Metal-tag Labeling Coupled with Multiple Reaction Monitoring-Mass Spectrometry for Absolute Quantitation of Proteins

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Examination of the sample loss when desalting with Zip Tip

To evaluate the sample loss caused by desalting with Zip Tip, we choose one signature peptide (AGYTAIVSHR) from enolase in Thermoanaerobacter tengcongensis and another peptide (IEAIPQIDK) used for "Accuracy of the Method" as model peptides. With the peptide (AGYTAIVSHR) from enolase in Thermoanaerobacter tengcongensis, due to no ε-primary amino group in C terminal, metal labeling was directly carried out. 20 μg of the peptide was reconstituted in 20 μL of 0.2 M TEAB buffer at pH 8.0, and 60 μL of ACN and 1000 nanomoles of the DOTA-NHS ester were added, followed by incubation for 1 h at room temperature. Next, the DOTA-peptide solution was adjusted to pH 6.0 with 0.1 M NH_4OAc and devided into two equal aliquots, and 1.2-fold molar excess of either a $TbCl_3$ or $TmCl_3$ solution to the amount of the DOTA-NHS ester was added to each aliquot, followed by incubation at 25 ℃ for 1 h. For evaluation by MALDI-TOF MS analysis performed on a 4800 Proteomics Analyzer (AB SCIEX, USA), each sample was first desalted with Waters Sep-Pak Vak (packed with 50 mg C18 packing materials, 78 μm, Waters Corporation, USA) and two fractions were collected as model samples. 10 μL of each Tb-labeled peptide and Tm -labeled peptide (chosen as internal standard) was pooled，and 1 μL of the mixture was analyzed on MALDI-TOF MS. As shown in Figure S1A, the ratio of peak area of Tb-labeled peptide to that of Tm -labeled peptide is 92.41 %. Then about 2 μg of Tb-labeled peptide solution was further filtered on Zip Tip and mixed with Tm -labeled peptide solution that was not filtered, this pooled solution was analyzed on MALDI-TOF MS. As shown in Figure S1B, the ratio of peak area of Tb-labeled peptide to that of Tm -labeled peptide is 87.08 %, and the recovery is 96.3 % (87.08 %/90.41 %) which suggest that little sample loss was caused when desalting with Zip Tip. In practical application, the analytes and their corresponding internal standards are prepared in same experimental conditions, therefore, about 2 μg of

Tb-labeled peptide solution and 2 μg of Tm -labeled peptide solution were separately filtered on Zip Tip, then mixed and analyzed on MALDI-TOF MS. As shown in Figure S1C, the ratio of peak area of Tb-labeled peptide to that of Tm -labeled peptide is 92.84 %, and the recovery is 102 % (92.84 %/90.41 %) which further suggest that little sample loss was caused when desalting with Zip Tip. With another peptide (IEAIPQIDK) used for "Accuracy of the Method", due to ε-primary amino group in C terminal, 40 μg of the peptide was first modified via guanidination as described in "Labeling of Peptides" in "EXPERIMENTAL SECTION", then desalted with Waters Sep-Pak Vak, then dried and dissolved in 40 μL 0.2 mol/L pH 8.0 TEAB and finally divided into equal aliquots. The following steps are the same as those done for the peptide (AGYTAIVSHR) described above. As shown in Figure S1D, the ratio of peak area of Tb-labeled peptide to that of Tm -labeled peptide is 93.53 %, then as shown in Figure S1E, the ratio of peak area of Tb-labeled peptide to that of Tm -labeled peptide is 90.02 %, and the recovery is 96.25 % (90.02 %/93.53 %), and at last as shown in Figure S1F, the ratio of peak area of Tb-labeled peptide to that of Tm -labeled peptide is 94.88 %, and the recovery is 101 % (94.88 %/93.53 %). The recovery results are similar to those obtained with peptide (AGYTAIVSHR), and further show that desalting with Zip Tip has little influence on the quantitation accuracy of proteins by using MRM MS with labeled internal standards.

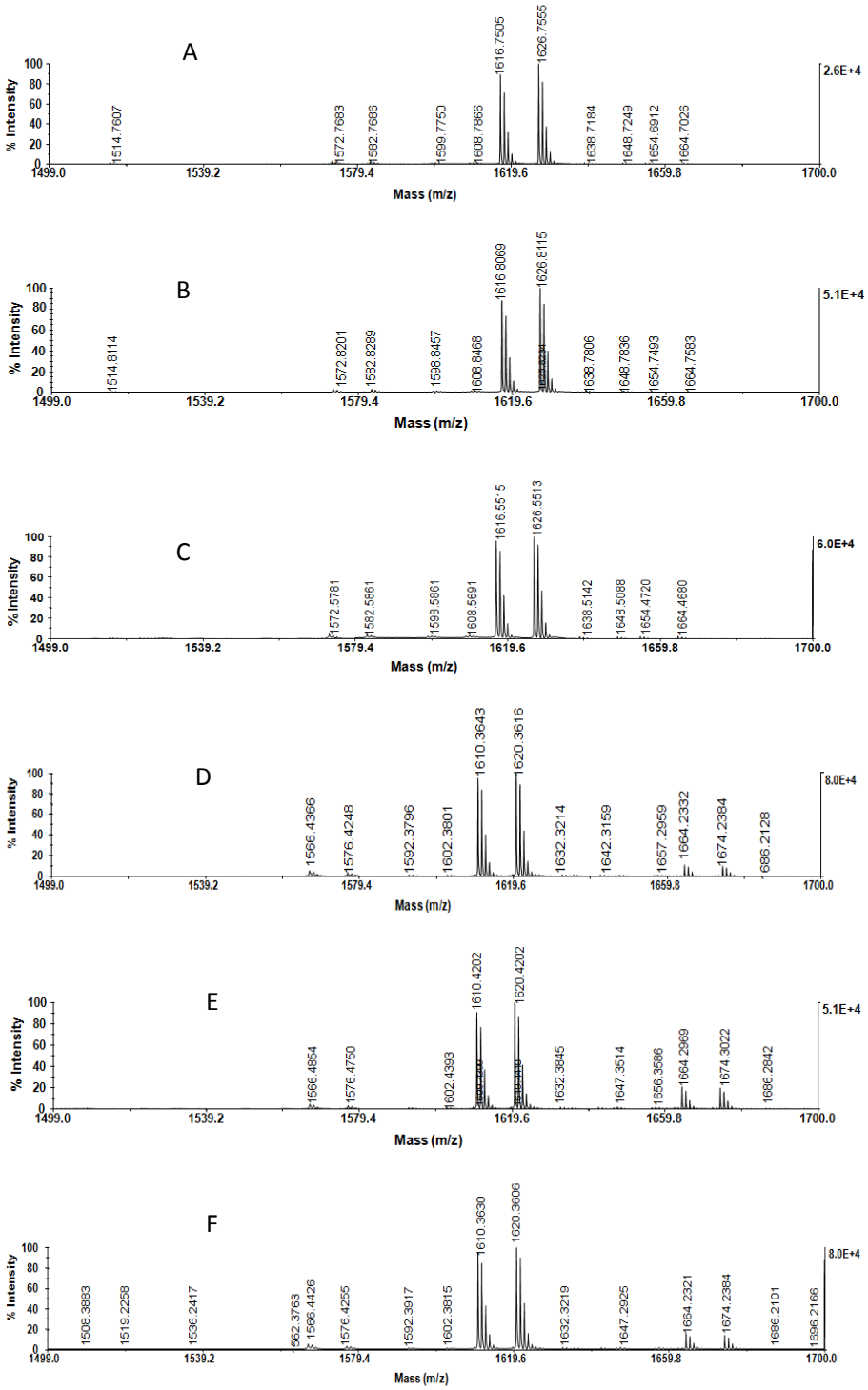

Figure S1 The spectra of Tb- and Tm-DOTA-AGYTAIVSHR and Tb- and Tm -DOTA-IEAIPQIDK analyzed on MALDI-TOF MS

A, the spectrum of Tb- and Tm-DOTA-AGYTAIVSHR desalted separately with Waters Sep-Pak Vak as a model sample and then mixed in the amount ratio of 1:1, D, the same as A for Tb- and Tm -DOTA-IEAIPQIDK; B, the spectrum of Tb- DOTA-AGYTAIVSHR desalted with Zip Tip and Tm-DOTA-AGYTAIVSHR without desalting with Zip Tip and then mixed in the amount ratio of 1:1, E, the same as B for Tb- and Tm -DOTA-IEAIPQIDK; C, the spectrum of both Tb- and Tm-DOTA-AGYTAIVSHR desalted with Zip Tip and ten mixed in the amount ratio of 1:1, F, the

same as C for Tb- and Tm -DOTA-IEAIPQIDK.

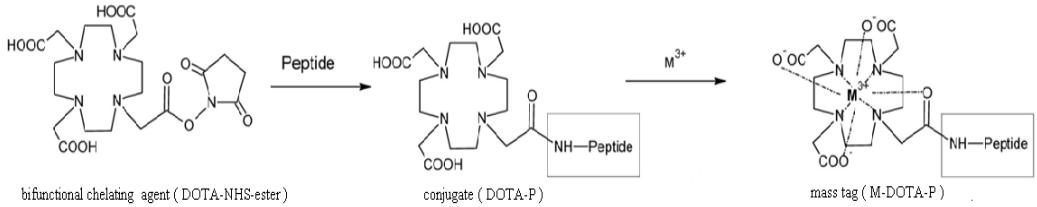

Figure S2. A scheme for DOTA-NHS ester coupling to primary amines of a peptide and then chelating lanthanide ions.

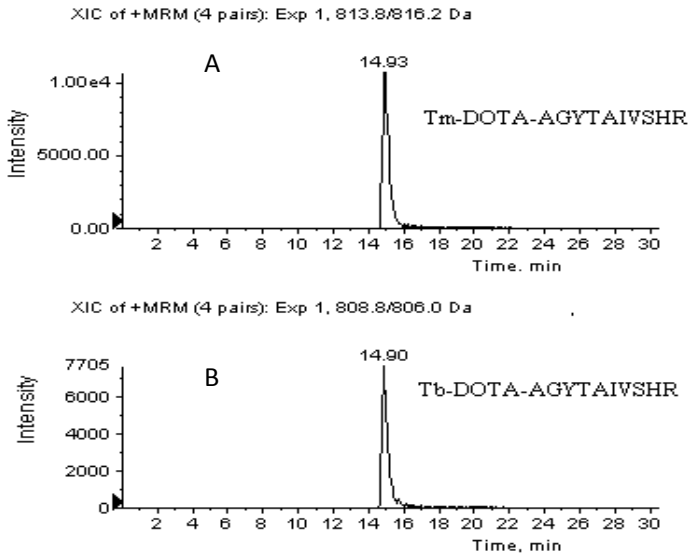

Figure S3. MRM chromatograms of a peptide (AGYTAIVSHR) by a nanoflow LC-MS/MS. A, the extracted MRM chromatogram (transition 813/816) of Tm-DOTA-P; B, the extracted MRM chromatogram (transition 808/806) of Tb-DOTA-P.

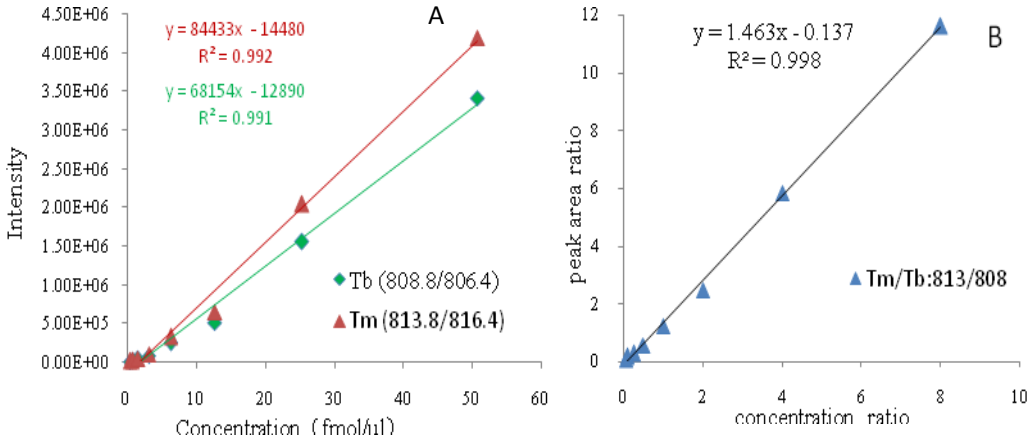

Figure S4. Limit of quantification and dynamic range.

A, a plot of changes in intensity with increase in sample concentration. Lowest analyte concentration from linear curve is considered as LOQ when relative standard deviation was measured below 20% . B, Linear dynamic range of the Tm-DOTA-AGYTAIVSHR and Tb-DOTA-AGYTAIVSHR analyzed by reversed phase liquid chromatography coupled with MRM MS. The horizontal abscissa: the ratio of concentrations(Tm-DOTA-P/Tb-DOTA-P), the vertical ordinate: peak area ratio (Tm-DOTA-P/Tb-DOTA-P).

Electronic Supplementary Material (ESI) for Analyst

Table S1. Labeling efficiencies of tryptic peptides from horse heart myoglobin with a metal-DOTA tag

Peptide sequence	Peptides m/z	After guanidination m/z	Guanidination efficiencies	DOTA-P m/z	Labeling efficiencies	Tm-DOTA-P m/z	Chelating efficiencies
ALELFR	748.3	—	—	1134.5	99.38%	1300.4	100%
LFTGHPETLEK	1271.4	1313.4	100%	1699.6	99.47%	1865.5	100%
HGTVVLTALGGILK	1378.5	1420.5	100%	1806.7	91.25%	1972.6	100%
VEADIAGHGQEVLIR	1606.5	—	—	1992.7	99.26%	2158.6	98.89%
GLSDGEWQQVLNVWGK	1815.5	1857.5	100%	2243.7	97.76%	2409.6	100%

Table S2. Accuracy of the method

Transitions	810.8 / 795.2	810.8 / 866.4
Added concentration(fmol/μL)	94.56	94.56
Tested concentration(fmol/μL)	88.75	81.65
RE (%)	-6.15	-13.66

Table S3. Transitions and MS conditions used for MRM MS analysis

Protein	Signature Peptides	Q1 (charge：+2)	Q3 (charge：+1	CE (V)	DP (V)
Enolase [Thermoanaerobacter tengcongensis MB4]	Tm-DOTA-AGYTAIVSHR	813.8	816.4	59	130
		813.8	681.1	60	130
	Tb-DOTA-AGYTAIVSHR	808.3	806.4	59	130
		808.3	671.1	60	130
	Tm-DOTA-SSIIDIYAR	795.3	812.3	49	110
		795.3	953.5	30	110
	Tb-DOTA-SSIIDIYAR	789.8	802.3	49	110
		789.8	943.5	30	110