Microsphere-based Detection of Biological Toxins and Signaling Molecules using Renewable Surface Microfluidic Platforms with Enhanced Mass Transport and Capture

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Biodetection of protein toxins and signaling molecules requires efficient capture from complex media followed by sensitive detection. To be useful in screening or clinical applications such detection methods must also be rapid. PNNL has developed bead-based approaches to capture and separate protein analytes from complex media and assemble fluorescently-labeled antibody sandwich complexes for detection. Bead sizes range from seven microns to ca. one hundred microns, depending on the assay approach, while labels include stable Alexa Fluor dyes and semiconductor quantum dot nanoparticles. We have found that perfusion of analyte samples through microscale packed columns of microspheres provides enhanced capture, while washing away unretained matrix components. Completion of labeling creates a selective fluorescent signal that may be read directly on the microcolumn as a sensor, or the beads may be released and transported downstream for fluorescent detection in a separated spatial location. Using our renewable column configuration, and flow cytometry detection, we have demonstrated botulinum toxin detection to levels as low as 1 pM in ten minutes. Work with toxin and cytokine analytes has shown that detection is both more sensitive and more rapid using microsphere columns compared with batch bead-based methods.