TOXIN DIAGNOSTICS – DEVELOPMENT OF NOVEL, FIELDABLE TECHNOLOGIES TO DIAGNOSE TOXIN EXPOSURE

Developing Novel Detection Methods For Use With Biosilica-immobilized Nanoscale Biosensors

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The biosilica shells (frustules) of diatoms are porous, hierarchically ordered structures with meso-to-nanoscale architectures, whose assembly can be genetically-modified for development of advanced materials for threat detection. Through biosynthesis and bioassembly under ambient culture conditions, bioengineered diatoms can be made to produce biosilica frustules functionalized with biosensor proteins. These genetically-functionalized frustules can be isolated in an active state and used for sensing and monitoring applications. While this technology sounds promising, diatom genetic engineering is an under-studied field. Prior work has focused on overcoming this deficit.

Sequencing of diatom genomes allows the identification and amplification of species-specific promoter-terminator systems that can be used to drive introduced fusion genes. In this work, the Thalassiosira pseudonana constitutive fcp promoter is used to drive both a drug resistance gene and a biosensor chimeric protein in the introduced DNA. The chimeric protein consists of a silica-targeting peptide, again from the host genome, and a functional protein of interest (e.g., nanobody for chemical, biological, or toxin detection). The trafficking and targeting functions of the silica-targeting peptide (Sil3T8 in T. pseudonana) can be uncoupled, allowing the targeting peptide to be attached to either end of the functional protein. This structural flexibility allows for more precisely oriented tethering to the diatom biosilica of the desired functional protein. Further, in this construct, rotational mobility and solvent accessibility through the silica pore network are retained while simultaneously protecting the protein component from chemical, physical, and thermal denaturation. In addition, recent advances in methods for introduction of foreign DNA into diatoms mean that the production of bioengineered diatom lines have become more rapid (weeks, not months) and more efficient (100-fold more transformants per million cells), and result in more consistent expression over time (necessary for scaling up culture volumes for production purposes).

The final hurdle for this technology will be development of a real-world sensing readout. Two types of assays that could prove useful are label-free systems and wash-free systems. Label-free sensing retains the highest specificity of the biosensor but relies on more specialized equipment. One example of a label-free system is surface-enhanced Raman scattering (SERS). Here, diatoms frustules act as photonic crystals, whose inherent photoluminescence is quenched upon antigen binding to the functionalized biosilica.

Wash-free systems like the case of fluorogen-activating proteins (FAPs) present an interesting and unexplored option for a chip-based sensing platform. FAPs are dimerized nanobody sensors that constrain the rotation of a fluorogen (e.g., malachite green), and induce a novel fluorescent signal. By incorporating the biosensor nanobody with the FAP domain, a wash-free sensor should be able to be constructed whereby when the antigen binds the sensor domain, the FAP domain is prevented from homodimerizing and the fluorogen signal is lost.

To summarize, previous work has created a green pathway for the production of biosilica functionalized with a biosensor through the genetic engineering of diatoms. Future work with functionalized diatom biosilica will explore the feasibility of various real-world sensing platforms, including label-free SERS and wash-free FAP systems.