**Selection and Optimization of Antibody Fragments for Biosensor Applications Using Phage Display**

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**Introduction:** We are involved in a systematic research program that utilize multiple phage display libraries and other modern protein/antibody engineering approaches to prepare a repertoire of novel interface biorecognition antibody fragment molecules for the rational design and fabrication of biochemically modified field effect transistor (BioFET) sensor surfaces at the molecular level. These molecules will primarily provide specific protein analyte binding capability to the BioFET sensor sensing channel. The ability to control/manipulate interfacial interactions at semiconductor-liquid surfaces/interfaces is of significant biomedical/biotechnological import, and is crucially essential for any investigation in the field of micro/nano-biosensor technology. The development of our BioFET sensor platform is aimed initially for detection of monokine induced by interferon-gamma (MIG/CXCL9) in transplant biology/rejection. We have preliminary results for selected single chain Fvs (scFvs) clones that specifically recognize our analyte of interest (MIG) from the naïve Tomlinson phage display libraries I and J (i.e., among other phage display libraries of antibody (Ab) fragments [Fab, single domains/minimal antigen-binding units] we will use).

**Materials and Methods:** Naïve phage display antibody libraries (Tomlinson I and J) in the phagemid/scFv format, and *E. coli* strains TG1 were obtained from the Medical Research Council (MRC), Cambridge, England. Biotinylated MIG was custom synthesized for us by Chemical Synthesis Services, Scotland, UK. NeutrAvidin coated magnetic particles were purchased from Spherotech, Inc., Libertyville, IL (USA). Libraries I and J were biopanned separately to ensure selecting the most MIG antigen binding clones. The biopanning procedure is essentially as described previously [1, 2]. Bound phages were eluted from the particles by suspension in 20 mM dithiothreitol (DDT)-PBS. An aliquot of the eluted/output phages was used to infect fresh exponentially growing culture of *E. coli* TG1 cells for subsequent amplification experiments as instructed in the Tomlinson (I+J) protocol, and used for further rounds of panning. Phagemid DNA sequencing of 44 randomly picked clones was performed using the QIAprep Spin M13 Kit (QIAGEN Inc., CA). Monoclonal phage/scFv ELISA was performed essentially as described on the MRC website [3]. Various control experiments to probe the specificity of binding of selected phage/scFv clones for MIG were performed.

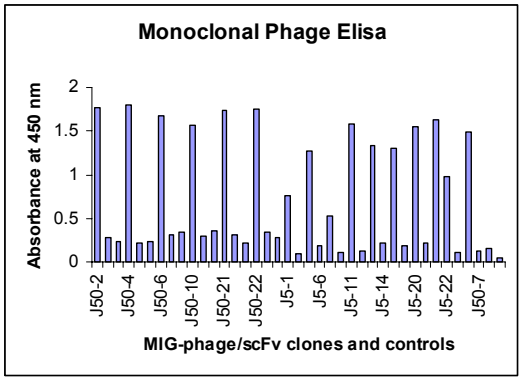


**Fig.1.** Monoclonal phage/scFv ELISA depicting binding activity between individual phage clones and biotinylated MIG (including non-MIG protein components of the bio-panning experiments).

**Results/Discussion:** Binding efficiency is measured as the fraction of input phage that binds the target molecule, MIG. In these experiments, phage titer was obtained after repeated buffer washes and elution. The concentration input phage used for each round of selection was kept constant at ~$10^{12}$ particles/mL. The results obtained showed that the relative eluate yield/output of binding phage particles was increased approximately 1000-fold and 100-fold from the first to the fourth round of selection for libraries J ($4 \times 10^6$ - $5 \times 10^9$) and I ($1.1 \times 10^6$ – $3.5 \times 10^8$), respectively. The results clearly indicate that isolation and enrichment of MIG binding phages was accomplished. The phagemid DNA sequences (results not shown) were examined by inspection to ensure that the selected MIG-binding scFv clones have full length VH and $V_\kappa$ inserts; the results show we succeeded in selecting clones incorporating complete sequences of scFvs. Results of the monoclonal phage/scFv ELISA is plotted in Fig.1. The plot clearly show that except for clone J5-9 with OD reading of ~0.5, there is significant difference in the absorbance reading in all other wells with the MIG antigen versus control wells (no MIG) with OD readings of only ~0.05-0.3. Thus, the results of the monoclonal phage/scFv ELISA demonstrate that positive clones with specific binding to biotinylated MIG have been selected from both Tomlinson libraries.

**Conclusion:** We have successfully selected a number of novel phage/scFv clone variants from the Tomlinson naïve libraries with specific binding affinity to MIG protein. We are currently evaluating selectants for MIG affinity and selectivity, and plan to engineer the size or topography and surface chemistry (for site-specific, oriented surface attachment) of selected antibodies to optimize them as molecular affinity interfaces in a MIG sensitive BioFET.

**References:** [1] Harrison JL, Williams SC, Winter G and Nissim A (1996). Meth. Enzymol. **267**:83-109; [2] Chames P, Hoogenboom HR and Henderikx P (2002). Meth. Mol. Biol. **178**:147-57; [3] http://www.geneservice.co.uk/products/proteomic/datasheets/tomlinsonIJ.pdf.