Supporting Information:

Distinguishing $_{D}$- and $_{L}$-Aspartic and Isoaspartic Acids in Amyloid β Peptides with Ultrahigh Resolution Ion Mobility Spectrometry

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METHODS

Materials and Sample preparation: To understand if our DTIMS or SLIM IMS systems were able to separate β-linked and D-form amino acids, Aβ(6-16) isoforms were selected for analysis. Aβ(6-16) is of great interest since its Asp7 residue is known to racemize and isomerize in senile plaques (His6-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys16), therefore understanding the variants present in clinical samples is of great importance for Alzheimer's studies. Four Aβ(6-16) isoforms with different variants of the Asp7 residue were chosen for analysis (the naturally existing L-Asp, and the three potential modifications D-Asp, L-*iso*Asp, and D-*iso*Asp). The four variants were purchased from New England Peptides Inc. (Gardner, MA) at >98% purity and to insure enantiomeric purity of each amino acid prior to synthesis, chiral GC-MS was performed (http://cat-online.com/enantiomeric%20purity.html). The purchased peptides were then prepared in (50:50) acetonitrile and water, and diluted to a final concentration of 1 μM prior to DTIMS-MS and SLIM IMS-MS analyses.

DTIMS-MS: The peptides were first analyzed using an Agilent 6560 ion mobility spectrometry-quadrupole time-of-flight mass spectrometer (DTIMS-QTOF MS)[1, 2]. For the DTIMS measurements, after the molecules were ionized with electrospray ionization, the ions were passed through the inlet glass capillary, focused by a high pressure ion funnel, and accumulated in a lower pressure ion funnel trap (IFT). The ions were then pulsed into the 89 cm-long IMS drift tube filled with ~ 4 torr of nitrogen gas, where they travelled under the influence of a weak electric field (10-20 V/cm). Ions exiting the drift tube were refocused by a rear ion funnel prior to QTOF MS detection and their arrival times (t_A) being recorded (arrival time distribution, ATD). The reduced mobility (the mobility scaled to standard temperature and pressure) can be determined from instrument parameters and is related to the collision cross sections (CCS, σ) of the analyte ion using kinetic theory[3]:

$$\sigma = 1.3\,(\frac{q^2E^2\,T}{\mu\,k_B p^2 N^2 l^2})^{1/2}\,(t_A - t_0)$$

where q is the ion charge, E is the voltage across the drift tube, T is the absolute temperature, μ is the reduced mass of the ion−nitrogen collision, and k_B is the Boltzmann constant, p is the pressure of the drift cell, N is the buffer gas number density at STP, l is the length of the drift tube, t_A is the arrival time and t_0 is the time after the drift cell. The CCS value provides information about the three-dimensional configurations of the analyte and all these quantities are either known constants or are measured for each experiment. The width of the ATD can be compared with the width calculated for a single analyte ion structure, which gives information on the distribution of conformations in the ATD [3]. All the CCS values were measured in triplicate using 7 voltage stepped fields and the relative standard deviations were below 0.5% for all cases.

SLIM IMS-MS: The $_D/_L$-forms of Asp and isoAsp in each peptide isomer were characterized by a recently developed approach using structures for lossless ion manipulation technology and IMS separations (SLIM IMS). SLIM IMS uses traveling waves and a compact serpentine ion drift path capable of efficient

ion selection, trapping and accumulations[4-9].The SLIM device is fabricated using two printed circuit boards with mirror-image 13 m long drift paths and a switch to allow the ions to travel multiple passes.

The multi-pass SLIM IMS module has a total length of 13.5 meter for one pass. A 822 kHz radio frequency (RF) waveform, 180º out-of-phase to adjacent RF electrodes, was used to create pseudopotentials preventing ion loss to surfaces and a static guard bias was used to confine ions laterally. A traveling wave (TW) potential was applied to subsets of eight electrodes and repeated across the entire ion path.[10] The TW voltages were then stepped one electrode at a time for ion propagation throughout the entire module. An ion switch was located in the end of the serpentine path and could be switched to either send ions to the MS detector or on another separation pass.

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