Orchestrating function: concerted dynamics, allostery, and catalysis in protein tyrosine phosphatases

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Highlights

- Protein tyrosine phosphatases (PTPs) have a structurally conserved catalytic domain, but differ in terms of dynamics, allostery, and function
- NMR spectroscopy, avant-garde X-ray crystallography, and other modern structural biology experiments give complementary insights into PTP dynamics and allostery
- Molecular dynamics (MD) simulations yield atomistic information about conformational motions, and sequence coevolutionary analyses reveal hypotheses about conserved vs. divergent allostery
- Mutations and small-molecule ligands, especially in large-scale libraries, provide useful and often high-throughput information about PTP allostery and ligandability
- The future is bright for experimentally and computationally characterizing catalytic motions and biologically relevant regulatory complexes for the broader family of PTPs in atomistic detail

Abstract

Protein tyrosine phosphatases (PTPs) are a family of enzymes that play critical roles in intracellular signaling and regulation. PTPs are conformationally dynamic, exhibiting motions of catalytic loops and additional regions of the structurally conserved catalytic domain. However, many questions remain about how dynamics contribute to catalysis and allostery in PTPs, how these behaviors vary among evolutionarily divergent PTP family members, and how mutations and ligands reshape dynamics to modulate PTP function. Recently, our understanding in these areas has expanded significantly, thanks to novel applications of existing methods and emergence of new approaches in structural biology and biophysics. Here we review exciting advances in this realm from the last few years. We organize our commentary both by experimental and computational methodologies, including solution techniques, avant-garde crystallography, molecular dynamics simulations, and bioinformatics, and also by scientific focus, including regulatory mechanisms, mutations and protein engineering, and small-molecule ligands such as allosteric modulators.

Introduction

Protein tyrosine phosphatases (PTPs) constitute a diverse enzyme family that is essential for regulating numerous cellular processes by dephosphorylating phosphotyrosine (pTyr) residues in various proteins. The human genome encodes 107 distinct PTP genes, classified into four major groups, including 37 classical PTPs [1]. These enzymes share structural and evolutionary relationships, forming coherent phylogenetic groupings that underline their functional similarities and diversification across various physiological contexts [2].

Previously, X-ray crystallographic studies provided detailed insights into the conserved architecture and structural diversity within the catalytic domains of classical PTP family members (**Fig. 1a**) [3]. These analyses revealed that despite a shared catalytic core characterized by a conserved α/β fold and active-site motif, significant variability exists in surface residues surrounding and distal from the catalytic site. Such variability likely contributes to the distinct substrate specificities and regulatory mechanisms of individual PTPs.

PTP catalytic domains exhibit intrinsic structural dynamics, notably involving specific loop regions whose movements directly influence catalytic activity (**Fig. 1a**). Most notably, the WPD loop transitions between open and closed states, positioning a catalytic aspartate residue that is essential for pTyr hydrolysis. Previous studies employing nuclear magnetic resonance (NMR) spectroscopy for different PTPs showed that these conformational transitions in the WPD loop directly correlate with catalytic turnover rates (k_{cat}) [4], underscoring the functional significance of dynamic loop transitions in PTPs (**Fig. 2**). Various studies also point to allosterism in multiple PTPs, emphasizing that functional dynamics are not limited to catalytic loops.

This review synthesizes recent advances in the study of PTP structural dynamics, particularly emphasizing the link between conformational fluctuations and functional modulation, including allosteric regulation. We discuss novel insights derived from a combination of structural biology, molecular biophysics, biochemistry, and computational methods. Collectively, these contemporary approaches enhance our view of PTPs as dynamic enzymes, revealing evidence for endogenous regulatory mechanisms and potential for targeted therapeutic interventions.

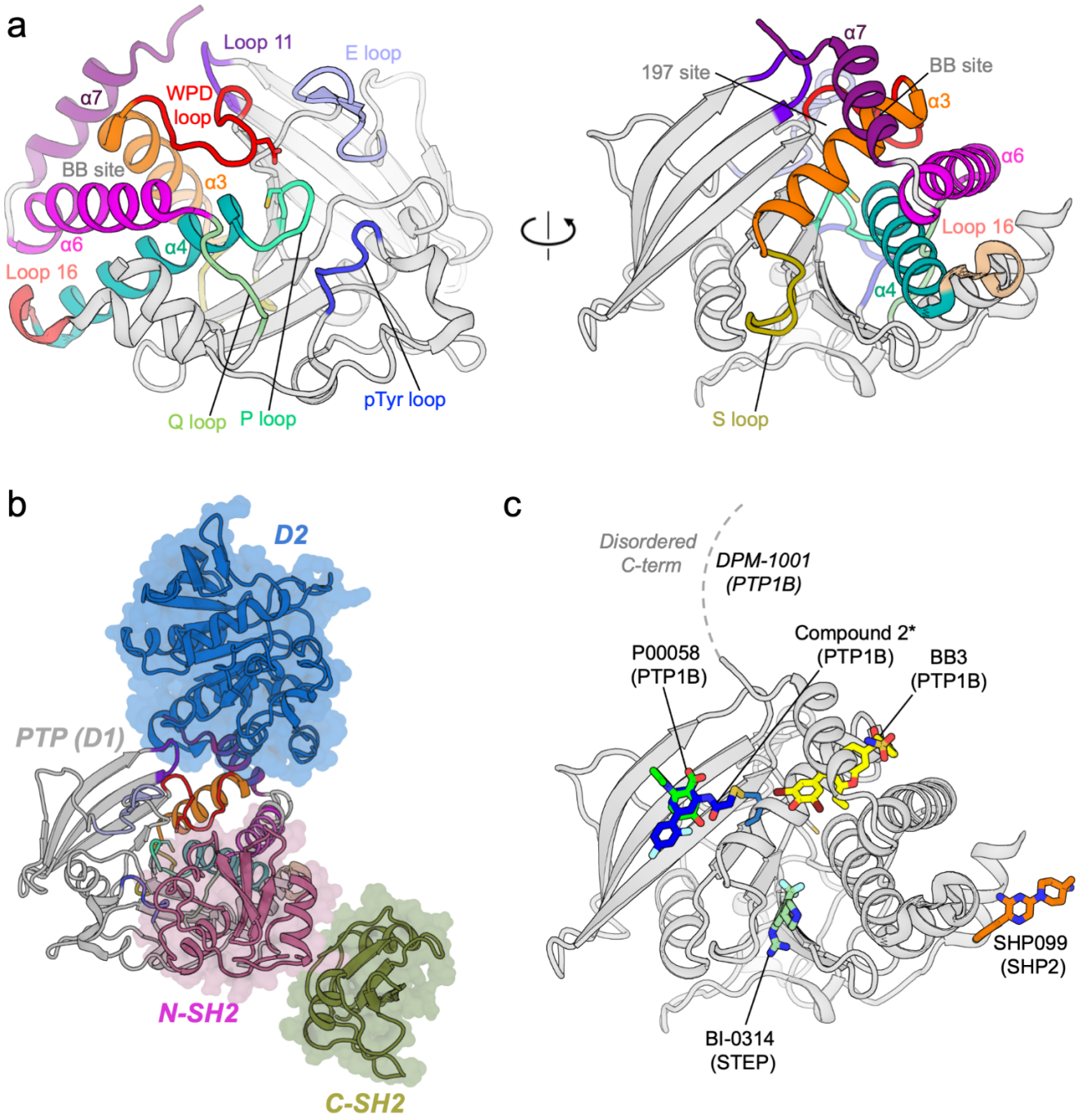

Figure 1: Structural overview of protein tyrosine phosphatases (PTPs).
a. Representative PTP catalytic domain from a crystal structure of PTP1B (PDB ID: 1sug) highlighting key structural elements relevant to catalysis and allostery. The phosphotyrosine (substrate-binding) loop (residues 45–50) is shown in blue, the E loop (residues 114–122) in light blue, loop 11 (L11, residues 150–153) in purple, the WPD loop (residues 177–186) in red, the α3 helix (residues 187–202) in orange, the S loop (203–210) in olive, the P loop (residues 214–219) in light cyan, the α4 helix (residues 220–238) in teal, loop 16 allosteric site (L16, residues 239–244) in light pink, the Q loop (residues 257–262) in light green, the α6 helix (residues 263–282) in magenta, and the α7 helix (residues 285–299) in dark purple. The “front” view (top) is complemented by a rotated “back” view (right). Allosteric BB site and 197 site are labeled in gray.

b. Structural organization of regulatory domains for selected PTPs. The catalytic (PTP) domain is shown in gray as in panel **a**. Additional regulatory domains are shown using separate crystal structures: a non-catalytic D2 domain in blue (PTPσ; PDB ID: 2fh7), and N-SH2 and C-SH2 domains in pink and green, respectively (SHP2; PDB: 2shp).
c. Binding sites for selected small-molecule allosteric modulators for PTP1B, SHP2, and STEP are shown using a representative crystal structure of PTP1B (PDB ID: 1sug) [69]. The BB3 inhibitor for PTP1B is shown in yellow (PDB ID: 1t49) [70], Compound 2 covalent inhibitor for PTP1B in blue (PDB ID: 6b95) [47], P00058 inhibitor for PTP1B in green (PDB ID: 7klx) [52], SHP099 inhibitor for SHP2 in orange (PDB ID: 5ehr) [71], and BI-0314 activator for STEP in green/cyan (PDB ID: 6h8s) [72]. The DPM-1001 inhibitor for PTP1B binds in the disordered C-terminus (dashed line, not to scale) [14]. *Compound 2 is covalently tethered to residue 197, which is mutated from a lysine to cysteine in PDB: 6b95; the K197C side chain is shown in sticks.

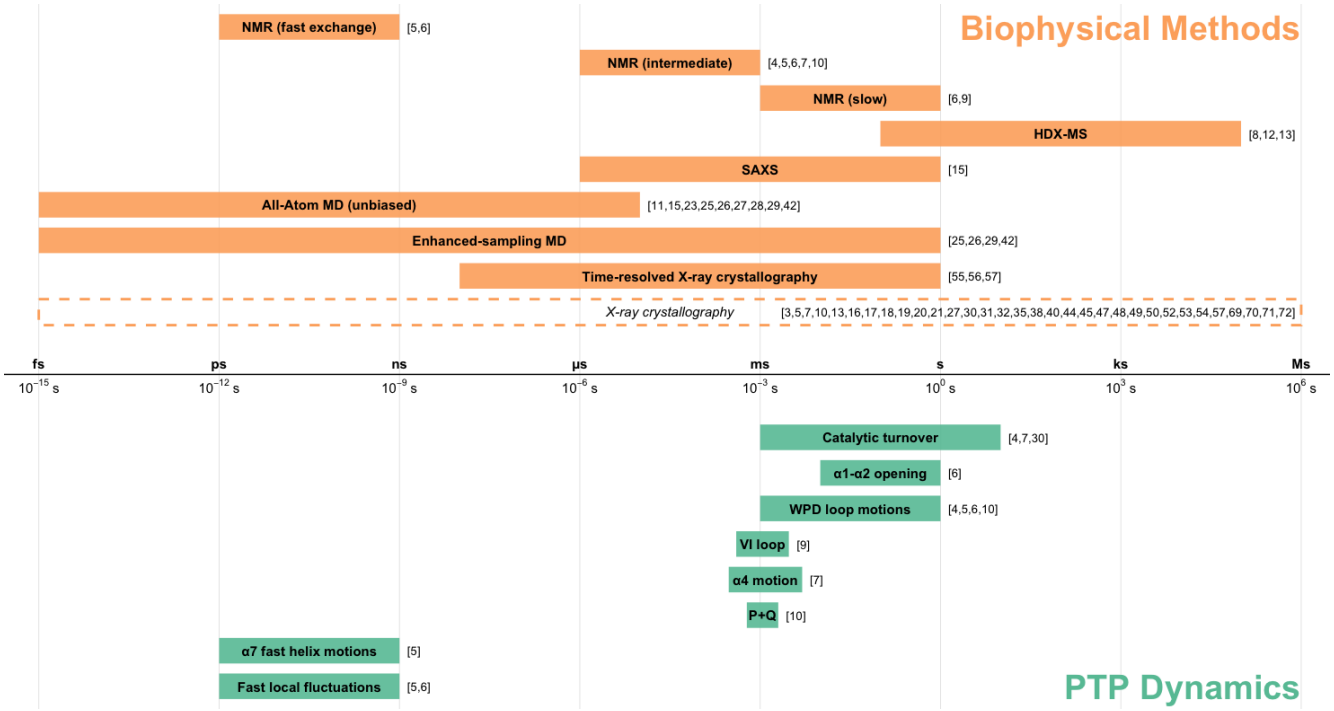

Figure 2: Timescales of relevant biophysical methods and PTP functional dynamics.
Comparison of temporal resolutions for biophysical methods (orange) and PTP conformational dynamics relevant to biological function (green). Time is shown on a logarithmic scale.
Top: Methods providing explicit kinetic timescale data include NMR spectroscopy (fast: ps–ns, intermediate: µs–ms, slow: ms–s), hydrogen-deuterium exchange mass spectrometry (HDX-MS, 0.1–100,000 s), small-angle X-ray scattering (SAXS, µs–s), all-atom molecular dynamics (MD, fs–µs), enhanced sampling MD (effective fs–s), and time-resolved crystallography (TRX, fs–s). Most X-ray crystallography (patterned bar) provides static snapshots without explicit kinetic resolution.
Bottom: Functionally relevant PTP dynamics include catalytic turnover rates (fastest: YopH ~ms, standard: PTP1B ~50 ms, slowest: PTEN ~5 s), α1-α2 'cap' opening (ms–s), WPD loop motions (µs–ms), variable-insert loop motions (µs–ms), α4-coordinated motions (µs–ms), P loop + Q loop concerted motions (µs–ms), α7 helix motions (ps–ns), and fast local fluctuations (fs–ns).
Relevant reference numbers from the text are indicated for each category of methods and dynamics.

Experimental biophysical methods

NMR spectroscopy

NMR spectroscopy is a powerful technique for measuring conformational dynamics in solution across a wide range of timescales (ps–s) (**Fig. 2**). Although NMR has limitations on protein size, PTP catalytic domains (~30–40 kDa) are generally amenable to NMR analysis.

A series of recent studies used NMR to probe dynamics in PTP1B. First, ^{15}N backbone relaxation NMR experiments showed fast (ps–ns) motions for allosteric regions including the critical α7 helix, but intermediate (μs–ms) motions for the catalytic WPD loop, revealing that dynamics at distinct timescales underlie different aspects of PTP1B function [5]. Second, ^{13}C-methyl side-chain relaxation experiments showed that several active-site loops (WPD, Q, E, and substrate-binding loops) undergo synchronized motions on an intermediate (μs–ms) timescale, and that the N-terminal helices α1' and α2' dynamically detach and reattach on a slow (ms–s) timescale (**Fig. 2**) [6]. Third, coevolutionary analysis identified residues distal from the active site that surprisingly increase PTP1B activity upon mutation; ^{13}C-methyl NMR experiments revealed a correlation between intermediate (μs–ms) dynamics and catalytic rate, suggesting that structurally distributed dynamics underlie catalysis in the broader PTP family [7].

Other work used NMR to study a protein-protein interaction (PPI) for PTP1B, documenting chemical shift perturbations upon binding of the adaptor protein Grb2 to the disordered C-terminus of PTP1B [8]. These data were complemented by other biophysical experiments (see later sections).

Beyond PTP1B, NMR of VHR, a human dual-specificity phosphatase (DUSP), revealed coordinated ms-timescale dynamics involving the WPD loop and a distal region; mutations in this region disrupted dynamic coupling and impaired catalytic efficiency [9]. These observations indicate that dynamic coupling between the active site and distal regions, regardless of their precise identity or location, is common to many different phosphatases.

Other studies contrast PTPs from humans and other species. NMR of the archaeal phosphatase SsoPTP revealed synchronized dynamics of several active-site loops; unlike for PTP1B, this synchronization was independent of active-site ligand binding [10]. Additionally, for the pathogenic low molecular weight PTP MptpA, NMR chemical shift perturbations identified an allosteric residue (Q75). MD simulations showed that mutation of this distal residue increased dynamics of catalytic loops, populating an active state that increases catalysis [11]. These results suggest that MptpA activity is allosterically prevented from maximal activity during infection. Such unique mechanisms could inform the design of inhibitors that selectively target pathogens without affecting human enzymes.

Other solution methods

While NMR spectroscopy reveals site-resolved information about dynamics across many timescales, it is challenging for long timescales (ms–min or longer) and large proteins. Hydrogen-deuterium exchange mass spectrometry (HDX-MS) complements NMR by providing information about protein conformational dynamics and/or solvent accessibility, at lower spatial resolution but across broader timescales, for larger systems including disordered regions (**Fig. 2**).

Recently, high-resolution local HDX-MS was used to characterize dynamics of PTP1B [12]. H/D exchange (HDX) was largely consistent with variability among crystal structures, with some outlier regions exhibiting unexpectedly high HDX in solution. In contrast to an active-site inhibitor, binding of an allosteric inhibitor induced surprisingly distinct and widespread changes in HDX.

HDX-MS was also used to study how human amino acid variants affect PTP1B dynamics and catalysis [13]. For the mutants with the greatest catalytic impact, changes in conformational heterogeneity along pathways to the active site were observed by HDX-MS and room-temperature crystallography. The results suggest that these mutations exploit a unique allosteric network in PTP1B.

Changes in HDX for the catalytic domain were also observed for a human variant in the disordered proline-rich region [13]. In another study, HDX-MS was used to validate the PPI between Grb2 and the disordered proline-rich region of PTP1B, demonstrating that Grb2 binding stabilized PTP1B dynamics globally [8]. These studies, alongside other reports of allosteric small-molecule inhibitors targeting disordered regions of PTP1B [14], indicate substantial allosteric communication between the dynamic disordered region and ordered catalytic domain.

Small-angle X-ray scattering (SAXS) complements other solution techniques by capturing large-scale conformational ensembles, which represent the distribution of conformational states accessible to the protein. SAXS and molecular dynamics (MD) simulations were used to demonstrate extensive conformational variability for the constitutively active E76K mutant of SHP2 [15], highlighting that active conformational states are not single static structures but rather dynamic ensembles.

X-ray crystallography

Solution methods provide valuable information about dynamics, but do not directly determine structures. By contrast, X-ray crystallography yields high-resolution all-atom structures, and can access distinct conformational states regardless of timescale that may pertain to function, although concerns may exist about artificial influence on protein conformations from the crystal lattice.

A traditional cryogenic-temperature (cryo) crystal structure of TCPTP with the α7 helix ordered revealed that α7 contributes to catalysis similarly in TCPTP and its close homolog PTP1B, by conditionally ordering upon WPD loop closure, albeit with subtle differences [16]. PTP1B and TCPTP thus are similar structurally, consistent with the recent report of a compound that potently inhibits both to the exclusion of all other PTPs [17] — yet they have distinct biological roles and disease associations, motivating future research to identify inhibitors that are specific for either PTP1B or TCPTP.

An exciting frontier of crystallography is using experimental perturbations like variable temperature to reveal alternative conformational states. For PTP1B, a structure at room temperature (RT) instead of cryo was determined using serial synchrotron crystallography [18]. The results revealed decoupling between the active-site WPD loop and the distal loop 16, adding nuance to our understanding of allosterism in PTP1B.

Expanding to different perturbations, another study compared temperature (T) vs. pressure (P) for STEP [19]. The high-P structure featured a conformation of the active-site E loop only seen previously in an allosterically activated structure, whereas the high-T structure adopted distinct active-like conformations at the catalytic Q and WPD loops. Interestingly, each structure had a distinct pattern of ordered water

molecules. Also, a dehydrated structure featured a more open conformation of an allosteric site, providing new opportunities for allosteric modulator design [20]. Together, these studies illustrate how crystallographic perturbations can reveal informative alternative conformations of PTPs.

Computational methods

MD simulations

Molecular dynamics (MD) simulations provide atomic-resolution windows into biomolecular motions. Although they use imperfect force fields and may require special approaches to access longer, functionally relevant timescales, MD simulations usefully complements experimental data for PTPs (**Fig. 2**) [5,7,11,21–24].

MD has been used to investigate WPD loop opening/closing motions in different ways. One study used long-timescale and weighted ensemble simulations of PTP1B to identify backbone dihedrals of the conserved PDFG motif (overlapping the eponymous WPD) as key to this motion [25]. Another study pinpointed two torsion angles involving the D-F bond as especially important for a friction-limited conformational change that is followed by diffusive loop motions [26].

In the context of drug discovery, long-timescale simulations for PTP1B revealed poses for weakly binding small-molecule fragments [27]. Another MD study showed that the PTP1B allosteric inhibitor amorphadiene binds to the disordered C-terminus [28], further validating allosterism in this region.

MD can reveal how structural differences between PTPs impact catalytic WPD loop dynamics. Simulations of PTP1B and YopH showed that prolines in PTP1B act as hinges to restrict motion, whereas the flexible loop of YopH samples more conformations including a rare hyper-open state [29]. Moreover, the adjacent E loop was shown to be flexible in PTP1B but rigid in YopH; these observations are consistent with previous reports of coupled dynamics between these two loops in PTP1B from NMR [6] and in HePTP from MD [30].

Other computational methods

Additional computational approaches can provide complementary insights into PTP dynamics. One study used network analysis of MD trajectories for three PTPs with divergent sequences and varying catalytic rates to reveal differences in correlated loop dynamics that underlie functional differences [23].

Aside from MD, dimensionality reduction for crystal structures reveals insights into conformational landscapes [31]. For STEP, dimensionality reduction showed that a high-temperature structure clustered with active-like structures, whereas a high-pressure structure was distinct from existing structures [19]. For PTP1B, dimensionality reduction for many structures from crystallographic fragment screening showed partial decoupling of the WPD loop and allosteric loop 16, and indicated a potential allosteric loop encompassing phosphorylation sites [32].

Sequence-based methods like statistical coupling analysis (SCA), which uses patterns of amino acid coevolution from multiple sequence alignments to predict conformational coupling in protein structures, can also provide insights into putatively coupled dynamics and allosteric networks. One study used SCA

to reveal allosteric sectors, one overlapping with a known allosteric network and the other unique [21]. Mutations to sector residues in multiple PTPs reduced k_{cat}, indicating conserved allostery. Another study identified coevolving residues distal from the active site that surprisingly increased catalysis upon mutation [7]. Together, these results suggest that some aspects of conformational dynamics are conserved within the PTP catalytic domain despite divergence in sequence and function. Nevertheless, the physical meaning of coevolving sectors remains unclear, both generally and for PTPs.

Regulatory domains, interactions, and mechanisms

The PTP catalytic domain is structurally conserved, but domain architecture varies between PTPs, providing for differences in endogenous regulation that are beginning to be unraveled (**Fig. 1b**).

For example, binding of the regulatory protein Grb2 to the disordered C-terminus of PTP1B allosterically alters NMR chemical shifts, decreases H/D exchange indicating decreased dynamics, and increases PTP1B catalysis [8]. Mutations to known allosteric residues [5] did not impact Grb2 activation, indicating a distinct allosteric mechanism.

TCPTP, the only other PTP with a C-terminal α7 helix, has a similar structure and mechanism involving α7 [16]. However, while both also have a disordered C-terminus, NMR shows that this tail in TCPTP uniquely wraps around the catalytic domain, blocking substrate binding; this autoinhibition is lifted upon binding of an integrin protein [33].

SHP2 is also allosterically regulated by autoinhibition, but by N-SH2 domain binding to the catalytic domain, blocking the active site (**Fig. 1b**). N-SH2 binding to a pTyr substrate relieves this autoinhibition, activating SHP2. A recent study identified a single ion pair that stabilizes this activated state; mutations to these residues resulted in unusual “hyperinhibited” SHP2 [34].

A distinct example of allosteric regulatory domains is found in RPTPα, whose non-catalytic D2 domain [2] inhibits activity in the catalytic D1 domain (**Fig. 1b**) [35]. Notably, this regulation is absent in the closest homolog, suggesting evolutionary divergence.

Finally, many PTPs are regulated by reversible oxidation of the catalytic cysteine, but in different ways. Some PTPs form a disulfide bond with a nearby “backdoor” cysteine; however, SHP2 and SHP1 have an additional backdoor cysteine that provides extra protection from oxidative inactivation at the expense of thermal stability, highlighting an evolutionary trade-off [36]. In a distinct mechanism, oxidized PTP1B is regulated *in vivo* by a redox-sensing loop that interacts with the regulatory protein 14-3-3ζ only in the oxidized state [37].

Amino acid sequence changes

Point mutations

Point mutations are powerful tools for interrogating connections between PTP structure, dynamics, and function, particularly when paired with other experimental and computational analyses [5–11,13–16,21,22,24,26,28,29,33–36,38–44].

Two complementary studies introduced mutations into the WPD loops of different PTPs and observed altered pH rate profiles for different mechanistic reasons. For the pathogenic YopH, effects were attributed to changes in WPD loop conformational preferences [45]. By contrast, for the human SHP1, effects were attributed to changes in active-site solvation and hydrogen-bonding networks [41].

Another study examined the disease-associated T42A mutation in the N-SH2 regulatory domain of SHP2 [22]. Biochemical assays showed bias for the mutant toward certain phosphopeptides. MD revealed that the mutation rearranges hydrogen bonds in the pTyr binding pocket, despite being distally located. These results demonstrate how an individual mutation in a PTP can allosterically dysregulate cellular signaling to cause disease.

At a larger scale, deep mutational scanning was used to characterize nearly all possible point mutants of SHP2 [24]. Many known disease mutations were gain-of-function, but others were unexpectedly neutral or loss-of-function. Screening full-length SHP2 and the isolated catalytic domain helped differentiate effects on intrinsic catalysis vs. interdomain autoinhibition, including residues at key autoinhibitory interfaces and those that bias coordinated WPD loop dynamics. This work highlights the variety of mechanisms by which SHP2 biological function can be disrupted by perturbed dynamics and/or allostery.

Protein engineering

Beyond individual point mutations, rational protein engineering can dissect the fundamental roles of specific structural elements in PTP dynamics, allostery, and function. Such approaches have the potential not only to tease apart the fundamental functional roles of specific structural elements in the PTP fold, but also to develop useful tools for basic research and biotechnology that enable illumination-based activation of PTP activity or recruit PTPs to specific targets in cells (e.g. PhosTACs).

To probe the factors controlling WPD loop stability, dynamics, and catalysis, chimeras were engineered that transpose this loop between the slower PTP1B and the faster YopH. The PTP1B scaffold with the YopH loop was less active than both parent enzymes, presumably due to altered WPD loop dynamics including differences in flexibility [40]. The reverse chimera exhibited an unusual hyper-open loop conformation [46] that was previously observed as a rare event for wild-type YopH [29], indicating a shift in the energy landscape of this important loop in a non-cognate structural context.

In another class of chimeric PTPs, blue-light-activated LOV domains were fused to the C-terminus of PTP1B [38]. Remarkably, the resulting proteins were allosterically regulated by light, with further enhancements to light-sensitivity by mutating interface residues. A subsequent study compared different architectures by varying the insertion point into the PTP domain [39]. The optimal insertion approach successfully regulated PTP1B and TCPTP but not SHP2, illustrating varying allosteric properties among PTPs.

Small-molecule ligands

Benign binders

Crystallographic small-molecule fragment screening generates many experimental ligand-bound protein structures. Although fragments typically bind weakly and are functionally benign, they can reveal low-occupancy protein conformations and provide footholds for downstream inhibitor design.

Previously, screening for PTP1B yielded 110 fragment-bound structures, including multiple allosteric sites [47]. Pre-clustering X-ray datasets [48] yielded 65 additional hits, including two novel sites [49]. One new fragment bound uniquely between the catalytic WPD and E loops; another allosterically induced closure of the WPD loop. Fragment hits re-screened at RT instead of cryo revealed new binding poses, binding sites, and allosteric responses [50]. Together, this wealth of structural data maps the ligandability and allosteric wiring of PTP1B.

Beyond PTP1B, crystallography of STEP revealed covalent ligands at cysteines that are (almost) unique to STEP among PTPs, and a novel citrate pose above the catalytic pocket, offering potential footholds for specific inhibitor design [20]. In addition to crystallography, STEP ligands have been identified from protein thermal shift screening assays, yielding both benign binders and inhibitors [51].

Allosteric modulators

Recent research has significantly advanced our understanding of how small-molecule allosteric modulators of PTPs achieve specificity and potency, e.g. by selectively targeting non-conserved allosteric sites.

Two recent allosteric inhibitors targeting PTP1B operate by distinct mechanisms. First, P00058 binds in the catalytic domain and induces conformational rearrangements extending to the distal WPD loop [52], thus reinforcing the structural and functional significance of this allosteric binding site [47]. Second, DPM-1001 binds to the disordered C-terminus, and exhibits efficacy in animal models of metabolic disorders [14].

For the oncogenic SHP2, selective, irreversible inhibitors were obtained that target the pathogenic mutation Y279C, providing a potent method for modulating mutant enzyme activity [43]. This parallels the successful therapeutic strategy for a pathogenic K-Ras mutation [53].

Finally, fragment screening by ^{19}F NMR identified diverse allosteric binders targeting VHR [54]. This approach revealed novel non-orthosteric binding sites that were validated by crystallography, providing opportunities for allosteric inhibitor development.

Future perspectives

Recent studies have used myriad techniques to explore the interplay between dynamics, allostery, and catalysis for several PTPs. Future work can expand to the broader PTP family and take advantage of several exciting methodological advances.

First, time-resolved X-ray crystallography (TRX) can record stop-motion movies of catalytic turnover, providing all-atom windows into concerted conformational motions accompanying catalysis. TRX is increasingly accessible at synchrotrons with ms time resolution [55,56], in line with PTP catalysis and loop motions [4]. TRX is now more generally feasible given advances in substrate delivery [55,56] and temperature jumps to activate protein motions [57]. TRX in crystals thus can serve as a powerful and highly complementary technique to e.g. advanced NMR methods such as CPMG relaxation dispersion experiments in solution.

Second, the cryo-electron microscopy (cryo-EM) “resolution revolution” sets the stage for determining structures of full-length PTPs in biologically relevant complexes, such as receptor-type PTPs in their native membrane environments or various PTPs in complex with substrate and/or regulatory proteins. Such experiments could use full-length PTPs, including regulatory domains, rather than just the catalytic domains that are more tractable for other methods like crystallography and NMR. In addition, n~~N~~ew algorithms can extract multiple conformational states from cryo-EM datasets [58] to reveal how PTP complexes temporally assemble and are allosterically regulated. Moreover, cryo-electron tomography (cryo-ET) is becoming attractive to examine PTP complexes in native cellular environments, e.g. in diseased vs. healthy human cell lines, for a larger-scale view of how “dynamics” connects to biological function.

Third, in the age of artificial intelligence, computational structural biology offers new possibilities for PTPs. Clustering algorithms to classify active vs. inactive states [59,60] could be used for PTPs, aided by new dimensionality reduction methods [32,61]. Such research would benefit from the wealth of available crystal structures for numerous PTPs [3,47,49,50]. Predicted AlphaFold structures [62,63] could complement experimental structures by (i) including all PTPs, (ii) modeling regions beyond the catalytic domain, and (iii) generating biologically relevant alternative conformations [64,65]. Moreover, experimental data can fruitfully integrate with computational methods, as seen with crystallographic density maps for modeling protein alternate conformations [66] and their networks of interactions [67], solution SAXS data for steering MD simulations [15], and multiple sequence alignments for predicting allosteric sectors [21]. Such integrative structural biology approaches have great potential for elucidating specific structural features or behavior in ways that are grounded by empirical observations.

Overall, structural biology is shifting its focus from static structures to ensembles, dynamics, and allostery [68]. PTPs are highly dynamic, allosteric enzymes with a plethora of endogenous regulatory mechanisms, many of which are yet to be understood, and a concomitant wealth of opportunities for exogenous modulation for therapeutic benefit. With the modern explosion of advances in techniques revealing protein dynamics, the future is bright for “tracking the movements” of these diverse, biomedically critical, fascinating enzymes.

Declaration of interest

The authors declare no conflict of interest.

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Data availability

No data were used for the research described in the article.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:
* of special interest
** of outstanding interest

1. Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, Mustelin T: **Protein tyrosine phosphatases in the human genome**. *Cell* 2004, **117**:699–711.

2. Andersen JN, Mortensen OH, Peters GH, Drake PG, Iversen LF, Olsen OH, Jansen PG, Andersen HS, Tonks NK, Møller NP: **Structural and evolutionary relationships among protein tyrosine phosphatase domains**. *Mol Cell Biol* 2001, **21**:7117–7136.

3. Barr AJ, Ugochukwu E, Lee WH, King ONF, Filippakopoulos P, Alfano I, Savitsky P, Burgess-Brown NA, Müller S, Knapp S: **Large-scale structural analysis of the classical human protein tyrosine phosphatome**. *Cell* 2009, **136**:352–363.

4. Whittier SK, Hengge AC, Loria JP: **Conformational motions regulate phosphoryl transfer in related protein tyrosine phosphatases**. *Science* 2013, **341**:899–903.

5. Choy MS, Li Y, Machado LESF, Kunze MBA, Connors CR, Wei X, Lindorff-Larsen K, Page R, Peti W: **Conformational rigidity and protein dynamics at distinct timescales regulate PTP1B activity and allostery**. *Mol Cell* 2017, **65**:644–658.e5.

6. Torgeson KR, Clarkson MW, Kumar GS, Page R, Peti W: **Cooperative dynamics across distinct structural elements regulate PTP1B activity**. *J Biol Chem* 2020, **295**:13829–13837.

7.** Torgeson KR, Clarkson MW, Granata D, Lindorff-Larsen K, Page R, Peti W: **Conserved conformational dynamics determine enzyme activity**. *Sci Adv* 2022, **8**:eabo5546.

Uses coevolutionary coupling analysis to identify amino acid positions in the PTP fold that are distal from the active site but influence catalytic activity. NMR chemical shifts and dynamics experiments show that mutations at these sites modulate PTP1B activity by altering μs–ms dynamics, not ps–ns dynamics or average structure. This work suggests that conformational dynamics underlying enzyme activity may be to some extent conserved, yet also tunable.

8. Chartier CA, Woods VA, Xu Y, van Vlimmeren AE, Johns AC, Jovanovic M, McDermott AE, Keedy DA, Shah NH: **Allosteric regulation of the tyrosine phosphatase PTP1B by a protein-protein**

interaction. *Protein Sci* 2025, **34**(1):e70016.

9. Beaumont VA, Reiss K, Qu Z, Allen B, Batista VS, Loria JP: **Allosteric impact of the variable insert loop in Vaccinia H1-related (VHR) phosphatase**. *Biochemistry* 2020, **59**:1896–1908.

10. Pinkston J, Jo J, Olsen KJ, Comer D, Glaittli CA, Loria JP, Johnson SJ, Hengge AC: **Significant loop motions in the SsoPTP protein tyrosine phosphatase allow for dual general acid functionality**. *Biochemistry* 2021, **60**:2888–2901.

11. Maschietto F, Zavala E, Allen B, Loria JP, Batista V: **MptpA kinetics enhanced by allosteric control of an active conformation**. *J Mol Biol* 2022, **434**:167540.

12.** Woods VA, Abzalimov RR, Keedy DA: **Native dynamics and allosteric responses in PTP1B probed by high-resolution HDX-MS**. *Protein Sci* 2024, **33**:e5024.

Reports a high-resolution local HDX-MS map of hydrogen-deuterium exchange, indicative of backbone dynamics and/or solvent exposure, for PTP1B. Apo exchange correlates with variability among many crystal structures, albeit with some outlier regions that are more dynamic in solution. Active-site vs. allosteric-site small-molecule inhibitors have distinct and surprisingly widespread effects on exchange across the catalytic domain. This work provides a unique window into the conformational dynamics of the archetypal PTP.

13.* Perdikari A, Woods VA, Ebrahim A, Lawler K, Bounds R, Singh NI, Mehlman TS, Riley BT, Sharma S, Morris JW, et al.: **Structures of human PTP1B variants reveal allosteric sites to target for weight loss therapy**. *bioRxiv* 2024, doi:10.1101/2024.08.05.603709.

Identifies human genetic variants of PTP1B associated with persistent thinness, then characterizes their effects on catalytic activity in cells and in vitro, and on conformational heterogeneity and/or dynamics in crystals via room-temperature crystallography and in solution via local HDX-MS. The results together suggest a previously unreported allosteric network that includes highly ligandable surface sites. This work illustrates how combining human genetics and structural biophysics can pave the way for downstream drug development, e.g. obesity therapeutics.

14. Krishnan N, Konidaris KF, Gasser G, Tonks NK: **A potent, selective, and orally bioavailable inhibitor of the protein-tyrosine phosphatase PTP1B improves insulin and leptin signaling in animal models**. *J Biol Chem* 2018, **293**:1517–1525.

15. Anselmi M, Hub JS: **Atomistic ensemble of active SHP2 phosphatase**. *Commun Biol* 2023, **6**:1289.

16. Singh JP, Lin M-J, Hsu S-F, Peti W, Lee C-C, Meng T-C: **Crystal structure of TCPTP unravels an allosteric regulatory role of helix α7 in phosphatase activity**. *Biochemistry* 2021, **60**:3856–3867.

17. Baumgartner CK, Ebrahimi-Nik H, Iracheta-Vellve A, Hamel KM, Olander KE, Davis TGR, McGuire KA, Halvorsen GT, Avila OI, Patel CH, et al.: **The PTPN2/PTPN1 inhibitor ABBV-CLS-484 unleashes potent anti-tumour immunity**. *Nature* 2023, **622**:850–862.

18. Sharma S, Ebrahim A, Keedy DA: **Room-temperature serial synchrotron crystallography of the human phosphatase PTP1B**. *Acta Crystallogr F Struct Biol Commun* 2023, **79**:23–30.

19.** Guerrero L, Ebrahim A, Riley BT, Kim M, Huang Q, Finke AD, Keedy DA: **Pushed to extremes: distinct effects of high temperature versus pressure on the structure of STEP**. *Commun Biol* 2024, **7**:59.

Compares the effects of complementary physical perturbations, temperature and pressure, in X-ray crystallography for STEP, revealing distinct effects on solvation and protein conformations, including

unique active-site interactions at high temperature and a unique active-site loop conformation at high pressure. Dimensionality reduction shows that different crystallographic perturbations stabilize distinct active-like vs. unique conformations. This study highlights new methods to access and visualize minor protein conformational states that may pertain to biological function.

20. Guerrero L, Ebrahim A, Riley BT, Kim SH, Bishop AC, Wu J, Han YN, Tautz L, Keedy DA: **Three STEPs forward: A trio of unexpected structures of PTPN5**. *bioRxiv* 2024, doi:10.1101/2024.11.20.624168.

21. Hjortness MK, Riccardi L, Hongdusit A, Zwart PH, Sankaran B, De Vivo M, Fox JM: **Evolutionarily conserved allosteric communication in protein tyrosine phosphatases**. *Biochemistry* 2018, **57**:6443–6451.

22. van Vlimmeren AE, Voleti R, Chartier CA, Jiang Z, Karandur D, Humphries PA, Lo W-L, Shah NH: **The pathogenic T42A mutation in SHP2 rewires the interaction specificity of its N-terminal regulatory domain**. *Proc Natl Acad Sci U S A* 2024, **121**:e2407159121.

23.* Welsh CL, Madan LK: **Allostery in protein tyrosine phosphatases is enabled by divergent dynamics**. *J Chem Inf Model* 2024, **64**:1331–1346.

Uses MD simulations and a variety of downstream analysis algorithms to dissect how differences in correlated structural dynamics underlie differences in catalytic rate for three PTPs with widely varying activities. This research highlights that variations in loop flexibility and structural rearrangements between different PTPs enable the modulation of their catalytic activity, and suggests that targeting these dynamic regions could offer novel strategies for regulating PTP function in therapeutic applications.

24. Jiang Z, van Vlimmeren AE, Karandur D, Semmelman A, Shah NH: **Deep mutational scanning of a multi-domain signaling protein reveals mechanisms of regulation and pathogenicity**. *bioRxiv* 2024, doi:10.1101/2024.05.13.593907.

25. Yeh CY, Izaguirre JA, Greisman JB, Willmore L, Maragakis P, Shaw DE: **A conserved local structural motif controls the kinetics of PTP1B catalysis**. *J Chem Inf Model* 2023, **63**:4115–4124.

26. Zinovjev K, Guénon P, Ramos-Guzmán CA, Ruiz-Pernía JJ, Laage D, Tuñón I: **Activation and friction in enzymatic loop opening and closing dynamics**. *Nat Commun* 2024, **15**:2490.

27.* Greisman JB, Willmore L, Yeh CY, Giordanetto F, Shahamadtar S, Nisonoff H, Maragakis P, Shaw DE: **Discovery and validation of the binding poses of allosteric fragment hits to protein tyrosine phosphatase 1b: From molecular dynamics simulations to X-ray crystallography**. *J Chem Inf Model* 2023, **63**:2644–2650.

Employs long-timescale and parallel MD simulations to elucidate the structural and sequence determinants of WPD loop opening/closing motions in PTP1B. Machine learning analyses of the trajectories reveal the PDFG motif, overlapping the signature WPD sequence, whose torsion angles dictate the WPD loop state. This motif recurs in other dynamic enzymes, such as the DFG motif in kinases, suggesting convergent evolution of a mechanism for structural switches.

28. Friedman AJ, Liechty ET, Kramer L, Sarkar A, Fox JM, Shirts MR: **Allosteric Inhibition of PTP1B by a Nonpolar Terpenoid**. *J Phys Chem B* 2022, **126**:8427–8438.

29. Crean RM, Biler M, van der Kamp MW, Hengge AC, Kamerlin SCL: **Loop dynamics and enzyme catalysis in protein tyrosine phosphatases**. *J Am Chem Soc* 2021, **143**:3830–3845.

30. Critton DA, Tautz L, Page R: **Visualizing active-site dynamics in single crystals of HePTP: opening of the WPD loop involves coordinated movement of the E loop**. *J Mol Biol* 2011,

405:619–629.

31. Yabukarski F, Biel JT, Pinney MM, Doukov T, Powers AS, Fraser JS, Herschlag D: **Assessment of enzyme active site positioning and tests of catalytic mechanisms through X-ray-derived conformational ensembles**. *Proc Natl Acad Sci U S A* 2020, **117**:33204–33215.

32. Saeed AA, Klureza MA, Hekstra DR: **Mapping protein conformational landscapes from crystallographic drug fragment screens**. *J Chem Inf Model* 2024, **64**:8937–8951.

33.** Singh JP, Li Y, Chen Y-Y, Hsu S-TD, Page R, Peti W, Meng T-C: **The catalytic activity of TCPTP is auto-regulated by its intrinsically disordered tail and activated by Integrin alpha-1**. *Nat Commun* 2022, **13**:94.

Uses a variety of biochemical and biophysical experiments to demonstrate that the intrinsically disordered C-terminal tail of TCPTP physically interacts with the enzyme's catalytic domain to downregulate catalytic activity; this autoinhibition is relieved upon binding of another regulatory protein, integrin-α1. This work demonstrates how the disordered regions of two closely related PTPs, TCPTP and PTP1B, function in dramatically different ways, illuminating the opportunities for evolutionary divergence of regulatory mechanisms among the PTP family in spite of apparent similarities.

34. Kim SH, Bulos ML, Adams JA, Yun BK, Bishop AC: **Single ion pair is essential for stabilizing SHP2's open conformation**. *Biochemistry* 2024, **63**:273–281.

35. Wen Y, Yang S, Wakabayashi K, Svensson MND, Stanford SM, Santelli E, Bottini N: **RPTPα phosphatase activity is allosterically regulated by the membrane-distal catalytic domain**. *J Biol Chem* 2020, **295**:4923–4936.

36. Yarnall MTN, Kim SH, Korntner S, Bishop AC: **Destabilization of the SHP2 and SHP1 protein tyrosine phosphatase domains by a non-conserved "backdoor" cysteine**. *Biochem Biophys Rep* 2022, **32**:101370.

37. Londhe AD, Bergeron A, Curley SM, Zhang F, Rivera KD, Kannan A, Coulis G, Rizvi SHM, Kim SJ, Pappin DJ, et al.: **Regulation of PTP1B activation through disruption of redox-complex formation**. *Nat Chem Biol* 2020, **16**:122–125.

38. Hongdusit A, Zwart PH, Sankaran B, Fox JM: **Minimally disruptive optical control of protein tyrosine phosphatase 1B**. *Nat Commun* 2020, **11**:788.

39. Hongdusit A, Liechty ET, Fox JM: **Analysis of Three Architectures for Controlling PTP1B with Light**. *ACS Synth Biol* 2022, **11**:61–68.

40. Shen R, Crean RM, Olsen KJ, Corbella M, Calixto AR, Richan T, Brandão TAS, Berry RD, Tolman A, Loria JP, et al.: **Insights into the importance of WPD-loop sequence for activity and structure in protein tyrosine phosphatases**. *Chem Sci* 2022, **13**:13524–13540.

41. Shen R, Brownless A-LR, Alansson N, Corbella M, Kamerlin SCL, Hengge AC: **SHP-1 variants broaden the understanding of pH-dependent activities in protein tyrosine phosphatases**. *JACS Au* 2024, **4**:2874–2885.

42. Anselmi M, Hub JS: **An allosteric interaction controls the activation mechanism of SHP2 tyrosine phosphatase**. *Sci Rep* 2020, **10**:18530.

43. Kim JY, Plaman BA, Bishop AC: **Targeting a pathogenic cysteine mutation: Discovery of a specific inhibitor of Y279C SHP2**. *Biochemistry* 2020, **59**:3498–3507.

44. Pádua RAP, Sun Y, Marko I, Pitsawong W, Stiller JB, Otten R, Kern D: **Mechanism of activating**

mutations and allosteric drug inhibition of the phosphatase SHP2. *Nat Commun* 2018, **9**:4507.

45. Shen R, Crean RM, Johnson SJ, Kamerlin SCL, Hengge AC: **Single residue on the WPD-loop affects the pH dependency of catalysis in protein tyrosine phosphatases**. *JACS Au* 2021, **1**:646–659.

46. Crean RM, Corbella M, Calixto AR, Hengge AC, Kamerlin SCL: **Sequence - dynamics - function relationships in protein tyrosine phosphatases**. *QRB Discov* 2024, **5**:e4.

47. Keedy DA, Hill ZB, Biel JT, Kang E, Rettenmaier TJ, Brandão-Neto J, Pearce NM, von Delft F, Wells JA, Fraser JS: **An expanded allosteric network in PTP1B by multitemperature crystallography, fragment screening, and covalent tethering**. *Elife* 2018, **7**:e36307.

48. Ginn HM: **Pre-clustering data sets using cluster4x improves the signal-to-noise ratio of high-throughput crystallography drug-screening analysis**. *Acta Crystallogr D Struct Biol* 2020, **76**:1134–1144.

49. Mehlman T, Ginn HM, Keedy DA: **An expanded trove of fragment-bound structures for the allosteric enzyme PTP1B from computational reanalysis of large-scale crystallographic data**. *Structure* 2024, **32**:1231–1238.e4.

50.* Skaist Mehlman T, Biel JT, Azeem SM, Nelson ER, Hossain S, Dunnett L, Paterson NG, Douangamath A, Talon R, Axford D, et al.: **Room-temperature crystallography reveals altered binding of small-molecule fragments to PTP1B**. *Elife* 2023, **12**:e84632.

Uses room-temperature crystallography to analyze how dozens of small-molecule fragments bind to PTP1B, revealing altered binding compared to traditional low-temperature methods. Distinct observations at room temperature include novel long-range allosteric protein conformational responses to ligand binding that were absent at cryogenic temperature. This study illustrates how non-standard approaches to crystallography can provide unique insights into accessible conformational states and allosteric mechanisms, and also provides promising new footholds for development of allosteric modulators for PTP1B.

51. Lambert LJ, Grotegut S, Celeridad M, Gosalia P, Backer LJ, Bobkov AA, Salaniwal S, Chung TD, Zeng FY, Pass I, et al.: **Development of a Robust High-Throughput Screening Platform for Inhibitors of the Striatal-Enriched Tyrosine Phosphatase (STEP)**. *Int J Mol Sci* 2021, **22**:4417.

52. Chirgadze YN, Battaile KP, Likhachev IV, Balabaev NK, Gordon RD, Romanov V, Lin A, Karisch R, Lam R, Ruzanov M, et al.: **Signal transfer in human protein tyrosine phosphatase PTP1B from allosteric inhibitor P00058**. *J Biomol Struct Dyn* 2022, **40**:13823–13832.

53. 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.

54. Wu J, Baranowski MR, Aleshin AE, Isiorho EA, Lambert LJ, De Backer LJS, Han YN, Das R, Sheffler DJ, Bobkov AA, et al.: **Fragment screening identifies novel allosteric binders and binding sites in the VHR (DUSP3) phosphatase**. *ACS Omega* 2025, **10**:4912–4926.

55. Pearson AR, Mehrabi P: **Serial synchrotron crystallography for time-resolved structural biology**. *Curr Opin Struct Biol* 2020, **65**:168–174.

56. Brändén G, Neutze R: **Advances and challenges in time-resolved macromolecular crystallography**. *Science* 2021, **373**:eaba0954.

57. Wolff AM, Nango E, Young ID, Brewster AS, Kubo M, Nomura T, Sugahara M, Owada S, Barad BA, Ito K, et al.: **Mapping protein dynamics at high spatial resolution with temperature-jump X-ray**

crystallography. *Nat Chem* 2023, **15**:1549–1558.

58. Tang WS, Zhong ED, Hanson SM, Thiede EH, Cossio P: **Conformational heterogeneity and probability distributions from single-particle cryo-electron microscopy**. *Curr Opin Struct Biol* 2023, **81**:102626.

59. Ung PM-U, Rahman R, Schlessinger A: **Redefining the protein kinase conformational space with machine learning**. *Cell Chem Biol* 2018, **25**:916–924.e2.

60. Modi V, Dunbrack RL Jr: **Defining a new nomenclature for the structures of active and inactive kinases**. *Proc Natl Acad Sci U S A* 2019, **116**:6818–6827.

61. Ginn HM: **Torsion angles to map and visualize the conformational space of a protein**. *Protein Sci* 2023, **32**:e4608.

62. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A, et al.: **Highly accurate protein structure prediction with AlphaFold**. *Nature* 2021, **596**:583–589.

63. Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, Ronneberger O, Willmore L, Ballard AJ, Bambrick J, et al.: **Accurate structure prediction of biomolecular interactions with AlphaFold 3**. *Nature* 2024, **630**:493–500.

64. Del Alamo D, Sala D, Mchaourab HS, Meiler J: **Sampling alternative conformational states of transporters and receptors with AlphaFold2**. *Elife* 2022, **11**:e75751.

65. Wayment-Steele HK, Ojoawo A, Otten R, Apitz JM, Pitsawong W, Hömberger M, Ovchinnikov S, Colwell L, Kern D: **Predicting multiple conformations via sequence clustering and AlphaFold2**. *Nature* 2024, **625**:832–839.

66. Wankowicz SA, Ravikumar A, Sharma S, Riley B, Raju A, Hogan DW, Flowers J, van den Bedem H, Keedy DA, Fraser JS: **Automated multiconformer model building for X-ray crystallography and cryo-EM**. *Elife* 2024, **12**:RP90606.

67. Raju A, Sharma S, Riley BT, Djuraev S, Tan Y, Kim M, Mahmud T, Keedy DA: **Mapping allosteric rewiring in related protein structures from collections of crystallographic multiconformer models**. *bioRxiv* 2025, doi:10.1101/2025.05.23.655529.

68. Lane TJ: **Protein structure prediction has reached the single-structure frontier**. *Nat Methods* 2023, **20**:170–173.

69. Pedersen AK, Peters G GÜH, Møller KB, Iversen LF, Kastrup JS: **Water-molecule network and active-site flexibility of apo protein tyrosine phosphatase 1B**. *Acta Crystallogr D Biol Crystallogr* 2004, **60**:1527–1534.

70. Wiesmann C, Barr KJ, Kung J, Zhu J, Erlanson DA, Shen W, Fahr BJ, Zhong M, Taylor L, Randal M, et al.: **Allosteric inhibition of protein tyrosine phosphatase 1B**. *Nat Struct Mol Biol* 2004, **11**:730–737.

71. Chen Y-NP, LaMarche MJ, Chan HM, Fekkes P, Garcia-Fortanet J, Acker MG, Antonakos B, Chen CH-T, Chen Z, Cooke VG, et al.: **Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases**. *Nature* 2016, **535**:148–152.

72. Tautermann CS, Binder F, Büttner FH, Eickmeier C, Fiegen D, Gross U, Grundl MA, Heilker R, Hobson S, Hoerer S, et al.: **Allosteric activation of striatal-enriched protein tyrosine phosphatase (STEP, PTPN5) by a fragment-like molecule**. *J Med Chem* 2019, **62**:306–316.