Electronic Supplementary Material (ESI) for Chemical Communications

Photosensitizer-incorporated G-quadruplex DNA-functionalized magenetic nanoparticles for simultaneous magnetofluorescent imaging and targeted cancer therapy

Experimental section

1. Materials

$FeCl_3 \cdot 6H_2O$, CH_3COONa, ethylene glycol (EG), ethanol and N,N-dimetylformamide (DMF) were purchased from Shanghai Chemical Factory (Shanghai, China).

Succinic anhydride (SA), tetraethoxysilane (TEOS), tris(2,2'-bipyridyl) dichlororuthenium(II) hexahydrate ($Ru(bpy)_3^{2+}$), trisodium citrate dehydrate, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), 3-(aminopropyl) triethoxysilane (APTS), 4-morpholineethanesulfonic acid (MES), 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin (TMPyP4) and N-hydroxysulfosucnimide sodium salt (sulfo-NHS) were obtained from Sigma-Aldrich.

The oligonucleotide used in this article was synthesized by Sangon Biotechnology Inc. (Shanghai, China). The sequence is as follows:

5'-NH_2-T_{10} GGT GGT GGT GGT TGT GGT GGT GGT GGT -3' Aptamer

5'- GGT GGT GGT GGT TGT GGT GGT GGT GGT -3'.

5'-NH_2-T_{10} TTA GGG TTA GGG TTA GGG TTA GGG TTA-3' Free-DNA

All the chemicals were used as received without further purification.

1.2. Apparatus

FT-IR analyses were carried out on a Bruker Vertex 70 FT-IR Spectrometer. SEM images were obtained with a Hitachi S-4800 FE-SEM. Transmission electron microscope (TEM) measurements were made on a HITACHI H-8100 EM with an accelerating voltage of 200 kV. Fluorescence spectra were recorded with a JASCO FP-6500 spectrofluorometer. Hysteresis loops were collected on a Quantum Design superconducting quantum interference device (SQUID) magnetometer (LakeShore 7307). X-ray diffraction (XRD) analysis was carried out on a D/Max 2500V/PC X-ray diffractometer using Cu (40 kV, 30 mA) radiation.

1.3Synthesis of spherical Fe_3O_4 particles

1 g of $Fe(NO_3)_3 \cdot 6H_2O$ and 2.8 g of sodium acetate were dissolved in 40 mL of ethylene glycol (EG) with stirring. After stirred for 30 min, the obtained solution was transferred to a Teflon-lined stainless-steel autoclave and heated at 200 ℃ for 8 h. Then the autoclave was naturally cooled to room temperature. The obtained black magnetite particles were washed with ethanol for several times, and dried in vacuum.

1.4 Synthesis of spherical Fe_3O_4/SiO_2(Ru(bpy))

0.10 g of obtained spherical Fe_3O_4 particles was treated using 0.1 M HCl solution by ultrasonication for 20 min. washed with deionized water, and then well dispersed in the mixture solution of 80 mL of ethanol, 20 mL of deionized water, and 1.0 mL of concentrated ammonia aqueous solution (28 wt.%) at ultrasonic condition . After this, 300μl of TEOS was added dropwise to the solution. After 3 h, then added 3mg $Ru(bpy)_3^{2+}$ and 600μl TEOS. 3 h later, the obtained particles were separated and washed with ethanol and water.

1.5 Synthesis and chemical modification of Fe_3O_4/SiO_2(Ru(bpy)) surface

To modify the silica surface with amino groups, 0.3 mL of APTS was added to 20 mL of toluene solution and stirred for 24 h. After the reaction, the prepared sample washed with ethanol and toluene. The FMN-NH_2 (50 mg) was reacted with succinic anhydride (0.5 g) in DMF (20 ml) under N_2 gas for 8 h with continuous stirring. By doing so, carboxyl groups were formed onto the FMN surface for conjugation of DNA. After a thorough water wash, the carboxylated nanoparticles were activated using EDC (10 mg/ml, 15 ml) and sulfo-NHS (10 mg/ml,15 ml) in a MES buffer (pH 6.0) for 15 min at room temperature with continuous stirring. Twenty microliters of PBS buffer (100mM, pH 7.4) was then added in the mixture, followed by the addition of aptamer or free DNA at room temperature with continuous stirring for 6 h and washing in PBS buffer (0.1 M, pH 7.4) to form the resultant DNA-conjugated nanoparticles (FMN-DNA). The concentration of DNA was calculated spectroscopy using an ε_{260} of 339700 $M^{-1}cm^{-1}$ in DNA bases. Quantification of the covalently bound oligonucleotide was carried out by the difference in absorption of DNA before and after reaction with nanoparticles. According our calculation, the immobilization efficiency of AS1411 was approximately 22.2 $\mu molg^{-1}$ FMNs. Then the no modified DNA was added to form dimer G-quadruplex structure in 28 mM Tris-HCl buffer (200 mM KCl, 4 mM $MgCl_2$). The dimeric G-quadruplex-archored FMNs were separated by magnet

and washed with Tris buffer for several times to remove the free DNA. The FMNs-apt-TMPyP4 (FMNs-apt-TMP) system was obtained by adding TMPyP4 into the FMNs-apt solution in Tris buffer. Then magnetic separation, and washed with Tris-HCl buffer for five times to remove extral TMPyP4. The concentration of TMPyP4 was calculated spectrophotometrically using an ε_{424} of 2.26×10^5 $M^{-1}cm^{-1}$. Quantification of TMPyP4 was carried out by comparing the difference of absorption intensity before and after reaction with FMNs-apt. According to our calculation, the quantity of porphyrin was 0.54 μmol mg^{-1}.

1.6 T_2-weighted imaging

For in vitro MRI, FMNs-apts were dispersed in water with concentrations in the range from 0 to 800 μg mL^{-1}. T_2-weighted images of FMNs-apt with varied concentrations were collected using 1.5 T human clinical scaner.

1.7 Cell culture

MCF7 and 3T3 cell lines were cultured in minimum essential medium (GIBCO; Grand Island, NY) supplement with 10% (v/v) fetal bovine serum, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 250 ng/mL amphotericin, at 37°C and in an atmosphere of 5% CO_2. The culture medium was changed on alternate days until confluence.The following quantities of cells was seeded onto 6-well culture plates: 5×10^4/well MCF7 and 5×10^4/well 3T3. After the cell lines were cultured for 24 h, the medium was replaced with fresh medium containing the FMNs drug and the prepared FMNs-apt complex at a concentration of 100 μg mL^{-1}. Cells were further cultured for 8 h to allow the uptake of the FMNs and the FMNs-apt complex.

1.8 Phototoxicity of the FMN-apt complex on the in vitro systems

The 5×10^3/well MCF7 cells that were suspended in culture medium were seeded onto 6-well plates and further cultured for 24 h at 37°C. The medium was replaced with fresh medium containing the free TMPyP4 drug and the prepared FMNs-apt-TMP complex. The concentration of pure TMPyP4 drug complex was with the equal dosage level of TMPyP4 in FMNs-apt-TMP. Cells were further cultured for 4 h to allow the uptake of TMPyP4 and the FMNs-apt-TMP complex. Cells were washed twice with PBS and then exposed to blue light equipment (PCI Biotech AS, Norway Lumisource) at a wavelength of 435 nm and a fluency rate of 200 mW cm^{-2} for 5 minutes. After light treatment, cells were reincubated in fresh medium for 36 h. Cell viability

was determined by trypan blue exclusion in a haemocytometer chamber. Control experiment was performed by cells cultured with FMNs-apt-TMP complex for 48h without light exposed.

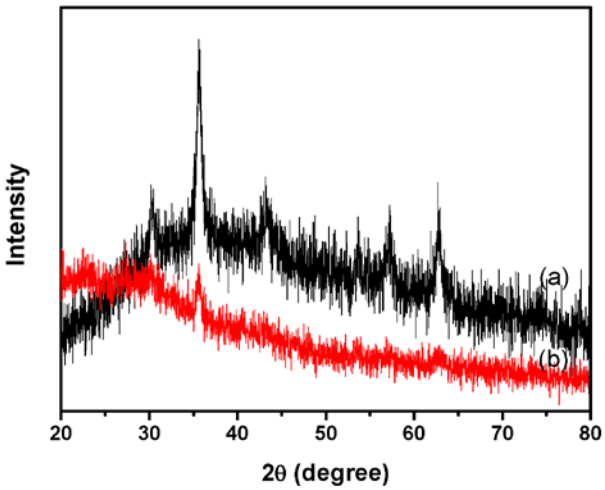

Fig. S1 XRD patterns of a) Fe3O4 b) Fe_3O_4/SiO_2 $(Ru(bpy)_3^{2+})$.

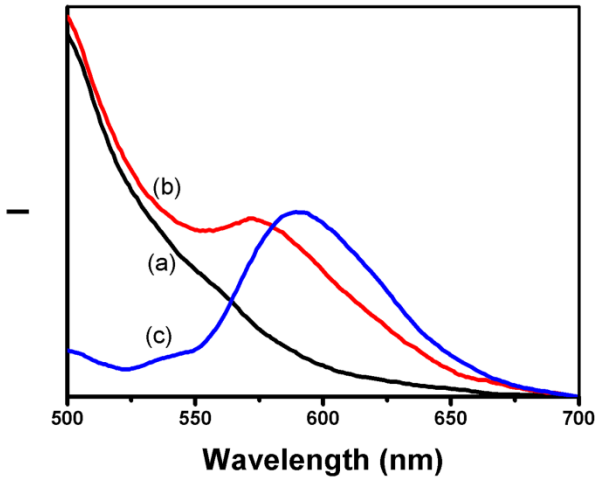

Fig. S2 Fluorescence images of a) Fe_3O_4 (blak), b) Fe_3O_4/SiO_2 $(Ru(bpy)_3^{2+})$ (red) and c) $Ru(bpy)_3^{2+}$ (blue).

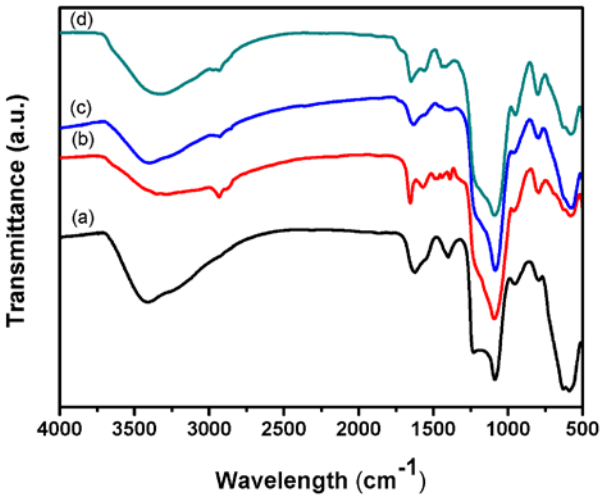

Fig. S3 FTIR spectra of the samples a) Fe_3O_4-Ru, b) Fe_3O_4-Ru -NH_2, c) Fe_3O_4-Ru -COOH and d) Fe_3O_4-Ru –DNA.

The FTIR spectrum of FMN-NH_2 exhibited absorption bands in the regions of amide I (1635 cm^{-1}), amide II (1560 cm^{-1}), and the emerging absorption band at around 1720 cm^{-1} in the sample FMN-COOH could be assigned to C=O stretching of the carboxyl groups contained within the attached succinic acid molecules. The efficient grafting of DNA onto the FMNs was validated by the appearance of an enhanced band at 1562 cm^{-1}, which was characteristic of acylamide vibration. Further, energy-dispersive X-ray (EDX) analysis of FMN-DNA nanoparticles confirmed the presence of phosphorus element, which indicated that DNA was covalently anchored on the surface of FMNs.

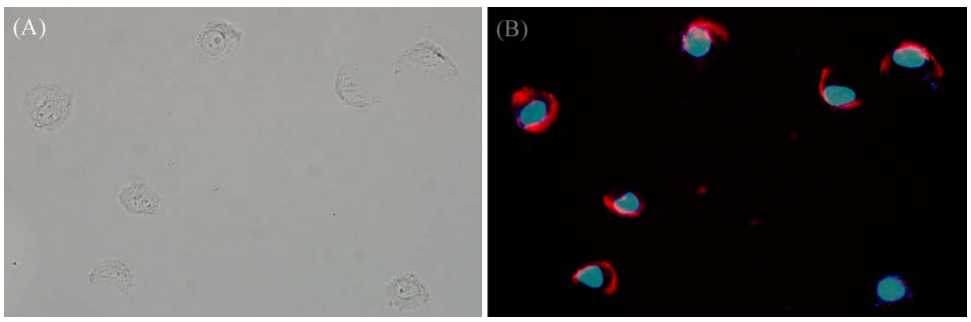

Fig. S4 Fluorescence images of MCF-7 cells treated with FMNs-apt. (A) bightfield, (B) fluoresent imaging with nuclear was washed by DAPI.

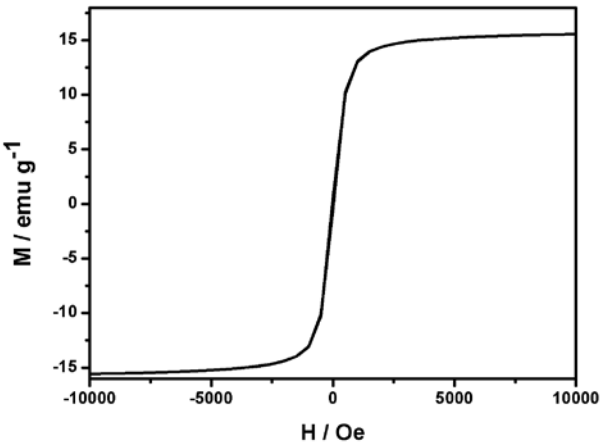

Fig. S5 Magnetization isotherm for FMNs-apt.

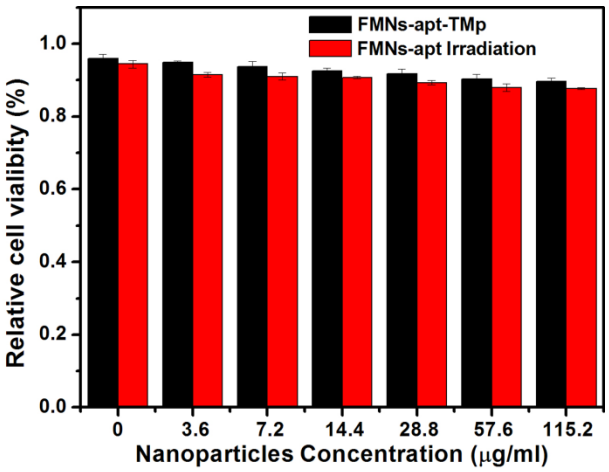

Fig. S6 The control of cytotoxicity without light irradiation of FMNs-apt-TMP and with light irradiation of FMNs-apt .

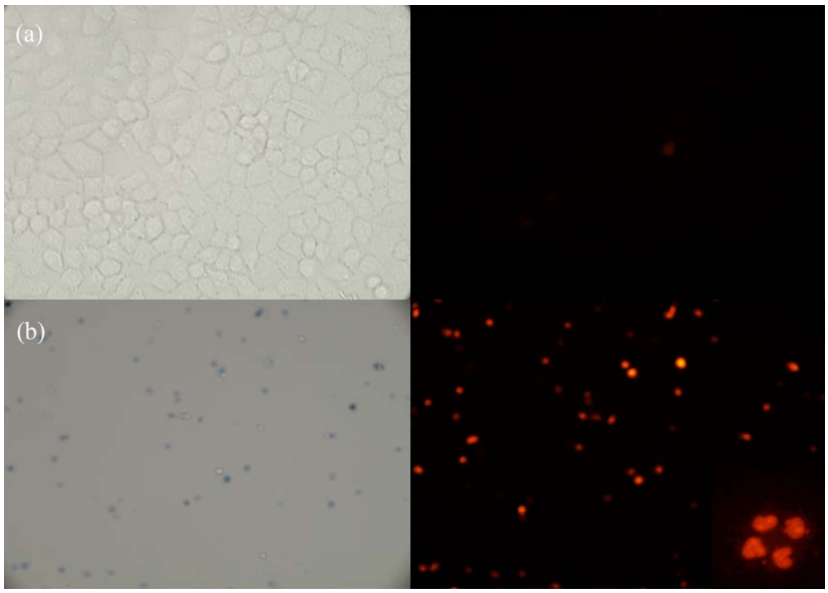

Fig. S7 Fluorescence images of MCF7 cells without (a) and with (b) FMNs-apt-TMP after irradiation in bright field and fluorescence.