Accelerating the Discovery of Therapeutic Antibodies Using High Throughput Array 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 array-based surface plasmon resonance (Array SPR) can facilitate the generation of high quality kinetic data from 384 antibodies in parallel, rapidly and 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.

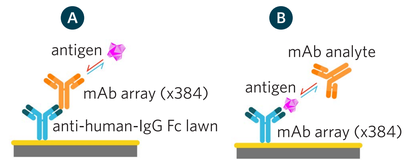

Figure 1. Assay formats described herein for (A) capture kinetics and (B) epitope binning

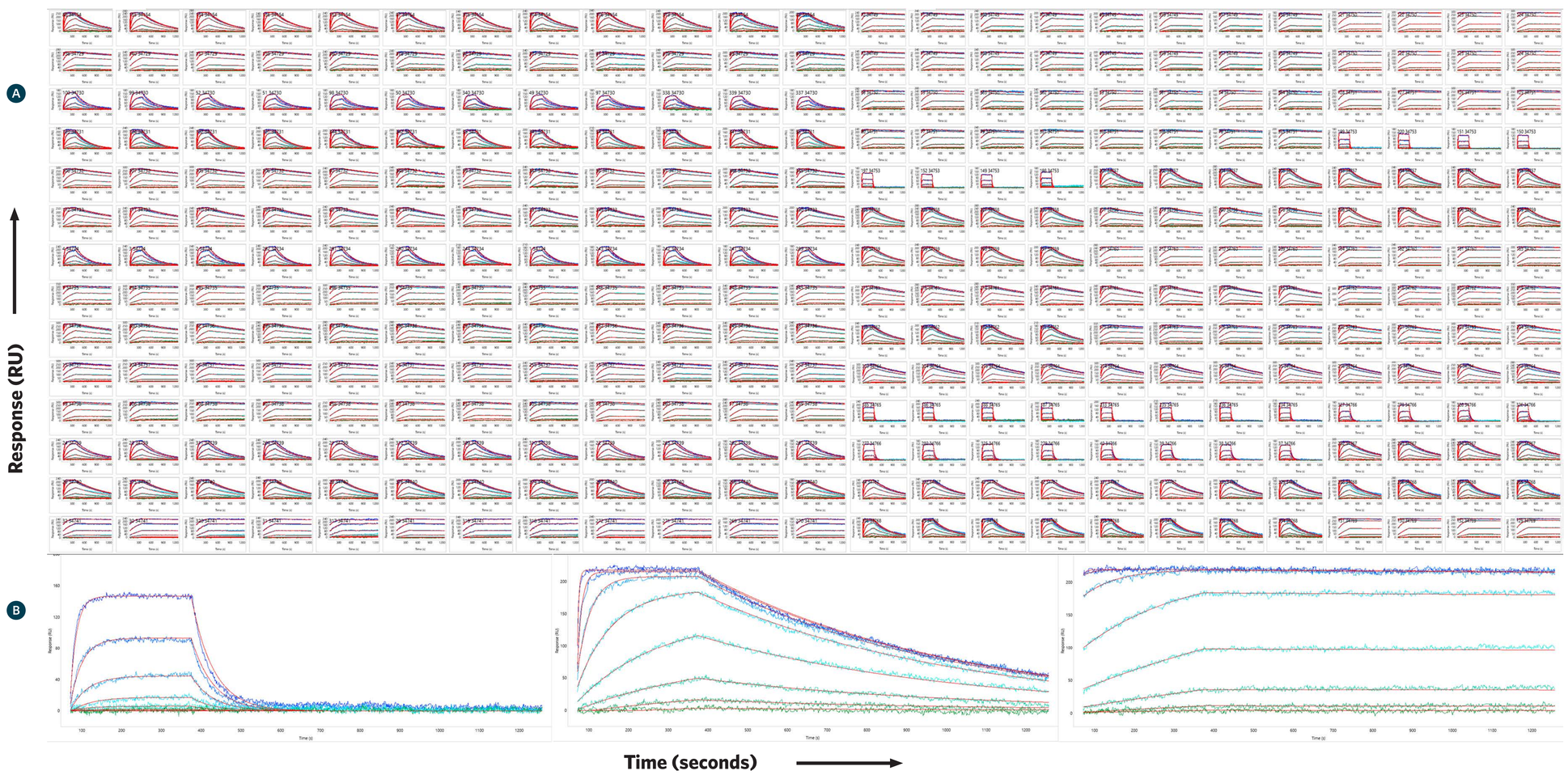

Figure 2. Simultaneous kinetic analysis of 384 antigen/antibody binding interactions using Array SPR. (A) The results from a single capture kinetics assay where a monovalent target (analyte) was titrated over 384 antibodies, each captured onto individual spots of a 384-array. Each panel represents the global kinetic analysis on a single spot; all 384 spots were analyzed in parallel (some spots are excluded). (B) Enlarged view of the data from 3 spots showing antibodies that bound their target with diverse kinetics (low, medium, and high affinities, from left to right), where the measured data are shown as a green-blue gradient and the global fit is shown in red.

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 Array 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 continuous flow microspotting (CFM) 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.

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, Array 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.

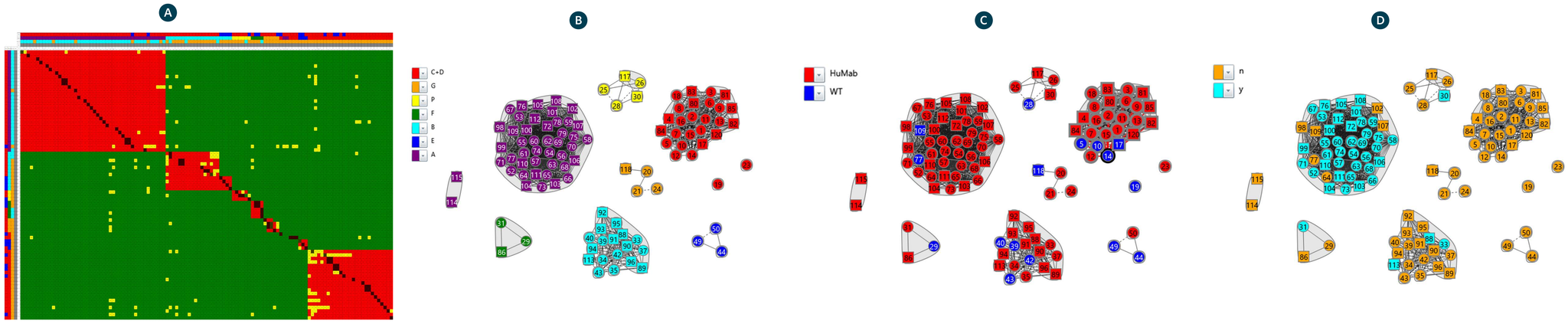

Figure 3. Epitope binning on a 192-mAb array using human progranulin as the model antigen (A) Heat map. Community plots colored by (B) subdomain, (C) mAb library, or (D) cross-reaction to mouse progranulin.

CONCLUSION

The "one-on-many" assay format offered by Array 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, and epitope binning 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 Array SPR technology to enable unattended analyses of, (1) 1152 mAbs in a capture kinetics format, or (2) a full 384 x 384 epitope binning assay. 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 Array SPR.

References: Ching *et al*, MABS 2017

We thank Adimab for providing the reagents for the kinetic analysis, which is part of an ongoing collaboration.