High Throughput Kinetic Characterization of Synthetic Recombinant Antibodies for Various Cell Surface Receptor Targets

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Introduction

Affinity and specificity are invaluable properties needed to assess the quality of a synthetic antibody (sAb) in relation to its target antigen. At IPI, we use bio-layer interferometry (BLI) to verify if antibodies bind to their specific antigen, and surface plasmon resonance (SPR) to obtain detailed kinetic parameters that allow us to gauge the affinity of the antibodies.

Methods

To assess antigen/antibody binding kinetics of sAbs in the early development of reagent antibodies, we utilize both bio-layer interferometry (BLI) and surface plasmon resonance (SPR) methods. Specifically, we used the BLI (Octet HTX) method for an initial assessment at two antigen screening concentrations (20, 100nM)m identifying sAb clones and discarding nonbinders. We then conducted further kinetic characterization using HT-SPR (Carterra), either capturing or directly coupling antibody candidates at different densities to a polycarboxylate dextran sensor chip. Antigens were then titrated from low-to-high concentrations until most ligand sites were occupied. To estimate k_a (association rate constant), k_d (dissociation rate constant) and K_D (affinity), the dose-dependent kinetic traces were fitted to a 1:1 Langmuir binding model. Using this kinetic information, we selected the high-affinity antibody binders for various downstream application development processes and used the data to optimize the clone selection process during antibody discovery.

High Throughput Biophysical Methods

BLI technology

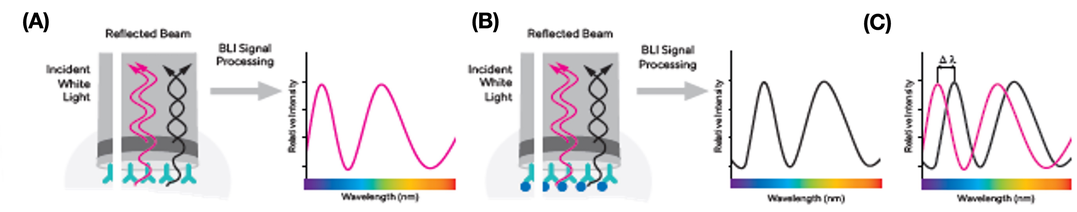

Figure 1: Schematic overview of the proprietary Octet® BLI technology, a label-free optical technique based on fluidic-free format in standard microtiter plates. A & B) Relative intensity of the light reflection pattern from the two surfaces on the biosensor. C) Octet® systems with BLI technology measure the difference in the reflected light's wavelength (Δλ) between the two surfaces (i.e., reference and sample) . *Figure source: Sartorius*

Carterra SPR technology

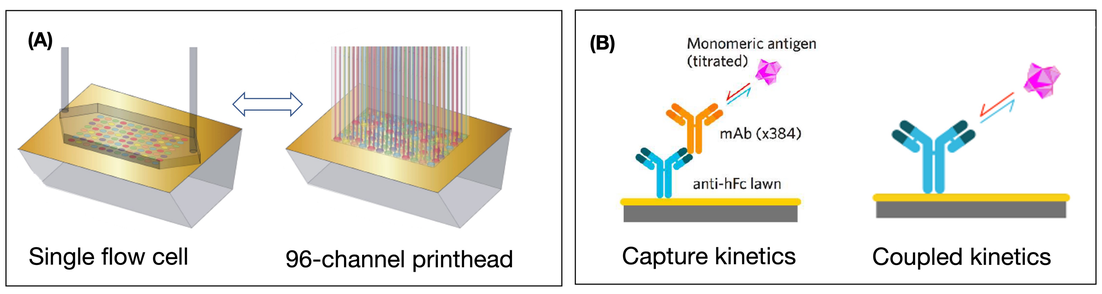

Figure 2: Schematic overview of the Carterra SPR technology. (A) Microfluidics have two different flow units, including a single flow cell and 96-channel printhead. The 96-channel printhead facilitates the printing of antibodies on an array of 384 reaction spots. The single flow cell facilitates antigen titrations for many antibodies on the surface (one-on-many interactions). (B) The antibody immobilization can be done with capture method i.e., using secondary antibody lawn and alternatively chemically coupled to the surface. *Figure source: Carterra*

Antibody biophysical validation workflow

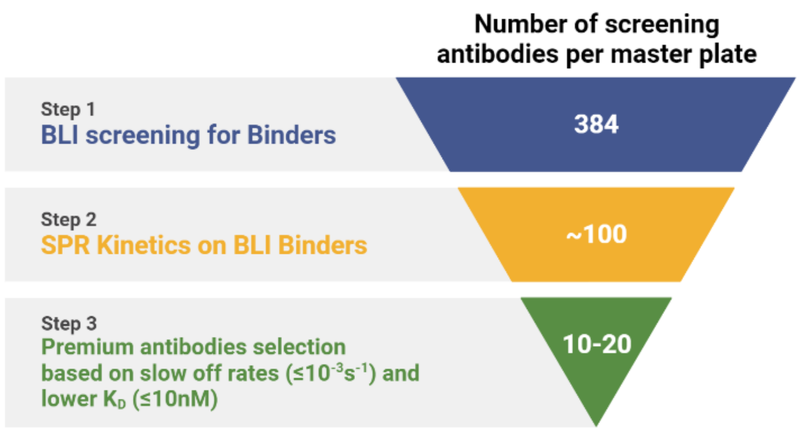

Figure 3: Schematic overview of the antibody biophysical validation workflow at IPI

- Step1: BLI (Octet HTX) method for an initial assessment at two antigen screening concentrations (20, 100nM).
- Step 2: Further kinetic characterization using HT-SPR (Carterra).
- Step 3: Sorting kinetic data based on slow off rates (≤$10^{-3}s^{-1}$) and lower K_D (≤10nM).

Results

Because experimental studies suggest that the intrinsic association rate constant for protein–protein interactions in normal salt conditions does not normally exceed 5 ×10^6 M^{-1} s^{-1}, the "off rate" is the key parameter for increasing affinity and a powerful indicator to select binders with high affinity. Here we show kinetic characterizations of ~2000 antibodies against 84 antigen targets from various antibody discovery campaigns on cell surface receptors and ligands.

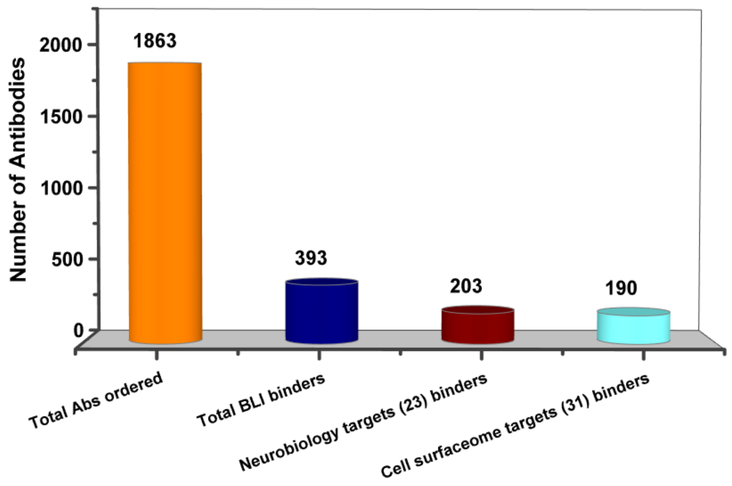

Figure 4: BLI screening of sAb binders

Graph shows the hit rate (21%) for the total ~2000 sAbs screened against 54 targets. The majority were bound to neurobiology (203 binders/23 targets) and cell surface targets (190 binders/31 targets).

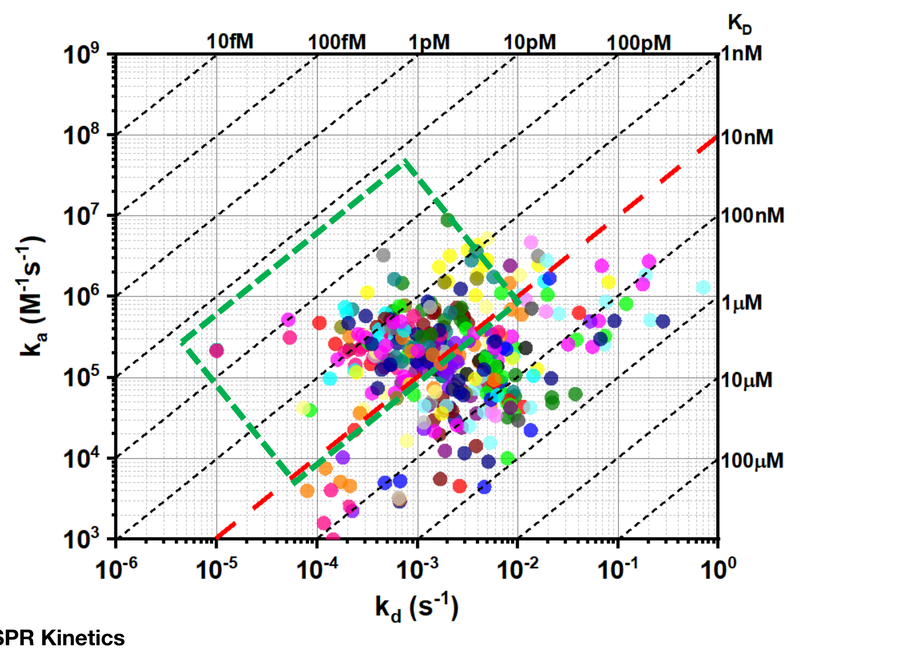

Figure 5: SPR Kinetics
Graph shows the results of kinetic analysis using HT-SPR (Carterra). We identified 244 high-affinity binders to 30 targets and then sorted the kinetic data based on slow off rates (≤$10^{-3}s^{-1}$) and lower K_D (≤10nM) for higher affinity binders (highlighted in green box with split line). The majority of high-affinity candidates bound to neurobiology (146 binders for 14 targets) and cell surface protein targets (98 binders for 16 targets).

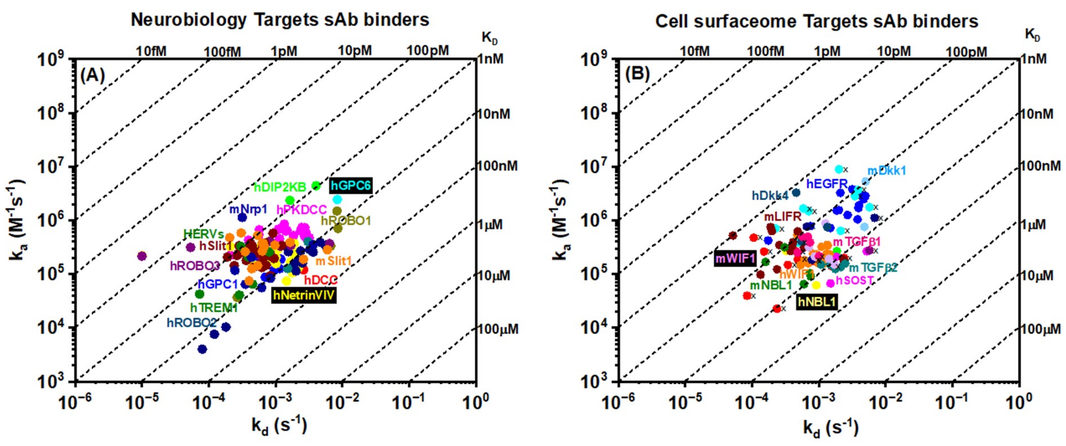

Figure 6: Isokinetic plots for various IPI's "pillar project" targets
A total 244 high affinity K_D (≤10nM) binders were identified, including (A) neurobiology (146 binders to 14 targets) and (B) cell surface protein targets (98 binders for 16 targets).

Conclusion

BLI and surface plasmon resonance methods can be combined to successfully discover novel antibody candidates. In addition, the large kinetic data set generated in these experiments can be used to optimize antibody discovery and selection processes.

Want To Collaborate With Us?

We have generated antibodies to more than 200 cell surface receptor targets.

We are open to collaborations from industry and academia.

Contact us at **https://proteininnovation.org/receptor-engineering/** or via **rob.meijers@proteininnovation.org**

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