Modulating Cell-uptake behavior of Au-based Nanomaterials *via* Quantitative Biomolecule Modification

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Supplementary Information

Table S1. the [Fe] and [Au] concentration of A, B and C samples analyzed by ICP-mass.

	[Au] (uM)	[Fe] (uM)
A	0.113	0.167
B	0.121	0.144
C	0.150	0.154

Magnetic properties of nanoparticles

After biomodification, the Tf-Au-Fe_3O_4 nanomaterials possess superparamagnetic properties owing to fast response toward the applied magnetic field. It can be used for the magnetic resonance image (MRI).

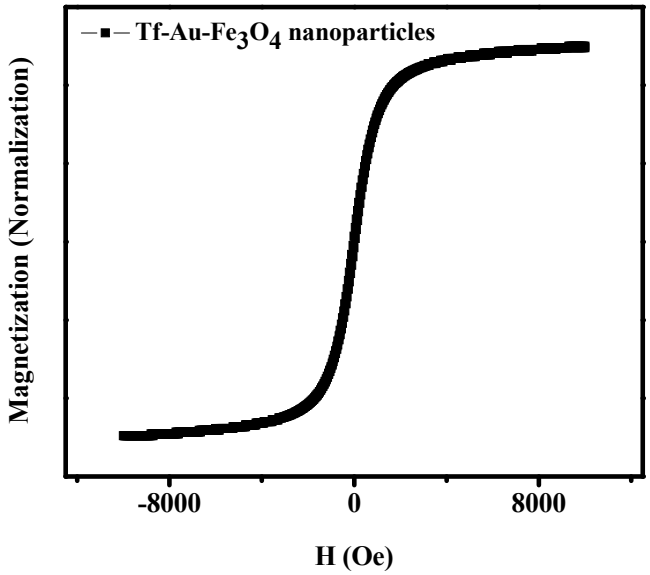

Figure S1. Magnetic hysteresis loop of Tf-Au-Fe_3O_4 nanoparticles.

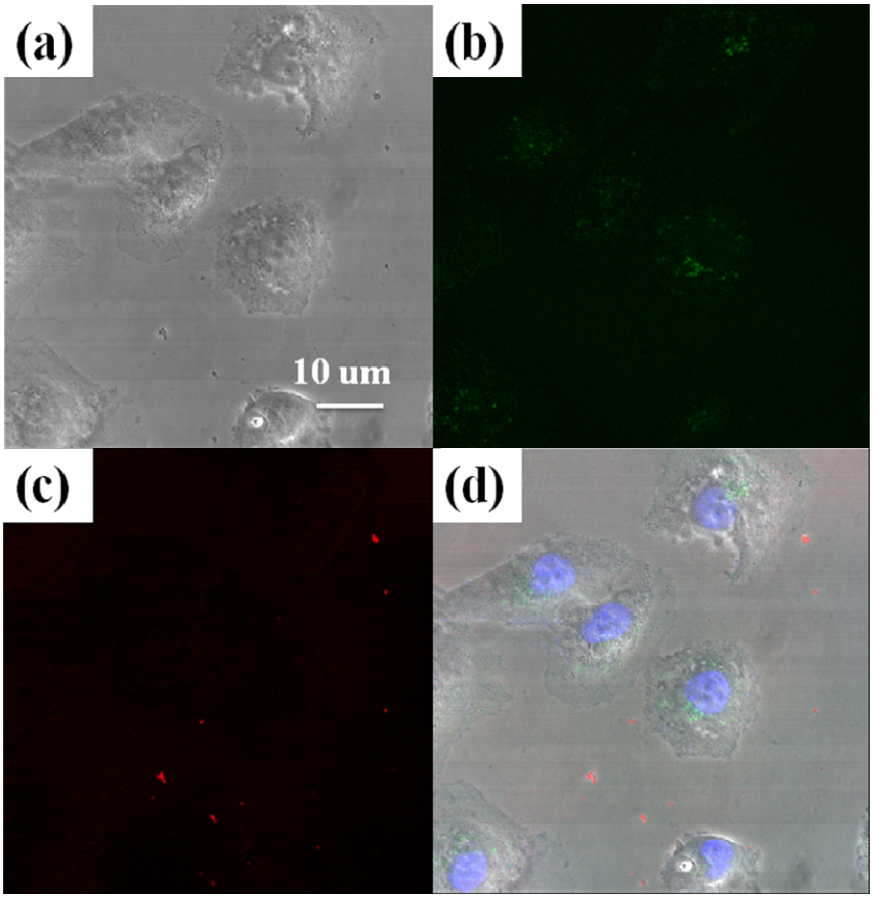

Figure S2. (a) The bright field image, (b) fluorescence image of FITC-ChromPure Human Albumin, (c) fluorescence image of rTf-Au-Fe_3O_4 nanoparticles and (d) merging image of them for specificity assay.

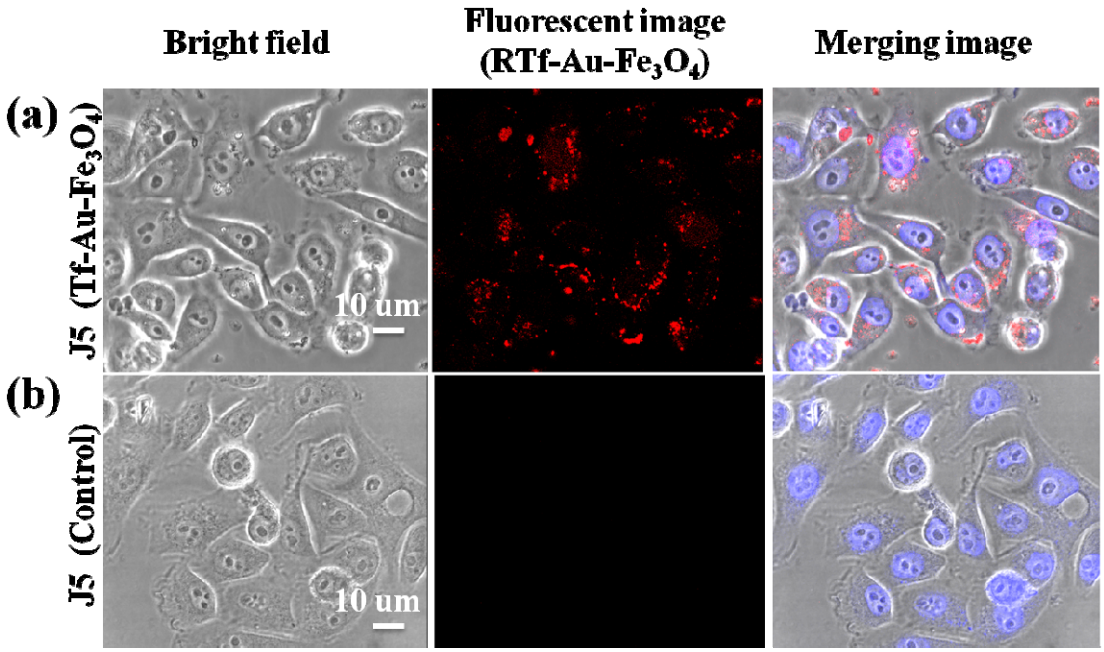

Figure S3. Confocal microscopy image of (a)-(b) J5 cancer incubated with/without rTf-Au-Fe_3O_4 nanoparticles in florescent and bright image mode. Note : the nanoparticles does not removed by washing before the paraldehyde is added for fixing cell. Nuclei of J5 cancer is stained by DAPI.

Magnetic Resonance image (MRI). The Au-Fe_3O_4 and Tf-Au-Fe_3O_4 nanoparticles, modified by various amount of iminothiolate activated Tf, were incubated with J5 cells overnight. The suspension solution was removed and J5 cells was washed using PBS for several times. the J5 cells are mixed and fixed with agarose. MRI machine performed to measure the cell images under the following conditions: receiver bandwith = ± 16 kHz, repetition time = 5000 ms, flip angle = 90 °C, echo time = 86 ms, field of view = 4 × 4 cm2, echo train length = 8, section thickness = 1 mm, 16 slices, scan time = 5min 25sec.

Magnetic Resonance image (MRI) of J5 cancer cell after treating with Tf-Au-Fe_3O_4 nanoparticles. To evaluate the performance of targeting efficiency of Tf-Au-Fe_3O_4 nanoparticles toward J5 cancer cell, it can be executed using MRI technique, as shown in Figure 3-9 (a) ~ (e). The magnetic hysteresis loop of as-prepared Tf-Au-Fe_3O_4 nanoparticles is measured, as shown in Figure 3-9 (f), revealing the prompt response of magnetization is observed in forward/reverse magnetic field. It indicates the sample (e) have superparamagnetic behavior under applied magnetic field and regarded as a good candidate for T_2 contrast reagent in MRI. As Fe_3O_4 nnaoparticles exposes to applied magnetic field, it rapidly responses an extra magnetic field to influences the T_2 relaxation time of proton. It indicates the contrast image can be enhanced after targeting by Tf-Au-Fe_3O_4 nanoparticles. Herein, with increasing the concentration of cell-internalized Tf-Au-Fe_3O_4 nanoparticles, the contrast of MRI is enhanced largely. In othe words, the targeting efficiency of nanoparticles must play an important role to determine the contrast degree of target cell. In our studies, the same dose of Fe_3O_4 for each Tf-Au-Fe_3O_4 nanoparticles were loaded and incubated with J5 cell over night. The suspension of free nanoparticles was removed by carefully washing cell for several times before MRI measurement. Finally, the J5 cells are

mixed and fixed with agarose. Au-Fe_3O_4 nanoparticles with various amount of Tf modification are used in MRI experiment for demonstrating that the targeting efficiency can improve image contrast, while same dose of Fe_3O_4 is used. In Figure 3-9 (a), the MRI image of J5 cancer cell without nanoparticles is utilized as reference. With incremental of Tf modification on Au-Fe_3O_4 nanoparticles, the contrast of J5 cancer cell is enhanced largely, as shown in Figure 3-9 (b) to (e). It indicates the high targeting efficiency can improved the contrast of MRI, which is consisted with the cell uptake experiments. On the other hand, the increment of nanoparticles concentration taken up by cell means the development in the targeting efficiency for enhancing the contrast of MRI. Figure 3-9 (f) reveals the relative intensity of sample a - e in Figure 3-9(a) - (e). Enhancement of MRI intensity for sample e is double as large as that for sample b, whose results is consisted with the result of cell uptake experiment, as shown in Figure 3-6(a). Hence, the increasing contrast is ascribed to the targeting efficiency of nanoparticles which is improved by large amount of Tf modification, while same dose of Fe_3O_4 nanoparticles is used. It indicates the loss possibility of nanoparticles in medium or circulation nanoparticlescould be reduced as using nanoparticles with high targeting efficiency.

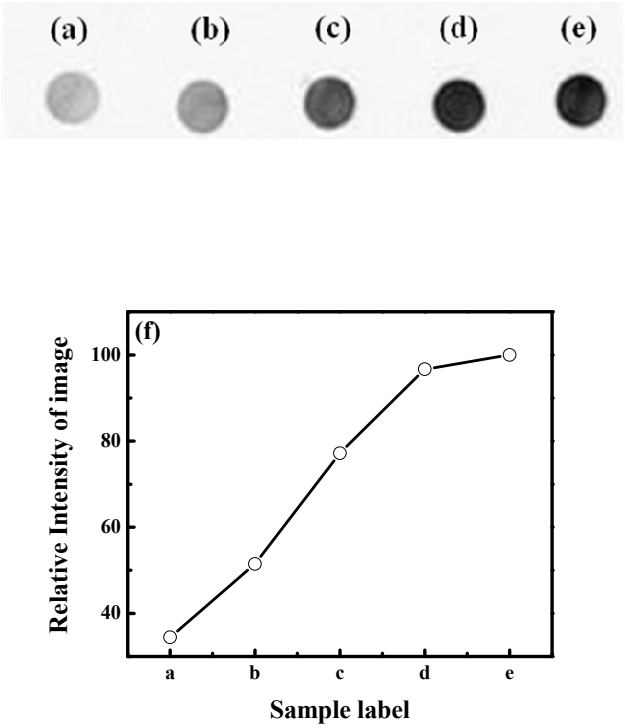

Figure S4. Magnetic Resonance Images of J5 cancer cell (a) without and (b) ~ (e) with treating of Tf-Au-Fe_3O_4 nanoparticles at different amount of Tf modification. (The detailed experiment steps for purifying Tf-Au-Fe_3O_4 nanoparticles are shown in Figure 3-4 (a), where the Tf are treated with 1, 2, 3, 5 mg/mL iminothiolane for (b) ~ (e), respectively). (f) The magnetic hysteresis loop of Tf-Au-Fe_3O_4 nanoparticles for (e). Note that they have same hysteresis. (f) The relative intensity of MRI for each sample(b-e), normalized by the value of sample (e). Label a indicates the only J5 cells is used.

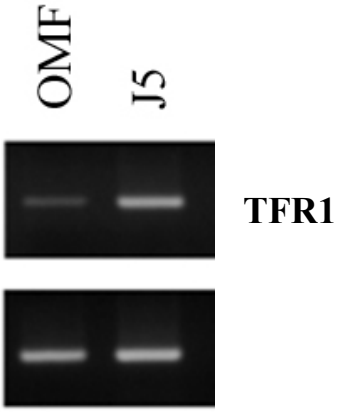

Figure S5. RT-PCR analysis for evaluating TFR1 gene expression of OMF and J5 cell

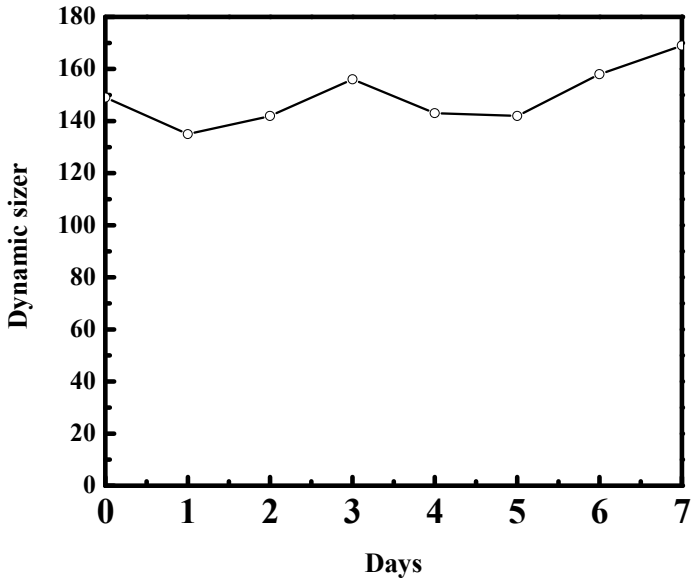

Figure S6. The dynamic size of Tf-Au-Fe_3O_4 nanoparticles for 1~7 day by zetasizer analysis.

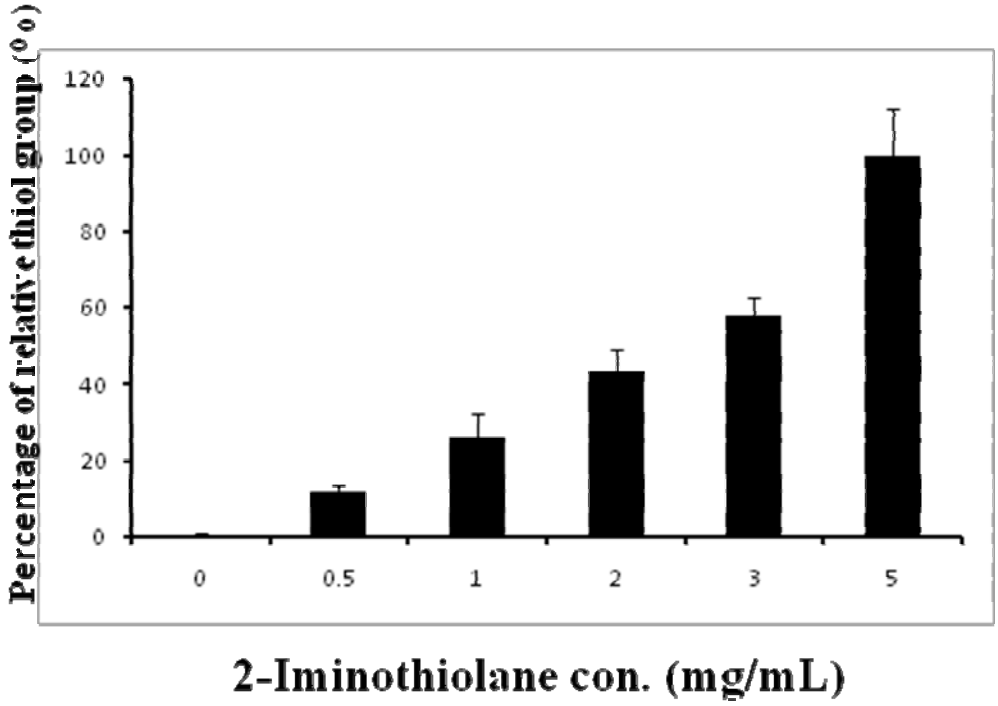

Figure S7. The percentage of relative thiol group of thiolated Tf using 0.5, 1. 2, 3 and 5 mg/mL, analyzed using Ellmann's test.