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BINDING AFFINITY IS NOT BINDING BEHAVIOR: REFRAMING LEARNED MOLECULAR RECOGNITION AROUND KINETICS, CONFORMATIONAL CHANGE, AND RESIDENCE TIME

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ABSTRACT

Equilibrium affinity remains a central descriptor of molecular recognition, yet it cannot by itself explain how rapidly a ligand reaches its target, which conformational states enable binding, how the complex reorganizes after encounter, which pathways control dissociation, or how long target engagement persists under changing biological conditions. This limitation is increasingly consequential for computational pharmaceutical science because models trained primarily on affinity labels may learn endpoint compatibility while overlooking the temporal and conformational behavior that connects molecular structure to pharmacological action. This Original Molecular-Modeling Perspective Article develops a proposed reframing of learned molecular recognition around a Dynamic Recognition Profile. The profile retains equilibrium affinity as one evidentiary component while separately representing conformational ensembles, recognition mechanisms, association and dissociation pathways, kinetic rate parameters, residence time, perturbation responses, pharmacological context, uncertainty, and provenance. The synthesis distinguishes conformational selection from induced fit without treating them as mutually exclusive, separates thermodynamic stability from kinetic barriers, and argues that trajectory, assay, structural, and perturbational evidence should remain distinguishable when incorporated into learned models. It further proposes that evaluation should move beyond one predictive error measure toward tests of ensemble fidelity, pathway reproducibility, kinetic calibration, perturbation sensitivity, transfer across chemical and target domains, and bounded decision usefulness. The contribution is conceptual rather than empirically validated. It does not establish that kinetic optimization is universally advantageous, that simulated pathways are mechanistically definitive, or that residence time alone determines efficacy, selectivity, or dosing. Reframing recognition as conditional behavior may nevertheless support more scientifically interpretable models and more disciplined translation from molecular prediction to pharmaceutical reasoning.

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Introduction

Molecular recognition is frequently compressed into an equilibrium quantity: a dissociation constant, inhibition constant, binding free energy, potency estimate, or ranking score. Such quantities are indispensable, but they answer a restricted question about the relative favorability or occupancy of states under specified conditions. They do not uniquely determine how a ligand approaches a target, whether access is conformationally gated, how long productive engagement takes to form, or how rapidly the complex disassembles. Drug-target interaction rates therefore provide a time-dependent dimension that conventional equilibrium potency does not contain [1]. This distinction matters wherever a model is expected to support more than retrospective affinity prediction. A system may rank compounds accurately while remaining unable to explain whether two

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affinity-equivalent ligands engage the same conformational states, follow the same pathway, cross the same kinetic barriers, or sustain target occupancy for the same duration.

Residence time sharpens this problem because it is related to the dissociation rate and the barriers governing escape from a bound state rather than simply to the depth of the equilibrium free-energy minimum [2]. A ligand can therefore remain bound for a comparatively long period without possessing uniquely superior equilibrium affinity, while another can achieve a similar equilibrium measurement through faster association and faster dissociation. The inverse relation between residence time and dissociation rate is useful, but even this description is incomplete when binding is multistep, when conformational changes introduce several metastable states, or when observed kinetics are not well represented by a single exponential process. A binding event should consequently be understood as a temporally ordered and context-sensitive sequence of molecular transitions, not merely as the occupation of one crystallographically defined pose.

Computational molecular modeling has historically treated thermodynamic and kinetic prediction as related but separable problems. Free-energy calculations characterize differences in state stability, whereas kinetic calculations require information about transition probabilities, barrier crossings, path ensembles, and time scales [3]. The distinction is not semantic. A model can reproduce an affinity ranking for the wrong mechanistic reasons, identify a plausible pose while missing the dominant access route, or infer a stable complex without representing the conformational bottleneck that controls dissociation. These risks are amplified in learned molecular models because labels such as affinity or activity can encourage representations optimized for endpoint association rather than dynamic recognition. Predictive accuracy under such labels should not be interpreted as evidence that the model has learned molecular mechanism, kinetic behavior, or causally relevant conformational change.

Available approaches to binding kinetics span enhanced sampling, milestoning, weighted ensembles, Markov-state modeling, accelerated dissociation, trajectory analysis, and machine-learning-assisted strategies, each with different assumptions, computational demands, and validation challenges [4]. Their diversity suggests that the scientific problem cannot be resolved by selecting one nominally superior method or optimizing one summary metric. The present perspective therefore proposes the Dynamic Recognition Profile as an evidence-preserving conceptual representation of ligand-target behavior. It distinguishes equilibrium state, conformational ensemble, recognition mechanism, pathway topology, association and dissociation kinetics, residence time, perturbation response, pharmacological context, uncertainty, and provenance. The profile is not proposed as a validated algorithm or universal descriptor. Its purpose is to organize what a time-dependent molecular model should represent, what evidence would support each component, and where interpretation must stop. Affinity, rate constants, and residence time are complementary but non-equivalent descriptions of recognition [1–3].

Why Equilibrium Affinity Captures Only Part of Molecular Recognition

Equilibrium affinity summarizes the balance between bound and unbound states once the system has reached the conditions under which the measurement is interpreted. This summary is scientifically valuable, but it is informationally lossy. It can conceal whether equilibrium was approached rapidly or slowly, whether binding occurred through one dominant route or several competing routes, and whether the observed complex required substantial reorganization after initial encounter. Drug-discovery practice has consequently treated kinetic profiling as complementary to affinity assessment rather than as a replacement for it [5]. The distinction should be retained in computational models. Affinity remains the equilibrium-state descriptor within the proposed Dynamic Recognition Profile, but it cannot stand in for a kinetic descriptor, a pathway representation, or a conformational-mechanism account.

The structural origin of this non-equivalence lies in the fact that equilibrium stability and transition barriers are shaped by overlapping but nonidentical molecular determinants. Persistent contacts, desolvation patterns, pocket rearrangements, water networks, steric restrictions, and conformational gating can alter the barriers to association or dissociation without proportionally changing the final bound-state free energy. Structure-kinetic relationships therefore depend not only on which interactions are present in a minimized complex but also on when those interactions form, how they reorganize, and which contacts must be disrupted before escape [6]. A static pose may identify stabilizing interactions while omitting the sequence through which they become accessible. Similarly, an affinity model may correctly recognize complementarity but fail to represent the slow rearrangement that generates a long-lived complex or the transient intermediate that accelerates dissociation.

A complete account must also resist reducing binding behavior to dissociation alone. Association kinetics can be influenced by diffusion, electrostatic steering, encounter-complex formation, solvent displacement, conformational preorganization, and the accessibility of compatible target states [7]. Two ligands with comparable dissociation rates may therefore differ in onset of engagement, while two compounds with similar residence times may achieve target occupancy through different combinations of association and dissociation behavior. In multistep systems, the experimentally reported association rate may itself represent several unresolved transitions. The proposed profile accordingly treats association, dissociation, and residence time as connected but separately documented variables. This separation prevents a model from interpreting a long residence time as a complete kinetic phenotype or from assuming that an equilibrium constant uniquely specifies its underlying rate constants.

Pharmacological interpretation adds a further boundary. Long residence time is not intrinsically desirable, and rapid association is not universally beneficial; the preferred kinetic pattern depends on target turnover, signaling dynamics, safety liabilities, desired onset, exposure duration, tissue distribution, and the consequences of prolonged occupancy [8]. Translation from in-vitro rate measurements to cellular or in-vivo behavior is similarly conditional on biological environment, target density,

rebinding, and drug exposure [9]. The central claim is therefore not that kinetics should displace affinity, but that affinity should be prevented from silently absorbing information it does not contain. Practical kinetic profiling requires joint consideration of residence time, structural determinants, and association behavior [5–7]. **Table 1** organizes the evidence, constructs, and interpretation boundaries needed to develop why equilibrium affinity captures only part of molecular recognition within the article's central argument.

Table 1. Evidence domains, core questions, scientific requirements, and interpretation boundaries for Why equilibrium affinity captures only part of molecular recognition

Construct or Evidence Domain	Core Question	Scientific Basis	Role in the Article	Failure or Overclaiming Risk	Boundary Statement
Equilibrium Affinity	How favorable is the bound state relative to the unbound state under specified conditions?	Equilibrium binding measurements, potency assays, or binding free-energy estimates	Provides the thermodynamic baseline of recognition	Treating one equilibrium value as a complete description of binding behavior	Affinity does not uniquely determine association rate, dissociation rate, pathway, conformational history, or residence time
Association Kinetics	How rapidly and through which early events does productive engagement form?	Time-resolved binding assays, encounter-complex analysis, access-pathway simulations, conformational-gating evidence	Represents onset, accessibility, and prebinding organization	Assuming that compounds with similar affinity engage a target at the same rate	Association behavior is conditional on experimental conditions, diffusion, target state, and model resolution
Dissociation Kinetics	How rapidly does a ligand leave the target, and which barriers control escape?	Dissociation measurements, rare-event simulation, transition-state analysis, pathway reconstruction	Characterizes persistence of engagement and unbinding barriers	Equating a slow dissociation rate with efficacy or therapeutic superiority	Dissociation rate is one molecular property and cannot establish downstream pharmacological benefit alone
Residence Time	For how long does the kinetically defined complex persist under the stated model or assay?	Inverse relation to a resolved dissociation rate or a suitable multistate kinetic formulation	Provides an interpretable duration descriptor	Applying a single-exponential residence-time interpretation to heterogeneous or multistep systems	Residence time is model- and condition-dependent and is not an intrinsic universal constant
Conformational Ensemble	Which target, ligand, solvent, and complex states are populated or accessible?	Experimental structural ensembles, spectroscopy, molecular simulation, state modeling	Supplies the state space within which affinity and kinetics are interpreted	Treating one structure as the unique recognition state	Computed or observed ensembles may remain incomplete and do not establish all biologically occupied states
Binding And Unbinding Pathways	Which intermediates, gates, bottlenecks, and transition states connect unbound and bound ensembles?	Trajectory ensembles, milestoning, metadynamics, weighted ensembles, interaction fingerprints	Connects molecular structure to temporal recognition behavior	Presenting one simulated trajectory as the dominant biological pathway	Pathway claims require replication, alternative methods, and perturbational support
Perturbation Response	Does ligand modification, mutation, or environmental change alter affinity, pathways, or rates as predicted?	Matched molecular series, mutagenesis, solvent or membrane changes, controlled simulation	Tests whether proposed structure-behavior relationships are falsifiable	Inferring a unique causal mechanism from a nonspecific perturbation	Perturbations can affect several coupled processes and must be interpreted cautiously
Pharmacological Context	Under what exposure, distribution, target-turnover, or signaling conditions might binding behavior matter?	Cellular engagement, target-occupancy models, pharmacokinetics, pharmacodynamics	Bounds the translation from molecular behavior to pharmaceutical use	Converting in-vitro residence time directly into efficacy, selectivity, or dosing claims	Molecular recognition data can support hypotheses but cannot establish therapeutic outcomes independently

Binding Pathways, Conformational Selection, Induced Fit, and Residence Time

Binding pathways describe the temporally ordered transitions through which molecular partners move between unbound, encounter, intermediate, and bound ensembles. These transitions can involve ligand translation and rotation, pocket opening, side-chain rearrangement, solvent displacement, ion movement, loop motion, domain shifts, or broader allosteric reorganization. Induced conformational changes may be detectable in molecular simulations, but their predictability is conditional on system properties, starting structures, force fields, sampling, and the time scales accessible to the calculation [10]. The existence of a ligand-dependent structural difference therefore does not by itself establish an induced-fit mechanism. A model must determine whether the relevant conformation was absent from the unbound ensemble, merely under-sampled, or already present at a low population before ligand encounter.

Conformational selection and induced fit are best treated as limiting descriptions within a continuum rather than as mutually exclusive labels. In conformational selection, a ligand preferentially associates with a pre-existing compatible target state and shifts its population. In induced fit, the initial complex is followed by ligand-dependent rearrangement that produces a more compatible or functionally distinct state. Molecular-state analysis has shown that both processes can participate in the same recognition system [11]. A ligand may first select an accessible substate, then induce local reorganization; alternatively, early ligand contact may alter the probability of larger conformational transitions. The proposed Dynamic Recognition Profile therefore represents mechanism as a conditional distribution over recognition routes rather than assigning one categorical mechanism to every complex. Such a representation should preserve the sequence and uncertainty of state transitions, especially where limited structural evidence cannot discriminate among competing explanations.

Residence time emerges from this pathway structure because dissociation requires the system to cross one or more kinetic barriers. Metadynamics studies of inhibitor dissociation illustrate how unbinding rates can be related to transition pathways and barrier-crossing events rather than inferred directly from the stability of the final complex [12]. Transition-state ensembles further provide information about the configurations that control movement between bound and unbound regions [13]. These observations motivate a distinction among the bound-state pose, the collection of metastable intermediates, and the transition-state configurations that dominate kinetic resistance. A model that predicts affinity from the endpoint pose may overlook a narrow exit channel, a transient hydrogen-bond network, a desolvation bottleneck, or an intermediate pocket that controls the dissociation rate. Conversely, a model may identify a plausible escape route while misrepresenting its statistical importance or rate contribution.

Scaled molecular dynamics and related accelerated approaches demonstrate that relative residence-time ranking and pathway analysis may be attempted prospectively, but their outputs remain conditional on the acceleration protocol, force field, starting state, replicate design, and relationship between accelerated and physical time [14]. These methods are therefore most informative when they provide convergent evidence across repeated trajectories, chemically related ligands, and experimentally measured kinetics. The conceptual implication is that learned molecular recognition should not treat a trajectory as an unlabeled sequence of coordinates or collapse an ensemble into one average time. It should represent pathway multiplicity, state occupancy, transition ordering, barrier uncertainty, and the provenance of every inferred kinetic quantity. Binding behavior is thus the joint product of accessible conformations, route selection, ligand-induced reorganization, and barrier-crossing dynamics. Affinity describes where equilibrium lies; the pathway architecture explains how the system reaches, occupies, and leaves that equilibrium region.

Learning Kinetic and Dynamic Behavior from Heterogeneous Evidence

Learning time-dependent molecular recognition requires evidence that differs not only in format but also in scientific meaning. Equilibrium assays describe state preference, kinetic assays estimate rates under specified conditions, structural methods observe selected conformations, and molecular simulations generate conditional trajectory ensembles. These sources cannot be treated as interchangeable labels for a single latent property. Accelerated unbinding simulations, for example, can yield both relative residence-time information and mechanistic hypotheses about dissociation routes, but the resulting trajectories remain conditioned by the simulation protocol and system representation [15]. An evidence-preserving model should therefore record whether a claim originates from direct measurement, experimentally constrained inference, conventional simulation, accelerated sampling, or statistical learning. Combining evidence is scientifically useful only when the model retains these distinctions rather than collapsing them into an undifferentiated training target.

Machine-learning models provide one route for mapping molecular and complex-level descriptors to unbinding kinetics, particularly when direct rare-event simulation is computationally demanding. Baseline work has shown that protein-ligand features can support prediction of unbinding behavior, while also revealing the limitations imposed by small, heterogeneous, and target-dependent kinetic datasets [16]. Predictive success in this setting does not demonstrate that the learned representation contains transition states, causal interactions, or physically valid pathways. A model may exploit scaffold identity, target-family correlations, assay conventions, or indirect affinity-related features. Evaluation must consequently distinguish interpolation within familiar chemical series from transfer to new ligands, binding modes, and target classes. It should also test whether uncertainty increases when the model moves beyond the conditions represented in its training evidence.

Simulation-derived evidence can enrich learned representations by exposing recurrent molecular transitions that are largely absent from static structural datasets. Ligand Gaussian accelerated molecular dynamics has been used to enhance repeated association and dissociation events and to support inference about free-energy landscapes, binding pathways, and kinetic observables [17]. Such trajectories can provide information about encounter states, solvent rearrangement, metastable intermediates, and escape channels. Yet simulation volume should not be confused with evidence diversity. Thousands of frames generated from one force field and one starting model remain samples from a constrained computational world. Temporal correlation, reweighting assumptions, incomplete state discovery, and uncertain mapping to physical time must be carried forward into any learned representation derived from these trajectories.

Hybrid strategies may help identify variables that organize high-dimensional recognition behavior without asserting that machine learning independently discovers mechanism. Machine learning has been combined with free-energy calculations to identify coordinates and interactions relevant to ligand dissociation [18]. Related work has integrated dimensionality reduction with infrequent metadynamics to estimate kinetic rates, locate transition-state regions, and examine molecular determinants of

dissociation [19]. These approaches support a revised role for learning: not to replace physical modeling or experimental kinetics, but to identify informative representations, stratify pathway ensembles, propose perturbations, and quantify uncertainty across heterogeneous evidence. Within the proposed Dynamic Recognition Profile, each inferred quantity should remain attached to its source, context, model assumptions, and validation status. The objective is an interpretable record of recognition behavior, not a single latent score that silently conflates affinity, motion, kinetics, and function.

A Revised Modeling Agenda for Time-Dependent Molecular Recognition

A revised modeling agenda should begin by rejecting the assumption that one computational strategy can resolve every dimension of molecular recognition. Existing kinetic approaches differ in the events they sample, the time scales they access, the state boundaries they require, and the quantities they estimate [20]. Some methods emphasize relative ranking, others seek absolute rates, and others are primarily useful for pathway discovery or mechanistic interpretation. Method choice must therefore follow the scientific claim. A model intended to rank dissociation rates within a congeneric series faces a different evidentiary burden from one intended to identify transition states, predict association mechanisms, or support pharmacokinetic-pharmacodynamic reasoning. The proposed agenda treats method selection as part of the provenance of a claim rather than as an invisible preprocessing decision.

Cross-method comparison should become a central evaluative practice. Weighted-ensemble and multiscale-milestoning approaches have been compared for ligand-binding kinetic ranking, illustrating that alternative rare-event frameworks can address related questions through different state definitions and sampling assumptions [21]. Agreement can strengthen confidence in a rank or pathway hypothesis, while disagreement can identify sensitivity to initialization, partitioning, sampling depth, or rate-estimation procedure. Neither agreement nor disagreement should be interpreted simplistically. Convergence between methods does not prove that a pathway is biologically dominant, and divergence does not automatically show that one method has failed. Instead, method disagreement should be represented as evidence about model-form uncertainty.

Thermodynamic and kinetic outputs must also remain separate even when they are derived from related trajectory data. Weighted-ensemble milestoning can estimate dissociation kinetics alongside free-energy information, but the two outputs rely on distinct statistical questions and may exhibit different error structures [22]. A revised architecture should therefore contain separate heads or inference layers for equilibrium state, association behavior, dissociation behavior, pathway topology, and conformational response. Shared molecular representations may be scientifically appropriate, but shared representation should not imply shared validation. An accurate free-energy estimate cannot validate an incorrect dissociation path, just as a useful rate ranking cannot establish that the model reproduces the correct conformational ensemble.

The proposed Dynamic Recognition Profile operationalizes this agenda as a structured, conditional representation. Pathway fingerprints can distinguish heterogeneous dissociation routes using time-dependent interaction patterns rather than a single averaged trajectory [23]. At the same time, the reliability and accessibility of kinetic simulations remain constrained by force-field quality, sampling design, machine-learning choices, and implementation complexity [24]. The profile therefore joins seven components without collapsing them: equilibrium state, conformational ensemble, recognition mechanism, pathway topology, kinetic parameters, perturbation response, and pharmacological context. Each component carries source-specific uncertainty, applicability boundaries, and model provenance. Alternative simulation and rate-estimation methods should be treated as complementary but method-dependent sources of evidence [20–22]. **Figure 1** presents the dynamic binding-behavior clock, showing how the article's key components and boundaries are connected within a revised modeling agenda for time-dependent molecular recognition.

Figure 1. Dynamic Binding-Behavior Clock.

The figure is an original conceptual synthesis that organizes the article's central contribution across equilibrium affinity captures only part of molecular recognition, binding pathways, conformational selection, induced fit, and residence time, binding pathways, conformational selection, induced fit, residence time. Arrows and grouping indicate proposed or evidence-

supported relationships rather than measured effect sizes. The figure does not represent a validated predictive model, regulatory determination, clinical recommendation, or deployment-ready system.

Evaluation against Structural Ensembles, Kinetics, And Perturbation Data

Evaluation must test whether a model represents the scientific object it claims to predict. For kinetic modeling, agreement with a measured dissociation rate is necessary but insufficient because rate estimates can be sensitive to charge representation, force fields, starting conformations, collective variables, and sampling protocol [25]. A model may obtain a plausible value through compensating errors or a target-specific calibration that does not transfer. Rate evaluation should therefore report error distributions, uncertainty coverage, chemical-series dependence, target-family dependence, and sensitivity to modeling assumptions. Where experimental replicates are available, measurement uncertainty should be separated from computational uncertainty rather than treating the reported assay value as an exact ground truth.

Pathway claims require a different form of evaluation. Random-acceleration molecular dynamics combined with interaction-fingerprint analysis provides one framework for generating and comparing dissociation routes [26]. Such representations can test whether repeated simulations recover similar contact-breaking sequences, intermediates, and exit channels. However, pathway reproducibility within one method does not establish physical correctness. Stronger support requires comparison across starting structures, force fields, sampling strategies, and perturbations expected to alter a proposed bottleneck. The most informative evaluation asks whether a model predicts how a pathway changes after a ligand substitution, pocket mutation, solvent alteration, or conformational constraint—not merely whether it reproduces a visually plausible movie.

Transferability must be stratified by binding mode and scientific use. Residence-time prediction across type I and type II kinase inhibitors illustrates that different binding modes can involve distinct conformational states and dissociation processes [27]. Performance within one inhibitor class should therefore not be generalized automatically to another class, even when the compounds share a target. Evaluation sets should distinguish interpolation among close analogues, extrapolation to new scaffolds, transfer across binding modes, and transfer across target families. Random train-test partitions are inadequate when they permit close structural analogues or related targets to appear on both sides of the evaluation boundary. A kinetic model should declare the domain in which its estimates are calibrated and should abstain or widen uncertainty when that domain is exceeded.

Integrated assessment should compare structural ensembles, affinity, rates, pathways, perturbation responses, and computational efficiency without collapsing them into one composite score. Multiscale milestoning combined with metadynamics has been evaluated against both residence-time behavior and binding free-energy information, demonstrating the value of testing distinct outputs separately [28]. Rapid simulation-based off-rate prediction further highlights the need to examine the trade-off between computational accessibility and evidentiary depth [29]. A faster prediction may be valuable for triage while remaining unsuitable for mechanistic interpretation or absolute-rate claims. **Figure 2** presents the evidence, validation, and translation boundary observatory for binding affinity is not binding behavior reframing, showing how the article's key components and boundaries are connected within evaluation against structural ensembles, kinetics, and perturbation data.

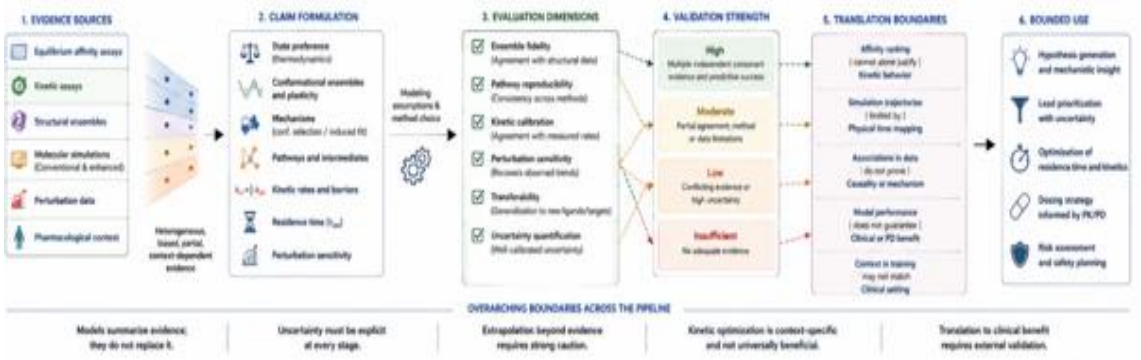

Figure 2. Evidence, Validation, and Translation Boundaries.

The figure is an original conceptual synthesis that shows how claims move from conceptual plausibility through evaluation, uncertainty assessment, and bounded pharmaceutical use. Arrows and grouping indicate proposed or evidence-supported relationships rather than measured effect sizes. The figure does not represent a validated predictive model, regulatory determination, clinical recommendation, or deployment-ready system.

Consequences for Efficacy, Selectivity, and Dosing Decisions

The pharmaceutical relevance of kinetic modeling lies not in the intrinsic desirability of one rate constant, but in the interaction between molecular engagement and biological exposure. Pharmacokinetic-pharmacodynamic models can incorporate association and dissociation kinetics to represent how changing drug concentration interacts with target occupancy over time [30]. This linkage is especially important when plasma or tissue concentration changes on a time scale comparable to target

binding or unbinding. Even then, a kinetic parameter is not a direct efficacy measure. Target engagement may be necessary but insufficient for downstream response, and the relationship between occupancy and effect can be nonlinear, delayed, amplified, desensitized, or limited by compensatory biology.

Distribution and rebinding further complicate translation. Target occupancy may be controlled not only by microscopic kon and koff but also by movement between compartments, local concentration, diffusion, target abundance, and repeated rebinding after dissociation [31]. Under some conditions, drug distribution may be slower than target binding; under others, dissociation or target turnover may become rate limiting. A residence-time estimate obtained in a simplified biochemical assay must therefore be interpreted within an explicit contextual model before it is used to reason about cellular persistence or dosing interval. The proposed context-transfer descriptor records which downstream processes have been measured, modeled, assumed, or omitted.

Experimental evidence indicates that residence time can affect in-vivo target occupancy through several pathways, including interactions with pharmacokinetic behavior, but the direction and magnitude of this effect remain target- and compound-dependent [32]. This conditionality is particularly important for selectivity. Kinetic selectivity can arise when a compound dissociates at different rates from on-target and off-target proteins, yet meaningful comparison requires compatible assay conditions and biologically relevant exposure. A long on-target residence time may support sustained engagement, but prolonged off-target occupancy may increase risk. Conversely, rapid reversibility may be desirable where physiological control or safety requires short-lived modulation. Kinetic optimization must therefore be framed as target-specific profile design rather than maximization of residence time.

Lead-optimization studies show that thermodynamic and kinetic profiling can provide complementary information when compounds with similar potency display different binding rates or residence behavior [33]. More broadly, an experimentally investigated disconnect between affinity and functional activation demonstrates that similar binding strength can coexist with different dynamic and functional outcomes [34]. Such evidence supports the article's central reframing while remaining bounded: kinetic or conformational differences may help explain functional divergence, but they do not establish that kinetics is the sole cause. Efficacy, selectivity, and dosing decisions require integration with pharmacology, exposure, safety, developability, and clinical evidence. **Table 2** organizes the evidence, constructs, and interpretation boundaries needed to develop consequences for efficacy, selectivity, and dosing decisions within the article's central argument.

Table 2. Evaluation, implementation, and research priorities arising from Binding Affinity Is Not Binding Behavior Reframing Learned Molecular Recognition around Kinetics

Component or layer	Inputs	Core function	Expected output	Uncertainty or limitation	Validation requirement
Equilibrium-state layer	Affinity measurements, potency values, free-energy estimates, bound-state structures	Characterize relative state preference under defined conditions	Equilibrium descriptor with assay and model provenance	Does not determine rates, pathways, or functional consequences	Matched equilibrium measurements and external chemical-series testing
Conformational-ensemble layer	Experimental structures, spectroscopy, cryo-EM, NMR, molecular simulations, predicted states	Represent accessible protein, ligand, solvent, and complex conformations	Ensemble distribution or state catalogue	Incomplete sampling and uncertain state populations	Comparison with multiple experimental observables and replicate simulations
Recognition-mechanism layer	State populations, time-resolved trajectories, association evidence, perturbations	Distinguish conformational selection, induced fit, and mixed mechanisms	Conditional mechanism hypothesis	Competing mechanisms may fit the same observations	Prospective perturbation and time-resolved testing
Pathway-topology layer	Binding and unbinding trajectories, contact maps, intermediate states, transition-state analyses	Represent alternative access and escape channels	Pathway ensemble with bottlenecks and intermediate states	Strong dependence on sampling method, starting structure, and coordinates	Cross-method replication and targeted mutational or chemical perturbation
Kinetic layer	kon, koff, residence time, kinetic traces, simulated rate estimates	Describe onset and persistence of engagement	Calibrated kinetic profile	Non-single-exponential behavior, assay dependence, model-form error	Matched kinetic assays, external validation, and uncertainty calibration
Perturbation-response layer	Matched molecular pairs, mutations, solvent changes, membrane context, temperature or ionic changes	Test whether proposed structure-behavior relationships respond as predicted	Directional perturbation hypothesis	Perturbations may influence several coupled mechanisms	Controlled prospective perturbation experiments
Evidence-integration layer	Assay, structural, simulation, sequence, trajectory, and contextual metadata	Combine heterogeneous evidence without erasing source semantics	Evidence-preserving Dynamic Recognition Profile	Missing metadata, incompatible conditions, data leakage, overfitting	Modality-specific validation and provenance audit
Context-transfer layer	Cellular engagement, distribution, rebinding, target turnover, PK and PD evidence	Connect molecular behavior to bounded target-occupancy reasoning	Context-dependent engagement or dosing hypothesis	Cross-scale uncertainty and incomplete biological representation	Mechanistic cellular and in-vivo validation
Decision-use layer	Dynamic Recognition Profile, uncertainty, medicinal-chemistry goals,	Support compound comparison or experimental prioritization	Bounded recommendation for further testing	More complex outputs may create false precision or decision burden	Prospective comparison with affinity-only decision processes

	safety and developability evidence				
Governance and reporting layer	Model versions, assay conditions, applicability domain, uncertainty decomposition, audit history	Make claims traceable and prevent misuse	Reproducible evidence record and explicit boundary statement	Reporting does not guarantee scientific validity	Independent review, version control, and predefined interpretation rules

Limitations and Boundary Conditions

The Dynamic Recognition Profile is a proposed conceptual contribution rather than an implemented architecture or validated prediction system. Its components are supported unevenly across targets, ligand classes, kinetic regimes, and evidence modalities. Association-rate data are less abundant than affinity measurements, absolute kinetic simulation remains demanding, and experimental structural ensembles incompletely resolve transient states. The profile may therefore be only partially populated for many systems. Missing components should be reported as unavailable or insufficiently supported rather than inferred automatically from affinity, docking, or structural similarity.

Mechanistic interpretation remains particularly vulnerable to overclaiming. Conformational selection and induced fit can coexist, and the same experimental observations may admit several state-transition models. A simulated pathway can be physically plausible without being statistically dominant in the biological environment. Likewise, a machine-learning explanation can identify features associated with kinetic variation without establishing that those features causally control the rate. Mechanistic claims require convergent trajectory evidence, perturbational tests, experimental kinetics, and explicit consideration of alternative explanations. Prediction, explanation, mechanism, and causality must remain separate evidentiary categories.

Pharmaceutical translation imposes additional boundaries. Residence time alone cannot establish efficacy, selectivity, safety, optimal dose, dosing interval, clinical utility, or regulatory acceptability. The value of a kinetic profile depends on target biology, compartmental exposure, target turnover, rebinding, signaling architecture, therapeutic objective, and off-target consequences. The proposed framework should not be used as an autonomous compound-selection or dosing tool. Its appropriate near-term role is to organize evidence, identify missing measurements, generate testable hypotheses, and clarify why an affinity-only conclusion may be incomplete.

Research Agenda and Implementation Priorities

The first research priority is the creation of matched, provenance-rich evidence resources. Affinity, kon, koff, structural states, assay conditions, functional responses, and perturbation outcomes should be collected for the same compounds wherever feasible. Raw kinetic traces, fitting assumptions, censoring, replicate variability, protonation states, temperature, buffer composition, membrane environment, and target construct should accompany derived labels. Evaluation splits should separate close-analogue interpolation from scaffold, binding-mode, and target-family transfer. Such infrastructure would make it possible to determine whether learned kinetic relationships reflect molecular behavior or merely reproduce experimental and chemical-series regularities.

The second priority is prospective multidimensional evaluation. Models should be asked to predict not only a kinetic rank but also which conformational states, pathway bottlenecks, or molecular interactions are expected to change after a predefined perturbation. These predictions should be recorded before new kinetic, structural, mutational, or functional experiments are performed. Cross-method simulation can be used to identify model-form uncertainty, while calibrated prediction intervals should distinguish measurement noise, sampling uncertainty, parameter uncertainty, and uncertainty arising from extrapolation. Failure should be reported by dimension: a model may rank koff acceptably while failing to recover pathway changes or ensemble shifts.

The third priority is staged implementation within discovery workflows. Initial use should focus on retrospective evidence organization and identification of affinity-equivalent compounds that merit kinetic measurement. A second stage may support prospective experiment prioritization within narrow, well-characterized target and chemical domains. Broader decision use should require demonstrated calibration, reproducible pathway analysis, matched pharmacological context, and evidence that the Dynamic Recognition Profile improves decisions relative to simpler affinity-based baselines. At every stage, outputs should remain reviewable by medicinal chemists, structural biologists, biophysicists, pharmacologists, and model developers. Expansion should follow demonstrated scientific usefulness rather than architectural complexity.

Conclusion

Binding affinity is a necessary descriptor of molecular recognition, but it is not a complete account of binding behavior. The proposed Dynamic Recognition Profile reframes learned recognition as a conditional relationship among equilibrium state, conformational ensemble, recognition mechanism, pathway topology, association and dissociation kinetics, residence time, perturbation response, and pharmacological context. Its central purpose is to prevent a single affinity label or benchmark metric from absorbing distinctions that matter scientifically and pharmaceutically. The profile remains conceptual and requires prospective, multidimensional validation. It cannot independently establish mechanism, efficacy, selectivity, safety, dose, or clinical utility. Its value lies in organizing evidence, exposing uncertainty, identifying missing experiments, and supporting more disciplined reasoning about how molecules approach, reorganize, occupy, and leave their targets over time.

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