Supplementary Information for:

Online Coupling of Liquid Chromatography with Mass Spectrometry by Desorption Electrospray Ionization (DESI)

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Additional information about experimental conditions and additional mass spectra are included in this Supplementary Information.

1. Experimental section

All experiments were carried out using a Thermo Finnigan LCQ DECA or DECA MAX ion trap mass spectrometer (San Jose, CA) and a Perkin Elmer HPLC system (Perkin Elmer, Shelton, CT) with an Agilent C18 column (250 mm × 4.6 mm i.d.). The DESI spray voltage was set at +5 kV and the nebulizing gas (N2) pressure used was 170 psi. Unless specified otherwise, the DESI spray solvent was 1% acetic acid in acetonitrile or in methanol/water (1 : 1 by volume) and sprayed at 10 μL min^{-1}.

2. Digestion of somatostatin 1-14

4 mg/mL digested somatostatin 1-14 (MW: 1637.9 Da) carried out using TPCK-treated trypsin with a ratio of 1:100 (enzyme/peptide) in 25 mM ammonium bicarbonate aqueous solution for 3 h at 38 ^{o}C incubation.

3. Separation of the tryptic digest of somatostatin 1-14

A binary solvent (solvent A: water containing 0.09% trifluoroacetic acid; solution B: acetonitrile/water (3:2 by volume) containing 0.09% trifluoroacetic acid) was employed. A 2-min elution with 100% A solution, 10-min linear gradient from 0 to 100 % solution B, 5-min elution with 100% solution B, 3-min linear gradient from 0 to 100 % solution A and 3-min elution with 100% solution A was used for HPLC separation. The volume of the digest loaded for separation was 5 μL. 4 mg/mL tryptic digested somatostatin 1-14 first underwent LC separation, an ASI adjustable splitter was used to reduce the flow rate to 5 μL/min and then electrochemical reduction prior to DESI ionization. The rest eluents were flowed through the UV detector (detection wavelength was set at 254 nm). A thin-layer μ-PrepCell^{TM} electrochemical flow cell equipped with a magic diamond (MD) electrode (diameters: 12 × 30 mm, Antec Leyden, Netherlands) as the working electrode (WE) was employed and a Roxy^{TM} potentiostat (Antec Leyden, Netherlands) was used to apply a potential of –1.5 V to the cell for triggering electrolytic reduction. The reduced species flowed out of the thin-layer cell via a short piece of fused silica capillary (i.d. 0.1 mm, 4.7 cm long) and then underwent DESI ionization.

4. Additional DESI mass spectra

Fig. S1 shows that the DESI also tolerates slow flow rate elution such as 4.5 μL/min. The separated three analyte compounds NE, NM and DA were clearly detected by DESI-MS, as shown in Fig. S1c, d and e, respectively.

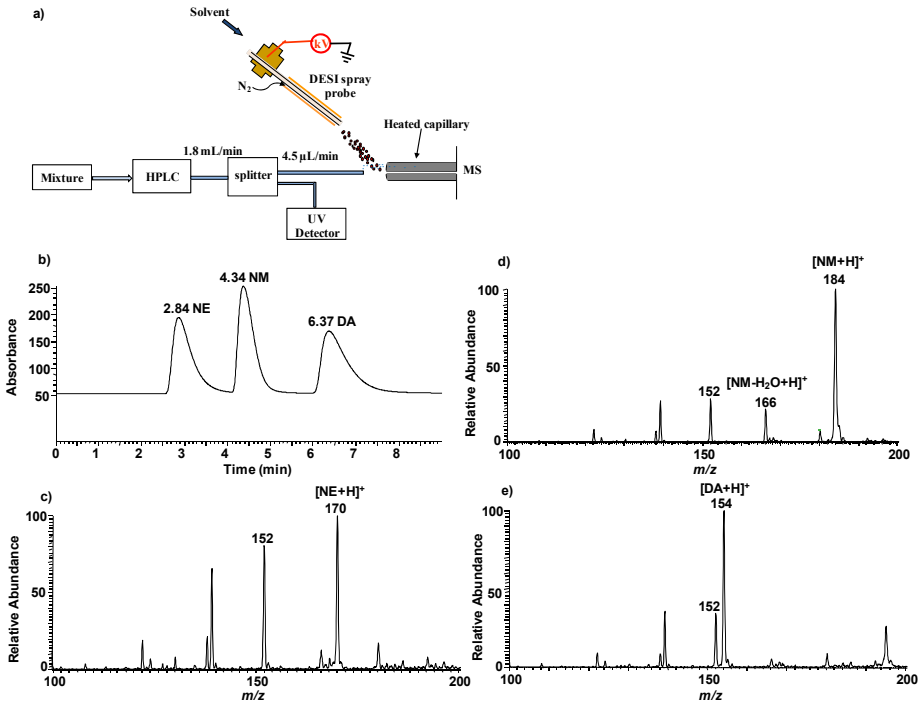

Fig. S1 (a) Scheme showing an apparatus for the coupled LC/MS using DESI interface, in which the eluent flow was reduced to 4.5 µL/min using a splitter; (b) acquired UV chromatogram (266 nm) using a C18 column and the isocratic mode; DESI-MS mass spectra showing the separated (c) NE, (d) NM and (e) DA.

Fig. S2 shows that the DESI-MS spectra of compounds NE, NM and DA after LC separation (isobaric elution as mentioned before in the text) and subsequent electrochemical oxidation prior to DESI ionization. In this experiment, a thin-layer ReactorCell^{TM} electrochemical flow cell equipped with a magic diamond (MD) electrode (i.d. 6 mm, Antec Leyden, Netherlands) as the working electrode (WE) was employed and a Roxy^{TM} potentiostat (Antec Leyden, Netherlands) was used to apply a potential of +1.6 V to the cell for triggering electrolytic oxidation. When the electrochemical cell was on, the ions corresponding to the oxidized products of NE and DA were seen at m/z 168 and 152, respectively. By contrast, no product ion resulting from oxidation of the electrochemically inactive NM was observed.

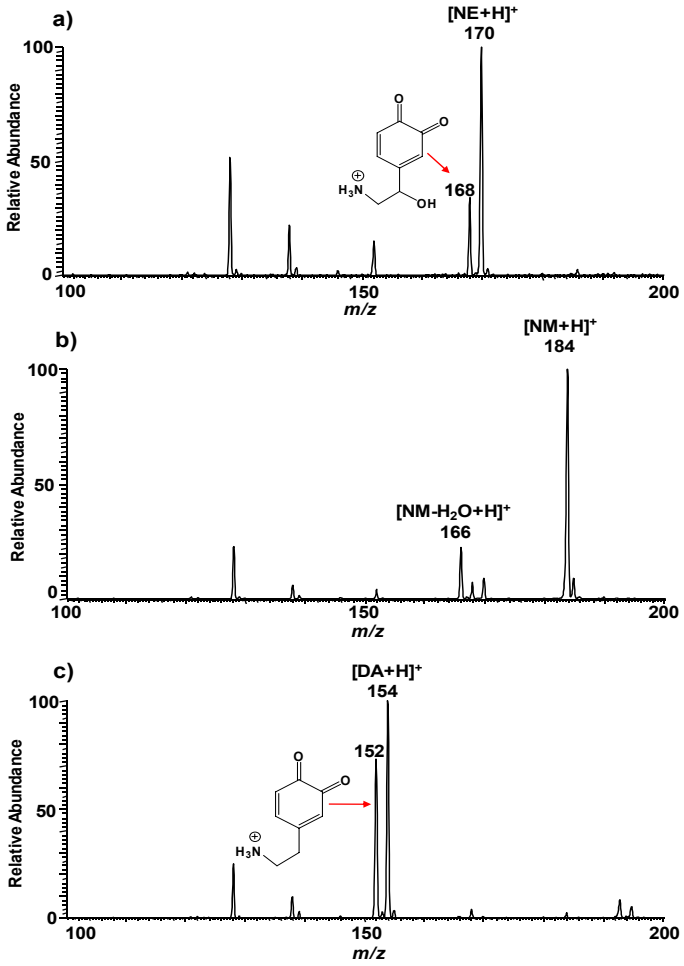

Fig. S2 Acquired DESI mass spectra showing the products after electrochemical oxidation of (a) NE, (b) NM and (c) DA following chromatographic separation. The structures of ions resulting from the oxidation of NE and DA were marked in (a) and (c), respectively.

Fig. S3 shows CID MS^2 spectra of a) *m/z* 933 and b) *m/z* 741, corresponding to the protonated peptide AGCK/TFTSC (this peptide has two chains of AGCK and TFTSC linked by a disulfide bond) and NFFWK, respectively, generated from the tryptic digestion of somatostatin 1-14. The fragment ion of *A1-A4/B*(y_1) in Fig. S3a refers to a fragment with y_1 ion of B chain linked with an intact A chain and this notation is applicable to other fragment ions. The fragment

ions observed in Fig. S3a and b are in agreement with the structures of peptides AGCK/TFTSC and NFFWK, respectively.

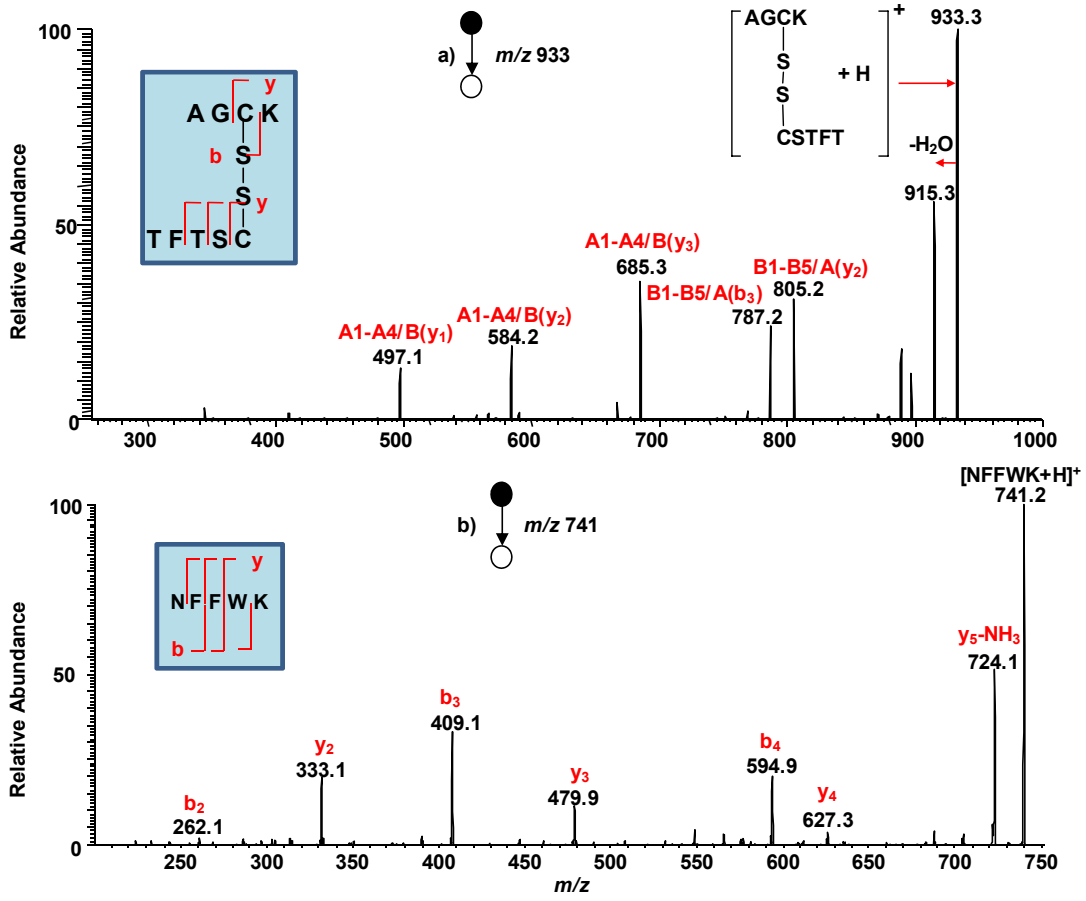

Fig. S3 CID MS^2 spectra of a) *m/z* 933 and b) *m/z* 741.

Fig. S4 shows CID MS^2 spectra of a) *m/z* 378 and b) *m/z* 558. Upon CID, the *m/z* 378.1 gives rise to b_2, b_3, b_4, y_1, and y_2 fragment ions, from which its sequence can be determined as either AGCK or GACK (cysteine is in 3rd position). Likewise, the *m/z* 558.0 dissociates into a_2, b_2, b_3, b_4, b_5, and y_3 ions, which reveals its sequence to be either TFTSC or FTTSC and the cysteine residue to be the 5th position in chain. Thus it can be seen that the sole disulfide bond of AGCK/TFTSC bridges the 3rd residue of one chain with the 5th residue of the other chain.

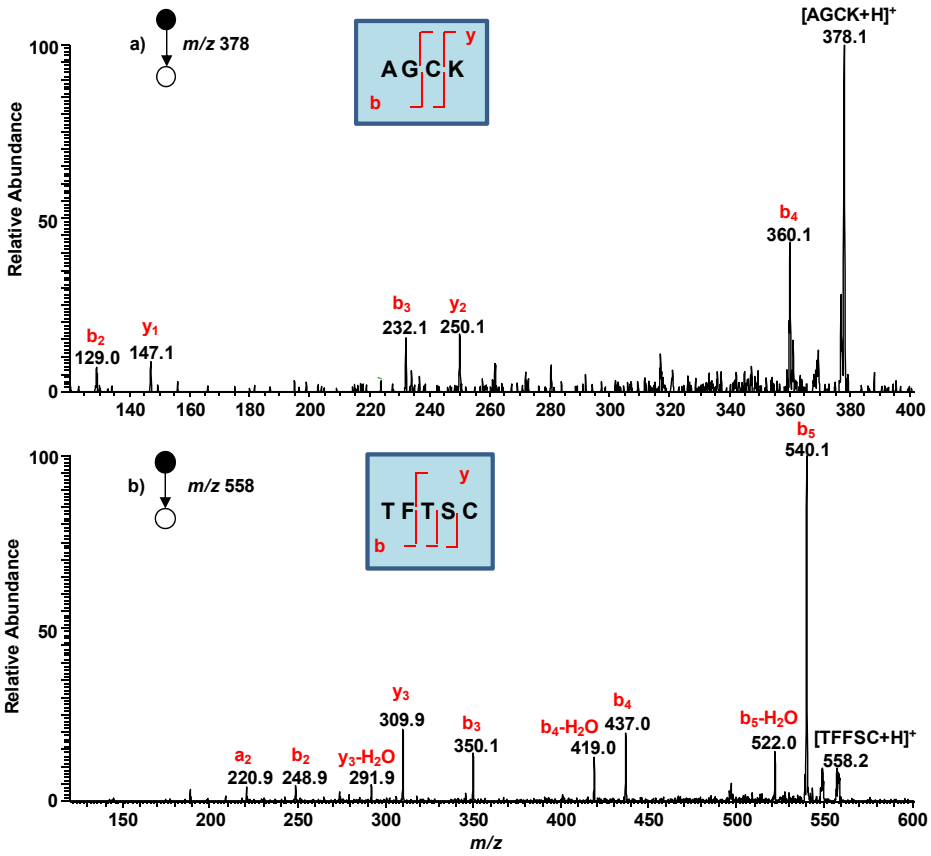

Fig. S4 CID MS^2 spectra of a) $[AGCK+H]^+$ (*m/z* 378) and b) $[TFTSC+H]^+$ (*m/z* 558).

Fig. S5 shows DESI-MS detection of another peptide in the tryptic digest of somatostatin 1-14, NFFWK, following chromatographic separation. Fig. S5a and b display the DESI mass spectra acquired when the cell was off and on, respectively. These two peptides did not undergo electrolytic reduction because of being lack of disulfide bonds.

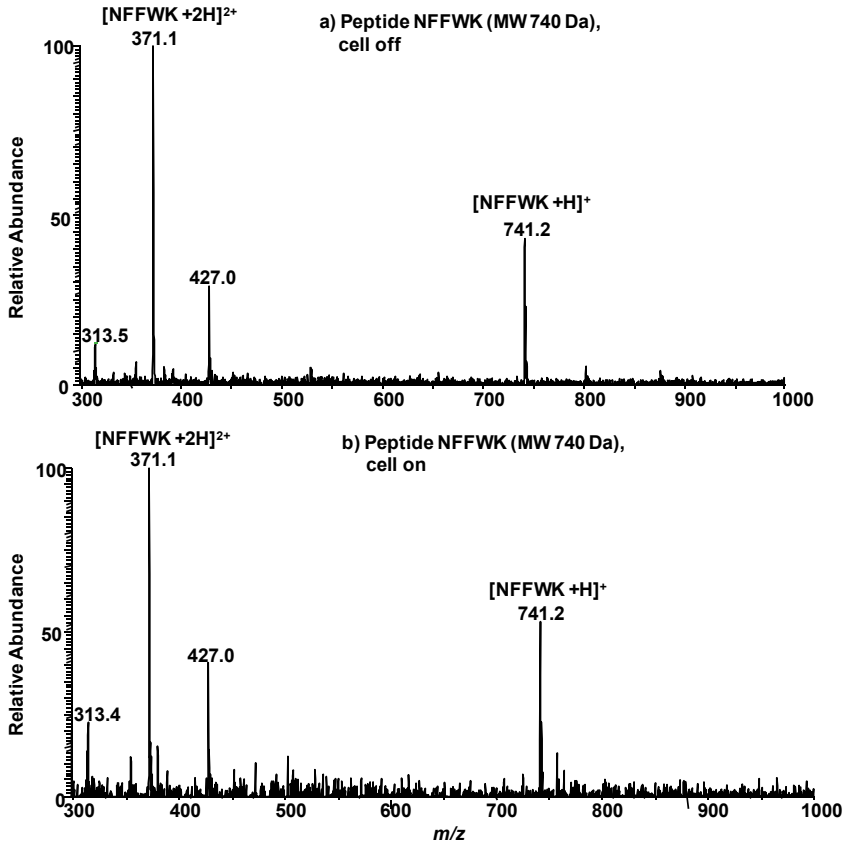

Fig. S5 DESI-MS mass spectra of the separated peptide NFFWK (a) with cell off and (b) with cell on (background was subtracted).