REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

Optimizing Cell-free Reaction Composition To Accelerate Toehold Switch Sensor Response Time

Steven Blum U.S. Army DEVCOM Chemical Biological Center **Stephanie Cole** U.S. Army DEVCOM Chemical Biological Center
Jennifer Lee Defense Threat Reduction Agency **Casey Bernhards** U.S. Army DEVCOM Chemical Biological Center
Daniel Phillips U.S. Army DEVCOM Chemical Biological Center **Katherine Rhea** U.S. Army DEVCOM Chemical Biological Center
Aleksandr Miklos U.S. Army DEVCOM Chemical Biological Center **Matthew Lux** U.S. Army DEVCOM Chemical Biological Center

Recent developments in the field of synthetic biology have made robust and rapidly deployable diagnostics for traditional and evolving biological threats achievable at low cost and with low burden for the Warfighter. Through the use of cell-free expression systems (CFEs), we are developing a threat detection platform which will mitigate the shortcomings of current state-of-the-art approaches such as PCR-based analyses and lateral flow immunoassays (LFAs). By lyophilizing CFEs on paper tickets and incorporating software-designed RNA toehold switches which recognize distinct threat agent nucleic acids, our proposed device can be programmed for novel threats, and be rapidly deployed to austere environments without the need for cold-chain storage or laboratory conditions for processing. While promising, a major challenge in pushing forward this technology is a need to accelerate assays to be comparable with LFAs for practical field use. Early versions of this approach required multiple hours to execute including sample preparation and target amplification, far from the 15-30 minutes typical for LFAs. Here we focus on optimization of the speed of the CFE reaction to close this gap.

Our current system utilizes an RNA toehold switch which regulates the expression of a lacZ gene, producing β-galactosidase, which in turn cleaves chlorophenol red-β-D-galactopyranoside (CPRG) substrate to produce an eye-readable colorimetric output. Initial testing with the commercially available PURExpress (NEB) CFE system produced positive identification outside of our targeted timeframe of 30 minutes. Given this, we sought to increase reaction speed on paper tickets in a number of ways. In addition to exploring various reporter methods, we developed a LacZα knockout lysate as an alternative to the PURExpress system and to reduce background signal typically present with E.coli-based lysates. We conducted comparison studies in liquid reactions on plate readers and in freeze dried reactions on paper. Once we determined that our in-house lysate produced significantly faster results, reaction optimization studies were conducted to determine the ideal colorimetric substrate concentration, lyophilized reaction to rehydration volume ratio, and total lysate percentage in reaction mixtures. Reaction additives to increase signal speed were also investigated, including supplementation with Triton X-100 and polyethylene glycol (PEG 8000), and by increasing the energy source adenosine 5`triphosphate (ATP). By combining our tested optimization strategies in our lysate system, we achieved a 20.9% reduction in time-to-signal in the control reactions and a 33.4% reduction in the toehold switch reactions, as compared to our initial lysate reaction conditions. With these efforts, the time to signal was reduced to ~25 min. This is a dramatic improvement over our preliminary testing utilizing the commercial system where the time to signal was ~1 hour for switch regulated reactions on paper.