



**HAL**  
open science

## **Cisplatin causes covalent inhibition of protein-tyrosine phosphatase 1B (PTP1B) through reaction with its active site cysteine: Molecular, cellular and in vivo mice studies**

Rongxing Liu, Wenchao Zhang, Panhong Gou, Jérémy Berthelet, Qing Nian, Guillaume Chevreux, Véronique Legros, Gautier Moroy, Linh-Chi Bui, Li Wang, et al.

### ► **To cite this version:**

Rongxing Liu, Wenchao Zhang, Panhong Gou, Jérémy Berthelet, Qing Nian, et al.. Cisplatin causes covalent inhibition of protein-tyrosine phosphatase 1B (PTP1B) through reaction with its active site cysteine: Molecular, cellular and in vivo mice studies. *Biomedicine and Pharmacotherapy*, 2022, 153, pp.113372. 10.1016/j.biopha.2022.113372 . hal-04281628

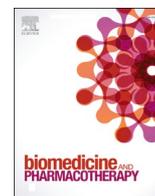
**HAL Id: hal-04281628**

**<https://cnrs.hal.science/hal-04281628>**

Submitted on 13 Nov 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# Cisplatin causes covalent inhibition of protein-tyrosine phosphatase 1B (PTP1B) through reaction with its active site cysteine: Molecular, cellular and *in vivo* mice studies

Rongxing Liu<sup>a</sup>, Wenchao Zhang<sup>a,b,c</sup>, Panhong Gou<sup>b,c,d</sup>, Jérémy Berthelet<sup>a,e</sup>, Qing Nian<sup>a,f</sup>, Guillaume Chevreux<sup>g</sup>, Véronique Legros<sup>g</sup>, Gautier Moroy<sup>a</sup>, Linh-Chi Bui<sup>a</sup>, Li Wang<sup>h</sup>, Jean-Marie Dupret<sup>a</sup>, Frédérique Deshayes<sup>a</sup>, Fernando Rodrigues Lima<sup>a,\*</sup>

<sup>a</sup> Université Paris Cité, CNRS, Unité de Biologie Fonctionnelle et Adaptative, F-75013 Paris, France

<sup>b</sup> Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

<sup>c</sup> Department of Lymphoma and Myeloma, The University of Texas MD Anderson Cancer Center, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

<sup>d</sup> Université Paris Cité, INSERM, Institut de Recherche Saint Louis, UMR 1131, F-75010 Paris, France

<sup>e</sup> Université Paris Cité, CNRS, Centre Epigénétique et Destin Cellulaire, F-75013 Paris, France

<sup>f</sup> Department of Blood Transfusion, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China and Chinese Academy of Sciences Sichuan Translational Medicine Research Hospital, Chengdu, China

<sup>g</sup> Université Paris Cité, CNRS, Institut Jacques Monod, F-75013 Paris, France

<sup>h</sup> Department of Hematology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

## ARTICLE INFO

### Keywords:

Protein tyrosine phosphatase 1B  
Cisplatin  
Redox sensitive cysteine  
Cysteine adduct  
EGFR signalling  
Drug target

## ABSTRACT

Protein tyrosine phosphatase 1B (PTP1B) is a critical regulator of different signalling cascades such as the EGFR pathway. The biological importance of PTP1B is further evidenced by knockout mice studies and the identification of recurrent mutations/deletions in PTP1B linked to metabolic and oncogenic alterations. Cisplatin is among the most widely used anticancer drug. The biological effects of cisplatin are thought to arise primarily from DNA damaging events involving cisplatin-DNA adducts. However, increasing evidence indicate that the biological properties of cisplatin could also rely on the perturbation of other processes such as cell signalling through direct interaction with certain cysteine residues in proteins. Here, we provide molecular, cellular and *in vivo* evidence suggesting that PTP1B is a target of cisplatin. Mechanistic studies indicate that cisplatin inhibited PTP1B in an irreversible manner and binds covalently to the catalytic cysteine residue of the enzyme. Accordingly, experiments conducted in cells and mice exposed to cisplatin showed inhibition of endogenous PTP1B and concomitant increase in tyrosine phosphorylation of EGFR. These findings are consistent with previous studies showing tyrosine phosphorylation-dependent activation of the EGFR pathway by cisplatin and with recent studies suggesting PTP1B inhibition by cisplatin and other platinum complexes. Importantly, our work provides novel mechanistic evidence that PTP1B is a protein target of cisplatin and is inhibited by this drug at molecular, cellular and *in vivo* levels. In addition, our work may contribute to the understanding of the pathways undergoing modulation upon cisplatin administration beyond of the established genotoxic effect of cisplatin.

## 1. Introduction

Phosphorylation of tyrosine residues by protein tyrosine kinases (PTKs) serves as a ubiquitous mechanism for cell signalling and plays an

important role in numerous biological processes such as proliferation, differentiation, migration and apoptosis [1]. Protein tyrosine phosphorylation is negatively regulated by the opposing action of protein tyrosine phosphatases (PTPs) that dephosphorylate phosphotyrosine

**Abbreviations:** PTP, protein tyrosine phosphatase; PTP1B, protein tyrosine phosphatase 1B; IAF, fluorescein 5-iodoacetamide; DTT, dithiothreitol; pNPP, *p*-nitrophenyl phosphate; EGFR, epidermal growth factor receptor; RP-UFLC, reverse phase ultra fast liquid chromatography; STAT, signal transducer and activator of transcription; FAM, carboxyfluorescein.

\* Correspondence to: Université Paris Cité, BFA, UMR 8251, CNRS, F-75013 Paris, France.

E-mail address: [fernando.rodrigues-lima@u-paris.fr](mailto:fernando.rodrigues-lima@u-paris.fr) (F. Rodrigues Lima).

<https://doi.org/10.1016/j.bioph.2022.113372>

Received 11 April 2022; Received in revised form 24 June 2022; Accepted 29 June 2022

Available online 6 July 2022

0753-3322/© 2022 The Authors. Published by Elsevier Masson SAS. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

(pTyr) residues. PTPs are thus key players of cellular signalling and responses [2,3]. Consequently, aberrant activity of PTPs is involved in different diseases such as diabetes, immune disorders and cancer [4–6]. PTP1B is a ubiquitous and abundant intracellular prototypic non receptor tyrosine phosphatase that is recognized as a major negative regulator of numerous signalling cascades of metabolic and/or oncogenic relevance such as the insulin or epidermal growth factor (EGF) pathways [6–8]. The critical biological role of PTP1B is further highlighted by the identification of recurrent mutations/deletions of PTP1B in certain cancer and by knock-out mice models showing metabolic and oncogenic alterations [9–14]. PTP1B activity depends upon a conserved catalytic cysteine residue that renders the enzyme redox sensitive [15–17]. Reversible oxidation and inhibition of PTP1B by reactive oxygen species (ROS) is considered as a critical physiological mechanism in the activation and the redox control of tyrosine kinase signalling as observed in the EGFR pathway [18,19]. In addition, chemical S-modification at the active site of PTP1B by environmental electrophiles (such as quinones or heavy metals) has been shown to inactivate the enzyme and drive aberrant EGFR tyrosine phosphorylation and signalling [20, 21].

Cisplatin is one of the most successful and widely used anticancer drug. The mechanism of action of cisplatin is thought to rely mainly on the formation of stable DNA adducts on guanine that lead to DNA damaging events and ultimately to cell death [22–24]. However, several studies indicate that cisplatin could also impact other biological processes such as DNA repair and cell signalling, most notably the EGFR pathway [23,25–29]. In addition, cisplatin could exert, at least in part, these biological effects through binding to peptide or proteins with subsequent structural and/or functional alterations [24,30,31]. In particular, the functions of certain cellular enzymes have been reported to be inhibited by cisplatin through binding to the thiol group of redox sensitive cysteines [26,32–35]. Interestingly, recent studies have pointed out that PTP1B could be inhibited by cisplatin albeit in depth molecular, cellular and *in vivo* evidences were lacking [70].

In this paper, we provide novel molecular, cellular and *in vivo* evidence that the chemotherapeutic drug cisplatin inhibits the enzymatic functions of PTP1B. Mechanistic and kinetics experiments using recombinant purified human PTP1B show that the inhibition is irreversible and involves the formation of a covalent adduct of cisplatin with the redox-sensitive catalytic cysteine residue of the enzyme. Further studies carried out in cells and in mice indicate that treatment with cisplatin leads to the irreversible inhibition of endogenous PTP1B and concomitant activation of EGFR signalling. Altogether, our findings identify PTP1B as a target of cisplatin and suggest that inhibition of PTP1B by cisplatin could contribute, at least in part, to biological effects of this widely used drug through aberrant PTP1B-dependent signalling. More broadly, our work provides a better understanding of the intricate network of pathways undergoing modulation upon cisplatin administration and that goes beyond the well-established genotoxic effect of cisplatin.

## 2. Materials and methods

### 2.1. Materials

Cisplatin, p-nitrophenyl phosphate (pNPP), 5-iodoacetamide fluorescein (5-IAF), N,N-dimethylformamide (DMF), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), HClO<sub>4</sub>, 1,4-dithiothreitol (DTT), sodium fluoride (NaF), sodium orthovanadate (Na<sub>3</sub>VO<sub>4</sub>), isopropyl β-D-thiogalactoside (IPTG), Triton X-100, lysozyme, His-select nickel resin, were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). Protease inhibitors were purchased from Roche (Meylan, France). PD-10 columns were from GE Healthcare (BUC, France). EGFR antibody (SAB4300352), β-actin antibody (SC-81178) and Protein G plus-agarose beads (SC-2002) were obtained from Santa Cruz Biotechnology (Heidelberg, Germany). PTP1B (FG6–1 G) and phospho-EGFR antibody (Tyr 1069, 09–310) were

obtained from Merck (Saint Quentin Fallavier, France). Oxidized PTP active site antibody (MAB2844) was obtained from R&D system (Minneapolis, USA). Xcalibur was purchased from Thermo Scientific Fisher (Boston, USA). Cisplatin was dissolved in DMF.

### 2.2. Cell culture

Human HaCaT or human A431 cells were cultured in DMEM medium (Invitrogen, Paris, France) with 10% fetal bovine serum (Sigma, France) at 37 °C with 5% CO<sub>2</sub>.

### 2.3. Expression and purification of recombinant human PTP1B

The complementary DNA (cDNA) coding for the shorter version of human PTP1B (1–321) was cloned into pET28a plasmid and was later transformed into *Escherichia coli* 21 (DE3). Bacteria were cultured at 37 °C under agitation until reaching an OD of 0.6, then grown overnight at 16 °C in the presence of 0.5 mM IPTG. Cells were harvested by centrifugation and resuspended in lysis buffer (20 mM Tris HCl, 100 mM NaCl, 0.5% Triton X-100, 1 mg/ml lysozyme and protease inhibitor, pH 7.5). The lysate was sonicated (10 s ON, 20 s OFF, 10 min, 20% power) on ice and pelleted (15,000 g, 30 min, 4 °C). Supernatant was incubated with His-select nickel resin in the presence of 10 mM imidazole for 2 h at 4 °C under agitation. Beads were then poured into a column, washed with washing buffer (20 mM Tris-HCl, 100 mM NaCl, 20 mM imidazole, pH 7.5) and PTP1B was eluted with elution buffer (20 mM Tris-HCl, 100 mM NaCl, 300 mM imidazole, pH 7.5). Protein elution was controlled by Bradford assay and eluted samples were incubated with 10 mM DTT on ice for 20 min. Purified PTP1B was buffer exchanged to 20 mM Tris-HCl, 100 mM NaCl, pH 7.5 using PD-10 column. Protein concentration and purity were assessed by Bradford reagent and SDS-PAGE. Proteins were stored at –80 °C until use.

### 2.4. Determination of PTP1B phosphatase activity using pNPP

The phosphatase activity of PTP1B was measured by pNPP assay as previously described [36,37]. Briefly, samples containing PTP1B (200 nM) were incubated with different concentrations of cisplatin in 100 mM sodium acetate buffer, 1 mM DTT, pH 6 for 30 min at 37 °C (total volume of 100 μl). 20 μl of each sample were taken and diluted 10 times with sodium acetate buffer containing 5 mM pNPP. The formation of the product (p-nitrophenol) was tracked by continuous measurement of the absorbance at 405 nm for 10 min at 37 °C using thermostatic microplate reader (BioTek, France) in a total volume of 200 μl. The final concentration of PTP1B after dilution was 20 nM.

### 2.5. Determination of recombinant PTP1B activity using FAM-conjugated tyrosine-phosphorylated STAT3 (pSTAT3) peptide by RP-UFLC

The tyrosine phosphatase activity of PTP1B was measured by RP-UFLC using FAM-conjugated peptide derived from the sequence of human STAT3 where tyrosine 704 is phosphorylated (701SAAP-pYLKTK<sub>709</sub>, Proteogenix, France) as previously described [36,38]. Briefly, samples containing PTP1B (200 nM) were incubated with different concentrations of cisplatin in 100 mM sodium acetate buffer, 1 mM DTT, pH 6 for 30 min at 37 °C (total volume of 100 μl). 20 μl of each sample were taken, diluted 10 times and incubated in sodium acetate buffer for 30 min at 37 °C in the presence of 50 μM FAM-conjugated pSTAT3 peptide (final concentration of PTP1B was 20 nM). The reaction was stopped with the addition of 50 μl HClO<sub>4</sub> (15%, v/v) and samples were injected into a Shim-pack XR ODS column (100 mm, 2 mm, 2.2 μm) (Shimadzu) at 40 °C. The mobile phase used for the separation consisted of two eluents: solvent A was water with 0.12% trifluoroacetic acid (TFA), and solvent B was acetonitrile with 0.12% TFA. Compounds were separated by an isocratic flow (80% A/20% B) rate of 0.6 ml/min. pSTAT3 peptide and its dephosphorylated form (product)

were monitored by fluorescence emission ( $\lambda = 530$  nm) after excitation at  $\lambda = 485$  nm and quantified by integration of the peak absorbance area.

## 2.6. Effects of reducing agent and buffer exchange on cisplatin-inhibited PTP1B

PTP1B (200 nM) was first incubated with cisplatin (25  $\mu$ M) in 100 mM sodium acetate buffer, pH 6 for 30 min at 37 °C (total volume of 100  $\mu$ l). Samples were either buffer exchanged (PD Spin Trap G-25; GE Healthcare) to sodium acetate buffer (100 mM, pH 6) or further incubated with 1 or 5 mM DTT (10 min at room temperature) before 10-fold dilution in sodium acetate buffer (100 mM, pH 6) and measurement of PTP1B residual activity using pNPP assay as described previously.

## 2.7. 5-IAF labeling and H<sub>2</sub>O<sub>2</sub> mediated oxidation of PTP1B catalytic cysteine

For IAF labeling, PTP1B (200 nM) was first incubated with cisplatin (25  $\mu$ M) in sodium acetate buffer (100 mM, 1 mM DTT, pH 6) at 37 °C for 30 min followed by another incubation at 37 °C for 10 min in the dark in the presence of 20  $\mu$ M 5-IAF. Samples were then split into two parts. One part was diluted 10-fold in sodium acetate buffer and used for measuring PTP1B residual activity using pNPP assay as described before; another part was separated by SDS-PAGE, transferred onto nitrocellulose membrane. 5-IAF fluorescence on PTP1B labelled cysteine residues was detected with a Fujifilm LAS 4000 detection system ( $\lambda_{exc} = 485$  nm,  $\lambda_{em} = 530$  nm). Membranes were later probed with anti-PTP1B antibody.

For the detection of PTP1B oxidized catalytic cysteine, PTP1B (200 nM) was incubated with cisplatin (25  $\mu$ M) or H<sub>2</sub>O<sub>2</sub> (250  $\mu$ M) at 37 °C for 30 min in sodium acetate buffer (100 mM, 1 mM DTT, pH 6) in a total volume of 100  $\mu$ l. Samples were then split into two parts. One part was diluted 10-fold in sodium acetate buffer and used for measuring PTP1B residual activity using pNPP assay as described above; the other part was separated by SDS-PAGE, transferred onto nitrocellulose membrane and probed with an antibody specific to the oxidized catalytic cysteine of PTPs. Membranes were washed thoroughly with PBS and further probed with anti-PTP1B antibody.

## 2.8. Effects of cisplatin on PTP1B in the presence of the competitive PTP inhibitor Na<sub>3</sub>VO<sub>4</sub>

PTP1B (200 nM) was incubated with 200  $\mu$ M Na<sub>3</sub>VO<sub>4</sub> in the presence or absence of cisplatin (25  $\mu$ M) in sodium acetate buffer (100 mM, 1 mM DTT, pH 6) at 37 °C for 30 min (total volume of 100  $\mu$ l). Samples were diluted 10-fold in sodium acetate buffer and PTP1B residual activity was quantified using pNPP assay as described before.

## 2.9. Mass spectrometry analysis of cisplatin binding to a PTP1B active site peptide containing the Cys215 residue

The peptide approach previously used to characterize oxidative modifications of Cys215 in PTP1B was carried out [39]. Briefly, the PTP1B peptide derived from the sequence of the enzyme catalytic domain (<sub>214</sub>HCSAGIGRS<sub>222</sub>, Proteogenix, Schiltigheim, France) (10  $\mu$ g, 0.5 mM) was incubated with cisplatin (2.5 mM) in H<sub>2</sub>O at 37 °C for 24 h in a total volume of 20  $\mu$ l. The peptide preparation (1  $\mu$ l) was injected into a BioResolve RP (450 Å, 2.7  $\mu$ m, 0.3 mm  $\times$  150 mm) Polyphenyl Column (Waters, Milford, USA) using an UltiMate 3000 RSLCnano chromatographic system fitted with a capillary flow meter from ThermoFisher Scientific (Waltham, MA, USA). Mobile phases A and B were respectively H<sub>2</sub>O/acetonitrile 98/2 and H<sub>2</sub>O/acetonitrile 20/80 acidified with 0.1% formic acid. The sample was eluted at a flow rate of 6  $\mu$ l/min using the following slope change points: 0–5 min hold at 6.3% B, 5 min slope until 25% B, 40 min slope until 56% B, 5 min wash at 100% B and finally 10 min hold at 6.3% B. The eluent was sprayed using the

conventional ESI ion source of a Q-Exactive Plus mass spectrometer from ThermoFisher Scientific operating in the positive ion mode. MS spectra were acquired at a resolution of 70,000 with a mass range of  $m/z$  250–1700 and an AGC target value of  $1 \times 10^6$  for a maximum ion accumulation time of 500 ms. MS/MS data were acquired at similar resolution, in data-dependent top2 acquisition mode with an AGC target value of  $5 \times 10^5$  for a maximum ion accumulation time of 250 ms. Parent ions were selected for fragmentation by Higher-energy C-trap Dissociation (HCD) with a dynamic exclusion time of 30 s. Raw data were processed with Thermo Xcalibur v3.0.63 and especially the isotope simulation tool embedded in the software was used to simulate mono-isotopic patterns obtained after reaction of cisplatin with the HCSA-GIGRS peptide of interest.

## 2.10. Kinetics of PTP1B inactivation by cisplatin

PTP1B (200 nM) was incubated with different concentrations of cisplatin in sodium acetate buffer (100 mM, 1 mM DTT, pH 6) at 37 °C. At different time points, aliquots were taken, diluted 10 times with sodium acetate buffer and then assayed for PTP1B residual activity using pNPP as described before. The inhibition rate of PTP1B by cisplatin can be represented as follows:  $\ln [\% \text{ residual activity}] = k_{obs} \times t$  (where  $t$  is time and  $k_{obs}$  is the apparent first-order inhibition rate constant). For each cisplatin concentration, the  $k_{obs}$  value can be calculated from the slope of the natural logarithm of the percentage of PTP1B residual activity as a function of time. The second-order rate constant ( $k_{inact}$ ) was determined from the slope of  $k_{obs}$  plotted as a function of cisplatin concentration ( $k_{obs} = k_{inact} \times [\text{cisplatin}]$ ). The reaction order between cisplatin and PTP1B could be determined by the slope of the linear plot of the natural logarithm of  $k_{obs}$  as a function of the natural logarithm of cisplatin concentration. The kinetic data were fitted and plotted using Prism 8.0.0 (GraphPad Software, La Jolla, USA).

## 2.11. Effects of cisplatin on EGFR tyrosine phosphorylation in cells

HaCaT or A431 cells were serum-starved overnight and then treated with different concentrations of cisplatin for 24 h at 37 °C, 5% CO<sub>2</sub>. Cells were then washed with PBS, harvested and lysed in lysis buffer (PBS, 1% Triton X-100, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM NaF and protease inhibitor) on ice for 30 min under agitation. Cell lysates were centrifuged at 15,000 g for 10 min at 4 °C, and the supernatants (whole cell extracts) were kept. Samples were separated by SDS-PAGE and transferred onto a nitrocellulose membrane. EGFR activation was then assayed by western blot using anti-phospho-EGFR antibody. Membranes were stripped and re-probed sequentially with anti-EGFR, anti-PTP1B and anti- $\beta$ -actin antibodies.

## 2.12. Immunoprecipitation of endogenous PTP1B

HaCaT or A431 cells were serum-starved overnight and treated with cisplatin (20  $\mu$ M) for 24 h at 37 °C, 5% CO<sub>2</sub>. Whole cell extracts were obtained as stated above, but without the addition of Na<sub>3</sub>VO<sub>4</sub> and NaF in lysis buffer. Endogenous PTP1B was immunoprecipitated by incubating 1 mg of whole cell extracts in the presence of 1  $\mu$ g of PTP1B antibody overnight at 4 °C under agitation. Samples were then rocked for 1 h at 4 °C in the presence of 30  $\mu$ l of protein G–Agarose. The immunobeads were harvested by centrifugation, washed three times with lysis buffer (PBS, 1% Triton X-100), and split in two. Half of the beads was incubated with 50  $\mu$ M FAM–phosphorylated STAT3 peptide in sodium acetate buffer (100 mM, 1 mM DTT, pH 6) at 37 °C for 2 h (total volume of 50  $\mu$ l) to determine endogenous PTP1B residual activity by RP-UFLC as described previously. The second part of immunobeads were incubated with non-reducing Laemmli sample buffer and heated at 100 °C for 10 min. Eluates were separated by SDS-PAGE, transferred onto nitrocellulose membrane and analyzed by western blot using an anti-PTP1B antibody.

### 2.13. Treatment of mice with cisplatin

C57BL/6 mice (12 weeks, female) were obtained from Janvier (Le Genest, France) and bred in pathogen-free conditions in the animal facility of the Institut de Recherche Saint-Louis (IRSL) (Hopital Saint-Louis, Universite de Paris). Mice ( $n = 3$ ) were treated by intraperitoneal injection of 7.5 mg/kg cisplatin in sodium chloride solution (0.9%). Control mice ( $n = 3$ ) were treated with vehicle only. After 5 h, liver tissues were taken and washed 3 times with cold PBS, cut into small pieces and washed again 3 times with cold PBS. Samples were lysed with lysis buffer (PBS, 1% Triton X-100, 1 mM  $\text{Na}_3\text{VO}_4$ , 1 mM NaF and protease inhibitor) for 30 min on ice under agitation. Lysates were centrifuged at 15,000 g for 10 min at 4 °C, supernatants were kept and analyzed for EGFR phosphorylation. Endogenous PTP1B was immunoprecipitated from lysates (without  $\text{Na}_3\text{VO}_4$  and NaF) and assayed for activity by RP-UFLC as described above. All the animal procedures performed were approved by the local Committee on the use and care of animal.

### 2.14. Statistical analysis

All data were reported as mean  $\pm$  standard deviation (S.D.) of three independent experiments. Quantification of western blot signal was carried out using ImageJ software (National Institutes of Health, USA). Data were analyzed by either t-test or one-way analysis of variance (ANOVA) using GraphPad Prism version 8.0.0. A threshold of  $p < 0.05$  was used to consider differences as statistically significant. (\*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ ).

## 3. Results

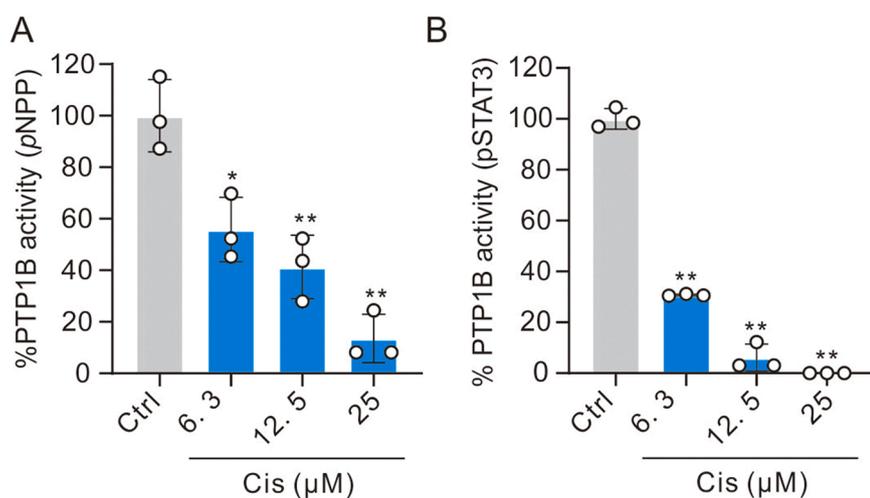
### 3.1. Cisplatin impairs the tyrosine phosphatase activity of human PTP1B enzyme in vitro

As stated above, although cisplatin is mainly known as a DNA-interacting drug, a limited number of cellular enzymes have nonetheless been shown to be targeted and inhibited by cisplatin at clinically relevant levels, *i.e.*  $< 100 \mu\text{M}$  [26,32,34,35,40–42]. We used purified recombinant human PTP1B to assess the effects of cisplatin on its activity. To this end, the enzyme was preincubated with different therapeutically-relevant concentrations of cisplatin (ranging from 0 to 25  $\mu\text{M}$ ) and residual PTP1B activity measured with the chromogenic prototypic phosphatase substrate pNPP as previously described [36,37]. As shown in Fig. 1 A, PTP1B was inhibited by cisplatin in a dose-dependent manner with almost full inhibition obtained at 25  $\mu\text{M}$  final concentration. These results were further confirmed with a

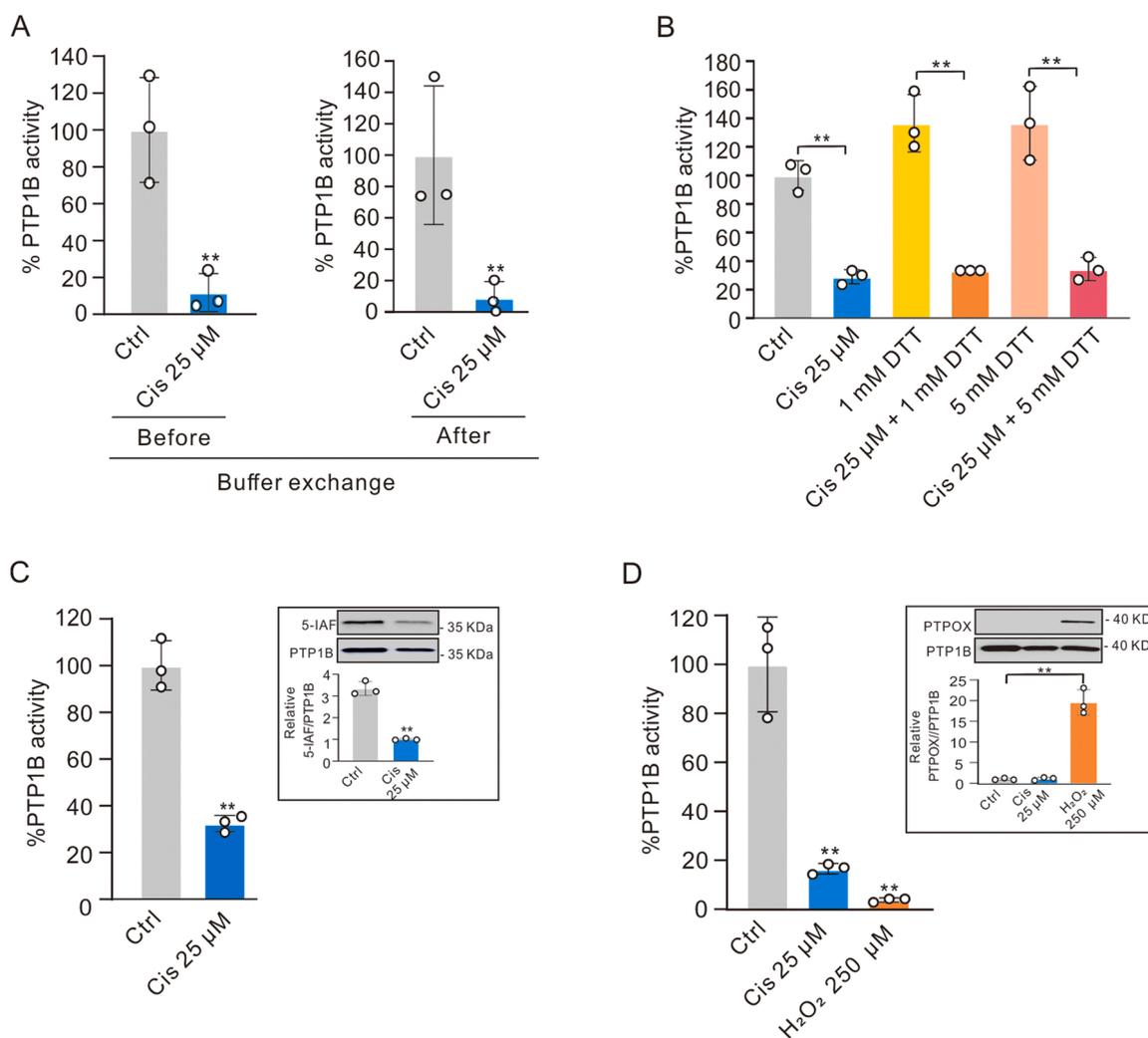
UFLC-based tyrosine phosphatase enzyme assay that uses a phospho-STAT3 peptide derived from the sequence of human STAT3 (701SAAPYLKTK709 where the Y705 is phosphorylated), a known substrate of PTP1B as described previously [36,38]. As expected, we found that the tyrosine-phosphatase activity of PTP1B towards the substrate peptide was inhibited by cisplatin in a similar manner as to what was observed with the pNPP assay (Fig. 1B).

### 3.2. PTP1B is irreversibly inhibited by cisplatin through the formation of a cisplatin adduct with its redox-sensitive active site cysteine residue

Cisplatin is known to be able to react with redox-sensitive cysteine within proteins to form irreversible covalent cisplatin adducts [24,43,44]. Moreover, it has been shown that the formation of these irreversible cysteine-cisplatin adducts could be responsible for the irreversible impairment of the catalytic activity of enzymes such as topoisomerase II,  $\text{Na}^+/\text{K}^+$  ATPase or glutathione transferase P1-1 [35,45,46]. We further analysed the inhibition of human PTP1B by cisplatin by preincubating the enzyme with cisplatin prior to dialysis and measurement of residual activity. As shown in Fig. 2 A, dialysis did not permit recovery of enzymatic activity of PTP1B previously exposed to cisplatin. Moreover, experiments carried out with DTT also showed no recovery of the PTP1B activity by this reducing agent (Fig. 2B). Altogether, these findings supported that inhibition of PTP1B by cisplatin proceeds through an irreversible mechanism, possibly through reaction with cysteine residues. We therefore investigated whether PTP1B cysteine residues were chemically modified by cisplatin. To this end, we used a method based on the labelling of free reduced cysteines by the sulfhydryl-reactive reagent iodoacetamide-fluorescein which has been used previously to detect the chemical modification of cysteine residues within proteins including PTP1B [47,48]. As shown in Fig. 2 C, inhibition of PTP1B by cisplatin was concomitant with the modification of cysteine residues as indicated by the decrease in IAF-labelling of the enzyme. Interestingly, the activity of PTP1B is known to rely on a redox-sensitive cysteine residue in the active site of the enzyme (Cys215) that can be reversibly or irreversibly oxidized by reactive oxygen species to sulfenamide, sulfenic, sulfinic and sulfonic species that inactivates the enzyme [15,16,18]. In addition, it has been shown that this active site cysteine residue could be covalently modified by certain electrophilic compounds with subsequent PTP1B inactivation [20,21]. We carried out western blots analyses using an antibody that specifically detects oxidized forms but not covalently adducted forms of the active cysteine residue of PTP1B or other PTPases [48,49]. As shown in Fig. 2D, we found that contrary to  $\text{H}_2\text{O}_2$ , inhibition of the enzyme by cisplatin was not due to oxidation of its catalytic cysteine residue thus supporting that cisplatin could impair PTP1B through a covalent cisplatin-cysteine



**Fig. 1.** Impairment of PTP1B phosphatase activity by cisplatin. PTP1B (200 nM) was incubated with increasing cisplatin concentrations (0  $\mu\text{M}$  (Ctrl), 6.3  $\mu\text{M}$ , 12.5  $\mu\text{M}$ , 25  $\mu\text{M}$ ) at 37 °C for 30 min and residual PTP1B activity was assayed. (A) PTP1B residual phosphatase activity determined with pNPP assay by following the absorbance of the solution at 405 nm. Data are represented as the mean of three independent experiments  $\pm$  SD. \* p-value  $< 0.05$ , \*\* p-value  $< 0.01$ . (B) PTP1B residual tyrosine phosphatase activity was measured using pSTAT3 peptide by RP-UFLC. Data are represented as the mean of three independent experiments  $\pm$  SD. \*\* p-value  $< 0.01$ .



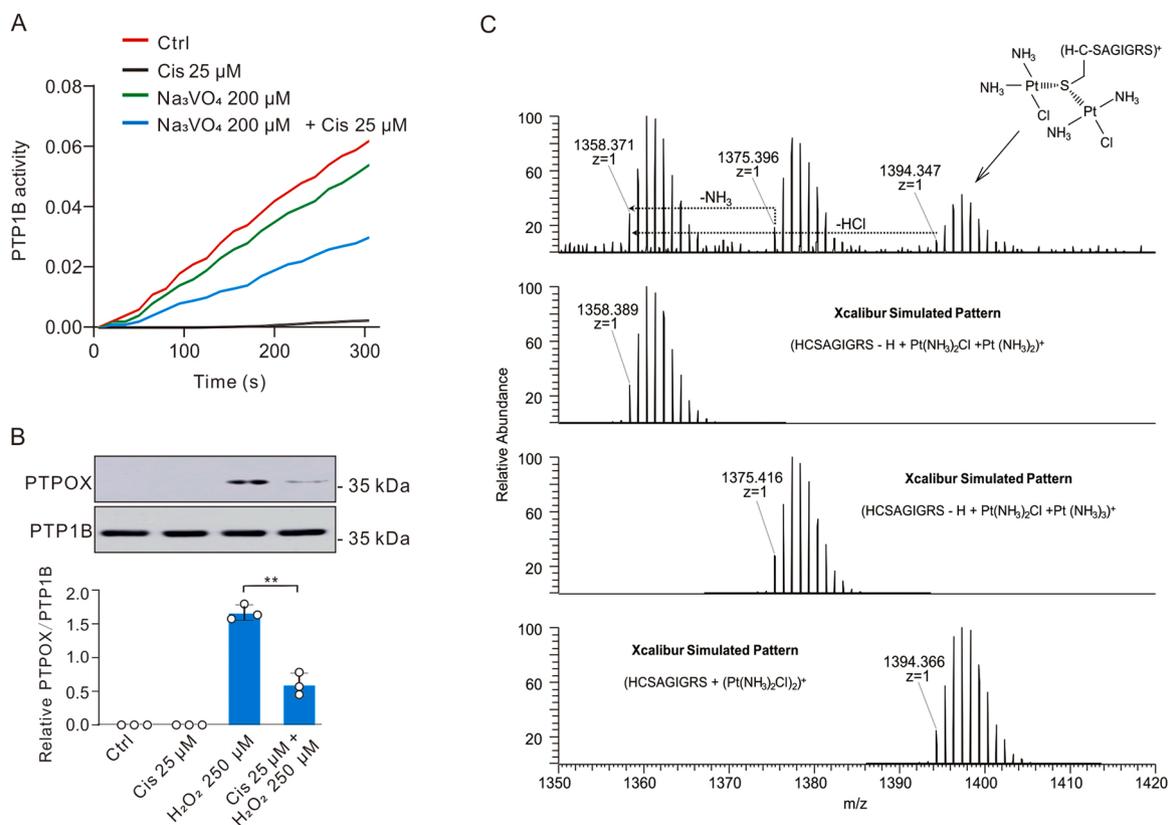
**Fig. 2.** Irreversibility of PTP1B inhibition by cisplatin and modification of cysteine residues. (A) PTP1B (200 nM) was incubated with cisplatin (25  $\mu$ M) at 37  $^{\circ}$ C for 30 min and then subjected to buffer exchange. A control (Ctrl) was done in absence of cisplatin. PTP1B residual activity was measured by pNPP before and after buffer exchange. Data are represented as the mean of three independent experiments  $\pm$  SD. \*\* p-value < 0.01. (B) PTP1B (200 nM) was incubated with cisplatin (25  $\mu$ M) at 37  $^{\circ}$ C for 30 min, followed by the addition of DTT (1 or 5 mM) and incubation at room temperature for 10 more minutes. A control (Ctrl) was done with PTP1B only. Other controls were done with PTP1B in presence of DTT. PTP1B residual activity in samples was measured by pNPP. Data are represented as the mean of three independent experiments  $\pm$  SD. \*\* p-value < 0.01. (C) PTP1B (200 nM) was incubated with or without (Ctrl) cisplatin (25  $\mu$ M) at 37  $^{\circ}$ C for 30 min, followed by the addition of 5-IAF (20  $\mu$ M) and further incubation at 37  $^{\circ}$ C for 10 more minutes. PTP1B residual activity was measured by pNPP assay and modification of PTP1B cysteine residues by 5-IAF was evidenced by fluorescence as described in *Materials and methods* section. Data are represented as the mean of three independent experiments  $\pm$  SD. \*\* p-value < 0.01. Membrane is representative of three independent experiments. (D) PTP1B (200 nM) was incubated with cisplatin (25  $\mu$ M), H<sub>2</sub>O<sub>2</sub> (250  $\mu$ M) or buffer (Ctrl) for 30 min at 37  $^{\circ}$ C. PTP1B residual activity and oxidation of PTP1B catalytic cysteine residue were detected by pNPP assay and western blot using an anti-oxidized PTP active site cysteine antibody, respectively. Data are represented as the mean of three independent experiments  $\pm$  SD. \*\* p-value < 0.01. Membrane is representative of three independent experiments.

adduct as reported previously for other enzymes [26,34,35].

Consistent with the data reported above, we conducted further experiments to test whether cisplatin-dependent inhibition of PTP1B could be due to covalent binding of cisplatin to the active-site cysteine residue of the enzyme. To this end, we carried out active-site protection assays using orthovanadate, a reversible competitive inhibitor of PTP1B that interacts with the catalytic cysteine residue within the active site of the enzyme [50]. This approach has already been used to identify chemicals that react with the catalytic cysteine of PTP1B and that inhibit its activity [37,48,51]. We observed that the presence of orthovanadate markedly slowed the inhibition of PTP1B by cisplatin thereby supporting that the inhibition process was active site directed and involved the catalytic cysteine residue (Fig. 3 A). In line with these results, we found that preincubation of PTP1B with cisplatin strongly decreased the oxidation of the catalytic Cys215 residue by H<sub>2</sub>O<sub>2</sub> as evidenced by western-blot using a specific antibody against oxidized forms of the

catalytic cysteine residue of PTP1B thus supporting that cisplatin reacted with this cysteine (Fig. 3B) [49,52].

To confirm that cisplatin can indeed react with the Cys215 in the active site of human PTP1B, mass spectrometry (MS) approaches were carried out. Unfortunately, we experienced technical difficulties for the MS analysis of purified PTP1B treated with cisplatin. Notably, peptides containing Cys215 could not be detected and more broadly the mass spectra were very complex. This is likely due to the myriad of different types (and masses) of cisplatin adducts that can be formed on cysteine residues [43,44,53]. Similar technical difficulties were previously reported for the reaction of cisplatin with cysteine residues of topoisomerase II $\alpha$  enzymes [45]. To circumvent this technical difficulties, MS experiments were conducted with a synthetic peptide (HCSAGIGRS) which is an active site sequence motif of PTP1B that contains the Cys215 residue. This peptide approach has been previously used to characterize oxidative modifications of Cys215 in PTP1B [39]. Consistent with the



**Fig. 3.** Analysis of PTP1B-cisplatin adduct on PTP1B active site cysteine. (A) PTP1B (200 nM) was incubated with  $\text{Na}_3\text{VO}_4$  (200  $\mu\text{M}$ ) at 37 °C for 30 min in the presence or absence of cisplatin (25  $\mu\text{M}$ ). Incubations of the enzyme in presence of cisplatin only were carried out. Incubation with PTP1B only was also done (Ctrl). PTP1B residual activity was measured by pNPP. (B) PTP1B (200 nM) was pre-incubated with cisplatin (25  $\mu\text{M}$ ) at 37 °C for 30 min prior to incubation with  $\text{H}_2\text{O}_2$  (250  $\mu\text{M}$ ) for 10 min. Control samples with PTP1B incubated with cisplatin or  $\text{H}_2\text{O}_2$  were also carried out. Oxidation of PTP1B catalytic cysteine residue was evidenced by western blot using an anti-oxidized PTP active site cysteine antibody. Membrane is representative of three independent experiments. (C) 10  $\mu\text{g}$  of PTP1B peptide derived from the sequence of the enzyme catalytic domain ( $_{214}\text{HCSAGIGRS}_{222}$ ) was incubated with cisplatin (2.5 mM) at 37 °C for 24 h prior to LC-MS/MS analysis. The combined non-deconvoluted signal of the modified peptide at charge state 1 + are shown (top panel). Thermo Xcalibur v3.0.63 and especially the isotope simulation tool embedded in the software was used to simulate isotopic patterns obtained after reaction of cisplatin with the HCSAGIGRS peptide (lower panels).

findings reported above, we found that cisplatin was able to covalently bind to the catalytic cysteine residue of PTP1B. Indeed, as shown in Fig. 3 C (upper panel) covalent adducts of cisplatin on the peptide were clearly identified. Notably, the highest monoisotopic mass at  $m/z$  1394.347 corresponded to the addition of two  $[\text{Pt}(\text{NH}_3)_2\text{Cl}]$  molecules on the free cysteine of the peptide. This type of cysteine-cisplatin adduct has already been identified in glutathione peptides treated with cisplatin [53]. The following peak at  $m/z$  1375.396 likely corresponded to another stoichiometry in solution with the addition of both  $[\text{Pt}(\text{NH}_3)_2\text{Cl}]$  and  $[\text{Pt}(\text{NH}_3)_3]$  while the last peak at  $m/z$  1358.371 could correspond to gas phase fragmentation with the loss of one HCl molecule. Simulations of the isotopic profiles of these species were generated using Xcalibur. They fitted very well the identified molecular compositions of the cisplatin-peptide adducts. Isotopic patterns are indeed very informative in this case with both chlorine and platinum atoms that display very specific isotopes resulting in unambiguous attribution of molecular compositions.

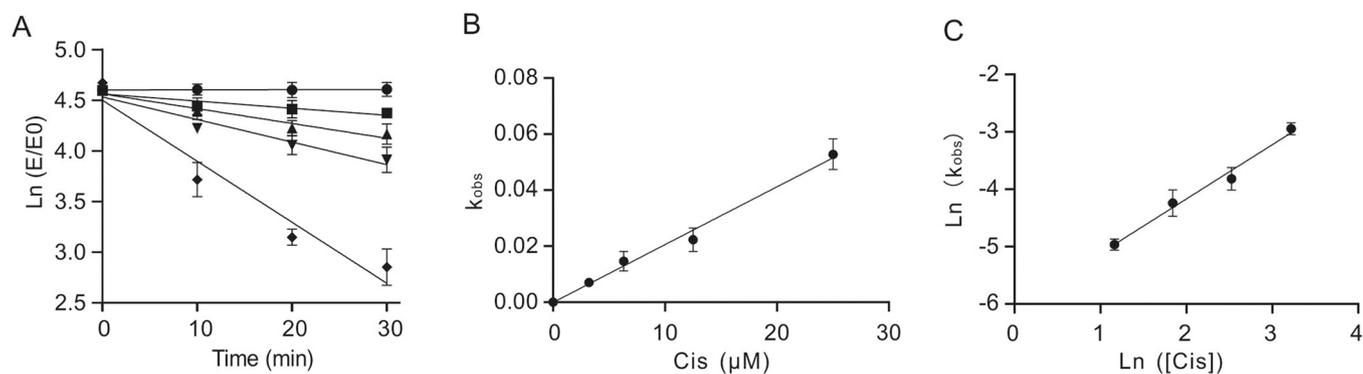
Formation of covalent adducts of cisplatin with cysteine residues has been shown to produce a broad absorption band between 220 and 350 nm corresponding to the cysteine-cisplatin adduct [35,54]. As shown in Supplementary Figure 1, incubation of the PTP1B active site peptide with cisplatin led to a time-dependent increase in absorbance between 220 and 350 nm thus supporting the formation of an adduct between the cysteine residue of the peptide and cisplatin. Finally, computational covalent docking approaches further supported that cisplatin can bind the catalytic cysteine residue within the active site of

PTP1B (Supplementary Figure 2).

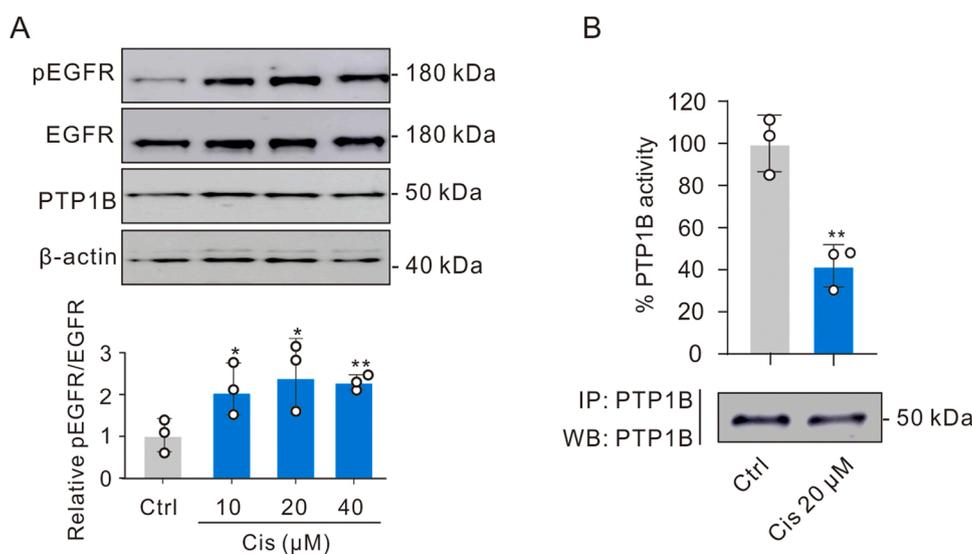
We carried out in depth kinetic analyses of PTP1B inhibition by cisplatin. Consistent with a process involving covalent modification of the active site cysteine of PTP1B, the enzyme was found to be inhibited in a time-dependent and dose-dependent manner (Fig. 4 A). The second-order rate inhibition constant ( $k_i$ ) was found to be close to 34  $\text{M}^{-1}\cdot\text{s}^{-1}$  (Fig. 4B). Stoichiometry of the PTP1B inhibition by cisplatin was close to 1 indicating that the inhibition relies on the reaction of one molecule of cisplatin with one molecule of enzyme (Fig. 4 C).

### 3.3. Cisplatin inhibits endogenous PTP1B and increases EGFR phosphorylation in human cultured cells and in mice

As stated previously, PTP1B is known to be a major regulator of the EGFR signaling pathway through its tyrosine phosphatase activity [18, 19,55]. In addition, covalent inhibition of PTP1B by electrophilic chemicals such as quinones is well known to increase EGFR tyrosine phosphorylation [20,21,56]. Interestingly, cisplatin has been shown to activate EGFR through increased tyrosine phosphorylation [25,27,57]. We thus exposed human HaCaT keratinocytes to cisplatin and monitored tyrosine phosphorylation of EGFR by western-blot as previously described [25,56]. HaCaT cells have been used in different studies aimed at analyzing PTP1B-dependent activation of EGFR phosphorylation and activation [56,58]. Consistent with earlier reports, we observed that exposure of the cells to cisplatin led to increased EGFR tyrosine phosphorylation (Fig. 5 A) [25,27,57]. Importantly and in line with these



**Fig. 4.** Kinetics of PTP1B inhibition by cisplatin. (A) PTP1B (200 nM) was incubated with different concentrations of cisplatin (circle represents 0  $\mu\text{M}$ , square represents 3.1  $\mu\text{M}$ , triangle represents 6.3  $\mu\text{M}$ , inverted triangle represents 12.5  $\mu\text{M}$ , diamond represents 25  $\mu\text{M}$ ) at 37 °C. At different time points, an aliquot of the mix was taken and PTP1B residual activity was determined with pNPP assay.  $k_{\text{obs}}$  were calculated from the slope of the natural logarithm of PTP1B residual activity as a function of time for each cisplatin concentration. Data are represented as the mean of three independent experiments + /- SD. (B)  $k_{\text{obs}}$  were plotted as a function of cisplatin concentration and the second order inhibition rate constant ( $k_i$ ) was determined from the slope of the linear regression (slope:  $2063 \pm 106 \text{ M}^{-1} \cdot \text{min}^{-1}$ ,  $R^2 = 0.97$ ). Data are represented as the mean of three independent experiments + /- SD. (C) Reaction order value was determined by a least-square procedure from the slope of the linear plot of the natural logarithm of  $k_{\text{obs}}$  as a function of the natural logarithm of cisplatin concentration (slope:  $0.95 \pm 0.068$ ,  $R^2 = 0.95$ ). Data are represented as the mean of three independent experiments + /- SD.



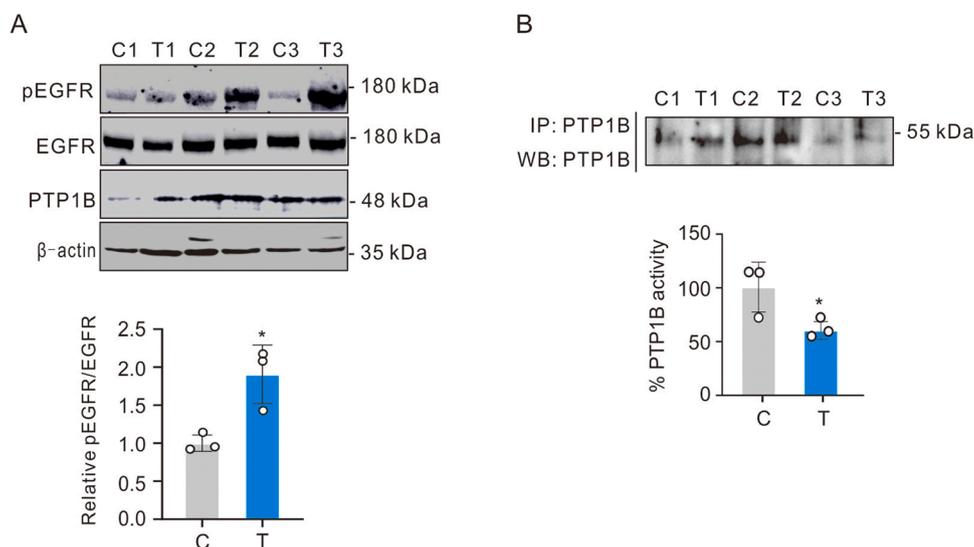
**Fig. 5.** Effects of cisplatin on EGFR tyrosine phosphorylation and on endogenous PTP1B activity in cells. (A) HaCaT cells were treated with different concentrations of cisplatin (0  $\mu\text{M}$ , 10  $\mu\text{M}$ , 20  $\mu\text{M}$ , 40  $\mu\text{M}$ ) for 24 h as described previously [25]. Total cell extracts were obtained and EGFR tyrosine phosphorylation and total EGFR protein expression were determined by western blot and quantified. PTP1B and  $\beta$ -actin amounts in cell extracts were also determined by western blot. Data are represented as the mean of three independent experiments + /- SD. \* p-value < 0.05, \*\* p-value < 0.01. Membrane is representative of three independent experiments. (B) HaCaT cells were treated with cisplatin (20  $\mu\text{M}$ ) for 24 h at 37 °C and endogenous PTP1B protein was immunoprecipitated from whole cell extracts. Residual PTP1B activity and PTP1B protein content were determined by RP-UFLC and western blot, respectively. Data are represented as the mean of three independent experiments + /- SD. \*\* p-value < 0.01. Membrane is representative of three independent experiments.

results, we found that the activity of cellular PTP1B was markedly impaired in the cells exposed to cisplatin (Fig. 5B). In line with this, endogenous EGFR activation (Supplementary Figure 3 A) and PTP1B activity inhibition (Supplementary Figure 3B) by cisplatin in A431 cells were further confirmed. Finally, we carried out investigations in mice (C57BL/6j,  $n = 3$ ) treated or not with a clinically-relevant dose of cisplatin *via* intraperitoneal injection (7.5 mg/kg). As shown in Fig. 6 A, treatment with cisplatin led to increased EGFR tyrosine phosphorylation in liver extracts of the treated mice. Accordingly, we observed a marked decrease in intrinsic tyrosine phosphatase activity of endogenous PTP1B when mice were treated with cisplatin (Fig. 6B). Taken together, these findings are consistent with the results reported above and support the notion that cisplatin is an inhibitor of PTP1B with subsequent impact on PTP1B-regulated signaling pathways such as EGFR [25,27,57].

#### 4. Discussion

Cisplatin is considered as one of the most potent anticancer drug in clinical use for the treatment of several cancers [23,62]. The biological

effects of this chemotherapeutic drug is thought to arise primarily from the formation of covalent adducts with purine DNA bases [24]. Yet, other key biological processes in particular protein phosphorylation-mediated cell signalling have been shown to be impacted by cisplatin [23,27,29,35]. We show here that the protein tyrosine phosphatase PTP1B, a key negative regulator of different protein tyrosine phosphorylation-dependent pathways, is impaired by cisplatin. In depth molecular and kinetic analyses using recombinant purified human PTP1B indicate that the inhibition of the tyrosine phosphatase activity of the enzyme is irreversible and occurs through the formation of covalent cisplatin-protein adducts with the active site cysteine residue of the enzyme (Cys 215). These data are in contrast with the results reported by Yuan et al. which suggested that cisplatin inhibits PTP1B reversibly [70]. However, reversible inhibition of PTP1B by cisplatin is unlikely. Indeed, the activity of PTP1B is known to rely on the redox status of this reactive active site Cys 215 residue [16,63]. Interestingly, the catalytic Cys 215 residue is prone to covalent modification by environmental electrophilic compounds such as naphthoquinones or aldehydes which have been shown to inhibit PTP1B



**Fig. 6.** Exposure of mice to cisplatin and subsequent effects on EGFR tyrosine phosphorylation and PTP1B activity in liver. (A) Mice were treated or not with cisplatin (7.5 mg/kg) for 5 h (C: control group; T: cisplatin treated group). Livers were taken, lysed and EGFR phosphorylation levels were determined by western blot. Data are represented as the mean of three independent experiments  $\pm$  SD. \* p-value  $< 0.05$ . Membrane is representative of three independent experiments. (B) Endogenous PTP1B protein was immunoprecipitated from liver whole cell extracts. Residual PTP1B activity and PTP1B protein content were determined by RP-UFLC and western blot, respectively. Data are represented as the mean of three independent experiments  $\pm$  SD. \* p-value  $< 0.05$ . Membrane is representative of three independent experiments.

irreversibly through covalent adduction [20,21,37]. Although cisplatin is mainly described as a DNA-interacting drug, it can covalently bind to certain reactive cysteine residues in proteins to form cisplatin adducts that can impact protein functions [24,35,43,64]. Of note, the binding of cisplatin to proteins is increasingly recognized as a mechanism whereby cisplatin could exert, at least in part, its biological effects [24,28,30,35]. In particular, certain cellular enzymes such as topoisomerase II, caspase 3 or  $\text{Na}^+/\text{K}^+$ -ATPase have been shown to be inhibited by cisplatin through covalent modification of important cysteine residues [26,32,34]. We found that the rate of inhibition of PTP1B by cisplatin ( $k_i = 34 \text{ M}^{-1} \cdot \text{s}^{-1}$ ) was very similar to the rates determined for the inhibition of human arylamine *N*-acetyltransferase 1 ( $12 \text{ M}^{-1} \cdot \text{s}^{-1}$ ) or GSTP1-1 ( $35 \text{ M}^{-1} \cdot \text{s}^{-1}$ ), two enzymes that are inhibited through the formation of cisplatin-cysteine adducts [33,35]. These rates are by far greater than rates of reaction of cisplatin with the cysteine residue of GSH, a known biological reducing agent that binds covalently to cisplatin ( $3.10^{-2} \text{ M}^{-1} \cdot \text{s}^{-1}$ ) or the thiol group of sodium-2-mercaptoethanesulfonate (mesna) a drug used to mitigate the toxic adverse effects of cisplatin ( $7.10^{-2} \text{ M}^{-1} \cdot \text{s}^{-1}$ ) [65,66]. Interestingly, the rate of reaction of cisplatin with DNA has been reported to be close to  $2 \text{ M}^{-1} \cdot \text{s}^{-1}$  [67]. In addition, the rate of inhibition of PTP1B by cisplatin is comparable to that reported previously for hydrogen peroxide ( $10 \text{ M}^{-1} \cdot \text{s}^{-1}$ ) and acrolein ( $87 \text{ M}^{-1} \cdot \text{s}^{-1}$ ), two known cellular inhibitors of PTP1B that modify the catalytic cysteine of the enzyme [37,68]. As stated above, PTP1B activity is a key negative regulator of different cell signalling cascades such as the EGFR pathway and inhibition of PTP1B activity is known to trigger increased EGFR tyrosine phosphorylation and activation [17–19,21]. In line with the results obtained with purified human PTP1B, we indeed observed that cisplatin was able to inhibit the activity of cellular PTP1B (Fig. 5B). In addition, these data are in agreement with studies conducted with different cell lines which showed that exposure to cisplatin leads to EGFR activation through increased tyrosine phosphorylation [25,27,57]. Experiments conducted in mice exposed to clinically-relevant concentrations of cisplatin further supported that cisplatin inhibits PTP1B and increases EGFR tyrosine phosphorylation. Interestingly, inhibition of PTP1B by chemical modification of its active site cysteine by certain electrophilic compound is known to trigger increased EGFR tyrosine phosphorylation and activation [19–21,69].

It is increasingly recognized that in addition to DNA, cisplatin exerts its biological effects by targeting proteins with subsequent alteration of cellular processes such as signalling pathways [24,28,32,35]. Interestingly, recent studies suggest that inhibition of PTP1B by platinum complexes could represent a novel anticancer mechanism [70]. In line with this, our work provides molecular, cellular and *in vivo* evidence that

cisplatin can impair the enzymatic functions of PTP1B thus supporting that PTP1B could be a protein target of cisplatin.

## Funding

This work was supported by running grants from Université Paris Cité, Centre National de la Recherche Scientifique (CNRS). RL, WZ, PG and QN were supported by PhD fellowships from the China Scholarship Council (CSC).

## CRedit authorship contribution statement

RL, JB, JMD and FRL designed research; RL, WZ, PG, JB, QN, VL, GM, and LCB performed research; All authors analyzed the data; RL, JB and FRL wrote the paper. All the authors commented on the paper.

## Conflict of interest statement

The authors declare no competing financial interests.

## Data Availability

Data will be made available on request.

## Acknowledgments

We thank the technical platform “BioProfiler-UFLC” (BFA Unit, Université Paris Cité) for provision of UFLC facilities.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.biopha.2022.113372](https://doi.org/10.1016/j.biopha.2022.113372).

## References

- [1] T. Pawson, Specificity in signal transduction: from phosphotyrosine-SH2 domain interactions to complex cellular systems, *Cell* 116 (2) (2004) 191–203.
- [2] N.K. Tonks, Protein tyrosine phosphatases: from genes, to function, to disease, *Nat. Rev. Mol. Cell Biol.* 7 (11) (2006) 833–846.
- [3] F. Bohmer, et al., Protein tyrosine phosphatase structure-function relationships in regulation and pathogenesis, *FEBS J.* 280 (2) (2013) 413–431.
- [4] T. Tiganis, A.M. Bennett, Protein tyrosine phosphatase function: the substrate perspective, *Biochem J.* 402 (1) (2007) 1–15.
- [5] X.M. Qi, et al., Targeting an oncogenic kinase/phosphatase signaling network for cancer therapy, *Acta Pharm. Sin.* B 8 (4) (2018) 511–517.

- [6] O. Villamar-Cruz, et al., Recent advances in PTP1B signaling in metabolism and cancer, *Biosci. Rep.* 41 (11) (2021).
- [7] P.D. Simoncic, C.J. McGlade, M.L. Tremblay, PTP1B and TC-PTP: novel roles in immune-cell signaling, *Can. J. Physiol. Pharm.* 84 (7) (2006) 667–675.
- [8] K.A. Pike, M.L. Tremblay, TC-PTP and PTP1B: Regulating JAK-STAT signaling, controlling lymphoid malignancies, *Cytokine* 82 (2016) 52–57.
- [9] M. Elchebly, et al., Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene, *Science* 283 (5407) (1999) 1544–1548.
- [10] S.G. Julien, et al., Protein tyrosine phosphatase 1B deficiency or inhibition delays ErbB2-induced mammary tumorigenesis and protects from lung metastasis, *Nat. Genet.* 39 (3) (2007) 338–346.
- [11] R. Banno, et al., PTP1B and SHP2 in POMC neurons reciprocally regulate energy balance in mice, *J. Clin. Invest.* 120 (3) (2010) 720–734.
- [12] J. Gunawardana, et al., Recurrent somatic mutations of PTPN1 in primary mediastinal B cell lymphoma and Hodgkin lymphoma, *Nat. Genet.* 46 (4) (2014) 329–335.
- [13] F. Jobe, et al., Deletion of Ptpn1 induces myeloproliferative neoplasm, *Leukemia* 31 (5) (2017) 1229–1234.
- [14] S. Le Sommer, et al., Deficiency in Protein Tyrosine Phosphatase PTP1B Shortens Lifespan and Leads to Development of Acute Leukemia, *Cancer Res* 78 (1) (2018) 75–87.
- [15] A. Salmeen, et al., Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate, *Nature* 423 (6941) (2003) 769–773.
- [16] Y.W. Lou, et al., Redox regulation of the protein tyrosine phosphatase PTP1B in cancer cells, *FEBS J.* 275 (1) (2008) 69–88.
- [17] N.K. Tonks, PTP1B: from the sidelines to the front lines!, *FEBS Lett.* 546 (1) (2003) 140–148.
- [18] S.R. Lee, et al., Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor, *J. Biol. Chem.* 273 (25) (1998) 15366–15372.
- [19] D.E. Heppner, A. van der Vliet, Redox-dependent regulation of epidermal growth factor receptor signaling, *Redox Biol.* 8 (2016) 24–27.
- [20] N. Iwamoto, et al., Chemical knockdown of protein-tyrosine phosphatase 1B by 1,2-naphthoquinone through covalent modification causes persistent transactivation of epidermal growth factor receptor, *J. Biol. Chem.* 282 (46) (2007) 33396–33404.
- [21] F. Zheng, et al., Redox toxicology of environmental chemicals causing oxidative stress, *Redox Biol.* 34 (2020), 101475.
- [22] K.J. Cullen, et al., Mitochondria as a critical target of the chemotherapeutic agent cisplatin in head and neck cancer, *J. Bioenerg. Biomembr.* 39 (1) (2007) 43–50.
- [23] S. Dasari, P.B. Tchounwou, Cisplatin in cancer therapy: molecular mechanisms of action, *Eur. J. Pharm.* 740 (2014) 364–378.
- [24] O. Pinato, C. Musetti, C. Sissi, Pt-based drugs: the spotlight will be on proteins, *Metalomics* 6 (3) (2014) 380–395.
- [25] M. Benhar, D. Engelberg, A. Levitzki, Cisplatin-induced activation of the EGF receptor, *Oncogene* 21 (57) (2002) 8723–8731.
- [26] B.B. Hasinoff, X. Wu, Y. Yang, Synthesis and characterization of the biological activity of the cisplatin analogs, cis-PtCl<sub>2</sub>(dextrazoxane) and cis-PtCl<sub>2</sub>(levrazoxane), of the topoisomerase II inhibitors dextrazoxane (ICRF-187) and levrazoxane (ICRF-186), *J. Inorg. Biochem.* 98 (4) (2004) 616–624.
- [27] T. Yoshida, et al., The anti-EGFR monoclonal antibody blocks cisplatin-induced activation of EGFR signaling mediated by HB-EGF, *FEBS Lett.* 582 (30) (2008) 4125–4130.
- [28] G.R. Tundo, et al., Effect of cisplatin on proteasome activity, *J. Inorg. Biochem.* 153 (2015) 253–258.
- [29] N.C. Schmitt, S. Trivedi, R.L. Ferris, STAT1 Activation Is Enhanced by Cisplatin and Variably Affected by EGFR Inhibition in HNSCC Cells, *Mol. Cancer Ther.* 14 (9) (2015) 2103–2111.
- [30] L. Messori, A. Merlino, Cisplatin binding to proteins: molecular structure of the ribonuclease A adduct, *Inorg. Chem.* 53 (8) (2014) 3929–3931.
- [31] M. Miyazawa, A.R. Bogdan, Y. Tsuji, Perturbation of Iron Metabolism by Cisplatin through Inhibition of Iron Regulatory Protein 2, *Cell Chem. Biol.* 26 (1) (2019) 85–97e4.
- [32] J.N. Shin, et al., Cisplatin inactivation of caspases inhibits death ligand-induced cell death in vitro and fulminant liver damage in mice, *J. Biol. Chem.* 280 (11) (2005) 10509–10515.
- [33] N. Ragunathan, et al., Identification of the xenobiotic-metabolizing enzyme arylamine N-acetyltransferase 1 as a new target of cisplatin in breast cancer cells: molecular and cellular mechanisms of inhibition, *Mol. Pharm.* 73 (6) (2008) 1761–1768.
- [34] J. Seflova, et al., Identification of cisplatin-binding sites on the large cytoplasmic loop of the Na<sup>+</sup>/K<sup>+</sup>-ATPase, *J. Enzym. Inhib. Med. Chem.* 33 (1) (2018) 701–706.
- [35] A. De Luca, et al., A structure-based mechanism of cisplatin resistance mediated by glutathione transferase P1-1, *Proc. Natl. Acad. Sci. USA* 116 (28) (2019) 13943–13951.
- [36] R. Duval, et al., A RP-UFLC assay for protein tyrosine phosphatases: focus on protein tyrosine phosphatase non-receptor type 2 (PTPN2), *Sci. Rep.* 5 (2015) 10750.
- [37] D.R. Seiner, J.N. LaButti, K.S. Gates, Kinetics and mechanism of protein tyrosine phosphatase 1B inactivation by acrolein, *Chem. Res Toxicol.* 20 (9) (2007) 1315–1320.
- [38] I.K. Lund, et al., Mechanism of protein tyrosine phosphatase 1B-mediated inhibition of leptin signalling, *J. Mol. Endocrinol.* 34 (2) (2005) 339–351.
- [39] V. Shetty, T.A. Neubert, Characterization of novel oxidation products of cysteine in an active site motif peptide of PTP1B, *J. Am. Soc. Mass Spectrom.* 20 (8) (2009) 1540–1548.
- [40] D.M. Townsend, M.H. Hanigan, Inhibition of gamma-glutamyl transpeptidase or cysteine S-conjugate beta-lyase activity blocks the nephrotoxicity of cisplatin in mice, *J. Pharm. Exp. Ther.* 300 (1) (2002) 142–148.
- [41] I. Tegeger, et al., Cisplatin tumor concentrations after intra-arterial cisplatin infusion or embolization in patients with oral cancer, *Clin. Pharm. Ther.* 73 (5) (2003) 417–426.
- [42] S.R. Martens-de Kemp, et al., DNA-bound platinum is the major determinant of cisplatin sensitivity in head and neck squamous carcinoma cells, *PLoS One* 8 (4) (2013), e61555.
- [43] T. Zimmermann, M. Zeizinger, J.V. Burda, Cisplatin interaction with cysteine and methionine, a theoretical DFT study, *J. Inorg. Biochem.* 99 (11) (2005) 2184–2196.
- [44] T. Minervini, et al., Fate of cisplatin and its main hydrolysed forms in the presence of thiolates: a comprehensive computational and experimental study, *Metallomics* 11 (4) (2019) 833–844.
- [45] B.B. Hasinoff, et al., Biochemical and proteomics approaches to characterize topoisomerase IIα cysteines and DNA as targets responsible for cisplatin-induced inhibition of topoisomerase IIα, *Mol. Pharm.* 67 (3) (2005) 937–947.
- [46] M. Huliciak, et al., Covalent binding of cisplatin impairs the function of Na<sup>+</sup>/K<sup>+</sup>-ATPase by binding to its cytoplasmic part, *Biochem. Pharm.* 83 (11) (2012) 1507–1513.
- [47] T.T. Wu, J.D. Castle, Tyrosine phosphorylation of selected secretory carrier membrane proteins, SCAMP1 and SCAMP3, and association with the EGF receptor, *Mol. Biol. Cell* 9 (7) (1998) 1661–1674.
- [48] R. Duval, et al., Benzoquinone, a leukemogenic metabolite of benzene, catalytically inhibits the protein tyrosine phosphatase PTPN2 and alters STAT1 signaling, *J. Biol. Chem.* 294 (33) (2019) 12483–12494.
- [49] R. Karisch, B.G. Neel, Methods to monitor classical protein-tyrosine phosphatase oxidation, *FEBS J.* 280 (2) (2013) 459–475.
- [50] G. Huyer, et al., Mechanism of inhibition of protein-tyrosine phosphatases by vanadate and pervanadate, *J. Biol. Chem.* 272 (2) (1997) 843–851.
- [51] J.N. LaButti, et al., Redox regulation of protein tyrosine phosphatase 1B by peroxyomnophosphate (=O<sub>3</sub>POOH), *J. Am. Chem. Soc.* 129 (17) (2007) 5320–5321.
- [52] J.M. Held, et al., Targeted quantitation of site-specific cysteine oxidation in endogenous proteins using a differential alkylation and multiple reaction monitoring mass spectrometry approach, *Mol. Cell Proteom.* 9 (7) (2010) 1400–1410.
- [53] D. Esteban-Fernandez, et al., Speciation analysis of platinum antitumor drugs in impacted tissues, *Talanta* 72 (2) (2007) 768–773.
- [54] T. Ishikawa, F. Ali-Osman, Glutathione-associated cis-diamminedichloroplatinum (II) metabolite and ATP-dependent efflux from leukemia cells. Molecular characterization of glutathione-platinum complex and its biological significance, *J. Biol. Chem.* 268 (27) (1993) 20116–20125.
- [55] T.H. Truong, K.S. Carroll, Redox regulation of epidermal growth factor receptor signaling through cysteine oxidation, *Biochemistry* 51 (50) (2012) 9954–9965.
- [56] V. Klaus, et al., 1,4-Naphthoquinones as inducers of oxidative damage and stress signaling in HaCaT human keratinocytes, *Arch. Biochem. Biophys.* 496 (2) (2010) 93–100.
- [57] A. Ahsan, et al., Role of epidermal growth factor receptor degradation in cisplatin-induced cytotoxicity in head and neck cancer, *Cancer Res* 70 (7) (2010) 2862–2869.
- [58] P. Baier, et al., Effect of liver regeneration after partial hepatectomy and ischemia-reperfusion on expression of growth factor receptors, *World J. Gastroenterol.* 12 (24) (2006) 3835–3840.
- [62] D. Wang, S.J. Lippard, Cellular processing of platinum anticancer drugs, *Nat. Rev. Drug Discov.* 4 (4) (2005) 307–320.
- [63] U. Schwertassek, et al., Reactivation of oxidized PTP1B and PTEN by thioredoxin 1, *FEBS J.* 281 (16) (2014) 3545–3558.
- [64] T. Zimmermann, Z. Chval, J.V. Burda, Cisplatin interaction with cysteine and methionine in aqueous solution: computational DFT/PCM study, *J. Phys. Chem. B* 113 (10) (2009) 3139–3150.
- [65] D. Hargman, J. Goodisman, A.K. Souid, Kinetic study on the reactions of platinum drugs with glutathione, *J. Pharm. Exp. Ther.* 308 (2) (2004) 658–666.
- [66] J.C. Dabrowiak, J. Goodisman, A.K. Souid, Kinetic study of the reaction of cisplatin with thiols, *Drug Metab. Dispos.* 30 (12) (2002) 1378–1384.
- [67] J. Jestin, B. Lambert, J.C. Chottard, Kinetic study of DNA binding of cisplatin and of a bicycloalkyl-substituted (ethylenediamine)dichloroplatinum(II) complex, *J. Biol. Inorg. Chem.* 3 (1998) 5.
- [68] J.M. Denu, K.G. Tanner, Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation, *Biochemistry* 37 (16) (1998) 5633–5642.
- [69] Y. Abiko, et al., Activation of PTP1B/EGFR signaling and cytotoxicity during combined exposure to ambient electrophiles in A431 cells, *J. Toxicol. Sci.* 46 (4) (2021) 177–185.
- [70] C. Yuan, et al., Potent and selective PTP1B inhibition by a platinum(II) complex: possible implications for a new antitumor strategy, *Chem. Commun. (Camb.)* 56 (1) (2020) 102–105.

### Further reading

- [1] K. Xu, H.K. Shu, Transcription factor interactions mediate EGF-dependent COX-2 expression, *Mol. Cancer Res* 11 (8) (2013) 875–886.
- [2] X. Tang, T.P.W. McMullen, D.N. Brindley, Increasing the low lipid phosphate phosphatase 1 activity in breast cancer cells decreases transcription by AP-1 and expressions of matrix metalloproteinases and cyclin D1/D3, *Theranostics* 9 (21) (2019) 6129–6142.
- [3] A. Sundqvist, et al., TGFbeta and EGF signaling orchestrates the AP-1- and p63 transcriptional regulation of breast cancer invasiveness, *Oncogene* 39 (22) (2020) 4436–4449.