The 1st International Electronic Conference on Antibiotics—The Equal Power of Antibiotics And Antimicrobial Resistance

Section: Antimicrobial Resistance Mechanisms and Intrinsic Microbial Factors Contributing to Resistance

Calcium Regulates Resistance of *Pseudomonas aeruginosa* to Polymyxin B

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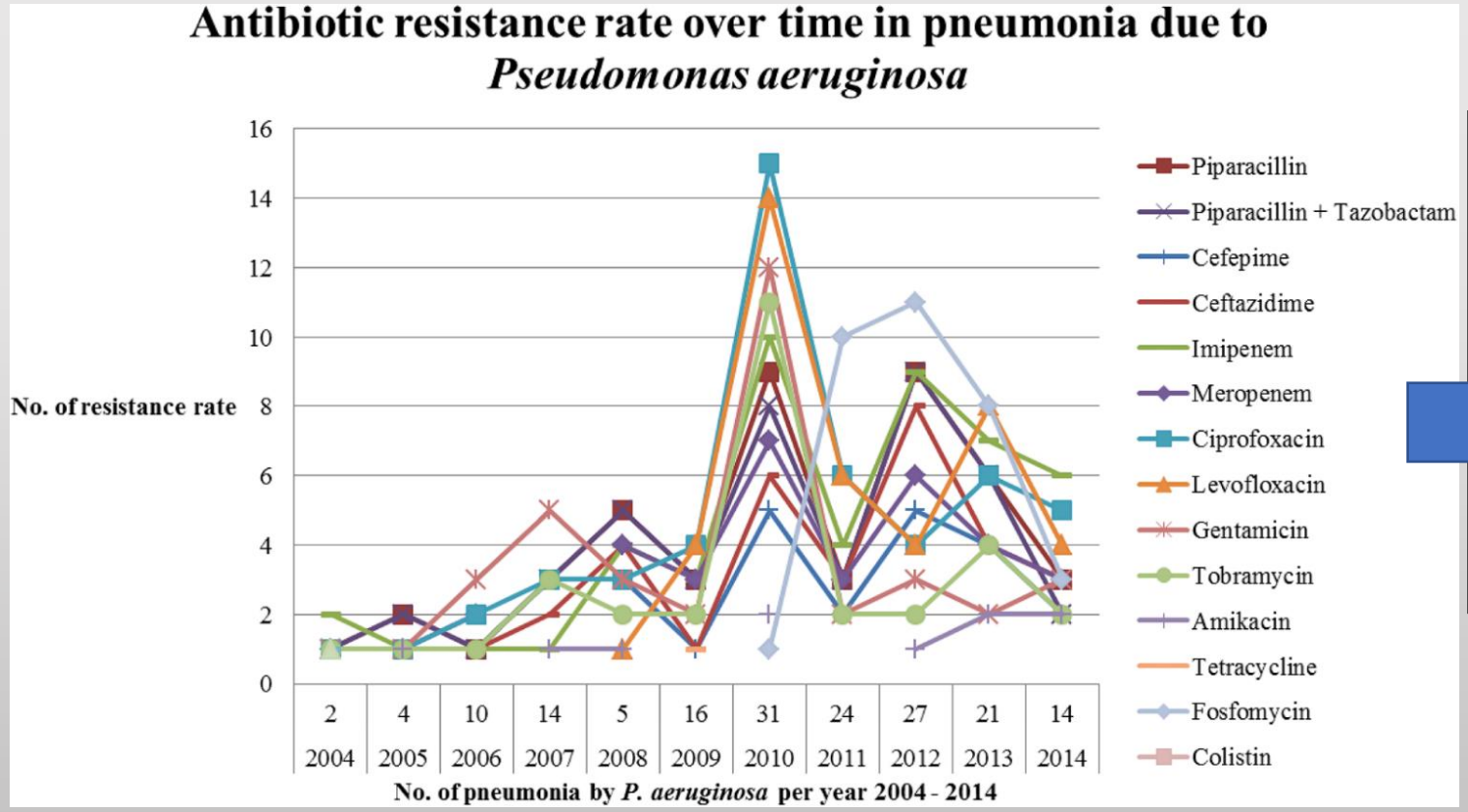
INTRODUCTION

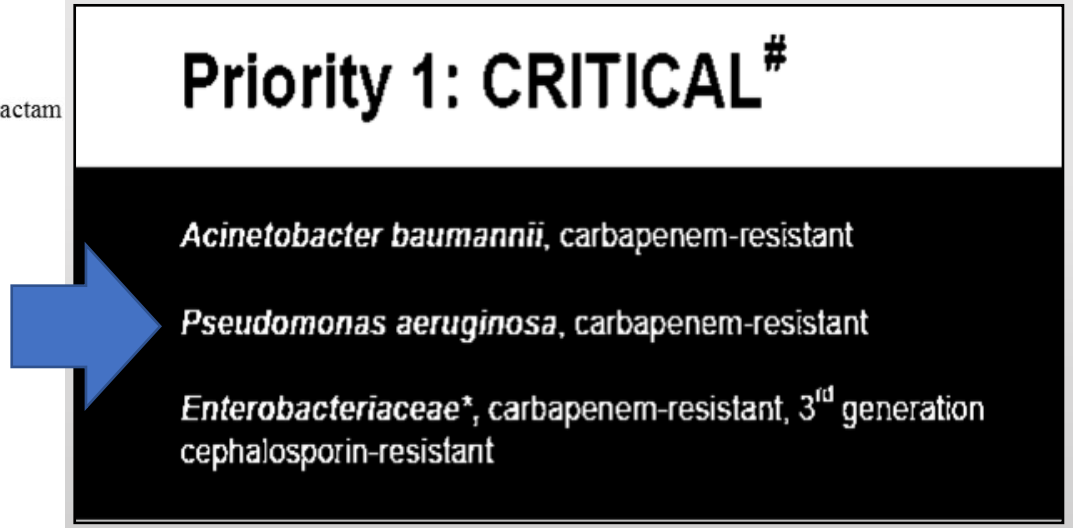
Pseudomonas aeruginosa

- Ubiquitous in nature
- A major cause of nosocomial infections (9 - 10% of all hospital infections)
- Causes high morbidity and mortality in cystic fibrosis (CF) patients and immunocompromised individuals

Multi-drug resistant *P. aeruginosa* is a growing global concern



Yayan *et al.*, 2015



WHO priority pathogen list for development of new antibiotics

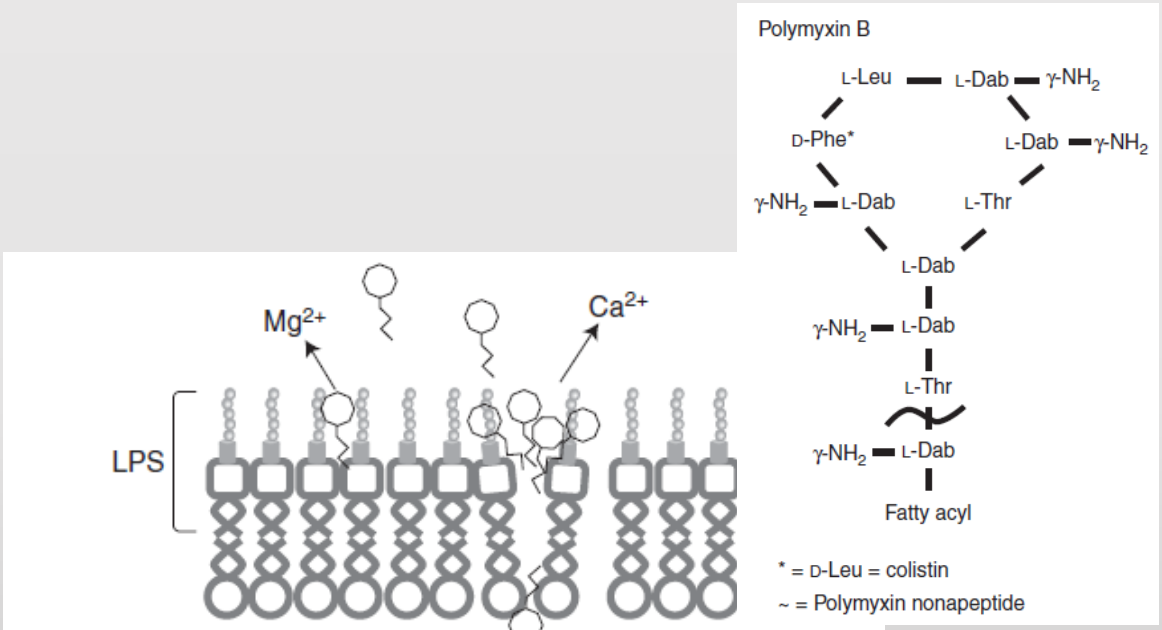
Last hope antibiotics to the rescue

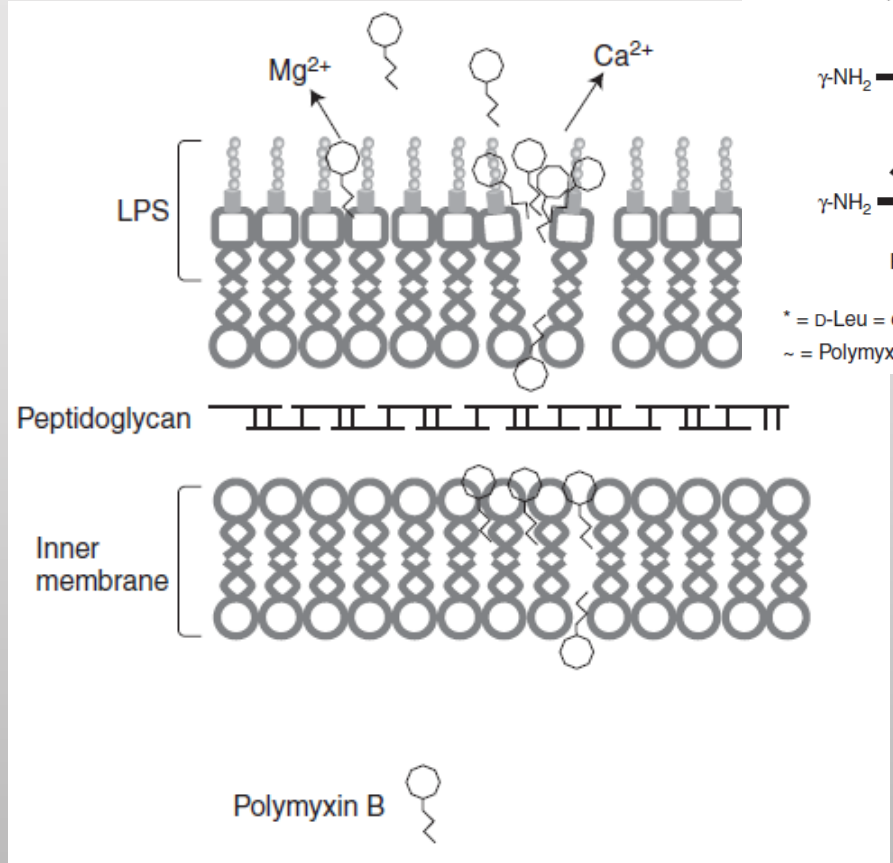
- Nature's peptides
- Small, cationic

  Polymyxin B (Pol B)

  Polymyxin E

  (Colistin)
- Binds to LPS promoting membrane permeabilization, results in cell lysis

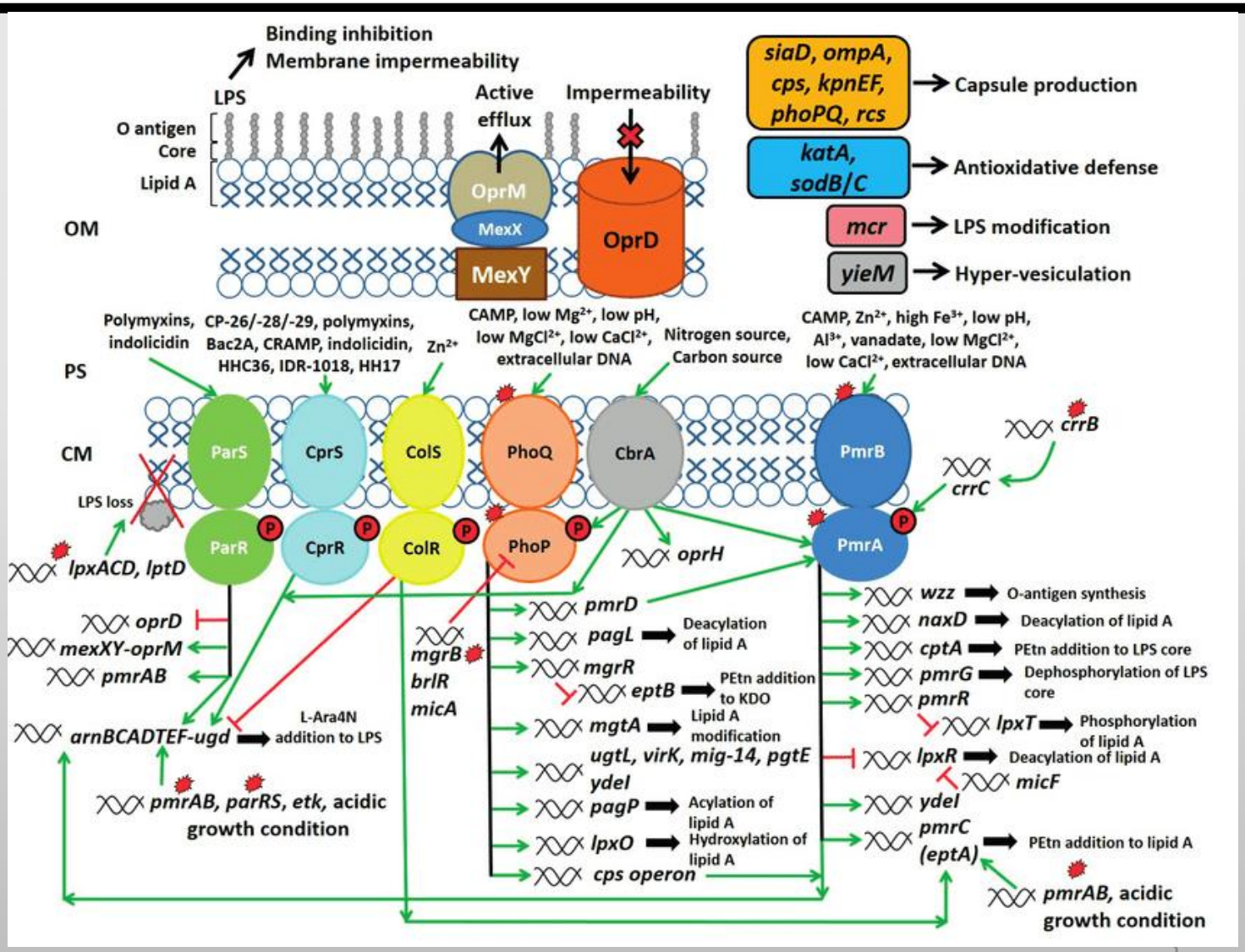




Trimble *et al.*, 2016

P. aeruginosa has multifactorial resistance mechanisms

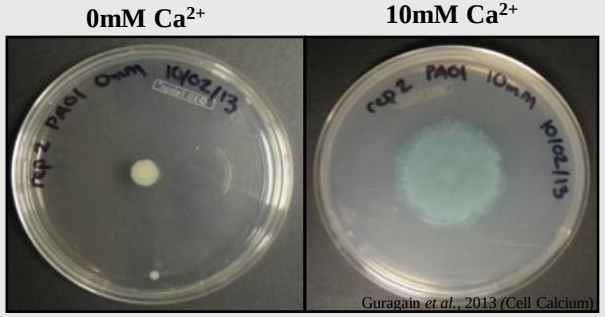
- Restricted uptake and efflux
- Drug inactivation
- Changes in targets



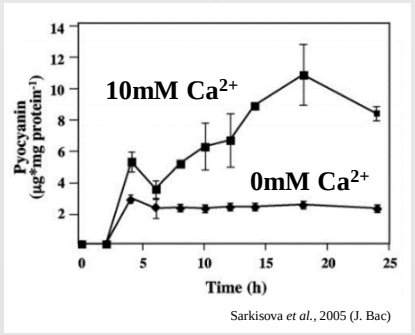
Patik and Kolar, 2019; *Czech Repub BIOMED PAP*

Ca enhances Polymyxin B resistance in *P. aeruginosa*

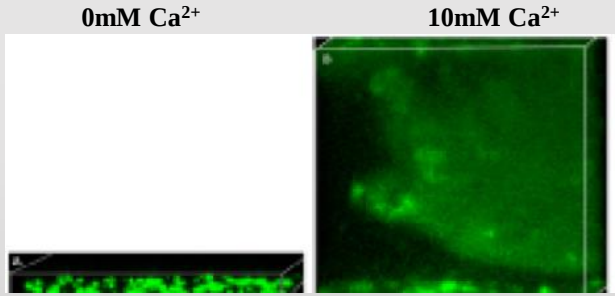
Swarming Motility



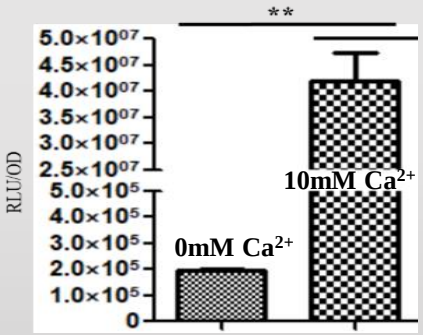
Pyocyanin production

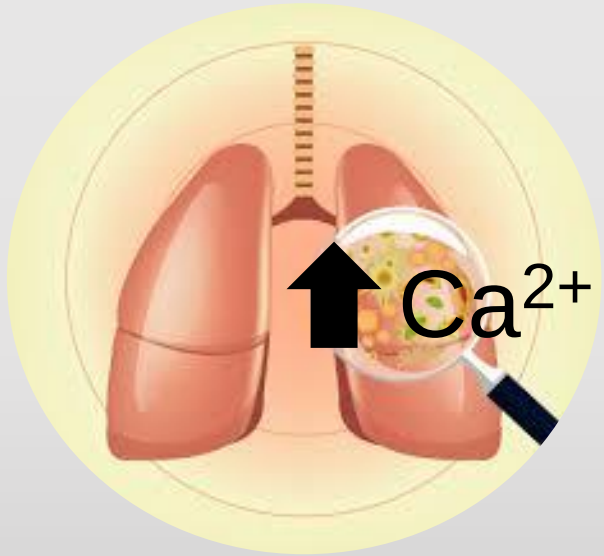


Biofilm formation



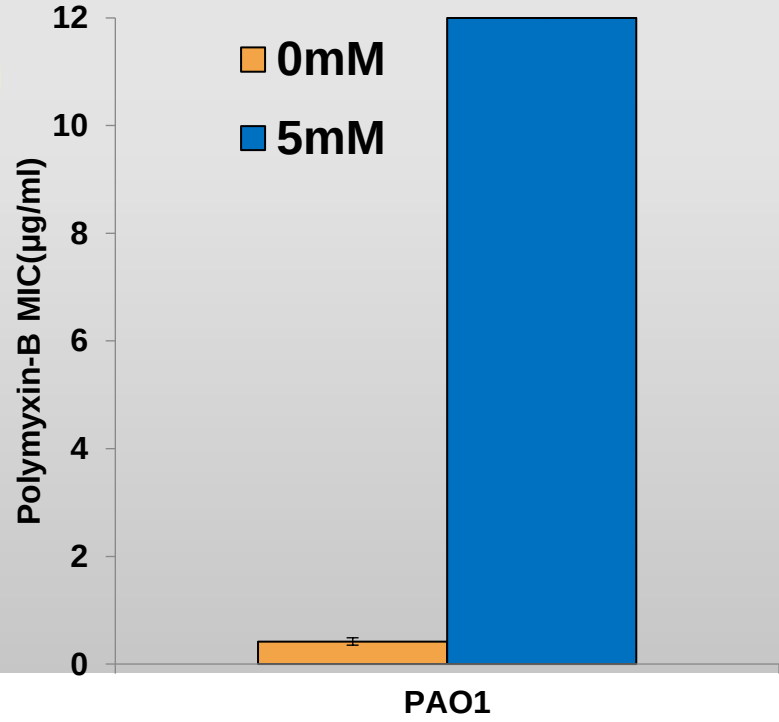
Pyoverdine Production





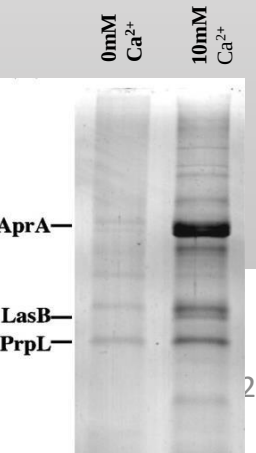
Resistance

Gentamycin
Tetracycline
Carbenicillin
Chloramphenicol
Tobramycin
Polymyxin B

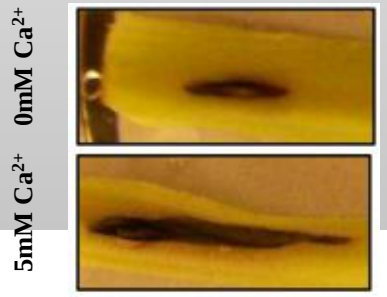


Increased resistance to Polymyxin B in PA01 in response to 5mM Ca

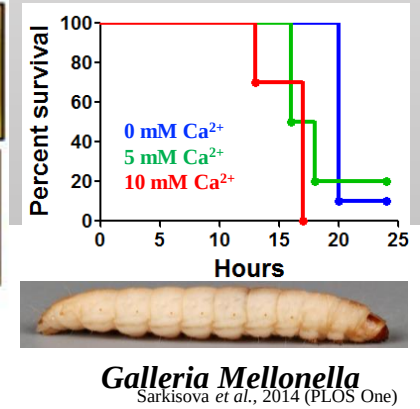
Extracellular Proteases



Virulence Assay



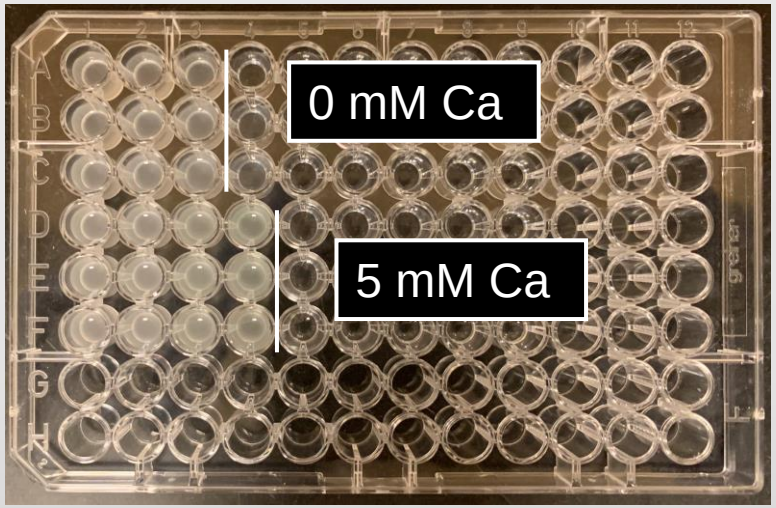
Lettuce Mid-Rib



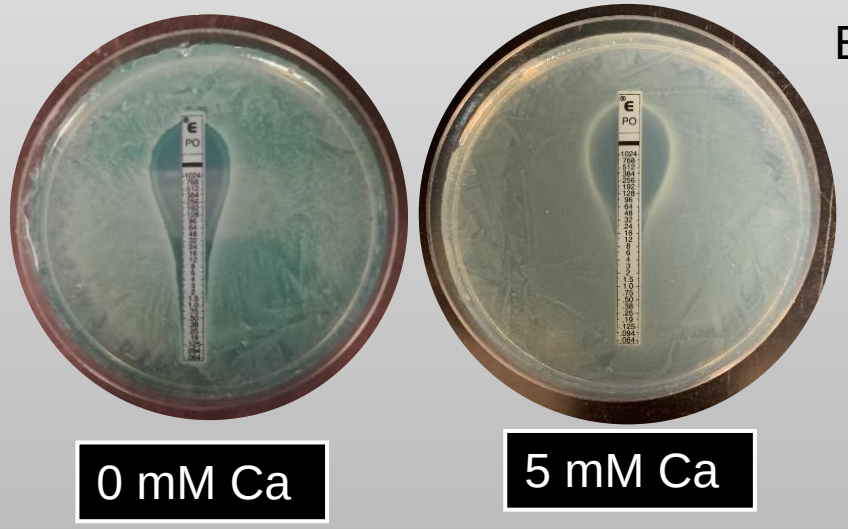
Galleria Mellonella

Sarkisova *et al.*, 2014 (PLOS One)

What are the mechanisms of Ca induced resistance to Polymyxin B in *P. aeruginosa?*



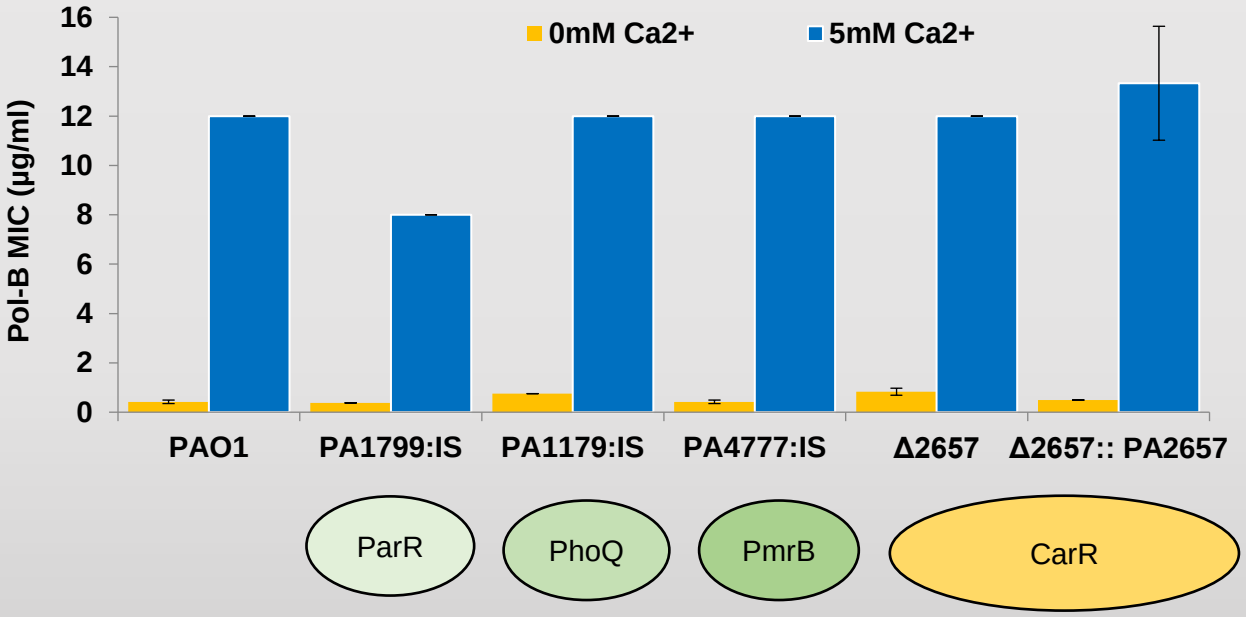
Broth dilution



E-test

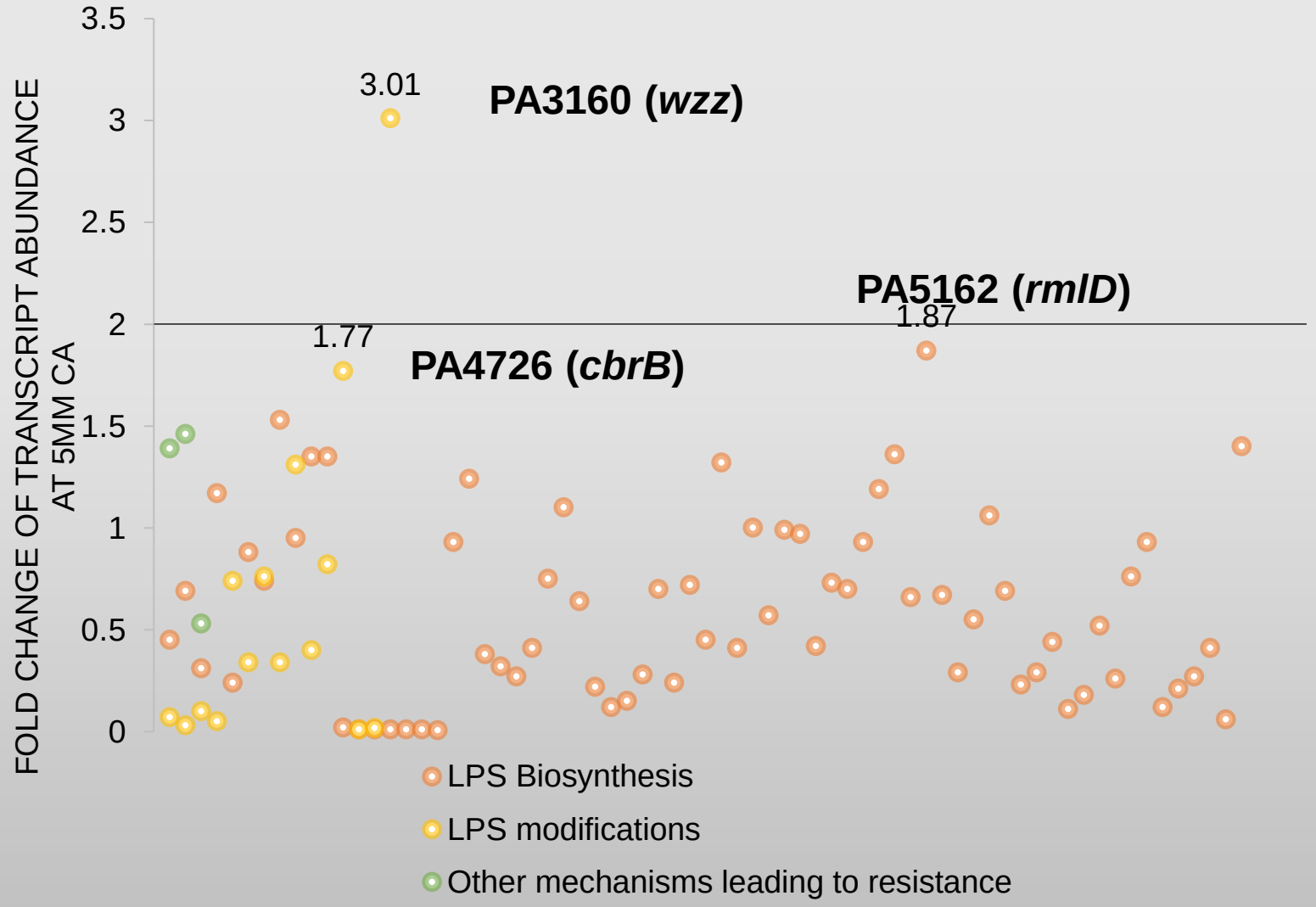
- Test the involvement of known mechanisms of resistance to Pol B in *P. aeruginosa*
- Identify Ca dependent mechanisms of Pol B resistance
- Characterize the membrane changes in response to Ca

Known Pol B resistance mechanisms do not play a role in Ca- induced resistance



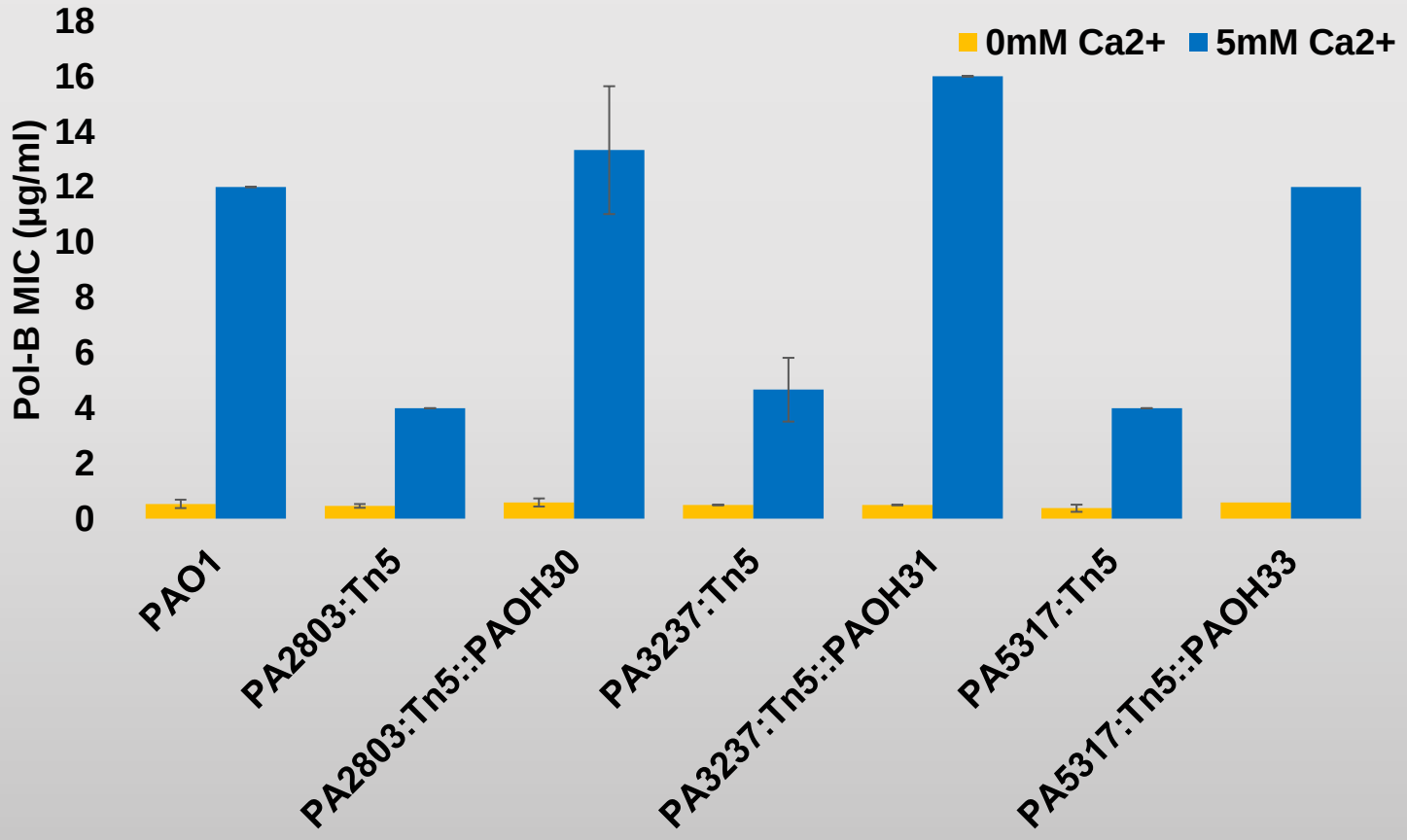
Modification	Gene	PolB resistance modulation	Transcriptional regulation by Ca
L-Ara4N or PEtn modification of lipid A	phoP	+	-
	phoQ	+	-
	pmrA	+	-
	pmrB	+	-
	parR	+	-
	parS	+	-
	cprR	+ (only in ΔphoQ)	No change
	cprS	+ (only in ΔphoQ)	-
	colR	+ (only in ΔphoQ)	No change
	colS	+ (only in ΔphoQ)	-
	cbrA	+	No change
	cbrB	No change	+
	arnB	+	-
	arnT	+	-
Deacylation of lipid A	pagL	+	No change
Phosphorylation of lipid A	waaP	+	-
Efflux pump	mexY	+	No change
	mexX	+	No change

Known Pol B resistance mechanisms do not play a role in Ca- induced resistance

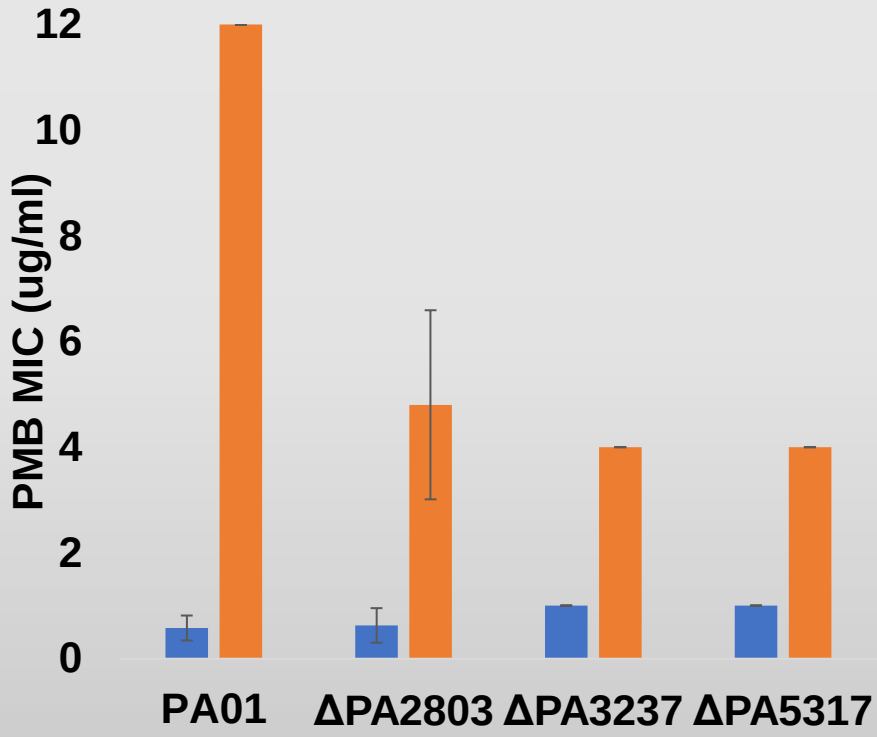


- Wzz: modulating O-antigen chain length
- RmlD: dTDP-4-dehydrorhamnose reductase
- CbrB: two-component response regulator

Three novel hypothetical proteins identified to be involved in Ca regulated polymyxin-B resistance

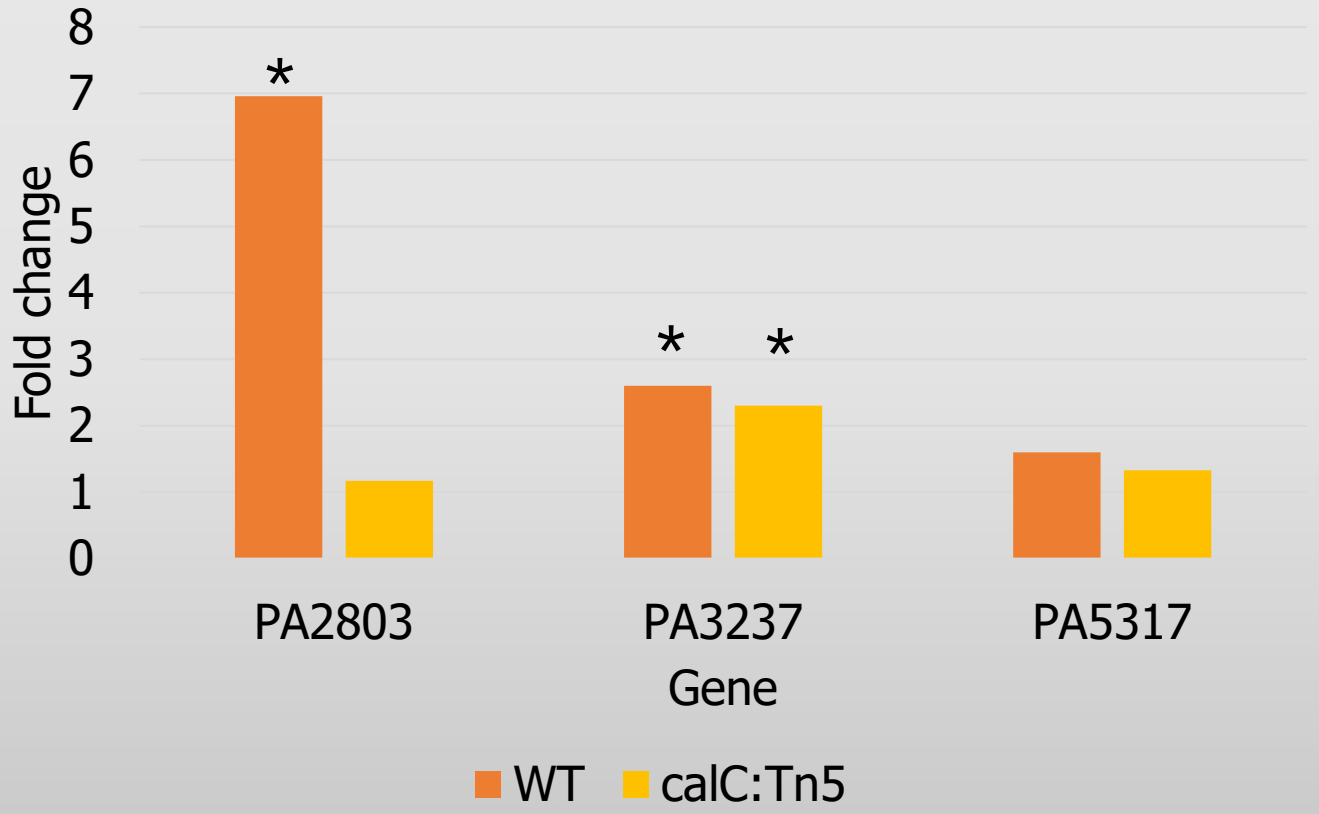


MIC of transposon mutants and in-trans complemented genes compared to WT



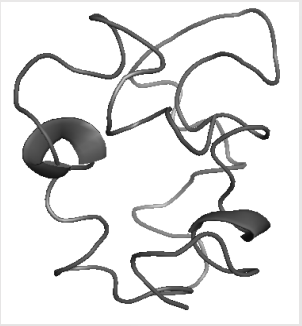
MIC of clean deletion mutants compared to WT

Increased transcript abundance of PA2803 and PA3237 in response to Ca



Transcript abundance in WT and *calC*:Tn5 background

Bioinformatics prediction of PA2803, PA3237 and PA5317



Hydrolase superfamily

PA2803

TM-helix

PA3237

Periplasmic ABC type transport domain

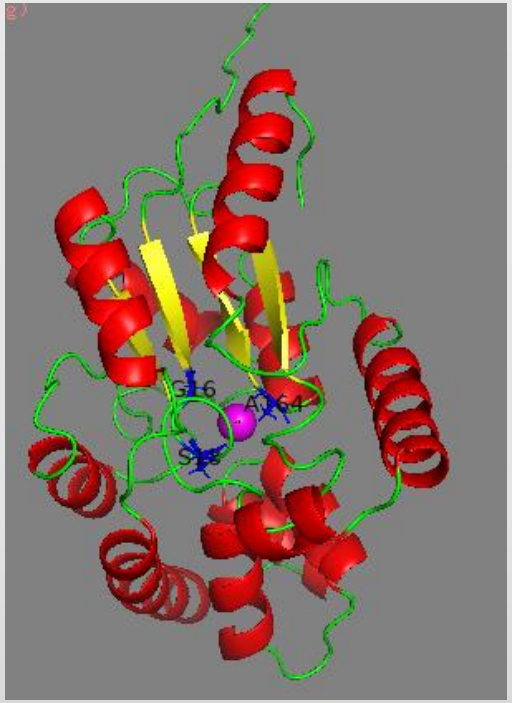
PBP2-DppA like region

PA5317

polypeptide binding sites

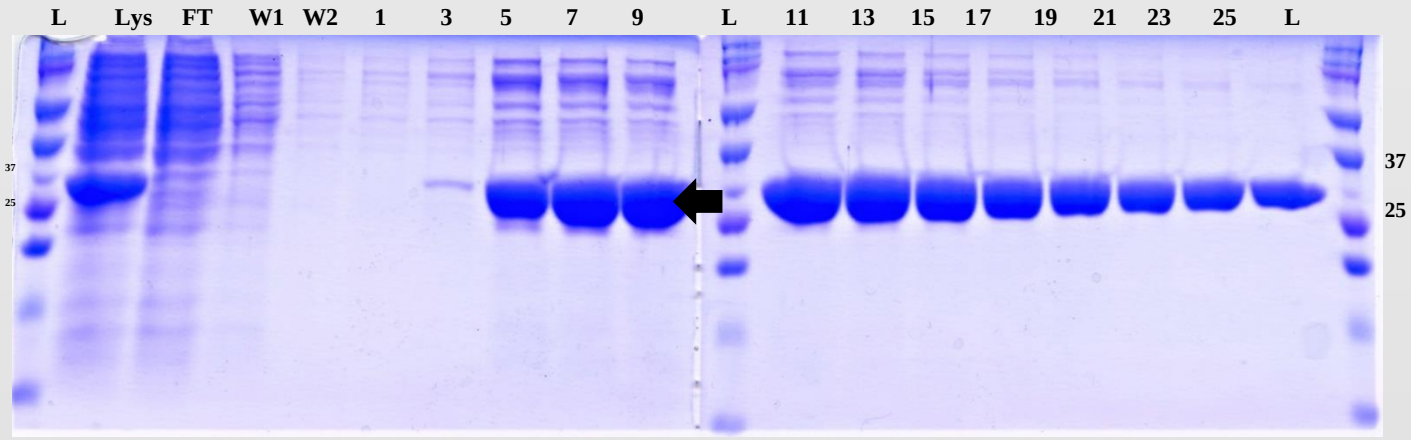
Structure prediction by i-Tasser.

Purification of PA2803

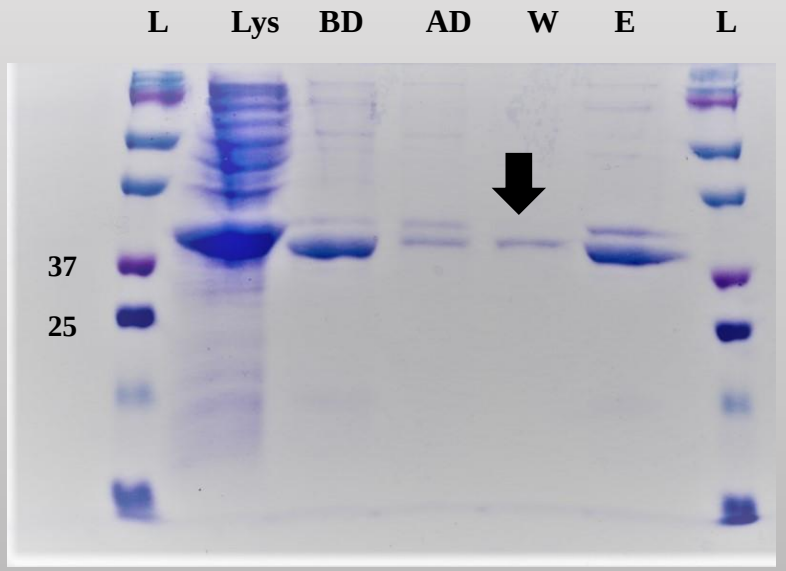


- A putative phosphonoacetaldehyde hydrolase- Phosphonatase
- Predicted Ca^{2+}/ Mg^{2+} binding
- Cytoplasmic (PSORTb 3.0)

Ni-NTA affinity purification



His-tag cleavage and affinity purification

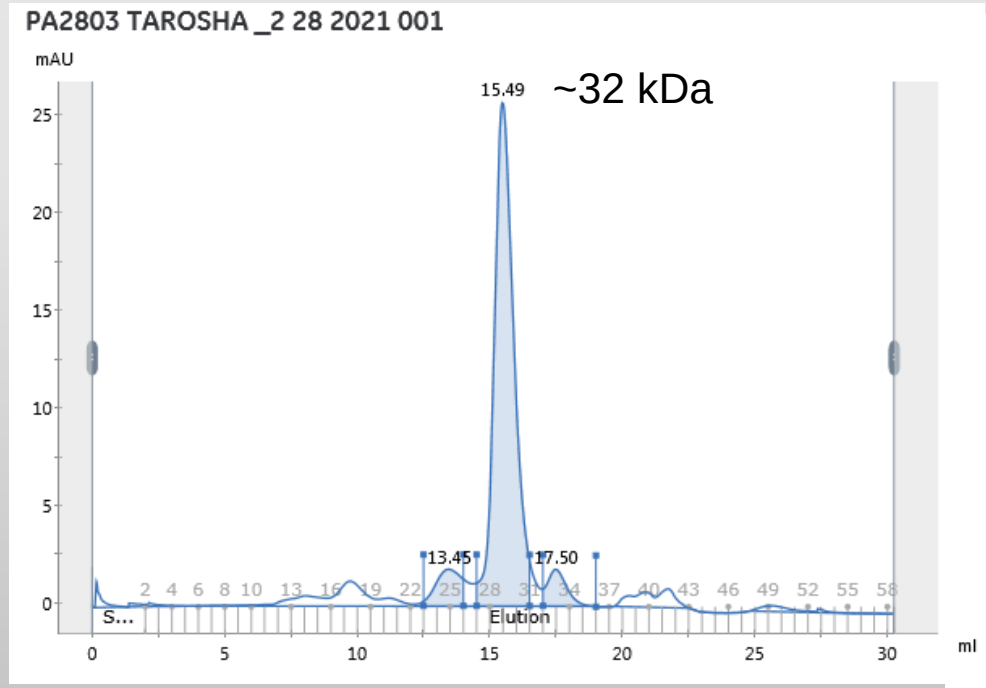


PA2803: ~29.7 kDA

L: Ladder
Lys: Lysate
BD: Before Digestion
AD: After Digestion (Flow through)
W: Wash
E: Eluent

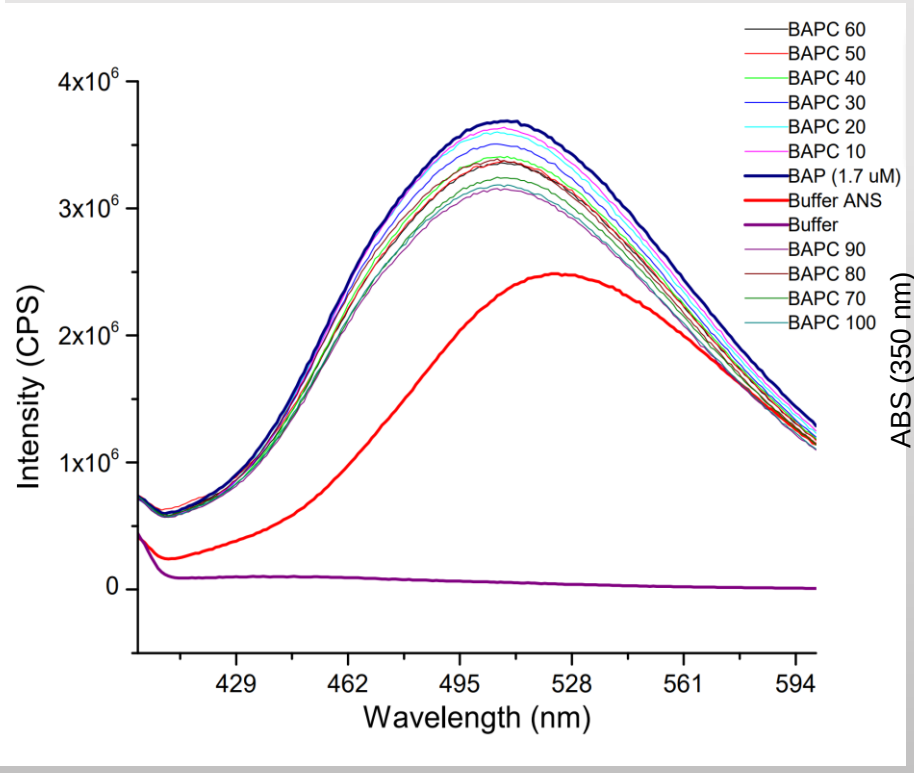
Characterization of PA2803

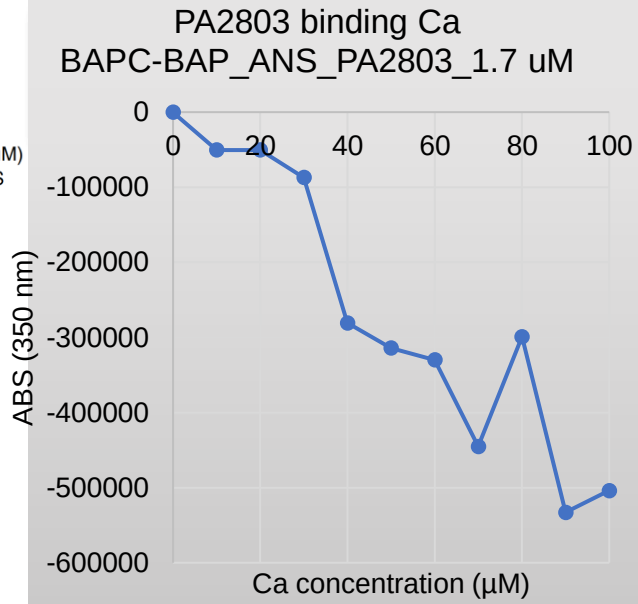
Determination of oligomeric state of PA2803



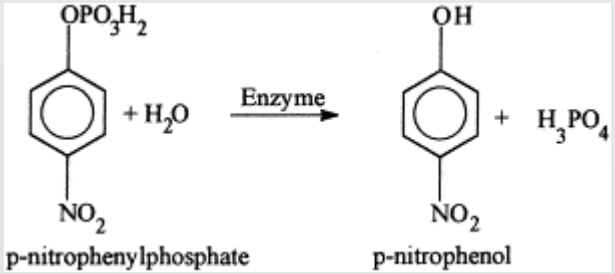
Size-Exclusion Chromatography of PA2803

Ca binding assay using ANS (8-anilino-1-naphthalene sulfonate)

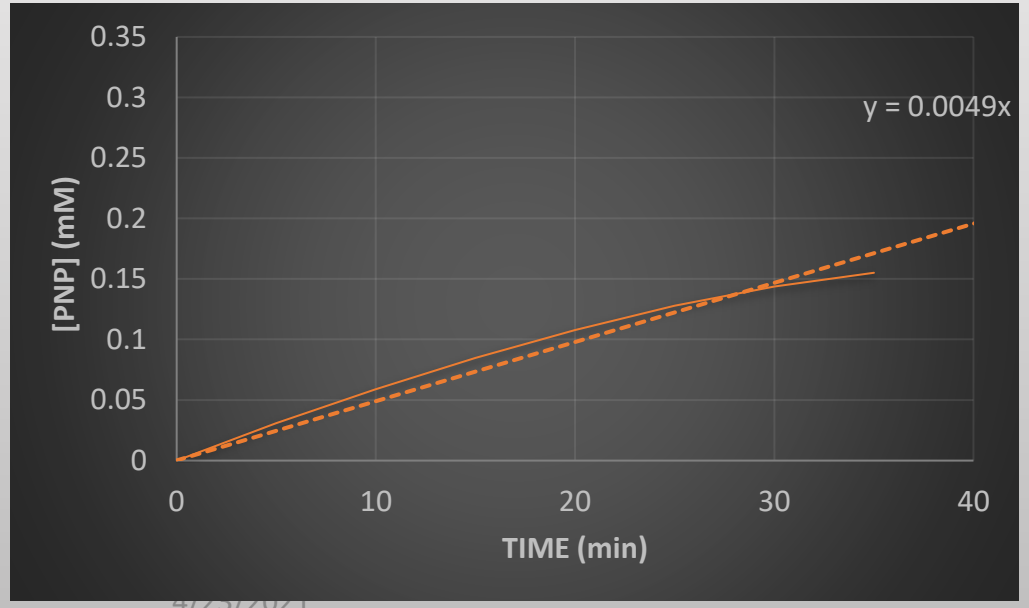




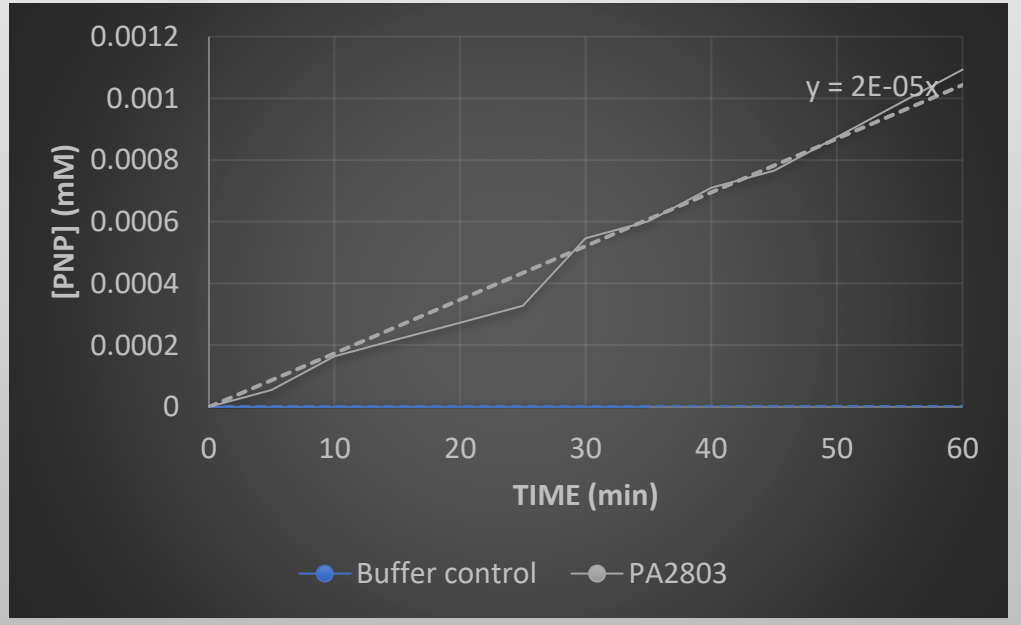
Phosphatase activity of PA2803



pNPP hydrolysis of Shrimp alkaline phosphatase

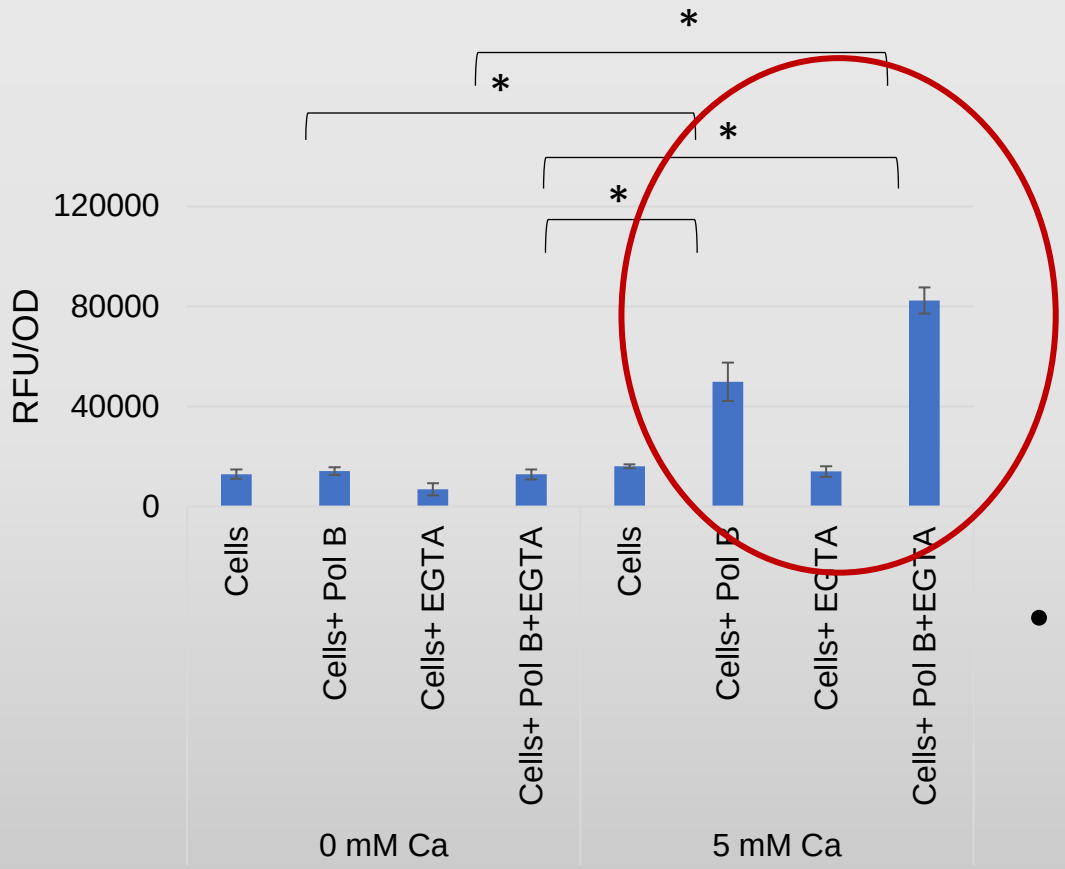


pNPP hydrolysis of PA2803

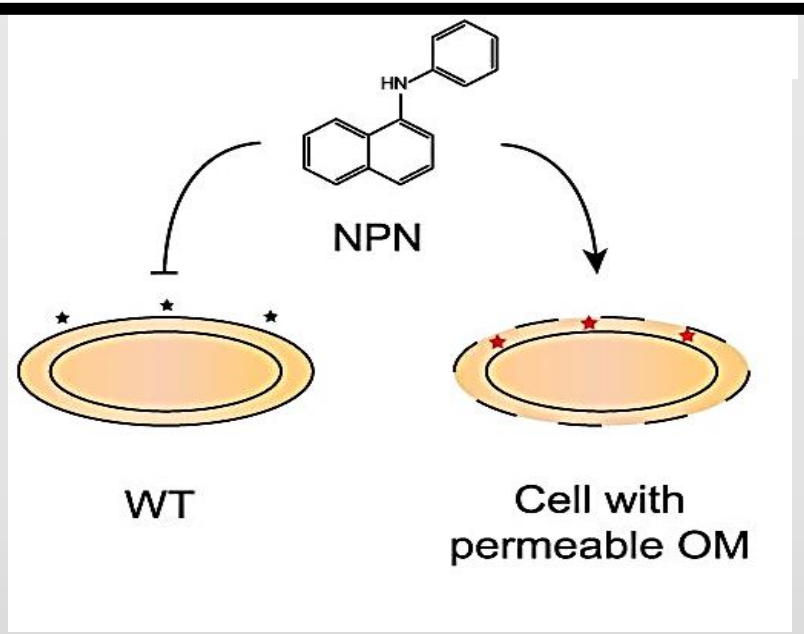


98.04 nM/min/µg of protein

Growth at elevated Ca levels increases the outer membrane permeability of *P. aeruginosa*

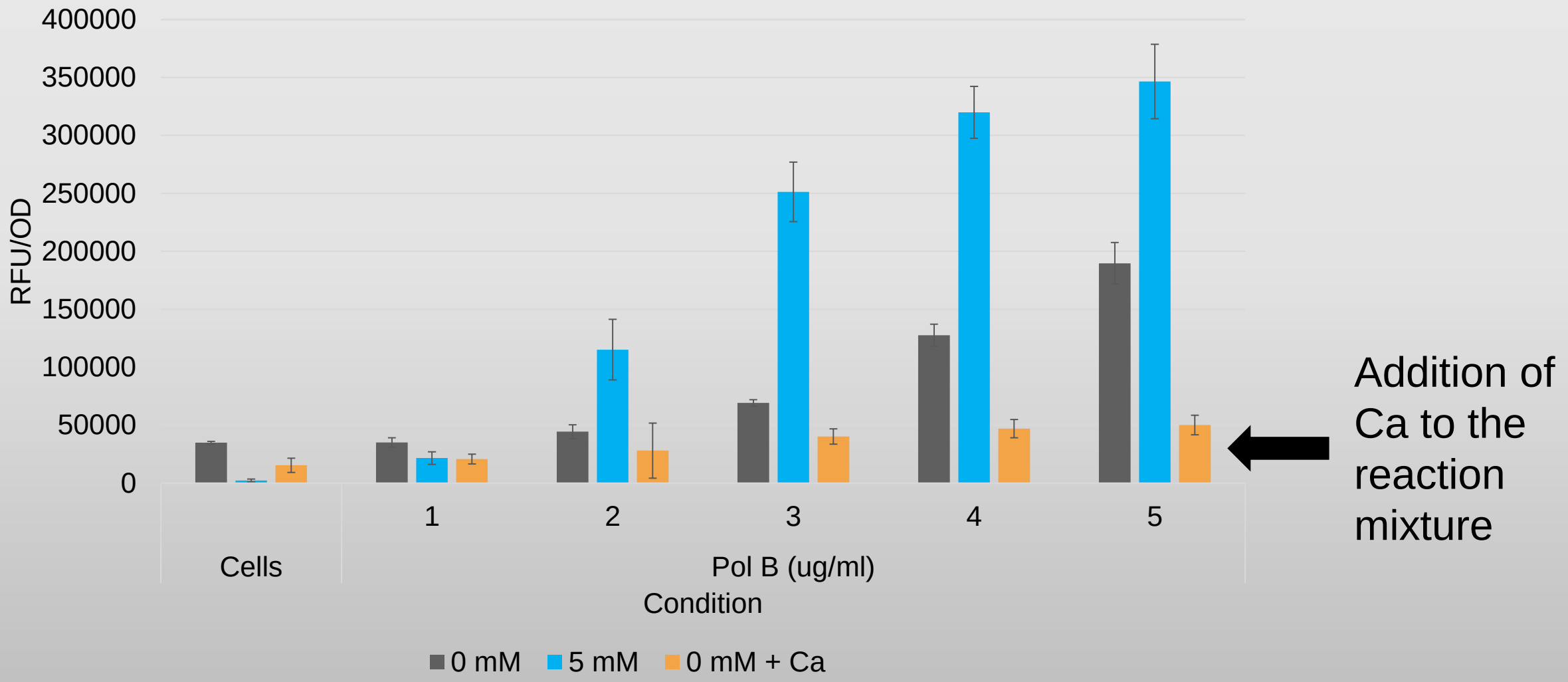


Outer membrane permeability of PAO1



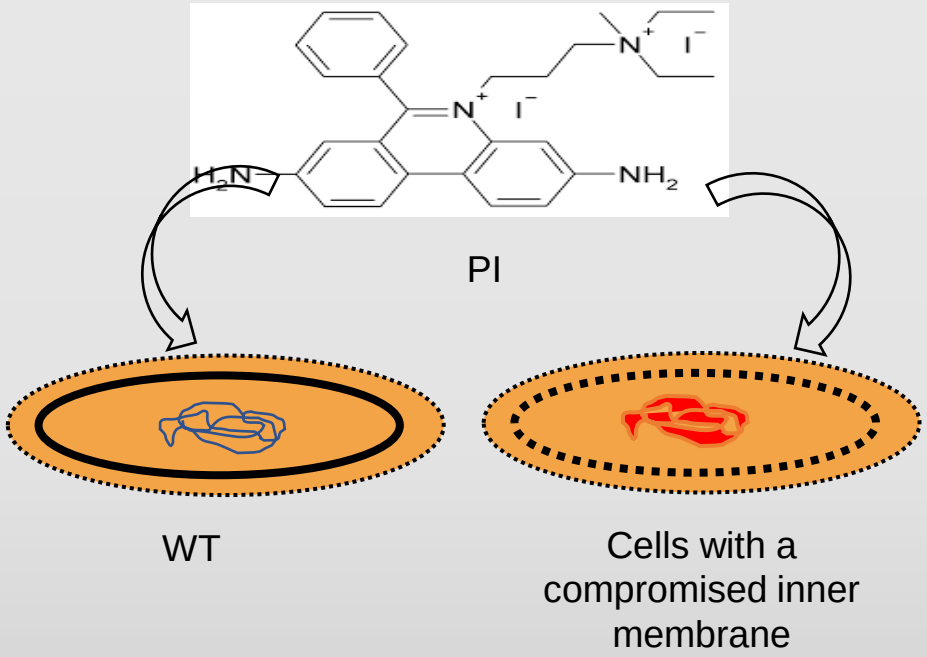
- The permeability of the outer membrane increases in a Pol B concentration dependent manner
- No significant change in permeability in mutants tested compared to WT

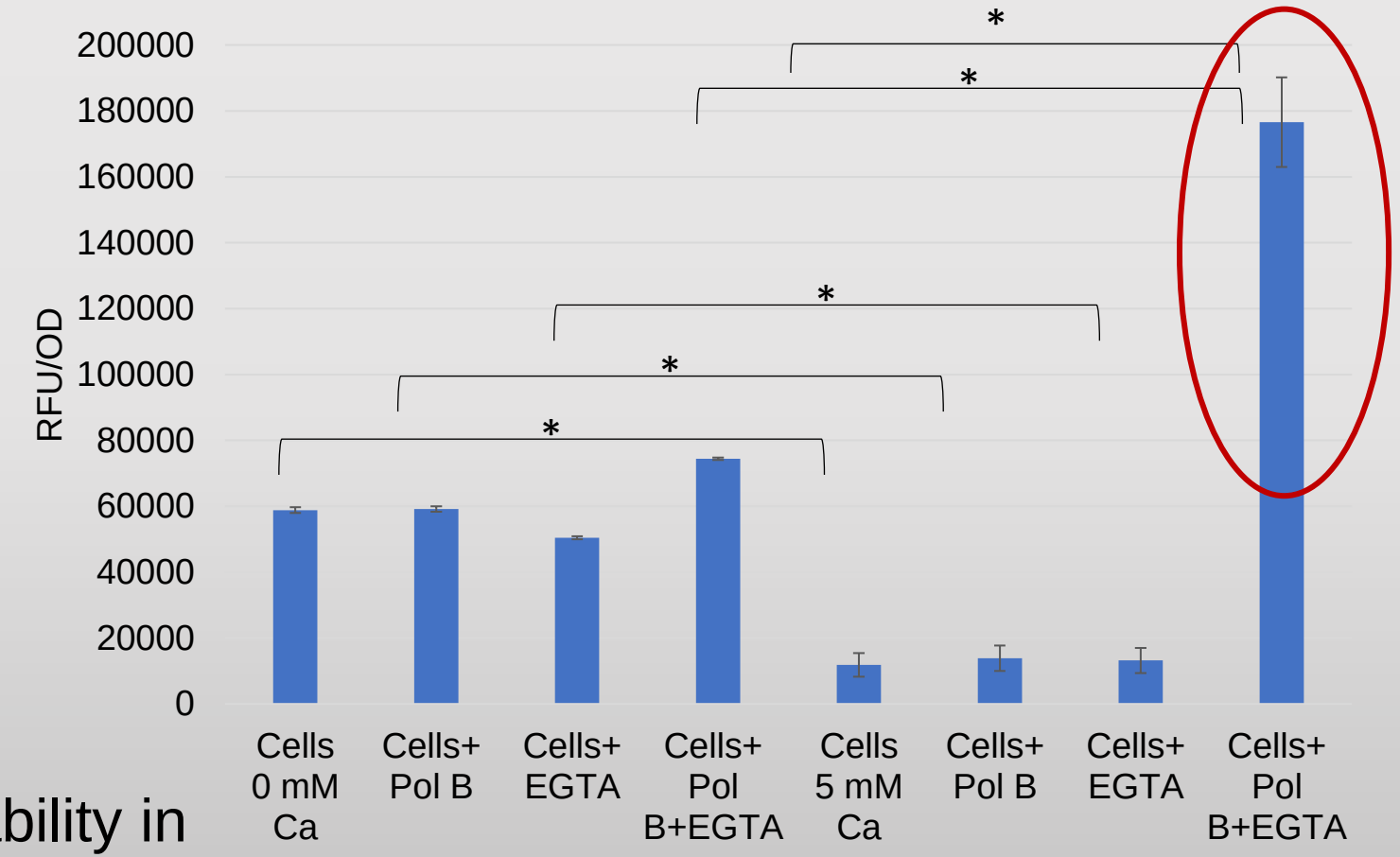
Short-term exposure to Ca does increase outer membrane permeability of PAO1



Addition of Ca to the reaction mixture

Growth at elevated Ca levels increases the inner membrane permeability of *P. aeruginosa*

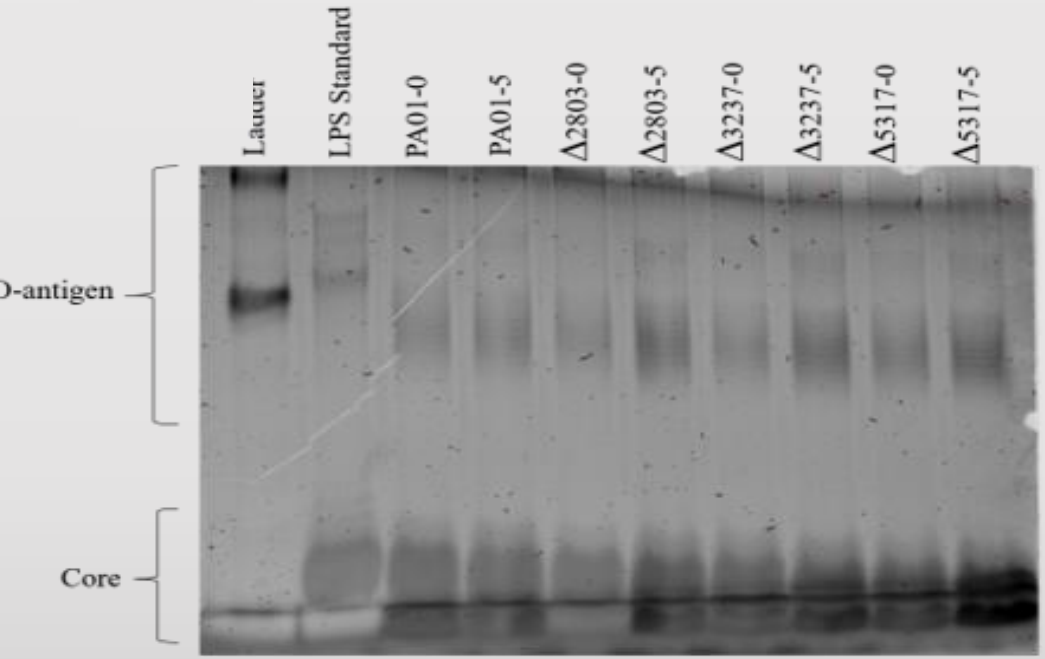




- No significant change in permeability in mutants tested compared to WT

Inner membrane permeability of PAO1

Differences in LPS and membrane-associated proteins at elevated Ca

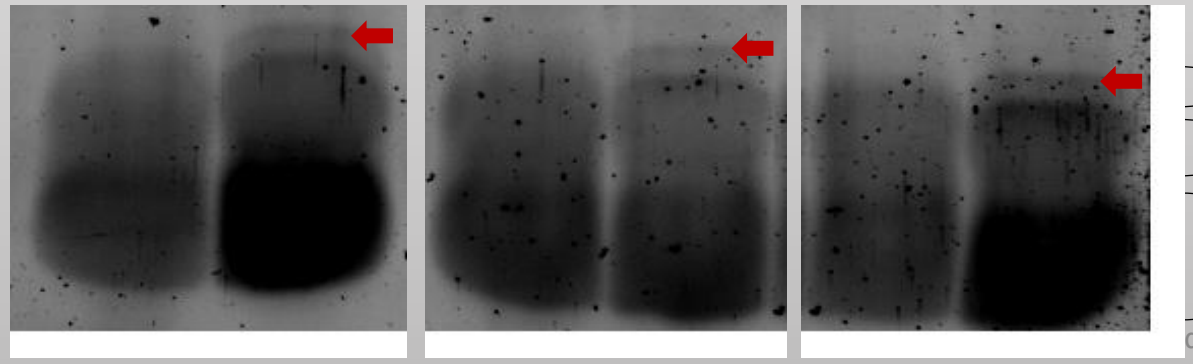


LPS was extracted as per Davis and Goldberg, 2012 and visualized by ProQ Emerald Staining Kit (Molecular Probes)

ΔPA2803 0 mM Ca 5mM Ca

ΔPA3237 0 mM Ca 5mM Ca

ΔPA5317 0 mM Ca 5mM Ca



Core+1

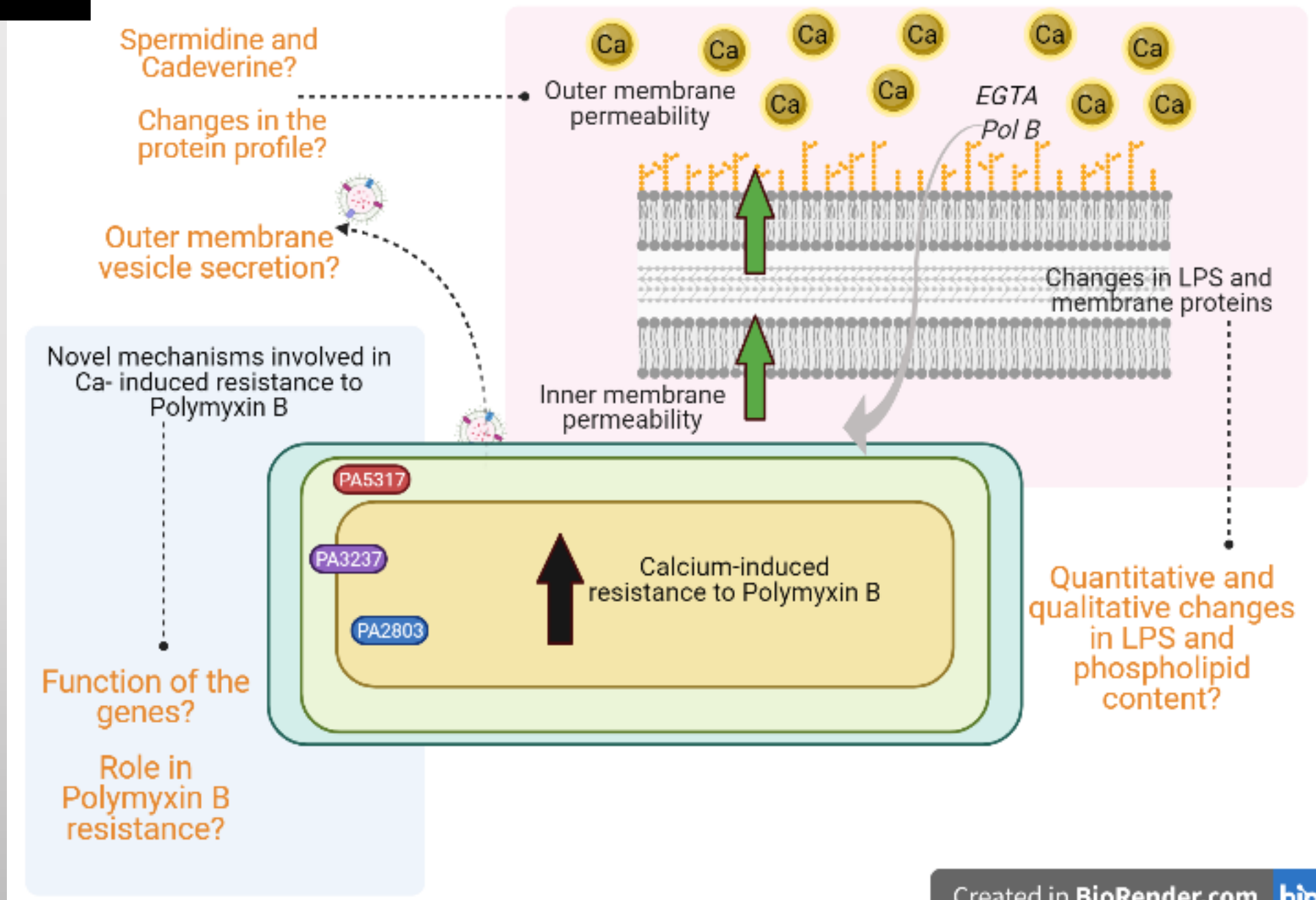
Uncapped core

Inner core

CORE

	Transcriptomic	Proteomic
OprM precursor		
Omp precursor CzcC		
Omp precursor PA1041		
Omp precursor PA1048		
Omp precursor PA1288		
Omp precursor PA2391		
Omp precursor PA2525		
Omp precursor PA2929		
Opr86		
OprG		
OprD		
OprH		
PhoP		
PmrB		
OpdT		
OprQ		
OpdO		
OpdQ		
OprE		
PagL		
MurG		
MrcB		

Summary


Spermidine and Cadeverine?
Changes in the protein profile?
Outer membrane vesicle secretion?
Novel mechanisms involved in Ca- induced resistance to Polymyxin B
Function of the genes?
Role in Polymyxin B resistance?
Ca
Outer membrane permeability
EGTA
Pol B
Changes in LPS and membrane proteins
Inner membrane permeability
PA5317
PA3237
PA2803
Calcium-induced resistance to Polymyxin B
Quantitative and qualitative changes in LPS and phospholipid content?
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Thank you!