A Preliminary Investigation of Phage Antibody Enrichment Using Reichert SPR Systems

Mark A. Sullivan, Ph.D[a], Mary M. Murphy, Ph.D[b] and Jeffrey J. Bornheim[b]
[a]University of Rochester, [b]Reichert Technologies–Life Sciences

Abstract

Surface plasmon resonance is an established technique for studying protein:protein interactions and has been the method of choice for determination of antibody affinity for the last 20 years. Developments such as phage display have led to the rapid production of numerous antibodies in a high-throughput environment that typically requires affinity analysis to select the proper candidates for further development. Since most display libraries are limited in terms of the number of sequences in the libraries (typically $1x10^9$ to $1x10^{10}$), a frequent result is that initial candidates for development are not of sufficiently high affinity, necessitating affinity improvement strategies. In this poster, we will demonstrate that, using a Reichert SPR system, we can improve the efficiency of phage antibody isolation and characterization and evaluate the potential for optimizing the selection of high affinity variants. The idea of using an SPR instrument to function as both a lab-on a chip, to permit semi-automated manipulation of phage preparations, as well as providing the SPR signal as a window on the binding and enrichment of high affinity variants of scFvs or Fabs (or other scaffolds) is appealing, and could lead to a significant competitive advantage in the expanding market for therapeutic antibodies

Goal

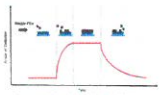
The goal is to demonstrate a novel use of the Reichert SR7000DC instrument to perform enrichment of phage antibodies using the fluidics system of the instrument. Phage antibody technology is a powerful method for obtaining specific antibodies directed at a defined target for a fraction of the time and cost of conventional monoclonal antibody technology. We theorized that if phage encoding a binding partner for a target immobilized on a sensor slide can be separated from phage displaying non-binding proteins, then it would be possible to employ the precise fluidic controls of the instrument to perform enrichments under conditions that could prove beneficial in selecting affinity variants.

Surface Plasmon Resonance

Label-Free technique for analyzing biomolecular interactions in *real time*

- Extremely precise mass sensor for quantitative binding measurements
- Flow based measurement
- Key Components
  - Sensor chip – ligand immobilization
  - Microfluidics – analyte delivery
  - Optical sensor – measurement

SPR is the Gold Standard for determining Kinetics



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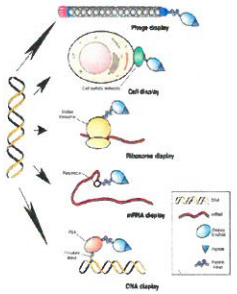
Display Technologies



There are a number of backbones to use for production of recombinant antibodies including ribosomes, cells, and DNA. Another way to do it is to use a bacteriophage (or "phage") as the backbone for construction of antibody libraries. Filamentous phages of E. coli can be used.

Advantages of Display Technology for Antibody Isolation

- Fast - typically **1 week** to isolate individual scFvs versus **months** for hybridomas
- Not subject to limitations of immunogenicity (self antigens)
- Avoids problems with immunodominance
- Alternative methods of antigen presentation
- Rapid production of proteins for analysis
- No animal use
- Yields DNA encoding antibody sequence
- Allows control of antigen presentation
- Use custom conditions during enrichment

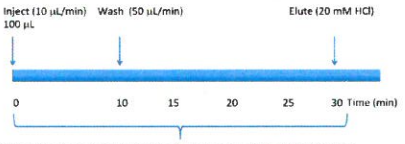
Human Phage Antibody Library

- "Naive" Library
  - Diversity ranges from ~$1x10^9$ to $1x10^{11}$ clones
  - Fab or scFv format
  - Uses pooled cDNA from many donors
  - Binding is driven by shape complementarity
    - Used to select antibodies against any target
    - Affinities generally 10-100 nM from $1x10^8$ sized libraries
- Many applications for therapeutics and diagnostics require higher affinities (< 1nM Kd)
  - Secondary libraries created by mutagenesis and re-selection can yield sub-nanomolar Kd
  - Most common method uses biotinylated antigen and magnetic beads to enrich for clones with slower off rates
  - Some methods employ very long incubations for off-rate selection (~1 week!)

Phage Antibody Enrichment on a Reichert Instrument

- Potential Advantages
  - Readily accessible fluidics system
  - Inexpensive components (tubing and sensor slides)
  - Outflow easily collected
  - Flexibility to employ different elution buffers, flow rates and temperatures
  - Capable of subtractive selection scheme

Monovalent Phage Injection

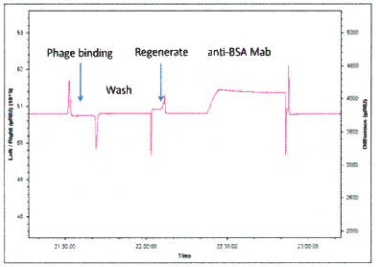


250 µL fractions of outflow collected and phage titers determined by transduction into *E. coli*.

Phage input: ~$1x10^8$

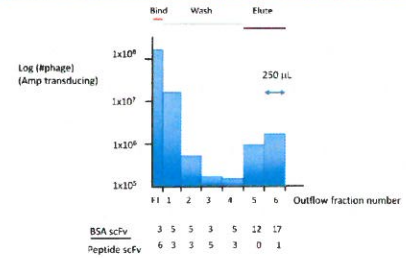
Phage output: ~$3x10^6$

Monovalent Phage Profile

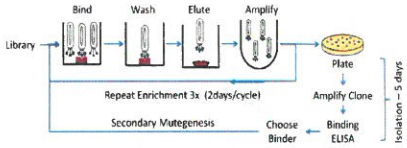


Phages present in the fractions were recovered by transduction of *E. coli* and samples of colonies in each fraction were subjected to colony PCR. The products were resolved by agarose gel electrophoresis.

Phage Titer of Fractions



Phage Display Cycle - Opportunity



1) Binder Selection w/SPR affinity in phage - skip protein expression
2) Eliminate rounds of enrichment - potential 6days->2days
3) Elution control to eliminate low affinity binders and improve reproducibility – fine control of temp, time, flowrate & conc
4) Specificity w/selective subtraction
5) Run up to 4 enrichments simultaneously

Future Directions

- Optimization of regeneration/elution buffer conditions to maximize affinity discrimination
  - Soluble target elution
  - Flow rate control
  - Temperature, pH, etc.
- Characterization of individual phage binding kinetics to rank order affinities
- Subtractive selection schemes
  - Immobilize homologs of target in left channel, actual target in right channel
  - Select phage that fail to bind the homolog but retain binding to the target
  - Switch inlet tubing to elute only phage in the right channel

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