# IM-MS interest group workshop

## Towards standard operating procedures?

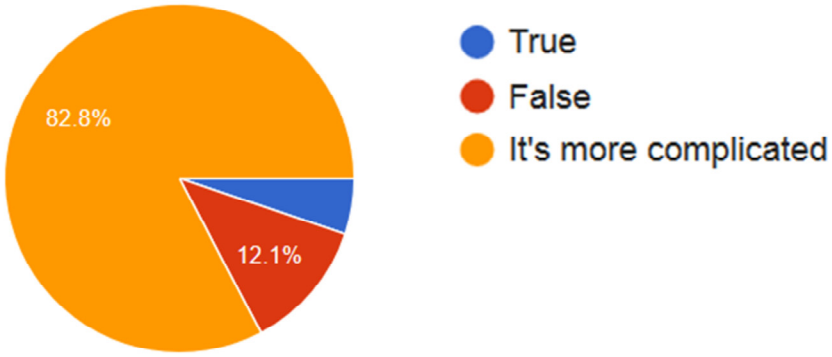
June 7, 2017

Valérie Gabelica, Brian Clowers, Stephen Valentine

## Survey responses

Ion mobility is an intrinsic property of an ion-neutral complex in the same fashion as m/z is an irreducible property of an ion

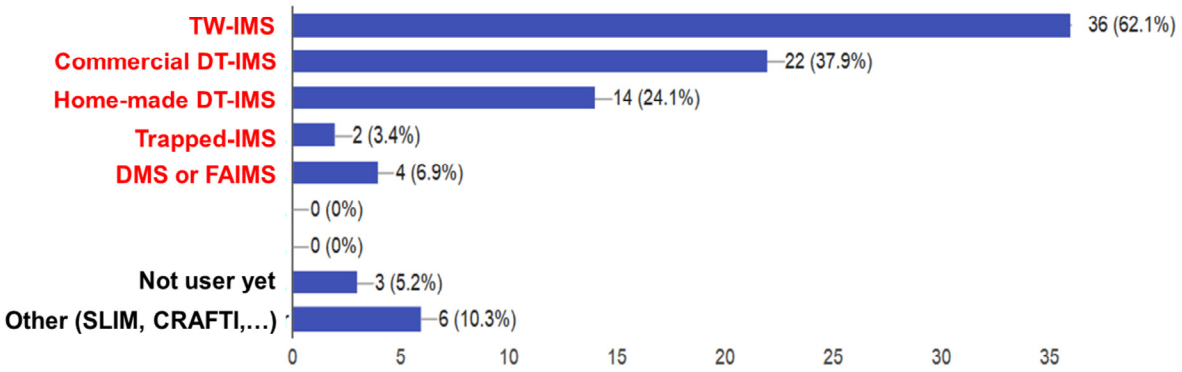
58 responses



## **Responders profile** (24-May-2017, 58 responses)

What instrument(s) do you use?

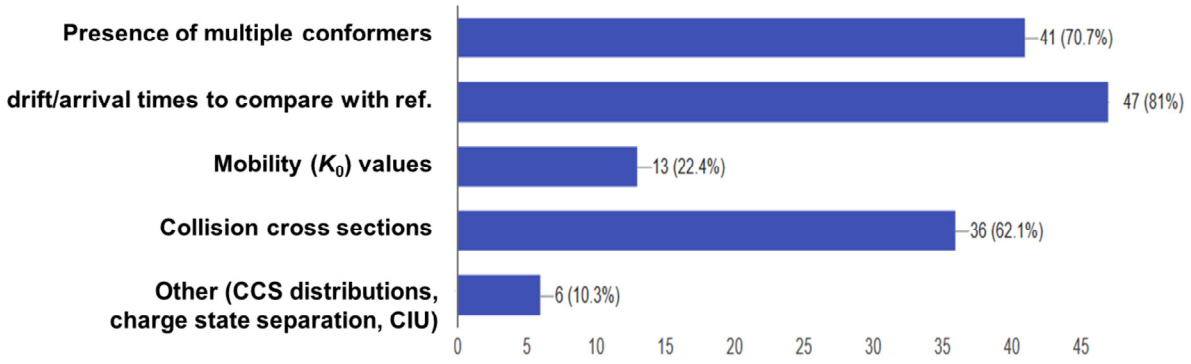
58 responses



## **Responders profile** (24-May-2017, 58 responses)

What parameters do you extract?

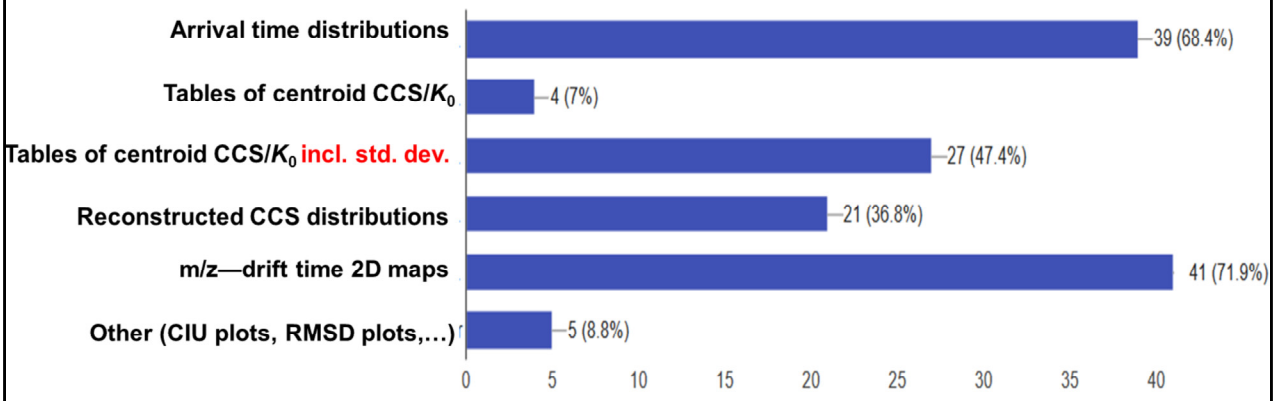
57 responses



## **Responders profile** (24-May-2017, 58 responses)

How do you present the data?

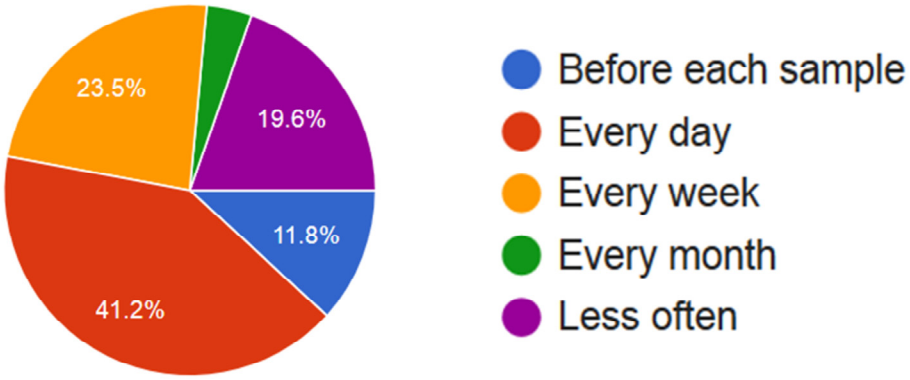
57 responses



## **Responders profile** (24-May-2017, 58 responses)

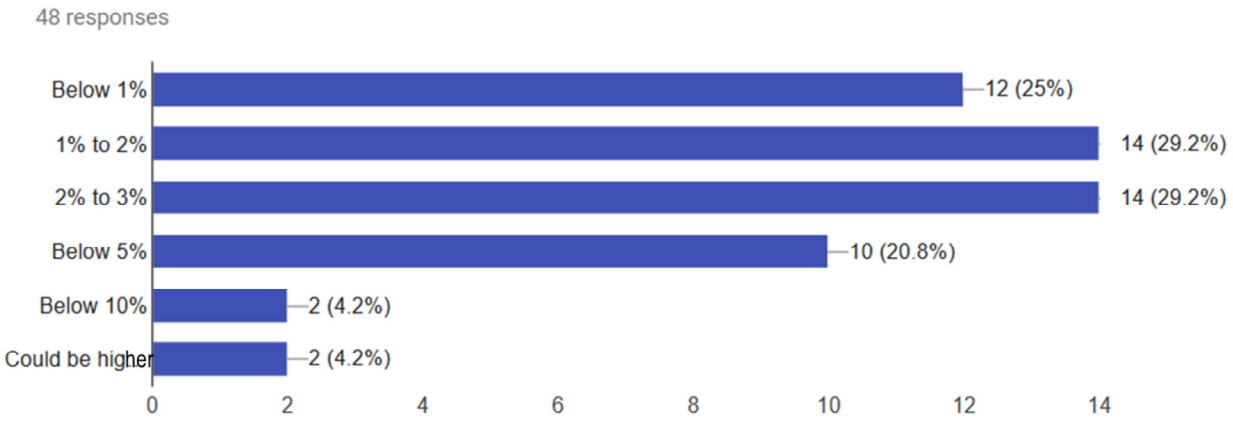
How often do you check the instrument performance for determining CCS or $K_0$?

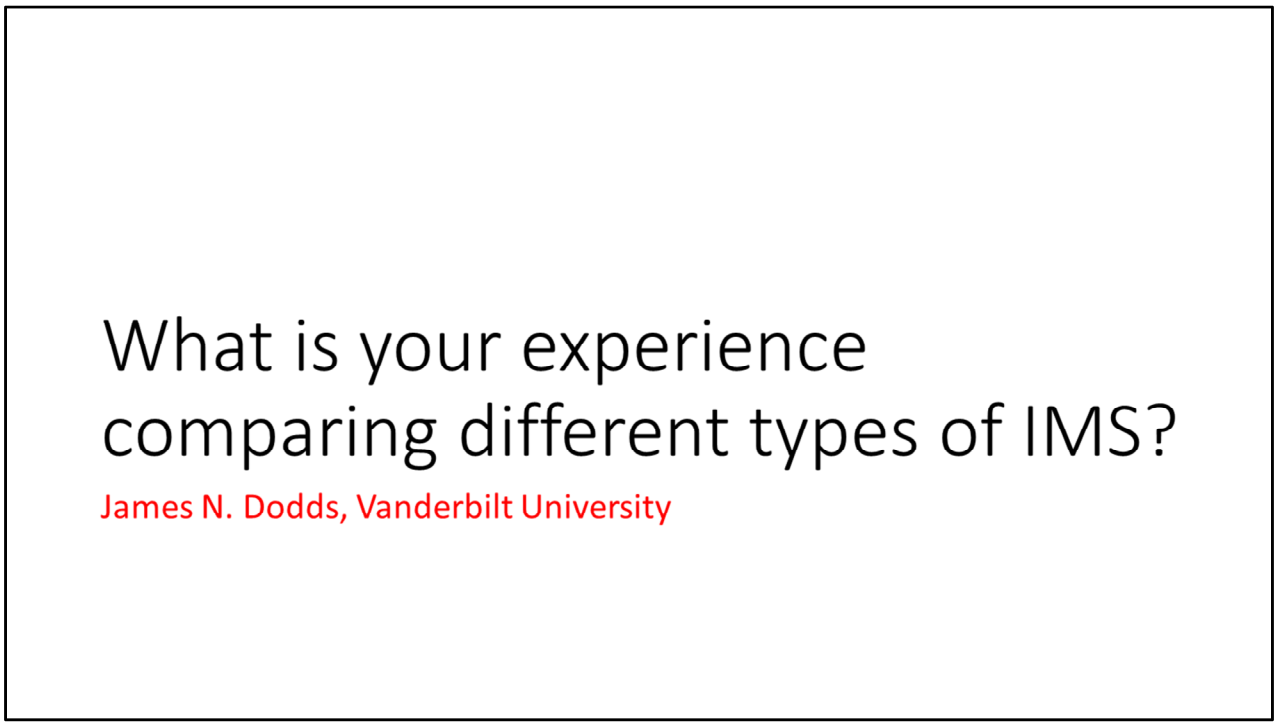
51 responses



## **Responders profile** (24-May-2017, 58 responses)

With your instruments, standards, and frequency of verification, what maximum deviation to true value do you tolerate for CCS/$K_0$?



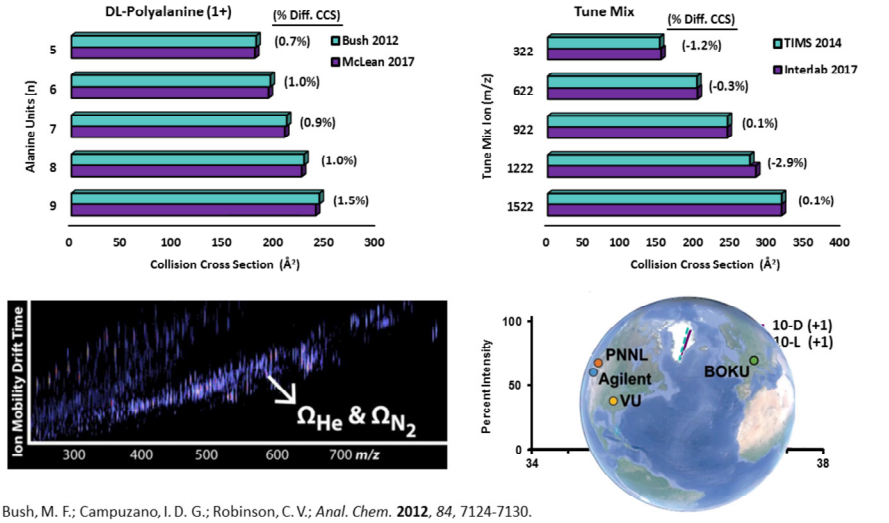



Workshop part I, discussion with the audience:

- (Shvartsburg:) About polyalanine, the problem is isomerization. Fresh fully poly(D-Ala) or poly(L-Ala) give the same CCS values, but not the isomers. Different IMS platforms work on different time scales in the gas phase, and conformations may have evolved differently.
- (Sobott:) When comparing different instrumental platforms, the source activation conditions may also lead to different conformer populations. So for flexible molecules it is hard to know how to get comparable results.
- (Barran:) The CCS depends on the gas. It is extremely important to mention which gas is used (or mixture of gases, in the case of Synapt G2 instrument series, with the helium cell and N2 in the T-wave). Recalls a proposed nomenclature that specifies the gas as subscript, e.g. $^{DT}CCS_{He}$ for a collision cross section measured by drift tube in helium, or $^{TWIMS}CCS_{N2\rightarrow He}$ for a collision cross section determined in a traveling wave IMS, measured in nitrogen but calibrated against helium data.
- All agree that it is extremely important to name the instrument and gas used.
- (Shvartsburg:) warns that the superscript (instrument type) is still too vague, and additional information should be source conditions and time scales of the ion mobility.
- (McLean:) Source conditions and time scales will play a role for large molecules, e.g. proteins, are intrinsically flexible and give multiple conformations. Small molecules usually give more simple results.

- (Gabelica:) Beware that small molecules can give surprising results sometimes, for example due to multiple charging sites coexisting, depending on the solvent composition or source geometry.

## CCS Calibrants

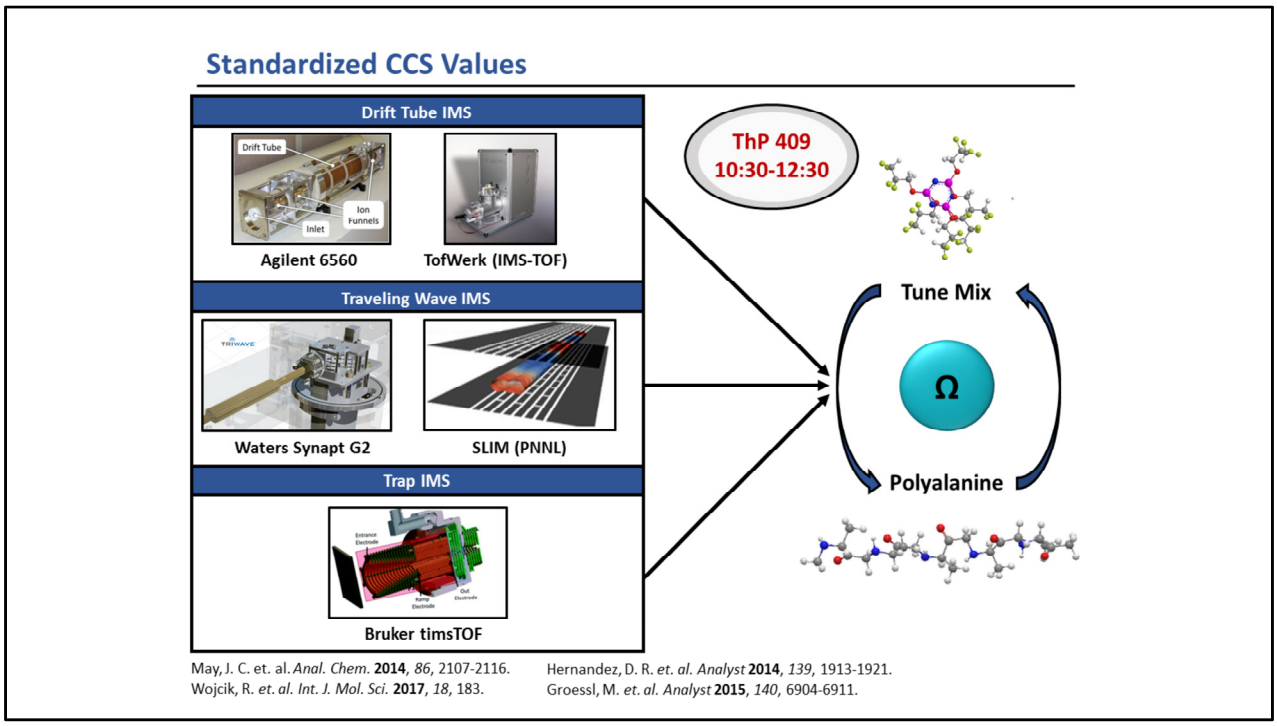


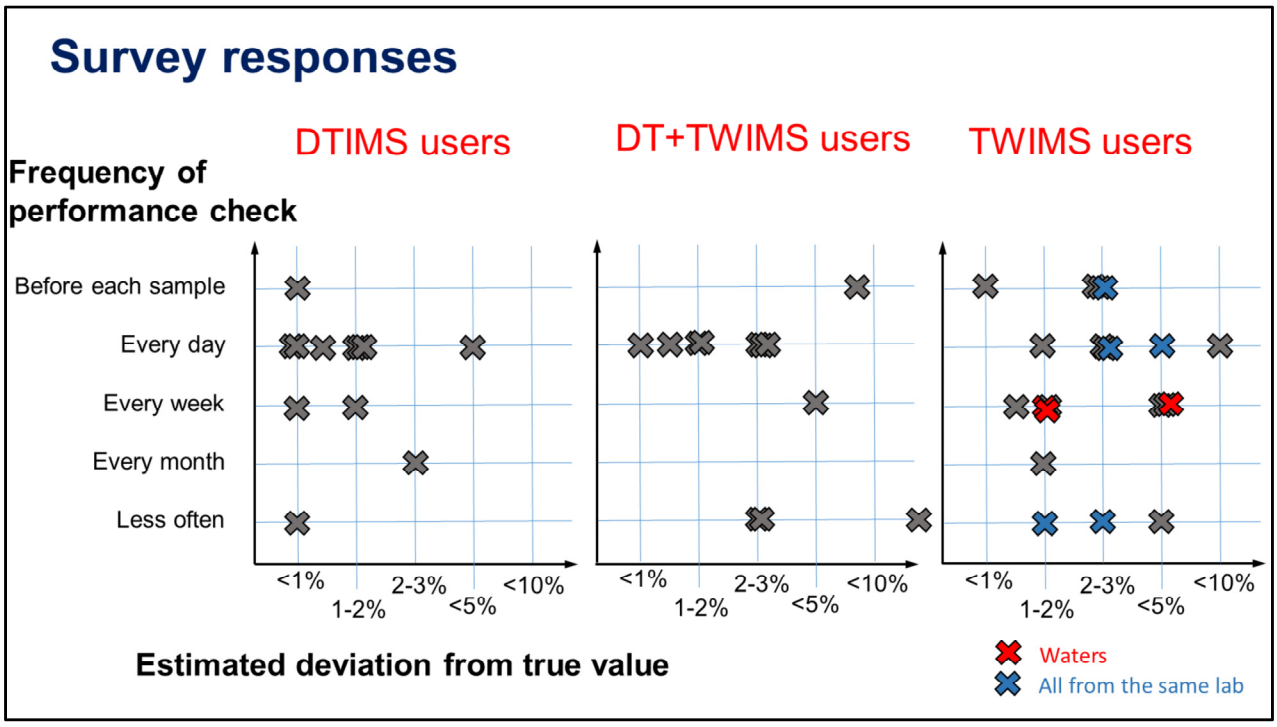

Bush, M. F.; Campuzano, I. D. G.; Robinson, C. V.; *Anal. Chem.* **2012**, *84*, 7124-7130.

Jiang, L. and Mehdi Moini. *J. Mass Spectrom.* **1992**, *3*, 842-846.

Hernandez, D. R.; DeBord, J. D.; Ridgeway, M. E.; Kaplan, D. A.; Park, M. A.; Fernandez-Lima, F. *Analyst* **2014**, *139*, 1913-1921.

Stow, S. M. et. al. "Interlaboratory Evaluation of Drift Tube CCS Measurements" *Anal. Chem.* Submitted. **2017.**


Standardized CCS Values
Drift Tube IMS
Drift Tube
Inlet
Ion Funnels
Agilent 6560
TofWerk (IMS-TOF)
Traveling Wave IMS
Waters Synapt G2
SLIM (PNNL)
Trap IMS
Bruker timsTOF
ThP 409
10:30-12:30
Tune Mix
Ω
Polyalanine
May, J. C. et. al. Anal. Chem. 2014, 86, 2107-2116.
Wojcik, R. et. al. Int. J. Mol. Sci. 2017, 18, 183.
Hernandez, D. R. et. al. Analyst 2014, 139, 1913-1921.
Groessl, M. et. al. Analyst 2015, 140, 6904-6911.


Survey responses
DTIMS users
DT+TWIMS users
TWIMS users
Frequency of performance check
Before each sample
Every day
Every week
Every month
Less often
<1%
1-2%
2-3%
<5%
<10%
Estimated deviation from true value
Waters
All from the same lab

## Survey responses (TWIMS users only)

| Standard used | Standards causing trouble |
|---|---|
| • cytochrome c, bradykinin, leu enk.<br>• Native protein ions from Bush database (2).<br>• Proteins for proteins (3)<br>• Proteins and Multiprotein complexes not included in the calibration worksheet.<br>• Several proteins above and below the MW of the protein we measure<br>• Polyalanine standards for protein/peptide<br>• Poly-alanine for metabolites and peptides for proteomic applications (2)<br>• Poly[D]alanine for Small molecules, proteins with $t_D$s that braket the $t_D$ of the analyzed system, and if possible with similar "conformation" | • Proteins, due to conformational dependence on voltages.<br>• Polyalanine not the best for small molecules but need to take time to identify a suitable mixture<br>• Most of them, the calibration procedure still contains a bias.<br><br>• **"What is the true value of a protein?"** |

## Survey responses (DTIMS users only)

| Standard used | Standards causing trouble |
|---|---|
| • Agilent tune mix (6)<br>• Tetraalkylammoniums (2)<br>• Phosphoric acid clusters<br>• Bradykinin, Ubiquitin (activation-sensitive) | • Folded proteins should not be used. ATD depends on the instrument tuning and on the charge state.<br>• flexible systems (activation-dependent) = proteins and many peptide<br>• Even within the same compound class as mine, I could not reproduce published data because pre-IMS activation conditions apparently differed.<br>• small sugars aggregate in the source and fall apart in the drift tube. |

## Survey responses (users of both DTIMS and TWIMS)

| Standard used | Standards causing trouble |
|---|---|
| • Agilent tunemix<br>• Agilent tunemix, polyalanine<br>• Leu-enk or polyalanine, primarily for peptides<br>• Tunemix, sometimes TAA salts<br>• In house calibration mix comprising small molecules and polymers. Typically to investigate drug-like small molecules, occasionally peptides. | • All! (incl. lipids because of varied backbone, peptides due to multiple conformations,…)<br>• Any protein (2)<br>• Proteins (up to 5%). For small molec. <1%.<br>• Larger proteins esp. with stepwave instruments (heating the conformers)<br>• Analytes having multiple conformations<br><br>**"The availability of chemical standards is troublesome"**<br><br>**"The community would do well to develop a set of rigid polymers to serve over a wide range of CCS values as standards."** |

# What are the problems and solutions for standardizing non-drift tube IMS instruments?

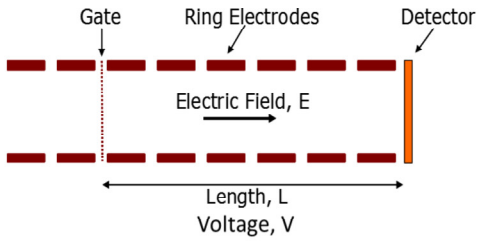
Kevin Giles, Waters

Workshop part II, discussion with the audience:

- (Giles:) After it was realized that He and N2 collision cross section cannot be converted easily (it depends on the molecule), there was a need for drift tube N2 collision cross sections as reference, especially for small molecules. Now many sets are available.
- (Giles:) Also distinguishes between the reproducibility of CCS measurement (which is needed for many applications, e.g. with databases) and the accuracy of CCS values (which is needed for fundamental interpretation of CCS in terms of structure).
- (Rutolo:) Reminds that the power law ($A.t_{d,corr}^{B}=CCS.sqrt(\mu)/z$) is just an empirical formula, which decently approximates more complicated functions. The residuals depend on the wave height and wave velocity.
- Many agree that rock solid molecules as calibrant (insensitive to gas-phase activation) would be most useful.
- (Shvartsburg:) Adds that in addition to being rigid, for TWIMS the calibrant should also be of similar nature as the analyte. This is because one calibrates for mobility (K) primarily, and the calibration should correct for minor variations of the pressure, field, and temperature. Different analyte chemical classes have different such responses.

## Non DT mobility separation

### Drift Tube Ion Mobility



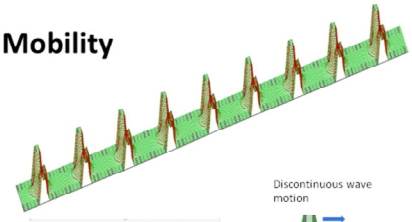

velocity = K.E  L/t = K.V/L  $K = L^2/(V.t)$

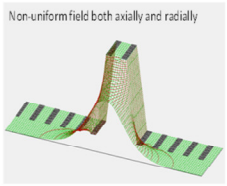
Mason Schamp Equation

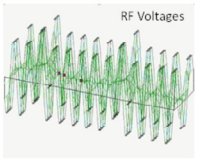
$$K = \left[\frac{(18\pi)^{1/2}}{16(k_b T)^{1/2}}\frac{e}{N}\right]\frac{z}{\mu^{1/2}}\frac{1}{\Omega}$$

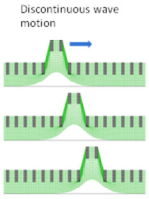
$$\underbrace{\Omega\frac{\mu^{1/2}}{z}}_{\text{(rCCS)}} = A_1 t$$

### Travelling Wave Ion Mobility





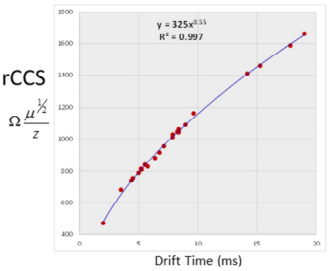




velocity = K.E(r,z,t)

No simple analytical solution

$$\Omega\frac{\mu^{1/2}}{z} = A_2 t^n \quad \text{(n ~0.5)}$$

Calibration possible



## Non DT mobility separation

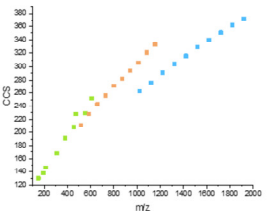
- **Calibration**
  - Availability of suitable calibrants to cover CCS ranges of interest
    - DT determined CCS values
      - Prof. D, Clemmer, Indiana U.
      - Prof M. Bush, U. of Washington
      - Prof J. McLean, Vanderbilt
- Ideal calibrants
  - Broad utility
    - Wide CCS range
    - Positive and negative ion modes
    - Wide m/z range
  - Conformationally stable
  - Good 'shelf life' (chemically stable)
  - Readily available
  - Easy to use

### Instrumental considerations

| Pre-mobility energetics | Mobility separation | Post-mobility transport characteristics | MS Analyser |
|---|---|---|---|

'Majormix' +1 charge state, $N_2$ CCS values

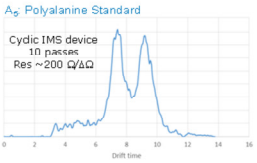
| Compound | m/z | Formula | |
|---|---|---|---|
| Acetaminophen | 152.07061 | C8H9NO2 | CCS=130.4 |
| Reserpine fragment | 195.08765 | C8H10N4O2 | CCS=138.2 |
| Sulfaguanidine | 215.05972 | C7H10N4O2S | CCS=146.8 |
| Sulfadimethoxine | 311.08085 | C12H14N4O4S | CCS=168.4 |
| Val-Tyr-Val | 380.21800 | C19H29N3O5 | CCS=191.7 |
| Verapamil | 455.29043 | C27H38N2O4 | CCS=208.8 |
| Terfenadine | 472.32101 | C32H41NO2 | CCS=228.7 |
| Polyalanine | 516.27764 | C21H37N7O8 | CCS=211.0 |
| Leucine Enkephalin | 556.27658 | C28H37N5O7 | CCS=229.8 |
| Polyalanine | 587.31475 | C24H42N8O9 | CCS=228.0 |
| Reserpine | 609.28066 | C33H40N2O9 | CCS=252.3 |
| Polyalanine | 658.35187 | C27H47N9O10 | CCS=243.0 |
| Polyalanine | 729.38898 | C30H52N10O11 | CCS=256.0 |
| Polyalanine | 800.42609 | C33H57N11O12 | CCS=271.0 |
| Polyalanine | 871.46321 | C36H62N12O13 | CCS=282.0 |
| Polyalanine | 942.50032 | C39H67N13O14 | CCS=294.0 |
| Polyalanine | 1013.53743 | C42H72N14O15 | CCS=306.0 |
| Polyalanine | 1084.57455 | C45H77N15O16 | CCS=321.5 |
| Polyalanine | 1155.61166 | C48H82N16O17 | CCS=333.6 |
| Ultramark 1621 | 1022.00341 | C20H18O6N3P3F28 | CCS=263.1 |
| Ultramark 1621 | 1121.99702 | C22H18O6N3P3F32 | CCS=276.5 |
| Ultramark 1621 | 1221.99064 | C24H18O6N3P3F36 | CCS=291.2 |
| Ultramark 1621 | 1321.98425 | C26H18O6N3P3F40 | CCS=304.0 |
| Ultramark 1621 | 1421.97786 | C28H18O6N3P3F44 | CCS=316.7 |
| Ultramark 1621 | 1521.97147 | C30H18O6N3P3F48 | CCS=329.0 |
| Ultramark 1621 | 1621.96509 | C32H18O6N3P3F52 | CCS=340.1 |
| Ultramark 1621 | 1721.95870 | C34H18O6N3P3F56 | CCS=351.3 |
| Ultramark 1621 | 1821.95231 | C36H18O6N3P3F60 | CCS=362.1 |
| Ultramark 1621 | 1921.94593 | C38H18O6N3P3F64 | CCS=372.6 |

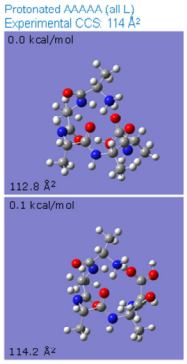



- Used in automatic CCS calibration routine on Synapt and Vion
- Also use 'Lock' mobility
- Measured CCS values usually within 2% of DT values
- Reproducibility better

## Non DT mobility separation

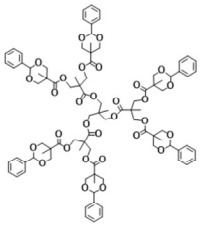
- **Calibrant study 1**
  - With Prof. B. Paizs, U. of Bangor, Wales
  - Poster WP 384


- **Calibrant study 2**
  - With Prof. S. Grayson, Tulane University and Polymer Factory, Sweden
  - Poster ThP 416

Polyester dendrimers based on the bis-MPA monomer



- Looking to extend the combined range of CCS and m/z calibration using these stable species

- Calibration for CCS
  - Good absolute agreement with DT values, good reproducibility (useful for fundamental studies and CCS libraries for screening)
  - Compatible with LC separation time-frames (useful for DT systems too)
  - Ongoing work to better characterise and develop calibrant species

## Survey responses

How do you evaluate if published CCS/$K_0$ values are trustworthy?

"Reputation of the group"

"Discuss with senior lab members"

"I generally trust the authors, especially if they have expertise"

"Values can be trustworthy only from a couple of research groups using drift tube"

"I always take published values with a grain of salt. Differences in measured values should be noted, but not taken to mean that one is "wrong," especially for proteins."

"Run the sample myself"

# What could be the guidelines for authors, reviewers, and editors of papers reporting IMS?

Perdita Barran, University of Manchester

Workshop part III, discussion with the audience:

- (Barran introduces:) The ion mobility community is currently at an early stage, similar to the X-ray crystallography community when the PDB did not exist. It is still often deemed not trustworthy. She advocates that the community should agree on international standards for reporting the data, and create a repository for collision cross section values.
- (Fjeldsted:) There should be a clear distinction between primary values from calibrated values. Calibrated values should clearly state the calibration method, calibration curve, and the origin of the primary values used.
- The survey and discussion during the workshop bring out several parameters that should be mentioned alongside any reported CCS value:
    - The drift gas
    - The instrument used
    - Particular conditions pertaining to ion mobility: the fields, the time scale of the mobility separation, the temperature
    - Any condition known to influence the ion population prior to IMS analysis: analyte preparation conditions, ion activation pre-IMS, charge state.
    - Representative arrival time distributions (showing whether peaks are broad or narrow with regard to the instrument resolving power, and whether there is one or multiple peaks)

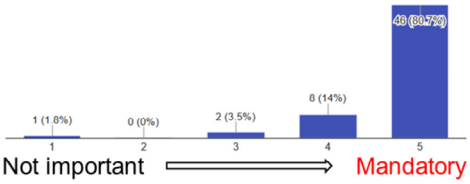
- Information on instrument calibration or performance verification. Include the source of the primary values of calibrants used.
- Number of independent replicates, and standard deviation.

## Survey responses

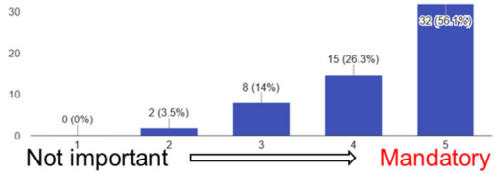
What parameters should also be reported alongside CCS/$K_0$ ?

**Highest scores**

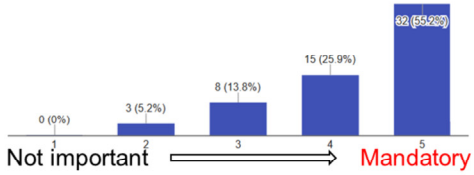
**Nature of the drift gas (4.71)**




**Std. Dev and # replicates (4.35)**



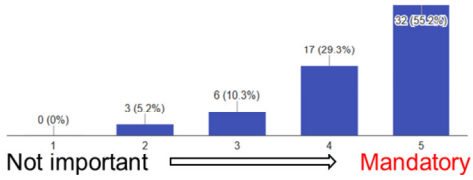

**Calibration details (4.31)**



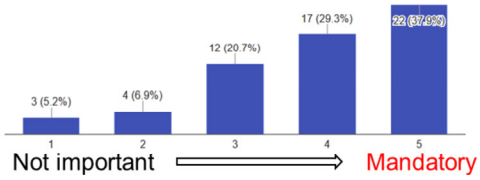
## Survey responses

What parameters should also be reported alongside CCS/$K_0$ ?

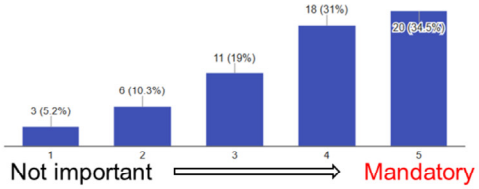
**IMS pressure (4.34)** High score



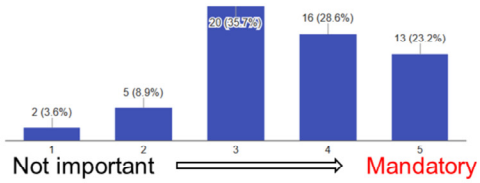

**Electric field (3.88)**




**IMS temperature (3.79)**



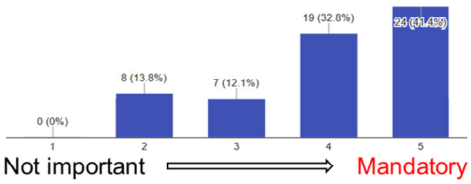

**E/N (3.59)** Lowest score



## Survey responses

What parameters should also be reported alongside CCS/$K_0$ ?

**Representative ATDs (4.02)**

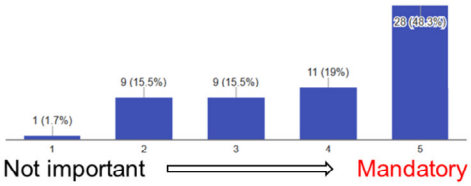



**Others:**

Charge state

Type of instrument

**Source/transfer tuning (3.97)**




**Analyte solution condt. (3.86)**