Supporting information

1. Materials and instruments

BSA, NADPH and other related bio-reagents were purchased from Dingguo Changsheng biotechnology (Beijing, China). Reduced glutathione (r-GSH) were purchased from Aladdin (Shanghai, China). Sodium selenite（Na_2SeO_3）were purchased from Xiya Reagent (Chengdu, China). Sodium borohydride and all other chemicals were purchased from Beijing Chemical Reagent Company (Beijing, China). $CdCl_2$•$2.5H_2O$ was provided by Northeast Normal University in Changchun, China. The double-distilled water (dH_2O) used throughout the experiments was produced by a Milli-Q system (Millipore, Bedford, MA, USA)

UV-visible absorption spectra were characterized using a Shimadzu 3600. Fluorescence spectroscopy was obtained with a Shimadzu 5301 PC spectrophotometer. Fourier transform infrared spectrometer (FT-IR Spectrometer) was carried out on a Brucker Vertex 80V. All optical measurements were performed at room temperature under ambient conditions. SEM images were conducted on a JEOL FESEM 6700F electron microscope. DLS was performed on a Zetasizer Nano ZS (Malvern Instruments). TEM images were collected using a JEOL JEM-2100F microscope.

2. Synthetic methods for the CdSe QDs

2.1 Preparation of SeNPs

The method of SeNP synthesis was modified from previous report.[1] Size controllable SeNPs were successfully produced by adding sodium selenite to solution containing BSA and GSH. Specifically, Selenite (0.1M, 500μl) was added into the 2.5ml solution containing BSA (10 mg) and GSH (15.5 mg). Within five minutes, the color of the solution turns from colorless to red, indicating the formation of SeNPs.

2.2 Synthesis of CdSe QDs

Unlike the general synthesis methods where Se^{2-} was produced by the reaction of sodium borohydride with selenium powder in an ice bath under the N_2 atmosphere for several hours, we use SeNPs as the source of active selenium. Specifically, SeNPs (400 μl) was added into the 14 ml

water-solution containing 24 mg sodium borohydride. Within three minutes, the colour of the solution became colorless and produced some foams, suggesting the formation of Se^{2-} group. After that, 0.1M Cd^{2+} (115 µl) was added into the Se^{2-} solution. In a second, the solution changed to yellow-green, hinting the formation of BSA-GSH-CdSe QDs. All the processes were acted within ten minute and did not need special equipment or skills (Scheme 1).

2.3 NADPH Detection

To study NADPH sensor function, NADPH (0.25 mM – 13 mM) was added into 400 µl PBS buffer (pH 7.4) containing BSA-GSH-CdSe QDs (0.82 mM), the fluorescence responses were recorded and analyzed carefully.

2.4 Detection of NADPH in biological background

Human blood samples were collected from volunteers. All the blood samples were centrifuged at 1000 g for 10 min at 4℃ before use. In parallel to BSA-GSH-CdSe QDs - PBS buffer system, 10% of PBS buffer was replaced by serum. The fluorescence responses of both systems toward NADPH were carefully compared.

Table S1 Summary of methods of synthesizing CdSe QDs at mild conditions

Selenium source	Reaction temperature	Reaction time	N_2 protection	pH adjustment	Ref.
Selenium powder + $NaBH_4$	RT	2 h	Yes	Yes	1
Selenium powder + Na_2SO_3	RT	20 h	No	Yes	2
Selenium powder + HNO_3	RT	2 h	Yes	Yes	3
Selenium powder + $NaBH_4$	RT	1.5 h	Yes	Yes	4
Selenium powder+ $NaBH_4$	40℃	41 h	Yes	Yes	5
Selenium powder+ $NaBH_4$	varies (0-100℃)	1 h	Yes	Yes	6
Selenium powder + Na_2SO_3	varies (55- 95℃)	4 h	Yes	No	7
Selenourea	RT	3 h	Yes	No	8
Na_2SeO_3	90℃	4 h	No	Yes	9
Selenium dioxide	100℃	1 h	No	Yes	10
Na_2SeO_3 → SeNPs	RT	8 min	No	No	this study

Table S2 Recovery for the determination of NADPH content in serum samples (Mean ± s; n = 3).

Type of samples	NADPH spiked (mM)	Recovery (%)
Human blood serum	0.25	102 ± 2.8
	0.75	98 ± 3.2
	1.5	99 ± 1.7

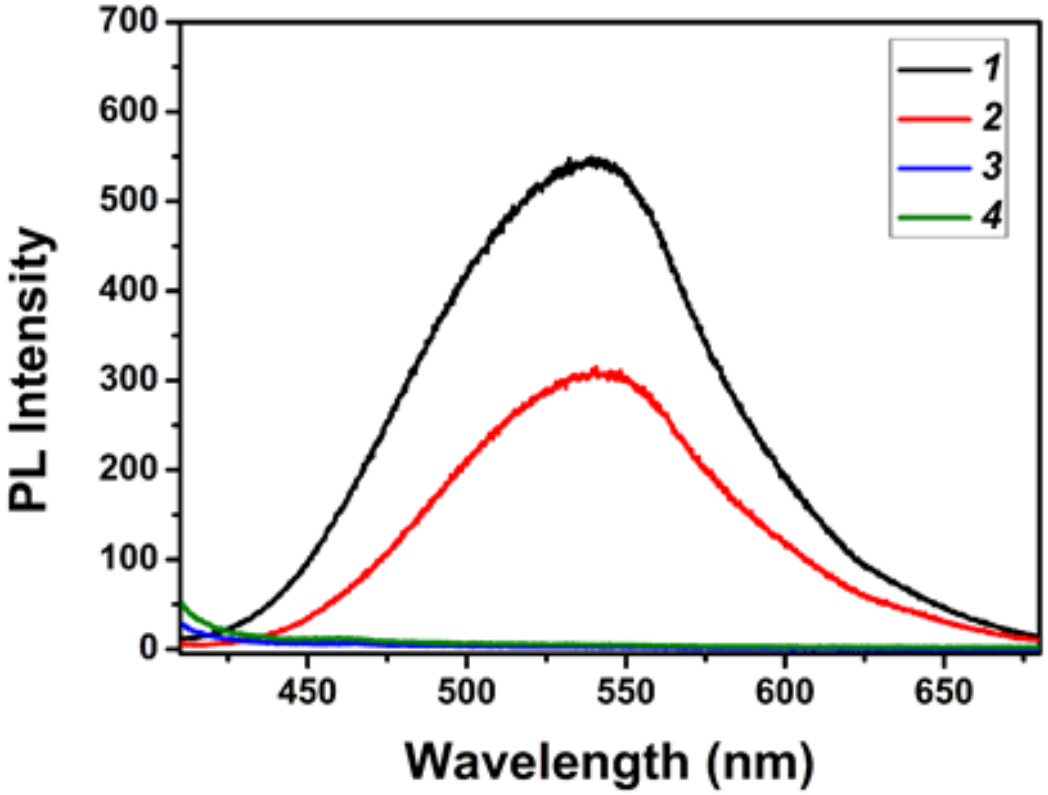

Fig. S1 Fluorescent spectra showed that the efficiency of CdSe QD synthesis at RT was largely dependent on the selenium sources.

1. CdSe QDs were synthesized by using the proposed approach with SeNPs as selenium source.

2. CdSe QDs were synthesized by using a modified approach with SeO_3^{2-} as selenium source. Specifically, selenite, BSA, GSH, and sodium borohydride were mixed and followed by 3-minute-incubation. When the solution was turned to colourless, hinting the Se^{2-} formation, Cd^{2+} was then added into the mixture, CdSe QDs were formed.

3. Selenite and sodium borohydride were mixed and followed by 3-minute-incubation. When the solution was turned to colourless, BSA, GSH, and Cd^{2+} were then added into the mixture.

4. Selenite and sodium borohydride were mixed and followed by 3-minute-incubation. When the solution was turned to colourless, Cd^{2+} was then added into the mixture.

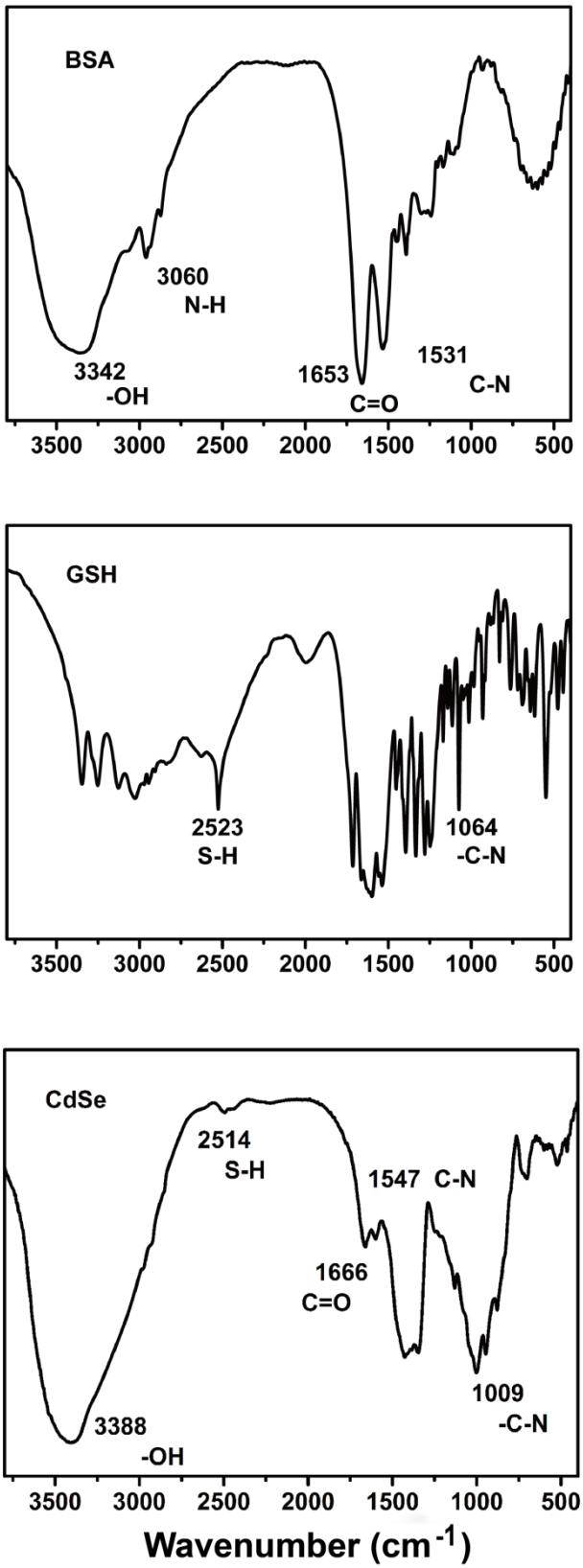

Fig. S2 The FT-IR spectra of BSA, GSH and BSA-GSH-CdSe QDs

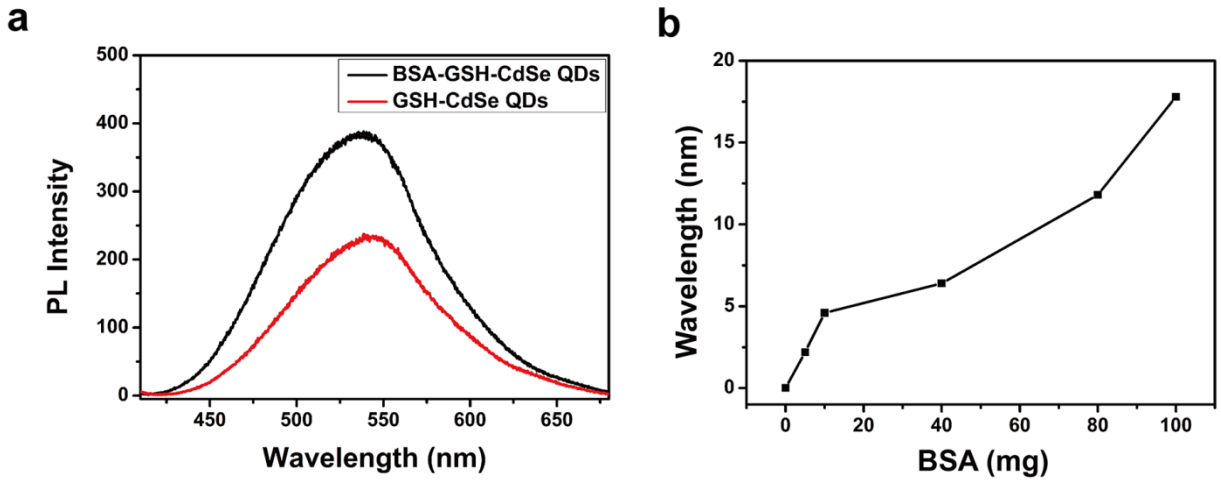

Fig. S3 a. Comparison of CdSe QD synthesis in the prescent/absence of BSA. b. The blue shift based on the changes of maximum fluorescence wavelength (535→482 nm) was observed alongwith the increase of BSA content in 2.5 ml solutions (0, 5, 10. 40, 80, 100 mg).

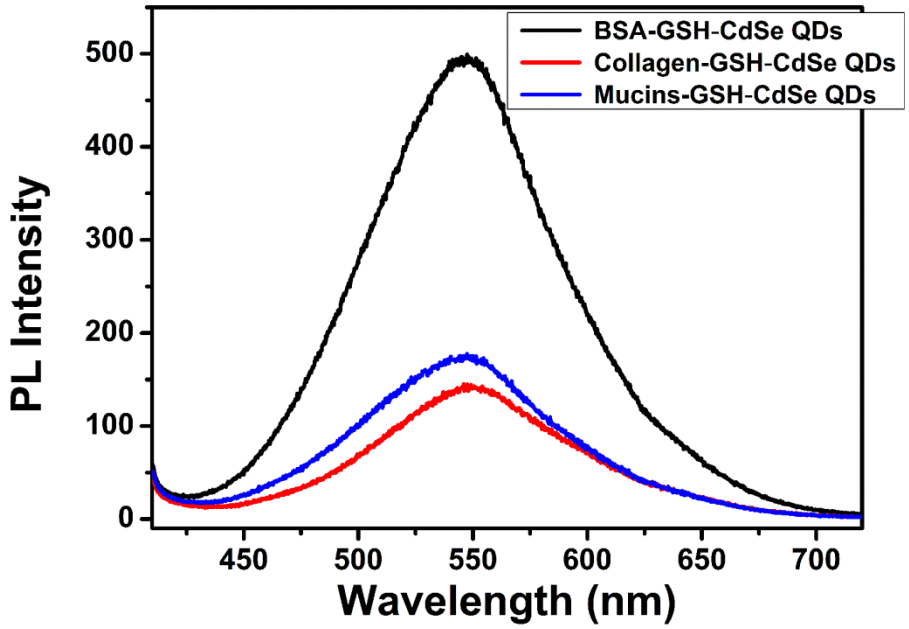

Fig. S4 The fluorescence spectroscopy of different proteins conjugated QDs using the same procedure, 10 mg proteins were added into 2.5 ml solution.

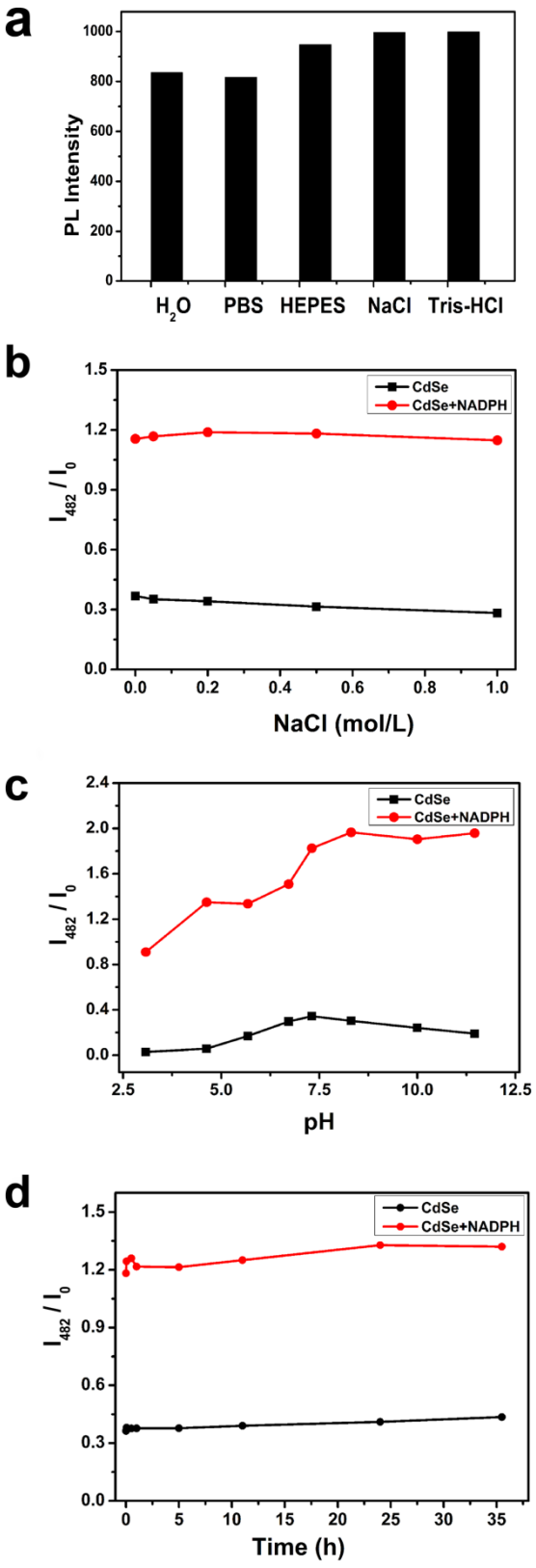

Fig. S5 The stability of BSA-GSH-CdSe QDs in different conditions. a. the fluorescence intensity of BSA-GSH-CdSe QDs in different buffers (BSA-GSH-CdSe QDs were lyophilized, and then

dissolved in different buffers. All the buffer solution was 20 mM (pH 7.4)). B. the stability of BSA-GSH-CdSe QDs toward the change of salt centration as indicated by the relative fluorescence intensity (NaCl concentration: 0, 10, 50, 100, 200, 300, 500 mM). c. the stability of BSA-GSH-CdSe QDs toward the change of pH as indicated by the relative fluorescence intensity. d. the stability of BSA-GSH-CdSe QDs toward longtime incubation at RT as indicated by the relative fluorescence intensity. I_{482} and I_0 refer to the fluorescent intensity at 482 and 535 nm, respectively.

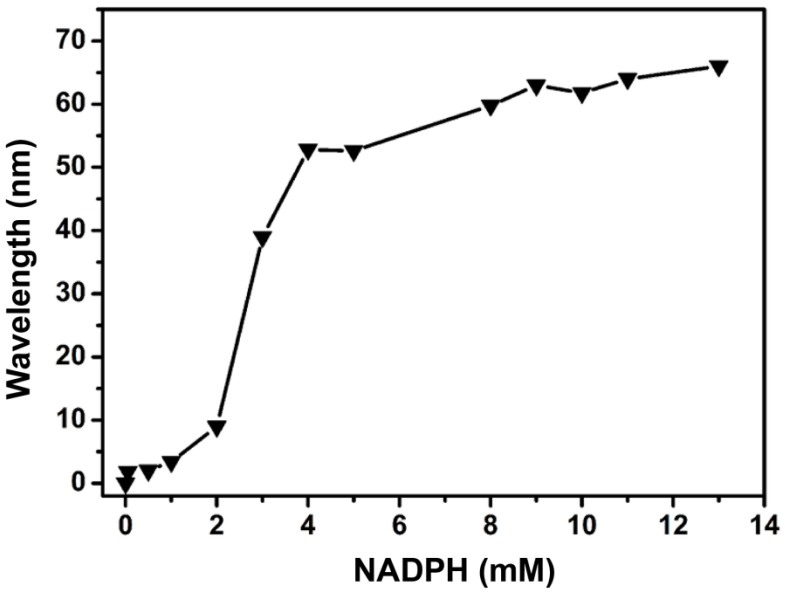

Fig. S6 The blue shift based on the changes of maximum fluorescence wavelength as resulted from adding different amount of NADPH (0 – 13 mM).

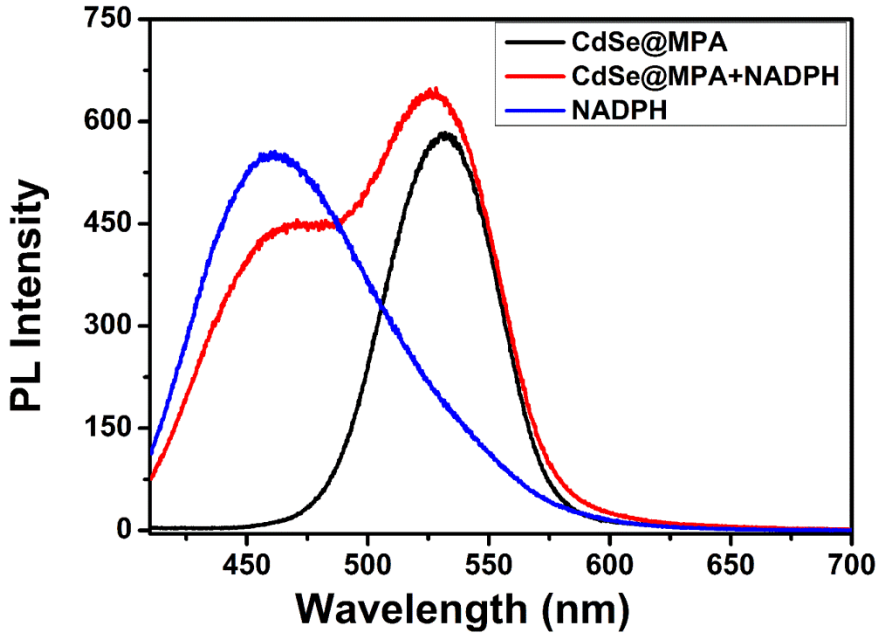

Fig. S7 The fluorescence spectra of MPA-CdSe QDs (synthesized by classical method (ref 40)), NADPH (5 mM) and their mixture.

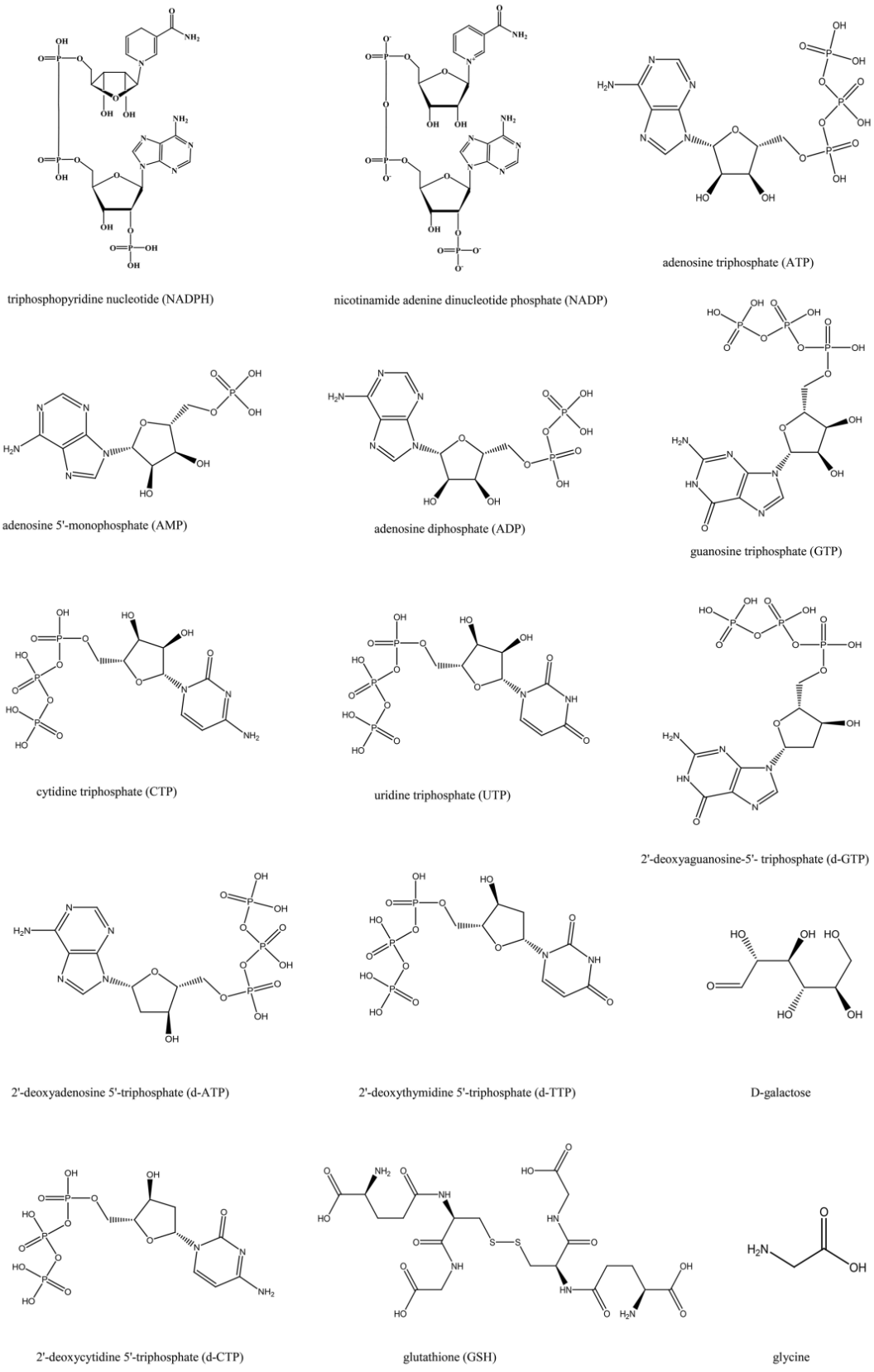

Fig. S8 Schematic chemical structures of NADPH and its derivatives or several other related compounds.

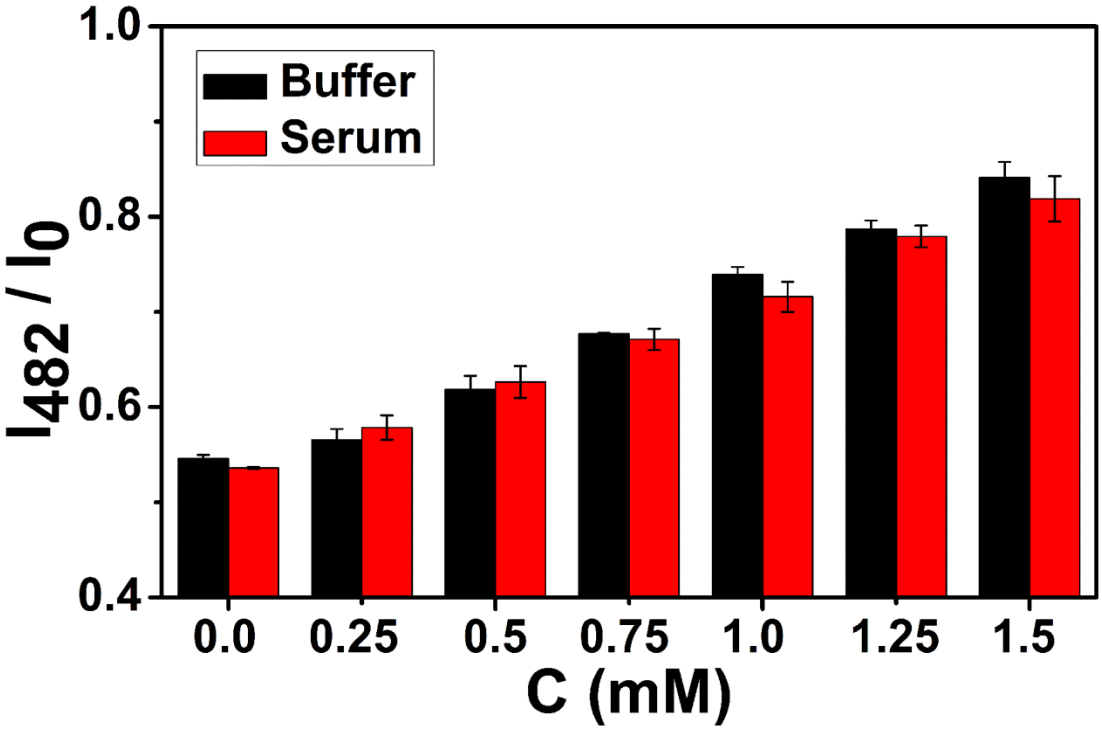

Fig. S9 The relative fluorescence intensity of BSA-GSH-CdSe QDs in the presence of different concentration of NADPH. Buffer refers to PBS (pH 7.4) which serves as a control. Serum refers to PBS buffer containing 10% serum. I_{482} and I_0 stand for the fluorescent intensity at 482 and 535 nm, respectively.

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