Electronic Supplementary Information

A dual-labelling strategy for integrated ICPMS and LIF for the determination of Peptides

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Chemicals

All chemicals and reagents used in this study were at least of analytical grade. Ultrapure water used throughout this study was prepared with Milli-Q system (Millipore Filter Co., Bedford, MA). Fluorescein isothiocyanate (FITC), Tris-(2-carboxyethyl)-phosphine (TCEP, ≥ 98%), vasopressin (Vas, ≥ 97%), somatostatin (Som, ≥ 98%), 4-Morpholineethanesulfonic acid (MES) and 4-Morpholonepropanesulfonic acid (MOPS) were all obtained from sigma-Aldrich (St. Louris, MO). GGYGGC was obtained from Sangon Biotech (Shanghai) Co., Ltd. 1,4,7,10-Tetraazacyclododecane-1,4,7-trisacetic acid-10-maleimidoethylacetamide (MMA-DOTA) was purchased from Macrocyclics (Dallas, TX). ^{153}Eu-enriched Eu_2O_3 (99.8%) was purchased from Cambridge Isotope Labratories, Inc. (Andover, MA). Concentrated HNO_3 (GR grade) and HPLC grade acetonitrile (ACN) were purchased from Merck KGaA (Darmstadt, Germany).

Natural isotopic abundance Eu solution for peptide labelling was prepared by dissolving the solid Eu_2O_3 (from Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, purity greater than 99.9999%) in concentrated HNO_3 at room temperature, and then evaporated gradually to dryness on an electric plate; the residue finally dissolved to a concentration of 500 mmol L^{-1} Eu solution using 2% HNO_3. The enriched isotopic stoke solution of ^{153}Eu used for species-unspecific isotope dilution analysis was prepared from solid $^{153}Eu_2O_3$ in the same way. And the final exact concentration of ^{153}Eu was determined via inverse isotope dilution analysis to be 2.80 μg L^{-1}.[1]

HPLC-ESI-MS. Chromatographic separations were performed on an Agilent 1100 series chromatographic system (Agilent Technologies, Palo Alto, CA) using a Zorbax 300SB-C18 column (1.0 I.D. × 150 mm in length, 3.5μm particle size). The derivatives were eluted with the following gradient program at the flow rate of 0.05 mL min^{-1}. The mobile phase A (0.05% TFA in ultrapure water) was maintained for 5 min for desalting and then mobile phase B (0.05% TFA in ACN) was linearly increased from 25% to 60% within 30 min. The column effluent was directly introduced into a Bruker Daltonics Esquire-LC ESI ion trap mass spectrometer (Bremen, Germany)

for structural analysis of the dual-labelled peptides. The operational parameters of the ESI-MS were as follows: ion mode, positive; m/z range measured 300-2000; capillary voltage, -3300 V; nebulizer gas, 70 psi; dry gas, 8 L min^{-1}; end plate offset, -500 V; dry temperature, 300 °C.

HPLC-ICPMS. An LC-20AD LC system (Shimadzu, Kyoto, Japan) equipped with the same C18 column was used in HPLC-ICPMS. The gradient elution program was also the same as HPLC-ESI-MS experiments. The effluent from the column was mixed with the enriched ^{153}Eu spike solution continuously pumped by a syringe pump (Cole-Parmer, East Bunker Court Vernon Hills, IL) through a three-way connector for on-line quantification using an ELAN DRC II CPMS (PerkinElmer, SCIEX, Canada) equipped with a concentric pneumatic nebulizer and a Cyclonic spray chamber. The operational parameters of ICPMS were as follows: RF power, 1200W; auxiliary gas, 1.0 L min^{-1}; nebulizer gas, 0.88 L min^{-1}; plasma gas, 15 L min^{-1}; dwell time, 100 ms; isotope monitored, ^{151}Eu and ^{153}Eu.

CE-LIF. The capillary electrophoretic analysis was carried out on a Beckman P/ACE capillary electrophoresis system equipped with a laser-induced fluorescence detector (the excitation wavelength were 488 nm) (Beckman Instruments, Palo Alto, CA, USA). A 40 cm length (30 cm from inlet end to the detection window) uncoated fused-silica capillary with 75 μm inner diameter was used. Unless indicated, the running buffer was Na_2CO_3-$NaHCO_3$ (200 mmol L^{-1}, pH 9.5). Samples were injected under pressure (0.5 psi) for 5s. The separation voltage was 5 kV at normal polarity. Prior to the runs, the capillary was rinsed with 0.1 mol L^{-1} NaOH solution, ultrapure water and the running buffer for 2 min each in sequence, respectively.

Subsequent labelling of -SH and -NH_2. We chose Vas as a model peptide to optimize the proposed dual-labelling conditions. Vas was dissolved in ultrapure water to get a stock solution containing 0.92 mmol L^{-1} Vas. The disulfide bond in Vas (10μL, 0.92 mmol L^{-1}) were reduced firstly with a fivefold molar excess (compared to the disulfide bond) of TCEP at 37 °C for 30 min in 40 μL 100 mmol L^{-1} MOPS buffer solution (pH6.8); then MMA-DOTA was added to label the nascent –SHs at 47 °C for 40 min. Under the best labelling conditions of pH, reaction temperature and time, the molar ratios (1:1, 3:1, 5:1, 7:1, 10:1) of MMA-DOTA to –SHs were investigated.

The next Eu-loading step was performed at pH 5.8 or lower because possible precipitation of Eu^{3+} would generate at high pH. Therefore, the Vas-MMA-DOTA solution was buffered by adding appropriate amount of 500 mmol L^{-1} MES buffer (pH 5.8). Subsequently, a 2-fold molar excess of Eu with respect to DOTA was added and reacted for 1 h at 37 °C. The excess free Eu^{3+} was depleted with EDTA and it was eluted within the dead volume during HPLC separation, making no interference with the determination of labelled peptides.

The following procedure was to label the -NH_2 in Vas with FITC. Typically, a pH of 9.0 is selected for the derivatization of -NH_2 with FITC.[2] Before conjugating, the pH of Vas-MMA-DOTA-Eu solution was adjusted to 9.0 with 200 mmol L^{-1} Na_2CO_3-$NaHCO_3$ so as to guarantee a basic condition. Finally, different amounts of FITC (3, 5, 10, 15, 25, 35, 50-fold in excess compared to -NH_2) were added to conjugate the -NH_2 at 50°C for 12 h. Besides, time-dependent labelling of -NH_2 (in Vas-MMA-DOTA-Eu) with FITC was also investigated. In the cases of Som and GGYGGC, the labeling conditions were the same as those of Vas.

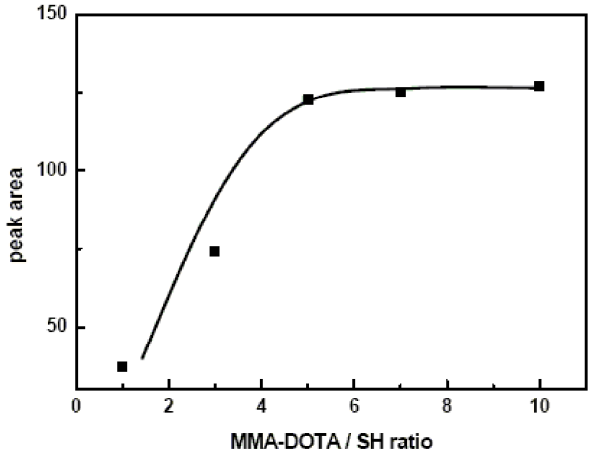

Fig. S1 Effect of the molar ratio of MMA-DOTA to -SH (in Vas) on the labelling efficiency of MMA-DOTA towards Vas. Reaction condition: 100 mmol L^{-1} MOPS (pH 6.8) at 47 °C for 40 min, 50 µmol L^{-1} Vas; HPLC-UV measurement ($\lambda = 214$ nm).

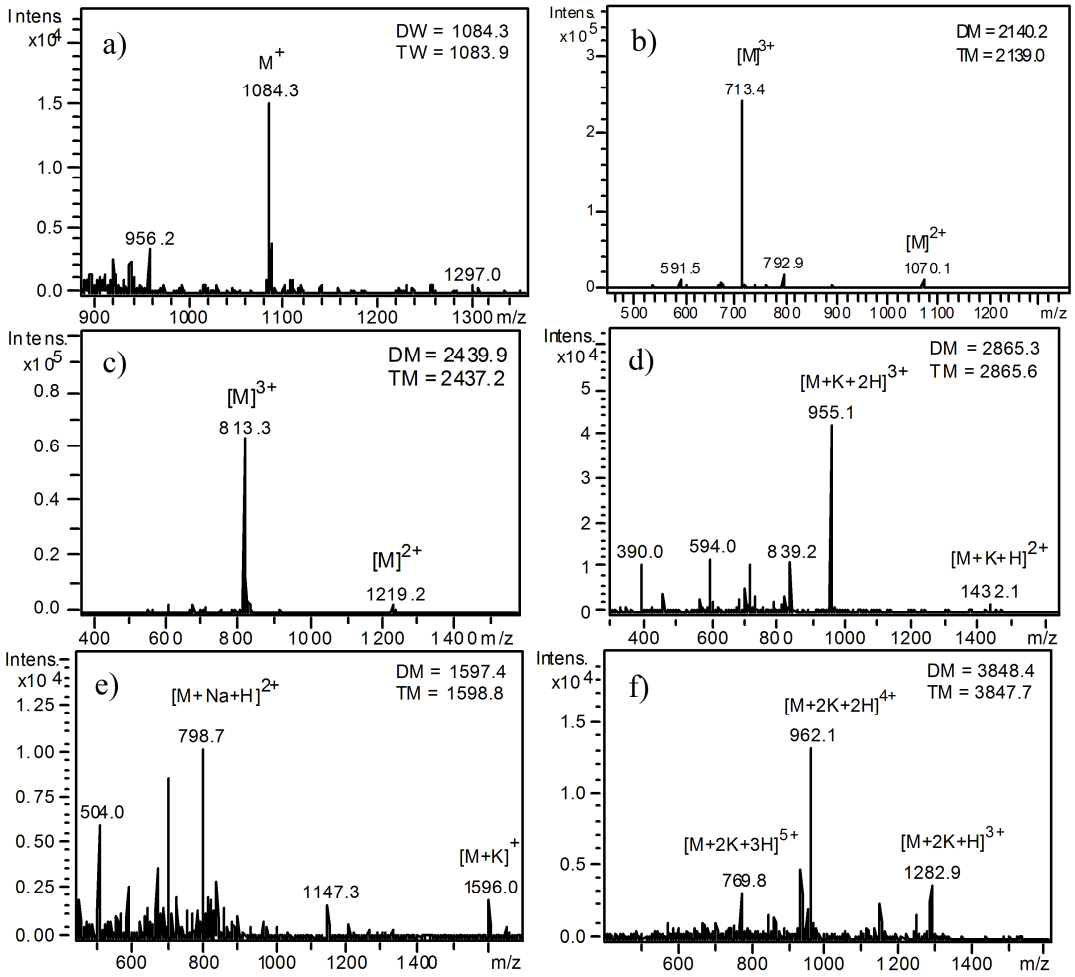

Fig. S2 ESI-MS spectra of a) Vas, b) MMA-DOTA labelled Vas, c) MMA-DOTA-Eu labelled Vas, d) MMA-DOTA-Eu and FITC labelled Vas, e) MMA-DOTA-Eu and FITC labelled GGYGGC, f) MMA-DOTA-Eu and FITC labelled Som. DM and TM in the MS spectra denote deconvolution and theoretical molecular weights, respectively.

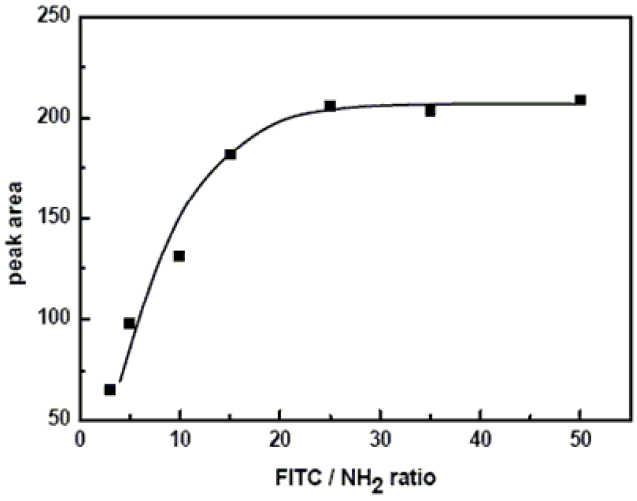

Fig. S3 Effect of the malor ratio of FITC to -NH_2 (in Vas-MMA-DOTA-Eu) on labelling efficiency of FITC-Vas-MMA-DOTA-Eu. Reaction conditions: 200 mmol L^{-1} Na_2CO_3-$NaHCO_3$ (pH 9.0) at 50°C, 50 μmol L^{-1} Vas. HPLC-UV measurement (λ = 254 nm).

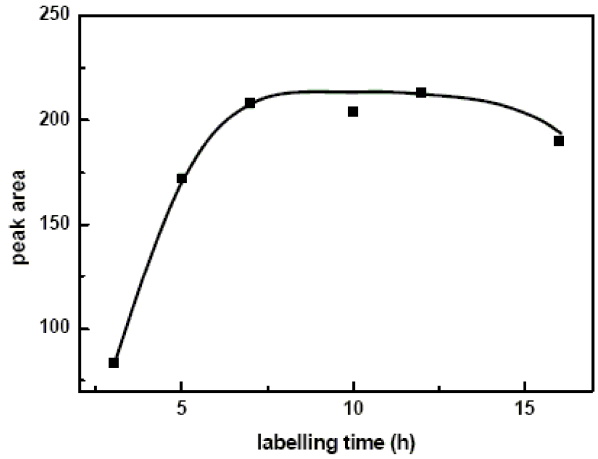

Fig. S4 Time-dependent labelling of -NH_2 (in Vas-MMA-DOTA-Eu) with FITC. Reaction conditions: the molar ratio of FITC to -NH_2 was 25:1, 200 mmol L^{-1} Na_2CO_3-$NaHCO_3$ (pH 9.0) at 50°C, 50 μmol L^{-1} Vas. HPLC-UV measurement (λ = 254 nm).

Quantification using HPLC coupled with species-unspecific isotope dilution ICPMS. An enriched ^{153}Eu spike solution was mixed continuously with the HPLC effluent so as to change the isotope ratio of ^{153}Eu/^{151}Eu. In order to keep the flow stable and match the ICPMS requirement as well as minimize the influence of ACN in the mobile phase, the spike solution was injected at a flow rate of 1 mL min^{-1} using an accurate syringe pump. The signal of ^{153}Eu and ^{151}Eu were monitored, realizing the absolute quantification of the peptides.

Optimization of CE-LIF conditions for separation and determination of the peptides. For the separation and quantification of three dual-labelled model peptides, Na_2CO_3-$NaHCO_3$ buffer was used as a running buffer. The effect of running buffer pH from 9.2 to 10.6 on the separation efficiency was studied. Moreover, different concentrations (50, 100, 150, 200 mmol L^{-1}) of Na_2CO_3-$NaHCO_3$ buffer solution at optimum pH for the separation were also studied, besides the

best separation voltage was investigated. On the basis of a series of experiments, the optimal CE conditions were obtained as follows: 200 mmol L^{-1} Na_2CO_3-$NaHCO_3$ (pH 9.5) used as the running buffer; the sample injected under 0.5 psi for 5s and CE separation voltage at 5 kV (normal polarity). A mixture containing completely dual-labelled Vas, GGYGGC and Som was analyzed by CE-LIF under the optimized conditions (Fig. S5 and S6). The results are shown in Fig. S7.

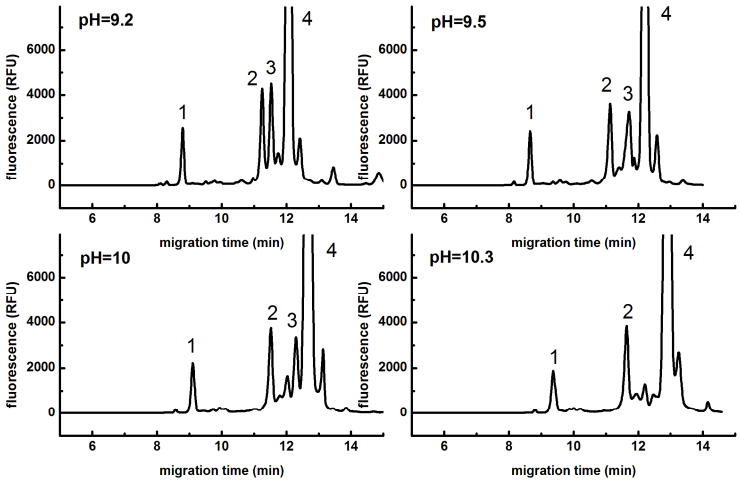

Fig. S5 pH effect of running buffer on the CE separation. CE conditions: uncoated fused-silica capillary, 40 cm × 75 μm ID; running buffer: 50 mmol L^{-1} Na_2CO_3-$NaHCO_3$; separation voltage: 5 kV; injection: 0.5 psi, 5s. Peak1, dual-labelled Vas; peak2, dual-labelled Som; peak3, dual-labelled GGYGGC; peak4, unreacted FITC.

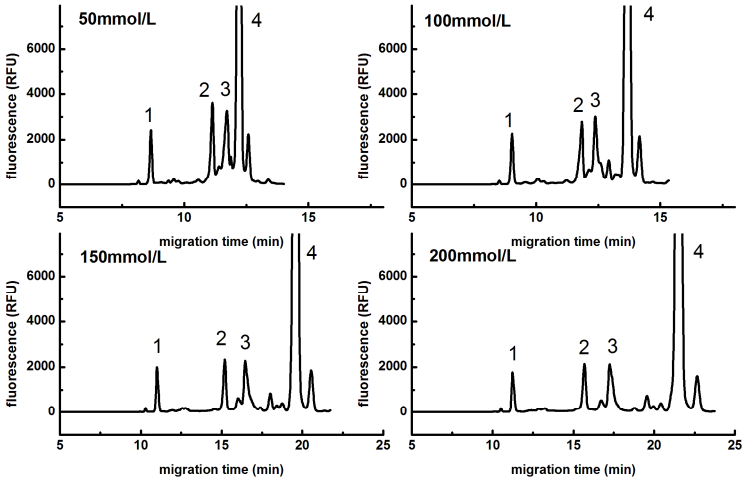

Fig. S6 Effect of running buffer concentration on the CE separation. Running buffer: Na_2CO_3-$NaHCO_3$ (pH 9.5). Other conditions were the same as those indicated in Fig. S5.

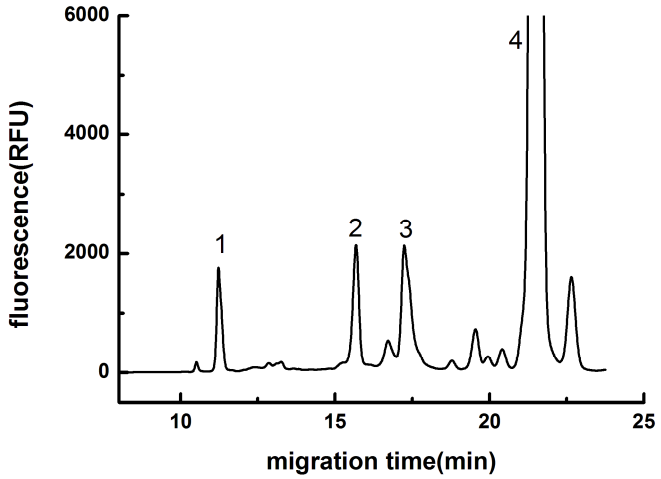

Fig. S7 Electrophoregram of the dual-labelled peptides. CE conditions: uncoated fused-silica capillary, 40 cm × 75 μm ID; running buffer: 200 mM Na_2CO_3-$NaHCO_3$ (pH 9.5); separation voltage: 5 kV; injection: 0.5 psi, 5s. Peak1, the dual-labelled Vas; peak2, the dual-labelled Som; peak3, the dual-labelled GGYGGC; peak4, unreacted FITC.

References

1 X. W. Yan, M. Xu, L.M.Yang and Q.Q. Wang. *Anal. Chem*. 2010, **82**, 1261.

2 Y.F. Cheng and N.J. Dovichi. *Science*. 1988, **242**, 562.