

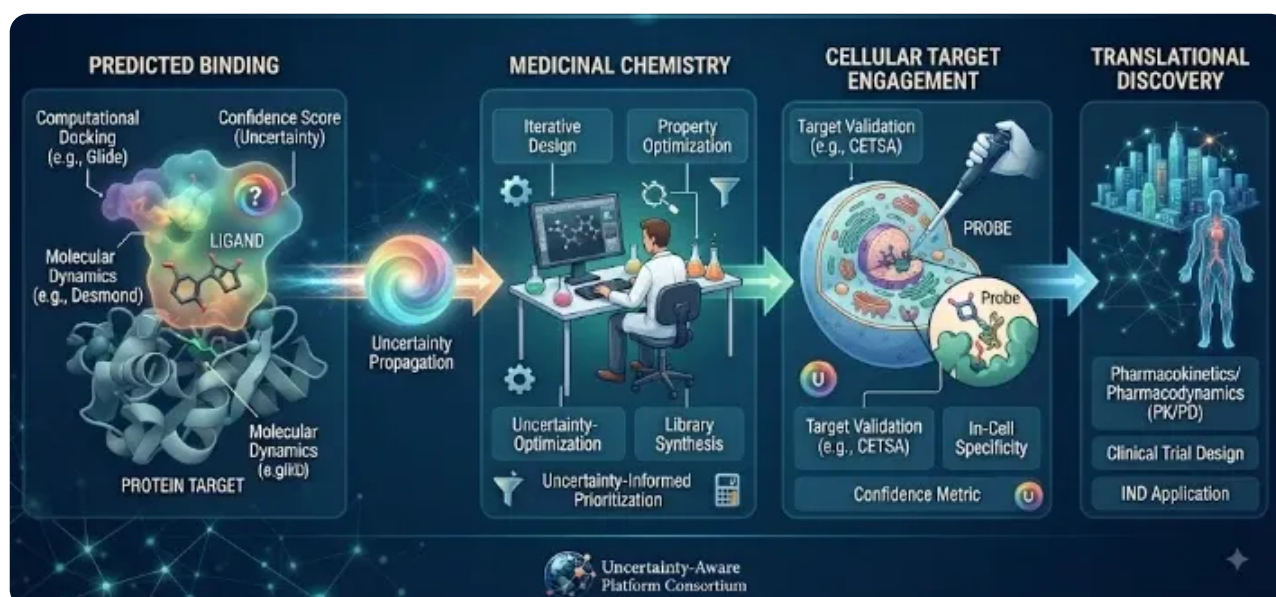
From Predicted Binding to Cellular Target Engagement: An Uncertainty-Aware Platform for Medicinal chemistry and translational discovery of new therapeutics

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Abstract

This article develops an uncertainty-aware platform that converts predicted molecular binding into experimentally verified cellular target engagement. It explains that docking, QSAR, or machine-learning scores can rank compounds efficiently but cannot establish intracellular exposure, occupancy, selectivity, residence time, or causal pharmacology. CETSA, live-cell engagement approaches, chemoproteomics, and measured intracellular concentration are presented as complementary tools for separating poor permeability from incorrect binding predictions. Model domain, binding pose, protonation, conformational state, permeability, target abundance, free concentration, residence time, and off-target profile are treated as interacting variables. Failure routes include prediction error, hidden binding modes, target mutation, polypharmacology, pathway bypass, transporter effects, and metabolite-driven activity. Unbound intracellular concentration, subcellular localization, plasma binding, metabolic stability, tissue penetration, and dose duration define meaningful exposure. Ensemble models, applicability domains, conformal prediction, Bayesian uncertainty, active learning, and multiparameter ranking help prioritize experiments. Prospective predictions, blinded compounds, inactive analogues, orthogonal engagement assays, genetic rescue, and exposure-response concordance are required for validation. The core message is that prediction becomes translationally useful only when uncertainty guides direct cellular testing.



Predicted Binding to Cellular Target Engagement presents a primary challenge for uncertainty-aware medicinal chemistry and translational target engagement: the ability to rank compounds efficiently in an unbiased manner based on predicted binding is not sufficient to determine cellular exposure, occupancy, selectivity or causal pharmacology. The better development strategy focuses on a quantifiable mechanism instead of one great assay result. That change puts chemistry, biology, exposure, resistance, safety and production as part of one translation argument.

The scientific basis is based on a platform that combines computational affinity predictions with direct binding of target molecules at the level of the cell and experiments that guide decision making. No single measurement can support the entire mechanistic claim; there are individual questions addressed by each component. In the absence of connected evidence, there is less risk of a mistake in interpretation being made between potency, binding, formulation performance and therapeutic understanding.

The idea behind the cellular target-engagement methods is that predictability of biochemical affinity and docking is poor and does not capture the cellular environment. CETSA is used to measure either thermal stabilization or destabilization that is associated with a ligand, and can be used in live cells for formats of bioluminescence resonance energy transfer for targets that are compatible. Chemoproteomic methods asks the question about many proteins. The ability to use these assays with measured intracellular concentration allows for the identification of weak engagement (due to poor permeability) from weak engagement (due to mis-prediction of binding).

Joint treatment is necessary for the design variables, because at the same time the

performance of the model domain, the binding pose, the protonation state, the conformational state, the cell permeability, the target abundance, the free concentration, the residence time and the off-target profile can change. Careful optimization of one aspect of the potency, stability, selectivity, delivery, or manufacturability can lead to an unnoticed cost in another. A multi-parameter decision rule is thus more realistic for candidate ranking than is a single numeric decision value.

Mechanistic confidence is also related to early failure route measurements. Prediction error, hidden binding mode, target mutation, polypharmacology, pathway bypass, transporter effects and metabolite-driven activity are all important escape mechanisms. When deliberately stress testing, these routes turn into design information to inform the next chemistry/ formulation/dosing/ model cycle.

Exposure is the link between nominal dose and the biological effect. The relevant quantities are as follows: unbound intracellular concentration, subcellular location of the target, occupancy of the target, plasma binding, metabolic stability, tissue penetration and duration of the dose. A candidate that is highly active but poorly exposed in the causal site may have good intrinsic activity but be poor to translate.

Realistic concentrations must be used for the direct engagement or functional perturbation. High doses may lead to secondary stress response, off-target effects or assay artefacts which simulate a desired mechanism. The concentration-response analysis related to exposure and occupancy can be used to determine the concentration range in which the proposed causal process is thought to operate.

The biological context is also influencing the interpretation in medicinal chemistry programs ranging from emerging targets to translational therapeutic discovery. Target dependence and effective exposure can vary depending on differences in genotype, cell state, tissue architecture, immune tone, metabolic environment and previous treatments. Representative model panels thus offer more convincing evidence than do repeated tests in a single system.

Adaptation and resistance should be measured in the future not explained in the past. When subjected to repeated pressure, such as serial passage, long-term selection, genetic perturbation, repeated dosing or environmental stress, the mechanisms that survive the pressure will be revealed. It's better to have a slightly less effective candidate with a better chance of getting away with it than a delicate screening leader.

Information on safety assessment is enhanced by association with mechanism. Issues like

proteome selectivity, normal cell engagement, target-family cross reactivity, cardiac and hepatic liabilities, reactive metabolites and therapeutic index are important. Relative to the desired engagement, there are unwanted biological responses that can be compared to determine if it is mechanism linked to pharmacology and/or broad cellular injury or formulation driven toxicity.

Manufacturing cannot be a downstream logistics problem since synthetic feasibility, analogue availability, chemical stability, solid form, compound purity, scale ability and assay ready product quality has an effect on the product given to biological systems. As long as the nominal product name stays the same, small changes in the process may result in changes in the composition or physical state, the level of impurities or the stability or dose reproducibility of the resulting product. Critical process attributes are directly correlated to mechanistic performance, which results in development gains.

Ensemble models, applicability domains, conformal prediction, Bayesian uncertainty, active learning and multiparameter ranking provide leverage to computational tools. Prediction is most useful when limits of uncertainty and applicability are still apparent, particularly in the vicinity of unusual biological states, sparse data sets, and novel chemical structures. Models which reveal uncertainty will lead to better experiment selections than models whose results are confident rankings without explicit statements of the location of the weakness in the evidence.

Prospective validation involves prospective predictions, blinded compounds, orthogonal engagement assays, inactive analogues, genetic rescue, exposure-response concordance and external series. Why orthogonal tests are important: repeated testing using the same assay family can be capable of yielding the same bias without reinforcing the causality. Consistency in independent approaches leads to better links between design choice and biological impact, and inconsistencies indicate potential changes in design assumptions.

Data integration should maintain the traceability of the data from the experiment stage to the development decision-making stage. Chemical structures, process conditions, assay metadata, exposure measurements, omics features, resistance observations and safety signals should be identified and analyzed in a stable manner (versioning). This discipline helps to avoid later interpretations going “off-piste” from the actual circumstances of a result.

Translation should not be a laboratory show, either, with decision metrics having to capture that. The scorecards that are useful include reproduction, exposure normalized activity, mechanistic confidence, resistance frequency, safety margin, stability and

manufacturing robustness. This allows both a balanced offer and trade-offs to be revealed up front prior to costly development investments.

In this context, the negative results have a significantly higher value. The edge of a hypothesis is determined by the failure in synthesis, failure to find target engagement, instable exposure, lack of rescue, emergence of resistance, failure of a batch, or loss of activity in realistic models. The record of those failures helps to design better experiments in the future and avoids reinvesting resources in interesting but unhelpful explanations.

Another filter is reproducibility across laboratories/batches. Even in the event of apparent strong statistical significance, a mechanism depending on one operator, one preparation, one cell line or one modeling choice is fragile. When exposed under conditions comparable to those in their own micro-environment, a plant's success can be replicated independently of the process of creating the durable biology.

There also needs to be a pragmatic perspective on clinical or deployment context in order to translate. A technically complex product may be worthless if it needs to be routed, is too burdensome to administer, requires complex diagnostics, is incompatible with storage, is too cumbersome to monitor, is too expensive, or if it can be used only at a certain time. Mechanism is important when it comes to product design, and impact is greater when it's used in the context where biological benefit needs to be realized.

The temporal design is an extra layer since biological systems will react differently to short pulses, prolonged exposure, washout and multiple dosing. Time-resolved measurements are able to distinguish primary engagement from downstream adaptation, and can be used to show if early activity remains following the diminution of the initiating signal. Sampling schedules should then reflect the anticipated uncertainty-aware medicinal chemistry/translational target engagement kinetics rather than just one convenient endpoint.

An integrated development record allows for better communication between medicinal chemistry, formulation science, pharmacology, computation, manufacturing and clinical planning. The record should be able to differentiate between known connections, likely ideas, non-resolved questions, and the next experiment with the most amount of information. This transparency speeds up iteration and will not create pressure to cover up for an initial assumption when there are conflicting data.

Creative reference conditions provide a perspective on novelty when benchmarking. Whether a new approach is better at predicting, mechanism, durability, and/or

manufacturability is determined by established compounds (known inactive controls), standard formulations, baseline models or accepted analytical methods. Head to head comparisons also reveal gains which are lost upon normalization for exposure, assay sensitivity, batch quality and/or experimental complexity.

The quality of the decision can be further enhanced by using predetermined stopping rules so that weak hypotheses do not use up additional cycles of synthesis and test. Progressive criteria ensure that resources focus on mechanisms that can be reproduced: the more the system is biologically and operationally complex, the more it is reproduced.

Prediction turns into a translationally helpful when the uncertainty decides which cellular experiment is to be given priority. Whether a candidate can win one assay or not is not the determining standard but whether the proposed mechanism is coherent throughout exposure, biological variation, resistance, safety and production. That standard is what gives discovery signal a more disciplined journey to a therapeutic concept that could contend with the complexity of the real world.