Supporting Information for

Development of a tumor-targetable heteropolymetallic lanthanide complex-based magnetoluminescent probe for dual-modal time-gated luminescence/magnetic resonance imaging of cancer cells in vitro and in vivo

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1. Experimental section

Synthesis of CDHH-NH₂

CDHH (100 mg, 0.20 mmol) and N-Boc-1,6-hexanediamine (51.3 mg, 0.24 mmol) were dissolved in anhydrous CH₂Cl₂ (5 mL), then NEt₃ (86.0 mg, 0.85 mmol) and DMAP (dimethylaminopyridine) (2.5 mg, 0.02 mmol) were added with stirring. The reaction mixture was stirred for 12 h at room temperature. After evaporation, the residue was dissolved in 20 mL CH₂Cl₂ and washed three times with saturated brines containing diluted sulphuric acid (1.0 M). The resulting solution dried with anhydrous Na₂SO₄ and evaporated to dryness. Then the residue was dissolved in 20 mL CH₂Cl₂ and followed by adding 5 mL trifluoroacetic acid dopewise. The reaction mixture was stirred for 24 h at room temperature. After evaporation, the residue was dissolved in 2 mL methanol and added dropwise to 20 mL of diethyl ether with stirring to afford a white precipitate. The precipitate was filtered and washed with water and diethyl ether thoroughly. The as-prepared crude product was dried under vacuum to afford the milk-white powder (98 mg, 90% yield). ¹H NMR (500 MHz, DMSO) δ 8.23 (d, J = 10 Hz, 2H), 8.01 (d, J = 10 Hz, 2H), 7.95 (d, J = 10 Hz, 2H), 7.90 (d, J = 10 Hz, 2H), 7.71 (t, 2H), 6.98 (s, 1H), 2.82-2.70 (m, 4H), 1.53-1.44 (m, 2H), 1.43-1.34 (m, 2H), 1.29-1.19 (m, 4H). ESI-MS (m/z): 941.2 ([M+H]⁺, 100%).

Synthesis of Gd-DO3A-CC

Gd-DO3ANH₂ (60.0 mg, 0.1 mmol) and Na₂CO₃ (10.6 mg, 0.1 mmol) were dissolved in 0.75 mL water. The mixture was added dropwise to 1.5 mL of acetone containing 46 mg cyanuric chloride (0.25 mmmol). The reaction mixture was stirred for 6 h at room temperature and diluted with 75 ml acetone to afford a white precipitate. The precipitate was filtered, washed with acetone, and dried under vacuum to afford the target compound as a white powder (70.9 mg, 95% yield). HPLC analysis: retention time, 4.38 min (purity, 93.6% integrated intensity); Sinochrom ODS-BP (5 μm) 250 mm × 4.6 mm C18 reverse-phase column; eluent, methanol/H₂O = 1/3 containing 0.1% trifluoroacetic acid; flow rate, 1.0 mL/min. The elution was monitored at 330 nm. ESI-HRMS (m/z): calcd. for [M-H]: 746.1219; found: 746.1195.

Synthesis of Gd-DO3A-CDHH

Gd-DO3A-CC (27.0 mg, 0.036 mmol) and Na₂CO₃ (3.8mg, 0.036 mmol) were dissolved in 0.5 mL water. The mixture was added dropwise to 1.5 mL of acetone containing 20.5 mg CDHH-NH₂ (0.036 mmmol). The reaction mixture was stirred for 6 h at room temperature and diluted with 50
mL tetrahydrofuran to afford a white precipitate. The precipitate was filtered, washed with
tetrahydrofuran, and dried under vacuum to afford the target compound as a white powder (43.8 mg,
92% yield). HPLC analysis: retention time, 7.48 min (purity, 94.7% integrated intensity);
Sinochrom ODS-BP (5 μm) 250 mm × 4.6 mm C18 reverse-phase column; eluent, methanol/H₂O =
1/3 containing 0.1% trifluoroacetic acid; flow rate, 1.0 mL/min. The elution was monitored at 330
nm. ESI-HRMS (m/z): calcd. for [M-H]: 1280.2952; found: 1280.2925.

**Synthesis of Tf-CDHH**

To the solution of 15.4 mg Tf dissolved in 2.0 mL of 0.05 M carbonate buffer at pH 8.0 was
added dropwise CDHH (1.0 mg, 2.0 μmol) dissolved in 400 μL of DMSO with stirring. After
stirring for 2.5 h at room temperature, the CDHH-conjugated Tf, Tf-CDHH, was separated from the
unreacted β-diketone by Sephadex G-50 column chromatography with 0.05 M NaHCO₃ of pH 8.0
as eluent.

**Synthesis of Eu(CDHH-DO3A-Gd)₃**

Gd-DO3A-CDHH (64.0 mg, 0.05 mmol) and europium(III) triflate (10.2 mg, 0.017 mmol) were
dissolved in 2.0 mL of DMSO-H₂O mixed solvent (v : v = 1:2), followed by adding 0.25 mL NaOH
(8 g/L) dopewise. The reaction mixture was stirred at room temperature for 4 h. After evaporation,
the residue was added to 40 mL of acetonitrile with stirring to afford a white precipitate. The
precipitate was filtered and washed with water and acetonitrile thoroughly. The as-prepared crude
product was dried under vacuum to afford the target compound as a white powder.

**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.

**MTT assay**

The cytotoxicity of **Tf-Eu-Gd** to HeLa cells was measured by the MTT test using the previously
reported method [1]. HeLa 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-Eu-Gd** probe (0, 100, 200, 300, 400, 500 mg/L) for 24 h at 37 °C in a 5% CO₂/95% 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-Eu-Gd** probe (200 μL in physiological saline solution, 4 g/L) via tail vein. Then the mice were continuously monitored by sequential *T₁*-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 further examine the biocompatibility of **Tf-Eu-Gd** probe, three KM mice (female, ~20 g bodyweight) were intravenously injected with 200 μL physiological saline solution containing 0.8 mg **Tf-Eu-Gd** 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.
Scheme S1. Reaction pathway for the synthesis of Tf-Eu-Gd.
Scheme S2. Reaction pathway for the synthesis of Eu(CDHH-DO3A-Gd)$_3$.

### 2. Table S1. Photophysical properties of Eu(CDHH-DO3A-Gd)$_3$ and Tf-Eu-Gd

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\lambda_{ex,max}$ (nm)</th>
<th>$\lambda_{em,max}$ (nm)</th>
<th>$\Phi^a$</th>
<th>$\tau$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu(CDHH-DO3A-Gd)$_3$</td>
<td>334</td>
<td>606</td>
<td>0.28</td>
<td>0.33</td>
</tr>
<tr>
<td>Tf-Eu-Gd</td>
<td>333</td>
<td>610</td>
<td>0.20</td>
<td>0.34</td>
</tr>
</tbody>
</table>

$a$ The quantum yields ($\Phi$) were calculated using equation, $\Phi_x = \frac{I_x \varepsilon_x c_x \Phi_{ref}}{I_{ref} \varepsilon_{ref} c_{ref}}$, where $\varepsilon$ is the extinction coefficient of the chelate at the excitation wavelength, $c$ is the concentration, $I$ denotes the total luminescence and subscripts $x$ and $\text{ref}$ refer to values of a chelate with unknown quantum yield and reference chelate, respectively.\[2\]

$N, N', N''-(4''-phenyl-2,2':6',2''-terpyridine-6,6''-diyl) \text{ bis(methylenenitro) tetraacetate–Eu}^{3+} \text{ (PTTA–Eu}^{3+})$ was chosen as a reference whose $\Phi_{\text{ref}} = 0.160$, $\varepsilon_{\text{ref}} = 14300 \text{ cm}^{-1}\text{mol}^{-1}\text{L}$. 


3. Characterization of Tf-Eu-Gd probe

![Polyacrylamide gel electrophoresis images of transferrin and Tf-Eu-Gd after stained with Coomassie Brilliant Blue R-250.](image1)

**Fig. S1** Polyacrylamide gel electrophoresis images of transferrin and Tf-Eu-Gd after stained with Coomassie Brilliant Blue R-250.

![Normalized phosphorescence spectra of the ligands Gd-DO3A-CDHH and Tf-CDHH in EtOH/MeOH (4:1, v/v) at 77 K, λ-ex=340 nm.](image2)

**Fig. S2** Normalized phosphorescence spectra of the ligands Gd-DO3A-CDHH and Tf-CDHH in EtOH/MeOH (4:1, v/v) at 77 K, λ-ex=340 nm.
Fig S3 MS spectrum of Eu(CDHH-DO3A-Gd)$_3$. 

Fig S4 MS spectrum of Tf-Eu-Gd.
4. Cytotoxicity and biocompatibility of Tf-Eu-Gd probe

![Cell viability graph](image)

**Fig. S5** Viabilities of HeLa cells after incubated with different concentrations of Tf-Eu-Gd for 24 h.

![Histological images](image)

**Fig. S6** Images of H&E stained main organs of the KM mice at 24 h after intravenous injection of physiological saline and Tf-Eu-Gd probe (200 μL, 4 g/L in physiological saline).

5. *In vivo* distribution of Tf-Eu-Gd
**Fig. S7** *In vivo* $T_1$-weighted MR images of KM mice at different time intervals after intravenous injection of Tf-Eu-Gd probe in longitudinal plane (TR = 500, TE = 19, recorded at 310 K under 0.5 T magnetic field).

**Fig. S8** Quantification results of liver contrast values in KM mice at different time intervals after injection of Tf-Eu-Gd probe.

### 6. References