

From Capture Strategy to Candidate Selection:

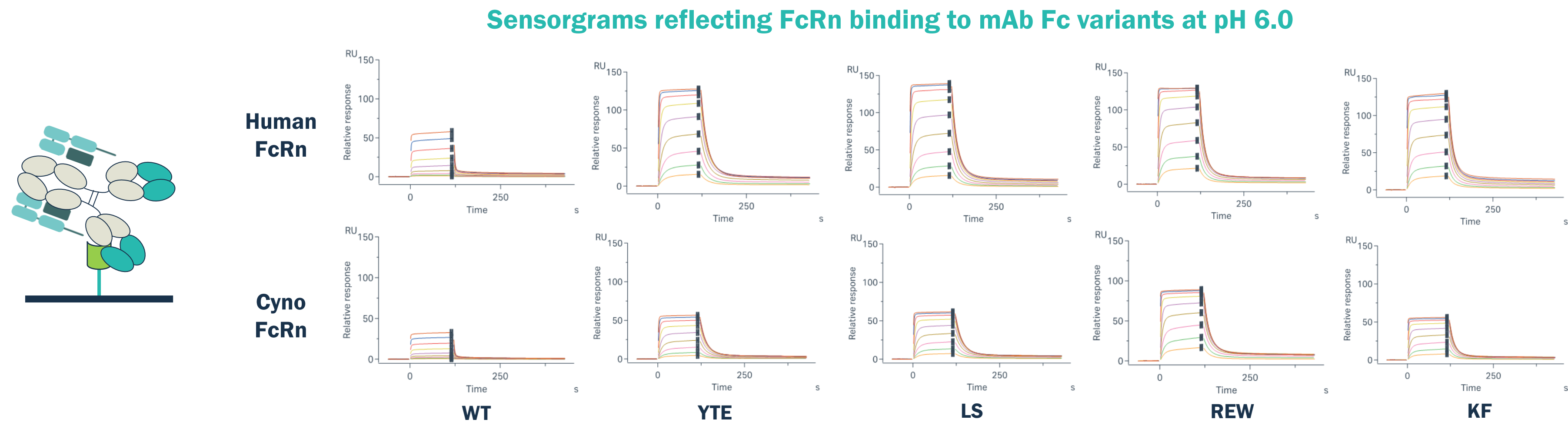
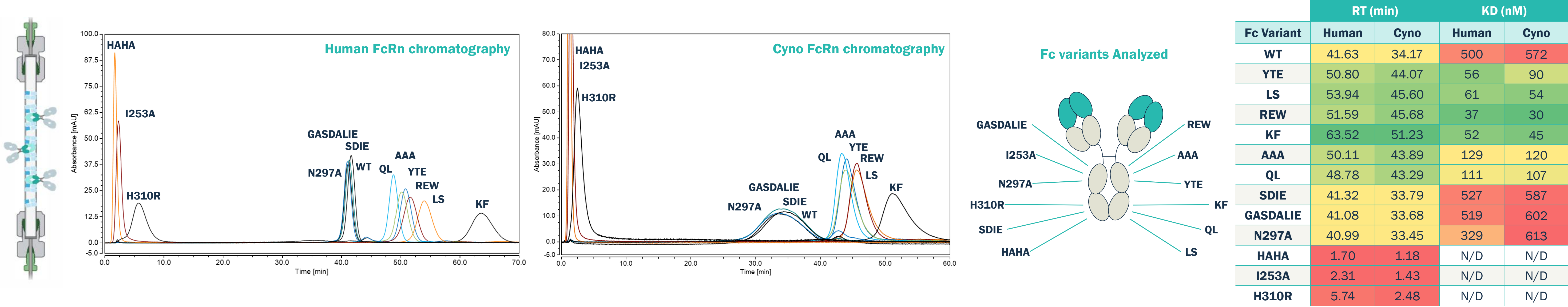
Optimizing SPR Assays for FcRn and FcγR Characterization in Antibody Discovery

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Surface plasmon resonance (SPR) plays a pivotal role in antibody discovery and engineering by enabling quantitative characterization of Fc–receptor interactions. However, robust affinity determination relies on selecting the appropriate capture strategy, as ligand orientation, receptor presentation and assay format can all influence kinetic measurements and data interpretation. We present a suite of SPR assay formats tailored for the characterization of Fc interactions including FcRn and Fcγ receptors. By applying receptor-specific capture strategies, we demonstrate how experimental design directly impacts affinity determination and assay performance. Combined with orthogonal approaches, these analytical workflows provide high-confidence data to support Fc engineering, improve candidate selection, and accelerate the development of next-generation antibody therapeutics.

Affinity characterization of Fc-variants using complementary SPR and chromatography approaches

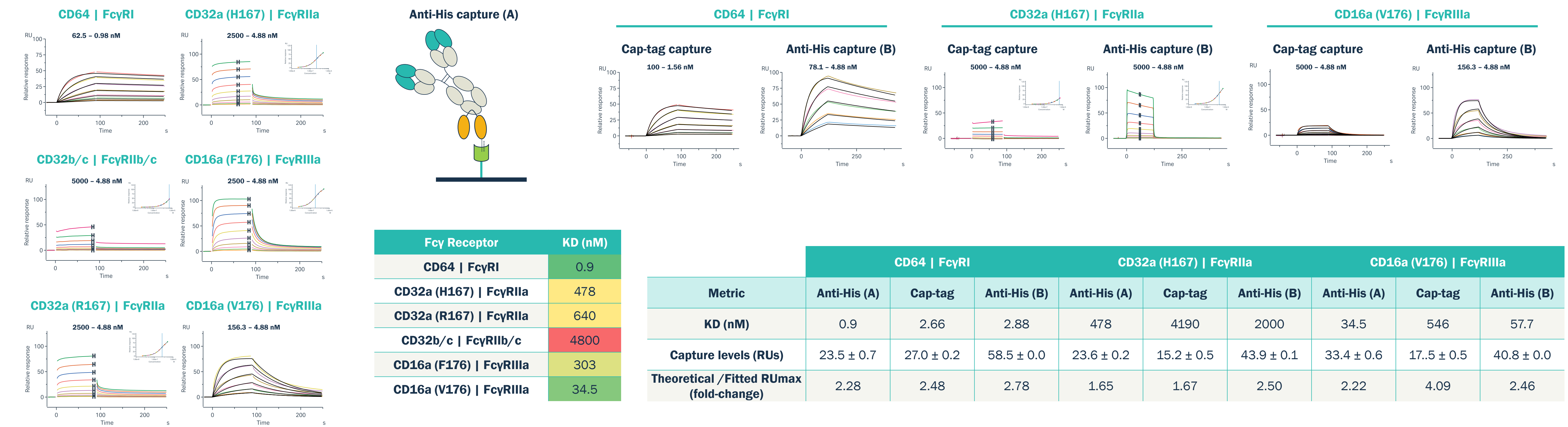
Thirteen Fc variants were evaluated for binding to human and cynomolgus monkey FcRn using two orthogonal analytical approaches: SPR and FcRn affinity chromatography.



- Fc variants engineered to enhance or reduce FcRn affinity exhibited the expected binding profiles toward both species and by both techniques.
- LS, YTE, REW, and KF displayed the strongest FcRn binding, whereas HAHA, I253A, and H310R showed no detectable binding.
- LS and REW exhibited different elution times between human and cynomolgus FcRn, demonstrating that the chromatographic method is sufficiently sensitive to discriminate among high-affinity Fc variants.

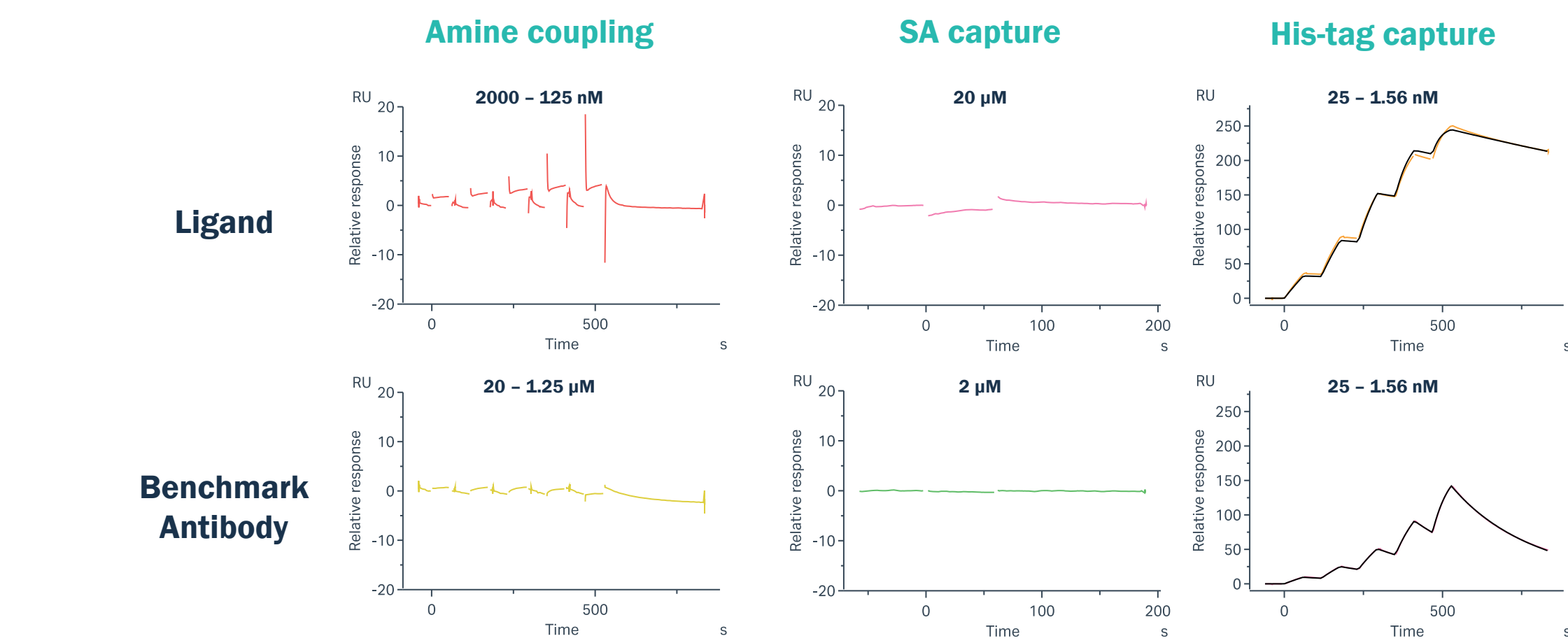
Affinity determination of Fc-gamma receptor and the impact of capture strategy

The affinity of one benchmark antibody was evaluated for binding to a panel of six different human Fcγ receptors, by SPR with receptors captured at low density using different capture strategies. Fcγ receptors exhibited the expected affinity range toward the WT Fc-containing antibody. However, affinity measurements were strongly dependent on the capture format, with up to 10-fold differences observed between capture strategies.



Variations in capture strategy dictate receptor activity

The activity of a dimeric receptor on the binding to its natural ligand and to a benchmark antibody was evaluated using different immobilization strategies. Both the natural ligand and the benchmark antibody bound exclusively to the dimeric receptor when captured through the His-tag.



Conclusions

- Results demonstrate the synergy of two orthogonal analytical approaches to assess the pivotal role of Fc engineering in modulating the FcRn interaction to optimize therapeutic antibody half-life.
- Sensitive SPR-based assays provide fast and accurate assessment of FcγR interactions in vitro and support the development of antibody variants with tailored effector functions.
- However, results highlight the critical impact of the antibody/antigen orientation in binding measurements and data interpretation.

References
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