## INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

# Evaluating Engineered Protease Chain Reactions As An Adaptable, Cross-platform Technology For Detecting New And Emerging Biological Threat Agents In Laboratory And Field Environments

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Protease chain reactions (ProCRs) use specially engineered proteases/inhibitor complexes to generate sensitive and highly specific signal cascades that can be leveraged to detect and measure the concentrations of bacteria, virus, and protein targets. The novel isothermal assays work by exploiting micro-environments in which transiently freed proteases function to cleave and activate other inhibited proteases, triggering a visually detectable chain reaction. ProCR kinetics are such that when antibody-bound proteases coalesce around soluble targets, the localized enzymes become more likely to lose their inhibitors and initiate robust signal cascades. This mechanism could allow Department of Defense laboratories to quickly reprogram, multiplex, and cross-link engineered cascades to detect and enzymatically integrate signals from new and emerging biothreat agents in field-forward environments. To explore the flexibility and utility of this innovative cross-platform technology, we evaluated the abilities of antibody-bound proteases to detect and quantify different protein, virus, and bacterial targets using various benchtop and hand-held-based detection modalities. Our study has highlighted several opportunities and challenges for adapting ProCR to meet the unique priorities and needs of the warfighter and broader biodefense community.