Peptide-based Epitope Mapping Using Minimally Consumptive High Throughput Array SPR

Introduction

Knowing the precise binding region, or epitope, targeted by a monoclonal antibody (mAb) on its specific target is of great interest for the discovery of therapeutic antibodies, enabling the identification of unique epitopes with biologically-relevant mechanism of action and building one's intellectual property portfolio. It is also tremendously useful in understanding host responses to pathogens in guiding the design of vaccines.

Array SPR is a biophysical technology that can significantly accelerate the specificity screening of mAbs via its "one-on-many" analyte-on-ligand assay format. In an Array SPR experiment, a solution partner (analyte) is injected over an immobilized array of hundreds of possible binding partners (ligands) enabling the analyte's binding specificity to be determined in a single injection. This configuration is particularly well-suited to epitope mapping of mAbs (as analytes) against an arrayed peptide library (as ligands), provided their epitopes (or partial epitopes) can be recapitulated on peptides, which generally lack a defined conformation.

To perform such an epitope mapping experiment, a custom biotinylated peptide library is prepared, typically consisting of an overlapping set of peptides spanning the antigen's primary sequence and immobilized onto discrete spots of a streptavidin-coated sensor chip. Herein, we describe how a panel of 96 mAb analytes, comprising 4 replicate sets of 22 unique clones and controls, was screened against a library of 384 biotinylated peptides, comprising 76 unique sequences arrayed onto 4 spots each with controls, in an overnight run (16 h) using <1 µg per mAb and <1 pmol per biotinylated peptide.

Method

Human and mouse forms of osteopontin (UniProt entries P10451 and P10923, respectively) were each synthesized as BioTides™ (JPT Peptide Technologies), using an overlapping set of 15-residue peptides, offset by 5 residues. A total library of 76 BioTides was prepared at standard scale (50 nmol per peptide) with a C-terminal biotin and an N-terminal glycine amide. BioTides were reconstituted to 1 g/l in DMSO and diluted 1000-fold to 1 µg/ml in HBS-EP+ (10 mM Hepes pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05% Tween-20).

To aid visualizing the spotted array, 1 µg/ml of an irrelevant biotinylated IgG was used as a "tracer". Printing was performed at room temperature (23°C) on a XanTec SAD200M (streptavidin-coated) sensor chip using Carterra's continuous flow microspotter (CFM) primed with HBS-EP+ as run buffer. BioTides were printed in batches using a 48-channel printhead for 5 – 15 min and the spots were traced for 5 min with 1 µg/ml irrelevant biotinylated IgG and washed with run buffer for 3 min. Eight consecutive prints were used to generate a 384-array representing quadruplicate spots of each BioTide (and tracer) and blank spots, representing tracer alone.

The printed chip was then docked in an MX96 SPR imager (IBIS, Netherlands) set at 25°C and primed with a run buffer of HBS-EP+ with 0.5 g/l BSA. A panel of 22 unique mAbs was prepared at approximately 5 µg/ml in run buffer and injected as analyte for 5 min in quadruplicate binding cycles, regenerating the surfaces with 4:1 v/v Pierce IgG Elution Buffer (pH2.8):5M NaCl after each mAb injection. Data were calibrated, zeroed, aligned, and referenced (using a global reference spot) in SPRINT and exported into Carterra's Epitope Software for analysis.

Results

BioTide-based epitope mapping has been described elsewhere[1], but here we expand the scope to a much larger array with minimal sample consumption and orders of magnitude higher throughput. **Figure 1** shows the assay format employed whereby 96 mAb analytes, comprising 22 unique clones prepared in replicate and dispensed into a 96-well plate, were injected serially over a 384-BioTide array. The "one-on-many" assay configuration not only offers a high throughput specificity screening method, but since SPR is a mass-based detection principle it gives excellent signal-to-noise detection sensitivity because the mAb analyte's molecular weight is about 100-fold higher than that of the immobilized peptides, allowing for clear mapping results.
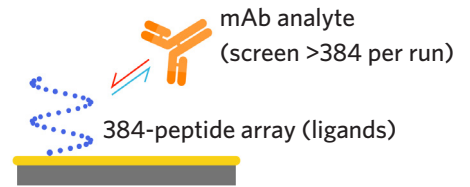

Figure 1: Assay orientation of a peptide-array based epitope mapping experiment.

Figure 2 shows a snapshot of the Epitope Software where the data are displayed as multiple panels. **Figure 2A** shows an overlay plot of the sensorgrams obtained for a single mAb analyte injected over the entire 384-peptide array; its specific binding to only a few peptides is clearly discerned above the baseline or "null" responses collected for all other peptide-coated spots, allowing it to be "mapped" specifically to those peptides. The horizontal line indicates the default global threshold setting (which can be user-adjusted) for defining "specific binding" with the vertical rainbow bar on the far left representing the range of observed binding responses from none/medium/high using a blue/pink/yellow palette. **Figure 2B** shows a partially sorted heat map for all measured 96 analyte x 384 ligand interactions (totaling 36,864 analyte/ligand pairs, plus buffer analyte blank control), with each cell representing the binding assignment for each analyte/ligand interaction, using the color palette described above. **Figure 2C** panel shows a dendrogram option for visualizing the clustering of the mAb analytes, based on their binding specificity for the arrayed peptides. Clearly, some mAbs show unique binding specificities, whereas others belong to families sharing the same binding specificity. **Figure 2D** shows a stacked plot representation of the mapping results, where the peptides contributing to a given mAb analyte's binding specificity are identified by name and clustered into families.
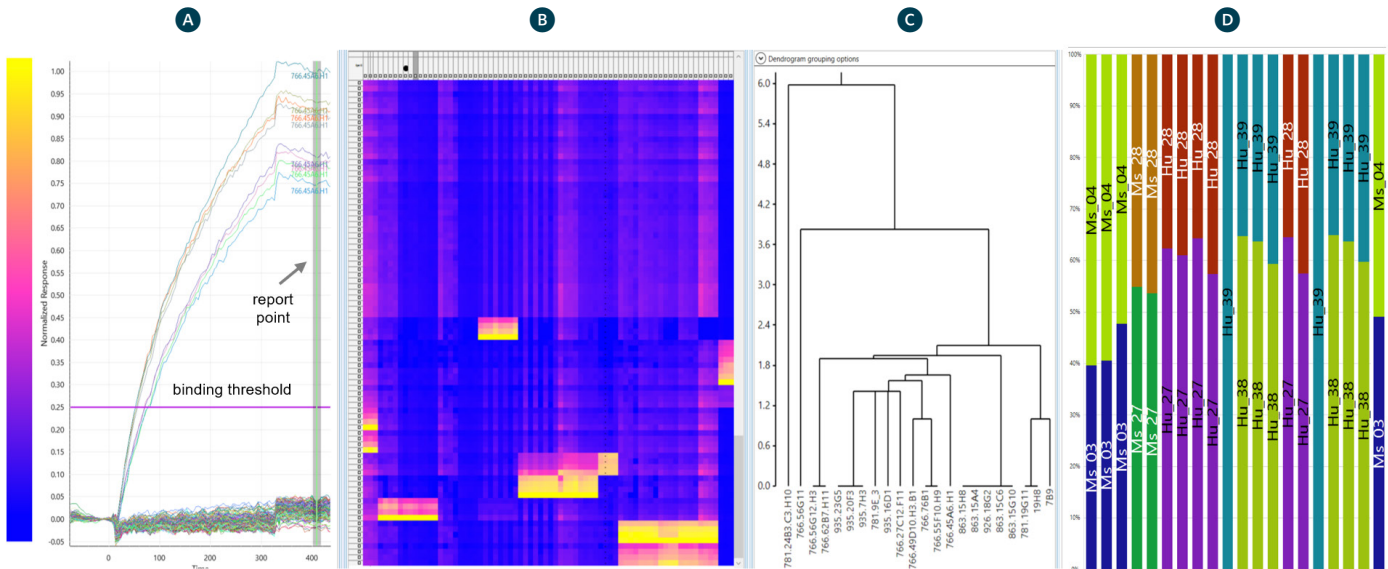

Figure 2: Snapshot of the results of a peptide-array based epitope mapping experiment in the Epitope Software. The binding results can be viewed in multiple panels: (A) sensorgram overlay plot, (B) heat map with peptides in rows and mAbs in columns, (C) dendrogram, showing how the mAbs cluster into distinct epitope families, and (D) stacked plot showing the peptides contributing to each mAb analyte's binding specificity, totaled to 100%.

Figure 3A shows the full sorted heat map resulting from the experiment and **Figure 3B** summarizes how the mAbs clustered into well-defined epitope groups. The 22 studied mAbs were thus mapped into ten epitope groups. Since we had fewer than 384 peptides in our library, we spotted each one four times within the array, and since we had fewer than 96 unique mAbs, we injected each one 4-8 times as analyte. This allowed us to generate replicates per mAb/peptide pair within the experiment, which manifested as "blocks" of color within the heat map and reinforced our confidence in the mapping assignments, because the results were highly reproducible, both spot-to-spot and injection-to-injection. The peptide array regenerated easily with acid, as expected because peptides can often tolerate quite harsh regeneration conditions without compromising their integrity.
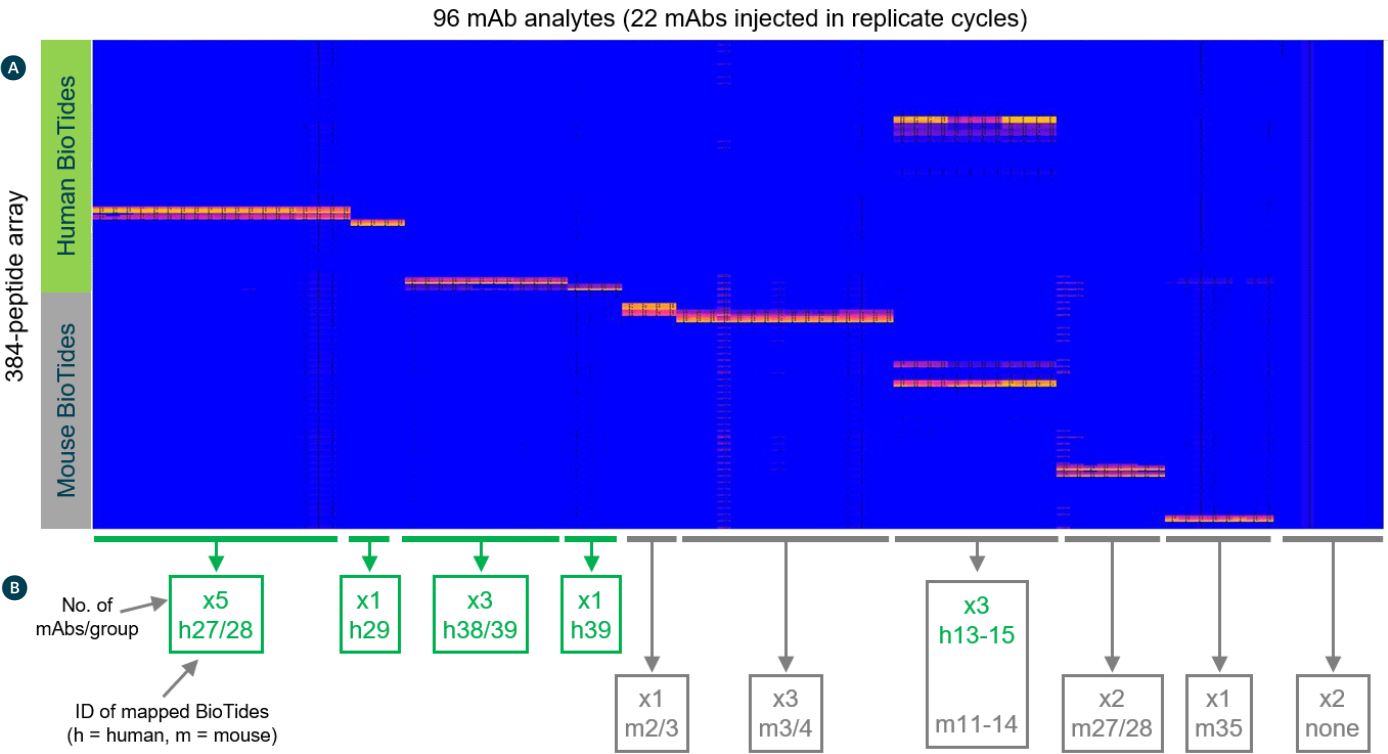

Figure 3: Summary of the mapping results. (A) Entire sorted heat map and (B) deduced epitope groups. The epitope groups are shown in boxes, with the number of mAbs per group indicated by the "x5" etc. and the mapped peptides from the human and mouse sequences referred to by h1, h2, h3... and m1, m2, m3... respectively. Thus, the 22 mAbs fell into ten groups; 4 groups were specific for the human sequence (shown in green), 4 were specific for the mouse sequence (shown in grey), and one was human/mouse cross-reactive. Two mAbs did not bind any peptide consistent with their binding conformationally-sensitive epitopes that could not be recapitulated on peptides.

Summary

The "one-on-many" assay format offered by Array SPR is ideally suited to specificity screening applications, as demonstrated here in the context of epitope mapping a panel of mAbs against a peptide library. Carterra's next generation LSA instrument expands the unattended throughput of this application from 96 to > 384 mAb analytes, by accommodating a 384well microplate for analytes, enabling a 384 x 384 "mAb x peptide" "analyte x ligand" interaction matrix to be explored in a standard run taking about 48 h and using <1 µg per mAb and <1 pmol per biotinylated peptide. An advantage of this assay format is that the sample consumption on a per mAb and per peptide basis does not scale, regardless of the size of the mAb panel or the peptide library, because analyzing one mAb binding to one immobilized peptide would consume the same amount of material as analyzing 384 unique mAbs (as analytes) against a 384-peptide library (arrayed on the surface). Analysis of these large data sets is simplified with Carterra's intuitive and powerful software tools, facilitating heat map generation, sorting and visualization of the mAb behaviors and clustering into epitope families. Additionally, the real-time sensorgram data are only a click away to enable in-depth analysis of nuanced behaviors and user-defined threshold settings.

When combined with epitope binning studies against the whole antigen in solution phase, epitope mapping results can provide a complementary tool for defining a mAb's precise epitope and enabling exquisite resolution between near-identical epitopes. Conversely, if mAbs are found to map to arrayed peptides but fail to bind to the solution antigen, this may suggest that they bind artificial epitopes that are not exposed on the native antigen, and are likely biologically irrelevant. In this case, screening a panel of mAbs against a peptide array can quickly identify mAbs that bind only linear (and possibly denatured) epitopes and not conformational ones.

References

1. Abdiche, YN, et al, Expanding the ProteOn XPR36 biosensor into a 36-ligand array expedites protein interaction analysis. Anal Biochem. Apr 1;411(1):139-51. doi: 10.1016/j.ab.2010.12.020 (2011).

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