

**Organization:** Biopraxis, Inc.

**Title:** Analysis and Simulation of Integrated Microsystems for Detection of Chemical and Biological Agents

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### **Project Goals**

The development of bio-microsystems requires a detailed understanding of fundamental transport and biomolecular binding processes. High-fidelity modeling of these processes, in turn, requires empirical data. The few tools that are available for collecting empirical data on molecular recognition processes are labor-intensive, yield limited information, and often require the use of labels such as fluorophores, which can alter the chemistry of the recognition process. Although many CBW agents are low-molecular-weight compounds (e.g., bioregulators and many peptide and nonpeptide toxins, as well as all of the CW agents), it is extremely difficult to use conventional techniques to study the binding of small molecules directly. And, finally, although competitive binding and displacement will affect bio-microsystem performance substantially, especially in complex environments, tools capable of studying such processes in real time do not exist.

Biopraxis is developing a new, reagentless biochip technology that will make it possible to monitor biomolecular recognition processes in a microfluidic device in quasi-real time; and to directly monitor the competitive binding and displacement interactions of cross-reactive sample constituents. Under the DARPA SBIR Phase I, Biopraxis proposed to:

- Show that the technology can directly monitor the binding of low-molecular-weight targets with immobilized biomolecules;
- Show that the intensity of the signal that is produced by the binding event increases as the incubation continues;
- Show that the binding interaction can be monitored in quasi-real time;
- Evaluate the impact of mass transport effects on the observed binding rates; and
- Demonstrate that the technology can be used to determine the impact of unrelated species on binding interactions.

## **Technical Approach**

To date, the Biopraxis technology has been studied for agent detection and specific identification. Preliminary experiments have shown the feasibility of detecting agents ranging from cyanide to anthrax spores. The methods used to prepare the biochips are still very crude, producing biomolecule 'pixels' that vary in size, shape, thickness, and percent denatured protein. Nevertheless, studies have shown that signals collected from an area of the protein spot ~1micron in diameter can be used to individually identify agents when the chips are incubated in samples containing cross-reactive constituents.

Under the Phase I SBIR, Biopraxis proposed to begin developing the technology as a sophisticated tool for studying biomolecular recognition, using a model system comprising the aflatoxins B<sub>1</sub> and G<sub>1</sub> (312 and 328 Da, respectively), and the enzymes glutathione transferase, lipoxygenase, and RNA polymerase. This model system was chosen for six reasons. (1) Aflatoxins B<sub>1</sub> and G<sub>1</sub> are such low-molecular weight targets (i.e., 312 and 328 Da, respectively) that their binding interactions cannot readily be monitored directly using any other technology. (2) The toxins are so closely-related that both cross-react with any biomolecule capable of binding either one. However, their relative affinities for various biomolecules can be quite different; and their toxicities differ substantially. It is therefore important to be able to distinguish between them. (3) Crude methods have already been developed for immobilizing the three enzymes on the biochip. (4) The three enzymes interact with aflatoxins via diverse mechanisms, and are therefore anticipated to exhibit different binding properties for the aflatoxins. (5) Glutathione transferase is known to be inhibited by a number of divalent metal cations and low-weight nitroaromatics commonly found on the battlefield, which can therefore be studied as potential interferants. And (6) since the aflatoxins are known BW agents, data developed under Phase I will be directly applicable to the development of BW detection bio-microsystems.

## **Recent Accomplishments**

In all previous studies, the chips have been incubated in 20-25microliters of solution, and then removed, excess solution carefully wicked away, and the signals collected from the dry chip. For the technology to be used to collect data on binding kinetics, the signals must be collected from the submerged chip while the binding interaction is taking place.

During the first three months of the program, efforts were primarily focused on (a) showing that the technology can directly monitor aflatoxin binding with immobilized biomolecules; and (b) showing that the intensity of the signal that is produced by the binding event increases as the incubation continues. A simple experimental protocol was developed, in which the chip was placed in the bottom of a small weigh boat; a few microliters of aflatoxin solution were deposited on the chip; a glass cover slip was placed over the drop; and the signal was collected through the cover slip. There were a number of mechanical problems with this very simplistic set-up; e.g., vibrations caused the cover slip to shift if not carefully balanced, and the droplet of solution would gradually dry and wick away from the protein spot as the signal collection continued.

Nevertheless, studies with lipoxygenase and RNA polymerase clearly showed that aflatoxin binding to the biomolecule immobilized on the submerged chip could be monitored directly, without the use of labels or tags. (Efforts are under way to improve the set-up, e.g., by adjusting the relative size of the cover slip and droplet, and/or by using a sealant to prevent evaporation.)

The next major objective was to show that the intensity of the aflatoxin signal changes in intensity as the incubation continues. Signals were collected from a ~1micron diameter area on the submerged chip once every 10minutes for an hour or more. As expected, the aflatoxin signals increased steadily in intensity during the first 30-40min of incubation. Intriguingly, studies with aflatoxin G<sub>1</sub> indicated that while the aflatoxin continued to bind to the biomolecules thereafter (i.e., the binding sites had not yet been saturated, presumably due to mass transport limitations), the chemistry of the enzyme-aflatoxin complex changed. The reason for the change has not been elucidated, but may be due, e.g., to an aging phenomenon such as that seen when cholinesterase binds nerve agents.

To study biomolecular recognition, quantitative binding information must be generated in real-time; i.e., a reasonable number of data points must be collected between the time the immobilized biomolecule is first exposed to the target and the time its binding sites are saturated and/or transport phenomena begin to control the rate at which binding can take place. Task 3 studies were initiated during the three-month period, using an RNA polymerase chip incubated in aflatoxin G<sub>1</sub>. Signals were collected once every ~3min for 32min, and then again at longer intervals for the next 30min. Despite the simplistic nature of the experimental setup, plotting signal intensity vs time produced an S-shaped curve over the first 50min of the incubation, with the signals collected from 9min to 27min falling on a straight line.

Preliminary batch incubation studies indicate Task 5 will be successful, as well. The binding of 2,4-dinitrophenol or 2-nitroaniline to glutathione S-transferase could readily be detected. More importantly, when biochips were incubated in solutions containing an aflatoxin/nitroaromatic mixture, the binding of both the aflatoxin and the nitroaromatic could be readily observed.

### **Six-Month Milestones**

The program is a Phase I SBIR. The Quarterly Report has been submitted. The remainder of the program will be completed, and the Phase I Final Report submitted by late June.

### **Team Member Organizations**

Biopraxis, Inc., is conducting the Phase I effort. CFD Research Corporation (CFDRC), another small business, will join Biopraxis on Phase II, if awarded, to develop detailed three-dimensional computational models (based on the CFDRC's CFD-ACE+ code) capable of simulating the impact of competitive binding and displacement, specific and nonspecific interferences, and biomolecule

degradation on molecular recognition parameters. Stanford University will adapt its microfluidic device technology for integration with Biopraxis' biochip transducer.