



Searching for Allosterism in Plant-Derived Chemicals Using Structural Dynamics, Pocket Plasticity, and Mechanism Prioritization

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ABSTRACT

Natural-product discovery is still frequently organized around the assumption that biologically useful ligands should be sought against structurally evident pockets and ranked primarily by predicted binding compatibility. That logic is incomplete for allosteric discovery because regulatory sites can be state-dependent, transient, structurally cryptic, or functionally meaningful only through their coupling to distant protein regions. This theory article develops a dynamics-centered framework for searching plant-derived chemical space against moving rather than singular protein structures. The analysis integrates conformational ensembles, pocket plasticity, cryptic-site discovery, residue-communication analysis, natural-product three-dimensional complexity, and mechanism-oriented molecular prioritization. The central proposal is that candidate allosteric discovery should be treated as a conditional matching problem between ligand properties and dynamically available regulatory microenvironments. Pocket visibility, persistence, communication coupling, ligand complementarity, and predicted perturbational consequences should therefore be kept analytically distinct rather than compressed into a single docking score. Plant-derived molecular complexity is treated as a potentially useful source of shape, stereochemical, and interaction diversity, not as evidence that natural products are intrinsically enriched for allosteric activity. The resulting theory further separates predicted pocket occupancy from functional allosteric control and requires prospective experimental falsification. Its applicability is limited by conformational sampling, force-field error, uncertain pocket energetics, model dependence, target-class differences, and the possibility that a dynamically detectable cavity is neither ligandable nor regulatory. The framework is proposed as a testable discovery logic, not a validated scoring system or implementation-ready pipeline.

Key Words: *Allosterism, Natural products, Molecular dynamics, Cryptic pockets, Protein conformational ensembles, Pocket plasticity*

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INTRODUCTION

Allosterism is commonly described spatially: a ligand binds away from a canonical active or orthosteric site and alters protein function. That description is useful but insufficient for discovery because it can make an allosteric site appear to be merely another pre-existing pocket waiting to be docked. Contemporary allosterism instead encompasses

changes in conformational populations, energetic coupling, communication networks, and state-dependent responsiveness that may or may not produce a conspicuous structural rearrangement [1]. A search strategy built around one experimentally resolved or computationally predicted structure therefore interrogates only one projection of the regulatory landscape.

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Natural products make this limitation especially consequential. Natural allosteric modulators have been identified across multiple biological targets, demonstrating that natural-product chemistry can support genuine regulatory-site mechanisms [2]. Yet this observation does not establish that plant-derived molecules are generally enriched for allosteric behavior. Natural-product drug discovery is chemically broad and pharmacologically productive, but its translation is also complicated by structural complexity, sourcing, characterization, target assignment, and mechanism attribution [3]. Those considerations argue against replacing an orthosteric bias with an equally simplistic “natural products are allosteric” bias.

The theoretical problem is therefore one of search representation. If the protein is represented as a single receptor conformation and the ligand is ranked principally by static complementarity, dynamic regulatory opportunities remain systematically underdescribed. The alternative proposed here is to treat allosteric discovery as a conditional search through protein-state space. A plant-derived molecule becomes interesting not merely when it fits a cavity, but when its chemical and three-dimensional properties are compatible with a dynamically available site whose perturbation is plausibly coupled to function. This reframing does not discard docking, predicted structures, or canonical binding sites. It changes their evidential role: they become components of a broader mechanism-oriented search rather than stand-ins for mechanism itself.

Allosteric recognition begins with protein motion

Protein motion is not a secondary complication added after ligand binding; it can be constitutive to the physical basis of allosteric regulation. Allosteric signaling can arise through shifts among conformational populations, redistribution of fluctuations, and altered coupling between regions of a protein or molecular assembly [4]. The experimentally relevant state is therefore not always the most populated unliganded conformation, nor does allosteric transmission necessarily require a large structural displacement.

This ensemble perspective also explains why allosteric effects are highly contextual. Mutations, state changes, local structural perturbations, and target architecture can modify whether and how a distant site communicates with a functional region [5]. Accordingly, the existence of geometric separation between two pockets says little by itself about regulatory coupling.

Molecular dynamics and network-based analyses provide one computational route for interrogating this problem by examining state populations, residue interactions, correlated motions, and candidate communication pathways [6]. Such analyses can make hidden coupling hypotheses visible, but they do not convert covariance into causation. The proposal advanced here is narrower: motion should be treated as a search variable. Candidate allosteric sites should be evaluated in relation to the states in which they appear, the communication structures with which they are associated, and the perturbations that could plausibly shift those structures.

The distinction between established allostery principles and the search extension proposed in this article is summarized in **Table 1**.

Table 1. From established dynamic allostery principles to a proposed search logic for plant-derived ligands

Established principle	Evidence-supported interpretation	Proposed discovery extension
Proteins populate conformational ensembles	Regulatory behavior need not be encoded by one dominant structure	Search over relevant states rather than treating one structure as the complete receptor
Allosteric effects depend on structural and mutational context	The same site or perturbation need not behave equivalently across systems	Treat target state and molecular context as explicit conditions of prioritization
MD and network analysis can expose candidate communication patterns	Distal coupling can be interrogated computationally	Use communication evidence as a mechanistic filter after site detection
Binding and allosteric control are conceptually distinct	A ligandable pocket is not automatically a regulatory site	Require later evidence that occupancy perturbs a functionally coupled network

Note: The right-hand column is the proposed theoretical extension developed in this article; it is not a validated scoring rule.

Figure 1 expresses the corresponding shift from a static-site search to a state-conditioned model of allosteric recognition.

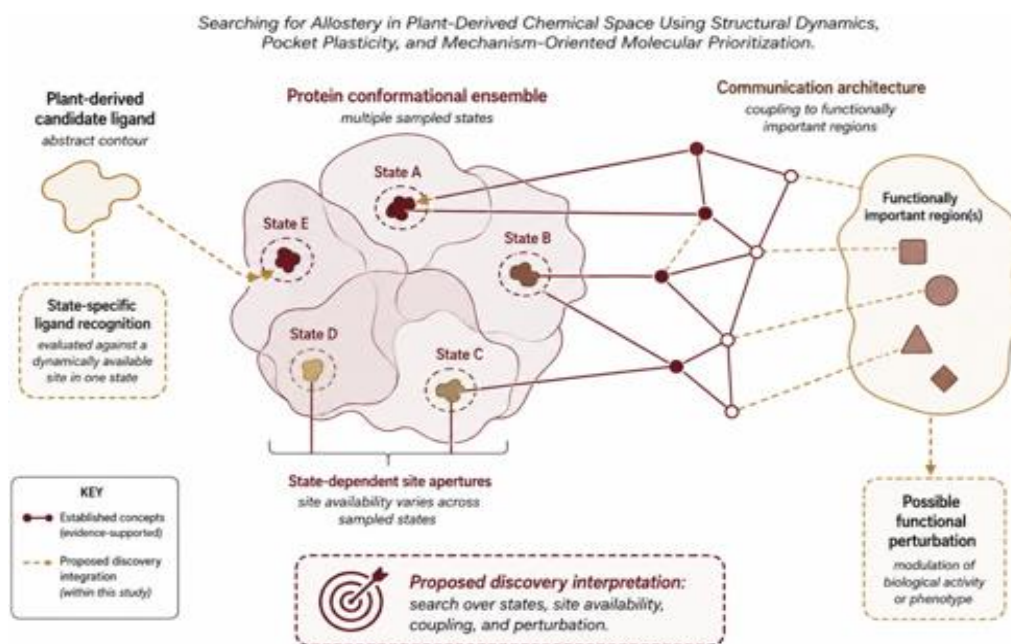


Figure 1. Protein-state dependence as the starting condition for allosteric discovery

Pocket plasticity changes what counts as a druggable site

A binding site can be chemically relevant without being continuously visible. Cryptic sites are a clear example: ligandable cavities may be absent or poorly formed in an apo structure and become accessible after local side-chain or backbone rearrangement [7, 8]. This means that “druggable site” is partly an ensemble-dependent designation. A protein surface that appears unpromising in one state may contain a chemically actionable microenvironment in another.

Recent computational work has consequently shifted from identifying only static depressions toward sampling and characterizing state-dependent pockets, while emphasizing that cryptic-site discovery remains sensitive to the sampling method, representation, and criterion used to define the pocket [9]. Tools designed to track pocket flexibility likewise demonstrate that cavity geometry can change substantially across protein conformations and that transient pockets can be detected from these changes [10]. For the present theory, three variables must therefore remain distinct. Visibility concerns whether a pocket is detectable in a particular structure. Persistence concerns how often or how stably a pocket occurs across the sampled ensemble. Regulatory relevance concerns whether occupying or perturbing that site is coupled to a functional region. A site may score highly on any one of these properties while failing on the others. This separation prevents a common inferential shortcut: converting the observation that a cavity opens into the conclusion that an allosteric druggable site has been found.

Why rigid structures underestimate plant-derived chemical space

Static protein representations can be valuable starting points, but their limitations become consequential when the discovery question itself depends on state change. Predicted or experimentally determined single structures can underrepresent alternative states important for allosteric recognition [11]. Benchmarking of ligand-induced structural prediction further shows that reproducing conformational changes associated with both orthosteric and allosteric binding remains challenging in some target systems [12]. The appropriate conclusion is not that static structures are unusable, but that they should not be treated as exhaustive when the site of interest is dynamically generated.

A parallel representation problem occurs on the ligand side. Contemporary computational approaches can generate extremely large natural-product-like libraries, demonstrating the scale at which this chemical space may be searched [13]. Large enumerated space, however, is not equivalent to complete pharmacologically relevant representation. Three-dimensional form, stereochemistry, accessible conformers, intramolecular interactions, and functional-group presentation can determine which protein microenvironments a complex molecule can actually occupy.

Experimental chemical-biology work with natural-product-like macrolides illustrates how stereochemically rich and three-dimensional molecular designs can produce biologically active chemotypes that are poorly described by simplistic flat-space notions of similarity [14]. That

evidence does not demonstrate preferential recognition of allosteric sites. The theoretical extension proposed here is conditional: when a protein exposes a noncanonical site with unusual shape, depth, polarity distribution, or state dependence, the three-dimensional diversity of plant-derived chemistry may provide matching opportunities that a rigid ligand-versus-rigid receptor search fails to prioritize.

Conformational ensembles as the search substrate

If allosteric opportunities are state-dependent, the computational search substrate should be an ensemble rather than a single receptor snapshot. Molecular dynamics can reveal cryptic conformations and hidden pockets not represented in a starting structure, although the resulting ensemble remains dependent on force field, sampling duration, initial state, and convergence [15]. The ensemble should therefore be viewed as a model of accessible states, not a complete catalogue of everything the protein can do. Structure-prediction methods can nevertheless contribute usefully when treated as seeds for further state exploration. AlphaFold-derived structures have been used to accelerate cryptic-pocket discovery when combined with molecular simulation rather than interpreted as final receptor states [16]. Enhanced-sampling methods extend the same principle by deliberately increasing exploration of conformational transitions that might be rare on straightforward simulation timescales. Normal-mode-guided enhanced sampling has, for example, been applied to cryptic-pocket exploration in KRAS G12D [17], whereas SWISH-X uses biased-solvent sampling to facilitate hidden-pocket discovery in proteins and protein–protein interfaces [18].

These approaches should not be collapsed into a single hierarchy of “best” sampling methods. They introduce different priors, biases, collective variables, and computational costs. The proposed theory therefore assigns the ensemble a more modest role: it generates candidate state hypotheses. A pocket becomes more interesting when its appearance is reproducible across defensible sampling strategies or structurally plausible state transitions, but persistence alone still does not establish allosteric function.

Detecting persistent, transient, and communication-coupled pockets

Dynamic-site discovery requires more than asking whether a cavity exists. One useful pre-sampling strategy is to predict where cryptic pockets are likely to form. PocketMiner, for example, uses graph-based learning to prioritize regions capable of pocket formation from a single protein structure [19]. Property-oriented platforms such as CavityPlus provide complementary geometric,

physicochemical, pharmacophoric, and allosteric-site analyses [20], while PASSer applies machine-learning approaches specifically to candidate allosteric-site prediction [21]. These methods differ in training labels and underlying definitions, so their outputs should not be treated as directly interchangeable measures of the same physical quantity.

The stronger theoretical distinction is between persistent, transient, and communication-coupled sites. Persistence is an ensemble property: a cavity remains sufficiently formed across a meaningful subset of sampled states. Transience is not necessarily a weakness; a short-lived cavity may still be chemically exploitable if ligand binding stabilizes a relevant state. Communication coupling adds a different requirement—evidence that the region is connected to a functional site through structural, energetic, dynamical, or evolutionary relationships.

Combined computational and experimental work has shown that analysis of reversed allosteric communication can uncover cryptic regulatory sites that can subsequently be tested experimentally [22]. Structural information can also be integrated with coevolutionary signals to improve allosteric-site inference [23]. MD-based residue–interaction networks provide another means of examining candidate coupling between distant protein regions [24], and computational approaches can identify plausible communication pathways across protein structures [25].

These results justify communication analysis as an evidence layer, but not as mechanistic proof. Correlation, path centrality, or residue-network connectivity can arise without causal signal transmission. The three-site categories are therefore proposed here as an organizing typology, not as mutually exclusive biological classes. A site may be transient yet strongly communication-coupled, persistent but mechanistically irrelevant, or predicted to lie on a communication route without ever becoming chemically accessible.

Matching natural product shape complexity to dynamic sites

Plant-derived chemistry offers a broad range of ring systems, stereochemical arrangements, oxygenation patterns, aromatic surfaces, hydrogen-bonding motifs, and conformational constraints. Studies of plant-derived molecules acting at G protein-coupled receptors illustrate pharmacological diversity across receptor systems and reinforce the need to interpret natural-product activity in a target-specific manner [26]. Such diversity alone, however, does not justify treating plant origin as a mechanistic predictor.

More compelling is direct evidence that a natural product can occupy a regulatory site and alter function. The discovery of a natural-product modulator targeting the

regulatory site of endoplasmic-reticulum aminopeptidase 1 provides an example in which noncanonical-site recognition is tied to a measurable biological consequence [27]. It remains an exemplar, not evidence that regulatory-site modulation is a general property of natural products. The proposed matching principle is therefore conditional. Ligand shape complexity matters only relative to the geometry and chemistry of the state-dependent site being considered. Rigidity may preserve a complementary three-

dimensional arrangement and reduce conformational ambiguity, but excessive rigidity can prevent adaptation to a plastic cavity. Flexibility can permit accommodation across related states, but can also increase entropic cost and complicate pose assignment. Polyfunctionality can create distributed interaction opportunities, yet can equally produce promiscuity or nonspecific binding.

Table 2 formalizes these dependencies as a construct architecture rather than a compound-scoring system.

Table 2. Proposed construct architecture for matching plant-derived molecular complexity to dynamic regulatory sites

Construct	Protein-side meaning	Ligand-side meaning	Evidential boundary
State-conditioned complementarity	Site geometry exists only in particular conformations	Three-dimensional shape must be evaluated against the relevant state	Static fit does not establish dynamic accessibility
Persistence compatibility	Site recurs or remains open across sampled states	Ligand need not rely on an implausibly rare geometry	Persistence does not establish regulation
Communication compatibility	Candidate site is connected to a functional region	Bound ligand is positioned to perturb that network	Network coupling remains inferential
Shape complexity	Dynamic site presents noncanonical spatial constraints	Stereochemistry, ring topology, rigidity and conformational range shape recognition	Natural-product complexity does not imply allosteric preference
Regulatory-site precedent	Nonorthosteric occupation produces a measurable functional consequence	A natural product can realize this mechanism in a defined system	Target-specific evidence cannot be generalized class-wide

Note: The constructs and their integration are proposed here. They are analytical dimensions, not validated numerical criteria.

Figure 2 integrates the preceding sections into a manuscript-derived typology of the matching problem.

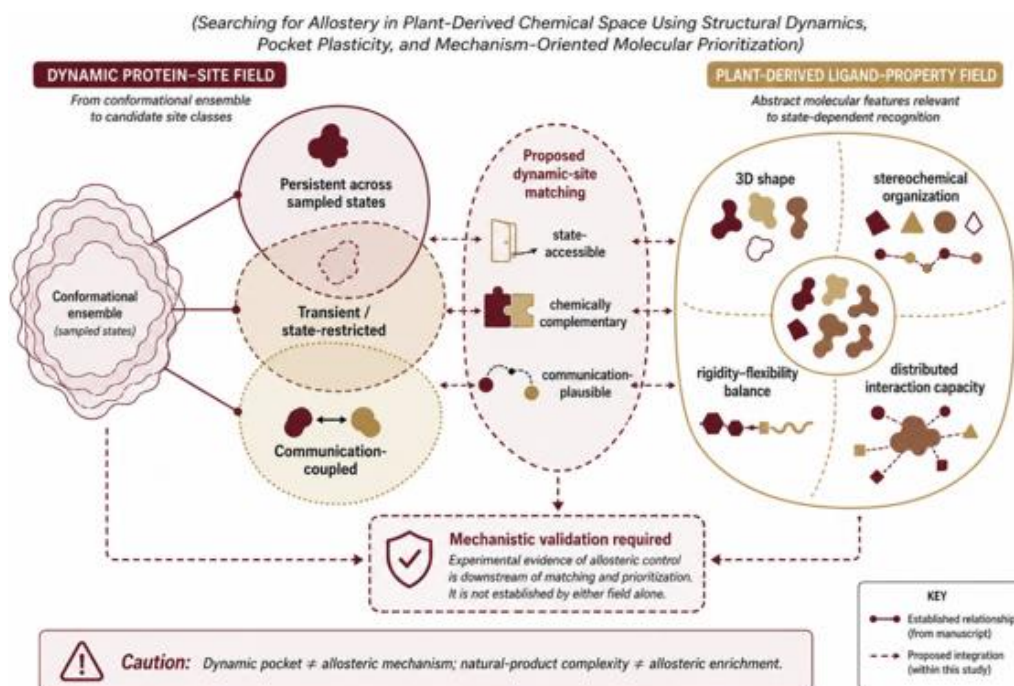


Figure 2. Conditional matching between dynamic pocket states and plant-derived molecular complexity

Beyond docking score: prioritizing mechanistic perturbation

Docking remains useful for generating poses, filtering large libraries, and testing geometric compatibility, but its score is not a direct measure of binding mechanism,

allosteric coupling, or functional consequence [28]. For a dynamic regulatory site, a high-ranking pose is therefore best treated as a structural hypothesis. The question that follows is not simply whether the ligand can occupy the pocket, but whether occupancy is compatible with the

receptor state in which the pocket exists and whether that state is connected to a functionally relevant response.

Ligand-aware complex-prediction methods provide one route beyond rigid receptor treatment by allowing receptor geometry to adjust in relation to the ligand [29]. Such adaptation can improve structural hypothesis generation, but conformational accommodation is not itself evidence of allostery. A receptor may reorganize locally around a ligand without altering a distant functional region, while an important allosteric effect may occur through changes in populations or fluctuations that are poorly represented by a single optimized complex.

A mechanism-oriented ranking should therefore interrogate perturbation after pose generation. MD-based communication analysis can compare how ligand-associated trajectories redistribute candidate paths or interaction networks, providing hypotheses about which bound states may be mechanistically consequential [30]. The relevant signal is not “network change” in the abstract, but a reproducible alteration that links the candidate pocket to residues, domains, interfaces, or conformational transitions tied independently to function.

Functional precedent further shows why affinity-centered ranking is incomplete. Structure-based identification of a CB1 allosteric modulator produced pathway-biased consequences, illustrating that an allosteric ligand can alter signaling quality rather than simply increase or decrease an undifferentiated response [31]. This is target-specific evidence, not a general prediction rule, but it shows that downstream phenotype belongs inside the discovery logic. Likewise, structure-based discovery of positive allosteric modulators of the A1 adenosine receptor demonstrates a progression from nonorthosteric structural hypotheses to functional confirmation [32]. For plant-derived screening, the proposed implication is to prioritize candidates whose poses remain plausible in relevant states, whose occupancy is compatible with communication evidence, and whose predicted perturbation can be translated into an experimentally discriminating functional hypothesis.

A dynamics-to-allostery prioritization theory

The proposed theory organizes candidate evidence along separable axes rather than collapsing it into a composite score. The first axis is state availability: is the candidate pocket physically represented in defensible conformational states? The second is pocket behavior: is the site persistent, transient but plausibly stabilizable, or otherwise condition-dependent? The third is communication plausibility: do structural, dynamical, energetic, or evolutionary analyses connect the region to a functional locus, recognizing that these signals remain inferential [23]? The fourth is ligand complementarity: does a plant-derived molecule present the three-dimensional shape, stereochemical arrangement,

rigidity–flexibility balance, and interaction pattern appropriate to that state-specific microenvironment?

A fifth axis is predicted perturbational consequence. Communication-path analysis can be used after a candidate pose is generated to ask whether ligand occupancy is associated with a change in the network linking the site to functionally important regions [30]. This ordering is proposed, not mandatory: some targets may have strong experimental allosteric annotation before pocket detection, whereas others may begin with a cryptic-site hypothesis. What matters is that geometric fit and communication evidence remain separately inspectable so one weak dimension cannot be hidden by strength in another.

Crucially, the axes should not be averaged simply to rescue weak candidates. A pocket that is repeatedly visible but lacks credible communication should remain a pocket hypothesis; a chemically attractive ligand that requires an unsupported receptor state should remain a pose hypothesis; and a strong network perturbation without plausible occupancy should remain a dynamics hypothesis. This veto-like interpretation preserves mechanistic transparency and makes negative evidence informative. It also allows prioritization to be adapted to target class without implying that one universal numerical weighting can transfer across enzymes, receptors, channels, or protein–protein interfaces. The theory therefore favors evidence profiles and explicit failure points over a single rank-ordering function.

The final axis is validation readiness. A useful candidate should imply observations that can fail: loss or alteration of modulation after site perturbation, measurable target engagement, state-sensitive structural or dynamic responses, and a functional phenotype consistent with the proposed direction of control. Existing structure-to-function allosteric discovery provides precedent for keeping functional validation downstream of structural plausibility [32]. The theory therefore defines a prioritization architecture, not a validated hierarchy of weights, and it remains falsifiable by candidates that satisfy its computational conditions but fail experimental tests.

Distinguishing allosteric binding from allosteric functional control

Occupancy of a nonorthosteric pocket is not equivalent to functional allostery. Cooperativity can depend on kinetic as well as equilibrium context, so the apparent relationship between ligand binding, orthosteric ligand behavior, and response may vary with timescale and assay design [33]. A mechanistic claim must therefore specify what is modulated: affinity, efficacy, kinetics, conformational population, pathway preference, catalytic behavior, or another defined functional endpoint.

Natural-product regulatory-site modulation of ERAP1 provides a concrete example in which noncanonical-site binding is connected to a measurable functional consequence [27]. Its importance here is evidential: the case crosses the boundary from pocket occupation to target-specific biological modulation. It does not establish that structurally similar natural products, other targets, or other regulatory pockets will behave equivalently. Accordingly, computational prioritization should terminate in a hypothesis about functional control, not in the declaration that allostery has been discovered. Positive allosteric modulation established through structure-based discovery still required functional testing beyond the binding-site model [32]. Negative results are equally informative because they can identify sites that are ligandable but nonregulatory, ligands that bind without effective coupling, or computational communication signals that do not survive perturbational testing.

Experimental routes from predicted pocket to mechanistic validation

Solution-state structural and dynamics measurements can test whether ligand binding is accompanied by changes in conformational protection, populations, or dynamics at regions beyond the candidate pocket [34-36]. These observations are valuable because the theory predicts state redistribution and communication, but a distal response alone does not establish causal allostery. Experimental validation should triangulate site engagement, structural or dynamic response, and a defined functional consequence. HDX-MS can map ligand-associated changes in exchange behavior that report on altered protection and protein dynamics, while interpretation must account for the multiple structural and kinetic processes contributing to exchange [34]. Such data are most informative when mapped against the proposed site and independently implicated functional regions rather than interpreted as a global “allostery signature.”

NMR can interrogate conformational populations and dynamics with complementary sensitivity to state exchange and distal responses [35]. High-resolution HDX-MS studies of PTP1B further show that native dynamics and allosteric responses can be experimentally resolved in a defined system [36]. For the proposed framework, stronger validation would combine these approaches where appropriate with direct binding or target-engagement measurements, functional assays, and perturbations such as site-directed mutagenesis. No single modality is universally decisive.

False allostery, overinterpreted dynamics, and other failure modes

The most immediate failure mode is to mistake pocket appearance for actionable regulation. A cryptic cavity may be dynamically real yet remain an infeasible drug target because opening energetics, ligandability, or chemical tractability are unfavorable [37]. Conversely, a tractable cavity may have no meaningful functional coupling. The theory therefore rejects a binary sequence in which “cryptic” automatically implies “allosteric” and “allosteric” automatically implies “druggable.”

Structural-model error creates a second route to false confidence. Predicted or static structures can fail to reproduce ligand-induced states relevant to recognition [12]. Sampling can also generate rare or force-field-dependent geometries that appear compelling when viewed retrospectively. Reproducibility across trajectories or methods, physical plausibility, and sensitivity analyses should therefore be treated as safeguards rather than as proof.

A third failure mode is interpretive: apparent disagreement between binding and function may reflect kinetic context rather than computational error or biological inconsistency [33]. Other hard cases include ligands with multiple binding sites, promiscuous natural products, covalent or reactive chemistry, proteins with poorly characterized functional states, and systems in which allosteric communication is distributed rather than dominated by a single pathway. These cases limit any simple site-to-mechanism narrative.

Scope of the theory

The theory is most applicable to proteins for which alternative conformational states, cryptic or transient pockets, and state-dependent communication can be meaningfully interrogated. Cryptic-pocket discovery itself remains method- and system-dependent [9]; therefore the framework does not imply that every target requires extensive molecular dynamics or that every allosteric campaign should begin from an unobserved site.

Nor does the proposal replace docking or static structural analysis. Docking remains valuable when the receptor state is appropriate to the question and its scoring limitations are respected [28]. The added requirement is epistemic separation: state availability, pocket feasibility, ligand complementarity, communication, and functional consequence should be represented as distinct evidential claims.

This is a proposed discovery theory rather than a validated scoring system. Its strongest prospective test would compare it with simpler baselines on targets containing known allosteric sites and difficult negatives, using stereochemically explicit plant-derived molecules and prespecified success criteria. Failure to outperform simpler approaches, failure of predicted coupled pockets to survive

perturbation, or systematic dependence on one sampling protocol would count against the theory rather than be absorbed as implementation detail.

CONCLUSION

Searching plant-derived chemical space for allostery requires a representation of the protein that admits motion, state dependence, pocket plasticity, and long-range coupling. The central proposal is not that natural products are intrinsically allosteric, nor that dynamics can reveal hidden drug targets automatically. It is that allosteric discovery becomes more coherent when pocket visibility, persistence, ligandability, communication plausibility, ligand complementarity, predicted perturbation, and functional validation are kept distinct.

This reframing changes what counts as a promising computational hit. A candidate is valuable not because one score is high, but because it supports a sequence of testable mechanistic propositions while exposing where uncertainty remains. Plant-derived structural complexity may broaden the set of state-specific microenvironments that can be productively explored, but that advantage is conditional and target-specific.

The theory is therefore deliberately falsifiable. Dynamic pockets that cannot be engaged, engaged pockets that do not couple to function, and computational perturbations that disappear under orthogonal testing are failures of the corresponding hypothesis, not successes awaiting reinterpretation. The practical objective is to search the moving protein without confusing motion with mechanism—and to use computation to generate sharper experiments rather than to substitute for them.

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