Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Supporting information for

Glucose-, pH- and thermo-responsive Nanogels Crosslinked by Functional Superparamagnetic Maghemite Nanoparticles as Innovative Drug Delivery Systems

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1. Supplemental experimental part

Scanning electron microscopy (SEM) was used to study the surface of the RCL nanogels with a JSM 840A microscope at 4 kV in high vacuum conditions. A drop of the RCL nanogels aqueous solution was deposited on a glass substrate, and the sample was dried at RT under air overnight. Then the sample was sputtered with gold in a cathode evaporator under argon atmosphere.

^{1}H nuclear magnetic resonance (NMR) spectra of the PVAc-*b*-PNVCL and PVOH-*b*-PNVCL macromolecules were recorded with a 250 MHz Bruker spectrometer in DMSO-d_6 at 50 °C.

Size-exclusion chromatography (SEC) in THF (flow rate of 1 mL/min) was performed at 40 °C using a Waters 600 liquid chromatograph equipped with a 410 refractive index detector and styragel HR columns (four columns HP PL gel 5 µm, 10^5 Å, 10^4 Å, 10^3 Å and 10^2 Å), calibrated with polystyrene standards. SEC in DMF (0.025 M LiBr, flow rate of 1 mL/min) was performed at 55 °C with a Waters 600 liquid chromatograph equipped with a 410 refractive index detector and styragel HR columns (HR1, 100-5000; HR3, 500-30,000; HR4, 5000-500,000; HR5, 2000-4000,000), calibrated with polystyrene standards.

Fourier transform infrared spectra (FTIR) of the bare γ-Fe_2O_3 NPs, γ-Fe_2O_3-APTES NPs and γ-Fe_2O_3-CNPBA NPs were performed with a Perkin Elmer FTIR instrument. Samples were mixed and grinded with potassium bromide, and then compressed for IR analysis.

X-ray photoelectron spectroscopy (XPS) was used to characterize the surface-functionalized γ-Fe_2O_3 NPs with a VG Scientific 220 i-XL ESCALAB spectrometer at 100 W (10 kV and 10 mA), which is equipped with a non-monochromatised MgKα source (hv = 1253.6 eV). A pressure of 10^{-7} Pa was maintained in the chamber during analysis. The analyzed area was *ca*. 150 µm in diameter. Full spectra (0-1150 eV) were obtained with constant pass energy of 150 eV and high-resolution spectra at 40 eV. Charge neutralization was required for insulating samples. The peaks were referenced to C1s peak at 284.7 eV.

2. Supplemental results

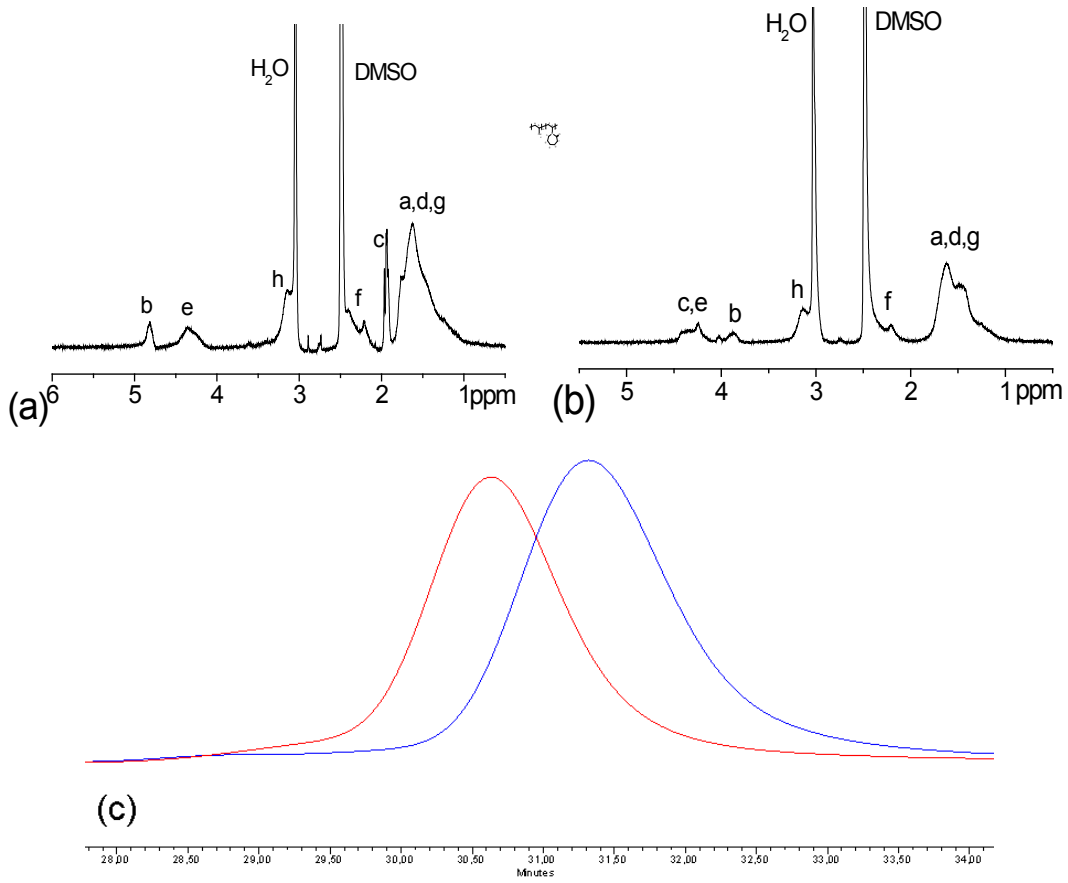


Figure S1. ^{1}H NMR spectrum of PVAc-*b*-PNVCL copolymer (a, DMSO-d_6, 353 K) and PVOH-*b*-PNVCL (b, DMSO-d_6, 353 K), SEC traces of PVAc-Co(II) macro-initiator (blue, M_n = 4.42×10^4 g/mol, M_n/M_n = 1.04) and PVAc-*b*-PNVCL copolymer (red, M_n = 5.67×10^4 g/mol, M_n/M_n = 1.06) in DMF (c, PS calibration standard).

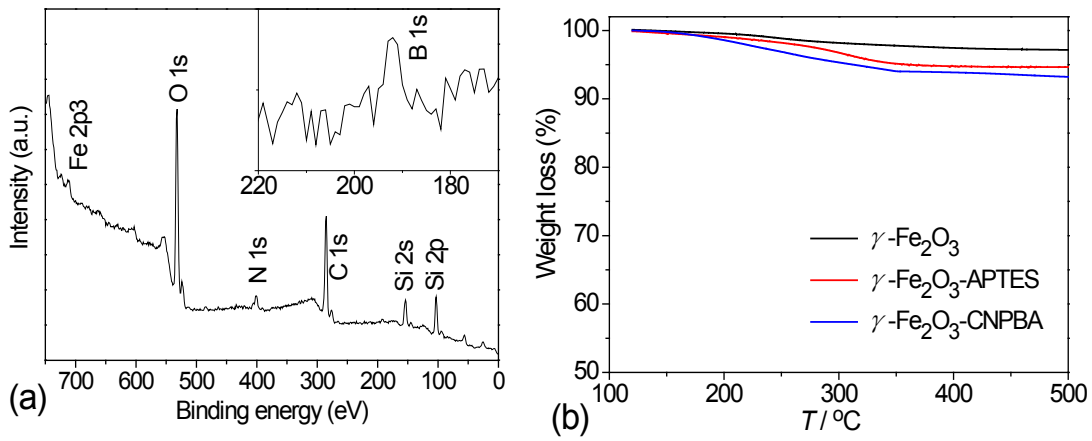


Figure S2. XPS spectrum of the γ-Fe_2O_3-CNPBA NPs, inset: partially-magnified spectrum in the range of 220~170 eV, and B1s signal could be detected at *ca*. 192 eV (a), and TGA traces of the γ-Fe_2O_3 NPs at each step of surface modification (b)

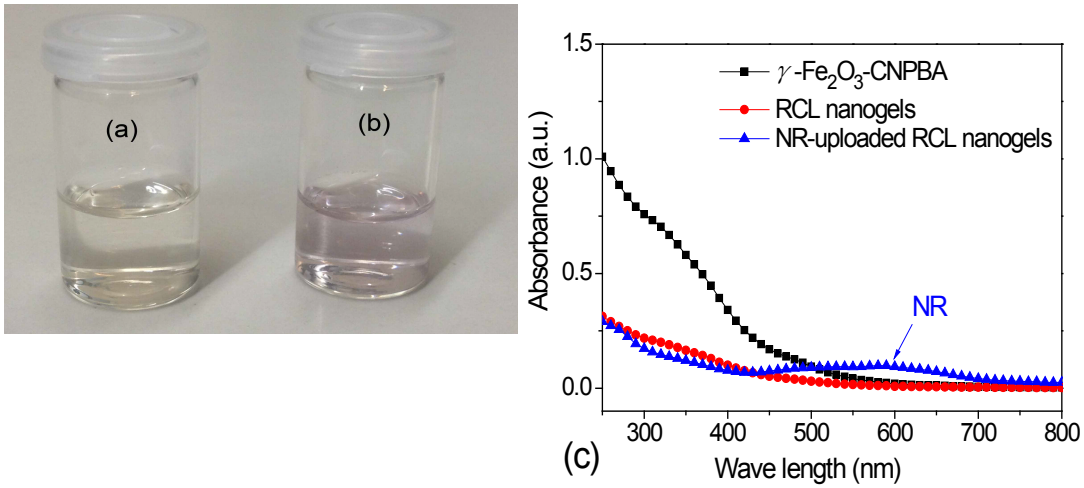
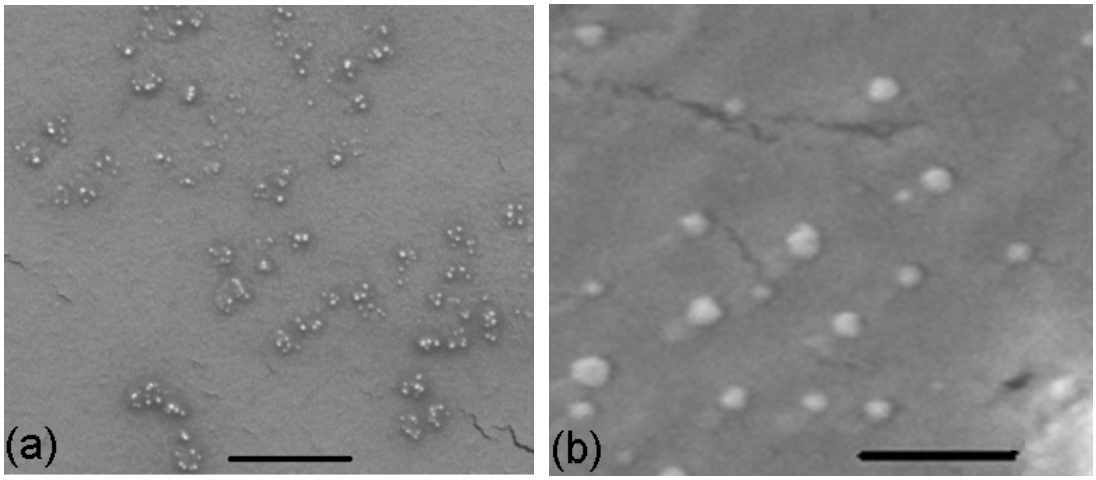


Figure S3. Pictures of RCL nanogels (a) and NR-encapsulated RCL nanogels (b), and UV/*vis* spectra of the γ-Fe_2O_3-CNPBA NPs, RCL nanogels and NR-uploaded RCL nanogels (c)



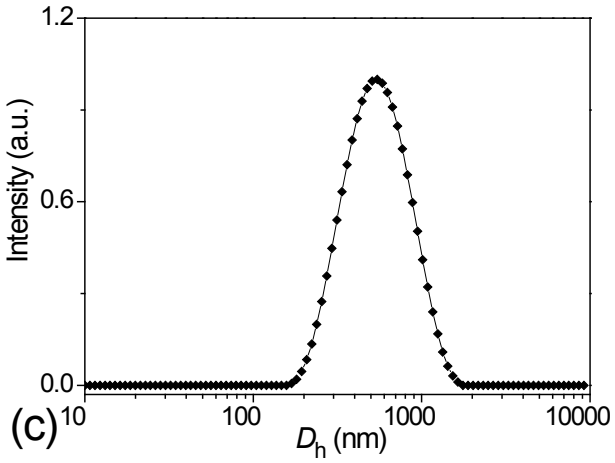


Figure S4. Representative SEM image of the RCL nanogels (scale bar: 2000 nm), magnified SEM image of the RCL nanogels (scale bar: 500 nm) (a); intensity-averaged size distribution of the RCL nanogels from DLS analysis (b).

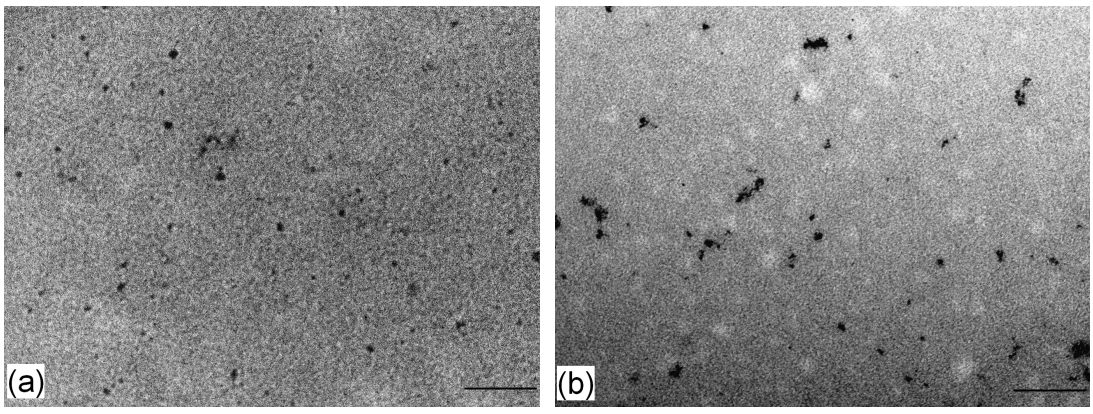


Figure S5. TEM images of the RCL nanogels after 6-h treatment of glucose (10 mM, pH 7.4, 25ºC) (a, scale bar: 100 nm), and 6-h treatment of acidic pH (pH 5.0, no glucose, 25ºC) (b, scale bar: 100 nm).

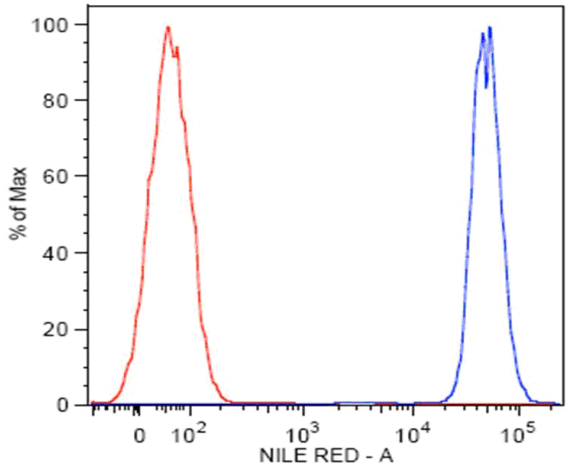


Figure S6. fluorescence-activated cell sorting (FACS) histogram of the untreated MEL-5 cells (red) and treated cells (blue) after 12-h incubation with NR-loaded RCL nanogels (100 μg/mL), and the log of red fluorescence intensity (Nile Red-A on *x*-axis) is plotted against the number of cells (counts on *y*-axis).

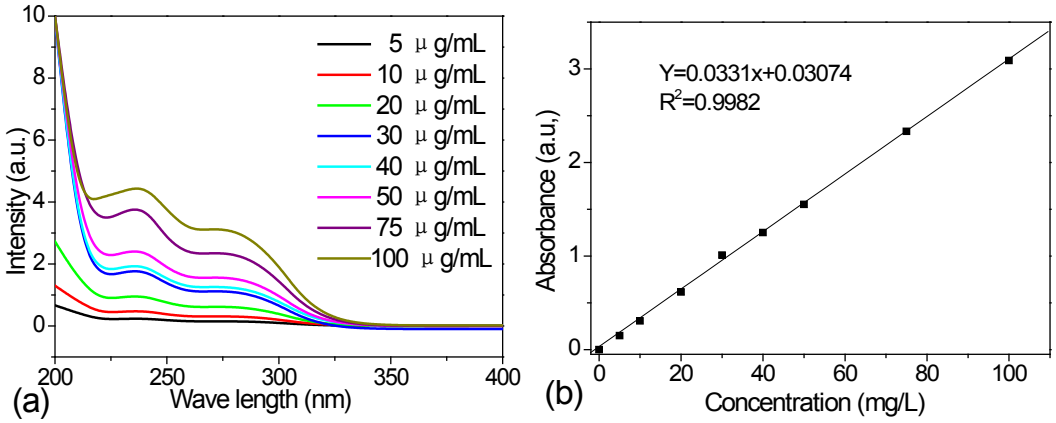


Figure S7. UV/*vis* absorption spectra of tamoxifen/methanol solutions with tamoxifen different concentrations (a)**;** and linear fitting of absorbance at 280 nm *vs*. concentration from 0 to 100 μg/mL (b).

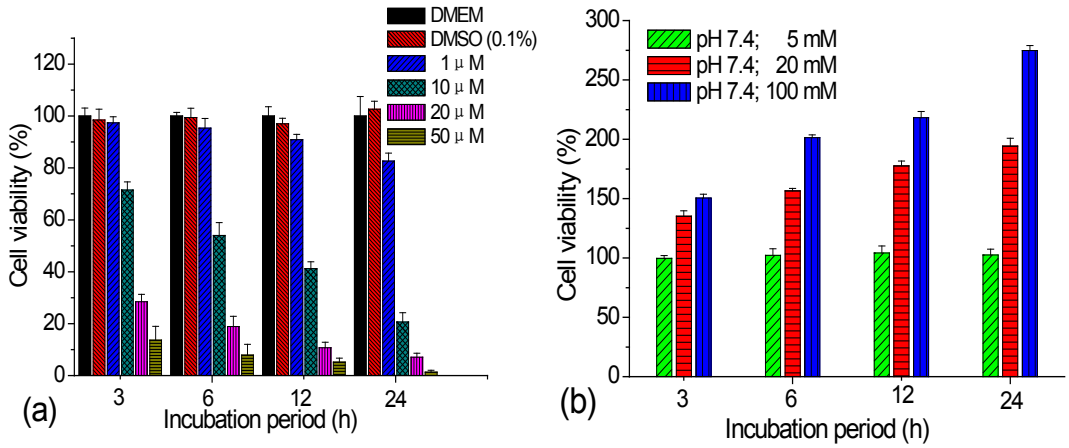


Figure S8. Cell viability of MEL-5 cells in DMEM complete medium (pH 7.4) with different tamoxifen concentrations (a), and cell viability of MEL-5 cells in DMEM buffer (pH 7.4) with different glucose concentrations (b); percentage viability was expressed relatively to the un-treated cells (control at pH 7.4, 5 mM glucose, 100% viability). Results were all presented as mean value ± standard deviation (n = 5).

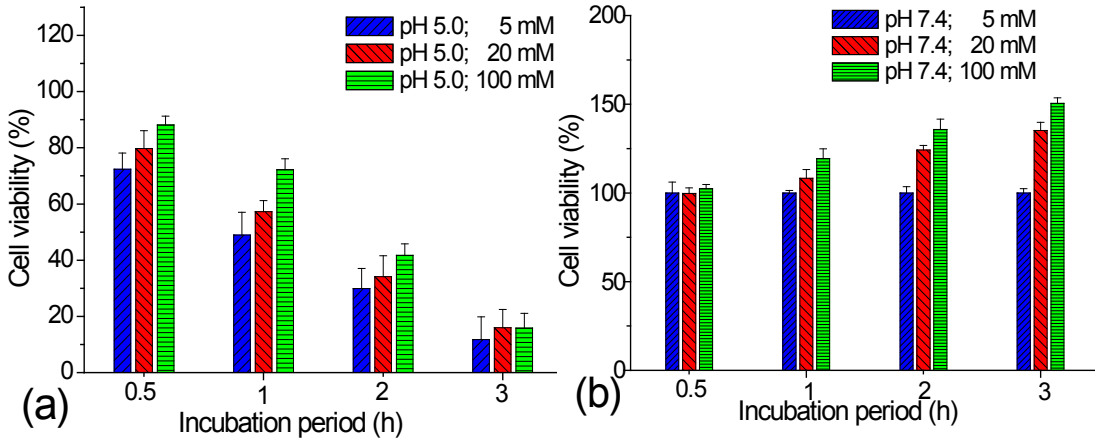


Figure S9. Cell viability of MEL-5 cells in DMEM buffer at pH 5.0 (a) and pH 7.4 (b) with different glucose concentrations; percentage viability was expressed relatively to the un-treated cells (control at pH 7.4 and 5 mM glucose, 100% viability). Results are presented as mean value ± standard deviation (n = 5).