IBIS White paper # 6.140522

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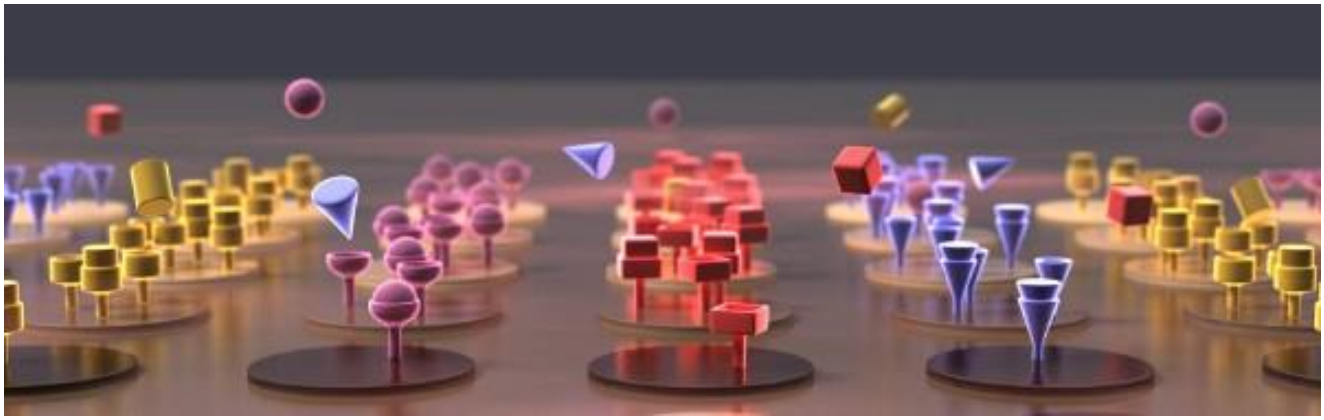

IBIS White paper

Epitope Binning

 # 6.140522

An important step in the engineering of biotherapeutics is to characterize and group a library of monoclonal antibod-ies by the epitope binding regions generated against a specific antigen. This "epitope binning" enables the maintenance of epitope diversity and provides important information to broaden intellectual property protection. IBIS Technologies and Wasatch Microfluidics are pleased to announce a label free, array-based SPR package that enables high throughput epitope binning of 96 antibodies directly from supernatant using only 150 µL of each clone, overcoming the sample consumption, throughput limitations, and/or labeling restrictions of current methods. IBIS and Wasatch have developed a powerful integrated hardware and software platform that delivers the full potential of label-free, high throughput antibody binning, enabling you to...BIN THE FRIDGE.

Fundamentals of Epitope Binning

Advances in antibody engineering allow for the rapid generation of large libraries of antibodies with therapeutic potential. This compounds the need for high-throughput analytical techniques to characterize these antibodies and their interaction with potential therapeutic targets. Epitope coverage and affinity are critical in the lead identification process [1]. Multiplexed label-free biosensor platforms can be used to characterize antibody-antigen interactions with greater throughput, but the sample requirements and operating time can be unwieldy [2]. Leveraging the power of the microarray format of SPR array imaging, IBIS Technologies and Wasatch Microfluidics have developed a powerful yet simple epitope binning workflow.

What is Epitope Binning?

In epitope binning, antibodies are tested in a pairwise combinatorial manner, and antibodies that compete for the same binding region are grouped togethering into bins. Epitope binning experiments are performed generally using three different protocols: tandem, premix, and classical sandwich blocking (see Fig. 1) [3]. The array SPR system is particularly powerful for the premix and sandwich assay formats.

In a classical sandwich assay, one antibody is immobilized onto the surface, the antigen is flowed over the capture antibody, and then the secondary antibody is flowed over the antibody/protein complex. In a premix binning assay, an antibody is immobilized on the surface and a premixed solution of the second antibody and antigen is flowed over the antibody. In tandem, the target protein is immobilized on the surface and the two antibodies compete to bind. In each of these techniques, antibodies that block one another can be identified.

By testing whether antibodies block one another's binding to their antigen in a pairwise fashion, a competitive blocking profile is created for each antibody relative to the others in the library. The blocking information determines into which "bin" the antibodies are placed. Each method has unique advantages and disadvantages, and provides the reseachers with different information.

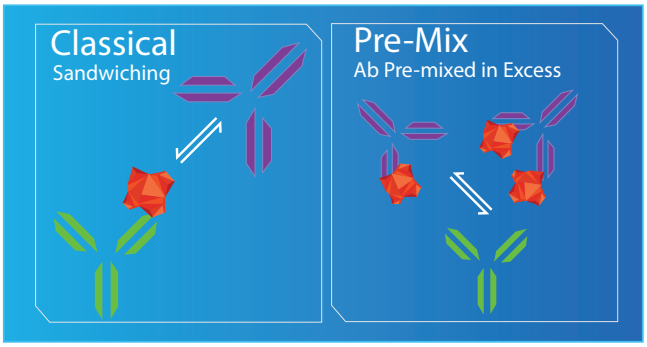

Figure 1. Array SPR enables high throughput classical and premix binning.

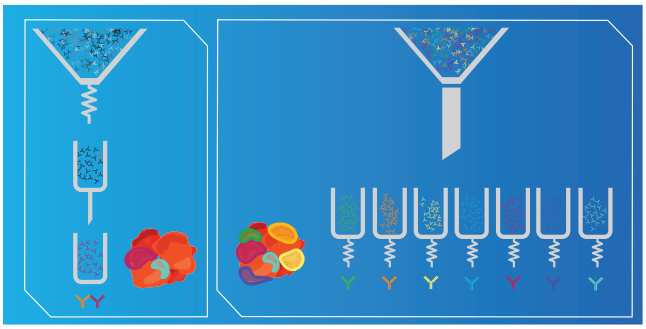

Figure 2. "See The World in Color." (Left panel) When affinity or other criterion are used as the primary selection tool, one can bias the results to a small number of epitopes. This limits the probability that functional epitopes are represented in the selected panel, or potentially misses candidates with otherwise desirable properties whose affinity could be matured.

(Right panel) When all candidates are grouped according to epitope first, epitope diversity is maintained and the best performing antibodies in each bin can then be selected.

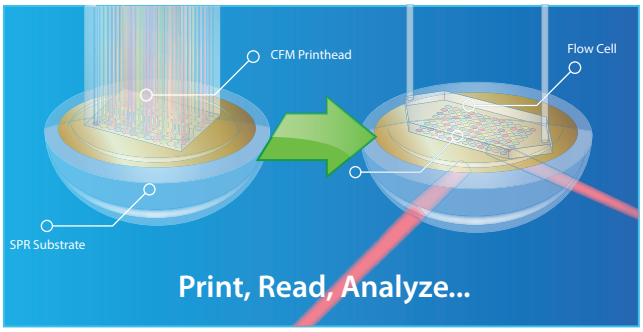

Figure 3. Pairing continuous flow printing with SPR imaging enables quick "Print, Read, Analyze…" of antibodies with limited sample consumption.

Why Perform Epitope Binning?

A significant challenge in engineering antibodies is that both the antibody and the epitope can possess properties that hinder engineering efforts. Further compounding the problem is the fact that "you can't engineer the epitope!" Both of these challenges beg for epitope characterization prior to drug candidate screening. Commonly, however, antibody affinity remains the primary selection mechanism. By using affinity for selection, the result can be the selection of a panel of antibodies that may bind to only one or a few epitopes, as affinity can often correlate with epitope (see Fig. 2, left panel). This epitope bias can be problematic if the functional epitopes are not represented by those selected with the highest affinity.

Characterizing the target protein's epitopes at an earlier stage facilitates understanding of function and mechanism of action which in turn allows for a more guided development for therapeutic antibodies.

Antibodies that target similar epitopes often share a similar function. Conversely, functional antibodies that target different epitopes may imply different mechanisms of action. The ability to generate this information early in the drug discovery process, from small sample amounts and without the need for labeling, enables researchers to "eliminate the funnel" by reducing the number of potential candidates while maintaining epitope diversity (see Figure 2, right panel)[4].

Best Way to do Epitope Binning?

Previously, epitope binning has required significant time, sample and manpower for experimental setup and data analysis. This has limited its application to small numbers of samples later in the development process. IBIS and Wasatch have developed a turnkey, high-throughput SPR solution that has a simple software and hardware setup (96x96 binning from two standard 96 well plates), small sample requirements
(total of 150uL of each clone, purified or serum-free supernatant), and streamlined data analysis (custom software tools for data management, heat map generation and bin allocations). Wasatch's epitope binning platform consists of three integrated components that combine to give
a unique, highly adaptive and high-quality array-based antibody binning solution: Flow printing of microarrays, SPR imaging of the microarrays, and custom software tools for experiment setup and data analysis.

Continuous Flow Microspotting

The Continuous Flow Microspotter™ (CFM) is a patented microfluidic printing technology that uses flow to deposit up to 96 biomolecules onto a sensor surface as an array of distinct spots. Unlike other systems which deposit droplets of material en-masse, the CFM uses flow to cycle molecules back and forth over the surface, yielding optimal binding from crude or dilute solutions. As a result, the CFM enables up to 10,000 fold enhancements in sensitivity and printing at concentrations 1,000 fold more dilute than competing technologies.

Scanning SPR Array Imaging

By using a hemispheric prism and CCD camera detection, the IBIS MX96 is able to simultaneously monitor up to 96 real-time interactions and associated reference spots for each analyte injection (see Fig. 3), delivering 9,216 interactions in a single automated run. For every time point, 96 minima are calculated and converted to a shift in response (see Fig. 4 & 5).

SPRi Express Setup Tool

The EST (Express Setup Tool) was designed to expedite the setup of array-based biosensor experiments. Sample species entry, selection, and tracking are all managed in a simple interface for a variety of array-based experiment types (see Fig. 6).

The EST was designed for deployment on the IBIS MX96, thereby simplifying and decoupling the experimental setup. It can be run from standard Windows computers, allowing researchers to plan and visualize experiments away from the interaction lab.

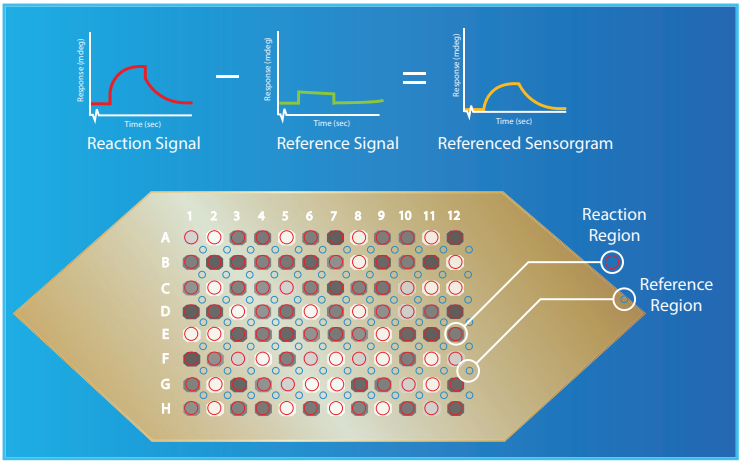

Figure 4. SPR array layout and data collection. For each time point and array location, SPR mimima are calculated and converted to a shift in response (see Fig. 5). Additionally, reference responses are calculated from an interstitial reference array. The simultaneous collection of all this data is displayed in real time as sensorgrams.

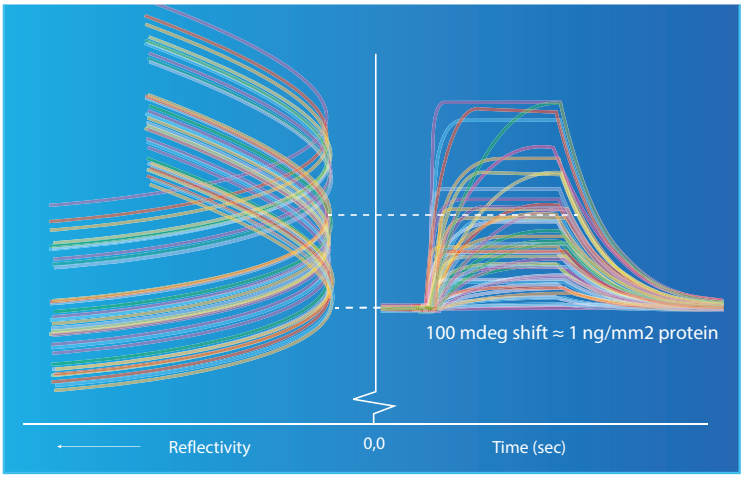

Figure 5. The shift in response of each SPR dip is converted to a sensorgram.

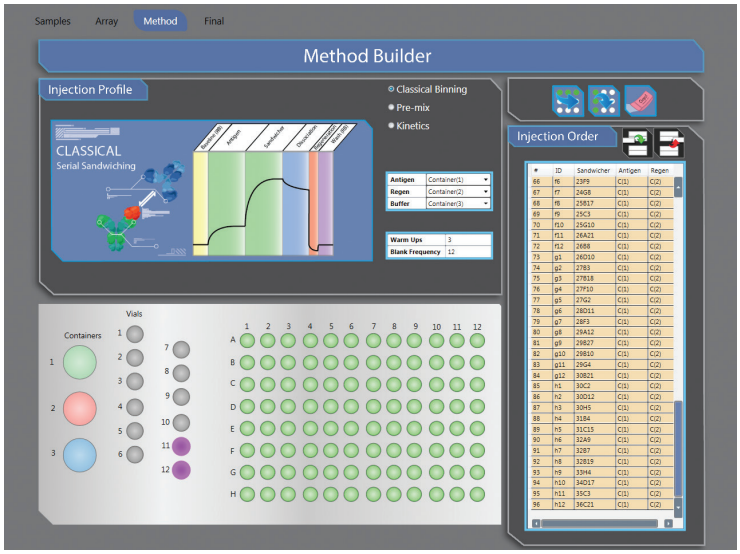

Figure 6. Screen capture from Wasatch's SPRi Express Setup Tool.

Binning Tool

The antibody binning tool was designed to process the massive data sets enabled by experiments run in an SPRi format, where 96 ligands can be tested against 96 analytes. The primary functionalities are:

- Read in up to 9,216 sensorgrams based on classical or premix binning assay formats.
- Quickly explore and curate the data. Plotting of injections, ligand spots, or other groups of interactions is possible from any point in the program, enabling the viewing of the raw data alongside the heat map generation, sorting and bin allocations.
- Define measurement points and generate a heat map showing blocking and sandwiching interactions with a high degree of automation and user control
- Sort the heat map into like-behaved antibodies and assign them to bins using custom algorithms and software automation.

Prior to the development of this software tool, the data analysis of even small panels of epitope binning could be a time intensive and laborious process (see Fig 7). Now a data set of 9,216 interactions can be binned in minutes!

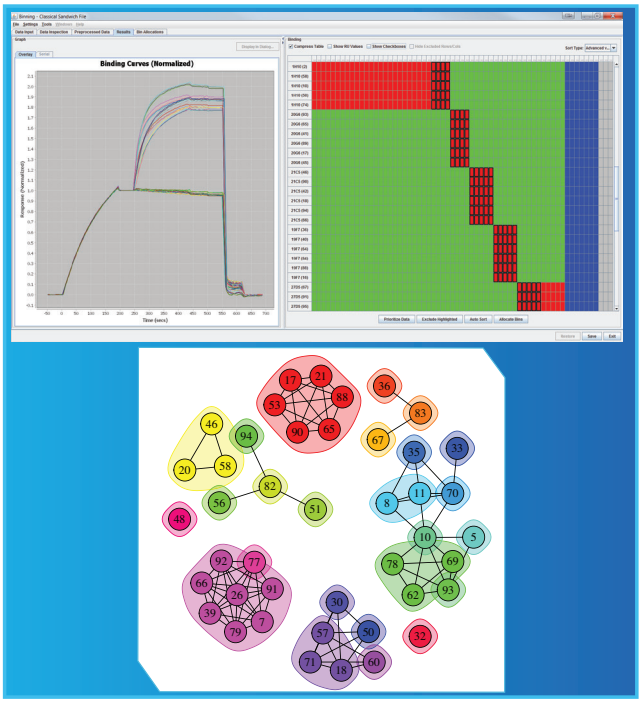

Figure 7. Wasatch has developed an Epitope Binning software package that generates a heat map and uses custom algorithms and software automation to sort it into like-behaved antibodies and assign them to bins. Viewing of the raw data is possible at any point in the program. Proprietary node plots can also be generated to help visualize relationships between mAb blocking profiles, with a variety of grouping algorithms for highlighting the relationships between mAbs.

Summary

The power of array-based SPR can now be harnessed for the label free performance of high-throughput epitope binning. IBIS's and Wasatch's epitope binning package allows researchers the ability to use limited quantities of samples, quickly interrogate large panels of antibodies for epitope blocking, "eliminate the funnel" while maintaining epitope diversity, avoid epitope bias that can occur with initial selection based on affinity alone, and generate valuable information that can be used to protect intellectual property. Array SPR can be used to conduct epitope binning experiments with minimal sample amounts in either a classical sandwich assay or premix assay format [3]. The combined CFM flow printer, SPR array imager, and custom software package make high-throughput binning a practical reality.

References

[1] P. Estep, F. Reid, C. Nauman, Y. Liu, T. Sun, J. Sun, and Y. Xu, "High throughput solution-based measurement of antibody-antigen affinity and epitope binning," MAbs, vol. 5, no. 2, pp. 270–278, Apr. 2013.

[2] Y. N. Abdiche, K. C. Lindquist, A. Pinkerton, J. Pons, and A. Rajpal, "Expanding the ProteOn XPR36 biosensor into a 36-ligand array expedites protein interaction analysis," Anal. Biochem., vol. 411, no. 1, pp. 139–151, Apr. 2011.

[3] Y. N. Abdiche, K. C. Lindquist, D. M. Stone, A. Rajpal, and J. Pons, "Label-free epitope binning assays of monoclonal antibodies enable the identification of antigen heterogeneity," J. Immunol. Methods, vol. 382, no. 1–2, pp. 101–116, Aug. 2012.

[4] Y. Abdiche, A. Miles, J. Eckman, D. Foletti, T.J. Van Blarcom, Y.A. Yeung, J. Pons, A. Rajpal, "High-Throughput Epitope Binning Assays on Label-Free Array-Based Biosensors Can Yield Exquisite Epitope Discrimination That Facilitates the Selection of Monoclonal Antibodies with Functional Activity," PLoS One 9(3): e92541, Mar. 2014.