Chemical Biology approach to identification of a small-molecule inhibitor of BamA function

March 2020

D. M. Rothman on behalf of team

Acknowledgements

Princeton University

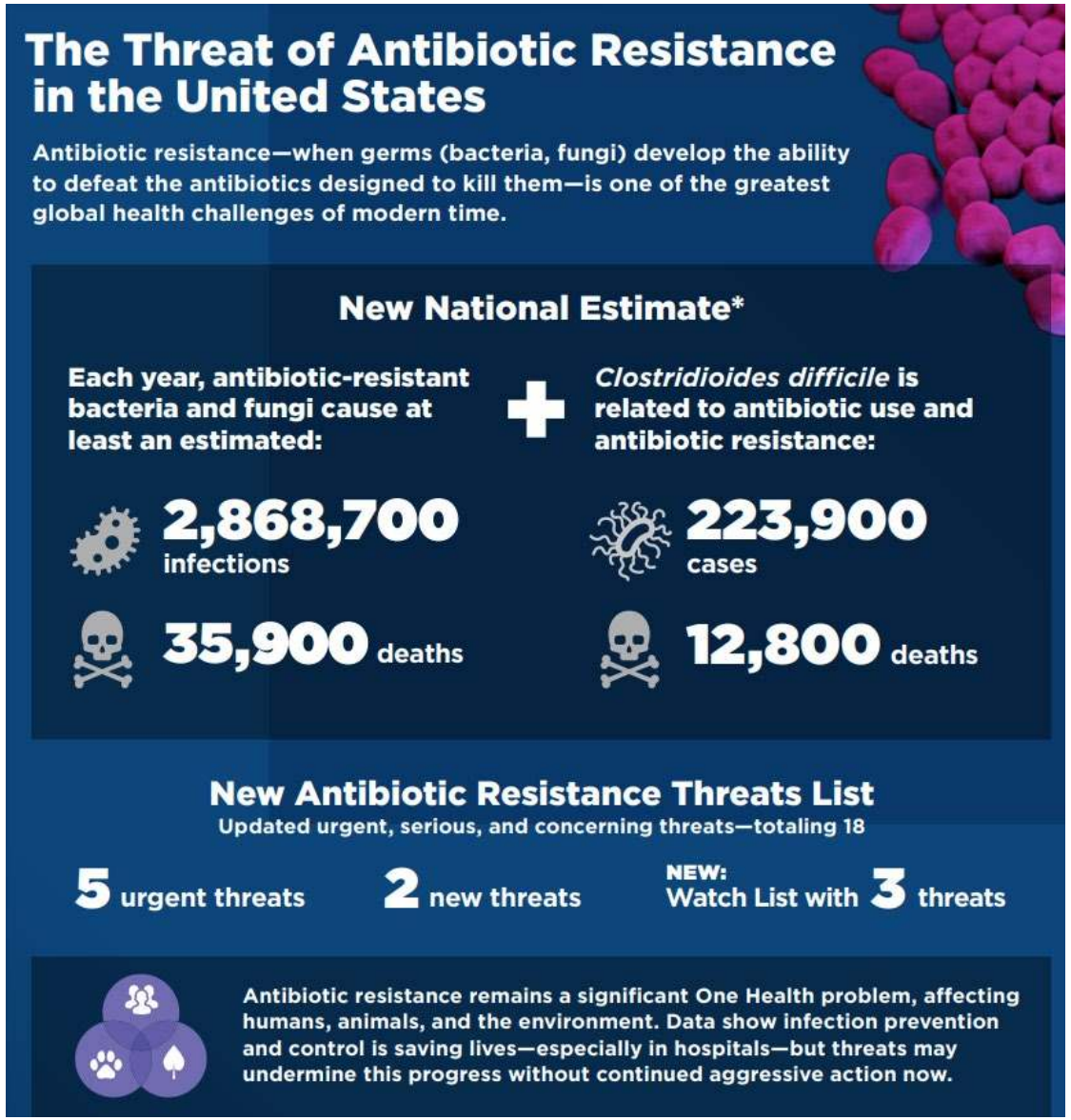
Elizabeth M. Hart
Angela M. Mitchell
Anna Konovalova
Marcin Grabowicz
Jessica Sheng
Thomas J. Silhavy

Merck

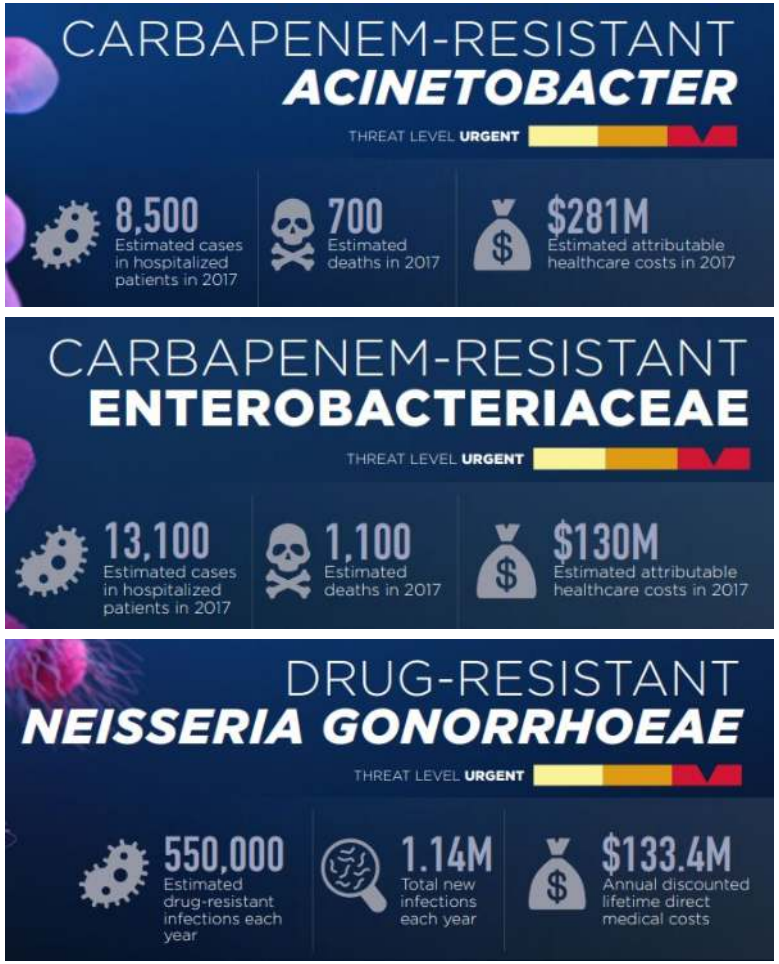
Xiaoqing Han
Frances P. Rodriguez-Rivera
Adam G. Schwaid
Juliana C. Malinverni
Carl J. Balibar
Smaranda Bodea
Qian Si
Hao Wang
Michelle F. Homsher
Ronald E. Painter
Anthony K. Ogawa
Holly Sutterlin
Terry Roemer
Todd A. Black
Deborah M. Rothman
Scott S. Walker

MERCK
INVENTING FOR LIFE

Gram-negative Challenge



Antibiotic resistance remains an increasing threat to public health and three of the five urgent threat pathogens are gram-negative bacteria



Images: cdc.gov

MERCK
INVENTING FOR LIFE

Gram-negative Challenge

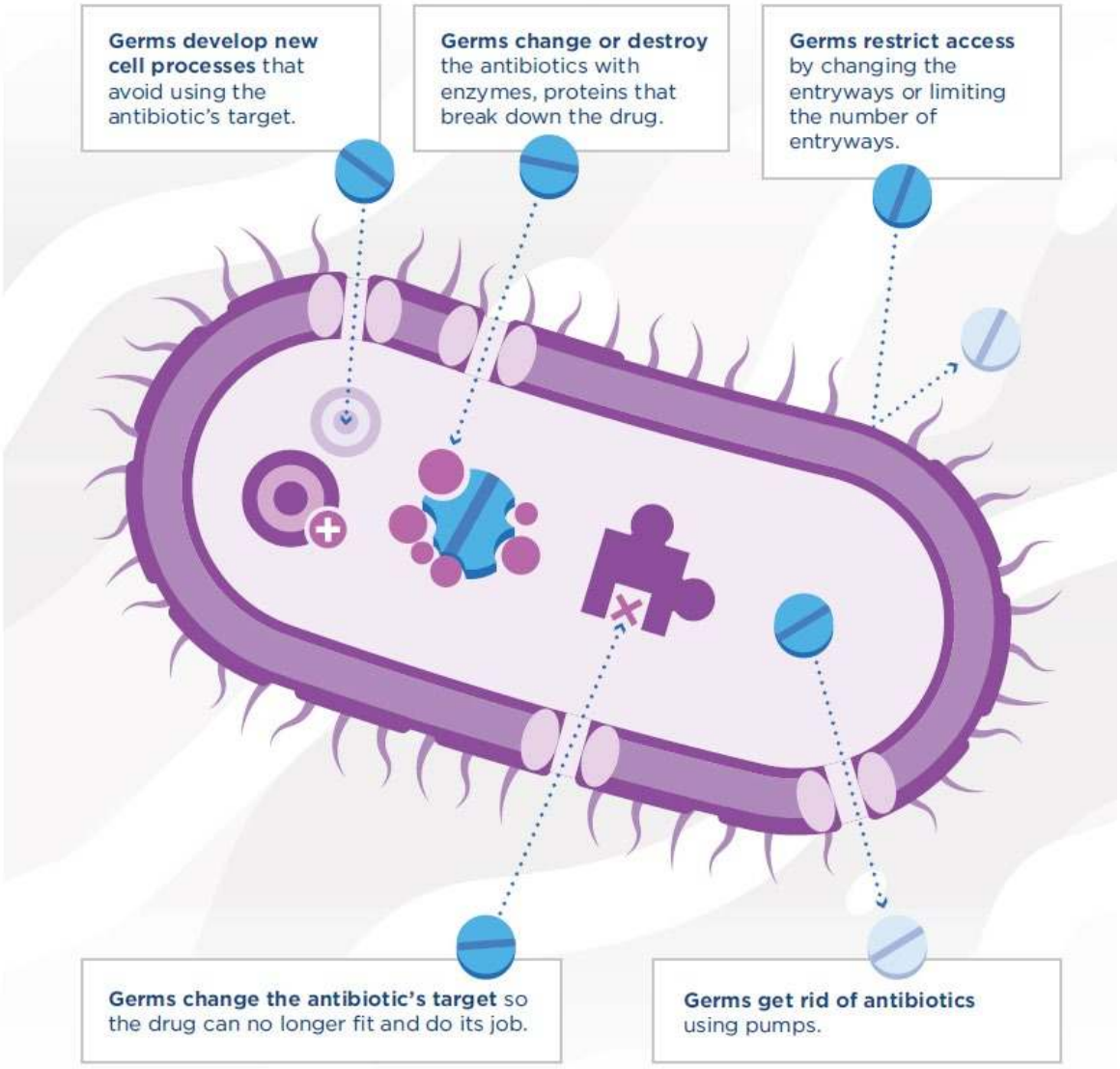
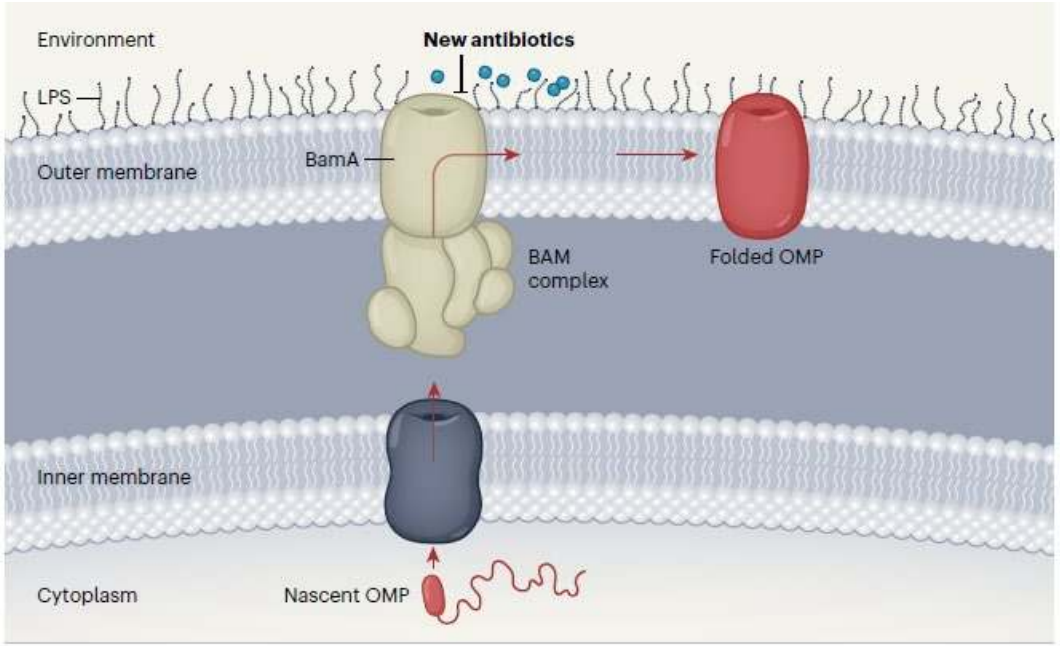


Image: cdc.gov

Gram-negative antibiotic discovery is additionally challenged by the Outer Membrane (OM)

- Asymmetric bilayer (Outer Leaflet = lipopolysaccharides, Inner Leaflet = phospholipids)
- MDR efflux pumps

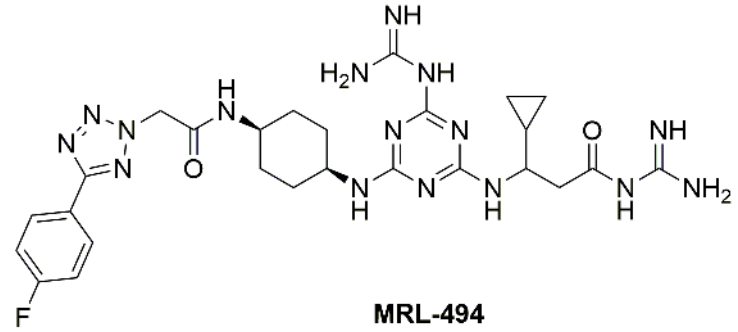
Targeting the OM as a strategy



New antibiotics target bacterial envelope
M. C. Sousa, Nature **2019** v576 p389

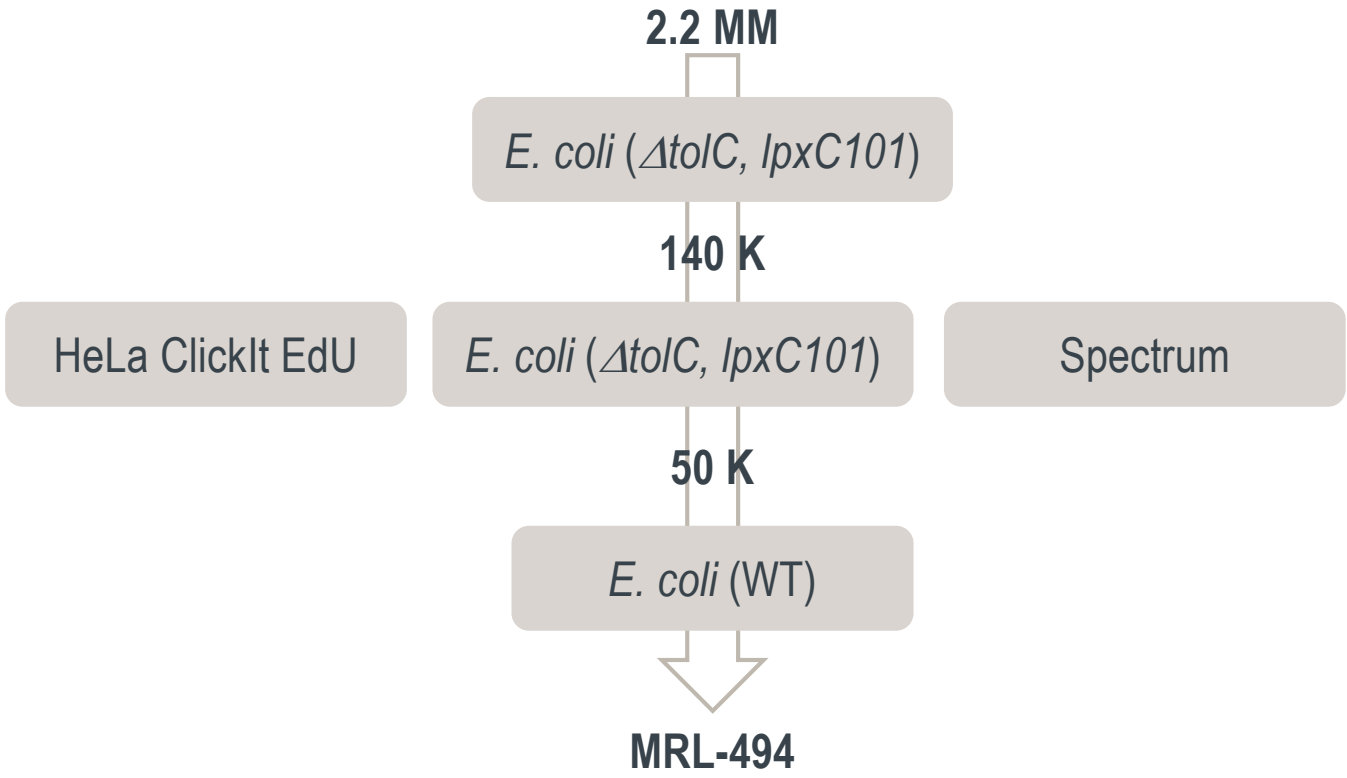
MERCK
INVENTING FOR LIFE

Approach and Identification of MRL-494



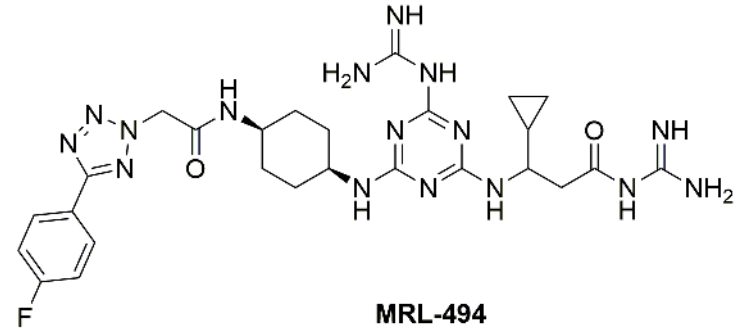
MRL-494

Hypothesis: compounds with similar activity on efflux and permeability competent and impaired cells may evade efflux and the OM permeability barrier due to chemical nature and/or the surface location of their target



MERCK
INVENTING FOR LIFE

Profiling MRL-494



MRL-494

Spectrum activity of MRL-494

Classification	Description	MIC (μM)
Gram-negative	E. coli (WT)	25
	E. coli (ΔtolC)	25
	E. coli (ΔtolC, lpxC101)	25
	K. pneumoniae	100
	A. baumannii (WT)	200
	A. baumannii (ΔlpxC)	200
	P. aeruginosa (WT)	100
	P. aeruginosa (efflux deficient)	100
Gram-positive	Staphylococcus aureus (methicillin-resistant)	12.5
	Bacillus subtilis rpoB18	25
HeLa	Mammalian – ClickIt EdU (EC50)	> 99

Not efflux substrate
No permeability issue

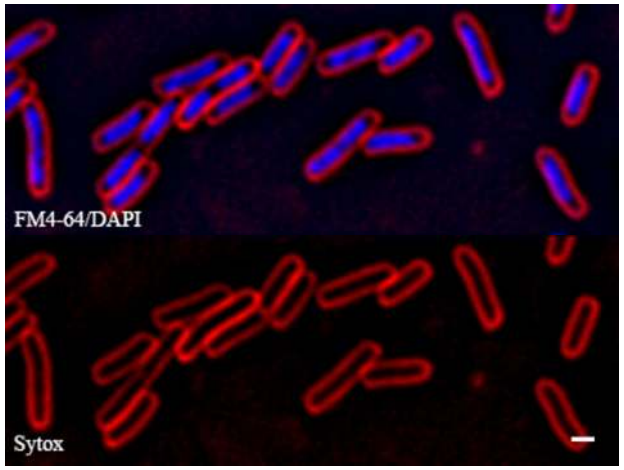
MERCK
INVENTING FOR LIFE

Initiating MOA Deconvolution

FM4-64: membrane
DAPI: nucleic acid
Sytox: nucleic acid, non-permeable
2hr compound exposure

Bacterial Cytological Profiling (Linnaeus)

2% DMSO



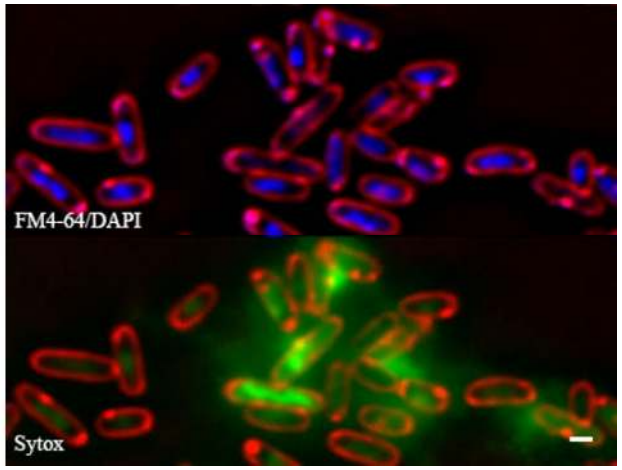
1X MIC

- Membrane foci at the cell poles
- Some abnormally long

3X MIC

- Some cells lysing

5X MIC



- Cells permeabilized
- Cells lysing

The compound does not closely match known controls.
Suggests novel MOA.

MERCK
INVENTING FOR LIFE

MRL-494 impacts OM and decreases OMP abundance

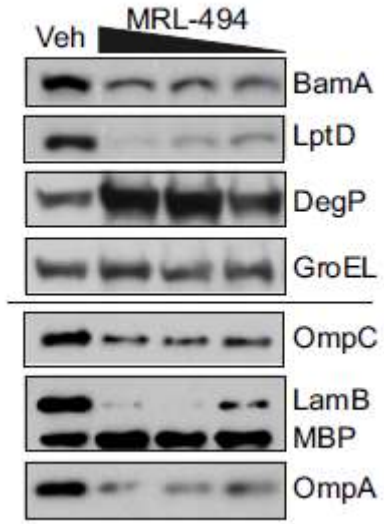
MRL-494 potentiates rifampicin

Condition	*E. coli* MIC (uM)	
	WT	lpxC101
Rifampicin	25.000	< 0.049
Rifampicin + 6.25 uM MRL-494	0.195	---

Rifampicin: RNA polymerase inhibitor which does not penetrate OM

lpxC101: mutation which decreases LPS on OM and potentiates rifampicin activity

MRL-494 decreases OMPs



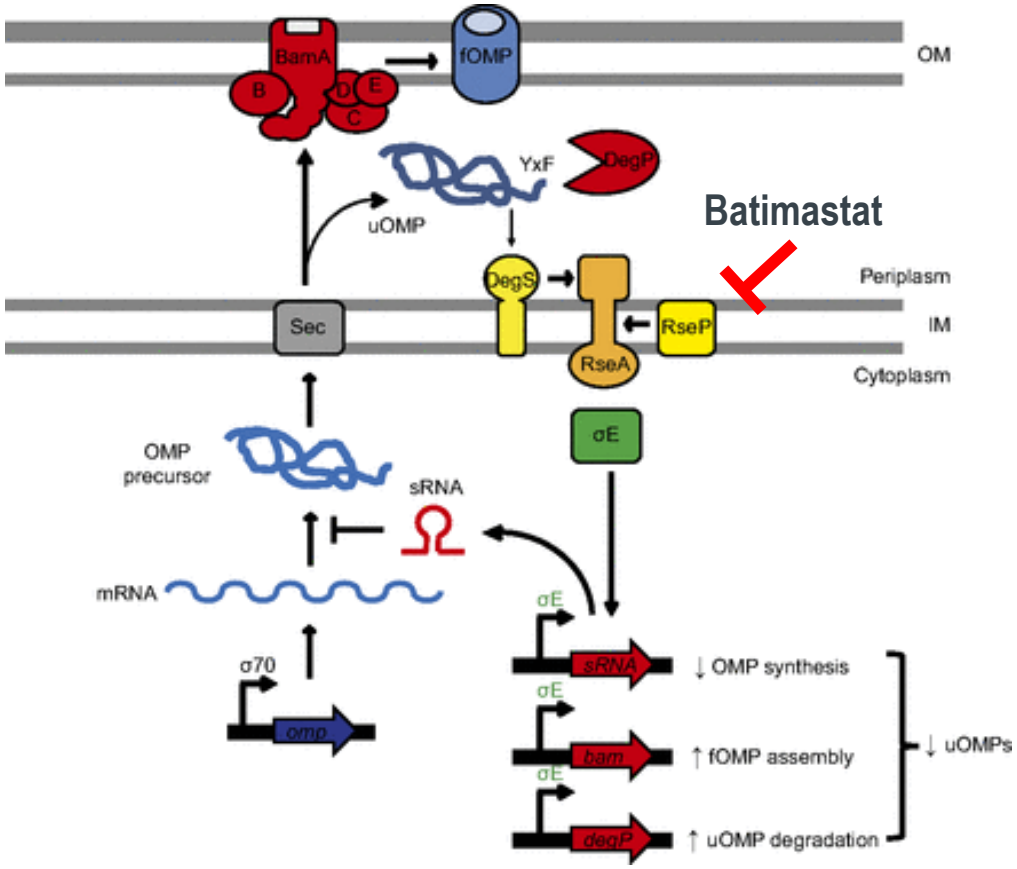
1.5h treatment

Observe increase in DegP
Indication of extracellular envelope stress

MERCK
INVENTING FOR LIFE

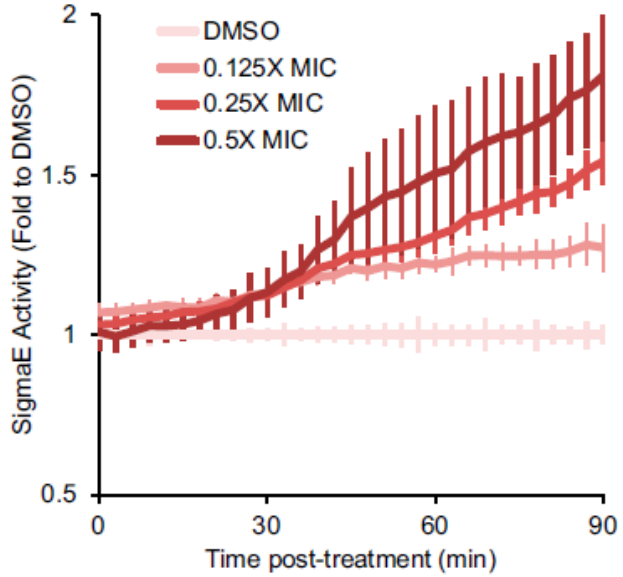
MRL-494 increases stress response

Batimastat targets RseP and inhibits the σE pathway



PNAS 2018 115 (28) E6614

MRL-494 increases σ^E activity

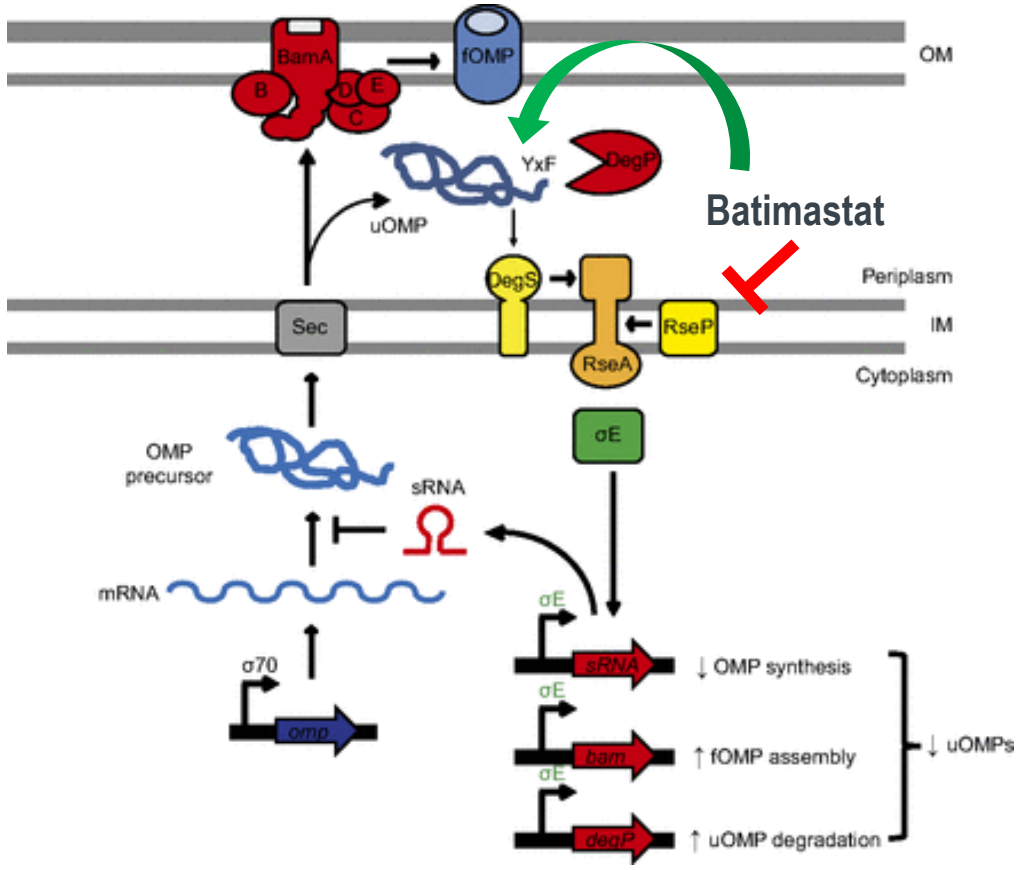


σ^E-GFP readout

MERCK
INVENTING FOR LIFE

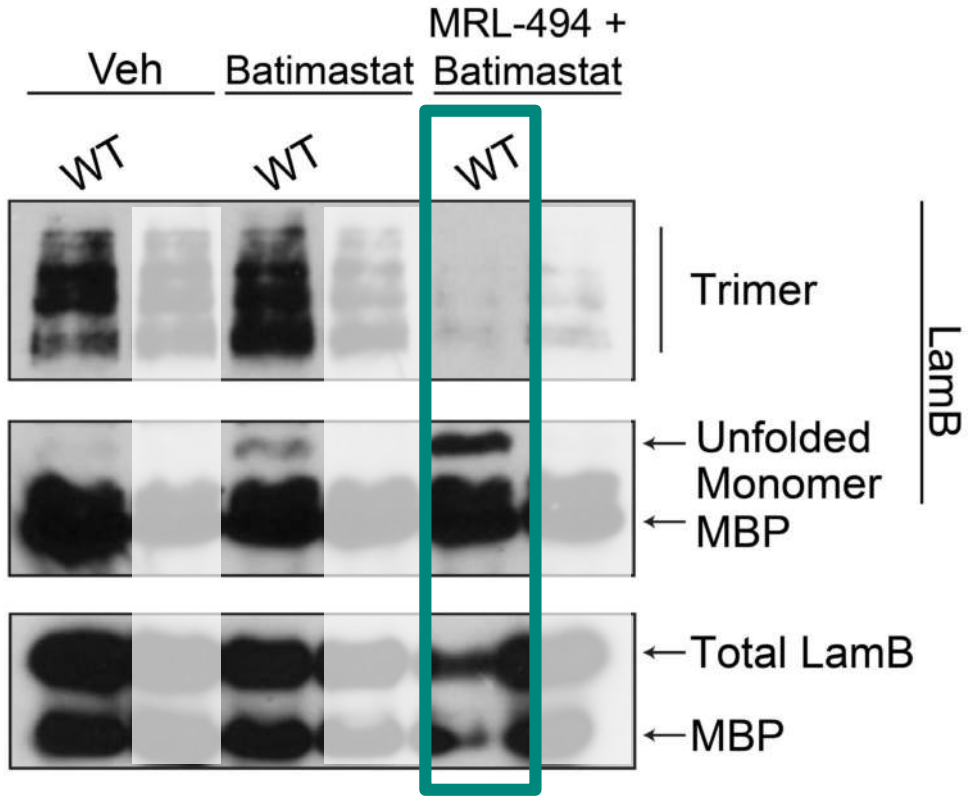
MRL-494 inhibits OMP biogenesis

Batimastat targets RseP and inhibits the σE pathway



PNAS 2018 115 (28) E6614

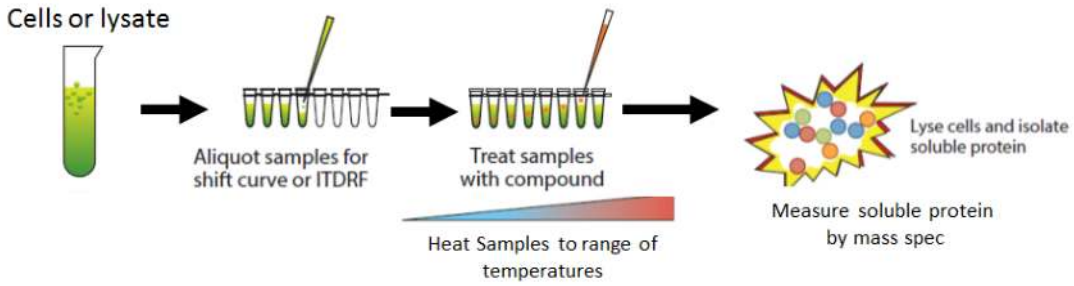
Batimastat potentiates MRL-494

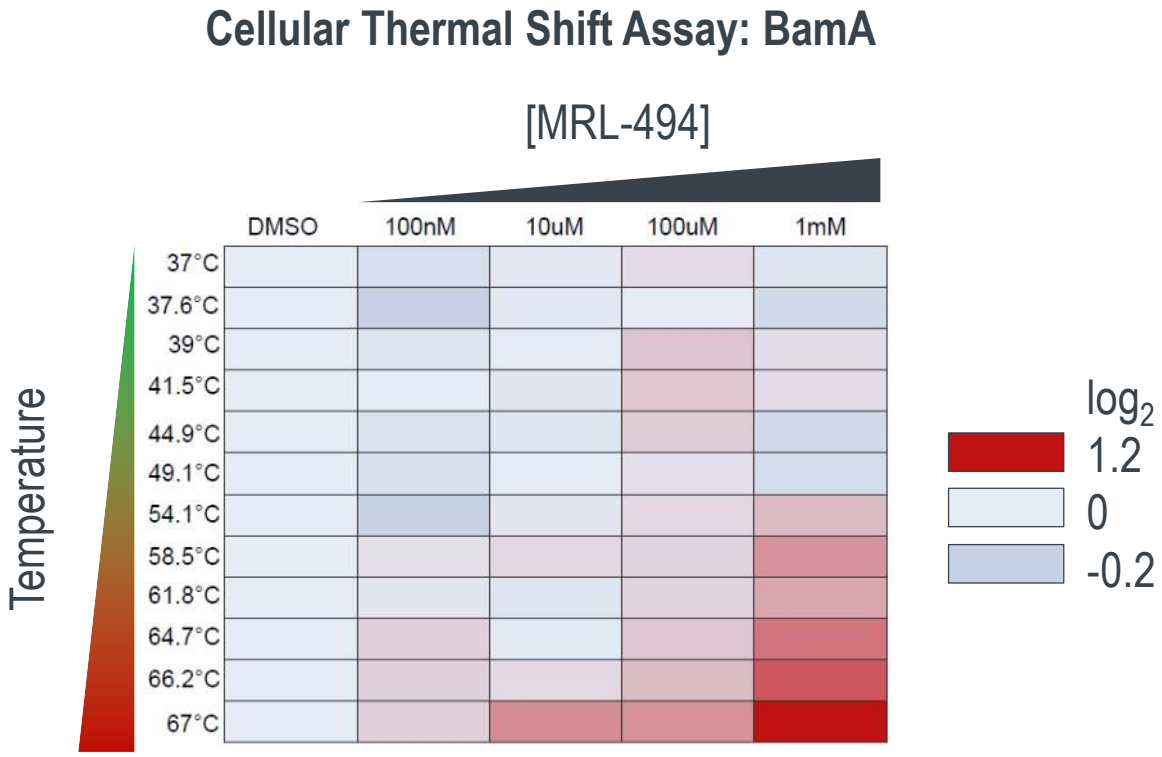


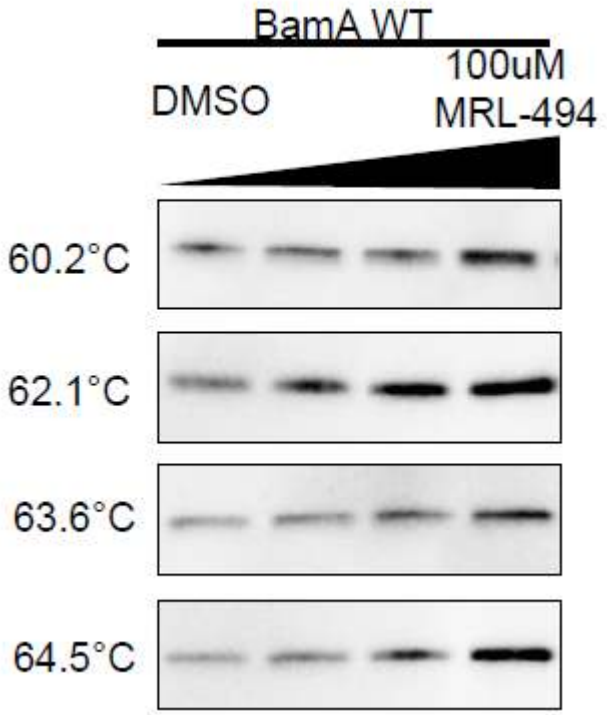
- MRL-494 prevents formation of trimer & decreases total amount of LamB
- Batimastat increases accumulation of unfolded OMP

MERCK
INVENTING FOR LIFE

MRL-494 stabilizes BamA by 2D-CETSA™



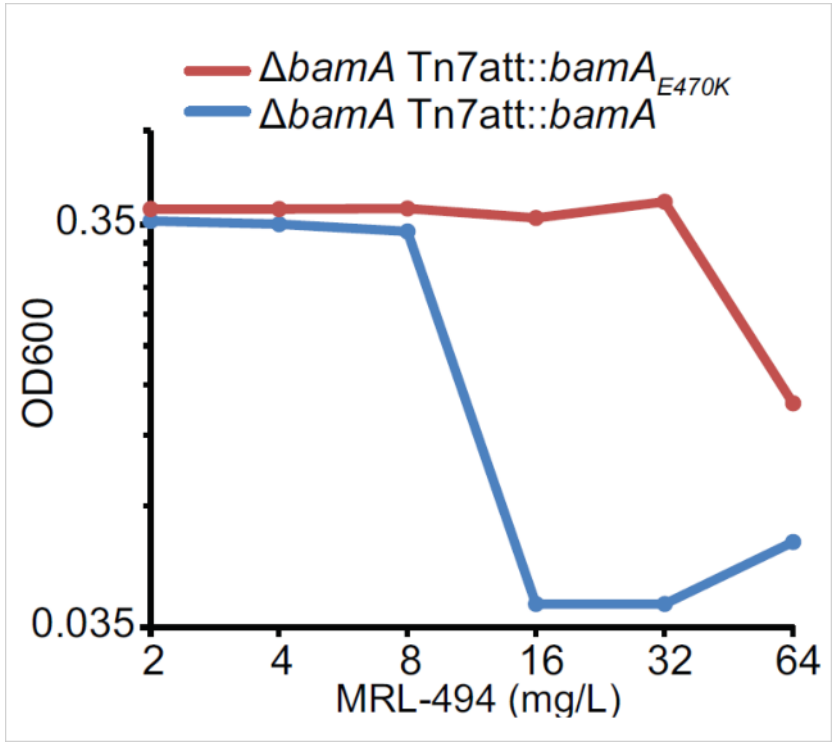




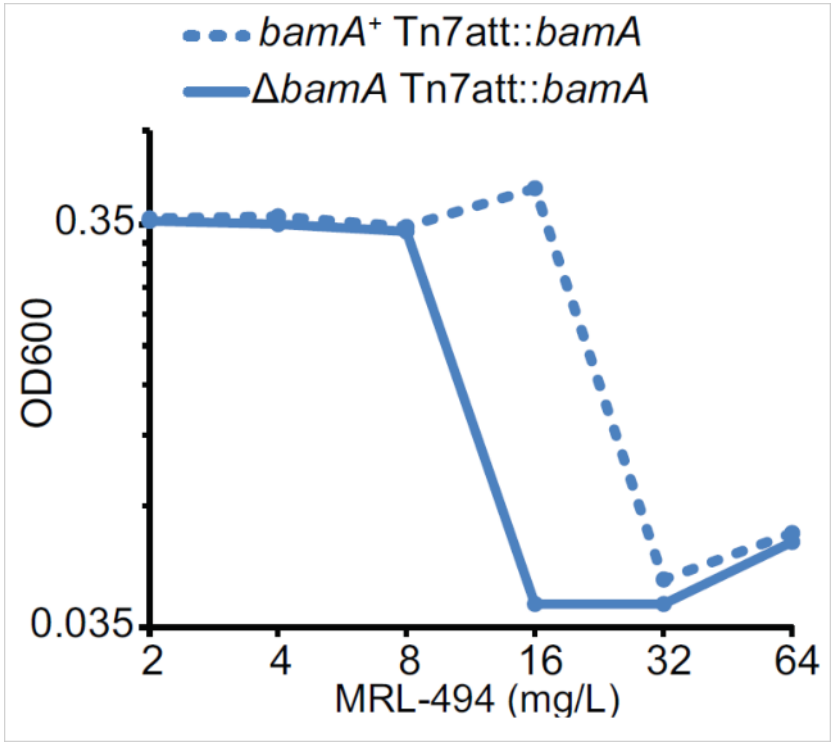
MERCK
INVENTING FOR LIFE

BamA genetic status impacts resistance to MRL-494

BamA^{E470K} confers resistance



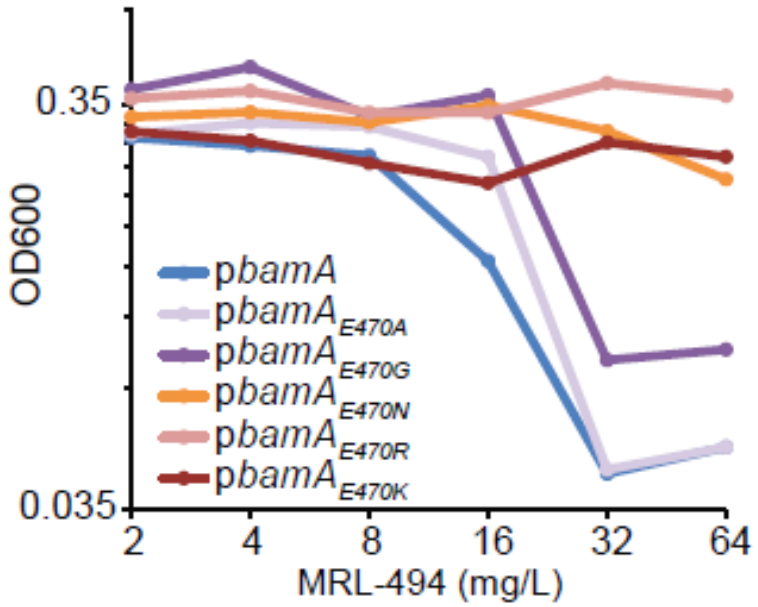
BamA WT OE confers resistance

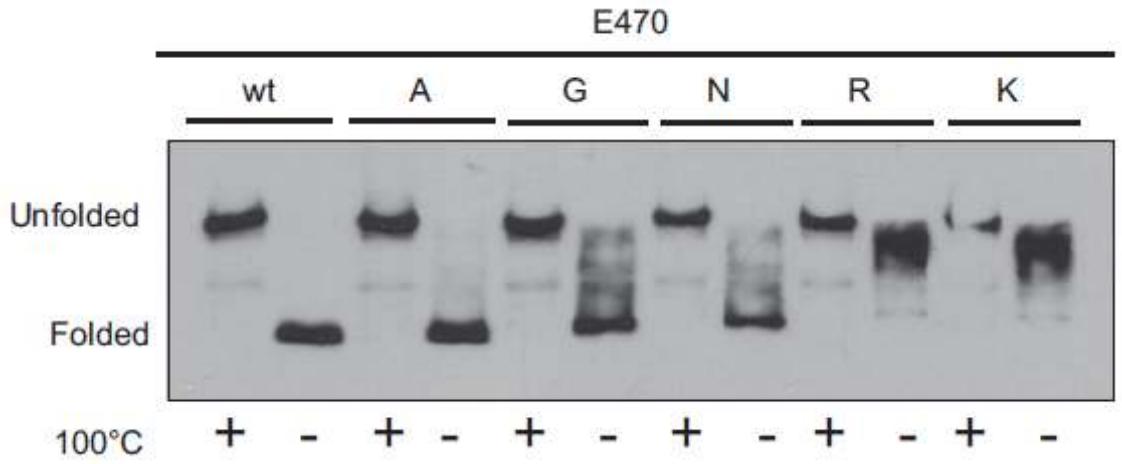


MERCK
INVENTING FOR LIFE

BamA^{E470} Mutation

Observed 470 amino acid charge impacts MRL-494 activity and BamA stability

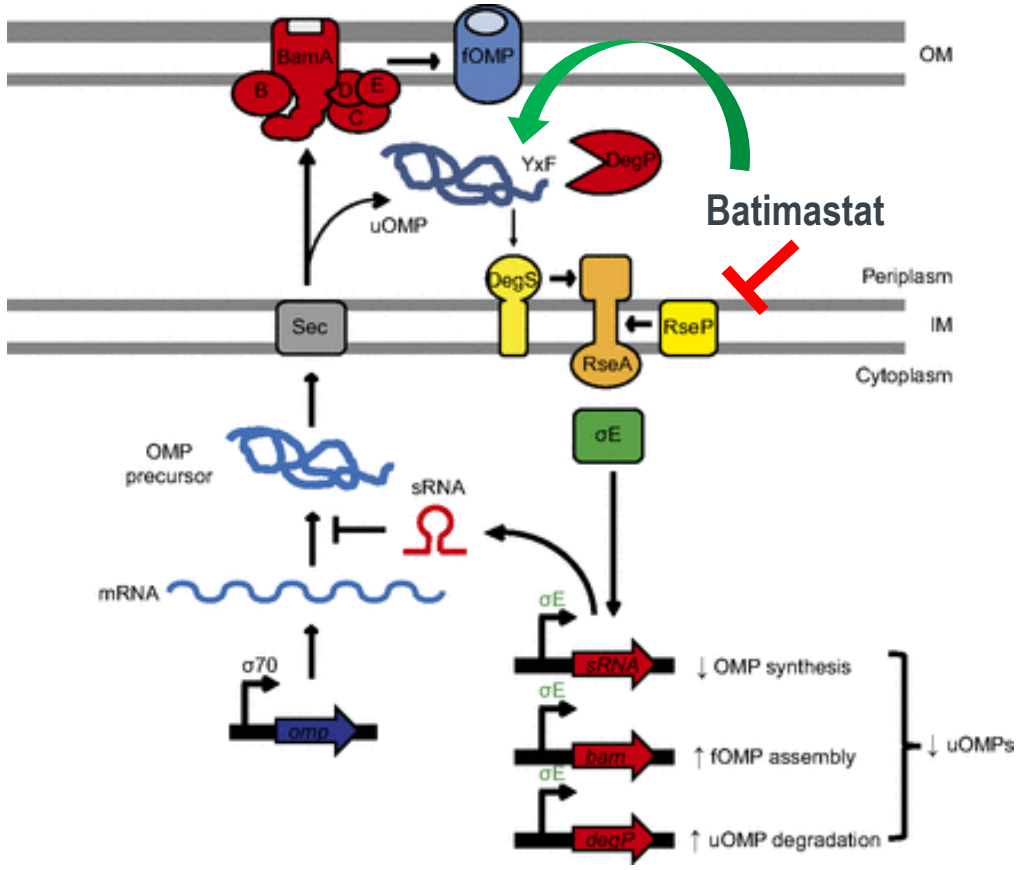




MERCK
INVENTING FOR LIFE

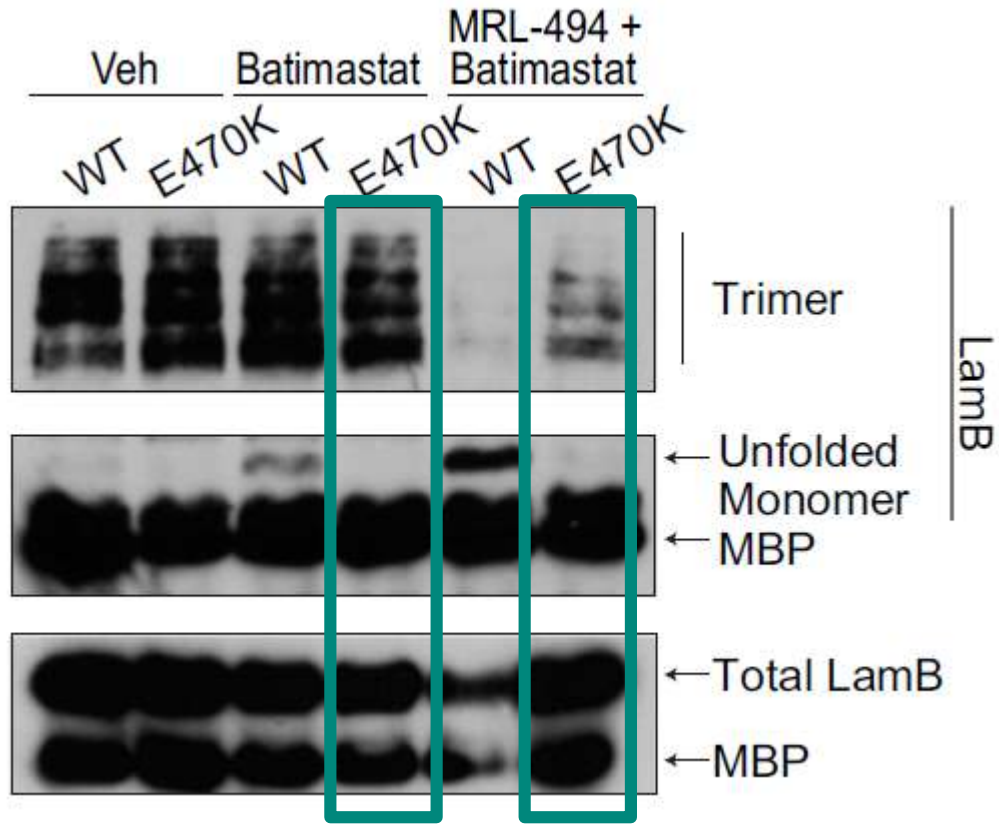
BamA^{E470} Mutation

Batimastat targets RseP and inhibits the σE pathway



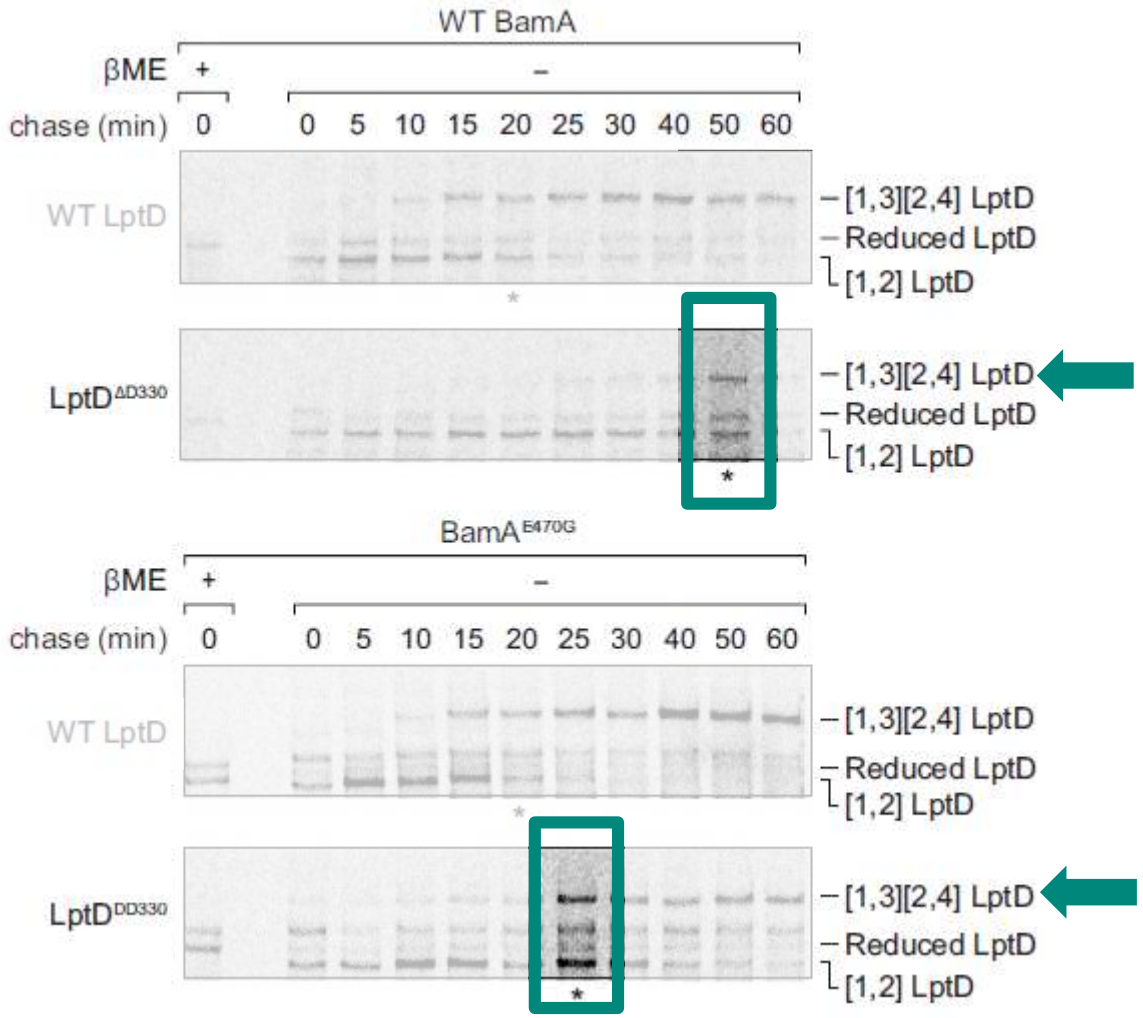
PNAS 2018 115 (28) E6614

E470K mutant suppresses batimastat/MRL-494



MERCK
INVENTING FOR LIFE

BamA^{E470} mutation may act as hypermorph



Lee et al. eLife 2019;8:e49787

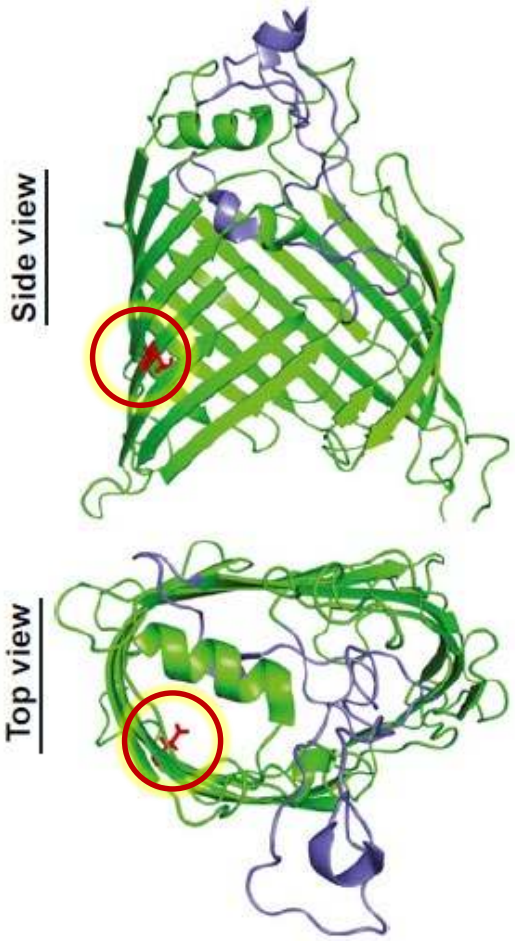
Pulse chase experiment recently reported from Kahne, et. al.

- * = approximately 50% of the LptD has converted to the mature form, containing the [1,3][2,4] disulfide bond configuration
- BamA WT ~ 50 min
  BamA^{E470G} ~ 25 min

MERCK
INVENTING FOR LIFE

Summary

- ✓ MRL-494 identified as tool which functionally inhibits BamA in Gram-negative bacteria
- ✓ BamA^{E470K} identified as mutant which demonstrates MIC shift for MRL-494
- ✓ BamA^{E470K} does not have impaired function in the assembly of wild-type OMPs under laboratory conditions
- ✓ BamA^{E470K} does have both altered conformation and activity
  - ➢ Correlates with resistance to MRL-494
    Neither the conformational change nor the altered activity sufficient to explain the MRL-494 resistance
- ✓ Recent publication demonstrates opportunity for how MRL-494 could be a mechanistic tool to inform on new biology – how would different mutations impact MRL-494 activity?



MERCK
INVENTING FOR LIFE

THANK YOU

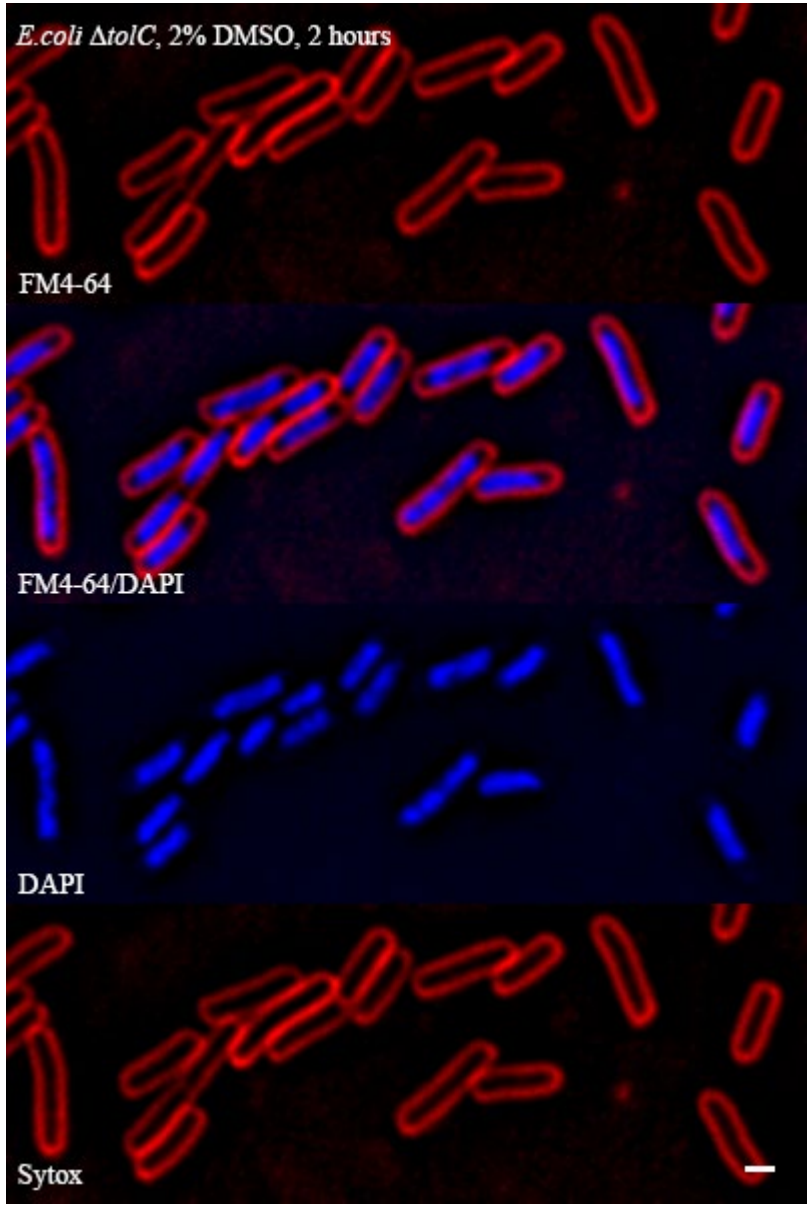
MERCK

Bacterial Cytological Profiling
Linnaeus

- For BCP, we grew samples of *E.coli ΔtolC* in rich media (LB) at 30^0C to an OD_{600} ~0.15. These cultures were then split and each sample treated with the appropriate concentration of test compound.
- We collected samples after 30 minutes or 2 hours of exposure, stained and then imaged them.
- Below, we have included images showing the predominant phenotype produced by each of the test compounds at 2 hours of exposure.

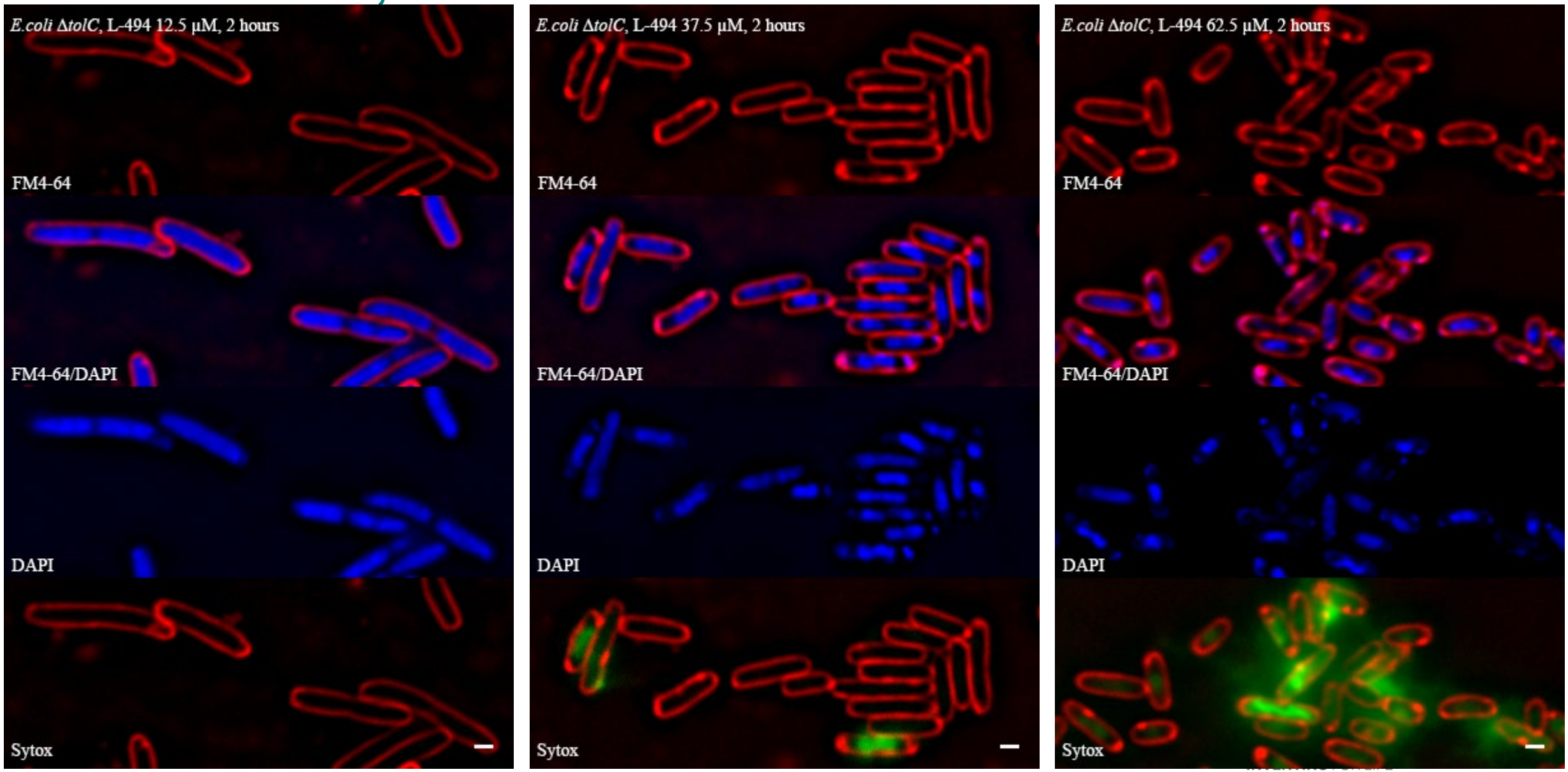
	Merck Δ*tolC* MIC (µM)	Linnaeus Δ*tolC* MIC (µM)
L-494	12.5	12.5

2% DMSO Control



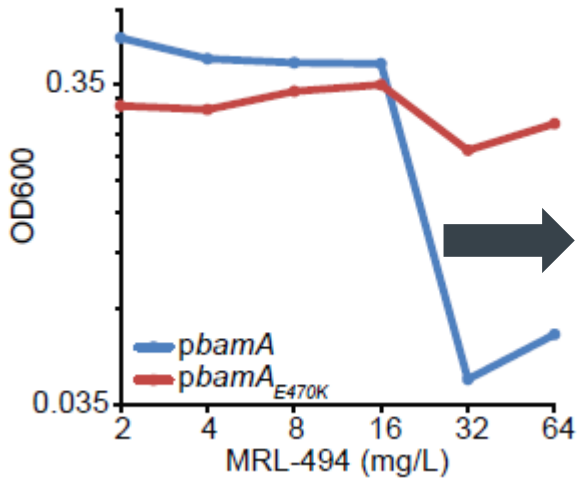
MERCK
INVENTING FOR LIFE

L-494 at 1X, 3X and 5X the MIC



BamA^{E470K} shifts MRL-494 potency

BamA^{E470K} shifts MIC to solubility limit



BamA^{E470K} shifts MIC in ΔbamB