INSIGHTIA JOURNAL OF RESEARCH AND INNOVATION
OPEN-ACCESS JOURNAL (ISSN: XXX)

Review
Volume 1, Issue 2, 2026, 03
https://doi.org/10.33/IJRI12.03

Cryptic Binding Pockets and Allosteric Vulnerabilities in Disease-Associated Proteins: From Structural Dynamics to Next-Generation Therapeutics

Vipul Agarwal [1,*], **Vikas Kumar Chaudhari** [2,*]

[1,2] Moradabad Educational Trust, Group of Institutions, Faculty of Pharmacy, Moradabad-244001, UP., India.
* Correspondence: vipulpharm@gmail.com (VA); vikaschaudhary679@gmail.com (VKC)

Received: 22.07.2026; Accepted: 18.08.2026; Published: 14.09.2026

Abstract: Protein structure is increasingly understood as a dynamic ensemble rather than a single rigid entity. Within this ensemble, transient cavities can emerge that are absent or poorly defined in conventional ligand-free structures. These cryptic binding pockets provide opportunities to modulate proteins for which conventional orthosteric drug discovery has been unsuccessful. Because many cryptic pockets are spatially separated from catalytic or orthosteric sites, their exploitation frequently overlaps with the pharmacological concept of allostery. Structural fluctuations, loop displacement, side-chain rearrangement, hydration changes, and domain motions can therefore generate previously unrecognized ligandable states. Experimental approaches including fragment-based crystallography, nuclear magnetic resonance, hydrogen–deuterium exchange mass spectrometry, covalent tethering, and cryogenic electron microscopy are increasingly complemented by molecular dynamics, enhanced sampling, Markov state modeling, mixed-solvent simulations, and machine-learning-based pocket prediction. These approaches have revealed therapeutically relevant vulnerabilities in KRAS, mutant p53, BACE-1, muscarinic receptors, SARS-CoV-2 proteins, Ebola virus VP35, kinases, and other disease-associated proteins. The KRAS G12C switch-II pocket provides a landmark example in which a mutation-associated cryptic site was transformed into a clinically validated therapeutic opportunity. More recent developments demonstrate that cryptic-pocket discovery can also support fragment-derived, covalent, macrocyclic, allosteric, and targeted-degradation strategies. Nevertheless, transient occupancy, uncertain ligandability, resistance, species differences, and incomplete links between binding and functional modulation remain important barriers. Integration of dynamic structural biology with artificial intelligence, chemical biology, medicinal chemistry, and translational pharmacology is likely to define the next stage of cryptic-pocket-enabled drug discovery.

Keywords: Cryptic Pocket; Allostery; Protein Dynamics; KRAS; Mutant p53; BACE-1.

1. Introduction

Conventional structure-based drug discovery frequently begins with a static representation of a protein and searches for cavities that can accommodate drug-like molecules. This approach has been highly productive, but it can underestimate the number and diversity of ligandable states because proteins continually sample conformations that differ from the experimentally dominant structure [1,2]. A cryptic binding pocket may therefore be regarded as a ligandable cavity that is absent, occluded, or insufficiently developed in the ground-state structure but becomes accessible through conformational rearrangement [3–5]. The concept is particularly important for proteins considered poorly druggable because they lack deep orthosteric pockets.

The distinction between cryptic and allosteric sites is useful. A cryptic site is defined primarily by its structural emergence, whereas an allosteric site is defined by its functional relationship to another region of the protein. Consequently, a cryptic cavity can be orthosteric, allosteric, or functionally silent. In practice, many disease-relevant cryptic sites are allosteric because the structural rearrangement that opens the pocket alters a distant catalytic, regulatory, or protein-interaction site [6–10]. The pharmacological consequences may include inhibition, activation, alteration of ligand affinity, changes in signaling bias, or stabilization of a specific protein state [31–33].

Allostery has traditionally been described using equilibrium models such as the Monod–Wyman–Changeux framework and related conformational-selection concepts [38,39]. Modern structural biology instead supports a continuum in which proteins fluctuate among many substates, with ligand binding shifting the populations of pre-existing conformations while also contributing to local structural adaptation [40,41]. This view is particularly relevant to cryptic pockets because pocket opening may occur infrequently, requiring specialized sampling or experimental trapping.

The therapeutic importance of this concept has become evident in oncology, infectious disease, neurodegeneration, and receptor pharmacology. KRAS, historically difficult to target directly, provided a striking example when an inducible pocket beneath the switch-II region was exploited for mutant-selective inhibition [25–29]. Similar principles have been applied to mutant p53, BACE-1, muscarinic GPCRs, SARS-CoV-2 protease, and viral RNA-binding proteins [16–24,42–46]. Thus, the study of cryptic pockets is shifting the focus of medicinal chemistry from static shape complementarity toward dynamic vulnerability.

2. Structural and Biophysical Basis of Cryptic Pocket Formation

Protein dynamics occur over a broad temporal spectrum ranging from picosecond side-chain fluctuations to millisecond and slower domain rearrangements. Cryptic pockets emerge when these motions alter solvent accessibility, packing interactions, loop positions, side-chain rotamers, or interdomain contacts sufficiently to produce a cavity that can stabilize a ligand [2,4,7,11].

Two classical concepts are particularly relevant. In conformational selection, the unliganded protein already samples an open or binding-competent state and the ligand preferentially stabilizes that subpopulation. In induced fit, ligand association promotes subsequent structural rearrangement. Experimental and simulation studies indicate that cryptic-site formation commonly incorporates elements of both mechanisms [4,11,38–41]. The relative contribution depends on the energy landscape, pocket hydration, local flexibility, and ligand chemistry.

Water is also a major determinant of cryptic-pocket behavior. Opening a cavity can involve desolvation, rearrangement of water networks, and changes in hydrophobic packing. Consequently, a geometrically visible cavity is not necessarily a druggable cavity. Indeed, many transient pockets detected by simulations do not support high-affinity binding [4]. This distinction emphasizes the importance of coupling pocket detection to energetic, chemical, and functional validation.

An important mechanistic feature is allosteric coupling. A cryptic cavity may be mechanically connected to a catalytic site through a network of residues, correlated motions, hydrogen-bond pathways, or changes in solvent exposure. Markov-state modeling and residue-

network analysis have shown that small local rearrangements can propagate through such networks to alter distal functional sites [10,12,13]. The resulting allosteric vulnerability may therefore be more important pharmacologically than the pocket volume itself. Fig. 1 illustrates this dynamic principle.

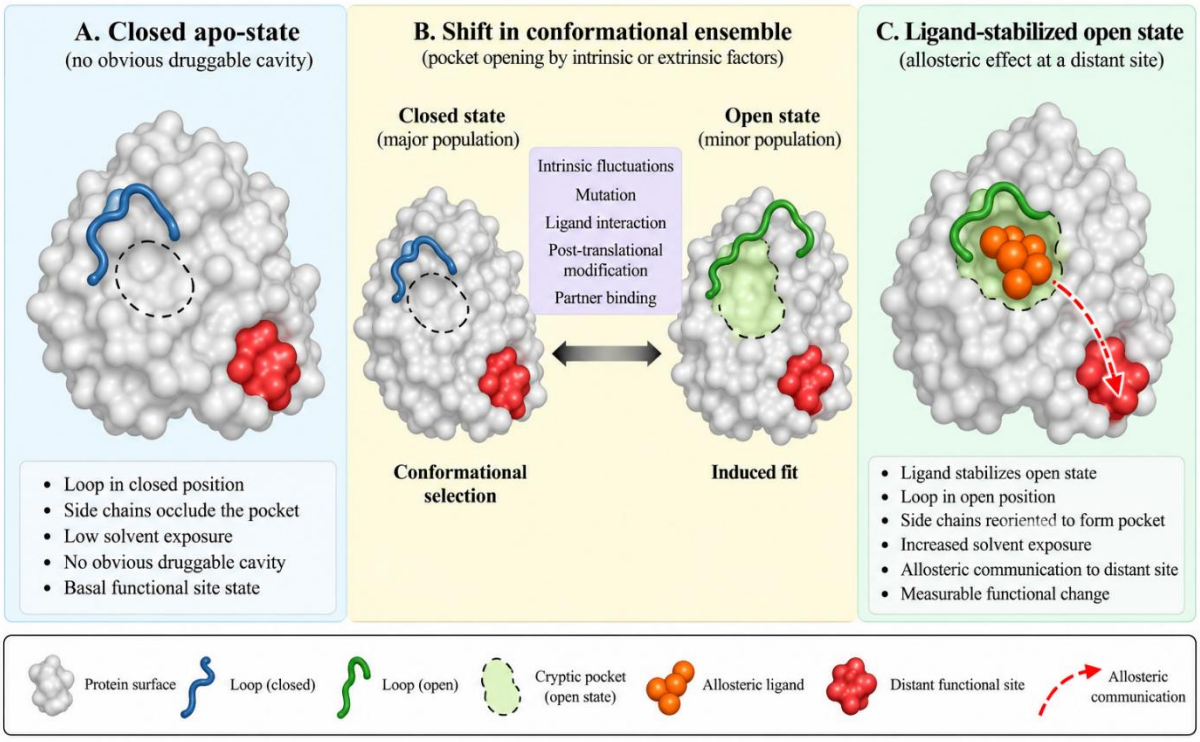

Figure 1. Structural emergence of a cryptic allosteric pocket. Conceptual schematic showing: (A) a closed apo-state in which no obvious druggable cavity is present; (B) intrinsic protein fluctuations, mutation, ligand interaction, post-translational modification, or partner binding shifting the conformational ensemble toward a pocket-open state; and (C) stabilization of the open state by a ligand, producing a measurable allosteric change at a distant functional site. The figure should distinguish conformational selection from induced-fit contributions and indicate changes in loop position, side-chain orientation, solvent exposure, and allosteric communication.

3. Experimental Discovery of Cryptic Pockets

3.1 X-ray crystallography and fragment screening

X-ray crystallography remains one of the most direct methods for identifying cryptic pockets because ligand-bound structures provide atomic-level information about cavity formation and ligand recognition. Fragment-based crystallographic screening can capture weak interactions that are difficult to observe by conventional biochemical assays. In SARS-CoV-2 M^pro, extensive X-ray screening identified multiple ligandable regions, including noncanonical sites that expanded the landscape available for antiviral development [16,17]. Such studies demonstrate how fragment binding can stabilize otherwise low-population conformations. Mutant p53 Y220C provides another prominent example. Structural studies showed that the mutation generates a crevice containing a transient hydrophobic subpocket whose opening and closing are linked to side-chain and backbone dynamics [18]. Subsequent work confirmed that ligand design can deliberately exploit this transient state rather than simply occupying the most obvious surface cavity [47,48].

3.2 Nuclear magnetic resonance

NMR spectroscopy is particularly valuable when cryptic-pocket formation involves low-population excited states that are difficult to capture crystallographically. Chemical-shift perturbation, relaxation-dispersion, paramagnetic relaxation enhancement, and related methods

can detect structural exchange and ligand-induced changes in local environments. NMR can therefore provide evidence that a pocket-opening state exists in solution, complementing crystallographic snapshots [5,8].

3.3 HDX-MS and related biophysical methods

Hydrogen–deuterium exchange mass spectrometry provides a lower-resolution but broader view of conformational changes by monitoring changes in solvent accessibility. It can identify regions that become more exposed or protected after mutation or ligand binding and is particularly useful for mapping long-range allosteric effects. Combining HDX-MS with crystallography or MD can distinguish local pocket formation from larger conformational transitions.

3.4 Covalent tethering

Covalent fragment approaches can trap otherwise fleeting states by reacting with a suitably positioned cysteine. Tethering approaches were central to the early discovery of the KRAS G12C switch-II pocket and have subsequently been refined for broader cryptic-site mapping [25,26,49]. A 2026 study further showed that disulfide tethering can reveal cryptic pockets across oncogenic KRAS variants and identify residue-dependent opportunities for isoform selectivity [7].

3.5 Cryo-EM and ensemble structural biology

Cryo-EM is increasingly capable of separating heterogeneous conformational states in large molecular assemblies. Although atomic resolution is not always achieved, classification of multiple states can reveal alternative domain arrangements that are difficult to capture by X-ray crystallography. Serial crystallography and time-resolved approaches may eventually provide direct visualization of pocket-opening trajectories rather than isolated end states [50].

4. Computational Discovery and Dynamic Pocket Mapping

Classical molecular dynamics simulations can reveal transient cavities that are absent in the experimental structure. However, cryptic openings may be too rare for conventional trajectories. Enhanced-sampling strategies therefore play an important role.

Metadynamics, accelerated MD, Gaussian accelerated MD, weighted-ensemble simulations, replica-exchange approaches, and water-focused sampling methods can increase exploration of high-energy conformations [11,14,20,21]. In particular, SWISH was developed to improve sampling of hydrophobic interfaces and cryptic cavities and has been applied across several pharmacologically relevant proteins [21]. Markov state models provide another conceptual advance because they convert many short trajectories into an ensemble description of long-timescale transitions. Bowman and colleagues demonstrated that equilibrium fluctuations can reveal numerous potential cryptic allosteric sites in proteins even in the absence of ligand [10,11]. Exposon analysis subsequently extended this concept by identifying coordinated changes in solvent exposure that can indicate functionally important conformational transitions [13].

Mixed-solvent molecular dynamics and fragment-mapping approaches provide complementary chemical information. Probe molecules or cosolvents can occupy transient cavities and thereby reveal regions with favorable interaction potential. FTMap and related fragment-mapping methods can identify energetic hotspots that may guide ligand design [51]. MixMD has likewise been used to locate hidden binding regions and discriminate pockets with potentially useful ligandability [52]. Artificial intelligence is now transforming the scale of this

analysis. CryptoSite demonstrated that sequence, structural, and dynamic descriptors could be used to predict cryptic binding sites [9]. PocketMiner subsequently used a graph neural network to predict where pockets are likely to open from a single protein structure, reporting a ROC-AUC of 0.87 and a computational speed more than 1000-fold greater than extensive MD workflows in its benchmark [1,2]. Structure prediction models such as AlphaFold2, RoseTTAFold, and ColabFold provide high-quality starting structures for large-scale analysis, although their outputs should not be interpreted as exhaustive conformational ensembles [22–24]. Their greatest value may therefore lie in combination with MD, experimental structures, ensemble docking, and dynamic ML models rather than as standalone predictors of cryptic pockets.

5. Disease-Associated Examples

5.1 KRAS and the switch-II pocket

KRAS represents the most influential demonstration of cryptic-pocket drug discovery. The G12C mutation introduces a cysteine that can be selectively exploited by electrophilic ligands. Ostrem et al. demonstrated that covalent compounds bind a newly revealed pocket beneath the switch-II region and allosterically alter nucleotide preference and effector interactions [29]. The switch-II pocket subsequently became the structural basis for a series of mutant-selective KRAS inhibitors, ultimately leading to clinically approved agents [27,28]. Structural studies have also shown that pocket composition and surrounding residues influence isoform selectivity, while resistance can arise from secondary mutations affecting noncovalent contacts within the pocket [25–29]. Recent work has broadened the concept beyond KRAS G12C. Conserved cryptic-pocket architecture across Ras, Rho, and Rab-family proteins suggests that dynamic pockets may provide a general route to targets previously considered inaccessible [3].

5.2 Mutant p53 Y220C

The Y220C mutation creates a destabilizing surface crevice in the p53 DNA-binding domain. Early fragment screening and MD demonstrated that the cavity is dynamic and contains additional transient interaction sites [47]. Subsequent crystallography resolved open and closed pocket states, while targeted ligand design showed that a transient subpocket could be exploited to improve affinity markedly [18]. More recent work has continued to develop Y220C-directed reactivators, including mechanistic and translational studies of mutant-selective stabilizers [48]. The p53 example illustrates an important principle: a cryptic pocket need not merely inhibit a target. It may instead **stabilize a disease-associated protein in a more functional conformational state**, thereby converting structural rescue into a therapeutic strategy.

5.3 BACE-1 and neurodegenerative disease

BACE-1, an enzyme implicated in amyloid precursor protein processing, provides an example of cryptic-pocket-assisted multitarget design. Virtual screening against a previously underexplored pocket at the edge of the catalytic cleft produced hybrid molecules capable of interacting with both the catalytic site and a secondary cryptic region [6]. The resulting strategy illustrates how cryptic pockets can be incorporated into bitopic or hybrid ligands rather than treated as isolated binding sites.

5.4 GPCRs and receptor pharmacology

GPCRs illustrate why dynamic pockets can improve subtype selectivity. Muscarinic receptors possess highly conserved orthosteric sites, creating challenges for selective targeting. MD and experimental studies identified a dynamic "vestigial" pocket whose formation contributes to subtype-selective allosteric modulation [15]. More broadly, allosteric GPCR ligands can display positive, negative, or pathway-biased modulation, expanding pharmacological control beyond conventional agonism and antagonism [31–33,42]. Such behavior is especially valuable for CNS drug discovery, where excessive or constitutive orthosteric activation can produce dose-limiting effects.

5.5 Viral proteins

Viral proteins are particularly attractive cryptic-pocket targets because their mutation rates and protein–protein interfaces can make orthosteric inhibition difficult. Enhanced sampling simulations have revealed cryptic sites in SARS-CoV-2 M^pro, including distal pockets that may support alternative antiviral mechanisms [17]. Large-scale simulations across the SARS-CoV-2 proteome further demonstrated that dynamic structural states can substantially expand the apparent ligandable surface [19]. The Ebola virus VP35 example is especially informative because the protein lacks obvious pockets at its RNA-binding interface. Adaptive sampling and machine-learning approaches identified a cryptic pocket allosterically coupled to the dsRNA-binding region, followed by experimental thiol-labeling and mutational validation [45].

5.6 Kinases and other signaling proteins

Kinases contain numerous regulatory pockets in addition to the conserved ATP site. The Abl myristate pocket provides a classical example of an allosteric site that can be exploited to regulate kinase conformation [38]. Broader kinase profiling has shown that small molecules can recognize diverse conformations and allosteric regions across the kinome [37]. Fragment-based strategies have also been applied to identify allosteric kinase ligands [40]. Other disease-associated targets, including STAT3 and heat-shock proteins, have similarly revealed opportunities for modulating conformational states outside canonical catalytic sites [41,42]. These examples suggest that cryptic-pockets approaches may be particularly valuable for signaling proteins dominated by conserved orthosteric motifs.

6. Drug-Discovery Strategies for Cryptic and Allosteric Vulnerabilities

Cryptic pockets can be addressed using several complementary modalities.

6.1 Fragment-based discovery

Fragments are particularly suited to cryptic sites because their small size allows them to probe shallow or partially formed cavities. Iterative fragment growing, linking, and merging can transform weak interactions into high-affinity ligands [6,18,30]. Fragment screening is often most powerful when X-ray, NMR, and computational ensembles are used together.

6.2 Covalent drug discovery

When a cryptic pocket exposes a reactive nucleophile, covalent chemistry can strongly stabilize the desired state. KRAS G12C is the best-established example [29]. More broadly, covalent fragment screening can map ligandable residues and identify otherwise inaccessible pockets [30].

6.3 Allosteric modulation

Once a cryptic pocket has been structurally characterized, classical allosteric medicinal chemistry can be applied. Depending on the target, ligands may act as positive allosteric

modulators, negative allosteric modulators, partial modulators, or functional-selectivity agents [31–33]. This can provide greater control than simple occupancy of a catalytic site.

6.4 Peptides and macrocycles

Extended or irregular cryptic pockets may be poorly suited to conventional small molecules but accessible to cyclic peptides or macrocycles. These modalities can make multiple contacts simultaneously and can stabilize protein–protein interfaces. The principal limitations remain permeability, metabolic stability, and formulation.

6.5 Targeted degradation

Cryptic-pocket ligands can also provide recognition elements for targeted protein degradation. The PROTAC concept demonstrated that ligand binding can be repurposed to recruit an E3 ubiquitin ligase and induce selective degradation [35,36]. In this context, even a modest-affinity cryptic ligand could potentially be valuable if it creates an efficient degrader geometry.

6.6 AI-assisted design

AI can contribute at nearly every stage: pocket prediction, conformational-state classification, virtual screening, ligand generation, property prediction, and prioritization of experimental candidates. A particularly useful strategy is a closed-loop workflow in which ML identifies candidate regions, MD characterizes pocket dynamics, fragment screening provides experimental evidence, and medicinal chemistry optimizes ligands. Fig. 2 summarizes this integrated discovery strategy.

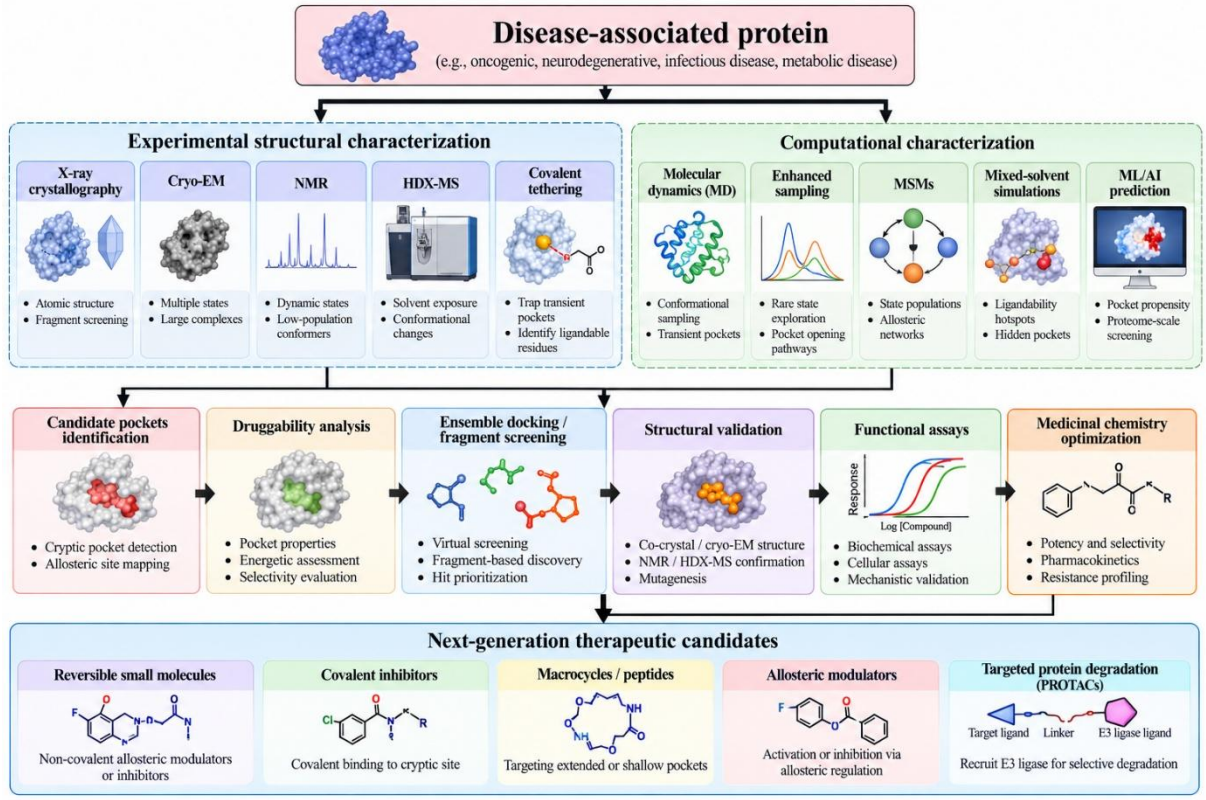

Figure 2. Integrated experimental–computational workflow for cryptic-pocket discovery.

The schematic should begin with a disease-associated protein and branch into experimental structural characterization (X-ray, cryo-EM, NMR, HDX-MS, covalent tethering) and computational characterization (MD, enhanced sampling, MSMs, mixed-solvent simulations, ML/AI). Candidate pockets should then undergo druggability analysis, ensemble docking or fragment screening, structural validation, functional assays, and medicinal-

chemistry optimization. The final stage should generate reversible, covalent, allosteric, macrocyclic, or targeted-degradation candidates.

7. Druggability, Selectivity, Kinetics, and Resistance

The identification of a cavity is only the first step. The critical question is whether the transient site is **ligandable**. Some simulated pockets are too shallow, short-lived, solvent-exposed, or energetically unfavorable to support meaningful drug binding [4]. Consequently, pocket volume alone is a poor predictor of therapeutic utility. Selectivity can be a major advantage of cryptic and allosteric sites. Orthosteric pockets are frequently conserved between protein paralogs, whereas secondary pockets may contain nonconserved residues that permit isoform-specific interactions [31–33]. However, lower sequence conservation can also complicate preclinical pharmacology because an allosteric ligand may behave differently across species [31]. Kinetics are equally important. A pocket may open slowly but remain ligand-bound for prolonged periods once stabilized. Conversely, a rapidly exchanging cryptic site may produce weak cellular activity even when equilibrium affinity appears attractive. These kinetic relationships require explicit evaluation during lead optimization. Resistance represents another challenge. KRAS G12C has demonstrated that mutations within or around an allosteric pocket can alter noncovalent interactions and impair inhibitor binding [29]. For dynamic pockets, resistance may therefore emerge not only through direct contact-residue mutation but also by shifting the conformational ensemble so that the ligand-compatible state becomes less populated.

Finally, binding does not guarantee functional control. A cavity may be structurally accessible yet biologically disconnected from the relevant signaling pathway. Functional assays, mutational validation, and cell-based phenotyping are therefore essential to distinguish a **druggable pocket** from a merely observable conformational cavity [10,13].

8. Translational Pathway and Next-Generation Therapeutics

The translational value of cryptic pockets depends on a chain of evidence extending from structural discovery to disease-relevant pharmacology. A practical development pathway includes target validation, dynamic pocket identification, ligandability assessment, hit discovery, structural confirmation, optimization, cellular activity, pharmacokinetics, pharmacodynamics, safety, and ultimately clinical evaluation. The KRAS experience demonstrates that this pathway can proceed from a mechanistic observation to a clinically useful therapeutic class within a comparatively short period [27–29]. Conversely, other cryptic targets remain at the discovery stage because suitable chemical matter or functional validation has not yet been established. Future progress will likely depend on **multiscale integration**. Structural biology should be combined with computational ensembles; MD should be informed by experimental restraints; AI predictions should be calibrated against experimentally validated datasets; and medicinal chemistry should optimize not only affinity but also residence time, pathway modulation, selectivity, and cellular exposure. The continued development of targeted degradation may broaden the concept further. Rather than requiring a cryptic ligand to completely inhibit a protein, degrader-based strategies can use a transient binding event as a molecular handle for eliminating the entire target [35,36]. Similarly, molecular-glue approaches may exploit cryptic interfaces that become favorable only after a small molecule stabilizes a new protein–protein interaction. Fig. 3 presents this translational framework.

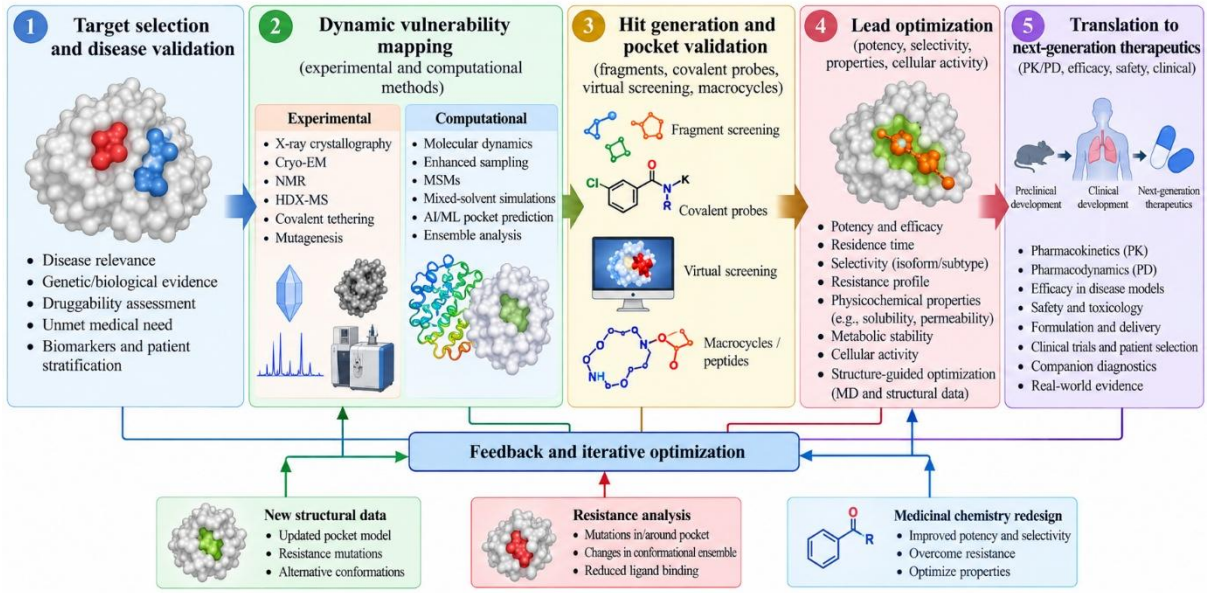

Figure 3. From cryptic pocket to next-generation therapeutic. A five-stage schematic: (1) Target selection and disease validation; (2) dynamic vulnerability mapping using experimental and computational methods; (3) hit generation and pocket validation using fragments, covalent probes, virtual screening, or macrocycles; (4) lead optimization emphasizing potency, residence time, selectivity, resistance profile, physicochemical properties, and cellular activity; and (5) translation through PK/PD, efficacy, toxicology, formulation, and clinical development. Feedback loops should connect resistance analysis and new structural data back to pocket redesign.

9. Current Challenges and Future Opportunities

Several developments are likely to determine the next phase of the field. First, dynamic structural prediction should move beyond single-state models toward experimentally anchored **conformational ensembles**. Second, AI systems trained on validated cryptic-pocket datasets may become increasingly useful for identifying vulnerabilities from sequence and structure at proteome scale [1,22–24,34]. Third, cryo-EM, serial crystallography, NMR, HDX-MS, and chemical-probe technologies should increasingly be integrated rather than treated as independent workflows. An important future direction is the combination of cryptic-pocket analysis with disease-specific variation. Mutations, post-translational modifications, oligomerization, membrane association, and protein–protein interactions can all alter conformational ensembles. Thus, the most useful pocket for therapy may exist only in a disease-associated state and not in the wild-type protein. Another promising area is the application of these concepts to difficult protein classes, including transcription factors, RNA-binding proteins, signaling adaptors, membrane proteins, and protein–protein interaction surfaces. The Ebola VP35 study demonstrates that a cryptic pocket can create a pharmacological entry point even when the disease-relevant interface is apparently flat [45]. Similar principles may help expand the ligandable landscape of other proteins traditionally regarded as undruggable. Ultimately, the field is moving from the question “Where is the binding pocket?” toward “Which conformational state contains a therapeutically exploitable vulnerability?” This shift has implications not only for discovery chemistry but also for target validation, biomarker development, resistance prediction, and precision medicine.

Table 1. Representative experimental and computational approaches for cryptic-pocket discovery and validation

Method	Primary principle	Typical output	Major strength	Major limitation	Role in drug discovery

X-ray crystallography	Captures ligand-bound or alternate structural states	Atomic-resolution pocket/ligand structure	Direct structural validation	Crystallization may bias conformational states	Fragment screening and structure-guided optimization
Cryo-EM	Separates heterogeneous conformational states	Multiple protein conformations	Useful for large complexes	Resolution and ensemble interpretation can be limiting	Mapping alternate states and interfaces
NMR	Detects low-population excited states and ligand interactions	Residue-level dynamics	Sensitive to transient states	Molecular-size constraints	Validation of pocket opening and allostery
HDX-MS	Measures changes in solvent accessibility	Regional conformational changes	Broad coverage with modest sample requirements	Lower spatial resolution	Mapping dynamic/allosteric effects
Covalent tethering	Traps reactive residues in transient pockets	Covalent fragment hits	Excellent for hidden ligandable hotspots	Requires compatible nucleophile chemistry	Discovery and mapping of cryptic sites
Conventional MD	Samples intrinsic conformational fluctuations	Transient cavities and motions	Atomistic mechanistic information	Rare events may be undersampled	Initial dynamic pocket identification
Enhanced sampling	Accelerates rare conformational transitions	Pocket-opening pathways	Accesses otherwise inaccessible states	Requires careful method selection	Discovery of cryptic conformations
MSM analysis	Reconstructs conformational-state networks	State populations and transitions	Connects dynamics to allostery	Requires extensive trajectories	Identification of rare functional states
Mixed-solvent MD	Uses chemical probes to reveal favorable regions	Ligandability hotspots	Provides chemical context	Computationally demanding	Mapping hidden binding surfaces
ML/AI pocket prediction	Learns cryptic-pocket signatures	Pocket propensity scores	Proteome-scale screening	Training-data dependence	Target prioritization
Ensemble docking	Screens ligands against multiple protein states	Candidate chemotypes	Accounts for conformational heterogeneity	Docking can mis-rank poses	Hit identification and optimization

10. Conclusions

Cryptic binding pockets represent a mechanistic bridge between protein dynamics, allostery, and modern drug discovery. Their emergence demonstrates that the ligandable surface of a disease-associated protein cannot always be inferred from a single static structure. Experimental structural biology, molecular dynamics, enhanced sampling, fragment screening, covalent chemistry, and AI-based prediction are converging to reveal these transient vulnerabilities. The strongest validation has come from systems such as KRAS, mutant p53, BACE-1, GPCRs, SARS-CoV-2 proteins, and Ebola VP35, where hidden or inducible pockets have provided new routes to inhibition, stabilization, or functional modulation. The next generation of therapeutics will likely emerge from integrating dynamic structural biology, AI-guided conformational analysis, chemical biology, and precision medicinal chemistry rather

than relying exclusively on canonical active sites. Cryptic-pocket drug discovery therefore offers a framework for converting structural dynamics from a complication of drug design into a source of therapeutic selectivity and vulnerability.

Author Contributions

Vipul Agarwal, and Vikas Kumar Chaudhari : Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Visualization, Writing Original Draft, Writing Review & Editing, Validation, Supervision, and Final Approval of the Manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

No new data were created or analyzed in this study. Data sharing is not applicable.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Full form
AI	Artificial Intelligence
Cryo-EM	Cryogenic Electron Microscopy
FBDD	Fragment-Based Drug Discovery
GPCR	G-Protein-Coupled Receptor
HDX-MS	Hydrogen–Deuterium Exchange Mass Spectrometry
MD	Molecular Dynamics
ML	Machine Learning
MSM	Markov State Model
NMR	Nuclear Magnetic Resonance
PK/PD	Pharmacokinetics/Pharmacodynamics
PPI	Protein–Protein Interaction
PROTAC	Proteolysis-Targeting Chimera
SBDD	Structure-Based Drug Discovery
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
X-ray	X-ray Crystallography

References

1. Zhang S, Bowman GR. Decrypting cryptic pockets with physics-based simulations and artificial intelligence. *Curr Opin Struct Biol*. 2026;96:103215.

2. Meller A, et al. Predicting the locations of cryptic pockets from single protein structures using the PocketMiner graph neural network. *Nat Commun*. 2023;14:1177. doi:10.1038/s41467-023-36699-3.

3. Vithani N, Rao A, Vishwanath DR, Briggs JM, Amaro RE. Exploration of cryptic pockets using enhanced sampling along normal modes: a case study of KRAS G12D. *J Chem Inf Model*. 2024;64:8258–8273.

4. Kuzmanic A, Bowman GR, Juarez-Jimenez J, Michel J, Gervasio FL. Investigating cryptic binding sites by molecular dynamics simulations. *Acc Chem Res*. 2020;53:2075–2086. doi:10.1021/acs.accounts.0c00443.

5. Raich L, et al. Discovery of a hidden transient state in all bromodomain families. *Proc Natl Acad Sci USA*. 2021;118:e2109762118. doi:10.1073/pnas.2109762118.

6. Pont C, et al. From virtual screening hits targeting a cryptic pocket in BACE-1 to a nontoxic brain permeable multitarget anti-Alzheimer lead with disease-modifying and cognition-enhancing effects. *Eur J Med Chem*. 2021;225:113779. doi:10.1016/j.ejmech.2021.113779.

7. Balius TE, et al. Disulfide tethering reveals cryptic pockets in oncogenic KRAS. *Commun Chem*. 2026;9:187. doi:10.1038/s42004-026-01992-x.

8. Wenthur CJ, Gentry PR, Mathews TP, Lindsley CW. Drugs for allosteric sites on receptors. *Annu Rev Pharmacol Toxicol*. 2014;54:165–184. doi:10.1146/annurev-pharmtox-010611-134525.

9. Cimermancic P, et al. CryptoSite: expanding the druggable proteome by characterization and prediction of cryptic binding sites. *J Mol Biol*. 2016;428:709–719. doi:10.1016/j.jmb.2016.01.029.

10. Bowman GR, Geissler PL. Equilibrium fluctuations of a single folded protein reveal a multitude of potential cryptic allosteric sites. *Proc Natl Acad Sci USA*. 2012;109:11681–11686. doi:10.1073/pnas.1209309109.

11. Oleinikovas V, Saladino G, Cossins BP, Gervasio FL. Understanding cryptic pocket formation in protein targets by enhanced sampling simulations. *J Am Chem Soc*. 2016;138:14257–14263. doi:10.1021/jacs.6b05425.

12. Bowman GR, Bolin ER, Hart KM, Maguire BC, Marqusee S. Discovery of multiple hidden allosteric sites by combining Markov state models and experiments. *Proc Natl Acad Sci USA*. 2015;112:2734–2739. doi:10.1073/pnas.1417811112.

13. Porter JR, Moeder KE, Sibbald CA, Zimmerman MI, Hart KM, Greenberg MJ, Bowman GR. Cooperative changes in solvent exposure identify cryptic pockets, switches, and allosteric coupling. *Biophys J*. 2019;116:818–830. doi:10.1016/j.bpj.2018.11.3144.

14. Knoverek CR, Amarasinghe GK, Bowman GR. Advanced methods for accessing protein shape-shifting present new therapeutic opportunities. *Trends Biochem Sci*. 2019;44:351–364. doi:10.1016/j.tibs.2018.11.007.

15. Hollingsworth SA, et al. Cryptic pocket formation underlies allosteric modulator selectivity at muscarinic GPCRs. *Nat Commun*. 2019;10:111. doi:10.1038/s41467-018-07868-w.

16. Günther S, et al. X-ray screening identifies active site and allosteric inhibitors of SARS-CoV-2 main protease. *Science*. 2021;372:642–646. doi:10.1126/science.abf7945.

17. Sztain T, Amaro RE, McCammon JA. Elucidation of cryptic and allosteric pockets within the SARS-CoV-2 main protease. *J Chem Inf Model*. 2021;61:3495–3501. doi:10.1021/acs.jcim.1c00140.

18. Joerger AC, Bauer MR, Wilcken R, Baud MGJ, Harbrecht H, Exner TE, Boeckler FM, Spencer J, Fersht AR. Exploiting transient protein states for the design of small-molecule stabilizers of mutant p53. *Structure*. 2015;23:2246–2255. doi:10.1016/j.str.2015.10.016.

19. Zimmerman MI, et al. SARS-CoV-2 simulations go exascale to predict dramatic spike opening and cryptic pockets across the proteome. *Nat Chem*. 2021;13:651–659. doi:10.1038/s41557-021-00707-0.

20. Bowman GR, Geissler PL. FAST conformational searches by balancing exploration/exploitation trade-offs. *J Chem Inf Model*. 2014;54:3720–3728. doi:10.1021/ci500391v.

21. Comitani F, Gervasio FL. Exploring cryptic pocket formation in targets of pharmaceutical interest with SWISH. *J Chem Theory Comput*. 2018;14:3321–3331. doi:10.1021/acs.jctc.8b00263.

22. Jumper J, et al. Highly accurate protein structure prediction with AlphaFold. *Nature*. 2021;596:583–589. doi:10.1038/s41586-021-03819-2.

23. Baek M, et al. Accurate prediction of protein structures and interactions using a three-track neural network. *Science*. 2021;373:871–876. doi:10.1126/science.abj8754.

24. Mirdita M, et al. ColabFold: making protein folding accessible to all. *Nat Methods*. 2022;19:679–682. doi:10.1038/s41592-022-01488-1.

25. Morstein J, et al. Targeting Ras-, Rho-, and Rab-family GTPases via a conserved cryptic pocket. *Cell*. 2024;187:6379–6392.

26. Taveras AG, et al. Ras oncoprotein inhibitors: the discovery of sotorasib. *Cancer Discov*. 2020;10:972–974. doi:10.1158/2159-8290.CD-20-0362.

27. Mullard A. FDA approves first KRAS inhibitor. *Nat Rev Drug Discov*. 2021;20:247. doi:10.1038/d41573-021-00006-2.

28. Maurer T, et al. Small-molecule ligands bind to a distinct pocket in RAS and inhibit SOS-mediated nucleotide exchange activity. *Proc Natl Acad Sci USA*. 2012;109:5299–5304. doi:10.1073/pnas.1201159109.

29. Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature*. 2013;503:548–551. doi:10.1038/nature12796.

30. Liu JJ, et al. Fragment-based covalent ligand discovery. *Nat Chem Biol*. 2014;10:953–959. doi:10.1038/nchembio.1654.

31. Conn PJ, Lindsley CW, Meiler J, Niswender CM. Opportunities and challenges in the discovery of allosteric modulators of GPCRs for treating CNS disorders. *Nat Rev Drug Discov*. 2014;13:692–708. doi:10.1038/nrd4308.

32. Melancon BJ, et al. Allosteric modulation of GPCR signaling: a therapeutic paradigm shift. *Cell*. 2020;183:605–620.

33. Wenthur CJ, Gentry PR, Mathews TP, Lindsley CW. Drugs for allosteric sites on receptors. *Annu Rev Pharmacol Toxicol*. 2014;54:165–184.

34. Huang T, Kehribar ES, Bao G. Targeting the undruggable proteome: designing chemistries to harness allostery. *Nat Rev Drug Discov*. 2023;22:451–465.

35. Sakamoto KM, et al. Protacs: chimeric molecules that target proteins to the Skp1–Cullin–F box complex for ubiquitination and degradation. *Proc Natl Acad Sci USA*. 2001;98:8554–8559.

36. Winter GE, Buckley DL, Paulk J, Roberts JM, Souza A, Dhe-Paganon S, Bradner JE. Phthalimide conjugation as a strategy for in vivo target protein degradation. *Science*. 2015;348:1376–1381. doi:10.1126/science.aab1433.

37. Fabian MA, et al. A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat Biotechnol*. 2005;23:329–336. doi:10.1038/nbt1068.

38. Hantschel O. Kinase inhibition in CML: translating allosteric inhibition. *Hematology Am Soc Hematol Educ Program*. 2017;2017:225–230. doi:10.1182/asheducation-2017.1.225.

39. Changeux JP, Edelstein SJ. Conformational selection or induced fit? 50 years of debate resolved. *F1000 Biol Rep*. 2011;3:19. doi:10.3410/B3-19.

40. Long MJC, et al. Application of the scaffold-hopping strategy in fragment-based discovery of STRADα kinase allosteric inhibitors. *Bioorg Med Chem*. 2021;29:115228. doi:10.1016/j.bmc.2020.115228.

41. Lee J, et al. MD simulations reveal cryptic pocket in heat shock protein 70. *J Phys Chem Lett*. 2020;11:3545–3550. doi:10.1021/acs.jpclett.0c01151.

42. Saikia N, et al. Fragment-based discovery of allosteric binders to STAT3. *ACS Chem Biol*. 2016;11:108–115. doi:10.1021/acschembio.5b00707.

43. Zhou Q, Huang J. Gαs/citronellal complex illustrates cryptic allosteric pocket in GPCR. *Cell Res*. 2020;30:947–949. doi:10.1038/s41422-020-0351-5.

44. Schames JR, Henchman RH, Secker AD, McCammon JA. Discovery of a novel binding trench in HIV integrase. *J Med Chem*. 2004;47:1879–1881.

45. A cryptic pocket in Ebola VP35 allosterically controls RNA binding. *Nat Commun*. 2022;13:2436.

46. Vithani N, et al. SARS-CoV-2 Nsp16 activation mechanism and a cryptic pocket with pan-coronavirus antiviral potential. *Biophys J*. 2021;120:1017–1028. doi:10.1016/j.bpj.2021.03.024.

47. Wilcken R, Wang G, Boeckler FM, Fersht AR. Kinetic and structural studies of p53 Y220C rescue by small molecules. *J Am Chem Soc*. 2012;134:6810–6816.

48. Mavridi D, et al. Targeting the p53 cancer mutants Y220C, Y220N, and Y220S with the small-molecule stabilizer rezatapopt. *Cell Death Dis*. 2026;17:268. doi:10.1038/s41419-026-08492-9.

49. Rothenberg DA, et al. Chemical proteomic mapping of cysteine ligandability and covalent ligand discovery. *Nat Chem Biol*. 2016;12:145–151.

50. Lynch ML, Snell EH, Bowman SEJ. Structural biology in the time of COVID-19: perspectives on methods and milestones. *IUCrJ*. 2021;8:1–16. doi:10.1107/S2052252521003948.

51. Kozakov D, Grove LE, Hall DR, Bohnuud T, Mottarella SE, Luo L, Xia B, Beglov D, Vajda S. The FTMap family of web servers for determining and characterizing ligand-binding hot spots of proteins. *Nat Protoc*. 2015;10:733–755. doi:10.1038/nprot.2015.043.

52. Smith RD, Carlson HA. Identification of cryptic binding sites using MixMD with standard and accelerated molecular dynamics. *J Chem Inf Model*. 2021;61:1287–1299. doi:10.1021/acs.jcim.0c01002.

Publisher's Note & Disclaimer

liability for any injury, damage, or loss to persons or property that may result from the use of any ideas, methods, instructions, or products mentioned in the content. Readers are encouraged to independently verify any information before relying on it, and the publisher assumes no responsibility for any consequences arising from the use of materials contained in this publication.