SUPPORTING INFORMATION

Thiol-mediated recovery of catalytic activity from oxidized protein tyrosine phosphatases

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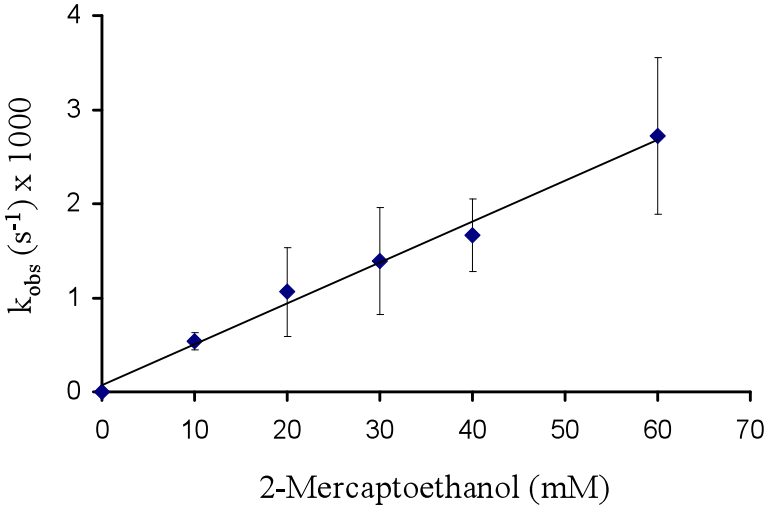
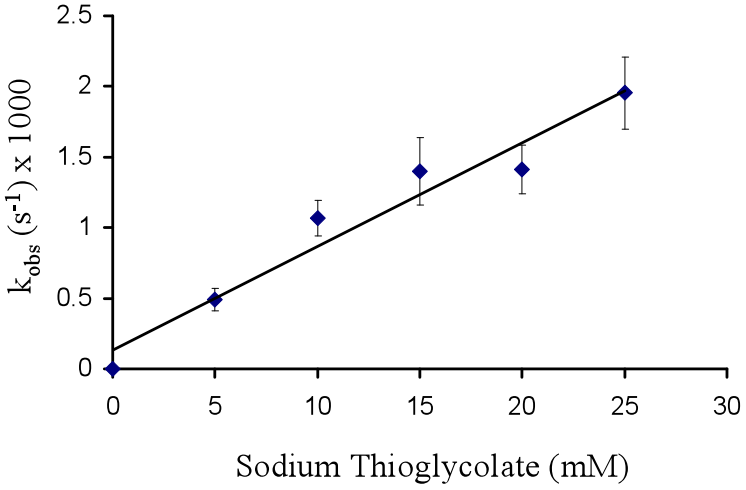
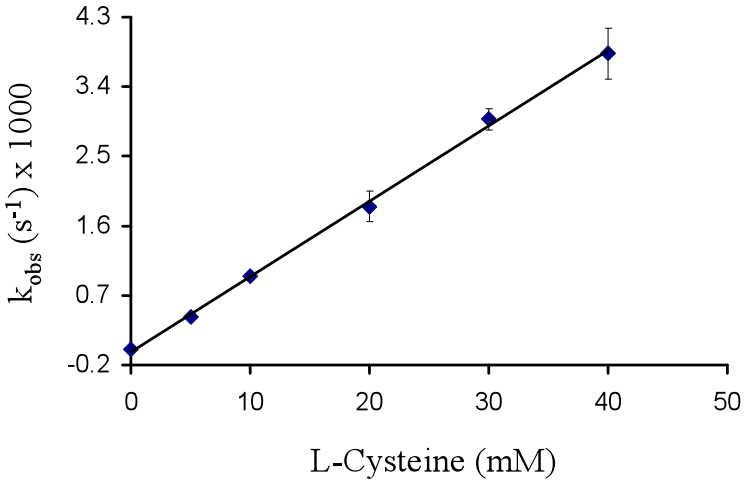
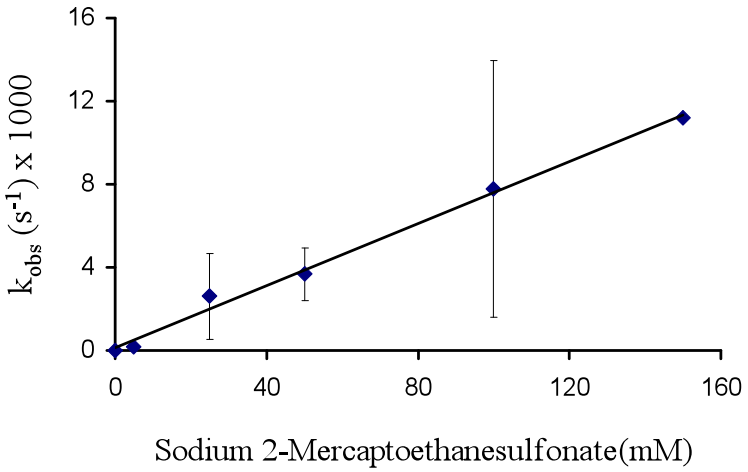
Contents

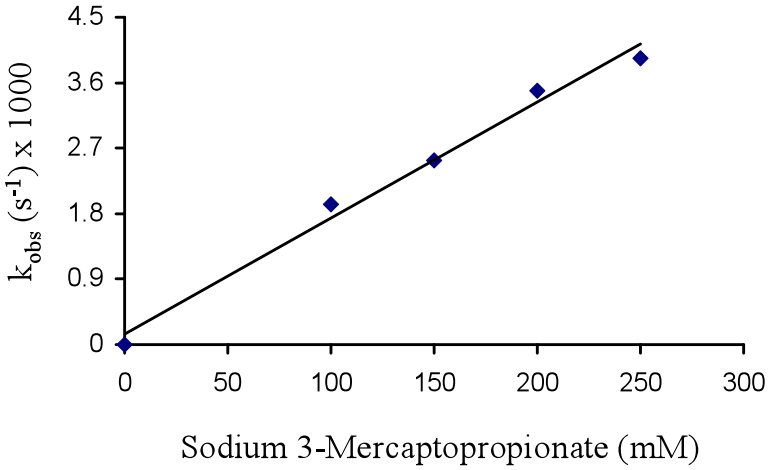
Pseudo First-Order Rate Constants vs Concentration of Monothiol: Reactivation of $PTP1B_{ox}$






4.3
3.4
2.5
1.6
0.7
-0.2
k_{obs} (s^{-1}) x 1000
0
10
20
30
40
50
L-Cysteine (mM)


16
12
8
4
0
k_{obs} (s^{-1}) x 1000
0
40
80
120
160
Sodium 2-Mercaptoethanesulfonate(mM)


4.5
3.6
2.7
1.8
0.9
0
k_{obs} (s^{-1}) x 1000
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50
100
150
200
250
300
Sodium 3-Mercaptopropionate (mM)

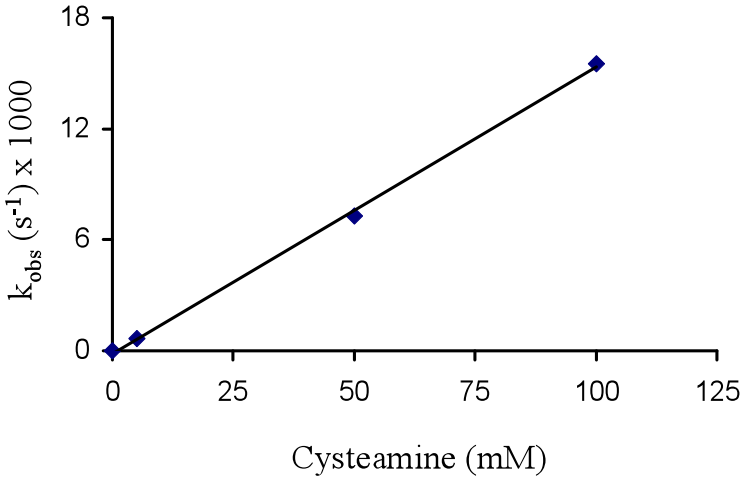


Figure S1. Pseudo first-order rate constants of reactivation of oxidized PTP1B versus the concentration of thiol in excess. For reactivation of oxidized PTP1B, plots of the pseudo first-order rate constants versus thiol concentration afford straight lines with intercepts at the origin. This suggests a bimolecular process in the rate-determining step of the reaction.

Reactivation of Oxidatively-Inactivated PTP1B by the Dithiol 2,3-dimercaptopropanol (BAL)

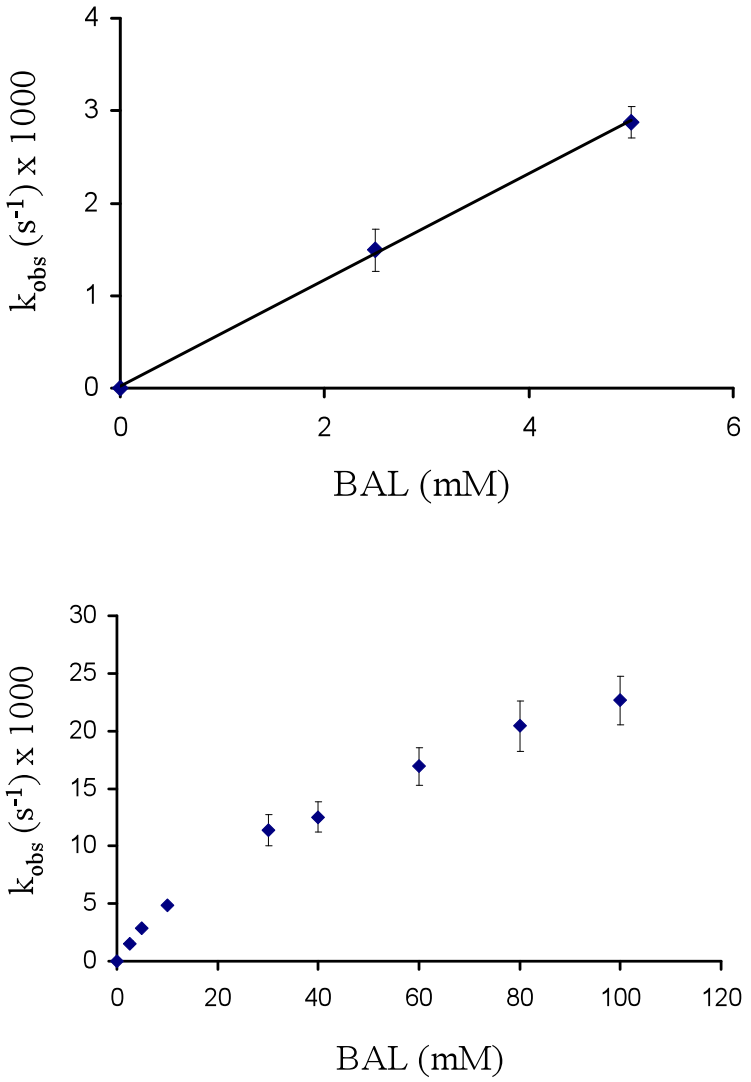
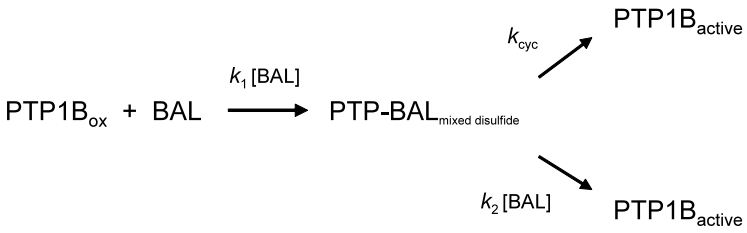


Figure S2. Pseudo first-order rate constants versus concentration of dithiol BAL: biphasic kinetics in the reactivation of oxidized PTP1B. For reactivation of oxidized PTP1B, a plot of the pseudo first-order rate constants versus BAL concentration affords a straight line which intercepts the origin in the low (0-5 mM)

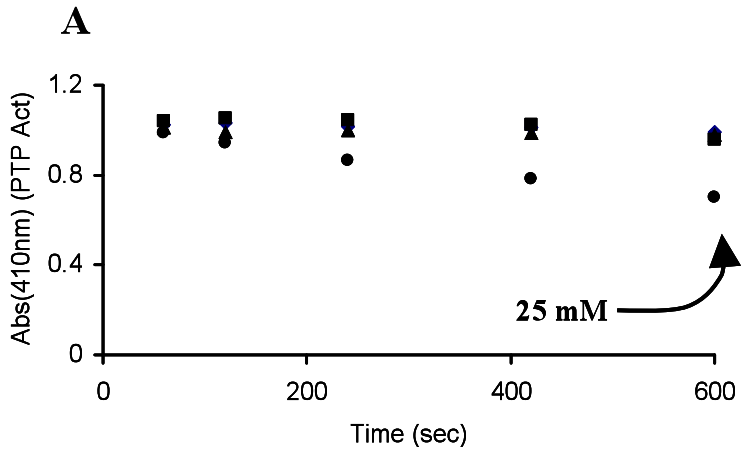
concentration regime (Figure S2, top). However, at high concentrations of BAL (~30 mM and higher), a second linear region is revealed, of shallower slope and a non-origin intercept (Figure S2, bottom). We ascribe this kinetic behavior to the following: at low concentrations of BAL, initial attack of an equivalent of BAL on the oxidized PTP is rate-limiting (k_1[BAL], Scheme S1 below), thus affording second-order kinetics (first-order in BAL concentration). At these **low** concentrations of BAL, intramolecular, 4-membered ring closure (k_{cyc}, Scheme S1) is sufficiently fast to kinetically "outcompete" the second process which also leads to active enzyme (attack of a second equivalent of BAL on the PTP-BAL mixed disulfide). Thus, at low concentrations of BAL, k_1[BAL] is "cleanly" rate-limiting. However, at high concentrations of BAL, the latter process whereby a second equivalent of BAL attacks the PTP-BAL mixed disulfide to release active enzyme (k_2[BAL], Scheme S1), kinetically "overtakes" the rate of intramolecular, 4-membered ring closure. Thus, the rate-limiting step in this case (at high concentrations of BAL) becomes the BAL concentration-dependent attack on the PTP-BAL mixed disulfide (k_2[BAL]). This kinetic scenario requires that k_1 be greater than k_2 in the Scheme below:

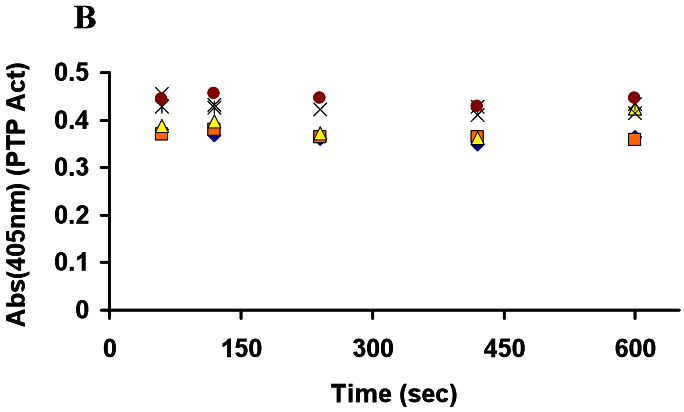


Scheme S1. **Postulated mechanisms and "kinetic diagram" of reactivation kinetics for recovery of PTP activity from oxidized PTP1B by BAL.** Structures of all relevant oxoforms of PTP1B and of mixed disulfides resulting from attack of

thiol(ate) on oxidized PTP1B are given in the manuscript. In the Scheme, only forward arrows are shown strictly to simplify the diagram, and are not intended to suggest absolute irreversibility of the associated chemical steps.

Disulfides Generated During Thiol-Mediated Reactivation of Oxidized PTP1B Do Not Effectively Inactivate the Native Enzyme





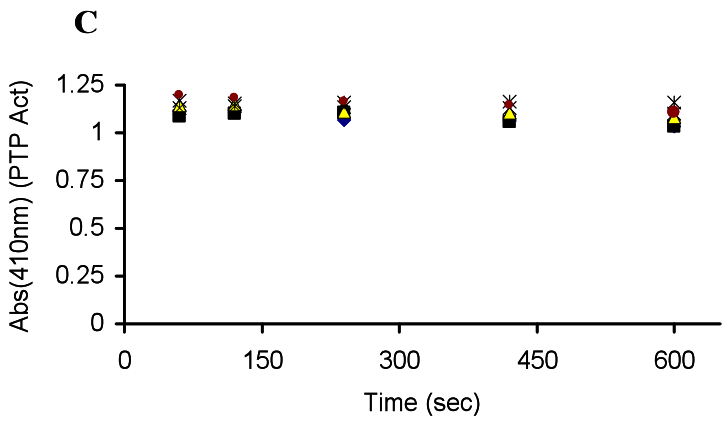


Figure S3. PTP1B activity vs time in the presence of excess disulfide. Native PTP1B (350 nM) was treated with an excess of disulfide reagent under identical assay conditions as those used in reactivation kinetics studies, and PTP activity monitored as a function of time. **(A)** 2-mercaptoethanol disulfide (0 μM, 50 μM, 5 mM, and 25 mM). **(B)** Glutathione disulfide (GSSG: 0 mM (red circles), 0.5 mM (asterisks), 1 mM (X), 4 mM (yellow triangles), 8 mM (orange squares), 10mM (blue diamonds). **(C)** Trans-4,5-Dihydroxy-1,2-dithiane (oxidized DTT: 0, 1, 3, 6, 9, and 12 mM).

2-Mercaptoethanol Disulfide Does Not Effectively Inactivate Native SHP2

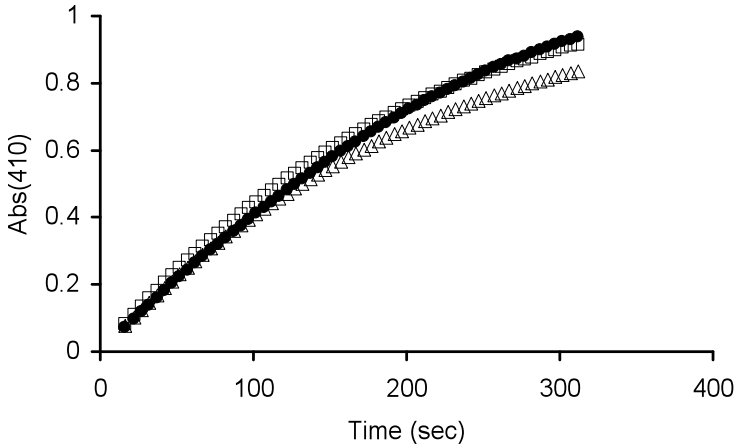
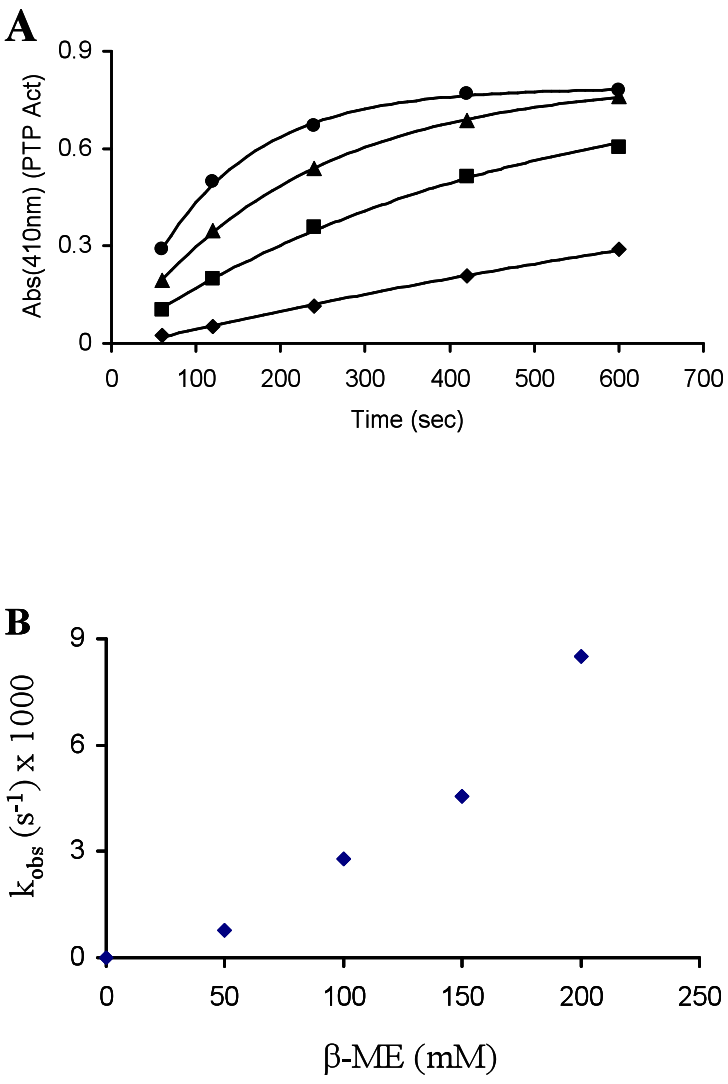


Figure S4. Inactivation of native SHP2 by 2-mercaptoethanol disulfide.

Native SHP2 (20 nM) was introduced to a solution of 20 mM pNPP and 0, 10, or 20 mM 2-mercaptoethanol disulfide (solid circles, open squares, and open triangles, respectively, in assay buffer the same as that reported for continuous spectrophotometric data in the manuscript). The release of 4-nitrophenol was followed at 410 nm. These concentrations of disulfide reagent were found to be inept at inactivating native SHP2, in similar fashion to that observed for native PTP1B in discontinuous assays (*vide supra*).

Reactivation of Oxidatively-Inactivated SHP2 by the Monothiol 2-Mercaptoethanol Also Exhibits Kinetics Second-Order in Thiol



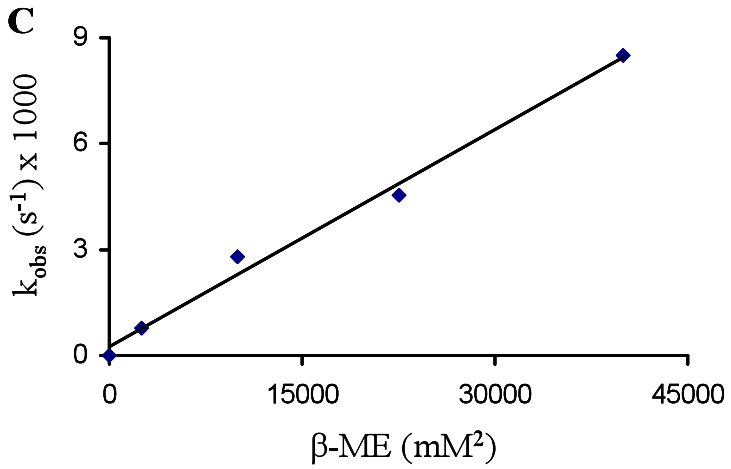


Figure S5. Reactivation of oxidatively-inactivated SHP2 by 2-mercaptoethanol. (A) Time-course of recovery of SHP2 activity by 50 mM, 100 mM, 150 mM, and 200 mM β-ME (bottom traces to top, respectively). (B) Pseudo first-order rate constants plotted as a function of β-ME concentration reveals an upward-rising curve inconsistent with an overall, simple second-order process. (C) Pseudo first-order rate constants plotted as a function of the square of β-ME concentration affords a straight line passing through the origin. In similar fashion to the "kinetic profile" observed for GSH-mediated reactivation of oxidized SHP2, this data suggests a rate law involving the term $k[\mathrm{RSH}]^2$.

Reactivation of Oxidatively-Inactivated PTP1B by the Phosphine-Based Reagent Tris(2-carboxyethyl)phosphine (TCEP)

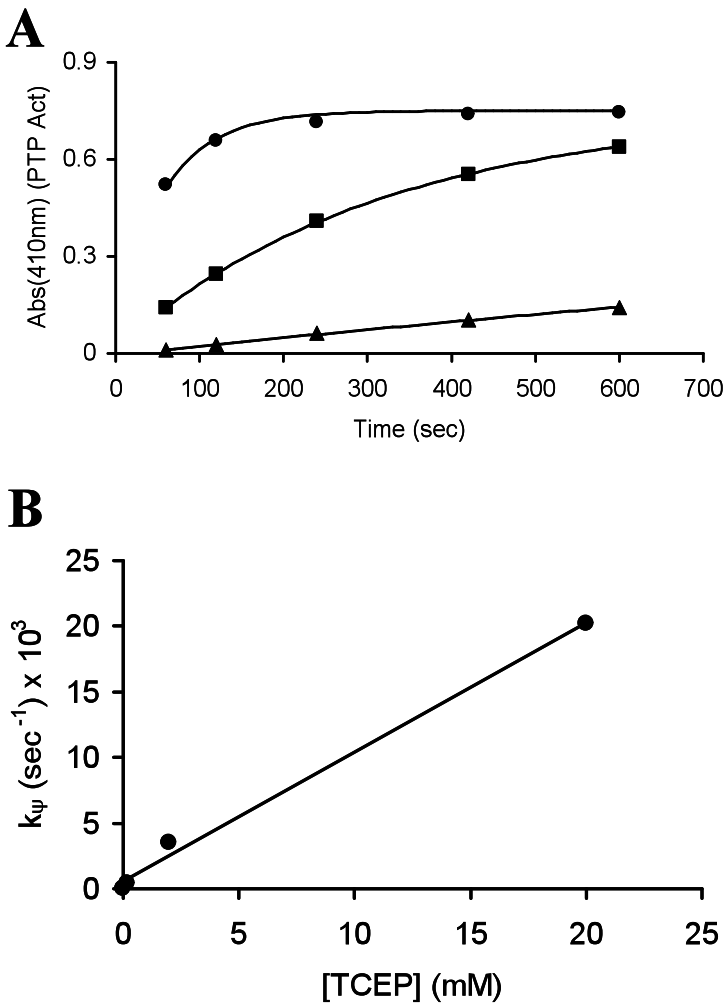


Figure S6. Reactivation of oxidatively-inactivated PTP1B by tris(2-carboxyethyl)phosphine (TCEP). (A) Time-course of recovery of PTP1B catalytic activity by 20 mM, 2 mM, or 0.2 mM TCEP (traces top to bottom, respectively). (B) A replot of the pseudo first-order rate constants versus concentration of TCEP affords a

straight line intersecting the origin, indicative of a simple second-order process in the rate-determining step; the relevant rate for this process being $1.5 \pm 0.5\ M^{-1}\ s^{-1}$.