## Folate-conjugated cross-linked magnetic nanoparticles as potential magnetic resonance probes for in vivo cancer imaging

Hee-Man Yang,<sup>‡</sup><sup>*a*</sup> Chan Woo Park,<sup>‡</sup><sup>*b*</sup> Pan Kee Bae,<sup>*c*</sup> Taebin Ahn,<sup>*b*</sup> Bum-Kyoung Seo,<sup>*a*</sup> Bong Hyun Chung<sup>*c*</sup> and Jong-Duk Kim<sup>\*<sup>*b*</sup></sup>

<sup>a</sup> Decontamination & Decommissioning Research Division, Daedeok-daero 989-111, Yuseong-gu, Daejeon 305-353, Republic of Korea. Fax: +82-42-868-8667; Tel: +82-42-868-4537; E-mail: <u>hmyang@kaeri.re.kr</u>

<sup>b</sup> Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology, 291 Daehak-roYuseong-gu, Daejeon 305-701, Republic of korea. Fax:+82-42-350-3910; Tel:+82-42-350-3961;

<sup>c</sup> BioNanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology(KRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon, Republic of Korea. Fax: +82-42-861-1759; Tel: +82-42-860-4442; E-mail:

<sup>\*</sup> Corresponding author. Tel.: +82-42-350-3961. Fax: +82-42-350-3910. E-mail: <u>kjd@kaist.ac.kr</u>.

*‡ These authors contributed equally to this work.* 

## Experiment

## Synthesis of as-synthesized iron oxide nanocrystals

 $Fe_3O_4$  nanocrystals were produced by a seed-mediated growth method using 6 nm sized nanocrystals synthesized using the thermal decomposition method.<sup>42</sup> Briefly, for the synthesis of 6 nm  $Fe_3O_4$ , iron (III) acetylacetonate (0.706 g, 2 mmol), 1,2-hexadecanediol (2.5845 g, 10 mmol), oleic acid (1.902 mL, 6 mmol), oleylamine (1.974 mL, 6 mmol), and benxyl ether (20 mL) were mixed under a nitrogen atmosphere. The mixture was heated to 200 °C for 2 h and further heated to 300 °C for 1 h under reflux. After being cooled to room temperature, excess ethanol was used to wash the reactant. Nanocrystals were collected by centrifugation.



Fig. S1. <sup>1</sup>H NMR spectra of folate-PEG-NH<sub>2</sub> in DMSO-d<sub>6</sub>.



Fig. S2. UV-visible absorption spectra of folate conjugated PEG (Fol-PEG-NH<sub>2</sub>).



**Fig. S3.** <sup>1</sup>H NMR spectra of PSI-*g*-C<sub>12</sub>-PEG-folate in DMSO-d<sub>6</sub>.



**Fig. S4.** (a) TEM image and (b) size histogram of as-synthesized iron oxide nanocrystals. A scale bar is 50 nm.



Fig. S5. X-ray diffraction patterns of as-synthesized Fe<sub>3</sub>O<sub>4</sub> nanocrystals using a Rigaku D/max-RB apparatus and a Cu K $\alpha$  source ( $\lambda = 0.154$  nm); powder samples were analyzed.



Fig. S6. Size distribution of the F-CLMNPs in water at 37 °C determined by DLS.



Fig. S7. TEM image of F-CLMNPs in water. Scale bar is 500 nm



**Fig. S8.** Thermogravity analysis results of as-synthesized iron oxide nanocrystals and folate-conjugated CLMNPs (F-CLMNPs).



**Fig. S9.** Magnetization curve of as-synthesized iron oxide nanocrystals and folate conjugated CLMNPs at room temperature (F-CLMNPs).



**Fig. S10.** Fluorescence microscopy images of A549 and KB cells treated with 0.025, 0.05, and 0.1 mg/ml of F-CLMNPs or CLMNPs for 2 h.



**Fig. 11.** Flow cytometry results of A549 and KB cells treated with 0.025, 0.05, and 0.1 mg/ml of F-CLMNPs and CLMNPs. Gray histograms represent control cells, value in the top middle part of each panel represents the mean fluorescence intensity (MFI) of nanoparticle treated cells.



**Fig. S12.** Prussian blue staining of A549 and KB cells treated with 0.2 mg/mL of F-CLMNPs and CLMNPs.



**Fig. S13.** In vivo T<sub>2</sub>-weighted MR images taken at 0 h and 3 h postinjection of CLMNPs to a mouse bearing KB tumor on its back.