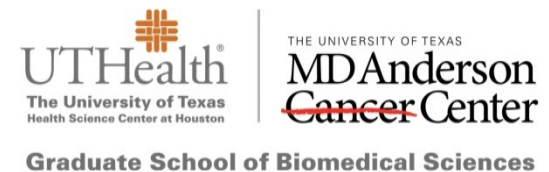
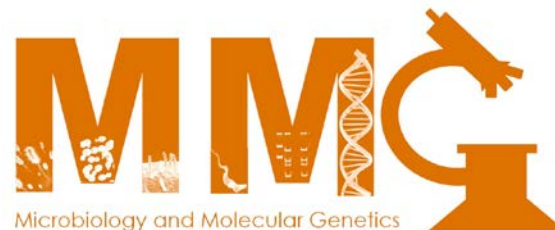


Mechanisms of Daptomycin Resistance and the Seesaw Effect in Multi-Drug Resistant Enterococci

NAME OF STUDENT

Candidacy Exam

August 25, 2017








VANCOMYCIN-RESISTANT ENTEROCOCCUS (VRE)



THREAT LEVEL
SERIOUS ○○○○○

This bacteria is a serious concern and requires prompt and sustained action to ensure the problem does not grow.

 **20,000**
DRUG-RESISTANT ENTEROCOCCUS INFECTIONS

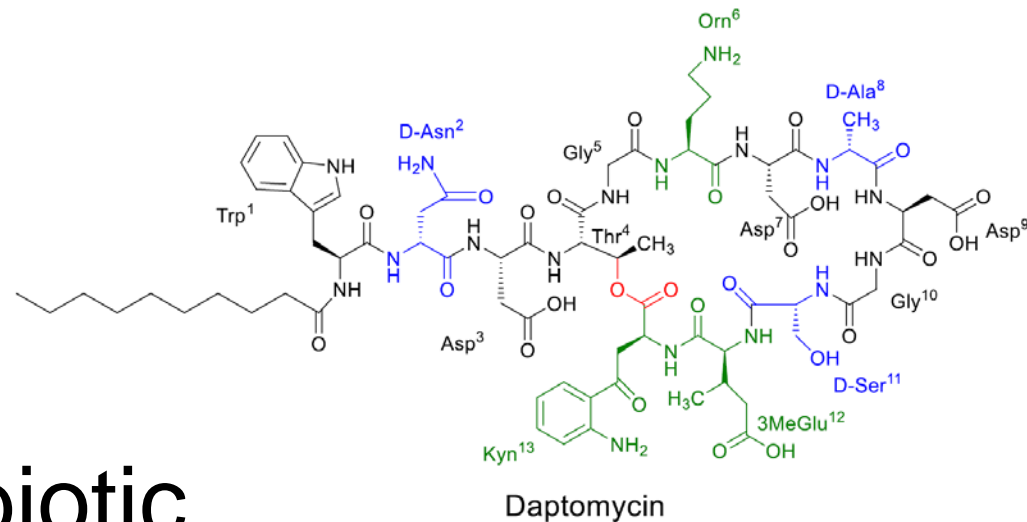
 **1,300**
DEATHS FROM DRUG-RESISTANT ENTEROCOCCUS INFECTIONS

 **66,000**
ENTEROCOCCUS INFECTIONS PER YEAR

 SOME ENTEROCOCCUS STRAINS ARE RESISTANT TO VANCOMYCIN
LEAVING FEW OR NO TREATMENT OPTIONS 

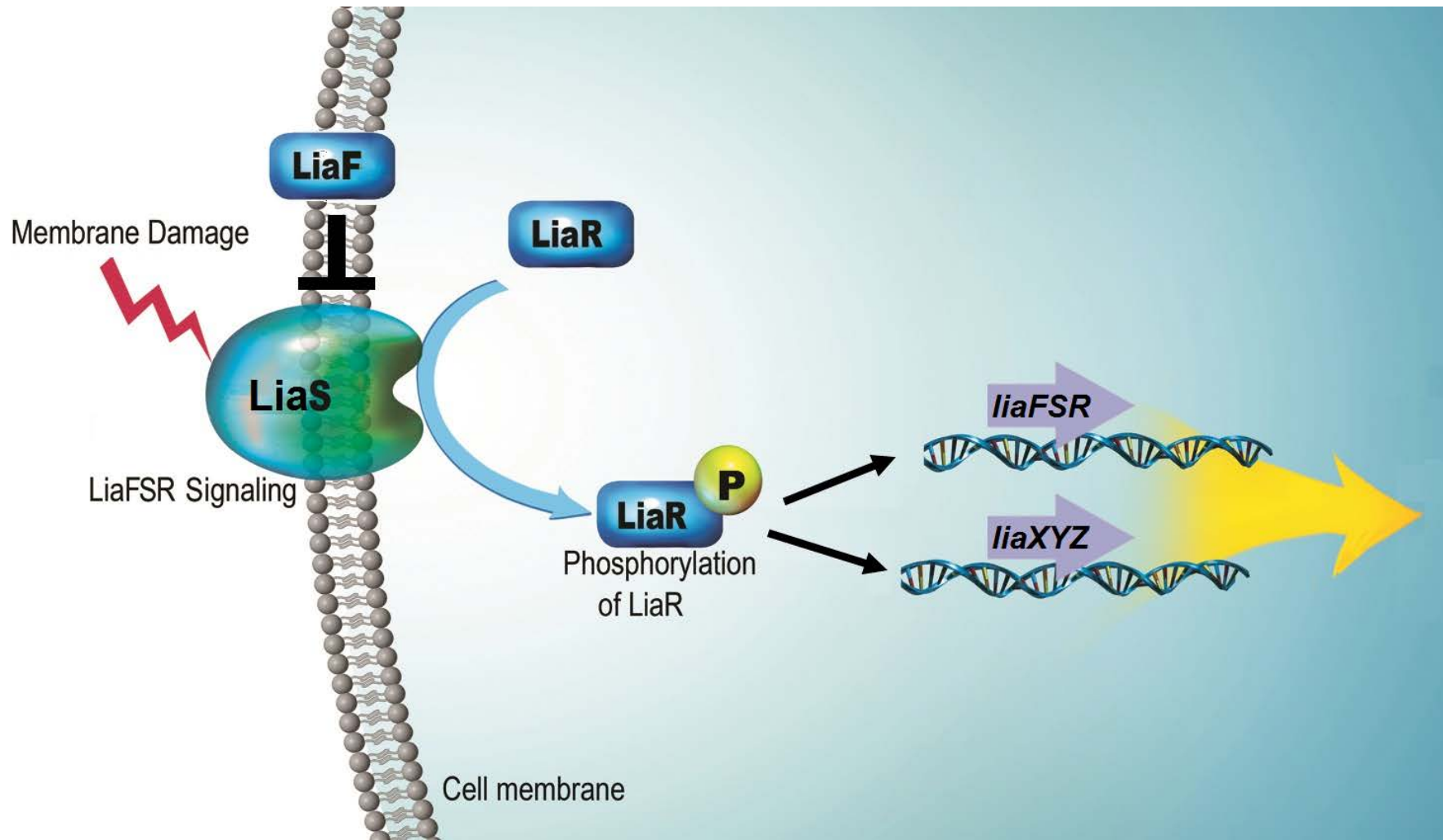
- Major nosocomial pathogen
- Endocarditis, bacteremia, UTIs, meningitis
- High intrinsic resistance to antibiotics (aminoglycosides, cephalosporins, beta-lactams)
- High genetic plasticity

Daptomycin

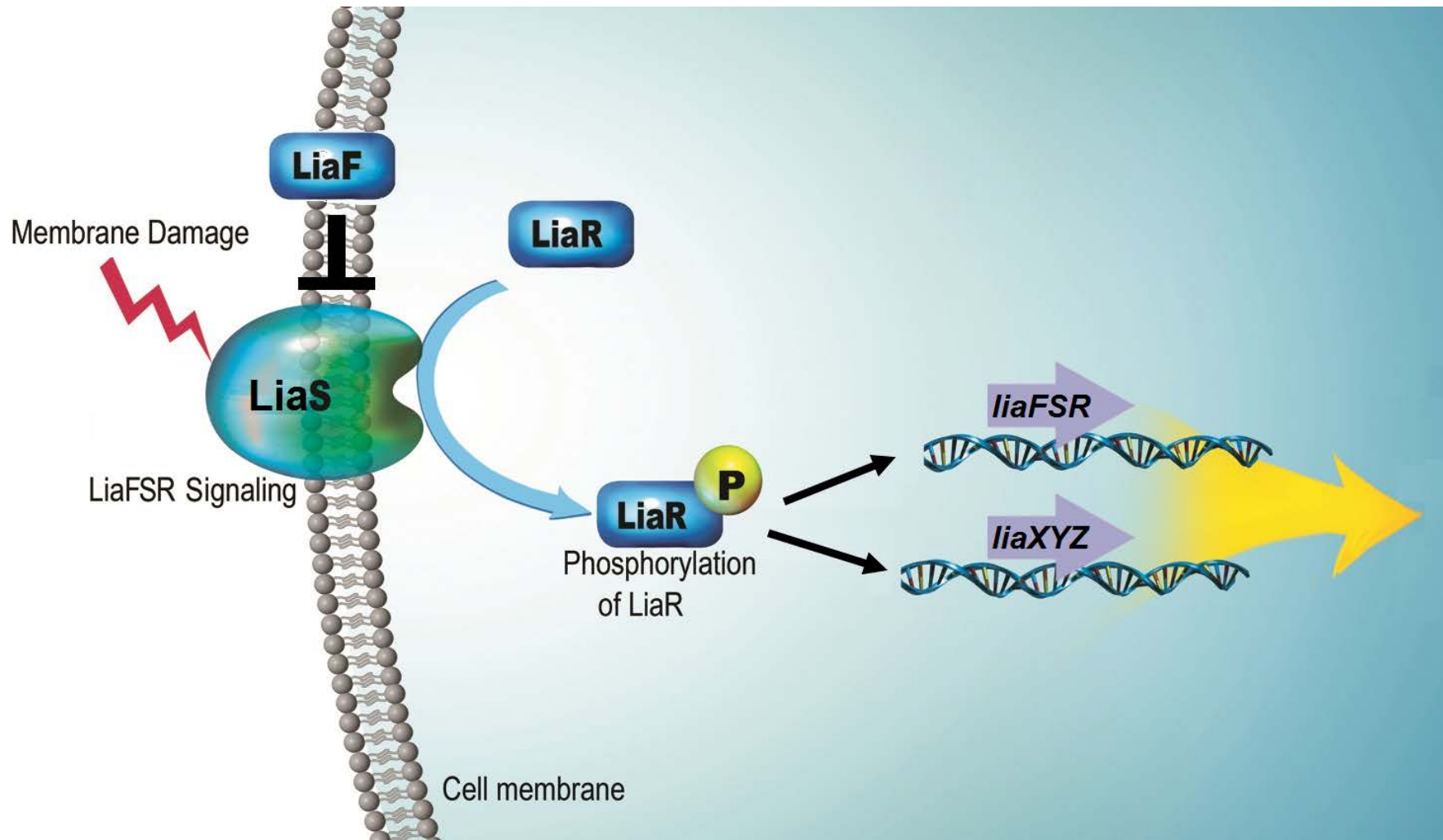


- Lipopeptide antibiotic
- Used as a "last resort" for MDR-enterococcal infections (Breakpoint MIC= 4 μ g/ml)
- Observed clinical resistance in VRE
- Disrupts cell membrane integrity

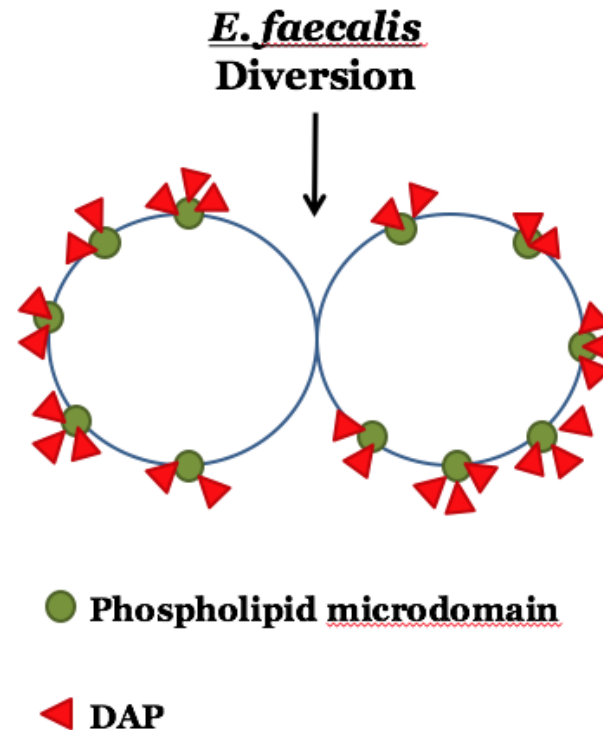
The LiaFSR system regulates DAP-R in enterococci



liaXYZ are effectors of the LiaFSR stress response

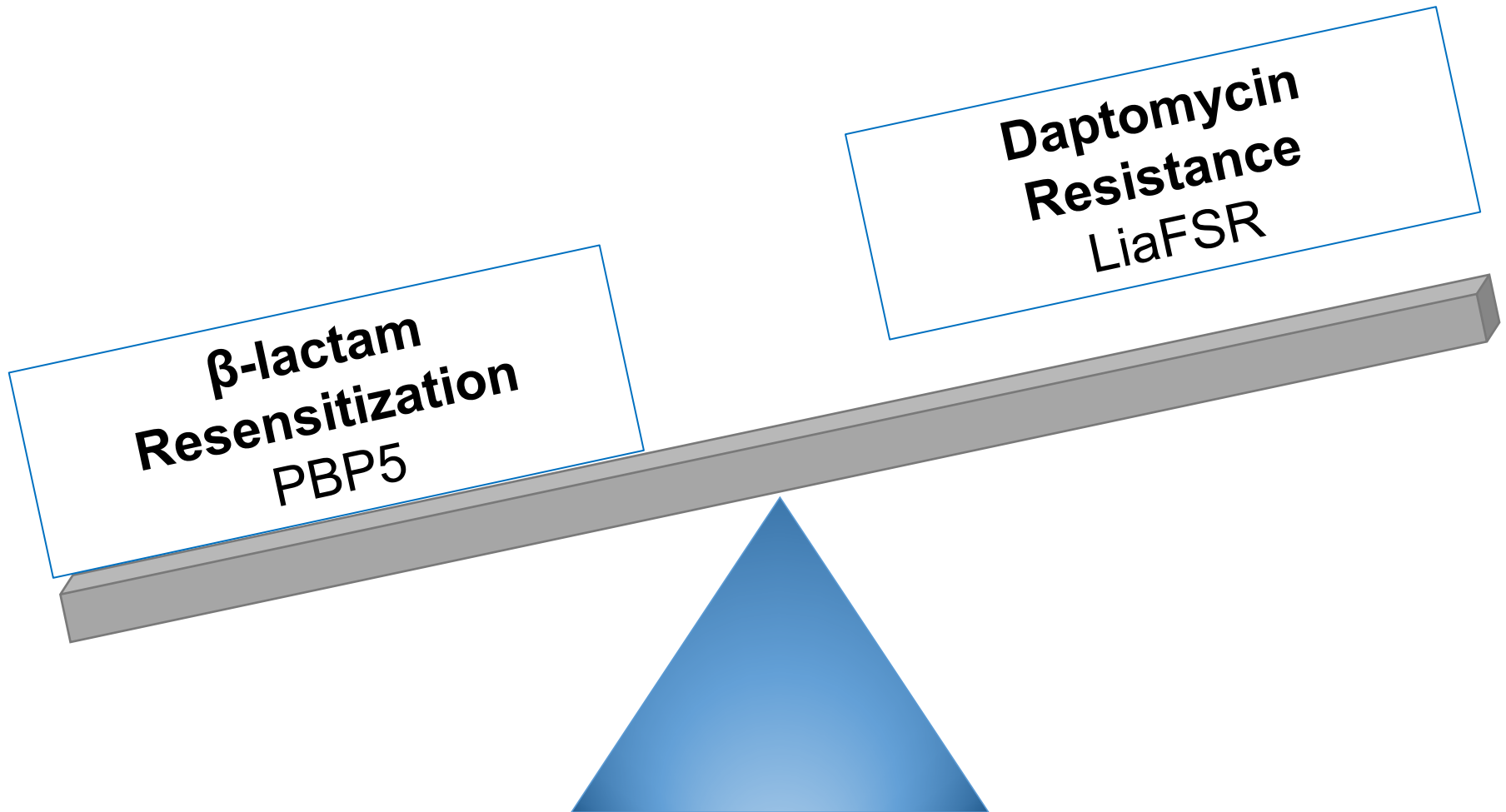


DAP-R leads to redistribution of anionic phospholipids



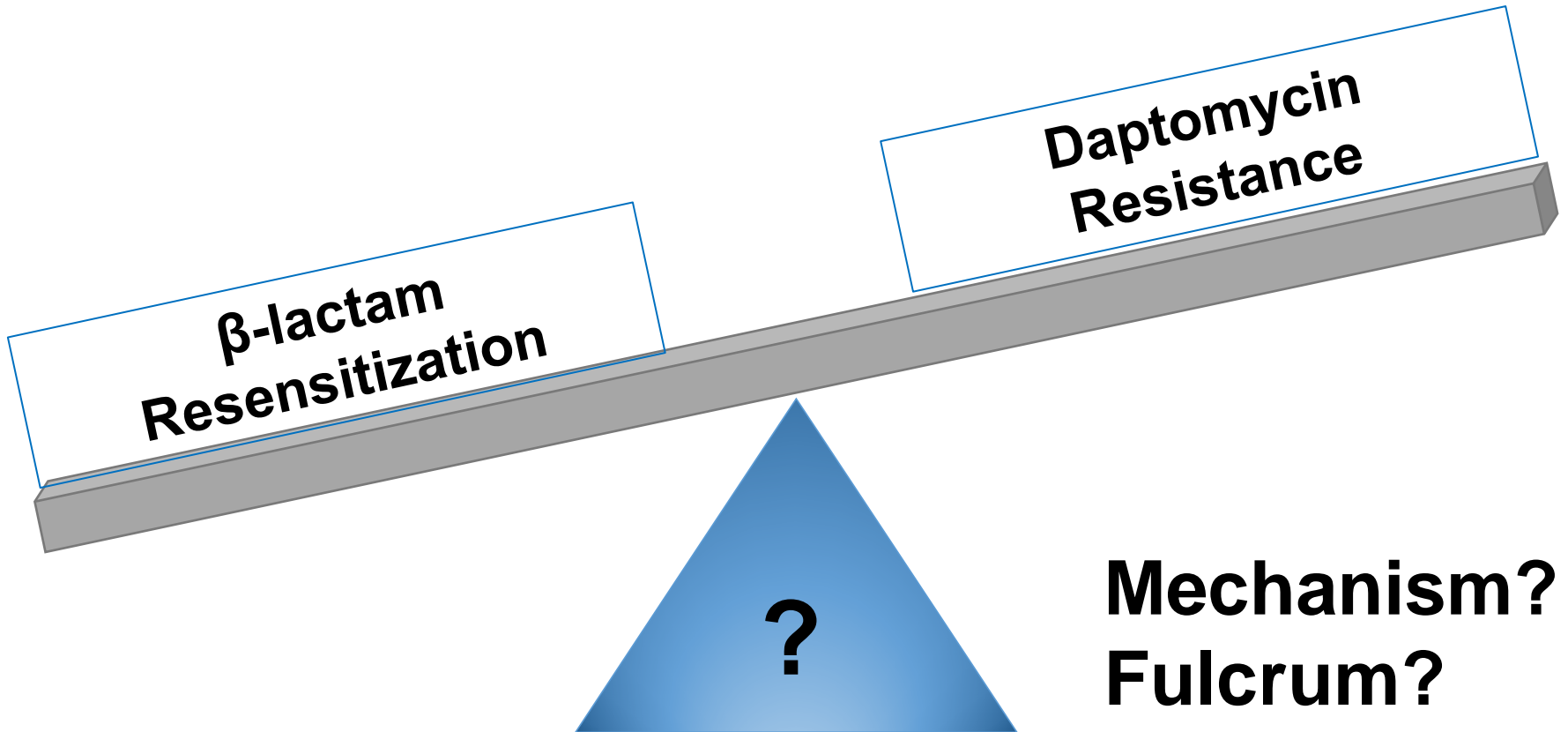
NAO Staining= Visualization of enriched anionic PL microdomains (Cardiolipin)

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



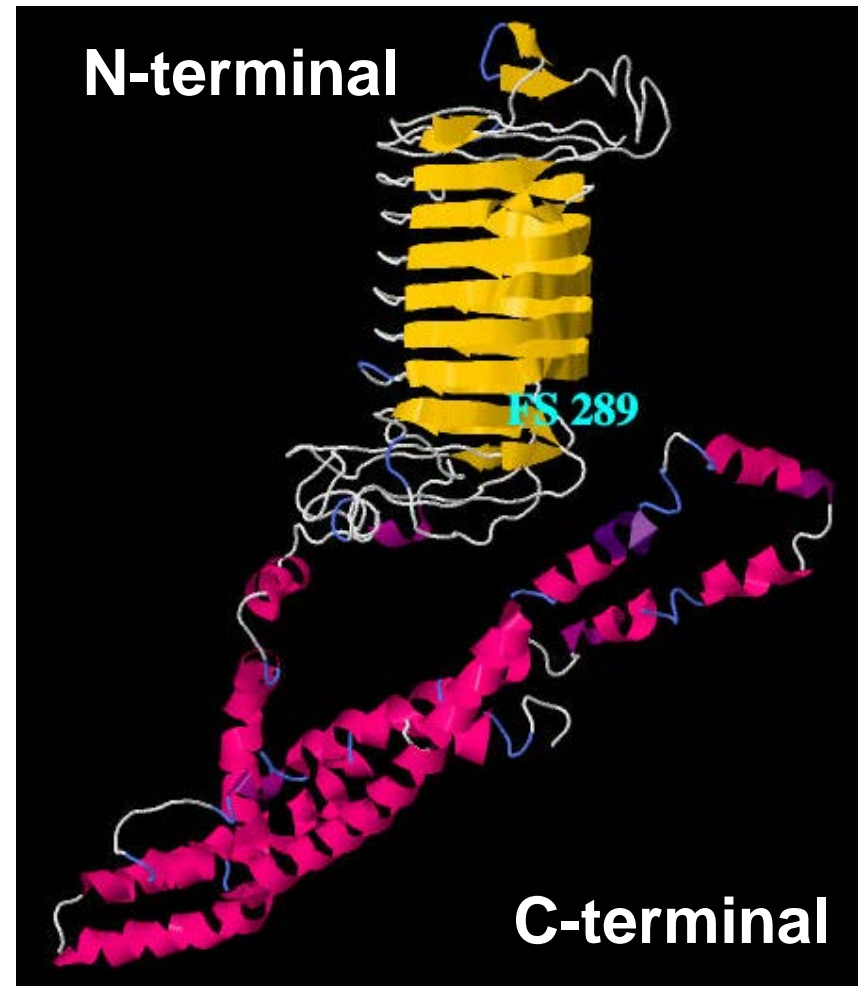
The Seesaw Effect- *Efs*, *Efm*, *MRSA*

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



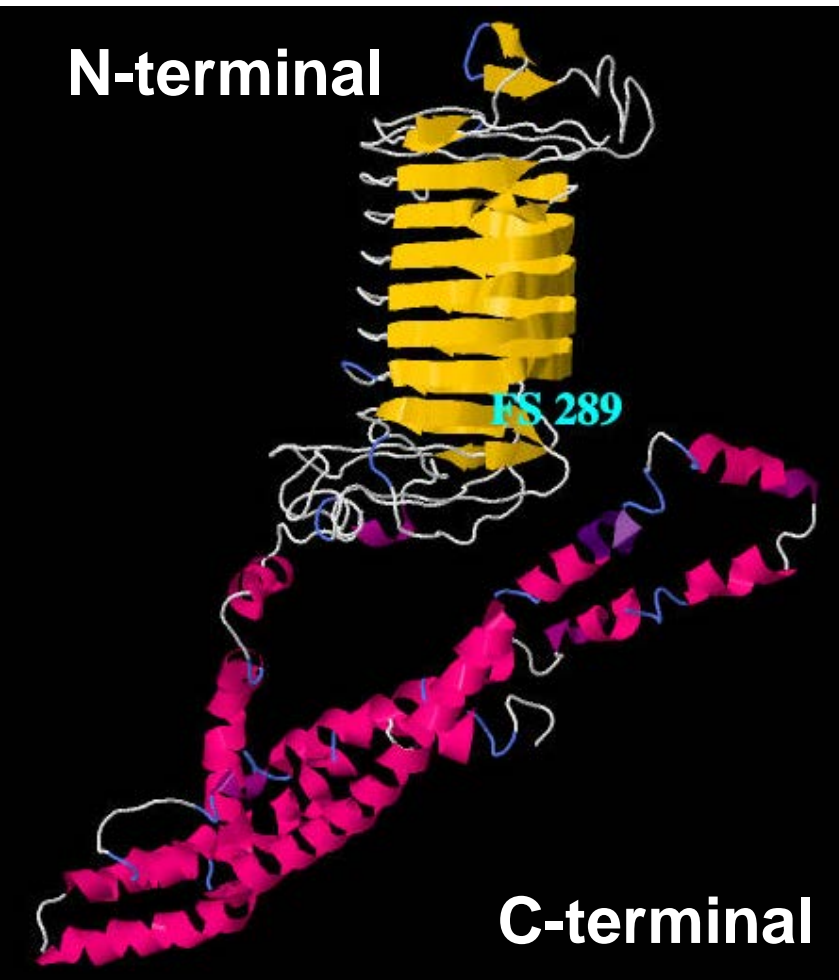
Overall Goal: Characterize LiaX and determine its role in antibiotic resistance

- 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

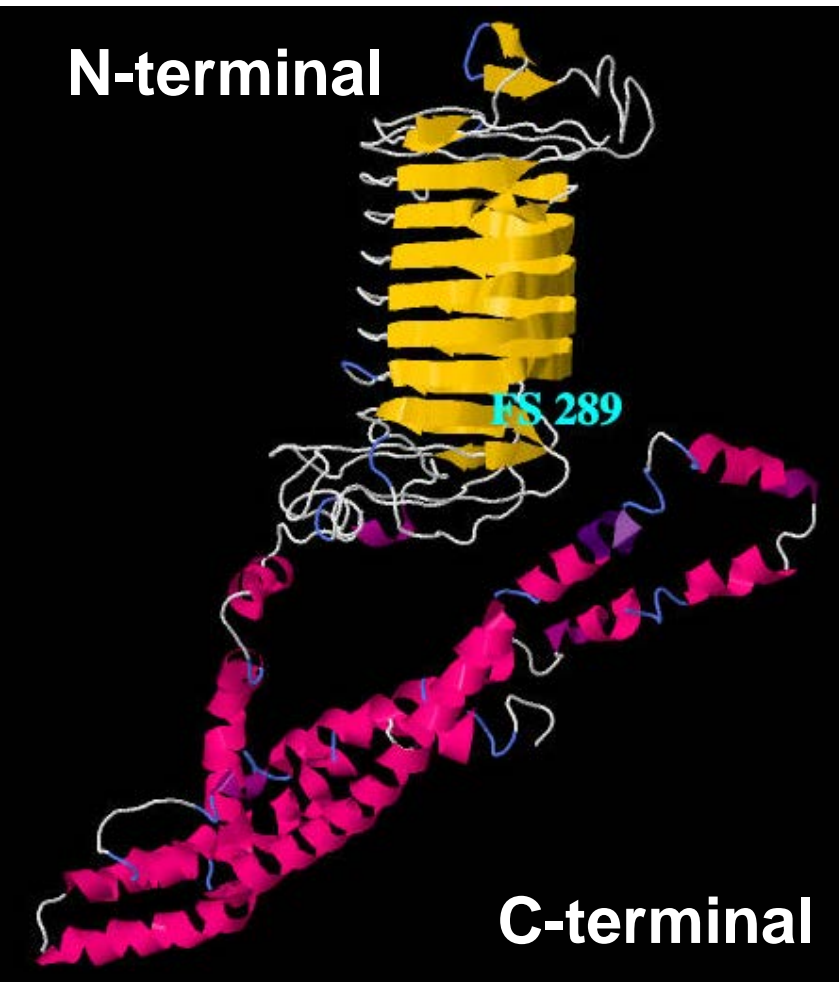


Overall hypothesis

LiaX is a multifunctional protein that
→ Regulates daptomycin resistance through negative inhibition of liaYZ

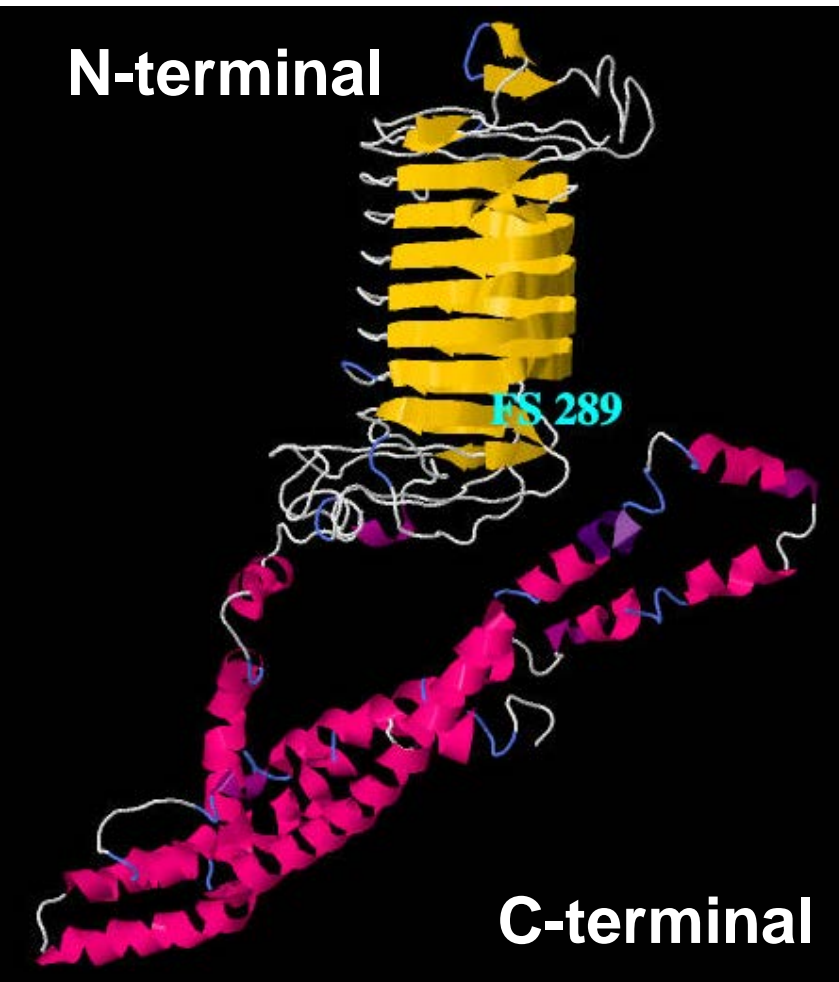


Overall hypothesis



- LiaX is a multifunctional protein that
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 - Activates the liaFSR system in the presence of extracellular stress

Overall hypothesis



- 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

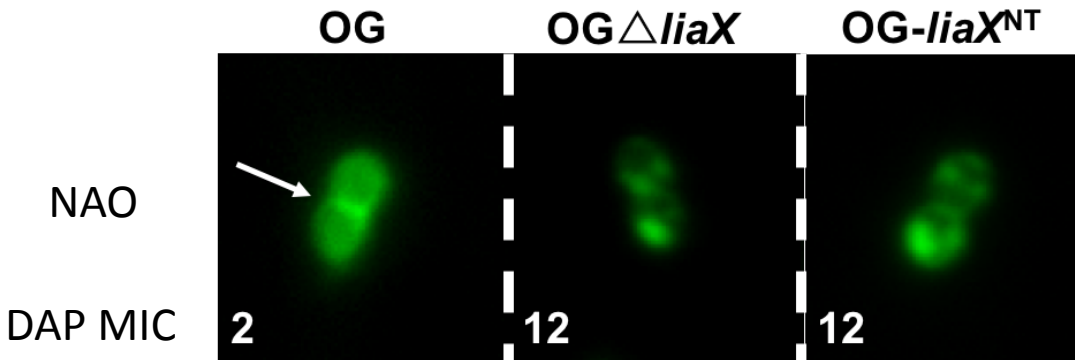
Aim 1: Characterize the localization of LiaX as it pertains to the CE stress response to AMPs

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*

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

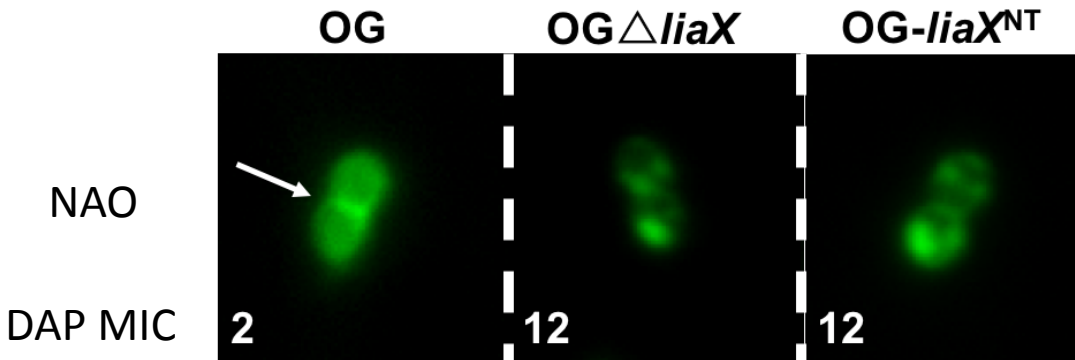
1. Evaluate LiaX protein levels and localization under DAP stress and upon the development of resistance
2. Determine the role of LiaX in resistance to AMPs *in vitro* and *in vivo*
3. Assess if extracellular LiaX can protect DAP-S strains from antibiotic attack by activating the liaFSR stress response

Aim 1 Preliminary Data



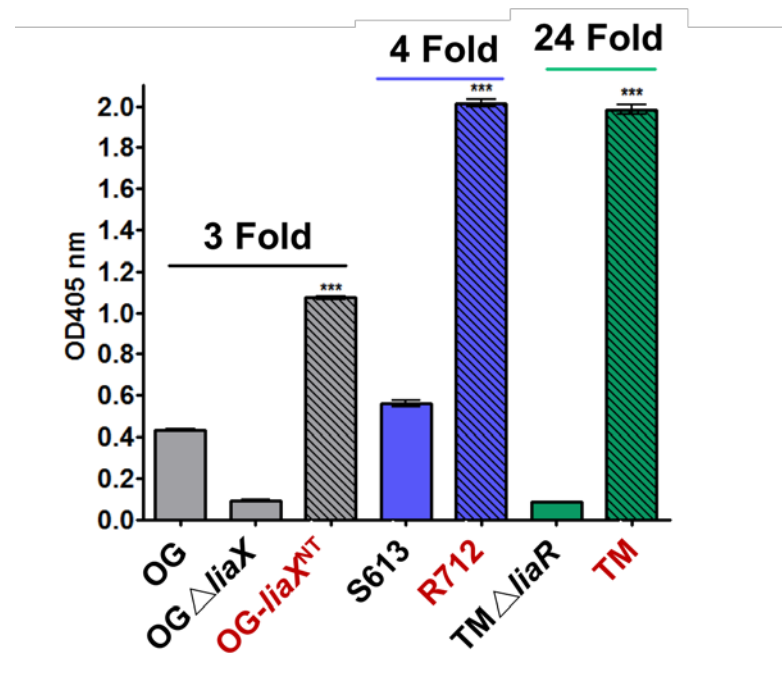
**LiaX (with the Ct alone)
negatively regulates
DAP-R and CM
remodeling**

Aim 1 Preliminary Data

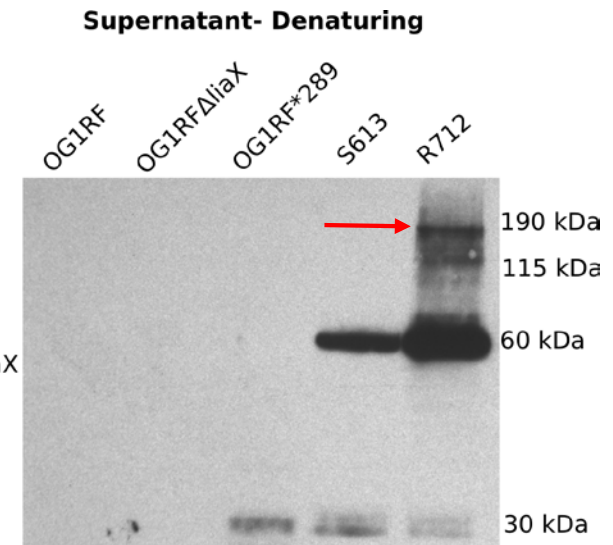
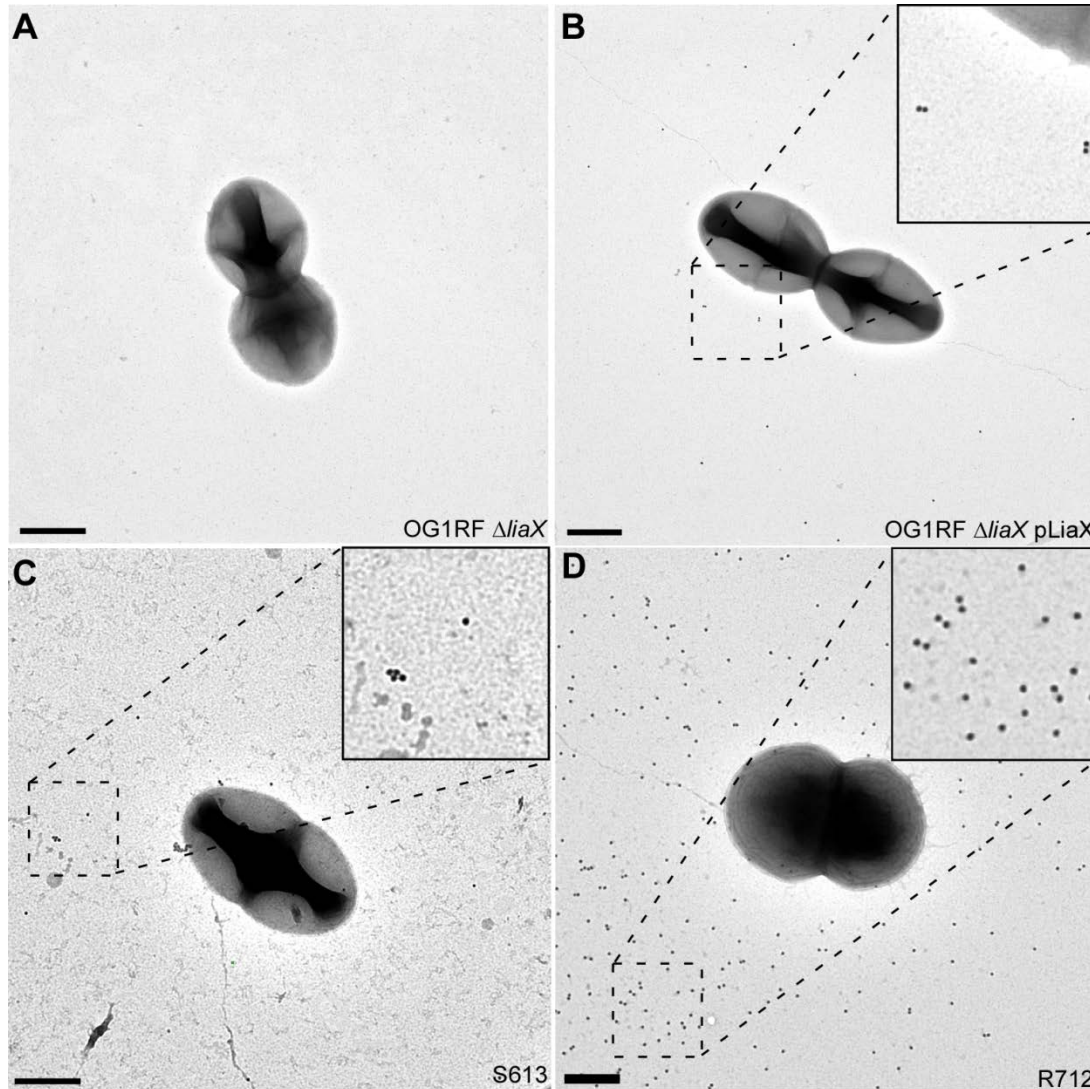


LiaX (with the Ct alone)
negatively regulates
DAP-R and CM
remodeling

Whole-cell ELISA

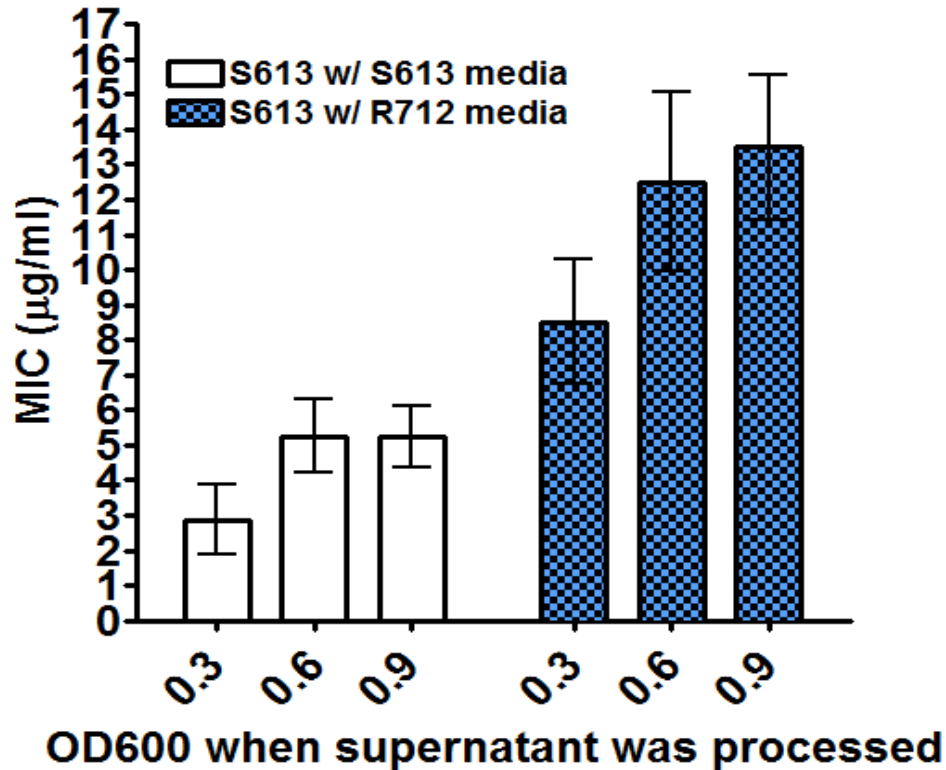


Extracellular LiaX in DAP-R strains



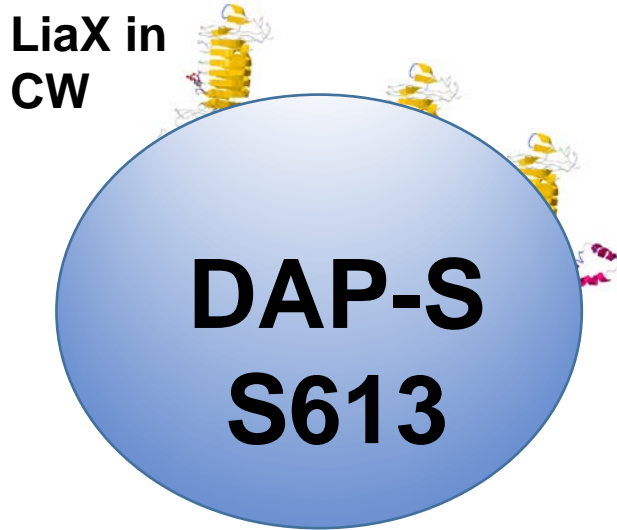
Scale bar 0.5 μ M. Secondary antibody conjugated to 18 nM gold particles.

DAP-R spent media protects DAP-S strain



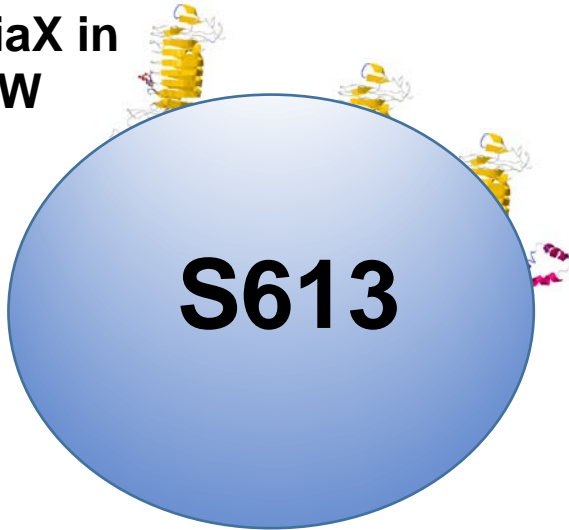
LiaX binds DAP ($K_d = 0.05 \mu\text{M}$)

Localization hypothesis



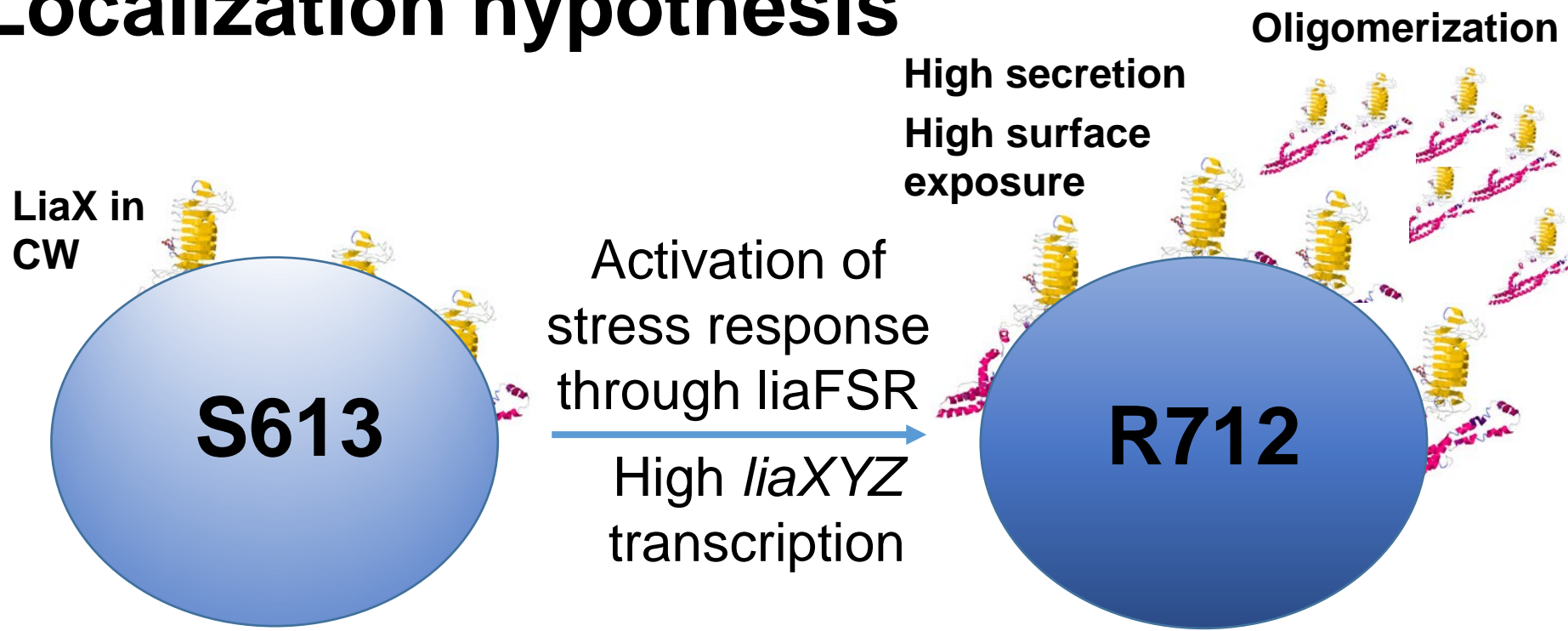
Localization hypothesis

LiaX in
CW



Activation of
stress response
through *liaFSR*
→
High *liaXYZ*
transcription

Localization hypothesis



LiaX in CW

S613

Activation of stress response through liaFSR

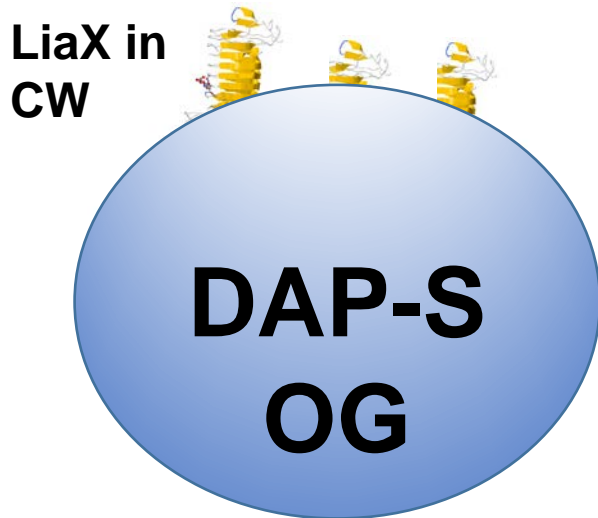
High *liaXYZ* transcription

High secretion
High surface exposure

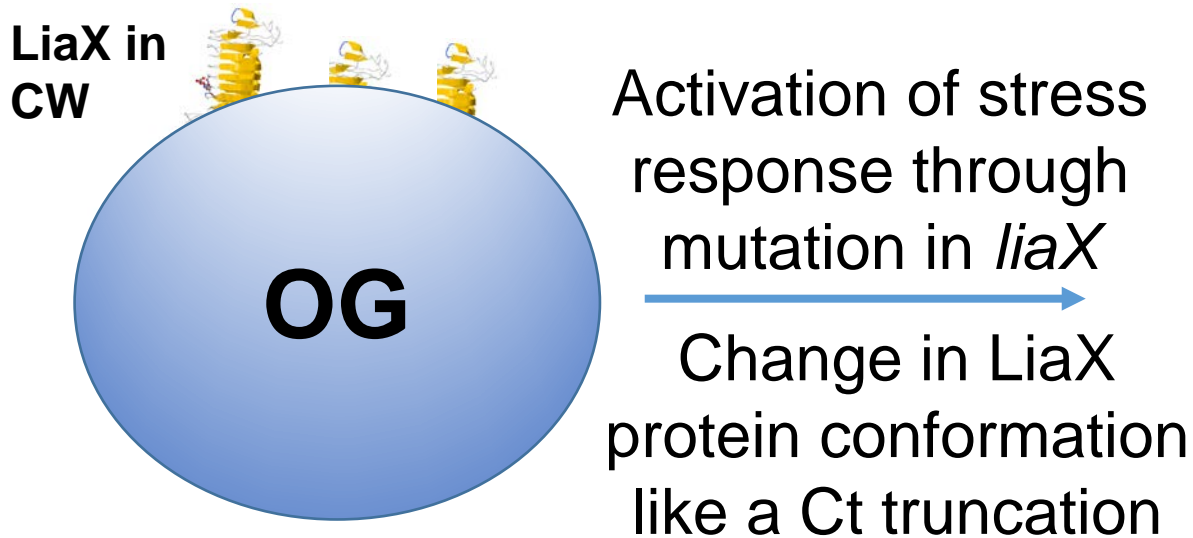
Oligomerization

R712

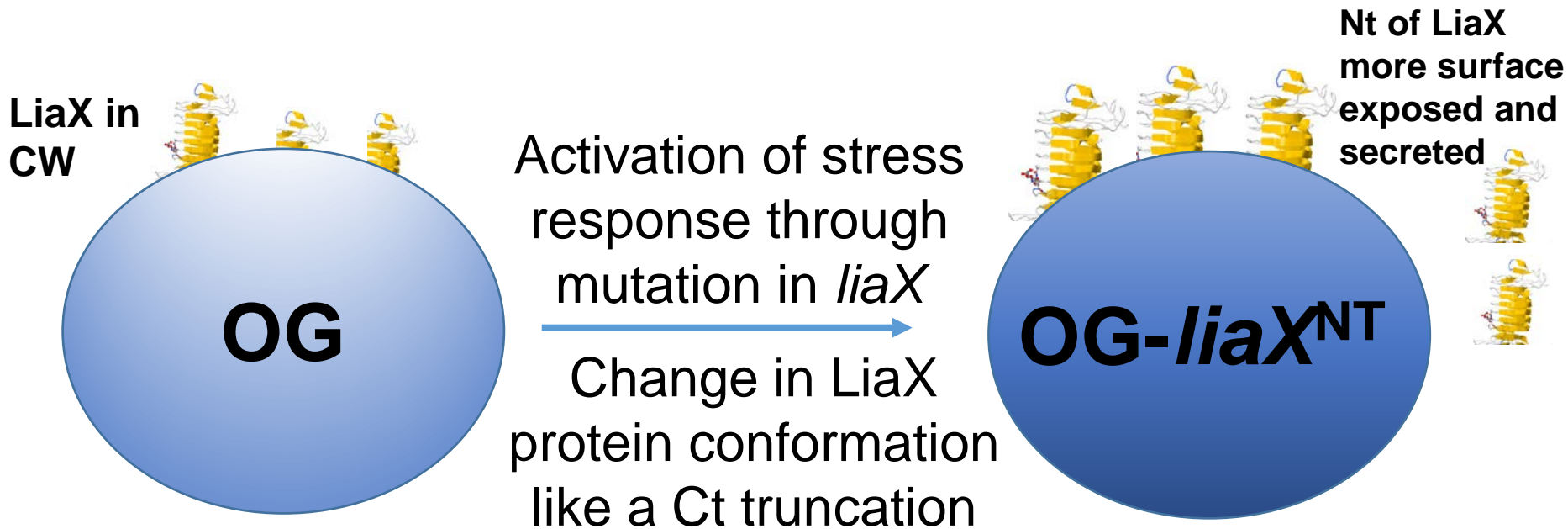
Localization hypothesis



Localization hypothesis



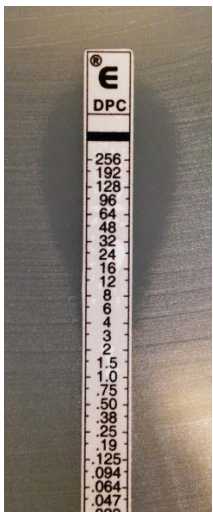
Localization hypothesis



AMP resistance hypothesis

DAP-R

MIC 8



Δ LiaR



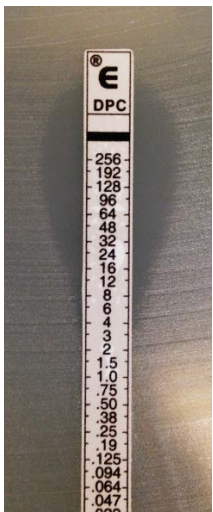
Susceptible
DAP
LL37
Nisin
HBD 3
Broad
spectrum

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

AMP resistance hypothesis

DAP-R

MIC 8

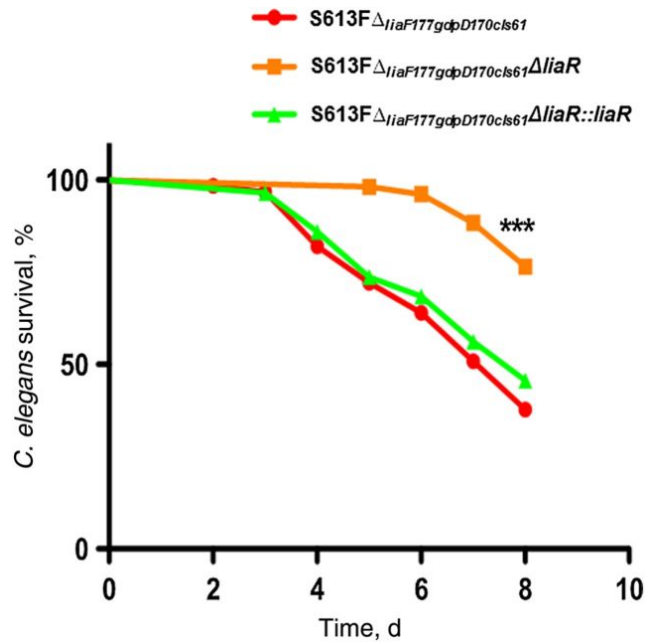


$\Delta LiaR$



Susceptible
DAP
LL37
Nisin
HBD 3
Broad
spectrum

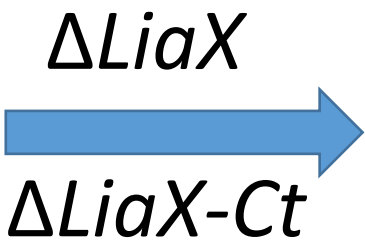
Reyes J, et al. *J Infect Dis*, 2015; Panesso D, et al. *Antimicrob Agent Chemother*, 2015



AMP resistance hypothesis

DAP-S

MIC 2

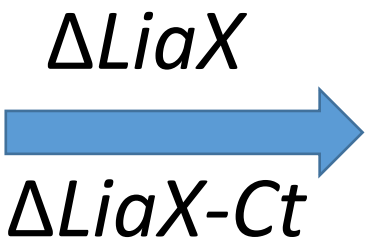


Resistant

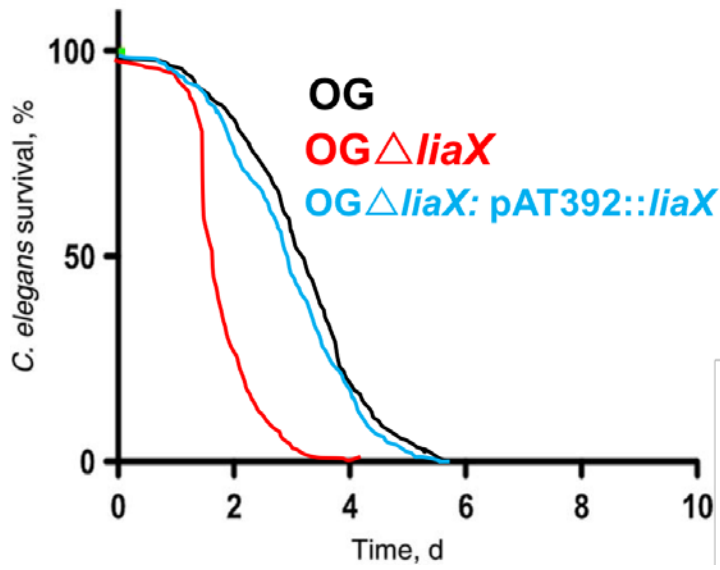
- DAP
- LL37
- Nisin
- HBD3
- Broad Spectrum

AMP resistance hypothesis

DAP-S
MIC 2



Resistant
DAP
LL37
Nisin
HBD3
Broad
Spectrum

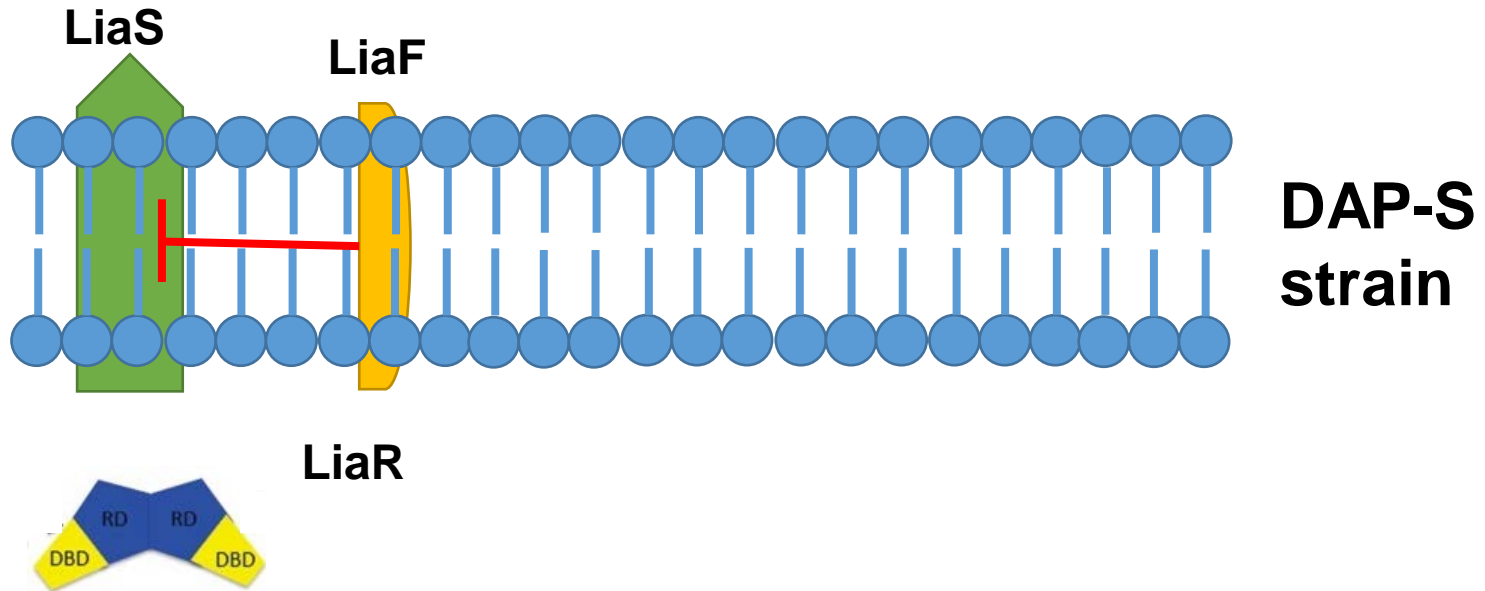


DAP attack on a DAP-S strain

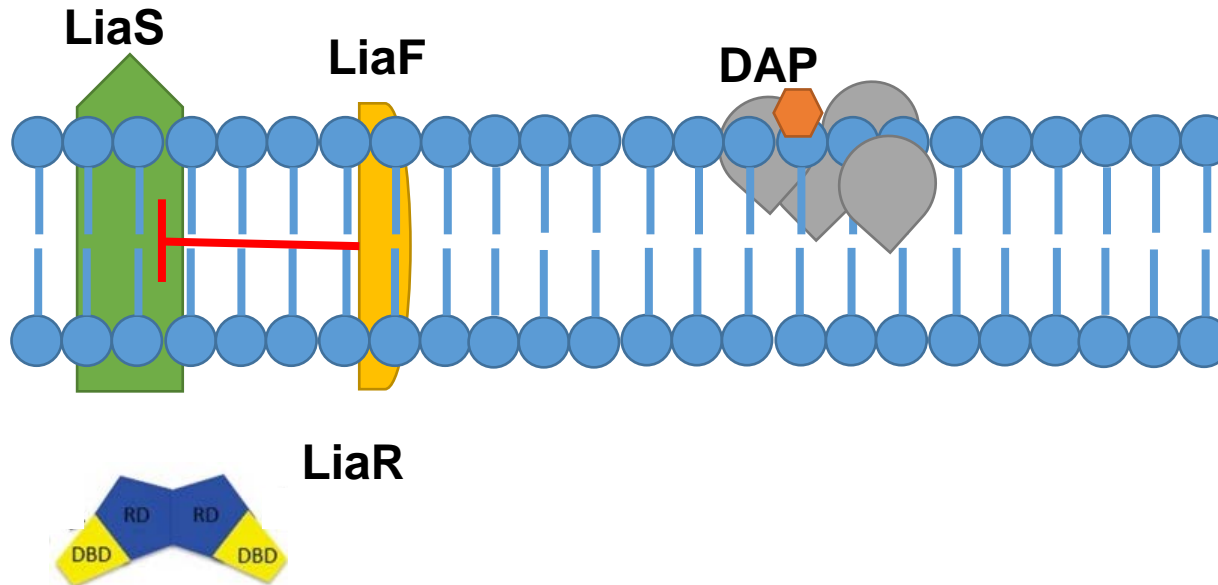
DAP



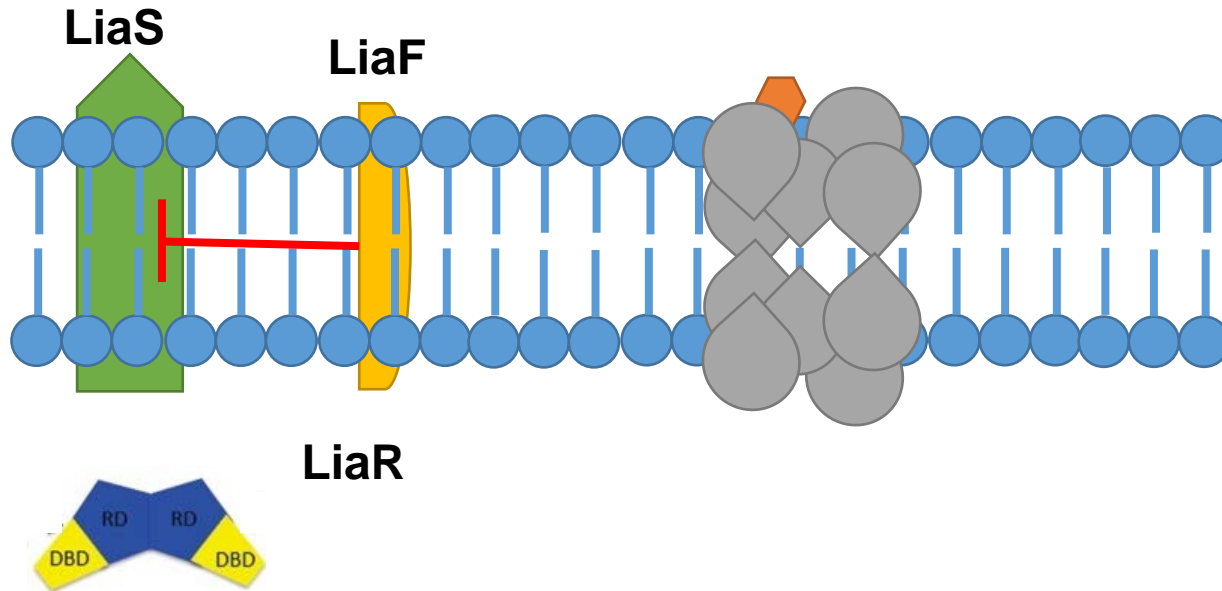
 Ca²⁺



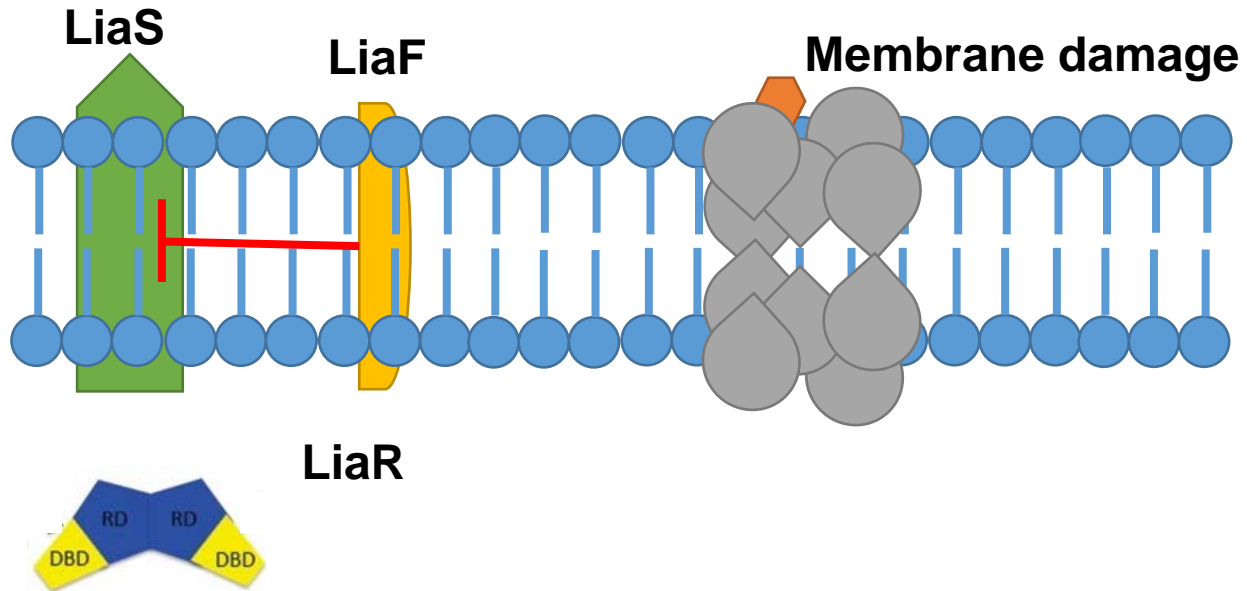
DAP insertion

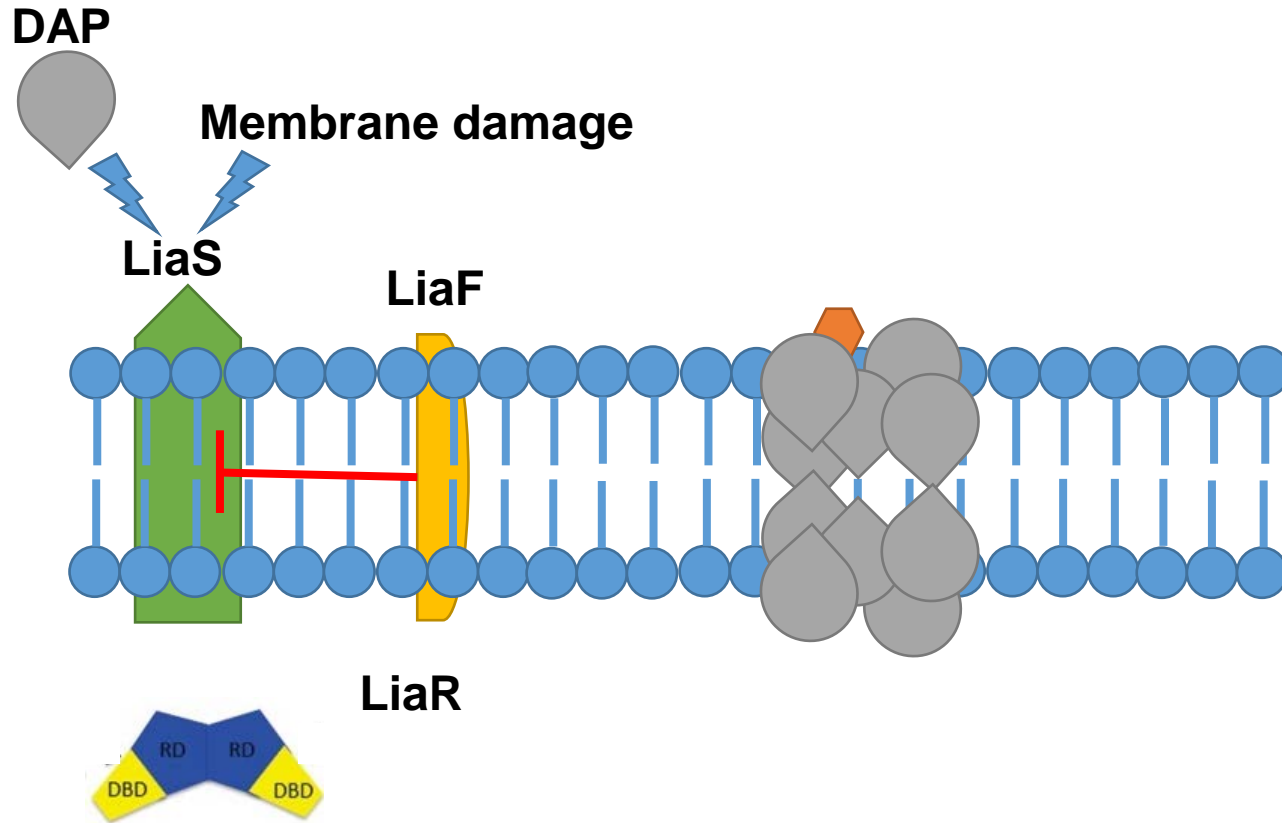


Oligomerization

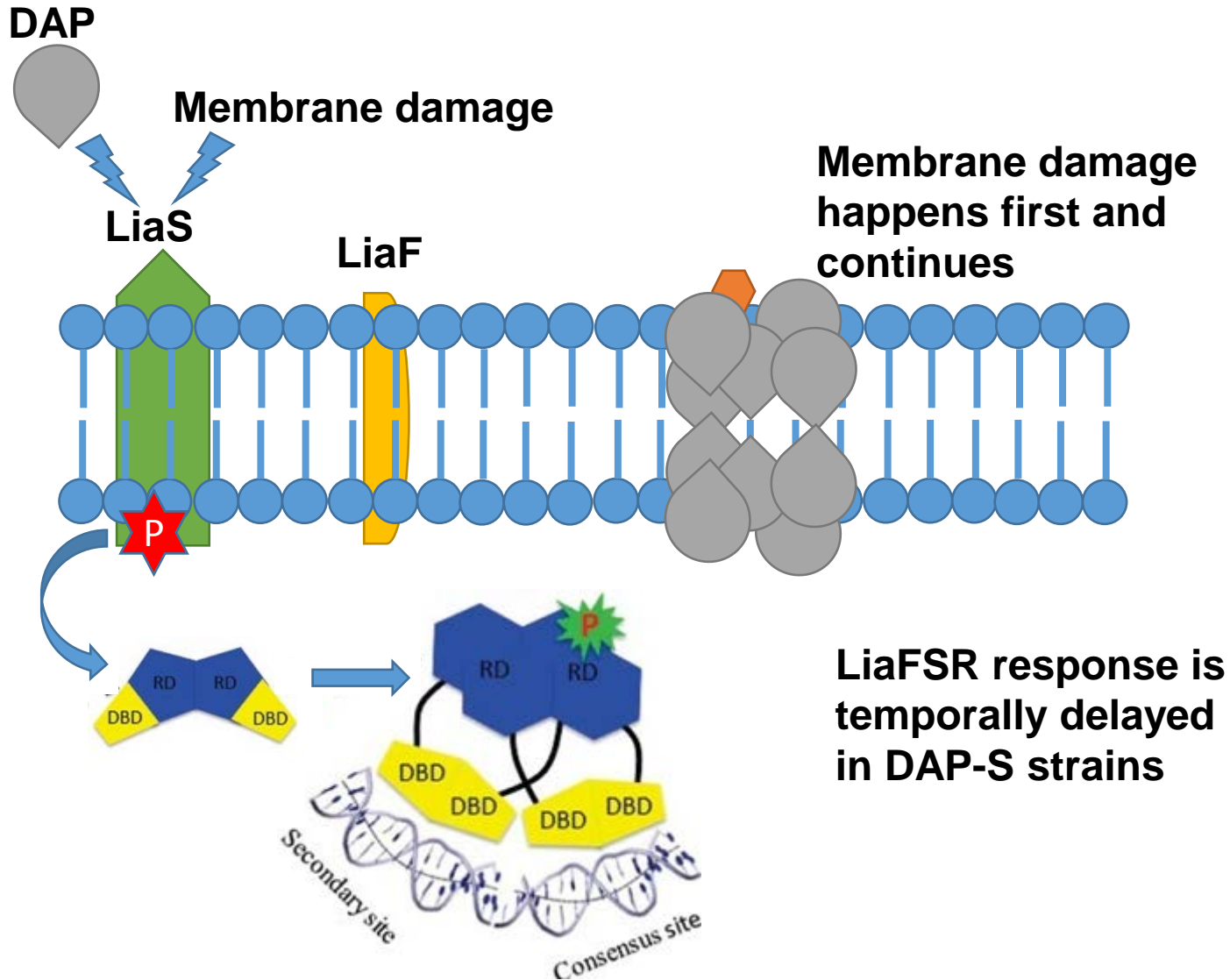


Damage begins



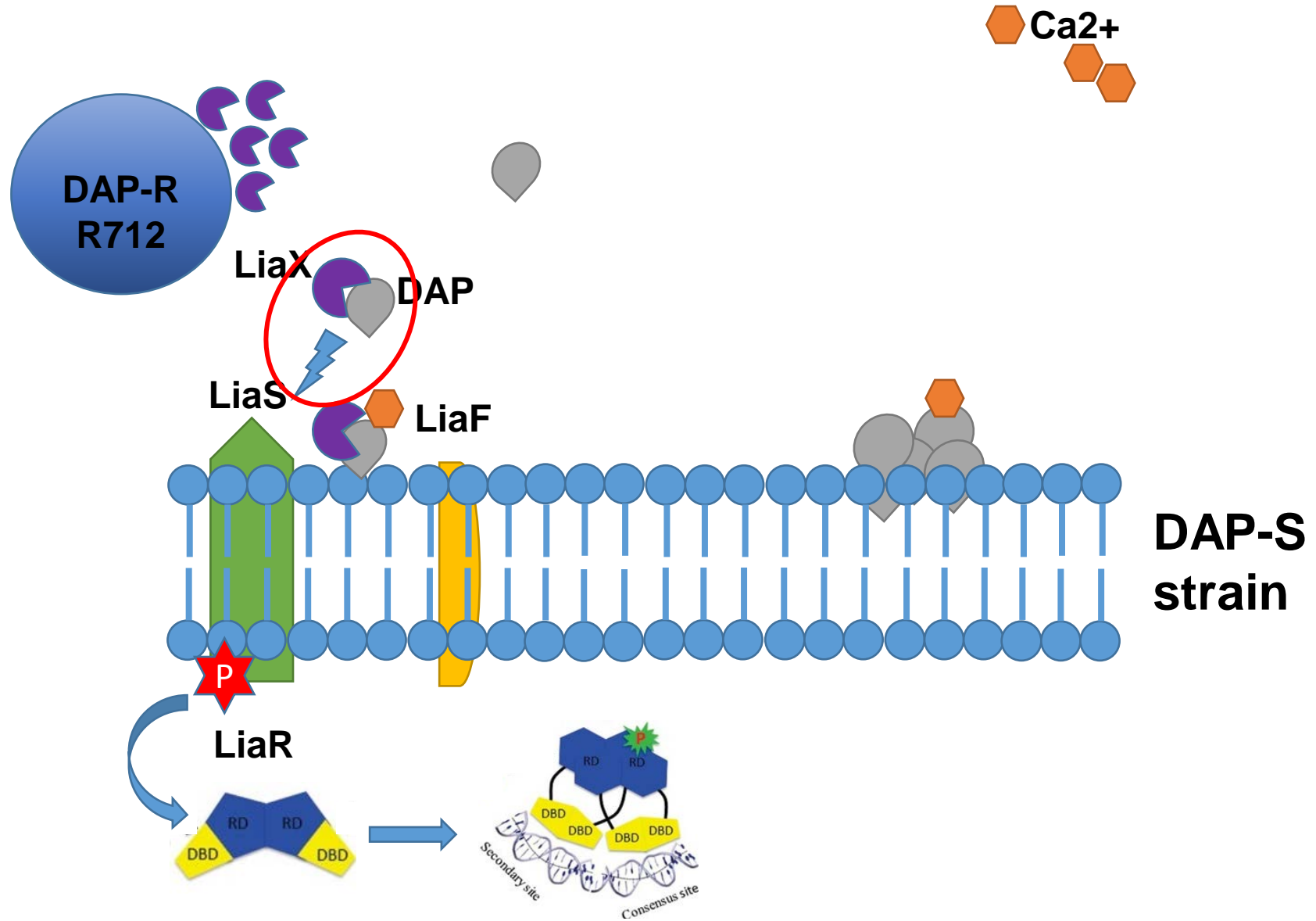


Time to cell death < Time to mount a response



Extracellular protection hypothesis

LiaX-DAP complex activates stress response before cell death

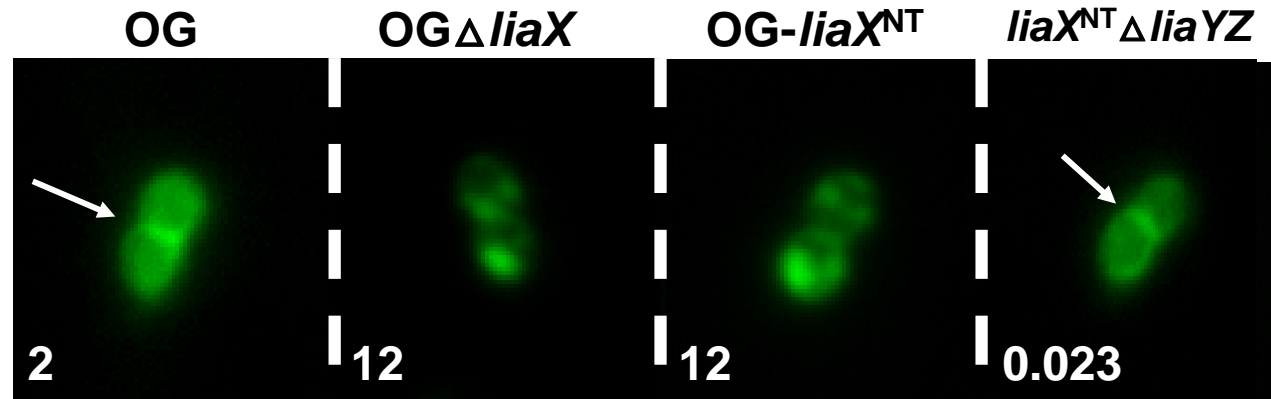


Aim 2: Dissect the role of LiaX in regulating DAP-R through protein interactions

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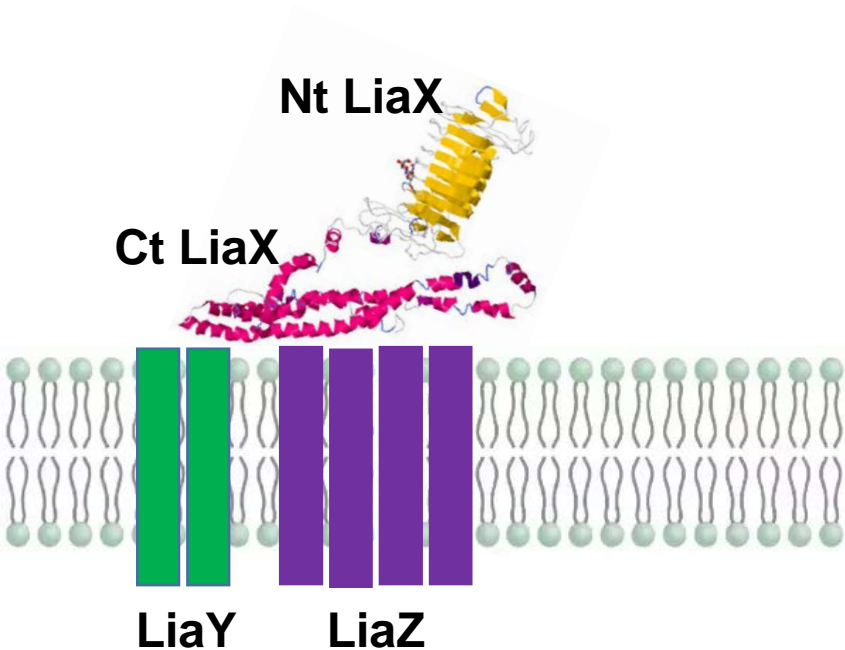
1. Characterize the liaX interactome in DAP-R and DAP-S strains
2. Study the liaX and liaYZ interaction as mechanism of regulation of DAP-R

Aim 2 Preliminary Data



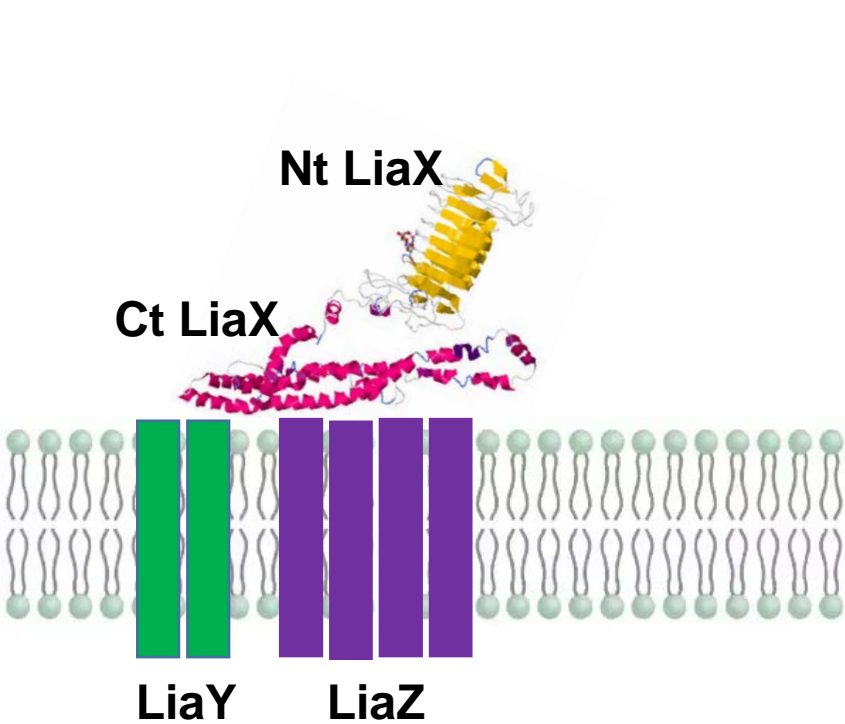
**LiaX regulates
DAP-R by
inhibiting *liaYZ***

Aim 2 hypothesis- LiaX and LiaYZ interaction

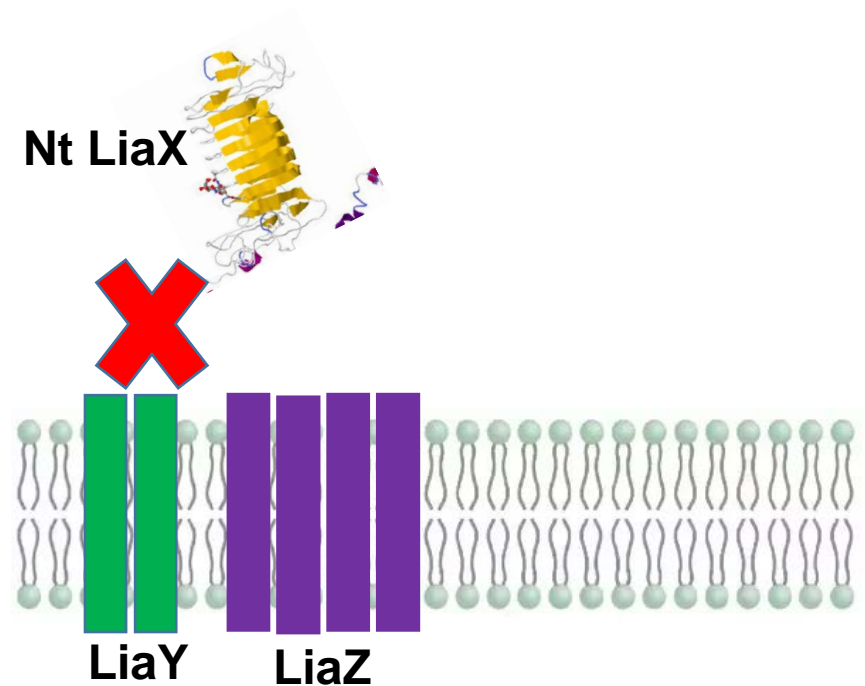


**Full length LiaX in
DAP-S strains**

Aim 2 hypothesis- LiaX and LiaYZ interaction

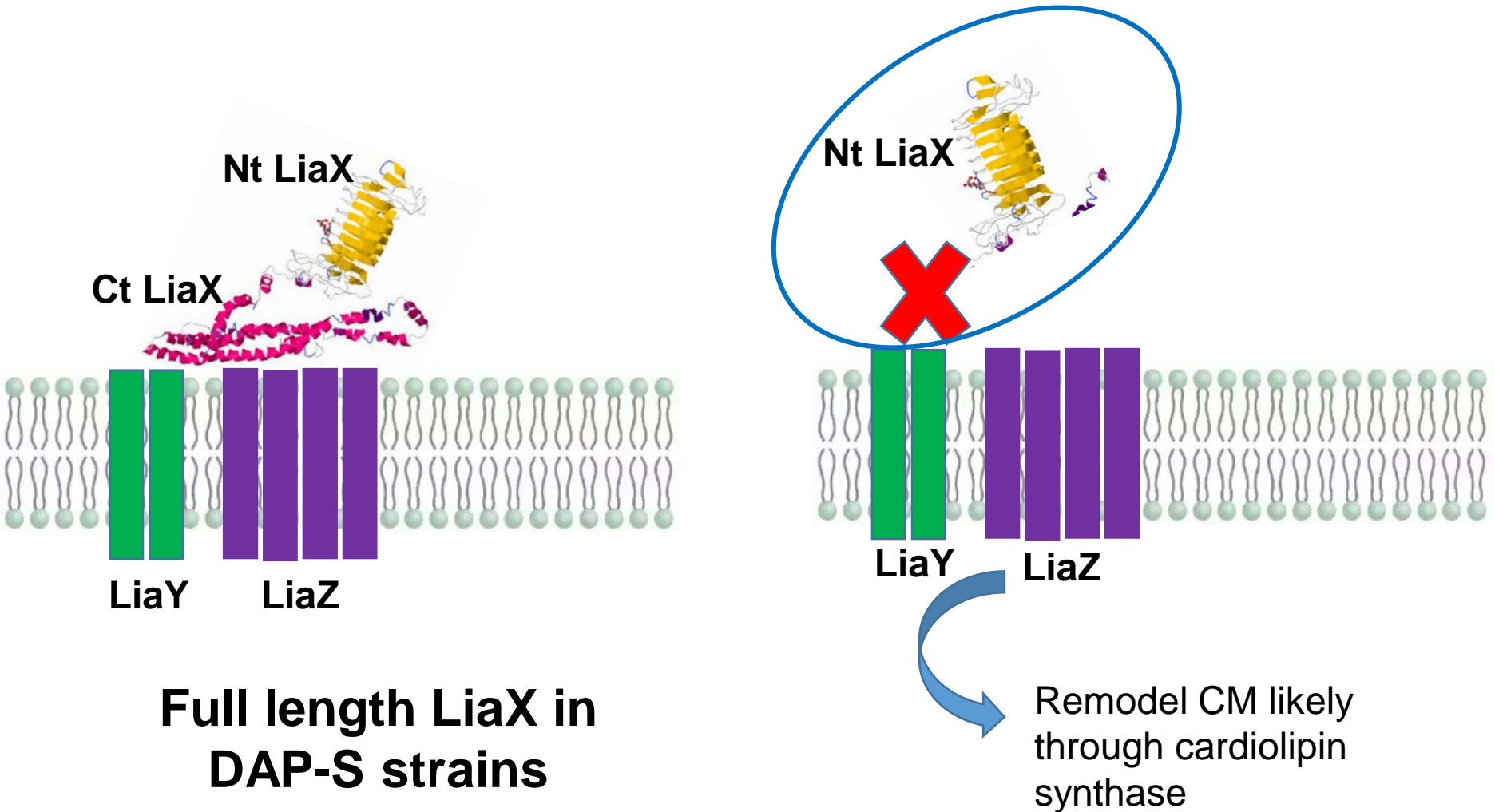


**Full length LiaX in
DAP-S strains**



**DAP-R strain with Ct
truncation of LiaX**

Aim 2 hypothesis- LiaX and LiaYZ interaction



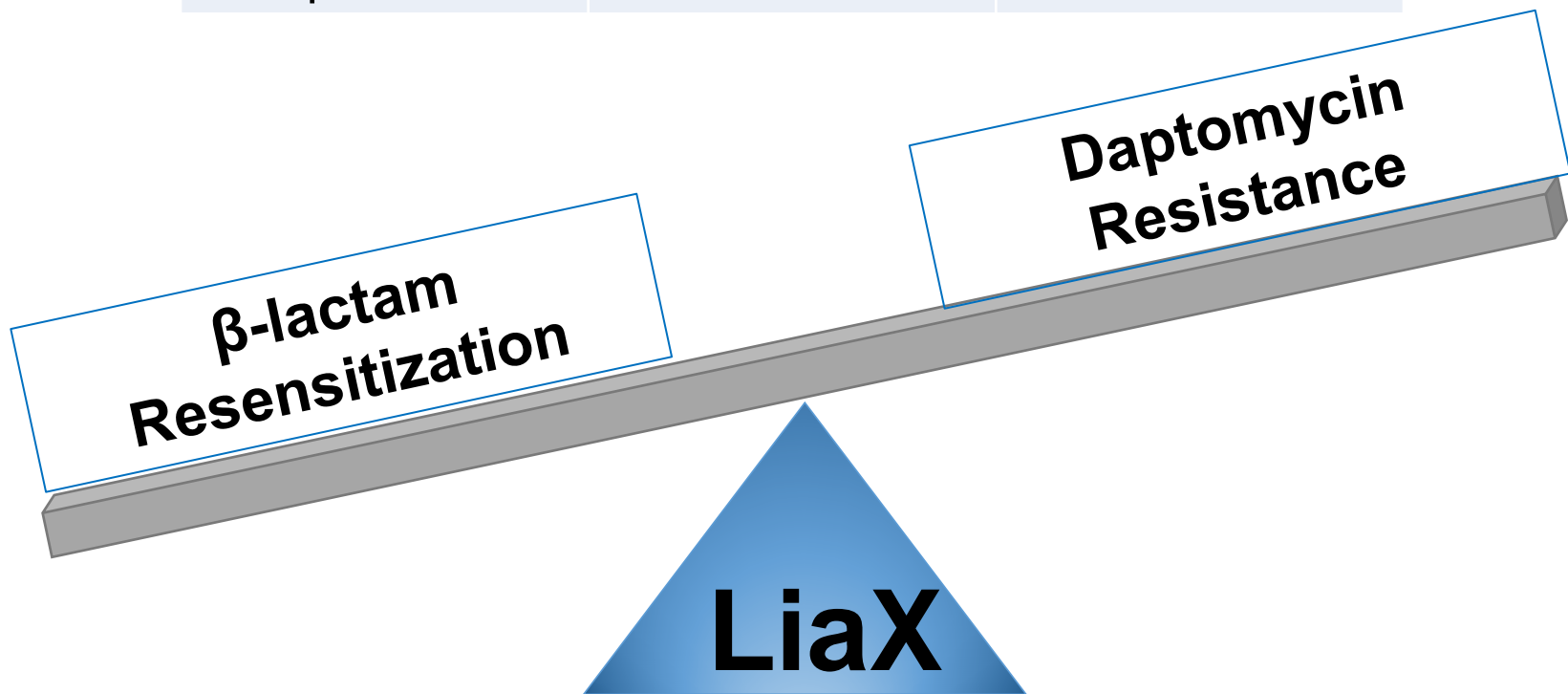
**Aim 3: Elucidate the role of LiaX
in mediating the seesaw effect
through interaction with PBP5**

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

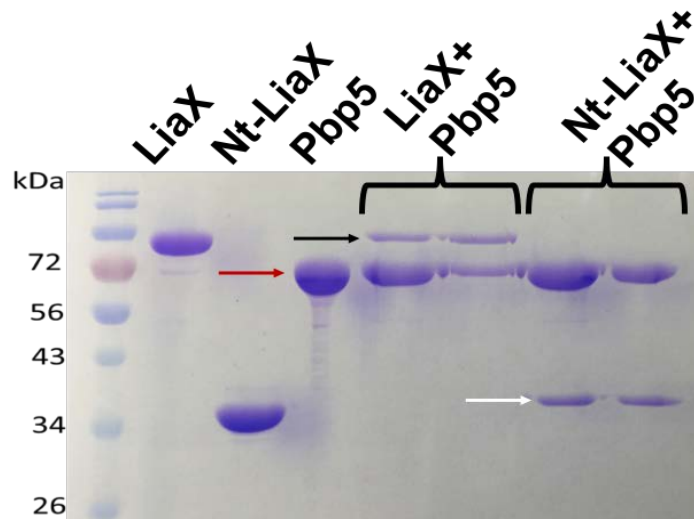
1. Study PBP5-liaX colocalization in DAP-S strains and PBP5 mislocalization in DAP-R strains
2. Assess PBP5 protein levels and β -lactam binding to PBPs in DAP-R strains

Aim 3 Preliminary Data

Strain	DAP MIC (ug/ml)	Ceftriaxone MIC
OG	2	32
OG Δ <i>liaX</i>	12	6
OG- <i>liaX</i> ^{NT}	12	6
Complements	4	32



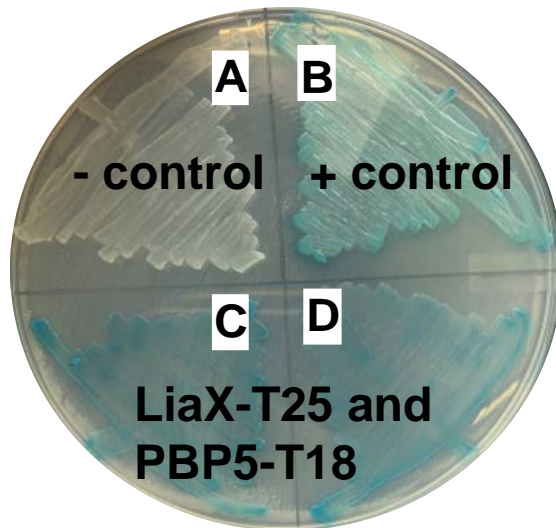
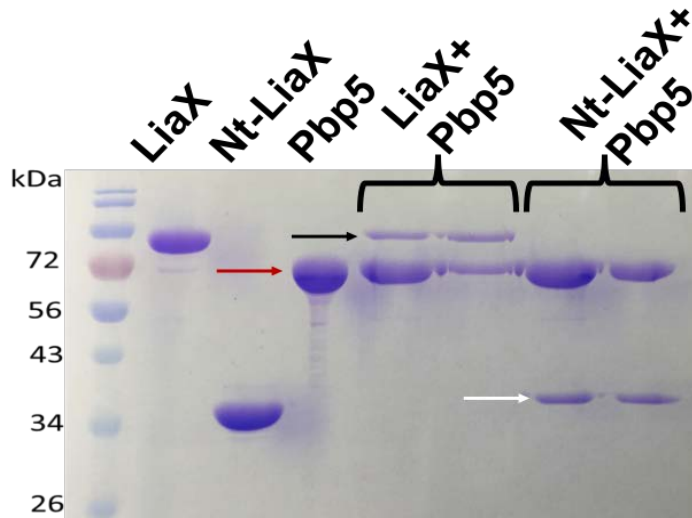
LiaX-Pbp5 pull down



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

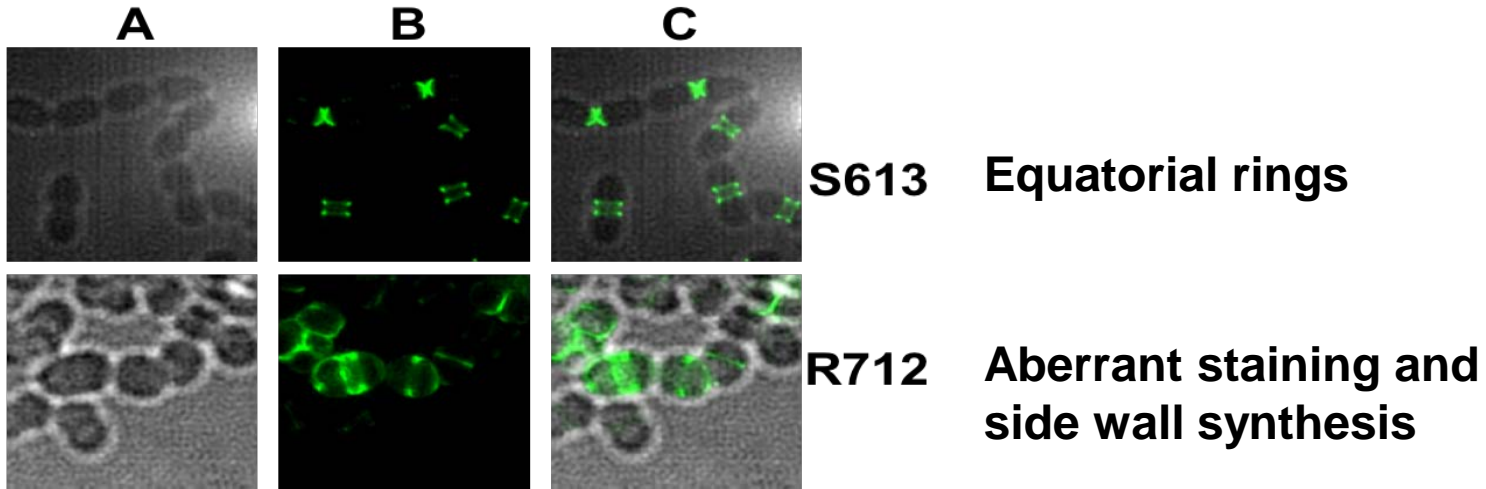
Controls: no bait, GFP used as bait/ prey

Pull-down and Bacterial 2hybrid show interaction



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

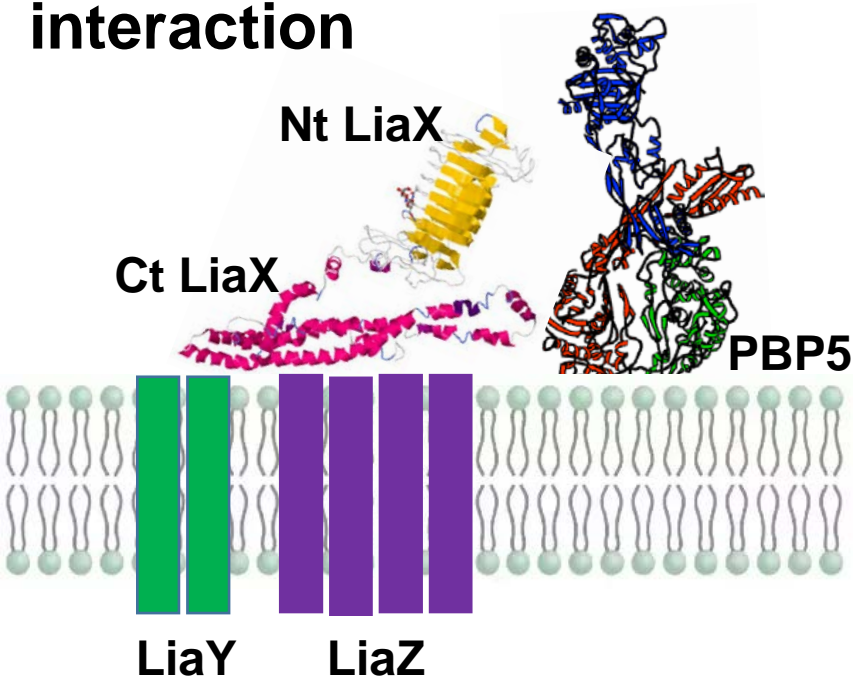
PG synthesis mislocalized



NADA Staining of nascent PG synthesis

Aim 3 hypothesis

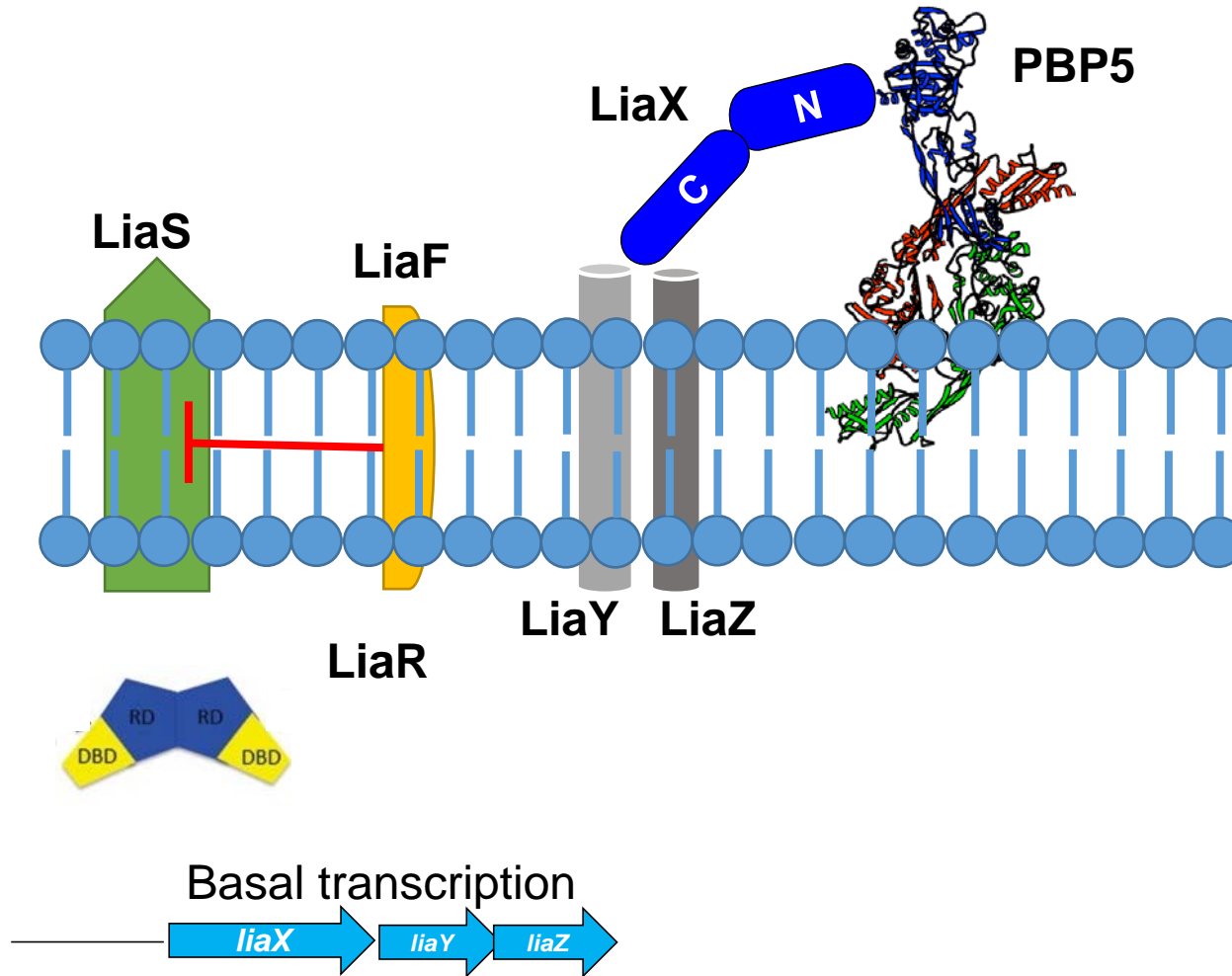
LiaX- PBP5
interaction



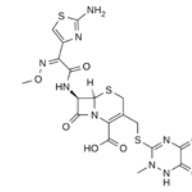
Full length LiaX in
DAP-S strains

Model of the LiaFSR and LiaX mediated stress response

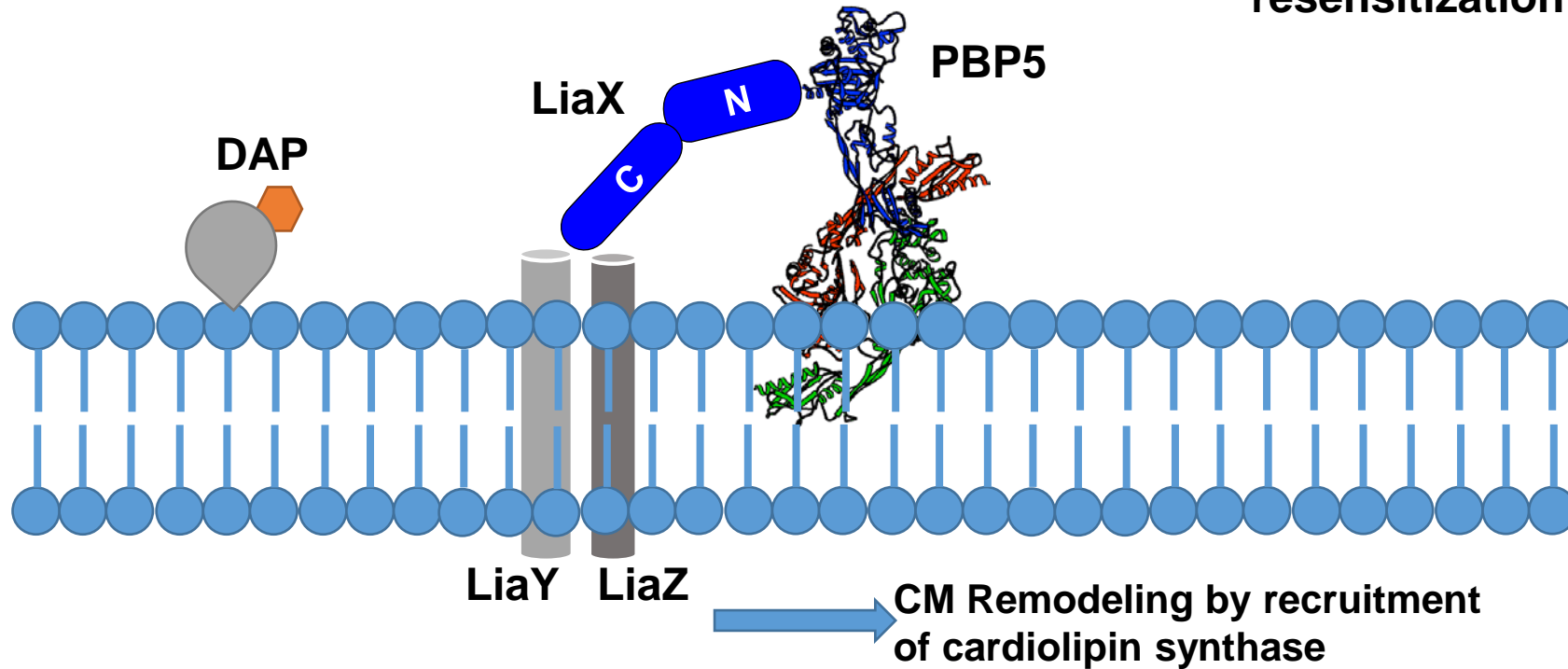
Absence of stress (“OFF”)



“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

