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Engineering Enzyme Selectivity through Conformational Dynamics: Integrating Kinetic Mechanisms, Protein Design, and Computational Modeling

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Abstract

Engineered enzyme selectivity is frequently judged by improved substrate preference or product distributions, yet the kinetic origin of those changes is often unresolved. Conformational dynamics offers a mechanistic explanation when mutations redistribute catalytically competent states, change interconversion rates, or alter access to competing reaction channels. This critical review distinguishes the measurable endpoints of substrate discrimination from assignments to binding, chemical conversion, product release, and protein-state exchange. Evidence from evolved hydrolases, isomerases, aminotransferases, designer catalysts, and computationally engineered biocatalysts is examined according to the strength of kinetic, structural, spectroscopic, and prospective design tests. Several studies link altered ensembles to functional improvement, but equivalent endpoint activity can also conceal different rate-controlling steps. Computational sequence-to-function models can broaden experimental search without independently demonstrating a conformational mechanism. A conditional design synthesis therefore couples explicit selectivity objectives with matched-substrate kinetics, transient measurements, orthogonal dynamics probes, and held-out sequence or substrate evaluation. The proposed interpretation prioritizes falsifiable alternatives and recognizes stability, expression, assay environment, and model sampling as possible confounders. Dynamics becomes a useful design variable when its predicted state change, measured kinetic consequence, and substrate-dependent outcome are jointly supported; absent that convergence, selectivity improvement remains an important engineering result without a uniquely identified cause.

Keywords: Substrate discrimination, Conformational ensembles, Pre-steady-state kinetics, Directed evolution, Multistate protein design, Enzyme specificity prediction

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Introduction

A redesigned enzyme can appear more selective because one substrate turns over faster, another is excluded, or the distribution of products shifts. Those outcomes are useful for catalysis but not mechanistically interchangeable. In a quantitative comparison, preference must be defined for a particular enzyme concentration, reaction mixture, substrate pair, and product analysis. Such specificity becomes especially consequential when an enzyme is engineered for a new substrate while retaining some native activity, or when a desired stereoisomer must compete with alternatives.

Two investigations illustrate why a dynamics-focused explanation requires more than an improved endpoint. Evolution of human kynureninase produced trajectories with distinct substrate selectivity and conformational or kinetic profiles despite convergent catalytic gains (Karamitros *et al*., 2024). By contrast, multistate design of an aminotransferase shifted a hinge-associated conformational equilibrium, with altered substrate discrimination supported by both steady-state activity and structural/spectroscopic measurements (St-Jacques *et al*., 2023). These are complementary examples, not evidence that every specificity gain is caused by the same protein motion.

The central question is therefore when observed or predicted conformational change can explain engineered discrimination better than alternative hypotheses concerning substrate association, chemistry, release, abundance, or stability. Recent multireaction scans of promiscuous enzymes emphasize that mutational effects can depend on which substrates are considered (Vanella *et al*., 2026). This review concentrates on enzyme-level mechanisms and their experimental identification. It does not generalize to cellular pathway productivity or pharmacological residence times.

Review Approach and Evidence Boundaries

A targeted, question-driven search was conducted on 1 November 2026 through publicly accessible web discovery, journal publisher pages, and PubMed-linked records. Query combinations included “enzyme substrate selectivity conformational dynamics kinetic mechanism,” “enzyme engineering conformational ensemble catalytic efficiency product selectivity,” “pre-steady-state kinetics engineered enzyme substrate discrimination,” “molecular dynamics enzyme design prospective experimental validation,” and “enzyme selectivity epistasis kinetic trade-off.” Citation

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chaining and targeted searches for counterexamples, methodological limits, and independently tested design claims supplemented these concepts.

The curated evidence roster consists of 31 distinct DOI-identified peer-reviewed journal publications, selected for complementary mechanistic or methodological roles rather than as an exhaustive sample. Publication identity and bibliographic fields were checked, but access to methods-level details was uneven and not every source received a completed full-text claim-locator assessment. No complete database export, exact screened-hit denominator, formal risk-of-bias scoring, or systematic-review flow is available; therefore, none is reported.

Interpretation is tiered: a fitted steady-state endpoint documents an assay-dependent phenotype; transient kinetics constrains steps of the catalytic cycle; spectroscopy or structural ensembles characterize populations and motions; perturbation of a proposed state tests a mechanism; and prospective tests ask whether an explanatory prediction transfers to new sequences or substrate contexts. Theoretical models are used for hypotheses, not mistaken for independent experimental validation. Molecular simulations and machine-learning scores are considered predictions until matched to observed measurements.

Kinetic Definitions and Mechanistic Identifiability

Even a minimal pathway, $E + S \rightleftharpoons ES \rightarrow EP \rightarrow E + P$, contains association, dissociation, chemical transformation, and product release. An observed kcat or KM reflects the network under specified conditions, not an unambiguous elementary rate. When enzyme states interconvert, additional hidden steps can make different mechanisms consistent with the same measured steady-state curve. Mechanistic identifiability improves when complementary experiments constrain independent observables rather than repeatedly measuring the same endpoint.

Substrate Discrimination, Catalytic Efficiency, and Product Distributions

At low substrate concentration, the specificity constant kcat/KM (units M^{-1} s^{-1}) describes the initial rate of productive encounter under a Michaelis–Menten model. A two-substrate selectivity factor can be written as (kcat/KM)A/(kcat/KM)B when the substrates are assayed under comparable conditions; this dimensionless ratio is not equivalent to the ratio of equilibrium dissociation constants KD,A/KD,B. K. A. Johnson (2019) argues for reliable collection and global interpretation of steady-state data and cautions against treating KM as a simple affinity measurement. Theoretical analysis similarly shows that selection accuracy can emerge from kinetic discrimination rather than solely from equilibrium binding differences (Banerjee *et al*., 2017).

Product selectivity requires a different denominator. Enantiomeric excess, for example, measures a product-composition imbalance and is influenced by competing pathways, conversion, and workup; a preferred product can arise without greater absolute turnover. In the kinetic resolution of near-symmetric esters, machine-learning-guided carboxylesterase engineering required a substrate-specific apparent enantioselectivity assay (Dou *et al*., 2024). A substrate-aware amidase design study likewise targeted enantioselectivity rather than a universal catalytic efficiency metric (Li *et al*., 2024). By contrast, a broad panel of stereodivergent cyclopropanases demonstrated that product preference and substrate scope have to be assessed together, not substituted for each other (Shen *et al*., 2026).

Single-substrate screens may conceal competitive inhibition, substrate depletion, or shifted preferences in mixtures. Substrate-multiplexed protein engineering demonstrates the design value of explicitly observing promiscuous reactivity under multiple substrate challenges (McDonald *et al*., 2022). Adding a recruitment domain can alter which molecules reach an active site without establishing that its internal chemical barrier or conformational exchange was changed (Park *et al*., 2023). **Table 1** links each readout to the narrower inference it can support.

Table 1. Selectivity readouts and their mechanistic interpretability

Measured question	Representative readout	What it establishes	What remains unidentified
Which substrate is efficiently converted?	kcat/KM for A and B, M^{-1} s^{-1}	Low-concentration catalytic discrimination (K. A. Johnson, 2019).	Separate association, chemistry, or release constants.
Which ligand binds more tightly?	KD or dissociation/association constants	Binding equilibrium or binding kinetics.	Productive catalysis and competing downstream steps.
Which product dominates?	Product fractions; ee or de, %	Product partitioning in a specified reaction (Dou *et al*., 2024).	Absolute catalytic rate and unique conformational cause.
What changes in mixtures?	Multiplexed activity and substrate competition	Preference under simultaneous substrate exposure (McDonald *et al*., 2022).	Intrinsic specificity outside the tested mixture.
How general is selectivity?	Substrate panel and product-specific outcomes	Performance across stated structural contexts (Shen *et al*., 2026).	Transfer to untested substrate classes.

Note. Conceptual measurement comparison; example citations identify evidence, not universal numerical benchmarks. Define conversion, active enzyme normalization, temperature, pH and substrate concentration in any quantitative comparison.

Conformational Ensembles and Rate-Controlling Steps

A protein conformation is not itself a catalytic step. State populations, exchange rates, and their coupling to chemical events are distinct quantities that can be measured on different time scales. Evolution from phosphotriesterase toward an arylesterase function involved altered conformational substate preferences, providing evidence that enzyme evolution can change the distribution of structures accessible to catalysis (Campbell *et al*., 2016). Directed evolution restored productive conformational dynamics in an

enzyme isomerase, with exchange measurements supporting a functional role for dynamics (Otten *et al*., 2018). Neither observation licenses the general claim that greater flexibility always improves selectivity.

Designer Kemp eliminases offer a contrasting direction of change. Room-temperature crystallography associated laboratory evolution with better active-site preorganization and restriction of catalytic-residue motion (Broom *et al*., 2020). An independent analysis of the evolved energy landscape linked productive-state enrichment to improved catalysis (Otten *et al*., 2020); subsequent dynamic-network work on designer enzymes examined how evolved interactions can support chemical conversion (Bunzel *et al*., 2021). Thus, both selective stabilization and useful conformational exchange are plausible, depending on the states and steps involved. Ligand-driven closed-complex formation provides a mechanistic route by which binding energy can help assemble a reactive configuration, as emphasized by Richard (2022).

Multi-observable tests are critical. Adenylate kinase calculations and biophysical observations connect collective motions to active-site rearrangement, but transferring a particular motion–chemistry relationship to other enzymes requires an independent test (Ojeda-May *et al*., 2021). NMR studies of histone deacetylase 8 variants provide relationships between intrinsic conformational exchange and enzymatic function, while their relevance to engineered substrate discrimination remains indirect (Shukla *et al*., 2023). Importantly, parallel kynureninase trajectories show that distinct kinetic pathways can converge on similar catalytic activity without converging on identical selectivity (Karamitros *et al*., 2024). The operative claim should specify which substrate-dependent step changed, how that step was observed, and whether a competing explanation was excluded.

Engineering and Modeling Strategies

Protein engineering can perturb conformational states indirectly by mutations in or beyond an active site, or explicitly by optimizing multiple structural models. Neither location nor algorithm guarantees a mechanistic interpretation: the experimental comparison must connect sequence intervention to a defined selectivity outcome and, separately, connect a structural or dynamical change to the rate process hypothesized to control that outcome. Distinguishing these two tests prevents a useful design success from being overinterpreted as proof of a unique molecular explanation.

Mutational Perturbations and Experimental Discrimination

Distal mutations can affect active-site access and chemical conversion through long-range communication, as suggested by combined experimental and computational analysis of cytochrome P450 catalytic traits (Acevedo-Rocha *et al*., 2021). A β-lactamase study associated altered hinge dynamics with an evolutionary shift from promiscuity toward specificity (Modi *et al*., 2021). These mechanisms are enzyme-specific hypotheses whose strongest tests combine controlled perturbations with discrimination among steps, rather than merely correlating a changed structure with a final activity ratio.

The value of transient kinetics is particularly clear in OXA-48 β-lactamase evolution. Fröhlich *et al*. (2024) compared variants using burst-phase kinetics and other assays, finding that evolution could shift the influential step from substrate binding toward chemical conversion. This explains why mutations that appear weak individually may show marked epistasis in combination; binding affinity alone would miss the changing kinetic bottleneck. In human kynureninase, flexibility-focused mutations altered the chemical step, supporting a more direct rate-based intervention than a statement of unspecified "dynamic enhancement" (Karamitros *et al*., 2022).

Stereoselective engineering of a Morita–Baylis–Hillman biocatalyst involved catalytic-site rearrangements that can be tested against enantioselective product formation (Crawshaw *et al*., 2022). A substrate recruitment domain represents a deliberately different design mechanism: it affects substrate positioning and encounter rather than requiring a change in the active-site conformational equilibrium (Park *et al*., 2023). Multi-substrate mutational scanning of D-amino-acid oxidase extends this logic to substrate-dependent mutational effects (Vanella *et al*., 2026). These contrasts limit claims of a common dynamics mechanism across all designs.

Combinatorial evolution adds another layer of inference. Fitness-landscape analysis of several enzyme families indicates widespread background dependence, but epistasis alone does not identify a conformational route (Buda *et al*., 2023). Empirical enrichment of productive mutational paths can improve engineering efficiency without resolving every transition within the catalytic cycle (Patsch *et al*., 2024). Together, these studies recommend separating expression, stability, and functional screening from purified active-enzyme kinetics and testing the same substitution across informative backgrounds when a causal claim is made.

Computational Predictions and Prospective Testing

Computational design is most persuasive when its target observable is defined before the experiment. Ensemble-based design of a Kemp eliminase reproduced selected features of evolution and produced activity gains, linking structural ensembles to prospective laboratory evaluation (Broom *et al*., 2020). More directly aligned with substrate discrimination, multistate design of an aminotransferase favored a reactive closed conformation and altered its non-native substrate preference; crystallographic and NMR observations supported the intended population shift (St-Jacques *et al*., 2023). Still, its specificity measurement and its conformational evidence are complementary observations rather than an exhaustive assignment of every elementary rate constant.

Predictions of enzymatic parameters or enzyme–substrate compatibility answer related but narrower questions. CatPred estimates kinetic quantities for enzyme–ligand combinations with attention to model evaluation and uncertainty (Boorla & Maranas, 2025); CataPro was evaluated for kinetic prediction and engineering utility (Wang *et al*., 2025). A graph model for enzyme specificity prospectively tested selected halogenase substrate predictions (Cui *et al*., 2025). Such results can prioritize candidates, but algorithmic confidence is not a measured

conformational state, and predictive accuracy on similar sequences cannot automatically be transferred to a dissimilar enzyme family.

Experimental assessment of neural-network-generated enzymes documents whether designed sequences function, an indispensable check that is nonetheless different from testing a state-exchange hypothesis (S. R. Johnson *et al*., 2025). Autonomous enzyme engineering can change substrate preference efficiently without resolving the kinetic steps responsible for it (Singh *et al*., 2025). Machine-learning-guided carboxylesterase and amidase programs additionally connect substrate-dependent product preference to experimentally measured selections (Dou *et al*., 2024; Li *et al*., 2024). These programs should report sequence overlap, training/test separation, assay transfer, and negative outcomes before claiming broad generalization.

Mechanism-aware modeling offers a complementary route. De novo catalytic-motif scaffolding can be judged by experimentally tested enzyme function while leaving assumptions about state dynamics open (Braun *et al*., 2026). Multistate design of cyclopropanases explicitly considered alternative stereochemical routes and evaluated activity across substrate panels (Shen *et al*., 2026). These are credible tests of design objectives, but success with a product distribution is not, by itself, proof that an enzyme moved between the states anticipated by a model. The matched evidence dimensions for several approaches are compared in **Table 2**.

Table 2. What engineering and modeling evaluations actually test

Intervention class	Computational target	Experimental discrimination	Unresolved mechanism
Ensemble redesign	Relative state favorability; candidate sequence	Aminotransferase kinetics plus NMR/X-ray (St-Jacques *et al*., 2023).	Which individual substrate-dependent step limits flux.
Evolution-informed design	Conformations and productive active-site geometry	Kemp eliminase catalytic efficiency/structures (Broom *et al*., 2020).	Generalization to new substrate pairs.
Parameter prediction	kcat, KM or substrate compatibility	Held-out data and enzyme/substrate tests (Boorla & Maranas, 2025; Cui *et al*., 2025).	Causal dynamical explanation.
Stereochemical multistate design	Competing product-forming states	Product selectivity across substrates (Shen *et al*., 2026).	Direct time-resolved state transitions.
Autonomous sequence search	Assay-defined fitness objective	Measured substrate preference in selected proteins (Singh *et al*., 2025).	Rate-step attribution; assay portability.

Note. Rows describe distinct kinds of validation; they are not rankings. "Held-out" means evaluation on data withheld from model fitting where reported; it must not be assumed for unverified experiments.

Figure 1 locates these results within a deliberately qualitative reaction-and-ensemble map. A redistribution detected by NMR or crystallography can support a targeted state hypothesis, whereas parallel substrate routes still require kinetic discrimination. In particular, comparisons of conformationally distinct kynureninase trajectories and β-lactamase rate-step shifts demonstrate why apparently similar endpoint improvement need not imply a single underlying pathway (Fröhlich *et al*., 2024; Karamitros *et al*., 2024).

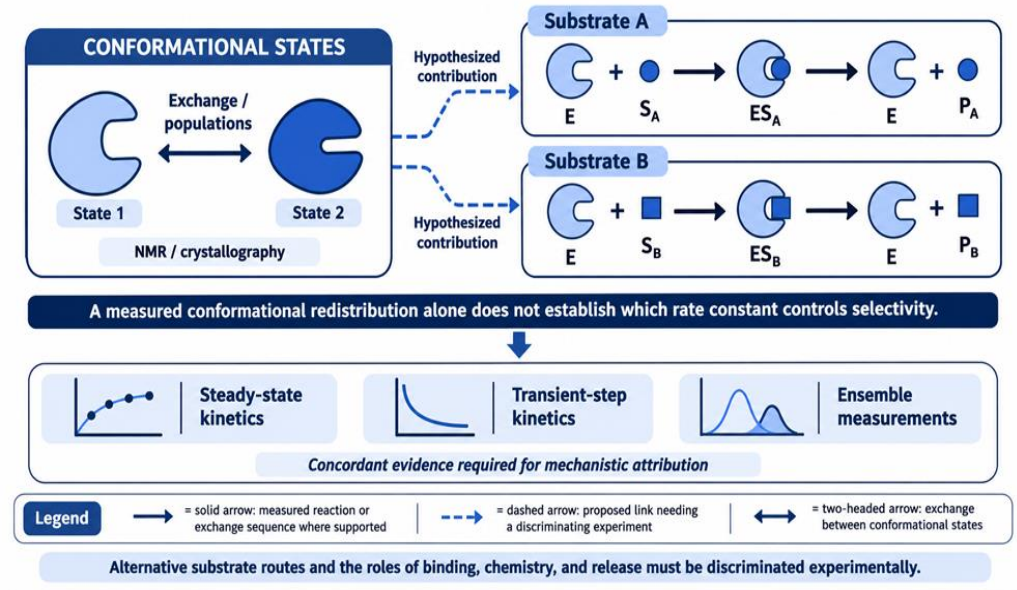


Figure 1. Ensemble redistribution and alternative kinetic routes to substrate selectivity

Note. Original qualitative synthesis informed by conformational design and rate-step studies. Paired-state arrows signify exchange; solid substrate arrows indicate schematic reaction sequences, not separately fitted elementary steps; dashed arrows indicate a proposed state-to-route

influence requiring a discriminating experiment. The illustration does not encode measured barrier heights or demonstrate that any specific conformation causes product selectivity.

A Mechanism-Guided Design and Validation Synthesis

A selectivity program should first declare the comparison that would count as success: competing substrates at specified concentrations, or a defined product distribution at stated conversion. The baseline and engineered enzyme should then be tested at matched temperature, pH, ionic composition, and active enzyme concentration, with stability and expression characterized separately. This order prevents an increase in soluble protein or reduced side-product recovery from being mistaken for altered intrinsic specificity. It also sets an explicit falsification criterion: a gain disappearing after active-enzyme normalization cannot be attributed confidently to an altered catalytic state.

The next decision is which elementary processes could produce the observed difference. Association or access effects call for direct binding and competition measurements; changes in chemical conversion motivate stopped-flow, rapid-quench, isotope, or intermediate-specific tests when technically feasible. A burst phase can reveal a disparity between early turnover and later cycling, but its interpretation depends on the proposed reaction scheme. Fröhlich *et al*. (2024) show why the controlling step cannot be presumed constant across evolved variants. When exchange is suspected, structural snapshots must be combined with a time-sensitive measurement of populations or transitions, not treated as a movie of the catalytic cycle.

The proposed causal test is a matched perturbation series rather than a single high-performing mutant. Choose substitutions predicted to favor or disfavor a specified enzyme state; include controls designed to affect stability or substrate contact without the targeted state shift. The prediction must specify which substrate-specific kinetic observable should change, in which direction, and under what assay conditions. In aminotransferase redesign, concordance between intended state redistribution and measured substrate preference provides an instructive basis for this strategy (St-Jacques *et al*., 2023). A causal assignment nevertheless remains conditional until alternative changes in binding, chemistry, and release are sufficiently constrained.

Computational models should be used to rank these competing predictions before choosing the next variants. Ensemble calculations can nominate shifts in accessible states, while predictive models can prioritize sequences or substrate panels; neither output can substitute for the corresponding measurement. A useful prospective test withholds sequence backgrounds and substrate contexts that were not used to calibrate the design. When predictions fail, the next iteration should distinguish inadequate structural sampling from an incorrectly specified rate-limiting mechanism, rather than labeling every discrepancy a dynamics failure. **Table 3** records the tests that would discriminate or weaken each explanation.

Table 3. Mechanism-specific validation tests and evidence that would challenge a proposed explanation

Competing claim	Discriminating observation or control	What would support the claim	Finding requiring revision
The apparent gain is intrinsic	Normalize to active enzyme; assess soluble yield and stability.	The selectivity difference persists at matched active-enzyme concentration.	Gain vanishes after normalization or depends on recovery.
Substrate encounter changed	Measure competition, binding kinetics and substrate-dependent initial rates.	Changed association or access predicts the substrate-specific effect.	Binding differs, but does not track the functional discrimination.
A chemical step changed	Test transient turnover, isotopes or quenchable intermediate states.	A step-rate change explains the phenotype in a stated kinetic model (Fröhlich *et al*., 2024).	Another step or product accumulation controls the observed flux.
State exchange directs selectivity	Measure state populations or exchange; perturb the nominated state.	State, kinetic readout and substrate preference respond concordantly (St-Jacques *et al*., 2023).	State shifts occur without predicted rate or selectivity changes.
Predictions generalize	Prospectively test new sequence backgrounds and substrate classes.	Predeclared selectivity predictions hold on genuinely held-out examples.	Predictions fail outside training-related conditions.

Note. Original prospective design synthesis, not a table of extracted effect sizes. Proposed measurements should be selected for the enzyme-specific mechanism; no single assay uniquely identifies all steps. Source citations indicate supporting examples, not universal validation thresholds.

Figure 2 organizes this prospective synthesis as a branch-and-retest decision map. Its arrows denote proposed experimental order, not an experimentally proven universal engineering sequence. Alternative explanations remain open until a targeted perturbation alters the predicted state and the relevant kinetic and selectivity readouts together; nonconcordant results return the design to competing explanations rather than automatically validating a conformational mechanism.

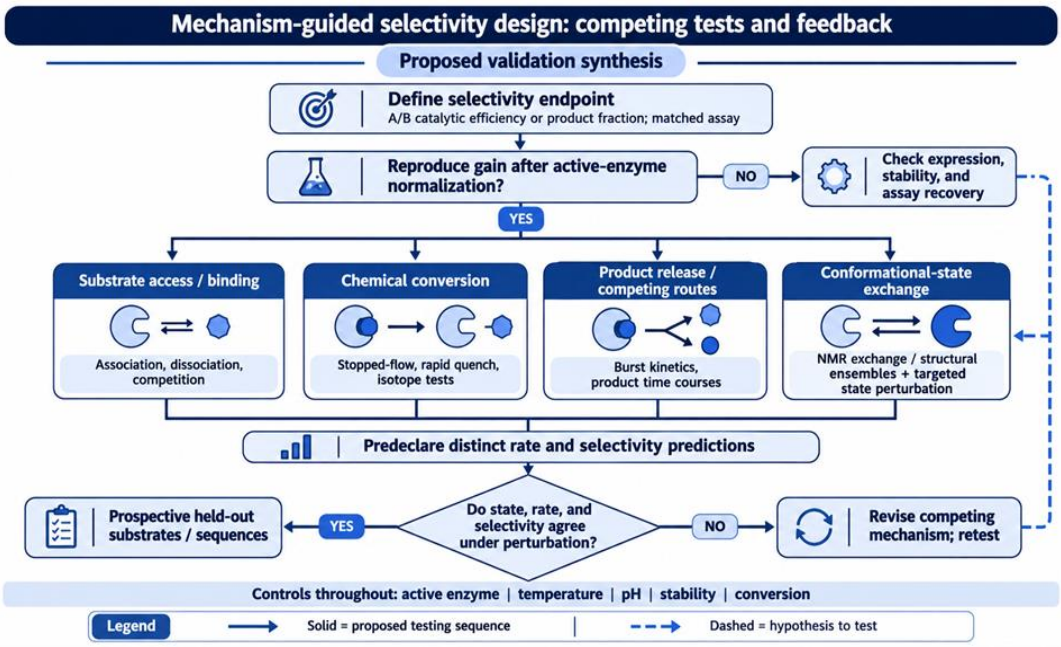


Figure 2. Conditional tests and feedback in mechanism-guided enzyme selectivity engineering

Note. Original prospective synthesis; this is a decision aid, not a measured biological pathway. Solid connectors indicate a proposed testing order, and dashed connectors indicate conditional feedback after an unresolved result. Binding, chemistry, product release and conformational exchange are competing or coupled hypotheses; a positive decision requires independent agreement among the relevant kinetic, state and selectivity readouts.

Results and Discussion

The most informative evidence does not support a universal preference for either increased motion or a rigid active site. Directed evolution has restored productive exchange in some systems (Otten *et al*., 2018), while more ordered catalytic conformations have also accompanied successful designer-enzyme optimization (Broom *et al*., 2020). The direction of the structural change matters less than whether it is tied to a substrate-dependent rate or partitioning process. This view explains why a well-designed enzyme can succeed functionally while the proposed conformational explanation remains unconfirmed.

A second tension concerns generality. Long-range mutational interactions in a cytochrome P450 enzyme are consistent with coordinated access-channel effects (Acevedo-Rocha *et al*., 2021), but they cannot establish the same coupling topology in an unrelated catalyst. Likewise, recruitment-domain engineering changes how substrates encounter the catalytic site and offers an alternative to active-site ensemble optimization (Park *et al*., 2023). Different mechanisms may even deliver equivalent endpoint selectivity. Comparing purified-enzyme measurements across substrate panels, instead of optimizing one screening condition, is consequently central to judging transferability.

Engineering success should therefore be reported at two levels. The performance claim specifies the substrate panel, conversion, product balance, and activity–stability trade-off. The explanatory claim specifies the rate model, conformational observable, perturbation, and competing mechanisms that were actually tested. This separation makes negative and mixed results useful: a failed state-specific prediction narrows the design hypothesis without erasing a reproducible improvement in catalysis. It also discourages extrapolation from algorithmic ranking scores to kinetic causality.

Limitations

The targeted evidence selection is not exhaustive and does not establish publication-wide frequencies of positive or negative effects. The roster privileges mechanistic and engineered-enzyme reports, while inaccessible methods, unreported controls, and outstanding correction/version details constrain claim-level certainty. Accordingly, apparent agreement among studies cannot be interpreted as an independent replication count.

Methodologically, steady-state ratios compress multi-step networks; rapid kinetic traces may require nonunique fitted models; structural measurements can enrich a state without resolving its exchange rate. Simulations sample finite trajectories and may miss rare productive states, whereas learned models may benefit from homologous training sequences or closely related test substrates. Differences in enzyme activation, immobilization, pH, solvent, temperature, and analytical conversion can change the observed phenotype. None of these uncertainties is repaired by combining incompatible measurements into a single selectivity score. More convincing future comparisons will report raw assay context, uncertainty and model alternatives, and prospective failures alongside successful variants.

Conclusion

Conformational dynamics can guide enzyme selectivity engineering when a specified state or transition makes a testable prediction about substrate discrimination or product partitioning,

and that prediction survives independent kinetic and perturbational checks. Improvements in endpoint activity, binding affinity, or predicted structural populations alone leave several causal routes unresolved. A practical sequence is to define comparable selectivity readouts, isolate competing kinetic explanations, design discriminating mutations or conditions, and validate the resulting state–rate–function relationship on new substrates or sequence backgrounds. This approach preserves valuable engineering outcomes while making mechanistic claims proportionate to the evidence.

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References

Acevedo-Rocha, C. G., Li, A., D'Amore, L., Hoebenreich, S., Sanchis, J., Lubrano, P., Ferla, M. P., Garcia-Borràs, M., Osuna, S., & Reetz, M. T. (2021). Pervasive cooperative mutational effects on multiple catalytic enzyme traits emerge via long-range conformational dynamics. *Nature Communications*, *12*(1), 1621. doi:10.1038/s41467-021-21833-w

Banerjee, K., Kolomeisky, A. B., & Igoshin, O. A. (2017). Accuracy of substrate selection by enzymes is controlled by kinetic discrimination. *The Journal of Physical Chemistry Letters*, *8*(7), 1552–1556. doi:10.1021/acs.jpclett.7b00441

Boorla, V. S., & Maranas, C. D. (2025). CatPred: A comprehensive framework for deep learning in vitro enzyme kinetic parameters. *Nature Communications*, *16*(1), 2072. doi:10.1038/s41467-025-57215-9

Braun, M., Tripp, A., Chakatok, M., Kaltenbrunner, S., Fischer, C., Stoll, D., Bijelic, A., Elaily, W., Totaro, M. G., Moser, M., et al. (2026). Computational enzyme design by catalytic motif scaffolding. *Nature*, *649*(8095), 237–245. doi:10.1038/s41586-025-09747-9

Broom, A., Rakotoharisoa, R. V., Thompson, M. C., Zarifi, N., Nguyen, E., Mukhametzhanov, N., Liu, L., Fraser, J. S., & Chica, R. A. (2020). Ensemble-based enzyme design can recapitulate the effects of laboratory directed evolution in silico. *Nature Communications*, *11*(1), 4808. doi:10.1038/s41467-020-18619-x

Buda, K., Miton, C. M., & Tokuriki, N. (2023). Pervasive epistasis exposes intramolecular networks in adaptive enzyme evolution. *Nature Communications*, *14*(1), 8508. doi:10.1038/s41467-023-44333-5

Bunzel, H. A., Anderson, J. L. R., Hilvert, D., Arcus, V. L., van der Kamp, M. W., & Mulholland, A. J. (2021). Evolution of dynamical networks enhances catalysis in a designer enzyme. *Nature Chemistry*, *13*(10), 1017–1022. doi:10.1038/s41557-021-00763-6

Campbell, E., Kaltenbach, M., Correy, G. J., Carr, P. D., Porebski, B. T., Livingstone, E. K., Afriat-Jurnou, L., Buckle, A. M., Weik, M., Hollfelder, F., et al. (2016). The role of protein dynamics in the evolution of new enzyme function. *Nature Chemical Biology*, *12*(11), 944–950. doi:10.1038/nchembio.2175

Crawshaw, R., Crossley, A. E., Johannissen, L., Burke, A. J., Hay, S., Levy, C., Baker, D., Lovelock, S. L., & Green, A. P. (2022). Engineering an efficient and enantioselective enzyme for the Morita–Baylis–Hillman reaction. *Nature Chemistry*, *14*(3), 313–320. doi:10.1038/s41557-021-00833-9

Cui, H., Su, Y., Dean, T. J., Yu, T., Zhang, Z., Peng, J., Shukla, D., & Zhao, H. (2025). Enzyme specificity prediction using cross-attention graph neural networks. *Nature*, *647*(8090), 639–647. doi:10.1038/s41586-025-09697-2

Dou, Z., Chen, X., Zhu, L., Zheng, X., Chen, X., Xue, J., Niwayama, S., Ni, Y., & Xu, G. (2024). Enhanced stereodivergent evolution of carboxylesterase for efficient kinetic resolution of near-symmetric esters through machine learning. *Nature Communications*, *15*(1), 9057. doi:10.1038/s41467-024-53191-8

Fröhlich, C., Bunzel, H. A., Buda, K., Mulholland, A. J., van der Kamp, M. W., Johnsen, P. J., Leiros, H.-K. S., & Tokuriki, N. (2024). Epistasis arises from shifting the rate-limiting step during enzyme evolution of a β-lactamase. *Nature Catalysis*, *7*(5), 499–509. doi:10.1038/s41929-024-01117-4

Johnson, K. A. (2019). New standards for collecting and fitting steady state kinetic data. *Beilstein Journal of Organic Chemistry*, *15*, 16–29. doi:10.3762/bjoc.15.2

Johnson, S. R., Fu, X., Viknander, S., Goldin, C., Monaco, S., Zelezniak, A., & Yang, K. K. (2025). Computational scoring and experimental evaluation of enzymes generated by neural networks. *Nature Biotechnology*, *43*(3), 396–405. doi:10.1038/s41587-024-02214-2

Karamitros, C. S., Murray, K., Kumada, Y., Johnson, K. A., D'Arcy, S., & Georgiou, G. (2024). Mechanistic conformational and substrate selectivity profiles emerging in the evolution of enzymes via parallel trajectories. *Nature Communications*, *15*(1), 7068. doi:10.1038/s41467-024-51133-y

Karamitros, C. S., Murray, K., Winemiller, B., Lamb, C., Stone, E. M., D'Arcy, S., Johnson, K. A., & Georgiou, G. (2022). Leveraging intrinsic flexibility to engineer enhanced enzyme catalytic activity. *Proceedings of the National Academy of Sciences*, *119*(23), e2118979119. doi:10.1073/pnas.2118979119

Li, Z.-L., Pei, S., Chen, Z., Huang, T.-Y., Wang, X.-D., Shen, L., Chen, X., Wang, Q.-Q., Wang, D.-X., & Ao, Y.-F. (2024). Machine learning-assisted amidase-catalytic enantioselectivity prediction and rational design of variants for improving enantioselectivity. *Nature Communications*, *15*(1), 8778. doi:10.1038/s41467-024-53048-0

McDonald, A. D., Higgins, P. M., & Buller, A. R. (2022). Substrate multiplexed protein engineering facilitates promiscuous biocatalytic synthesis. *Nature Communications*, *13*(1), 5242. doi:10.1038/s41467-022-32789-w

Modi, T., Risso, V. A., Martinez-Rodriguez, S., Gavira, J. A., Mebrat, M. D., Van Horn, W. D., Sanchez-Ruiz, J. M., &

Ozkan, S. B. (2021). Hinge-shift mechanism as a protein design principle for the evolution of β-lactamases from substrate promiscuity to specificity. *Nature Communications*, *12*(1), 1852. doi:10.1038/s41467-021-22089-0

Ojeda-May, P., Mushtaq, A. U., Rogne, P., Verma, A., Ovchinnikov, V., Grundström, C., Dulko-Smith, B., Sauer, U. H., Wolf-Watz, M., & Nam, K. (2021). Dynamic connection between enzymatic catalysis and collective protein motions. *Biochemistry*, *60*(28), 2246–2258. doi:10.1021/acs.biochem.1c00221

Otten, R., Liu, L., Kenner, L. R., Clarkson, M. W., Mavor, D., Tawfik, D. S., Kern, D., & Fraser, J. S. (2018). Rescue of conformational dynamics in enzyme catalysis by directed evolution. *Nature Communications*, *9*(1), 1314. doi:10.1038/s41467-018-03562-9

Otten, R., Pádua, R. A. P., Bunzel, H. A., Nguyen, V., Pitsawong, W., Patterson, M., Sui, S., Perry, S. L., Cohen, A. E., Hilvert, D., et al. (2020). How directed evolution reshapes the energy landscape in an enzyme to boost catalysis. *Science*, *370*(6523), 1442–1446. doi:10.1126/science.abd3623

Park, R., Ongpipattanakul, C., Nair, S. K., Bowers, A. A., & Kuhlman, B. (2023). Designer installation of a substrate recruitment domain to tailor enzyme specificity. *Nature Chemical Biology*, *19*(4), 460–467. doi:10.1038/s41589-022-01206-0

Patsch, D., Schwander, T., Voss, M., Schaub, D., Hüppi, S., Eichenberger, M., Stockinger, P., Schelbert, L., Giger, S., Peccati, F., et al. (2024). Enriching productive mutational paths accelerates enzyme evolution. *Nature Chemical Biology*, *20*(12), 1662–1669. doi:10.1038/s41589-024-01712-3

Richard, J. P. (2022). Enabling role of ligand-driven conformational changes in enzyme evolution. *Biochemistry*, *61*(15), 1533–1542. doi:10.1021/acs.biochem.2c00178

Shen, Z., Siriboe, M. G., Ren, X., Dayananda, T., Jenkins, J. L., Khare, S. D., & Fasan, R. (2026). Computational design of generalist cyclopropanases with stereodivergent selectivity. *Nature Communications*, *17*(1), 1620. doi:10.1038/s41467-026-68327-1

Shukla, V. K., Siemons, L., & Hansen, D. F. (2023). Intrinsic structural dynamics dictate enzymatic activity and inhibition. *Proceedings of the National Academy of Sciences*, *120*(41), e2310910120. doi:10.1073/pnas.2310910120

Singh, N., Lane, S., Yu, T., Lu, J., Ramos, A., Cui, H., & Zhao, H. (2025). A generalized platform for artificial intelligence-powered autonomous enzyme engineering. *Nature Communications*, *16*(1), 5648. doi:10.1038/s41467-025-61209-y

St-Jacques, A. D., Rodriguez, J. M., Eason, M. G., Foster, S. M., Khan, S. T., Damry, A. M., Goto, N. K., Thompson, M. C., & Chica, R. A. (2023). Computational remodeling of an enzyme conformational landscape for altered substrate selectivity. *Nature Communications*, *14*(1), 6058. doi:10.1038/s41467-023-41762-0

Vanella, R., Boult, S., Küng, C., & Nash, M. A. (2026). Decoding the substrate specificity landscape of a promiscuous enzyme through multi-substrate mutational scanning. *Nature Communications*, *17*(1), 3245. doi:10.1038/s41467-026-69913-z

Wang, Z., Xie, D., Wu, D., Luo, X., Wang, S., Li, Y., Yang, Y., Li, W., & Zheng, L. (2025). Robust enzyme discovery and engineering with deep learning using CataPro. *Nature Communications*, *16*(1), 2736. doi:10.1038/s41467-025-58038-4