Supporting Information

The Biological Buffer, Bicarbonate/CO_2, Potentiates H_2O_2-Mediated Inactivation of Protein Tyrosine Phosphatases

Haiying Zhou[§], Harkewal Singh,[§] Zachary Parsons[§], Sarah M. Lewis[§], Sanjib Bhattacharya[§], Derrick R. Seiner[§], Jason N. LaButti[§], Thomas J. Reilly[†], John J. Tanner,[§,‡,]* and Kent S. Gates[§,‡,]*

[§]Department of Chemistry, [‡]Department of Biochemistry, and [†]Department of Veterinary Pathobiology and Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO 65211

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*Department of Chemistry, 125 Chemistry Building, University of Missouri-Columbia, Columbia, MO 65211. E-mail: gatesk@missouri.edu and tannerjj@missouri.edu, phone: (573) 882-6763; (573) 884-1280; fax: (573) 882-2754

Materials. Reagents were purchased from the following suppliers: sodium acetate, tris(hydroxymethyl)aminomethane (Tris), 2-[bis-(2-hydroxyethyl)-amino]-2-hydroxymethyl-propane-1,3-diol (Bis-tris), 4-nitrophenyl phosphate disodium salt hexahydrate (PNPP), *5*,5'-dithiobis(2-nitrobenzoic acid) (DTNB), *L*-glutathione reduced (GSH), D,L-dithiothreitol (DTT), ammonium bicarbonate, ethylenediaminetetraacetic acid, disodium salt dehydrate (EDTA), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), diethylenetriaminepentaacetic acid (DTPA), sodium hydrogen phosphate, papain (cat# P-3125), and N^{α}-benzoyl-L-arginine 4-nitroanilide hydrochloride (cat# B3279) were obtained from Sigma-Aldrich (St. Louis, MO). Sodium chloride, sodium hydroxide, potassium bicarbonate, sodium phosphate, and sodium bicarbonate were obtained from Fisher. Zeba mini and micro centrifugal buffer exchange columns, and Tween-80 were purchased from Pierce Biotechnology (Rockford, IL). Amicon Ultra centrifugal filter devices were purchased from Millipore (Milford, MA). The enzyme consisting of amino acids 1-322 of human PTP1B was expressed and purified as described previously[1] and the concentration of active enzyme in stock solutions was determined as described by Pregel et al.[2] "Thiol-free" PTP1B was prepared by two sequential Zeba mini centrifugal buffer exchange columns according to manufacturer protocol. The buffer exchange columns were equilibrated before use in sodium acetate (100 mM), Tris (50 mM), Bis-tris (50 mM, pH 7), DTPA (10 mM), and Tween 80 (0.05%). The thiol-free enzyme was further diluted, if necessary, with sodium acetate (100 mM), Tris (50 mM), Bis-tris (50 mM, pH 7), DTPA (10 mM), and Tween 80 (0.05%) to achieve final stock concentrations of 600 nM–8 μM active enzyme.

PTP1B 1-321 and 1-298 constructs. For the crystallographic studies, PTP1B catalytic domain constructs having cleavable polyhistidine tags were engineered. The coding sequence for residues 1-321 was subcloned into pKA8H vector using *Nde*I and *BamH*I site such that the *N*-terminal His_8 tag was cleavable using tobacco etch virus protease (TEVP). Briefly, the plasmid containing PTP1B coding sequence was amplified using PCR. The PCR product was gel purified using a 1% DNA agarose gel. The purified PCR product was ligated into pZErO vector at 16 °C. The resulting ligation product was transformed into DH5α and plated onto LB agar plates supplemented with 40 μg/mL kanamycin and the plate was incubated at 37 °C overnight. Following incubation, four single colonies were picked and grown in LB media supplemented with 40 μg/mL kanamycin. These cultures were incubated at 37 °C and 250 rpm overnight in an incubator-shaker and further used for plasmid preparation. The isolated plasmids were excised with *Nde*I and *BamH*I and gel purified as described above. These inserts were ligated into pKA8H vector (cut with *Nde*I/*Bam*HI and purified) followed by transformation into DH5α. The transformants were plated on LB agar plates supplemented with 50μg/mL ampicillin. Four single colonies were picked again for plasmid preparation and the clone was verified by DNA sequencing.

A shorter version of PTP1B including residues 1-298 was created by inserting a stop codon into the aforementioned plasmid. The QuickChange kit (Stratagene) was used for this purpose, and the clone was confirmed by DNA sequencing.

Expression and Purification of PTP1B (1-298 domain). The PTP1B (1-298) plasmid was transformed into *E. coli* BL21AI cells and plated on LB Agar containing ampicillin (50 μg/mL). The plate was incubated at 37 ºC overnight and a single colony

of the transformant was picked to inoculate 10 mL starter culture made of 1% tryptone and 0.5% yeast extract. This was incubated at 37 ºC with constant shaking at 250 rpm, overnight. The starter culture was used to inoculate 1 L of auto-induction media,[3] and the cells were allowed to shake constantly for two hours at 37 ºC and 250 rpm. After two hours of cell growth, the temperature was reduced to 25 ºC, and 0.2 % arabinose was added to the media. Cells were harvested after 20-21 hours by centrifugation at 4 ºC and 3500 rpm and resuspended in Buffer A (20 mM Tris, 150 mM NaCl, 10% glycerol pH 7.5). The cell pellet was quick-frozen into liquid nitrogen for later use.

Frozen cells were thawed at 4 ºC in the presence of the following protease inhibitors: 10 μM leupeptin, 1 μM pepstatin A, 1 mM PMSF. Cells were stirred for 15-20 minutes at 4 ºC followed by disruption using sonication. Unbroken cells and debris were removed by centrifugation for 60 min at 17,000 rpm. The supernatant was collected and subjected to a second centrifugation step for 30 min at 17,000 rpm. The resulting supernatant was used for further purification by immobilized metal-ion affinity chromatography (Ni^{2+}-charged HiTRAP; GE Healthcare). The fractions were eluted using buffer B (Buffer A supplemented with 1 M imidazole). Fractions containing PTP1B were pooled and mixed with TEVP (1 mg of TEVP per 40 mg of PTP1B) and 1 mM THP. The sample was incubated for 8 h at 20 ºC and then dialyzed against buffer A. The dialyzed protein was again loaded onto the Ni^{2+} charged column using buffer A. Tag-free PTP1B was collected in both the flow-through and by elution in 3% buffer B. The purified protein was dialyzed into 10 mM Tris, 25 mM NaCl, 1 mM EDTA, 1 mM THP pH 7.5. Finally, the protein was distributed into thin-walled PCR tubes, quick-frozen in liquid nitrogen, and stored at –80 ºC.

SHP2 expression and purification. The SHP2 encoding plasmid was transformed in to BL21AI cells and plated on LB–Agar supplemented with kanamycin (40 μg/ml). The plate was incubated at 37 ˚C overnight and a single colony of the transformant was picked to inoculate a 10 mL starter culture made of 1% tryptone and 0.5% yeast extract. This was incubated at 37 ºC with constant shaking at 250 rpm, overnight. The starter culture was used to inoculate 1 L of autoinduction[3] media and cells were allowed to shake constantly for 2 h at 37 ˚C and 250 rpm. After 2 h of cell growth, the temperature was reduced to 18 ˚C and 0.2 % arabinose was added to the media. Cells were harvested after 28 h at 4 ˚C and 3500 rpm by centrifugation and resuspended in 10 mM HEPES, 250 mM NaCl pH 7.5. The cell pellet was flash frozen in liquid nitrogen and was stored at –80 ˚C.

Frozen cells were thawed at 4 ºC and ruptured using a sonicator. Unbroken cells and debris was removed by centrifugation for 60 min at 17,000 rpm. The supernatant was collected and subjected to a second centrifugation step for 30 min at 17,000 rpm. The resulting supernatant was used for further purification by immobilized metal-ion affinity chromatography (Ni^{2+}-charged HiTRAP; GE Healthcare), followed by anion-exchange chromatography (HiTRAP Q; GE Healthcare). The purified enzyme was dialyzed into 10 mM HEPES, 250 mM NaCl, and 1 mM DTT at pH 7.5.

Supplemental Method 1. Enzyme Inactivation Assays. Thiol-free PTP1B was added to a mixture containing various concentrations of H_2O_2, various concentrations of $KHCO_3$ and buffer to achieve final concentrations of PTP1B (0.3 μM), sodium acetate (100 mM), Tris (50 mM), Bis-tris (50 mM, pH 7), DTPA (5 mM), and Tween 80

(0.025%). After mixing, the reactions were incubated at 25 ˚C. Assays without potassium bicarbonate employed 125-750 μM H_2O_2; assays containing 2 and 3.5 mM potassium bicarbonate employed 62.5-375 μM H_2O_2; assays containing 7 mM potassium bicarbonate employed 35-214 μM H_2O_2; assays containing 14.4 mM potassium bicarbonate employed 19-115 μM H_2O_2; assays containing 25 mM potassium bicarbonate employed 11-68 μM H_2O_2; assays containing 35 mM potassium bicarbonate employed 7-46 μM H_2O_2; assays containing 50 mM potassium bicarbonate employed 6-37 μM H_2O_2. The final pH of the inactivation mixtures did not vary more than ± 0.2 units from that of the starting buffer. Minor variations in pH cannot account for the bicarbonate effects reported here, as previous work shows that minor pH variations in the range 6.5-8.0 do not substantially alter the rate at which H_2O_2 alone (no bicarbonate) inactivates a PTP enzyme (see Figure 4 in ref 26). Enzyme activity remaining at various time points (30 s, 60 s, 90 s, 120 s, 150 s, and 180 s) was assessed by a method similar to those described previously.[4,5] Specifically, an aliquot (10 μL) of the inactivation reaction into an assay mixture (490 μL) containing Bis-tris (50 mM, pH 6.0), NaCl (100 mM), DTPA (10 mM), and *p*-nitrophenyl phosphate (*p*-NPP, 20 mM), followed by incubation at 30 ˚C for 10 min. The activity assay was quenched by addition of NaOH (500 μL of a 2 *N* solution in DI water) and the amount of *p*-nitrophenol released during the assay determined by measurement of the absorbance of *p*-nitrophenolate at 410 nm at 24 ˚C. Inactivation reactions including additives such as tryptophan (1 mM), sodium phosphate (50 mM), mannitol (100 mM), or desferal (1 mM) were performed as described above and the additives were present in the inactivation mixture prior to addition of the enzyme.

Inactivation assays involving SHP-2 were carried out in an identical manner except somewhat lower enzyme concentrations were employed (~ 0.2 μM).

The value of $\ln(A/A_0)$, where A is the enzyme activity remaining at time = t and A_0 is the enzyme activity at time = 0, was plotted against t and the pseudo-first-order rate constant for inactivation at each concentration of H_2O_2 (k_{obs}) calculated from the slope of the line. The apparent second-order rate constant for the inactivation process was obtained from the slope of a replot of k_{obs} versus H_2O_2 concentration.[4,6] The results of enzyme inactivations carried out under various conditions are shown below in Figures S1-S20. To investigate the stability of bicarbonate solutions under our reaction conditions, we added basic $BaCl_2$ to a mock assay solution and measured the resulting $BaCO_3$ precipitate.[7] This analysis revealed that the solutions retained the desired bicarbonate concentration over the course of a typical assay.

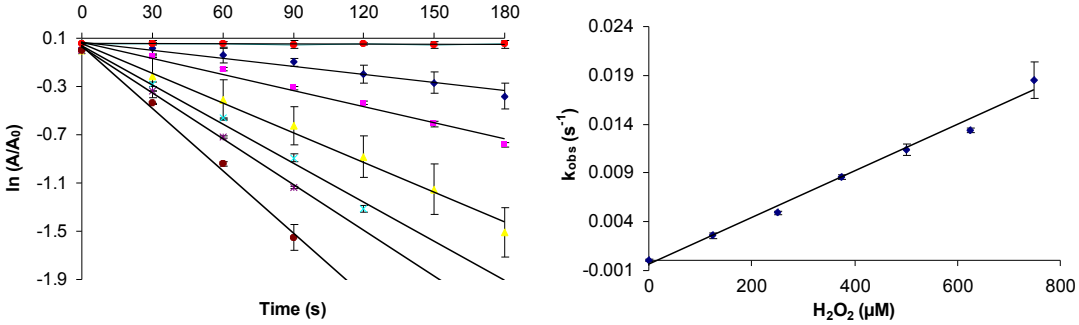


Figure S1. Inactivation of PTP1B by H_2O_2 alone. The lines correspond to 0, 125, 250, 375, 500, 625, 750 μM H_2O_2 from top to bottom. $k_{app} = 24 \pm 3\ M^{-1}\ s^{-1}$.

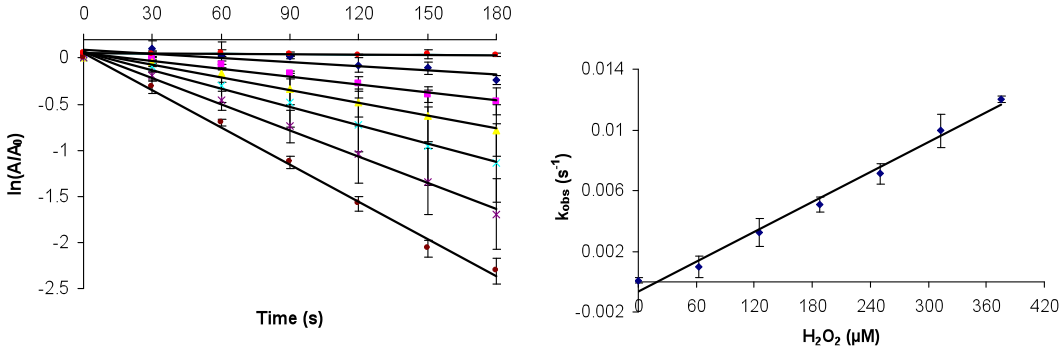


Figure S2. Inactivation of PTP1B by H_2O_2-$KHCO_3$ (2 mM). The lines correspond to 0, 62, 125, 188, 250, 312, 375 μM H_2O_2 from top to bottom. k_{app} = 33 ± 5 M^{-1} s^{-1}.

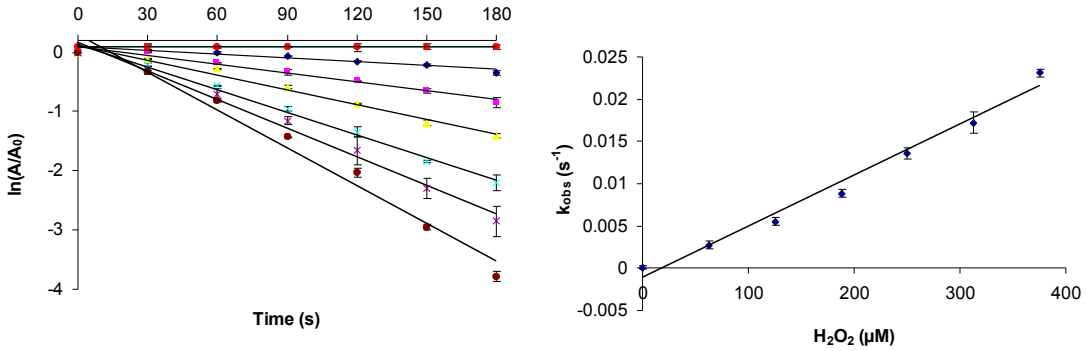


Figure S3. Inactivation of PTP1B by H_2O_2-$KHCO_3$ (3.5 mM). The lines correspond to 0, 62, 125, 188, 250, 312, 375 μM H_2O_2 from top to bottom. k_{app} = 61 ± 2 M^{-1} s^{-1}.

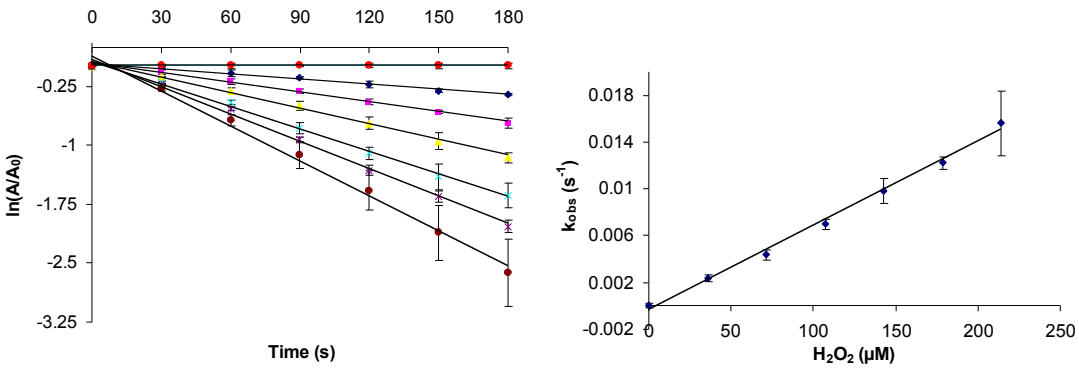


Figure S4. Inactivation of PTP1B by H_2O_2-$KHCO_3$ (7 mM). The lines correspond to 0, 36, 71, 107, 143, 179, 214 μM H_2O_2 from top to bottom. $k_{app} = 74 \pm 14$ M^{-1} s^{-1}.

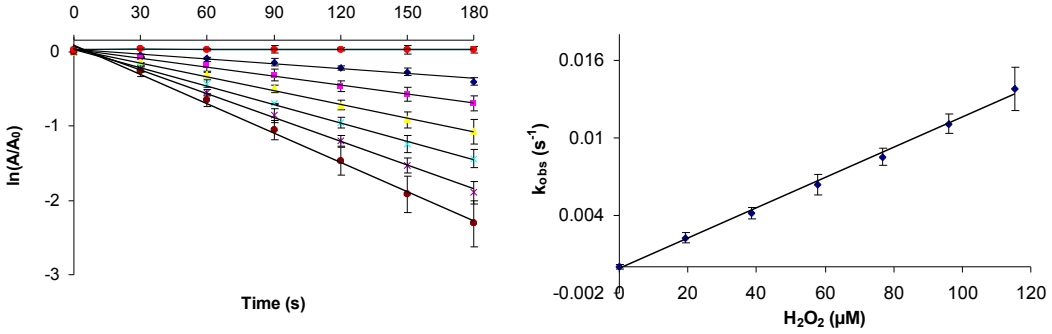


Figure S5. Inactivation of PTP1B by H_2O_2-$KHCO_3$ (14.4 mM). The lines correspond to 0, 19, 38, 58, 77, 96, 115 μM H_2O_2 top to bottom. $k_{app} = 117 \pm 11$ M^{-1} s^{-1}.

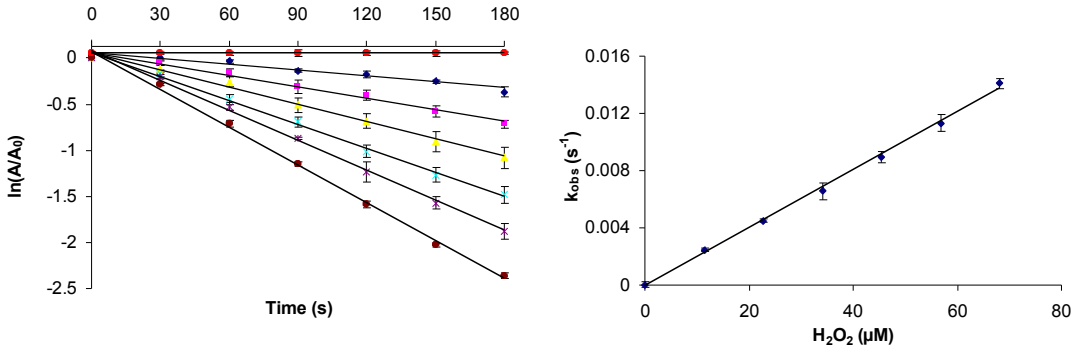


Figure S6. Inactivation of PTP1B by H_2O_2-$KHCO_3$ (25 mM). The lines correspond to 0, 11, 23, 34, 45, 57, 68 μM H_2O_2 from top to bottom. $k_{app} = 202 \pm 4$ M^{-1} s^{-1}.

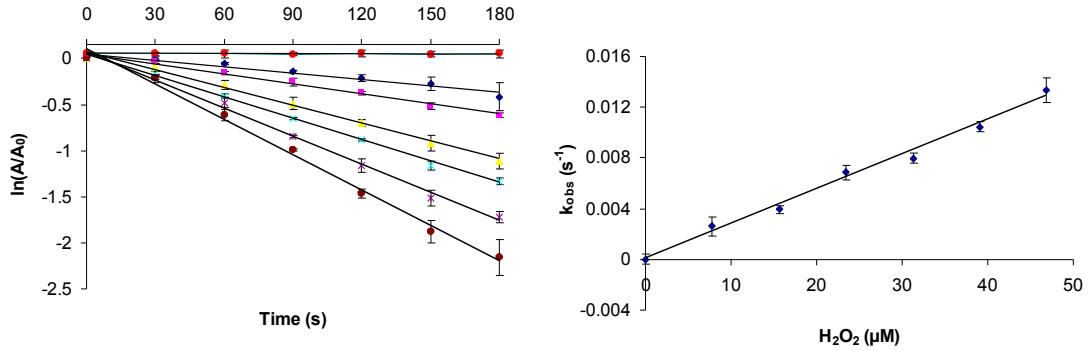


Figure S7. Inactivation of PTP1B by H_2O_2-$KHCO_3$ (35 mM). The lines correspond to 0, 8, 16, 23, 31, 39, 47 μM H_2O_2 from top to bottom. $k_{app} = 274 \pm 15$ M^{-1} s^{-1}.

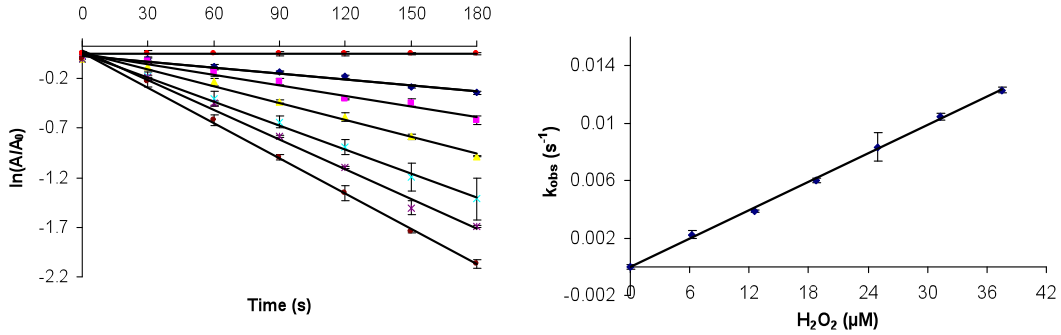


Figure S8. Inactivation of PTP1B by H_2O_2-$KHCO_3$ (50 mM). The lines correspond to 0, 6, 12, 19, 25, 31, 38 μM H_2O_2 from top to bottom. $k_{app} = 330 \pm 11$ M^{-1} s^{-1}

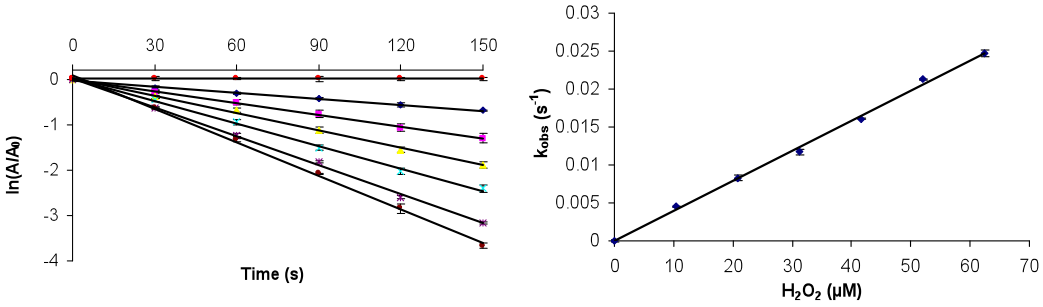


Figure S9. Inactivation of PTP1B by H_2O_2-$KHCO_3$ (25 mM) at 37 ˚C (rather than 25 ˚C which was used for the assays shown above). The lines correspond to 0, 10, 21, 31, 42, 52, 62 μM H_2O_2 from top to bottom. $k_{app} = 396 \pm 10$ M^{-1} s^{-1}. At 37 ˚C, in the presence of bicarbonate salts ($NaHCO_3$), a slow inactivation of the enzyme occurring with an apparent rate constant of 0.04 M^{-1} s^{-1} is observed even in the absence of H_2O_2.

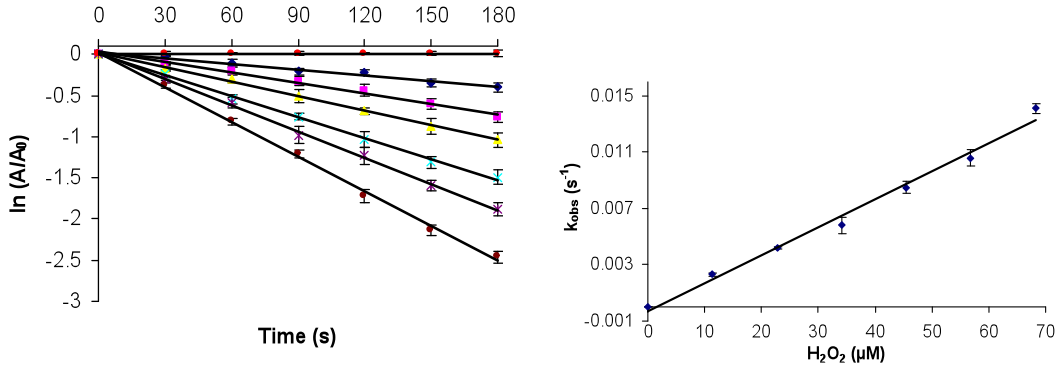


Figure S10. Preincubation of H_2O_2-$KHCO_3$ (25 mM) for 2 h prior to addition of PTP1B does not significantly change inactivation rates, relative to assays without preincubation. The lines correspond to 0, 11, 23, 34, 45, 57, 68 μM H_2O_2 from top to bottom. k_{app} = 199 ± 7 M^{-1} s^{-1} (compare to Figure S6, H_2O_2-$KHCO_3$ (25 mM) *without* preincubation, k_{app} = 202 ± 4 M^{-1} s^{-1}).

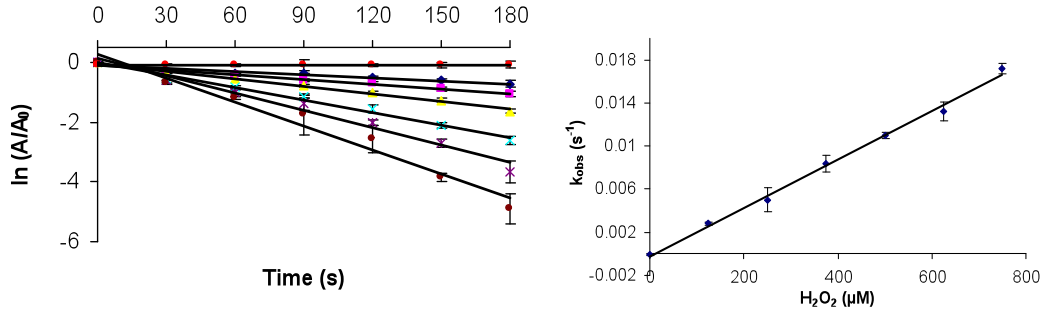


Figure S11. Inactivation of PTP1B by H_2O_2 in presence of sodium chloride (25 mM). The lines correspond to 0, 125, 250, 375, 500, 625, 750 μM H_2O_2 from top to bottom. k_{app}= 23 ± 4 M^{-1} s^{-1} (compare to inactivation of PTP1B by H_2O_2 *without* added NaCl, Figure S1, k_{app} = 24 ± 3 M^{-1} s^{-1}).

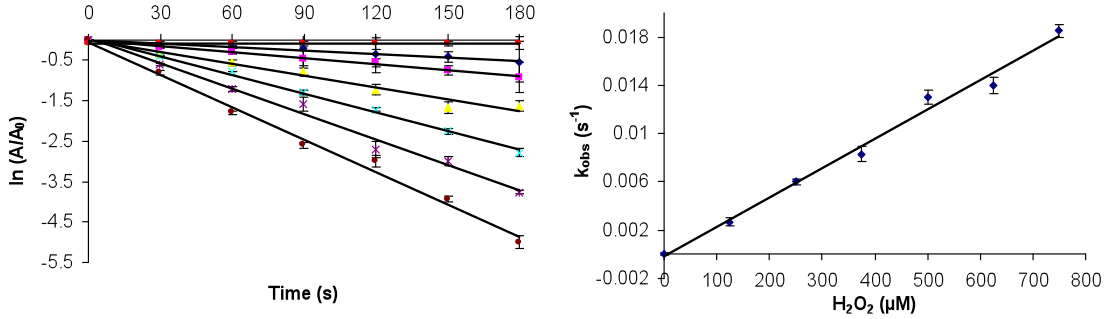


Figure S12. Inactivation of PTP1B by H_2O_2 in presence of potassium chloride (25 mM). The lines correspond to 0, 125, 250, 375, 500, 625, 750 μM H_2O_2 from top to bottom. k_{app}= 25 ± 4 M^{-1} s^{-1} (compare to inactivation of PTP1B by H_2O_2 *without* added KCl, Figure S1, k_{app} = 24 ± 3 M^{-1} s^{-1})

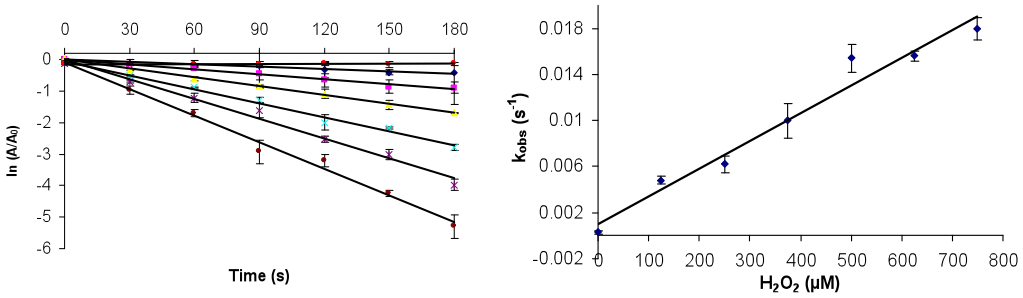


Figure S13. Inactivation of PTP1B by H_2O_2 in presence of magnesium chloride (2 mM). The lines correspond to 0, 125, 250, 375, 500, 625, 750 μM H_2O_2 from top to bottom. k_{app} = 24 ± 5 $M^{-1}s^{-1}$ (compare to inactivation of PTP1B by H_2O_2 *without* added $MgCl_2$, Figure S1, k_{app} = 24 ± 3 M^{-1} s^{-1}).

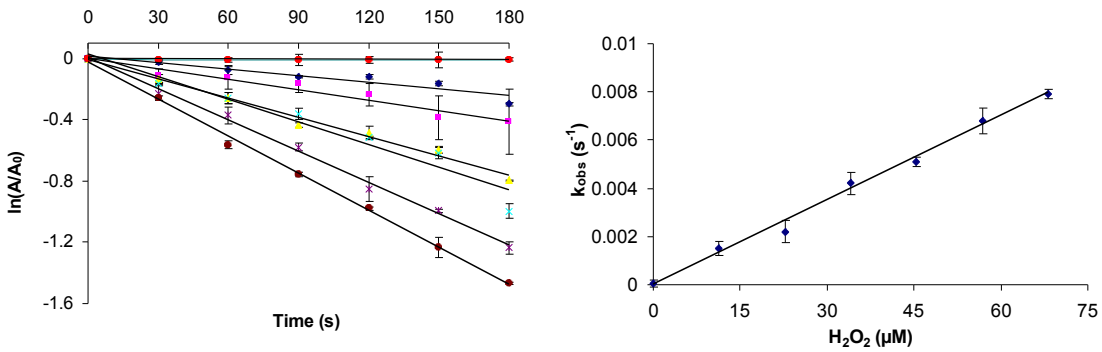


Figure S14. Inactivation of PTP1B by H_2O_2-$KHCO_3$ (25 mM) in presence of the competitive inhibitor sodium phosphate (50 mM). The lines correspond to 0, 11, 23, 34, 45, 57, 68 μM H_2O_2 from top to bottom. k_{app} = 117 ± 10 M^{-1} s^{-1} (compare to inactivation of PTP1B by H_2O_2-$KHCO_3$ (25 mM) without added sodium phosphate, Figure S6, k_{app} = 202 ± 4 M^{-1} s^{-1}).

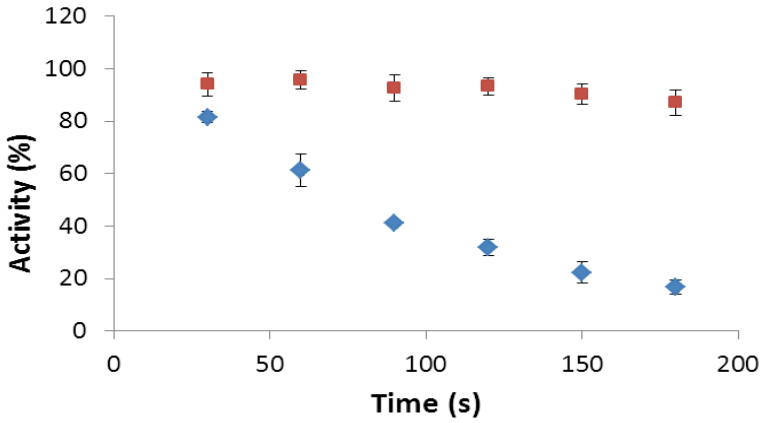
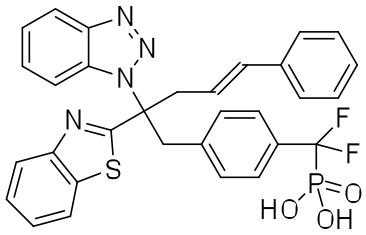


Figure S15. Inactivation of PTP1B (1.2 μM) by H_2O_2-$KHCO_3$ (60 μM-25 mM) in the presence and absence of the competitive inhibitor, **S1** (50 nM). Enzyme activity was measured as described. The upper points (red squares) depict the time course for the enzyme treated with H_2O_2-$KHCO_3$ (60 μM/25 mM) in the presence of **S1** (50 nM). The lower points (blue diamonds) depict the time course for the enzyme treated with H_2O_2-$KHCO_3$ (60 μM/25 mM) without **S1**. The IC_{50} of **S1** against PTP1B is 47 nM.[8]



S1

In principle, the inactivation of PTP1B by peroxides may proceed by either one-electron or two-electron oxidation mechanisms.[9,10] A one-electron process would involve metal-mediated reduction of the peroxide bond to yield a highly reactive oxygen radical.[9,10] In the present case, a radical-mediated inactivation process seemed unlikely given that the reactions were performed in a radical-scavenging buffer (Tris/bis-Tris) that contained a chelator (diethylenetriamine pentaacetic acid) that inhibits metal-dependent conversion of peroxides to oxygen-centered radicals.[9] Nonetheless, we examined this issue and found that the presence of an additional trace metal chelator, desferal (1 mM) or radical scavengers such as mannitol (100 mM) and tryptophan (1 mM) had no significant effect on the inactivation of PTP1B by hydrogen peroxide in the presence of bicarbonate (Figures S16-S18). These results are consistent with the involvement of peroxymonocarbonate in the enzyme inactivation process, as this agent has previously been shown to act as a two-electron oxidant of sulfhydryl groups.[11]

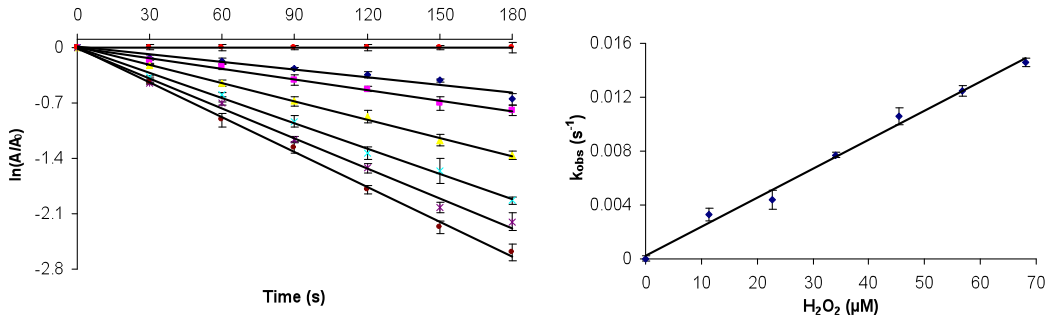


Figure S16. Inactivation of PTP1B by H_2O_2-$KHCO_3$ (25 mM) in presence of desferal (1 mM). The lines correspond to 0, 11, 23, 34, 45, 57, 68 μM H_2O_2 from top to bottom. k_{app} = 215 ± 14 M^{-1} s^{-1} (compare to inactivation of PTP1B by H_2O_2-$KHCO_3$ (25 mM) without added desferal, Figure S6, k_{app} = 202 ± 4 M^{-1} s^{-1}).

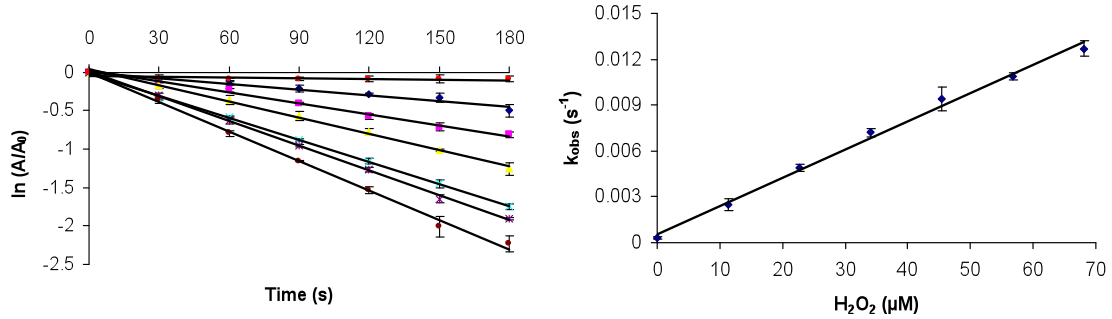


Figure S17. Inactivation of PTP1B by H_2O_2-$KHCO_3$ (25 mM) in presence of mannitol (100 mM). The lines correspond to 0, 11, 23, 34, 45, 57, 68 μM H_2O_2 from top to bottom. k_{app}= 184 ± 18 M^{-1} s^{-1} (compare to inactivation of PTP1B by H_2O_2-$KHCO_3$ (25 mM) without added mannitol, Figure S6, k_{app} = 202 ± 4 M^{-1} s^{-1}).

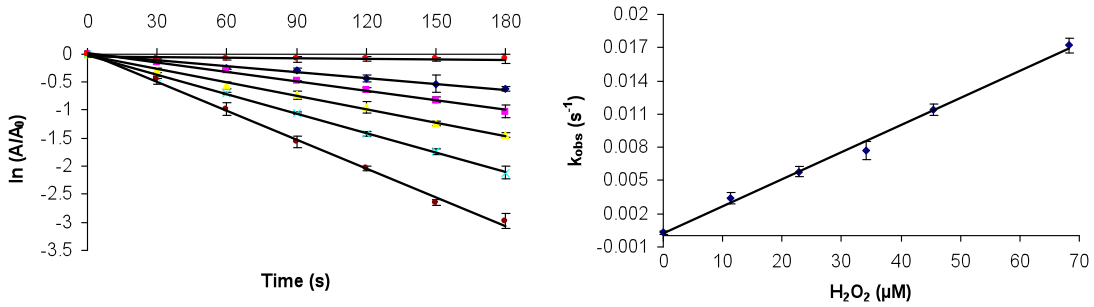


Figure S18. Inactivation of PTP1B by H_2O_2-$KHCO_3$ (25 mM) in presence of tryptophan (1 mM). The lines correspond to 0, 11, 23, 34, 45, 68 μM H_2O_2 from top to bottom. k_{app} = 244 ± 23 M^{-1} s^{-1} (compare to inactivation of PTP1B by H_2O_2-$KHCO_3$ (25 mM) without added tryptophan, Figure S6, k_{app} = 202 ± 4 M^{-1} s^{-1}).

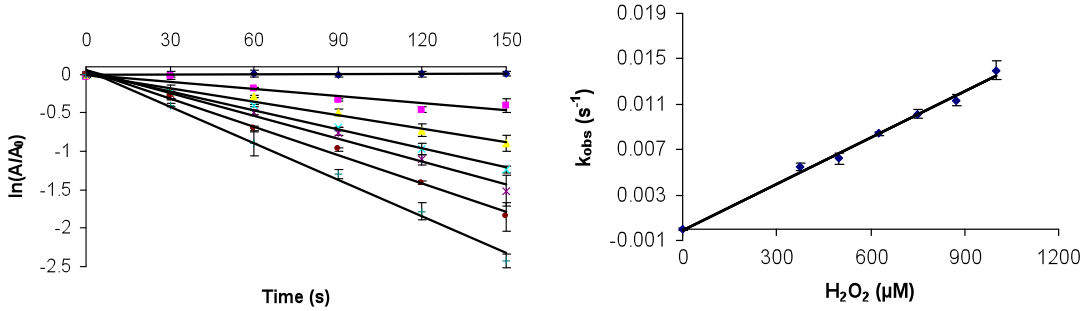


Figure S19. Inactivation of SHP-2 by H_2O_2. The lines correspond to 0, 375, 500, 625, 750, 875, 1000 μM H_2O_2 from top to bottom. k_{app}= 15 ± 2 M^{-1} s^{-1}.

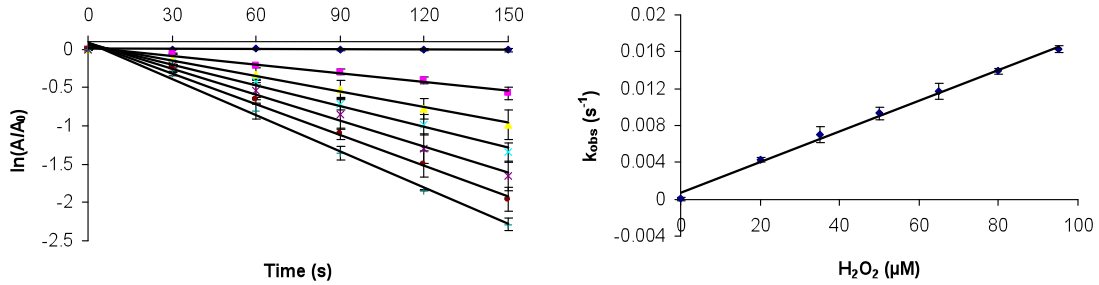


Figure S20. Inactivation of SHP-2 by H_2O_2 in presence of potassium bicarbonate (25 mM). The lines correspond to 0, 20, 35, 50, 65, 80, 95 μM H_2O_2 from top to bottom. k_{app} = 167 ± 12 M^{-1} s^{-1}.

Supplemental Method 2. Continuous Assay for Inactivation of PTP1B by H_2O_2 and Various Bicarbonate Salts. Thiol-free PTP1B was added to a cuvette containing substrate, buffer, bicarbonate, and H_2O_2 to achieve final concentrations of p-nitrophenyl phosphate (10 mM), sodium acetate (100 mM), Bis-Tris (50 mM), Tris (50 mM), bicarbonate salt (14.4 or 25 mM), and H_2O_2 (200 μM). Immediately following addition of enzyme to the cuvette, the reaction was mixed by inversion (3x), and enzyme-catalyzed release of *p*-nitrophenol was monitored at 410 nm, and 25 ºC. Data points were taken every 2 s. The results of these assays are shown in Figure S21, below.

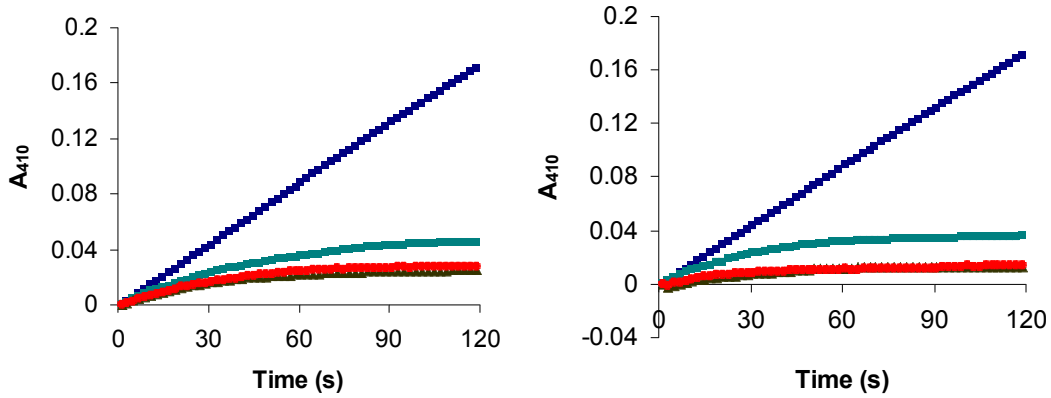


Figure S21. Comparison of the ability of $KHCO_3$, $NaHCO_3$, and NH_4HCO_3 to enhance the inactivation of PTP1B by H_2O_2. **Left side:** Control enzyme (no H_2O_2 or HCO_3^-, upper, blue line), inactivation of PTP1B by H_2O_2-$NaHCO_3$ (14.4 mM, upper, teal green curve), inactivation of PTP1B by H_2O_2-NH_4HCO_3 (14.4 mM, red curve), and inactivation of PTP1B by H_2O_2-$KHCO_3$ (14.4 mM, bottom, dark green curve). **Right side:** Control enzyme (no H_2O_2 or HCO_3^-, upper, blue line), inactivation of PTP1B by H_2O_2-$NaHCO_3$ (25 mM, upper, teal green curve), inactivation of PTP1B by H_2O_2-NH_4HCO_3 (25 mM, red curve), and inactivation of PTP1B by H_2O_2-$KHCO_3$ (25 mM, bottom, dark green curve). Inactivation by H_2O_2-NH_4HCO_3 and H_2O_2-$KHCO_3$ are comparable. Inactivation by $NaHCO_3$ is less effective, perhaps due to the lower solubility of this bicarbonate salt.

Supplemental Method 3. Gel Filtration of PTP1B Inactivated by H_2O_2-$KHCO_3$. Thiol free PTP1B (0.35 μM) was incubated for 3 min at 24 ˚C with H_2O_2 (70 μM) and $KHCO_3$ (25 mM) in a mixture containing NaOAc (100 mM), Bis-Tris (50 mM), Tris (50 mM), Surfact-Amps® 80 (0.025% v/v), DTPA (5 mM) pH 7.0. An aliquot was removed from this solution and the remaining enzyme activity was measured as described above. The remainder of the solution was subjected to buffer exchange via centrifugal gel filtration using a Zeba micro spin column according to manufacturer protocol. Exchange buffer contained: sodium acetate (100 mM), Tris-HCl (50 mM), Bis-tris (50 mM), DTPA (10 mM), Surfact-Amps® 80 (0.05 % v/v) at pH 7. Following buffer exchange, the resulting enzyme-containing filtrate was assayed for PTP activity as described above. The results are shown in Figure S22, below.

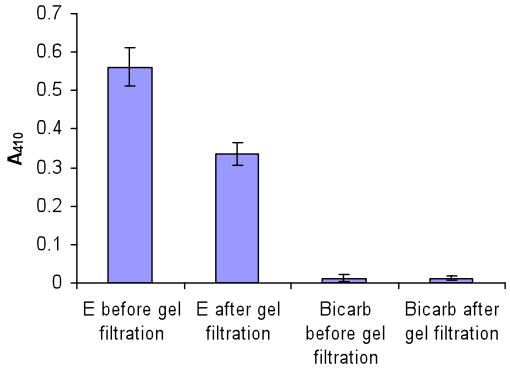


Figure S22. Activity of H_2O_2-$KHCO_3$-inactivated PTP1B does not return following gel filtration. The first and second bars show the activity of control, untreated enzyme before and after gel filtration. The second two bars show the "activity" of inactivated enzyme before and after gel filtration.

Supplemental Method 4. Dialysis of PTP1B Inactivated by H_2O_2-$KHCO_3$. Thiol free PTP1B (0.35 μM) was incubated for 3 min at 24 ˚C with H_2O_2 (70 μM) and $KHCO_3$ (25 mM) in a mixture containing sodium acetate (100 mM), Bis-Tris (50 mM), Tris (50 mM), Surfact-Amps® 80 (0.025% v/v), DTPA (5 mM) pH 7.0. An aliquot was removed from this solution and the remaining enzyme activity was measured as described above. The remainder of the solution was loaded onto a Slide-A-Lyzer MINI Dialysis Unit and dialyzed for 100 min at 4 ˚C against dialysis buffer consisting of Tris (50 mM), Bis-Tris (50 mM), NaOAc (100 mM), DTPA (10 mM), and Surfact-Amps® 80 (0.05% v/v) detergent at pH 7.0. Following dialysis, the solution inside the dialysis chamber was assayed for activity as described above. The results are shown in Figure S23, below.

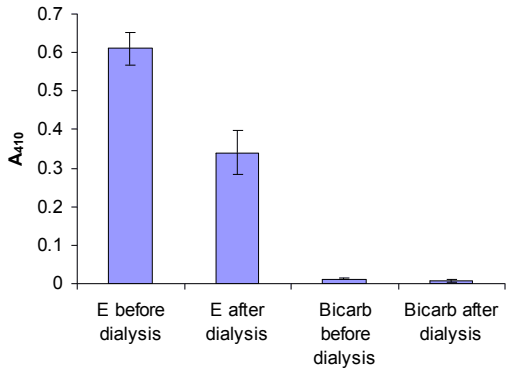


Figure S23. Activity of H_2O_2-$KHCO_3$-inactivated PTP1B does not return following dialysis. The first and second bars show the activity of control, untreated enzyme before and after dialysis. The second two bars show the "activity" of inactivated enzyme before and after dialysis.

Supplemental Method 5. Treatment of PTP1B Inactivated by H_2O_2 or H_2O_2-$KHCO_3$ with Dithiothreitol (DTT). Thiol free PTP1B (0.64 μM) was incubated for 3 min at 24 ˚C with H_2O_2 (375 μM) in a mixture containing NaOAc (100 mM), Bis-Tris (50 mM), Tris (50 mM), Surfact-Amps® 80 (0.025% v/v), DTPA (5 mM) pH 7.0. An aliquot was removed from this solution and the remaining enzyme activity was measured as described above. To the remainder of the solution was added DTT to a final concentration of 50 mM. The resulting mixture was incubated at 25 ˚C for 30 min and then assayed for PTP activity as described above. The results of this assay are shown below in Figure S24.

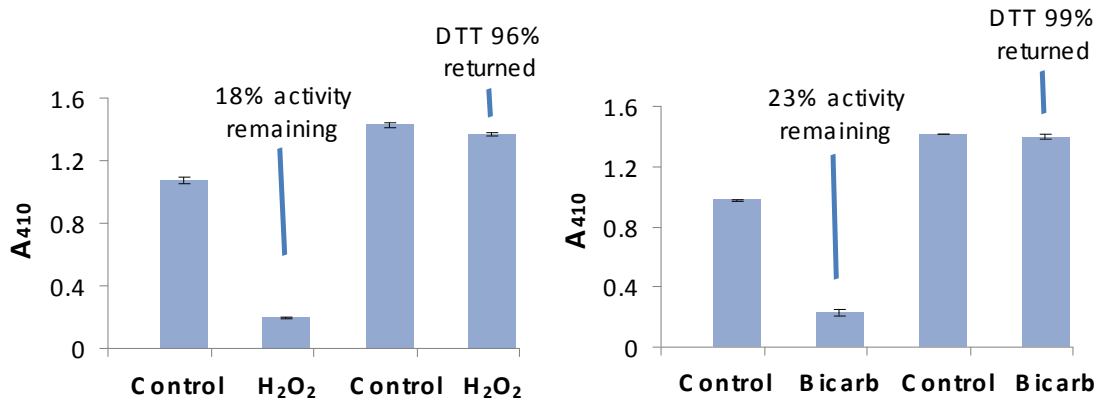


Figure S24. Treatment with DTT leads to the recovery of activity of oxidatively-inactivated PTP1B. In each plot, the first and second bars correspond to the activity of control, untreated enzyme and inactivated enzyme, respectively (no DTT treatment). The second two bars show the activity of control, untreated enzyme and inactivated enzyme, following treatment with DTT as described above.

Supplemental Method 6. Catalytic activity of SHP-2 inactivated by H_2O_2-$KHCO_3$ can be recovered by treatment with dithiothreitol (DTT). Thiol-free SHP-2 (0.45 μM) was inactivated by treatment with H_2O_2 (150 μM) and $KHCO_3$ (25 mM) in assay buffer containing sodium acetate (100 mM), bis-Tris (50 mM, pH 7.0), Tris (50 mM), DTPA (50 μM), Tween-80 (0.00025%), and *p*-NPP (10 mM, 1 mL final volume). The reaction was carried out in a quartz cuvette and the enzyme activity was continuously monitored by following the release of *p*-nitrophenol at 410 nm. When enzyme inactivation was complete, as indicated by a plateau in the production of *p*-nitrophenol, DTT (5 μL of a 1 M in H_2O, to yield a final concentration of 5 mM DTT) was added to the cuvette using a long pipette tip, the solution mixed by pumping the pipette three

times, and the recovery of enzyme activity observed by monitoring the absorbance increase at 410 nm. The results are shown below in Figure S25, below.

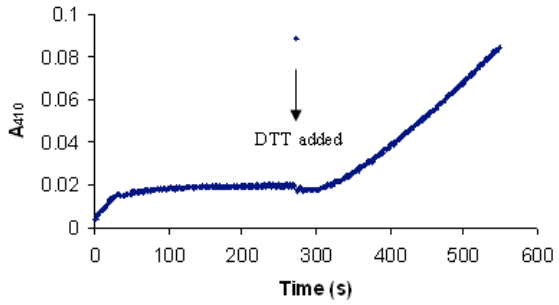


Figure S25. Catalytic activity of SHP-2 inactivated by H_2O_2-$KHCO_3$ can be recovered by treatment with dithiothreitol (DTT).

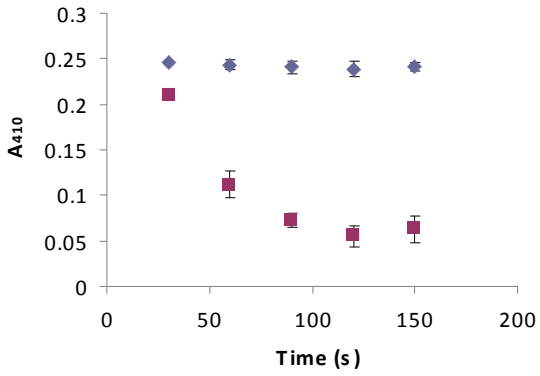


Figure S26. Inactivation of SHP-2 by H_2O_2-$KHCO_3$ (430 μM/25 mM) in presence of the competitive inhibitor sodium phosphate (50 mM). Enzyme activity was measured as described above in Supplemental Methods 1. The upper points (blue diamonds) depict the loss of activity in enzyme treated with H_2O_2-$KHCO_3$ (430 μM/25 mM) in the presence of sodium phosphate (50 mM). The lower points (red squares) depict the loss of activity in enzyme treated with H_2O_2-$KHCO_3$ (430 μM/25 mM) *without* sodium phosphate.

Supplemental Method 7. Inactivation of the Cysteine Protease Papain by H_2O_2 and H_2O_2-$KHCO_3$. Papain (10 mg/mL) was activated by incubation with DTT (2 mM) for

30 min at 25 ˚C in sodium phosphate buffer (50 mM, pH 7) containing EDTA (2.5 mM) as described previously.[12] Thiol was removed from the activated enzyme as described for PTP1B in the Materials Section. The buffer exchange columns were equilibrated with sodium phosphate (50 mM, pH 7), EDTA (2.5 mM). The resulting "thiol-free" papain was used immediately in inactivation assays. Typical inactivation assays contained various concentrations of H_2O_2 either with, or without, $KHCO_3$ (25 mM) along with papain (1 mg/mL), sodium phosphate (50 mM, pH 7), EDTA (2.5 mM). At various times an aliquot (100 µL) of the inactivation reaction was transferred into a cuvette containing substrate solution (900 µL) to give final concentrations of N^{α}-benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA,[13] 1 mM), sodium phosphate (45 mM), EDTA (2.25 mM), and DMSO (10% v/v). Enzyme activity was measured by monitoring the initial rate of release of 4-nitroaniline at 410 nm (first 30 s). The percent remaining activity was calculated based on comparison to a control assay containing no H_2O_2 or $KHCO_3$. The results are shown below in Figures S27 and S28.

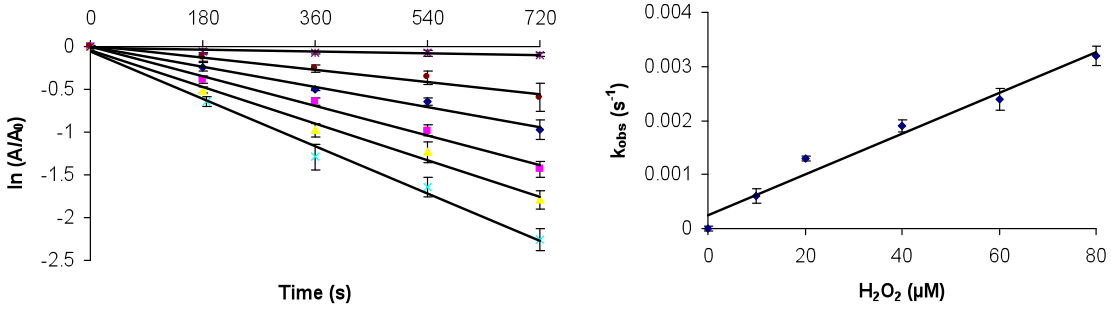


Figure S27. Inactivation of Papain by H_2O_2 alone. The lines correspond to 0, 10, 20, 40, 60, 80 µM H_2O_2 from top to bottom. $k_{app} = 43 \pm 7\ M^{-}1\ s^{-1}$. This value is comparable to

published literature values for the inactivation of cysteine proteases by H_2O_2 in the absence of substrate.[14,15]

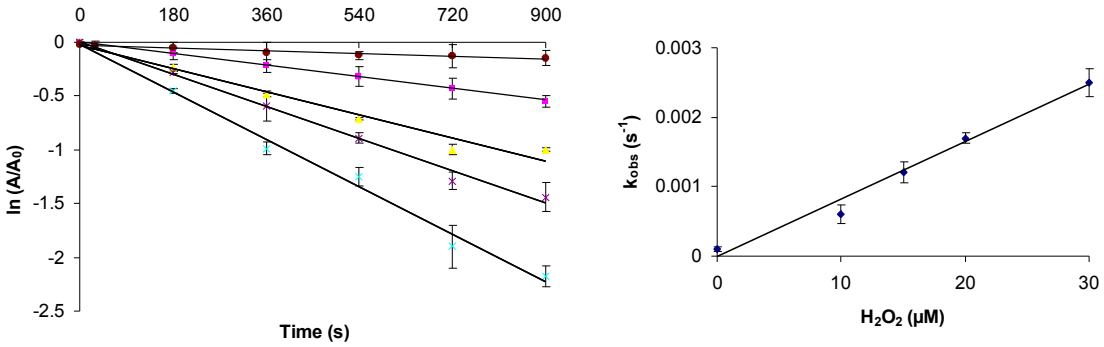


Figure S28. Inactivation of Papain by H_2O_2-$KHCO_3$ (25 mM). The lines correspond to 0, 10, 15, 20, 30 μM H_2O_2 from top to bottom. $k_{app} = 83 \pm 9\ M^{-1}\ s^{-1}$.

Supplemental Methods 8. Crystal structure determination. Crystallization trials were performed at 4º C using sitting drop vapor diffusion and a 10 mg/mL stock solution of PTP1B (1-298). Diffraction quality crystals were grown using 11-18 % PEG3000, 0.1 M HEPES pH 7.0-8.0, 0.2 M magnesium acetate, and 2 mM TCEP as reported previously.[16]

Crystals of inactivated PTP1B were prepared by soaking PTP1B crystals at room temperature. The crystals were first cryoprotected using 20% PEG3000, 0.1 M HEPES pH 7.0, 0.2 M magnesium acetate, and 20% PEG 200, and then transferred to a solution containing the cryobuffer supplemented with 25 mM $KHCO_3$ and 50 μM H_2O_2. The soak time was varied from 20 to 45 min. Crystals were picked up with Hampton loops and plunged into liquid nitrogen.

A 1.7 Å resolution X-ray diffraction data set was collected at ALS beamline 4.2.2

and processed using d*TREK (Table S1).[17] The crystals have space group $P3_121$ with unit cell dimensions of a = 88.4 Å and c = 104.3 Å; there is 1 molecule in the asymmetric unit. Molecular replacement phasing as implemented in PHENIX AutoMR was used.[18] The search model was derived from a native PTP1B structure (PDB code 2f71)[19] with following residues omitted: 46-49, 180-188, and 215-221. Note that this list includes the active site Cys215 and flexible active site loops whose conformations are sensitive to the oxidation state of Cys215 and the presence of bound ligands. The model from molecular replacement was improved with iterative rounds of model building in Coot[20] and refinement in PHENIX.[21]

The 1.7 Å resolution electron density map clearly showed the formation of the cyclic sulfenyl amide involving Cys215 and Ser216 (Figure S29). The conformations of the P-loop and other flexible active site loops are identical to those described previously for crystals soaked in H_2O_2.[22,23] For example, electron density for the P-loop is shown in Figure S29.

Table S1. X-ray data collection and refinement statistics[a]

Wavelength (Å)	1. 0000
Data collection resolution (Å)	44.21 - 1.70 (1.76 - 1.70)
No. of Observations	571812
No. of unique reflections	52290
$R_{merge}(I)$	0.063 (0.552)
Average I/σ	12.6 (2.8)
Completeness (%)	100.0 (100.0)
Redundancy	10.94 (11.00)
Refinement resolution (Å)	44.21 - 1.70 (1.76 - 1.70)
R_{cryst}	0.197 (0.290)
R_{free}[b]	0.210 (0.303)
No. of protein residues	282
No. of protein atoms	2265
No. of water molecules	170
Average B-factor (Å^2)	
Protein	32.1
Water	38.0
Mg^{+2}	35.0
rmsd[c]	
Bonds (Å)	0.006
Angles (deg)	1.051
Ramachandran plot[d]	
Favored (%)	98.21
Allowed (%)	1.79
Outliers (%)	0.00
Coordinate error (Å)[e]	0.21
PDB code	3SME

[a]Values for the outer resolution shell of data are given in parenthesis.
[b]5 % random test set.
[c]Compared to the parameters of Engh and Huber.[24]
[d]The Ramachandran plot was generated with RAMPAGE.[25]
[e]Maximum likelihood-based coordinate error estimate.

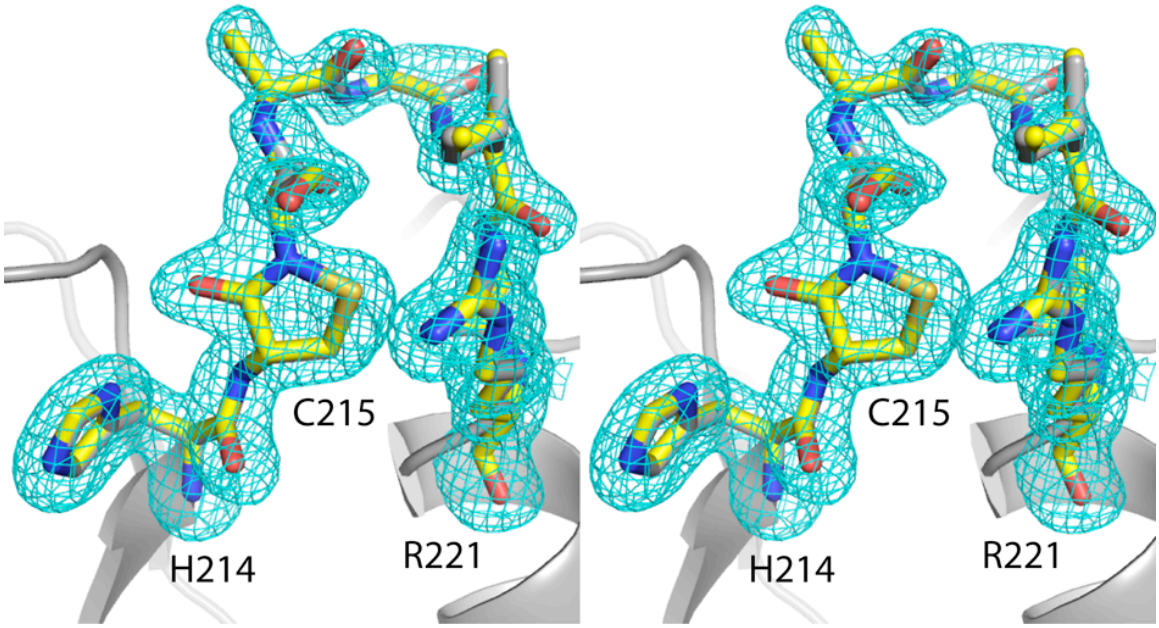


Figure S29. Stereographic view of PTP inactivated by 25 mM $KHCO_3$ and 50 μM H_2O_2 (gray) or H_2O_2 alone (yellow, PDB code 1OEM). The P-loop is shown in sticks (residues 214-221). The cage represents a simulated annealing σ_A-weighted F_o-F_c omit map contoured at 3.0σ. Prior to map calculation, the P-loop was omitted and simulated annealing refinement was performed using PHENIX.

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