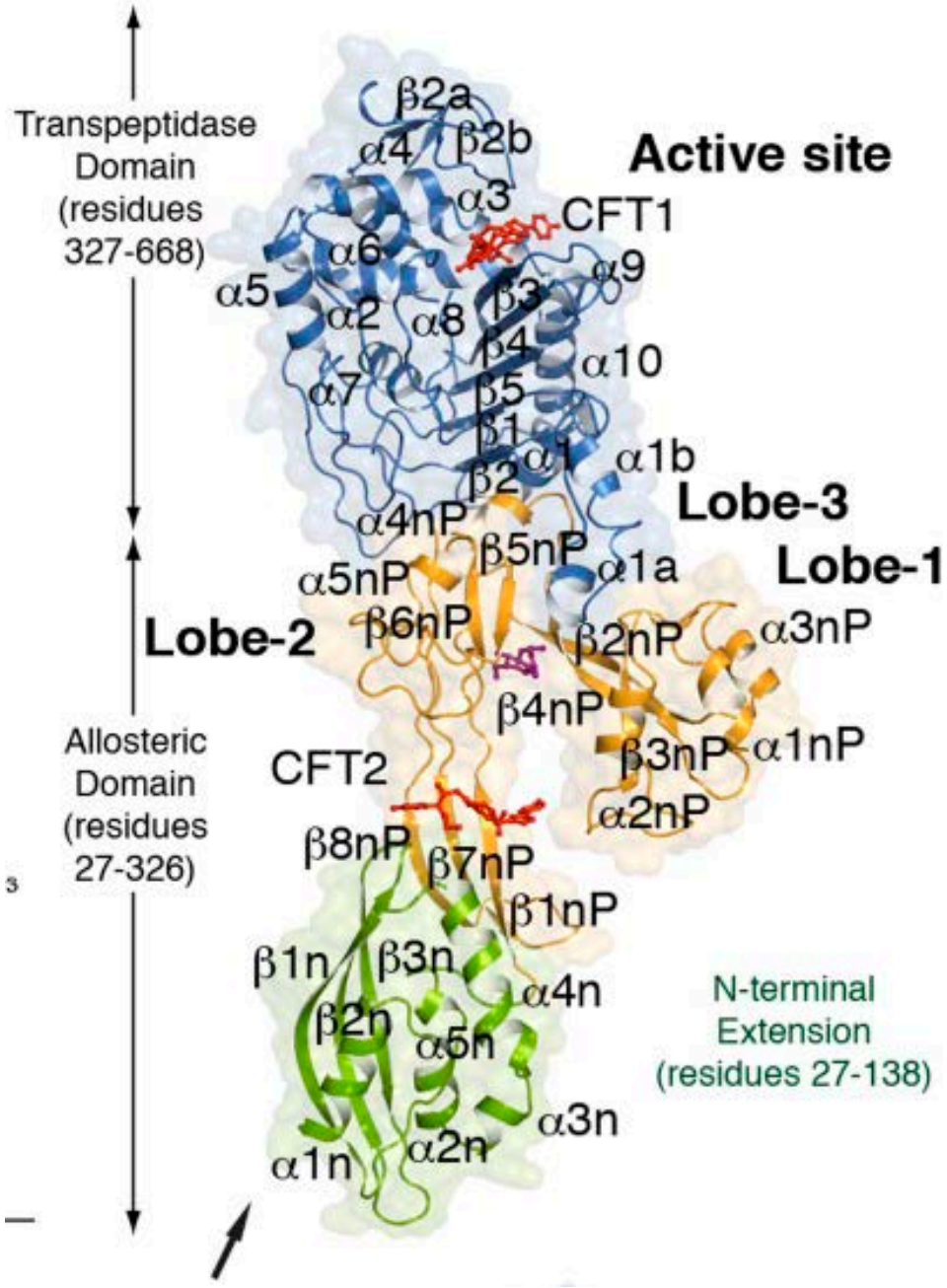


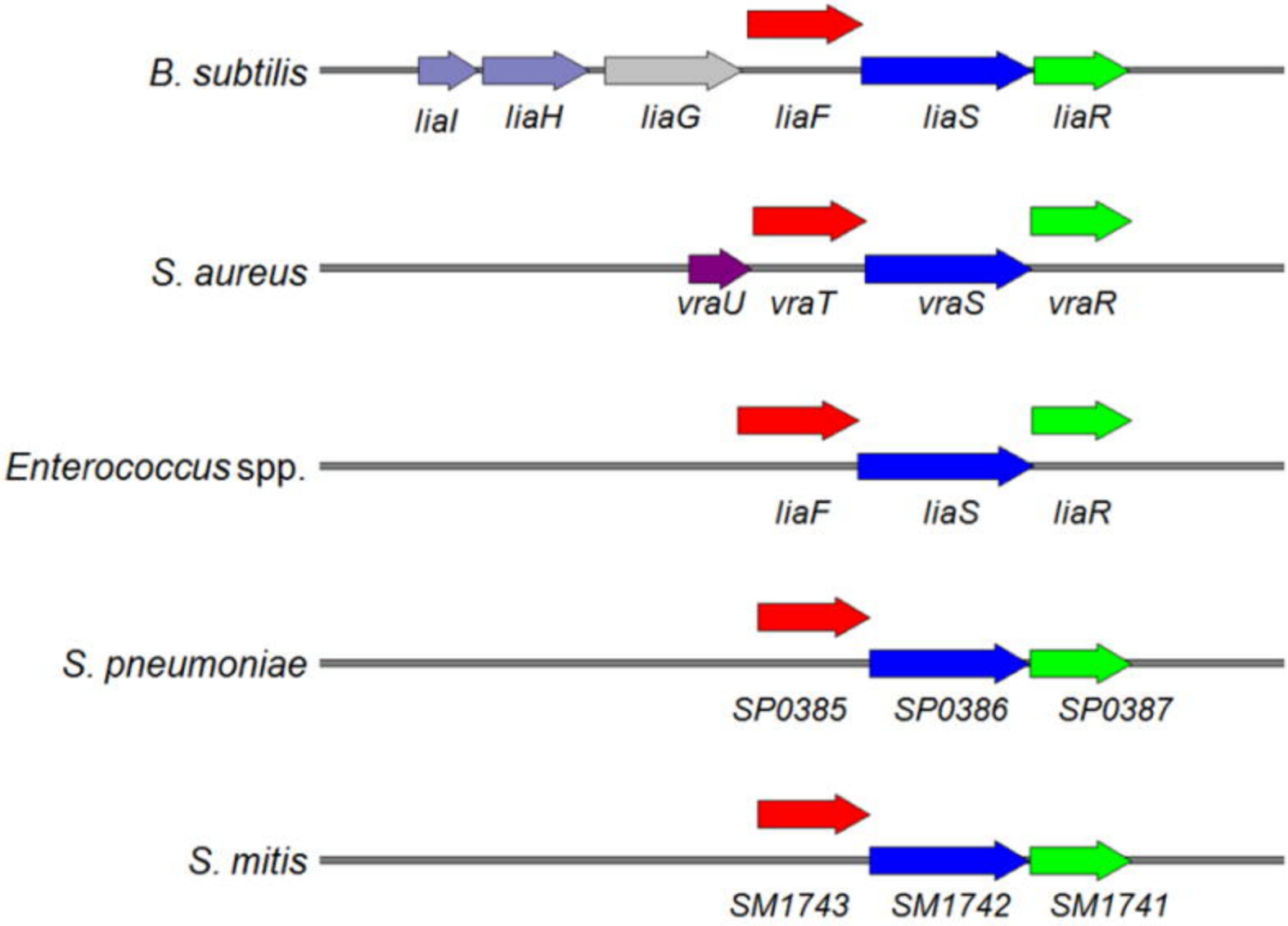
B-lactam resensitization

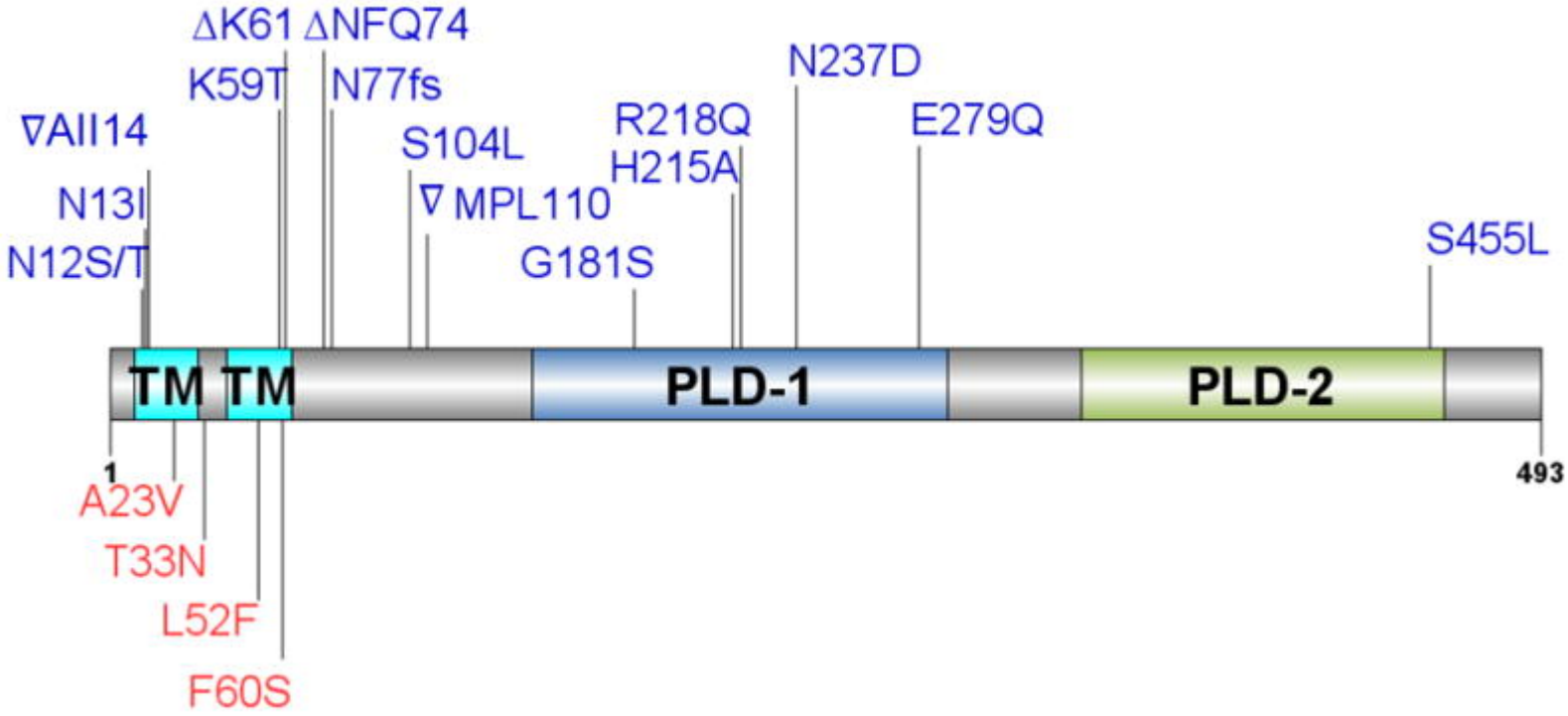


This project aims to

1. Dissect the mechanism by which LiaX regulates the CE stress response
2. Identify the mechanism for the LiaX modulation of the see-saw effect in enterococci
3. Study the DAP “resistome” --> expose many new therapeutic targets


Transpeptidase Domain (residues 327-668)
Allosteric Domain (residues 27-326)
Active site
Lobe-1
Lobe-2
Lobe-3
CFT1
CFT2
β2a
α4
β2b
α3
α6
α5
α2
α8
β3
α9
β4
α10
α7
β5
β1
α1
α1b
β2
α4nP
β5nP
α1a
α5nP
β6nP
β2nP
α3nP
β4nP
β3nP
α1nP
α2nP
β8nP
β7nP
β1nP
β1n
β3n
α4n
β2n
α5n
α3n
α2n
α1n
N-terminal Extension (residues 27-138)


B. subtilis
liaI
liaH
liaG
liaF
liaS
liaR
S. aureus
vraU
vraT
vraS
vraR
Enterococcus spp.
liaF
liaS
liaR
S. pneumoniae
SP0385
SP0386
SP0387
S. mitis
SM1743
SM1742
SM1741


ΔK61
ΔNFQ74
K59T
N77fs
N237D
∇AII14
R218Q
E279Q
S104L
H215A
N13I
∇MPL110
S455L
N12S/T
G181S
TM
TM
PLD-1
PLD-2
1
493
A23V
T33N
L52F
F60S