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Review

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Structure-Based Virtual Screening of Protein Tyrosine Phosphatase Inhibitors: Significance, Challenges, and Solutions

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Phosphorylation, a critical mechanism in biological systems, is estimated to be indispensable for about 30% of key biological activities, such as cell cycle progression, migration, and division. It is synergistically balanced by kinases and phosphatases, and any deviation from this balance leads to disease conditions. Pathway or biological activity-based abnormalities in phosphorylation and the type of involved phosphatase influence the outcome, and cause diverse diseases ranging from diabetes, rheumatoid arthritis, and numerous cancers. Protein tyrosine phosphatases (PTPs) are of prime importance in the process of dephosphorylation and catalyze several biological functions. Abnormal PTP activities are reported to result in several human diseases. Consequently, there is an increased demand for potential PTP inhibitory small molecules. Several strategies in structure-based drug designing techniques for potential inhibitory small molecules of PTPs have been explored along with traditional drug designing methods in order to overcome the hurdles in PTP inhibitor discovery. In this review, we discuss druggable PTPs and structure-based virtual screening efforts for successful PTP inhibitor design.

Keywords: Allosteric targeting, cancers, diabetes, drug designing, molecular docking, protein tyrosine phosphatases

Introduction

Protein phosphorylation is a prevalent and pivotal mechanism in cell physiology. Dephosphorylation of amino acid residues counteracts the effects of protein phosphorylation, thereby controlling biological functions. This reversible modification is critical for the proper control of a wide range of cellular activities, including cell cycle, proliferation and differentiation, metabolism, motility, cytoskeletal organization, neuronal development, cell-cell interactions, gene transcription, and immune responses. All these processes are concomitantly orchestrated by protein kinases and phosphatases [1–3]. Phosphorylation usually occurs at serine and threonine residues (Ser/Thr) in eukaryotic cells. In contrast, tyrosine (Tyr) phosphorylation rationalizes 0.1–0.5%, but upon growth factor stimulation gradually increases up to 2% [4, 5]. Even though Tyr phosphorylation shows meager occurrence compared with Ser/Thr phosphorylation, it is a prerequisite in constituting key cellular signaling mechanisms, such as receptor tyrosine kinase (RTK) signaling, epidermal growth factor signaling, and cytokine-like cell surface receptor signaling [6-9]. Perhaps both Ser/Thr phosphatases and Tyr phosphatase constitute similar activity "dephosphorylation," but structurally differentiate in catalytic and active site domains and mechanism of action. Contradictorily, Ser/Thr and Tyr protein kinases share similarity in catalytic domain sequences [10–12].

Based on substrate specificity and functional diversity, protein phosphatases are classified into three superfamilies as summarized in Table 1. To date, available information of nearly 500 protein tyrosine phosphatases (PTPs) from genome sequencing data have been obtained throughout the eukaryotic genome, and so far, 107 PTPs are well characterized and explored in the human genome [13]. Based on structures, catalytic activities, and specificities,

Table 1. Classification of protein phosphatases.

Name	Catalytic site residues	Target substrates	Representative phosphatases
Protein serine/threonine phosphatases	DXH…DXXD…N	Serine/threonine residues in proteins	PP1, PP2A, PP2B, PP2C
Protein tyrosine phosphatases	(H/V)C(X)$_5$R(S/T)	Tyrosine residues in proteins	Class I PTPs: PTPRS, PTP1B
		Serine/threonine residues in proteins	Class II PTPs: MKPs, MTMRs, PTENs, SSHs, LMW-PTPs
		Lipids	Class III PTPs: CDC25A–C
HAD-related protein phosphatases	DXDXT/V	Serine/threonine residues in proteins	RNA Pol II C-terminal domain phosphatases
		Tyrosine residues in proteins	Class IV PTPs: Eyas

conventional PTPs are broadly categorized into four major classes (Table 1) [14]. Class I is the largest PTP class, which consists of 99 members subdivided into 2 major subfamilies. The first subfamily consists of 38 members, further subdivided into 17 receptor and 21 non-receptor types; for example, receptor-type tyrosine-protein phosphatase S (PTPRS) and protein-tyrosine phosphatase 1B (PTP1B), respectively. The second subfamily is very broad and it is composed of 61 dual specific phosphatase members, including mitogen-activated protein kinase (MAPK) phosphatases (MKPs), myotubularin-related phospholipid phosphatases (MTMRs), phosphatase and tensin homologs (PTENs), slingshot protein phosphatases (SSHs), and low-molecular-weight protein tyrosine phosphatases (LMW-PTPs). Three CDC25 phosphatases lie in class III PTPs [15]. The above classification is illustrated in Table 1. Four Eyes Absent phosphatases (Eyas) have different catalytic motifs from the other PTPs, and lie in class IV PTPs.

As a result of advances in X-ray crystallography, nuclear magnetic resonance, and transmission electron microscopy technologies, exquisite changes have been observed in protein structural biology. It left many milestones in proteomics by determining high-resolution structures for target or receptor protein complexes [16–18]. In addition, implementations of computational technology in biological sciences have accelerated protein structure prediction using computational methods [19, 20]. Consequently, many proteins and enzymes are able to reveal three-dimensional (3D) structures that have opened the doors for structure-based drug design (SBDD) [21, 22]. A finely constructed 3D structure of a receptor offers flexibility to design potential ligands. This phenomenon makes SBDD a promising tool for designing novel inhibitors for targeted receptors [23–25]. Owing to the structural complexities of PTPs, SBDD is assumed to be a favorable technique for designing novel inhibitors for them. Virtual screening (VS) is a renowned technique in the drug designing world. Hence, the combination of robustness of SBDD and high-throughput VS is referred to as structure-based virtual screening (SBVS). This is a potent and suitable method to develop inhibitors for challenging targets like PTPs.

Herein, we exclusively review the complexity of conserved domains and their influence on catalytic activities of PTPs, challenges in targeting PTPs, diseases associated with defective PTPs, and pragmatic strategies such as a combinatorial approach using SBDD and VS for designing PTP inhibitors. Additionally, we discuss contemporary techniques, alternative methods, and emerging strategies.

Table 2. Protein tyrosine phosphatases and their representative implicated diseases.

PTP	Disease/activity	Reference
PTP1B	Breast cancer, colon cancer, type II diabetes	[141]
RPTPσ	Stem cell regeneration, neuronal cell growth	[142]
SHP2	Noonan syndrome, lung cancer, hepatocellular carcinoma	[143]
CDC25B	Non-Hodgkin's lymphoma, prostate cancer, gastric cancer	[144]
MKP1	Non-small-cell lung cancer, breast cancer, prostate cancer, renal cancer	[52]
PRL3	Hepatocellular carcinoma, colorectal cancer, gastric cancer	[145]
PTPMT1	Diabetes, oncogene transformation	[64]

Significance of Protein Tyrosine Phosphatases in Disease

Phosphorylation is critical for the success of several inter- and intra-cellular signaling events. To date, many studies have presented the implications of tyrosine phosphatases in numerous human diseases. Here, we attempt to emphasize various classes of thoroughly studied PTPs pertinent to diseases, and checkpoints for designing their inhibitor molecules.

Protein Tyrosine Phosphatase 1B

PTP1B is the non-receptor type tyrosine phosphatase encoded by the gene, protein tyrosine phosphatase, non-receptor type 1 (*PTPN1*). It has a pivotal role in numerous signal transduction pathways. Its major role has been denoted in insulin receptor- and leptin receptor-mediated signaling pathways [26]. From the literature and experimental evidence, it has been known that PTP1B-deficient mice showed increased sensitivity for insulin and were affected with type II diabetes. In contrast, PTP1B expression reversed the condition. This supports the theory that PTP1B can act as a successful negative regulator of the insulin receptor [27, 28]. Since PTP1B activity is a key component for cellular functions like cell adhesion and for metabolic processes like insulin receptor signaling, it has been extensively studied for the past decade. It has also been identified to have a pivotal role in breast and colon cancers, because its dephosphorylation property influences the epidermal growth factor receptor and downstream genes that participate in breast cancer [29]. In human breast cancer cells, it dephosphorylates inhibitory tyrosine residues on Src kinases in response to integrin signaling, and the dephosphorylation enhances the Src kinase activity. Ras-extracellular signal-regulated kinase (ERK) activation in conjunction with Src activation contributes to the HER2/Neu-mediated breast cancer pathway. Apart from the role of PTP1B in breast cancer by activation of Src kinases, recent genetic studies elucidated its role in colon cancer and renal cancers [30]. Owing to the primitive role of PTP1B in numerous metabolic and pathological processes at certain levels, there is necessity for inhibitors of PTP1B in order to develop therapeutic drugs of diabetes and cancer.

Src Homology 2 Domain-Containing Phosphatase 2 (SHP2)

Another non-receptor type tyrosine phosphatase, SHP2, is encoded by the protein tyrosine phosphatase non-receptor type 11 (*PTPN11*) gene and contains two Src homology 2 domains with auto-inhibitory activity and one PTP-signature domain. PTPN11/SHP2 is associated with genetic diseases such as Noonan syndrome, Leopard syndrome, and cancers (breast cancer, colorectal cancer, and acute myeloid leukemia) [31]. Synergistic expressions of SHP2 contribute to activation of different intercellular signaling pathways and tumorigenesis in response to various other triggers such as growth factors and cytokines. SHP2 plays an important role in Ras-mitogen-activated protein kinase (MAPK)-signaling activation by promoting Gab2/Sos1 complex formation, as seen in evidence from the literature [32]. SHP2 mediates MAPK activation, thereby further contributing to the activation and regulation of various proliferative pathways like Janus-kinase, and signal-transducer and activator of transcription (STAT) [33]. Mutations in SHP2 led to 10–35% hyperactivation, and concomitant downstream action on oncoproteins, resulting in malignancies, as seen from studies on xenograft models. A study on human breast cancer cells showed that hyperactivated SHP2 led to activation on ERK pathways and thereby facilitated expression of downstream transcription factors such as v-myc, zinc finger E-box1 (ZEB1), and c-myc. These factors could promote the expression of an emerging oncogenic driver (LIN28B) in cancer stem cells [34]. Overexpressed LIN28B initiated growth of breast cancer-inducing cells, but knock-down of SHP2 depreciated the breast cancer cell growth and metastasis. Unlike in breast cancer, SHP2 has shown a contradictory role in the case of hepatocellular carcinoma (HCC) [35]. The tumor suppressor role of PTPN11/SHP2 in hepatocytes is unexpected because SHP2 has predominantly been denoted as a proto-oncogene and its role is defined as a tumor proliferator [36]. Interestingly, knock-out of SHP2 in hepatocytes led to inflammatory signal enhancement by an excited STAT3 pathway and subsequently induction of HCC [37]. Since SHP2 has cell-specific regulation of cancer, there is a need to develop novel inhibitors against it for each type of cancer.

Receptor Protein Tyrosine Phosphatase Sigma (PTPRσ)

PTPRσ is encoded by the gene receptor-type tyrosine-protein phosphatase S (PTPRS), which belongs to a receptor-type family since it has two tandem cytoplasmic and one transmembrane domain. Active PTPRσ is a major regulator in diverse cell functions, such as cell growth, mitosis, differentiation, and transformation. Its differential expression is important for promoting essential developmental processes, such as axon genesis and neuron regeneration [38]. Neurotrophic receptors of tropomyosin receptor kinase (Trk) protein family members of receptor tyrosine kinases have pivotal roles in neuron development and

survival of sensory neuronal growth [39]. PTPRσ is well studied among all 21 receptor protein tyrosine phosphatases (RPTPs), and plays a role in axon guidance. Thus, researchers have focused on a study of the signaling events of PTPRσ in Trk-mediated downstream pathways and its effects on neurite growth and sensory neurons outgrowth [40]. PTPRσ dephosphorylates TrkA, TrkB, and TrkC, but differentially interacts with Trk proteins. A study using PC12 neuronal cells has shown that downregulation of PTPRσ escalates neurite growth and TrkA activation [41]. PTPRσ was observed at higher expression than other PTPRs in expression profiles of receptor PTPs in hematopoietic stem cells (HSCs). This signifies that PTPRσ plays a role in the repopulation of HSCs [42]. By virtue of PTPRσ's dynamic balancing of tyrosine phosphatase activity, it inhibits Ras-related C3 botulinum toxin substrate 1 (RAC1) signaling by a dephosphorylating p250GAP member of Rho GTPases [43]. PTPRσ dephosphorylation activity activates p250GAP, thereby inhibiting RAC signaling. PTPRσ, along with its co-expressing leukocyte antigen-related tyrosine phosphatase (LAR) and neurite outgrowth inhibitor (nogo) receptors, plays a critical role in speeding recovery by simulating axon growth from spinal cord injury by modulating chondroitin sulfate peptidoglycans [44].

M-Phase Inducer Phosphatase 2 (CDC25B)

CDC25B is cell division cycle 25 homolog B and a dual specificity phosphatase belonging to the PTP super-family. CDC25 phosphatases have a prime role in cell cycle progression due to "ON and OFF" switching properties of isoforms by orchestrating cyclin-dependent kinase (CDK) activities [45]. CDC25 phosphatases act as positive regulators of the cell cycle by selective dephosphorylation of Thr14 and Tyr15 inhibitory phosphate residues of CDK and constitute mitotic phase inductions [46]. The enzymes are called "cell division cycle" phosphatases based on the roles of the proteins. CDC25B has been thought to have an exclusive role in dose-dependent oncogene transformation [46]. Elevated co-expression of M-phase inducer phosphatase 1 (CDC25A) and CDC25B was detected in various diseases like prostate cancer, gastric cancer, non-Hodgkin's lymphoma, and Alzheimer's disease. Importantly, concomitant overexpression of CDC25A and CDC25B impacted cell cycle checkpoint control, consequently leading to an oncogenic condition [47]. Additionally, an interesting study described its role in neuron degeneration associated with Alzheimer's disease [48]. In brain samples from Alzheimer's patients, it was observed that CDC25B rapidly dephosphorylated Thr14 and Tyr15 of cell division cycle protein 2 homolog (CDC2)/CyclinB. As a consequence, prematurely activated cyclin kinase resulted in accumulation of M phase phosphoepitopes, and mitotic phase structural modifications led to neuronal degeneration in Alzheimer's disease [49]. From more observations of the elevated CDC25A expression along with CDC25B, these insights are implying that inappropriate expression of CDC25B is associated with particular diseases. Hence, CDC25B could be a promising drug target for many diseases.

Mitogen-Activated Protein Kinase Phosphatase 1 (MKP1)

MKP1 is also known as dual specificity phosphatase-1 (DUSP1). It dephosphorylates Thr and Tyr residues in MAPKs [50]. MAPK signaling is exclusively associated with cell proliferation, differentiation, and transformation; thus, its misregulation results in inflammation and carcinogenesis. Misregulation effects of MKP1 have been properly observed in non-small-cell lung and renal cancers [51]. MKP1 has an extensive role in non-small-cell lung cancer by modulating the p38/JNK pathway [52]. An essential role of MKP1 was proved in repressive response for treatment with cisplatin due to its dual specificity activity. MKP1 dephosphorylates and inactivates JNK. Consequently, it protects from cisplatin impact [53]. Knock-out of MKP1 increased the sensitivity of animals to cisplatin. Thus, MKP1 suppression in cancer treatment is useful to improve the positive effects of anticancer drugs.

Phosphatase of Regeneration Liver 3 (PRL3)

PRL3 is encoded by the gene protein tyrosine phosphatase type IVA 3 (*PTP4A3*), and belongs to the novel family of PTPs. It contains a C-terminal prenylated domain [54]. PRL3 is exclusively involved in cell migration, invasion, and metastasis. Along with phosphatase of regeneration liver 1 (PRL1), it plays a major role in progression to metastasis [55]. PRL3 is implicated in ovarian cancer and melanoma. Elevated levels of PRL3 are related to carcinomas such as gastric cancer, colorectal cancer, and hepatocellular carcinoma [56, 57]. Dynamic imbalance of PRL3 can affect various stages of cell proliferation, invasion, and motility. Initially, in cell proliferation, PRL3 is related to degradation of p53 by ubiquitination and proteasome degradation that thereby induces the carcinogenesis [58]. Furthermore, PRL3 is involved in invasion and metastasis through hyperactivation of the epidermal growth factor receptor. Consequently, it activates the RTK, PI3K/AKT, Src/STAT, and RAS/MAPK pathways [59]. The PRL3-mediated aberrant RTK signaling pathway contributes to rapid progression of malignancy and metastasis. The positive

results by inhibition of PRL3 were shown in colon and gastric cancers, stating that PRL3 could be targeted as a cancer therapeutic drug target [60].

Protein Tyrosine Phosphatase Mitochondria 1 (PTPMT1)

PTPMT1 is a DUSP that is extensively localized to the mitochondria inner membrane. PTPMT1 is a key regulator of cardiolipin (CL) biosynthesis, since PTPMT1 activity is the checkpoint for the control of CL metabolism. It is indirectly associated with CL-mediated diseases, such as Parkinson's disease, Alzheimer's disease, non-alcoholic fatty liver disease, diabetes, and cancer [61, 62]. CL is vital for mitochondrial respiration and cytochrome *c* release. PTPMT1 balances CL biosynthesis through its phosphatase activity [63]. Similarly, downregulation or knock-down of PTPM1 exhibited decisive results against cancer cell growth [64]. PTPMT1 is detected to have a role in embryonic development along with its natural activity in mitochondrial-based biosynthetic pathways, and it is also identified that PTPMT1 contributes to dephosphorylating phosphatidylglycerol phosphate to phosphatidylglycerol, which is a precursor of CL [65]. Recent studies showed that PTPM1 ablation regulated CL levels, which thereby led to activation of mitochondrial-mediated apoptosis. This was observed in the presence of the PTPMT1 inhibitor "Alexidin" [66, 67]. Similar phenomena were observed in glucose level regulation; a study revealed that PTPMT1 activity on succinate dehydrogenase influenced glucose metabolism. This action was relevant to diabetes. Inhibition of PTPM1 resulted in the lowering of sugar levels [68].

Structural Overview of Protein Tyrosine Phosphatases

Owing to the advances in structural biology, many proteins have been characterized as 3D structures. Some of these solved structures included PTPs [69]. Typical PTPs possess more than 400 residues. The hallmark that defines the PTPase family is the conserved catalytically active site (H/V)C(X)5R(S/T) that surrounds a 250-amino-acid-residue region, and other catalytically significant structures (WPD, Q-loop, p-Tyr-loop, and E-loop). These structures are conserved in about >85% eukaryotic PTPs [70]. To constitute phosphatase activity, PTPs require these conserved loop structures for various activities like substrate recognition, binding, and catalysis [71]. By virtue of the structural conservation of PTPs, in this review we focused on catalytically important structural components and their functional significance. Among PTPs, PTP1B has an elegantly modeled structure. Hence, we considered it to be an ideal representative example of PTP structures in general, and focused the remainder of the review on its features.

pTyr-Loop

The pTyr-loop (NXXKNRY) is a moderately conserved loop structure that is present in all classical tyrosine phosphatases and also considered a substrate-recognition loop (Fig. 1). The conserved residues, NRY, are involved in substrate recognition and loop stabilization. The tyrosine residue (Y) has a notable role in defining active-site cleft depth, recognizes the pTyr residue, and facilitates access to the active site through electrostatic interactions [72]. The highly conserved arginine (R) residue is implicated in loop stabilization, which is important for constituting the catalytic activity by interacting with adjacent oxygen atoms. An adjacent moderately conserved asparagine (N) residue strengthens the interaction between the pTyr residue of the substrate and active site residues by forming hydrogen bonds [73].

PTP-Loop

The PTP-loop is a highly conserved loop structure of the PTPase domain and exhibits in most PTPs. Conserved PTP active site motif $(H/V)C(X)_5R(S/T)$ lies in this PTP-loop (Fig. 1). The arginine (R) in the loop helps to create a positive charge in the active site pocket [74]. A charged active site stabilizes the thiolate anion of the cysteine (Cys) residue of the PTP-loop to cut down the pKa for efficient catalysis and increases the Cys's nucleophile activity [75]. It enhances the affinity for phosphate ions. The low pKa values of Cys influence its nucleophilic mechanism. It is sensitized to oxidation and nitrosylation, which result in abolishing the phosphatase activity [76, 77].

WPD-Loop

The WPD-loop generally exists in an upstream region of the conserved active site in all classical PTPs. Conserved tryptophan (W), proline (P), and aspartate (D) residue repeats offer the name as WPD-loop (Fig. 1); it is a movable and flexible structure in the PTP domain [78]. W and D are much more conserved compared with proline, and W has an important role in loop flexibility. D acts as a general acid/base catalyst in catalysis. An adjacent glycine acts as a hinge residue and helps energetically in the loop motion [79, 80]. Owing to its flexible quality, it acts as a "regulatory switch" of the PTP-loop, and the WPD-loop coordinates the closed and open conformations of the PTP-loop. Upon substrate binding to the active site, the WPD-

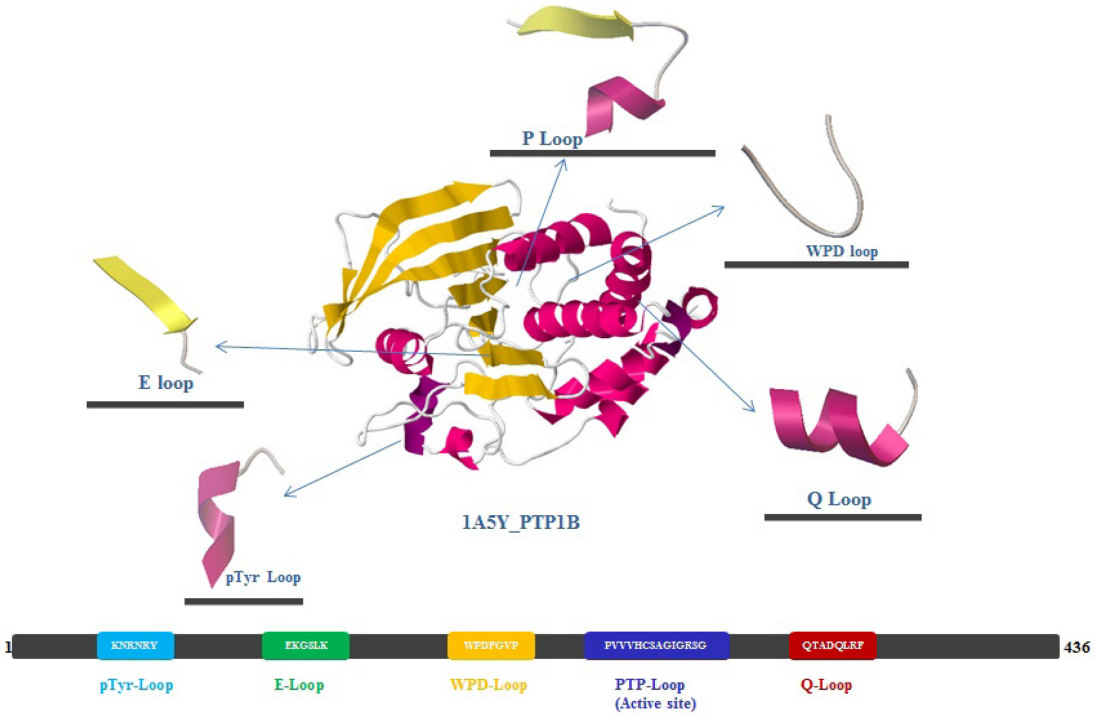


Fig. 1. An illustrative structure of protein tyrosine phosphatase 1B (PTP1B).
The upper image is the backbone of the PTP1B (PDB:3A5K) crystal structure with loops highlighted in the 3D structure. The lower structure represents the arrangement of loops in the protein structure with corresponding colors and contributing residues.

loop moves closer and the catalytic acid/base residue D accelerates the catalytic action [81]. Mutational studies demonstrated that the decrease in loop flexibility with W mutation and D alterations resulted in a lower catalytic activity [71]. A WPD-loop is missing in CDC25s and MTMRs. It is placed differently to the downstream region of the PTP-loop in LMW-PTPs [82].

Q-Loop

The Q-loop is the other less-conserved structure of the PTP domain, and appears in all classical PTPs with a moderately conserved glutamine (Q) residue [83]. A conserved Q residue in the Q-loop (QTXXQY) moves towards the active-site signature motif (Fig. 1), and forms hydrogen bonds with an active site water molecule and substrate oxygen anion. It contributes to Cys nucleophilic activity and thereby helps in cysteinyl phosphate hydrolysis. In addition, the Q residue combines with the D residue of the WPD-loop and activates water molecules. It helps in catalysis, and also helps to maintain the WPD-loop in its active conformation during catalysis [84, 85]. Interestingly, DUSPs do not possess the conserved glutamine, and LMW-PTPs do not contain a similar loop structure [82, 86].

E-Loop

The E-loop is immediately contiguous to the PTP-loop and WPD-loop and is conserved throughout human PTPs. The E-loop contains 100% conserved glutamate (E) and about 90% conserved lysine (K) residues (Fig. 1). An E residue forms a hydrogen bond with a PTP-loop side chain arginine in the active form, and stabilizes the guanidium group to enhance the accessibility of the phosphate group [87, 88]. On the other hand, the less-conserved K residue has a similarly important role in WPD-loop stabilization in its closed conformation. The K residue forms a hydrogen bond with a catalytic aspartate (D) and maintains its closed conformation under the influence of the substrate [79]. The E-loop is extensively studied in hematopoietic PTPs (HePTPs) and a kinase interaction motif PTP family member. E-loop differs from classical PTPs in HePTPs in terms of sequence and structure [89].

Challenges in Targeting PTPs

Since impaired functionality of PTPs has been acknowledged in many diseases, researchers have focused on PTPs as druggable targets. Although PTPs are perceived

as promising drug targets, few challenges have been presented. Structural complexity is a major hurdle to overcome in the development of PTP inhibitors. Broadly, PTPs are grouped into four major classes. Alternatively, PTPs can be differentiated into Cys-based PTPs and Asp-based PTPs based on general acid/base catalytic residues. The active site and allied loop structures are highly conserved among all PTPs except for Asp-based PTPs [90]. The high conservation in the catalytic domains of PTPs is a big hurdle in targeting the malfunctioned ones [91]. Moreover, the positively charged active site, which binds to phosphotyrosine residues, possibly attracts negatively charged moieties during small molecule screening. This leads to false-positive inhibitor predictions. Falsely predicted redox compounds may affect the nucleophilic cysteine by oxidation when the active site is in a reduced state, which influences the phosphatase potentiality [76, 77, 92].

Classical PTPs share common functional features [93]. In addition, a single phosphatase will be expressed in multiple pathways. We cannot target one phosphatase, since selective targeting for common functional PTPs will affect undesired pathways and may lead to a dead end in terms of drug development [94, 95]. For this reason, there have been challenges in the developments of PTPs as drug targets [73]. To achieve the fruitfulness in targeting PTPs for treatment of threatening diseases, we are expected to overcome the basic hurdles and to think in new dimensions. Therefore, we herein discuss the novel ways of targeting and alternative methodologies that will help in passing the bottlenecks to predict potential inhibitors for targetable PTPs.

Structure-Based Virtual Screening

In recent decades, remarkable changes have been pursued in structural biology due to advancements in structural genomics and proteomics. Cutting edge technologies like X-ray crystallography and nuclear magnetic resonance in structure determination have changed the face of genomics and proteomics, and the structures of many biological molecules have been obtained, which establish the new era in the drug discovery process [96, 97]. Further computational applications in the biological field have boosted the drug designing process, made possible the screening of thousands of compounds instantly, and provided opportunity to select potential candidates against the desired biological target [98]. Since high-throughput screening (HTS) is burdened with false-negatives frequently, SBDD has emerged as the promising tool for the drug industry, as it uses the finely tuned target structure against lead compounds and can visualize the binding process [99]. We believe that the combination of SBDD and VS could be the paramount technique to design small molecules for complicated drug targets like kinases, G-protein-coupled receptors, and cancer targets [100].

Virtual screening is the integral part of modern drug discovery, but the sheer number of resulting compounds from VS and HTS are not clinically viable owing to its limitations. The solidarity of HTS results does not elucidate the crucial properties like binding energies and intermolecular forces. Most of the screened compounds could not be able to pass the ADMET bottleneck at the final stage [101]. On the other hand, SBDD could offer clear information of binding pockets and visualization of protein-ligand binding complex interactions with detailed binding energy data. One on one, both VS and SBDD has its own advantages in favor of drug designing; hence, in this review, we discuss the benefits and limitations of SBVS in the perspective of PTPs. On the other side, SBVS incorporates several steps such as target preparation, decoy set preparation, docking, post-docking analysis, scoring, and ranking (Fig. 2); in each level, the difficulties endure when dealing with structurally conserved molecules like PTPs [102, 103]. Here we want to emphasize the capable edge of SBVS, and challenges and prospective in successive ligand design in respect to PTPs.

SBVS Implementation

The SBDD and VS combinatorial approach has some similarity in the workflow in the means of target preparation and ligand preparation, and the uniqueness of the approach lies in the docking and post-docking steps. Basically, the prerequisite in SBDD is the receptor/target structure that may be X-ray crystallized or systematically modeled.

Structure preparation. The fundamental step of SBVS starts with target structure preparation, which is an inevitable step in this approach. Almost all tyrosine phosphatases possessing 3D structures and a few PTPs like SHP2, MKP1, and PRL3 are crystallized on its catalytic sites. The preeminent problem in targeting PTPs is a structural similarity. The problem starts from the initiation level, and our challenging motive behind selecting the SBVS approach for tyrosine phosphatases is its structure complexity [104].

Basically, in structure preparation, special attention is required in the bond orders, active site waters, cofactors, metal ions, topologies, tautomeric forms, ionization and protonation states, missing atoms, side chain atoms, and partial charges. Several programs are available to find the

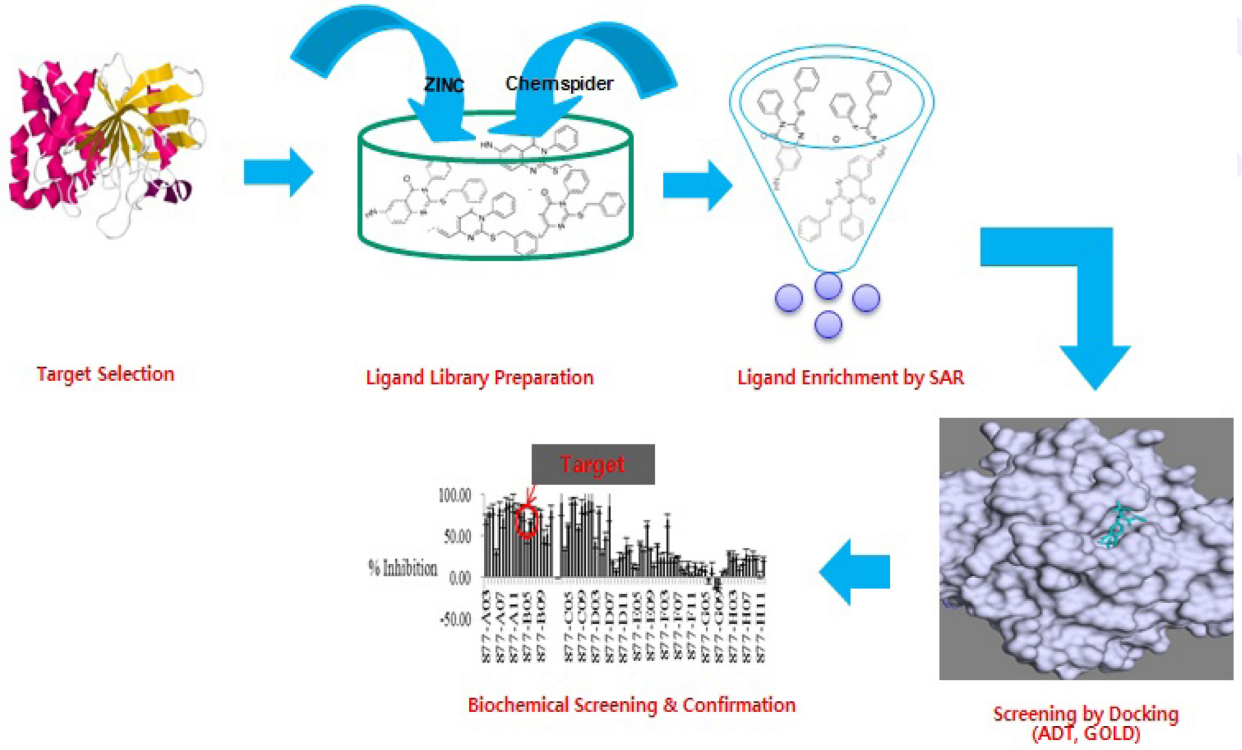


Fig. 2. A conceptual figure of structure-based virtual screening, illustrating the schematic design and typical drug discovery steps in the search for a potential protein tyrosine phosphatase inhibitor.

structure quality, such as PDBsum, Ramachandran plot, WHATIF, and Prosa-web [105–107]. PTPs are known to be identified by their signature catalytic site, so that focusing on the active site is vulnerable. This is the primary and ultimate challenge, but results can be achieved by targeting other functional sites [104].

Binding site identification. This is the extended crucial step to the previous step in this approach, because the binding site has a major part in deciding the fate of the result and is playing the exclusive role in PTP targeting. In SBVS, the binding site projects the protein-ligand interaction phenomenon, post-docking dynamics, hydrogen bond formation, and free energies of the complex [108]. The best calculated pharmacophores of the binding site determine the fate of the novel inhibitor. In PTPs, the five functional sites are the pTyr-loop signature site, PTP-loop, WPD-loop, Q-loop, and E-loop. Among them, the Q- and E-loops are less conserved and decisive for protein function [109]. On the other hand, finding the novel binding sites using randomly selected fragment-based chemical probes can improve the specificity [110]. Numerous online tools and web servers are available to calculate and identify the probable binding sites in target proteins, such as CASTp, QsiteFinder, metapocket, LigAsite, and MSpocket [111–113].

Ligand library preparation. Ligand library preparation is the important step for VS. For library construction, compounds will usually be collected from various sources such as natural ligands, public repositories like ZINC and drug-bank, and commercial vendor sites. Every drug-like compound or every ligand must obey the limitations of "Lipinski's rule of five" (logP values, molecular weight, number of H-bond donors and acceptors). Generally, natural drug-like compounds fail to cross this obstacle [114–116]. More precisely, to improve the quality, medicinal chemists propose a rule of 3 for compounds in screening libraries, such as molecular weight <300 daltons, H-bond donors or acceptors not more than 3, and logP value not greater than 3, and finally no more than 3 rotatable bonds [117].

After satisfying the basic criteria, we need to optimize the decoy sets. Fortunately, PTPs have information of chemical probes, which helps to develop novel candidates based on prior experiences. For PTP1B, researchers have designed the small molecules using VS and biochemical screening, but not many inhibitors have succeeded as

commercial drugs. Further studies on native substrates like chemical probes seem to be successful with a rational SBDD. The 1,2,5-thiadiazolidin-3-one-1,1-dioxide template as a pTyr mimic substrate showed satisfactory results [118]. Similarly decoy sets will be optimized to minimize the number of screening compounds. Likewise, some web-based and standalone tools help in designing new and knowledge-based ligands, such as LUDI, MEGA, LEGEND, and LigBuilder [119, 120]. Additionally, structure activity relationship studies are useful to improvise the quality and affinity of novel ligands [121].

Docking

Docking is the decisive step in inhibitor design, which helps in visualizing the interaction patterns and binding energies of protein-ligand complexes. There are no prescribed tools for SBDD, but constructed algorithms and scoring functions differentiate the usefulness of the tools for this approach.

Recently, a large number of docking tools evolved based on searching methods and scoring functions. Some of the major tools used are Autodock [122], GOLD, Discovery Studio, FlexX, ICM, and UCSF Dock [123]. Generally, these tools are based on stochastic search algorithms such as a genetic algorithm (Autodock, Gold), geometric matching (Dock), and an exhaustive search algorithm (Glide). Apart from docking algorithms, the output will depend on target topologies, rotamers, and ligand polarities. Software packages like Discovery Studio (Biovia, USA) and Schrodinger's small molecule designing suite (Schrodinger, USA) provide end-to-end solutions for SBDD. Open source tools such as the Autodock and Dock programs have been widely used for SBDD. Additionally, scoring functions play important roles in ranking the best poses and selecting novel ligands. Autodock ranks the best posed molecules based on binding free energies using stochastic-based scoring functions. Hence, researchers can successfully used Autodock for the screening of PTP1B inhibitors [124].

Scoring Functions

Scoring functions are mostly used for post-docking analyses. Estimations of protein-ligand fitting and molecular contacts form the focus of drug design processes. Generally, scoring functions are used to calculate the noncovalent interactions. Scoring function encompasses basic characteristics like speed and veracity. Four major types of scoring functions are typically used to describe protein-ligand fitness [125].

Force-field analysis. These functions use force fields to estimate the intermolecular non-bonded interactions like van der Waals and the electrostatics of each atom in binding complexes. When an experiment is carried out in the presence of water, desolvation energies will be taken into account and solved by Poisson–Boltzmann or generalized Born and surface area (PBSA or GBSA) implicit solvation methods. The van der Waals and electrostatic forces and hydrophobicity are optimized to each force field to calculate the binding efficacies of the protein-ligand complex [126]. Energies of binding molecules are estimated by Lennard-Jones terms. Electrostatics are drawn from distance-dependent dielectric constants of columbic formulation of charged interactions, and similarly van der Waals interactions are obtained from hydrogen bond energies and solvation energies. The Medusa Score tool best handles the virtual screening scoring job with the above-mentioned properties [127].

Empirical scoring function. Empirical scoring function is the faster one among all; it calculates the various interactions between interacting atoms of binding molecules and change in solvent-accessible surface area. The empirical terms, such as van der Waals interaction energies, hydrogen-bonding energy, electrostatic energy, hydrophobicity, desolvation energy, and entropy are optimized according to the developing algorithms. Potentially evolved empirical scoring functions fit for SBVS are ID-Score and PLANTS (Protein-Ligand ANT System). ID-Score covers a vast variety of descriptors such as electrostatic interactions, van der Waals interactions, hydrogen-bonding interaction, metal-ligand binding interaction (which is not explored in many scoring functions), π-π stacking interaction, entropic loss effect, desolvation effect, shape matching, and surface property matching, which reduces false positives and redundant molecules [128]. ID-Score uses support vector regression algorithm, and it is outperformed compared with other existing empirical scoring function tools. PLANTS works on the ant colony optimization algorithm [129] and coordinates with various empirical values. It functions well when tested with GOLD from experimentally driven structures [130]. In the context of complex structures like PTPs, we require robust scoring functions to achieve better results.

Knowledge-based scoring function. As mentioned, in name, these scoring functions use statistical structure geometrical data from databases like PDB and differentiate the deviated structures. This method calculates the interactions by potential of mean force, and estimates energies between frequent interactions of certain atom types irrespective of other coordinates [131]. It also uses the

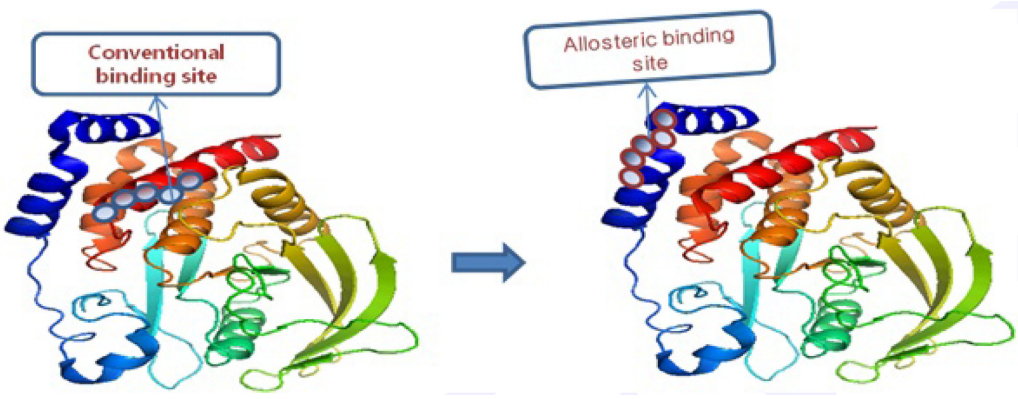


Fig. 3. Schematic illustration of a strategy for showing differences between traditional and allosteric docking modes.

changes in the solvent-accessible surface of binding complexes. The main limitation of this method is calculating the atoms' random state by relying on their standard state irrespective of intermolecular interactions and other parameters. The software tool Drug Score overcomes the limitation and calculates the potentials of distance-dependent pairs, solvent-accessible surfaces, and solvation independent of protonation states, and has succeeded in differentiating structures with RMSD value < 2Å compared with native structures [132].

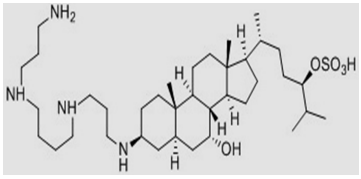
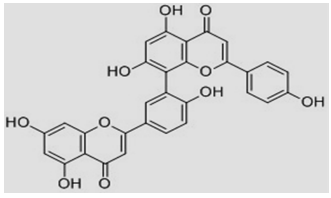
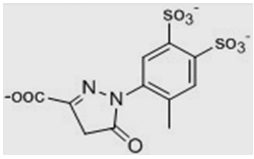
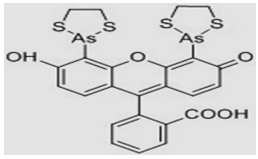
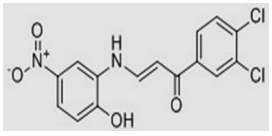
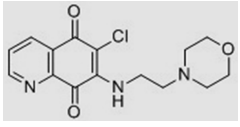
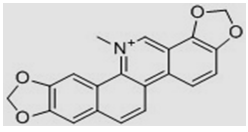
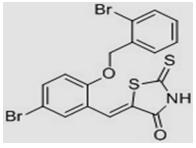
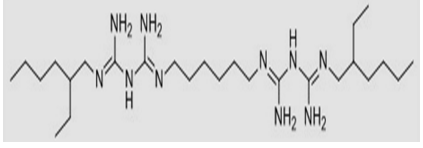
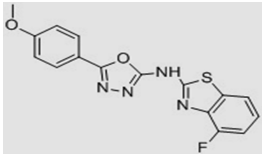
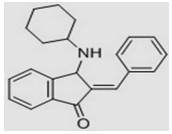
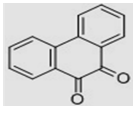
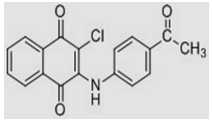
Machine-learning (ML) scoring function. ML-based scoring functions are distinct from classical scoring functions. As these work in feed-forward mode, predetermined forms do not infer with the protein-ligand complexes. ML-based scoring functions use predominantly machine learning algorithms that support vector machines, random forest, multivariate adaptive regression splines, and *k*-nearest neighbors to calculate the optimized parameters chosen by the user. A minor constraint of ML scoring function is the availability of relevant structure data. Comparatively, ML scoring functions predict better than commercially available tools like GOLD, FlexX, and Surflex. ML-based scoring functions outperformed classical scoring functions by 87% in identifying native poses of screened complex data taken from the PDBbind 2007 database [133]. From the observations, ML scoring functions are suitable to incorporate with SBVS, and conserved structures like PTPs could be easily scrutinized in bounded poses from near-native poses with minor deviations [134].

Prospective

Many researchers are focusing on PTP targeting owing to its critical role in innumerable diseases. Thus, numbers of inhibitors are populating everyday, but negligible numbers of compounds are evolving into commercial drugs. Conserved structural features and multitasking ability are confronting the novel inhibitor development for PTPs. Despite of conventional drug targeting, few researchers are concentrating on substitute novel approaches alike allosteric targeting and fragment-based drug design (FBDD). Allosteric docking and FBDD are emerging drug discovery techniques, which differ from traditional methods and are supportive for designing effective inhibitors (Fig. 3). Allosteric targeting is an option that can be used as part of a combinatorial technique for structure-based drug discovery concepts that are more likely for targeting highly conserved molecules such as PTP [135].

Traditional drug designing methodologies aim precisely to design effector candidates, which bind the active site to modulate the activity of target molecules. This phenomenon is not suitable for ubiquitous targets like PTPs and most oncoproteins, since the majority of these proteins or targets are key players in vital cellular functions [136]. Allosteric binding or targeting exclusively concentrates to design the inhibitors aimed to barely bind in the active site. Several studies have suggested that allosteric targeting exhibits profound results. The inhibitor designed for phosphoinositide-dependent kinase-1 (PDK1) successfully interrupted the activity of downstream kinases by binding at the conserved allosteric PDK1 interacting fragment (PIF) binding site or PIF pocket [137]. A study by Lee *et al.* [138] on PTP1B strategically confronted the phosphatase activity without targeting the conserved active site; in this experiment, bioflavonoids were used as ligands and docking results denoted that amentoflavone was preferentially bound to allosteric backbone residue Phe280 by hydrogen bond and adjacent Glu276 by water-mediated hydrogen bond to

Table 3. Protein tyrosine phosphatases with traditional and allosteric inhibitors.

Target	Traditional inhibitor	Allosteric inhibitor	References
PTP1B			[143, 146]
SHP2			[147, 148]
RPTPσ		NA	[149]
CDC25B		NA	[150]
MKP1		NA	[151]
PRL3		NA	[152]
PTPMT1		NA	[153]
DUSP3			[154, 155]
PTPRC1			[145, 156]

counter the PTP1B phosphatase activity [139]. Likewise, ideal studies are emphasizing the significance of allosteric docking, in order to develop persuasive inhibitors for remaining PTPs [140]. Taken from the PTP1B result, in similar fashion, other PTPs such as SHP2, CD45, and DUSP3 (VHR) also underwent allosteric docking for potential distantly binding inhibitory compounds (Table 3).

In conclusion, in recent decades, structural proteomic-

based research is evolving in a new dimension and concentrating on the root cause of diseases, which revealed that abnormal post-translational modifications (PTM) are key players in numerous human diseases. The major PTM across eukaryotes is phosphorylation, which is counter-balanced by the phosphatases. Among protein phosphatases, PTPs are associated with key intra and intercellular mechanisms. Owing to PTP's activity being closely associated with the prime biological mechanism, abnormal expression of PTPs has been observed in threatening diseases like diabetes and numerous cancers, such as breast cancer, prostate cancer, non-small-cell lung cancer, and gastric cancer. As a consequence, PTPs are thought to be promising targets for drug development, but structural challenges and complex functionality hinder the potential target discovery.

Recently, researchers have devised various biochemical and in silico methods to find PTP inhibitors, but their efforts have unfortunately not led to the development and approval of many of these compounds. Recent advances in structural biology changed the face of drug discovery by enabling the precise prediction of target structures for evaluation as part of the drug discovery process. Additionally, computer-aided drug design as a supplement to high-throughput biochemical screening has greatly accelerated potential compound identification. In this review, we addressed the structures and mechanisms of typical PTPs, implications for diseases, and challenges in targeting of appropriate compounds. Furthermore, we discussed the pros and cons of SBVS, and useful scoring functions to design potential small molecules to inhibit PTPs. We have concluded that structure-based development in silico can be a proxy for real-time drug development and may constitute a way to bypass bottlenecks that occur downstream in the investigational pipeline. Owing to robust prediction of every detail of the protein-ligand complex, we predict that SBVS would be a promising technique to design successful inhibitors for highly conserved structures like PTPs.

Although SBVS can be used for the design of PTP inhibitors, the limitations currently outweigh the advantages. A few of the bottlenecks include structural conservation of the proteins, limited entities for analysis, and no prescribed tools and tailored scoring functions. Further advancement in recognizing non-regular binding sites and successfully confronting the activity of receptors using classical docking techniques force as to look into additional advanced methods, such as the allosteric docking strategy. Allosteric docking was found to be appropriate to overcome obstacles presented by structurally conserved PTPs. A study of Lee *et al*. [138] on PTP1B clearly denoted how distant binding sites contribute to the development of novel inhibitory compounds. Other experiments related to PDK1 have disclosed the potential of allosteric docking to structurally challenging targets. In conclusion, well-characterized drug targets are prerequisites for the wise implementation of SBVS, and allosteric docking will be an effective supplementary strategy for designing novel inhibitors of PTPs.

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