Supporting Information for

Bioconjugates of versatile β-diketonate-lanthanide complexes as probes for time-gated luminescence and magnetic resonance imaging of cancer cells *in vitro* and *in vivo*

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1. Experimental section

SDS-PAGE electrophoresis

Samples were mixed with an equal volume of 5× SDS-PAGE loading buffer containing 50 mM Tris-HCl (pH 6.8)，10% SDS (w/v), 2.5% mercaptoethanol (v/v) and 10% glycerol (w/v). Then, the mixture was boiled for 5 min and cooled before loading on the 12% polyacrylamide gel. The electrophoresis was run at a constant voltage of 80 V for 2 h. After the electrophoresis, gels were stained with Coomassie Blue (0.25%) for 1 min and then destained with distilled water for 15 min. Each set of experiment was repeated for three times with the same procedure.

Quantum yield measurement

The relative luminescence quantum yields of the complexes were measured with *N*,*N*,*N*′,*N*′-(4′-phenyl-2,2′:6′,2″-terpyridine-6,6″-diyl) bis(methylenenitrilo) tetraacetate–Eu^{3+} (PTTA–Eu^{3+}) as the reference (Φ = 0.16 in water). The excitation was performed at 330 nm, and the quantum yield is calculated using the following equation.[1]

$$\Phi_{unk} = \Phi_{std} \frac{(F_{unk}/A_{unk})}{(F_{std}/A_{std})} \left(\frac{\eta_{unk}}{\eta_{std}} \right)^2$$

In the equation, Φ_{unk} and Φ_{std} are the radiative quantum yields of the sample and standard, F_{unk} and F_{std} are the integrated emission intensities of the corrected spectra for BHHBCB-Eu^{3+} (or Tf-BHHBCB-Eu^{3+}) and PTTA–Eu^{3+}, respectively; A_{unk} and A_{std} are the absorbance of BHHBCB-Eu^{3+} (or Tf-BHHBCB-Eu^{3+}) and PTTA–Eu^{3+} at the excitation wavelength (λ_{ex} = 330 nm, A_{unk} and A_{std} < 0.05), respectively; and η_{unk} and η_{std} are the indices of refraction of BHHBCB-Eu^{3+} (or Tf-BHHBCB-Eu^{3+}) and PTTA–Eu^{3+}, respectively. All the measurements were performed for five times and the results were averaged.

MTT assay

The cytotoxicity of Tf-BHHBCB-Ln^{3+} to MCF-7 cells was measured by the MTT test using the previously reported method.[2] MCF-7 cells cultured in Dulbecco's modified Eagle Medium (DMEM) were washed with an isotonic saline solution (140 mM NaCl, 10 mM glucose, and 3.5 mM KCl), and then incubated with different concentrations of Tf-BHHBCB-Eu^{3+} probe (0, 32, 64, 128, 320, 640, 1280 mg/L) for 24 h at 37 °C in a 5% CO_2/95% air incubator. After the culture medium was

removed, the cells were further incubated with the isotonic saline solution containing 500 μg/mL of MTT for 4 h in the incubator. After the supernatants were removed, the cells were dissolved in DMSO, and then the absorbance at 490 nm was measured.

In vivo distribution and toxicity evaluation

After three KM mice (female, ~20 g bodyweight) were anesthetized by 1.5% isoflurane in oxygen, they were injected with Tf-BHHBCB-Gd^{3+} probe (200 μL in physiological saline solution, 17 g/L) via tail vein. Then the mice were continuously monitored by sequential T_1-weighted MRI on a NMI20-030H-I Analyzing and Imaging system. In each experiment, the MR intensity analysis of ROIs was performed using the Horos V3.3.1 software for Mac. To qualify the signal enhancement, we calculated the SNR by the equation $SNR = SI_{tumor}/SD_{noise}$ where SI represents signal intensity and SD represents s.d. And the relative enhancement signal intensity (ΔSNR) was calculated according to the following formula: $\Delta SNR = (SNR_{post} - SNR_{pre}) / SNR_{pre} \times 100\ \%$, where SNR_{post} and SNR_{pre} are SNR of RIOs at postinjection and preinjection, respectively.

To further examine the biocompatibility of Tf-BHHBCB-Eu^{3+} probe, three KM mice (female, ~20 g bodyweight) were intravenously injected with 200 μL physiological saline solution containing 3.3 mg Tf-BHHBCB-Eu^{3+} probe. After 24 h, they were sacrificed by dislocating cervical vertebra and the main organs (heart, liver, spleen, lung and kidney) were surgically dissected. The collected organs were fixed with 4% formaldehyde in PBS and embedded in paraffin. Then the standard hematoxylin and eosin (H&E) staining was carried out for histological analysis.

Statistical analysis

All the experiments were performed three times and the values were presented as the mean ± SD. Statistical comparison between two groups was determined by Student's test. All statistical analyses were conducted with Excel ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). A value of $P < 0.05$ was considered statistically significant.

2. Table S1. Comparisons between BHHBCB-Eu^{3+} and Tf-BHHBCB-Eu^{3+}

Complex	$\lambda_{ex,max}$ (nm)	$\lambda_{em,max}$ (nm)	Φ (%)	τ (ms)	q [a]
BHHBCB-Eu^{3+}	330	606	72	0.49	0.39
Tf-BHHBCB-Eu^{3+}	327	606	40	0.52	0.43

[a] Using the luminescence lifetimes of BHHBCB-Eu^{3+} and Tf-BHHBCB-Eu^{3+} in H_2O and D_2O buffers, the average number (q) of water molecules in the first coordination sphere of Eu^{3+} ion was calculated from the equation of $q=1.2(1/\tau_{H2O} - 1/\tau_{D2O} - 0.25)$.[3]

3. Characterization of Tf-BHHBCB-Ln^{3+} probe

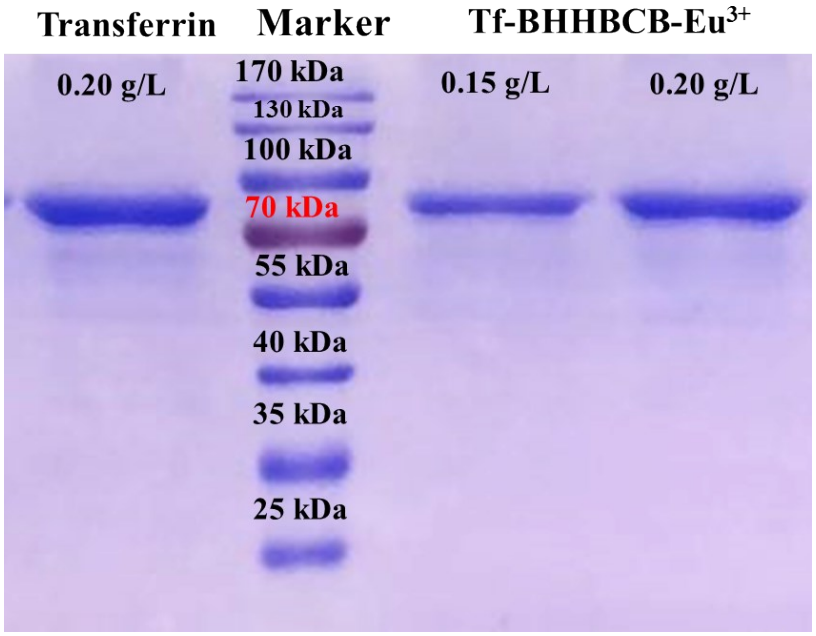

Fig. S1 Polyacrylamide gel electrophoresis images of transferrin (0.20 g/L) and Tf-BHHBCB-Eu^{3+} (0.15, 0.20 g/L) after stained with Coomassie Brilliant Blue R-250.

4. Cytotoxicity and biocompatibility of Tf-BHHBCB-Ln^{3+} probe

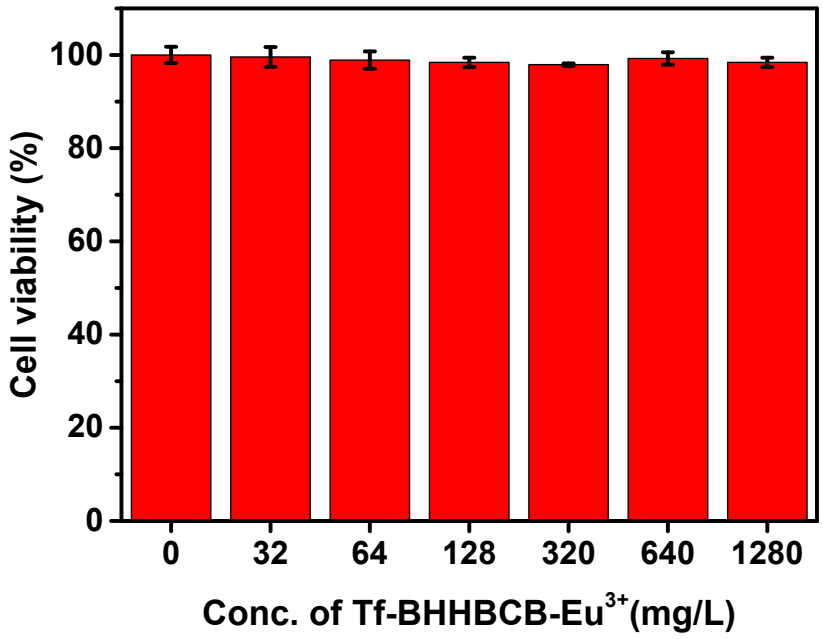

Fig. S2 Viabilities of MCF-7 cells after incubated with different concentrations of Tf-BHHBCB-Eu^{3+} for 24 h.

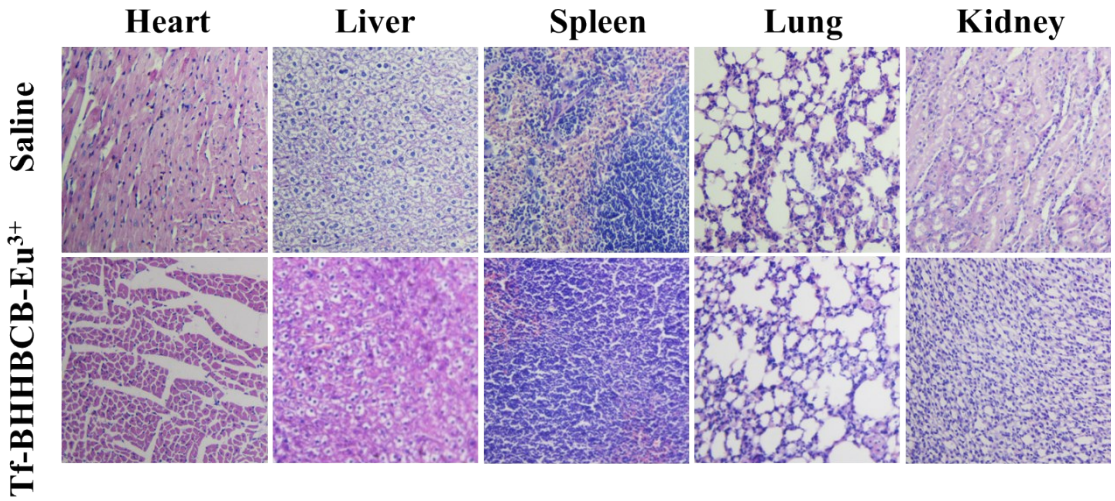

Fig. S3 Images of H&E stained main organs of the KM mice after intravenous injection of physiological saline and Tf-BHHBCB-Eu^{3+} probe (200 μ, 17 g/L in physiological saline) for 24 h.

5. *In vivo* distribution of Tf-BHHBCB-Gd^{3+}

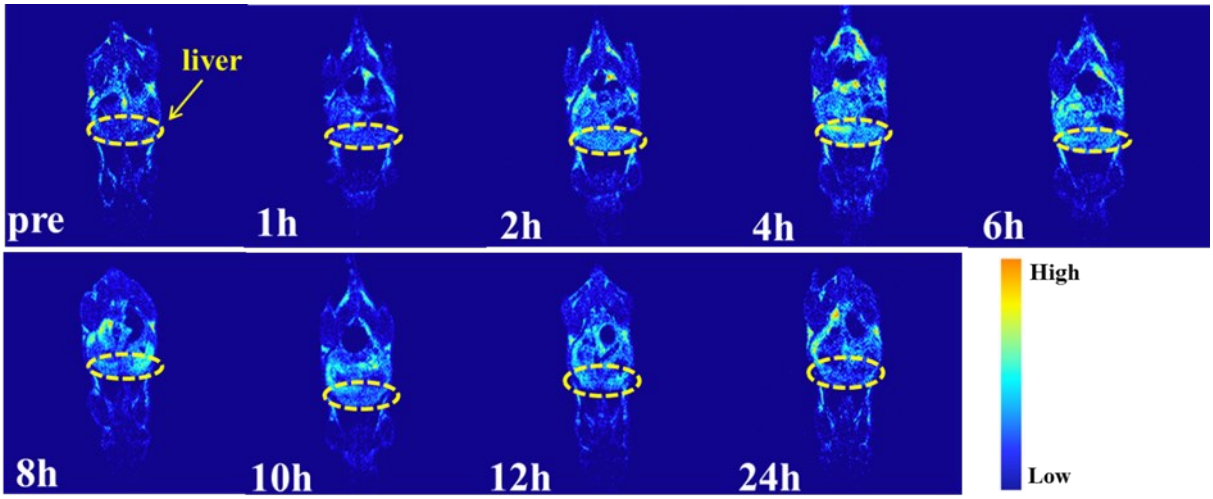

Fig. S4 *In vivo* T_1-weighted MR images of KM mice at different time intervals after intravenous injection of Tf-BHHBCB-Gd^{3+} probe in longitudinal plane (TR = 500, TE = 19, recorded at 310 K under 0.5 T magnetic field).

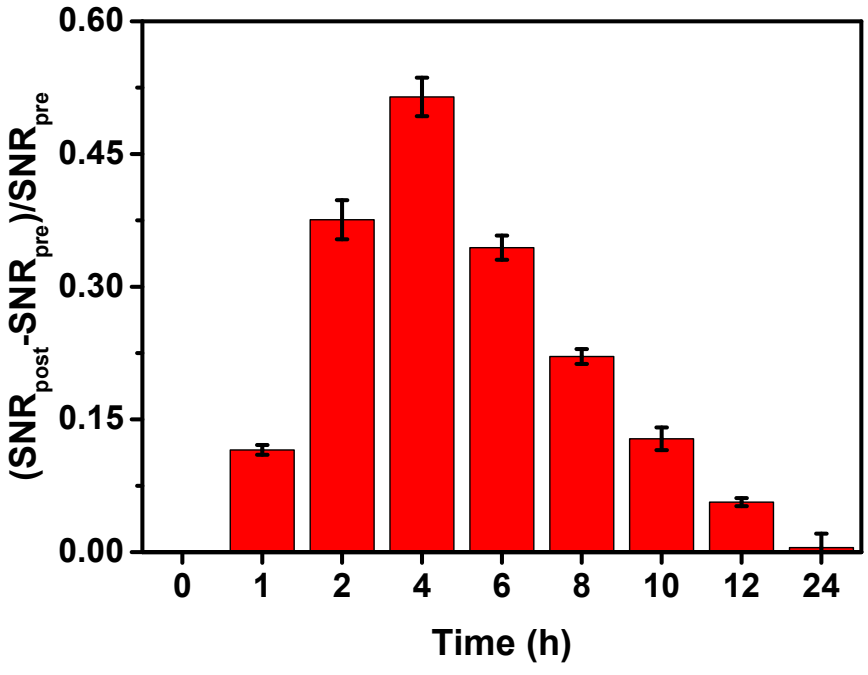

Fig. S5 Quantification results of liver contrast values in KM mice at different time intervals after injection of Tf-BHHBCB-Gd^{3+} probe.

6. References

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2. U. Schatzschneider, J. Niesel, I. Ott, R. Gust, H. Alborzinia and S. Wölfl, *ChemMedChem*, 2008, **3**, 1104-1109.
3. B. Song, G. Wang, M. Tan and J. Yuan, *J. Am. Chem. Soc.*, 2006, **128**, 13442-13450.