Electronic Supplementary Information

Aggregation-Induced Phosphorescence Enhancement of Mn-Doped ZnS Quantum Dots: the Role of Dot-Dot Distance

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Contents

1. Experimental Section

1.1. Reagents. Glucose oxidase (GOD), Bovine serum albumin (BSA), Pepsin and Glutathione (GSH) were purchased commercially from Sigma-Aldrich (Shanghai, China). Poly(ethylene imine) (PEI) 600, 1800, 10000 and 70000, Insulin and Thioglycolic acid (TGA) were purchased commercially from Aladin (Shanghai, 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. Ultra-pure water (resistance >18.2 MΩ.cm) purified by a purification system (PCUJ-10, Chengdu Pure Technology Co., Ltd., Chengdu, China), was used for all experiments and making buffers.

1.2. Instruments. Phosphorescence, fluorescence and light-scattering spectra of the prepared Mn-doped ZnS (Mn-ZnS) quantum dots (QDs) were performed using an F-7000 spectrofluorometer (Hitachi, Japan) equipped with a plotter unit and a quartz cell (1 cm × 1 cm). Fluorescence lifetime and phosphorescence lifetime were measured on a Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon) with a SpectraLED (280 nm, S-280, Horiba Scientific) and a DeltaDiode (280 nm, D-280, Horiba Scientific) as the excitation source and a picosecond photon detection module (PPD-850, Horiba Scientific) as the detector respectively. For instance, the time range was set at 88 ms for phosphorescence lifetime measurements, and the time range was set at 1.6 μs for fluorescence lifetime measurements. Absorption spectra were recorded using a UV-1750 UV/Vis spectrophotometer (Shimadzu, Japan). TEM and 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). Dynamic light scattering and Zeta potential measurements of QDs was obtained using particle size analyzer ZEN3690 (Malvern Instruments Ltd.). Stop-flow fluorescence spectrum was carried out on Chirascan-plus spectrophotometer (Applied Photophysics Limited, UK). The luminescence and scattering images of QDs (under UV irradiation 302 nm and green laser) were taken with a Nikon D300S digital camera.

1.3. Synthetic methods. For the synthesis of Mn-doped ZnS quantum dots (Mn-ZnS QDs) with six positively charged and four electronegative kinds of ligands is similar to our previous work.[1,2] Every ligand-capped Mn-ZnS QDs has different synthetic conditions, concrete details as following:

1.3.1. TGA-capped Mn-ZnS QDs. For preparation of TGA-mediated Mn-ZnS QDs, 45 μL TGA

(2 mM), 40 μL $MnAc_2$ (1 mM), 38 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 8.0, 0.1 M) were added to 293 μL ultrapure water for five minutes in room temperature. Then 34 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent TGA-capped Mn-ZnS QDs were thus obtained.

1.3.2. GSH-capped Mn-ZnS QDs. For preparation of GSH-mediated Mn-ZnS QDs, 30 μL GSH (2 mM), 40 μL $MnAc_2$ (1 mM), 38 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 7.5, 0.1 M) were added to 308 μL ultrapure water for five minutes in room temperature. Then 34 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent GSH-capped Mn-ZnS QDs were thus obtained.

1.3.3. Insulin-capped Mn-ZnS QDs. For preparation of insulin-mediated Mn-ZnS QDs, 40 μL insulin (0.1 mM), 40 μL $MnAc_2$ (1 mM), 38 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 7.5, 0.1 M) were added to 298 μL ultrapure water for five minutes in room temperature. Then 34 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent insulin-capped Mn-ZnS QDs were thus obtained.

1.3.4. Pepsin-capped Mn-ZnS QDs. For preparation of pepsin-mediated Mn-ZnS QDs, 70 μL pepsin (5 mg mL^{-1}), 40 μL $MnAc_2$ (1 mM), 38 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 7.5, 0.1 M) were added to 268 μL ultrapure water for five minutes in room temperature. Then 34 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent pepsin-capped Mn-ZnS QDs were thus obtained.

1.3.5. BSA-capped Mn-ZnS QDs. For preparation of BSA-mediated Mn-ZnS QDs, 40 μL BSA (10 mg mL^{-1}), 40 μL $MnAc_2$ (1 mM), 38 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 7.0, 0.1 M) were added to 298 μL ultrapure water for five minutes in room temperature. Then 34 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent BSA-capped Mn-ZnS QDs were thus obtained.

1.3.6. GOD-capped Mn-ZnS QDs. For preparation of GOD-mediated Mn-ZnS QDs, 30 μL GOD

(10 mg mL^{-1}), 40 μL $MnAc_2$ (1 mM), 38 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 6.5, 0.1 M) were added to 308 μL ultrapure water for five minutes in room temperature. Then 34 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent GOD-capped Mn-ZnS QDs were thus obtained.

1.3.7. PEI600-capped Mn-ZnS QDs. For preparation of PEI600-mediated Mn-ZnS QDs, 45 μL PEI600 (0.5 mM), 40 μL $MnAc_2$ (1 mM), 38 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 6.5, 0.1 M) were added to 293 μL ultrapure water for five minutes in room temperature. Then 34 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent PEI600-capped Mn-ZnS QDs were thus obtained.

1.3.8. PEI1800-capped Mn-ZnS QDs. For preparation of PEI1800-mediated Mn-ZnS QDs, 45 μL PEI1800 (0.2 mM), 40 μL $MnAc_2$ (1 mM), 38 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 6.5, 0.1 M) were added to 293 μL ultrapure water for five minutes in room temperature. Then 34 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent PEI1800-capped Mn-ZnS QDs were thus obtained.

1.3.9. PEI10000-capped Mn-ZnS QDs. For preparation of PEI10000-mediated Mn-ZnS QDs, 50 μL PEI10000 (0.2 mM), 40 μL $MnAc_2$ (1 mM), 38 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 6.5, 0.1 M) were added to 288 μL ultrapure water for five minutes in room temperature. Then 34 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent PEI10000-capped Mn-ZnS QDs were thus obtained.

1.3.10. PEI70000-capped Mn-ZnS QDs. For preparation of PEI70000-mediated Mn-ZnS QDs, 35 μL PEI70000 (0.2 mM), 40 μL $MnAc_2$ (1 mM), 38 μL $ZnAc_2$ (20 mM) and 50 μL Tris-HCl solution (pH = 6.5, 0.1 M) were added to 313 μL ultrapure water for five minutes in room temperature. Then 34 μL Na_2S (20 mM) was quickly injected into the precursor solution above followed gently vortexing in room temperature. Phosphorescent PEI70000-capped Mn-ZnS QDs were thus obtained.

2. Characterization of prepared Mn-ZnS QDs

2.1. Optical characterization

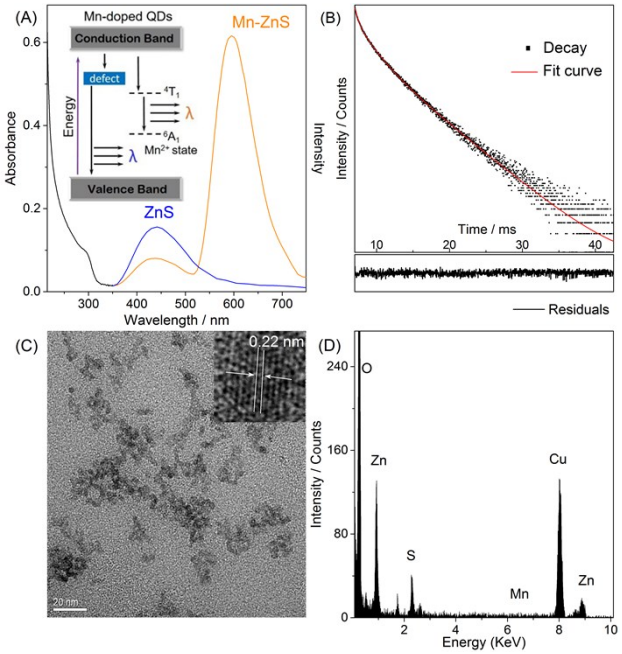

Figure S1. Characterization of the as-prepared Mn-ZnS QDs with 5% Mn^{2+}: (A) UV-vis absorption spectra (black line), fluorescence emission spectra (orange line) of Mn-ZnS QDs, and fluorescence emission spectra (blue line) of ZnS QDs (Ex: 290 nm), the inset is electronic transitions involved in the short and long lived emission of Mn-ZnS QDs; (B) phosphorescence decay curve of GSH-Mn-ZnS QDs; (C) TEM image of the Mn-doped ZnS QDs, with a high-magnification TEM image shown in the inset; and (D) the EDX of the Mn-doped ZnS QDs.

2.1. Calculation of phosphorescence and fluorescence lifetime. Phosphorescence and fluorescence lifetime measurements were performed on a Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon) with SpectraLED (280 nm, S-280, Horiba Scientific) and DeltaDiode (280 nm, D-280, Horiba Scientific) as the excitation source respectively and a picosecond photon detection module (PPD-850, Horiba Scientific) as the detector. The Phosphorescence and fluorescence time range were set at 44 ms and 1.6 μs respectively. The average lifetime was calculated using the equation below:

$$\tau = \sum f_i \tau_i = f_1\tau_1 + f_2\tau_2 + f_3\tau_3 \quad (1)$$

Where τ_i is the lifetime and f_i is the contribution factor of τ_i to τ, which can be collected from the phosphorescence lifetime measurements after proper fitting. Here, the data are then fitted with the third order exponential decay.

3. Interaction of prepared Mn-ZnS QDs with protamine

3.1. Particle size analysis of ligand-capped Mn-ZnS QDs with protamine

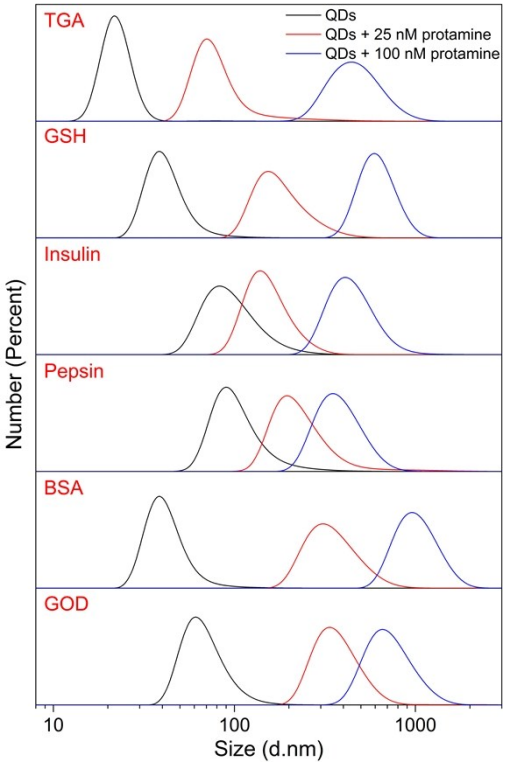

Figure S2 Dynamic light scattering (DLS) analysis of the prepared several ligands-capped Mn-ZnS QDs with different concentration of protamine. The reactive solutions were prepared in 0.01 M Tris-HCl buffer of pH 9.0.

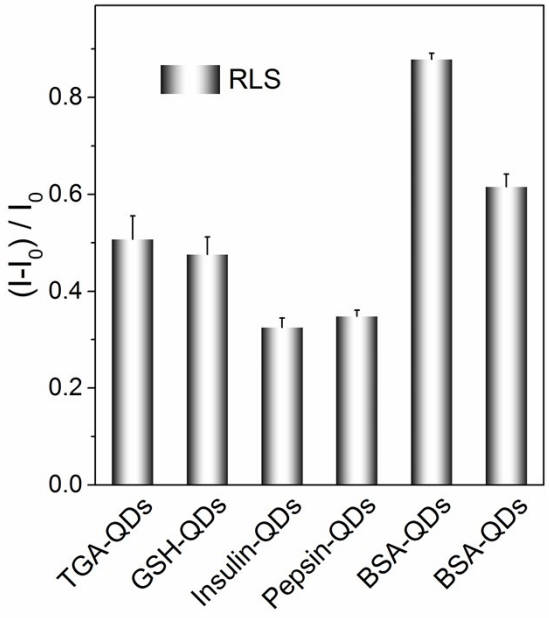

Figure S3 Increased resonant light scattering (RLS) of the prepared several ligands-capped Mn-ZnS QDs with protamine.

3.2. Luminescence, light-scattering and UV-Vis absorption spectra and lifetime of ligand-capped Mn-ZnS QDs with protamine

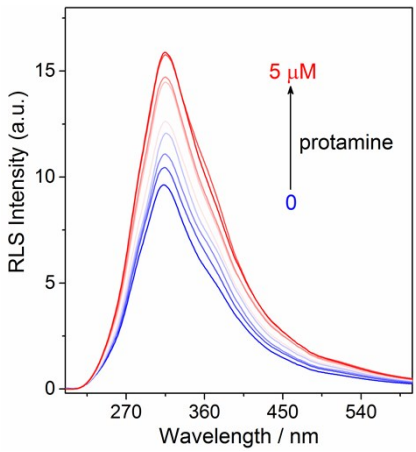

Figure S4 Light-scattering spectra of TGA-capped Mn-doped ZnS QDs in the presence of various concentrations of protamine.

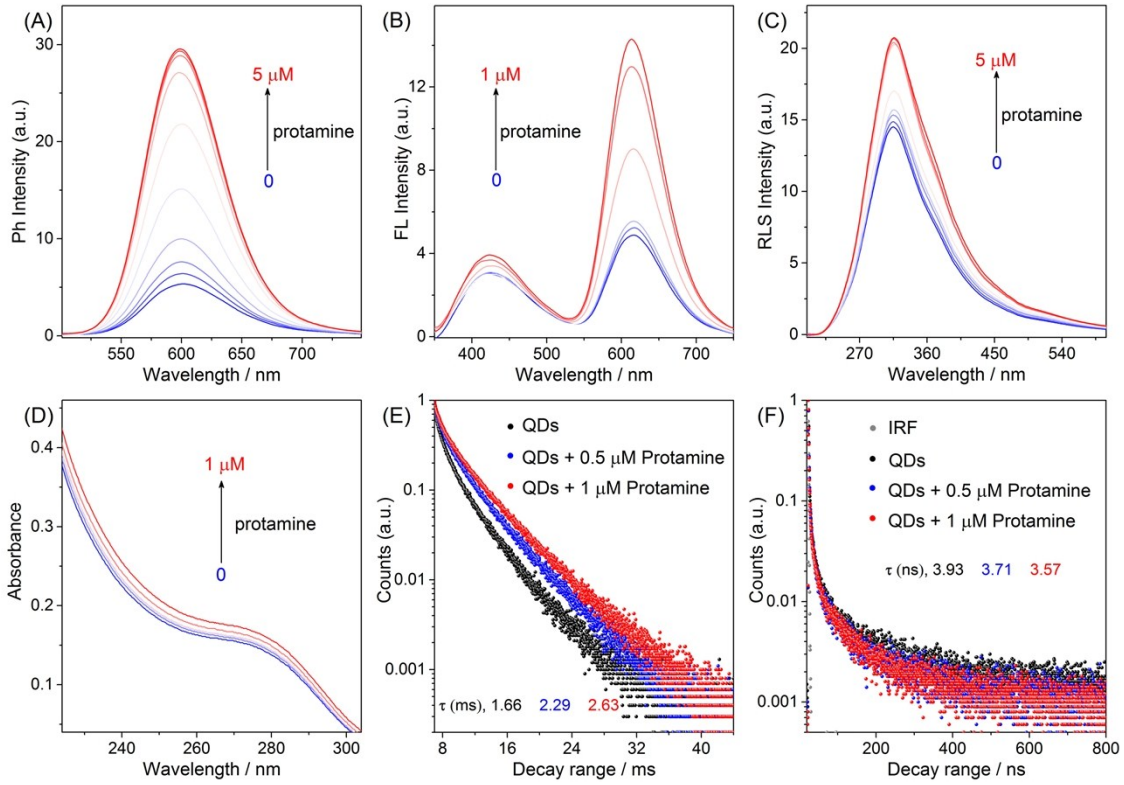

Figure S5 (A) Phosphorescence, (B) fluorescence, (C) light-scattering and (D) UV-Vis absorption spectra, (E) phosphorescence and (F) fluorescence lifetime of GSH-capped Mn-doped ZnS QDs in the presence of various concentrations of protamine.

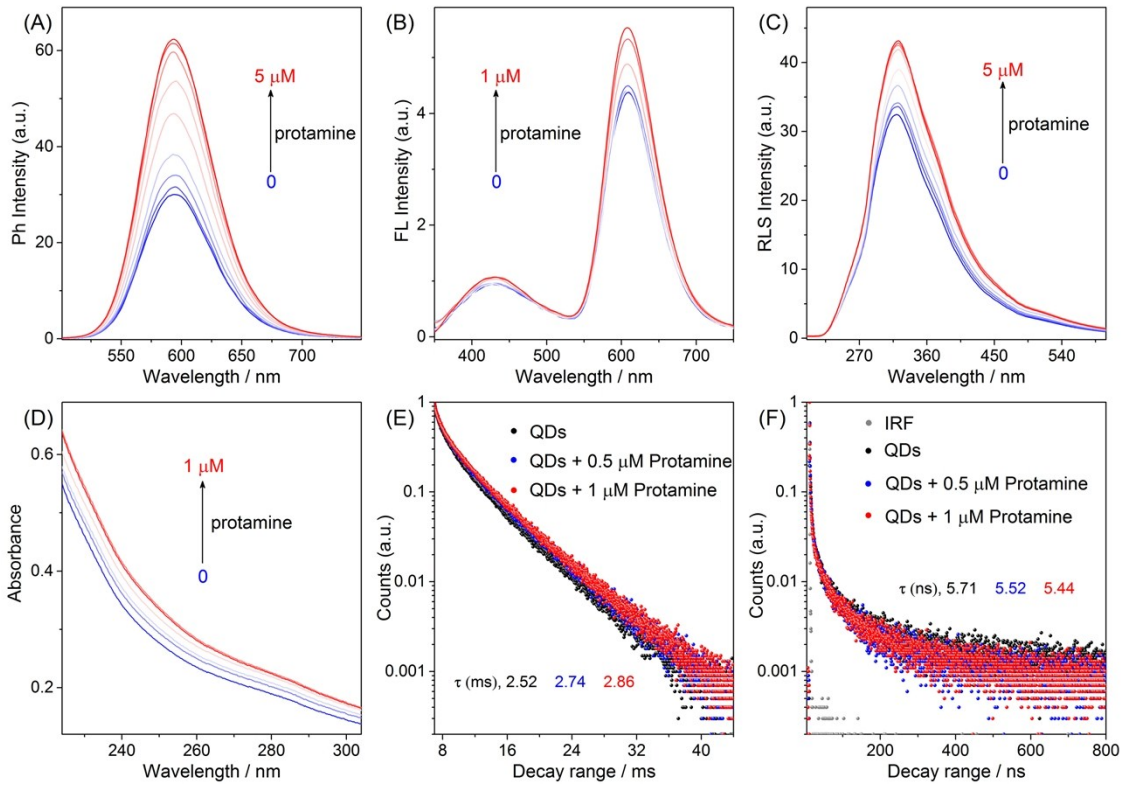

Figure S6 (A) Phosphorescence, (B) fluorescence, (C) light-scattering and (D) UV-Vis absorption spectra, (E) phosphorescence and (F) fluorescence lifetime of Insulin-capped Mn-doped ZnS QDs in the presence of various concentrations of protamine.

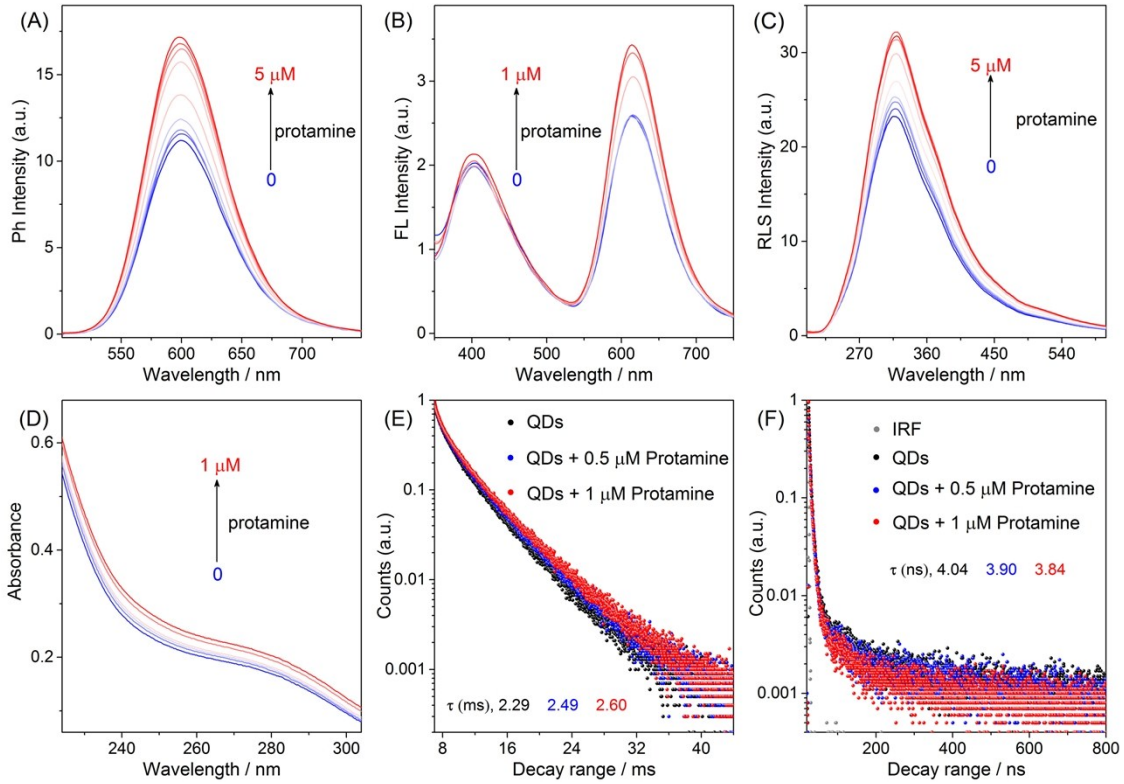

Figure S7 (A) Phosphorescence, (B) fluorescence, (C) light-scattering and (D) UV-Vis absorption spectra, (E) phosphorescence and (F) fluorescence lifetime of Pepsin-capped Mn-doped ZnS QDs in the

presence of various concentrations of protamine.

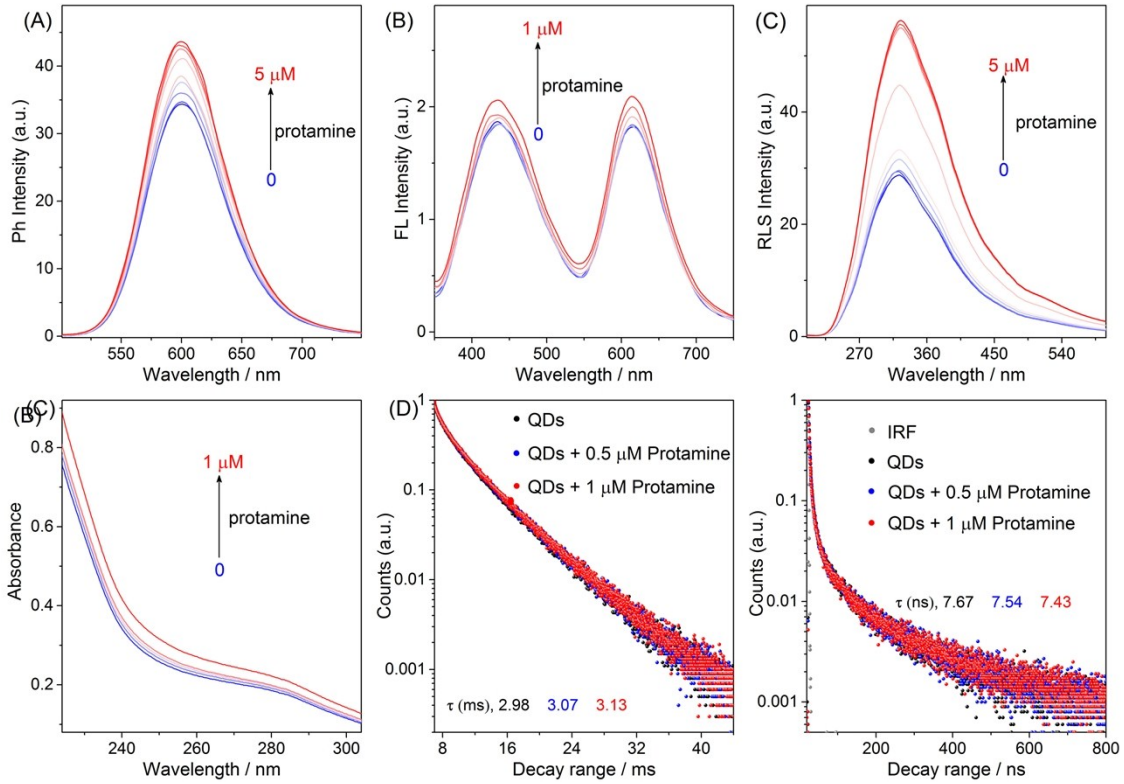

Figure S8 (A) Phosphorescence, (B) fluorescence, (C) light-scattering and (D) UV-Vis absorption spectra, (E) phosphorescence and (F) fluorescence lifetime of BSA-capped Mn-doped ZnS QDs in the presence of various concentrations of protamine.

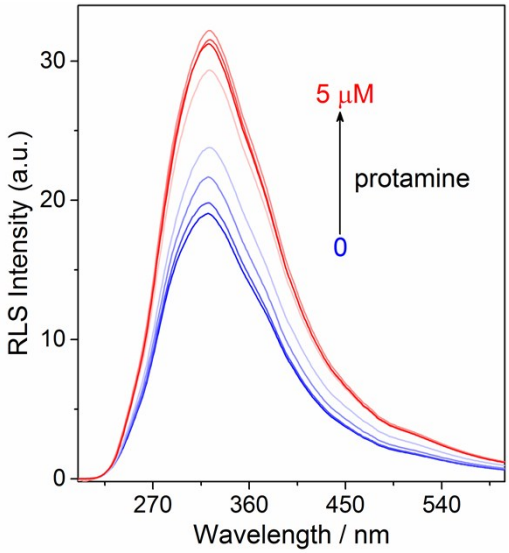

Figure S9 Light-scattering spectra of GOD-capped Mn-doped ZnS QDs in the presence of various concentrations of protamine.

4. The approximate distance of dot-dot of TGA and GSH-capped QDs

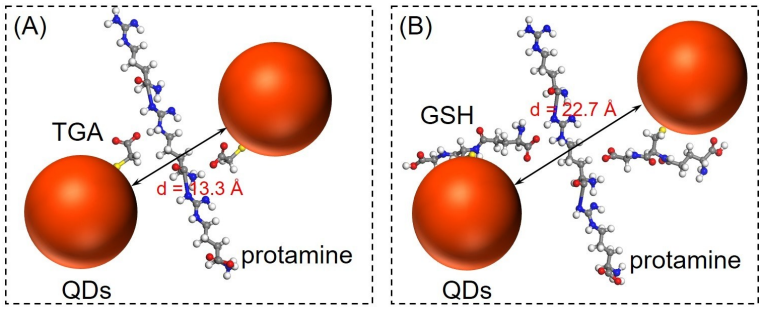

Figure S10 The approximate distance of dot-dot of (A) TGA and (B) GSH-capped QDs by numerical simulation respectively.

5. Fluorescence and Phosphorescence lifetime parameters of Mn-doped ZnS QDs

Table S1. Luminescence lifetime parameters of Mn-doped ZnS QDs

Sample	TGA-capped Mn-doped ZnS QDs								GOD-capped Mn-doped ZnS QDs							
	Defect-related emission (ns)				Dopant emission (ms)				Defect-related emission (ns)				Dopant emission (ms)			
	τ_i	f_i	$\tau_{average}$	χ^2	τ_i	f_i	$\tau_{average}$	χ^2	τ_i	f_i	$\tau_{average}$	χ^2	τ_i	f_i	$\tau_{average}$	χ^2
QDs	0.98	0.76	2.34	1.06	0.05	0.36	1.20	1.35	2.05	0.87	8.67	1.27	0.42	0.25	2.88	1.16
	4.55	0.19			0.98	0.40			18.18	0.11			2.21	0.39		
	14.62	0.05			3.28	0.24			171.4	0.02			5.37	0.36		
QDs + 0.5 μM protamine	0.86	0.82	1.89	1.16	0.30	0.27	2.06	1.13	2.48	0.87	8.28	1.38	0.33	0.23	2.92	1.14
	4.60	0.14			1.66	0.46			21.11	0.10			2.10	0.40		
	14.77	0.04			4.47	0.27			169.2	0.03			5.39	0.37		
QDs + 1 μM protamine	0.84	0.87	1.56	1.14	0.33	0.25	2.52	1.21	2.37	0.86	8.13	1.27	0.35	0.24	2.97	1.14
	4.34	0.11			1.99	0.43			18.95	0.11			2.22	0.39		
	14.45	0.02			4.95	0.32			150.4	0.03			5.47	0.37		

$\tau_{average} = \sum f_i \tau_i = f_1\tau_1 + f_2\tau_2 + f_3\tau_3$

Table S2. Parameters of TGA-capped Mn-doped ZnS QDs

Sample	Defect-related emission (ns)				Dopant emission (ms)			
	τ_i	f_i	$\tau_{average}$	χ^2	τ_i	f_i	$\tau_{average}$	χ^2
QDs	0.98	0.76	2.34	1.06	0.05	0.36	1.20	1.35
	4.55	0.19			0.98	0.40		
	14.62	0.05			3.28	0.24		
QDs + protamine	0.83	0.87	1.54	1.13	0.44	0.25	2.61	1.11
	4.28	0.10			2.09	0.42		
	15.38	0.03			4.92	0.33		
QDs + Zn^{2+}	0.93	0.89	1.61	1.28	0.11	0.28	1.89	1.34
	4.87	0.10			1.35	0.43		
	17.48	0.01			4.35	0.29		
QDs + Zn^{2+} + protamine	0.92	0.88	1.58	1.19	0.42	0.24	2.70	1.09
	4.28	0.10			2.18	0.45		
	15.41	0.02			5.12	0.31		
QDs + S^{2-}	0.83	0.82	1.87	1.14	0.07	0.45	0.60	1.34
	3.80	0.12			0.64	0.41		
	12.68	0.06			2.26	0.14		
QDs + S^{2-} + protamine	0.76	0.85	1.50	1.22	0.27	0.34	1.52	1.13
	3.49	0.11			1.34	0.44		
	12.15	0.04			3.86	0.22		

$\tau_{average} = \sum f_i\tau_i = f_1\tau_1 + f_2\tau_2 + f_3\tau_3$

Table S3. Parameters of GOD-capped Mn-doped ZnS QDs

Sample	Defect-related emission (ns)				Dopant emission (ms)			
	τ_i	f_i	$\tau_{average}$	χ^2	τ_i	f_i	$\tau_{average}$	χ^2
QDs	2.47	0.87	8.26	1.40	0.43	0.25	2.88	1.16
	20.83	0.10			2.21	0.40		
	167.9	0.03			4.26	0.35		
QDs + protamine	2.38	0.86	8.17	1.28	0.36	0.23	2.94	1.15
	19.35	0.11			2.23	0.42		
	153.4	0.03			5.50	0.35		
QDs + Zn^{2+}	2.08	0.87	8.76	1.28	0.35	0.23	3.02	1.08
	19.20	0.10			2.27	0.41		
	177.5	0.03			5.55	0.36		
QDs + Zn^{2+} + protamine	2.11	0.86	9.15	1.23	0.42	0.24	3.05	1.14
	19.46	0.11			2.36	0.40		
	175.0	0.03			5.56	0.36		
QDs + S^{2-}	2.57	0.90	7.35	1.43	0.05	0.30	2.38	1.29
	23.12	0.09			1.50	0.34		
	166.9	0.01			5.12	0.36		
QDs + S^{2-} + protamine	2.32	0.90	6.51	1.34	0.06	0.28	2.45	1.20
	20.65	0.09			1.51	0.34		
	163.2	0.01			5.08	0.38		

$\tau_{average} = \sum f_i\tau_i = f_1\tau_1 + f_2\tau_2 + f_3\tau_3$

183

6. Kinetics of Mn-doped ZnS QDs reactive with protamine

Stopped-flow measurements were performed with a stopped-flow CS/SF module coupled to Applied Photophysics fluorescence detector. In a standard experimental setup, the solution of QDs was placed in Syringe 1, while Syringe 2 contained protamine at different concentrations. Fluorescence measurements were initiated by mixing the contents of the two syringes in equal volumes (total volume = 200 μL) in a stopped-flow chamber. All experiments were carried out in 10 mM Tris-HCl buffer, pH 7.0, at 25 °C. Fluorescence intensities were recorded with an excitation wavelength of 290 nm and emission at 600 nm. For each experiment, measurements from 7 to 13 injections were accumulated and the average of these traces were used for data analysis. To ensure reproducibility, each experiment was repeated at least three times.

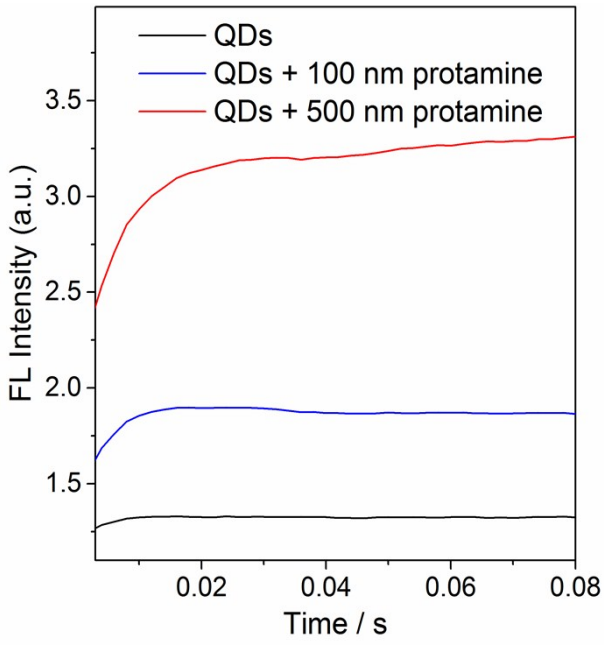

Figure S11 Kinetics of Mn-doped ZnS QDs reactive with protamine, monitored by stopped-flow experiments. The reactive solutions were prepared in 0.01 M Tris-HCl buffer of pH 9.0.

7. Interferences of relevant metal ions and other biomolecules

Table S4 Effect of co-existing substances on the quenched RTP intensity of Mn-doped ZnS QDs by 100 nM protamine.

Existing substances	Concentration	Changed RTP intensity (%)
HSA	100 μM	-4.0
Glucose	1 mM	-0.7
L-cysteine	1 μM	-7.4
GSH	1 μM	-4.0
Ascorbic acid	1 μM	-6.4
Dopamine	5 μM	-7.3
Amantadine Hydrochloride	20 μM	-3.9
Acetylcholine	50 μM	-6.1
Phenylalanine	100 μM	-5.0
Alanine	100 μM	0.6
Glycine	100 μM	-0.5
Glutamic acid	100 μM	-1.9
Arginine	100 μM	5.2
Lysine	100 μM	4.5
Proline	100 μM	-5.6
Valine	100 μM	-2.4
Histidine	20 μM	-5.6
Ca^{2+}	100 μM	3.7
Cu^{2+}	100 nM	-8.4
Fe^{3+}	100 nM	-7.3
K^{+}	1 mM	-2.2
Mg^{2+}	50 μM	4.4
Mn^{2+}	200 nM	-5.9
Na^{+}	1 mM	-5.6
Zn^{2+}	2 μM	1.3

8. Analytical results of protamine in serum samples

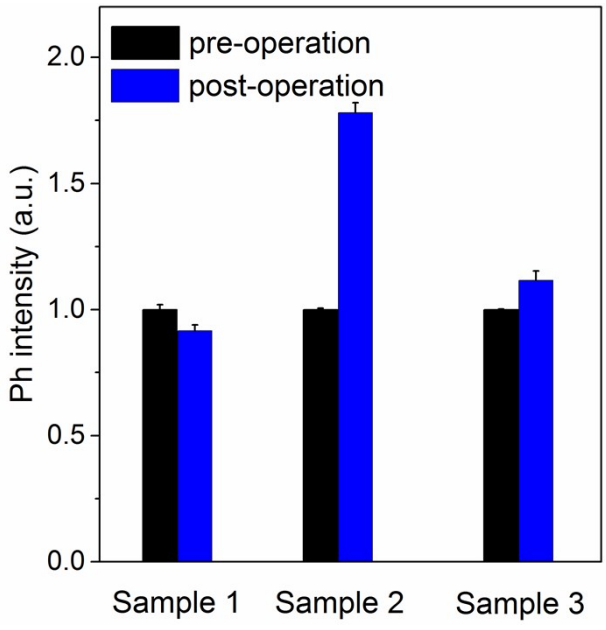

Figure S12 Analytical results of protamine in serum samples of pre-operation and post-operation of cardiovascular patients. The phosphorescence intensity of QDs with 1% serum of pre-operation is normalized to 1 for the calculation in all cases. The reactive solutions were prepared in 0.01 M Tris-HCl buffer of pH 9.0.

9. References

(1) Wu, P.; Zhang, J.; Wang, S.; Zhu, A.; Hou, X. *Chem. Eur. J.* **2014**, *20*, 952-956.

(2) Zhang, J.; Zhu, A.; Zhao, T.; Wu, L.; Wu, P.; Hou, X. *J. Mater. Chem. B* **2015**, *3*, 5942-5950.