Self-Assembled Mn-doped ZnSe quantum dots/methyl viologen nanohybrids as an OFF-ON fluorescent probe for time-resolved fluorescence detection of tiopronin

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Chemicals. 3-Mercaptopropionic acid (MPA, 99%) and 1,1׳-dimethyl-4,4׳-bipyridinium chloride (MV^{2+})$^{2+}$ were from Fluka. Zn(NO$_3$)$_2$ (99%), MnCl$_2$ (99%), NaBH$_4$ (96%), sodium oleate (99%), and selenium powder (99.999%, about 200 mesh) were obtained from Shanghai Reagent Company. Tiopronin, methimazole and 2-mercaptobenzothiazole were from Jiangsu Zhongkang Pharmaceutical Science and Technology Company. Ethanol (CH$_3$CH$_2$OH, anhydrous) was of analytical grade and used without further purification. Other chemicals were of analytical grade. Phosphate buffer solution (PBS, 25 mM, pH=7.4) was prepared by mixing the solutions of K$_2$HPO$_4$ and NaH$_2$PO$_4$. The ultra pure water with 18.2MΩ (Millipore Simplicity, USA) was used in the experiments. The serum samples were collected from healthy volunteers. No further pretreatment and deproteinization procedures were needed in the sample preparation.

Characterizations. X-ray diffraction (XRD) measurements were performed on a Shimadzu XRD-6000 powder X-ray diffractometer, using Cu Kα (λ = 1.5405 Å) as the incident radiation. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was performed on a Perkin-Elmer Optima 3000 DV after dissolving the aqueous Mn-QDs in 5% hydrochloric acid. Transmission electron microscopy (TEM and HRTEM) samples were prepared by dropping the samples dispersed in water onto carboncoated copper grids with excess solvent evaporated. TEM images were recorded on a Shimadzu

JEM-2010 CX with an accelerating voltage of 100 kV. HRTEM images were recorded on a JEM-2010 F with an accelerating voltage of 200 kV. The measurement of the infrared spectroscopy was performed using a Nicolet IR100 infrared spectrometer. Fluorescence measurements were performed using a Shimadzu RF-5301 PC fluorescence spectrometer. Electron paramagnetic resonance (EPR) measurements were performed using an X-band (9.7747 GHz) EMX-10/12 spectrometer (Bruker) at room temperature. Cylindrical quartz tube containers were used to insert the powders into the microwave cavity. The fluorescence life time of the Mn-QDs was measured with a FLS 920 time-resolved spectroscopy (Edinberge). The time-resolved fluorescence spectrum was performed on a LS-55 fluorometer (Perkin Elmer). All of the measurements were performed at room temperature.

Synthesis of the Mn-QDs. The MPA-capped Mn-QDs were prepared as our previous report.[S1] A microwave synthetic system (CEM Discover) was used for the preparation of the Mn-QDs. The reaction temperature, pressure and time were programmed in the reaction. The synthesis of the Mn-QDs was performed in a high-strength cylindrical digestion vessel consisting of a special kind of glass. The volume of vessel used in the reaction was 80 ml. In a typical synthesis, 3.0 g sodium oleate was added to the mixture of 15 ml water and 5 ml ethanol, and the pellucid solution was obtained by stirring intensively for 5 min. 10ml of aqueous solution containing 0.25g of zinc nitrate and 0.02g of manganese chloride was added to the sodium oleate solution, which was stirred intensively for 5 min under nitrogen atmosphere. Then 5 ml freshly prepared NaHSe solution was added to the above reaction mixture and stirred for 10 min under nitrogen atmosphere. The molar ratio of Zn^{2+}:Se^{2-} was equal to 3:1. The mixture was transferred to an 80 ml cylindrical digestion vessel under agitation. Under microwave irradiation (260 W), the reaction was maintained at 170 °C and 175 psi for 40 min. In this procedure, metal ions (Zn^{2+}, Mn^{2+}) firstly underwent an ion exchange process to form metal oleate. In the addition of Se^{2-} ion, the reaction between metal oleate and Se^{2-} generated metal selenide precursors, which were interacted to form Mn-QDs. Along with the process, the Mn-QDs were endowed with hydrophobic surfaces coated with the alkyl ligands of oleate. The spontaneous separation of the Mn-QDs from the bulky solution occurred due to the weight of the Mn-QDs and the incompatibility between the hydrophobic surface and their hydrophilic surrounding. The Mn-QDs collected at the bottom of the container were redispersed in 50 mL of chloroform. After centrifugation at 10 000 rpm, the transparent upper Mn-QDs solution was collected. The solution was dried in vacuum and the flaxen Mn-QDs powders about 80 mg were obtained. The flaxen Mn-QDs coated with the original alkyl ligands were dissolved in 2 ml chloroform and treated with 200 μL MPA. The mixture was shaken for 60 min under sonication. The chloroform solution gradually became turbid because the original ligands with a long hydrophobic alkyl chain were replaced by hydrophilic carboxyl chain of MPA. The MPA-coated Mn-QDs precipitate was isolated by centrifugation. Excess MPA was further removed by washing the precipitate with chloroform for three times. The final precipitate was dried in vacuum and the flaxen Mn-QDs powders were obtained. The powders can be dispersed to PBS solution(pH 7.4）. The content of the incorporated Mn^{2+} in the Mn-QDs was determined by ICP-AES after dissolving the Mn-QDs in 5% hydrochloric acid solution. In the typical synthesis, the Mn:Zn mass ratio of 10% in the reaction solution gave the incorporated Mn^{2+} ratio of 0.84% in the prepared Mn-QDs.

Self-Assembly Mn-QDs/MV^{2+} nanohybrids. To the above 8mg/L Mn-QDs solution, and different concentration MV^{2+} solution was added to aggregate the nanohybrids through electrostatic self-assembly.

Time-resolved fluorescence detection of Tiopronin. 300 μL Mn-QDs/MV^{2+} nanohybrids solution, 300 μL of PBS solution (pH 7.4), 200μL of serum solution of 50-fold dilution containing given concentrations of tiopronin were sequentially added to a 1 mL calibrated test tube. The mixture was then diluted with PBS solution and mixed thoroughly. The time-resolved fluorescence measurements were carried out with the excitation wavelength of 350 nm, delay time of 0.2 ms, gate time of 0.4 ms, and cycle time of 20 ms in the absence or presence of a series of tiopronin solutions.

Figure S 1 compared the fluorescence spectra of the Mn-QDs in human serum PBS solution contained tiopronin using the conventional fluorometry and time-resolved fluorometry. In the conventional fluorescence spectra, the emissions of both human serum and tiopronin from 400nm to 650 nm interfered with the emission peaks at 450nm and 585 nm of the Mn-QDs (curve a, b and c in Figure S 3).

The fluorescence interferences led to a serious limitation in analytical accuracy and testing sensitivity. However, in the time-resolved fluorescence spectra, the short-lived fluorescences of both tiopronin and human serum from 400 nm to 650 nm were disappeared by delaying detection time, and thus, no fluorescence interference (curve d) was observed.

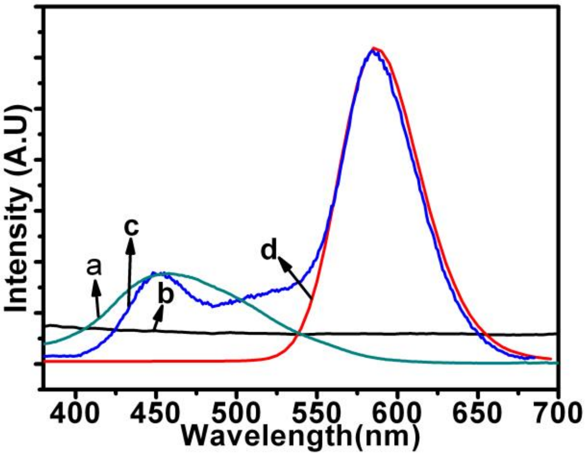

Figure S 1. Conventional fluorescence spectra of human serum (a), tiopronin (b), the mixture of tiopronin, Mn-QDs and human serum (c) and time-resolved fluorescence spectra of the mixture (d) in PBS solution (pH=7.4). Conventional fluorescence measurement condition: Excitation wavelength: 350nm; Time-resolved fluorescence measurement condition: Excitation wavelength: 350nm, delay time 0.2 ms, gate time 0.4 ms, and cycle time 20 ms.

Table S1. Effect of co-existing substances on the determination of 1.5 μM 5-FU

Substance	Concentration(mM)	Change in the determination (%)
Na^+	150	+3.1
K^+	10	+1.5
Ca^{2+}	2	-2.5

Mg^{2+}	1	-3.3
Glucose	6	+2.0

S1 Zhu. D., Jiang. X.X., Zhao. C.E., Sun.X.L., Zhang. J.R. and Zhu.J.J, *Chem. Commun.*, **2010**, *46*, 5226.