Electronic Supplementary Material (ESI) for Polymer Chemistry.
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Electronic Supplementary Information for

Hyperbranched Poly (glycerol) as T_1 Contrast Agent for Tumor-targeted Magnetic Resonance Imaging *in Vivo*

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Synthesis of glycidyl azide and alkynyl-FA

Synthesis of glycidylazide. Glycidyl azide was synthesized according to the reported method.[1] Epichlorohydrin (4.0 mL, 51 mmol) and sodium azide (4.0 g, 61 mmol) was added to a mixed solution of 3.5 mL acetic acid, 5 mL ethanol and 15 mL H_2O. The mixture was stirred vigorously at room temperature for 24 h. Afterward, brine (25 mL) was added and the mixture was extracted with EtOAc (3×40 mL). The combined organic phase was dried over Na_2SO_4 and concentrated with rotary evaporator to provide 1-azido-3-chloropropan-2-ol as colorless oil (5.9 g, 85%). The chemical structure was confirmed by ^{1}H NMR spectrum (shown in Figure S1). Furthermore, 55 mL sodium hydroxide solution (1 N) was added to 1-azido-3-chloropropan-2-ol and then the mixture was stirred at room temperature for 30 min. After that, the suspension was extracted with CH_2Cl_2 (3×30 mL). The combined organic phase was washed with brine (20 mL) and dried over Na_2SO_4. Finally, concentration in vacuum provided the colorless oil (3.5 g, 73%). The chemical structure was confirmed by ^{1}H NMR spectrum (shown in Figure S2).

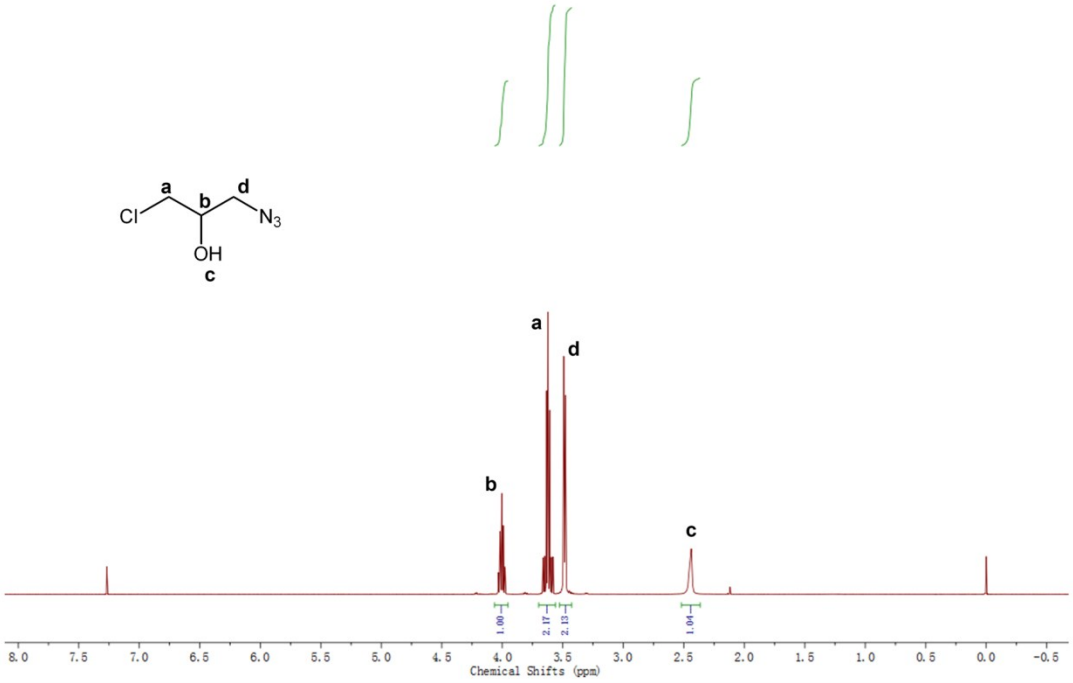

Figure S1. ^{1}H NMR spectrum of 1-azido-3-chloropropan-2-ol (recorded in $CDCl_3$).

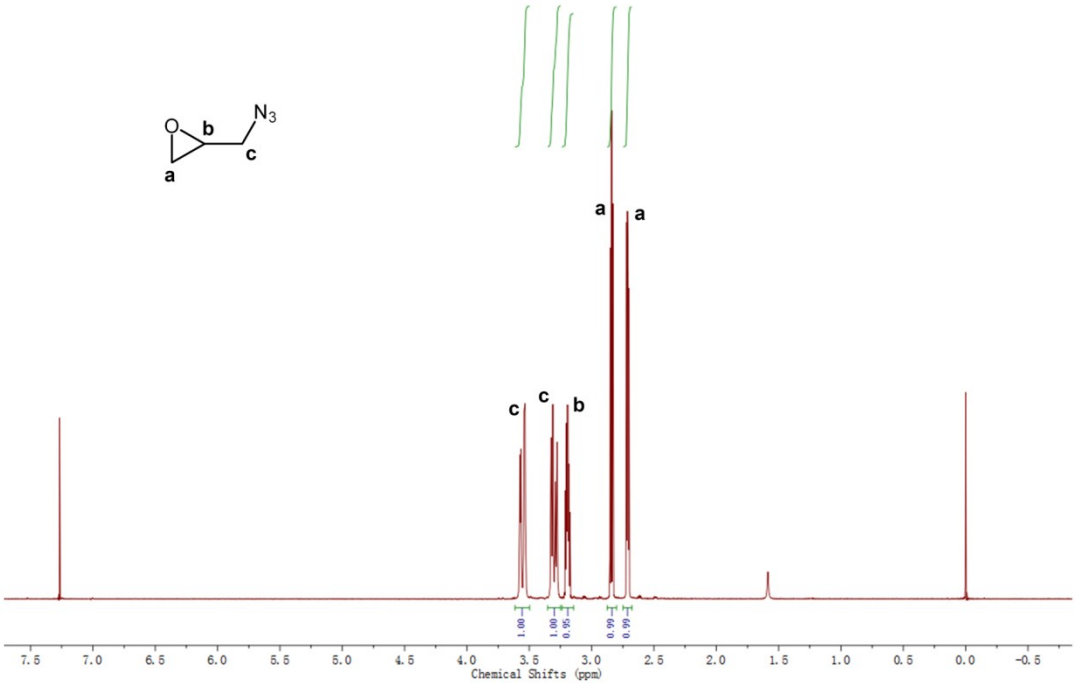

Figure S2. ^{1}H NMR spectrum of glycidyl azide (recorded in $CDCl_3$).

Synthesis of alkynyl-FA. Propargyl amine functionalized FA was synthesized via the reported method.[2] FA (0.15 g, 0.34 mmol) was dissolved in 50 mL DMF under vigorous stirring, and the un-dissolved solid was removed by filtration. Then, a mixture of EDC (0.13 g, 0.68 mmol) and NHS (0.0783 g, 0.68 mmol) in 3 mL DMF was added to the above solution, and the reaction mixture was stirred at room temperature for 4 h. Afterward, propargylamine (88 μL, 1.37 mmol) was added to the mixture solution, which was stirred for another 24 h. Followed by precipitation in excess diethyl ether, the solid was extensively washed with water, ethanol and acetone each for 3 times to achieve final product (112 mg, 69%).The chemical structure was confirmed by ^{1}H NMR spectrum (shown in Figure S3).

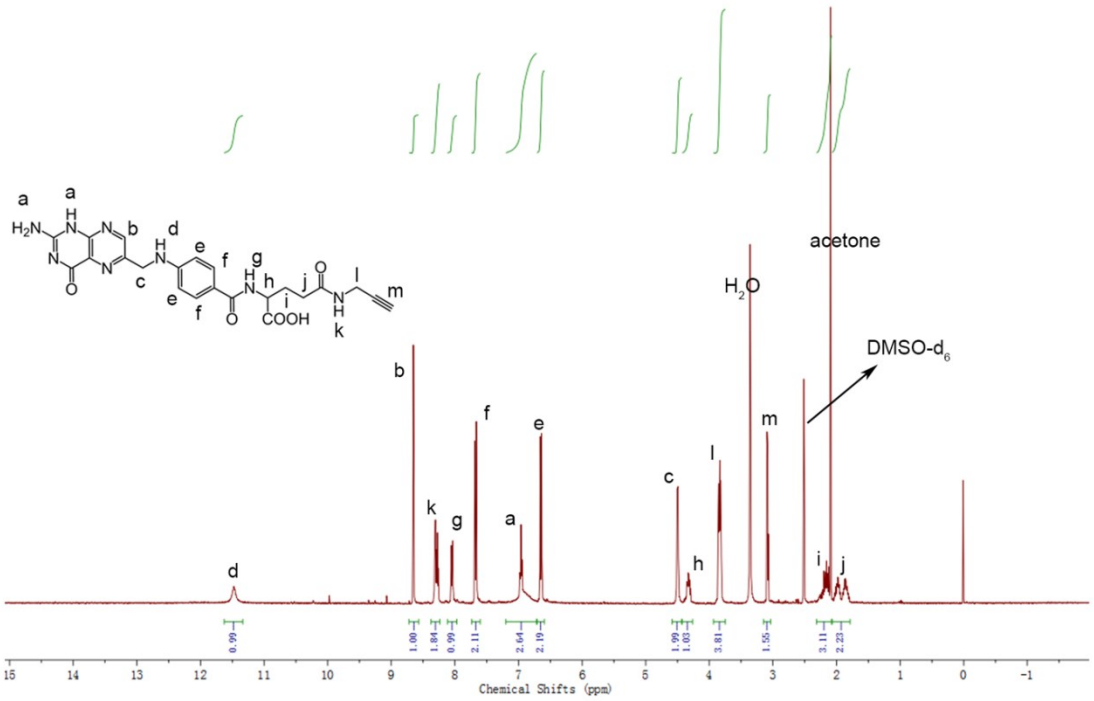

Figure S3. ^{1}H NMR spectrum of alkynyl-FA (recorded in DMSO-d_6).

Characterization and analysis of chemical structures

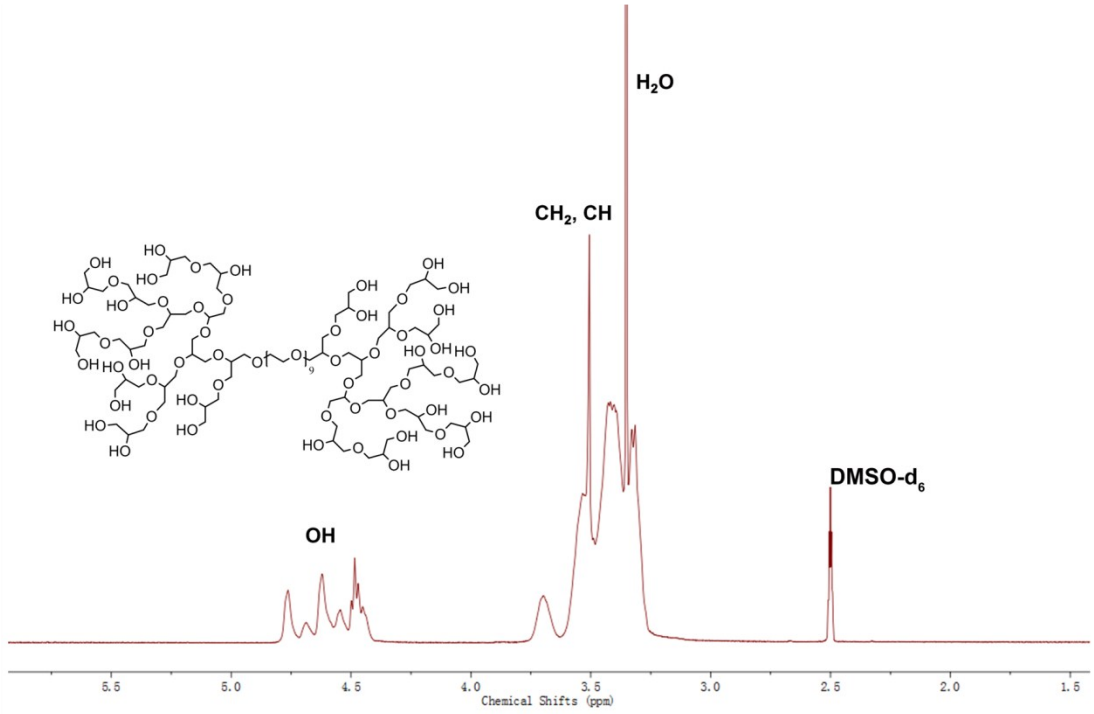

Figure S4. ^{1}H NMR spectrum of *h*PG-PEG-*h*PG (recorded in DMSO-d_6).

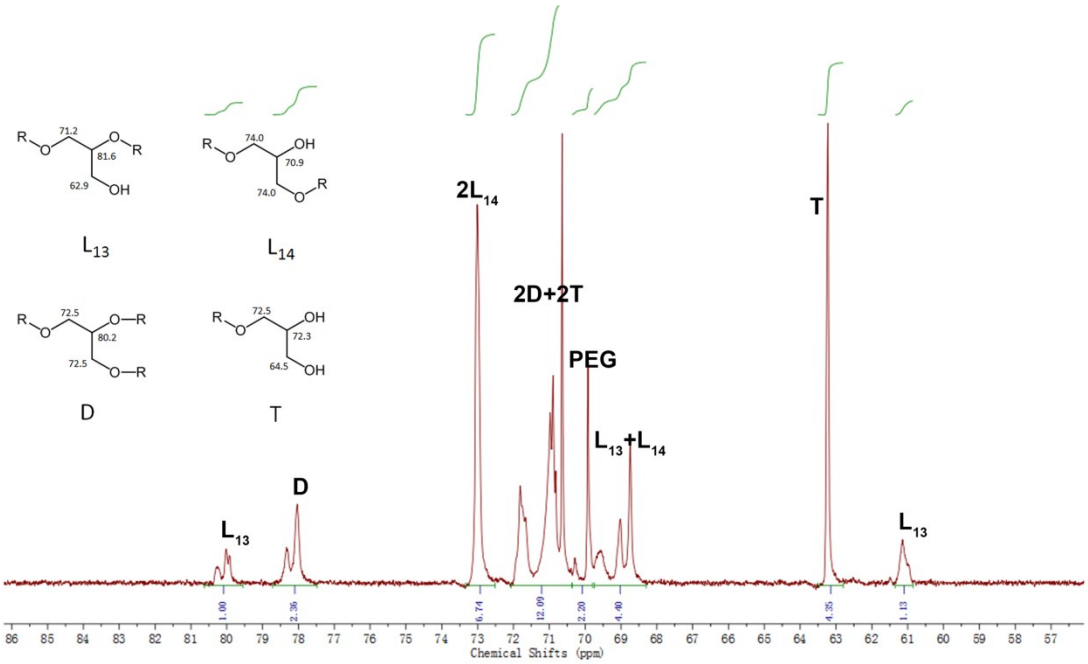

Figure S5. Quantitative ^{13}C NMR spectrum of *h*PG-PEG-*h*PG (recorded in DMSO-d_6).

The degree of branching (DB) of *h*PG is calculated according to the Equation (1) shown below, and the value was 0.52.

$$DB = \frac{2D}{2D + L_{13} + L_{14}}$$ (Equation 1)

The number of structural units is calculated according to the integrated area of peak (PEG) and peak (structural units), and the formula is shown in Equation (2).The results are listed in the Table S1below. The molecular weight based on NMR ($M_{n,NMR}$) could be calculated according to Equation (3), which turns out to be 7107 g/mol.

$$Number = \frac{A_{unit} \times 18}{A_{PEG}}$$ (Equation (2))

$$M_{n,NMR} = M_n(PEG) + (L_{13} + L_{14} + D + T) \times 74$$ (Equation (3))

Table S1: Number of structural units calculated from quantitative ^{13}C NMR spectrum of *h*PG-PEG-*h*PG.

	L_{13}	**L_{14}**	**D**	**T**
Integrated areas	1	3.37	2.36	4.35
Number of structural unit	8.18	27.57	19.30	35.59

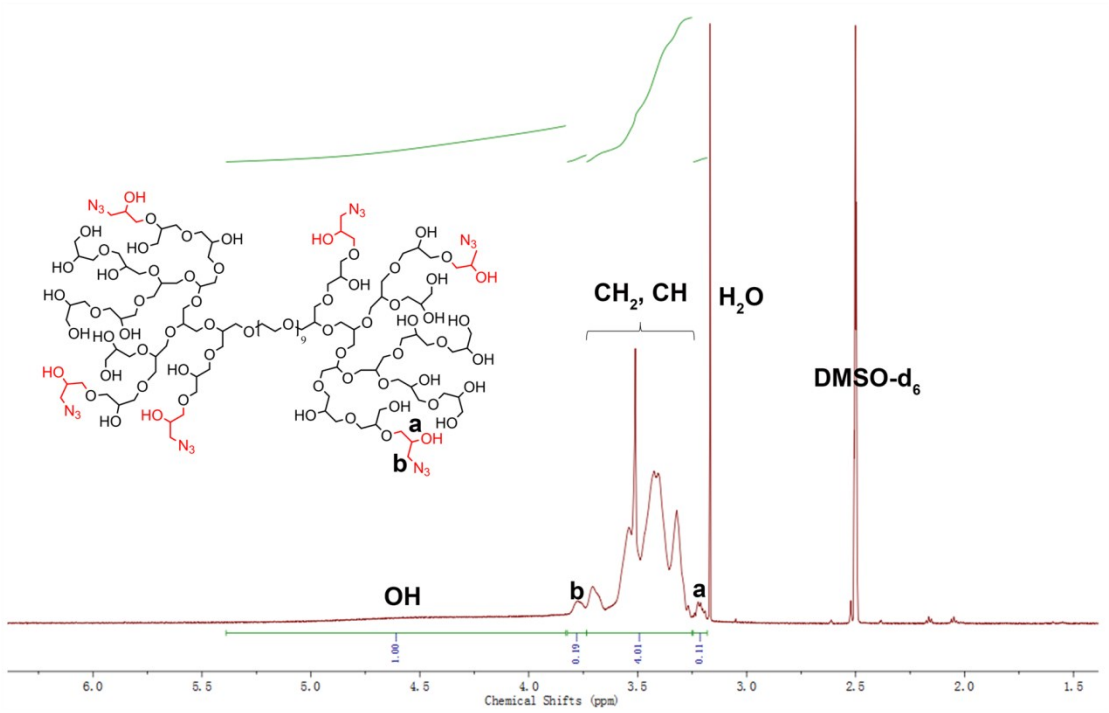

Figure S6. ^{1}H NMR spectrum of *h*PG-PEG-*h*PG-N_3 (recorded in DMSO-d_6).

Based on the number of structural units, the number of hydroxy group on one macromolecule is calculated according to Equation (4), which turns out to be 107. The number of azido group is calculated according to the integrated area of peak (hydroxyl) and peak (a), and the formula is shown in Equation (5), which turns out to be 12.

$$Number(hydroxyl) = L_{13} + L_{14} + T \times 2 \text{ (Equation (4))}$$

$$Number(azido) = \frac{107 \times A_a}{A_{hydroxyl}} \text{ (Equation (5))}$$

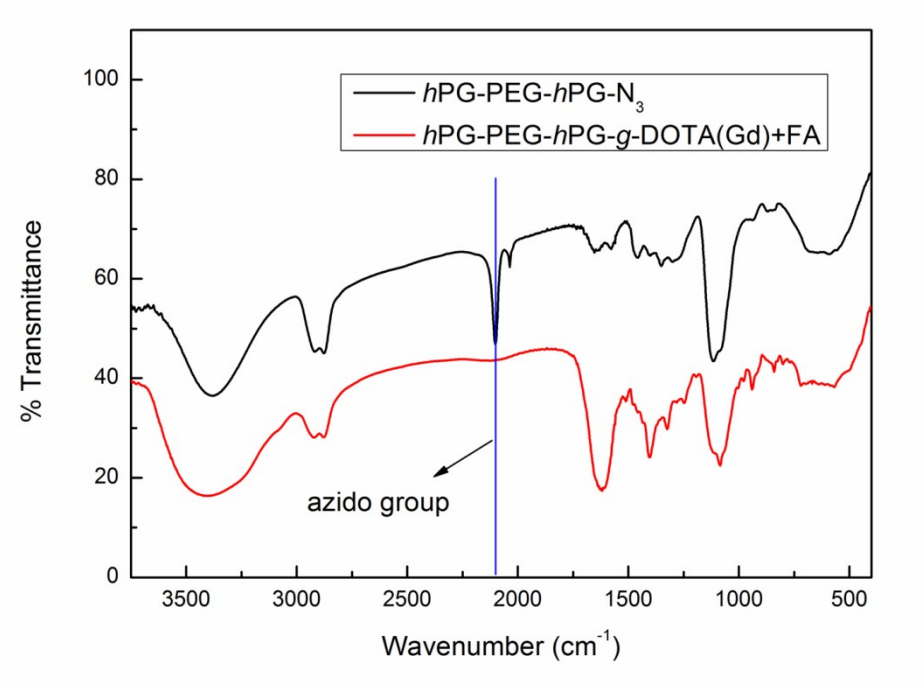

Figure S7. FT-IR absorption spectra of*h*PG-PEG-*h*PG-N_3 and *h*PG-PEG-*h*PG-*g*-DOTA(Gd)+FA.

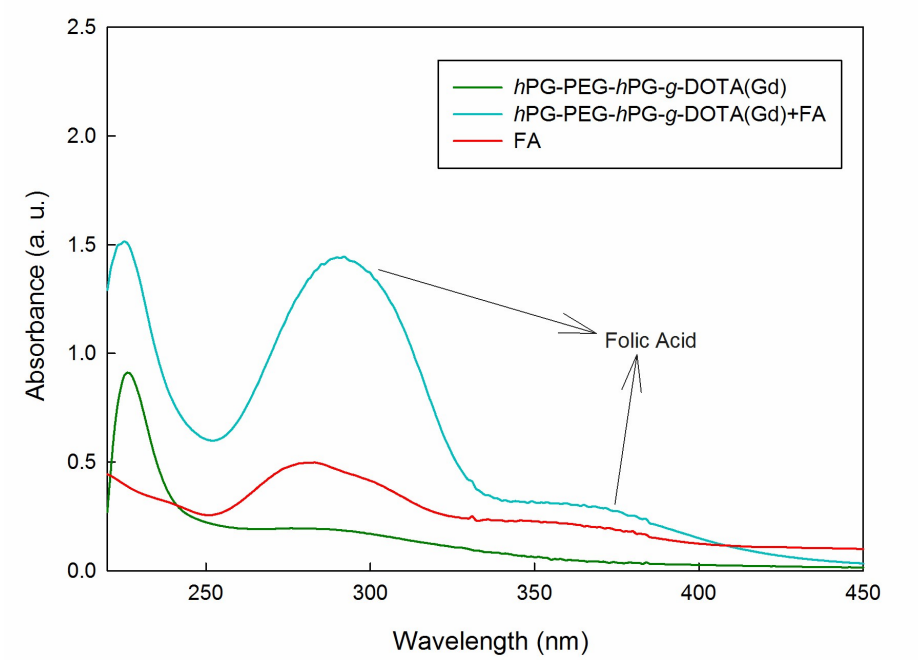

Figure S8. UV-vis absorption spectra of *h*PG-PEG-*h*PG-*g*-DOTA(Gd)+FA and *h*PG-PEG-*h*PG-*g*-DOTA(Gd). The characteristic spectrum of folic acid was also recorded as a comparison.

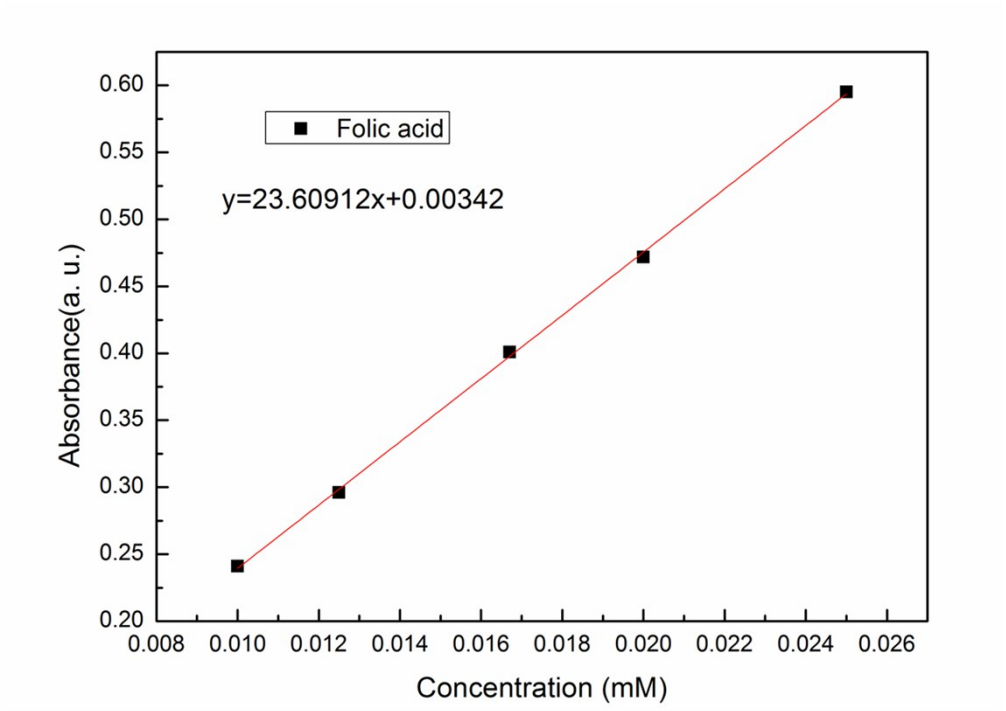

Figure S9. The standard curve of FA at the wavelength of 280 nm determined by UV-vis absorption spectra.

According to the gadolinium concentration determined by ICP-OES and UV-vis absorption spectra of hPG-PEG-hPG-*g*-DOTA(Gd)+FA sample, when the gadolinium concentration is 0.0486 mM, the intensity of UV-vis absorption is 0.587 at the wavelength of 280 nm. Therefore, the concentration of FA could be calculated to be 0.0247 mM based on the standard curve of FA (Figure S9). The ratio between gadolinium concentration and FA concentration is about 2:1. Since the number of azido group for "click chemistry" is 11, the amount of gadolinium chelate and FA ligand on each macromolecule is about 7 and 4, respectively.

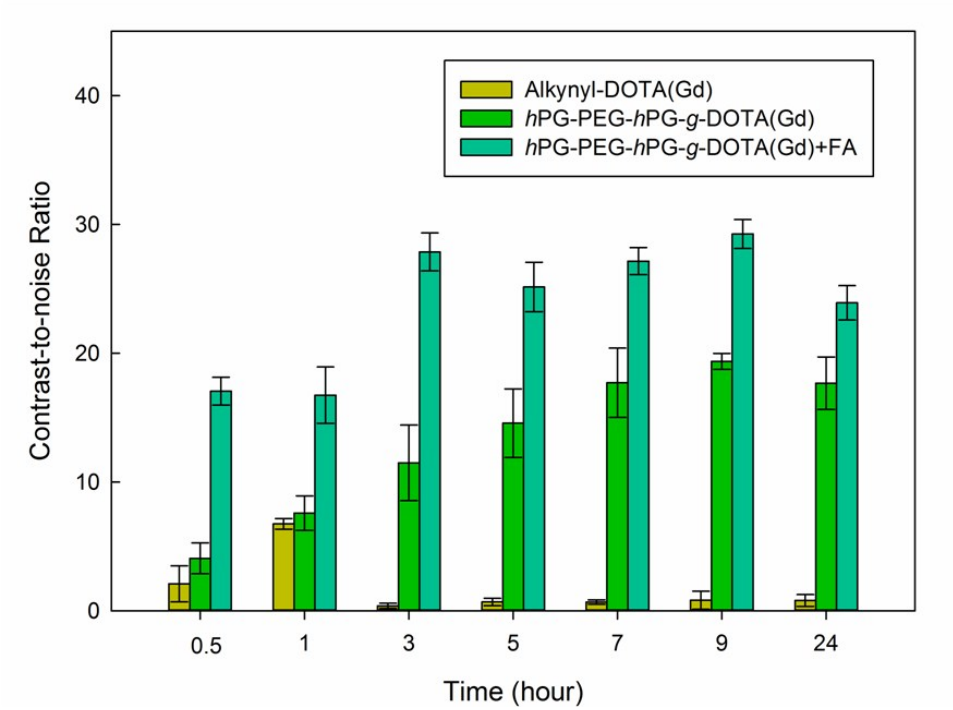

Figure S10. Contrast to noise ratio (CNR) at tumor region after mice (n=3) intravenously injected with alkynyl-DOTA(Gd), *h*PG-PEG-*h*PG-*g*-DOTA(Gd) and *h*PG-PEG-*h*PG-*g*-DOTA(Gd)+FA, respectively, at a gadolinium dose of 0.1 mmol/kg. The CNR was calculated using the following formula: ($signal_{tumor}$-$signal_{muscle}$)/standard deviation of noise.

References

1 E. G. Gharakhanian and T. J. Deming, *Biomacromolecules*, 2015, **16**, 1802-1806.

2 Z. Luo, X. Ding, Y. Hu, S. Wu, Y. Xiang, Y. Zeng, B. Zhang, H. Yan, H. Zhang and L. Zhu, *ACS nano*, 2013, **7**, 10271-10284.