Supporting Information *for*

Thiol-Ene Click Reaction-Induced Fluorescence Enhancement by Altering Radiative Rate for Assaying Butyrylcholinesterase Activity

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range of 33.3 – 400 μM.

14. **Figure S13**. (a) PL intensity of BChE assay solution (DCPDA 300.0 μM, pH 7.4 HEPES buffer, 5.0 U/L BChE) vs substrate (BTCh) concentration in the range of 0.1 – 0.6 mM during the incubation time of 0 – 30 min. (b) Reaction rate K plotted against substrate concentration [S] for the BChE assay.
15. **Figure S14.** Calibration curve between enzymatic rates K and BChE activity using Ellman's method.
16. **Figure S15.** (a) Time-course enzymatic reaction kinetics of BChE assay in the presence of different concentrations of tacrine ranging from 0.0 to 5.0 μM. (b) Calibration curve between 1/K (min) and tacrine concentration in the range of 1.0 – 5.0 μM.
17. **Figure S16.** Time-course enzymatic reaction kinetics of Ellman colorimetric BChE assay in the presence of different concentrations of tacrine ranging from 0.0 to 5.0 μM.
18. **Table S1**. Comparison between established BChE assay and traditional Ellman method in determining BChE levels of human serum samples.
19. **Cartesian Coordinates**

1. Computational Methods.

All the calculations were performed using Gaussian 09 package.[1] The geometries of all compounds (DCHQ, DCPDA and the product of DCPDA and TGA) were fully optimized with PCM solvent model using THF as the solvent using density functional theory at B3P86/6-31+G(d,p) level.[2] NBO calculations were performed at the same level, and the visualization of HOMO and LUMO were conducted with Gview program.[3] Excited state calculations were run at wB97XD/6-31+G(d,p) level with time-dependent density functional theory (TD-DFT).[4] Electron density difference maps were generated using Gview program.

References

(1) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09, Revision A.02*, Gaussian Inc., Wallingford CT, **2009**.

(2) Zhao, Y.; Truhlar, D. G. *Theor. Chem. Acc.* **2008**, *120*, 215−241.

(3) Weinhold, F. *J. Comput. Chem.* **2012**, *33*, 2363−2379.

(4) Adamo, C.; Jacquemin, D. *Chem. Soc. Rev.* **2013**, *42*, 845−856.

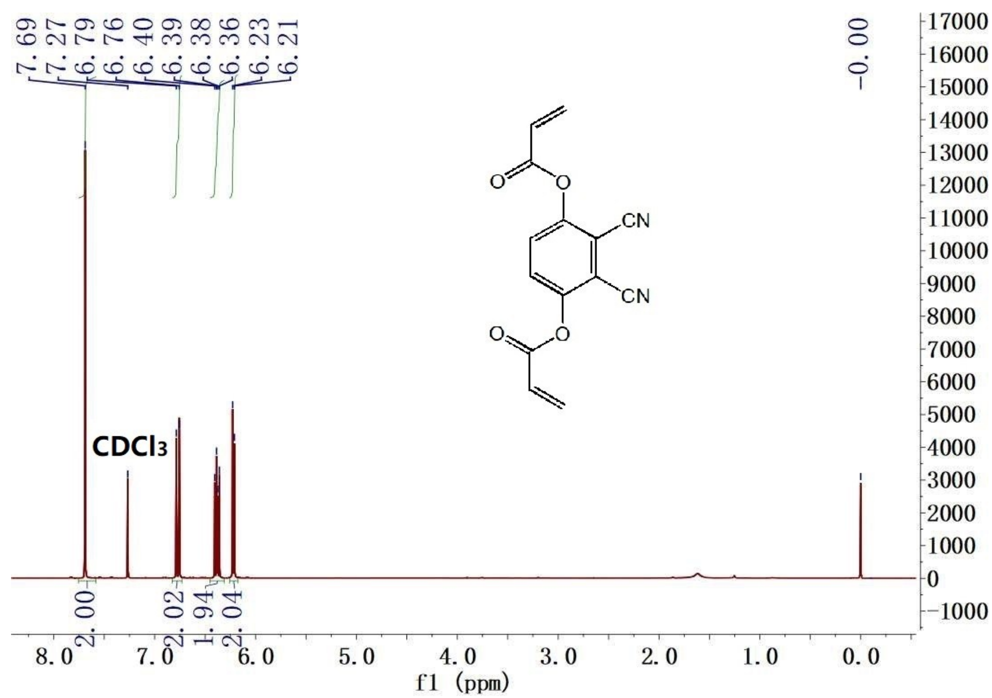

Figure S1. [1]H NMR spectrum of 2,3-dicyano-1,4-phenylene diacrylate (DCPDA) in $CDCl_3$.

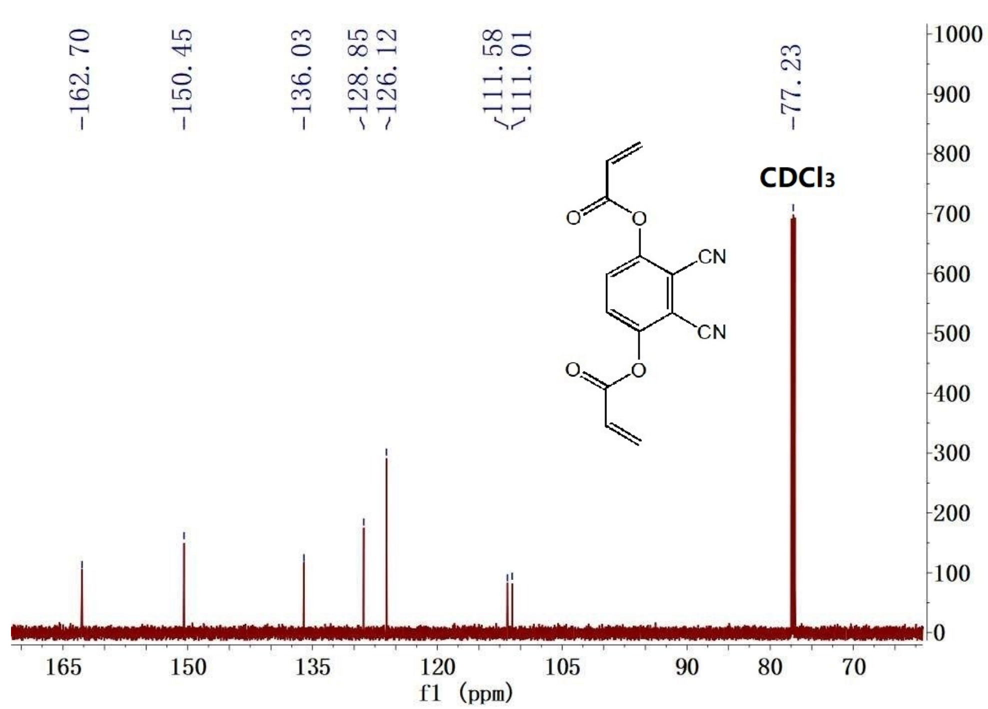

Figure S2. [13]C NMR spectrum of 2,3-dicyano-1,4-phenylene diacrylate (DCPDA) in $CDCl_3$.

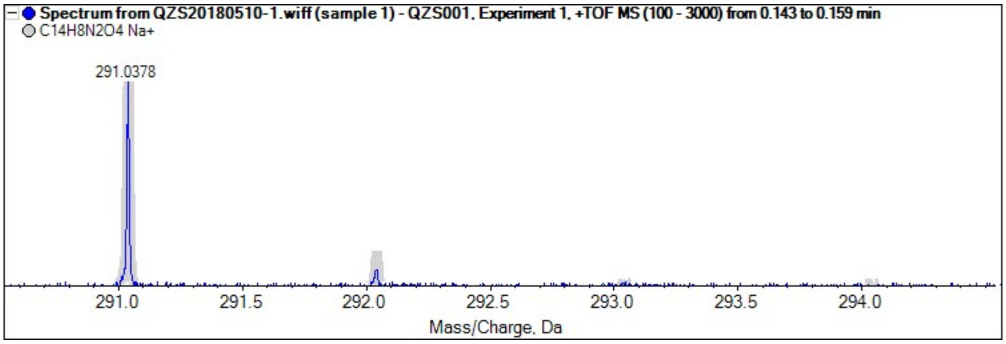

Figure S3. High-resolution mass spectrum of 2,3-dicyano-1,4-phenylene diacrylate (DCPDA).

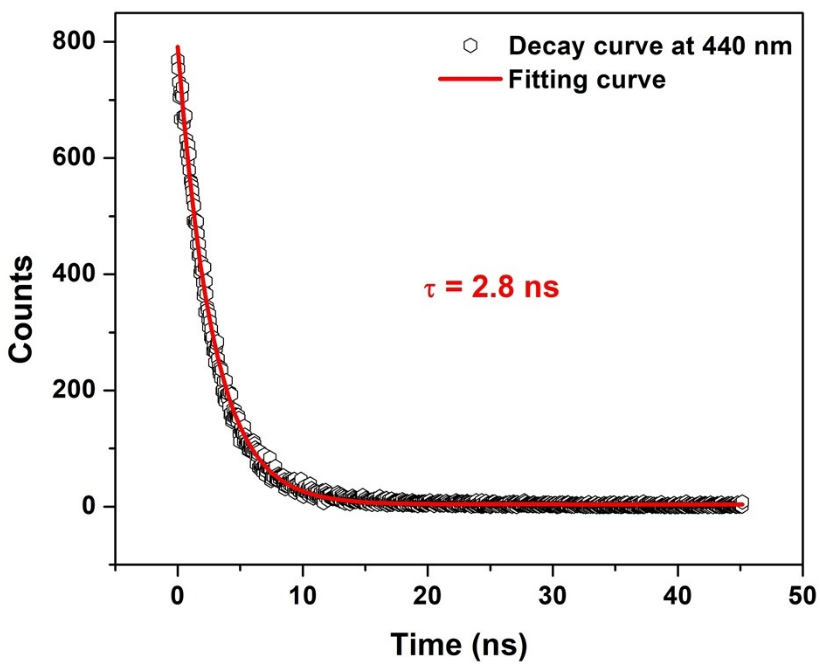

Figure S4. Time-resolved PL decay curve of 2,3-dicyano-1,4-phenylene diacrylate (DCPDA) at 440 nm.

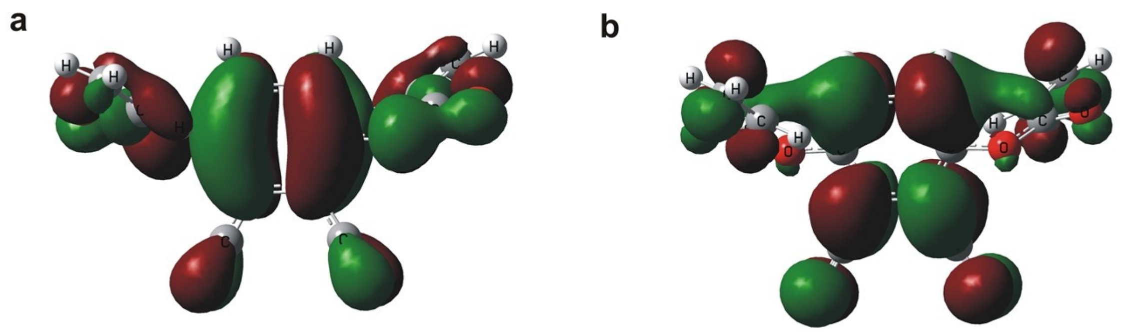

Figure S5. (a) HOMO of DCPDA computed at B3P86/6-31+G(d,p) level. (b) LUMO of DCPDA computed at B3P86/6-31+G(d,p) level.

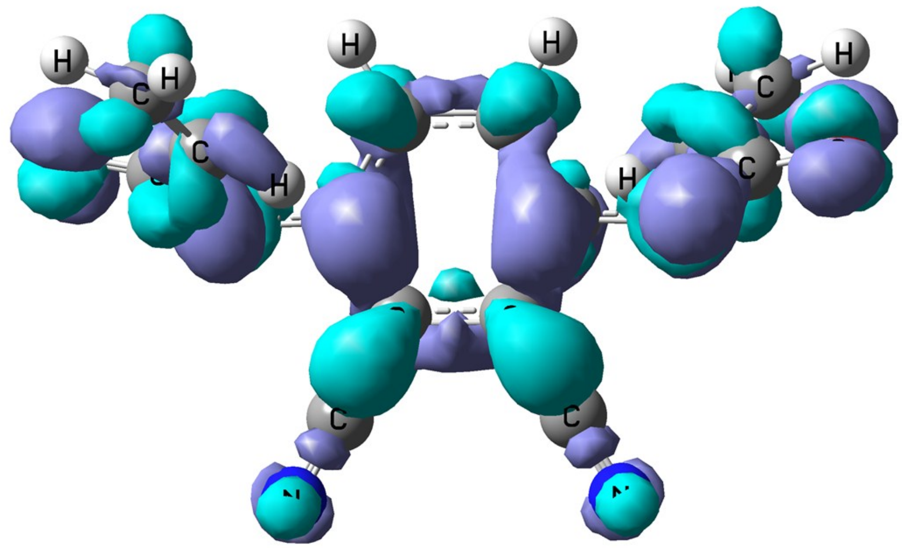

Figure S6. Electron density difference map between S_0 and S_1 of DCPDA. Isovalue for density is 0.001.

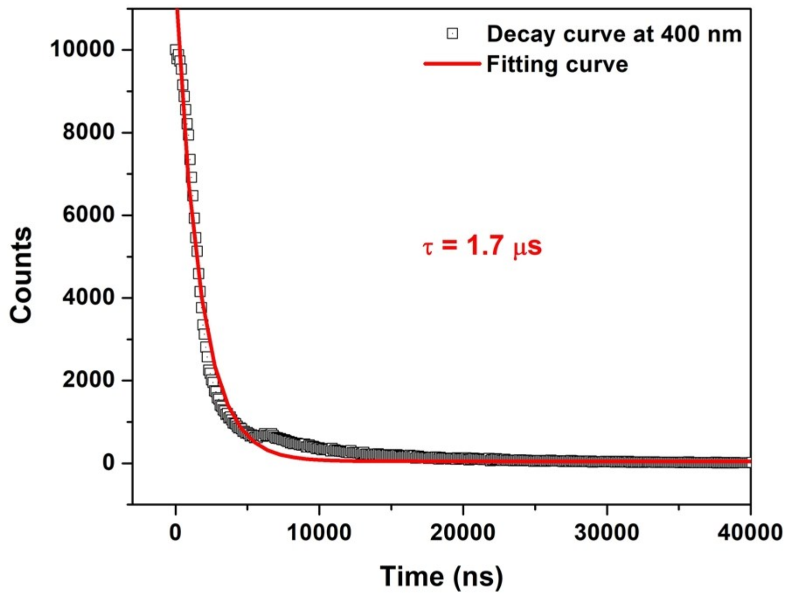

Figure S7. Time-resolved PL decay curve of 2,3-dicyano-1,4-phenylene diacrylate (DCPDA) at 400 nm at 77 K.

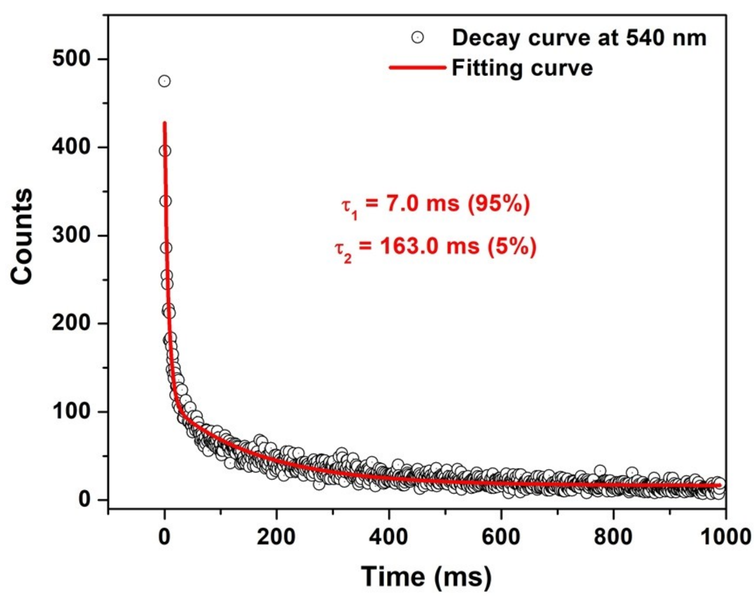

Figure S8. Time-resolved PL decay curve of 2,3-dicyano-1,4-phenylene diacrylate (DCPDA) at 540 nm at 77 K.

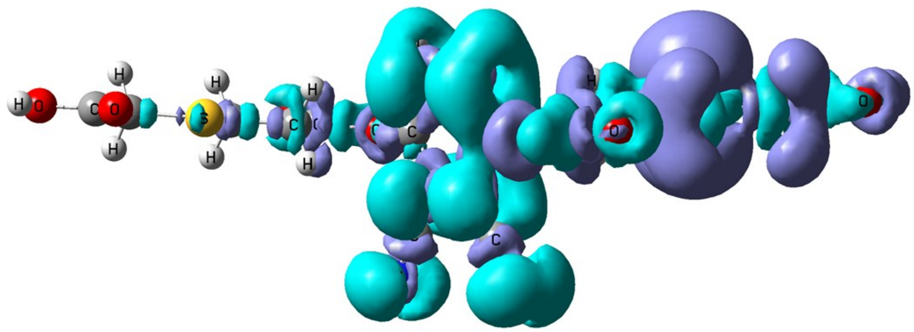

Figure S9. Electron density difference map between S_0 and S_1 of the product of DCPDA and TGA. Isovalue for density is 0.001.

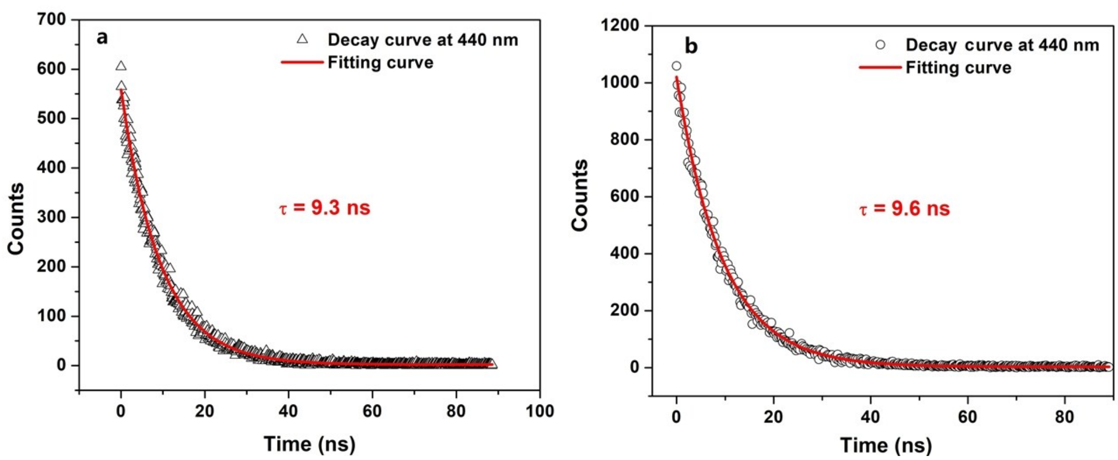

Figure S10. Time-resolved PL decay curves of the products of DCPDA with GSH (a) and TGA (b) at 440 nm.

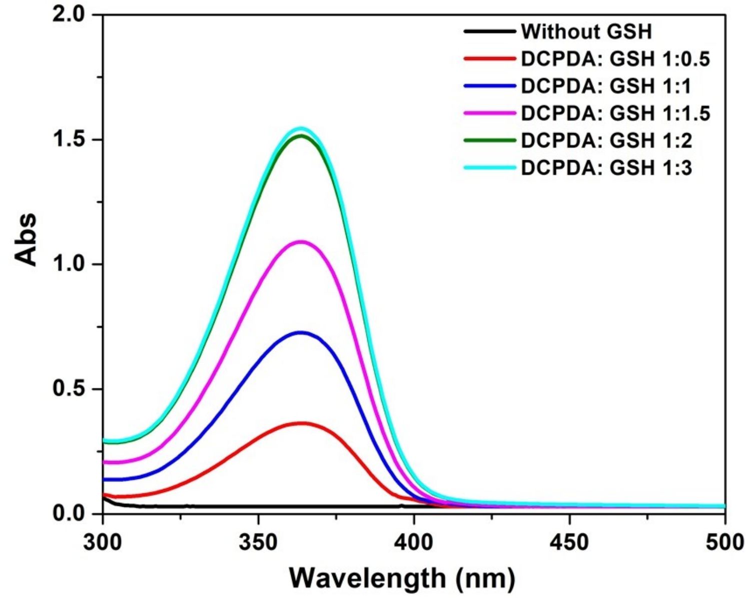

Figure S11. The change of UV-visible spectra of DCPDA in the presence of different ratios of GSH.

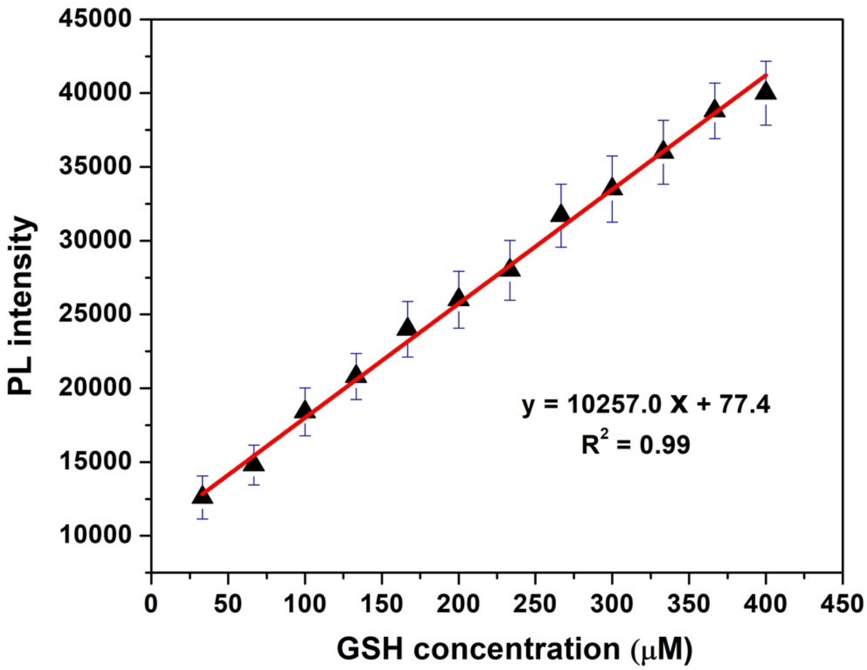

Figure S12. Calibration curve between PL intensity and GSH concentration in the range of 33.3 – 400 μM.

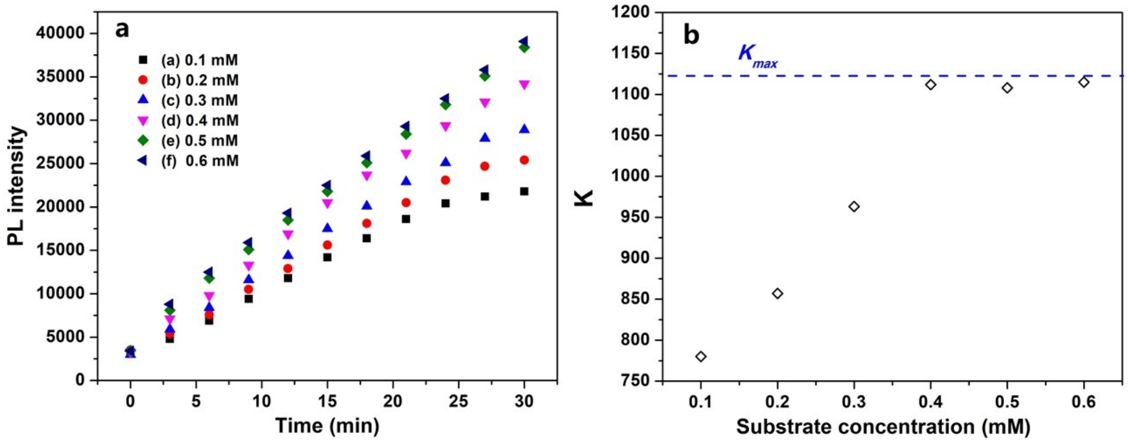

Figure S13. (a) PL intensity of BChE assay solution (DCPDA 300.0 μM, pH 7.4 HEPES buffer, 5.0 U/L BChE) vs substrate (BTCh) concentration in the range of 0.1 – 0.6 mM during the incubation time of 0 – 30 min. (b) Reaction rate K plotted against substrate concentration [S] for the BChE assay.

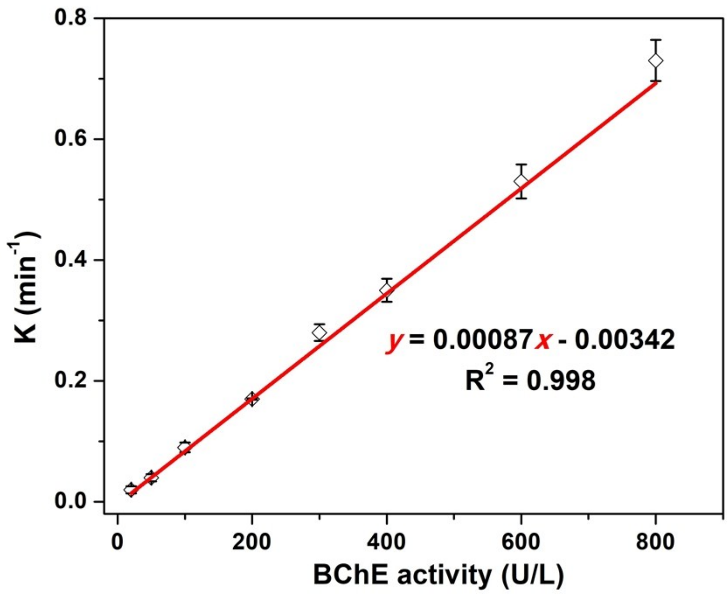

Figure S14. Calibration curve between enzymatic rates K and BChE activity using Ellman's method.

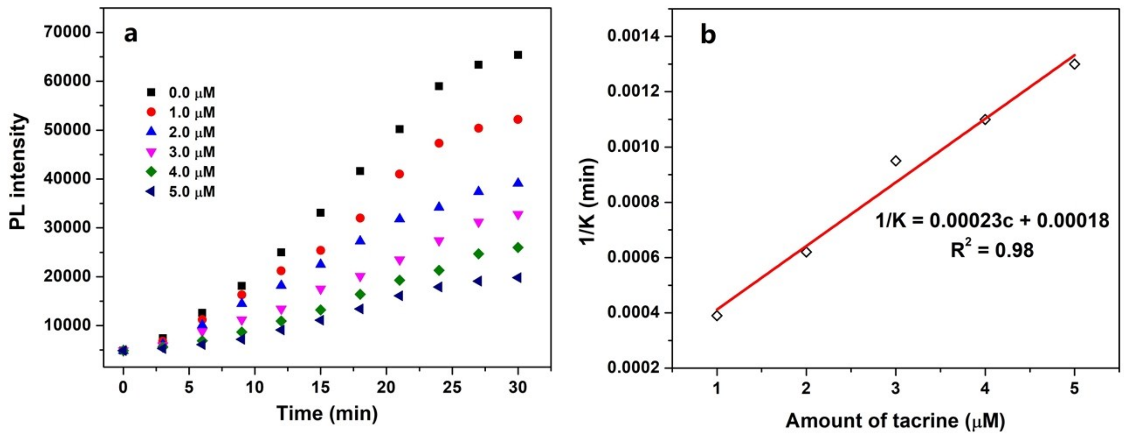

Figure S15. (a) Time-course enzymatic reaction kinetics of BChE assay in the presence of different concentrations of tacrine ranging from 0.0 to 5.0 μM. (b) Calibration curve between 1/K (min) and tacrine concentration in the range of 1.0 – 5.0 μM.

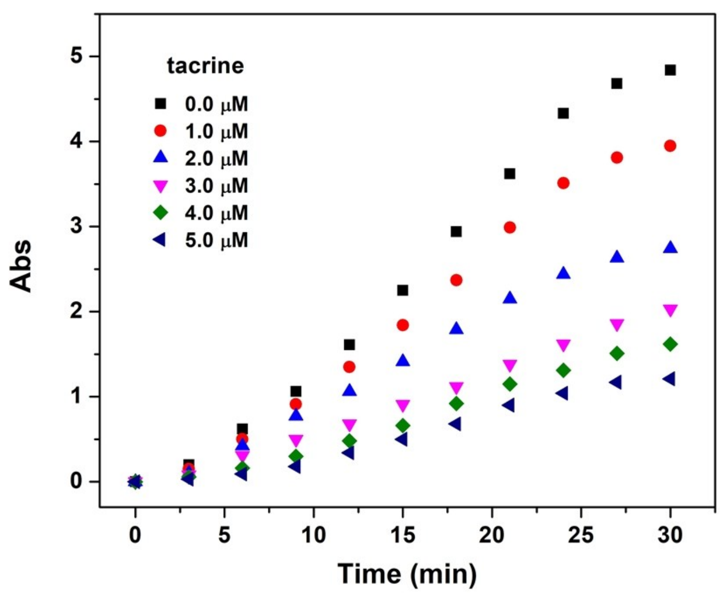

Figure S16. Time-course enzymatic reaction kinetics of Ellman colorimetric BChE assay in the presence of different concentrations of tacrine ranging from 0.0 to 5.0 μM.

Table S1. Comparison between established BChE assay and traditional Ellman method in determining BChE levels of human serum samples.

Sample number	measured BChE level using established method (U/L)	measured BChE level using Ellman method (U/L)
1	2.03 ± 0.03	2.08 ± 0.03
2	4.03 ± 0.02	4.06 ± 0.03
3	8.01 ± 0.01	8.05 ± 0.04

Cartesian Coordinates

2,3-dicyanohydroquninone (DCHQ)

C	-0.10545400	-0.03464700	0.00030400
C	1.27899800	-0.03472100	0.00069200
C	1.99706900	1.16617400	0.00002900
C	1.29571200	2.37786600	-0.00095600
C	-0.12190600	2.37794100	-0.00132600
C	-0.82339600	1.16632600	-0.00071700
H	-0.64046100	-0.97912500	0.00082200
H	1.81390500	-0.97925500	0.00150800
C	-0.84444900	3.60442500	-0.00232900
N	-1.43614000	4.60591800	-0.00316200
C	2.01839300	3.60426800	-0.00159000
N	2.61019400	4.60569600	-0.00210400
O	-2.17097000	1.22675100	-0.00119600
H	-2.55502800	0.34000500	-0.00052600
O	3.34465000	1.22645100	0.00030700
H	3.72860700	0.33966100	0.00077300

2,3-dicyano-1,4-phenylene diacrylate (DCPDA)

C	-0.12353300	0.21279200	-0.15063900
C	1.25791300	0.23068500	-0.02396900
C	1.93053300	1.44122200	0.13543300
C	1.22488200	2.64948400	0.13789800
C	-0.18002800	2.63256900	-0.01480600
C	-0.84114300	1.40778700	-0.15868500
H	-0.65149200	-0.72954400	-0.24614100
H	1.82061800	-0.69616000	-0.04266800
C	-0.92669100	3.84691800	-0.02356200
N	-1.53064600	4.83965300	-0.03332000

C	1.92568000	3.88059500	0.29726500
N	2.49334600	4.88589600	0.42957300
O	-2.20752300	1.42264300	-0.20215500
O	3.29501900	1.49922400	0.19624700
C	-2.93030500	0.86789900	-1.24700300
O	-4.09289600	0.62730000	-1.03805000
C	4.02540700	0.83337500	1.16864200
O	5.19651100	0.65540400	0.94518400
C	-2.22007400	0.68587200	-2.52583800
C	-2.81042000	0.03090400	-3.52988400
H	-1.22833900	1.11056200	-2.63798900
H	-2.31282600	-0.09126500	-4.48566100
H	-3.80240100	-0.39425800	-3.41201900
C	3.30938700	0.46601200	2.40379400
C	3.90564700	-0.30706100	3.31615400
H	2.30771800	0.85129400	2.56144400
H	3.40351700	-0.56726700	4.24149600
H	4.90701900	-0.69305600	3.15197600

The product of DCPDA and TGA

C	-0.04139400	-0.61190800	-0.23563100
C	1.32837200	-0.58677100	-0.01195200
C	1.97609500	0.62417200	0.21538100
C	1.26330300	1.82862500	0.19208100
C	-0.12950400	1.80392000	-0.04406800
C	-0.76557500	0.57671900	-0.26434900
H	-0.55757500	-1.55356100	-0.38671500
H	1.90349800	-1.50587100	-0.01085300
C	-0.88742000	3.01148200	-0.06231500
N	-1.50546900	3.99524900	-0.08330800
C	1.94434100	3.06160700	0.41319300
N	2.50133300	4.06425200	0.59951300
O	-2.13075600	0.55625700	-0.39963800
O	3.33995200	0.66665200	0.36159300
C	-2.76195100	0.45315000	-1.62515400
O	-3.95976900	0.36220800	-1.61593600
C	3.96960500	0.43470800	1.57059300
O	5.17084800	0.44362000	1.57097100
C	-1.88913800	0.48036300	-2.84984400
H	-1.18769000	-0.36101800	-2.80224700
H	-1.27513800	1.38885500	-2.82606800

C	-2.72017200	0.41691600	-4.12202600
H	-3.37551100	1.28904300	-4.18922700
H	-3.34118400	-0.48238900	-4.12102700
C	3.08912000	0.20073400	2.76724900
H	2.51932100	-0.72231200	2.60277600
H	2.34974500	1.00754600	2.83243900
C	3.90538100	0.10950400	4.04734900
H	4.45010100	1.04256600	4.21337900
H	4.62959300	-0.70593800	3.97799500
S	-1.58798600	0.38123000	-5.55044400
C	-2.84233900	0.28221700	-6.85691400
H	-3.50940300	1.14984100	-6.82747900
H	-3.46187500	-0.61285200	-6.73678700
C	-2.18604600	0.22753300	-8.20998900
O	-0.99158700	0.22038300	-8.41729500
O	-3.10389700	0.18383700	-9.18667200
H	-2.63717100	0.14722400	-10.03841200
S	2.76357600	-0.19084500	5.43675800
C	3.99102300	-0.17523300	6.77201700
H	4.51401400	0.78598300	6.81044600
H	4.74561000	-0.95376500	6.61948100
C	3.32430500	-0.40986600	8.10055500
O	2.13839500	-0.59116200	8.27517600
O	4.22198400	-0.39445600	9.09665700
H	3.74903800	-0.54853400	9.93163800