Supporting Information

Correlating Resolving Power, Resolution and Collision Cross Section: Unifying Cross Platform Assessment of Separation Efficiency in Ion Mobility Spectrometry

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Comments on Modeling Ion Mobility Distributions Presented in this Work

In this Supporting Information we describe each step in the process to fit Gaussian distributions to existing publication data (see Figure S1). We also include additional figures related to IM separations in TWIMS related to the discrepancy between reporting time based resolving power versus CCS based R_p (see Figures S2 and S3). Lastly we include descriptive citations for the 22 sources examined in this work and provide links to the non-peer reviewed sources. Finally, we discuss each previously reported IM separation in detail and any pertinent information that provides context for the empirical measurements (*e.g.* cited resolving power, CCS, and chemical identity of molecules/isomers that are separated).

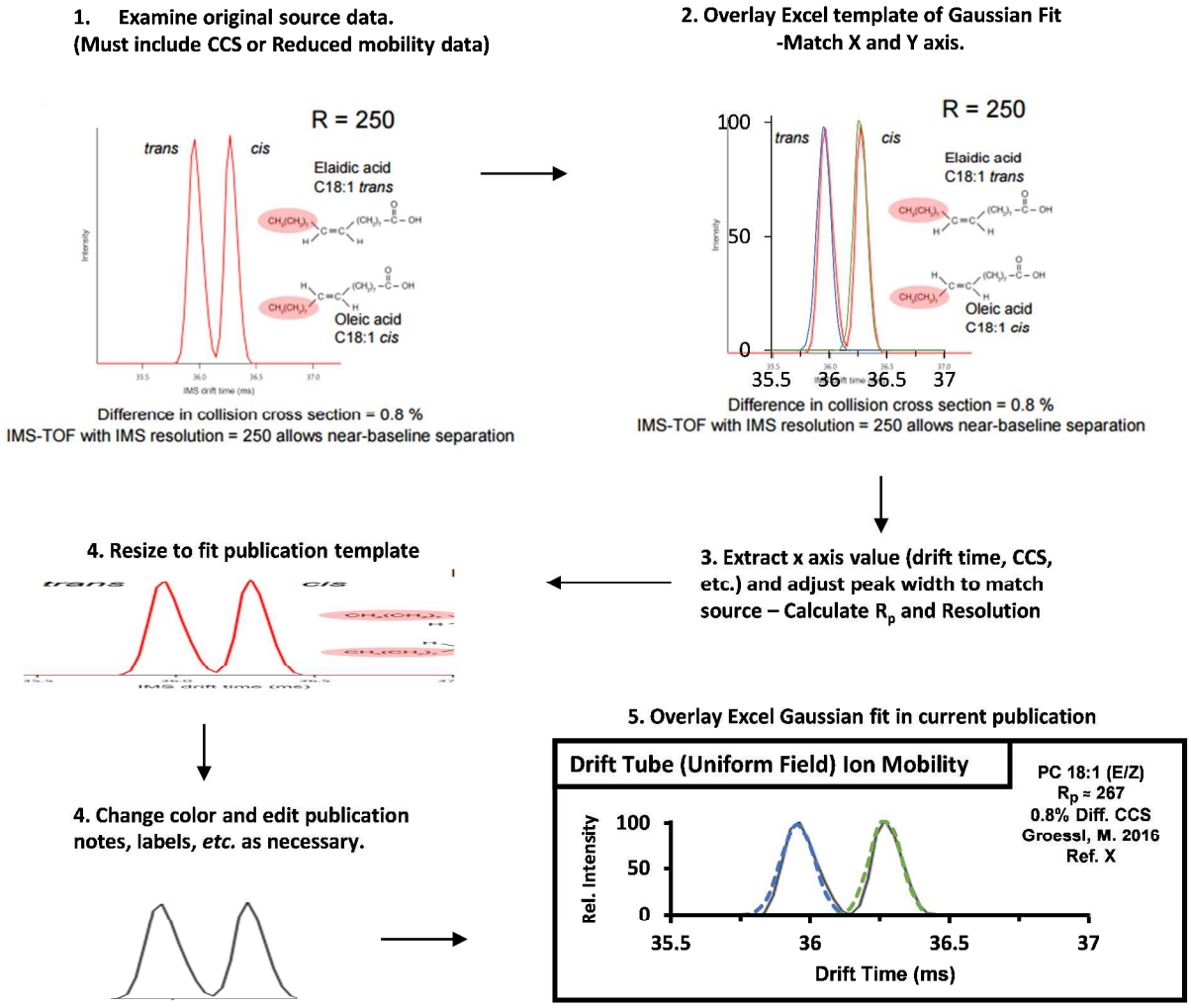

Figure S1. Workflow for overlaying Gaussian fits to published spectra. This method was applied for all sources referenced in Figures 2, 3, and 4. Two-peak resolution values (R_{pp}) were calculated by using eqn 7 in the manuscript along with relevant information noted from the source publication (*i.e.* drift time, CCS, and FWHM). Letter abbreviations added to delinate figures in caption (here **A**). Reproduced/Adapted with permission from Ref. A with permission from Michael Groessl, primary author (ASMS 2016, see Ref. A).

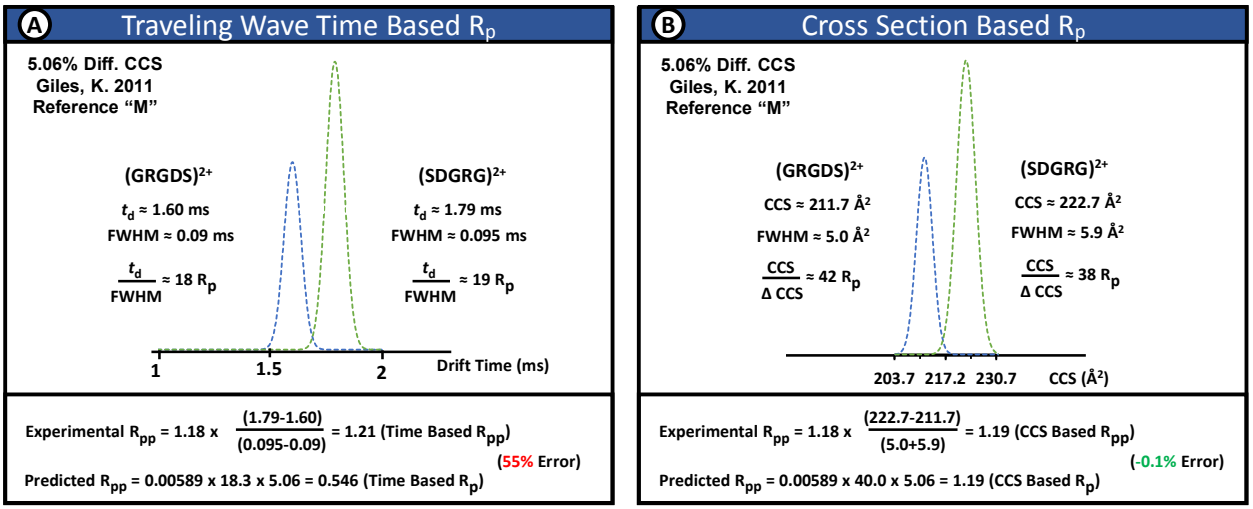

Figure S2. Comparison of resolving power in both the **(A)** time based dimension (t_d/fwhm) and **(B)** cross section space (CCS/ΔCCS) for traveling wave instruments as noted in Reference M. Giles and coworker's experimental results ($R_{pp} \approx 1.21$) have much closer agreement with R_p calculated in the CCS domain as opposed to R_p determined from the time domain.

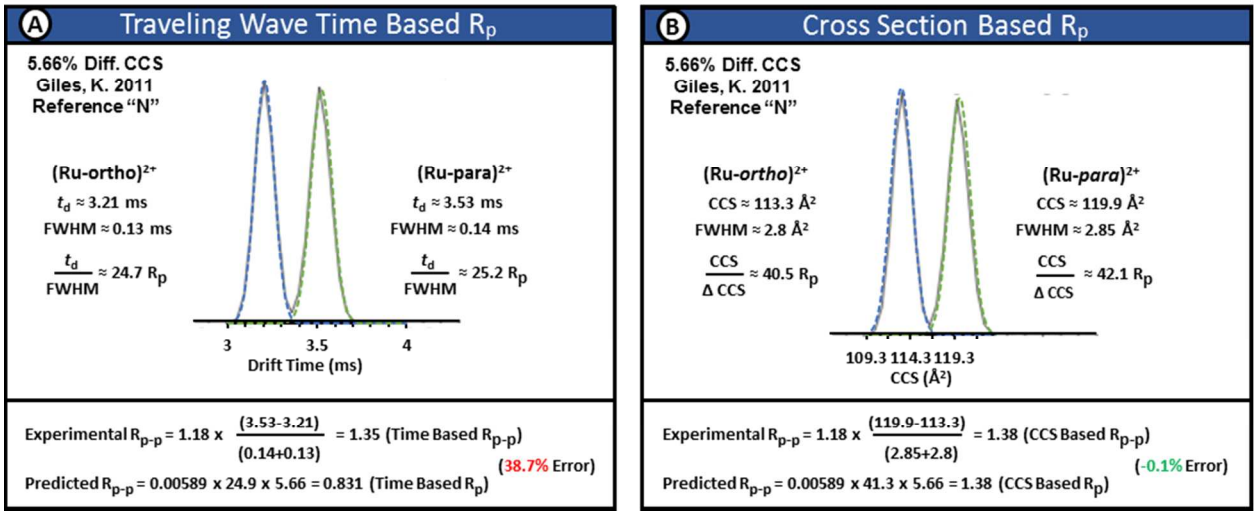

Figure S3. Separation of Ruthenium complexes (*ortho*/*para* isomers) as described in reference "N". **(A)** Time based resolving power, which does not accurately reflect the separation efficiency of the device. **(B)** Cross section based R_p is a more accurate depiction of TWIMS selectivity. Reproduced/Adapted with permission from Ref. 6. Wiley and Sons, 2011.

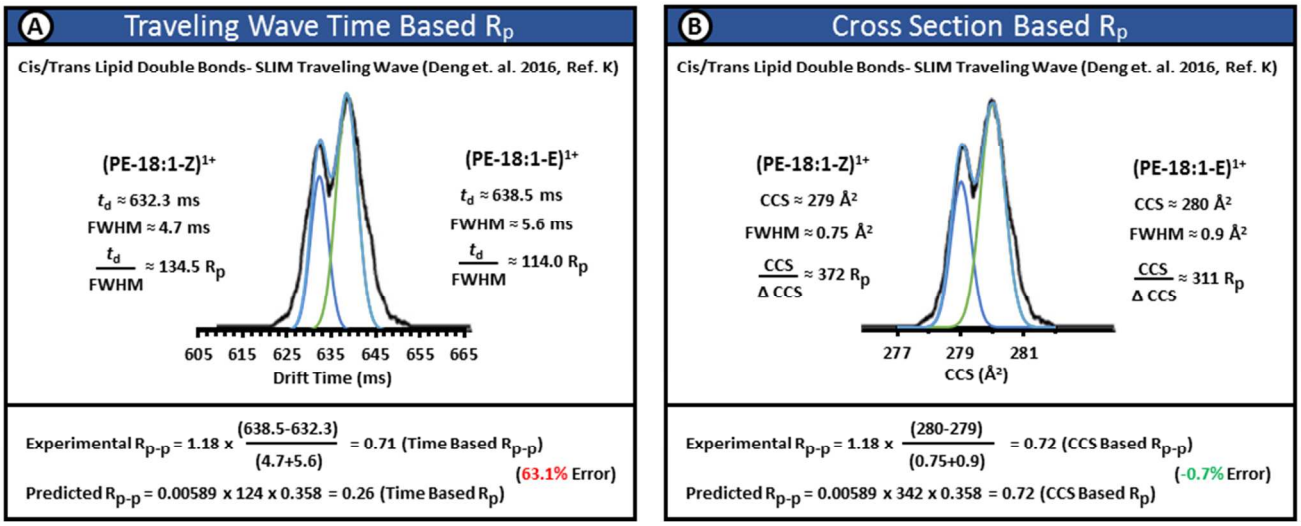

Figure S4. Separation of *cis/trans* lipid isomers in the SLIM traveling wave currently undergoing development by Smith and coworkers at PNNL. As with the commercial TWIMS devices, time based resolving power is not an accurate descriptor of separation efficiency **(A)**. Conversion to CCS based R_p **(B)** is more reflective of the analytical selectivity of this device. Reproduced/Adapted with permission from Ref. 48 in main text, Wiley and Sons, 2016.

Table S1. TWIMS separation parameters calculated by defining the separation equations in terms of the CCS.

	First Author	Reference Point	Percent Difference in CCS (%)[1]	Experimental Resolving Power (R_p)[2]	Experimental Resolution (R_{pp})[3]	Predicted Resolution (R_{pp})[4]	Percent Error in Resolution (%)[5]	Dispersion Axis Used in the Calculations
TWIMS	Deng, L.	K	0.4	342	0.72	0.72	-0.7	t_d → CCS
	Giles, K.	L	1.5	476	4.34	4.34	0.1	t_d → CCS
	Giles, K.	M	5.1	40	1.19	1.19	-0.1	t_d → CCS
	Giles, K.	N	5.7	41	1.38	1.38	0.1	t_d → CCS
	Hofmann, J.	O	5.9	43	1.49	1.49	0.0	t_d → CCS

1. Calculated from equation 4.
2. Calculated from equation 1 using the CCS.
3. Calculated from equation 2 using the CCS as the dispersion axis.
4. Calculated from equation 3 using the CCS-based definition for R_p.
5. Calculated from equation 5.

Table S2. Tabulated relationship between CCS-based resolving power and the percent difference in collision cross section.

Resolving Power	% Difference in CCS resolvable at Half Height
400	0.36
375	0.38
350	0.40
325	0.43
300	0.47
275	0.51
250	0.56
225	0.63
200	0.70
175	0.81
150	0.94
125	1.13
100	1.41
75	1.88
50	2.82
25	5.63

Increasing Ion Mobility Selectivity

Increasing Difficulty of Isomer Separation

Appendix S1. Extended information regarding the Reference Sources used in Figures 1-5 in the manuscript.

The letter of each reference corresponds to the labels in Figures 1-4 and Tables 2 and 3 in the manuscript. Resolving powers noted in each publication are verified using the Gaussian fit procedure described in Figure S1, and the % differences in CCS for each source are provided after each reference. If the source material was not taken from an official publication source (*i.e.* peer reviewed journal article), a source link has been provided for reference.

(A) Groessl, M.; Graf, S.; Lisa, M.; Holcapek, M.; Sampaio, J.; Dick, B.; Vogt, B.; Knochenmuss, R. "Analysis of Isomeric Lipids by High Resolution Ion Mobility Mass Spectrometry". *63rd Annual ASMS Conference on Mass Spectrometry and Allied Topics*, St. Louis, MO (2015) - Poster.

Isomers are PC 18:1Δ*cis*/18:1Δ9*cis* and PC 18:1Δ9*trans*/18:1Δ9*trans*. An apDTIMS instrument was used and operated with temporal (Hadamard) multiplexing. CCS values used to determine the % difference (0.4%) was obtained from this poster. The resolving power determined for this separation is *ca.* 330 ($t_d/\Delta t_d$), which is substantially higher than what is stated in the referenced source (>250).

Link: http://www.tofwerk.com/wp-content/uploads/2015/06/Tofwerk_WP262_asms2015.pdf (accessed March 1, 2017)

(B) Groessl, M.; Graf, S. "Separation of Isomers in Lipidomics and Metabolomics Experiments by High Resolution Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS)" *64th Annual ASMS Conference on Mass Spectrometry and Allied Topics*, San Antonio, TX (2016) - Poster.

Isomers are Elaidic acid (C18:1 *trans*) and Oleic acid (C18:1 *cis*). An apDTIMS instrument was used and operated with temporal (Hadamard) multiplexing. The CCS values used to determine the % difference (0.8%) were obtained from this reference source. Resolving power stated in the source is 250, and the average found in this study is 268.

Link: http://www.tofwerk.com/wp-content/uploads/2016/06/TOFWERK_ASMS2016_TP469.pdf (accessed March 1, 2017)

(C) Groessl, M.; Graf, S. "Separation of Isomers in Lipidomics and Metabolomics Experiments by High Resolution Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS)" *64th Annual ASMS Conference on Mass Spectrometry and Allied Topics*, San Antonio, TX (2016) - Poster.

Isomers are leucine and isoleucine. An apDTIMS instrument was used and operated with temporal (Hadamard) multiplexing. While the CCS values are not specifically stated in the poster, we have analyzed these isomers in our laboratory with a DTIMS instrument operated under pure nitrogen conditions (reference "H"). Our CCS results indicate that the % difference in CCS is 1.2%. A resolving power values was not provided in the poster. We estimate a resolving power of 251.

Link: http://www.tofwerk.com/wp-content/uploads/2016/06/TOFWERK_ASMS2016_TP469.pdf (accessed March 1, 2017)

(D) Asbury, G. R.; Hill, H.H.; J. "Evaluation of Ultrahigh Resolution Ion Mobility Spectrometry as an Analytical Separation Device in Chromatographic Terms" *Journal of Microcolumn Separations 12*, 172-178 (2000). Figure 5, middle panel.

Isomers are leucine and isoleucine. An apDTIMS instrument was used. While the CCS values are not specifically stated in the paper, we use the 1.2% CCS obtained in our laboratory. Of note, the high % error noted in Table 2 (*ca.* 20%) is likely related to the fact that we are using a nitrogen-based CCS value, whereas the original data was obtained in ambient air. Resolving power in the reference source is stated in terms of theoretical plates (96,615 and 76,209), however our calculated resolving power indicates *ca.* 130 ($t_d/\Delta t_d$). Our calculated resolution (via eqn. 2) is 0.76, whereas stated in the publication is 0.668. This discrepancy between R_{pp} values likely arises from the fact that the reference source uses the "peak width at base" definition for two-peak resolution (eqn. 4 from the above reference, reproduced below) as opposed to the half-height definition used in this current study.

$$R_{pp} = (t_{d2} - t_{d1})/(w_{b1} + w_{b2})2$$

(E) Groessl, M.; Klee, S.; Graf, S.; "High Resolution Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS) for Separation of Isomers in Natural Products and Complex Mixtures" *64th Annual ASMS Conference on Mass Spectrometry and Allied Topics*, San Antonio, TX (2016) - Poster.

Isomers are Datiscetin and Kaempferol (flavonoid positional isomers). An apDTIMS instrument was used and operated with temporal (Hadamard) multiplexing. The CCS values (149.2 $Å^2$ and 151.2 $Å^2$, respectively) were obtained from the reference source, which corresponds to a % difference in CCS of 1.3%. Resolving power for this separation is not specifically stated in the publication. We estimate a resolving power of 187 ($t_d/\Delta t_d$).

Poster (Accessed March 1, 2017).

(F) Pierson, N.A.; Chen L.; Valentine, S. J.; Russell, D. H.; Clemmer, D. E. "Number of Solution States of Bradykinin from Ion Mobility And Mass Spectrometry Measurements" *Journal of the American Chemical Society 133*, 13810-13813 (2011). Figure 2, 90:10 dioxane:water spectrum.

Conformers are for triply protonated bradykinin ($[M + 3H]^{3+}$). The analysis was conducted on the 90:10 dioxane: water spectrum for conformers "E" and "F" that are near half-height separated. A reduced pressure DTIMS instrument was used. Cross sections for these conformers are not explicitly stated in the publication, but we estimate 325.5 $Å^2$ and 331 $Å^2$ for "E" and "F", respectively, based on extrapolation to the published x axis. Resolving powers are not stated in this publication. We estimate a resolving power of 66 (CCS/ΔCCS).

(G) Tang, X.; Bruce, J. E.; Hill, H. H. "Design and Performance of an Atmospheric Pressure Ion Mobility Fourier Transform Ion Cyclotron Resonance Mass Spectrometer" *Rapid Communications in Mass Spectrometry 21*, 1115-1122 (2007). Figure 6, mixture.

Isomers are doubly protonated phosphorylated peptides (YLpSRSGR and YLSRpSGR) with m/z 459.71. The reported reduced mobilities (K_0) are 1.16 and 1.18 cm^2/Vs, respectively. An apDTIMS instrument interfaced to an FT-ICR was used. Due to the direct relationship between CCS and K_0, we used the reduced mobilities to calculate the % difference in CCS (see below).

$$\%\ \text{Diff. in CCS} = \frac{1.18 - 1.16}{\text{Avg K}_o\ (1.17)} \text{x } 100 = \ 1.71\%$$

Resolving powers were not reported in this publication. We estimate an average resolving power of 62 ($t_d/\Delta t_d$).

(H) Dodds, J. N.; May, J. C.; McLean, J. A. "Investigation of the Complete Suite of Leucine and Isoleucine Isomers: Toward Prediction of Ion Mobility Separation Capabilities" *Analytical Chemistry 89,* 952-959 (2016). Figure 3B.

Isomers are L-isoleucine and L-norleucine, which have CCS values of 133.5 and 136.6 Å^2, respectively. This results in a 2.30% difference in CCS and DTIMS instrument resolving power was determined to be 58 (CCS/ΔCCS).

(I) Gaye, M. M.; Nagy, G.; Clemmer, D. E.; Pohl, N. L. B. "Multidimensional Analysis of 16 Glucose Isomers by Ion Mobility Spectrometry" *Analytical Chemistry 88,* 2335-2344 (2016). Figure 2.

Conformers are for metal-adducted D-mannose ([Mn^{II}+(L-phe-Gly-H)+D-mannose]$^+$) with an m/z of 456. Measurements were obtained on a reduced pressure DTIMS instrument. The published CCS values for each conformer were 119.1 and 122.2 Å^2, respectively. We estimate that the resolving power averaged 83 (CCS/ΔCCS).

(J) Adamov, A.; Mauriala, T.; Teplov, V.; Laakia, J.; Pedersen, C. S.; Kotiaho, T.; Sysoev, A. A. "Characterization of a High Resolution Drift Tube Ion Mobility Spectrometer with a Multi-Ion Source Platform" *International Journal of Mass Spectrometry 298,* 24-29 (2010). Figure 4B.

Multiple distributions of 2,6-DtBP (2,6-di-tert-butylpyridine) generated using a corona discharge-APCI source and measured on an apDTIMS instrument. Our reduced mobility value (1.49 $cm^2V^{-1}s^{-1}$) after fitting differed slightly from reported value (1.47 $cm^2V^{-1}sec^{-1}$) although the reported resolving power (92, $t_d/\Delta t_d$) agrees with estimate for this peak (82, $K_o/\Delta K_o$). The lower abundance mobility peak is slightly less resolved (we estimate a R_p of. 72 $K_o/\Delta K_o$), and this affects the average resolving power we report in the manuscript (77, $K_0/\Delta K_0$).

(K) Deng, L.; Ibrahim, Y. M.; Baker, E. S.; Aly, N. A.; Hamid A. M.; Zhang, X.; Zheng, X.; Garimella, S. V. B.; Webb, I. K.; Prost, S. A.; Sandoval, J. A.; Norheim, R. V.; Anderson, G. A.; Tolmachev, A. V.; Smith, R. D. "Ion Mobility Separations of Isomers based upon Long Path Length Structures for Lossless Ion Manipulations Combined with Mass Spectrometry" *ChemistrySelect 1*, 2396-2399 (2016). Figure 2C.

Isomers are cis/trans Lipids with variations in double bonds. A SLIM-based TWIMS instrument was used. PE(18:1(9Z)/18:1(9Z))+H)$^+$ at 279 Å^2 and PE(18:1(9E)/18:1(9E))+H)$^+$ at 280 Å^2 are reported in the above communication, and we base our 0.4% difference in CCS on this information. The resolving power is not reported in this publication, but we estimate 124 ($t_d/\Delta t_d$). After the conversion to cross section space, we calculated an average R_p of 341 (CCS/ΔCCS).

(L) Giles, K.; Ujma, J.; Wildgoose, J.; Green, M. R.; Richardson, K.; Langridge, D.; Tomczyk, N. "Design and Performance of a Second-Generation Cyclic Ion Mobility Enabled Q-TOF" *65th*

Annual ASMS Conference on Mass Spectrometry and Allied Topics, Indianapolis, IN (2017) – Poster.

Isomers are the singly-charged reverse sequence peptides, GRGDS (+1) and SDGRG (+1). The CCS values are reported on the poster as 205.3 and 208.5 Å^2, respectively. Although the time based resolving power would be about 208 ($t_d/\Delta t_d$, reported in Table 1 of the main text), cross section based resolving power agrees with equation 4 of the manuscript, and CCS/ΔCCS which we calculate as ≈ 476, which is fairly consistent with his findings (550, CCS/ΔCCS). This discrepancy may be parted related to the fact that small variations in our fitted Gaussian can affect the FWHM with small variations, yet produce a decent bit of variation in resolving power. (*i.e.* calculating Rp based on FWHM gets more challenging as FWHM decreases).

Link: http://www.waters.com/webassets/cms/library/docs/2017asms_giles_cyclic.pdf (Accessed August 29, 2017)

(M) Giles, K.; Williams J. P.; Campuzano, J. "Enhancements in Travelling Wave Ion Mobility Resolution" *Rapid Communications in Mass Spectrometry 25*, 1559-1566 (2011). Figure 2, bottom panel.

Isomers are the doubly-charged reverse peptides, GRGDS (+2) and SDGRG (+2), with reported cross sections of 211.7 and 222.7 Å^2, respectively. A second generation TWIMS instrument was used. Time based resolving power for this separation is 18 ($t_d/\Delta t_d$). However, the authors note that cross section based R_p is 45, and our analysis agrees with this finding, with an estimated R_p of 40 (CCS/ΔCCS) found in this current study.

(N) Giles, K.; Williams J. P.; Campuzano, J. "Enhancements in Travelling Wave Ion Mobility Resolution" *Rapid Communications in Mass Spectrometry 25*, 1559-1566 (2011). Figure 3.

Isomers examined here are Ruthenium *ortho*- and *para*-terphenyl compounds. A second generation TWIMS instrument was used. The reported cross sections are 113.3 Å^{2}and 119.9 Å^2 and the resolving power we obtain is 41 (CCS/ΔCCS). This differs somewhat from the authors' findings of 32. Regardless, either of these values seems much more appropriate in comparison to the time based R_p, which we calculate as 25 ($t_d/\Delta t_d$).

(O) Hofmann, J.; Hahm, H. S.; Seeberger, P. H.; Pagel, K. "Identification of Carbohydrate Anomers using Ion Mobility-Mass Spectrometry" *Nature 526,* 241-244 (2015). Figure 2C, third panel (isomers 6 and 3).

Isomers are synthesized carbohydrates with varied bond linkages (α-1→3 and α-1→4). A second generation TWIMS instrument is used. The cross sections for each of these isomers in nitrogen are reported in Extended Data Table 2 for the $[M-H]^-$ ion, which for isomer 6 and 3 are 219.9 and 233.2 Å^2, respectively. Although the resolving power is not stated in the publication, our time based R_p is estimated at 26 ($t_d/\Delta t_d$), which is consistent with source M and N which utilize the same instrumentation model. In similar fashion to source M and N, after conversion to cross section the resolving power increases, here to 43 (CCS/ΔCCS).

(P) Silveira, J. A.; Ridgeway, M. E.; Park, M. A. "High Resolution Trapped Ion Mobility Spectrometry of Peptides" *Analytical Chemistry 86*, 5624-5627 (2014). Figure 2B.

Conformers are for substance P(+3), labeled "B1" and "B1" [*sic*] in the publication, with corresponding CCS values reported as 493 and 498 Å^2, respectively. The resolving power we obtain using our fitting analysis agree with the R_p range provided in the publications (154 to 183, K/ΔK), with an estimated R_p of 178 (CCS/ΔCCS) found in this current study.

(Q) Commercial vendor (Bruker) brochure for the timsTOF™ instrument, "1844502 – timsTOF™. Flexibility to Empower Your Ideas" (2016). Figure 4C.

Isomers are Morin and Quercetin (m/z 303.05). The instrument is a production model TIMS. The axis for this TIMS separation is illustrated in terms of reciprocal reduced mobility ($1/K_0$), although the specific K_0 values for each isomer are not explicitly stated, we determine through visual extrapolation that the inversed reduced mobilities are approximately 0.785 and 0.793 Vs/cm^2, respectively. Resolving powers are not stated, but we estimate the R_p to be around 113 ($K_o^{-1}/\Delta K_o$).

Link: https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/Separations_MassSpectrometry/Literature/Brochures/1844502_timsTOF_brochure_05-2016_ebook.pdf (Accessed March 1, 2017)

(R) Commercial vendor (Bruker) brochure for the timsTOF™ instrument, "1844502 – timsTOF™. Flexibility to Empower Your Ideas" (2016). Figure 2.

Isomers are the carbohydrates, raffinose and maltotriose, reported at baseline resolution in the "imeX Ultra" mode, which is the high mobility resolution mode of the instrument. Although the reduced mobilities of these compounds are not stated in the brochure, we visually extrapolate values of 1.01 and 1.03 Vs/cm^2 for raffinose and maltotriose, respectively. These correlate well with our previous measurements for these carbohydrates. Using these K_0 values, we estimate the R_p to be 177 ($K_0^{-1}/\Delta K_0$), which is comparable to the 185 quoted in the brochure.

Link: https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/Separations_MassSpectrometry/Literature/Brochures/1844502_timsTOF_brochure_05-2016_ebook.pdf (Accessed March 1, 2017)

(S) Barnett, D. A.; Ells, B.; Guevremont, R.; Purves, R. W. "Separation of Leucine and Isoleucine by Electrospray Ionization-High Field Asymmetric Waveform Ion Mobility Spectrometry-Mass Spectrometry" *Journal of the American Society for Mass Spectrometry 10*, 1279-1284 (1999). Figure 4, lower trace.

Isomers are leucine and isoleucine. The FAIMS separation was conducted in compressed air device, and we use CCS values obtained in our laboratory, which utilized high purity nitrogen as the buffer gas. CCS values for deprotonated isoleucine and leucine ions are 129.8 and 131.1 Å^2, respectively. We determine R_p to be 130 (CCS/ΔCCS).

(T) Lee, S.; Ewing, M. A.; Nachtingall, F. M.; Kurulgama, R. T.; Valentine, S. J.; Clemmer, D. E. "Determination of Cross Sections by Overtone Mobility Spectrometry: Evidence for Loss of Unstable Structures at Higher Overtones" *Journal of Physical Chemistry B 114*, 12406-12415 (2010). Figure 2, panel 2 (21st harmonic frequency number).

Conformers are for ubiquitin (+10) obtained from OMS. CCS values were used as reported in the publication from OMS measurements (Table 1), and were 1857.6 and 1806.9 $Å^2$ (ΔCCS = 2.8%) for the two ubiquitin conformers observed. The authors note that these values differ slightly in the CCS values obtained from their DTIMS experiments (1590 and 1660 $Å^2$). Using the frequency axis, we estimate the resolving power to be on average 66 (Hz/ΔHz*)* at the 21st harmonic, which exhibited the highest resolution for ubiquitin (+10) conformers in this work.

(U) Glaskin, R. S.; Valentine, S. J.; Clemmer, D. E. "A Scanning Frequency Mode for Ion Cyclotron Mobility Spectrometry" *Analytical Chemistry 82*, 8266-8271 (2010). Figure 4, third inset.

The compounds investigated in this work are two peptides (KATNE and KTER) obtained from a cytochrome *c* digest, which are not isobaric, but are closely-spaced in the IM spectrum reported. Our information for this separation is taken from Table 1 in the publication, where peak 1 is KATNE (K_0 circular = 6.88 cm^2/Vs) and peak 2 is KTER (K_0 circular = 6.70 cm^2/Vs), and these values were used to calculate the percent difference in CCS (2.7%). Similar to Source T, these values differ somewhat between the values obtained by conventional DTIMS which are listed in the same table. Resolving power for this separation was estimated to be 85 (Hz/ΔHz), (6% error with predicted R_{pp}) however if the frequency axis is converted to K_o as in the TWIMS separations resolving power is ca. (95 $K_o/\Delta K_o$ yielding 0.2% error).

(V) Glaskin, R. S.; Valentine, S. J.; Clemmer, D. E. "A Scanning Frequency Mode for Ion Cyclotron Mobility Spectrometry" *Analytical Chemistry 82*, 8266-8271 (2010). Figure 4, first inset.

The compounds investigated in this work are two peptides (TGQAPGFTYTDANK and TEREDLIAYLK) obtained from a cytochrome *c* digest. Information for this separation is taken from Table 1, where peak 16 is TGQAPGFTYTDANK (K_0 circular = 3.67 cm^2/Vs) and peak 17 is TEREDLIAYLK (K_0 circular = 3.59 cm^2/Vs), and these values were used to calculate the percent difference in CCS (2.2%). Resolving power for this separation was estimated to be 145 ($K_o/\Delta K_o$), which is significantly lower than what is reported in the publication (417, Hz/ΔHz). We note that we obtain a similar resolving power using the Gaussian peak analysis (440, Hz/ΔHz) when we use the frequency domain as reported in the publication.