Supporting Information

Synthesis of bio-templated clickable quantum dots and dual-emitting organic/inorganic complex for ratiometric fluorescence visual assay of blood glucose

Yeling Yang[a], Guobin Mao[a], Xinghu Ji*[a], Zhike He*[a]

a College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China;

***Corresponding authors**

E-mail: zhkhe@whu.edu.cn; Fax: +86 27-68754067; Tel: +86 27-68756557; http://orcid.org/0000-0002-0082-4056

Materials and Reagents

Tellurium powder (Te), cadmium chloride hemi(pentahydrate) ($CdCl_2 \cdot 2.5H_2O$), zinc chloride ($ZnCl_2$), sodium telluritewere (Na_2TeO_3), sodium hydroxide (NaOH), hydrochloric acid (HCl), dextran and ascorbic acid were obtained from Shanghai Chemical Reagents Company (Shanghai, China). Sucrose, maltose and fructose were supplied by Gibco BRL. Dimethyl sulfoxide (DMSO), N-acetyl-L-cysteine (NAC), tris(hydroxymethyl)aminomethane (Tris), sodium borohydride ($NaBH_4$), 5(6)-carboxyfluorescein-N-hydroxysuccinimide ester (5(6)-FAM-NHS ester), mercaptosuccinic acid (MSA), glycerin, dibenzocyclooctyne-N-hydroxysuccinimidyl ester (DBCO-NHS ester), glucose and GOx were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrafiltration tubes (30 kD and 50 kD) were purchased from Millipore (U. S. A.). Human serum of the health patients and diabetes was supplied from Zhongnan Hospital of Wuhan University (Wuhan, China). All chemical reagents were of analytical reagent grade and utilized without further purification. All solutions were prepared with ultrapure water, which was obtained using a Millipore water purification system. The oligonucleotides that one teminal modified by N_3 and the opposite end modified by phosphorothioate were supplied by Sangon Biotechnology Co. Ltd. (Shanghai, China).

The sequences were listed as follow:

ps-po DNA-N_3:

G*G*G*G*G*AAAAACCTGACTTTGCAATATCCAGAGACCTCCTAT-N_3 (5'-3', * indicates the phosphorothioate linkage)

Characterization Methods

The UV-vis absorption spectra of probes were obtained by a UV-2250 spectrophotometer (Shimadzu, Japan). The fluorescence spectra of samples were recorded on RF-6000 PC spectrophotometer (Shimadzu, Japan). A JEM 2100 electron microscope was used to characterize the CdZnTeS QDs and FAM@GOx-QDs complex (JEOL, Japan). The hydrodynamic diameter of samples was measured on the ZEN3600 zetasizer instrument (Malvern, U.K.). Powder X-ray diffraction (XRD) analysis was obtained by a PANalytical X'Pert Pro MPD diffractometer (Netherlands). The elemental analysis was measured by zeiss sigma field emission scanning electron microscope (Carl zeiss, Japan). An ESCA Lab electron spectrometer (Thermal Fisher Scientific, U.S.A.) was used performing the X-ray photoelectron spectroscopy (XPS) characterizations of DNA functionalized CdZnTeS QDs. Photos of the FAM@GOx-QDs for the visual detection of glucose were obtained by a smartphone (Honor v20) under an ultraviolet lamp (16 W, λ_{ex} = 365 nm).

Synthesis of CdZnTeS QDs and N3-CdZnTeS QDs

The CdZnTeS QDs co-stabilized by NAC and MSA was prepared via a one-step method. In this method, Na_2TeO_3 was used as Te source. NAC and MSA were used as reducing agent, S source and surface ligand. In a typical synthesis, $ZnCl_2$ (6.25 mM), $CdCl_2 \cdot 2.5H_2O$ (6.25 mM), MSA (12.5 mM) and NAC (12.5 mM) were dissolved in 38 mL ultrapure water. The NaOH solution (1.0 M) was stepwise added to the mixed solution until the pH was adjusted to 9.0. Then, the stock solution can be obtained by adding Na_2TeO_3 to the above mixed solution. For the preparation of CdZnTeS QDs, 2 mL of stock solution was moved to a Teflon-lined stainless steel autoclave. Then, the stock solution was heated at 200 °C for 28 min and cooled down in a water bath at room temperature. For the preparation of N_3-CdZnTeS QDs, the ps-po DNA-N_3 (50 nmol) was added to 2 mL stock solution. Then, the mixture was moved to a Teflon-lined stainless steel autoclave and heated at 200 °C for 28 min.

The CdZnTeS QDs and the N_3-CdZnTeS QDs were purified by the ultrafilter of molecular weight cutoff 30 kDa and molecular weight cutoff 50 kDa, respectively. All QDs were centrifugation at 8000 rpm for 10 min and washed 4 times with ultrapure water.

Preparation of FAM@GOx-QDs and GOx-QDs

The FAM@GOx-QDs was constructed via the formation of amide between the primary amine groups of GOx and the carboxyl groups of DBCO-NHS ester and 5(6)-FAM-NHS ester. GOx was dissolved in phosphate buffered solution (PB, pH 7.4, 10 mM) to obtain a solution of 20.0 $mg \cdot mL^{-1}$. The DBCO/FAM-GOx was prepared by adding equimolar quantities of DBCO-NHS ester and 5(6)-FAM-NHS ester that were dissolved in DMSO (20 μL) to the GOx solution and shaking overnight. An Amicon Ultra-4 centrifugal filter

device (MW cut off of 30 kDa) was used for the purification of the mixture. The DBCO-GOx was prepared by adding DBCO-NHS ester to GOx solution (20.0 mg·mL^{-1}). DBCO-NHS ester (1mg) was dissolved in DMSO (20 μL). Similarly, the mixture was shaken overnight and purified with an Amicon Ultra-4 centrifugal filter device (MW cut off of 30 kDa). The DBCO-GOx and DBCO/FAM-GOx were both centrifugation at 8000 rpm for 10 min and washed 4 times with ultrapure water. The purified DBCO-GOx and DBCO/FAM-GOx were stored at -20 ° C.

For the preparation of FAM@GOx-QDs, the DBCO/FAM-GOx and the N_3-CdZnTeS QDs were mixed in 400 μL phosphate buffered solution (pH 7.4, 10 mM) and incubated at room temperature with continuous gentle shaking. After shaking 12 h, the FAM@GOx-QDs was purified with an Amicon Ultra-4 centrifugal filter device (MW cut off of 50 kDa). The preparation of GOx-QDs was similar to that of FAM@GOx-QDs. The purified probes were stored at 4 °C.

Glucose Detection

In order to perform glucose detection in Tris buffer, 20 nM of FAM@GOx-QDs and different amounts of glucose dissolved in Tris buffer (20 mM, pH 7.8) were incubated for 30 min at room temperature. The total volume of the solution was 300 μL. Afterwards, the fluorescence spectra measurements were performed using 360 nm as an excitation wavelength. In addition, for the visual assay of glucose concentrations, 200 nM of FAM@GOx-QDs and different amounts of glucose dissolved in 20 mM of Tris buffer were incubated for 20 min. The total volume of the solution was 200 μL. Then, the photos were obtained under the 16W ultraviolet lamp (UV lamp) with excitation wavelength of 365nm.

For blood glucose concentration determination, the human blood was firstly centrifugated at 5000 rpm for 10 min to remove blood plasma. Then, an ultrafilter with a 30 cutoff was used to remove the proteins in serum. The serum centrifugation was performed at 8000 rpm for 10 min. Last, the pretreated serum was diluted 10 times. 400 μL of mixed solution containing 300 nM of FAM@GOx-QDs and 5 μL of human serum from volunteers was incubated for 30 min. Fluorescence spectra were recorded using 360 nm as an excitation wavelength. For visual blood glucose assay, 200 nM of FAM@GOx-QDs complex was incubated with diluted serum for 20 min and the photos were taken under a 365 nm UV lamp. The total volume of the mixture was 200 μL.

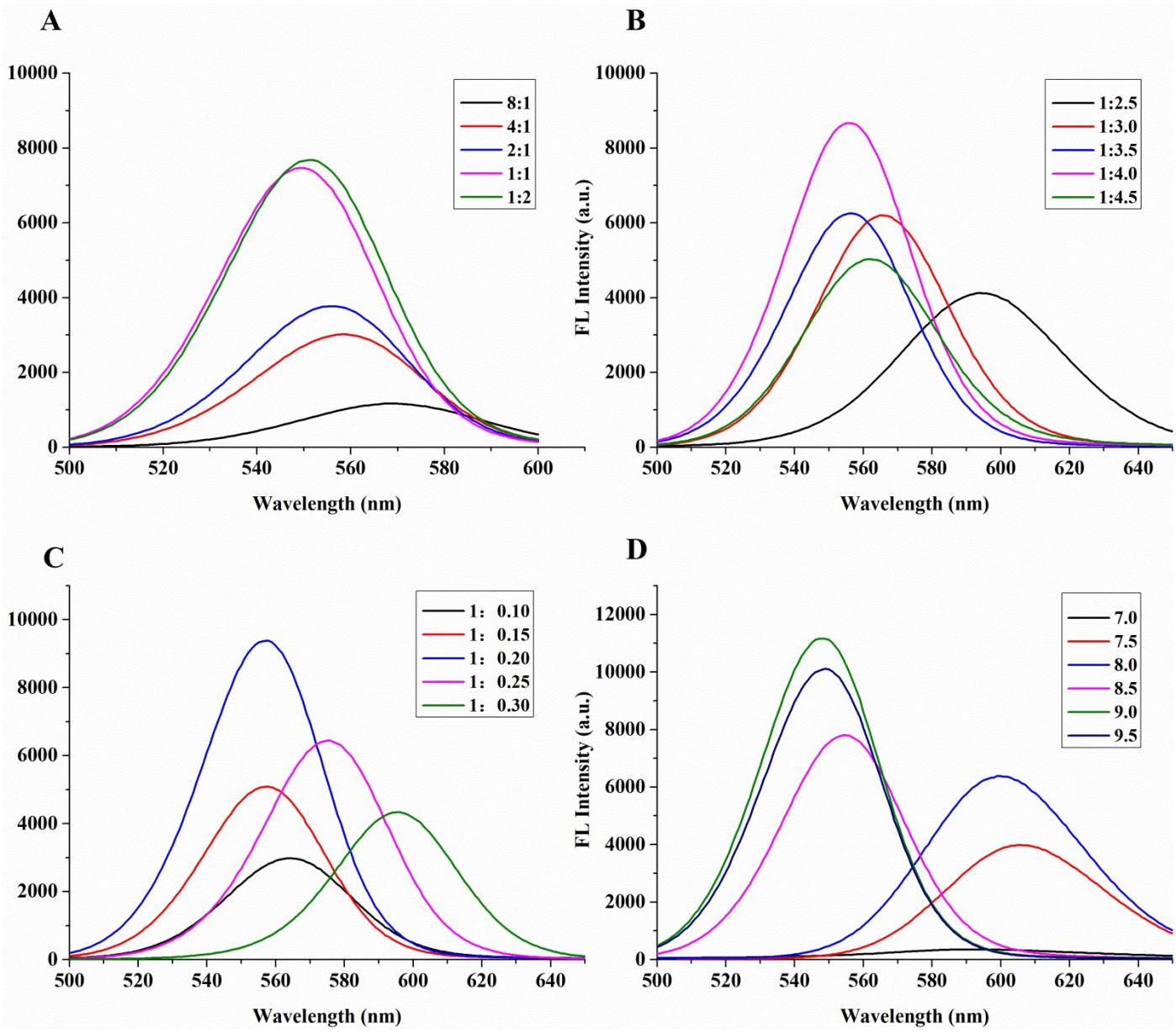

Figure S1. Fluorescence spectra of CdZnTeS QDs at different MSA/NAC (A), the $CdCl_2$/ sulfhydryl compounds (B), $CdCl_2/Na_2TeO_3$ (C) molar ratios and different pH (D).

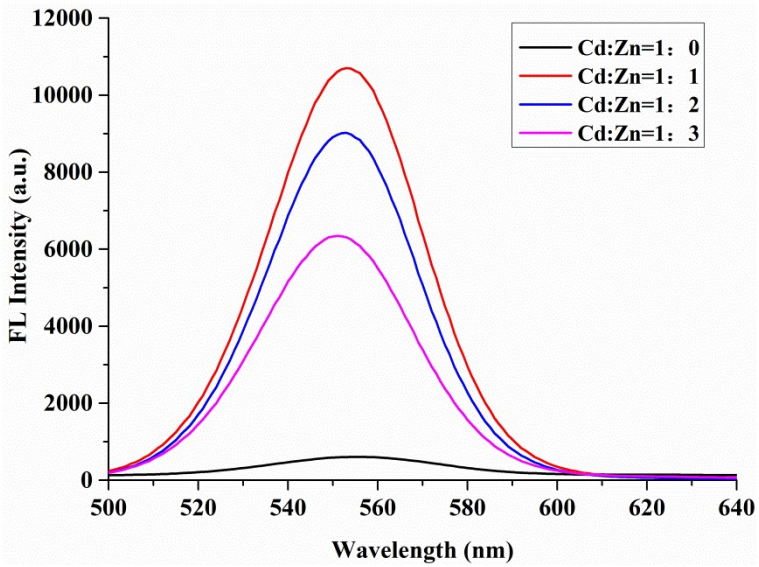

Figure S2. Fluorescence spectra of CdZnTeS QDs at different $CdCl_2$/ $ZnCl_2$ molar ratios.

Table S1. Optical properties of CdZnTeS QDs obtained at various reaction time.

Reaction time (min)	λ_{em} (nm)	FWHM (nm)	QYs (%)
20	506	32	34.5
22	540	45	73.7
24	561	46	64.5
26	594	50	62.8
28	632	54	54.2
30	667	63	50.3

FWHM: full width at half maximum.

QYs: quantum yields.

Table S2. The results of element analysis of C, N and O in CdTe: Zn^{2+}QDs and CdZnTeS QDs.

Sample	C (%)	N (%)	O (%)	C/N ratio	C/O ratio
CdTe:Zn^{2+}	50.10	8.90	30.54	5.62	1.64
CdTeZnS	41.15	1.41	38.84	29.18	1.06

Note: the results represent atomic percentages of the corresponding element in sample.

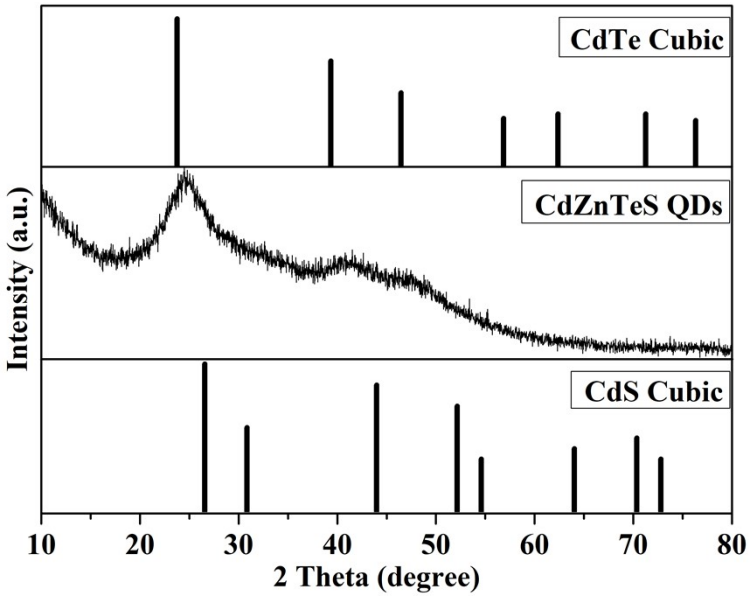

Figure S3. XRD of CdZnTeS QDs co-stablized by NAC and MSA and the line spectra of cubic CdTe and CdS diffraction.

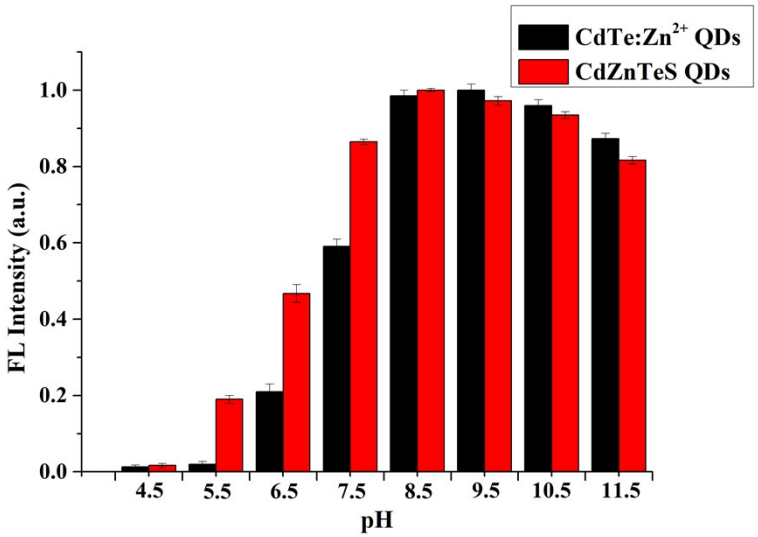

Figure S4. Fluorescence properties of NAC stabilized CdTe: Zn^{2+}QDs and NAC and MSA co-stabilized CdZnTeS QDs at different pH.

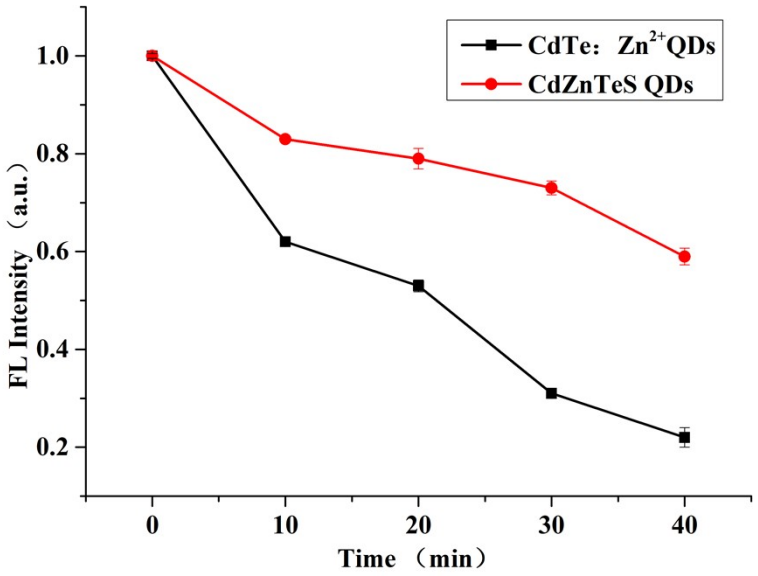

Figure S5. The photostability of CdTe: Zn^{2+} QDs and CdZnTeS QDs (UV lamp 16W, 365nm).

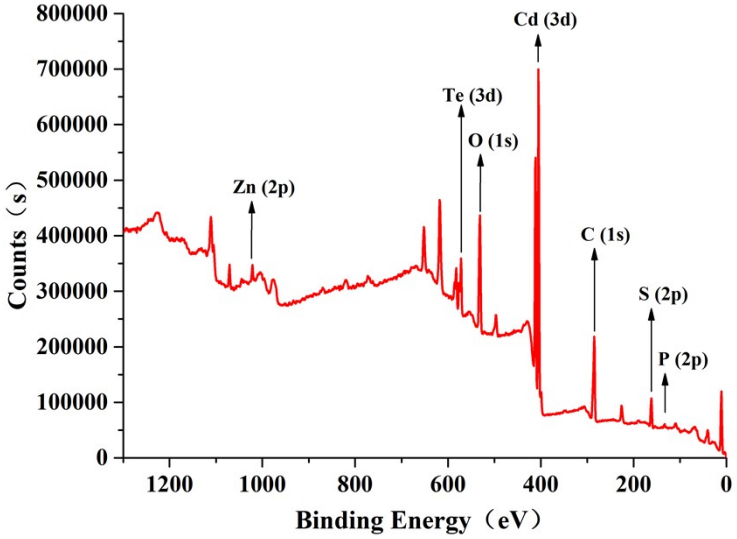

Figure S6. XPS of DNA-passivated CdZnTeS QDs.

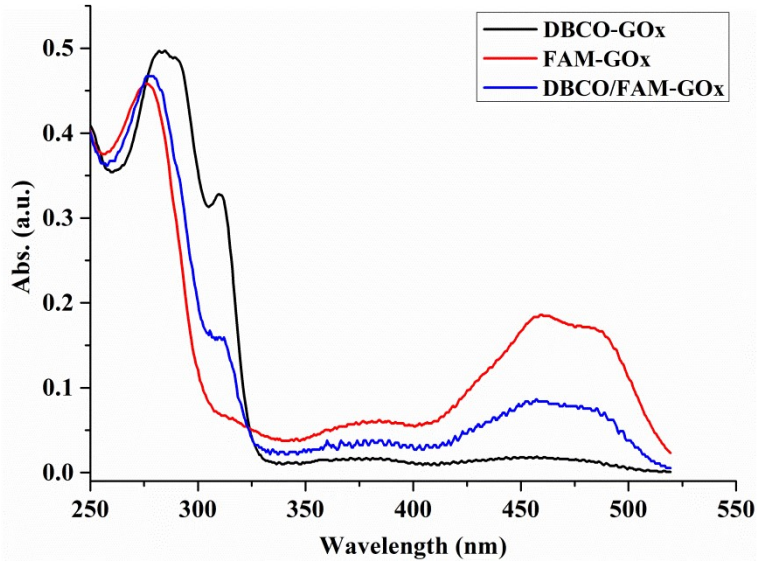

Figure S7. UV-vis absorbance spectra of DBCO-GOx, FAM-GOx and DBCO/FAM -GOx.

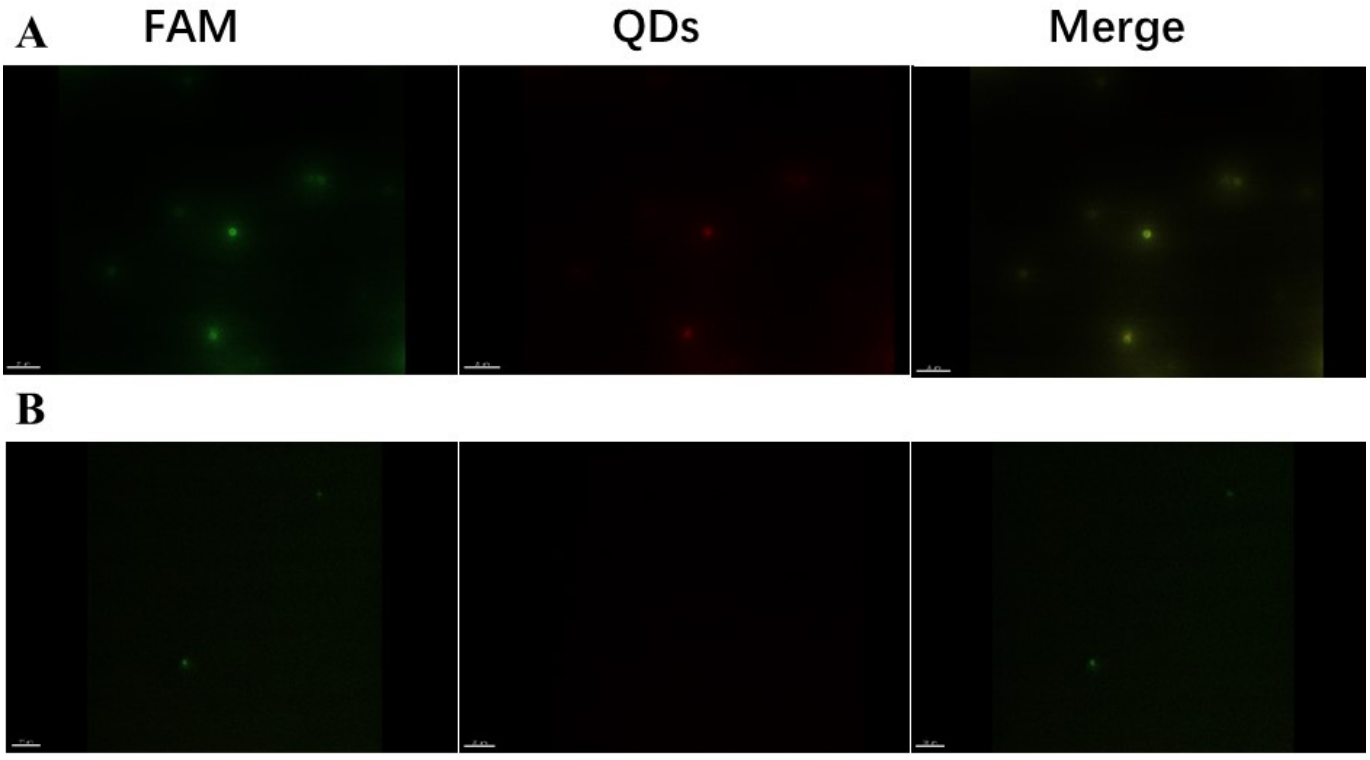

Figure S8. Confocal images of the FAM@GOx-QDs complex before (A) and after (B) incubation with glucose. Scale bar: 5 μm.

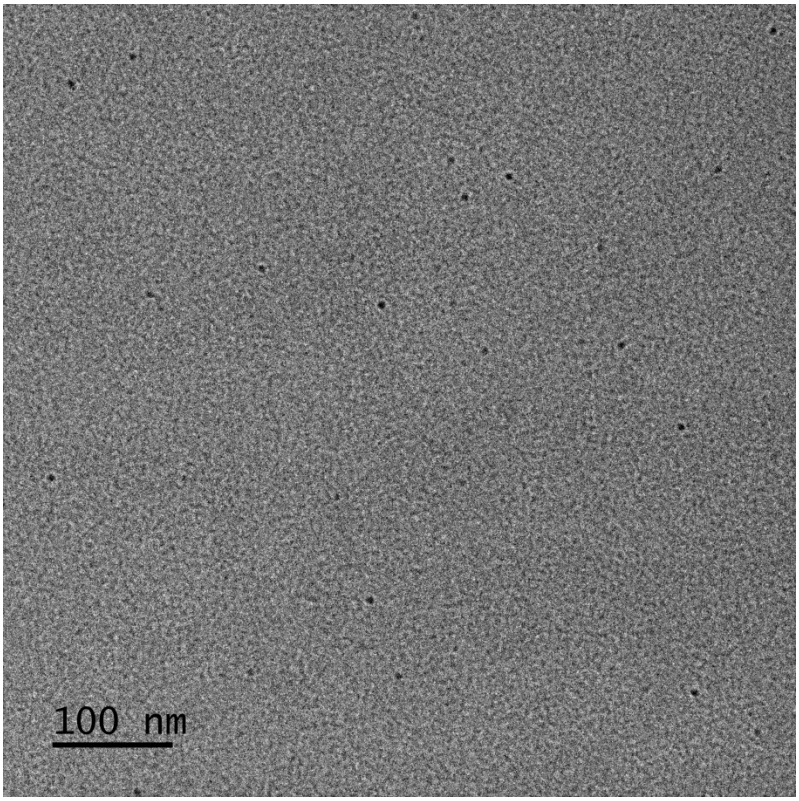

Figure S9. Transmission electron microscopy (TEM) images of FAM@GOx-QDs complex.

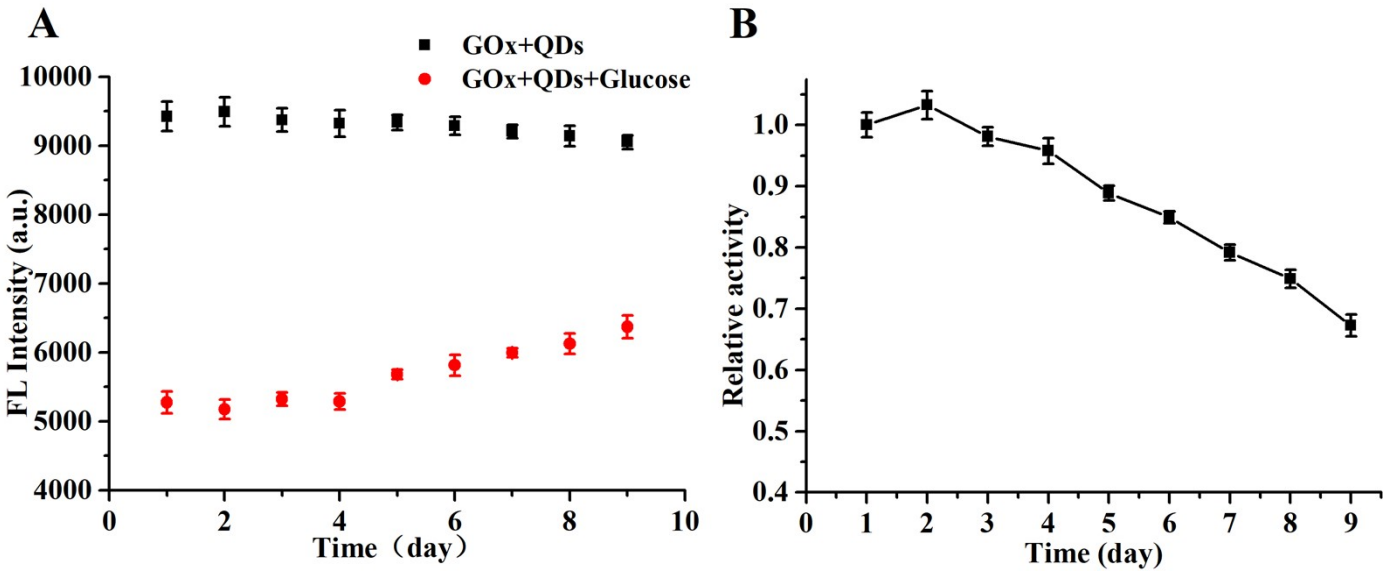

Figure S10. (A) Fluorescence intensity of the QDs adding GOx under different storing time before and after the addition of glucose. (B) The enzyme activity of GOx in the mixture of QDs and GOx under different storing time.

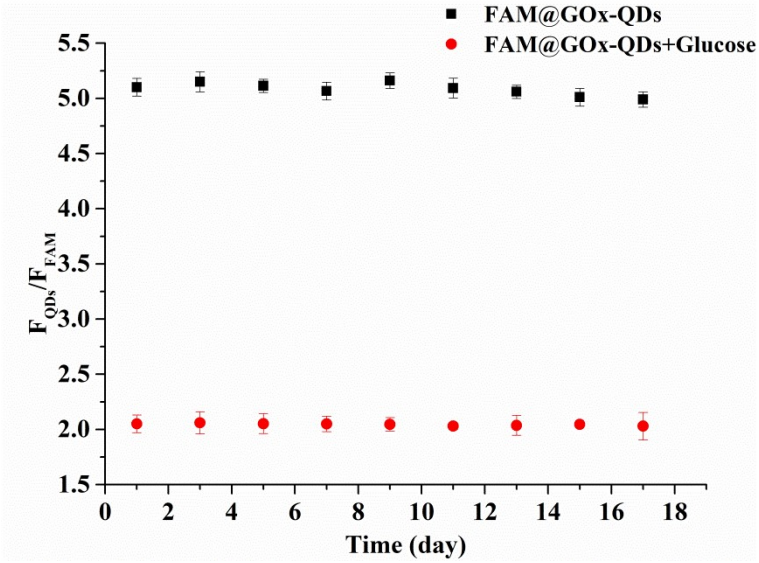

Figure S11. Fluorescence properties of the FAM@GOx-QDs complex under different storing time before and after the addition of glucose.

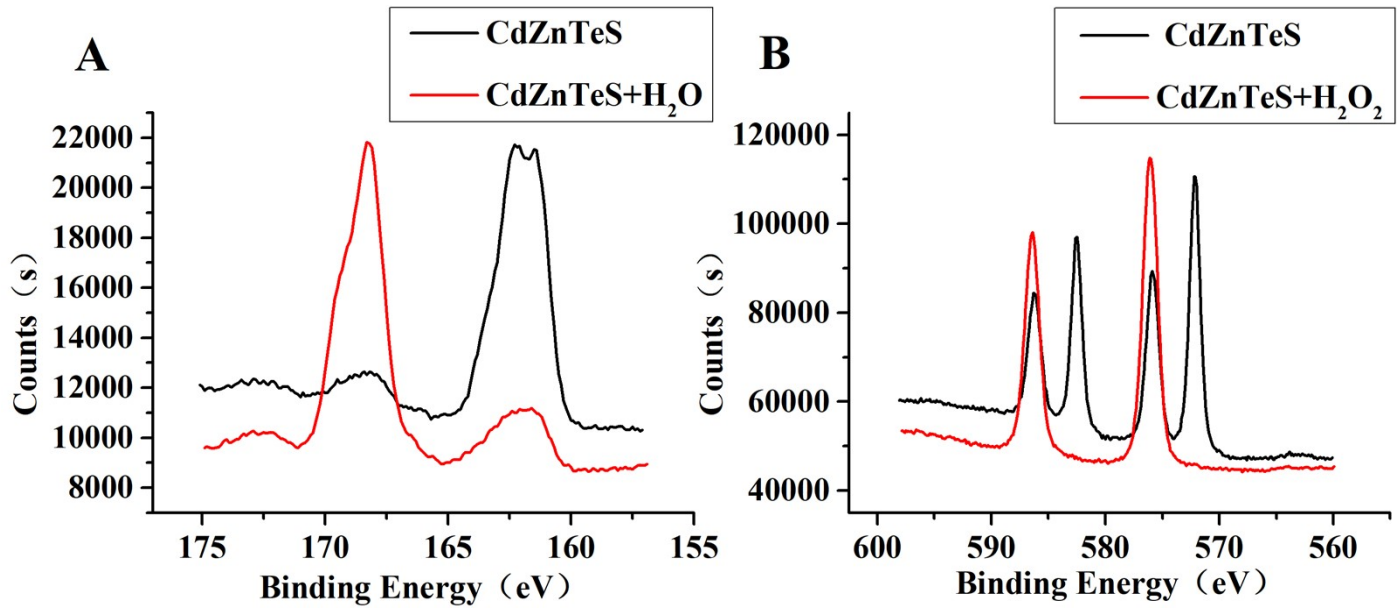

Figure S12. The high-resolution S peaks (A) and Te peaks (B) from XPS spectra of CdZnTeS QDs in the absence and present of excess H_2O_2

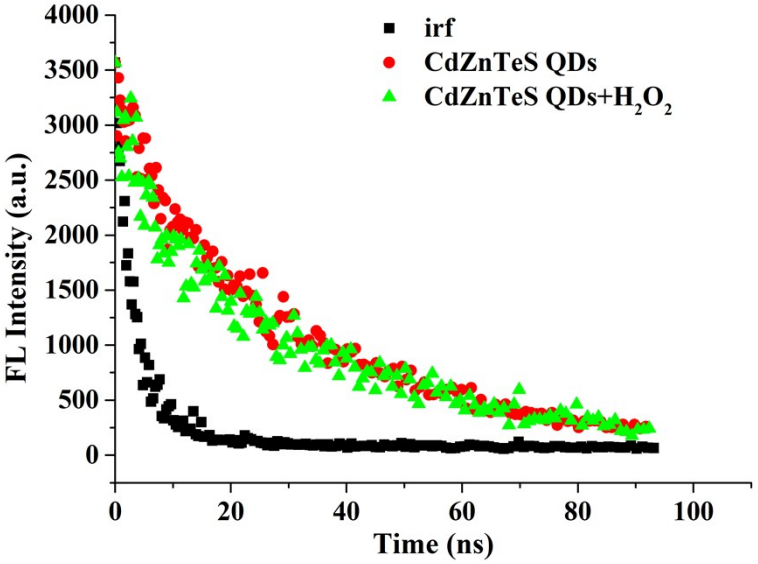

Figure S13. Fluroescence lifetime decay of CdZnTeS QDs in the absence and present of H_2O_2

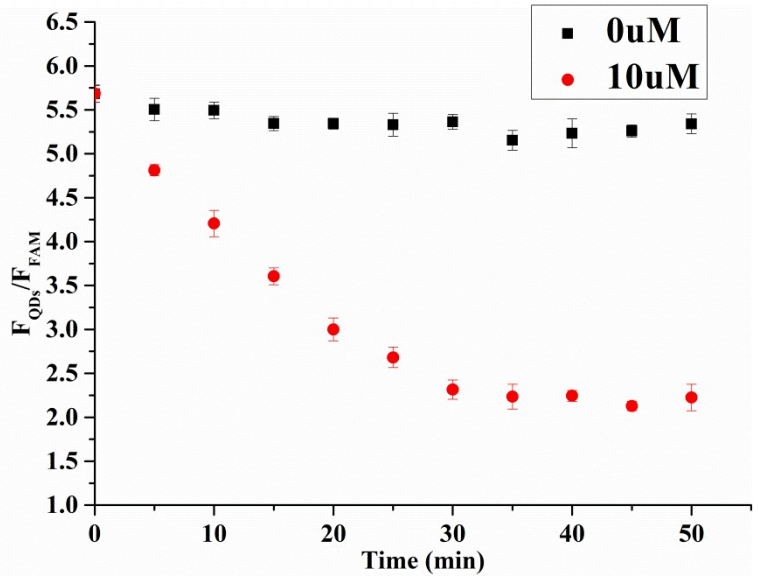

Figure S14. The kinetic curve of glucose (10 μM) reacting with FAM@GOx-QDs complex.

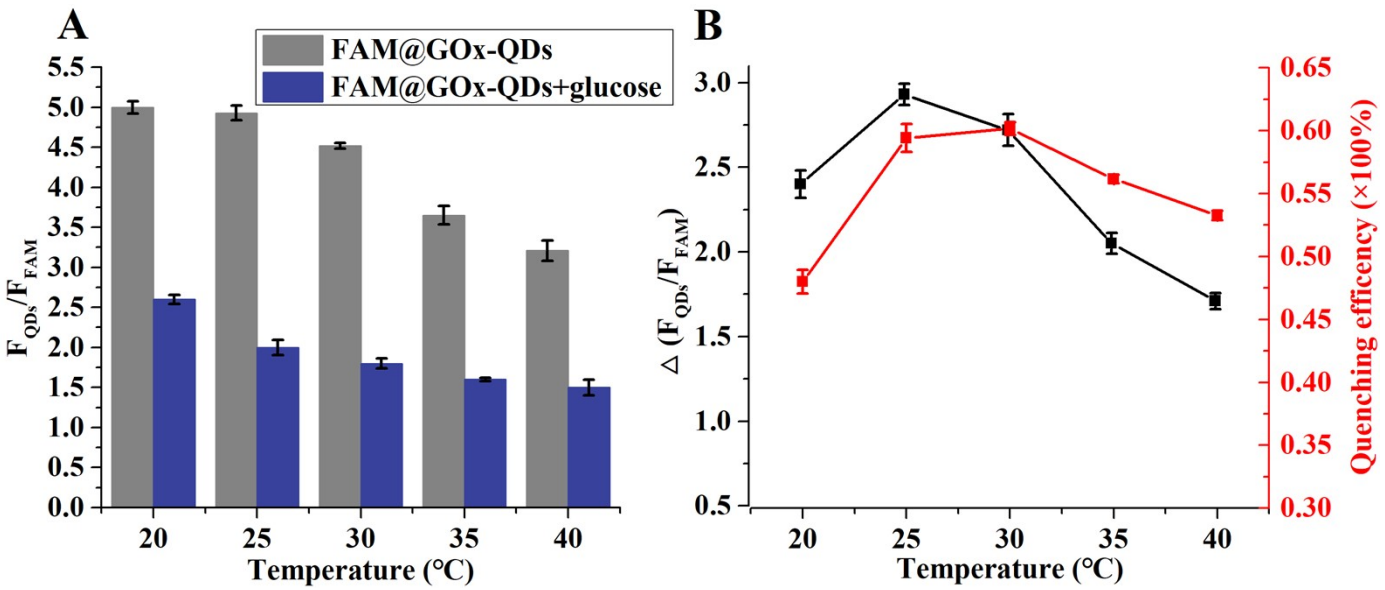

Figure S15. (A) Fluorescence properties of the FAM@GOx-QDs complex at different temperature before and after the addition of glucose. (B) The fluorescence quenching efficiency of the FAM@GOx-QDs complex in the presence of glucose corresponding to temperature.

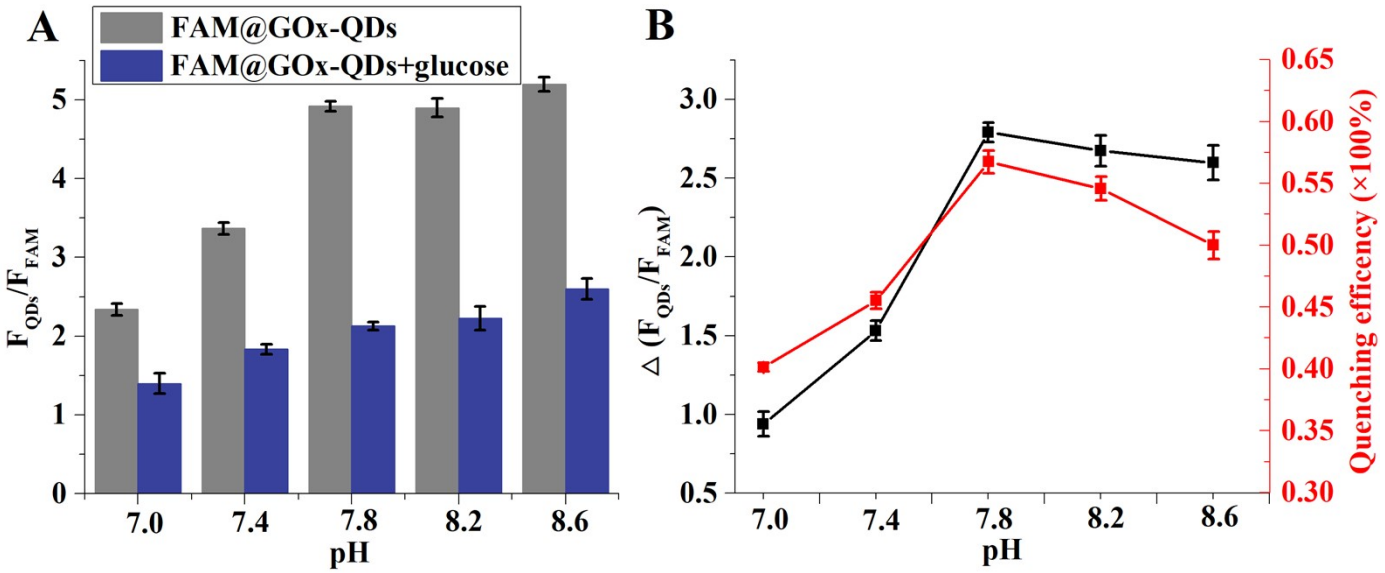

Figure S16. (A) Fluorescence properties of the FAM@GOx-QDs complex at different pH before and after the addition of glucose. (B) The fluorescence quenching efficiency of the FAM@GOx-QDs complex in the presence of glucose corresponding to pH.

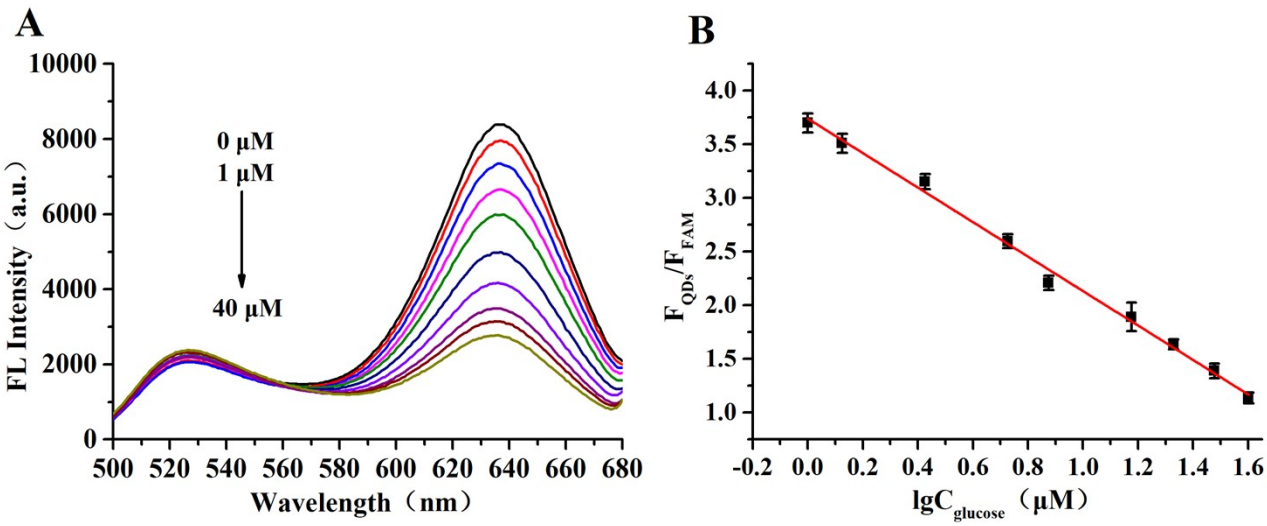

Figure S17. (A) Fluorescence spectra of the CdTeZnS QDs adding FAM-GOx and different concentrations (1-40.0 μM) of glucose. (B) Calibration curve for glucose established on mixture of FAM-GOx and QDs detection platform. The detection of limit is 0.22 μM.

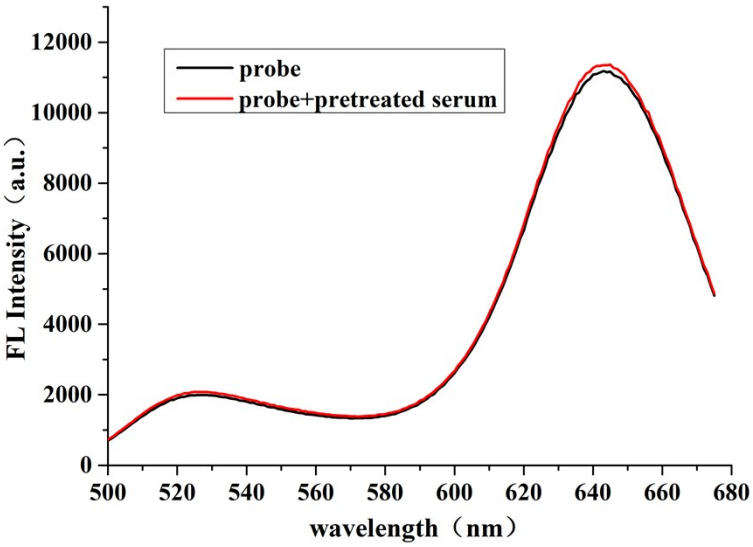

Figure S18. Fluorescence spectra of the FAM@GOx-QDs complex before and after added the pretreated serum.

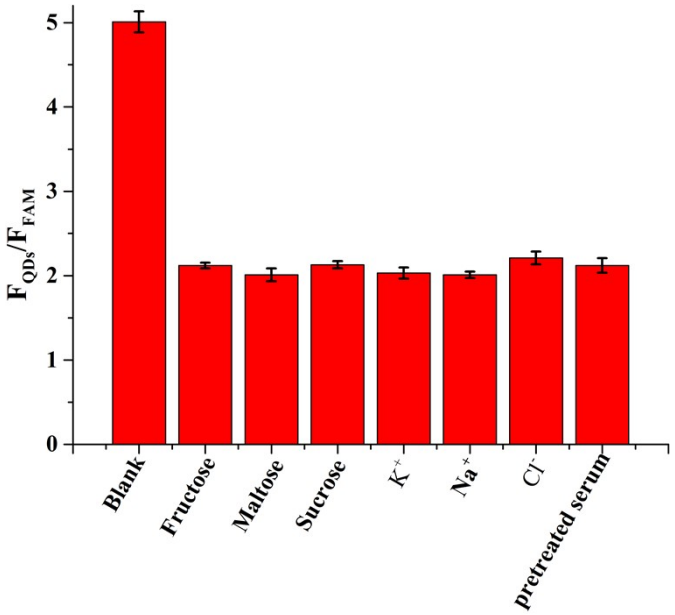

Fig. S19 Selectivity of the fabricated sensor for glucose detection in real samples. The pretreated serum sample was diluted for glucose detection (Final concentration of glucose is about 9.5 μM). The pretreated serum was added to buffer solution, in which the concentration of other interferents is 95 μM.

Table S3. Various Previously Reported Sensors for Glucose

materials	method	analytical range (μM)	LOD (μM)	time (min)	ref.
0D/2D Au_xPd_{100-x} nanocomposites	colorimetric	5-400	0.85	45	1
Fe-GDY/GOx	colorimetric	5-160	0.89	25	2
GOx-DNA@ mLDHs	colorimetric	5-250	0.42	240	3
Ag NPs@PCN-224	fluorescence	5-80	0.078	40	4
thiol-functionalized carbon dots	fluorescence	0.1-10	0.03	40	5
GOx-QDs complex	fluorescence	0.1-20	0.017	30	6
Ru−C_3N_4 nanosheets	ratiometric fluorescence	1-500	0.1	40	7
Eu-MOF	ratiometric fluorescence	0.1-4	0.064	60	8
CDs@SiO_2@QDs/Ag NP	ratiometric fluorescence	2-200	0.59	90	9
Rox-DNA functionalized CdZnTeS QDs	ratiometric fluorescence	2-100	0.042	60	10
FAM@GOx-QDs	ratiometric fluorescence	0.3-30	0.035	30	our work

Table S4. Results of glucose detection in human blood samples.

No.	Clinical outcome	Automatic analyzera (mM)	F_{QDs}/F_{FA} M	This method (mM)	RSD (%, n=3)
1	Diabetic	7.68	2.24	7.59	2.7
2	Diabetic	7.49	2.25	7.49	2.1
3	Diabetic	9.55	2.07	9.46	2.9
4	Diabetic	10.31	2.00	10.36	2.1
5	Diabetic	7.97	2.18	8.20	3.3
6	Diabetic	10.15	2.03	9.97	2.9
7	Diabetic	7.41	2.24	7.59	3.2
8	Diabetic	12.37	1.86	12.40	3.7
9	Diabetic	8.99	2.13	8.75	2.8
10	Diabetic	13.74	1.79	13.59	2.7
11	Diabetic	6.47	2.42	6.02	3.5
12	Diabetic	16.44	1.66	16.08	3.1
13	Diabetic	17.52	1.63	16.7	2.9

a The glucose determination was performed in the hospital for clinical analysis.

1. S. Cai, Z. Fu, W. Xiao, Y. Xiong, C. Wang and R. Yang, *ACS Appl. Mater. Interfaces*, 2020, **12**, 11616-11624.

2. J. Liu, X. Shen, D. Baimanov, L. Wang, Y. Xiao, H. Liu, Y. Li, X. Gao, Y. Zhao and C. Chen, *Appl. Mater. Interfaces*, 2019, **11**, 2647-2654.

3. H. Shen, Z. Zhou, W. He, H. Chao, P. Su, J. Song and Y. Yang, *Appl. Mater. Interfaces*, 2021, **13**, 14995-15007.

4. P. Du, Q. Niu, J. Chen, Y. Chen, J. Zhao and X. Lu, *Anal. Chem.*, 2020, **92**, 7980-7986.

5. J. Chen, J. Yan, B. Dou, Q. Feng, X. Miao and P. Wang, *Sensor. Actuat. B: Chem*, 2021, **330**, 129325.

6. G. Mao, Y. Ma, G. Wu, M. Du, S. Tian, S. Huang, X. Ji, Z. He, **2021**. *Anal. Chem*. **93**, 777-783.

7. W. Deng, Y. Peng, H. Yang, Y. Tan, M. Ma, Q. Xie and S. Chen, *Appl. Mater. Interfaces*, 2019, **11**, 29072-29077.

8. Y. Cui, F. Chen and X. B. Yin, *Biosens. Bioelectron.*, 2019, **135**, 208-215.

9. H. Lu, C. Yu, S. Quan and S. Xu, *Analyst*, 2019, **144**, 1153-1158.

10. G. Mao, Q. Cai, F. Wang, C. Luo, X. Ji and Z. He, *Anal. Chem.*, 2017, **89**, 11628-11635.