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

A Platform For Rapid Design And Discovery Of Bres For Emerging Threats

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In a world of global pandemics and increased biological weaponization, the need for rapid and accurate diagnostics is more pertinent than ever. One component common to all sensing platforms is the bio-recognition element (BRE), which sits at the interface between sensor and the sample matrix and is responsible for detecting the presence or absence of the target molecule. While this role has traditionally been filled by antibodies, aptamers are a relatively new class of BRE gaining favor due to their thermal stability, low immunogenicity, and ease of synthesis. Aptamers are short, unique sequences of oligonucleotides (DNA/RNA) or amino acids (peptides) that adopt a specific three-dimensional structure with the potential for target binding. Aptamer selection, however, is very time-consuming and requires many rounds of both positive and negative selection followed by sequence amplification and purification, with no guarantee that the selected sequence will bind the target with high affinity and specificity. To improve this process, we have established a platform for high-throughput aptamer selection designed to rapidly respond to emerging threats and newly discovered biomarkers. Here, we use in silico structural and computational modeling to generate large libraries of sequences with predicted binding to a target of interest. These libraries are screened for target binding using microarrays capable of holding up to 1 million unique sequences. This approach is unique in that all sequences can be assessed in parallel with no secondary amplification step needed. Binding sequences identified on the microarray are then down-selected for comprehensive structure and binding characterization using techniques such as bio-layer interferometry (BLI), isothermal titration calorimetry (ITC), surface plasmon resonance (SPR), ELISA, and/or nuclear magnetic resonance (NMR). This process can be repeated, as necessary, until final selection of an aptamer with high binding affinity and specificity to the target of interest is achieved. Four main advantages of this pipeline are 1) the speed to aptamer discovery, 2) compatibility with a range of sample matrixes, including minimally invasive sample types such as sweat, saliva, urine and interstitial fluid, 3) applicability to diverse target molecules, and 4) sequence customization for sensor integration. Here we show results for a range of targets including viral proteins, biotoxins, and small molecule biomarkers. Selected aptamers have target-binding affinity at levels relevant for operational sensing and have been transitioned into a variety of sensing platforms. As new biological threats or biomarkers are identified, we believe this platform will enable the rapid discovery of BREs required for development of sensors and diagnostics to new emerging threats.

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