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Designing Therapeutic Enzymes across Competing Biological Constraints: A Conceptual Framework Integrating Catalytic Performance, Glycosylation, Tissue Uptake, and Immune Recognition in Protein Engineering

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Abstract

Therapeutic enzymes are often engineered through molecular objectives that can be measured efficiently in vitro, including catalytic activity, substrate selectivity, folding stability, and expression yield. Yet therapeutic performance emerges only after these molecular properties interact with glycosylation, systemic persistence, receptor-mediated distribution, tissue access, intracellular trafficking, and host immune recognition. Optimizing a single measurable attribute can therefore improve one component of performance while leaving another unchanged or even impaired. This article develops a non-empirical conceptual account of therapeutic-enzyme design as a multicriteria biological problem in which intrinsic catalytic competence is necessary but insufficient for therapeutic quality. The analysis distinguishes five linked but non-equivalent levels: catalytic and conformational performance; glycosylation-dependent stability and distribution; tissue and cellular uptake; intracellular functional availability; and immune compatibility during repeated exposure. Evidence from enzyme engineering, glycoprotein design, lysosomal-enzyme trafficking, protein delivery, and enzyme-replacement therapy is used to identify where these dimensions reinforce or constrain one another. Particular attention is given to situations in which assay-defined improvements fail to predict physiological performance because of receptor biology, organ-specific uptake, intracellular localization, or immunogenicity. The resulting account does not prescribe a universal optimal enzyme architecture. Instead, it frames therapeutic engineering as the identification of context-dependent combinations of molecular and biological properties whose adequacy must be judged at the scale at which the therapy is intended to function. This perspective provides a basis for evaluating therapeutic-enzyme candidates without reducing their quality to catalytic performance alone.

Keywords: Therapeutic enzymes, Protein engineering, Glycosylation, Tissue uptake, Intracellular delivery, Immunogenicity

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Introduction

Enzymes occupy an unusual position among biological therapeutics because their intended clinical effect depends on a chemically defined function that can often be measured with exceptional precision outside the organism. Catalytic turnover, substrate affinity, specificity, temperature dependence, and conformational stability can therefore become attractive optimization targets during early engineering. Therapeutic-enzyme development, however, imposes additional requirements that do not arise from catalysis alone. Engineered enzymes may need prolonged circulation, resistance to extracellular degradation, delivery to a restricted tissue compartment, receptor-mediated internalization, localization to an organelle, compatibility with repeated dosing, or reduced recognition by the host immune system. Contemporary therapeutic-enzyme engineering consequently extends well beyond modification of the active site or improvement of catalytic output (Michailidou, 2023).

Delivery technologies illustrate why this distinction matters. Encapsulation, conjugation, nanomaterial-assisted transport, and molecular targeting can alter where an enzyme travels, how long it remains accessible, and whether it reaches a biologically relevant compartment (Torres-Herrero *et al.*, 2024). These interventions can improve exposure without altering the catalytic mechanism, while an enzyme with outstanding catalytic properties may remain therapeutically ineffective if it is rapidly cleared or fails to reach the target tissue. A clinically relevant design therefore represents the interaction of molecular function with a sequence of biological filters rather than the preservation of one favorable biochemical property.

The same problem is visible in strategies developed to extend the half-life of therapeutic proteins. PEGylation, protein fusion, glycoengineering, lipidation, and related approaches may increase persistence or alter biodistribution, but the resulting molecule can also acquire changes in receptor interaction, tissue penetration,

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manufacturability, or immunogenicity (Chen *et al.*, 2026). The direction of these effects is not uniform across therapeutic classes. A modification that is favorable for a circulating replacement protein may be counterproductive for a lysosomal enzyme that depends on receptor-mediated cellular uptake. Likewise, an intervention that improves plasma exposure can reduce the fraction of administered protein that reaches a required intracellular compartment.

Biodistribution is therefore not a secondary consequence of potency but an independent component of therapeutic behavior. Protein size, charge, receptor binding, vascular permeability, target-mediated disposition, Fc-related interactions where relevant, and tissue-specific clearance mechanisms can all reshape the relationship between administered dose and local exposure (Conner *et al.*, 2020). For therapeutic enzymes, this distinction is particularly important because catalytic activity is exerted only where enzyme and substrate physically coincide under conditions that preserve the relevant conformation and molecular state.

This article examines therapeutic-enzyme design through five connected constraints: catalytic performance, molecular stability, glycosylation-dependent distribution, tissue and intracellular access, and immune recognition. The central proposition is conditional rather than universal: catalytic performance remains indispensable, but it becomes therapeutically meaningful only when the enzyme persists, distributes, enters the relevant biological compartment, and retains sufficient functional availability without provoking immune responses that substantially alter subsequent exposure or activity. The following sections first separate catalytic competence from therapeutic quality, then examine how glycosylation reshapes stability and distribution before later sections address cellular uptake, immune recognition, and their integration into a multicriteria design account.

Catalytic Performance as an Incomplete Measure of Therapeutic Enzyme Quality

Catalytic performance provides an intuitive starting point for enzyme engineering because it can often be quantified under controlled conditions. Yet a catalytic assay isolates only part of the phenotype of a protein molecule. A mutation may alter turnover while simultaneously affecting folding efficiency, conformational stability, expression, aggregation, substrate discrimination, or sensitivity to the surrounding physicochemical environment. High-throughput mapping of D-amino-acid oxidase variants has demonstrated that catalytic activity and folding stability can be evaluated as related but separable properties across thousands of missense substitutions (Vanella *et al.*, 2024). The relevance of this observation to therapeutic design lies in the structure of the problem rather than in direct quantitative transfer from that enzyme: a candidate selected only for improved activity can occupy an unfavorable region of the broader protein-fitness landscape.

The stability-function relationship is especially important because catalytic optimization frequently depends on structural changes that increase conformational flexibility or alter interaction networks around the active site. Such changes can improve reaction-related behavior while reducing stability under another condition. Conversely, mutations introduced to stabilize a protein can constrain conformational transitions that are important for function. Protein-engineering research therefore treats stability and function as interacting objectives whose relationship can range from antagonistic to mutually compatible depending on the protein and the structural location of the change (Teufl *et al.*, 2022). The practical implication for therapeutic enzymes is that neither stability nor catalytic activity should be treated as an unconditional surrogate for the other.

This does not mean that therapeutic-enzyme engineering requires maximizing every molecular property simultaneously. Some loss of intrinsic activity may be acceptable if the resulting molecule survives substantially longer in the relevant environment or reaches a greater fraction of its intended substrate pool. In another therapeutic context, high catalytic efficiency may be indispensable because the accessible substrate concentration is low or because the administered enzyme reaches only a small fraction of the target compartment. The appropriate molecular state therefore depends on the biological environment in which catalysis must occur.

The engineering process itself also affects which combinations of properties are discovered. Directed-evolution studies have shown that enriching productive mutational trajectories can accelerate movement through sequence space by reducing the burden of variants that are nonfunctional or otherwise unfavorable (Patsch *et al.*, 2024). Such work highlights a methodological issue for therapeutic-enzyme development: a screening system defines what counts as an improved variant. If the screen measures only reaction velocity under permissive conditions, variants with desirable catalytic behavior but unfavorable stability, expression, or environmental sensitivity may be preferentially retained. A more informative design strategy therefore requires parallel or sequential measurements that capture the dimensions most likely to constrain the intended therapeutic context.

Specificity creates another layer of complexity. Protease engineering provides a clear example because a useful therapeutic or biotechnological protease must often achieve not simply high catalytic activity but activity toward a defined substrate set while avoiding undesired cleavage events. Modern protease-engineering strategies consequently integrate directed evolution, display systems, high-throughput screening, and structure-guided methods to evaluate specificity alongside activity (Martinusen *et al.*, 2025). Although proteases represent only one class of therapeutic enzyme, the general lesson is broader: the relevant objective is the pattern of function in context, not maximal catalytic output in isolation.

The resulting distinction can be stated without assigning a universal ranking to the underlying properties. Catalytic activity asks whether the enzyme can perform the required chemical transformation. Stability asks whether a sufficiently functional molecular population persists under the conditions encountered before and during action. Specificity asks whether catalytic activity is restricted appropriately. Expression and folding affect how reliably the desired protein state can be produced. Each property can influence therapeutic usefulness, but none establishes it independently.

These distinctions support a comparison of the major molecular constraints that precede tissue-level considerations. **Table 1** organizes the molecular design states by the type of measurement that defines them and by the biological information that remains unresolved after each measurement.

Table 1. Molecular performance states that constrain interpretation of therapeutic-enzyme quality

Engineering state	Primary measurement focus	Biological property directly interrogated	Therapeutically relevant question still unresolved	Typical failure if treated as sufficient	Discriminating next-level assessment
High apparent catalytic activity	Turnover, reaction rate, substrate conversion	Ability to catalyze the target reaction under assay conditions	Does the active molecular population persist under physiological exposure?	Selection of a highly active but unstable or rapidly inactivated variant	Stability and retained activity under relevant physicochemical conditions
Increased conformational stability	Folding, thermal or chemical stability, resistance to unfolding	Persistence of a folded molecular state	Is the stabilized conformation still catalytically and biologically competent?	Stabilization that restricts functional conformational dynamics	Joint activity–stability characterization
Altered substrate specificity	Desired versus off-target substrate conversion	Selective catalytic recognition	Does selectivity remain appropriate in the cellular substrate environment?	Unanticipated activity toward biologically accessible off-target substrates	Context-specific substrate and cellular-function testing
Improved expression or folding yield	Soluble production, folding efficiency, recoverable protein	Manufacturability of the intended molecular state	Does the produced state possess the required post-translational and distribution properties?	High-yield production of a biologically suboptimal form	Product-state, glycoform and functional characterization
Engineered multi-property variant	Parallel measurement of activity, stability and specificity	Compatibility of several intrinsic objectives	Does the molecular improvement survive systemic, tissue and cellular constraints?	Treating an optimized molecular phenotype as equivalent to therapeutic optimization	Biodistribution, uptake and functional-availability assessment

Table 1 emphasizes that each molecular measurement resolves one part of the design problem while leaving downstream biological questions open. A joint activity–stability phenotype is therefore more informative than a catalytic measurement alone, but even this expanded molecular description does not establish where the enzyme will distribute or whether it will enter the cells in which the target substrate is located. Those questions become especially sensitive to glycosylation.

Glycosylation, Protein Stability, and Biological Distribution

Glycosylation expands therapeutic-enzyme design beyond amino-acid sequence because a nominally identical polypeptide can exist as multiple molecular species that differ in glycan composition, occupancy, branching, terminal residues, or site-specific combinations. Advanced analysis of glycosylated biotherapeutics has made this heterogeneity increasingly visible at the intact-protein level, demonstrating that therapeutic glycoproteins cannot always be represented adequately as a single chemically uniform entity (Schachner *et al.*, 2024). Analytical resolution alone does not establish which glycoform is clinically preferable, but it changes the object being optimized: "the enzyme" may in practice be a distribution of molecular states.

Glycans can affect folding, stability, solubility, receptor interaction, biological activity, safety, and pharmacokinetic behavior (Rocamora *et al.*, 2023). Their importance is therefore not restricted to product characterization. A glycan pattern can influence how long a therapeutic molecule remains in circulation and which cells remove it from plasma. For an enzyme intended to act intracellularly, those same interactions may determine whether clearance represents therapeutic delivery or nonproductive loss. The functional meaning of a glycosylation change thus depends on the tissue and receptor system through which the enzyme must pass.

Production platform sets an upstream boundary on this design space. Bacterial and cell-free glycoengineering systems increasingly permit construction of glycoproteins with defined or customized glycan structures, expanding the range of biopharmaceutical states that can be generated deliberately (Palma *et al.*, 2024). At the same time, bacterial glycoprotein engineering remains constrained by pathway complexity, control of glycan assembly, host compatibility, scalability, and the requirement to produce glycan structures appropriate to the intended biological use (Li *et al.*, 2025). These constraints make glycoengineering a manufacturing problem and a biological-design problem simultaneously.

Plant-based expression systems provide a different route to recombinant glycoproteins and have been engineered to generate increasingly mammalian-like N-glycan patterns (Kao *et al.*, 2024). Their relevance to therapeutic-enzyme design does not imply that one host is intrinsically superior. Rather, each host determines a characteristic set of accessible post-translational states and process constraints. The appropriate production platform is therefore conditional on which glycan features are necessary for stability, receptor recognition, circulation, or downstream processing of the specific enzyme.

Glycosylation can also alter immune interactions. Glycan structures participate in recognition pathways that influence immune-cell behavior, and glycoengineering is increasingly being used deliberately in antigen-specific and other immunotherapeutic strategies (Li *et al.*, 2024). Evidence from those systems cannot be transferred quantitatively to enzyme-replacement therapy, but it establishes an important design principle: glycosylation can participate in immune recognition as well as pharmacokinetics. Chemical neoglycosylation similarly demonstrates that addition or modification of carbohydrate structures can alter properties such as solubility, stability, and circulatory persistence, although effects depend on the glycan, conjugation chemistry, site of modification, and underlying protein (Walther & Zelikin, 2021).

Lysosomal replacement enzymes provide one of the clearest demonstrations that glycan design can create competing biological outcomes. In engineered α-galactosidase A, manipulation of N-glycans altered the balance among receptor-dependent uptake, circulation, and organ distribution in a Fabry-disease model (Tian *et al.*, 2019). The critical point is not that one glycoform defines a universally optimal solution. Increased recognition by mannose-6-phosphate or related uptake pathways can be beneficial when receptor-mediated internalization into target cells is limiting, yet rapid uptake by tissues that are not the principal therapeutic target can reduce circulating availability for other organs. Conversely, glycan structures that prolong systemic persistence may weaken uptake through receptors required for lysosomal delivery.

This trade-off also depends on the cellular machinery that generates mannose-6-phosphate recognition signals. TMEM251, also described as GCAF, has been shown to regulate lysosome biogenesis through the mannose-6-phosphate pathway, with loss of the protein disrupting modification and trafficking of lysosomal enzymes (Zhang *et al.*, 2022). Such findings reinforce the distinction between producing an enzymatically active polypeptide and producing a molecular state that carries the post-translational information required for appropriate intracellular targeting.

More recent mechanistic work has further clarified how LYSET regulates GNPTαβ, the phosphotransferase complex responsible for initiating mannose-6-phosphate tagging of lysosomal enzymes. LYSET contributes to the stability and Golgi retention of this machinery, linking glycan-processing fidelity to the cellular systems that determine lysosomal-enzyme sorting (Yang *et al.*, 2026). These mechanisms arise from cell biology rather than therapeutic outcome studies, so they do not by themselves establish the clinical performance of any engineered replacement enzyme. They do, however, identify points at which production-cell biology can alter the molecular signals later interpreted by therapeutic target cells.

The resulting design problem is therefore relational. A glycosylation strategy cannot be judged solely by whether it increases molecular stability, extends circulation, strengthens receptor binding, or resembles a preferred analytical profile. Its value depends on how those effects combine in the intended disease context. A lysosomal enzyme may benefit from high-affinity uptake into one tissue while suffering premature clearance in another. A glycan pattern that increases systemic exposure may improve access to poorly perfused or difficult-to-reach compartments while decreasing entry through a receptor on which intracellular trafficking depends. The same design variable can therefore carry opposite consequences at different biological scales.

This relationship is summarized in **Table 2** by treating glycosylation strategies as changes in receptor and disposition behavior rather than as a simple progression toward a single preferred glycoform.

Table 2. Functional consequences that must be evaluated when glycosylation is engineered in therapeutic enzymes

Glycosylation-related design state	Principal molecular change	Receptor or recognition process potentially affected	Possible effect on stability or circulation	Possible distribution consequence	Critical measurement needed before therapeutic interpretation
Increased lysosomal-targeting glycan content	Greater abundance or accessibility of uptake-relevant carbohydrate signals	Mannose-6-phosphate and related cellular uptake pathways	May increase receptor-mediated removal from circulation	Greater uptake in receptor-rich tissues but potentially reduced availability elsewhere	Parallel circulation, organ-distribution and intracellular-localization measurements
Increased terminal sialylation	Altered terminal glycan composition	Reduced recognition by selected clearance pathways	Potentially longer systemic persistence	Greater opportunity for distribution beyond rapidly clearing tissues	Tissue-resolved exposure together with retained cellular uptake

High-mannose or other clearance-prone glycoforms	Greater presentation of mannose-rich structures	Mannose-sensitive uptake/clearance mechanisms	Potentially shorter circulating persistence	Preferential uptake into tissues expressing relevant receptors	Receptor-specific uptake and organ-level biodistribution
Chemically introduced glycans	Added carbohydrate moieties at engineered or accessible sites	Depends on glycan identity, attachment site and presentation	Can alter solubility, stability and residence time	May reshape exposure without guaranteeing productive uptake	Site-specific structural characterization plus functional uptake testing
Production-host-specific glycoform distribution	Host-dependent glycan processing	Multiple lectin and receptor systems	Variable product stability and clearance behavior	Host-derived glycan differences may redirect disposition	Intact and site-specific glycoform analysis linked to biological assays
Engineered mannose-6-phosphate biosynthetic machinery	Modified generation or preservation of lysosomal-targeting signals	Lysosomal-enzyme sorting and uptake pathways	Indirect effect through product-state composition	Alters capacity for productive lysosomal targeting after administration	Product glycan characterization, receptor uptake and lysosomal-delivery assessment

The central implication is that glycosylation should be interpreted as a control over several coupled biological processes rather than as a secondary decoration of an already optimized enzyme. The therapeutic relevance of a glycan state emerges through the interaction of product stability, circulation, receptor recognition, organ distribution, cellular internalization, and subsequent trafficking. This prepares the next analytical step: even a well-distributed enzyme remains therapeutically incomplete if exposure does not become productive uptake and functional availability within the required cellular compartment.

Tissue Uptake, Intracellular Delivery, and Functional Availability

Molecular optimization becomes therapeutically relevant only when sufficient active enzyme reaches the anatomical and intracellular site at which substrate processing is required. Long-term Pompe-disease experience with avalglucosidase alfa illustrates this distinction at clinical scale: sustained treatment outcomes must be judged in patients rather than inferred from biochemical activity alone (Kishnani *et al.*, 2025). The observation does not assign benefit to a single molecular feature, but it confirms that therapeutic performance emerges from exposure, uptake, intracellular delivery, disease state, and time.

Tissue barriers can dominate this chain. Receptor-targeted transport vehicles have enabled lysosomal-enzyme delivery across the blood–brain barrier in mice (Ullman *et al.*, 2020), while related Fc-based transport systems have demonstrated brain delivery in mice and nonhuman primates (Kariolis *et al.*, 2020). Pabinafusp alfa extends this principle into human MPS-II by coupling iduronate-2-sulfatase to transferrin-receptor-mediated transport; reductions in cerebrospinal-fluid heparan sulfate show that engineered access can alter CNS exposure, although the single-arm design prevents a universal claim about neurocognitive benefit (Okuyama *et al.*, 2021).

Access to tissue is still not equivalent to productive intracellular availability. Protein therapeutics may require endocytosis, endosomal escape, organelle targeting, or preservation of activity after internalization (Su *et al.*, 2024). Polypeptide conjugation can modify solubility, persistence, and delivery, but these gains remain cargo- and chemistry-dependent (Zhang *et al.*, 2025). Lipid-nanoparticle delivery of superoxide dismutase and catalase in a sepsis model further demonstrates that intracellular enzyme delivery is technically achievable, without establishing that the same strategy is suitable for replacement enzymes in other diseases (Koide *et al.*, 2025). Albumin-based systems (Murphy *et al.*, 2025) and newer route-specific biologic-delivery approaches (Dela Ahator *et al.*, 2026) reinforce the same principle: exposure must be interpreted through the barrier that limits the intended therapy.

A candidate enzyme should therefore be evaluated across successive but non-interchangeable states—circulating exposure, tissue access, cell entry, correct subcellular localization, and retained catalytic function. **Table 3** expresses these states as conditional trade-offs rather than as a single optimization score.

Table 3. Cross-scale trade-offs that determine productive therapeutic-enzyme availability

Design change	Potential gain	Competing loss or uncertainty	Measurement needed to discriminate the outcome
Higher intrinsic catalytic activity	Greater reaction capacity per active molecule	No benefit if exposure or uptake remains limiting	Activity under relevant conditions plus tissue/cellular exposure
Longer systemic persistence	Greater opportunity for tissue distribution	Reduced receptor-mediated uptake or altered tissue partitioning	Pharmacokinetics together with organ- and cell-level uptake
Stronger uptake-receptor engagement	Increased internalization into receptor-rich cells	Faster systemic clearance or sequestration in non-target tissues	Receptor-specific uptake plus whole-body biodistribution

Carrier or conjugate addition	Improved stability, solubility, or barrier transport	Steric interference, altered trafficking, or new immune liabilities	Retained activity, trafficking, exposure, and immune characterization
Enhanced intracellular delivery	Greater enzyme concentration in target cells	Incorrect organelle localization or post-entry inactivation	Subcellular localization plus functional substrate correction

Immune Recognition and the Competing Constraints of Therapeutic Optimization

Repeated administration introduces a constraint that cannot be inferred from catalytic performance or initial biodistribution: the host can change in response to the therapeutic protein. Immune-tolerance strategies used with enzyme-replacement therapies show that anti-drug responses depend on disease, treatment history, immune status, and timing (İnci *et al.*, 2024). Immunogenicity is therefore a dynamic property of the drug–host system rather than a fixed attribute of the enzyme sequence alone.

Clinical evidence also cautions against treating antibody reduction as a sufficient therapeutic endpoint. In a prospective Pompe-disease cohort, immune-tolerance induction substantially reduced anti-rhGAA antibody burden, yet motor and respiratory recovery was not consistently restored (Chen *et al.*, 2024). Advanced disease, treatment timing, and irreversible tissue injury can therefore remain limiting even when one immune constraint improves. Fabry-disease literature similarly distinguishes transient or clinically minor antibodies from neutralizing responses capable of reducing effective enzyme exposure (Gómez-Cerezo *et al.*, 2025). Mechanistic work on neutralizing antibodies further shows why antibody presence, neutralizing capacity, pharmacokinetic consequence, and clinical outcome should not be collapsed into one category (Lenders & Brand, 2021).

Pegunigalsidase alfa provides a useful boundary case because half-life extension, dosing interval, exposure, and immunogenicity are evaluated together. In the BRIGHT study, no de novo anti-drug antibodies were observed during the reported treatment period, but the small open-label cohort does not establish absence of future immunogenicity or superiority over other designs (Holida *et al.*, 2025). Clinical recommendations for late-onset Pompe disease likewise use longitudinal functional, respiratory, tolerability, and treatment-response criteria rather than a single biochemical variable when deciding whether to start, switch, or stop enzyme replacement (Schoser *et al.*, 2024). BRIDGE provides complementary switch evidence for pegunigalsidase alfa while retaining the limitations of a small, open-label study (Linhart *et al.*, 2023).

These observations make immune compatibility part of therapeutic engineering rather than an after-the-fact safety screen. **Table 4** therefore defines decision criteria according to the biological question each criterion resolves, without imposing universal thresholds.

Table 4. Decision criteria for evaluating therapeutic-enzyme designs across interacting biological constraints

Evaluation criterion	Question resolved	Evidence that would support advancement	Evidence that would require qualification or redesign
Catalytic competence	Can the enzyme perform the required chemistry?	Retained target reaction under relevant conditions	Activity limited to permissive assay conditions
Molecular persistence	Does a functional protein state survive long enough to act?	Stability compatible with intended exposure	Rapid unfolding, aggregation, or activity loss
Distribution adequacy	Does systemic exposure reach the required tissue?	Target-tissue exposure without dominant non-target sequestration	High plasma exposure with poor target access
Productive cellular delivery	Does tissue exposure become functional intracellular availability?	Cell entry and correct compartment localization with retained function	Uptake without correct trafficking or activity
Immune compatibility	Can repeated exposure preserve effective treatment?	Manageable immune response without major loss of effective exposure	Neutralizing response, accelerated clearance, or intolerance
Cross-domain balance	Are gains in one dimension preserved across the others?	Improvement without a clinically material countervailing loss	Better single-axis performance accompanied by failure elsewhere

A Multicriteria Framework for Therapeutic Enzyme Engineering

The evidence across Sections 2–5 supports a multicriteria account in which therapeutic quality reflects the compatibility of several biological states rather than one maximized molecular property. Activity–stability mapping shows why intrinsic function must be evaluated jointly (Vanella *et al.*, 2024); glycoengineering demonstrates that molecular state can redirect circulation and receptor-dependent uptake (Tian *et al.*, 2019); transport-vehicle studies show that tissue access may require a separate engineering solution (Ullman *et al.*, 2020); and immune-tolerance evidence shows that the host response can change effective exposure after treatment has begun (Chen *et al.*, 2024). These dimensions interact,

but they are measured at different biological scales and should remain analytically distinct.

The central design question is consequently whether a proposed modification improves the constraint actually limiting therapeutic function in the intended indication. A glycan alteration is favorable only when its effects on stability, circulation, receptor uptake, and intracellular delivery are jointly acceptable (Rocamora *et al.*, 2023). Longer persistence is useful only when the enzyme remains capable of reaching and functioning in the target compartment (Dela Ahator *et al.*, 2026). Likewise, reducing anti-drug antibodies does not establish therapeutic recovery when irreversible disease burden or inadequate cellular delivery remains limiting (Chen *et al.*, 2024). The objective is not to assign fixed weights to these dimensions, but to determine which constraint dominates under defined conditions and which measurements can discriminate a genuine improvement from a redistribution of failure.

This reasoning produces a nonlinear design landscape rather than a sequence in which success at one level guarantees success at the next. **Figure 1** represents the relationships while preserving the evidentiary distinction between molecular measurements, distributional observations, intracellular function, and patient-level consequences.

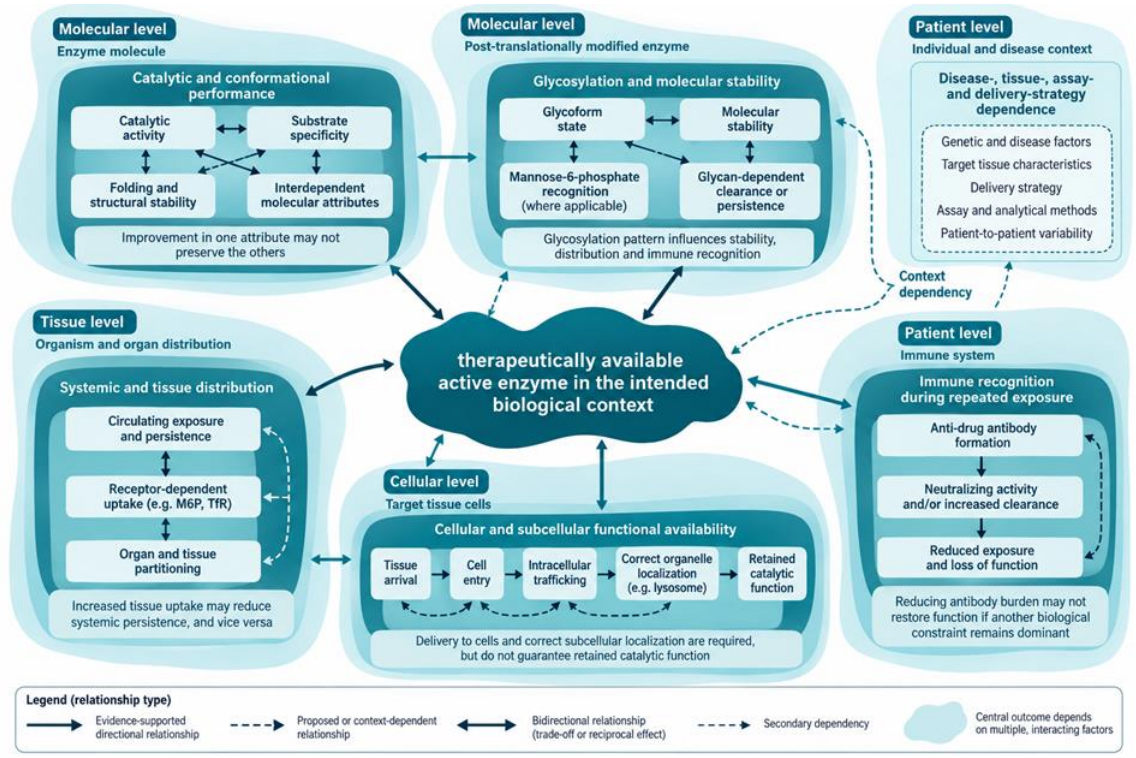


Figure 1. Where therapeutic enzyme performance is gained or lost across molecular, distributional, cellular, and immune constraints

Conclusion

Therapeutic-enzyme quality cannot be inferred from catalytic performance alone. A useful design must remain structurally competent, possess an appropriate glycosylation state, reach the required tissue, enter the relevant cellular compartment, preserve activity after delivery, and remain sufficiently compatible with repeated exposure. These properties can reinforce one another, but they can also generate trade-offs whose direction depends on the enzyme, disease, tissue, production system, and treatment context.

The resulting framework favors discriminating measurements over universal rankings. An engineered change is therapeutically meaningful when it improves the limiting biological constraint without creating a more consequential failure elsewhere. Catalytic performance therefore remains a necessary molecular foundation, but its therapeutic value is determined by the biological system in which catalysis must ultimately occur.

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