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Drug–Target Affinity Emerges from Conformational Populations Rather Than a Single Bound Structure

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ABSTRACT

Drug–target affinity is commonly interpreted through the geometry of a representative bound complex, yet an experimentally resolved or computationally predicted structure does not specify how frequently that conformation is occupied, how it is reached, or how alternative protein, ligand, and solvent states contribute to binding. This Perspective argues that affinity is more appropriately interpreted as an ensemble property emerging from coupled conformational populations and their redistribution upon ligand recognition. The argument does not make structural snapshots dispensable, nor does it reduce binding free energy to receptor populations alone. Instead, it separates structural realizability, state population, solvent and energetic contributions, transition kinetics, and simulation-dependent inference. Evidence from conformationally flexible targets, alternative ligand poses, alchemical free-energy calculations, enhanced-sampling methods, and binding-kinetic simulations shows why structurally similar complexes can encode different thermodynamic or kinetic behavior and why simulation-derived populations remain conditional on sampling and energy models. A proposed ensemble-based interpretation is developed in which affinity claims are evaluated against state identity, statistical population, transition connectivity, energetic representation, and observable-specific validation. This formulation turns conformational heterogeneity from a complication surrounding a preferred structure into a mechanistically testable component of affinity interpretation.

Keywords: Conformational ensembles, Protein–ligand binding, Binding free energy, Molecular dynamics, Binding kinetics, Enhanced sampling

INTRODUCTION

A drug–target complex is often communicated as a structure: a ligand occupies a pocket, interactions are enumerated, and the resulting geometry becomes the physical explanation for affinity. This representation is indispensable for identifying steric complementarity, hydrogen-bond networks, electrostatics, and medicinal-chemistry opportunities. It is nevertheless incomplete whenever the observed complex is only one member of a heterogeneous molecular population.

Experiments and simulations on HSP90 provide a direct example. Ligand-dependent changes in binding-site conformation were associated with different thermodynamic and kinetic behavior, showing that structural flexibility can influence measurable binding properties rather than merely decorate a fixed recognition event [1]. Association simulations of the Mdm2–PM1 system likewise demonstrate that binding kinetics can depend on

transition processes extending well beyond the information contained in an endpoint complex [2]. Neither result implies that a crystal structure is misleading. It implies that structural identity and statistical occupancy answer different questions.

The central argument developed here is therefore narrower than the claim that “everything is an ensemble.” Drug–target affinity should be interpreted through the populations and interconversions of relevant protein, ligand, and solvent states, while retaining separate accounting for energetic-model quality, sampling completeness, and the distinction between thermodynamic and kinetic observables. The aim is a mechanistic interpretation in which a bound structure remains a high-information state description but no longer acts as a surrogate for the whole binding landscape.

The limits of single-structure affinity interpretation

Single-structure reasoning becomes problematic when structural observability is silently converted into thermodynamic importance. An experimentally resolved pose establishes that a molecular arrangement is physically realizable under the conditions used to obtain it; it does not by itself establish its solution population, the population of its precursor states, or whether another state contributes substantially to recognition. Ensemble docking makes this distinction operational. Reduced receptor ensembles can preserve useful docking performance while changing which conformations are presented to the ligand, demonstrating that computational recognition depends partly on receptor-state representation rather than a unique rigid target [3]. Docking scores, however, remain ranking quantities and should not be interpreted as equilibrium state populations or binding free energies. A second limitation concerns the pathway inferred between structures. Adaptive-sampling comparisons have shown that the sampling strategy can alter state discovery, estimated transition probabilities, and the apparent mechanism of conformational change [4]. Consequently, a trajectory that connects two experimentally compatible endpoints does not automatically establish that the reconstructed pathway is the biologically dominant one. The distinction is especially important when a simulated “mechanism” is inferred from rarely sampled intermediates. Single-structure interpretation is also incomplete in a different direction: not every omitted contribution is conformational. Interfacial water can alter protein–ligand binding energetics through solvent-mediated effects that are not recoverable from protein geometry alone [5]. A population-based account therefore fails if it simply replaces one privileged protein structure with several protein structures while leaving ligand strain, hydration, interaction energetics, and statistical weighting implicit. The required conceptual move is from a structural object to an explicitly defined thermodynamic and kinetic ensemble, not from one picture to many pictures.

Conformational ensembles and population redistribution

An ensemble interpretation begins with a distinction between states present before binding and states whose populations change because of ligand engagement. Enhanced-sampling methods make this distinction computable but also reveal its dependence on methodological choices. Dynamically optimized collective variables can accelerate calculations of binding free energies and residence times, yet the variables used to drive rare transitions necessarily define which motions are made accessible efficiently [6]. Ensemble-based replica-exchange alchemical calculations similarly show why repeated sampling is valuable for characterizing uncertainty rather than treating one trajectory as a deterministic molecular history [7].

Population heterogeneity exists on the ligand side as well. Nonequilibrium candidate Monte Carlo combined with molecular dynamics has been used to improve transitions among alternative conformations of bound ligands in kinase systems, demonstrating that a single bound pose can conceal slowly interconverting ligand substates [8]. At a broader thermodynamic scale, absolute binding free-energy calculations across pharmaceutically relevant targets explicitly construct bound and unbound configurational ensembles rather than deriving affinity from one minimized complex [9]. Even such ensemble calculations remain vulnerable to slow coordinates outside a convenient protein-state description. Buried water rearrangements can impede convergence [10], while force-field combinations and simulation conditions can materially influence calculated affinity [11].

The population concept is therefore useful only when the state definition remains visible. Machine-learning-assisted molecular-dynamics analyses can identify binding-relevant conformational substates, but the recovered partition depends on descriptors and dimensionality-reduction choices [12]. In Abl–imatinib simulations, initial ligand engagement with an autoinhibited receptor state was followed by substantial target rearrangement toward a crystallographic-like complex [13]. That sequence is particularly instructive because it resists a simple choice between conformational selection and induced fit: pre-existing-state recognition and post-engagement reorganization can coexist within one binding process.

The interpretation proposed here is thus population redistribution, not population determinism. A ligand can preferentially engage one part of a pre-existing ensemble, stabilize newly favored substates, alter transitions among previously accessible states, or participate in a mixture of these processes. The relevant causal language should remain conditional unless experimentally constrained. Simulation identifies plausible state populations and pathways under a defined model; it does not, by itself, prove that every reconstructed population is occupied with the same weight in the biological system.

This distinction between sampled conformations, redistributed populations, and observable affinity is difficult to preserve in linear prose alone. **Figure 1** therefore separates the trajectory level from the population-inference level and from thermodynamic or kinetic interpretation, while keeping sampling and energetic uncertainty visible rather than hiding them inside a single “ensemble” box.

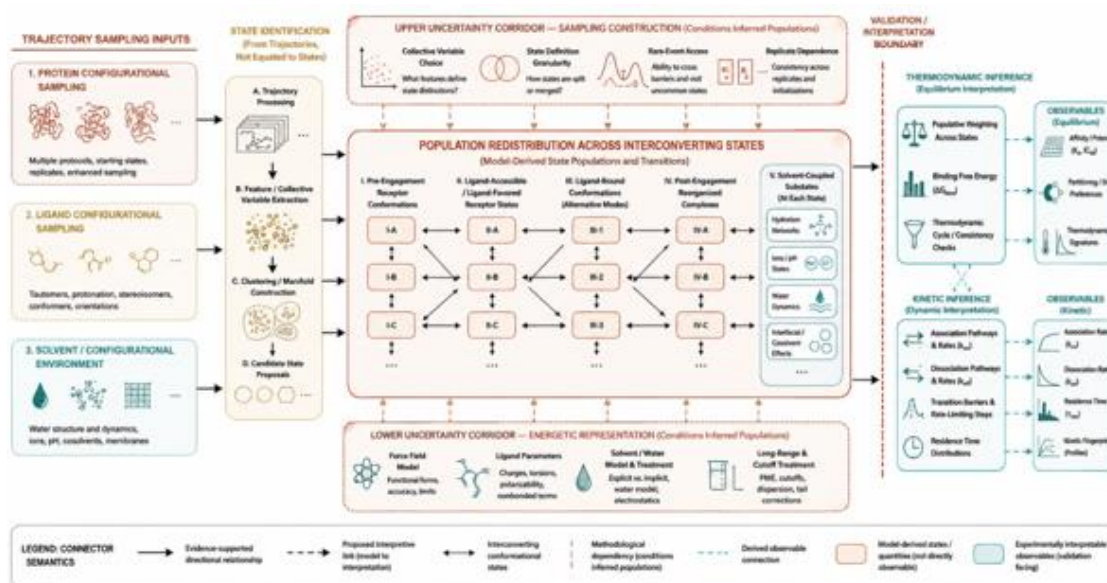


Figure 1. Population Redistribution Separates Sampled Conformations from Thermodynamic and Kinetic Interpretation

Binding-competent and binding-incompetent states

The labels *binding-competent* and *binding-incompetent* are useful only if competence is defined relative to a specified molecular event. A receptor conformation can be poorly suited to direct occupancy of the final ligand pose yet remain relevant because it accesses an encounter configuration or rapidly interconverts with a pocket-ready state. Conversely, a geometrically plausible pocket does not become thermodynamically important merely because docking can place a ligand within it. Competence is therefore conditional on the observable being explained.

At the receptor level, conformational-state analysis can identify substates that are more compatible with substrate or ligand recognition, but these classifications inherit the state definitions used in the analysis [12]. At the ligand level, slowly interconverting bound conformations can coexist within a nominally identical complex [8]. The Abl–imatinib example further shows why competence should not be equated with resemblance to the final crystallographic complex: a receptor state can participate meaningfully in recognition before substantial subsequent reorganization [13].

This motivates three distinctions. Recognition competence concerns whether a molecular state can participate in productive initial engagement. Bound-state competence concerns whether a state contributes materially to the equilibrium bound ensemble. Pathway competence concerns whether a state or transient configuration connects regions of the landscape relevant to association or dissociation. These distinctions are proposed analytical categories rather than experimentally validated universal state classes. A conformation may satisfy one without satisfying the others.

The practical consequence for medicinal chemistry is that apparent structural incompatibility should not automatically be treated as functional exclusion. A minor receptor state may be chemically important if a ligand preferentially stabilizes it; an apparently favorable bound conformation may be negligible if its statistical weight is vanishingly small; and an intermediate may matter kinetically without dominating equilibrium occupancy.

Table 1 makes these cross-scale distinctions explicit so that state classification is linked to an appropriate decision consequence and a visible transfer boundary rather than being treated as a binary property of a structure.

Table 1. When a Conformation Qualifies as Binding-Competent Across Recognition, Bound-State, and Access Contexts

scenario	Basis for calling the state competent or noncompetent	Molecular or chemical state being evaluated	Consequence for affinity interpretation	Transfer boundary	Failure signature if the classification is wrong	Experimental counterpart	Prospective test	Ensemble-affinity endpoint
Pre-existing pocket-ready receptor substate	Ligand can engage without first requiring a large receptor rearrangement	Apo or weakly perturbed receptor subpopulation	May contribute to conformational selection and to the effective population available for binding	Computational occupancy is not automatically the solution population	Affinity is attributed to a pose that appears only under one sampling regime	Conformation-sensitive structural or kinetic measurements	Reproduce the state with independent sampling and perturb its population experimentally	Proposed : recognition-relevant population
Productive encounter state followed by receptor reorganization	Initial engagement is compatible with later movement toward a stable complex	Ligand-associated intermediate and reorganizing receptor	Final pose alone cannot identify the complete recognition mechanism	Pathway recovered in simulation may not be the dominant biological route	End-state agreement is mistaken for pathway validation	Time-resolved or state-sensitive binding measurements	Test whether perturbations that alter the precursor state change association behavior	Proposed : transition-connected recognition state
Alternative bound ligand substate	Distinct ligand conformations are kinetically accessible within the bound ensemble	Multiple ligand poses within a common pocket environment	Affinity may integrate more than one bound microstate	Pose frequency depends on ligand sampling efficiency and energy model	One pose appears artificially dominant because torsional transitions are undersampled	Structural heterogeneity or spectroscopy where feasible	Increase ligand-state sampling while holding receptor representation constant	Proposed : bound-ensemble contributor
Geometrically plausible but statistically unsupported state	Favorable local contacts exist without evidence of appreciable population	Docked or minimized complex	Structural plausibility alone is insufficient to assign thermodynamic importance	Docking score and occupancy are different quantities	High-ranked pose fails to persist or reproduce across ensemble calculations	Orthogonal binding-mode evidence	Compare against population-aware or free-energy calculations	Exclude from population-weighted interpretation until supported

Access-relevant transient state	State contributes to ligand entry, exit, or pocket opening without dominating the final complex	Intermediate along an access or dissociation route	May influence kinetics more strongly than equilibrium affinity	Access-path relevance does not establish a free-energy contribution of the same magnitude	Residence behavior changes while endpoint structure remains similar	Association/dissociation kinetics	Perturb pathway-lining interactions without changing final contacts	Proposed : kinetic-pathway contributor or
Long-lived closed or misaligned state	State blocks productive geometry or access over the timescale relevant to recognition	Receptor substate outside the immediately productive region	Can reduce the effective population available for binding	“Incompetent” remains timescale- and ligand-dependent rather than absolute	State is labelled inactive despite rapid interconversion or ligand-specific rescue	Conformational exchange measurements	Change ligand chemistry or receptor-state population and reassess competence	Proposed : conditionally inaccessible population

Free-energy landscapes and affinity

The ensemble interpretation becomes thermodynamically meaningful only when populations are connected to free energy. Absolute binding free-energy calculations across pharmaceutically relevant targets illustrate the relevant statistical-mechanical principle: affinity is obtained by integrating configurational ensembles of the coupled receptor–ligand system and appropriate reference states, not by assigning an energy to one minimized pose [9]. The most populated structural basin is therefore not automatically synonymous with the complete binding free energy, because multiple substates can contribute and because unbound-state statistics also matter. Landscape reconstruction is especially sensitive to slow variables. Buried waters can reorganize on timescales that impede convergence and can change the energetic balance among apparently similar complexes [10]. Force-field selection introduces a separate boundary: comparison of protein, ligand, and solvent parameter combinations shows that calculated binding free energies can vary with the energetic model and simulation protocol [11]. A disagreement between computed and experimental affinity should consequently not be diagnosed automatically as missing conformational sampling. Population error and energy-model error are distinguishable possibilities. Replicated ensemble calculations are useful because they expose stochastic variability that a single calculation can conceal [7]. Yet numerical convergence across replicas is still weaker than mechanistic validation. Several replicas can agree while sharing the same force-field bias or omitted slow degree of freedom. For the present framework, a free-energy landscape is therefore an inferred statistical object whose credibility depends jointly on state coverage, weighting, energetic representation, and reproducibility.

This distinction also clarifies why “the bound structure explains affinity” is often too strong. Structural contacts can rationalize local energetic contributions, whereas affinity belongs to a difference between statistical ensembles. A structure becomes mechanistically persuasive when its interactions are connected to appropriately sampled populations and when alternative explanations—particularly solvent reorganization, ligand strain, or model error—have been evaluated rather than silently absorbed into a conformational narrative.

Kinetic and thermodynamic coupling

Thermodynamic affinity and binding kinetics interrogate the same molecular landscape but do not encode the same information. Reviews of biased molecular-dynamics approaches emphasize that prediction of association, dissociation, and residence times requires explicit treatment of rare transitions and coarse-grained kinetic reconstruction [14]. Accordingly, two ligands with similar equilibrium affinity need not share the same association route, dissociation barrier, or residence behavior.

Structure–kinetics analyses of opioid analogues illustrate this distinction at chemical-series scale. Metadynamics and machine-learning analysis identified relationships between ligand substitutions and dissociation behavior while also exposing the difficulty of obtaining absolute kinetic timescales from incomplete transition sampling [15]. The proper inference is therefore not that kinetics supersedes affinity, but that a free-energy difference alone cannot uniquely specify the barriers and pathways connecting bound and unbound populations.

Kinetic interpretation is also unusually sensitive to how states are initialized and discretized. Deep-learning-assisted weighted-ensemble simulations show that the initial distribution of configurations can influence convergence of rare-event sampling [16]. Related free-energy work on a model protein–protein complex demonstrates how slowly rearranging interaction and water networks create sampling problems even when the nominal endpoint states are defined [17]. Rebalanced replica-exchange protocols likewise show that changing the sampling construction alters the accessible conformational exploration [18], while comparisons of equilibrium and nonequilibrium alchemical approaches caution against assigning methodological superiority without matched assessments of convergence and reliability [19]. These results are not interchangeable demonstrations of binding kinetics, but together they establish an important boundary: reconstructed barriers and populations are conditional on the state model and sampling protocol used to obtain them.

Energetic representation provides another source of coupling. Local quantum-mechanical reparameterization has been shown to alter calculated dissociation kinetics in milestoning applications, indicating that a refined pathway is not sufficient if the forces governing transitions are poorly represented [20]. Conversely, methods that predict ligand-specific receptor conformations demonstrate why state definition can precede kinetic analysis: the relevant receptor geometry itself may depend on ligand identity [21]. Such predicted structures should not be read as equilibrium populations, but they can define alternative hypotheses about which regions of conformational space require kinetic interrogation.

Recent enhanced-sampling approaches increasingly attempt to recover thermodynamic and kinetic observables within a common simulation framework. LiGaMD3, for example, was developed to improve repeated binding and unbinding sampling for small molecules and flexible peptides and to estimate both binding thermodynamics and kinetics in benchmark systems [22]. This convergence of objectives is conceptually valuable because it discourages treating affinity and residence time as competing explanations. They are different projections of a shared landscape.

The mechanistic implication is that a ligand's observed affinity can emerge from population weighting even when kinetic routes differ substantially, while kinetic selectivity can arise from transition barriers that contribute little information to a single equilibrium structure. An ensemble-based account must therefore preserve both the statistical weights of states and the connectivity between them. Collapsing either dimension into a preferred bound pose removes information needed to distinguish thermodynamic stabilization from kinetic trapping, altered association access, or slow dissociation.

Reconciling molecular-dynamics evidence across sampling strategies

No simulation protocol observes “the ensemble” independently of how conformational space is explored. Current assessments of binding and unbinding methods emphasize that enhanced sampling, rare-event techniques, and machine-learning-assisted approaches trade throughput against assumptions about state definition, bias, reweighting, and dynamical reconstruction [23]. Agreement between methods should therefore be evaluated at the level of states, populations, and observables rather than inferred from similar endpoint structures.

A useful reconciliation exercise must also preserve method-specific failure signatures. Weighted-ensemble calculations can depend on how initial configurations populate the chosen state space [16]. Slowly rearranging contact and hydration networks can limit apparently well-defined free-energy transformations [17], and replica-exchange design can change which protein conformations are visited efficiently [18]. Equilibrium and nonequilibrium alchemical approaches likewise require comparison under matched reliability criteria rather than by method label alone [19]. Thus, concordance is strongest when different algorithms reproduce the same chemically meaningful state partition and observable despite differing routes through configurational space.

Sampling deficiencies can be coordinate-specific. Alchemical enhanced sampling has reduced hysteresis and replicate dispersion when slowly transitioning ligand degrees of freedom limit relative binding free-energy calculations [24]. Such gains establish improved precision under the tested protocol, not automatic mechanistic correctness. Energetic representation remains an independent axis: neural-network potentials can alter affinity predictions even when the broad conformational problem is unchanged [25].

A reconciled interpretation should consequently ask whether independent sampling constructions discover compatible metastable regions, whether their relative populations are stable to reasonable state definitions, whether thermodynamic observables converge, and whether kinetic pathways remain consistent when initialization or biasing choices change. Disagreement is informative when localized: it can identify insufficient state coverage, unstable weighting, an inadequate potential, or pathway-specific uncertainty rather than being compressed into one generic “simulation error.”

This discipline is especially important in prospective compound comparison, where a mechanistic narrative should survive altered starting structures, independent replicas, and reasonable model perturbations before guiding chemical interpretation.

This separation is easier to inspect visually than through method-by-method comparison. **Figure 2** places simulation choices upstream of inference and shows where agreement can strengthen interpretation without converting computational convergence into biological validation.

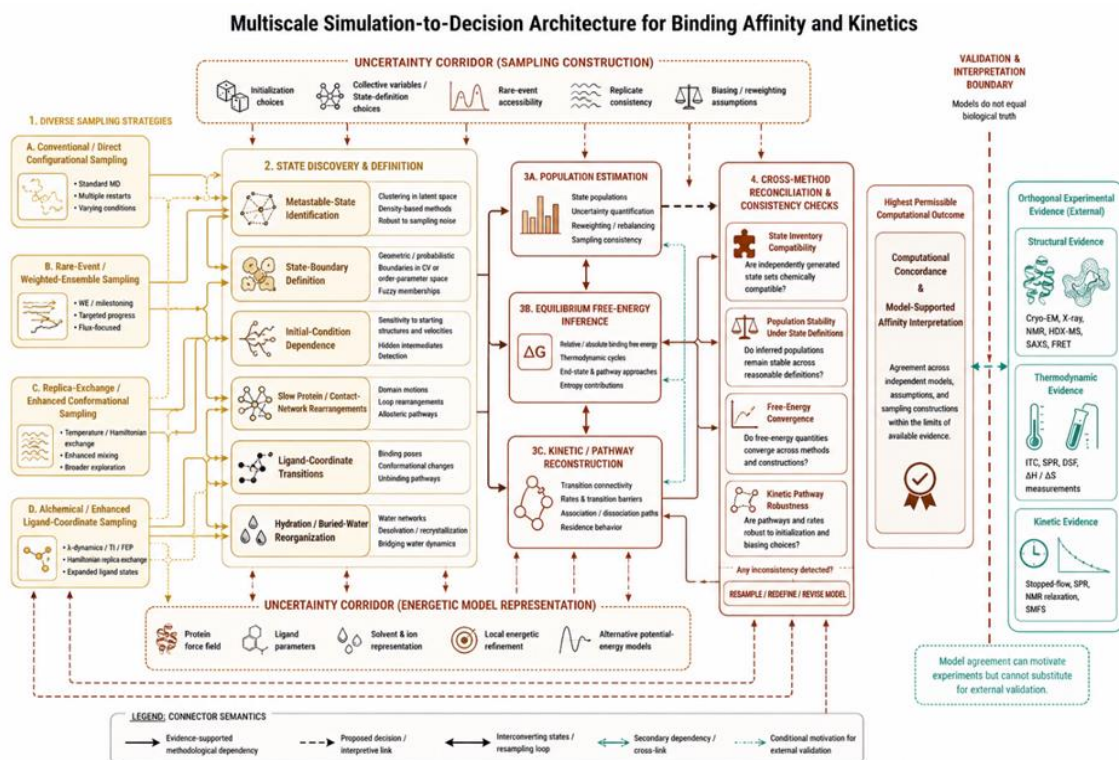


Figure 2. Sampling Agreement Has Distinct Boundaries Before Affinity Becomes a Mechanistic Inference

An ensemble-based affinity interpretation framework

The proposed framework treats affinity interpretation as a sequence of evidence states rather than a search for one privileged structure. First, structural observations define realizable configurations. Second, sampling establishes candidate states and their connectivity. Third, reproducible population estimates determine which states carry statistical weight under a specified model. Fourth, energetic calculations connect those populations to equilibrium observables, while kinetic analyses interrogate barriers and pathways separately. None of these stages substitutes for the next.

The framework therefore distinguishes population uncertainty from potential-energy uncertainty. Changing the energetic representation can change predicted affinity without changing the nominal state inventory [25]. Conversely, broader sampling can expose states while leaving their relative energies inaccurate. A mechanistic claim becomes stronger only when the inferred state organization, population weighting, observable calculation, and orthogonal validation are mutually compatible.

This ordering also prevents a common inference error: using a high-quality ligand-specific structure as evidence that the corresponding receptor state is highly populated. Structure-prediction methods can generate ligand-conditioned receptor conformations [21], but they do not turn generated structures into equilibrium probabilities. Likewise, a kinetic model can identify plausible barriers without establishing the equilibrium weight of every intermediate. The framework therefore asks which quantity has actually been estimated before allowing a structural, thermodynamic, or kinetic interpretation to cross into another evidential level.

Table 2 converts this logic into decision states. Its purpose is not to rank methods but to specify what inference is permissible at a given level of information and what observation should force reassessment.

Table 2. Decision States for Interpreting Affinity When Structural Populations and Simulation Reliability Diverge

Proposed decision state	Molecular or chemical state available	Observable or model output	Manuscript-specific consequence	Unresolved uncertainty	Permissible action	Reassessment trigger
Structural anchor	One or several plausible complex geometries	Pose or experimentally resolved structure	Describe interactions without assigning population dominance	State occupancy and transitions	Generate explicit ensemble hypotheses	Alternative conformers or inconsistent ligand behavior
Sampled but population-unstable	Multiple metastable configurations	State inventory with sampling-sensitive weights	Treat heterogeneity as plausible, not quantitatively resolved	State definition and coverage	Expand or alter sampling	Population shifts across reasonable protocols
Population-stable but energy-sensitive	Reproducible state organization	Stable weights with model-dependent affinity	Separate conformational support from energetic confidence	Force field, solvent, ligand energetics	Compare energetic representations	Affinity changes despite stable state coverage
Thermodynamically supported, kinetically unresolved	Converged equilibrium estimate	Affinity or free-energy agreement	Permit equilibrium interpretation without pathway claims	Association and dissociation barriers	Add kinetic or rare-event analysis	Different kinetic mechanisms fit the same equilibrium result
Cross-domain concordance	Compatible structural, population, thermodynamic, kinetic, and orthogonal observations	Multiple mutually consistent observables	Support the strongest ensemble-based interpretation available	Remaining system and transfer boundaries	Use as a mechanistic working model	New ligand, target state, or experiment breaks concordance

Table note. All decision states are proposed interpretive categories. They are not validated scores, universal thresholds, or evidence grades.

Testable computational predictions

The framework yields falsifiable expectations rather than a claim of prospective validation. For buried or gated sites, improving pathway-access sampling should change mechanistic consistency when access states are genuinely limiting; prior-informed adaptive sampling provides a concrete strategy for testing this proposition [26]. If the final bound pose is unchanged while access-state populations or transition pathways shift, kinetics should be affected more readily than equilibrium structural interpretation.

A second prediction is that metastable-state representations can outperform reliance on a single nominal minimum when ligand recognition depends on minority conformations. Multiple independent simulations of aptamer–small-molecule recognition provide an example in which metastable structures improved identification of ligand-favorable configurations [27]. Transfer to protein targets remains a hypothesis requiring target-specific validation. A further prediction concerns perturbational consistency. If a ligand is proposed to gain affinity by stabilizing a minor receptor state, then experimentally or computationally shifting the pre-binding population of that state should alter the inferred binding behavior in the predicted direction without requiring a different final pose. The HSP90 evidence establishes that ligand-dependent conformational flexibility can accompany distinct thermodynamic and kinetic behavior [1], but the proposed perturbational test is stricter: it asks whether state-population manipulation tracks the affinity mechanism rather than merely correlating with it.

For medicinal-chemistry use, this distinction matters most when two analogues preserve the same contact pattern. The framework predicts that such analogues can still diverge through altered state weighting, ligand torsional populations, hydration, or transition barriers. A failure to reproduce those differences after exhaustive state and energy-model checks would count against an ensemble-based explanation rather than being reclassified automatically as hidden conformational complexity.

More generally, disagreement among state populations, free energies, and kinetics should be diagnostically partitionable. If changing sampling alters populations while the potential is held fixed, sampling insufficiency is implicated; if changing the energetic model alters affinity despite stable state coverage, energetic error is implicated. These are proposed diagnostic tests, not established universal decision rules.

CONCLUSION

Drug–target affinity cannot be reduced safely to the geometry of a single bound complex when relevant protein, ligand, and solvent states are heterogeneous and interconverting. The stronger interpretation is population-based but explicitly conditional: state identity, occupancy, energetic representation, and kinetic connectivity must remain distinct. A structure defines a realizable molecular arrangement; an ensemble supplies statistical context; thermodynamics assigns equilibrium consequence; kinetics describes access and escape. The proposed framework is therefore an interpretive discipline rather than a validated predictive model. Its value will depend on whether independent sampling, energetic perturbation, kinetic analysis, and orthogonal experiments can falsify or refine the population assignments on which an affinity mechanism is based.

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