Minimally disruptive optical control of protein tyrosine phosphatase 1B

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Protein tyrosine phosphatases regulate a myriad of essential subcellular signaling events, yet they remain difficult to study in their native biophysical context. Here we develop a minimally disruptive optical approach to control protein tyrosine phosphatase 1B (PTP1B)—an important regulator of receptor tyrosine kinases and a therapeutic target for the treatment of diabetes, obesity, and cancer—and we use that approach to probe the intracellular function of this enzyme. Our conservative architecture for photocontrol, which consists of a protein-based light switch fused to an allosteric regulatory element, preserves the native structure, activity, and subcellular localization of PTP1B, affords changes in activity that match those elicited by post-translational modifications inside the cell, and permits experimental analyses of the molecular basis of optical modulation. Findings indicate, most strikingly, that small changes in the activity of PTP1B can cause large shifts in the phosphorylation states of its regulatory targets.
The enzymatic phosphorylation of tyrosine residues is centrally important to cellular function. It controls the location and timing of cellular differentiation, movement, proliferation, and death\(^1\)–\(^4\); its misregulation can cause cancer, diabetes, and neurodegenerative diseases, among other disorders\(^5\)–\(^7\). Methods to control the activity of phosphorylation-regulating enzymes without interfering with their native structure or cellular organization could, thus, enable detailed analyses of the mechanisms by which cells process essential chemical signals\(^8\)–\(^9\).

Optogenetic actuators—genetically encoded proteins that undergo light-induced changes in conformation—provide a powerful means of controlling enzyme activity over time and space. As protein fusion partners, they have enabled optical manipulation of biomolecular transport, binding, and catalysis with millisecond and submicron resolution\(^10\)–\(^11\). Common strategies to integrate optogenetic actuators into enzymes include (i) attachment near an active site, where they control substrate access\(^12\)–\(^13\), (ii) insertion within a catalytic domain, where they affect activity-modulating structural distortions\(^14\), and (iii) fusion to N- or C-termini, where they direct subcellular localization\(^15\) or guide domain assembly\(^16\). These approaches have generated powerful tools for stimulating phosphorylation-mediated signaling networks; unfortunately, their reliance on disruptive structural modifications has tended to limit their use in biochemical studies of native regulatory effects, for example, spatially dependent protein–protein interactions or, more notably, shifts in activity that match, rather than artificially exceed, those caused by post-translational modifications of an enzyme under study.

Protein tyrosine phosphatase 1B (PTP1B) is an important regulatory enzyme for which minimally disruptive architectures for photocontrol could prove particularly informative. This enzyme, which catalyzes the hydrolytic dephosphorylation of tyrosine residues, helps regulate insulin, leptin, and epidermal growth factor signaling and participates in a diverse set of spatially distributed signaling processes\(^17\). PTP1B has two intriguing biophysical traits that make it particularly amenable to optogenetic study: (i) Its catalytically essential WPD loop undergoes cyclic, open-and-close motions that control the rate of phosphotyrosine hydrolysis at the active site\(^18\), and its C-terminal a7 helix modulates these motions through an allosteric network that extends over 25 Å across the protein (Fig. 1a). An architecture for photocontrol that makes use of this network could afford changes in activity that preserve interactions between PTP1B and its regulatory targets. (ii) PTP1B undergoes several post-translational modifications outside of its active site that cause modest, yet physiologically influential shifts in its activity (i.e., 1.7–3.1-fold\(^19\)–\(^20\); Supplementary Table 1). An optogenetic construct that affords similar changes in activity could help determine whether or not they—rather than the specific post-translational modifications that cause them—are sufficient to influence cellular physiology.

In this study, we used a protein-based light switch to place the native allosteric regulatory system of PTP1B under optical control. This conservative optogenetic design preserved the native structure and subcellular localization of PTP1B, permitted changes in activity that match those caused by post-translational modifications inside the cell and, when paired with a FRET-based biosensor, enabled spatiotemporal control and measurement of intracellular PTP1B activity. An optogenetic analysis carried out with this system showed that small, transient changes in PTP1B activity can cause large shifts in insulin receptor (IR) phosphorylation. The optogenetic tools developed in this study thus complement existing methods for studying protein tyrosine kinases (PTKs)—which, unlike PTPs, possess many light-sensitive analogs and FRET-based biosensors\(^21\)–\(^22\)—and, more broadly, demonstrate an optogenetic approach for studying regulatory enzymes in their native biophysical context.

## Results

### Allosteric photocontrol of PTP1B.

We sought to place PTP1B under optical control by using LOV2, the light-sensitive domain from phototropin 1 of *Avena sativa*, to toggle the conformation of its a7 helix. LOV2 derives its optical activity from a noncovalently bound flavin mononucleotide (FMN) that, when exposed to blue light, forms an intermolecular carbon-sulfur bond that destabilizes the N- and C-terminal helices of the protein (Fig. 1a)\(^23\)–\(^25\). We hypothesized that attachment of the N-terminal Aα helix of LOV2 to the C-terminal a7 helix of PTP1B would couple (i) light-induced unwinding of the Aα helix to (ii) destabilization of the a7 helix and disruption of WPD loop motions (Fig. 1b). To our satisfaction, several PTP1B-LOV2 chimeras—each generated by fusing the Aα and a7 helices at a different crossover point in a primary sequence alignment—exhibited light-dependent catalytic activity on 4-methylumbelliferyl phosphate (4MUP; Fig. 1c). Fusion of the Jα helix of LOV2 to the N-terminus of PTP1B, by contrast, did not confer photosensitivity (Supplementary Figs. 1a, b), a result consistent with the large distance between its N-terminus and active site (Fig. 1a).

To enhance the dynamic range (DR = Vo-dark/Vo-light) of our most light-sensitive chimeras (i.e., construct 7, where DR = 1.9), we used two approaches: First, we attempted to improve communication between the LOV2 and PTP1B domains by reducing the length of the linker between them; similar changes have improved photoswitching in other light-sensitive fusions\(^26\). Unfortunately, shorter linkers (which, through residue deletions, could also alter the phases of the fused helices) tended to reduce DR; we chose one variant with an unaltered DR—chimera 7.1—for further optimization. Next, we attempted to increase the stability of the dark state over the light state by adding stabilizing mutations to flexible helices. (For the Jα and Aα helices, we used established stabilizing mutaitions\(^23\)–\(^25\); for a7, we replaced solvent-exposed residues with alanine, which has a high helix propensity\(^26\). Stabilizing mutations in the Jα helix have amplified DRs of previous LOV2 fusions\(^25\)–\(^27\). Intriguingly, for our chimeras, mutations in the Jα helix improved activity, but reduced photosensitivity; several mutations in the Aα and a7 helices, by contrast, increased the DR (Fig. 1c). Overall, the effects of amino acid substitutions in these two helices were non-additive and reached a maximum DR of 2.2 on 4MUP. We chose a single high-DR chimera—7.1(T406A), termed PTP1BPS—for further study.

We assessed the structural integrity of the PTP1B domain within PTP1BPS by using X-ray crystallography to examine its dark-state conformation. Intriguingly, although crystals of PTP1BPS were yellow and turned clear when exposed to blue light—a behavior indicative of the presence of LOV\(^12\)–\(^28\)–

diffraction data permitted placement and refinement of only PTP1B (Supplementary Figs. 2 and 3). Detection of LOV2 was likely impeded by two interrelated crystallographic features: (i) a disordered a7 helix, which is unresolvable in apo structures of PTP1B\(^29\), and (ii) variability in the orientation of LOV2 within the crystal lattice (Supplementary Note 1). Despite this structural disorder, aligned catalytic domains of PTP1BPS and wild-type PTP1B had a root-mean-square deviation of 0.30 Å (Fig. 1d). Crystallographic results, thus, suggest that LOV2 does not alter the native conformation of the catalytic domain of PTP1B.

We explored the mechanism of photomodulation by using kinetic assays to examine the influence of LOV2 on PTP1B-mediated catalysis. In brief, we measured the activity of PTP1BPS on p-nitrophenyl-phosphate (pNPP) in the presence and absence of blue light (455 nm), and we used the initial rates to construct...
dark- and light-state Michaelis–Menten curves (Fig. 1e). These curves indicate that blue light reduces \( k_{\text{cat}} \) by 2.5-fold but leaves \( K_m \) unaltered. Data collected under repeated illumination, in turn, shows that changes in \( k_{\text{cat}} \) are reversible (Supplementary Fig. 4c).

The isolated influence of LOV2 on \( k_{\text{cat}} \) indicates that this photoswitch does not interfere with substrate binding and, importantly, is consistent with a mechanism in which LOV2 allosterically modulates WPD loop motions, which control the rate of hydrolysis.

To assess the maximum achievable DR for our system, we removed the \( \alpha_7 \) helix of PTP1B; that is, we used a \( \alpha_7 \)-less variant as a model for a maximally photoswitched form of the enzyme. Intriguingly, helix removal lowered \( k_{\text{cat}} \) by 2.9-fold, suggesting that PTP1B\(_{\text{Ps}}\) has a DR that is 85% of the maximum value for a photoswitch does not interfere with substrate binding and, substrate-independent.

**Biophysical analysis of photoscontrol.** Although we designed our chimeras to exploit conformational changes in the N-terminal \( \alpha' \) helix of LOV2, the results of crystallographic and spectroscopic analyses of this photoswitch indicate that its N- and C-terminal helices work together to transduce conformational
changes across the protein. To examine the contribution of both helices to the photoresponse of chimera 7.1, one of our most light-sensitive chimeras, we introduced disruptive mutations (i.e., we added charged residues at buried sites). For both helices, disruptive mutations reduced light-dependent catalytic activity as effectively as C450M, lower its sensitivity to photoactivation, and WPD loop 29. To confirm the contribution of allosterism to photocontrol, we modified chimera 7.1 with a mutation known to exert such an effect: Y152A/Y153A. This modification reduced DR by ~25%, a disruption distinct from the conservative/benign effects of alanine substitutions in the a7 helix (Fig. 2a). The sensitivity of DR to mutations in the L11 loop indicates that the native allosteric network of PTP1B is, indeed, necessary for optogenetic control of its catalytic activity.

We hypothesized that our most photoswitchable chimeras might exhibit large changes in secondary structure between light and dark states—changes that result largely from ordered-to-disordered transitions of the A’α, Ja, and a7 helices. To test this hypothesis, we used circular dichroism (CD) spectroscopy to compare optically induced shifts in α-helical content (Δδ222 = [CD222-dark − CD222-light]/CD222-dark, or the fractional change in MRE at 222 nm) to disruptive mutations in LOV2 and (ii) the insensitivity of recovery kinetics to the latter of which is not affected by W491; unmeasurable. The discrepancy between these constants is smallest for PTP1B (i.e., 7.1(T406A)). The plotted data depict the mean, associated data points, and SE for n = 6 independent reactions. Source data are provided as a Source Data file.

Fig. 2 Analysis of allosteric communication in PTP1B. a Mutations that either prevent adduct formation in LOV2 (C450M), destabilize the A’α and Ja helices (I532E, I539E, and ΔJa), or disrupt the allosteric network of PTP1B (Y152A/Y153A) reduce the photosensitivity of 7.1 and, with the exception of I532E and C450M, lower its specific activity. The plotted data depict the mean, SE, and associated constants for DR for n = 6 independent experiments. Exposure of PTP1B to 455 nm light reduces its α-helical content (i.e., the mean residue ellipticity [MRE] at 222 nm). An analysis of different chimeras indicates that light-induced changes in α-helical content (i.e., Δδ222 = [CD222-dark − CD222-light]/CD222-dark, or the fractional change in MRE at 222 nm) are necessary, but not sufficient for light-sensitive catalytic activity (i.e., high DR). Mutations correspond to variants of 7.1. Chimeras with large values of Δδ222 in blue; the dashed line indicates Δδ222 for equimolar amounts of free PTP1B and LOV2. Error bars denote SE for n = 6 independent reactions. d, e Thermal recovery of (d) α-helical content (i.e., the change in MRE at 222 nm normalized by the full change over 250 s) and (e) tryptophan fluorescence (i.e., the change in fluorescence normalized by the full change over 250 s) of PTP1B constructs (Fig. 2c). The one-way dependence of DR on Δδ222 indicates that changes in α-helical conformation are necessary, but not sufficient for photostability.

We speculated that chimeras with large changes in α-helical content (Δδ222) but light-insensitive catalytic activities (low DRs) might emerge from weak conformational coupling between the LOV2 and PTP1B domains. To study this coupling, we carried out two experiments. In the first, we examined the thermal recovery of LOV2 from the light state by illuminating PTP1B constructs (Fig. 2c). The one-way dependence of DR on Δδ222 indicates that changes in α-helical conformation are necessary, but not sufficient for photostability.

In the second experiment, we examined the thermal recovery of PTP1B by measuring the return of tryptophan fluorescence in the dark (Fig. 2e). A link between the conformation of LOV2 and α-helical content is supported by (i) the sensitivity of Δδ222 to disruptive mutations in LOV2 and (ii) the insensitivity of Δδ222 to the catalytic response of PTP1B (i.e., activity-modulating structural changes in PTP1B, which presumably differ between high- and low-DR chimeras, do not affect Δδ222). In the second experiment, we examined the thermal recovery of PTP1B by measuring the return of tryptophan fluorescence in the dark (Fig. 2e). A link between the conformation of PTP1B and tryptophan fluorescence is supported by (i) the existence of six tryptophan residues in PTP1B (Fig. 2f) and (ii) the insensitivity of recovery kinetics to the removal of W491, the only tryptophan in LOV2 (Fig. 2g).
Preparation of a natively localized variant of PTP1BPS. Inside the cell, PTP1B possesses a C-terminal region—a disordered proline-rich domain followed by a short membrane anchor—that localizes it to the endoplasmic reticulum (ER; Fig. 3a)33. To examine the influence of this region on photocontrol, we attached the bulk of it (all but the hydrophobic ER anchor) to the C-terminus of PTP1BPS and assayed the extended chimera in vitro. This construct (PTP1BPS*) exhibited a reduced DR, which was not improved by the addition of stabilizing mutations to the Jα helix (Figs. 3b, c); nonetheless, it remained photoswitchable. A construct with the full-length C-terminus of PTP1B (everything including the ER anchor; PTP1BPS**), in turn, conferred native localization in COS-7 cells (Fig. 3d).

We completed our characterization of the elongated forms of PTP1BPS by examining the influence of LOV2 on interactions mediated by the disordered C-terminal region. Briefly, we compared the susceptibilities of PTP1B1-405 and PTP1BPS* to inhibition by DPM-1001, an inhibitor that binds preferentially to this region (Supplementary Fig. 5)34. To our surprise, IC50’s differed by ~30%; this small difference indicates that LOV2 does not preclude regulatory interactions with the disordered region. Importantly, DPM-1001 also binds weakly to the catalytic domain, likely by binding near the α7 helix35; IC50’s for PTP1B1,321 and PTP1BPS were, thus, much higher than IC50’s for the full-length constructs and exhibited a greater sensitivity to LOV2. This finding suggests that LOV2 may affect weak interactions that occur at its point of attachment (though, this region is not an established target for post-translational modifications).

An optogenetic probe for studying intracellular signaling. To examine the function of PTP1B-LOV2 chimeras in living cells, we sought a genetically encodable sensor for PTP1B activity. Several previously developed sensors for PTKs could plausibly support such a function; we chose a sensor for Src kinase36, an enzyme with an orthogonal activity to PTP1B37. This biosensor consists of an SH2 domain, a flexible linker, and a substrate domain (i.e., WMEDYDYVHLQG, a peptide derived from p130cas), all sandwiched between two fluorescent proteins (FPs). Src-mediated phosphorylation of the substrate domain causes it to bind to the SH2 domain, reducing Förster resonance energy transfer between the FPs (FRET; Fig. 4a); PTP1B-mediated dephosphorylation of the substrate domain, in turn, reverses this effect and increases FRET. To enhance the compatibility of the sensor with the blue light necessary to stimulate LOV2, we replaced CFP and YPet—the original FPs—with mClover3 and mRuby3, which have longer excitation wavelengths38. As expected, incubation of the modified sensor with Src reduced FRET and increased the donor/acceptor emission ratio; simultaneous incubation with Src and PTP1B (or Src and EDTA), by contrast, prevented this increase (Fig. 4b).

We began our imaging studies by co-expressing the biosensor with PTP1B-LOV2 chimeras in COS-7 cells. These cells are large and flat and, thus, facilitate imaging of subcellular regions39; previous studies have used them to examine PTP1B-mediated signaling events40,41. Whole-cell irradiation of cells expressing PTP1BPS with 457 nm light increased the biosensor signal in both the nucleus and cytosol by ~7%, a change larger than the 3–4%...
increase afforded by the dark-state mutant (Fig. 4c and Supplementary Fig. 6); the response of the biosensor in cells expressing PTP1BPS**, by contrast, was nearly imperceptible when compared with the dark-state analog (Supplementary Fig. 7). Our results thus indicate that transient inactivation of PTP1B allows background signal caused by photobleaching to increase within a secondary region located far from the first (Fig. 4e and Supplementary Fig. 8); both irradiated and secondary regions maintained a similar increase in signal for at least 30 s after irradiation. In cells expressing PTP1BPS**, irradiation near the nucleus produced a similar change in signal, while irradiation near the plasma membrane (PM) failed to do so (Fig. 4e and Supplementary Figs. 9–11a). In all cases, dark-state mutants produced no detectable effect. Our results thus indicate that localized inactivation of PTP1BPS** and nucleus-proximal PTP1BPS** can produce a measurable cell-wide increase in the phosphorylation state of their targets.

The ER is a vesicular network that extends unevenly from the nucleus of the cell. To determine if the reduced activity of PTP1BPS** near the PM results from the low abundance of ER in this region, we used BFP-Sec61β, a genetically encoded ER
label, to measure the subcellular distribution of ER. The fluorescence of 5-µm circular regions located near the PM was 2.7-fold lower than the fluorescence of equivalently sized regions located near the nucleus (Supplementary Figs. 11b, c); this discrepancy suggests that the diffuse distribution of PTP1B-C** at the PM limits its activity on membrane-proximal targets.

Cells rely on complex networks of biomolecular interactions to transmit, filter, and integrate chemical signals. The biochemical repercussions of changes in the activity of any single regulatory enzyme are, thus, difficult to assess with artificial biosensors alone. To evaluate the influence of modest changes in PTP1B activity on the phosphorylation state of a native regulatory target, we generated HEK293T/17 cells that stably express PTP1B-C** and used an enzyme-linked immunosorbent assay (ELISA) to measure shifts in IR phosphorylation caused by transient illumination (455 nm, enzyme-linked immunosorbent assay (ELISA) to measure shifts in IR phosphorylation caused by transient illumination (455 nm, 10 min). To our surprise, illumination increased IR phosphorylation to levels that rivaled those elicited by high concentrations of both an allosteric inhibitor (BBR) and insulin (Fig. 4g). These optically derived shifts—which, by our best estimate, exceeded a 20-fold increase over background levels (Supplementary Note 2)—did not occur in cells shielded from light or in cells stably expressing PTP1B-C** (C450M); IR phosphorylation levels in these two varieties of cells were indistinguishable from those of the wild-type cell line. Our findings thus suggest that PTP1B-C** leaves native phosphorylation levels intact but enables large shifts in target phosphorylation under blue light.

**Discussion**

The study of PTPs has long suffered from a paucity of tools for probing and measuring their intracellular activities. In this study, we developed a photoswitchable variant of PTP1B and used it to exert spatiotemporal control over the phosphorylation state of a genetically encoded biosensor in living cells. Transient irradiation of the full-length, natively localized construct near the nucleus but not the PM produced cell-wide changes in sensor phosphorylation. Importantly, the allosteric photoswitchable control system afforded shifts in activity that reach—by our best estimate—70–85% of the maximum achievable dynamic range and match physiologically influential shifts in activity caused by post-translational modifications of PTP1B. Their use in the present system.

**Methods**

**Cloning and molecular biology.** We constructed PTP1B-LOV2 chimeras by fusing PTP1B and LOV2 at crossover points in a primary sequence alignment. In brief, we used the Needleman-Wunsch algorithm to align the C-terminus of PTP1B (residues 285–305) with the N-terminus of LOV2 (residues 387–410), and we selected eight matching aligned residues as fusion points for the two domains (Fig. 1b). To assemble chimeric genes, we amplified DNA encoding PTP1B and LOV2 from pET21b and pTriEx-PA-Rac1 plasmids, respectively. The pET21b plasmid was a kind gift from the Tonks Group (Cold Spring Harbor Laboratory). We purchased pTriEx-PA-Rac1 from Addgene (Addgene). We joined the two amplified segments with overlap extension PCR (oePCR; see Supplementary Table 3 for primers) and ligated the final chimeric product into pET16b for protein expression.

We generated additional constructs with standard techniques. To build single-site mutants and truncation variants, we amplified parent plasmids with appropriate mutagenic primers (Supplementary Table 4). To construct PTP1B-C**, and PTP1B-C**, we amplified C-terminal regions of PTP1B (residues 299–405 and 299–435, respectively) from pGEX-2T-PTP1B (Addgene) and used Gibson assembly to join them to the C-terminus of PTP1B-C** (50 °C for 1 h; see Supplementary Table 5 for primers). Finally, to construct GFP-tagged versions of PTP1B-C**, PTP1B-C**, and PTP1A**, we amplified these genes from their parent plasmids (see Supplementary Table 5 for primers) and ligated the PCR product into pAcGFP1-C1 (Clonetech, Inc.) at the NcoI and BamHI sites of the MCS for protein expression.

We developed a biosensor for PTP1B by replacing the fluorescent proteins of a biosensor for Src kinase with mClover3 and mRuby3. In brief, we amplified DNA encoding the following components: (i) the central segment of the Src kinase—Src SH2 domain, interdomain linker, and substrate domain (i.e., WEDDYDVVEHLOQ, a peptide derived from p130cas)—from its parent plasmid (a Kras-Src FRET biosensor, Addgene), (ii) genes for mClover3 and mRuby3 (plasmids pNCS-mClover3 and pNCS-mRuby3, respectively), and (iii) the backbone of pAcGFP1-C1 (Clonetech, Inc.), respectively, and (ii) the backbone of pAcGFP1-C1 (Clonetech, Inc.). After amplification, we joined all segments with Gibson assembly (50 °C for 1 h; see Supplementary Table 6 for primers).

For live-cell experiments, we integrated the modified biosensor and PTP1B-LOV2 chimeras into pAcGFP1-C1 by using protocols described above. In short, we amplified DNA encoding (i) PTP1B-C**, or PTP1B-C**, (ii) a ribosomal skipping peptide sequence (F2A-GSG, GSGATENIELQQQADGVIDENPPGP), (iii) the modified biosensor, and (iv) the pAcGFP1-C1 backbone, and we joined the segments with Gibson assembly (50 °C for 1 h; see Supplementary Tables 6 and 7 for primers and DNA fragments, respectively).
Protein expression and purification. We overexpressed PTP1B1,2,3,4, PTP1B1-321, PTP1B1-405, LOV2a-L457, PTP-LOV2 chimeras, Src153-356, and the modified bio- sensors by employing the following steps: (i) We subcloned the polyhistidine-tagged versions of each construct into a PET16b plasmid. We positioned the tag at the N-terminus of Src and the FRET-based biosensor and the C-terminus for all other proteins. For Src, we also added a gene for Cdc37, a chaperone that facilitates protein folding in bacteria42. (ii) We transformed E. coli BL21(DE3) cells and cultured each plasmid-expressing cell line in Luria-Bertani (LB) media (1% tryptone, 0.5% yeast extract, 5% glucose). (iii) We used one colony from each plasmid-expressing cell line to inoculate a 20-mL culture (250 g/L tryptone, 50 g/L yeast extract, and 10 µL DNase). After mixing to homogeneity, we rocked the lysis mixtures for 1 h at room temperature (~22 °C). We used a light plate to estimate initial rates to a Michaelis–Menten equation on the secondary structure of PTP1B-LOV2 chimeras by using a circular dichroism spectroscopy.

Initial analysis of photoswitching. We screened PTP-LOV2 chimeras for light-dependent catalytic activity by measuring their activity on 4MUP in the presence and absence of light. We carried out the following steps: (i) We resuspended the microplate wells—hereafter referred to as the “light plate” and “dark plate”—with 100-μL reactions consisting of buffer (50 mM HEPES, 0.5 mM TCEP, pH 7.5), substrate (500 μM 4MUP), and enzyme (5 nM); at 4, 8, 12, 16, and 20 min after initiating the reaction, we measured the production of p-nitrophenol (405 nm) on a SpectraMax M2 plate reader; and we used DataGraph to fit initial rates to a Michaelis–Menten model of enzyme kinetics. Final values of kcat and Km reflect the mean of independent estimates determined from three Michaelis–Menten curves; error bars reflect the standard error of these estimates.

We examined the inhibitory effect of DPM-1001 on PTP1B-mediated hydrolysis of pNPP as follows: (i) We carried out the aforementioned pNPP reactions in the presence of different concentrations of DPM-1001 (0, 20, 40, 60 μM for PTP1B1,2,3 and PTP1B1-321; 0, 100, 200, 400 μM for PTP1B1-405 and PTP1B1-405p). (ii) We used MATLAB’s “nlfit” and “nlinfit” functions to fit (a) initial-rate measurements collected in the absence of inhibitors to a Michaelis–Menten model and (b) initial-rate measurements collected in the presence and absence of inhibitors to four models of inhibition (i.e., competitive, noncompetitive, uncompetitive, and mixed inhibition58). We used the Akaike information criterion to assign probabilities to each model. (iii) We used an F-test to compare the fits to a (a) mixed model, which has two parameters, and (b) each nested single-parameter model with the lowest sum of squared errors for a given dataset. DPM-1001 exhibited mixed inhibition for all constructs (p < 0.01, two-tailed Student's t test). (iv) We estimated IC50 by using the best-fit kinetic model to determine the inhibitor concentration required to reduce initial rates by 50% on 15 μM pNPP. This high substrate concentration minimizes the contribution of nonlinearity to IC50. We used the MATLAB function “nlparci” to determine the confidence intervals of kinetic parameters and propagated those intervals to estimate the corresponding confidence on IC50.

We compared the activities of PTP1B1,2,3 and PTP1B1-321 on pNPP by using a continuous assay. Briefly, we incubated 100-μL reactions consisting of buffer (50 mM HEPES, 0.5 mM TCEP, pH 7.5), pNPP (0.2, 0.5, 1, 2.5, 5, 10, and 15 μM), and enzyme (25 nM); we measured the production of p-nitrophenol at 5-s intervals for 270 s (SpectraMax M2 plate reader); we used DataGraph to fit initial rates to a Michaelis–Menten model. Finally values of kcat and Km reflect the mean of independent estimates determined from three Michaelis–Menten curves; error bars reflect the standard error of these estimates.

Finally, we repeatedly evaluated the reversibility of our LOV2-based light switch by illuminating 25 μM of PTP1B1-2,3 or PTP1B1-321 on pNPP by using a continuous assay. We incubated 100-μL reactions consisting of buffer (50 mM HEPES, 0.5 mM TCEP, pH 7.5) for 10 s and by, subsequently, monitoring its activity on 5 μM pNPP after 5 min in the dark. To minimize error, we repeated this experiment three times with seven with times (Supplementary Fig. 2).

X-ray crystallography. We prepared crystals of PTP1B1-2,3 by using hanging drop vapor diffusion. To begin, we prepared a concentrated solution of PTP1B1-2,3 (400 μM PTP1B1-2,3, 50 mM HEPES, pH 7.3) and a crystallization solution (100 mM HEPES, 200 mM magnesium acetate, and 14% polyethylene glycol 8000, pH 7.3). We mixed the two solutions in 1:2, 1:3, and 1:6 ratios (protein: crystallization) to form 7–9 μl droplets for crystal growth; and we incubated the droplets over reservoirs filled with crystallization solution at 4 °C in the dark. Long hexagonal crystals with a yellow hue appeared after 1–3 weeks. Prior to freezing, we soaked all crystals in cryoprotectant (100 mM HEPES, 200 mM magnesium acetate, and 25% polyethylene glycol 8000, pH 7.5) overnight.

We collected X-ray diffraction through the Collaborative Crystallography Program of the Berkeley Center for Structural Biology. We performed integration, scaling, and merging of XRD data with the xia2 software package, and we carried out molecular replacement with the Phaser graphical user interface, followed by one round of PDB-REDO.64 The crystallographic data collected in this study are reported in Table 1.

Circular dichroism spectroscopy. We examined the influence of photomodulation on enzyme kinetics by measuring the activities of PTP1B1-2,3 and PTP1B1-321 on pNPP in the presence and absence of light (i.e., we used dark and light plates as described above). Briefly, we prepared 100-μL reactions consisting of buffer (50 mM HEPES, 0.5 mM TCEP, pH 7.5), substrate (0.2, 0.5, 1, 2.5, 5, 10, and 15 mM pNPP), and enzyme (5 nM); we mixed the two solutions in 1:2, 1:3, and 1:6 ratios (protein: substrate) after initiating the reaction, we measured the production of p-nitrophenol (405 nm) on a SpectraMax M2 plate reader; and we used DataGraph to fit initial rates to a Michaelis–Menten model of enzyme kinetics. Final values of kcat and Km reflect the mean of independent estimates determined from three Michaelis–Menten curves; error bars reflect the standard error of these estimates.

For a phosphopeptide (DADEpYLIPOQQ from EGFR) we added a gene for Cdc37, a chaperone that facilitates protein folding in bacteria42. (ii) We transformed E. coli BL21(DE3) cells and cultured each plasmid-expressing cell line in Luria-Bertani (LB) media (1% tryptone, 0.5% yeast extract, 5% glucose). (iii) We used one colony from each plasmid-expressing cell line to inoculate a 20-mL culture (250 g/L tryptone, 50 g/L yeast extract, and 10 µL DNase). After mixing to homogeneity, we rocked the lysis mixtures for 1 h at room temperature (~22 °C). We used a light plate to estimate initial rates to a Michaelis–Menten equation on the secondary structure of PTP1B-LOV2 chimeras by using a circular dichroism spectroscopy.

Initial analysis of photoswitching. We screened PTP-LOV2 chimeras for light-dependent catalytic activity by measuring their activity on 4MUP in the presence and absence of light. We carried out the following steps: (i) We resuspended the microplate wells—hereafter referred to as the “light plate” and “dark plate”—with 100-μL reactions consisting of buffer (50 mM HEPES, 0.5 mM TCEP, pH 7.5), substrate (500 μM 4MUP), and enzyme (5 nM); we added a plastic cover to each plate. (ii) We added a plastic cover to each plate. (iii) We added a plastic cover to each plate. (iv) We exchanged solution over an anion exchange column, and eluted the protein of interest with a 0–100% gradient of imidazole (50 mM Tris-HCl, 0.5 mM TCEP, 500 mM imidazole, pH 7.5). For further purification, we exchanged the protein into HEPS buffer (50 mM HEPES, 0.5 mM TCEP, pH 7.5), flowed the exchanged solution over an anion exchange column, and eluted the final protein with 0–100% gradient of NaCl (50 mM HEPES, 0.5 mM TCEP, 500 mM NaCl, pH 7.5). We purchased all columns (2/10 HiPrep desalting), HitHiTrap HP [Ni], and HiPrep Q HP 16/10 [anion exchange]) from GE Healthcare, Inc. We confirmed the purity of final solutions with SDS-PAGE, and we stored each protein in storage buffer (50 mM HEPES, 0.5 mM TCEP, 20 v/v% glycerol, pH 7.5) at ~80 °C.
Fluorescence spectroscopy. To examine the influence of photomodulation on the conformation of PTP1B within PTP1B-LOV2 chimeras, we use fluorescence spectroscopy to measure optically induced changes in tryptophan fluorescence. In brief, we prepared 60 μM solutions of protein (50 mM HEPES, 0.5 mM TCEP, pH 7.5) in a Helma ultra-micro quartz cuvette (Thomas Scientific, Inc.), 12.5 μl of Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s protocols. At 10–12 h after transfection with plasmid DNA, we imaged the cells in Opti-MEM media at 37 °C.

Preparation of cells for imaging experiments. For live-cell imaging experiments, we grew COS-7 cells (ATCC CRL-1651, seeded from a freezer stock) in DMEM media supplemented with 10% FBS, 100 units/ml penicillin, and 100 units/ml streptomycin for 24 h to achieve 70–90% confluency, and we seeded them on a 20-mm glass-bottom cell culture dish (MatTek). At 10–20 h after seeding, we depleted endogenous PTP1B by transfecting the cells with 25 nM of a PTP1B siRNA silencer (AM16794, Thermo Fisher Scientific, Inc.), 12.5 μl Dharmako, and 10% FBS. At 5 h after adding siRNA, we washed cells with 1X PBS buffer, replaced this buffer with DMEM media supplemented as above (with FBS and antibiotic), and transfected the cells with 2000 ng of plasmid DNA and 6 μl of 2000 reagent (Invitrogen) according to the manufacturer’s protocols. At 10–12 h after transfection with plasmid DNA, we imaged the cells in Opti-MEM media at 37 °C.

Confocal microscopy. We carried out all imaging experiments with a x100 1.45 NA objective on a Nikon A1R confocal scanning microscope supplemented with an environmental chamber (37 °C, 75% humidity, and 5% CO2; Pathology Devices, Inc.). To localize both GFP-tagged PTP1B-LOV2 chimera and PTP1B-Scelβ, we illuminated Cos-7 cells with a 488-nm laser (0.57 mW/μm² with a pixel dwell time of 2.2 μs) and imaged them with a 525/50 nm bandpass filter. The plasmid bearing BFP-Scelβ (pTagBFP-C1) was a kind gift from the lab of Gia Voical of the University of Colorado, Boulder.

Table 1 Data collection and refinement statistics for X-ray crystallographic analysis of PTP1BPs-

| Parameter | Value |
|-----------|-------|
| Data collection | P3,21 |
| Cell dimensions | a, b, c (Å) 89.254, 89.254, 105.747 |
| α, β, γ (°) | 90.000, 90.000, 120.000 |
| Resolution (Å)a | 52.87-1.89 (1.92-1.89) |
| Rmergea | 0.071 (2.550) |
| Rpım,a | 0.025 (0.893) |
| completeness (%)a | 100.0 (100.0) |
| Multiplicitya | 9.0 (9.1) |
| Refinement | Resolution (Å) 44.67-1.89 |
| No. reflections | 37270 |
| W Prot/Refl | 0.17922/0.21175 |
| No. atoms | Protein | 2351 |
| Ligand/ion | 1 |
| Water | 224 |
| B-factors | Proteinb | 21.7 |
| Ligand/ionb | 50.8 |
| Waterb | 47.4 |
| R.m.s.d. deviations | Bond lengths (Å) | 0.008 |
| Bond angles (°) | 1.488 |

aValues in parentheses are for highest-resolution shell. We examined one crystal.

bValues correspond to means of six independent measurements (i.e., six individual cells).

For whole-cell imaging studies, we imaged individual cells with a 457-nm laser focused over the breadth of the cell (0.14 mW/μm² with a pixel dwell time of 4.8 μs). To examine the photoresponse of the biosensor after activation, we illuminated the field of view with a 488 nm laser (0.57 mW/μm²) and imaged the entire cell with 525/50 nm and 600/50 nm bandpass filters for 1 min (resonant scanning mode with 518.1 nm frame time). We estimated the average change in donor/acceptor emission ratio between 0 and 60 s after activation by calculating the interquartile average of measurements from 11 individual cells.

For localized activation studies, we focused 405-nm light over 5-μm circular regions (0.49 mW/μm² with a pixel dwell time of 4.8 μs) and imaged the photoresponse of the biosensor by illuminating at 488 nm (0.57 mW/μm² with a 30 nm activation filter) and imaging with 525/50 nm and 600/50 nm bandpass filters for 1 min. We estimated the average change in donor/acceptor emission ratio within circular regions, in turn, by calculating the difference in 5-s averages starting (i) 5 s before activation and (ii) 35 s after activation; final estimates of changes in donor/acceptor emission reflect the mean and standard error from six independent measurements (i.e., six individual cells).

The 488-nm light used to image our FRET-based biosensor could plausibly stimulate LOV2, which absorbs at 488 nm (although less so than at 405 and 457 nm)32. The results of Fig. 4e, however, indicate that such activation does not occur. In brief, irradiation at 405-nm light causes a transient increase in FRET for cells expressing PTP1BPs and PTP1BPs**, but not for cells expressing light-insensitive analogs of these two constructs; accordingly, 488-nm light does not activate LOV2 (at least, not fully) under our imaging conditions (if it did so, irradiation at 405 nm would not elicit further activation). The insensitivity of LOV2 to 488-nm light likely results from both (i) the low extinction coefficient of LOV2 at 488 nm and (ii) the insufficient combination of power and pixel dwell time of the 488-nm laser.

Preparation of cells for ELISA. We prepared HEK293T/17 cells (ATCC CRL-11268) stably expressing PTP1BPs**, or PTP1BPs(C450M) by following standard protocols. In brief, we grew the cells in 75 cm² culture flasks (Corning) with DMEM media supplemented with 10% FBS, 100 units/ml penicillin, and 100 units/ml streptomycin. When cells achieved 60–80% confluency, we harvested them with 2000 ng of plasmid DNA (pGFP-C1 with PTP1BPs** or PTP1BPs(C450M), but no GFP) linearized with the ApaLI restriction enzyme (New England Biolabs) and (ii) 6 μl of Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s protocols. We passaged the cells in our growth media (as above) supplemented with 1.5 μg/ml puromycin, and we replaced the media every day for 10 days. We passaged the cells seven times before freezing them for further use.

ELISA of insulin receptor phosphorylation. We examined IR phosphorylation in HEK293T/17 cells exposed to various conditions by using an enzyme-linked immunosorbent assay (ELISA). To begin, we used siRNA to deplete cells stably expressing PTP1BPs**, or PTP1BPs(C450M) of endogenous PTP1B (see above) and starved all cells for 48 h with FBS-free media. After starvation, we exposed all cells to one of several conditions for 10 min: (i) 455-nm light (we irradiated the culture flask with ~450 mW light, SLS-0301-C, Mightex Systems, Inc.), (ii) sustained darkness (i.e., we wrapped the culture flask in aluminum foil), (iii) 300 μM of BBR (3-(3,5-dibromo-4-hydroxybenzoyl)-2-ethyl-N-[4-[(2-thiazolylamino)sulfonyl]phenyl]-6-benzofuransulfonamide, Cayman Chemical), an allosteric inhibitor of PTP1B, (iv) 10 nM human insulin (Sigma), and (v) 1.5% DMSO. After these perturbations, we incubated each sample with lysis buffer (Cell Signaling Technology) supplemented with 1X halt phosphatase inhibitor cocktail and 1X halt protease inhibitor cocktail (Thermo Fisher Scientific, Inc.) for 10 min, spun the cells down, and measured IR phosphorylation by using the PathScan® Phospho-Insulin Receptor β (panTyr) Sandwich ELISA Kit (Cell Signaling Technology, #7082).

We carried out the ELISA by using the manufacturer’s prescribed steps: (i) We diluted the entirety of each lyophilized antibody—a detection antibody (phosphorytosine mouse detection mAb, #12982) and a secondary detection antibody (anti-mouse IgG, HRP-linked antibody; #13304)—into 11-mL of antibody-specific diluent (detection antibody diluent; #13339; HRP diluent; #13351). (ii) We used lysis buffer to dilute each sample of cell lysate to 30 μg/ml total protein (based

$$\Delta C_\text{AR} = e^{-kt}$$

$$W_{\text{NO}} = W_t - W_0$$

$$W_{\text{500}} = W_{\text{250}} - W_0$$

$$W_e = e^{-kt}$$
on absorbance at 280 nm). (iii) We prepared 100 µl of 1X, 2X, 4X, and 8X dilutions of lysate from each sample (1X corresponds to no dilution, 2X corresponds to a 1:1 dilution in lysis buffer and cell lysate, and so on), and incubated each 100 µl sample in a single well of an antibody-coated 96-well plate (insulin receptor β rabbit mAb coated microwells; #18872) at 4°C overnight. (iv) We washed the cells four times with 200 µl of 1X wash buffer and incubated the washed cells with 100 µl of detection antibody at 37°C for 1 h. (v) We washed the cells four times as before and incubated the cells with 100 µl of HRP-linked secondary antibody solution at 37°C for 30 min. (vi) We washed the cells four times and incubated them with 100 µl of TMB substrate at 37°C for 10 min. (vii) We added 100 µl of STOP solution and measured absorbance at 450 nm using SpectraMax M2 plate reader.

**Statistical analysis.** We used an F-test to compare one- and two-parameter models of inhibition to one another. For all other analyses, we determined statistical significance by using a two-tailed Student’s t test.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The source data underlying Figs. 1c, 1e, 1f, 2a, 2b, 2c, 2d, 2e, 2g, 3b, 3c, 4b, 4c, 4e, and 4g also group data by type (photoswitching experiments, kinetic analyses, FRET-based studies, etc.). The crystal structure determined in this study is available from the RCSB Protein Data Bank (PDB entry 6npt). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp). Table 1 provides the refinement statistics for this structure. Plasmids harboring important genes used in this study are available from Addgene: LOV2 entry6ntp).

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Author contributions

J.M.F. conceived of research. A.H. and J.M.F. designed experiments. A.H. carried out cloning, protein expression, kinetic measurements, crystal growth, spectroscopic analyses, and imaging experiments. B.S. collected X-ray diffraction data. B.S. and P.Z. provided guidance on structural refinement. A.H. and J.M.F. analyzed all data. A.H. and J.M.F. wrote the paper.

Competing interests

A.H. and J.M.F. are inventors on a PCT application that includes data from this paper. This patent focuses on the use of genetically encoded systems to build biologically active agents, which include light-sensitive enzymes. J.M.F. is a co-founder and consultant of Think Bioscience, which develops therapeutics but does not currently focus on the assembly of light-sensitive enzymes. The remaining authors declare no competing interests.

Additional information

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