Electronic Supplementary Information

Silica Nanoparticle Coated Perfluorooctyl Bromide for Ultrasensitive MRI

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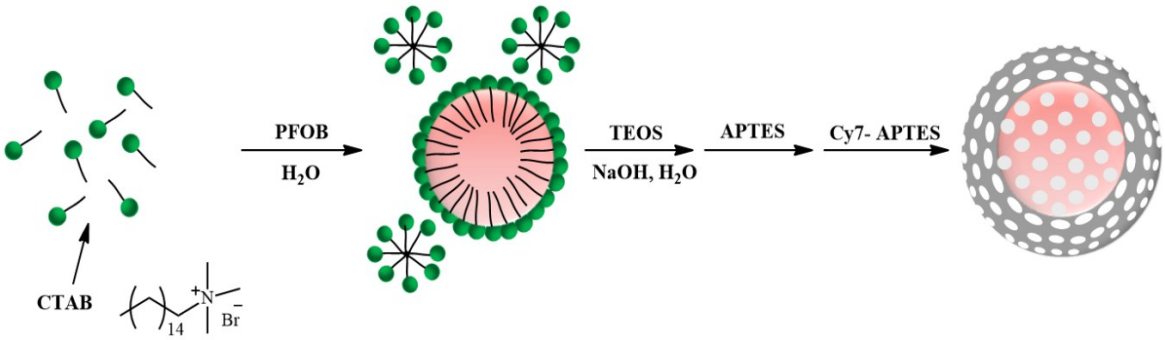
CONTENTS

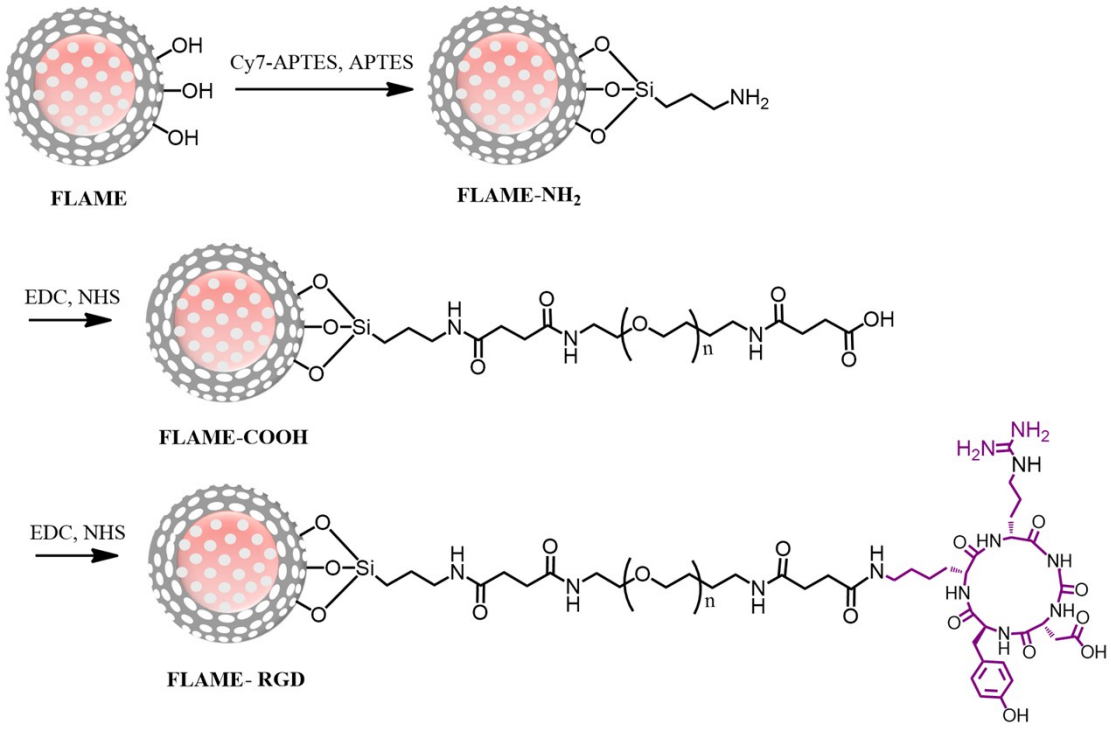
1. Materials

(3-Aminopropyl)trimethoxysilane(APTES) (97%), n-cetylammonium bromide (CTAB) (≥99.0%), 1-Bromoheptadecafluorooctane （98%）, were purchased from J&K Scientic Ltd. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl), N-Hydroxysuccinimide (NHS), Peptide cyclo-(Arg-Gly-Asp-D-Tyr-Cys) (c-(RGDyC)) was purchased from GL Biochem (Shanghai, China). The aforementioned reagents were used as received without further purification. Cy7-Cl was prepared according to the previous report.[s1]

2. Synthesis and characterization



Scheme S1 Synthetic scheme of FLAME



Scheme S2. Synthetic scheme of FLAME-RGD

2.1 Synthesis of FLAME

CTAB (50 mg) was first dissolved in deionized water (6 mL). PFOB (1 mL) was added to the aqueous CTAB solution and the resulting solution was stirred for 2 h at 50°C. The aqueous CTAB-PFCE solution was filtered through a 0.45-µm syringe filter to remove large aggregates or contaminants. The aqueous CTAB-PFCE solution was added to a solution of deionized water (23 mL) and 2 M sodium hydroxide (0.15 mL), and heated to 70°C with stirring. Then, TEOS (0.25 mL) was added dropwise and the resulting solution was stirred for 4 h. The synthesized materials were centrifuged (3000 rpm, 10 min), and H_2O (20 mL) three times and dispersed in 40 mL of H_2O.

2.2 Synthesis of FLAME-NH_2

FLAME dispersed in H_2O (40 mL) was heated to 80°C with stirring. Then, APTES (100 µL) was added slowly into the resulting solution and stirred for 4 h under N_2. The resulting materials were washed with H_2O three times. The resulting materials were centrifuged (3000× rpm, 10 min) and washed with H_2O (20 mL) three times. FLAME-NH_2 was subsequently dispersed in 20 mL of H_2O.

2.3 Synthesis of FLAME-Cy7

Cy7-Cl (5.0 mg) was reacted with APTES (10 µL) in MeOH (10 mL) at 40 °C for 2 h. Then, FLAME-NH_2 in H_2O were added and stirring for 3 h under an N_2 atmosphere. The resulting materials were separated by centrifugation (3000 × rpm, 10 min) and washed with H_2O (5 mL) three times. FLAME-Cy7 was dispersed in 10 mL of H_2O.

2.4 Synthesis of FLAME-COOH

FLAME-NH_2 or FLAME-Cy7 dispersed in H_2O (20.0 mL) was heated to 40°C while stirring. Then, Carboxyl-PEG (0.50 g), EDC.HCl (0.5 g) and NHS (0.1 g) were and stirred for 1 d under an N_2 atmosphere. The resulting materials were separated by centrifugation (3000 × rpm, 10 min) and washed with H_2O (5 mL) three times. FLAME-COOH was dispersed in 10 mL of H_2O.

2.5 Synthesis of FLAME-RGD

FLAME-COOH dispersed in H_2O (10.0 mL) was heated to 40°C while stirring. Then, RGD (10 mg), EDC.HCl (0.5 g) and NHS (0.1 g) were and stirred for 1 d under an N_2 atmosphere. The resulting materials were separated by centrifugation (3000 × rpm, 10 min) and washed with H_2O (5 mL) three times. FLAME-RGD was dispersed in 10 mL of H_2O.

2.6 Characterization of FLAME and FLAME-RGD

The size distribution and ζ-potential of FLAME and FLAME-RGD were measured by Dynamic light scattering (DLS; Nano ZS 90, Malvern, UK; Figure S3, S7, S8) and transmission electron microscopy (TEM; Nano ZS 90, Malvern, UK; Figure s3). FLAME and FLAME-RGD nanoparticles were suspended in water at 25°C for the DLS measurements. For TEM measurements, fresh prepared FLAME nanoparticles were suspended in water and loaded on copper mesh a day before test. TEM size distribution was measured by image analysis software (Nano Measurer 1.2). The size distribution was then fitted to the Gaussian function:

$y=y_0+A \times \exp(-0.5 \times ((x-x_c)/w)^2)$.

Xe atoms were mainly accommodated in the PFOB liquid core. The diameter of PFOB liquid core was also measured by image analysis software (Nano Measurer 1.2). The volume equivalent diameter of PFOB liquid core was given by $D = \left(\sum_{i=1}^{N} (d_i)^3 \Big/ N \right)^{\frac{1}{3}}$

Where D is volume equivalent diameter of PFOB liquid core, d_i is the diameter of the i-th PFOB liquid core in TEM image, N is the total number of PFOB liquid core in TEM image. From the calculation result, the equivalent diameter of PFOB liquid core is 253 nm.

N_2 adsorption/desorption isotherms of FLAME were recorded by Porosity Measuring Equipment (ASAP2010, Micromeritics; Figure S4a). Fluorescence spectra of FLAME were measured by fluorescence spectrophotometer (FS5, Edinburgh, UK; Figure S4b). The slit width was 5.0 nm for both excitation and emission. FLAME nanoparticles were suspended in water for the fluorescence spectra measurements.

3. ^{129}Xe exchange kinetics of FLAME

3.1 Hyper-CEST spectra

Multiple CEST spectra acquired with different saturation pulse strengths could be employed to quantify multiple parameters in exchanging system.[S3,S4] To quantify the exchange kinetics of ^{129}Xe in FLAME, the Hyper-CEST spectra were acquired by ^{129}Xe NMR experiments using 5 s saturation pulse with Five different saturation pulse strengths (2/3/4/6/8 μT). The entire CEST spectra contain 65 saturation offsets in the frequency range of 40-250 ppm. Additional ^{129}Xe NMR measurements without saturation were acquired for data normalization. The normalized Hyper-CEST spectra were then fitted to the Bloch-McConnell (BM) equation for the case of hyperpolarized nuclei.[S4]

3.2 BM equation

Without loss of generality, it is assumed the saturation pulse is applied along the x-axis. In the rotating lab frame, we use superscripts a and b to show parameters of bulk pool (free ^{129}Xe in solution) and dilute pool (captured ^{129}Xe), respectively. The BM equation in matrix form are the following:[S5]

$$\frac{dM(t)}{dt} = A \cdot M$$

Where

$$A = \begin{vmatrix} -R_2^a - k_{ab} & \Delta\omega_a & 0 & k_{ba} & 0 & 0 & 0 \\ -\Delta\omega_a & -R_2^a - k_{ab} & \omega_1 & 0 & k_{ba} & 0 & 0 \\ 0 & -\omega_1 & -R_1^a - k_{ab} & 0 & 0 & k_{ba} & R_1^a M_0^a \\ k_{ab} & 0 & 0 & -R_2^b - k_{ba} & \Delta\omega_b & 0 & 0 \\ 0 & k_{ab} & 0 & -\Delta\omega_b & -R_2^b - k_{ba} & \omega_1 & 0 \\ 0 & 0 & k_{ab} & 0 & -\omega_1 & -R_1^b - k_{ba} & R_1^b M_0^b \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{vmatrix}$$

and

$$M(t) = \left| M_x^a(t) \quad M_y^a(t) \quad M_z^a(t) \quad M_x^b(t) \quad M_y^b(t) \quad M_z^b(t) \quad 1 \right|^T$$

Where M_0^a and M_0^b denote the thermal equilibrium magnetizations of the free ^{129}Xe and captured ^{129}Xe, respectively. f_B is given by M_0^b/M_0^a, which is defined as the ratio of capture ^{129}Xe and free ^{129}Xe. R_1^a, R_2^a, R_1^b, and R_2^b represent the longitudinal and transverse relaxation rates of free ^{129}Xe and captured ^{129}Xe, respectively. k_{ab} is the exchange rate from free ^{129}Xe to captured ^{129}Xe and vice versa, and $k_{ab} = f_B k_{ba}$. $\Delta\omega_a$ and $\Delta\omega_b$ are given by $\omega_a - \omega_{rf}$ and $\omega_b - \omega_{rf}$, where ω_a and ω_b are the Larmor frequencies of free ^{129}Xe and captured ^{129}Xe, respectively, and ω_{rf} is the frequency of the saturation pulse. ω_1 is the amplitude of the saturation pulse. $M_x^a(t)$, $M_y^a(t)$, $M_z^a(t)$, $M_x^b(t)$, $M_y^b(t)$, $M_z^b(t)$ represent the magnetization of free ^{129}Xe and captured ^{129}Xe in x/y/z directions at time t.

The solution of the above BM equation can be given by

$$M(t) = e^{At_{sat}} \cdot M(0)$$

t_{sat} is the saturation time and $M(0)$ is the initial value of $M(t)$ at $t = 0$.

For the case of hyperpolarized nuclei

$$M(0) = \left| 0 \quad 0 \quad P \cdot M_0^a \quad 0 \quad 0 \quad P \cdot M_0^b \quad 1 \right|^T$$

Where P represents the signal enhancement factor of ^{129}Xe via the optical pumping techniques.

The simulated Hyper-CEST spectra are given by

$$Z(\omega_{rf}) = \frac{M_z^a(\omega_{rf})}{P \cdot M_0^a}$$

3.3 Fitting to BM equation

Due to the strong signal enhancement of hyperpolarization, the magnetization of HP ^{129}Xe is always much larger than the thermal equilibrium magnetization.[s3] For simplicity's sake, the value of P is set to 10,000; After Xenon gas delivery to the sample, several ^{129}Xe NMR spectra were acquired with different delay times (T_d) to estimate the R_1^a value of the free ^{129}Xe in solution. For the case of hyperpolarized nuclei, the relationship between signal intensity and

delay time can be given by

$$I(T_d) = I(0) \cdot e^{-R_1^a T_d} \quad (1)$$

$I(T_d)$ is the signal intensity with delay time T_d, $I(0)$ is the signal intensity without delay time. The R_1^a value of 0.0069 s^{-1} was obtained by fitting the NMR signal intensity for different delay times to equation 1 (Figure S5).[S4]

The multiple Hyper-CEST spectra were fitted to the BM equation (Figure S6); the value of R_1^a was kept constant, while other parameters were varied in the fitting. The fitted parameter values were listed in Table S1.

4. *In vitro* study of FLAME-RGD

4.1 Cell culture

The non-small-cell lung cancer A549 cells and breast cancer McF7 cells were purchased from the cell bank of Chinese Academy of Sciences (Shanghai, China). The tumor cells were cultured in IMDM (Iscove's Modified Dulbecco's Medium, Boster, China), supplemented with 10% fetal bovine serum (Boster, China), 100 U/mL penicillin (Boster, China) and 100 U/mL streptomycin (Boster, China) in a humidified air with 5% CO^2 at 37 ^oC.

4.2 Cytotoxicity assay

The cytotoxicity of FLAME-RGD on A549 cells was determined by the standard methylthiazolytetrazolium (MTT) assay. A549 cells were plated into 96-well culture plates and incubated overnight. FLAME-RGD samples were diluted with RPMI-1640 media to different concentration (The concentration of PFOB, C_{PFOB}, is from 0.05 to 12.9 mM) and added to the wells. The concentration of PFOB in FLAME-RGD was determined by measuring the ^{19}F NMR signal intensity of PFOB at -83 ppm (^{19}F NMR signal intensity of trifluoroethanol at -76 ppm as internal standard). After incubated with cells for 2 h at 37 ^oC, the sample in each well was washed 2 times with PBS, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added to the culture medium (final concentration, 0.5 mg/mL). 200 μL DMSO was added to each well to thoroughly dissolve the formazan crystals after removal of the medium. Then the cell viability was determined by reading optical densities at 490 nm using ELISA plate reader (Molecular Devices, USA). The cells cultured with RPMI-1640 media were used as control. Date are presented as mean $\pm$ SD, n>3.

4.3 Cellular uptake study

A549 cells and McF7 cells were seeded in a 6-well chamber slide at a density of 2×10^5 /mL and incubated for 12 h which was then treated with FLAME-RGD in blocking buffer (1% bovine serum albumin in DPBS) at 0 °C for 2 h. The concentration of PFOB in FLAME-RGD was 25.8 mM determined by measuring the ^{19}F NMR signal intensity of PFOB at -83 ppm (^{19}F NMR signal

intensity of trifluoroethanol at -76 ppm as internal standard). According to the density (1.93 g/cm3), molar mass (498.96 g/mol) of PFOB and the equivalent diameter of PFOB liquid core (~250 nm), the concentration of FLAME-RGD was about 169.28 pM. After washed with PBS for 3 times, cells were fixed with 4% paraformaldehyde for 10 min at room temperature. Fixed cells were stained with DAPI for 10 min and washed with PBS 3 times. Finally, cells were mounted on slides in fluoromount with coverslips imaged under confocal laser scanning microscope (A1R/A1, Nikon, Japan). All captured microscopic images were analyzed using ImageJ software. The fluorescence of DAPI and FLAME-RGD is defined as blue and red, respectively. The Average red fluorescent intensity were 37.97 for A549 cells and 11.12 for MCF7 cells.

4.4 NMR/MRI Sample Preparation

The A549 cells and McF7 cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin within a standard 5 % CO_2 incubator at 37 °C. Cell monolayers were labeled with FLAME- RGD as previous report.[s2] In brief, cells were washed twice with cold blocking buffer (1% BSA in DPBS) and pre-cool in ice for 15 min. Then cells were incubated with FLAME-RGD in cold blocking buffer for 2 h in ice. The concentration of PFOB in FLAME-RGD was about 25.8 mM determined by measuring the ^{19}F NMR signal intensity of PFOB at -83 ppm. According to the density (1.93 g/cm3), molar mass (498.96 g/mol) of PFOB and the equivalent diameter of PFOB liquid core (~250 nm), the concentration of FLAME-RGD was about 169.28 pM. The cells were washed three times with PBS at room temperature followed by trypsinization and re-suspension. After centrifugation, the cell pellet was resuspended in RPMI-1640 to achieve cell densities of 10-15 million cells/ml. The cell solutions were transferred into a 10-mm NMR phantom for NMR measurements.

4.5 ^{129}Xe NMR and MRI experiments

All the ^{129}Xe NMR and MRI experiments were obtained on a 400 MHz Bruker AV400 wide-bore spectrometer (Bruker Biospin, Ettlingen, Germany), Xe (26.4% natural abundance of ^{129}Xe) gas mixture consisting of 10% N_2 , 88% He, and 2% Xe was flowed through a homebuilt Hyperpolarizer and then bubble into the NMR sample tube at the rate of 0.1 standard liters per minute, the temperature of the sample tube was 298 K controlled by VT unit and the pressure was 51 PSI. In both NMR and MRI experiments, the hyperpolarized gas mixture was directly bubbled into the sample tube for 20s and signal acquisition began 3 s later after bubbles collapse. NMR spectra for direct detection were acquired using 4 signal average and processed using 10 Hz line broadening filter, the ratio of capture ^{129}Xe to free ^{129}Xe was 0.017 calculated by the ratio of signal intensity of ^{129}Xe@FLAME to signal intensity of ^{129}Xe@solution. According to the Ostwald solubility of Xe in PFOB and water, and the equivalent diameter of PFOB liquid core (~250 nm), the concentration was about 28.77 pM for NMR direct detection. For Hyper-CEST NMR experiments, continuous wave (CW) saturation pulses of 5 s were used for selectively saturation and spectrum for each saturation offset was acquired in

a single scan. I MRI experiments, 10 s CW saturation pulses with field strength of 6.5 μT were employed. The saturation frequency is measured relative to dissolved ^{129}Xe resonance (195 ppm). The on-resonance frequency was at 106 ppm, while the off-resonance frequency was at 284 ppm. ^{129}Xe Hyper-CEST MR images were acquired using RARE (Rapid Acquisition with Refocused Echoes) sequence with a RARE factor of 8, 30 × 30 mm^2 field of view, 32 × 32 matrix size, echo time: 3 ms. The MR images were processed using in-house written Matlab scripts (R2014a, MathWorks, Natick, MA). The 32*32 image matrix was interpolated into 64*64 matrix and segmented using the 0.2*maximum value of off-resonance image as the threshold. CEST effect of each pixel was then calculated by the formula (CEST effect = (Off_{Res} - On_{Res}) / Off_{Res}) pixel by pixel.

5. Supplementary table and figures

ω_a(ppm)	$R^a_{1(s^{-1})}$	$R^a_{2(s^{-1})}$	ω_b(ppm)	$R^b_{2(s^{-1})}$	f_B	$k_{ba(s^{-1})}$
194	0.0069	2.2	106	764	0.0063	131

Table S1. parameters for BM equation fitting

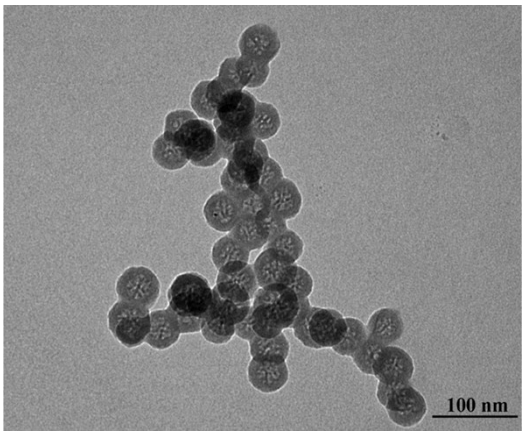


Figure S1. TEM image of MSNs. Fresh prepared MSNs were suspended in water and loaded on copper mesh a day before test.

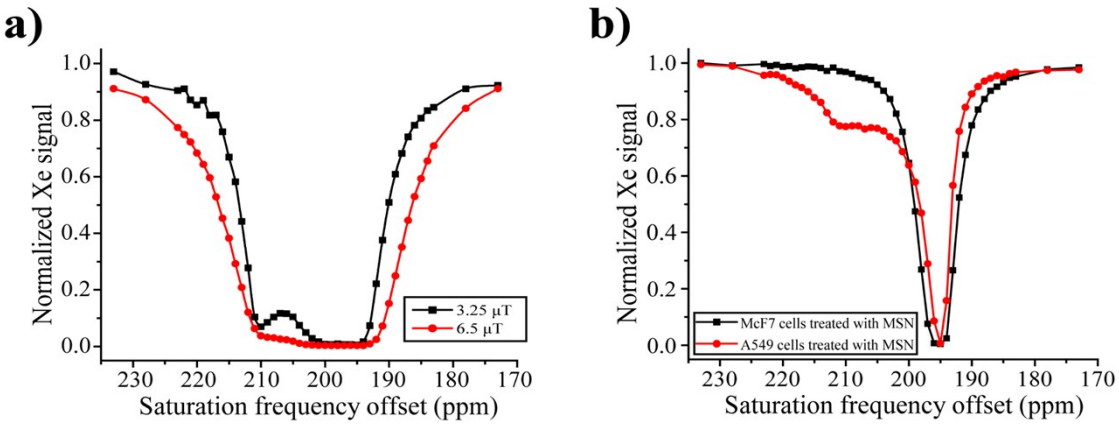


Figure S2. a) Hyper-CEST spectra of MSNs with concentration of 0.5 mg/ml in PBS (pH 7.4) with different saturation pulse (3.25/6.5 µT, 10 s). b) Hyper-CEST spectra of cells treated with MSNs-RGD (saturation pulse: 3.25 µT, 10 s). 0.5 mg/ml MSNs-RGD was incubated with A549 or McF7 cells for 2 h on ice. The cells were washed, trypsinized, resuspended in 2 mL of media and transferred to NMR tube for NMR analysis, the final density of cells is about 2.5× 10^6 cells/ml determined by hemocytometer.

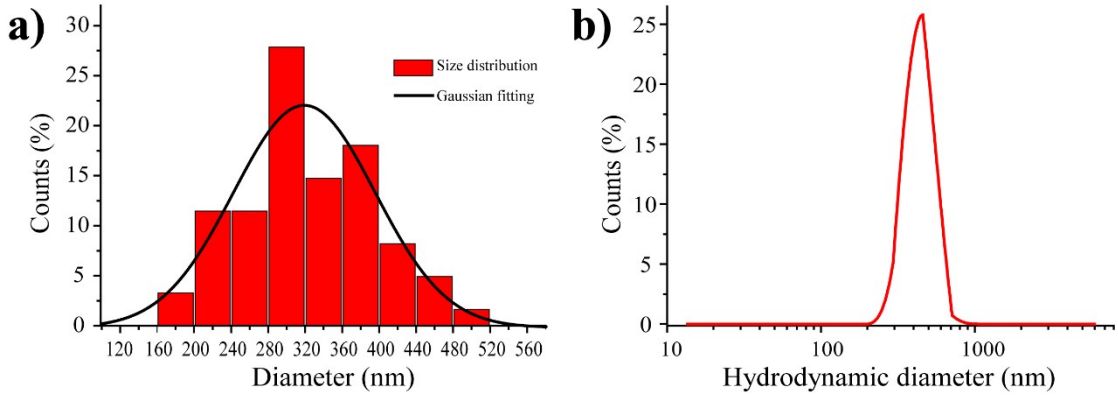


Figure S3. a) size distribution and gaussian fitting of FLAME by transmission electron microscopy (TEM). The average diameter of FLAME is 325 nm and the Standard deviation is 73 nm. b) Hydrodynamic diameter of the FLAME by Dynamic light scattering (DLS), the average hydrodynamic diameter of FLAME is 396 nm.

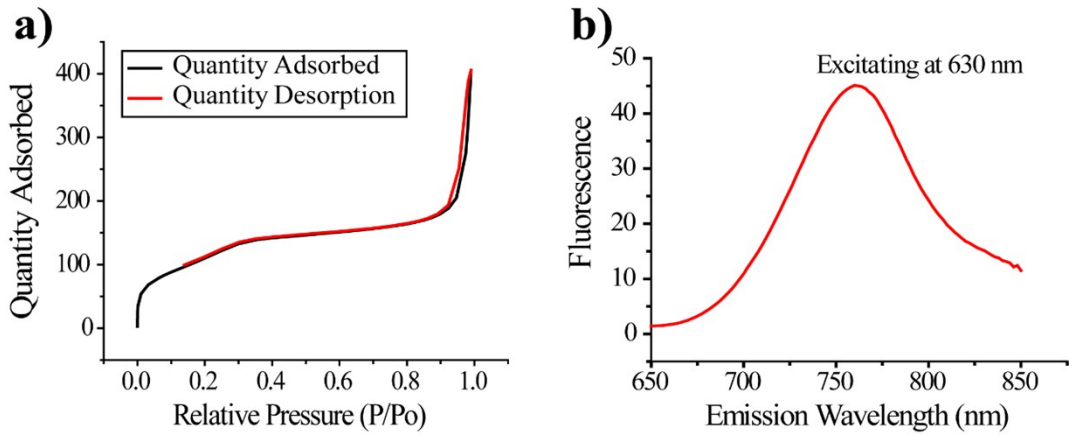


Figure S4. a) N_2 adsorption/desorption isotherms of FLAME. The N_2 adsorption/desorption isotherms of FLAME revealed a typical mesoporous structure with a Brunauer–Emmett–Teller(BET) surface area of 364.8278 m^2g^{-1}, pore volume of 0.63 $cm^3\ g^{-1}$, and the average pore diameter of 6.9 nm. b) Excitation and fluorescence spectra of FLAME in phosphate buffered saline (pH 7.4). The slit width was 5.0 nm for both excitation and emission. FLAME nanoparticles were suspended in water for the fluorescence spectra measurements.

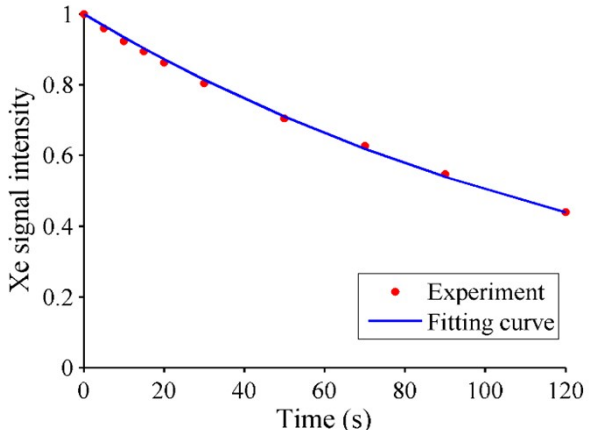


Figure S5. Longitudinal relaxation rates R_1^a of ^{129}Xe in solution. The R_1^a value of 0.0069 s^{-1} was measured by fitt[illegible]quation 1.

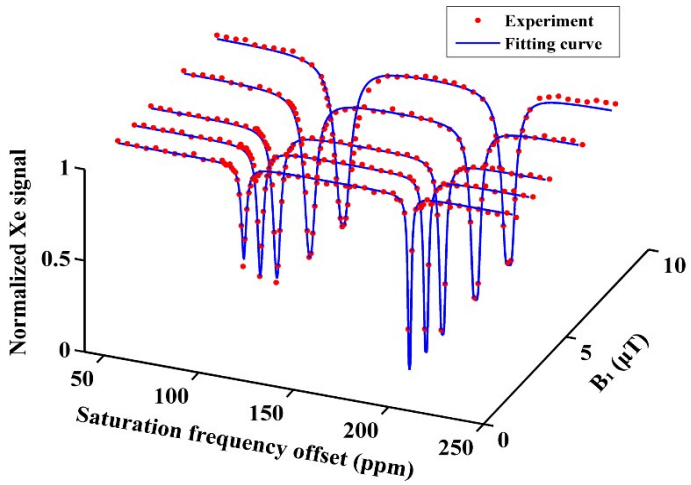


Figure S6. Fitting result of Multiple Hyper-CEST spectra. The fitted parameter values are listed in Table S1. The value of R_1^a was kept constant in the fitting, while other parameters in Table

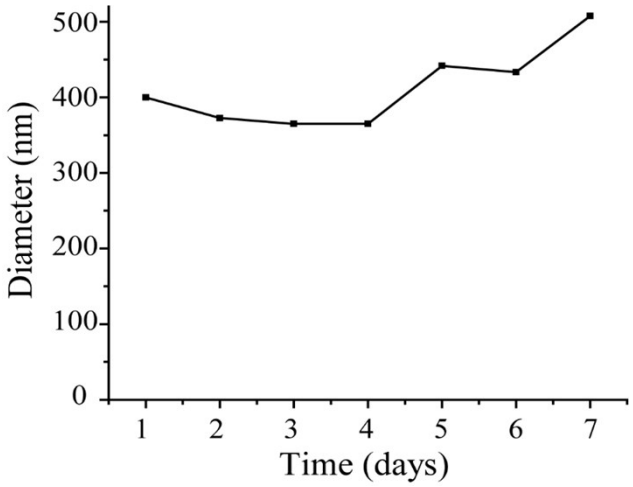


Figure S7. Dynamic light scattering (DLS) data of FLAME-RGD over the course of seven days at 25℃. The slight growth of the average hydrodynamic diameter may be due to the slight ag

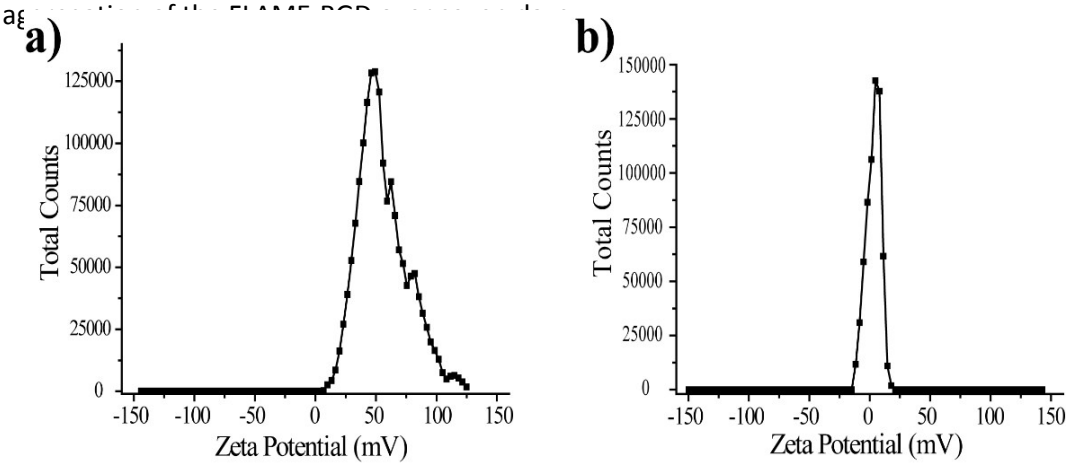


Figure S8. The Zeta potential distribution curves of FLAME and FLAME-RGD. The carboxyl-PEG on the surface of FLAME-RGD reduced the Zeta potential of FLAME-RGD

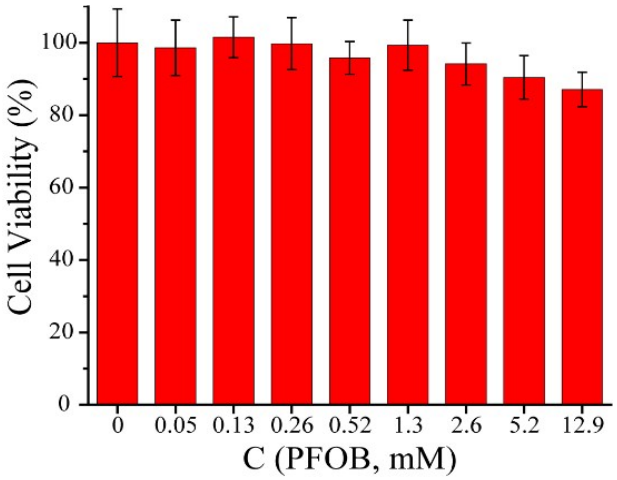


Figure S9. Cytotoxicity assay of FLAME-RGD on A549 cells. A549 cells were incubated with FLAME-RGD at varied concentration (The concentration of PFOB, CPFOB, is from 0.05 to 12.9 mM). The cells cultured with RPMI-1640 media were used as control. Date are presented as mean ± SD.

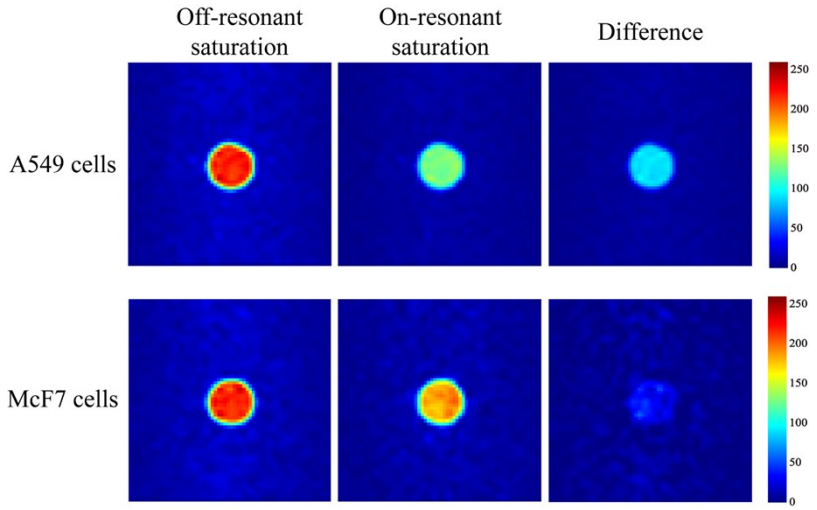


Figure S10. ^{129}Xe MR images of A549 cells and McF7 cells treated with FLAME-RGD using selective saturation pulse(6.5 μT, 10 s). The saturation pulse was applied at 284 ppm for off-resonance Hyper-CEST images and 106 ppm for on-resonance Hyper-CEST images.

6. Supplementary references

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