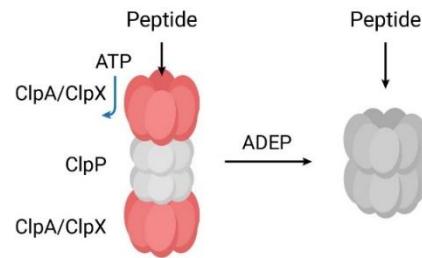
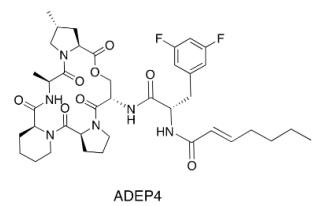


# Discovering New Antibiotics from Unlikely Sources

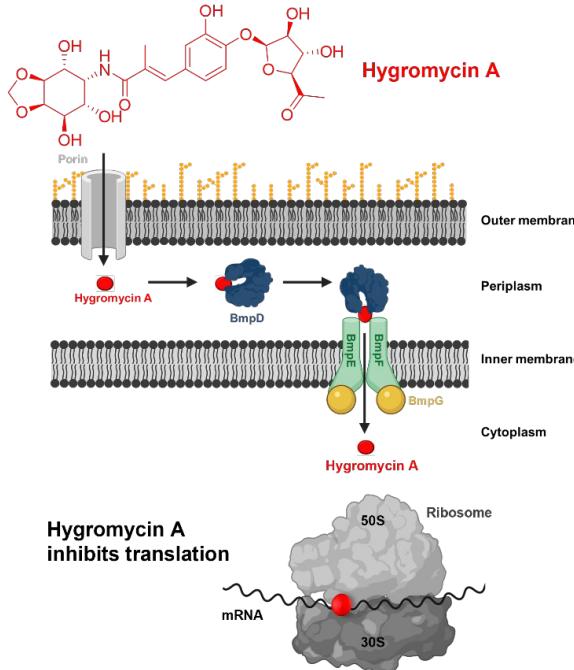
**Kim Lewis**



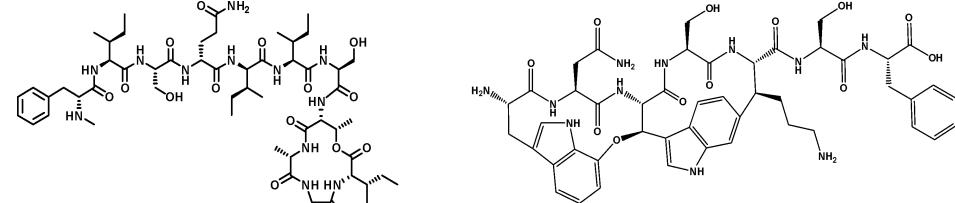
Northeastern University  
*Antimicrobial Discovery Center*



Conlon et al., Nature 2013

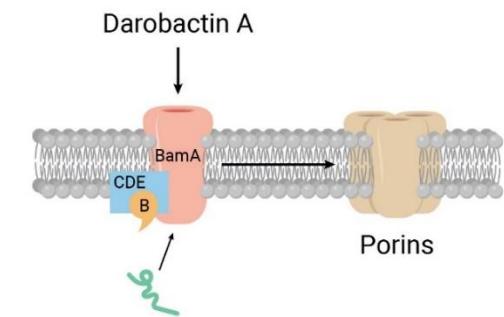


Leimer et al., CELL 2021



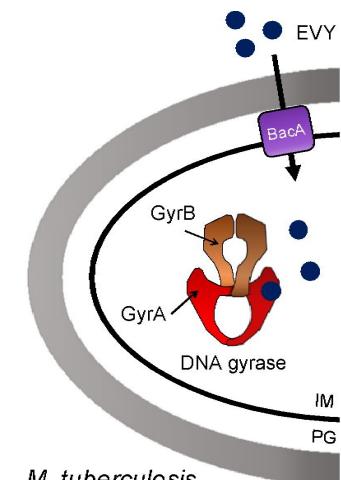
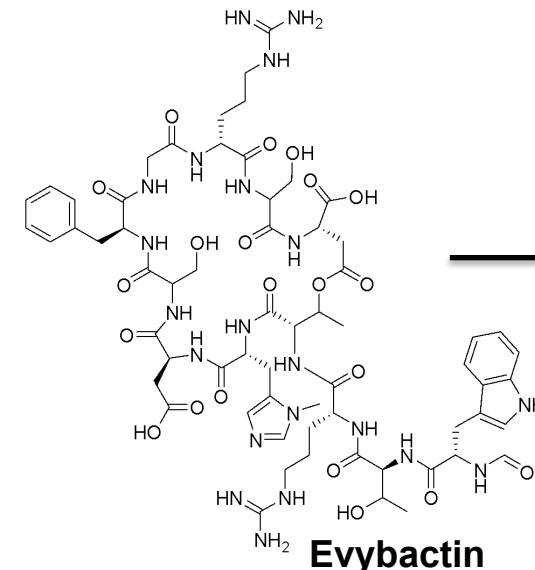
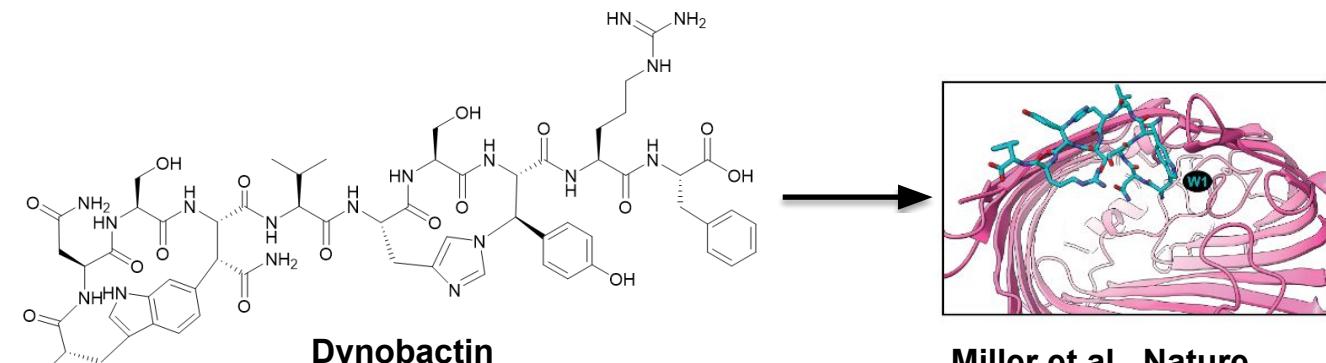
Ling et al., Nature 2015

Shukla et al., Nature 2022



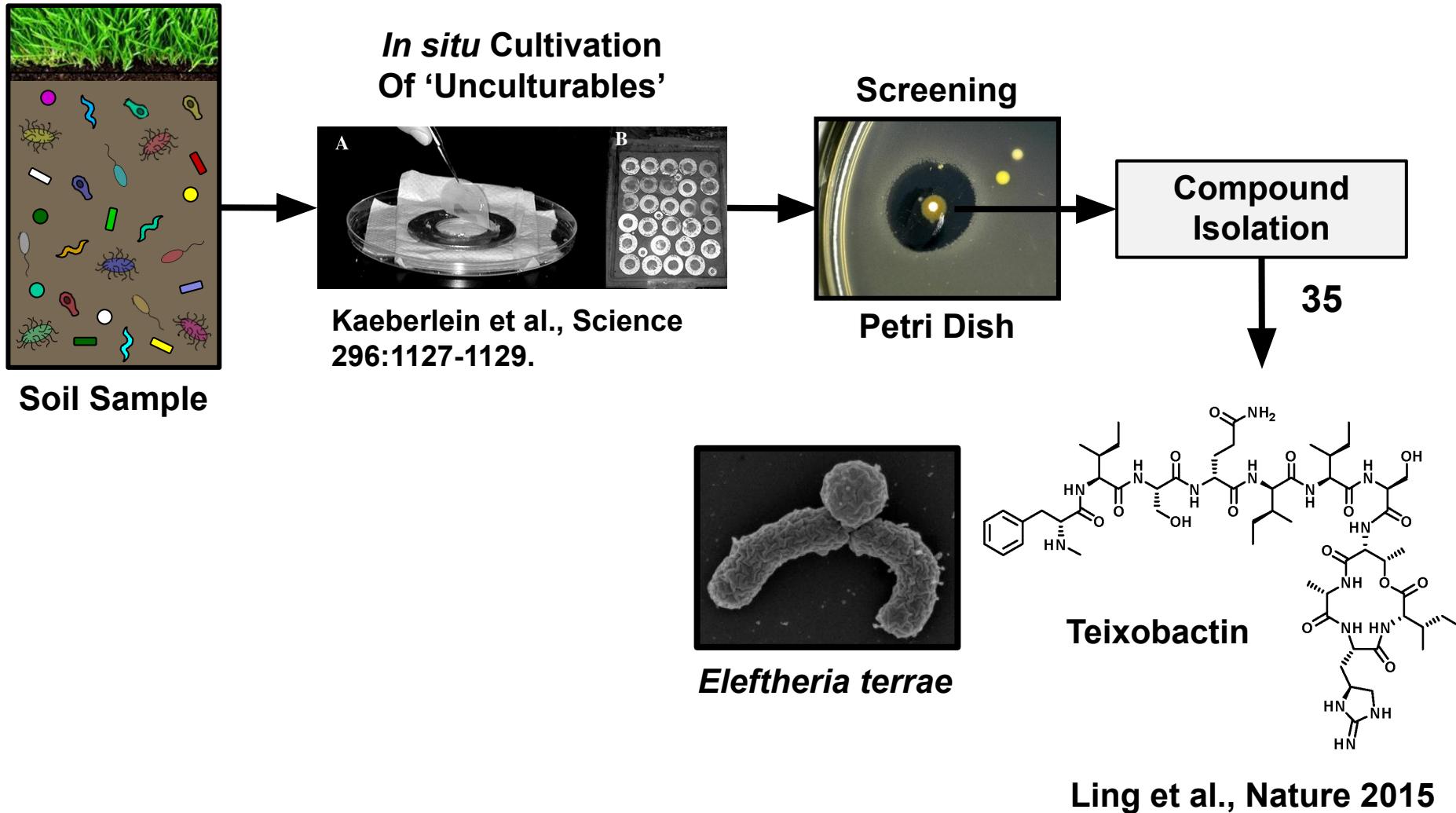
Imai et al., Nature 2019

Kaur et al., Nature 2021



Imai et al., Nature Chem. Biol. 2022

# Tapping into Uncultured Bacteria



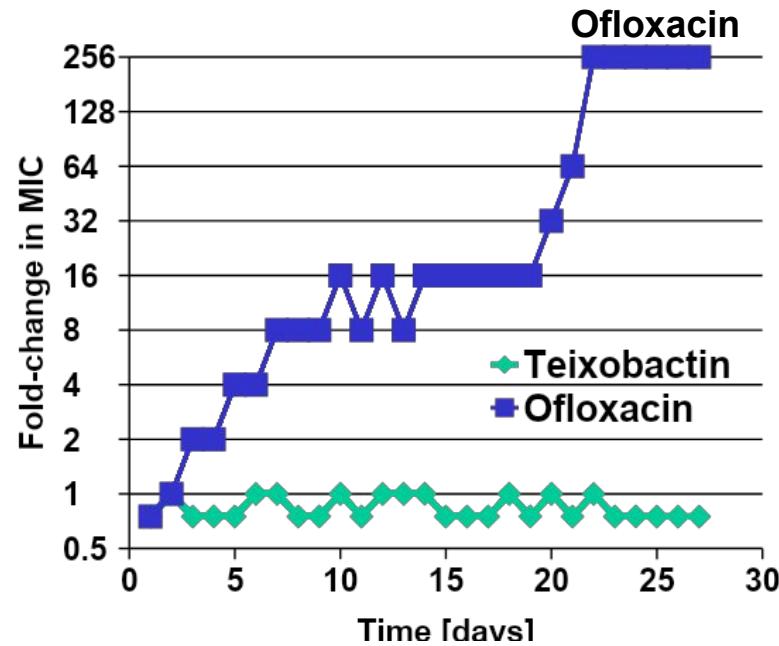
# Teixobactin Spectrum

(MIC,  $\mu\text{g ml}^{-1}$ )

Organism	Teixobactin
<i>S. aureus</i> (MSSA)	0.25
<i>S. aureus</i> +10% serum	0.25
<i>S. aureus</i> (MRSA)	0.25
<i>Enterococcus faecalis</i> (VRE)	0.5
<i>Enterococcus faecium</i> (VRE)	0.5
<i>Streptococcus pneumonia</i>	$\leq 0.03$
<i>Streptococcus pyogenes</i>	0.06
<i>Streptococcus agalactiae</i>	0.12
Viridans Group Streptococci	0.12
<i>B. anthracis</i>	$\leq 0.06$
<i>Clostridium difficile</i>	0.005
<i>Propionibacterium acnes</i>	0.08
<i>M. tuberculosis</i> H37Rv	0.125
<i>Haemophilus influenzae</i>	4
<i>Moraxella catarrhalis</i>	2
<i>Escherichia coli</i>	25
<i>Escherichia coli</i> (asmB1)	2.5
<i>Pseudomonas aeruginosa</i>	>32
<i>Kebsiella pneumoniae</i>	>32

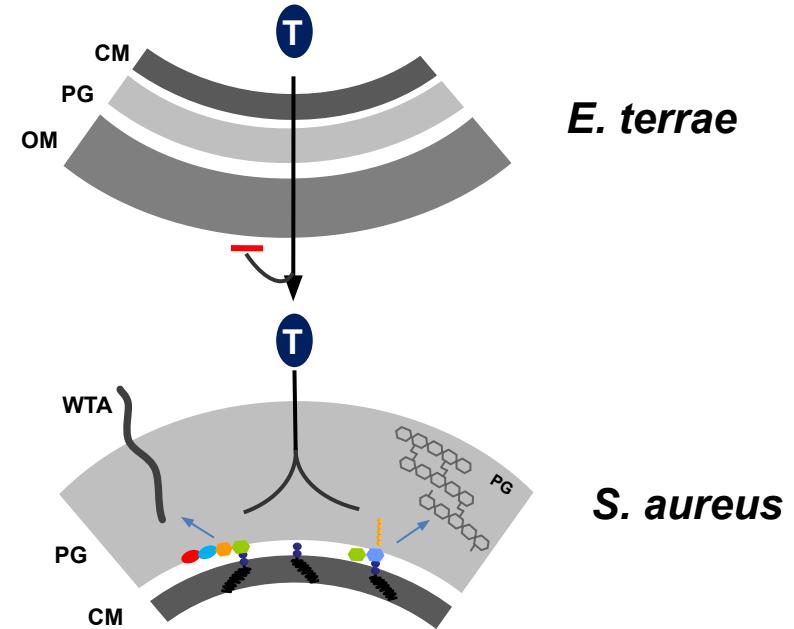
IC50 >100  $\mu\text{g/ml}$

# Antibiotic With No Detectable Resistance



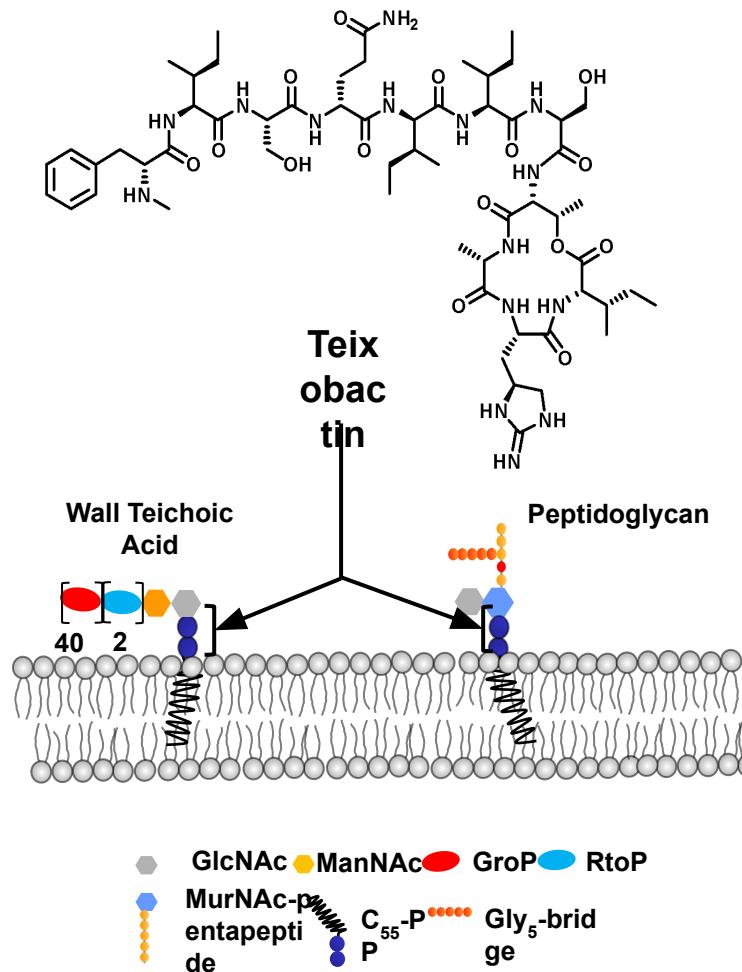
Antagonist	S. aureus growth		
	Lipid II	UDP-MurNAc-pentapeptide	UDP-GlcNAc
Teixobactin	+	-	-
Vancomycin	+	nd	nd

(+) antibiotic activity antagonized, (-) antibiotic activity unaffected, (nd) not determined

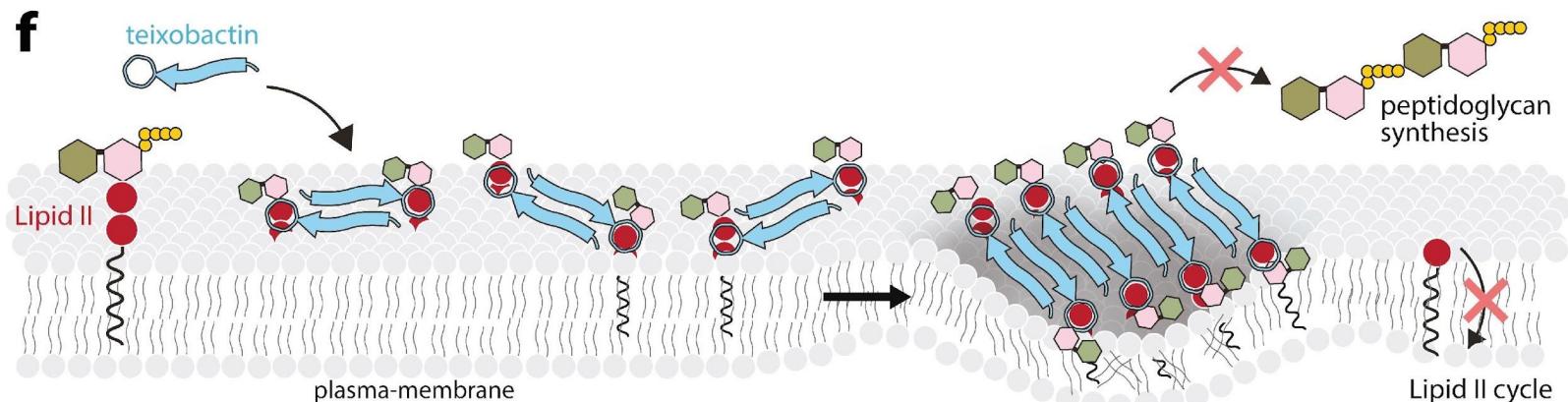
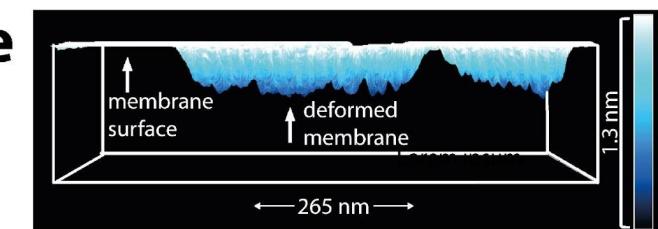
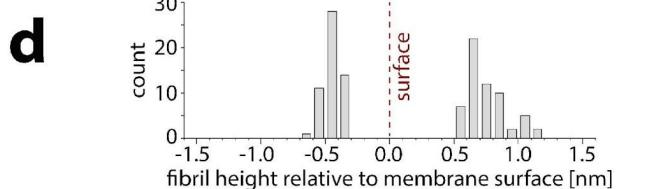
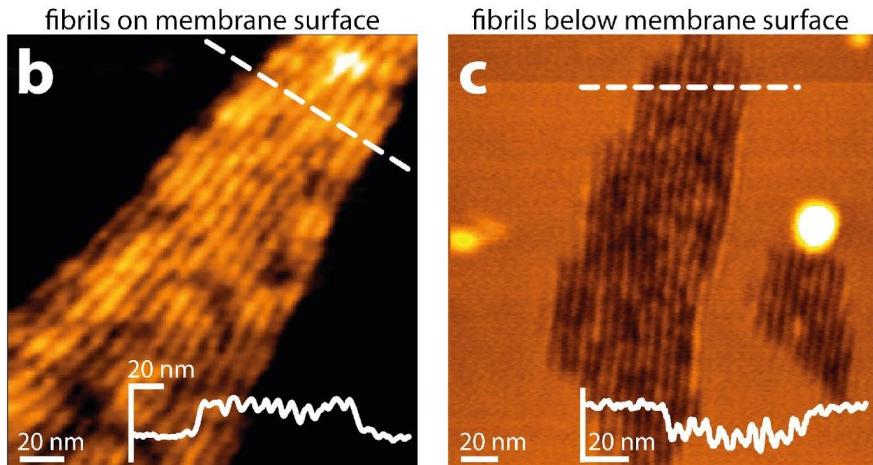
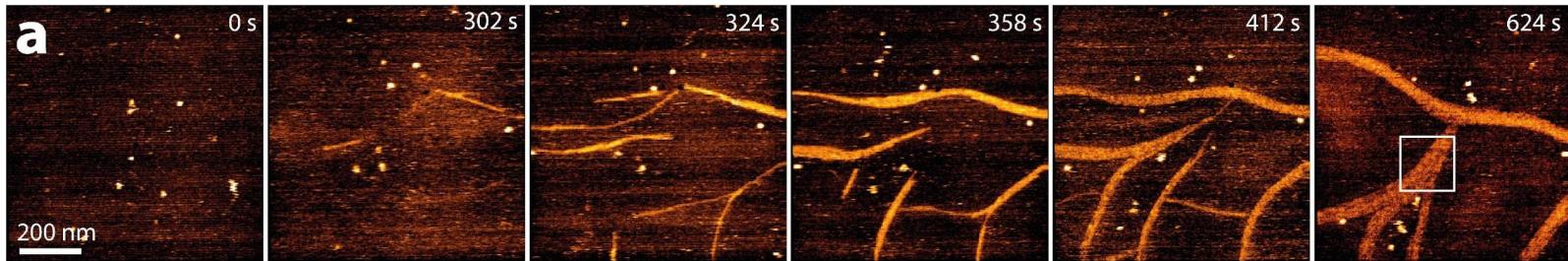


Ling, Schneider et al., Nature 2015

# Antibiotic With No Detectable Resistance



# Antibiotic With No Detectable Resistance

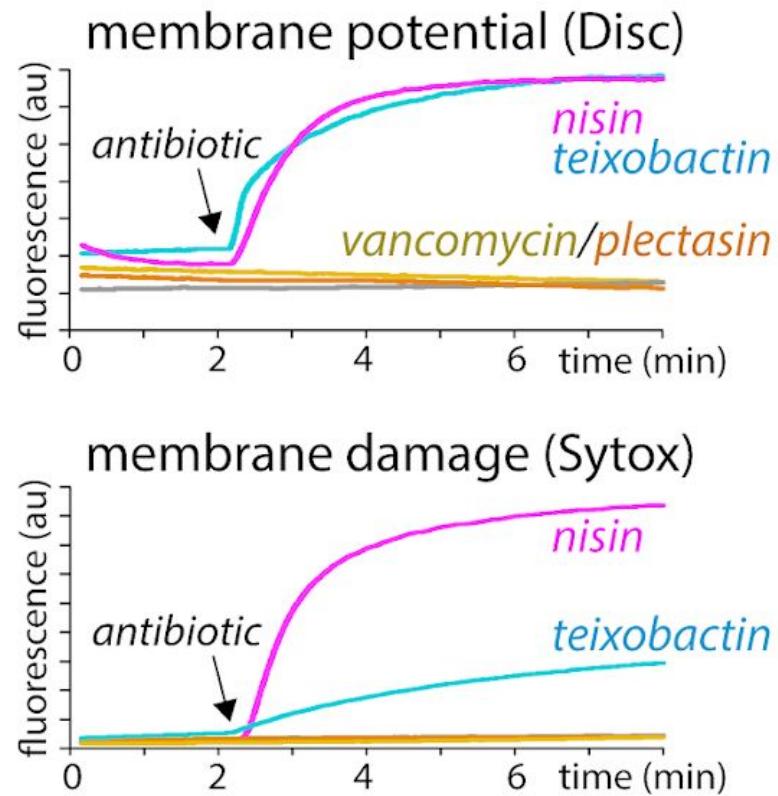


Rhem Shukla

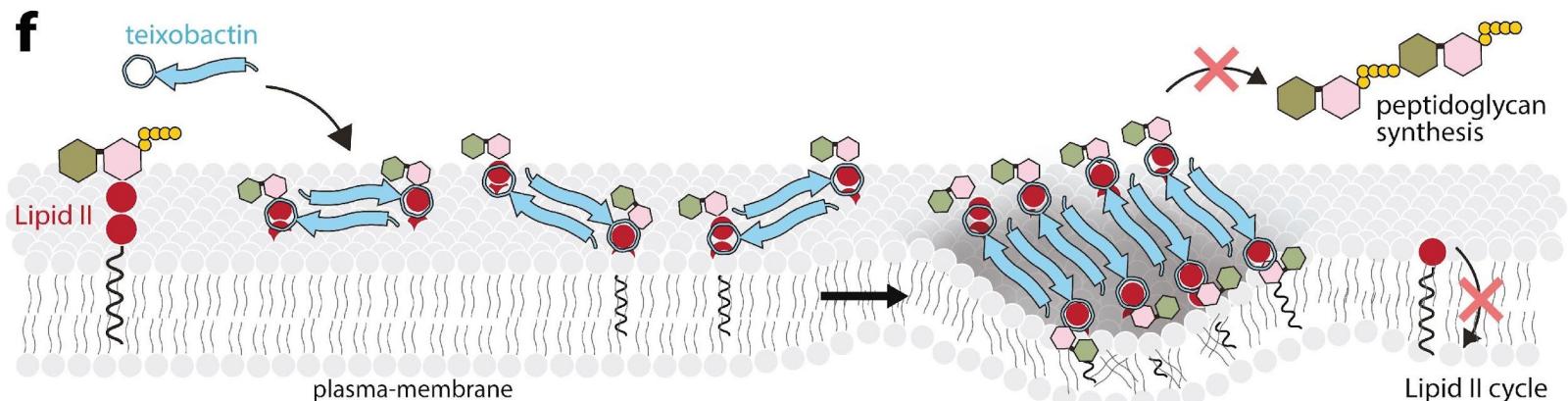
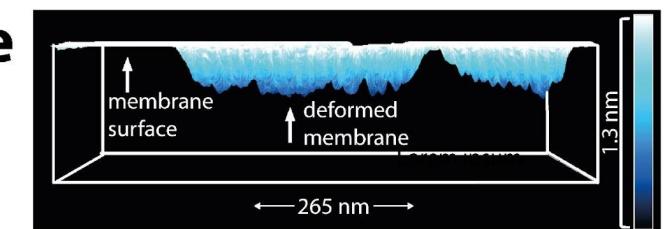
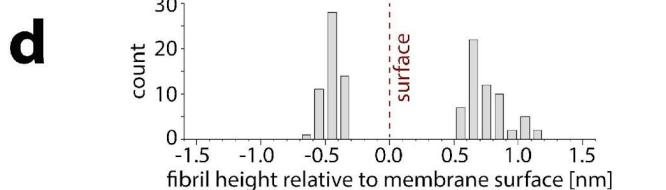
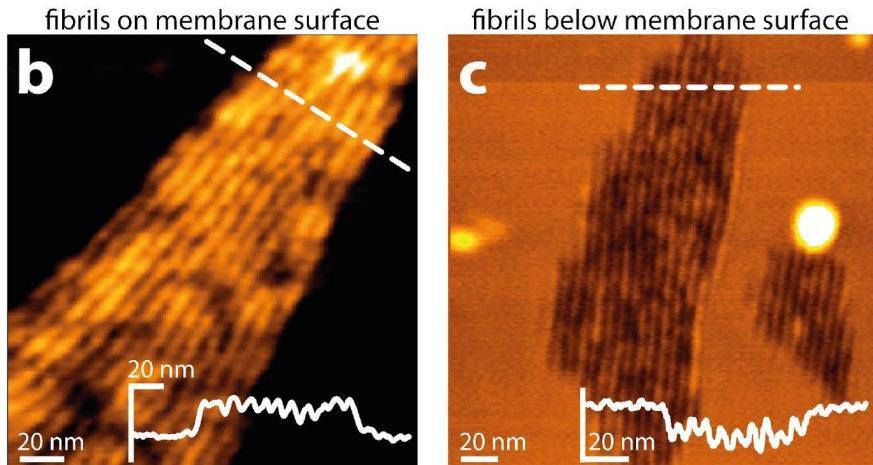
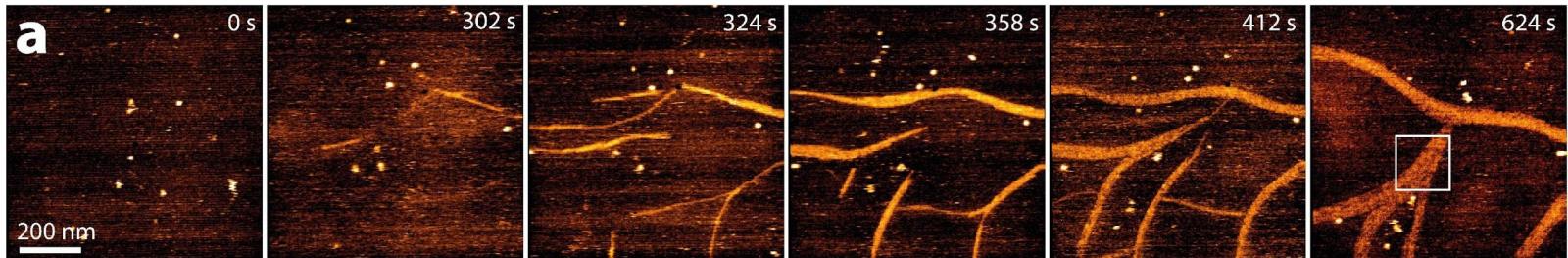


Markus Weingarth

# Antibiotic With No Detectable Resistance



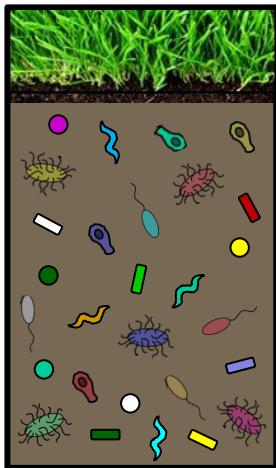
# Antibiotic With No Detectable Resistance



Rhem Shukla

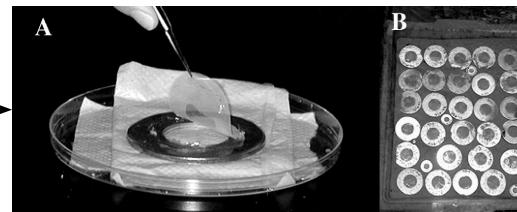


Markus Weingarth



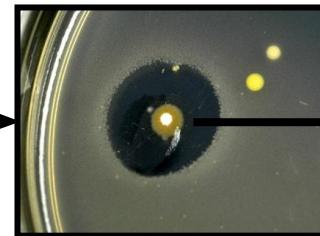
Soil Sample

*In situ* Cultivation  
Of 'Unculturables'



Kaeberlein et al., Science  
296:1127-1129.

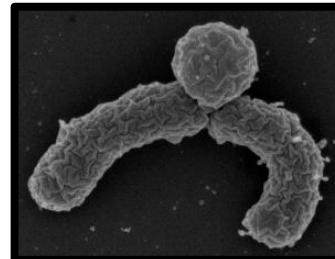
Screening



Petri Dish

Compound  
Isolation

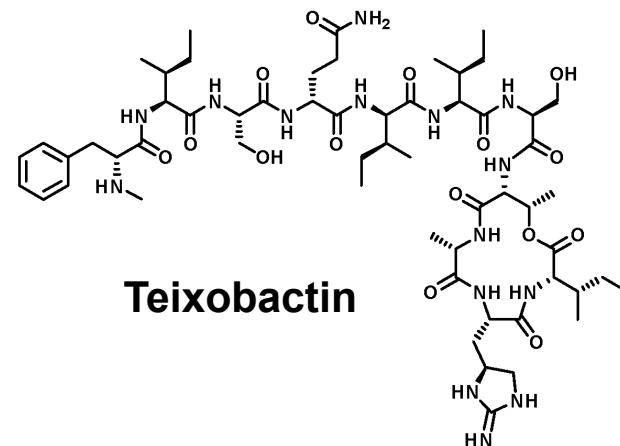
35



*Eleftheria terrae*

Problem: 10 years, 2 Gram (+) Leads

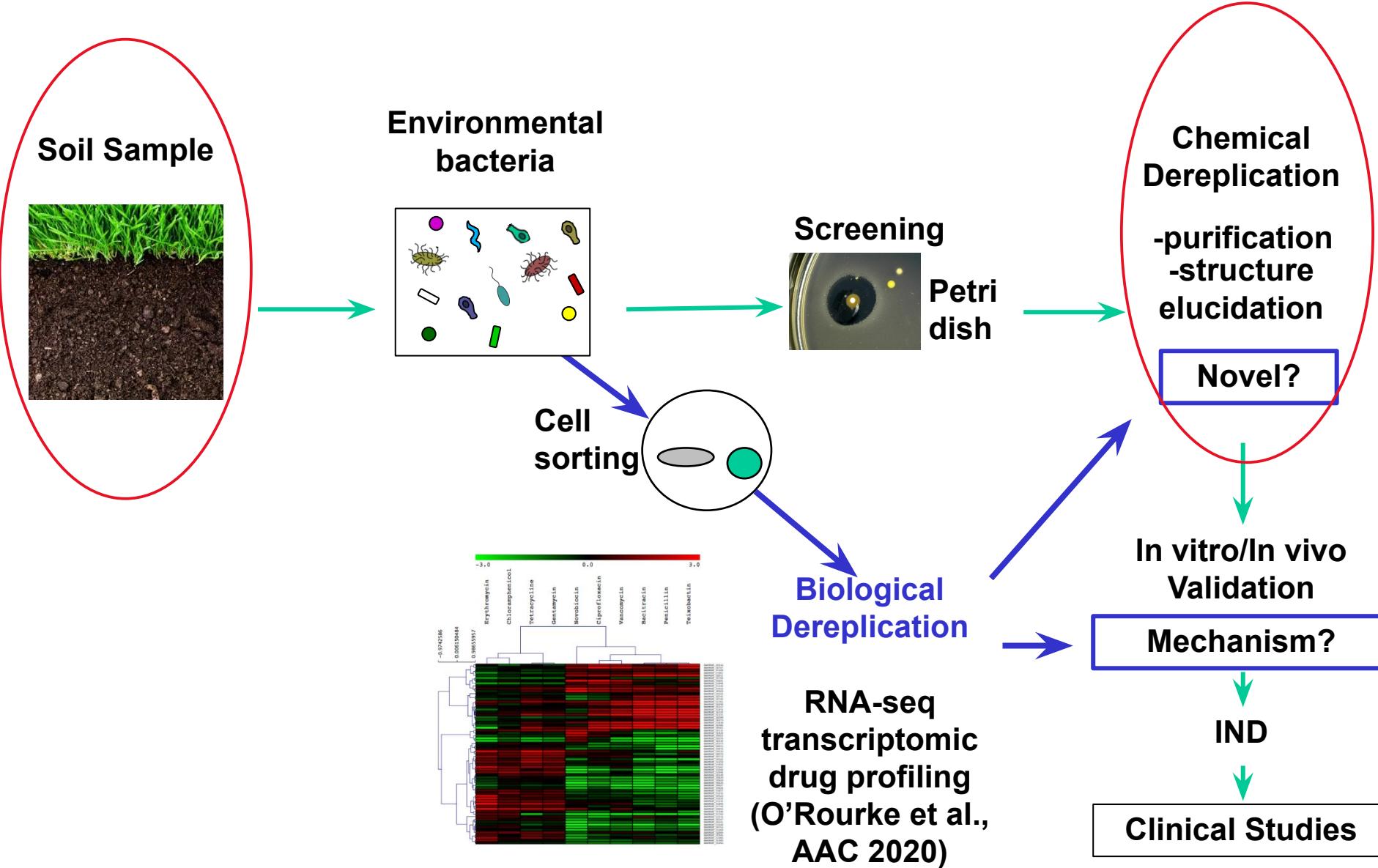
Hit rate: G-/G+  $\leq 1/10$



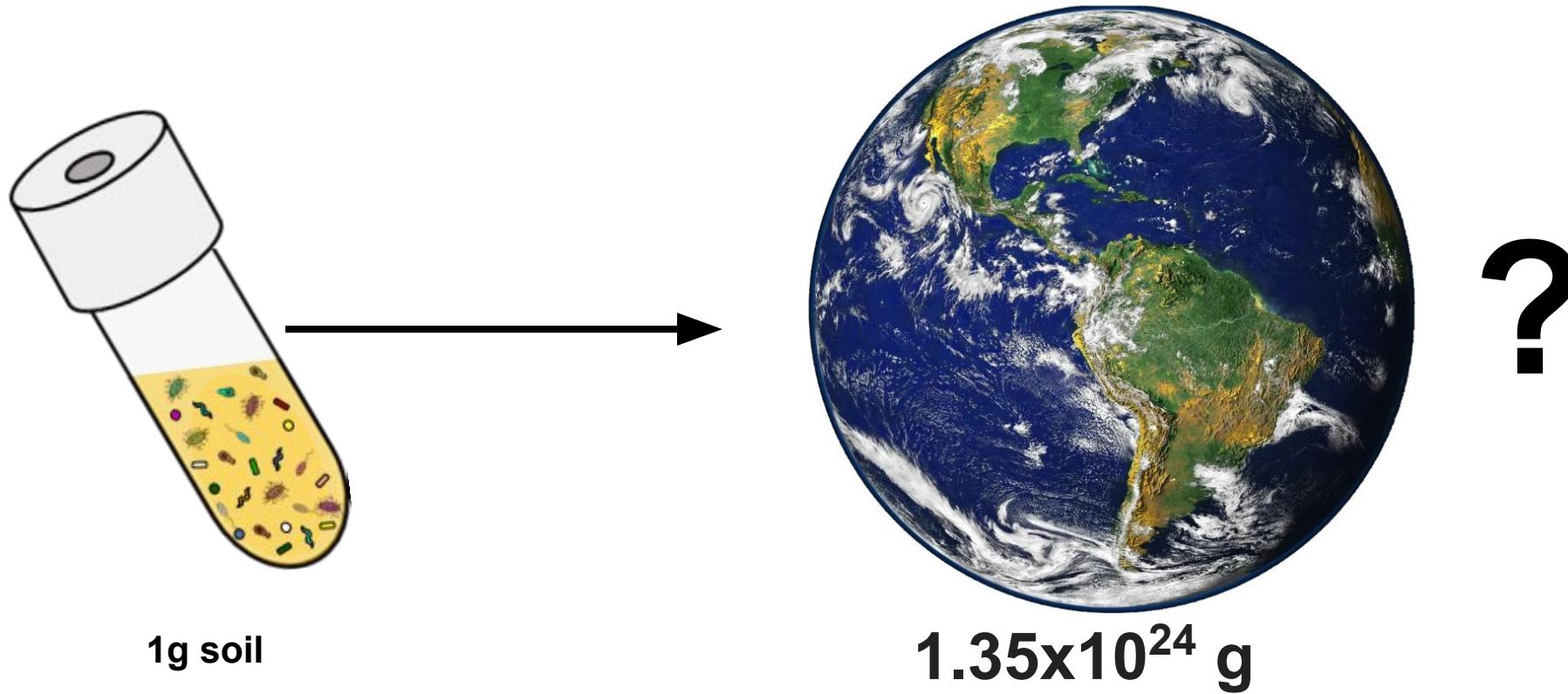
Teixobactin

Ling et al., Nature 2015

# Drug Discovery Pipeline

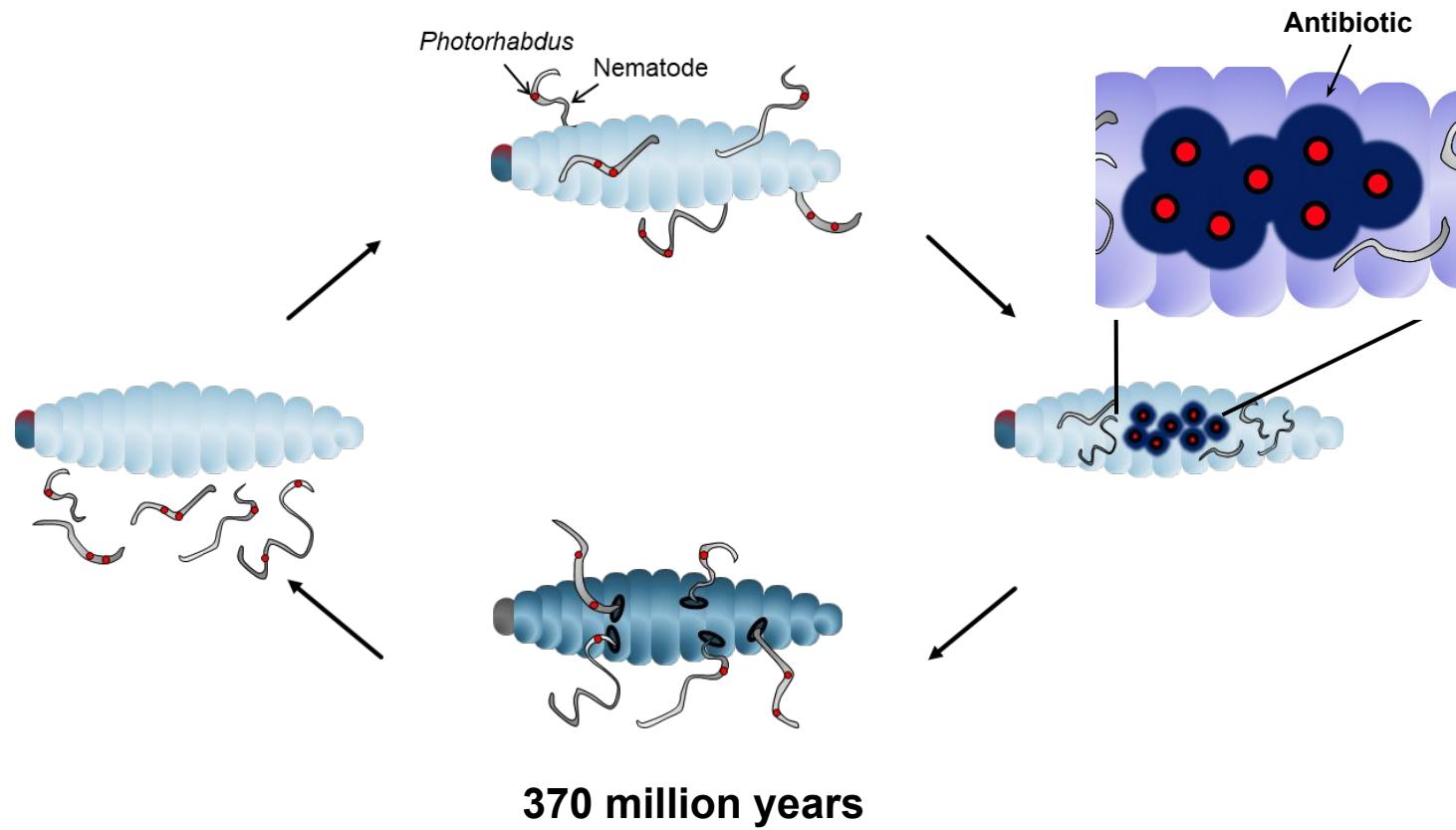


# A Problem Of Scale

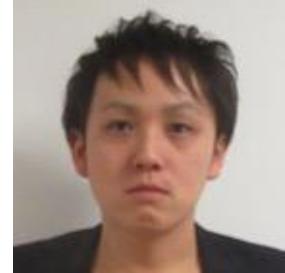


# Screen of screens: Targeting producers with similar requirements for antibiotics to humans

- Active against Gram negative pathogens
- Low toxicity
- Good PK
- in vivo efficacy

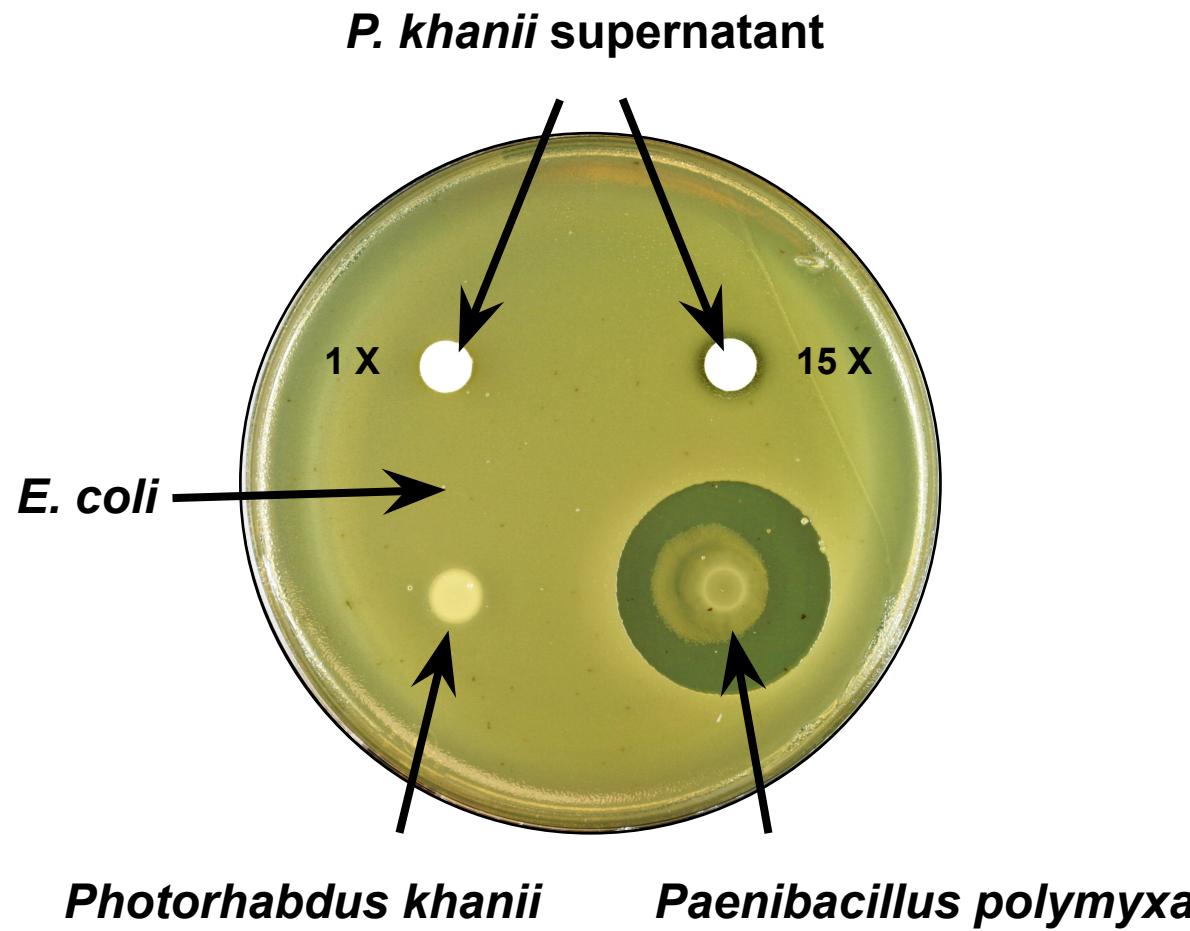


# Screen of screens: Targeting collectors of antibiotics

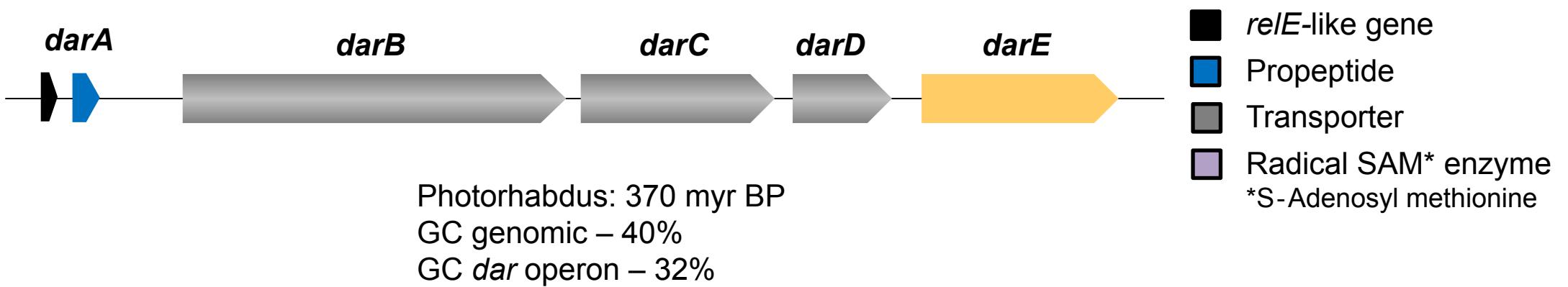


Yu Imai

Pilot screen, 27 species



# Darobactin



# Spectrum of Darobactin

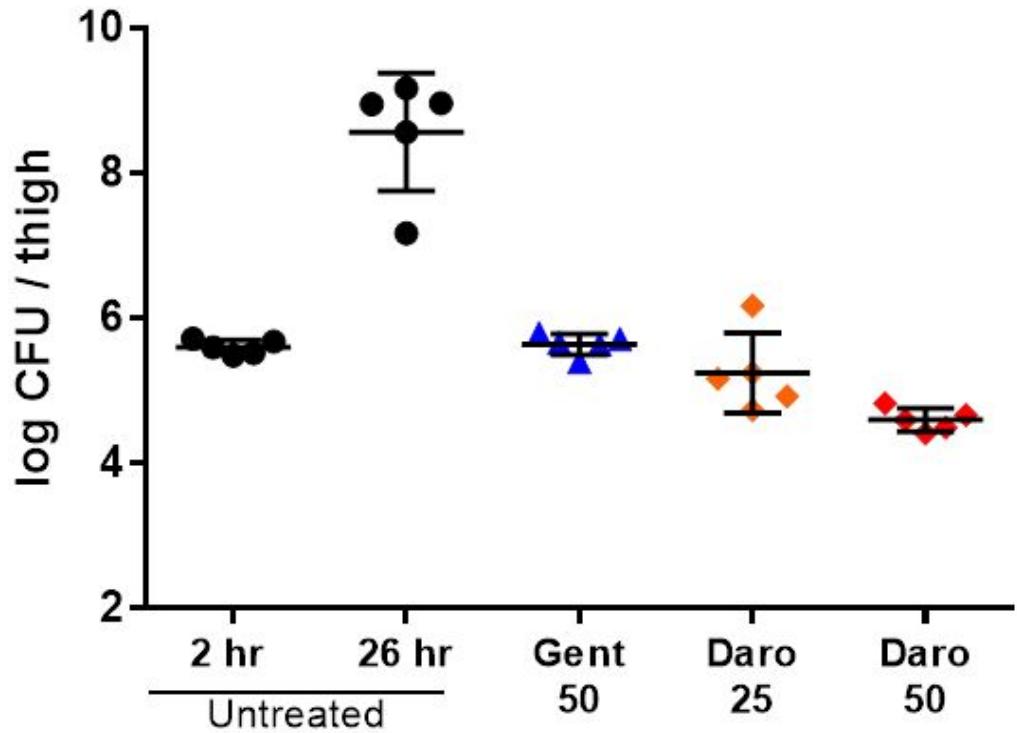
Organism and genotype	Concentration ( $\mu\text{g ml}^{-1}$ )		Organism and genotype	Concentration ( $\mu\text{g ml}^{-1}$ )	
	Darobactin	Ampicillin		Darobactin	Ampicillin
<b>Pathogenic bacteria (MIC)</b>					
<i>Pseudomonas aeruginosa</i> PAO1	2	>128	<i>Staphylococcus aureus</i> HG003	>128	0.5
<i>Pseudomonas aeruginosa pmrB</i> 523C>T	2	>128	<i>Clostridium bifermentans</i> KLE 2329 <sup>a,b</sup>	>128	1
<i>Pseudomonas aeruginosa</i> JMI 1045324	16	ND	<i>Mycobacterium tuberculosis</i> mc26020	>128	16
<i>Shigella sonnei</i> ATCC 25931 <sup>a</sup>	2	4	<i>Bifidobacterium longum</i> ATCC BAA-999 <sup>a</sup>	>128	0.25
<i>Klebsiella pneumoniae</i> ATCC 700603	2	128	<i>Bacteroides fragilis</i> ATCC 25285 <sup>a</sup>	>128	128
<i>Klebsiella pneumoniae</i> ESBL JMI 1052654	2	>128	<i>Bacteroides xylanisolves</i> KLE 2253 <sup>a,b</sup>	>128	1
<i>Escherichia coli</i> ATCC 25922	2	8	<i>Bacteroides vulgatus</i> KLE 2303 <sup>a,b</sup>	>128	2
<i>Escherichia coli</i> AR350 ( <i>mcr-1</i> )	2	>128	<i>Bacteroides nordii</i> KLE 2369 <sup>a,b</sup>	>128	4
<i>Escherichia coli</i> ESBL JMI 1043856	2	>128	<i>Lactobacillus reuteri</i> ATCC 23272 <sup>a</sup>	>128	1
<i>Escherichia coli</i> ATCC BAA-2340 (KPC)	2	>128	<i>Enterococcus faecalis</i> KLE 2341 <sup>a,b</sup>	>128	4
<i>Escherichia coli</i> MG1655 +10% serum	2	4	<i>Faecalibacterium prausnitzii</i> KLE 2243 <sup>a,b</sup>	>128	64
<i>Salmonella</i> Typhimurium LT2 ATCC 19585 <sup>a</sup>	4	2	<i>Stenotrophomonas maltophilia</i> KLE 11416 <sup>a,b</sup>	>128	>128
<i>Moraxella catarrhalis</i> ATCC 25238 <sup>a</sup>	8	<0.25	<b>Human cell line (IC<sub>50</sub>)</b>		
<i>Acinetobacter baumannii</i> ATCC 17978	8	64	HepG2	>128	>128
<i>Enterobacter cloacae</i> ATCC 13047 <sup>a</sup>	32	>128	FaDu	>128	>128
<i>Proteus mirabilis</i> KLE 2600 <sup>a,b</sup>	64	>128	HEK293	>128	>128

ND, no data. ESBL, extended spectrum  $\beta$ -lactamase. <sup>a</sup>Cultivated under anaerobic conditions. <sup>b</sup>Human stool isolate, K.L. laboratory collection.

# Mouse Thigh Infection



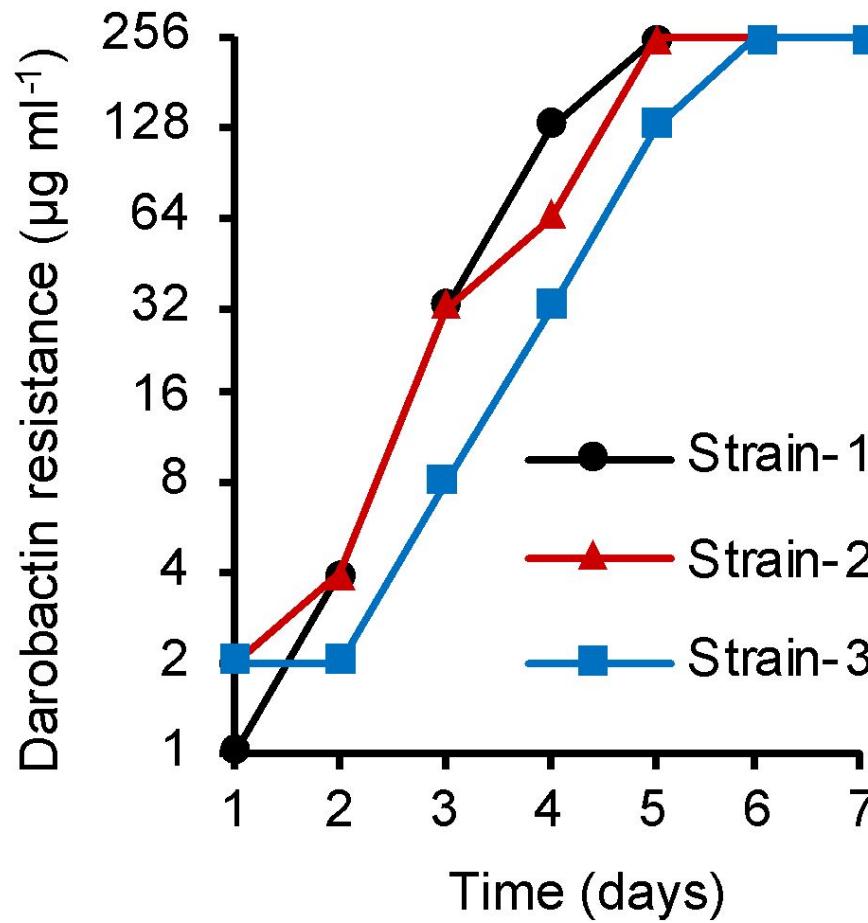
Kirsten  
Meyer



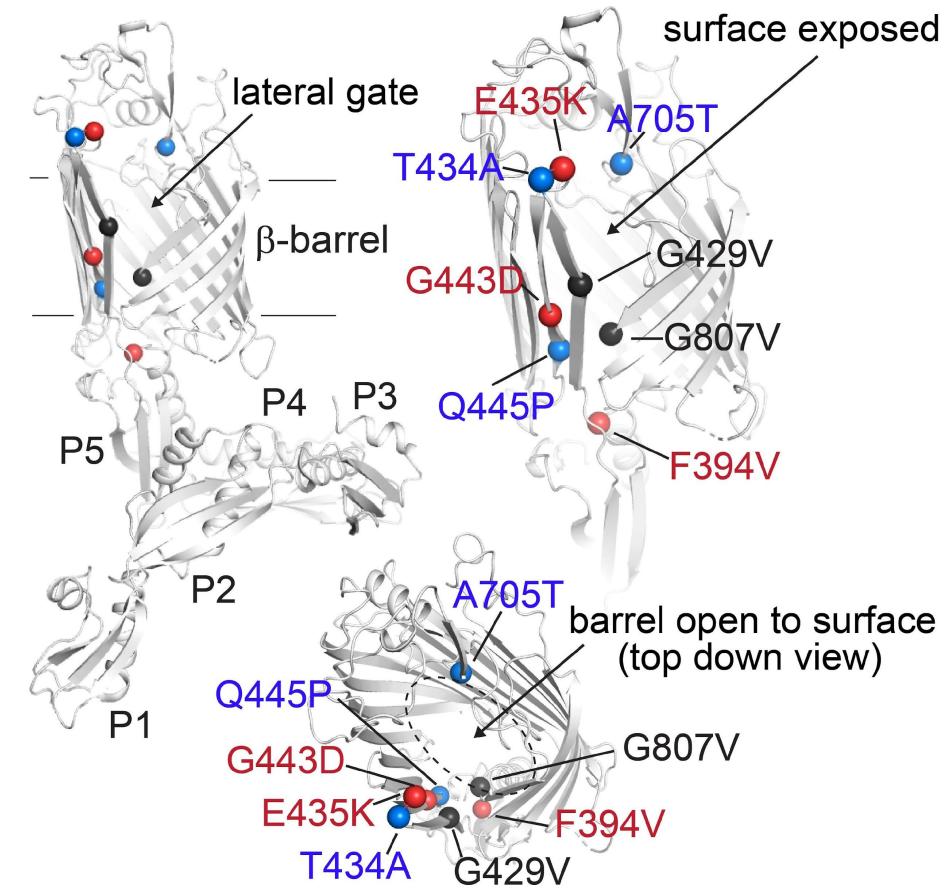
*E. coli* AR350, mcr-1 polymyxin resistant, neutropenic thigh model

# Darobactin Targets BamA

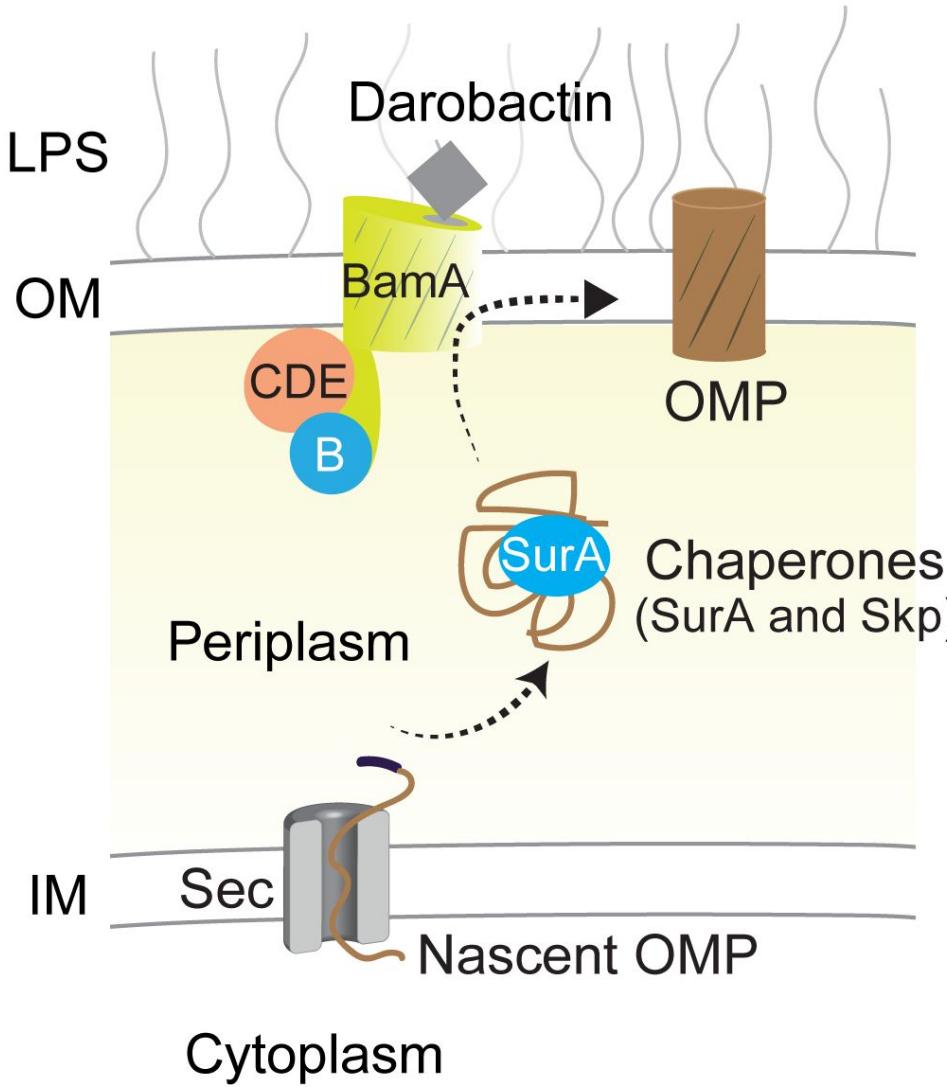
a

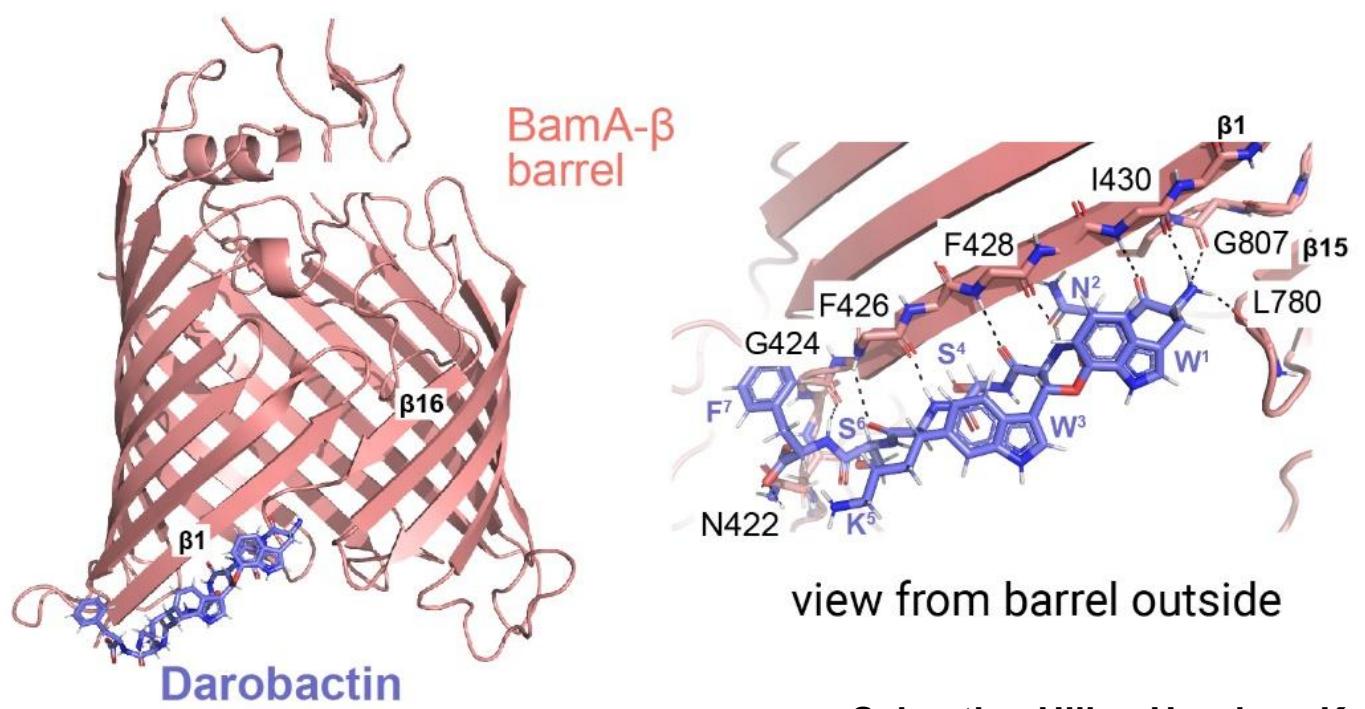


b



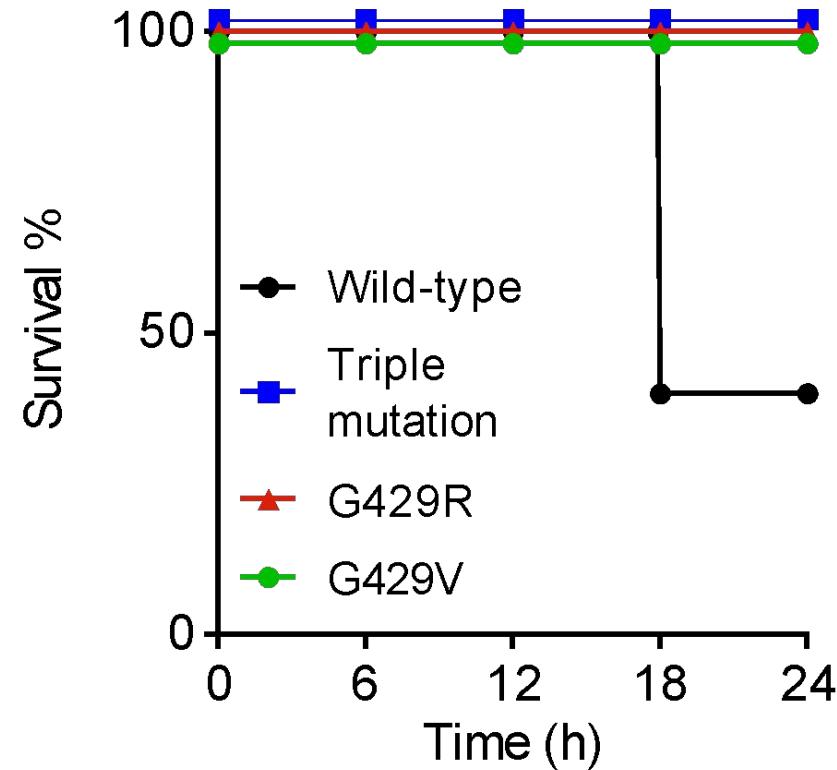
# BAM complex



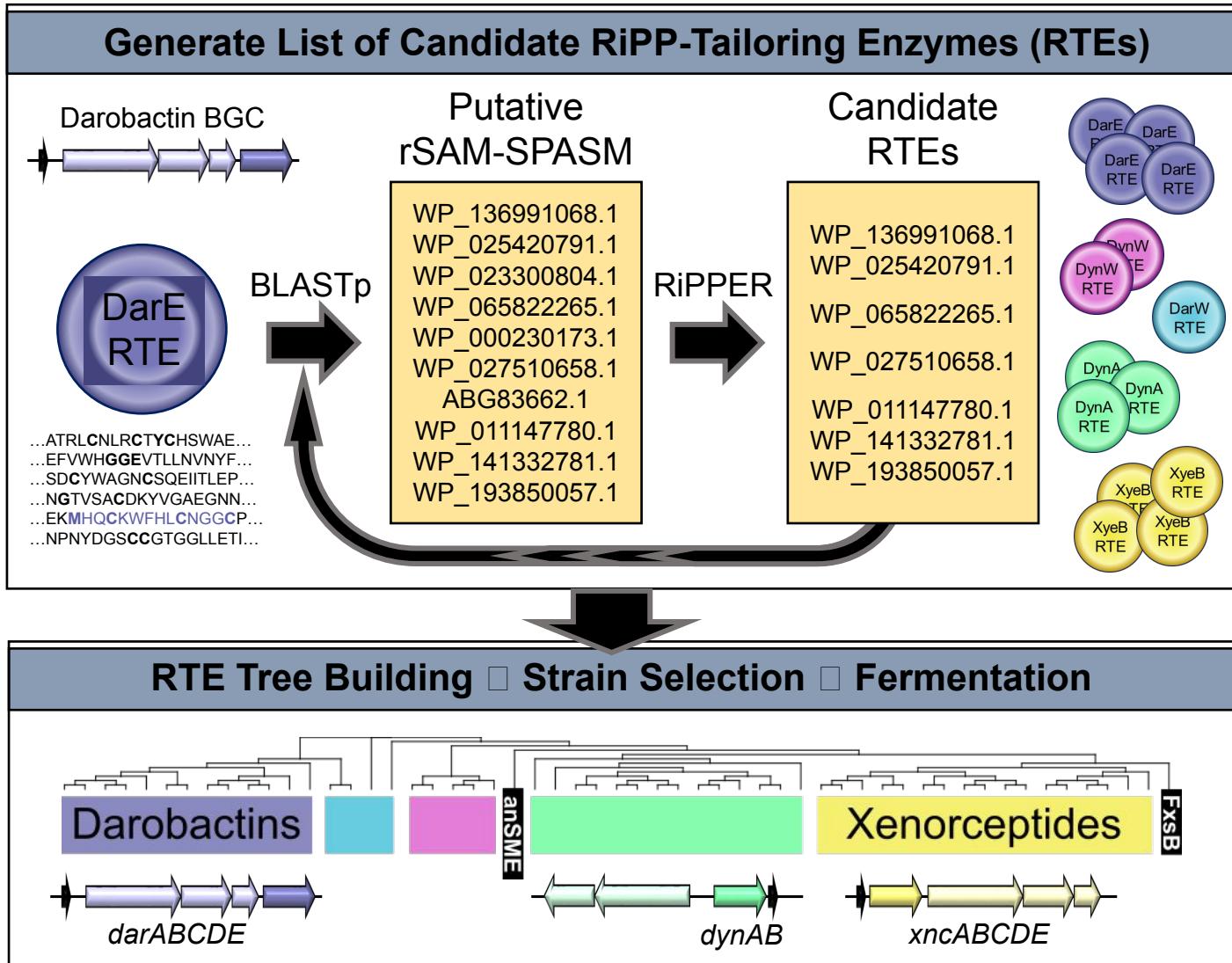


Sebastian Hiller, Hundeep Kaur et al., Nature 2021

# *bamA* Mutants of *E. coli* Lose Virulence

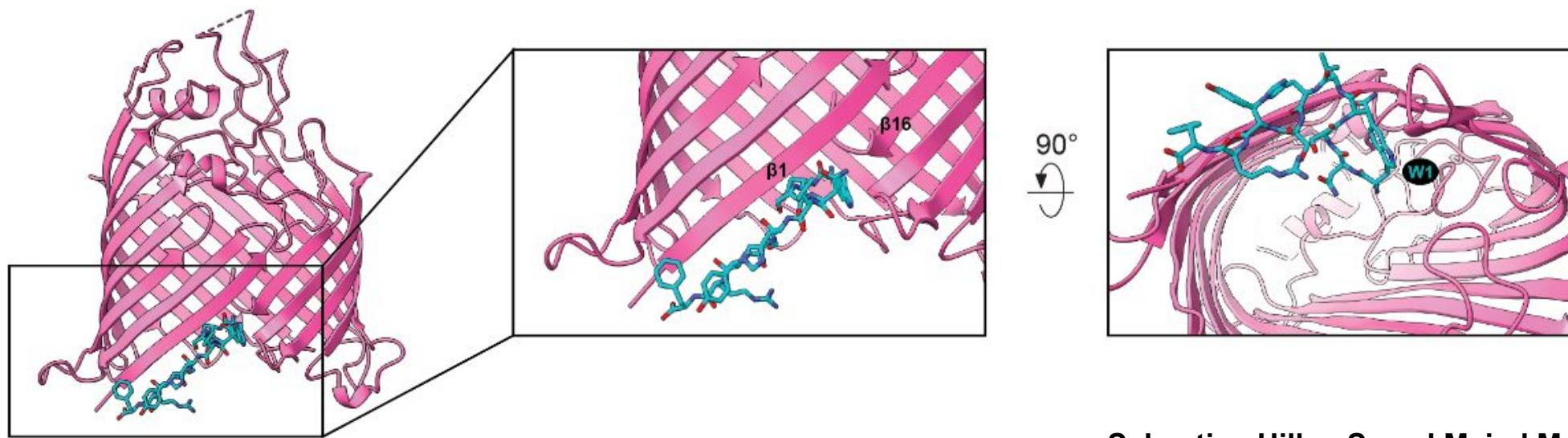
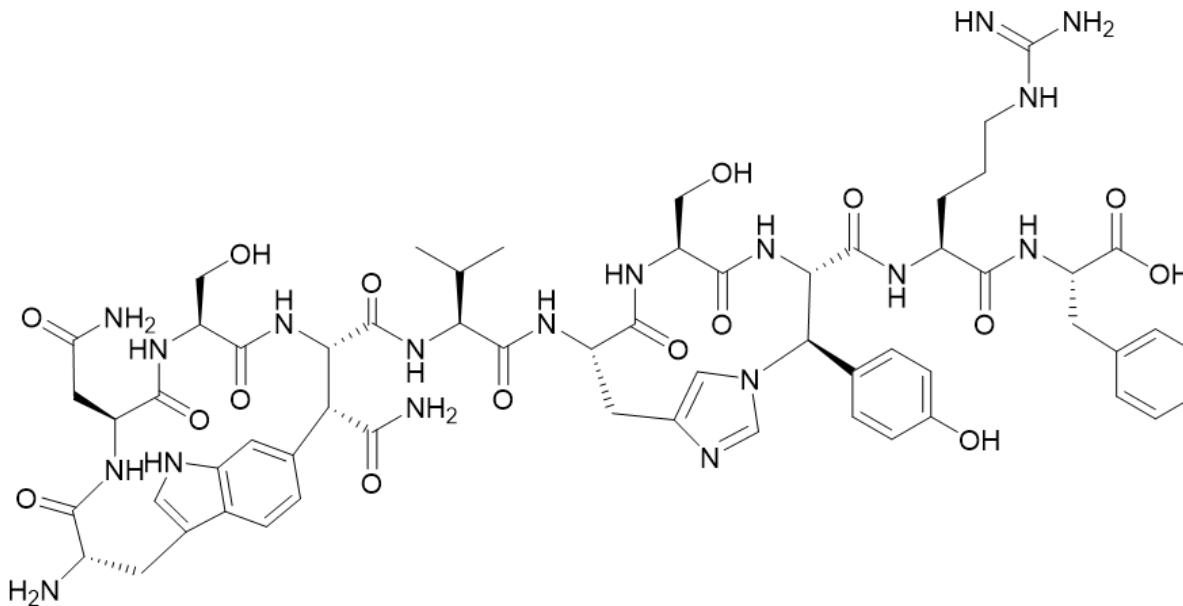


# Computational Discovery



Ryan Miller

# Dynobactin A



Sebastian Hiller, Seyed Majed Modaressi



**Tony D'Onofrio  
Stephanie Gao  
Nikita Gupta  
Oleg Vasilieva  
Blake Caravas  
Andrei Musetesku  
Patil Pankaj  
Akira Ilinishi**

**Stephanie Gao  
Rachel Bargabos  
Nadja Leimer  
Tom Curtis  
Sangkeun Sun  
Monique Theriaut  
Vladimir Tikhonov  
Nadja Leimer**

**Tahmina Kamrunessa  
Michael Gates  
Akira Ilinishi  
Sanika Vaidya  
Norman Pitt  
Viktoria Holubnycha  
Raleb Taher  
Tamara Jordan**



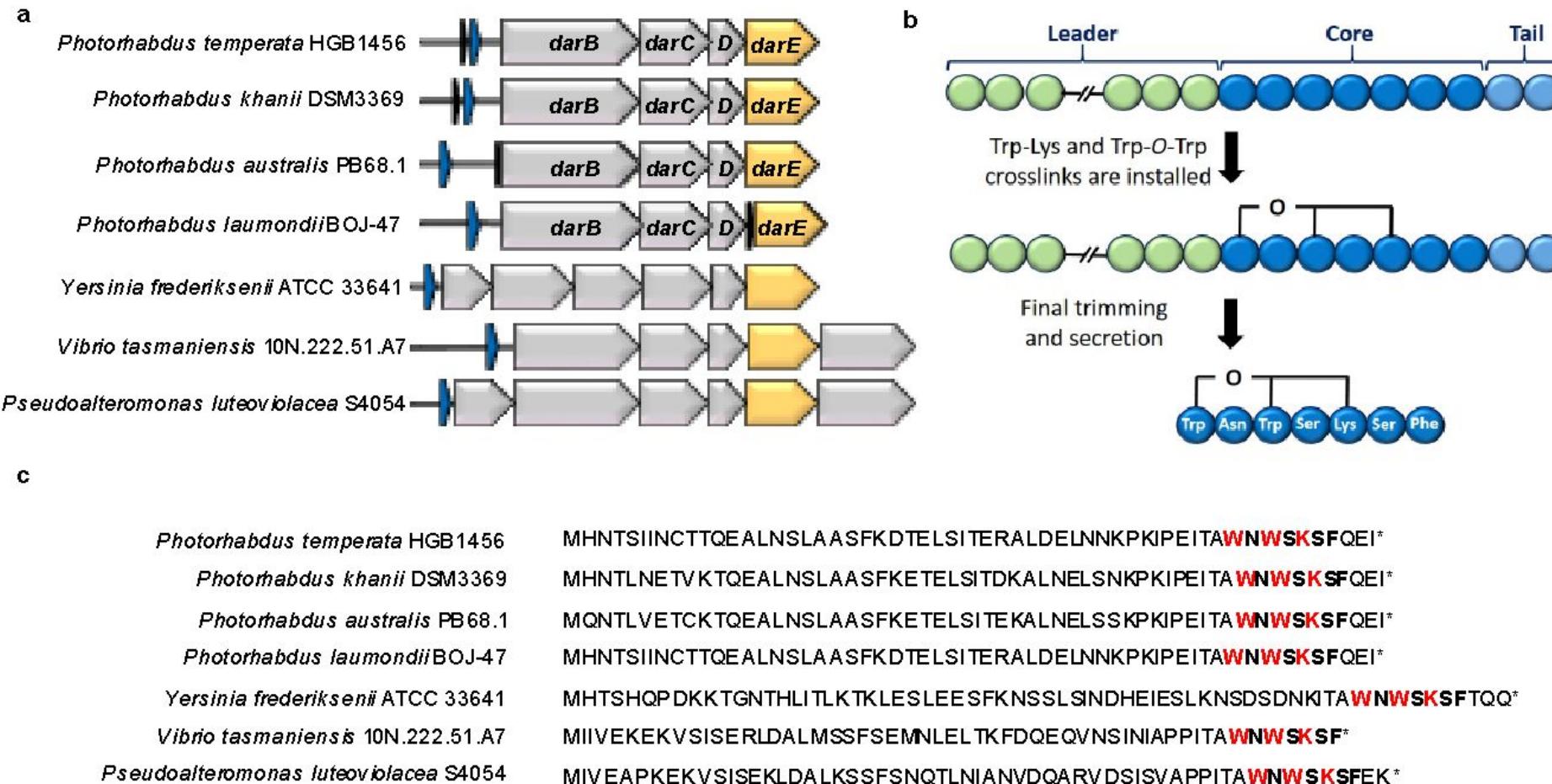
**Collaborators:**

**Sebastian Hiller, University of Basel  
Johan Paulsson, HMS  
Richard E. Lee, St. Jude Children's Hospital  
Linden Hu, Tufts  
Chris Dupont, JCVI  
Amy Spoering, NovoBiotic  
Michael LaFleur, Arietis  
Phil Strandwitz, Holobiome**



**SCHMIDT FUTURES**

# Darobactin

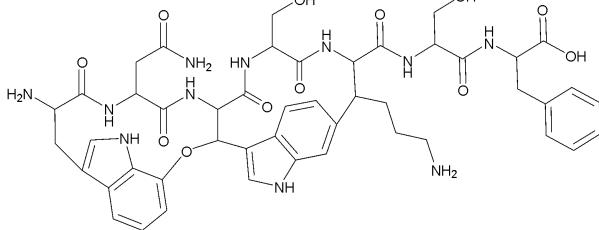


# Analogs By Homology Search



**Robbie Green**

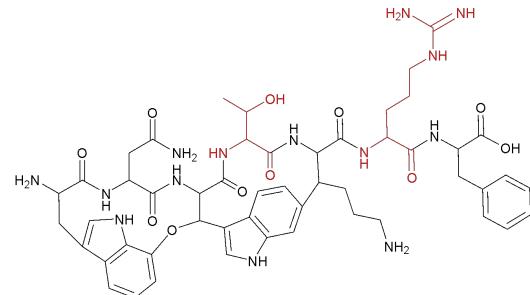
**Darobactin A: wNWSKSF**



[Darobactin A Producing Organism](#)

*Photobacterium temperata*  
*Photobacterium asymbiotica*  
*Photobacterium luminescens*  
*Photobacterium namnaonensis*  
*Photobacterium khanii*  
*Photobacterium australis*  
*Photobacterium laumondii*  
*Yersinia rohdei*  
*Yersinia frederiksenii*  
*Yersinia massiliensis*  
*Yersinia enterocolitica*  
*Yersinia kristensenii*  
*Yersinia intermedia*  
*Yersinia aldovae*  
*Vibrio crassostreae*  
*Pseudoalteromonas luteoviolacea*

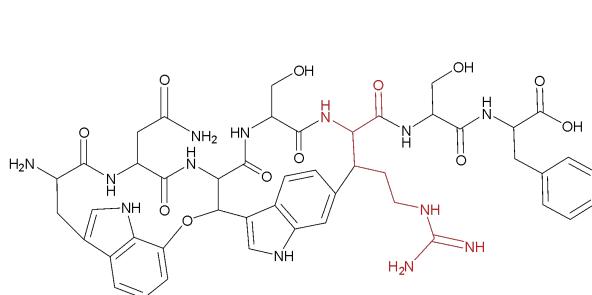
**Darobactin B: wNWTKRF**



[Darobactin B Producing Organism](#)

*Photobacterium asymbiotica*  
*Photobacterium australis*

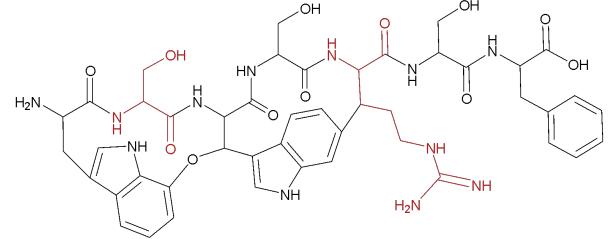
**Darobactin D: wNWSRSF**



[Darobactin D Producing Organism](#)

*Yersinia pseudotuberculosis*  
*Yersinia enterocolitica*  
*Yersinia frederiksenii*  
*Yersinia aldovae*  
*Yersinia pekkanenii*  
*Yersinia intermedia*  
*Yersinia similis*

**Darobactin C: wSWSRSF**



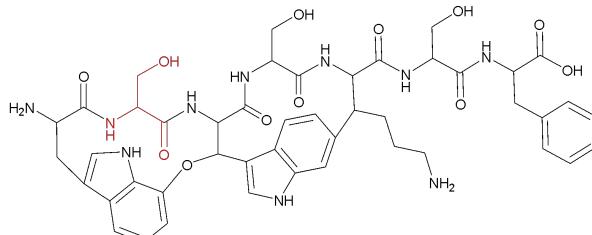
[Darobactin C Producing Organism](#)

*Yersinia pseudotuberculosis*  
*Yersinia pestis*

**Aminoglycosides: streptomycin - tobramycin, 20 years**

**Darobactins: 1 day**

**Darobactin E: wSWSKSF**



[Darobactin E Producing Organism](#)

*Yersinia bercovieri*