

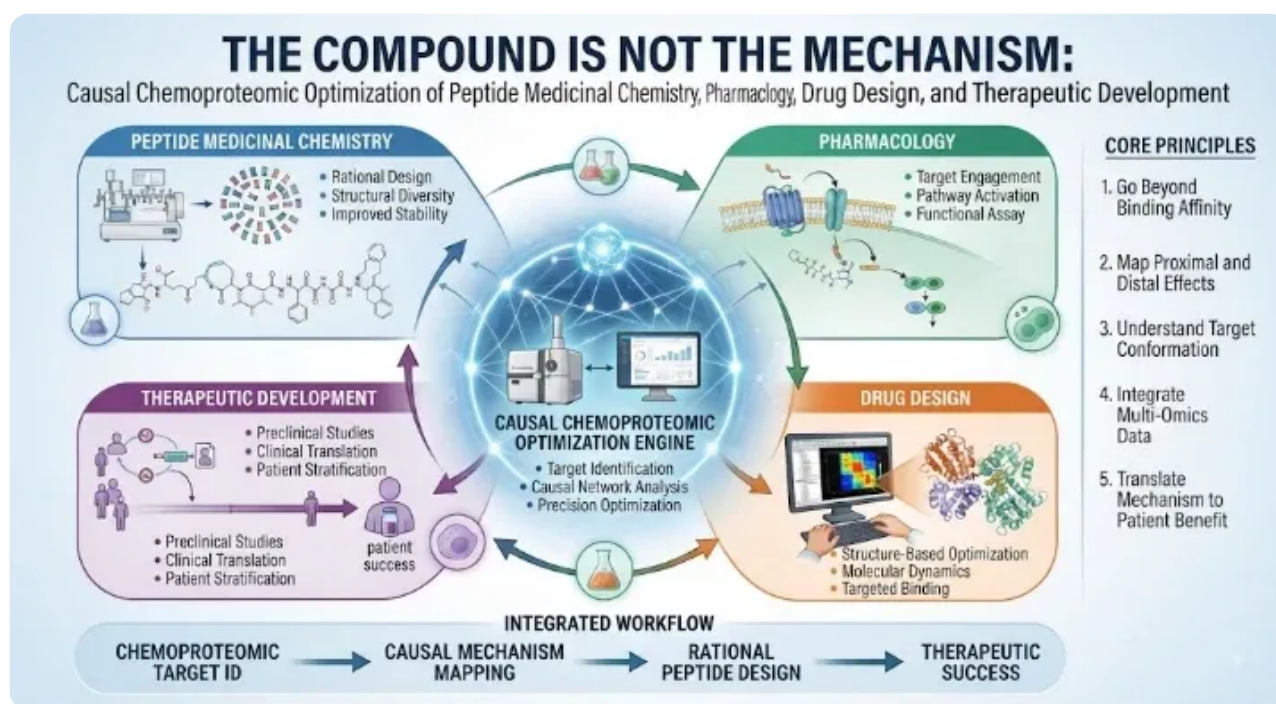
The Compound Is Not the Mechanism: Causal Chemoproteomic Optimization of Peptide Medicinal Chemistry, Pharmacology, Drug Design and Therapeutic Development

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Abstract

This article explains why peptide activity cannot be used as a shortcut for peptide mechanism. A therapeutic peptide may bind a specific receptor or protein interface, perturb membranes, enter cells through multiple routes, or interact with several proteins at concentrations used in phenotypic assays. The article builds a causal framework around target binding, membrane effects, intracellular entry, pathway modulation, and downstream phenotype. Chemoproteomic capture, crosslinking, competition experiments, orthogonal biophysics, cellular target-engagement assays, genetic loss-of-function, and rescue studies are presented as complementary tools for distinguishing causal interactions from collateral binding. Strong evidence requires dose-linked engagement and functional changes that weaken when the proposed target is removed and recover when the mechanism is restored. Target expression, disease subtype, tissue penetration, protease activity, and concomitant therapy are highlighted as context variables. Risks include nonspecific adsorption, probe artifacts, proteolysis, membrane disruption, and target redundancy. Purity, sequence fidelity, conformational control, labeling chemistry, formulation, and prospective modeling are integrated into the workflow. The article concludes that chemistry, engagement, and phenotype must converge before a mechanism is accepted.



The validation of a causal mechanism for peptide therapeutics is no longer just a question of potency, delivery or association, as the contemporary development of a therapeutic depends more and more on the understanding of the reason for a result and the time at which the result is reliable. The heart of this transformation is a mechanistic network of binding to target, membrane interactions, entry into the cell, modulation of cell pathway and the resulting cell phenotype. So that network is important, because a peptide can bind multiple biomolecules or it can disrupt membranes and so nominal target affinity is not a valid indicator of the mechanism that leads to the efficacy. A more robust development strategy, then, considers the role of mechanism, context and uncertainty as interdependent design variables and not as distinct steps in the development process.

Peptide activity does not necessarily imply peptide mechanism. Some sequences interact with a particular receptor or protein surface while others have a disruptive effect on membranes, enter cells by several pathways and/or bind multiple proteins in concentrations utilized in phenotypic assays. The chemical proteomic capture, crosslinking, competition, biophysical affinity measurements, and genetic perturbation are complementary tools to capture those interactions. Strong causal attribution would be achieved when the dose was correlated with the change of target engagement, loss of the proposed target correlated with a decrease in the phenotype, and reengagement of the proposed target with dose or orthogonal modulation would correlate with recovery of the expected biological response.

The actual implication is that the discovery workflow will be different, with

chemoproteomic capture, crosslinking, competition experiments, genetic loss-of-function and rescue studies, orthogonal biophysics and cellular target-engagement assays. None of these tools is definitive by itself, as each of the assays is only part of a larger biological or clinical process. Independent measurements gain confidence when they all point to a similar causal narrative over the dose, time, model system and the relevant biological context. Discordant results are still relevant because inconsistency can reveal something that needs to be explored before continuing costly development efforts and before there is a huge development.

When considering decision making, the main question is which interaction is causally connected with therapeutic activity and which interactions are tolerable or harmful “collateral” binding. To answer the question, it’s necessary to explicitly compare the intended mechanism to likely alternate mechanisms, such as off-target activity, parallel pathways, differential exposure, or measurement bias. Perturbation experiments are particularly useful since they show whether the observed effect is due to a proposed component. Reliability and confidence in the mechanism must be derived from challenge tests and not from repeated confirmation within a single convenient assay.

Furthermore, almost all results obtained from causal mechanism validation studies for peptide therapeutics would vary depending on the context. Relevant variations are target expression, disease subtype, tissue penetration, protease activity and concomitant therapy. If an intervention is effective in one set of these circumstances, it may be less effective, more toxic or have a different effect, in another. When useful, stratification can be based on variables associated with mechanism or exposure, as descriptive categories do not often provide insight into why treatment performance varies.

Risk assessment is given the same consideration, due to non-specific adsorption, probe modification artifacts, proteolysis, concentration dependent membrane effects and target redundancy. Early safety work is best to be integrated with the target biology, carrier behavior, exposure, metabolites, or analytical bias associated with the mechanism of efficacy. This method decreases the risk of safety coming across as a late checklist isolated in discovery logic. It also facilitates the ability to make more sound trade-offs made when efficacy will need to be enhanced through increasing exposure, modulation of more pathways, providing longer persistence in more sensitive tissues.

The added level of complexity comes with translational development, which optimized lead based on the known cellular mechanism – not only on assay potency. With preclinical success, experiments can be more informative if they replicate clinically

relevant timing, concentration ranges, biological barriers, treatment history and sources of heterogeneity. If a convenient model can easily be extended to a complex model, that is not very useful, as it is more useful if there is a series of models to address specific questions of uncertainty. Each step thus requires a specific choice – whether to proceed, to redesign, to limit the population to be studied, to change the dose, to gather another kind of mechanistic data.

Mechanism experiments and product quality is equally important as manufacturing and analytical control as purity, sequence fidelity, conformational control, labelling chemistry and formulation affects both. Explanations based on biology are of little value when the thing being tested is not the same in different batches, or when the test is conducted with inadequate characterization of the inputs. Critical attributes must therefore have predetermined ranges, sensitive measurements and documentation of the relationship of process conditions to biological performance. In studies involving complex molecules, nanoparticles, biomaterials or data-rich studies, exposure to drugs or their metabolites, signal and apparent response of treatment can vary subtly depending on the molecule, the nanoparticle, the biomaterial type, or the amount of data.

The separation between prediction and confirmation is also enhanced in the data analysis aspect of CV in peptide therapeutics. The outputs of computational models, machine learning, causal estimators and mechanistic simulations can be used to rank hypotheses, combine high-dimensional measurements, and reveal uncertainty, but outputs of models do not equal outputs of biology. Helpful models set up assumptions, define how well they have been calibrated, identify areas of low information, and suggest what experiment to perform to get information that will influence a decision. Prospective validation is crucial because there are patterns that are likely to be fit in the past but not in the future due to new populations, new batches or new biological conditions.

Another significant change is the time factor; many processes that play a key role in causal mechanism validation of peptide therapeutics change following the initial exposure. When the final result is favorable, early response, adaptation, clearance, immune programming, tissue remodeling, adherence or resistance can take various paths. Longitudinal measurement determines if an intervention is associated with a stable state, a temporal perturbation or a delayed liability. Time-resolved data also can facilitate causal interpretation by allowing the hypothesis of the proposed mechanism to be evaluated before the downstream phenotype is seen, instead of after it is known.

Experimental design is more efficient if each study is associated with a specific

uncertainty budget. Questions can be prioritized based on their ability to alter the identity of which interaction is causally related to therapeutic activity, as well as which interactions are either tolerated or deleterious (collateral) effects, and not just because of novelty. Replication is then done across complementary models, to see if the conclusion is based on one assay, one laboratory condition, or one population. This discipline drives the efficient use of optimization time and enables negative results to be informative as the mechanism space is further reduced with each failed iteration and the next design cycle can be optimised accordingly.

The regulatory or clinical credibility also relies on traceability of claims to measures. The development package for causal mechanism validation of peptide therapeutics is strengthened if there are connections between the experimental observations, processing decisions, model assumptions, protocol deviations and material properties from early experiments to the translation experiments. This traceability allows an easy separation of biological variation from technical variation, and facilitates post hoc evaluation of efficacy or safety claims. It also establishes a more logical foundation to use when revising findings, should the input change based on new data.

More precision is required in communication around this field also. The term 'targeted', 'selective', 'durable', 'safe', 'predictive', or 'personalised' do not mean a great deal without the inclusion of a measurement, a comparator, a population and uncertainty range. The reporting of negative results is more useful, and exploratory findings can be avoided if they are not reported. This precision enables common standards and guidelines for researchers, developers, clinicians, and regulators to evaluate if a claim is based on direct measurement, calibrated inference or an untested assumption.

This strongest direction for causal mechanism validation is not a single new technology, but a tighter loop between chemistry and/or formulations, mechanistic measurement, quantitative modelling, safety, manufacturing control and clinically relevant context. Rapid progress is achieved if assumptions are exposed as early as possible and mechanisms that work are tested intentionally to ensure their success. With that logic, peptide drug discovery is provided with a tighter connection between chemistry, engagement and phenotype. In the field, the most important innovation is a system that learns from every trial and trial-and-error process, and that always interprets the causes of the results and maintains a focus on the ability to replicate and apply the findings in the real world.