# Accelerating the Discovery of Therapeutic Antibodies Using High Throughput SPR

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## INTRODUCTION

Throughput, speed, resolution, and sample consumption are typically key limiting- factors for detailed kinetic characterization early in antibody discovery campaigns. Here, we show that high throughput surface plasmon resonance (SPR) can be used to rapidly generate high quality kinetic data from 384 antibodies in parallel with minimal sample consumption. Additionally, epitope binning assays can be performed routinely on up to 384 antibodies per array, providing unprecedented throughput that allows for early assessment of your library's epitope coverage with exquisite epitope discrimination, facilitating the identification of clones targeting unique epitopes. The ability to characterize binding kinetics, affinity, and epitope specificity on large antibody panels with minimal sample consumption at early stage research is highly advantageous in drug discovery because it helps to accelerate library-to-lead triage.

## CARTERRA LSA

The LSA is a disruptive technology for antibody screening and characterization. It provides both throughput and flexibility via the automated choreography of two microfluidic modules (**Figure 1**) that deliver samples in different ways: the Single Flow Cell (left) covers the entire chip surface while the 96-Channel Printhead (right) addresses 96 spots in parallel and can be docked four times to create 384 spots per array. Flowing analyte in the Single Flow Cell over a 384-ligand array therefore enables the simultaneous analysis of 384 binding interactions.

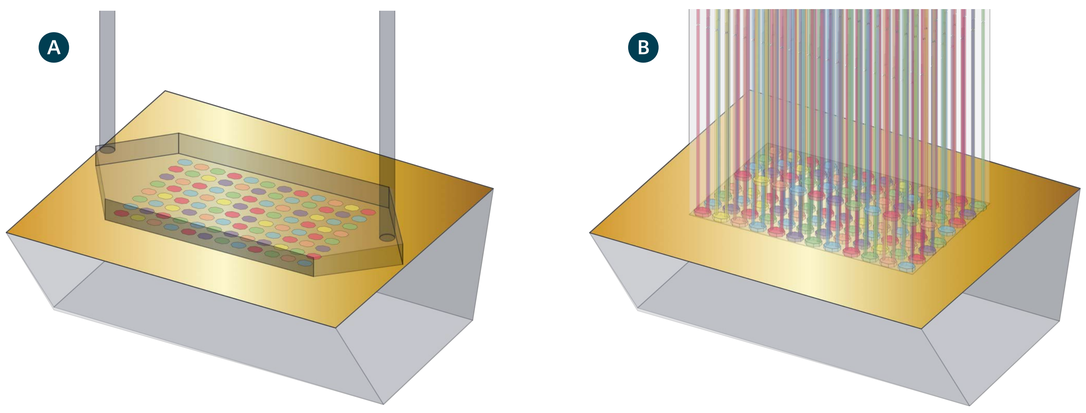
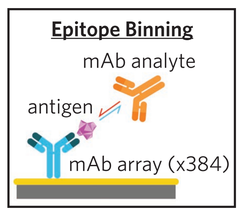



**Figure 1. Carterra LSA flow cells: (A) Single Flow Cell and (B) 96-Channel Printhead**

## HIGH THROUGHPUT EPITOPE BINNING




Competition or epitope binning assays can be used to test whether two antibodies block one another's binding to their specific target antigen. If two antibodies 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 antibodies in a pairwise and combinatorial manner means that these assays scale geometrically with the size of the antibody panel, which has limited the application of these assays to small panels of antibodies. In contrast, the LSA's 384 ligand array format is an efficient way of surveying the epitope landscape of a large panel of antibodies. By coupling up to 384 antibodies onto individual spots of an array (to provide the ligands), the pairwise binding of a series of solution antibodies (or analytes) can be tested to explore a 384 x 384 comprehensive pairwise analysis, using < 5 µg per antibody (in the role of both ligand and analyte) and approximately 100 µg antigen. Currently, this requires 4 separate experiments, each delivering 96 analytes to the chip - data files can be merged in Carterra's Epitope Software for data analysis and the generation of heat maps and network/community plots to visualize the epitope clustering. **Figure 2** shows an example of the results obtained from an epitope binning experiment performed on a 192-array **[Ching et al]**.

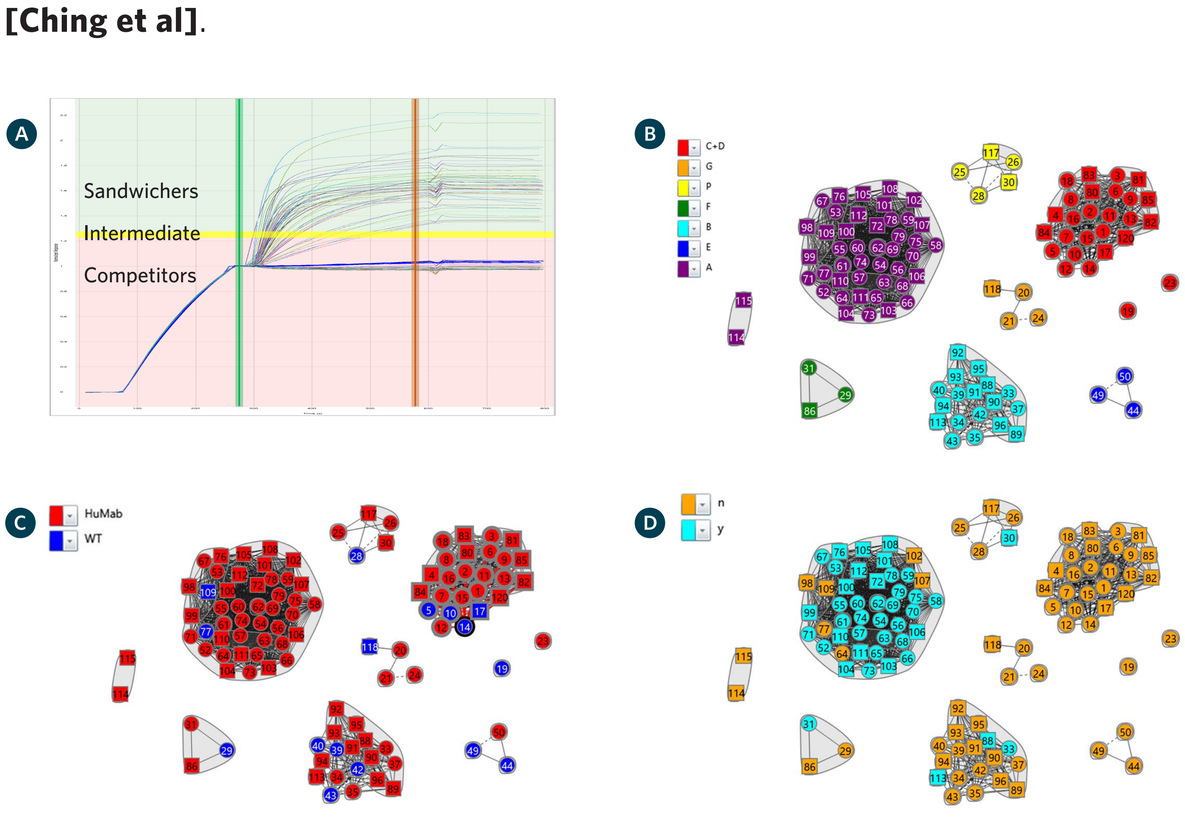



**Figure 2. Epitope binning on a 192-antibody array using human progranulin as the model antigen (A) Sensorgram data. Community plots colored by (B) subdomain, (C) antibody library, or (D) cross-reaction to mouse progranulin.**

In this experiment, the epitope diversity of 105 antibodies produced from the immunization of transgenic chickens was benchmarked against 16 antibody standards produced in wild-type chickens. Furthermore, by merging data from orthogonal sources (e.g., subdomain mapping, antibody 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 antibodies with uniquely desirable properties.

## HIGH THROUGHPUT KINETICS

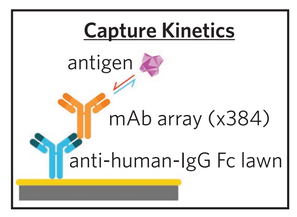



**Figure 3** Illustrates the high data quality in unprecedented throughput that can be achieved when capture kinetics is performed in high throughput. As a model system, we used a panel of 43 unique antibodies that bound specifically to the same monomeric target antigen. A sensor chip was coated with an anti-human-IgG Fc antibody and used to capture the antibodies onto 384 individual spots via Carterra's flow printing technology. Each antibody 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 antibody and only 2 µg of antigen, the binding kinetics and affinities of the 43 unique antibodies was analyzed in a highly parallel manner and with statistical confidence by reporting the mean ± standard deviation of 8 – 16 measurements (spots) per antibody.

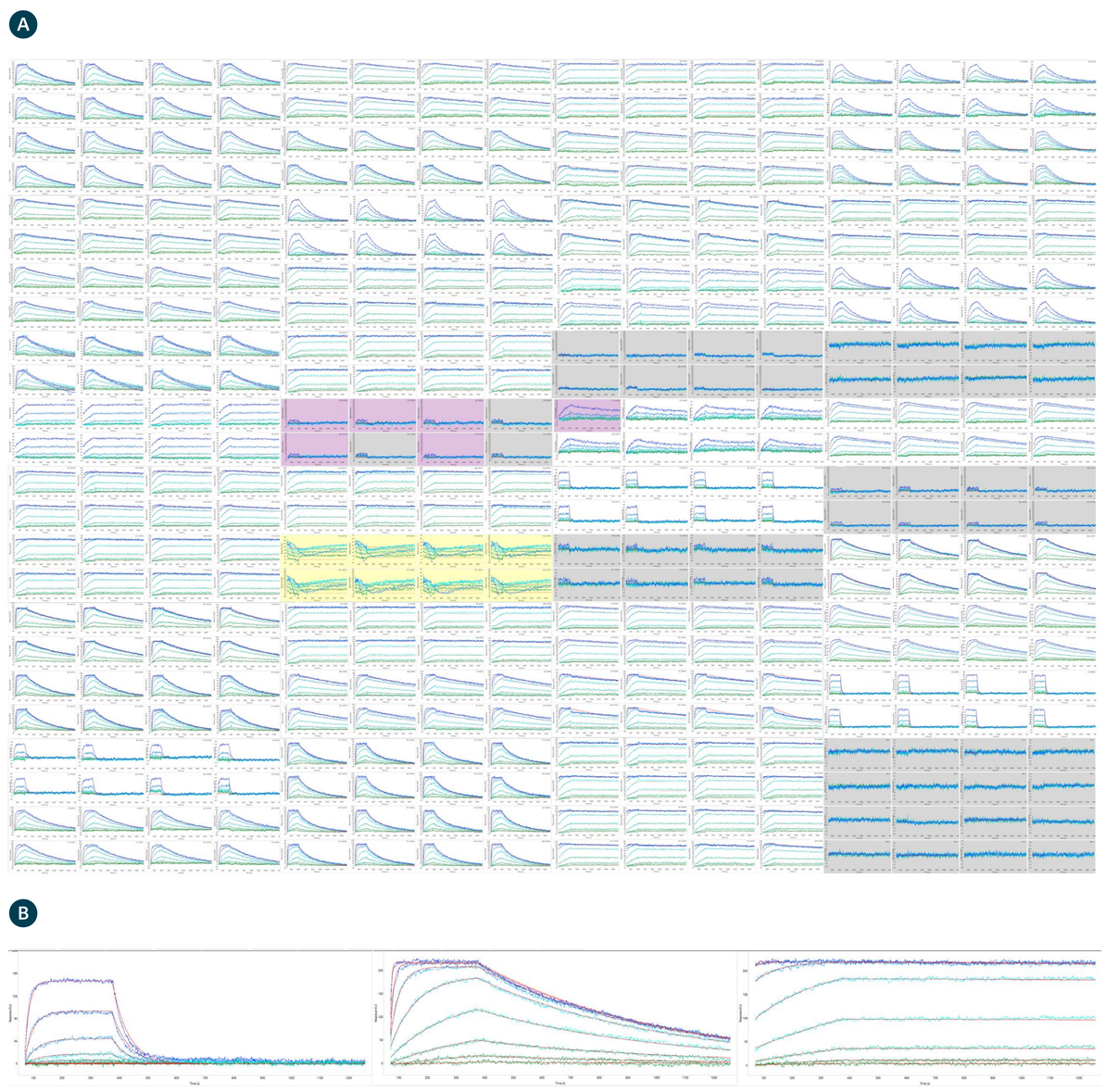



**Figure 3. Kinetic analysis of 384 antigen/antibody binding interactions in parallel using Carterra's LSA. (A) The results from a capture kinetics assay where a monovalent antigen (analyte) was titrated over 384 antibodies, captured onto individual spots. Each tile represents the global kinetic analysis on a single spot. Carterra's kinetic analysis software automatically flags poorly performing spots and assigns those tiles a color (grey = inactive/barely binding; yellow = heterogenous binding; and purple = insufficient onrate information). (B) Examples of spots for antibodies showing diverse binding kinetics, where the measured data are shown as a green-blue gradient and the global fit is shown in red.**

## CONCLUSION

The one-on-many assay format offered by Carterra's LSA platform using high throughput SPR is ideally suited to expanding the throughput of many standard applications that are relevant to the screening and characterizing of antibodies whether they are destined for use as therapeutics, reagents, or as probes/ diagnostics to inform vaccine design. Performing binding kinetics and epitope binning on a 384-ligand array significantly accelerates data acquisition compared with traditional platforms and consumes orders of magnitude less sample, because analytes are delivered across the entire array, resulting in a facile set up.

**References: Ching et al. Chickens with Humanized Immunoglobulin Genes Generate Antibodies with High Affinity and Broad Epitope Coverage to Conserved Targets. MABS 2017**

**We thank Adimab for providing the reagents for the kinetic analysis, which are part of a published study, accepted in PLoS One 2020 [Brown et al.]**