Supporting Information

Ratiometric Photoelectrochemical Microsensor Based on Small-Molecule Organic Semiconductor for Reliable in Vivo Analysis

Yunhui Xiang,[a] Yao Kong,[b] Wenqi Feng,[a] Xiaoxue Ye,[*b] and Zhihong Liu,[*a]

[a.] *College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China. E-mail: zhhliu@whu.edu.cn*

[b.] *College of Chemistry and Chemical Engineering, Hubei university, Wuhan 430062, China. E-mail: yexxchem@hubu.edu.cn*

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Experimental section

Reagents and Solutions. Benz[cd]indol-2(1H)-one, Cyclohexanone, 2-Hydroxy-4-Methoxybenzaldehyde, Phosphorus oxychloride, 2,4-Dinitrobenzenesulfonyl chloride, Acetyl chloride, 3-Bromopropyne, 3-(azidopropyl)triethoxysilane, N-methyl-D-aspartic acid (NMDA), 4,4'-Diisothiocyanatostilbene-2,2'-disulfonic acid disodium salt hydrate (DIDS), ascorbate (AA), dopamine (DA), uric acid (UA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT), epinephrine (E), and norepinephrine (NE) etc. were all purchased from Sigma and the solutions were prepared just before use. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. All aqueous solutions were prepared by using ultrapure water with a resistivity of 18.25 MΩ (purified by Milli-Q system supplied by Millipore). The pH of the buffer solution was controlled by a digital pH meter (FE20, Mettler-Toledo). Artificial cerebrospinal fluid (aCSF) was prepared as reported previously.[1] In the selectivity test, different reactive oxygen species (ROS) and reactive nitrogen species (RNS) were produced as follows: Hydroxy radical (·OH) was generated through Fenton Chemistry (Fe^{2+}/H_2O_2 = 1: 6). Peroxynitrite ($ONOO^-$) stock solution was prepared by a reported method: HCl (0.6 M, 10 mL) was added to a vigorously stirred solution of $NaNO_2$ (0.6 M, 10 mL) and H_2O_2 (0.7 M, 10 mL) in deionized H_2O at 0 ℃, immediately followed by the rapid addition of NaOH (1.5 M, 20 mL). Other ROS and RNS were prepared according to literature methods.[2]

Apparatus and Measurements. Mass spectrometry was performed on Ultimate3000&Compact (Bruker, Germany), LTQ Orbitrap Elite (Thermo Fisher Scientific, America) and Biotech Axima TOF2 (Shimadzu, Japan) mass spectrometers. SEM images and were taken using field emission gun Hitachi S-4800 (Japan) scanning electron microscope operated at 4.0 kV. UV-Vis. spectra were recorded using a UV 2550 UV-Vis spectrophotometer (Shimadzu, Japan). NIR-II fluorescence spectra were excited by an 808-nm laser (Beijing Hi-Tech Optoelectronic Co., Ltd.) and recorded with a fluorometer (Fluorolog-3, Horiba Jobin Yvon, France). Confocal microscopy images of U87 cells were conducted on Zeiss LSM 880 Microscope. In vivo NIR-II fluorescence images were acquired by In-Vivo Master NIR-II fluorescence imaging system (Grand Imaging Technology Co. Ltd., Wuhan).

Method

Optical responses of CyOR in solution. CyOR were dissolved in DMSO and then diluted with PBS/MeCN mixed solution for optical spectra measurement. The final concentration of the **CyOR** were all 10 μM. The absorption spectra and fluorescence spectra of **CyOH**, **CyOMe** and **CyOAc** in 0.1 M PBS/MeCN (pH = 7.4) containing 1% DMSO were recorded. The absorption changes of **CyOH** in 0.1 M PBS/MeCN (6:4) with different pH values (pH = 3.5, 4.0, 4 5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.4, 8, 8.5 and 9.0) containing 1% DMSO were recorded. The absorption changes of **CyOThiols** upon the addition of 500 μM Cys in 0.1 M PBS/MeCN (6:4, pH = 7.4) containing 1% DMSO were recorded every 1 min of mixing. The fluorescence changes of **CyOThiols** upon the addition of different concentrations of Cys in 0.1 M PBS/MeCN (6:4, pH = 7.4) containing 1% DMSO were recorded after 2 min of mixing. The absorption changes of **CyOALP** upon the addition of 500 $U \cdot L^{-1}$ ALP in 0.1 M PBS/MeCN (8:2, pH = 7.4) containing 1% DMSO were recorded every 1 min of mixing.

Pretreatment of titanium wires. The titanium wires were soaked in concentrated nitric acid for 12 h, soaked in piranha solution for five minutes and then cleaned with ultrapure water for three times. Next, the hydroxylated titanium wires were placed in an absolute ethyl alcohol solution containing 1 mM 3-(azidopropyl)triethoxysilane for 24 h. Finally, the obtained titanium wires were washed three times with absolute ethyl alcohol.

Preparation of microelectrodes CyOH/TiWE**, CyOMe**/TiWE**, CyOAc**/TiWE**, CyOThiols**/TiWE **and CyOALP**/TiWE. The obtained titanium wire was cut into 1 cm length for the next step of microelectrode preparation. 1 mm length of titanium wire was reserved and the rest part was sealed into the glass capillary to obtain microelectrode TiWE. Next, TiWEs were placed in a solution of DMF containing 0.1 mM copper acetate, 1.2 mM AA and 1mM **CyOH** for 12 h. The obtained electrode was cleaned three times with DMF to obtain **CyOH**/TiWE. Similarly, the same process was used for the preparation of **CyOMe**/TiWE, **CyOAc**/TiWE, **CyOThiols**/TiWE and **CyOALP**/TiWE.

Photoelectrochemical experiment. The photoelectrochemical experiments were performed on an electrochemical workstation (CHI 660E, Chenhua Co. Ltd, Shanghai, China) with a two-electrode system, where the platinum wire was used as the counter and reference electrode, TiWE was used as the working electrode. A coaxial optical fiber containing 750-nm and 808-nm beam was employed as light sources with spots size of 1 cm^2 (60 $mW \cdot cm^{-2}$).

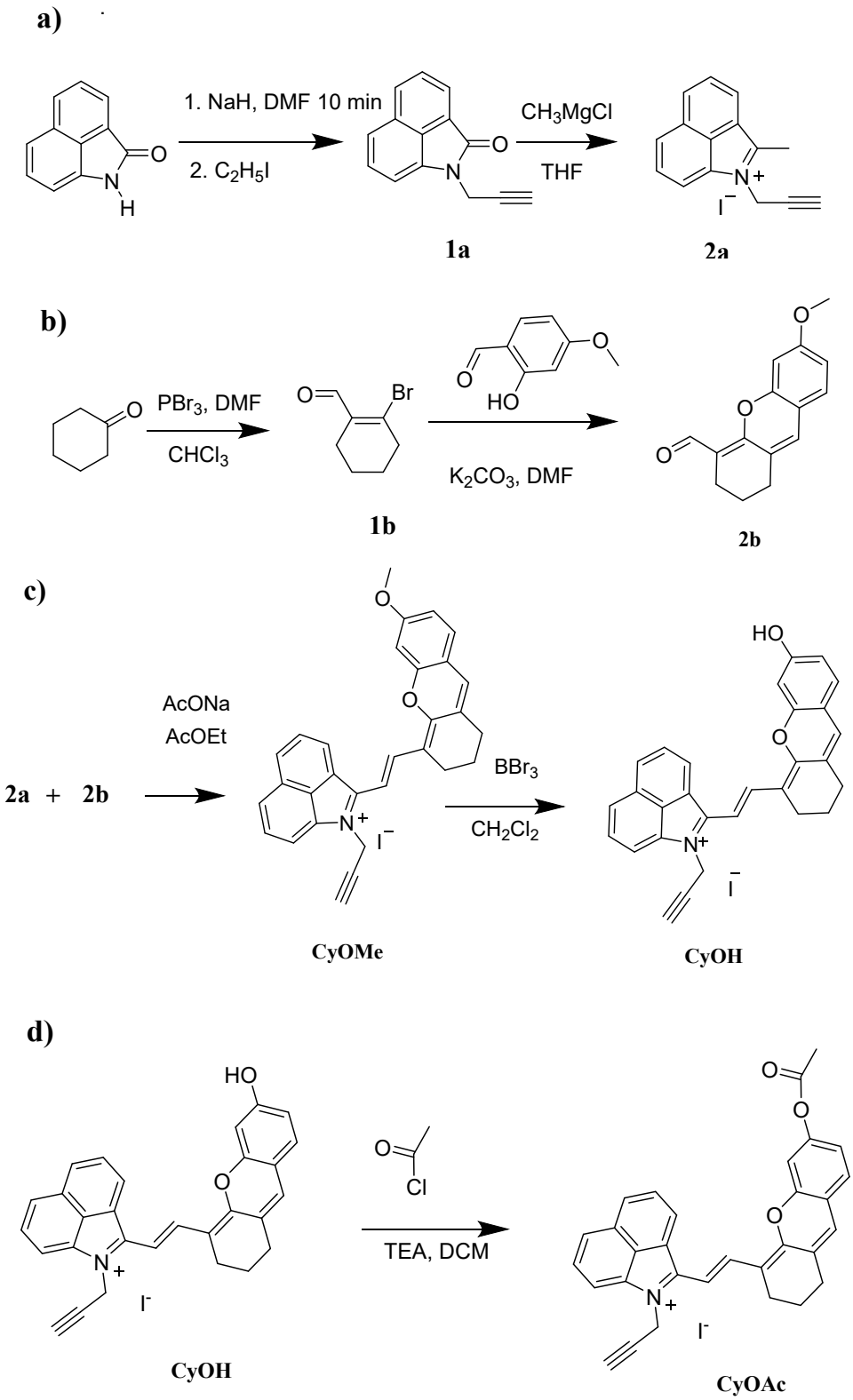
Cell culture and biocompatibility test. U87 cells were used for cytotoxicity experiment. The cells were seeded on the **CyOThiols** coated titanium sheet substrates (1.0 cm × 1.0 cm × 100 μM), and cultured in Dulbecco's modified Eagle's medium (DMEM) in a petri dish at 37 ℃ under 5% CO_2 atmosphere for 3 days. The DMEM contained 10% of fetal bovine serum (FBS), and 100 $U \cdot mL^{-1}$ penicillin/streptomycin. Calcein-AM (live/green cytoplasmic stain), Propidium Iodide (PI) (dead/red nucleic stain) were added to the medium at a final concentration of 3 $\mu g \cdot mL^{-1}$ following the anufacturer's protocols to stain these cells before fluorescence imaging. After staining for 15 min, the residual dyes were washed off. The microphotographs were taken on the confocal fluorescence microscope.

Mice model of drug-induced cytotoxic edema. Local drug delivery by exogenous microinfusion of NMDA was constructed with a silica capillary (4 cm length, 50 μm in inner diameter, 375 μm in outer diameter) bound with the **CyOThiols**/TiWE in parallel. Infusion solution was delivered from a gastight syringe and pumped through tetrafluoroethylene hexafluoroproene (FEP) tubing by PHD 2000 infusion pumps.

(kd Scientific LEGATO130, USA). NMDA local microinfusion was performed at a perfusion rate of 1.0 μL·min^{-1} for 1 min. In inhibitor treatment, the mixture containing 500 μM NMDA and 5 mM DIDS was applied at a perfusion rate of 1.0 μL·min^{-1} for 1 min.

Mice model of drug-induced epilepsy. KA-induced different stages seizure rats are ignited by injecting different doses of KA. Symptoms of epilepsy in rats were graded by Racine. Racine II stage epilepsy was induced by an injection of 30 mg·kg^{-1} KA while Racine III stage epilepsy was induced by injection of 50 mg·kg^{-1} KA. Rats with facial clonus and rhythmic nodding were classified into Racine II stage. While, rats with facial clonus, rhythmic nodding and myoclonus of forelimbs, but no upright position of hind limbs were classified into Racine III stage. The rats with corresponding symptoms (Racine II stage or Racine III stage) were selected as the experimental group. The control group were intraperitoneal injection with a volume of saline equal to that of the KA injection.

***In Vivo* Experiments.** All animal studies were performed according to the Guidelines for the Care and Use of Laboratory Animals of the Chinese Animal Welfare Committee and approved by the Institutional Animal Care and Use Committee, Wuhan University Center for Animal Experiment, Wuhan, China. The animals were housed on a 12:12 h light-dark schedule with food and water ad libitum. The animals were anaesthetized with isoflurane (4% induction, 2% maintenance) through a gas pump (RWD R520, Shenzhen, China) and positioned onto a stereotaxic frame during in vivo experiment. The implanted microelectrodes were implanted into the cortex (AP = 2.3 mm, *L* = 2.4 mm from bregma, *V* = 1.5 mm from skull) or hippocampus (AP = 3.3 mm, L = 1.5 mm from bregma, V = 2.8 mm from skull) using standard stereotaxic procedures. Platinum wire (100 μm) was embedded in subcutaneous tissue on the brain and used as both counter and reference electrode. The **CyOThiols**/TiWE was respectively driven by 808 nm (60 mW cm^{-2}) and 750 nm (60 mW cm^{-2}) coaxial laser at a bias voltage of 0 V (Vs Pt^0).



Scheme S1. Synthetic routes **CyOMe**, **CyOH** and **CyOAc**.

Synthesis of 1a. NaH (3.6 g, 150 mmol) was added into a solution of benzo[cd]indol-2(1H)-one (8.5 g, 50.2 mmol) in 150 mL of anhydrous DMF under Ar atmosphere. Then the mixture was cooled to 0 °C, and 3-Bromopropyne (7.4 g, 62.2 mmol) was added dropwise. The mixture was stirred at room temperature for 3 h and then extracted with ethyl acetate, washed with brine, and concentrated. The crude product was then purified by column chromatography to give a yellow solid of 1a (9.6 g, 46.3 mmol 92.2 %). 1H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 7.0 Hz, 1H), 8.03 (d, J = 8.1 Hz, 1H), 7.72 (dd, J = 8.1, 7.1 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.50 (dd, J = 8.4, 7.1 Hz, 1H), 7.13 (d, J = 7.0 Hz, 1H), 4.74 (d, J = 2.5 Hz, 2H), 2.28 (t, J = 2.5 Hz, 1H).

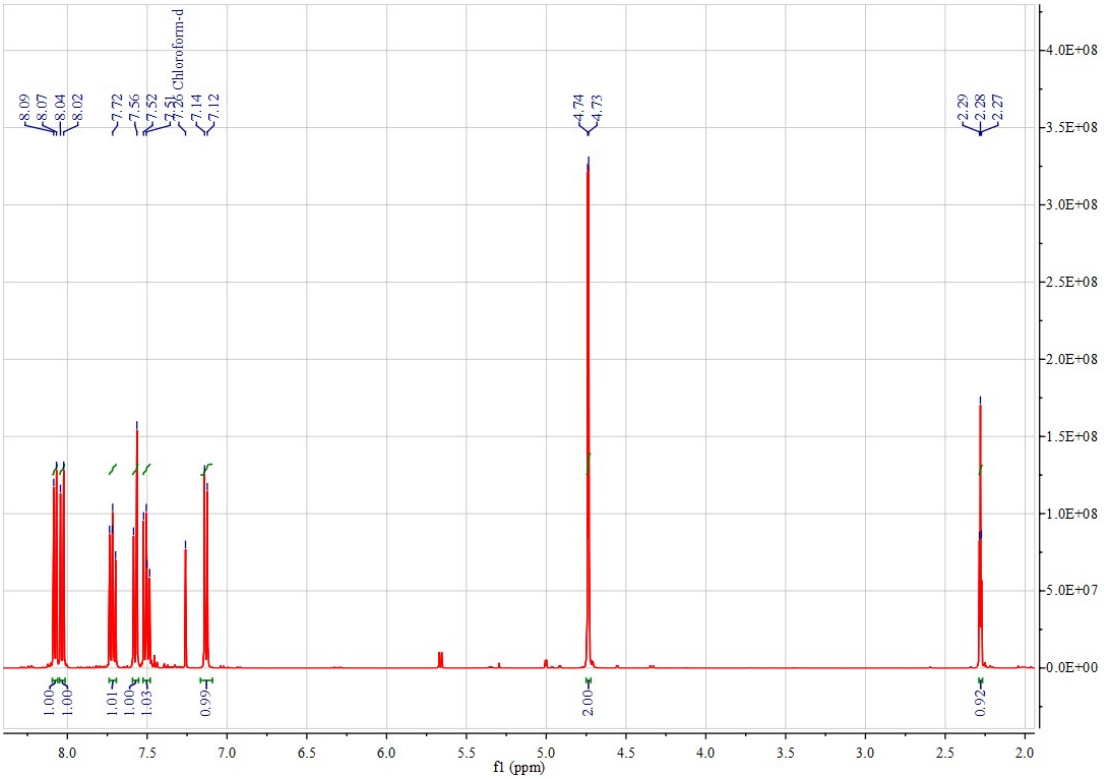


Figure S1. ^1H NMR spectrum (in $CDCl_3$) of an equivalent mixture of compound 1a.

Synthesis of 2a. Methylmagnesium chloride (3 M solution in THF, 4 mL, 12 mmol) was added dropwise to a solution of 1a (2 g, 9.7 mmol) in 40 mL anhydrous THF under Ar atmosphere. After stirring at 60 °C for 1 h, the mixture was cooled down and hydrochloric acid (2 M, 12 mL, 24 mmol) was added to the mixture. Then THF was removed by vacuum distillation, and KI solution (1 M, 10 mL) was added to obtain orange solid. The crude product was filtered, washed with water and ethyl acetate, and dried. (2.4 g, 7.2 mmol, 74%). 1H NMR (400 MHz, DMSO) δ 7.70 (dd, J = 11.9, 7.7 Hz, 2H), 7.54 (t, J = 7.6 Hz, 1H), 7.34 (t, J = 7.7 Hz, 1H), 7.15 (d, J = 8.3 Hz, 1H), 6.67 (d, J = 7.2 Hz, 1H), 5.16 (s, 1H), 4.71 (s, 1H), 4.65 (d, J = 1.7 Hz, 2H), 3.18 (s, 1H).

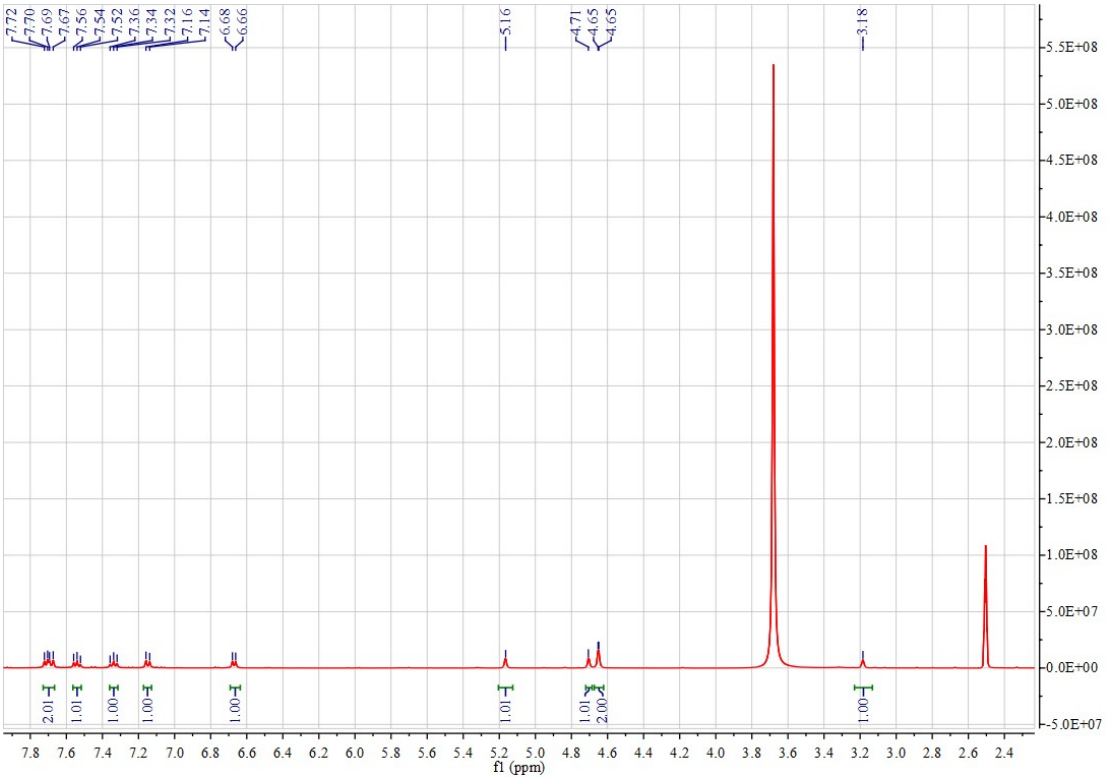


Figure S2. [1]H NMR spectrum (in DMSO) of an equivalent mixture of compound **2a**.

Synthesis of 2-bromocyclohex-1-ene-1-carbaldehyde (1b). PBr_3 (12.4 ml, 130.5 mmol) was dropwise added to a mixture of DMF (11.2 mL) and chloroform (50 mL) at 0 °C. Then the mixture was stirred for 30 min before the addition of cyclohexanone (5 mL, 48.4 mmol). The resulting solution was stirred for 16 h at room temperature, before it was poured into 50 mL of ice water, neutralized with solid $NaHCO_3$, and extracted with dichloromethane. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuum to provide a yellow oil, compound 1b (7.8 g, 41.2 mmol, 81 %), which was used directly in the next step.

Synthesis of 6-methoxy-2, 3-dihydro-1H-xanthene-4-carbaldehyde (2b). 2-hydroxy-4-methoxybenzaldehyde (5.8g, 38 mmol) and K_2CO_3 (5.8, 42 mmol) were added to compound 1b (7.8 g, 41.3 mmol) in DMF (40 mL) at 25 ℃. The mixture was stirred for 16 h at 37 ℃. Then, 200ml water was added to the reaction system, and the product was extracted with 400 mL (2 × 200 mL) ethyl acetate (AcOEt). The organic layer was washed with H_2O (2 × 150 mL) and dried over Na_2SO_4, filtered and concentrated in vacuum. Purification by silica gelcolumn chromatography (AcOEt/Petroleum ether (PE) = 20:1) provided compound 2b as a deep yellow solid (5.6 g, 60.8 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.30 (s, 1H), 7.07 (d, J = 9.1 Hz, 1H), 6.66 – 6.63 (m, 3H), 3.83 (s, 3H), 2.57 – 2.53 (m, 2H), 2.43 (t, J = 6.1 Hz, 2H), 1.73 – 1.67 (m, 2H).

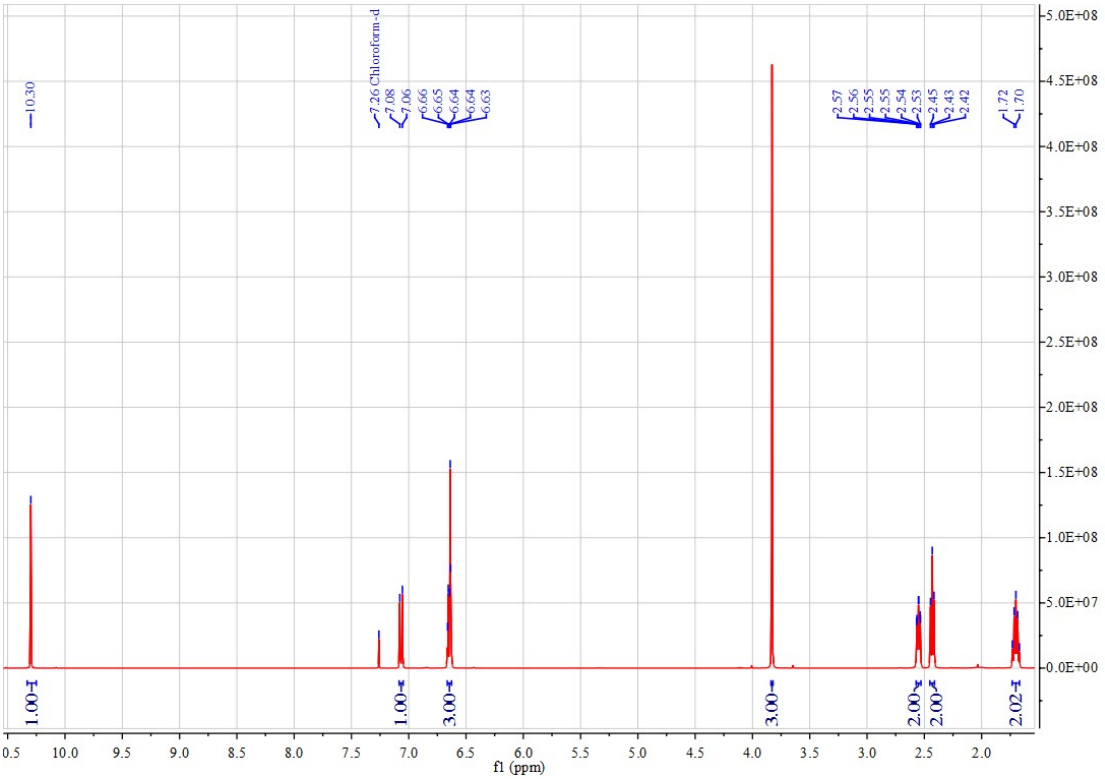


Figure S3. ^{1}H NMR spectrum (in $CDCl_3$) of an equivalent mixture of compound **2b.**

Synthesis of CyOMe. Anhydrous NaOAc (300 mg, 3.7 mmol) and compound 2a (1.0 g, 3.0 mmol) were added to a solution of compound 2b (0.75g, 3.1 mmol) in 10 mL anhydrous Ac_2O. After stirring for 2 h at 70 ℃, the deep green solution was obtained. Then the mixture was cooled to room temperature, and 100 mL of diethyl ether was added. The black precipitate was filtered and washed with 100 mL water. The solid with a metallic luster was recrystallized in EtOH to give **CyOMe** (1 ^{1}H NMR (400 MHz, DMSO) δ 9.02 (d, *J* = 13.4 Hz, 1H), 8.73 (d, *J* = 7.3 Hz, 1H), 8.38 (d, *J* = 7.9 Hz, 1H), 8.08 (t, *J* = 7.5 Hz, 1H), 7.94 (s, 1H), 7.89 (d, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 7.1 Hz, 1H), 7.76 – 7.68 (m, 2H), 7.51 (d, *J* = 1.5 Hz, 1H), 7.16 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.94 (d, *J* = 13.4 Hz, 1H), 5.46 (s, 2H), 4.01 (s, 3H), 3.62 (s, 1H), 2.83 (d, *J* = 4.5 Hz, 4H), 1.95 – 1.85 (m, 2H). HRMS calcd for $C_{30}H_{24}NO_2^+$ ([M]) 430.1802 found 430.1796. $C_{30}H_{24}INO_2$ Wt 557.4315

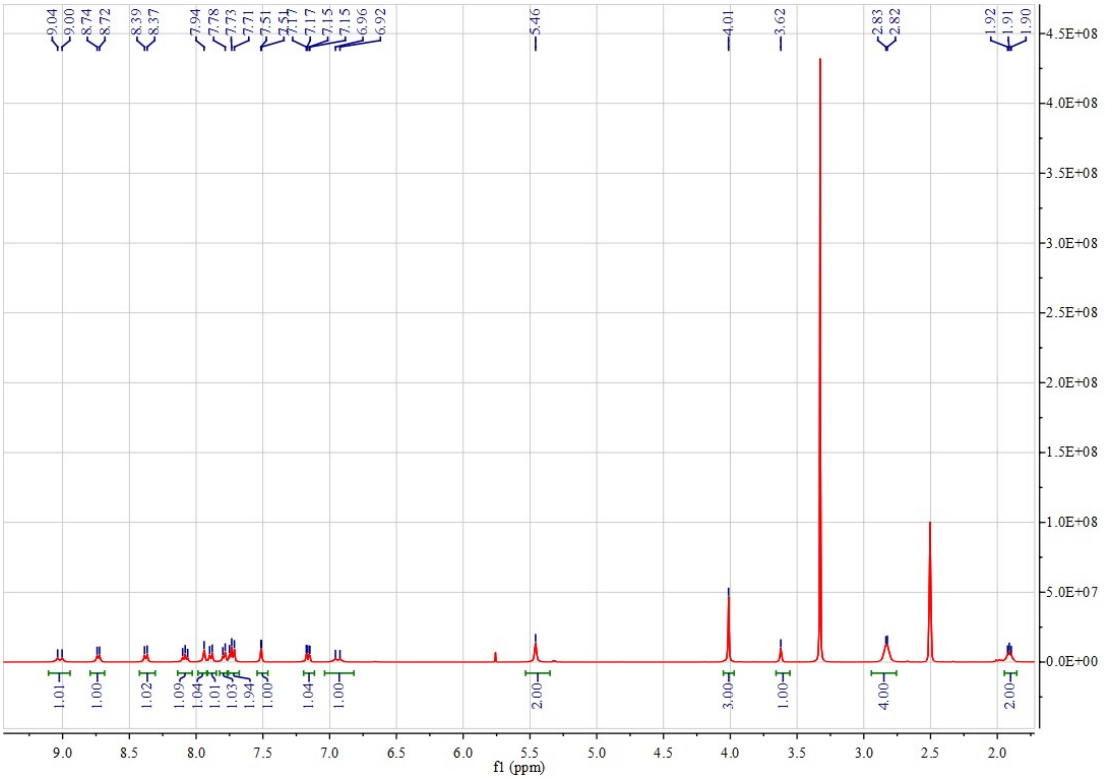


Figure S4. ^{1}H NMR spectrum (in DMSO) of an equivalent mixture of compound **CyOMe**.

Synthesis of CyOH. 6 mL BBr_3 (3M solution in CH_2Cl_2, 18mmol) was added to an anhydrous CH_2Cl_2 solution containing **CyOMe** (1g, 1.8 mmol) at 0 ℃. The mixture was stirred for 16 h at 25 ℃. Quenching with a saturated solution of $NaHCO_3$ at 0 ℃ was followed by extraction of the aqueous layer with CH_2Cl_2/MeOH (4:1). The organic layer was then washed with H_2O, dried over Na_2SO_4, filtered and concentrated in vacuo. Purification by silica gel column chromatography (CH_2Cl_2/MeOH = 100:1~10:1) afforded **CyOH** as blue-green solid (0.9 g, 1.65 mmol, 91.7 %). 1H NMR (400 MHz, $CDCl_3$&MeOD) δ 8.62 (d, J = 13.8 Hz, 1H), 8.10 (d, J = 6.7 Hz, 1H), 7.88 – 7.82 (m, 1H), 7.67 (d, J = 7.2 Hz, 1H), 7.45 (s, 1H), 7.38 (d, J = 7.0 Hz, 2H), 7.27 (s, 3H), 6.97 (d, J = 6.2 Hz, 1H), 6.75 – 6.65 (m, 2H), 6.32 (d, J = 13.3 Hz, 1H), 2.64 (s, 4H), 2.44 (s, 1H), 1.83 (d, J = 4.0 Hz, 2H). HRMS calcd for $C_{29}H_{22}NO_2^+$ ([M]) 416.1643 found 416.1643.

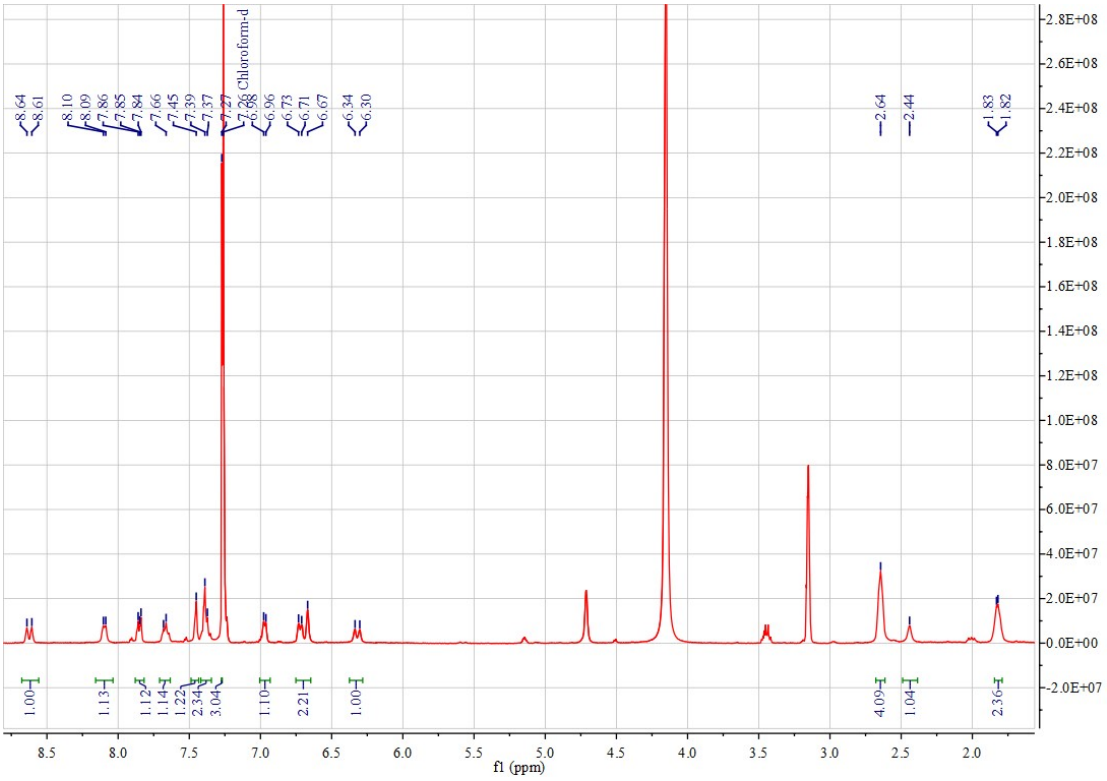


Figure S5. [1]H NMR spectrum (in $CDCl_3$&MeOD) of an equivalent mixture of compound **CyOH**.

Synthesis of CyOAc. CyOH (54 mg, 0.1 mmol) and 15 µL triethylamine were dissolved in CH_2Cl_2. The mixture was stirred at 0°C for 10 min, and then acetylchloride 10 µL (~ 0.3 mmol) dissolved in 2 ml CH_2Cl_2 (30.1 mg 0.25 mmol) was added slowly. After overnight reaction, the solvent was removed under reduced pressure. The resulting residue was purified by a silica gel column (CH_2Cl_2/C_2H_5OH = 10:1) to afford compound **CyOAc** as a green solid (37 mg, 0.064 mmol isolated yield: 64 %). ^{1}H NMR (400 MHz, DMSO) δ 9.12 (s, 1H), 8.87 (d, *J* = 7.5 Hz, 1H), 8.52 (d, *J* = 8.1 Hz, 1H), 8.15 (t, *J* = 7.8 Hz, 1H), 8.07 (d, *J* = 8.3 Hz, 1H), 7.99 (d, *J* = 7.4 Hz, 1H), 7.84 (dd, *J* = 8.1, 7.6 Hz, 1H), 7.78 – 7.76 (m, 2H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.27 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.10 (d, *J* = 14.5 Hz, 1H), 5.57 (d, *J* = 2.3 Hz, 2H), 3.64 (t, *J* = 2.4 Hz, 1H), 2.84 (s, 4H), 2.37 (s, 3H), 1.95 – 1.87 (m, 2H). HRMS calcd for $C_{31}H_{24}NO_3^+$ ([M]) 458.1751 found 458.1755.

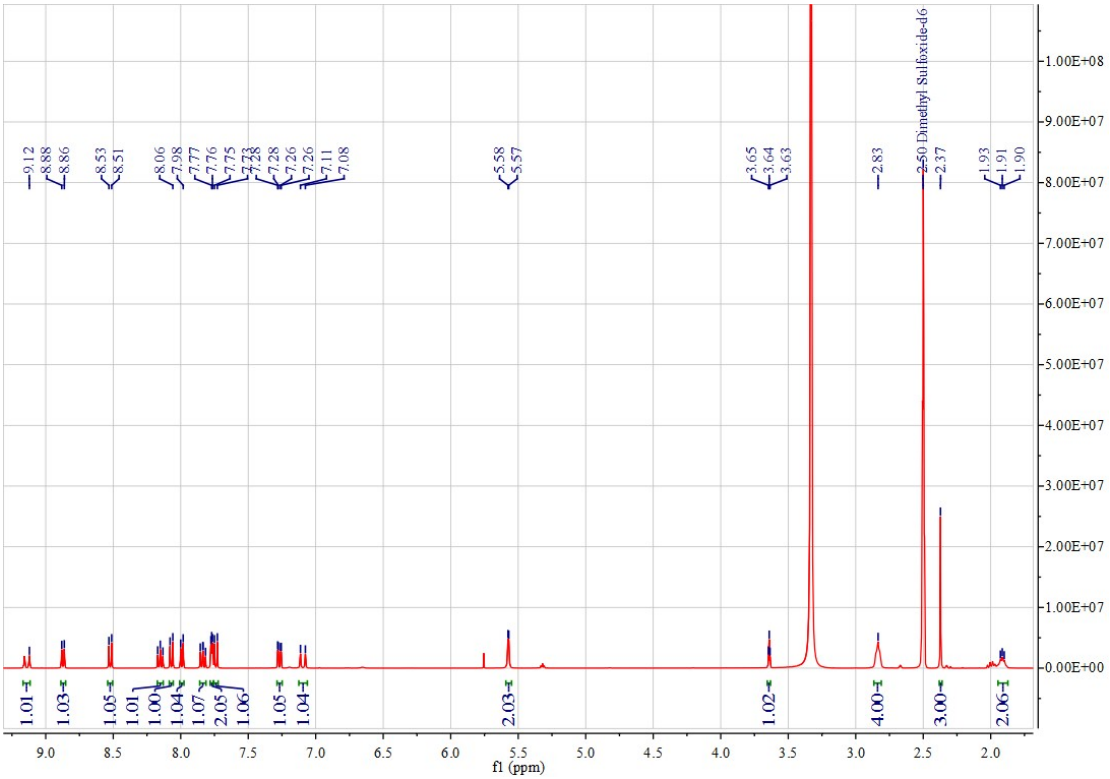
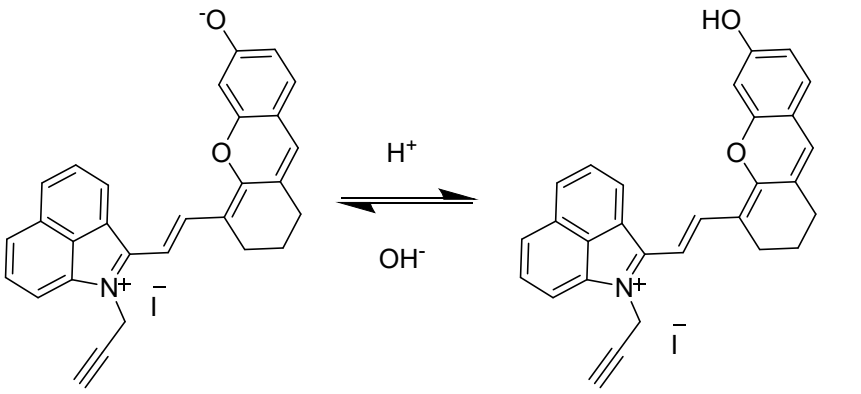


Figure S6. ^{1}H NMR spectrum (in DMSO) of an equivalent mixture of compound **CyOAc**.



Deprotonated form (CyO^-) **Protonated form (CyOH)**

Scheme S2. Protonation and deprotonation of **CyOH**.

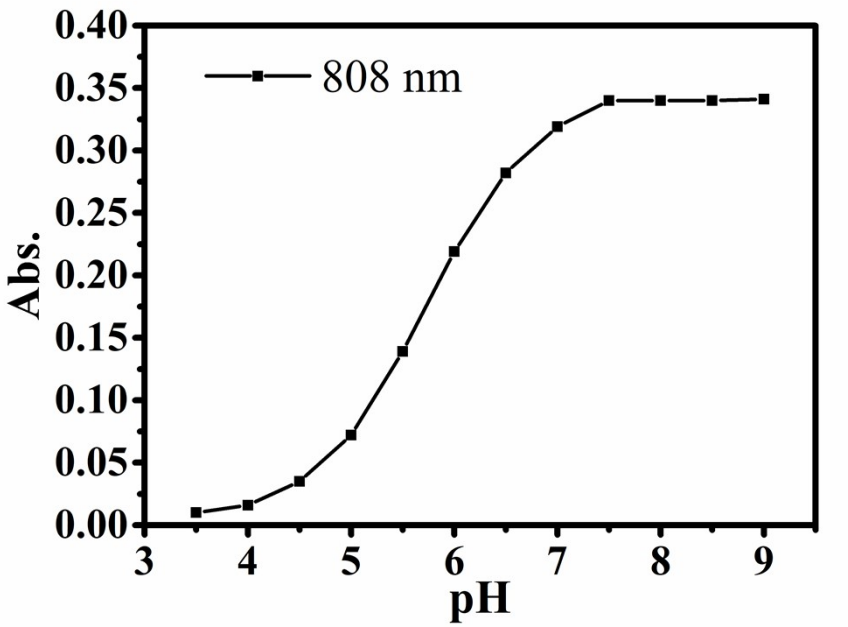


Figure S7. Absorption of **CyOH** versus pH values in PBS/MeCN (6:4, pH = 3.5, 4.0, 4 5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.4, 8, 8.5 and 9.0) at 808 nm excitation.

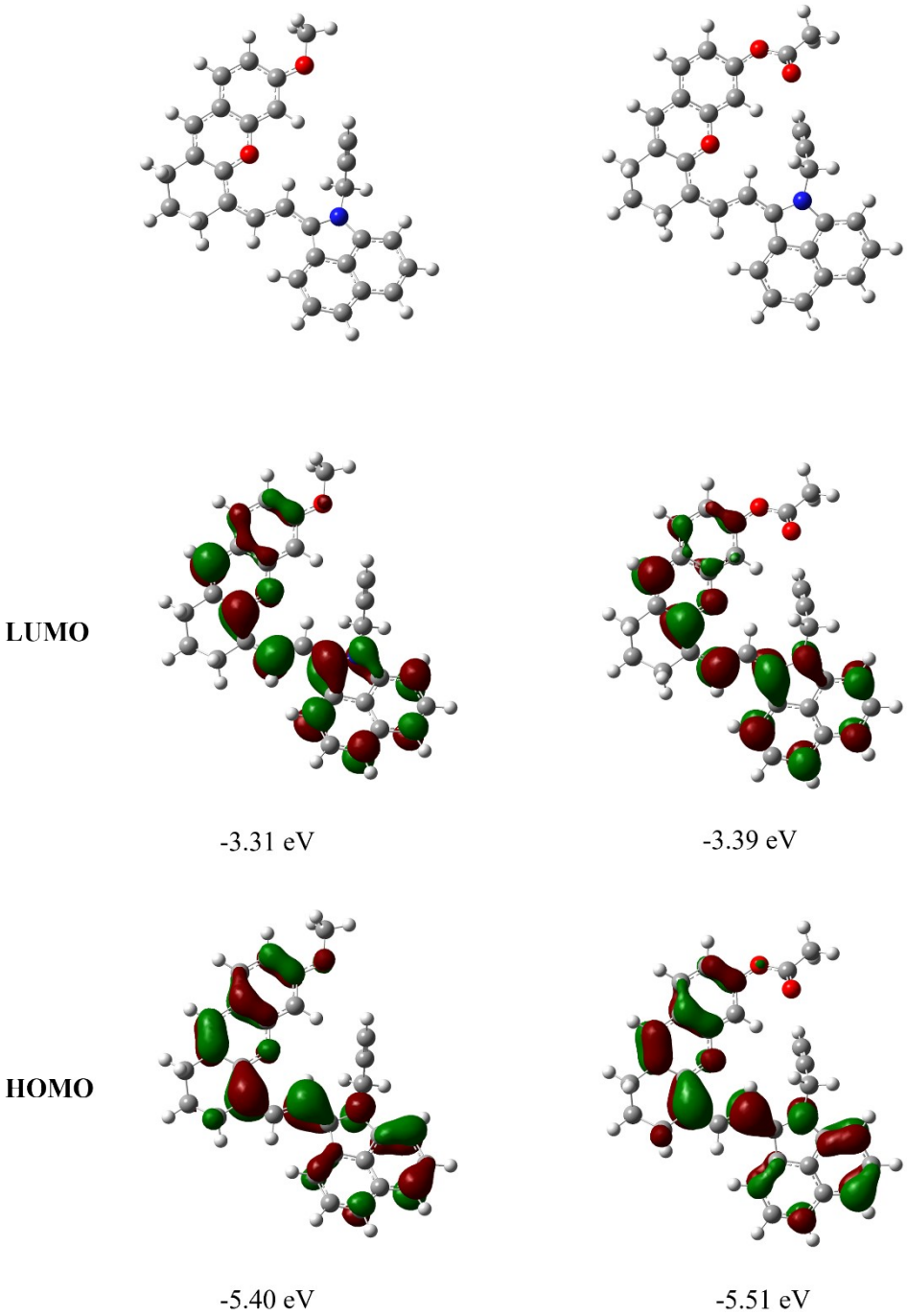


Figure S8. DFT optimized structures and molecular orbital plots (LUMO and HOMO) of **CyOMe** (left column) and **CyOAc** (right column) in H_2O. In the ball-and-stick representation, carbon, nitrogen, and oxygen atoms are colored in gray, blue, and red, respectively.

Table S1. The effect of O-linked functional group on the HOMO/LUMO.

	-O^- of **CyO$^-$**	-OMe of **CyOMe**	-OH of **CyOH**	-OAc of **CyOAc**
HOMO	8.05%	2.20%	1.78%	0.87%
LUMO	3.46%	0.84%	0.76%	0.52%
HOMO-LUMO	4.59%	1.36%	1.02%	0.35%

Table S2. The HOMO-LUMO Gaps of **CyOAc**, **CyOH**, **CyOMe** and **CyO$^-$** determined by DFT calculations.

	CyO$^-$	**CyOMe**	**CyOH**	**CyOAc**
HOMO-LUMO Gap (eV)	2.087	2.092	2.099	2.124

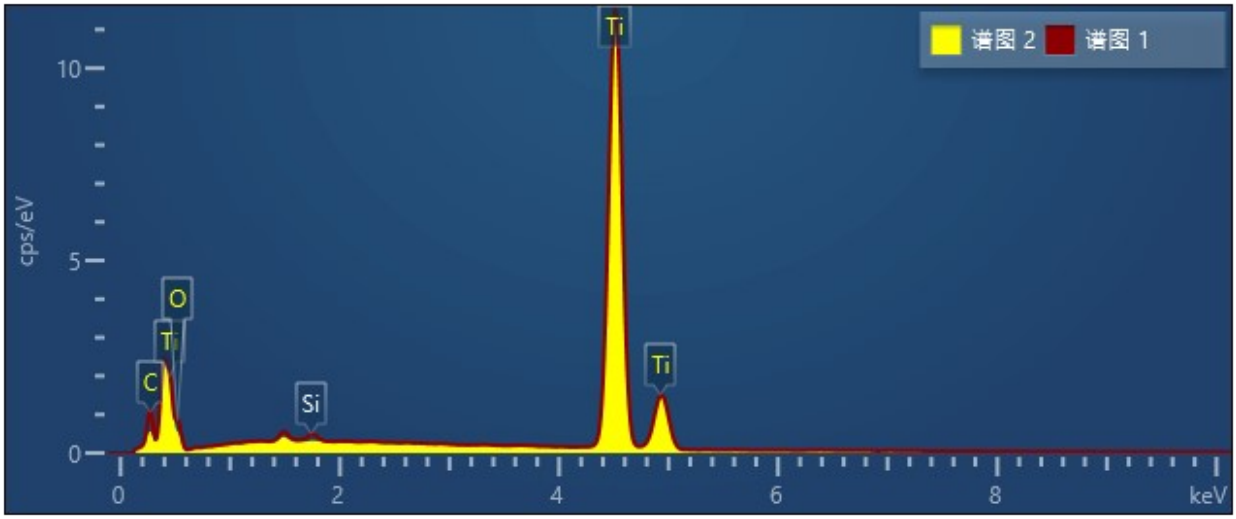


Figure S9. EDS analysis of titanium wire (yellow) and TiWE (red).

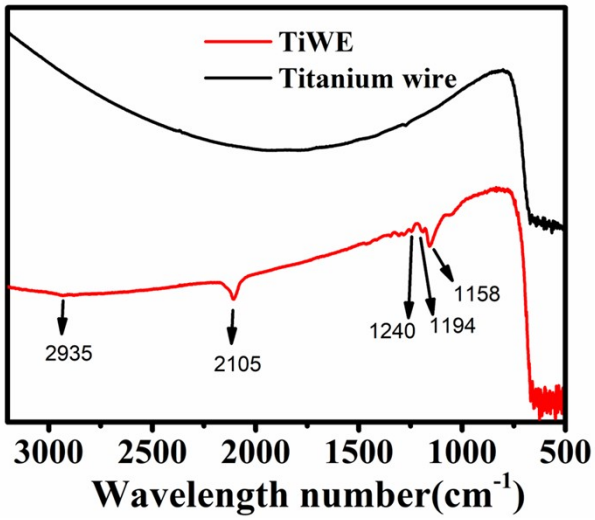


Figure S10. IS-FTIR spectra of hydroxylated titanium wire (black) and TiWE (red).

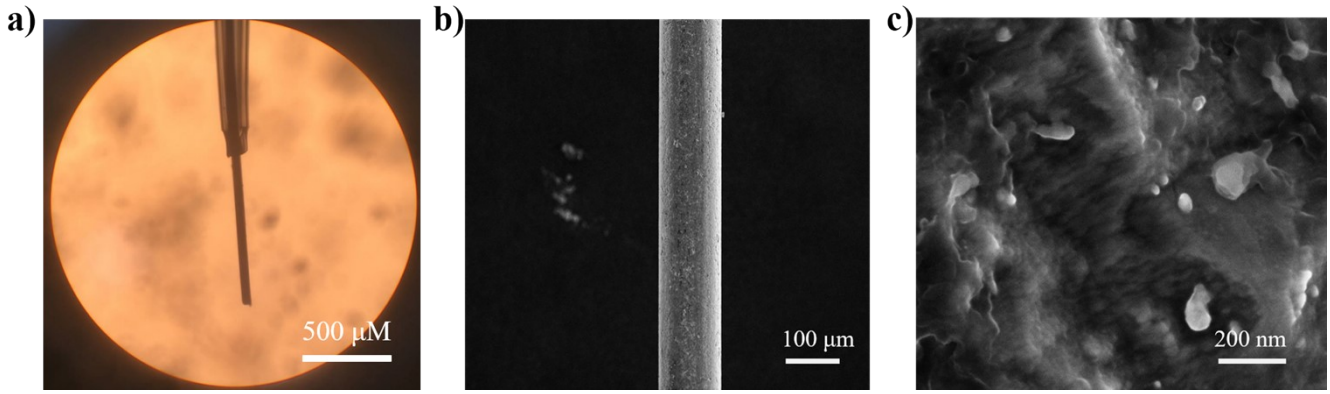


Figure S11. Optical microscope image of TiWE (a), and SEM images of TiWE with low-magnification (b) and high-magnification (c).

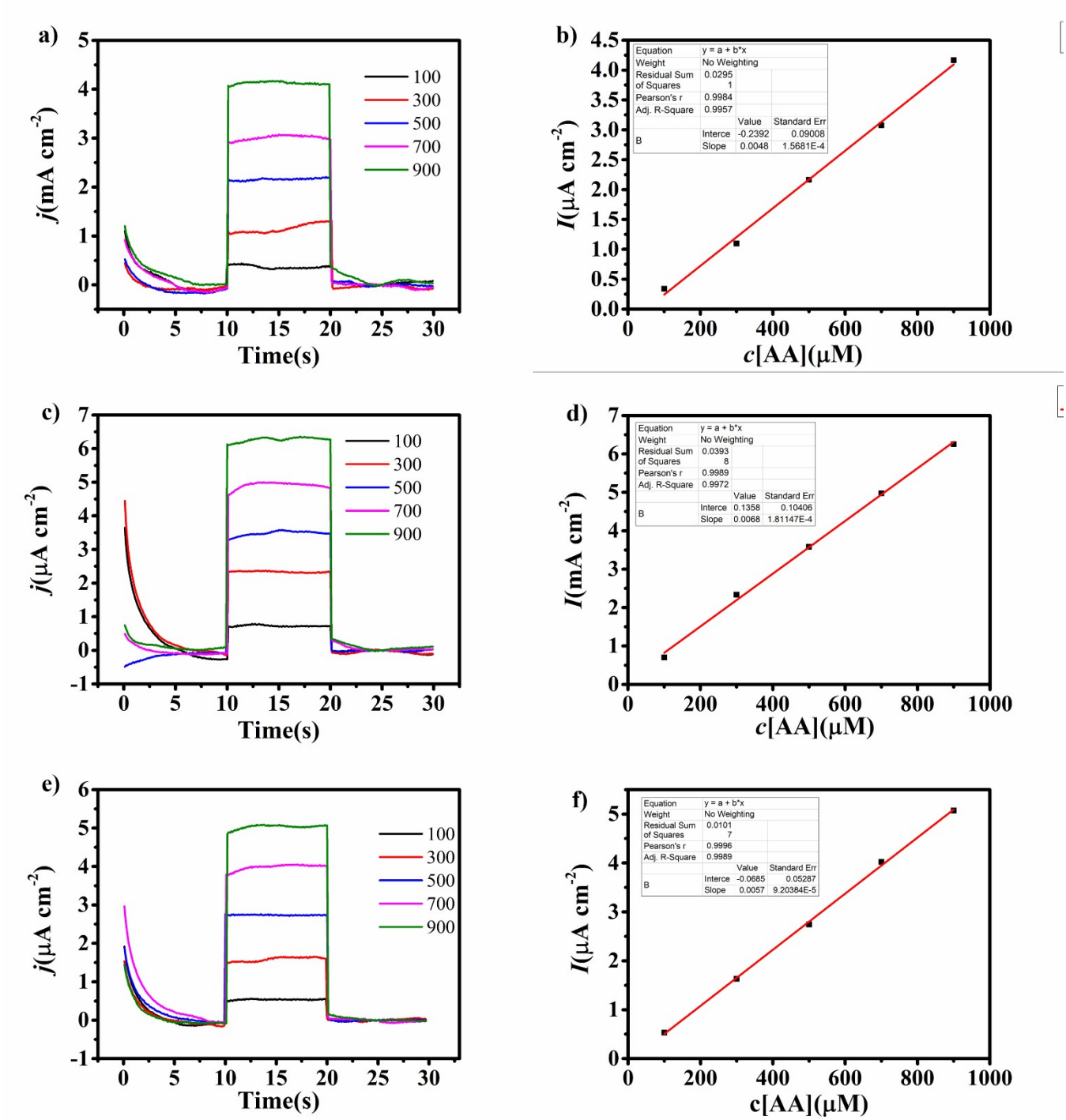
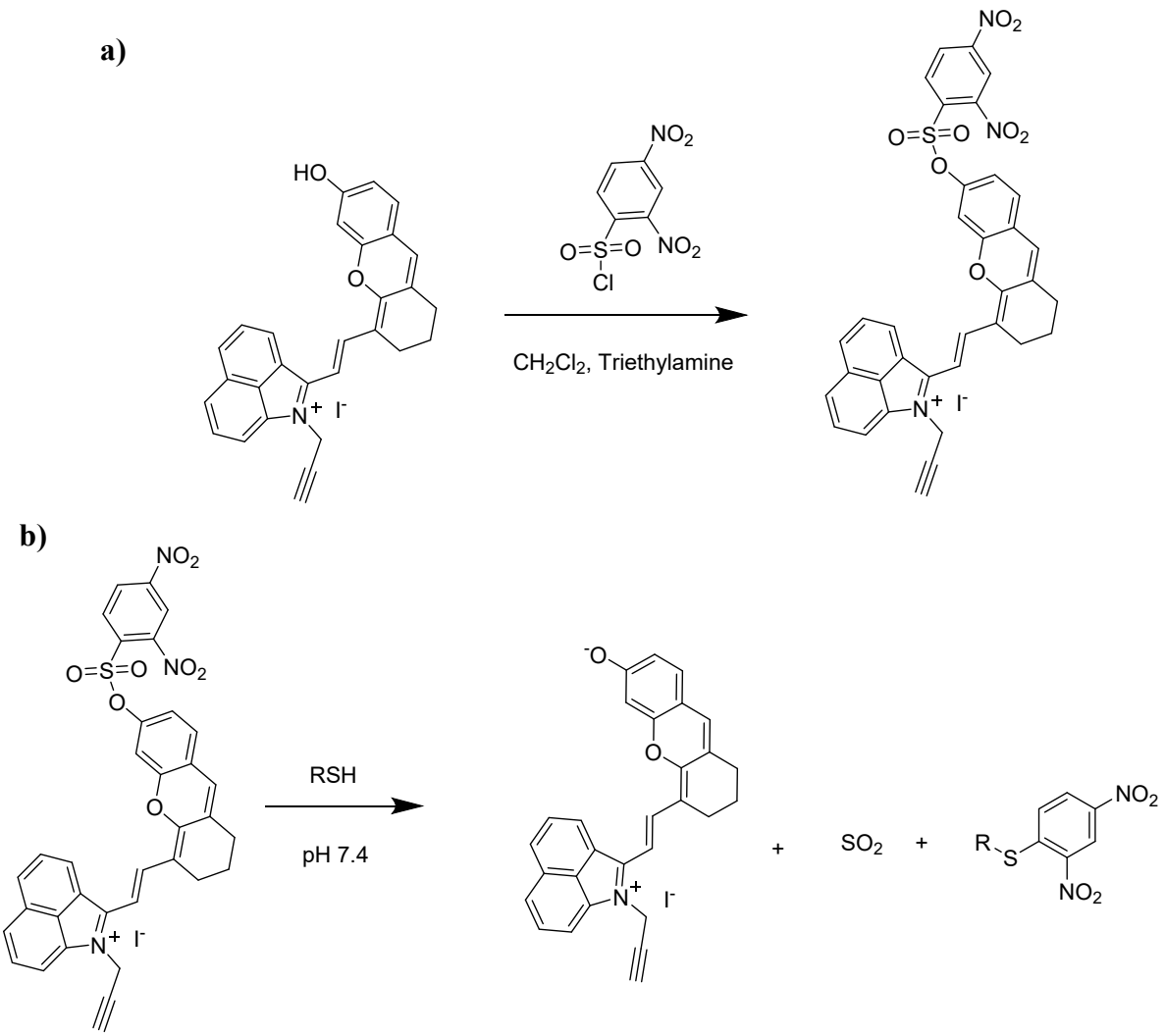


Figure S12. The photocurrent profiles of **CyOH**/TiWE (a), **CyOMe**/TiWE (c) and **CyOAc**/TiWE (e) under 750-nm laser excitation in the presence of varying amounts of AA, and the corresponding dependence of photocurrent intensities of **CyOH**/TiWE (b), **CyOMe**/TiWE (d) and **CyOAc**/TiWE (f) on the concentration of AA.



Scheme S3. Synthetic routes of **CyOThiols** (a) and its response to thiols (b).

Synthesis of CyOThiols. CyOH (54 mg, 0.1 mmol) and 15 μL triethylamine were dissolved in CH_2Cl_2. The mixture was stirred at 0°C for 10 min, and then 2, 4-dinitrobenzenesulfonyl chloride dissolved in 2 ml CH_2Cl_2 (66.7 mg 0.25 mmol) was added slowly. After stirring at room temperature for 24 hours, the solvent was evaporated at reduced pressure. The resulting residue was purified by a silica gel column (CH_2Cl_2/C_2H_5OH = 100:1~10:1) to afford compound **CyOThiols** as a green solid (46.4 mg, 0.06 mmol, isolated yield: 60 %). ^{1}H NMR (400 MHz, DMSO) δ 9.07 (d, *J* = 14.6 Hz, 1H), 8.99 (d, *J* = 2.8 Hz, 1H), 8.82 (d, *J* = 7.5 Hz, 1H), 8.59 – 8.46 (m, 2H), 8.10 – 7.97 (m, 3H), 7.90 (d, *J* = 2.3 Hz, 1H), 7.80 (dd, *J* = 19.6, 11.1 Hz, 3H), 7.49 (d, *J* = 9.2 Hz, 1H), 7.30 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.09 (d, *J* = 14.6 Hz, 1H), 5.56 (t, *J* = 10.0 Hz, 2H), 3.65 (t, *J* = 2.4 Hz, 1H), 2.91 – 2.72 (m, 4H), 1.93 (dd, *J* = 15.4, 9.9 Hz, 2H). HRMS calcd for $C_{35}H_{24}N_3O_8S^+$ ([M]) 646.1279 found 646.1279.

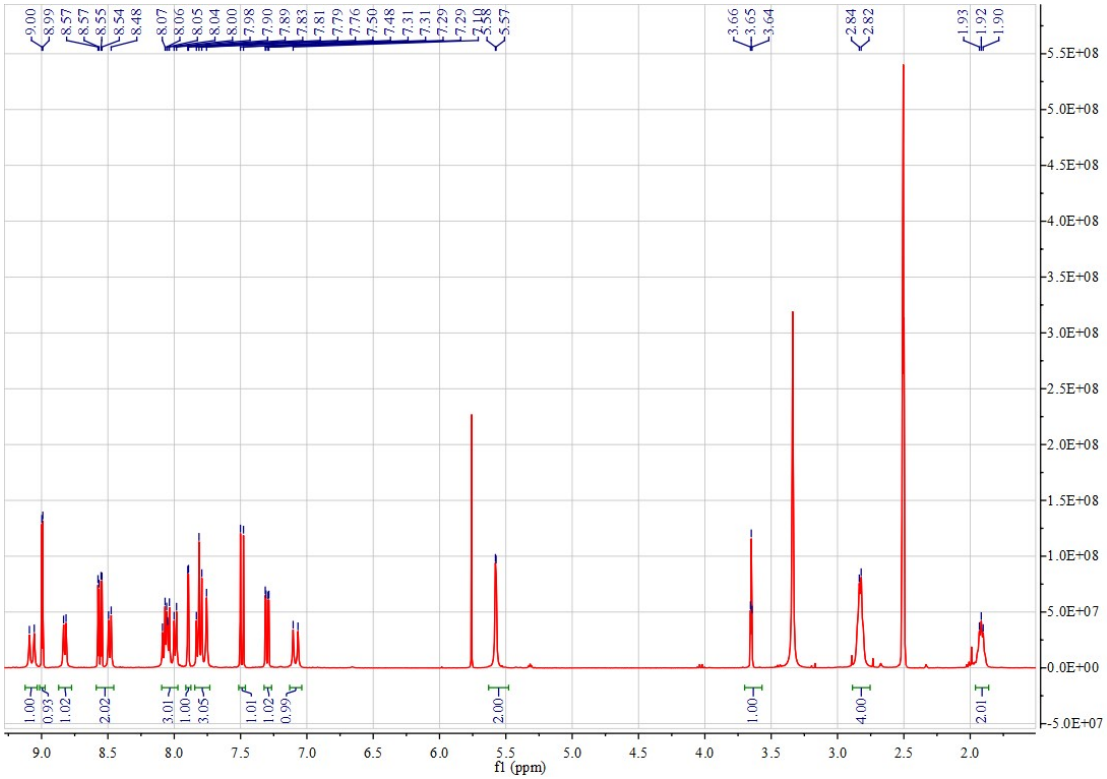


Figure S13. ^{1}H NMR spectrum (in DMSO) of an equivalent mixture of compound **CyOThiols**.

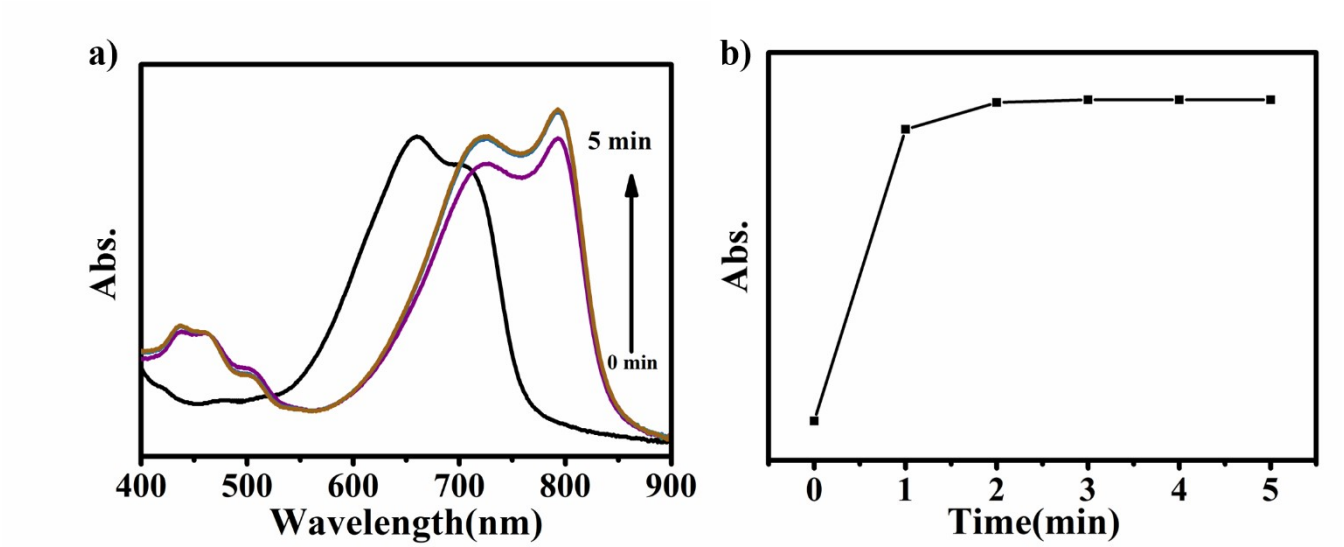


Figure S14. Time-dependent absorption spectra (a) and plots of absorbance at 808 nm (b) of **CyOThiols** in the presence of 500 μM Cys in aCSF.

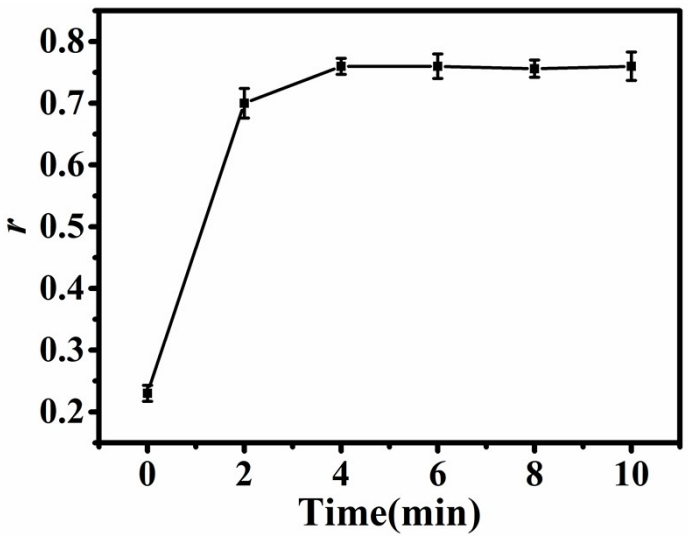


Figure S15. The reaction time-dependent ratiometric signal ($r = j_{808}/j_{750}$) of **CyOThiols**/TiWE toward 500 μM Cys in aCSF containing 250 μM AA (n=3, S.D.).

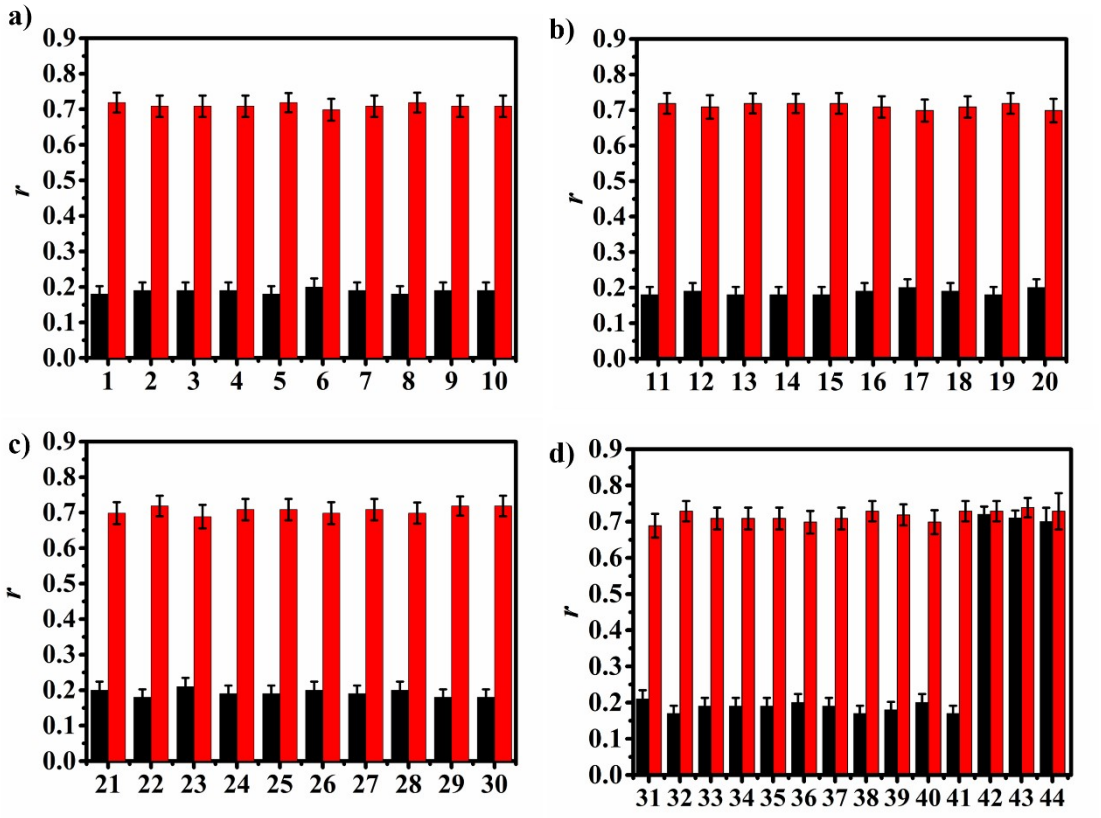


Figure S16. Selectivity (black) and competition (red) tests for Cys (1 mM) with **CyOThiols**/TiWE: (a) 1, Cu^{2+}; 2, Ca^{2+}; 3, Na^{+}; 4 Mg^{2+}; 5, K^{+}; 6, Cu^{+}; 7, Mn^{2+}; 8, Fe^{3+}; 9, Zn^{2+}; 10, Fe^{2+} (3 mM for K^{+}, 150 mM for Na^{+}, 1 mM for $Ca^{2+}$and Mg^{2+}, 50 μM for Mn^{2+} and 10 μM for others metal ions); (b) 11, Glu; 12, Gly; 13, His; 14, Leu; 15, Iso; 16, Try; 17, Val; 18, Met; 19, NMDA; 20, DIDS; (20 μM for Glu, Gly, His, Leu, Iso, Try, Val, Met, 50 μM for Lys, Arg, and Thr; and 1 mM NMDA and DIDS); (c) 21, AA; 22, UA; 23, DA; 24, ATP; 25, glucose; 26, O_2; 27, H_2O_2; 28, NADPH; 29, NADH; 30, ALP; (30 μM for H_2O_2; 200 μM for AA, 10 mM for glucose, 1000 $U \cdot L^{-1}$ for ALP and 50 μM for other species); (d) 31, NO_3^-; 32, Cl^-; 33, HCO_3^-; 34, $S_2O_3^{2-}$; 35, SO_4^{2-}; 36, ClO^-; 37, $ONOO^-$; 38, OH; 39, O_2^-; 40, GSH; 41, Cys; 42, Hcy, (100 μM for NO_3^-, 0.1 M for Cl^-, 50 mM for HCO_3^-, 1 mM for GSH and Hcy, 10 μM for other species).

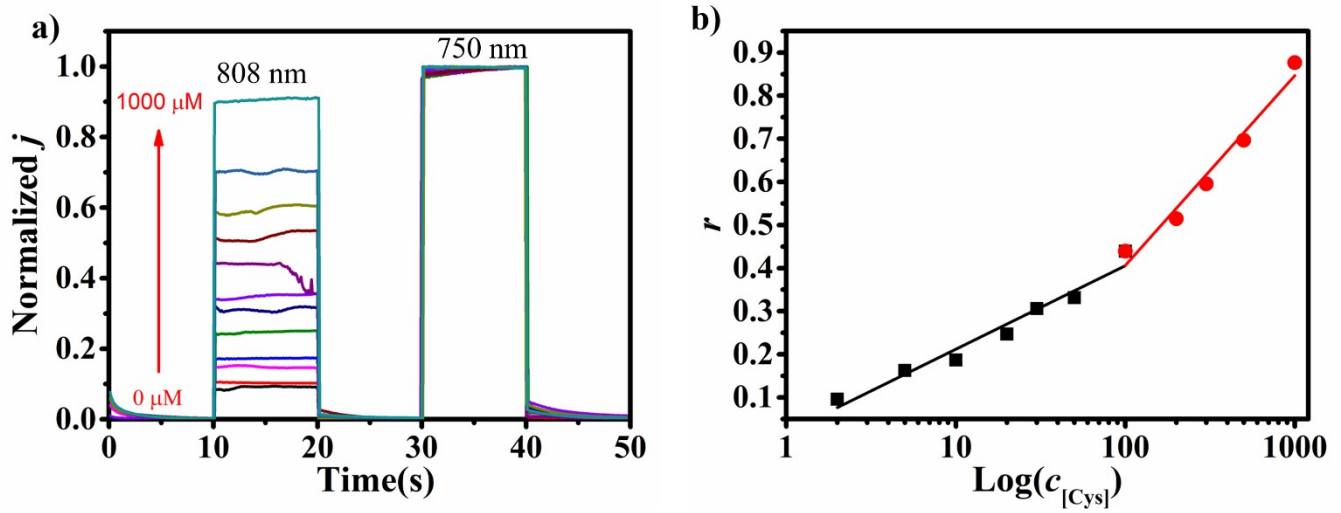


Figure S17. (a) Normalized photocurrent intensities of **CyOThiols**/TiWE in aCSF containing 250 μM AA toward different concentrations of Cys. (b) Linear fitting curves between the ratiometric signal ($r = j_{808}/j_{750}$) and the log concentration of Cys.

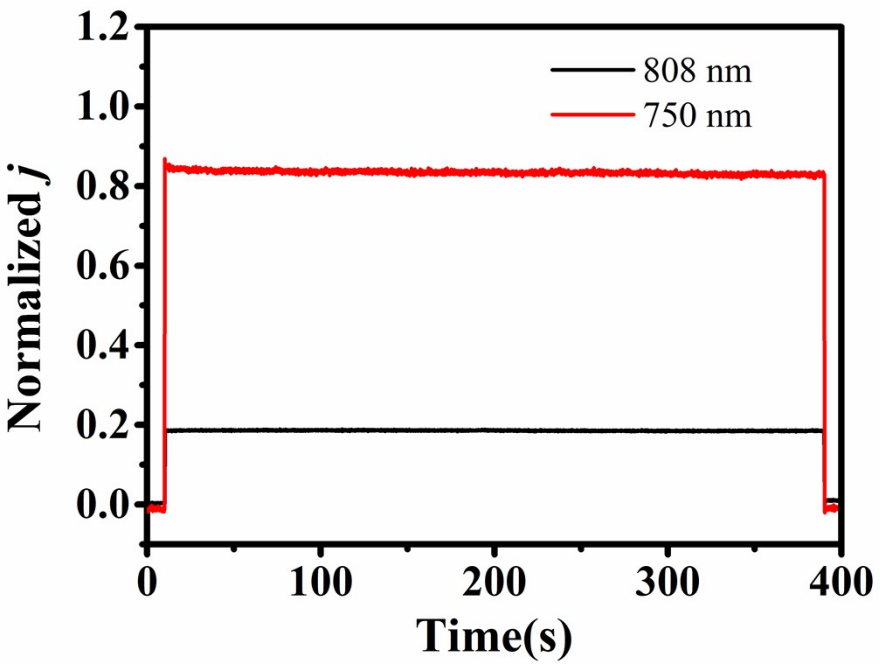


Figure S18. Photocurrent stability of **CyOThiols**/TiWE under 808-nm and 750-nm light irradiation for 380s in aCSF containing 250 μM AA.

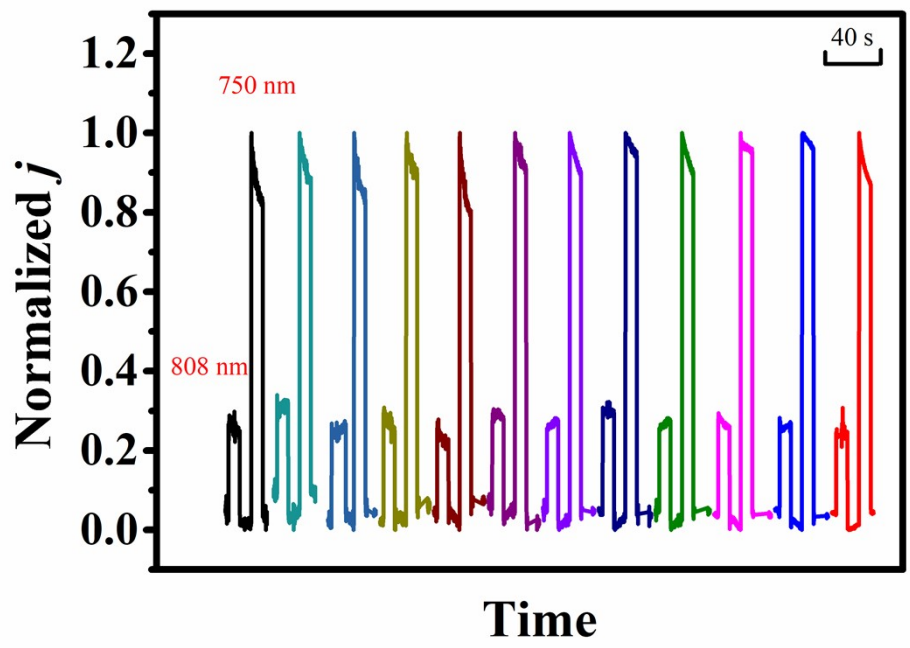


Figure S19. Photocurrent responses obtained at 12 independent **CyOThiols**/TiWE under 808-nm and 750-nm light excitation in the same rat brain.

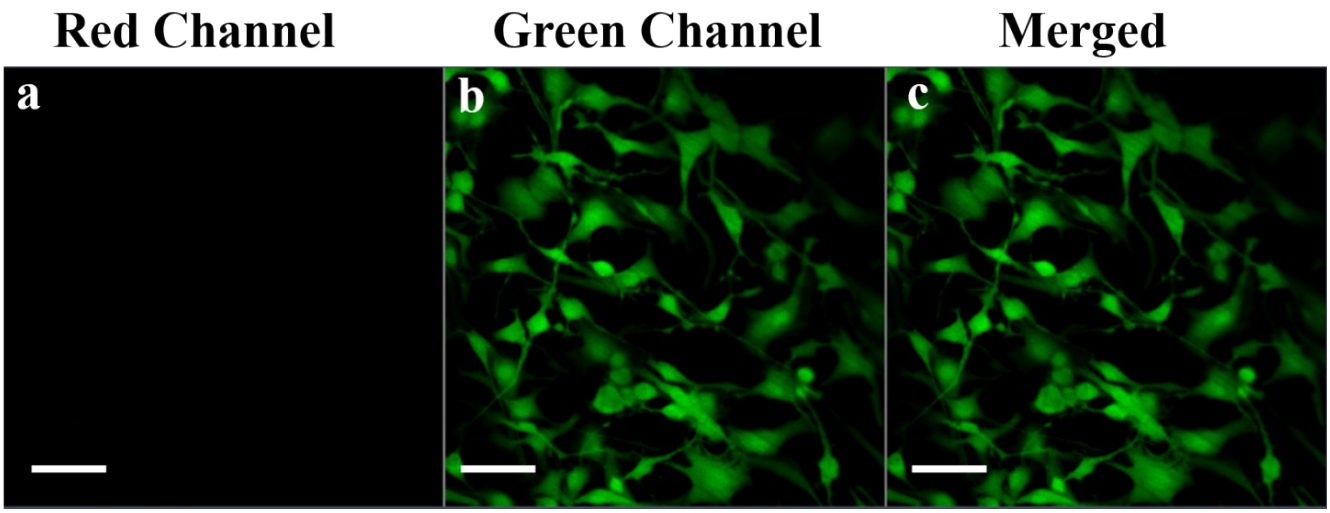


Figure S20. Confocal fluorescence (a,b) and merged (c) images of cultured cells on the titanium sheet modified by **CyOThiols**: (a) with Propidium Iodide (red, dead cells) stain; (b) with Calcein-AM (green, live cells) stain. (c) Merged image from (a) and (b). Scale bar: 200 μm.

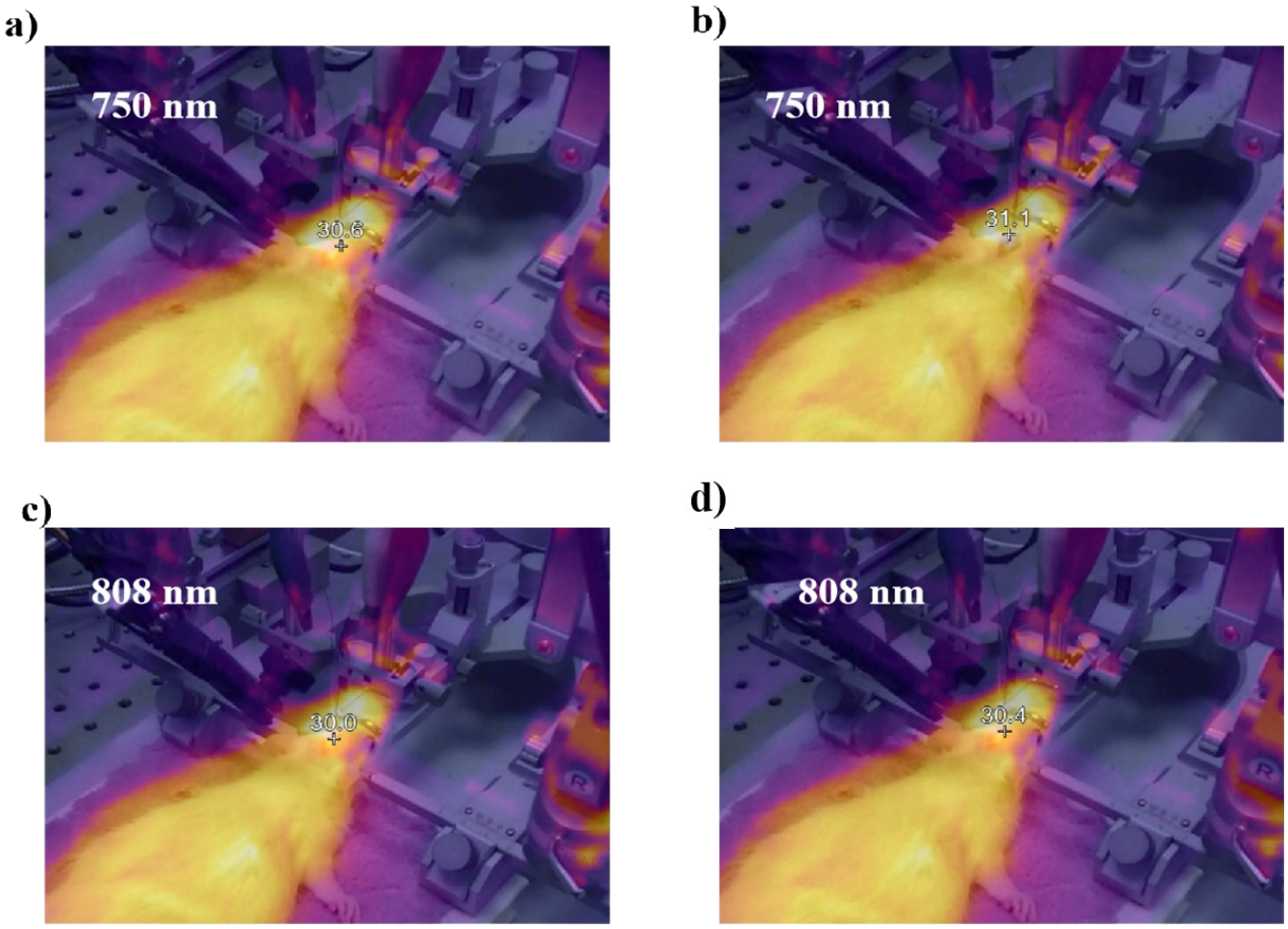


Figure S21. Infrared thermal image of rat head before (a), (c) and after (b), (d) the irradiation with 750-nm laser and 808-nm laser for 10 s, respectively.

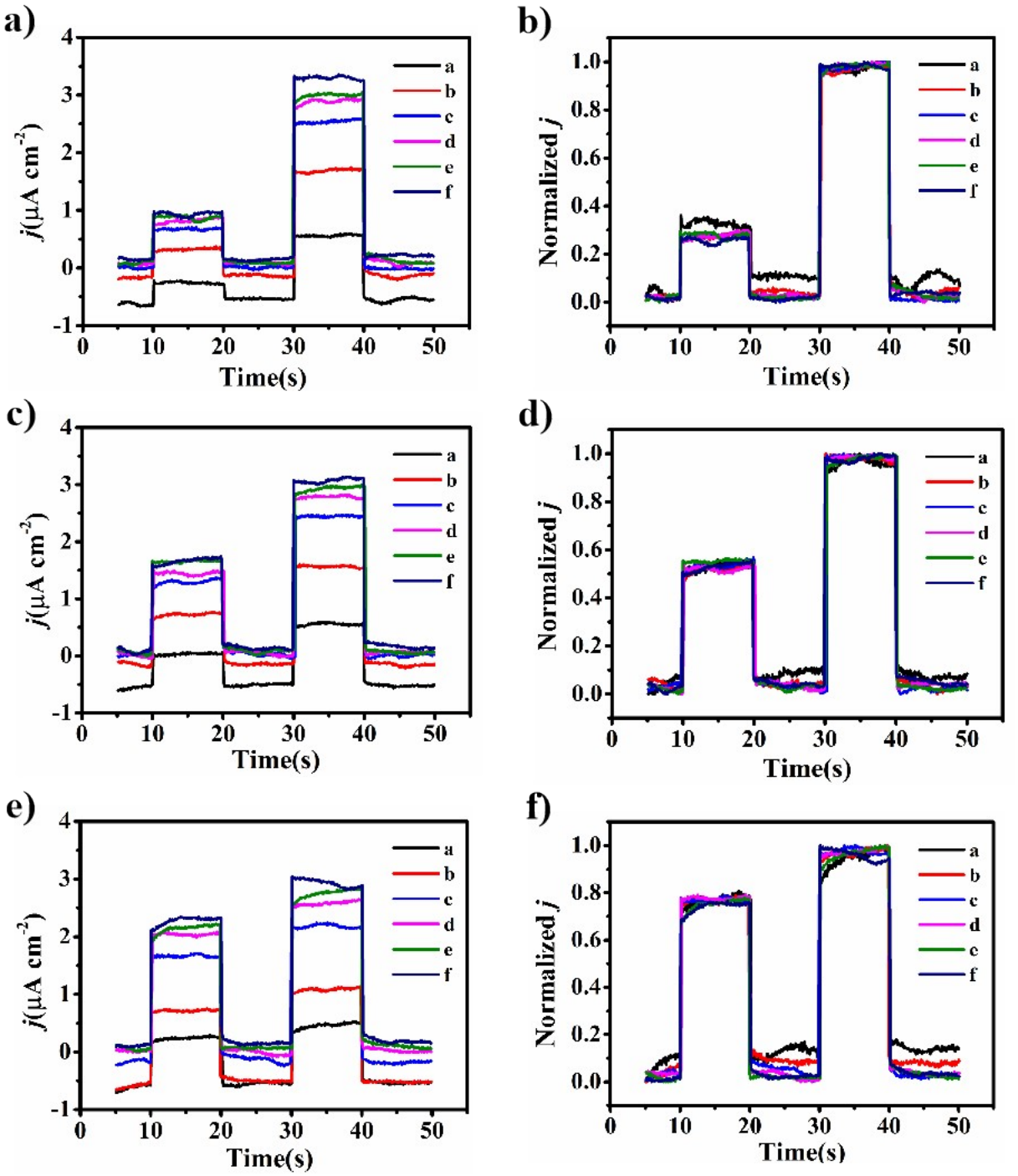


Figure S22. Effects of AA concentration on PEC signals of **CyOThiols**/TiWE under 808-nm (left column) and 750-nm (right column) excitation. Curves a ~ f: 100, 200, 300, 400, 500 and 600 μM AA in aCSF). Figures a), c) and e): without normalization; figures b), d) and f): normalized to 750-nm intensities. The concentrations of Cys were 10 μM, 70 μM and 500 μM in Figure a) and b), c) and d), and e) and f), respectively.

Table S3. Effects of AA concentration on recovery of Cys at different concentrations.

c[AA](μM)	Group1		Group2		Group3	
	500μM Cys	**Recovery (%)**	**70 μM Cys**	**Recovery (%)**	**10 μM Cys**	**Recovery (%)**
100.00	534.47	106.89	76.34	109.05	10.55	105.53
200.00	516.19	103.24	67.46	96.38	9.01	90.13
300.00	538.19	107.64	75.42	107.74	9.35	93.46
400.00	548.16	109.63	65.62	93.74	9.14	91.42
500.00	476.05	95.21	75.26	107.52	9.12	91.24
600.00	462.88	92.58	75.41	107.73	9.56	95.65

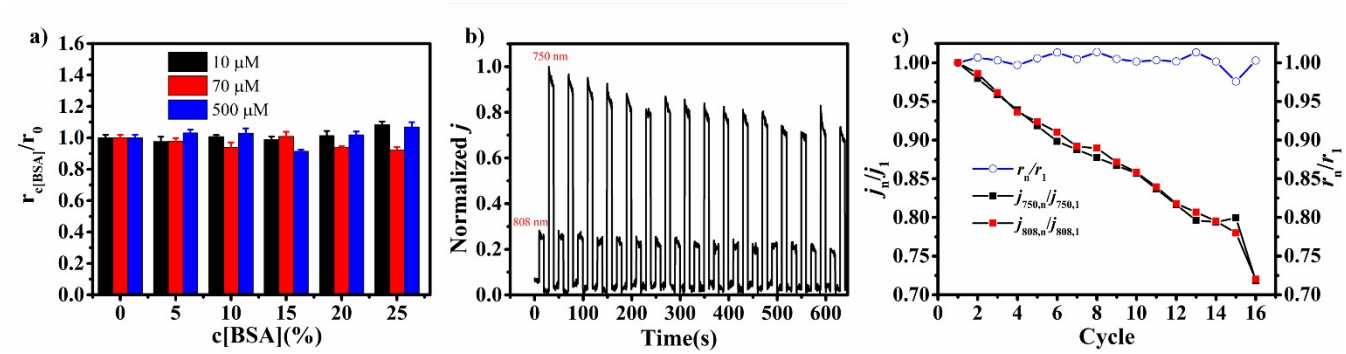


Figure S23. (a) Effects of BSA concentration (0%, 5%, 10%, 15%, 20% and 25%) on **CyOThiols**/TiWE. r_0 represents the value of j_{808}/j_{750} obtained in aCSF without BSA, while $r_{c[BSA]}$ indicates the value of j_{808}/j_{750} obtained in aCSF containing varying concentrations of BSA. (b) Photocurrent signals obtained at one photoelectrode excited 16 cycles under 808-nm and 750-nm in the cortex of normal rat brain. (c) Photocurrent intensities value and *r* value obtained in the curve of (b), where n (n = 1, 2, …, 16) represents the number of cycles.

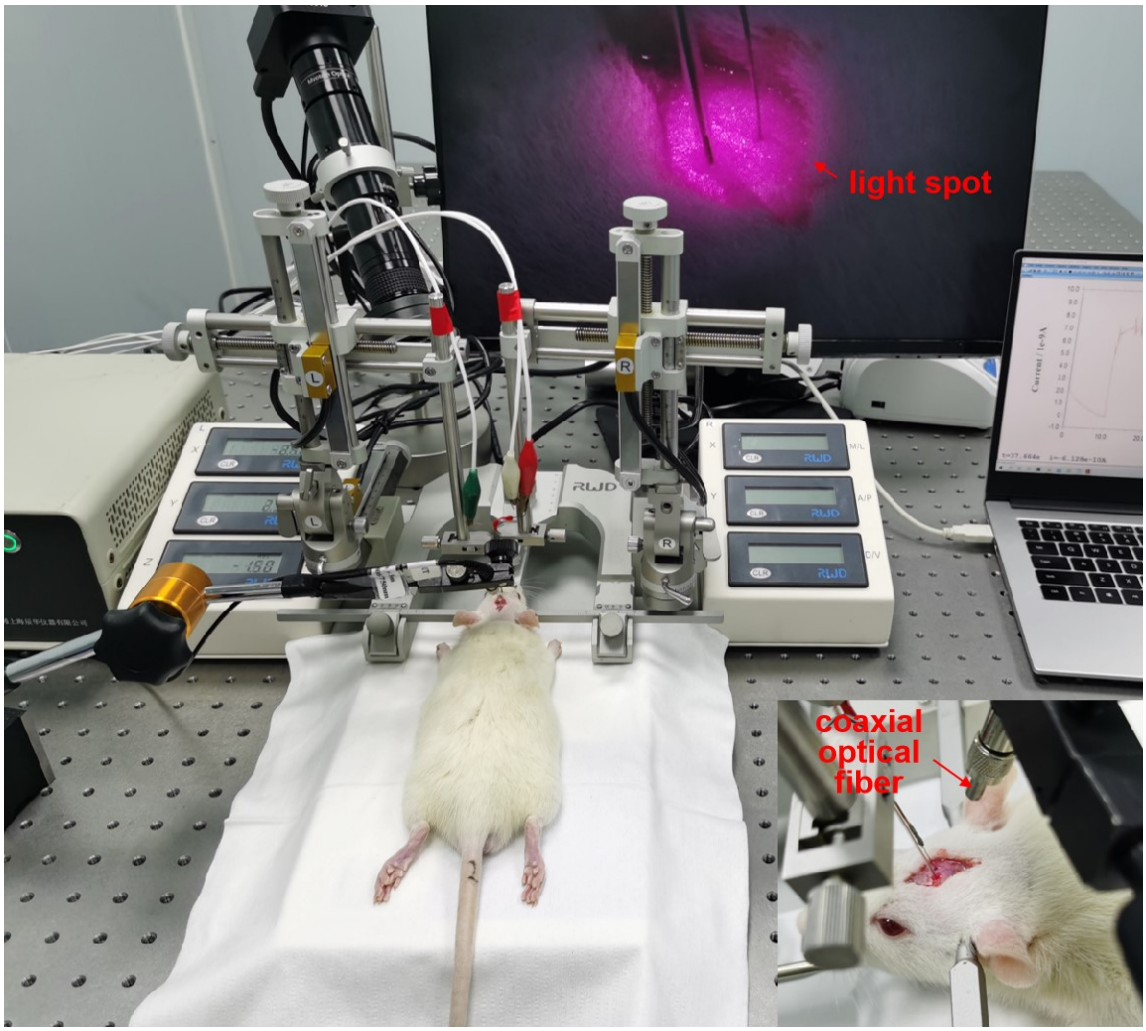


Figure S24. *In vivo* experiment equipment diagram and laser exposure location diagram.

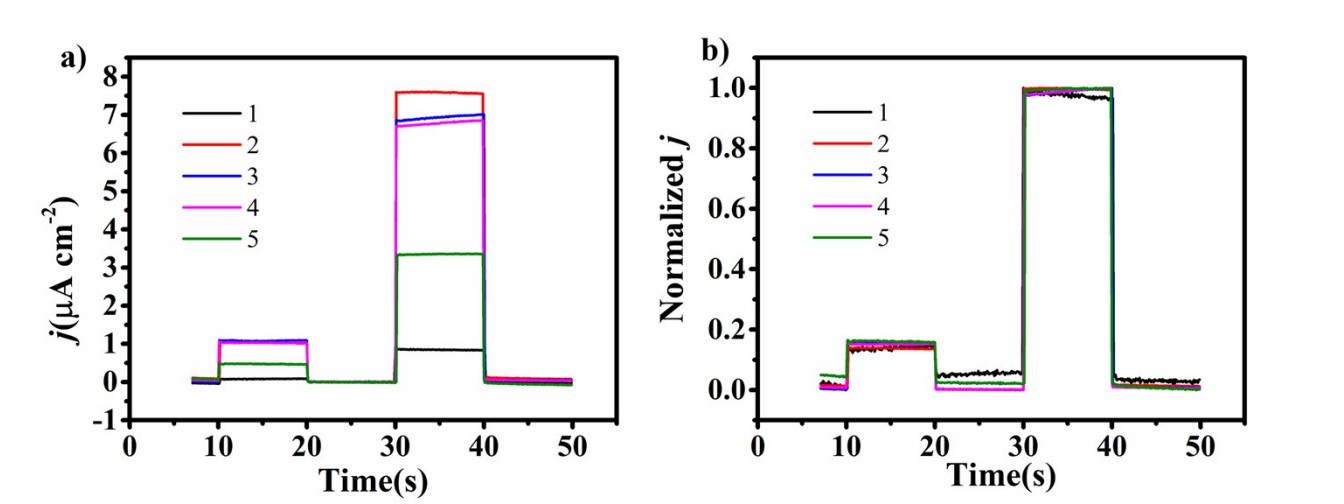


Figure S25. Photocurrent intensities (a) and normalized photocurrent intensities (b) recorded by a same **CyOThiols**/TiWE at five randomized distances between electrode and optical fiber. Left column: 808-nm excitation; right column: 750-nm excitation.

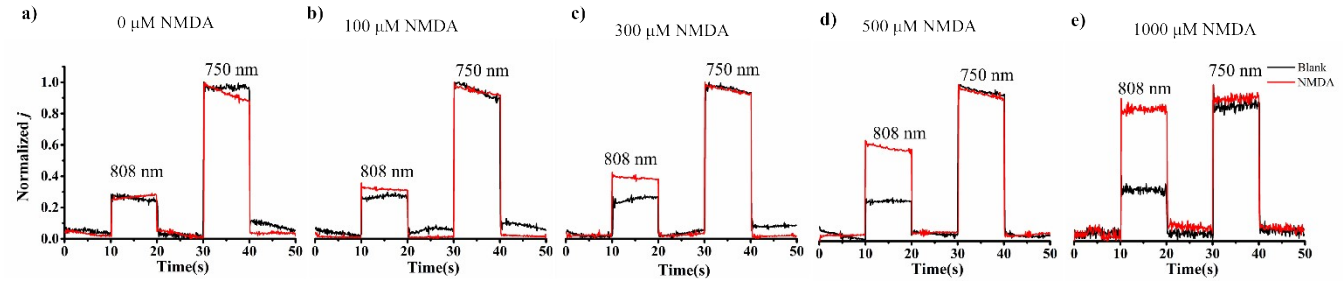


Figure S26. Normalized photocurrent intensities of **CyOThiols**/TiWE pre- (black) and post- (red) local microinjection with 0 μM(a), 100 μM (b), 300 μM (c), 500 μM (d) 1000 μM (e) NMDA in rat brains under 808-nm and 750-nm excitation.

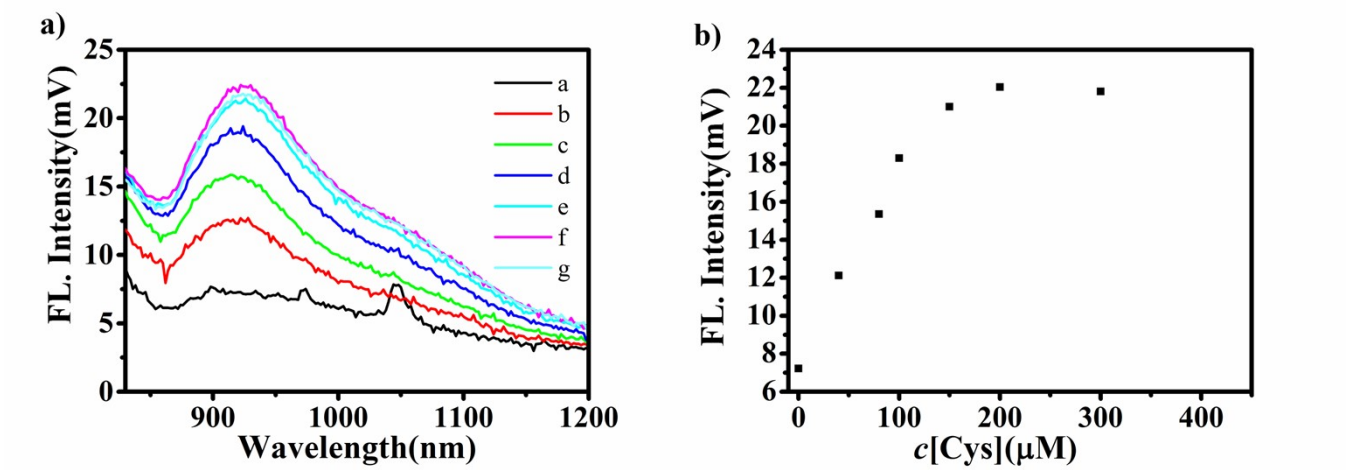


Figure S27. (a) Fluorescence spectra of **CyOThiols** (10 μM) in response to Cys (a-g: 0, 40, 80, 100, 150, 200 and 300 μM) in PBS buffer (pH 7.4, 40% MeCN). (b) Titration curve of **CyOThiols** to Cys. **CyOThiols** and Cys were incubated together at 37 ℃ for 2 minutes and the fluorescence was collected under 808-nm laser excitation.

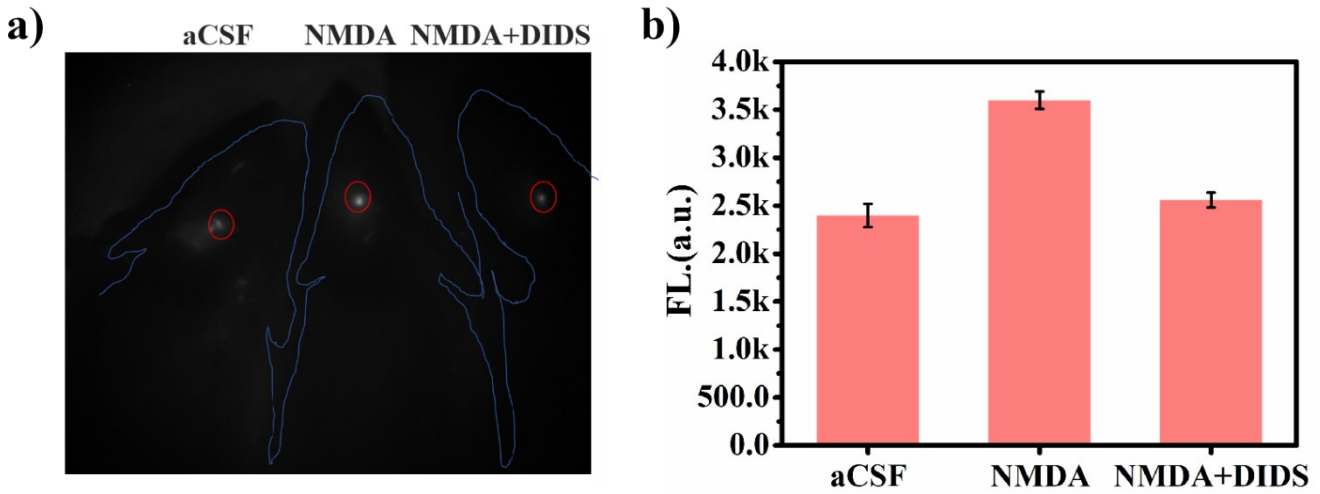
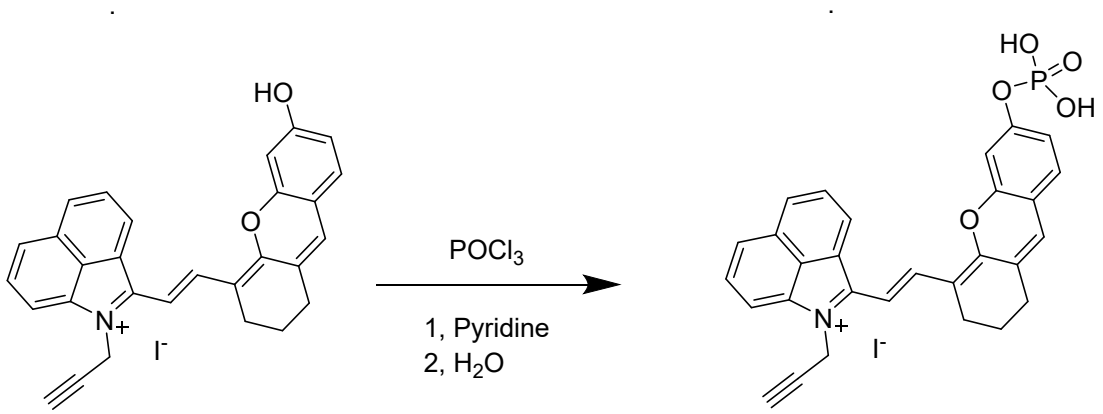


Figure S28. (a) *In vivo* fluorescence images of living rat brains and (b) fluorescence intensity of the injection point: The living rat brains were co-injected with aCSF + 10 μM **CyOThiols**, 500 μM NMDA + 10 μM **CyOThiols** and 500 μM NMDA + 5mM DIDS + 10 μM **CyOThiols** (1 μL min^{-1} for 60 s, n=3) respectively. NIR-II fluorescence images were gained under 808 nm excitation with 1000 nm long-pass filter 2 min after co-injection. Exposure time was 300 ms.



CyOH **CyOALP**

Scheme S4. Synthetic routes of **CyOALP**.

Synthesis of CyOALP. CyOH (109 mg, 0.2 mmol) was dissolved in 5 mL pyridine and then phosphorus oxychloride (62 mg, 0.4 mmol) was added. After overnight reaction, 2mL saturated salt-water was added to the reaction system. The precipitated solids were recrystallized with dichloromethane to afford compound **CyOALP** as a green solid (78.5 mg, 0.126 mmol, isolated yield: 63 %). ^{1}H NMR (400 MHz, DMSO&MeOD) δ 8.76 (d, *J* = 14.3 Hz, 1H), 8.43 (d, *J* = 7.5 Hz, 1H), 8.18 (t, *J* = 7.6 Hz, 1H), 8.05 (d, *J* = 7.7 Hz, 1H), 7.72 (dd, *J* = 14.2, 6.7 Hz, 4H), 7.64 – 7.61 (m, 1H), 7.56 (d, *J* = 8.6 Hz, 1H), 7.15 (dd, *J* = 8.6, 1.9 Hz, 1H), 6.81 (d, *J* = 14.3 Hz, 1H), 5.38 (d, *J* = 2.0 Hz, 2H), 3.52 (t, *J* = 2.2 Hz, 1H), 2.78 (dd, *J* = 9.1, 2.9 Hz, 2H), 2.71 (dd, *J* = 5.2, 3.5 Hz, 2H), 1.92 – 1.81 (m, 2H). HRMS calcd for $C_{28}H_{25}NO_5P^+$ ([M]) 496.1308 found 496.1301.

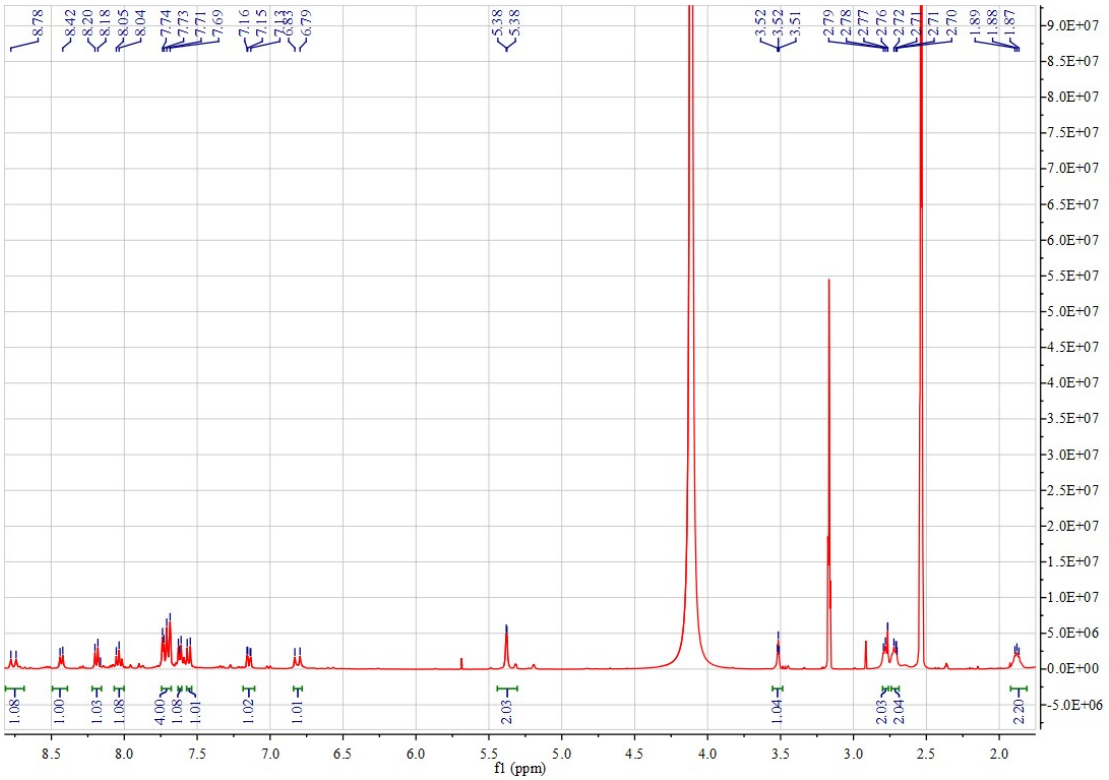
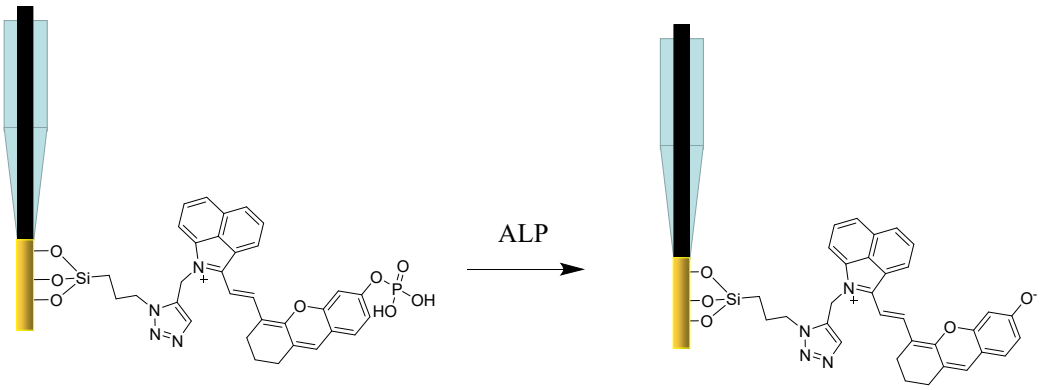


Figure S29. ^{1}H NMR spectrum (in DMSO&CD_3OD) of an equivalent mixture of compound **CyOALP**.



Scheme S5. Recognition reaction of ALP by **CyOALP**/TiWE.

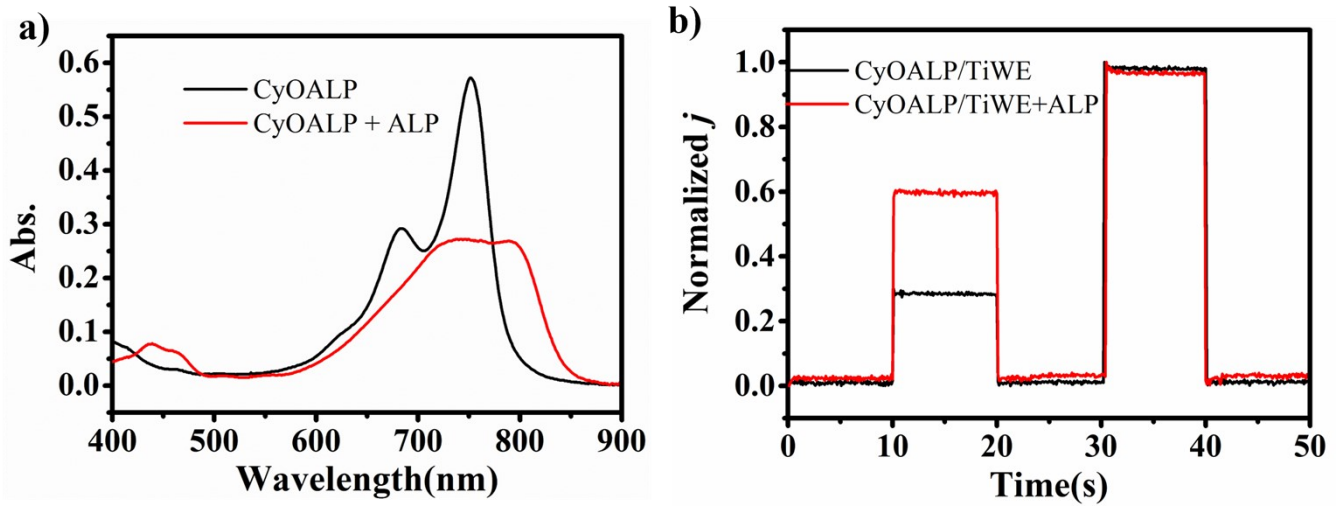


Figure S30. (a) Absorption spectra of 6 μM **CyOALP** in pH 7.4 PBS/MeCN (6:4) before and after reacting with 200 $U \cdot L^{-1}$ ALP for 5 min. (b) Normalized photocurrent intensities of **CyOALP**/TiWE in 0.1 M PBS containing 250 μM AA driven by 808-nm (left column) and 750-nm (right column) light before and after reacting with 500 $U \cdot L^{-1}$ ALP for 5 min.

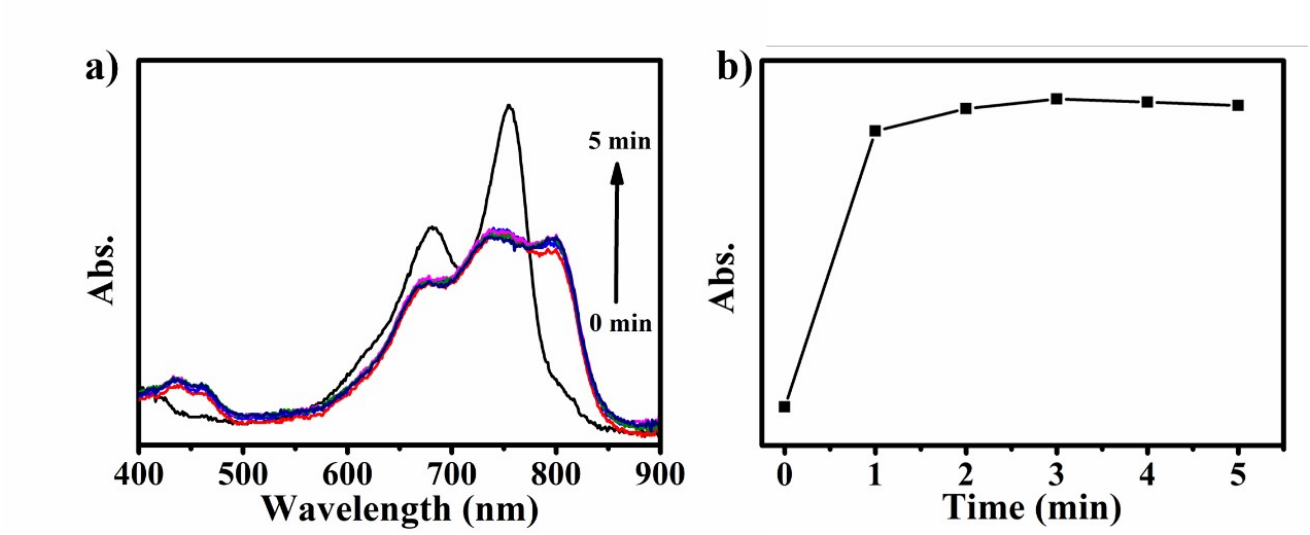


Figure S31. Time-dependent absorption spectra (a) and plots of absorbance at 808 nm (b) of **CyOALP** toward 500 $U \cdot L^{-1}$ ALP in aCSF containing 250 μM AA (n=3, S.D.).

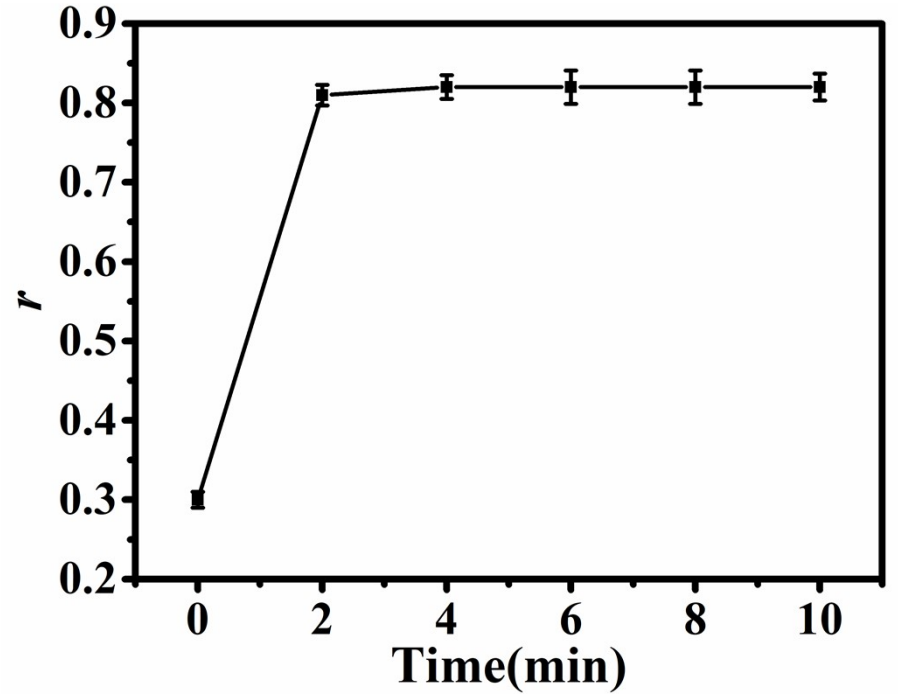


Figure S32. Time dependence of *r* values (j_{808}/j_{750}) of the **CyOALP**/TiWE toward 500 U·L^{-1} ALP in aCSF containing 250 μM AA (n=3, S.D.).

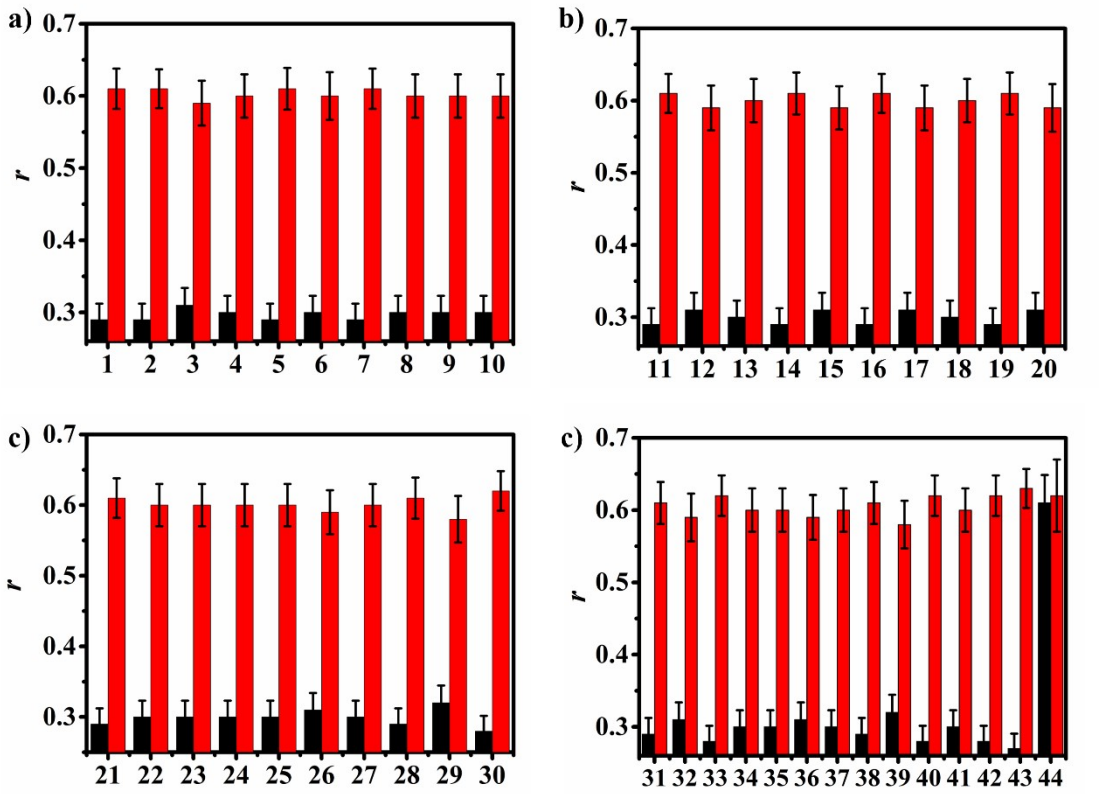


Figure S33. Selectivity (black) and competition (red) tests for ALP (500 $U \cdot L^{-1}$) obtained with **CyOALP**/TiWE: (a) 1, Cu^{2+}; 2, Ca^{2+}; 3, Na^{+}; 4 Mg^{2+}; 5, K^{+}; 6, Cu^{+}; 7, Mn^{2+}; 8, Fe^{3+}; 9, Zn^{2+}; 10, Fe^{2+} (3 mM for K^{+}, 150 mM for Na^{+}, 1 mM for $Ca^{2+}$and Mg^{2+}, 50 µM for Mn^{2+} and 10 µM for others metal ions); (b) 11, Glu; 12, Gly; 13, His; 14, Leu; 15, Iso; 16, Try; 17, Val; 18, Met; 19, NMDA; 20, DIDS; (20 µM for Glu, Gly, His, Leu, Iso, Try, Val, Met, 50 µM for Lys, Arg and Thr; 1 mM for NMDA and DIDS); (c) 21, NO_3^-; 22, Cl^-; 23, HCO_3^-; 24, $S_2O_3^{2-}$; 25, Na_2S_2; 26, SO_4^{2-}; 27, ClO^-; 28, $ONOO^-$; 29, OH; 30, O_2^- (100 µM for NO_3^-, 0.1 M for Cl^-, 50 mM for HCO_3^-, 1 mM for GSH and Hcy, 10 µM for other species); (d) 31, AA; 32, UA; 33, DA; 34, ATP; 35, glucose; 36, H_2O_2; 37, NADPH; 38, NADH; 39, GSH; 40, Cys; 41, Hcy, 42, Horseradish Peroxidase, 43, galactosidase 44, ALP, (500 $U \cdot L^{-1}$ Horseradish Peroxidase and galactosidase; 30 µM for H_2O_2; 200 µM for AA, 10 mM for glucose, 1000 $U \cdot L^{-1}$ for ALP and 50 µM for other species).

Reference

1 Liu, Z. *et al. Angew. Chem., Int. Ed.* 2021, doi:10.1002/anie.202101468.
2 Dong, H., Zhou, Q., Zhang, L. & Tian, Y. *Angew. Chem., Int. Ed.* 2019, **58**, 13948-13953.