Crystallography materials and methods for PTP1B-Compound **8e** and **22i**.

Co-crystallization of PTP1B with inhibitors

A 6-10 mg/mL preparation of PTP1B in 10 mM Tris pH 7.5, 25 mM NaCl, 0.2 mM EDTA and 3 mM DTT, was used for crystallization. Crystals were grown by the sitting, as well as the hanging drop, vapor diffusion methods. A 1:10 (PTP1B:inhibitor) molar ratio mixture was prepared at least one hour prior to crystallization. Two μL PTP1B-inhibitor solution was mixed with 2 μL reservoir solution consisting of 0.1 M Hepes buffer pH 7.5, 0.3-0.4 M Na-acetate or Mg-acetate, 12-16% (w/v) polyethylene glycol 8000 and/or 4% (v/v) glycerol. The reservoir volume was 1 mL. Crystals grew to the size of 0.3-0.6x0.1-0.3x0.1-0.3 mm within 2-3 days.

Data collection.

All diffraction data collections were performed at 100 K. The following cryo conditions were used: to the hanging or sitting drop 3 μL of 50% (v/v) glycerol (containing 0.5 mmol inhibitor) were added. The crystal was removed from the drop after 5-30 min and transferred to 50% glycerol (containing 0.5 mmol inhibitor) and flash frozen. Data were collected using a Mar345 image plate detector at the MAX-lab synchrotron facilities at Lund University. A 1° oscillation per image was used for 60 images. The space group was determined to be P3121 for the crystals obtained with compound **8e** and P43212 for all the crystals obtained with compound **22i**. Data processing was performed using Denzo, Scalepack and the CCP4 program suite.[1,2]

Refinements.

As P3121 contains a polar axis and, thus, possesses more than one indexing possibility, a molecular replacement solution using Amore[3] was determined prior to the refinements. Further, a molecular replacement solution was also calculated for the P43212 space group. A high resolution PTP1B structure was used as a starting model (PDB file: 1PTV),[4] with ligand and water molecules omitted from the structure. All refinements were performed with CNX (Asselrys Inc.). Interchanging cycles of model building using Quanta X-build (Asselrys Inc.) and refinement were performed. The $2F_o$-F_c maps were inspected by the use of X-ligand (Asselrys Inc.) at a 1 sigma level for densities that could correspond to the structures of the inhibitors. In both cases, a well-suited inhibitor electron density was identified in the active site pocket (see below). No other densities were identified to fit the inhibitors. Water molecules were inserted using the X-solvate program (Asselrys Inc.) based on 1.5σ $2F_o$-F_c electron density maps. For further details see Table 1.

References

1. Otwinowski, Z. and Minor, W. Processing of X-ray diffraction data collected in oscillation mode. In *Methods Enzymol, ed.;* Carter, C. W. and Sweet, R. M., Eds. Academic Press, New York, 1997; vol. 276, pp 307- 326.

2. Collaborative Computational Project, N. 4. The CCP4 Suite: Programs for protein crystallography. *Acta Crystallogr., Sect. D* **1994**, *50*, 760-763.

3. Navaza, J. Amore - an automated package for molecular replacement. *Acta Crystallogr., Sect. A* **1994**, *50*, 157-163.

4. Jia, Z. C., Barford, D., Flint, A. J., and Tonks, N. K. Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatases 1B. *Science* **1995**, *268*, 1754-1758.

Table 1. Statistics of X-ray data and structure refinements.

Table 1.

Protein	PTP1B	PTP1B
Ligand	Compound **8e**	Compound **22i**
Space group	P3121	P43212
Unit cell parameters	a=b=88.2 c=104.1 Å	a=b=97.74 c=92.347Å
Completeness (all data)	98.9% (30-1.89 Å)	94.3 % (30-2.0 Å)
Completeness (high res.)	99.8% (1.91-1.89 Å)	98.8% (2.03-2.00 Å)
Multiplicity (all data)	3.5 (30-1.89 Å)	4.4 (30-2.0 Å)
Rmerge (all data)	8.2% (30-1.89 Å)	4.3% (30-2.0 Å)
Rmerge (high res.)	49% (1.91-1.89 Å)	44.7% (2.03-2.00 Å)
$\langle I/\sigma(I)\rangle$ (all data)	14.3 (30-1.89 Å)	20.7 (30-2.0 Å)
$\langle I/\sigma(I)\rangle$ (high res.)	2.7 (1.91-1.89 Å)	2.2 (2.03-2.00 Å)
Unique reflections	37618	29166
Atoms in structure	2764	2683
R-factor[1]	20.5%	22.7%
R-free[2]	21.9%	28.5%
R.m.s. deviations from idealised geometry's		
Bond lengths (Å)	0.006	0.006
Bond angles (°)	1.2	1.3

[1]Crystallographic R-factor – calculated using all data. [2]Rfree= $\sum_{(hkl)\in T} ||Fo| - |Fc|| \Big/ \sum_{(hkl)\in T} |Fo|$

where T is a test set containing a random 5% of the observations omitted from the refinement process.