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When Catalytic Efficiency Fails to Predict Cellular Function: A Theoretical Framework Linking Enzyme Kinetics, Substrate Availability, Macromolecular Crowding, and Pathway-Level Constraints

Helena Voss*, Erik Sandberg, Karin Åkesson

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Abstract

Catalytic efficiency is often used to compare enzymes, parameterize metabolic models, and infer how molecular variation may influence biological output. Yet catalytic performance measured under purified or standardized conditions does not map uniquely onto function inside cells. This theoretical analysis examines why that translation becomes unreliable when substrate availability, apparent in-vivo turnover, macromolecular crowding, spatial organization, proteome allocation, compartmental capacity, and pathway demand intervene between intrinsic catalytic potential and realized flux. Evidence from enzyme-constrained modeling, quantitative proteomics, metabolomics, cellular biophysics, and metabolic regulation supports a multilevel interpretation in which catalytic efficiency remains informative but becomes conditionally predictive. The central implication is that failure of a kinetic parameter to predict cellular function need not indicate defective enzymology; it may reveal that another biological constraint has become limiting. A mechanistically useful interpretation therefore requires identification of the controlling scale, experimental context, and competing limitation before catalytic efficiency is assigned explanatory priority.

Keywords: Enzyme kinetics, Catalytic efficiency, Metabolic flux, Substrate availability, Macromolecular crowding, Proteome allocation

Introduction

Enzyme kinetics provides one of the most powerful quantitative languages for relating molecular properties to biochemical reaction capacity. Genome-scale metabolic modeling has consequently moved beyond purely stoichiometric feasibility by incorporating enzyme abundance and catalytic constraints, improving the ability of models to exclude biologically implausible flux states and reproduce observed phenotypes (Sánchez *et al*., 2017). Machine-learning approaches to enzyme turnover numbers have extended this strategy by exploiting biochemical, structural, and network information where direct kinetic measurements are unavailable (Heckmann *et al*., 2018). These developments reinforce a basic proposition: cellular metabolism cannot be understood without some representation of catalytic capacity. They do not, however, establish that a catalytic parameter measured for an isolated enzyme is itself sufficient to predict the functional contribution of that enzyme inside a cell.

The gap becomes evident when finite cellular resources are considered. Protein efficiency and proteome allocation can determine which metabolic strategies are economically feasible for a cell even when alternative reactions are chemically possible (Chen & Nielsen, 2019). Conversely, apparent in-vivo enzyme turnover inferred from simultaneous flux and protein-abundance measurements can remain reproducible across defined cellular states while differing from values obtained under purified assay conditions (Heckmann *et al*., 2020). A kinetic parameter therefore enters cellular physiology through an allocation system: the enzyme must be expressed at an adequate abundance, occupy the appropriate compartment or molecular state, encounter substrate, and operate in a pathway whose upstream supply and downstream demand permit its capacity to be used.

Direct comparison of kinetic quantities strengthens this concern. In yeast, in-vitro turnover numbers showed limited correspondence with apparent in-vivo activities across metabolic enzymes, and part of the discrepancy was attributable to the provenance and experimental context of kinetic measurements (Chen & Nielsen, 2021). This observation should not be read as evidence that purified-enzyme measurements lack biological value. Instead, it identifies a translation problem. A measured maximum turnover number describes catalytic behavior under a defined assay regime; cellular function reflects the fraction of that potential realized under a particular intracellular state. Differences between the two may arise from substrate undersaturation, regulatory interactions, molecular crowding, transport, compartmentation, enzyme

Helena Voss*
Department of Biochemistry, Uppsala University, Uppsala, Sweden.

Erik Sandberg
Department of Molecular Biology, Lund University, Lund, Sweden.

Karin Åkesson
Department of Cell Biology, University of Gothenburg, Gothenburg, Sweden.

***E-mail:** helena.voss@uu.se

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assembly, competing protein-resource demands, or constraints elsewhere in the pathway.

The expansion of predicted kinetic databases does not remove this distinction. Deep-learning estimates of turnover numbers can improve enzyme-constrained model reconstruction and extend kinetic coverage to enzymes and organisms for which experimental values are sparse (Li *et al*., 2022). Their usefulness remains dependent on the question being asked: predicting a plausible catalytic parameter is different from demonstrating that the parameter is rate controlling in a particular cell. The problem addressed here is therefore not whether catalytic efficiency matters, but when it deserves predictive priority. The analysis develops a conditional account in which intrinsic or assay-derived catalytic performance is interpreted alongside cellular enzyme state, substrate accessibility, physicochemical environment, proteome allocation, and pathway-level constraints. The framework is intended to distinguish cases in which catalytic efficiency should track cellular function from cases in which another biological limitation can dominate the phenotype.

Materials and Methods

The Interpretive Limits of Catalytic Efficiency in Cellular Environments

Enzyme-constrained genome-scale models formalize an important distinction between metabolic feasibility and catalytic feasibility. By restricting reaction flux according to enzyme availability and turnover capacity, such models identify states that an unconstrained stoichiometric network would otherwise permit (Domenzain *et al*., 2022). Resource-allocation modeling extends the same logic to ribosomes, protein pools, membrane occupancy, or other limited cellular resources, while also exposing a persistent methodological problem: kinetic parameters are incomplete, heterogeneous, and frequently measured outside the physiological state to which the model is applied (Kerkhoven, 2022). Catalytic capacity is therefore an informative constraint, but its interpretation depends on what quantity has actually been measured or inferred.

This distinction is especially important for turnover numbers. Condition-integrated estimation strategies have shown that kinetic parameters inferred from cellular data can be improved by using observations collected across multiple physiological states (Wendering *et al*., 2023). Machine-learning approaches likewise predict turnover values for enzymes lacking direct characterization and can improve downstream estimates of protein allocation (Kroll *et al*., 2023). Neither class of parameter is equivalent to a purified maximal turnover measurement. An apparent cellular turnover rate combines information about reaction flux and enzyme abundance under a defined physiological state, whereas a predicted turnover value reflects statistical inference from available training information. Treating these quantities as interchangeable would obscure precisely the biological context that a theory of cellular enzyme function must explain.

Quantitative proteomics provides a further bridge between catalytic potential and cellular realization. In *Chlamydomonas reinhardtii*, integration of proteome measurements with metabolic modeling generated reaction-level in-vivo turnover proxies that improved predictions of enzyme usage (Arend *et al*., 2023). Contemporary enzyme-constrained modeling protocols similarly distinguish the biochemical parameterization of a reaction from the proteome and physiological constraints under which that reaction is evaluated (Chen *et al*., 2024). These approaches are valuable because they turn catalytic capacity into a testable systems-level variable. Their outputs nonetheless remain conditional on the accuracy of enzyme abundance measurements, flux estimates, reaction assignments, optimization assumptions, and the biological state represented by the model.

The cellular proteome also changes systematically with environmental demand. Multi-omic analysis of glucose-limited yeast showed growth-rate-dependent redistribution of protein investment and implicated substrate saturation and post-translational regulation in the relationship between enzyme abundance and metabolic flux (Xia *et al*., 2022). Whole-cell modeling further demonstrated that compartment-specific limits can alter predicted metabolic strategies: mitochondrial and cytosolic protein capacities need not impose equivalent restrictions under different nutrient conditions (Elsemman *et al*., 2022). Thus, even an accurately characterized enzyme may possess unused catalytic capacity because the cell cannot allocate sufficient protein to its pathway, because another compartment becomes limiting, or because metabolite supply does not support operation near the enzyme's assay-defined maximum.

Resource-balance analysis reaches a similar conclusion from a different direction. Estimated apparent turnover and resource requirements vary across nutritional and respiratory contexts rather than behaving as invariant cellular constants (Dinh & Maranas, 2023). Experimental and modeling work on yeast growth regulation likewise indicates that resource allocation is adjusted as extracellular substrates and signaling conditions change (Nguyen *et al*., 2023). The same catalytic machinery may therefore participate in distinct cellular states with different feasible outputs. Across these studies, condition-sensitive allocation repeatedly emerges as a determinant of realized function (Xia *et al*., 2022; Dinh & Maranas, 2023; Nguyen *et al*., 2023). This does not imply that intrinsic catalysis becomes irrelevant. It means that catalytic efficiency can become nonlimiting: once an enzyme has more potential capacity than the surrounding system can use, further differences in that capacity need not translate proportionally into pathway output.

This distinction is central to interpreting apparent contradictions between kinetic assays and cellular phenotypes. Four quantities should remain analytically separate: a purified catalytic parameter obtained under specified assay conditions; an apparent in-vivo turnover inferred from cellular flux and protein abundance; the amount and molecular state of enzyme available to the reaction; and the pathway-level output that ultimately contributes to phenotype. Agreement among them is biologically informative, but disagreement is also informative because it localizes the need for an additional constraint. The interpretive mistake is to assume in advance that the kinetic parameter and the cellular output occupy the same explanatory level.

The distinction can be expressed more concretely by separating what common kinetic and cellular measurements contain—and what they omit.

Table 1 summarizes the measurement classes and their boundaries for cellular interpretation.

Table 1. Measurement classes that separate intrinsic enzyme performance from realized cellular catalytic capacity

Analytical unit	What the quantity primarily represents	Context incorporated directly	Important determinants left outside the quantity	Appropriate cellular interpretation	Principal failure mode if overextended
Purified maximal turnover	Catalytic cycling capacity of a defined enzyme preparation under specified assay conditions	Assay temperature, pH, buffer, substrate regime, enzyme preparation	Cellular abundance, competing ligands, compartmentation, crowding, pathway demand, protein allocation	Upper or context-specific catalytic potential	Treating assay-defined potential as an in-cell operating rate
Apparent cellular turnover	Flux realized per measured enzyme abundance in a defined cellular state	Cellular enzyme abundance and estimated pathway flux	Hidden isozyme contributions, inactive protein fractions, unresolved compartmentation, model uncertainty	State-dependent effective use of enzyme capacity	Reinterpreting an apparent rate as an invariant molecular constant
Predicted turnover	Statistically inferred catalytic parameter for an incompletely characterized enzyme–reaction pair	Features represented in the prediction model	Unrepresented cellular regulation, physiological state, novel chemistry outside training domain	Parameter estimate for model construction or hypothesis generation	Treating computational prediction as direct biochemical measurement
Enzyme abundance	Quantity of protein assigned to a cellular process	Expression and degradation outcomes at sampling time	Fraction catalytically competent, substrate saturation, inhibition, localization, molecular interactions	Available protein investment	Assuming additional protein produces proportional additional flux
Substrate-saturation state	Degree to which available substrate can exploit catalytic capacity	Local substrate conditions when measured adequately	Compartmental gradients, channeling, transport, competing reactions	Modifier of the fraction of catalytic capacity actually used	Inferring flux solely from enzyme kinetic potential
Compartmental/ proteome capacity	Cellular resource available to an enzyme or pathway relative to competing functions	Protein-space and allocation constraints represented by the system	Unmeasured regulation and dynamic reallocation	Systems-level ceiling on realizable catalytic output	Assigning a local enzyme as limiting when another resource is binding

The relationships summarized in **Table 1** imply that cellular prediction requires information to cross several biological levels. A catalytic parameter may constrain an enzyme, but pathway output additionally depends on whether sufficient enzyme is present, whether it is catalytically competent, and whether surrounding cellular conditions permit its capacity to be exploited. The corresponding multilevel structure is summarized in **Figure 1**.

Figure 1 illustrates the multilevel constraints linking catalytic potential to cellular throughput.

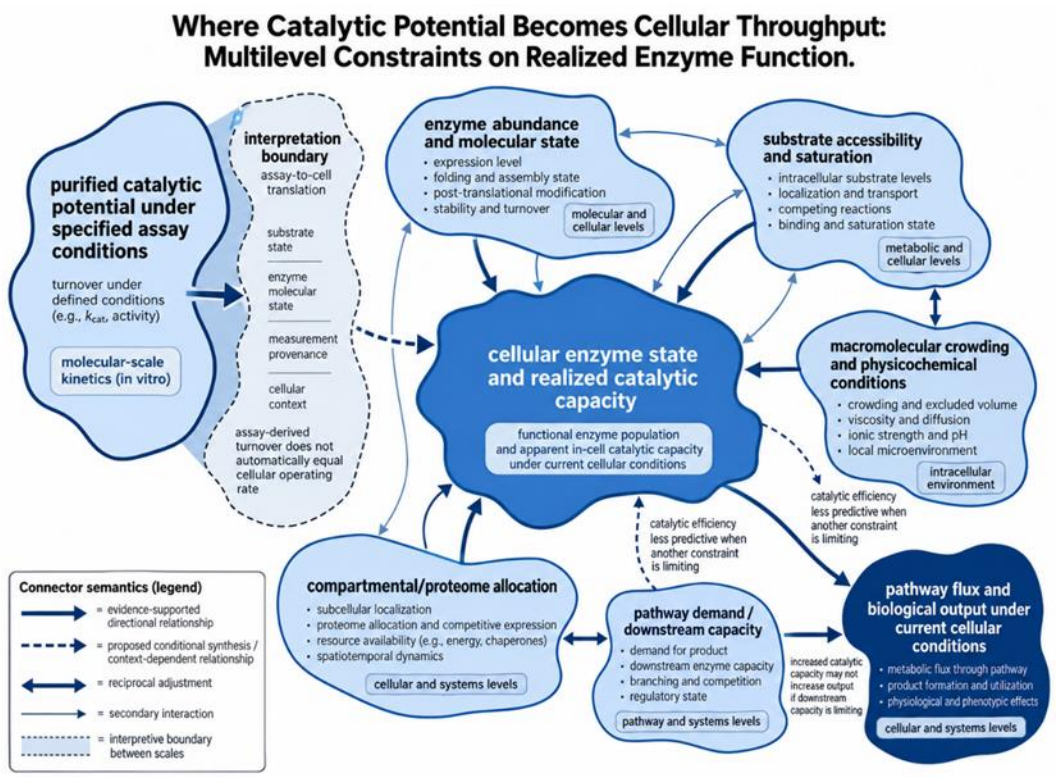


Figure 1. Where Catalytic Potential Becomes Cellular Throughput: Multilevel Constraints on Realized Enzyme Function

Substrate Accessibility, Macromolecular Crowding, and Effective Catalytic Capacity

The fraction of catalytic potential expressed in a cell depends first on whether an enzyme encounters its substrate under conditions that allow the reaction to use that potential. Multi-omic perturbation studies in *Escherichia coli* indicate that substantial flux adjustment can occur through local metabolite concentrations even when global enzyme-expression programs do not explain the magnitude of metabolic change (Kochanowski *et al*., 2021). A complementary theoretical treatment of enzyme–substrate allocation shows why this is plausible: increasing investment in enzyme can yield little additional benefit if substrate remains scarce, while maintaining excessive substrate pools also carries cellular costs (Dourado *et al*., 2021). Catalytic performance and substrate supply are therefore jointly relevant quantities. An enzyme cannot operate near its intrinsic capacity when the local chemical state does not support that rate.

Substrate concentration can also carry information about pathway demand. Kinetic analysis of central carbon metabolism identified reaction contexts in which metabolite concentrations respond systematically to upstream flux because thermodynamic and kinetic properties make the substrate pool sensitive to reaction throughput (Euler & Mahadevan, 2022). Such relationships are reaction specific. They do not justify a universal assumption that metabolite concentration predicts flux. Instead, they show that substrate availability can become an active component of flux regulation and therefore a potential explanation for why enzyme-centered predictions succeed in one reaction but fail in another.

Spatial organization adds another layer: transient or stable enzyme assemblies can alter effective substrate availability by changing the distance and probability of intermediate transfer between consecutive reactions (Sweetlove & Fernie, 2018).

The concept of metabolic channeling illustrates the danger of confusing molecular proximity with demonstrated function. Channeling generates specific predictions about intermediate transfer, competition, leakage, and kinetic behavior, and those predictions can be evaluated experimentally (Pareek *et al*., 2021). Evidence of protein–protein interaction alone is insufficient to establish that an intermediate is functionally transferred within an enzyme assembly. Reviews of proposed metabolons make this distinction explicit, showing that structural association, colocalization, and functional channeling occupy different evidentiary levels (Zhang & Fernie, 2020). For the present argument, channeling is therefore a conditional mechanism by which the substrate actually experienced by an enzyme may differ from a bulk concentration measurement. It should not be inserted as a default explanation whenever an enzyme complex is observed.

Macromolecular crowding complicates the relationship further because the intracellular medium is not chemically equivalent to an inert concentrated solution. Modern biophysical work emphasizes that crowding involves both excluded-volume effects and nonspecific chemical interactions between macromolecules (Speer *et al*., 2022). These interactions can favor or oppose one another, producing stabilization, destabilization, altered association, or modified reaction kinetics depending on the molecules involved. Cellular experiments likewise show that

changes in crowding can reshape mesoscale diffusion and phase behavior in a size-dependent manner rather than imposing a uniform viscosity penalty on every molecular species (Delarue *et al*., 2018). A single scalar notion of "crowding" is therefore inadequate for predicting enzyme function.

Physiological perturbation provides evidence that such physical effects can propagate to cell-level behavior. Under mechanical confinement, yeast cells exhibit increased intracellular crowding together with reduced macromolecular mobility, protein production, and growth, consistent with a feedback between physical compression and biosynthetic capacity (Alric *et al*., 2022). Conceptual work on crowding argues that cells may respond to such changes through distributed physicochemical effects on biochemical reactions rather than through a dedicated molecular crowding sensor (Holt & Delarue, 2023). This possibility is important because it makes crowding a property of the operating environment of many enzymes simultaneously. A kinetic discrepancy produced under such conditions need not originate in an altered active site; it may arise because diffusion, molecular association, substrate encounter, or global biosynthetic capacity has shifted.

The broader intracellular environment reinforces this interpretation. The cytoplasm combines high macromolecular occupancy with active processes, structural heterogeneity, transient assemblies, and energy-dependent material properties that are absent from dilute biochemical assays (Bonucci *et al*., 2023). Cells also regulate pH, ionic composition, osmotic state, hydration, crowding, and energetic variables within organism-specific ranges, so biochemical reactions occur inside a maintained physicochemical system rather than an unconstrained solution (Poolman, 2023). These variables interact, and evidence remains substantially stronger for some organisms and molecular scales than for others. The appropriate inference is therefore conditional: purified kinetics supplies a reference state, while cellular performance reflects how much of that capacity remains accessible after the intracellular environment has imposed its local constraints.

Several crowding studies converge on the further point that the effect cannot be assigned a universal sign (Speer *et al*., 2022; Bonucci *et al*., 2023; Holt & Delarue, 2023). Excluded volume may favor compact states or molecular association, whereas nonspecific chemical interactions may oppose those effects; changes in diffusion can influence encounter rates differently according to molecular scale. Consequently, the relevant question is not whether crowding is "good" or "bad" for catalytic efficiency. It is whether the cellular physicochemical state changes the relationship among enzyme concentration, available substrate, molecular encounters, and pathway flux enough to make a purified kinetic comparison misleading.

The mechanistic conditions developed in this section can be organized according to what becomes limiting between an enzyme's intrinsic catalytic potential and pathway-level output.

Table 2 summarizes conditions that decouple intrinsic catalytic potential from pathway-level function.

Table 2. Mechanistic conditions that decouple intrinsic catalytic potential from pathway-level function

Cellular condition	Immediate constraint on realized catalysis	Quantity that may cease to track pathway output	Mechanistic discriminator	Expected interpretive pattern	Boundary on inference
Local substrate undersaturation	Insufficient substrate occupancy of available enzyme	Purified catalytic efficiency or maximal turnover	Manipulate substrate supply while holding enzyme abundance approximately stable	Flux changes disproportionately to enzyme quantity	Requires knowledge of the substrate concentration experienced by the enzyme, not only bulk abundance
Thermodynamically sensitive reaction state	Reaction driving force couples substrate/product concentrations to feasible flux	Enzyme abundance alone	Joint metabolite and flux measurements across controlled states	Metabolite pools covary with flux in reaction-specific patterns	Not every reaction is sufficiently close to thermodynamic constraint for this behavior
Functional substrate channeling	Local transfer alters intermediate accessibility relative to bulk solution	Bulk metabolite concentration	Demonstrate pathway-specific transfer or leakage behavior beyond colocalization	Effective substrate access differs from freely diffusing expectation	Protein association or proximity alone does not establish channeling
Excluded-volume-dominated crowding	Steric occupancy alters accessible volume and molecular association	Dilute-solution kinetic parameter	Vary crowding while controlling chemical identity as far as feasible	Catalytic behavior shifts without a change in enzyme sequence or abundance	Synthetic crowders do not necessarily reproduce cellular crowding
Nonspecific enzyme–crowder interactions	Weak chemical interactions modify	Simple crowding-volume estimate	Compare crowders with different interaction	Direction and magnitude of kinetic	Effects observed for one enzyme–crowder system

	enzyme or substrate behavior		chemistry at comparable physical occupancy	change depend on crowder identity	cannot be generalized automatically
Mesoscale cytoplasmic crowding	Diffusion and material properties change selectively with molecular size and cell state	Assay-derived expectations of molecular encounter	Combine intracellular mobility measurements with biochemical or flux readouts	Cellular function changes despite unchanged nominal enzyme identity	Small solutes, proteins, particles, and assemblies need not respond identically
Physicochemical homeostasis shift	pH, ions, hydration, energy state, and crowding jointly alter reaction conditions	Any kinetic parameter measured under unmatched buffer conditions	Measure relevant intracellular physicochemical variables alongside enzyme function	Several kinetic determinants may change simultaneously	Attribution to a single variable requires orthogonal perturbation
Proteome or compartmental limitation	Finite protein and spatial resources cap the amount of pathway capacity that can be deployed	Intrinsic catalytic improvement at one enzyme	Quantify pathway proteome allocation and competing resource use	Additional catalytic potential produces little output when another resource binds	Limiting resource can change with nutrient state, compartment, or time

Table 2 highlights why a cellular enzyme cannot be classified as functionally strong or weak from an isolated kinetic parameter alone. A high catalytic potential can remain unused because substrate is inaccessible, because the physical environment alters effective molecular behavior, or because the pathway lacks the resource or downstream capacity required to exploit it. Conversely, apparently modest intrinsic kinetics may be sufficient when enzyme abundance, substrate availability, and network organization keep the reaction away from a catalytic ceiling. The analytical task is thus to identify which constraint is binding in the biological state under study. the section "An Integrated Theoretical Framework for Pathway-Constrained Enzyme Function" will use these distinctions to specify the conditions under which enzyme limitation, substrate limitation, physicochemical limitation, and pathway-level limitation should generate different observable patterns.

An Integrated Theoretical Framework for Pathway-Constrained Enzyme Function

The evidence supports a conditional interpretation of catalytic performance. Cellular function should track intrinsic catalytic potential most closely when active enzyme capacity is itself the dominant limitation: sufficient enzyme is present, substrate is accessible, the physicochemical environment permits turnover, and upstream and downstream pathway capacities can accommodate additional flux. Functional decomposition shows that flux and protein investment are distributed across interdependent metabolic functions (Mori *et al*., 2023), while physiological models show that finite proteome allocation constrains how catalytic capacity is deployed (Scott & Hwa, 2023). Catalytic efficiency is therefore potentially predictive, but only within a cellular state that can express it.

Four limiting regimes follow from this synthesis. In an enzyme-limited state, changes in active enzyme capacity should covary with pathway output after relevant contextual variables are controlled. In a substrate-limited state, catalytic capacity remains underused because substrate supply or reaction thermodynamics restrict turnover. In a physicochemical-limited state, crowding, molecular interactions, ionic conditions, or compartmental properties alter realized activity. In a pathway/resource-limited state, downstream capacity or competing resource demands prevent a local catalytic advantage from propagating to system output. These regimes may overlap and change with time. Proteome reallocation and flux redistribution can proceed with different transition kinetics (Yuan *et al*., 2024), while direct crowding experiments show that kinetic parameters can change in different directions with crowder identity and interaction state (Xu *et al*., 2023). The framework therefore predicts shifting limitation rather than a permanent property of an enzyme.

Figure 2 represents this interpretation as a boundary map in which the relevant scientific question is not whether catalytic efficiency matters, but whether enzyme capacity is the binding constraint in the cellular state being examined.

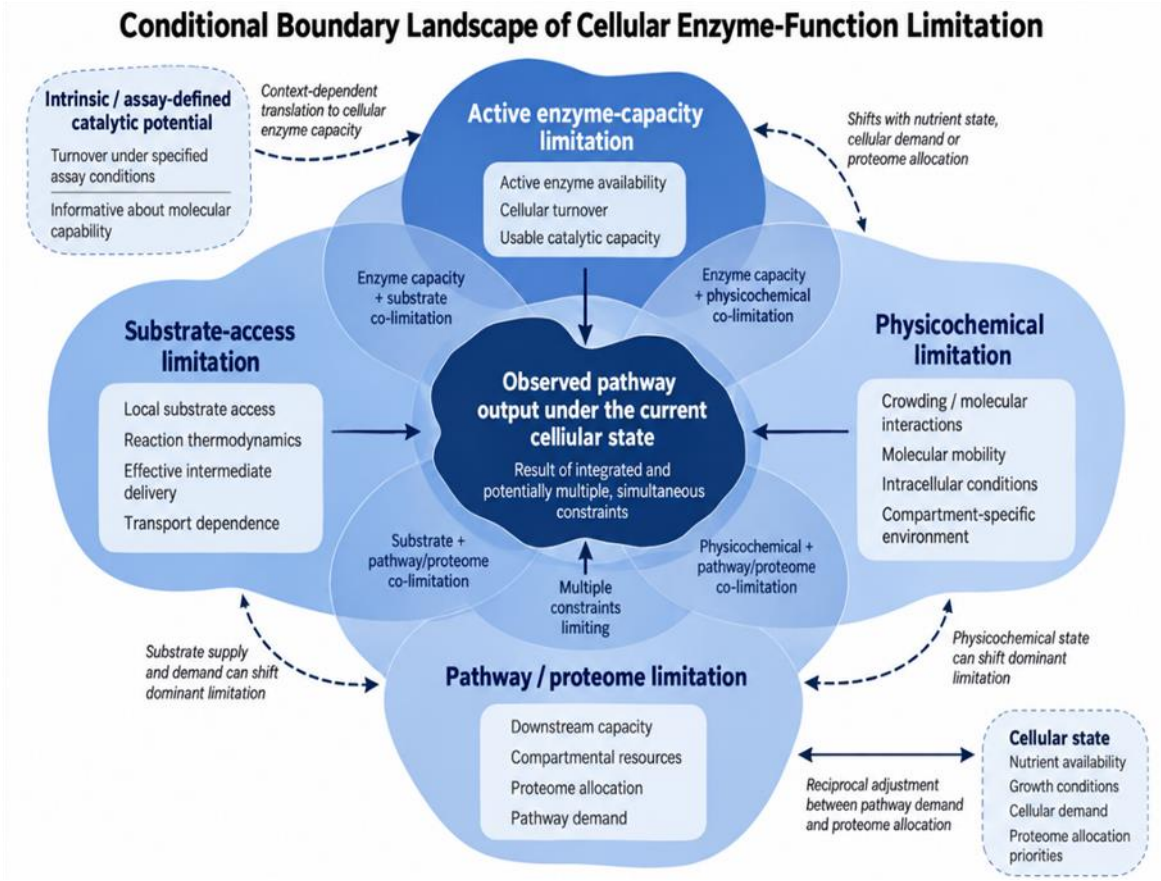


Figure 2. Conditional Boundary Map of Limitation in Cellular Enzyme Function

Results and Discussion

Model Predictions, Boundary Conditions, and Experimental Discrimination

The framework is useful only if its alternatives can be separated empirically. An enzyme-limited interpretation predicts concordance among active enzyme capacity, cellular turnover and pathway output; disagreement between purified and cellular rates instead signals the need to examine contextual constraints (Heckmann *et al*., 2020; Chen & Nielsen, 2021). A substrate-limited interpretation predicts that changing substrate supply, transport, or metabolite state can alter flux without a proportional change in enzyme abundance, consistent with metabolite-mediated regulation and reaction-specific flux sensing (Kochanowski *et al*., 2021; Euler & Mahadevan, 2022). Claims of substrate channeling require stronger evidence because molecular proximity alone does not establish functional transfer (Pareek *et al*., 2021).

A physicochemical interpretation requires matched measurements of intracellular state because crowding can produce different effects depending on molecular interactions (Speer *et al*., 2022). A pathway/resource explanation predicts weak response to local catalytic improvement when downstream capacity, compartmental resources, or proteome allocation is already limiting. Enzyme-constrained and whole-cell models can formulate these competing possibilities (Domenzain *et al*., 2022; Elsemman *et al*., 2022), while functional decomposition helps identify the metabolic functions consuming limiting resources (Mori *et al*., 2023). Condition-specific kinetic and allocation data may strengthen such discrimination across changing states (Arend *et al*., 2023; Wendering *et al*., 2023; Yuan *et al*., 2024).

The principal boundary is transferability. Much of the quantitative evidence derives from microbial metabolism, whereas mammalian tissues and strongly compartmentalized systems may introduce additional regulation. Apparent turnover also inherits uncertainty from flux and abundance estimates, and optimization models embed assumptions about cellular objectives. The framework would therefore be weakened if supposedly distinct limiting regimes produced no distinguishable response to appropriately targeted perturbations. Its purpose is to organize falsifiable alternatives, not to assign causality from a diagram or kinetic parameter alone.

Conclusion

Catalytic efficiency predicts cellular function best when enzyme capacity is the dominant limitation and the surrounding biological system can exploit that capacity. Divergence is expected when substrate accessibility, physicochemical conditions, proteome allocation, compartmental capacity, or downstream pathway constraints dominate instead. The useful distinction is therefore between intrinsic catalytic potential and the fraction of that

potential realized in a particular cellular state. Mechanistic interpretation requires identifying the controlling scale, preserving competing explanations, and testing whether perturbing the proposed limiting factor changes pathway output as predicted.

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References

Alric, B., Formosa-Dague, C., Dague, E., Holt, L. J., & Delarue, M. (2022). Macromolecular crowding limits growth under pressure. *Nature Physics*, *18*(4), 411–416. doi:10.1038/s41567-022-01506-1

Arend, M., Zimmer, D., Xu, R., Sommer, F., Mühlhaus, T., & Nikoloski, Z. (2023). Proteomics and constraint-based modelling reveal enzyme kinetic properties of *Chlamydomonas reinhardtii* on a genome scale. *Nature Communications, 14*, Article 4781. doi:10.1038/s41467-023-40498-1

Bonucci, M., Shu, T., & Holt, L. J. (2023). How it feels in a cell. *Trends in Cell Biology*, *33*(11), 924–938. doi:10.1016/j.tcb.2023.05.002

Chen, Y., & Nielsen, J. (2019). Energy metabolism controls phenotypes by protein efficiency and allocation. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(35), 17592–17597. doi:10.1073/pnas.1906569116

Chen, Y., & Nielsen, J. (2021). In vitro turnover numbers do not reflect in vivo activities of yeast enzymes. *Proceedings of the National Academy of Sciences of the United States of America, 118*(32). doi:10.1073/pnas.2108391118

Chen, Y., Gustafsson, J., Tafur Rangel, A., Anton, M., Domenzain, I., Kittikunapong, C., Li, F., Yuan, L., Nielsen, J., & Kerkhoven, E. J. (2024).

Delarue, M., Brittingham, G. P., Pfeffer, S., Surovtsev, I. V., Pinglay, S., Kennedy, K. J., Schaffer, M., Gutierrez, J. I., Sang, D., & Poterewicz, G., et al. (2018). mTORC1 controls phase separation and the biophysical properties of the cytoplasm by tuning crowding. *Cell, 174*(2), 338–349.e20. doi:10.1016/j.cell.2018.05.042

Dinh, H. V., & Maranas, C. D. (2023). Evaluating proteome allocation of *Saccharomyces cerevisiae* phenotypes with resource balance analysis. *Metabolic Engineering, 77*, 242–255. doi:10.1016/j.ymben.2023.04.009

Domenzain, I., Sánchez, B., Anton, M., Kerkhoven, E. J., Millán-Oropeza, A., Henry, C., Siewers, V., Morrissey, J. P., Sonnenschein, N., & Nielsen, J. (2022). Reconstruction of a catalogue of genome-scale metabolic models with enzymatic constraints using GECKO 2.0. *Nature Communications, 13*, Article 3766. doi:10.1038/s41467-022-31421-1

Dourado, H., Mori, M., Hwa, T., & Lercher, M. J. (2021). On the optimality of the enzyme-substrate relationship in bacteria. *PLOS Biology, 19*(10). doi:10.1371/journal.pbio.3001416

Elsemman, I. E., Rodriguez Prado, A., Grigaitis, P., Garcia Albornoz, M., Harman, V., Holman, S. W., van Heerden, J., Bruggeman, F. J., Bisschops, M. M. M., Sonnenschein, N., et al. (2022). Whole-cell modeling in yeast predicts compartment-specific proteome constraints that drive metabolic strategies. *Nature Communications, 13*, Article 801. doi:10.1038/s41467-022-28467-6

Euler, C., & Mahadevan, R. (2022). On the design principles of metabolic flux sensing. *Biophysical Journal, 121*(2), 237–247. doi:10.1016/j.bpj.2021.12.022

Heckmann, D., Campeau, A., Lloyd, C. J., Phaneuf, P. V., Hefner, Y., Carrillo-Terrazas, M., Feist, A. M., Gonzalez, D. J., & Palsson, B. O. (2020). Kinetic profiling of metabolic specialists demonstrates stability and consistency of in vivo enzyme turnover numbers. *Proceedings of the National Academy of Sciences of the United States of America, 117*(37), 23182–23190. doi:10.1073/pnas.2001562117

Heckmann, D., Lloyd, C. J., Mih, N., Ha, Y., Zielinski, D. C., Haiman, Z. B., Desouki, A. A., Lercher, M. J., & Palsson, B. O. (2018). Machine learning applied to enzyme turnover numbers reveals protein structural correlates and improves metabolic models. *Nature Communications, 9*, Article 5252. doi:10.1038/s41467-018-07652-6

Holt, L. J., & Delarue, M. (2023). Macromolecular crowding: Sensing without a sensor. *Current Opinion in Cell Biology, 85*, Article 102269. doi:10.1016/j.ceb.2023.102269

Kerkhoven, E. J. (2022). Advances in constraint-based models: Methods for improved predictive power based on resource allocation constraints. *Current Opinion in Microbiology, 68*, Article 102168. doi:10.1016/j.mib.2022.102168

Kochanowski, K., Okano, H., Patsalo, V., Williamson, J., Sauer, U., & Hwa, T. (2021). Global coordination of metabolic pathways in *Escherichia coli* by active and passive regulation. *Molecular Systems Biology, 17*(4). doi:10.15252/msb.202010064

Kroll, A., Rousset, Y., Hu, X. P., Liebrand, N. A., & Lercher, M. J. (2023). Turnover number predictions for kinetically uncharacterized enzymes using machine and deep learning. *Nature Communications, 14*, Article 4139. doi:10.1038/s41467-023-39840-4

Li, F., Yuan, L., Lu, H., Li, G., Chen, Y., Engqvist, M. K. M., Kerkhoven, E. J., & Nielsen, J. (2022). Deep learning-based kcat prediction enables improved enzyme-constrained model reconstruction. *Nature Catalysis, 5*(8), 662–672. doi:10.1038/s41929-022-00798-z

Mori, M., Cheng, C., Taylor, B. R., Okano, H., & Hwa, T. (2023). Functional decomposition of metabolism allows a system-level quantification of fluxes and protein allocation towards specific metabolic functions. *Nature Communications, 14*, Article 4161. doi:10.1038/s41467-023-39724-7

Nguyen, V., Xue, P., Li, Y., Zhao, H., & Lu, T. (2023). Controlling circuitry underlies the growth optimization of *Saccharomyces cerevisiae*. *Metabolic Engineering, 80*, 173–183. doi:10.1016/j.ymben.2023.09.013

Pareek, V., Sha, Z., He, J., Wingreen, N. S., & Benkovic, S. J. (2021). Metabolic channeling: Predictions, deductions, and

evidence. *Molecular Cell*, *81*(18), 3775–3785. doi:10.1016/j.molcel.2021.08.030

Poolman, B. (2023). Physicochemical homeostasis in bacteria. *FEMS Microbiology Reviews*, *47*(4). doi:10.1093/femsre/fuad033

Sánchez, B. J., Zhang, C., Nilsson, A., Lahtvee, P. J., Kerkhoven, E. J., & Nielsen, J. (2017). Improving the phenotype predictions of a yeast genome-scale metabolic model by incorporating enzymatic constraints. *Molecular Systems Biology*, *13*(8), Article 935. doi:10.15252/msb.20167411

Scott, M., & Hwa, T. (2023). Shaping bacterial gene expression by physiological and proteome allocation constraints. *Nature Reviews Microbiology*, *21*(5), 327–342. doi:10.1038/s41579-022-00818-6

Speer, S. L., Stewart, C. J., Sapir, L., Harries, D., & Pielak, G. J. (2022). Macromolecular crowding is more than hard-core repulsions. *Annual Review of Biophysics*, *51*, 267–300. doi:10.1146/annurev-biophys-091321-071829

Sweetlove, L. J., & Fernie, A. R. (2018). The role of dynamic enzyme assemblies and substrate channelling in metabolic regulation. *Nature Communications*, *9*, Article 2136. doi:10.1038/s41467-018-04543-8

Wendering, P., Arend, M., Razaghi-Moghadam, Z., & Nikoloski, Z. (2023). Data integration across conditions improves turnover number estimates and metabolic predictions. *Nature Communications*, *14*, Article 1485. doi:10.1038/s41467-023-37151-2

Xia, J., Sánchez, B. J., Chen, Y., Campbell, K., Kasvandik, S., & Nielsen, J. (2022). Proteome allocations change linearly with the specific growth rate of *Saccharomyces cerevisiae* under glucose limitation. *Nature Communications*, *13*, Article 2819. doi:10.1038/s41467-022-30513-2

Xu, S., Wang, J., & Dong, J. (2023). Nonspecific interaction and overlap concentration influence macromolecular crowding effect on glucose oxidase activity. *International Journal of Biological Macromolecules*, *241*, Article 124525. doi:10.1016/j.ijbiomac.2023.124525

Yuan, H., Bai, Y., Li, X., & Fu, X. (2024). Cross-regulation between proteome reallocation and metabolic flux redistribution governs bacterial growth transition kinetics. *Metabolic Engineering*, *82*, 60–68. doi:10.1016/j.ymben.2024.01.008

Zhang, Y., & Fernie, A. R. (2020). Metabolons, enzyme-enzyme assemblies that mediate substrate channeling, and their roles in plant metabolism. *Plant Communications*, *2*(1), Article 100081. doi:10.1016/j.xplc.2020.100081