Advances in Mass Spectrometry:
ION MOBILITY

Background literature:
- Wyttenbach and Bowers, Top Curr Chem **225**, 207-232 (2003)
- Clemmer et al., Annu Rev Anal Chem **1**, 293-327 (2008)
- Gabelica and Marklund, Curr Opinion Chem Biol **42**, 51-59 (2018)

Anouk M. Rijs
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Free Electron Lasers for Infrared eXperiments

Master course Advances in MS - lecture 4

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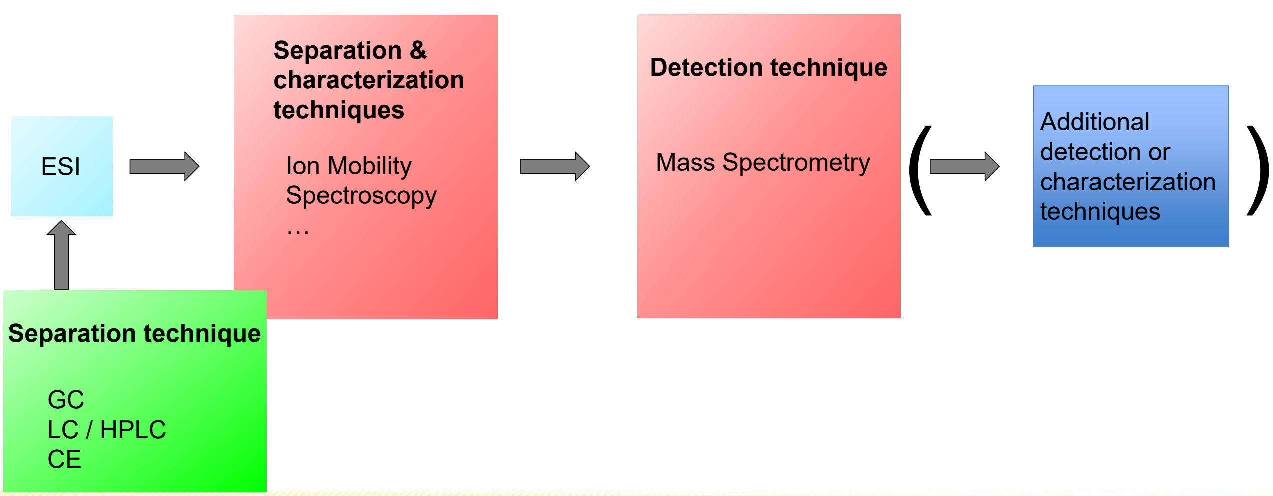
Hyphenated Mass Spectrometry



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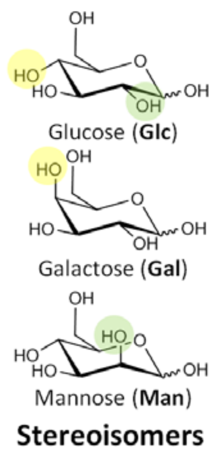
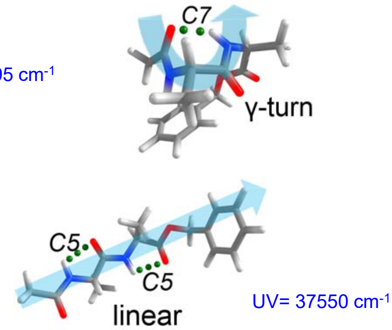
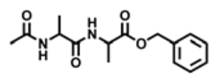
What if just mass spectroscopy is not enough?



Isobaric molecules:
- different chemical structure
- same mass
- same fragmentation?

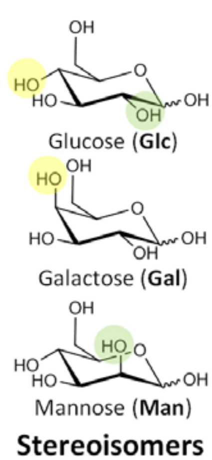


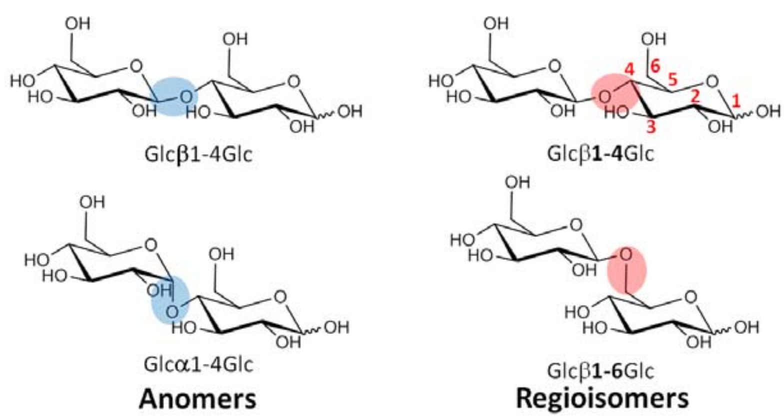

Ac-Ala-Ala-OBn (B)

Isomeric molecules:
- same chemical formula
- same mass
- different conformation
- same fragmentation?

FELIX — Rijs, Barran, Compagnon, Eyers, Flitsch Anal. Chem. 89, 4540–4549 (2017). — Bakels, Rijs, Faraday Discussions (2019) DOI: 10.1039/C8FD00208H — Radboud University

Is tandem-MS specific enough?



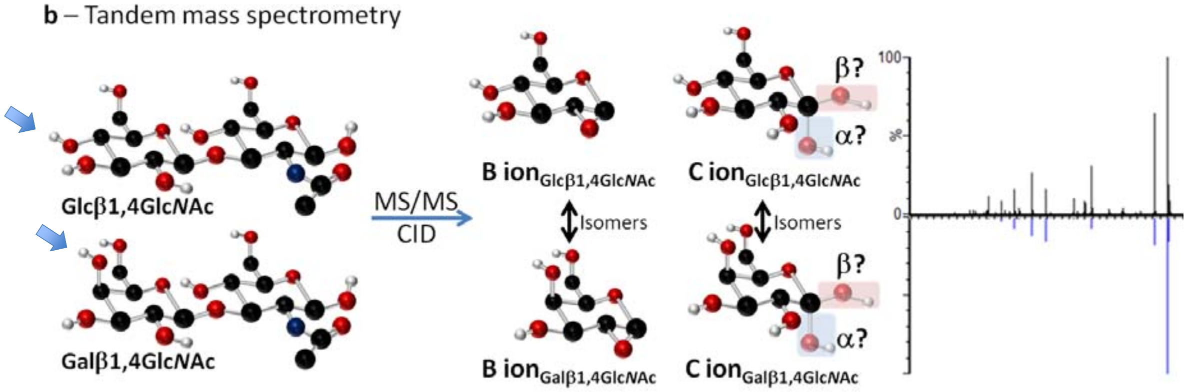


MW= 180.156 g/mol

FELIX — C. J. Gray, B. Schindler, L. G. Migas, M. Pičmanová, A. R. Allouche, A. P. Green, S. Mandal, M. S. Motawie, R. Sánchez-Pérez, N. Bjarnholt, B. L. Møller, **A. M. Rijs**, P. E. Barran, I. Compagnon, C. E. Eyers, and S. L. Flitsch, *Anal. Chem.* **89**, 4540–4549 (2017). — Radboud University

When tandem-MS is not enough….

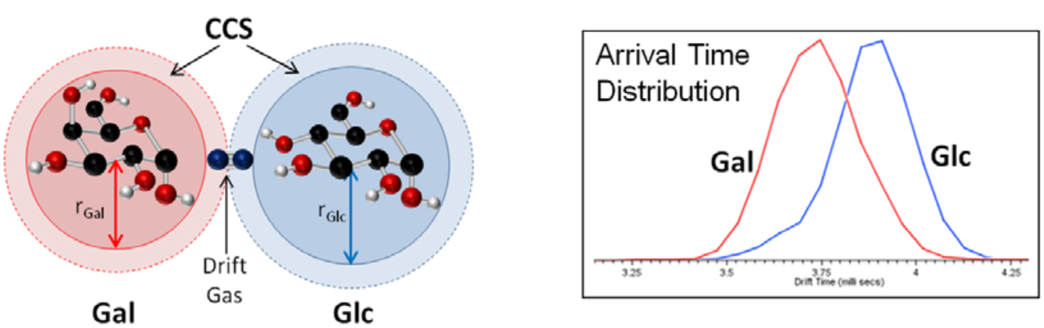
b – Tandem mass spectrometry



FELIX C. J. Gray, B. Schindler, L. G. Migas, M. Pičmanová, A. R. Allouche, A. P. Green, S. Mandal, M. S. Motawie, R. Sánchez-Pérez, N. Bjarnholt, B. L. Møller, **A. M. Rijs**, P. E. Barran, I. Compagnon, C. E. Eyers, and S. L. Flitsch, *Anal. Chem.* **89**, 4540–4549 (2017). Radboud University

…. Ion mobility might bring the solution!

c – Ion mobility spectrometry



FELIX C. J. Gray, B. Schindler, L. G. Migas, M. Pičmanová, A. R. Allouche, A. P. Green, S. Mandal, M. S. Motawie, R. Sánchez-Pérez, N. Bjarnholt, B. L. Møller, **A. M. Rijs**, P. E. Barran, I. Compagnon, C. E. Eyers, and S. L. Flitsch, *Anal. Chem.* **89**, 4540–4549 (2017). Radboud University

Course Layout – ion mobility mass spectrometry

1) Background into IM-MS (what?, history, fundamental principles)
2) Obtaining mobilities and CCS values
3) Instrumentation
4) Various types of ion mobility
5) Separation or characterization?
6) Applications / papers

Background literature:

- book
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Special thanks to prof. Perdita Barran (University of Manchester) for sharing her IM slides.
For research by the Barran group: https://www.mbc.manchester.ac.uk/barrangroup/

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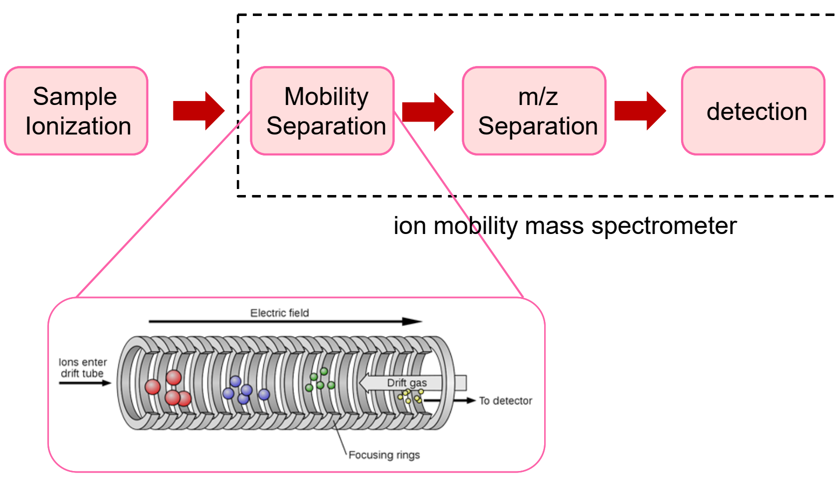
Typical set-up



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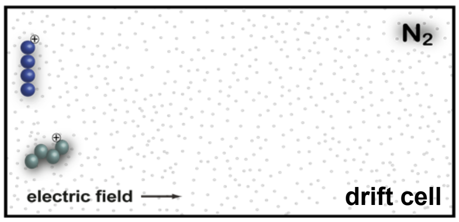
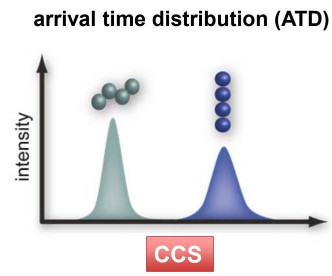
Ion Mobility: the general principle





- Gas-phase Separation of isobaric species
- Drift time converts to collision cross-section (**CCS**)
  = **Size information**

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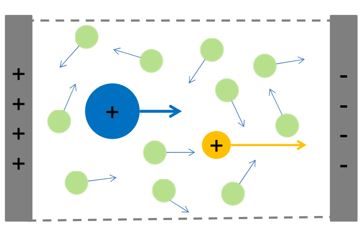
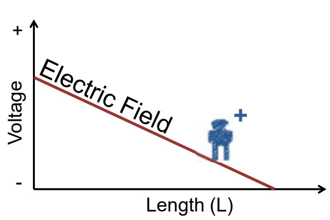
What is the basis for Ion Mobility separation?



- Electric Field “pulls”
- Gas Collisions “impede”
- Ratio of ion velocity (v) to the applied field (E) is Mobility (K)
- Applied over a length (L)



$$K = \frac{v}{E} \quad \left(K = \frac{L}{tE}\right)$$

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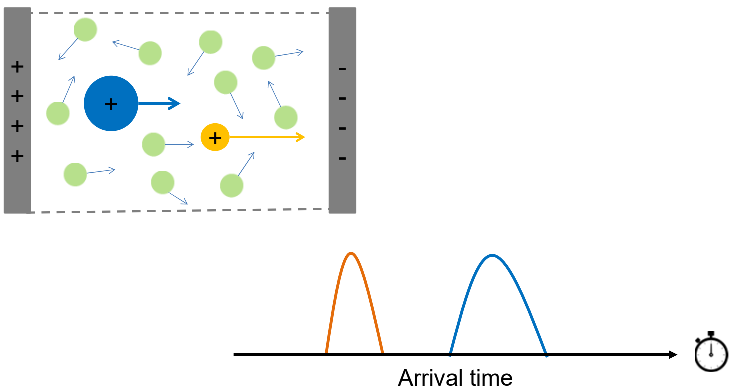
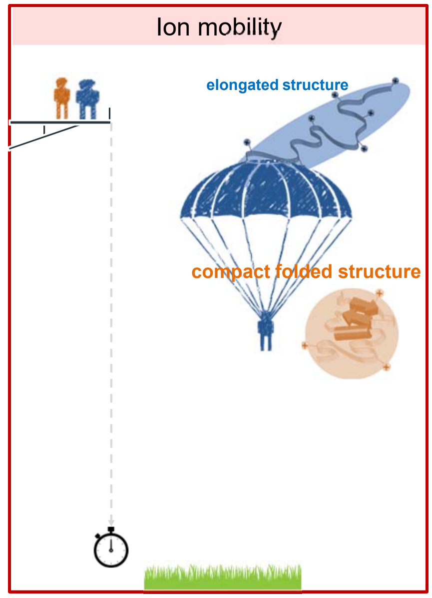
Ion Mobility: conformational seperation





- Same mass, charge, electric field, pressure and temperature
- Different arrival times ~ different conformations!

Animation: Bruno Belllina (UoM)

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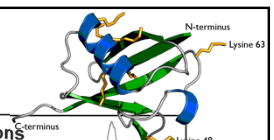
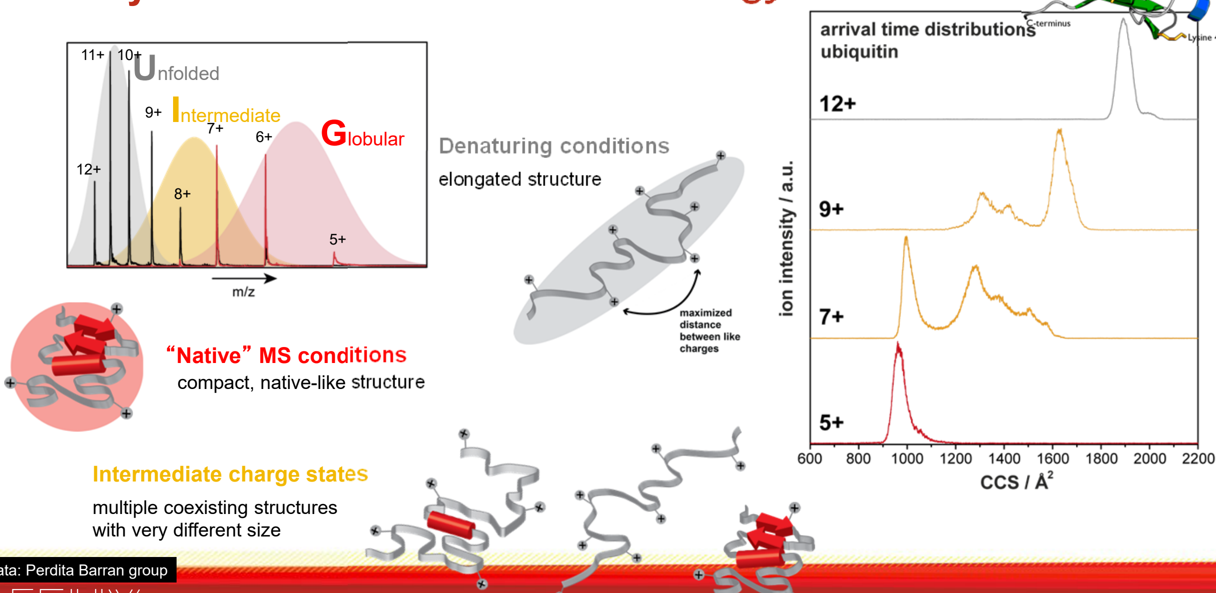
Why is IM relevant to structural biology?





Data: Perdita Barran group

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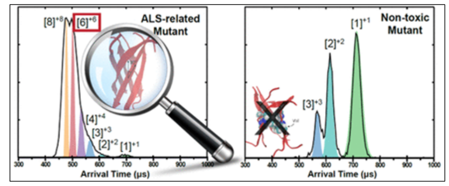
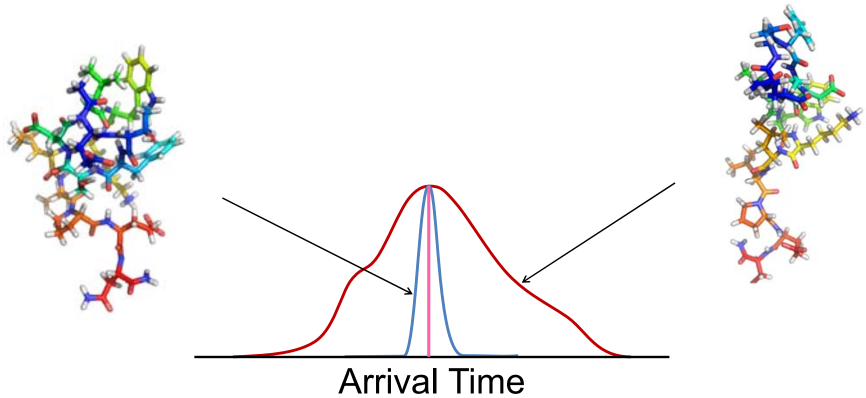
Arrival Time Distribution of Gaseous Ions





MD Movies: Terakawa, Takada, Biophys J. 2011 September 21; 101(6): 1450–1458.

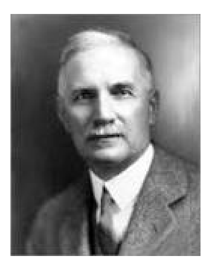
Historical Background

- Which came first IMS or MS? Dates?
- *Answer*: IMS is ~ 15 years older than MS
- John Zeleny B.Sc. (1898) VI. *On the ratio of the velocities of the two ions produced in gases by Röntgen radiation; and on some related phenomena* - Philosophical Magazine Series 5, 46:278, 120-154, DOI:10.1080/14786449808621173

We are thus led to suppose, as in liquids, that the observed velocity difference is due to an inequality in the size of the two ions. Why the two ions, even if they are formed of groups of molecules, should in a simple gas be of a different size is a question to which definite answers cannot be given in the present state of our knowledge, or rather ignorance, of the relation between matter and electricity, but is one which must be borne in mind in considerations of this relation.

In conclusion, I desire to express my best thanks to Prof. J. J. Thomson for many valuable suggestions.

Cavendish Laboratory,
April 12, 1898.



Timeline of developments in IM-(MS)

- **1890s** ***J. Zeleny,*** *J. J. Thomson, E. Rutherford*
  Interest in charge carriers gas after recent X-Ray discovery
- **1903** *P. Langevin*
  Theoretical work; basis of todays theory
- **1913** F.W. Aston, *J. J. Thomson*
  Development of first mass spectrometers
- **1950/60s** Advances through military/security, pollution, and space research
  Combination of Ion mobility and mass spectrometry (**IM-MS**) - study of ion-molecule reactions
- **1960/70s** *Mason, McDaniel*
  Study of collision phenomena in ionized gases, *book: "Transport Properties of Ions in Gases"*
- **1990s** *Bowers, Kebarle, Hill, Russell*
  Metal ions and clusters. Later: biopolymers and biomolecules
  Advances in structural biology (*Jarrold, Clemmer, …*)
- **2007** 1st commercial IM-MS setup (Waters Synapt HDMS)
  since then larger molecules, complexes, many new instruments more accurate calculations, …

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Timeline of developments in IMS

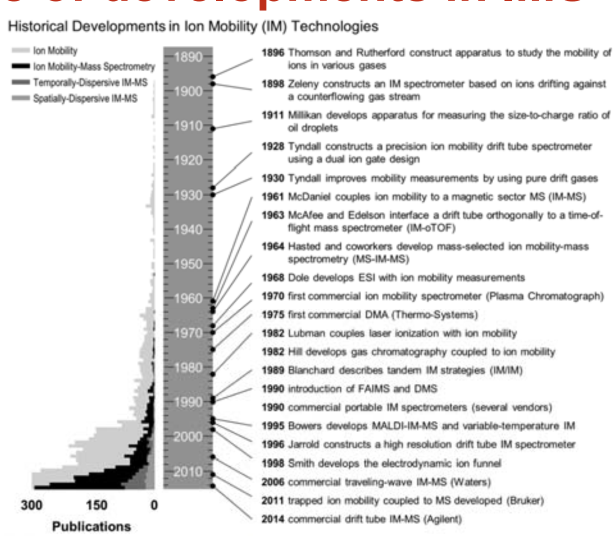

Figure 1. (Left) Histogram of the number of publications published per year in ion mobility and ion mobility-mass spectrometry. Note that the scale is truncated at 300 to highlight the number of publications specifically utilizing IM-MS. Further distinction is made to discriminate the frequency of publication for both time and space-dispersive IM-MS publications. (Right) Historical milestones in the development of ion mobility and IM-MS instrumentation.

May, McLean
Analytical Chemistry **87**, 1422 (2015)

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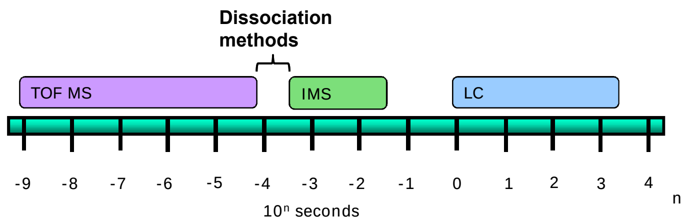
Applications of IMS

- **Fundamental research:** molecular structure, reactions, dynamic, kinetic
- **Industry (pharmaceutical, semiconductor, petrochemical, …):** quality control / security, food security
- **Medical applications:** breath analysis, cancer recognition
- **Security / military:** Detection of narcotics, explosives, warfare agents
- **Environmental monitoring**

● IMS timescale: Combination with orthogonal methods



Waters Corporation

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Course Layout – ion mobility mass spectrometry

1) Background into IM-MS (what, history, fundamental principles)
2) **Obtaining mobilities and CCS values -> Theory**
3) Instrumentation
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Theory of IMS

- **Definition of mobility *K***

  v_d – drift velocity

  E – electric field

$$v_\mathrm{d} = KE \qquad \text{speed} = \frac{\text{distance}}{\text{time}}$$

- **Drift time t_d**

  L – drift length

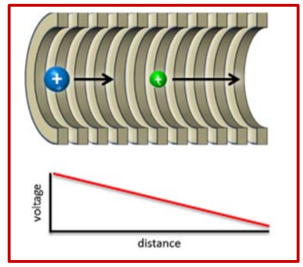
  t_0 – time offset

$$t_\mathrm{d} = \frac{L}{KE} + t_0$$

- **Ion mobility K**

$$K = \frac{3}{16}\sqrt{\frac{2\pi}{\mu k_B T}}\frac{ze}{N\Omega}$$

CCS



q – charge ze

N – drift-gas number density

μ – reduced mass (ion + drift-gas molecule)

k_B – Boltzmann constant

T – drift gas temperature

P – drift gas pressure

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Theory of IMS

- **Definition of mobility *K***

  v_d – drift velocity

  E – electric field

$$v_\mathrm{d} = KE \qquad \text{speed} = \frac{\text{distance}}{\text{time}}$$

- **Drift time t_d**

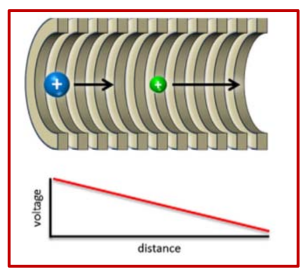
  L – drift length

  t_0 – time offset

$$t_\mathrm{d} = \frac{L}{KE} + t_0$$

- **Collision cross section Ω** (Mason-Schamp equation)

$$\Omega = \frac{3q}{16N}\sqrt{\frac{2\pi}{\mu\, k_\mathrm{B}T}}\frac{1}{K_0}$$



$$K_0 := \frac{P}{P_0}\frac{T_0}{T}\;K$$

reduced mobility

q – charge ze

N – drift-gas number density

μ – reduced mass (ion + drift-gas molecule)

k_B – Boltzmann constant

T – drift gas temperature

P – drift gas pressure

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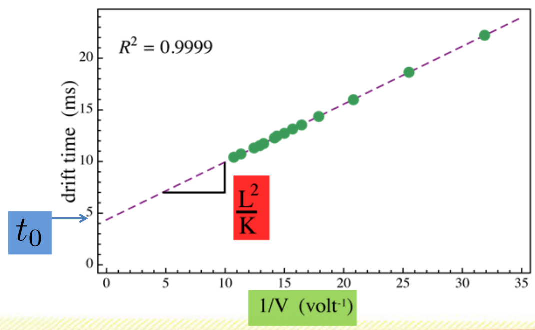
Theory of IMS – Ω determination

- **Definition of mobility *K***
  v_d – drift velocity
  E – electric field
- **Drift time t_d**
  L – drift length
  t_0 – time offset

$$v_d = KE \qquad \text{speed} = \frac{\text{distance}}{\text{time}}$$

$$t_d = \frac{L}{KE} + t_0 = \frac{L^2}{K}\frac{1}{V_d} + t_0$$



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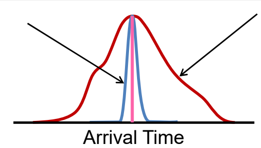
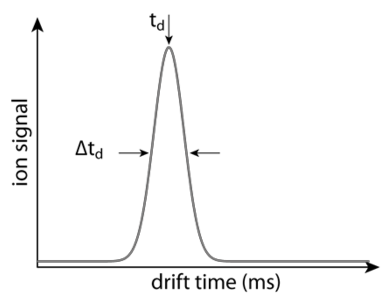
Theory of Ion Mobility: Resolution



- **Resolving power *R* (drift tube)**



$$R_{DT} = \frac{t_d}{\Delta t} = \frac{1}{4}\sqrt{\frac{q}{k_B \ln 2}}\sqrt{\frac{V_d}{T}}$$

Example:

$$R_{DT} = 100$$
$$\Delta\Omega = 1\%$$

OTHER OPTION??
= INCREASE L

- **Increase *R* by increasing V_d or lower *T***
  - **Limited by**
    (low field condition)
    $$\frac{1}{2}Mv_d^2 \ll \frac{3}{2}k_B T$$
    → increase pressure *P*

and t_0

$$t_d = \frac{L^2}{K}\frac{1}{V_d} + t_0$$

and
electrical discharges

http://scienceblogs.com/

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Drift Tube Ion Mobility

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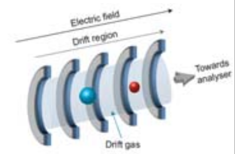
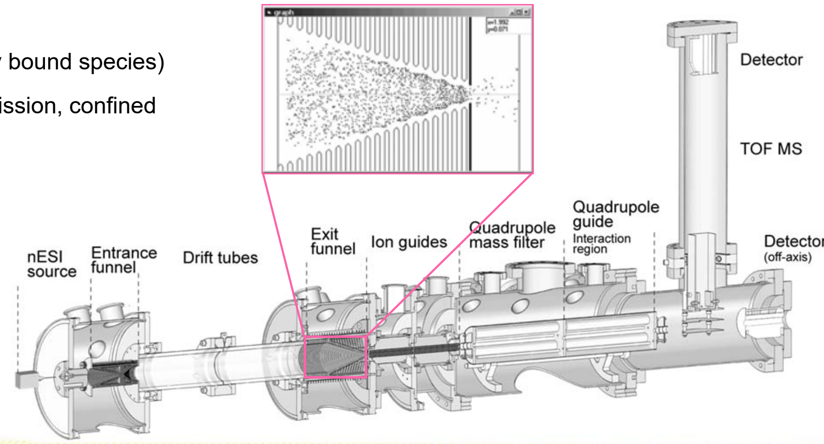
Drift time ion mobility (DTIM / DTIMS)



- **Conventional drift-tube setup**
- 2-10 mbar drift-gas pressure
- $R_{\mathrm{DT}}(80\ \mathrm{cm}) \approx 40$
- "soft" conditions (allows weakly bound species)
- **Ion funnels:** increased transmission, confined ion clouds allows for larger L

$$R_{\mathrm{DT}} \propto \sqrt{\frac{V_{\mathrm{d}}}{T}} \propto \sqrt{LE}$$

- ➢ Increase drift length *L*
- ➢ Higher *R* at constant *E* and p



Lanucara, Holman, Gray, Eyers, Nature Chemistry **6**, 281 (2014)
Proteomics, **2015**, doi: 10.1002/pmic.201400480, Mass Spectrometry Reviews, **2009**, *29*, 294-312

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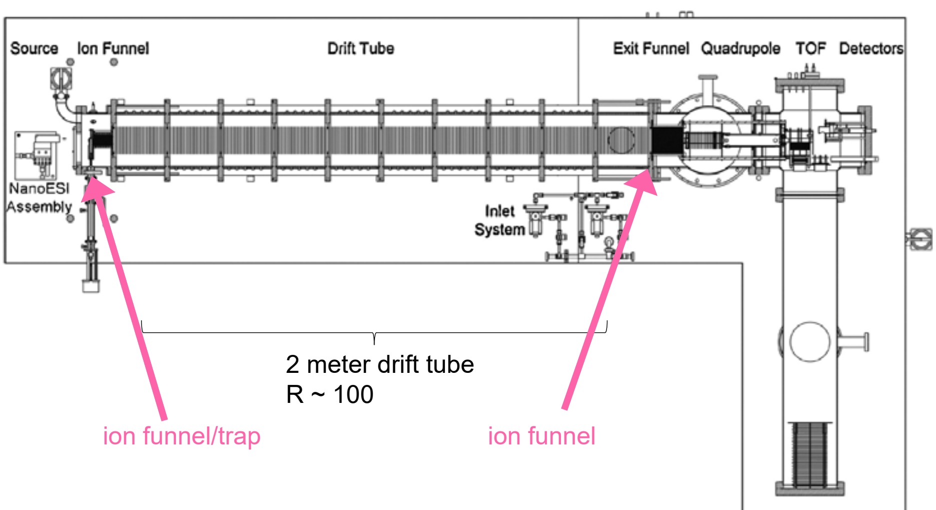
Higher resolution ion mobility set-ups



Ion funnels are essential for drift-tube setups

Bowers group; International Journal of Mass Spectrometry, 2009, *287*, 46-57

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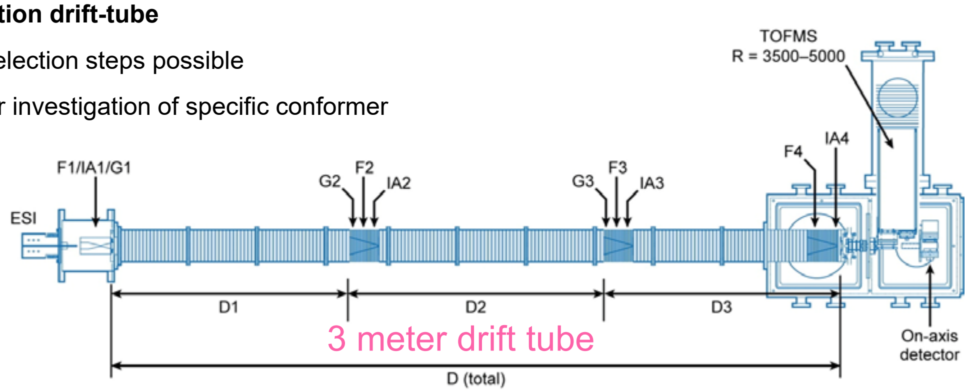
Tandem IMS (and higher resolution)

- **Higher resolution drift-tube**
- Multiple IMS selection steps possible
- Enables further investigation of specific conformer



Clemmer group; Annual Review of Analytical Chemistry, **2008**, *1*, 293-327

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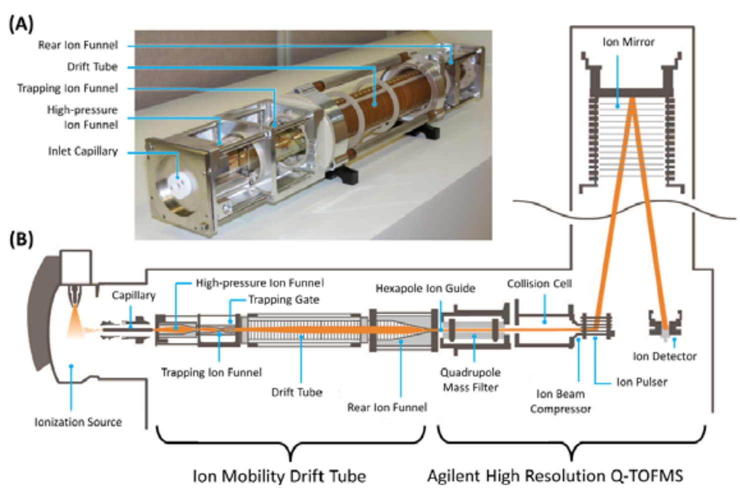
Commercial DTIMS

- Agilent 6560 IM QTOF
- Launched 2013
- 80 cm drift tube
- Linear field
- Resolution ~60
- Capable of multiple injections
- Trapping time variation



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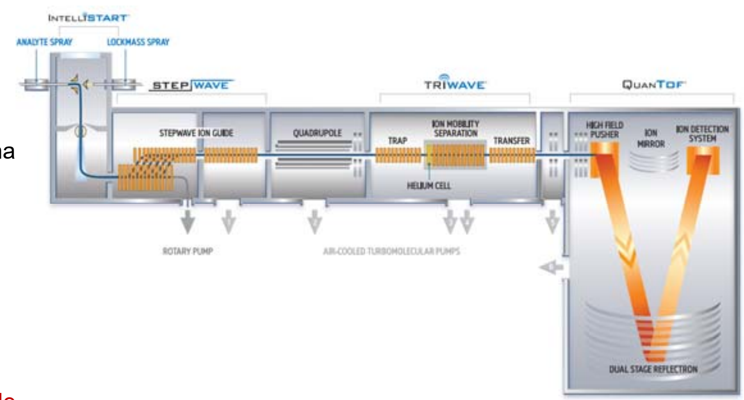
Traveling Wave Ion Mobility

Commercial TWIMS: Synapt

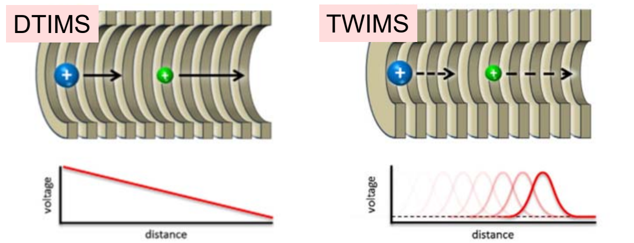
- Developed by Waters
- Ions radially confined by RF
- Buffer gas in drift cell within ‘Triwave’
- $\Omega \propto t_D^x$
- Ions of high K roll over the wave less often tha species of low K = Shorter transit
- Mobility ‘based’ separation



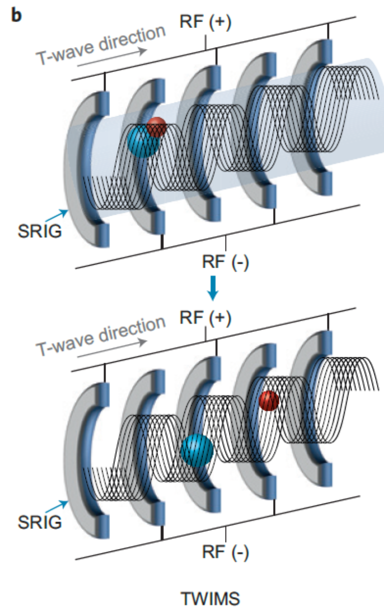
- Commercially available
- High sensitivity
- High mass resolution
- Pre- and post- IM activation possible
- Ω determination requires calibration

- Collision Cross Sections in Å² taken in N_2
- Scaled to Helium CCS
- Need for calibration

Travelling wave ion mobility (TWIMS)



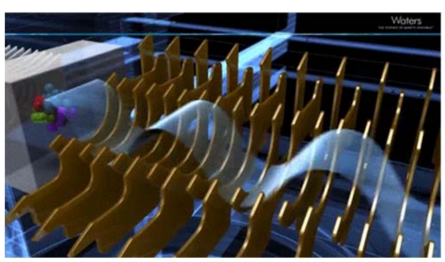
- Series of ring electrodes (SRIG)
- Electric field small region DT, wave pushes ion to detector
- Traveling wave upon which ion surf and traverse the IM cell
- Separation:
  - Higher mobility ions: carried by the wave
  - Lower mobility ions: roll over (longer time to move through)



Biochimica et Biophysica Acta, **2011**, *1811*, 935-945 and Eyers et al., Nature Chemistry **6**, 281 (2014)

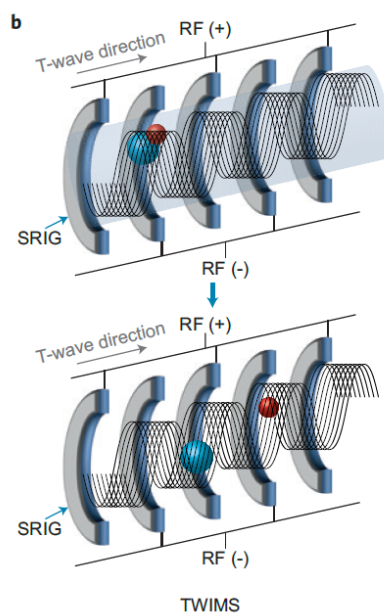
*Stacked Ring Ion Guide

Travelling wave ion mobility (TWIMS)



www.youtube.com/user/WatersCorporation

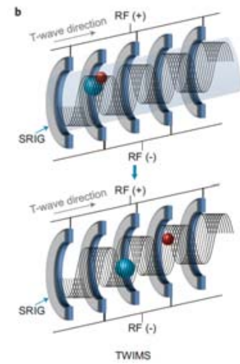
- Series of ring electrodes (SRIG)
- Electric field small region DT, wave pushes ion to detector
- Traveling wave upon which ion surf and traverse the IM cell
- Separation:
  - Higher mobility ions: carried by the wave
  - Lower mobility ions: roll over (longer time to move through)
- Use for **separation** and CCS **characterization after calibration**



Biochimica et Biophysica Acta, **2011**, *1811*, 935-945 and Eyers et al., Nature Chemistry **6**, 281 (2014)

*Stacked Ring Ion Guide

Travelling wave ion mobility (TWIMS)



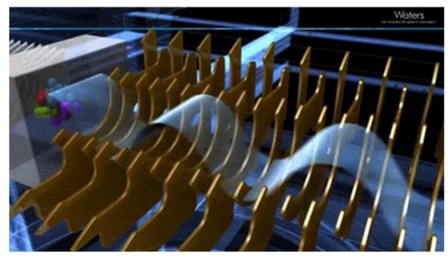
TWIMS features:

- **Compact design - high resolution**

  $R_{\mathrm{DT}}(80\ \mathrm{cm}) \approx 40$

  $R_{\mathrm{TW}}(25\ \mathrm{cm}) \approx 40$

- **No direct CCS (Ω) information**
  - non-uniform electric fields, not able to calculate CCS (Ω)
  - Calibration required for Ω
  - Calibration requires defined conditions:
    - gas type and pressure
    - travelling wave speed and height
    - temperature
    - ion – similar properties (size, charge, shape)



Biochimica et Biophysica Acta, **2011**, *1811*, 935-945 and Eyers et al., Nature Chemistry **6**, 281 (2014)

www.youtube.com/user/WatersCorporation

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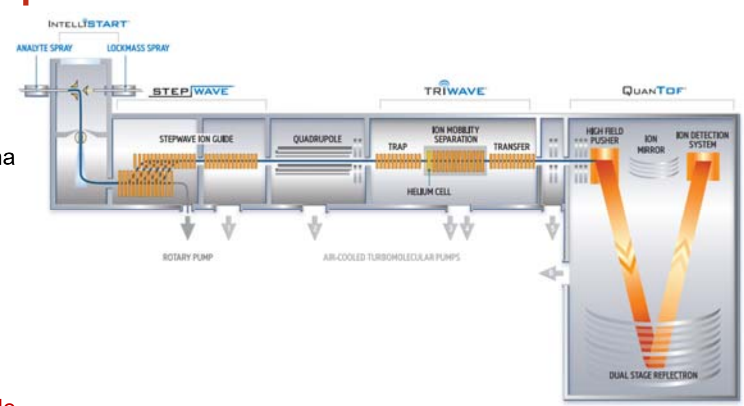
Commercial TWIMS: Synapt

- Developed by Waters
- Ions radially confined by RF
- Buffer gas in drift cell within 'Triwave'
- $\Omega \propto t_D^{\,x}$
- Ions of high K roll over the wave less often tha species of low K = Shorter transit
- Mobility 'based' separation



- Commercially available
- High sensitivity
- High mass resolution
- Pre- and post- IM activation possible
- Ω determination requires calibration

- Collision Cross Sections in Å² taken in N_2
- Scaled to Helium CCS
- Need for calibration

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Trapped Ion Mobility

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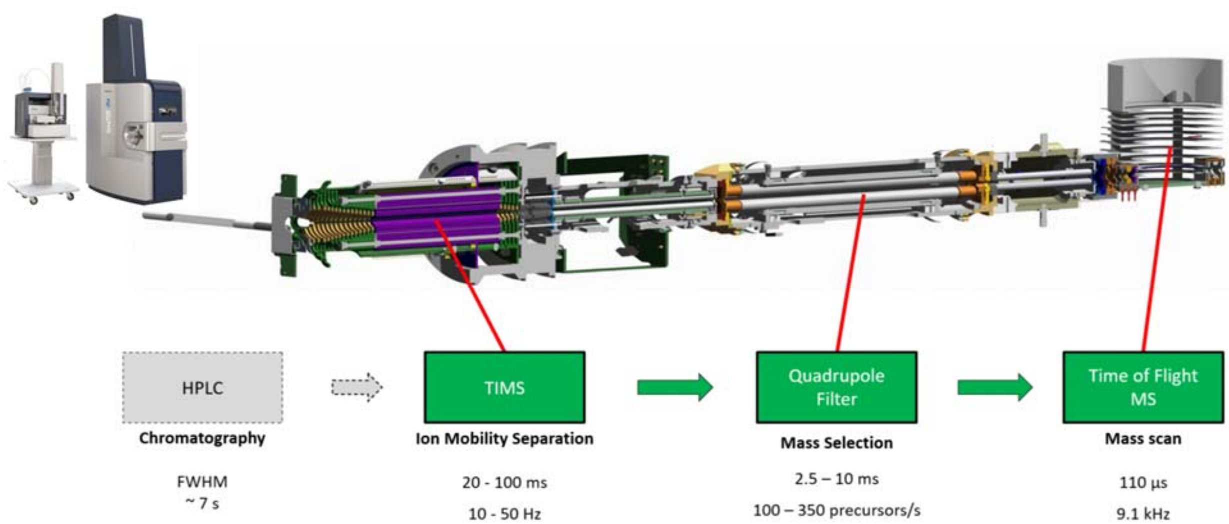
Trapped Ion Mobility Spectrometry: TIMS



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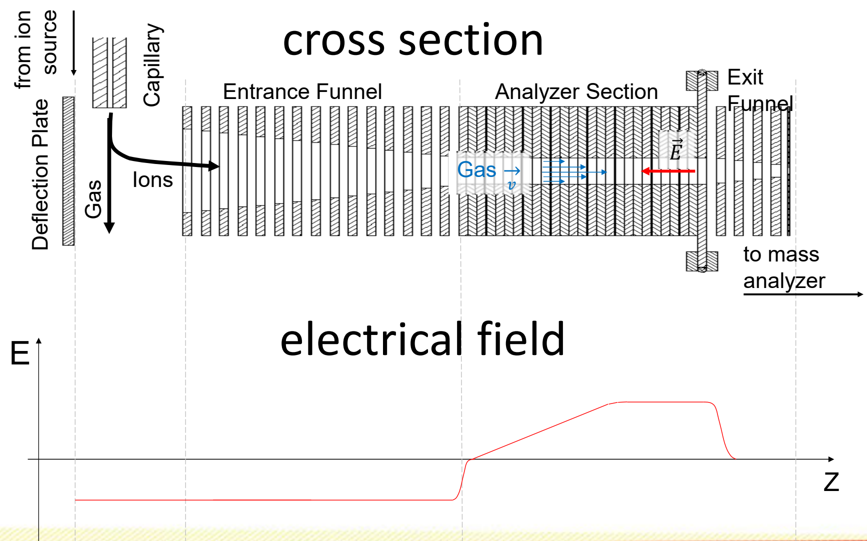
TIMS Separation



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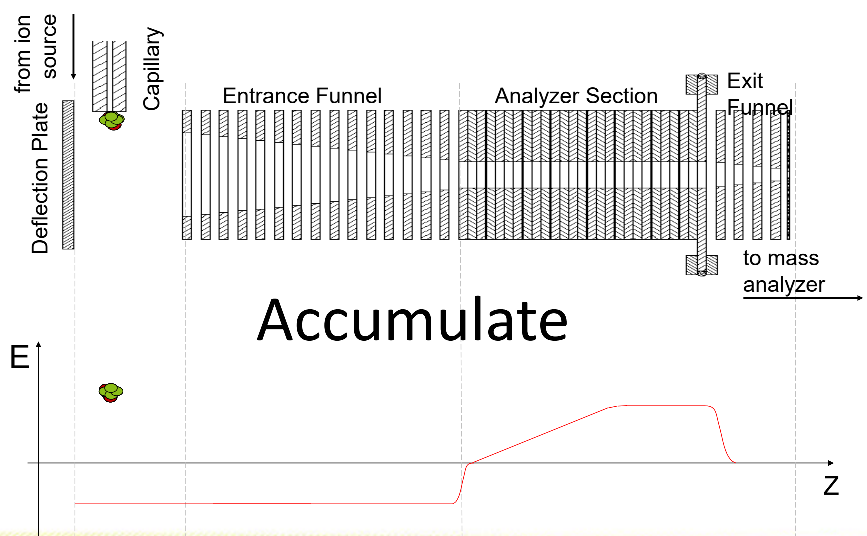
TIMS Separation



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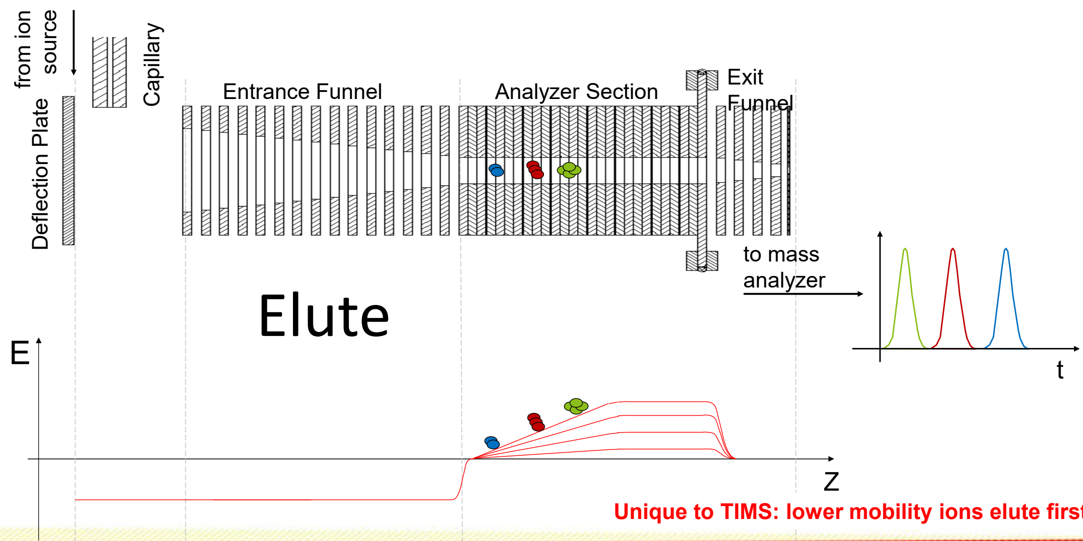
TIMS Separation



Unique to TIMS: lower mobility ions elute first

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Other forms of Ion Mobility

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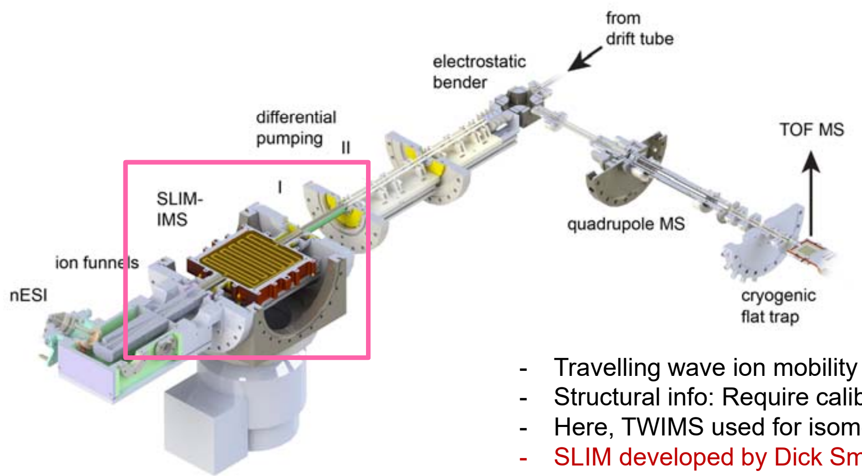
SLIM: Structures for Lossless Ion Manipulation



- Travelling wave ion mobility
- Structural info: Require calibration for CCS
- Here, TWIMS used for isomer preselection
- SLIM developed by Dick Smith et al. at PNNL
- Exceptionally high resolution

Experiment and slides from Thomas Rizzo (EPFL)

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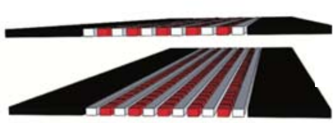
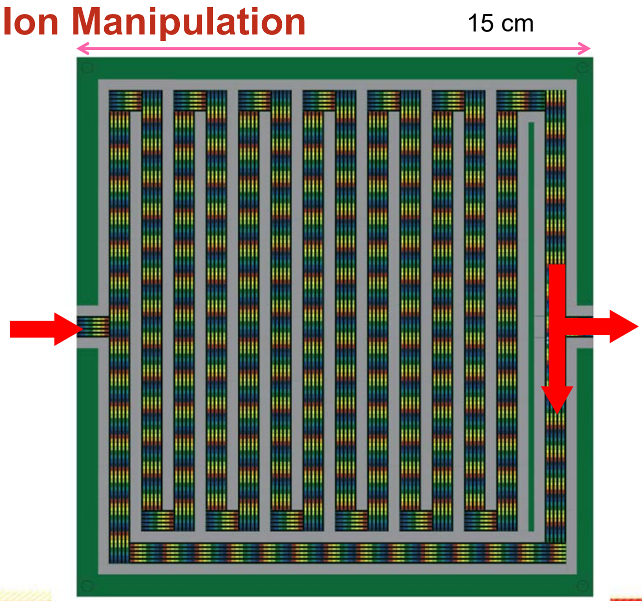
SLIM: Structures for Lossless Ion Manipulation

- PCB based electrodes
- Compact, cheap
- High ion throughput





→211 SLIM segments
1.8 m drift length (*L*)
$R_{TW} \propto \sqrt{nL}$
n - number of laps

SLIM: A. M. Hamid et al., *Anal. Chem.* **2015**, *87*, 11301

Experiment and slides from Thomas Rizzo (EPFL)

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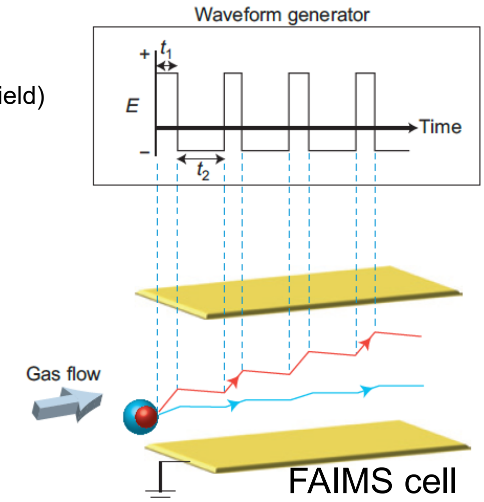
Field-asymmetric ion mobility (FAIMS)

- Ion introduced in alternating asymmetric field (low and high field)
- Ions have different drift time towards the two electrodes
  = different *K* of ions in low- and high electric fields
  = separation

- FAIMS
  - High sensitivity
  - Separation of otherwise inseparable species
  - Possible to upgrade existing setup
- No CCS (Ω) information
- Prediction very difficult
- “Hot” experimental conditions



FAIMS

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Comparison of the three main types of IMS

REVIEW ARTICLE — NATURE CHEMISTRY DOI: 10.1038/NCHEM.1889

Table 1 | Comparison of the three main types of IMS.

	DTIMS	TWIMS	FAIMS
Advantages	Rotationally averaged collisional cross-section (CCS; Ω), that is, ‘shape’ can be measured (Å²) Can be used to separate species of very similar mobility; high resolving power (>100 as defined by Ω/ΔΩ measured at FWHM)[157]	Rotationally averaged CCS can be determined Can be used for mobility separation of product ions generated either by collision-induced dissociation or by electron-transfer dissociation	High resolving power (≤ ~100 as defined by Ω/ΔΩ at FWHM)[14] Relatively straightforward to transfer the ion mobility device between different mass spectrometers
Disadvantages	The geometric configuration of current commercial DTIMS-MS instruments means that they can only be used to separate analytes immediately post-ionization Gating-type instruments are susceptible to ion losses when transferred from atmospheric pressure during ionization to the reduced pressure required for analysis	CCS determination requires calibration of the drift time through the TWIMS cell, ideally using a calibrant of similar physical and chemical properties Relatively low resolving power (≤~45 as defined by Ω/ΔΩ at FWHM)[152] Ion heating can occur as ions are injected into the TWIMS cell which may affect gas-phase conformation. Unless carefully controlled, the process of measurement may therefore perturb analyte structure	CCS cannot be determined The geometric configuration of a FAIMS-MS instrument means that it can only be used to separate analytes immediately post-ionization The percentage of ions detected relative to those generated following ionization (that is, the duty cycle) is relatively low when operated under conditions where the CV is ramped (CV scanning mode), reducing sensitivity

CV: compensation voltage; FWHM: full width at half maximum.

Eyers et al., Nature Chemistry **6**, 281 (2014)

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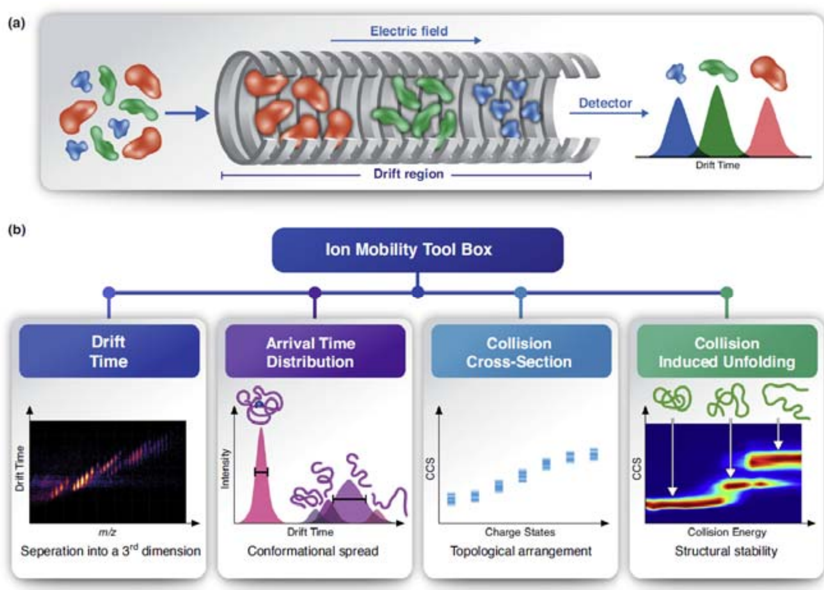
Course Layout – ion mobility mass spectrometry

1) Background into IM-MS (what, history, fundamental principles)
2) Obtaining mobilities and CCS values
3) Instrumentation & various types of ion mobility
4) Separation or characterization? ➡ **Discussion in tutorial !**
5) **Applications / papers**

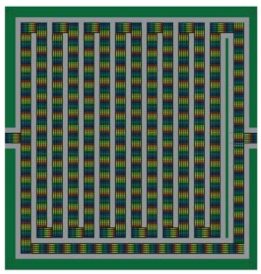
Ion Mobility Tool Box: structural characterization of proteins



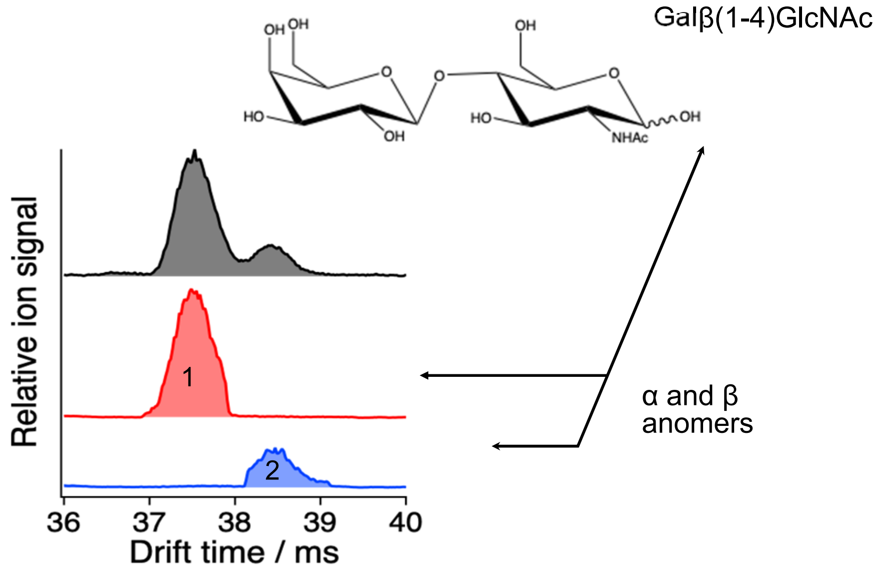
Ben-Nissan and Sharon, Current Opinion in Chemical Biology **42**, 25-33 (2018)

Drift time

AIM = Conformer separation



13 m drift
(7 cycles)



from Thomas Rizzo (EPFL)

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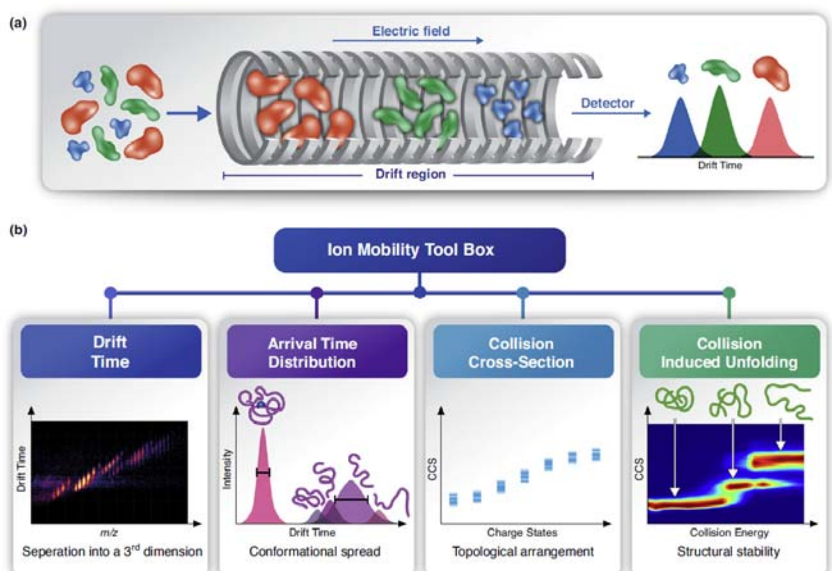
Ion Mobility Tool Box: structural characterization of proteins



Ben-Nissan and Sharon, Current Opinion in Chemical Biology **42**, 25-33 (2018)

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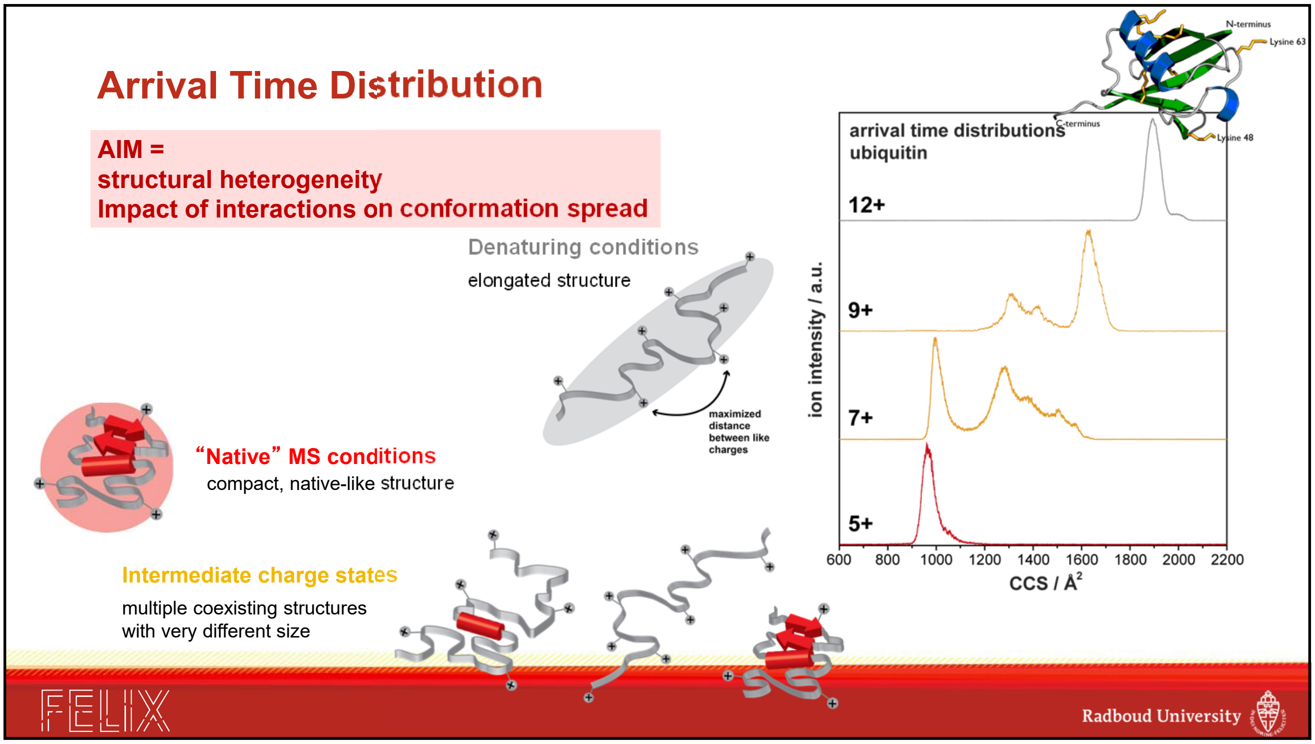
Arrival Time Distribution
AIM =
structural heterogeneity
Impact of interactions on conformation spread
Denaturing conditions
elongated structure
maximized distance between like charges
"Native" MS conditions
compact, native-like structure
Intermediate charge states
multiple coexisting structures
with very different size
arrival time distributions
ubiquitin
N-terminus
Lysine 63
C-terminus
Lysine 48
12+
9+
7+
5+
ion intensity / a.u.
600
800
1000
1200
1400
1600
1800
2000
2200
CCS / Å²
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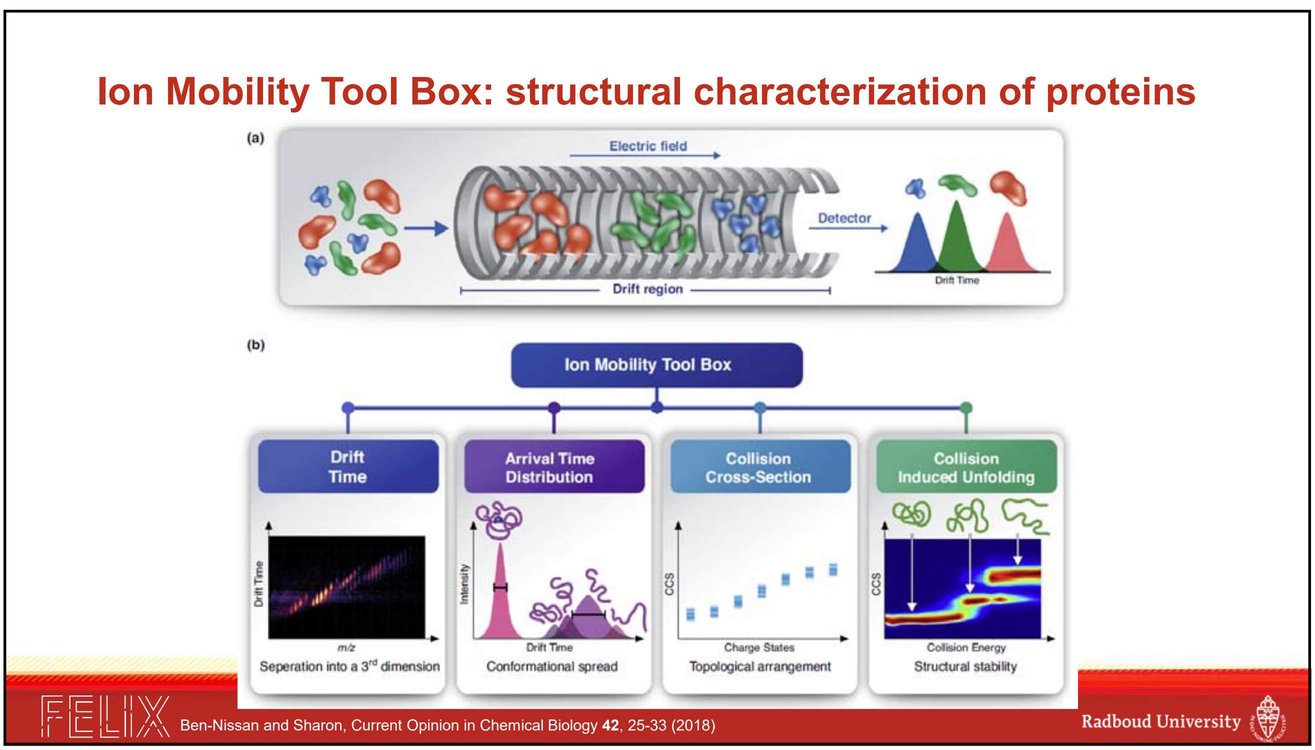
Ion Mobility Tool Box: structural characterization of proteins
(a)
Electric field
Detector
Drift region
Drift Time
(b)
Ion Mobility Tool Box
Drift Time
Drift Time
m/z
Seperation into a 3rd dimension
Arrival Time Distribution
Intensity
Drift Time
Conformational spread
Collision Cross-Section
CCS
Charge States
Topological arrangement
Collision Induced Unfolding
CCS
Collision Energy
Structural stability
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Ben-Nissan and Sharon, Current Opinion in Chemical Biology 42, 25-33 (2018)
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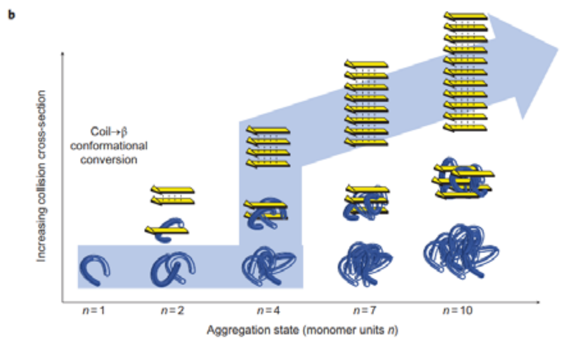
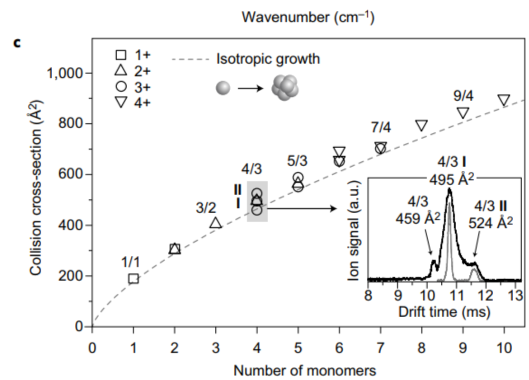
Collision Cross Section

AIM =
Structure elucidation
unravel structural features





Bowers, Nature Chemistry 3, 172 (2011)

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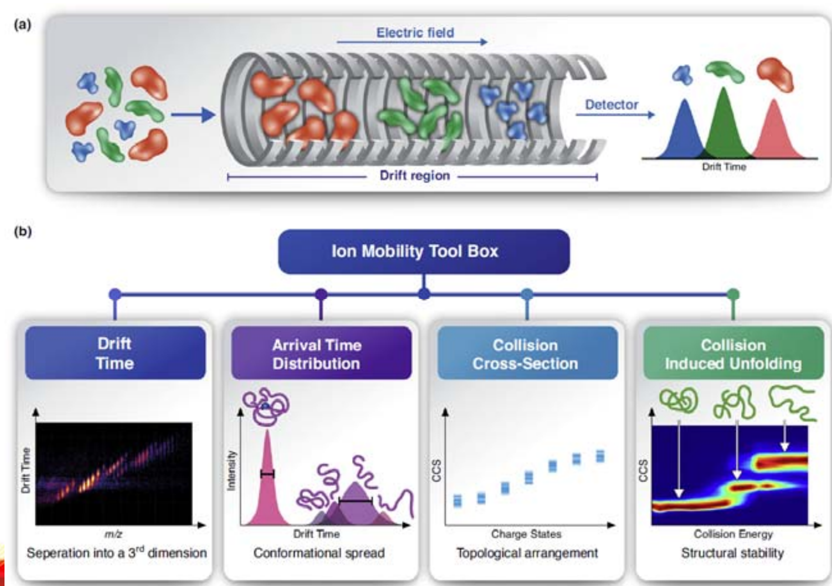
Ion Mobility Tool Box: structural characterization of proteins



Ben-Nissan and Sharon, Current Opinion in Chemical Biology **42**, 25-33 (2018)

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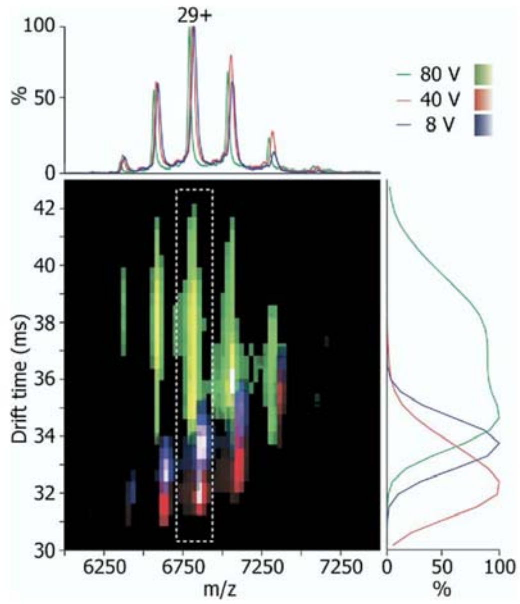
Collision Induced Unfolding

AIM =
conformational stability
Protein unfolding, organization

- 8V minimum voltage for ion transmission
  - native MS
- 40V
  - MS: removal of salts and water (slightly lighter than blue)
  - IM: protein collapses
- 80V
  - MS: more removal water, salts
  - IM values larger = Unfolding of the protein complex



Justin Benesch, J.Am.Soc. Mass Spectrom **20,** 341-348 (2009)

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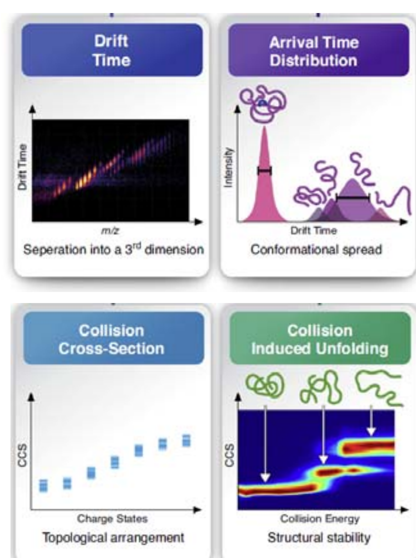
Summary

1) Background into IM-MS (what, history, fundamental principles)
2) Obtaining mobilities and CCS values
   = for drift tube via calculations
   = for TWIMS (SLIM): via calibration
3) Instrumentation, types of ion mobility
   = DTIMS, TWIMS, SLIM, FAIMS, etc
4) Different experiments
   = drift time
   = arrival time distribution
   = collisional cross section
   = collisional induced unfolding



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