Supplementary Imformation

Multifunctional imaging probe based on gadofulleride nanoplatform

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1. Materials, instruments and methods.

Materials and instruments: Endohedral metallofullerene Gd@C_{82} was synthesized and purified as reported,[1] other reagents and organic solvents were all commercially available and used as received. Water was purified with Millipore Milli-Q Synthesis purifier (18.0 MΩ cm, Barnstead). Dynamic Light Scattering (DLS) was carried out on a Malvern Instruments Zetasizer Nano ZSI at a 173° scattering angle. X-ray photoelectron spectroscopy analysis was performed on ESCALab220i-XL (VG Instruments). The relaxation times were measured at 0.5 T with NMI20 Analyst (Shanghai Niumag Corporation, Shanghai, China).

Methods:

XPS characterization: The chemical structure of f-Gd@C_{82} was characterized using X-ray photoelectron spectroscopy (XPS). As shown in Fig. S1, the main line of the XPS spectrum exhibits a broad and asymmetric line-shape. Through the curve fitting, C1s peaks centered at binding energy of 284.8, 286.2 and 288.3 eV appear and should assign for C-C, C-O and C=O bonds, respectively, suggesting the existence of carboxyl groups and hydroxyl groups on the carbon cage. The intensities of these C1s components in f-Gd@C_{82} were estimated from area integrals under each line to be 68.8%, 16.4%, and 14.8%, respectively. Thus, f-Gd@C_{82} can be designated as Gd@$C_{82}(OH)_{\sim 24}(CH_2CH_2COOH)_{\sim 22}$.

Longitudinal relaxivity measurement and MR imaging: The longitudinal relaxiation times were measured in distilled water at 0.5 T (Niumag NMI20-Analyst) at 37 ^{o}C, using an inversion recovery (IR) sequence. Solutions in different Gd concentrations were prepared by diluting the stock solution with deionized water. The concentrations of Gd were determined by ICP-AES. The measurement parameters at IR sequence were shown as follows: T_R= 3000 ms, D_1 = 1 ms, Add D_1 = 100 ms, N_{IR} = 40 per acquisition, the number of acquisition = 4.

MR Images were performed on a Niumag NMI20-Analyst (22 MHz, 0.5 T, 37 ^{o}C). The magnet was equipped with the standard gradient set and 15 mm internal diameter volume coil. Aqueous solution of EMF-FA-FITC in 0.1 mM was prepared. Meanwhile, a sample of distilled water and an aqueous solution of commercial Gd-DTPA in 0.5 mM were used as control. The measurement conditions were as follows: T_1-weighted sequence: Spin echo, T_R = 1500 ms, T_E = 17 ms, D_0 = 400 ms.

Fluorescent image of EMF-FA-FITC: The purified EMF-FA-FITC shows distinct fluorescent signals in view field under excitation wavelength at 488 nm, whereas the control sample that collected after dialyzing free 5-FITC under the same condition shows no fluorescent signal because 5-FITC in the low molecular weight can be completely removed during dialysis process.

Cell culture: The Hela cells were cultured with Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, USA) supplemented with 10 % fetal bovine serum (Hyclone Company, South Logan, UT), penicillin (100 μg/mL), and streptomycin (100 μg/mL) (Gibco, Grand Island, N. Y. USA) in 5% CO_2 at 37 ^{o}C in a humidified incubator.

TEM characterization: Cells with incubation of EMF-FA-FITC and EMF-FITC for 3 h, respectively, were washed by Hank's balanced salt solution (HBSS) to remove all noninternalized particles and fixed overnight in 4 % glutaraldehyde and then in 4 % paraformaldehyde at 4 ^{o}C. After that, cells were centrifugated under 1000 rpm for 5 minutes, and washed with 0.1 M phosphate-buffered saline (PBS) three times. All the cells were fixed in 3 % glutaraldehyde containing blood plasma in small amount overnight at 4 ^{o}C for coagulating. Afterwards the cells were post-fixed with 1 % osmium tetraoxide for 2 h at room temperature in 1 mm^3 masses. The cells were then dehydrated in a graded series of ethanol and acetone and embedded in Epon812. Ultra-thin section were cut by ultramicrotome (UC6, Leica Ltd. Co, Germany), and transferred onto 200-mesh copper grids, strained with uranyl acetate and lead nitrate and observed with a H-7650 TEM (Hitachi Ltd. Co, Japan).

2. Synthesis details.

Synthesis of f-Gd@C$_{82}$: In a typical synthesis procedure, functionalized Gd@C$_{82}$ was synthesized and purified similar to literature reported.[2] In brief, Gd@C$_{82}$ (ca. 3 mg) in 5 mL of *o*-dichlorobenzene was added succinic acid peroxide (3.2 mg, 5 equiv.). The resultant solution was deaerated with flowing argon and heated at 84^{o}C overnight. Then, 15 mL of 0.2 M NaOH aqueous solution was added to extract the water-soluble product. Two layers were obtained; the top layer was deep brown and the bottom layer was colorless. The top layer was concentrated, and the residue was purified via a Sephadex G-25 (Pharmacia) size-exclusion gel column with pure water as eluent to obtain a narrow brown band with pH = 6-7.

Synthesis of FA-ED (ethylenediamine modified folic acid): 88.4 mg of folic acid (0.2 mM) was dissolved in 5 mL of DMSO in a round-bottomed flask under stirring at 40 ^{o}C. A combination of 2.0 molar equivalent of DCC and NHS were then added to the solution, which was stirred for 12 h. The resulting solution was centrifuged at 10,000 for 1 min and the supernatant was filtered using a syringe filter with pore size of 0.22 μm. Then 5 molar equivalent of ethylenediamine was added to the filtrate and stirred for 3 h. The solution was precipitated using 50 mL of acetone/ether mixture (V/V = 3:7). The precipitation was collected through Buchner funnel and washed with ether.

Synthesis of EMF-FA: For further conjugation, f-Gd@C$_{82}$ (ca. 4 mg, 0.002 mM) was dissolved in 10 mL of PBS buffer (pH = 7.4) under stirring. Then 19.1 mg of EDC and 11.5 mg of NHS were added to the solution to activate the carboxyl groups of gadofulleride. After 10 min, FA-ED in 500 μL of DMSO solution was added to the solution while stirring. After 4 h, the resulting solution was dialyzed with NaOH solution (pH = 9) and distilled water, respectively, yielding the pure EMF-FA.

Synthesis of EMF-FA-FITC: The as-prepared EMF-FA solution was evaporated and then dissolved in 10 mL of MES buffer (pH = 6.5). 19.1 mg of EDC and 11.5 mg of NHS were added to the solution while stirring. After 10 min, 5-FITC (5 mg/mL) in 7 μL of DMF solution was added to the stirring solution and the resulting solution was striring in the dark for 3h, and dialyzed with distilled water subsequently, yielding the pure EMF-FA-FITC.

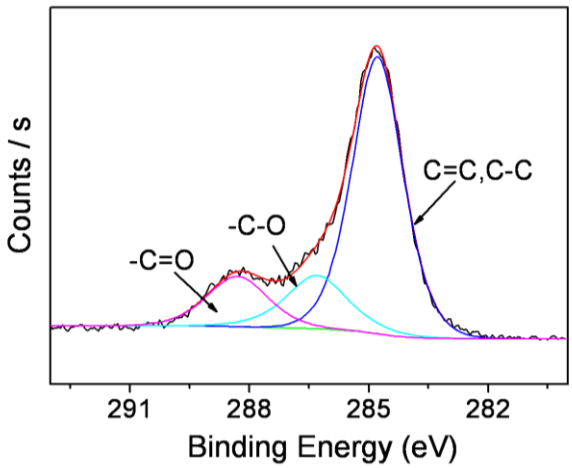

Fig. S1 Curve fitting of C1s binding energy in f-Gd@C_{82}.

Table S1. Fitting of C1s binding energy

Bond (C1s)	Binding Energy (eV)	Area	FWHM (eV)	%GL (%)
C-C,C=C	284.8	9886.5	1.6	80
C-O	286.4	2356.4	1.8	80
C=O	288.2	2134.2	1.8	80

Combined with the area integral of C_{1s} binding energy under different chemical environment, the average molecular formula can be designed as $Gd@C_{82}(OH)_{24}(CH_2CH_2COOH)_{22}$.

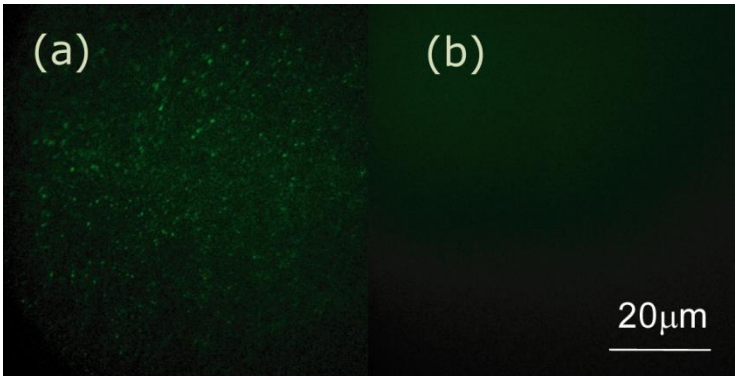

Fig. S2 Fluorescent image of EMF-FA-FITC (a) and control (b) under excitation of 488 nm.

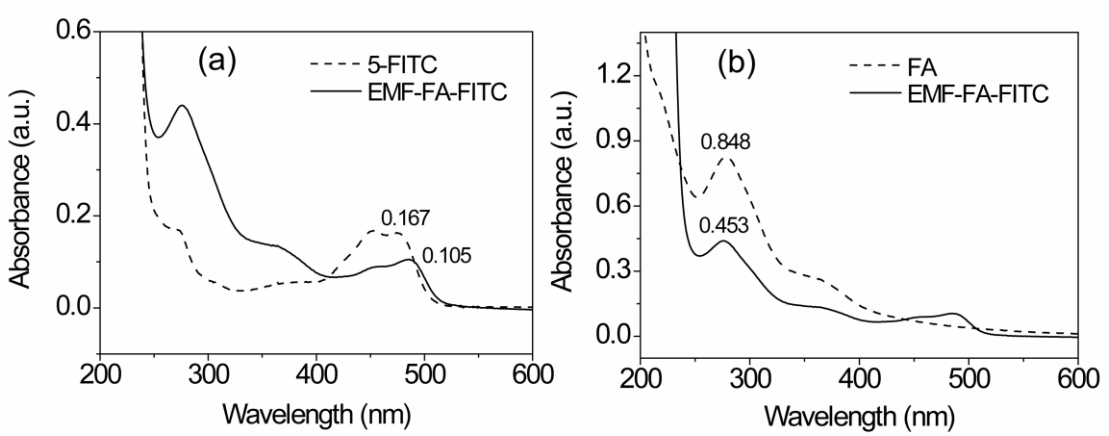

Fig. S3 Relative UV absorbance of 5-FITC (a) and FA (b), the concentration of 5-FITC, FA and EMF-FA-FITC were 0.0088mM, 0.022mM and 0.003 mM, respectively, for the estimation of conjugated FA and FITC ratio on f-G@C_{82}.

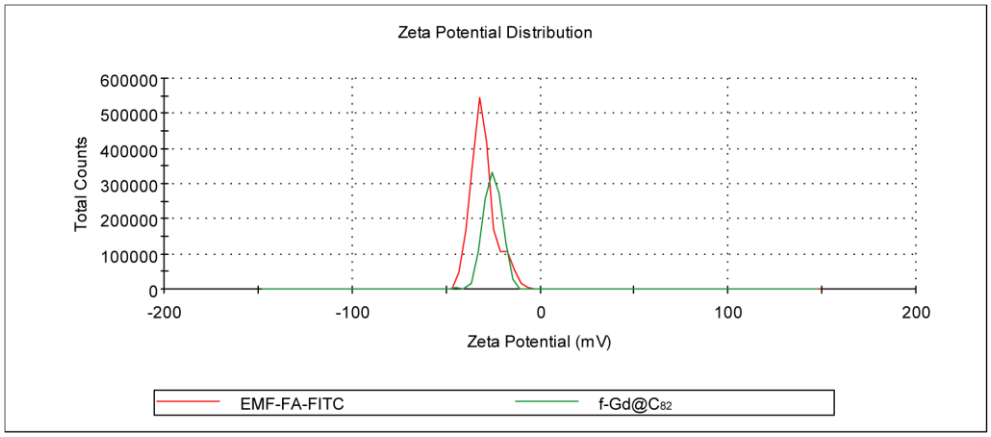

Fig. S4 Zeta potential of f-G@C_{82} (green) and EMF-FA-FITC (red)

References:

1 D. W. Cagle, S. J. Kennel, S. Mirzadeh, J. M. Alford and L. J. Wilson, *Proc. Natl. Acad. Sci.USA*, 1999, **96,** 5182.

2 C. Y. Shu, F. D. Corwin, J. F. Zhang, Z. J. Chen, J. E. Reid, M. H. Sun, W. Xu, J. H. Sim, C. R. Wang, P. P. Fatouros, A. R. Esker, H. W. Gibson and H. C. Dorn, *Bioconjugate Chem.*, 2009, **20**, 1186.