

Supporting information

for

A Cell Penetrating Protein Designed for Bimodal Fluorescence and Magnetic Resonance Imaging

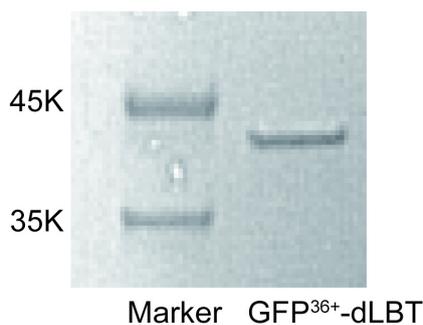


Figure S1. The SDS-PAGE electrophoresis gel shows the purified GFP³⁶⁺-dLBT protein.

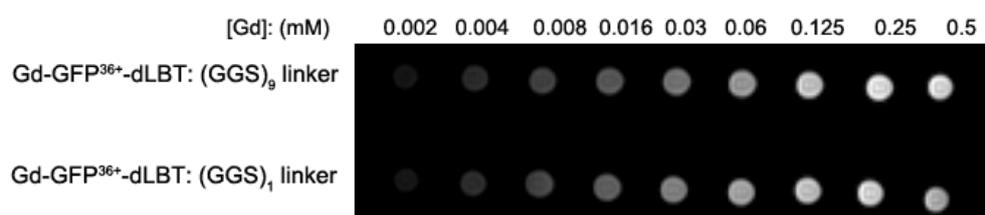


Figure S2. Comparison MR images of the GFP³⁶⁺-dLBT protein with different length of linkers at different concentrations of gadolinium. Upper: the fusion protein with a (GGS)₉ linker. Lower: the fusion protein with a (GGS)₁ linker.

Table S1. Gd(III) in Gd-DTPA, Gd-GFP³⁶⁺-dLBT and Gd-GFP³⁶⁺ measured with ICP-MS.

Gadolinium complex	Sample prepared (μM)	Gd measured		Binding ratio*
		μg/ml	μM	
Gd-DTPA	50	8.3±0.5	53±3	1
	100	15.8±1.3	100±8	
Gd-GFP ³⁶⁺ -dLBT**	50	17.6±0.6	112±4	2
	100	32.9±2.2	209±14	
Gd-GFP ³⁶⁺ **	50	0.12±0.006	0.76±0.038	~ 0.01
	100	0.18±0.016	1.1±0.1	

* Binding ratio: the number of gadolinium atoms bound to each molecule.

** Both GFP³⁶⁺-dLBT and GFP³⁶⁺ proteins contain a (His)₆ tag.

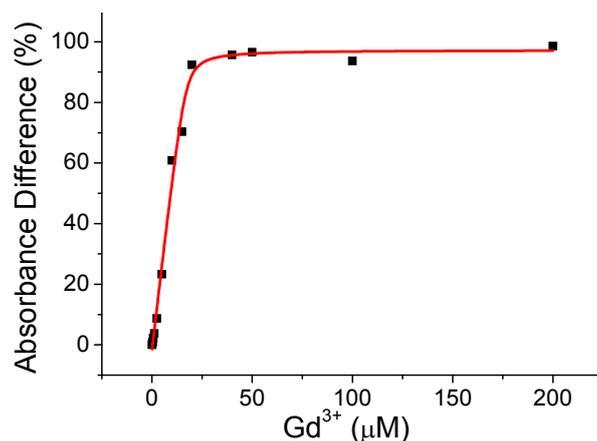


Figure S3. UV-vis titration of 10 μM GFP^{36+} -dLBT with Gd^{3+} . The binding constant was obtained by non-linear fitting of the absorbance at 280 nm. Data are the average values of three titrations.

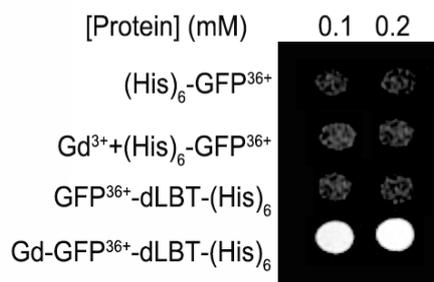


Figure S4. T_1 -weighted MR images of $(\text{His})_6\text{-GFP}^{36+}$ and GFP^{36+} -dLBT- $(\text{His})_6$ proteins. Gd^{3+} was loaded to $(\text{His})_6\text{-GFP}^{36+}$ or GFP^{36+} -dLBT- $(\text{His})_6$ proteins by dialysis and the excess Gd^{3+} in solution was removed by subsequently dialyzed against the buffer without Gd^{3+} .

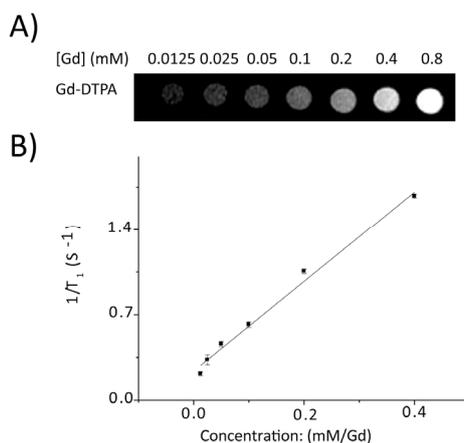


Figure S5 Relaxivity measurement of Gd-DTPA. (A) T_1 -weighted MR images with an inversion recovery sequence (TR 300 ms, TE 14 ms). (B) Longitude relaxation rates ($1/T_1$) as a function of concentration. Relaxivity rates r_1 were obtained by linear fitting the measured data. Error bars indicate standard deviation.

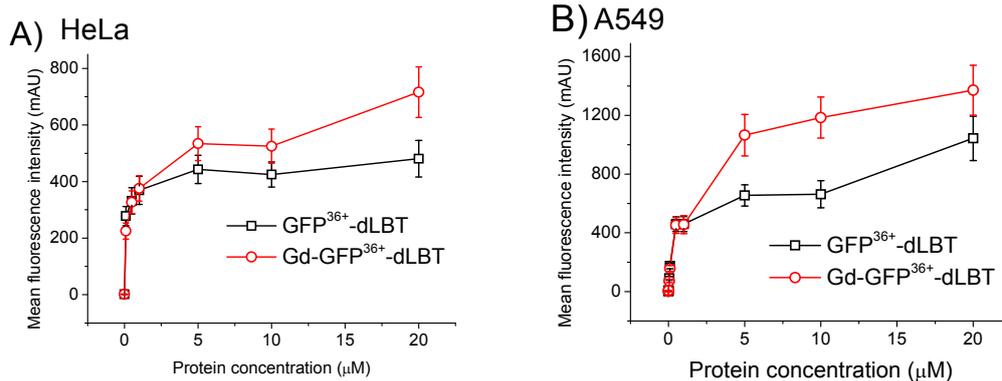


Figure S6. Flow cytometry analysis of the cellular uptake of GFP³⁶⁺-dLBT and Gd-GFP³⁶⁺-dLBT. (A) HeLa cells; (B) A549 cells. Cells were washed 5 times to remove membrane bound protein. Symbols denote Gd-GFP³⁶⁺-dLBT (circle) or GFP³⁶⁺-dLBT (square).

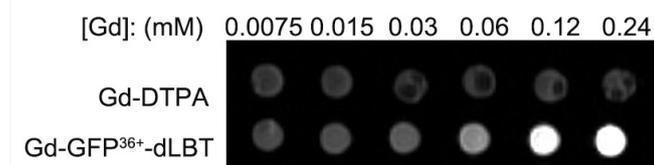


Figure S7: T₁-weighted MR images of 2×10⁶ HepG2 cells treated with Gd-DTPA or Gd-GFP³⁶⁺-dLBT at different concentrations. Cells were washed 5 times to remove the free protein.



Figure S8. Representative T₁-weighted MR images of kidney before (0 h) and after (1 h and 3 h) the injection with Gd-GFP³⁶⁺-dLBT via tail vein. Circles indicate the kidney area. Arrows indicate the enhanced MR signal compared to the control.

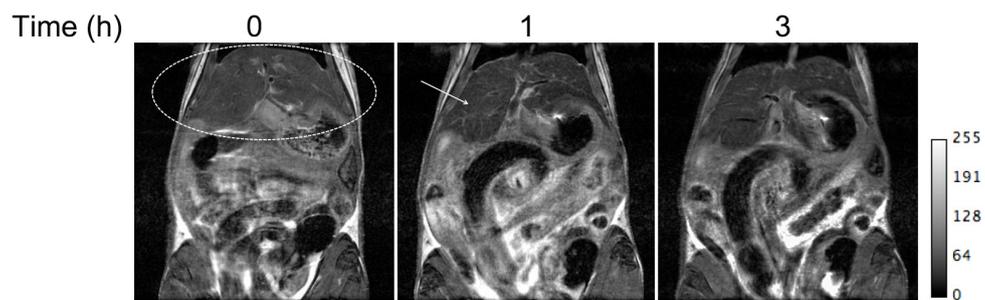


Figure S9. Representative T_1 -weighted MR images of liver before (0 h) and after (1 h and 3 h) the injection of $Gd-GFP^{36+}$ -dLBT via tail vein. Circles indicate the liver area. Arrows indicate the enhanced MR signal compared to the control.