



Replacing Single-Point Potency with Dose–Time Response Surfaces for Computational Prioritization of Natural Product Candidates

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ABSTRACT

Natural-product discovery commonly compresses pharmacological activity into potency values obtained at selected concentrations and observation times. Such summaries are useful for screening, but they can conceal whether activity is rapid or delayed, transient or persistent, growth-suppressive or lethal, and whether apparent potency changes as the biological system evolves. This article develops an original pharmacodynamic framework in which candidate response is represented as a dose–time surface rather than a single potency estimate. The framework treats concentration and observation time as explicit coordinates, preserves uncertainty and assay context, and separates scalar feature extraction from direct learning of the full response landscape. It further proposes that candidate prioritization should depend on the therapeutic objective: rapid suppression, durable effect, recoverability, maximal effect, or exposure-compatible response may imply different preferred regions of the same surface. Dynamic phenotypes are interpreted descriptively unless orthogonal evidence establishes mechanism, and in vitro surface geometry is not equated with target engagement, clinical exposure, or therapeutic efficacy. The framework also identifies design requirements for surface construction, including longitudinal or repeated measurements, adequate concentration coverage, replication, normalization, and uncertainty-aware interpolation. Its central contribution is a proposed decision architecture for comparing candidates whose apparent potency rankings diverge across time. The approach remains conceptual and requires prospective testing against simpler ranking strategies, with held-out compounds and biological contexts, before any claim of improved predictive or translational performance is justified.

Key Words: Dose–time response, Natural products, Pharmacodynamics, Drug prioritization, Response surfaces, Machine learning

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INTRODUCTION

Natural products continue to supply chemically and biologically distinctive starting points for drug discovery, but their value depends on more than identifying a compound that crosses an activity threshold in a convenient assay [1]. Early prioritization often asks a scalar question—how much compound is required to produce a specified effect?—because scalar potency is easy to rank, model, and communicate. The difficulty is not

that such measurements are intrinsically invalid. It is that a single concentration or fitted potency value obtained at one observation time is only a narrow sample of a pharmacological process that unfolds across concentration, time, cellular state, and assay conditions. For structurally unusual or mechanistically diverse natural products, discarding that structure in the response data may be especially costly when candidate numbers must be reduced before deeper characterization.

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Temporal context can materially change the phenotype recorded after drug treatment, as high-throughput deep phenotyping has shown for time-dependent drug sensitivity [2]. Dynamic response can also emerge from cell-cycle-specific rate effects that are poorly represented by a terminal population measurement [3]. More broadly, the definition of the response label itself affects what computational models learn and how their outputs are interpreted [4]. At single-cell resolution, apparently coherent population-level dose responses may additionally conceal heterogeneous thresholds and subpopulations [5]. These observations support a limited but important proposition: pharmacological response is not exhausted by one potency number, even when that number is measured accurately.

This article therefore proposes dose–time response surfaces as a richer representation for computational prioritization of natural-product therapeutic candidates. The purpose is not to replace every screening endpoint with maximal experimental complexity. Rather, the proposed framework asks when a second dimension—observation time—contains decision-relevant information that would be lost by scalar ranking. It treats the response trajectory as the object of interest and reserves mechanistic interpretation for cases in which orthogonal evidence justifies it. The resulting framework is intended to organize experimental design, feature extraction, direct surface learning, rank crossover, uncertainty, and exposure-aware interpretation without claiming that surface-based ranking has already been prospectively validated.

How single-point screens collapse dose and time

A single-point screen fixes concentration and observation time by design, then reports what happened at that coordinate. Even a fitted IC50 usually remains conditional on the chosen assay window, readout, growth state, normalization procedure, and tested concentration range.

Cross-center experiments have shown that drug-response estimates can vary even when laboratories use coordinated mammalian-cell protocols, demonstrating that assay context is part of the measurement rather than a negligible background variable [6]. Thus, a scalar response can be precise within a protocol while still being incomplete as a description of the underlying pharmacology.

Standardization also does not eliminate all experimental variability. Large collections of repeated cell-culture measurements retain substantial dispersion despite highly standardized conditions [7]. This matters because a rank order based on closely spaced potency estimates may reflect a mixture of biological difference and measurement uncertainty. A dose–time representation does not remove those sources of error, but it can make their location visible: uncertainty may be concentrated at late times, at steep portions of the concentration axis, or in regions where few observations support interpolation. The proposed framework therefore treats replicate structure and uncertainty as properties of the surface, not as annotations added after ranking.

Normalization choices further influence what the response means. Growth-rate-aware drug-response metrics can improve consistency when baseline proliferation differs across cell populations [8]. Whole-curve analysis can also assess the statistical credibility and classification of a dose response rather than accepting a fitted point estimate without regard to curve quality [9]. These methods do not by themselves create a dose–time model, but they show why the input to such a model should preserve the provenance of each measurement: concentration, time, response scale, control behavior, normalization, and uncertainty.

Table 1 organizes the distinct forms of information that are compressed when a dynamic pharmacological process is represented by a single screening value.

Table 1. What a single potency value preserves and what it leaves unresolved

Construct	What the scalar can represent	Information lost or conditional	Consequence for prioritization	Evidence status
Assay response	Effect under one defined protocol	Dependence on laboratory, readout, and protocol context [6]	Small rank differences may not be portable across conditions	Evidence-supported
Replicate precision	Central estimate at the sampled coordinate	Heteroscedastic or region-specific uncertainty [7]	Ranking should separate response magnitude from confidence	Evidence-supported; ranking implication proposed
Growth-normalized sensitivity	Response adjusted for baseline proliferation [8]	Other temporal state changes and recovery behavior	Normalization improves interpretation but does not replace time	Evidence-supported
Curve-level potency	Concentration dependence within one observation window	Evolution of the curve at earlier or later times	A well-fitted curve can remain temporally incomplete	Proposed synthesis

Curve credibility	Statistical support for a dose-response relation [9]	Mechanism and durability of the observed effect	Curve quality is necessary but insufficient for candidate priority	Evidence-supported; implication proposed
Dynamic response	—	Onset, delay, persistence, reversal, and time-specific rank changes	These dimensions require explicit temporal sampling	Proposed construct

The compression process is summarized in **Figure 1**, where the reported scalar is treated as a projection of a larger measured response space rather than as a complete pharmacological state.

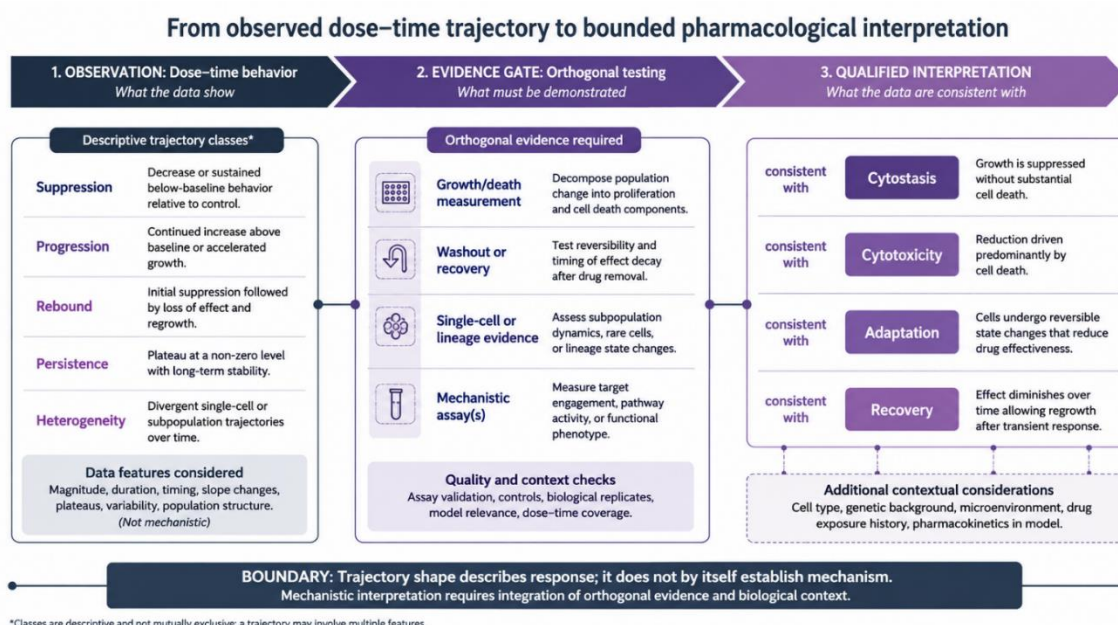


Figure 1. From a dynamic experimental response space to a compressed potency label

Potency changes with observation time

Dose, response, and time are coupled dimensions of pharmacodynamics rather than interchangeable summaries. Quantitative pharmacology has emphasized that the same nominal dose can produce different effects depending on when response is observed and how the underlying system accumulates, adapts, turns over, or recovers [10]. A potency estimate at an early observation and one obtained later therefore need not answer the same biological question. Their difference may arise because a compound acts slowly, damage accumulates, cells enter a different state, or the assay captures a downstream process whose kinetics lag behind the initiating perturbation. Recent experimental and mathematical work reinforces this time-conditioned view of sensitivity. Human-cell studies have shown that drug sensitivity can vary with temporal state and that mathematical models can help identify drivers of those changes [11]. Such findings should not be conflated with elapsed-exposure kinetics when the experimental variable is time of day, but they establish a broader principle: measured sensitivity can be conditional on temporal context. The framework proposed here applies that principle specifically to elapsed

observation time after treatment and makes time an explicit coordinate rather than a nuisance variable.

The natural-product connection is especially important because prolonged observation can alter which compounds appear active as cellular state changes. Time-dependent proteomic and drug-response analysis in expanding cancer cells identified temporal changes in sensitivity and included natural-product activity within that evolving response landscape [12]. This does not establish a universal temporal signature for natural products. It does justify asking whether candidates with similar early potency remain equivalent later. In the proposed surface view, potency becomes a trajectory-dependent descriptor: ranking at one time is local, whereas prioritization considers whether the pharmacologically relevant ordering persists across the observation window.

Early, delayed, transient, and persistent response phenotypes

Longitudinal assays make it possible to distinguish trajectories that can converge to similar endpoint values. At organoid resolution, time-resolved measurement platforms have revealed heterogeneous treatment responses and resistant subpopulations that pooled

terminal measurements can obscure [13–15]. For the present framework, these observations motivate four descriptive phenotypes: an early response appears soon after treatment; a delayed response emerges only after a measurable lag; a transient response appears and then weakens; and a persistent response remains evident across the observed interval. These labels are proposed as computational descriptors of trajectory shape, not as universal biological classes.

Mechanistic studies of drug persistence reinforce the need for that caution. Rare-cell variability and drug-induced reprogramming can create reversible resistance states, while persister populations can arise from lineages with distinct cycling programs [16, 17]. A persistent or recovering trajectory therefore cannot automatically be interpreted as genetic resistance, cytostasis, or successful adaptation. Multiple mechanisms can generate superficially similar time courses, and an observation window can end before recovery becomes apparent. The surface should preserve the phenotype first; causal labeling requires additional evidence.

For candidate prioritization, the distinction matters because the preferred phenotype depends on the intended pharmacological objective. Rapid but transient suppression may be valuable when exposure can be sustained, whereas a slower response may be preferable if it becomes deeper or more durable within achievable exposure. Conversely, apparent persistence may be misleading if it reflects an irreversible assay signal rather than continuing pharmacological action. The proposed framework therefore treats onset, delay, persistence, reversal, and recovery as separable descriptive dimensions whose value is conditional on context rather than inherently favorable or unfavorable.

Constructing dose–time response surfaces

A dose–time response surface can be expressed conceptually as $R(d,t)$, where (d) denotes administered concentration or dose and (t) denotes observation time after treatment. Explicit dose–time modeling has already been demonstrated with recursive approaches that predict pharmacological response jointly across these dimensions [18]. The present framework does not prescribe that particular model. Instead, it adopts the representation principle: measurements should be positioned within a common dose–time coordinate system, and interpolation between them should state its assumptions and uncertainty. Existing full-curve modeling also shows that pharmacological responses need not be reduced to one fitted scalar before computational analysis. Multi-output Gaussian-process approaches can predict complete dose–response curves and exploit relationships among curve outputs [19]. Extending that logic from a one-dimensional

response function to a dose $\times$ time field is proposed here, not claimed as an established superiority result. A surface could be represented by splines, Gaussian processes, functional models, neural representations, or mechanistic equations, provided the model does not impose monotonicity when the observed biology contradicts it or conceal poorly sampled areas behind visually smooth interpolation.

Probabilistic response-surface work further demonstrates the value of representing uncertainty when measurements are sparse or noisy [20]. Although such methods have been developed for drug-combination dose surfaces rather than elapsed time, one methodological principle transfers cautiously: interpolation is a prediction, not an observation. A dose–time surface should therefore distinguish measured coordinates from modeled regions and preserve replicate variability. Nonmonotonic responses, incomplete plateaus, delayed effects, and recovery should remain visible rather than being forced into a conventional sigmoid. The goal is a pharmacodynamically interpretable landscape that shows both response structure and where confidence in that structure weakens.

Extracting pharmacodynamic features beyond ic_{50}

A response surface can be summarized without collapsing back to a single potency estimate. Longitudinal growth-rate metrics in organoid assays demonstrate that dynamic normalization can distinguish behavior that conventional endpoint measurements may miss [21]. In the proposed framework, candidate features could analogously describe onset, maximal observed effect, persistence, recovery after peak response, or integrated response over an exposure-relevant region. These remain candidate descriptors rather than universally validated metrics, and every feature should retain its assay definition and observation window. Uncertainty should accompany any derived feature. Statistical frameworks using probabilistic dose–response modeling show that pharmacological conclusions can change when uncertainty is represented rather than ignored [22]. A candidate with a marginally stronger estimated feature but substantially poorer precision should therefore not automatically outrank a better-characterized alternative. Separating response magnitude from confidence is consequently proposed as a core prioritization rule.

Recent work also reiterates that IC_{50} can vary with the observation window and normalization approach, while alternative indices can be constructed to reduce particular forms of temporal dependence [23]. Such alternatives remain context dependent and do not eliminate the information contained in longitudinal measurements. The practical distinction is therefore between feature extraction

and surface retention: derived features provide compact, interpretable quantities for decision-making, whereas the complete surface preserves dose–time interaction structure for later comparison and modeling.

Distinguishing cytostasis, cytotoxicity, adaptation, and recovery

A declining or flattened viability trajectory does not by itself specify what happened to the cells. Real-time quantitative phase imaging has shown that drug responses can be resolved into temporally evolving single-cell features rather than a terminal viability value alone [24]. Such measurements support a descriptive distinction between reduced proliferation, loss of viable cells, altered morphology, and heterogeneous trajectories. The proposed framework therefore interprets surface geometry first as a functional phenotype. Mechanistic terms should be attached only when the assay directly measures the relevant process or when orthogonal experiments provide adequate support.

Growth inhibition and cell death are particularly important to separate because similar net population responses can arise from different combinations of these processes. Drug

GRADE demonstrates that population growth and death can be decomposed to reveal drug- and cancer-subtype-specific response profiles [25]. Within a dose–time surface, an apparently durable low-response region could consequently reflect sustained cytostasis, progressive killing, or a mixture of both. These alternatives are not interchangeable for candidate prioritization: one may require continuing exposure, whereas another may continue to deepen after sufficient damage has accumulated.

Adaptation adds a further temporal distinction. Drug-tolerant colorectal cancer cells can enter a reversible diapause-like state that supports survival during chemotherapy [26]. This evidence does not justify assigning the same mechanism to every recovering trajectory, but it shows why post-treatment behavior matters. A candidate that produces strong suppression followed by rapid rebound is pharmacodynamically different from one that produces comparable suppression without recovery over the observed interval. **Table 2** therefore separates interpretations that a response surface can support descriptively from stronger biological labels requiring additional measurements.

Table 2. Distinguishing similar dose–time patterns without conflating pharmacological states

Observed pattern	Surface-level interpretation	Evidence needed for stronger biological label	Prioritization implication
Reduced expansion with preserved cells	Growth suppression consistent with cytostasis	Direct proliferation or cell-cycle measurement	Durability may depend on maintained exposure
Progressive loss of viable cells	Response consistent with cytotoxicity	Orthogonal death assay or fate tracking	Depth and time-to-kill may both matter
Suppression followed by rebound	Recovery or adaptation-like trajectory	Washout, lineage, state, or mechanistic assays	Early potency may overstate durability
Persistent suppression	Sustained observed effect	Extended observation plus growth/death discrimination	Persistence matters only relative to the intended objective
Mixed subpopulation trajectories	Heterogeneous response	Single-cell or clonal resolution	Mean response may conceal resistant fractions

Note: These are proposed descriptive distinctions. Longitudinal phenotyping provides the observational basis [24]. Growth/death decomposition supports separating cytostasis from cytotoxicity [25]. Reversible tolerant states establish that suppression and later recovery need not represent permanent resistance [26].

A surface-based candidate prioritization framework

The central proposal is to rank natural-product candidates according to a defined pharmacological objective applied to the response surface rather than according to the lowest scalar potency by default. This requires a fit-for-purpose computational design. Artificial-intelligence applications in clinical pharmacology have emphasized data quality, interpretability, validation, and alignment between a model and its intended decision [27]. Those principles apply equally to preclinical prioritization: a richer representation is useful only when the ranking question is specified before the model is optimized.

The proposed framework therefore separates four analytical layers. First, the experimental layer records

dose, time, response, normalization, replication, and assay context. Second, the representation layer retains either the complete surface or explicitly defined dynamic features. Third, the decision layer states which behavior is valued—for example, rapid onset, sustained suppression, deep maximal response, limited recovery, or response within an exposure-relevant region. Fourth, the confidence layer records uncertainty and extrapolation. Computational experiments in exposure-response modeling show that preprocessing, validation strategy, sample characteristics, and model choice can materially alter machine-learning conclusions [28], supporting the need to keep these layers explicit rather than hiding them within one composite score.

Interpretability becomes particularly important when candidate ranks reflect several dynamic properties. Interpretable machine-learning workflows have been applied to nonlinear exposure-response relationships in pharmacometrics [29]. By analogy, a surface-based ranking should remain decomposable: investigators should be able to identify whether a candidate is favored because it acts earlier, remains active longer, reaches a deeper effect, or is supported by lower uncertainty. The exact weighting of these properties is proposed to be objective-specific rather than universal.

A surface-based decision also requires an explicit comparison rule. The proposed default is not to average every coordinate indiscriminately, because that would recreate scalar compression at a later stage. Instead, the decision region should be prespecified from the biological use case and applied consistently across candidates. Coordinates outside experimentally supported or exposure-relevant regions may still be displayed, but they should not silently dominate the ranking. Where several objectives matter simultaneously, a transparent multi-criterion profile or sensitivity analysis is preferable to an opaque composite score. This preserves legitimate disagreement among objectives: the candidate favored for rapid onset need not be the candidate favored for durable suppression.

Comparing compounds whose potency rankings cross over time

Two compounds may exchange rank as observation time changes. One may appear more potent early but plateau, whereas another develops a slower, deeper response. Uncertainty-aware EC50 prediction illustrates that rankings based on estimated potency should account for confidence in the fitted response rather than treating every point estimate as equally reliable [30]. The surface framework extends that reasoning: a rank crossover should be interpreted only where both candidates are adequately measured or modeled and where the difference is meaningful relative to uncertainty.

A crossover is not itself evidence that one compound is globally superior. It identifies a decision boundary. If the intended exposure is brief, an early-response region may be most relevant; if sustained exposure is feasible, later behavior may legitimately dominate. Natural-product candidates can therefore receive different conditional rankings without contradiction. The proposed rule is to report the region of preference and the pharmacological objective that makes it preferable rather than compressing all crossings into one context-free league table.

This approach also avoids treating potency order as a permanent compound property. A rank is conditional on the response definition, observation window,

concentration region, biological system, and uncertainty accepted by the decision. Surface-based comparison is therefore most informative when it reveals *why* two rankings disagree. Such disagreement becomes analytically useful rather than an inconvenience to be averaged away.

Machine learning directly on response surfaces

Feature extraction is not the only computational option. Multiplex single-cell pharmacotranscriptomics demonstrates that high-dimensional drug responses can be represented and analyzed without first reducing each treatment to a single scalar endpoint [31]. Functional regression similarly shows that dosage-dependent response can be modeled as a function rather than as unrelated point labels [32]. These studies do not validate direct dose–time surface learning, but they establish computational precedents for treating pharmacological response as structured functional information.

The proposed extension is to learn from the surface itself, whether represented as a functional output, structured grid, latent representation, or probabilistic field. Such models should preserve missingness, observation density, and uncertainty rather than interpreting interpolated coordinates as measured data. Direct surface learning should also be compared with simpler feature-based models. Additional dimensionality is justified only if it improves a prespecified decision while remaining interpretable and valid outside the training configuration.

A practical comparison would therefore place scalar potency, engineered dynamic features, and full-surface representations on the same evaluation task. Better reconstruction of the experimental surface would not by itself establish better candidate prioritization. The clinically or experimentally relevant question is whether the richer representation preserves information that changes a downstream decision in a reproducible and defensible way.

Experimental designs that efficiently populate the surface

A useful surface does not require indiscriminate measurement of every dose–time combination. Label-free longitudinal imaging can repeatedly characterize single-cell drug responses without destructive endpoint sampling [33], illustrating how temporal density may be increased while reusing experimental units. Where repeated observation is technically valid, this can shift resources toward concentration coverage and replication rather than requiring a separate experimental unit for every time point. Design should nevertheless follow the pharmacological question. Concentrations must span regions capable of revealing weak, steep, saturated, or nonmonotonic behavior; observation times must extend far enough to

detect plausible delay or recovery; and controls must be time matched. Sparse or adaptive designs may be efficient, but model-filled regions should remain visibly distinct from observations. No universal grid density is proposed because adequate sampling depends on response kinetics, assay precision, biological variability, and the intended ranking decision.

Efficiency also depends on where information changes most rapidly. A nearly flat region does not necessarily warrant the same sampling density as a region containing a steep concentration transition or rapid temporal inflection. Adaptive refinement around such regions is conceptually attractive, particularly when probabilistic interpolation indicates where uncertainty is large. However, model-guided acquisition should be evaluated against a prespecified fixed design because adaptive sampling can otherwise reinforce an incorrect early surface assumption. Replication remains necessary wherever technical or biological variation could alter the candidate order.

Linking in vitro dose–time behavior to exposure profiles

A functional surface should not be promoted into a mechanistic or translational claim by visual resemblance alone. Cellular target engagement and downstream pharmacology are experimentally separable layers, as demonstrated by approaches that explicitly couple engagement measurements to subsequent functional effects [34]. Consequently, a surface showing delayed or persistent activity cannot identify a molecular target, prove engagement, or establish pathway causality. Orthogonal engagement and mechanistic experiments remain necessary when those claims matter for prioritization.

Translation also requires exposure. Model-informed precision dosing emphasizes that concentration changes over time and that pharmacological decisions depend on relationships among dose, exposure, covariates, and response [35]. An in vitro surface uses nominal or measured experimental concentration as one coordinate; it is not an in vivo pharmacokinetic model. Its translational relevance is therefore conditional: regions of interest should eventually be compared with plausible exposure trajectories, protein binding, tissue distribution, metabolism, and dosing constraints appropriate to the candidate.

This distinction is particularly important for natural products, whose physicochemical and metabolic diversity may make nominal in vitro concentration a poor proxy for exposure at the biological site of interest. The proposed framework thus uses exposure compatibility as a later decision filter rather than embedding an assumption of clinical attainability into the surface itself. A favorable in

vitro trajectory may prioritize further investigation, but it cannot establish therapeutic efficacy.

The translational sequence is consequently one of progressive constraint. First, the surface identifies where and when a functional response occurs. Next, experimental pharmacology asks whether that region reflects reproducible and mechanistically interpretable activity. Pharmacokinetic reasoning then asks whether relevant concentrations and durations are attainable in the intended biological compartment. Failure at any stage should revise the candidate interpretation rather than being concealed by a favorable original potency rank.

Interpretation limits and validation needs

More information does not automatically produce a better predictor. Drug-response prediction can appear successful under evaluation specifications that exploit dataset structure rather than the intended generalization problem [36]. A dose–time model should therefore be evaluated under splits that reflect its actual use, including genuinely held-out compounds and, where relevant, held-out biological contexts. Comparison against single-point potency, conventional curve features, and simpler dynamic summaries is essential because a complex surface model must justify its additional experimental and computational cost.

Validation should also separate surface reconstruction from prioritization. A model may reproduce measured surfaces accurately yet fail to choose better candidates, while a simpler representation may preserve the decision-relevant ordering. The validation endpoint should therefore match the intended use, and uncertainty calibration should be evaluated separately from average predictive accuracy so that weak rank differences are not presented as decisive. Other limitations remain intrinsic. Surface estimates depend on assay quality, normalization, sampling density, interpolation assumptions, and observation duration. Closely ranked candidates may be indistinguishable within uncertainty, while apparent persistence can disappear beyond the experimental window. Nonmonotonic surfaces may be real or may arise from measurement artifacts, and concentration-dependent mechanism changes can make one global interpretation inappropriate. A smooth computational surface should never conceal these difficult cases.

Mechanistic interpretation requires orthogonal evidence, and in vitro concentration–time behavior cannot substitute for pharmacokinetic or pharmacodynamic validation. Prospective evaluation should ask whether the proposed representation changes a meaningful downstream decision, not simply whether additional measurements improve curve fitting. The framework should therefore be treated as

a testable organization of pharmacodynamic information rather than an implementation-ready ranking standard.

CONCLUSION

Single-point potency answers a useful but narrow question: what response is observed at a particular concentration, or what concentration corresponds to a chosen effect within a particular assay window? Candidate prioritization often requires more. The framework developed here proposes treating dose and observation time as joint coordinates so that onset, depth, persistence, recovery, heterogeneity, and uncertainty remain available to computation rather than being discarded before ranking.

Its central principle is conditional ranking. A candidate should be favored because its response trajectory matches a stated pharmacological objective, not because one isolated potency estimate is numerically smallest. Full surfaces and derived features are complementary representations, and either may be appropriate depending on data density, interpretability, and decision requirements. Dynamic phenotype must remain distinct from mechanism, and in vitro response must remain distinct from exposure and therapeutic effect.

The value of the framework is therefore empirical rather than assumed. Prospective experiments must determine whether surface-based representations improve decision-relevant prioritization over simpler alternatives under realistic uncertainty and held-out contexts. Until then, the appropriate claim is narrower: preserving the response trajectory creates a more explicit basis for asking what information a potency ranking retains, what it discards, and which distinctions deserve validation.

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