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Structural characterization of a pathogenic mutant of human protein tyrosine phosphatase PTPN2 (Cys216Gly) that causes very early onset autoimmune enteropathy

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Abstract

PTPN2 is an important protein tyrosine phosphatase (PTP) that plays a key role in cell signaling. Deletions or inactivating mutations of PTPN2 have been described in different pathologies and underline its critical role in hematopoiesis, autoimmunity, and inflammation. Surprisingly, despite the major pathophysiological implications of PTPN2, the structural analysis of this PTP and notably of its pathogenic mutants remains poorly documented. Contrary to other human PTP enzymes, to date, only one structure of PTPN2 (wild-type form) has been reported. Here, we report the first crystal structure of a pathogenic mutant of PTPN2 (Cys216Gly) that causes an autoimmune enteropathy. We show in particular that this mutant adopts a classical PTP fold. More importantly, albeit inactive, the mutant retains its ability to bind substrates and to adopt the characteristic catalytically competent closed form of PTP enzymes. This novel PTPN2 structure may serve as a new tool to better understand PTP structures and the structural impacts of pathogenic mutations. Moreover, the C216G PTPN2 structure could also be helpful to design specific ligands/inhibitors.

KEYWORDS
crystal structure, pathogenic mutation, protein tyrosine phosphatase, PTPN2

1 | INTRODUCTION

PTPN2 is a ubiquitous protein tyrosine phosphatase (PTP) that serves as negative regulator of cytokines and

Abbreviations: PTP, protein tyrosine phosphatase; PTPN2, PTP non-receptor type 2.

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growths factors signaling.[1] PTPN2 is known to act as a tumor suppressor in T-cell leukemia and lymphoma.[2] Moreover, several studies have implicated PTPN2 in diabetes, autoimmune, and inflammatory diseases.[3–5] Knock-out mice models further support the key role of PTPN2 in hematopoiesis and inflammation.[6,7] More recently, PTPN2 was identified as a major target for cancer immunotherapy.[8–11] Despite the physiological and pathophysiological importance of PTPN2, this tyrosine phosphatase and its disease-associated mutated forms remain poorly studied at both molecular and structural levels.[1]

We have recently reported a germline de novo missense variant (C216G) in human PTPN2 as a novel cause of autoimmune enteropathy due to PTPN2-dependent aberrant activation of the JAK/STAT pathway.[12] Cys216 in PTPN2 corresponds to an evolutionarily conserved active site residue in PTPs that makes the nucleophilic attack on the phosphor-tyrosyl residue of the substrates (Figure 1a,b). Although mutations of this active site Cys residue are known to affect the activity of PTP enzymes, they can also convert them into inactive enzymatic forms that however retain the ability to bind substrates.[13–15]

Numerous structures of PTP enzymes (mostly their catalytic domains) have been described.[16] Notably, more than 200 structures of human PTP1B (including mutants and ligand bound forms), the closest homolog of PTPN2 (75% amino acid sequence identity between their catalytic domains, that is, residues 1–314), are available in the Protein Data Bank (PDB). Surprisingly, to date, only one PTPN2 structure (catalytic domain of WT PTPN2, residues 1–314) has been reported thus limiting the structural knowledge of this key PTP enzyme.[17] Therefore, to understand the structural bases of the pathogenic C216G mutation of PTPN2 and, more broadly, to better characterize the structure of this PTP, we carried out crystal structure studies of the C216G mutant.

2 | RESULTS AND DISCUSSION

As observed for other PTP enzymes, the full length PTPN2 (415 amino acids) is not easily amenable to crystallization, therefore we used the truncated form of C216G PTPN2 corresponding to the PTP catalytic domain (residues 1–314) that has been used previously to crystalize WT PTPN2.[17] Similar truncated forms have also been commonly used for the determination of the crystal structures of the catalytic domain of other PTPs, notably human PTP1B the closest homolog of PTPN2.[16] As shown in Figure 1c, both catalytic domains of WT and C216G PTPN2 (residues 1–314) were readily expressed and purified to homogeneity from *Escherichia coli* thus indicating that the C216G mutation has no gross impact on the quality of expression and solubility of the protein. Purified WT PTPN2 (catalytic domain, residues 1–314) was found to be active toward a tyrosine-phosphorylated peptide substrate (SAAPYLKTK) derived from human STAT3, whereas the C216G PTPN2 mutant was inactive (Figure 1d, left panel). Similar results were obtained with a generic nonpeptide phosphatase substrate (pNPP) (Figure 1d, right panel). In addition, upon transfection in HEK293 cells, the C216G PTPN2 mutant failed to dephosphorylate cellular tyrosine-phosphorylated proteins in contrast to the WT form (Figure 1e). Overall, these results are in agreement with our previous studies obtained with the full forms of WT and C216G PTPN2.[12] We next crystallized the C216G PTPN2 protein, which allowed us to determine the structure of this PTPN2 mutant at a resolution of 2.4 Å (Table 1; coordinates are deposited in the PDB under the PDB ID 6ZZ4). C216G PTPN2 was found as a dimer in the crystal as observed previously for other PTPs structures including human PTP1B (Figure 2a).[18–20] This contrasts with the unusual packing reported for WT PTPN2 (PDB code 1L8K), where one molecule of the enzyme (via its the DDQ loop) is inserted in the active site of a neighboring molecule, resulting in a continuous row of WT PTPN2 molecules.[17] The mechanism underlying this unusual crystal packing of WT PTPN2 structure (with multimerization of PTPN2 molecules) remains unclear and might point to crystal packing artifacts, suggesting that the WT PTPN2 structure reported so far might not reflect, at least in part, a biologically relevant structure of the enzyme.[17]

As observed for WT PTPN2, the overall structure of C216G PTPN2 adopts the characteristic PTP fold with, notably, the important structural/functional loops that are found in PTP enzymes (Figures 1a and 2b). The structure of the mutant clearly shows the replacement within the P-loop of the evolutionary conserved catalytic cysteine residue by a glycine and further supports that the mutation has no major effect on the overall structure of the enzyme as underlined by the RMSD value of 0.54 Å (over 254 Cα) between WT and C216G PTPN2 structures (Figure 2b,c). More in depth comparison shows nonetheless significant structural differences in the WPD and K122 loops (Figure 2c), whereas other important loops, including the P-loop that harbors the C216G mutated residue (and two other essential catalytic residues Arg221 and Ser223) have highly similar conformations (Figure 2c,d: P-loop panel; Figure S1). While in WT PTPN2, the WPD loop adopts a classical "open" conformation,[17] in C216G PTPN2 this loop is in a "closed" form (Figure 2d: WPD loop panel). In PTP enzymes, notably PTPN2 or PTP1B, the WPD loop acts as a flexible gate that upon substrate binding makes a

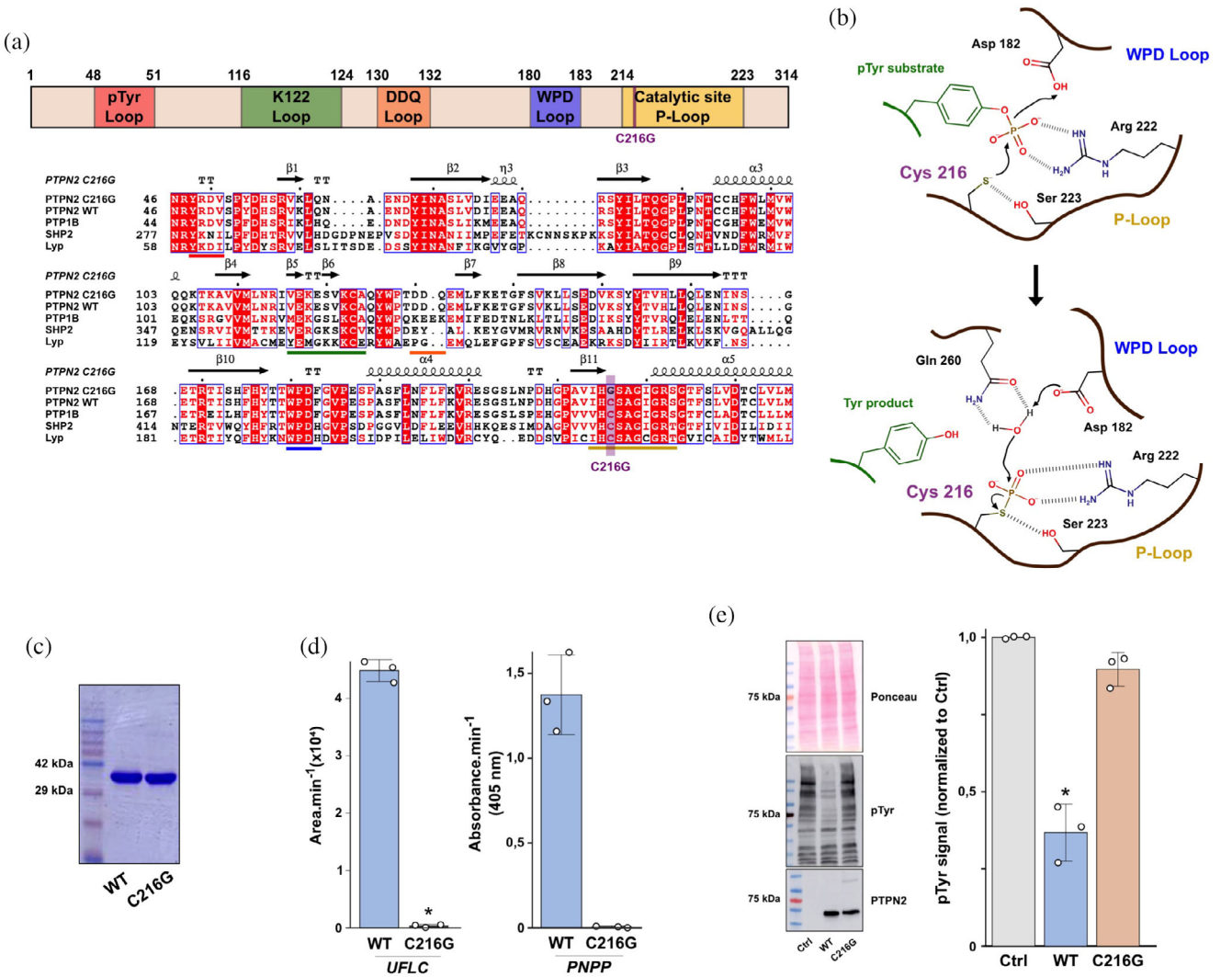

FIGURE 1 Expression, purification, and enzymatic characterization of C216G PTPN2 catalytic domain (residues 1–314). (a) Top panel: Schematic representation of the different key domains of PTPN2 1–314. Location of the C216G mutation is highlighted in purple. pTyr loop is shown in red, K122 loop in green, DDQ loop in orange, WPD loop in blue, and P-loop (catalytic site) in yellow. Bottom panel: Sequence alignment of human PTPN2, PTP1B, SHP2 (PTPN11), and Lyp (PTPN22) enzymes (Uniprot database). Key domains of PTPN2 are underlined using the same color scheme as top panel. Location of the conserved C216 residue is highlighted in purple. (b) Two step mechanism of PTPN2 phosphatase activity. C216 residue is essential for the phosphatase activity: the thiolate anion will perform a nucleophilic attack on the phosphate group of the phosphorylated tyrosine substrate, and this phosphate group will later react with a molecule of water to regenerate the catalytic cysteine. Catalytic residues are labeled in black and the phosphorylated tyrosine is displayed in green. Location of the WPD loop and P-loop are depicted in blue and yellow, respectively. (c) 3 μg of purified recombinant WT or C216G 1–314 PTPN2 were analyzed by SDS-PAGE and stained by Coomassie blue. The migration of the molecular weight markers are displayed in Lane 1. (d) Left panel: Recombinant WT or C216G 1–314 PTPN2 (10 nM) activity was assessed by UFLC using a pSTAT3 fluorescent peptide. Barplots and error bars represent the means and the *SD* of three independent experiments. *: *p*.value < .05 compared with control (WT). Right panel: recombinant WT or C216G 1–314 PTPN2 (400 nM) activity was assessed by absorbance (405 nm) using pNPP, a chromogenic phosphatase substrate. Barplots and error bars represent the means and the *SD* of three independent experiments. *: *p*.value < .05 compared with control (WT). (e) HEK293T cells transfected with WT or C216G Flag-PTPN2 plasmids and 50 μg of cellular protein extracts were separated by SDS-PAGE and transferred onto a nitrocellulose membrane. Flag-PTPN2 protein was detected using an anti-Flag antibody and the global level of tyrosine phosphorylation using an anti-Pan-pTyr antibody. Ponceau staining was carried out to ensure equal protein loading. pTyr signal was then normalized by Ponceau staining, and the resulting ratio normalized by control (Ctrl). Barplots and error bars represent the means and the *SD* of three independent experiments, respectively. *: *p*.value < .05 compared with control (Ctrl)

conformational switch movement of around 10 Å from an "open" to a catalytically competent "closed" form (Figure 2d: WPD loop panel).[16] This is consistent with the presence in the active site pocket of the C216G PTPN2 structure of a phosphate group that mimics a phosphorylated tyrosine substrate (in particular through

TABLE 1 Data collection and refinement statistics

Resolution range	43.59–2.432 (2.519–2.432)
Space group	C 1 2 1
Unit cell	142.89, 55.98, 101.42, 90, 120.74, 90
Total reflections	178,268 (16,822)
Unique reflections	25,796 (2,475)
Multiplicity	6.9 (6.8)
Completeness (%)	98.25 (94.49)
Mean I/sigma(I)	10.97 (2.38)
Wilson B-factor	45.48
R-merge	0.1178 (0.6409)
R-meas	0.1276 (0.6941)
R-pim	0.04844 (0.2634)
CC1/2	0.996 (0.878)
CC*	0.999 (0.967)
Reflections used in refinement	25,770 (2,468)
Reflections used for R-free	1,288 (123)
R-work	0.2087 (0.2559)
R-free	0.2531 (0.3338)
CC (work)	0.931 (0.860)
CC (free)	0.921 (0.753)
Number of non-hydrogen atoms	4,760
Macromolecules	4,557
Ligands	30
Solvent	173
Protein residues	556
RMS (bonds)	0.009
RMS (angles)	1.24
Ramachandran favored (%)	97.10
Ramachandran allowed (%)	2.90
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.00
Clashscore	6.20
Average B-factor	50.48
Macromolecules	50.51
Ligands	60.60
Solvent	47.84
Number of TLS groups	2

contacts with the side chains of the important catalytic residues Arg222 and Asp182) (Figure S1b). Other PTP structures with a phosphate group in the active site have been previously reported and showed evidence of a closed WPD-loop.[21] Further structural comparison between C216G PTPN2 and the structures of PTP1B in its closed (RMSD 0.52 Å over 264 Cα) and open (RMSD 0.6 Å over 260 Cα) forms (PDB codes 15AY and 1T4J, respectively) further support that the C216G PTPN2 structure is in a "closed" form while WT PTPN2 structure was obtained in an open form (Figure S1c: WPD loop panel).[17] This further indicates that the C216G mutation does not impair binding of ligands in the active site that induce closure of the WPD loop and make the enzyme adopt a "catalytically competent" conformation.[16] Accordingly, it is known that inactive PTP enzymes which are mutated on their catalytic cysteine (equivalent to Cys216 in PTPN2) may retain the ability to bind substrates.[14,19] In addition, protein tyrosine pseudophosphatases have been described.[15] These proteins are atypical PTPs that are catalytically inactive through mutations within their active site (notably the catalytic cysteine residue) and/or important domains required for catalysis.[22] These inactive PTPs maintain a classical 3D fold and the ability to bind phosphorylated proteins and have been suggested to act as signaling regulators.[15] Thermal shift assay experiments with a tyrosine-phosphorylated peptide substrate (SAAPYLKTK) derived from human STAT3 further supported that albeit inactive, C216G PTPN2 was able to bind substrates (Figure S1g). This suggests that C216G PTPN2 could function as tyrosine pseudophosphatase.

The most striking structural difference between WT PTPN2 and C216G mutant form is observed in the K122 loop (also known as E-loop) (Figure 2d: E117-R222 salt bridge panel and K122 loop panel; Figure S1c: K122 loop panel). In PTPs structures, the K122 loop (residues 116–123 in PTPN2) adopts a conformation that allows the conserved Glu119 residue to form a salt bridge with the side chain of the invariant catalytic residue Arg222 of the P-loop (Figure 2d: E117-R222 salt bridge panel). This ionic bond is observed in all classical PTPs structures and serves to stabilize the structure of the P-loop in a position that favors binding of the tyrosine phosphate to the P-loop.[16,17] The C216G PTPN2 structure shows that the conformation of the K122 loop adopts a conformation that allows the formation of the key Glu117-Arg222 salt bridge (Figure 2d: E117-R222 salt bridge panel). This is in sharp contrast with the conformation of the K122 loop in the WT PTPN2 form, where the Glu117 residue is at a distance of 13 Å of the Arg222 residue thus impairing the formation of the Glu117-Arg222 ionic bond (Figure 2d: E117-R222 salt bridge panel). Moreover, the overall conformation of the K122 loop in WT PTPN2 is significantly different from the one of C216G mutant with the side chains of Lys118, Lys122, and Glu119 moving more than 10 Å between the two PTPN2 structures (Figure 2d: K122

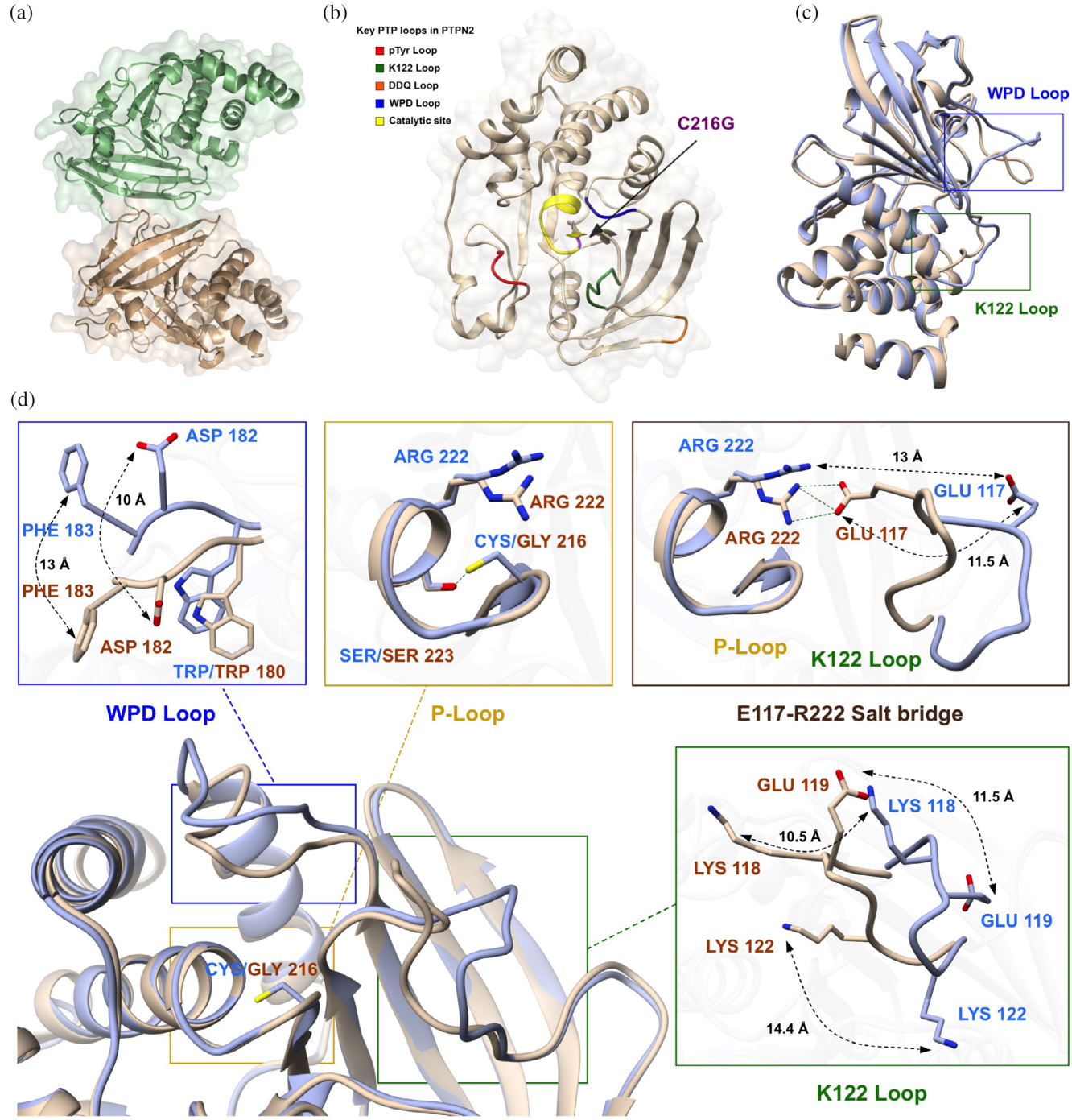

FIGURE 2 Overall structure and comparison of C216G PTPN2 and WT PTPN2. (a) Ribbon representation of the dimer of C216G PTPN2. Each monomer is represented with a different color. Protein surface is represented by transparency. (b) Ribbon representation of the Cα trace of C216G PTPN2. The positioning of each key domain of PTPN2 is displayed as follows: pTyr loop is shown in red, K122 loop in green, DDQ loop in orange, WPD loop in blue, and P-loop (catalytic site) in yellow. Location of the C216G mutation is shown with a purple arrow. Protein surface is represented by transparency. (c) Structural alignment of WT (blue) and C216G (beige) PTPN2 monomers. Regions of interest where differences are significant between the two structures are highlighted by boxes (K122 loop is displayed in green and WPD loop in blue). (d) Close-up view of the structural alignment between WT (blue) and C216G (beige) PTPN2. Each region of interest is zoomed in to have a more detailed view of the differences in the involved residues (P-loop [catalytic site] is shown in yellow, K122 loop in green, WPD loop in blue, and the interaction between the P-loop and the K122 loop is displayed in brown). Hydrogen bonds between Arg 222 and Glu 117 residues are depicted with green dashed lines. Distances between residues are represented with black double-heads dashed arrows. Residues are labeled in blue for WT PTPN2 and in brown for C216G mutant

loop panel). These significant differences of the structure of the K122 loop between the WT and mutant PTPN2 forms and the absence of the key Glu117-Arg222 salt bridge in WT PTPN2 are not because the two PTPN2 structures are in the closed (C216G PTPN2) or in the open form (WT PTPN2). Indeed, the conformation of the K122 loop in PTPs is not related to their closed or open state status.[16] This is further illustrated in Figure S1c (K122 loop panel) where the conformation of the K122 loop of C216G PTPN2 and PTP1B in its closed or open form are highly similar (with the side chains of the Glu117 and Lys122 of C216G PTPN2 and their PTP1B counterparts displaying positions), whereas the K122 loop of WT PTPN2 adopts a completely different conformation. As stated above, this unusual conformation of the K122 loop of WT PTPN2 compared to other PTPs (notably human PTP1B) could be due to crystallization artifacts that impacted the packing of the PTPN2 molecules in the crystal.[17]

In this work, we determined the first structure of an inactive mutant of PTPN2 (C216G PTPN2) which causes an intestinal autoimmune disorder.[12] We show that the overall structure of the C216G mutant is similar to WT PTPN2 structure and displays the characteristic PTP fold. More importantly, we found that, albeit inactive, the C216G PTPN2 mutant retains the ability to bind substrates and can adopt the characteristic catalytically competent PTP closed form.[16] This suggests that the C216G PTPN2 mutant could, at least partially, act as a pseudophosphatase.[22] Comparison of the C216G PTPN2 and WT PTPN2 structures also pointed out conformational differences in the K122 loop between the two forms of the enzyme that supports that the WT PTPN2 structure deposited in the PDB may not correspond (at least in part) to a biologically relevant structure of WT PTPN2.[17] While additional work will be needed to ascertain this point, we believe that this novel PTPN2 structure may help to understand the impact of pathogenic variants on PTPN2 conformation and may serve as a new tool for the study of PTP structures and design of specific ligands/inhibitors.

3 | MATERIALS AND METHODS

Detailed materials and methods can be found as supplementary material file.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Nadine Cerf-Bensussan, **Ahmed Haouz**, and **Fernando Rodrigues Lima**: Conceived the project. All authors performed experiments or/and analyzed the data. **Jérémy Berthelet** and **Fernando Rodrigues Lima**: Wrote the manuscript with input from all authors.

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SUPPORTING INFORMATION

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