Supplementary Material (ESI) for *Nanoscale*

Electronic supplementary information

Highly fluorescent magnetic quantum dot probe with superior colloidal stability[#]

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Experimental Procedure:

Synthesis:

High quality hydrophobic CdSe-ZnS and γ-Fe_2O_3 have been synthesized according to published method.[3,4] For the synthesis of CdSe-ZnS, CdSe of different size has been synthesized first by high-temperature carboxylate precursor root in octadecene solvent. Next, ZnS shell was grown at 200°C in octadecene via the alternate injection of Zn stearate and elemental S. Hydrophobic γ-Fe_2O_3 was synthesized by high temperature pyrolysis of iron carboxylate in octadecene in presence of long chain amine/acid as capping ligand and methyl morpholine N-oxide as oxidizing agent. After synthesis all the particles have been purified from free surfactants using the standard precipitation-redispersion method and finally dissolved in cyclohexane.

MQD has been synthesized via reverse micelle based optimized polyacrylate coating chemistry developed for the synthesis of functionalized quantum dot/Au/Ag nanoparticles.[5a] The only difference is that two hydrophobic nanoparticles (CdSe-ZnS and γ-Fe_2O_3) have been introduced in the reverse micelle instead of one nanoparticle. In a typical synthesis, 10 mL of Igepal-cyclohexane reverse micelle has been prepared by mixing 1 mL Igepal into 9 mL of cyclohexane and then 2 mL cyclohexane solution of CdSe-ZnS (16 mg/mL) and 0.3 mL cyclohehane solution of γ-Fe_2O_3 (70 mg/mL) have been added to it. Next, 13 mg N-(3-aminopropyl) methacrylamide hydrochloride (dissolved in 100 μL water), 36 μL poly (ethylene glycol) methacrylate (dissolved in 100 μL water) and 3 mg methylene bis acryleamide (dissolved in 200 μL water by 10 minutes sonication) have been mixed and optically clear solution has

been formed. This solution has been taken in a three naked flask and put under oxygen free atmosphere by purging nitrogen for 15 minutes and finally, ammonium persulfate solution (3 mg dissolved in 100 μL water) has been injected as radical initiator to start the polymerization. The polymerization has been continued at room temperature for one hour and then particles have been precipitated by adding minimum ethanol and are washed with ethanol 3-4 times. Finally particles have been solubilized in water. Quantum yield (QY) was measured using integrated fluorescence intensity of CdSe-ZnS and reference (for fluorescein QY = 97% and for rhodamine 6G QY = 100%). The magnetization (M) measurements were carried out at different temperature using a superconducting quantum interference device (SQID) magnetometer.

Functionalization and labeling study:

Oleyl functionalization has been achieved using glutaraldehyde as conjugation reagent as reported earlier.[5a] First, glutaraldehyde conjugate of oleylamine has been prepared in ethanol by mixing them in 1:1 molar ratio and after 15 minutes they were treated with amine functionalized MQD solution in carbonate buffer of pH 10.00. After one hour of reaction this solution was mixed with 100 μL of 0.2 M $NaBH_4$ solution to reduce the imine bonds formed by reaction between aldehyde and amine. Next, solution has been dialyzed overnight against distilled water using 12–14 kDa molecular weight cut-off (MWCO) membrane. Vancomycin functionalization of MQD has been performed by N-(3-dimethylaminopropyl-N-ethylcarbodiimide (EDC) coupling chemistry.[8] A 2 mL amine functionalized polymer coated MQD solutions has been mixed with 0.2 mL of aqueous solution of vancomycin (2 mg/mL) and then pH has been adjusted to 6.0 using MES (4-morpholineethanesulfonic acid) buffer solution. Next, aqueous EDC (0.1 M) and N-hydroxysuccinimide (NHS) (0.1 M) have been prepared separately and 0.2 mL of each solution has been added to MQD solution and incubated overnight. The resulting solution has been dialyzed overnight to remove excess reagents and preserved at 4°C.

COS-7 cell lines have been sub-cultured in 24 well cell culture plates for overnight so that cells were attached on well plate. Next, they were mixed with 100 μL of oleyl functionalized MQD solution and kept at incubation chamber for 1-2 hs. Next, they were washed with PBS buffer solution for 3 times and finally PBS buffer or cell culture medium was added for imaging study. EAC cell have been collected from peritoneal cavity of adult female mice and 1 mL of this cell suspension (~ 10^7 cell /mL) has been mixed with 100 μL of oleyl functionalized MQD solution. After 30 minutes labeled cells were separated from unbound MQD via centrifuging at 2000 rpm, resuspended in PBS buffer solution and used for imaging and separation study. Bacillus subtilis has been taken and inoculated in nutrient agar medium. After overnight growth, the bacteria slant was dissolved with NaCl solution and the fresh bacterial culture solution has been diluted with a final concentration of ~1.22 $\times 10^8$ CFU/mL. One mL of this bacterial suspension has been mixed with 100 μL of vancomycin functionalized MQD solution and after 30 minutes of incubation labeled bacteria has been separated from unbound MQD via centrifuging at 2000 rpm, resuspended in PBS buffer solution and used for imaging and separation study.

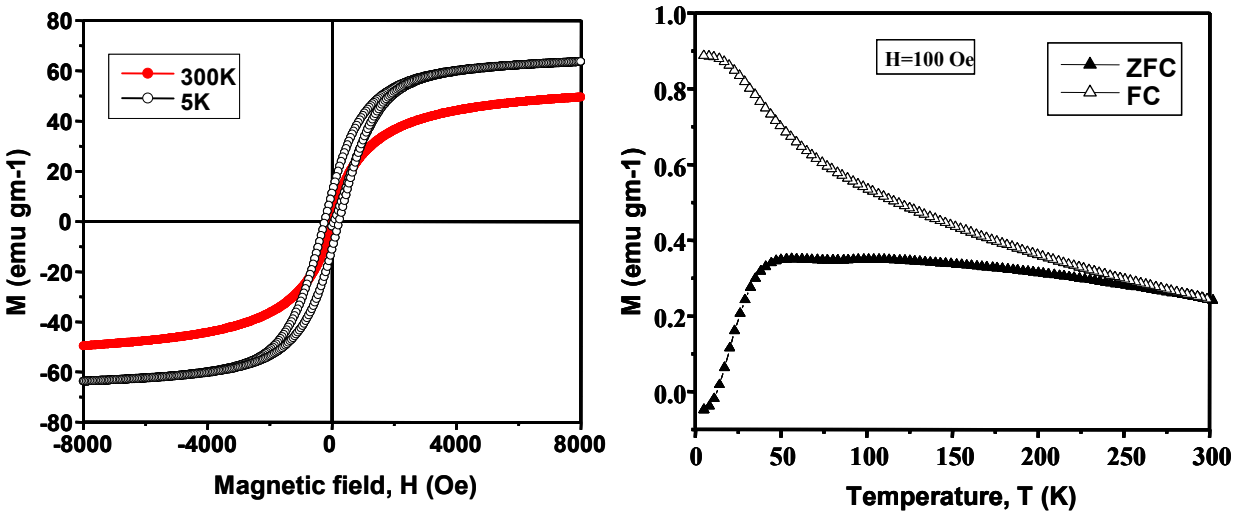

Figure S1. Magnetic property of 10 nm hydrophobic γ-Fe_2O_3. Left panel shows the hysteresis loop at 5 and 300 K. Right panel shows temperature dependent zero-field-cooled (ZFC) and field-cooled (FC) magnetization curves.

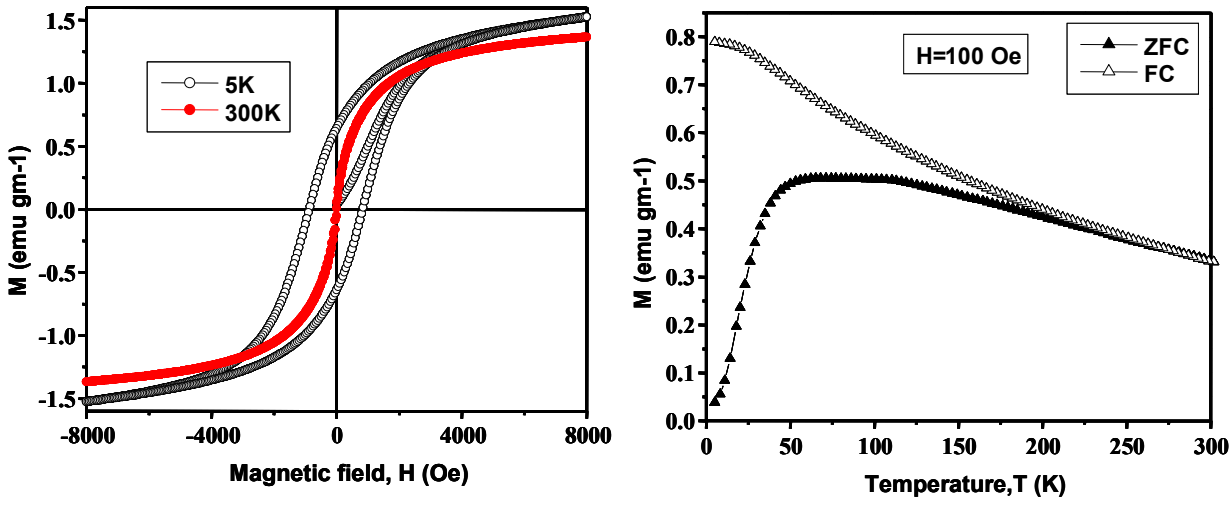

Figure S2. Magnetic property of MQD made of 10 nm γ-Fe_2O_3 and red emitting CdSe-ZnS. Left panel shows the hysteresis loop at 5 and 300 K. Right panel shows temperature dependent zero-field-cooled (ZFC) and field-cooled (FC) magnetization curves.

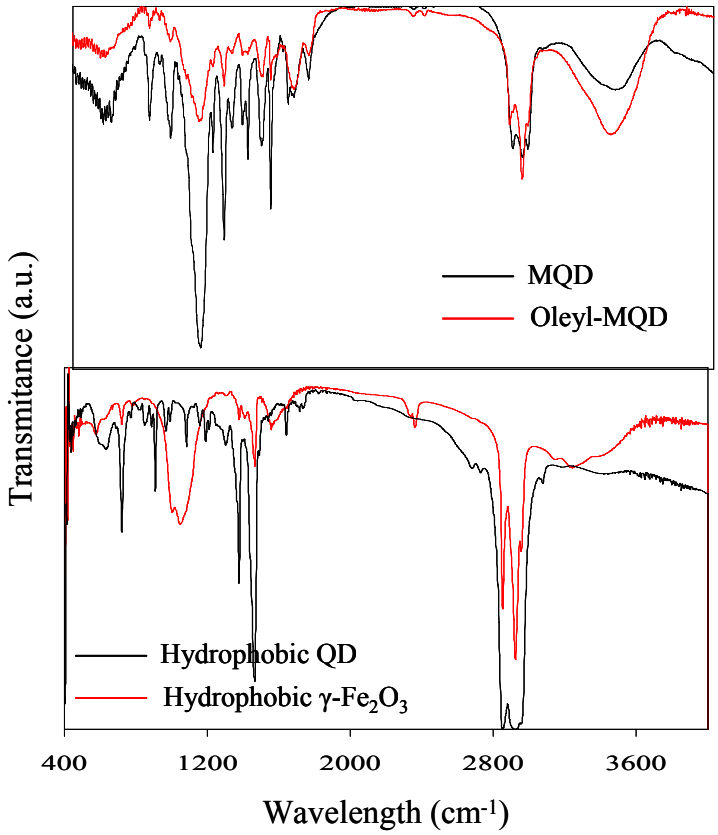

Figure S3: FTIR spectra of hydrophobic QD, hydrophobic γ-Fe_2O_3, MQD and MQD after functionalization with oleyl group. Hydrophobic particle shows strong peaks at 2920-2850 cm^{-1} and 1300-1600 cm^{-1} due to stretching and bending vibrations of CH_3 and CH_2 coming from coated

hydrophobic surfactants. Those peaks are weak in MQD after polymer coating, with the appearance of peak of primary amine group at 1525 cm^{-1}. After oleyl functionalization in MQD the primary amine peak at 1525 cm^{-1} becomes weaker with the appearance of stronger band at around 3100 cm^{-1} due to oleyl group.

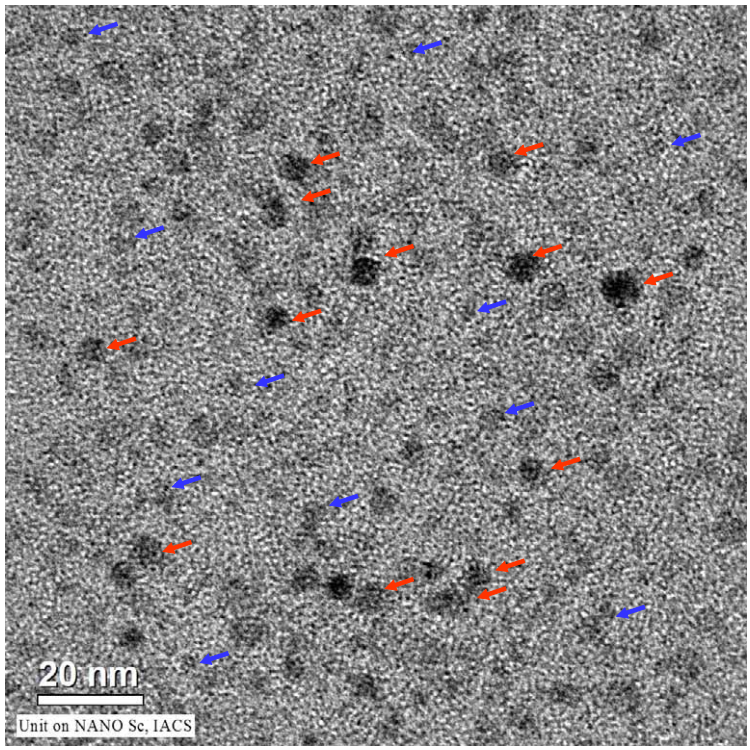

Figure S4. Representative transmission electron microscopic (TEM) image of MQD structure showing the presence of both 5-6 nm size γ-Fe_2O_3 (red arrow) and 3 nm size green CdSe-ZnS component (blue arrow).

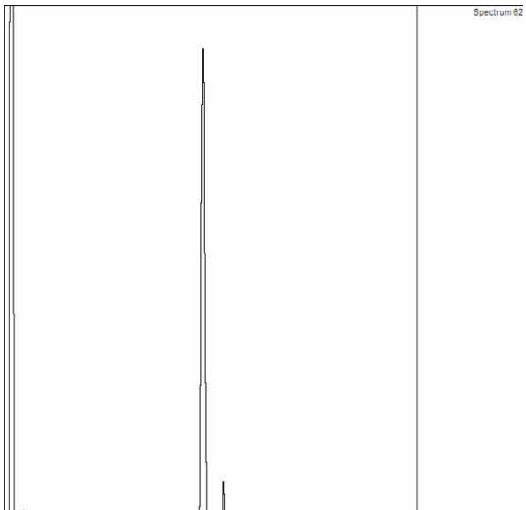

Figure S5. Energy-dispersive X-ray (EDX) spectra of MQD. Presence of Fe, Cd, Zn, Se peaks suggest the composite nature of nanoparticle.

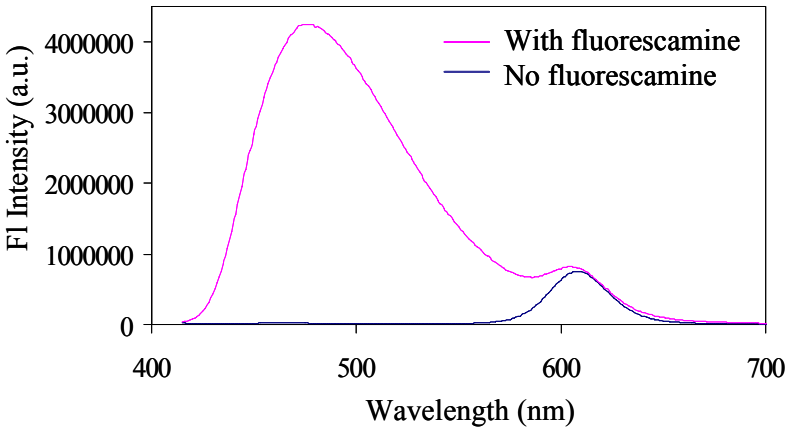

Figure S6. Results of fluorescamine test to determine primary amine on the surface of MQD. Appearance of strong fluorescence peak at ~485 nm indicates the presence of primary amine.

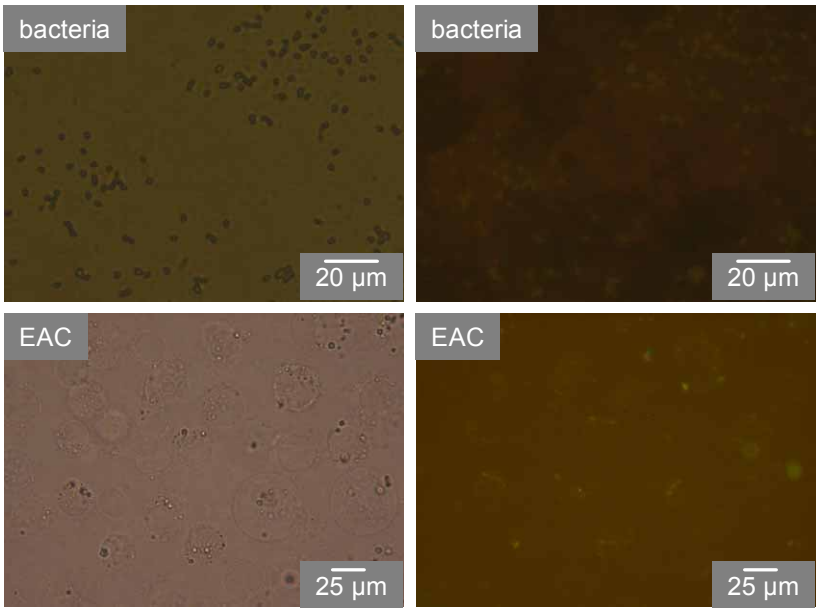

Figure S7. Labeling study with as synthesized water soluble MQD without any oleyl/vancomycin functionalization. Labeled cells/bacteria are imaged under bright field (left panels) and fluorescence mode (right panels). Insignificant fluorescence signal indicates no labeling.