Loop Dynamics and Enzyme Catalysis in Protein Tyrosine Phosphatases

Rory M. Crean,† Michal Biler,‡ Marc W. van der Kamp,§ Alvan C. Hengge*,‡ and Shina C. L. Kamerlin*,†

† Science for Life Laboratory, Department of Chemistry − BMC, Uppsala University, BMC, Box 576, S-751 23 Uppsala, Sweden.
§ School of Biochemistry, University of Bristol, Biomedical Sciences Building, University Walk, Bristol BS8 1TD, United Kingdom.
‡ Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322-0300, United States.

*Corresponding Authors
E-mail: alvan.hengge@usu.edu and lynn.kamerlin@kemi.uu.se
Abstract

Protein tyrosine phosphatases (PTPs) play an important role in cellular signalling and have been implicated in human cancers, diabetes, and obesity. Despite shared catalytic mechanisms and transition states for the chemical steps of catalysis, catalytic rates within the PTP family vary over several orders of magnitude. These rate differences have been implied to arise from differing conformational dynamics of the closure of a protein loop, the WPD-loop, which carries a catalytically critical residue. The present work reports computational studies of the human protein tyrosine phosphatase 1B (PTP1B), and YopH from *Yersinia pestis*, for which NMR has demonstrated a link between both their respective rates of WPD-loop motion and catalysis rates, which differ by an order of magnitude. We have performed detailed structural analysis, both conventional and enhanced sampling simulations of their loop dynamics, as well as empirical valence bond simulations of the chemical step of catalysis. These analyses revealed the key residues and structural features responsible for these differences, as well as the residues and pathways that facilitate allosteric communication in these enzymes. Curiously, our wild-type YopH simulations also identify a catalytically incompetent hyper-open conformation of its WPD-loop, sampled as a rare event, previously only experimentally observed in YopH-based chimeras. The effect of differences within the WPD-loop and its neighbouring loops on the modulation of loop dynamics, as revealed in this work, may provide a facile means for the family of PTP enzymes to respond to environmental changes and regulate their catalytic activities.

**Keywords:** Protein Tyrosine Phosphatases • Loop Dynamics • Enhanced Sampling Simulations • Empirical Valence Bond • Enzyme Catalysis
Introduction

Protein tyrosine phosphatases (PTPs) are a superfamily of regulatory enzymes that play a key role in cellular signaling.\(^1\) As a result, these enzymes have been implicated in a wide variety of disorders, including type 2 diabetes\(^2\) and cancer,\(^3\) and have therefore been the subject of substantial biomedical research effort as potential drug targets.\(^4\) Members of this superfamily share a unique HCXXGXXRRS(T) “P-loop” signature motif at their active sites (HC(X\(_3\))R, where X is any residue). They catalyze dephosphorylation via a two-step "ping-pong" mechanism that is shared among PTPs (Figure 1), in which the thiol group of the conserved cysteine of the PTP signature motif (Cys215 using protein tyrosine phosphatase 1B - PTP1B - numbering) first acts as a nucleophile, in order to form a covalently bound thiophosphate enzyme intermediate, which is then hydrolyzed through nucleophilic attack by an active site water molecule.\(^5\) Further catalytic assistance is provided by the active site arginine present on the P-loop - Arg221 using PTP1B numbering. Furthermore, critical to this process is acid-base catalysis via a conserved aspartic acid, Asp181 in PTP1B, which acts as a general acid to protonate the leaving group in the first (cleavage) step of the PTP-catalyzed reaction, and subsequently as a general base to activate the nucleophilic water molecule for hydrolysis of the phospho-enzyme intermediate in the second step of the reaction mechanism. This aspartic acid lies on a conserved loop, the WPD-loop, which undergoes a large (~10 Å) conformational change upon substrate binding, from a catalytically inactive "open" conformation, to a catalytically active "closed" conformation, which brings this aspartate into position for acid/base catalysis (Figure 1). The nucleophilic water in the second step is positioned for attack by a glutamine on the “Q-loop” (Figure 1C).
The dynamics of WPD-loop motion have been the subject of significant research effort.\textsuperscript{6,8-15} In particular, a detailed NMR study\textsuperscript{9} of two related PTPs, the human phosphatase PTP1B\textsuperscript{1} as well as YopH, a virulence factor from \textit{Yersinia},\textsuperscript{16} found an important role for the conformational dynamics...
of this loop in regulating phosphoryl transfer in these two enzymes, with rates of loop closure that mimicked the rates of phosphoester bond cleavage. Despite PTP1B and YopH having relatively low sequence conservation (20.6% sequence identity as determined by the T-Coffee webserver\cite{17}), they and other members of the PTP superfamily share highly superimposable active sites, the same mechanisms and transition states, and the same rate-limiting chemical steps.\cite{12} This is significant because catalytic rates across the superfamily vary by several orders of magnitude.\cite{12} Further evidence for the role of loop motion in regulating PTP catalysis comes from NMR dynamics studies of several PTP1B point variants, in which a correlation was observed between the rate of loop opening/closure and the rates of both chemical steps.\cite{14}

Whilst YopH is the one of the most proficient PTPs,\cite{18} with $k_{\text{cat}}$ values $\approx 1300\ \text{s}^{-1}$ for the hydrolysis of monoester dianions at its pH optimum,\cite{19} PTP1B’s rate of catalysis is significantly slower, with $k_{\text{cat}}$ values of $\sim 40\ \text{s}^{-1}$ at its pH optimum.\cite{20} Finally, a combined kinetics, isotope effect and X-ray crystallography study has shown that the precise molecular details of WPD-loop movement differ between PTP1B and YopH,\cite{21} as does the tolerance of the two loops to mutations, again strengthening the notion that differences in WPD-loop dynamics are important for regulating catalysis.

Despite extensive experimental and computational studies,\cite{6,9,10,13,14,21-23} it remains unclear why the catalytic rates of different members of the PTP superfamily are so different from each other, although the high structural conservation between these enzymes strongly points towards differences in the rates and dynamics of WPD-loop motion within the superfamily. This study focuses specifically on PTP1B and YopH, two of the best-characterized members of the PTP superfamily.\cite{5,6,9,10,12-14,21-24} In particular, our goal in this work is to understand why the rates of WPD-loop motions differ so significantly between the two enzymes,\cite{9} as well as exploring their
correlation with the first chemical step of catalysis, as described experimentally in ref. 9. We are also interested in understanding whether WPD-loop closure proceeds as a rigid-body motion, or whether loop motion is more flexible with multiple conformational states (as observed in our recent related work on triosephosphate isomerase\textsuperscript{25}), and whether there are differences in flexibility between the two enzymes.

Furthermore, a recent study on WPD-loop chimeras of YopH and PTP1B (using the YopH scaffold with varying segments of the PTP1B WPD-loop sequence transposed in), demonstrated that some of these chimeras could adopt several different “hyper-open” WPD-loop conformations, in which the C-terminal portion of the WPD-loop is extended.\textsuperscript{12} While no crystal structures of the native enzymes show such conformations, it is unknown whether these conformations exist in solution and, if so, what, if any, functional relevance these hyper-open conformations have. We note, in this context, that an atypical catalytically inactive open state has also been observed in three other PTPs from different subgroups (STEP, LYP and GLEP1),\textsuperscript{26} further supporting that these hyper-open conformations are not artefactual but rather have functional relevance for the superfamily as a whole. In addition, while it is clear that WPD-loop closure is essential for the rapid first step of catalysis, it is unclear whether further protein motions occur later in the mechanism.

We note that while both enzymes have been the subject of significant computational work, in particular for drug discovery efforts, advanced studies of their loop dynamics have been limited in scope\textsuperscript{10,13,27} and there currently exists no comparative computational study of the two systems. To this end, in the present study, we have performed extensive conventional molecular dynamics (MD) simulations coupled with advanced enhanced sampling simulations. Using Hamiltonian replica exchange molecular dynamics (HREX-MD)\textsuperscript{28} and parallel tempering metadynamics...
simulations performed in the well-tempered ensemble (PT-MetaD-WTE), we characterize the free energy landscapes of loop motion in both enzymes, and how these loop dynamics are regulated by the remainder of the protein scaffold. We further complement our approach by performing empirical valence bond (EVB)\textsuperscript{29,30} simulations on both chemical steps of catalysis, to provide insight into the link between loop dynamics and the regulation of the phosphoryl transfer reaction catalyzed by these enzymes. Our study provides key insights into both the dynamical and chemical aspects of PTP catalysis, and therefore broadens our insight into these enzymes from a fundamental enzymology perspective, as well as providing valuable input for future drug discovery and enzyme engineering efforts on these biomedically critical enzymes.

Methodology

Crystal Structure Analysis

Crystal structures of PTP1B and YopH were obtained from the RCSB Protein Data Bank (PDB).\textsuperscript{31} Structures with missing residues within the residue range Glu2-Ile281 for PTP1B and Ser187-Lys456 for YopH were not taken forward for further analysis, to allow Principal Component Analysis (PCA) to be performed over these regions. This resulted in a total of 231 and 16 structures of PTP1B and YopH being included in PCA respectively, with 3 additional YopH-PTP1B chimera structures also included. A complete list of all PDB structures used for the analysis of each enzyme is provided in Table S1. PCA was then performed on these structures using the CPPTRAJ\textsuperscript{32} module that is part of the AmberTools18\textsuperscript{33} suite of programs, by first reformatting all structures into Amber-readable format (through a combination of shell scripts and the pdb4amber program within AmberTools18\textsuperscript{33}), and subsequently performing PCA on the $C_\alpha$ carbon atoms of the residues selected for the analysis. PCA was performed by first generating an average structure
based on all the X-ray crystal structures used, and then performing root mean square (RMS) fitting of all structures to this average structure for the subsequent PCA. Several different RMS fitting procedures were used depending on the analysis being performed (for example, whether the PCA was being performed on both PTP1B and YopH simultaneously or independently), and the specific residues chosen for all analyses are provided in the Supplementary Methodology.

System Preparation for Conventional and Enhanced Sampling Molecular Dynamics Simulations

A total of six crystal structures\(^5,7,34,35\) were used in this study to generate the starting points for simulations of the unliganded forms, Michaelis complexes with \(p\)-nitrophenyl phosphate (\(p\)NPP) and covalent phospho-enzyme intermediates for PTP1B and YopH, with their WPD-loops in both their open and closed conformations (see Table S2 for a full list of structures used, and the modifications required for preparation of each system). In brief, simulations at the \(p\)NPP bound Michaelis complexes were prepared by modifying structures with a peptide inhibitor bound, while simulations of the phospho-enzyme intermediates were prepared by modifying structures with a vanadate ion bound to the active site (by replacing the vanadate ion with a phosphate group). Simulations of both the unliganded enzymes and the Michaelis complexes were performed with the catalytic aspartic acid (Asp181 in PTP1B and Asp356 in YopH, respectively) protonated and the cysteine nucleophile deprotonated, whilst simulations of the phospho-enzyme intermediate were performed with the catalytic aspartic acid on the WPD-loop in its standard deprotonated form (protonation states selected to be in line with the chemical mechanism depicted in Figure 1). Care was taken to ensure the protonation and tautomerisation of all other residues were kept consistent for all simulations, based on a combination of empirical screening using PROPKA\(^{36}\) v3.1 and visual inspection. Based on a predicted \(pK_a\) of 8.27 for both the WPD-loop open and closed conformations (using both conformations available in PDB ID: 6B90), Glu102 of PTP1B was
protonated in all our simulations. Finally, tautomerisation states and any required Asn or Gln side chain “flips” were predicted using the MolProbity\textsuperscript{37} server, with the results visually inspected to ensure consistency across all simulations (tautomerisation states used for all simulations are provided in Table S3). For all conventional and enhanced sampling MD simulations, structures were solvated in an octahedral water box, with the box size selected such that no protein atom was within 10 Å of the box boundary, and Na\textsuperscript{+} or Cl\textsuperscript{−} counterions were added as required to ensure overall charge neutrality of the system. System solvation and other simulation preparations for our EVB simulations are described separately in the section \textbf{Empirical Valence Bond Simulations}.

\textit{Ligand Parameterization for Conventional and Enhanced Sampling Molecular Dynamics Simulations}

Partial charges for the ligand, pNPP (modelled in its dianion form), were calculated using the standard restrained electrostatic potential (RESP) protocol, using Antechamber.\textsuperscript{38} The electrostatic potential of pNPP was determined \textit{in vacuo} at the HF/6-31G(d) level of theory, using Gaussian 16 Rev. A.03,\textsuperscript{39} after having performed geometry optimization at the same level of theory. All other force field terms for pNPP were then described using the general Amber force field 2 (GAFF2),\textsuperscript{40} with relevant parameters provided in Table S4. In the case of the phosphorylated cysteine residue, geometry optimization of this residue was performed at the MP2/6-311G(d,p) level of theory, using capping acetyl (ACE) and N-methylamide (NME) capping groups, and implicit solvent, using the conductor-like polarizable continuum model (CPCM).\textsuperscript{41} As with pNPP, charges were determined in vacuo at the HF/6-31G(d) level of theory, using Gaussian 16 Rev. A.03.\textsuperscript{39} Further, the charges of the main chain and capping group atoms were kept constant (in order to match the charges of deprotonated cysteine from the Amber ff14SB force field\textsuperscript{42}), and only the side chain charges were allowed to change. All other parameters were taken directly from the ff14SB force
field, where possible, and any remaining missing terms were obtained using GAFF2. The parameters used to describe the covalent phospho-enzyme intermediate are provided in Table S5. These parameters were then used for all conventional and enhanced sampling molecular dynamics simulations. In the case of the EVB simulations, these simulations were performed using the OPLS-AA force field for consistency with previous related studies, and therefore separate EVB parameters were derived as described in the section Empirical Valence Bond Simulations.

Conventional Molecular Dynamics Simulations

All conventional MD simulations were performed using the GPU-accelerated version of Amber16, with the protein and water molecules described using the ff14SB force field and the TIP3P water model, respectively. Simulations of all 12 different systems of interest here (unliganded, pNPP bound Michaelis complexes and phospho-enzyme intermediates, starting from both open and closed conformations of the WPD-loops of both PTP1B and YopH) were performed for 25 x 200 ns each, in the NPT (300 K, 1 atm) ensemble. In order to prepare for production quality MD simulations, we used a previously described minimization, heating and equilibration procedure, that is described in full in the Supplementary Methodology. All production quality MD simulations were performed using a 2 fs time step, with the SHAKE algorithm used to constrain all bonds containing hydrogen atoms. The temperature and pressure during the simulations were regulated using Langevin temperature control (collision frequency of 1 ps$^{-1}$), and a Berendsen barostat (pressure relaxation time of 1 ps), respectively. All simulations used an 8 Å direct space non-bonded cutoff, with long range electrostatics being evaluated using the particle mesh Ewald algorithm. Four distance and one angle restraint(s) were applied during simulations of the Michaelis complex to maintain the pNPP substrate in a catalytically competent
configuration throughout the MD simulations (see Table S6 for a full list of restraints applied). Equivalent restraints were put in place for both PTP1B and YopH simulations, and we note that no restraints were placed between the substrate and the WPD-loop to ensure full conformational freedom of this loop. These restraints were only applied to the Michaelis complex simulations to prevent dissociation of pNPP from the active site upon WPD-loop opening, and were used for both the conventional MD simulations and the parallel tempering metadynamics simulations.

*Hamiltonian Replica Exchange Molecular Dynamics Simulations*

Hamiltonian replica exchange molecular dynamics (HREX-MD),\(^{28}\) which has been described in detail elsewhere (see ref. 54), is an enhanced sampling simulation technique that provides an unbiased approach to increase the rate of sampling of the free energy landscape for a region of interest. In the present work, HREX-MD simulations were performed on the unliganded enzyme structures of PTP1B and YopH, using the systems prepared as described in the section *Preparation of Conventional and Enhanced Sampling Molecular Dynamics Simulations*. All HREX-MD simulations were performed using the Amber ff99SB-ILDN\(^ {55}\) force field and TIP3P\(^ {48}\) water model as implemented into GROMACS 2018.4,\(^ {56}\) interfaced with PLUMED v2.5\(^ {57}\) (the AMBER ff99SB-ILDN was chosen over the AMBER ff14SB for HREX-MD simulations as only force fields embedded into the GROMACS software can be used for this simulation methodology). Following a standard minimization, heating and equilibration procedure (described in detail in the *Supplementary Methodology*), two production quality HREX-MD simulations of 500 ns per replica (over 8 replicas, see below) were performed for PTP1B and YopH each, with one simulation starting from structures with the WPD-loop in its closed conformation, and the other starting from a loop-open conformation to maximize the potential sampling of the conformational space. All HREX-MD simulations were performed in the NPT ensemble, using a 2 fs time step
and the P-LINCS algorithm\(^5\) to restrain all bonds to hydrogen atoms. A 10 Å non-bonded interaction cut-off was used to evaluate long range electrostatic interactions, using the Particle Mesh Ewald (PME) algorithm.\(^5\) The simulations were performed using Langevin temperature control\(^5\) (collision frequency of 1 ps\(^{-1}\)) and with pressure regulated using a Parrinello-Rahman barostat\(^5\) (pressure relaxation time of 1 ps).

When setting up the HREX-MD simulations, a generous definition of the WPD-loop was used to define the residues included in the "hot region" of the simulations (i.e., residues 175-191 in the case of PTP1B, and 349-365 in the case of YopH). The Hamiltonian of the hot region was then modified by scaling the partial charges by √\(\lambda\), the Lennard Jones parameters by \(\lambda\), and the dihedral terms by \(\lambda\) (with the exception of the first and last residues of the "hot region", which were scaled by √\(\lambda\)). A total of 8 replicas, with \(\lambda\) values exponentially scaled between 1.0-0.6 (\(\lambda\) values of 1.0, 0.930, 0.864, 0.803, 0.747, 0.694, 0.645, 0.6 were used), resulting in an effective temperature range of 300-500 K. Exchanges between replicas were attempted every 1 ps, achieving an average exchange rate of 40% for PTP1B and 38% for YopH. Subsequent analysis was performed solely on the neutral replicas (\(\lambda=1\)).

*Parallel Tempering Metadynamics Simulations*

Parallel tempering metadynamics performed in the well-tempered ensemble (PT-MetaD-WTE)\(^6\) is a form of metadynamics simulation well suited to describe complex reaction coordinates such as protein conformational changes. During conventional metadynamics\(^6\) simulations, two or three collective variables (CVs) are normally chosen to describe the process under investigation, with each CV being biased by a history dependent Gaussian-type bias. In PT-MetaD-WTE,\(^6\) multiple metadynamics simulations are propagated in parallel with each replica assigned a different temperature (in our case temperatures of 300, 312.62, 325.78, 339.47, 353.75,
368.63, 384.13 and 400.29 K were used) and exchanges between adjacent replicas are attempted periodically. By performing simulations in the well-tempered ensemble (WTE), we are able to enhance the energy fluctuations of each replica whilst still keeping the average close to the canonical value. This in practice means one can use a much smaller number of replicas over a given temperature range and still achieve a reasonable exchange rate as compared to standard PT-MetaD.\textsuperscript{50,61} PT-MetaD-WTE simulations were performed with GROMACS 2018.4,\textsuperscript{56} interfaced with PLUMED v2.5,\textsuperscript{57} using the Amber ff14SB\textsuperscript{42} force field and the TIP3P\textsuperscript{48} water model (systems were setup with “tLeap” in AmberTools\textsuperscript{18} and then converted to a GROMACS compatible format using the program “parmed”, also in AmberTools\textsuperscript{18}).

Following initial structure preparation (see the section \textbf{Preparation of Conventional and Enhanced Sampling Molecular Dynamics Simulations} and equilibration for NPT simulations (see the \textbf{Supplementary Methodology}), PT-MetaD-WTE simulations were performed on all of the unliganded, \textit{p}NPP-bound Michaelis complex, and phospho-enzyme intermediate states of both PTP1B and YopH (i.e., 6 systems in total). After the equilibration of each replica to its target temperature, PT-MetaD-WTE simulations were performed in two stages. First, a 10 ns long PT-MetaD simulation was performed with a bias potential placed on the potential energy of the system. In this step the bias factor, gaussian height and gaussian deposition rates was set to 60, 0.6 kcal mol\textsuperscript{-1} and 1 ps for both PTP1B and YopH respectively. The gaussian widths were set to 100 kcal mol\textsuperscript{-1} and 110 kcal mol\textsuperscript{-1} for PTP1B and YopH simulations respectively. In the second step, the bias on the potential energy was retained (in order to run simulations in the WTE) but no additional gaussians were deposited onto this CV. Instead, three CVs were chosen to describe WPD-loop motion (see below) for the production PT-MetaD-WTE simulations, which were run for between 700-800 ns per replica. Simulations were stopped once they had been observed to be converged,
which was assessed by the analysis of the time evolution of the free energy profiles (Figures S1 and S2), alongside the clear observation of diffusive dynamics along each CV (see Figures S3 and S4).

Exchanges between replicas were attempted every 1 ps, and all six PT-MetaD-WTE simulations had exchange rates between 21-29%. The 3 CVs chosen (Figure S5 and Tables S7 and S8) were used to enable sampling of the free energy landscape of WPD-loop motion. CV1 was the interloop distance-RMSD (DRMSD) of the Cα atoms of the WPD- and P-loops, with the closed crystal structures used as the reference structure. CV2 and CV3 describe the motions in the central and C-terminal portions of the WPD-loop respectively through a center of mass distance measurement between the WPD-loop residues to atoms on the P- or Q-loops (which are highly rigid). Gaussians with an initial height of 0.2 kcal mol⁻¹ were deposited every 2 ps, with the gaussian height gradually reduced over the course of the simulation by using a bias factor of 12. Gaussians widths were set to 0.1, 0.3 and 0.3 Å respectively, and wall potentials were used to prevent the sampling of non-relevant states (walls were placed to prevent sampling of states beyond those seen in the HREX-MD simulations, see Supplementary Information). All analysis was performed on the replica simulated at 300 K and simulations were reweighted and projected onto unbiased CVs using the approach described by Tiwary and Parrinello. The minimum free energy pathway (MFEP) analysis was performed using the Minimum Free Energy Path Surface Analysis (MEPSA) approach.

Analysis of Conventional and Enhanced Sampling Molecular Dynamics Simulations

Unless stated otherwise, all analysis of all conventional and enhanced sampling molecular dynamics simulations was performed using the CPPTRAJ module that is part of the AmberTools suite of programmes. All hydrogen bonds formed between the WPD-loop and
the remainder of the enzyme were identified using a donor-acceptor distance cut-off of 3.5 Å, and a donor-hydrogen-acceptor angle of $180 \pm 45^\circ$. Only hydrogen bonds with an occupancy of $>1\%$ of the cluster simulation time were included in the subsequent analysis. PCA on the trajectories was performed in the same manner as described in the structural analysis section (RMS fit to a stable region of the enzyme, generate average structure, RMS fit to the average structure and then perform PCA on the $C_\alpha$ carbons of this trajectory).

Dynamic cross correlation matrices (DCCMs) and average inter-residue distance matrices of the PT-MetaD-WTE trajectories were computed using CPPTRAJ$^{32}$ for the $C_\alpha$ of every residue. Prior to the DCCM calculation, the same RMS fitting procedure as used for PCA calculations was applied. Shortest path maps (SPM)$^{66}$ were determined using the DCCM and average inter-residue distance matrices for the simulations of PTP1B and YopH with $p$NPP-bound to the active site, using the available python script.$^{66}$

**Empirical Valence Bond Simulations**

The EVB$^{29}$ approach has been used extensively to describe phosphoryl transfer reactions,$^{25,46,67}$ including in computational studies of PTP mechanisms.$^{68,69}$ In this work, we have performed EVB simulations of both chemical steps of the reactions catalyzed by PTP1B and YopH (Figure 1). Our starting points for these simulations were PDB IDs: 3I7Z$^5$ and 1QZ0$^{35}$ to describe the cleavage step, and 3I80$^5$ and 2I42$^7$ to describe the hydrolysis step in the reactions catalyzed by PTP1B and YopH, respectively.

In brief, system preparation and initial equilibration for EVB simulations was performed as described in the **Supplementary Methodology**. Each system / reaction step was simulated using 30 individual replicas. Each replica was first equilibrated for 30 ns at the approximate transition state ($\lambda = 0.5$), with the subsequent EVB trajectories propagated downhill from the transition state.
in both the reactant and product directions, following our previous work. Each EVB simulation was performed in 51 individual mapping windows of 200 ps in length per trajectory. This led to total cumulative equilibration and EVB simulation timescales of 1.8 and 0.612 µs per enzyme over all individual replicas and both reaction steps (cleavage and hydrolysis), respectively. We note that the active site microenvironment in PTPs causes a substantial reduction in the pKₐ of the catalytic cysteine relative to free cysteine in solution, to experimentally determined values of 4.67 in YopH and 5.6 in a related PTP, vaccinia H1-related PTP (VHR). This means that no thermodynamic correction would need to be applied for the deprotonation of the active site cysteine as the deprotonated form will dominate at ambient pH.

All EVB simulations were performed using the Q6 simulation package and the OPLS-AA force field. All EVB parameters necessary to reproduce our work, as well as a detailed description of the computational methodology and subsequent simulation analysis can be found in the Supplementary Methodology.

Results and Discussion

*Analysis of Available Experimental Structural Information*

In order to lay the groundwork for our subsequent simulations, as our starting point, we performed a detailed analysis of the conformational diversity of the WPD-loops of PTP1B and YopH based on structures available in the PDB. Our focus was on distinguishing between different closed and open conformations of the loop, including a non-productive "hyper-open" conformation of the WPD-loop observed in YopH-PTP1B chimeras in which some WPD-loop residues of YopH were replaced with those of PTP1B.
We performed combined principal component analysis (PCA) on the WPD-loop conformations of a total of 250 structures of PTP1B, YopH and YopH-PTP1B chimeras (231, 16 and 3 structures each respectively), as described in the Methodology section (Figure 2). This analysis produces orthogonal eigenvectors (principal components, PCs) that describe the variance of the different structures, with the PCs produced ranked by the amount of variance they describe in the given dataset. PCA was performed in Cartesian coordinate space on the C\_\alpha carbons of the WPD-loops of all structures studied. We then projected the coordinates of each crystal structure onto the first three PCs (Figure 2), which describe 96.2\% of the total variance observed in the data, with all remaining PCs providing a relatively small contribution (Figure S6). Analysis of the PC projections and each PCs' corresponding mobility plot (Figures 2C-F) demonstrates that the first principal component, PC1, describes the open-to-closed transition of the WPD-loop. This is further validated by the well-defined partition of the open and closed crystal structures of PTP1B and YopH along PC1 (we note here that PDB ID: 1QXK\textsuperscript{75} represents a semi-closed conformation of PTP1B, induced by the presence of an active site inhibitor). PC2, on the other hand, appears to largely describes differences in the structure of the residues that anchor to the C-terminal portion of the WPD-loop (the \(\alpha\)4- and \(\alpha\)3-helices of PTP1B and YopH respectively), thus partitioning the PTP1B, YopH and YopH-PTP1B chimeras into three clearly defined groups. Unlike PC2, PC3 describes variance primarily in the central portion of the WPD-loop, but, like PC2, it does appear to describe structural differences between PTP1B, YopH and YopH-PTP1B WPD-loops, and not the open-to-closed transition.
**Figure 2.** Principal component analysis (PCA) on the WPD-loops of the crystal structures of PTP1B, YopH and the YopH-PTP1B chimeras (for details see main text). (A, B) Projections of crystal structures onto the first three principal components (PCs), with the total variance described by each PC indicated in brackets in the axis labels. (C) Relative mobility of each residue in each PC projection, with residue numbers and names provided above and below respectively for both PTP1B and YopH. (D–F) Projection of PCs 1-3 onto a representative structure of PTP1B. The color gradient on the WPD-loop indicates moving from negative (red) to positive (blue) values along the given PC (as seen in panels A, B). The catalytic cysteine (yellow) and aspartic acid are shown as spheres for reference, and the locations of the β10-strand and α4-helix on PTP1B (which precede and follow the WPD-loop residues respectively) are indicated in all three panels.

In summary, our PCA calculations on available structural data suggest that the open-to-closed transition of both the WPD-loops of PTP1B and YopH can largely be described by a single PC. To further investigate this observation, we performed PCA analysis on the WPD-loops of the structures of PTP1B and YopH individually (**Figure S7**). In both cases, we observe the first PC to correspond to loop opening/closure, and to be able to describe the large majority of the variance.
between the different structures (96.7% and 91.9% of the variance in the data for PTP1B and YopH, respectively, see Figure S6). Interestingly, a comparison of the mobility plots for the individual PC1 projection data (Figure S8) shows that the majority of changes in both WPD-loops are focused on the central portion of the WPD-loop (the two residues either side of PTP1B residue G183, and the equivalent YopH residue T358). However, PTP1B shows a smaller second peak, towards the C-terminus of the WPD-loop, which is of particular interest in light of the fact that both residues in this second peak (E186 and S187) flank proline residues, and are therefore likely to have restricted mobility. These insights may provide some rationale as to why the movement of the WPD-loop of YopH is so much faster than that of PTP1B. That is, the bi-modal distribution of mobility over multiple PTP1B residues (as opposed to the mono-modal distribution observed in YopH), combined with the increased number of pre- or post-proline residues that show significant mobility over PC1 (describing loop motion, of which there are 3 in PTP1B and only 1 in YopH), would point towards likely slower loop motion in PTP1B than in YopH.

Hamiltonian Replica Exchange Molecular Dynamics Simulations

Despite the fact that our PCA (which was performed on a broad range of crystal structures) suggests that the differences in dynamics of the WPD-loops of both PTP1B and YopH can be well described by a single PC (i.e., a vector), this does not mean that the conformational change from the open-to-closed conformations of the loop is simple in solution. That is, we have previously shown that even in the textbook example of the closure of the catalytic loop of triosephosphate isomerase, which has been often argued to occur as a two-state rigid-body loop motion, the loop dynamics are complex, exhibiting high flexibility and sampling of multiple conformational substates. To further explore the loop dynamics of PTP1B and YopH, we therefore turned to both conventional and enhanced sampling molecular dynamics (MD) simulations, including HREX-
MD simulations of WPD-loop motion in the unliganded-enzyme forms of PTP1B and YopH (8 µs simulation time per PTP, see the Methodology section). A comparison of the conformations sampled by both enzymes’ WPD-loops in our HREX-MD simulations suggests that the WPD-loop of YopH has both higher mobility and/or a larger accessible conformational space than PTP1B (Figures 3A and B). This is supported by Cα RMSF calculations of both enzymes, which show the WPD-loop of YopH to have significantly greater mobility than that of PTP1B, alongside being generally being more mobile in the remainder of the enzyme (Figure S9).

Figure 3. Extensive sampling of the free energy landscapes of the WPD-loops of PTP1B and YopH using HREX-MD simulations. (A, B) Snapshots from the HREX-MD simulations of PTP1B (A) and YopH (B), showing the diverse conformations sampled by the WPD-loop during the simulations. The WPD-loop residues are colored from red (most
flexible) through white and to blue (least flexible) according to their calculated $C_\alpha$ RMSF (shown in graphical form in Figure S9). This shows that the WPD-loop in YopH is more flexible and samples a broader range of conformational space than that of PTP1B. The catalytic Asp on the WPD-loop is shown as a sphere on this plot for reference. (C, D) 2D PMF plots projected onto the first two PCs of the WPD-loop for (C) PTP1B and (D) YopH, respectively (PCA was performed on the two enzymes separately). The corresponding X-ray crystal structures are projected onto each plot as small grey dots, with structures corresponding to closed, open and hyper-open states indicated. (E) The percentage of snapshots which are in an $\alpha$-helical configuration for the WPD-loops and subsequent $\alpha$-helices of PTP1B and YopH. (F) Representative structure of the “hyper-open” conformation of the WPD-loop adopted by YopH in which residues up to T358 are in an $\alpha$-helical conformation. The hyper-open conformation is colored cyan, whilst the open and closed state conformations are colored in dark blue and orange for reference. Two key side chain interactions which help stabilize this configuration are shown (see main text for further details).

In order to characterize the WPD-loop conformations sampled during our HREX-MD simulations, we performed PCA on each PTP individually (Figures 3C and 3D), and used the resulting statistics to calculate 2D potential of mean force (PMF) plots (1D PMF plots showing converged sampling of WPD-loop motion for both PTPs are provided in Figure S10). PC1 for both PTP1B and YopH largely corresponds to WPD-loop opening and closure as evidenced by the groupings of closed and open X-ray structures along PC1 for both PTPs (Figure 3C and D). It is also clear that both PTPs can sample a wide variety of open conformations which can also be notably “more open” (i.e., further from the crystal structure closed conformation) than the open crystal structures.

Interestingly, as we observed in the structural PCA performed on the WPD-loop residues (discussed above, Figure S8), the mobilities obtained for PC1 from the HREX-MD data also show a clear mono-modal vs bi-modal distribution of mobilities for the WPD-loop residues of YopH and PTP1B respectively (Figure S11). PC2 in PTP1B corresponds to changes in the central portion of the WPD-loop, and to a lesser extent the N-terminal residues, whilst PC2 in YopH describes a
broader movement of the WPD-loop from the “open” state to another energy minima, referred to as the “hyper-open” conformation. In this hyper-open conformation, the α-helix connected to the C-terminal portion of the WPD-loop is extended by four residues (normally beginning at Ser362 in the WT closed and open states, in contrast with Thr358 in the hyper-open conformation, see Figures 3E and F), and has previously only been observed in the crystal structures of two YopH-PTP1B chimeras, in which the YopH WPD-loop is partially swapped for that of PTP1B.12

These results therefore suggest that this hyper-open loop conformation is already sampled in the WT-YopH structure, albeit as a rare event (only ~1.1% of simulation time as determined from analysis of the % α-helical content of the WPD-loop residues, see Figure 3E). The WPD-loop in the two hyper-open crystal structures (PDB IDs: 6DR112 and 6DT612) slightly differ from one another (WPD-loop backbone RMSD of 1.38 Å) and our simulations of WT-YopH show both crystallographically observed hyper-open conformations can be readily sampled (Figure S12). Specifically, the snapshot with the lowest RMSD to each PDB has an RMSD of 0.95 Å and 1.03 Å to PDB IDs: 6DR112 and 6DT612 respectively (see Figure S12). Our PMF profile of the YopH WPD-loop (Figure 3D) also demonstrates that conformational exchange between the closed and hyper-open conformations occurs via passing through the open state, which (unlike what the names may suggest) is not obvious from the crystal structures alone. Finally, we calculated how the hydrogen bonding network of the WPD-loop differs for the closed, open and hyper-open states of YopH (Figure S13). Alongside the new interhelical hydrogen bonds formed through the extended α-helix, the analysis showed the side chain carbonyl of the Gln357 caps the positive dipole at the end of the helix, as well as a high occupancy hydrogen bond between Thr358 and Asp452 on the α6-helix (as depicted in Figure 3F).
Comparison of the WPD-loop sequences (see Figure 1B) helps rationalize why WT-YopH but not WT-PTP1B appears to be able to adopt this hyper-open conformation. That is, Pro188 in PTP1B (equivalent to Glu363 in YopH) likely acts as a “helix-breaker”, preventing the extension of the α-helix beyond residue Ser187 as seen in our simulations of WT-PTP1B (see Figure 3E). The lack of a proline at this position of the WPD-loop in YopH (and in both chimeras crystallized with hyper-open conformations12) may therefore provide the necessary conformational flexibility to form this extended helix conformation. It is interesting to note that a recent NMR dynamics study of the PTP1B point variant P188A identified 2 exchange processes for the WPD-loop as opposed to 1 for WT-PTP1B and the 4 other point variants included in the study.14 Taken together, our simulations suggest this second/new process identified in the P188A PTP1B variant may correspond to conformational exchange between the open and (now accessible) hyper-open states of the WPD-loop.

Parallel Tempering Metadynamics Simulations

In order to obtain deeper insight into the free energy landscape underlying WPD-loop dynamics, we extended our study by performing parallel tempering metadynamics simulations in the well-tempered ensemble (PT-MetaD-WTE),60,61 simulating the unliganded, pNPP-bound Michaelis complexes, and phospho-enzyme intermediate states of both PTP1B and YopH. Here, PT-MetaD-WTE (which combines temperature based replica exchange with metadynamics simulations) is particularly useful method for sampling complex reactions coordinates such as the protein conformational change simulated here,79-81 as any slow degrees of freedom not well described by the chosen metadynamics CVs will still have their sampling enhanced through the use of temperature based replica exchange.
For all simulations of both PTP1B and YopH, we used 8 replicas spanning a temperature range of 300-400 K and 3 collective variables (CVs) to describe WPD-loop motion (See **Methodology section**). Analysis of time evolution of the free energy profiles and the observation of diffusive dynamics along each CV (**Figure S1-S4**) demonstrated good convergence of all six systems after between 700-800 ns of simulation time per replica. Whilst our CVs allow for efficient sampling of the WPD-loop’s accessible conformational space, they do not clearly distinguish between the open and closed WPD-loop states (2 out of the 3 of the CVs are combinations of main and side chain atom distances, See **Figure S5** for further explanation). We therefore reweighted our PT-MetaD-WTE simulations, projecting the underlying free energy landscape onto the inter-distance RMSD between the WPD- and P-loops (i.e. CV1) and the fraction of native contacts\(^82\) between the WPD- and P-loops (**Figure 4**). This allowed us to clearly distinguish between the closed and open state(s) of the WPD-loop alongside constructing a minimum free energy pathway (MFEP) between both states. We caution that the transition state (TS) barrier obtained for complex conformational changes like this is highly sensitive to the reaction coordinate(s) used to project the free energy landscape, and should be considered an approximation of the TS.\(^83,84\) Instead, in the following sections we use our obtained MFEPs to describe the structural features along the loop opening/closing process.
Figure 4. Changes in the WPD-loop free energy landscape over the catalytic cycle of PTP1B and YopH as determined by our PT-MetaD-WTE simulations. (A-C) PMFs of the unliganded, pNPP bound, and phospho-enzyme intermediates states of PTP1B and (D-F) the same but for YopH. The x-axis is the metadynamics simulations collective variable 1 (CV1), which is the inter-distance RMSD (DRMSD) between the WPD-loop and P-loop C\textalpha{} carbons (using the closed conformation as the reference state). The y-axis is the fraction of native contacts formed between the C\textalpha{} carbons of the WPD-loop and P-loop (values decreasing from 1 mean a move away from the reference state, the closed X-ray structure, defined in detail in the Supplementary Methodology). In all cases the minimum free energy pathway (MFEP) obtained from moving between the closed and open states is plotted as a black line. The minimum energy values of the closed and open states are indicated, alongside the size of the pseudo TS barrier (grey diamond) between the two states. A representative X-ray structure of the open (PTP1B: 6B90 and YopH: 1YPT) conformation is shown as a red triangle. Note that the closed state (PTP1B: 6B90 and YopH: 2I42) has coordinates (0,1).

Clearly and consistent with our HREX-MD simulations, both PTP1B and YopH (in all three states) can sample a broad range of conformational space, including conformations notably more open than their corresponding “open” X-ray crystal structure. Further, these simulations show a
clear population shift towards the closed state when \( pNPP \) is bound or in the phospho-enzyme intermediate state (\textit{i.e.}, this state becomes less energetically unfavorable than in the case of the unliganded enzymes, and in some cases even very slightly energetically favored over the corresponding open state). These results are consistent with prior NMR and crystal structure data, in which substrate binding or the presence of a phosphate group mimic (to mimic the phospho-enzyme intermediate state) shifted the WPD-loop equilibrium towards favoring the loop closed state for both PTPs (we note that whilst for YopH with \( pNPP \)-bound, the open state is still energetically preferred, but there has nonetheless been a clear population shift towards the closed state when compared to unliganded YopH).\(^9\)

We further analyzed our PT-MetaD-WTE simulations in order to understand what mechanism(s) drive the observed population shift (Figure 4) towards the closed WPD-loop conformation for both PTPs when bound to either the substrate, \( pNPP \), or when in the phospho-enzyme intermediate state. One driving force will of course be from direct interactions between either the substrate or the thiol-phosphate group and the side chain of the WPD-loop Asp when in the closed state (see \textit{e.g.} Figure 1). Our simulations, however, identify a secondary and indirect mechanism by which the binding of a phosphate group induces a population shift towards the closed state through preorganization of the E-loop towards a productive WPD-loop closed state (Figure 5). That is, for both PTPs, the E-Loop and P-Loop are coordinated to one another through a highly evolutionarily conserved (among PTPs, Figure S14) salt bridge between the Arg residue on the P-Loop and the Glu residue on the E-Loop (R211/E115 and R409/E290 for PTP1B and YopH respectively). This P-Loop Arg is responsible for coordinating the reacting phosphate group (Figure 5A and B), with the E-Loop glutamic acid being responsible for helping to lock the arginine side chain into its catalytic configuration. For both PTPs, we observe the binding of a
phosphate group (either from the substrate or in the phospho-enzyme intermediate state) to stabilize this salt bridge (Figure 5A and B) and ultimately rigidify the E-Loop (Figure 5C and D). This also helps to induce a population shift towards the closed state by preventing the P-Loop arginine from sampling side chain rotamers that would block productive WPD-loop closure.

**Figure 5.** Differences in the structural stability of the E-loops of PTP1B and YopH from our PT-MetaD-WTE simulations. (A,B) Normalized histograms (sum of all points for each histogram = 1) of the P-Loop arginine to the E-loop glutamic acid salt bridge distance from each our simulations of (A) PTP1B and (B) YopH, for the unliganded, pNPP bound, and phospho-enzyme intermediates states of both enzymes. The figures show representative conformations for each protein with and without the salt bridge formed. (C,D) RMSFs of the C$_\alpha$ atoms of the E-loop residues, obtained from our PT-MetaD-WTE simulations of (C) PTP1B and (D) YopH in all three states simulated.
Representative structures of the conformational sampling/diversity of the E-loops of (E) PTP1B and (F) YopH, in the unliganded states of each enzyme. E-loop residues are colored mapped from red (most flexible) through white and to blue (least flexible) according to their calculated Cα RMSF (as shown in graphical form in panels C and D).

From comparing the relative structural stabilities of the E-loops of PTP1B and YopH (Figures 5C-F), it is clear that the E-loop of YopH is notably more stable than that of PTP1B, and even contains some degree of secondary (α-helical) structure. Further, in the case of YopH, we observe no instances of the salt bridge breaking when a phosphate group is present, whilst for PTP1B in all three simulated states, the salt bridge is observed to be broken for at least some of the simulation time (Figures 5A and B). Our results show that even with a phosphate group bound, the PTP1B E-loop can still undergo large conformational changes to break the salt bridge (Figures 5A and E). These observations are particularly noteworthy given that a recent NMR dynamics study has suggested the observed $k_{cat}$ for PTP1B does not reflect the isolated open to closed transition of the WPD-loop but one in which this motion occurs in concert with other, cooperative fluctuations, involving the E-loop in particular.\textsuperscript{15}

Identification of Residues Directly Correlated to WPD-Loop Motion

To complement our enhanced sampling simulations, we performed structural bioinformatics analysis on the resulting PT-MetaD-WTE trajectories in order to explore potential pathways of allosteric communication throughout both PTPs. As we are particularly interested in how the motion of the WPD-loop is regulated by the remainder of the protein, we computed dynamic cross correlation matrices (DCCMs) for both PTPs (Figure S15) to identify which residues correlated with the motion of the WPD-loop (Figure 6). DCCMs measure the degree of correlated motion between residues (using the Cα carbon of each residue for the measurement) over the course of the
simulation. Motions between atoms are assigned values between +1 (perfectly positively correlated motion) and −1 (perfectly anti-correlated motion), and 0 indicating no correlation between residues. Interestingly, the residues identified to correlate with WPD loop motion for PTP1B include those that make up both of the known allosteric drug binding sites on PTP1B (BB and K197 allosteric sites, see Figure 6). These results are of particular interest, given the relatively limited information on YopH allostery and the potential application of inhibitors towards YopH.16,86,87

Figure 6. Residues in PTP1B and YopH that are directly correlated with WPD-loop motion identified from our BEX-METAD simulations of PTP1B and YopH with pNPP-bound. Correlated residues (shown as red spheres) were identified by calculating dynamic cross correlation matrices (DCCMs) for both PTPs and identifying any residue with a correlation cut-off value >|0.3| to any WPD-loop residue. The WPD-loop, P-loop and Q-loop are colored in blue, green and magenta respectively, and key secondary structure regions are also labelled. The two known allosteric drug binding sites (BB and 197) are also depicted with a representative drug bound in each position (using PDB IDs: 1T49 and 6B95 respectively). Truncated DCCM plots of PTP1B and YopH in all three simulated states are provided in Figure S16.
We further note that comparison of the truncated DCCM plots obtained for unliganded, pNPP-bound and the phospho-enzyme intermediate simulations of PTP1B and YopH (Figure S16) are largely similar, with the unliganded state generally showing slightly higher correlation than the remaining two states. However, the same sets of residues are indicated for each state, suggesting the same allosteric network is active in all three states. Specifically, for PTP1B, we identified residues on the α3-, α6- and α7-helices; the β5-, β6- and β7-strands, and the E-Loop (~residues 109-129). For YopH, we identified residues on the α1-, α4- and α7-helices; the β5-, β6- and β7-strands, and the E-Loop (~residues 284-304). Interestingly, despite their close spatial proximity and catalytic importance (see Figure 1), the motion of the P-loop is not notably correlated with WPD-loop motion in either PTP1B or YopH (Figure 6). In contrast to the P-loop, however, and of interest considering the data presented in Figure 5, we find the motions of the WPD-loop and E-loop to be correlated with one another.

Identification of Key Allosteric Communication Pathways and Residues

Following our observations that similar residues in both PTP1B and YopH were directly correlated to WPD-loop motion, we next computed the Shortest Path Maps (SPMs) for PTP1B and YopH in their pNPP-bound Michaelis complexes (Figure 7), as this is likely the most therapeutically relevant state for targeting by allosteric inhibitors. In the SPM approach, dynamic cross correlation maps (DCCMs) and average inter-residue distance matrices are computed, and used to calculate the shortest (communication) pathways between all nodes (here, a residue is a node) in the network. Nodes and edges (pathways between nodes) that are often used for communication between residues can then be identified, and these residues can therefore be considered as being important for regulating the global conformational dynamics of the enzyme.
Figure 7. Identification of key residues and pathways utilized for allosteric communication in (A) PTP1B and (B) YopH, determined using the Shortest Path Maps (SPM) method. SPM for both PTPs were calculated based on our PT-METAD-WTE simulations of the pNPP-bound Michaelis complexes of each enzyme. The size of the spheres and edges are proportional to the number of pathways found through the residue (spheres) or between two residues (edges), with a larger size indicating increased pathways and therefore increased importance in allosteric communication. For PTP1B, non-WPD or P-loop mutations found on the SPM that are known to alter PTP1B activity by >50% are shown as purple spheres, with mutations not found on the allosteric map colored red. For mutations not found, the closest heavy atom distance between a SPM residue and the mutated residue is indicated. The two known allosteric drug binding sites (BB and K197) are also depicted with a representative drug bound in each position using PDB IDs 1T49\(^6\) and 6B95\(^6\) respectively). (C) Structure based sequence alignment of PTP1B and YopH, with all aligned residues marked with either a “:” or “.”. Residues marked with a “:” indicating the two aligned residues are within 5 Å of one another (Ca-Ca distance). All residues in PTP1B and YopH found on the SPM are highlighted in
blue, with those known to affect enzyme activity (same criteria as in A) highlighted in purple if on the SPM or in red if not on the SPM. Boxes are used to highlight regions that have a high frequency of SPM residues in both PTPs. Structural alignment was performed using TM-align. PDB IDs 6B90 and 2I42 were used to describe PTP1B and YopH, respectively.

This approach was recently shown to be able to consistently identify distal residues mutated over the course of a directed evolution trajectory in order to enhance the catalysis of a retro-aldolase enzyme. It offers insights into key residues and pathways used for allosteric communication, both of which would be highly valuable for drug discovery efforts targeting allosteric inhibitors of PTPs (for reviews describing interest in this area, see e.g. 4,89,90). We took advantage of the extensive characterization of PTP1B allostery available in the literature to compare our SPM generated for PTP1B to known important mutations and allosteric binding sites (Figures 7A and C). In PTP1B, 68 residues (of 299 in total) are included in the SPM. Of the eleven non-WPD- or P-loop mutations that were shown to alter $k_{cat}$ or $K_m$ by >50% (as compared to the WT, see Table S8 for all mutations identified), eight are identified by SPM as being important for allosteric communication. Further, of the three remaining mutations (K197, L232 and M282) that significantly alter $k_{cat}$ or $K_m$, all are within 4 Å (closest heavy atom distance) of a residue included in the network. Finally, residues that form both of PTP1B’s known allosteric binding sites (Figure 7A) were identified in the SPM. These results (alongside subsequent comparison to experimental data, see below) therefore provide us with confidence that our SPM analysis can identify residues key for allosteric communication in PTP1B, and therefore also in YopH.

Following from this, our SPM analysis of PTP1B identifies a central “highway” of key residues (H175, M109 and H214) which contain four major branches spouting off from this highway (Figure 7A). One of these branches begins from the $\alpha$7-helix (H296 and E297), passing through Loop 11 residues (including Y152 and Y153) towards the N-terminus of the WPD-loop (H175).
The observations that allosteric activation of the α7-helix is communicated through Loop 11 through to the N-terminus of the WPD-loop is consistent with prior NMR and structural studies, and the SPM analysis allows us to characterize the key residues and pathways involved in this allosteric activation. Known allosteric mutations A69V and R56V form another branch to the central highway, passing through residues on the β3-strand (T84), P-loop (H214) and Loop 9 (M109) before reaching the N-terminus of the WPD-loop. Note that analogous mutations at equivalent positions in other PTPs (SHP2 and TC-PTP) also significantly alter activity. Another branch starts between the α1 and α2-helices, and passes through the C-terminal region of the α6-helix, followed by the Q-loop before finally reaching the central highway residue H214 on the P-loop. Mutations G259Q (on the Q-loop) and R254A (on the α5-helix) likely interfere with this pathway of communication. The final branch identified begins at F135, travels through the α3-helix (i.e. residues the G92 and C93) and Loop 3 (i.e. P87 and G85) before reaching H214.

In the case of YopH, 71 residues (of 282 in total) are included in the SPM. Given the limited information on YopH allostery and the above observations that our SPM results were able to identify distal mutation sites in PTP1B, including those conserved among other PTPs, we performed a structure-based sequence alignment of the two PTPs (Figure 7C). Comparison of the SPM residues identified in PTP1B and YopH reveals a reasonably high level of conservation between the two PTPs, with 35 of the 69 PTP1B SPM residues conserved in YopH. Notably, five structurally conserved regions in both PTPs show a high frequency of SPM residues, including those that make up the BB-site (Figure 7C), suggesting some of the allosteric pathways known in PTP1B are also present in YopH, and that therefore YopH could possibly be targeted in a similar manner as has successfully been applied to PTP1B.
Evaluation of the Stability of the Michaelis Complexes Formed During PTP Catalysis

Our structural analysis and enhanced sampling MD simulations have enabled us to extensively characterise the dynamical properties of both PTPs, with a particular focus on WPD-loop motion. To characterise the stability of the reactant complexes for both chemical steps of PTP catalysis, we performed 25x200 ns MD simulations (5 μs cumulative simulation time) of both the pNPP-bound Michaelis complex and the phospho-enzyme intermediate, starting simulations from the closed (catalytically competent) state. Simulations with pNPP bound were performed with restraints between pNPP and several residues on the P-loop (see the Methodology section), to ensure pNPP was consistently bound to the active site throughout these simulations. Histograms of the hydrogen bond (H-bond) distance between the aspartic acid on the WPD-loop and pNPP (Figure 8A) show some sampling of non-productive states for both PTP1B and YopH, which arise primarily from the side chain of the aspartic acid on the WPD-loop “swinging out” to form H-bonds with either the solvent and/or nearby residues (Figure S17). Whilst simulations of the phospho-enzyme intermediate have no restraints in place, we observed a water molecule to be consistently coordinated to the phosphate group (Figure S17), likely because the phosphate is highly charged and has adequate room to coordinate a water molecule (note that there is extensive experimental evidence that the substrate binds as a phosphodianion, see e.g. refs. 5,19,91,92). We therefore evaluated the stability of the H-bonds formed by the catalytic aspartic acid and the coordinating glutamine on the Q-loop to the nucleophilic water molecule (Figures 8B and C). Analysis of Figure 8 suggests the active site of YopH is better configured to stabilize the reactant complexes formed for both steps of PTP catalysis. Note, however, that exchanges between productive and non-productive conformations occurred in both PTPs and for both reactant
complexes on the nanosecond timescale, suggesting that the differences identified here may not contribute significantly to the experimentally observed rates for either step.

**Figure 8.** Histograms of hydrogen bonding (H-bond) distances for key interactions required for the formation of the Michaelis complexes for both the (A) cleavage and (B, C) hydrolysis steps of PTP catalysis. The chemical structure embedded into each panel represents the donor-acceptor distance measurement made (in all cases, these are oxygen-oxygen distances). We note that as the aspartic acid oxygens in panel B are equivalent, the closest oxygen atom was identified at each snapshot and used to generate the histogram. Histograms were obtained from 25x200 ns long MD simulations of each PTP, starting from the closed (catalytically competent) state. All histograms have a bin size of 0.2 Å. A dotted line is placed at 3.5 Å on each graph, to indicate the approximate point at which an H-bond can no longer be considered formed.

**Empirical Valence Bond Simulations**

Both PTP1B and YopH catalyze the turnover of their substrates using the same two-step mechanism shown in **Figure 1**, involving nucleophilic attack of an active site cysteine on the substrate to form a phospho-enzyme intermediate (cleavage) followed by nucleophilic attack of a water molecule to hydrolyze the phospho-enzyme intermediate (hydrolysis).\(^5\) YopH achieves this more efficiently than PTP1B, with turnover numbers that differ by ~1 order of magnitude (\(k_{\text{cat}}\) of ~1300 s\(^{-1}\) for YopH,\(^{19}\) compared to ~40 s\(^{-1}\) for PTP1B\(^{20}\) at their pH optima). We note that the experimental rates for the first chemical step are similar, with a caveat that these were obtained at
a pH of 6, a more optimal pH for PTP1B catalysis than YopH. The main difference is observed in the rates for the subsequent, rate-limiting hydrolysis of the phospho-enzyme intermediate (Table 1). This difference is curious given the similarity in the active sites of the two enzymes, and therefore, in a final step, we have used the EVB approach in order to model the cleavage and hydrolysis reactions catalyzed by PTP1B and YopH, respectively (Figure 1).

**Table 1.** Calculated activation ($\Delta G^\ddagger$) and reaction free energies ($\Delta G_0$), obtained using the empirical valence bond approach, as well as relevant corresponding experimental observables, for both steps of catalysis for both PTPs.

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<th>$\Delta G^\ddagger$</th>
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<td>PTP1B</td>
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<td>YopH</td>
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<td>2.8 ± 0.3</td>
<td>343$^{93}$</td>
<td>3.5</td>
<td>5.8</td>
<td>12.96</td>
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<tr>
<td>PTP1B</td>
<td>14.2 ± 0.2</td>
<td>-10.3 ± 0.3</td>
<td>28$^{14}$</td>
<td>3.5</td>
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<td></td>
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<td>48$^{9,12,34}$</td>
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<td>24.4$^{21}$</td>
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<tr>
<td>YopH</td>
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<td>-10.4 ± 0.3</td>
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<td>601$^{94}$</td>
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$^a$ All calculated values are averages and standard errors of the mean over 30 individual EVB trajectories per system, as described in the **Methodology** section. Both experimental and calculated activation and reaction free energies are presented in kcal mol$^{-1}$. Shown here are also the corresponding kinetics ($k$, s$^{-1}$) and activation free energies ($\Delta G^\ddagger_{\text{exp}}$) derived from the experimentally observed rates using the Eyring equation.

We prepared our EVB simulations as described in the **Methodology** section using the EVB parameters presented in the **Supplementary Methodology**. The resulting activation and reaction
free energies are provided in Table 1 and Figure 9, and the structures of key stationary points from our simulations are provided in Figure 10 for PTP1B, and Figure S18 for YopH. We note that normally, we would have directly modelled the hydrolysis step using the structure of the phospho-enzyme intermediate obtained from the endpoint of EVB simulations of the cleavage step. However, in between the cleavage and hydrolysis steps, the side chain rotamer of a conserved glutamine on the Q-loop (residues Q262 and Q446 for PTP1B and YopH respectively, see Figure 1) changes from originally pointing away from the active site to coordinating the nucleophilic water molecule for the hydrolysis step (see detailed discussion in ref. 5). We do not observe this displacement at the end point of our EVB simulations of the cleavage reaction (Figures 10 and S18), likely due to the product (p-nitrophenol) still being located in the active site of the enzyme. As this rotamer change is observed in PDB IDs: 3I80\textsuperscript{10} and 2I42\textsuperscript{12} (PTP1B and YopH, respectively), which were crystallized in the presence of vanadate ion as a proxy for the phospho-enzyme intermediate, we modelled the two reaction steps as discrete processes with the appropriate starting crystal structure for each process (see the Supplementary Methodology), and also with the p-nitrophenol leaving group removed from the starting point for the hydrolysis reaction, as this would be expected to depart rapidly from the active site.\textsuperscript{95} This gives rise to the six key stationary points presented in Figures 10 and S18. We note here that for comparison, we also modelled the cleavage step using the end-point of our EVB simulation (which does not include the motion of Gln446 into the active site in the case of YopH). In doing so, we obtain an identical activation free energy in the case of PTP1B (14.2 ± 0.4 kcal mol\textsuperscript{-1}), whereas in YopH we obtain a higher activation free energy (17.6 ± 0.3 kcal mol\textsuperscript{-1}) when Gln446 is not in a catalytically competent conformation, in agreement with structural data.
Figure 9. A Comparison of the calculated ($\Delta G^\ddagger_{\text{calc}}$) and experimental ($\Delta G^\ddagger_{\text{exp}}$) activation free energies for the non-enzymatic, PTP1B- and YopH-catalyzed hydrolysis of pNPP. Shown here are separate data for each of the cleavage and hydrolysis steps shown in Figure 1. Data is presented in kcal mol$^{-1}$ as average values and standard error of the mean over 30 individual EVB trajectories obtained as described in the Supplementary Methodology section. The raw data for this figure is presented in Tables 1 and S9.

Figure 10. Representative structures of (A) the Michaelis complex, (B) the transition state for the cleavage step, (C, D) the phospho-enzyme intermediate, (E) the transition state for the hydrolysis step, and (F) the final product complex,
for the PTP1B-catalyzed hydrolysis of pNPP (see Figure S18 for equivalent YopH results). Note that while states C and D are essentially identical, we modelled the two reaction steps (see Figure 1) as discrete processes, using separate starting structures with appropriate transition state analogues bound as described in the main text and the 

Supplementary Methodology section. The structures shown here are the centroids of the top ranked cluster obtained from RMSD clustering of 30 individual EVB trajectories of each stationary or saddle point, performed as described in the Supplementary Methodology. Average reacting distances for each catalytic step are also shown. The orientation used was selected as it is the clearest angle from which to see all key reacting atoms in a 2-D representation.

Based on the energies presented in Table 1 and Figure 9, it can be seen that for the cleavage step, we calculate an activation free energy that is 2.6 kcal mol$^{-1}$ lower in YopH than in PTP1B, whereas for the hydrolysis step, the barrier is more similar, and only 0.7 kcal mol$^{-1}$ lower in YopH than in PTP1B. In both cases, we obtain a higher barrier for the hydrolysis step than the cleavage step, again in agreement with experimental data. While our PTP1B calculations give generally good quantitative agreement with experiment, our YopH calculations underestimate either the expected activation free energy compared to experiment for the cleavage step, or the difference between PTP1B and YopH for the hydrolysis step (see Table 1). However, a direct comparison to experiment is not straightforward. That is, it has been shown experimentally that catalysis in these enzymes is correlated with WPD-loop motions, and therefore it is quite possible that the two processes (chemistry and loop motion) are coupled. In such a case, one cannot reliably use the Eyring equation to obtain the experimental activation barrier for the chemical process on the enzyme, because the temperature effect on the rate of catalysis is reflecting other temperature-dependent events besides the phosphoryl transfer. The fact that we obtain relatively similar barriers for the hydrolysis step, for example, would be expected from the fact that these active sites are practically identical and superimposable; it in turn suggests that the difference in reaction rates is being determined by a non-chemical event. We also note that the large catalytic effects (~16 kcal
mol$^{-1}$ for cleavage and 20 kcal mol$^{-1}$ for hydrolysis) observed for both enzymes and both chemical steps are well reproduced by our simulations (Figure 9).

Taking these limitations into account, we have explored structural changes observed in our EVB simulations of the different reaction steps and systems. In terms of transition state geometries (Table S10), we observe very similar P-O distances to either the leaving group in the cleavage or nucleophile in the hydrolysis step between the non-enzymatic and enzymatic reactions (irrespective of enzyme). However, we observe a slight contraction in the $S_{\text{Cys}}$-P distances between the sulfur atom of the cysteine side chain and the phosphorus atom of the phosphate group in both PTP1B and YopH, compared to the non-enzymatic reaction. This difference becomes clear when considering Pauling bond orders of the transition state ensembles (determined from the calculated distances provided in Table S10 and the equilibrium bond distances used in our EVB simulations, using the relationship $r = r_e - 0.6\ln(n)$, see the Supplementary Methodology). These are 0.42, 0.58 and 0.63 for the non-enzymatic reaction and the PTP1B and YopH catalyzed reactions, respectively, in the cleavage step, and 0.53, 0.76 and 0.80 in the hydrolysis step. For the P-O$_{\text{NPP}}$ distance in the cleave step, the differences are much smaller, whereas for the hydrolysis step, the P-O$_{\text{H2O}}$ bond orders follow a similar trend to the $S_{\text{Cys}}$-P distances (0.50 to 0.40 to 0.38 for the three different reactions, respectively). From this analysis based on Pauling bond orders (see full data in Table S10), it is clear that (aside from the differences between the non-enzymatic and enzyme catalyzed reactions), the main differences between PTP1B and YopH are observed in the sulfur-phosphorus distances, for both reaction steps.

We also applied our EVB simulations to determine the per residue electrostatic contributions to TS stabilization (Figure S20 and Table S11). For both PTPs, many of the residues that provide TS stabilization for the cleavage reaction are destabilizing in the hydrolysis reaction, whilst
residues which provide TS stabilization for the hydrolysis reaction are destabilizing in the cleavage reaction. As an example, K120 on PTP1B and R404 in YopH both coordinate the catalytic aspartic acid in their respective enzymes, and each provide the largest contribution to TS stabilization for the cleavage step (Figure S20), where they help stabilize the buildup of negative charge on the aspartic acid at the TS. In contrast, both K120 and R404 destabilize the hydrolysis step, in which pushing positive charge onto the aspartic acid when it is required to accept a proton is unfavorable. This observation suggests that the active sites of PTPs have been subjected to competing evolutionary interests towards barrier reduction for both chemical steps. Further, we have previously observed symmetrical roles of residues between reaction steps for the enzyme β-phosphoglucomutase,\textsuperscript{67} which also undergoes a ping-pong reaction mechanism. This is likely a common feature among ping-pong reaction mechanisms, as the second reaction step is the reverse of the first.

It is also interesting to note that many of the key residues that provide substantial TS (de)stabilization for either or both of PTP1B’s reactions are located on the E-loop (residues: R112, E115, K116 and K120, see Figure S20). This would suggest that the conformational sampling of the E-loop is essential for productive catalysis and supports the proposition of Torgeson et al.,\textsuperscript{15} in which the conformational sampling of the WPD-loop and other active site loops including the E-loop control the observed \( k_{\text{cat}} \) (see discussion surrounding Figure 5).

In order to examine the solvent accessibility of reacting atoms in the active site, we monitored the average number of water molecules within 4 Å of the reacting atoms (Table S10). These values are similar between PTP1B and YopH for both reacting steps, but up to two additional water molecules (not including the nucleophilic water molecule) enter the active site at the transition state for the hydrolysis step compared to the cleavage step, which may partially account for the
slightly higher barrier to the hydrolysis step (Table 1), although the higher barrier could also simply be due to the fact that the hydrolysis step (leaving group S-alkyl) is intrinsically more challenging to catalyze with a non-enzymatic barrier of ~35 kcal mol\(^{-1}\) compared to ~29.5 kcal mol\(^{-1}\) for the non-enzymatic equivalent of the cleavage of \(p\)NPP.\(^{96-98}\)

From this combined analysis, we see that there are subtle changes in transition state geometries and solvent exposure of the active site between the different systems, that can likely account for the differences in calculated activation free energies between the different reaction steps and enzymes shown in Table 1. However, more significantly, our data indirectly suggests that the observed differences in rate between the two enzymes are linked to changes in WPD-loop dynamics, which has been already suggested based on experimental work.\(^9\) Modeling the direct coupling between the conformational change and the chemical step of catalysis would be extremely computationally challenging and out of the scope of the present work; however, clear differences are indicated in the dynamical behavior of the WPD-loops of PTP1B and YopH based on our enhanced sampling simulations, that further support this observation.

Overview and Conclusions

PTPs regulate a myriad of biological pathways, and as such, their catalytic rates will have been subjected to strict evolutionary pressures. Despite a shared catalytic mechanism and transition states for the chemical steps, PTP catalytic rates vary by orders of magnitude and NMR has demonstrated linkage between the rate of WPD-loop motion and catalysis in YopH and PTP1B.\(^9,12\) These differences likely apply throughout the classical PTP family. The results reported here provide an understanding of the basis for the differing conformational dynamics between YopH and PTP1B, and insights into the origins of their respective catalytic activities. To the best of our
knowledge, PTPs are the first enzyme family known that carries out the same reaction at highly different rates modulated by differences in their protein dynamics. An understanding of these differences also allows for consideration of their broader evolutionary implications.

Our structural analysis of PTP1B and YopH (Figure 1) identified a single principal competent (PC, i.e. vector) that describes the WPD-loop open-to-closed transition. Two additional hinge points flanked by proline residues in the WPD-loop of PTP1B provide a structural rationale for its ~50-fold slower loop motion compared to YopH. This was confirmed by HREX-MD simulations, which demonstrate that whilst both PTPs sample a vast array of conformations, YopH samples more conformations and at a faster rate than PTP1B. Furthermore, HREX-MD simulations (Figure 3) identified YopH to be able to adopt a “hyper-open” WPD-loop conformation, previously only observed in the crystal structures of two WPD-loop swapped YopH-PTP1B chimeras, although these conformations are rare events that are infrequently sampled in wild-type YopH. This implies that the swapping of WPD-loop residues in the chimeras did not cause the hyper-open conformations observed in their crystal structures, but merely stabilized this conformation allowing it to be crystallized, and also, presumably, to be populated to a higher degree, resulting in reduced catalytic activity. In contrast, wild-type PTP1B does not adopt this hyper-open conformation in our simulations, and likely cannot do so, due to the role of P188 acting as a “helix-breaker”. The proposition that PTP1B cannot form this state is consistent with a P188A PTP variant showing two WPD-loop exchange processes, as compared to one for wild-type PTP1B and other point variants. The functional role (if any) of this hyper-open conformation and its prevalence among other PTPs is currently unknown, although we note an atypical, catalytically inactive hyper-open conformation has also been observed in three other PTPs from different subgroups. This suggests that although the precise functional role of this hyper-open state is
unknown, it carries some sort of functional significance for PTPs as a whole. It is possible this unproductive state is preferentially (de)stabilized by the binding of regulators or changes in the cellular environment, allowing a means for control of catalysis.

Our PT-MetaD-WTE simulations (Figure 4) were able to reproduce the experimentally observed population shift towards the closed WPD-loop state in the presence of substrate and in the phospho-enzyme intermediate state. This population shift is induced not only through direct interactions between the ligand/thiol-phosphate group and the WPD-loop acid, but also through stabilising a salt bridge between the side chains of a highly conserved P-loop arginine and E-loop glutamic acid (Figure 5).

We utilised correlation-based methods to identify the key residues and pathways for allosteric communication in both PTP1B and YopH (Figure 6 and 7). Agreement of these predictions with a substantial body of experimental allosteric data for PTP1B (both in the form of known allosteric inhibitor binding sites and allosteric mutations) lends confidence to the predictions of analogous potential allosteric regions in YopH, which has been significantly less well characterised in this regard. Comparison of the Shortest Path Maps (SPMs) produced for both PTP1B and YopH showed a high degree of conservation of the residues playing significant roles in allosteric communication. Given the relatively low sequence similarities between PTP1B and YopH (20.6% sequence identity) this raises the likelihood that these conserved regions are also present in other PTPs, which would be consistent with a recent study that identified evolutionarily conserved mechanisms of allosteric communication among several PTPs.23

Our MD simulations of the reactant state showed local fluctuations of reacting side chain atoms on the nanosecond timescale (Figure 8), demonstrating that the large-scale closure of the WPD-loop is not the only prerequisite for efficient catalysis. Additionally, modest differences are found
in the computed activation barriers between PTP1B and YopH for the initial cleavage step, and very similar computed activation barriers for the rate-determining hydrolysis step (Table 1). While not surprising given the similar active sites and transition states, the experimentally measured rates are very different. Despite these small differences (not surprising given the similar active sites), the experimentally measured rates are very different. This further strengthens the notion that protein motions contribute to the differences in $k_{cat}$ in the PTP family. Our analysis of per-residue contributions to TS (de)stabilization for PTP1B identified a key role of many E-loop residues (Figure S20), suggesting that correct conformational sampling of this loop is essential for catalysis. Further, our PT-MetaD-WTE simulations show the motion of the WPD- and E-loops are correlated with one another for both PTPs (Figure 5).

In summary, whilst PTP1B and YopH are chemically and mechanistically indistinguishable in their chemical steps of catalysis, there are clear differences in their WPD-loop and E-loop dynamics. If altered WPD-loop dynamics are primarily responsible for regulating PTP catalysis, then this raises biological questions as to how and why nature “chose” this approach. It is possible this allows PTPs to respond to changes in their local environment, such as changes in temperature, pH, viscosity, or crowding. It also provides a means for allosteric regulation by small molecules or proteins that affect WPD-loop motions. Physiologically crucial PTPs like PTP1B must function at rates that meet the physiological requirements of the organism, where the fastest rate is not necessarily optimal. It is likely not a coincidence that YopH has evolved to become the fastest PTP yet characterized, given its role in facilitating Yersinia infection where “running wild” in the invaded host is beneficial. Future work could focus on how different (cellularly relevant) environmental conditions can alter the loop dynamics of PTPs. Furthermore, given the recently renewed interest in the allosteric inhibition of PTPs, understanding the similarities and
dissimilarities in the allosteric regulation of human PTPs may prove valuable in the design of selective allosteric inhibitors.

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