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Why Kinetic Pathways Matter More Than Endpoint Stability for Next-Generation in Silico Pharmacology

Daniel Fischer^{1*}, Laura Meier¹, Stefan Koch², Thomas Braun³

¹Department of Kinetic Pathways and In Silico Pharmacology, Faculty of Pharmacy, University of Freiburg, Freiburg, Germany.

²Department of Endpoint Stability and Drug Action, Faculty of Pharmacy, Technical University of Munich, Munich, Germany.

³Department of Next-Generation Drug Discovery, Faculty of Pharmacy, University of Kiel, Kiel, Germany.

*Email: daniel.fischer@pharmazie.uni-freiburg.de

ABSTRACT

Computational pharmacology has become increasingly effective at predicting bound poses, relative affinities, and equilibrium free-energy differences, yet these endpoint quantities describe only part of molecular recognition. A ligand can reach the same apparent bound state through different association routes, populate distinct encounter or metastable complexes, cross different rate-limiting barriers, and leave through dissociation pathways that are not simply the reverse of association. These features matter because pharmacological engagement is inherently temporal. Two compounds with similar equilibrium stability can therefore differ substantially in association rate, dissociation rate, residence time, sensitivity to conformational gating, and susceptibility to mutation-dependent changes in kinetic behavior. This Mechanistic Perspective argues that next-generation in silico pharmacology should treat kinetic pathways as an additional level of molecular description rather than as a secondary annotation to endpoint stability. The proposed pathway-centred view separates trajectory generation, pathway identification, barrier interpretation, rate estimation, and experimental validation so that mechanistic plausibility is not confused with quantitatively validated kinetics. Evidence from enhanced sampling, milestoning, weighted-ensemble methods, accelerated molecular dynamics, pathway-search approaches, and data-driven reaction-coordinate learning shows that route-resolved kinetic modeling is increasingly feasible, but predictive performance remains strongly system- and method-dependent. The key opportunity is therefore not to replace thermodynamic calculations, but to integrate equilibrium and kinetic representations while preserving uncertainty, competing mechanisms, and validation boundaries. Such integration could make molecular simulation more informative for kinetic selectivity, resistance analysis, and medicinal-chemistry optimization without overstating current prospective predictive capability.

Keywords: Drug-target residence time, Binding kinetics, Dissociation pathways, Conformational gating, Enhanced sampling, Molecular simulation

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INTRODUCTION

Drug discovery has traditionally privileged equilibrium descriptors because affinity, potency, and structural complementarity are experimentally accessible and computationally tractable. These quantities remain indispensable, but they do not fully describe how a drug reaches, occupies, and leaves its target. Pharmacological effect can depend on association and dissociation rates, target residence time, rebinding, target turnover, exposure history, and downstream persistence; consequently, kinetic parameters may add information that is not contained

in an equilibrium affinity value alone [1,2]. The relevant question is therefore not whether kinetics should replace thermodynamics, but when endpoint stability becomes an incomplete representation of the molecular process that determines temporal engagement.

Computationally, this distinction is demanding because equilibrium observables and kinetic observables require different kinds of sampling. A stable bound minimum may be well characterized even when the transition pathways connecting it to solvent remain effectively invisible on ordinary molecular-dynamics timescales [3]. Contemporary kinetic methods increasingly address this gap, but the field still lacks sufficiently broad standardized benchmarks, blind prospective comparisons, and consensus about which level of success constitutes useful validation [4]. This Perspective therefore advances a proposed pathway-centred inference architecture in which endpoint stability, pathway identity, barrier structure, rate estimation, and decision utility remain analytically separate. The central claim is deliberately conditional: when temporal engagement is mechanistically important, a pathway-resolved description can expose information that an endpoint description cannot, but pathway visualization alone does not establish quantitative or pharmacological validity.

Endpoint stability and its blind spots

Endpoint stability usually refers, explicitly or implicitly, to the energetic favorability of a ligand-associated state. In computational practice, it can be represented through a bound pose, docking score, relative or absolute binding free energy, or a structural ensemble concentrated around a thermodynamic basin. Such measures answer a meaningful question: how favorable is one state relative to another? They do not, by themselves, answer how rapidly that state is reached, how many intermediate states intervene, what barrier controls entry or exit, or whether the same apparent endpoint is kinetically accessible through different routes.

Experimental kinase profiling illustrates why this distinction matters. Across the drugged kinome, ligand-target interactions can display substantial variation in association and dissociation behavior, demonstrating that a target-ligand pair has a kinetic phenotype that is not reducible to a single equilibrium descriptor [5]. The implication is not that affinity becomes uninformative. Rather, two interactions can occupy comparable equilibrium territory while differing in how long the complex persists or how readily it reforms after dissociation.

The blind spot becomes more consequential when the target itself changes. Resistance-conferring substitutions can affect not only endpoint contacts but also transition routes, conformational accessibility, or the ease of escape from a binding pocket. Altered residence time has therefore been identified as one mechanism that can contribute to resistance in selected drug-target systems [6]. Conversely, pharmacological reviews emphasize that residence time is not automatically superior to potency or affinity; its importance depends on the biological context, including exposure, target turnover, receptor state, rebinding opportunities, and the temporal coupling between occupancy and downstream response [7].

The appropriate interpretation is therefore asymmetric. Endpoint stability is sufficient for some questions but incomplete for others. It is particularly vulnerable when medicinal-chemistry decisions depend on temporal selectivity, mutation-sensitive persistence, slow conformational rearrangement, or dissociation barriers that vary independently of the final bound-state energy. In those settings, a kinetic pathway is not decorative mechanistic detail. It becomes part of the state description required to explain why apparently similar endpoints behave differently over time.

Association pathways

Association is often idealized as a single transition from unbound ligand plus target to a bound complex. At molecular resolution, however, recognition can begin with diffusional encounter, orientational filtering, transient surface contacts, desolvation, pocket entry, side-chain rearrangement, and protein conformational change. Multiscale calculations that combine Brownian dynamics with molecular dynamics show that association-rate prediction can therefore be decomposed into a long-range encounter problem and a shorter-range molecular recognition problem [8]. This decomposition is conceptually important because a favorable final pose does not guarantee rapid access to that pose.

The protein conformational landscape can further determine which association mechanism dominates. Direct kinetic analysis of lactose binding to galectin-3 showed that ligand-dependent stabilization of a protein transition state could shift pathway flux from conformational selection toward induced fit [9]. The significance lies in the rate network rather than in terminology. Conformational selection and induced fit need not be mutually exclusive labels assigned after structural observation; they can represent competing kinetic routes whose relative flux changes when ligand interactions modify transition-state energetics.

Controlled synthetic systems reinforce the general mechanistic principle while also defining a transfer boundary. Engineered molecular switches can be constructed so that induced-fit and conformational-selection architectures generate different response kinetics [10]. These systems do not establish that pharmaceutical targets obey the same quantitative rules. They do show that identical or similar endpoint states need not imply equivalent temporal behavior when the order of binding and conformational rearrangement differs.

This Perspective therefore proposes pathway identity as a distinct level of molecular annotation. For association, pathway identity should describe the sequence and relative flux of encounter, conformational, and pocket-entry events rather than merely identify a starting and ending structure. Whether this annotation becomes useful for medicinal chemistry depends on reproducibility and experimental kinetic support, but the distinction itself prevents a common inferential collapse: treating accessibility to a stable endpoint as though it were encoded by endpoint stability.

Metastable encounter complexes

Between freely diffusing ligand and the final bound state lies a region of the energy landscape that is often sparsely characterized. Weighted-ensemble simulations and related path-sampling methods demonstrate that binding and unbinding can involve intermediate configurations whose occupancy and connectivity influence kinetic ranking even in comparatively simple host-guest systems [11]. These intermediates become especially relevant when they partition flux toward productive binding, delayed entry, recrossing, or escape.

Milestoning analyses of CDK8 ligand recognition provide a more pharmacologically relevant example. Transient states and barriers distinguished related inhibitors and were sufficiently informative to support a targeted chemical modification with experimentally evaluated kinetic consequences [12]. Such evidence suggests that an intermediate state can contain design information not obvious from inspection of the final crystallographic or modeled complex. A substituent may have little effect on endpoint geometry yet alter the probability of crossing a transient barrier or stabilizing an intermediate encountered during association or dissociation.

Peptide-GPCR simulations likewise reveal alternative receptor-associated locations and multiple binding or unbinding routes [13]. These observations should not be overinterpreted. A free-energy minimum is not automatically a kinetically important intermediate, and a transiently populated configuration is not necessarily on the dominant reactive pathway. Population, lifetime, connectivity, and flux all matter.

For that reason, this Perspective proposes a restrained functional distinction among encounter states, metastable pathway states, productive bound states, and escape-compatible states. These labels are analytical, not claims that every system contains four discrete classes. Their purpose is to prevent structural intermediates from being assigned mechanistic importance merely because they appear in trajectories. A metastable state becomes pharmacologically meaningful only when evidence links it to pathway choice, barrier formation, rate modulation, or experimentally discriminable kinetic behavior.

Binding barriers

If endpoint stability describes the depth of a basin, kinetics depends critically on the barriers separating basins. A deep bound state can dissociate rapidly if the relevant escape barrier is low, whereas a moderately favorable bound state can persist if leaving requires a rare conformational fluctuation or traversal of a high-energy route. Computational studies of PYK2 and p38 MAP kinase inhibitors illustrate how barrier topology and pathway geometry can therefore contribute information beyond endpoint energetics [14, 15].

The crucial qualification is that identifying a plausible barrier is not equivalent to predicting the correct rate. In the PYK2 study, closely related ligands followed different calculated dissociation routes, yet quantitative agreement with experimental dissociation rates varied markedly. Two calculated rates were within approximately two orders of magnitude of experiment, whereas another was at least three orders of magnitude too fast. This is a valuable negative result because it separates mechanistic interpretability from numerical validation. A simulation can reveal chemically sensible exit contacts and still misestimate the absolute timescale because of incomplete sampling, reaction-coordinate construction, force-field error, state decomposition, or kinetic-estimator assumptions.

Related metadynamics studies of CDK8 inhibitors provide a complementary level of success. Simulated dissociation behavior could distinguish short- and long-residence-time inhibitors within a congeneric series [16]. Relative discrimination is valuable for prioritization, but it answers a narrower question than accurate prediction of absolute k_{off} .

The resulting hierarchy is central to the proposed framework. Route plausibility, relative kinetic ranking, and absolute kinetic prediction are three distinct evidentiary claims. A method may succeed at the first, the first two, or all three. Collapsing them into a single statement that a simulation “predicts residence time” obscures both scientific progress and remaining uncertainty.

Dissociation routes

Dissociation is particularly informative because the bound state is known while the escape process may involve many rare alternatives. Data-driven analysis of enhanced-sampling trajectories has shown that ligand unbinding trajectories can be separated into distinct route families rather than forced into one canonical pathway [17]. Such classification changes the unit of interpretation. Instead of asking only for a single reaction coordinate, one can ask whether several dynamically distinct channels carry appreciable flux and whether those channels respond differently to chemical modification or target mutation.

Quantitative benchmarks show that route-resolved methods can succeed strongly under defined conditions. Multiscale milestoning combined with metadynamics reproduced experimentally anchored residence-time behavior for a series of eight long-residence-time TTK inhibitors, including useful absolute and rank-order performance [18]. This demonstrates that pathway-resolved kinetics can move beyond qualitative visualization. It does not establish comparable accuracy for unrelated targets, ligands, or residence-time regimes.

Mutation studies add another dimension. Accelerated molecular-dynamics analyses of protein-protein complexes show that mutations can alter relative off-rates while changing dissociation contacts and exit mechanisms [19]. Because these are protein-protein systems, they should not be treated as direct evidence for small-molecule drug dissociation. They nevertheless support an important mechanistic principle: perturbations can redirect the kinetic route itself rather than simply raise or lower the stability of an unchanged path.

At a different evidentiary level, pathway-search approaches can rapidly enumerate putative ligand-exit routes across multiple protein systems [20]. These tools are useful for hypothesis generation, especially when exhaustive rare-event simulation is impractical, but a geometrically or energetically plausible route is not yet a validated kinetic channel. Rate estimation, pathway probability, and experimental support must follow separately.

The distinction is consequential enough to make dissociation routes the central visual anchor of the article. Rather than positioning trajectories as direct evidence of mechanism, the proposed architecture inserts explicit inference and validation gates between simulated motion and medicinal-chemistry interpretation.

A pathway-centred analysis is therefore most informative when the reader can see where trajectory evidence ends and kinetic inference begins. **Figure 1** separates those levels so that route discovery, rate reconstruction, mechanistic interpretation, and validation cannot be mistaken for interchangeable outputs.

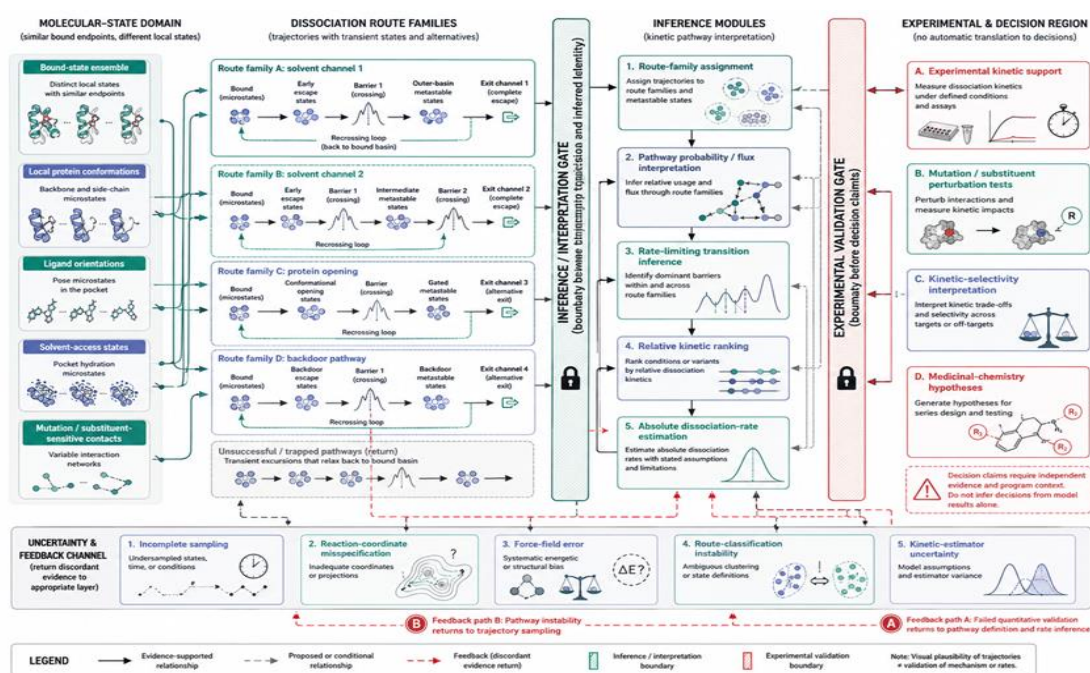


Figure 1. Dissociation-route ensembles as the missing layer between molecular trajectories and kinetic inference

Conformational gating

Kinetic pathways are shaped not only by ligand motion but also by the availability of protein states through time. Conformational gating occurs when access to, progression through, or escape from a binding pathway depends on a protein transition. The same concept can therefore operate at several stages. A pocket may open before productive association, a ligand may stabilize a transition that redirects association flux, or closure around a bound ligand may suppress dissociation. Experimental work on galectin-3 directly demonstrates that ligand-dependent transition-state stabilization can alter the balance between conformational-selection and induced-fit pathways [9]. Slow dissociation can also emerge when the protein reorganizes around the ligand after binding. The ligand-trapping concept emphasizes that conformational closure can raise the effective difficulty of escape even when the endpoint interaction energy alone does not explain a prolonged residence time [21]. This does not imply that every long-residence-time complex is trapped. Electrostatic interactions, desolvation, local steric barriers, multistep escape, and rebinding can produce similar kinetic outcomes without the same gating mechanism. GPCRs illustrate a further complication because the “bound state” can contain multiple receptor conformations, cooperative interactions, and state-dependent transitions. Mathematical modeling and molecular-dynamics analyses indicate that residence-time interpretation in such systems can depend on receptor-state architecture and cooperativity rather than on a simple binary ligand-bound versus ligand-unbound model [22]. Alternative GPCR-associated states observed in enhanced-sampling simulations provide compatible structural context for this multistate view, although those simulations do not by themselves establish the same kinetic mechanism in every receptor-ligand pair [13].

For the present Perspective, conformational gating is therefore treated as a proposed three-part analytical distinction: access gating, in which protein state availability controls entry to a productive pathway; transition gating, in which conformational rearrangement redistributes pathway flux after encounter; and escape gating, in which a post-binding conformational state alters the probability or barrier of dissociation. The classification is intended to organize evidence, not to claim three universal mechanisms.

The principal interpretive difficulty is that several evidence patterns can look similar at an endpoint. A more closed bound structure may indicate true kinetic trapping, but it may also be an equilibrium consequence with little influence on the rate-limiting transition. A simulated conformational change may appear necessary only because the chosen reaction coordinate or sampling method failed to discover an alternative path. Likewise, a long measured residence time may be consistent with gating without identifying which conformational transition controls the timescale.

These ambiguities are difficult to reconcile in prose because the same endpoint observation can support different mechanistic hypotheses depending on trajectory coverage, kinetic measurement, and perturbational evidence. **Table 1** therefore separates the observable pattern from the mechanistic inference it can and cannot justify.

Table 1. Reconciling conformational-gating evidence when similar bound endpoints imply different kinetic mechanisms

Evidence configuration		Interpretive alternatives	Adjudication requirement
Gating comparison anchor / Molecular or conformational scenario	Observable or model output / Sampling or data support / Mechanistic interpretation that is justified	Competing explanation viable	Consequence for the pathway-centred synthesis
Gating comparison anchor: Access-gated recognition Molecular or conformational scenario: Productive pocket state is available only intermittently before stable binding	Observable or model output: Association trajectories concentrate in a subset of protein conformations Sampling or data support required: Repeated entry events or independent kinetic evidence showing state-dependent accessibility Mechanistic interpretation that is justified: Protein-state availability contributes to the association timescale	Slow diffusion, desolvation, orientational restriction, or incomplete sampling	Endpoint pose cannot establish how frequently the productive state is kinetically reachable
Gating comparison anchor: Ligand-stabilized transition gating Molecular or conformational scenario: Ligand interactions change the relative flux	Observable or model output: Microscopic rates or trajectory populations shift between alternative association mechanisms Sampling or data support required: Time-resolved kinetic network or well-converged path sampling	Parallel pathways may coexist without one fully replacing the other	Pathway identity becomes a ligand-dependent property rather than a fixed target property

through conformational-selection and induced-fit routes	Mechanistic interpretation that is justified: Ligand chemistry alters pathway usage through conformational energetics		
Gating comparison anchor: Metastable-state redistribution Molecular or conformational scenario: Ligand occupies transient receptor-associated states before the dominant bound basin	Observable or model output: Recurrent intermediate states with measurable lifetime or pathway connectivity Sampling or data support required: Evidence that intermediate occupancy contributes appreciable reactive flux Mechanistic interpretation that is justified: An intermediate participates in recognition or escape	The state may be an off-pathway minimum or sampling artifact	Metastability matters only when linked to connectivity, flux, or kinetic consequence
Gating comparison anchor: Escape gating by structural closure Molecular or conformational scenario: Protein reorganizes after binding into a state less permissive to ligand exit	Observable or model output: Long residence time accompanied by recurrent closed conformations or delayed opening events Sampling or data support required: Dissociation trajectories plus perturbation or kinetic evidence linking opening to escape Mechanistic interpretation that is justified: Conformational closure contributes to slow dissociation	Strong local contacts, solvent rearrangement, rebinding, or a different hidden barrier	Bound-state stability and escape accessibility must be evaluated separately
Gating comparison anchor: State-dependent receptor kinetics Molecular or conformational scenario: Multiple receptor conformations or cooperative states remain kinetically populated during ligand engagement	Observable or model output: Residence behavior changes with receptor state, coupling condition, or conformational ensemble Sampling or data support required: State-resolved kinetics, validated modeling, or orthogonal structural evidence Mechanistic interpretation that is justified: Apparent residence time depends on a multistate kinetic architecture	A simpler binary model may still explain a restricted assay condition	Kinetic descriptors inherit the biological state definition used to measure or model them
Gating comparison anchor: Apparent gating from model insufficiency Molecular or conformational scenario: A simulation shows one mandatory conformational transition before entry or exit	Observable or model output: One dominant transition appears across limited trajectory data Sampling or data support required: Independent trajectories, alternative coordinates, sensitivity analysis, and external kinetic comparison Mechanistic interpretation that is justified: No strong mechanistic claim until pathway robustness is demonstrated	Reaction-coordinate bias, insufficient sampling, force-field error, or missed alternative routes	Proposed caution: a visually coherent gating mechanism must not be promoted to validated mechanism without discriminating evidence

The reconciliation grid makes the central boundary explicit. Conformational motion becomes mechanistically informative only when it alters pathway accessibility, pathway flux, or the rate-limiting process in a way that survives alternative explanations. Structural change alone is insufficient. The same discipline will be carried forward into kinetic selectivity and method selection: the objective is not to maximize mechanistic detail, but to identify which dynamic distinctions are necessary for the pharmacological question and which remain unvalidated interpretations.

Kinetic selectivity

Selectivity is usually framed as a difference in equilibrium affinity across targets, but time adds another axis. Two targets can show similar endpoint affinity yet differ in association or dissociation kinetics, allowing occupancy to diverge during a dosing interval. Kinetic selectivity should therefore be treated as a context-dependent difference in temporal engagement, interpreted with exposure, target abundance, rebinding, and downstream persistence rather than as an intrinsic advantage.

Screening-scale kinetic prediction is becoming more feasible. ModBind was developed as a comparatively rapid simulation-based predictor of binding and off-rates [23]. Such methods matter because the most expensive rare-event protocols cannot be deployed for every analogue, but greater throughput does not eliminate applicability-domain constraints. A larger-scale SILCS-based workflow combined pathway-derived energetic information with machine learning for dissociation-rate prediction across hundreds of ligand measurements and multiple proteins [24]. This supports the idea that kinetic-pathway information can become a screening variable, while leaving generalization beyond represented targets and chemical space unproven.

Pathway-centred simulation strategies

A pathway-centred workflow should define the inference target before selecting the algorithm. Milestoning can decompose rare events into transitions among interfaces, but its rate estimates depend on state definition, milestone placement, sampling, and reconstruction assumptions [25]. SEEKR2 shows how molecular dynamics and Brownian dynamics can be combined within a multiscale milestoning framework to reduce the burden of long-timescale simulation [26].

Accelerated approaches make a different trade-off. LiGaMD enhances repeated ligand binding and unbinding while permitting recovery of thermodynamic and kinetic observables [27], and LiGaMD3 extends the approach to additional molecular classes including flexible peptides [28]. These advances do not make thermodynamic and kinetic convergence interchangeable; each requires its own audit.

Rate estimation also depends on the statistical treatment of biased trajectories. Short-time infrequent metadynamics was proposed to improve kinetic inference where conventional estimators can become unreliable [29], while the broader metadynamics literature emphasizes collective-variable quality, biasing conditions, and transition-state perturbation as central validity requirements [30]. τ -random-acceleration molecular dynamics occupies another useful niche: accelerated dissociation can support comparative residence-time ranking and reveal exit mechanisms without assuming that every accelerated time is itself an absolute physical off-rate [31].

Data-driven reaction-coordinate learning offers a further strategy. A state-predictive information-bottleneck approach coupled to enhanced sampling produced quantitative residence-time estimates across a defined six-complex benchmark [32]. The result is promising but domain-bound. The proposed architecture is therefore method-agnostic: trajectory coverage, kinetic estimator, and external validation are separate layers, and weakness in any one limits the final claim.

Experimental validation

Validation should identify exactly what succeeded. A route can be chemically plausible without yielding the correct rate; a method can rank a congeneric series while missing absolute timescales; and quantitative performance on one target need not transfer elsewhere. The PYK2 study illustrates the first problem because mechanistically informative pathways coexisted with substantial absolute-rate error for one ligand [14]. Conversely, the TTK benchmark demonstrated strong quantitative performance within a defined inhibitor series [18], and the learned-reaction-coordinate study reported encouraging residence-time recovery in its benchmark set [32].

This Perspective therefore proposes three non-equivalent validation levels. Pathway-consistent denotes route or transition assignments supported by orthogonal evidence; kinetically discriminative denotes reproducible ranking or separation of kinetic classes; and quantitatively kinetic denotes experimentally anchored rate estimates with a defined applicability domain. None of these labels alone implies pharmacological or clinical validity.

Retrospective agreement is also not prospective validation. The CDK8 work connected transient-state analysis to an experimentally evaluated chemical modification [12], while newer faster methods broaden the possibility of screening [23]. The larger SILCS-kinetics benchmark extends scale [24], but current field analyses still identify standardized datasets, blind challenges, and prospective benchmarking as important needs [4]. Decision use should therefore sit beyond an explicit experimental-validation boundary.

Implications for drug optimization

Kinetic simulation should complement rather than replace affinity optimization. Binding free energy addresses thermodynamic favorability; kinetic analysis asks whether access, persistence, or escape creates a temporally distinctive interaction. Residence-time and translational binding-kinetics literature support treating these as complementary dimensions [1], whose relevance depends on target biology, exposure, receptor state, and downstream persistence [2].

Pathway-resolved models may nevertheless reveal design levers hidden from endpoint inspection. Substituents can alter transient contacts, intermediate lifetimes, opening transitions, or escape barriers without dramatic changes in the final pose. Mutation-dependent kinetic resistance motivates attention to such transitions [6], while CDK8 provides a specific example in which transient-state analysis informed chemical modification [12]. Pathway-derived features can also support larger-scale dissociation-rate models [24].

The proposed decision rule is conservative: use endpoint stability for thermodynamic favorability, pathway analysis for mechanistic hypotheses, kinetic models for temporal predictions, and experiments to establish

whether those predictions are accurate enough for the intended decision. The objective is calibrated integration, not replacement of affinity by residence time.

CONCLUSION

Next-generation in silico pharmacology becomes more informative when the bound endpoint is not treated as a complete description of recognition. Association routes, metastable states, barriers, dissociation channels, and conformational gating can alter temporal engagement without necessarily producing a conspicuous change in the final bound structure.

The central contribution of this Mechanistic Perspective is a proposed separation of endpoint stability, pathway identity, kinetic ranking, absolute rate prediction, experimental validation, and medicinal-chemistry decision use. A plausible trajectory is not automatically a validated mechanism; a correct ranking is not an absolute-rate prediction; and an accurate molecular rate is not by itself evidence of therapeutic superiority.

Kinetic pathways matter most when they reveal a mechanistically relevant temporal distinction that endpoint stability cannot resolve. Integrating thermodynamic and kinetic computation while preserving uncertainty, competing pathways, system boundaries, and explicit validation gates provides a more defensible route toward reliable computational pharmacology.

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