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APPLICATION NOTE

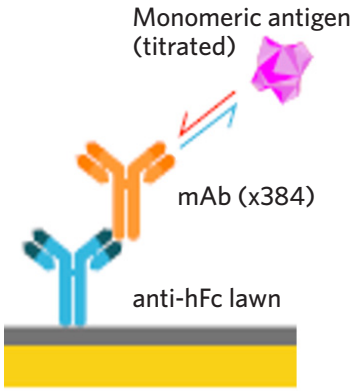
No Compromise Kinetics™ with Array SPR

Introduction

Throughput, speed, resolution, and sample consumption are typically key limiting factors for detailed kinetic characterization early in monoclonal antibody (mAb) discovery campaigns. Here, we show that Array SPR can facilitate the generation of high quality kinetic data from a large panel of clones rapidly and with minimal sample consumption. In this example of a single day's run, 384 independent kinetic measurements were made on an array comprised of 43 unique mAbs, each immobilized at 8-16 capacities, using a capture approach which does not require purified antibodies. This method required <1 µg per mAb and only 2 µg of the recombinant monomeric antigen. The array format provided well-described and highly reproducible kinetic measurements for clones spanning a 10,000-fold affinity range for their target antigen. These data clearly demonstrate the efficiency and quality of kinetic analysis that is possible using Array SPR.

Method

A capture kinetic analysis of a large panel of mAbs binding their specific monomeric antigen (as analyte) was performed using Array SPR. A moderate density (~1,000 Response Units) anti-human IgG-Fc capture "lawn" on a XanTec HC30M chip (30nm polycarboxylate) was prepared via amine coupling. To prepare the lawn, the chip was activated with 133mM EDC and 33.3mM S-NHS in 100mM MES pH 5.5 and goat anti-Human IgG Fc (Southern Biotech) was coupled for 10 minutes at 25µg/mL in 10mM sodium acetate pH 4.5 and quenched using 1M ethanolamine HCl pH 8.5. Carterra's continuous flow microspotting (CFM) technology was used to capture a panel of mAbs for 5 minutes at 2 µg/mL to create a 384-mAb array with 8-16 individual spots per clone. A purified recombinant monomeric form of the specific antigen targeted by the mAbs was injected over the entire printed array at 7 concentrations in a 3-fold dilution series ranging from 0.4-300nM using a 5-minute association phase and a 25-minute dissociation phase per analyte concentration. Running buffer was 10mM HEPES pH7.4, 150mM NaCl, 3mM EDTA, 0.05% Tween 20 with 0.5 mg/mL BSA. Binding data were double referenced by subtracting the responses from an interspot (local reference) surface and the responses from a buffer analyte injection and globally fit to a 1:1 Langmuir binding model for estimation of k_a (association rate constant), k_d (dissociation rate constant), and K_D (affinity) using the Carterra™ Kinetics software.



Results

The results of a capture kinetic experiment performed on a 384-mAb array using 0.4-300nM monomeric antigen as analyte, are shown as a "tile view" in **Figure 1**, where each panel represents the binding responses (colored by analyte concentration, with a blue/green palette) and global fit (in red) obtained for the antigen interacting with a single mAb-coated spot. Since we had fewer than 384 unique mAbs to study, we arrayed each mAb onto 8-16 individual spots within the array to highlight the spot-to-spot reproducibility of the array format, which shows that the results are invariant of a spot's address within the array. The use of multiple spots per clone also meant that the apparent kinetic rate and affinity constants of each antigen/mAb interaction could be reported with statistical confidence (**Figure 2**). The use of a wide analyte concentration range enabled us to characterize clones across a broad affinity range, with apparent K_D values from <39pM to >222nM. The analysis discerned clones varying ~73-fold in their apparent association rate constant (k_a) and >7000-fold in their dissociation rate constant (k_d), as shown by the histoplots in **Figure 2**. Examples of three clones showing diverse binding kinetics and affinities are shown in **Figure 3**. **Figure 4** summarizes the kinetic diversity of the studied panel, in terms of an isoaffinity plot, where the three clones highlighted in **Figure 3** are shown as blue symbols.

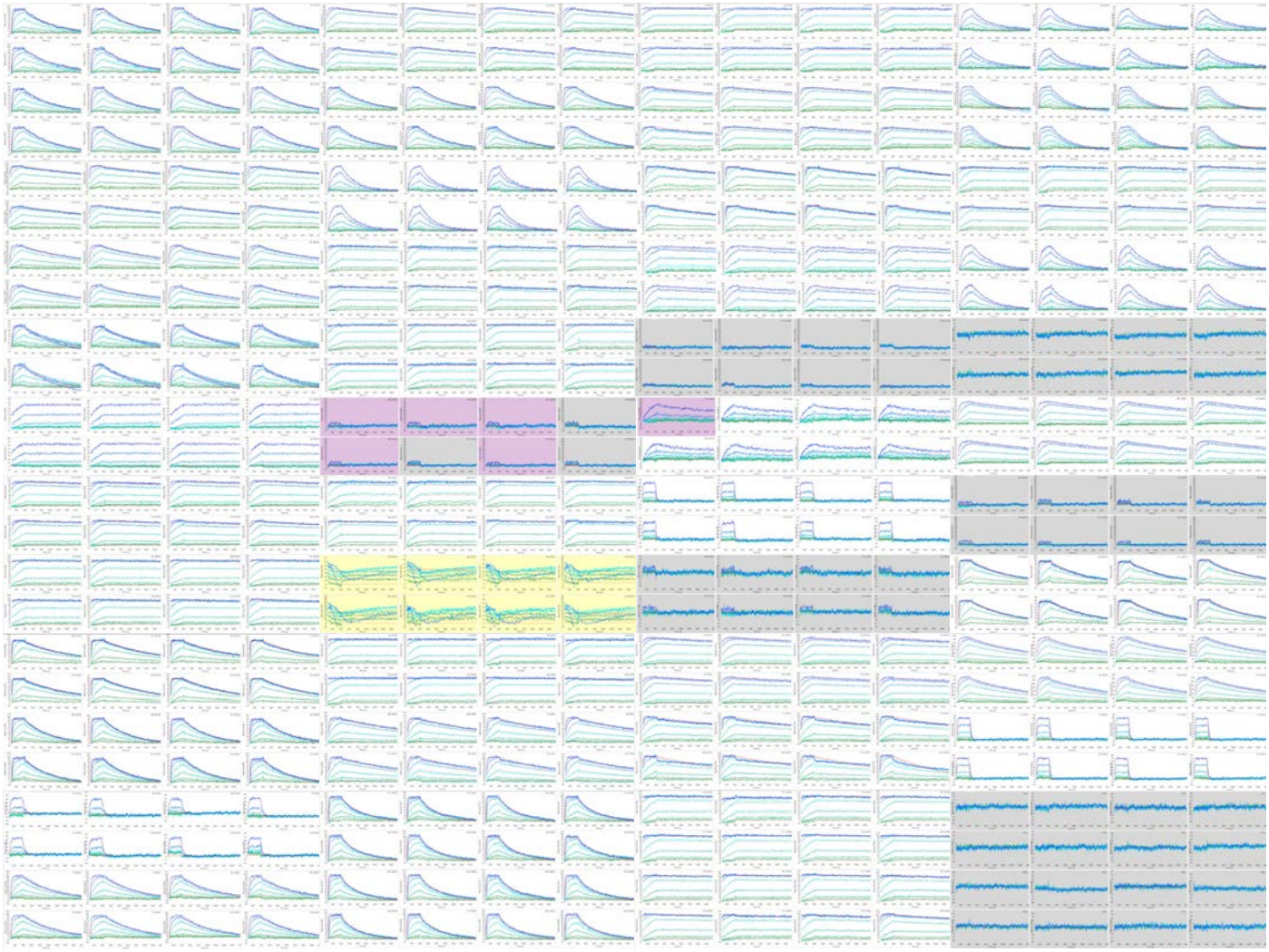


Figure 1. Capture kinetics was performed on 384 arrayed mAbs simultaneously using Array SPR. The array comprised 43 unique clones each captured onto 8-16 individual spots of an anti-human-IgG Fc-coated chip and their specific monomeric antigen was injected as analyte from 0.4-300nM over the entire array. Data with potential quality issues was automatically flagged by the software. Samples where the standard deviation of the residuals was >10% of the calculated R_{max}, which indicates a poor fit, are highlighted in yellow. Samples where the observed binding level was < 50% calculated R_{max} are highlighted in purple. Grey highlighting indicates barely binding or non-binder clones.

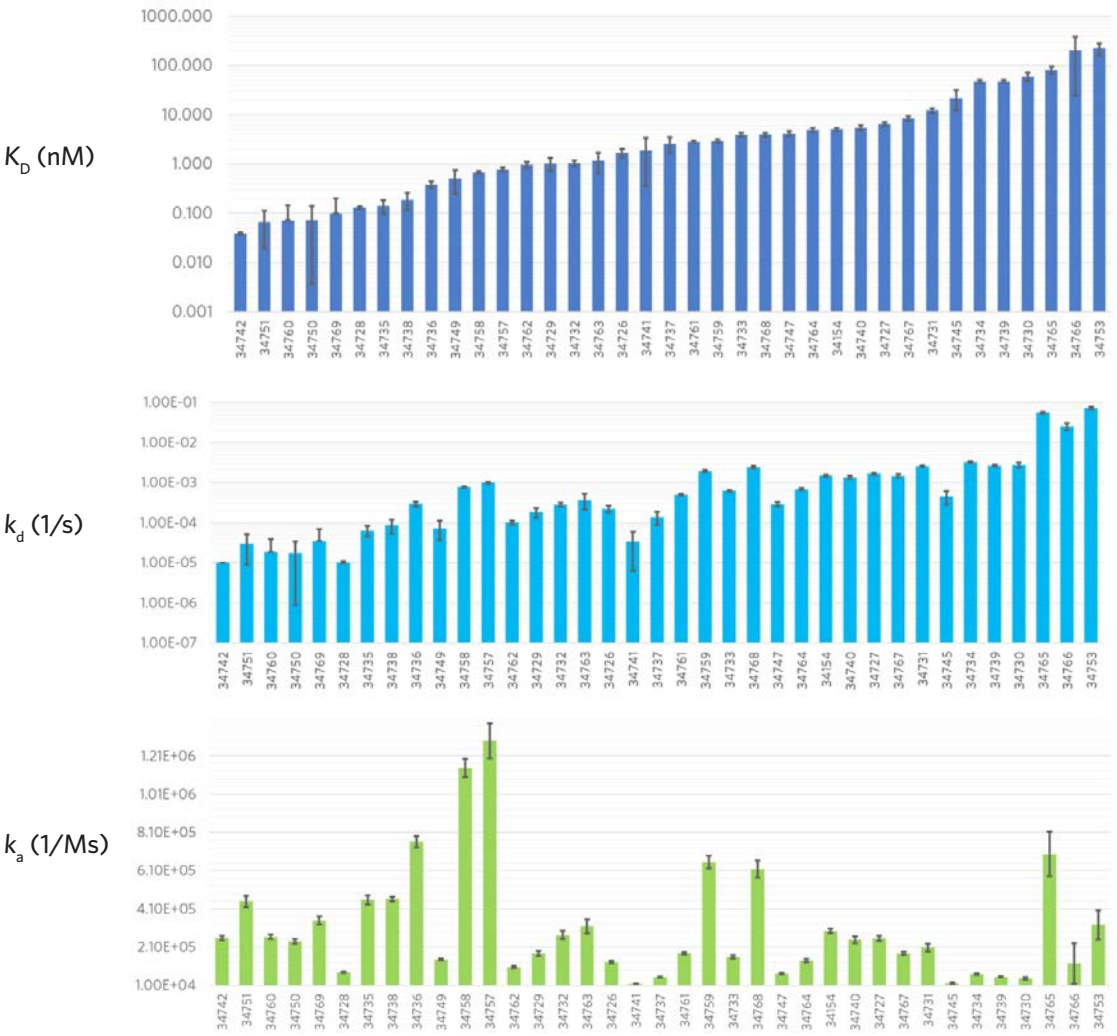


Figure 2. Affinity (K_D) values and kinetic rate constants (k_a and k_d) from Figure 1 are reported as mean values from 8-16 replicates per clone and error bars reflect the standard deviation.

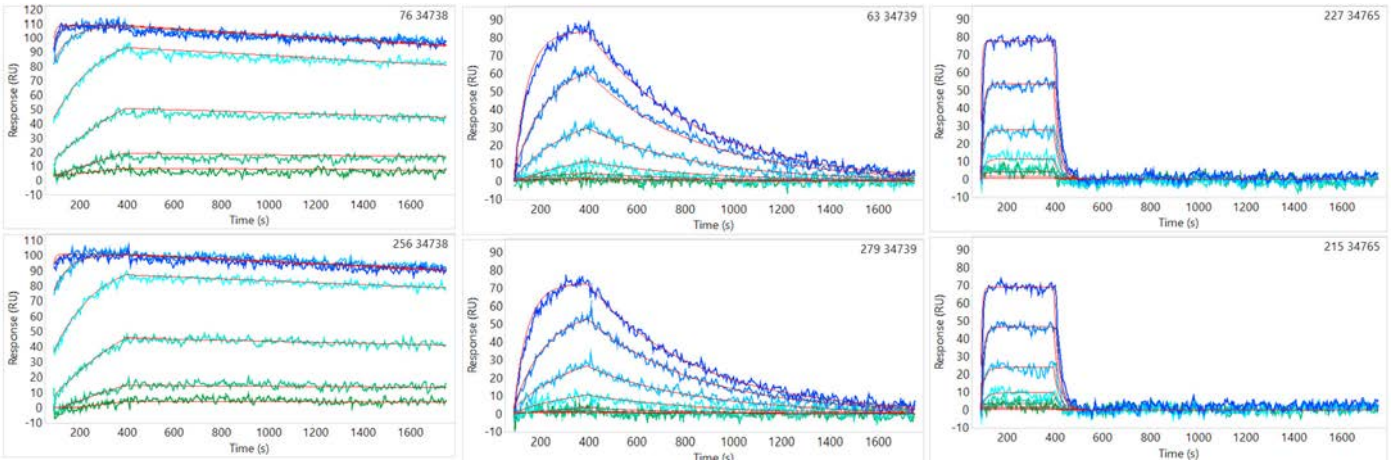


Figure 3. Example of duplicate spots for three clones with diverse kinetics (slow, medium, and fast dissociation rates, from left to right).

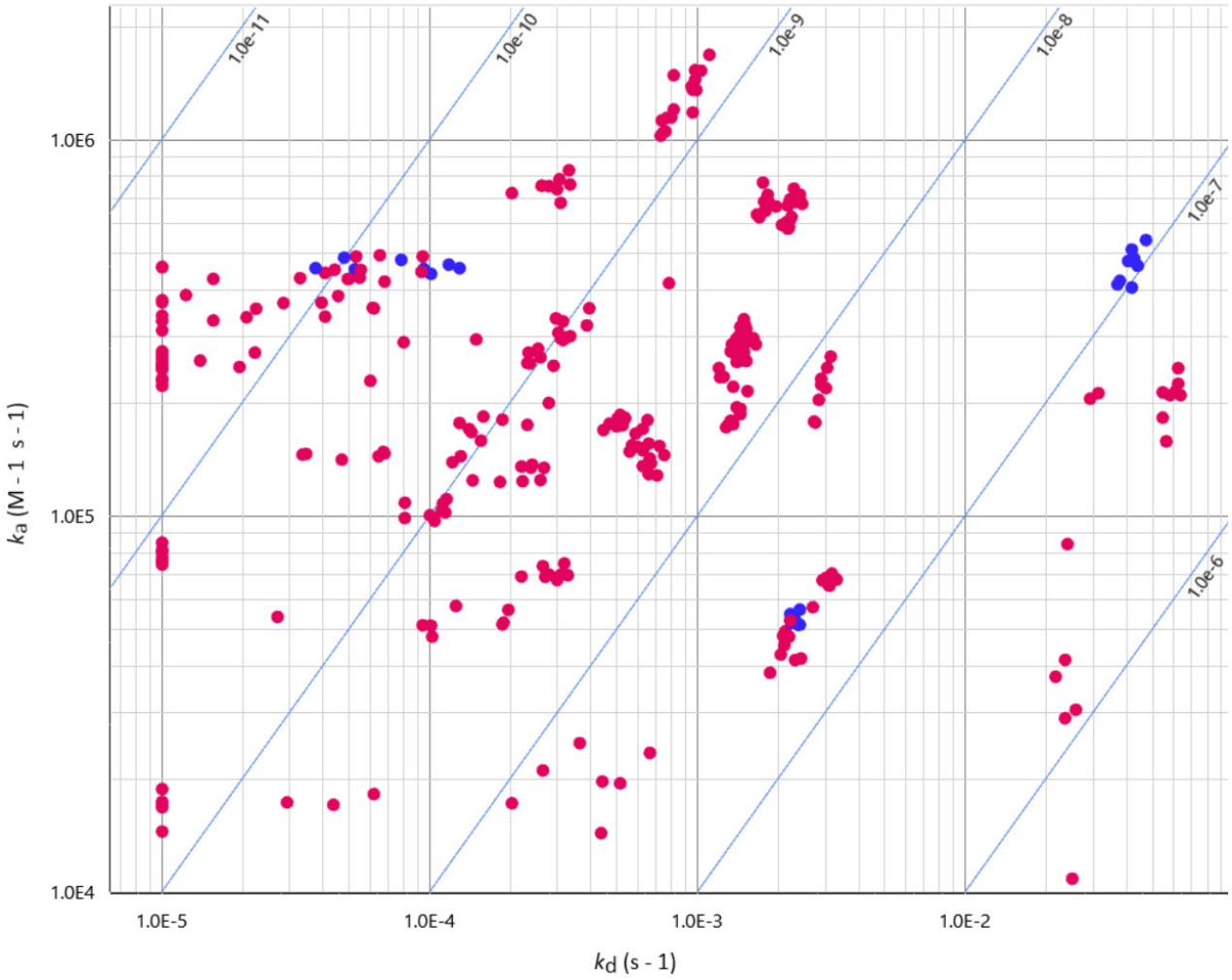


Figure 4. Iso-affinity plot, depicting the relationship between the association rate constant (k_a, y-axis) and the dissociation rate constant (k_d, x-axis) for all replicate mAb measurements (non-binders and clones flagged as poor fits in Figure 1 are excluded). The diagonal lines represent lines of equal or "iso" affinity (K_D). The blue dots represent the replicate measurements for the three clones shown in Figure 3. Note that a k_d value of 1x10-5 (1/s) was used as a limit in the analysis, for clones showing barely any detectable dissociation within the allowed dissociation phase (of 25 min), as much longer dissociation phases would need to have been monitored to provide sufficient signal decay to accurately estimate slower dissociation rates.

Summary

Array SPR facilitates the rapid generation of kinetic screening data from up to 384 samples in parallel. The use of Carterra's CFM technology to print the 384-spot array allows for the efficient immobilization of mAbs to a capture surface from low concentration (and unpurified) samples. These reloadable arrays yield high quality and reproducible binding kinetics of clones with diverse affinities and binding rate constants. Capture kinetic experiments performed by Array SPR take significantly less time and consume significantly less antigen than would be required for any other method to complete an analysis of this scale. Additionally, analyzing these large data sets is quick and easy using Carterra's powerful and intuitive Kinetics software, with a typical analysis taking only a few minutes.

This is part of collaborative work with Adimab, whom we thank for supplying the mAb panel.

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