Zeng and Anderson

Rapid and sensitive LC-MS approach to quantify non-radioactive transition metal impurities in metal radionuclides

Supporting information:

Reagents. All solvents and reagents were purchased from commercial sources and used without additional purifications. The stable-isotope enriched *DL*-Phenylalanine-2,3-$^{13}C_2$ (50 atom% ^{13}C) and ethylene-d_4-diamine (98 atom % D) were purchased from CDN Isotopes (Quebec, Canada). Fmoc-Cl and formic acid (LC-MS Grade) were obtained from Fisher Scientific (Hanover Park IL). 4-Methyltrityl chloride resin and Fmoc-DL-Phe-OH were obtained from Chem-Impex International (Wood Dale, IL). LC-MS grade acetonitrile and water were obtained from Sigma Aldrich (St. Louis, MO). DOTA-NHS ester was obtained from Macrocyclics (Dallas, TX). Chelex@100 resins were obtained from Bio-Rad (Hercules, CA). Ultrapure Water was produced using a Thermo Scientific Barnstead Nanopure® Ultrapure Water Systems. $^{64}Cu^{2+}$ in 0.1 M HCl was obtained from University of Wisconsin. All other chemicals and reagents were obtained from Sigma Aldrich (St. Louis, MO). The reactions were conducted under N_2 atmosphere, unless described otherwise.

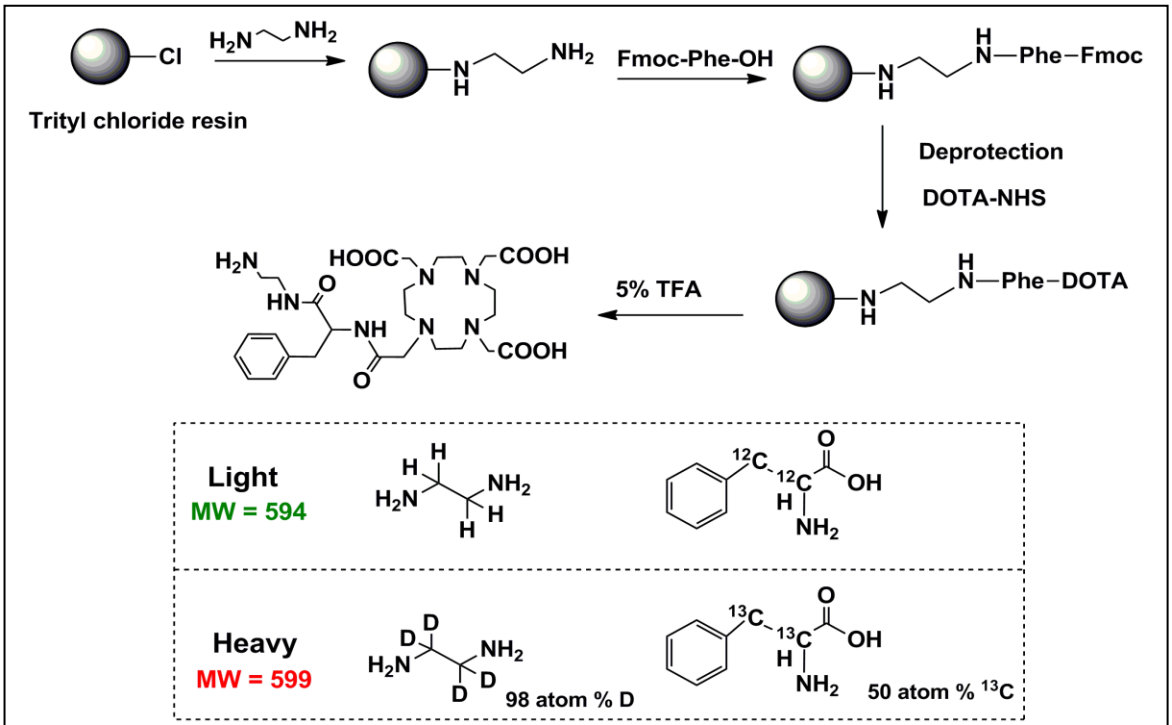
Instrumentation. ^1H NMR spectra were recorded on Brucker DRX 300 MHz. Waters LCT Premier mass spectrometer and 2695 Alliance HPLC were used for all the LC-MS experiments, with isocratic elution (Acetonitrile: H_2O = 12: 88, with 0.1% formic acid). Waters 1525 binary pump HPLC system was used for the EdF-DOTA agent purification using two elution buffers (0.1 v% TFA in de-ionized water as elution buffer A and 0.1 v%

TFA in acetonitrile as elution buffer B). Two Phenomenex HPLC columns, C18(2) Luna (250*10 mm) and C18(2) Luna (150 * 4.6 mm), were used for the HPLC and LC-MS experiments respectively.

Removal of trace amount of metal contamination from the ligand chelation. Before experiments of the EdF-DOTA ligand chelation, reagents and vessels used must be free from trace amount of metal contamination according to a previously reported protocol.[1] To remove trace amount of metal contaminants from reaction tubes, caps and pipette tips, the following procedure was used: 1) soak the tubes, caps and/or tips in 2.0 M nitric acid (diluted from concentrated nitric acid with ultrapure H_2O) overnight, and then drained; 2) wash with absolute ethanol and then drained; 3) wash with diethyl ether and then drain; 4) dry at room temperature under moderate nitrogen flow overnight. To remove trace amount of metal contaminants from reaction buffers, HPLC elution buffer and other solutions, the procedure is as follows: 1) add Chelex resin (10 g/L) to the buffers and solutions; 2) stir over night at room temperature; 3) filter through a Corning 1-liter filter system (pore size 0.2 mm).

Synthesis of Fmoc-($^{13}C_2$)Phe-OH. The title compound was synthesized through a procedure slightly modified from previously reported one[2] by using the $^{13}C_2$-encoded phenylalanine instead of native phenylalanine: *DL*-phenylalanine-2,3-$^{13}C_2$ (50 atom% ^{13}C) (660 mg, 3.97 mmol) was stirred vigorously in 20 mL 5% K_2CO_3 with ice bath cooling. A solution of Fmoc-Cl (1.2 g, 4.2 mmol) in 10 mL tetrahydrofuran (THF) was added slowly, and then the reaction mixture was stirred for 3 h while it was allowed to warm up to room temperature gradually. After the reaction was complete,

THF was removed in *vacuo*. The residue was poured into 80 mL 1 N HCl, and then extracted with 70 mL dichloromethane (DCM) three times. The combined organic solvent was washed with 100 mL brine, and dried over anhydrous Na_2SO_4. The product was purified by silica gel chromatography with ethyl acetate/hexane (1:1) to obtain the title compound (1.17 g, 76%) as a white powder. The ^{1}H NMR matched that of the previously synthesized Fmoc-DL-Phenylalanine.[3] ^{1}H NMR (400 MHz, CDCl3) δ 3.09-3.14 (m, 1H), 3.20-3.24 (m, 1H), 4.18-4.22 (t, 1H), 4.33-4.38 (m, 1H), 4.43-4.47 (m, 1H), 4.70-4.74 (m, 1H), 5.17-5.21(d, 1H), 7.14-7.16 (d, 2H), 7.22-7.42 (m, 7H), 7.53-7.56 (m, 2H), 7.75-7.77 (d, 2H); ESI-MS: $(M+H)^+$ = 389.51.



Scheme S1: solid-phase synthesis of the EdF-DOTA agents

Synthesis of the ^{13}C/^{2}H labeled EdF-DOTA. The synthesis is shown in **Scheme S1.** 4-Methyltrityl chloride resin (20 μmol) was incubated with ethylenediamine (100 μmol) and triethylamine (TEA, 200 μmol) in 1 mL dimethylformamide (DMF) for 2 h,

and was then washed thoroughly with DMF and DCM respectively. Fmoc-($^{13}C_2$)Phe-OH (100 μmol) was then coupled to the resin by mixing with PyBrOP (100 μmol) and DIPEA (200 μmol) in 1 mL DMF for 2 h at room temperature. Afterwards, the Fmoc group was removed with 20% piperidine in DMF (1.0 mL*3). After washing with DMF and DCM, the resin was incubated with the mixture of DOTA-NHS (60 μmol) and DIPEA (250 μmol). Finally, $^{13}C/^{2}H$ labeled EdF-DOTA was cleaved from the resin with 20% TFA in DCM. The purification of EdF-DOTA was performed using a Phenomenex Luna C18(2) (250*10 mm) column with the following elution conditions: 4.0 mL/minute flow rate; 0–5 minutes 100% A, 5-25 minutes 100% A to 70% A, 25-30 minutes 70% A to 10% A. Under these condition, the EdF-DOTA agent was eluted out at ~18.4 min. The identity and purity of the purified $^{13}C/^{2}H$ EdF-DOTA was confirmed by LC-MS using a Phenomenex C18(2) Luna (150*4.6 mm) column with the following elution conditions: 1.5 mL/minute flow rate; 0–5 minutes 100% A, 5-20 minutes 100% A to 60% A, 25-30 minutes 10% A. The peak at ~10.1 min corresponded to pure $^{13}C/^{2}H$ labeled EdF-DOTA (calculated, m/z $(M+H)^+$, 599.67; observed, m/z $(M+H)^+$ = 599.66).

Synthesis of native EdF-DOTA. The light-tagged agent was synthesized and purified in a similar manner using native ethylenediamine and phenylalanine. Its identity and purity were confirmed under the same LC-MS conditions, and the peak at ~10.1 min corresponded to pure native EdF-DOTA. (Calculated, m/z $(M+H)^+$, 594.68; observed, m/z $(M+H)^+$ = 594.69)

Preparation of internal standard solutions: Native Fe(III)DOTA-EdF (10 μM) was prepared by incubating 500 μM native EdF-DOTA ligand with 100 μM Fe^{3+} solution in 0.1 M NH_4OAc buffer (pH = 6.80) at 60 °C for 30 minutes and diluted 5-fold with 0.1 M NH_4OAc buffer (pH = 6.80). Other four internal standards were prepared in a similar manner using Ni^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}, respectively. The internal standard solutions were stored at -80 °C.

Quantification of the $^{13}C/^{2}H$ labeled EdF-DOTA with Cu-64. Copper-64 (in 0.1 N HCl) obtained from Wisconsin was immediately neutralized and buffered with 0.1 M NH_4OAc (pH = 6.8), to a final concentration of 20 μCi/μL (decay corrected to the time of receipt). After one week when the Cu-64 was completely decayed, 10 μL of the stock solution (200 μCi at the time of receiving activity) was mixed with 10 μL of 0.5 mM $^{13}C/^{2}H$ labeled EdF-DOTA and 55 μL of 0.1 M NH_4OAc buffer (pH = 6.80). The mixture was incubated at 22 °C for 10 (or 30) min or at 60 °C for 30 min, and then the five internal standards (10 μM) prepared earlier were added (5.0 μL for each standard, 25 μL in total) and mixed thoroughly. The entire mixture was transferred and loaded for LC-MS analysis. As described in **Fig. 2**, the relative amount of a particular heavy complex can be quantified by comparing its total ion counts with the corresponding native complexes.

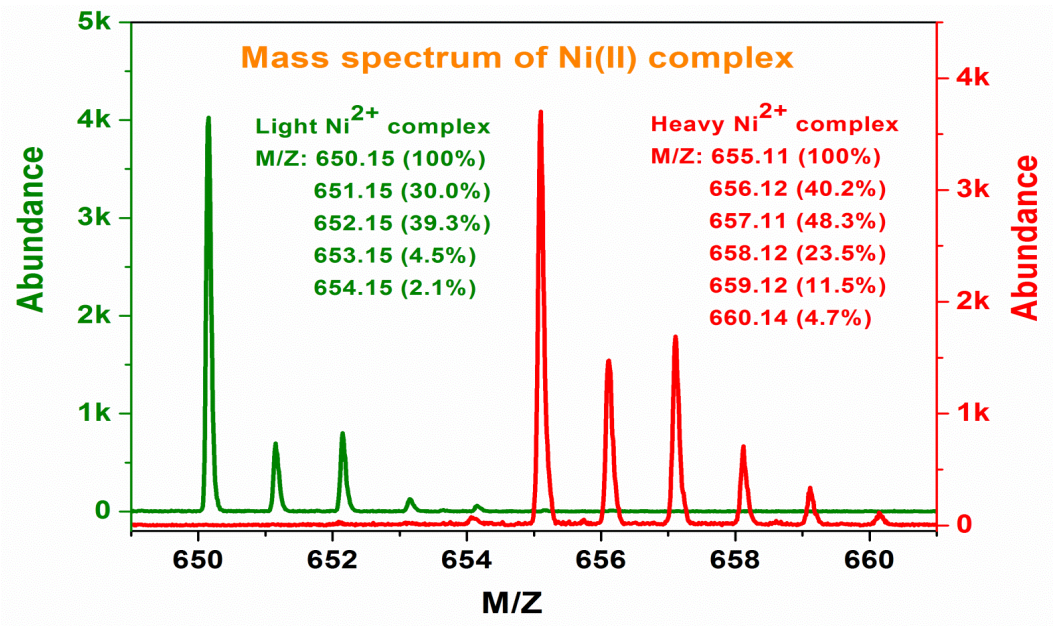


Figure S1: MS spectrum of EdF-(Ni^{2+})DOTA complexes.

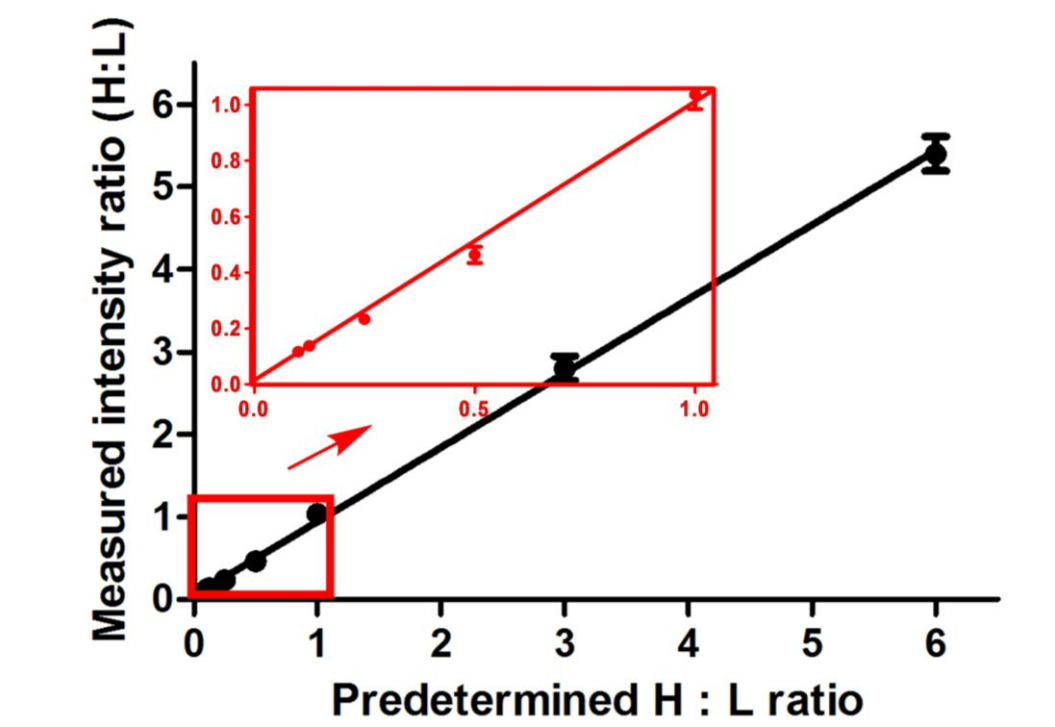


Figure S2: linear regression relationship between the measured Cu(II) ratios (y-axis) and the normalized predetermined Cu(II) ratios (x-axis) (standard error bars are shown)

References:

1. T. J. Wadas and C. J. Anderson, *Nat Protoc*, 2006, **1**, 3062-3068.
2. H. Ishiyama, K. Yoshizawa and J. Kobayashi, *Tetrahedron*, 2012, **68**, 6186-6192.
3. W. L. Scott, J. Alsina, C. O. Audu, E. Babaev, L. Cook, J. L. Dage, L. A. Goodwin, J. G. Martynow, D. Matosiuk, M. Royo, J. G. Smith, A. T. Strong, K. Wickizer, E. M. Woerly, Z. Zhou and M. J. O'Donnell, *J Comb Chem*, 2009, **11**, 14-33.