

Near-infrared and lysosome-targetable fluorescent probe based on phenoxazinium for detection of hydrogen peroxide

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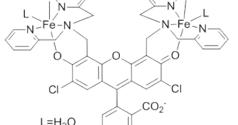
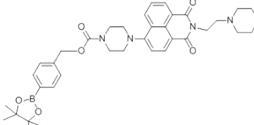
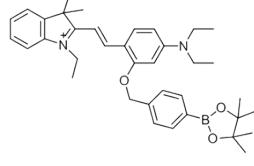
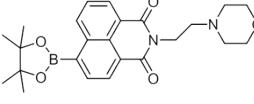
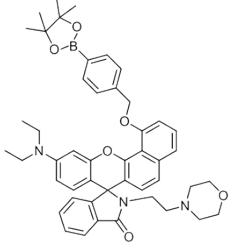
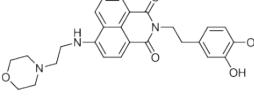
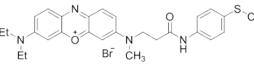
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Table. S1 Properties of the reported lysosome targeted probes for H₂O₂.

| Probes | Signaling mode | $\lambda_{Em,max}^a$ | LOD ^b | Response time | Working solution | references |
|---|------------------------|----------------------|------------------|---------------|--|---|
|  | Turn-on | 536 | 29 μ M | 10 min | PIPES (pH 7.0) | <i>Chem. Commun.</i> 2012, 48, 5449 |
|  | Turn-on | 528 | ---- | 30 min | PBS / DMF (99:1, pH 7.4) | <i>Sci Rep.</i> 2015, 5, 8488 |
|  | Turn-on | 584 | 0.23 μ M | 10 min | Acetate buffer (pH 5.0) | <i>Anal. Chem.</i> 2016, 88, 5865 |
|  | Turn-on ((two photon)) | 550 | 1.21 μ M | 160 s | PBS / DMF (1:1, pH 7.4) | <i>Biosens Bioelectron.</i> 2016, 79, 237 |
|  | Turn-on | 606 | 0.06 μ M | 9 min | Acetate buffer (pH 4.5) | <i>Analyst.</i> 2017, 142, 4522 |
|  | Turn-on | 537 | 0.22 μ M | 60 min | PBS / DMSO (199:1, pH 7.4) PBS / DMSO (9:1, pH 7.4) | <i>Chem. Commun.</i> 2017, 53, 3701 |
|  | Turn-on | 676 | 0.21 μ M | 25 min | DMSO (9:1, pH 7.4) | <i>This work</i> |

^a Reported in nm. ^b The reported limit of detection (LOD) of the corresponding probes.

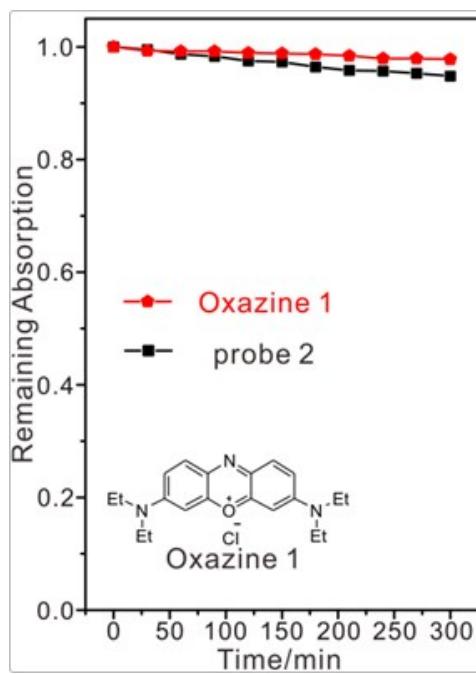


Fig. S1 Photofading behaviors of probe **2** and Oxazine **1** in acetonitrile.

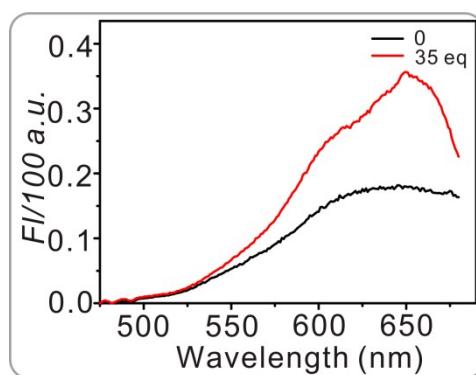


Fig. S2 Excitation spectra of probe **2** ($10 \mu\text{M}$, $\lambda_{\text{em}} = 675 \text{ nm}$, slit width: $3 \text{ nm}/1.5 \text{ nm}$) towards the H_2O_2 in PBS buffer (20 mM , $\text{pH}=7.4$) containing 10% DMSO.

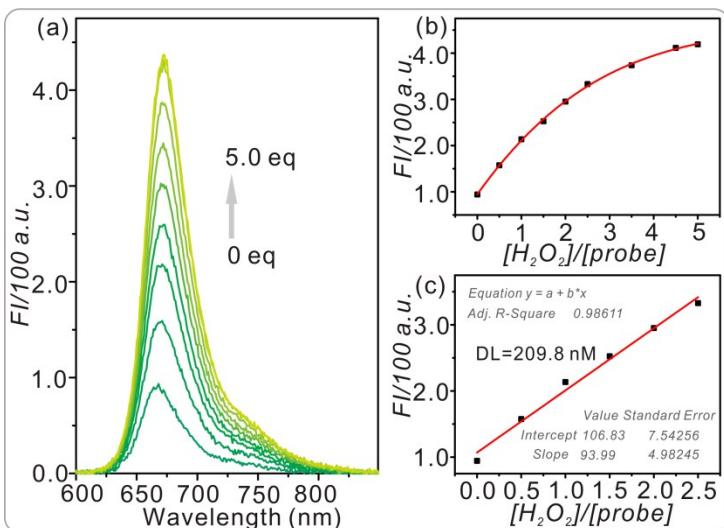


Fig. S3 Detection limit test of probe **2** (1 μM) toward different H_2O_2 concentration in PBS buffer (20 mM, pH=7.4). (a) Emission spectra ($\lambda_{\text{ex}} = 590 \text{ nm}$, slit width: 5 nm/3 nm). (b) Plot of the fluorescence intensity upon addition of H_2O_2 (0–2.5 μM).

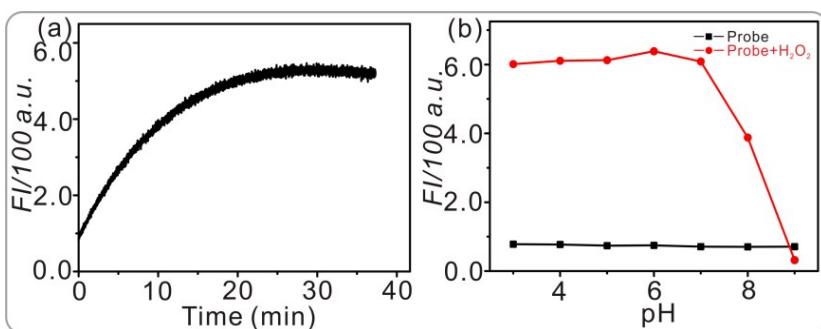


Fig. S4 The time course response and pH-dependent of the probe **2** (10 μM) to H_2O_2 (35.0 equiv.) in PBS buffer (20 mM, pH=7.4). (a) Time course response. (b) The pH-dependent response for free probe and probe + H_2O_2 ; All data represent the fluorescence intensity at 676 nm ($\lambda_{\text{ex}} = 590 \text{ nm}$, slit: 3/3 nm).

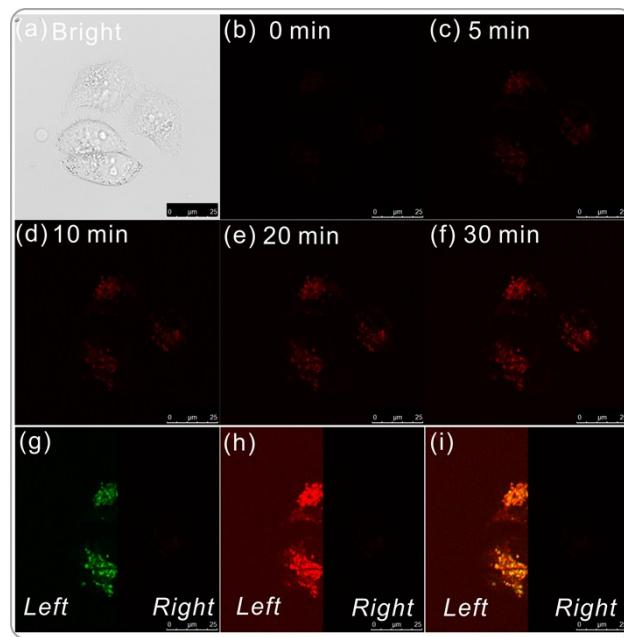


Fig. S5 Fluorescence confocal images of HeLa cancer cells with probe **2** (10 μ M) and LysoTracker Green DND-26 (50 nM). (a) Bright-field transmission image; (b) Confocal image (red channel) with probe **2** before treatment of H₂O₂; (c–f) confocal images of cells with probe **2** after treatment of H₂O₂ (10 mM) for 5 min, 10 min, 20 min, 30 min; (g) left, confocal images (green channel) of cells with LysoTracker Green DND-26; right, the right half of (b); (h) left, enhanced background light of (b); right, the right half of (b); (i) merged images of (g) and (h).

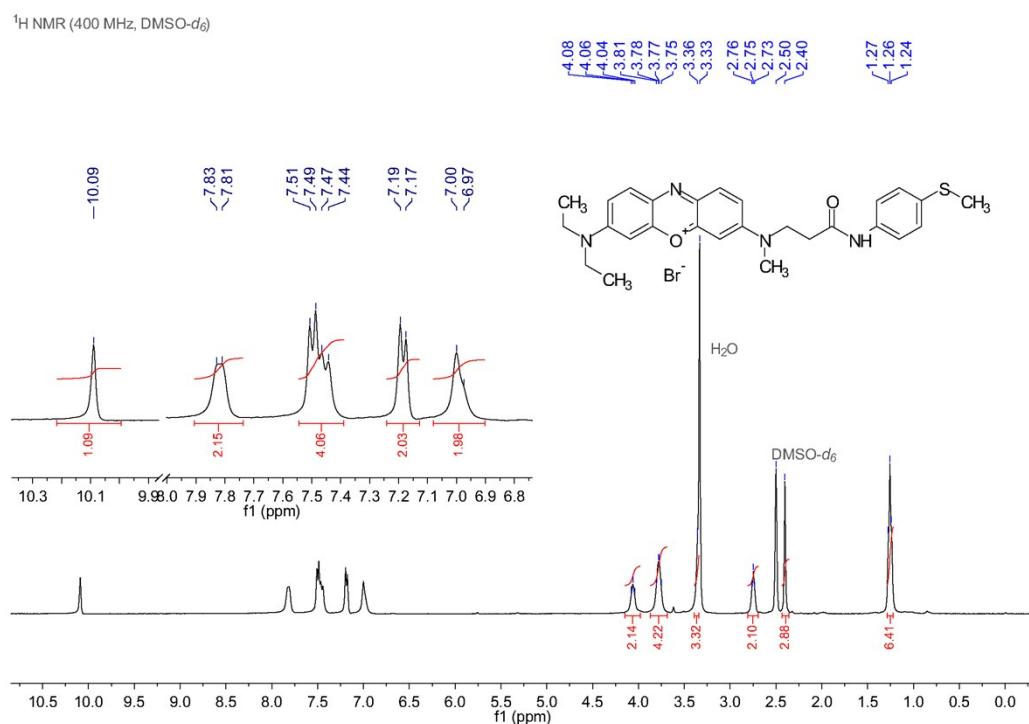
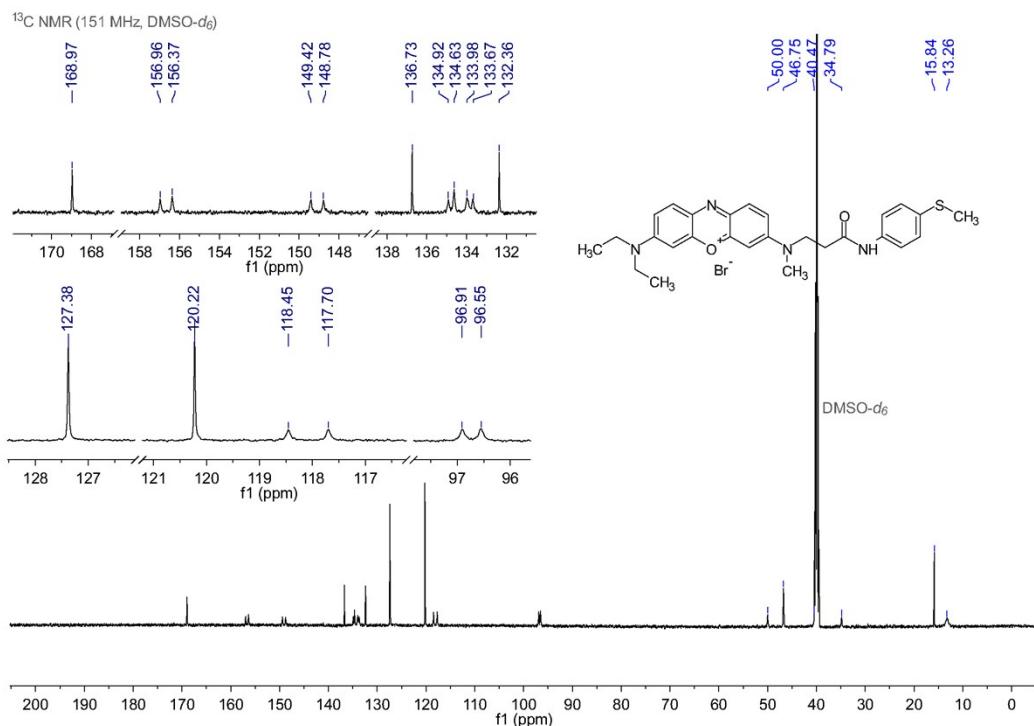
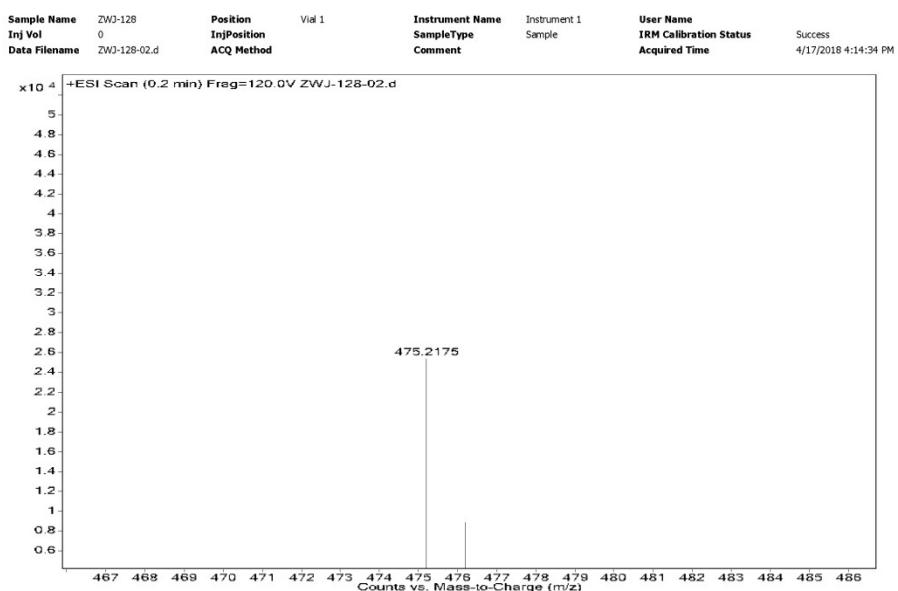


Fig. S6 ¹H-NMR spectrum of compound **2**.

**Fig. S7** ¹³C-NMR spectrum of compound 2.**Fig. S8** HRMS(ESI⁺) spectrum of compound 2.

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|---------------|--------------|-------------|--------|-----------------|--------------|------------------------|
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| Inj Vol | 0 | InjPosition | | SampleType | Sample | IRM Calibration Status |
| Data Filename | zwj-131-03.d | ACQ Method | | Comment | | Acquired Time |

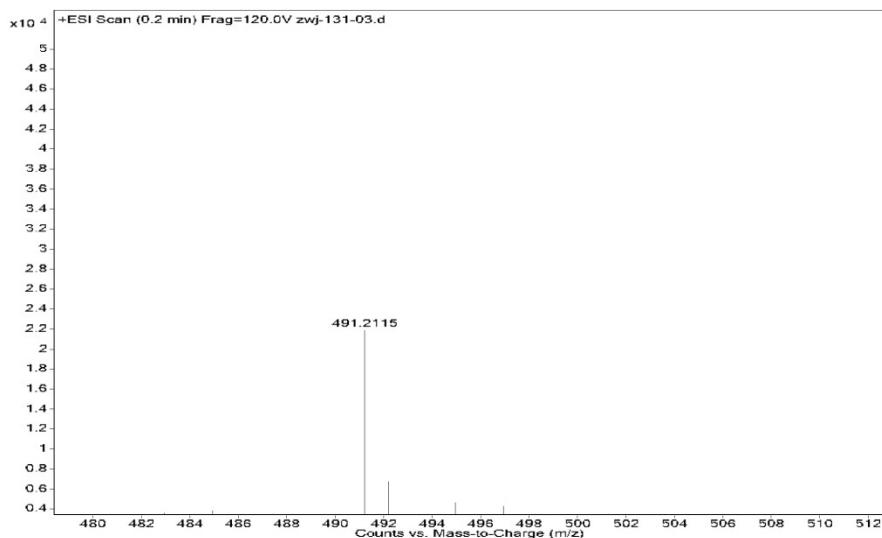


Fig. S9 Mass spectrum of **2** + H₂O₂ adduct.