



Cryptic Pockets Expand the Druggable Space of Natural Product Pharmacology Through Conformational Sampling, Transient Site Detection, and Structure-Guided Ligand Discovery

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ABSTRACT

Static protein structures can conceal ligand-binding opportunities that emerge only when conformational fluctuations expose otherwise inaccessible regions. Cryptic pockets therefore challenge structure-centered definitions of druggability, but their discovery creates a second problem: transient-site detection is not equivalent to ligandability, functional relevance, or pharmacological usefulness. This conceptual framework integrates protein dynamics, molecular simulation, experimental hidden-state evidence, natural-product chemical-space reasoning, and structure-guided ligand discovery into an evidence-bounded strategy for cryptic-pocket pharmacology. The analysis distinguishes cryptic pockets from merely small or shallow cavities, separates pocket opening from persistence and druggability, and treats computational detection as an evidence-generating step rather than structural confirmation. It further proposes that the stereochemical and three-dimensional complexity represented in natural-product and natural-product-like chemical space may complement some conformationally generated binding environments, while emphasizing that preferential cryptic-pocket recognition by natural products has not been demonstrated. The principal contribution is a proposed pocket-before-ligand framework in which candidate sites are prioritized according to evidence for accessible opening, recurrence, structural and physicochemical coherence, functional connectivity, and orthogonal validation before ligand ranking is allowed to dominate. The framework also separates docking plausibility, target engagement, functional modulation, allosteric interpretation, and downstream pharmacology. Major boundaries include rare-state sampling, force-field and model dependence, uncertain opening probabilities, incomplete experimental observability, and sparse direct evidence connecting natural-product shape complexity to cryptic-site preference. Cryptic pockets expand actionable structural space only when dynamic detectability is converted into experimentally constrained pharmacological opportunity.

Key Words: Cryptic pockets, Natural products, Conformational sampling, Molecular dynamics, Druggability, Structure-Guided discovery

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INTRODUCTION

Protein structures used in drug discovery are often treated as sufficiently complete descriptions of the molecular

surface available to small molecules. For many targets this approximation is productive, but it becomes less secure when ligand-relevant cavities form only in low-population or ligand-stabilized conformations. Contemporary cryptic-

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pocket research therefore treats an experimentally resolved protein structure as one member of a conformational ensemble rather than as a complete inventory of druggable surface. Recent computational developments increasingly exploit this ensemble view to identify binding opportunities that are absent, occluded, or poorly formed in dominant apo structures [1].

A cryptic pocket is not simply an overlooked surface depression. Its defining characteristic is conformational hiddenness: a site that is absent or insufficiently formed in an unbound structure becomes substantially more binding-competent after protein rearrangement, often in association with ligand binding [2]. This distinction prevents an indiscriminate expansion of “druggable space” in which every transient cavity produced by simulation is promoted to a pharmacological opportunity. Molecular dynamics may generate many temporary depressions, but only a subset is expected to provide sufficiently coherent interaction environments for meaningful ligand discovery. Accordingly, cryptic-site discovery should be interpreted as a sequence of evidential questions rather than a contest in pocket detection. Structural analyses of feasible cryptic targets indicate that the mechanism and extent of site opening influence whether an exposed state can plausibly accommodate drug-sized ligands [3]. Detection therefore does not establish druggability.

The framework developed here treats dynamic protein structure as an expansion of pharmacological search space only when pocket opening can be separated from persistence, ligandability, functional relevance, and experimental validation. Within this logic, natural products are not assumed to possess privileged access to cryptic pockets. Instead, their three-dimensional and stereochemical diversity motivates a narrower, testable proposition: certain dynamically generated binding environments may reward molecular shapes underrepresented in flatter screening collections. The article consequently adopts a pocket-before-ligand

principle in which chemical-space matching begins only after the structural opportunity has acquired sufficient evidential credibility.

What makes a pocket cryptic rather than merely small?

The distinction between a cryptic pocket and a small constitutive pocket begins with state dependence. A small cavity may be shallow, solvent-exposed, or difficult to occupy while remaining recognizable in the apo protein. A cryptic pocket instead requires conformational movement that creates, uncovers, or substantially improves a ligand-compatible site. Structural comparisons indicate that regions later forming cryptic pockets may already contain binding hot spots and increased local flexibility in unbound structures [4]. These features may signal latent recognition potential, but they do not demonstrate that opening will occur or that the resulting cavity will be ligandable.

Crypticity is also mechanistically heterogeneous. Structural analysis has shown that pocket opening can range from side-chain rearrangement to loop movement and larger backbone displacement [5]. The discovery implications differ accordingly. A site masked mainly by a rotameric barrier may become accessible under modest local sampling, whereas a pocket requiring coordinated displacement of a larger structural segment may remain kinetically remote.

Recent simulation of targets undergoing extensive pocket-forming conformational changes further suggests that local interaction networks can stabilize closed states and that disruption of selected intramolecular contacts can facilitate larger rearrangements [6]. The relevant construct is therefore not pocket size alone but the relationship among hidden geometry, the motions necessary to expose it, and the accessibility of those motions.

The distinctions developed in this section are summarized in **Table 1** without treating any structural category as a direct measure of pharmacological value.

Table 1. Structural distinctions for interpreting hidden and conformationally generated protein cavities

Construct	Defining structural condition	Evidence needed for assignment	What the designation does not establish
Constitutive small pocket	Cavity already present in an apo structure	Reproducible apo-state geometry	Crypticity, affinity, or druggability
Incipient cryptic region	Apo surface contains structural precursors to a later pocket	Apo–holo comparison and local structural evidence	Spontaneous or frequent opening
Cryptic pocket	Binding-compatible cavity emerges through conformational change	State-resolved structural evidence	Useful ligandability or functional importance
Locally gated cryptic pocket	Opening dominated by limited local rearrangement	Evidence identifying the gating motion	Easy access for drug-sized ligands
Extensively gated cryptic pocket	Opening requires coordinated segment or backbone motion	Ensemble evidence spanning the transition	High native population or tractable sampling

Conformational fluctuations as pocket-opening events

Once crypticity is defined through state dependence, the mechanistic question becomes how the ligand-compatible

conformation is reached. A simple division between induced fit and conformational selection may be insufficient. Long-timescale simulations of small-molecule binding to interleukin-2 demonstrated a pathway in which protein motion and ligand interactions jointly shaped access to a cryptic protein–protein-interface pocket [7]. Such observations support an ensemble-based interpretation in which ligands may encounter pre-existing fluctuations, stabilize partially formed states, and subsequently reorganize local structure. They do not establish a universal kinetic mechanism for cryptic recognition.

The pharmacological importance of an opening event depends on what the newly accessible state controls. At CB1, a cryptic extension identified through molecular dynamics was incorporated into ligand design, and subsequent structural and pharmacological experiments connected occupancy of the region with altered signaling and peripheral selectivity in the studied ligand series [8]. This provides strong target-specific evidence that a low-accessibility structural state can become a medicinal-chemistry variable, but it does not demonstrate that cryptic-pocket targeting generally improves selectivity or therapeutic index.

A different relationship appears in Ebola VP35, where adaptive simulations identified a cryptic pocket linked to the double-stranded RNA-binding interface and experimental perturbations supported allosteric communication between the hidden site and protein function [9]. Together, these studies show that cryptic-pocket recognition can involve different combinations of ligand-access dynamics, state stabilization, and functional coupling [7–9].

The proposed framework therefore separates pocket opening from functional consequence. A cavity may become geometrically accessible without controlling a relevant biological process, whereas a functionally connected site may still be too unstable, chemically unfavorable, or rarely populated to justify ligand screening. Conformational mobility creates opportunity; it does not determine pharmacological importance by itself.

Detecting transient sites through molecular simulation

Molecular simulation is particularly attractive because cryptic-pocket discovery is intrinsically an ensemble problem. Conventional molecular dynamics can reveal conformations absent from a starting structure, while enhanced methods attempt to cross barriers unlikely to be traversed on accessible simulation timescales. Methodological analyses emphasize both this opportunity and its central limitation: the observation of an open state is conditional on force field, sampling duration, starting conformation, solvent model, and acceleration strategy

[10]. Simulation therefore explores conformational possibility rather than directly confirming biological occupancy.

Mixed-solvent molecular dynamics adds chemically informative probes to the sampling process. In a study of proteins with known cryptic sites, MixMD identified several hidden regions, whereas larger rearrangements could require accelerated sampling [11]. This demonstrates why failure to observe opening under one protocol cannot be equated with absence of a pocket. The converse is equally important: accumulation of probe molecules in a simulation does not prove that the corresponding state is naturally populated or pharmacologically tractable.

Enhanced-sampling methods address different conformational barriers. SWISH-X extends pocket exploration to demanding systems including protein–protein interfaces by increasing the accessible conformational space [12]. Normal-mode-guided enhanced sampling has similarly been applied to KRAS G12D to explore known and candidate cryptic conformations that may be difficult to recover from ordinary trajectories [13]. These strategies deliberately reshape sampling efficiency, so they are particularly useful for hypothesis generation while requiring caution when their generated populations are interpreted thermodynamically.

Detection must also consider time. A water-informed method for dynamic pocket characterization was developed to distinguish changing pocket states across trajectories rather than evaluating only isolated conformations [14]. CrypToth combines mixed-solvent simulations with topological analysis to prioritize cryptic regions using probe preference and structural changeability [15].

These developments motivate a three-level distinction. A cavity can appear in an individual frame, recur in independent trajectories, or persist long enough to present a chemically coherent interaction environment. Only the first condition establishes computational detectability. Recurrence addresses reproducibility, whereas persistence begins to address whether the pocket represents more than a transient geometric fluctuation. None of these levels alone establishes druggability.

Experimental evidence for hidden pocket states

Simulation can miss genuine openings, but it can also generate conformations whose biological relevance is uncertain. Cryptic-pocket claims therefore become stronger when hidden states are constrained experimentally. Disulfide tethering provides one route. Screening engineered cysteine variants of oncogenic KRAS identified known and previously unrecognized ligandable regions, with selected hits subsequently

examined using competition experiments, computation, and NMR [16]. Such evidence supports physical accessibility and fragment recognition at particular regions but does not mean that native noncovalent ligands will reproduce tethering behavior.

NMR offers a complementary approach because it can interrogate conformational equilibria in solution. In Bcl-xL, an NMR-based strategy characterized a cryptic-site equilibrium and demonstrated that perturbation of the protein could alter the population of a binding-competent state [17]. This type of evidence is particularly important when the open conformation is sparsely populated and may be poorly represented by conventional structural determination.

The evidential implication is hierarchical rather than method-specific. Structural comparison can demonstrate an alternative pocket geometry. NMR can constrain conformational exchange or state populations. Tethering and fragment methods can test physical ligand access. Mutational or biochemical perturbation can investigate whether the site is connected to function. No single modality is obligatory for every protein, but increasingly strong claims require evidence capable of addressing distinct alternative explanations.

The proposed framework therefore treats computational pocket discovery as provisional until at least one orthogonal observation supports either the state or its accessibility to ligands. Stronger statements such as mechanism, allostery, or functional control require further evidence beyond simple engagement.

Why natural product shape complexity may fit cryptic recognition

Natural products occupy chemically diverse space shaped by biosynthetic constraints, stereochemistry, ring architecture, and dense functional-group organization, and they remain important sources of bioactive compounds and medicinal-chemistry inspiration [18]. Contemporary natural-product discovery increasingly integrates isolation and characterization with computational analysis, target-based screening, structural biology, synthetic modification, and modern pharmacological evaluation [19]. These developments make natural-product chemical space compatible with structure-guided cryptic-pocket discovery, but compatibility does not establish preferential recognition.

The more specific hypothesis concerns molecular shape. Cryptic pockets may expose irregular three-dimensional surfaces following conformational rearrangement, potentially creating interaction vectors absent from dominant apo conformations. Natural-product-like fragments provide an experimentally relevant bridge: crystallographic screening against a kinase demonstrated

that three-dimensional, natural-product-inspired fragments could identify chemotypes beyond conventional flatter fragment space and generate distinctive structural recognition modes [20].

This evidence supports the value of three-dimensional chemical matter in structure-based discovery; it does not demonstrate that natural products or natural-product-like compounds preferentially occupy cryptic sites. The article therefore proposes a conditional matching hypothesis rather than a class advantage.

Once a cryptic pocket has credible evidence for opening, recurrence, and a coherent interaction environment, stereochemically rich scaffolds may provide complementary shapes for nonplanar subpockets, spatially separated interaction regions, or conformationally selective recognition. The proposition remains explicitly testable. Existing evidence establishes natural-product chemical diversity, the growing integration of computational strategies into natural-product discovery, and the ability of natural-product-like three-dimensional fragments to access unusual recognition space [18–20]. It does not establish cryptic-site enrichment.

A decisive experiment would compare appropriately matched natural-product, natural-product-like, and conventional libraries against independently validated cryptic and constitutive pockets under equivalent screening, engagement-confirmation, and counter-screening conditions.

Ranking pockets before ranking ligands

Once a transient cavity has been detected, the next decision should concern the pocket rather than the ligand library. Candidate sites require more than geometric visibility. Proteome-scale characterization demonstrates that binding pockets can be compared through combinations of shape, physicochemical environment, and ligand-related descriptors that extend beyond simple cavity volume [21]. For cryptic sites, such descriptors should be supplemented by state-dependent evidence: whether opening recurs, whether the pocket maintains coherent boundaries, and whether the structural transition is compatible with function.

Prediction confidence also depends on benchmark construction. CryptoBench formalizes apo–holo differences and illustrates why cryptic-site predictors should be evaluated on conformations that do not already expose the target pocket [22]. Learned models nevertheless remain useful for candidate generation. PocketMiner demonstrated that graph neural networks can infer likely cryptic-site residues from single protein structures [23]. Such outputs can prioritize investigation but do not directly estimate open-state probability or pharmacological tractability.

The proposed ranking logic therefore places several questions ahead of ligand score: Is opening reproducible? Does a chemically coherent cavity persist? Is the site structurally compatible with drug-sized ligands? Is there evidence of functional connectivity? Is its detection supported by a benchmark or orthogonal method appropriate to the starting state?

Figure 1 integrates these distinctions into the article's central pocket-before-ligand architecture. The reader should understand the figure as an evidence-gated framework rather than a linear discovery pipeline: protein motion creates candidate states, simulation and experiment constrain their credibility, chemical-space matching follows pocket qualification, and validation can return an apparently promising site to an earlier interpretive state.

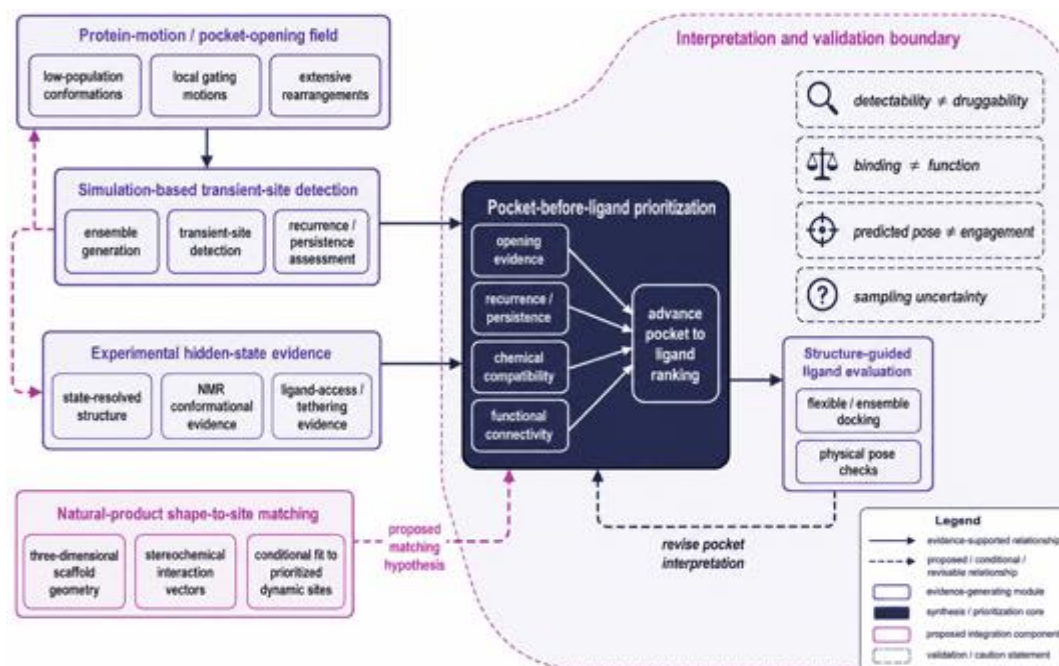


Figure 1. From hidden conformational opportunity to evidence-qualified cryptic-pocket prioritization

Table 2 makes the decision boundaries explicit without converting them into numerical thresholds.

Table 2. Conceptual distinctions required before a transient pocket advances to ligand ranking

Decision question	Evidence-bearing construct	Permitted inference	Premature inference
Is a site detectable?	Reproducible structural or computational pocket signal	Candidate site warrants investigation	Site is ligandable
Does opening recur?	Similar pocket state across structures or trajectories	Opening is not an isolated frame	Native-state population is known
Is the site chemically coherent?	Stable geometry and plausible interaction environment	Chemical matching is justified	High-affinity ligands necessarily exist
Is prediction trustworthy?	Evaluation against appropriate apo-state or independent benchmarks	Method has relevant validation support	Generalization to a new target is established
Is the pocket functionally connected?	Independent structural, mutational, biochemical, or pharmacological evidence	Site may influence target behavior	Binding will produce useful pharmacology

A cryptic-pocket natural product discovery framework

The proposed framework begins with ensemble generation rather than ligand docking. Candidate conformations may originate from experimentally determined alternative structures, conventional molecular dynamics, enhanced sampling, or structure-prediction ensembles. Large-scale resources such as CryptoBank demonstrate that apo–holo comparisons can support the identification and prediction of cryptic regions across extensive structural datasets [24].

Such resources enlarge the candidate space, but database-derived crypticity remains constrained by which conformations and protein classes have been experimentally observed.

A second gate asks whether a candidate state is credible enough for pocket-level analysis. Structure-prediction systems can contribute alternative conformations, but their outputs should not automatically be interpreted as thermodynamic ensembles. Analysis of AlphaFold3

predictions showed that cryptic-pocket conformations can depend strongly on ligand inclusion and learned structural priors [25]. Agreement among simulation, structure prediction, and experiment can therefore strengthen confidence, whereas disagreement should remain visible rather than being collapsed into one score.

The framework consequently proposes that evidence should travel with the pocket state. A candidate conformation should retain its provenance: how it was generated, whether independent trajectories recover it, which structural features define opening, and what experimental observations constrain its interpretation. This approach prevents a downstream docking score from erasing uncertainty introduced during state generation.

Pocket qualification then proceeds through distinct gates. Opening must be observed or predicted; recurrence must distinguish reproducible states from isolated frames; physicochemical structure must be sufficiently coherent for chemical matching; functional connectivity should be investigated where mechanistic prioritization matters; and contradictory evidence should be allowed to demote the site.

Only after these questions are addressed does chemical-space matching begin. Natural-product and natural-product-like collections enter as one source of three-dimensional chemical matter rather than as privileged ligands. Their potential value depends on the geometry and interaction topology of the qualified pocket.

The framework is intentionally recursive. Failure of a ligand to retain a physically plausible pose, engage the proposed pocket, perturb the predicted conformational state, or produce the expected functional effect should return the pocket to an earlier evidential stage. This architecture is proposed here and requires prospective comparison with conventional static-structure discovery before any improvement in success rate or predictive performance could be claimed.

Docking into rare conformations

Docking against a rare conformation changes both the receptor model and the interpretation of a successful pose. Ligand-conditioned generative approaches such as DynamicBind demonstrate that protein–ligand complex prediction can incorporate receptor conformational adjustment rather than treating the protein as rigid [26]. Such flexibility is relevant to cryptic sites because the ligand-compatible state may differ substantially from the dominant apo conformation. It also creates more opportunities for a model to generate an attractive but incorrect complex.

Receptor selection is therefore part of the cryptic-pocket hypothesis. Docking across several independently supported open states is preferable to selecting one visually

compelling snapshot, because ligand rank may otherwise reflect arbitrary frame selection rather than a reproducible interaction environment. Consistency across qualified conformations can strengthen prioritization, whereas severe pose dependence should motivate further state sampling.

Structure-guided medicinal chemistry provides a stronger downstream test than docking alone. In DsbA, a cryptic-pocket fragment was exploited through parallel synthesis, direct-to-biology screening, and structural characterization, demonstrating that a hidden pocket can support iterative ligand optimization in a specific target [27]. The significance of this example is not that every sampled cavity can support medicinal chemistry, but that a sufficiently qualified cryptic site can eventually become a conventional structure-guided discovery object.

Predicted poses must also survive physical scrutiny. PoseBusters showed that contemporary AI docking methods can produce poses with favorable structural overlap while retaining chemically or physically implausible features and limited generalization to novel sequences [28]. Rare-state docking should consequently incorporate stereochemical, steric, geometric, and energetic checks in addition to ranking scores. A docking pose remains a molecular hypothesis until independent evidence supports binding.

Coupling pocket opening to functional mechanism

The mechanistic value of a cryptic pocket depends on what changes when the pocket is occupied. CB1 illustrates one possibility: ligands designed to exploit a cryptic extension generated target-specific structural and signaling consequences within the studied chemical series [8]. VP35 illustrates another, in which perturbation of a hidden site affected a distal RNA-binding interface [9]. These studies justify evaluating functional connectivity but also demonstrate why “cryptic” cannot be used interchangeably with “allosteric.”

The proposed framework separates three events. Pocket opening is a conformational event. Ligand engagement is a molecular-recognition event. Functional modulation is a biological consequence requiring independent evidence. A ligand can stabilize a hidden conformation without producing a relevant functional effect, and an observed functional effect can arise from interactions unrelated to the proposed cryptic site.

Mechanistic interpretation becomes stronger when multiple relationships converge: the ligand engages the predicted location; the interaction stabilizes or shifts the relevant conformational state; perturbation of the site influences the predicted functional pathway; and pharmacological activity changes consistently with that mechanism. Even this convergence should be interpreted

at the level directly demonstrated rather than extrapolated automatically to therapeutic efficacy.

The same distinction constrains selectivity claims. A hidden pocket may provide structural features absent from conserved orthosteric sites, creating an opportunity for selective recognition. The CB1 study supports that possibility in one receptor system [8]. However, structural uniqueness does not guarantee pharmacological selectivity because off-target conformational states, exposure, metabolism, pathway bias, and concentration remain independent determinants.

Distinguishing druggability from detectability

Detectability asks whether a cavity can be identified. Druggability asks whether that cavity can support a productive ligand-discovery program. Structural feasibility analyses indicate that cryptic pockets differ substantially in the extent and type of opening required and in the resulting binding environment [3]. A cavity that repeatedly appears in simulation may therefore remain a poor target if its open state is chemically incoherent, energetically inaccessible, or incompatible with relevant protein function.

Multidimensional pocket descriptors can support analysis of shape and physicochemical properties beyond raw cavity volume [21]. Yet descriptor similarity is not equivalent to pharmacological equivalence. For cryptic pockets, druggability also depends on whether the open state can be accessed and stabilized without unrealistic protein deformation.

The practical consequence is a two-stage decision. Detection methods nominate pockets. Independent structural, chemical, and functional evidence determines whether those pockets warrant substantial ligand investment. The proposed framework deliberately prevents a high computational pocket score from bypassing this second stage.

Accordingly, “detected,” “recurrent,” “persistent,” “ligandable,” “functionally connected,” and “validated” should be treated as different evidential states. Collapsing these terms creates apparent certainty precisely where cryptic-pocket discovery is most vulnerable to overinterpretation.

Validation of predicted cryptic-site binding

Validation should test both the ligand and the hidden state. Solution NMR can provide information on protein–ligand interactions and conformational ensembles that may not be available from static structures alone, making it valuable for structure-based drug discovery involving dynamic states [29]. Other systems may be more suitable for crystallography, cryogenic structural methods, tethering, mutational analysis, biochemical competition, or

complementary biophysical measurements. No single validation technology is universal.

The strongest validation logic is therefore convergent. Tethering can establish physical accessibility at a hidden region, structure-guided medicinal chemistry can link ligand modification to an experimentally resolved recognition mode, and NMR can provide solution-state interaction or conformational evidence [16, 27, 29]. These approaches address different potential errors and should not be treated as interchangeable confirmations.

Mechanistic validation requires another evidential step. Direct engagement should be separated from a functional response that could arise through unrelated ligand properties. Perturbations designed to alter pocket opening, site-specific competition, conformational measurements, and target-relevant functional assays can strengthen attribution when they produce mutually compatible results. The framework therefore avoids a binary concept of validation. Evidence can support existence of an open state, accessibility to ligand, site-specific engagement, conformational stabilization, functional coupling, or downstream pharmacological modulation. Each level permits a stronger claim than the previous one, but none should be inferred automatically from another.

Failure modes and sampling limits

Rare conformational states are difficult not only to discover but also to quantify. A recent comparison of AI-based ensemble methods and physics-based simulations against experimentally characterized cryptic-pocket systems found that relative changes in pocket opening can be informative while absolute probabilities for rare open states remain difficult to predict reliably [30]. This limits attempts to rank candidate pockets using apparently precise population estimates.

Sampling can fail in opposite directions. Conventional trajectories may never cross a relevant barrier, creating false negatives. Enhanced approaches can overemphasize coordinates chosen by the investigator or reshape the observed ensemble. Mixed solvents can reveal interaction hotspots while perturbing the protein surface. Structure-prediction models may reproduce learned alternative conformations without generating a thermodynamically faithful ensemble [25].

Docking can compound these uncertainties. A receptor state produced by uncertain sampling may subsequently receive a high-scoring but physically implausible ligand pose [28]. Each layer can therefore amplify uncertainty from the previous layer if evidence provenance is discarded.

Dataset construction creates an additional risk. Models assessed on holo-like conformations or proteins closely related to their training examples may appear more

effective than when challenged with genuinely apo structures of unfamiliar targets [22]. Evaluation should therefore distinguish pocket memorization, structural similarity, and true generalization.

The framework treats disagreement as informative. If independent simulations fail to reproduce an opening, if structure prediction requires the ligand to recover the pocket, if experimental measurements do not support accessibility, or if ligand engagement fails despite favorable docking, the candidate should be demoted rather than protected by the strongest positive result. A cryptic-pocket workflow becomes scientifically useful only when negative evidence can overturn computational enthusiasm.

Limitations

This framework is conceptual and has not been prospectively compared with conventional static-pocket discovery. Its individual components are supported unevenly across target classes. Some cryptic pockets have strong structural, computational, ligand-binding, and functional evidence, whereas other conclusions rely on methodological benchmarks or target-specific demonstrations. Universal sampling lengths, persistence thresholds, pocket scores, or validation sequences cannot therefore be specified.

The natural-product component represents the largest evidential gap. Current evidence supports the structural diversity of natural products and demonstrates that natural-product-like three-dimensional fragments can access unusual recognition space, but it does not show class-wide enrichment at cryptic pockets. The proposed shape-matching relationship remains a falsifiable research proposition rather than an established pharmacological principle.

Transferability is also constrained by experimental tractability. Membrane proteins, intrinsically disordered regions, multimeric complexes, and targets with extremely rare transitions may require different methods for both sampling and validation.

Finally, cryptic-pocket accessibility is only one determinant of lead quality. Exposure, selectivity, metabolic stability, synthetic accessibility, toxicity, target biology, formulation behavior, and pharmacokinetics lie outside the structural core of the framework. Success at pocket discovery cannot substitute for these later requirements.

CONCLUSION

Cryptic pockets expand drug-discovery thinking by replacing a static inventory of binding sites with an evidence-qualified view of conformational opportunity. The important consequence is not that every transient

cavity becomes druggable, but that some pharmacologically relevant surfaces may become apparent only when protein motion is considered.

The proposed framework organizes this opportunity around a pocket-before-ligand rule. Conformational sampling and transient-site detection generate candidates; recurrence, structural and chemical coherence, experimental hidden-state evidence, and functional connectivity determine whether those candidates merit chemical investment. Natural-product shape complexity enters only after structural qualification and remains a hypothesis about chemical-space complementarity rather than a demonstrated class advantage.

This ordering also constrains interpretation. A detected pocket is not necessarily persistent; a persistent pocket is not necessarily ligandable; a plausible pose is not target engagement; and engagement is not mechanism or therapeutic value. Progress therefore depends on preserving these distinctions while allowing evidence from simulation, structural biology, chemical biology, medicinal chemistry, and pharmacology to converge.

The druggable proteome may be larger than deposited crystal structures suggest, but the scientifically useful expansion lies in experimentally constrained dynamic states, not in the number of cavities a computational method can produce.

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