Supporting Information

Aqueous Based Ultra-Small Magnetic Cr doped CdSe Quantum Dots as a Potential Dual Imaging Probe in Biomedicine

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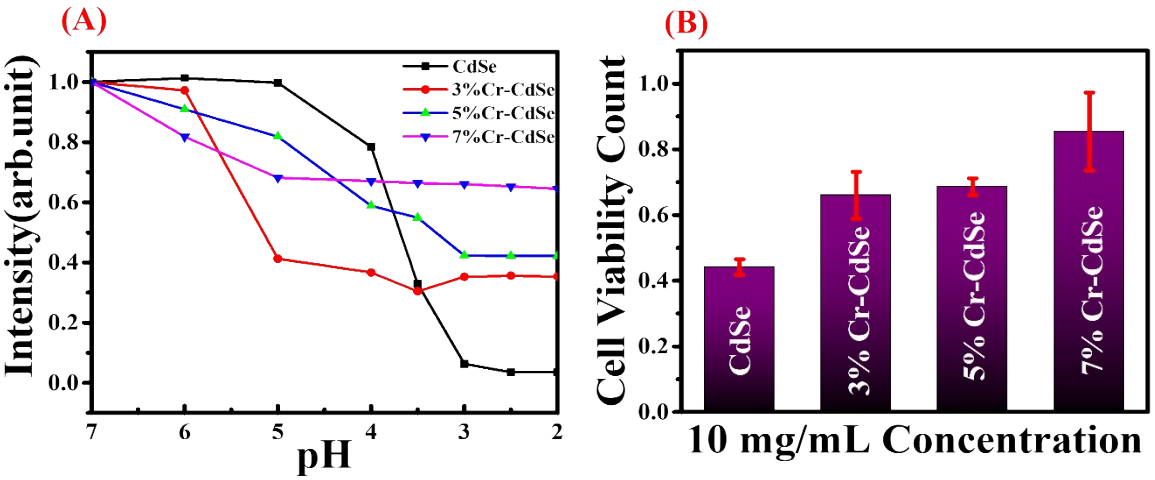
1. Methodology

1.1 Cell uptake study

All cell culture reagents were obtained from Gibco (New York, USA) unless otherwise stated. HEK293T cells of passage 20 were used for all the uptake experiments. Briefly, 50,000 cells were seeded on coverslips in 24-well tissue culture plates and maintained in complete Dulbecco's Modified Eagle Medium (DMEM), comprising 1X DMEM, 10% Fetal Bovine Serum (FBS) and 1% Penicillin-Streptomycin (P/S) for 24 hours. Following that, 1mg/ml of either CdSe, 3% Cr-CdSe, 5% Cr-CdSe or 7% Cr-CdSe were added to the respective wells and incubated for 24 hours. Following the 24-hour incubation, the QD-containing media was aspirated, and the wells were washed gently thrice with 1X Phosphate-buffered saline (PBS). The cells were then fixed using 3.7% paraformaldehyde (Nacalai-Tesque, Kyoto, Japan) and incubated at 37℃ for 15 minutes. The cells were washed again thrice with 1X PBS following removal of the paraformaldehyde. The coverslips containing cells were then mounted using Prolong antifade gold mounting medium (Thermo Fisher Scientific, Waltham, MA, USA), and



stored at -20℃ till imaging with confocal microscope.

Figure S1: The stability and MTT study of Cr doped CdSe at different concentration (0%,35,5%,75). **Figure S1(A)** shows the stability of Cr doped CdSe at different pH from 2-7 and it observed that compared to undoped sample (CdSe), doped samples (Cr-CdSe) show high stability in acid medium. **Figure S1(B)** shows the MTT assay performed on HEK293T cells recorded after 4 hours incubation and observed that Cr doping increases the no. of cell viability increases.

1.2 MTT Study

For cell viability assays, HEK293 cells were used. In brief, 15,000 cells were seeded in 100uL per well in a 96-well cell culture plate. All the cells were grown in complete growth medium (1X Dulbecco's Modified Eagle Medium, 10% fetal bovine serum, 1% penicillin-streptomycin) (Gibco, Thermofisher, NY, USA). After cells were adhered to the wells for about 24 hours, 1mg/mL of either CN1, CN2, CN3 or CN4 were added to the wells. The nanoparticles were incubated with the cells for 4 hours, before performing the MTT viability assay. After the incubation, nanoparticles were removed and replaced with 0.5mg/mL MTT dye diluted in complete growth medium. After 2 hours of incubation with the MTT, it was removed, and the formazan crystals formed were solubilized with pure DMSO (Sigma Aldrich, MO, USA). The absorbance readings of the wells were taken with the Synergy microplate reader at 570nm.

1.3 *In Vivo* imaging

C.elegans N2 strain worms were grown and maintained in Nematode Growth Medium (NGM) plates (Agar, sodium chloride, peptone, sterilized by autoclaving for 50 minutes and added the working concentrations -0.025 M KPO_4, 0.001 M $MgSO_4$, 0.001 M $CaCl_2$, and 5 µg/mL) inoculated with *E.coli* strain OP50 as food source. The worms were synchronized by bleaching and the synchronized L1 stage worms were seeded onto a 90 mm OP50 petri-plate. After reaching the adult stage worms were washed from the plates with the help of M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 ml 1 M $MgSO_4$, H_2O to 1 L and sterilized by autoclaving in order to remove the bacteria). The washed worms were incubated with 100 µL of various concentrations of the nanoparticles on a rotator. After the incubation the worms were washed with M9 buffer to remove excess nanoparticles. The worms were fixed with 4% paraformaldehyde for 5 minutes and again washed with M9 buffer. The fixed worms were placed on a 3% agarose pad soaked in M9 buffer and covered with coverslip. Following which the worms were visualized, and images were taken with Nikon Eclipse Ti2 with 10X objective, and the images were processed with the NIS Elements AR software (Nikon).

1.4 MRI Method

50 µL of each sample was mixed with 50 µL of 1% agarose to prepare serially diluted Cr-CdSe samples. Similarly, HEK293T cell pellets incubated for 4 h with Cr-doped CdSe semiconductors were redissolved in PBS buffer and mixed with equal volume of 1% agaose to

prepare samples for relaxation measurements. All samples were siphoned into glass capillaries and left at room temperature to solidify prior to imaging. The capillaries with different concentrations for each sample were fixed in 30 mm NMR tubes for scanning through MRI. 1 mm thick slices for each sample were identified using gradient-enhanced localizer scan. Rapid Acquisition with Relaxation Enhancement (RARE) experiments were conducted with variable repetition time to plot T1 data for different concentrations. The magnetization signal was plotted against repetition time to calculate T1 relaxation times at each concentration. 1/T1 was plotted against the sample concentration to calculate relaxivity values for different samples. Each scan was acquired as a 256 x 256 pixelated image.

2.Equations and Tables

2.1 Tauc's plot Equation

The bandgap of synthesized magnetic doped semiconductors can be calculated using Tauc's equation given by

$$(\alpha.h\nu)^{1/\gamma} = \mathbf{B\ (h\nu - E_g)}$$

Were, h – Planck constant,

α-absorption coefficient,

ν- photon frequency,

Eg- bandgap,

B- constant and

γ-electron transition factor

2.2 Lifetime Equation

To calculate the decay time of the magnetic semiconductor using given equation

$$(\tau 1 \times a1) + (\tau 2 \times a2) + (\tau 3 \times a3)/100$$

Were, τ1-radiative recombination.

τ2- non-radiative recombination

τ3- auger recombination and

a1, a2, a3 are respective percentage of the recombination.

2.3 Debye's Scherrer Equation

The crystalline grain size is calculated using Debye's Scherrer equation.

$$D = k\lambda/\beta\cos\theta$$

Were, D- crystallin grain size.

k- Scherrer constant

λ- wavelength of x-rays

β- Full width at half maximum

θ- diffraction angle

The lattice strain of the Cr-CdSe samples is calculated using below equations

$$\varepsilon = \beta/4\tan\theta$$

Were, ε- lattice strain

β- Full width at half maximum

θ- diffraction angle

2.4 Mean fluorescence quantification

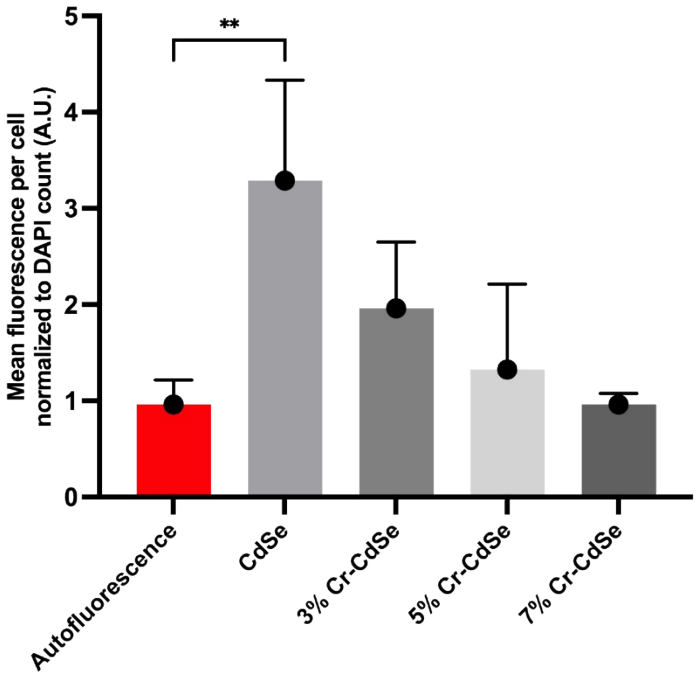


Figure S2: Mean fluorescence quantification compared with auto fluorescence as Cr doping increases.

Figure S2 demonstrate the fluorescence quantification (normalized to number of cells as determined by DAPI count) of the compounds as compared to auto fluorescence (no compound treatment). From the attached Figure S2 it was observed that the mean fluorescence was decrease to 7% Cr-CdSe when compared to 0% Cr-CdSe. 3% Cr-CdSe and 5% Cr-CdSe followed the trend. The decrease in the mean fluorescence is due to the introduction of more traps states which leading to more non-radiative recombination Additionally, the trends in mean fluorescence of Cr doped CdSe after cellular uptake and PL property of the magnetic semiconductor outside of the cell are in good agreement. The standard equation used for fluorescence quantification was taken from the raw pixel data obtained from the ImageJ software.

Mean fluorescence normalized to total cell number (DAPI count) (relative fluorescence units) = Total mean fluorescence/number of cells.

Sample	Crystalline Grain Size (nm)	Lattice Strain ε
CdSe	0.74	0.2
3%Cr-CdSe	0.67	0.22
5%Cr-CdSe	0.62	0.24
7%Cr-CdSe	0.56	0.26

Table S1. Grain size calculation of Cr-CdSe magnetic semiconductor.

3. Instrumentation

3.1 UV-Visible spectroscopy: Absorbance and PL Measurements were recorded through the TECAN spark M model microplate reader. By using XENON lamp source, the absorbance and fluorescence intensity scan modes with greiner-96 pate.

3.2 Time resolved Photoluminescence: Time resolved photolumiscence were carried out using time correlated single photon counting by TCSPC instrument (Horiba Jobin Y von IBH)

using laser diode (Delta Diode-425L) peak power 230mW as excitation source with wavelength at 420nm. IBH DAS6 software is used for decay analysis.

3.3 Powder X-ray diffraction: Powder x-ray diffraction measurement is recorded using Empyrean PANalytical X-ray diffractometer with Cu-Kα x-radiation of wavelength 1.5406Å at 40kV and 30mA power. The sample were prepared by using drop casting the Cr doped CdSe solution on cleaned 2 × 2 cm glass substrate and using High score 4.1 software to analysis XRD data.

3.4 X-ray photoelectron spectroscopy: The surface composition of sample measured using X-ray photoelectron spectroscopy (XPS) was measurement using Mg Kα (1253.6eV) radiation (PHI VersaProbe III). The measurement was supported with detection angle of 45° C, the energies pass through analyzer for survey spectra of 55eV and detailed spectra of 280eV. The charging effects of sample were neutralized with electrons from a flood gun of current 20μA and the surface spot size of the sample is 5 × 5μm for XPS survey.

3.5 Transmission Electron Spectroscopy: The JEOL-JEM 2100 high resolution transmission electron microscope to measure the bright filed TEM images under high voltage 200kV. The Cr doped CdSe sample of 5-10μL were drop casted on the copper grid (200 mesh, from TED PELLA) and allowed to dry. ImageJ software is used to analysis the TEM images.

3.6 Confocal Microscopy: The Zeiss laser scanning microscope 800 (LSM 800) was used for all confocal imaging studies. Briefly, the 63X oil magnification was used to image all the slides. The 561nm laser line was used to image all the cells treated with CdSe and doped CdSe samples. All scans were taken at 0.5X zoom, with double averaging, and 6 scan speed. Both brightfield and fluorescent images were captured for all samples.

3.7 Magnetic Resonance Imaging: The MRI experiments were conducted on a Bruker Avance III HD 600 MHz (14.1 T) NMR spectrometer fitted with a MicWB40 microimaging probe in combination with the Micro2.5 Gradient System. A quadrature coil with an inner diameter of 30 mm (MICWB40 RES 600 1H 040/030) was used to transmit/receive the MR signals.