Electronic Supplementary Information

Interaction mechanisms of CdTe quantum dots with proteins possessing different isoelectric points

Zhisong Lu, [a,b] *Weihua Hu,* [a,b] *Haifeng Bao,* [a,b] *Yan Qiao* [a,b] *and Chang Ming Li,** [a,b]

a School of Chemical and Biomedical Engineering, Nanyang Technological University, 70 Nanyang Drive, Singapore 637457, Singapore.

b Centre for Advanced Bionanosystems, Nanyang Technological University, 70 Nanyang Drive, Singapore 637457, Singapore.

*: Author to whom correspondence should be addressed. Tel.: +65 67904485; Fax: +65 67911761. E-mail: ecmli@ntu.edu.sg (C.M. Li).

Electronic Supplementary Information

Experimental Section

Materials. GOD, human Hb and Cyt C were purchased from Sigma-Aldrich. Proteins were dissolved in 0.1 M phosphate buffer (KH_2PO_4 – K_2HPO_4) and directly used in experiments without further purifications. Cadmium chloride, trisodium citrate dehydrate, sodium tellurite, sodium borohydride, mercaptosuccinic acid (MSA), cysteamine, and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were bought from Sigma – Aldrich. All other chemicals were of analytical grade and used as received. Deionized water (resistance over 18 MΩ cm) from a Millipore Q water purification system was used in all experiments.

Synthesis and Characterization of QDs. Highly fluorescent MSA-capped CdTe nanocrystals were synthesized according to Bao's route[1-3]. Transmission electron microscopy (TEM) images were taken with a JEM 2100 (JEOL, Japan) electron microscope operating at 200 kV. TEM samples were prepared by dropping QDs or QD-protein complex on copper grids, drying overnight at room temperature. Freshly synthesized CdTe QDs with an emission at 610 nm were used in following assays. Characterization results show that the nanocrystals possess uniform size, good optical and physical properties, and the surfaces are coated with carboxyl groups (Fig. S1). The full width at half maximum (FWHM) of the PL spectrum indicates a wide particle size distribution, which is in agreement with the size distribution histogram in Fig.S1A. The molar concentration of QDs was determined based on its adsorption according to Peng's report[4].

Electronic Supplementary Information

SPR Experiments. Real-time SPR measurements were conducted using an autolab SPRINGLE surface plasmon resonance system (Eco Chemie BV, Netherlands). Fresh gold-evaporated glass disks were directly used in the test. Firstly, 0.1% cysteamine solution was added into the cuvette and incubated at room temperature for 2 h to form a self-assembled monolayer on the gold surface. After washing with deionized water, a 100 μM QDs + 2 mM EDC solution was injected into the cuvette to covalently bond QDs on the gold disk in one step. Once the binding reached to the equilibrium, the disk was washed and then incubated with deionized water for 30 min to hydrolyte EDC caused intermediates and to regenerate carboxyl groups on QDs surfaces. QD-immobilized gold disks were applied in the protein adsorption tests. A 1.2 μM protein phosphate buffer solution was dropped into the cuvette to allow the adsorption of proteins on the QD modified gold surface. The binding process was real-time monitored using SPR binding curve. Since an SPR angle alteration of 100 millidegrees corresponds to a surface concentration of about 1 ng/mm^2 for most proteins. The surface molecular densities of adsorbed proteins can be calculated according to the following equation:

$$Surface\ protein\ molecular\ density(proteins/mm^2) = \frac{\Delta SPR\ angle \times 10^{-9}}{100 \times protein\ molar\ mass} \times 6.02 \times 10^{23} \qquad (S1)$$

Atomic force microscopy. Surface morphologies of gold disks were captured after each immobilization and adsorption process with tapping-mode AFM (SPM 3100, Veeco Instruments Inc., USA) at ambient condition.

Photoluminescence spectrometry. 1 μM freshly synthesized QDs were mixed in different protein solutions (pH 7.4) with the concentration from 0 to 1.2 μM,

Electronic Supplementary Information

respectively. After incubation at 37 ^{o}C for 2 h, PL spectra of all solutions were obtained using Aminco Bowman II luminescence spectrometer (Thermo Electron, USA) with an illumination source at 400 nm.

Hydrodynamic size and zeta potential measurements. Both hydrodynamic size and zeta potential were recorded using Nano-ZS instrument (Malvern Instruments Ltd., UK). Hydrodynamic sizes of QDs, proteins and QDs-protein combinants were measured at 25 ^{o}C with an equilibration time of 3 min. As to zeta potential measurements, QD and protein solutions were centrifuged at 2,000 g for 5 min before the study. Each sample was tested three times at 25 ^{o}C with an equilibration time of 5 min. Since there is a concentration requirement for both hydrodynamic size and zeta potential measurements, in this experiment the concentration of QDs and proteins are 10 μM and 1μM, respectively.

Protein structures. Protein sequences and structures are available from NCBI structure database (MMDB id of GOD: 56200; MMDB id of Hb: 42825; MMDB id of Cyt C: 58016). The images were exported from the Cn3D software (www.ncbi.nih.gov/Structure/CN3D/cn3d.shtml) in a space filled model with surface charges.

Electronic Supplementary Information

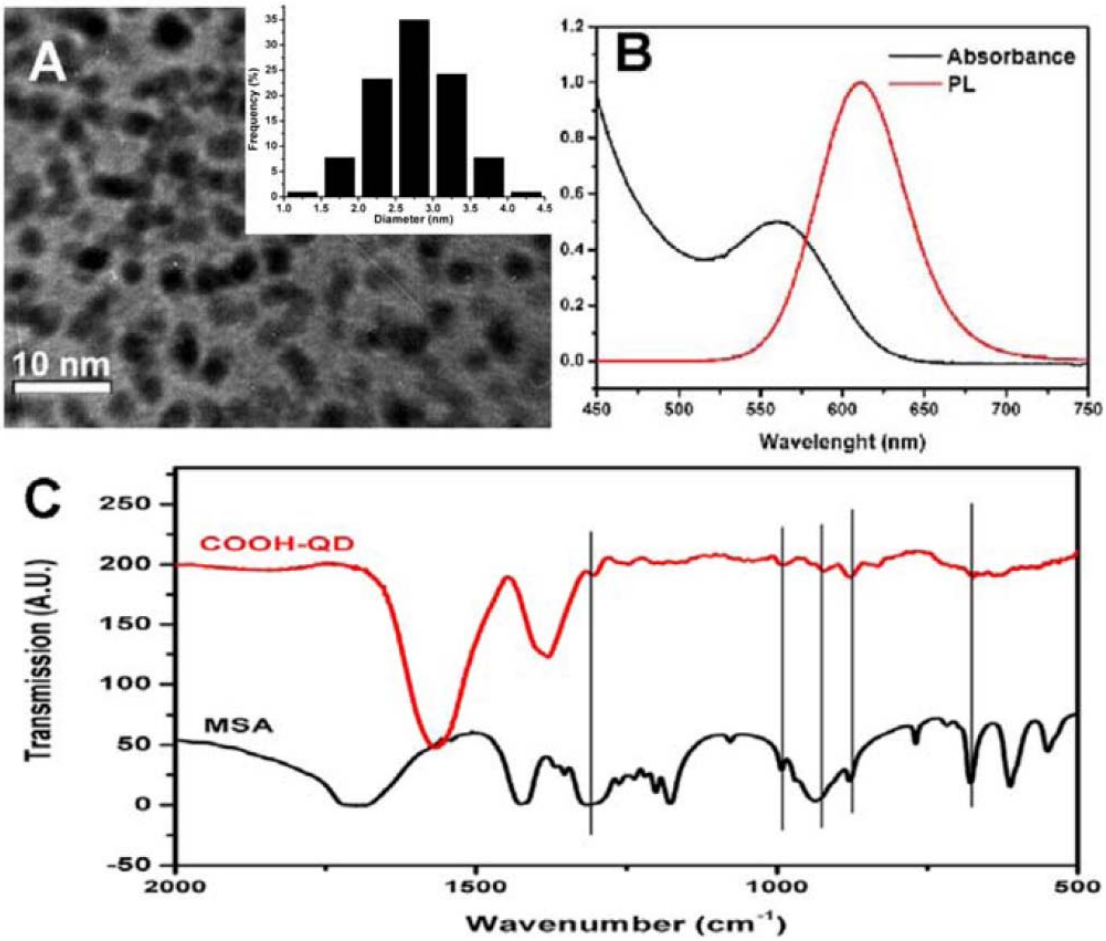


Figure S1. Physical and optical properties of as synthesized MSA-capped QDs. (A) A TEM image of a large population of MSA coated QDs; (B) UV-vis and photoluminance spectra of synthesized MSA coated QDs; (C) FTIR spectra of MSA and synthesized MSA coated QDs.

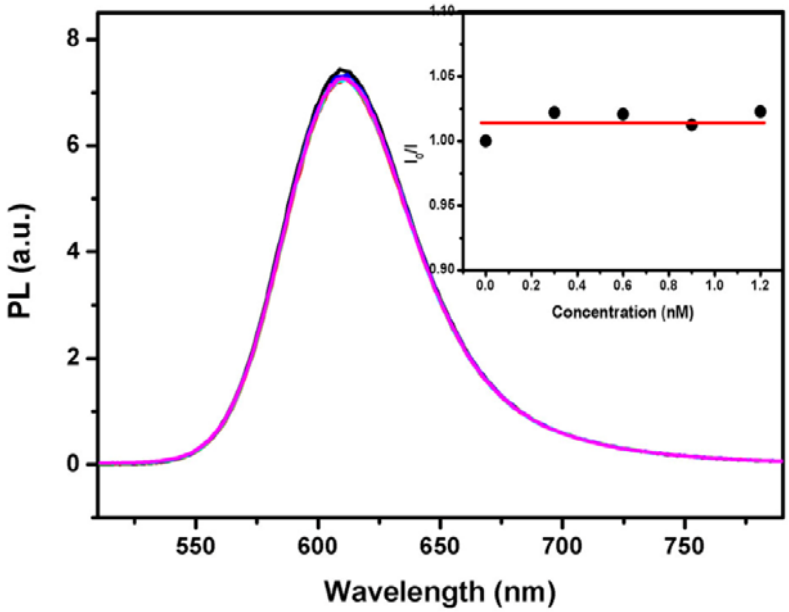
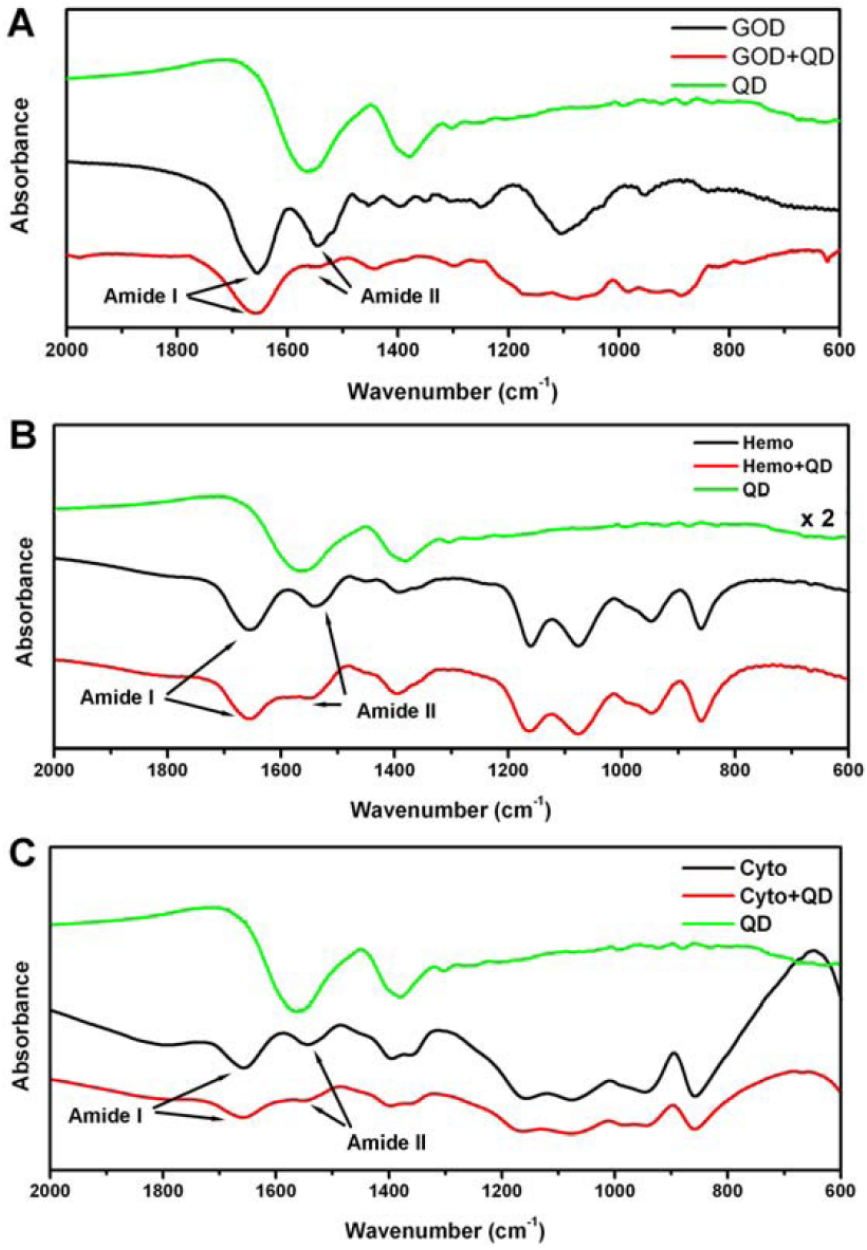


Figure S2. PL spectra of QDs – GOD mixtures with different protein concentrations. Inset corresponds to Stern – Volmer plots.

Electronic Supplementary Information

1



2

3 **Figure S3**. (A) FTIR spectra of QD, GOD and QD-GOD mixture; (B) FTIR spectra of QD, Hemo
4 and QD-Hemo mixture; (A) FTIR spectra of QD, Cyt C and QD-Cyt C mixture

5

Electronic Supplementary Information

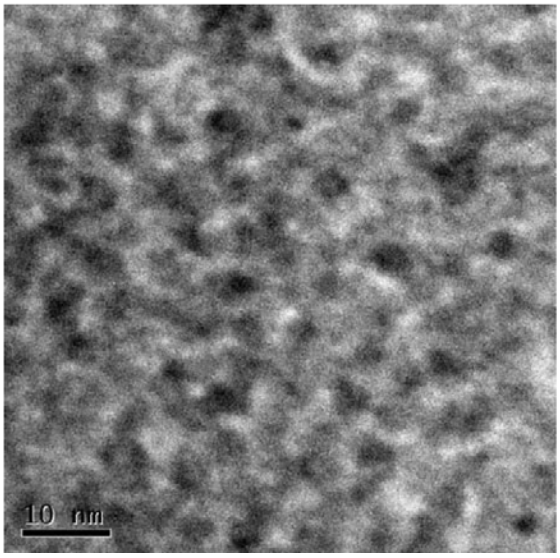


Figure S4. A TEM image of Hemo-QD complex

Table S1. The surface density of each protein on the QDs immobilized SPR gold disk

	molar mass (kDa)	□SPR angle (m^o)	□Mass (ng/mm^2)	□Mole ($mol/mm^2 \times 10^{-14}$)	Surface density ($Proteins/mm^2 \times 10^9$)
GOD	160.0	90 ± 18	0.90 ± 0.18	0.50 ± 0.11	3.20 ± 0.66
Hb	64.5	800 ± 160	8.00 ± 1.60	12.00 ± 2.50	70 ± 15
Cyt C	12.4	200 ± 69	2.00 ± 0.69	16.00 ± 5.56	100 ± 33

References

(1) H. F. Bao, E. K. Wang and S. J. Dong, *Small* 2006, **2**, 476.

(2) X. D. Cao, C. M. Li, H. F. Bao, Q. L. Bao and H. Dong, *Chem. Mater.* 2007, **19**, 3773.

(3) E. B. Ying, D. Li, S. J. Guo, S. J. Dong and J. Wang, *PLoS ONE*, 2008, **3**, e2222.

(4) W. W. Yu, L. H. Qu, W. Z. Guo and X. G. Peng, *Chem. Mater.* 2003, **15**, 2854.

(5) S. Suire, M. C. Maurel and F. Guillou, *Eur. J. Biochem.* 1996, **239**, 52.