Rational Design of an α-Ketoamide-Based Near-Infrared Fluorescent Probe Specific for Hydrogen Peroxide in Living Systems

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Supporting Information

ABSTRACT: Hydrogen peroxide, an important biomolecule, receives earnest attention because of its physiological and pathological functions. In this Article, we present the rational design, characterization, and biological application of a mitochondria-targetable NIR fluorescent sensor, Mito-NIRHP, for hydrogen peroxide visualization. Mito-NIRHP utilizes a unique reaction switch, α-ketoamide moiety, to turn on a highly specific, sensitive, and rapid fluorescence response toward hydrogen peroxide coupled with the intramolecular charge transfer strategy. Mito-NIRHP is competent to track endogenously produced hydrogen peroxide in both living cells and living animals. In addition, utilizing Mito-NIRHP, overgeneration of hydrogen peroxide during ischemia-reperfusion injury was directly visualized at both cell and organ levels.

Hydrogen peroxide (H₂O₂) is a main member of the reactive oxygen species (ROS) family and is involved in a wide range of physiological and pathological processes. To date, H₂O₂ has been acknowledged as an oxidative stress marker in aging and disease, a defense agent in immune response to pathogen invasion, and a messenger molecule related to cell proliferation, migration, and differentiation. There is increasing convincing evidence that aberrant production of H₂O₂ contributes to various pathological processes, such as diabetes, cancers, cardiovascular disorders, and neurodegeneration, etc.

To achieve the complete elucidation of H₂O₂ biofunction, an analytical tool capable of monitoring its generation, distribution, and dynamic fluctuation in living systems is desirable. Fluorescence imaging based on small-molecule fluorescent probes has emerged as an efficient methodology to visualize the spatial and temporal distribution of biomolecules in biological specimens due to its real-time, sensitive, and noninvasive characteristics. Compared with other ROS, H₂O₂ is a relatively stable species and exhibits mild reactivity. Therefore, fluorescent probes for H₂O₂ are liable to be interfered upon by highly reactive species, such as peroxynitrite. Up to now, numerous fluorescent sensors for H₂O₂ have been constructed based on different chemical strategies, including H₂O₂-mediated hydrolysis of sulfonic esters, H₂O₂-triggered conversion of aryboronates to phenols, transformation from benzil to benzoic acid, and so on. However, the probes based on sulfonic esters hydrolysis also respond to other ROS and thiols besides H₂O₂. Also, it has been reported that several boronate-based probes react more slowly with H₂O₂ as compared to other reactive species (e.g., peroxynitrite and hypochlorite). Some of them have been used to detect peroxynitrite or benzoyl peroxide instead of H₂O₂. Therefore, there still has been a large demand to develop a new fluorescent probe which features high specificity, excellent sensitivity, and fast response toward H₂O₂.

In this paper, we identify the α-ketoamide moiety as a novel reaction switch specific for H₂O₂. Integration of this unique moiety into the hemicyanine fluorophore afforded a fluorescence “off–on” probe, Mito-NIRHP, for highly specific and sensitive detection of H₂O₂ which features NIR absorption/emission and mitochondrial localization. The practical utility of Mito-NIRHP in biological contexts is demonstrated by imaging basal and endogenous H₂O₂ levels in living cells and in living animals. Moreover, Mito-NIRHP is utilized as an efficient molecular tool to prove that H₂O₂ is overgenerated during ischemia-reperfusion injury (IRI) at both cell and organ levels, corroborating the close relationship between H₂O₂ and this type of organism damage process.

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EXPERIMENTAL SECTION

Materials and Instruments. Unless otherwise stated, all reagents for synthesis were purchased from commercial suppliers and were used without further purification. The solvents were purified by conventional methods before use. All synthesis was carried out under an argon atmosphere, magnetically stirred, and monitored by thin-layer chromatography (TLC). Flash chromatography was performed using silica gel (100–200 mesh). 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenylnitrosoazobromide (MTT) and phosphor myristate acetate (PMA) were purchased from Sigma Chemical Company. Rotenone was from Bide Pharmatech. Ltd. (Shanghai, China). Mito-Tracker Green was purchased from Thermo Fisher Scientific (Shanghai, China). The MGC AnaeroPouch, oxygen indicator, and anaerobic bag were purchased from Mitsubishi Gas Chemical Co. (Tokyo, Japan). All the cells were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Male Kunming mice (20 g) were purchased from the School of Medicine at Shandong University. All animal experiments were in agreement with the guidelines of the Institutional Animal Care and Use Committee. Sartorius Gas Chemical Co. (Tokyo, Japan). All the cells were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Male Kunming mice (20 g) were purchased from the School of Medicine at Shandong University. All animal experiments were in agreement with the guidelines of the Institutional Animal Care and Use Committee. Sartorius Gas Chemical Co. (Tokyo, Japan). All the cells were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Male Kunming mice (20 g) were purchased from the School of Medicine at Shandong University. All animal experiments were in agreement with the guidelines of the Institutional Animal Care and Use Committee. Sartorius Gas Chemical Co. (Tokyo, Japan). All the cells were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Male Kunming mice (20 g) were purchased from the School of Medicine at Shandong University. All animal experiments were in agreement with the guidelines of the Institutional Animal Care and Use Committee.

Analytical Chemistry

Synthesis of Compound 1. This analog was synthesized according to the above procedure starting from pyruvic acid instead of 2-(4-nitrophenyl)-2-oxoacetic acid. 1H NMR (400 MHz, DMSO-d6): δ 10.93 (s, 1H), 8.56 (d, J = 16 Hz, 1H), 8.09 (s, 1H), 7.84 (d, J = 8 Hz, 1H), 7.78 (d, J = 8 Hz, 1H), 7.74 (d, J = 8 Hz, 1H), 7.55 (s, 1H), 7.53 (s, 1H), 7.49–7.46 (m, 1H), 7.42 (s, 1H), 6.62 (d, J = 16 Hz, 1H), 4.47 (s, 2H), 2.71 (s, 2H), 2.67 (s, 2H), 2.45 (s, 1H), 1.81 (s, 2H), 1.76 (s, 6H), 1.38 (s, 3H). HRMS (ESI): calcd for C35H33N2O3 (M+), 467.2329; found, 467.2318.

Synthesis of Compound 2. This analog was synthesized according to the above procedure starting from 2-oxo-2-phenylacetic acid instead of 2-(4-nitrophenyl)-2-oxoacetic acid. 1H NMR (400 MHz, DMSO-d6): δ 11.50 (s, 1H), 8.61 (d, J = 16.0 Hz, 1H), 8.08 (d, J = 8 Hz, 2H), 8.05 (s, 1H), 7.82–7.77 (m, 2H), 7.73 (d, J = 8 Hz, 1H), 7.66–7.59 (m, 4H), 7.55 (t, J = 8 Hz, 1H), 7.47 (s, 2H), 6.65 (d, J = 16 Hz, 1H), 4.48 (d, J = 4 Hz, 2H), 2.75 (s, 2H), 2.70 (s, 2H), 1.85 (s, 2H), 1.76 (s, 6H), 1.39 (s, 3H). HRMS (ESI): calcd for C35H32N3O5 (M+), 574.2385; found, 574.2365.

HepG2 cells were seeded at a density of 1 × 10⁶ cells mL⁻¹ in high-glucose DMEM supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin. Cultures were maintained in a humidified incubator MCO-15A (SANYO, Japan) at 37 °C, in 5% CO₂/95% air. Cells were passaged and plated on 18-mm glass coverslips in a culture dish. The cells were excited at 633 nm with a laser and the emission was collected between 660 and 750 nm.

Animal Models and in Vivo Imaging. Before imaging, the Kunming mice were fasted for 12 h to avoid possible food fluorescence interference to the dye fluorescence. The mice were divided into three groups. The first group was given an injection of Mito-NIRHP (20 μM, 100 μL) into the peritoneal cavity as the negative control. The second group was given an injection of rotenone (2.5 mM, 100 μL, thus 5 mg/kg of animal weight) into the peritoneal cavity followed by injection of Mito-NIRHP (20 μM, 100 μL) at the same region after 1 h. The third group was successively treated with rotenone (2.5 mM, 100 μL) for 1 h, NAC (20 mM, 100 μL, thus 16 mg/kg of animal weight) for 1 h, and Mito-NIRHP (20 μM, 100 μL) at the same region. Before imaging, the mice were anesthetized with 4% chloral hydrate (15 mL/kg) by intraperitoneal injection. Whole body images of the mice were then acquired using an IVIS Lumina III system with 660-nm excitation and 710-nm emission channel.

Confocal Imaging during Oxygen-Glucose Deprivation/Reperfusion (OGD/R). HepG2 cells were washed twice with PBS and then cultured with DMEM without glucose that had been pregassed with 95% N₂/5% CO₂ for 10 min to remove residual oxygen. The dishes were placed into an airtight AnaeroPouch bag, which provided near anaerobic conditions with an O₂ concentration <1% (monitored with the oxygen indicator) and a CO₂ concentration of about 5% within 1 h of incubation at 37 °C. Cells were exposed to these conditions for 5 h to produce OGD. After this, the cultures were washed with
PBS twice, and then incubated again in high-glucose DMEM at 37 °C in 95% air/5% CO₂ (reperfusion) for another 1 h. The H₂O₂ of HepG2 cells produced during above-described OGD/R process was recorded by adding 10 μM Mito-NIRHP and incubating for 10 min, and then imaged with 633-nm excitation and emission collected between 660 and 750 nm.

**Animal Model for Ischemia-Reperfusion Injury (IRI).** Before imaging, the Kunming mice were fasted for 12 h to avoid possible food fluorescence interference to the dye fluorescence. The mice were anesthetized with 4% chloral hydrate (15 mL/kg) by intraperitoneal injection and a laparotomy was performed to expose the kidneys. The right renal ischemia was induced by clamping the corresponding vascular pedicle. An hour later, the ischemic kidney was reperfused by removal of the clamp. Meanwhile, the left kidney was given no operation as the negative control. The mouse was reperfused by removal of the clamp. Meanwhile, the left kidney was reperfused by removal of the clamp. The mouse was reperfused by removal of the clamp. Meanwhile, the left kidney was reperfused by removal of the clamp. The mouse was reperfused by removal of the clamp. Meanwhile, the

**RESULTS AND DISCUSSION.**

**Design, Synthesis, and Screening of Fluorescent Probes for H₂O₂.** Unlike many other ROS that proceed via one-electron transfer or electrophilic oxidation pathways, H₂O₂ serves as a potent nucleophile, especially when deprotonated.⁹ This characteristic opens a direction to the selective detection of H₂O₂ versus other ROS. Meanwhile, previous literature reported that α-ketoacid¹⁶,³⁷ and α-ketocarbonyl¹³,³⁸ both reacted with H₂O₂ smoothly. As an analog to them, α-ketoamide was expected to possess similar reactivity to H₂O₂. We predicted that the α-ketoamide moiety might firstly undergo a direct nucleophilic attack by hydroperoxide anion, then experience a Baeyer–Villiger rearrangement, and finally hydrolysis to carboxylic acid, carbon dioxide, and amine (Scheme 1). Incorporating α-ketoamide moiety onto a suitable fluorophore may generate a specific probe for H₂O₂. Hence, an amino (−NH₂) containing NIR fluorophore, hemicyanine (Cy-NH₂), was selected because of its good biocompatibility, high quantum efficiency, and suitability for living organisms.⁵,⁵⁹,⁶⁰ We envisioned that the probe itself gave almost no fluorescence emission in the absence of H₂O₂. Upon reaction with H₂O₂, the fluorophore Cy-NH₂ was released and showed significant fluorescence enhancement attributing to the intramolecular charge transfer (ICT) process (Scheme 2). On the basis of this assumption, a series of fluorescent probe candidates for H₂O₂ were constructed (Scheme 2) and their reactivity toward H₂O₂ was tested.

Compound 1 was synthesized first (Scheme S1, Supporting Information). Its reactivity was investigated by examining the fluorescence enhancement (λₑx/λₑm = 670/704 nm) after incubation with H₂O₂ (PBS buffer, 50 mM, 1% methanol, pH 7.4) at 37 °C for 1 h. Unfortunately, 1 showed minor fluorescence enhancement after reaction (Figure S1a). Subsequently, compound 2 was synthesized and exhibited one-fold fluorescence increment after incubation with H₂O₂ for 1 h (Figure S1b). This positive result inspired us to design and synthesize a new probe that could readily react with H₂O₂ with high turn-on ratio. Compared with the methyl group of 1, the phenyl group of 2 serves as an electron-withdrawing substituent and enhances the electrophilicity of the adjacent carbonyl group, which can remarkably accelerate the reaction progress. Hence, we introduced a strongly electron-withdrawing nitro group onto the phenyl ring to give Mito-NIRHP. As anticipated, Mito-NIRHP showed a significant fluorescence increment after incubation with H₂O₂ for 10 min (Figure S1c), which apparently outperformed 1 and 2. Therefore, Mito-NIRHP was identified and selected for the comprehensive evaluation in both chemical and biological contexts.

**Specificity of Mito-NIRHP toward H₂O₂.** Before spectral analysis, the specificity of Mito-NIRHP toward H₂O₂ was first evaluated (Figures 1 and S2). As can be seen, the fluorescence could not be triggered on after Mito-NIRHP was incubated with a panel of ROS/RNS/RSS: (1) 100 μM H₂O₂; (2) 100 μM NO; (3) 100 μM ONOO⁻; (4) 100 μM NO; (5) 100 μM ClO⁻; (6) 100 μM TBHP; (7) 100 μM OH; (8) 30 μM O₂⁻; (9) 5 mM Cys; (10) 5 mM GSH; (11) 100 μM Hcy. Therefore, Mito-NIRHP was synthesized and exhibited one-fold fluorescence enhancement after reaction (Figure S1a). Subsequently, compound 2 was synthesized and exhibited one-fold fluorescence increment after incubation with H₂O₂ for 1 h (Figure S1b). This positive result inspired us to design and synthesize a new probe that could readily react with H₂O₂ with high turn-on ratio. Compared with the methyl group of 1, the phenyl group of 2 serves as an electron-withdrawing substituent and enhances the electrophilicity of the adjacent carbonyl group, which can remarkably accelerate the reaction progress. Hence, we introduced a strongly electron-withdrawing nitro group onto the phenyl ring to give Mito-NIRHP. As anticipated, Mito-NIRHP showed a significant fluorescence increment after incubation with H₂O₂ for 10 min (Figure S1c), which apparently outperformed 1 and 2. Therefore, Mito-NIRHP was identified and selected for the comprehensive evaluation in both chemical and biological contexts.

**Figure 1.** Fluorescence responses of 10 μM Mito-NIRHP toward various ROS/RNS/RSS: (1) 100 μM H₂O₂; (2) 100 μM NO; (3) 100 μM ONOO⁻; (4) 100 μM NO; (5) 100 μM ClO⁻; (6) 100 μM TBHP; (7) 100 μM OH; (8) 30 μM O₂⁻; (9) 5 mM Cys; (10) 5 mM GSH; (11) 100 μM Hcy.
to react with certain probes for H2O2. Satisfactorily, neither of these two switched on the fluorescence of Mito-NIRHP. Considering the abundant contents in cells and the potential reactivity to aromatic nitro group, reactive sulfur species (RSS) including cysteine (Cys), glutathione (GSH), hydrogen sulfide (H2S), and homocysteine (Hcy), were also investigated. Encouragingly, no fluorescence increment was observed even when the concentrations of Cys and GSH were as high as 5 mM (Figure 1). Additionally, other biological relevant species including amino acids (Ser, Pro, Thr, Gly, and Val), cations (Na+, K+, Ca2+, Zn2+, Cu2+, Co2+, Mg2+, Fe2+, and Fe3+), anions (NO3−, NO2−, CH3COO−, S2O32−, PO43−, CO32−, SO42−, and SO32−), and vitamin C, did not trigger an obvious fluorescence enhancement (Figure S2). These results collectively implied the high specificity of Mito-NIRHP toward H2O2.

Spectral Properties and Analytical Parameters of Mito-NIRHP. On the basis of the good specificity of Mito-NIRHP to H2O2, the spectral properties of Mito-NIRHP in the absence and presence of H2O2 were then examined. Mito-NIRHP itself displays a blue color with an absorption maximum at 598 nm ($\epsilon = 17500$ L·mol⁻¹·cm⁻¹, Figure S3). However, after addition of different concentrations of H2O2, the solution gradually changed to blue–green with the appearance of a new absorption band of absorption maximum at 670 nm ($\epsilon = 22500$ L·mol⁻¹·cm⁻¹, Figure S3). This indicated the generation of Cy-NH2. The reaction kinetics was investigated through fluorescence spectral analysis. Upon addition of H2O2, the fluorescence intensity of Mito-NIRHP rapidly increased and then reached a maximum (Figure S4), which was faster than the reported H2O2 probes based on arylboronate moiety. The fluorescence spectra variation of Mito-NIRHP upon H2O2 addition showed that the fluorescence intensity gradually increased with the increasing concentration of H2O2 (Figure 2a). This is resulted from the 10-fold higher quantum yield of Cy-NH2 ($\Phi = 0.636$) than Mito-NIRHP ($\Phi = 0.066$). An excellent linear correlation ($R^2 = 0.994$) between the fluorescence intensities (at 704 nm) and H2O2 concentrations in range of 0 to 50 μM was obtained (Figure S3). The limit of detection (LOD) value was calculated to be as low as 26 nM based on S/N = 3, which hinted that the probe was sensitive enough to image basal and endogenous H2O2 in intact biosamples. In addition, Mito-NIRHP kept weakly fluorescence-emitting in pH 6.0–9.0, and exhibited dramatic fluorescence enhancement to H2O2 in pH 7.4–8.6, which was well consistent with the physiological mitochondrial pH range (Figure S5), suggesting the potential suitability of Mito-NIRHP in mitochondria.

Verification of the Reaction Mechanism. The reaction mechanism between Mito-NIRHP and H2O2 was verified and confirmed by HRMS analysis. After the incubation of Mito-NIRHP and H2O2 for 30 min, the peaks at m/z 397.2283 and 166.0153 were both detected out in the reaction mixture, which agreed well with the fluorophore Cy-NH2 and the byproduct 4-nitrobenzoic acid, respectively (Scheme S2 and Figure S6). The result testified to the proposed reaction mechanism in Scheme 1.

Imaging the Basal and Endogenously Produced H2O2 in Mitochondria. The aforementioned encouraging properties of Mito-NIRHP in chemical system prompted us to investigate its capability of detecting H2O2 in living cells. Prior to being applied in cell imaging, the MTT assay was performed to assess the biocompatibility of Mito-NIRHP (Figure S7). The IC50 value for HepG2 cells was calculated to be 96 μM, implying the low cytotoxicity of Mito-NIRHP to culture cells and its potential utility in biological systems.

The subcellular distribution of Mito-NIRHP was mapped. The HepG2 cells were costained with Mito-NIRHP and Mitotracker Green (MTG), a commercially available mitochondrial marker. As displayed in Figure S8, the images of red channel and green channel merged well, and the intensity profiles of linear regions of interest (ROIs) across a HepG2 cell of the two channels tended toward synchronization. Moreover, the Pearson’s colocalization coefficient of Mito-NIRHP and MTG was calculated to be 0.96, indicating that Mito-NIRHP was organelle-specifically trapped in mitochondria.

Subsequently, the capability of Mito-NIRHP to image basal level of H2O2 in living cells was investigated (Figure 3 and Figure S9). It was shown that the probe-loaded HepG2 cells displayed observable signal because of the fluorescence enhancement induced by basal intracellular H2O2 (Figure 3a).
Meanwhile, the fluorescence was apparently attenuated in the presence of N-acetylcysteine (NAC), a widely used antioxidant for H$_2$O$_2$ elimination (Figure 3b). The outcome confirmed that Mito-NIRHP was sensitive enough to image the basal intracellular H$_2$O$_2$.

After that, Mito-NIRHP was utilized to image the fluctuation of H$_2$O$_2$ in living cells. Phorbol myristate acetate (PMA), a well-established H$_2$O$_2$ inducer through a cellular inflammation response, and rotenone, an inhibitor of complex-I of the mitochondrial electron-transport chain, were employed as stimulators to initiate the up-regulation of intracellular H$_2$O$_2$. The fluorescence images were acquired and are shown in Figure 4 and Figure S10. As can be seen, the Mito-NIRHP loaded HepG2 cells gave weak fluorescence emission (Figure 4a). By contrast, a significant fluorescence increase was observed after the cells were pretreated with PMA (Figure 4b) or rotenone (Figure 4c). Meanwhile, these two fluorescence increases were both efficiently blocked by NAC (Figure 4d and 4e). These results suggested that Mito-NIRHP was capable of detecting endogenously stimulated H$_2$O$_2$ in the mitochondria.

**Visualizing Endogenous H$_2$O$_2$ in Animal Models.** The NIR absorption/emission property endows Mito-NIRHP with the feasibility of visualizing endogenous H$_2$O$_2$ in living animals. In brief, Kunming mice were divided into three groups. The first group was given an injection of Mito-NIRHP into the peritoneal cavity as the negative control. The second group was given an injection of rotenone into the peritoneal cavity and then stained with Mito-NIRHP (10 μM, 10 min) before imaging. (a) Control. (b) PMA (1 μg/mL, 30 min). (c) Rotenone (100 nM, 30 min). (d) PMA (1 μg/mL, 30 min) and then NAC (20 mM, 1 h). (e) Rotenone (100 nM, 30 min) and then NAC (20 mM, 1 h). (f) Relative fluorescence intensities of (a)–(e). The images were acquired using a confocal microscope with 633-nm excitation and 660–750-nm collection. Scale bar: 10 μm.

**Imaging H$_2$O$_2$ during IRI.** IRI occurs when the blood supply to an organ is disrupted and then restored. It presents remarkable clinical significance since it is closely associated with ischemic diseases, surgery, and organ transplantation. Previous reports proved that ROS initiated oxidative damage, cell death, and aberrant immune responses in ischemia-reperfusion tissue/organ. H$_2$O$_2$, as a prominent member of ROS family, is supposed to play a predominant role in IRI. Herein, an in vitro ischemia-reperfusion model was established in which HepG2 cells were exposed to oxygen-glucose deprivation/reperfusion (OGD/R). Mito-NIRHP was employed to measure the H$_2$O$_2$ fluctuations during OGD/R. Figure 6A and Figure S11 exhibit that cells in OGD/R group emitted enhanced fluorescence intensity in comparison with the control group, indicating an elevated level of H$_2$O$_2$ during the intracellular OGD/R process. After that, an IRI animal model was constructed and the generated H$_2$O$_2$ was also detected.

**Figure 4.** Fluorescence images of endogenously stimulated H$_2$O$_2$ in HepG2 cells. Cells were treated with stimulators or scavenger and then stained with Mito-NIRHP (10 μM, 10 min) before imaging. (a) Control. (b) PMA (1 μg/mL, 30 min). (c) Rotenone (100 nM, 30 min). (d) PMA (1 μg/mL, 30 min) and then NAC (20 mM, 1 h). (e) Rotenone (100 nM, 30 min) and then NAC (20 mM, 1 h). (f) Relative fluorescence intensities of (a)–(e). The images were acquired using a confocal microscope with 633-nm excitation and 660–750-nm collection. Scale bar: 10 μm.

**Figure 5.** Fluorescence imaging (pseudocolor) of H$_2$O$_2$ in Kunming mice. (a) Only the Mito-NIRHP (20 μM, 100 μL) was injected as control. (b) Mouse pretreated with rotenone for 1 h and then injected with Mito-NIRHP. (c) Mouse successively injected with rotenone, NAC, and Mito-NIRHP. (d) Relative fluorescence intensities of (a)–(c). The excitation filter was 660 nm, and the emission filter was 710 nm.

**Figure 6.** Fluorescence imaging of H$_2$O$_2$ produced during IRI in HepG2 cells (A) and organs (B). (A) Cells stained with 10 μM Mito-NIRHP for 10 min (a) and cells exposed to OGD for 5 h followed by 1 h reperfusion, and then stained with 10 μM Mito-NIRHP for 10 min (b). (c) Relative fluorescence intensities of (a) and (b). Scale bar: 10 μm. (B) The isolated right kidney (suffered from IRI) and left kidney (negative control) of a Kunming mouse (a). (b) Relative fluorescence intensities of (a).
mouse was anesthetized and its peritoneal cavity was opened to expose the kidneys. The right renal ischemia was induced by clamping the corresponding vascular pedicle. An hour later, the ischemic kidney was reperfused by removal of the clamp. Meanwhile, the left kidney was given no operation as the negative control. The mouse was injected with Mito-NIRHP via tail vein. After 10 min, the kidneys were isolated and imaged (Figure 6B). It was obvious that the injured right kidney showed much stronger (approximately 3-fold higher) fluorescence signal than the left one, indicating that H2O2 was indeed overproduced during the IRI process. The direct visualization of the H2O2 up-regulation by Mito-NIRHP in IRI model at both cell and organ levels indicated that H2O2 is closely related to this type of organism damage.

■ CONCLUSION

In summary, a new NIR probe specific for H2O2 has been developed utilizing the unique reaction of α-ketoamide moiety and H2O2 in combination with an ICT mechanism. The proposed probe, Mito-NIRHP, demonstrates favorable characteristics in terms of high specificity, high sensitivity, rapid response, and mitochondrial localization, enabling the tracking of endogenously produced H2O2 in both living cells and living animals. In particular, with the aid of Mito-NIRHP, the visualization of H2O2 up-regulation was realized in ischemia-reperfusion injury models, presenting the direct evidence that there is a close relationship between H2O2 and ischemia-reperfusion injury. Thus, we anticipate that the superior properties of Mito-NIRHP will make it of great potential use in efficient H2O2 monitoring. Meanwhile, the robust reaction switch, α-ketoamide moiety, might be installed to alternative fluorophores to create a variety of H2O2 sensors for diverse bioanalytical and biomedical applications.

■ ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b01256.

Experimental details, supplementary data, and synthesis route and characterization of compounds (PDF)

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Notes

The authors declare no competing financial interest.

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