Supplementary Information for:

γ-Cyclodextrin/folate complex-functionalized quantum dots for tumor-targeting and site-specific labeling

Mei-Xia Zhao,[a] Hai-Feng Huang,[b] Qing Xia,[*b] Liang-Nian Ji[a] and Zong-Wan Mao[*ab]

Characterization of γ-CD/FA inclusion complex

Scheme 1 in main text presents the synthetic strategy that γ-CD/FA inclusion complex. FA was included into γ-CD via hydrophobic action. The UV-vis spectra of the γ-CD, γ-CD/FA and FA are shown in Figure S6. It can be found that γ-CD there is no absorbance spectrum in 240-500 nm. However, the γ-CD/FA complex there is a similar absorbance spectrum in the position or width of the absorbance bands with FA, suggesting that the γ-CD/FA is present stably.

ESI-MS spectrometry of γ-CD/FA is a more important method for the investigation into the formation of complexation in DMSO solution. Figure S7A shows the MS spectra of the sample exhibited the molecular ion peak at m/z 1737.8 that can be assigned to the inclusion complex $[\gamma\text{-CD/FA+H}]^+$ and the peak at m/z 1319.3 belonging to the host $[\gamma\text{-CD+Na}]^+$, the other peaks at m/z: 442.0, 464.1, and 882.9, 937.0, which were assigned to $[FA+H]^+$, $[FA+Na]^+$, and its

dipolymer 2[FA+H]$^+$, 2[FA+Na]$^+$. Particularly, the MS/MS spectrum (Figure S7B) showed the molecular ion fragment peaks at m/z 1760.1 and m/z 1319.2 which were assigned to the complex [γ-CD/FA+Na]$^+$ and the host [γ-CD+Na]$^+$, respectively. The results indicate that the complexation binding between the FA and γ-CD exists.

The ROESY spectroscopy has been become an essential method in the study of the interaction between host CDs and guest molecules, since two protons are closely located in space if an NOE cross-peak is detected between the relevant protons in the NOESY or ROESY spectrum.[1,2] Therefore, it gives information about the part of the guest included inside the CD cavity, the mode of penetration, i.e. either from narrower or wider rim side, the depth of penetration and orientation of the guest.

To access the complexation of γ-CD and FA, ^{1}H NMR and 2D ROESY NMR spectra were used to investigate. Figure S8 shows the H^1 NMR spectrum of γ-CD/FA inclusion complex. Some changes in the chemical shift of the FA protons in γ-CD/FA were observed relative to those in γ-CD: the Ha, Hb, and Hc protons shifted downfield by approximately 0.12, 0.13, and 0.17 ppm, respectively, which indicated that a complex had formed between the γ-CD and FA. To further confirm the above observation, the inclusion complexation of γ-CD/FA was investigated by means of ROESY NMR spectroscopy. The ROESY spectrum for ROESY cross-peaks are indicative of specific proximity relationships between adjacent protons (the distance between protons is generally in the region of 4-5 Å).[3] Therefore, once the guest molecule is included into the γ-CD cavity, the NOE correlation signals between the protons of the guest and the interior

protons of the γ-CD cavity (H3 and H5) can be observed. In an observed ROESY NMR spectrum (Figure S9), interactions between the protons of FA and the protons inside of cyclodextrin were found, exhibiting clear NOE cross-peaks between the FA protons (Ha, Hb, Hc) and H3, H5 of γ-CD, which indicated that FA can be included into the cavity of γ-CD. It can be inferred from these cross-peaks that the protons of FA is embedded into the central cavity from the narrow side of the γ-CD cavity.

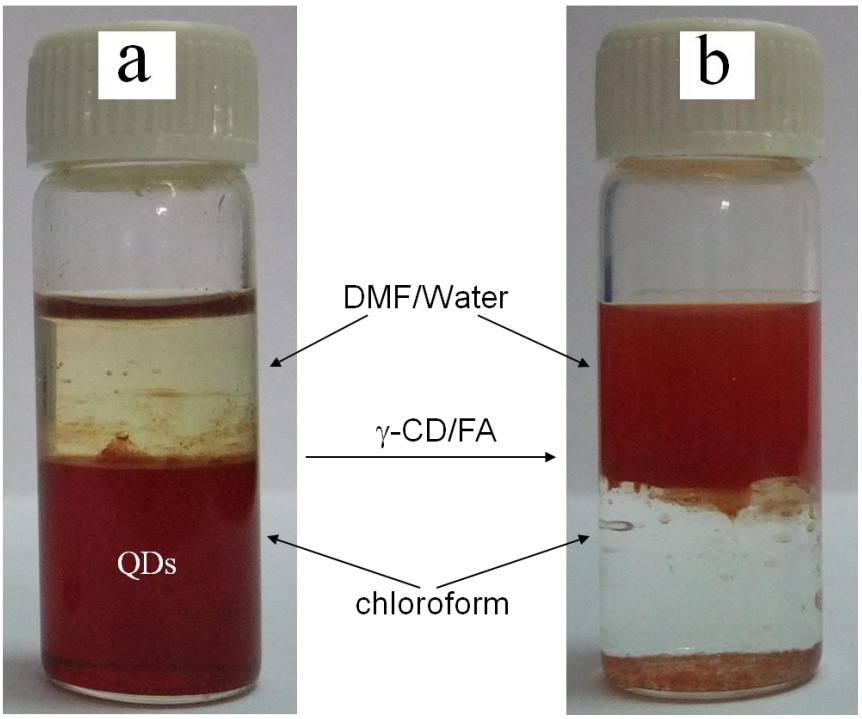

Figure S1. Photographs of the phase transfer experiments using quantum dots. The QDs in the chloroform phase (a) are transferred to the DMF/H_2O phase (b) upon complexation of γ-CD/FA inclusion complex.

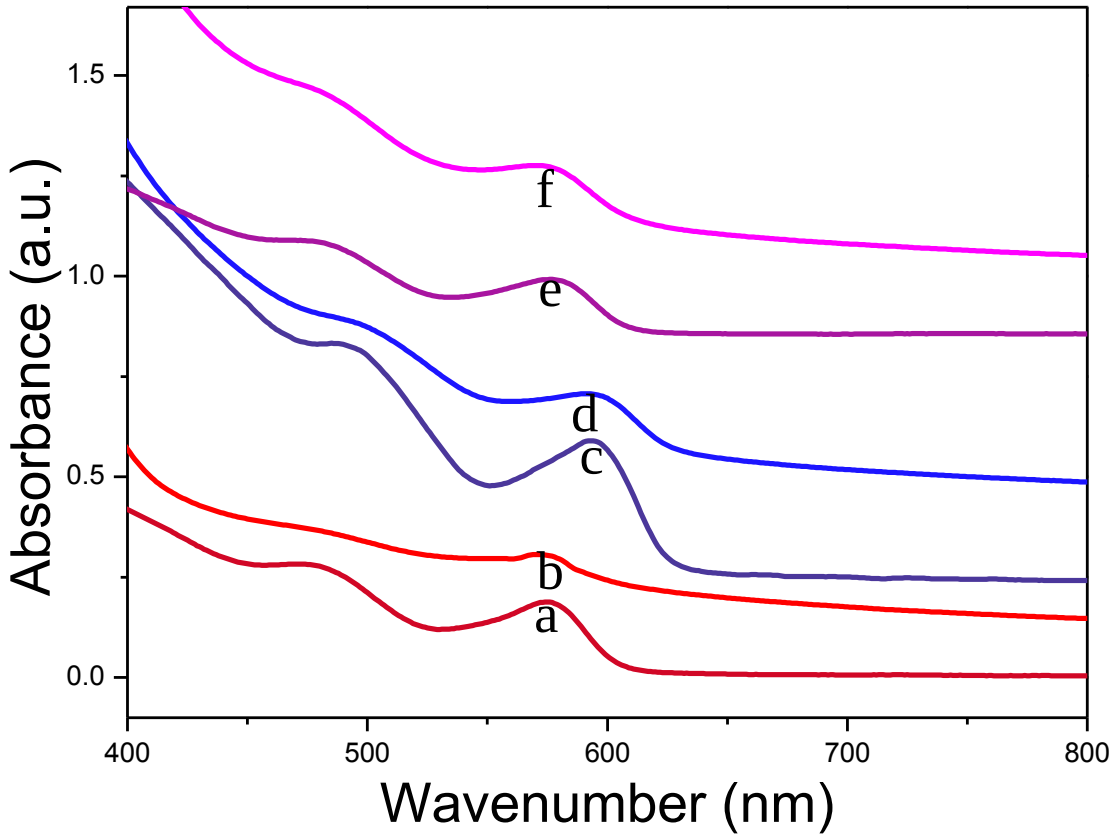

Figure S2. UV-vis spectra of TOPO-coated CdSe QDs (a), TOPO-coated CdSe/CdS QDs (c), TOPO-coated CdSe/ZnSe QDs (e) in chloroform, and γ-CD/FA-coated CdSe QDs (b), γ-CD/FA-coated CdSe/CdS QDs (d), γ-CD/FA-coated CdSe/ZnSe QDs (f) in PBS.

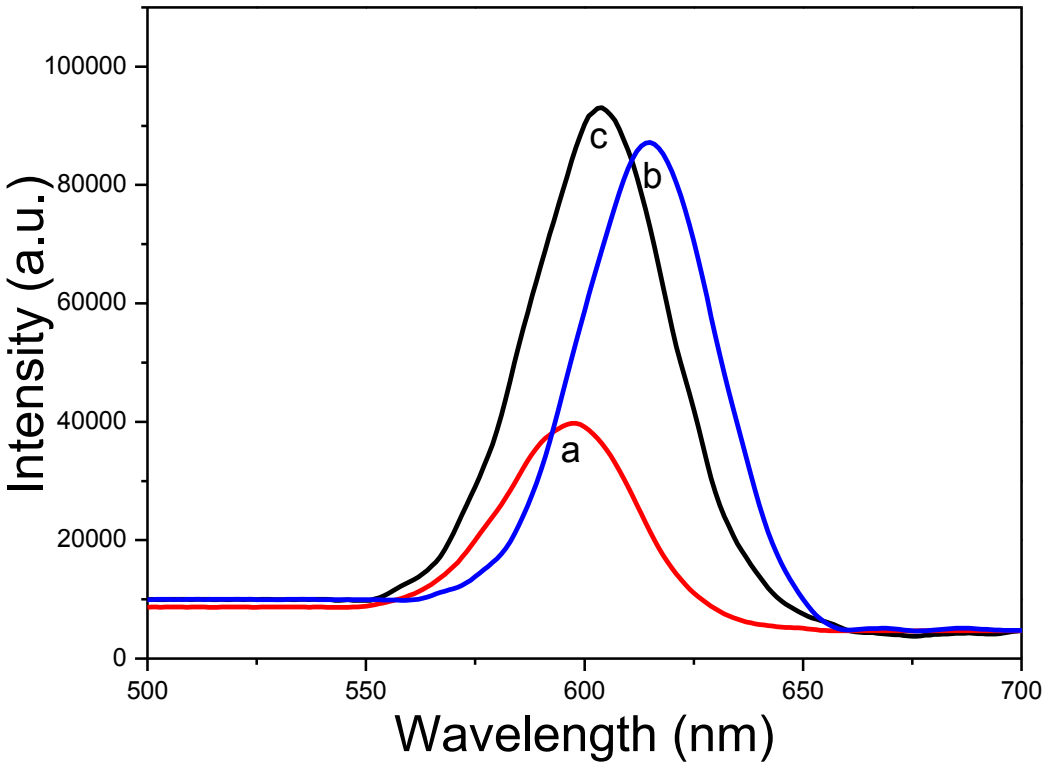

Figure S3. The luminescence spectra of γ-CD/FA-coated CdSe QDs (a), γ-CD/FA-coated CdSe/CdS QDs (b), and γ-CD/FA-coated CdSe/ZnSe QDs (c) in PBS. Each sample was excited at wavelength with an absorbance value of 0.1.

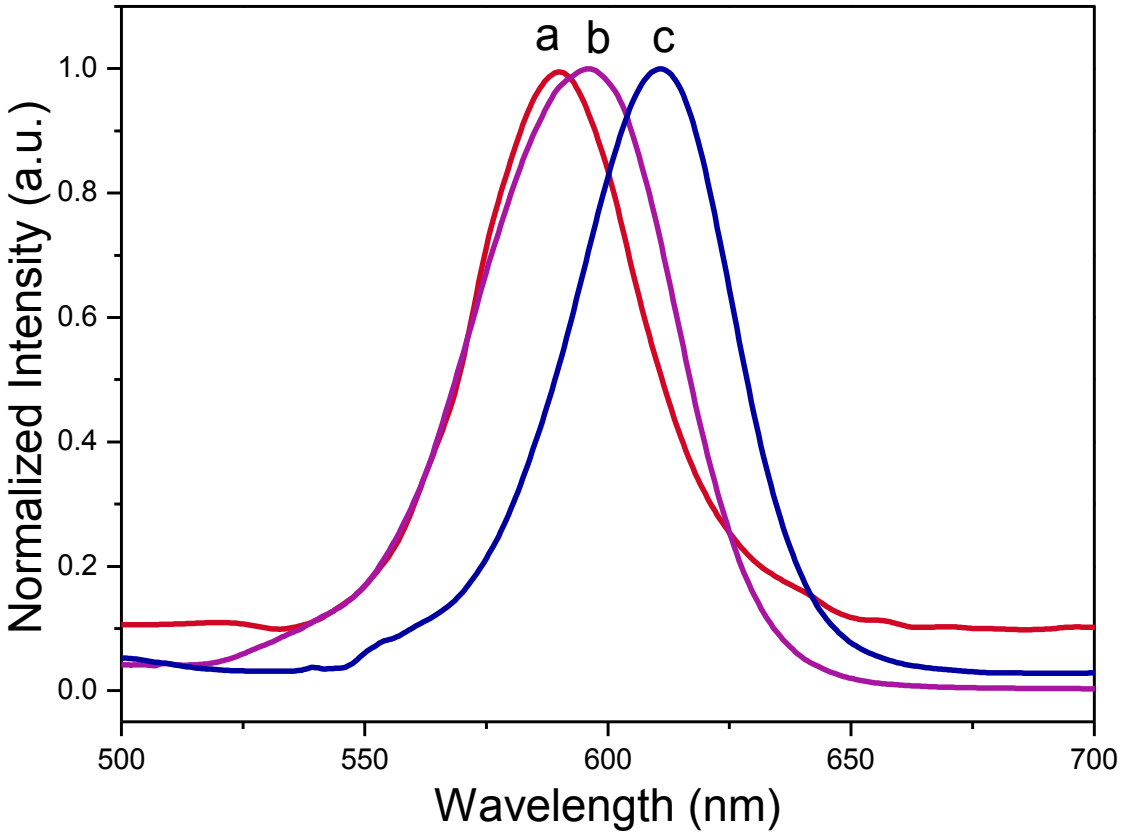

Figure S4. The luminescence spectra of three original QDs CdSe (a) CdSe/ZnSe (b) CdSe/CdS (c) in chloroform. Each sample was excited at wavelength with an absorbance value of 0.1.

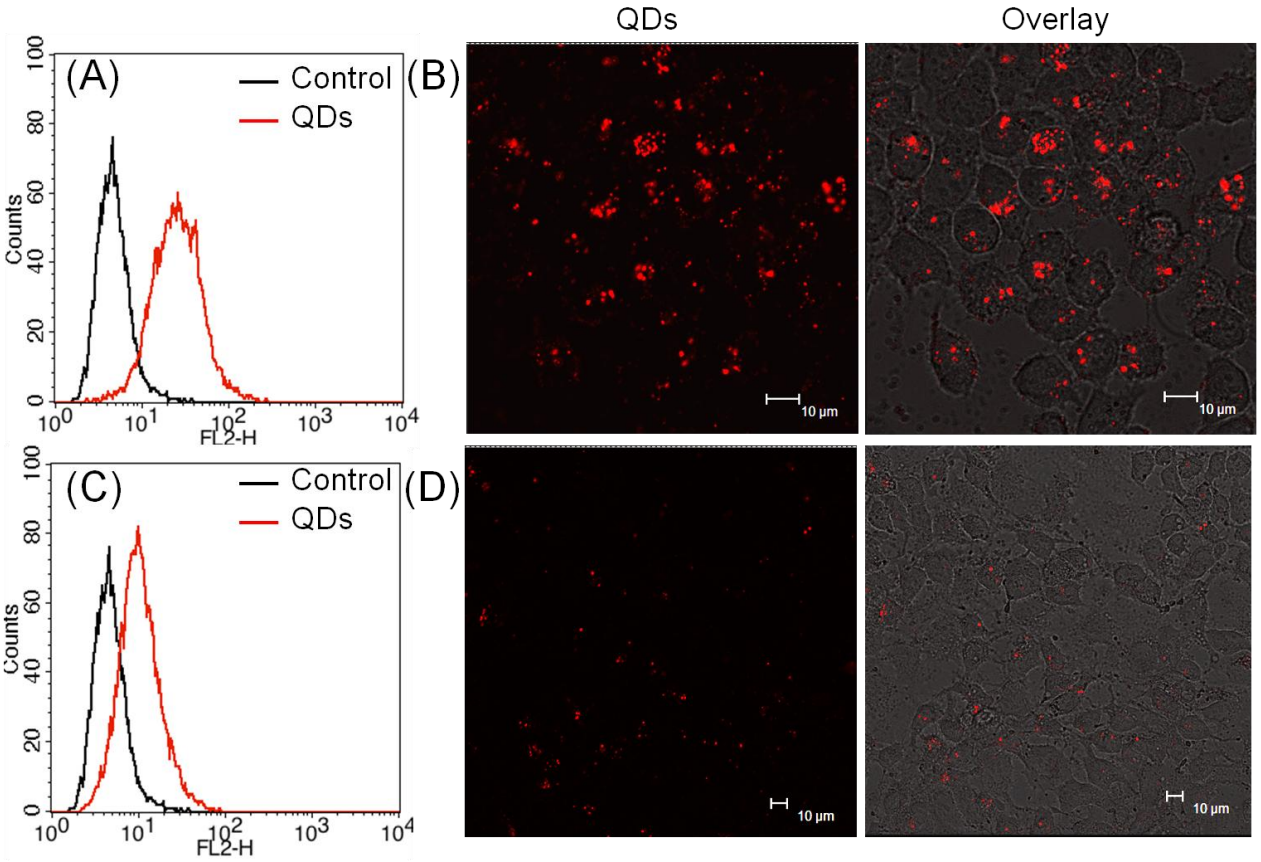

Figure S5. Targeted cellular uptake folate-targeted γ-CD/FA-coated CdSe/ZnSe QDs incubated with FR(+) HeLa cells (A,B) and FR(–) ECV cells (C,D) under same conditions; γ-CD/FA-coated CdSe/ZnSe QDs solutions at 50 μg/mL concentration were used.

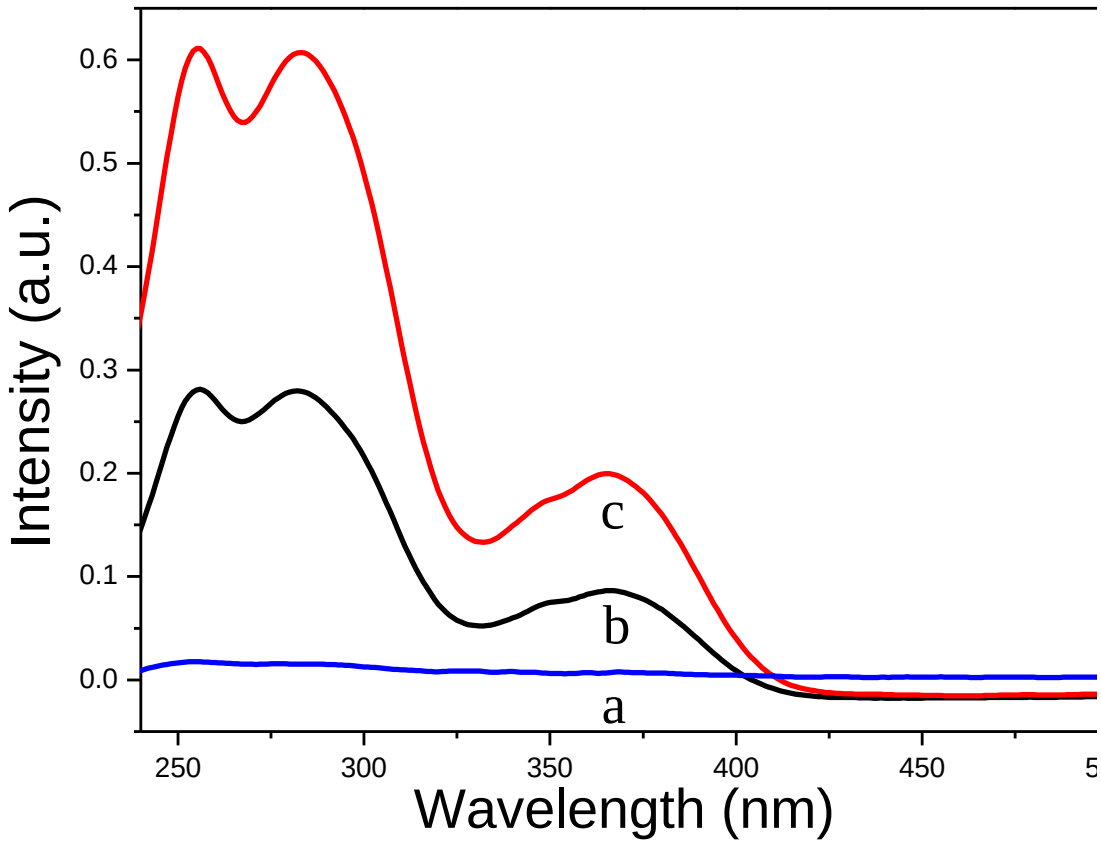

Figure S6. UV-vis spectra of γ-CD (a) γ-CD/FA inclusion complex (b) and FA (c) in 1.0 mM NaOH solution at 25 °C. Concentration of samples: 1.0×10^{-5} M.

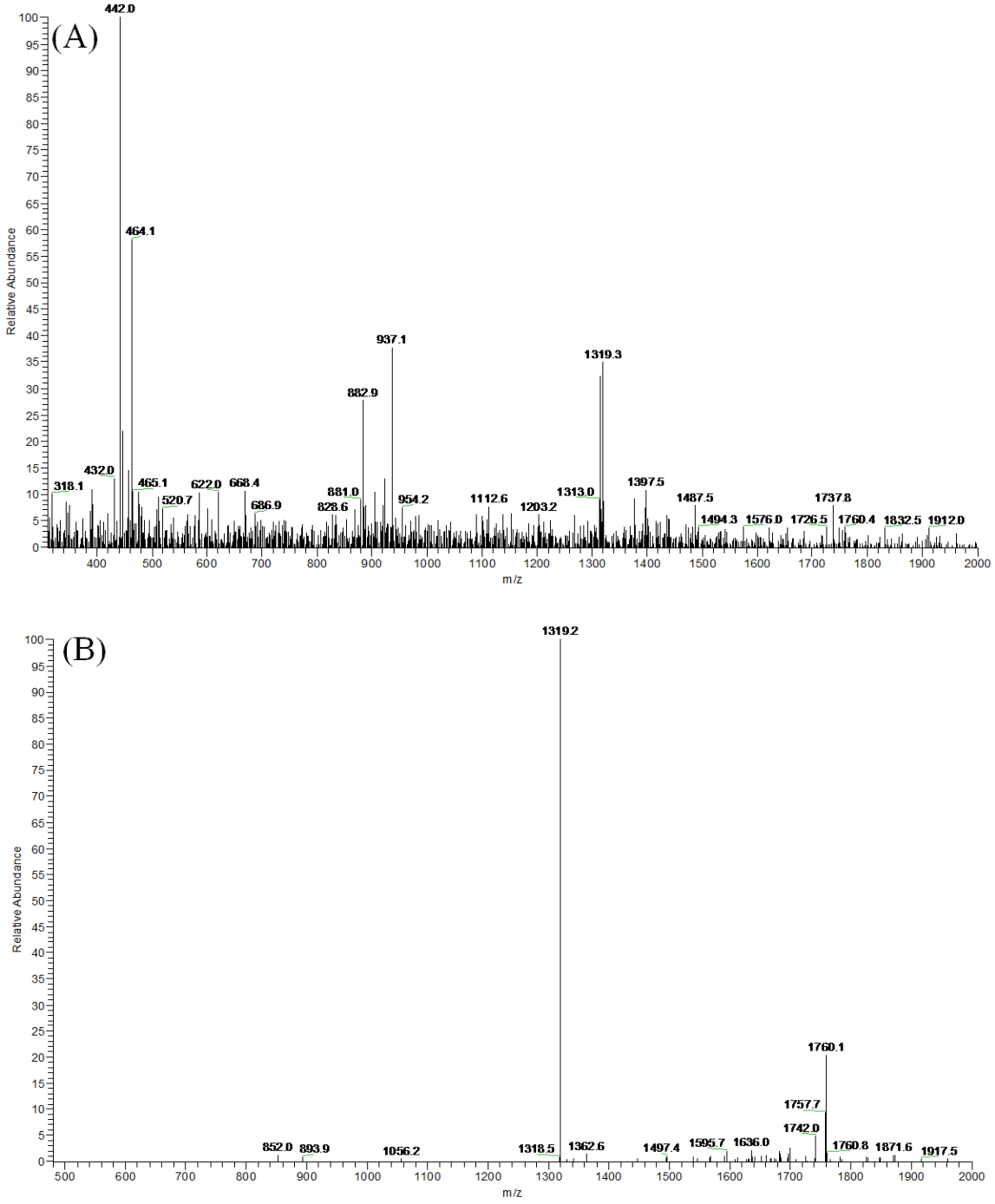

Figure S7. The MS spectra (A) and the MS/MS spectra (B) of γ-CD/FA inclusion complex.

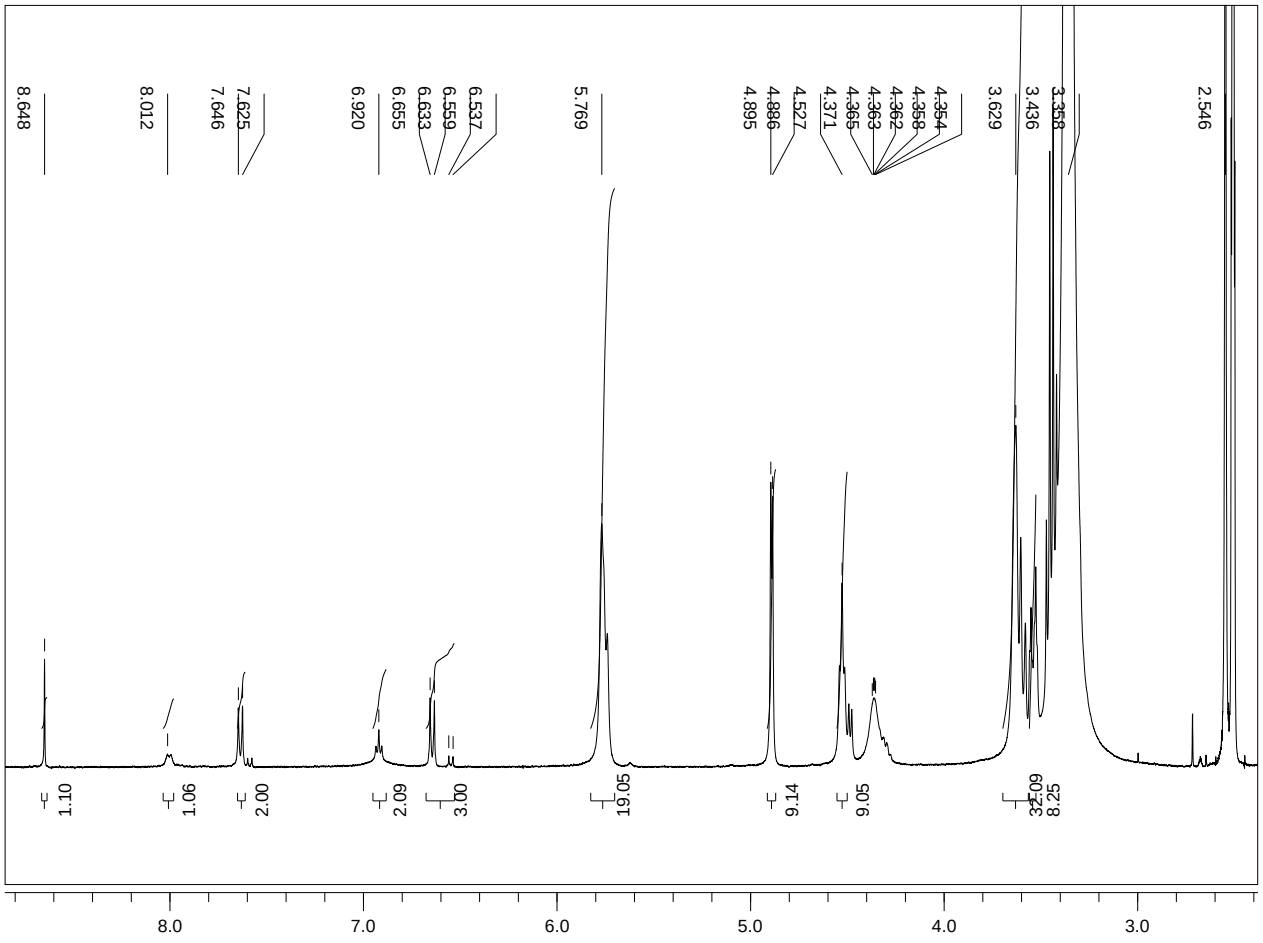

Figure S8. The H^1 NMR spectrum of γ-CD/FA inclusion complex.

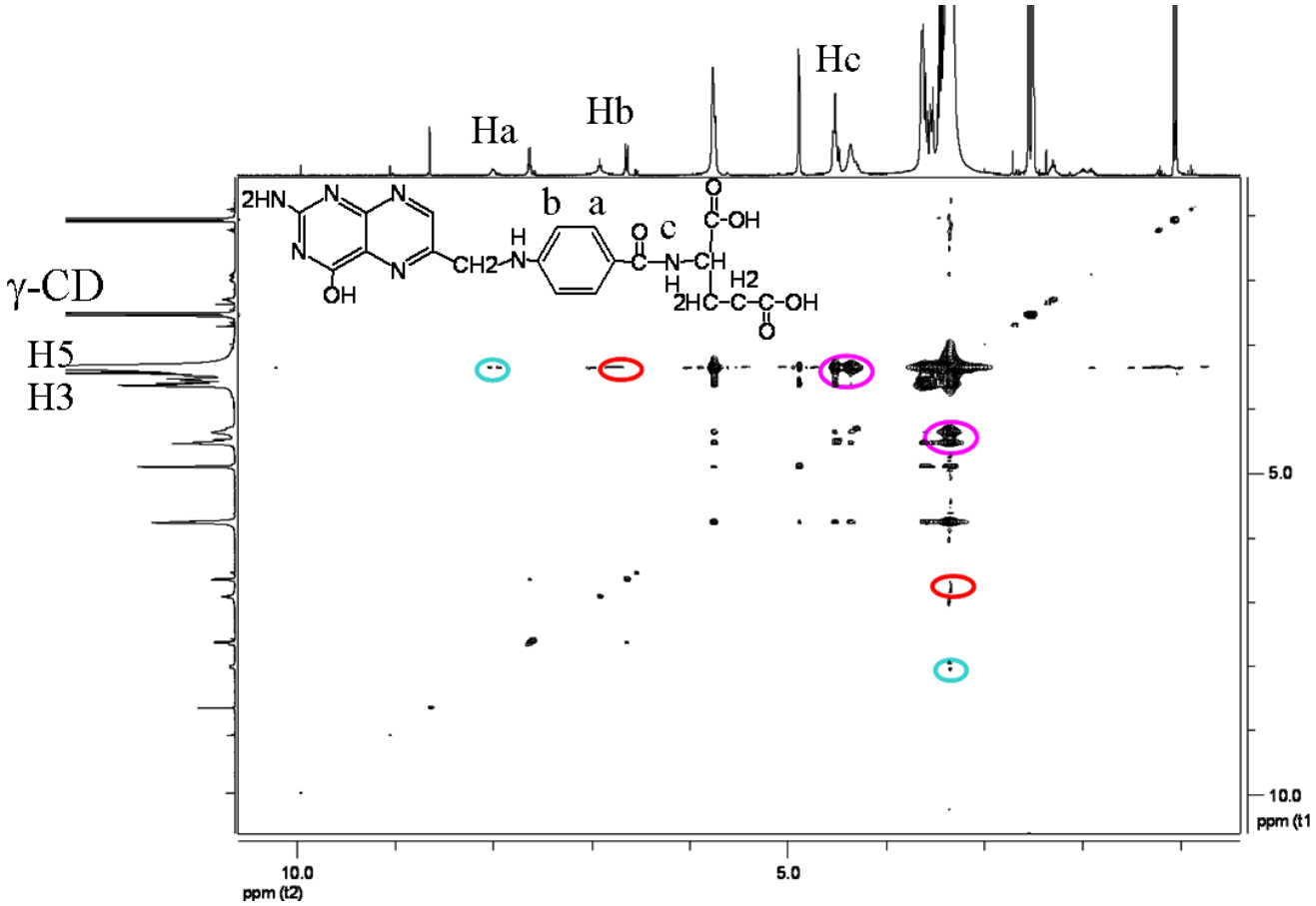

Figure S9. Part of the NOESY spectrum of γ-CD/FA inclusion complex.

Table S1 Summary of the optical properties and the cytotoxicity activity of γ-CD/FA-functionalized QDs.

Sample	Emission Peak (nm)	Φ(%)	Estimated size (nm)	τ_{av} (ns)	IC_{50} (μg/mL)[a]		
					Chang′s liver	HepG2	HeLa
γ-CD/FA-coated CdSe	597	~32.5	~4.1	~5.5	>200	156.2±5.4	90.3±3.1
γ-CD/FA-coated CdSe/CdS	615	~50.7	~4.7	~7.5	>200	164.4±3.3	130.6±5.2
γ-CD/FA-coated CdSe/ZnSe	604	~63.4	~4.3	~9.2	>200	184.5±4.7	161.4±4.5

[a] IC_{50} values are given in μg/mL, and the data are presented as mean values ± standard deviations, and cell viability is assessed after 48 h of incubation.

References for Supplementary Information:

1 K. Kano and H. Hasegawa, *J. Am. Chem. Soc*., 2001, **123**, 10616.

2 H. Yamamura, M. V. Rekharsky, Y. Ishihara, M. Kawai and Y. Inoue, *J. Am. Chem. Soc*., 2004, **126**, 14224.

3 H. J. Schneider, F. Hacket and V. Rüdiger, *Chem. Rev.*, 1998, **98**, 1755.