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Regulatory Mechanisms and Novel Therapeutic Targeting Strategies for Protein Tyrosine Phosphatases

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Abstract

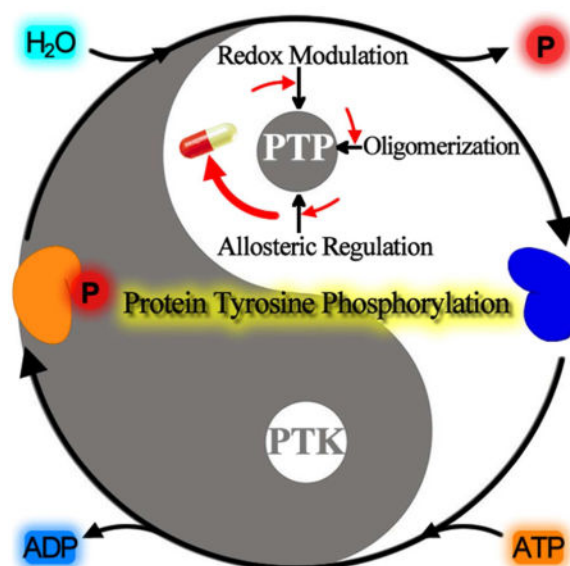
Appropriate level of protein phosphorylation on tyrosine is essential for cells to react to extracellular stimuli and keep cellular homeostasis. Faulty operation of signal pathways mediated by protein tyrosine phosphorylation causes numerous human diseases, which presents enormous opportunities for therapeutic interventions. While the importance of protein tyrosine kinases in orchestrating the tyrosine phosphorylation networks and in target-based drug discovery has long been recognized, the significance of protein tyrosine phosphatases (PTPs) in cellular signaling and disease biology has historically been underappreciated, due to a large extent an erroneous assumption that they are largely constitutive and housekeeping enzymes. Here, we provide a comprehensive examination on a number of regulatory mechanisms including redox modulation, allosteric regulation, and protein oligomerization, in controlling PTP activity. These regulatory mechanisms are integral to the myriad PTP-mediated biochemical events and reinforce the concept that PTPs are indispensable and specific modulators of cellular signaling. We also discuss how disruption of these PTP regulatory mechanisms can cause human diseases and how these diverse regulatory mechanisms can be exploited for novel therapeutic development.

Graphical Abstract

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Notes

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1. Introduction

The phosphorylation of protein represents a major post-translational modification that serves an essential role in controlling protein function.¹ Latest estimations based on phosphoproteomic analyses suggest that under the appropriate conditions the bulk of cellular proteins are phosphorylated at one or more amino acid residues.² The level of protein phosphorylation is determined by the opposing actions of protein kinases and protein phosphatases, which catalyze the addition or removal of the phosphate to or from proteins. Variation in the extent of protein phosphorylation brings about changes in protein activity, protein localization, protein–protein interaction, and protein stability, enabling cells to respond precisely to alterations in their environment and control almost all cellular processes. Not surprisingly, aberrant protein phosphorylation is known to disrupt key cellular pathways and is a well-established feature of many human diseases. In the human genome there are greater than 500 protein kinases, of which approximately 90 are Tyr specific and the remaining kinases prefer either Ser or Thr residues.³ All protein kinases share a conserved three-dimensional structure for the kinase domain and catalyze the phosphoryl transfer reaction with the same chemical mechanism. Interestingly, nature has evolved multiple strategies for protein dephosphorylation, utilizing distinct families of protein phosphatases that employ different catalytic mechanisms.

The protein tyrosine phosphatase (PTP) superfamily has 103 enzymes⁴ that catalyze substrate dephosphorylation through a covalent enzyme intermediate, involving a thiophosphate linkage from the active site cysteine residue.⁵ Strikingly, the human genome encodes a relatively small number of catalytic subunits for the classical Ser/Thr protein phosphatases (15 for the PPP family and 16 for the PPM family), which hydrolyze phospho-substrates through a metal-dependent mechanism.^{6–7} The relatively smaller number of Ser/Thr catalytic subunits combined with their broad *in vitro* activity has led to the misconception that protein phosphatases lack substrate specificity. We now know that the

PPP family of Ser/Thr protein phosphatases function as obligatory oligomers inside the cell by associating with one or two regulatory subunits that modulate their substrate specificity, intracellular localization and overall activity of the holoenzymes.⁸ For example, PP1 typically functions as heterodimeric holoenzymes of one catalytic subunit and one regulatory subunit. With three different PP1 catalytic subunits and more than 200 PP1 regulatory subunits already reported, there exists a diverse array of functionally distinct PP1 holoenzymes inside the cell.⁹ Similarly, PP2A usually works as heterotrimeric holoenzymes containing a structural scaffold A subunit, a regulatory B subunit, and a catalytic C subunit. Potentially, more than 70 heterotrimeric PP2A complexes can be generated by combinatorial association of the two A subunits, dozens of B subunits and two C subunits.¹⁰ Unlike the PPP phosphatases, the PPM family members are generally believed to operate as monomers. Although PPP and PPM phosphatases share no sequence homology, they exhibit a similar three-dimensional structure in the phosphatase domain and utilize an analogous catalytic mechanism with a dinuclear (Fe^{3+} and Zn^{2+}) metal-activated nucleophilic water molecule for phosphate monoester hydrolysis.¹¹ Recent studies indicate that there are more than 40 novel protein phosphatases having the haloacid dehalogenase (HAD) fold.¹² Notable members of the HAD family protein phosphatases include Fcp and Scp, which remove phosphates from the C-terminal domain of RNA polymerase II,^{13–14} and the Eyes absent (Eya) family of transcriptional co-activators that are vital for cell proliferation and organ formation.^{15–17} Unlike other classes of protein phosphatases, the HAD phosphatase catalyzed dephosphorylation reaction proceeds through an aspartyl phosphoenzyme intermediate.¹⁸

As is apparent from the above discussion, the overall number of protein phosphatases in the human genome is comparable to that of protein kinases. Given the dynamic and reversible nature of protein phosphorylation, this may be a necessary prerequisite for the cell to fine-tune and limit the extent and duration of signaling events through the opposing activity of specific protein kinases and phosphatases. However, while the role of protein kinases in controlling protein phosphorylation has long been recognized, the importance of protein phosphatases in signaling has historically been underappreciated, due to a large extent an erroneous assumption that the corresponding protein phosphatase activity needed to modulate the protein phosphorylation status may largely be constitutive.^{6,19} This is particularly disappointing despite ample evidence documenting that protein phosphatases in their own right are specific and crucial regulators of signal transduction and function as essential partners with protein kinases to orchestrate cellular responses to a wide range of biological stimuli.^{20–21} For example, a large-scale RNA interference study²² identified a significant number of survival kinases, as expected (11% of the total), but also exposed a positive role for protein phosphatases in cell survival, with 32% of the total promoting survival. An additional 5% of the total were identified as ‘death phosphatases’ for their apparent roles as tumor suppressors. This study provides a powerful demonstration that regulation of protein phosphatase activity is a key point of control in maintaining cellular homeostasis. As an effort to counter the misguided belief that protein phosphatases are constitutive and housekeeping enzymes, we will focus this review on a number of regulatory mechanisms identified for the PTPs in controlling the pleiotropic roles that these enzymes play in cell signaling. We will also discuss how disruption of these PTP regulatory

mechanisms causes human diseases and how these diverse regulatory mechanisms can be exploited for novel therapeutic development.

2. Protein Tyrosine Phosphatases

2.1. Classification of PTP Family Members

Encoded by 103 genes in humans, the PTPs are composed of a large family of phosphatases with their structural diversity and functional complexity rival those of protein tyrosine kinases (PTKs).⁴ Together with the PTKs, the PTPs regulate a plethora of cellular activities, including proliferation and differentiation, survival, migration, metabolism, and the immune response.^{21,23} Not surprisingly, perturbed activities of either PTKs or PTPs often results in abnormal protein tyrosine phosphorylation, which has been associated with the etiology of various human diseases, including cancer, diabetes, and autoimmune dysfunctions.^{24–26} Accordingly, cellular processes coerced by dysregulated protein tyrosine phosphorylation present unique opportunities for targeted therapeutic modality.^{27–28} Drug discovery and development endeavors on the PTKs have already produced over two-dozen small molecule inhibitors and numerous antibodies that are used in the clinic today,²⁹ further substantiating the concept that dysfunctional signaling events regulated by protein tyrosine phosphorylation can be manipulated for novel medicine. Naturally, there is considerable interest in selectively modulating disease biology, through targeting of the PTPs, for novel therapeutic applications.^{30–33}

The main structural feature that characterizes the PTPs is the active site sequence (I/V)HCXAGXGR(S/T), also named the P-loop, housed within a conserved catalytic domain.⁵ Amino acid sequence examinations and substrate specificity studies suggest that the PTPs may be classified into: 1) the classical pTyr specific PTPs, 2) the dual specificity phosphatases (DSPs), 3) the Cdc25 phosphatases, and 4) the low molecular weight (LMW) PTPs (Figure 1). The pTyr specific PTP subfamily comprises 38 members, which may be further categorized into intracellular (17) and receptor-like (21) PTP enzymes (Figure 1). The intracellular PTPs, represented by PTP1B and SHP2, harbor a PTP domain and a variety of amino or carboxyl terminal extensions with either targeting or regulatory properties.^{34–36} The receptor-like PTPs, typified by CD45³⁷ and PTP α ,³⁸ normally possess an extracellular ligand-binding segment, followed by a transmembrane region, and one or two cytoplasmic PTP domains. The DSPs consist of 61 members with diverse substrate specificity. Many of the dual specificity phosphatases are capable of dephosphorylating pSer/pThr as well as pTyr residues.³⁹ Interestingly, PTEN and Myotubularins are also able to remove 3 position phosphate from phosphatidylinositol 3, 4, 5-triphosphate (PIP3) and phosphatidylinositol 3, 4-bisphosphate (PIP2), respectively.^{40–41} Laforin, another member of the DSP subfamily, is a glycogen phosphatase.⁴² The Cdc25 phosphatases (Cdc25A, B, and C) are responsible for taking off the inhibitory phosphates from Thr14 and Tyr15 in cyclin-dependent kinases, which activates the kinases and drives cell cycle progression.^{43–44} With the exception of PTP signature motif, the Cdc25 phosphatases have no significant amino acid sequence similarity with the classical PTPs, DSPs, or LMW-PTPs. The PTP signature motif in Cdc25 is flanked by two CH2 domains (~25 residues) and the X-ray crystal structures of the Cdc25 phosphatase domain resembles rhodanese fold.⁴⁵ The LMW-PTP, encoded by *Acp1*, is an 18

kDa cytosolic enzyme found ubiquitously in both prokaryotic and eukaryotic organisms.⁴⁶ There is little sequence homology between LMW-PTP and other PTPs beyond the active site signature motif. Interestingly, the active site sequence in LMW-PTP is positioned near the N-terminus, whereas it is localized near the C-terminal end of catalytic domains in all other PTPs and DSPs.

2.2. Key Structural Features of PTPs

Despite variation in primary structure and difference in substrate specificity, structural studies reveal that the catalytic domains in pTyr specific PTPs and DSPs are evolutionarily related and share a similar tertiary fold consisting of several α -helices decorated on both sides of a central β -sheet (Figure 2A).^{47–49} Moreover, major structural features, such as the active site P-loop and the general acid/base containing WPD loop, that are important for substrate binding and catalysis are conserved among all members of the PTP superfamily.^{50–52} The PTP active site is situated within a ~ 9 Å deep cleft for pTyr specific PTPs and a ~ 6 Å deep cleft for the DSPs (Figure 2B). The P-loop sequence (I/V)HCXAGXGR(S/T) sits at the bottom of the active site crevice. The inward orientation of the P-loop amides creates a positive electrostatic potential to accommodate the negatively charged phosphoryl group in the substrate. The bound phosphoryl moiety is further stabilized by a α -helix dipole, positioned NH₂-terminal to the P-loop, and the active site Arg residue (Figure 2A). The PTP active site is formed by several surface loops, which contain residues important for substrate recognition and catalytic turnover. One of the surface loops (referred as the WPD loop in the pTyr specific PTPs, see Figure 2) holds an essential Asp residue (Asp181 in PTP1B), which is required for general acid/base catalysis.⁵³ This Asp is usually positioned 30–40 residues upstream of the PTP signature motif (I/V)HCXAGXGR(S/T) in both pTyr specific and dual specificity phosphatases. One key structural feature for the classical pTyr specific PTPs is that substrate binding stimulates a pronounced conformational change of the WPD loop, which brings Asp181 into close proximity to the phenolic oxygen of the pTyr substrate in order to render general acid catalysis.^{48,54} A second loop ($\alpha 1$ – $\beta 1$ loop), called the pTyr recognition loop, contains residues that engage in specific interactions with the peptide backbone and pTyr in the substrate.^{54–56} Together with the P-loop, WPD-loop, and $\alpha 5$ – $\alpha 6$ loop (also called Q-loop, Figure 2), the $\alpha 1$ – $\beta 1$ pTyr recognition loop surrounds the active site and defines a 9 Å deep pTyr-binding pocket (Figure 2B), which may provide the structural basis for the classical PTP's specificity for pTyr.^{54,57} DSPs lack the corresponding $\alpha 1$ – $\beta 1$ loop, resulting in an approximately 3 Å shallower active site, which may explain why DSPs can dephosphorylate both pTyr and pSer/pThr.^{49,58}

2.3. PTP Catalytic Mechanism

Extensive mechanistic and biochemical investigations have demonstrated that all PTPs employ the same mechanism for catalysis.⁵ The PTP catalyzed substrate dephosphorylation proceeds via a two-step chemical mechanism (Figure 3) involving a covalent thiophosphoryl enzyme intermediate (E-P), which results from the nucleophilic attack on the phosphorus atom in the substrate by the side chain of the active site Cys residue (Cys215 in PTP1B).^{59–61} In the first chemical step, the formation of the phosphoenzyme intermediate E-P is aided by the general acid (Asp181 in PTP1B) to defuse the build-up of a negative charge on the ester oxygen of the leaving group.^{53,62} In the second chemical step, E-P hydrolysis is

initiated by the general base Asp181 mediated nucleophilic attack by a water molecule, regenerating the free enzyme and yielding inorganic phosphate. The EP formation and hydrolysis steps are further catalyzed by the PTPs through preferential stabilization of the transition states with the guanidinium group of active site Arg (Arg221 in PTP1B).^{63–65} Finally, kinetic and structural studies suggest that the conserved Gln in the Q-loop (Gln262 in PTP1B) is important for the precise positioning of both the general base Asp181 and the water nucleophile which are essential for optimal E-P hydrolysis.^{66–67} The overall catalytic mechanism of substrate dephosphorylation is shared by the PTPs, DSPs and the more distantly-related Cdc25 phosphatases and LMW-PTP.^{5,68}

3. PTP Regulatory Mechanisms

To ensure proper level of protein tyrosine phosphorylation for cellular signaling, PTP activity is carefully organized through a number of regulatory mechanisms, including differential gene expression, confined subcellular localization, ligand binding, limited proteolysis, and post-translational modifications.⁶⁹ PTPs can also be regulated through alterations in subcellular location, as exemplified by the observation that Cdc25C phosphatase can move between the cytoplasm and the nucleus, which appears to be an important regulatory mechanism.⁷⁰ Here, we focus on additional regulatory mechanisms employed by the cell to modulate the intrinsic activity of PTPs. These include control of PTP activity by reversible active site Cys oxidation, allosteric mechanisms, and protein oligomerization. We will also discuss how inappropriate operation of these regulatory mechanisms may lead to pathological states and how they may be exploited for therapeutic purposes.

3.1. Redox Regulation

An increasingly appreciated mechanism of PTP regulation is reversible oxidation of the active site Cys through stimulus-mediated production of reactive oxygen species (ROS). Although initially regarded as toxic byproducts, controlled ROS production such as hydrogen peroxide (H_2O_2) is widely accepted as an important regulatory mechanism for cellular signaling processes.^{71–72} It has been demonstrated that engagement of cell surface receptors by ligands as diverse as insulin,⁷³ EGF,⁷⁴ PDGF⁷⁵ or TGF- β ⁷⁶ induces rapid production of intracellular H_2O_2 . Production of H_2O_2 requires NADPH oxidases in a regulated manner to govern cellular responses to a wide range of physiological stimuli (Figure 4).⁷⁷ Importantly, it has been shown that intracellular H_2O_2 production is also required for growth factor-induced activation of receptor PTKs and tyrosine phosphorylation dependent cellular signaling. Evidence suggest that H_2O_2 can influence tyrosine phosphorylation-mediated cellular processes through transient and reversible oxidation of the PTP active site Cys (Figure 4).^{78–82} Indeed, reversible oxidation of the active site Cys thiol group by reactive oxygen species has been established as a viable mechanism of PTP regulation in a number of physiological contexts.^{83–86} Both cytosolic and receptor-like PTPs are known to subject to ROS mediated inactivation.

As discussed above, an important feature for the PTP reaction is the involvement of the active site Cys for nucleophilic catalysis (Figure 3). Given the positively charged

electrostatic potential in the PTP active site pocket, the pKa of the active site sulfhydryl group is ~ 5 ,⁸⁷ which is substantially lower than a normal pKa for a typical Cys side chain in proteins (~ 8.5). Consequently, the sulfhydryl group within the PTP active site Cys must exist as a thiolate anion at physiological conditions. This not only increases the active site thiolate's nucleophilicity but also makes it more susceptible to oxidation. Indeed, biochemical experiments show that the catalytic Cys can be converted to sulfenic acid (Cys-SOH) in the presence of H_2O_2 , which effectively quenches the PTP reaction since the oxidized Cys-SOH is not capable of performing nucleophilic catalysis in PTP reaction (Figure 4).^{78,80,88} This provides a mechanistic basis for why H_2O_2 production promotes cell surface receptor mediated tyrosine phosphorylation. The resulting sulfenic acid could be rapidly reduced back to thiolate through reaction with small molecule cellular thiols⁸⁹ or the thioredoxin system⁹⁰, leading to full restoration of PTP activity. Accordingly, oxidation of the PTP active site Cys by H_2O_2 is transient and reversible and constitutes a dynamic regulatory mechanism of PTP activity.

For some time now, H_2O_2 has been viewed as the major ROS mediating reversible sulfhydryl oxidation within the cell. To that end, most investigations on PTP redox mechanisms have been carried out with H_2O_2 . However it is important to recognize that, although H_2O_2 is able to oxidize the PTPs, it is not very reactive in chemical sense.⁹¹ Indeed, rate constants observed for H_2O_2 -mediated PTP inactivation fall in the range of $10\text{--}20\text{ M}^{-1}\text{s}^{-1}$,^{78,92-93} suggesting that PTP inactivation will be a rather slow process ($t_{1/2} > 10\text{ h}$) at the physiological concentrations of H_2O_2 ($0.1\text{--}1\text{ }\mu\text{M}$) thought to be existent upon growth factor stimulation.⁹⁴⁻⁹⁵ Strikingly, ROS-induced inactivation of PTP typically happens rapidly ($2\text{--}5\text{ min}$) inside the cell, which overlaps well with the temporary rise in H_2O_2 concentration upon exposure to growth factors.^{73,75,79,83} The disagreement between the kinetics for robust PTP inactivation observed during early cellular signaling events and the seemingly sluggish *in vitro* reactivity of H_2O_2 toward the PTPs may be reconciled if one assumes that H_2O_2 can undergo either spontaneous or enzymatic conversion to more reactive ROS capable of mediating rapid intracellular PTP inactivation (Figure 4).⁹⁴⁻⁹⁵ One recent study shows that the biological buffer bicarbonate/ CO_2 can potentiate H_2O_2 's ability to inactivate PTPs.⁹⁶ The proposed mechanism involves oxidation of the active site Cys by peroxyphosphate produced by the reaction of H_2O_2 with $\text{HCO}_3^-/\text{CO}_2$ (Figure 4). Peroxyphosphate (${}^2\text{-O}_3\text{POOH}$) was also found an exceptional strong inactivator of PTP1B with a $k_{\text{inact}}/K_{\text{I}}$ of $65,553\text{ M}^{-1}\text{ s}^{-1}$, which is approximately 7,000 times more potent than H_2O_2 .⁹⁷ Similar to H_2O_2 , PTP1B inactivation by peroxyphosphate is also active site directed, and can be easily reversed by incubating the modified PTP1B with dithiothreitol. Another study suggests that hydroxyl radical, likely produced from Fenton reaction (H_2O_2 reduction mediated by Fe(II) or Cu(I)), may also serve as a more physiologically relevant ROS for effecting PTP inactivation (Figure 4).⁹³ Indeed, hydroxyl radical was shown to inactivate PTPs through active site directed oxidation of the essential Cys to sulfenic acid, which can be reduced by small molecule thiol compounds. Importantly, it was shown that hydroxyl radical is kinetically more efficient than H_2O_2 to carry out oxidative inactivation of the PTPs. Thus, the second-order rate constants measured for the hydroxyl radical-induced PTP inactivation are at a minimum 2-3 orders of magnitude higher than those caused by H_2O_2 under the same reaction conditions. Hence at a physiologically

relevant concentration of 1 μM H_2O_2 , the half-life for the hydroxyl radical-induced PTP inactivation will be closer in time scale typically expected for cellular signaling, which takes place within 2–5 min upon growth factor stimulation. Other reactive oxygen species including superoxide anion ($\text{O}_2^{\cdot-}$)⁸⁰ and peroxidized lipids⁹⁸ have also been implicated as physiologically relevant oxidizing agents in PTP regulation (Figure 4). Collectively, these studies establish redox regulation of PTP activity as a new layer of control over protein tyrosine phosphorylation mediated cellular events. From a kinetic perspective, either peroxyphosphate or hydroxyl radical could serve as an endogenous hydrogen peroxide-derived redox regulator of cellular PTP activity under physiological or pathophysiological conditions. Further experiments are needed to conclusively identify the ROS responsible for the redox control of PTP activity *in vivo*.

3.2. Allosteric Mechanisms

3.2.1. MKP3 activation—A number of allosteric regulatory mechanisms have been reported for the control of PTP substrate specificity and phosphatase activity. A good case in point is how mitogen-activated protein kinase phosphatases (MKPs), which make a distinct sub-family of DSPs, recognize their substrates, the mitogen-activated protein (MAP) kinases. The MAP kinases are key players in relaying extracellular signals from cell membrane to the nucleus.^{99–101} There exist three main MAP kinase cascades: the ERK1/2 pathway which answers to stimuli that provoke cell proliferation and differentiation, as well as oncogenic transformation; the c-Jun N-terminal protein kinase (JNK) pathway; and the p38 pathway, both of which are stimulated in response to environmental stresses and cytokine mediated inflammations. While activation of protein kinases generally necessitates phosphorylation of a single amino acid within a structurally conserved activation loop,¹⁰² the MAP kinases are activated by phosphorylation of both the Thr and Tyr residues in the TxY motif (where x is Glu in ERK, Pro in JNK, and Gly in p38) within the activation loop.^{103–105} The MKPs are responsible for the inactivation of MAP kinases by removing the phosphate from both the pTyr and pThr in the MAP kinase activation loop. Earlier studies indicate that the MKPs exhibit distinct *in vivo* preferences for various MAP kinases as substrates.¹⁰⁶ The best studied member of the MKP family is MKP3 (also known as DUSP6), which is known for its absolute substrate specificity for ERK1/2 over all other MAP kinases.^{107–108} Interestingly, MKP3 displays extremely low phosphatase activity towards small aryl phosphate substrates (e.g., $k_{\text{cat}}/K_{\text{m}} = 2.0 \text{ M}^{-1}\text{s}^{-1}$ for *p*-nitrophenyl phosphate and pTyr)¹⁰⁹ and the bisphosphorylated peptide derived from the ERK2 activation loop (DHTGFLpTEpYVATR, $k_{\text{cat}}/K_{\text{m}} = 5.0 \text{ M}^{-1}\text{s}^{-1}$)¹¹⁰. Moreover, with the bisphosphorylated ERK2 activation loop-derived peptide as a substrate, MKP3 can only hydrolyze pTyr but not pThr. On the contrary, kinetic studies with the bisphosphorylated ERK2, the physiological substrate for MKP3, revealed that the $k_{\text{cat}}/K_{\text{m}}$ value for the MKP3-catalyzed dephosphorylation of ERK2/pTpY ($3.8 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$) is more than 10^6 -fold higher than those measured for the hydrolysis of *p*NPP or the ERK2-derived bisphosphorylated peptide.¹¹¹ Moreover, MKP3 can efficiently dephosphorylate both the pTyr and pThr in the activation loop of ERK2. In fact, the MKP3 catalyzed ERK2/pTpY dephosphorylation proceeds via an ordered, distributive mechanism in which MKP3 first associates with the bisphosphorylated ERK2/pTpY, removes the phosphate from the pTyr, and releases the monophosphorylated ERK2/pT, which then binds to a second MKP3

molecule for dephosphorylation, yielding the completely dephosphorylated ERK2. Given the vast difference in intrinsic reactivity (10^5 -fold) between pTyr and pThr, it is quite noteworthy that MKP3 can promote pThr and pTyr hydrolysis with the same efficiency.

A series of biochemical and structural experiments were carried out to delineate the biochemical basis for the extraordinary substrate specificity of MKP3 for pERK2. The fact that small molecule and peptide substrates are weakly dephosphorylated suggests that substantial engagement of specific interactions between MKP3 and its physiological substrate pERK2 must occur. Like other members of the MKP subfamily, the MKP3 structure is comprised of an N-terminal regulatory segment harboring a kinase interaction motif (KIM) and a highly conserved C-terminal phosphatase domain.¹⁰⁶ The crystal structure of the phosphatase domain of MKP3 revealed a distorted active site pocket that is incompatible for catalysis.^{58,112} Unlike other members of the PTP family, residues Cys293 and Arg299 within MKP3 active site are misaligned and the general acid Asp262 is found away from the catalytic site (Figure 5A). A critical clue came from the finding that ERK2 binding to MKP3 leads to a strong increase in MKP3 activity towards pNPP.¹¹³ Importantly, although the N-terminal domain of MKP3 is not needed for the MKP3-catalyzed pNPP reaction,¹¹⁰ it is required for ERK2 binding.¹¹⁴ To furnish biochemical understanding of the molecular basis for the ERK2 binding-induced MKP3 activation, detailed kinetic analyses of the MKP3-catalyzed aryl phosphate hydrolysis were conducted in the presence and absence of ERK2.^{109,115} Without ERK2, the hydrolysis of aryl phosphate substrates catalyzed by MKP3 shows little dependence on pH, viscosity, and leaving group properties. In the presence of ERK2, MKP3 exhibits increased phosphatase activity and higher affinity for oxyanions. Furthermore, general acid/base catalysis is restored for the MKP3 catalyzed reaction in the presence of ERK2. Taken together, the results indicate that, in the absence of ERK2, the rate-limiting step for the MKP3 catalyzed reaction probably corresponds to a substrate ERK2-induced conformational (allosteric) change in MKP3 that involves active site reorganization and closure of the general acid loop. ERK2 binding to MKP3 most likely serve to facilitate repositioning of key active site residues and accelerate WPD loop closure in MKP3 such that one of the chemical steps becomes rate-limiting. Binding of a nonphysiological substrate to MKP3 does not generate enough energy to trigger such a conformational reorganization within MKP3 for efficient catalysis. This rationalizes why MKP3 is unable to dephosphorylate pThr and displays weak activity toward the phosphopeptide derived from the ERK2 activation loop.

The studies described above indicate that conformational rearrangement within the MKP3 active site, induced by specific interactions between MKP3 and ERK2, is obligatory for MKP3 to attain full activity against pERK2. To define the structural basis for ERK2 recognition by MKP3 and ERK2 binding-induced MKP3 activation, systematic mutational and deletion analyses combined with X-ray crystallography and hydrogen-deuterium exchange mass spectrometry studies were executed. Results from mutational and biochemical experiments suggest that ERK2 recognition and ERK2-induced MKP3 activation involve multiple regions of MKP3.¹¹⁶ As expected, MKP3's kinase interaction motif (KIM; residues ⁶⁴RRLQKGNLPVR⁷⁴) is a major contributor to high affinity binding to ERK2. In addition to KIM, a unique sequence motif in the middle of MKP3 also plays a role in binding ERK2. However, these two structural elements in MKP3 are not

indispensable for the ERK2-induced MKP3 activation. A third binding site for ERK2 is found within the C-terminus of MKP3 (residues 348–381). Although C-terminus deletion or mutation of the putative ERK2 specific docking motif ³⁶⁴FTTP³⁶⁷ decrease MKP3's affinity for ERK2 by only a modest amount, this C-terminal region is absolutely required for ERK2 binding-induced MKP3 activation. Similar analyses revealed that MKP3 activation by ERK2 also engages two distinct binding sites in ERK2.¹¹⁷ One site encompasses the so called common docking (CD) site (Figure 5B) in MAP kinases, which is comprised of several negatively charged residues (e.g., Asp316 and Asp319 in ERK2) that may interact with the positively charged residues in KIM through electrostatic interactions.^{118–120} It was shown that the common docking site, which is comprised of ERK2 residues Glu-79, Tyr-126, Arg-133, Asp-160, Tyr-314, Asp-316, and Asp-319, plays an important role in high affinity binding to MKP3 but is not essential for ERK2-provoked MKP3 activation. It turns out that ERK2-mediated activation of MKP3 requires ERK2 residues Tyr-111, Thr-116, Leu-119, Lys-149, Arg-189, Trp-190, Glu-218, Arg-223, Lys-229, and His-230 in the kinase substrate-binding (SB) region, which is distal to the ERK2 common docking (CD) site (Figure 5B).¹¹⁷

The three-dimensional structure of ERK2 in complex with the KIM sequence from MKP3 revealed that the common docking site (or the KIM docking site), situated in a non-catalytic segment opposite to the kinase active site pocket, is formed by a highly acidic patch and a hydrophobic groove, which are ideally positioned to interact with both the basic and Phi(A)-X-Phi(B) residues in the KIM motif (Figure 5C).¹²¹ Hydrogen-deuterium exchange mass spectrometry experiments showed that the KIM sequence in MKP3 and an MKP3-specific peptide fragment (¹⁰¹NSSDWNE¹⁰⁷) associate with ERK2's common docking site defined by amino acid residues in L₁₆, L₅, β₇–β₈, and α_d-L₈-α_e, localized opposite to the kinase active site (Figure 5B). On top of this “tethering” effect, additional binding interactions between the ³⁶⁴FTTP³⁶⁷ motif in MKP3 and the ERK2 substrate-binding pocket, created by residues in the kinase activation lip, the P+1 site (β₉-α_f loop), L₁₃ (α_f-α_g loop), and the MAP kinase insert (L₁₄-α_{1L14}-α_{2L14}), are crucial for the ERK2-induced allosteric activation of MKP3 and formation of a productive enzyme-substrate complex by which the MKP3 active site is correctly juxtaposed to catalyze ERK2/pTpY dephosphorylation (Figure 5B).¹²² Collectively, the results demonstrate that specific interactions between the two proteins reside in structural elements that are remote from the active site of MKP3 and the ERK2 activation loop, through a bipartite recognition process. Accordingly, MKP3's intrinsic substrate specificity is coupled to the ability of the ERK2 substrate to bring about productive reorientation of the MKP3 active site, which affords a powerful strategy to ensure high fidelity in down-regulating ERK2 activity. More recent crystal structure and NMR studies provide additional evidence on how residues outside the canonical KIM motif in MKPs interact with specific MAP kinases and contribute further to MAP kinase selectivity and signaling pathway fidelity.^{123–124}

3.2.2 SHP2 molecular switch—The Src homology 2 (SH2)-domain containing PTP-2 (SHP2), encoded by the *Ptpn11* gene, functions as a positive mediator in signal transduction.^{125–127} Results from biochemical and genetic studies reveal that SHP2 operates upstream of Ras, which functions as an essential signaling node that mediates growth factor-induced cell

growth, migration, and survival.^{128–132} SHP2 is a ubiquitously expressed cytoplasmic PTP composed of two tandem SH2 domains at its NH₂-terminus (N-SH2 and C-SH2), a central PTP catalytic domain, and a C-terminal tail (Figure 6A). Numerous studies show that the phosphatase activity of SHP2 is necessary for full activation of the Ras- ERK1/2 pathway.^{125,133} Interestingly, SHP2 is an allosteric enzyme modulated by an elegant ‘molecular switch’ regulatory mechanism (Figure 6A).^{35,134} SHP2 is auto-inhibited in the basal state by an intramolecular interaction between its N-SH2 domain and the PTP domain, which blocks the phosphatase active site for substrate binding.^{125,135} Upon growth factor receptor activation, the SHP2 N-SH2 domain binds specific pTyr motifs in receptor-PTKs, or more commonly scaffold proteins, which localize SHP2 to the vicinity of its substrates and simultaneously weakens the N-SH2/PTP domain interaction, enabling the PTP domain to bind and dephosphorylate SHP2 substrates.^{136–137}

Structural analyses suggest that although the N-SH2 domain’s pTyr peptide-recognition site is distinct and remote from the binding interface between the N-SH2 and PTP domain, the two binding sites are linked via an allosteric switch associated with the two different N-SH2 domain conformations: an active (**A**) pTyr-containing peptide-bound state (Figure 6B&C, cyan) and an inactive (**I**) PTP domain bound state (Figure 6B&C, gray). In comparison with the crystal structures of the isolated N-SH2 domain, both with and without a bound pTyr peptide,^{138–139} the N-SH2 residues that interact with the SHP2 PTP domain (namely amino acids within β sheet β D’, β E, and β F, as well as helix α B) have experienced a concerted conformational movement of approximately 2 Å (Figure 6B). It appears that molecular motion of the α B helix in the PTP-binding site of N-SH2 can trigger a conformational change in the EF loop, which together with the adjacent BG loop makes the SH2 domain phosphopeptide binding site for residues C-terminal to the pTyr residue (Figure 6C). Consequently, the two N-SHP2 domain conformations have mutually exclusive properties. The **I** N-SHP2 domain conformation displays surface complementarity with the PTP catalytic site, but its phosphopeptide-binding site is distorted due to the shift of the EF loop towards the BG loop (Figure 6C). Conversely, in the phosphopeptide binding competent state (**A**) the PTP domain recognition surface (constituted by residues in β D’, β E, β F, and α B) is disrupted. This surface protrudes to and conflicts with PTP active site pocket at (**A**) state, thereby abolishing the N-SH2/PTP intramolecular interaction (Figure 6D). The allosteric conformation transition of the N-SH2 domain from the **I** to **A** state is regulated by pTyr-containing peptides derived from growth factor receptors or adapter proteins that bind to the N-SH2 domain. Thus binding of phosphopeptides to the N-SH2 domain promotes intramolecular “dissociation” of the N-SH2 domain from the PTP domain, shifting the SHP2 equilibrium to an open conformation in which the steric block of the phosphatase active site is relieved and SHP2 becomes catalytically active.

The importance of SHP2 in cell signaling is underlined by mutations in *Ptpn11*, which are linked to numerous human diseases. For example, germline mutations in *Ptpn11* are associated with 50% Noonan syndrome (NS), an autosomal dominant developmental disorder manifested with features such as short stature, facial dysmorphia, and cardiac defects.^{140–141} Germline mutations in *Ptpn11* are also found in 90% of LEOPARD syndrome (LS) cases.¹⁴² Both LS and NS are linked to heightened risk of malignancy.

^{141,143–144} In addition, somatic mutations in *Ptpn11* have been detected in patients with juvenile myelomonocytic leukemia (35%), acute myeloid leukemia (4%), myelodysplastic syndrome (10%), and acute lymphoid leukemia (7%).^{145–149} In addition to pediatric leukemia, *Ptpn11* somatic mutations are also detected in adult acute myeloid leukemia (6%) and in solid tumors ranging from lung adenocarcinoma, melanoma, neuroblastoma, hepatocellular carcinoma and colon cancer.^{150–151} SHP2 has also been implicated in gastric carcinoma inflicted by *Helicobacter pylori*, which contains a virulence factor CagA, which, upon tyrosine phosphorylation by host Src kinases, can provoke SHP2 activation.¹⁵² Finally, due to the universal requirement of SHP2 in multiple receptor tyrosine kinase-mediated signal pathways, blocking SHP2 phosphatase activity may serve as a novel approach for malignancies caused by aberrant activation of receptor tyrosine kinases, some of which respond poorly to tyrosine kinase targeted monotherapy.¹⁵³ Together, the existing biochemical and genetic evidence strongly identify SHP2 as the first bona fide oncoprotein in the PTP superfamily. There is increasing interest in therapeutic targeting of SHP2 for novel anti-cancer agents.³²

Most of the NS or neoplasia-associated *Ptpn11* mutations alter residues clustered at the N-SH2 and PTP domain binding interface (Figure 7A).^{150,154} These substitutions were thought to alter the allosteric mechanism of SHP2 to favor the activated state, resulting in gain-of-function properties.^{155–159} Indeed, studies have shown that these *Ptpn11* mutations induce leukemia through cell-autonomous mechanisms that depend on SHP2 phosphatase activity.^{160–163} In contrast, LS associated *Ptpn11* mutations have been found exclusively in the PTP phosphatase domain (Figure 7B). Recent experiments indicated that LS *Ptpn11* mutations manifest in significantly reduced SHP2 phosphatase activity and are therefore regarded as loss-of-function genetic defects.^{159,164–166} These biochemical and genetic observations created a conundrum: how can *Ptpn11* mutations with opposite effects on SHP2 phosphatase activity elicit similar overlapping phenotypes? The answer to this question came from the realization that disease-causing *Ptpn11* mutations modify not only the phosphatase activity of SHP2 but also its molecular switch regulatory mechanism that could dictate phenotypic outcome.^{167–168}

Detailed kinetic studies^{167–168} show that the full-length SHP2 phosphatase activity is less than 5% of the that of its catalytic domain, which is consistent with the finding from SHP2 crystal structures that SHP2 exists in an autoinhibited, closed conformation.³⁵ In line with the expectation that gain-of-function SHP2 mutants exist in an open, constitutively active state, full-length D61Y and E76K proteins have kinetic parameters indistinguishable to those of the catalytic domain of SHP2. By contrast, the phosphatase domains of LS SHP2 mutants display k_{cat} values that are 10- to 930-fold lower than that of the wild-type SHP2 counterpart, suggesting that LS mutants may be catalytically compromised. Importantly, the turnover numbers for full-length LS mutants are still 2 to 26-fold lower than those of their corresponding phosphatase domain alone.^{167–168} These results suggest that, similar to the wild-type SHP2, full-length LS mutants are also in a closed, autoinhibited conformation. Given the diverse nature of the SHP2 LS mutations, three-dimensional structures for all major LS mutants (Y279C, A461T, G464A, T468M, Q506P, and Q510E) were determined in an effort to fully delineate the molecular underpinnings for the disease.^{167–169} These crystal structures reveal why LS-causing SHP2 mutants have reduced phosphatase activity

(Figure 8). The structural observations indicate that catalytic impairments induced by the LS mutations range from decreased substrate-binding to misalignment of active site residues and diminished transition state stabilization. Interestingly, the structures also show that, like the wild-type enzyme, all LS mutants are found in an auto-inhibited, closed conformation, in agreement with the prediction based on results from the kinetic experiments. Importantly, although the LS mutants adopt a closed conformation, these structures also disclose that the LS mutations weaken the autoinhibitory structure between the interfaces of the N-SH2 and PTP domains. Additional biophysical and computational analyses suggest that in comparison to native SHP2, the LS mutants display a greater tendency to switch from an autoinhibited closed conformation to a catalytically competent open state.^{167–168}

Based on thermodynamic principles and the predicted negative cooperativity between the two ligand binding sites (for pTyr peptide and the PTP domain) in the N-SHP2 domain, it follows that diminished interaction between the N-SH2 domain and the PTP domain must necessarily lead to increased association between the N-SH2 domain and its pTyr peptide ligands. Indeed, LS mutants exhibit greater affinity for upstream pTyr peptide motifs and are preferentially (as compared to native SHP2) activated by growth factor receptors or scaffolding proteins (Yu et al., 2013; 2014). Moreover, SHP2-associated LS mutants indeed display enhanced binding affinity for Grb2-associated binder-1 (Gab1)¹⁷⁰ and stay longer on the scaffolding protein.^{167–168} Via its N-SH2 domain association with the pTyr-sequence motif in Gab1, SHP2 is directed to its physiological substrate(s) to drive Ras-ERK1/2 pathway activation.^{171–172} Accordingly, the LS mutants are more efficient in dephosphorylating Paxillin/pY118,^{167–168} a physiological substrate of SHP2 required for EGF-stimulated ERK1/2 activation.¹⁷³ SHP2/Gab1 binding and SHP2-mediated dephosphorylation of Paxillin/pY118 are two recognized signaling events shown to be important for EGF-induced ERK1/2 phosphorylation.^{171,173} Given the LS mutants' higher affinity for Gab1 and increased Paxillin/pY118 dephosphorylation by the LS SHP2 mutants, LS mutations are expected to engender a gain-of-function impact on ERK1/2 activation. Indeed, compared to cells expressing native SHP2, the ERK1/2 activity is more elevated and sustained after EGF stimulation in cells expressing the LS mutants.^{167–168} Furthermore, the biophysical outcome of the weakened association between the N-SH2 domain and the PTP phosphatase domain in the LS mutants is the augmented ability of the N-SH2 domain to bind the pTyr-peptide motifs in their physiological adaptor proteins under much lower concentrations of stimuli, thus effectively reducing the threshold for growth factor induced Ras/ERK1/2 pathway activation. As expected, the LS-associated SHP2 mutants induced ERK1/2 activation require considerably lower EGF concentration, which could confer significant developmental advantage under growth factor limiting conditions.^{167–168} Additional studies in *Drosophila* reveal that ubiquitous expression of SHP2/Y279C and SHP2/T468M, two of the most frequent LS-causing SHP2 alleles, leads to gain-of-function phenotypes due to heightened EGF mediated Ras-ERK1/2 pathway activation.¹⁷⁴ These gain-of-function phenotypes observed for the LS mutants are similar to those observed with transgenic flies harboring NS-associated SHP2 mutants.¹⁵⁸ Notably, experiments with SHP2/Y279C or SHP2/R468M transgenic fly suggest that LS mutant's residual phosphatase activity is required for the gain-of-function developmental phenotypes.¹⁷⁴ Expression of the most common LS-associated SHP2 mutants in embryos of zebrafish results in

hyperactivation of ERK1/2 signaling, which leads to defective early heart development.¹⁷⁵ Collectively, these structural and functional investigations provide strong evidence that catalytically deficient SHP2 mutants could still display *gain-of-function* phenotypes since they could preferentially engage upstream activator proteins with extended resident times, thus enabling prolonged substrate dephosphorylation by the mutant phosphatases and sustained activation of the Ras-ERK1/2 pathway.

3.3 Protein Oligomerization

3.3.1. Dimerization of transmembrane receptor-like PTPs—Protein dimerization forms the molecular basis for ligand-dependent catalytic activation of many receptor PTKs.¹⁷⁶ In addition to receptor PTKs, an emerging mechanism for controlling the activity of intracellular protein kinases is to switch between inactive and active states by allosteric coupling with kinase domain surface residues involved in homo- or hetero-dimerization.¹⁷⁷ Upon realization that resistance to Raf kinase-targeted therapeutics often stems from dimerization-dependent mechanisms,¹⁷⁸ there is huge interest in gaining a better understanding of dimerization-induced allostery in order to develop improved therapeutic strategies.¹⁷⁷ In the case of PTPs, protein oligomerization also represents an important regulatory mechanism, which was first recognized for transmembrane receptor-like PTPs. In addition to a ligand binding N-terminal domain situated outside the cell and a transmembrane segment, the majority of receptor PTPs also contains two cytoplasmic PTP domains. The PTP domain closer to the plasma membrane, named D1, harbors most, if not all, of the catalytic activity, while the plasma membrane distal second PTP domain, D2, is predominantly inactive but appears to be important for the function of the receptor PTP as a whole.^{179–180} In an analogous manner to receptor PTKs, the phosphatase activity of receptor PTPs can also be regulated by ligand-induced dimerization. A model linking ligand induced dimerization and receptor PTP phosphatase activity was suggested based on the three-dimensional structure of the membrane-proximal D1 domain of PTP α .¹⁸¹ In the X-ray crystal structure, the plasma membrane-proximal PTP α D1 domain forms a symmetrical dimer, in which a structurally restricted N-terminal helix-turn-helix ‘wedge’ from one monomer was found inserted into the active site pocket of the second (Figure 9). This observation led to the proposal that the phosphatase activity of receptor PTPs is inhibited in the dimeric state due to reciprocal occlusion of the respective active site pockets.¹⁸¹ Interestingly, the X-ray crystal structures of the tandem D1 and D2 domain constructs of LAR¹⁸² and CD45¹⁸³ showed no evidence of dimerization even though the wedge motif was also present in these structures.^{182–183} Moreover, the D1 and D2 domains were positioned in such a way that both of their active site pockets were accessible. Indeed, the steric hindrance manifested by the D2 domain precludes the wedge-mediated D1 domain dimerization. Nonetheless, these structural observations may not accurately reflect the situation inside the cell since the relative D1 and D2 orientation and the accessibility of D1 domain active site to the helix-turn-helix wedge motif could be influenced by conformational changes in other parts of the molecule upon binding of a ligand to the extracellular fragment of the receptor PTP. In fact, biochemical and cell-based analyses of PTP α revealed that multiple structural elements in the D2 domain, the extracellular segment, and the transmembrane region make contribution to dimer formation inside the cell.^{184–185}

Using reconstitution of TCR signaling¹⁸⁶ or rescue of Src family kinase activity¹⁸⁷ as readouts, it was shown that forced dimerization of mutant receptor PTPs results presents loss of function phenotypes. Moreover, alteration of residues in the wedge motif attenuated dimerization-induced inhibition, indicating that this motif is important for the inhibitory effect of receptor PTP dimerization.^{187–188} Results from measurements using fluorescence resonance energy transfer (FRET) microscopy and cross-linking of the receptor inside the cell suggest that dimerization of PTP α is controlled not only by the wedge motif in D1 but also by structural features in the transmembrane and ectodomains.^{184–185} Furthermore, the D2 domain is also shown to play a role in regulating receptor PTP dimerization.^{184,189} Also, there is ample data supporting that dimerization of receptor PTPs occur inside cells and that receptor PTP dimerization inhibits the phosphatase activity.^{190–193} A clear example of receptor PTP regulation by a physiological extracellular molecule is furnished by the observation pleiotrophin inhibits the intrinsic phosphatase activity of its receptor, PTPbeta/zeta.¹⁹⁴ Taken together, it appears that dimerization represents an important regulatory mechanism for receptor-like PTPs. Further studies will shed more light on the potential functional relevance of receptor PTP dimerization and of D1-wedge interactions.

3.3.2. Oligomerization of Intracellular PTPs—Recent studies suggest that protein oligomerization may also represent an emerging mechanism for the regulation of intracellular PTPs. The dual specificity phosphatase VH1 (Vaccinia virus H1 gene product) can attenuate host cell antiviral response by catalyzing STAT1 dephosphorylation. The VH1 crystal structure shows a novel dimeric arrangement (Figure 10A), with the two active sites positioned ~39 Å from each other.¹⁹⁵ It appears that VH1 dimer formation involves a domain swap of the N-terminal helix and that specific recognition of STAT1 by VH1 requires the dimeric structure, which prevents STAT1 nuclear translocation, thereby blocking INF γ -mediated antiviral responses.¹⁹⁶ The Vaccinia H1-related (VHR) phosphatase is a member of the DSP family shown to be important for the progression cell-cycle and tumorigenesis.¹⁹⁷ Functional analysis of the “variable insert” unique to VHR by incorporating para-benzoylphenylalanine, a photo-cross-linkable amino acid, led to the discovery that VHR can dimerize inside cells.¹⁹⁸ Further experiments indicate that VHR dimerization can lead to occlusion of its active site, blocking substrate accessibility. Evidence suggests that the glycogen phosphatase laforin can also form a dimer (Figure 10B), although the biological relevance of laforin dimerization is still the subject of active debates.^{199–202} The three-dimensional structure, along with complementary biophysical data, suggests that laforin can exist as an antiparallel dimer, which is mediated by residues in its phosphatase domain.²⁰³ Importantly, it was shown that the Lafora disease associated mutation F321S can interrupt dimerization of laforin and reduces its phosphatase activity toward glycogen. Myotubularin related protein 2 (MTMR2), a phosphoinositide lipid phosphatase, can also form as a homodimer (Figure 10C), which is important for its function inside the cell.²⁰⁴ Interestingly, while the laforin phosphatase domain contributes to dimer formation, the dimer interface for MTMR2 and VH1 phosphatases is formed via ancillary C-terminal coiled-coil domain and a swapped N-terminal α helix, respectively.^{196,204}

The importance of protein homodimerization in regulating PTP activity was further emphasized by the recent findings that homodimerization of PTEN is important for its

phosphatase activity toward the physiological substrate phosphatidylinositol 3, 4, 5-triphosphate.²⁰⁵ As a major tumor repressor, malfunction PTEN has been implicated in the pathogenesis of both hereditary and sporadic cancers.²⁰⁶ Strikingly, several cancer-associated catalytically impaired PTEN mutants were shown to form heterodimers with native PTEN and hamper its catalytic activity in a dominant-negative fashion.²⁰⁵ As a consequence, cells and tissues expressing heterozygous PTEN^(C124S/+) and PTEN^(G129E/+) mutants display increased sensitivity to PI3K/Akt pathway activation in comparison to native and PTEN^(+/-) heterozygous counterpart. In addition, catalytically inactive PTEN mutant knock-in mice are found to be more prone to tumor formation and exhibit phenotypes reminiscent of complete loss of PTEN. Further structural and biophysical studies reveal that PTEN can form homodimers in solution, which is stabilized by the C-terminal tail, and that C-terminal tail phosphorylation interferes with this stabilization.²⁰⁷ These results provide new insight into the cellular regulatory mechanism of PTEN phosphatase activity.

Finally, PRL (Phosphatase of Regenerating Liver) phosphatases provide another striking example where protein oligomerization may play a crucial role in regulating PTP function. PRLs constitute a novel class of C-terminal prenylated phosphatases within the PTP superfamily, with three closely related members (PRL1, 2 and 3, also referred to PTP4A1, PTP4A2, and PTP4A3).^{208–211} PRL1 was initially discovered as an immediate early gene induced upon partial hepatectomy.²¹² PRL2 and PRL3 were subsequently discovered by their sequence similarity to PRL1. Unlike many other PTPs that antagonize the action of protein kinases, the PRLs rank among the most oncogenic PTPs and function to promote activation of both ERK1/2^{213–217} and Akt^{218–221}, two of the key pathways that are abnormally up-regulated in cancer. PRLs are overexpressed in many cancer cell lines, and those with high level PRL expressions show increased proliferation, migration and anchorage-independent cell growth.^{212–213,222–225} Moreover, cells overexpressing PRLs form tumors in mice with high metastatic potential,^{215,226–228} whereas reduction in PRL expression decreases cell proliferation and migration as well as tumorigenesis *in vivo*.^{215,217,229–233} Importantly, PRL level is elevated in various human cancers where PRL overexpression is strongly correlated with late stage metastasis as well as poor clinical outcomes.^{224,230,234–241} Recent functional studies using engineered genetic mouse models indicate that deletion of PRL2 or PRL1 causes placental insufficiency, hematopoietic stem cell renewal, and spermatogenesis due to increased PTEN level and decreased Akt activity.^{220–221,242–243} The ability of PRLs to activate Akt and down-regulating the tumor repressor PTEN provides a biochemical basis for their oncogenic potential. Taken together, the current data implicate PRLs as novel molecular markers and therapeutic targets for metastatic cancers. Consequently, PRLs have garnered considerable interest for drug discovery.²¹¹

Early structural studies uncovered that PRL1 crystallizes as a homotrimer (Figure 11).^{92,244} The PRL1 homotrimer features a three-fold rotational symmetry with the C-terminal tail of each monomer facing the same side of the trimer (Figure 11A). Since the C-terminal tail of PRL contains a prenylation motif, it is most likely that the side of the trimer facing the plasma membrane is made up by the C-residues. Accordingly, the active site of PRL1 is pointed away from the trimer interface and opposite to the C-terminal plasma membrane binding region (Figure 11A&B). PRL1 trimer formation is mediated by both polar and

hydrophobic interactions. The sum of buried accessible surface area at the dimer interface is 1130–1150 Å² for each monomer, which account for ~29% of the whole surface area of each PRL1 monomer.⁹² In the PRL1 homotrimer structure, helix α_5 of one PRL1 monomer (amino acids 124–134) makes contacts with helix α_1 , the $\beta_1 - \beta_2$ hairpin, and the $\alpha_3 - \beta_5$ loop from another monomer (Figure 11C). Remarkably, Arg138 in helix α_5 inserts into the hydrophobic pocket formed by Lys39 and Tyr40 in helix α_1 , making a specific salt bridge with Glu36. On the other hand, Pro96 from the $\alpha_3 - \beta_5$ loop protrudes into a hydrophobic pocket bordered by residues Leu146 in helix α_6 , Val130 and Tyr126 in helix α_5 (Figure 11D). Additional interactions include six hydrogen bonds made by residues Asp128, Gln131, Arg134, and Gln135 with main chain amides or carbonyls in the $\alpha_3 - \beta_5$ loop, the $\beta_1 - \beta_2$ hairpin, and the side chain of Arg18. Since residues (Arg18, Glu36, Pro96, Met124, Tyr126, Asp128, Val130, Arg138, and Leu146) observed at the dimer interface are conserved among all PRLs, trimer formation is likely a general property for all PRL phosphatases.

Subsequent biochemical experiments established that all PRLs have the ability to form trimers in solution and inside the cells.^{92,214,216,244} The PRLs are known to be prenylated at CaaX motif in their C-termini,^{222,245–246} which serves to attach them to plasma membrane. This anchoring effect is expected to increase the local PRL concentration in cell membrane and significantly enhance the propensity of PRL trimerization. Thus PRL1 homotrimers observed in the crystal structures may have direct relevance to intact PRL1 inside the cell since its C-terminal prenylation motif sits on the membrane-facing surface.⁹² Consequently, PRL1 trimerization may operate as a novel regulatory mechanism for PRL function. Indeed, it has been shown that trimer formation is required for PRL-mediated cell proliferation and motility because disruption of PRL trimer formation by mutations of residues in the dimer interface results in a loss-of-function phenotype.^{214,247} Overexpression of PRLs, as observed in numerous cancer cell lines, is expected to escalate trimer formation. Thus it is possible that the increased proliferation and metastasis observed in cancer cells with elevated PRL expression is the consequence of increased PRL trimerization.

4. Therapeutic targeting of PTP regulatory mechanisms

4.1. Targeting the oxidized active site Cys

Selective oxidation of the active site thiolate in PTPs by ROS initially produces the sulfenic acid form (Cys-SOH), but high concentrations of oxidant can cause irreversible generation of sulfinic (Cys-SO₂H) or sulfonic acid (Cys-SO₃H) derivatives (Figure 4). To prevent irreversible oxidation, the sulfenic acid product can form covalent adduct with residues in close proximity of the catalytic Cys. For example, it has been shown that sulfenic acid is capable of reacting with a backbone amide to yield a cyclic sulfenyl amide^{88,248–249} or with an adjacent thiol to form an intramolecular disulfide^{92,250} (Figure 4). These secondary reaction adducts can be reversed by reduction, and serve as stabilizing mechanisms to shield the active site Cys from further oxidation. Interestingly, the ability of the reversibly oxidized active site thiol group to transform into a cyclic sulfenyl-amide intermediate presents an exciting opportunity to target the PTPs. Structural studies reveal that this sulfenyl-amide formation induces a large conformational change in the active site of PTP1B which transiently blocks substrate binding and catalysis.^{88,249} There is considerable interest in

targeting PTP1B for diabetes and obesity treatment, given that fact that PTP1B deficient mice are healthy, exhibit increased insulin sensitivity, and do not develop obesity when they are fed with high-fat diet.^{251–252} To demonstrate whether stabilization of the oxidized form could be a viable approach for therapeutic targeting of the PTPs, single-chain variable antibody fragments that specifically recognizes the cyclic sulfenamide form of PTP1B were generated against the active-site double mutant PTP1B-C215A/S216A, which adopts a conformation similar to the oxidized PTP1B.^{253–254} When expressed in mammalian cells, clone scFv45 immunoprecipitated oxidized PTP1B after H₂O₂ or insulin stimulation and colocalized with the oxidized PTP1B. Importantly, expression of scFv45, but not a non-targeting intrabody, promotes insulin-induced tyrosine phosphorylation of the insulin receptor, its substrate IRS1, and downstream Akt phosphorylation.²⁵³ These results provide the needed proof of principle that strategies to stabilize the oxidized, inactive PTP1B could present a new therapeutic approach to target PTP1B. To that end, a number of small molecule compounds, including sulfone-stabilized carbanions and 1,3-diketone derivatives (Figure 12), have been identified for covalent capture of the cyclic sulfenamide intermediate in oxidized PTP1B.^{255–256} These sulfone carbon acids could be used as building blocks for the construction of therapeutic agents that inhibit PTP1B phosphatase activity via transient trapping of the cyclic sulfenamide produced during growth factor signaling. In addition, the dimedone scaffold (Figure 12) when incorporated into an appropriate binding module has also been demonstrated to exhibit selectivity for the sulfenic acid generated in PTP active site.²⁵⁷ More recently, a novel immunochemical approach consisting of an antibody designed to recognize the conserved sequence of the PTP active site (VHCDMDSAG) harboring the catalytic cysteine modified with dimedone has been developed to directly profile oxidized PTPs.²⁵⁸ It is anticipated that future work in this area will lead to novel therapeutic molecules that can covalently capture the oxidized forms of PTPs generated inside the cell under disease conditions.

4.2. Targeting allosteric activation of MKP3

In order to develop an improved understanding of the roles of MKPs in health and diseases, potent and selective small molecule probes will be invaluable. Unfortunately the shallower active sites for the DSPs have evaded many traditional high throughput screening and medicinal chemistry efforts. A zebrafish based chemical screen led to the identification of a small molecule inhibitor for MKP3, named (E)-2-benzylidene-3-(cyclohexylamino)-2,3-dihydro-1H-inden-1-one (BCI) (Figure 12), which blocks MKP3 catalyzed dephosphorylation of a small aryl phosphate substrate in the presence of ERK1/2 and enhances the expression of fibroblast growth factor target genes in zebrafish embryos.²⁵⁹ Further structure and activity study and molecular modeling suggest that the BCI series of molecules inhibit MKP3 by binding the crevice located between helix α 7 and the general acid loop in MKP3, preventing the re-positioning of Asp262 to act as a general acid during catalysis.²⁶⁰ Treatment of patient-derived pre-B acute lymphoblastic leukemia cells with BCI resulted in ERK1/2 hyperphosphorylation and induced cell death, suggesting potential for targeting MKP3 in ALL indications.²⁶¹ These MKP3 inhibitors can be useful in helping reveal the role of MKP3 in normal physiology and in diseases.

4.3. Targeting the SHP2 molecular switch mechanism

The above discussion on SHP2 highlights the importance of the allosteric mechanism, namely the intramolecular switch between its N-SH2 domain and the PTP domain, not only in controlling SHP2-mediated signaling under normal physiological conditions but also in pathogenic settings where the molecular switching mechanism is perturbed. Recent progress in targeting SHP2 for drug discovery suggests that the unique allosteric mechanism may also be exploited to develop compounds that selectively inhibit SHP2 relative to other PTPs for therapeutic purposes.^{262–263} Utilizing the full-length SHP2 construct and screening for compounds that can block SHP2 activation by an N-SH2 domain binding pTyr peptide, the Novartis team identified several small molecule compounds that stabilize the auto-inhibited SHP2 conformation.²⁶³ Medicinal chemistry optimization afforded SHP099 (Figure 12)), which has a K_d of 70 nM for SHP2 and exhibits no significant activity against other PTPs. The three-dimensional structure of SHP2 complexed with SHP099 shows that the inhibitor binding to a tunnel-like region at the intersection of the C-SH2, N-SH2, and PTP domains (Figure 13A). In essence, SHP099 acts like “molecular glue” to lock SHP2 in the inactive conformation and prevents its enzymatic activation. In receptor PTK-driven cancer cells SHP099 was shown to suppress Ras–ERK1/2 signaling and attenuate cell proliferation.²⁶² SHP099 was also efficacious in mouse tumor xenograft models.²⁶² These results with the allosteric inhibitor SHP099 together with similar results generated with active site directed SHP2 inhibitors^{264–267} provide the proof-of-concept that pharmacological modulation of SHP2 activity represents a promising therapeutic intervention for cancer treatment.

4.4. Targeting receptor PTP dimerization

The potential impacts of receptor PTP dimerization on its phosphatase activity raise the prospect of developing small molecules that can either stabilize or disrupt the dimeric structure.²⁶⁸ The recent finding that a monoclonal antibody targeting the extracellular regions of CD148/DEP-1 can block endothelial cell proliferation and angiogenesis serves as an example for this approach.²⁶⁹ Based upon the dimerization model of regulation, several ‘wedge domain’ peptides have been identified as inhibitors of receptor PTPs.²⁷⁰ A more recent case is provided by targeting PTP σ , a receptor for chondroitin sulphate proteoglycans, with a cell-penetrating wedge peptide that can bind PTP σ and lessen the chondroitin sulphate proteoglycans-induced inhibition. Administration of the PTP σ cell-penetrating wedge peptide mimetic inhibits PTP σ promoted innervation and functional restoration in mice following spinal cord injury.^{271–272} Treatment with the PTP σ cell-penetrating wedge peptide mimetic also promotes cardiac innervation in a mouse model of myocardial infarction.²⁷³ Finally, *in silico* screening for compounds predicted to bind the interface between CD45’s D1 and D2 domains led to compound 211 (Figure 12).²⁷⁴ Compound 211 increased Tyr394 phosphorylation in Lck, a CD45 substrate in Jurkat, but not CD45-null (J. 45) cells, and blocked early and proximal TCR signaling.²⁷⁴ Compound 211 also dose-dependently reduced inflammation in the delayed-type hypersensitivity mouse model.²⁷⁴

4.5. Targeting PRL3 trimerization

The PRL phosphatases have recently emerged as compelling molecular targets for cancer drug discovery. However, the fairly flat PRL active site pocket and its structural similarity to

other PTPs pose significant challenge for PRL inhibitor development. Indeed, active site directed PRL inhibitors disclosed from the literature are neither sufficiently potent nor selective, and thus are not appropriate for *in vivo* functional study and drug development.²¹¹ Recent reports suggest that intracellular PTPs such as PRLs, can be targeted with antibodies.^{275–277} Given the functional requirement of PRL trimerization, pharmacologic disruption of PRL trimerization represents an innovative approach for the treatment of human cancers with elevated PRL expression. By targeting the unique, noncatalytic trimerization interfaces that are not related to any other PTP family members, such PRL detrimerizers would be highly specific to the PRL. Starting from a stepwise computer-based virtual screening approach, Cmpd-43 (Figure 12) and several of its analogs were identified that are capable of preventing PRL trimerization.²⁴⁷ Biochemical and structural studies establish that Cmpd-43 can effectively block PRL1 trimerization. Interestingly, PRL1 crystallized as a monomer in the presence of Analog-3 (Figure 12), a close derivative of Cmpd 43.²⁴⁷ The crystal structure of PRL1-Analog-3 shows that the compound makes direct contact with residues Tyr14, Met124, Phe132, Asp128, Thr13, Tyr14, Lys15, Asn16, and Arg159 in the trimer interface of PRL1 and obstructs its trimerization (Figure 13B). Cmpd-43 can also specifically attenuate the activation of both ERK1/2 and Akt and inhibit PRL-induced cell proliferation and migration. Importantly, Cmpd-43 exhibits excellent anti-cancer efficacy both in tumor cell lines and in a melanoma xenograft mouse model.²⁴⁷ This study provides pharmacological validation that trimerization is important for PRL1 function and that targeting PRL trimerization is a viable approach for therapeutic development.

5. Concluding Remarks

We have made great strides in delineating the biological functions of a number of PTP family members.^{20,24,278} These PTPs have been demonstrated to control a wide range of physiological processes, including cell proliferation, migration, metabolism, survival and immune response. As might be expected for enzymes that participate in such an important capacity in cellular signaling, PTP activity must be tightly regulated. That expectation is further reinforced by the examples presented here showing how various regulatory mechanisms, such as oxidation of active site Cys, allostery and oligomerization, are utilized to control PTP activity. Moreover, a growing body of work also implicates perturbation of PTP regulatory mechanisms in the pathogenesis of human diseases, ranging from metabolic disorders to cancer. Structure and function investigation has yielded critical insights into the regulatory mechanisms that control the PTP activity. Promising case studies covered in this review indicate that targeting PTP regulatory mechanisms could provide a new way forward to developing a broad array of PTP modulating agents, one that exploits the remarkable diversity of PTP regulatory elements while avoiding the challenges of pursuing the PTP active site.^{30–33}

It is important to point out that comparing to PTKs, the study of PTPs is still in its early stages as the majority of the PTP superfamily remain largely uncharacterized. It is anticipated that application of state-of-the-art CRISPR/Cas9 gene editing techniques²⁷⁹ and novel genetically modified animal models^{280–281} will continue to provide new functional perspectives for the remaining PTP family members and reveal new linkage of PTP dysfunctions to the causes of human diseases. As more potent and specific PTP inhibitors

become available,^{31,282} these small molecule chemical probes are expected to play increasingly important role in interrogating the biological functions of PTPs in both normal physiological processes and pathological conditions. Further understanding of the biochemical underpinnings of PTP regulation shall continue to offer new perspectives on the importance of these enzymes in both health and disease. Continued and persistent efforts to exploit PTP regulatory mechanisms will bring a new dimension to the armory of PTP-based drug discovery, ultimately delivering novel medicines for various human diseases.

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Abbreviations

PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
DSP	dual specificity phosphatase
pTyr	phosphotyrosine
MAP	mitogen-activated protein
MKPs	mitogen-activated protein kinase phosphatases
PRL	phosphatase of regenerating liver
SHP2	The Src homology 2 (SH2)-domain containing protein tyrosine phosphatase-2
ROS	Reactive oxygen species

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Biographies

Zhong-Hong Yu is an Assistant Research Scientist in the Department of Medicinal Chemistry and Molecular Pharmacology at Purdue University. He received his bachelor's degree in Chemistry in 2001 and his Ph.D. degree in Chemical Biology in 2007 from Nankai University in China. His training and research during the Ph.D. program were in the area of computer-assisted drug design. He started his postdoctoral research in 2008 at Indiana University School of Medicine with Professor Zhong-Yin Zhang and moved to Purdue University in 2016. Dr. Yu's current research focuses on the discovery of small molecular inhibitors for specific PTPs by integrated computational and experimental approaches, including high throughput virtual screening, molecular docking, molecular dynamics simulation, enzyme kinetics, mutagenesis, and X-ray crystallography. He also utilizes these techniques to investigate the structure and function of PTPs implicated as therapeutic targets and understand the molecular basis of how these PTPs cause disease.

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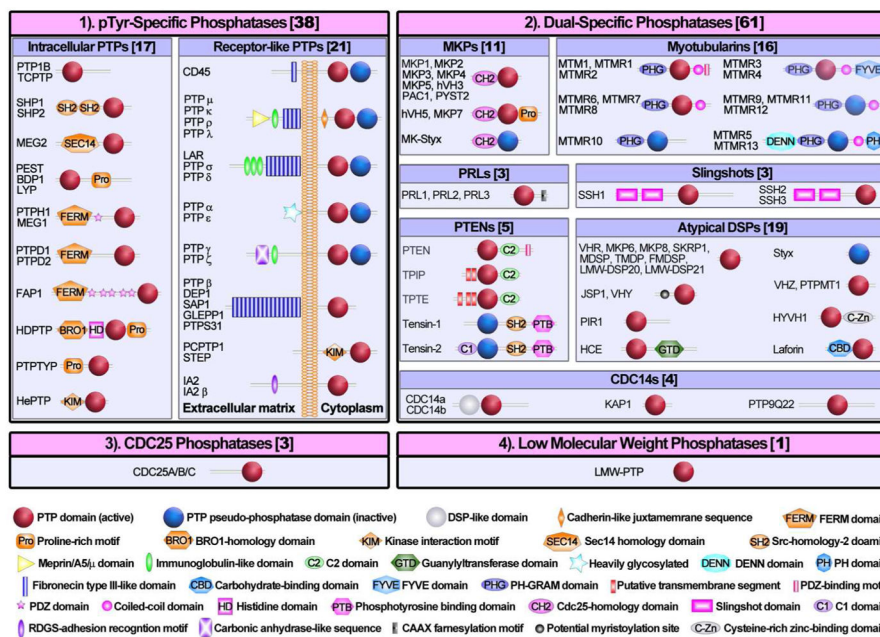


Figure 1. Classification and schematic representation of the PTP superfamily.

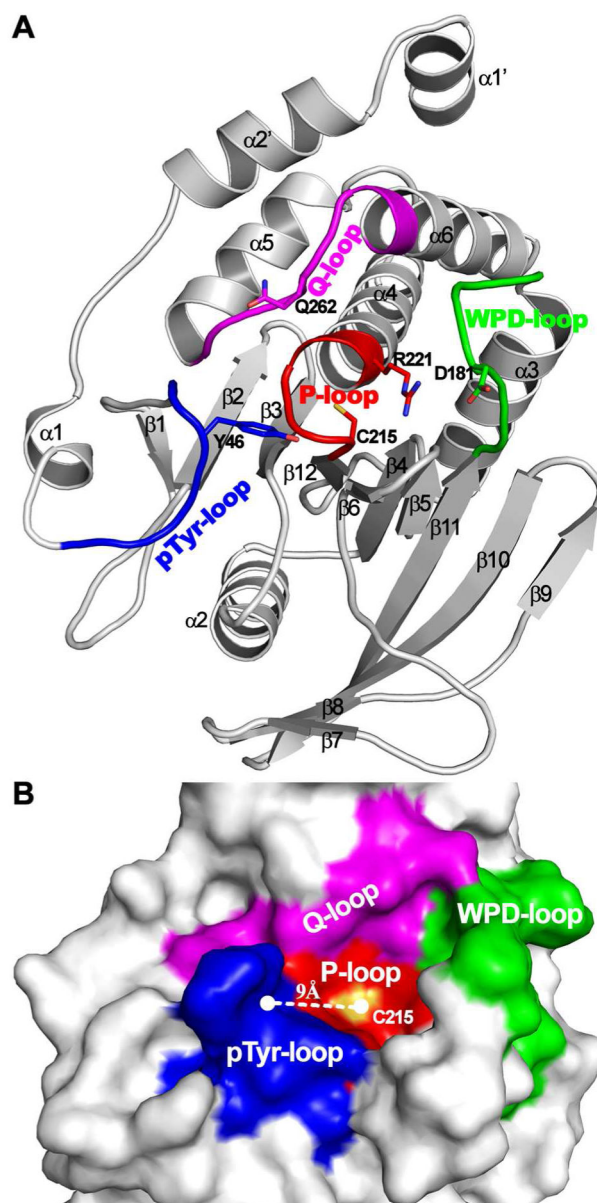


Figure 2.

Key structure features of PTPs depicted on the crystal structure of PTP1B. (A) Overall structure of PTP1B catalytic domain in ribbon mode. All α -helix and β -sheet are labeled, and the four loop fragments (i.e. P-loop, WPD-loop, Q-loop and pTyr-loop) constituting active site pocket are highlighted. Essential residues for catalysis are shown in stick and labeled. (B) The representation of active site pocket on the surface of PTP1B. The surface contributed by the P-loop, WPD-loop, Q-loop and pTyr-recognition loop are colored in red, green, magenta and blue.

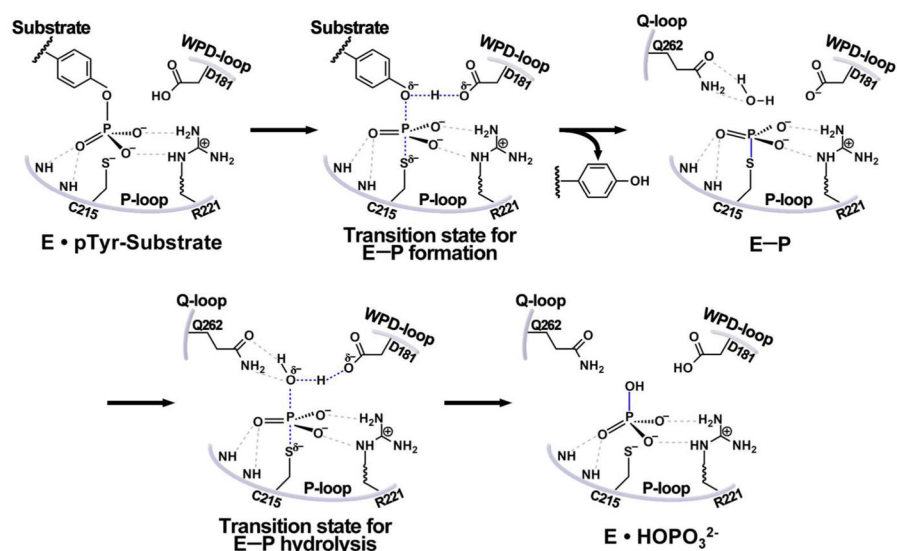


Figure 3. The catalytic mechanism for PTP-mediated dephosphorylation reaction. The gray dash lines indicate H-bonds which position the phosphate group and nucleophilic water, the blue dash lines show broken/formed bond in the transition state, and the blue solid line represent newly formed bond.

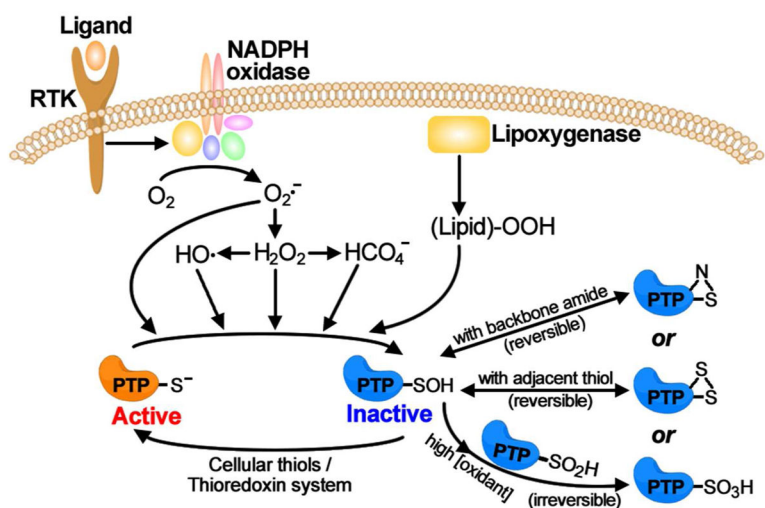


Figure 4. Redox regulation of PTP activity by ROS. Intracellular ROS, including H₂O₂, O₂⁻, •OH and peroxidized lipids, converts PTP catalytic Cys to sulfenic acid and inactivates the PTPs. The sulfenic acid form could be further irreversibly oxidized to the sulfinic or sulfonic acid forms, or reversibly converted to a cyclic sulfenyl amide or intramolecular disulfide form.

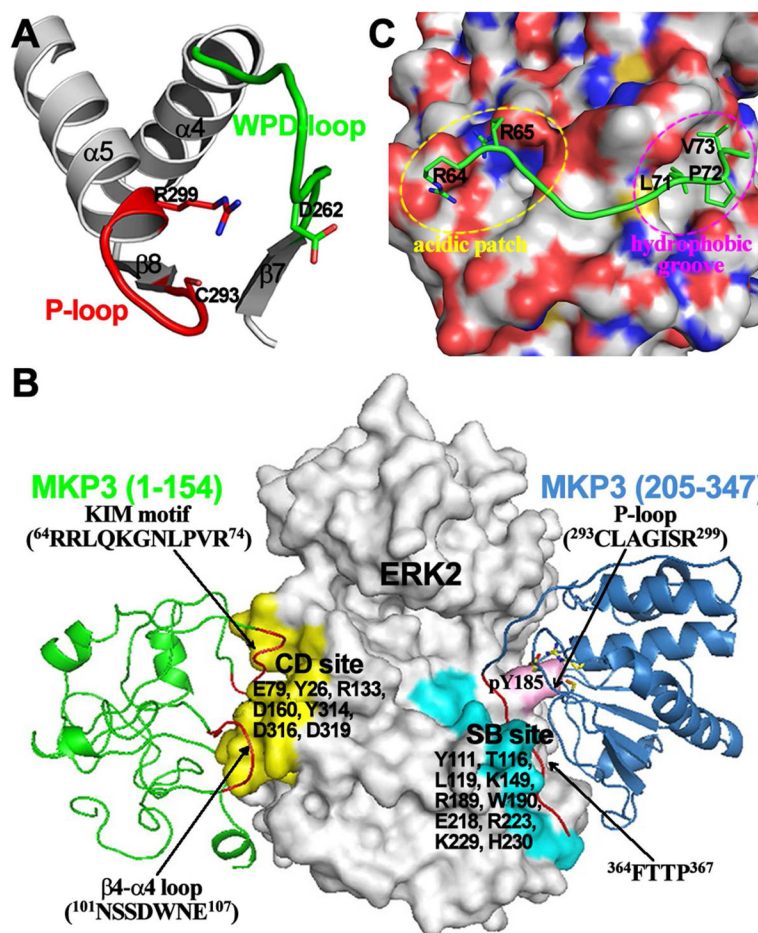
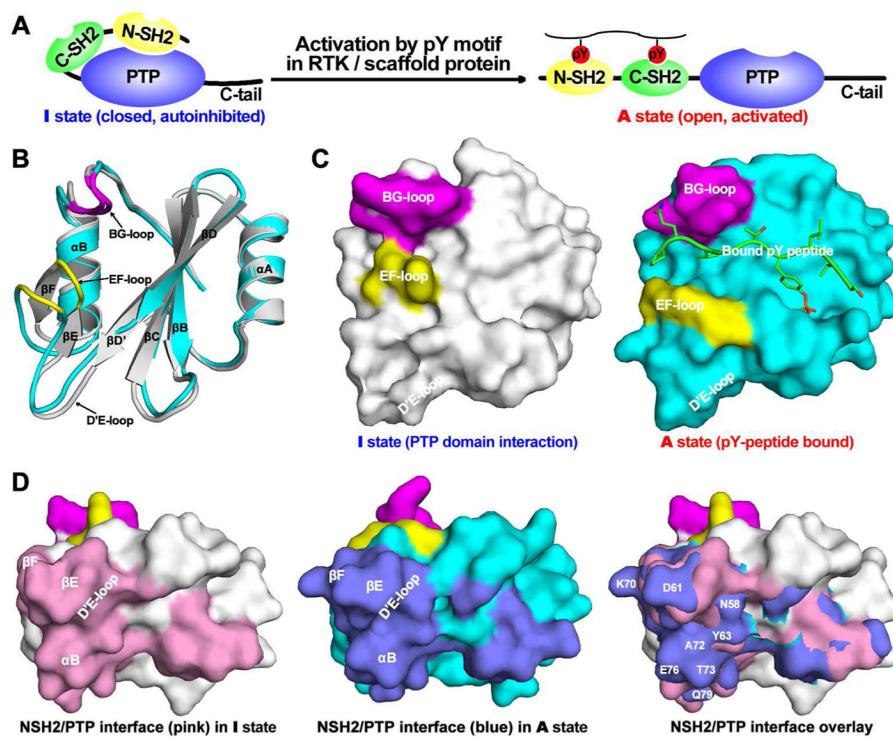


Figure 5. Allosteric activation of MKP3 by MKP3-ERK2 interaction. (A) Distorted MKP3 active site in the X-ray crystal structure of the MKP3 phosphatase domain. (B) Key interaction elements presented in a structural model of MKP3-ERK2 complex. (C) Crystal structure revealed that a peptide from KIM sequence of MKP3 binds at the common docking site of ERK2, which consists of an acidic patch and a hydrophobic groove.

**Figure 6.**

Allosteric regulation of SHP2. (A) The schematic representation of SHP2 structure and allosteric regulation. (B) The comparison of N-SH2 domain conformation at **I** (gray) and **A** (cyan) state. (C) The pY peptide binding surface in N-SH2 domain at **I** and **A** state. BG- and EF-loop are depicted in purple and yellow, respectively. (D) The N-SH2/PTP interaction surface in the N-SH2 domain at the **I** and **A** state.

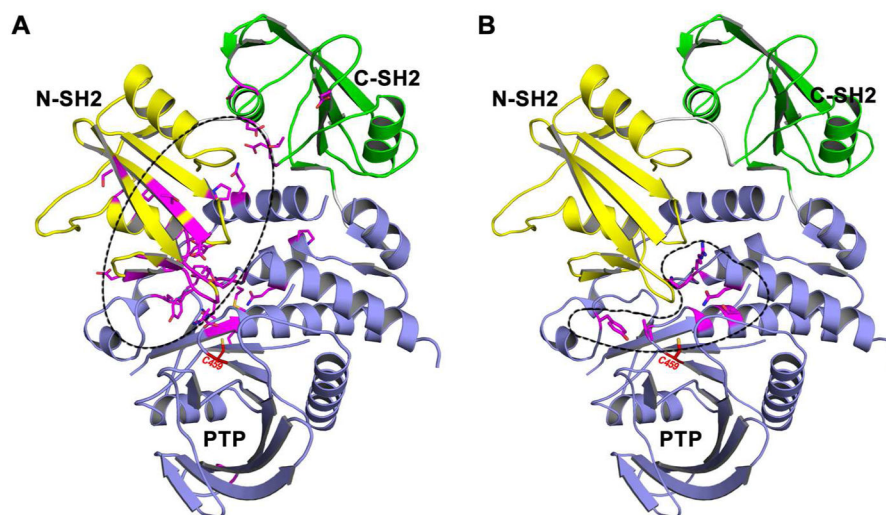
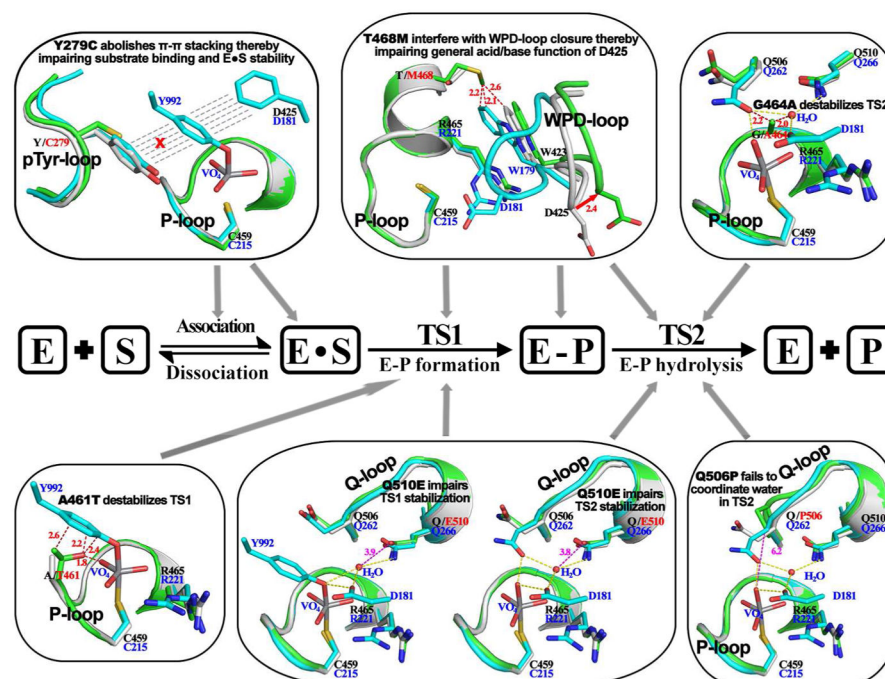


Figure 7. Disease associated SHP2 mutations. (A) NS/cancer-associated SHP2 mutations mainly reside at the interface of N-SH2 and PTP domains. (B) LS-associated SHP2 mutations only appear within the PTP domain.

**Figure 8.**

LS SHP2 mutations reduce SHP2 phosphatase activity by disturbing different step(s) in the catalytic process. In this figure, SHP2 wild-type (gray) and mutant (green) were superimposed onto PTP1B (cyan) structure representing transition state 1 or 2 to show mutation-induced disturbance at each specific step. Residue numbers are shown in blue for PTP1B and black for SHP2. Red dash lines represent mutation induced steric conflicts.

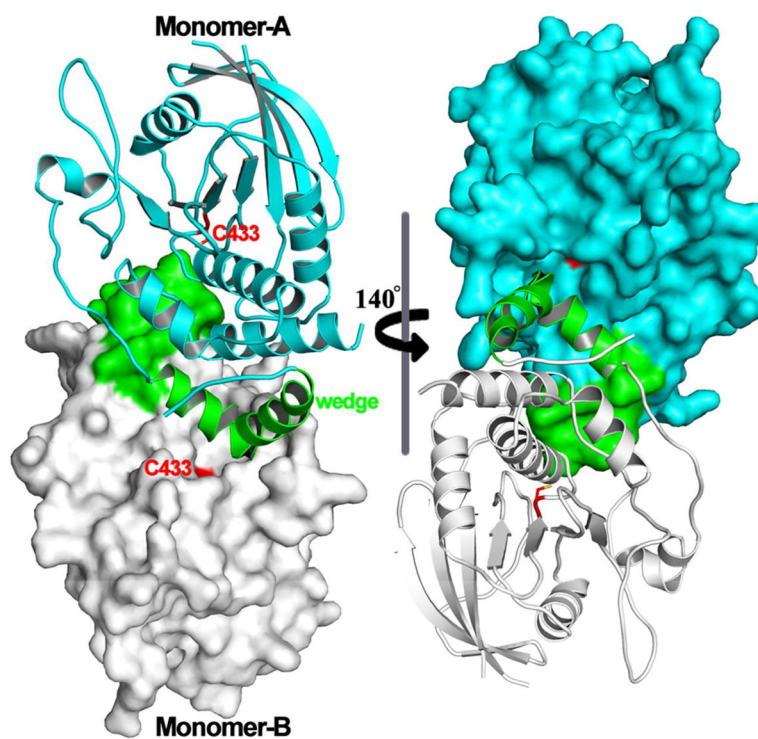


Figure 9. Dimeric structure of PTP α D1 domain. The N-terminal helix-turn-helix 'wedge' and catalytic Cys are highlighted in green and red respectively.

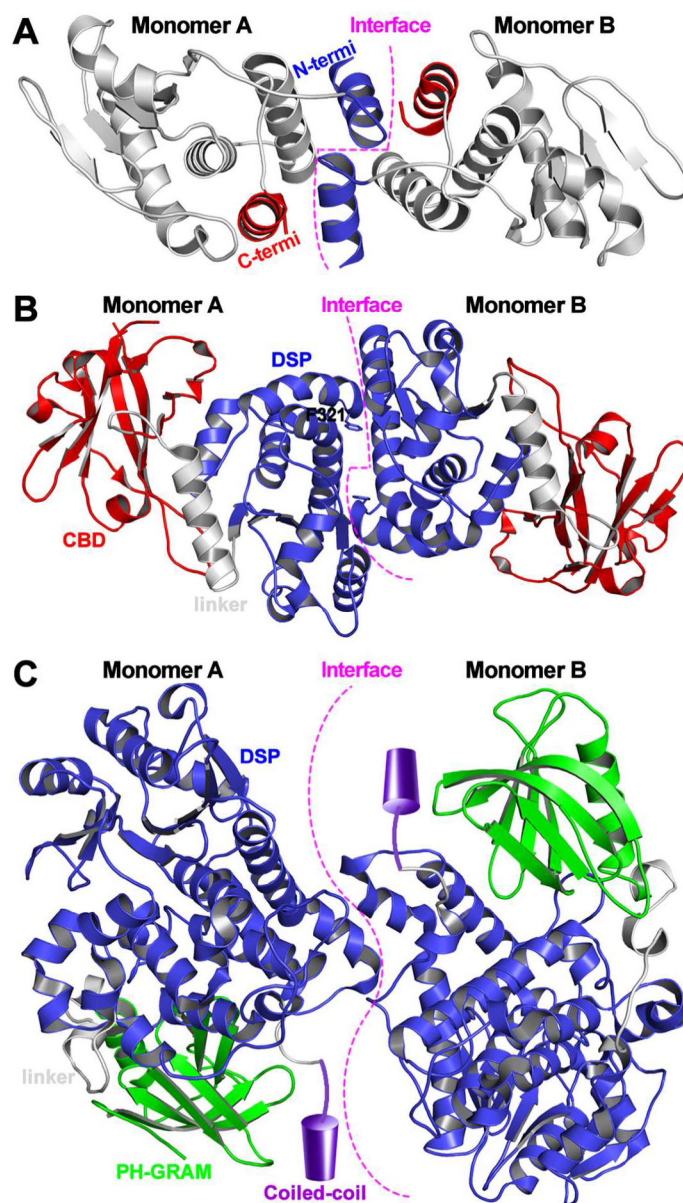


Figure 10. Dimeric structures of (A) VH1, (B) laforin, and (C) MTMR2. In the MTMR2 dimer presentation, the coiled-coil domain is not observed in the crystal structure and delineated as a cylinder to show its relative location.

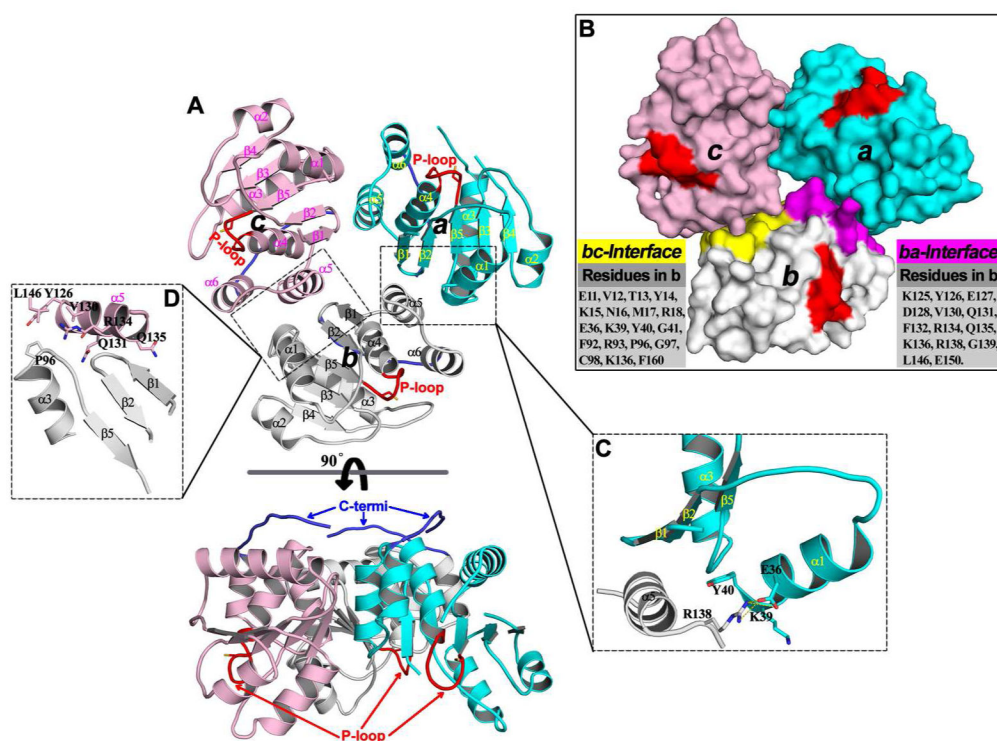


Figure 11. Trimeric arrangement of PRL1 in the crystal structure. (A) Overall structure of the PRL1 trimer in two orientations. In each monomer, the secondary structures are labeled; the P-loop and C-terminal tail are highlighted in red and blue, respectively. (B) Surface representation of the PRL1 trimer. Two dimer interfaces in monomer b are highlighted in purple (ba-interface) and yellow (bc-interface), and all residues involved in these interfaces in monomer b are listed accordingly. The close-up view of key interactions are respectively shown in (C) and (D) for ba- and bc-interface.

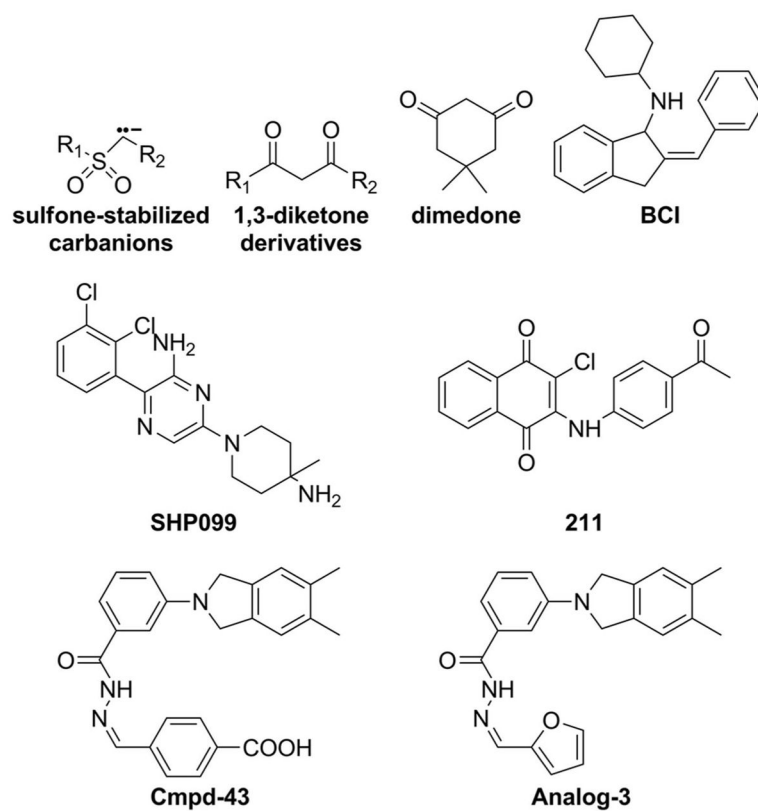


Figure 12. Structures of small molecule PTP inhibitors discussed in this review, which target the regulatory mechanisms unique to each PTP.

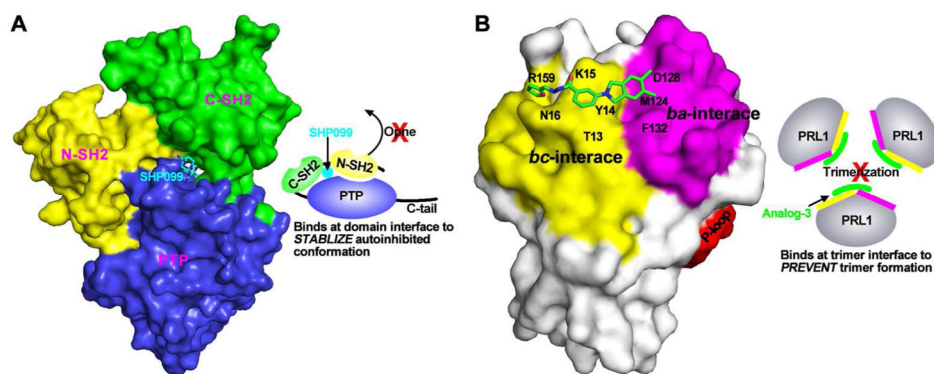


Figure 13.
The binding modes for two allosteric PTP inhibitors. (A) SHP099 binds at the inter-domain interfaces of SHP2 to stabilize the autoinhibited conformation. (B) Analog 3 binds at the trimer interfaces of PRL1 to prevent trimer formation.