RNA Binding Chaperone Hfq as a Novel Target for Antibacterial Drug Development

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Abstract

Objective. The continued rise of antibiotic resistance in nearly all bacterial strains demands urgent development of clinically efficacious antibacterial therapeutics with novel mechanisms of action. It is hypothesized that inhibition of the RNA-binding protein Hfq, which mediates gene expression of virulence factors, drug resistance mechanisms, and quorum sensing receptors in bacteria, will translate to greater susceptibility to the current antibiotic arsenal.

Methods. Two libraries of peptide-based inhibitory probes were designed based on the binding interfaces of Hfq. The first strategy leveraged the knowledge that Hfq exists dynamically in monomeric, hexameric, and high molecular weight forms; the homo-hexamer being required for Hfq-mediated gene expression. Based upon findings from site-directed mutagenesis, six putative Hfq hexamerization inhibitors were evaluated in vitro using gel electrophoresis and against wildtype E. coli. In the second strategy, a library of eight putative inhibitors of Hfq-sRNA binding was constructed and evaluated in vitro in a fluorescence-based assay and against wildtype E. coli.

Results. In an assay evaluating E. coli sensitivity, the tripeptide tyrosine-lysine-histidine (YKH) exhibited inhibition of bacterial growth at high concentration (1 mM). When dosed in combination with ampicillin (AMP), YKH exhibited nearly complete attenuation of E. coli growth compared to AMP or YKH alone denoting sensitization. Conversely, probes designed to inhibit Hfq-sRNA binding failed to demonstrate inhibition of E. coli growth or sensitization to currently available antibiotics.

Conclusions. Albeit at a high dose, preliminary findings mark the first demonstration of Hfq hexamerization inhibition in the field. This is a significant first step in the validation of Hfq as a next generation target for antibacterial drug discovery.

Antibiotic Resistant Infection & Hfq:

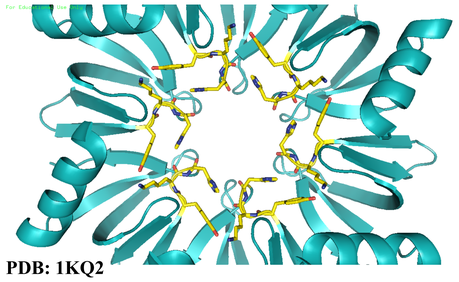
US Incidence & Fatalities:

2013: 2m infections, 23,000 deaths

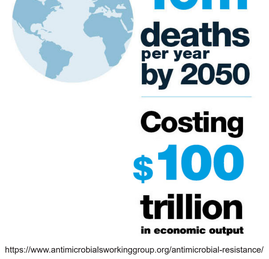
2019: 2.8m infections, 35,000 deaths

<5% pharmaceutical investment

CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019



PDB: 1KQ2



Role of Hfq in Pathogenic Bacteria:

Context:

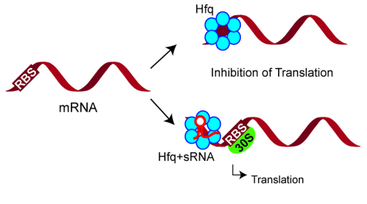
Regulatory small, non_coding RNAs (sRNAs) have been identified in bacteria that modulate gene expression. All appear to regulate gene expression via the accessory protein Hfq.

Species: Gram- and Gram+ (>50% Strains)

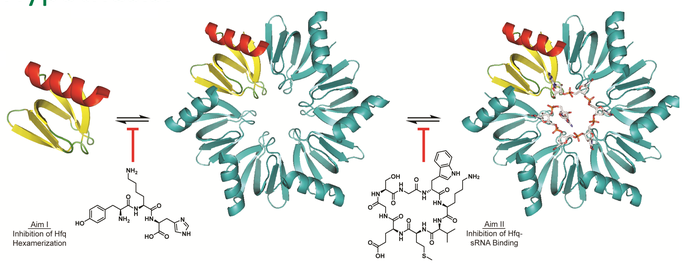
Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhimurium, Staphylococcus aureus, Yersinia pestis, Enterohemorrhagic *E. coli, Bacillus subtilis,Salmonella enterica, Shigella flexneri,*

Virulence Factors:

- Toxin Production
- Environmental Stress Tolerance (Macrophage Survival, Acid, Mammalian Hormones, Heat, Peroxide, Limited Nutrients)
- Drug Efflux Pumps
- Biofilm Formation
- Quorum Sensing Receptor Expression



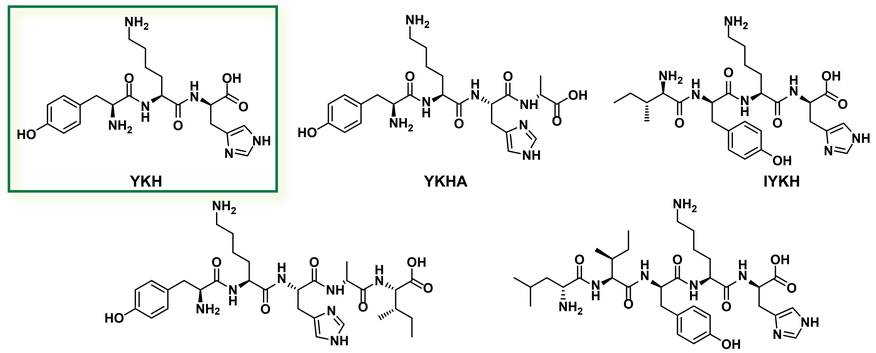
Study Hypothesis:



The central hypothesis of this research is that modulation of the sRNA-mRNA-Hfq complex will translate to reduced virulence-associated gene expression.

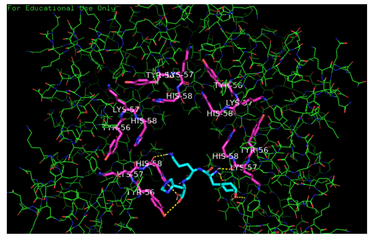
Library of Putative Hfq Hexamerization Inhibitors:

Sequence of YKH: L_{46}NSQGKQHLI**YKH**AI**STYTVE**$_{66}$

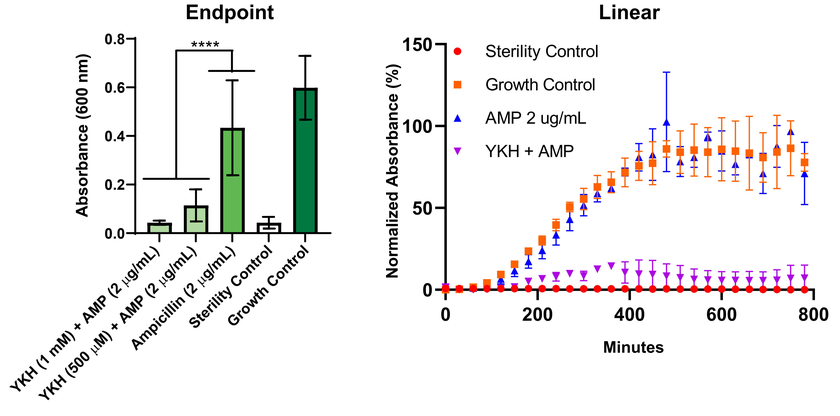


Docked YKH Exhibits Lowest Energy Conformation:

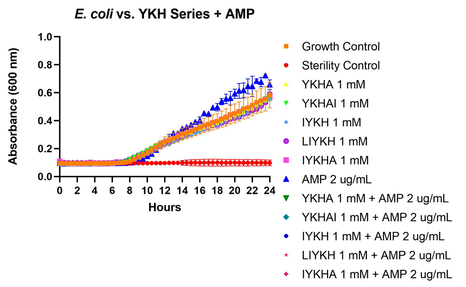
Software: AutoDock 4 (Scripps Research), PyMOL



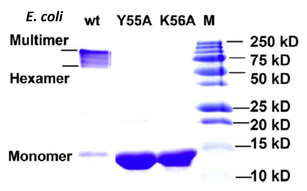
YKH Exhibits Synergistic Inhibition of *E. coli* Growth with AMP:



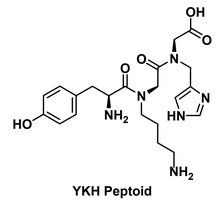
Other YKH Peptides Show No Growth Inhibition:



Future Directions:

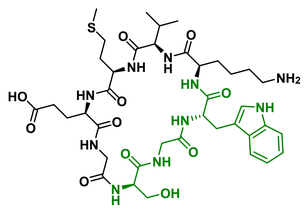


J. Mol. Biol. 2012, 417, 406-412.

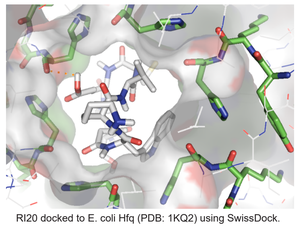


YKH Peptoid

Proposed Hfq-sRNA Binding Inhibitors:

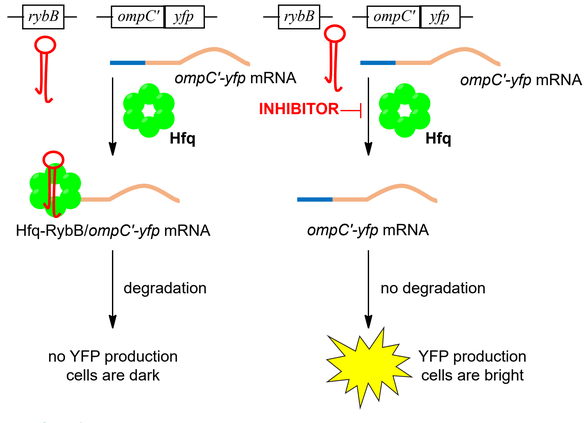


Placeholder Sequence for SICLOPPS Strategy: Screen Truncated Analogues



RI20 docked to E. coli Hfq (PDB: 1KQ2) using SwissDock.

Fluorescent Assay for Screening Inhibitors of Hfq-sRNA Binding:



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