**Supporting information**

# Enhancing MRI imaging through high loading of superparamagnetic nanogels with high sensitivity to the tumor environment

Jinfeng Liao,[§a] Liangyu Zhou,[§b] Yongzhi Wu,[a] Zhiyong Qian[c] and Pei Li *[b]

## Synthesis of MPS-γ-$Fe_2O_3$ nanoparticles

$FeCl_2 \cdot 4H_2O$ (1.99 g) and $FeCl_3$ (3.25 g) were dissolved in 20 ml DI-water separately. These two solutions were then mixed under vigorous stirring to give a homogeneous solution. $NH_3 \cdot H_2O$ (0.6 M, 200 mL) and $NH_3 \cdot H_2O$ (25%, 30 mL) were added sequentially. The color of the mixture changed from transparent to black dispersion immediately after adding ammonium hydroxide solution. The mixture was continuously stirred at room temperature for 1 hour, followed by heating it to 120℃ in an oil bath for 8 hours. At the end of the reaction, the black dispersion changed to brown color. After cooling down to room temperature naturally, the brown dispersion was purified by centrifugation at 19,000 rpm for 30 minutes three times.

After purification, 50 mL $HNO_3$ (4 M) was quickly added to 30 mL of stable brown magnetic dispersion to maintain a pH value of around 3. The dispersion was diluted with DI water (200 mL) and heated to reflux. Trisodium citrate dehydrate (11.7 g) dissolved in 20 mL DI-water was then added to the dispersion, followed by heating the solution to reflux for 1 hour. The final dispersion was purified by dialysis in a dialysis tube (10 KDa) with a daily change of DI water for 3 days. The citrate-coated nanoparticles were then collected by centrifugation at a speed of 19,000 rpm for 2 hours, and then re-dispersed in 50 mL di-water.

The purified citrate-coated γ-$Fe_2O_3$ nanoparticles (20 mL, 2.92 w/w %) were mixed with 80 mL methanol in a three-necked water-jacketed flask, followed by stirring at 40 ℃ for 1 hour. $NH_3 \cdot H_2O$ (1.8 mL, 25 w/w %) was subsequently added to the mixture and stirred at 40℃ for 30 minutes. TEOS (0.687 mL) was then added and

allowed to react for 24 hours at 40°C. Finally, MPS (3.64 mL) was charged to this mixture and continued the reaction for additional 24 hours at the same temperature. The final product of the vinyl-coated γ-$Fe_2O_3$ (MPS-γ-$Fe_2O_3$) nanoparticles was purified by repeated centrifugation (three times) at the speed of 18,000 rpm for 2 hours. The MPS-γ-$Fe_2O_3$ nanoparticles were subsequently purified by dialysis with a daily change of ethanol (5 days). The purified MPS-γ-$Fe_2O_3$ nanoparticles were re-dispersed in 50 mL ethanol and the dispersion was stored at ambient temperature for further use.

**Table S1.** Effect of reaction time on properties of magnetic nanogel*.

| Reaction time (h) | 2 | 8 | 24 |
|---|---|---|---|
| Monomer conversion (%) | 56 | 76 | 71 |
| Iron oxide content (%) | 68 | 63 | 63 |
| Particle size at 25 ℃ (nm) | 280 ± 3 | 475 ± 10 | 510 ± 2 |
| Particle size at 50 ℃ (nm) | 294 ± 4 | 247 ± 12 | 436 ± 4 |
| Size distribution (PDI)<br>(at 25 and 55 ℃) | 0.14 (25℃)<br>0.20 (55℃) | 0.19 (25℃)<br>0.30 (55℃) | 0.06 (25℃)<br>0.20 (55℃) |
| Surface charge at pH 5.3<br>(δ-potential) (mV) | + 33.7 | +38.3 | +43.3 |
| Shrinking volume (%)<br>(from 25 to 55 ℃) | 37 | 61 | 60 |

* Reaction conditions: The weight ratio of chitosan to monomer was 1.98:1 using step-wise feeding method at 80℃.

**Table S2.** Effect of chitosan to monomer weight ratio on properties of magnetic nanogel*

| Chitosan to monomer weight ratio | 3.46:1 | 1.98:1 | 0.82:1 |
|---|---|---|---|
| Monomer conversion (%) | 56 | 71 | 71 |
| Iron oxide content (%) | 60 | 63 | 60 |
| Particle size at 25 ℃ (nm) | 452 ± 4 | 510 ± 2 | 457 ± 5 |
| Particle size at 50 ℃ (nm) | 378 ± 5 | 436 ± 4 | 388 ± 9 |
| Shrinking volume (%)<br>(from 25 to 55 ℃) | 62.7 | 60 | 50 |
| PDI range<br>(at 25 and 55 ℃) | 0.17 (25℃)<br>0.21 (55℃) | 0.06 (25℃)<br>0.20 (55℃) | 0.06 (25℃)<br>0.20 (55℃) |
| Surface charges at pH 5.3<br>(δ-potential) (mV) | + 43.5 | +43.3 | +36.4 |

*Reaction conditions: The polymerization was conducted at 80℃ for 8 hours via a step wise feeding method.

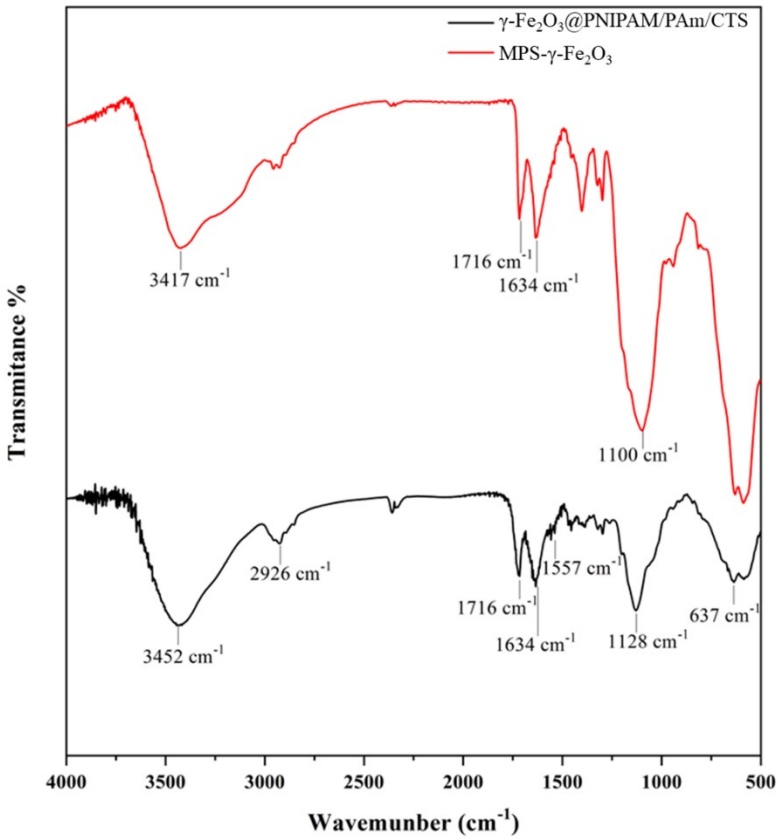


**Figure S1.** The FTIR spectra of MPS-γ-$Fe_2O_3$ (red) and γ-$Fe_2O_3$@PNIPAM/PAm/CTS (black) nanogels.

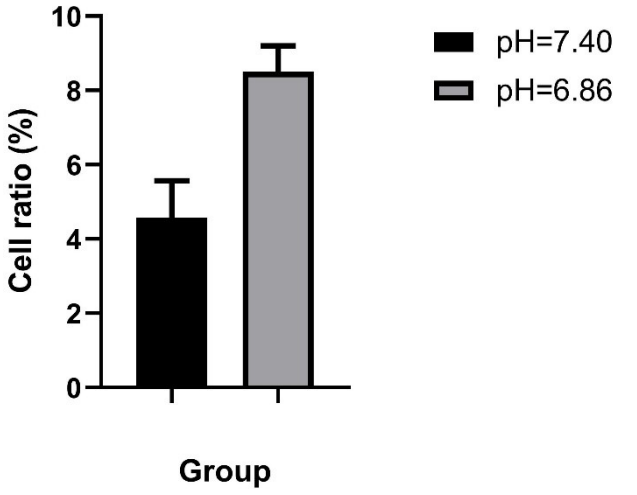


Figure S2. A quantitative comparison of the cellular uptake ratio of γ-$Fe_2O_3$@PNIPAM/PAm/CTS nanogels at different pHs.

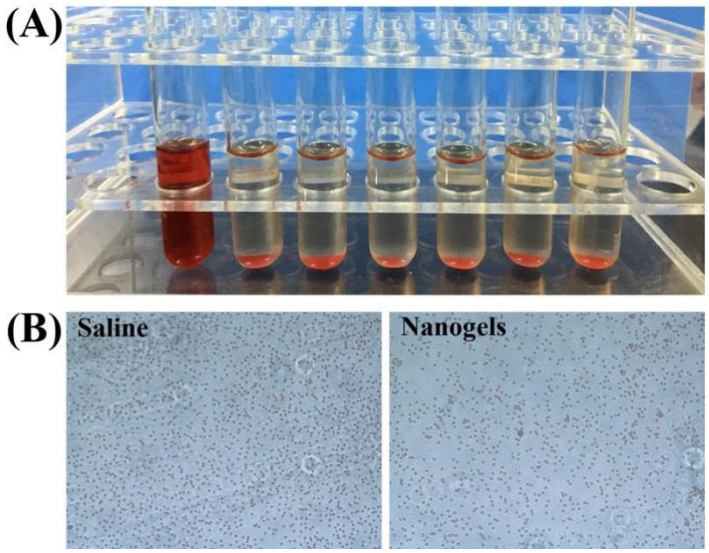


**Figure S3.** (A) The hemolytic analysis of γ-$Fe_2O_3$@PNIPAM/PAm/CTS nanogels (From left to right: $H_2O$, 8 mg/mL nanogels, 6 mg/mL nanogels, 3 mg/mL nanogels, 2 mg/mL nanogels, 1 mg/mL nanogels, saline); (B) The RBC morphological observation by the microscope (100×).

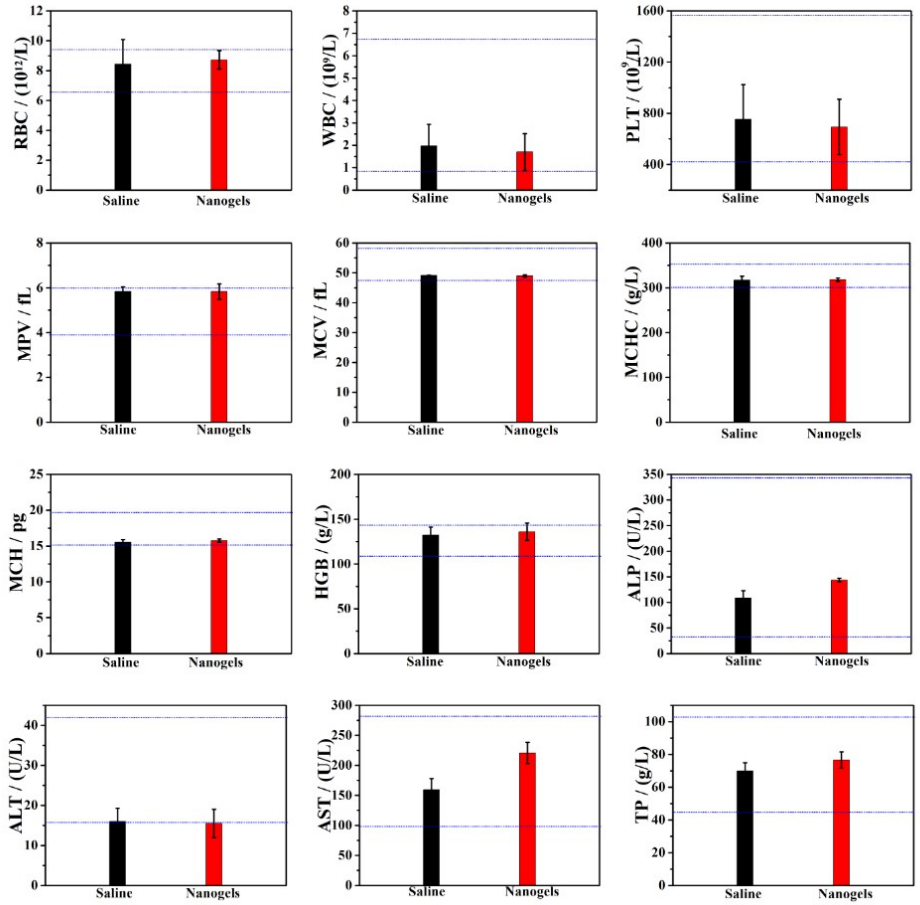


**Figure S4.** Blood routine tests and blood biochemistry tests for mice treated with saline and γ-$Fe_2O_3$@PNIPAM/PAm/CTS nanogels. The blue region represents the normal range from the literature. Abbreviation: RBC, red blood cell; WBC, white blood cell; PLT, platelet count; MPV, mean platelet volume; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; HGB, hemoglobin; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; TP, total protein.