Electronic Supplemental Material for

Atmospheric-Pressure Ionization and Fragmentation of Peptides by Solution-Cathode Glow Discharge

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Experimental Section (continued)

ESI-Tandem MS

All peptides analyzed with SCGD-MS were also subjected to more conventional ionization and fragmentation approaches on two different mass spectrometers. In both cases, solutions containing 1 µg/mL of each peptide were dissolved in HPLC-MS grade water. Flow injection of a 5-µL plug of peptide solution was carried to a heated ESI source (HESI II probe, Thermo Scientific, San Jose, CA) with 50 µL/min carrier flow of 50% methanol, 25% water, and 0.1% TFA.. The carrier solvent flow was delivered by a binary liquid chromatograph pump (model HPG-3400RS, Thermo Scientific, San Jose, CA) after passing through a vacuum degasser (model SRD-3600, Thermo Scientific, San Jose, CA).

Low-resolution ESI tandem mass spectra were acquired with a Thermo Scientific LTQ XL linear ion trap (San Jose, CA). The source was operated at 4 kV with a sheath gas flow rate and gas-heater temperature of 20 (manufacturer's units) and 50 °C, respectively, to cope with the relatively high liquid flow rates. Spectra were collected in automatic gain-control mode with a maximum ion-trap injection time of 100 ms and 1 microscan per spectrum. The key experimental parameters for positive-ion detection were: capillary temperature 310 °C; capillary voltage 20 V; tube lens 85 V. Tandem mass spectrometry (MS^n) was performed *via* collision-induced dissociation (CID) after isolation of either the MH_2^{2+} of MH_3^{3+} ion. These tandem mass spectra were obtained with an isolation window of 1.5 Th and 20% relative collision energy.

High-resolution ESI mass spectra were acquired with an Exactive Plus Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany). Fragmentation of all ions (*i.e.* without isolation) was performed through in-source collision-induced dissociation with energies between 20 and 40 eV. The in-source CID occurs within the vacuum system of the mass spectrometer through a DC voltage offset between the entrance ion optics and the transfer optics. All spectra were recorded with a mass resolving power setting of 140,000 and an automatic gain control (AGC) target value of 1×10^6 ions. To ensure high mass accuracy, better than 1 ppm, the instrument was mass calibrated daily and a lock mass of *m/z* 371.10124, due to polysiloxane, was used throughout.

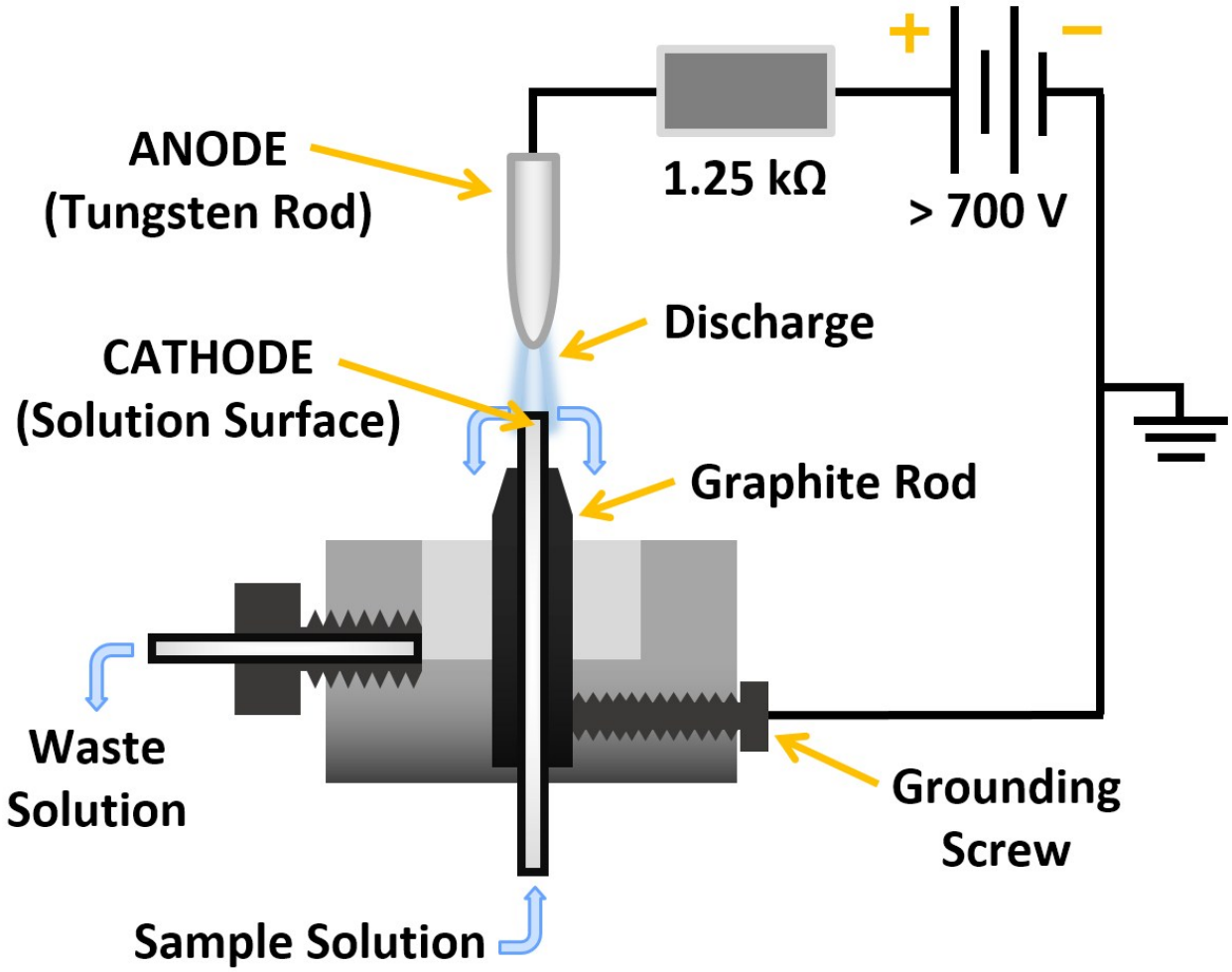

Figure S1. Schematic diagram of the compact solution-cathode glow discharge cell utilized for mass spectrometry experiments.

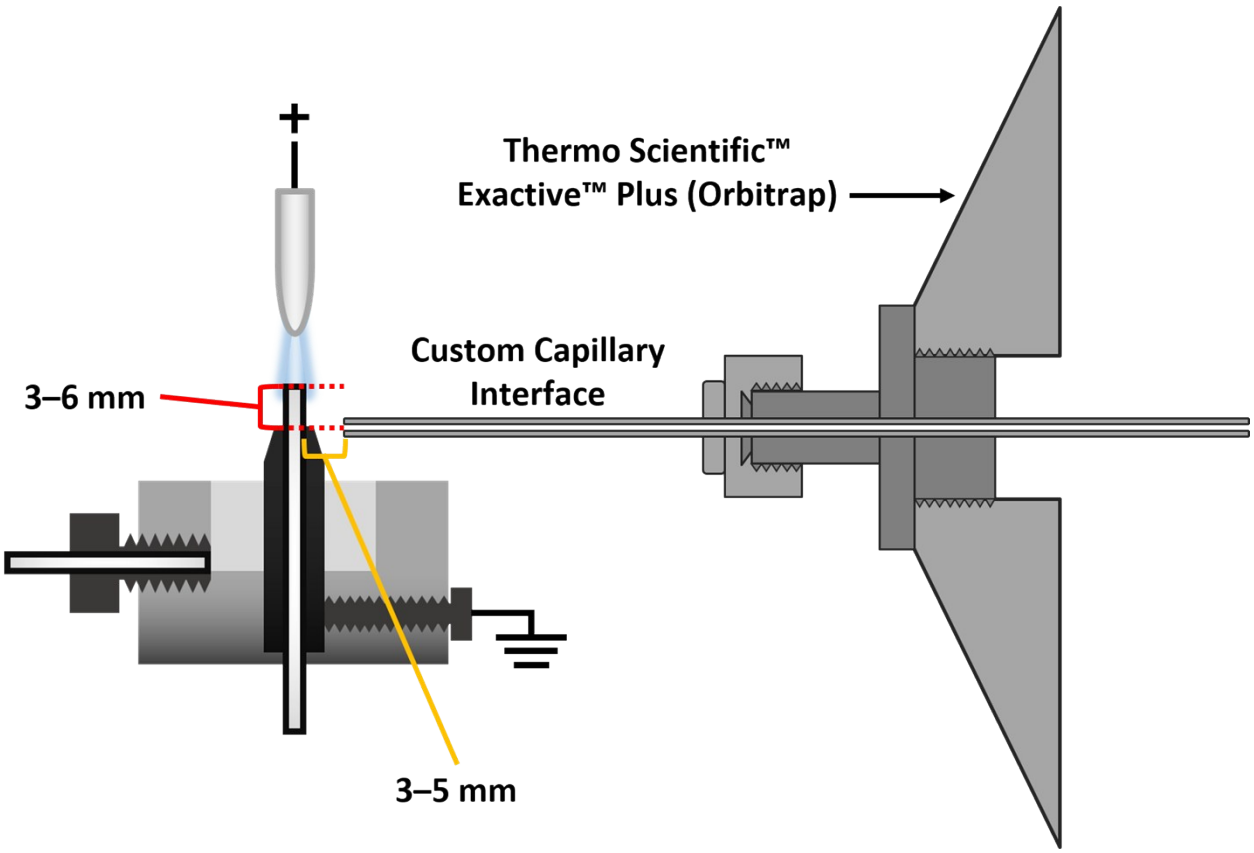

Figure S2. Diagram of the inlet capillary position with respect to the SCGD.

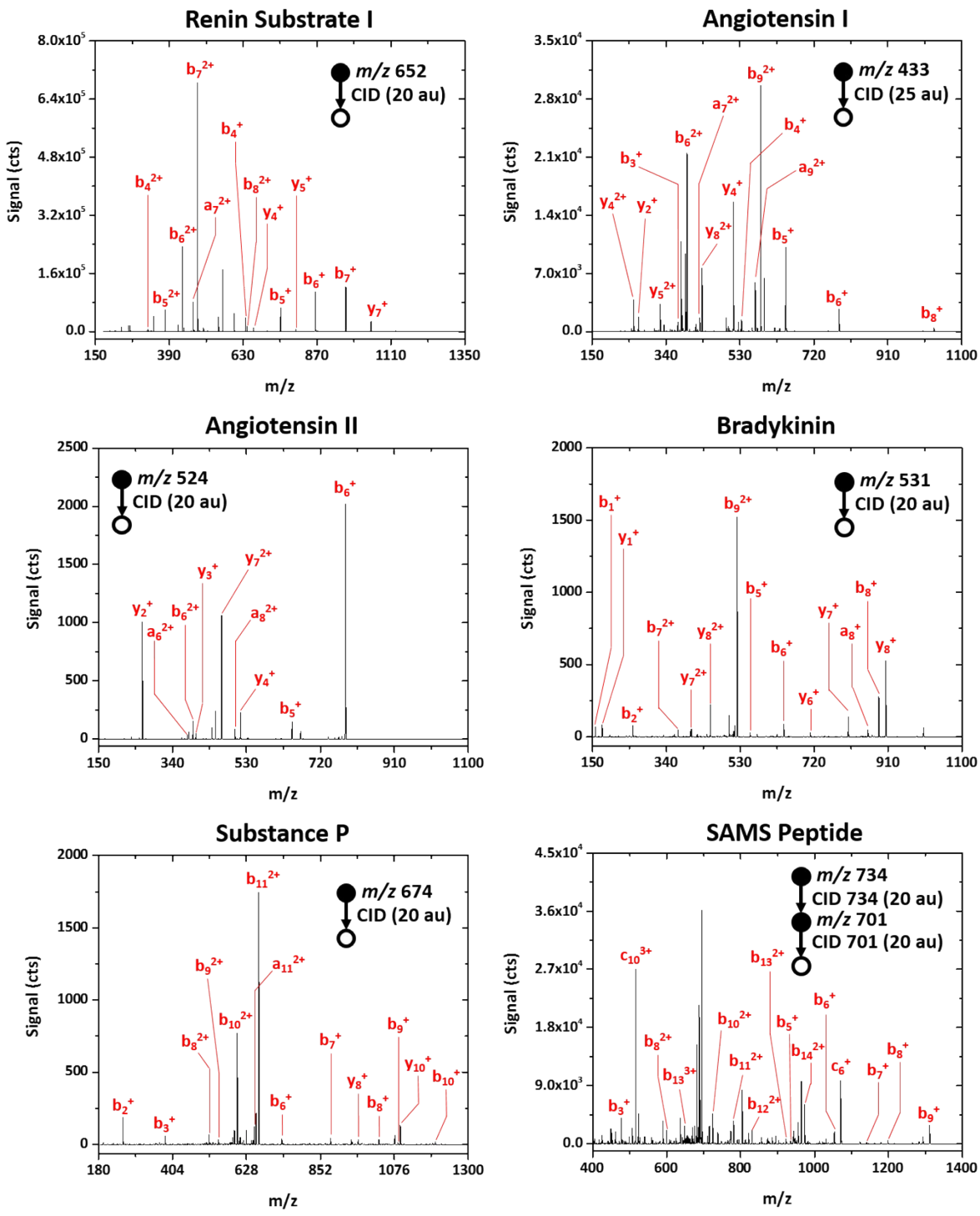

Figure S3. MS/MS spectra of various peptides obtained with ESI-CID. For each peptide, the target parent ion was the doubly protonated, doubly charged molecular ion, except in the case of angiotensin I and SAMS peptide, where the triply protonated, triply charged precursor was selected. Sequence coverage, compared to SCGD-MS, is summarized in Table 1.