Electronic Supplementary Information (ESI)

A Highly Selective Ratiometric Near-infrared Fluorescent Cyanine Sensor for Cysteine with Remarkable Shift and Its Application Bioimaging

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Table of Contents

Table S1. Chemical shifts of alkene-H in **IR-780**, **CyAK** and **CyAC**

Figure S1. Time dependence of **CyAC** (5.0 μM) with the addition of 50 μM Cys in absorption and emission spectra

Figure S1. Absorbance responses of CyAC with all kinds of amino acids

Figure S3. (A) Excitation spectra of **CyAC** (5.0 μM) with the titration of Cys (λ_{ex}=780 nm); (B)Absorption spectra and (C) Emission spectra change of **CyAC** as a function of concentration Cys (0-25μM)

Figure S4. Spectra proprieties of **CyAC** (5.0 μM) with 50 μM Hcy

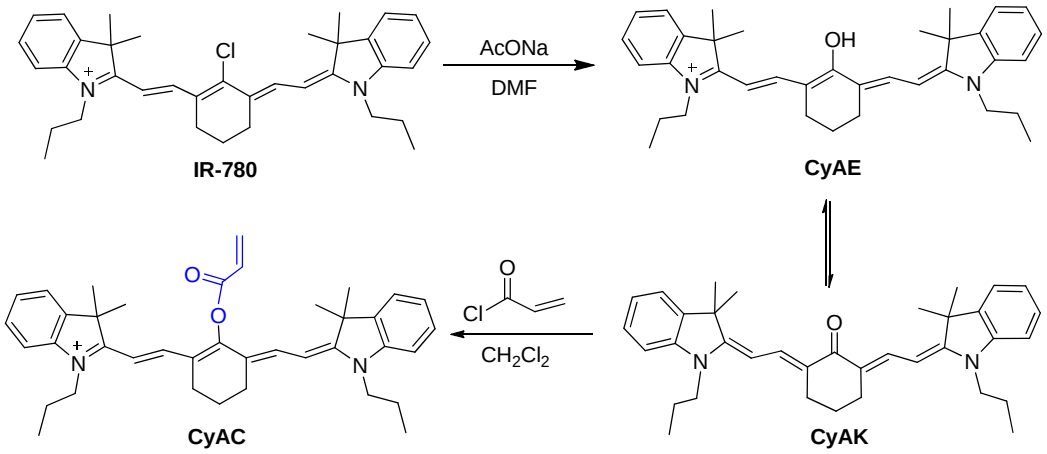
Figure S5. Spectra proprieties of **CyAC** (5.0 μM) with 50 μM GSH

Figure S6. Kinetic Studies of **CyAC** with Cys, Hcy, and GSH.

General Information: Unless otherwise noted, materials were obtained from Aldrich and were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker AM-300 spectrometers. ^{1}H NMR and ^{13}C NMR in $CDCl_3$ were measured on a Bruker AM-300 spectrometer with tetramethylsilane (TMS) as internal standard. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). UV−vis spectra were obtained using a Scinco 3000 spectrophotometer (1 cm quartz cell) at 25 °C. Fluorescence spectra were recorded on RF-5301/PC (Shimada) fluorescence spectrophotometer (1 cm quartz cell) at 25 °C. Deionized water was used to prepare all aqueous solutions.

Methods for cell culture and fluorescent imaging

Human breast carcinoma (MCF-7) cells were seeded on 18 mm-glass coverslips (Marienfeld, Lauda-Koenigshofen, Germany) at density 2×10^5 cells and cultured in McCoy's 5a media with 10% bovine calf serum and 26 mM sodium carbonate at 37 ºC in a humidified incubator containing 5% CO_2 and 95% air. In order to induce oxidative stress, cells were rinsed three times with phosphate-buffered saline (PBS) and incubated in glucose-free DMEM (Dulbecco's Modified Eagle Media) without antibiotics and bovine calf serum for 2 h.[17] After the incubation, MCF-7 cells were rinsed with PBS and then incubated with 5 μM of CyAc for 30 min at RT. The treated cells were washed with PBS and mounted onto a glass slide with ClearMount™ aqueous mounting medium (Invitrogen). To visualize the NIR fluorescence a zenon lamp (Hamamatsu, Shizuoka, Japan; 75 watt) and cy7 filter cube (Semrock, Rochester, NY; Ex. 660 - 750 nm/Em. 760 - 855 nm) was used in comparison with Hg^{2+} lamp (Nikon; 100 watt) and Nikon filter cube (G-2A; Ex. 510 - 560 nm/Em. 590 nm) for 535 nm absorption peak of **CyAc**. Fluorescent images of the mounted cells were obtained by using an inverted microscope (Nikon Eclipse TE2000-U) at various magnifications (100 × to 200 ×).



Cyanine-Keto (CyAK)

A solution of IR-780 (300 mg, 0.45 mmol) and sodium acetate (100 mg, 1.2 mmol) in in anhydrous *N, N*-dimethylformamide (15 ml) was heated about 80 ^{o}C for 6 hr under N_2 atmosphere. After mixture solution was cooled and then quenched with solid carbon dioxide, a crude mixture was filtered and the solution was concentrated by rotary evaporation to obtain red oil product. Finally, ketone dye **CyAK** (150 mg) was isolated by silica chromatography eluting with CH_2Cl_2/CH_3OH, up to 15% of methanol. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ = 1.02 (t, *J* = 7.5 Hz, 6H, $NH(CH_2)_2C\underline{H_3}$), 1.68 (s, 12H, -$CH_3$), 1.73-1.90(m, 6H, $NHCH_2C\underline{H_2}CH_3$ and cyclohexane-*H*), 2.62 (t, *J* = 5.7 Hz, 4H, cyclohexane-*H*), 3.65 (t, *J* = 7.2 Hz, 4H, $NHC\underline{H_2}CH_2CH_3$), 5.48 (d, *J* = 13.5 Hz, 2H, alkene-H), 6.69 (d, *J* = 7.8 Hz, 2H, Ph-H), 6.91(t, *J* = 7.5 Hz, 2H, Ph-H), 7.18-7.22 (m, 4H, Ph-H), 8.18 (d, *J* = 13.5 Hz, 2H, alkene-H). ^{13}C-NMR (75 MHz, $CDCl_3$, ppm): *δ* = 11.75, 19.75, 22.59, 25.84, 28.79, 44.11, 46.52, 92.52, 106.71, 120.37, 121.74, 126.49, 127.59, 132.83, 139.66, 144.40, 162.37, 186.30.
Mass: $[C_{36}H_{44}ON_2 + H]^+$ (FABs) positive mode Calculated : **521.3532**; Find: **521.3530**.

Cyanine-Acryoyl (CyAC)

To a solution of CyAC (100 mg, 0.19 mmol) and *N,N*-Diisopropylethylamine (0.3ml) in 15 mL of CH_2Cl_2 at 0 ^{o}C, then acryloyl chloride (0.1 ml, mixed with 5 mL of CH_2Cl_2) was added dropwise and kept stirring at this temperature 30 min. Then the mixture was warmed to rt and stirred overnight. The solution was diluted with CH_2Cl_2 (30 mL) for extraction operation. The solvent was removed in vacuo to obtain a crude mixture deep blue solid. Finally, **CyAC** was isolated by silica chromatography eluting with CH_2Cl_2/CH_3OH, from 100/1 to 20/1 as a deep blue solid (40 mg, 38 % yield). ^{1}H NMR (300 MHz, CD_3OD, ppm): δ = 1.05 (t, *J* = 7.5 Hz, 6H, $NH(CH_2)_2C\underline{H_3}$), 1.61 (s, 12H, -$CH_3$), 1.75-1.86 (m, 6H,

NHCH$_2$C$\underline{H_2}$CH$_3$ and cyclohexane-*H*), 2.71(t, *J* = 6.0 Hz, 4H, cyclohexane-*H*), 4.13 (t, *J* = 7.5 Hz, 4H, NHC$\underline{H_2}$CH$_2$CH$_3$), 6.23 (d, *J* = 14.0 Hz, 2H, alkene-H), 6.14 (d, 1H, J_1 = 1.2 Hz, J_2= 10.5 Hz, alkene-H), 6.66 (m, 1H, alkene-H), 6.87 (dd, 1H, J_1 = 1.2 Hz, J_2= 17.3 Hz, alkene-H),7.27 (td, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 2H, Ph-H), 7.31(d, *J* = 7.5 Hz, 2H, Ph-H), 7.42 (td, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 2H, Ph-H), 7.48 (d, J_1 = 7.5 Hz, 2H, Ph-H), 7.79(d, *J* = 14.0 Hz, 2H, alkene-H).^{13}C-NMR (75 MHz, CD_3OD, ppm): δ = 10.28, 20.44, 20.73, 23.83, 27.03, 45.17, 49.05, 78.10, 100.23, 110.86, 121.41, 122.07, 125.00, 126.75, 128.48, 134.78, 140.27, 141.04, 142.31, 159.54, 163.54, 172.34.

Mass: $[C_{39}H_{47}O_2N_2I - I]^+$ (FABs) positive mode Calculated: **575.3638;** Find: **575.3635**

Reaction CyAC with the addition of Cys for CyAK

CyAC (20 mg) and Cys (10eq) was mixed in ethanol (5ml), and stirred for 3 hr at room temperature. The color of solution changed from deep blue to red. Next, we checked our product using thin layer chromatography (TLC) compared with above CyAK. Moreover, the final product was purified by silica chromatography eluting with CH_2Cl_2/CH_3OH. The final product CyAK is also confirmed by ^{1}H NMR. ^{1}H NMR (300 MHz, CD_3OD, ppm): δ = 1.02 (t, *J* = 7.5 Hz, 6H, NH(CH$_2$)$_2$C$\underline{H_3}$), 1.65 (s, 12H, -CH$_3$), 1.84-2.02(m, 6H, NHCH$_2$C$\underline{H_2}$CH$_3$ and cyclohexane-*H*), 2.61(t, *J* = 5.7 Hz, 4H, cyclohexane-*H*), 3.77 (t, *J* = 7.2 Hz, 4H, NHC$\underline{H_2}$CH$_2$CH$_3$), 5.61 (d, *J* = 13.5 Hz, 2H, alkene-H), 6.86 (d, *J* = 7.8 Hz, 2H, Ph-H), 6.95 (d, *J* = 7.5 Hz, 2H, Ph-H), 7.22 (t, *J* = 7.8 Hz, 2H, Ph-H), 7.27(d, *J* = 7.5 Hz, 2H, Ph-H), 7.99(s, 1H, -OH), 8.20(d, *J* = 13.5 Hz, 2H, alkene-H).

Table S1. Chemical shifts of alkene-H in **IR-780**, **CyAK** and **CyAC in $CDCl_3$**

Compound	**IR-780**	**CyAK**	**CyAC**
δ (ppm)	H_e 8.45	H_a 8.18	H_c 7.79
	H_f 6.32	H_b 5.48	H_d 6.23

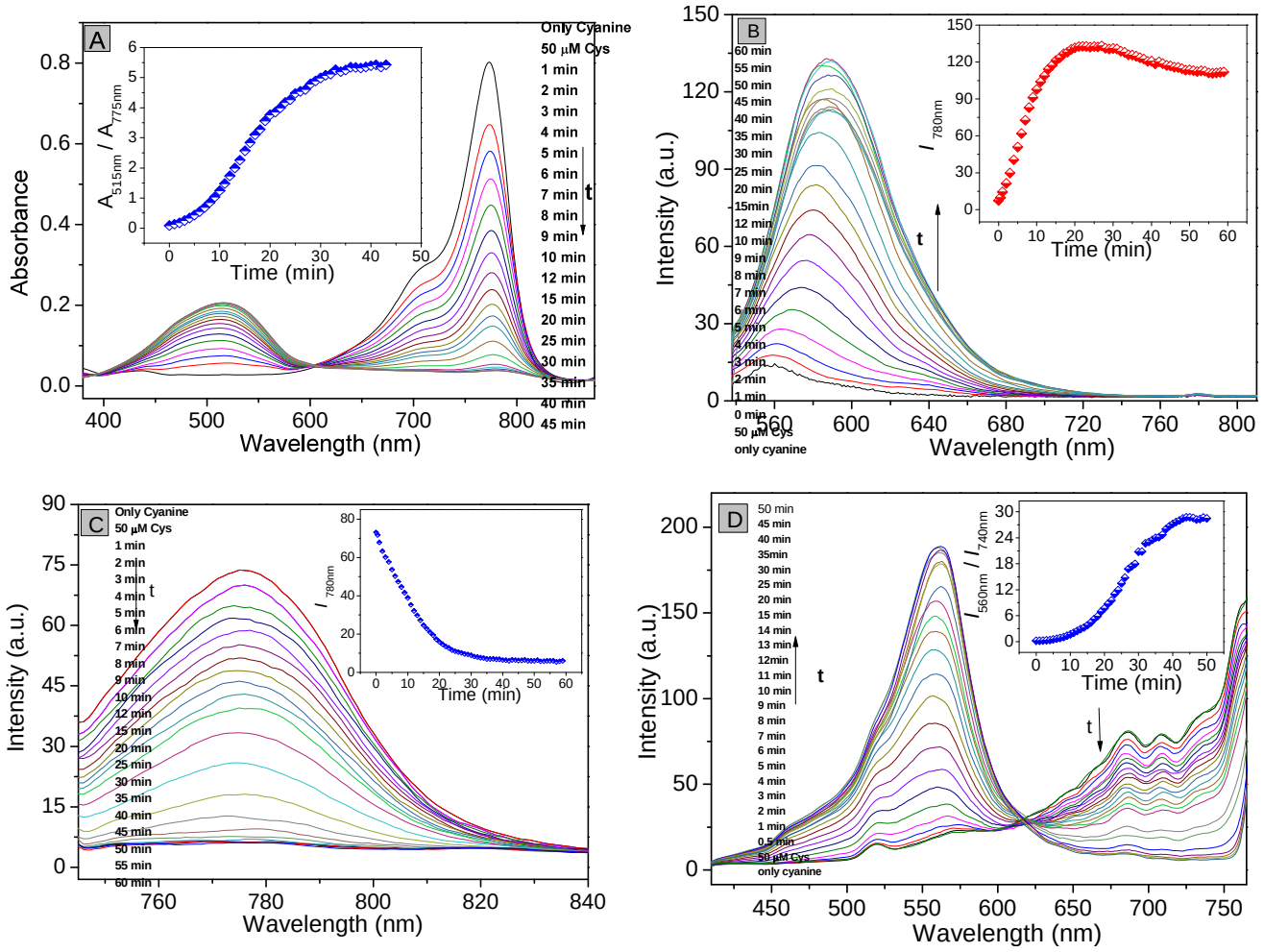


Figure S1. Spectra properities of **CyAC** (5.0 μM) with 50 μM Cys in mixture solution of EtOH-HEPES (1-9, 0.01M, pH = 7.4). (A) Absorption spectra; Inset: A_{515nm}/A_{775nm} with addition of 50μM Cys depending on time; (B) Emission spectra (λ_{ex}=520 nm); Inset: I_{585nm} with addition of 50μM Cys depending on time; (C) Emission spectra (λ_{ex}=720 nm); Inset: I_{780nm} with addition of 50μM Cys depending on time; (D) Excitation spectra with 50 μM Cys (λ_{ex}=780 nm); Inset: $I_{560\ nm}$ / $I_{740\ nm}$ with addition of 50μM Cys depending on time.

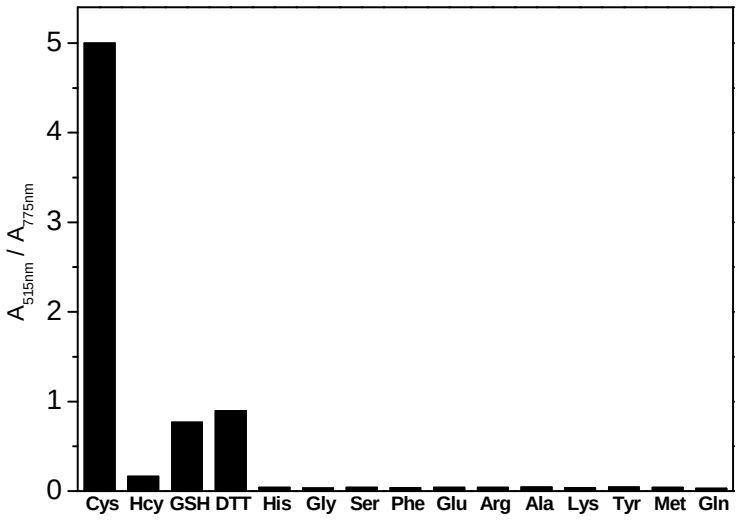


Figure S2. Absorbance responses of CyAC with all kinds of amino acids (50 μM), Cys, Hcy, GSH, Gly, Phe, Ser, Glu, Lys, Arg, His, Ala, Gln, Met, Tyr in mixture solution of EtOH-HEPES (1-9, 0.01M, pH = 7.4), after Each spectrum was recorded at 30 min after the addition to **CyAC**.

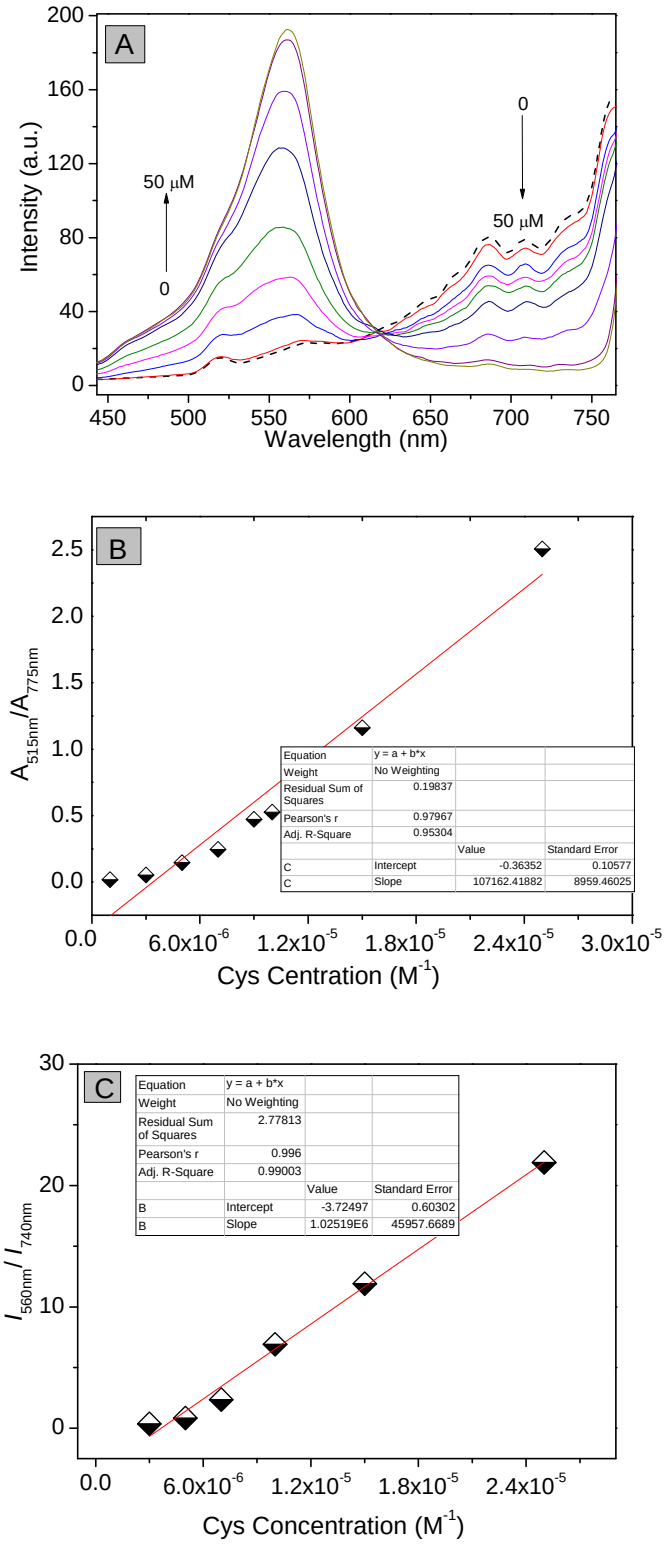


Figure S3.(A) Excitation spectra of **CyAC** (5.0 μM) with the titration of Cys (1, 3, 5, 7, 10, 15, 25, 50 μM) (λ_{ex}=780 nm) in mixture solution of EtOH-HEPES (1-9, 0.01M, pH 7.4); (B)Absorption spectra and (C) Emission spectra change of **CyAC** as a function of concentration Cys (0-25μM) in mixture

solution of EtOH-HEPES (1-9, 0.01M, pH = 7.4) . Each spectrum was recorded at 30 min after the addition of Cys to **CyAC**.

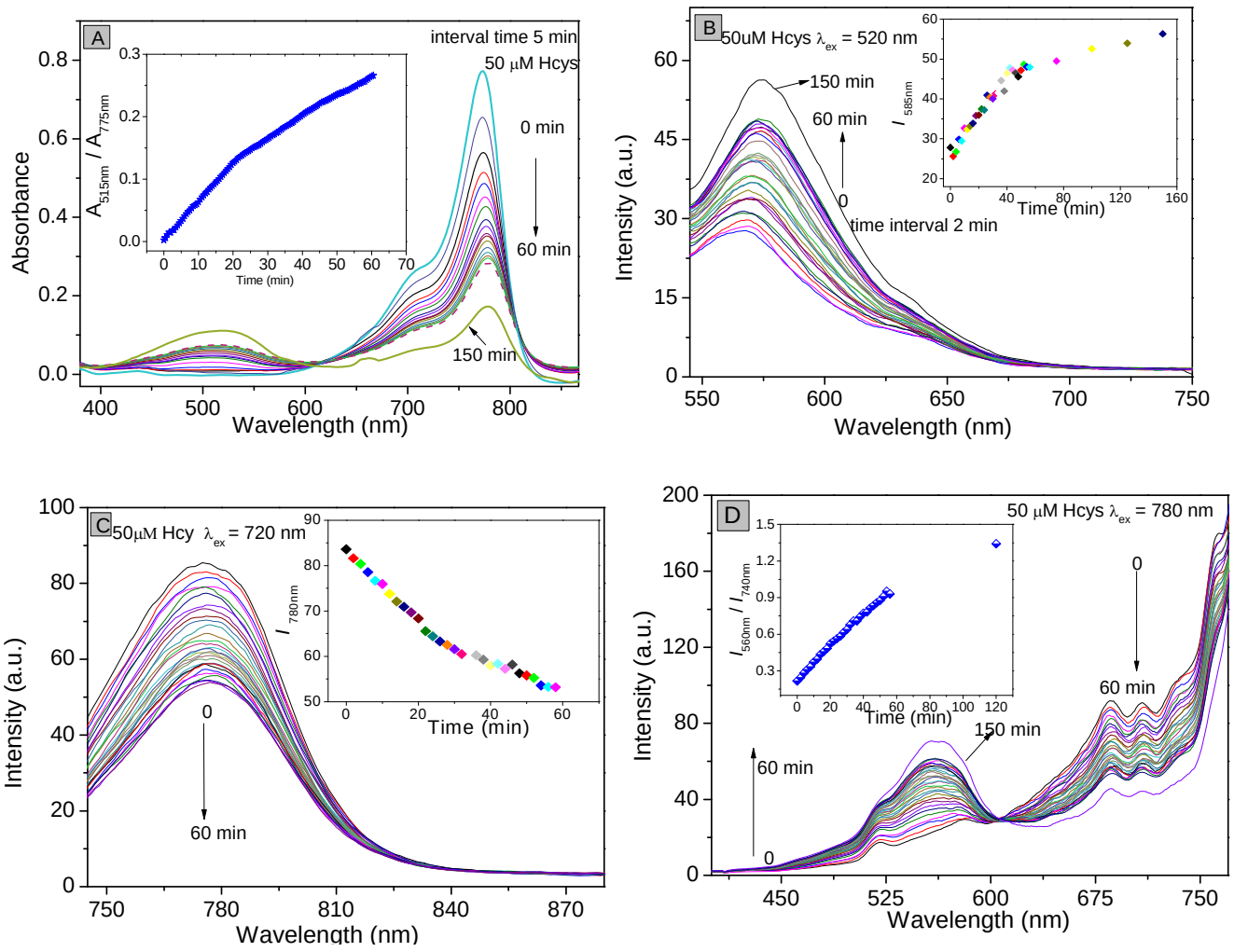


Figure S4. Spectra properities of **CyAC** (5.0 μM) with 50 μM Hcy in mixture solution of EtOH-HEPES (1-9, 0.01M, pH = 7.4). (A) Absorption spectra. Inset: A_{515nm}/A_{775nm} with addition of 50μM Hcy depending on time; (B) Emission spectra. Inset: I_{585nm} with addition of 50μM Hcy depending on time; (C) Emission spectra with 50 μM Hcy (λ_{ex}=720 nm); Inset: I_{780nm} with addition of 50μM Hcys depending on time; (D) Excitation spectra with 50 μM Hcy (λ_{ex}=780 nm); Inset: $I_{560\ nm}$ / $I_{740\ nm}$ with addition of 50μM Hcy depending on time.

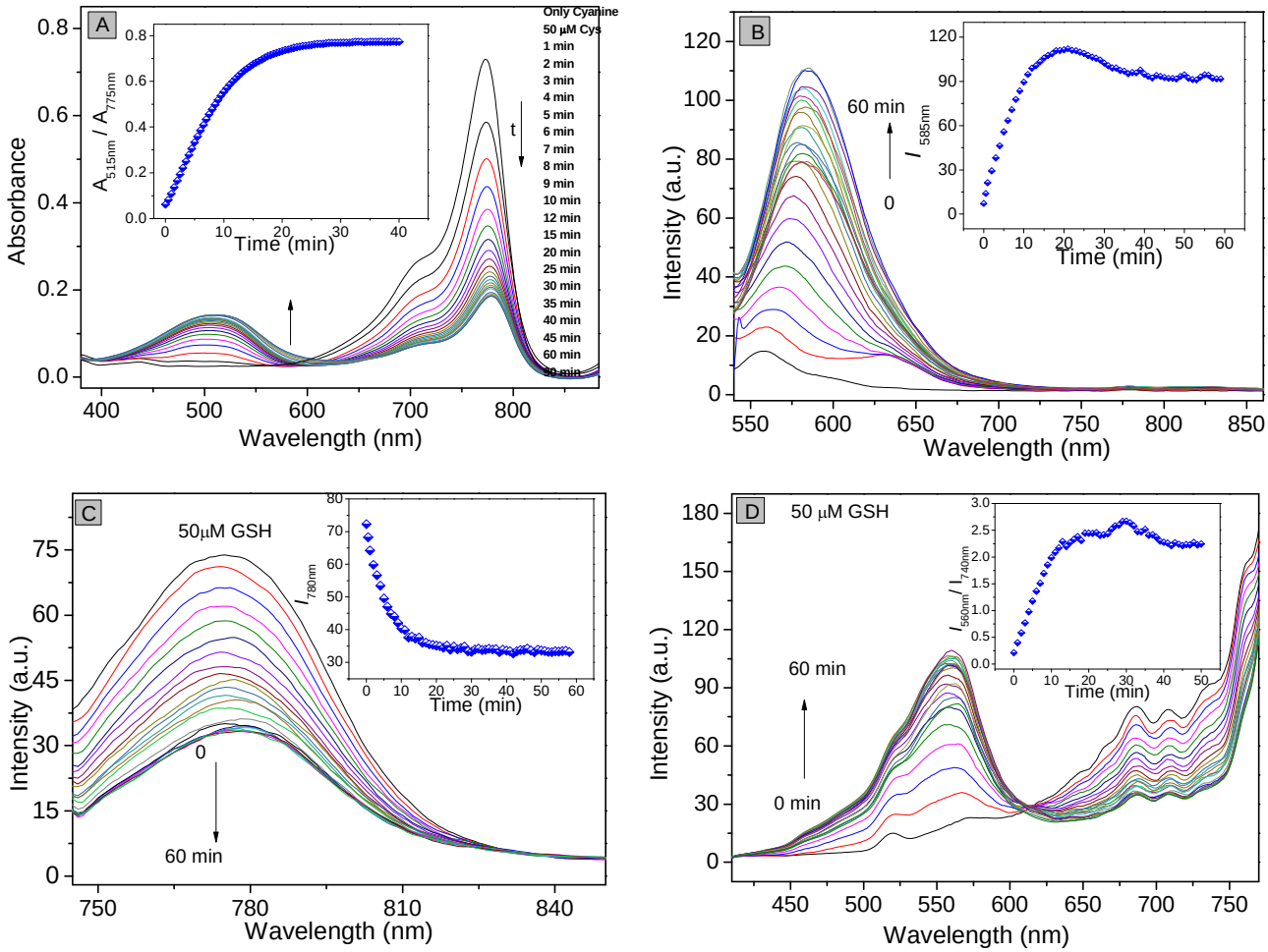


Figure S5. Spectra properities of **CyAC** (5.0 μM) with 50 μM GSH in mixture solution of EtOH-HEPES (1-9, 0.01M, pH = 7.4). (A) Absorption spectra. Inset: A_{515nm}/A_{775nm} with addition of 50μM Hcy depending on time; (B) Emission spectra. Inset: I_{585nm} with addition of 50μM GSH depending on time; (C) Emission spectra with 50 μM GSH (λ_{ex}=720 nm); Inset: I_{780nm} with addition of 50μM GSH depending on time; (D) Excitation spectra with 50 μM GSH (λ_{ex}=780 nm); Inset: $I_{560\,nm}/I_{740\,nm}$ with addition of 50μM GSH depending on time.

Kinetic Studies:

The reaction of sensor **CyAC** (5.0 x 10^{-6} M) with Cys, Hcy and GSH in mixed buffer solution of EtOH/H_2O (v/v, 90/10; 10 mM HEPES pH =7.4). Fluorescence intensity ratio $I_{560\ nm}$ / $I_{740\ nm}$ in excitation spectra was monitored using λ_{ex} = 780 nm as excitation wavelength. The apparent rate constant for the reaction was determined by fitting the fluorescence intensities of the samples to the pseudo first-order equation:

$$Ln((R_{max} - R_t) / R_{max}) = - k' t \qquad (S1)$$

Where R_t and R_{max} are the fluorescence intensities ratio $I_{560\ nm}$ / $I_{740\ nm}$ at times t and the maximum value obtained after the reaction was complete. k' is the apparent rate constant. Figure S7 is the pseudo first-order plot for the reaction of **CyAC** with Cys, Hcy and GSH. The negative slope of the line provides the apparent rate constant: k'.

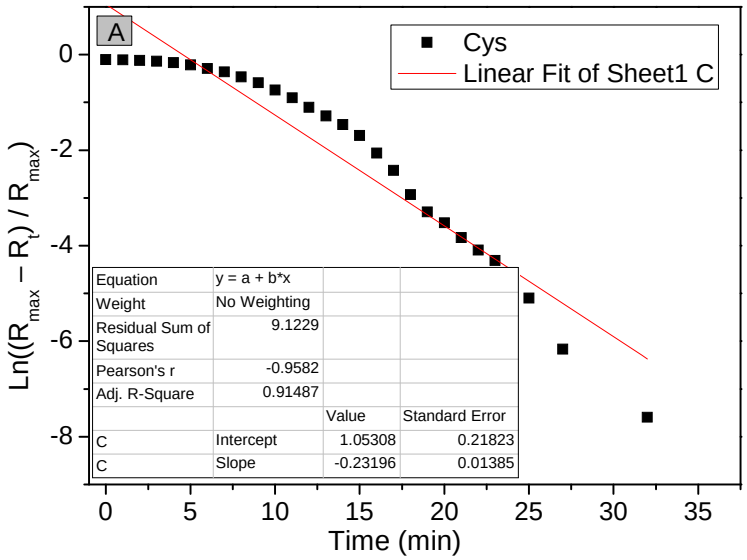


Figure S6A. Pseudo first-order kinetic plot of reaction of **CyAC** (5 x 10^{-6} M) with Cys (5 x 10^{-5} M), slope = -0.23 so k' = -0.23 min^{-1}.

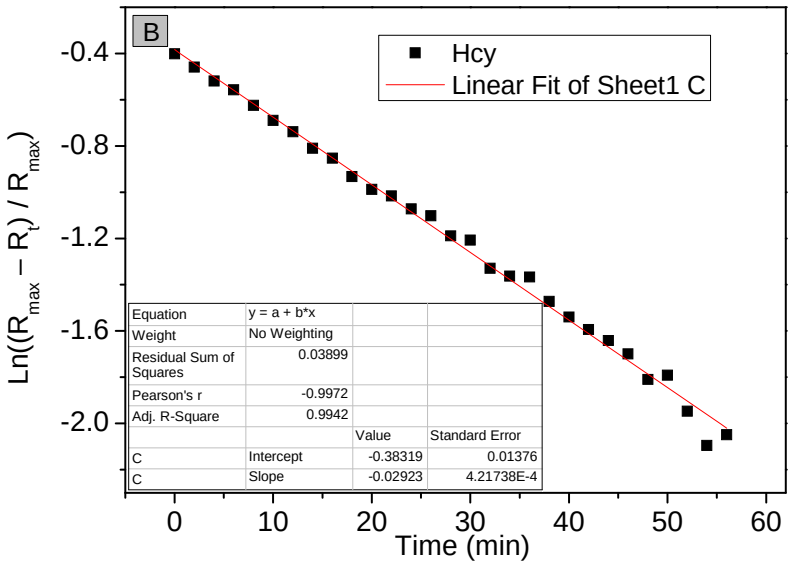


Figure S6B. Pseudo first-order kinetic plot of reaction of **CyAC** (5×10^{-6} M) with Hcy (5×10^{-5} M), slope = -0.029, so k' = -0.029 min^{-1}.

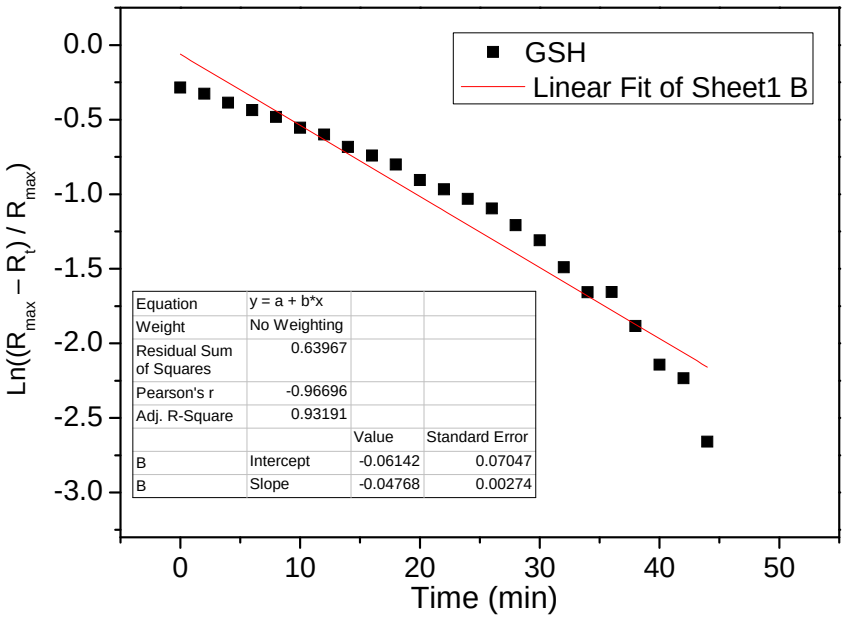
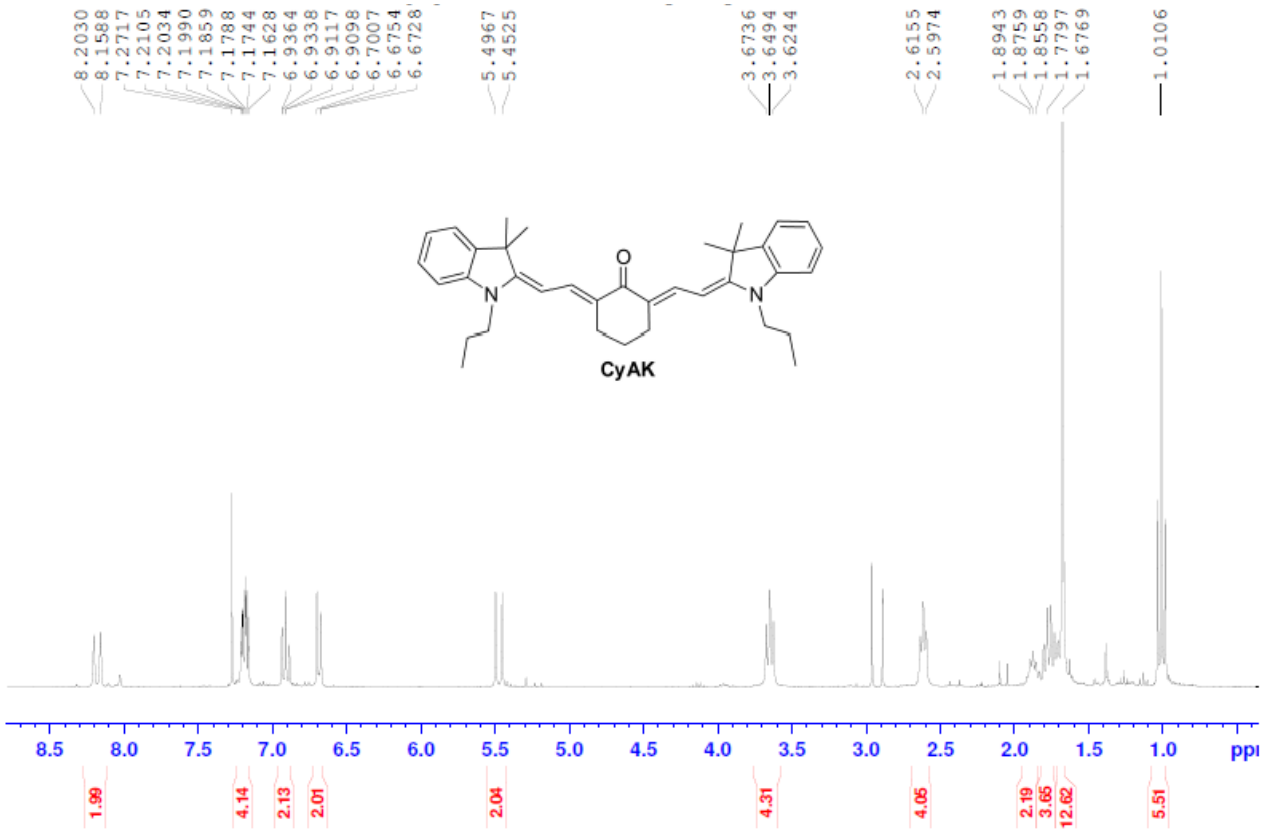
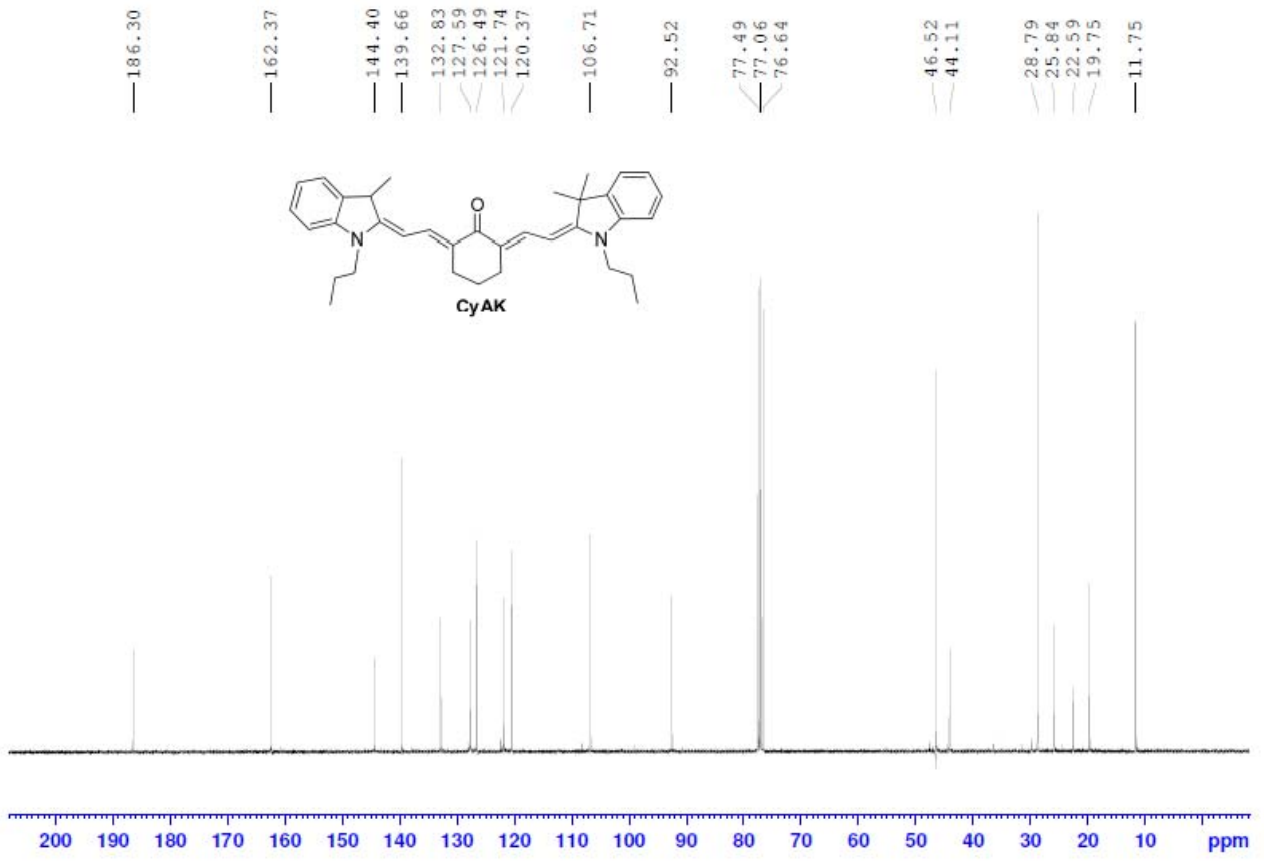
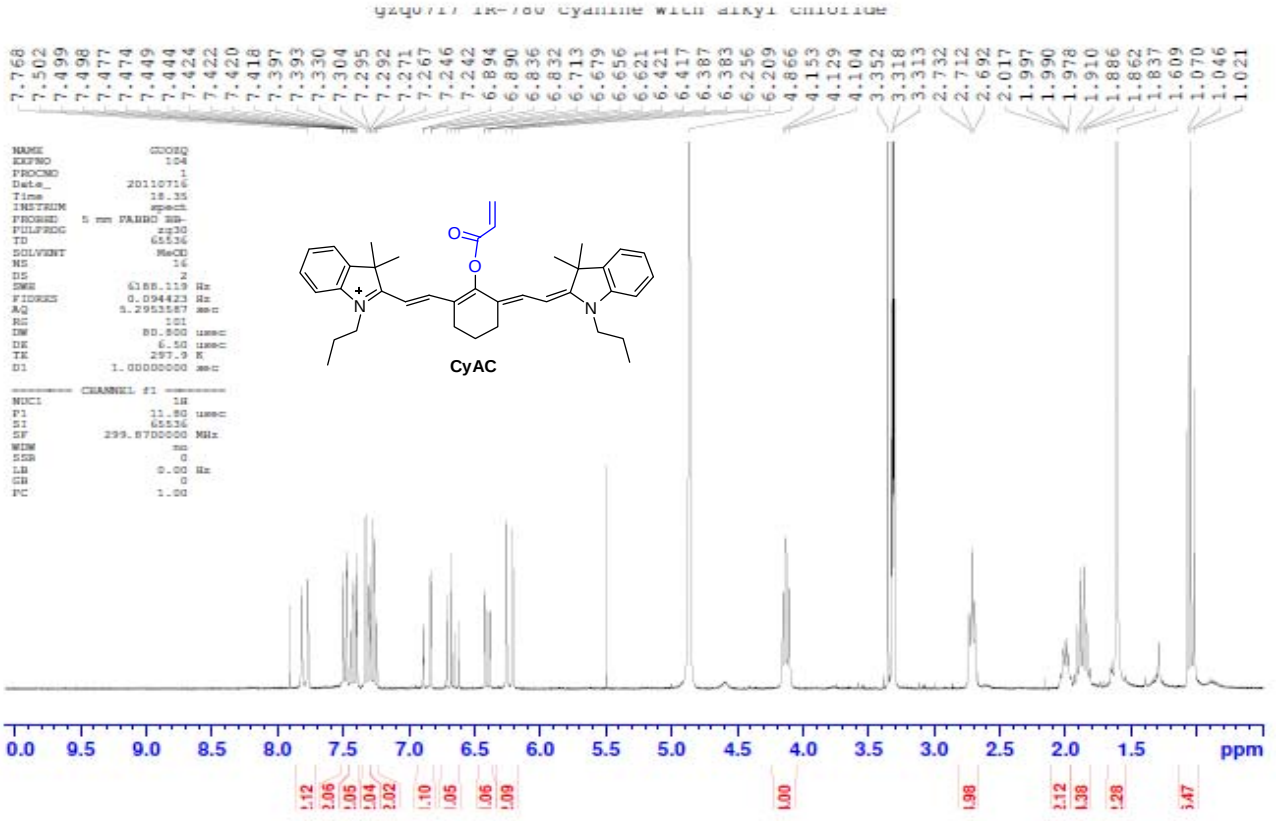
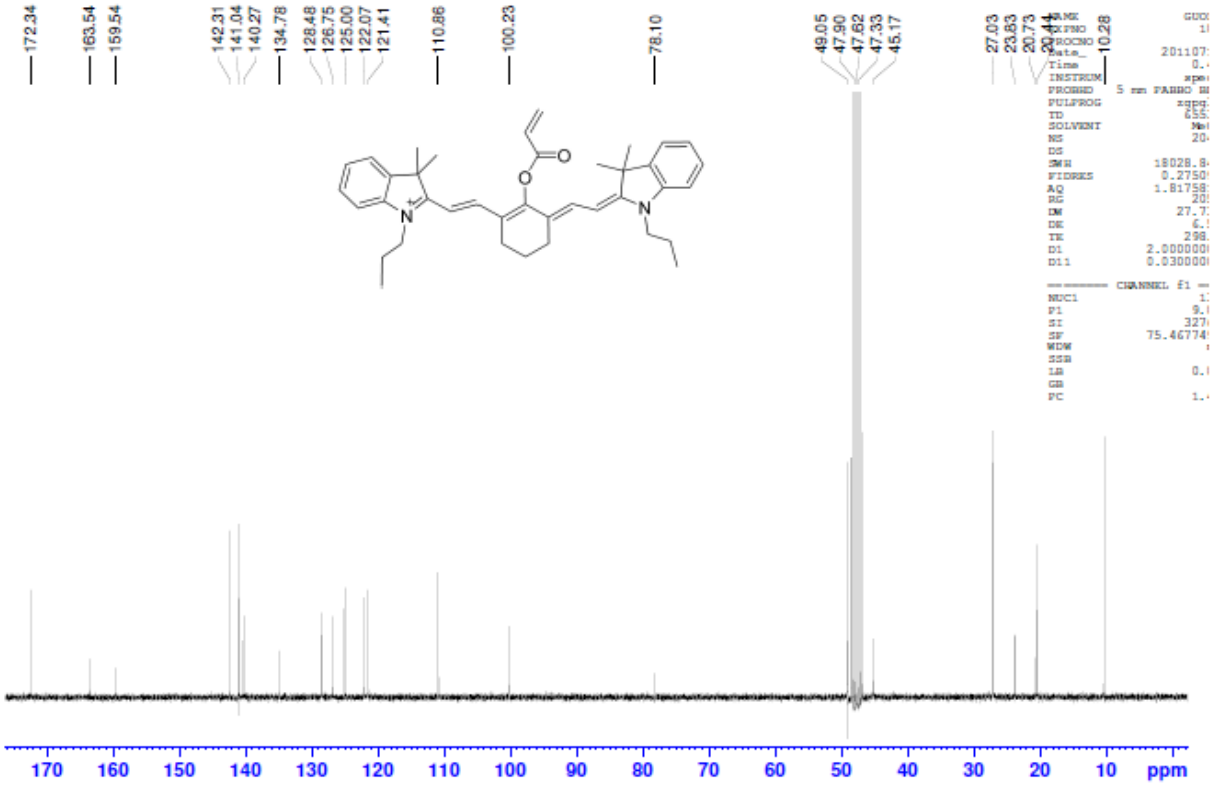


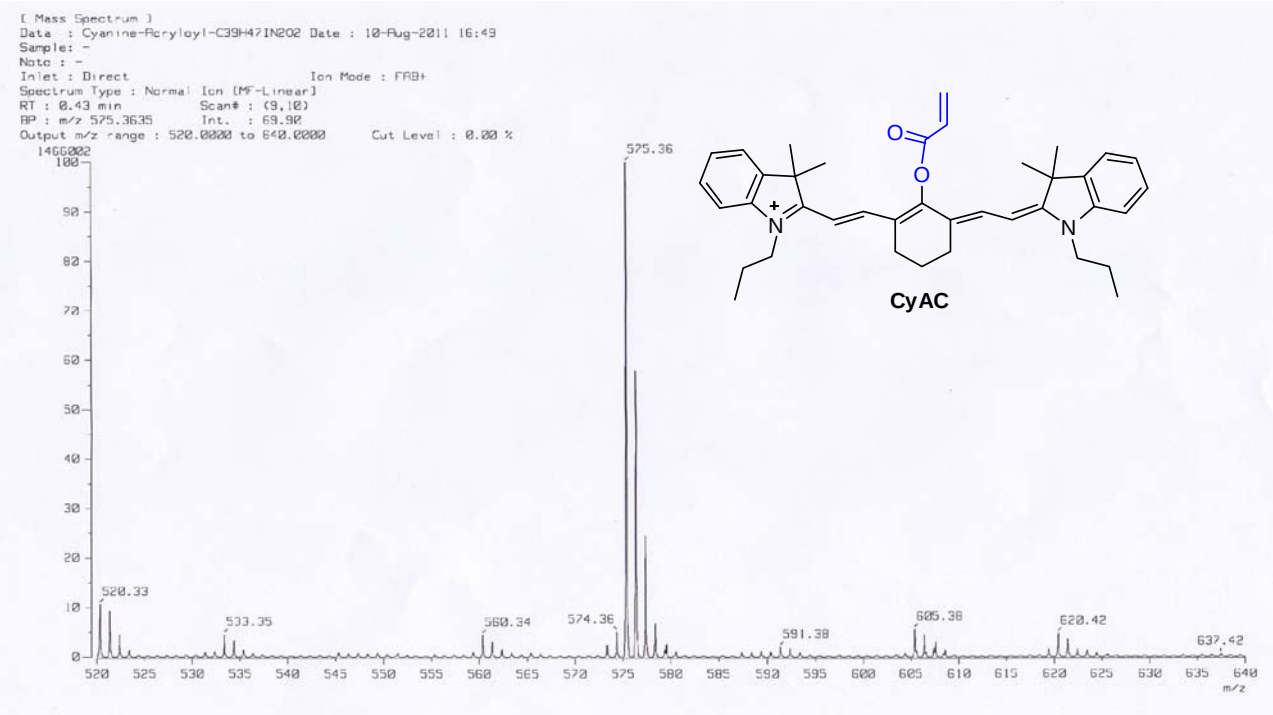
Figure S6C. Pseudo first-order kinetic plot of reaction of **CyAC** (5×10^{-6} M) with GSH (5×10^{-5} M), slope = -0.047, so k' = -0.047 min^{-1}.











Page: 1

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Mass Tolerance : 1000ppm, 1mmu if m/z < 1, 3mmu if m/z > 3
Unsaturation (U.S.) : -0.5 - 200.0

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577.3769	24.5			

Page: 1

[Theoretical Ion Distribution]
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576.3671	44.8931	**********
577.3703	10.2353	******
578.3734	1.5807	*
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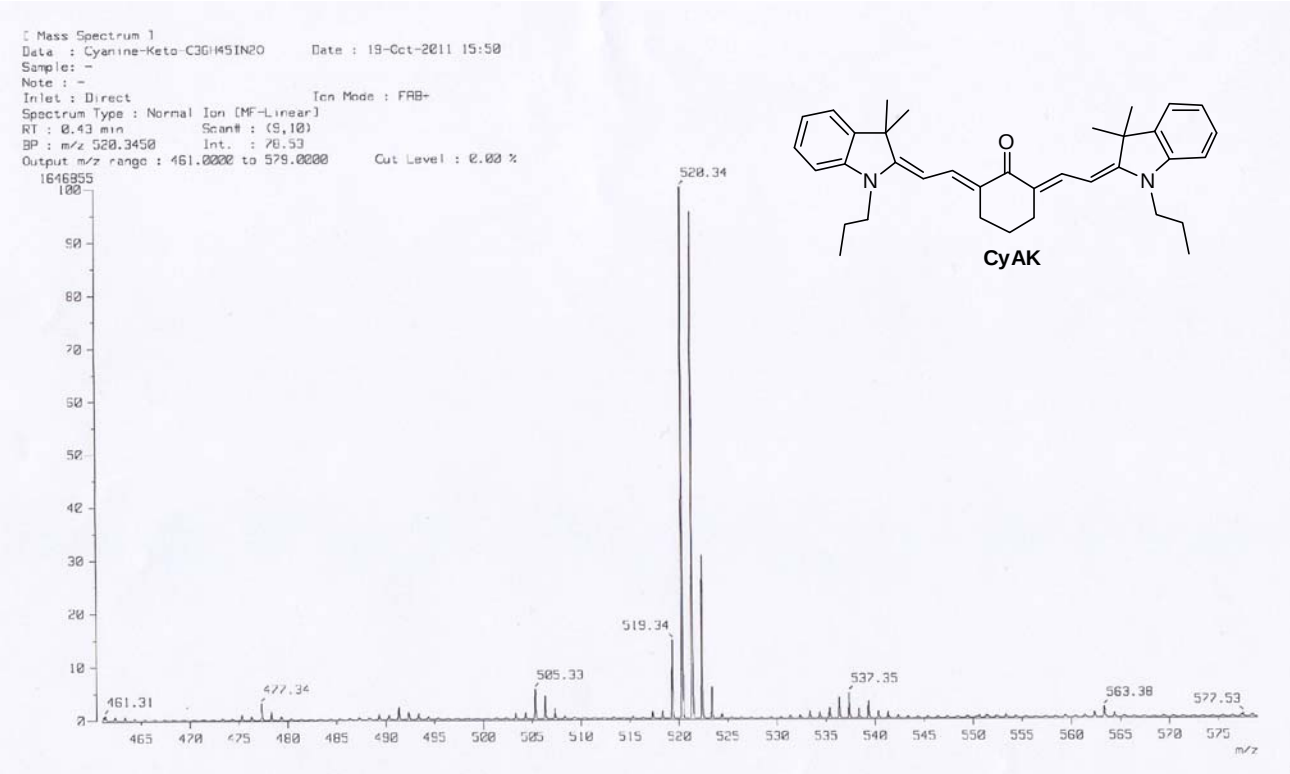


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Unsaturation (U.S.) : -0.5 - 200.0

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  521.3530    95.4     -0.3 /  -0.2    15.5  C 36 H 45 O N 2

  522.3582    30.7
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[ Theoretical Ion Distribution ]                                Page: 1
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