Optimization of Time-Resolved Fluorescence Assay for Detection of Eu-DOTA-labeled Ligand-Receptor Interactions

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Supporting information

QC and ligand purification

The purity of the products was checked by analytical PR-HPLC using a Waters Alliance 2695 Separation Model with a Waters 2487 dual wavelength detector (220 and 280 nm) on a reverse phase column (Waters Symmetry C18, 4.6 × 75 mm, 3.5 μm). Peptides were eluted with a linear gradient of aqueous CH_3CN/0.1% TFA at a flow rate of 0.3 mL/min. Purification of ligands was achieved on a Waters 600 HPLC using a reverse phase column (Vydac C18, 15–20 μm, 22 × 250 mm). Peptides were eluted with a linear gradient of CH_3CN/0.1% TFA at a flow rate of 5.0 mL/min. Separation was monitored at 230 and 280 nm. Size exclusion chromatography was performed on a borosilicate glass column (2.6 × 250 mm, Sigma, St. Louis, MO) filled with medium sized Sephadex G-25 or G-10. The compounds were eluted with an isocratic flow of 1.0 M aqueous acetic acid. The pure compounds were dissolved in DI water or DMSO at approximately 1-5 mM concentrations. The accurate concentration was determined by HPLC at 280 nm. A solution of D-Trp (0.5 mM) in water or DMSO, accordingly, was co-injected as an internal standard. Structures were characterized by ESI (Finnigan,

Thermoquest LCQ ion trap instrument) or MALDI-TOF (Bruker Reflex-III, α-cyanocinnamic acid as a matrix) mass spectrometry. For internal calibration an appropriate mixture of standard peptides was used with an average resolution of 8,000–9,000. High resolution mass measurements were carried out on a FT-ICR IonSpec 4.7T instrument.

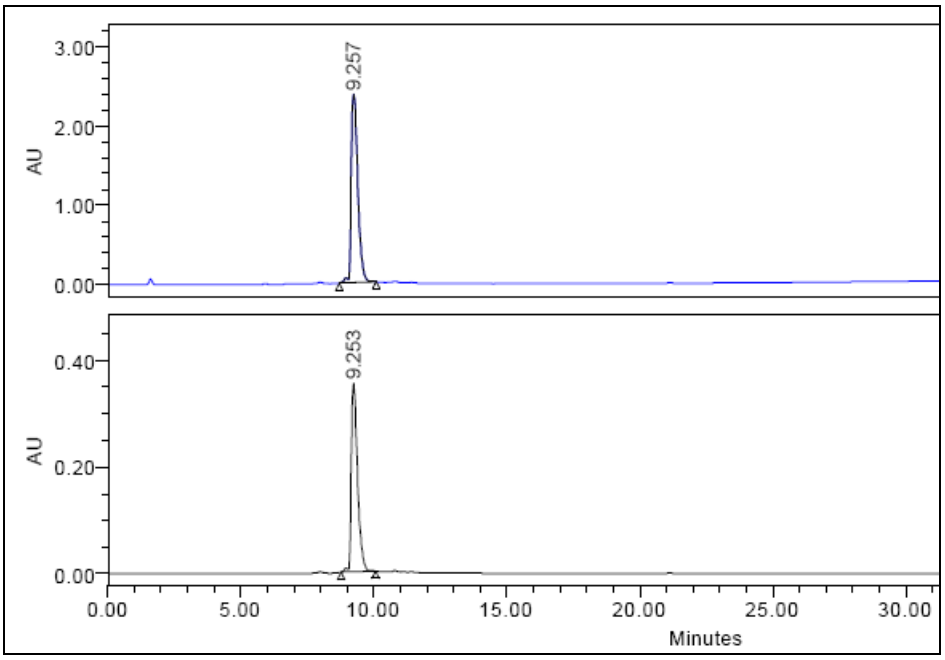


Fig. S1. HPLC profile of purified Eu(III)-DOTA-PEG9-NDP-α-MSH

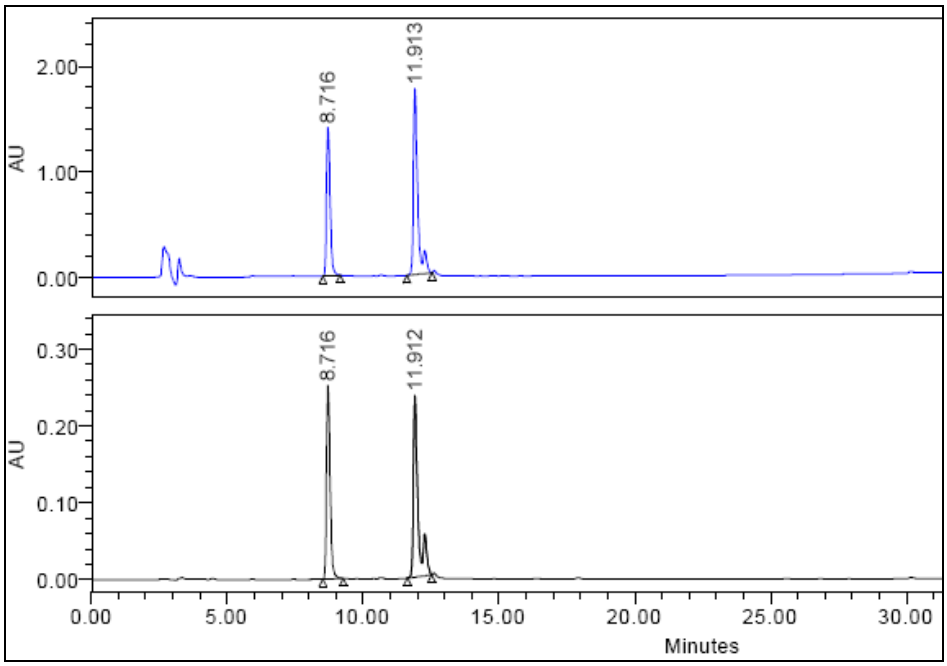


Fig. S2. HPLC profile of purified Eu(III)-DTPA-PEG9-NDP-α-MSH (R_t=11.9 min) and Trp standard (0.5 mM; R_t=8.7 min).

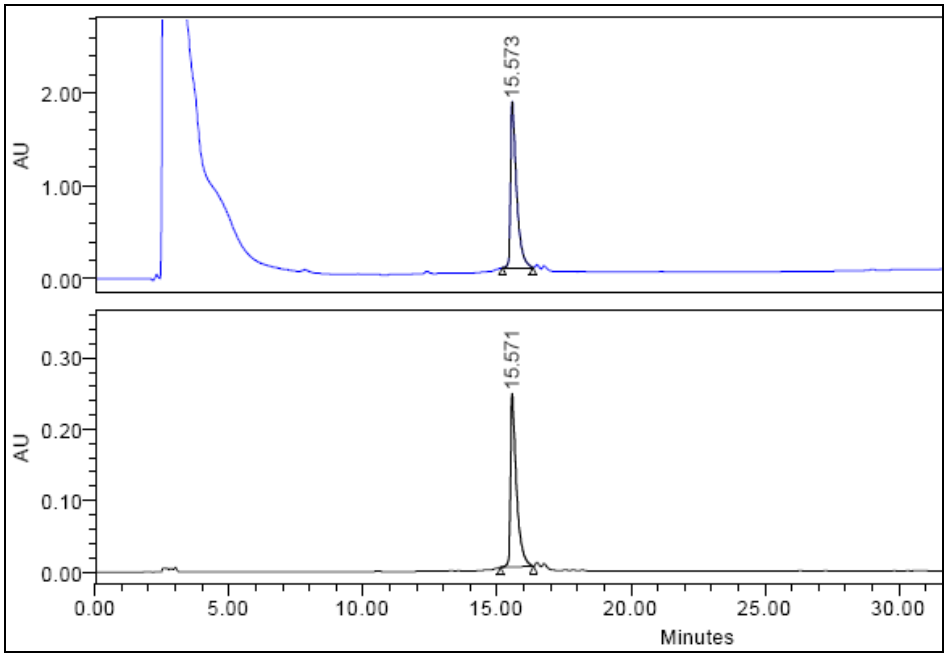


Fig. S3. HPLC profile of purified MSH(7)-PEGO-$[ProGly]_6$-Lys[PEG9-Eu(III)-DOTA]-PEGO-CCK(6).

Table S1. Summary of analytical data for the peptides

Peptide	Retention time R_t (min)	m/z (FT-ICR) Calculated	Observed
Eu(III)-DOTA-PEG9- NDP-α-MSH	9.2	762.66	762.66 $(M+3)^{3+}$
Eu(III)-DTPA-PEG9- NDP-α-MSH	11.9	758.98	758.98 $(M+3)^{3+}$
MSH(7)-PEGO-$[ProGly]_6$-Lys[PEG9-Eu(III)-DOTA]-PEGO-CCK(6)	15.5	1030.24	1030.24 $(M+4)^{4+}$

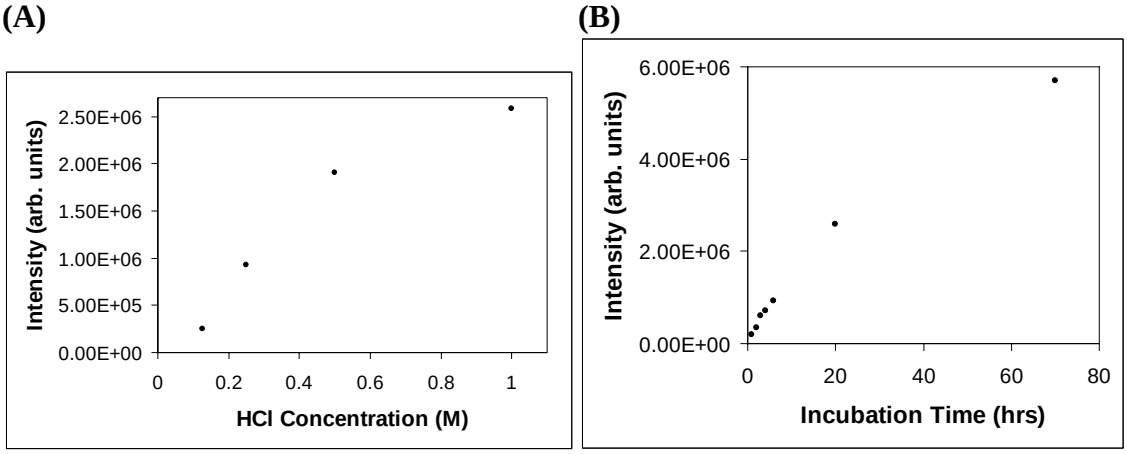


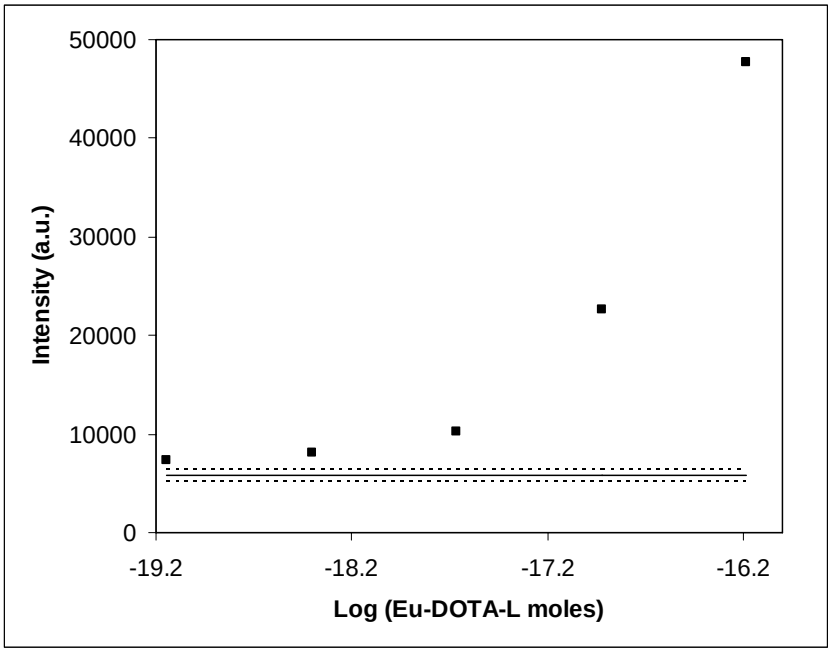
Fig. S4. Eu(III) ion release kinetics of Eu(III)-DOTA-PEG9-NDP-α-MSH. (A) HCl acid strenght dependancy and (B) incubation time depencancy using 1.0 M HCl.

Table S2. Assessment of ligand-receptor binding DELFIA assay quality

Assay	Compound	S:N	Z'-factor
Saturation binding	Eu(III)-DOTA-L	7.5	0.6
	Eu(III)-DTPA-L	6	0.5
Competitive binding	Eu(III)-DOTA-L	15.4	0.79
	Eu(III)-DTPA-L	8.9	0.64

S:N and Z'-factor were calculated near the K_d values of the saturation binding assays.

(A)



(B)

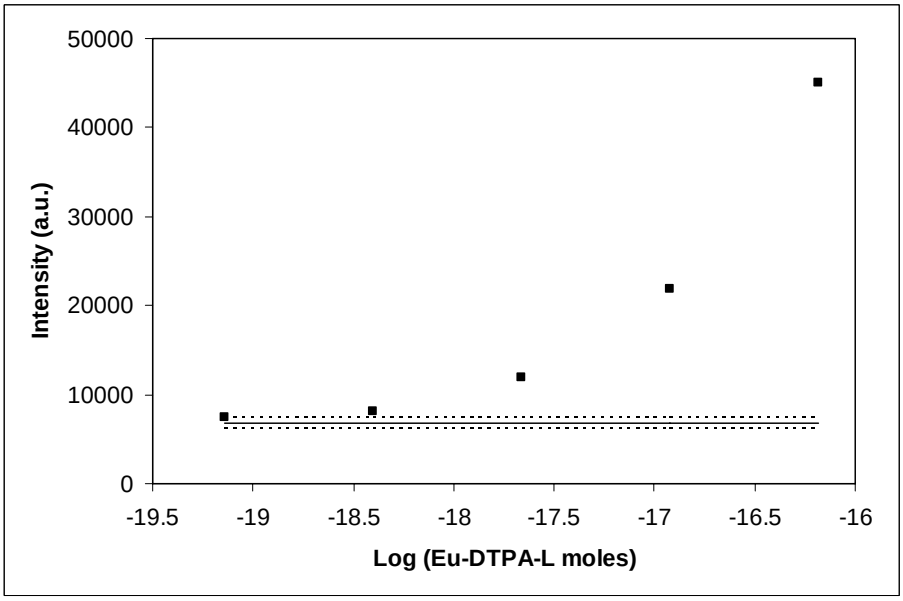


Fig S5. Detection limit of (A) Eu(III)-DOTA-L using modified DELFIA and (B) Eu(III)-DTPA-L using traditional DELFIA (L = PEG9-NDP-α-MSH). Serial dilutions of ligands were prepared in dH_2O. The background luminescence (solid line) was determined by

averaging 12 background samples. The dotted lines represent the standard error of the background.

Table S3. Evaluation of signal-to-noise ratio (S:N) of Eu(III)-labeled ligands using DELFIA ((L = PEG9-NDP-α-MSH)

Concentration (moles/well)	S:N (Eu-DOTA-L)	S:N (Eu-DTPA-L)
3.61×10^{-16}	36	37
3.61×10^{-17}	18	20
3.61×10^{-17}	3	6
3.61×10^{-18}	1.7	1.4
3.61×10^{-19}	0.8	0.45