Electronic Supplementary Information

Glucose Oxidase-Directed, Instant Synthesis of Mn-doped ZnS Quantum Dots in Neutral Media with Retained Enzymatic Activity: Mechanistic Study and Biosensing Application

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1. Experimental Section

Materials and chemicals

Glucose oxidase (GOD, > 100 IU mg^{-1}, Shanghai Sangon), bovine serum albumin (BSA) and papain (Sigma-Aldrich), lysozyme and pepsin (Newprobe Biotechnology Co., Beijing), horseradish peroxidase (HRP, > 160 IU mg^{-1}, Aladin), 3,3',5,5'-tetramethylbenzidine (TMB, Aladin, Shanghai, China), D-glucose and H_2O_2 (Kelong Chemical, Chengdu, China), $ZnAc_2 \cdot 7H_2O$, $MnAc_2 \cdot 4H_2O$, and $Na_2S \cdot 9H_2O$ (Aladin) and Tris (Sinopharm Chemical Reagent Co., Ltd) were used in this work. 1-Acetylimidazole (NAI, 98.0%), N-succinimidyl N-methylcarbamate (NHS, 97.0%), and diethylpyrocarbonate (DEPC, 99.0%), N-ethylmaleimide (99.0%), methylamine (40% w/w aq. solution), and 1-(3-dimethylaminopropyl)-3- ethylcarbodiimide hydrochloride (EDC, 98.0%) were purchased from Aladin (Shanghai, China). Ultrapure water (18.2 MΩ cm) from a water purification system (PCUJ-10, Chengdu Pure Technology Co., Ltd., Chengdu, China).

Synthesis of GOD-Mediated Mn-doped ZnS QDs

For preparation of GOD-mediated Mn-doped ZnS QDs, 30 μL GOD (10 mg mL^{-1}) , 20 μL $MnAc_2$ (1 mM), 39 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 6.5, 0.1 M) were added to 325 μL ultrapure water for five minutes in room temperature. Then 36 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent GOD-capped Mn-doped ZnS QDs were thus obtained.

Characterization

Phosphorescence, fluorescence and phosphorescence lifetime were performed using an F-7000 spectrofluorometer (Hitachi, Japan) equipped with a plotter unit and a quartz cell (1 cm × 1 cm). The voltage of the photomultiplier tube (PMT) was set at 700 V. The excitation wavelength was set at 310 nm for phosphorescence, fluorescence and phosphorescence lifetime measurements. Absorption spectra were recorded using a UV-1700 UV/Vis spectrophotometer (Shimadzu, Japan). HRTEM images of QDs were obtained using a Tecnai G2 F20 S-TWIN transmission electron microscope at an accelerating voltage of 200 kV (FEI Co., USA). The polyacrylamide gel electrophoresis (PAGE) was performed using JY-SCZ2+ electrophoresis apparatus (Jun Yi-Dong Fang, Beijing, China). Circular dichroism (CD) spectrum was performed using JASCO J-A500-150 spectrometer. The polyacrylamide gel images and luminous QDs images (under UV irradiation 302 nm) were taken with a Nikon D300S digital camera.

2. Optimization of the Synthetic conditions for Mn-ZnS QDs

In this work, we employed the GOD as the template for directing the synthesis of Mn-doped ZnS QDs in one second and in neutral aqueous media at room temperature. The doping amount of Mn^{2+} plays a key roles in the process, so various amounts of $Mn(Ac)_2$ adding to $Zn(Ac)_2$ solution in the presence of GOD were studied. As shown in Figure S1A, the emission color of the QDs changed immediately after incorporation of Mn^{2+}. When reached 2.5% dopant amount, the highest

phosphorescence QDs was obtained (Figure S1B) and therefore used in further investigations.

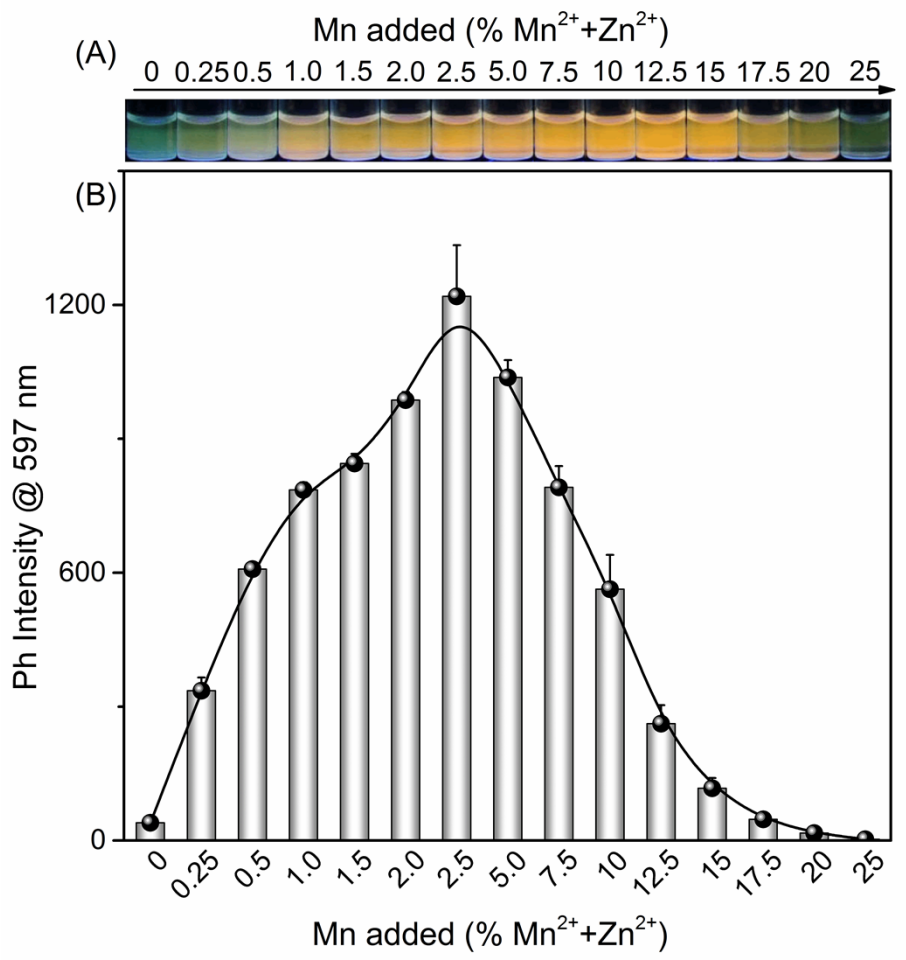

Figure S1. (A) Fluorescent photographs of UV-excited GOD-Mediated Mn-doped ZnS QDs with increasing Mn^{2+} concentration (λ_{ex} = 302 nm). (B) Phosphorescence intensity of GOD-mediated Mn-doped ZnS QDs at 597 nm mineralized in the presence of indicated amount of Mn^{2+} (λ_{ex} = 310 nm).

In order to achieve higher phosphorescence, the concentration of S^{2-} and pH of the buffer solution were investigated. As shown in Figure S2, when the ratio of S^{2-} : (Mn^{2+} + Zn^{2+}) was set at 0.9, the highest phosphorescence was obtained. It is worth nothing that the amount of S^{2-} was not equal to that of cations. It is possible that excessive amount of S^{2-} would increase the degree of surface defects, which may

result in decreasing the light intensity of QDs. One can also see from Figure S3 that the absorbance of QDs increased to the maximum when the S^{2-} ratio was set at 0.9. Investigations on the pH of synthetic media revealed that 0.01 M, pH 6.5 Tris-HCl buffer was optimal for synthesis of Mn-ZnS QDs (Figure S4).

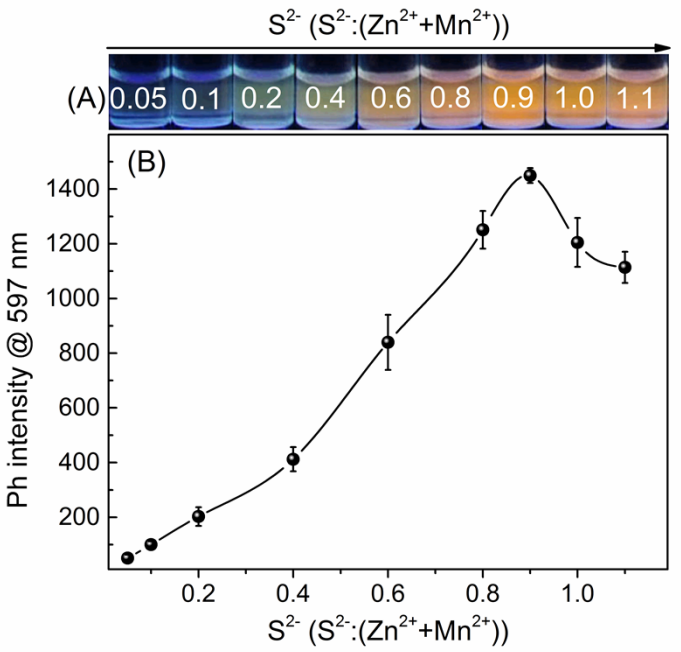

Figure S2. (A) Fluorescent photographs of UV-excited GOD-mediated Mn-doped ZnS QDs with increase of concentration of S^{2-} (λ_{ex} = 302 nm). (B) Phosphorescence intensity of GOD-mediated Mn-doped ZnS QDs at 580 nm mineralized in the presence of indicated amount of S^{2-} (λ_{ex} = 310 nm). The slit width was 10 and 10 nm for excitation and emission, respectively.

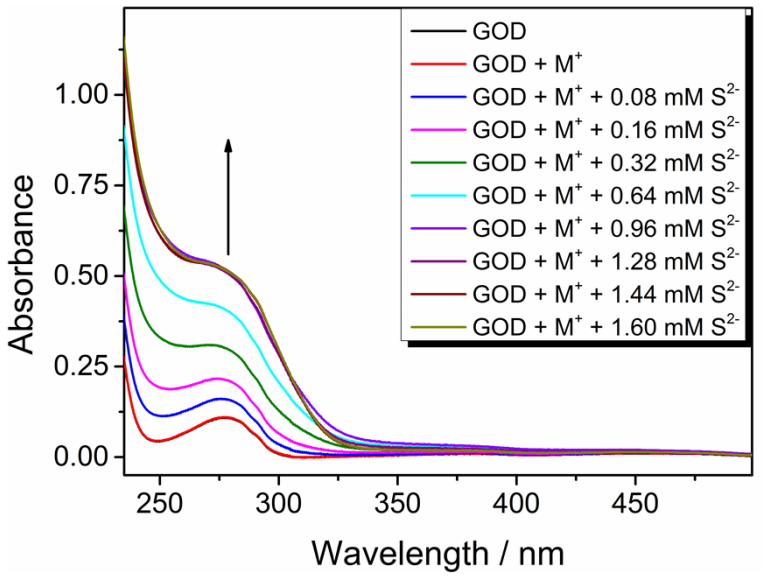

Figure S3. Absorption spectra of GOD-Mediated Mn-ZnS QDs synthesized at different amount of S^{2-}.

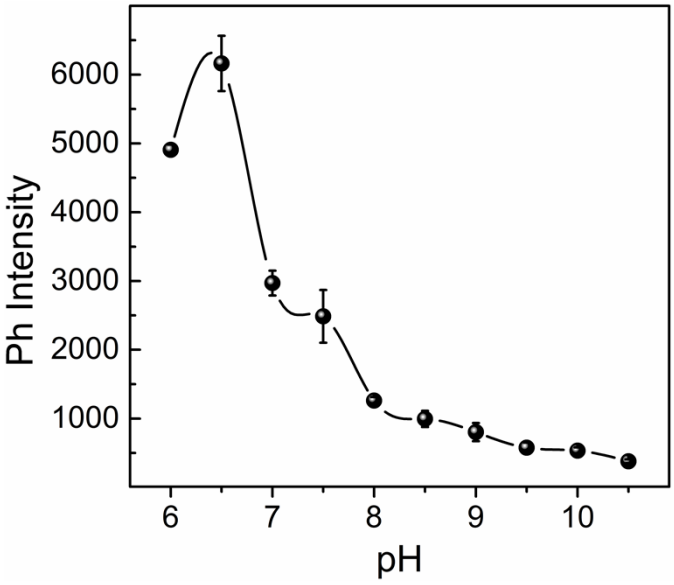

Figure S4. Optimization of pH in the synthetic conditions for GOD-mediated Mn-doped ZnS QDs (Tris-HCl 0.01 M).

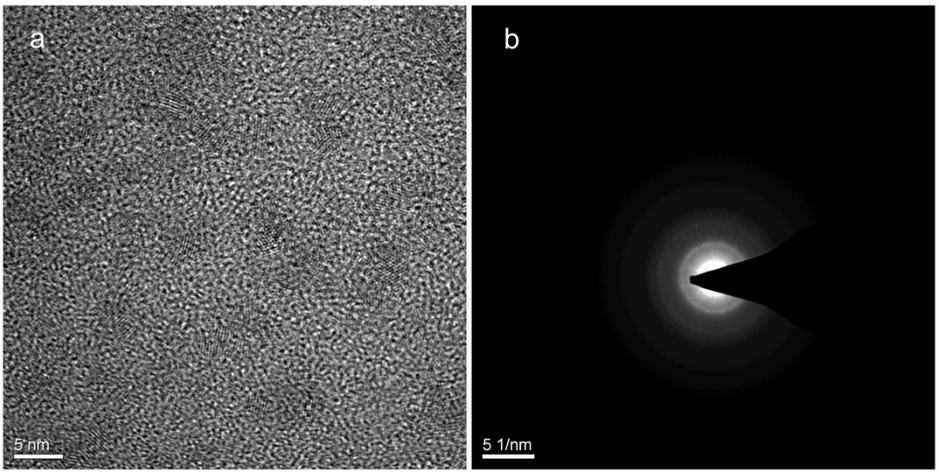

Figure S5. High-magnification TEM image (a) and selected area diffraction (SAD, b) of the as-prepared Mn-doped ZnS QDs.

3. Enzymatic activity assay

To carry out the enzymatic activity assay, we obtained a standard curve of H_2O_2 and absorbance of TMB firstly. Then the free and QDs-bound GOD enzymes (2 mg mL^{-1}, 20 μL) were mixed in 3 mL of phosphate-buffered saline (PBS,10 mM, pH = 6.5) containing 10 μL of HRP (2 mg mL^{-1}), 20 μL TMB (5 mg mL^{-1}, dissolving in ethanol) and various concentrations of glucose as a substrate separately. The absorbance was recorded at 652 nm. The value of the K_m, which gives an indication of the enzyme-substrate kinetics, was determined by the analysis of the slope of enzymatic reactions. The K_m value for an enzymatic reaction determines the affinity of the enzyme for the substrate. These parameters have been estimated using the Lineweaver-Bruke plot:

$$\frac{1}{v} = \frac{K_m}{V_{\max}} \frac{1}{[S]} + \frac{1}{V_{\max}}$$

$$V_{\max} = k_{\text{cat}}[E]_0$$

Table S1 Kinetic parameters of free GOD and GOD after directing the synthesis of QDs.

GOD conditions[a]	K_m (mM)	k_{cat} (1/s)	k_{cat}/K_m (1/M*s)
Free GOD	1.3	25.66	1.97×10^4
25 °C, instant, pH 6.5	1.0	22.84	2.28×10^4
37 °C, 4 days, pH 6.5	1.1	21.02	1.91×10^4
50 °C, 3 h, pH 6.5	3.2	41.62	1.30×10^4
60 °C, 3 h, pH 6.5	7.1	24.47	3.43×10^3
70 °C, 3 h, pH 6.5	∞	0	0
25 °C, instant, pH 6	1.5	28.62	1.95×10^4
25 °C, instant, pH 7	1.6	23.35	1.46×10^4
25 °C, instant, pH 8	5.0	50.99	1.02×10^4
25 °C, instant, pH 9	5.2	49.3	9.42×10^3
25 °C, instant, pH 10	11.2	95.32	8.54×10^3
25 °C, instant, pH 11	12.4	83.07	6.68×10^3

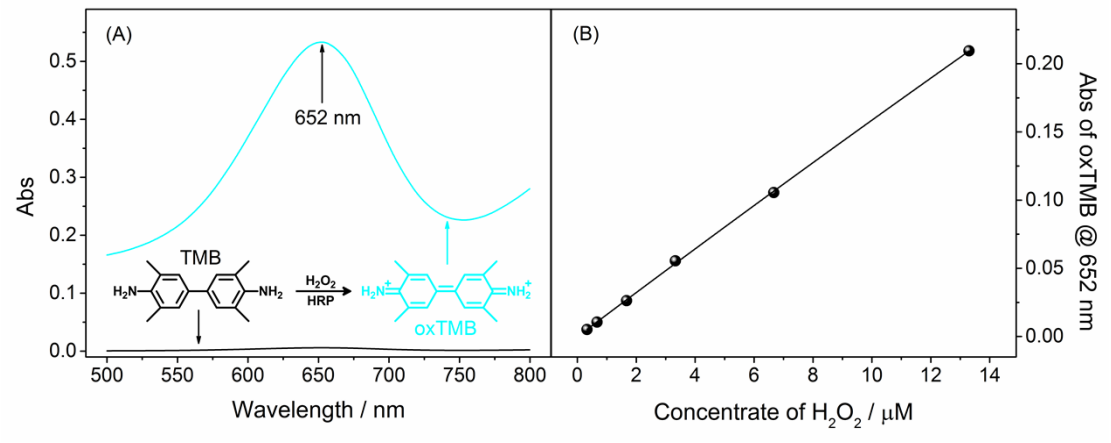

Figure S6. (A) Absorption spectra of TMB and oxidized TMB; and (B) calibration curve of H_2O_2 *versus* absorbance of oxidized TMB (@652 nm).

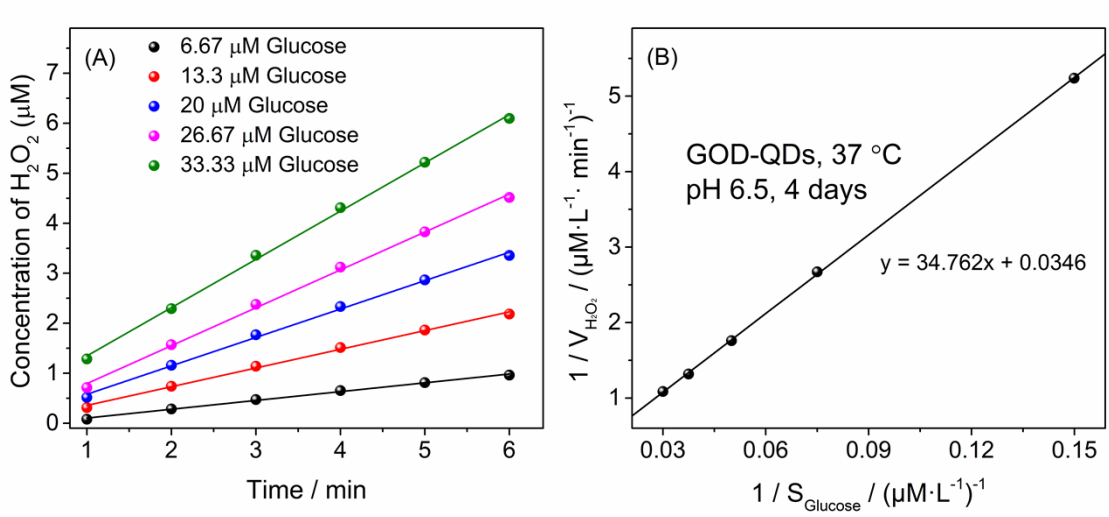

Figure S7. (A) The generation velocity of H_2O_2 (measured *versus* the calibration curve shown in Figure S6B) in the presence of different amounts of glucose catalyzed by GOD after directing the synthesis of Mn-ZnS QDs (GOD: 4 μg/mL, HRP: 6.7μg/mL; synthetic conditions: 37 °C, pH 6.5 Tris-HCl buffer, and 4 days); and (B) the corresponding Lineweaver-Burk plot.

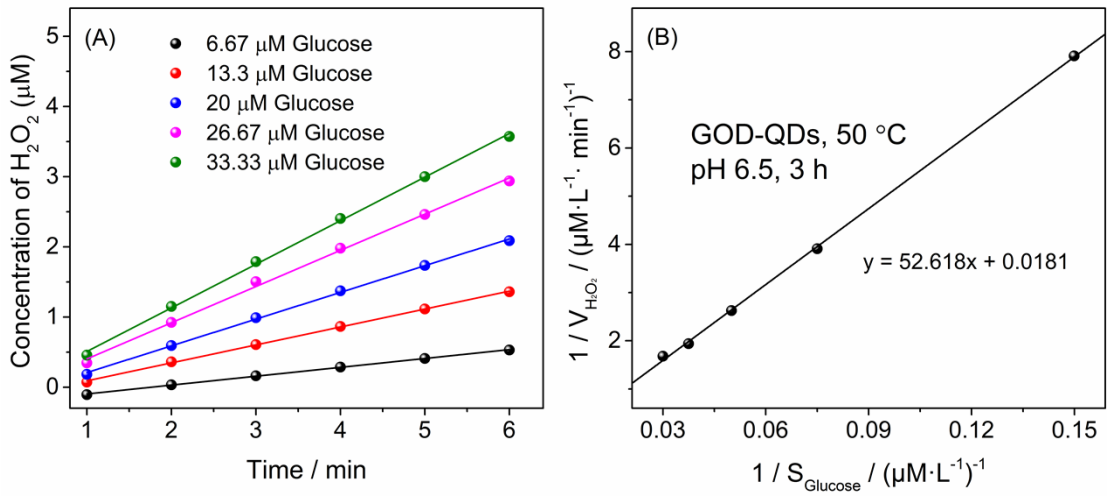

Figure S8. (A) The generation velocity of H_2O_2 (measured *versus* the calibration curve shown in Figure S6B) in the presence of different amounts of glucose catalyzed by GOD after directing the synthesis of Mn-ZnS QDs (GOD: 4 μg/mL, HRP: 6.7μg/mL; synthetic conditions: 50 °C, pH 6.5 Tris-HCl buffer, and 3 h); and (B) the corresponding Lineweaver-Burk plot.

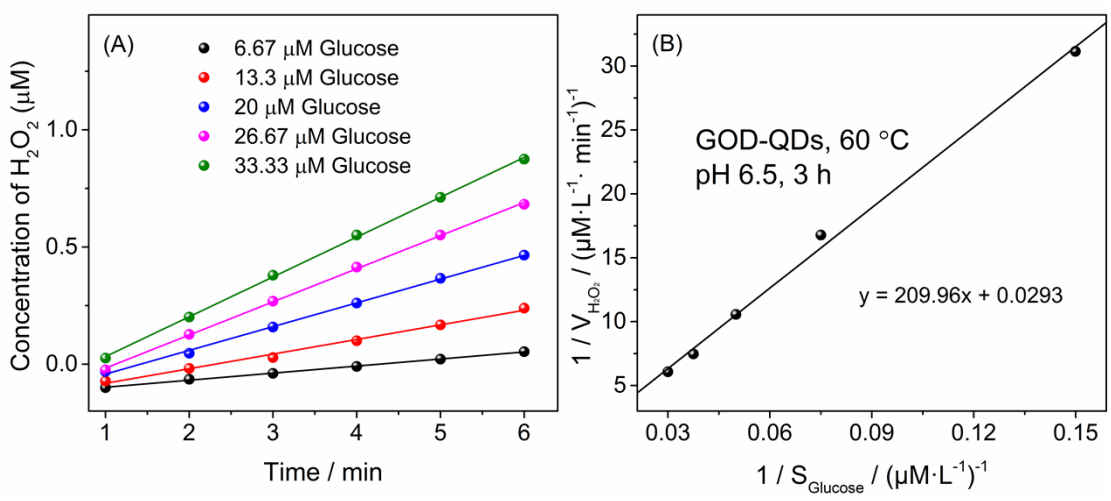

Figure S9. (A) The generation velocity of H_2O_2 (measured *versus* the calibration curve shown in Figure S6B) in the presence of different amounts of glucose catalyzed by GOD after directing the synthesis of Mn-ZnS QDs (GOD: 4 μg/mL, HRP: 6.7μg/mL; synthetic conditions: 60 °C, pH 6.5 Tris-HCl buffer, and 3 h); and (B) the corresponding Lineweaver-Burk plot.

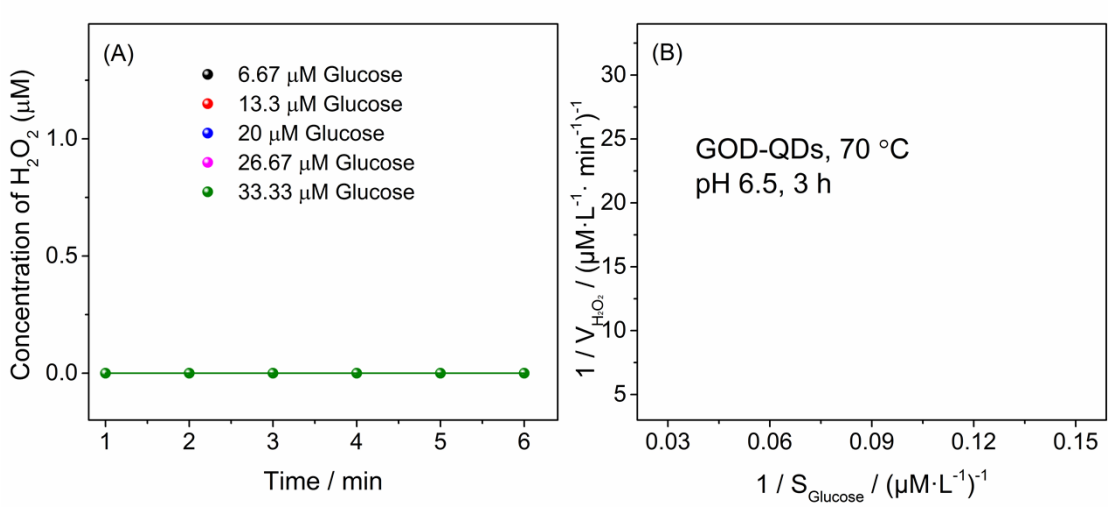

Figure S10. (A) The generation velocity of H_2O_2 (measured *versus* the calibration curve shown in Figure S6B) in the presence of different amounts of glucose catalyzed by GOD after directing the synthesis of Mn-ZnS QDs (GOD: 4 μg/mL, HRP: 6.7μg/mL; synthetic conditions: 70 °C, pH 6.5 Tris-HCl buffer, and 3 h); and (B) the corresponding Lineweaver-Burk plot. At such conditions, the enzymatic activity of GOD was almost completely lost. Therefore, no H_2O_2 was generated and no TMB oxidation was observed.

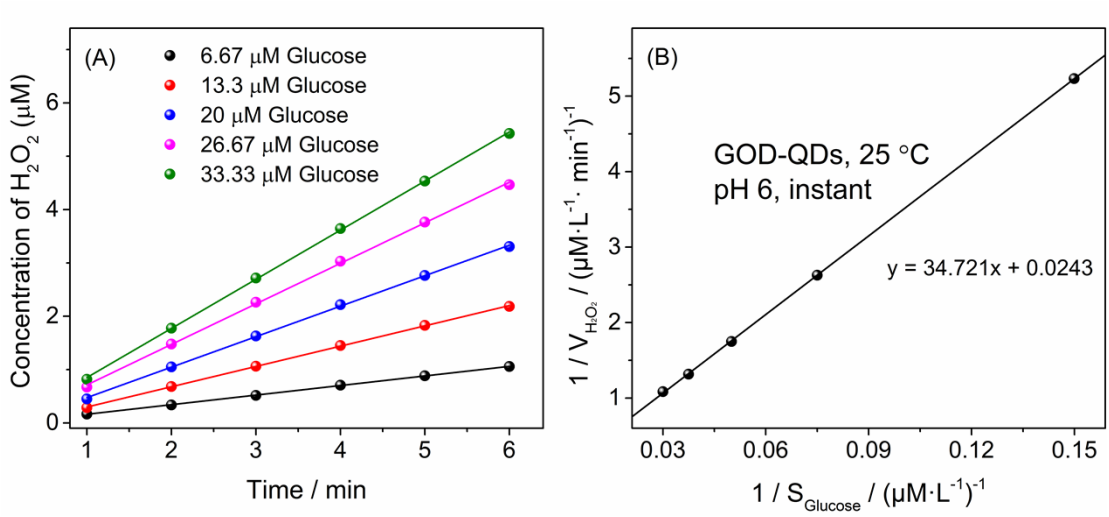

Figure S11. (A) The generation velocity of H_2O_2 (measured *versus* the calibration curve shown in Figure S6B) in the presence of different amounts of glucose catalyzed by GOD after directing the synthesis of Mn-ZnS QDs (GOD: 4 μg/mL, HRP: 6.7μg/mL; synthetic conditions: 25 °C, pH 6, Tris-HCl buffer, and instant); and (B) the corresponding Lineweaver-Burk plot.

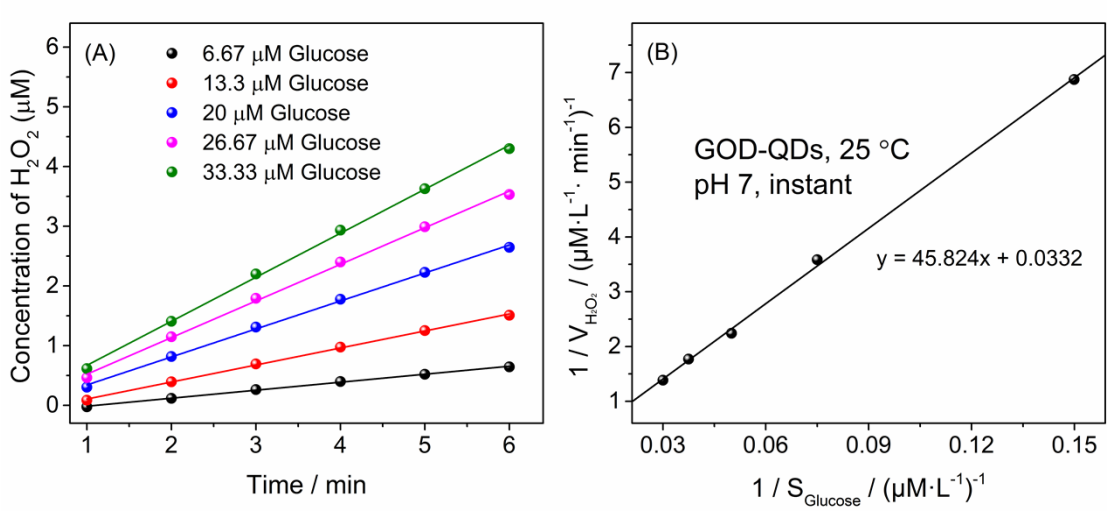

Figure S12. (A) The generation velocity of H_2O_2 (measured *versus* the calibration curve shown in Figure S6B) in the presence of different amounts of glucose catalyzed by GOD after directing the synthesis of Mn-ZnS QDs (GOD: 4 μg/mL, HRP: 6.7μg/mL; synthetic conditions: 25 °C, pH 7, Tris-HCl buffer, and instant); and (B) the corresponding Lineweaver-Burk plot.

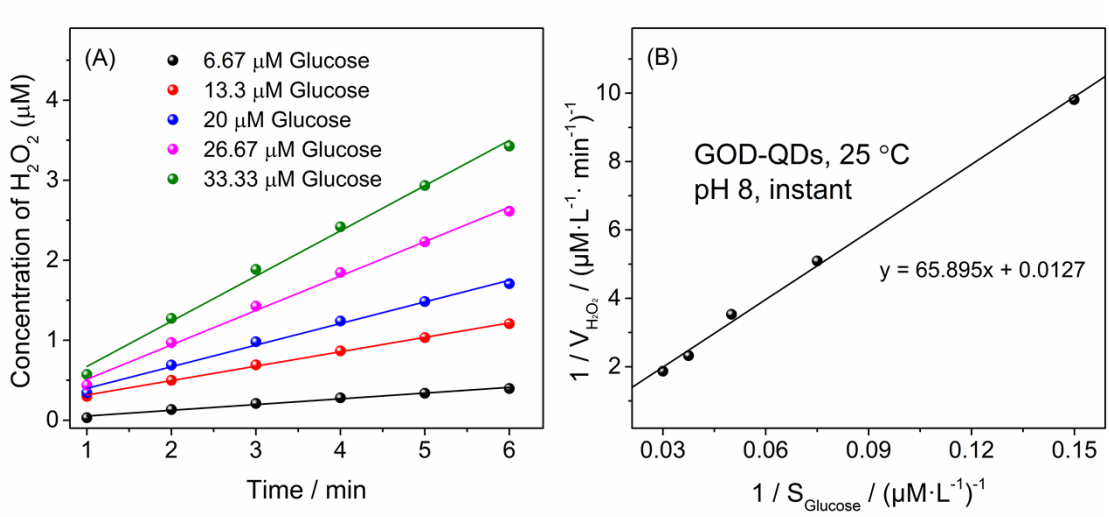

Figure S13. (A) The generation velocity of H_2O_2 (measured *versus* the calibration curve shown in Figure S6B) in the presence of different amounts of glucose catalyzed by GOD after directing the synthesis of Mn-ZnS QDs (GOD: 4 μg/mL, HRP: 6.7μg/mL; synthetic conditions: 25 °C, pH 8, Tris-HCl buffer, and instant); and (B) the corresponding Lineweaver-Burk plot.

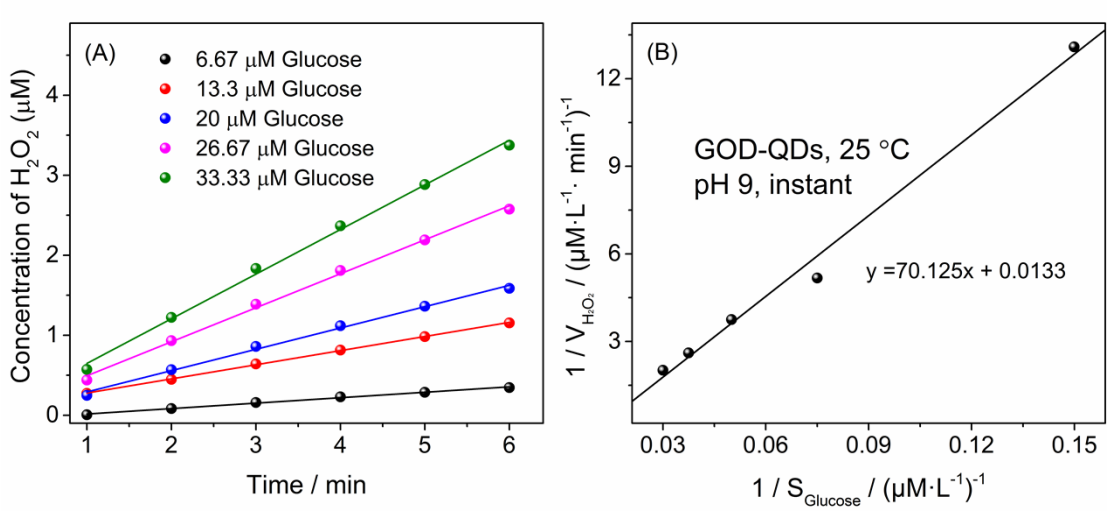

Figure S14. (A) The generation velocity of H_2O_2 (measured *versus* the calibration curve shown in Figure S6B) in the presence of different amounts of glucose catalyzed by GOD after directing the synthesis of Mn-ZnS QDs (GOD: 4 μg/mL, HRP: 6.7μg/mL; synthetic conditions: 25 °C, pH 9, Tris-HCl buffer, and instant); and (B) the corresponding Lineweaver-Burk plot.

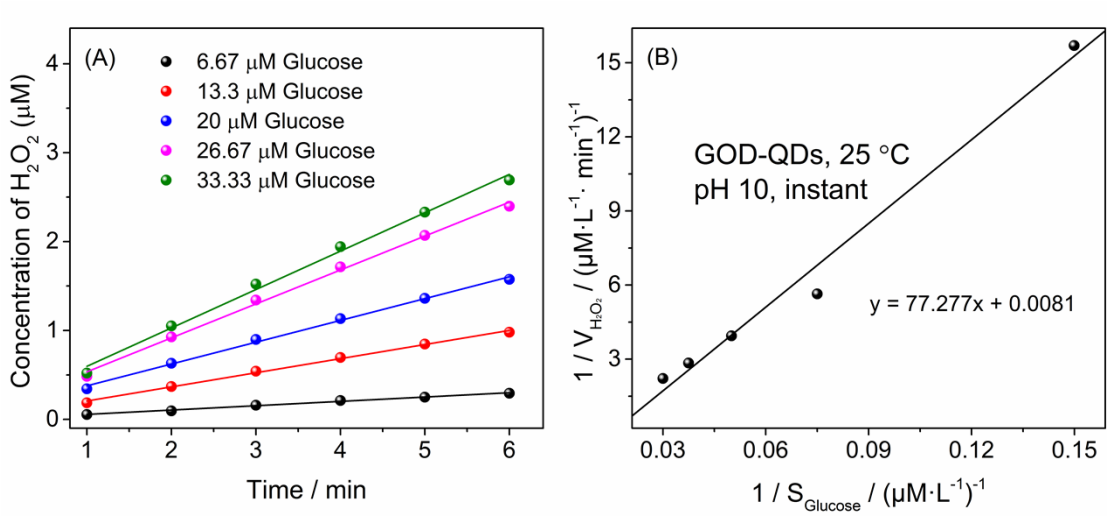

Figure S15. (A) The generation velocity of H_2O_2 (measured *versus* the calibration curve shown in Figure S6B) in the presence of different amounts of glucose catalyzed by GOD after directing the synthesis of Mn-ZnS QDs (GOD: 4 μg/mL, HRP: 6.7μg/mL; synthetic conditions: 25 °C, pH 10, Tris-HCl buffer, and instant); and (B) the corresponding Lineweaver-Burk plot.

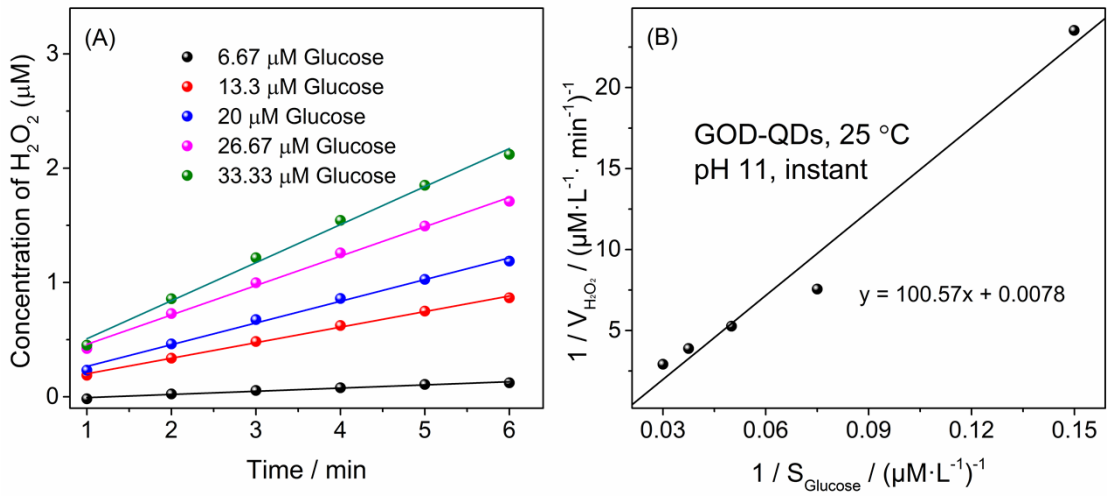

Figure S16. (A) The generation velocity of H_2O_2 (measured *versus* the calibration curve shown in Figure S6B) in the presence of different amounts of glucose catalyzed by GOD after directing the synthesis of Mn-ZnS QDs (GOD: 4 μg/mL, HRP: 6.7μg/mL; synthetic conditions: 25 °C, pH 11, Tris-HCl buffer, and instant); and (B) the corresponding Lineweaver-Burk plot.

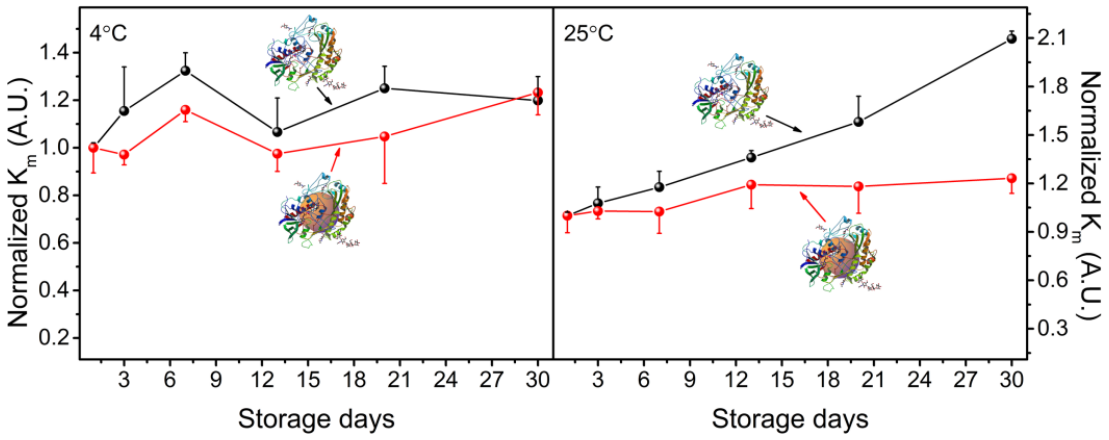

Figure S17. Normalized K_m of free GOD or GOD-QDs was been monitored under 4°C and 25°C storage for one month, respectively.

Table S2. Enzymatic conformation information of free GOD or GOD after various synthetic conditions using the self-consistent method from the CD spectra.

Conformation / Various GOD	α-helix	β-sheet	β-turn	Polypro II helix	Random coil	total
Free GOD	53.6	8.0	5.1	3.3	32.5	100
GOD-M^+, pH 6.5	52.3	8.0	4.3	3.1	33.4	100
GOD-QDs, pH 6.5, instant	55.8	7.2	6.5	2.7	24.9	100
GOD-QDs, pH 6.5, 4 days	54.3	10.8	4.9	2.4	27.9	100
GOD-QDs, 50°C, 3 h	41.5	14.5	11.0	4.4	32.0	100
GOD-QDs, 60°C, 3 h	47.0	7.3	10.6	3.9	32.0	100
GOD-QDs, 70°C, 3 h	68.1	5.2	3.6	2.7	20.7	100
GOD-QDs, pH 6, instant	58.3	6.3	6.8	2.5	23.6	100
GOD-QDs, pH 7, instant	60.6	5.6	6.9	1.9	22.3	100
GOD-QDs, pH 8, instant	63.1	9.3	1.6	5.5	22.0	100
GOD-QDs, pH 9, instant	64.5	3.5	7.0	1.0	24.6	100
GOD-QDs, pH 10, instant	65.3	7.9	3.9	3.2	21.2	100
GOD-QDs, pH 11, instant	68.1	5.2	3.6	2.7	20.7	100

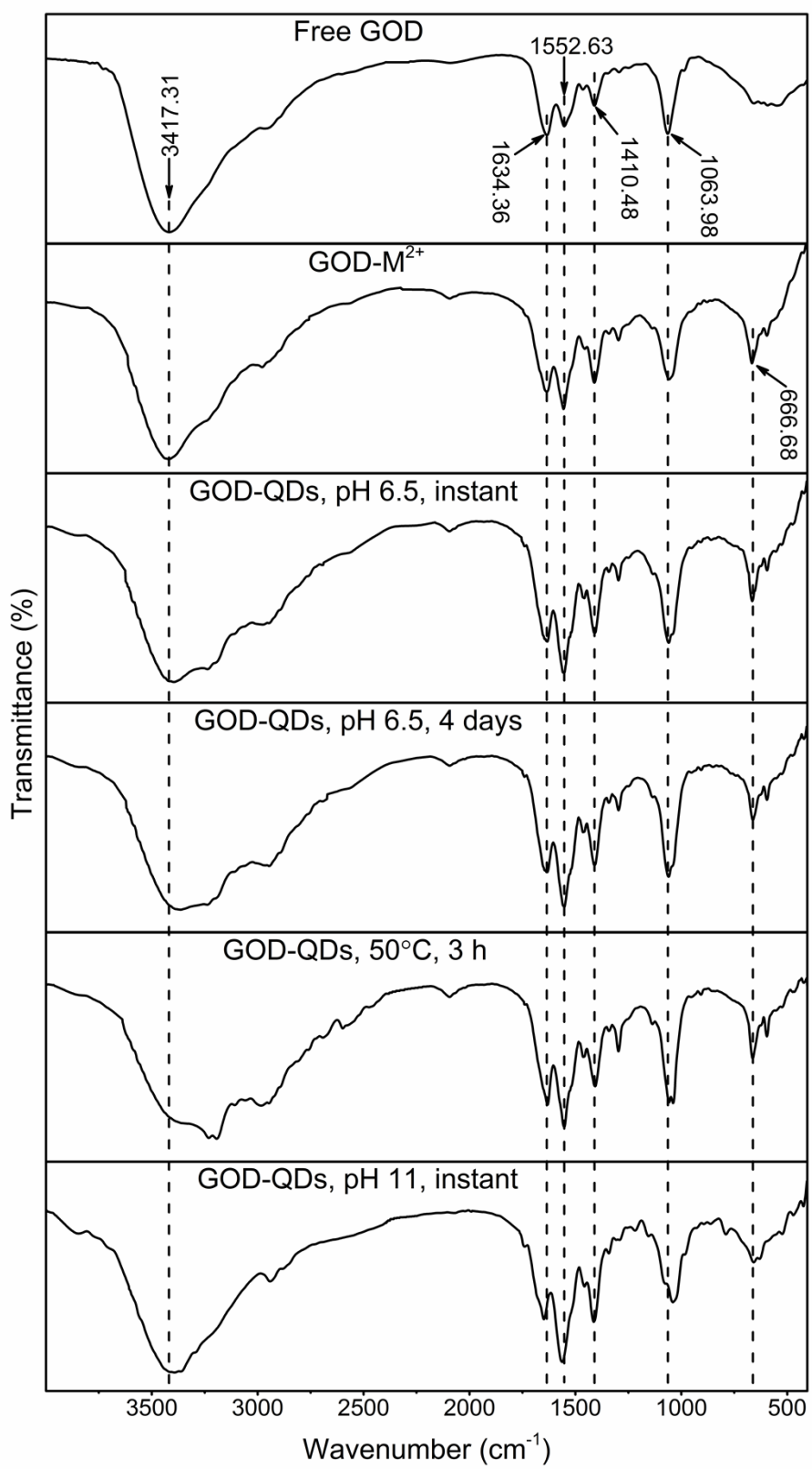

Figure S18. FT-IR spectra of free GOD, GOD-metal ions and GOD-capped Mn-ZnS QDs.

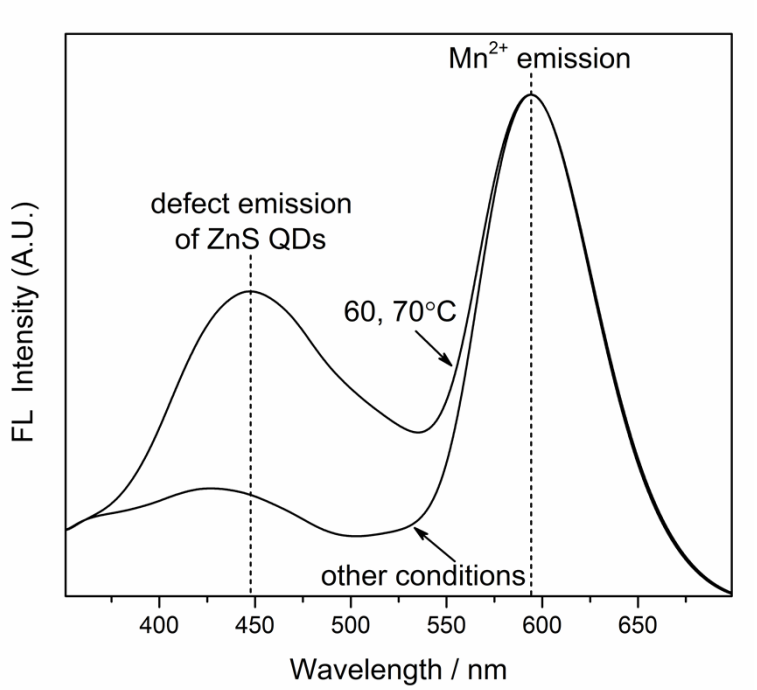

Figure S19. Fluorescence emission spectra (Ex: 310 nm) of GOD-Mn-ZnS QDs synthesized at 60 and 70 °C and other conditions.

4. Photostability investigation

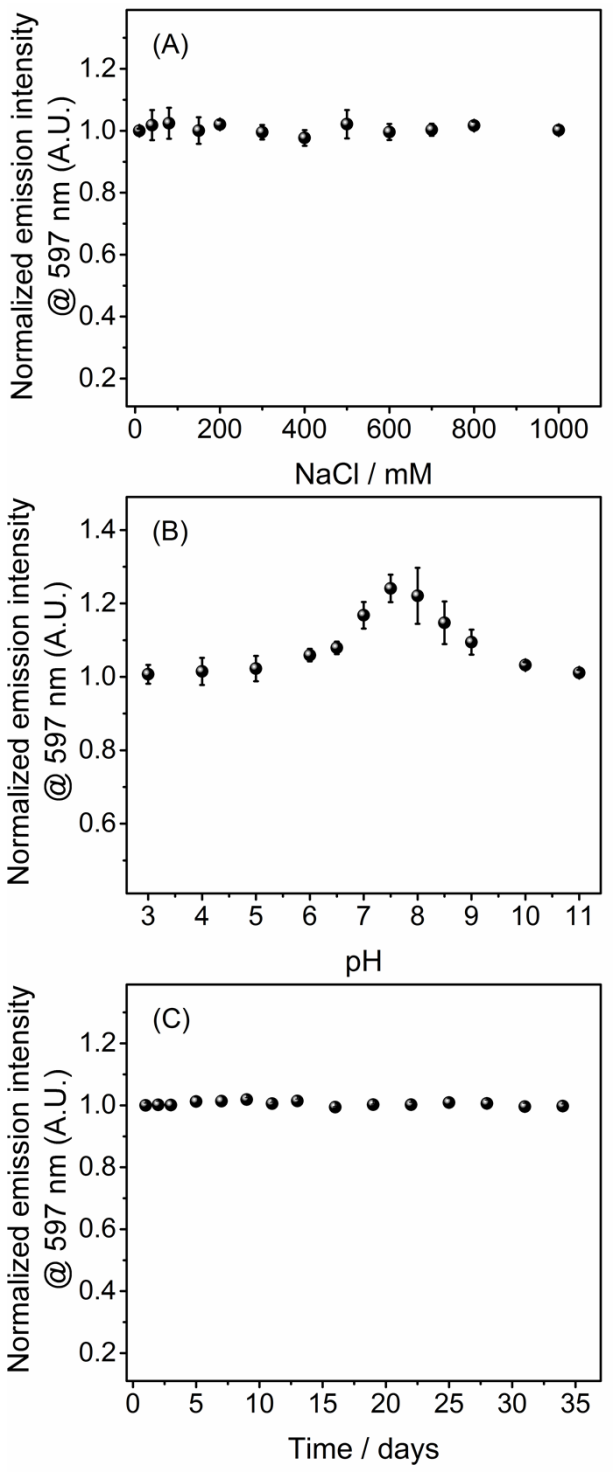

Figure S20. Investigation of the stability of GOD-capped Mn-ZnS QDs against salinity (a), pH (b), and storage time (c) based on its phosphorescence intensity.

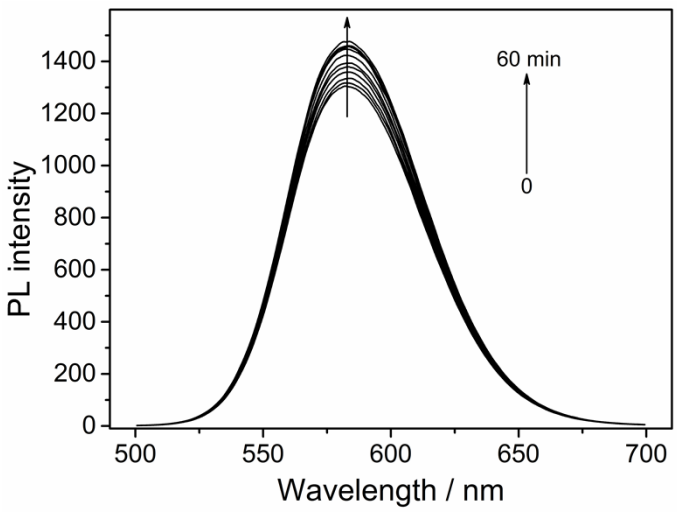

Figure S21. Photostability investigation of GOD-capped Mn-ZnS QDs upon continuously irradiated with 310 nm UV light for varied time. The emission spectra were recorded at different time points (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min).

5. Investigation of role of GOD in synthesis of Mn-doped ZnS QDs

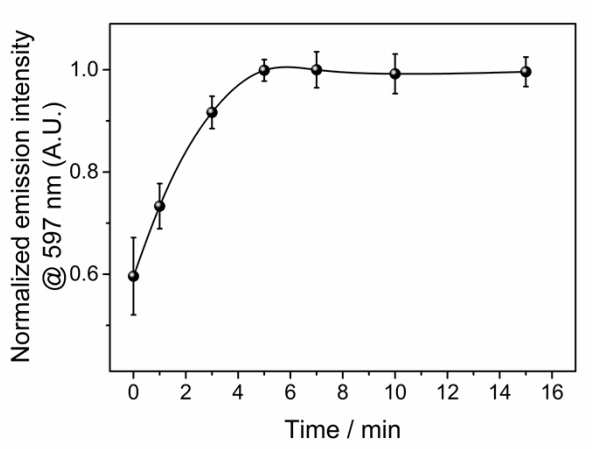

Figure S22. Effect of biomineralization time of GOD and cation precursors (Zn^{2+} and Mn^{2+}) on the phosphorescence intensity of the eventual Mn-ZnS QDs.

Polyacrylamide gel electrophoresis

12 μL free GOD (1.8 mg mL^{-1}) and GOD-QDs sample were mixed with 4 μL 4 × loading buffer and then loaded into polyacrylamide gel respectively. 1×Tris-Gly buffer was used as the running buffer. The stacking gel was 5% and separation gel

12%. The gel was run for 40 min at constant voltage of 100 V in stacking gel and then run for 3 h at constant voltage of 120 V in separation gel. The gel was subsequently stained with 0.25% Coomassie brilliant blue R-250 in 50% ethanol and 10% acetic acid for 1 h and destained in 25% ethanol and 8% acetic acid for 24 h in an orbital shaker (TS-2, QILINBEIER) with the speed of 80 rpm before the digital image acquisition.

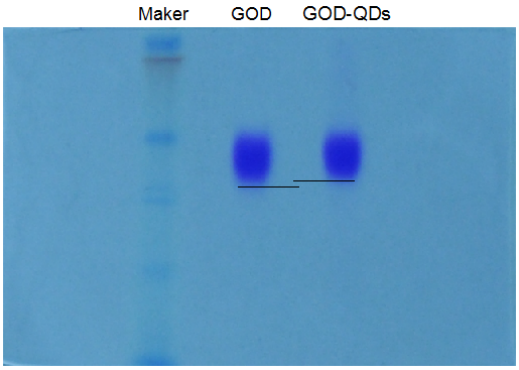

Figure S23. Image of the polyacrylamide gel electrophoresis of free GOD and GOD-QDs. The blue bands is the Coomassie brilliant blue-stained image.

Chemical modification of amino acid residues in GOD molecules[S2]

GOD powder was dissolved in ultrapure water to the concentration of 20 mg mL^{-1}; DEPC was diluted in anhydrous ethanol; N-ethylmaleimide was dissolved in 1/100 (v / v) DMSO / H_2O solution; NHS was dissolved in DMSO; EDC was dissolved in water; NAI was dissolved in 1/2 (v / v) DMSO/H_2O solution; and methylamine (40% w / w aq. Soln) of 12.88 M was directly used. All the materials above were freshly prepared for GOD modification. The total reaction volume of each chemical modification reaction is 500 μL. The GOD concentration is 10 mg mL^{-1}.

One equivalent of DEPC was used to modify the imidazole groups of histidine; 10 equivalents of NHS were used to modify the amino groups (-NH_2) of lysine and

the N terminus; 10 equivalents of N-ethylmaleimide were used to modify the thiol groups (-SH) of cysteine; 10 equivalents of EDC and 50 equivalents of methylamine were used to modify the carboxylic groups (-COOH) of glutamic acid, aspartic acid, and the C terminus; 10 equivalents of NAI were used to modify the hydroxyl groups (-OH) of tyrosine, serine, and threonine.

After each reaction, the modified GOD product was purified thrice with an YM-30 ultrafilter via centrifugation at 8000 rpm for 10 min. The purified product was then recovered to the 500 μL in ultrapure water for QDs synthesis. The protocol for QDs synthesis with chemically modified GOD is the same as unmodified GOD. Below is the specific chemical modification strategies.

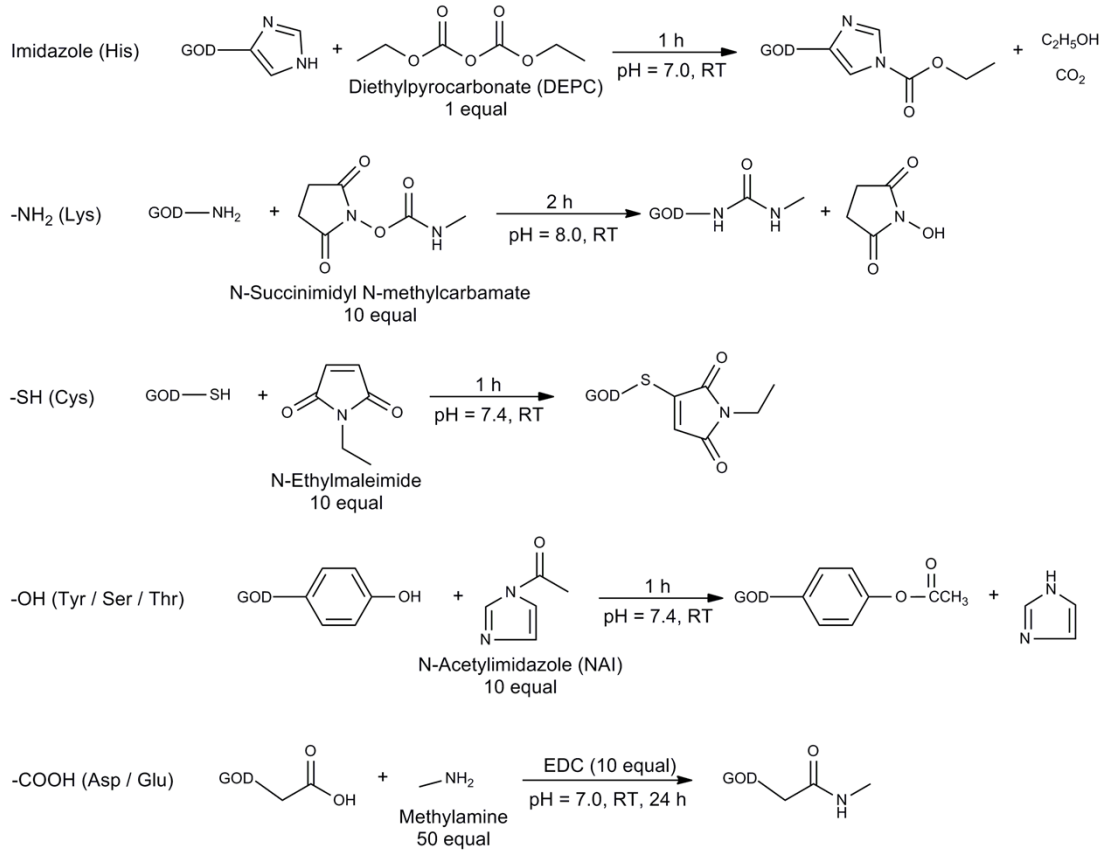

Figure S24. Chemical reactions for modification of five types of amino acid residues (imidazole, $-NH_2$, -SH , -OH, and -COOH).

GOD sequence (1230 aa)

Chain A

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1    MAILNSMYNN VSPLQCTSPF LGGPQLTDVC SASNGELFLA LLNFFVATSP VIGEPCQRVH
61   SSRIPDLSYD FIVVGGGAAR AVVAGRLSEV SNWKVLLLEA GPDEPAGAEI PSNLQLYLGG
121  DLDWKYYTTN ESHACLSTGG SCYWPRGKNL GGTTLHHGMA YHRGHRKDYE RWVQQGAFGW
181  SWDEVMPYYL KSENNTELSR VGTKYHRSGG LMNVERFPYQ PPFAWKILKA AEEAGFGVSE
241  DLSGDRINGF TVAQTISRNG VRLSSARAFI TPFENRSNLH VIVNATVTKV RTLNKRATGV
301  NVLINGRRRI IFARREVILS AGSVNTPQLL MLSGIGPKEH LRSLGIPVVV DLPGVGENLH
361  NHQSFGMDFS LNEDFYPTFN QTNVDQYLYN QTGPLSSTGL AQVTGIWHSN LTTPDDPDIQ
421  IFFAGYQAIC KPKLKIADLS AHDKQAVRMS ALNVQPTSKG RITLNSKDPL DPPVIWSNDL
481  ATEHDRSVMI QAIRVVQKLV NTTVMRDLGV EFQKIELKQC DEFVEDSDDY WNCVIQYNTR
541  AENHQTGTAK MGPSYDPMAV VSPRLKVHGI RGLRVADASV QPQVISGNPV ASVNMVGERA
601  ADFIKEDWGE LLQLL
```

Chain B

```
1    MAILNSMYNN VSPLQCTSPF LGGPQLTDVC SASNGELFLA LLNFFVATSP VIGEPCQRVH
61   SSRIPDLSYD FIVVGGGAAR AVVAGRLSEV SNWKVLLLEA GPDEPAGAEI PSNLQLYLGG
121  DLDWKYYTTN ESHACLSTGG SCYWPRGKNL GGTTLHHGMA YHRGHRKDYE RWVQQGAFGW
181  SWDEVMPYYL KSENNTELSR VGTKYHRSGG LMNVERFPYQ PPFAWKILKA AEEAGFGVSE
241  DLSGDRINGF TVAQTISRNG VRLSSARAFI TPFENRSNLH VIVNATVTKV RTLNKRATGV
301  NVLINGRRRI IFARREVILS AGSVNTPQLL MLSGIGPKEH LRSLGIPVVV DLPGVGENLH
361  NHQSFGMDFS LNEDFYPTFN QTNVDQYLYN QTGPLSSTGL AQVTGIWHSN LTTPDDPDIQ
421  IFFAGYQAIC KPKLKIADLS AHDKQAVRMS ALNVQPTSKG RITLNSKDPL DPPVIWSNDL
481  ATEHDRSVMI QAIRVVQKLV NTTVMRDLGV EFQKIELKQC DEFVEDSDDY WNCVIQYNTR
541  AENHQTGTAK MGPSYDPMAV VSPRLKVHGI RGLRVADASV QPQVISGNPV ASVNMVGERA
601  ADFIKEDWGE LLQLL
```

Figure S25. Amino acid sequences of GOD (from the database of the National Center for Biotechnology Information).

BSA sequence (585 aa)

```
1   DTHKSEIAHR FKDLGEEHFK GLVLIAFSQY LQQCPFDEHV KLVNELTEFA KTCVADESHA
61  GCEKSLHTLF GDELCKVASL RETYGDMADC CEKQEPERNE CFLSHKDDSP DLPKLKPDPN
121 TLCDEFKADE KKFWGKYLYE IARRHPYFYA PELLYYANKY NGVFQECCQA EDKGACLLPK
181 IETMREKVLT SSARQRLRCA SIQKFGERAL KAWSVARLSQ KFPKAEFVEV TKLVTDLTKV
241 HKECCHGDLL ECADDRADLA KYICDNQDTI SSKLKECCDK PLLEKSHCIA EVEKDAIPEN
301 LPPLTADFAE DKDVCKNYQE AKDAFLGSFL YEYSRRHPEY AVSVLLRLAK EYEATLEECC
361 AKDDPHACYS TVFDKLKHLV DEPQNLIKQN CDQFEKLGEY GFQNALIVRY TRKVPQVSTP
421 TLVEVSRSLG KVGTRCCTKP ESERMPCTED YLSLILNRLC VLHEKTPVSE KVTKCCTESL
481 VNRRPCFSAL TPDETYVPKA FDEKLFTFHA DICTLPDTEK QIKKQTALVE LLKHKPKATE
541 EQLKTVMENF VAFVDKCCAA DDKEACFAVE GPKLVVSTQT ALA
```

Papain sequence (347 aa)

```
1   MAMIPSISKL LFVAICLFVY MGLSFGDFSI VGYSQNDLTS TERLIQLFES WMLKHNKIYK
61  NIDEKIYRFE IFKDNLKYID ETNKKNNSYW LGLNVFADMS NDEFKEKYTG SIAGNYTTTE
121 LSYEEVLNDG DVNIPEYVDW RQKGAVTPVK NQGSCGSCWA FSAVVTIEGI IKIRTGNLNE
181 YSEQELLDCD RRSYGCNGGY PWSALQLVAQ YGIHYRNTYP YEGVQRYCRS REKGPYAAKT
241 DGVRQVQPYN EGALLYSIAN QPVSVVLEAA GKDFQLYRGG IFVGPCGNKV DHAVAAVGYG
301 PNYILIKNSW GTGWGENGYI RIKRGTGNSY GVCGLYTSSF YPVKN
```

Pepsin sequence (339 aa)

```
1   VEEGKKTHPY NPAAKFYSSG TESMTNDADL SYYGTISIGT PPQSFSVIFD TGSSNLWVPS
61  VYCNSTACEN HNQFNPSQSS TFQWGNQSLS IQYGTGSMTG FLGSDTVEVG GISVANQVFG
121 LSQTEASFMT YMQADGILGL AFQSIASDNV VPVFNTMITE GLVSEPIFSV YLSGNSEQGS
181 EVVFGGTDST HYTGTITWIP LSSATYWQIN MDSVTINGQT VACSGGCQAI IDTGTSLIVG
241 PTTDINNLNS WVGASTDQSG DAIVNCQNIP SMPDVTFTLN GNAFTVPASA YVSQSSSGCM
301 TGFGQGGTMQ LWILGDVFIR EYYAVFNAQT QNIGLAKSA
```

Lysozyme sequence (147 aa)

```
1   MKALIILGFL FLSVAVQGKV FERCELARTL KKLGLDGYKG VSLANWLCLT KWESSYNTKA
61  TNYNPSSEST DYGIFQINSK WWCNDGKTPN AVDGCHVSCS ELMENDIAKA VACAKHIVSE
121 QGITAWVAWK SHCRDHDVSS YVQGCTL
```

Figure S26. Amino acid sequences of BSA, papain, pepsin, and lysozyme (from the database of the National Center for Biotechnology Information).

Table S2. The characteristics of five model proteins

Proteins / Amino acid	BSA (66 kDa)	Pepsin (35 kDa)	Papain (23 kDa)	Lysozyme (14 kDa)
His	17	3	3	4
Asp / Glu	40 / 59	14 / 11	15 / 20	7 / 7
Lys	59	4	21	12
Tyr / Ser / Thr	20 / 28 / 34	13 / 44 / 34	28 / 24 / 15	5 / 14 / 8
Cys	35	6	8	8

Table S3. Occurrences of critical amino acid residues in BSA, pepsin, papain and lysozyme sequence

Amino acids (number of aa)	His (imidazole)	Asp / Glu (-COOH)	Lys ($-NH_2$)	Tyr / Ser / Thr (-OH)	Cys (-SH)
BSA (%)	2.9	17	10.1	14	6
Pepsin (%)	0.9	7.4	1.2	26.8	1.8
Papain (%)	0.9	10.1	6.1	19.4	2.3
Lysozyme (%)	2.7	9.5	8.2	18.4	5.4

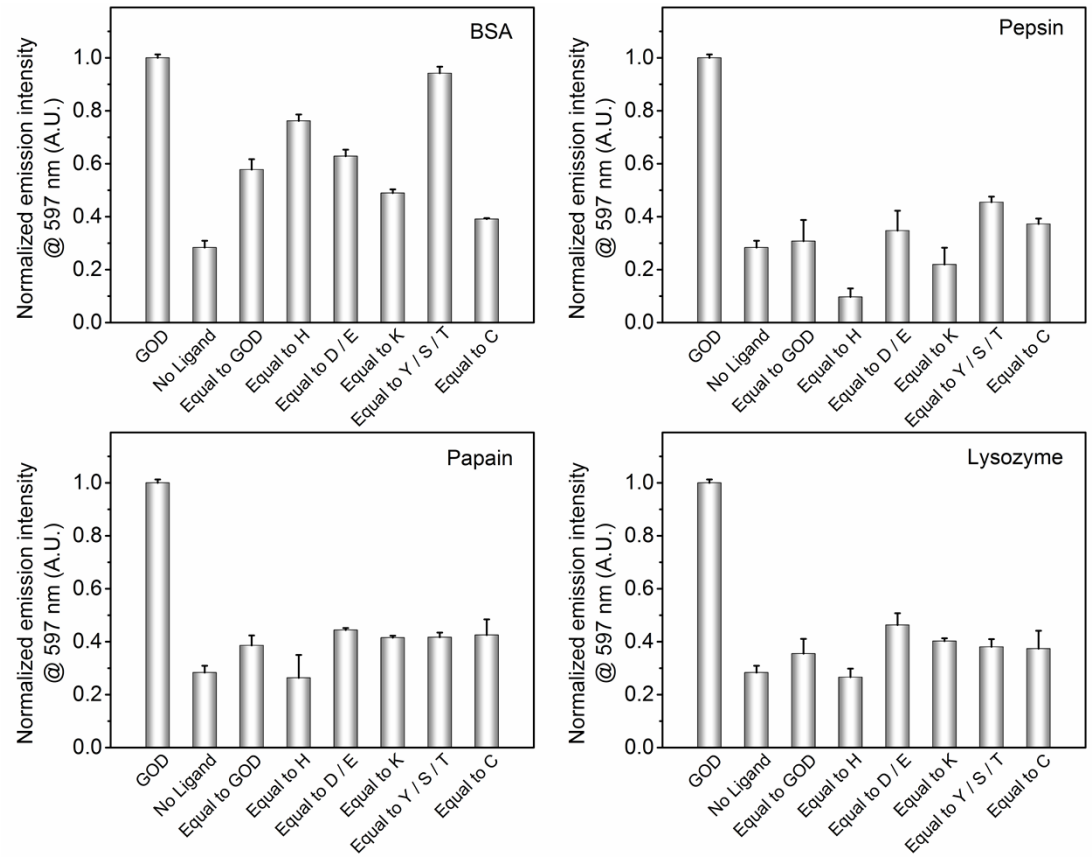

Figure S27. Normalized phosphorescence intensity of Mn-ZnS QDs directed with BSA, pepsin, papain, and lysozyme with the conditions of six sets experiments (Tris-HCl, pH 6.5, 0.01M, and instant). GOD-directed synthesis and QDs synthesized without ligands were performed as control.

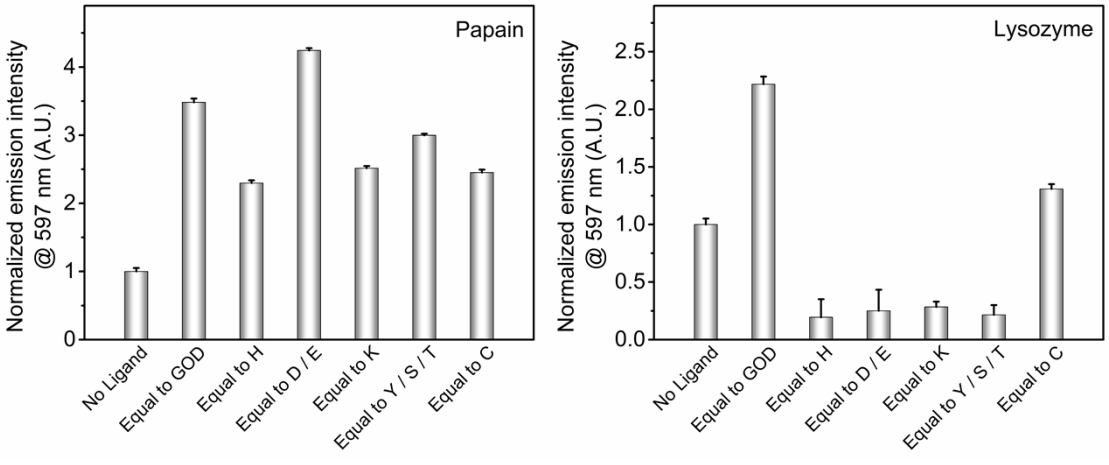

Figure S28. Normalized phosphorescence intensity of Mn-ZnS QDs directed with papain and lysozyme with the conditions of six sets experiments (NH_4HCO_3 solution, 0.02 M, pH = 12, adjusted by using NaOH solution).

5. Analytical performance of GOD-Mn-ZnS QDs for glucose sensing

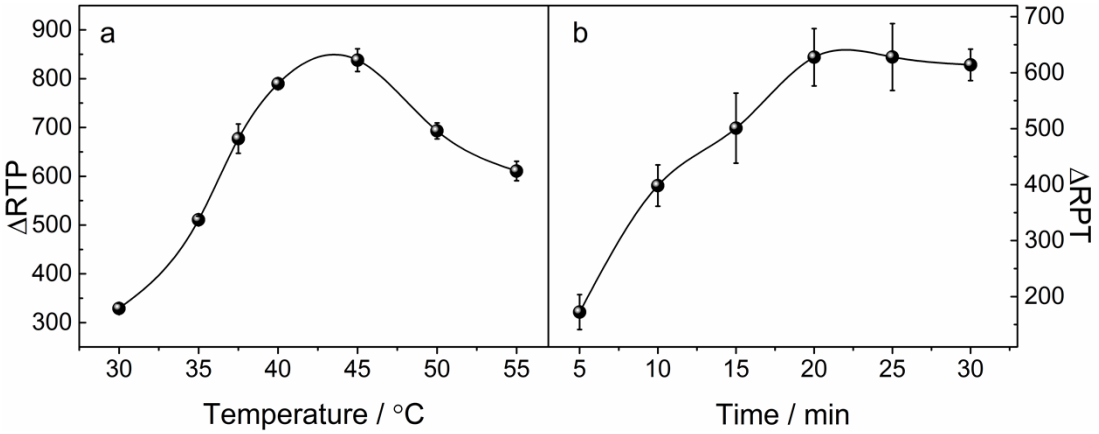

Figure S29. a) Effect of the temperature of GOD-mediated Mn-doped ZnS QDs incubating with 200 μM glucose and b) incubation time.

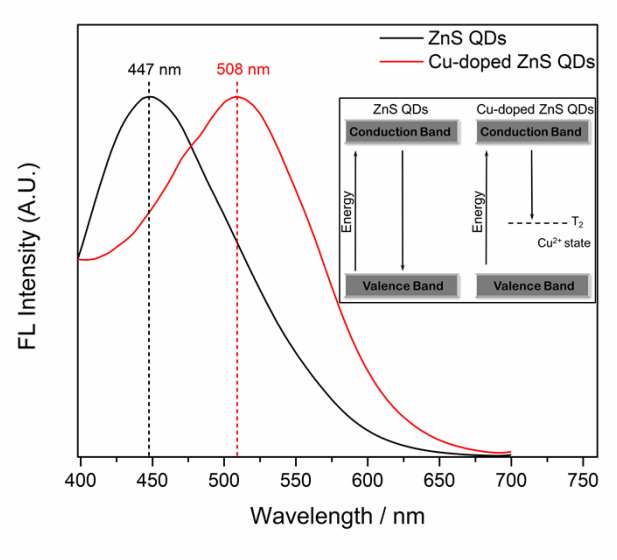

Figure S30. Fluorescence Emission spectra (Ex: 310 nm) of GOD-ZnS QDs and GOD-Cu-doped ZnS QDs. Doping Cu into ZnS host resulted in res-shifting of the host emission, which is arisen from the recombination from the conduction band to the Cu2+ T2 state (inset of Figure S30).

Table S4. Effect of existing substances on GOD-mediated Mn-doped ZnS QDs

Existing substances	Concentration (M)[a]	Changed in the RTP intensity (%)
Maltose	1×10^{-2}	-2.0
Fructose	1×10^{-2}	-3.4

Sucrose	1×10^{-2}	-5.0
Alanine	4×10^{-3}	-2.2
Arginine	4×10^{-4}	2.0
Histidine	1×10^{-3}	-3.6
Lysine	5×10^{-4}	3.6
Glycine	1×10^{-3}	-3.2
Valine	1×10^{-3}	-4.6
Histidine	1×10^{-3}	-3.6
Lysine	5×10^{-4}	3.6
Ascorbic acid	2.5×10^{-5}	-3.9
Glutathione	2×10^{-4}	0.3
L-cysteine	5×10^{-4}	3.0
Glutamic acid	2×10^{-3}	-3.0
Glycine	1×10^{-3}	-3.2
Tyrosine	2×10^{-4}	-3.8
Phenylalanine	5×10^{-4}	-5.7
Leucine	2×10^{-3}	-4.2
Proline	5×10^{-4}	-1.5
Serine	1×10^{-3}	-4.6
Tryptophan	2×10^{-4}	-5.2
Aspartic acid	2×10^{-4}	-5.4
Oxalic	1×10^{-3}	4.4
Methionine	2×10^{-4}	-5.4
Threonine	2×10^{-4}	-3.1
HSA	3×10^{-7}	3.1
Citrate	2.5×10^{-5}	3.4
K^+	1×10^{-2}	0.6
Na^+	4×10^{-2}	-2.5
Fe^{3+}	2×10^{-6}	0.3
Zn^{2+}	1×10^{-5}	4.1
Mg^{2+}	7.5×10^{-4}	-7.2
Co^{2+}	1×10^{-6}	-1.1
Mn^{2+}	1×10^{-6}	1.4
Cu^{2+}	3×10^{-7}	-0.7
Ca^{2+}	5×10^{-4}	1.0

References

[S1] G. M. Dalpian and J. R. Chelikowsky, *Phys. Rev. Lett.*, 2006, **96**, 226802.

[S2] He, X. W.; Gao, L.; Ma, N. *Sci. Rep.*, 2013, 3, 2825.