Tackling biofilms and drug resistance: Targeting bacterial response regulators to resensitize multidrug resistant bacteria to antibiotics

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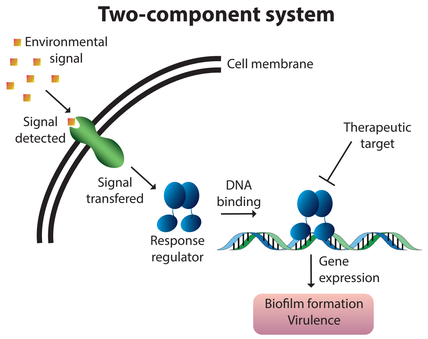
Abstract

The 2-aminoimidazole (2-AI) class of small molecules can inhibit the formation of bacterial biofilms, disperse existing biofilms, and even resensitize multidrug resistant bacteria to antibiotics. These compounds are active against both Gram-positive and Gram-negative bacteria, making them a powerful weapon against biofilms and the increasing threat of antibiotic resistance. We have identified bacterial response regulator proteins as a cellular target of 2-AI compounds. Response regulators are the transcriptional regulatory component of canonical two-component systems. Two-component systems allow a bacterium to detect and respond to changes in its environment, and often trigger quorum sensing, virulence factors, and initiation of biofilm formation. As such, response regulators have been highly sought after as therapeutic targets but have yet to be successfully exploited. The dynamic nature of response regulators makes structure-based drug design challenging. Here, we explore the interaction between response regulators and 2-AI compounds as a means of addressing multidrug resistance by using 2-AI compounds as adjuvant therapies. In this work, we focus on the response regulator BfmR, the master biofilm regulator from *Acinetobacter baumannii*. Using a combination of structural, biochemical, and microbiological techniques we are investigating the structure and function of BfmR and its interactions with 2-AI compounds. Understanding the impact of 2-AI small molecules on response regulators and their mechanism of interaction will lead to the development of more potent compounds that will serve as adjuvant therapies with broad-range antibiotics.



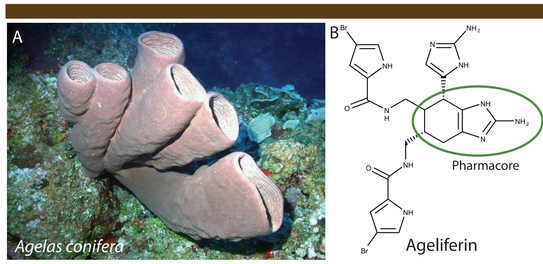


Figure 1) Ageliferin prevents bacterial colonization. The marine sea sponge, *Agelas conifera* (A), protects itself by secreting the bioactive compound, ageliferin (B). Ageliferin is toxic and not well suited as a drug. A library of ageliferin derivatives has been generated based on the 2-aminoimidazole ring acting as the pharmacore.

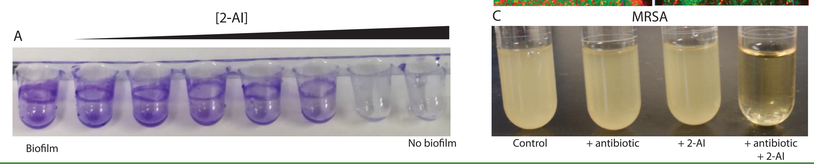


Figure 2) 2-AIs inhibit biofilm formation, disperse preformed biofilms, and resensitize bacteria to antibiotics. (A) Increasing concentrations of 2-AI inhibits the formation of *A. baumannii* biofilms. (B) Adding 2-AI to preformed *Pseudomonas aeruginosa* biofilms results in a significant depletion of biomass. (C) Multidrug resistant bacteria, like MRSA, become susceptible to antibiotic treatement upon addition of 2-AI.

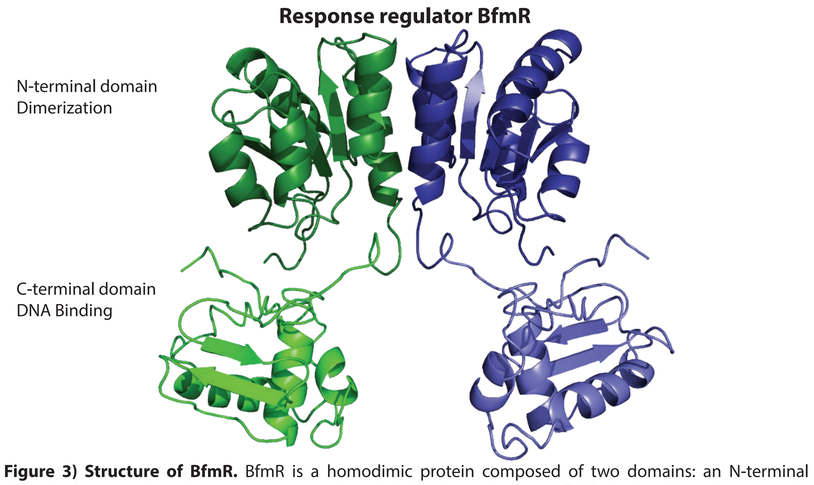


Figure 3) Structure of BfmR. BfmR is a homodimic protein composed of two domains: an N-terminal dimerization domain which houses the putative histidine kinase site, and a DNA binding C-terminal domain. Due to the flexible nature of the linker that connects the two domains, structural information has been limited. We generated a hybrid structure of BfmR using X-ray crystallography for the N-terminal domain, NMR for the C-terminal domain, and chemical crosslinking data bring the domains together.

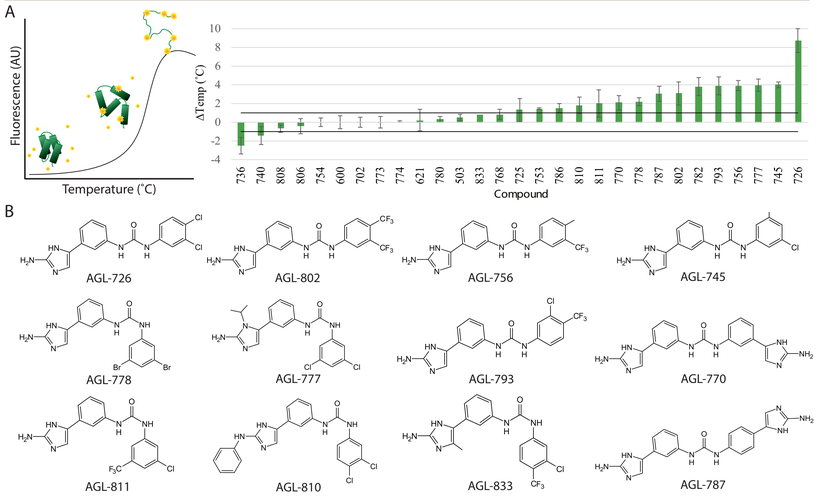


Figure 4) 2-AIs bind the BfmR response regulator. (A) A fluorescence-based thermal shift assay was employed to quickly screen a library of compounds for binding interactions with response regulators. An increase in the protein's melting temperature (T_m) correlates to an increase in protein stability, and suggests the formation of a protein–ligand complex. (B) 15 compounds (2 not shown due to patent pending) significantly increased the T_m of BfmR, suggesting binding interactions.

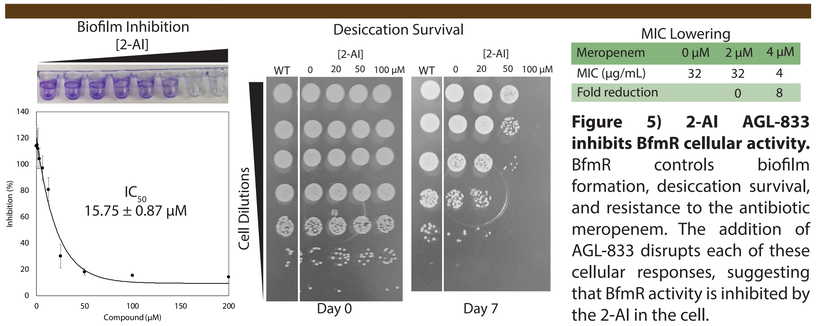


Figure 5) 2-AI AGL-833 inhibits BfmR cellular activity. BfmR controls biofilm formation, desiccation survival, and resistance to the antibiotic meropenem. The addition of AGL-833 disrupts each of these cellular responses, suggesting that BfmR activity is inhibited by the 2-AI in the cell.

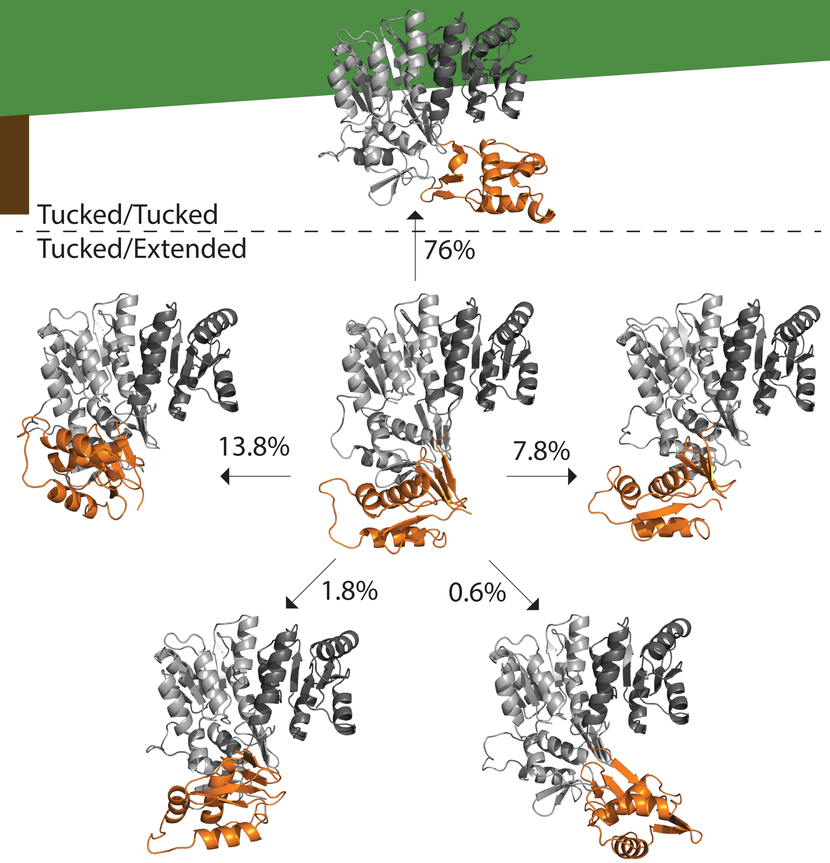


Figure 6) Dynamics of response regulators in solution. PmrA (a homologue to BfmR) binds DNA (center) in a "tucked/extended" state in the crystal structure. Molecular dynamics simulations reveal that the protein quickly relaxes to a "tucked/tucked" state. On a 250 ns time scale, 76% of the population is in a "tucked/tucked" state. Variations on the "tucked/extended" state are sampled for very short periods of time and account for small portions of the population at a given time.

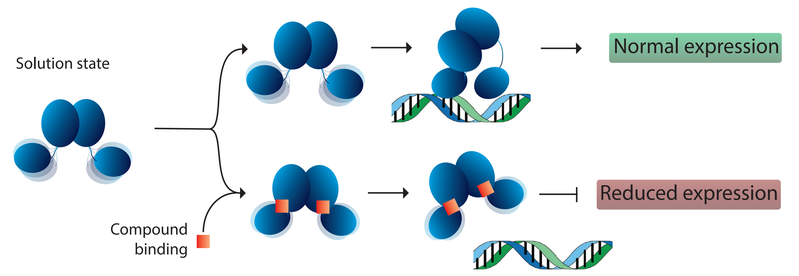


Figure 7) Model for 2-AI mechanism of response regulator inhibition. In solution, response regulators are predominantly in the "tucked" conformation. In the absence of inhibitor, the response regulator is free to move the C-terminal domains. This assists in binding the target DNA and allows the N-terminal domain to "kneel" over one of the DNA-binding domains. Binding of a 2-AI compound likely increases the interactions between the N- and C-terminal domains, impacting the protein's ability to position both DNA-binding domains on the promotor substrate and "kneel" over the N-terminal domain.

Conclusions

- Targeting response regulators represents a promising approach for combating drug resistances
- Inhibition of response regulators would shut off a bacteria's ability to detect external signals, removing its means of protection.
- Biochemical and cellular data confirm that BfmR is a cellular target of 2-AI compounds.
- Understanding the structural and functional interactions between response regulators and 2-AIs will allow us to design more potent compounds.