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Self-assembled Mn-doped ZnSe quantum dot–methyl viologen nanohybrids as an OFF–ON fluorescent probe for time-resolved fluorescence detection of tiopronin†

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The improvement of analytical accuracy and *S*/*N* (signal to noise ratio) based on conventional homogeneous fluorometry was always limited because of high background noise from complicated sample ingredients, buffer solution and lysates. Herein, a self-assembled Mn-doped ZnSe quantum dot–methyl viologen nanohybrid as an OFF–ON fluorescent probe is presented for the time-resolved fluorescence detection of tiopronin, showing high *S*/*N* and selectivity with a low detection limit of 0.1 μmol L^{-1} and a linear response ranging from 4 to 160 μM. We anticipate that this probe will have promising applications for sensitive biosensing and drug analysis.

Time-resolved fluorometry has become a strong tool for immunoassay and biological research because it can effectively eliminate short-lived scattering light and background noise from the specific fluorescence signal of the long-lived fluorescence probe, which results in the high sensitivity and wide dynamic range.[1] The probe is key in this technique, and the ideal probe should have long fluorescence life time, large Stoke's shift and narrow emission peaks. At present, a lanthanide chelate is mainly used as the probe for time-resolved fluorometry despite its shortcomings such as weak luminescence and photobleaching.[2] Recently, Mn-doped ZnSe or ZnS quantum dots attracted increasing attention because of their charming characteristics such as long fluorescence life time (extends to 400 μs), strong photoluminescence and high resistance to photobleaching, from the sharp $^4T_1(^4G) \rightarrow {}^6A_1(^6S)$ emission of the Mn^{2+}.[3] Some researchers have shown that Mn-doped ZnSe and ZnS quantum dots can be applied as the probe for time-resolved fluorometry.[4] Self-assembly refers to the spontaneous organization of molecules, molecular clusters, and aggregate structures into two-dimensional arrays or three-dimensional networks by attractive forces (*e.g.* hydrogen bonding, acid/base proton transfer and electrostatic forces) or chemical bond formation.[5] After the QDs are organized into well-defined aggregates with chemical cross-linkers, they offer desirable collective properties that are not found in the individual components.[6–8] Herein, we report the self-assembled nanohybrids from 3-mercaptopropionic acid (MPA)-capped Mn-doped ZnSe quantum dots (Mn-QDs) and methyl viologen (1,1′-dimethyl-4,4′-bipyridinium chloride, abbreviated as MV^{2+}), which are used as an OFF–ON fluorescent probe for time-resolved fluorescence detection of tiopronin in a serum solution. Tiopronin [*N*-(2-mercaptopropionyl)-glycine] was a kind of drug widely applied to prevent kidney damage and to treat hepatic disorders, rheumatoid arthritis as well as cataracts.[9] In the past detection of tiopronin was difficult because of the absence of chromophores in its structure.[14]

Fig. 1(A) depicts the mechanism of the self-assembled Mn-QD–MV^{2+} nanohybrids as an OFF–ON fluorescent probe for detection of tiopronin. MV^{2+} with two quaternary ammonium groups was added to the Mn-QD solution, which acted as linkers between the individual Mn-QDs through electrostatic self-assembling. These linkers enabled the Mn-QDs to form spontaneously the spherical Mn-QD–MV^{2+} nanohybrids in aqueous solution. MV^{2+} (a strong electron transfer agent) in the nanohybrids caused the Mn-QD fluorescence in the OFF state due to an efficient electron transfer process,[10] and then the assembled nanohybrids almost didn't have any emission. However, the tiopronin added could effectively bond with MV^{2+} and make the Mn-QDs partly release from the Mn-QD–MV^{2+} nanohybrids, which restored the native fluorescence of the Mn-QDs to the ON state. Because tiopronin was widely used as a strong stabilizing agent in synthesizing Ag and Au nanoparticles,[15] its adsorption on the Mn-QDs might also partially restore the native fluorescence of the Mn-QDs. Fig. 1(B) illustrates the principle of the time-resolved fluorometry technique for detecting tiopronin. After short-lived background fluorescence from tiopronin and human serum disappeared by a delay in measuring time, the

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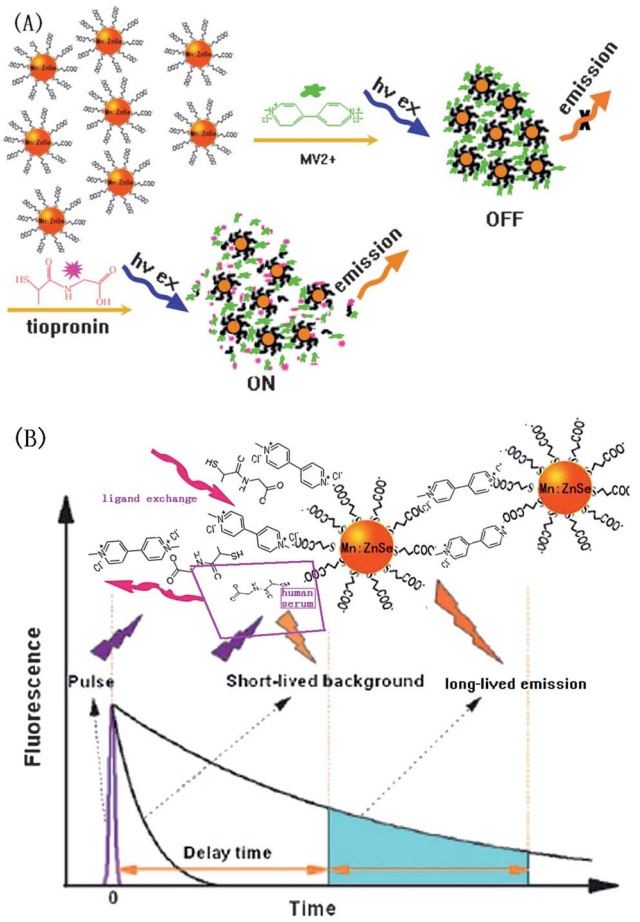

Fig. 1 (A) Schematic illustration of fabrication of Mn-QD–MV^{2+} nanohybrids for the detection of tiopronin. (B) The principle of detection for tiopronin based on the time-resolved fluorometry technique.

long-lived fluorescence from Mn-QDs was then determined to eliminate the fluorescent interference. The detailed experiments are elaborated in the ESI.†

The MPA-capped Mn-QDs were prepared as in our previous report.[11] The assemblies between the Mn-QDs and MV^{2+} could be shown by high-resolution transmission microscopy (Fig. 2). Fig. 2(A) shows good monodispersity of the Mn-QDs with particle sizes ranging from 4.5 nm to 5.5 nm. While the HRTEM image from Fig. 2(B) for the nanohybrids exhibited spherical aggregates with a mean diameter of 50 nm, only a few individual nanoparticles of the Mn-QDs could be identified, which indicated that the self-assembly between Mn-QDs and MV^{2+} was successful. After addition of tiopronin, the spherical nanohybrids became loose due to combination of tiopronin and MV^{2+}, which could make the linkage of the linker-MV^{2+} partly invalid, as shown in Fig. 2(C).

The bonding of MV^{2+} onto the surface of the Mn-QDs and the ligand exchange after the addition of tiopronin were also examined by the measurement of the zeta potential. In comparison with that of the Mn-QDs (−27 mV), the zeta potential of the self-assembled Mn-QD–MV^{2+} nanohybrids changed to −12 mV, demonstrating that the negative charge of the Mn-QDs was partially neutralized by the positive charge of

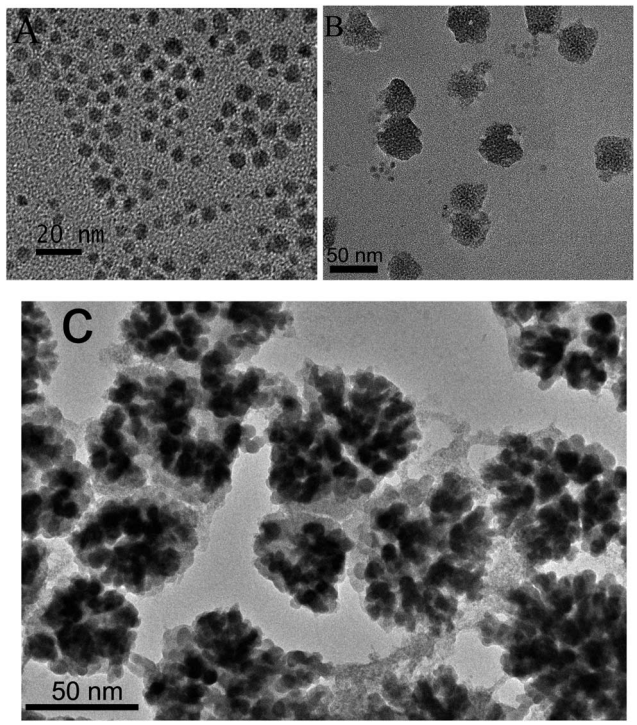

Fig. 2 (A) The HRTEM image of Mn-QDs. (B) The HRTEM image of Mn-QD–MV^{2+} nanohybrids prepared from MPA capped Mn-QDs (8 mg L^{-1}) and MV^{2+} (8 μM). (C) The HRTEM image of the nanohybrids after adding tiopronin (160 μM).

the MV^{2+} during the self-assembling process. However, the zeta potential was restored to −23 mV after the addition of tiopronin, which indicated that tiopronin could combine effectively with MV^{2+}. Besides the interaction with MV^{2+}, of course, the negatively charged tiopronin might adsorb onto the surface of Mn-QDs and contribute to the zeta potentials. The above results were also proved by the infrared (IR) spectroscopy. As shown in Fig. 3(A), the bonding of MV^{2+} onto the surface of the Mn-QDs was confirmed by the two new vibration peaks (1640 cm^{-1} for stretching vibrations of the C=C bond and 3039 cm^{-1} for stretching vibrations of the C–H bond from MV^{2+}) in the infrared (IR) curve, while these peaks were not present in the IR curve from Mn-QDs only. Fig. 3(B) also indicates that tiopronin could combine effectively with MV^{2+} and bind with the Mn-QDs by the disappearance of the peak around 1640 cm^{-1} of MV^{2+} and the appearance of the new peak of 1610 cm^{-1} for stretching

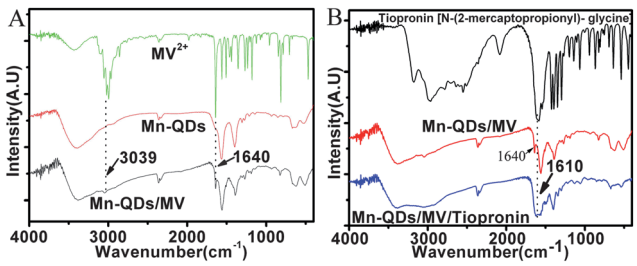

Fig. 3 (A) IR spectra of the MV^{2+}, the Mn-QDs capped with MPA and the nanohybrids from Mn-QDs and MV^{2+}; (B) IR spectra of tiopronin, nanohybrids from Mn-QDs and MV^{2+} before and after loading tiopronin.

vibrations of the C=O bond from tiopronin after loading tiopronin onto the Mn-QD–MV^{2+} nanohybrids.

The Mn-QDs displayed the photoluminescence (PL) emission peak at 585 nm with a long lifetime of 0.4 ms, ascribed to the transition of Mn $^4T_1(^4G) \rightarrow {}^6A_1(^6S)$ as shown in Fig. 4(A). And the PL emission disappeared when the added MV^{2+} concentration was up to 8 μM, showing that the fluorescence was in the OFF state because electrons from surface traps of Mn-QDs were efficiently transferred to the MV^{2+} acceptors. Subsequently, the fluorescence intensity was restored (ON state) upon addition of tiopronin to the solution as shown in Fig. 4(B). As shown in Fig. 4(B), after the addition of 160 μM tiopronin, the fluorescence is restored to 53% of the former intensity for the same Mn-QD solution without MV^{2+}. Increasing amounts of tiopronin could not bring out higher fluorescence intensity. The advantage of the time-resolved fluorometry for detecting tiopronin in human serum is demonstrated in Fig. S1 (see the ESI†). In the conventional fluorescence spectra, the emissions of both human serum and tiopronin from 400 nm to 650 nm interfered with the emission peaks at 450 nm and 585 nm of the Mn-QDs (curves a, b and c in Fig. S1†). 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 disappeared due to a delay in detection time, and thus, high accuracy and S/N were obtained. A linear calibration plot of the increased fluorescence intensity against the concentration of tiopronin was observed in the range of 4 to 160 μM with a correlation coefficient of 0.995. The regression equation was $(F - F_0) = 46.37 + 5.654c_q$, where F and F_0 are the fluorescence intensities in the presence and absence of tiopronin, respectively, and c_q is the concentration of the tiopronin. The precision for seven replicate detections of 50 μM tiopronin was 4.8% (RSD) and the detection limit (3δ) of the present probe for tiopronin was 0.1 μM.

To further confirm the selectivity of the probe to tiopronin, interference experiments were carried out in the presence of other competitors such as methimazole and 2-mercaptobenzothiazole thiol drugs. As shown in Fig. 5(A), the ability of the probe to detect tiopronin was much higher than that for methimazole and 2-mercaptobenzothiazole. The recovered fluorescence intensities for the addition of 180 μM methimazole and 2-mercaptobenzothiazole are only equivalent to 8.3% and 10.4% intensity for the addition of the same concentration of tiopronin, respectively. Fig. 5(B) revealed that the addition of methimazole or 2-mercaptobenzothiazole to a tiopronin solution only slightly decreased the fluorescence responses of the probe. These results suggested that the Mn-QD–MV^{2+} nanohybrid probe had high selectivity toward tiopronin and the presence of other similar thiol drugs only produced a small perturbation on the measurements. Moreover, the probe also showed significant tolerance to the presence of glucose and representative electrolyte ions involved, namely, Na^+, K^+, Ca^{2+} and Mg^{2+}. The determination of tiopronin at 1.5 μM was unaffected by 150 mM Na^+, 10 mM K^+, 2 mM Ca^{2+}, 1 mM Mg^{2+} and 6 mM glucose, a normal concentration level in most detection situations, especially, in biological organisms (see Table S1, ESI†).

Some methods have been developed for the determination of tiopronin in biological fluids, including high-performance liquid chromatography (HPLC) coupled with fluorescence detection (HPLC-FLD),[12] ultraviolet detection (HPLC-UV)[13] and mass spectrometry (LC-MS).[14] Owing to the absence of chromophores in the structure of tiopronin, it has to be derivatized with a special reagent that contains chromogenesis groups in all the reported HPLC-FLD and HPLC-UV methods.[13,14] Although mass spectrometry is a good choice for the determination of compounds without chromophores, derivatization reagents, such as methyl acrylate (MA), were needed to stabilize the active thiol group of tiopronin and to achieve better chromatographic retention before tiopronin was analyzed by LC-MS.[14] Compared with these methods, the time-resolved fluorometry was facile and rapid for the detection of tiopronin (usually within 5 min) because the troublesome sample pretreatment could be avoided.

In conclusion, a facile electrostatic self-assembly method was successfully applied in the fabrication of the Mn-QD–MV^{2+} nanohybrids to develop a novel OFF–ON fluorescence probe for the time-resolved fluorescence quantitative detection of

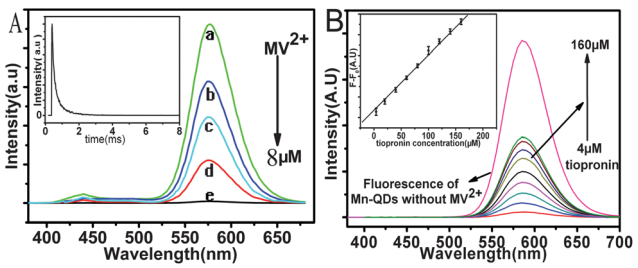

Fig. 4 (A) The MV^{2+} concentration-dependent fluorescence spectra of the prepared Mn-QD–MV^{2+} nanohybrids. The concentration of Mn-QDs was 8 mg L^{-1}. The MV^{2+} concentrations were (a) 0; (b) 2 μM; (c) 4 μM; (d) 6 μM; and (e) 8 μM. The inset is the decay curve of fluorescence emission of the Mn-QDs. Conventional fluorescence measurement condition: excitation wavelength: 350 nm. (B) Time-resolved fluorescence spectra of the Mn-QD–MV^{2+} nanohybrids with different concentrations of tiopronin and the Mn-QD solution without MV^{2+}. The inset is the calibration curve for tiopronin. Time-resolved fluorescence measurement conditions: excitation wavelength: 350 nm, delay time: 0.2 ms, gate time: 0.4 ms, and cycle time: 20 ms. Error bars mean the standard deviation. Each point is an average value of three independent measurements.

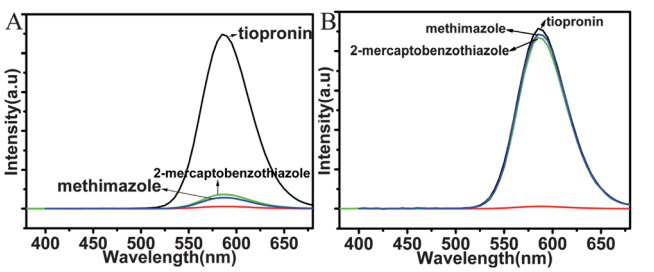

Fig. 5 Interference experiments for the measurement of tiopronin: (A) the Mn-QD–MV^{2+} nanohybrid probe ($C_{MV^{2+}}$ = 8 μM) was exposed to methimazole (180 μM), 2-mercaptobenzothiazole (180 μM) and tiopronin (180 μM), respectively and (B) fluorescence response of the nanohybrid probe ($C_{MV^{2+}}$ = 8 μM) to tiopronin (180 μM) after addition of methimazole (180 μM) and 2-mercaptobenzothiazole (180 μM) sequentially.

tiopronin. This probe not only effectively detects tiopronin with high *S*/*N*, but also shows high selectivity despite other related thiol drug, glucose and representative electrolyte ion interferences. We anticipate that this probe will be applied to other drugs or biological molecules.

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