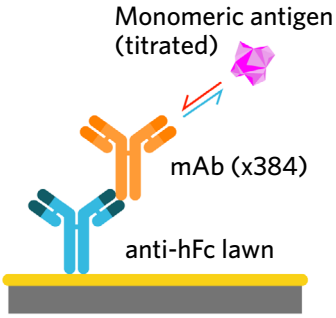
carterra®

APPLICATION NOTE

High Throughput SPR Kinetics

- **High throughput with simple set up.**
- **Extremely reagent efficient. Antigen usage does not scale with the number of clones.**
- **Highly reproducible kinetics. Allows for true replicates and error estimation.**
- **Analyze broad kinetic ranges from a single concentration series. No need to optimize concentration series for different kinetic ranges.**
- **High data quality.**



Capture Kinetics

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 the Carterra LSA can enable 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, with each clone included 8-12 times. This capture approach does not require purified antibodies. This method required <1 µg per mAb and only 7 µg of the recombinant monomeric antigen. The array format provided well-described and highly reproducible kinetic measurements for clones spanning a 5,000-fold affinity range for their target antigen. These data clearly demonstrate the efficiency and quality of kinetic analysis that is possible using the LSA.

Method

The Carterra LSA was used to perform a capture kinetic analysis of a large panel of mAbs binding their specific monomeric antigen (as analyte). A moderate density (~3,000 Response Units) anti-human IgG-Fc capture "lawn" on an 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. The anti-human IgG-Fc capture surface was then used to capture a panel of mAbs for 10 minutes at 2 µg/mL to create a 384-mAb array with 8-12 individual captures per clone. 8 replicate immobilizations required 1 µg of each mAb, and this sample was recovered after the capture step.

A purified recombinant monomeric form of the specific antigen targeted by the mAbs was injected over the captured antibody array at 8 concentrations in a 3-fold dilution series ranging from 460 pM to 1 µM using a 5-minute association phase and a 15-minute dissociation phase per analyte concentration. A total of only 7 µg of antigen was required to analyze 384 ligand interactions with an 8 point concentration series starting at 1 µM. 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 panel using 460 pM to 1 µM 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 replicate. Since we had fewer than 384 unique mAbs to study, we immobilized each individual mAb in multiple replicates across the array to highlight the reproducibility and demonstrate that the results are invariant of a ligand's physical location within the array. The use of multiple replicates 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 <134 pM to 670 nM. This approach would be slow and consume orders of magnitude more antigen on traditional biosensors. The analysis discerned clones varying ~30-fold in their apparent association rate constant (k_a) and >5000-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.

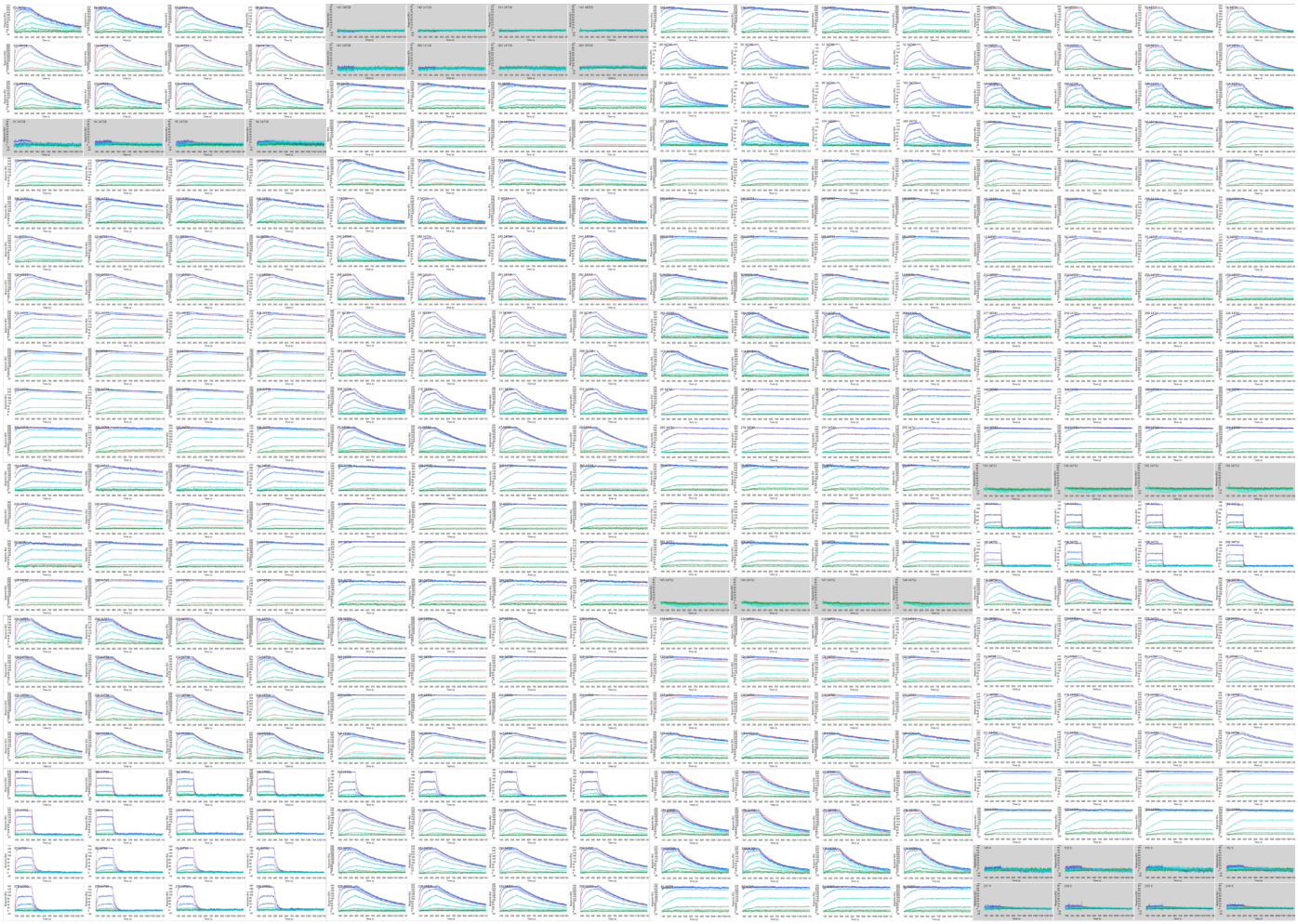


Figure 1: Capture kinetics was performed on 384 ligands simultaneously using the LSA. The array comprised 43 unique clones each captured onto 8-12 individual locations of an anti-human-IgG Fc-coated chip. The specific monomeric antigen was injected as analyte from 0.46 nM to 1 µM over the entire ligand set. Ligands with low or no binding were automatically flagged by the software and are shown as grey.

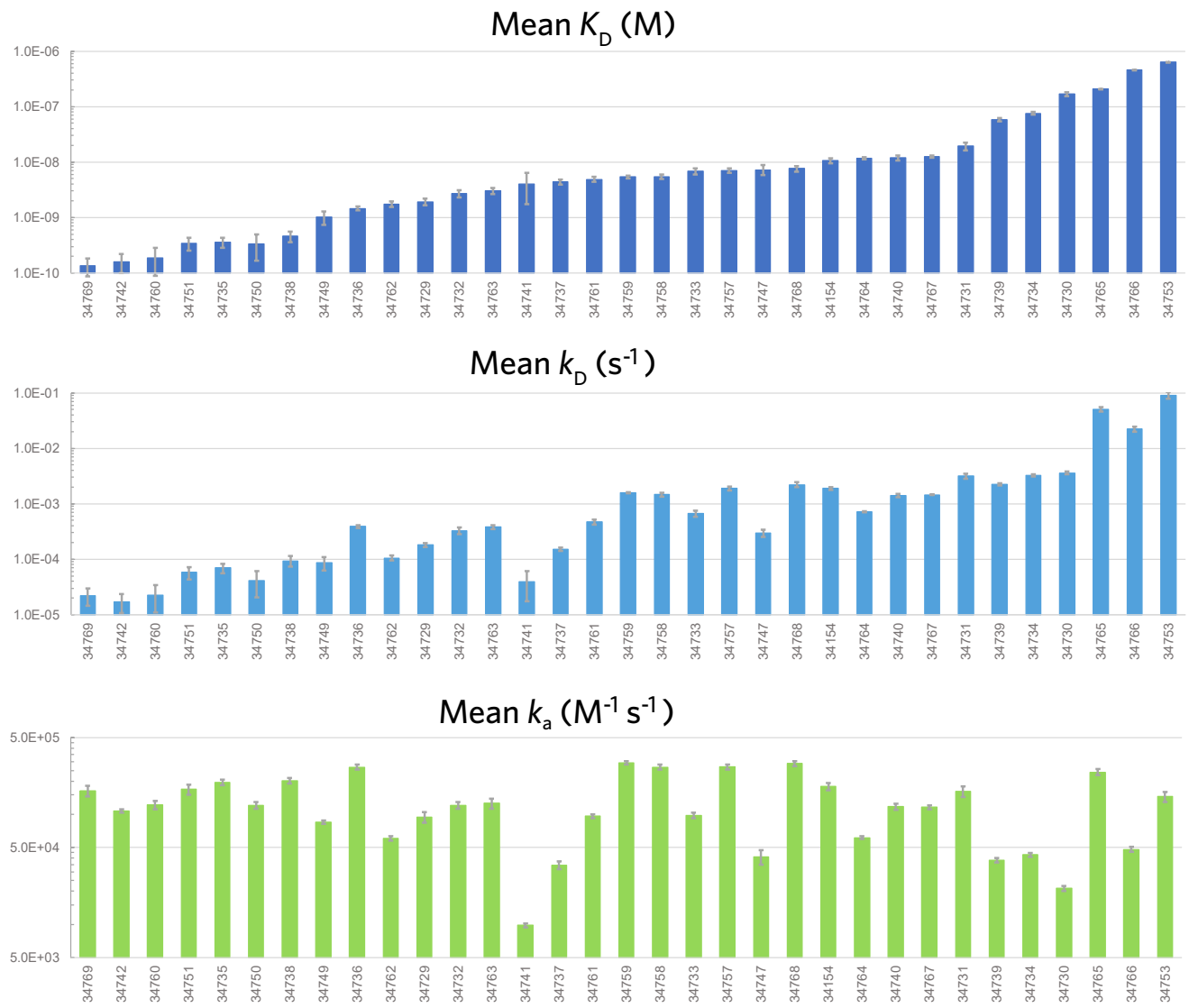


Figure 2: Affinity (K_D) values and kinetic rate constants (k_a and k_d) from Figure 1 are reported as mean values from 8-12 replicates per clone and error bars reflect the standard deviation.

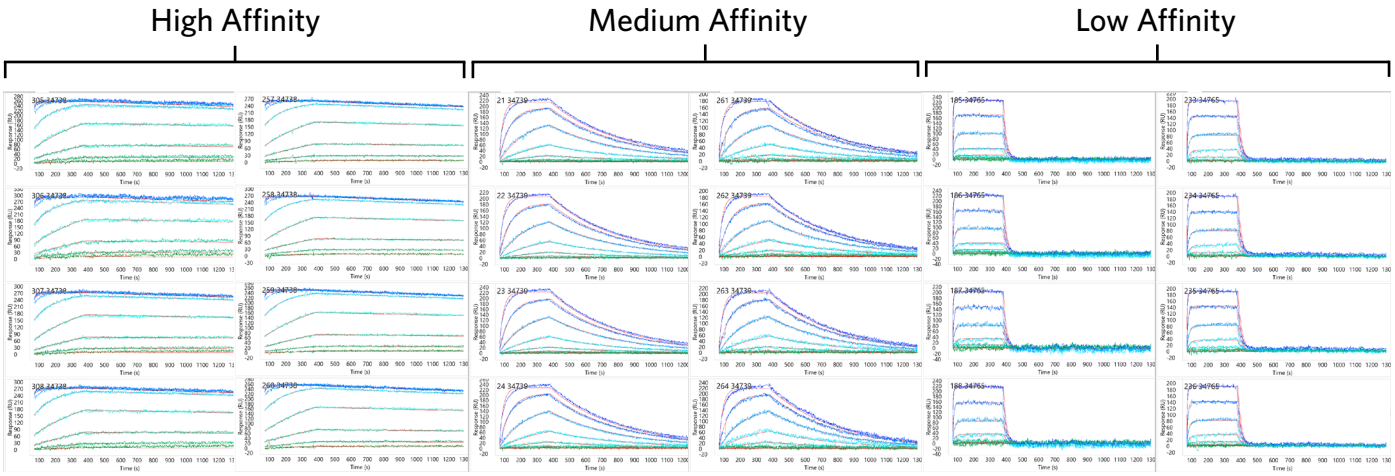


Figure 3: Example of replicate spots for three clones with diverse kinetics (slow, medium, and fast dissociation rates, from left to right).

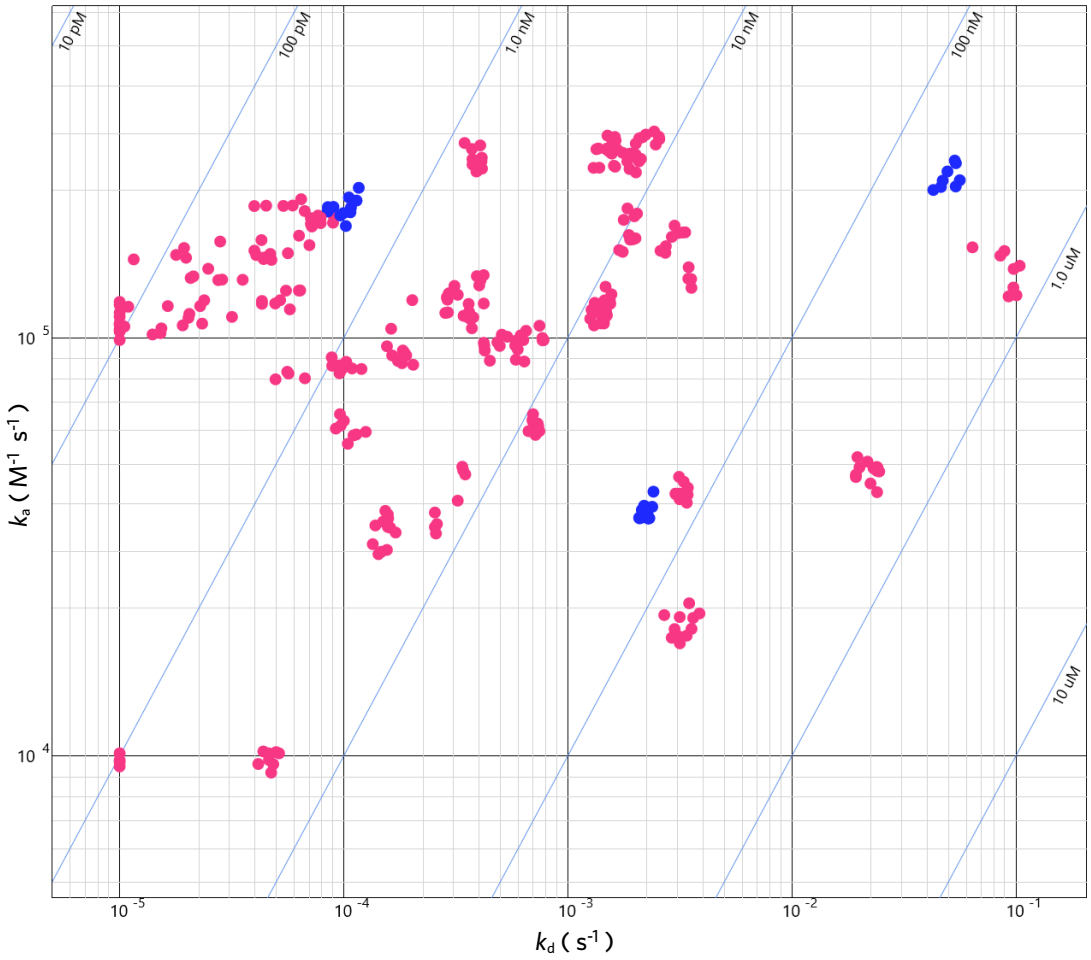


Figure 4: Iso-affinity plot, depicting the relationship between the association rate constant (k_a, y-axis) and the dissociation rate constant (k_d, x-axis) for all replicate mAb measurements (non-binders in Figure 1 are excluded). The diagonal lines represent lines of equal or "iso" affinity (K_D). The blue dots represent the replicate measurements for the three clones shown in Figure 3. Note that a k_d value of $1x10^{-5}$ (s^{-1}) was used as a limit in the analysis, for clones showing barely any detectable dissociation within the allowed dissociation phase (of 15 min), as much longer dissociation phases would need to have been monitored to provide sufficient signal decay to accurately estimate slower dissociation rates, which is beyond the scope of a capture-based kinetic format.

Summary

The Carterra LSA facilitates the rapid generation of kinetic screening data from up to 384 samples in parallel. The unique design of the 96-channel flow cell allows efficient capture of up to 384 ligand mAbs in batches of 96 at a time, using 4 serial prints, on to the surface from low concentration (and unpurified) samples and allows for nearly complete sample recovery. These reloadable surfaces yield high quality and reproducible binding kinetics of clones with diverse affinities and binding rate constants. Capture kinetic experiments performed by high throughput SPR take significantly less time and consume dramatically 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 and provides sophisticated tools for visualization and data summary plots.

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

carterra®
info@carterra-bio.com
825 N 300 W Suite C309
Salt Lake City, UT 84103
www.carterra-bio.com