Ranomics

WHITE PAPER · CASE STUDY

Engineering pH-Dependent Antibodies Using Yeast Surface Display

A case study in alternating dual-selection FACS screening of a one-million-variant scFv library

RANOMICS, INC.
AI-Driven Protein & Cell Engineering
ranomics.com

01 EXECUTIVE SUMMARY

Identifying pH-Dependent Antibodies from a Million-Variant Library

pH-dependent antibodies that bind their target at acidic pH and release at physiological pH are critical tools in antibody recycling, receptor-mediated endosomal release, and next-generation therapeutic formats. Identifying binders with this conditional phenotype from large combinatorial libraries presents a significant challenge: conventional single-condition screening cannot distinguish pH-sensitive binders from constitutive binders or non-binders.

In this case study, we demonstrate Ranomics' yeast surface display (YSD) platform applied to the discovery of pH-dependent scFv antibodies. Starting from a computationally designed library of over one million variants, we applied six rounds of alternating positive selection (pH 6.0, binding enrichment) and negative selection (pH 7.4, non-binder depletion). Antigen concentration was reduced by roughly an order of magnitude across the three paired cycles, progressively tightening the affinity threshold.

10^6	6	63	94.8%
scFv variants in starting library	FACS sorts across three paired cycles	high-confidence pH-dependent binders	of final candidates carry histidine substitutions

> Across the campaign, 63 candidates emerged as high-confidence pH-dependent binders. Two histidine substitutions at distinct CDR positions recur as convergent mutational hotspots — consistent with the known role of histidine protonation in pH-dependent molecular switching.

This white paper details the complete workflow from library construction through hit identification, demonstrating the power of iterative dual-selection on yeast display for discovering conditional binders with defined molecular mechanisms.

02 THE CHALLENGE

Why pH-Dependent Binders Are Hard to Find

The core difficulty in pH-dependent antibody discovery is that it requires simultaneous optimization of two opposing properties: strong binding under one condition and weak or absent binding under another. Standard affinity-based selections enrich for the tightest binders regardless of pH sensitivity, and the resulting hit pool is dominated by constitutive binders rather than the conditional ones being sought.

Single-round FACS or panning against the target at acidic pH will recover binders, but cannot distinguish those that also bind at neutral pH from those that release. The pH-dependent phenotype occupies a narrow slice of sequence space, and isolating it demands a selection strategy that explicitly rewards binding at one pH while penalizing binding at the other.

Library diversity compounds the problem. A 1,000,000-member scFv library spans an enormous combinatorial space, and the fraction of variants with genuine pH-switch behavior may be small. Without iterative enrichment and stringency escalation, these rare variants are lost in the noise of the initial library.

03 OUR APPROACH

Alternating Dual-Selection on Yeast Surface Display

Ranomics' yeast surface display platform uses the EBY100 Saccharomyces cerevisiae strain with Aga2p-based surface anchoring, enabling simultaneous readout of surface expression (via Myc tag detection) and target binding (via fluorescently labeled antigen). The platform supports two-color and multi-color FACS, allowing parallel interrogation against multiple antigens or buffer conditions on a per-cell basis.

For this campaign, we constructed an scFv library with approximately 1,000,000 unique variants, achieving 15% display efficiency on the yeast surface. The library was subjected to six sequential FACS sorts organized as three paired cycles of positive and negative selection:

Cycle 1 — high antigen concentration

Sort 1: Positive selection at pH 6.0 — collect double-positive (displaying + binding) cells.
Sort 2: Negative selection at pH 7.4 — remove cells that retain binding at neutral pH.

Cycle 2 — antigen reduced

Sort 3: Positive selection at pH 6.0 with reduced antigen — increase affinity stringency.
Sort 4: Negative selection at pH 7.4 — further deplete constitutive binders.

Cycle 3 — lowest antigen concentration

Sort 5: Positive selection at pH 6.0 at highest stringency.
Sort 6: Negative selection at pH 7.4 — final depletion of non-pH-dependent binders.

Reducing antigen concentration by roughly an order of magnitude across the three cycles increases the affinity threshold required for positive selection, ensuring that only high-affinity pH-dependent binders survive the full gauntlet. After the final sort, genomic DNA was extracted from all sorted populations and subjected to next-generation sequencing for quantitative enrichment analysis.

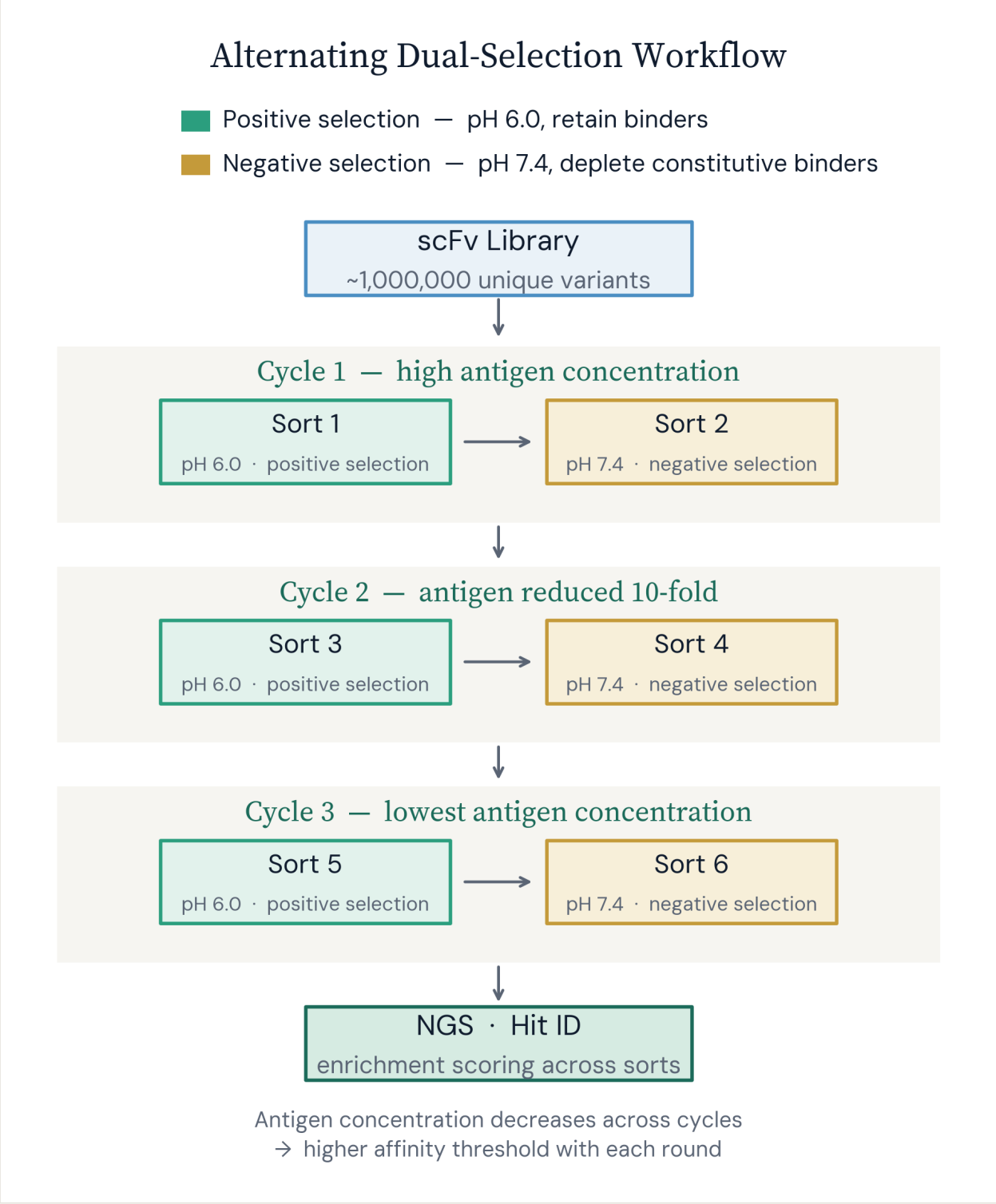

Figure 1. Alternating dual-selection workflow. Three paired cycles of positive (pH 6.0) and negative (pH 7.4) FACS selection with antigen concentration reduced by roughly an order of magnitude across cycles.

Each sort reads two fluorescent channels per cell: surface display (via the C-terminal Myc tag) and antigen binding (via a fluorescently labeled antigen added at the target pH). The

double-positive upper-right quadrant defines the sort gate at each round. Gate stringency tightens cycle over cycle as antigen concentration is reduced.

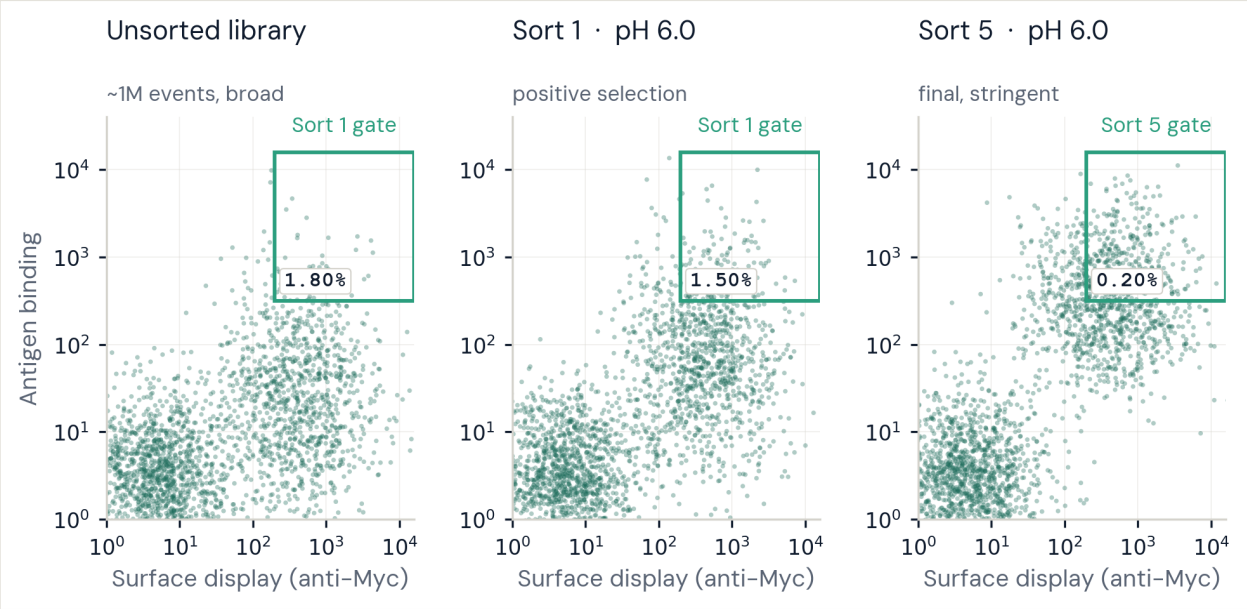

Figure 2. Representative two-color FACS dot plots across the selection campaign. Left: the unsorted starting library shows a broad distribution across surface display and antigen binding channels. Middle: Sort 1 positive-selection gate captures double-positive cells at pH 6.0 and high antigen concentration. Right: by Sort 5 the displaying population is tightly concentrated in the upper-right quadrant, reflecting three orders of magnitude of enrichment. Axes are log fluorescence intensity; gate stringency tightens with each positive sort.

04 RESULTS

Progressive Enrichment Reveals pH-Dependent Candidates

The starting library contained over one million unique variants, but the first FACS sort (display-positive and antigen-binding at pH 6.0) collapses this diversity by roughly three orders of magnitude: only a small fraction of the library displays the scFv correctly and engages antigen at acidic pH. NGS of the sorted populations then follows the variants that cleared this initial display-and-binding filter.

From the starting population and the six sorted populations, 640 designs achieved sufficient read depth across all seven samples to support quantitative enrichment analysis — this is the subset used throughout the remainder of this white paper.

Enrichment scores are computed per design, per paired cycle, as the log-fold change in relative read frequency between the positive and negative selection rounds of that cycle:

> $\text{Enrichment\_cycle} = \log_2 (\text{f\_positive} \ / \ \text{f\_negative})$
> where f_positive and f_negative are the relative read frequencies (per-design read count ÷ total reads in that sort) of a given design in the positive and negative sorts of the cycle. Scores > 0 indicate the design was retained preferentially at pH 6.0 and depleted at pH 7.4 — the pH-dependent phenotype.

Each design therefore carries three enrichment scores across the campaign (one per cycle), and a cumulative score summing all three cycles. Candidates with consistently positive cycle-level scores and a high cumulative score constitute the high-confidence pH-dependent pool.

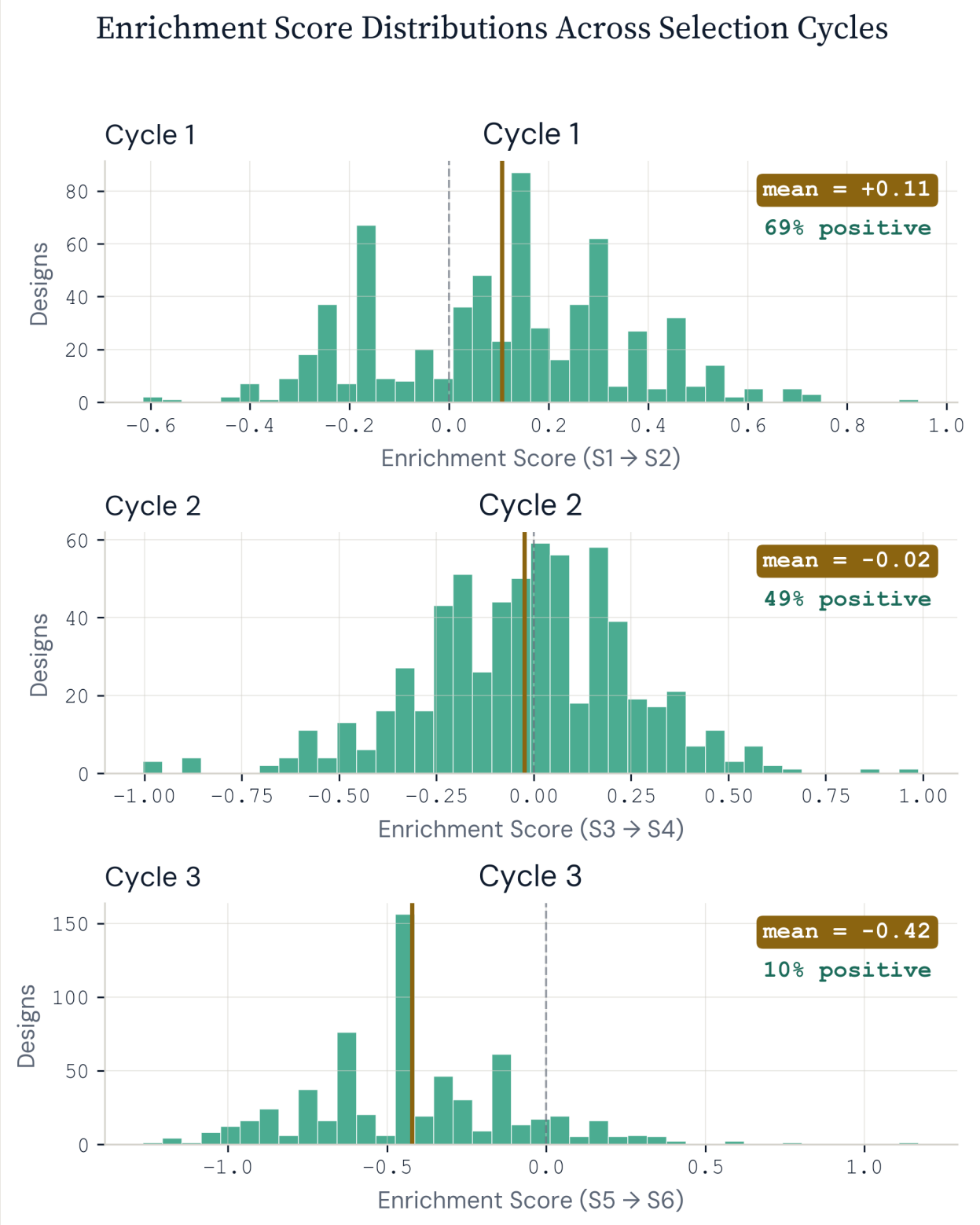

Figure 3. Enrichment score distributions across three selection cycles. Mean enrichment shifts from +0.11 (Cycle 1) to -0.42 (Cycle 3), reflecting progressive elimination of non-pH-dependent variants. The fraction of designs with positive enrichment falls from 69% to 10%.

The enrichment score distributions (Figure 3) show clear progressive selection pressure. In Cycle 1, 69% of the 640 analyzed designs scored positive, reflecting the initial broad capture of binders at the highest antigen concentration. By Cycle 3 at the most stringent conditions, 63 designs (10% of the analyzed subset) maintained positive enrichment. Combined with the three-order-of-magnitude library collapse imposed by Sort 1, these 63 candidates represent the high-confidence pH-dependent binder pool emerging from the full 10^6-variant starting library.

The mean enrichment score shifted from +0.11 in Cycle 1 to −0.02 in Cycle 2 to −0.42 in Cycle 3, confirming that the alternating selection strategy progressively eliminated constitutive binders while retaining pH-sensitive variants. The downward drift reflects the intended behavior of the assay, not a loss of diversity: it is the constitutive binders that are being depleted cycle over cycle.

Tracking individual candidates across all six sorts reveals the sawtooth signature of pH-dependent binding (Figure 4): read counts rise during positive selection at pH 6.0 and fall during negative selection at pH 7.4, with a net upward trend across cycles — the conditional phenotype the campaign was designed to isolate.

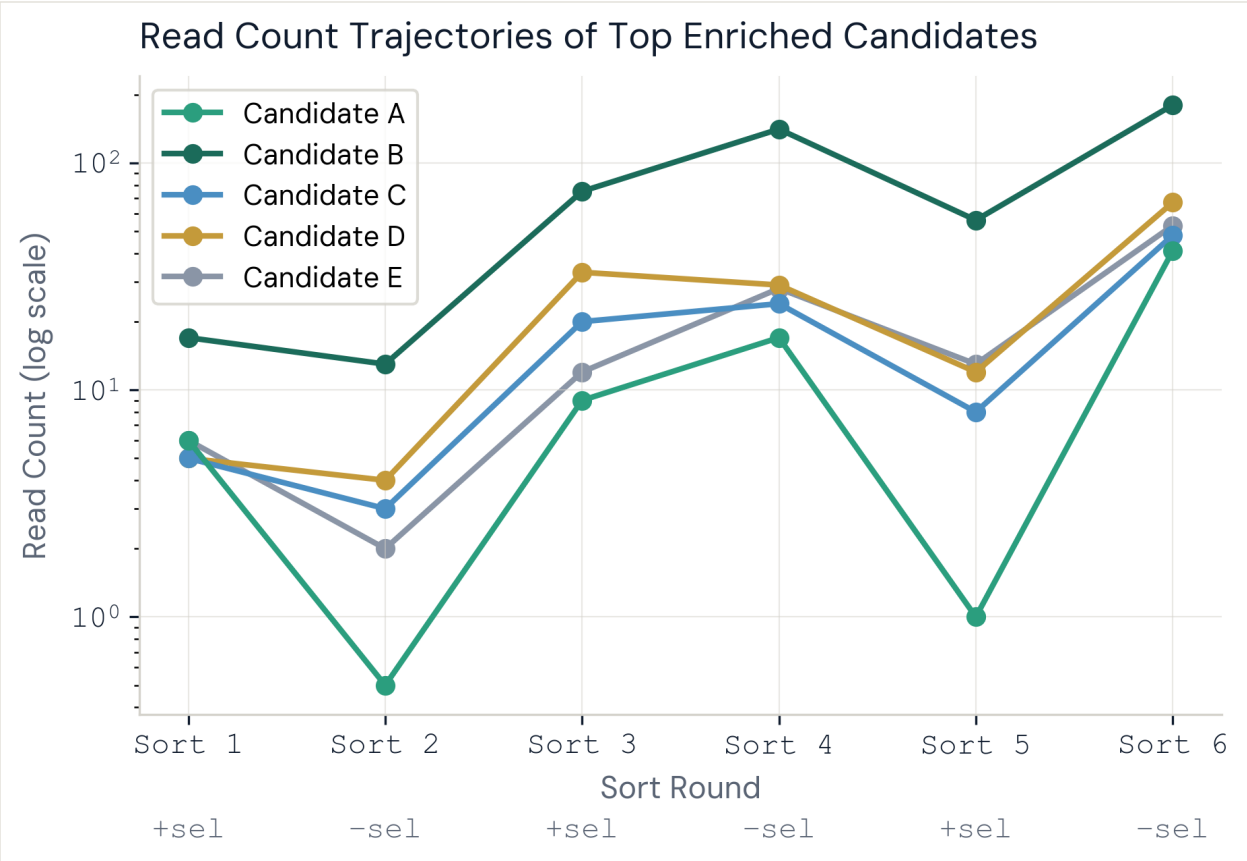

Figure 4. Read count trajectories of the top five enriched candidates across all six sorts. The sawtooth pattern reflects enrichment during positive selection (odd sorts) and partial depletion during negative selection (even sorts), with a net upward trend indicating pH-dependent binding.

05 MECHANISTIC VALIDATION

Histidine Enrichment Confirms a pH-Switch Mechanism

Analysis of the mutation profiles across the 640 analyzed designs revealed a striking convergence on histidine substitutions. 94.8% of these designs carry at least one histidine mutation in their variable domains. Among the top 20 candidates by cumulative enrichment score, two positions dominate:

> The first histidine hotspot (H-1) appears in 11 of the top 20 candidates; a second hotspot (H-2) appears in 7 of the top 20. These convergent hotspots are consistent with the known biophysical role of histidine (pKa ~6.0) as a molecular pH switch in antibody-antigen interfaces.

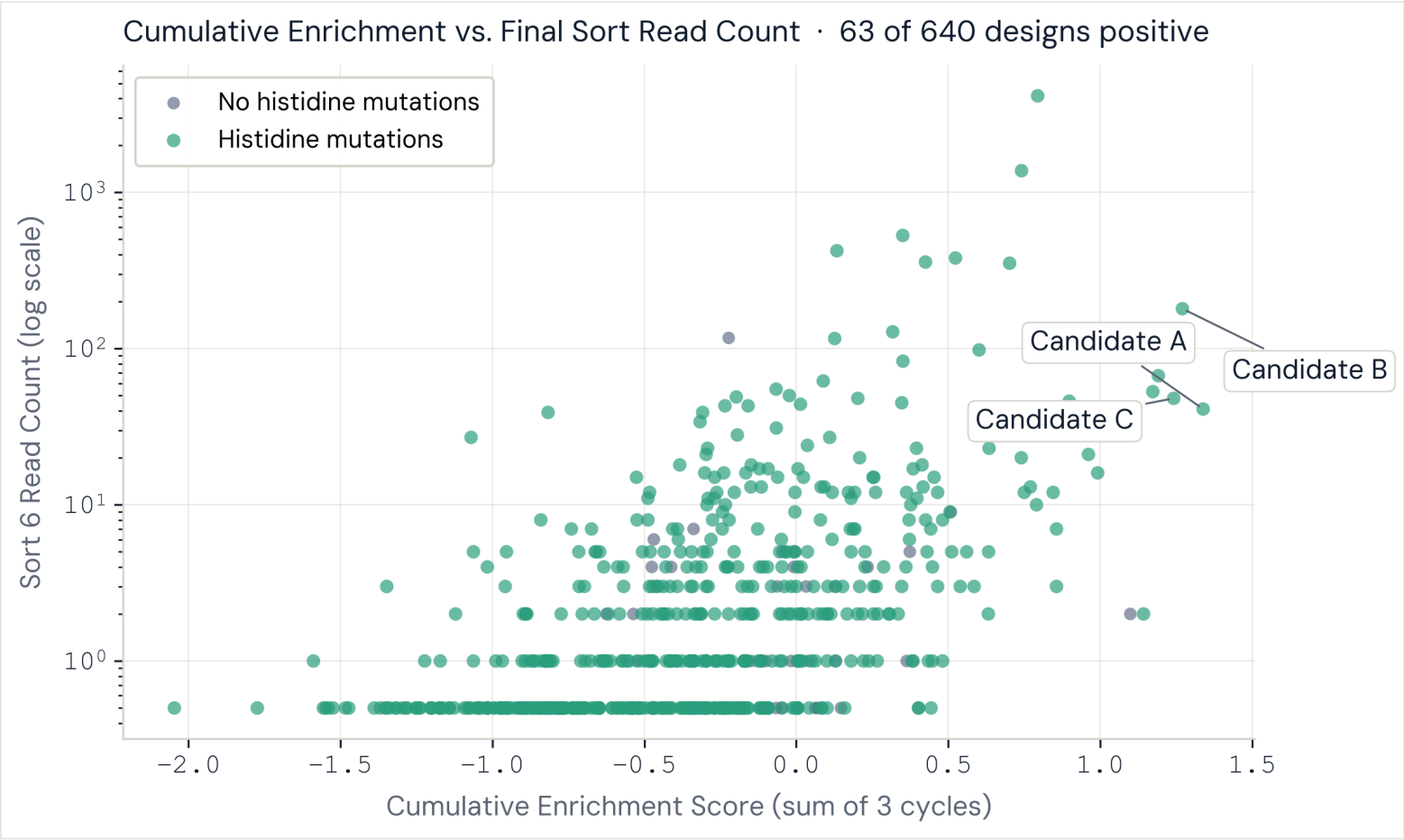

Figure 5. Cumulative enrichment score versus final sort read count. 63 designs (9.8% of the analyzed subset) with positive final-cycle enrichment represent the high-confidence pH-dependent candidate pool. Nearly all enriched designs carry histidine mutations, consistent with a pH-switch mechanism.

The histidine enrichment pattern provides orthogonal validation of the selection outcome: the selected candidates are not only behaviorally pH-dependent (as shown by FACS enrichment), but their sequence signatures point to the precise molecular mechanism (histidine protonation at pH 6.0) responsible for conditional binding. This convergence of functional and mechanistic evidence strongly supports the quality of the selected candidates.

06 CONCLUSIONS

Platform Capabilities and Broader Applications

This case study demonstrates Ranomics' end-to-end yeast surface display capabilities for discovering antibodies with complex conditional binding phenotypes. The key outcomes:

- Successfully screened a 1,000,000-variant scFv library using alternating dual-selection FACS.
- Identified 63 high-confidence pH-dependent binder candidates from the 640 designs with NGS read depth sufficient for quantitative tracking across all six sorts.
- Confirmed a pH-switch mechanism through convergent histidine mutation analysis.

The alternating positive/negative selection strategy is broadly applicable beyond pH-dependent antibodies. The same framework can be adapted for:

- Affinity maturation — progressive stringency selection from existing leads.
- Specificity campaigns — positive selection against the target, negative selection against off-target antigens.
- Conditional binders — temperature-dependent, ion-dependent, or ligand-dependent binding phenotypes.
- Bispecific discovery — alternating selection against two different targets.

Ranomics combines computational protein design with high-throughput experimental validation to accelerate antibody and protein engineering campaigns. Our yeast display platform supports custom library construction, multi-parameter FACS selection, and deep NGS-based hit identification — delivering characterized candidates ready for downstream development.

07 ABOUT RANOMICS

AI-Driven Protein & Cell Engineering

Ranomics is a contract research organization specializing in AI-driven protein and cell engineering. We apply computational protein design to optimize biophysical and biochemical properties — including molecular size, thermostability, conditional binding behavior, and catalytic activity — and pair it with high-throughput experimental platforms including yeast surface display, deep mutational scanning, and next-generation sequencing, delivering engineered proteins and binders ready for downstream development.