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Dynamic Druggability of Proteins: How Conformational States, Post-Translational Modifications and Cryptic Sites Expand Chemical Space

Anugya Gupta [1,*], Km. Monika [2]

[1] Department of Pharmaceutical Chemistry, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, 244001, India; anugyapharm@gmail.com.
[2] Pharmacy academy, IFTM University, Moradabad-244102, Uttar Pradesh, India; iammonika1994@gmail.com.
* Correspondence: anugyapharm@gmail.com (A.G.)

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Abstract: Protein druggability has traditionally been assessed from static three-dimensional structures by identifying cavities that can accommodate drug-like molecules. This structure-centric view is increasingly being replaced by an ensemble-based concept in which proteins are recognized as dynamic systems that interconvert among multiple conformational states. Such structural plasticity can transiently create cavities, alter allosteric communication, expose buried residues, and change the physicochemical properties of ligand-binding surfaces. Cryptic binding sites represent a particularly important consequence of this behavior because they may be poorly developed or invisible in ligand-free structures but become accessible following loop, helix, domain, or side-chain rearrangements. In parallel, post-translational modifications (PTMs), including phosphorylation, acetylation, methylation, glycosylation, and other chemical modifications, can reshape protein energy landscapes and generate distinct proteoforms with altered ligandability. Experimental approaches such as X-ray crystallography, NMR spectroscopy, hydrogen-deuterium exchange mass spectrometry (HDX-MS), fragment screening, and covalent tethering can capture these transient states, whereas molecular dynamics (MD), enhanced sampling, mixed-solvent simulations, Markov state models, and machine-learning methods can predict them. Recent applications to KRAS, β-lactamases, protein–protein interfaces, and other challenging targets demonstrate that dynamic pockets can provide selective and mechanistically distinct opportunities for medicinal chemistry. This review summarizes the molecular basis of dynamic druggability, explains how PTMs and cryptic sites expand accessible chemical space, discusses current experimental and computational strategies, and outlines future directions for integrating conformational ensembles, proteoform diversity, hydration, and artificial intelligence into next-generation drug discovery.

Keywords: Protein Dynamics; Druggability; Cryptic Pockets; Post-Translational Modifications; Allostery; Molecular Dynamics

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1. Introduction

The conventional description of proteins as relatively rigid three-dimensional structures has been enormously useful for structural biology and structure-based drug design. Nevertheless, proteins continuously sample multiple conformations over timescales ranging from femtoseconds to seconds or longer. These motions are not simply thermal noise; they are closely linked to molecular recognition, catalysis, signaling, assembly, and regulation [1–4]. A protein structure determined by X-ray crystallography or cryo-electron microscopy therefore

represents one or a limited subset of an underlying conformational ensemble rather than a complete description of its molecular behavior. Recent work continues to emphasize that conformational ensembles are central to protein function and to the design of molecules that regulate it.

Ligand recognition can involve both conformational selection and induced fit. In conformational selection, a ligand preferentially binds a pre-existing minor state and shifts the population toward that state. In induced fit, initial recognition is followed by conformational rearrangement that improves molecular complementarity. Real systems often contain contributions from both mechanisms [5,6]. Thus, the relevant drug target may not be a single structure but a collection of states connected through a free-energy landscape.

This ensemble perspective leads to a broader definition of **dynamic druggability**. A target should not be considered ligandable solely because a stable cavity is visible in one structure; conversely, the absence of an obvious cavity should not automatically imply that the protein is undruggable. Transient cavities, alternative conformations, PTM-dependent states, and cryptic sites can all provide new opportunities for small molecules.

A classic example is interleukin-2 (IL-2), in which small molecules were shown to bind a dynamic surface at a protein–protein interaction interface. Fragment assembly and subsequent optimization demonstrated that a relatively featureless surface could become a chemically tractable site through conformational adaptation [7,8]. These observations helped establish the conceptual foundation for targeting protein surfaces that are incompletely represented by static structures.

2. From Static Druggability to Dynamic Druggability

Conventional druggability metrics generally examine pocket volume, enclosure, hydrophobicity, polarity, hydrogen-bonding capacity, and related physicochemical properties. Computational tools such as SiteMap, Fpocket, FTMap, and related algorithms have made such evaluations rapid and scalable [24–27]. However, these assessments may be strongly dependent on the particular protein conformation used as input.

Dynamic druggability extends the concept by asking additional questions: Does a ligand-binding cavity emerge in another conformational state? How frequently is that state populated? How long does the pocket remain open? Is the opening associated with loop movement, helix displacement, domain rearrangement, or side-chain motion? Does the resulting cavity contain energetically favorable hotspots? And can a ligand bind strongly enough to stabilize the open state?

The distinction is important because not all transient pockets are equally useful. Analysis of structurally characterized cryptic sites suggests that pockets generated by substantial backbone rearrangements, particularly loop or hinge motions, are more promising than very shallow pockets generated solely by rapid side-chain fluctuations [9,10,55].

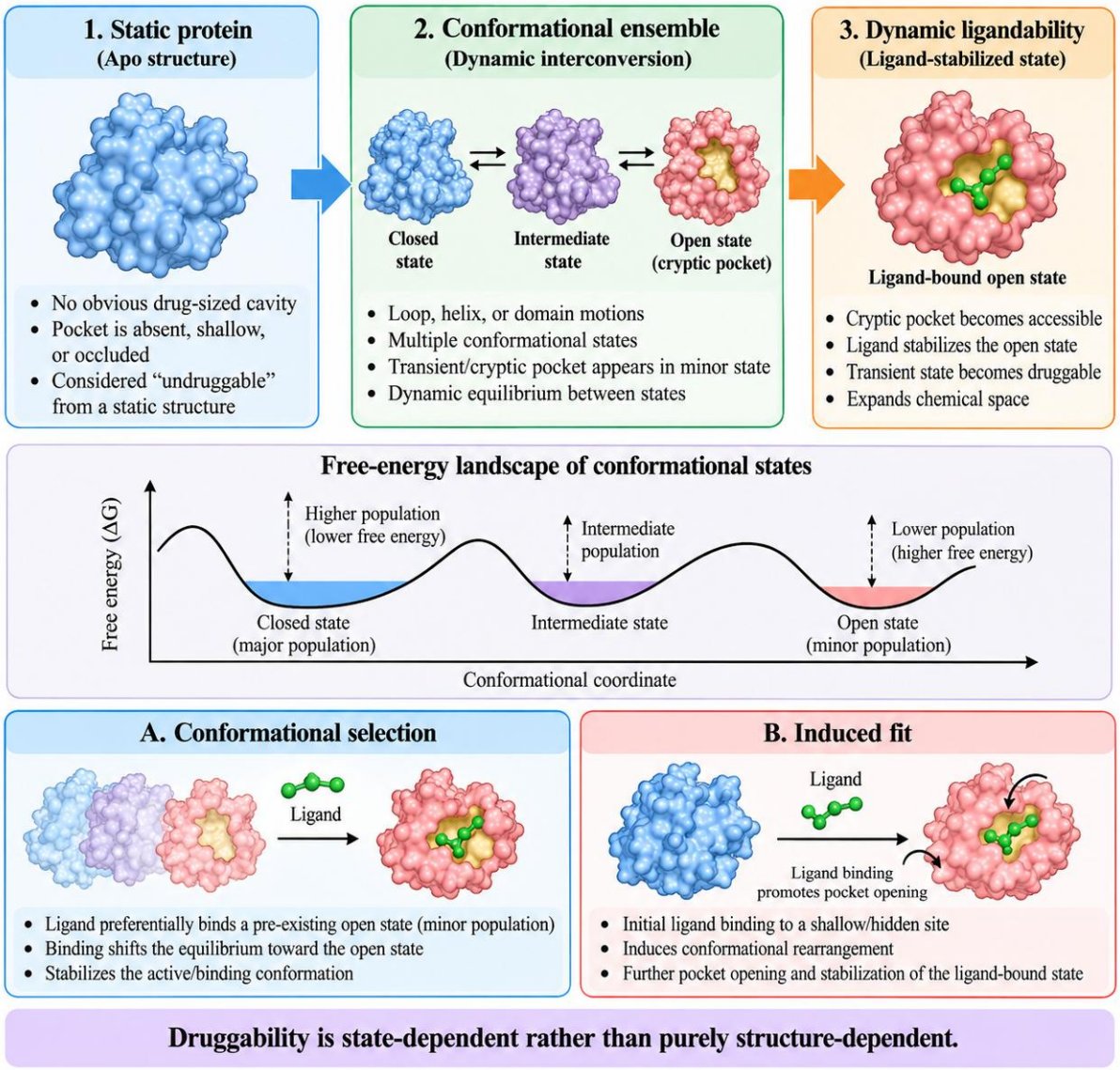


Figure 1. Dynamic Druggability as an Ensemble Property of Proteins.

The Fig. 1 should additionally show two parallel mechanisms: **conformational selection**, in which the ligand selects a pre-existing open state, and **induced fit**, in which ligand binding promotes further pocket opening. A free-energy landscape beneath the structures can depict the relative populations of closed and open states. The overall message should be that **druggability is state-dependent rather than purely structure-dependent**.

3. Cryptic Binding Sites Expand the Druggable Proteome

A cryptic binding site can be broadly described as a ligandable region that is absent, poorly formed, or undetectable in an unbound structure but becomes recognizable in another conformational state, often upon ligand binding [9,10]. The definition is inherently ensemble-dependent because a single "apo" structure cannot capture all conformations accessible to the unbound protein.

The CryptoSite study provided an important framework by comparing apo–holo protein pairs and characterizing residues associated with cryptic pockets. The analysis showed that cryptic sites tend to retain evolutionary conservation but are often more flexible and less hydrophobic than conventional ligand-binding sites [10]. Importantly, computational prediction suggested that the potentially druggable proteome could be substantially larger when hidden sites were included.

Several structural mechanisms can generate cryptic pockets. A loop can move away from a surface, a helix can rotate or translate, two domains can separate, or secondary-structure elements can reorganize. Conversely, a binding site may also arise when protein segments move closer together around a ligand. These structural mechanisms produce chemically distinct pockets with different depths, polarities, and hydration patterns [9–13].

The TEM-1 β-lactamase system illustrates the value and complexity of this phenomenon. Long-timescale simulations and Markov state models revealed an allosteric pocket that was partially open for a substantial fraction of the simulated ensemble, although it was not readily apparent in the conventional apo crystal structure [11,12]. Subsequent experimental and computational studies demonstrated that molecules could interact with such hidden sites and alter enzyme activity [13].

Cryptic pockets can also occur in proteins associated with disease. Their importance has been demonstrated for protein–protein interfaces, kinases, enzymes, and oncogenic signaling proteins. Rather than requiring a conventional orthosteric cavity, such targets can sometimes be manipulated by stabilizing a rare structural state. Consequently, cryptic-site discovery expands chemical space both geometrically and functionally.

4. Conformational Dynamics and Allosteric Regulation

Allostery is fundamentally a communication process through which ligand binding at one region of a protein changes function at another region. Although classical models emphasized large structural transitions, NMR and computational studies have shown that allostery can also arise from changes in conformational dynamics, populations, and correlated motions [3,4,22].

A protein may contain multiple states whose relative populations determine its activity. An allosteric ligand does not necessarily create a completely new structure; instead, it may selectively stabilize an already accessible state. This population-shift model is particularly relevant to dynamic druggability because a minor state may possess a pocket that is unsuitable for binding in the dominant conformation. [2–6]

The practical importance is considerable. Orthosteric sites are often highly conserved among protein families, making selectivity difficult. Allosteric sites can be less conserved and can provide opportunities for subtype, isoform, or disease-state selectivity [22,23]. Hidden allosteric pockets can therefore provide an alternative to highly competitive active sites.

The recognition of dynamic allostery also changes how screening campaigns should be designed. Docking against only one crystal structure may miss ligandable states, whereas ensemble docking can evaluate several conformations sampled experimentally or computationally. The objective is not simply to maximize the number of structures but to identify conformations that contain physically plausible and energetically favorable binding opportunities.

5. Computational Discovery of Transient and Cryptic Pockets

5.1 Conventional Molecular Dynamics

Molecular dynamics simulations provide atomistic trajectories in which side chains, loops, secondary structures, and domains can move continuously. Analysis of such trajectories can identify transient cavities that are absent from starting structures [18,19]. MD has therefore become a major tool for linking protein flexibility with druggability. However, conventional MD remains limited by timescale and sampling. Rare transitions may require microseconds,

milliseconds, or longer, whereas routine simulations may sample only a small fraction of the relevant conformational landscape. As a consequence, failure to observe pocket opening does not prove that the pocket cannot form.

5.2 Markov State Models

Markov state models (MSMs) address part of the sampling problem by combining many shorter trajectories and clustering them into metastable conformational states. Transition probabilities can then be estimated between states, allowing investigators to characterize the kinetics and relative populations of rarely observed conformations [11,12,21]. The TEM-1 β-lactamase work is an influential example. MSM analysis identified both known and previously unrecognized cryptic states and provided a quantitative framework for describing their accessibility. Subsequent experiments demonstrated that simulations could guide the selection of structures for small-molecule screening [12,13].

5.3 Enhanced Sampling

Enhanced-sampling methods such as metadynamics, accelerated MD, replica exchange, and Gaussian accelerated MD increase exploration of conformational space by reducing barriers or reweighting the energy landscape [15,20]. These approaches are particularly useful when pocket opening is a rare event. SWISH and related approaches have been used to examine cryptic pocket formation in systems including β-lactamases. Such methods have shown that a combination of ligand interaction and intrinsic fluctuations can contribute to pocket formation, supporting a spectrum between conformational selection and induced fit [15].

5.4 Mixed-Solvent Molecular Dynamics

Mixed-solvent MD introduces low concentrations of small organic probes into the aqueous environment. Probe occupancy can identify hydrophobic hotspots and transient cavities, thereby providing a computational analogue of fragment-based experimental mapping [14,44]. Such simulations can reveal both known binding sites and previously hidden pockets. The method has been applied to proteins including HIV-1 protease, where probe enrichment identified catalytic and potential allosteric hotspots [44].

5.5 Pocket Mapping and Druggability Algorithms

Computational solvent mapping approaches such as FTMap detect regions where multiple probe molecules preferentially cluster. The presence, strength, connectivity, and organization of hotspots can then be used to assess whether a site is likely to bind larger ligands [27]. Fpocket and related geometric approaches provide rapid cavity detection, while other algorithms integrate physicochemical and evolutionary information [25,26].

5.6 Machine Learning and AI-Based Prediction

Machine learning is increasingly being used to prioritize dynamic druggability. CryptoSite established an early machine-learning framework for predicting cryptic sites from structural features [10]. More recently, PocketMiner used a graph neural network trained on molecular-dynamics-derived conformational information to predict where cryptic pockets are likely to form. It demonstrated rapid prediction from individual structures and provided a large-scale assessment of potential cryptic sites across the human proteome [16]. These developments suggest an emerging workflow in which machine learning is used to prioritize candidate proteins or residues, MD is used to characterize pocket formation, and experiments are used to establish whether the predicted state is actually ligandable.

6. Fragment-Based Discovery and Expansion of Chemical Space

Fragment-based drug discovery (FBDD) is particularly well suited to dynamic targets because small fragments can probe weak, shallow, and incompletely formed interactions that larger compounds may not initially access [28,29]. Fragment screening by X-ray crystallography, NMR, surface plasmon resonance, and related methods have become an important strategy for challenging targets.

Fragments can also function as structural probes. When a fragment binds a weakly formed cavity, the resulting complex may stabilize a previously rare protein conformation. This creates an iterative cycle: **transient state → fragment binding → pocket stabilization → structural characterization → fragment optimization.**

The approach is especially useful for protein–protein interfaces, which frequently contain dynamic hotspots rather than classical enzyme-like cavities [28,47,48], also tabulated in Table 1. A key consequence is that dynamic druggability expands **chemical space** rather than merely adding more targets. The same protein can contain multiple conformationally distinct pockets, each capable of recognizing different chemotypes. These sites may support orthosteric inhibitors, allosteric modulators, covalent ligands, molecular glues, or bifunctional compounds.

Table 1. Mechanisms and technologies contributing to dynamic protein druggability.

Dynamic mechanism or approach	Molecular basis	Representative methods	Drug-discovery opportunity	Main limitation
Conformational selection	Ligand stabilizes a pre-existing minor state	NMR, MD, MSM	Target rare but functional conformers	Rare-state sampling
Induced fit	Ligand promotes additional structural rearrangement	X-ray, MD, kinetic studies	Stabilization of ligand-induced pockets	Difficult mechanistic interpretation
Loop/hinge motion	Flexible backbone segment opens a cavity	MD, enhanced sampling, fragment screening	Often creates substantial drug-sized pockets	Energetic cost of opening
Domain motion	Relative movement of domains creates an interface pocket	Cryo-EM, MD, MSM	New allosteric and PPI sites	Large conformational space
PTM-induced remodeling	Chemical modification changes charge, sterics, or interactions	Mass spectrometry, NMR, MD	State-selective or proteoform-selective ligands	Cellular heterogeneity
Mixed-solvent probing	Organic probes reveal transient hotspots	MixMD, FTMap	Identification of weakly formed pockets	Probe-dependent bias
Fragment screening	Small ligands sample shallow or cryptic hotspots	X-ray, NMR, SPR	Expansion of chemotypes and fragment vectors	Weak affinity and hit validation
Machine learning	Structural/dynamic patterns predict pocket formation	CryptoSite, PocketMiner, graph neural networks	Proteome-scale prioritization	Dependence on training data
Hydration-aware analysis	Water structure contributes to pocket formation and affinity	MD, water mapping, free-energy methods	Improved optimization of cryptic pockets	Difficult quantitative prediction

7. Post-Translational Modifications as Regulators of Druggability

PTMs add another layer of structural diversity to the protein ensemble. Phosphorylation, acetylation, methylation, glycosylation, ubiquitination, lipidation, and related modifications can alter electrostatics, hydrogen-bond networks, solvent exposure, local flexibility, and long-range communication [30–35].

7.1 Phosphorylation

Phosphorylation introduces a negatively charged phosphate group that can create or disrupt salt bridges, alter local secondary structure, change protein stability, or shift conformational populations [31]. These effects can propagate beyond the modified residue and influence distal ligand-binding or interaction sites. Computational analyses have shown that phosphorylation can modulate dynamic networks and allosteric coupling. More recent work has directly examined how phosphorylation changes conformational dynamics in signaling proteins, illustrating that the chemical modification can alter not only a local site but also the broader energy landscape [32,33].

7.2 Acetylation

Lysine acetylation removes a positive charge and changes hydrogen-bonding properties. Non-histone acetylation is widespread and can influence enzyme activity, protein interactions, localization, and structural stability [34]. From a drug-discovery perspective, acetylation can change both target recognition and the architecture of downstream binding sites. The bromodomain family provides a particularly clear illustration of chemical recognition of a PTM. Bromodomains contain defined acetyl-lysine recognition pockets, demonstrating that a PTM can itself become a pharmacologically relevant molecular feature [36]. BET bromodomain inhibitors such as JQ1 showed that small molecules can selectively recognize this PTM-dependent binding environment [37,38].

7.3 Glycosylation

Glycosylation can modulate protein folding, conformational stability, accessibility, and intermolecular recognition. The enormous structural diversity of glycans generates a large proteoform landscape that is not captured by the amino-acid sequence alone [35]. Computational and experimental analysis of glycosylated proteins has shown that specific glycans can reshape the conformational energy landscape and influence ligand-binding regions.

7.4 PTM Crosstalk and Proteoform-Specific Druggability

The functional proteome is composed of multiple proteoforms generated by PTMs and their combinations [40]. Consequently, one genetically encoded protein may possess different druggable surfaces depending on cellular state. This creates the concept of **proteoform-selective druggability**. A molecule might preferentially recognize a phosphorylated, acetylated, glycosylated, oxidized, or otherwise modified state. Such selectivity could distinguish disease-associated protein states from normal counterparts. Hsp90 provides an informative model in which multiple PTM sites can participate in allosteric communication and collective dynamics [39]. This suggests that future drug-discovery campaigns should not automatically treat the unmodified recombinant protein as the only relevant target, also shown in Fig 2.

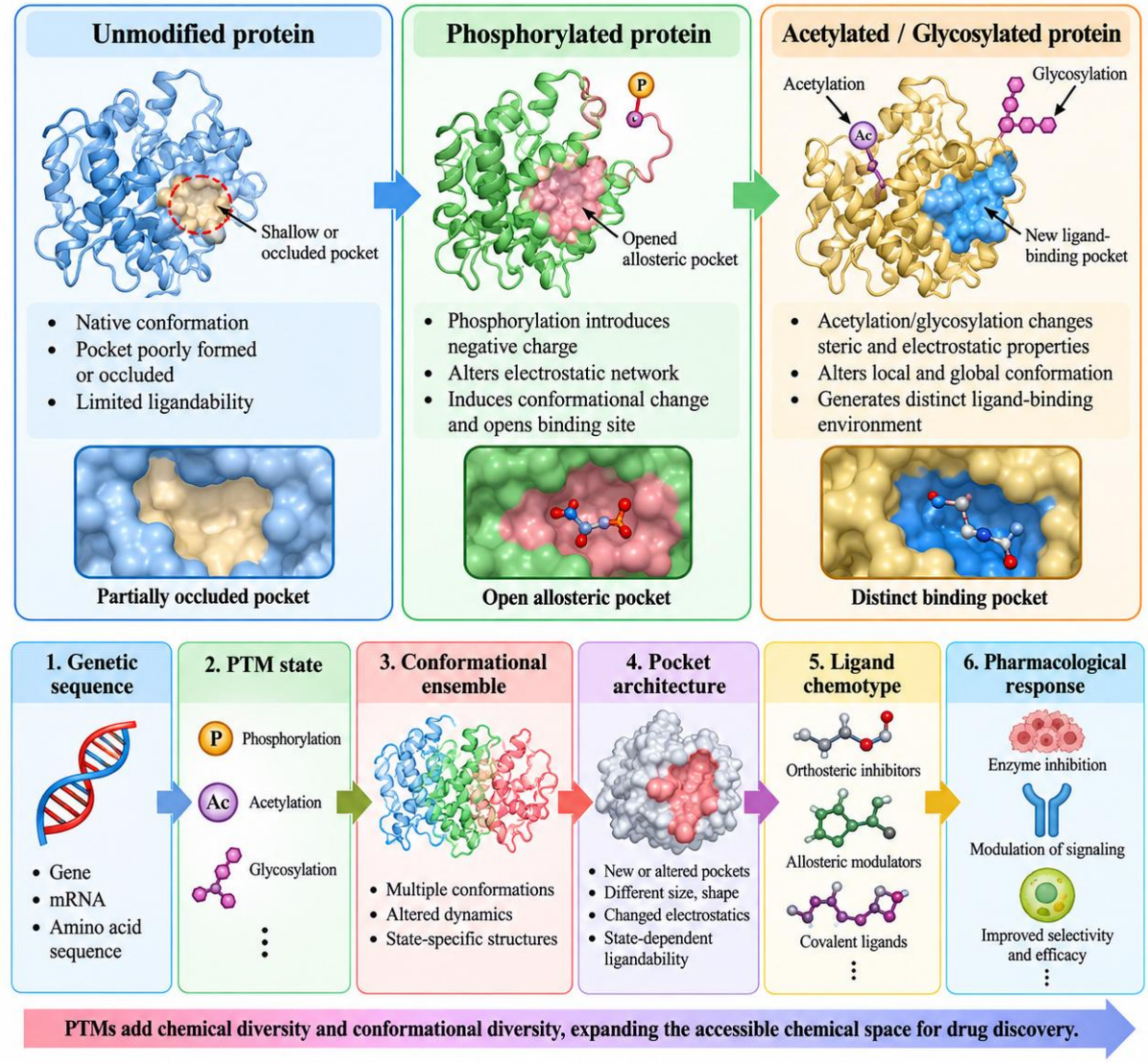


Figure 2. Post-Translational Modifications Expand the Protein Conformational and Chemical Landscape

8. Disease-Relevant Examples of Dynamic Druggability

8.1 KRAS

KRAS represents one of the most compelling examples of how dynamic and cryptic pockets can alter perceptions of druggability. KRAS(G12C) contains a switch-II pocket that became exploitable by covalent inhibitors despite the longstanding difficulty of targeting KRAS directly [49,50]. The pocket is dynamic and closely coupled to the nucleotide cycle.

The emergence of this site enabled allele-selective inhibition and showed that a transient or shallow pocket can still support effective pharmacology when conformational state, covalent chemistry, and reaction kinetics are considered together. The development of MRTX1133 against KRAS(G12D) further illustrates how non-covalent recognition can exploit a mutant-specific binding environment. Structural and biochemical analyses demonstrated highly selective interaction with KRAS(G12D), extending the concept of state- and mutation-dependent druggability to another difficult target [51].

8.2 β-Lactamases

TEM-1 and related β-lactamases demonstrate that hidden pockets can occur far from the catalytic site and can be coupled to enzyme activity. MD, MSM, enhanced sampling, and experiments have together revealed cryptic allosteric sites and conformational pathways that

affect catalytic behavior [11–15]. This example also illustrates an important caveat: **the existence of a transient cavity does not prove that it is a therapeutically useful site**. The pocket must be sufficiently populated, appropriately shaped, chemically favorable, kinetically accessible, and capable of supporting strong and selective binding.

8.3 Protein–Protein Interaction Interfaces

Large protein–protein interfaces are often considered challenging because they lack deep traditional cavities. However, local hotspots, conformational rearrangements, and cryptic grooves can create exploitable small-molecule binding regions [46–48]. IL-2 is a classical example, while additional success has been achieved against a growing range of signaling and regulatory protein interactions.

9. Experimental Approaches for Validation

Computational prediction must ultimately be connected to experimental evidence. X-ray crystallography provides direct structural validation when a fragment or ligand stabilizes an open state. NMR spectroscopy can characterize conformational exchange and low-population states that may be difficult to detect crystallographically [52–54]. Relaxation-dispersion NMR is particularly useful for identifying invisible or excited states and relating them to ligand binding and functional transitions. Such measurements can provide information on kinetics, populations, and exchange pathways rather than simply producing a single static structure.

HDX-MS provides a complementary solution-phase technique that can detect changes in solvent accessibility and protein dynamics after ligand binding or modification [52,53]. It can be applied to proteins that are difficult to crystallize and is increasingly integrated into drug-discovery workflows. Fragment screening, covalent tethering, limited proteolysis, cryo-EM, mutagenesis, and functional assays can further establish whether a predicted cryptic site is biologically meaningful. The strongest evidence for dynamic druggability therefore arises from **convergent validation**: **prediction → conformational characterization → ligand binding → structural confirmation → mutagenesis → functional modulation.**

10. Hydration and the Physical Basis of Dynamic Druggability

Pocket formation is not governed solely by protein geometry. Water molecules occupy, reorganize, and leave binding sites during conformational transitions. Therefore, a newly opened cavity can be attractive or unattractive to ligands depending on the energetic cost of desolvation and the properties of the water network. Modern computational approaches increasingly treat hydration as a central variable in pocket characterization. This is particularly important for cryptic sites because a pocket that appears geometrically suitable may remain poorly ligandable if it is highly hydrated or requires excessive energetic expenditure to open.

Recent analyses emphasize that water can influence both enthalpic and entropic components of ligand binding and that transient pockets may possess distinctive hydration patterns that should be incorporated into structure-based design [45,55]. Thus, future definitions of druggability should ideally combine **geometry, conformational population, pocket lifetime, hotspot strength, hydration, electrostatics, and ligand accessibility** rather than relying on pocket volume alone.

11. Integration of AI, Structural Prediction, and Dynamic Drug Discovery

AlphaFold has dramatically increased access to protein structural models, covering a much larger fraction of the human proteome than experimental structures alone [41,42]. However, a predicted structure should not automatically be interpreted as a complete representation of protein conformational space. AlphaFold primarily provides a structural prediction rather than a comprehensive kinetic ensemble.

The next generation of dynamic drug discovery will therefore likely combine structure prediction with ensemble generation, MD simulations, AI-based pocket prediction, experimental structural biology, and proteomics. PocketMiner already provides an example of this direction by learning from MD-derived conformational information to infer cryptic pocket propensity from a single protein structure [16]. More broadly, recent work on protein dynamics and allostery argues for integrating simulation and experimental data rather than relying exclusively on either paradigm. A practical future workflow may be: **AlphaFold/experimental structure → ensemble generation → pocket prediction → dynamic scoring → fragment/covalent screening → structural validation → medicinal chemistry optimization (Fig. 3).**

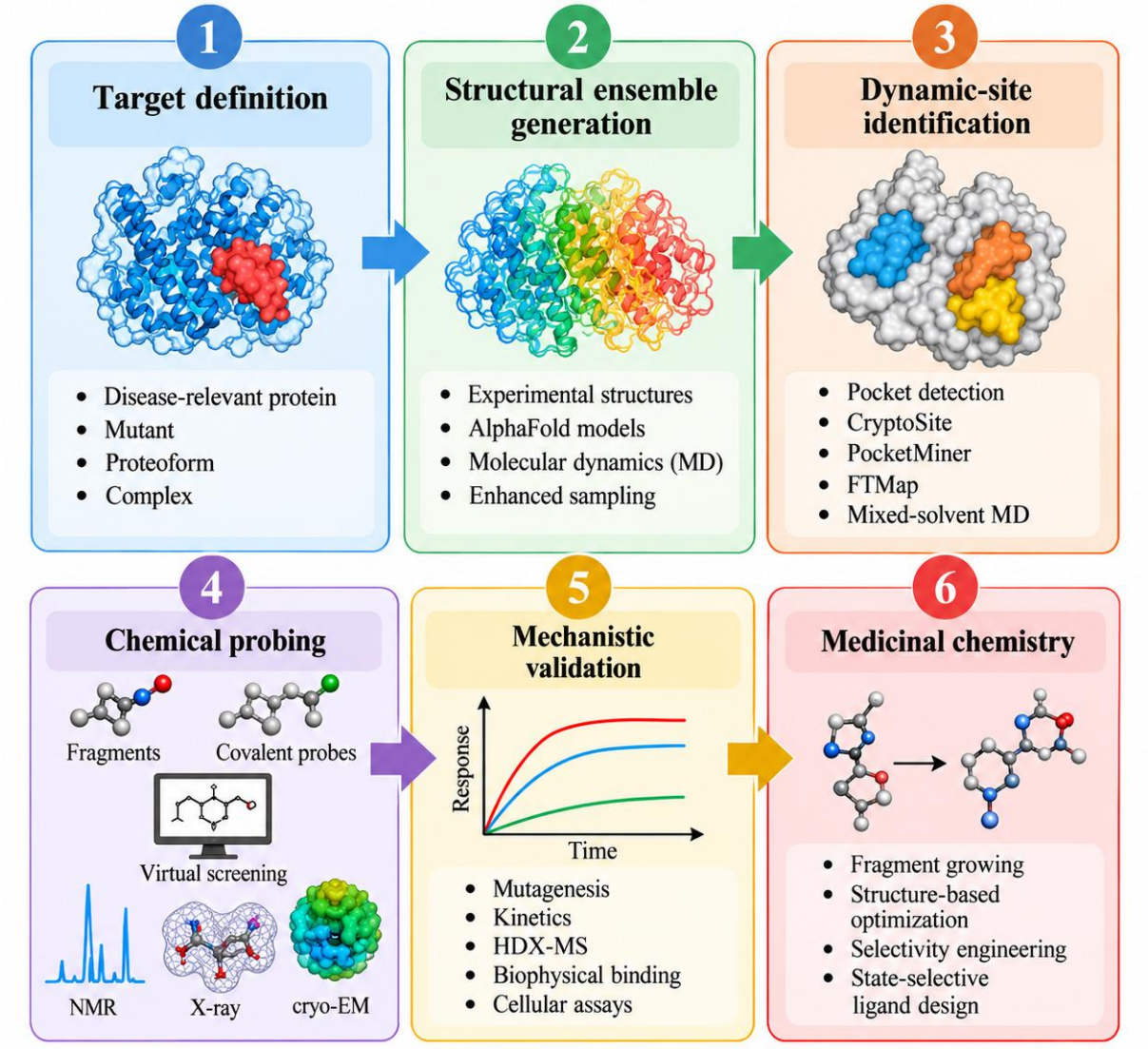


Figure 3. Integrated Workflow for Dynamic Druggability and Chemical-Space Expansion.

Above the workflow, show three interacting dimensions: **Conformation + PTM/proteoform + cryptic pocket = expanded chemical space**

The final output should be represented as **new chemotypes, new binding modes, new selectivity windows, and new pharmacological mechanisms**.

12. Challenges and Future Perspectives

Dynamic druggability offers substantial opportunities but also introduces new technical challenges. First, conformational sampling remains incomplete. A simulation that does not observe a particular pocket cannot prove that the site never forms. Conversely, a transient computational cavity may be an artifact of insufficiently restrained or over-biased simulations [14,15,18–21]. Second, cryptic-site prediction and druggability are not identical. A predicted cavity must still possess sufficient affinity, selectivity, kinetic accessibility, and functional coupling. Recent analyses indicate that cryptic pockets generated primarily by very rapid side-chain movement may be less useful than pockets created through larger backbone or loop rearrangements [55]. Third, PTM-dependent druggability creates biological heterogeneity. The relevant modification may vary between tissues, disease stages, cellular compartments, or treatment conditions. The experimentally purified protein therefore may not reproduce the conformational ensemble present in vivo. Fourth, medicinal chemistry must account for conformational entropy. Stabilizing a rare open state can be energetically expensive, and the ligand must compensate for that cost through favorable interactions. Water displacement, desolvation, induced strain, and conformational entropy all influence the final binding free energy [45,55].

Finally, AI approaches need high-quality training data. Predictions are only as reliable as the structural and dynamic examples used to train the models. Integration with experimentally validated datasets will therefore be essential. Despite these challenges, the conceptual shift is clear. The relevant question is no longer simply: **“Does the protein have a pocket?”.** It is increasingly: **“Which protein state contains a chemically exploitable site, how is that state populated, and can a ligand stabilize it selectively?”**. This change fundamentally broadens the design space available to medicinal chemists.

13. Conclusions

Dynamic druggability provides an expanded framework for understanding why proteins that appear poorly ligandable in static structures can nevertheless support useful small-molecule interactions. Conformational ensembles create transient cavities and alternative allosteric states; PTMs generate chemically distinct proteoforms; cryptic pockets expose binding opportunities that are invisible in canonical structures; and computational and experimental technologies increasingly allow these features to be detected and exploited. The growing success of difficult targets such as KRAS demonstrates that dynamic recognition can be translated into selective pharmacology. Ultimately, dynamic druggability should be viewed as a multidimensional property emerging from the interaction of protein conformation, chemical modification, solvent organization, ligand kinetics, and cellular context. Combining these dimensions is likely to expand both the number of druggable proteins and the diversity of chemical matter that can be designed against them. Future discovery programs that integrate AI-based prediction, long-timescale simulations, PTM-aware structural modeling, fragment screening, hydration analysis, and rigorous experimental validation may therefore transform previously difficult targets into tractable opportunities for precision therapeutics.

Author Contributions

Anugya Gupta and Km. Monika: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Visualization, Writing Original Draft, Writing Review & Editing, Validation, Supervision, and Final Approval of the Manuscript.

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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
MD	Molecular Dynamics
PTM	Post-Translational Modification
FBDD	Fragment-Based Drug Discovery
MSM	Markov State Model
NMR	Nuclear Magnetic Resonance
HDX-MS	Hydrogen–Deuterium Exchange Mass Spectrometry
X-ray	X-ray Crystallography
cryo-EM	Cryogenic Electron Microscopy
PPI	Protein–Protein Interaction
Fpocket	Fast Pocket Detection Algorithm
FTMap	Fast Fourier Transform-Based Molecular Interaction Mapping
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
GNN	Graph Neural Network
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
IL-2	Interleukin-2

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