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Enhancing Nanobody Specificity: Experimental Evidence of the Performance of the Locuaz Binder Design Platform

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Developing high-affinity binders against specific antigens is challenging and requires extensive experimental screening. Computational methods can accelerate this process, but their success depends on efficiently exploring the mutation space and accurately assessing binding affinity changes upon mutation. The LOCUAZ platform uses an evolutionary algorithm to generate nanobody (Nb) libraries with enhanced target specificity. As a case study, LOCUAZ was applied to reprogram Nb specificity toward the lysozyme active site. Microscale thermophoresis (MST) was used to determine the binding affinities, confirming that the two highest-affinity mutants (60–80 nM) bind the protein target. Enzyme inhibition assays validated their binding to the active site at pH conditions where a critical Nb histidine is deprotonated. LOCUAZ was further employed to differentiate between two HER2 isoforms with distinct functions: P105, a breast cancer biomarker, and P100, which inhibits tumor growth. They differ by a short C-terminal tail. Optimized Nbs were expressed and tested against a peptide library mimicking P105 terminal sequence. Preliminary MST binding assays revealed moderate affinity but high selectivity for the epitope. These results demonstrate the LOCUAZ effectiveness in designing high-specificity nanobodies, offering a computationally guided approach to antibody fragment engineering.