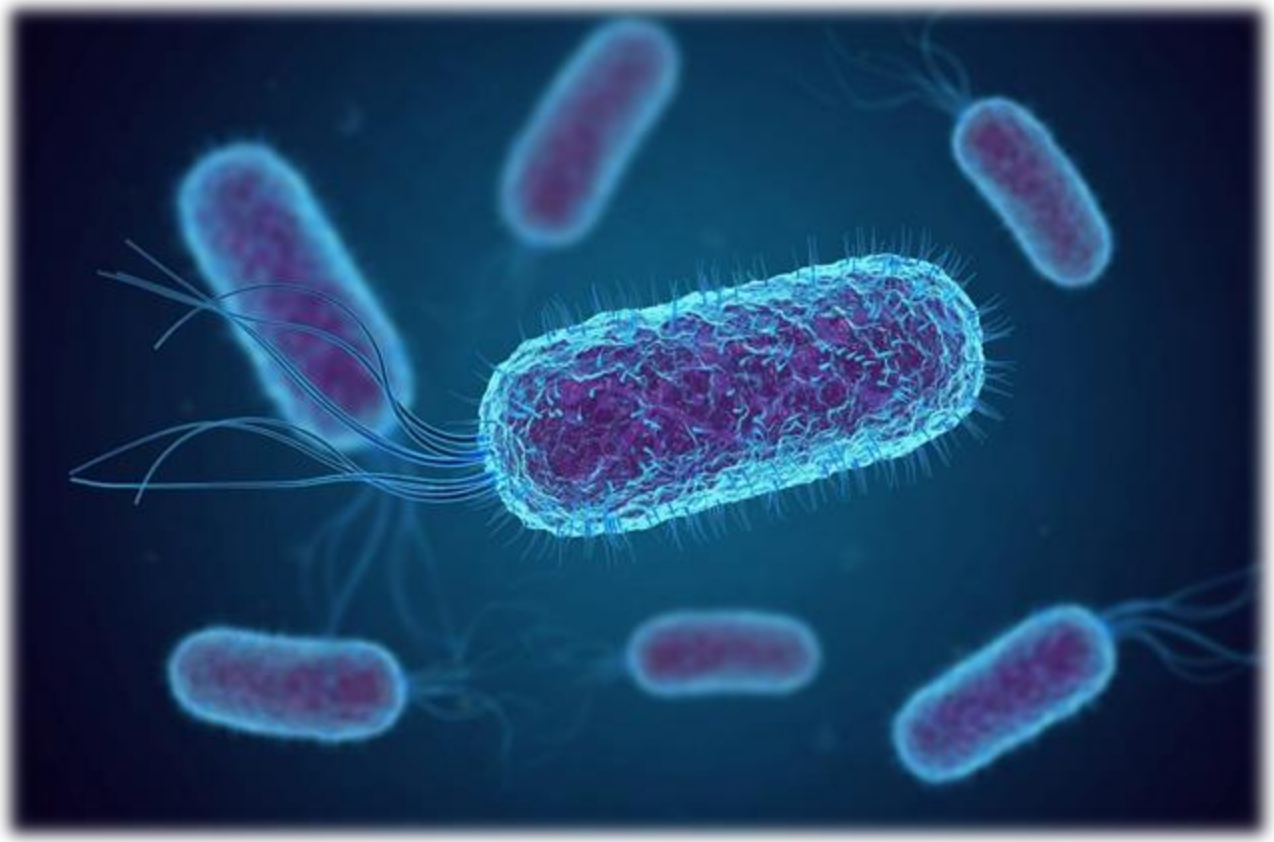
A Tale of Two Tails: PasT Toxin Exhibits Dual Functions

Taylor Blackburn, M.S.
Emory University
Department of Chemistry
ARTDTP T32

The impact of pathogenic Escherichia coli

- Extraintestinal pathogenic *E. coli* (ExPEC) is the most common gram-negative bacterial pathogen in humans



Poolman and Wacker, J. Infect. Dis. 2015

The impact of pathogenic Escherichia coli

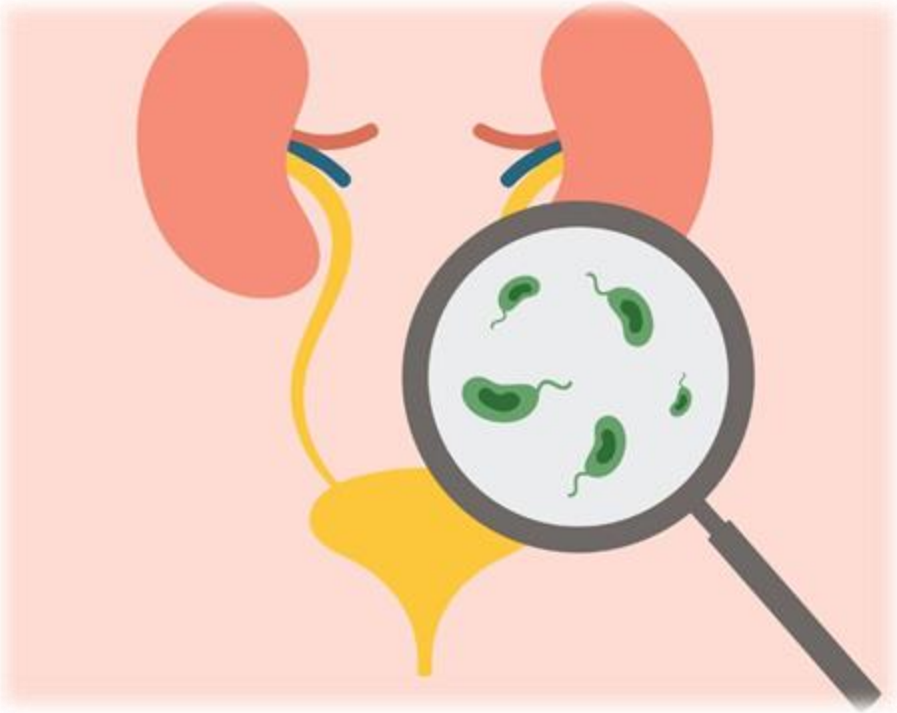
- Extraintestinal pathogenic *E. coli* (ExPEC) is the most common gram-negative bacterial pathogen in humans
- ExPEC cause a wide variety of infections, have high incidence rates of clinical syndromes, and are increasingly exhibiting antimicrobial resistance

Clinical Syndrome	ExPEC	Commensal *E. coli*
Uncomplicated UTI	Major cause	Minor cause
Complicated UTI	Major/minor cause	Major/minor cause
Prostatitis	Major cause	Minor cause
Spontaneous bacterial peritonitis	Major cause	Minor cause
Pneumonia	Major cause	Minor cause
Neonatal meningitis	Major cause	Minor cause
Community acquired bacteremia	Major cause	Minor cause
Nosocomal bacteremia	Major cause	Minor cause

Table adapted from Russo and Johnson, Microbes Infect. 2003

The impact of pathogenic Escherichia coli

- Extraintestinal pathogenic *E. coli* (ExPEC) is the most common gram-negative bacterial pathogen in humans
- ExPEC cause a wide variety of infections, have high incidence rates of clinical syndromes, and are increasingly exhibiting antimicrobial resistance
- Uropathogenic *E. coli* (UPEC) are a subset of ExPEC that are the major causative agent of urinary tract infections (UTIs)



Whelan et. al., Microorganisms 2023

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- 400 million cases & 230,000 deaths globally in 2019
- Most common among the elderly & women
- Nearly 50% of all women will experience a UTI

Whelan et. al., Microorganisms 2023

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- Major burden on healthcare system
- 6% of U.S. medical visits & $1.6 billion USD cost annually

Whelan et. al., Microorganisms 2023

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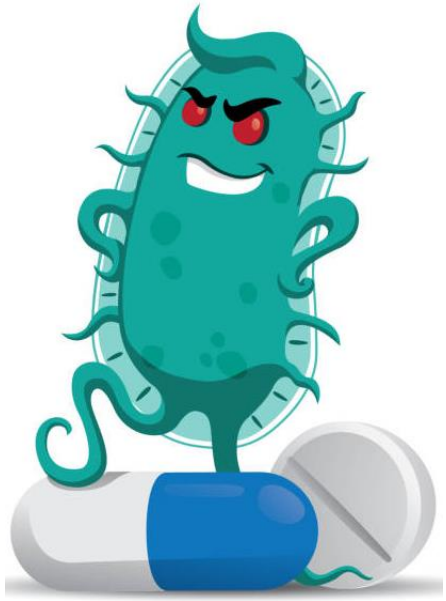

➢ Propensity to reoccur, despite antibiotic therapy

Whelan et. al., Microorganisms 2023

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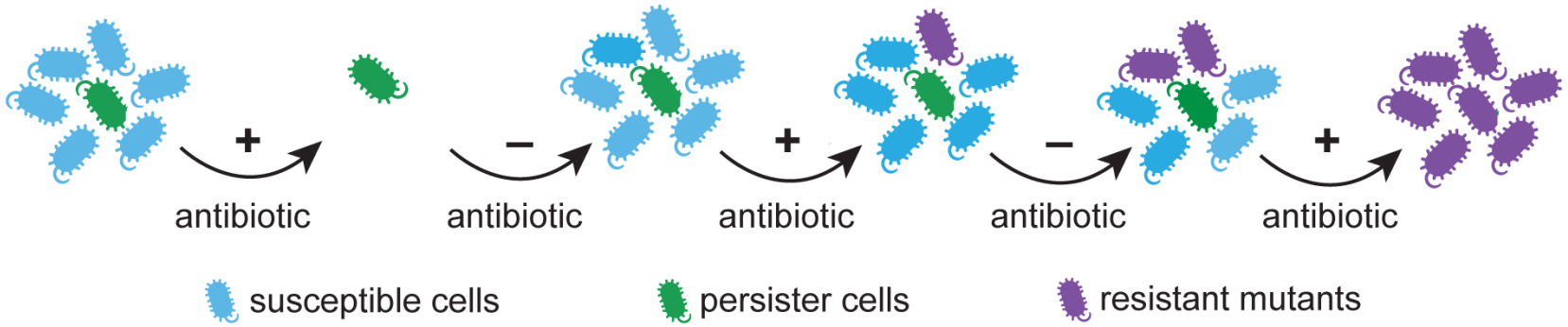
How do UPEC cause chronic and recurrent UTIs?

Toxin-Antitoxin mediated persister cells

- UPEC are able to form metabolically quiescent cells called persister cells



Toxin-Antitoxin mediated persister cells

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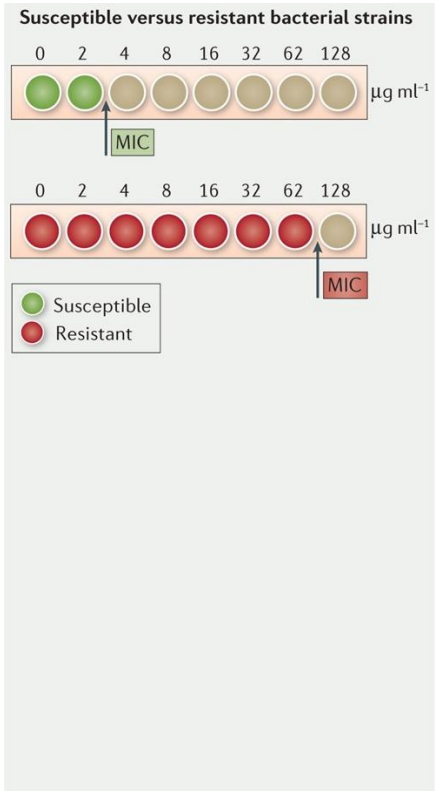
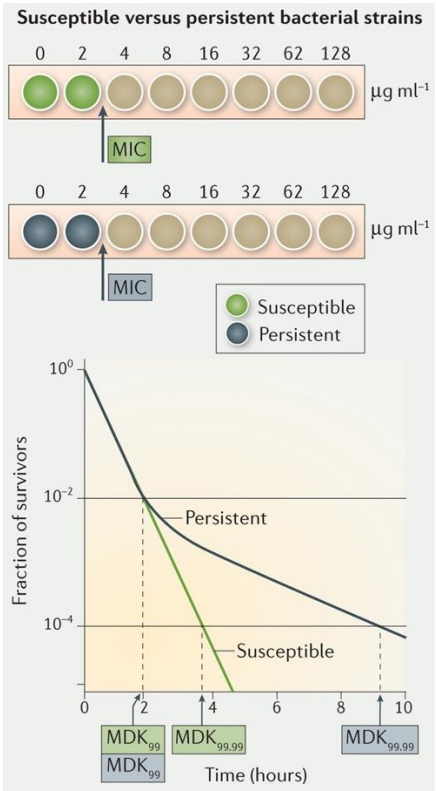
- Persisters are genetically identical to their susceptible parent population, but persistence has been shown to be sufficient for establishment of resistance mutations





Brauner et al., Nat. Rev. Microbiol. 2016; Levin-Reisman et al., Science 2017

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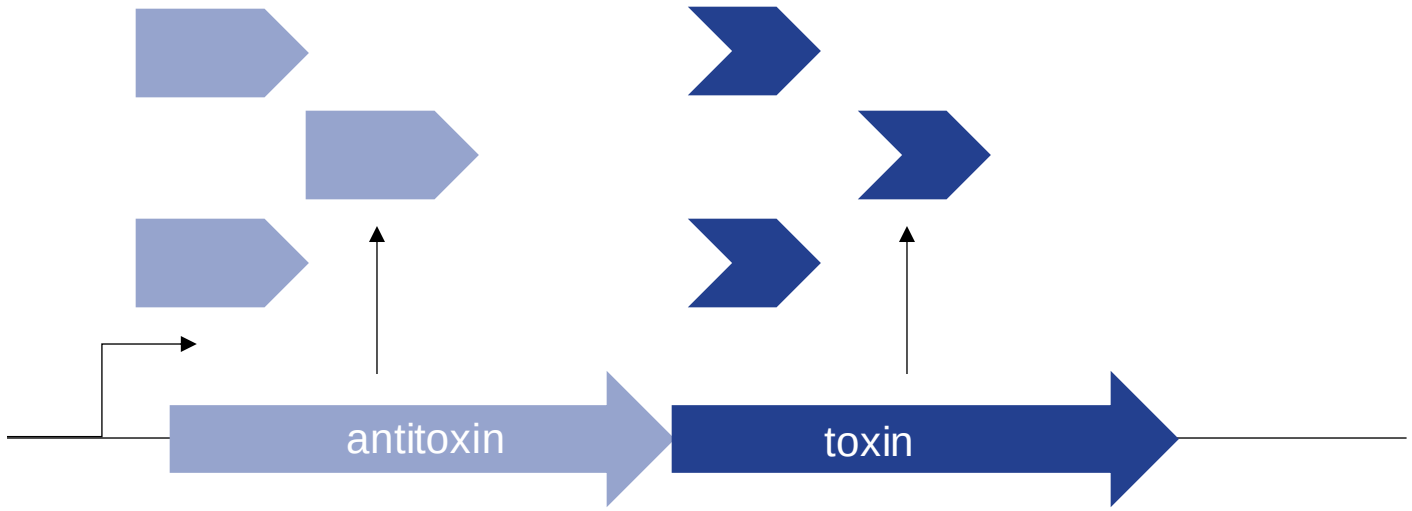
- Formation of persisters can be mediated by two-component genetic modules called toxin-antitoxin (TA) systems



Wang and Wood, Appl. Environ. Microbiol. 2011

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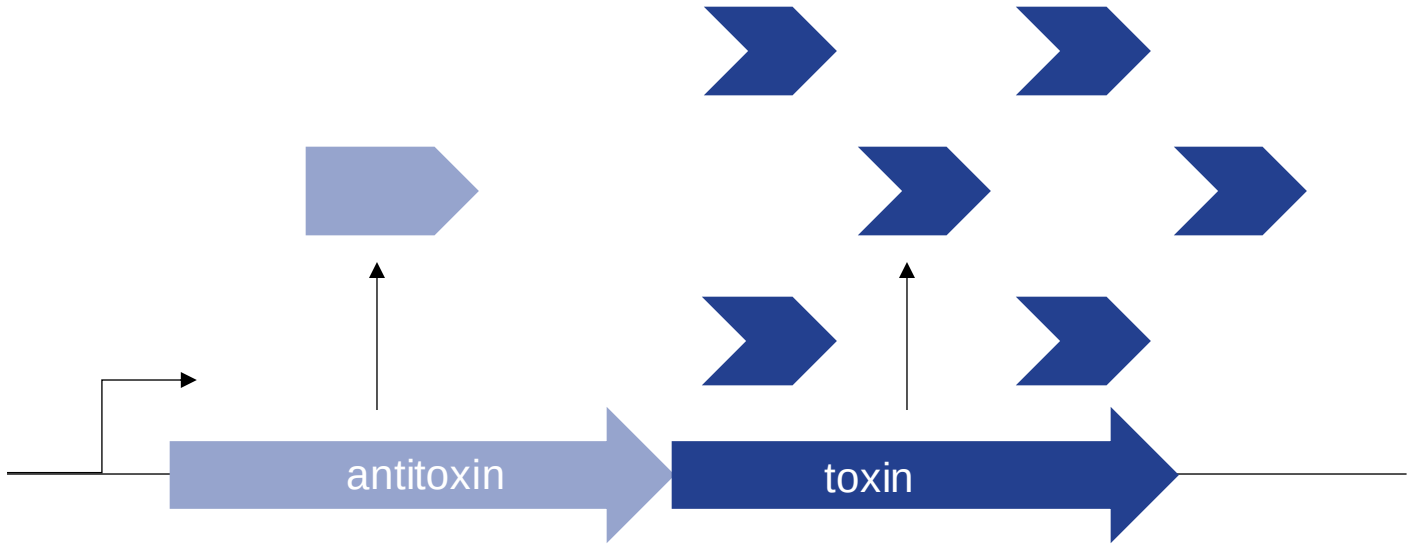

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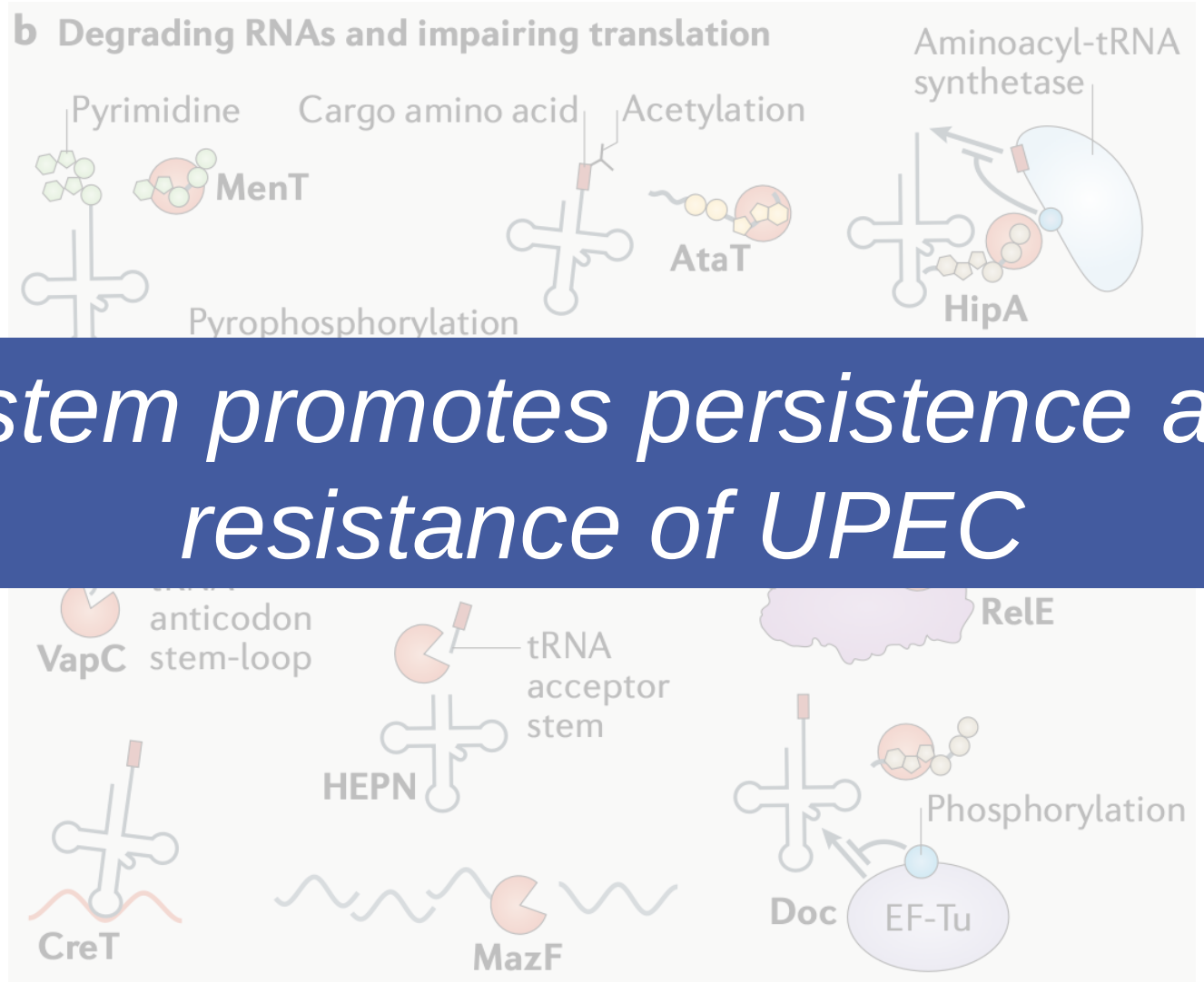
- Currently 8 classes of TA systems defined by mechanism of the antitoxin, with diverse cellular targets

Toxin-Antitoxin mediated persister cells

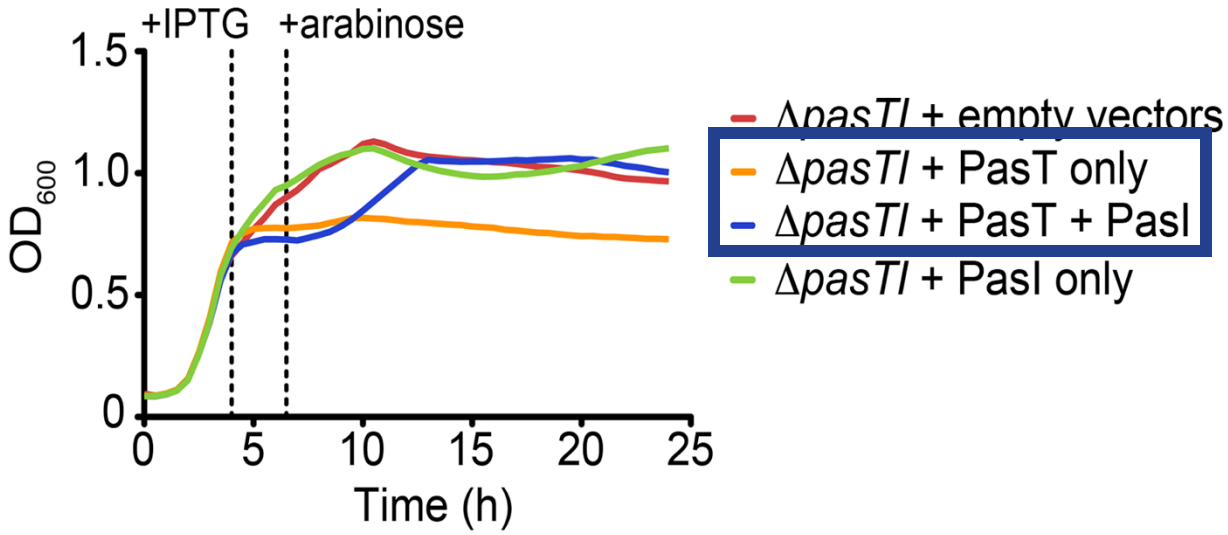


PasTI system promotes persistence and stress resistance of UPEC

Jurénas et. al., Nat. Rev. Microbiol. 2022

PasTI system promotes UPEC persistence

- Type II TA system PasTI identified in UPEC by persister and growth assays



Norton and Mulvey, PLoS Pathog. 2012

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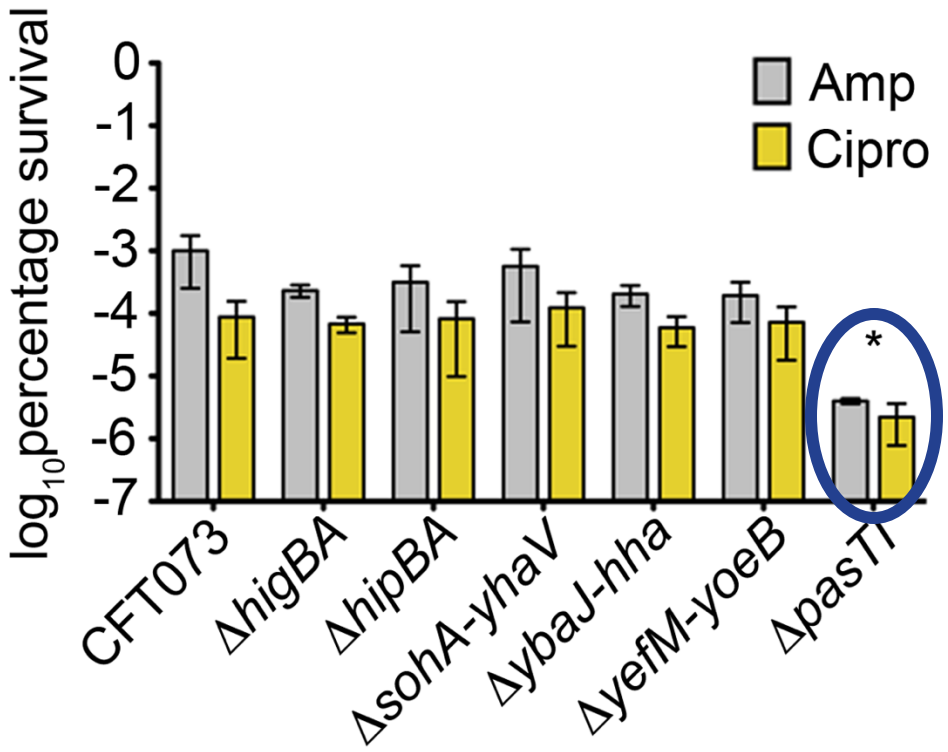

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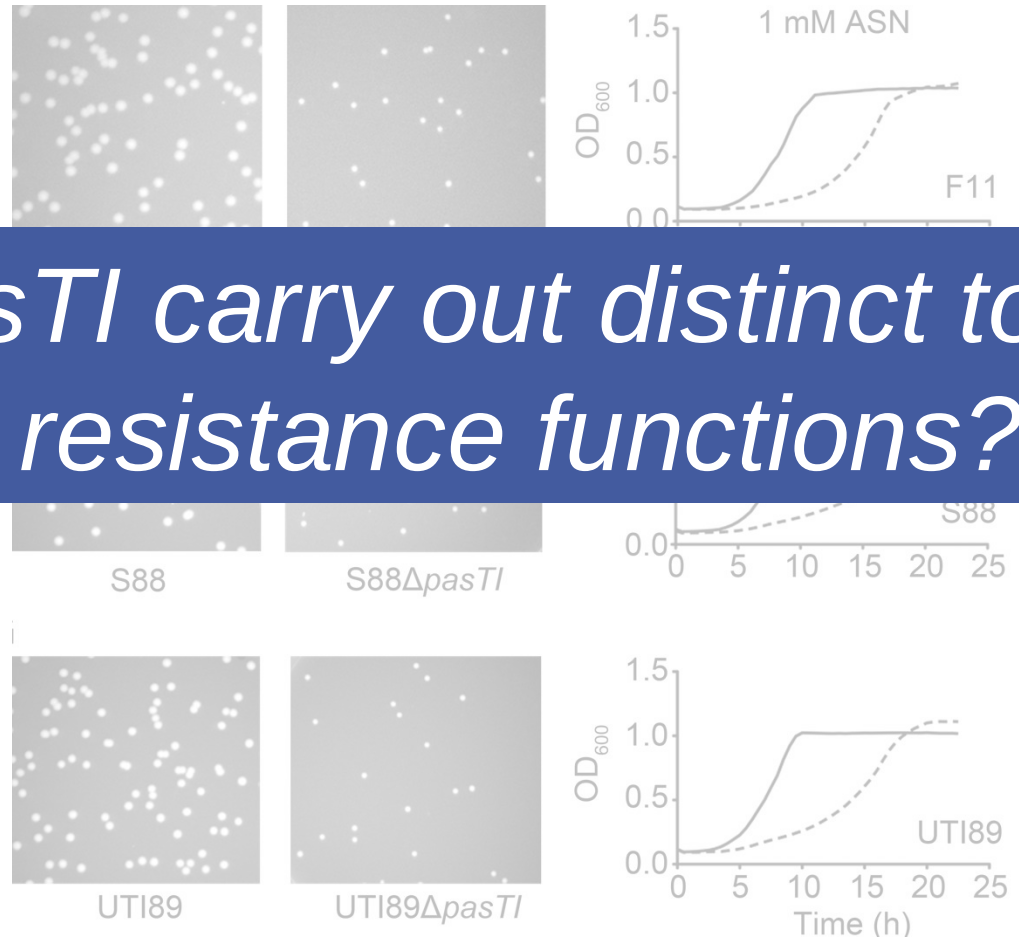
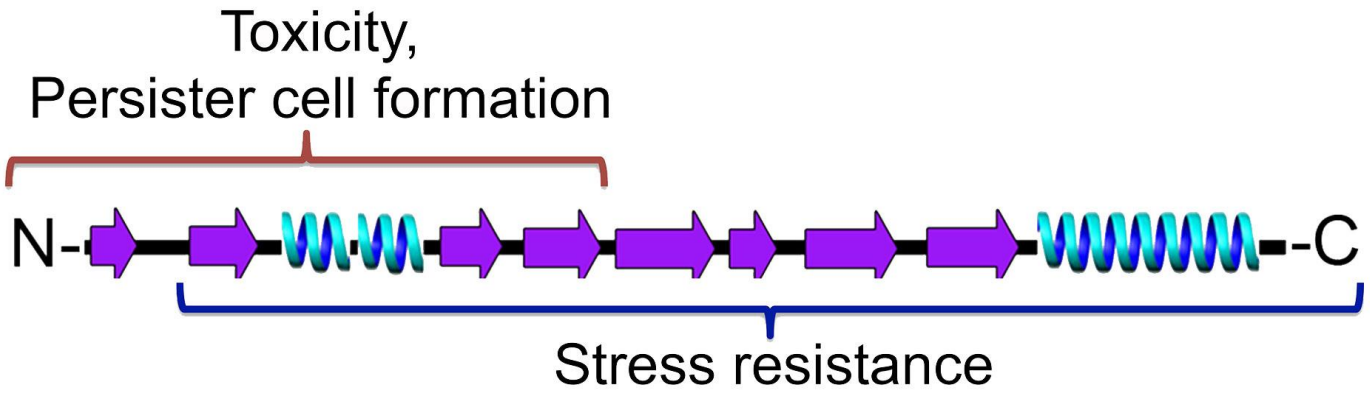


How does PasTI carry out distinct toxic and stress resistance functions?

Norton and Mulvey, PLoS Pathog. 2012

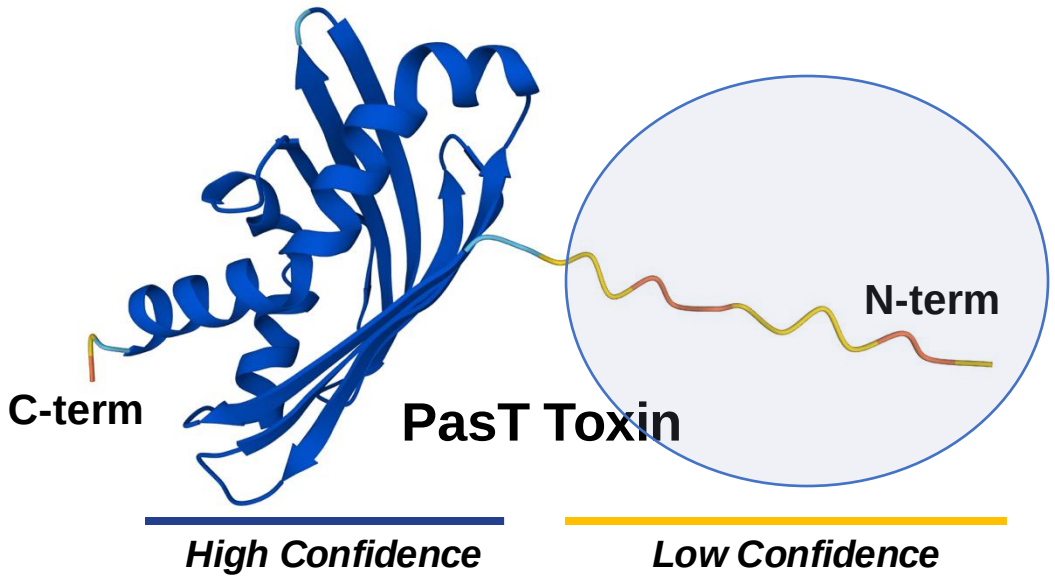
Dual functions mapped to PasT termini

- Stress resistance and toxic functions of toxin PasT mapped to termini



Dual functions mapped to PasT termini

- Stress resistance and toxic functions of toxin PasT mapped to termini
- Predicted: disordered N-term and START domain fold C-term



PasT N-term contains key residues

- Residues in the N-term of PasT vary among toxic and nontoxic versions of the protein

Bacterium	PasT Sequence	T	SR	P
ExPEC	1—G—E——158 (+6, +11)	++	+	+
N. meningitidis	-21——178	-	+	-
Y. pestis	+12——145	-	+	-
S. typhimurium	1—R—G——158 (+6, +11)	±	+	-

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ExPEC (G6R/E11G)	1—R—G——158 (+6, +11)	-	+	-
STm (R6G/G11E)	1—G—E——158 (+6, +11)	++	+	+

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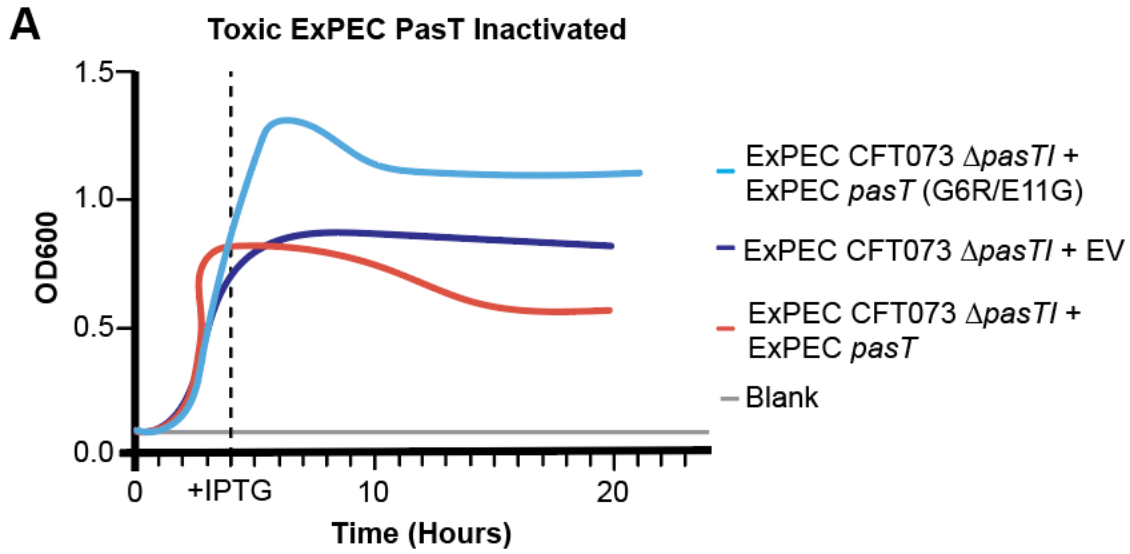
- Residues G6 and E11 can be mutated to inactivate toxic ExPEC PasT



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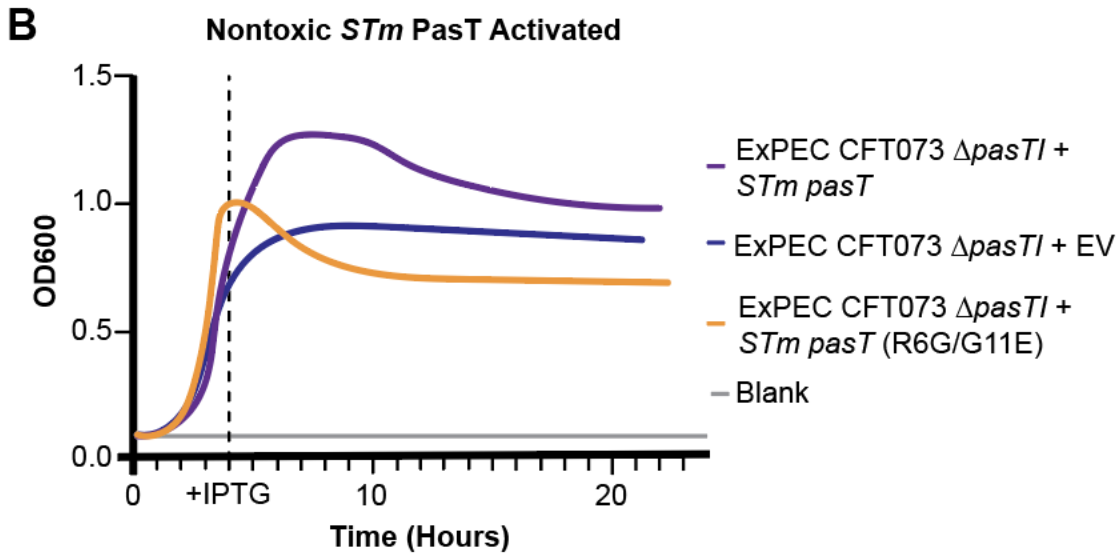

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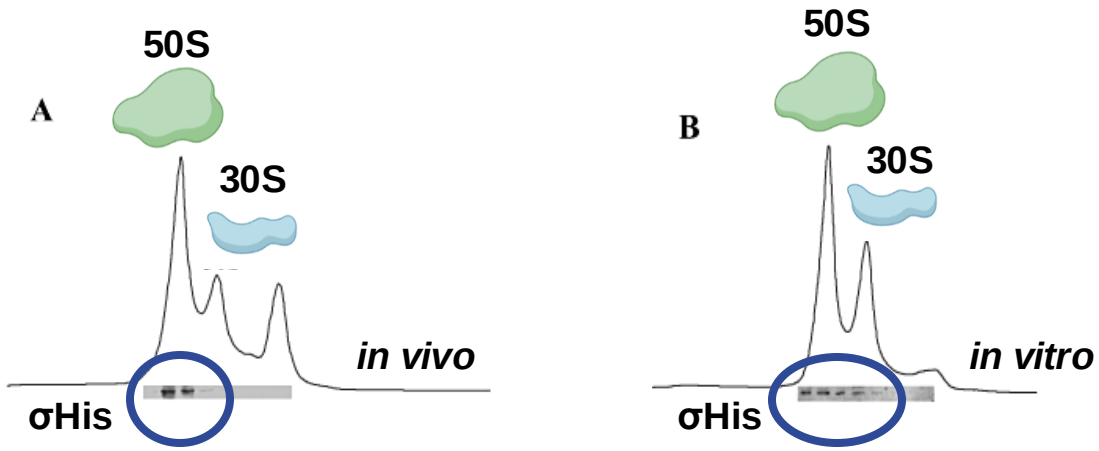

Figure adapted from Zhang et al., Mol. Microbiol. 2011

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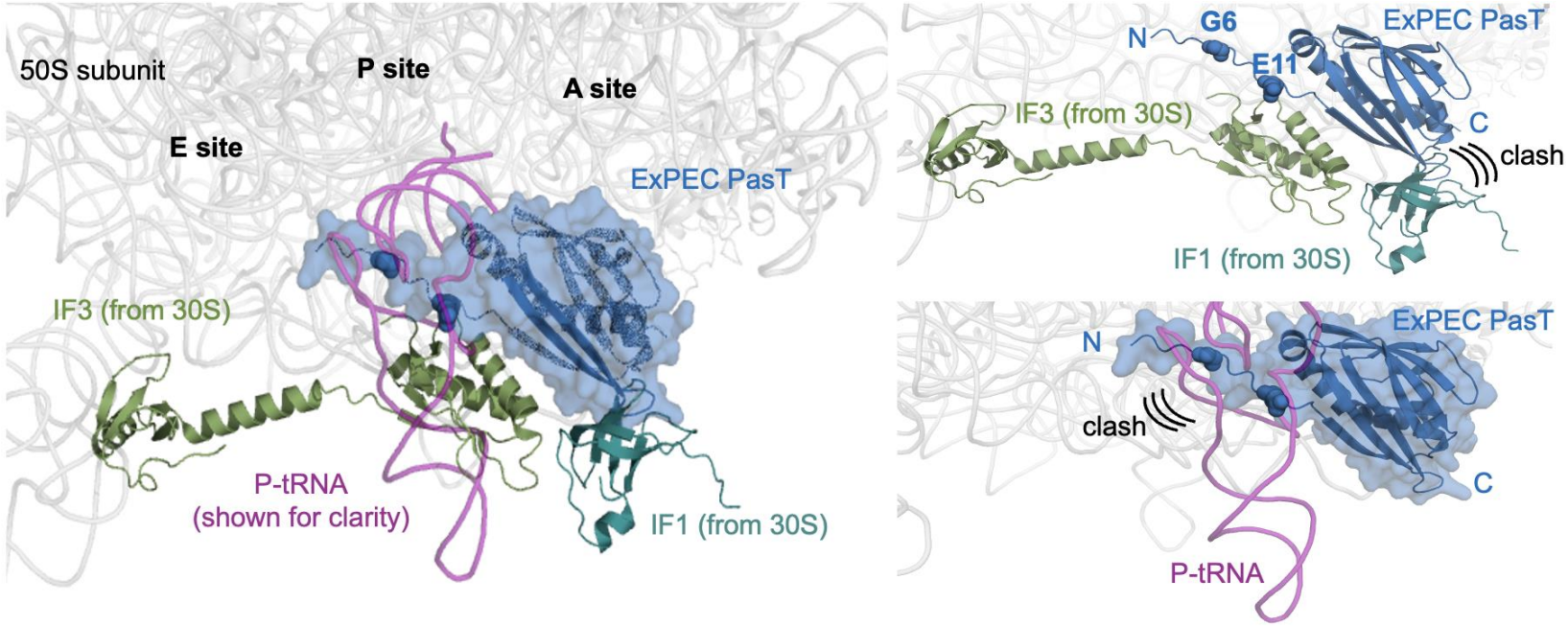
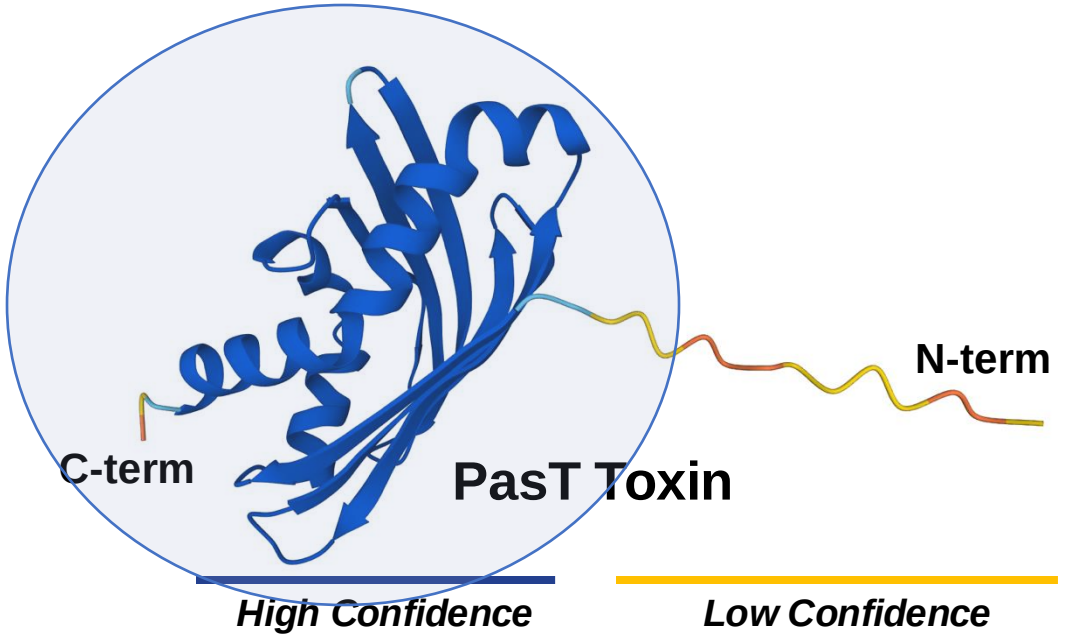
- N-term binding to ribosomal P-site predicted mechanism of toxicity



PasT C-term adopts START domain fold

- The C-term of PasT adopts a Steroidogenic Acute Regulatory Protein-related Lipid Transfer (START) fold



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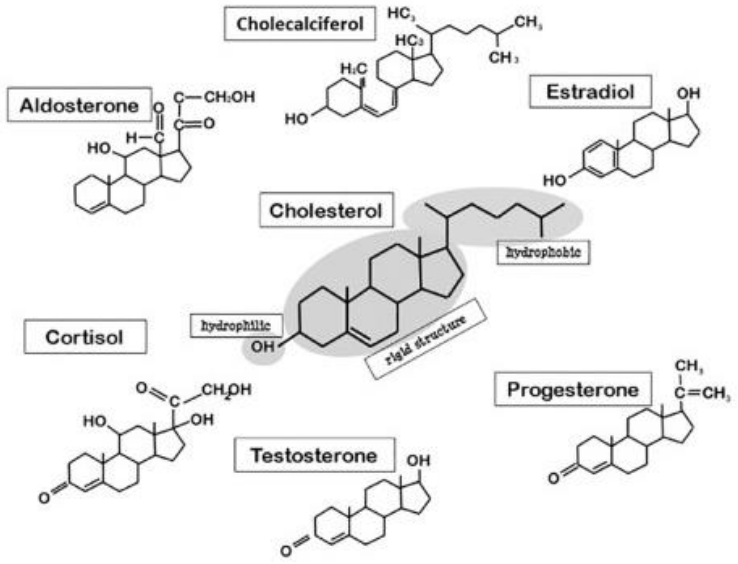
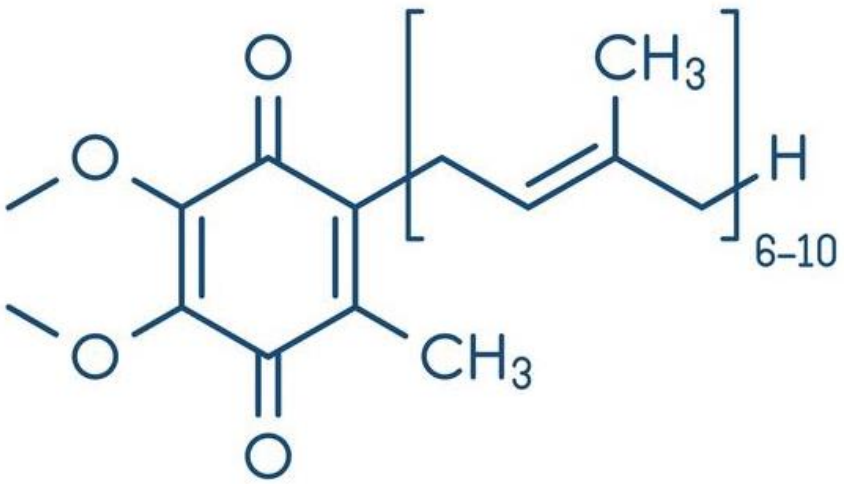
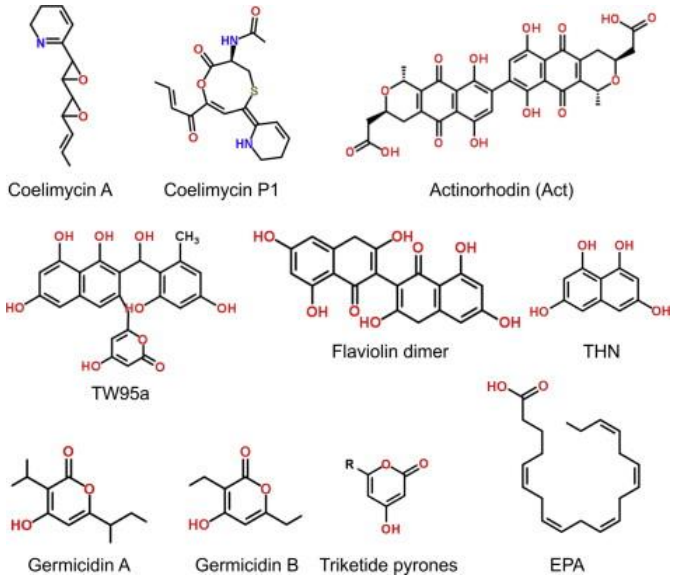
Cholesterol



Ubiquinone (CoQ_{10})

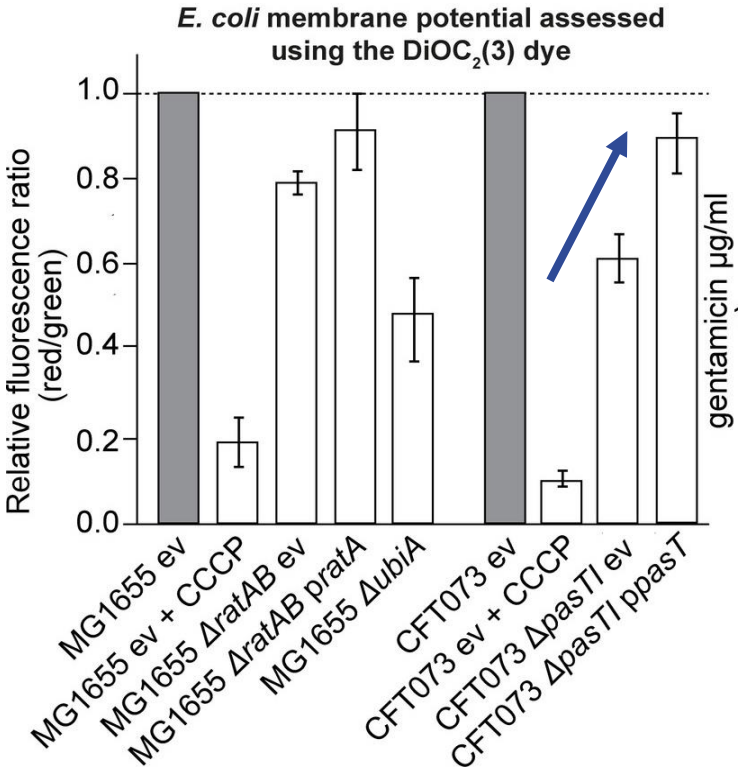


Polyketides



PasT C-term adopts START domain fold

- The C-term of PasT adopts a Steroidogenic Acute Regulatory Protein-related Lipid Transfer (START) fold
- Likely interacts with the cellular membrane (at least transiently)



Fino et al., Microbiology Open 2020

PasT exhibits dual functions

- PasT toxin of the PasTI system exhibits dual toxic and stress resistance functions
- PasT likely has multiple cellular targets
- The PasTI system supports persister cell development of UPEC

Future Questions:

What is the cellular target of the C-term START domain?

How does PasI antitoxin stop the toxic function of PasT?

What selective pressures drove the emergence of a toxic N-term in ExPEC PasT?

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Questions?