



Binding Kinetics in Natural Product Pharmacology and the Importance of Association, Dissociation, Residence Time, and Target Engagement for Lead Selection

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ABSTRACT

Natural-product lead selection remains strongly influenced by equilibrium potency and affinity, yet these descriptors do not determine how rapidly target occupancy develops, how persistently a ligand remains bound, or whether binding survives the transition from purified systems to cellular and in vivo environments. This state-of-the-art review examines association rate, dissociation rate, residence time, kinetic selectivity, and target engagement as complementary dimensions of natural-product pharmacology. The evidence indicates that association can be shaped by desolvation, access routes, and intermediate binding states, whereas dissociation can reflect local interactions, conformational organization, and escape barriers. Direct natural-product kinetic evidence remains limited, but macrocyclic Gαq inhibitors demonstrate that closely related natural-product-derived structures can display distinct residence behavior. Target-engagement, thermal-profiling, chemical-proteomic, biophysical, live-cell, and computational approaches provide additional information but measure different observables and should not be treated as interchangeable kinetic evidence. The review proposes a kinetics-aware lead-selection logic in which temporal binding is considered only when it resolves a pharmacologically relevant uncertainty and remains compatible with selectivity, exposure, biological context, and functional duration. Longer residence is therefore not treated as intrinsically advantageous. Major limitations include sparse matched natural-product kinetic datasets, assay dependence, imperfect transfer between purified and biological systems, and uncertainty in computational rate estimation. Progress will require orthogonal measurement, explicit kinetic models, cross-target profiling, and prospective tests of whether kinetic information improves decisions beyond conventional potency- and exposure-based prioritization.

Key Words: Binding kinetics, Natural products, Residence time, Target engagement, Kinetic selectivity, Lead selection

eJPPR 2025; 15(4):75-84

HOW TO CITE THIS ARTICLE: De Smet W, Van Dam L, Janssens P. Binding Kinetics in Natural Product Pharmacology and the Importance of Association, Dissociation, Residence Time, and Target Engagement for Lead Selection. Int J Pharm Phytopharmacol Res. 2025;15(4):75-84. <https://doi.org/10.51847/m47QprFmhi>

INTRODUCTION

Equilibrium affinity and potency remain foundational to pharmacology, but they compress a temporally evolving molecular process into a static or endpoint quantity. Association and dissociation kinetics provide different information: (k_{on}) describes the rate at which productive

target binding develops, whereas (k_{off}) describes how rapidly the formed complex is lost. Residence time is consequently related to persistence of the bound state under an appropriate kinetic model. Contemporary pharmacology increasingly recognizes that these variables can provide information not contained in an equilibrium measurement [1].

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Received: 10 March 2025; **Revised:** 12 July 2025; **Accepted:** 12 July 2025

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The translational significance of that information is conditional rather than automatic. A favorable kinetic phenotype can matter only if it is expressed within the relevant concentration range, cellular compartment, target-turnover regime, and pharmacodynamic timescale. Binding kinetics therefore complements rather than supersedes potency, pharmacokinetics, and biological context [2].

Natural products make this distinction particularly consequential. They remain major sources of drugs, probes, and mechanistic scaffolds and occupy chemically diverse regions of bioactive space [3]. Yet natural-product optimization still frequently moves from activity to affinity, target identification, selectivity, and developability without asking whether the temporal behavior of binding changes the interpretation of an otherwise promising lead. The central question of this review is therefore not whether slow dissociation is universally desirable, but when association, dissociation, residence behavior, and target engagement provide information capable of changing a lead-selection decision.

From equilibrium binding to time-resolved target interaction

Contemporary natural-product discovery combines improved isolation, dereplication, screening, target identification, structural characterization, and medicinal chemistry [4]. Within that workflow, binding kinetics represents an additional analytical dimension rather than a

separate discovery philosophy. The relevant comparison is between what equilibrium and time-resolved measurements can establish. Equilibrium affinity reports the relative distribution of bound and unbound states under defined conditions; it does not specify the temporal route by which the system reached that distribution or how rapidly the complex will disappear when free ligand concentration changes.

This distinction is particularly plausible in natural-product chemical space because natural-product-derived drugs and chemical probes display substantial structural, stereochemical, and physicochemical diversity [5]. Such diversity may create varied encounter routes, desolvation requirements, conformational constraints, or exit barriers. However, chemical complexity by itself cannot establish a kinetic advantage.

Target identification creates another boundary. Modern natural-product studies increasingly combine labeled, label-free, proteomic, computational, and orthogonal strategies to establish which proteins interact with a bioactive compound [6]. A confirmed target is necessary for meaningful kinetic interpretation, but target identification does not disclose the rates of association or dissociation. The state-of-the-art synthesis adopted here therefore admits multiple evidence classes while preserving their inferential limits.

Table 1 formalizes the eligibility logic used to distinguish direct kinetic evidence from contextual evidence that supports—but does not itself constitute—a kinetic claim.

Table 1. Evidence eligibility for a state-of-the-art synthesis of time-resolved natural-product pharmacology

Evidence category	Eligible contribution	Interpretive requirement	Inference not permitted
Direct kinetic experiments	Measured association, dissociation, or time-dependent binding	Assay conditions and kinetic model must be explicit	Rates treated as context-free constants
Structure–kinetic studies	Links defined structural changes with temporal binding	Restrict mechanism to the studied scaffold–target system	Universal structural rules
Natural-product analogue studies	Tests kinetic consequences within related compounds	Preserve chemical-family and target boundaries	General natural-product superiority
Target-engagement studies	Shows interaction in cellular or proteomic context	State the assay-specific observable	Engagement converted into (k_{on}) or (k_{off})
Structural/dynamic evidence	Supports hypotheses about stabilization or escape	Interpret with kinetic measurements where available	Static structure treated as rate measurement
Computational kinetics	Predicts pathways, barriers, or kinetic quantities	State sampling and model limitations	Prediction described as experimental measurement
PK–occupancy–PD evidence	Connects binding persistence with biological duration	Consider exposure, rebinding, and turnover	Residence time treated as the sole determinant

Note: Categories define evidence eligibility and inferential boundaries; they are not a hierarchy of methodological quality.

Figure 1 makes the central conceptual distinction visible: equivalent equilibrium descriptions can arise from—and

subsequently generate—different temporal binding behavior.

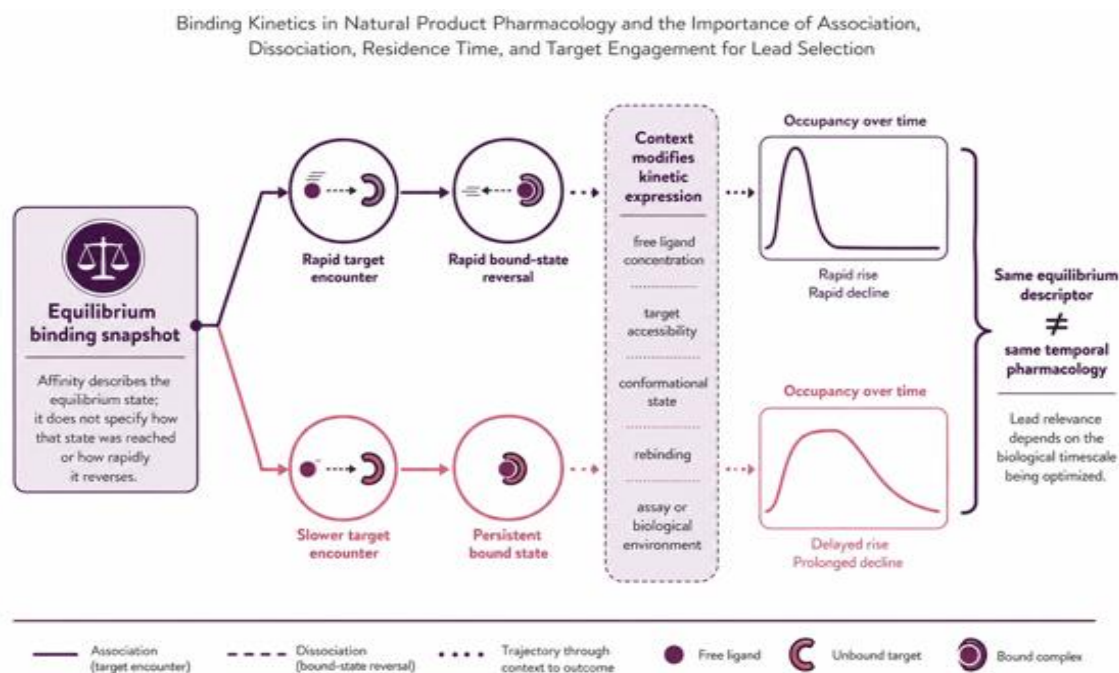


Figure 1. Equilibrium equivalence can conceal divergent temporal binding trajectories

Association rate as an underexamined determinant of target access

Association rate is often treated as secondary to residence time, yet target access can be pharmacologically limiting when exposure is brief or when competing ligands, transport processes, or changing compartments restrict the opportunity for productive binding. Drug-discovery analyses therefore increasingly treat (k_{on}) as an independent kinetic variable rather than as a mathematically convenient component of equilibrium affinity [7].

Association need not be a simple diffusion-controlled collision. Ligand desolvation, access-channel geometry, electrostatics, membrane partitioning, and receptor conformational gating can shape how rapidly a productive complex forms. Work on Hsp90 inhibitors provides a direct physical example: changes in ligand desolvation were linked to differences in on-rate and overall residence behavior within a related inhibitor series [8]. This finding does not establish desolvation as a universal determinant, but it demonstrates that energetic costs incurred before the fully bound state can influence kinetic optimization.

For natural products, association deserves particular attention when scaffolds are large, conformationally restricted, amphiphilic, or capable of multiple transient contacts. These features may create slow encounter without necessarily producing weak equilibrium affinity. Conversely, a rapid association rate may be of little value if exposure is insufficient or if the same kinetic advantage applies to an unwanted target. Association should therefore be interpreted as part of a target- and context-specific

temporal phenotype, not as an independently maximized property.

Dissociation rate and the persistence of the bound state

Association defines arrival; dissociation defines persistence. The molecular path between a fully bound complex and complete separation may include rearrangement of local contacts, conformational changes, partial escape states, and transient rebinding. Evidence from bitopic β 2-adrenoceptor ligands supports the participation of a metastable binding site in the overall binding pathway, illustrating that ligand entry and exit can involve intermediate states rather than a single elementary step [9].

Natural-product pharmacology provides unusually informative direct evidence through the FR900359/YM family of macrocyclic G α q inhibitors. Structure-affinity and structure-residence-time analysis demonstrated that closely related macrocycles can differ in persistence even when they belong to the same mechanistically coherent series [10]. This finding is important because it shows that residence behavior can be chemically tunable within natural-product-derived space rather than being an immutable property of a scaffold class.

Dissociation rate should nevertheless be interpreted mechanistically. Slow disappearance of a bound complex may reflect genuinely high exit barriers, but apparent slow dissociation can also be influenced by rebinding, heterogeneous states, assay design, or inappropriate model fitting. Consequently, a small (k_{off}) is best treated as an observation requiring explanation. For lead selection, the central question is whether persistent binding

occurs at the intended target, under relevant conditions, for a duration that supports rather than conflicts with the intended pharmacology.

Residence time: useful descriptor, frequent oversimplification

Residence time offers a convenient temporal description of persistence and is often approximated as $(1/k_{\text{off}})$ for a simple reversible one-step interaction. Its intuitive appeal can also encourage overinterpretation. Structural NMR work on adenosine A2A receptor ligands demonstrates that residence differences can be associated with distinct receptor–ligand structural behavior, strengthening the case for interpreting persistence through dynamic molecular mechanisms rather than treating residence time as an isolated numerical endpoint [11].

At the same time, target-family profiling shows that kinetic behavior varies widely across drug–target pairs [12]. A “long” residence time therefore has no target-independent meaning. What matters is its relationship to the competing timescales of drug exposure, target turnover, signaling, tissue redistribution, and off-target occupancy.

Three distinctions are especially important. First, long residence does not guarantee sustained functional effect if downstream signaling terminates independently. Second, slow dissociation may be undesirable when the same persistence occurs at an adverse off-target. Third, residence time inferred from a simplified model may conceal multistate binding or experimental artifacts.

The useful role of residence time is therefore comparative and contextual. It can discriminate otherwise similar leads or help explain temporal pharmacology, but it should not become a universal optimization target. A kinetic measurement becomes valuable when it resolves a biologically meaningful question, not merely because it produces an additional descriptor.

Slow-onset and time-dependent natural product binding

Slow onset or delayed equilibration can arise from slow association, conformational adjustment, membrane access, multistep binding, or other processes. These mechanisms should not be inferred from a time-dependent functional response alone. Matched-target studies such as the FAK/PYK2 analysis show that structural dynamics can produce distinct kinetic behavior between closely related targets, emphasizing that temporal binding is often target-specific rather than a generic property of a ligand [13].

This point is important for natural products because phenotypic assays may reveal delayed or durable responses before the underlying target interaction is fully characterized. Contemporary target-engagement technologies can help determine whether a proposed

molecular interaction is detectable in increasingly biological settings [14]. However, engagement is a distinct evidentiary layer.

CETSA and thermal proteome profiling are particularly relevant because they can support target identification and engagement studies for natural products without always requiring derivatization of the active molecule [15]. Their readout is a ligand-associated change in protein thermal behavior, not a direct association or dissociation rate.

Accordingly, slow-onset natural-product pharmacology should be decomposed experimentally. Direct kinetic assays can test whether binding itself is slow. Target-engagement measurements can determine whether the interaction survives cellular complexity. Functional time courses can then establish whether kinetic persistence corresponds to biological duration. Only concordance across these layers supports an interpretation in which slow or sustained pharmacology originates from the binding process itself.

Structural determinants of kinetic behavior

Natural products frequently contain structural features that plausibly influence kinetics: macrocyclic topology, conformational preorganization, dense stereochemistry, heterogeneous polar surfaces, and extended hydrophobic contacts. Direct evidence remains scaffold-specific. Mechanistic investigation of FR900359-related Gαq inhibitors linked their persistent binding behavior to interactions within the target pocket, including lipophilic contributions associated with the binding and unbinding process [16]. Combined with analogue-based residence measurements, this supports structure–kinetic reasoning within a defined natural-product family.

The broader inference must remain restrained. Macrocyclization does not inherently confer slow dissociation, and lipophilicity does not constitute a general route to useful residence time. Structural properties can alter association, binding-site organization, membrane behavior, and off-target interactions simultaneously.

Experimental target discovery introduces another structural complication. Chemical proteomics can accelerate identification of natural-product targets, but installation of affinity tags, reactive handles, or other probe elements can modify the parent molecule’s physicochemical and pharmacological behavior [17]. A probe-derived target assignment therefore cannot automatically establish the native compound’s kinetic phenotype.

The proposed medicinal-chemistry principle is conditional: structural features should generate hypotheses about encounter and escape, but kinetic measurements within matched analogue series should determine whether those hypotheses are valid. Structural explanation is

strongest when modification, measured rates, and mechanistic evidence converge without requiring extrapolation beyond the studied chemical series.

Measuring kinetics in complex natural product systems

Kinetic measurements are only as interpretable as the experimental system and binding model used to generate them. Live-cell fluorescence approaches can quantify time-dependent ligand binding at cell-surface receptors while retaining features absent from purified systems, including membrane composition, local receptor environment, and competing cellular processes [18]. Their added biological context is accompanied by tracer dependence, receptor-expression effects, nonspecific fluorescence, and assumptions required for kinetic fitting. Label-free biophysical approaches provide a complementary route. Biolayer interferometry can extract association and dissociation information from time-

resolved sensor responses and is increasingly applied across drug-discovery workflows [19]. Yet immobilization, mass transport, analyte behavior, surface density, and model choice can influence apparent kinetic parameters. Natural products with limited solubility, aggregation propensity, strong nonspecific adsorption, or unusual optical properties may intensify these problems. No assay should therefore be treated as automatically definitive. A robust kinetic phenotype is more convincing when it survives changes in assay principle or biological context. Disagreement between methods should not simply be averaged away: it can reveal transport limitation, tracer perturbation, multistate binding, compound interference, or an inappropriate kinetic model.

Table 2 organizes the experimental domains represented in the evidence base according to what each can directly measure and where interpretation becomes less transferable.

Table 2. Experimental domains represented in the binding-kinetics evidence base and the information each contributes

Evidence domain	Representative use	Primary observable	Distinctive contribution	Principal limit
Direct kinetic binding	Drug–target rate profiling [7]	Association/dissociation	Separates affinity into temporal components	Purified-system dependence
Structure–kinetic chemistry	Hsp90 desolvation analysis [8]	Rate changes across analogues	Links modification with kinetics	Scaffold-specific mechanism
Target-family profiling	Drugged kinome survey [12]	Cross-target kinetic profiles	Reveals kinetic selectivity	Defined target-family scope
Natural-product macrocycles	Gaq inhibitor series [10]	Affinity and residence behavior	Direct natural-product kinetic evidence	Limited chemical family
Live-cell binding	Fluorescent receptor kinetics [18]	Cellular time-dependent binding	Retains cellular constraints	Tracer/expression effects
Label-free biophysics	Biolayer interferometry [19]	Binding sensorgrams	Orthogonal kinetic measurement	Immobilization/mass transport

Note: The domains are complementary rather than interchangeable; apparent rate constants should be interpreted within the architecture that produced them.

Figure 2 translates these methodological distinctions into a proposed challenge sequence for determining whether an

apparent natural-product kinetic phenotype is sufficiently robust to influence interpretation.

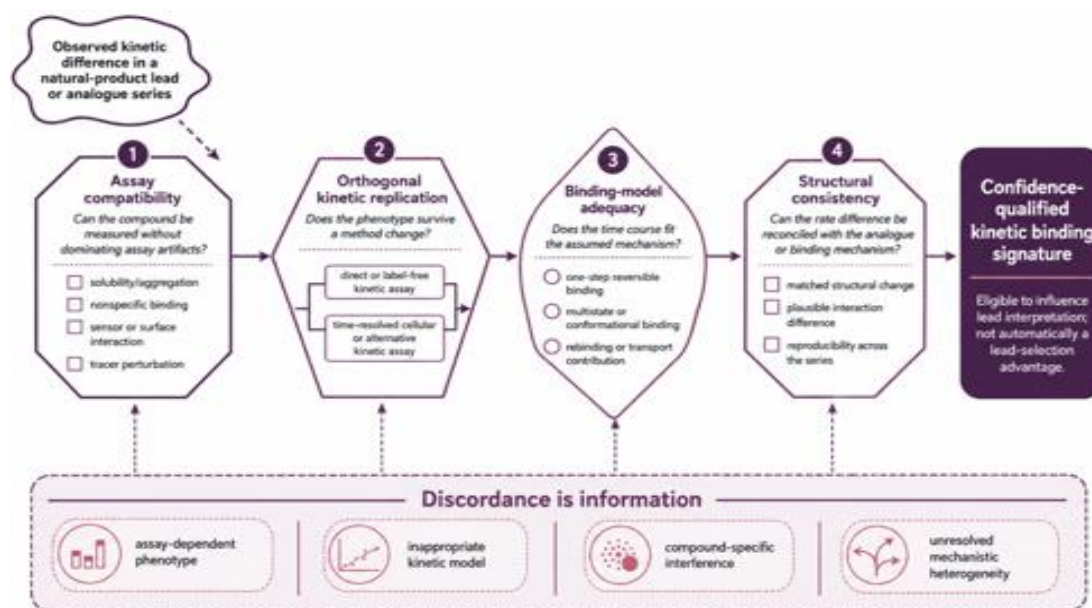


Figure 2. From an apparent kinetic phenotype to an interpretable natural-product binding signature

Computational estimation of association and dissociation processes

Binding and unbinding events can occur on timescales that make brute-force molecular simulation impractical. Enhanced-sampling approaches therefore aim to accelerate transitions while preserving sufficient physical information to characterize pathways, thermodynamics, and, under appropriate conditions, kinetic behavior. LiGaMD3 illustrates this direction by extending Gaussian accelerated molecular dynamics to improve calculations of binding thermodynamics and kinetics for benchmark small-molecule and peptide systems [20].

For natural products, computational kinetics is attractive because it can interrogate molecular events that experimental rate constants alone do not reveal: transient encounter states, conformational gating, alternative exit routes, solvent rearrangement, and local barriers. These predictions can help prioritize structural modifications or explain why matched analogues differ.

The evidentiary distinction is nevertheless essential. A simulated escape pathway is a model-based mechanistic hypothesis, not direct observation of dissociation. Estimated (k_{on}) or (k_{off}) values depend on force fields, sampling completeness, state definitions, treatment of solvent and ions, and conversion from accelerated dynamics to physical time. Natural-product flexibility, macrocyclization, stereochemical complexity, protonation, and membrane partitioning can further complicate transfer.

Computational kinetics is therefore most valuable when it generates experimentally testable differences rather than when it simply reproduces a fitted rate retrospectively. Prospective prediction across matched natural-product

analogue series, followed by blinded experimental kinetic measurement, would provide a stronger test of practical utility.

Cellular target engagement versus purified-system kinetics

Purified-system kinetics and cellular target engagement occupy different positions in the evidentiary chain. A biochemical experiment can resolve association and dissociation with high temporal precision while excluding membrane transport, intracellular sequestration, protein competition, metabolism, and dynamic target abundance. Conversely, cellular engagement can demonstrate that an interaction remains detectable under biological constraints without necessarily resolving the elementary binding rates. The translational importance of this distinction is illustrated by analyses showing that drug-target residence time affects in vivo occupancy through multiple pathways rather than through a simple one-to-one relationship [21]. Free concentration, repeated association, rebinding, distribution, and target turnover can all influence the persistence of occupancy.

For natural-product pharmacology, this means that neither purified kinetics nor cellular engagement should be privileged as universally “more real.” They answer different questions. Purified kinetics establishes the temporal properties of a defined molecular interaction under controlled conditions. Cellular engagement tests whether the proposed interaction survives a more complex environment. In vivo occupancy introduces exposure and biological turnover.

A defensible mechanistic chain therefore moves by concordance rather than substitution: direct rates should remain rates, engagement should remain assay-specific

evidence of interaction, and occupancy should remain a context-dependent state. Translational confidence increases when these layers tell a compatible temporal story, not when one measurement is assumed to stand in for all the others.

When binding kinetics change lead ranking

Kinetic characterization is most valuable when it changes a decision that affinity or potency alone leaves unresolved. Controlled manipulation of residence behavior at the vasopressin V2 receptor provides a useful example: an optical kinetic switch was used to alter ligand residence across in vitro, ex vivo, and in vivo models, demonstrating within that system that kinetic differences can propagate into pharmacological behavior [22]. This does not establish slow dissociation as a universal route to improved efficacy. Medicinal-chemistry studies at the same receptor further demonstrate that affinity and kinetic behavior can be optimized as partly separable properties within a congeneric series [23]. This distinction provides a practical criterion for natural-product programs. If two analogues have similar potency but differ in onset, duration, off-target persistence, or cellular engagement, kinetics can supply a mechanistically interpretable discriminator.

Kinetic data are less useful when they merely confirm a decision already dominated by exposure, selectivity, toxicity, or synthetic feasibility. They can also reverse an apparently favorable ranking: a lead with durable intended-target binding may become less attractive if the same persistent behavior occurs at a safety-relevant off-target. The proposed decision rule is therefore conditional. Measure kinetics when temporal behavior represents an unresolved development variable; interpret rates across the intended and competing targets; then determine whether the kinetic distinction survives biological context. Kinetic characterization should resolve uncertainty, not become an obligatory numerical layer added to every natural-product screening cascade.

Linking kinetic behavior to functional duration

The duration of target binding and the duration of pharmacological effect are related only when the relevant biological timescales align. Structural interrogation of histamine H1 receptor ligands demonstrates that binding-site changes can tune kinetic behavior within a defined receptor system [24]. Such studies make temporal medicinal chemistry experimentally tractable, but they do not establish that a longer-lived complex necessarily produces a longer or more desirable functional response. Small-molecule optimization therefore benefits from considering thermodynamic and kinetic profiles together

[25]. Affinity determines equilibrium preference; kinetics defines how rapidly that preference is approached and reversed. Exposure determines whether adequate free ligand reaches the target, while signaling and target turnover determine how binding is translated into function. For natural products, the translational question should consequently be framed as temporal compatibility. A slow (k_{off}) may be useful when exposure falls quickly but prolonged occupancy is desirable. Rapid association may be more important when target capture must occur during a short exposure window. Persistent binding may be irrelevant if signaling is rapidly desensitized or the target is replaced. It may be harmful if prolonged occupancy occurs at competing targets.

Functional duration should therefore be modeled as an emergent property of binding kinetics, exposure, target biology, and downstream response rather than being assigned directly to residence time. This preserves the mechanistic value of kinetic information without converting it into an unsupported efficacy surrogate.

Current evidence gaps in natural product pharmacology

The central gap is the scarcity of matched natural-product datasets in which structure, affinity, (k_{on}), (k_{off}), cellular engagement, exposure, and functional duration are characterized together. Computational approaches to ligand-binding kinetics are advancing rapidly, but challenges of sampling, cost, and system dependence remain substantial [26]. These limitations are likely to be especially important for flexible, macrocyclic, amphiphilic, or highly stereochemically complex natural products.

A second gap is methodological comparability. Force-field choice, sampling strategy, machine-learning data quality, benchmark selection, and usability remain recognized constraints in kinetic simulation [27]. Experimental studies have analogous problems: assay architecture, target construct, immobilization, tracer choice, concentration regime, fitting model, and temperature can all influence apparent rates.

The field also lacks sufficient negative evidence. Published examples often emphasize unusually persistent compounds or successful mechanistic explanations, creating a risk that kinetic optimization appears more universally tractable than it actually is. Studies in which analogue modification fails to alter kinetics, or in which predicted and measured rates disagree, are equally informative.

Table 3 distinguishes domains in which the evidence is comparatively mature from those where natural-product-specific translation remains underdeveloped.

Table 3. Where the evidence is strong, transferable, or still incomplete in natural-product binding kinetics

Evidence domain	Current support	Remaining uncertainty	Priority
General kinetic pharmacology	Rates add information beyond equilibrium affinity [1]	Universal superiority of any profile	Prospective ranking studies
Natural-product SKR	Gαq macrocycles show distinct residence behavior [10]	Generalization across NP classes	Diverse matched analogue series
Kinetic selectivity	Related targets can diverge temporally [13]	Transfer to NP polypharmacology	Multi-target kinetic panels
Cellular engagement	CETSA/TPP supports NP target work [15]	Direct rate inference	Combined rate/engagement studies
Computational kinetics	Enhanced sampling can estimate kinetic behavior [20]	Prospective accuracy in complex NP space	Blinded experimental benchmarks
Occupancy integration	Persistence interacts with PK and occupancy [21]	Predicting functional duration	Integrated exposure–occupancy–PD studies

Note: An unresolved domain indicates an evidence boundary rather than evidence that the proposed relationship is absent.

Methodological priorities for the next generation of studies

The next generation of studies should prioritize integration rather than accumulation of isolated residence-time measurements. First, matched analogue series should be designed so that structural changes are interpretable and not accompanied by uncontrolled shifts in aggregation, permeability, ionization, or target identity. Second, direct kinetic measurements should be repeated with an orthogonal method whenever compound properties make one platform especially vulnerable to artifacts.

Third, studies should report the assumed binding model rather than only the fitted rate constants. Time courses that are incompatible with a one-step reversible mechanism should trigger alternative models instead of being forced into apparently precise (k_{on}) and (k_{off}) values. Fourth, cross-target kinetics should be incorporated when selectivity is a development question, because intended-target persistence is pharmacologically incomplete without knowledge of competing-target persistence.

Computational studies require analogous discipline. Prospective prediction, external benchmarking, uncertainty reporting, and explicit state definitions would be more informative than retrospective explanation alone. Cellular target engagement should then test whether the kinetic hypothesis survives increasing biological complexity.

Finally, kinetic-aware ranking itself requires validation. A prospective study should compare conventional prioritization based on potency, affinity, selectivity, physicochemical properties, and exposure with an otherwise equivalent strategy that additionally

incorporates kinetic information. Only if later pharmacological evidence is better anticipated by the kinetic-aware strategy would the proposed framework acquire predictive support.

Review limitations

Natural-product target discovery has advanced through both labeled and label-free strategies, but these approaches introduce different biases and often establish target relationships without directly measuring binding rates [28]. This limits the amount of natural-product-specific evidence that can support broad conclusions about association, dissociation, or residence behavior. Several concepts discussed here are therefore informed by stronger general drug-target kinetic evidence and then transferred cautiously to natural-product lead selection.

Residence time also has conceptual limitations. Critical analysis has warned that it can become misleading when treated independently of free concentration, target turnover, rebinding, pharmacokinetics, and the simplifying assumptions of common kinetic models [29]. The present synthesis accordingly does not equate long residence with desirable pharmacology.

Evidence heterogeneity creates an additional limitation: purified biochemical rates, receptor assays, thermal proteomics, chemical proteomics, simulations, occupancy measurements, and functional studies interrogate different levels of the system. Numerical values across these modalities should not be treated as directly interchangeable.

Table 4 converts these limitations into proposed quality priorities for future natural-product kinetic studies.

Table 4. Proposed quality priorities for next-generation studies of natural-product binding kinetics

Priority	Minimum requirement	Rationale	Failure prevented
Confirm target identity	Orthogonal evidence for native-compound binding	Kinetics requires the correct target	Rates assigned to probe artifacts

State kinetic model	Binding scheme, assumptions, concentration regime	Rates are model-dependent	Precise but misleading parameters
Use orthogonal kinetics	Method with different dominant artifacts	Tests reproducibility	Platform-specific lead decisions
Preserve native chemistry	Parent/probe comparison when modified [28]	Derivatization may alter behavior	Probe kinetics generalized to parent
Profile relevant off-targets	Intended plus plausible competing targets	Tests kinetic selectivity	Persistent off-target binding overlooked
Add biological context	Cellular engagement where feasible	Tests transfer from purified systems	In vitro rates treated as occupancy
Integrate free exposure	Unbound concentration with temporal binding [21]	Duration depends on exposure and kinetics	Residence-time determinism
Report discordance	Fit uncertainty, failed models, negative findings	Defines reliability	Selective reporting
Test ranking prospectively	Compare kinetic-aware and conventional selection	Tests added decision value	Proposed framework treated as validated

Note: These priorities are proposed from the review synthesis; they are not validated reporting standards or regulatory criteria.

CONCLUSION

Binding kinetics extends natural-product pharmacology from the question of how strongly a ligand binds to the questions of how rapidly binding develops, how persistently it survives, and whether that temporal behavior remains relevant in biological context. Association, dissociation, residence time, kinetic selectivity, target engagement, exposure, and functional duration are related but non-equivalent evidentiary dimensions.

Current evidence supports kinetic-aware investigation more strongly than it supports any claim that natural products possess an intrinsic kinetic advantage. Direct natural-product structure–kinetic datasets remain limited, and computational, structural, target-engagement, and proteomic approaches should complement rather than substitute for measured rates.

The proposed lead-selection principle is therefore conditional: use kinetics when temporal behavior can resolve a real pharmacological uncertainty; establish the mechanism where possible; test the phenotype across targets and biological contexts; and reject kinetic optimization when it conflicts with exposure, selectivity, safety, or the intended timescale of action. Prospective natural-product datasets are required before this framework can be considered predictive..

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

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