INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Nanobody Engineering For Novel Diagnostics

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Nanobodies, single-domain antibody fragments produced naturally by camelid species, represent the smallest antibody fragments which retain the ability to bind to an antigen. These fragments have many advantages including their small size, high stability, and low cost of expression. Additionally, as single-domain fragments, nanobodies do not require light-chain pairing, simplifying their incorporation into bispecific constructs. Recently, nanobodies have generated great interest for use in a multitude of applications, including diagnostics and therapeutics, for both of which we are actively developing novel nanobodies and engineering strategies.
As diagnostics, nanobodies can be applied to rapidly and reliably detect numerous biothreats, including infectious bacteria, viruses, and toxins. In our project, Cascade Amplification for Biological Sensors (CABS), we are developing nanobodies for diagnostic applications in a low-cost, portable, and highly accurate detection system. This novel detection method, while as accurate as a polymerase chain reaction, paves a new pathway into detection systems through nanobody-driven fluorescent protein amplification. Rather than working to alter the heavily studied PCR, we have utilized nanobodies to create a new technology that gives extreme accuracy without the need for complex reagents or machines, in a fraction of the time. This system utilizes pairs of nanobodies which bind distinct regions (epitopes) on fluorescent proteins to initiate a visibly-detected complementation cascade. Applying pairs of nanobodies in this manner enables greater sensitivity and specificity of biothreat detection. Through this project we have worked to discover and affinity mature novel nanobodies which target fully assembled split fluorescent proteins, and methods and results will be presented herein. Desired nanobodies are selected using fluorescence-activated cell sorting (FACS) of yeast displayed libraries and characterized by flow cytometry. These techniques are also applied for selecting nanobodies specific to a desired epitope and binning for validation. Nanobodies tagged with components of split fluorescent proteins are also produced, purified, and characterized as soluble proteins using a low-cost system. Our data on binding characteristics, affinity maturation, split fluorescent protein tag and linker optimization for three different split fluorescent proteins (GFPtri, RFPtri, and Calgreentri) developed at LANL indicates that the sensor system is compatible with multiple fluorescent proteins and nanobody pairs. Data on the modification of nanobodies identified in the literature and de novo selection of nanobodies toward novel fluorescent proteins will also be discussed. In our selections, we demonstrate the application of advanced sorting techniques. Such methods enable us to select nanobodies which recognize only the fully assembled forms of the tripartite fluorescent proteins. The advanced conformational detection provided by these engineered nanobodies critically contributes to the sensitivity of the overall CABS detection method. These nanobodies are incorporated into the amplification sensor and used for detection of model biothreats, creating a fast, highly accurate system that can be readily deployed for a warfighter to use in the field with minimal to no prior training required. The nanobody complementation cascade represents the final component of CABS development. The techniques developed in this project demonstrate the contributions of nanobodies to warfighter health and biosecurity.
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DTRA Grant Cascade Amplification Binding Assay