Supporting Information

Title: A simple aptamer-functionalized gold nanorods based biosensor for sensitive detection of MCF-7 breast cancer cells

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Experimental Section

Preparation of the Biosensor Assay. Gold nanorods (GNRs) were synthesized according to our previously reported the seed-mediated surfactant-directed approach in a two-step procedure.[1,2] The functionalization of GNRs with Mucin 1 protein (MUC-1) aptamer and the detection of MCF-7 cells proceeded as follows. After the excess cetyltrimethylammonium bromide (CTAB) had been removed, the GNRs were concentrated to *ca.* 1.6 mM by sonication and centrifugation before use. Before functionalization, the disulfide bonds between MUC-1 aptamers were reduced by dithiothreitol (DTT) treatment. DTT treatment was done by mixing 20 μL of 100 μM aptamer solution (Table S1) with 80 μL of 0.15 M DTT, in the presence of 0.18 M phosphate buffer saline (PBS, pH=8.0) for 2 h at room temperature (RT). The aptamer was further purified by passing through a desalting NAP-5 column with 500 μL of the effluent collected. The freshly deprotected aptamer solution was mixed immediately with isometric GNRs concentrations and allowed to stand for 0.5 h, followed by treatment with 10 mM sodium hexametaphosphate solution and allowed to react at RT under continuous shaking for 24 h. Then, 0.4 mg mL^{-1} aqueous solution of mPEG-thiol (Mw=5000) was added to the functionalization system to block the active sites of the surface of GNRs overnight. After washing twice, the aptamer-functionalized GNRs were redispersed in

deionized water. Finally, the aptamer-functionalized GNRs were directly added into the above assay system to capture MCF-7 cells, and the mixture were incubated for a certain period at 37 ℃ in a humidified atmosphere containing 5% CO_2. After three times of centrifugation and washing with PBS (pH=7.4), the conjugations of aptamer-functionalized GNRs and MCF-7 cells were then diluted in PBS, whose LSPR signal were read out for the following analysis.

Specificity Experiment. Female nu/nu athymic mice (7 weeks old) were obtained from Charles River Laboratories. The mice (five per cage) were housed in cages equipped with air filter lids and maintained under pathogen-limiting conditions. MCF-7 cells (1×10^7/mL) were inoculated into the mammary fat pads of the mice. Approximately two weeks later, tumor masses and tumor adjacent tissues were collected from mice, cut into small pieces, and incubated with collagenase D/DNase I mixture (1670 ManDI U/ml of collagenase D, 0.2 mg/ml DNase in HBSS buffer) at 37 ºC for 30 min with gentle shaking. Subsequently, incubating solutions were pipetted up and down multiple times to disaggregate tumor cells and filtered with a 70 μm strainer. Single cells were suspended with PBS for successive detection by MUC-1 aptamers functionalized GNRs. All animal studies were conducted in compliance with the guidelines of NBCDSER and the Beijing Institute of Transfusion Medicine.

Table S1 The related DNA sequences used in this work

APTAMERS	LENGTH	SEQUENCE (5'-3')
COMPLEMENTARY	35-MER	SH(C6)-AAA AAA AAA AGC AGT TGA TCC TTT GGA TAC CCT GG
COMPLEMENTARY WITH FITC(FAM)	35-MER	SH(C6)-AAA AAA AAA AGC AGT TGA TCC TTT GGA TAC CCT GG-(C3)FITC
NON-COMPLEMENTARY	35-MER	SH(C6)-AAA AAA AAA ACG AGA ACA TCC AAA CCT TTG GGA CC
NON-COMPLEMENTARY WITH FITC(FAM)	35-MER	SH(C6)-AAA AAA AAA ACG AGA ACA TCC AAA CCT TTG GGA CC-(C3)FITC

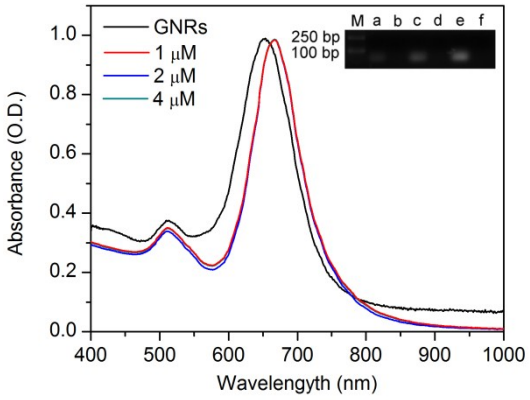

Fig. S1 The LSPR spectra of GNRs before and after functionalization by aptamer at three different concentrations after blocking; Inset: AGE of aptamer solution (a. 1 μM, c. 2 μM, e. 4 μM), and the supernatant after incubation with GNRs at different concentrations (b. 1 μM, d. 2 μM, f. 4 μM).

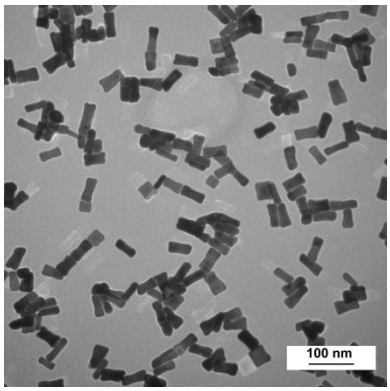

Fig. S2 TEM image of GNRs probes after functionalized by 1 μM MUC-1 aptamer.

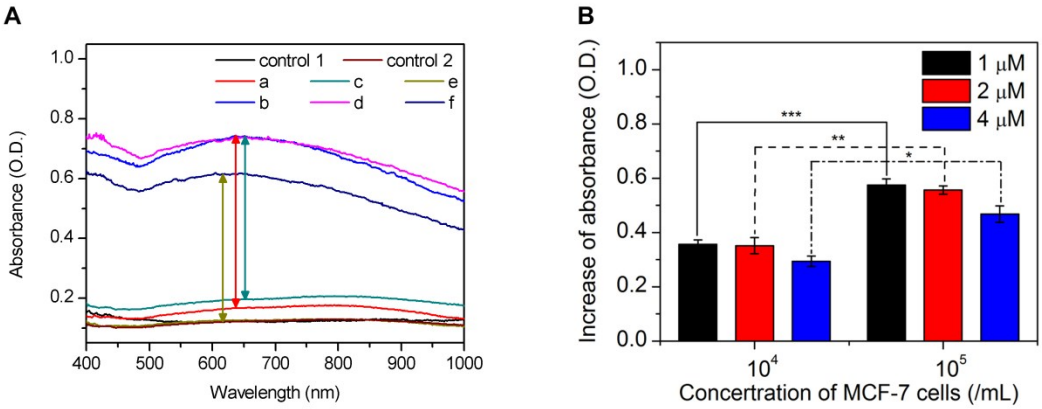

Fig. S3 (A) The LSPR spectra of MCF-7 cells after incubation with GNRs modified by 1 (b) , 2 (d), 4 (f) μM MUC-1 aptamer or HepG-2 cells incubated with GNRs modified by 1 (a), 2 (c), 4 (e) μM MUC-1 aptamers; the LSPR spectra of the MCF7 and HepG2 cells themselves were set as control 1 and control 2; (B) The increased absorbance of MCF-7 cells after incubation with aptamers functionalized GNRs (***$p < 0.001$). Values are expressed as mean ± SD, student's *t*-test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

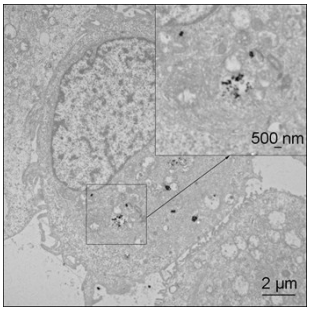

Fig. S4 TEM microimages of aptemer-functionalized GNRs after incubating with MCF-7 cells, there are still some GNRs probes internalized via endocytosis.

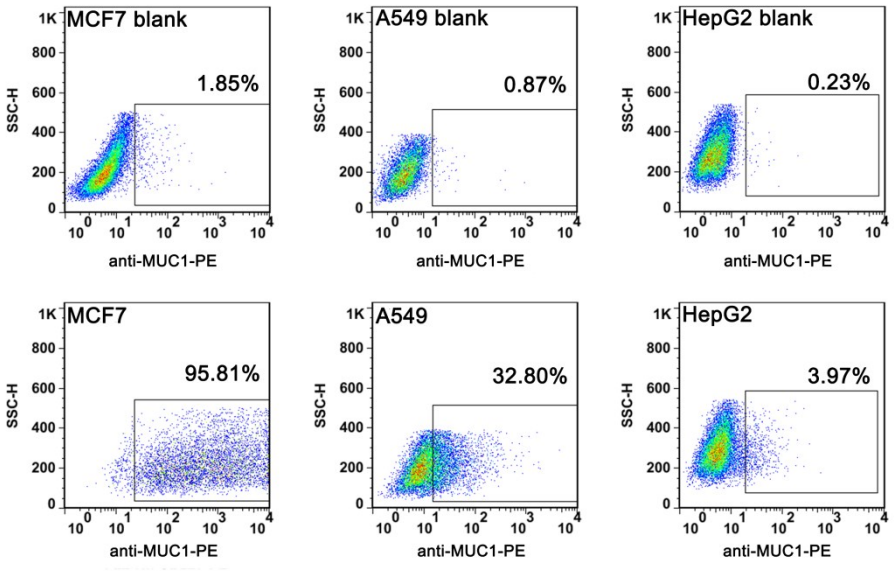

Fig. S5 FACS results in the detection of the surface MUC1 protein in MCF7, A549 and HepG2 cells.

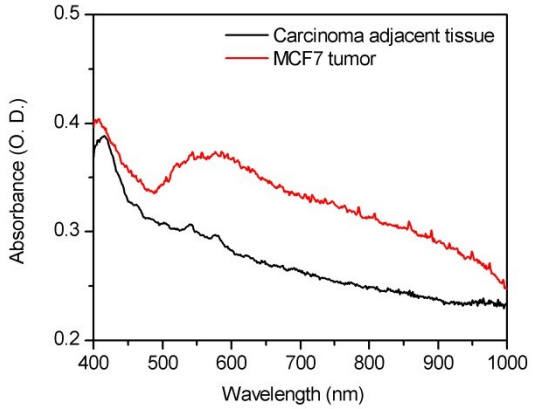

Fig. S6 The LSPR spectra of the biosensor assay in differentiating MCF7 cells and carcinoma adjacent tissue in transplanted breast cancer in nu/nu mice.

References:

1 X. Wang, Y. Li, H. Wang, Q. Fu, J. Peng, Y. Wang, J. Du, Y. Zhou and L. Zhan, *Biosens. Bioelectron.*, 2010, **26**, 404- 410.

2 X. Wang, Y. Li, J. Wang, Q. Wang, L. Xu, J. Du, S. Yan, Y. Zhou, Q. Fu, Y. Wang and L. Zhan. *Analyst.*, 2012, **137**, 4267- 4273.