Supplementary Material

Folate Receptor-Targeting Gold Nanoclusters as Fluorescence Enzyme Mimetic Nanoprobes for

Tumor Molecular Colocalization Diagnosis

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Figure. S1. ¹H NMR spectra of NCs and the NCs-FA nanoprobes.



Figure. S2. Effects of pH (a), salt (b), photo-irradiation (c) and storage time (d) on the fluorescence intensity of the NCs and the NCs-FA nanoprobes.



Figure. S3. Cytotoxicity studies of NCs and the NCs-FA nanoprobes. (a) HepG2, (b) MCF-7 and (b) 293T tumor cells in 96-well plates were incubated with NCs and the NCs-FA nanoprobes in a wide range of concentrations for 24 h. Error bars represent the standard deviation of five measurements.



Figure. S4. Histological changes in the heart, liver, spleen, lung and kidney of the rat 30 days after intravenous injection of a high dose of the NCs-FA nanoprobes. These organs are stained with H&E. The control shows the organ images of the rat without injection of the NCs-FA nanoprobes.



Figure. S5. Effects of salt (a) and photo-irradiation (b) on the catalytic activity of the NCs and the NCs-

FA nanoprobes.



Figure. S6. Effect of H_2O_2 (a, b) and TMB (c, d) concentration on the reaction rate of TMB oxidation catalyzed by the NCs-FA nanoprobes (a, c) and HRP (b, d).



Figure. S7. (a) Peroxidase activity of the NCs-FA nanoprobes with (right tube) or without (left tube) the •OH scavenger ethanol. The NCs-FA nanoprobes catalyzed the oxidation of peroxidase substrate TMB in the presence of H_2O_2 to give a colored product. (b) Absorbance of the TMB reaction solution catalytically oxidized by the NCs-FA nanoprobes with or without the •OH scavenger ethanol.



Figure. S8. Western blot of FR expression in H460, MCF-7, MDA-MB-231, SKOV3 and HepG2, actin was used as a loading control.



Figure. S9. The tumor targeting capability of the NCs-FA nanoprobes in tumor cell. The absorption values at 652 nm after 600 s depend on the number of MCF-7 cells. The data were represented as mean \pm standard deviation, n=5/group.



Figure. S10. Tumor-targeting investigation of the NCs-FA nanoprobes in nude mice bearing MCF-7 and HepG-2 tumor xenograft monitored by NIR fluorescence imaging system. (a) NIR Fluorescence images of the MCF-7 tumor-bearing mice model after administration of the NCs-FA nanoprobes within 48 h. (b) NIR Fluorescence images of the HepG-2 tumor-bearing mice model after administration of the NCs-FA nanoprobes within 48 h. (c) The MCF-7 tumor-bearing mice and (d) HepG-2 tumor-bearing mice were sacrificed and performed a thoracotomy at 6 h post injection. Red dotted circle indicate the tumor site.



Figure. S11. False-positive and false-negative of IHC. The section from MCF-7 and HepG-2 tumor tissues were stained with IHC, and fixated 1 min, 10 min and 30 min, respectively. HepG-2 tumor section showed negative staining, negative staining and positive staining with HRP-peroxidase staining for 1 min, 10 min and 30 min. On the contrary, MCF-7 tumor section showed negative staining, positive staining and positive staining in and 30 min.