

An Integrated icIEF–SPR Workflow for Functional Characterization of Monoclonal Antibody Charge Variants

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Abstract

Biomolecular charge variant characterization has been a challenge that involves a cumbersome and time-consuming process. This study presents a novel and efficient method for determining binding kinetics and based on the charge heterogeneity of biological molecules. USP mAb 003 was selected as for evaluation owing to its well-characterized charge heterogeneity and suitability as a benchmarking material. First, the charge heterogeneity of USP mAb 003 was analyzed using imaged capillary isoelectric focusing (icIEF) on the CEInfinite systems by Advanced Electrophoresis Solutions, followed by the collection of individual charge variant fractions on the same system. Next, the collected fractions were tested for their ability to bind to FcγRI and FcγRIIIa, as a model to measure how therapeutic antibodies interact with immune cell receptors to evaluate their safety, efficacy, and mechanism of action. To accomplish this, Digital surface plasmon resonance (Digital SPR) was used to measure the binding of each fraction and Fc receptor, requiring only 2 μL of each. Digital SPR offers a key advantage over conventional SPR platforms due to its low sample volume requirements and higher throughput for fractionated samples. Overall, this poster highlights the streamlined fractionation and characterization of charge variants in biotherapeutics. This will help identify critical charge species that impact binding potency to inform the control steps needed in the process development and manufacturing of a biotherapeutic.

Digital SPR: Label-free Binding Analysis in a Droplet

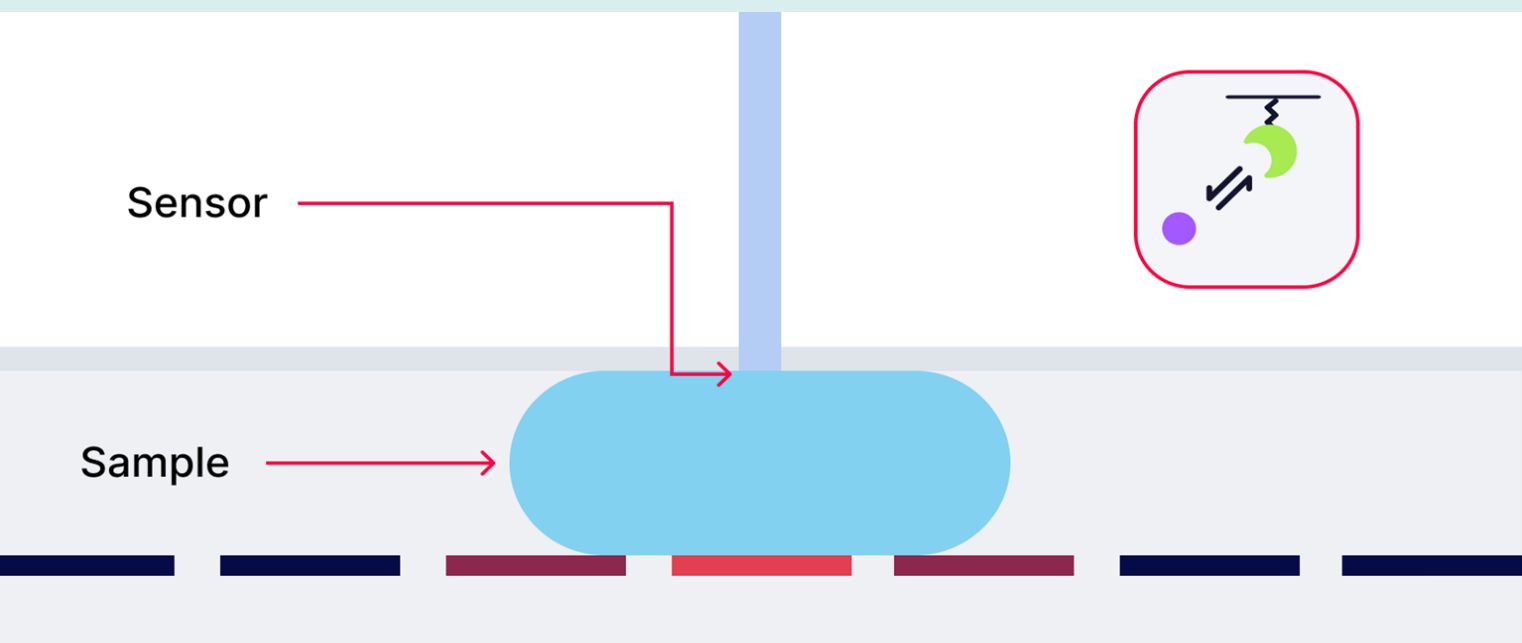
Nicoya’s Digital SPR systems use digital microfluidics (DMF) to deliver automatically diluted sample droplets to SPR sensors for effortless real-time characterization of biomolecular interaction analysis including quantitation, screening, epitope binning and binding kinetics.



- 4, 8 or 16 independently addressable channels for high throughput.
- Complete kinetics analysis using only 2 μL of crude or pure sample.
- No manual dilutions, tagging, degassing, cleaning, or strenuous assay set up.
- Powerful epitope binning, data visualization and analysis software.

What is DMF? (Digital Microfluidics)

DMF is a liquid-handling technology capable of accurately controlling and manipulating discrete nanoliter-sized droplets across an array of electrodes. The fluidics are contained within a disposable cartridge, allowing Digital SPR to overcome the major limitations associated with increasingly complex fluidic systems present in traditional label-free instruments.



Unraveling Charge Heterogeneity with Imaged Capillary Isoelectric Focusing (icIEF)

In icIEF, an electric field creates a stable capillary pH gradient. Proteins migrate by net charge until electrically neutral, focusing into distinct zones. Specific charge variants can then be isolated using preparative icIEF fractionation. IcIEF was performed on USP mAb 003, selected because of its well-characterized charge heterogeneity and suitability as a benchmarking material, using CEInfinite and an extended-length (13.5 cm) fractionation capillary cartridge. The extended capillary simultaneously improves the resolution of complex charge proteoforms while increasing preparative yield and fraction purity.

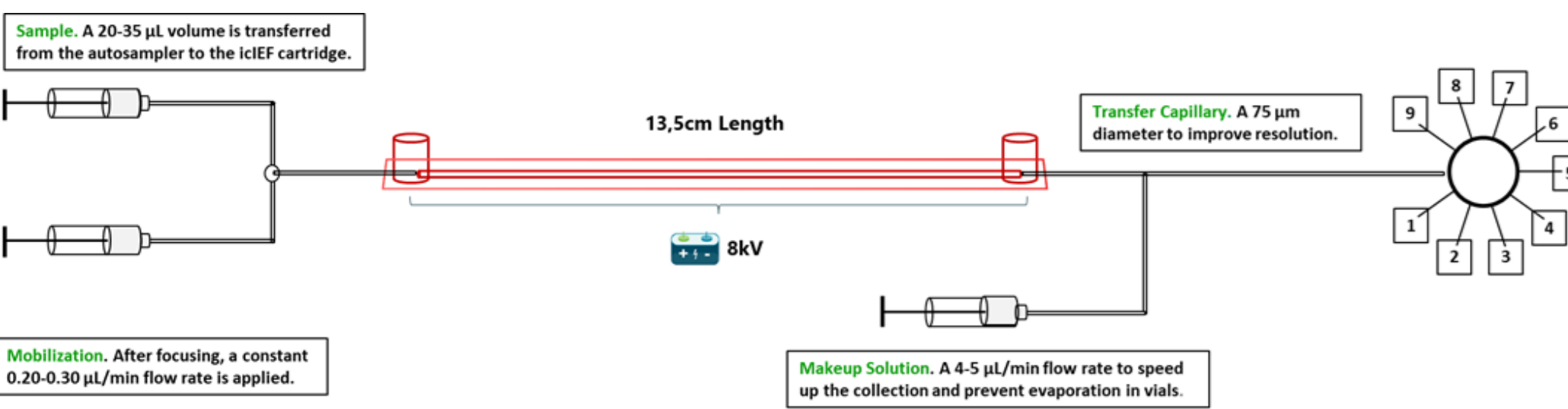


Figure 1. Schematics of ultra-high yield icIEF fractionation facilitated by extended-length capillary

The 13.5 cm capillary cartridge increased fractionation yield approximately fourfold compared with the standard 5 cm capillary cartridge while also improving fraction purity through enhanced separation resolution, as shown in Figure 2A.

The reproducibility of the fractionation system supports reliable pooling across multiple collection sessions, as demonstrated in Figure 2B.

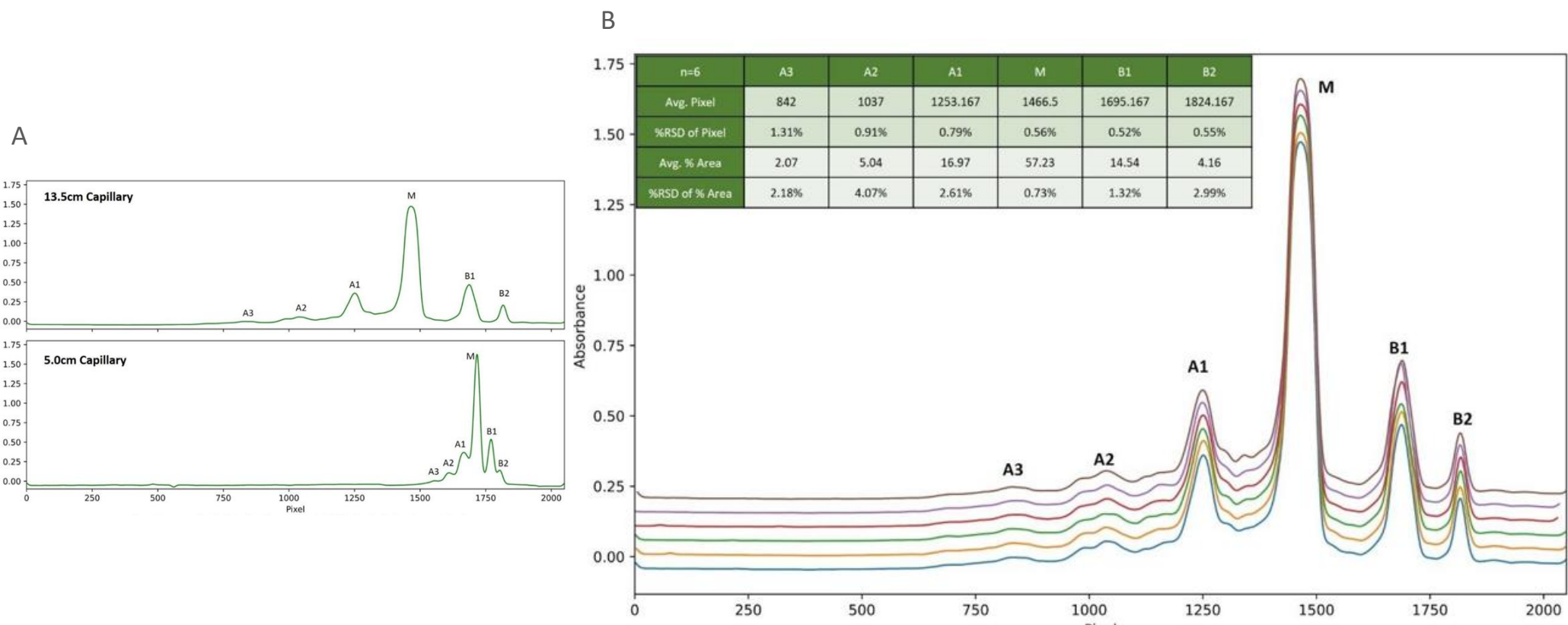


Figure 2. Improved separation performance using the extended-length capillary cartridge. A) Separation resolution of USP 003 charge variants in icIEF fractionation using the extended 13.5 cm length capillary cartridge. B) Repeatability of icIEF fractionation over six consecutive runs using the extended-length 13.5 cm length capillary cartridge.

The preparative icIEF workflow operates on a 45-min cycle per run, with periodic analytical icIEF analyses performed to monitor fraction purity throughout the collection process. Figure 3 shows the high yield and purity of charge variants from USP mAb 003 using the extended-length capillary icIEF protocol.

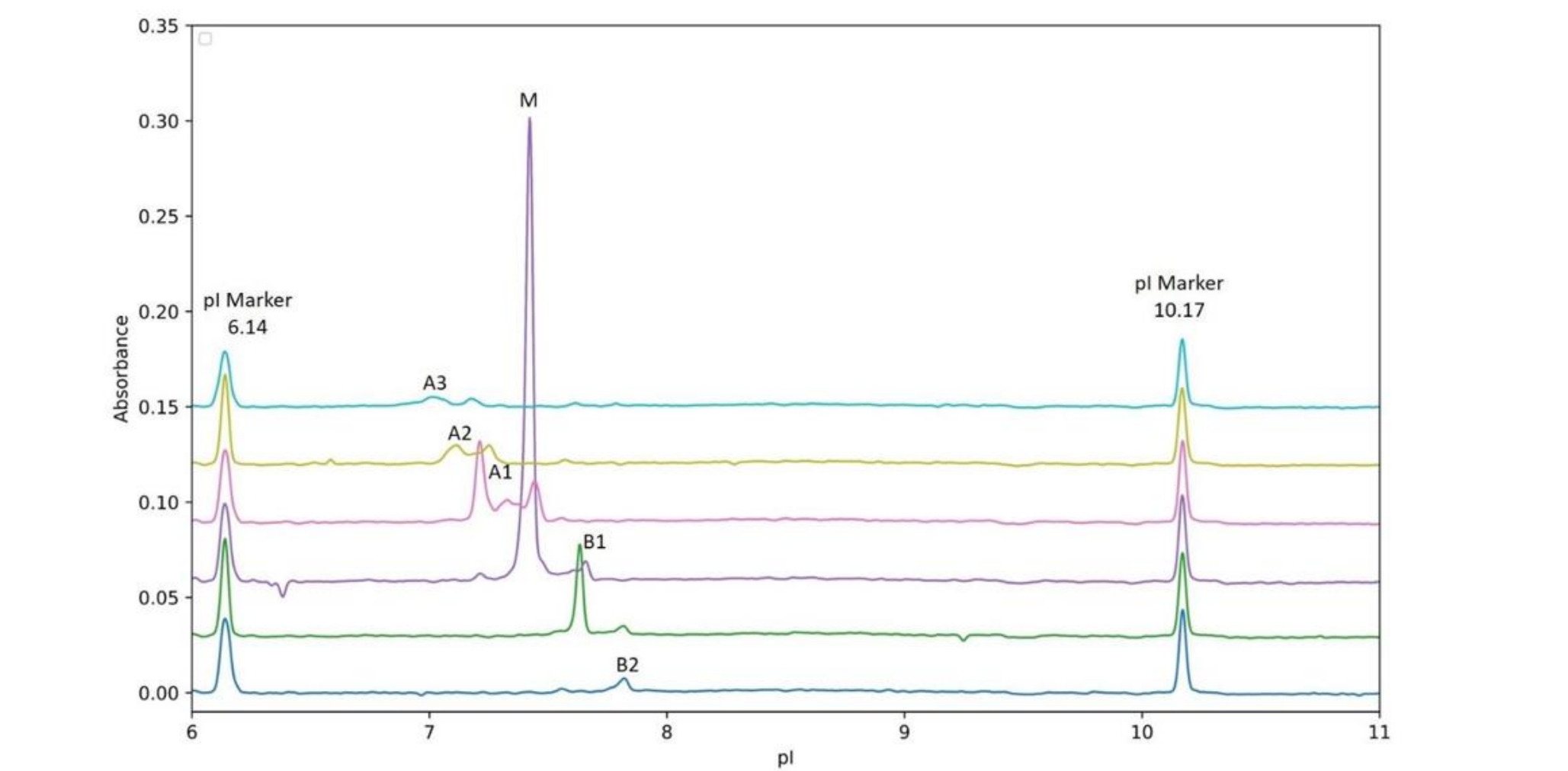


Figure 3. Analytical icIEF profiles of reinjected USP mAb 003 fractions demonstrating the purity of the collected charge-variant fractions.

Kinetic Characterization of USP mAb 003

SPR analysis was performed to measure the real-time interaction between isolated charge variants of the USP mAb 003 and Fc gamma receptors (FcγRI and FcγRIIIa), requiring only 2 μL of each collected fraction. Digital SPR offers powerful time, labor and reagent savings for running traditional multi-cycle (MCK) or single-cycle kinetics (SCK) on up to 72 unique interactions per cartridge. For each analyte, Digital SPR measures five (5) unique concentrations by automatically performing 3X serial dilutions on cartridge.

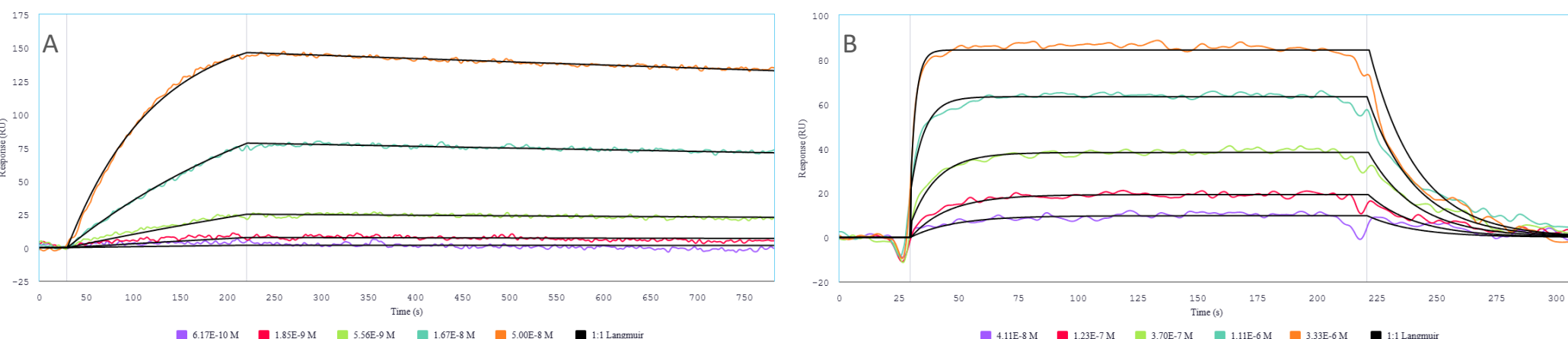


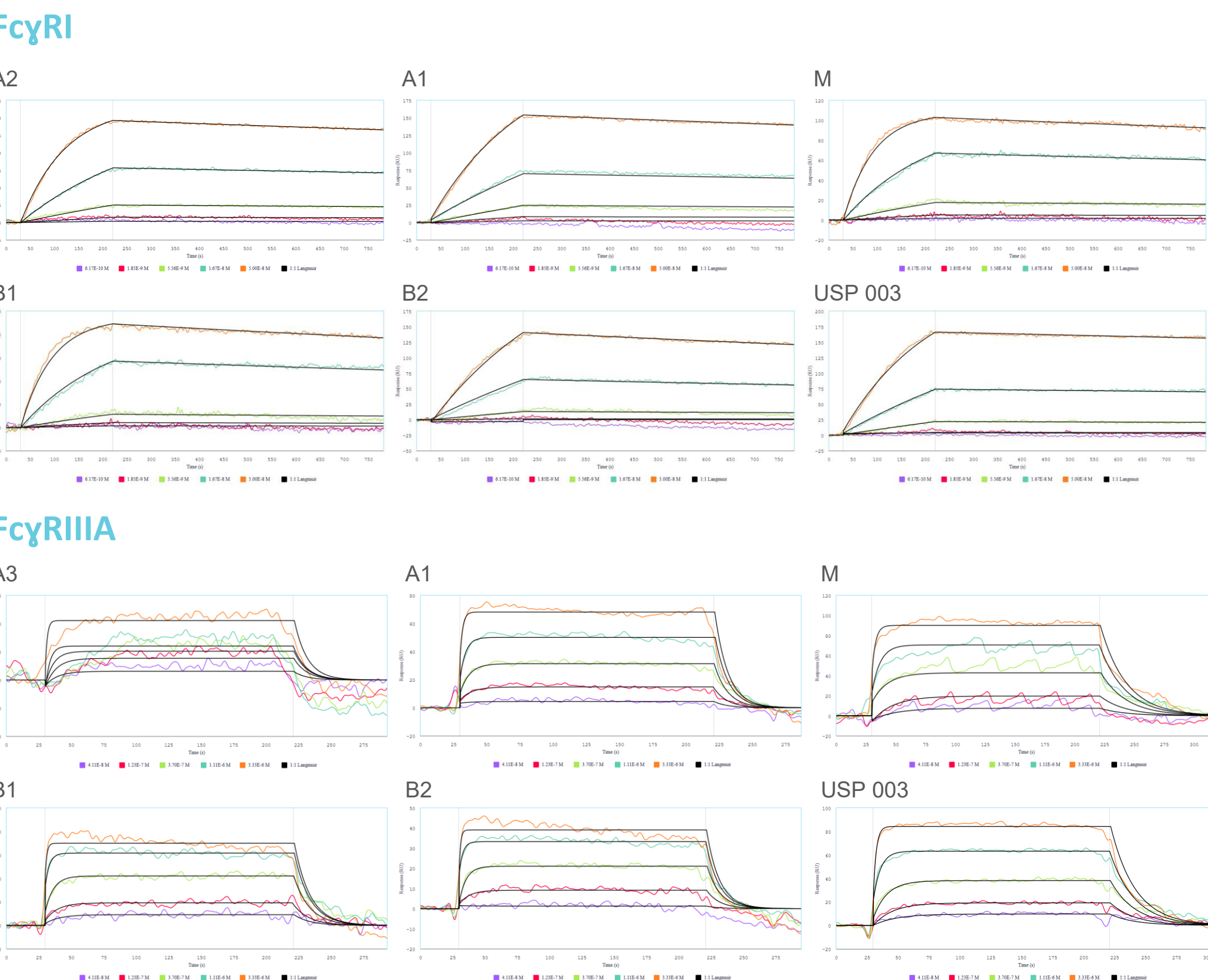
Figure 4. SPR analysis for collected charge variants from USP mAb 003. Representative kinetic fits for binding of USP mAb 003 fractions to (A) FcγRI, and (B) FcγRIIIa, captured on Nicoya's Protein A sensor surface, using multi-cycle kinetics (MCK). The FcγR analyte concentrations are indicated in the figure. The black curves represent the Langmuir 1:1 binding model fits.

All fractions showed strong binding to FcγRI, and weaker binding to FcγRIIIa, consistent with previously reported IgG1–Fcγ receptor interactions. The acidic (A), main (M), basic (B), and unfractionated (U) fractions displayed comparable association rate constants (k_a), dissociation rate constants (k_d), and equilibrium dissociation constants (K_D) for both receptors

Table 1. Surface plasmon resonance (SPR) analysis of FcγRI and FcγRIIIa binding kinetics for the USP-3 charge-variant fractions.

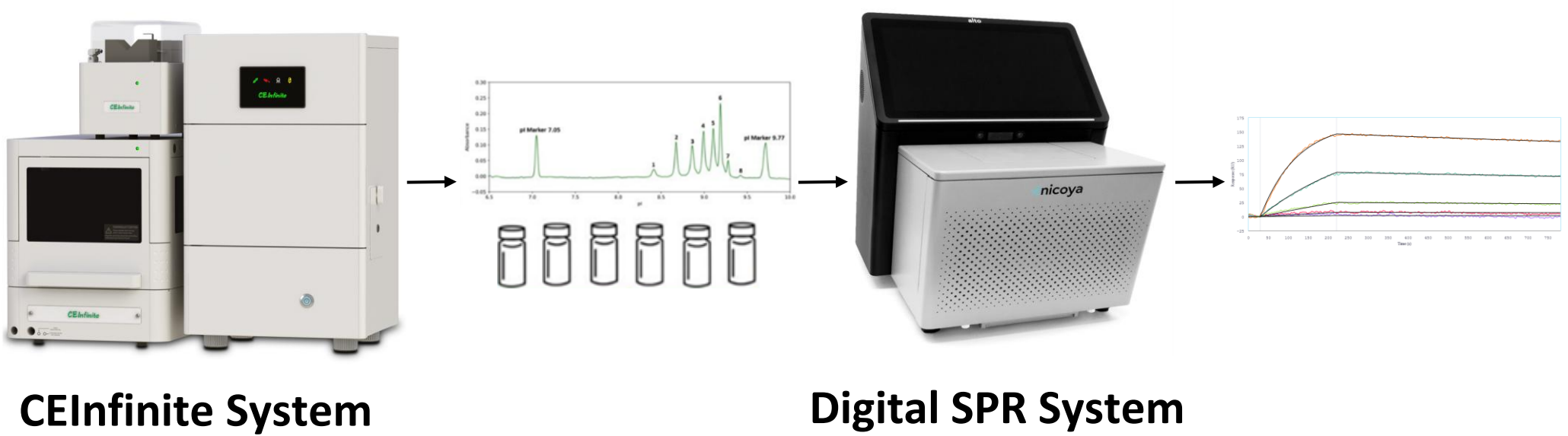
Ligand	FcγRI			FcγRIIIa		
	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	K_D (M)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	K_D (M)
A3	3.47E+05	1.93E-04	5.56E-10	1.68E+05	1.00E-01	5.95E-07
A2	1.55E+05	2.07E-04	1.35E-09	ND		
A1	1.14E+05	2.24E-04	1.96E-09	1.51E+05	7.49E-02	5.43E-07
B1	3.32E+05	2.43E-04	7.31E-10	1.80E+05	9.14E-02	5.29E-07
B2	1.34E+05	1.68E-04	1.25E-09	2.42E+05	9.40E-02	3.90E-07
M	2.21E+05	1.77E-04	7.89E-10	1.08E+05	7.50E-02	6.52E-07
USP003	7.63E+04	9.75E-05	1.46E-09	1.05E+05	7.51E-02	7.29E-07

Figure 5. SPR analysis for collected charge variant fractions from USP mAb 003 against FcγR and FcγRIIIa. Binding affinity and kinetics measured for different USP 003 fractions with FcγRI (top section), and FcγRIIIa (bottom section). The figure displays the data for the USP 003 acidic (A3, A2, A1), main (M), basic (B1, B2) and unfractionated peaks (USP 003).



Integrated icIEF-SPR Protocol for Protein Charge-Variant Characterization.

The charge heterogeneity of USP mAb 003 was analyzed using icIEF on the CEInfinite system, followed by the collection of individual charge variant fractions. The collected fractions were then tested on Digital SPR to determine their binding kinetics to FcγRI and FcγRIIIa.



Conclusions

The characterization of charge heterogeneity is a crucial regulatory requirement throughout the development, manufacturing, and quality control (QC) of therapeutic proteins. Accurate resolution and interpretation of charge-based microheterogeneity have become increasingly important for assessing structural integrity, functional activity, and manufacturing consistency. This study presents an integrated icIEF and SPR protocol that combines analytical charge profiling and charge-variant fractionation performed on the CEInfinite platform, followed by downstream potency assessment using Digital SPR. All workflows can be performed on a single icIEF instrument through rapid exchange of capillary cartridges. **The truly unique combination of Alto's low sample-volume requirements and high-throughput capabilities, combined with CEInfinite's high-yield fractionation of product charge variants, creates an efficient and high-impact workflow for biologically relevant characterization of all variants.** This will help identify critical charge species that impact binding potency to inform the control steps needed in the process development and manufacturing of a biotherapeutic.



Learn more about Digital SPR