Supplementary information for

A colorimetric and near infrared fluorescent probe with high sensitivity and selectivity for acid phosphatase and inhibitor screening

Yongqian Xu,*[a] Benhao Li,[b] Liangliang Xiao,[a] Shiguo Sun*[a] and Yi Pang[c]

[a]College of Sciences, Northwest A&F University, Yangling, P. R. China, 712100, xuyq@nwsuaf.edu.cn

[b]School of Chemistry & Chemical Engineering, Shaanxi Normal University, Xi'an, P. R. China, 710062

[c]Department of Chemistry & Maurice Morton Institute of Polymer Science, The University of Akron, Akron, OH, 44325

Materials

BSA, lysozyme, trypsin, Rnase A, pepsinum were purchased from Xiaan Wolsen Bio. Reagents Co. (Xiaan, China) and were used as received. Acid phosphatase (ACP) from potato was bought from Energy Chemical. **SQ** was synthesized and purified as reported previously. [S1] K_2MoO_4 and $(NaPO_3)_6$ was bought from Alfa Aesar.

Measurements

Absorption and emission spectra were collected by using a Shimadzu 1750 UV-visible spectrometer and a RF-5301 fluorescence spectrometer (Japan), respectively.

Sample Preparation and Titration

Stock solutions of acid phosphatase, BSA, lysozyme, trypsin, Rnase A, pepsinum (1.0×10^{-2} M) were prepared in distilled water and diluted in phosphate buffer solution (pH=7.4) for titration experiments. Stock solutions of $(NaPO_3)_6$ and K_2MoO_4 (1.0×10^{-4} M) were prepared in distilled water and diluted in phosphate buffer solution (pH=7.4) to 5.0×10^{-6} M for titration experiments. Stock solution of **SQ** (5.0×10^{-4} M) was prepared in ethanol and diluted in phosphate buffer solution (pH=7.4) to 5.0×10^{-6} M for titration experiments. Acid phosphatase and other analytes were added to phosphate buffer solution of **SQ**, UV and fluorescence spectra were monitored after 3 h.

Calculation of concentrations of the hydrolyzed $(NaPO_3)_6$ and unhydrolyzed $(NaPO_3)_6$ as function of time[2]

For accurate evaluation of the enzymes' kinetics, concentrations of unhydrolyzed substrate $(NaPO_3)_6$ at different times were calculated by fluorescence intensity data based

on Eq. S1:

$$[S]t = [S]0\left[\left(\frac{I0}{Iq} - 1\right)/\left(\frac{I0}{Iq} - 1\right)\right] \quad \text{Eq. S1}$$

where $[S]_t$ is the substrate $(NaPO_3)_6$ concentration at time *t*; $[S]_0$ is the initial substrate $(NaPO_3)_6$ concentration; I_0 is the fluorescence intensity of the **SQ**; I_q is the fluorescence intensity quenched by the substrate $(NaPO_3)_6$ before addition of the enzyme; and I_t is the fluorescence intensity at time *t*.

The hydrolyzed substrate $(NaPO_3)_6$ concentrations at different time were derived from Eq. S2:

$$[hydrolyzed\ (NaPO3)6] = [S]0 - [S]t \quad \text{Eq. S2}$$

where $[S]_0$ is the initial substrate $(NaPO_3)_6$ concentration and $[S]_t$ is the substrate concentration at different time.

Calculation of kinetic parameters

Kinetic parameters with ACP were calculated from maintaining different $(NaPO_3)_6$ concentration assays within the scope of 10 μM. The Lineweaver-Burk plot was achieved using double reciprocal data of initial rate *V vs* substrate $(NaPO_3)_6$ concentration $[S]_0$ as in Eq. S3

$$\frac{1}{V} = \frac{Km}{Vmax[S]0} + \frac{1}{Vmax} \quad \text{Eq. S3}$$

where *V* is the initial rate and calculated from the range of the plots in Figure 2a; $[S]_0$ is the initial substrate $(NaPO_3)_6$ concentration; V_{max} is the maximal rate; K_m is the Michaelis constant.

Calculation of detecting limit

Detecting limit DL = $K \times S_{b1}/S$, where K=3, S_{b1} is the standard derivation of the blank solution and *S* is the slope of the calibration curve. [S3]

Preparation of human blood samples

The procedure for preparation of human blood samples is followed the reported literature.[S4] Human blood samples were collected from healthy volunteers treated in the local Medical Hospital. All samples were obtained by venipuncture and collected in heparinized vacutainer tubes. Then, a 200 μL aliquot of the blood was deproteinized by mixing immediately with 400 μL of cold 10% Cl_3CCOOH. After vortex mixing, the mixture was centrifuged at 8000 rpm for 10 min. A total of 400 μL of the supernatant was

collected. The obtained supernatant was ready for assays.

S1. a) P. F. Santos, L. V. Reis, I. Duarte, J. P. Serrano, P. Almeida, A. S. Oliveira and L. F. V. Ferreira, *Helv. Chim. Acta*, 2005, **88**, 1135; b) Y. Xu, Z. Li, A. Malkovskiy, S. Sun and Y. Pang, *J. Phys. Chem. B*, 2010, **114**, 8574.

S2. Y. H. Xie, Y. Tan, R. X. Liu, R. Zhao, C. Y. Tan and Y. Y. Jiang, *ACS Appl. Mater. Interfaces*, 2012, **4**, 3784.

S3. A. Hakonen, Anal. Chem., **2009,** *81,* 4555.

S4. D. Tian, Z. Qian, Y. Xia and C. Zhu, *Langmuir*, 2012, 28, 3945.

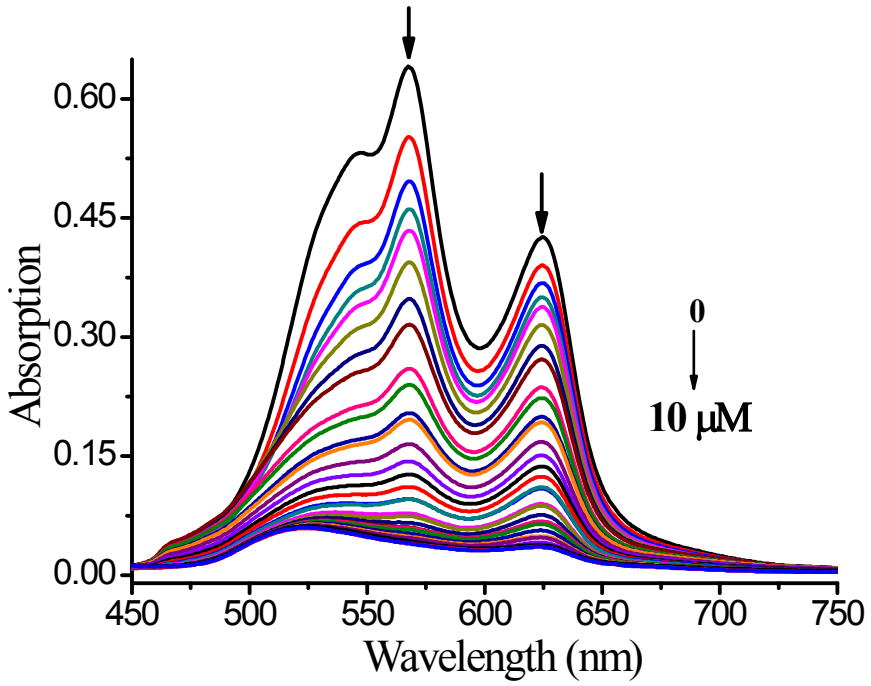

Fig. S1 UV-Vis spectra change of **SQ** (5 μM) in phosphate buffer (5 mM, pH =7.4) upon addition of $(NaPO_3)_6$ (0-10 μM).

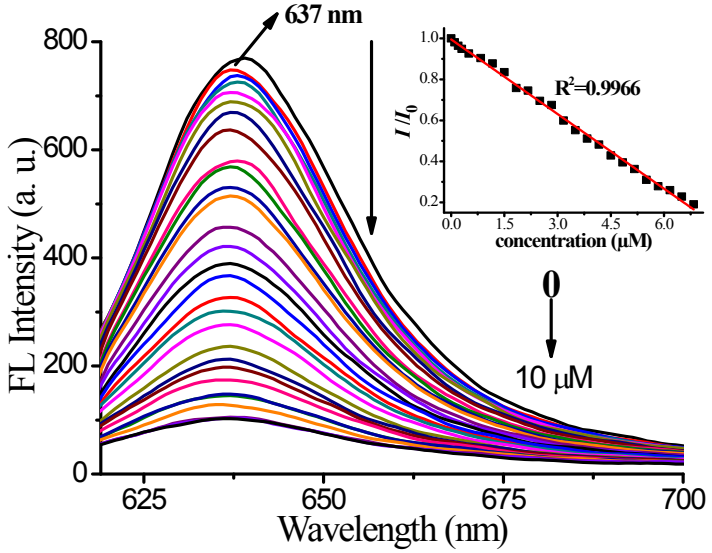

Fig. S2 Fluorescence spectra change of **SQ** (5 μM) in phosphate buffer (5 mM, pH =7.4) upon addition of $(NaPO_3)_6$ (0-10 μM). The arrow indicates the change of the fluorescence intensity with the $(NaPO_3)_6$ concentrations (λ_{ex}=600 nm). Inset: plot of the relative fluorescence intensity (I/I_0) of solution to $(NaPO_3)_6$ concentrations, where I and I_0 stand for the fluorescence intensity at 637 nm in the absence and presence of $(NaPO_3)_6$.

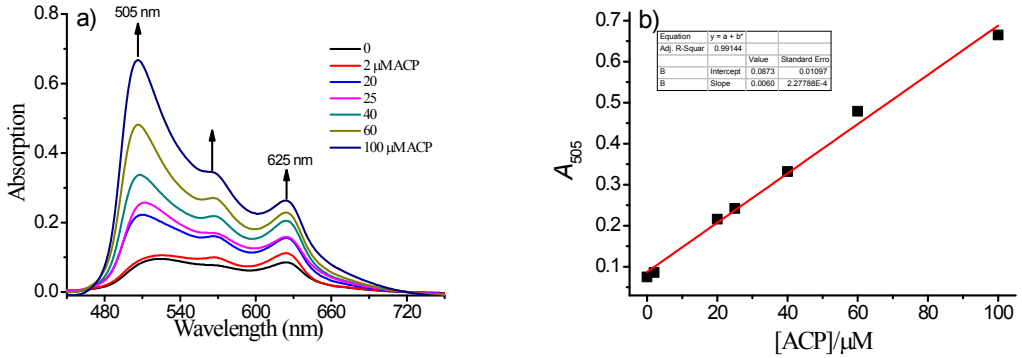

Fig. S3 UV-Vis spectra change (a) and intensity change (A_{505}) (b) of **SQ** (5 μM) in phosphate buffer (5 mM, pH =7.4) in the presence of $(NaPO_3)_6$ (10 μM) upon addition of ACP.

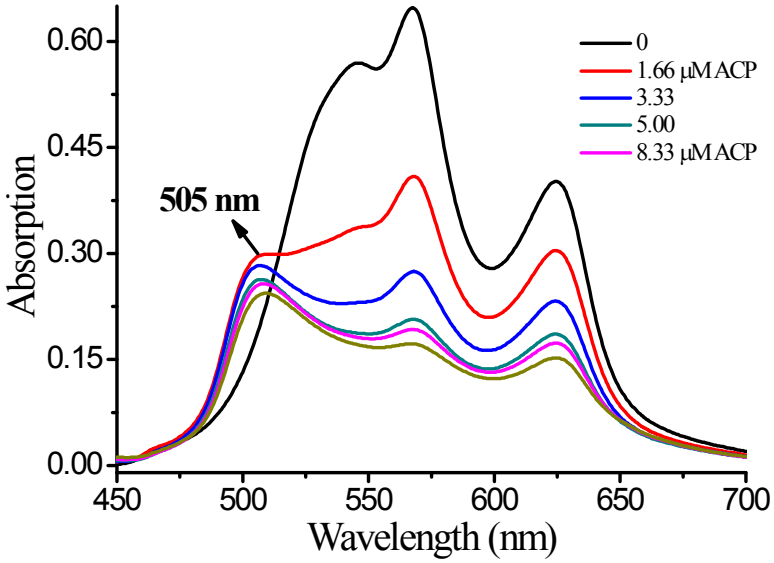

Fig. S4 UV-Vis spectra change of **SQ** (5 μM) in phosphate buffer (5 mM, pH =7.4) in the absence of $(NaPO_3)_6$ upon addition of ACP (0-8.33 μM).

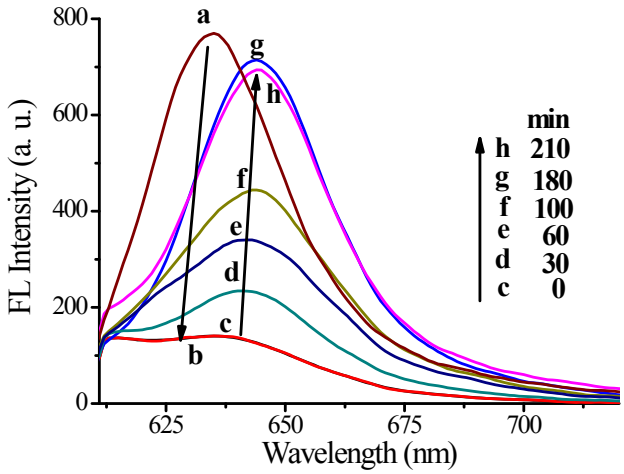

Fig. S5 Fluorescence spectra change of **SQ** (5 μM) in the presence of $(NaPO_3)_6$ (10 μM) and ACP (0.53 μM) in phosphate buffer (5 mM, pH =7.4), the excitation wavelength is 600 nm. Plot a, the spectrum of 5 μM **SQ**, plot b, after titration of 10 μM $(NaPO_3)_6$; plots c-h: spectra after addition of 0.53 μM ACP and collected as a function of time (0, 30, 60, 100, 180, and 210 min, respectively).

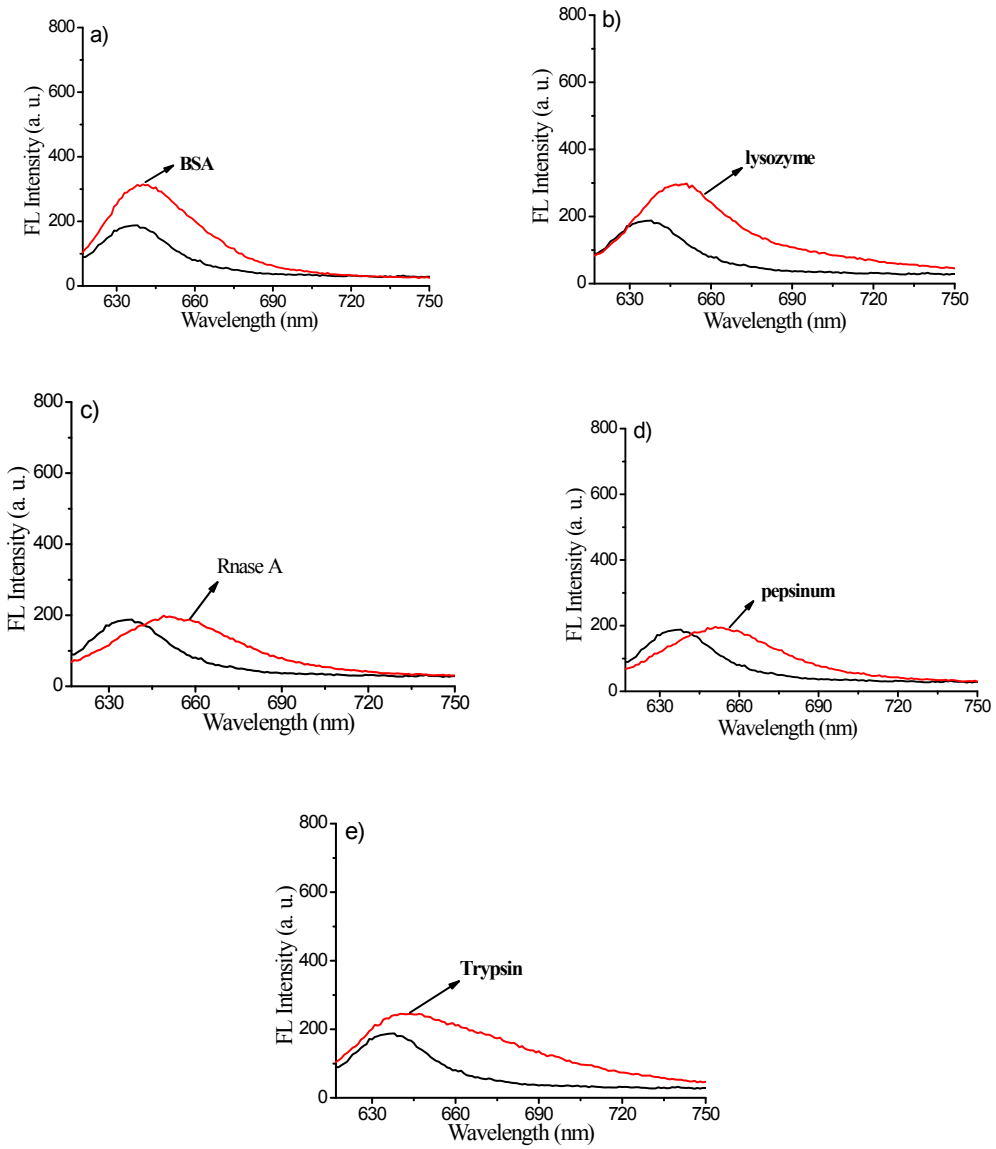

Fig. S6 The fluorescence spectra change of **SQ** (5 μM) in phosphate buffer (5 mM, pH =7.4) in the presence of $(NaPO_3)_6$ (10 μM) upon addition of 0.53 μM of BSA (a), lysozyme (b), Rnase A (c), pepsinum (d) and Trypsin (e), respectively.

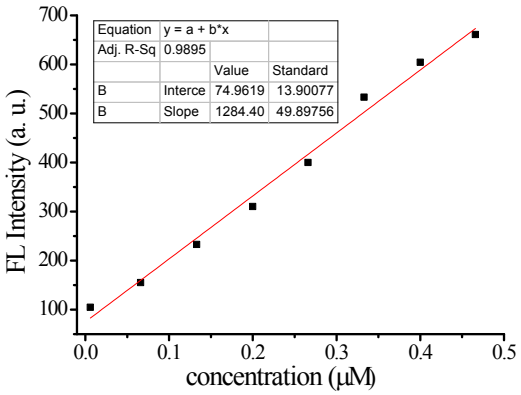

Fig. S7 Fluorescence intensity change of **SQ** (5 μM) at 644 nm in phosphate buffer (5 mM, pH =7.4) in the presence of $(NaPO_3)_6$ (10 μM) upon addition of ACP (S_{b1} = 2.098 and S = 1284.4).

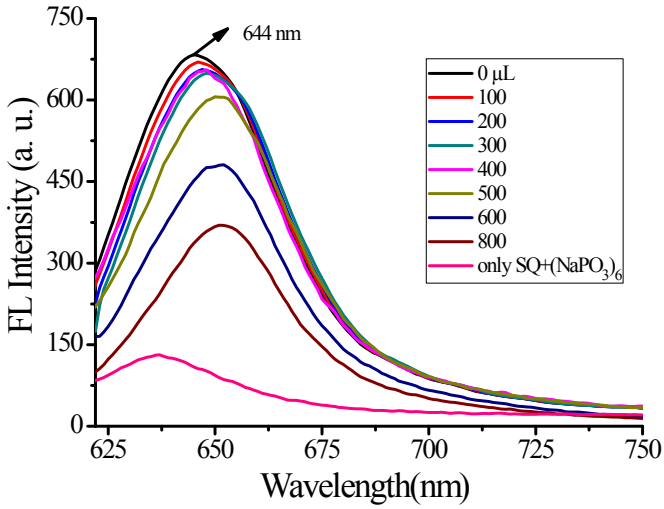

Fig. S8 Fluorescence spectra change of **SQ** (5 μM) in 3 mL phosphate buffer (5 mM, pH =7.4) with $(NaPO_3)_6$ (10 μM) and ACP (0.53 μM) in the presence of different amounts of human serum samples.

In diluted human serum samples, **SQ**-$(NaPO_3)_6$ shows turn-on fluorescence response to ACP. As human serum samples were diluted 7.5 times with phosphate buffer (5 mM, pH =7.4), the fluorescence response to ACP is similar to that in pure phosphate buffer (5 mM, pH =7.4). These results suggest that **SQ**-$(NaPO_3)_6$ can be used for ACP detection in serum samples.

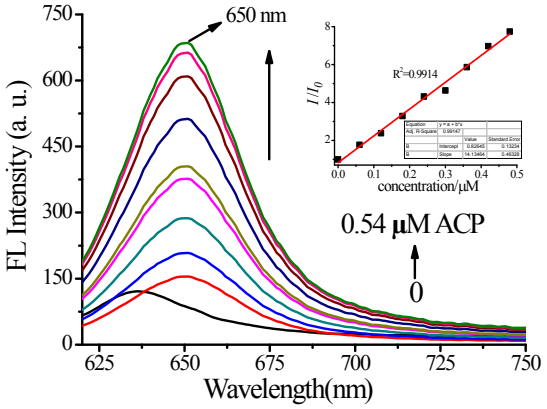

Fig. S9 Fluorescence spectra change of **SQ** (5 μM) in 3 mL phosphate buffer (5 mM, pH =7.4) with $(NaPO_3)_6$ (10 μM) and 400 μL human serum samples upon addition of ACP (0-0.54 μM). Inset: The relative fluorescence intensity change at 650 nm with increasing concentration of ACP from 0 to 0.54 μM.

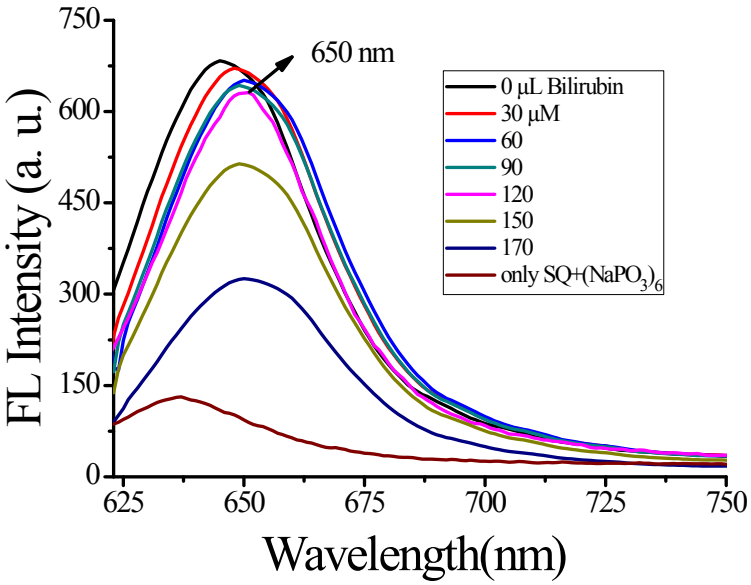

Fig. S10 Fluorescence spectra change of **SQ** (5 μM) in 3 mL phosphate buffer (5 mM, pH =7.4) with $(NaPO_3)_6$ (10 μM) and ACP (0.53 μM) in the presence of different concentration of bilirubin.

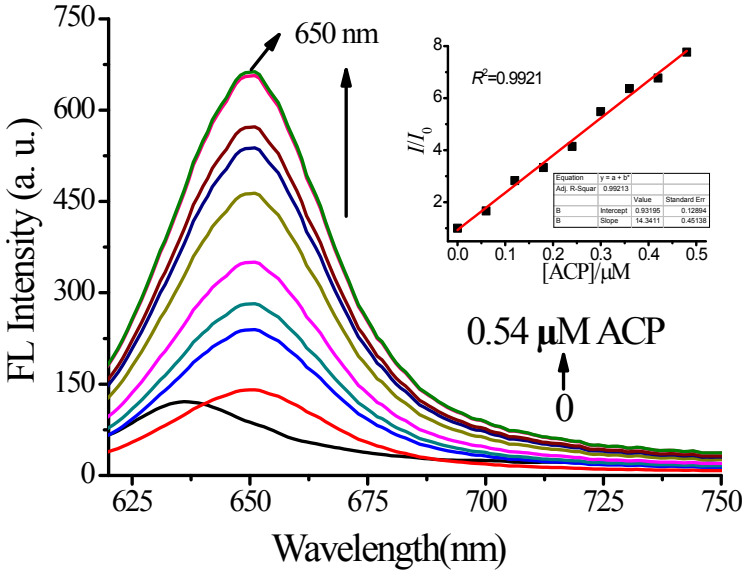

Fig. S11 Fluorescence spectra change of **SQ** (5 μM) in 3 mL phosphate buffer (5 mM, pH =7.4) with $(NaPO_3)_6$ (10 μM) and bilirubin (120 μM) upon addition of ACP (0-0.54 μM). Inset: The relative fluorescence intensity change at 650 nm with increasing concentration of ACP from 0 to 0.54 μM.

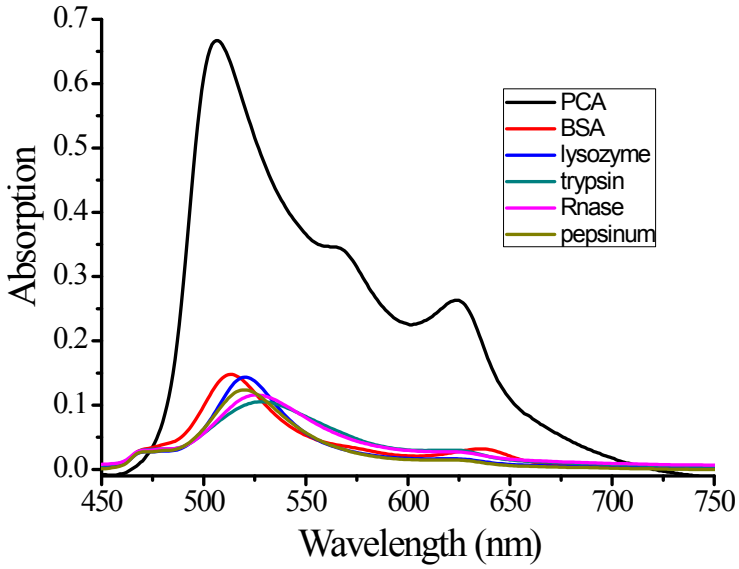

Fig. S12 The UV-Vis spectra change of probe **SQ** (5 μM) in phosphate buffer (5 mM, pH =7.4) with $(NaPO_3)_6$ (10 μM) in the presence of BSA, lysozyme, Rnase A, pepsinum and Trypsin (0.53 μM), respectively.

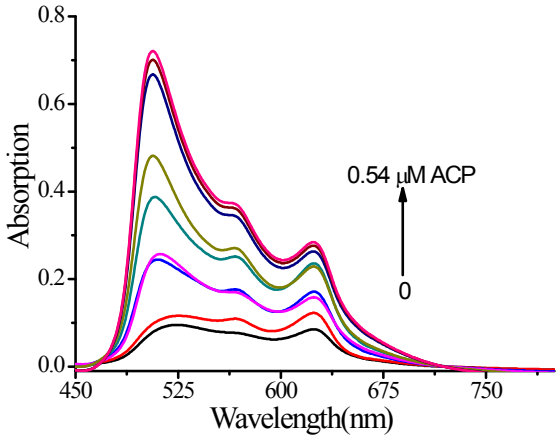

Fig. S13 UV-Vis spectra change of **SQ** (5 μM) in 3 mL phosphate buffer (5 mM, pH =7.4) with $(NaPO_3)_6$ (10 μM) and 400 μL human serum samples upon addition of ACP (0-0.54 μM).

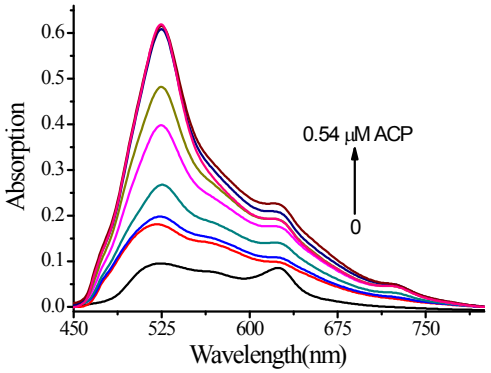

Fig. S14 UV-Vis spectra change of **SQ** (5 μM) in 3 mL phosphate buffer (5 mM, pH =7.4) with $(NaPO_3)_6$ (10 μM) and bilirubin (120 μM) upon addition of ACP (0-0.54 μM).