Proposed Role of Conserved Motifs in the Classic, Tyrosine-specific PTPs

Motif Residue in PTP1B	Conservation by (i) amino acid identity (upper) (ii) amino acid similarity (lower	Conser- vation in 3D	Proposed roles of the residues
Motif 1 40-46	NxxK**N**R**Y** Nxx**(K/R)N**R**Y**	Medium	**pTyr-recognition loop: restricts substrate specificity to pTyr** Asn 44 Coordinates Asn68 which links Arg257 Arg 45 Putative substrate binding site, electrostatic attraction of ligand Tyr 46 Hydrophobic packing with phosphotyrosine residue of substrate
Motif 2 53-59 53-59	D**xxRVxL** D**xxR(V/I)xL**	Low	**Conserved secondary structure (β1 sheet), surface exposed** Arg 56 H-bonds to Asp65 Ile 57 Hydrophobic core cluster (residues 57, 67, 69, 82, 98) Leu 59 Hydrophobic core
Motif 3 65-69 65-70	D**YINA** D**YINA(N/S)**	Medium	**Core structure** Tyr 66 Coordinates Asn44 through hydrogen bonding Ile 67 Hydrophobic core cluster (residues 57, 67, 69, 82, 98) Asn 68 H-bonds with Arg257 Ala 69 Hydrophobic core cluster (residues 57, 67, 69, 82, 98)
Motif 4 82-87 81-87	**IAxQGP** **(F/Y)(I/V)**Ax**QGP**	High	**Core structure surrounding the PTP-loop** Ile 82 Hydrophobic core cluster (residues 57, 67, 69, 82, 98) Ala 83 Packs/surrounds the PTP-loop Gln 85 H-bonds with highly buried water molecule Gly 86 Packs/surrounds the PTP-loop Pro 87 Packs/surrounds the PTP-loop
Motif 5 91-100 91-101	T**xxDFWxMxW** T**xxDFWx(M/L/V)x(W)(E/Q)**	Medium	**Conserved secondary structure (α2 helix)** Asp 94 Contributes to conserved subdomain at the 'back side' Phe 95 Energetically favored T-stacking arrangement with invariant Trp96 Trp 96 H-bonds to backbone of invariant Tyr124 Met 98 Hydrophobic core cluster (residues 57, 67, 69, 82, 98) Trp 100 Contributes to conserved subdomain at the 'back side'
Motif 6 107-111 107-111	IV**M**xT **(I/L/V)(V/I)M**xT	Medium	**Hydrophobic core structure** Ile 107 Hydrophobic core structure packs with invariant Trp96 Val 108 Hydrophobic core structure packs with invariant Trp96 Met 109 Packs with invariant Trp125 Thr 111 Packs with PTP-loop
Motif 7 120-126 120-126	K**CxxYWP** K**CxxYWP**	Low	**Hydrophobic core structure** Lys 120 Interacts with Asp181 (ligand induced) Tyr 124 H-bonds with His214, stabilizing T-stacking arrangement with Trp125 Trp 125 Favored T-arrangement of aromatic ringsystem with Tyr124
Motif 8 179-185 176-185	**WP**DxGx**P** **(Y/F)xxWP**DxGx**P**	Low	**WPD-loop, surface exposed, movable, contains general acid** Trp 179 Center of movable WPD-loop, mediating motion of loop Pro 180 H-bonds to NH2 of Arg221, mediating motion of loop Asp 181 General acid catalyst Gly 183 Energetically favorable in loop motion (acts as hinge) Pro 185 Energetically favorable in loop movement (no backbone H-bonding)
Motif 9 210-223 210-223	P**xxVHCSAGxGR**T**G** P**xx(V/I)HCSAGxGR(T/S)G**	High	**PTP-loop surrounding active site Cys. 7 successive main-chain nitrogens coordinates 3 phosphate oxyanions** Pro 210 Structural hydrophobic core His 214 Lowers pKa of Cys215 Cys 215 Nucleophile Ser 216 H-bonds with Tyr46 stabilizing its interaction with substrate Ala 217 Phosphotyrosine binding, nonpolar interaction with substrate phenyl Gly 218 Phosphotyrosine binding Gly 220 Phosphotyrosine binding Arg 221 H-bonds with phosphate oxygens (transition-state stabilization) Thr 222 Lowers pKa of Cys215
Motif 10 262-269 261-269	**QTxxQ**Y**xF** **(V/I/L)QTxxQYxF**	Low	**The Q-loop: Interaction with active site water molecule** Gln 262 H-bonds with scissilie oxygen and active site water molecule Gln 266 H-bonds with active site water molecule Tyr 267 Defines α6' helix structure Phe 269 Defines α6' helix structure

Sequence conservation (Column 2) was calculated according to: (i) amino acid identity (upper) and (ii) amino acid similarity (lower).
Consensus substitution groups are defined as: 1, DN; 2, EQ; 3, ST; 4, KR; 5, FYW; 6
100% conservation: Underscored bold-type letters; >90% conservation: Bold-type letters; >80% conservation: Non-bold letters

Proposed role of single conserved PTP residues that reside outside the 10 PTP motifs

Amino acid in human PTP1B	Conserved by amino acid identity	Proposed roles of the residues
Glu 19	E (>80%)	Definition of α2' helix structure
Glu 115	**E** (100%)	Conserved H-bonds with Arg221
Arg 157	R (>80%)	Definition of β10 sheet
Arg 169	R (>80%)	Definition of β11 sheet
Leu 192	L (>80%)	Definition of the α3 helix structure
Arg 254	**R** (>90%)	H-bonds with PTP-loop
Arg 257	**R** (100%)	H-bonds with PTP-loop lowering pKa of Cys215