

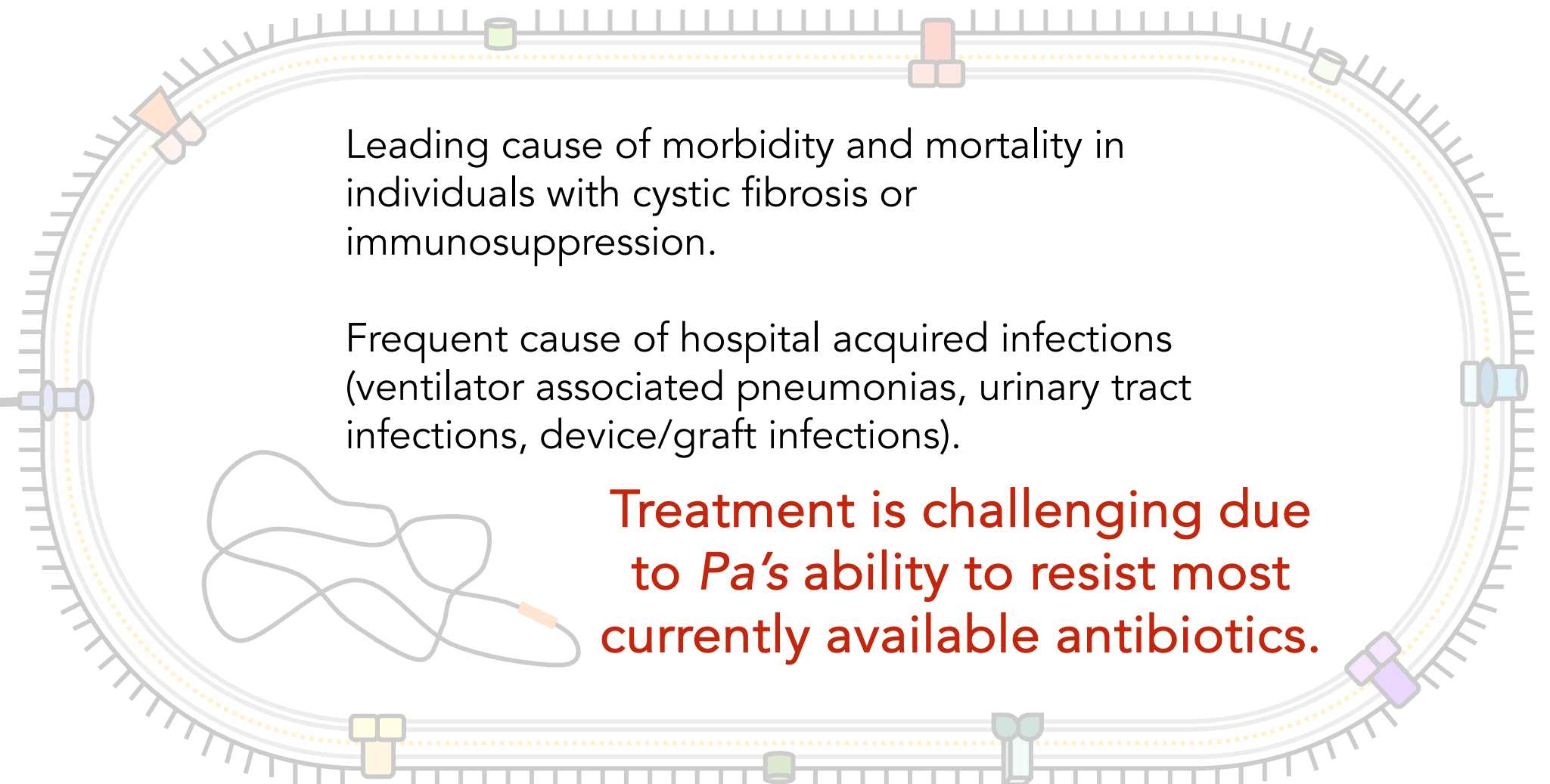
# Intrinsic antibiotic resistance in *Pseudomonas aeruginosa*

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Medicine (Infectious Diseases) & Microbial Pathogenesis

Yale University School of Medicine, New Haven CT

# *Pseudomonas aeruginosa* is an opportunistic MDR pathogen



Leading cause of morbidity and mortality in individuals with cystic fibrosis or immunosuppression.

Frequent cause of hospital acquired infections (ventilator associated pneumonias, urinary tract infections, device/graft infections).

**Treatment is challenging due to *Pa's* ability to resist most currently available antibiotics.**

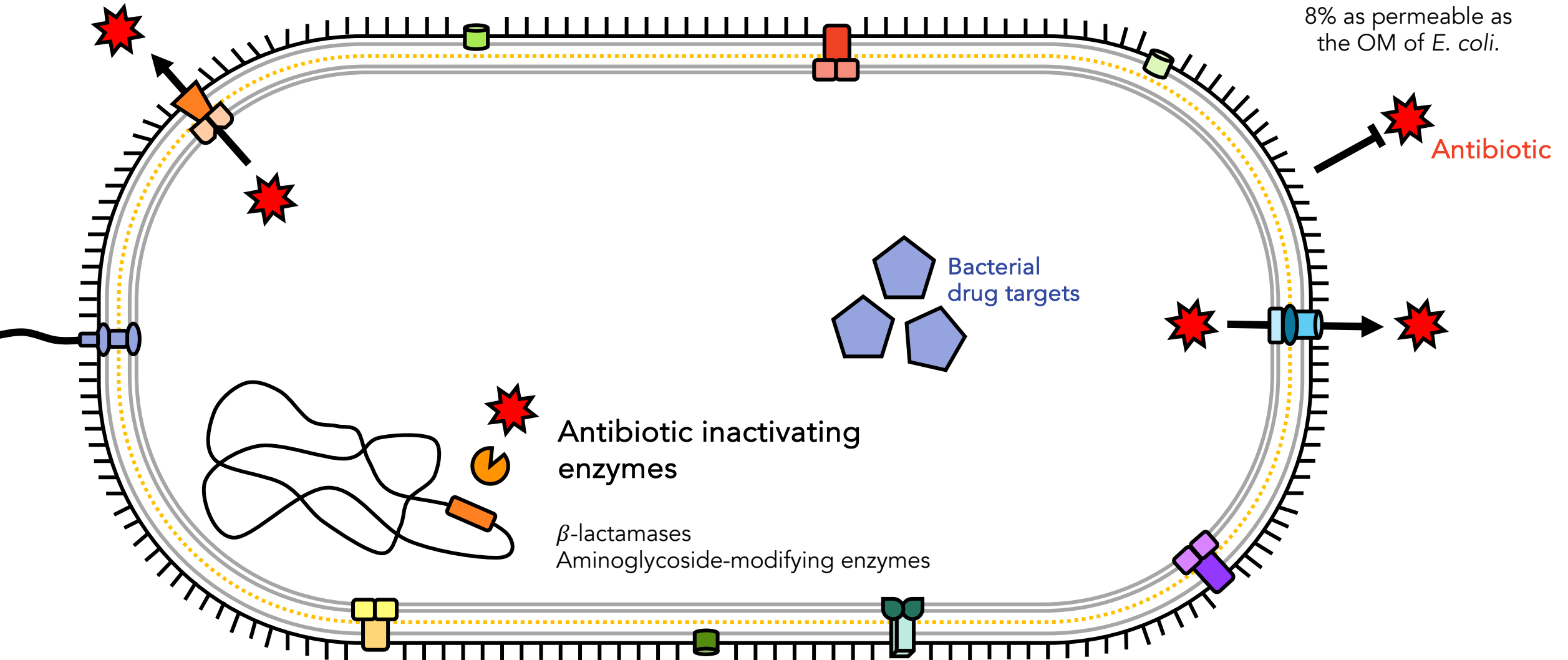
# *P. aeruginosa* is intrinsically resistant to many types of antibiotics

Active efflux

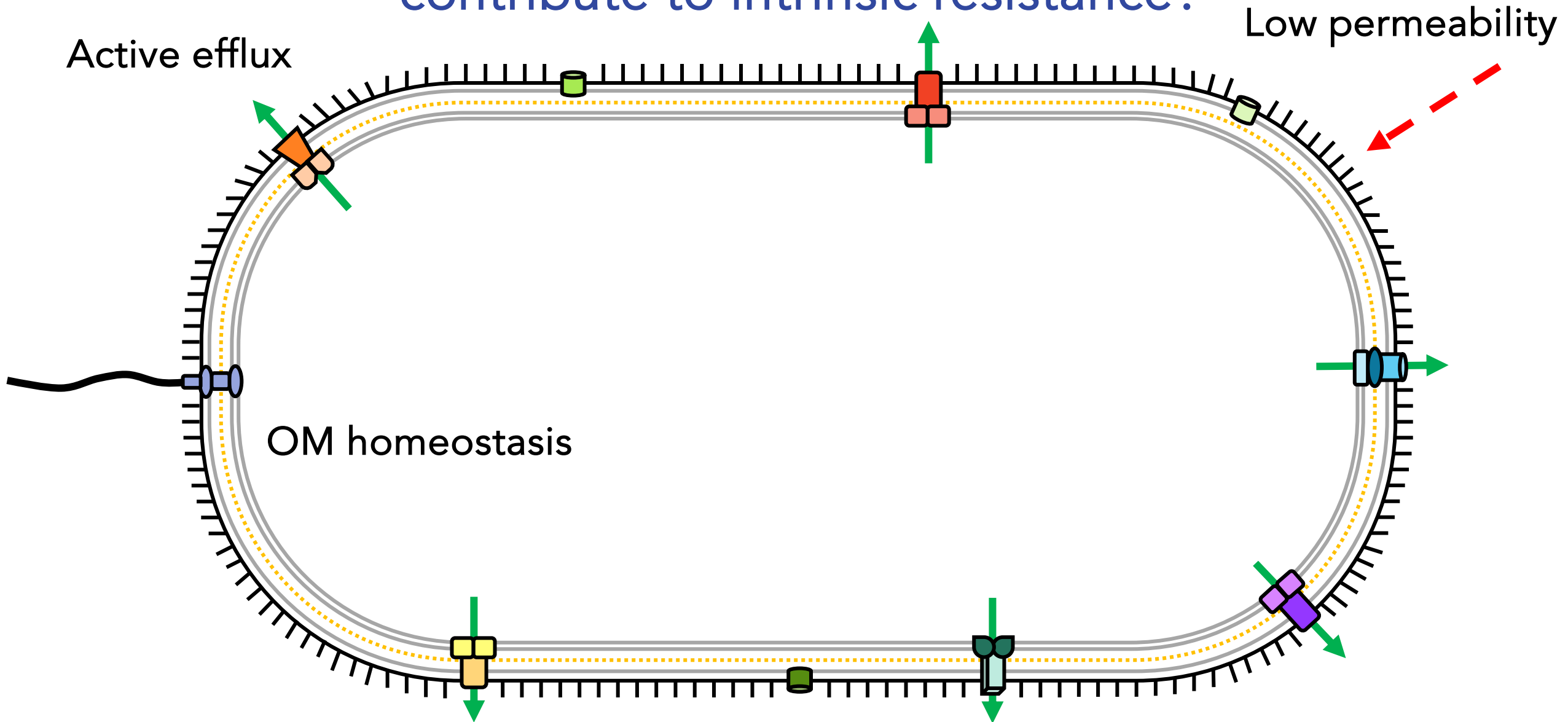
12 RND family systems

Restricted outer membrane permeability

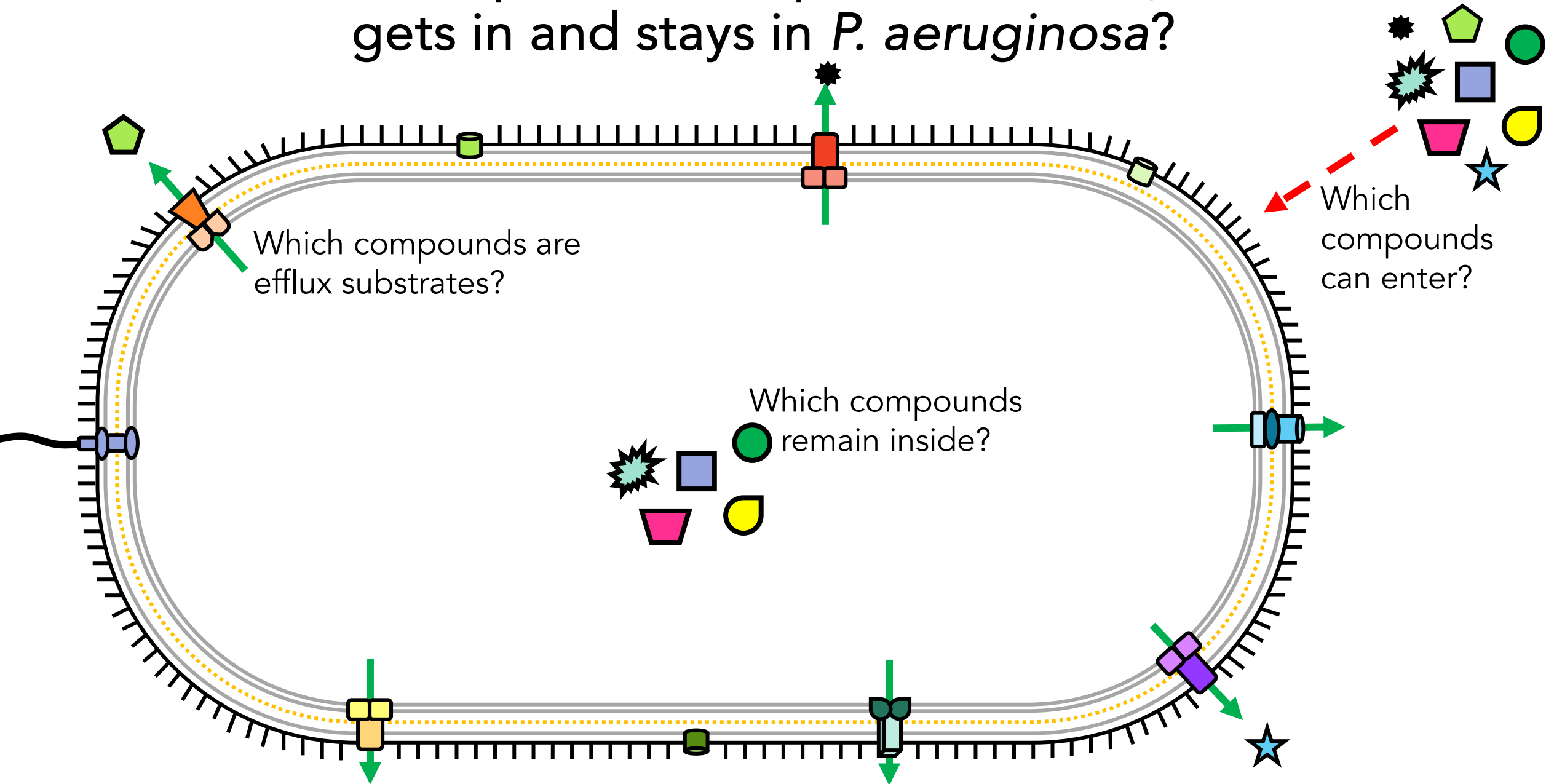
8% as permeable as the OM of *E. coli*.



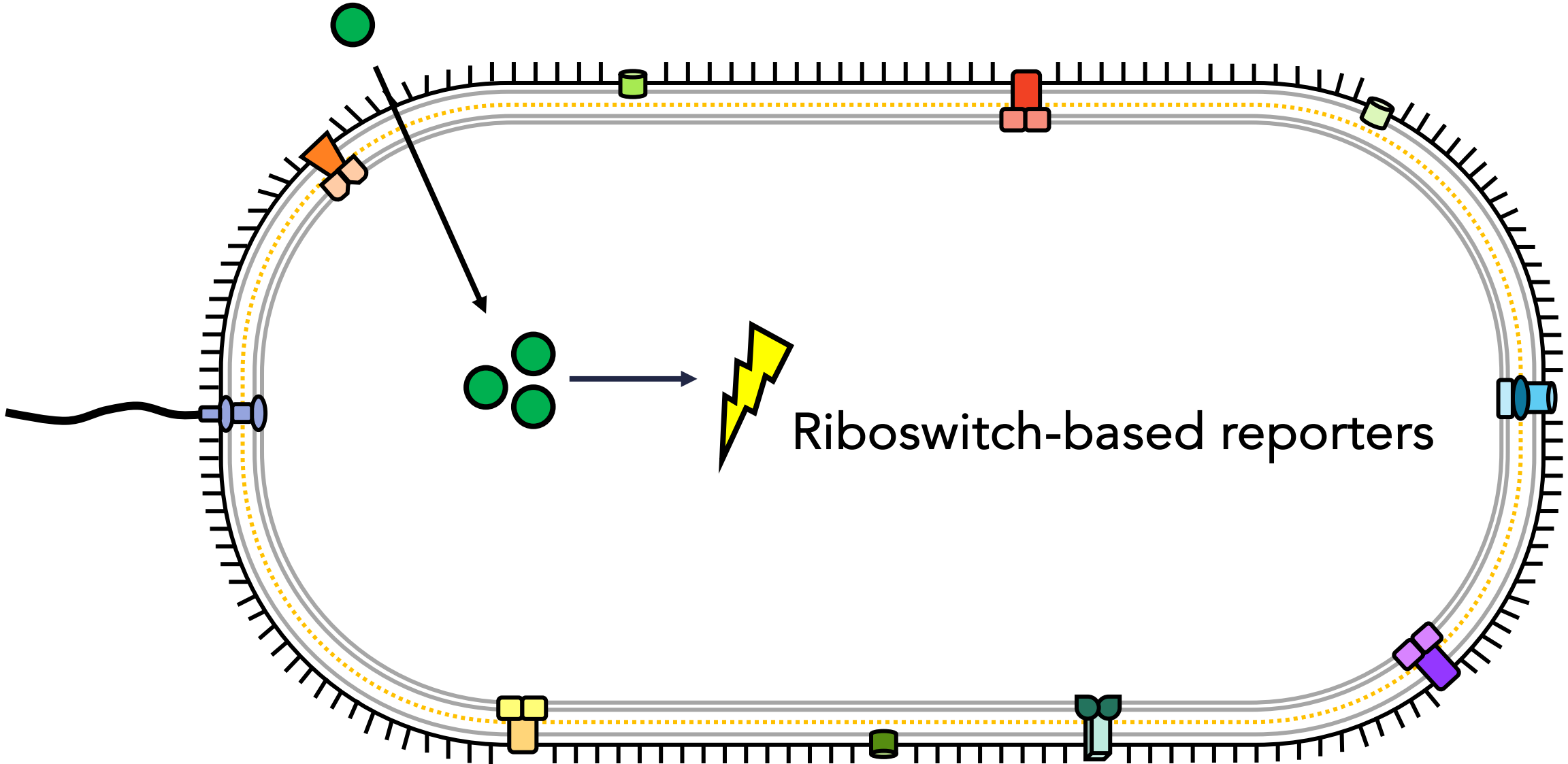
# Can we identify all *Pa* gene products that contribute to intrinsic resistance?



# Can we map chemical space to identify what gets in and stays in *P. aeruginosa*?



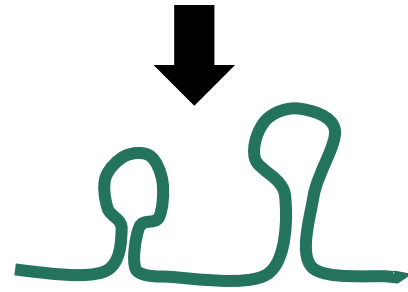
# 1. Detecting compounds that perturb cell homeostasis



# Riboswitches (RNA elements)



Specialized RNA structures located in the 5'UTR of certain bacterial mRNAs.



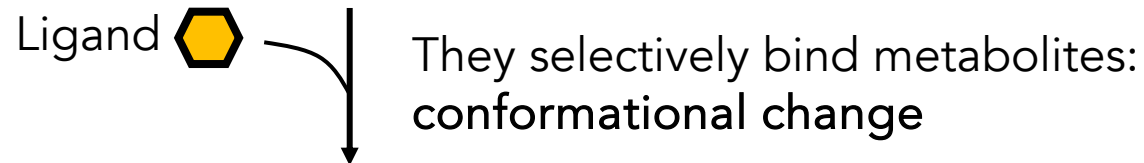
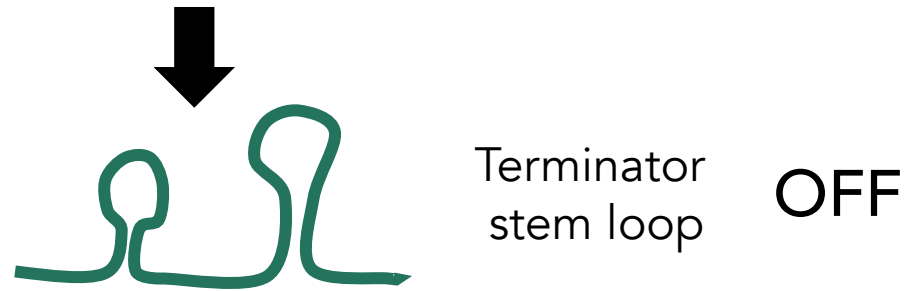
Terminator stem loop

OFF

# Riboswitches (RNA elements)



Specialized RNA structures located in the 5'UTR of certain bacterial mRNAs.

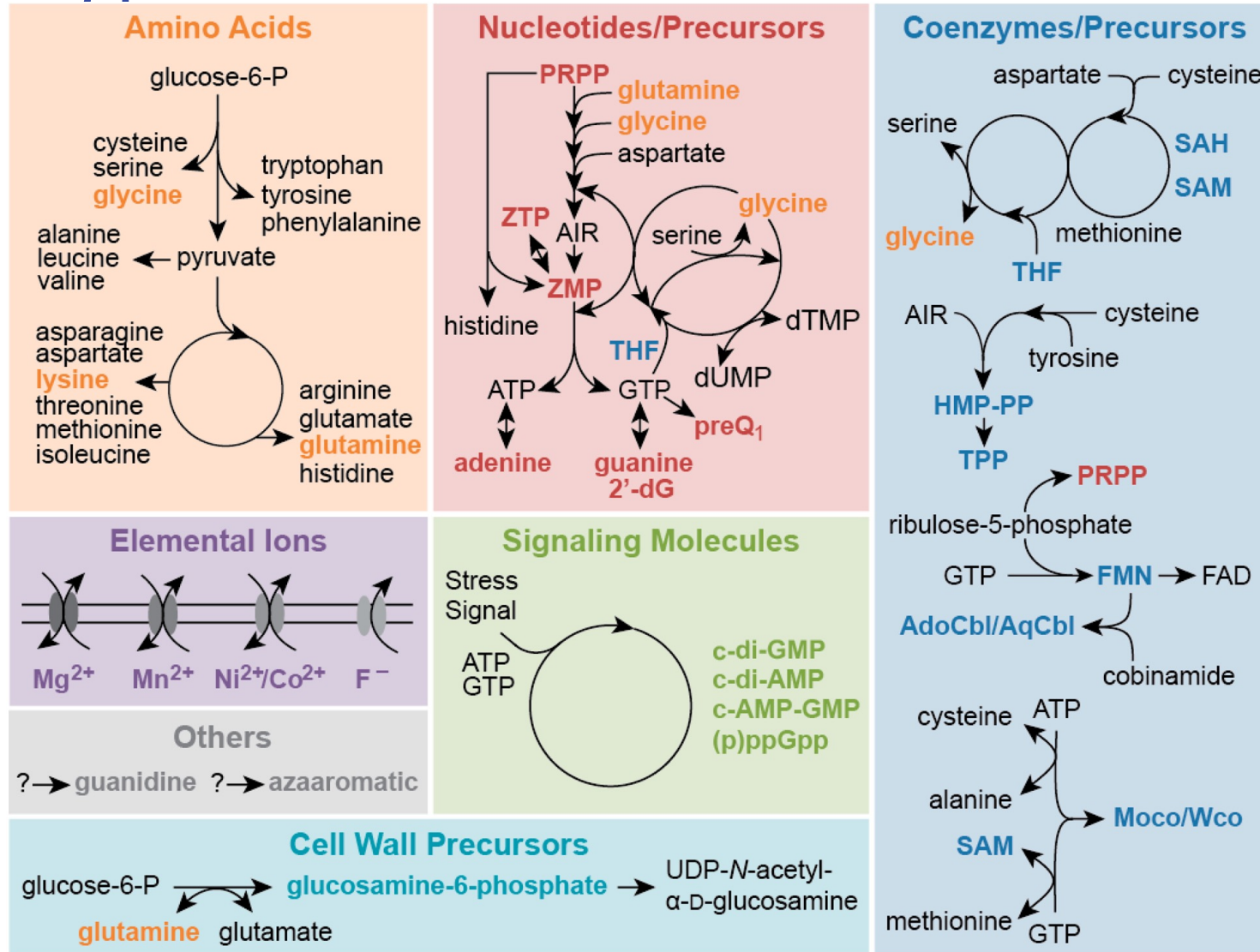


Gene expression regulation

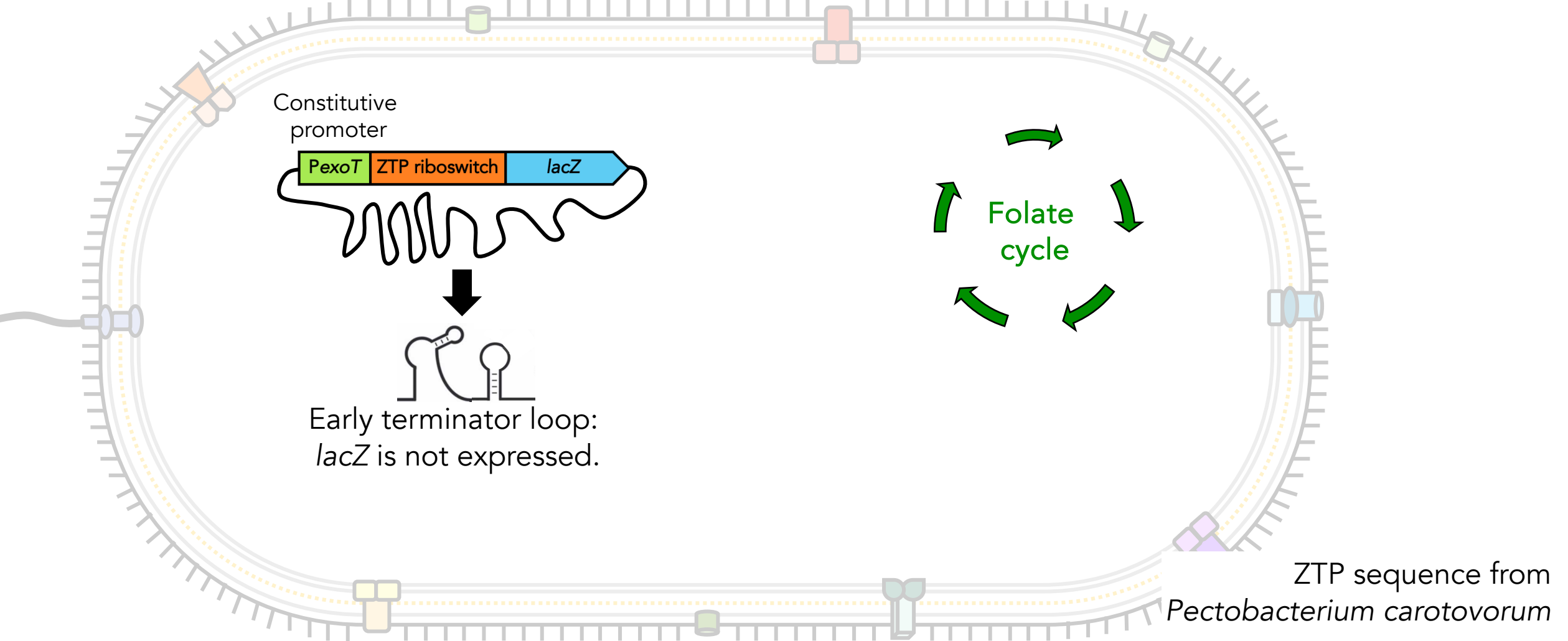
(genes essential for viability and virulence)



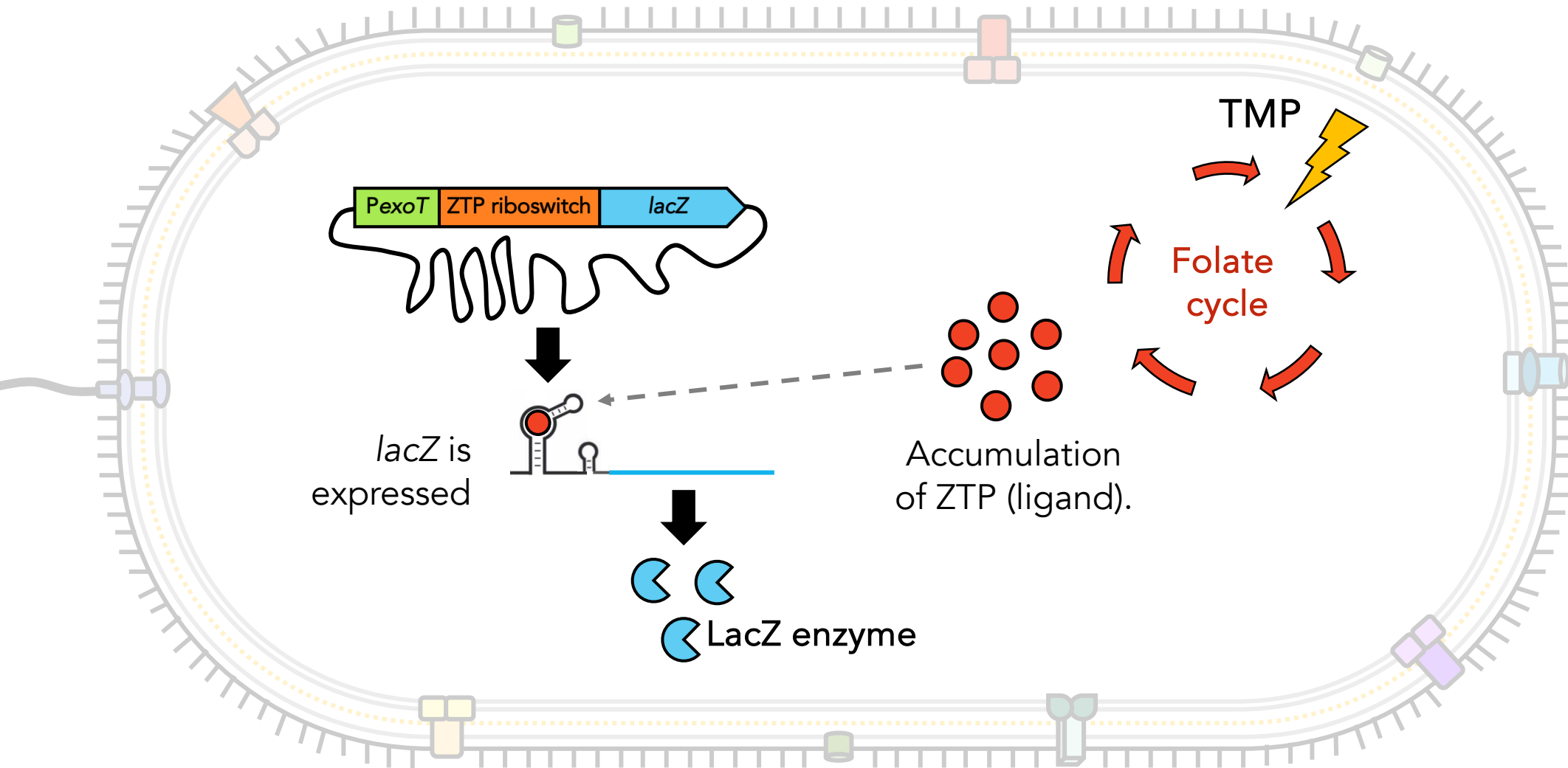
# Riboswitches monitor diverse biochemical pathways and signaling processes



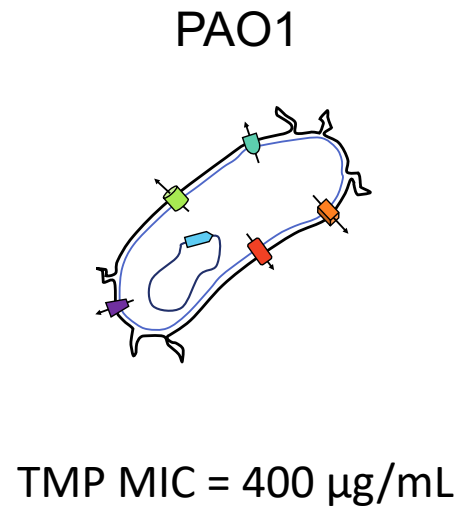
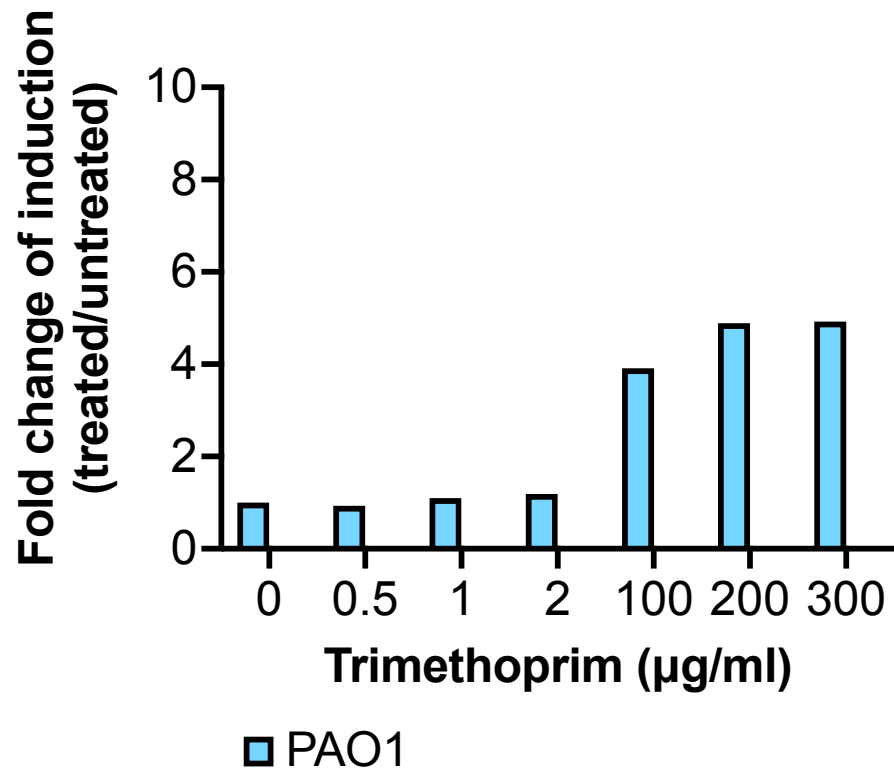
# ZTP riboswitch reporter in *P. aeruginosa*



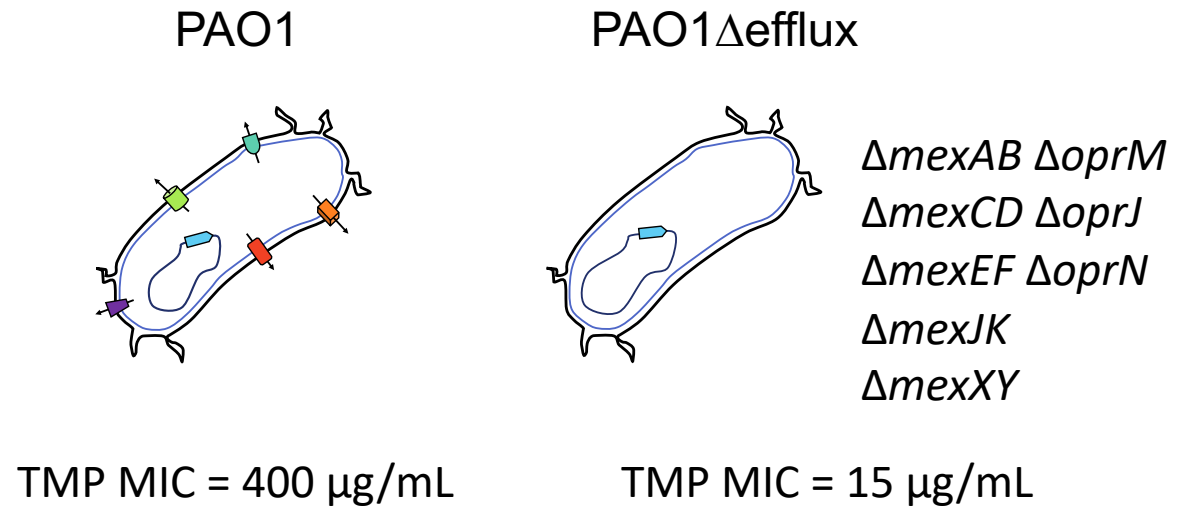
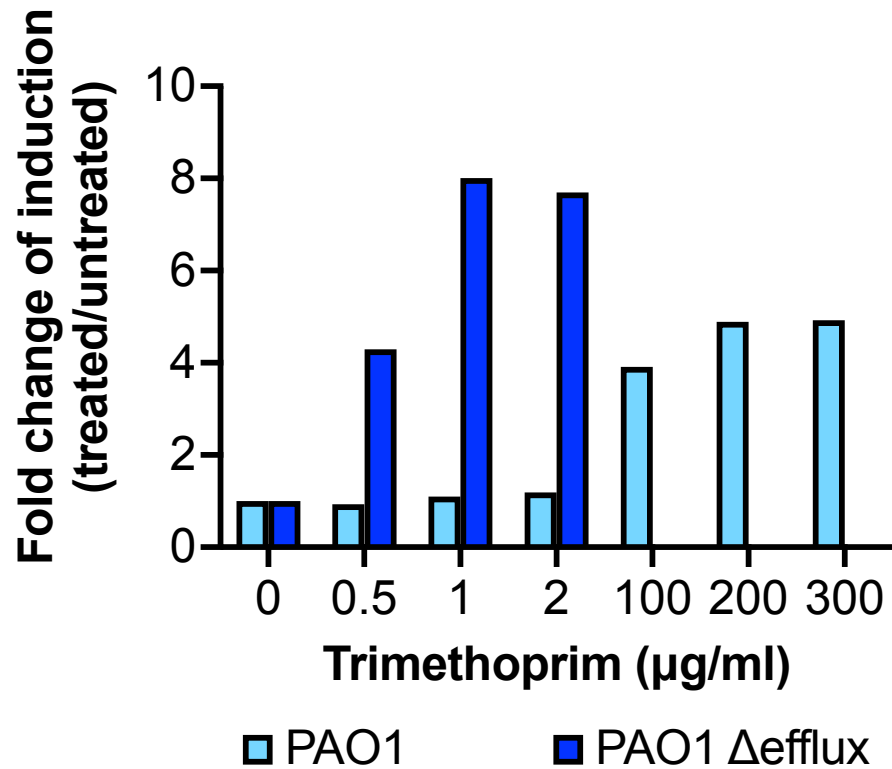
# ZTP riboswitch reporter in *P. aeruginosa*



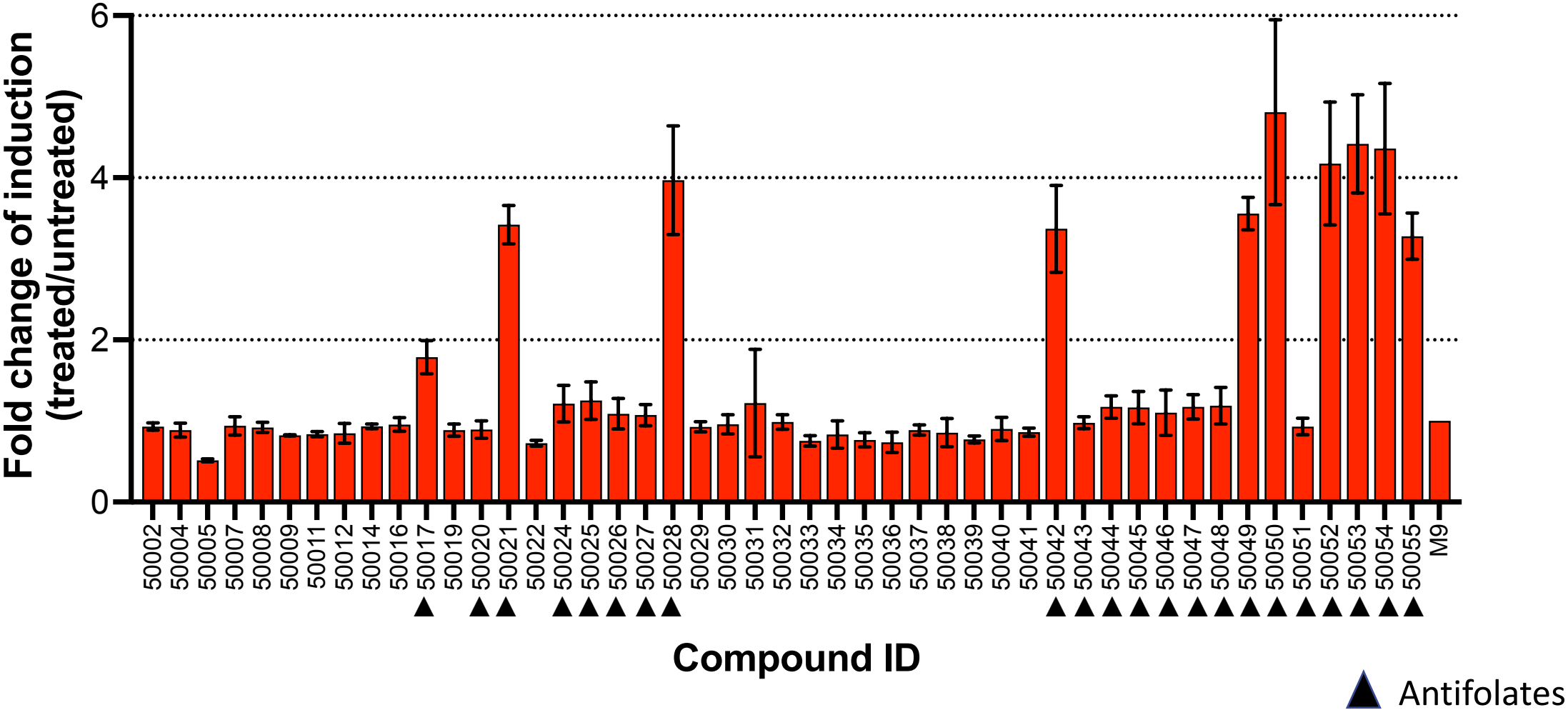
# Riboswitch responses to trimethoprim (TMP) in the presence vs. absence of efflux



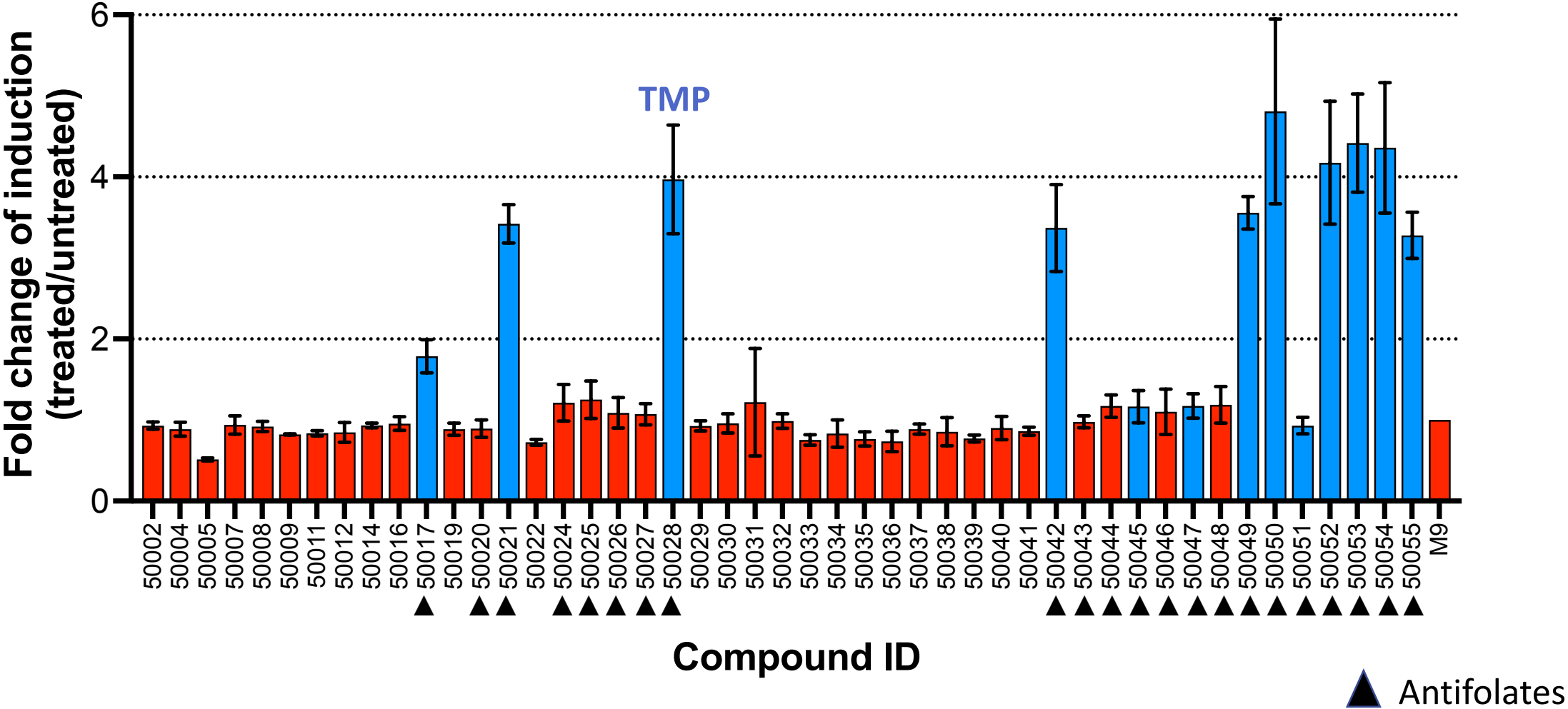
# Riboswitch responses to trimethoprim (TMP) in the presence vs. absence of efflux



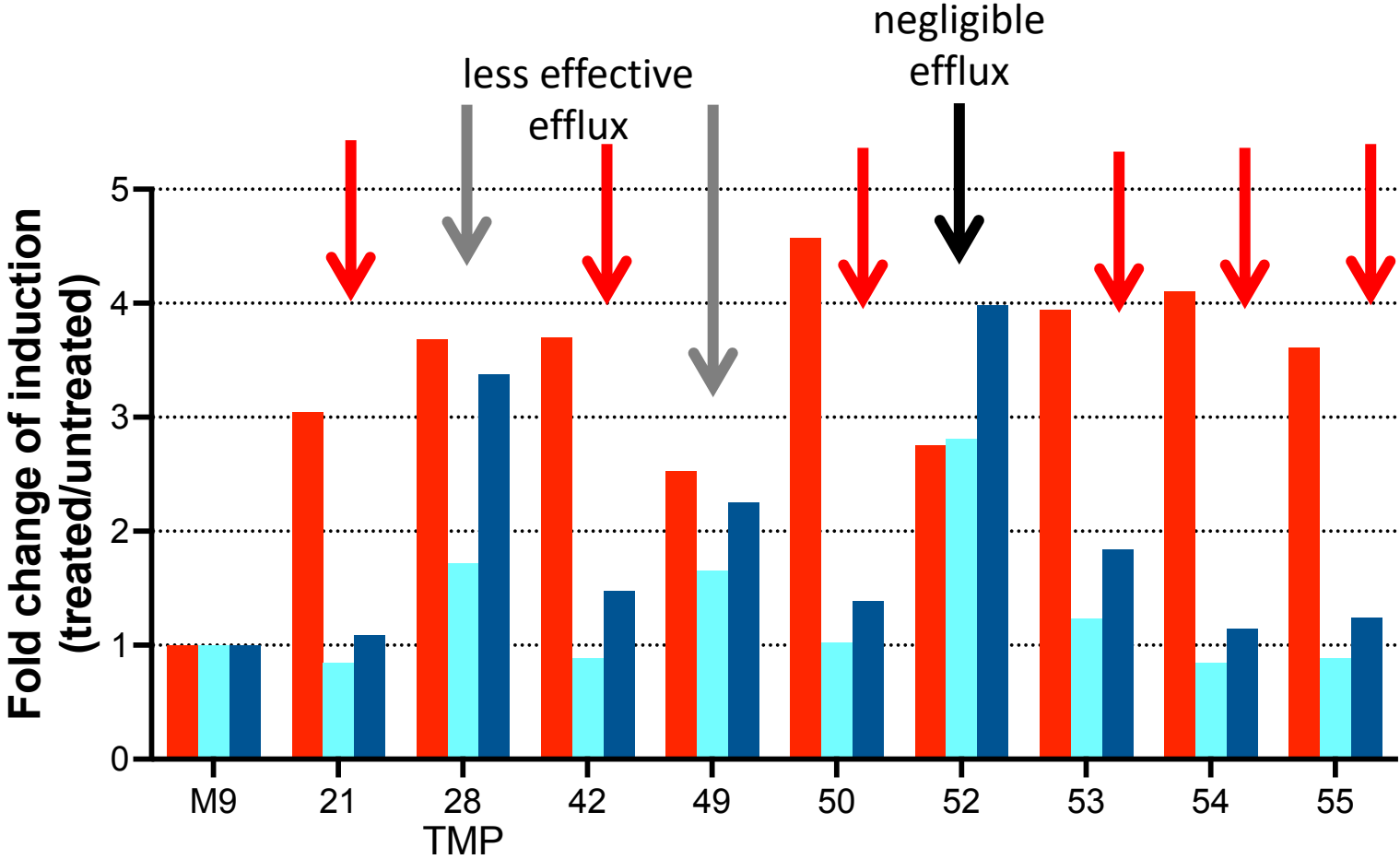
# Miniscreen using the ZTP-*lacZ* reporter in PAO1 $\Delta$ efflux



# Miniscreen using the ZTP-*lacZ* reporter in PAO1 $\Delta$ efflux picks up only dihydrofolate reductase inhibitors



# Efflux-proficient vs. efflux-deficient strain responses

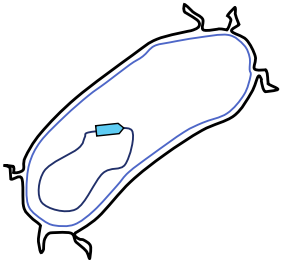


■ PAO1  $\Delta$ efflux (@50  $\mu$ M)

■ PAO1 (@50  $\mu$ M)

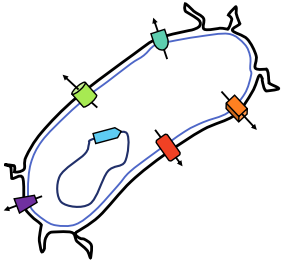
■ PAO1 (@100  $\mu$ M)

PAO1  $\Delta$ efflux



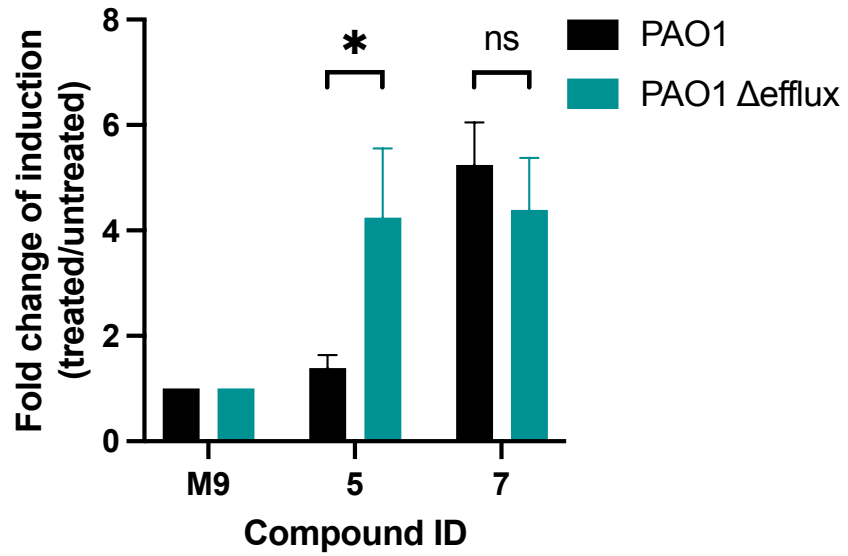
versus

PAO1

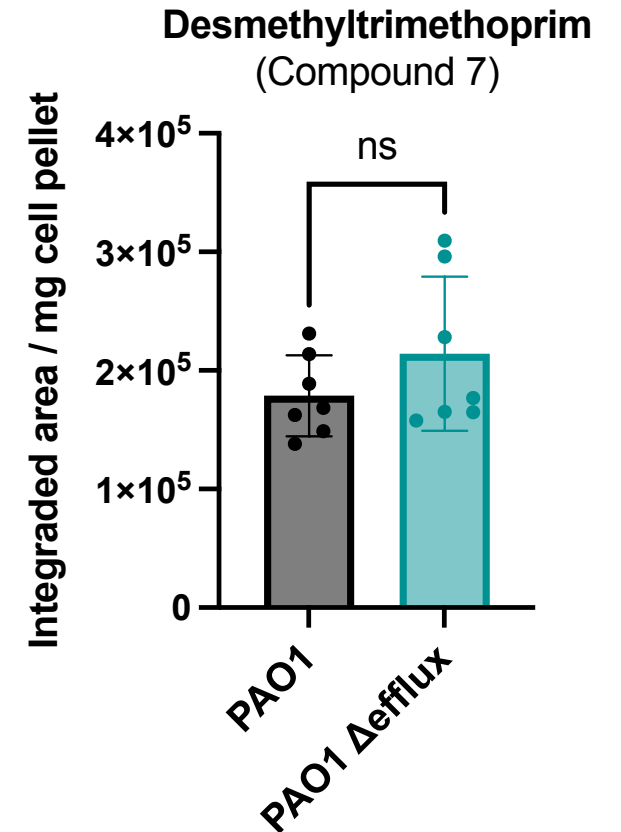
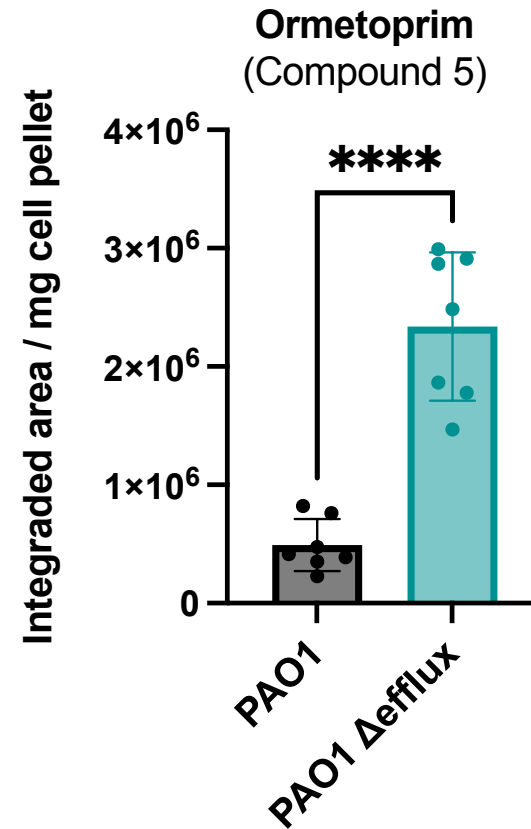
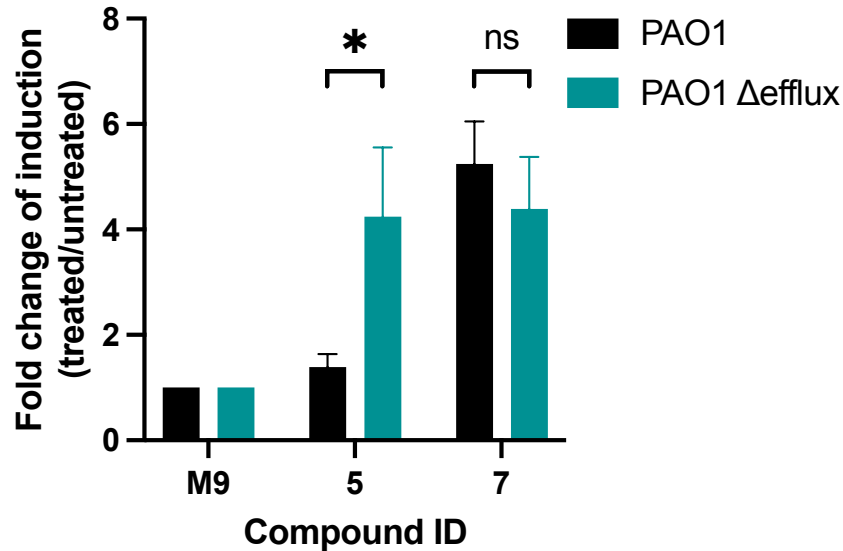




# Beta-galactosidase assay vs LC-MS:

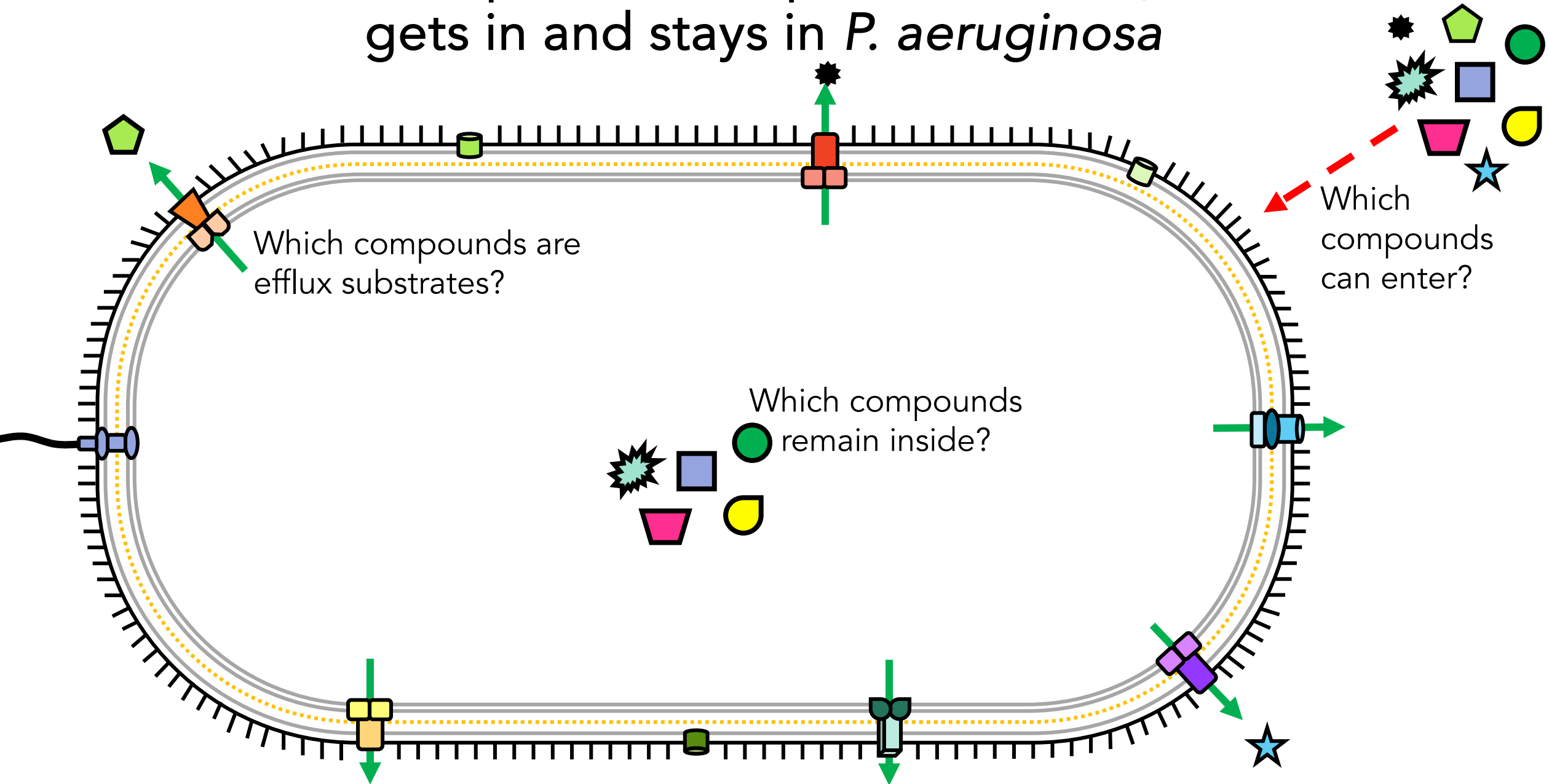


# Beta-galactosidase assay vs LC-MS:



The levels of *lacZ* induction reflect levels of compound accumulation within the cells.

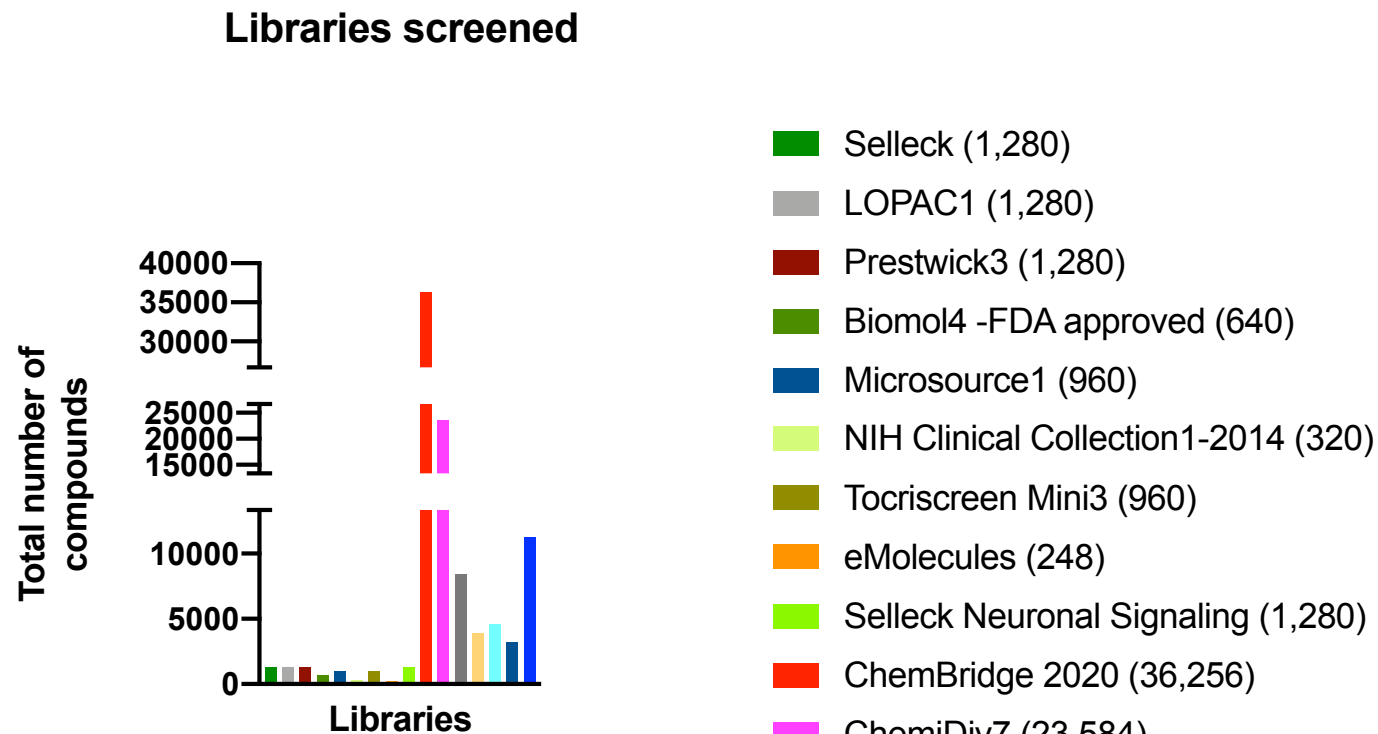
# HTS to map chemical space to identify what gets in and stays in *P. aeruginosa*



# ZTP Riboswitch High-Throughput Screen



L2 Diagnostics

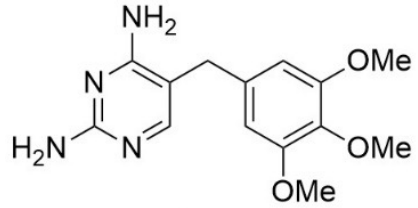


- Selleck (1,280)
- LOPAC1 (1,280)
- Prestwick3 (1,280)
- Biomol4 -FDA approved (640)
- Microsource1 (960)
- NIH Clinical Collection1-2014 (320)
- Tocriscreen Mini3 (960)
- eMolecules (248)
- Selleck Neuronal Signaling (1,280)
- ChemBridge 2020 (36,256)
- ChemiDiv7 (23,584)
- ActiMol TimTec 1 (8,448)
- Life Chemicals 1 (3,872)
- Maybridge 4 (4,576)
- Maybridge 5 (3,168)
- ChemDiv 6 (11,264)

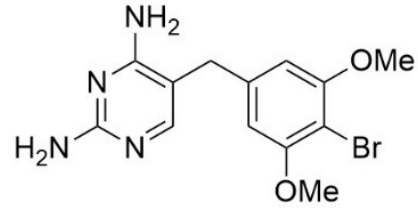
**98,840 compounds  
total**

# HTS Hits

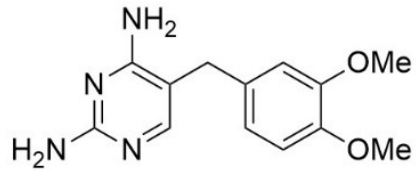
## Exported



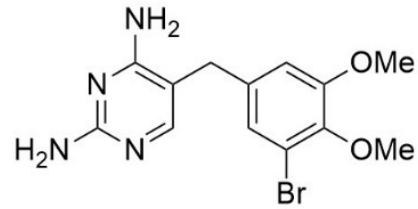
**trimethoprim (TMP)**



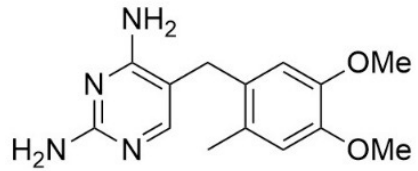
**bromdimoprim (8)**



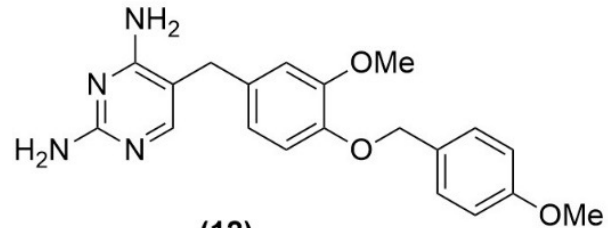
**diaveridine (3)**



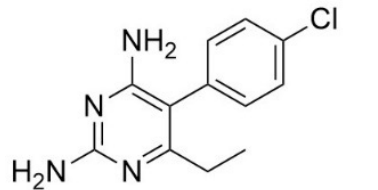
**(9)**



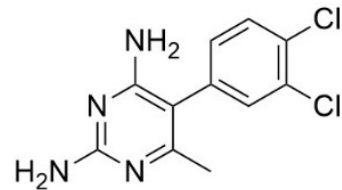
**ormetoprim (5)**



**(12)**

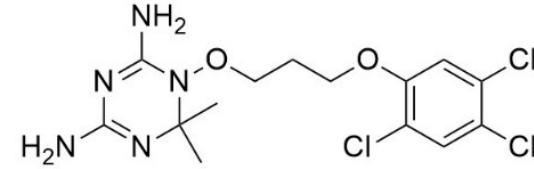


**pyrimethamine (2)**

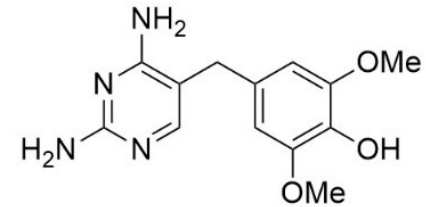


**metoprine (10)**

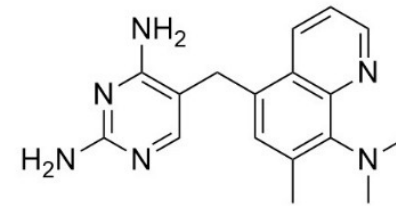
## Not exported



**WR99210 (4)**



**desmethyltrimethoprim (7)**

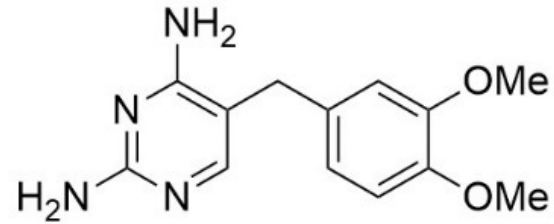


**baquiloprim (11)**

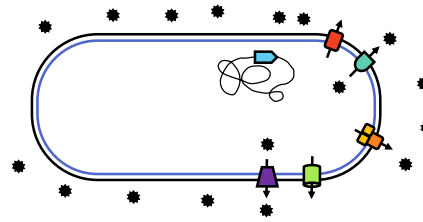
+3

+1

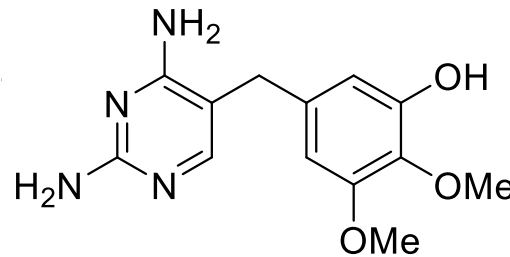
# Identifying structural modification that enhance absorption and retention



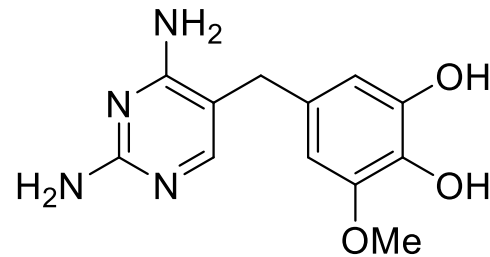
diaveridine (3)



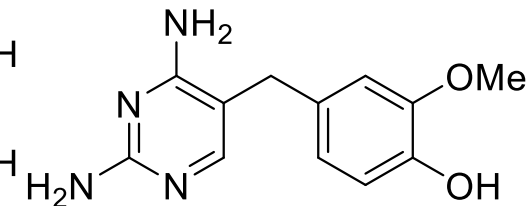
## Diaveridine Analog



L2-50172



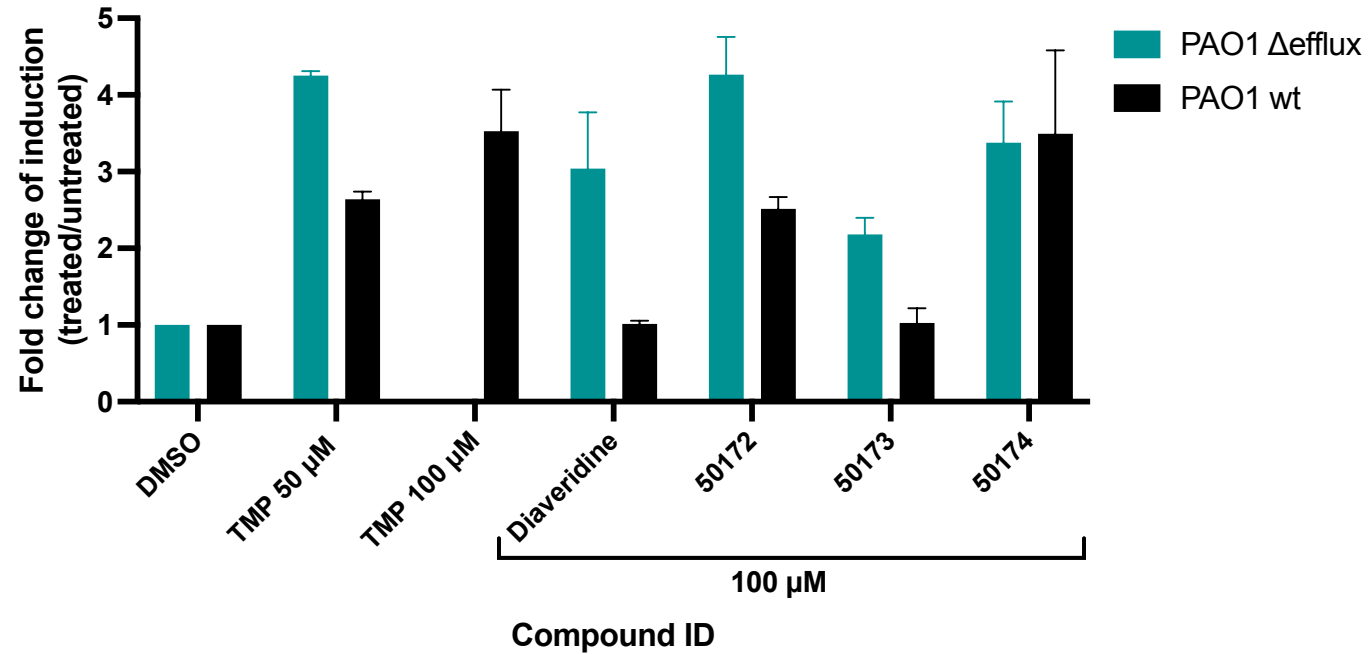
L2-50173



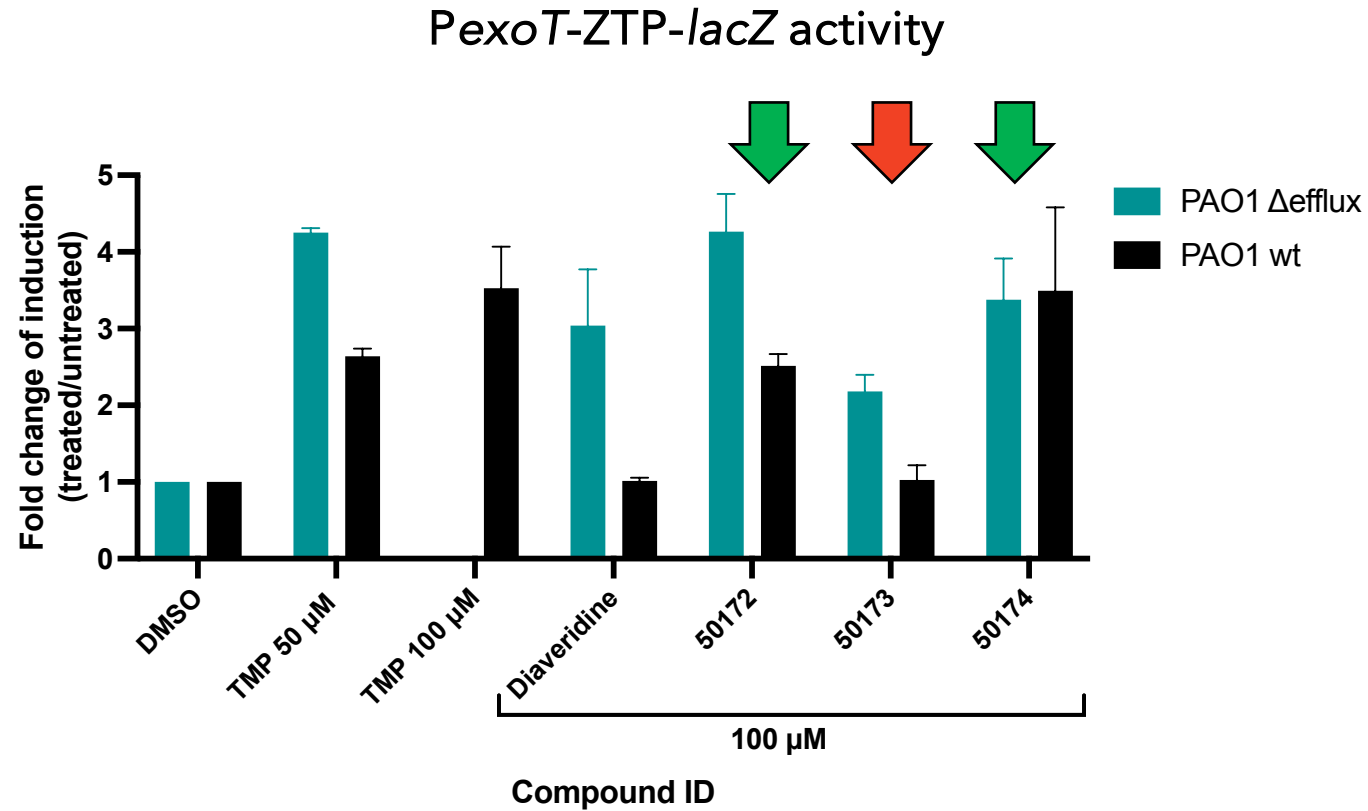
L2-50174

# HTS hit derivatives

*PexoT*-ZTP-*lacZ* activity



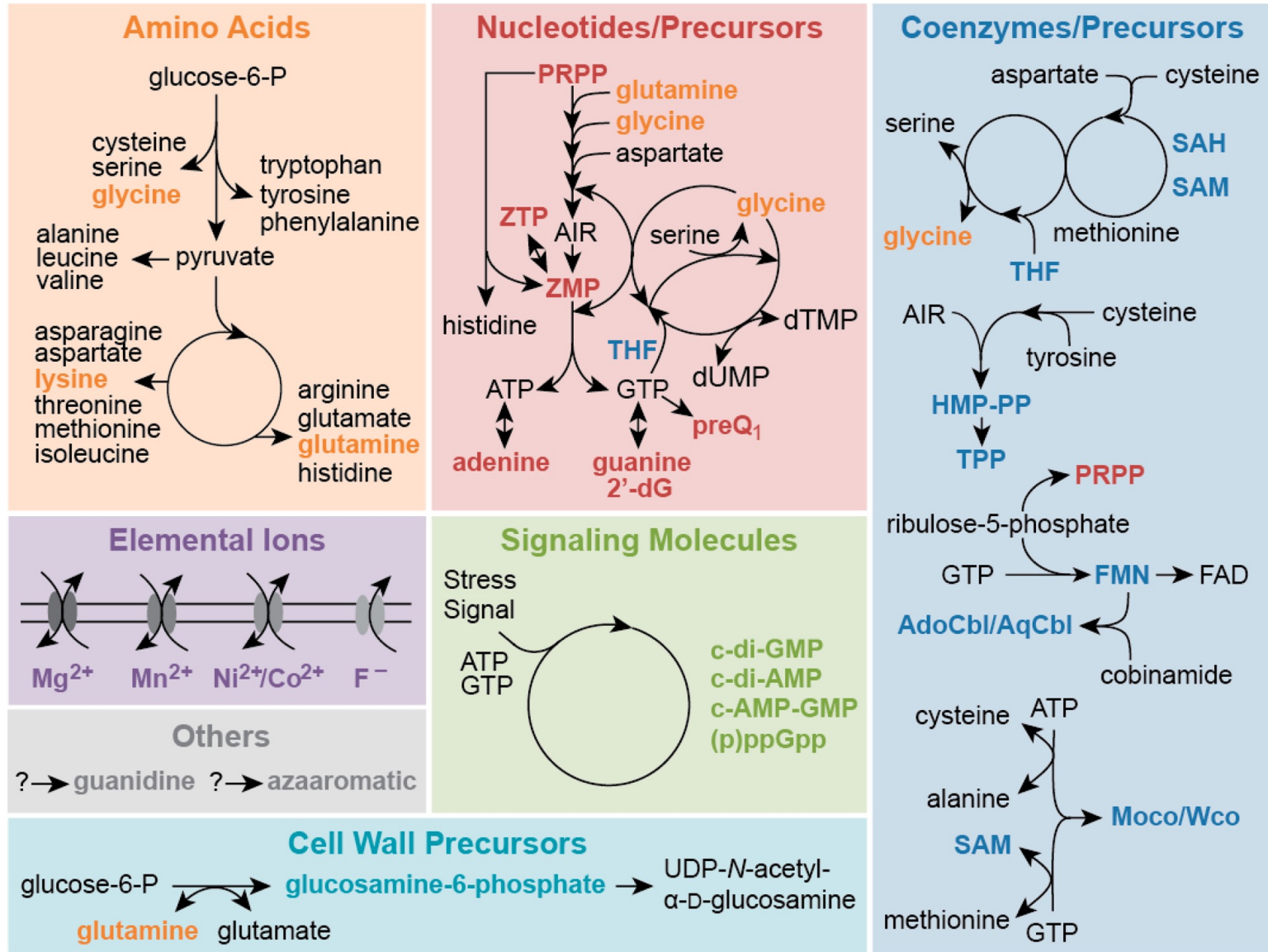
# HTS hit derivatives show altered activity and retention



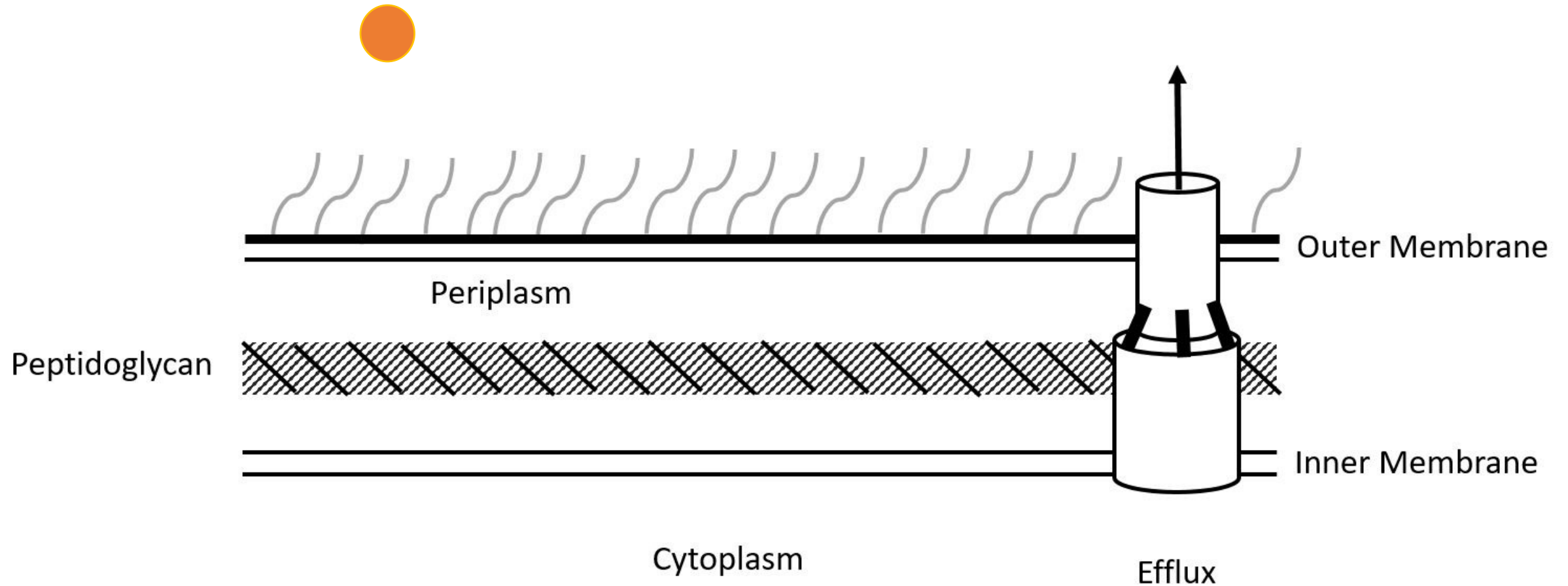


# Next steps:

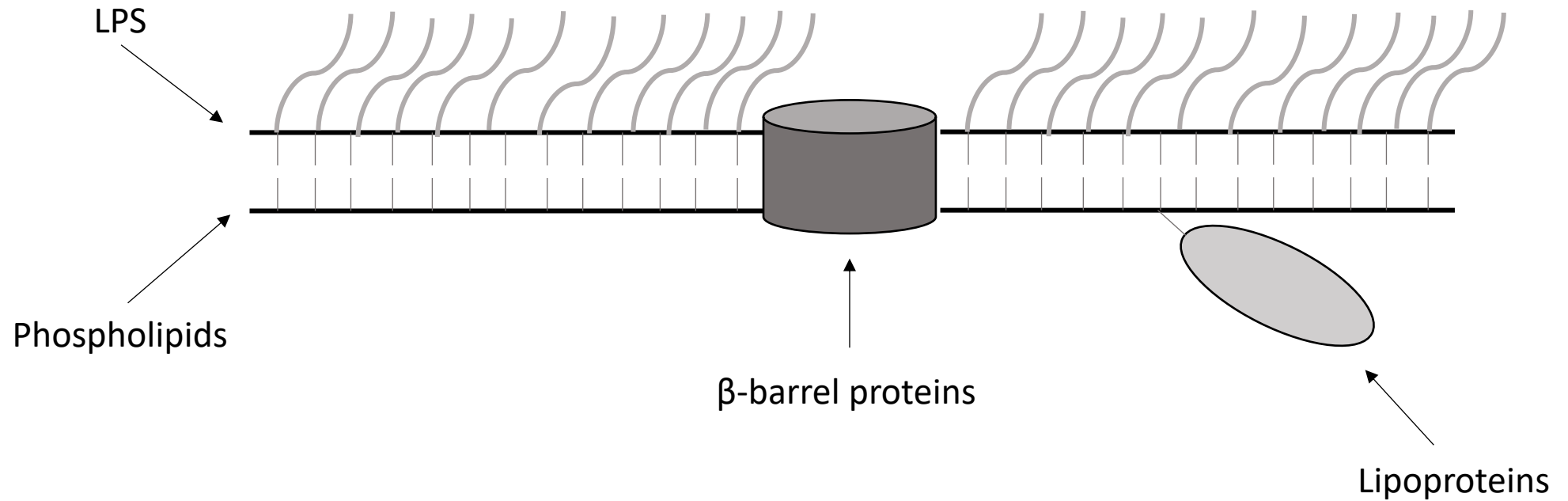
1. Medicinal chemistry campaign for two novel folate pathway inhibitors
2. Screening with additional ready-to-go riboswitch reporters: SAH, TPP, glutamine.



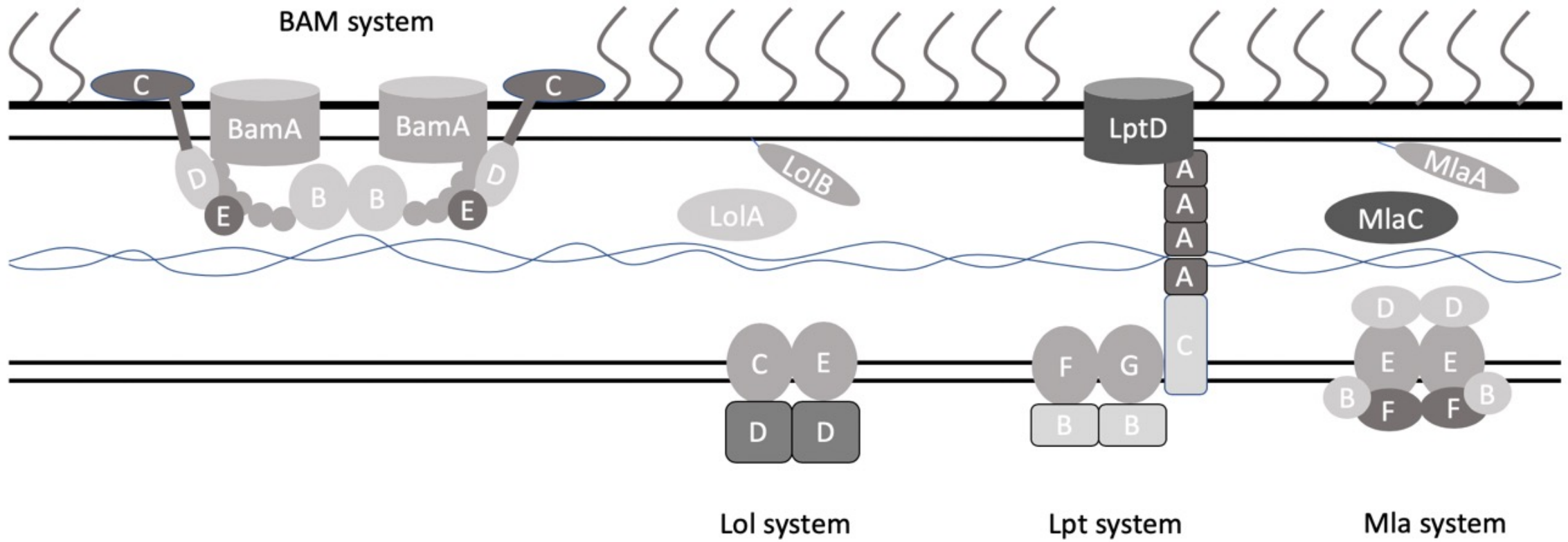
What keeps molecules from getting across the OM?



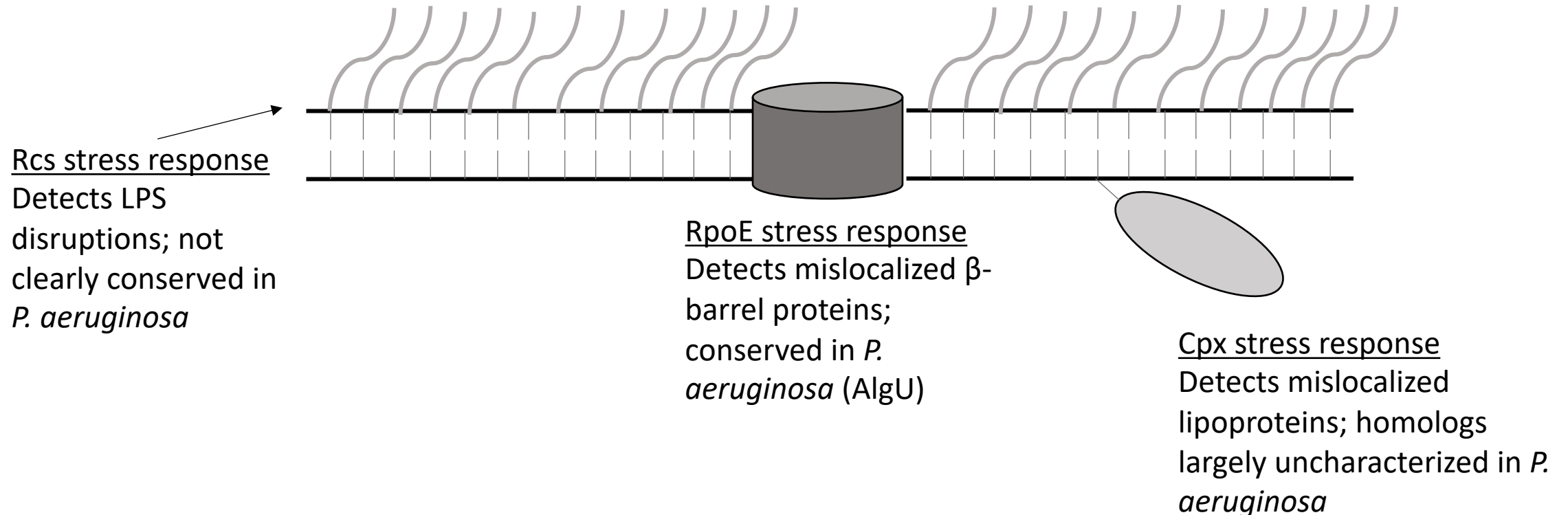
# Building the outer membrane: lessons from *E. coli*



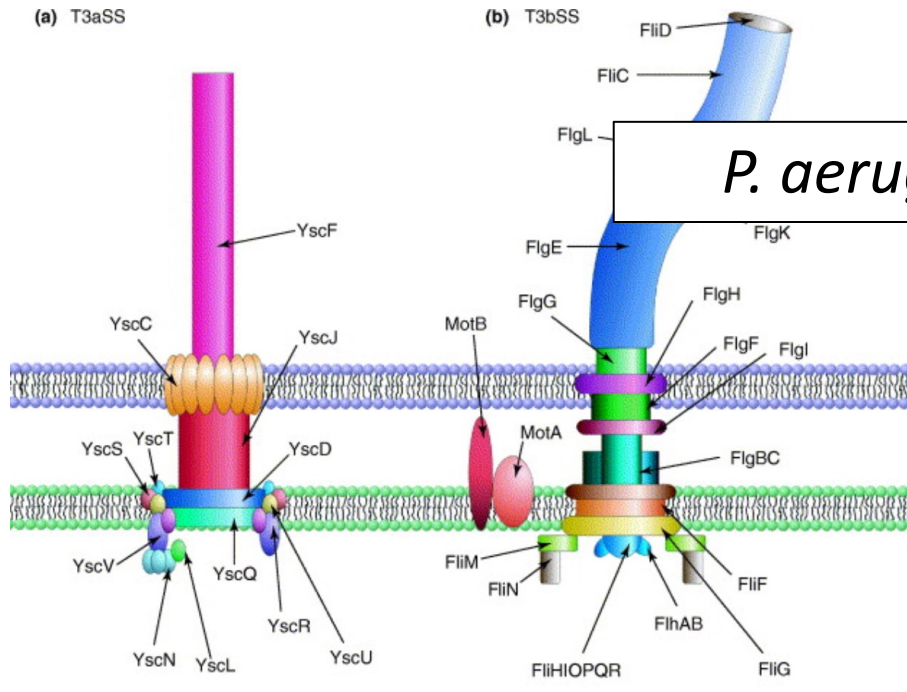
# Conserved machines for OM biogenesis



# Stress responses to disruption of each outer membrane component



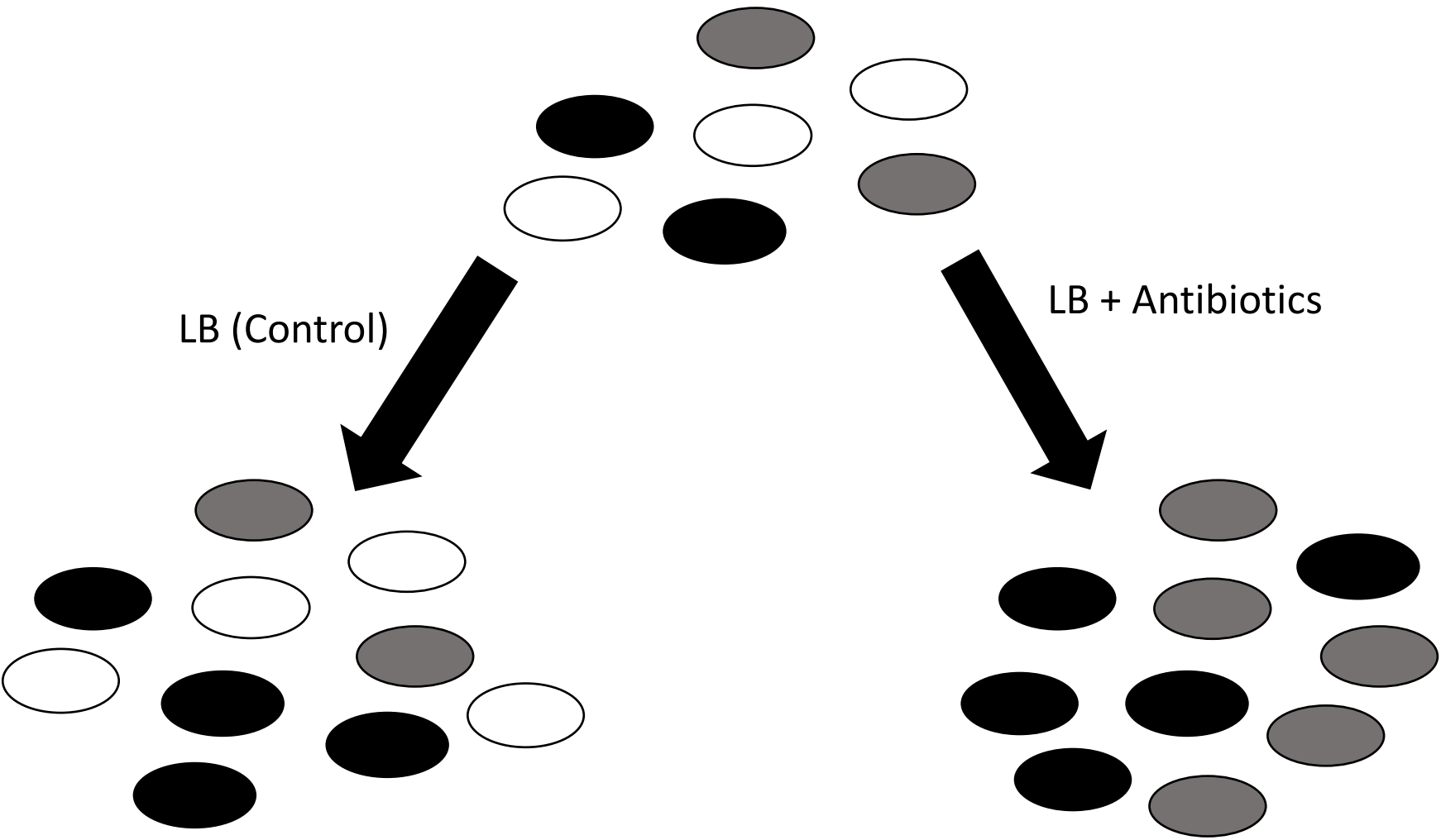
# The Psp system maintains cell envelope integrity during assembly of large structures



*P. aeruginosa* lacks a Psp system.

- A screen for outer membrane-mediated antibiotic resistance determinants

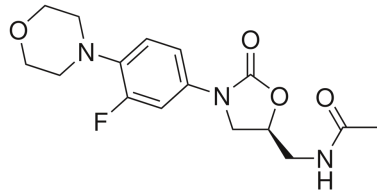
# Using InSeq to identify genes that impact growth in antibiotics





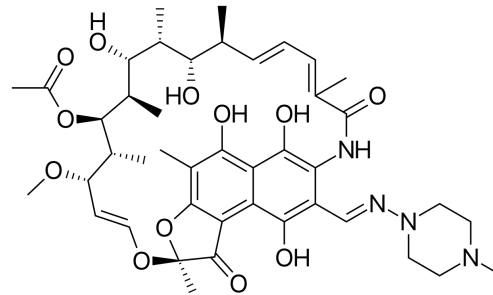
# Selecting antibiotics

## Linezolid



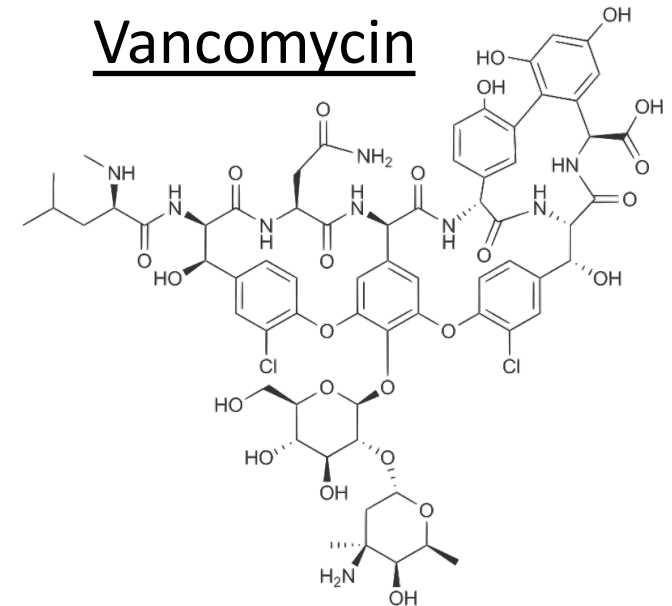
Mechanism of action:  
Binds ribosome and  
disrupts translation

## Rifampicin



Mechanism of action:  
Binds RNA polymerase  
and disrupts transcription

## Vancomycin

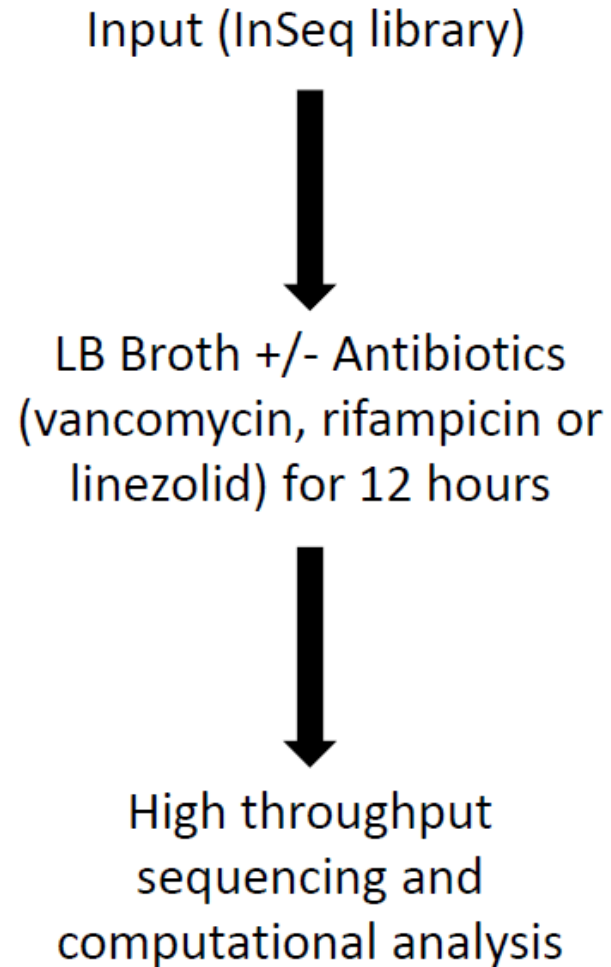


Mechanism of action:  
Binds d-Alanine and  
inhibits PG crosslinking

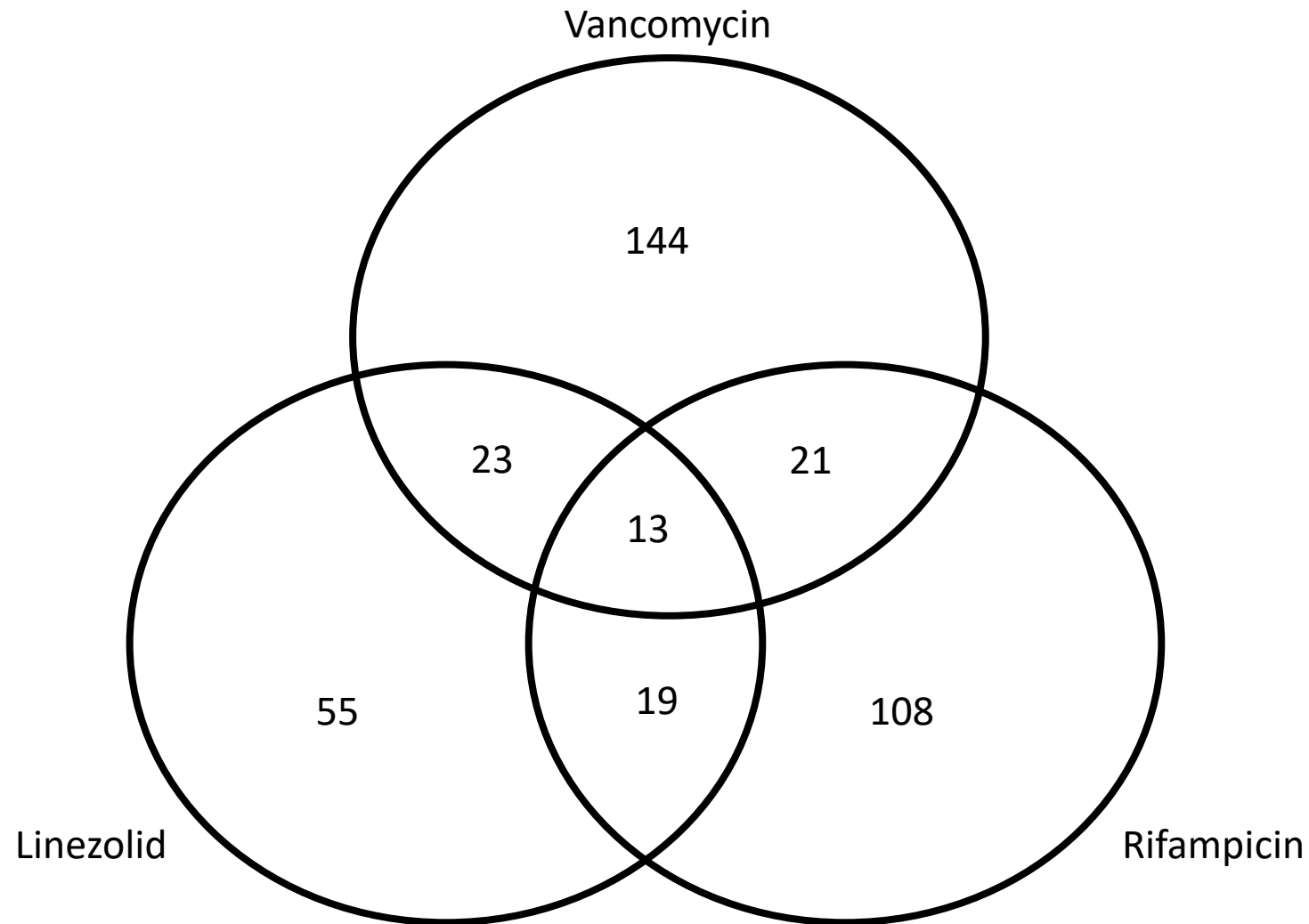
### HYPOTHESIS:

*P. aeruginosa* resistance to one or more of these antibiotics is outer-membrane dependent.

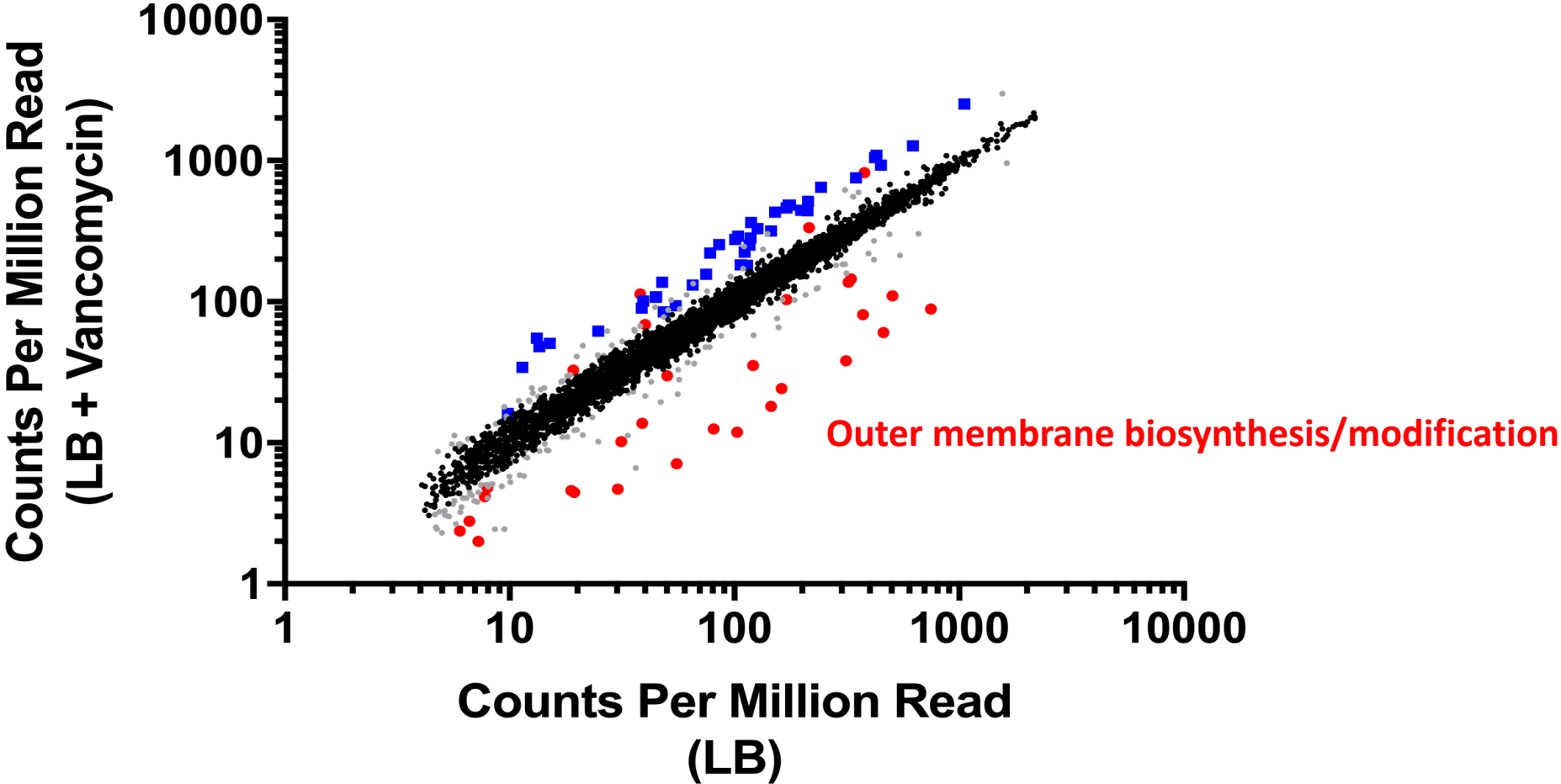
# An INSeq screen for determinants of intrinsic antibiotic resistance



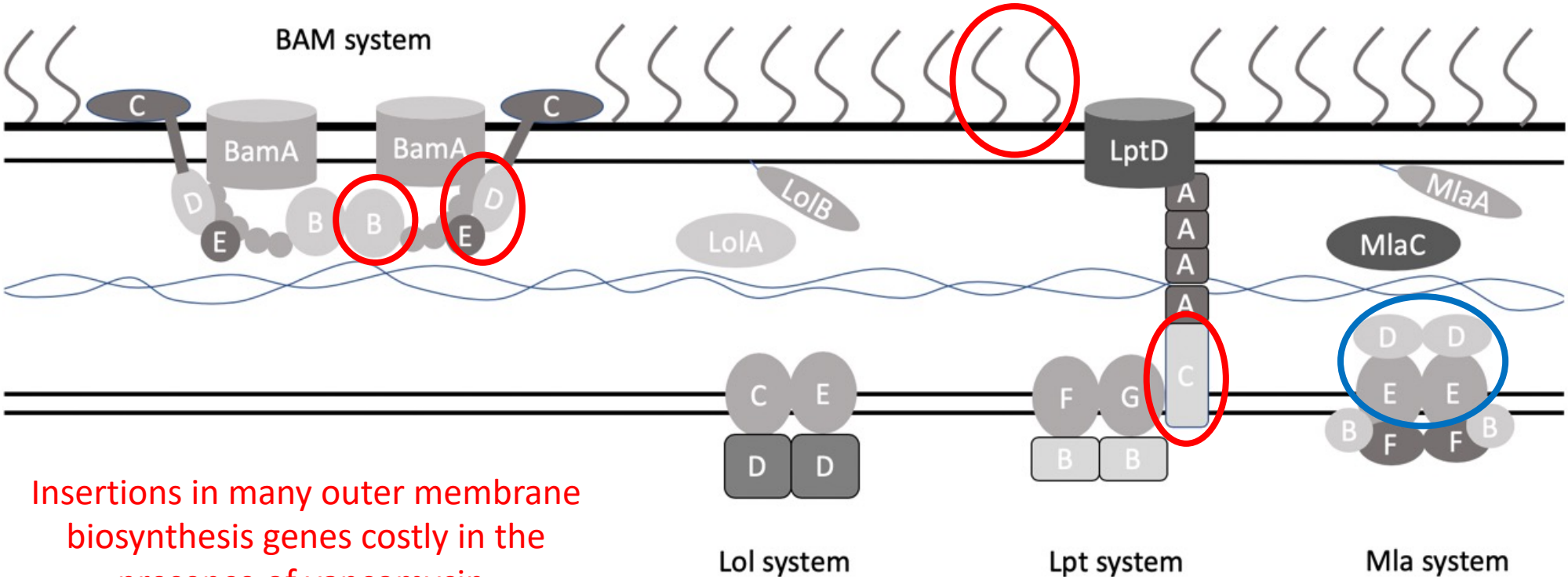
Majority of hits are unique to one antibiotic



# The outer membrane barrier confers fitness in vancomycin



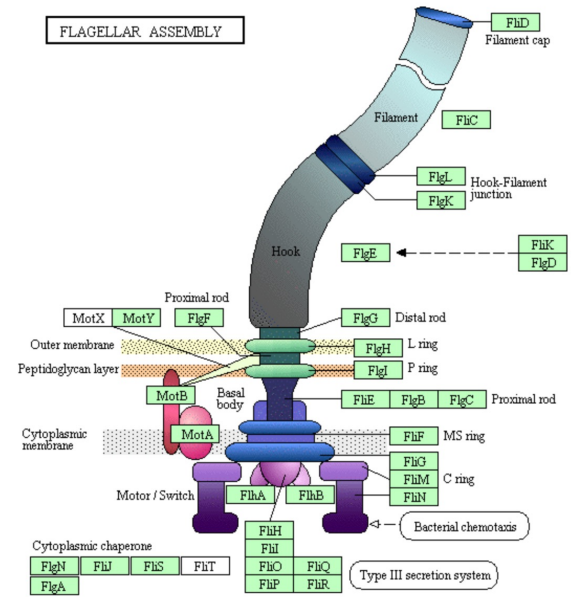
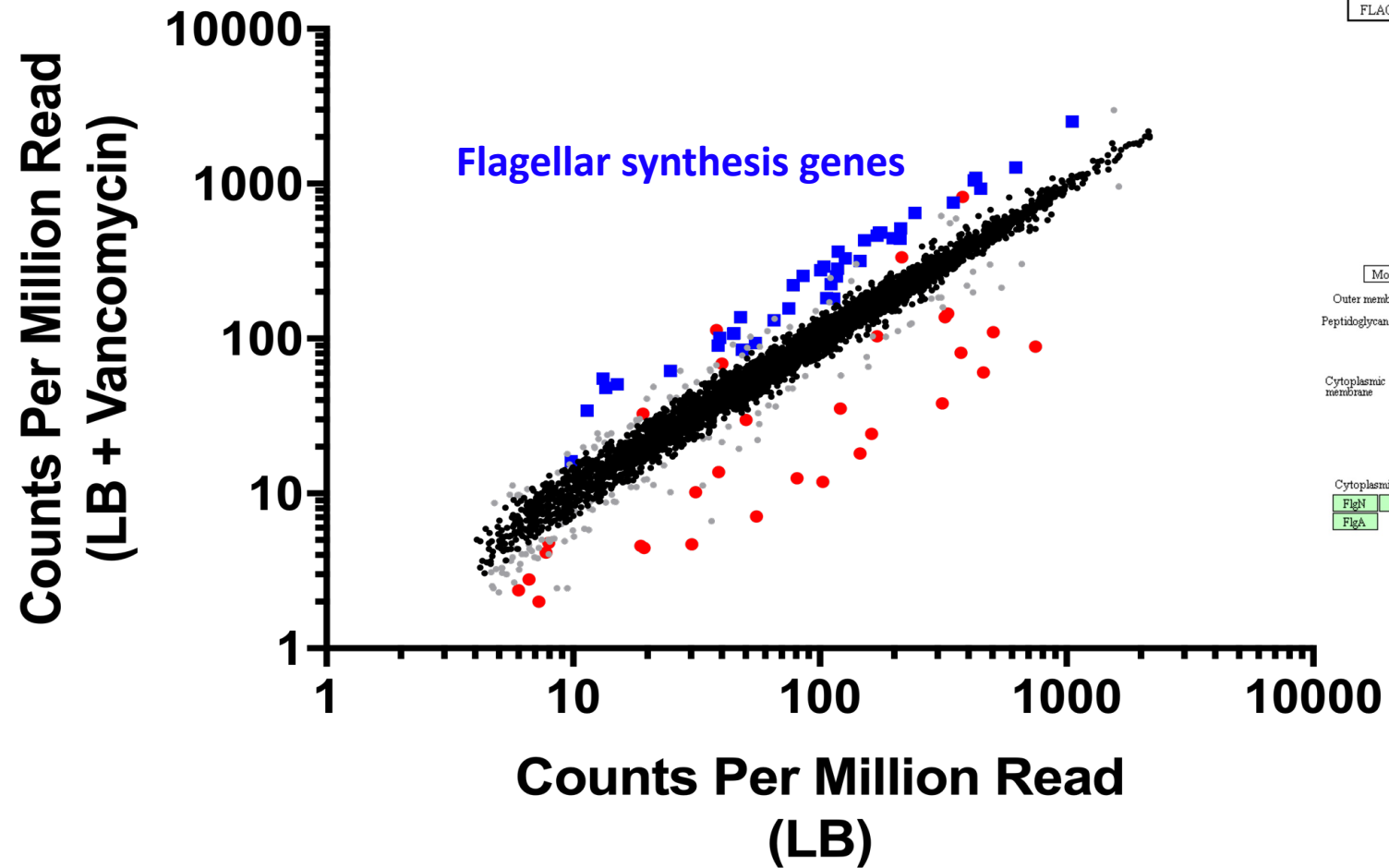
# Independent contributions of multiple OM homeostasis systems to vancomycin fitness



Insertions in many outer membrane biosynthesis genes costly in the presence of vancomycin.

Insertions in Mla genes advantageous in the presence of vancomycin.

# Mutation of flagellar genes increases fitness in vancomycin



# *P. aeruginosa* commonly loses its flagellum in chronic infections

- Nonmotile mutants frequently isolated from CF patients
- One selective pressure against flagellum: recognition by TLR5 and NLRC4
- Does flagellum impact cell envelope barrier function?



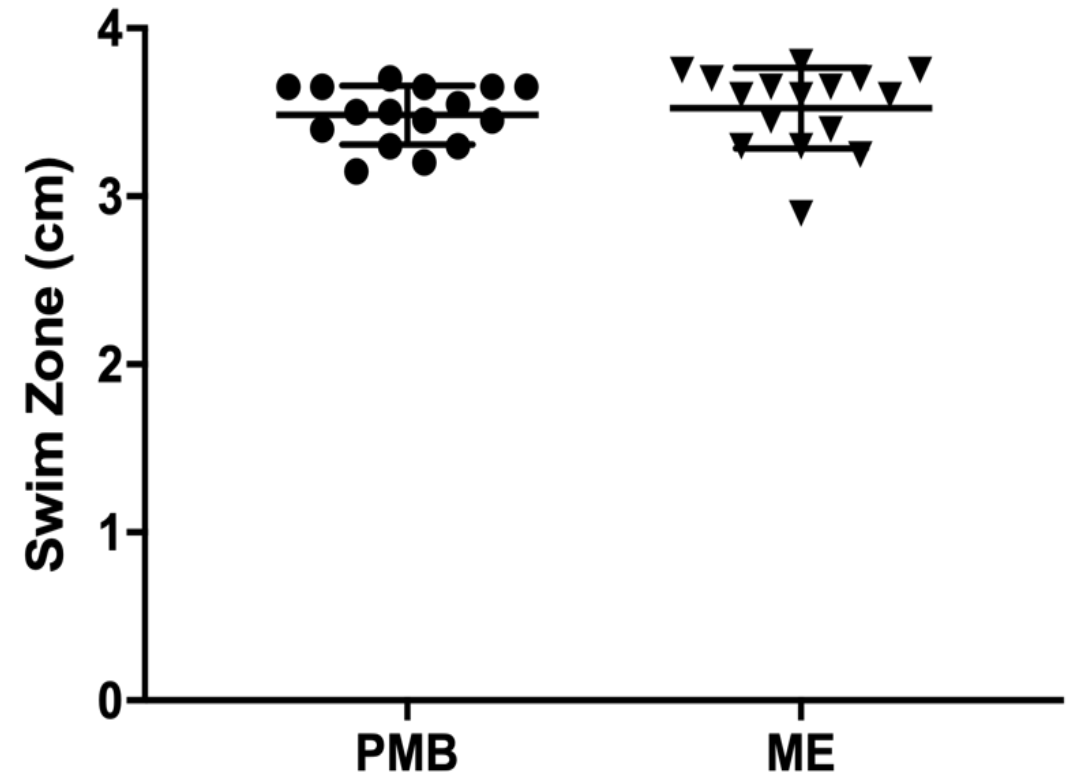
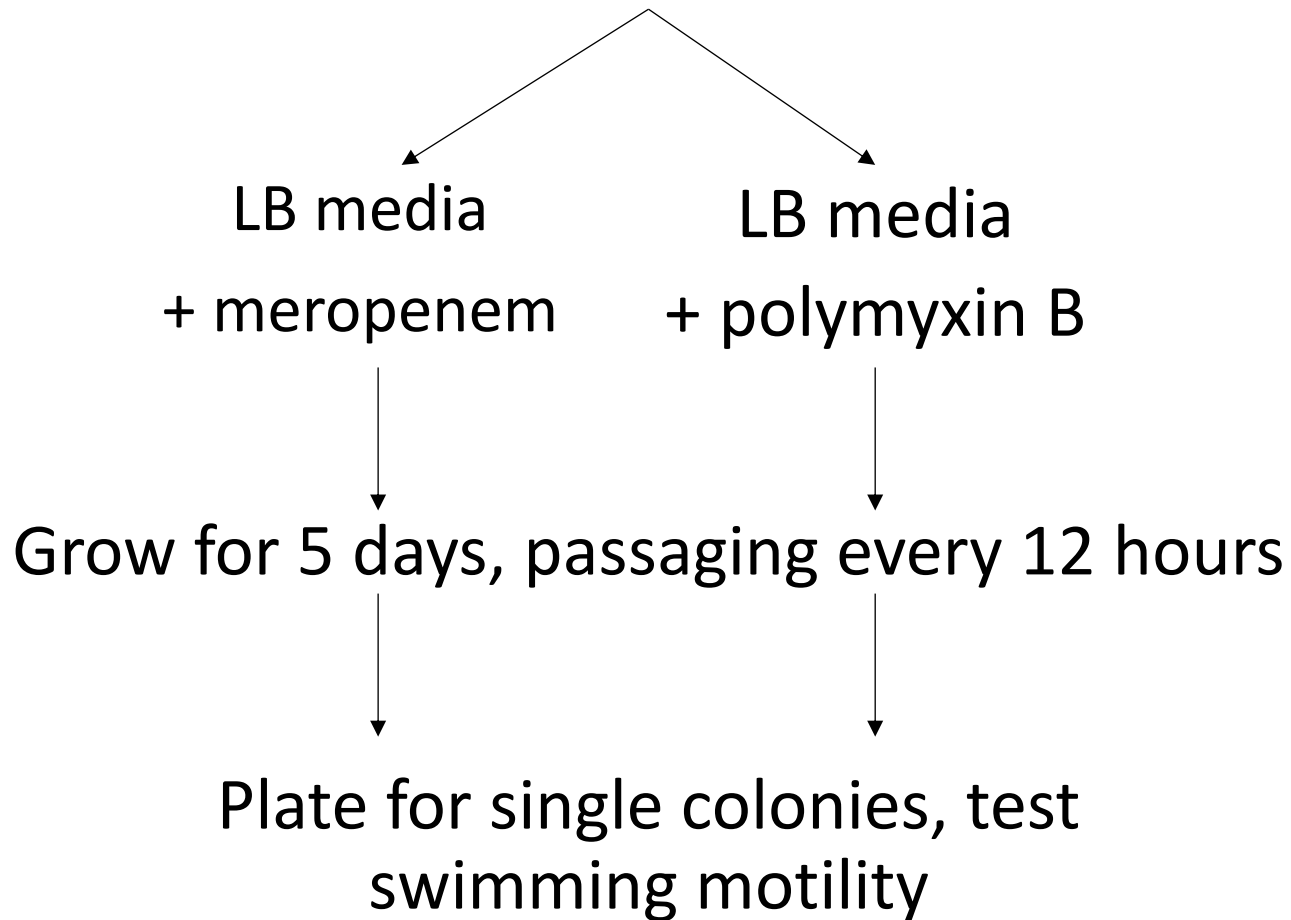
Image:  
Shiwei Zhu,  
Laboratory of Jun Liu,  
Yale University



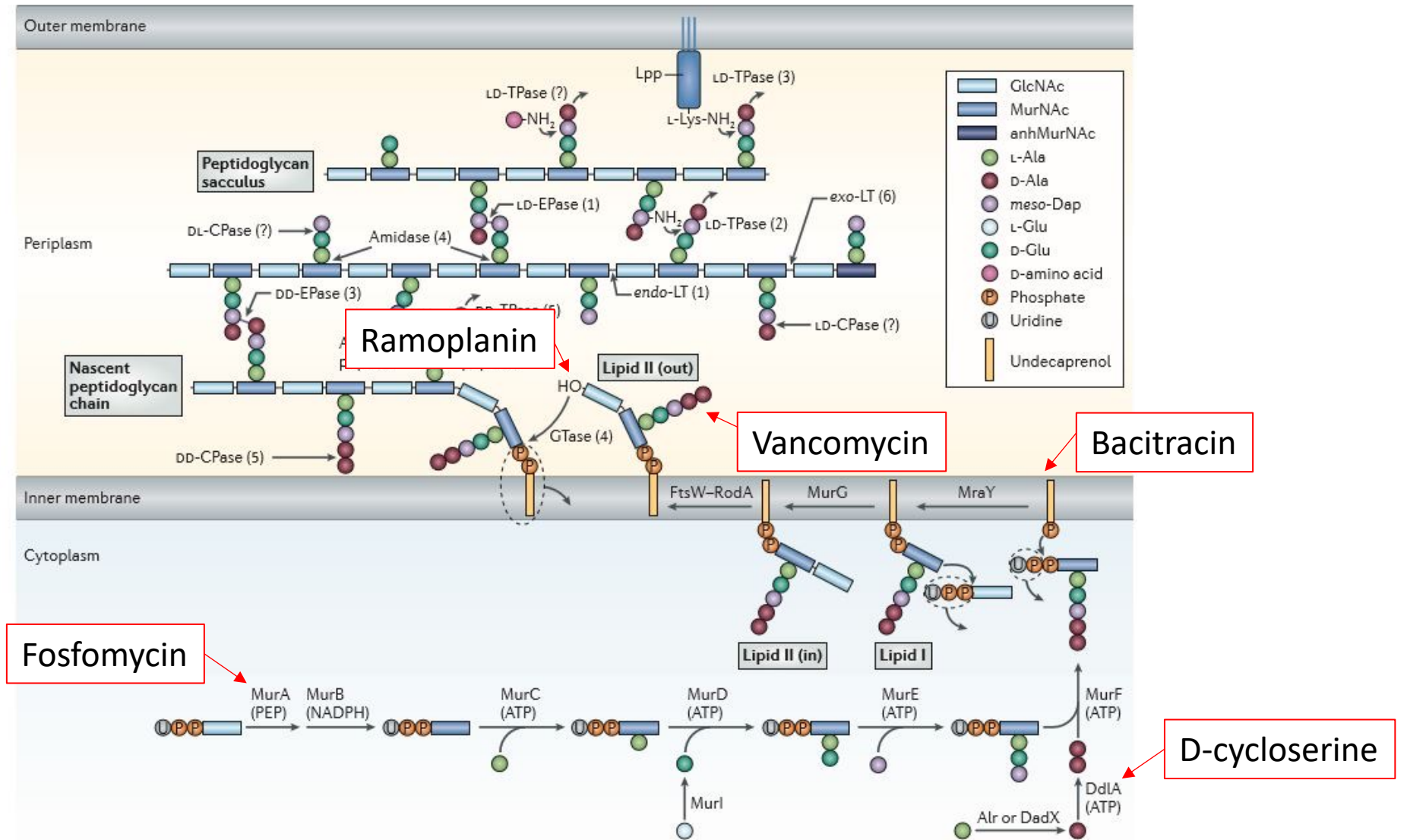


Loss of motility does not evolve after prolonged exposure to other antibiotics

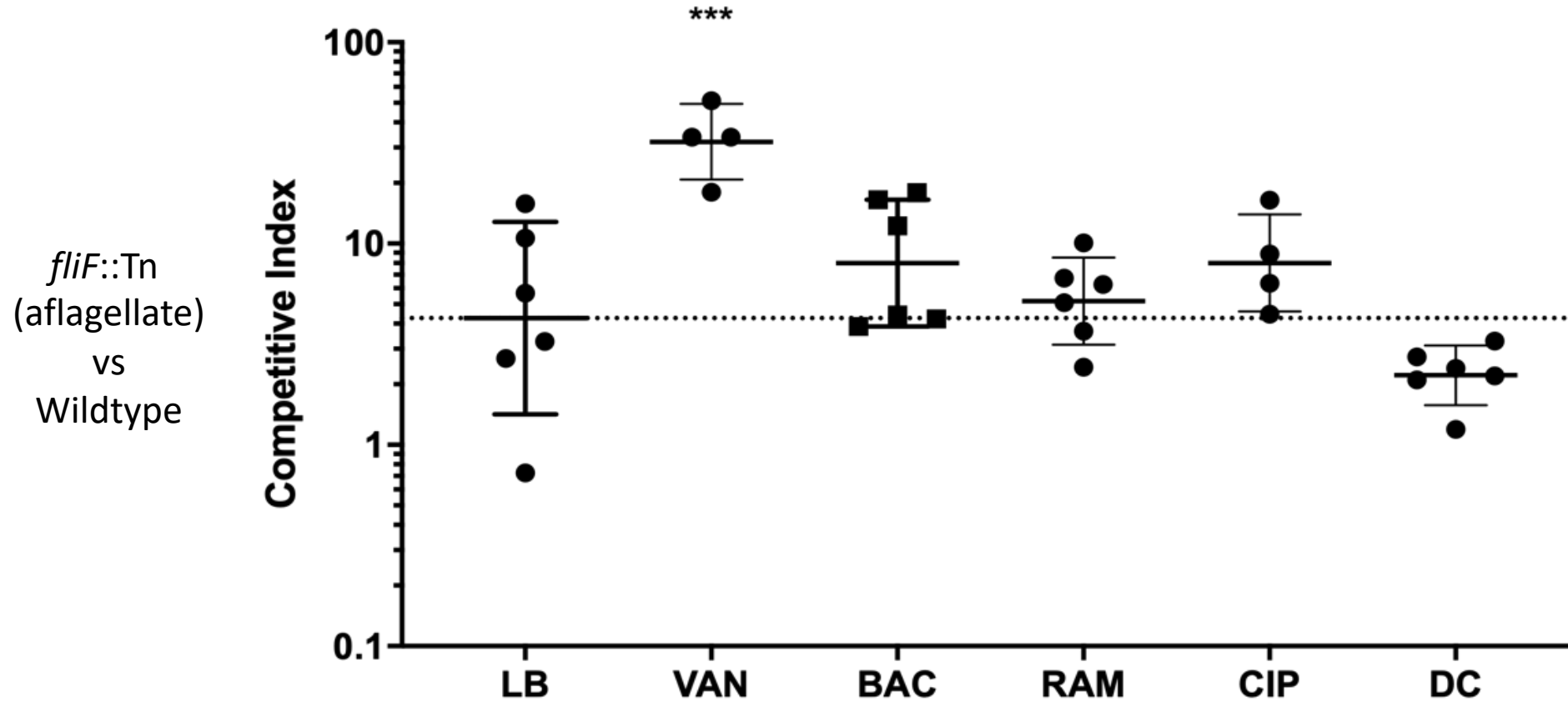
Wildtype *P. aeruginosa*



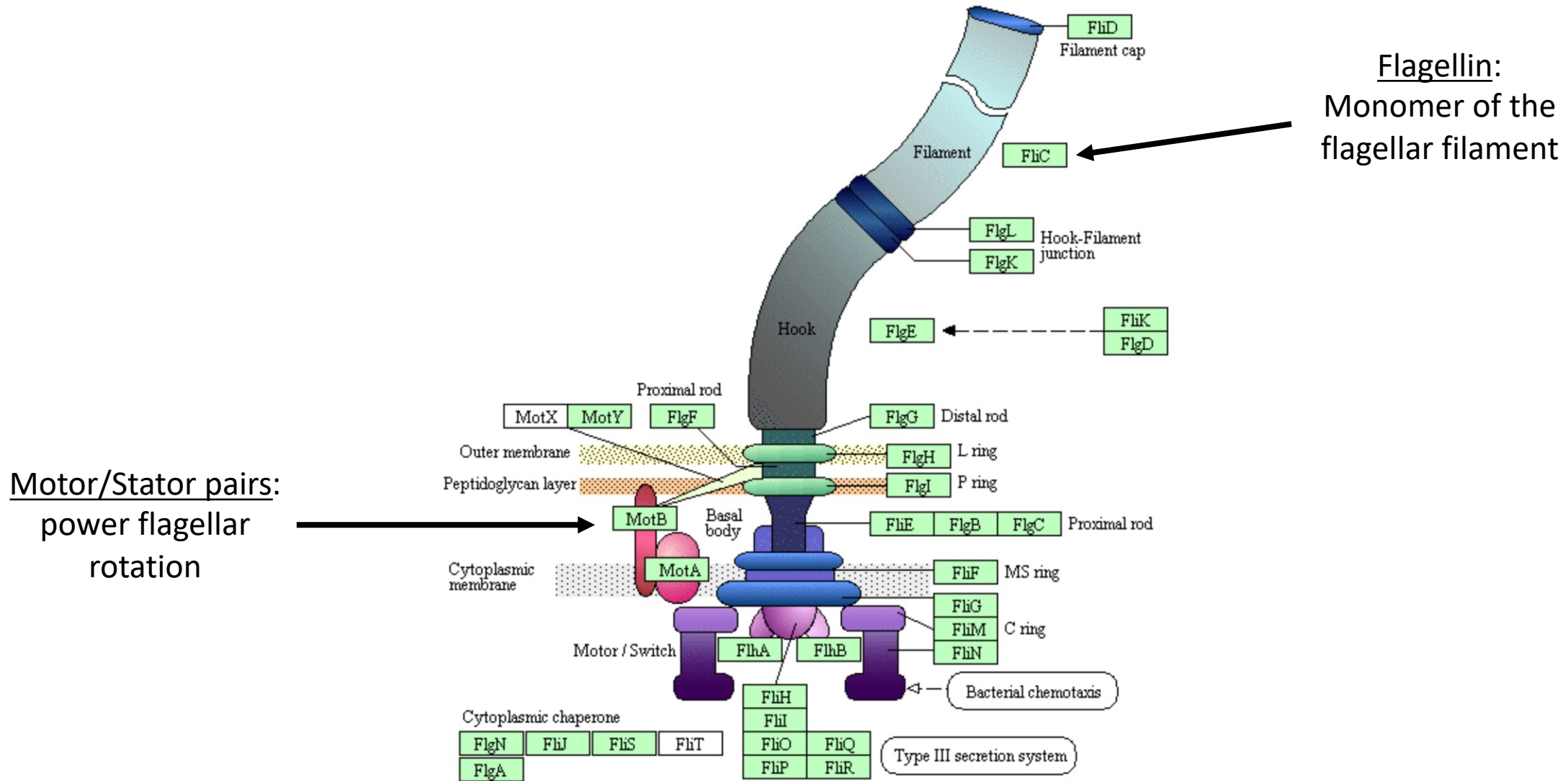
# Testing additional PG-targeting antibiotics



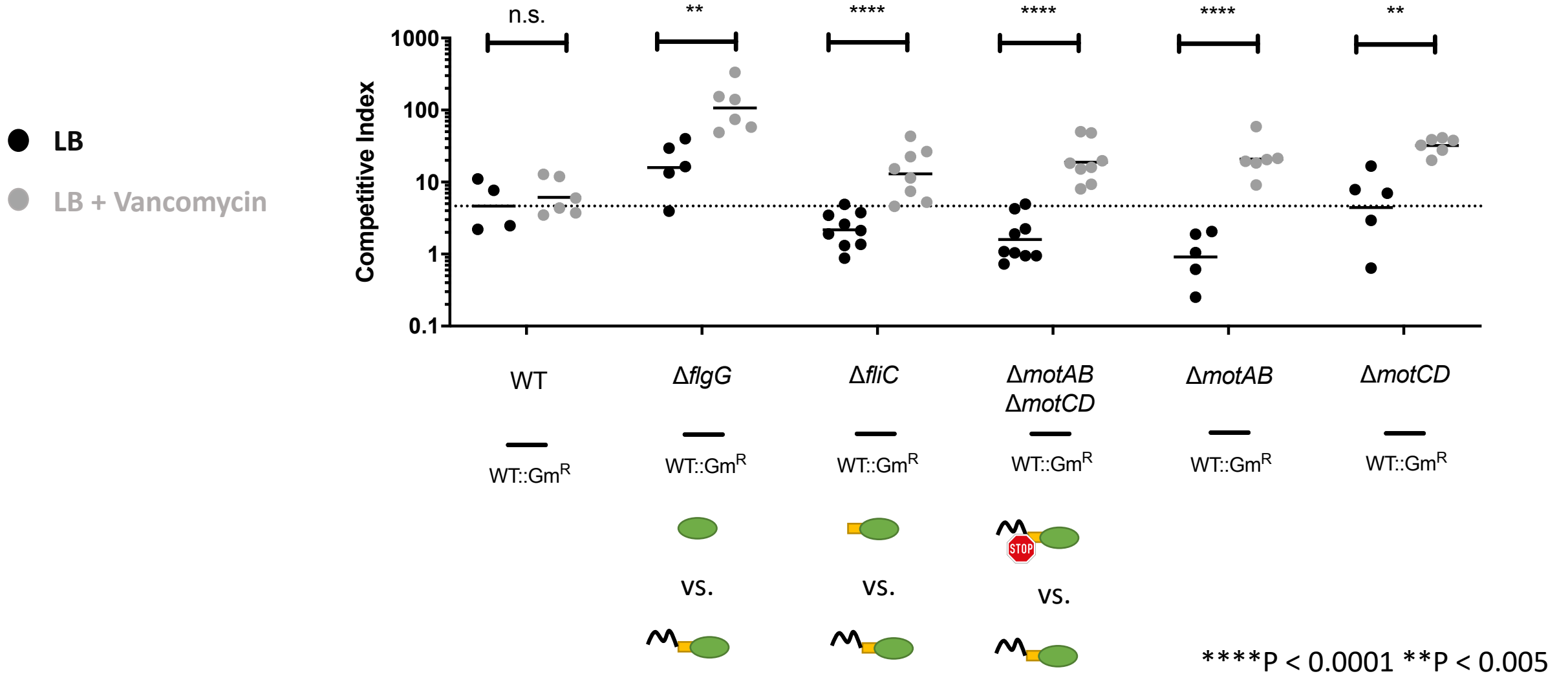
The fitness advantage of flagellar loss is unique to vancomycin



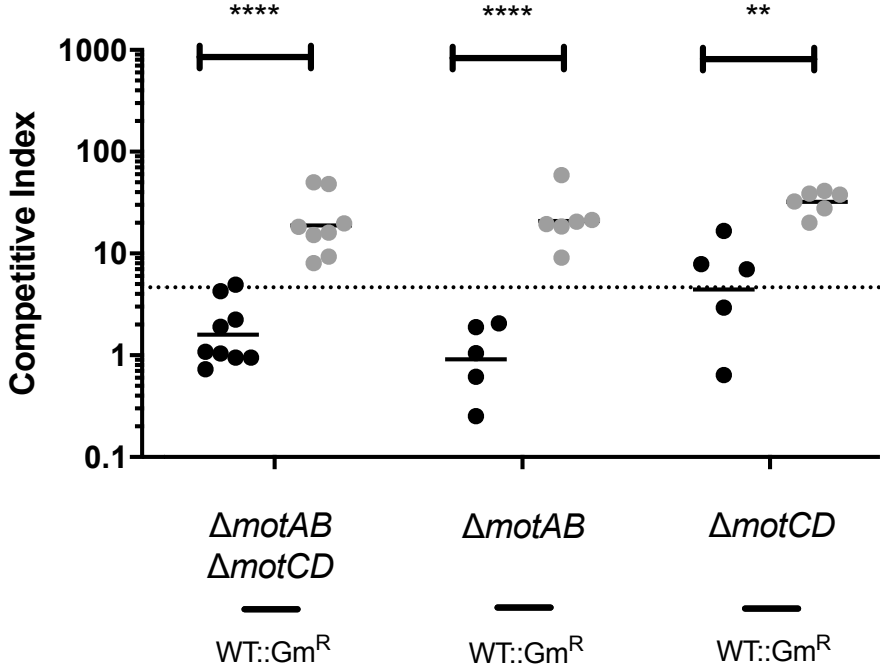
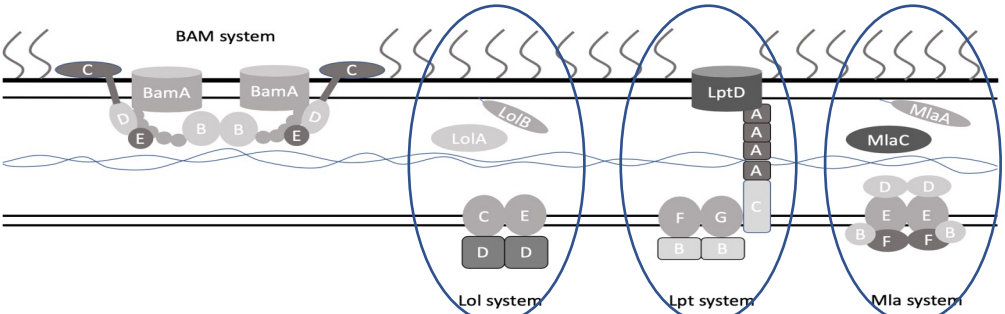
# Which flagellar components decrease fitness in vancomycin?



Loss of motility is sufficient to confer a fitness advantage in the presence of vancomycin.



# Does motility's reliance on proton motive force hinder intrinsic vancomycin resistance?



Does the flagellum carry a similar cost in other motile bacteria?

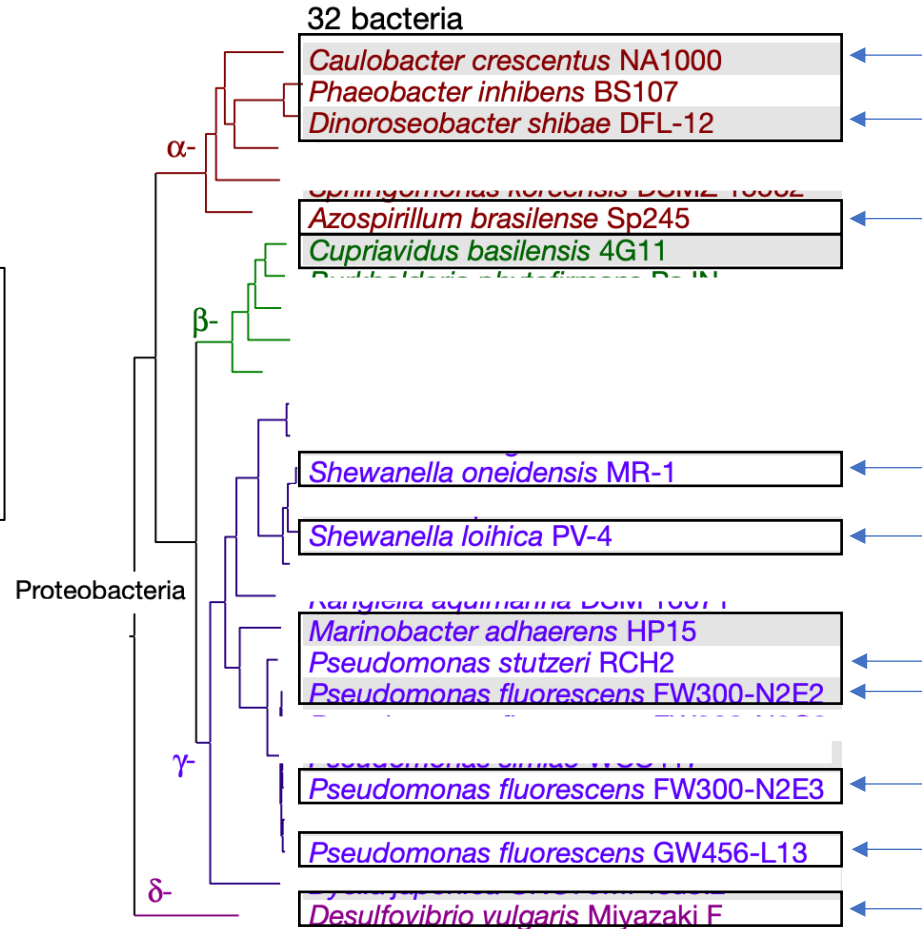
## **Mutant phenotypes for thousands of bacterial genes of unknown function**

Morgan N. Price<sup>1</sup>, Kelly M. Wetmore<sup>1</sup>, R. Jordan Waters<sup>2</sup>, Mark Callaghan<sup>1</sup>, Jayashree Ray<sup>1</sup>, Hualan Liu<sup>1</sup>, Jennifer V. Kuehl<sup>1</sup>, Ryan A. Melnyk<sup>1</sup>, Jacob S. Lamson<sup>1</sup>, Yumi Suh<sup>1</sup>, Hans K. Carlson<sup>1</sup>, Zuelma Esquivel<sup>1</sup>, Harini Sadeeshkumar<sup>1</sup>, Romy Chakraborty<sup>3</sup>, Grant M. Zane<sup>4</sup>, Benjamin E. Rubin<sup>5</sup>, Judy D. Wall<sup>4</sup>, Axel Visel<sup>2,6</sup>, James Bristow<sup>2</sup>, Matthew J. Blow<sup>2\*</sup>, Adam P. Arkin<sup>1,7\*</sup> & Adam M. Deutschbauer<sup>1,8\*</sup>

- TN-Seq study of 32 bacteria in a wide range of conditions
  - 11 flagellated species tested with vancomycin

# A cost for polar flagella in vancomycin?

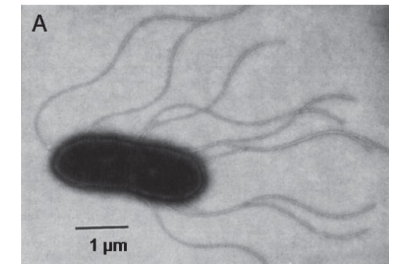
Evidence for a widespread vancomycin-dependent cost associated with flagellar motility in Proteobacteria.



Tested in Vancomycin

← flagellum or chemotaxis genes costly in vancomycin

All but *C. basilensis* assemble polar flagella; *A. brasilense* assembles both polar and lateral flagella





# Conclusions

The Gram-negative bacterial cell envelope is highly conserved – but there are still unique mechanisms underlying its biogenesis, homeostasis and response to stress that are not understood.

*LET'S GET TO WORK!*

# Acknowledgments

## Kazmierczak Lab

Carrie Flynn

Casey Grun

**Veronica Urdaneta, PhD**

Roxanne Morris

Kristen Ramsey

Deanna Hausman

Rebecca Colon-Rios

***Ethan Rundell***

*Christina Lin*

*Daniel Zhitnitsky, PhD*

*Maren Schniederberend, PhD*

*Ruchi Jain, PhD*

## Collaborators

Ron Breaker (Yale)

Alan Sutherland (L2 Diagnostics)

Andy Goodman (Yale)

## Funding

R01 AI36794 (BK, RB, AS)

R21 AI123984 (BK)

F31 AI131531 (ER)