INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Leveraging Allosteric Transcription Factors To Detect Small Molecule Threats

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Microcystin is a hepatotoxin produced by harmful cyanobacteria globally. Cyanobacteria, also known as blue-green algae, are single-celled, photosynthetic organisms that can accumulate to dangerous levels, causing harmful algal blooms in aquatic environments. Toxin production by cyanobacteria is influenced heavily by anthropogenic influences such as the presence of fertilizer or other organic runoff (phosphate or nitrate waste products) into bodies of water. Microcystin (MC-LR) is a well-studied cyanotoxin due to its wide distribution and high toxicity. This specific cyanotoxin inhibits eukaryotic protein serine/threonine phosphatases 1 and 2A and causes oxidative stress in liver tissue. Tools are necessary to aid scientists and warfighters in detecting MC-LR and other small molecule toxins in freshwater systems to monitor freshwater and drinking water systems. For example, allosteric transcription factors (aTFs) have the ability to act as both sensors and switches, making them useful tools in the field of synthetic biology as well as in vitro biosensing. Transcription factors can act as small molecule-induced regulatory proteins that bind to DNA and regulate DNA transcription in bacteria. Additionally, transcription factors can form binding pockets that, when bound to a ligand such as a small molecule toxin, cause the transcription factor to undergo a conformational change, therefore releasing from DNA. Previously, an aTFs library was designed at the University of Wisconsin-Madison to utilize aTF-based biosensing by designing a library of engineered aTF-containing unique individual RNA barcodes within Escherichia coli. In this study, we aimed to determine whether microcystin-LR can bind in any of the aTF binding pockets within the library to evaluate if engineered transcription factors are a feasible detection system for small molecule targets. The E. coli aTF library was incubated with 100μM of MC-LR to allow for differential expression of the reporter constructs. Deep sequencing results indicated that numerous aTFs within the library were able to bind to MC-LR, indicated by differential expression of the corresponding RNA barcode. This preliminary data demonstrates that aTFs could be used as sensors to detect small molecule toxins in the future. This collaboration between DEVCOM CBC and University of Wisconsin-Madison highlights the importance of using basic research principles to expand into the field of toxin detection.

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