

Rapid and High-Throughput SPR Characterization of VHH Discovery Leads Across Related Polymerase Targets for LAMP Dx

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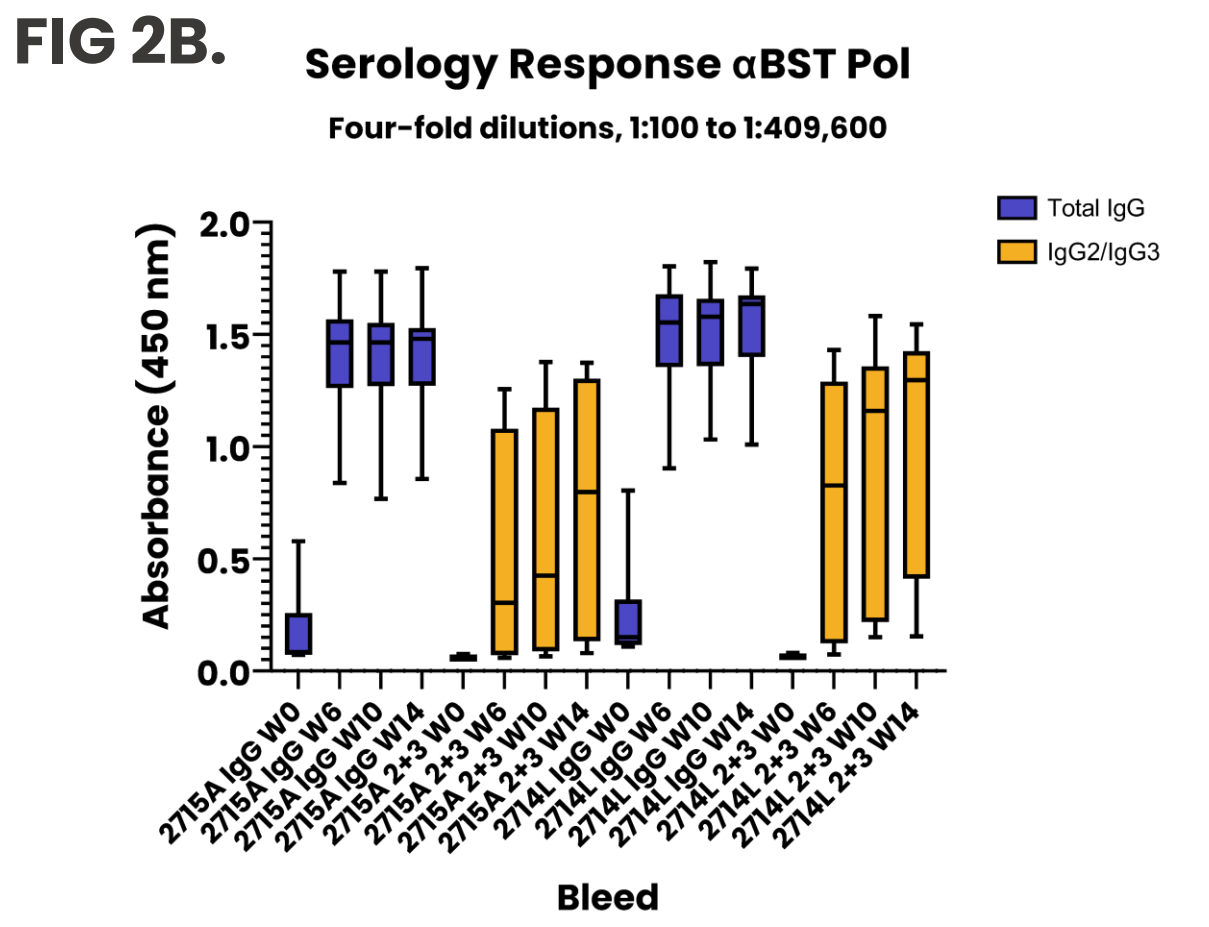
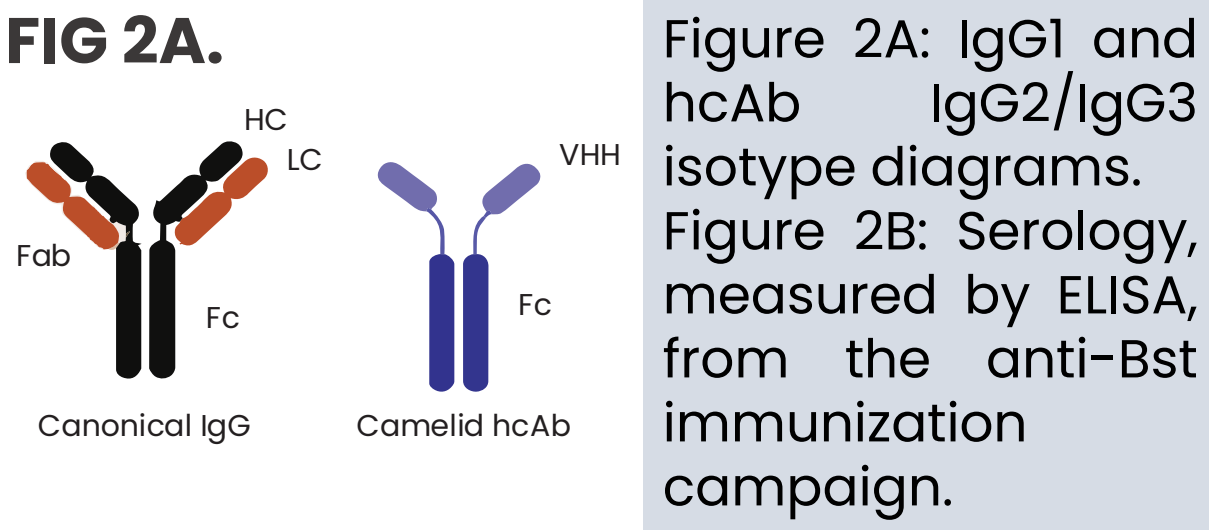


Discovery of Anti-Bst-Polymerase VHH Clones

Conditional selection of antibody-antigen interactions allows for in situ binding selectivity in protein engineering applications. Single-domain antibodies represent ideal candidates for conditional selection. Here, we present a potentially-generalizable strategy for generating temperature-specific inhibitors of enzymes. Bst polymerase enzyme was immunized into camelids, and the immune-derived aBST VHH repertoire was isolated and displayed on the surface of phage. VHH antibody libraries were enriched for temperature-specific binding interactions. Lead molecules were characterized for T_m and enzyme inhibition, as well as by SPR. The final VHH candidate set reversibly binds Bst polymerase in LAMP assay conditions, significantly improving background amplification.

Immunization and Library Construction

Bst polymerase was used to generate immune repertoires from one llama and one alpaca in a 14-week protocol. PBMCs were isolated on days 70 and 98, and were preserved at time of isolation in RNeasy Lysate. Serology was tested by adsorbing Bst polymerase, then applying plasma in a dilution series. Antibody titers were measured for both total IgG and for IgG2/IgG3 hcAb isotypes. hcAb titers for the llama were approximately 1:400,000, and hcAb titers for the alpaca were approximately 1:100,000. A total of 7.3 x 10⁸ PBMCs were lysed and used for library construction. The theoretical diversity of the library is approximately 2.5 x 10⁸ VHH proteins.



Conditional Selections in Phage Display

The aBst VHH library was displayed on the surface of filamentous M13KO7 bacteriophage and precipitated to 10¹³ pfu/mL. 5 x 10¹² pfu were incubated with adsorbed Bst polymerase in duplicate reactions before washing. Duplicate reactions were eluted either by incubation with triethylamine pH 11 or by incubation at 60 °C. Phage eluate was recovered and amplified in *E. coli*, then precipitated before panning subsequent rounds. In the third round of selections, the thermal elution track was subdivided such that one reaction was eluted at 60 °C and a separate, identical reaction was eluted at 50 °C. Phage outputs after each round of selections, as well as the initial immune-derived library, were minipreped and sent for NGS analysis (Azenta Amplicon-EZ). The phage outputs from each round 3 output were separately transformed into non-amber-suppressor strains of *E. coli* for off-phage VHH screening by ELISA. NGS results were analyzed by Geneious Biologics. Unique clonotypes were defined by 85% CDR3 amino acid identity or differing CDR3 amino acid length.

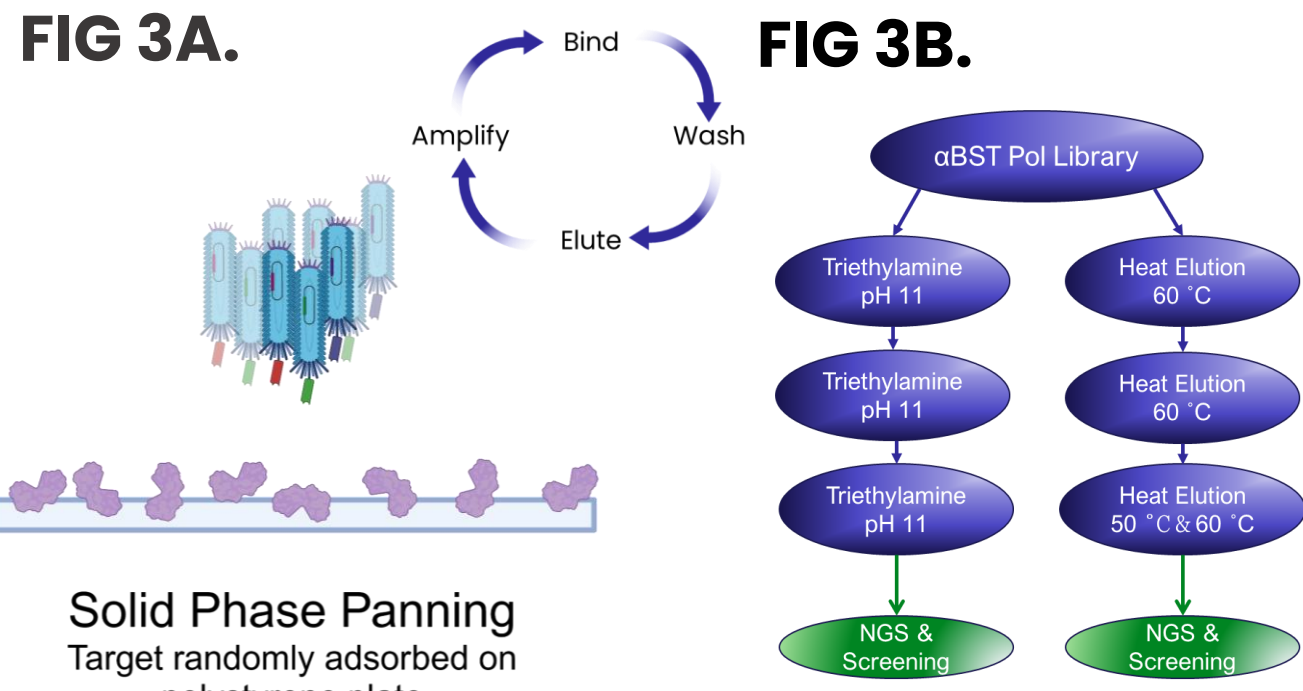


Figure 3A: Phage display target antigen strategy. Solid phase adsorption was used to present Bst polymerase in random orientations on the surface. Figure 3B: Parallel panning selection strategies created the potential for novel enrichment profiles. Elution was varied between parallel selections, using either TEA or heat to elute the bound phage from the target.

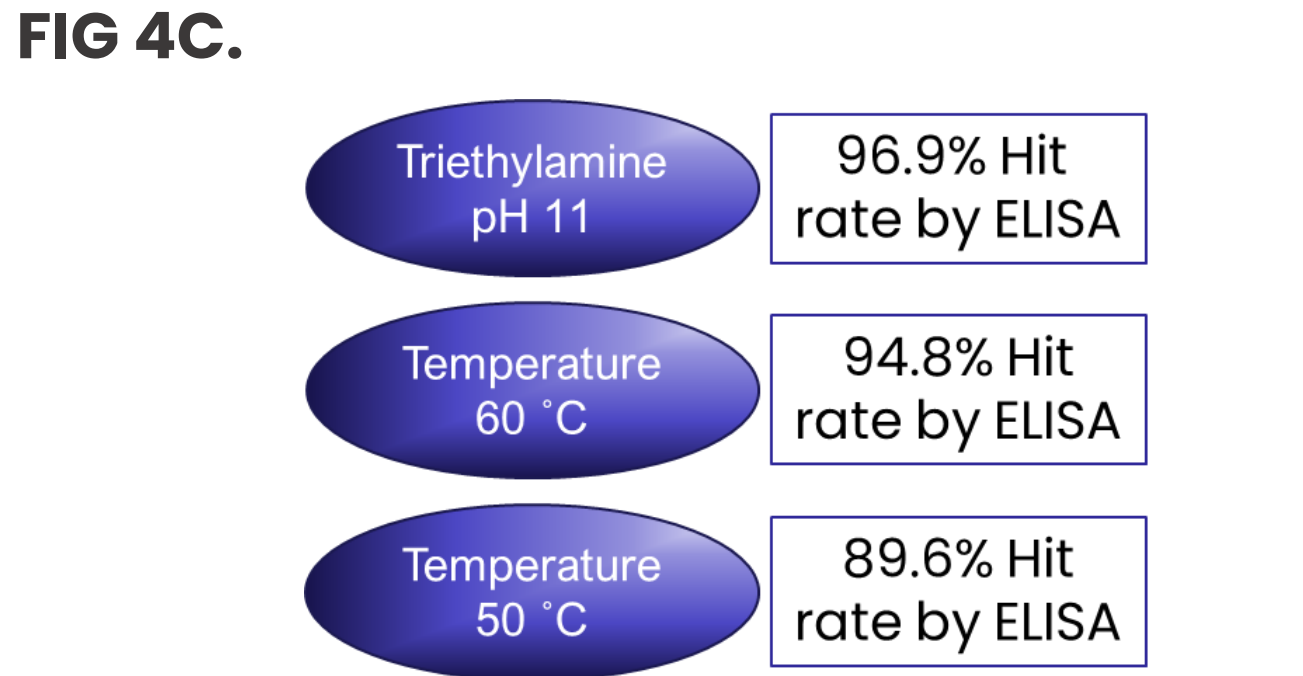
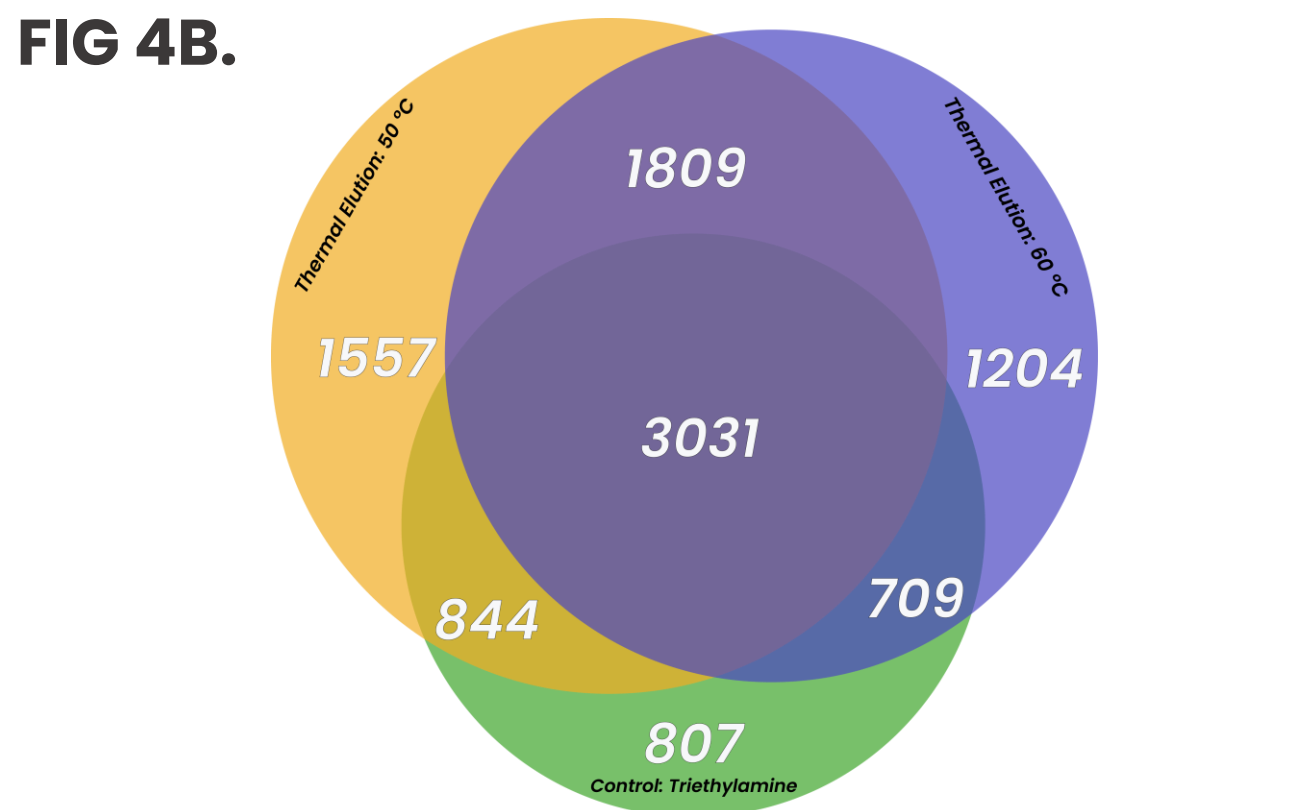
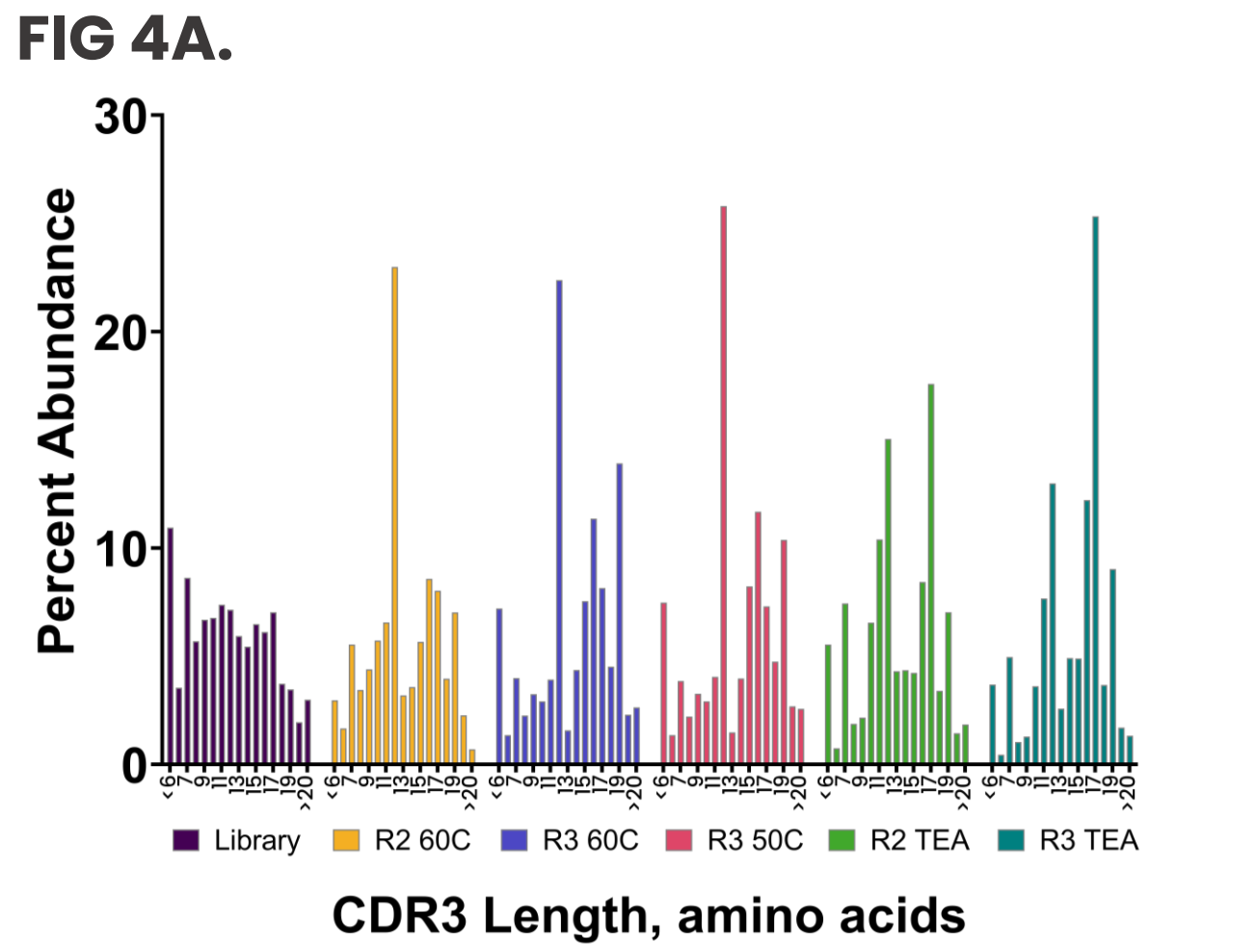


Figure 4A: Loop lengths of CDR3 in amino acids, grouped by panning elution condition and round. Unique panning tracks create novel enrichment patterns in loop length. Figure 4B: Quantitative Venn diagram of CDR3 amino acid cluster identities from Round 3 outputs, with identical amino acid sequence overlaps accounted for as a function of panning elution condition. Each panning track contributes novel clonotypes to the final pool. Figure 4C: Single-point ELISA hit rates from crude periplasmic VHH, with percent hit rate by panning elution condition.

NanoStart™ Antibody Characterization – SPOC HT-SPR Platform

Random clonal VHH were expressed as periplasmic extracts. They were measured for binding to adsorbed Bst polymerase by ELISA, detected by anti-VHH conjugates. ELISA hits were sequenced, and unique sequences were consolidated for high-throughput SPR binding kinetics analysis using SPOC platform, where each chip can display up to 1152 unique proteins with integrated SPR sensing at every spot. Each sensorgram captures the complete binding event in real time: association, dissociation, and full kinetic parameters (K_D , k_{on} , k_{off} , R_{max} , $t_{1/2}$).

A subset of hits with diverse clonotypes were selected to be tested using SPOC platform for affinity measurements, rank ordering, and cross-reactivity assessments. SPOC Biosciences synthesized VHHs directly on SPR biosensor chips for binding kinetics and affinity measurements. VHHs were directly synthesized on the SPR biosensor chip and screened against 5 concentrations of Bst and Gsp polymerases using a customized Carterra LSA-XT SPR instrument. On-chip synthesized VHHs were screened simultaneously to assess the cross-reactivity against both polymerases. Clones that were cross-reactive were prioritized for further characterization.

Figure 5A: SPOC in vitro transcription and translation in nanowells. Figure 5B: SPOC biosensor chips at different protein densities, supporting 1152 up to 2400 proteins on a single biosensor.

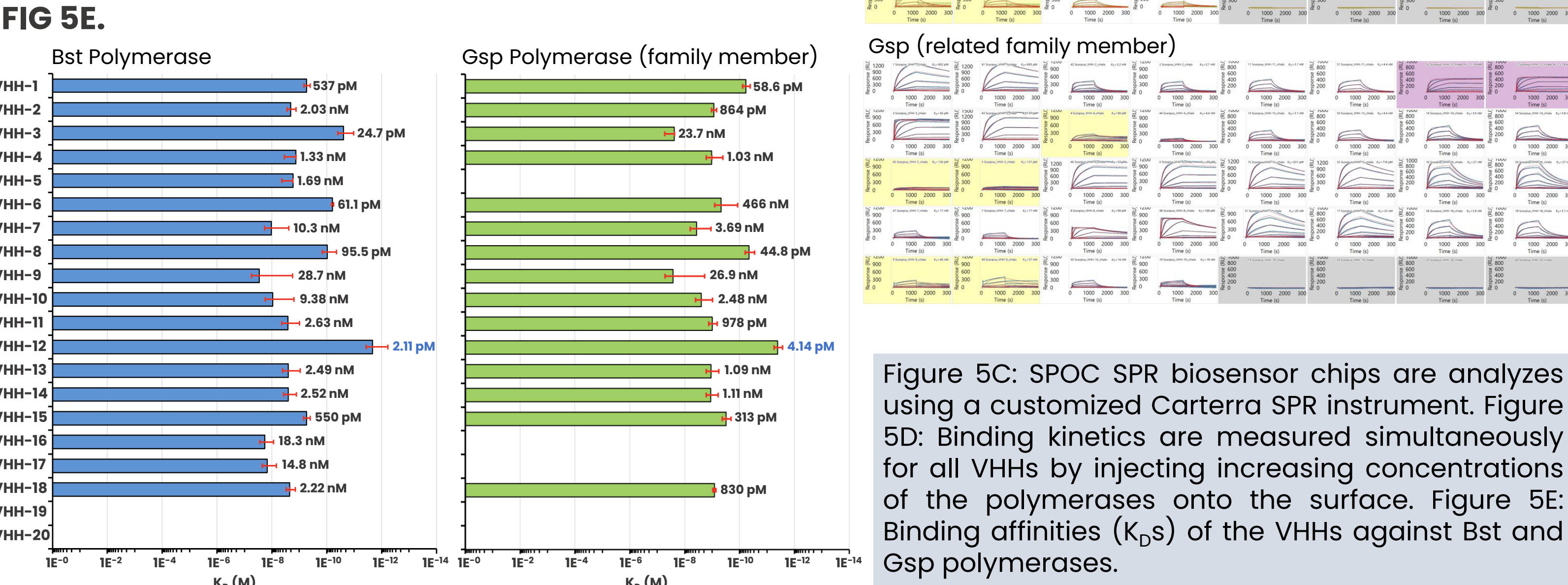
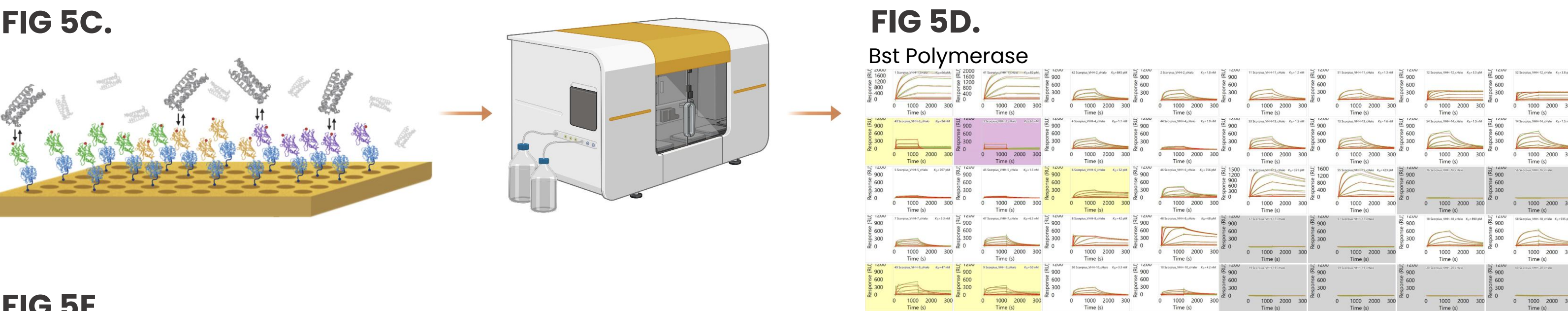
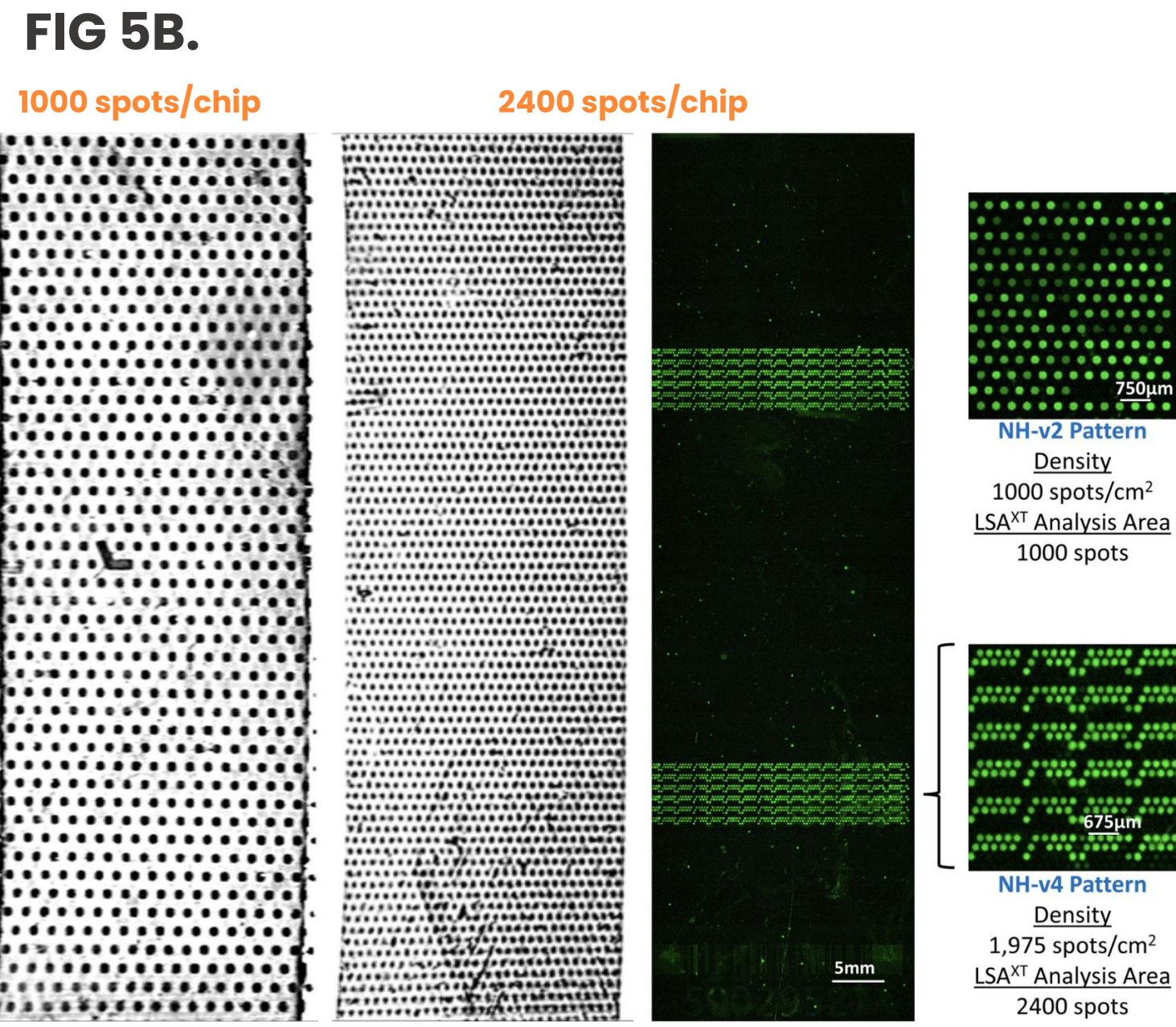
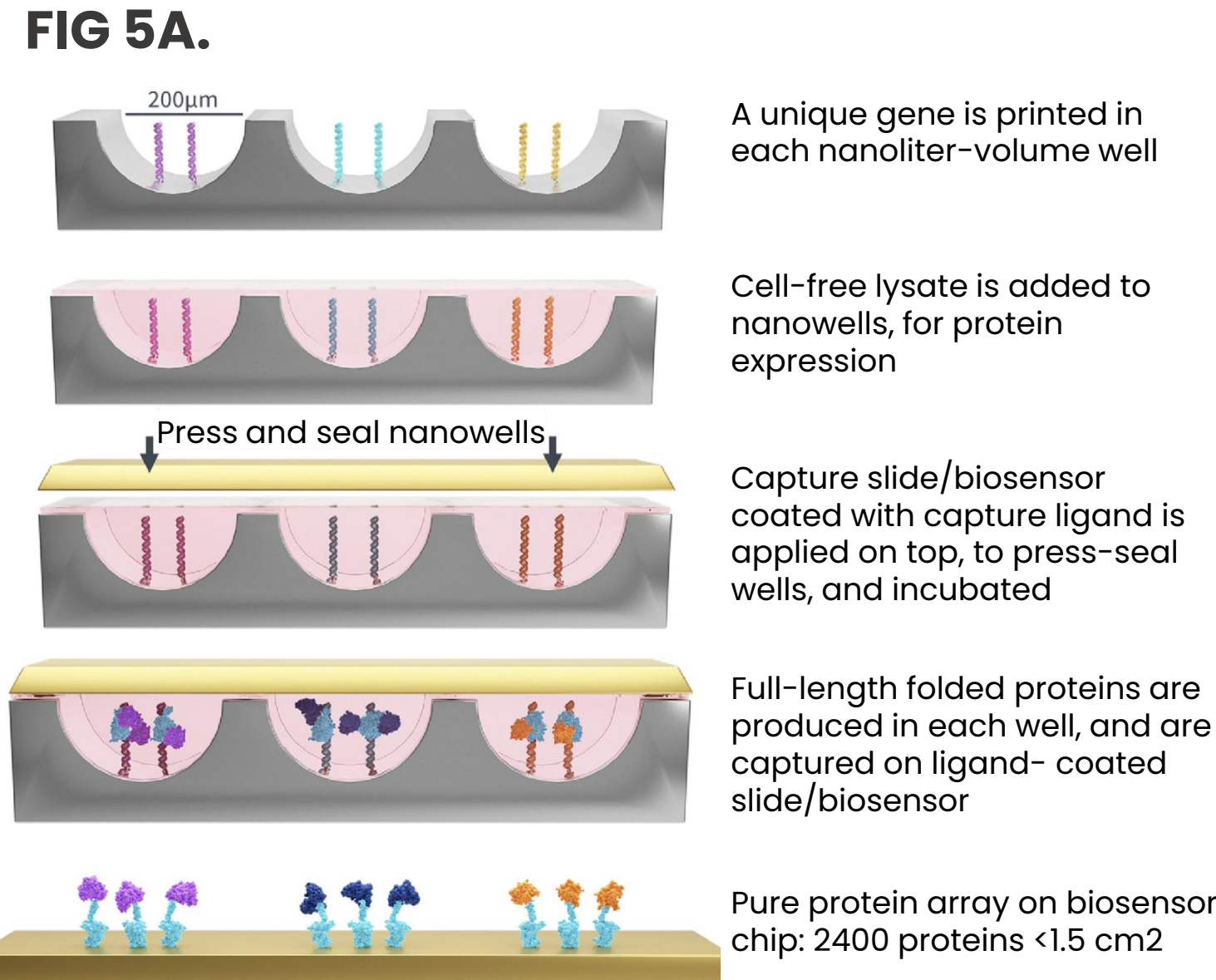


Figure 5C: SPOC SPR biosensor chips are analyzed using a customized Carterra SPR instrument. Figure 5D: Binding kinetics are measured simultaneously for all VHHs by injecting increasing concentrations of the polymerases onto the surface. Figure 5E: Binding affinities (K_D s) of the VHHs against Bst and Gsp polymerases.

Final Clone Selection

NanoStart™ VHH candidates were selected based on a combination of SPR kinetic profiles measured against both Bst and Gsp polymerases and inhibition performance in LAMP reactions (data not shown). Kinetic profiles against the two polymerase targets informed the prioritization for functional evaluation. The inhibitory profiles of VHH-3, VHH-6, and VHH-15 significantly affected their utility in an isothermal amplification capacity, with affinity of the VHH-enzyme interaction likely playing a central role. VHH-13 showed the least impact on processivity among the panel, which may reflect its comparatively lower affinity.

Conclusions & Future Directions

Screening on-chip synthesized VHH candidates simultaneously against two structurally related polymerases on a single SPOC biosensor chip, run on a customized Carterra SPR instrument, compressed what would otherwise be a multi-week characterization campaign into a single experiment. Cross-reactivity profiling, affinity rank-ordering, and full kinetic parameter extraction (k_{on} , k_{off} , K_D , R_{max} , and $t_{1/2}$) were captured in parallel across the entire panel, enabling confident prioritization of clones before committing resources to functional assays. SPOC HT-SPR platform supports titer assessment, polyreactivity screening, aggregation propensity, and stress condition testing on the same chip in the same run, providing a breadth of developability information that traditionally requires separate assays and separate timelines with cumbersome expression and purification steps for each of the candidates.

This consolidation is particularly consequential at the early screening stage, where decisions made on incomplete characterization data often determine which candidates survive to later rounds. The approach demonstrated here is not specific to polymerase targets. The same workflow applies to any program where conditional binding behavior, cross-reactivity against related family members, or early developability flags need to be resolved before advancing a lead panel. Future work will explore applying this multiplexed characterization framework earlier in the discovery funnel, where high-throughput kinetic and developability data can directly guide which sequences advance, reducing the number of candidates that reach costly downstream assays without the binding and stability profiles to justify it.