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When Biochemical Potency Misrepresents Pharmacological Persistence: A Theoretical Analysis of Inhibitor Binding Kinetics, Target Resynthesis, and Pathway Recovery Following Transient Drug Exposure

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Abstract

Biochemical potency is often summarized by equilibrium measurements that describe how strongly an inhibitor suppresses or binds a target under a defined assay condition. Such measurements do not, by themselves, specify how long pharmacological effects persist once free drug concentrations decline. This theoretical analysis examines when that mismatch becomes consequential. Evidence from drug-target kinetic studies, target-engagement methods, covalent and reversible-covalent inhibition, target-turnover models, clinical Bruton tyrosine kinase pharmacodynamics, and post-washout experimental systems was integrated without generating new empirical data. The analysis separates four temporally distinct processes: disappearance of free inhibitor, loss or persistence of target occupancy, replacement of inhibited target by newly synthesized protein, and recovery of downstream pathway function. Published evidence supports a conditional rather than universal relationship between biochemical potency and persistence. Slow dissociation, irreversible or slowly reversible engagement, and sustained target occupancy can extend effects beyond plasma exposure, but their influence depends on target turnover, biological context, intracellular accessibility, and pathway architecture. Conversely, rapid target resynthesis or compensatory pathway activity can shorten functional persistence even when inhibitor-target engagement is durable. Target-level recovery and pathway-level recovery therefore need not occur on the same time scale. Pharmacological persistence is best interpreted as an emergent temporal property of interacting exposure, binding, target-renewal, and network-recovery processes rather than as a direct extension of equilibrium potency. Mechanistic claims about persistence should therefore specify which temporal process was measured and which remained inferred.

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Introduction

Biochemical potency measurements are indispensable for ranking compounds and establishing whether a molecular target can be modulated under controlled conditions, yet they compress time into an equilibrium or protocol-defined estimate. Pharmacodynamic duration, by contrast, unfolds after exposure changes. A drug-target complex may persist after free drug concentrations fall, while a covalently modified target may remain inhibited until replacement protein appears. Target abundance itself is dynamic, with synthesis and degradation rates that vary by tissue, disease state, and biological condition. These observations make pharmacological persistence a temporal problem in which binding and target turnover can become partly independent of the potency value obtained at a single assay time point (Gabrielsson & Hjorth, 2023; Knockenhauer & Copeland, 2024).

This distinction has become experimentally tractable. Medicinal-chemistry programs increasingly optimize association and dissociation behavior rather than treating affinity as the only binding attribute (Qiao *et al.*, 2025). In parallel, target-engagement methods can characterize whether a compound binds the intended protein in biochemical, cellular, or in-vivo settings, using assay families that differ in temporal resolution and mechanistic information (St John-Campbell & Bhalay, 2025). These developments allow a more precise question than whether a compound is simply potent: which process maintains target perturbation after free drug exposure becomes transient or negligible?

Covalent pharmacology sharpens the question because bond formation adds another time-dependent step. Reversible covalent kinase inhibitors demonstrate that covalency itself does not define permanent engagement (Zhao & Bourne, 2025), while the expanding repertoire of covalent warheads shows that intrinsic electrophile reactivity, target geometry, and reversibility must be considered jointly (Hillebrand *et al.*, 2024). Apparently durable inhibition may consequently arise from slow noncovalent dissociation, slowly reversible covalent chemistry, effectively irreversible modification, repeated rebinding during residual exposure, or combinations of these mechanisms. Compounds with

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similar biochemical potency can therefore differ substantially in how inhibition is sustained after the original exposure condition changes.

A theoretical account must also retain the strongest counterargument to kinetic simplification. Residence time can become misleading when considered outside pharmacokinetic context, because continued target-site exposure and rebinding may sustain occupancy even when intrinsic dissociation is relatively fast, whereas rapid loss of drug from the relevant compartment can constrain the influence of a slow molecular dissociation process (Folmer, 2018). The central problem is consequently not to replace potency with residence time as a superior scalar. It is to identify the conditions under which equilibrium potency ceases to predict persistence because several biological clocks—drug exposure, inhibitor-target complex lifetime, target replacement, and pathway recovery—become non-equivalent. The following analysis first separates the binding-level variables and then examines target resynthesis as an independent route toward pharmacological recovery.

Materials and Methods

Biochemical Potency, Binding Residence Time, and the Limits of Equilibrium-Based Interpretation

Equilibrium affinity and kinetic binding descriptors answer different questions. Affinity summarizes the thermodynamic tendency of ligand and target to occupy a bound state under defined conditions, whereas association and dissociation rates describe how rapidly that state is entered and left. Residence time is therefore a kinetic descriptor of complex persistence and cannot be inferred uniquely from affinity alone (Bernetti *et al.*, 2019). Mechanistic pharmacokinetic-pharmacodynamic models make this distinction explicit by allowing time-varying exposure, binding kinetics, and target turnover to contribute separately to target occupancy (Daryaee & Tonge, 2019). The separation becomes particularly important when the system is not at equilibrium or when biological response evolves on a time scale comparable with binding or target renewal.

Kinetic measurements can directly reveal slow dissociation that conventional endpoint potency assays obscure. Label-free biosensing strategies have been used to estimate affinity and residence time for high-affinity kinase-inhibitor complexes whose dissociation is difficult to resolve with standard formats (Quinn *et al.*, 2018). Such assays establish that a complex can be kinetically long-lived under the tested conditions, but they do not establish persistent cellular signaling inhibition. Immobilized targets, assay buffers, protein constructs, ligand depletion, and the absence of intracellular transport or target renewal create a boundary between a measured molecular rate and an inferred pharmacological duration.

Structure-kinetic studies further demonstrate why equilibrium similarity does not guarantee kinetic similarity. Histamine H1-receptor ligands can acquire markedly different residence-time behavior through structural modification (Kuhne *et al.*, 2025). At heteromeric nicotinic acetylcholine receptors, ligands with broadly comparable pharmacological properties can nevertheless exhibit heterogeneous association-dissociation fingerprints (Holmgård *et al.*, 2025). An equilibrium phenotype may therefore be compatible with more than one kinetic mechanism. The significance of a kinetic difference in a living system remains conditional on whether that binding process continues to be influential once exposure, cellular context, receptor abundance, and signaling dynamics are considered.

More direct evidence arises from perturbational systems in which kinetics can be varied within related chemical series. Optical switching of a vasopressin V2-receptor antagonist altered residence time together with downstream functional behavior, while related V2-receptor antagonists showed substantial kinetic divergence despite comparatively smaller changes in affinity (Cao *et al.*, 2023; Gu *et al.*, 2023). These studies support the proposition that binding duration can become pharmacologically consequential in a defined system. They do not establish a universal hierarchy in which residence time is inherently more informative than affinity; the V2-receptor findings remain dependent on the tested ligands, receptor system, exposure conditions, and biological models.

Binding mechanism adds another layer of temporal information. Methionine-selective nuclear magnetic resonance has distinguished inhibitor-induced conformational states in full-length Src, showing that kinase inhibition can correspond to different protein-state interactions (Fernández *et al.*, 2024). For covalent probes, observed target engagement depends jointly on noncovalent affinity, intrinsic electrophile reactivity, ligand size, concentration, and incubation time (Petri *et al.*, 2025). A single potency measurement is consequently vulnerable to protocol dependence in such systems. A concentration producing strong inhibition after prolonged incubation may reflect a different mechanistic balance from the same concentration tested after brief exposure, even though both observations can be expressed using the language of biochemical potency.

Covalent-allosteric inhibition further illustrates why a mechanistic category cannot substitute for direct evidence of pharmacological duration. Combining an allosteric recognition element with covalent chemistry may produce useful selectivity or prolonged engagement for some targets, but performance remains dependent on molecular recognition, accessible nucleophiles, chemical reactivity, and biological setting (Tao *et al.*, 2025). Similarly, optimized MerTK inhibitors with demonstrated in-vivo target engagement show that biochemical activity can translate into target binding within an organism (Frey *et al.*, 2024). Such engagement establishes biological access to the target, but it does not isolate dissociation kinetics as the cause of downstream duration. Exposure, target renewal, and network state remain plausible determinants.

Some systems nevertheless reveal a stronger relationship between kinetics and functional duration. In experimental models of vasopressin V2-receptor antagonism, residence time was more closely associated with ex-vivo and in-vivo inhibitory efficacy than affinity within the studied compound series (Zhang *et al.*, 2022). Structural and kinetic analysis of FAK inhibitors has likewise linked slow dissociation and selectivity over PYK2 to ligand-induced conformational differences supported by

crystallographic, mutational, kinetic, and simulation evidence (Berger *et al.*, 2021). These studies justify a conditional interpretation: binding kinetics can become more informative than equilibrium potency when the kinetic step remains influential in the full biological system, but that influence requires demonstration rather than assumption.

The analytical consequence is that biochemical potency, binding kinetics, target engagement, and pharmacological persistence occupy distinct inferential levels. Their relationships are summarized in **Table 1**; the separation prevents a measurement obtained at one biological level from being treated as direct evidence for persistence at another.

Table 1. Analytical distinctions among potency, binding kinetics, target engagement, and pharmacological persistence

Analytical unit	What is directly characterized	Principal temporal information	What it can legitimately support	What remains unresolved
Equilibrium biochemical potency	Concentration-dependent inhibition or binding under a specified assay condition	Usually limited to the assay endpoint and incubation protocol	Relative activity under that assay configuration	How rapidly binding forms or dissociates; persistence after exposure falls
Association–dissociation kinetics	Rates of entering and leaving the inhibitor-target complex	Explicit molecular binding time scale	Whether kinetically distinct complexes can exist despite similar equilibrium behavior	Whether the kinetic difference remains rate-limiting in cells or organisms
Reversible or irreversible covalent engagement	Combination of recognition, chemical reaction, reversibility, and occupancy	Reaction- and reversal-dependent engagement duration	Whether covalent chemistry changes the lifetime of target modification	How quickly active target is regenerated and how signaling adapts
Cellular or in-vivo target engagement	Fraction or state of target bound or modified in its biological context	Exposure-dependent occupancy over sampled times	Whether biochemical activity translates into target binding in the relevant system	Whether downstream function persists for the same interval
Pharmacological persistence	Duration of functional effect after changing or terminating exposure	Integrated system-level recovery time	Net consequence of exposure, binding, target renewal, and network recovery	Which component is causally rate-limiting without additional measurements

Target Resynthesis and the Temporal Dynamics of Pharmacological Recovery

Durable inhibitor-target engagement has a finite biological consequence if uninhibited target is continually synthesized. This point is clearest for effectively irreversible inhibitors, for which dissociation of the originally modified complex may contribute little to recovery while pharmacological activity still wanes because newly synthesized target enters the active pool. A mechanistic BTK pharmacokinetic-pharmacodynamic analysis explicitly connected target engagement and target turnover with in-vivo response, illustrating why recovery after covalent inhibition cannot be inferred from drug elimination alone (Daryaee *et al.*, 2017). Target resynthesis therefore constitutes a recovery route mechanistically distinct from inhibitor dissociation.

Clinical BTK pharmacology provides direct evidence that this process matters in humans. In chronic lymphocytic leukemia, de novo BTK synthesis affected the temporal pattern of covalent target occupancy, while dosing frequency altered occupancy and pathway inhibition (Sun *et al.*, 2020). These observations do not establish that a particular schedule is universally superior or that differences in occupancy necessarily determine long-term clinical outcomes. They demonstrate something narrower and mechanistically important: once existing target molecules have been covalently inhibited, appearance of newly synthesized target can determine how rapidly pharmacologically available protein re-emerges.

Dose interruption makes this distinction especially informative because continuing drug exposure is reduced as an alternative explanation. Following interruption of acalabrutinib, free BTK regenerated through new protein synthesis, while downstream pharmacodynamic measures did not recover in parallel (Alsadhan *et al.*, 2020). The divergence shows that restoration of target availability and restoration of pathway function are separate events. Newly available target may appear while selected downstream states remain suppressed, and downstream activity may also depend on processes beyond the abundance of the original target. A single occupancy measurement therefore cannot specify the complete recovery state of the signaling system.

Quantitative systems pharmacology provides one means of representing these competing processes when direct sampling of all relevant tissues is impractical. A clinical-data-informed BTK model incorporated systemic and intracellular pharmacokinetics, target binding, and BTK turnover to simulate occupancy across biological compartments and treatment-interruption scenarios (Demin *et al.*, 2025). Its importance here is structural: exposure, binding, and protein renewal can be represented as distinct temporal processes whose relative influence changes during and after treatment. Predictions for compartments lacking direct measurements remain model outputs, however, and cannot be

treated as observations merely because the model reproduces available clinical data.

Washout experiments in bacterial systems provide an independent mechanistic test of the target-renewal principle. Translation of inhibitor residence time into post-antibiotic activity depended on target vulnerability and on the capacity of the system to regenerate active target after compound removal (Basu *et al.*, 2021). This evidence cannot provide quantitative predictions for human pharmacology because bacterial physiology, target vulnerability, and network organization differ fundamentally from mammalian therapeutic settings. It nevertheless demonstrates experimentally that a long-lived inhibitor-target complex does not guarantee equally long functional suppression if enough active target can be restored.

These observations permit recovery after transient exposure to be partitioned according to the event that restores pharmacological capacity. **Table 2** separates changes in exposure, engagement, target abundance, and system state so that recovery is not reduced to a single decay process.

Table 2. Mechanisms that can restore pharmacological capacity after transient inhibitor exposure

Recovery mechanism	Biological event that restores activity	Relationship to prior target engagement	Condition under which it may dominate	Principal interpretation hazard
Declining target-site exposure	Free inhibitor concentration falls below that required to sustain new binding	Existing complexes may remain occupied or modified	Binding is rapidly reversible and rebinding becomes negligible	Attributing recovery to fast dissociation when local exposure was the limiting process
Dissociation or reversible bond cleavage	Previously occupied target becomes available again	Directly terminates engagement of the same target molecule	Target turnover is slow relative to complex reversal	Treating an in-vitro dissociation rate as the whole-system recovery rate
Target resynthesis or replacement	Newly synthesized uninhibited protein expands the active target pool	Can restore function while originally engaged molecules remain inhibited	Protein turnover is rapid or biological state accelerates renewal	Assuming effectively irreversible engagement implies irreversible pharmacology
Pathway re-equilibration	Existing signaling architecture moves toward its pre-inhibition state	May lag behind restoration of target availability	Downstream states retain temporal memory or require sequential resetting	Using a single downstream marker as a surrogate for global recovery
Compensatory or bypass activation	Alternative signaling routes restore output without recreating the original target state	Can permit functional recovery despite continuing engagement	Network redundancy or adaptive regulation is sufficiently strong	Misclassifying network escape as loss of target engagement

The interaction among inhibitor exposure, occupancy, protein renewal, and downstream recovery is therefore better represented as partially independent temporal layers than as a single decay trajectory. **Figure 1** places the molecular binding process within a broader recovery landscape in which target resynthesis can erode occupancy-based persistence while downstream signaling retains its own temporal behavior.

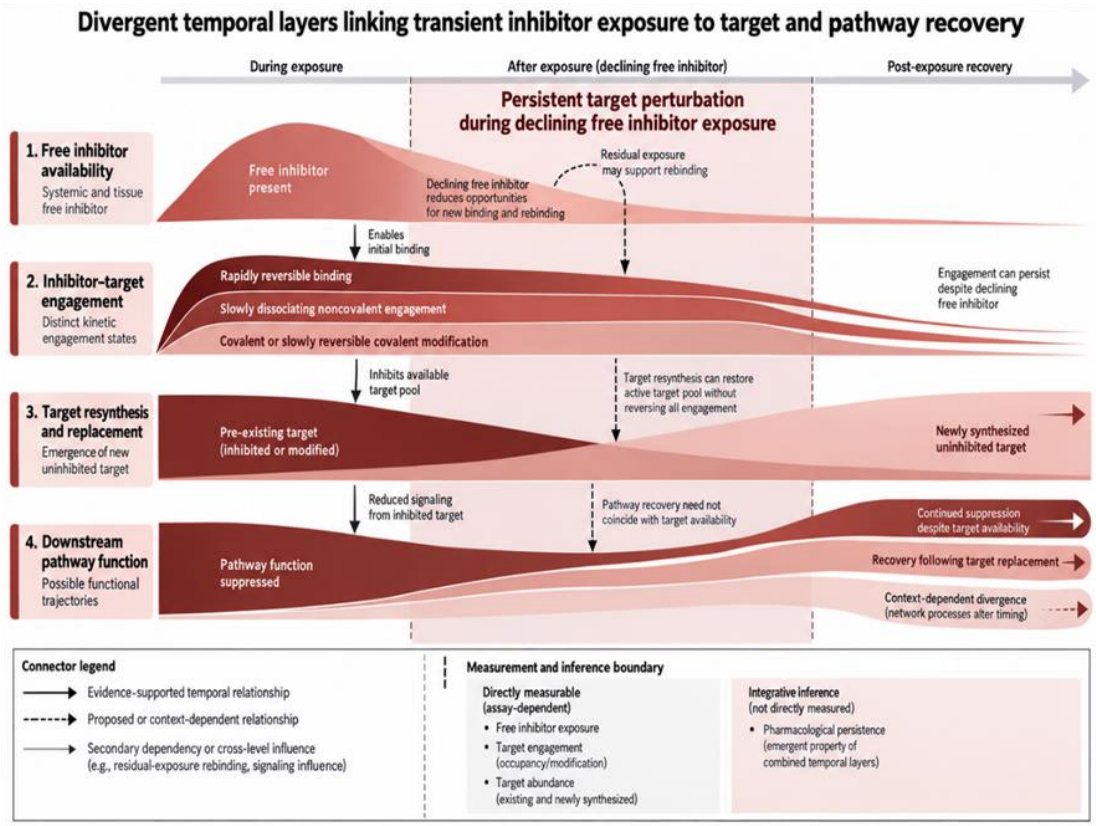


Figure 1. Divergent temporal layers linking transient inhibitor exposure to target and pathway recovery

Pathway Recovery Following Transient Inhibitor Exposure

Recovery of a signaling pathway after transient inhibition cannot be inferred solely from the return of unoccupied target. Pathways contain feedback loops, alternative signaling routes, transcriptional responses, and state-dependent regulatory mechanisms that can restore downstream function even while direct target inhibition remains substantial. KRASG12C systems illustrate this distinction particularly well. Following conformation-specific KRASG12C inhibition, a subset of cells can synthesize new KRASG12C that enters a drug-insensitive active state, while receptor-mediated activation of wild-type RAS can independently restore MAPK signaling (Ryan *et al.*, 2020; Xue *et al.*, 2020). These mechanisms occupy different causal levels: one replenishes pharmacologically available target, whereas the other bypasses the inhibited target through network reorganization.

Direct measurement of target occupancy is therefore valuable because it separates failure of engagement from failure of downstream control. Quantitative mass spectrometry has been used to measure covalent KRASG12C engagement in mouse tumors while simultaneously relating occupancy to MAPK pharmacodynamic effects (Tran *et al.*, 2023). Such measurements can show whether a compound continues to modify its molecular target after exposure, but correspondence between occupancy and pathway suppression remains an empirical question. The same distinction applies to covalent EGFR inhibition. Fluorosulfate-based inhibitors have demonstrated site-specific covalent engagement together with sustained cellular inhibition after washout (Tang *et al.*, 2023). These observations establish that target modification can outlast removal of extracellular compound in a defined system, yet they do not imply that all downstream functions must remain suppressed for the same interval.

Pathway recovery can also become progressively independent of the original binding event during prolonged treatment. Acquired resistance to KRASG12C inhibition has been associated with nongenetic changes involving ITGB4-linked AKT–mTOR signaling and WNT/β-catenin-associated states (Mohanty *et al.*, 2023). These mechanisms differ from immediate post-washout recovery because they emerge through sustained selection or adaptation rather than simply through dissociation or target replacement. Their relevance to pharmacological persistence lies in demonstrating that continued molecular engagement does not guarantee durable system-level control when the network can alter its dependency structure.

A related example is provided by YAP1/TAZ–TEAD signaling. Functional-genetic studies have shown that activation of this transcriptional program can compensate for suppression of KRAS effector signaling and contribute to both primary and acquired resistance (Edwards *et al.*, 2023). Such compensation creates a form of functional recovery in which the inhibited molecular target need not return to its original state. Consequently, the duration of target engagement and the duration of phenotypic suppression may

diverge because the pathway has acquired an alternative route to a similar downstream output.

Conversely, highly efficient covalent inactivation does not eliminate the need to identify what ultimately terminates the pharmacological effect. Covalent FGFR4 inhibitors based on suitably positioned electrophiles can achieve strong time-dependent inactivation together with cellular activity (Schwarz *et al.*, 2024). The kinetic efficiency of target inactivation remains distinct from the rate at which active FGFR4 is replaced or downstream pathway function is restored. Mutation-dependent binding kinetics reveal the opposite problem. In Abl kinase, clinically relevant mutations can preserve near-wild-type affinity while accelerating imatinib dissociation, demonstrating that equilibrium affinity may conceal a kinetically important resistance mechanism (Lyczek *et al.*, 2021). Together, these examples show that persistence can be shortened either because target engagement itself becomes unstable or because the biological system regains function despite continued engagement.

The mechanistic alternatives become clearer when target-level and pathway-level recovery are represented separately. **Figure 2** organizes persistent and rapidly reversible pharmacological effects according to the process most capable of terminating inhibition, while retaining the distinction between measured evidence, mechanistic interpretation, and context-dependent inference.

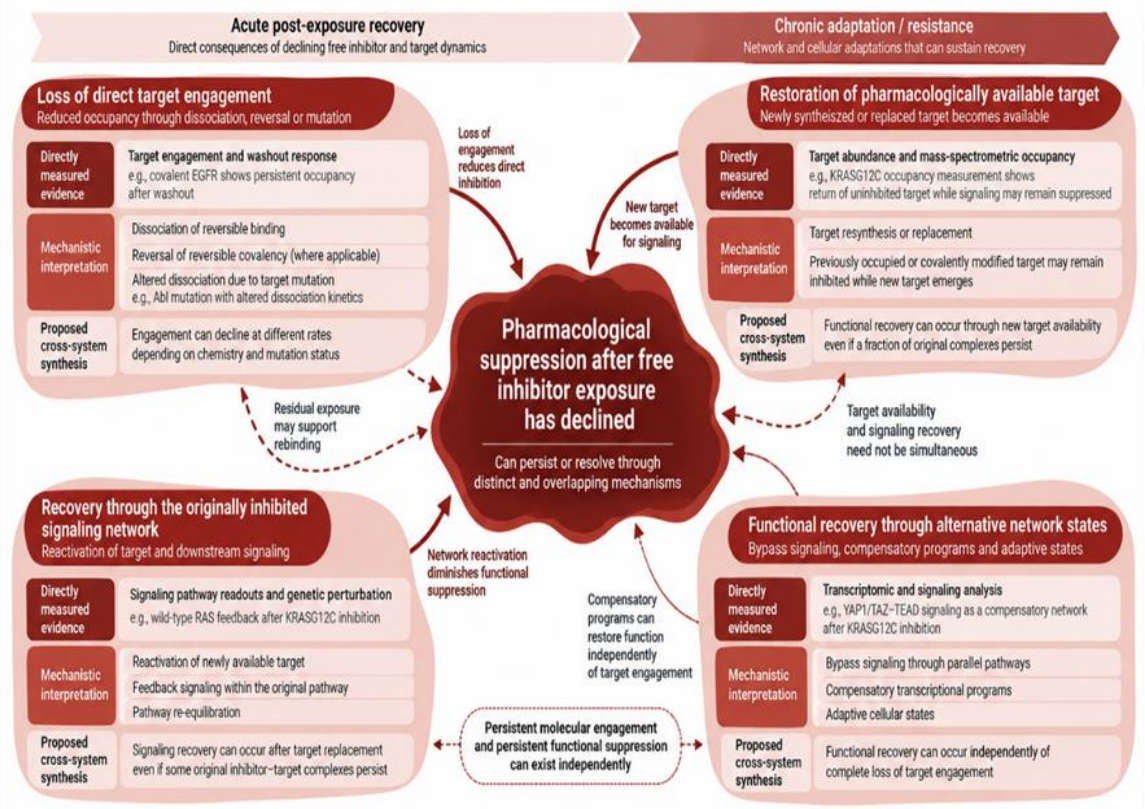


Figure 2. Mechanistic routes that uncouple target engagement from functional persistence

An Integrated Theoretical Model of Pharmacological Persistence

The evidence supports a view of pharmacological persistence as the outcome of several competing temporal processes rather than the direct extension of any single biochemical measurement. The first process is decline of pharmacologically relevant free drug. The second is persistence or disappearance of inhibitor-target engagement. The third is replacement of inhibited target by newly synthesized active protein. The fourth is recovery of downstream pathway function. Each process can become rate-limiting, but no evidence reviewed here supports a universal hierarchy among them. Binding kinetics are therefore most informative when they remain influential after pharmacokinetic exposure, target turnover, and network adaptation have been considered (Knockenhauer & Copeland, 2024).

Target turnover provides a particularly important reason why effectively irreversible molecular engagement need not generate equally persistent pharmacology. An inhibited protein can remain permanently modified while the biological system progressively replaces it with uninhibited protein (Gabrielsson & Hjorth, 2023). Under such conditions, the relevant recovery event is not dissociation of the original drug-target complex but expansion of a newly synthesized active target pool. Conversely, slowly reversible binding may remain pharmacologically important where target renewal is slow and residual exposure is insufficient to dominate occupancy. The relative importance of these processes is thus conditional on the temporal organization of the target system.

This account also explains why residence time can be highly predictive in one pharmacological system and much less

informative in another. The critique that residence time can become misleading when considered independently of pharmacokinetics remains decisive (Folmer, 2018). Yet the V2-receptor evidence demonstrates that residence time can outperform affinity as a predictor of inhibitory efficacy within a defined compound series and biological context (Zhang *et al.*, 2022). These positions are compatible once the question changes from whether residence time is intrinsically important to whether dissociation remains one of the slowest consequential processes in the particular system.

Target replacement introduces another conditional transition. BTK studies show that covalent occupancy can progressively decline because newly synthesized target appears even though previously modified protein remains inhibited (Daryaee *et al.*, 2017). Clinical interruption data further show that recovery of target availability and downstream signaling can diverge temporally (Alsadhan *et al.*, 2020). The resulting implication is that pharmacological persistence cannot be assigned to occupancy alone. If pathway output remains suppressed after free target begins to return, downstream processes have acquired temporal persistence of their own. If signaling recovers despite substantial continuing target engagement, feedback or bypass has become dominant instead.

A useful theoretical distinction therefore concerns the process that terminates inhibition. In some systems, pharmacological activity ends when free drug disappears sufficiently to prevent rebinding. In others, dissociation restores the same target molecules to an active state. Covalent systems may instead recover predominantly through target resynthesis. Networked signaling systems can recover through feedback, pathway re-equilibration, or compensation that does not require restoration of the original target state. These termination routes are not mutually exclusive, and their relative contribution can change during treatment.

The model therefore predicts no fixed sequence in which exposure decline must be followed by occupancy loss, target restoration, and pathway recovery. Published observations already demonstrate departures from such ordering. Target renewal can erode occupancy during continued treatment (Sun *et al.*, 2020), while pathway adaptation can proceed through newly synthesized target or alternative RAS signaling (Xue *et al.*, 2020). In bacterial systems, translation of long residence time into post-washout activity depends on target vulnerability and resynthesis (Basu *et al.*, 2021). The common theoretical feature is not one privileged kinetic variable but competition among processes operating on different time scales.

This formulation also gives pharmacological persistence an experimentally discriminable meaning. Persistence should refer to the interval during which a specified biological effect remains detectably altered after the exposure condition capable of sustaining new inhibition has changed. The causal mechanism cannot be assigned from that duration alone. Determining whether persistence reflects residual exposure, slow dissociation, covalent modification, slow target renewal, delayed pathway recovery, or compensatory adaptation requires measurements positioned at the relevant biological levels.

Results and Discussion

Model Predictions, Confounding Mechanisms, and Translational Boundaries

The principal implication of this analysis is that biochemical potency should not be asked to answer a temporal question for which it was not designed. Equilibrium or endpoint potency remains useful for comparing activity under standardized conditions, but it cannot by itself specify how inhibition evolves after exposure changes. The same caution applies to residence time and target occupancy. Each is informative within a defined level of measurement, and each becomes misleading when interpreted as a complete surrogate for system-level persistence. Target-engagement methods are therefore most valuable when selected according to the mechanistic question and biological context rather than treated as interchangeable demonstrations of efficacy (St John-Campbell & Bhalay, 2025).

Post-exposure experiments provide a particularly strong way to discriminate competing mechanisms because they perturb the system at the point where equilibrium assumptions become least reliable. Serial measurements during washout or scheduled treatment interruption can test whether free drug, occupancy, target abundance, and downstream function recover together or separate into distinct trajectories. Clinical acalabrutinib interruption demonstrates the value of this design by revealing different recovery behavior for free BTK and downstream pharmacodynamic measures (Alsadhan *et al.*, 2020). The bacterial washout experiments similarly show that target vulnerability and renewal can determine whether long residence time translates into sustained function (Basu *et al.*, 2021). Such experiments do not require a universal quantitative model to be informative; their main value is mechanistic separation.

Measurement alignment is equally important. Direct mass-spectrometric measurement of covalent KRASG12C occupancy illustrates how engagement can be evaluated independently of pathway readouts (Tran *et al.*, 2023). A convincing persistence analysis would ideally align target-site exposure, direct engagement, target abundance or renewal, and downstream functional measurements on the same temporal axis. This is an article-specific evidentiary proposal rather than an established reporting standard. Its purpose is to reduce ambiguity about which biological process is responsible when pharmacological activity persists or recovers.

Several confounders can mimic the effect expected from slow dissociation. Continued intracellular exposure can maintain binding after plasma concentrations decline. Rebinding can prolong apparent occupancy without requiring an exceptionally slow intrinsic dissociation rate. Target abundance may change because of synthesis, degradation, trafficking, or disease-associated regulation. Assay conditions can alter apparent covalent engagement through changes in incubation time and concentration. The broader caution that residence-time arguments become unreliable when separated from pharmacokinetics therefore remains applicable even when kinetic measurements are technically precise (Folmer, 2018).

The reverse problem also occurs: rapid functional recovery can arise while target engagement remains substantial. KRASG12C systems show that newly synthesized target and wild-type RAS feedback can restore signaling through distinct mechanisms (Xue *et al.*, 2020). Nongenetic adaptive states provide additional routes to sustained resistance (Mohanty *et al.*, 2023), while YAP1/TAZ–TEAD signaling demonstrates how alternative transcriptional programs can compensate for inhibition of the original oncogenic pathway (Edwards *et al.*, 2023). These examples are oncology-specific and should not be generalized as universal recovery mechanisms. They establish the more limited principle that pathway architecture can shorten or redirect pharmacological persistence independently of direct target occupancy.

Chronic resistance must also remain conceptually separate from acute pharmacological recovery. Resistance can arise through selection, stable transcriptional adaptation, genetic change, or altered cellular dependency over prolonged treatment. Acute post-washout recovery instead asks what happens when an inhibitor is removed or substantially reduced over a shorter interval. The two phenomena may share mechanisms, but they are not interchangeable. Mutation-dependent changes in Abl inhibitor dissociation demonstrate that altered kinetics can contribute to resistance (Lyczek *et al.*, 2021), whereas adaptive signaling studies demonstrate that resistance can also develop without a primary failure of molecular binding.

The proposed account would be weakened if carefully controlled studies showed that equilibrium biochemical potency alone consistently predicts post-exposure duration after relevant exposure, binding kinetics, target abundance, and pathway state are measured. It would likewise be weakened if target resynthesis failed to influence recovery in systems where covalent occupancy should otherwise persist, or if pathway recovery always tracked target availability with no evidence of feedback, delay, or bypass. These are useful falsification conditions because they prevent the theory from treating every discordant observation as another unspecified temporal mechanism.

Translation across targets and tissues requires particular restraint. The bacterial target-turnover evidence cannot provide quantitative predictions for mammalian systems. Receptor kinetic studies cannot establish that the same kinetic determinants dominate kinase signaling. Oncology models provide unusually rich examples of feedback and adaptation but may overrepresent highly plastic networks. Quantitative systems pharmacology can bridge missing measurements, yet simulated occupancy in unsampled tissues remains dependent on model assumptions (Demin *et al.*, 2025). Pharmacological persistence should therefore be investigated as a target-, tissue-, exposure-, and pathway-specific property before being elevated into a compound-level attribute.

A practical consequence is that the most informative experimental design depends on the suspected terminating process. When dissociation is hypothesized to dominate, direct kinetic measurements and controlled exposure are necessary. When target replacement is suspected, serial target-abundance and occupancy measurements become essential. When pathway recovery appears earlier or later than occupancy recovery, downstream signaling and compensatory-network measurements are required. The theoretical value of the present account lies in specifying these alternatives rather than assigning all durable pharmacology to one preferred molecular metric.

Conclusion

Biochemical potency can misrepresent pharmacological persistence when the duration of drug action is controlled by processes that an equilibrium or endpoint potency measurement does not capture. Binding association and dissociation, reversible or irreversible target engagement, target resynthesis, and downstream pathway recovery operate on separable time scales, and the slowest or most consequential of these processes can differ across biological systems.

The central implication is conditional. Residence time can be highly informative when inhibitor dissociation remains an important determinant of occupancy and function, but it cannot replace pharmacokinetics, target turnover, or direct biological measurement. Similarly, covalent engagement can prolong molecular inhibition without guaranteeing durable pathway suppression when active target is rapidly replaced or network compensation restores function.

Pharmacological persistence is therefore best treated as a multilevel temporal property rather than a fixed attribute implied by biochemical potency. Determining its mechanism requires identifying which event actually terminates inhibition after exposure changes. Aligning measurements of exposure, target engagement, target abundance, and downstream function offers the clearest route for distinguishing persistent molecular binding from persistent pharmacological effect.

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