

## Construction of a Single-chain Fv Antibody for a Bacterial Target-specific Biosensor

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The highly specific interaction between antibodies and their target antigen can be manipulated to construct an antibody-based biosensor that will allow for rapid identification of specific contaminants in a liquid sample. To avoid potential complications with the expression of a full length antibody molecule in bacterial cultures such as protein folding and glycosylation, a single-chain antibody gene is being constructed that is based on the antigen-recognizing domain only. This molecule will be significantly smaller and less complex than the full-length immunoglobulin and will facilitate manipulations of the gene itself that will directly affect the specificity of the antibody for its target antigen.

For detection of bacterial targets, a displacement biosensor is under development. The biosensor will consist of a single-chain Fv antibody (scFv) conjugated to a magnetic bead and bound to a solid support through a minimal antibody-antigen affinity for a substance on the support surface. The scFv will be engineered to demonstrate a high degree of affinity for a bacterial target antigen allowing it to dissociate from the solid support in the presence of this antigen. The biosensor displacement from the solid support will be detectable using a separate device which measures miniscule changes in mass. Since the scFv-bead conjugate is significantly larger than a single bacterium, displacement of even a few of the biosensors should be immediately detectable, compared to changes in mass that would occur with the binding of the target bacterium alone.

Our work is currently in a proof-of-concept stage. The target of our biosensor is a *Pseudomonas* species which serves as a pathogen stimulant. We have isolated immunoglobulin genes for the heavy and light antibody chains that demonstrate an acceptable level of affinity for the target bacterial antigens, and combined them into an scFv. Conjugation to a high mass bead has been performed with full-length antibodies and should be easily accomplished using the abbreviated scFv molecule. Mutational studies of the scFv gene will be conducted to improve specificity for the target antigens and to diminish specificity for the antigen bound to the solid support of the detector. Improvements in the scFv for its target antigens will be determined through surface plasmon resonance and phage display.

These preliminary studies will lead to future improvements that will allow for rapid detection of potential bio-terrorism threats such as *Bacillus anthracis* or *Clostridium botulinum* based on isolation of highly specific single-chain Fv antibodies. Although initial work focuses on a single target antigen, we are hoping to improve the capabilities of the biosensor by combining several scFv that will identify a number of target antigens into a single biosensor unit that can readily be used in the field.