Electronic Supplementary Information

Quantum Dot-FRET-based Detection of Vitamin B12 at Picomolar Level

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Experimental Section

A. Materials: Zinc acetate dihydrate (99%, Merck), Sodium sulphide (58%, Merck), Manganese acetate tetrahydrate (Merck), Vitamin B12($\geq$ 98%, Sigma), Quinine sulfate (Fluka), sulphuric acid (Merck), Tryptophan (Merck), Lysine (Merck), Arginine (Merck), Glycine (Merck), Salts of Na+, K+, Ca^{2+} and Mg^{2+}(Merck), and Bovine Serum Albumin (Merck), were purchased and used directly without any purification. Mili-Q grade water was used in all experiments.

B. Synthesis of Mn^{2+}-doped ZnS QDs: The Mn^{2+}-doped ZnS QDs were synthesized in an water medium.[1-3] The ligand free QDs were synthesized by mixing 5.0 mM of zinc acetate dihydrate, 5.0 mM of sodium sulphide flakes and 2.5 mM of manganese acetate tetrahydrate in the molar ratio 2:2:1 in 50.0 mL of Mili-Q water under stirring condition and the resulting mixture was refluxed for 3 h at 100° C. Next, the milky white colloidal dispersion was centrifuged at a speed of 20,000 rpm for 10-15 minutes. The pellet (following centrifugation) was washed with water and redispersed again in 50.0 mL of water. The centrifugation process was repeated 2 times and the resulting pellet was finally dispersed in 200.0 mL Mili-Q water and sonicated for 10 minutes. The subsequent dispersion was used for further experiments.

C. Preparation of VB12 solution: VB12 of strength 10^{-5} mg/mL was prepared by followed by diluting 1 mg/mL stock solution of VB12 and used for further experiment.

D. Sensing of VB12: The VB12 detection by Mn^{2+}-doped ZnS QD was performed followed by 2.0 μL sequential addition of solution of VB12 (10^{-5} mg/mL in water) to an aqueous dispersion of 3.0 mL solution of Mn^{2+}-doped ZnS QD (having absorbance of 0.3 at 320 nm). The highest amount of VB12 used here is 54.1 pM. The emission spectra (at an excitation wavelength of 320 nm) of the Mn^{2+}-doped ZnS QD was monitored to describe the quenching in the emission

intensity of Mn^{2+}-doped ZnS QD (at 587 nm) upon VB12 addition. Each measurement was given time of 5 minutes. The limit of detection (LOD) was estimated by using $3\sigma/K_{slope}$ plot; where σ represents standard deviation of the emission intensity (at 587 nm) of the Mn^{2+}-doped ZnS QD and K_{slope} signifies the slope obtained from the plot of change in emission intensity of Mn^{2+}-doped ZnS QD at 587 nm against concentration of VB12.

E. Centrifugation Experiment: The highest amount of VB12 (54.1 pM) added 3.0 mL Mn^{2+}-doped ZnS QD (having absorbance of 0.3 at 320 nm) solution was centrifuged at a speed of 20000 rpm and the obtained pellet was redispersed in the same amount of solvent to record the emission spectrum (at an excitation wavelength of 320 nm) of the dispersion.

F. Selectivity experiment: The selectivity experiment was tested by using higher concentration (in µM scale) of interfering amino acids (such as tryptophan (Trp), lysine (Lys), arginine (Arg), glycine (Gly)), metal ions (such as Mg^{2+}, Ca^{2+}, K^+, and Na^+), and bovine serum albumin (BSA) protein. The aforementioned interfering substances were added to 3.0 mL solution of Mn^{2+}-doped ZnS QD (with absorbance of 0.3 at 320 nm) the emission spectra (at an excitation wavelength of 320 nm) was recorded. The change in the intensity of Mn^{2+}-doped ZnS QD (at 587 nm) was observed to define the effect of interfering substances and thus selectivity of Mn^{2+}-doped ZnS QD towards VB12 in presence of the aforementioned interfering substances.

G. Instruments: A Perkin Elmer Lambda 45 UV-Vis Spectrophotometer was used to record the UV-vis spectra. The luminescence was recorded using a Horiba Fluoromax-4 spectrofluorimeter. XRD study was performed using Brucker D2 Advance X-ray diffractometer with Cu Kα radiation at 1.5418 Å. A JEOL JEM 2100 electron microscope operating at a maximum accelerating voltage of 200 KV was used for TEM, HRTEM analysis. IFFT analysis was performed using Gatan digital software. Zeta potentials of the samples were measured by using Malvern Zetasizer Nano-ZS instrument.

H. FRET Calculation: The details of the calculation of FRET distance and energy transfer efficiency are described as follows.[4-5]

In brief, the Förster distance R_o, which is the distance at which FRET is 50% efficient, is calculated using the following equation:

$$R_o{}^6 = 8.8 \times 10^{-25} \kappa^2 \eta^{-4} \varnothing \, J(\lambda) \qquad (1)$$

where $\kappa 2$ = orientation factor between the emission dipole of Mn^{2+}-doped ZnS QD $\kappa=2/3$ for the current case) and the absorption dipole of VB12, η = refractive index ($\eta =1.33$) of the medium, ϕ = quantum yield of Mn^{2+}-doped ZnS QD in absence of VB12 ($\phi=0.07$), and J (λ) = overlap integral of the emission spectrum of Mn^{2+}-doped ZnS QD and the absorption spectrum of VB12. J (λ) is calculated using the given formula:

$$J(\lambda) = \frac{\int FD(\lambda)\epsilon(\lambda)\lambda^4 d\lambda}{\int FD(\lambda)d\lambda} \qquad (2)$$

$= 2.19213 \times 10^{14} \, M^{-1} cm^{-1} nm^4 = 2.2 \times 10^{-14} \, M^{-1} cm^3$

Where, FD(λ) = corrected emission intensity of Mn^{2+}-doped ZnS QD with total intensity normalized to unity, $\varepsilon(\lambda)$ = extinction coefficient of VB12. As per equations (2) and (3), the value of J (λ) was found to be $2.2 \times 10^{-14} \, M^{-1} cm^3$. The Fröster distance ($R_0$) was calculated to be 2.33 nm. Importantly, the energy transfer efficiency was calculated using the following equation:

$R_0^6 = 8.8 \times 10^{-25} \, k^2 N^{-4} \Phi J$
$= (8.8 \times 10^{-25} \times 2/3 \times (1.33)^{-4} \times 0.039 \times 2.19213 \times 10^{-14}) \, cm^6$
$= 1.6 \times 10^{-40} \, cm^6$
$R_0 = 2.33$ nm

$$E = 1 - \frac{F_{DA}}{F_D} \qquad (3)$$

E = 1 - 0.207 = 0.793

Where, F_{DA} = emission intensity of Mn^{2+}-doped ZnS QD in presence of VB12 and F_D = emission intensity of Mn^{2+}-doped ZnS QD in absence of VB12. The FRET efficiency was estimated to be 79.3%.

$$E = \frac{1}{1+(r_0/R_0)^6} \qquad (4)$$

Where, r_0 = distance between Mn^{2+}-doped ZnS QD and VB12. The value of r_0 is estimated to be 1.86 nm.
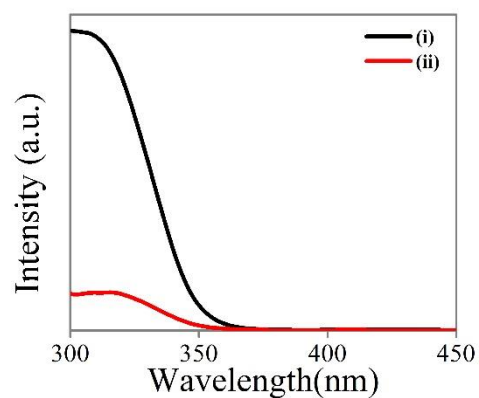
Fig. S1. Excitation spectra of (with emission wavelength fixed at 587 nm) of Mn^{2+}-doped ZnS QDs and (ii) VB12 added Mn^{2+}-doped ZnS QDs.
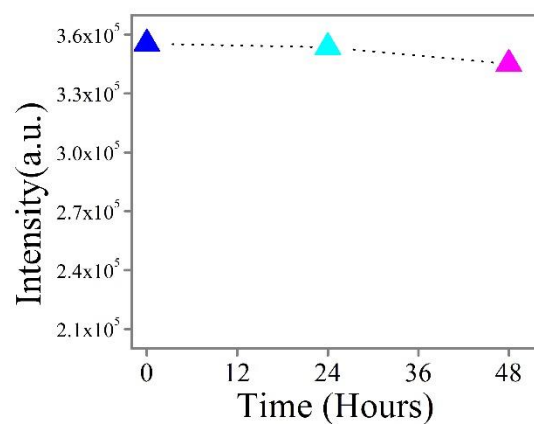

Fig. S2. Luminescence stability of Mn^{2+}-doped ZnS QDs (in water dispersion; having absorbance of 0.3 at 320 nm at 25 °C) for 48 hours.

Table S1. Quantum yield of Mn^{2+}-doped ZnS QDs.

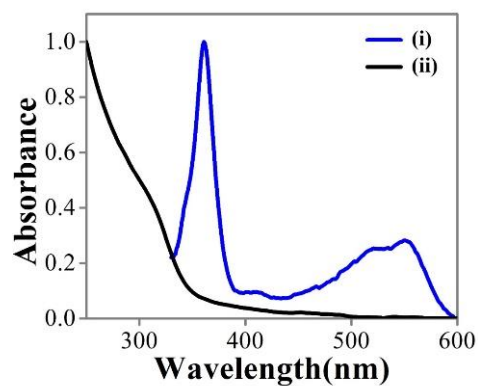| Samples	QY	Reference
Mn^{2+}-doped ZnS QDs	0.07	Quinine sulphate in 0.1 M H_2SO_4

Fig. S3. No spectral overlap between the (i) UV-vis spectrum of VB_{12} and (ii) UV-vis spectrum of Mn^{2+}-doped ZnS QDs. There is no overlap between the two spectra.
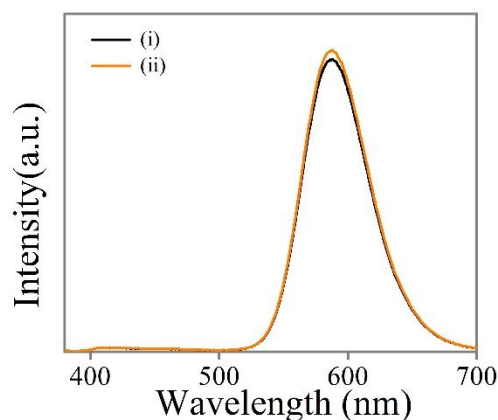

Fig. S4. Emission spectra (λ_{ex} = 320 nm) of (i) Mn^{2+}-doped ZnS QDs (pH=5.9) and (iii) Mn^{2+}-doped ZnS QDs at pH=6.09, the pH of which adjusted to VB12 added Mn^{2+}-doped ZnS QDs.
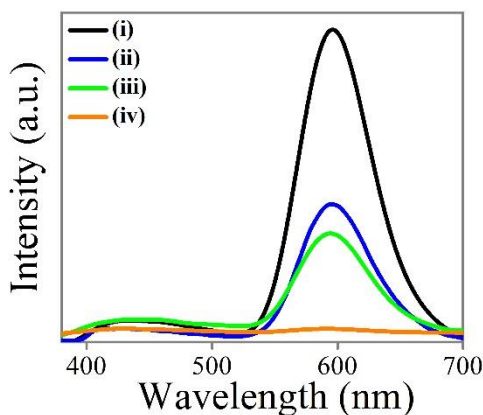

Fig. S5. Emission spectra (λ_{ex} = 320 nm) of (i) Mn^{2+}-doped ZnS QDs (ii) highest amount of VB12 added Mn^{2+}-doped ZnS QDs, (iii) pellet (following centrifugation and redispersion into same amount of solvent) and (iv) supernatant (after centrifugation) of highest amount of VB12 added Mn^{2+}-doped ZnS QDs.
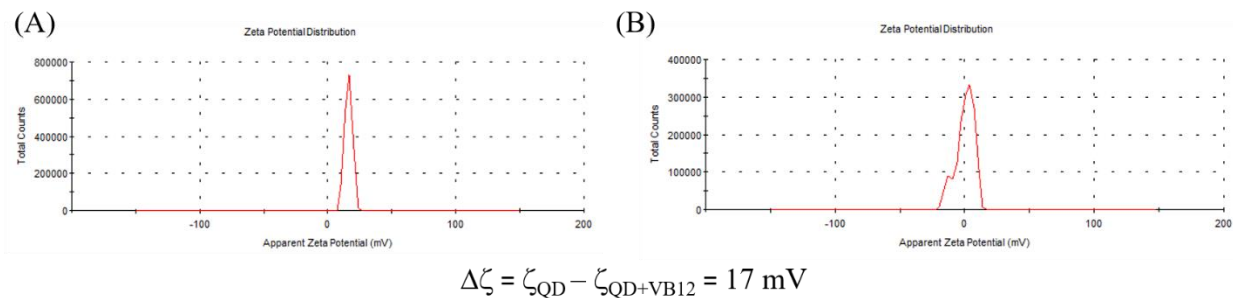
$$\Delta\zeta = \zeta_{QD} - \zeta_{QD+VB12} = 17 \text{ mV}$$

Fig. S6. Zeta potential curves of (A) Mn^{2+}-doped ZnS QDs and (B) VB12 added Mn^{2+}-doped ZnS QDs.

References.
1. S. Pramanik, S. Bhandari, S. Roy and A. Chattopadhyay, *J. Phys. Chem. Lett.* 2015, **6**, 1270-1274.
2. S. Pramanik, S. Roy, A. Mondal and S. Bhandari, *Chem. Commun.*, 2019, **55**, 4331-4334.
3. S. M. Shet, M. Bisht, S. Pramanik, S. Roy, S. Kumar, S. K. Nataraj, D. Mondal and S. Bhandari, *Adv. Opt. Mater.* 2020, 1902022.
4. A. Jaiswal, P. Sanpui, A. Chattopadhyay and S. S. Ghosh, *Plasmonics* 2011, **6**, 125.
5. P. Maity, T. Gayathri, S. P. Singh, and H. N. Ghosh, *Chem. Asian J.* 2019, **14**, 1688.