Accelerate Kinetic Screening and Epitope Characterization of Antibody Libraries with High Throughput SPR

Daniel Bedinger, Ph.D.
Carterra Inc., 825 N 300 W Suite C309, Salt Lake City, UT 84103

INTRODUCTION

Throughput, speed, resolution, and sample consumption are typically key limiting factors for performing detailed kinetic and epitope characterization of monoclonal antibody (mAb) libraries destined for use as therapeutics. In addition, mAb immune responses can be used to survey the epitope landscape of pathogens and inform the design of more effective immunogens for vaccines. Here, we demonstrate three core applications of High Throughput Surface Plasmon Resonance (HT-SPR) (**Figure 1**), (A) capture kinetics, (B) epitope binning, and (C) epitope mapping, that together provide a comprehensive characterization of your mAb library with minimal sample consumption, enabling more confident decisions to be made earlier in the research process and obviating the need for preliminary ELISA screening. We show that HT-SPR can facilitate the generation of high quality kinetic data from 384 mAbs in parallel, using < 1 µg per mAb and only 2 µg of antigen. Epitope binning in a 384-mAb array format allows for a rapid assessment of the depth and breadth of your library's epitope diversity, providing exquisite resolution between near-identical clones, using < 5 µg per mAb and about 100 µg antigen. Finally, a large panel of mAbs can be mapped against a 384-peptide library, if their epitopes can be (partially) recapitulated on peptides, using 1 µg per mAb and <0.1 µg per peptide.

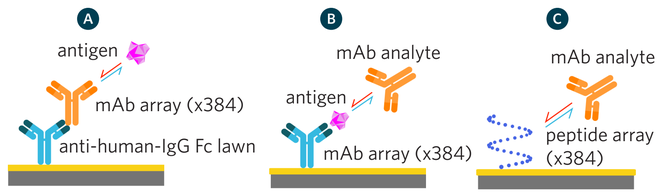
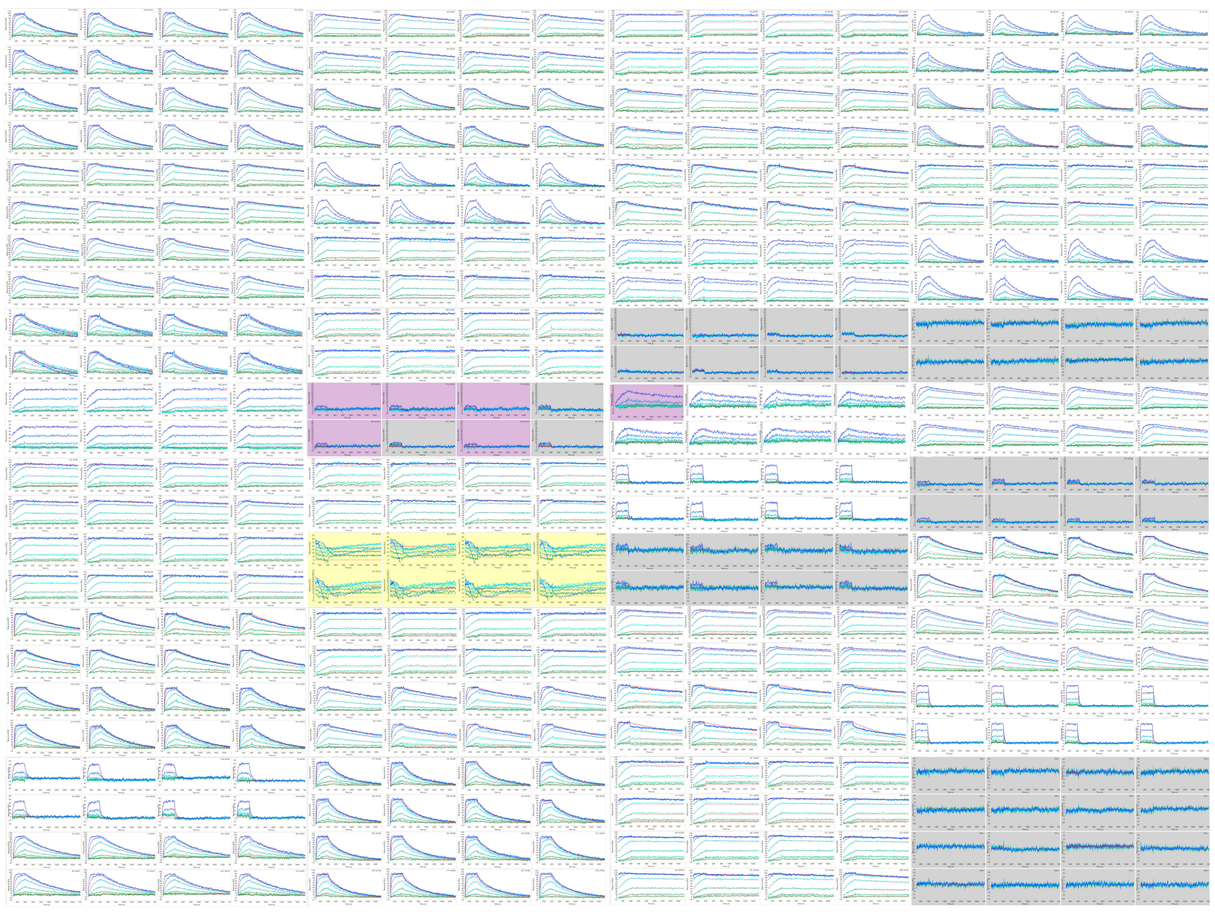


Figure 1. Assay formats described herein for (A) capture kinetics, (B) epitope binning, and (C) epitope mapping on a peptide array.



HIGH THROUGHPUT KINETICS

Figure 2 illustrates the high data quality in unprecedented throughput that can be achieved when capture kinetics is performed in a 384-array format using HT-SPR. As a model system, we used a panel of 43 unique mAbs that bound specifically to the same monomeric target antigen. A sensor chip was coated with an anti-human-IgG Fc antibody and used to capture the mAbs onto 384 individual spots via Carterra's flow printing technology. Each mAb was captured onto multiple spots to fill out the 384-spotted array and then the specific antigen was titrated over the entire array as a monovalent analyte using a wide concentration spanning 0.4 nM – 300 nM. Thus, in a single unattended run that consumed < 1 µg per mAb and only 2 µg of antigen, the binding kinetics and affinities of the 43 unique mAbs was analyzed in a highly parallel manner and with statistical confidence by reporting the mean ± standard deviation of 8 – 16 measurements (spots) per mAb.

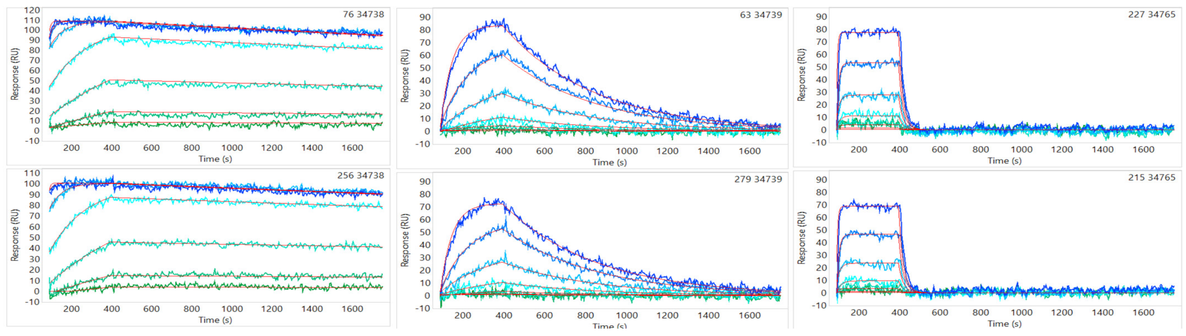


Figure 2. High Throughput Kinetics (On the left) stamp collection view of the results (measured data and global fits) obtained for a monovalent antigen interacting with 43 unique mAbs captured onto replicate spots within a 384-mAb array. The Kinetic Software automatically highlights the "good, bad, and ugly" fits (different colored panels) to facilitate data curation. (Above) Zoomed in view of three clones, on duplicate spots, with diverse kinetics (high, medium, and low affinities, from left to right).

HIGH THROUGHPUT EPITOPE BINNING

Competition or "epitope binning" assays can be used to test whether two mAbs block one another's binding to their specific target antigen. If two mAbs block one another, we infer that their epitopes overlap, whereas if they do not, we infer that their epitopes are non-overlapping and discrete from one another. Testing mAbs in a pairwise and combinatorial manner means that these assays scale geometrically with the size of the mAb panel, which has limited the application of these assays to small panels of mAbs. In contrast, HT-SPR's 384-mAb format is an efficient way of surveying the epitope landscape of a large panel of mAbs. By coupling up to 384 mAbs onto individual spots of an array (to provide the "ligands"), the pairwise binding of a series of solution mAbs (or "analytes") can be tested to explore a 384 x 384 comprehensive pairwise analysis, using < 5 µg per mAb (in the role of both ligand and analyte) and approximately 100 µg antigen. **Figure 3** shows an example of the results obtained from an epitope binning experiment performed on a 192-array **[Ching et al]**. In this experiment, the epitope diversity of 105 mAbs produced from the immunization of transgenic chickens was benchmarked against 16 mAb standards produced in wild-type chickens. Furthermore, by merging data from orthogonal sources (e.g., subdomain mapping, mAb library, or cross-reaction to the mouse target), the binning results provided an epitope-centric way of navigating the totality of data to facilitate the selection of mAbs with uniquely desirable properties.

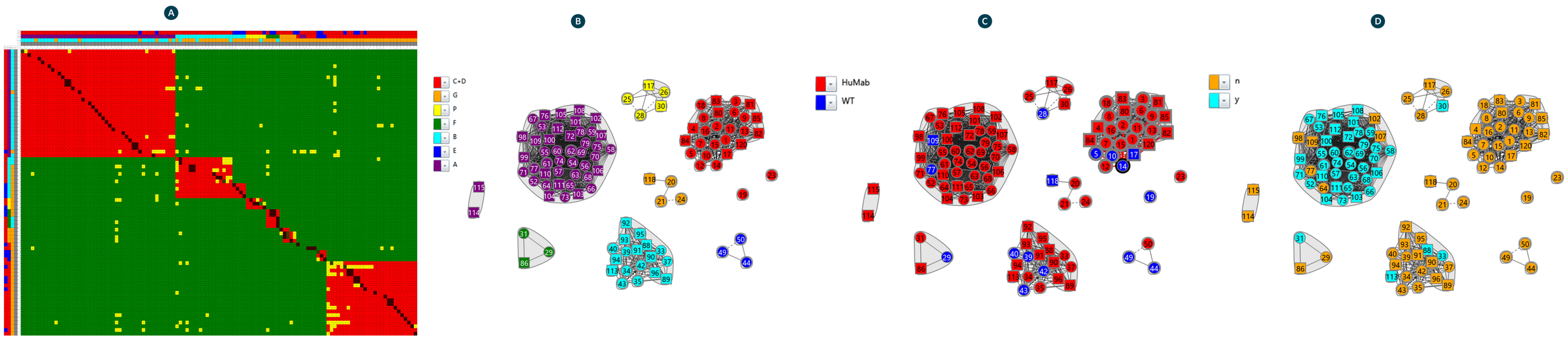
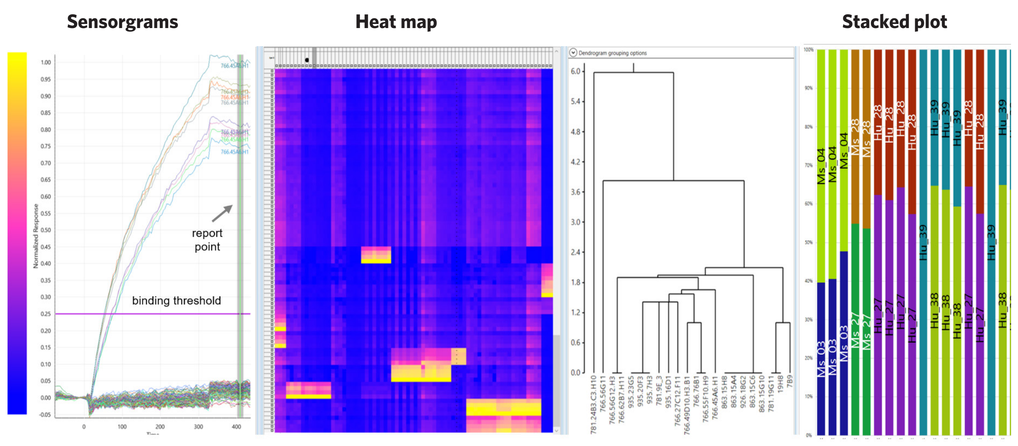


Figure 3. Epitope binning on a 192-mAb array using human progranulin as the model antigen (A) Heat map. Community plots colored by (B) subdomain, (C) mAb library, or (D) cross-reaction to mouse progranulin.

EPITOPE MAPPING ON A 384-PEPTIDE ARRAY

While mAbs generally bind conformational epitopes, some mAbs may recognize linear forms of their epitopes, as recapitulated on peptides. The "one-on-many" assay format offered by HT-SPR is ideally suited to binding specificity studies, such as epitope mapping of mAbs to peptide libraries **[Abdiche et al]**. In this type of experiment, hundreds of mAbs (which do not have to be purified) can be screened one after another (as analyte) over a 384-peptide array, providing high detection sensitivity owing to the much larger mass (100x) of the mAb analyte compared to the peptide ligand. **Figure 4** shows an example of the data obtained for an experiment where 96 mAb analytes (comprising 24 unique mAbs, injected in quadruplicate cycles) were screened over a 384-peptide array (comprising 76 unique biotinylated peptides plus controls, spotted in quadruplicate). The positive binding signals for peptides that are recognized by a given mAb analyte are clearly discerned from background (all other, non-binding peptides), facilitating mapping assignments and allowing mAbs to be clustered into epitope groups.



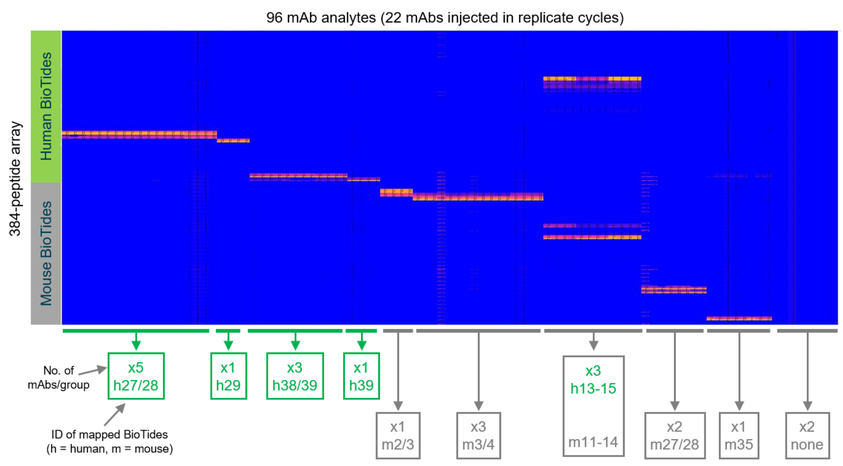


Figure 4. Epitope mapping on a 384-peptide array (A) Snapshot of the results as analyzed in the Epitope Software Tool showing sensorgrams, heat map, dendrogram, and stacked plot analyses (B) Sorted heat map showing how the mAbs cluster into different epitope groups based on their peptide specificities.

CONCLUSION

The "one-on-many" assay format offered by HT-SPR is ideally suited to expanding the throughput of many standard applications that are relevant to the screening and characterizing of mAbs whether they are destined for use as therapeutics or as probes/diagnostics to inform vaccine design. Performing binding kinetics, epitope binning, and epitope mapping on a 384-array significantly accelerates data acquisition compared with traditional platforms and consumes orders of magnitude less sample resulting in a facile set up. Carterra's LSA integrates flow printing with HT-SPR technology to enable unattended analyses of, (1) 1152 mAbs in a capture kinetics format, (2) a full 384 x 384 epitope binning assay, or (3) >384 mAbs screened for their binding specificity to a 384-peptide array. These assays are representative of the repertoire of assays that are achievable in high throughput without sacrificing data quality, enabling high confident measurements to be made at the earliest stages of research, allowing you to "see more, do more" with HT-SPR.

References: Ching et al, MABS 2017; Abdiche et al, Anal Biochem 2011

We thank Adimab for providing the reagents for the kinetic analysis.