



Original Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

When Molecular Dynamics Changes the Mechanistic Interpretation of Allosteric Drug Action

Hans Müller^{1*}, Anna Schmidt¹, Thomas Weber²

¹Department of Molecular Dynamics and Allostery, Faculty of Pharmaceutical Sciences, University of Bonn, Bonn, Germany.

²Department of Drug Action and Mechanistic Pharmacology, Faculty of Pharmacy, LMU Munich, Munich, Germany.

*Email: hans.mueller@uni-bonn.de

ABSTRACT

Allosteric drugs are commonly explained with structures: a ligand occupies a distal pocket, the protein adopts a different conformation, and activity changes at a functional site. Molecular dynamics (MD) complicates this account because it can reveal state populations, transient pockets, coupled fluctuations and communication routes that are absent from endpoint structures. Yet the appearance of motion in a trajectory does not itself establish a new mechanism. This Perspective asks a narrower question: when does MD change mechanistic interpretation rather than merely enrich structural description? Across recent examples, mechanistic reinterpretation becomes plausible when simulation changes an inference about which states are populated, how transitions occur, how distant regions become coupled, or why different ligands produce different functional consequences. The same evidence also exposes important limits. Communication networks depend on representation, transient states can be undersampled, pathway reconstructions can be method-sensitive, and single long trajectories can give a misleading impression of convergence. We therefore distinguish dynamic observation from mechanistic attribution and treat experimental concordance, sampling reproducibility and analysis robustness as constraints on interpretation. This approach positions MD not as an automatic mechanism generator, but as an inference-changing tool whose mechanistic weight depends on what it alters in the explanatory model and how well that alteration survives competing computational and experimental accounts.

Keywords: Protein allostery, Molecular dynamics, Conformational ensembles, Dynamic networks, Cryptic pockets, Allosteric drug design

INTRODUCTION

The pharmaceutical attraction of allostery is easy to state but harder to mechanize. A ligand can bind away from an orthosteric or catalytic site and alter function, yet the physical origin of that change need not be a single visible structural relay. Contemporary treatments describe allostery through changes in free-energy landscapes, kinetics and inter-residue communication, including cases in which signal propagation does not require a large conformational displacement. For drug design, this shifts attention from the geometry of a pocket alone toward the coupled behaviour of site, ligand, protein ensemble and functional output [1–3]. The resulting difficulty is inferential: a static structure may be correct about where a ligand binds while remaining incomplete about why that binding changes function.

Independent perturbation data reinforce the need to separate binding-site description from mechanistic coupling. Large-scale mapping of KRAS showed that distal perturbations can alter folding and partner-specific interaction

energetics across a protein landscape [4]. Such observations do not validate any particular MD-derived pathway, but they establish that mechanistically consequential coupling can extend beyond the immediate ligand-contact shell and can differ with the functional interaction being interrogated. A mechanistic account therefore has to explain not only occupancy of an allosteric site but also the state- and partner-dependent consequences of that occupancy.

This Perspective focuses on the point at which MD becomes interpretation-changing. The relevant threshold is not visual novelty. A trajectory that shows breathing, side-chain exchange or a transient hydrogen bond may add molecular detail without changing the causal story being told. By contrast, MD can alter attribution if the static account assumes one dominant state but the ligand changes the populated ensemble; if a supposedly fixed communication route is replaced by state-dependent coupling; if an apparently inaccessible pocket becomes populated in an intermediate; or if two ligands sharing a site generate different dynamical responses. These are different claims and require different standards of support.

The central argument developed here is therefore deliberately conditional. MD should carry greater mechanistic weight when it changes a consequential inference about state occupancy, transition structure, communication topology or ligand-dependent coupling, and when that changed inference remains compatible with sampling, methodological and experimental constraints. This is a proposed synthesis rather than a validated decision rule. Its purpose is to prevent two opposite errors: treating static structures as exhaustive mechanisms, and treating every simulation-derived motion as mechanistic evidence.

Allostery as dynamic redistribution

A useful starting point is to replace the image of an allosteric protein moving from one definitive structure to another with an ensemble whose populations and transition rates can be perturbed. This does not deny structural change. It changes what counts as the mechanism. In Pin1, ligand-specific magnetic-resonance measurements showed that different peptides altered the equilibrium between compact and extended interdomain arrangements differently; pCDC25c increased the population of extended states, whereas the apo and FFpSPR-bound forms were much more similar in that population balance [5]. The mechanistic implication is not merely that “the protein moves.” Ligand identity changes how pre-existing conformational possibilities are occupied and how perturbations are transmitted between domains.

The same logic becomes pharmacologically sharper when allosteric and orthosteric ligands coexist. In the M2 muscarinic receptor, cryo-EM, NMR and functional measurements showed that the positive allosteric modulator LY2119620 did not impose one context-free receptor state. Its effects depended on the co-bound agonist and were associated with distinct intracellular conformational ensembles and signalling consequences [6]. Mechanistic attribution to “the PAM” alone is therefore too coarse. The relevant unit is a ligand–receptor–state context in which orthosteric efficacy, allosteric occupancy and intracellular coupling can interact.

Convergence across evidence classes can strengthen such an interpretation without eliminating its boundaries. For the adenosine A1 receptor, MIPS521 was resolved at an extrahelical allosteric pocket in a receptor–adenosine–G-protein complex, while MD and ligand-kinetic experiments supported stabilization of that complex; the same study reported analgesic activity in a rat neuropathic-pain model [7]. These data connect structure, dynamics, kinetics and preclinical pharmacology more tightly than simulation alone could. They do not, however, justify transferring the rat efficacy result to humans or treating complex stabilization as a universal mechanism for A1-receptor PAMs.

Enhanced simulation can also change interpretation before experimental confirmation by altering what binding states are considered plausible. Gaussian accelerated MD of the A1 receptor captured spontaneous association of the PAMs PD81723 and VCP171 with extracellular allosteric regions and produced low-energy binding modes consistent with prior mutagenesis [8]. Here MD changes the structural hypothesis space: an initially unbound ligand can discover a candidate site and reshape agonist-associated receptor sampling. The inference remains provisional because agreement with mutagenesis is not identical to direct structural confirmation. Dynamic redistribution is therefore most useful mechanistically when the changed population or binding hypothesis is linked to a discriminating observation rather than inferred solely from trajectory appearance.

Hidden conformational states revealed by simulation

Static structures preferentially depict states that are sufficiently stable and experimentally accessible. Allosteric action, however, may depend on intermediates that are sparsely populated, short-lived or selectively stabilized by a ligand. Enhanced sampling of the A1 receptor identified intermediate and pre-active states between

experimentally observed endpoints, together with transient pockets and state-dependent energy-network organization [9]. Such results can overturn a two-state interpretation by suggesting that communication and pocket availability change along activation. They do not establish that every detected pocket is ligandable or that every simulated intermediate contributes measurably to pharmacology.

The stronger case arises when a hidden state yields a functional or experimentally testable consequence. Extensive MD and Markov-state modelling of the angiotensin II type 1 receptor identified a previously unresolved activation intermediate containing a cryptic pocket; mutagenesis and conformational biosensors were then used to interrogate features of the proposed activation landscape [10]. The mechanistic advance is not the mere existence of another cluster. It is the possibility that an intermediate state creates an allosteric opportunity that endpoint structures would not nominate and that perturbing this state can alter receptor activation.

Prospective structural validation provides an especially stringent version of this logic. Long-timescale MD simulations of fragment binding to protein tyrosine phosphatase 1B predicted binding poses and a rare pocket conformation that were subsequently observed by X-ray crystallography [11]. This sequence matters. Retrospective simulation can often be interpreted in the light of a known answer; prospective pose prediction followed by crystallographic confirmation instead tests whether a simulation-derived state is physically recoverable. Even this strong example remains bounded by the target, fragment chemistry and simulation protocol used.

A broader discovery workflow can combine multiple computational representations before experimental testing. An integrated residue-intuitive machine-learning and MD approach for the β 2-adrenoceptor identified a candidate allosteric site, proposed a modulator and used protein-structure-network analysis to formulate a negative-regulation mechanism that was followed by experimental assays [12]. Such integration can raise mechanistic confidence because the site hypothesis, dynamical response and functional test are not the same evidence class. It does not establish a transferable accuracy rate for the workflow across receptors. Hidden-state discovery changes mechanistic interpretation most convincingly when a state is reproducible enough to generate a prediction that an orthogonal method can challenge.

Communication networks and coupled motions

Once allostery is framed as redistribution across an ensemble, a second question follows: how is a perturbation at one region related to a response elsewhere? Network analysis offers a language for this problem by representing residues or structural units as nodes and encoding contacts, correlations, energetic couplings or learned dependencies as edges. MD-based network approaches differ substantially in how those edges are constructed, how state changes are compared and how long-range dependencies are inferred [13–15]. The important consequence is epistemic as much as computational: a communication pathway is an analysis-derived model of the trajectory, not a directly observed molecular wire.

Different network constructions answer different mechanistic questions. Neural relational inference has been used to learn latent long-range interactions from MD trajectories of Pin1, SOD1 and MEK1, explicitly addressing dependencies that simple spatial proximity can miss [14]. Difference contact network analysis instead emphasizes how contact architecture changes between functional states and is particularly useful when large-amplitude conformational change would obscure a single-network description [15]. These approaches need not identify the same “pathway,” because one asks which dependencies best account for dynamical relationships whereas the other asks which contact relationships differ between states.

This distinction prevents a common overinterpretation. Correlated motion does not, by itself, establish directional causal transmission; nor does the presence of a high-centrality residue prove that drug binding acts through that residue. Network topology can still be mechanistically valuable when perturbation, state comparison or experiment gives the inferred relationships a testable role. The most informative network is therefore not necessarily the most connected one, but the one whose construction matches the biological question and whose predicted dependencies remain stable under reasonable analytical alternatives.

MD is also not uniquely entitled to reveal allosteric communication. The structure-based Ohm method mapped predicted allosteric networks across a set of proteins and showed correspondence with NMR chemical-shift covariance evidence for CheY [16]. That result provides a useful comparator: if a static perturbation model and an MD-derived network imply the same mechanistic organization, MD may reinforce rather than change attribution. For MD to alter the mechanism, it should contribute information unavailable to the simpler representation—for example, state-dependent route switching, ligand-specific coupling, transient accessibility or kinetic ordering.

Ligand-dependent rewiring of allosteric pathways

The phrase “allosteric pathway” can misleadingly suggest that proteins contain a fixed chain of residues waiting to transmit every perturbation. Simulations of the open–closed transition of T4 lysozyme instead support a more distributed picture: a global transition emerged from cooperative changes among inter-residue contacts and was described as a fluctuation-driven transmission network [17]. Although T4 lysozyme is not a drug target exemplar for every pharmacological system, the study makes a mechanistic point that matters for allosteric ligands. Transmission may be an emergent property of a fluctuating network rather than passage along one immutable route.

Ligand dependence makes that point actionable for medicinal chemistry. In Hsp90, dynamics-derived information from ensemble docking and MD was used with machine learning to distinguish known allosteric activators from inhibitors [18]. The study does not establish prospective clinical prediction, but it shows that two compounds occupying an allosteric design space can carry different functional signatures in their dynamical effects. A binding pose alone may therefore fail to explain why chemically or spatially related ligands move function in opposite directions.

Not all rewiring needs to be localized to a discrete pocket-to-site path. A statistical thermodynamic treatment of small low-affinity ligands at membrane proteins showed how distributed ligand partitioning can shift conformational equilibria under concentration and stimulus dependence [19]. In such a regime, forcing the mechanism into a single high-affinity relay would erase the mechanism being proposed. The relevant variables become distributed occupancy and state free energies rather than one privileged chain of contacts.

These examples suggest a restrained definition of ligand-dependent rewiring. It should not mean that every edge in a network changes when a new compound is simulated. It means that ligand identity alters a relationship that matters to functional interpretation: the populated states, coupling between regions, accessibility of a pocket, transition preference or ensemble feature associated with activation versus inhibition. Rewiring is therefore a mechanistic claim only when the difference is larger than an analysis artifact and when its functional meaning is independently constrained.

Timescale and sampling dependence

Mechanistic stories derived from MD are unusually vulnerable to the way time is sampled. Long trajectories can look persuasive because they contain detailed motion, but trajectory length alone does not guarantee that relevant states have been visited in representative proportions. Ensemble simulations, Markov-state approaches and specialized transition-path methods each address different parts of this problem and impose different assumptions on the resulting mechanism [20–22]. Sampling strategy is therefore part of the mechanistic model, not a technical footnote.

Independent long-timescale simulations of SARS-CoV-2 protein–ligand systems demonstrated substantial trajectory-to-trajectory variability in contacts and other macroscopic observables, showing why one apparently well-behaved trajectory can be a poor basis for statistical inference [20]. The transferable lesson is qualitative rather than numerical: replicate ensembles reveal whether an interpretation is dominated by one stochastic realization. An allosteric route that appears only once, or a ligand-induced state shift that disappears across replicas, should carry less mechanistic weight than a pattern recoverable across independently initiated sampling. Markov-state models address a related but different problem. They replace the visual narrative of a continuous trajectory with an explicit set of metastable states and transition probabilities, making the discretization of conformational space and the kinetics between states part of the analysis [21]. This is valuable when the mechanistic claim concerns state populations or ordering, but it introduces its own dependence on state definition, lag time, sampling coverage and Markovian approximations. A clean state diagram can therefore be more interpretable than raw trajectories without becoming uniquely true.

Transition-path methods add another boundary. Linear-response-driven MD was developed to estimate apo-to-holo conformational pathways from an apo structure and ligand-binding information and was evaluated across several protein–ligand systems [22]. Such methods can expose plausible intermediates without requiring the final holo structure as an input, but the path is conditioned by the representation of the protein, the perturbation model and the sampling procedure. Mechanistic attribution should therefore distinguish “a pathway supported by this model” from “the unique pathway used by the molecule.”

The combined implication is that MD changes interpretation only within a support region defined by timescale, replicate behaviour and analytical stability. A transient state may be chemically meaningful yet too poorly sampled for population claims; a stable contact network may be reproducible yet too coarse to establish

directionality; an MSM may recover metastability while remaining sensitive to state construction. Conversely, agreement among independent replicas, state-based analysis and alternative pathway descriptions can narrow the space of plausible mechanisms even before an experiment is available. **Figure 1** compresses this sampling problem by treating mechanistic interpretation as nested support rather than as a binary property of having performed MD.

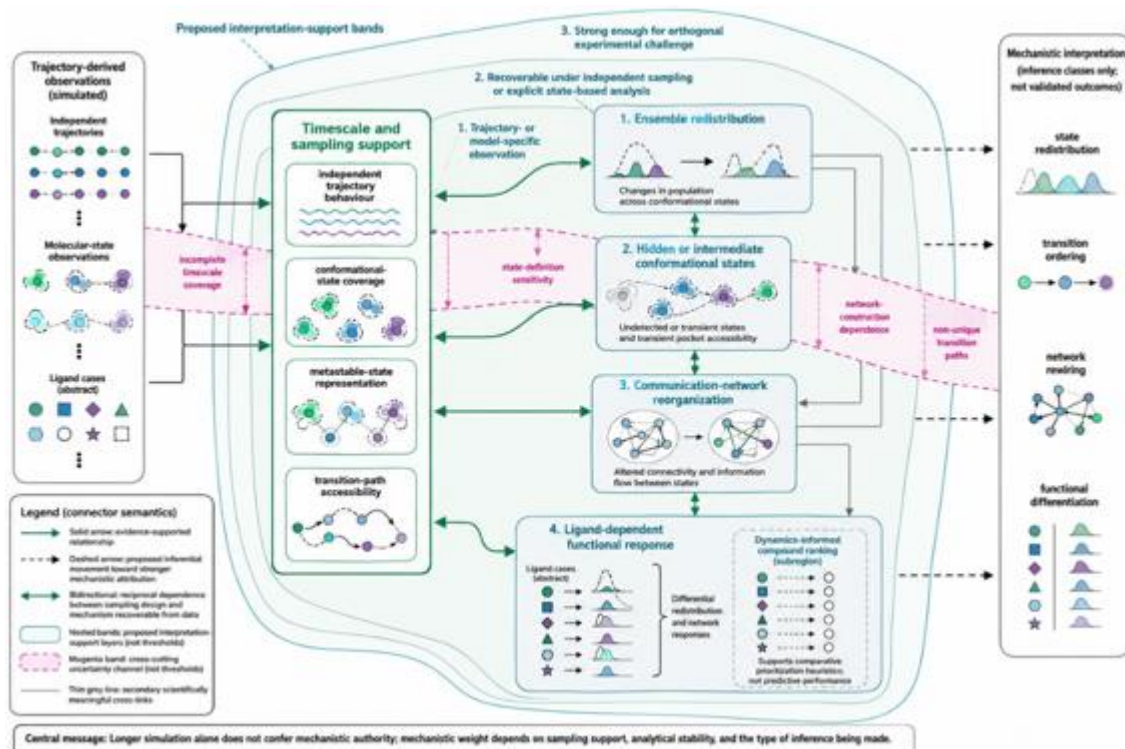


Figure 1. Sampling support determines when a simulated allosteric trajectory can carry mechanistic weight

Conditions under which molecular dynamics changes mechanistic attribution

MD changes mechanistic attribution only when it alters an explanatory variable that matters to pharmacological interpretation. The relevant change may concern which conformational state is populated, which transition is kinetically accessible, how distant regions are coupled, or why two ligands with similar binding geometries produce different functional effects. A broader computational ecosystem combining enhanced sampling, network analysis, artificial intelligence and experimental structural biology supports treating these evidence streams as complementary rather than interchangeable [23]. The proposed criterion here is therefore consequential reattribution: a simulation should change the mechanism only if removing the dynamic result would restore a materially different explanation of drug action.

That criterion requires three constraints. First, the changed inference must survive reasonable changes in sampling or representation; a state seen in one trajectory but not across independent sampling should not carry the same weight as a reproducible ensemble feature [20]. Second, the interpretation should remain distinguishable from simpler alternatives. If a structure-based network explains the same coupling pattern, MD may strengthen rather than replace the original account [16]. Third, the dynamic claim should generate an observable counterpart appropriate to its level: population redistribution should be challenged with ensemble-sensitive measurements, a cryptic pose with structural or binding evidence, and a proposed functional route with perturbations capable of discriminating among competing mechanisms.

These constraints define a proposed, non-numeric attribution sequence. A trajectory-level observation first becomes a candidate dynamic feature; it then becomes a mechanistically relevant pattern only when sampling and analysis do not make it disappear; and it changes attribution only when the surviving pattern changes the explanatory relationship between ligand, protein state and functional consequence. Failure at any step does not make the simulation useless. It changes the permissible claim—from mechanism, to hypothesis, to descriptive dynamics.

The distinction is easier to apply when the sequence, interruption points and validation counterparts are visible together rather than distributed across prose. **Table 1** therefore converts the proposed attribution logic into a sequence-aware ledger without turning it into a score or universal threshold.

Table 1. Sequence-aware ledger for deciding when MD evidence changes an allosteric mechanism rather than extending its description

case	Precursor interpretation and dynamic trigger	Governing condition for reattribution	Transfer boundary	Failure signature	Experimental counterpart	Proposed framework endpoint
Ensemble redistribution	A static state description is challenged by a reproducible ligand-conditioned population shift	The shift persists beyond one trajectory or one state partition	Applies to the tested ligand, protein state and sampling regime	Population change vanishes with replicate or state-definition changes	Ensemble-sensitive structural or spectroscopic measurement	Reattribute only the state-occupancy component
Hidden-state exposure	Endpoint structures omit an intermediate or pocket that becomes populated during simulation	The state is recurrent enough to support a discriminating prediction	Transient accessibility does not itself establish ligandability or druggability	State appears only under one protocol or lacks a stable physical interpretation	Structural, binding or targeted perturbation test	Treat as a mechanistic intermediate only after support exceeds trajectory occurrence
Communication rewiring	Ligand binding changes inferred long-range coupling or pathway organization	The network change survives defensible node, edge and state definitions	Network association does not establish directional causal transmission	High-centrality residues or routes change with analysis choices	Mutation, spectroscopy or other perturbation sensitive to the proposed coupling	Reattribute coupling architecture, not causality, unless independently tested
Functional divergence	Similar binding descriptions yield different dynamic responses associated with activation, inhibition or signalling context	Dynamic differences are reproducible and connected to measured function	Computational classification remains domain-specific and non-clinical	Functional label is predicted but the dynamic signature lacks robustness or external testing	Biochemical or cellular functional assay matched to the claimed effect	Use dynamics to refine ligand-mechanism ranking, not to claim universal predictive validity
Sampling-fragile mechanism	A compelling route or state is produced by a single long simulation	Independent sampling or state-based analysis recovers the decisive feature	Simulation duration cannot substitute for convergence	Mechanistic conclusion changes across replicas, discretizations or pathway methods	Replicate computation first; experiment only after a stable hypothesis exists	Downgrade from mechanism to hypothesis or description

Abbreviation: MD, molecular dynamics.

Experimental tests of dynamic allostery

Experimental testing should target the exact inference that MD changed. In PTP1B, biochemical screening established inhibitor activity and selectivity while MD and energy decomposition were used to suggest residues contributing to allosteric recognition [24]. The activity measurement and the residue-level mechanism are therefore different evidential statements; the former does not automatically validate the latter. Conversely, prospective crystallographic confirmation of simulation-predicted fragment poses provides a strong test when the changed claim concerns a hidden binding configuration [11].

Ensemble claims require ensemble-sensitive tests. NMR and other dynamic structural methods can reveal ligand-dependent conformational equilibria that static structures compress into a small number of endpoint models. Agreement between simulation and such measurements can support redistribution, whereas disagreement can

expose force-field, sampling or state-definition problems. The test must also have access to the relevant timescale and population; non-observation by an insensitive experiment is not equivalent to demonstrating absence.

Consequences for allosteric drug design

For medicinal chemistry, the practical consequence is to rank mechanisms as well as compounds. FASTDock illustrates how computational workflows can search beyond known allosteric sites and identify candidate ligandable regions and compounds [25]. Dynamic discovery should nevertheless remain separated from proof of pharmacological usefulness. A transient pocket can widen chemical search space without establishing affinity, selectivity, efficacy or safety.

Dynamic information is especially valuable when compounds with similar site assignments have different signalling consequences. The M2-receptor evidence shows that an allosteric modulator can interact differently with orthosteric agonist context [6], while Hsp90 work indicates that dynamics-derived descriptors can contain information relevant to functional ligand classification [18]. These findings justify using dynamics to refine compound prioritization within defined targets and datasets, not to claim target-independent predictive performance.

Experimental dynamic structural biology remains an important counterweight to simulation-only mechanisms. In GPCR drug discovery, solution NMR has been used alongside crystallographic and cryo-EM structures to resolve ligand-dependent conformational equilibria inaccessible to a single static representation [26]. The design implication is therefore convergence: simulation can expand the states and mechanisms considered, but medicinal-chemistry decisions become more defensible when dynamic hypotheses survive both computational perturbation and orthogonal measurement.

CONCLUSION

Molecular dynamics changes the mechanistic interpretation of allosteric drug action when it changes a consequential explanatory relationship rather than merely adding motion to a known structure. The most defensible reattributions involve reproducible state redistribution, hidden intermediates, altered communication architecture or ligand-dependent dynamic responses that remain stable under appropriate analysis and can be challenged experimentally. The proposed attribution logic is intentionally conditional: transient observations, analysis-sensitive networks and nonconverged pathways should remain hypotheses or descriptions. Used this way, MD does not replace structural or pharmacological evidence. It identifies when the mechanism itself must be reconsidered and where that reconsideration still requires validation.

ACKNOWLEDGMENTS: None

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

ETHICS STATEMENT: None

REFERENCES

1. Astore MA, Pradhan AS, Thiede EH, Hanson SM. Protein dynamics underlying allosteric regulation. *Curr Opin Struct Biol.* 2024;84:102768. doi:10.1016/j.sbi.2023.102768
2. Wu N, Barahona M, Yaliraki SN. Allosteric communication and signal transduction in proteins. *Curr Opin Struct Biol.* 2024;84:102737. doi:10.1016/j.sbi.2023.102737
3. Tee WV, Berezovsky IN. Allosteric drugs: New principles and design approaches. *Curr Opin Struct Biol.* 2024;84:102758. doi:10.1016/j.sbi.2023.102758
4. Weng C, Faure AJ, Escobedo A, Lehner B. The energetic and allosteric landscape for KRAS inhibition. *Nature.* 2024;626(7999):643-52. doi:10.1038/s41586-023-06954-0
5. Born A, Soetbeer J, Henen MA, Breitgoff F, Polyhach Y, Jeschke G, et al. Ligand-specific conformational change drives interdomain allostery in Pin1. *Nat Commun.* 2022;13(1):4546. doi:10.1038/s41467-022-32340-x

6. Xu J, Wang Q, Hübner H, Hu Y, Niu X, Wang H, et al. Structural and dynamic insights into supra-physiological activation and allosteric modulation of a muscarinic acetylcholine receptor. *Nat Commun.* 2023;14(1):376. doi:10.1038/s41467-022-35726-z
7. Draper-Joyce CJ, Bhola R, Wang J, Bhattarai A, Nguyen ATN, Cowie-Kent I, et al. Positive allosteric mechanisms of adenosine A1 receptor-mediated analgesia. *Nature.* 2021;597(7877):571-6. doi:10.1038/s41586-021-03897-2
8. Miao Y, Bhattarai A, Nguyen ATN, Christopoulos A, May LT. Structural basis for binding of allosteric drug leads in the adenosine A1 receptor. *Sci Rep.* 2018;8(1):16836. doi:10.1038/s41598-018-35266-x
9. Maria-Solano MA, Choi S. Dynamic allosteric networks drive adenosine A1 receptor activation and G-protein coupling. *Elife.* 2023;12:RP90773. doi:10.7554/eLife.90773
10. Lu S, He X, Yang Z, Chai Z, Zhou S, Wang J, et al. Activation pathway of a G protein-coupled receptor uncovers conformational intermediates as targets for allosteric drug design. *Nat Commun.* 2021;12(1):4721. doi:10.1038/s41467-021-25020-9
11. Greisman JB, Willmore L, Yeh CY, Giordanetto F, Shahamadtar S, Nisonoff H, et al. Discovery and validation of the binding poses of allosteric fragment hits to protein tyrosine phosphatase 1b: From molecular dynamics simulations to X-ray crystallography. *J Chem Inf Model.* 2023;63(9):2644-50. doi:10.1021/acs.jcim.3c00236
12. Chen X, Wang K, Chen J, Wu C, Mao J, Song Y, et al. Integrative residue-intuitive machine learning and MD approach to unveil allosteric site and mechanism for β 2AR. *Nat Commun.* 2024;15(1):8130. doi:10.1038/s41467-024-52399-y
13. Bernetti M, Bosio S, Bresciani V, Falchi F, Masetti M. Probing allosteric communication with combined molecular dynamics simulations and network analysis. *Curr Opin Struct Biol.* 2024;86:102820. doi:10.1016/j.sbi.2024.102820
14. Zhu J, Wang J, Han W, Xu D. Neural relational inference to learn long-range allosteric interactions in proteins from molecular dynamics simulations. *Nat Commun.* 2022;13(1):1661. doi:10.1038/s41467-022-29331-3
15. Yao XQ, Momin M, Hamelberg D. Elucidating allosteric communications in proteins with difference contact network analysis. *J Chem Inf Model.* 2018;58(7):1325-30. doi:10.1021/acs.jcim.8b00250
16. Wang J, Jain A, McDonald LR, Gambogi C, Lee AL, Dokholyan NV. Mapping allosteric communications within individual proteins. *Nat Commun.* 2020;11(1):3862. doi:10.1038/s41467-020-17618-2
17. Post M, Lickert B, Diez G, Wolf S, Stock G. Cooperative protein allosteric transition mediated by a fluctuating transmission network. *J Mol Biol.* 2022;434(17):167679. doi:10.1016/j.jmb.2022.167679
18. Marchetti F, Moroni E, Pandini A, Colombo G. Machine learning prediction of allosteric drug activity from molecular dynamics. *J Phys Chem Lett.* 2021;12(15):3724-32. doi:10.1021/acs.jpcllett.1c00045
19. Treptow W. Allosteric modulation of membrane proteins by small low-affinity ligands. *J Chem Inf Model.* 2023;63(7):2047-57. doi:10.1021/acs.jcim.2c01542
20. Bhati AP, Hoti A, Potterton A, Bieniek MK, Coveney PV. Long time scale ensemble methods in molecular dynamics: Ligand-protein interactions and allostery in SARS-CoV-2 targets. *J Chem Theory Comput.* 2023;19(11):3359-78. doi:10.1021/acs.jctc.3c00020
21. Sengupta U, Strodel B. Markov models for the elucidation of allosteric regulation. *Philos Trans R Soc Lond B Biol Sci.* 2018;373(1749):20170178. doi:10.1098/rstb.2017.0178
22. Punia R, Goel G. Computation of the protein conformational transition pathway on ligand binding by linear response-driven molecular dynamics. *J Chem Theory Comput.* 2022;18(5):3268-83. doi:10.1021/acs.jctc.1c01243
23. Agajanian S, Alshahrani M, Bai F, Tao P, Verkhivker GM. Exploring and learning the universe of protein allostery using artificial intelligence augmented biophysical and computational approaches. *J Chem Inf Model.* 2023;63(5):1413-28. doi:10.1021/acs.jcim.2c01634
24. Yang Y, Zhang L, Tian J, Ye F, Xiao Z. Integrated approach to identify selective PTP1B inhibitors targeting the allosteric site. *J Chem Inf Model.* 2021;61(9):4720-32. doi:10.1021/acs.jcim.1c00357
25. Ahmed F, Brooks CL 3rd. FASTDock: A pipeline for allosteric drug discovery. *J Chem Inf Model.* 2023;63(22):7219-27. doi:10.1021/acs.jcim.3c00895
26. Shimada I, Ueda T, Kofuku Y, Eddy MT, Wüthrich K. GPCR drug discovery: Integrating solution NMR data with crystal and cryo-EM structures. *Nat Rev Drug Discov.* 2019;18(1):59-82. doi:10.1038/nrd.2018.180