Design of a Multi-Use Photoreactor to Enable Visible Light Photocatalytic Chemical Transformations and Labeling in Live Cells

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Design of a Multi-Use Photoreactor to Enable Visible Light Photocatalytic Chemical Transformations and Labeling in Live Cells

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ABSTRACT

Despite the growing utilization of visible light photochemistry in both chemistry and biology, a general low-heat photoreactor for use across these different disciplines does not exist. Herein, we describe the design and utilization of a standardized photoreactor for visible light driven activation and photocatalytic chemical transformations. Using this single benchtop photoreactor, we perform photoredox reactions across multiple visible light wavelengths, a high throughput photocatalytic cross coupling reaction, and in vitro labeling of proteins and live cells. Given the success of this reactor in all tested applications, we envision that this multi-use photoreactor will be widely used in biology, chemical biology, and medicinal chemistry settings.

Keywords

Photoreactor, photochemistry, photoredox, multiplex capabilities, protein and live cell labeling

INTRODUCTION

Photochemistry has long been utilized by chemists to generate reactive species to achieve unique transformations otherwise inaccessible through thermodynamic means. Molecules are electronically excited (to singlet or triplet excited states) by light absorption, promoting the formation of species with new reactivity patterns relative to the ground state.[1] Small molecule based, photoactivation chemistry
has a rich history in organic synthesis particularly in driving complex small molecule rearrangements such as isomerizations and cyclizations.[2,3] The true power of photoactivation chemistry is best illustrated in Eschenmoser’s classic total synthesis of cobyric acid, the key organic component of vitamin B12, where sunlight was utilized to catalyze a macrocyclization of the A and D rings.[4]

Photoactivation has also enabled the use of photoaffinity labeling within chemical biology. For these applications, substrates including aryl azides, diazirines and benzophenones are activated by UV light (~300 nm) for covalent cross linking to biomolecules.[5,6] Probes based on these substrates have found applications in radiolabeling,[5] drug-antibody conjugation,[7] chemoproteomics,[8] target ID[9] and protein cross linking.[10] In some cases, the reactive intermediate used for these applications forms from the T1 excited state.[11] This state is formed in two steps: (I) direct excitation to S1 excited state using UV light then (II) Intersystem crossing (ISC) to form the reactive T1 state. Many times, there is a significant energy gap between the S1 and T1 energy states, therefore there is a large energy loss due to ISC.[12] If direct excitation to the T1 state was possible, a significantly lower energy light source could be utilized. However, quantum mechanics forbids direct excitation to the T1 state because spin is not conserved. Recognizing this inefficient use of energy, as well as the constraints due to quantum mechanics, organic chemists have made significant progress in utilizing photosensitization and photoredox chemistry to access reactive intermediates, particularly free radicals, using lower energy excitation sources.[13] Over the last decade, an unprecedented number of highly valuable synthetic transformations have been developed utilizing photoredox chemistry.[14]

Photoredox reactions within the flask have gone from obscure to commonplace in both industry and academia. This upheaval can largely be attributed to the development of a uniform tool set used to perform photoredox chemistry. This toolset includes both photocatalysts as well as a uniform photoredox reactor with efficient cooling and modular light intensity.[15] Commercial reactors have standardized and enabled the fine tuning of reaction conditions and more recently have become multiplex in design.
Multiplex or high throughput synthesis (HTS) enables speedy reaction optimization through screening multiple reaction conditions at once. Additionally, multiplex synthesis can empower medicinal chemists to generate multiple analogs in parallel, allowing efficient collection of SAR (Structure-Activity Relationship) data. Considering current reactor capabilities, chemists looking to utilize multiplex photoredox synthesis must choose between buying multiple singleton reactors, which can be expensive, or designing their own, which can introduce variabilities in light exposure and heating between devices. While some HTS photoredox reactors have been developed, they are solely engineered for small molecule transformations.\[16\] Thus, an ideal all-purpose photoreactor is currently lacking.

The boundaries of visible light photochemistry have been pushed into protein and live systems by emerging methodologies for peptide and biomolecule functionalization,\[17–20\] bioconjugation,\[21\] protein labeling,\[22,23\] and crosslinking.\[24\] Recently, we reported the development of a photocatalytic-based proximity labeling platform for mapping protein microenvironments on live mammalian cell surfaces.\[25\] The successful applications of photocatalysis within biological systems will continue to increase the utility of this approach to explore biology. However, photocatalytic conditions can limit live-cell applications leading to a need for suitable biocompatible tools and reagents. Surprisingly, a uniform visible light photoreactor for probing biological systems is non-existent. Relative to small molecules, proteins and living cells are significantly more sensitive to heat which can cause dramatic perturbations to a protein structure or physiological environment within the cell. Therefore, an ideal device would have efficient heat dissipation that can maintain samples at a low temperature. Furthermore, the optimal device would be compatible with multiple samples in order to ensure uniformity across a data set or multiple reactions. A final important feature of this new device would be the ability to function at different wavelengths. Although blue light is used in most photoredox methodologies, new methodologies that utilize lower energy green\[26\] and red light\[27,28\] have emerged. In addition to the chemical transformations achieved by these lower energy light sources, longer wavelengths have
significantly better penetration into living tissue.\textsuperscript{[29]} As such, a uniform device integrating all of these features would be widely employed within the medicinal chemistry, chemical biology, and biology communities. Herein we describe the design and development of a multi-use, low heat photoreactor to enable multiplex photoredox reactions, covalent labeling of biomolecules, and labeling within live cells (Figure 1). We showcase a proof of concept framework for known light-driven chemical transformations at different wavelengths, a model photoredox high throughput screen (HTS) Buchwald coupling on drug-like scaffolds, protein biotinylation, and live cell labeling for confocal microscopy imaging analysis.

![Figure 1](image)

**Figure 1:** Features desired in an ideal photoreactor which would enable chemical synthesis, bioconjugation, and cellular labeling reactions.

**RESULTS AND DISCUSSION**

**Photoreactor Design and Development**

To develop a photoreactor that enables multifunctional application in biology and chemistry settings, the reactor size needs to accommodate multiple reaction vials of different types and sizes (e.g. dram vials, microcentrifuge tubes, cell culture plates) with even light distribution across the entire reactor space. To achieve this goal, we designed an oven-styled photoreactor consisting of two chambers. The
outer chamber contains a pull-out lid for sample insertion onto interchangeable, translucent reaction trays into a cylindrical inner chamber containing low heat light chips (Figure 2a). To maximize light coverage, the inner chamber cylinder (Figure 2b) was designed using 6063 T5 aluminum polished to a mirror finish to minimize light absorbance and optimize the light scattering effect (Figure 2c). Four LED-based chips (20W/chip, 29.7 lm/w at 455nm, 99.5 lm/w at 555 nm, 24.5 lm/w at 660 nm) were arrayed in a staggered X position onto the inner chamber to create a consistent light distribution across the horizontal midline of the inner chamber and eliminate any shadowing effect from reaction samples (Figure 2b). To dissipate heat generated from the LEDs, heat sinks were physically attached to each light chip that extend into the outer chamber of the reactor (Figure 2d). This heat sink technology functions through absorbing heat away from the LED chips into the heat sink core and fins located in the outer chamber. While the device relies primarily on passive cooling within the heat sinks, two fans (80 mm, 2200 rpm at 100% output) were mounted to the inner wall of the outer chamber on the top and bottom center of the device (Figure 2e). The fans pull air from outside of the device to displace radiant heat within the inner and outer chambers and cool the heat sink fins. In order to facilitate easy removal and replacement of different light chips with varied wavelengths, each LED/heat sink assembly connects to the inner chamber via wireless magnetic contact to electrical contact pins. Finally, the device was designed to be controlled wirelessly through a mobile app via a Bluetooth (BLE) antenna connected inside of the outer chamber to control photoreactor light intensity (0-100%), light duration (hr/min/s), and cooling fan percent output (0-100%).
Figure 2: Biophotoreactor schematic diagrams. (A) Biophotoreactor with highlighted features. (B) Cutaway view of inner chamber with external components marked. (C) Internal view highlighting the reflective interior of inner chamber for efficient light distribution. (D) Internal view of inner and outer chambers showing heat flow. Red and blue arrows indicate hot and cool air flow, respectively. (E) Internal cutaway view showing inner and outer chamber with components marked.

Multiplex of Photocatalytic Chemical Transformations

Given the historical impact of photoredox catalysis in advancing the field of synthetic organic chemistry, we explored the utility of the photoreactor by first investigating three photocatalytic synthetic transformations at different visible light wavelengths. We began our photoreactor evaluation studies utilizing the protocol for the elegant photoredox-mediated iridium (1)/nickel dual catalyzed C-N coupling of trifluoromethyl bromobenzene (2) and morpholine (3) reported by Macmillan et al. Using a 2-dram vial that easily fits into a single spot of the 24-hole array tray of this new reactor, the reaction was performed under blue light (455 nm) irradiation using nickel(II) bromide glyme and {Ir(dF(CF3)ppy)2(dtbbpy)}PF6 (1) to successfully give the desired coupled product (4) in 68% yield (Figures 3a and 3d). To examine the uniformity of light distribution within the reactor, the same reaction was
performed simultaneously at all 24 vial spots of the reaction tray within the inner chamber. As expected, consistent yields were achieved across all 24 reactions (Figure S1). To highlight the readily interchangeable LED chips feature of the reactor, we next performed a photocatalytic dehalogenation reaction reported by Zeitler et al. In the presence of organic photocatalyst Eosin Y (5), under irradiation with lower energy green light in the photoreactor (555 nm), dehalogenation of an \( \alpha \)-bromoacetophenone (6) proceeded quantitatively (Figures 3b and 3d).\(^{[26]}\) Inspired by the red light-based photocatalytic fluoroalkylation of heteroarenes reported by Postigo et al.\(^{[27]}\), we set out to functionalize capped-tryptophan (9) using a interchangeable red light (660 nm) LED chip. In the presence of 1,1,1,2,3,3,3-heptafluoro-2-iodopropane (10), tryptophan easily underwent C-H functionalization to give excellent yield (80%) of the fluoroalkylated-tryptophan product using a red-light activated zinc phthalocyanine photocatalyst (8) (Figures 3c and 3d). The efficient coupling yield of the tryptophan amino acid under red light illumination showcases the potential opportunity to utilize this transformation in an \textit{in vivo} or tissue setting for tryptophan labeling. Finally, for all three wavelengths the internal heat temperatures were measured and observed to not exceed \( \sim 30 \, ^\circ \text{C} \) when run at 100% light intensity over time indicating the ability to maintain low temperature within the device (Figure 3e). The three model reactions tested within the integrated photoreactor at multiple LED wavelengths and the low temperatures maintained will permit the development and application of novel chemical transformations for profiling biological environments.
High Throughput Screening (HTS) capabilities have served a vital role in medicinal chemistry efforts to perform SAR studies for drug design. Therefore, the ease to quickly access various analogs of a bioactive compound is critical for the cycle time of synthesis to biological testing. To explore the ability of this new integrated photoreactor to enable parallel library synthesis (Figure 4a), we selected photocatalytic Buchwald C-N coupling reactions, one of the most frequently utilized reactions in drug discovery. Using an Iridium photocatalyst (1) (Figure 4b), the photoreactor enabled the concurrent synthesis of functionalized bioactive molecules (Figure 4c). All the coupled monomers feature elements commonly found in medicinal chemistry such as high fraction sp$^3$ containing monomers, basic amines and highly functionalized heterocyclic cores. The ability to rapidly screen multiple photoredox-based transformations involving different functional groups would be of large interest to medicinal chemistry groups to quickly access useful compound targets for SAR studies using a benchtop reactor. These results further highlight the utility of the photoreactor’s multiplex design for overcoming issues associated with concurrent running of multiple photoredox reactions that currently rely on the use of multiple singleton reactors, or the building of custom reactors using Kessil lamps or LED strips.
Figure 4: Photocatalytic reaction multiplexing. (A) Photoreactor with reaction tray loaded with dram vials for parallel synthesis. (B) Iridium catalysts used for library synthesis. (C) Parallel scope photoredox Buchwald C-N coupling reaction.

Visible Light Induced Protein Labeling

Successful application of the photoreactor for small molecule reactions led us to next explore protein labeling \textit{in vitro}. A photocatalytic and photoactivatable visible light induced protein labeling reaction were each selected to install biotin onto carbonic anhydrase (CA) over time (Figure 5a). For the photocatalytic protein labeling system we turned to Ru(bpy)$_3^{2+}$ that is known to oxidize tyrosine residues to form tyrosyl radicals in the presence of blue light and ammonium persulfate ((NH$_4$)$_2$S$_2$O$_8$).$^{[31]}$ This system has been utilized to develop protein cross linking,$^{[24]}$ and ligand-directed protein labeling techniques.$^{[22,23,32],[33]}$ Inspired by these applications, we utilized this ruthenium-based system to generate phenoxy radicals from biotin tyramide for protein biotinylation (Figure 5a, protein labeling A). We observed CA protein biotinylation upon blue light irradiation in the photoreactor that was dependent on the light exposure time (Figure 5b). The multiplex feature of the photoreactor (Figure 5c) enables the
ability to irradiate multiple labeling conditions simultaneously. Accordingly, altering the reaction conditions to remove the photocatalyst and/or visible light did not lead to protein labeling (Figure 5b).

Azido phenol probes have recently been used for protein proximity labeling through a technique called enzyme mediated activation of radical sources (EMARs) where a peroxidase is used to generate a radical labeling species. Since phenyl azides are known to undergo UV activated protein labeling, we wondered whether the presence of a hydroxy group on the ring system would make the probe sensitive to visible light activation for protein labeling as the hydroxy group has been shown to induce a bathochromic shift in absorption spectra when present on a benzene ring (Figure 5a, Protein labeling B). CA protein was mixed with the azido phenol biotin probe and irradiated with visible light to yield time dependent protein labeling (Figure 5b). Additionally, a light on/off protein labeling experiment shows that biotinylation using the azido phenol biotin probe only occurs upon addition of visible light, highlighting the utility of the photoreactor to maintain exquisite control over the protein labeling reaction (Figure 5d). As a final demonstration of the light box to facilitate protein labeling, Eosin Y was used to induce protein oxidation that could be trapped by biotin hydroxyl amine (Figure S2). Collectively, these results highlight the ability to achieve temporal control over protein labeling using the photoreactor and open the possibility to explore other light controlled protein labeling reactions.
Figure 5: Visible light-mediated protein labeling. (A) General reaction scheme for the photocatalytic and photoactivatable biotinylation of carbonic anhydrase (CA). (B) Western blot analysis shows time dependent biotinylation of (CA). (C) Biophotoreactor loaded with microcentrifuge tubes for reaction multiplexing. (D) Biotin-azido-phenol on/off light experiment showcasing light dependency of the protein labeling reaction (average of 3 independent experiments, error bars represent ± S.D.).

Live Cell Photochemical Labeling

To test the feasibility of using the photoreactor for labeling of live cells, we initially determined viability of the A375 melanoma cell line upon extended exposure to full intensity visible light and observed no reduction in viability (Figure S3). Encouraged by these results, we next investigated the ability to induce protein labeling via photoredox on live A375 cells. Given the successful protein labeling results observed with the Ru(bpy)$_3^{2+}$ photocatalyst in Figure 5b, we selected this system for live cell labeling.
Cellular biotinylation was monitored by confocal imaging using streptavidin Alexa Fluor 488 (AF 488) conjugate dye that binds biotinylated cells after washing unreacted biotin tyramide (Figure 6a). Gratifyingly, cellular biotinylation only occurred in the presence of Ru(bpy)$_3^{2+}$, (NH$_4$)$_2$S$_2$O$_8$, and visible light irradiation in the photoreactor (Figure 6b). In contrast, in the absence of visible light irradiation no cellular labeling was detected (Figure 6b). Similarly, removing (NH$_4$)$_2$S$_2$O$_8$ or both (NH$_4$)$_2$S$_2$O$_8$ and Ru(bpy)$_3^{2+}$ also led to minimal cellular labeling (Figure 6b). These results clearly show that the photoreactor is also capable of inducing labeling of live cells for downstream analysis and can allow for the screening of other light-activated chemistry reactions in live cell systems. To our knowledge, this photoreactor represents the first uniform reactor for protein or cellular labeling.

**Figure 6**: Live cell labeling in the multi-use photoreactor. (A) General reaction scheme for the biotinylation of A375 cells using Ru(bpy)$_3^{2+}$, (NH$_4$)$_2$S$_2$O$_8$, and biotin-tyramide. (B) Confocal microscopy images showing nuclei (blue) and biotinylation (green). Scale bars indicate 10 µm. Duplicate images are shown below each condition.
CONCLUSION

The use of visible light as a reagent in photocatalysis to generate reactive species in a controlled manner provides unique advantages for profiling biological environments due to the highly tunable temporal and spatial precision of light. Thus, for photocatalysis to bridge the gap from chemistry centric methods towards enabling biological investigation, a uniform photoreactor that is biocompatible and permits the use of cell culture plates and microcentrifuge tubes with efficient light distribution and low heat generation is a required piece of the photochemistry toolbox.

Herein, we disclosed the design and development of a multifunctional photoreactor that can be broadly used for visible light driven activation and catalytic reactions for proteins and live cells. The photoreactor LED was designed with heat dissipation sinks to reduce heat while efficiently and uniformly illuminating light within the reflective reaction chamber. Photocatalytic reactions at multiple visible light wavelengths (blue, green, and red) were easily achieved in the modular photoreactor through interchangeable light chips. The multiplexing capability of the photoreactor enabled high throughput library synthesis as shown through a relevant medicinal chemistry coupling reaction.

In addition to the demonstrated utility for chemical transformations, we showcased protein labeling methods as well as photocatalytic biotinylation of live A375 cells for confocal microscopy imaging. To our knowledge, this photoreactor represents the first uniform reactor for protein and live cell labeling in biological environments that we anticipate will enable the transfer of light-mediated chemical transformations, and the development of new ones, into cellular environments and tissues.
SUPPLEMENTAL INFORMATION

All supplementary figures, experimental details, and synthesis information are included in Supporting Information.

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AUTHOR CONTRIBUTIONS

Project was conceived and initiated by R.C.O. and O.O.F. All experiments were designed by N.B.B., R.C.O and O.O.F. and performed by N.B.B., K.A. R., T.R.R. The manuscript was written by N.B.B., R.C.O and O.O.F and proofread by all authors.

DECLARATION OF INTERESTS

N.B.B., K.A.R., T.R.R., S. W., E.C.H., L.R.R., R.C.O., O.O.F. are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. M.J.W. is an employee of Efficiency Aggregators. A patent on the photoreactor has been filed by Efficiency Aggregators.
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Supporting Information

for

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Figure S1: Light distribution across multiplex set up. All reactions reached full conversion.
Figure S2: Labeling of Carbonic Anhydrase using an Eosin Y photocatalyst and an oxyamine biotin probe.
Figure S3: A375 cell viability measurements during visible light irradiation over 60 minutes. Cell viability is not altered during light irradiation at any of the tested wavelengths.
General Information

All reagents were purchased from commercial suppliers and stored per the manufacturer’s guidelines. Biotin tyramide (LS-3500.1000) was purchased from Iris Biotech GMBH. BIOTIN-PEG3-OXYAMINE HCL was purchased from Fisher Scientific. Azido-phenol biotin probe ((4-azido-2-hydroxy-N-(6-(6-((3aS,4S,6aR)-2-oxohexahydro-1Hthieno[3,4-d]imidazol-4-yl)hexanamido)hexyl)benzamide)) was synthesized as described in the literature. An Agilent Technologies 1290 Infinity II HPLC attached to an Agilent Technologies 6130 Quadrupole LC/MS using a Supelco Column: Ascentis Express C18 HPLC column (5 cm x 2.1 mm x 2.7 µm) was used to collect LCMS data for small molecule compounds. The column was heated to 50°C; the gradient used was 0 min (2% B), 0.2 min (2% B), 1 min (50% B), 1.5 min (98% B), 2 min (98% B). Solvent A was Water and B was MeCN. 0.1% v/v acid modifier (TFA) was added to each solvent. NMR data was acquired using a Brunker 400 Ultrashield NMR. Spectra are internally referenced to residual solvent signals (CDCl₃ δ 7.26 (1H) and 77.16 (13C) or DMSO δ 2.50 (1H) and 39.52 (13C)). Data for ²H NMR are reported according to the following conventions: chemical shift (ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, q = quintet, br = broad, and combinations thereof), coupling constant J (Hz), integration. Magnetic stirring was induced when specified by placing a V&P Scientific Inc. Magnetic Tumble Stirrer VP 710 Series inside of the photoreactor.

High Throughput Screen

Three unique amine reactant stock solutions were prepared. Amine (4.5 equiv., 1.247 mmol), DABCO (5.4 equiv, 1.496 mmol) and DMA (2 mL) was added to a vial. A solution of (Ir[dF(CF₃)ppy]₂(dtbbpy))PF₆ (.0006 equiv) in DMA (4.5 µL) was added to the vial. A solution of NiBr₂•glyme (.15 equiv) in DMA (1 mL) was also added to the vial. The vial was placed under an atmosphere of nitrogen, cooled to – 78 °C, degassed via vacuum evacuation (3 min), backfilled with nitrogen, and warmed to room temperature. This process was repeated twice. To the vials was then added aryl bromide (0.277 mmol, 1 equiv). The vials were sealed, placed under vacuum for 30 seconds and backfilled with nitrogen 2x. Next 1mL of each amine reactant stock solution was added across the three unique aryl bromide vials under nitrogen. The 8 unique vials were sealed with parafilm, placed in the Biophotoreactor and irradiated. After 12 h, each reaction was analyzed by LCMS. Based on LCMS data reactions were either purified via mass directed reversed phase chromatography or discarded. Pure fractions were combined, dried and analyzed using NMR and LCMS.

General Western Blot Procedure

50 µL of Biorad 4x Laemmli loading buffer was added to each sample and incubated at 95°C for 10 min. During this time, a 12% Criterion TGX precast gel, (18 well, 30 µL, 1 mm.) was loaded into a gel box and filled with tris-glycine buffer. 10 µL of sample or molecular weight marker was added to each lane (Invitrogen iBright prestained protein ladder). The gel was run at 180V for 45 minutes. Afterwards, the gel was transferred onto a membrane (Invitrogen iBlot 2 PVDF) using the iBlot 2 device according to manufacturer’s instructions. The membrane was incubated with 50 mL of blocking solution (TBS-Tween containing 3% BSA) for one hour then 10 µL of IR Dye 800 CW Streptavidin antibody (Li-COR) was added, and the membrane was incubated overnight. The membrane was then washed with TBS-Tween 3x, followed by one wash with Milli-Q water. The membrane was imaged using the Li-COR Odyssey CLx. Afterwards, the membrane was stained with Li-COR Revert Total Protein Stain and incubated for 5-10 minutes. The membrane was then imaged again on the Li-COR.
Photoreactor Reaction Position Uniformity Test

To a solution of 4-bromobenzotrifluoride (460 µL, 3.328 mmol, 1.0 equiv), morpholine (460 µL, 4.928 mmol, 1.5 equiv), and DABCO (664 mg, 5.936 mmol, 1.8 equiv) in DMA (6 mL) was added (Ir[dF(CF3)ppy]2(dtbbpy))PF6 (0.732 mg, 0.0002 equiv) as a solution in DMA (26.4 µL). A solution of NiBr2•glyme (36 mg, 0.164 mmol, 0.05 equiv) in DMA (6 mL), which had been sonicated for 1 min, was then added. The vial was placed under an atmosphere of nitrogen, cooled to −78 °C, degassed via vacuum evacuation (3 min), backfilled with nitrogen, and warmed to room temperature. This process was repeated two times. Next a 1-dram vial was placed under vacuum for 30 seconds and backfilled with nitrogen. This process was repeated once. 0.45 mL of the stock solution was added to the vial and sealed with parafilm. This was repeated 23 more times. All the vials were placed in the Biophotoreactor and irradiated. After 12 h, LCMS was used to analyze % conversion (at 254.4 nm) of each vial and a map of light coverage was generated based on conversion.

| Row | Column 1       | Column 2       | Column 3       | Column 4       |
|-----|----------------|----------------|----------------|----------------|
| 6   | 100% Conversion| 100% Conversion| 100% Conversion| 100% Conversion|
| 5   | 100% Conversion| 100% Conversion| 100% Conversion| 100% Conversion|
| 4   | 100% Conversion| 100% Conversion| 100% Conversion| 100% Conversion|
| 3   | 100% Conversion| 100% Conversion| 100% Conversion| 100% Conversion|
| 2   | 100% Conversion| 100% Conversion| 100% Conversion| 100% Conversion|
| 1   | 100% Conversion| 100% Conversion| 100% Conversion| 100% Conversion|

Figure S4: Left, table showing percent conversion at each position in the photoreactor. Right, image of reaction tray to highlight positioning of each vial.

Internal Temperature Measurements

Thermal data was collected using the OM-CP-RHTemp101A Humidity and Temperature Data Logger with a thermocouple. Data was processed using the Omega Software. Temperature was recorded every 15 seconds for 18 hours for the Biophotoreactor with Blue, Green and Red LEDs. Operating temperature after heating period was determined by averaging temperature data points from 2 to 18 hours. Data was exported to MS Excel and plotted (see Figure 3e).
Light On/Off Experiment
600 µL of Carbonic Anhydrase (1 mg/mL) in PBS and 6 µL of biotin azide tag (25 mM) in DMSO were added to a microcentrifuge tube. The sample was irradiated with blue LED light for 5 minutes and afterwards left in the dark for 5 minutes. This process was repeated 4x. After each light or dark period, 60 µL aliquots were taken. 15 µL of loading buffer was added to each aliquot. The samples were then analyzed through western blot analysis. Densitometry was used to quantify biotinylated protein levels. Figure 5d shows average data from three independent experiments with error bars representing ± S.D.

Effect of Visible Light Irradiation on Cell Viability
Cell viability was measured with the CyQUANT MTT Cell proliferation Assay kit (V13154) following the manufacturer’s guidelines. Briefly, A375 cells were plated in 96 well plates at a density of 50k cells/well and grown overnight. The cells were irradiated in the corresponding wavelength of light for periods between 0 to 60 min in the biophotoreactor. Following irradiation, the culture medium was removed and replaced with 100 µL of fresh PBS + 10% HI FBS. 10 µL of 12 mM MTT stock solution was then added to each well. The plate was incubated at 37°C for 4 hours. Following incubation, all but 25 µL of medium was removed from the wells. 50 µL of DMSO was added to each well and mixed thoroughly with a pipette, avoiding bubbles. The plate was incubated at 37°C for 10 minutes. Each well was mixed thoroughly again, and the absorbance was measured at 540 nm.

Eosin Y Based Biotinylation of Carbonic Anhydrase
200 µL of Carbonic Anhydrase (1 mg/mL) in PBS, 2 µL of Eosin Y (500 µM) in PBS and 2 µL of biotin tag (25 mM) in DMSO were added to a microcentrifuge tube. This was repeated 4 times. One sample was prepared without Eosin Y as a control. Samples were irradiated with blue light in the Biophotoreactor with max fan for 0 sec, 15 sec, 1 min, 3 min and 5 min, respectively. The sample without photocatalyst was irradiated for 5 min. The samples were then analyzed through western blot using the General Western Blot Procedure.

Ruthenium Based Biotinylation of Carbonic Anhydrase
200 µL of Carbonic Anhydrase (1 mg/mL) in PBS, 2 µL of Ru(bpy)3(PF6)2 (100 µM) in DMSO, 2 µL of ammonium persulfate (25 mM) in PBS and 2 µL of biotin phenol tag (25 mM) in DMSO were added to a microcentrifuge tube. Samples were irradiated with blue light in the Biophotoreactor with max fan for 0 sec, 30 sec, 2 min, 7.5 min and 15 min respectively. The samples were then analyzed through western blot using the General Western Blot Procedure.

Photoactivated Biotinylation of Carbonic Anhydrase
200 µL of Carbonic Anhydrase (1 mg/mL) in PBS and 2 µL of biotin azide tag (25 mM) in DMSO were added to a microcentrifuge tube. Samples were irradiated with blue light in the Biophotoreactor with max fan for 0 sec, 5 min, 10 min, 20 min and 40 min respectively. The samples were then analyzed through western blot using the General Western Blot Procedure.
General Cell Culture Methods

A375 cells were purchased from Sigma-Aldrich (88113005-1VL) and cultured in 1x DMEM with GlutaMAX-I (Thermo Fisher Scientific: 10569-010) containing 10% HI FBS (Thermo Fisher Scientific: 10082-139), and 100 IU Penicillin/100µg/mL Streptomycin (Thermo Fisher Scientific: 15140-148). Cells were grown at 37°C with 5% CO₂ in tissue culture dishes. For passaging, cells were washed once with 1x DPBS and suspended using TrypLE Express Enzyme (1x) (Gibco: 12604021).

Confocal Microscopy Imaging of A375 cells

µ-Dish 35 mm, glass bottom dishes (ibidi: 81158) were rinsed 1x with 1 mL of 1x DPBS (Gibco: 14190144) and 1 mL of poly-L-lysine solution (Sigma: P4707-50ML) was added per dish and incubated for 30 min at room temperature. Dishes were washed 2x with 1 mL of 1x DPBS and 500,000 A375 cells were seeded in 400 µL of A375 culture media (see General Cell Culture Methods section) and incubated overnight at 37°C with 5% CO₂.

Reaction solutions were prepared in 1 mL of 1x DPBS at the following concentrations: 10 µm Ru(ppy)₃, 250 µm biotin phenol, 250 µm ammonium persulfate. Cell culture media was removed using a pipette and reaction solution was added. Samples were irradiated (or stored under tinfoil for the no light control) for 30 minutes. Afterwards the reaction mixture was removed and 1 mL of 1x DPBS was added using a pipette.

Cells were washed 1x with 1x DPBS and staining procedure for imaging was modified as reported previously. Briefly, 6% paraformaldehyde (PFA, Electron Microscopy Sciences: 15710) and 0.2% glutaraldehyde (Sigma-Aldrich: G5882-10X10ML) were prepared in 1x DPBS and added gently at equal ratios per dish (final concentration of 3% PFA and 0.1% glutaraldehyde in a total volume of 400 µL) and incubated for 10 min at 4°C. The dishes were washed 3x in Stain Buffer (BD Biosciences: 554656) and incubated overnight in 1 mL of Stain Buffer at 4°C. The following day, samples were stained with Alexa Fluor 488 Streptavidin (BioLegend: 405235) at a 1:200 dilution in 400 µL of Stain Buffer and incubated overnight at 4°C. The samples were washed 1x with 1 mL of Stain Buffer and Hoechst DNA dye (Cayman Chemical Company: 600332) was added at a 1:10,000 dilution in 400 µL of Stain Buffer per dish and incubated while protected from light for 10 min at room temperature. The dishes were washed 2x in Stain Buffer and fixed with 400 µL of a 3% PFA and 0.1% Glutaraldehyde solution in 1x DPBS for 5 min at room temperature, washed 2x in 1 mL of Stain Buffer, and imaged using a Zeiss LSM800 inverted, confocal microscope using a 63X oil immersion objective.
Photoreactor

The photoreactor (BPR200, Fisher, Product number: NC1558343 or available through Sigma-Aldrich) was designed, developed, and manufactured by Efficiency Aggregators (Richmond, TX, USA). The figures below highlight components and features of the photoreactor described in the manuscript.

**Figure S5:** Left, side view of photoreactor with control tablet (light off). Right, side view of photoreactor with control tablet (light on).

**Figure S6:** Left, side view of photoreactor outer chamber with lid open. Middle, top view of photoreactor with lid open. Right, bottom view of photoreactor.
Figure S7: Left, photoreactor inner chamber with reflective interior. Right, outside of inner chamber showing contact pins where LED chips connect.

Figure S8: Left, front view of LED Chip. Middle, side view of LED chip showing attached heat sink. Right, back view of LED chip showing heat sink core and fin.
Figure S9. Photoreactor control tablet and instructions on how to turn on the photoreactor. A) Opening the BPR app for the first time will launch a screen asking for various permissions. B) Upon making a choice, the main screen will be displayed. C) Click connect device and the device management screen will pop up. If the photoreactor is plugged in and on, its name should appear on screen after hitting the refresh button. D) Selecting the photoreactor will launch the control screen. E) Input the chosen fan and light intensity and select a reaction time. F) Hit start and irradiation will begin.

Figure S10: Light on Photoreactor for various wavelengths: Blue (λ=453nm), Green (λ=555nm), and Red (λ=660nm).
Figure S11: Open Photoreactor loaded with vials, microcentrifuge tubes and cell plates at various light intensities.
Synthesized Compounds

4-(4-(trifluoromethyl)phenyl)morpholine (4)

To a solution of 4-bromobenzotrifluoride (115 µL, 0.832 mmol, 1.0 equiv), morpholine (115 µL, 1.232 mmol, 1.5 equiv), and DABCO (166 mg, 1.484 mmol, 1.8 equiv) in DMA (1.5 mL) was added (Ir[dF(CF3)ppy]2(dtbbpy))PF6 (0.183 mg, 0.0002 equiv) as a solution in DMA (6.6 µL). A solution of NiBr2•glyme (9 mg, 0.041 mmol, 0.05 equiv) in DMA (1.5 mL), which had been sonicated for 1 min, was then added. The vial was placed under an atmosphere of nitrogen, cooled to –78 °C, degassed via vacuum evacuation (3 min), backfilled with nitrogen, and warmed to room temperature. This process was repeated two times, and the reaction vial was then sealed with parafilm, placed in the Biophotoreactor, and irradiated. After 12 h, the reaction mixture was analyzed using LCMS (100% conversion). The mixture was then purified through reversed phase chromatography furnishing product in a 68% yield. Spectra matches known compound.

\[ \text{1H NMR (400 MHz, CDCl3): } \delta 7.53 (d, J = 8.7 \text{ Hz}, 2\text{H}), 6.97 (d, J = 8.7 \text{ Hz}, 2\text{H}), 3.90 (t, J = 4.9 \text{ Hz}, 4\text{H}), 3.27 (t, J = 4.9 \text{ Hz}, 4\text{H}). \]

\[ \text{LCMS: Expected mass for C}_{11}\text{H}_{13}\text{F}_{3}\text{NO \ [M+H]}=232.1. \text{ Found=232.1.} \]

Acetophenone (7)

To a vial charged with a stir bar was added 2-bromo-1-phenylethan-1-one (100 mg, 0.5 mmol, 1 equiv), diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (139.9 mg, 0.55 mmol, 1.1 equiv), and Eosin Y (8.7 mg, 0.0125 mmol, 0.025 equiv). The vial was placed under vacuum and backfilled with nitrogen. This was repeated 2x. In a separate vial, DMF (2 mL, 0.25 M) was degassed via bubbling nitrogen through for 10 min. The degassed DMF was added to the other vial under nitrogen. Additionally, DIPEA (175 µL, 1 mmol, 2 equiv) was added. The top of the vial was sealed with parafilm, placed in the Biophotoreactor, and irradiated with green light. After 18 h, the reaction mixture was analyzed through TLC (Eulent Hex/EtOAc 85:15). TLC indicated 100% conversion of starting material to product. The reaction mixture was then transferred to a separatory funnel and extracted from water with DCM 3x. The combined organic layers were dried with magnesium sulfate and concentrated under reduced pressure. The crude residue was purified through normal phase chromatography (Hex/EtOAc 100:0 to 80:20). Pure fractions were identified through TLC, combined and concentrated under reduced pressure yielding acetophenone.

\[ \text{1H NMR (400 MHz, Chloroform-d) } \delta 7.98 – 7.94 (m, 2\text{H}), 7.60 – 7.53 (m, 1\text{H}), 7.50 – 7.43 (m, 2\text{H}), 2.61 (s, 3\text{H}). \text{ Spectra matches known compound.} \]
(S)-2-acetamido-N-methyl-3-(2-(perfluoropropan-2-yl)-1H-indol-3-yl)propenamide (11)

To a vial charged with a stir bar was added (S)-2-acetamido-3-(1H-indol-3-yl)-N-methylpropanamide (63.1 mg, 0.3 mmol, 1 equiv), ascorbic acid (79.3 mg, 0.45 mmol, 1.5 equiv), 2,4,6-collidine (59 µL, 0.45 mmol, 1.5 equiv), Zinc Phthalocyanine (1.73 mg, 0.003 mmol, 1 mol%) and MeCN/DMF (1:1, 0.12 M). Nitrogen was bubbled through the mixture for 15 min. Next, heptafluoro-2-iodopropane (128 µL, 0.9 mmol, 3 equiv) was added and nitrogen was bubble through the mixture for 3 min. Afterwards, the septum of the reaction vial was wrapped in parafilm. The mixture was then irradiated with red LED light and stirred for 22 hours. The mixture was extracted from brine using DCM (3X). The combined organic fractions were dried over magnesium sulfate and concentrated under reduced pressure. The crude residue was purified through normal phase chromatography (DCM/MeOH, 100:0 to 90:10 gradient). The pure fractions were combined and concentrated under reduced pressure furnishing (S)-2-acetamido-N-methyl-3-(2-(perfluoropropan-2-yl)-1H-indol-3-yl)propenamide (102.0 mg, 80% yield).

\[ \text{LCMS: Expected mass for C}_{17}\text{H}_{17}\text{F}_{7}\text{N}_{3}\text{O}_{2} [M+H]=428.1. \text{Found}=428.1. \]

3-(5-morpholinopyridin-2-yl)oxetane-3-carbonitrile (12)

Synthesized using the High Throughput Screen Procedure. 97% conversion, 66% yield. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 8.39 (d, \(J = 2.8\) Hz, 1H), 7.51 (d, \(J = 8.7\) Hz, 1H), 7.44 (dd, \(J = 8.8, 3.0\) Hz, 1H), 5.07 (d, \(J = 6.3\) Hz, 2H), 4.98 (d, \(J = 6.3\) Hz, 2H), 3.79 – 3.72 (m, 4H), 3.25 – 3.18 (m, 4H). \(^1\)C NMR (101 MHz, DMSO) \(\delta\) 146.45, 143.64, 136.88, 122.41, 121.15, 120.78, 78.33 (2C), 65.80 (2C), 47.37 (2C), 41.80. \text{LCMS: Expected mass for C}_{13}\text{H}_{16}\text{N}_{3}\text{O}_{2} [M+H]=246.1. \text{Found}=246.2.

4-(1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)morpholine (13)

Synthesized using the High Throughput Screen Procedure. 52% conversion, 35% yield. \(^1\)H NMR (400 MHz, Chloroform-\(d\)) \(\delta\) 8.23 (d, \(J = 2.6\) Hz, 1H), 8.16 – 8.12 (m, 2H), 7.66 (d, \(J = 4.0\) Hz, 1H), 7.60 – 7.51 (m, 1H), 7.46 (td, \(J = 7.1, 1.5\) Hz, 2H), 7.39 (d, \(J = 2.2\) Hz, 1H), 6.51 (d, \(J = 4.0\) Hz, 1H), 3.93 – 3.86 (m, 4H), 3.18 – 3.11 (m, 4H). \(^1\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 143.88, 142.85, 138.54, 137.06, 134.06, 129.13 (2C), 127.94 (2C), 127.48, 123.28, 117.02, 105.61, 66.63 (2C), 51.20 (2C). \text{LCMS: Expected mass for C}_{17}\text{H}_{18}\text{N}_{3}\text{O}_{3}\text{S} [M+H]=344.1. \text{Found}=344.1.
4-methyl-3-morpholinobenzonitrile (14)

Synthesized using the High Throughput Screen Procedure. 68% conversion, 9% yield. $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 7.32 (dd, $J = 7.8$, 1.4 Hz, 1H), 7.30 – 7.26 (m, 2H), 3.96 – 3.89 (m, 4H), 3.00 – 2.93 (m, 4H), 2.39 (s, 3H).

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 150.86, 138.69, 132.31, 127.63, 122.71, 118.85, 110.47, 66.87 (2C), 52.10 (2C), 18.52.

LCMS: Expected mass for C$_{12}$H$_{15}$N$_2$O [M+H]=203.1. Found=203.1.

3-(5-((6-hydroxy[3.3]heptan-2-yl)amino)pyridin-2-yl)oxetane-3-carbonitrile (15)

Synthesized using the High Throughput Screen Procedure. 86% conversion, 73% yield. $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 8.29 (d, $J = 2.8$ Hz, 1H), 7.44 (d, $J = 8.7$ Hz, 1H), 7.02 (dd, $J = 8.7$, 2.8 Hz, 1H), 5.20 (d, $J = 6.3$ Hz, 2H), 5.12 (d, $J = 6.3$ Hz, 2H), 4.31 – 4.20 (m, 1H), 3.85 (p, $J = 7.6$ Hz, 1H), 2.52 (tq, $J = 11.6$, 5.8, 4.9 Hz, 3H), 2.38 (dt, $J = 12.0$, 6.1 Hz, 1H), 2.07 – 1.93 (m, 4H).

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 143.86, 139.59, 134.48, 122.55, 121.16, 119.98, 79.14 (2C), 69.25, 63.38, 45.71, 45.50, 44.70, 42.70, 42.29, 29.16. LCMS: Expected mass for C$_{16}$H$_{20}$N$_3$O$_2$ [M+H]=286.1. Found=286.1.

6-((1-phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)amino)spiro[3.3]heptan-2-ol (16)

Synthesized using the High Throughput Screen Procedure. 43% conversion, 8% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.04 – 7.98 (m, 2H), 7.74 (d, $J = 2.5$ Hz, 1H), 7.71 – 7.65 (m, 2H), 7.58 (t, $J = 7.7$ Hz, 2H), 6.96 (d, $J = 2.2$ Hz, 1H), 6.61 (d, $J = 4.0$ Hz, 1H), 3.95 (p, $J = 7.5$ Hz, 2H), 3.67 (p, $J = 7.5$ Hz, 1H), 2.45 – 2.25 (m, 3H), 2.15 (dt, $J = 11.7$, 6.2 Hz, 1H), 1.88 – 1.73 (m, 4H).

$^{13}$C NMR (101 MHz, DMSO) $\delta$ 141.08, 139.68, 137.81, 134.35, 133.23, 129.45 (2C), 127.18 (2C), 126.75, 123.17, 110.01, 106.21, 61.42, 45.73, 45.44, 44.23, 42.23, 41.71, 28.35. LCMS: Expected mass for C$_{20}$H$_{22}$N$_3$O$_3$S [M+H]=384.1. Found=384.2.

3-((6-hydroxy[3.3]heptan-2-yl)amino)-4-methylbenzonitrile (17)

Synthesized using the High Throughput Screen Procedure. 99% conversion, 81% yield. $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 7.10 (d, $J = 7.6$ Hz, 1H), 6.97 (dd, $J = 7.6$, 1.4 Hz, 1H), 6.72 – 6.67 (m, 1H), 4.30 – 4.24 (m, 1H), 4.22 (s, 1H), 3.81 (p, $J = 7.5$ Hz, 1H), 2.59 – 2.44 (m, 3H), 2.36 (dt, $J = 11.4$, 5.9 Hz, 1H), 2.17 (s, 3H), 2.08 – 1.88 (m, 4H).

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 144.71, 130.83, 128.18, 121.80, 119.84, 113.59, 110.58, 63.42, 45.71, 45.42, 45.10, 42.90, 42.45, 29.15, 17.91. LCMS: Expected mass for C$_{15}$H$_{19}$N$_2$O [M+H]=243.1. Found=243.1.
3-(5-(2-oxa-6-azaspiro[3.3]heptan-6-yl)pyridin-2-yl)oxetane-3-carboxamide (18)

Synthesized using the High Throughput Screen Procedure. Nitrile was hydrolyzed to amide. 70% conversion, 32% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.87 (d, $J = 2.7$ Hz, 1H), 7.42 (d, $J = 8.5$ Hz, 1H), 6.91 (dd, $J = 8.5$, 2.9 Hz, 1H), 5.05 (d, $J = 6.2$ Hz, 2H), 4.96 (d, $J = 6.2$ Hz, 2H), 3.64 (s, 4H), 3.55 (s, 4H). $^{13}$C NMR (101 MHz, DMSO) $\delta$ 147.08, 141.20, 133.25, 121.32, 120.73, 118.52, 78.43 (2C), 62.91 (2C), 55.40 (2C), 42.09, 41.79. LCMS: Expected mass for C$_{14}$H$_{18}$N$_3$O$_3$ [M+H]=276.1. Found=276.1

4-methyl-3-(2-oxa-6-azaspiro[3.3]heptan-6-yl)benzonitrile (19)

Synthesized using the High Throughput Screen Procedure. 71% conversion, 7% yield. $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 7.12 – 7.06 (m, 2H), 6.70 (s, 1H), 4.86 (s, 4H), 4.13 (s, 4H), 2.28 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 132.21, 127.60 (weak signal), 123.62, 121.74 (weak signal), 119.53, 116.08, 110.35, 81.12 (2C), 62.92 (2C), 38.87, 19.92. LCMS: Expected mass for C$_{13}$H$_{15}$N$_2$O [M+H]=215.1. Found=215.1
Synthesized Compounds (Spectra)

4-(4-(trifluoromethyl)phenyl)morpholine (4)
Ret. Time: 1.35

Acetophenone (7)
(S)-2-acetamido-N-methy l-3-(2-(perfluoropropan-2-yl)-1H-indol-3-yl)propenamide (11)
3-(5-morpholinopyridin-2-yl)oxetane-3-carbonitrile (12)
4-(1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)morpholine (13)
4-methyl-3-morpholinobenzonitrile (14)
3-(5-((6-hydroxyspiro[3.3]heptan-2-yl)amino)pyridin-2-yl)oxetane-3-carbonitrile (15)
6-((1-phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)amino)spiro[3.3]heptan-2-ol (16)
3-((6-hydroxyspiro[3.3]heptan-2-yl)amino)-4-methylbenzonitrile (17)
3-(5-(2-oxa-6-azaspiro[3.3]heptan-6-yl)pyridin-2-yl)oxetane-3-carbonitrile (18)
4-methyl-3-(2-oxa-6-azaspiro[3.3]heptan-6-yl)benzonitrile (19)
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