Smarter selection: building better reagents through Array SPRi-based high-throughput biophysical characterization and RabMAb® technologies

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INTRODUCTION

The high-throughput Array SPRi system can be used to provide comprehensive epitope binning and kinetic screening information directly at the primary screening stage. In combination with RabMAb® hybridoma and B cell fusion technologies, this approach can quickly identify and develop high quality monoclonal antibodies (MAbs) for research and diagnostic applications. To demonstrate the value of this approach, we characterized a panel of MAbs corresponding to a subset of highly abundant B cells that circulate after immunization with a particular antigen. Crude and purified preparations of the panel were included in the assay to test assay performance at the very earliest stages of product development. High-throughput epitope binning and kinetic screening on the Array SPRi platform allowed us to profile the binding character of each MAb, and assess the breadth of epitope recognition represented in the panel using only 20 µL of each supernatant and a few micrograms of the target analyte.

RESULTS AND DISCUSSION

HT biophysical characterization using Array SPRi for kinetic ranking and epitope binning

A continuous flow microspotter (CFM) was used to amine couple 32 purified MAbs and their 32 corresponding crude preparations onto a dextran coated prism. A classical binning competition study was then performed in which the purified and crude preparations were injected at concentrations of 20 µg/mL and 1:10, respectively, in a pairwise, combinatorial sequence over the array to fully compete each species in the panel against every other in the presence of the target.

- Nearly 5,000 sensorgrams were generated that fully mapped the kinetic and epitopic landscapes of the panel.
- There was excellent agreement between kinetic data from crude preparations and purified MAbs (Figure 1).
- The blocking and sandwiching profiles from the epitope binning experiment were almost identical between the crude and purified samples (Figure 2).

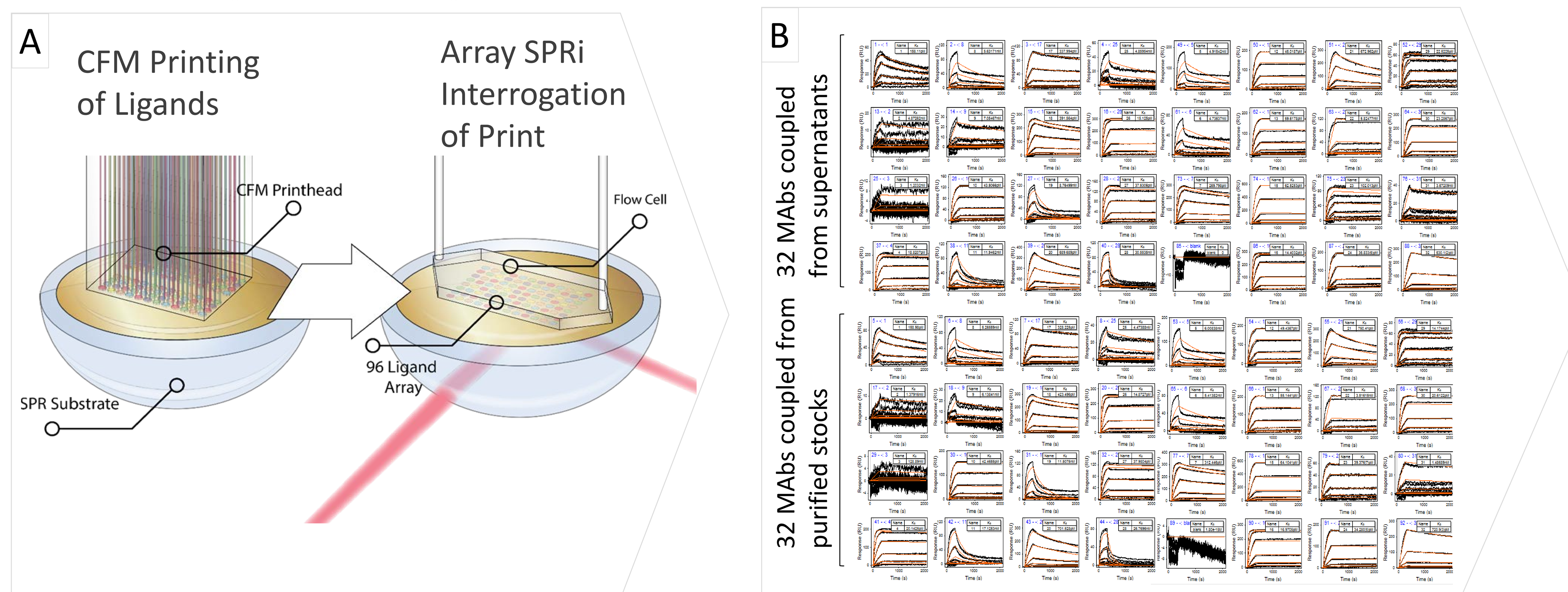

FIGURE 1. HIGH-THROUGHPUT KINETIC RANKING. A. Ligands are printed onto a dextran-coated prism using a CFM print head. The 96 SPRi array grid can be interrogated for antibody-ligand interaction on the sensor surface. **B.** A kinetic screen probed relative affinities within the antibody panel. The kinetic cycle was a 7 point, 3-fold concentration series from 100 nM, to cover the range of affinities represented. All species were fit to a 1:1 Langmuir, and off-rates for which insufficient decay was observed were fixed at 1x10-5 according to the 5% rule. For this reason some affinities reported are likely an underestimate of the true affinity, while others exhibited complex behaviors that could not be fitted to a 1:1 model.

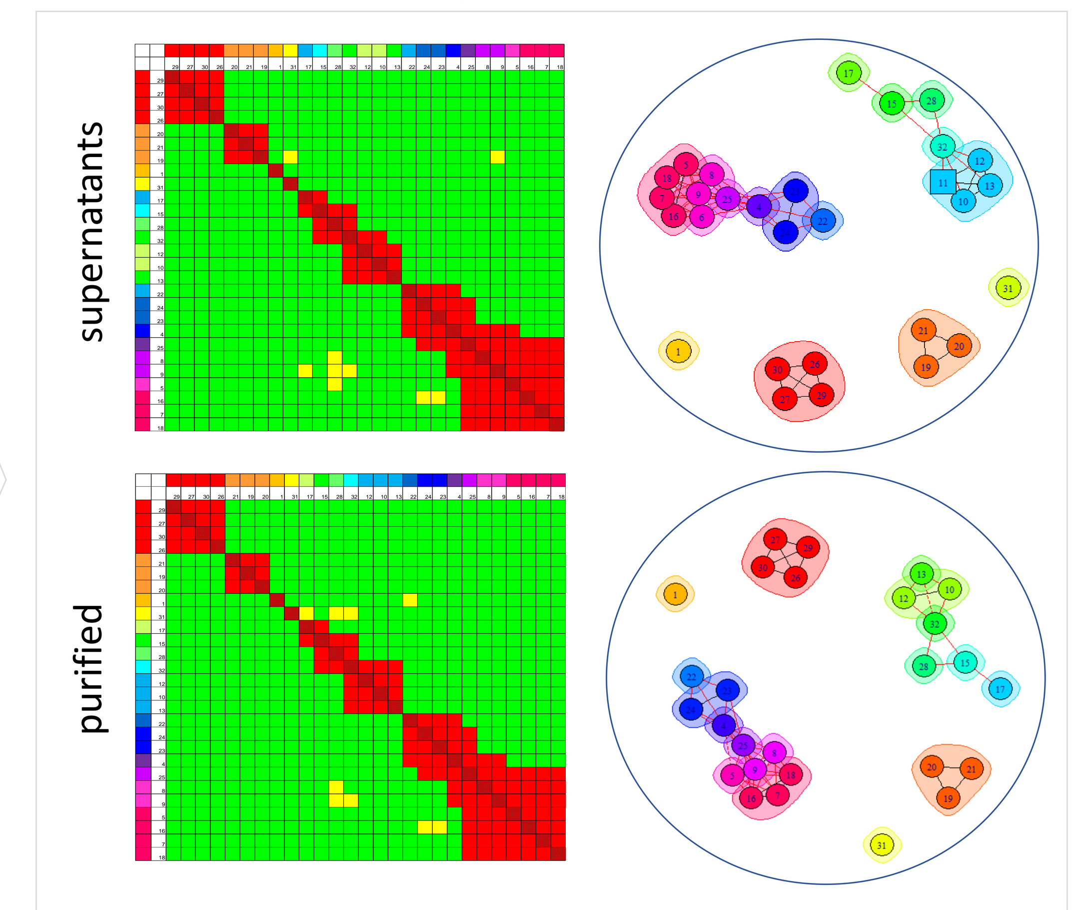

FIGURE 2. HT EPITOPE BINNING. The binding profile from the competition study were analyzed in the WM binning tool which sorts the heat maps to cluster blocking behaviors. Additionally, the software creates networks of the blocking interactions, which further help identify the unique diversity in the panel.

RabMAb® antibodies cover a wide range of epitope recognition and affinity

We next identified likely reagent candidates by applying epitopic diversity and affinity selective 'lenses' to find high affinity representatives of unique behaviors.

- Discreet subset analysis revealed 15 epitope bins, or unique blocking and sandwiching behaviors (Figure 3).
- Good correlation between heavy chain complementarity determining region (HCDR3) sequences and the binning data strongly indicates that IgG sequence similarity can predict epitopic specificity.
- Identically behaved species with diverse sequences, highlighting the complexity of the immune response and the value of early, direct characterization for the more informed selection of reagent candidates.

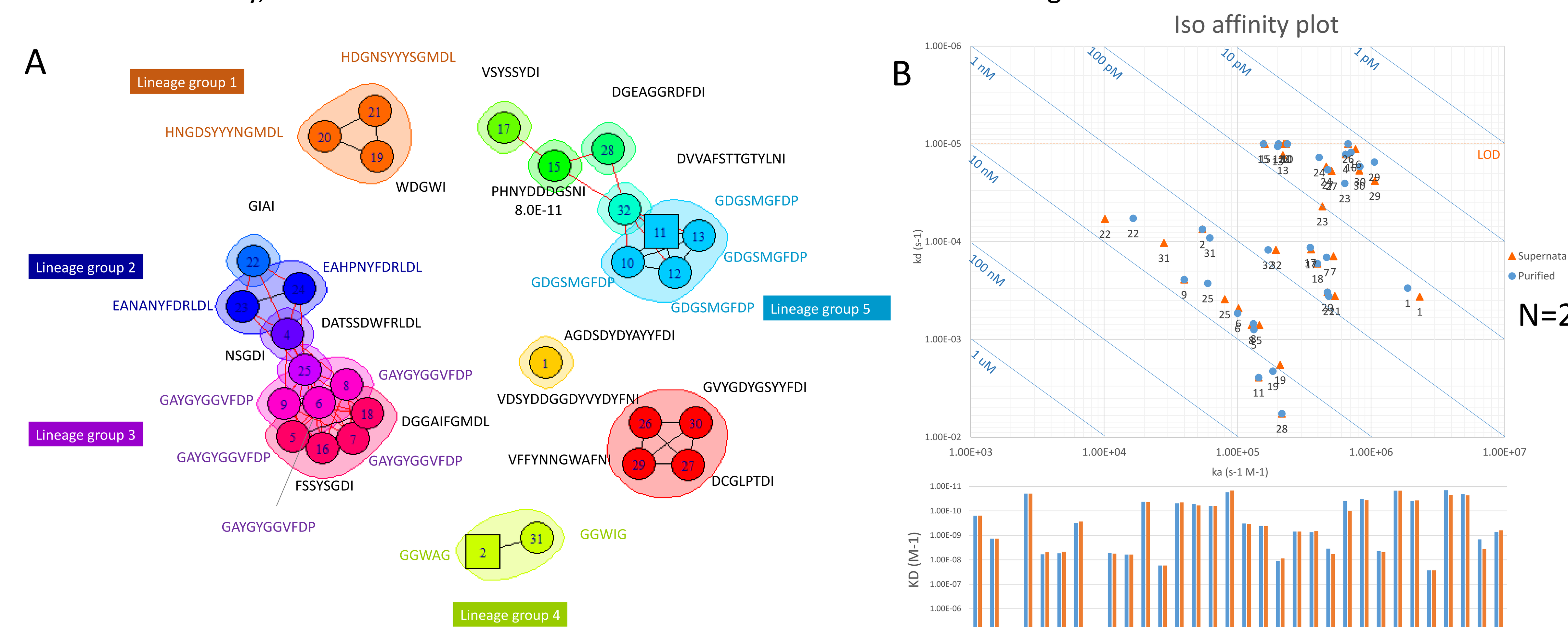

FIGURE 3. DIVERSITY IN EPITOPE RECOGNITION. A. Discreet subset analysis revealed 15 epitope bins, or unique blocking and sandwiching behaviors. The results are plotted as a network or clique plot in which each blocking interaction is identified by a line between participating nodes. RabMAb® antibodies with identical binding profiles are contained within a colored group. **B.** A quantitative assessment of affinity is an important predictive measure for the performance of the antibody. For the 22 species which fit 1:1 model, there was excellent agreement between pure and crude.

HIGHLIGHTS

- Array SPRi is a high-throughput epitope binning and kinetic ranking platform that can support large screening efforts.
- Excellent agreement between crude and purified samples indicates that the technology can be applied at the earliest stages in the antibody development pipeline.
- The wide range of epitope bins identifies targeting to discrete regions of the target protein without more extensive mapping efforts.
- Combinations of data identify likely product candidates for assay development such as binary pairs for an immunoassay.

REFERENCES

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