

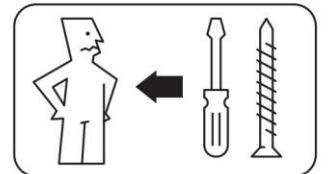
# Novel antibacterials targeting the transcriptional regulators PrfA and BrtA

Jörgen Johansson



UMEÅ UNIVERSITET

**SCREW THE SWEDES**



Made in Ireland

# Antibiotics – Miracle drugs saving billions of lives

## What do antibiotics allow us to do?

- Treatment of infectious diseases, like tuberculosis



# Antibiotic resistance is one of the biggest threats to global health

**What is AMR?**  
Antimicrobial Resistance (AMR) is the ability of microorganisms to resist antimicrobial treatments, especially antibiotics.

Excessive and inappropriate use of antimicrobial medicines and poor infection control practices have transformed AMR into a serious threat to public health worldwide. If trends continue we would revert to a world where simple infections are no longer treatable.

**Why is AMR a serious threat to public health?**

- 25 000 patients die annually in the EU alone as a result of infections caused by resistant bacteria.
- Globally this number could be as high as 700 000.
- 10 million deaths per year are projected between 2015 and 2050 if current infection and resistance trends are not reversed.
- Only 0.7 million of these additional deaths would occur in North America or Europe, with the largest numbers in Africa and Asia.

**What is the economic cost of AMR?**

AMR is estimated to cost the world economy around 100 billion dollars per year.

**Infection Raises Specter of Superbugs Resistant to All Antibiotics**

By SABRINA TAVERNIERE and DENISE GRADY MAY 26, 2016

A strain of the E. coli bacteria. Jason Gay/Centers for Disease Control and Prevention, via Associated Press

American military researchers have identified the first patient in the United States to be infected with bacteria that are resistant to an antibiotic that was the last resort against drug-resistant germs.

The patient is well now, but the case raises the specter of superbugs that could cause untreatable infections, because the bacteria can easily

**RELATED COVERAGE**

- U.S. Aims to Curb Peril of Antibiotic Resistance (SEPT. 16, 2014)
- Deadly CRE Germs Linked to Hard-to-Clean Medical Scopes (FEB. 24, 2015)

**RECENT COMMENTS**

- Wayne Dawson** May 26, 2016: Evolution by human selection... Darwin focused on our breeding of animals and plants, but clearly, our short sighted strategies risk...
- Impedimentus** May 26, 2016: Expect a chorus of deniers from Congress to assure us that there is really no problem. Expect big agriculture and big pharma to continue to...
- STAN CHUN** May 26, 2016: The Chinese had it right centuries ago when they believed that there is no such thing as a virus but simply the body out of balance and it...

**TB rates in parts of London 'worse than Iraq, Eritrea and Rwanda'**

Mayor Boris Johnson urged to 'get a grip' on fight against TB as rates in parts of city exceed 150 cases per 100,000 residents

Boris Johnson is being urged to "get a grip" on London's tuberculosis problem after a report revealed that parts of the capital have higher rates of the disease than Rwanda, Eritrea and Iraq.

There were more than 2,500 new cases of TB in London last year - about 40% of the UK's total - according to a report issued by the London assembly and presented to Johnson, the mayor.

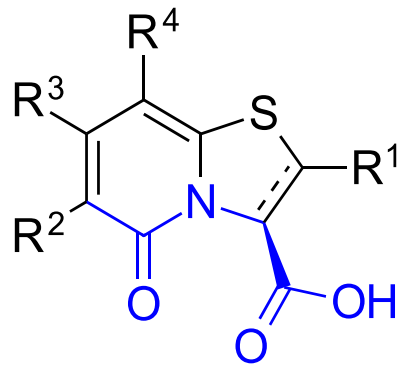
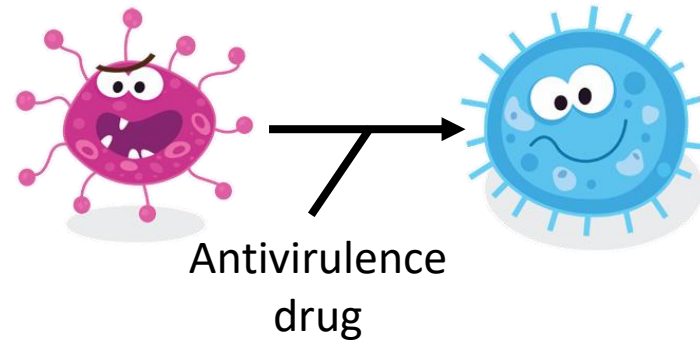
The study found a third of London boroughs exceed the World Health Organisation's (WHO) "high incidence" threshold with more than 40 cases per 100,000 people.

And some borough wards are recording markedly more - areas of Hounslow.

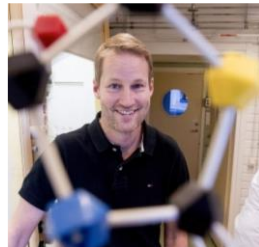
What are the alternatives to old antibiotics?

- Antivirulence drugs
- New antibacterials
- ??

# Antivirulence drug: Targeting bacterial virulence to disarm pathogens



Heterocyclic 2-pyridones  
> 1500 synthesized by  
group of Fredrik Almqvist



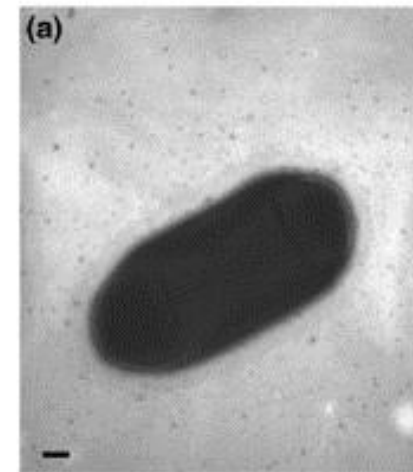
Fredrik Almqvist  
(Umeå Univ. Umeå)



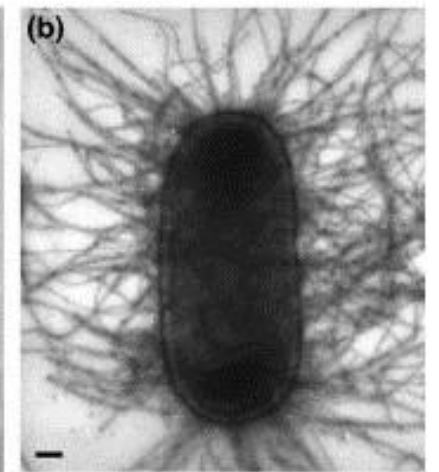
Scott Hultgren  
(Wash. Univ St. Louis, US)

Uropathogenic *E. coli*:

+ 2-pyridones



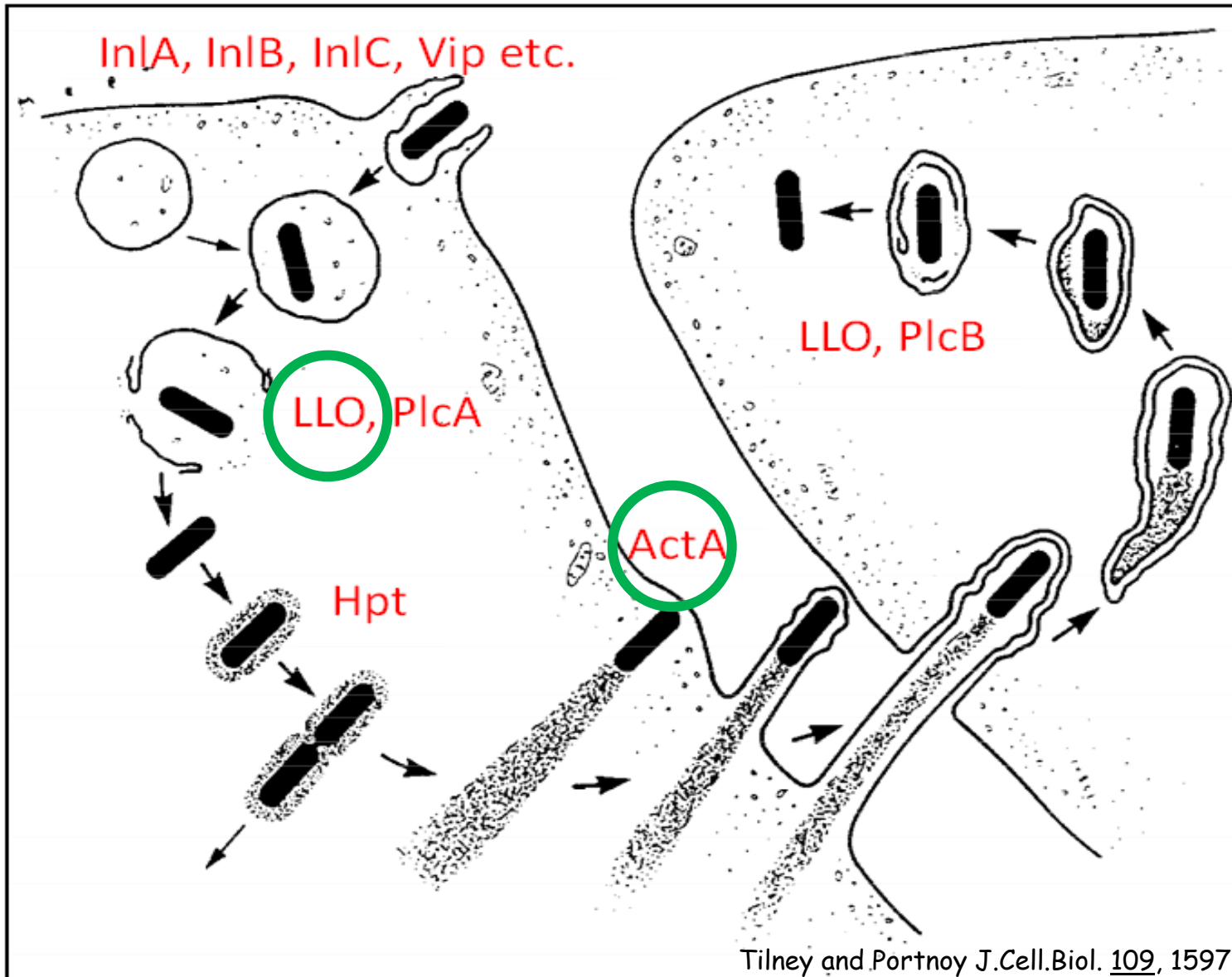
- 2-pyridones



Current Opinion in Pharmacology

**Pinkner *et al.*, PNAS, 2006**  
**Greene *et al.*, mBio, 2014**

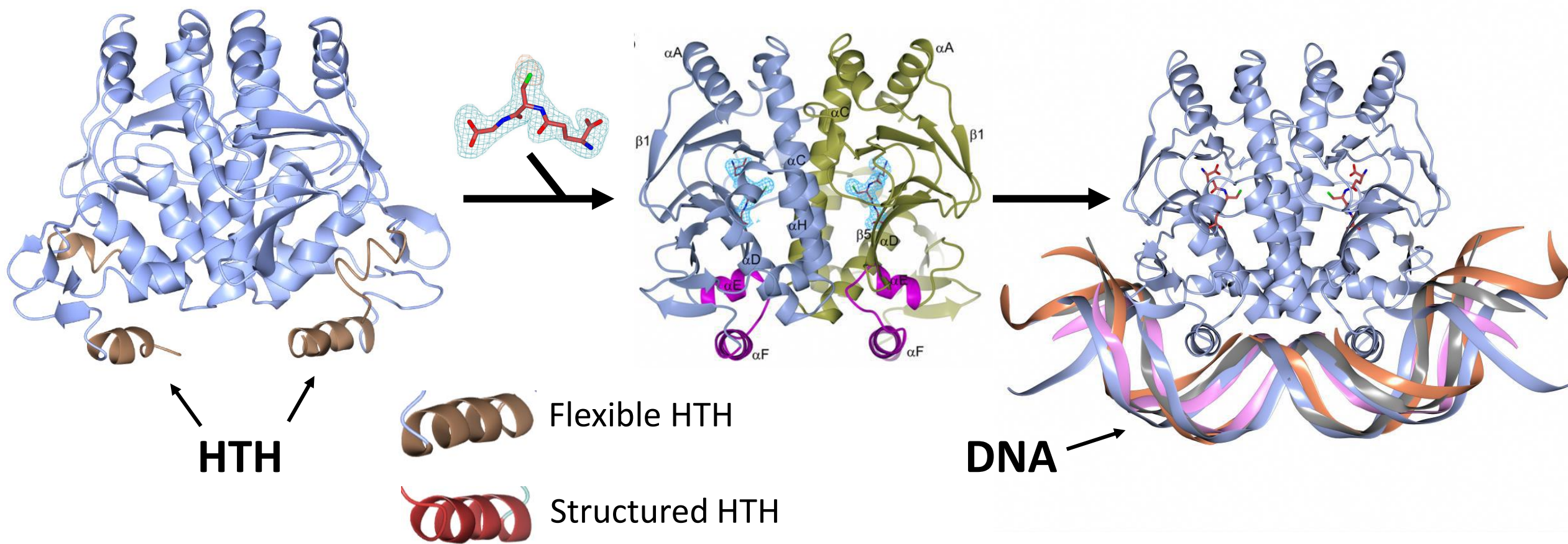
# Many virulence genes in *Listeria monocytogenes* are regulated by the transcriptional activator PrfA





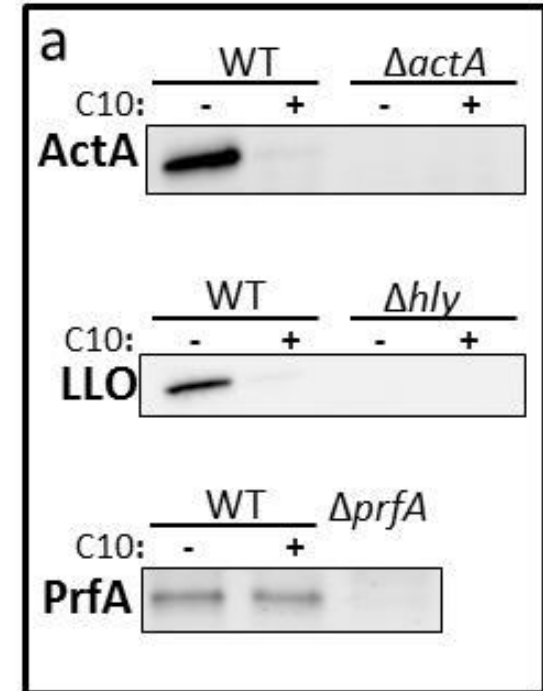
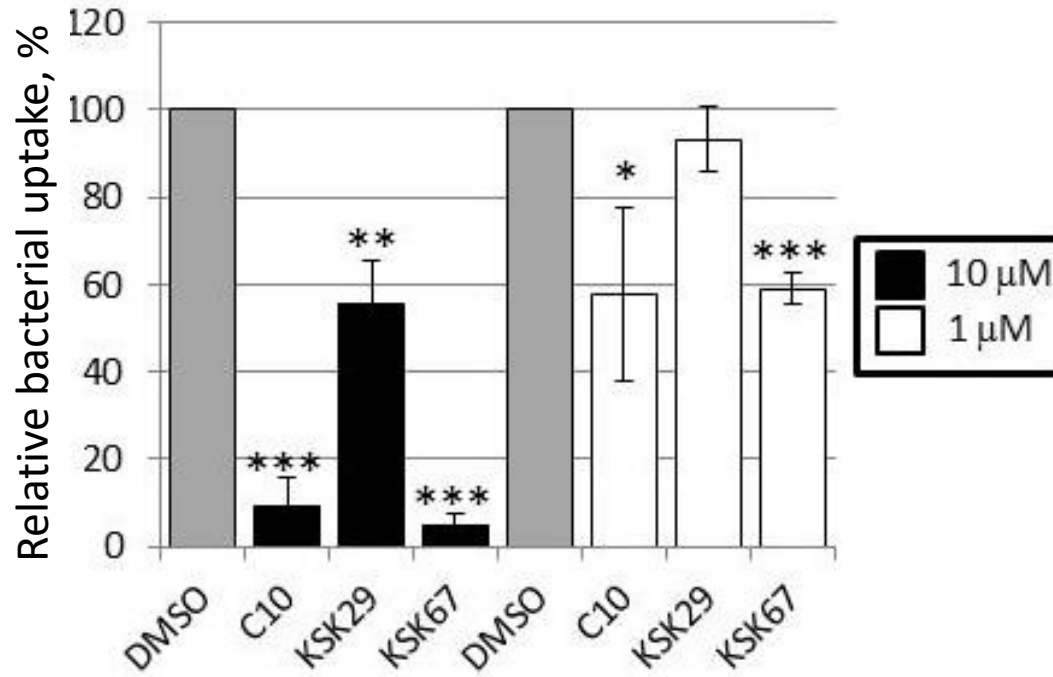
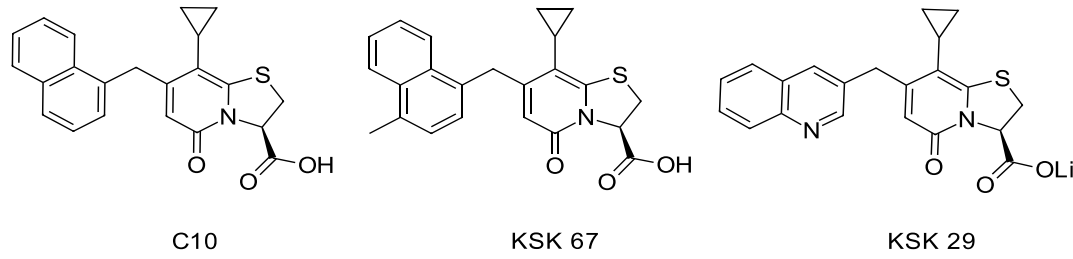
# Glutathione act as a co-factor to activate PrfA, restructuring the flexible HTH of PrfA to allow DNA-binding

Glutathione binds at the entrance of an intra-protein tunnel site



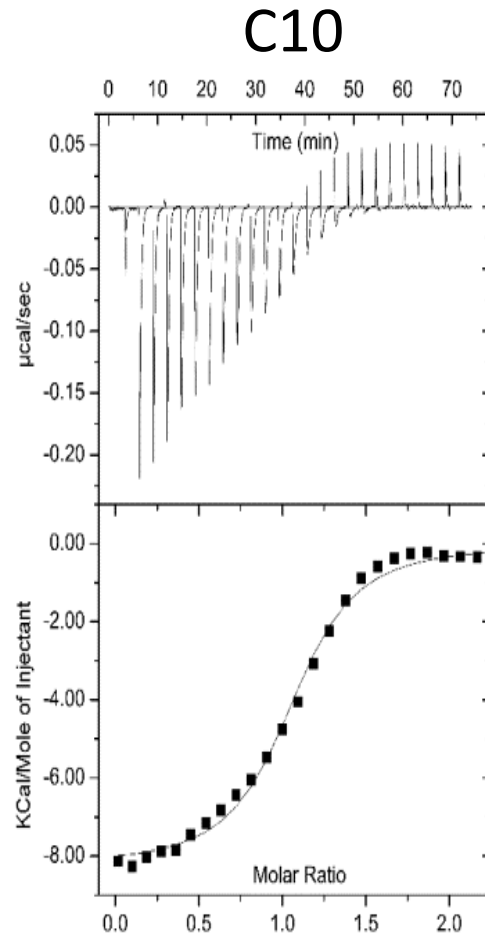
# Can 2-pyridones affect *L. monocytogenes* pathogenicity?

Large collaborative project with group Fredrik Almqvist and group Elisabeth Sauer-Eriksson at Umeå University



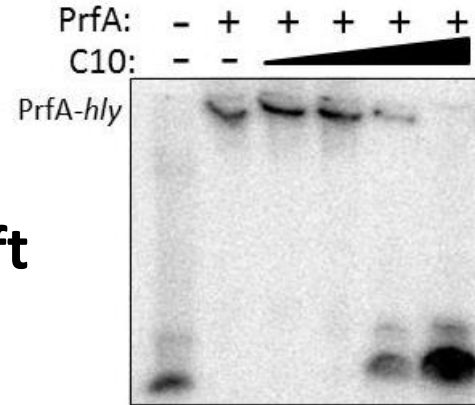
# The 2-pyridones bind directly to PrfA, preventing PrfA from binding DNA

Isothermal calorimetry

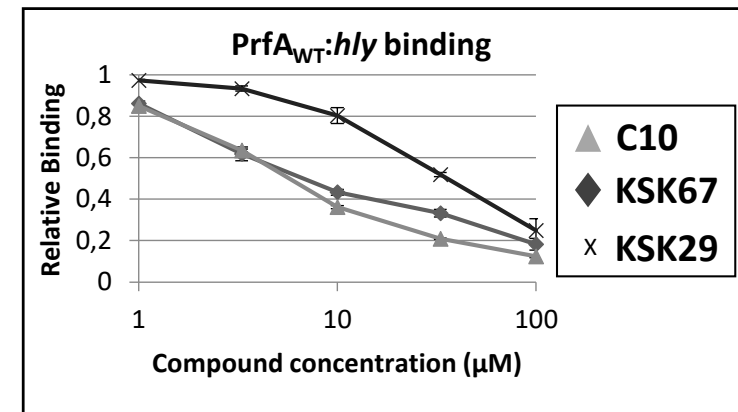


$K_D \sim 1.0 \mu\text{M}$

Gelshift



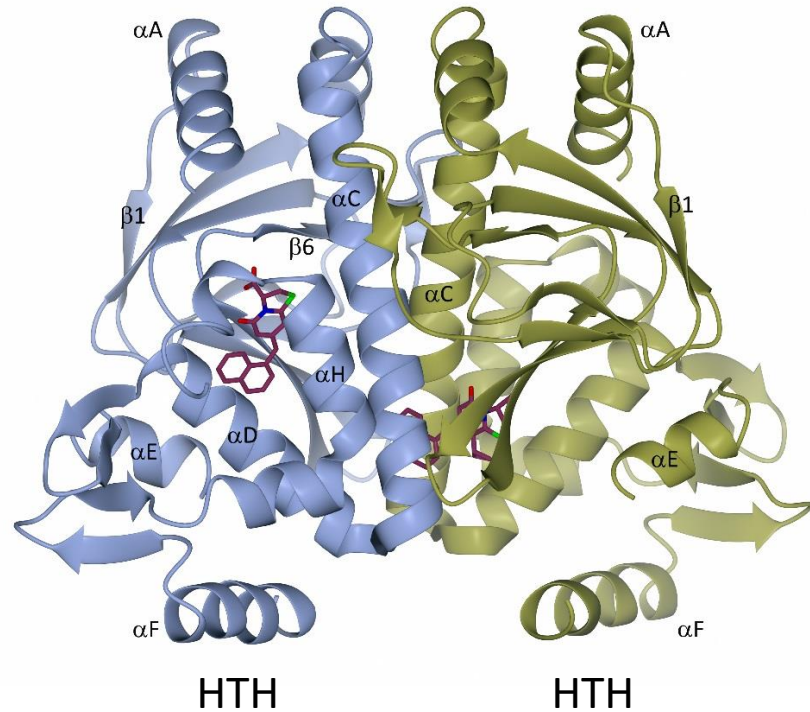
Surface Plasmon Resonance



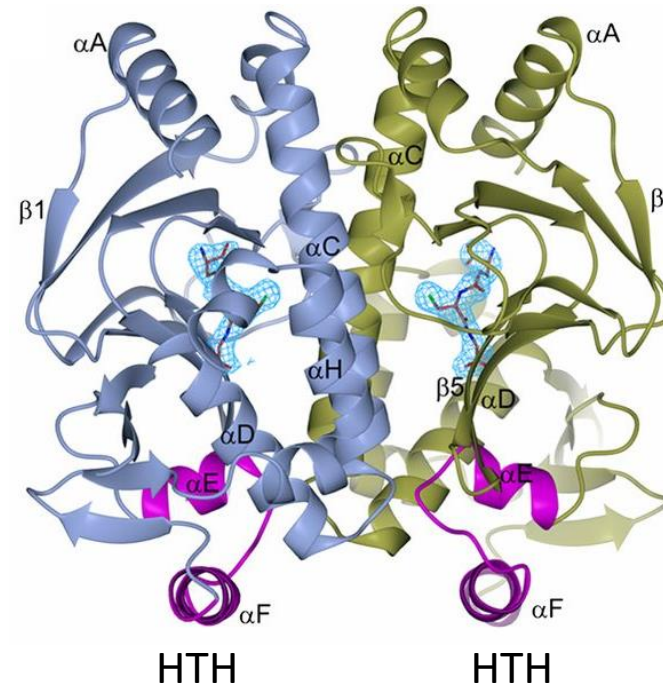


# How does the 2-pyridone:PrfA interaction look like?

With 2-pyridone

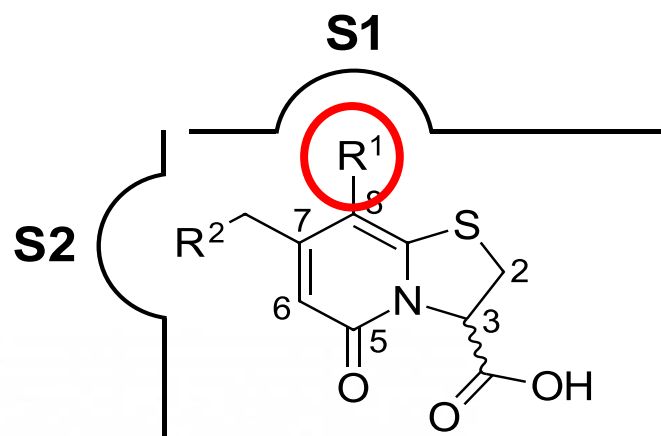
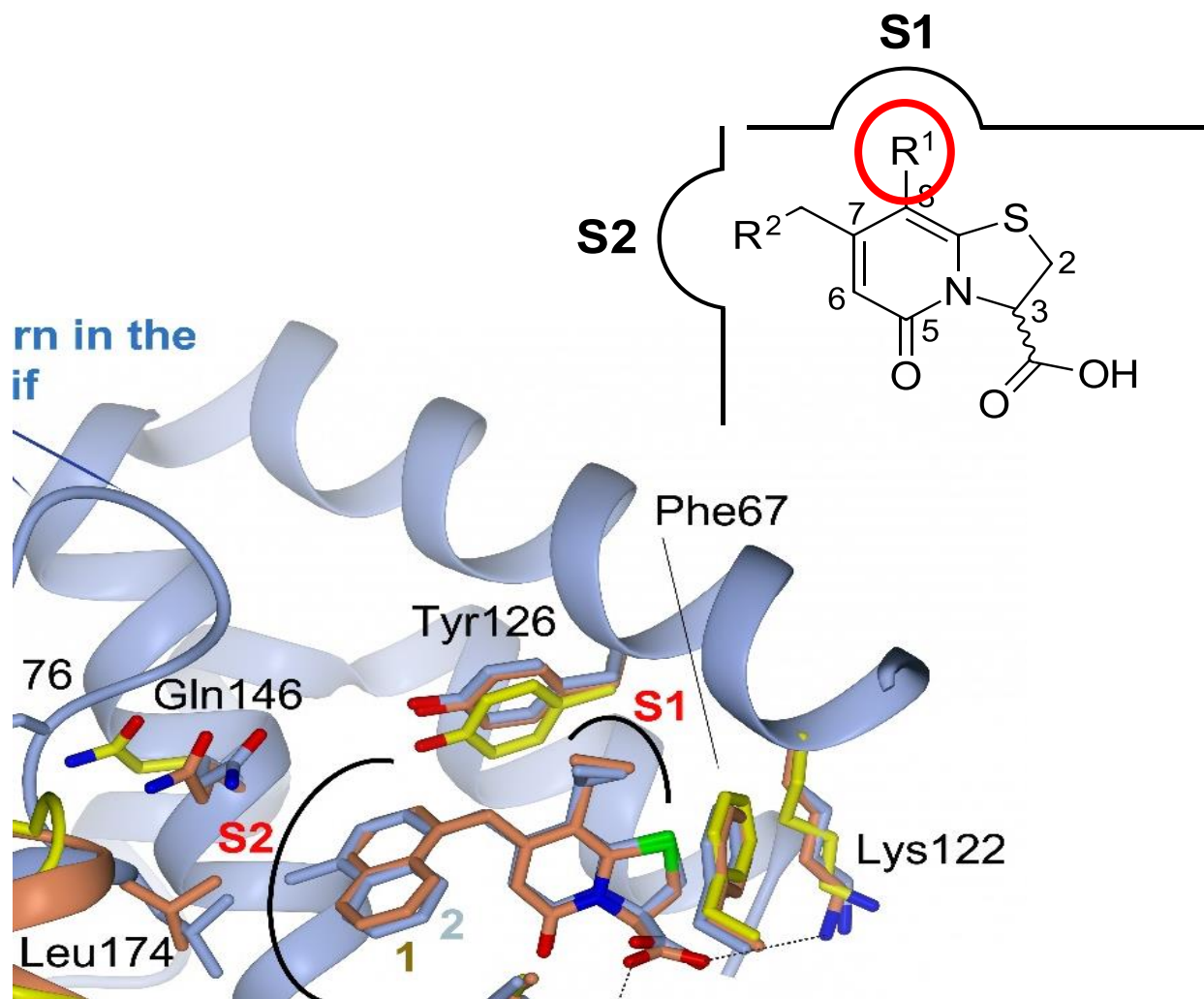


With Glutathione (co-activator)



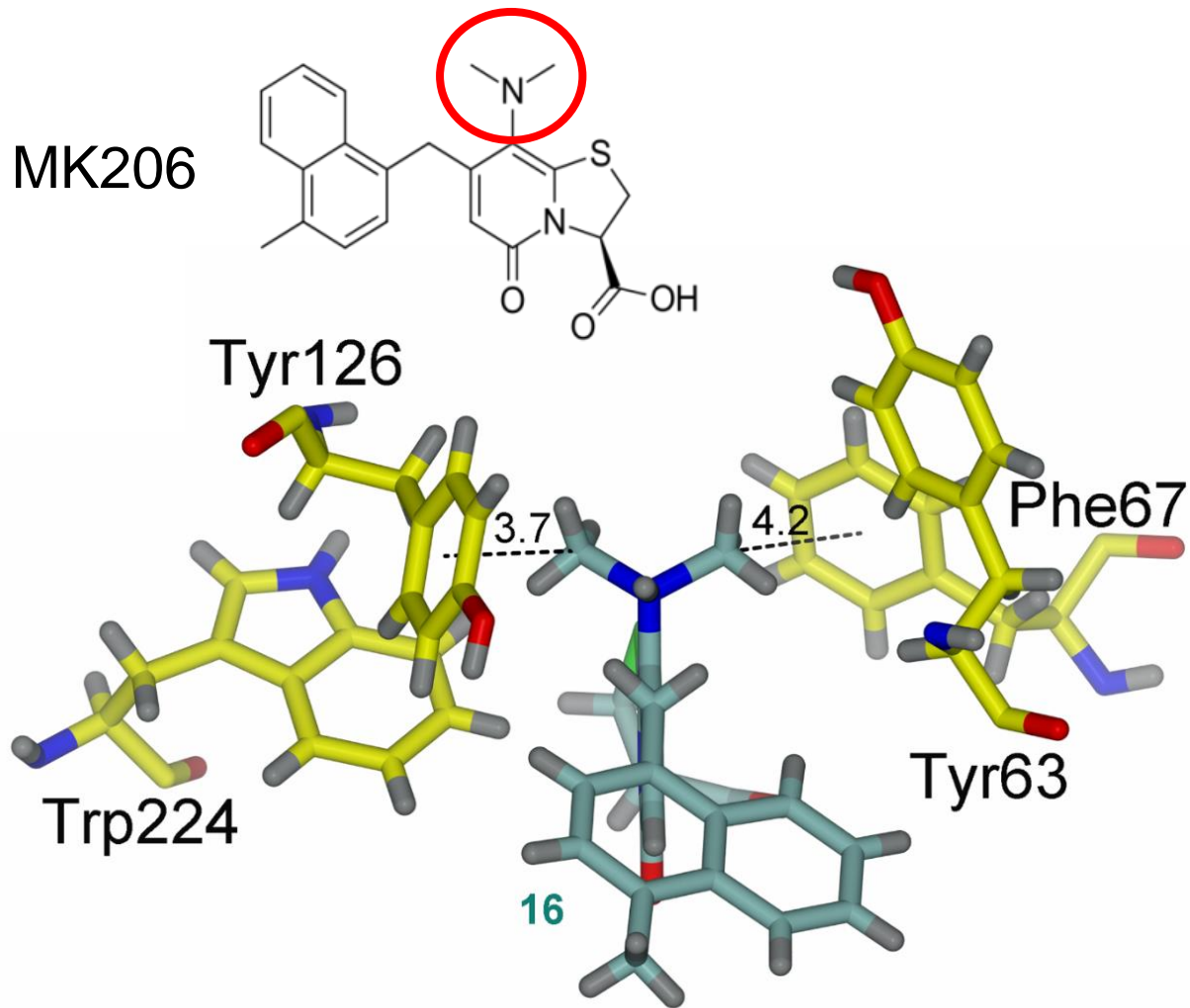
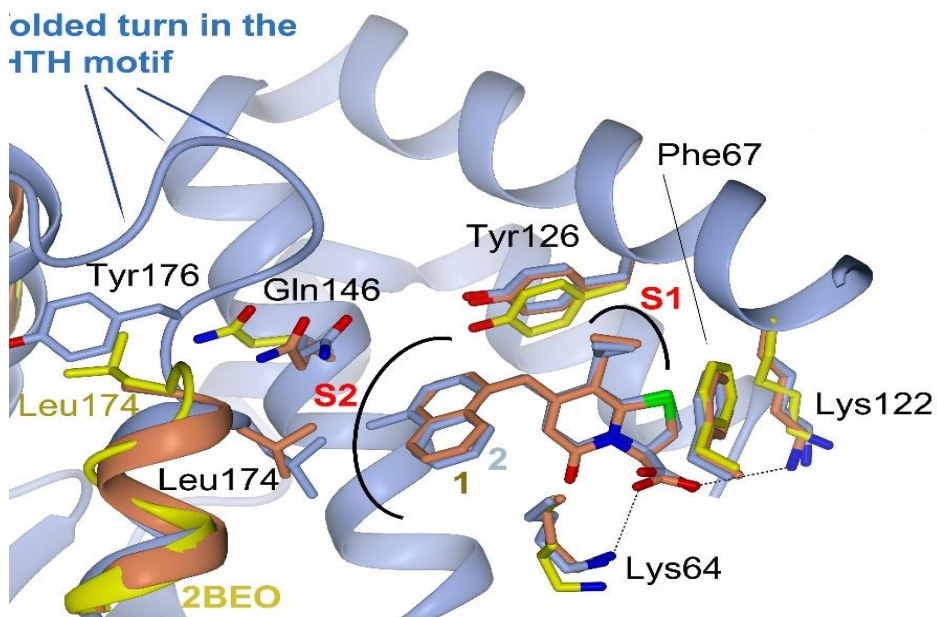
**2-pyridones binds to the same site as glutathione, but tilts the HTH to position preventing DNA-binding**

# Can the cyclopropyl group at position R1 be substituted by a more "sticky" group?



Substitutions (R1)	Effectiveness (Blocked uptake, IC <sub>50</sub> )
	~2 μM
	>20 μM
	>20 μM
	<0.5 μM

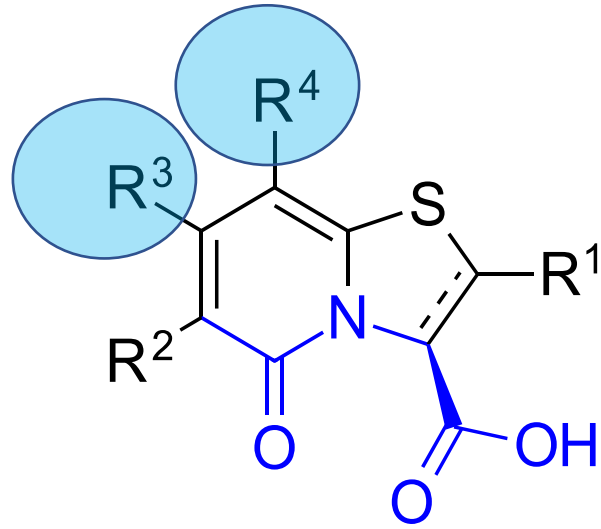
# Why does the dimethyl amine group show better properties compared with the cyclopropyl group?



A non-classical hydrogen bonding between the dimethylamino group of the compounds with the aromatic amino acids allow a strong interaction

# What about substituting other parts of the 2-pyridones?

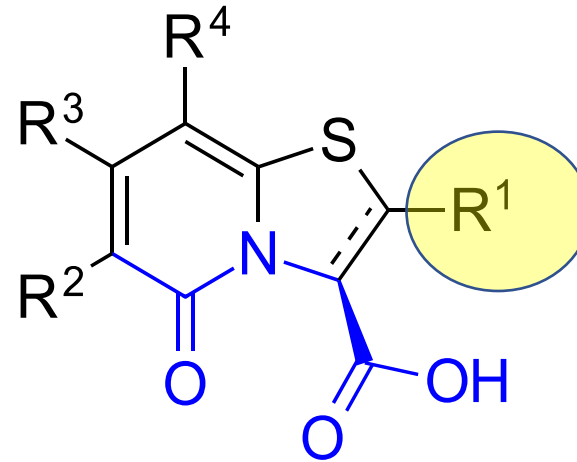
Virulence-blockers



Target



PrfA



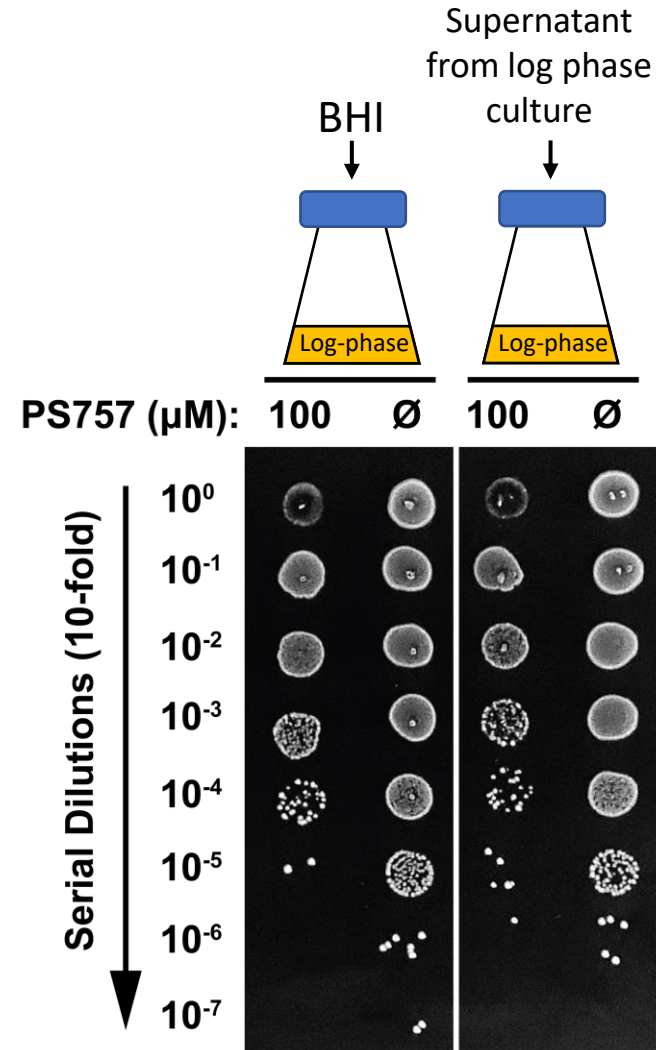
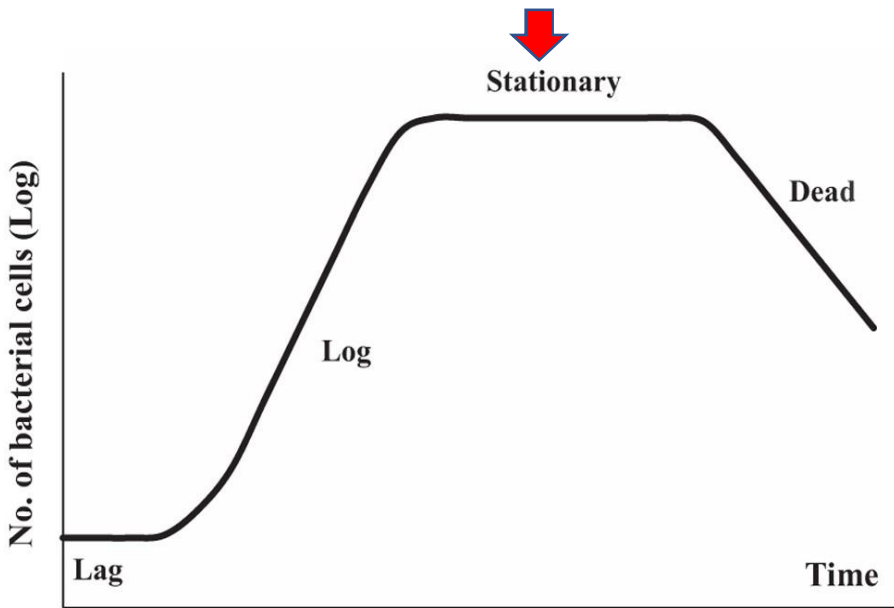
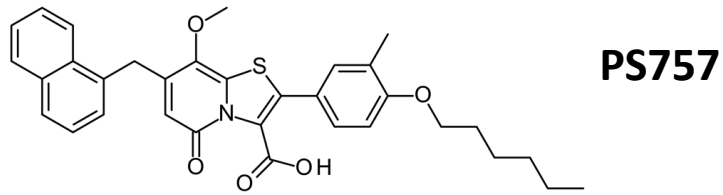
= GmPcides

Target



??

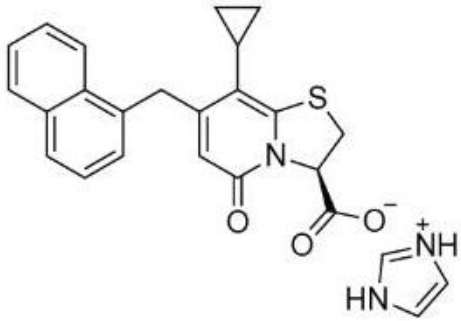
# GmPcides are bactericidal in stationary phase (non-growing) *Enterococcus*



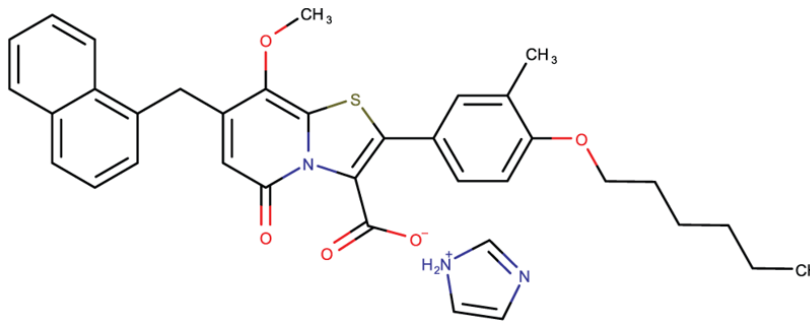


# GmPcides block *Listeria* virulence and binds PrfA

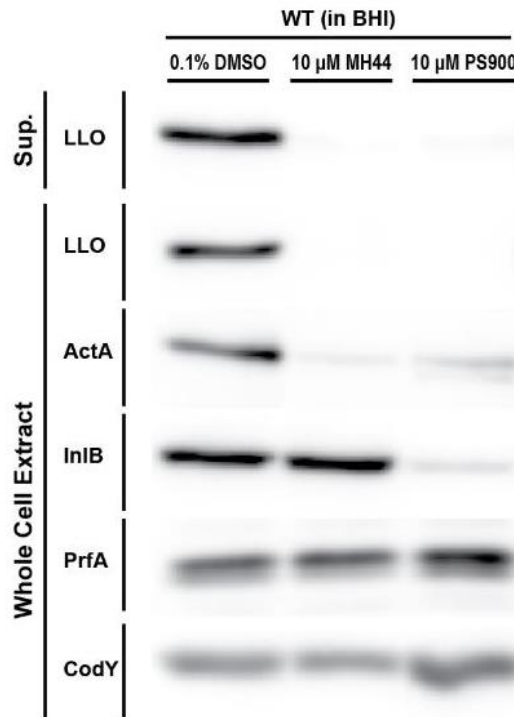
Virulence blocker (MH44)



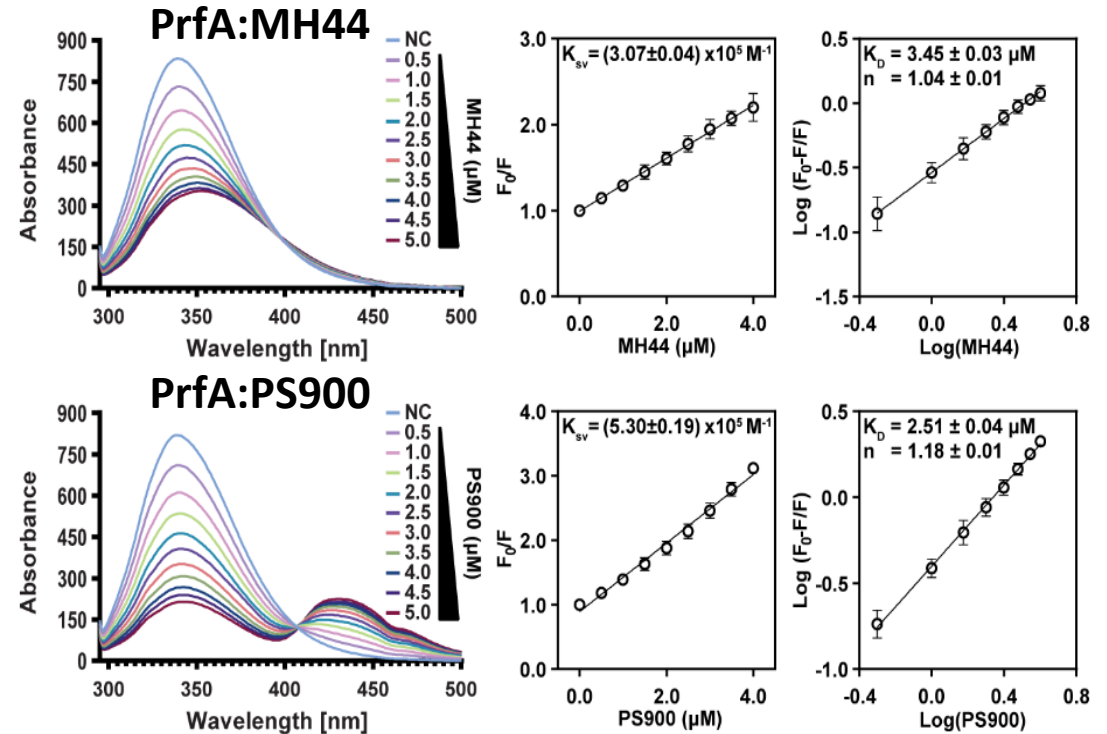
GmPcide (PS900)



***Listeria* virulence factor expression**



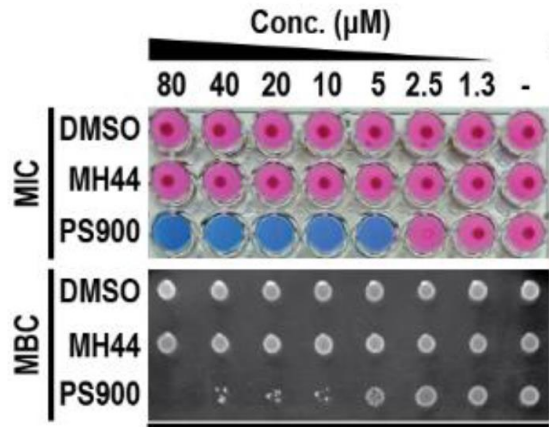
Fluorescence quenching spectra



**PS900 and MH44 bind PrfA equally well**

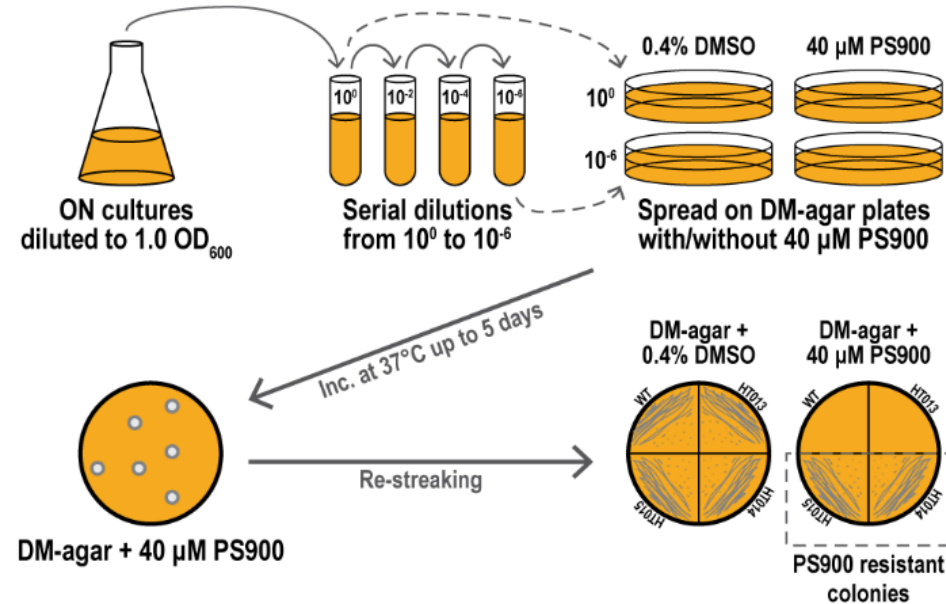
# PS900 (but not MH44) kill *L. monocytogenes* in defined minimal media

## Listeria growth and survival

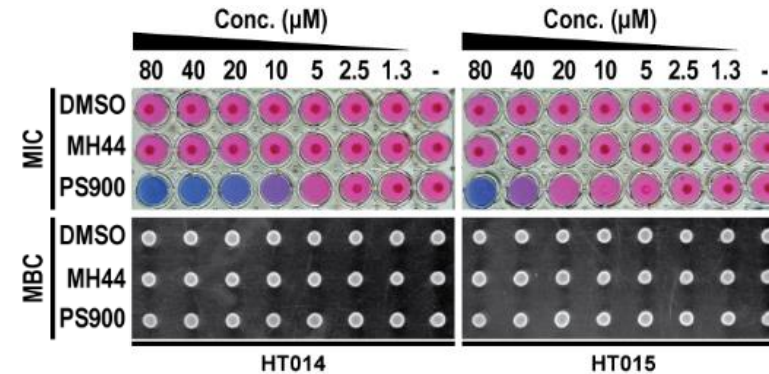


### Resazurin assay

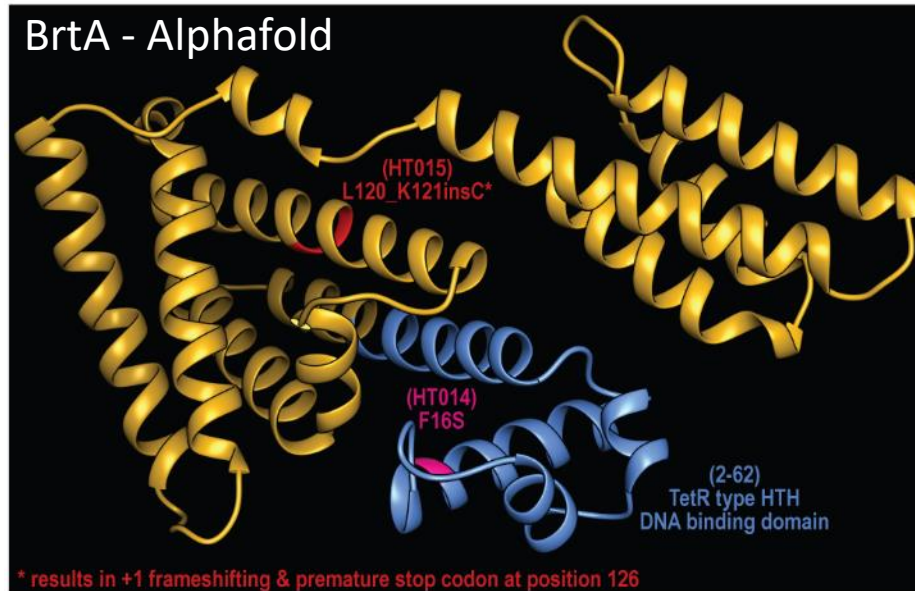
- Growth
- No growth



Two isolated mutants could grow at higher PS900 concentrations

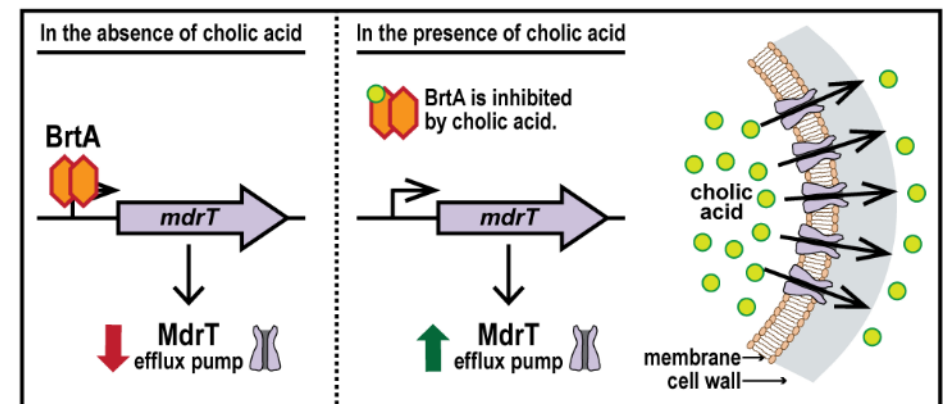


# HT014 and HT015 carry base-substitution mutations in the gene encoding the efflux repressor BrtA



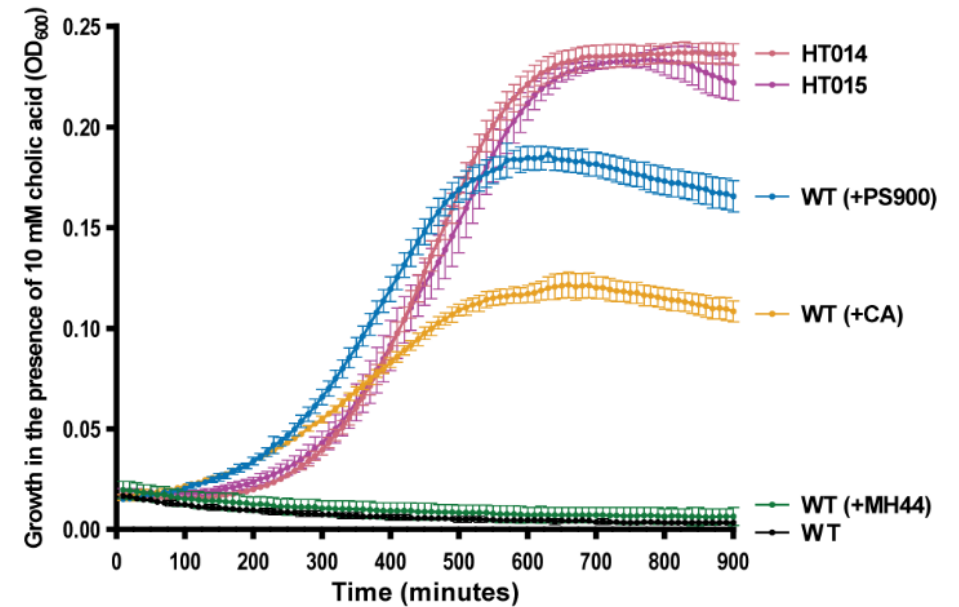
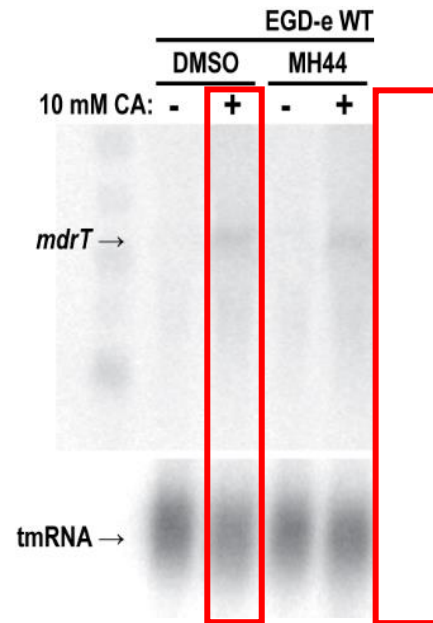
The amino acid substitutions in BrtA are located in the DNA-binding domain (HT014) or cause a premature translational termination (HT015)

BrtA is a transcriptional repressor of *mdrT* encoding the MdrT efflux pump

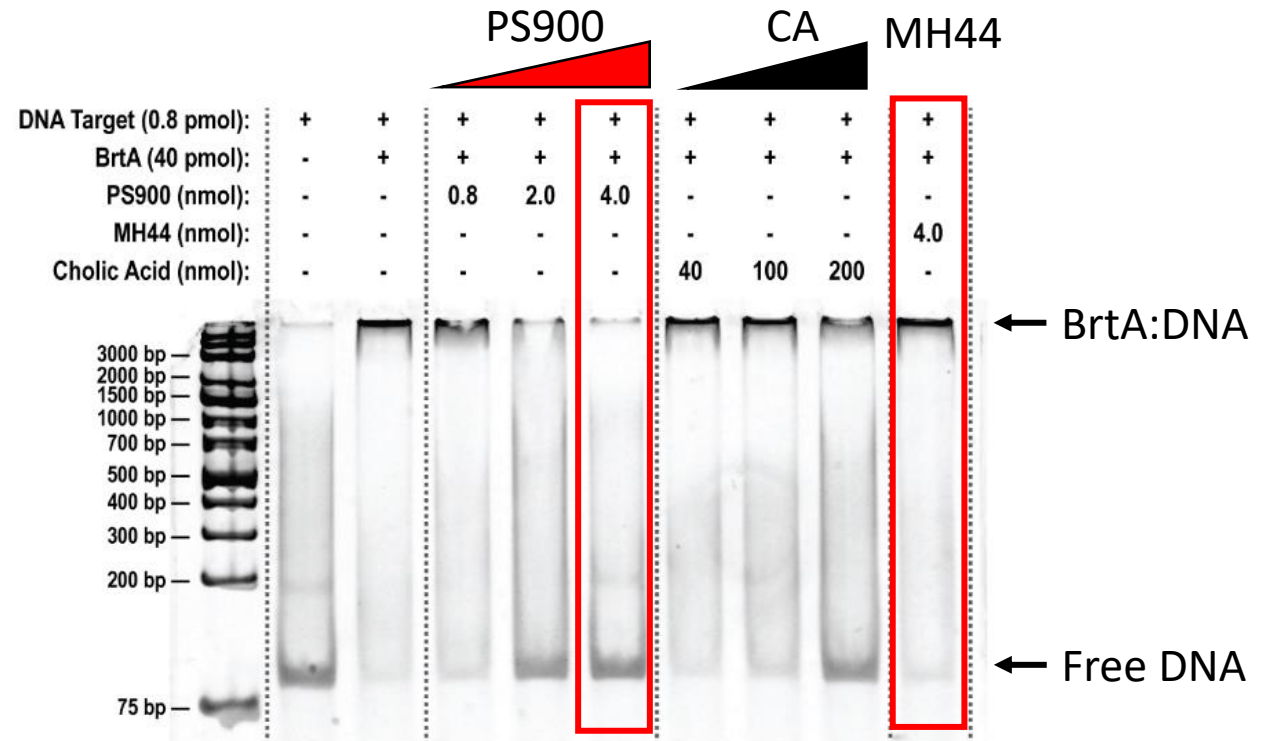
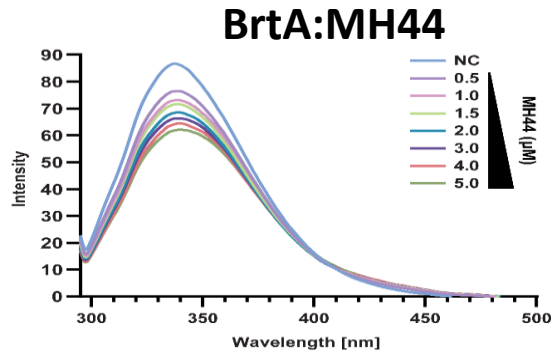
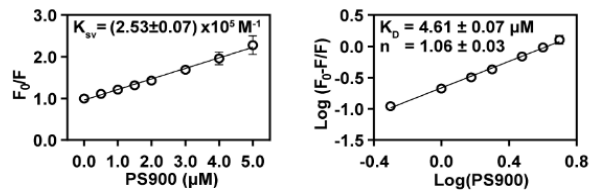
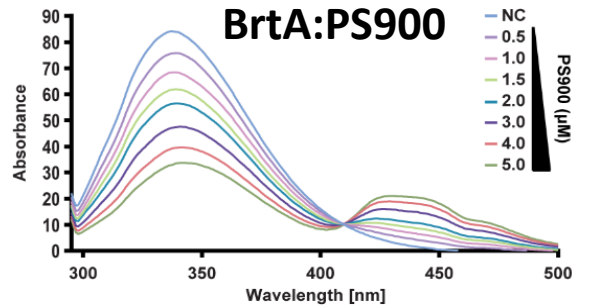


# PS900 induces *mdrT* expression which allow bacterial growth at elevated cholic acid levels

BHI

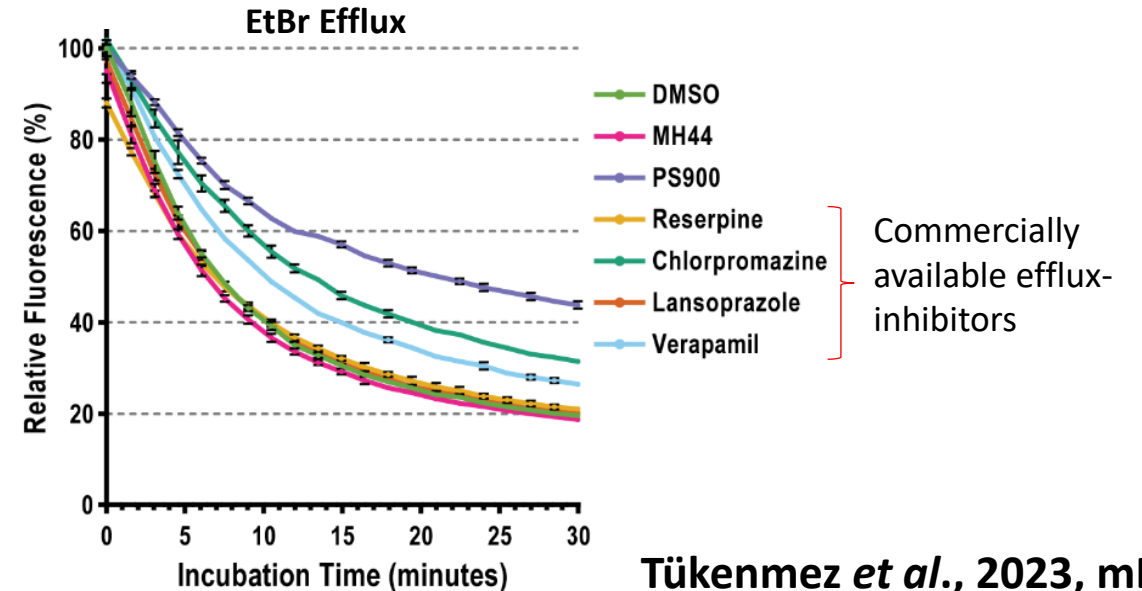
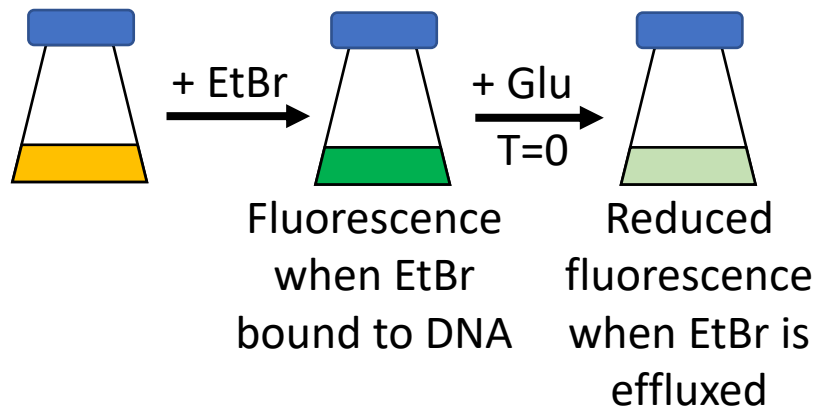
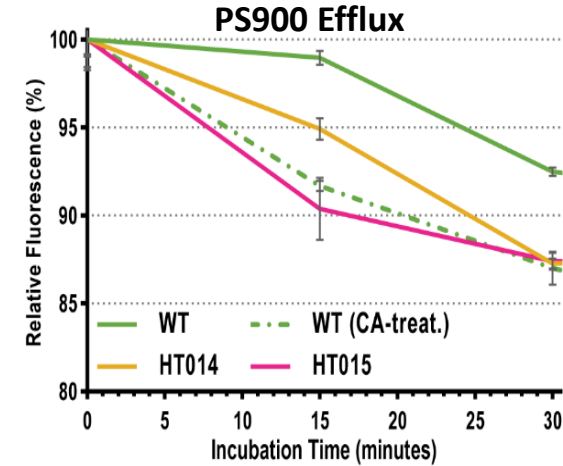
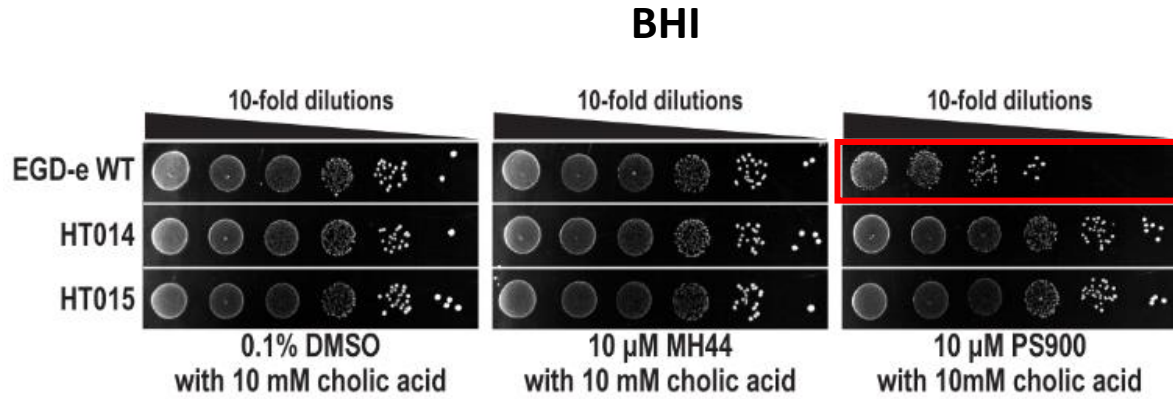


# PS900, but not MH44, can interact with BrtA and displace it from the *mdrT* promoter

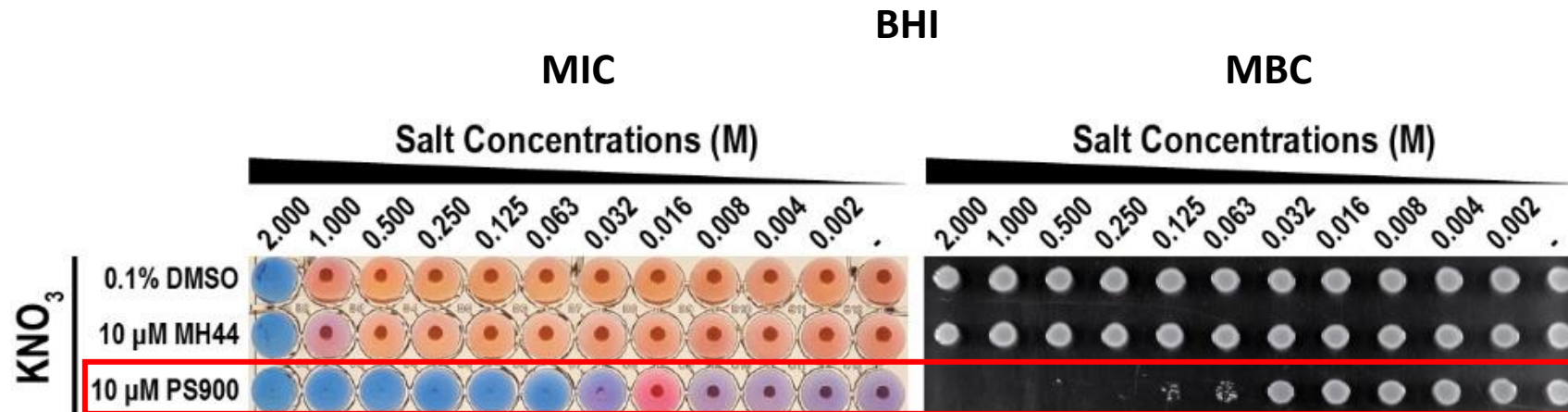




# Simultaneous addition of PS900 and MH44 is toxic for the bacterium, possibly because efflux is impaired

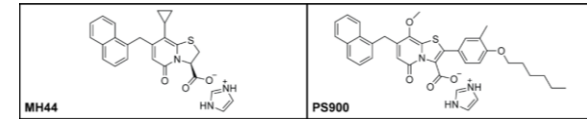


# PS900 potentiates sensitivity to osmotic salt stress, particularly Potassium Nitrate



# Summary:

- Virulence-blocking 2-pyridones bind PrfA and block its DNA binding capacity
- *Listeria* virulence can be inhibited by both MH44 and PS900 through PrfA-binding without affecting bacterial growth in BHI



- PS900 can block growth and kill *Listeria* in defined media
- PS900 binds and inactivates the transcriptional repressor BrtA, thereby inducing expression of the efflux pump MdrT
- PS900 sensitizes *Listeria* to osmotic salt stress

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**Group of Prof Elisabeth Sauer-Eriksson**

**Group of Prof Scott Hultgren**

**Group of Prof Mike Caparon**



**QureTechBio**  
Defeats bacterial virulence

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