Supporting Information

Investigation of the Complete Suite of Leucine and Isoleucine Isomers: Toward Prediction of Ion Mobility Separation Capabilities

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Comments on measuring CCS and Ion Mobility Distributions Presented in this Work

All IM-MS measurements in this study were obtained in positive ionization mode utilizing incremental steps of increasing drift field in the mobility portion of the Agilent 6560 IM-MS with nitrogen as the drift gas. After the non-mobility flight times (*i.e.* dead times) were subtracted at each voltage and the collision cross section for each analyte was calculated, the equation shown in Figure S3 was used to convert the time component of the experiment (x axis) to CCS. The optimal drift field occurs at 14.7 V/cm for the analytes examined in this study (see Figure S2), and hence the distributions at this drift field are presented in Figures S4 and S5. All theoretical (modeled) spectra are generated using Equation 4 of the main text.

Figure S1- CID fragmentation spectra for the leucine/isoleucine isomers investigated in this work. Fragmentation data was obtained using the QTOF stage of the IM-MS instrument after extracting the mobility of the precursor ion (m/z 132.1). Fragmentation energy was kept constant at 20 eV (laboratory frame).

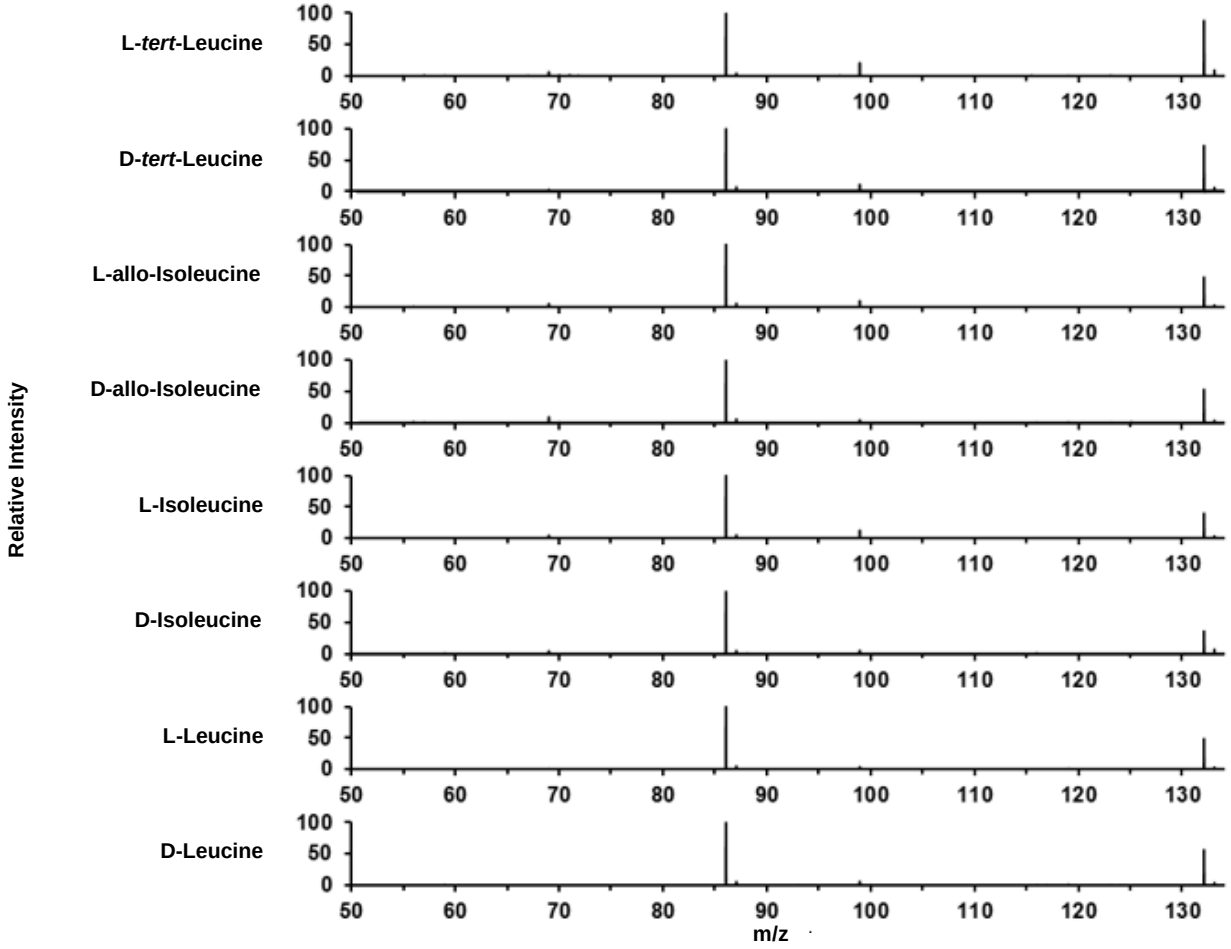

Figure S2- An overlaid IM spectrum obtained at different drift fields which demonstrates the separation of a mixture of L-norleucine and L-isoleucine for various applied drift fields. The optimal separation occurs at the maximum observed resolving power, here 61 resolving power at 14.7 V/cm, which results in a *ca.* 50% valley for this isomer pair.

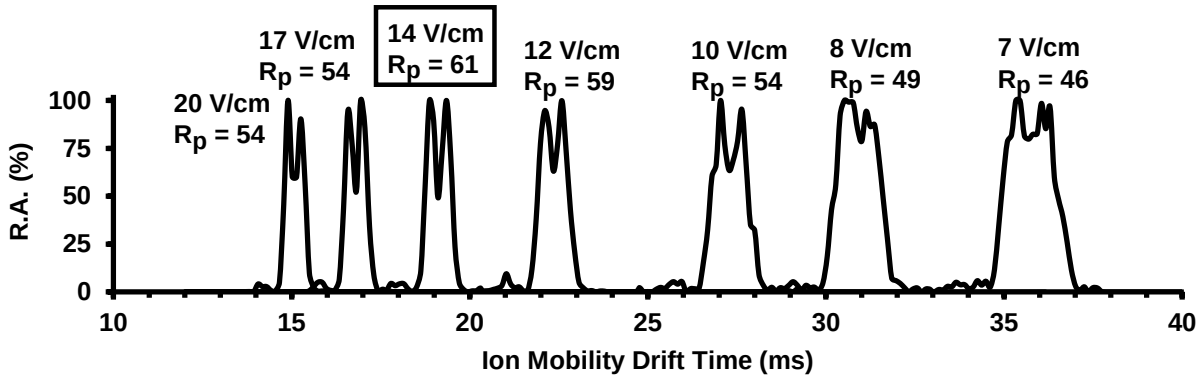

Table S1- Cross section data for each isomer of $C_6H_{13}NO_2$ investigated in this work. The standard deviation of the CCS for each measurement (RSD *ca.* 0.2%) is included in addition to the corresponding reduced mobility (k_o), drift time, FWHM, and resolving power.

Molecule	CCS (Å^2)	σ CCS (Å^2)	t_d (14.7 V/cm)	FWHM (ms)	K_o (cm^2/V s)	R_P
D-Leucine	135.2	0.3	19.14	0.32	1.634	59.8
L-Leucine	135.1	0.3	19.1	0.34	1.635	56.2
D-Isoleucine	133.3	0.3	18.9	0.33	1.658	57.3
L-Isoleucine	133.5	0.3	18.89	0.32	1.655	59.0
D-allo-Isoleucine	133.1	0.3	18.87	0.30	1.66	62.9
L-allo-Isolecuine	132.9	0.2	18.87	0.32	1.663	59.0
D-tert-Leucine	132.5	0.2	18.76	0.34	1.668	55.2
L-tert-Leucine	132.4	0.3	18.77	0.33	1.669	56.9
L-Norleucine	136.6	0.3	19.35	0.32	1.617	60.5
6-Aminocaproic Acid	129.3	0.2	18.27	0.30	1.709	60.9
N-N-Dimethylglycine	127.5	0.3	18.15	0.32	1.732	56.7

Figure S3- Linear correlation between drift time and collision cross section obtained from the Mason-Schamp equation (below) for isomers analyzed in this study. Drift times correlate to peak centroids at 14.7 V/cm in a uniform field instrument. Error bars indicate the uncertainty in the CCS measurement.

$$\Omega = \frac{3Ze_c}{16N} * \left(\frac{2\pi}{k_b T}\right)^{\frac{1}{2}} * \left(\frac{m_{ion}+m_{gas}}{m_{ion}*m_{gas}}\right)^{\frac{1}{2}} * \left(\frac{V*t_d}{L^2} * \frac{273.15℃}{T} * \frac{P}{760\ torr}\right)$$

Cross sections were obtained by calculating the corrected drift time and incorporating various laboratory parameters of the experiment including ion mass (m_{ion}), gas mass (m_{gas}), drift voltage (V), drift tube length (L), temperature (T) and pressure (P).

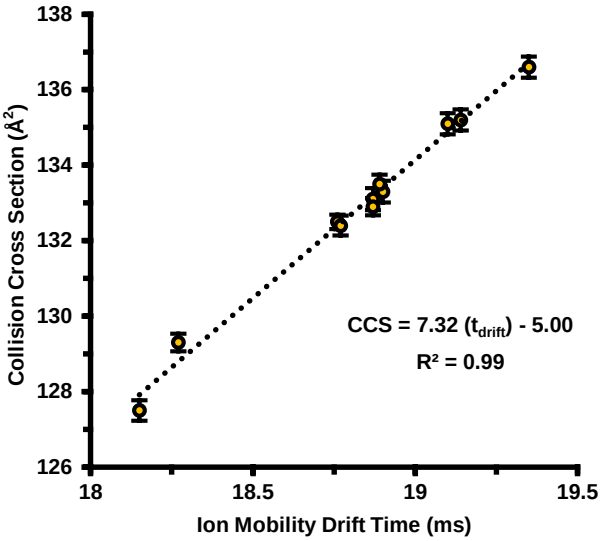

Figure S4- Individual drift spectra from Figure 2B with dotted lines to indicate the range of CCS data.

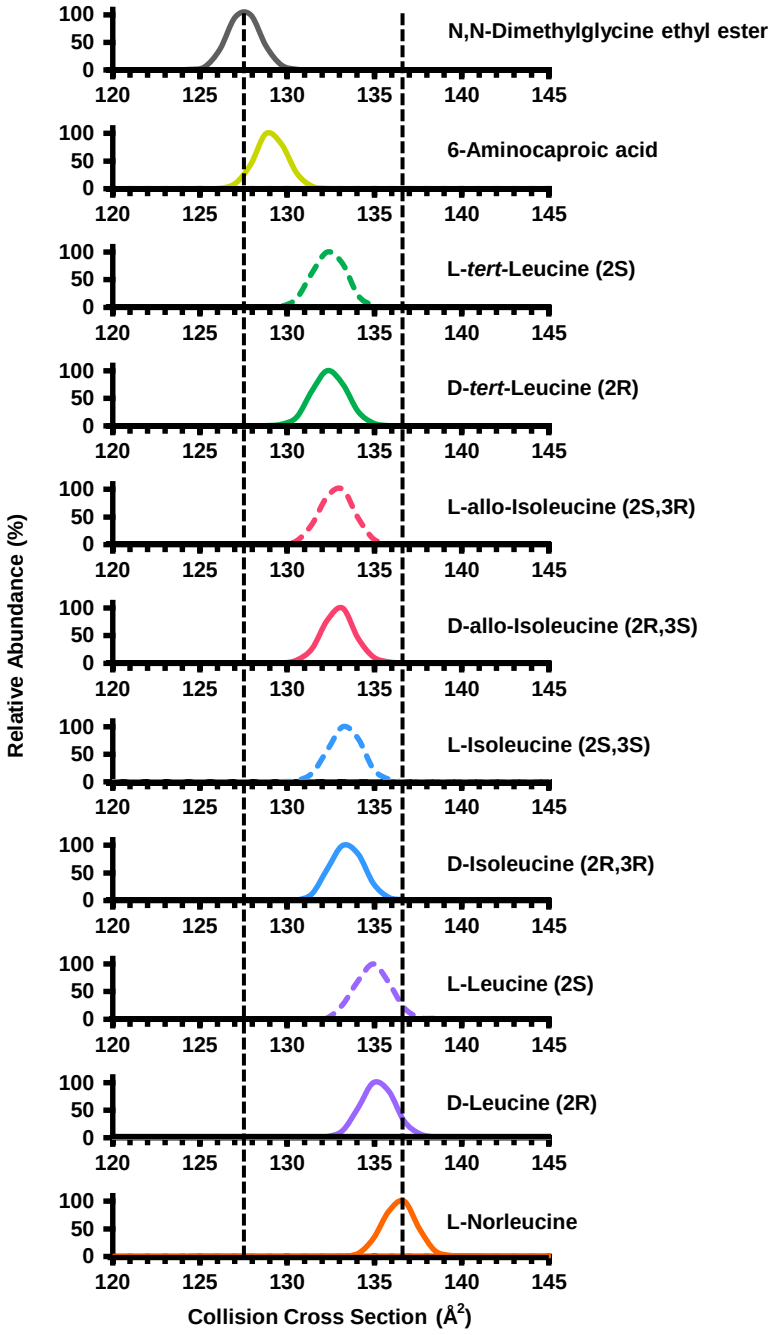

Figure S5- (A) Individual overlays and mixtures for panels B-IV and B-V from Figure 2. Note the discrepancy between separations of overlays (colored spectra) and analytical mixtures (black traces). (B) For comparison, panels VI and VII represent the enantiomer pairs for the other isomers overlaid to the left in (A). Another representative enantiomer comparison is depicted in VIII for reference.

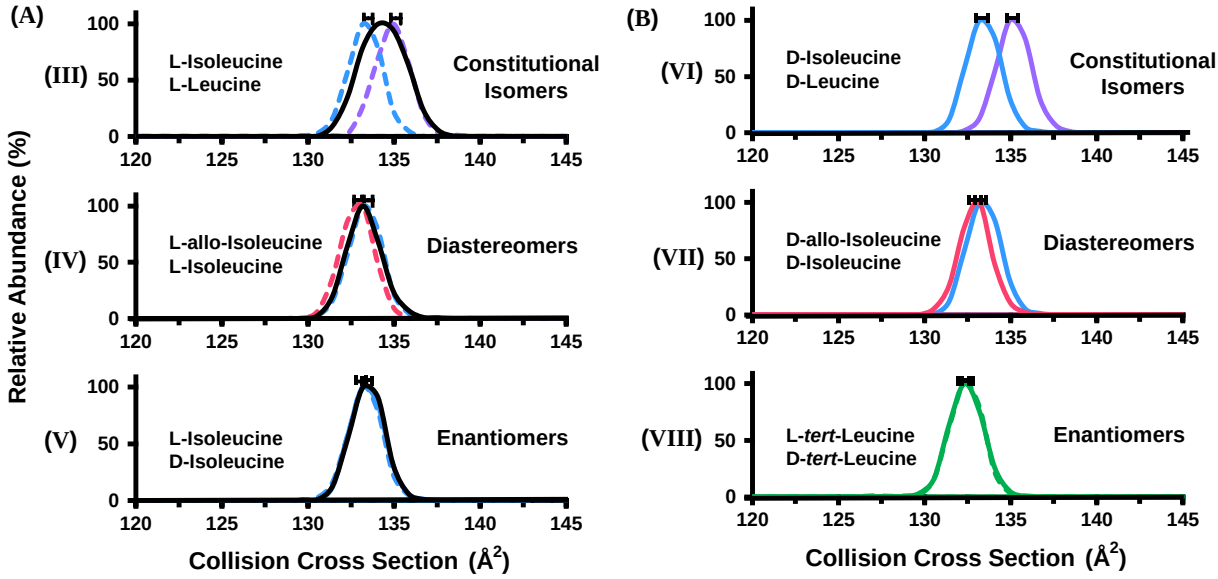

Figure S6- Comparison highlighting the effect of isomer abundance ratios on separation efficiency. Abundance ratios have significantly more of an impact on separation efficiency for more difficult separations (i.e. lower percent difference in CCS). For isomers that are 2-1.5% different in CCS both compounds are distinguishable in a 3:1 ratio at 100 resolving power. As the molecules become more structurally similar (similar cross sections, and hence lower percent difference) the isomer ratio becomes increasingly more important in order to observe both species in a mixture.