INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Cell-free Detection Of Small-molecules Using Nanobody Conjugates

David Garcia U.S. Army DEVCOM Chemical Biological Center **Justin Modica** Northwestern University **Eric Berns** Northwestern University **Aleksandr Miklos** U.S. Army DEVCOM Chemical Biological Center **Milan Mrksich** Northwestern University

Assay formats such as ELISAs have effectively employed antibodies for detection of small molecules, but often require numerous steps, complex protocols, and lack modularity, making them useful but ultimately unfieldable technologies for the warfighter. Nanobodies are small, single chain forms of antibodies and hold several advantages for use as biological sensors, including their compact size (~12-15 kDa), superior stability, and monomeric structure that make them amenable to rapid evolution towards desired targets. In this work, we have developed sensors incorporating nanobodies that bind to insecticides fipronil and cyanitriliprole that when fused to FRET-compatible fluorescent proteins produce an effective sensor for the insecticides. To reduce the complexity of the optics needed for measuring the sensor signal, the FRET pair was replaced with a split luciferase and the system was further tested in one-pot, lyophilized cell-free reactions containing the DNA for the fusion proteins, the organic dimerizer, and one of the small molecule targets. We found that both liquid and lyophilized cell-free reactions produced a visible signal presence of either of the nanobody targets. We hypothesize that the continued development of nanobody-based biological sensors will enable high-throughput production of fieldable detection tools for a variety of chemical and biological threats.

This research is funded by the Congressional Cell-Free Biomanufacturing initiative.