Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A Strategy to Construct Fluorescent Non-aromatic Small-Molecules: Hydrogen Bonds Contributing to the Unexpected Fluorescence†

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Figure S11. Emission spectra of PDH in ethanol/glycerol mixtures with different fractions of glycerol (λ_{ex}=410 nm). All of the concentration of PDH in mixtures is 1×10^{-4} mol/ L

Experimental Section

Cell imaging. Human neuroblastoma SH-SY5Y cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% FBS (fetal bovine serum) and incubated at 37 ℃ in air atmosphere (5% CO_2). For fluorescence imaging, the cells were seeded into glass bottom dishes with appropriate density. After 24 h, the cells were first incubated with 5 μM dye for 30 min at 37 ℃ and washed three times with PBS (pH 7.4) to remove excess extracellular dye.

Crystallographic information. Single colourless block crystals of P were used as supplied. A suitable crystal with dimensions 0.12 × 0.11 × 0.10 mm^3 was selected and mounted on a XtaLAB Synergy, Dualflex, HyPix diffractometer controlled by CrysAlisPro 1.171.39.46 (Rigaku OD, 2018). The crystal was kept at a steady T = 293(2) K during data collection. The structure was solved using SheLXL-2014/7(Sheldrick, 2014) and by using Olex2 (Dolomanov et al., 2009) as the graphical interface. The model was refined with ShelXL-2014/7 (Sheldrick, 2014) using full matrix least squares minimisation on $\boldsymbol{F^2}$. 5659 reflections measured, 1388 unique (Rint = 0.0400) which were used in all calculations.

Quantum chemistry calculation method. B3LYP/6-31G calculations were performed by Gaussian 09, Revision B.01.

Materials. Diethyl malonate, hydrazine hydrate, acethydrazide, oxalyldihydrazide, succinic hydrazide and glutaryl hydrazide were purchased from Aladdin Industrial Corporation (China). Methanol, ethanol, glycol, dimethyl sulfoxide (DMSO), acetonitrile (ACN), tetrahydrofuran (THF), dioxane, cyclohexane and N-hexane were purchased from Sinopharm Chemical Reagent Co., Ltd. All reagents are of analytical reagent grade and used as received.

Apparatus. Cell data of single crystal were measured by Rigaku XtaLAB Synergy diffractometer coupled to a Rigaku Hypix detector with Cu Kα radiation (λ = 1.54184 Å) from PhotonJet micro-focus X-ray sources (Japan). The fluorescence pictures of single crystal were recorded by Nikon A1MP confocal microscopy. Ultraviolet-Visible and photoluminescence spectra were recorded by Shimadzu UV2550 spectrophotometer and Fluoromax-4 spectrophotometer (Horiba JY, France) respectively. Absolute quantum yields were measured by Fluoromax-3 spectrophotometer (Horiba JY, France). Dilute solutions in water were placed in quartz cuvettes (1.0 cm pathlength) using Combined Measurement System for Infrared Fluorescence (NanologR FluoroLog-3-2-iHR320). MS spectra were recorded by

Liquid Chromatograph-Induced Coupled Plasma Mass Spectrometry (Nexion 300x, USA). The ^{1}H-NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer (Swiss), and solvent is DMSO-d6. Fluorescent lifetimes are measured by Steady State and Transient State Fluorescence Spectrometer (FLS980, Edinburgh Instruments, UK). Elemental analyses were determined Vario EL III elemental analyzer (Elementar, Ger.). Electrochemical measurements were carried out with a CHI760D voltammetric analyzer.

Table S1. Crystal data, date collections, and structure refinements of PDH

Compound	**P**
CCDC	CCDC 1968023
Formula	$C_3H_{10}N_4O_3$
$D_{calc.}$/ g cm^{-3}	1.458
μ/mm^{-1}	0.127
Formula Weight	150.15
Colour	colourless
Shape	block
Size/mm^3	0.12×0.11×0.10
T/K	293(2)
Crystal System	monoclinic
Space Group	$P2_1/c$
a/Å	9.1606(6)
b/Å	9.7305(6)
c/Å	7.8691(6)
α/°	90
β/°	102.839(7)
γ/°	90
V/Å^3	683.89(8)
Z	4
Z'	1
Wavelength/Å	0.71073
Radiation type	MoK_α
Θ_{min}/°	3.381
Θ_{max}/°	26.367
Measured Refl's.	5659
Ind't Refl's	1388
Refl's with I > 2(I)	1162
R_{int}	0.0400
Parameters	110
Restraints	0
Largest Peak	0.268
Deepest Hole	-0.180
GooF	1.050
wR_2 (all data)	0.0829
wR_2	0.0791
R_1 (all data)	0.0403
R_1	0.0330

Table S2. Information of hydrogen bonds in PDH crystal

D	H	A	d DA (Å)
N1	H1A	O1	3.2738(16)
N1	H1A	O2	2.9815(15)
N1	H1B	O1	3.0348(17)
N2	H2	O1	2.8210(15)
N3	H3	O1W	2.9033(16)
N4	H4A	O2	2.9778(15)
N4	H4B	O1W	3.1986(17)
O1W	H1WA	N1	2.9114(16)
O1W	H1WB	N4	2.8943(17)

Table S3. Kamlet-Taft parameters of various solvents of PDH solution and their absorption, excitation, emission wavelength, and absolute fluorescent quantum yield (Yf)

Solvent	**ε**	**α**	**β**	**π***	**$\lambda_{Abs.}$(nm)**	**$\lambda_{Ex.}$(nm)**	**$\lambda_{Em.}$(nm)**	**Y_f (%)**
EtOH	24.3	0.86	0.75	0.54	412	410	516	11.74
MeOH	33.0	0.98	0.66	0.60	413	410	525	13.76
Glycol	37.7	0.90	0.52	0.92	422	417	532	36.52
H_2O	80.0	1.17	0.47	1.09	422	426	482	-
ACN	38.0	0.19	0.4	0.75	420	408	475	3.83
Acetone	20.7	0.08	0.43	0.71	419	410	473	4.65
Methylene chloride	8.9	0	0.3	0.82	417	413	476	4.08
Dioxane	2.3	0	0.37	0.55	416	407	465	5.14
THF	7.6	0	0.55	0.55	415	410	467	5.68
DMSO	47.2	0	0.76	1.00	421	417	485	10.03
Cyclohexane	2.0	0	0		418	402	455	0.74

Herein, ε, α, β and π* representing dielectric constant, hydrogen-bond donation, hydrogen-bond acceptation ability[13] and polarizability of solvent.

Table S3. τ_1, τ_2 and τ of PDH in powders, acetone and ethanol

	τ_1 (ns)	τ_1%	τ_2 (ns)	τ_2 %	τ (ns)
powder	0.87	62.35	2.57	37.65	1.51
In ethanol	0.76	83.93	3.26	16.07	1.16
In acetonitrile	0.53	86.80	4.35	13.20	1.03

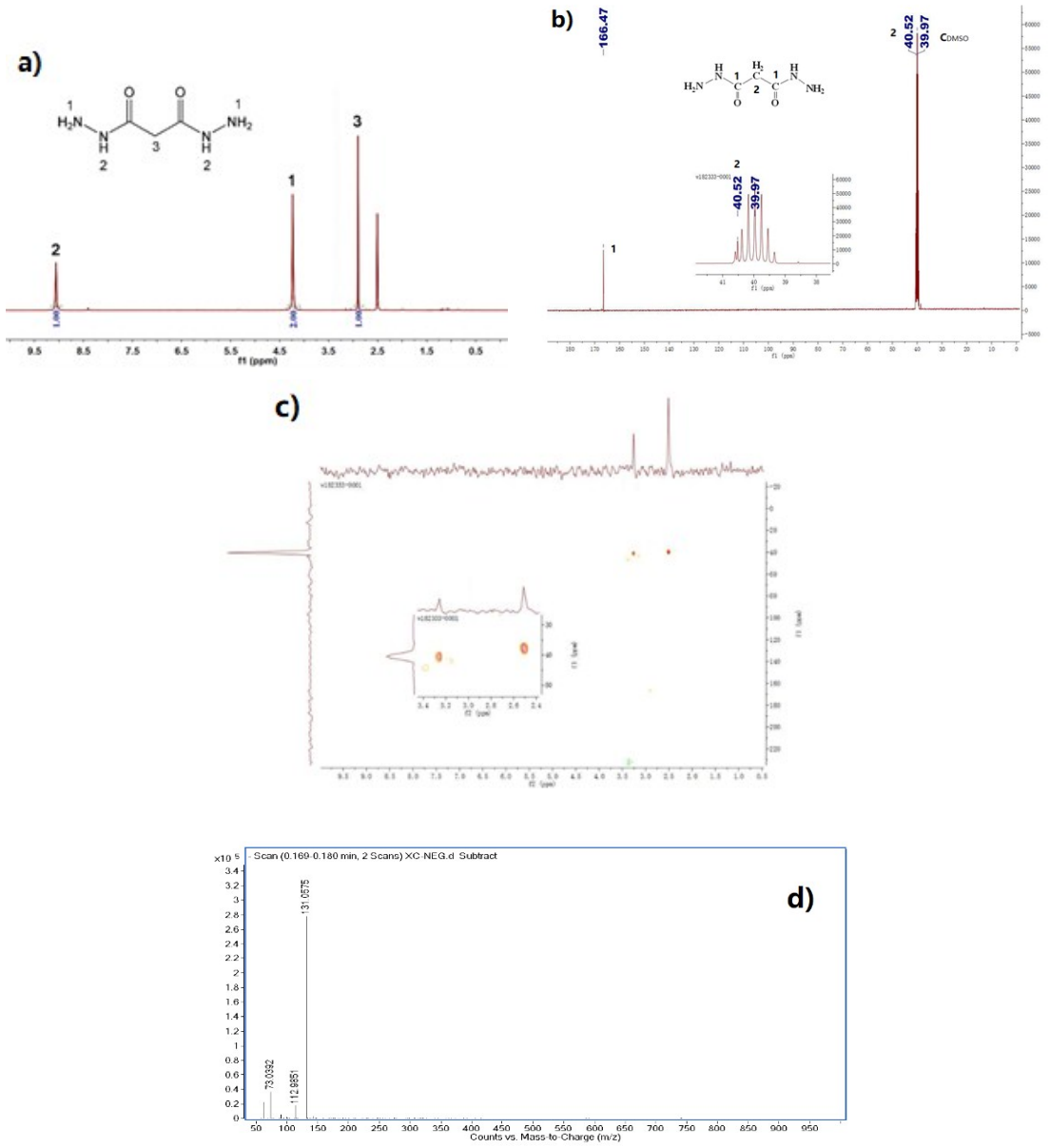


Figure S1. (a) ^{1}H NMR, (b) ^{13}C NMR spectra, (c) heteronuclear single quantum coherence (HSQC) spectrum of PDH in DMSO-d_6. (d) ESI-MS Spectrum of PDH. m/z=131 : $[M-H]^-$

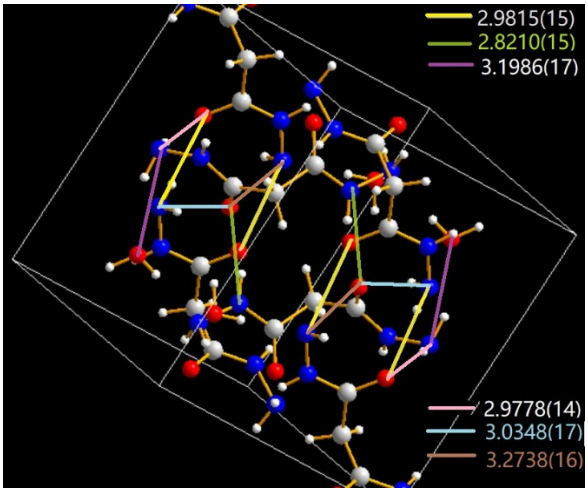


Figure S2. Hydrogen bonds in PDH crystal

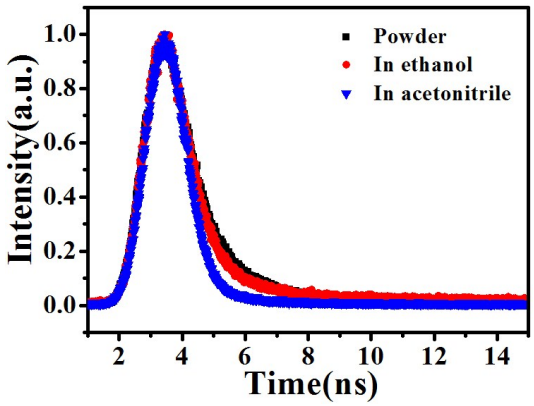
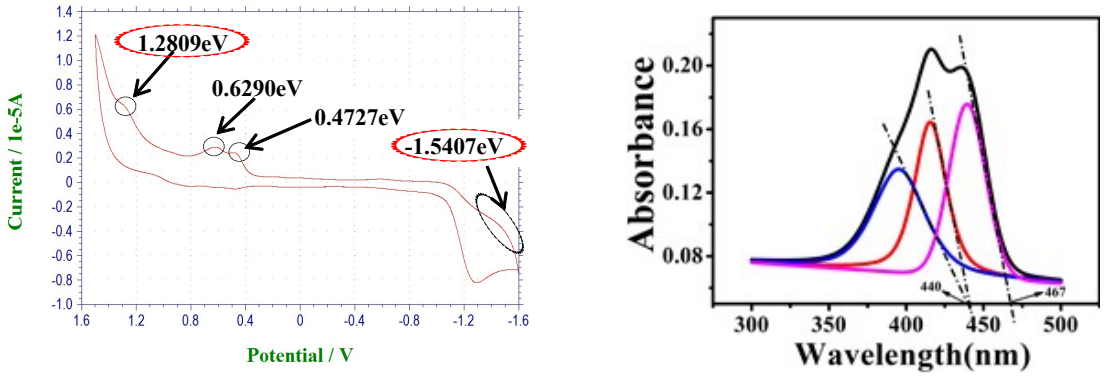


Figure S3. Fluorescent lifetime decay curves of PDH in powders, ethanol and acetonitrile



(a) (b)

Figure S4. (a) redox spectrum of cyclic voltammetry and (b) overlapping peaks of absorption spectrum of PDH in dichloromethane

Figure S4a illustrates that **PDH** indeed loses or gains electrons, though repeatability is not good because there are no obvious redox half-waves in the line below. From **Figure S4a**, energy gap is

$-E_{1/2}^{Oxd1} - (-E_{1/2}^{Red1}) = 1.2809 - (-1.5407) = 2.8217$ eV, corresponding $\lambda = hc/Eg = 1240 / 2.8217 = 439.5$ nm. where, *Eg* is the energy band gap of **PDH**, c is the speed of light, h is Planck's constant and l is obtained from the absorption spectra **Figure S4b**.

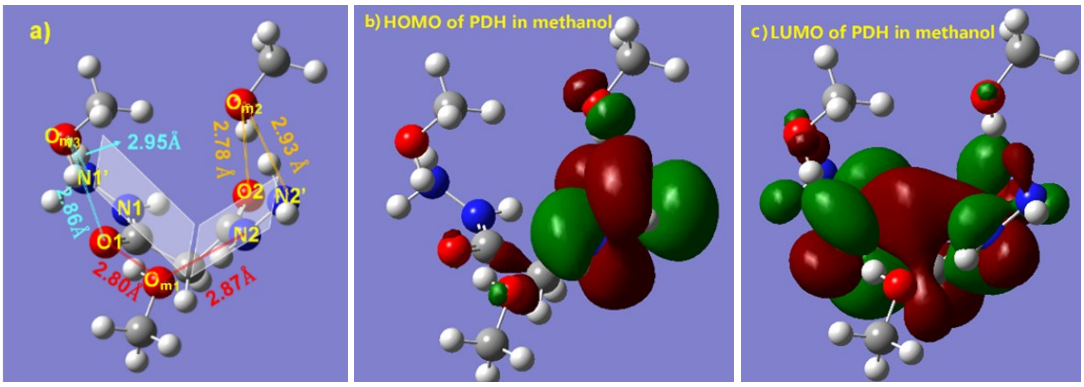


Figure S5. The optimized molecular structure of the PDH methanol solution calculated by RB3LYP TD-FC quantum chemistry calculation method

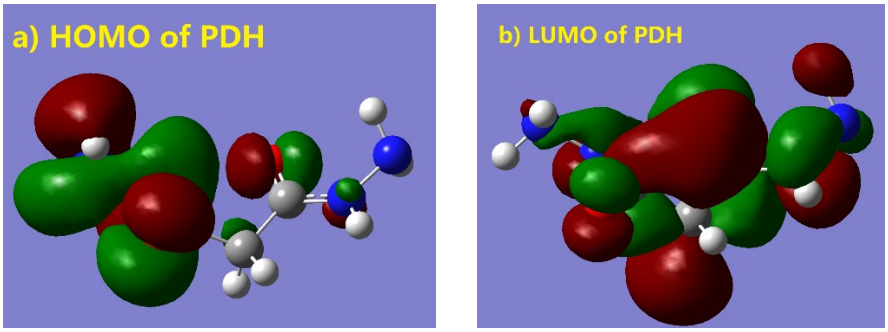


Figure S6. The optimized molecular structure of the PDH calculated by RB3LYP TD-FC quantum chemistry calculation method

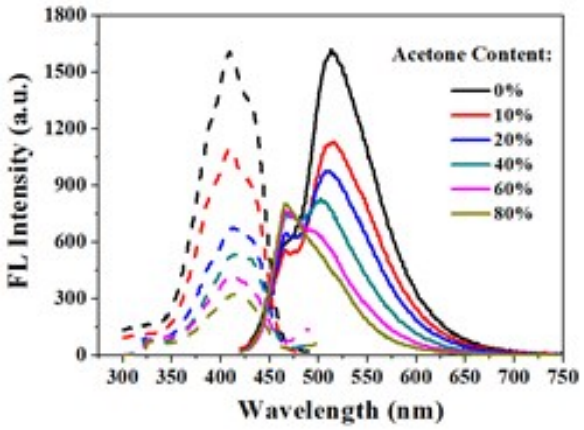


Figure S7. The variation of the emission spectra of PDH in EtOH/Acetone mixtures

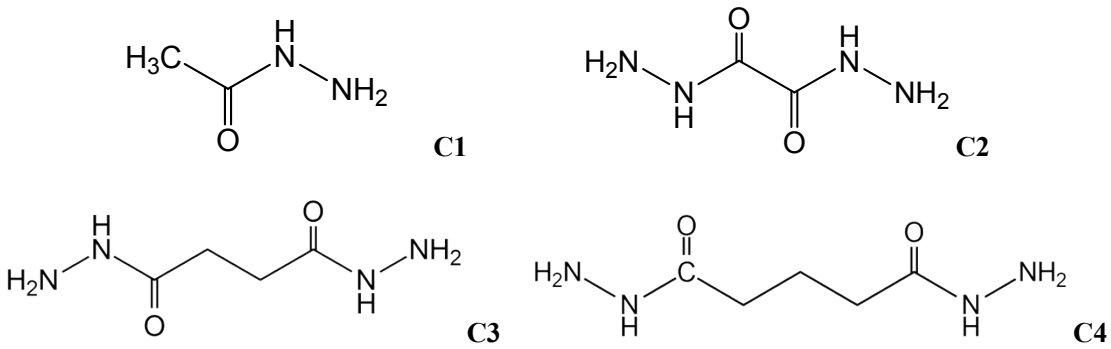


Figure S8. Molecular structures of acethydrazide (**C1)**, oxalyldihydrazide (**C2)**, Succinic hydrazide (**C3**) and glutaryl hydrazide (**C4**)

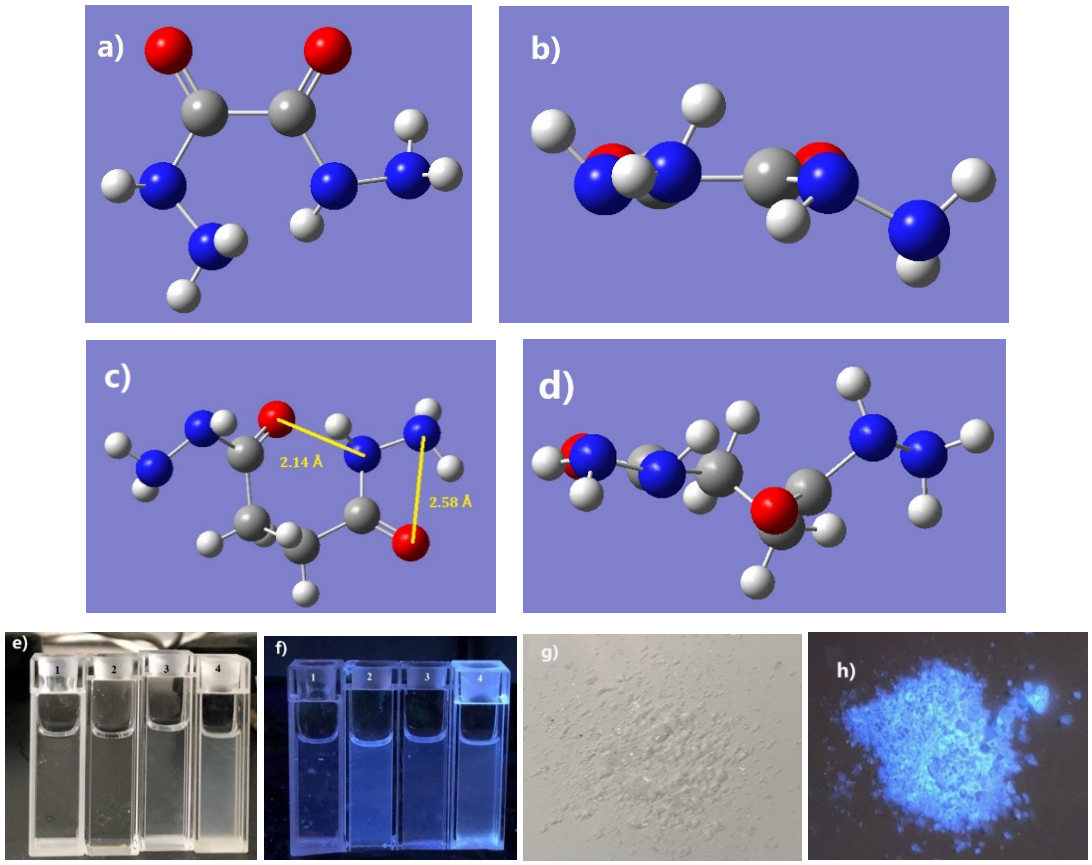


Figure S9. a) Top view, and b) front view of the optimized molecular structure of the **C2**, c) top view, and d) front view of the optimized molecular structure of **C3** calculated by RB3LYP TD-FC quantum chemistry calculation method. e) The picture under daylight and f) fluorescence picture of **C3** solution (in $1H_2O$, 2 DMSO, 3 DMF, 4 MeOH) excited at 365nm, g) the picture under daylight and h) fluorescence picture of **C3** solid excited at 365nm

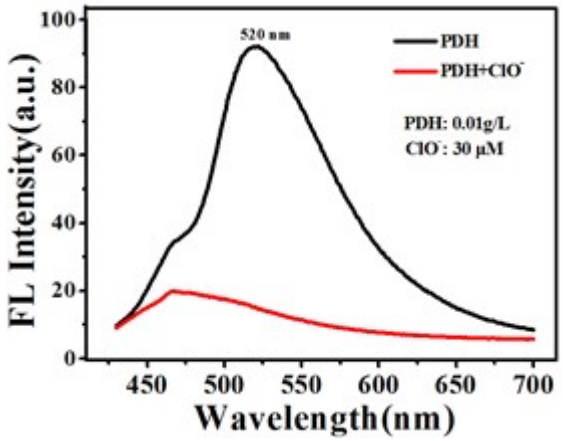


Figure S10. Emission spectra of PDH and PDH+ ClO^- (λ_{ex}=410 nm). The concentration of PDH and ClO^- are 0.01g/ L and 30μm respectively

PDH was dissolved in absolute ethanol and the concentration was adjusted to 0.01 g/L. 2 ml of PDH solution was added to a 3 ml quartz cuvette, and then NaClO (30.0 μM) was added. The mixture was incubated for 3 minutes prior to fluorescence measurement

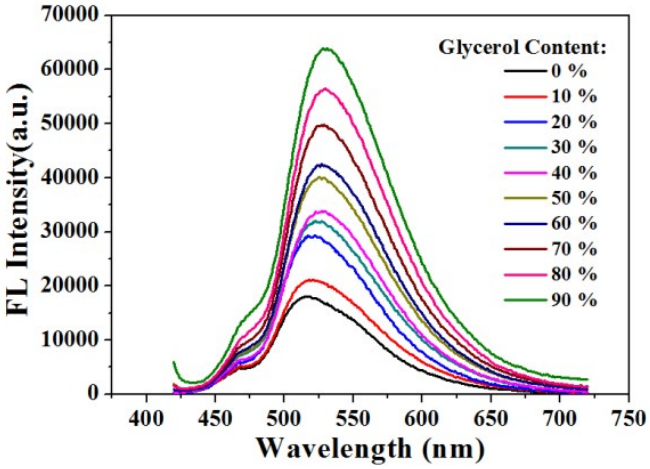


Figure S11. Emission spectra of PDH in ethanol/glycerol mixtures with different fractions of glycerol (λ_{ex}=410 nm). All of the concentration of PDH in mixtures is 1×10^{-4} mol/ L