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Supporting Information

Highly Water-Soluble Magnetic Iron Oxide (Fe_3O_4) Nanoparticles for Drug Delivery: Enhanced *InVitro* Therapeutic Efficacy of Doxorubicin and MION Conjugates

Muhammad Irfan Majeed[†], *Qunwei Lu*[§], *Wei Yan*[*†¶], *Zhen Li*[±], *Irshad Hussain*[*‡], *Muhammad Nawaz Tahir*[ʊ], *Wolfgang Tremel*[ʊ] *and Bien Tan*[†*]

[†]School of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, Wuhan 430074 (P.R. China), [§]School of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, (P.R. China), [±]Institute for Superconducting and Electronic Materials, Australian Institute of Innovative Materials, University of Wollongong, NSW 2500 (Australia), [‡]Department of Chemistry, LUMS School of Science and Engineering (SSE), Lahore 54792 (Pakistan), [ʊ] Institute for Inorganic and Analytical Chemistry, University of Mainz, Duesbergweg 10-14, 55128 Mainz, Germany, ¶ Faculty of Materials Science and Engineering, Hubei University, Wuhan 430062, P.R. China.

AUTHOR EMAIL ADDRESS: bien.tan@mail.hust.edu.cn;

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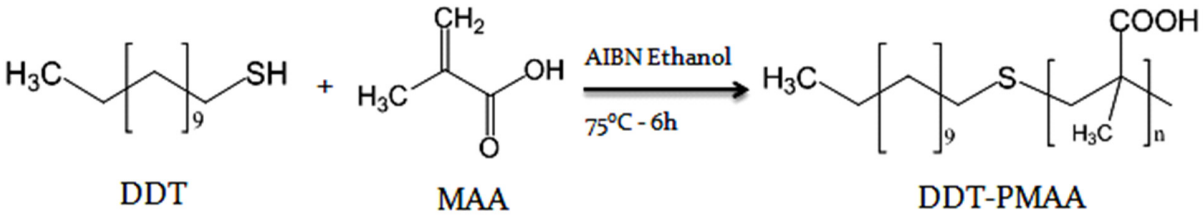
SI-1: Synthesis and Characterization of Polymer Ligand.

A series of polymer ligand DDT-PMAA, with four different molecular weights, was synthesized using (Dodecanethiol, DDT) as chain transfer agent by free radical polymerization of monomer methacrylic acid (MAA) as described previously. (Scheme S1) represents the polymer synthesis process. GPC elution curves of four different polymer samples are shown in (Fig. S1) and their molecular weights given in Table S1. As expected, the molecular weight of the polymer was decreased with the increase in concentration of chain transfer agent.



Scheme S1. Polymer Synthesis Process

DDT-PMAA was synthesized by the chain transfer method using thiol as chain transfer agent as described in previous works.[1, 2] Molar ratio of monomer to chain transfer agent was altered in order to prepare four different molecular weight polymer samples. In a typical preparation, for

0.5%DDT-PMAA, methacrylic acid (MAA, 5 g, 58 mmol), dodecanethiol (DDT, 0.05 g, 0.29 mmol) and 2,2´-azobisisobutyronitrile (AIBN, 0.095 g, 0.58 mmol) were added to EtOH (25 mL) in a three-necked round-bottomed flask, equipped with a reflux condenser and mechanical stirrer. The temperature of the reaction mixture was maintained at 75 °C for 5 h under Nitrogen with vigorous stirring. At the end of this period the reaction mixture was left to cool down to room temperature and then the products were isolated by precipitation into cold diethyl ether. The polymer was collected by filtration on a Buchner funnel, and the solvent and monomer residues were removed by evaporation to constant mass using a vacuum oven set at 45 °C. A fraction of low molar mass polymer, un-reacted monomer and some oligomers remained after reaction has been removed during the precipitation step. The yield obtained was 92% for this reaction. The other three polymer fractions 0.5%DDT-PMAA, 5%DDT-PMAA and 10%DDT-PMAA were prepared in the same way with yields 85, 71 and 48% respectively. The yield of the reactions was gradually lowered down which can be attributed to the higher level of chain transfer agent used.

SI-2: Characterization of Polymer Ligand.

^{1}HNMR Spectroscopy. ^{1}HNMR spectra were recorded on a 400 MHz Bruker AV400 spectrometer using d6-DMSO as a solvent in a 5mm quartz NMR tube at room temperature using the δ scale. The ^{1}HNMR spectra were consistent to the previous works.[1, 2] The molecular weights of the polymer ligands were calculated based on the ratio of monomer units attached to the terminal group in the ^{1}HNMR spectra and compared with molecular weights determined by GPC (data not given). Chemical structure of the polymer was confirmed by ^{1}HNMR spectroscopy and data as given under was consistent with our previous work. [1, 2]

DDT-PMAA (d_6 DMSO) δ (ppm): 0.9(b) CH_3, 1.24 $(CH_2)_9$ 1.7(b) CH_2 (backbone), 2.4 CH_2 (from DDT), 12.3(b) COOH

Gel Permeation Chromatography. The as synthesized polymer was soluble in EtOH, MeOH, H_2O and DMSO. In order to determine the molecular weight of all the four polymer ligands by GPC they were transferred to the THF by converting them into methyl esters using TMS-Diazomethane reagent according to the previous work.[3-5] GPC was performed with an Agilent 1100 instrument using refractive index detector (RID) and THF was used as eluent at a flow rate of 1.0 mL/min at 23 °C. The calculated molecular weights were based on a calibration curve for polystyrene standards of narrow polydispersity (Polymer Laboratories).

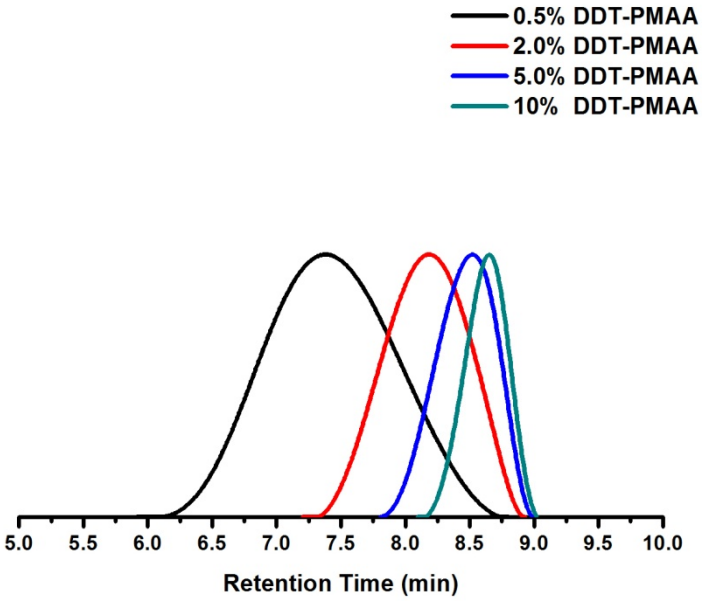


Fig. S1 Normalized GPC curves of DDT-PMAA synthesized using different monomer to DDT molar ratios.

Table S1. DDT-PMAA Ligands with Four Different Molecular Weights.

		Molecular Weights (g/mol)				
Sample #	**MAA/DDT (mol/mol)**	***GPC M_n***	***GPC M_w***	**Corrected M_n**	**PDI**	**Recovered Yield (%)**
1	100/0.5	16,970	25,930	14,624	1.52	92
2	100/2	6,165	9,008	5,330	1.46	85
3	100/5	3,598	4,754	3,123	1.32	71
4	100/10	2,619	3,154	2,281	1.20	48

SI-3: Synthesis of MIONs with Polymer Ligand.

Experimental Details. Briefly in a 500 mL four necked round bottom flask equipped with reflux condenser, thermometer and nitrogen supply, 300 mL of Milli-Q water was added. The water was purged with nitrogen gas to remove oxygen and was heated to reflux in oil bath with magnetic stirring. When temperature reached at 80 °C the polymer ligand (2%DDT-PMAA) was introduced to flask to make the aqueous solution of polymer ligand (1.5 mM pH = 4). When the temperature reached at 100 °C, the iron precursor solution comprising of $FeCl_3.6H_2O$ (1.62 mmol) and $FeSO_4.6H_2O$ (3.24 mmol) in 6 mL of conc. HCl was added and the 90 mL of conc. NH_4OH was added within 5 sec. Upon addition of iron precursor solution, the color of the reaction mixture became yellow and upon addition of ammonia solution the color of the reaction mixture turned to dark black suddenly indicating the formation of iron oxide NPs. The temperature of the reaction mixture dropped to 85 °C upon the addition of iron precursor and ammonia addition and it took ~15 min to raise the temperature to 100 °C again and then the reaction was allowed to continue for 2 h at this temperature with vigorous magnetic stirring and constant nitrogen bubbling. After 2 h the heating was stopped and reaction mixture allowed cooling down to room temperature under nitrogen and then solvent was removed by rotary evaporator and solution concentrated to 60 mL and dialyzed against Milli-Q water for 72 h using dialysis membrane with molecular weight cut-off value 14000 kDa. The dried NPs powder was obtained by evaporation of dialyzed NPs black suspension using rotary evaporator, washing with acetone and then drying in vacuum oven to a constant weight. The yield obtained was ~94%.

SI-4: Characterization of MIONs Prepared with Polymer Ligand.

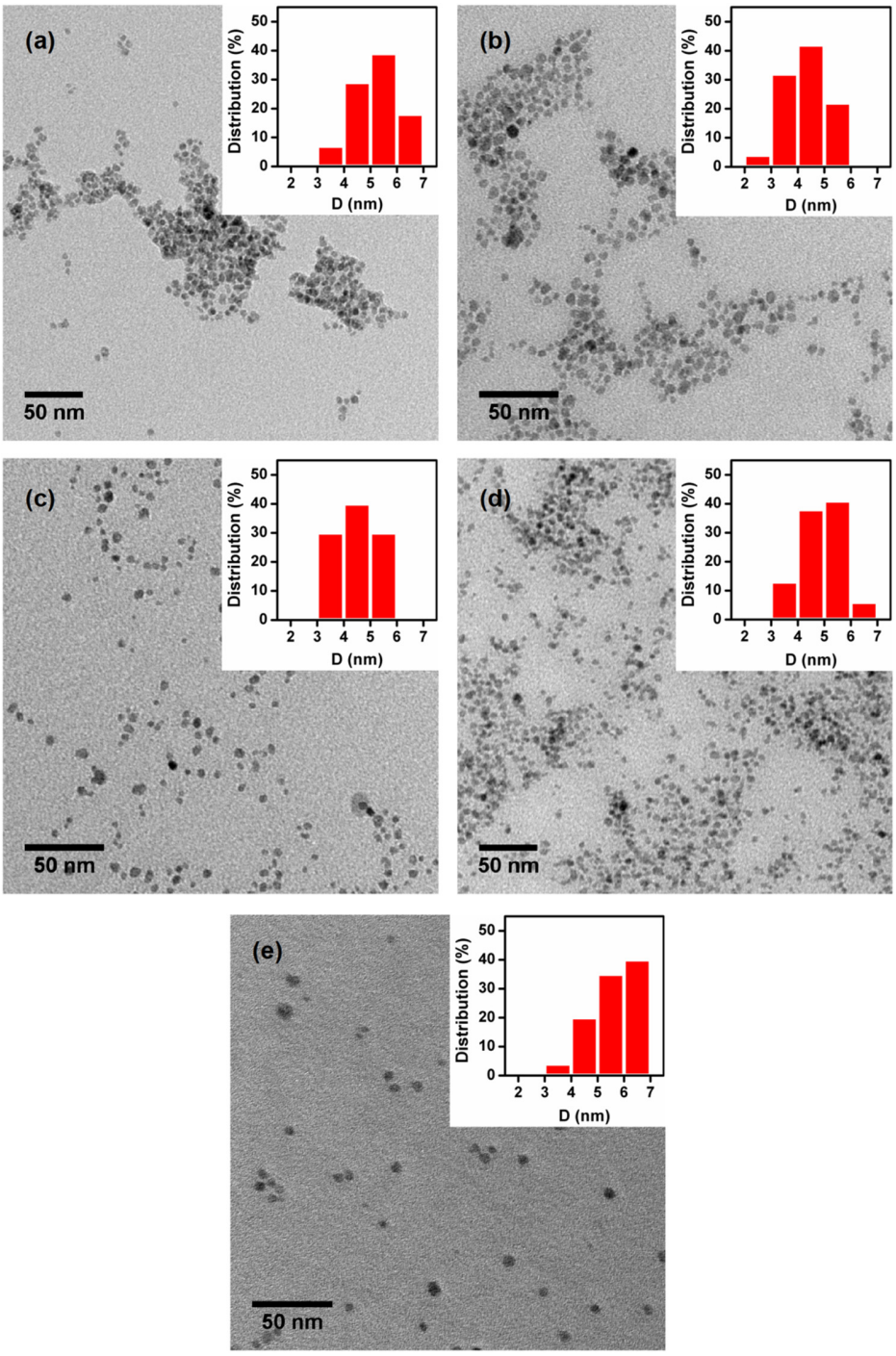


Fig. S2 TEM images with size distribution histograms of MIONs prepared with 0.5%DDT-PMAA, a, b, c, d and e for 0.25, 0.5, 1, 1.5 and 2 mM of polymer ligand respectively.

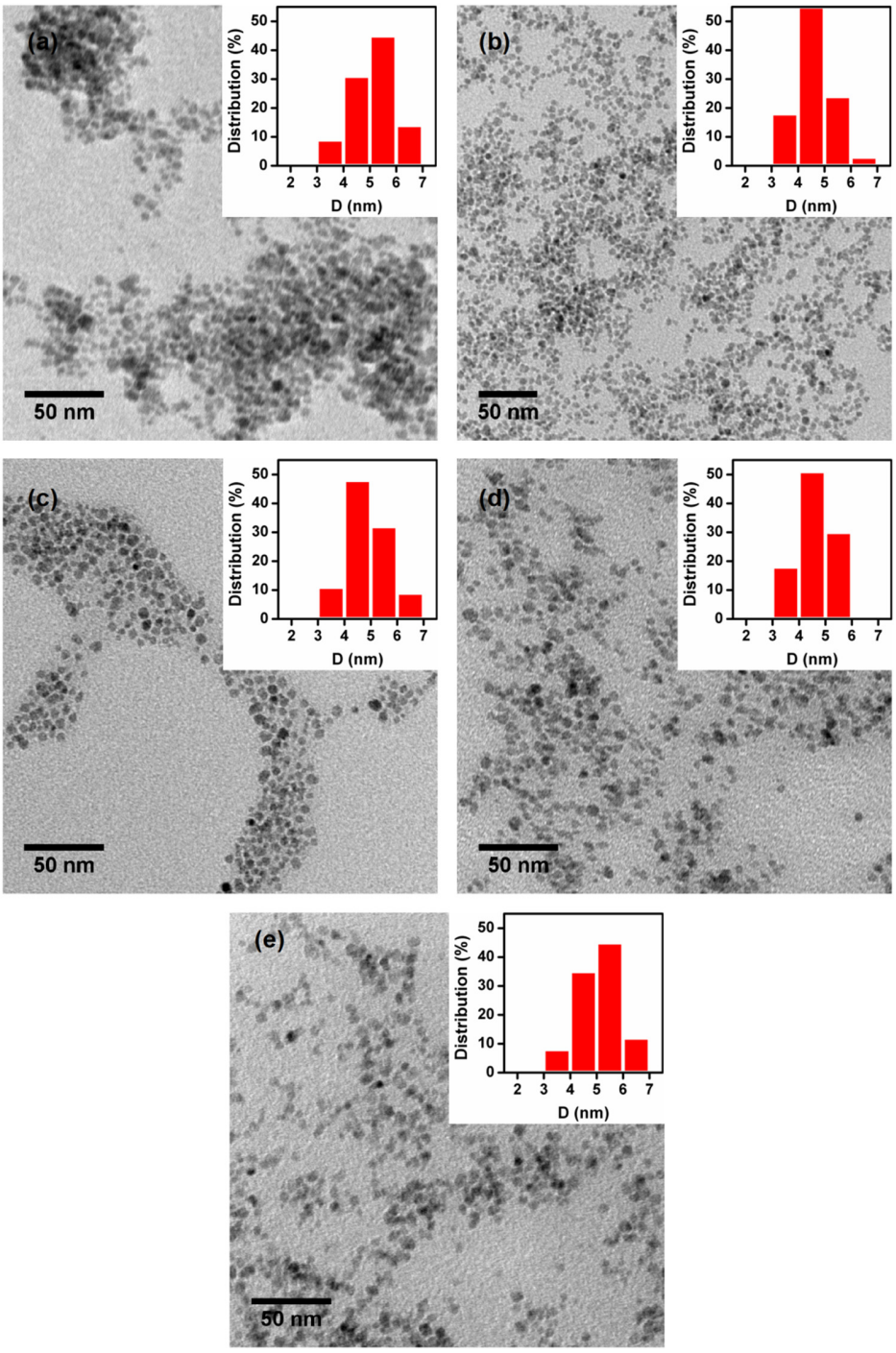


Fig. S3 TEM images with size distribution histograms of MIONs prepared with 2%DDT-PMAA, a, b, c, d and e for 0.25, 0.5, 1, 1.5 and 2 mM of polymer ligand respectively.

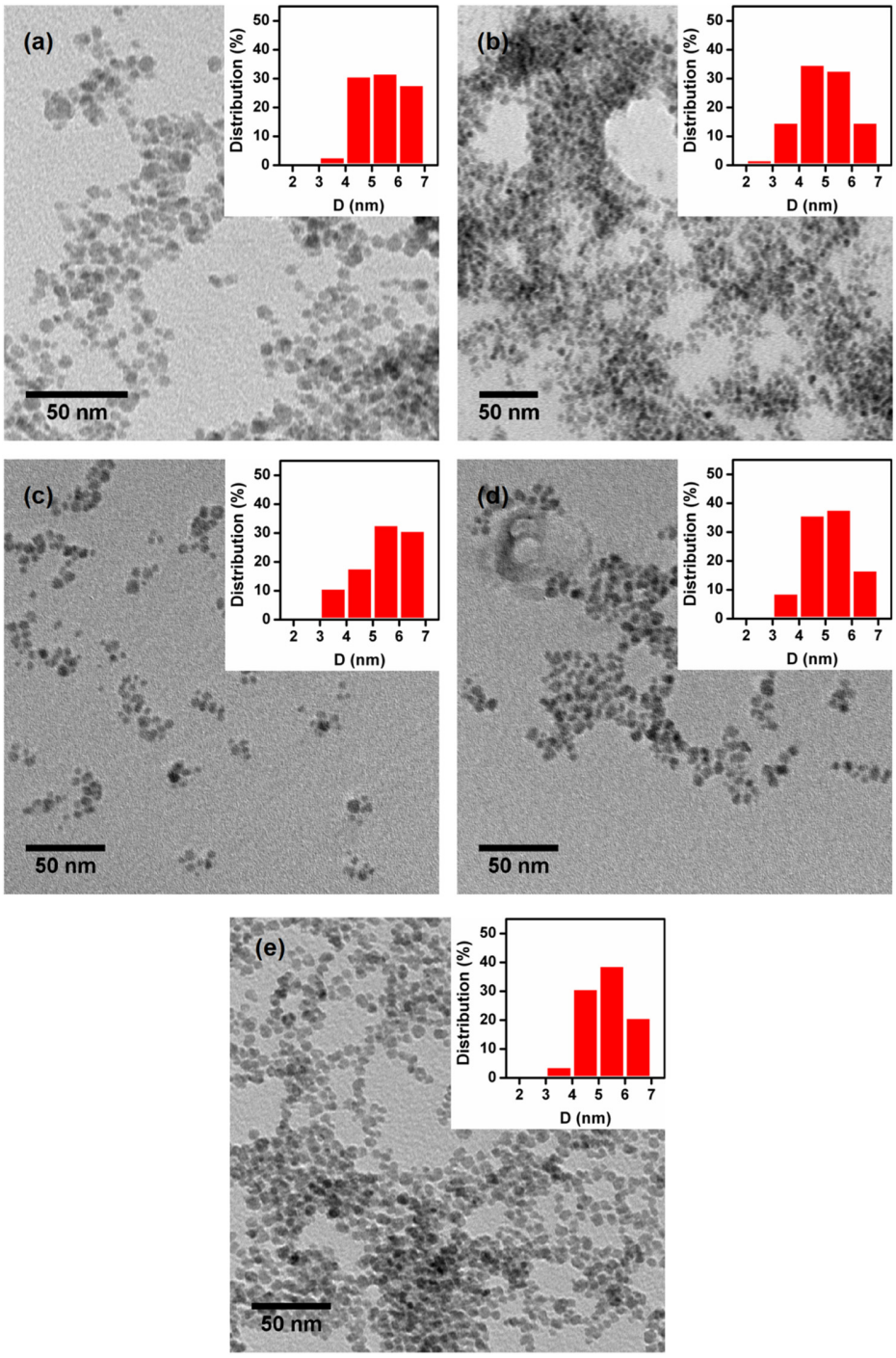


Fig. S4 TEM images with size distribution histograms of MIONs prepared with 5%DDT-PMAA, a, b, c, d and e for 0.25, 0.5, 1, 1.5 and 2 mM of polymer ligand respectively.

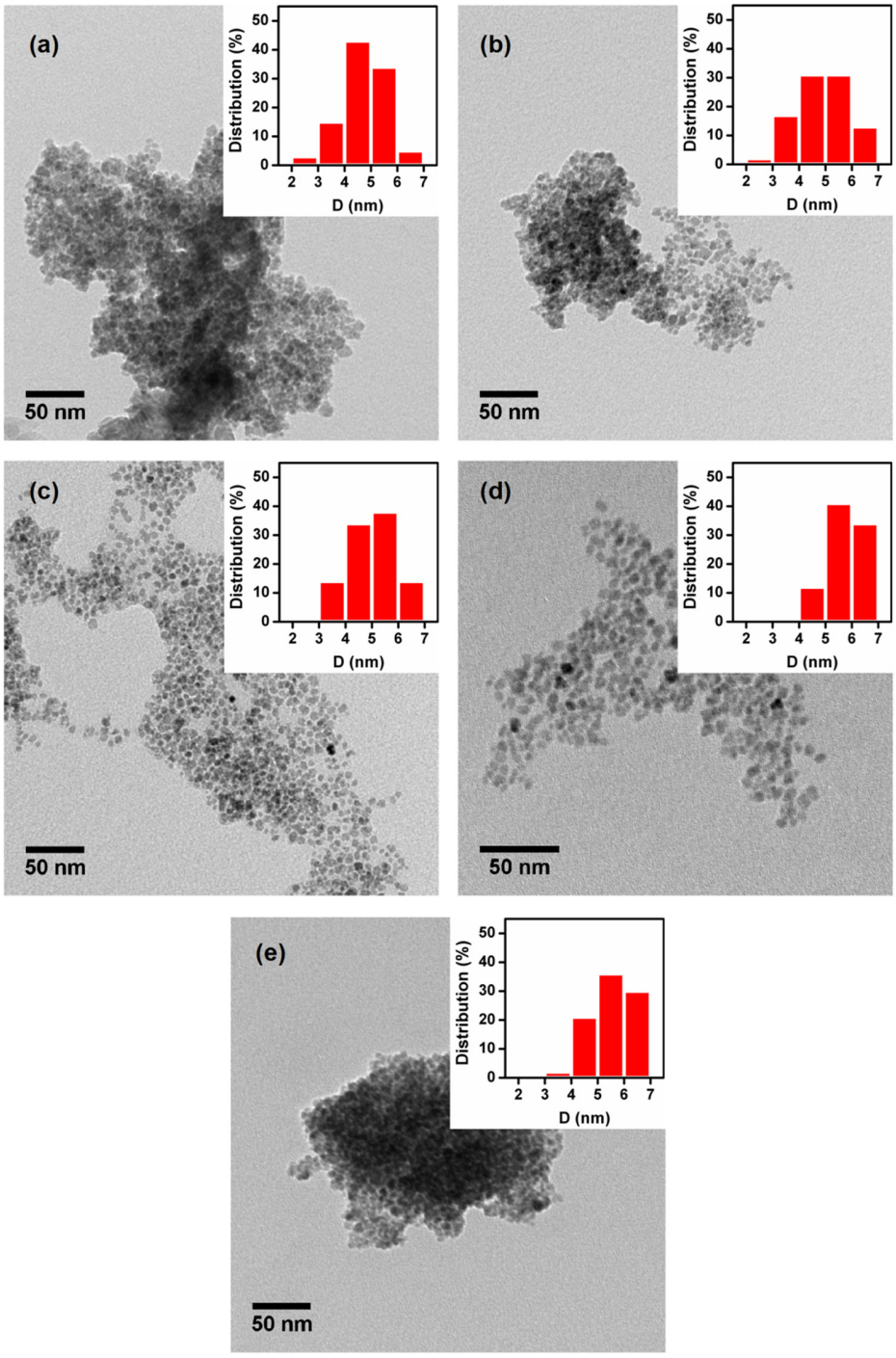


Fig. S5 TEM images with size distribution histograms of MIONs prepared with 10%DDT-PMAA, a, b, c, d and e for 0.25, 0.5, 1, 1.5 and 2 mM of polymer ligand respectively.

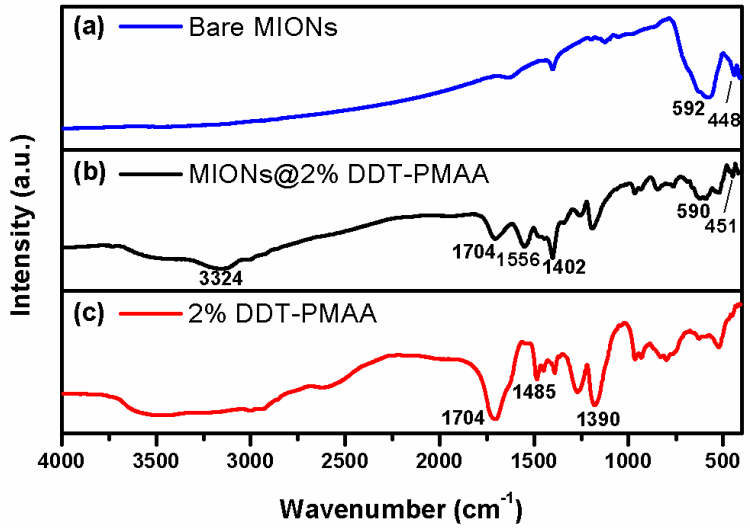


Fig. S6 FTIR spectra of 2%DDT-PMAA, MIONs with 2%DDT-PMAA (1.5 mM) and bare MIONs.

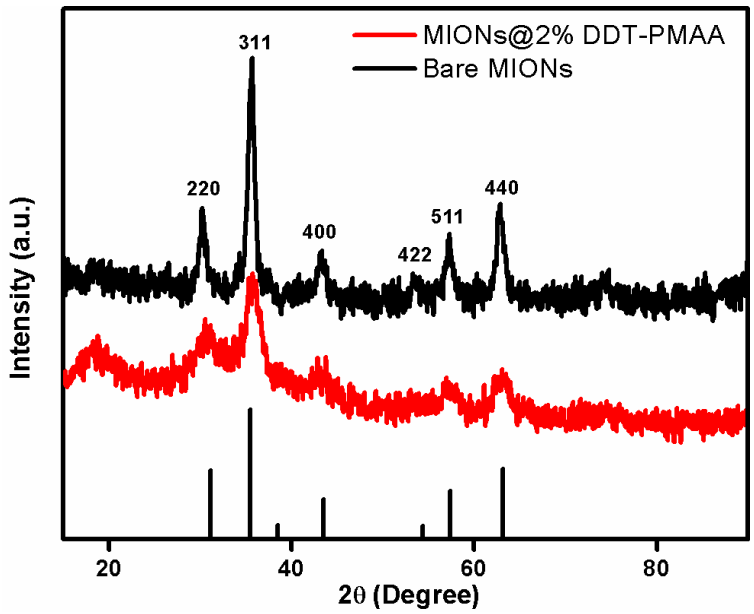


Fig. S7 XRD spectra of bare MIONs and MIONs prepared with 2%DDT-PMAA (1.5 mM).

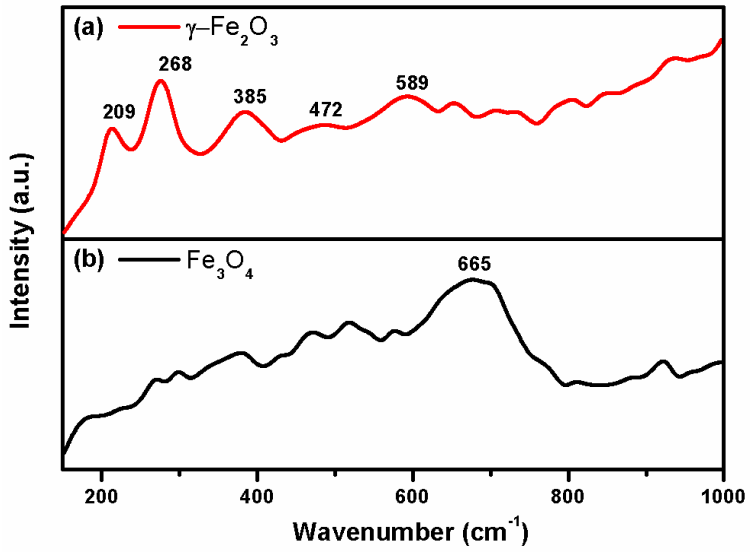


Fig. S8 Raman spectra of MIONs prepared using 2%DDT-PMAA (1.5 mM) with and without N_2-protection consisting of Fe_3O_4 and γ-Fe_2O_3 respectively.

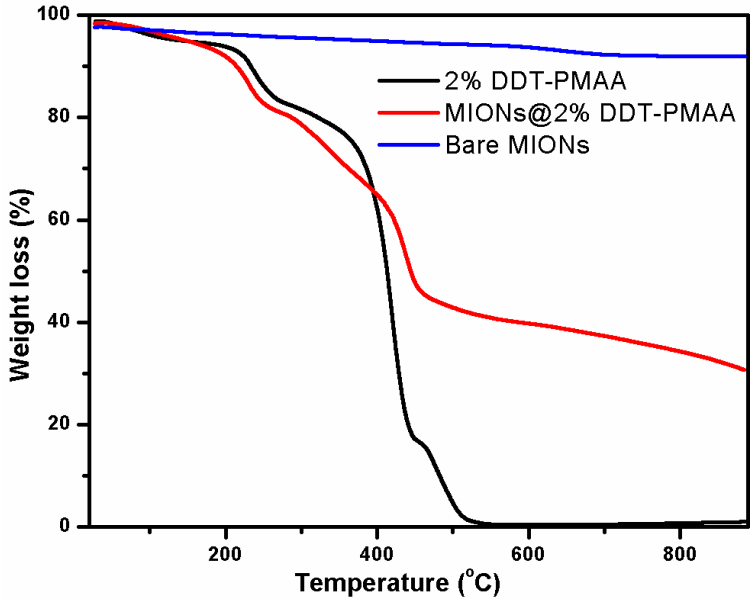


Fig. S9 TGA curves of 2%DDT-PMAA, MIONs with 2%DDT-PMAA (1.5 mM) and bare MIONs.

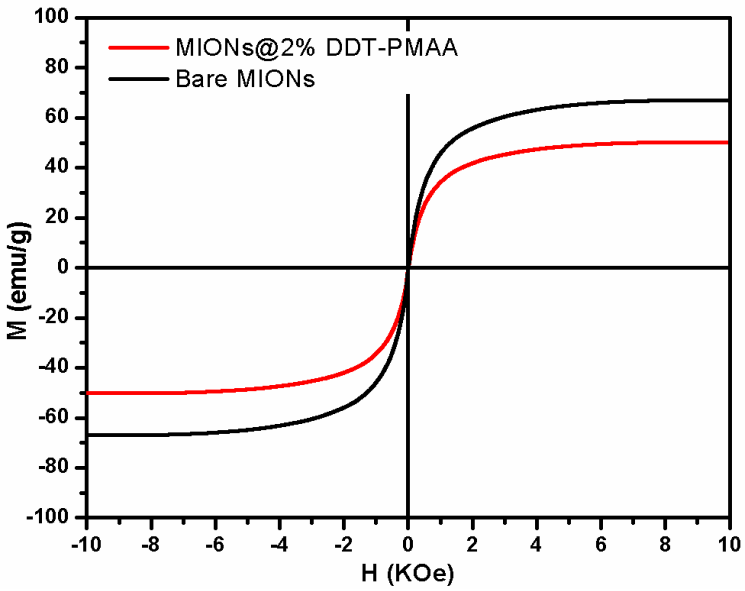


Fig. S10 Magnetization curves of MIONs prepared with 2%DDT-PMAA (1.5 mM) and bare MIONs without polymer ligand.

SI-5: pH and Salt Stability Analysis of MIONs.

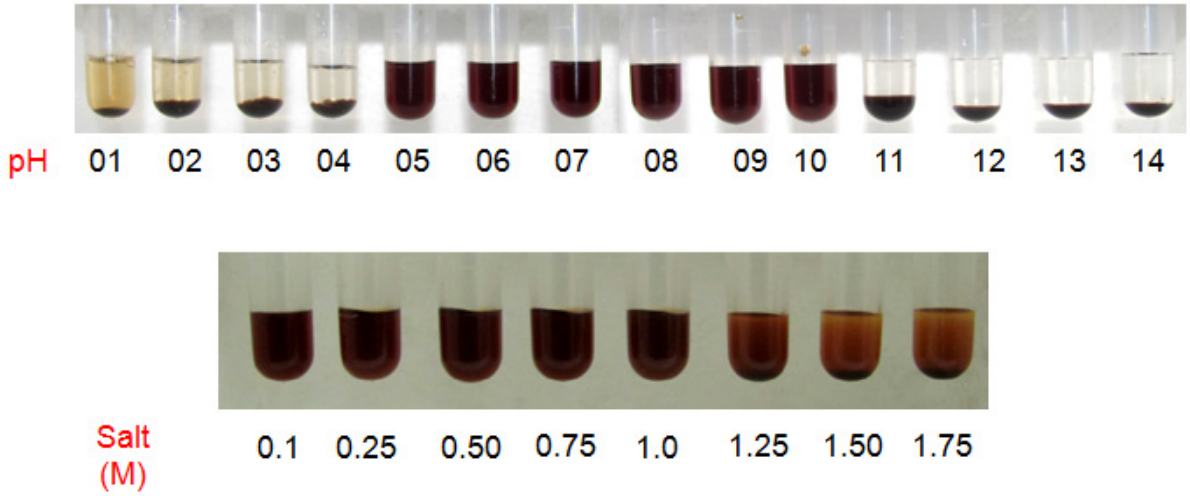


Fig. S11 pH and salt stability test performed on aqueous solutions of MIONs prepared with 2%DDT-PMAA (1.5 mM).

SI-6: Cytotoxicity Analysis of Polymer Ligand and MIONs.

Cell Culturing. Rat macrophage and HepG2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 µg mL^{-1} streptomycin and 100 U mL^{-1} penicillin, in a humidified incubator at 37 °C with a 5% CO_2 atmosphere.

In Vitro Cytotoxicity Assay. Cell viability was determined by MTT-assay. Rat macrophages were seeded into a 96-well plate with a cell density of 1×10^4 cells per plate and suspended in DMEM supplemented with 10% FBS and incubated for 24 h at 37 °C in a 5% CO_2 atmosphere. After that, the cell culture medium was replaced with fresh medium containing different concentrations of (25, 50, 100, 200, 500 and 1000 µg mL^{-1}) MIONs, DDT-PMAA and MIONs@DDT-PMAA in triplicate. A control experiment with only cell culture medium without any NPs or polymer was also carried out in each case. Plates were placed at 37 °C in a humidified 5% CO_2 incubator and MTT-assay was performed after 24, 48 and 72 h.

For MTT-assay, briefly cell culture media were aspirated and 20 µL MTT (5 mg mL^{-1}) [3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide] in FBS free DMEM was added to each well and incubated for another night at 37 °C in a humidified 5% CO_2 incubator. After incubation, MTT solution was removed and 150 µL DMSO was added in each vial to dissolve newly formed formazan crystals. The plates were placed on a swing bed for 10 min and then absorbance was recorded at 490 nm using a micro plate reader (Thermo Electron Corporation, USA). The absorbance was recorded in triplicate in each case and cell viability was calculated from their average values along with SD. Similarly, toxicity of MIONs@DDT-PMAA on HepG2 was determined by MTT-assay.

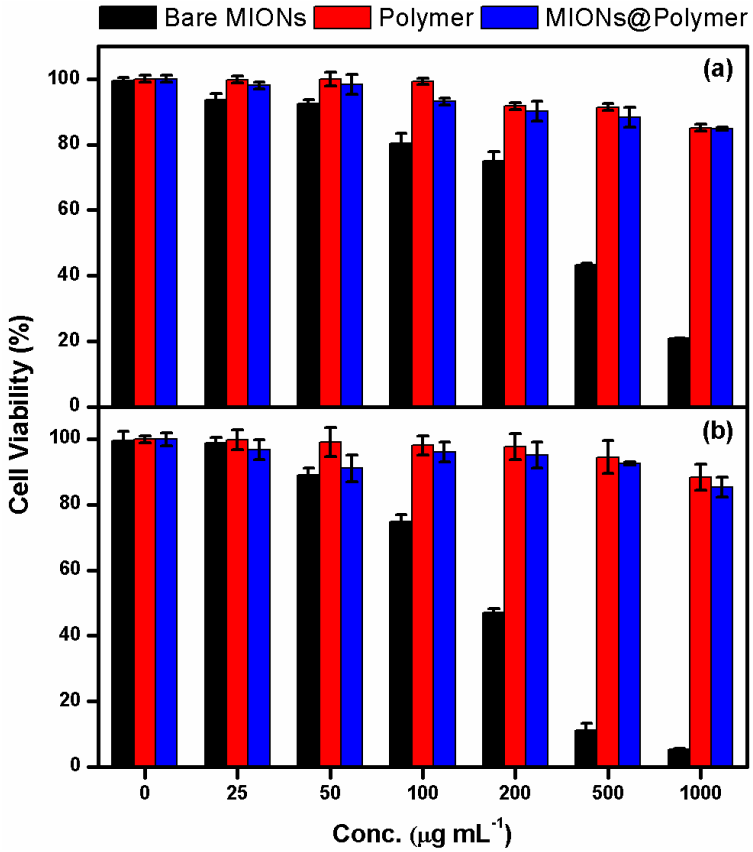


Fig. S12 Cell viability of rat macrophages determined by MTT-assay after 48 h (a) and 72 h (b) incubation with various concentrations of bare MIONs, 2%DDT-PMAA and MIONs@2%DDT-PMAA (1.5mM).

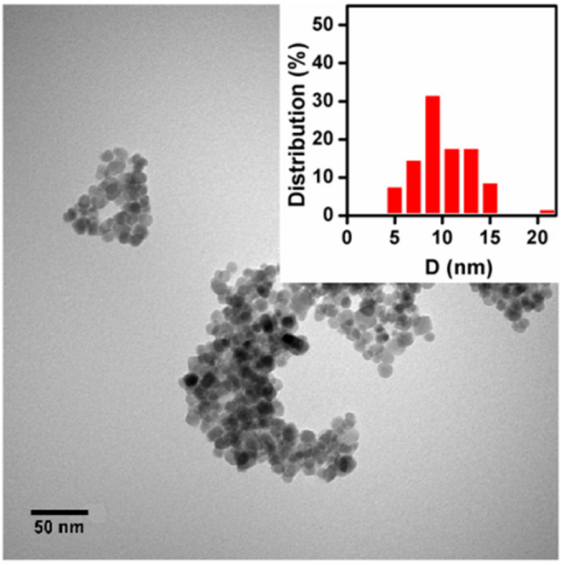


Fig. S13 TEM image of Bare MIONs with histogram (inset)

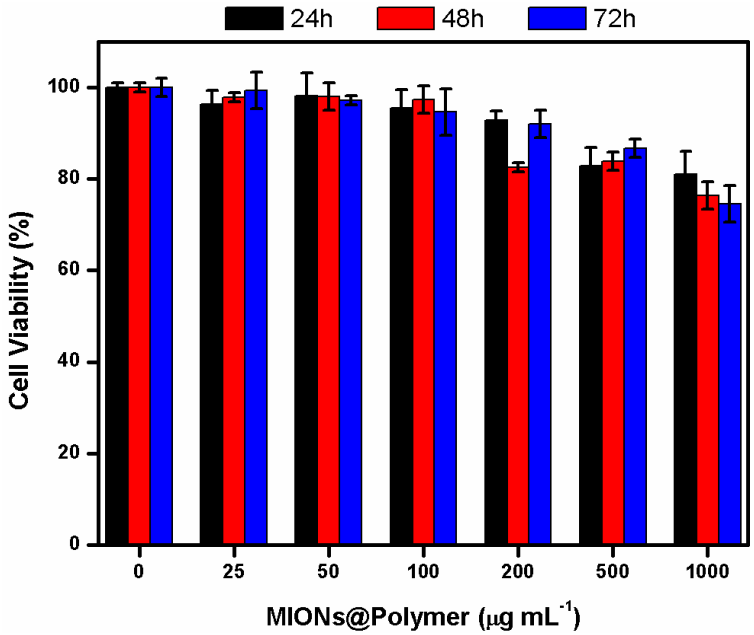


Fig. S14 Cell viability of HepG2 determined by MTT-assay after 24 h, 48 h and 72 h incubation with various concentrations of MIONs@2%DDT-PMAA (1.5m).

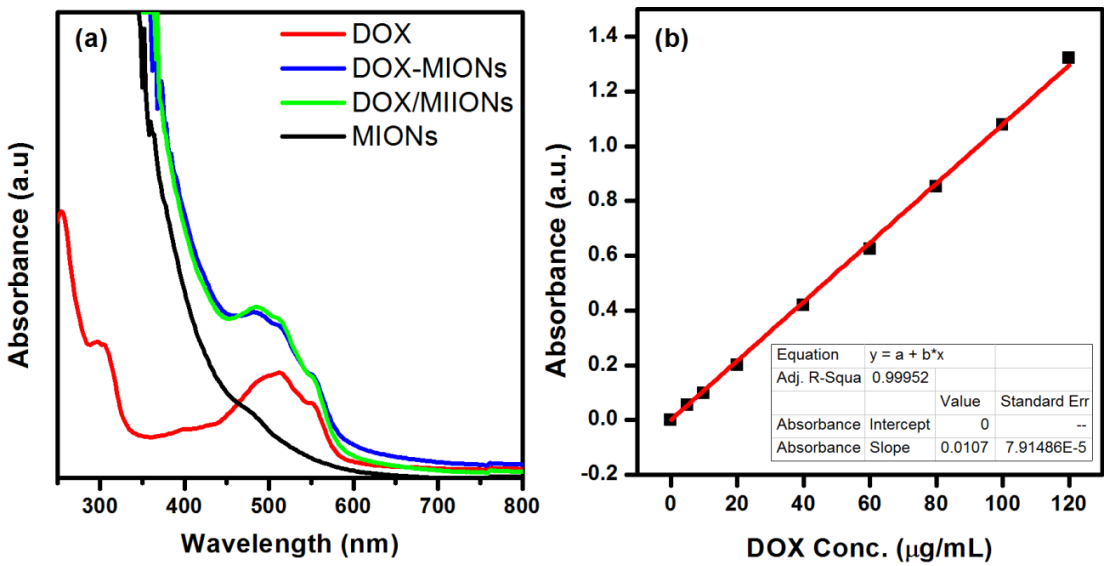


Fig. S15 UV-Visible spectra of DOX, MIONs and their conjugates (a) and UV-Visible Standard curve for Doxorubicin (b)

Fig. S16 Optical image of MIONs/DOX being separated with strong magnet

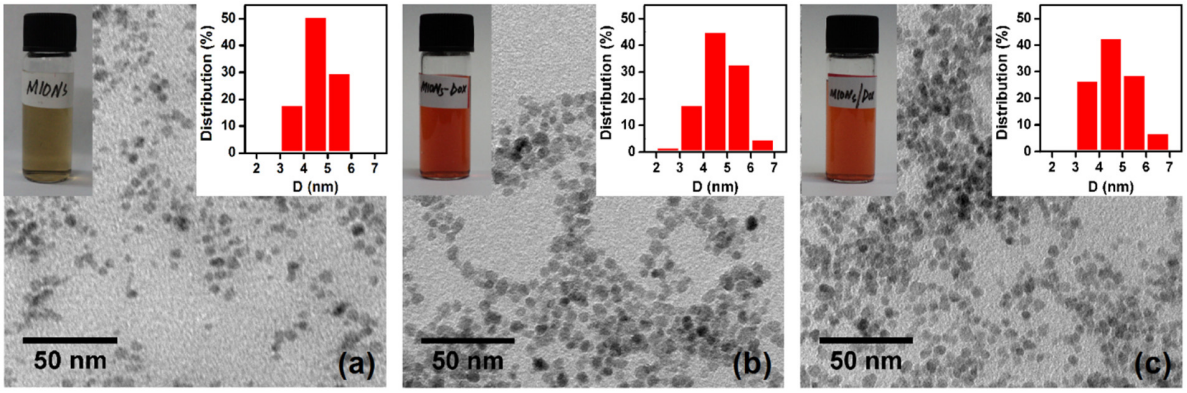


Fig. S17 TEM images of MIONs (a), MIONs-DOX (b) and MIONs/DOX (c) with size distribution histograms (upper right insets) and optical images of their redispersed aqueous solutions (upper left insets)

SI-7: References and Notes.

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