Structural analysis of human dual-specificity phosphatase 22 complexed with a phosphotyrosine-like substrate

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4-Nitrophenyl phosphate (*p*-nitrophenyl phosphate, pNPP) is widely used as a small molecule phosphotyrosine-like substrate in activity assays for protein tyrosine phosphatases. It is a colorless substrate that upon hydrolysis is converted to a yellow 4-nitrophenolate ion that can be monitored by absorbance at 405 nm. Therefore, the pNPP assay has been widely adopted as a quick and simple method to assess phosphatase activity and is also commonly used in assays to screen for inhibitors. Here, the first crystal structure is presented of a dual-specificity phosphatase, human dual-specificity phosphatase 22 (DUSP22), in complex with pNPP. The structure illuminates the molecular basis for substrate binding and may also facilitate the structure-assisted development of DUSP22 inhibitors.

1. Introduction

The protein tyrosine phosphatase (PTP) family consists of a large number of proteins that play critical roles in a variety of signal transduction pathways (Tonks, 2006, 2013). PTPs can be further divided into several subclasses based on their molecular structure and substrate preference for tyrosine, serine or threonine (Alonso *et al.*, 2004; Patterson *et al.*, 2009). In close coordination with the opposing kinases, PTPs act to regulate important cellular processes such as cell growth, differentiation and proliferation by removing phosphates from phosphorylated residues (Hunter, 1995). The molecular disruption of such tightly coordinated events can result in a range of maladies such as cancer, infectious diseases, immune disorders, diabetes and inflammation (Zhang, 2002; Tonks, 2006). Consequently, a number PTPs are emerging as validated drug targets for a variety of ailments (Combs, 2007; Blaskovich, 2009; He *et al.*, 2014).

PTPs possess a signature molecular motif, CX₅R, that forms the phosphate-binding loop in the active site (Denu & Dixon, 1998). The dephosphorylation reaction is initiated by a conserved catalytic cysteine residue that functions as a nucleophile by attacking the P atom of the substrate to yield a cysteinyl-phosphate intermediate (Pannifer *et al.*, 1998). A conserved arginine residue from the phosphate-binding loop initially acts to provide favorable binding interactions with the incoming substrate but also serves to stabilize the reaction intermediate (Denu & Dixon, 1998). A conserved aspartic acid residue located on the neighboring WDP loop (general acid loop) functions as a general acid/base by first transferring a proton to the oxygen of the leaving group in the tyrosine residue and then accepting a proton from the attacking water

during hydrolysis of the phospho-enzyme intermediate to regenerate the free enzyme (Denu *et al.*, 1995).

There are a variety of methods available to assay PTP activity (Montalibet *et al.*, 2005; Lorenz, 2011). One simple and widely adopted assay utilizes the chromogenic substrate 4-nitrophenol phosphate (*p*-nitrophenyl phosphate, pNPP). Upon enzymatic hydrolysis of the colorless pNPP substrate, the *p*-nitrophenolate ion product yields a yellow color that can be monitored by absorbance at 405 nm, thereby negating the need for a coupled enzyme system to quantify the liberated phosphate ion (Zhang *et al.*, 1992). Therefore, this assay is also well suited for incorporation into high-throughput screening of small molecule libraries to identify potential PTP inhibitors (Bahta *et al.*, 2012). Small molecule phosphotyrosine mimetics such as arylphosphates have also been demonstrated to serve as potential starting points for PTP inhibitor design. Indeed, several potent and specific inhibitors of *Yersinia pestis* YopH have been developed by substrate-activity screening of arylphosphates that were identified to bind to YopH with high affinity (Kim *et al.*, 2011; Bahta *et al.*, 2011, 2012). Subsequent structure-assisted optimization of these substrates and their conversion into nonhydrolyzable substrate analogs eventually yielded a YopH inhibitor with nanomolar affinity (Bahta *et al.*, 2011).

The dual-specificity phosphatase DUSP22 (JNK stimulatory phosphatase 1, JSP-1) is a 184-residue PTP that is broadly expressed in a variety of human tissues (Yokota *et al.*, 2007). Owing to its small size and its lack of an N-terminal non-catalytic domain, it is classified as an atypical dual-specificity phosphatase (DUSP; Patterson *et al.*, 2009). DUSPs are distinguished from the classical PTPs by their ability to dephosphorylate both tyrosine and serine/threonine residues. They share a similar mechanism with the classical PTPs, but the catalytic pockets of the DUSPs are much shallower than those of the classical PTPs (Denu & Dixon, 1995; Yuvaniyama *et al.*, 1996). The shallow active-site pocket allows DUSPs to accommodate either phosphotyrosine, phosphothreonine or phosphoserine (Schumacher *et al.*, 2002).

Many DUSPs are emerging as potential therapeutic targets for treating human diseases such as cancer (Ríos *et al.*, 2014). DUSP22 has been reported to activate the JNK (c-Jun N-terminal protein kinases) signaling pathway (Shen *et al.*, 2001) and may have potential as a target for therapeutic intervention in human diseases that are caused by aberrant regulation of this pathway. Specific DUSP22 inhibitors are needed to study the role of this enzyme in normal and diseased cells. However, to date there have been few drug-like inhibitors reported in the literature (Cutshall *et al.*, 2005; Zhang *et al.*, 2006, 2007; Kamila *et al.*, 2011). Furthermore, there are very few examples of complexes between DUSPs and small molecules in the Protein Data Bank (Jeong *et al.*, 2014). Here, we have designed a surface entropy reduction (SER) mutant (Goldschmidt *et al.*, 2014) of DUSP22 that yields well diffracting and reproducible crystals. With the incorporation of the active-site mutation C88S, we were able to solve the crystal structure of DUSP22 in complex with the pNPP substrate at 1.34 Å resolution, thereby revealing the structural

details of substrate binding. This structure may also serve as a useful starting point for the design of other arylphosphate analogs that could potentially be developed into dual-specificity phosphatase inhibitors.

2. Materials and methods

2.1. Cloning, protein expression and purification

Having failed to obtain crystals of the DUSP22 C88S mutant that were of sufficient quality for data collection, we introduced three additional amino-acid substitutions into the molecule in an effort to facilitate its crystallization by surface-entropy reduction (SER) mutagenesis. These mutations (E24A/K28A/K30A) were selected by manual inspection of an electron-density map from PDB entry 1wrm (Yokota *et al.*, 2007), seeking clusters of lysine and glutamic acid residues with weak electron density around the side-chain atoms. Human DUSP22 (Met1–Glu163) was amplified from cDNA (IMAGE clone ID 3942055, American Type Culture Collection, Manassas, Virginia, USA) by the polymerase chain reaction (PCR) using the following oligonucleotide primers: 5'-GAG AAC CTG TAC TTC CAG GGT GGT CCG ATG GGT AAT GGT ATG AAC AAG ATC CTG CCC-3' and 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTT ATT ATT CTG CAT CCT GCA ACG GGC TCT CTC CAT ATT C-3' (primer R). The PCR amplicon was subsequently used as the template for a second PCR with the following primers: 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC GGA GAA CCT GTA CTT CCA G-3' and primer R. The amplicon from the second PCR was inserted by recombinational cloning into the entry vector pDONR221 (Life Technologies, Carlsbad, California, USA) and the nucleotide sequence was confirmed experimentally. The catalytically inactive C88S mutation and the E24A/K28A/K30A surface-entropy reduction mutations were introduced using the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent, Santa Clara, California, USA). The open reading frame of DUSP22 (Met1–Glu163, E24A/K28A/K30A/C88S) with a recognition site for tobacco etch virus (TEV) protease and a three-amino-acid spacer added to its N-terminus (ENLYFQ/GGP) was moved by recombinational cloning into the destination vector pDEST-HisMBP to produce pJT294 (Sun *et al.*, 2011). This plasmid directs the expression of human DUSP22 (Met1–Glu163, E24A/K28A/K30A/C88S) as a fusion protein with *Escherichia coli* maltose-binding protein (MBP) with an intervening TEV protease recognition site and GGP spacer. The MBP contains an N-terminal hexahistidine tag for affinity purification by immobilized metal-affinity chromatography.

The fusion protein was expressed in *E. coli* strain Rosetta 2(DE3) (Novagen EMD Millipore Corporation, Billerica, Massachusetts, USA). Cells containing the expression plasmid were grown to mid-log phase (OD₆₀₀ of ~0.5) at 310 K in LB broth (Miller's formulation) containing 100 µg ml⁻¹ ampicillin, 30 µg ml⁻¹ chloramphenicol and 0.2% glucose. Overproduction of fusion protein was induced with isopropyl

β -D-1-thiogalactopyranoside at a final concentration of 1 mM for 4 h at 303 K. The cells were pelleted by centrifugation and stored at 193 K.

All procedures were performed at 277–281 K. 10 g *E. coli* cell paste were suspended in 100 ml ice-cold 25 mM HEPES pH 7.5, 100 mM NaCl, 25 mM imidazole buffer (buffer A) containing cOmplete EDTA-free protease-inhibitor cocktail tablets (Roche Molecular Biochemicals, Indianapolis, Indiana, USA). The cells were lysed with an APV-1000 homogenizer (Invensys APV Products, Albertslund, Denmark) at 69 MPa and centrifuged at 30 000g for 30 min. The supernatant was filtered through a 0.45 μ m polyethersulfone membrane and applied to a 10 ml Ni-NTA Superflow column (Qiagen, Valencia, California, USA) equilibrated in buffer A. The column was washed to baseline with buffer A and eluted with a linear gradient of imidazole to 250 mM. Fractions containing recombinant His₆-MBP-DUSP22 (Met1–Glu163, E24A/K28A/K30A/C88S) fusion protein were pooled, concentrated using an Amicon Ultracel 30 kDa cellulose membrane (EMD Millipore Corporation, Billerica, Massachusetts, USA), diluted with 25 mM HEPES pH 7.5, 100 mM NaCl buffer to reduce the imidazole concentration to about 25 mM and digested overnight at 277 K with His₆-tagged TEV protease (Kapust *et al.*, 2001). The digest was applied to a 25 ml Ni-NTA Superflow column equilibrated in buffer A, and recombinant DUSP22 (Met1–Glu163, E24A/K28A/K30A/C88S) emerged in the column effluent. The effluent was incubated overnight with dithiothreitol (10 mM), concentrated using an Amicon Ultracel 10 kDa cellulose membrane and applied to a HiPrep 26/60 Sephacryl S-100 HR column (GE Healthcare Bio-Sciences Corporation, Piscataway, New Jersey, USA) equilibrated in 20 mM HEPES pH 7.2, 200 mM NaCl, 2 mM tris(2-carboxyethyl)phosphine buffer. Fractions containing nearly pure recombinant protein were pooled, passed through a second Ni-NTA Superflow column, concentrated and re-chromatographed on the HiPrep 26/60 Sephacryl S-100 HR column. The peak fractions containing DUSP22 (Met1–Glu163, E24A/K28A/K30A/C88S) were pooled and concentrated to 20–25 mg ml^{−1} (estimated from the absorbance at 280 nm using a molar extinction coefficient of 18 450 M^{−1} cm^{−1} derived using the ExPASy ProtParam tool; Gasteiger *et al.*, 2003). Aliquots were flash-frozen in liquid nitrogen and stored at 193 K. The final product was judged to be >95% pure by SDS-PAGE. The molecular weight was confirmed by electrospray ionization mass spectroscopy.

2.2. Crystallization and data collection

DUSP22 (Met1–Glu163, E24A/K28A/K30A/C88S) (21 mg ml^{−1}) was incubated with 10 mM pNPP (Sigma-Aldrich, St Louis, Missouri, USA) dissolved in deionized water for 2 h on ice. Insoluble or precipitated compound was removed by centrifugation. The sample was screened for crystals using a Phoenix crystallization robot (Art Robbins Instruments, Sunnyvale, California, USA) and the commer-

Table 1

Data-collection and refinement statistics.

Values in parentheses are for the highest resolution shell.

Data collection	
X-ray source	22-BM, SER-CAT
Wavelength (Å)	1.0
Resolution (Å)	50.0–1.34 (1.36–1.34)
Space group	C2
Unit-cell parameters (Å, °)	$a = 89.8$, $b = 49.6$, $c = 39.9$, $\alpha = 90.0$, $\beta = 94.9$, $\gamma = 90.0$
Total/unique reflections	137570/37336 (1522)
Completeness (%)	95.5 (78.8)
$R_{\text{merge}}^{\dagger}$	0.035 (0.315)
$\langle I/\sigma(I) \rangle$	38.9 (2.8)
Multiplicity	3.7 (2.5)
Refinement statistics	
Resolution (Å)	44.7–1.34
$R_{\text{work}}^{\ddagger}$ (%)	14.0
$R_{\text{free}}^{\ddagger}$ (%)	16.5
No. of atoms	
Protein	1330
Water	193
<i>p</i> -Nitrophenolphosphate	14
Polyethylene glycol	7
Ethylene glycol	4
Mean <i>B</i> factor (Å ²)	
Protein	16.9
Water	40.4
<i>p</i> -Nitrophenolphosphate	19.0
Polyethylene glycol	37.7
Ethylene glycol	26.8
R.m.s. deviation from ideal geometry	
Bond lengths (Å)	0.009
Bond angles (°)	1.3
MolProbity validation	
Ramachandran plot	
Preferred regions (%)	96.7
Allowed regions (%)	3.3
Outliers (%)	0
MolProbity clash score	4.1 [93rd percentile]
MolProbity protein geometry score	1.5 [76th percentile]
PDB code	4woh

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $\langle I(hkl) \rangle$ is the mean intensity of multiply recorded reflections. $\ddagger R = \sum_{hkl} ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum_{hkl} |F_{\text{obs}}|$. R_{free} is the *R* value calculated for 5% of the data set that was not included in refinement.

Figure 1

Stereo image illustrating the superimposed structures of wild-type DUSP22 (PDB entry 1wrm, magenta ribbons) and the DUSP22 (Met1–Glu163, E24A/K28A/K30A/C88S) construct (PDB entry 4woh, green ribbons). The residues selected for SER mutagenesis are depicted in stick form.

cially available sparse-matrix crystallization screens MCSG Screens I–IV (Microlytic, Burlington, Massachusetts, USA) and Pi-minimal Screen (Jena Bioscience, Jena, Germany). All crystallization trials were conducted at 291 K. 20 numerous crystallization hits were observed and condition B5 from the MCSG I screen [0.1 M Tris–HCl pH 8.5, 0.2 M magnesium chloride, 25%(w/v) polyethylene glycol 3350] was selected for follow-up optimization screens. All optimization reagents were purchased from Hampton Research (Aliso Viejo, California, USA). Crystals used in X-ray diffraction experiments grew under optimized conditions in which 2 μ l DUSP22 (Met1–Glu163, E24A/K28A/K30A/C88S)–pNPP complex solution (21.1 mg ml^{−1}) was mixed with 2 μ l well solution [0.1 M Tris–HCl pH 8.5, 0.2 M magnesium chloride, 20%(w/v) polyethylene glycol 3350]. The drops were sealed over 0.5 ml crystallization well solution using a 15-well EasyXtal Tool (Qiagen, Valencia, California, USA) and incubated at 291 K. Crystals appeared within 3–5 d. A single crystal was retrieved from the crystallization drop with a LithoLoop (Molecular

Dimensions, Altamonte Springs, Florida, USA), transferred to a 2 μ l drop of mother liquor supplemented with 20%(v/v) ethylene glycol and 10 mM pNPP, and allowed to soak for 1 min. The crystal was captured with a LithoLoop and then flash-cooled by rapidly plunging it into liquid nitrogen.

Native X-ray diffraction data were collected from a crystal held at 100 K using a 1.0 Å wavelength and a MAR 225 detector on beamline 22-BM of the SER-CAT facilities at the Advanced Photon Source, Argonne National Laboratory. 180 frames of data were collected using 3 s exposures, an oscillation angle of 1.0° and a crystal-to-detector distance of 120 mm. The data were integrated and scaled with *HKL-3000* (Minor *et al.*, 2006). The crystal belonged to space group *C2*, with unit-cell parameters $a = 89.8$, $b = 49.6$, $c = 39.9$ Å, $\alpha = 90.0$, $\beta = 94.9$, $\gamma = 90.0^\circ$. The calculated Matthews coefficient of 2.39 Å³ Da^{−1} and solvent content of 48.6% indicated the presence of one molecule per asymmetric unit (Matthews, 1968, Kantardjieff & Rupp, 2003).

2.3. Structure solution and refinement

The crystal structure of DUSP22 (Met1–Glu163, E24A/K28A/K30A/C88S) in complex with pNPP was solved by molecular replacement using the coordinates of the wild-type DUSP22 structure (PDB entry 1wrm) as a search model after deleting all solvent and ligand atoms (Yokota *et al.*, 2007). Molecular replacement was performed using the automated molecular-replacement option with *Phaser* in *PHENIX* (McCoy *et al.*, 2007; Adams *et al.*, 2011). The search model was used to locate one molecule in the asymmetric unit with data extending to 3.0 Å resolution. Rigid-body refinement of the solution was performed with *phenix.refine* (Afonine *et al.*, 2012). Several rounds of model building and refinement with *REFMAC5* (Murshudov *et al.*, 2011) and *Coot* (Emsley *et al.*, 2010) were performed to adjust the positions of the side-chain residues while gradually incorporating data up to the maximum resolution of 1.34 Å. The atomic coordinates and ligand topology file for pNPP were constructed using *PRODRG* (Schüttelkopf & van Aalten, 2004). The location of the pNPP was clearly identified using σ_A -weighted $mF_o - DF_c$ difference electron-density maps (Read, 1997). Water and solvent molecules were located with *Coot* and were manually inspected and refined with *REFMAC5*. Anisotropic *B*-factor

Figure 2

Fit of the 4-nitrophenyl phosphate (pNPP) molecule to the σ_A -weighted $mF_o - DF_c$ electron-density map (green, contoured at the 3.0σ level; 1.34 Å resolution) before addition of the substrate to the refined model. C atoms are represented in gray, N atoms in blue, O atoms in red and P atom in orange.

Figure 3

Stereo image illustrating the fit of the active-site residues and pNPP to the final $2mF_o - DF_c$ electron-density map (blue, contoured at the 1.0σ level; 1.34 Å resolution). C atoms are represented in green (protein) and gray (pNPP), N atoms in blue, O atoms in red and P atom in orange. Hydrogen bonds are depicted as red dashed lines.

refinements were conducted during the final stages of refinement. Model quality was validated using *MolProbity* (Chen *et al.*, 2010) and secondary-structure elements were assigned using *ksdssp* (Kabsch & Sander, 1983). Structural alignments were performed using *PyMOL*.

3. Results and discussion

3.1. Overall structure

Crystals of DUSP22 (Met1–Glu163, E24A/K28A/K30A/C88S) were obtained in space group *C2* and the structure was refined to 1.34 Å resolution with an *R* factor of 14.0% and an *R*_{free} of 16.5%. Data collection and refinement statistics are presented in Table 1. The refined crystal structure includes the non-native residues GGP in the N-terminal tail (remaining after TEV protease cleavage) and residues 1–155. Residues 156–163 that constitute the C-terminus were not visible in the

Figure 4
LIGPLOT representation highlighting the interaction between the pNPP substrate and active-site residues.

electron-density maps and were therefore excluded from the final model. Superposition of the structure with that of wild-type DUSP22 (PDB entry 1wrm) reveals that there are no significant structural distortions of the helix and loop that contain the E24A/K28A/K30A mutations (Fig. 1).

3.2. Binding interactions of pNPP

The position of the bound pNPP substrate was clearly identified in the active site of the enzyme in the σ_A -weighted $mF_o - DF_c$ electron-density map (Fig. 2). The substrate is held in the active site by six hydrogen-bonding interactions between the phosphate O atoms and backbone amide N atoms in the phosphate-binding loop (Figs. 3 and 4). Additional hydrogen-bonding interactions are found between the phosphate O atoms of pNPP and the side-chain Ser88 O' atom (2.5 Å), Arg94 NE atom (3.0 Å) and Arg94 NH2 atom (2.9 Å). The side chain of Ser88, which is a cysteine in the wild-type enzyme, is located within 3.4 Å distance of the P atom of pNPP, and thus is within an appropriate distance to serve as the catalytic nucleophile in the wild-type enzyme (Zhou *et al.*, 1994). Arg94 also wraps around the phosphate head group in a cradle-like fashion to provide a stabilizing positive-charge interaction with the negatively charged phosphate. Furthermore, two water molecules are located within hydrogen-bonding distance (2.8 Å) of the general acid/base residue Asp57 on the general acid loop. However, both are located approximately 6.6 Å from the P atom of pNPP and therefore are not optimally positioned to engage in nucleophilic attack in the current structure.

Facing the active-site region of DUSP22 (Fig. 5*a*), the electrostatic surface charge distribution map (Fig. 5*b*) clearly highlights the predominately positively charged active-site cavity that serves to attract the negative charge of the incoming phosphate moiety. The hydrophobic side chains of residues Leu89–Val92 of the phosphate-binding loop provide a hydrophobic environment around the aryl ring. Negatively charged patches formed by Asp57 and Asp37 also surround

Figure 5
(*a*) Ribbon representation of DUSP22 with bound pNPP (highlighted in sticks, C atoms in yellow). (*b*) Electrostatic potential mapped onto the surface of DUSP22. Hydrophobic regions are in gray, negatively charged regions are red and positively charged residues are blue. The bound pNPP molecule is highlighted as sticks with C atoms in yellow.

the active-site pocket. The region labeled 'A' in Fig. 5(b), adjacent to the pNPP-binding site, is characterized by very shallow indentations with a mostly hydrophobic character and dispersed negatively charged and positively charged regions on the outer edges. A deeper groove labeled 'B' in Fig. 5(b), situated on the other side of the pNPP-binding site, is composed of both positively charged and hydrophobic patches which extend into a deeper cavity that is predominately negatively charged. Given the rather shallow surfaces found in region A, it may better from a strategic vantage point to target the groove extending into the region B cavity for the design of elongated or bidentate inhibitors.

3.3. Comparison with DUSP22–MES and VHR–SA3 complex structures

The original publication of the DUSP22 structure identified a bound MES buffer molecule in the active site (Yokota *et al.*, 2007). The superimposed DUSP22–pNPP and DUSP22–MES (PDB entry 1wrn) structures show that the overall structures are virtually the same (r.m.s.d. of 0.26 Å over 989 aligned atoms) and that the bound MES buffer occupies a very similar binding mode to that of pNPP (Fig. 6a). The sulfate head group of the buffer molecule occupies a very similar position to the phosphate group and displays the same hydrogen-bonding interaction patterns with residues of the phosphate-binding loop as seen with the phosphate moiety of pNPP. Additionally, the morpholine ring of the MES molecule occupies the same space as the 4-nitrophenyl ring of pNPP.

The only example of a dual-specificity phosphatase with a bound inhibitor found in the Protein Data Bank is that of

vaccinia H1-related (VHR) phosphatase complexed with the inhibitor SA3 (PDB entry 3f81; Wu *et al.*, 2009). In other cases, only phosphate, sulfate or buffer molecules have been found in the active sites of DUSPs (Jeong *et al.*, 2014). There is one example of a structure of VHR bound to a bisphosphorylated peptide (Schumacher *et al.*, 2002). SA3 is a bidentate inhibitor of VHR with an IC_{50} of 0.078 μ M. However, in the crystal structure of the VHR–SA3 complex only portions of the SA3 inhibitor could be fitted into visible electron density. The superimposed coordinates of the VHR–SA3 and DUSP22–pNPP complexes (r.m.s.d. of 0.60 Å over 538 atoms) show that the oxo-thioxothiazolidinyl-ethanesulfonic acid moiety binds in a similar fashion to that of pNPP (Fig. 6b). The sulfonic acid group is hydrogen-bonded to several amide backbone N atoms of the phosphate-binding loop. In the case of VHR, multidentate analogs of SA3 were designed to target surface features outside the substrate-binding pocket (Wu *et al.*, 2009). Therefore, it is feasible that the crystal structure of DUSP22 bound to pNPP may serve as a useful starting point for the structure-assisted design of inhibitors that target the areas surrounding the active site. The use of phosphotyrosine-like substrate analogs to identify PTP inhibitor leads has been successful for other PTPs such as *Y. pestis* YopH (Bahta *et al.*, 2011, 2012) and may serve as a beneficial strategy to identify novel DUSP inhibitors as well.

4. Conclusion

The structure of DUSP22 bound to the phosphotyrosine-like substrate pNPP has been solved at 1.34 Å resolution. Using a substrate-trapping mutant (CS88) of DUSP22, clear electron

Figure 6

(a) Stereo image of the superimposed active-site residues of the DUSP22–pNPP complex (C atoms in green) and the DUSP22–MES complex (PDB entry 1wrn; C atoms in magenta). (b) Stereo image of the superimposed active-site residues of the DUSP22–pNPP complex (C atoms in green) and the VHR–SA3 complex (PDB entry 3f81; C atoms in magenta).

density for the entire pNPP molecule was found in the active site, allowing the unambiguous identification of its mode of binding. This is the first structure of a DUSP in complex with a small molecule substrate analog and it provides a template for the structure-assisted design of inhibitors. Using information gleaned from the binding mode of the pNPP substrate and by examining small pockets surrounding the active site, it may be feasible to develop a strategy for the design of bidentate or multidentate inhibitors of DUSP22.

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