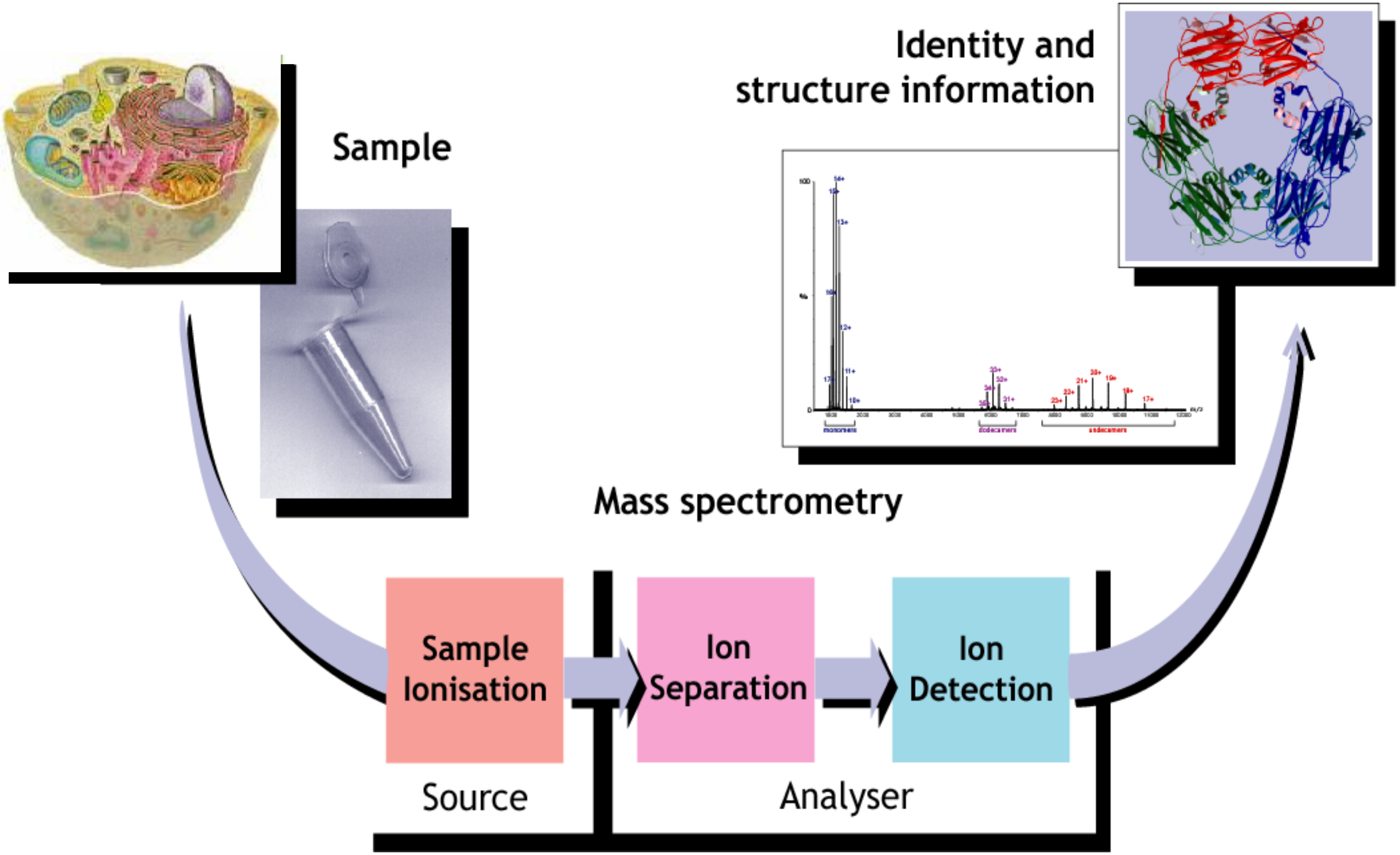
DEPARTMENT OF CHEMISTRY

GRADUATE COURSE IN MASS SPECTROMETRY: LECTURE 3

Collisions in vacuum: cooling, activating, and sizing ions

Professor Justin Benesch, 27th October 2016

Mass spectrometry overview



Sample

Identity and structure information

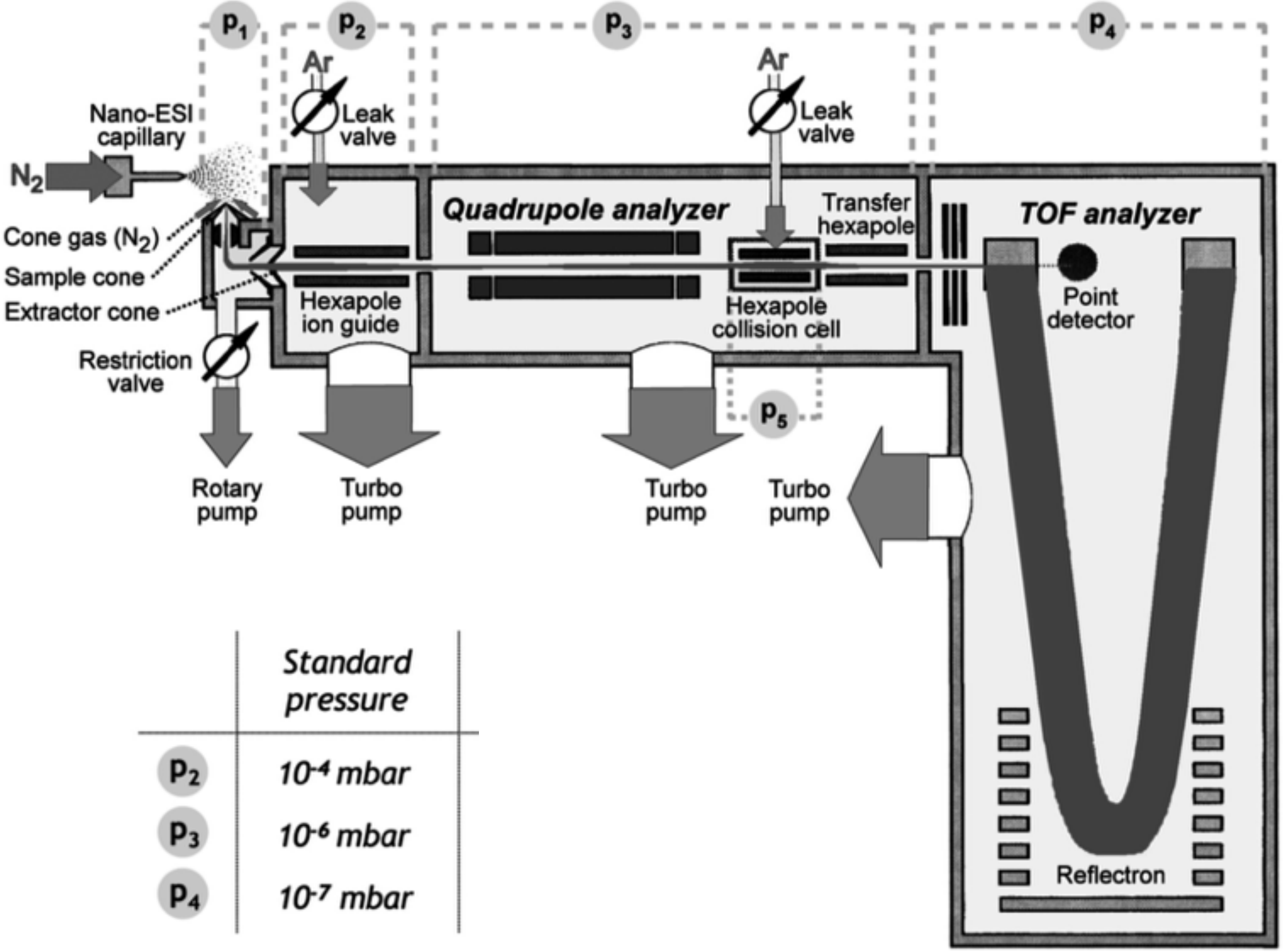
Mass spectrometry

Sample Ionisation → **Ion Separation** → **Ion Detection**

Source

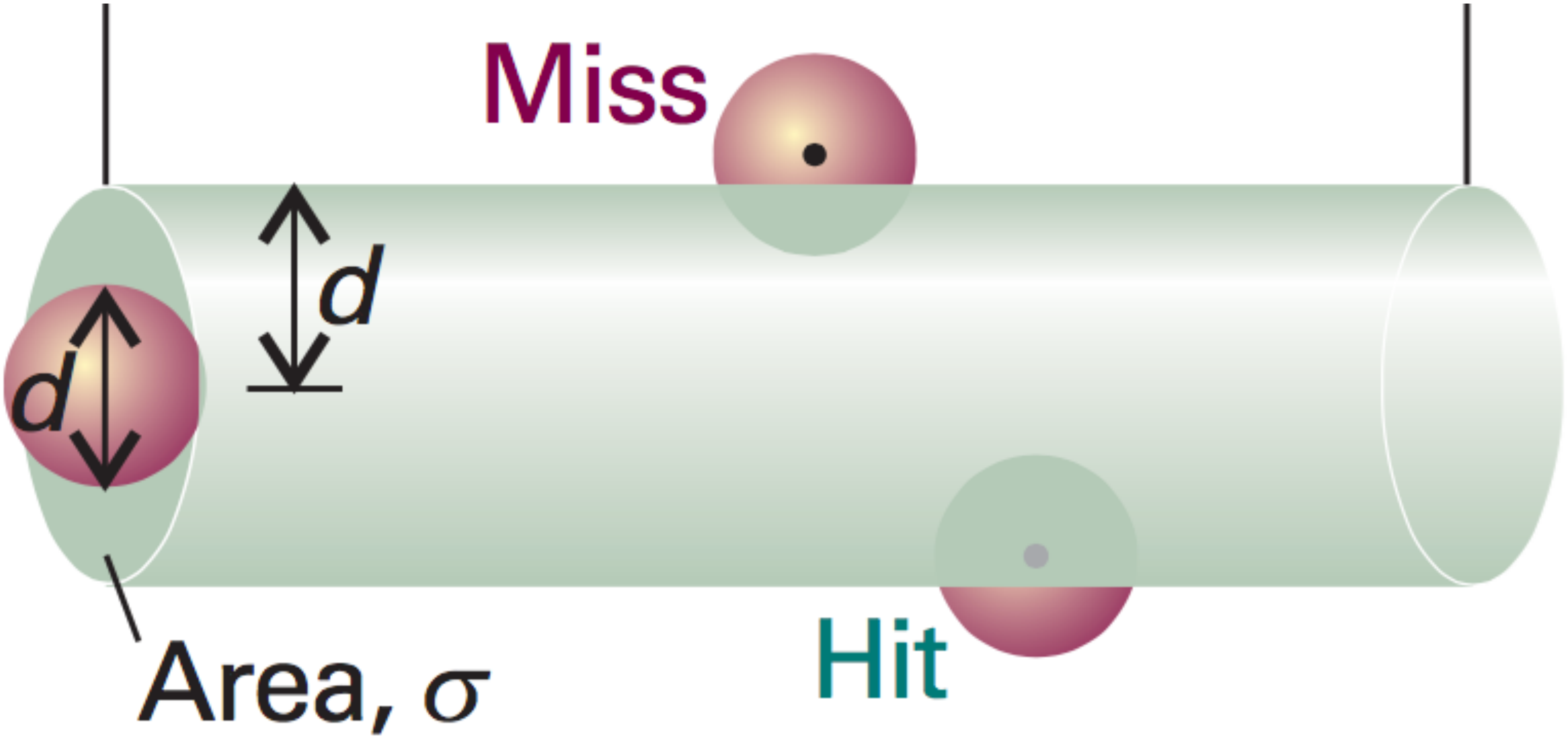
Analyser

From atmosphere to high vacuum



	Standard pressure
p_2	*10^{-4} mbar*
p_3	*10^{-6} mbar*
p_4	*10^{-7} mbar*

Origin of collisions



Mean free path and collision cross-section

$$\lambda = \frac{RT}{\sqrt{2}\,\sigma N_A p}$$

$$\sigma = \pi(R_{ion} + R_{gas})^2$$

- Hard-sphere model - classical mechanics view of collisions
- Mean free path: the average distance travelled by the ion between successive collisions

A more convenient expression

$$n = 102430(l\sigma p/T)$$

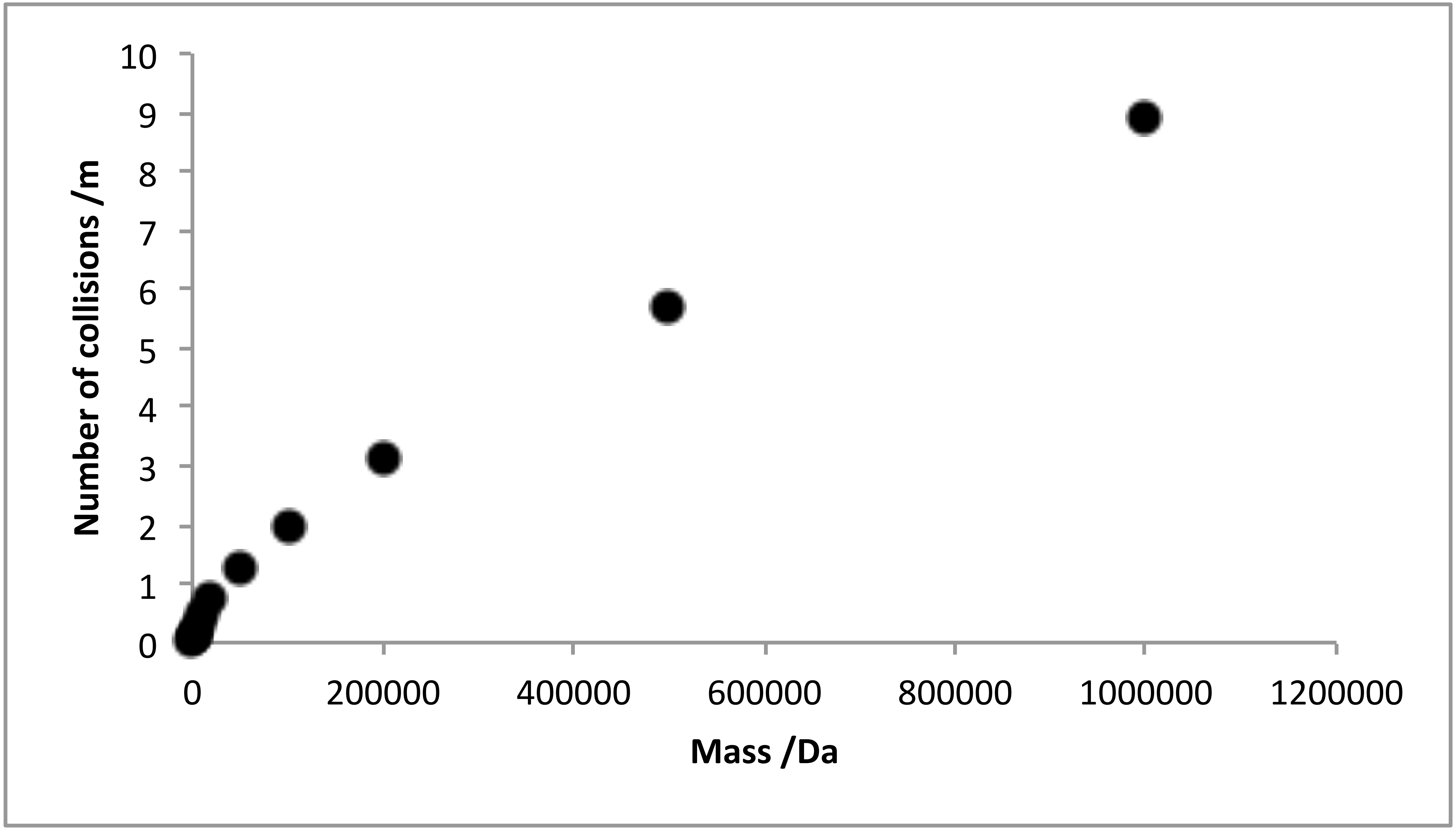
l is the length in m, σ the collision cross-section in Å^2, p is pressure in mbar, and T is temperature in K.

If nothing better:

$$\sigma = \pi(\sqrt[3]{3M_i/4\pi\rho} + R_g)^2$$

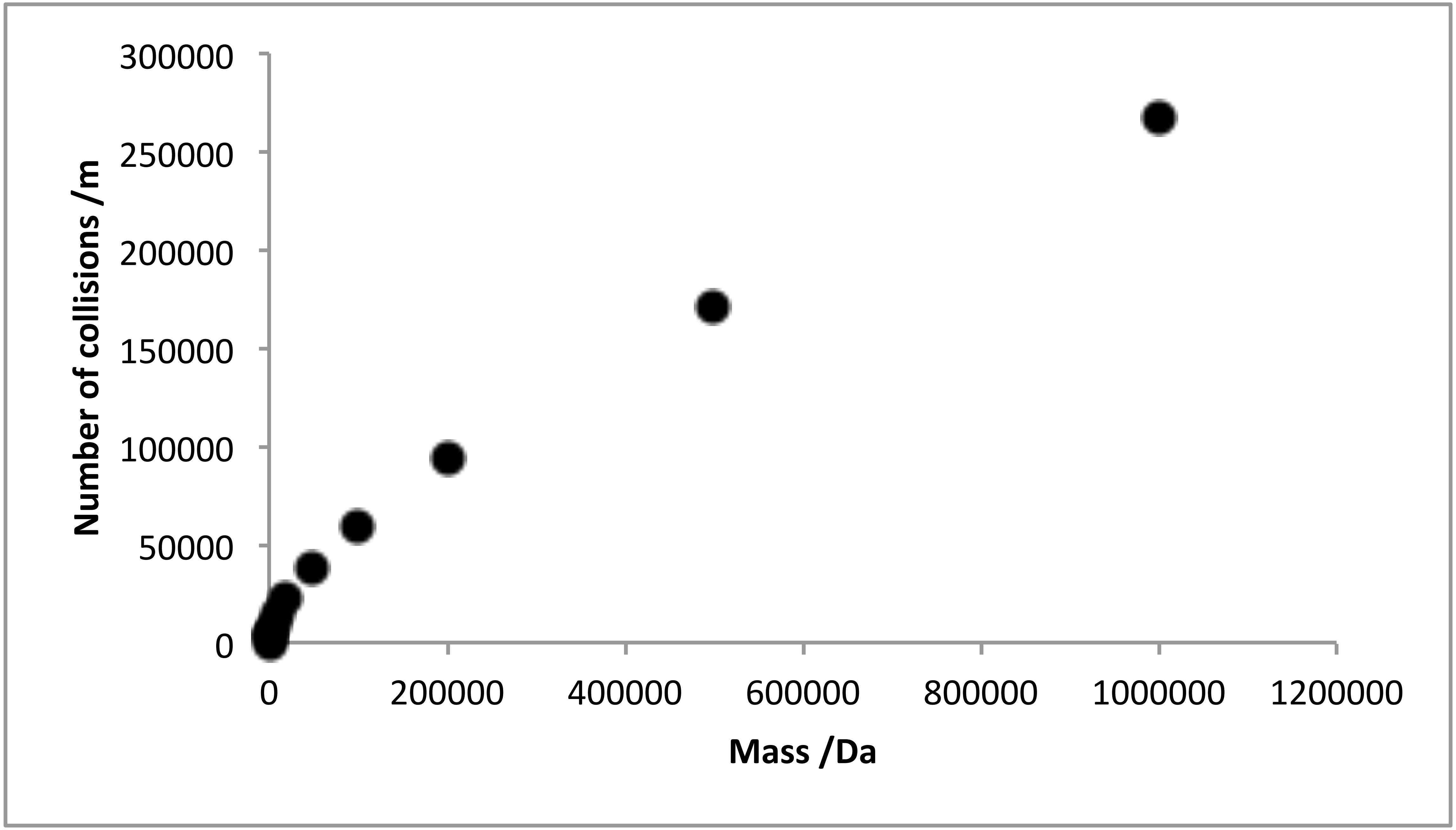
M_i is the mass of the ion, R_g is the radius of the gas, ρ is the density of the ion (for a protein, try 0.33 Da/ Å^3)

How many collisions are there?



- Calculation at typical ToF pressure - scattering

How many collisions are there?



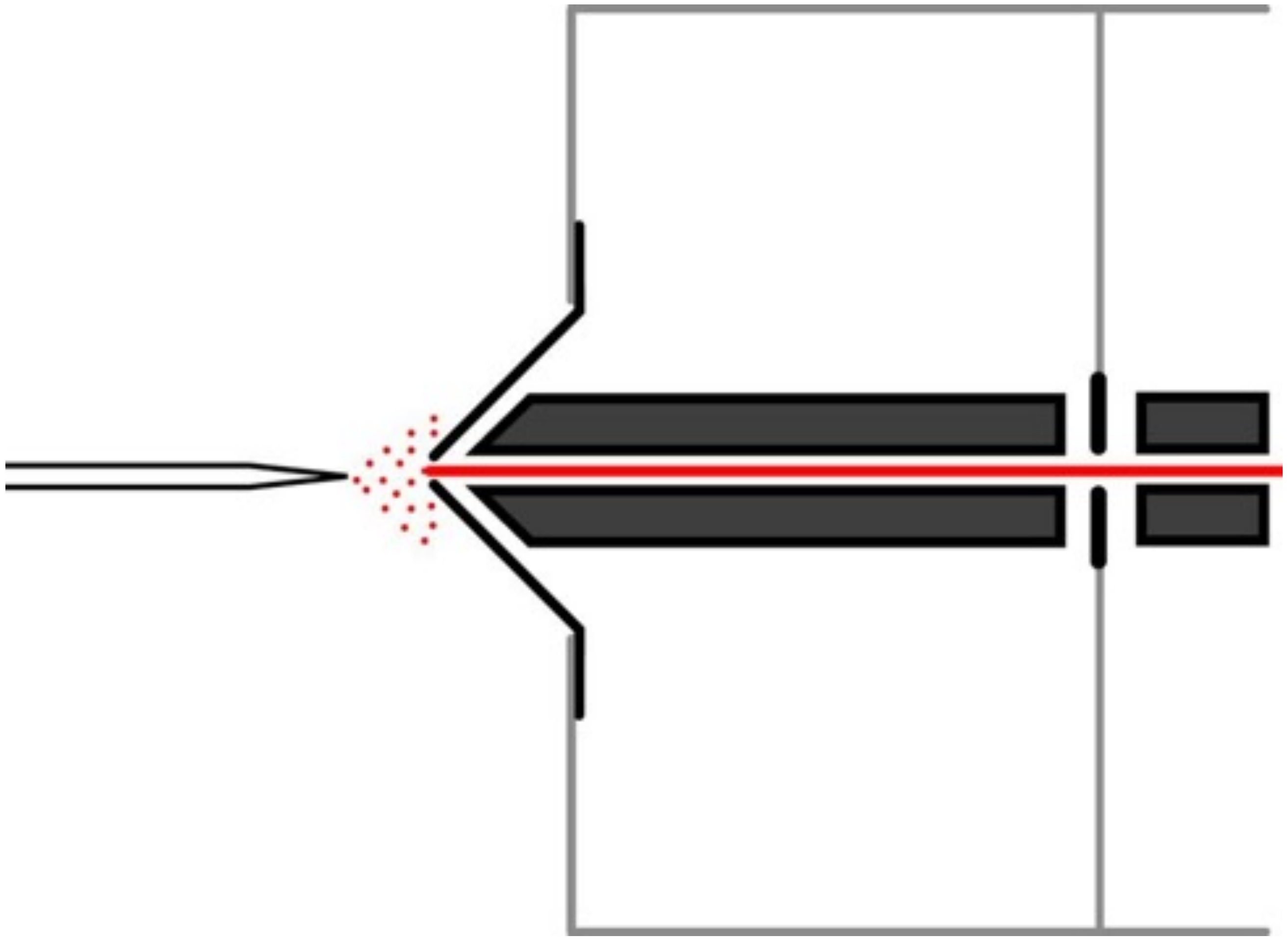
- Calculation at typical collision-cell pressure - can enable “chemistry”

Collisions are inevitable, and often intentional

- Scattering
- Collisional focussing and cooling
- Collisional activation
- Ion mobility spectrometry

Ion acceleration due to gas flow

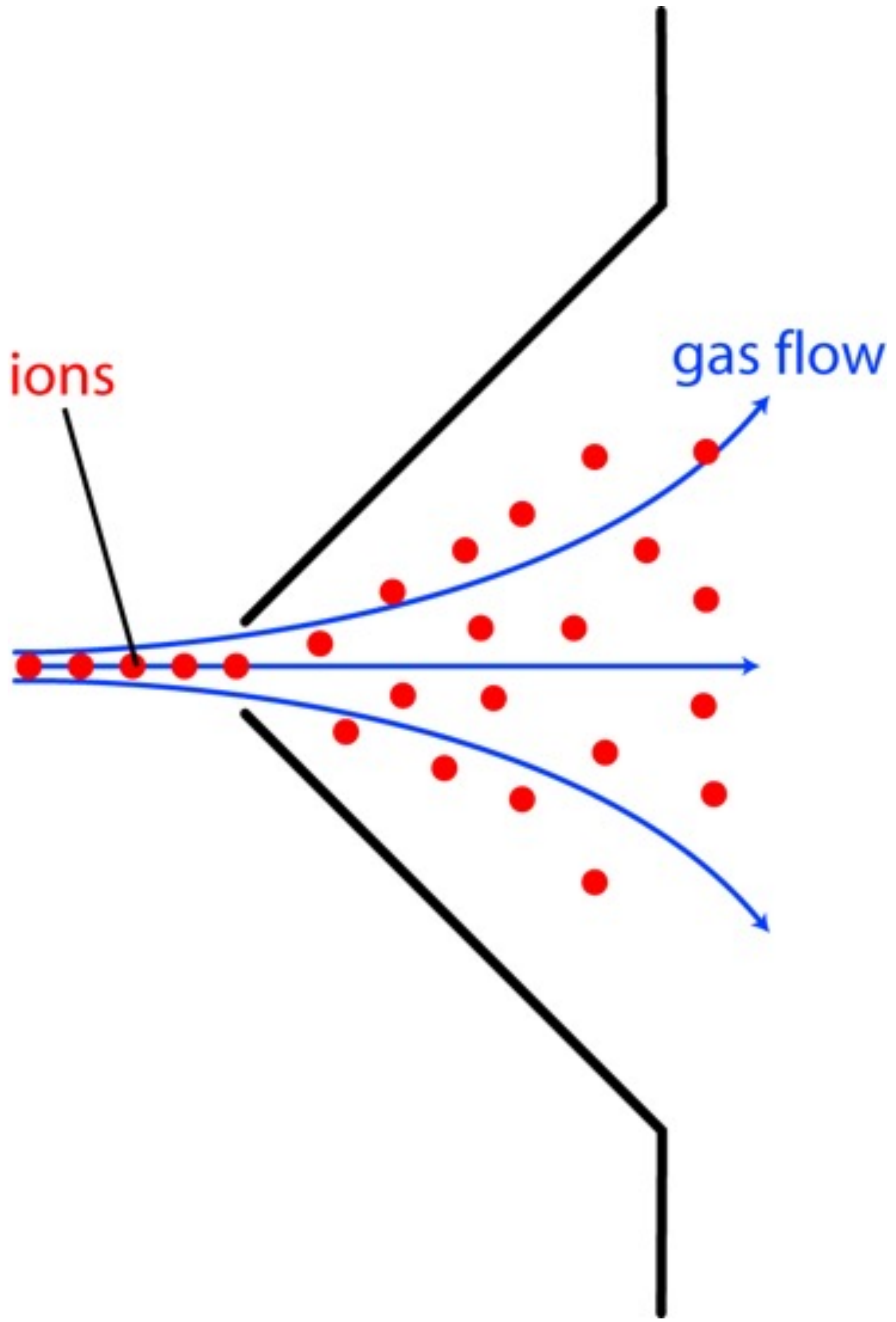
- Atmospheric pressure to rough vacuum
- Gas stream expands
- Ions accelerated to velocity of gas jet (~300m/s)
- 1MDa = ~ 1keV



Ion acceleration due to gas flow

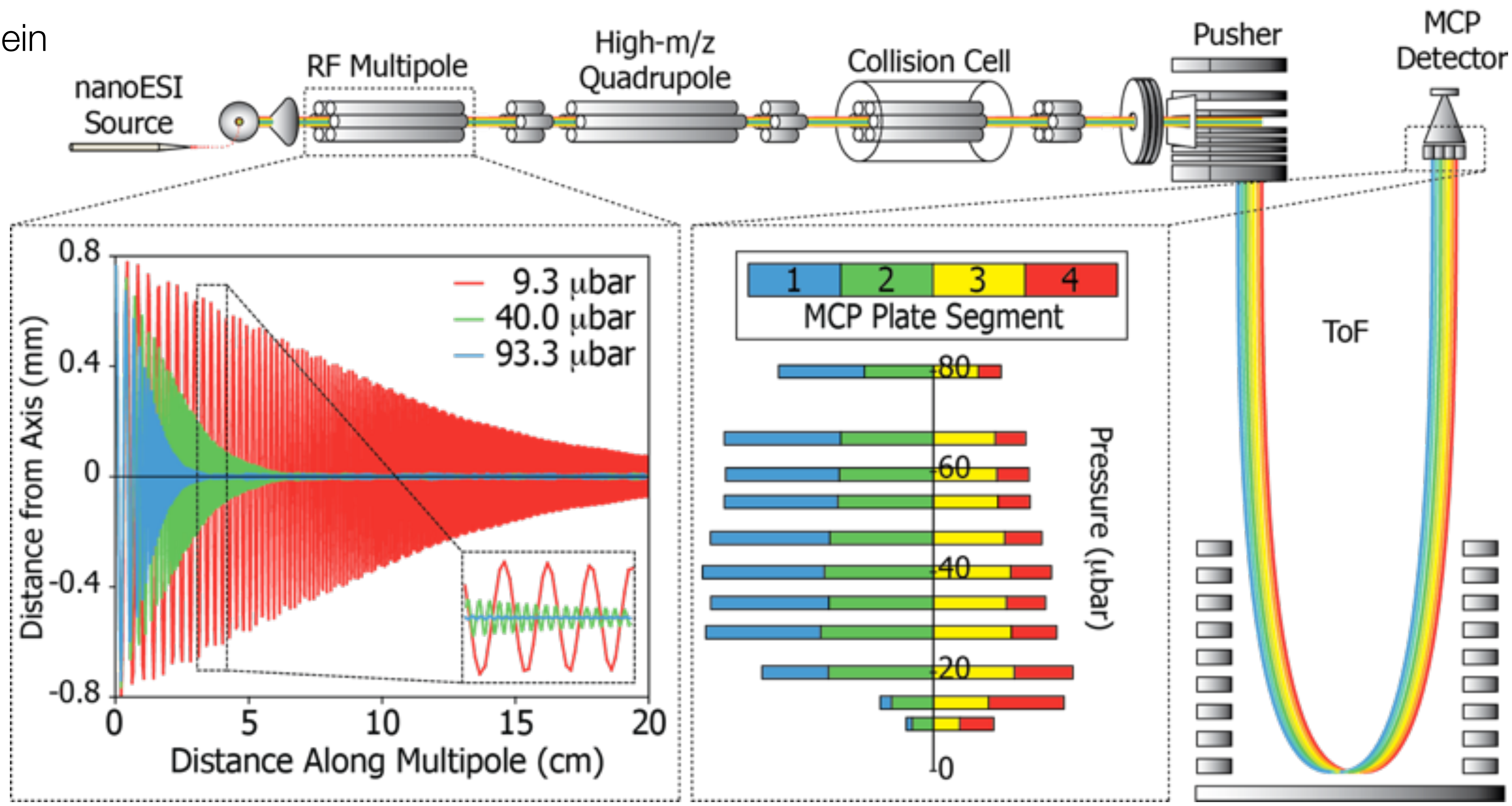
- ☐ Ions diverge due to gas expansion and Coulombic repulsion

- ☐ Focussing of large ions can is difficult due to high kinetic energies and low charge states



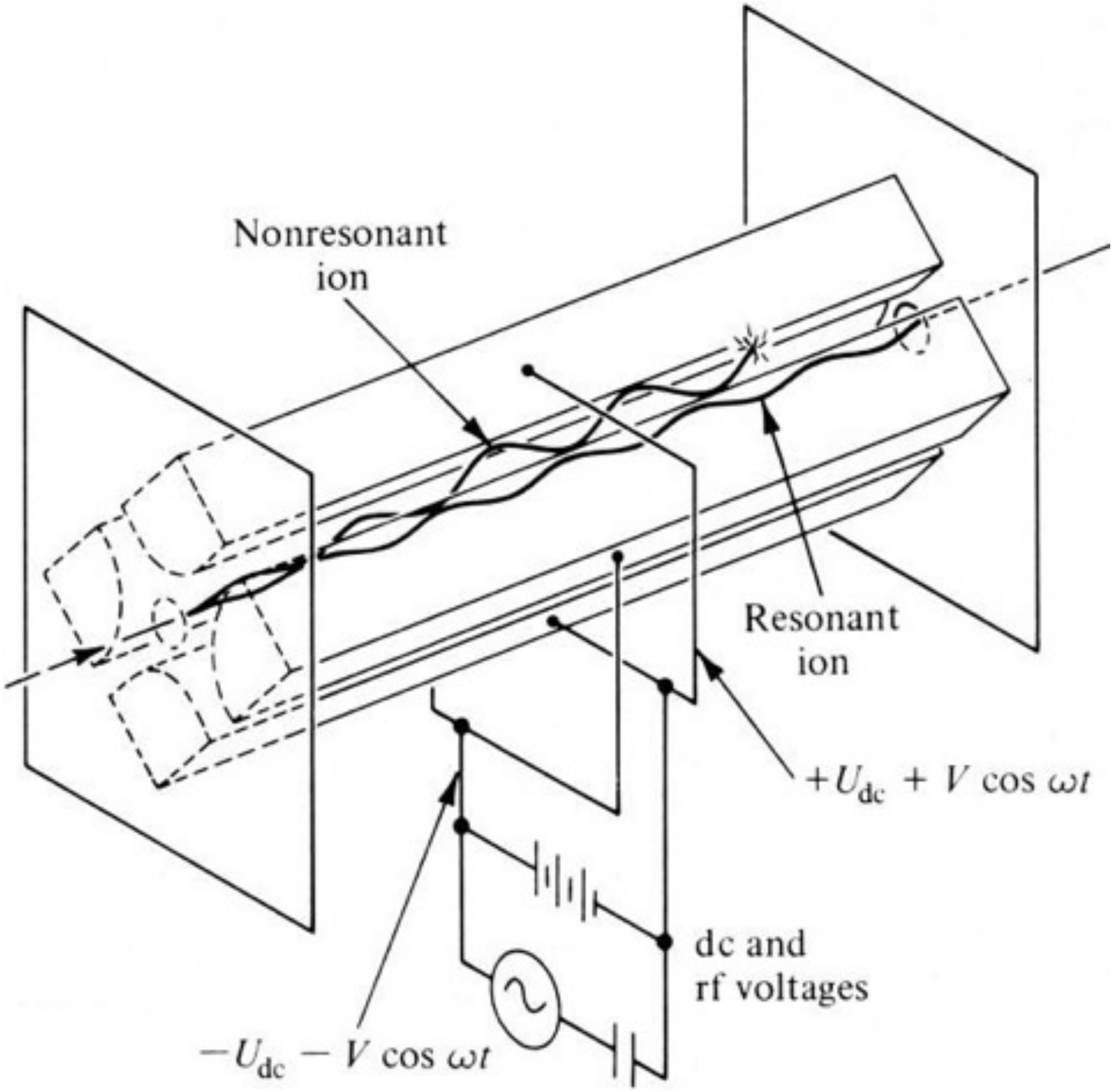
Collisional focussing

147 kDa Protein
Assembly



- Both axial and radial components of the ions’ velocity can be dampened by collisions with background gas

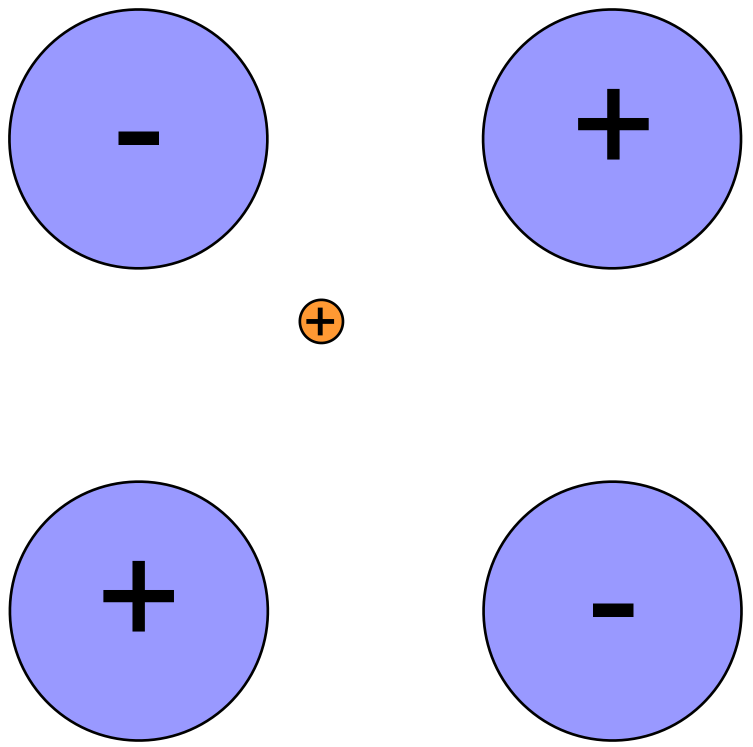
RF-only multipolar ion guides



- Operate multipole ion guide without the application of DC voltage (i.e. U=0)

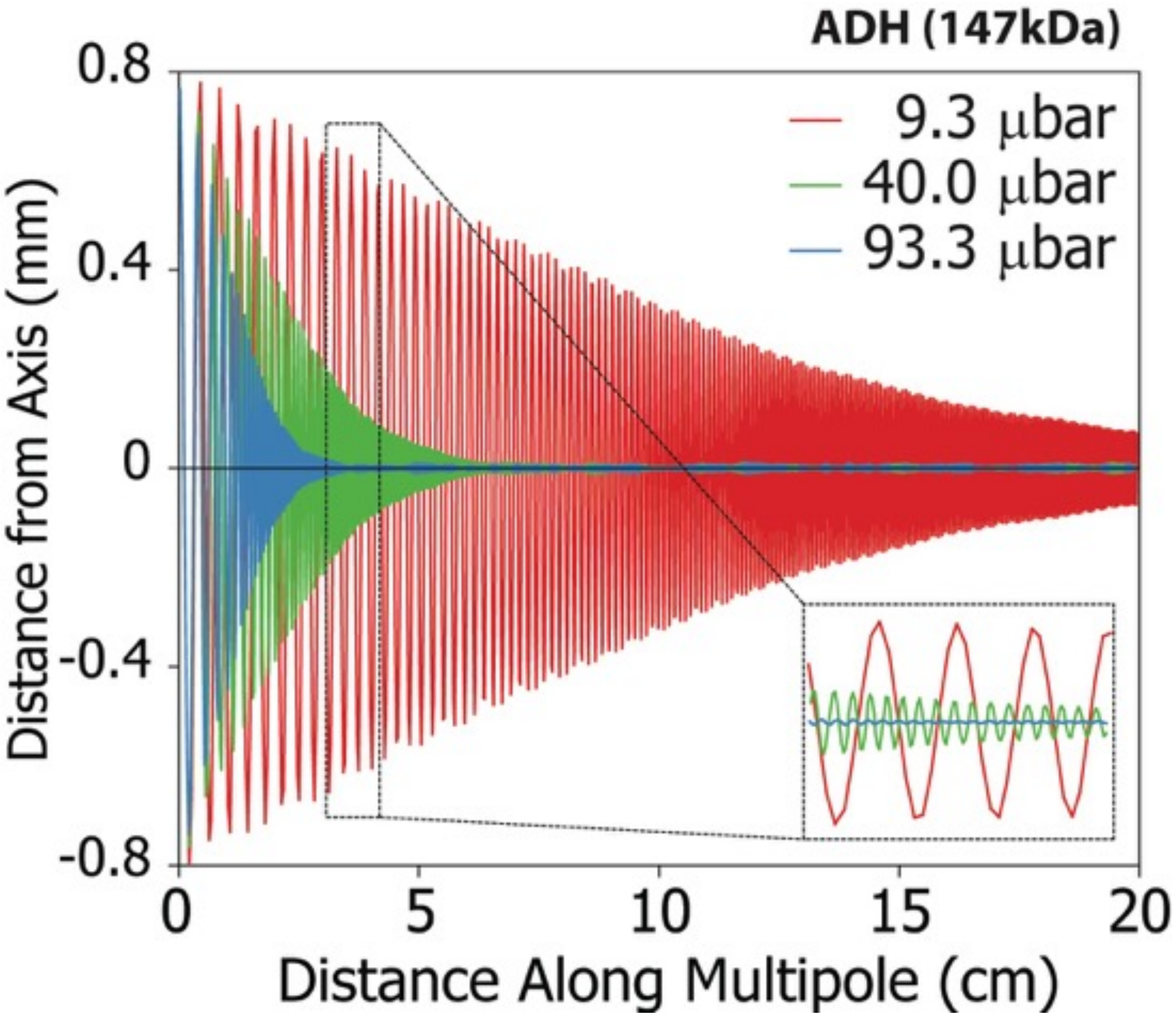
Focussing in multipole

- Focussing results in ions flying stably between the rods and through the apertures
- Focussing only works if the quadrupolar field is strong enough to overcome the momentum of the ion
- Could increase the field (RF amplitude) but increased chance of voltage breakdown
- Or, decelerating ions



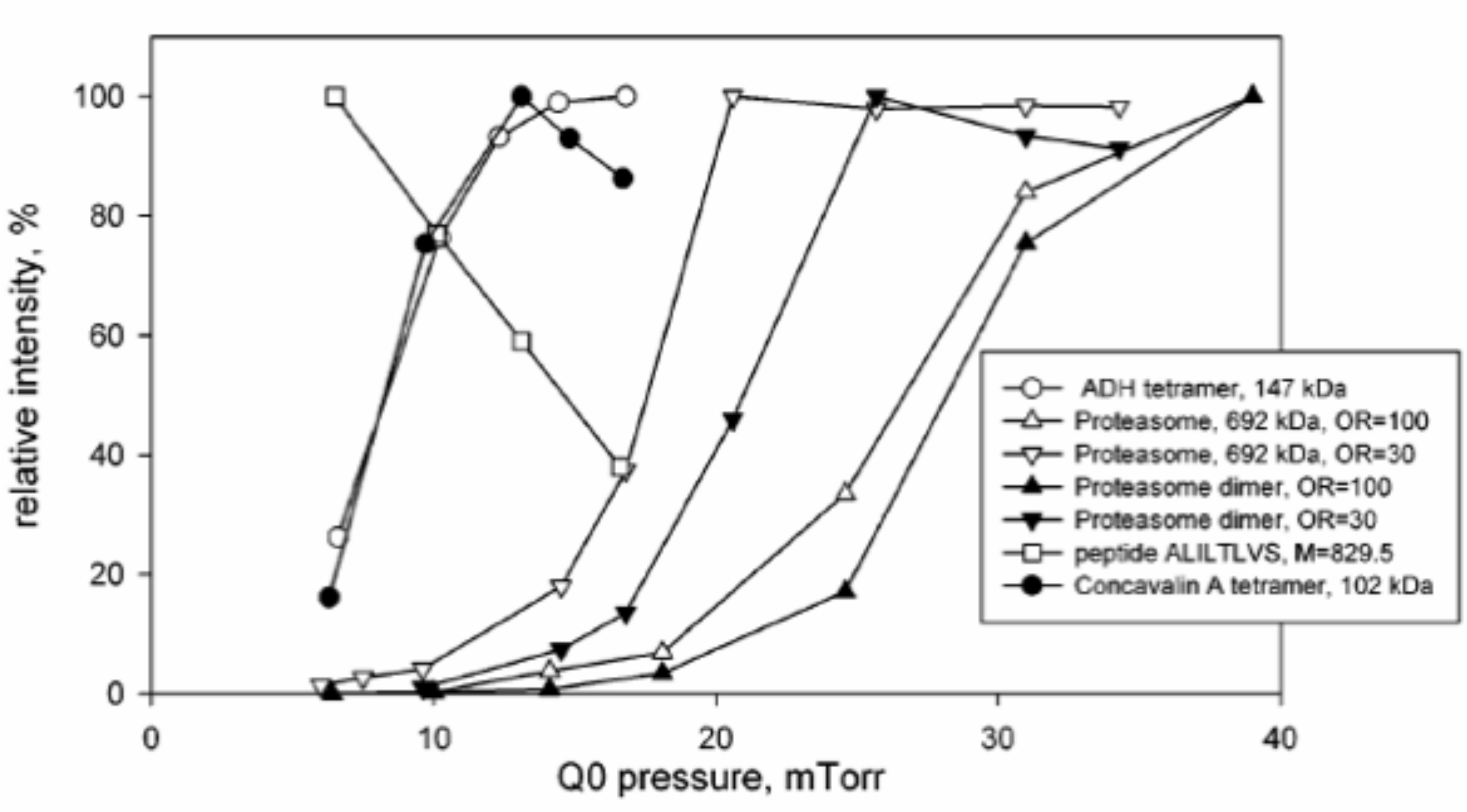
Collisional focussing

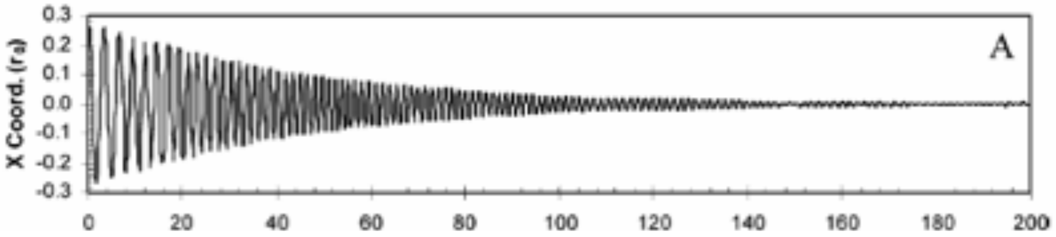
- Increase pressure in RF-only ion guide



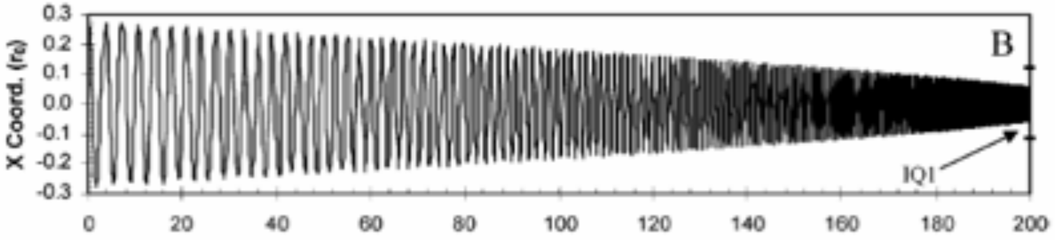
- If the pressure is too low, ions don't make it through the apertures
- Alternatives include increasing time spent in ion guide

Collisional focussing and scattering

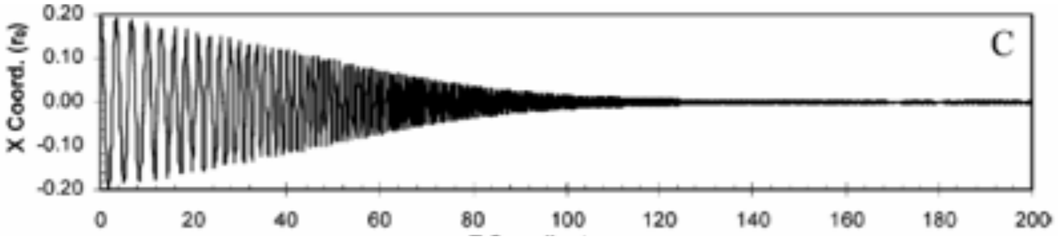




Myoglobin, 10 mBar



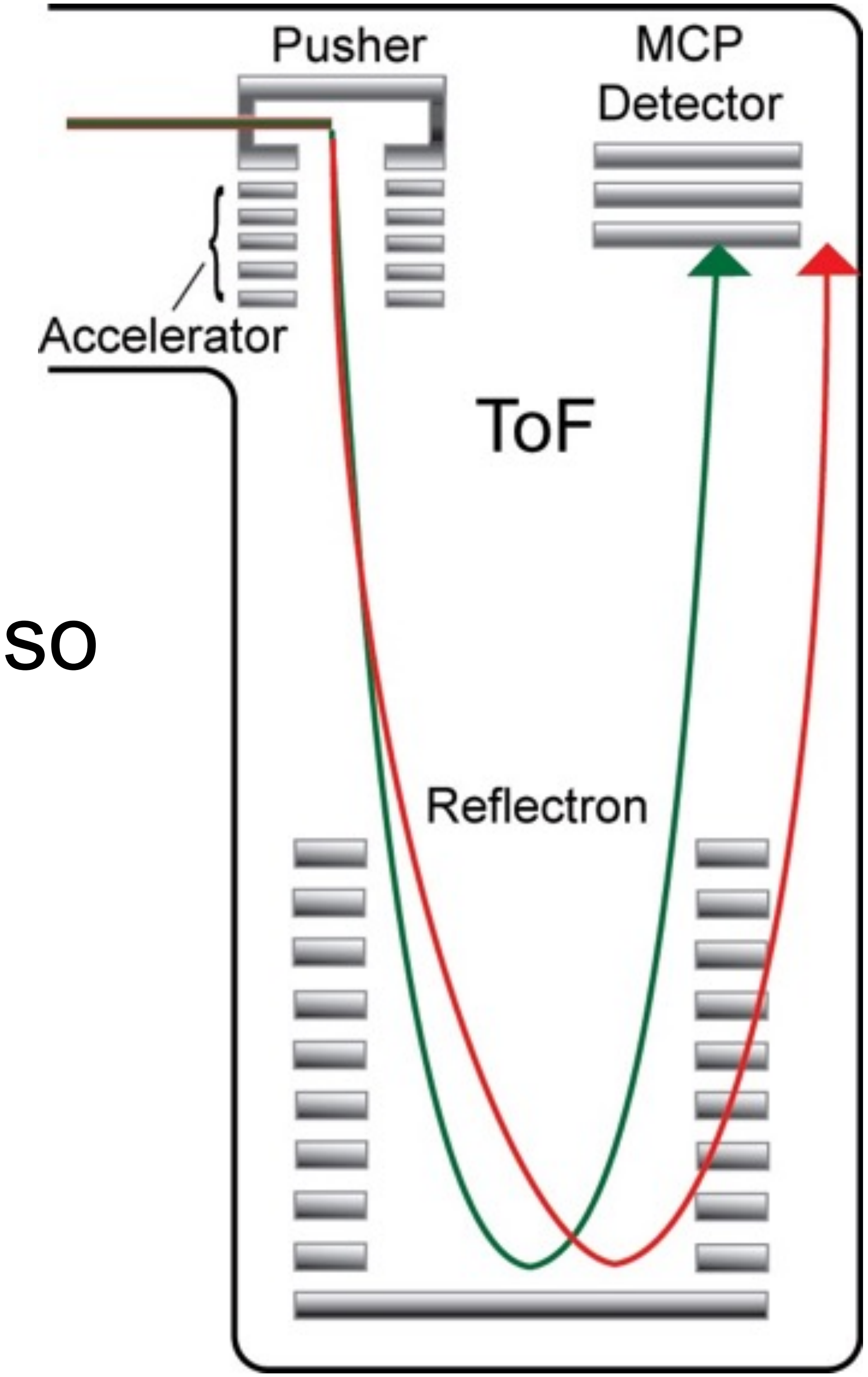
20S Proteosome, 10 mBar



20S Proteosome, 40 mBar

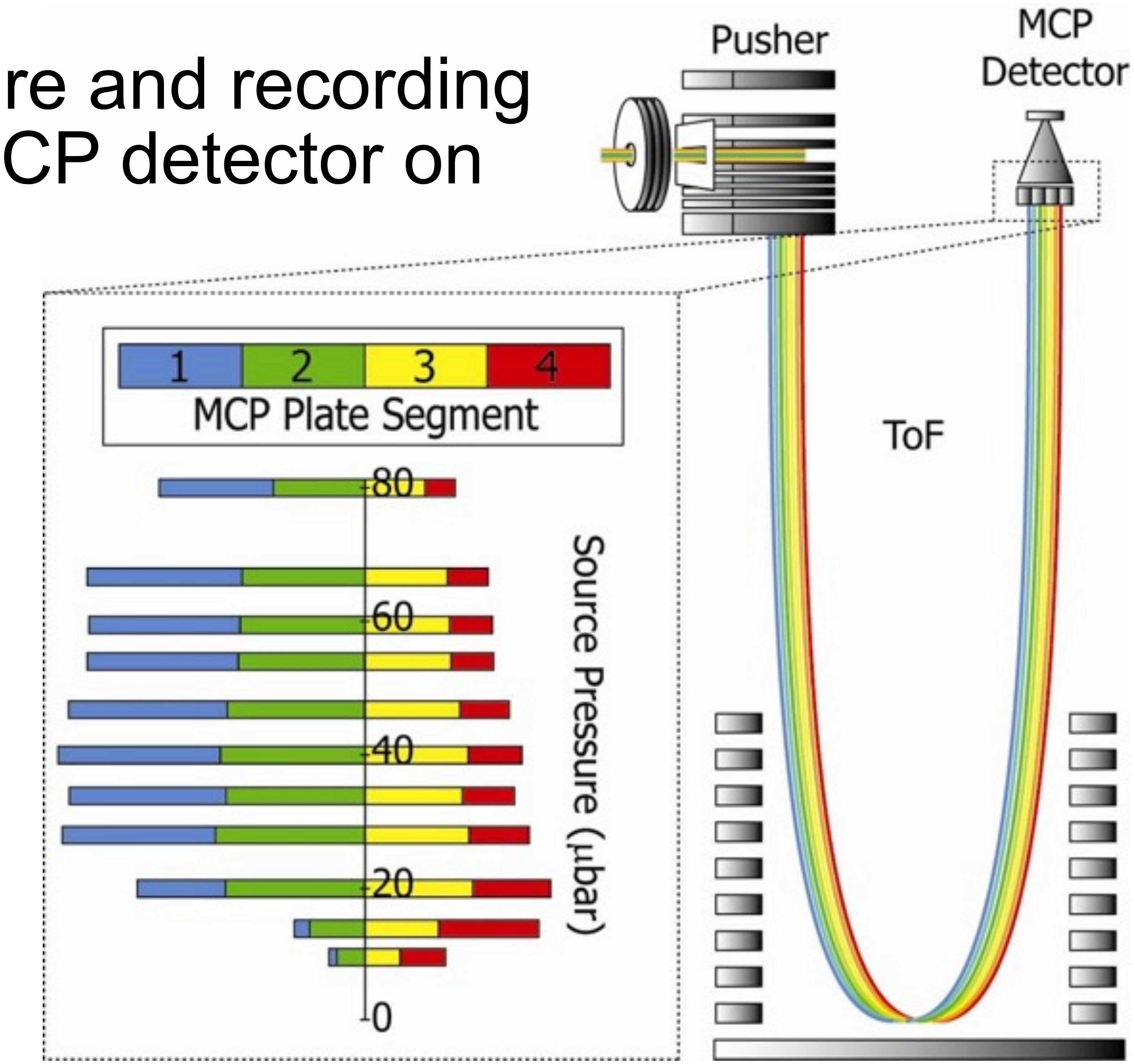
Focussing in ToF

- ☐ Excess velocity of ions can also incur ion losses in o-ToF



Focussing in ToF

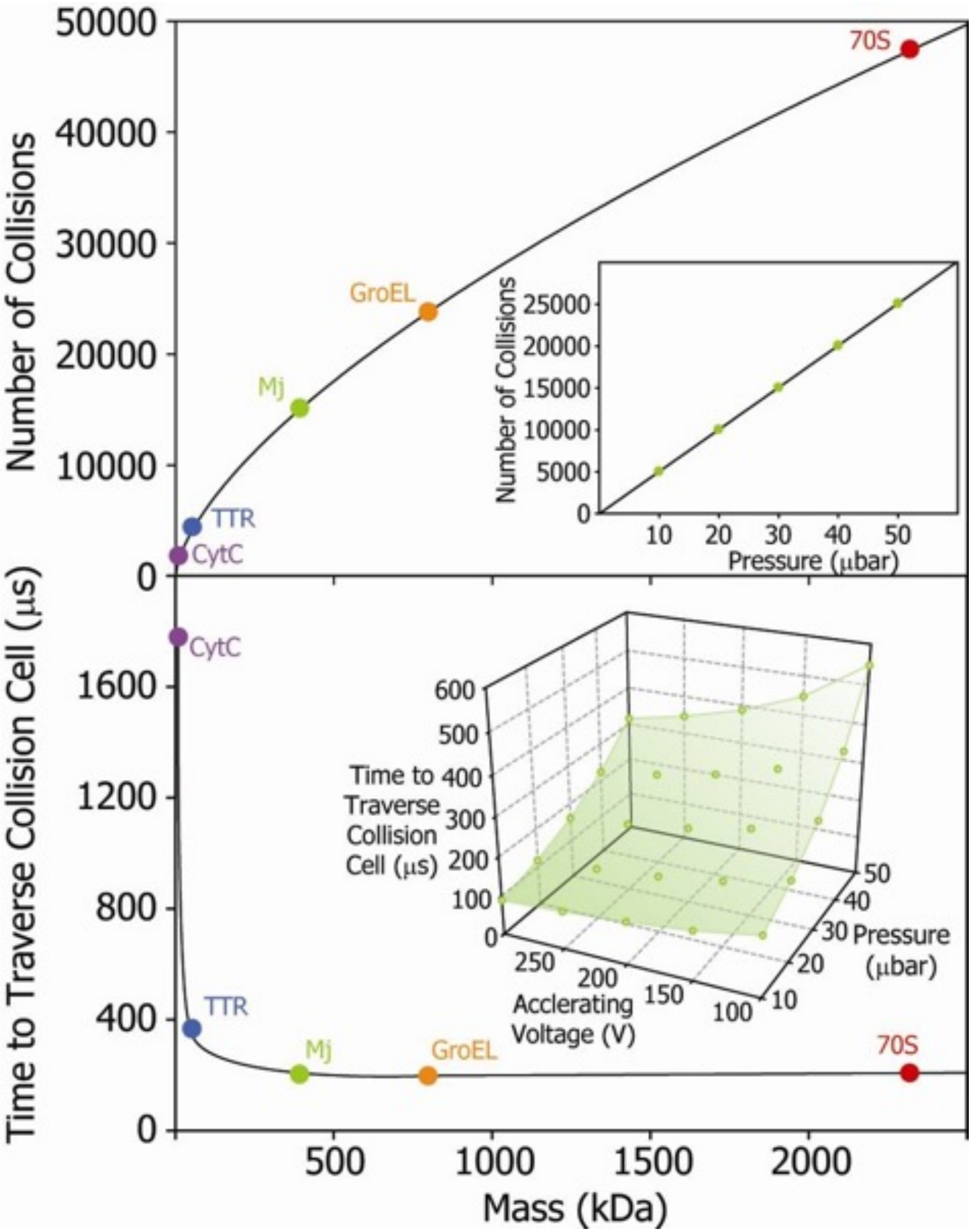
- ☐ Varying source pressure and recording signal across 4-part MCP detector on QStar



- ☐ Total signal reaches a maximum then decreases
- ☐ Signal moves across MCP
- ☐ Over/undershooting detector
- ☐ Ideal pressure

Collisional activation

- QToF Ultima collision cell, 200V acceleration, 30 μbar Ar in cell
- Activation takes place on the μs timescale, and ions experience 100s- 10000s of collisions



Energy transfer

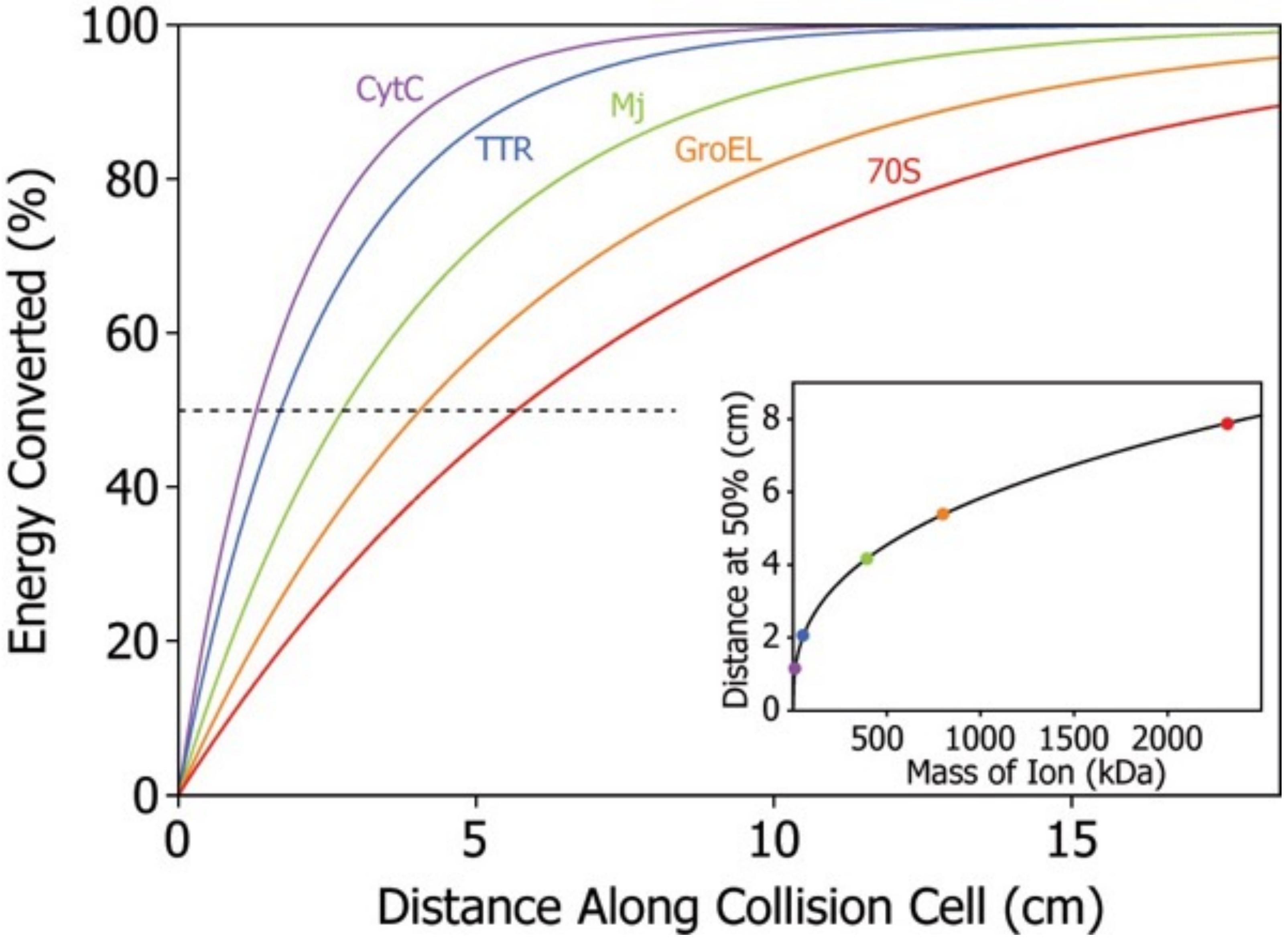
$$\Delta E_{Int} = zV_a\,(1 - [(M_i^2 + M_g^2)/(M_i+M_g)^2]^n)$$

M_i and M_g are the masses of the ion and gas respectively. z is the charge state of the ion, and V_a the accelerating voltage, and n the number of collisions

Energy accumulation

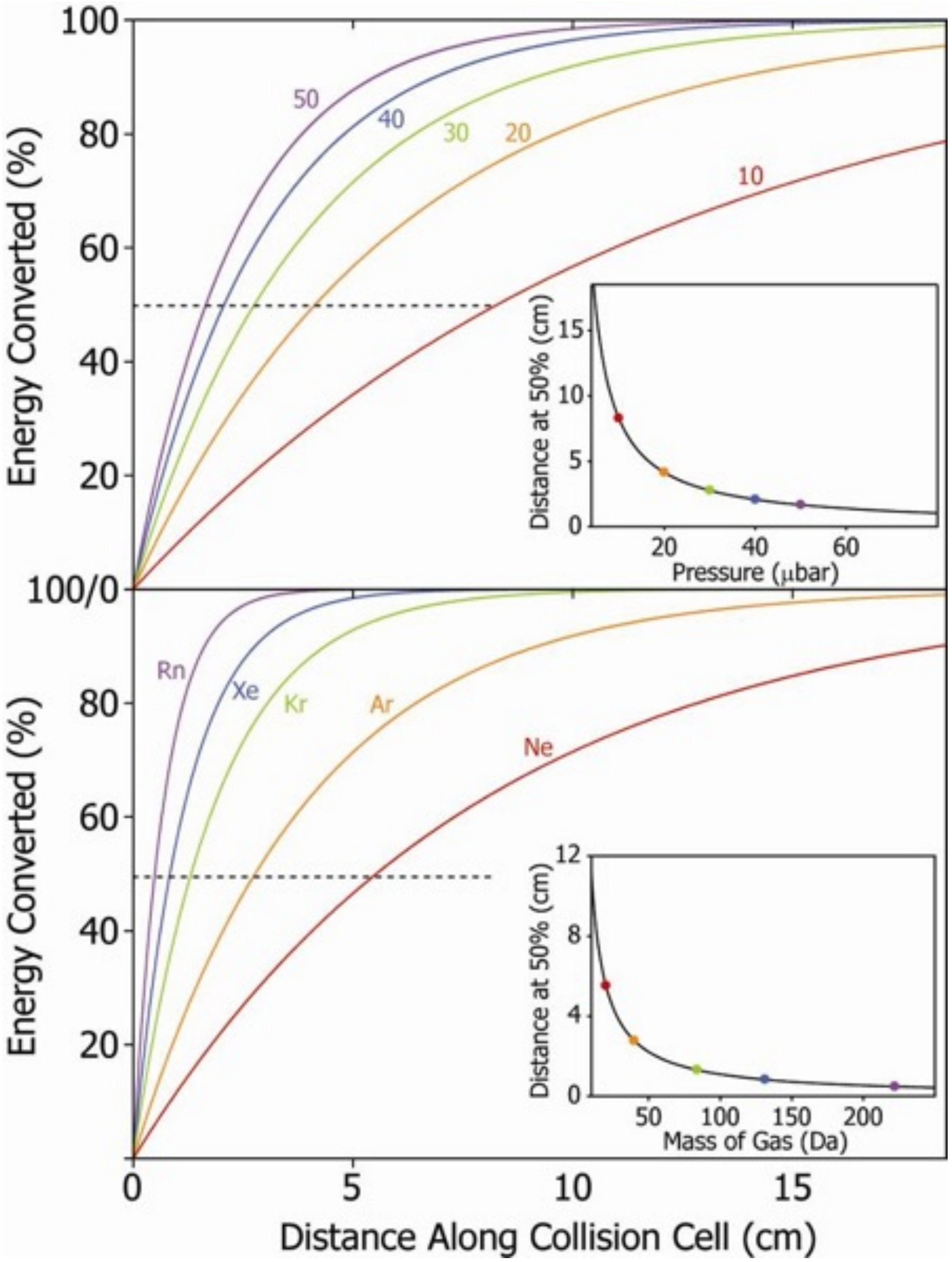
- QToF Ultima collision cell, 30μBar Ar in cell

- Conversion can be incomplete for very large species



Energy accumulation

- ☐ QToF Ultima collision cell, (390 kDa protein)
- ☐ More, and heavier gas preferable
- ☐ Diminishing returns



Why can ions stay intact?

- 10+ ion accelerated by 200 V = 2000 eV
- C-C bond approximately 3.6 eV
- Hydrogen bond approximately 0.25 eV

- Does not take into account internal vibrational redistribution of energy over degrees of freedom
- Vibrational modes = 3N-6
- Average number of atoms in amino acid residue = 16.2
- Rough calculation: N = 150*16 = 2400; 3N = 7200; So <0.3 eV/mode

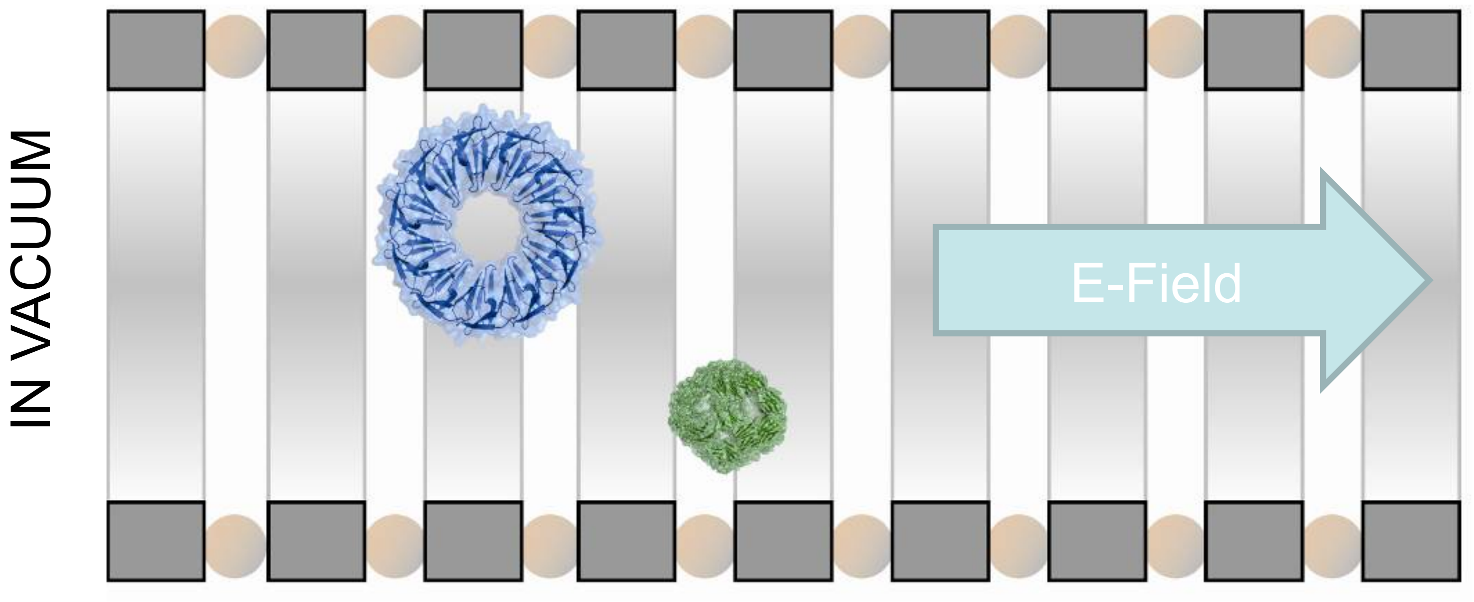
- RRKM theory

Ion mobility

The mobility (K) of an ion is its ability to traverse a region of gas under the influence of an electric field

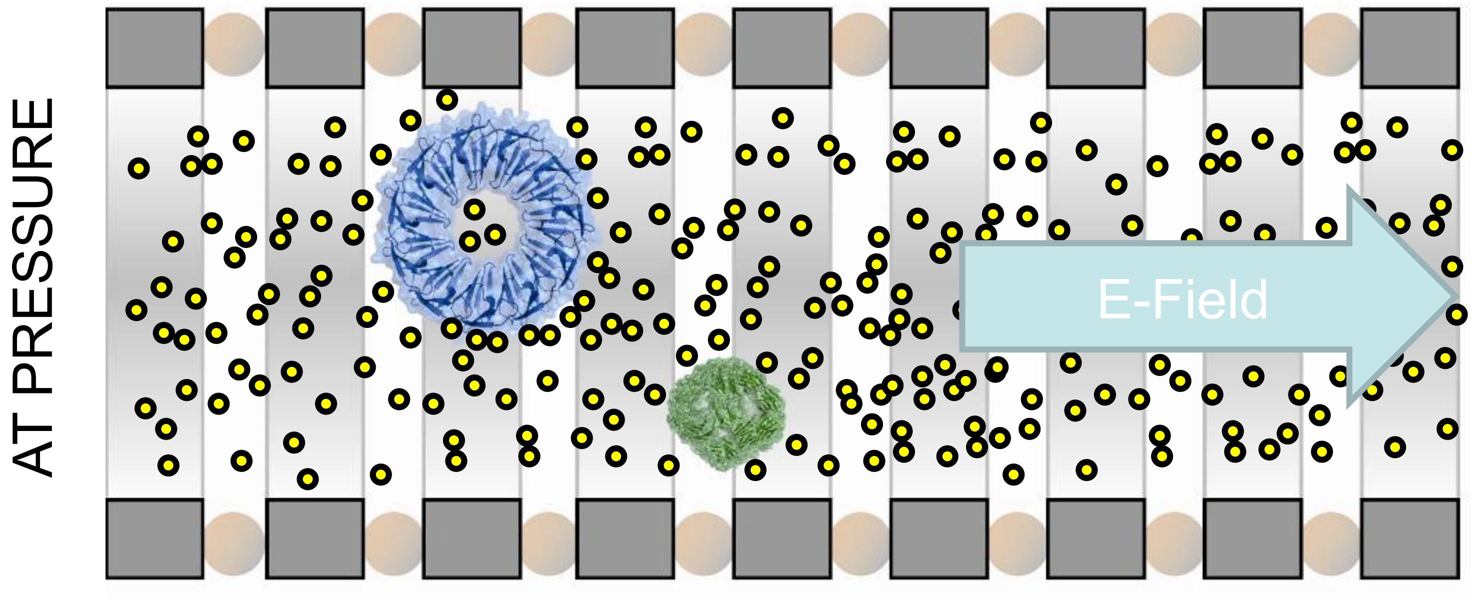
$$K = \frac{3}{16}\sqrt{\frac{2\pi}{\mu k T}}\frac{ze}{N\sigma}$$

Principles of IM separation



$$v = \sqrt{2zeEd/m}$$

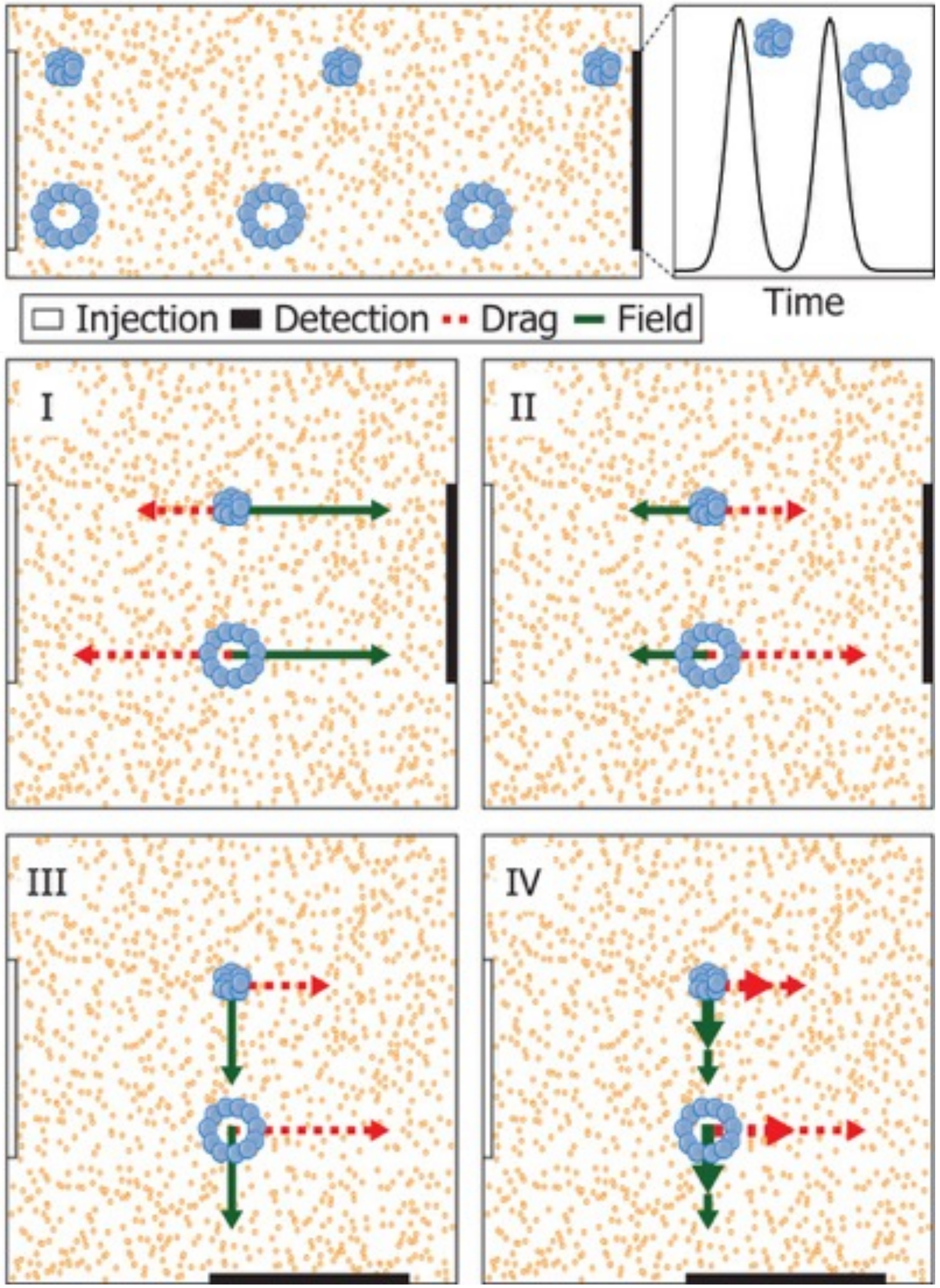
- Velocity depends on m/z



$$v = KE$$

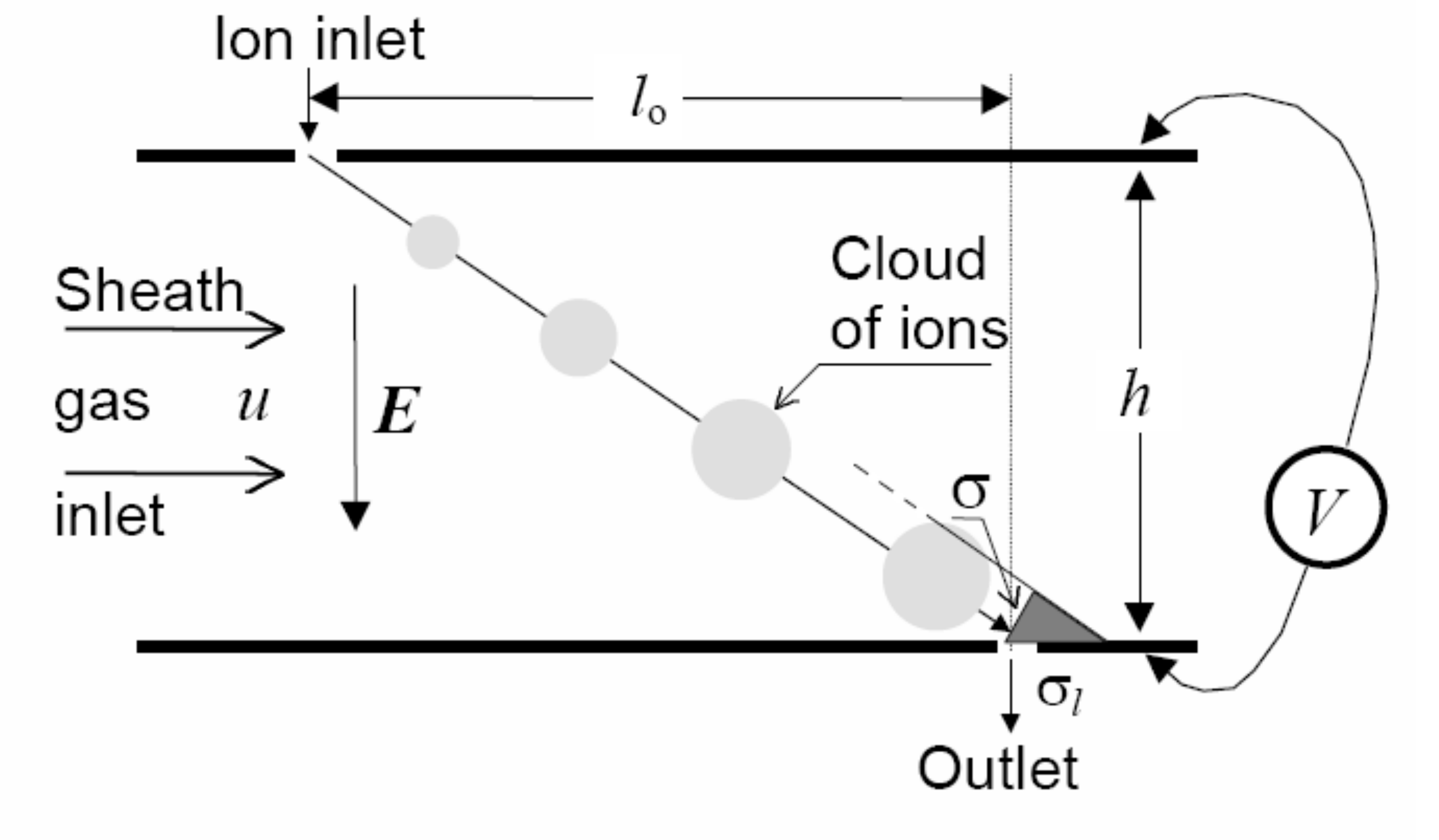
- Velocity depends on K

Different schemes for IM separation



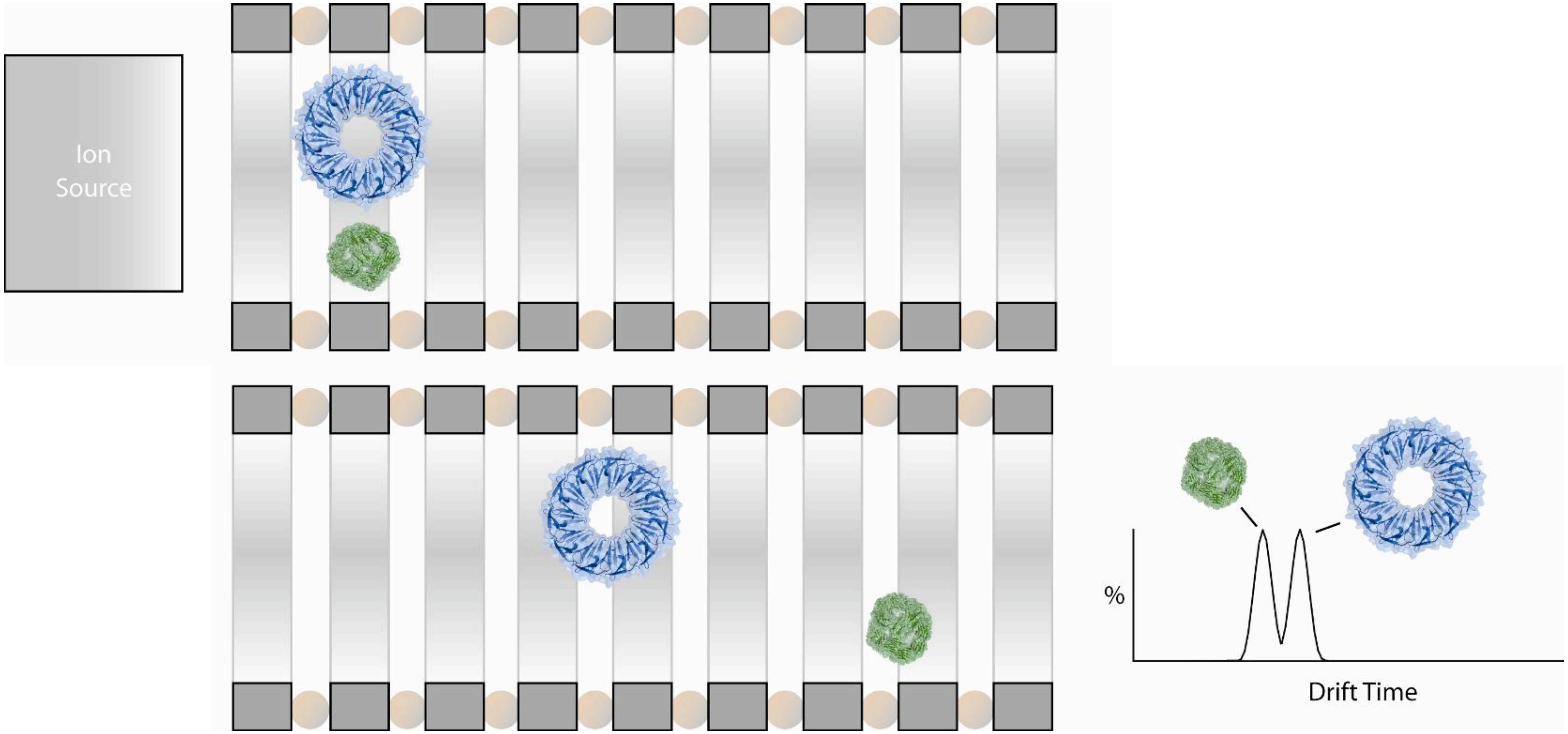
- IMS relies on opposition of effects of both the electric field and gas flow experienced by the ions
- Separation can be performed on axis, or off axis
- Electric field does not need to be constant

Some types of IM separation - DMA



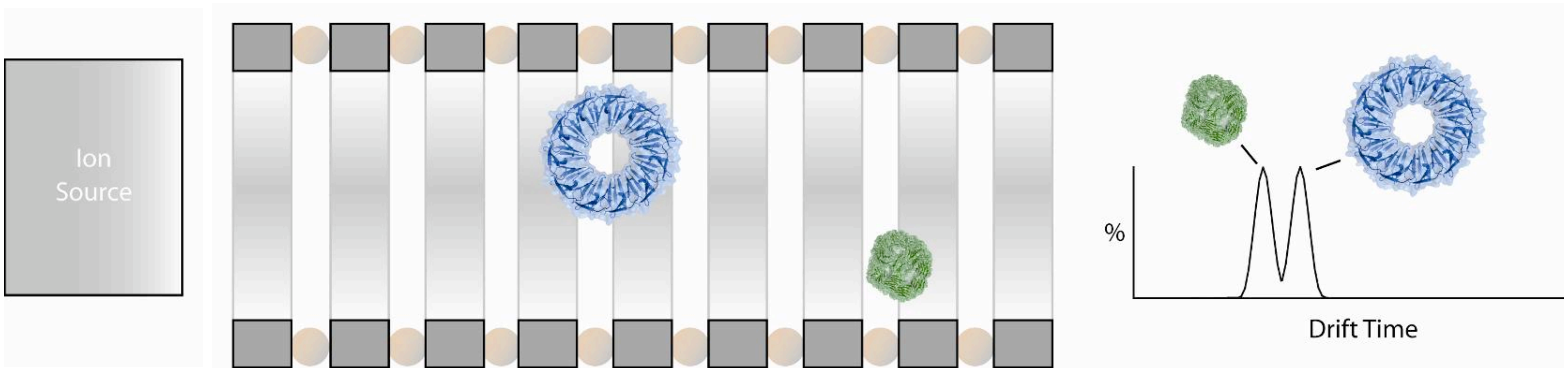
- "Differential mobility analyser" : can act as an ion mobility filter

Drift tube ion mobility spectrometry (DT-IMS)



- Separation of ions according to their ability to traverse a region of gas under the influence of a weak electric field
- Separation is based on ion ‘mobility’, unlike time-of-flight separation (mass)

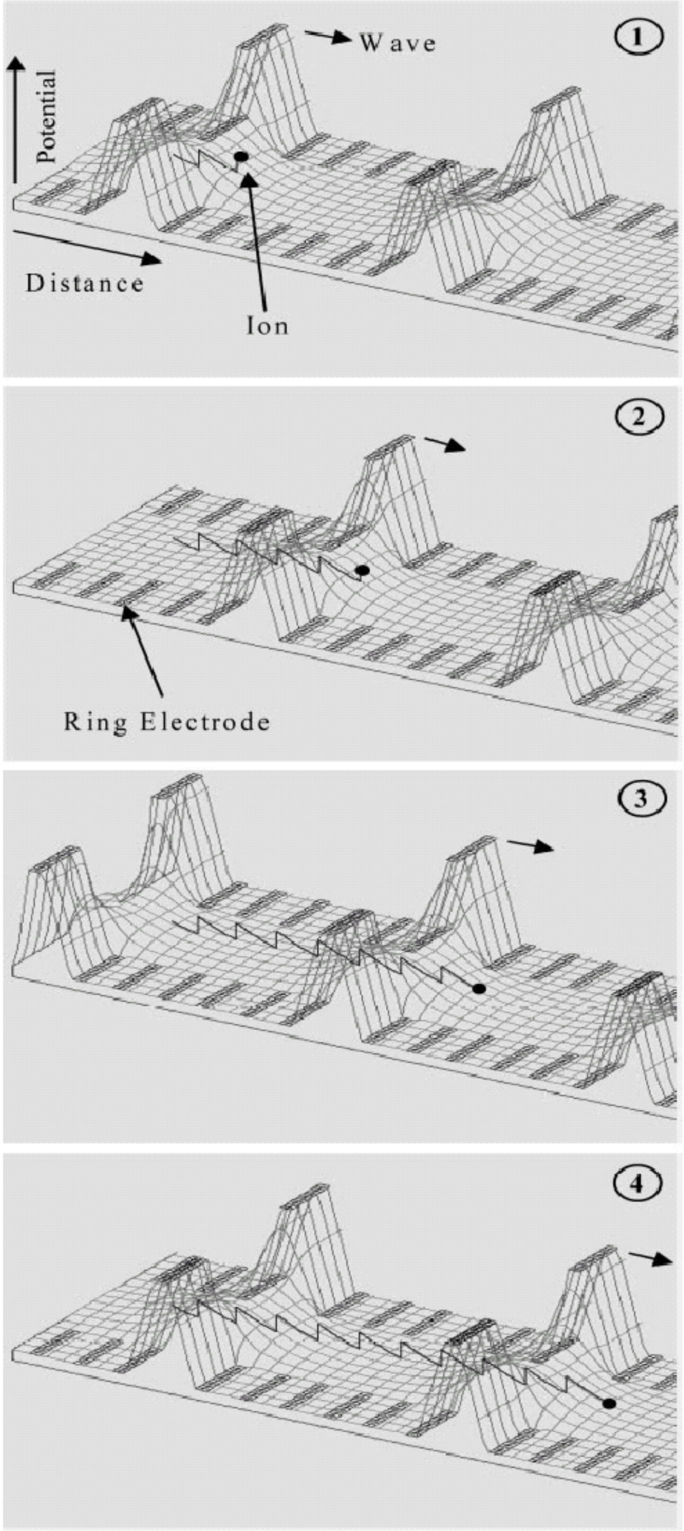
Some types of IM separation - Drift tube

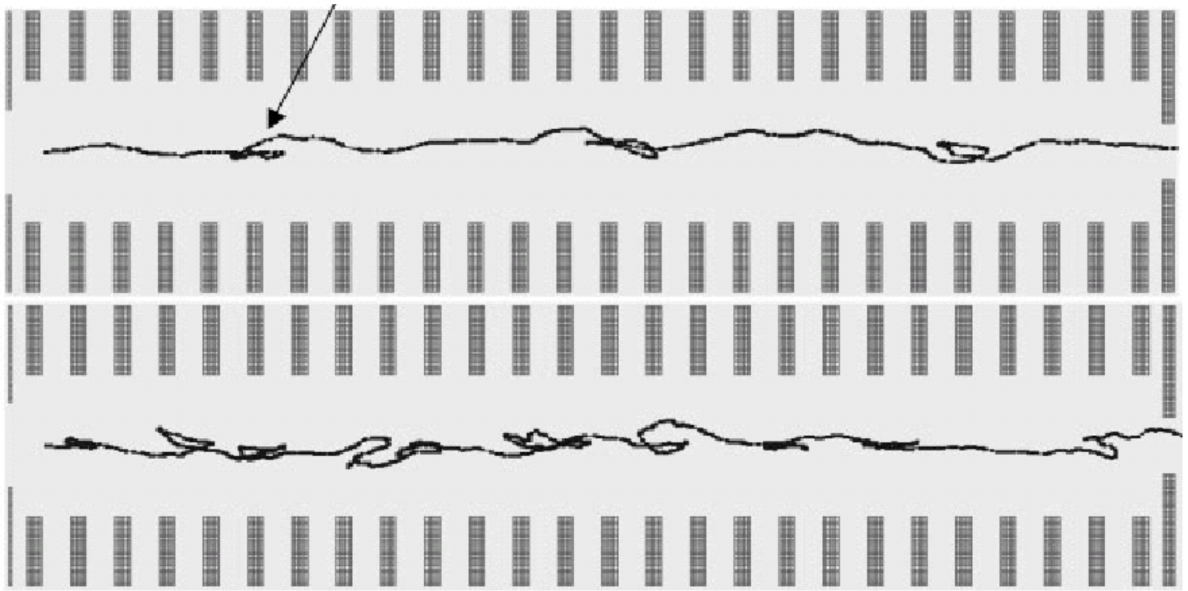


$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_b T)^{1/2}} \left[\frac{1}{m_I} + \frac{1}{m_N} \right]^{1/2} \frac{t_D E}{L} \frac{760}{P} \frac{T}{273.2} \frac{1}{N}$$

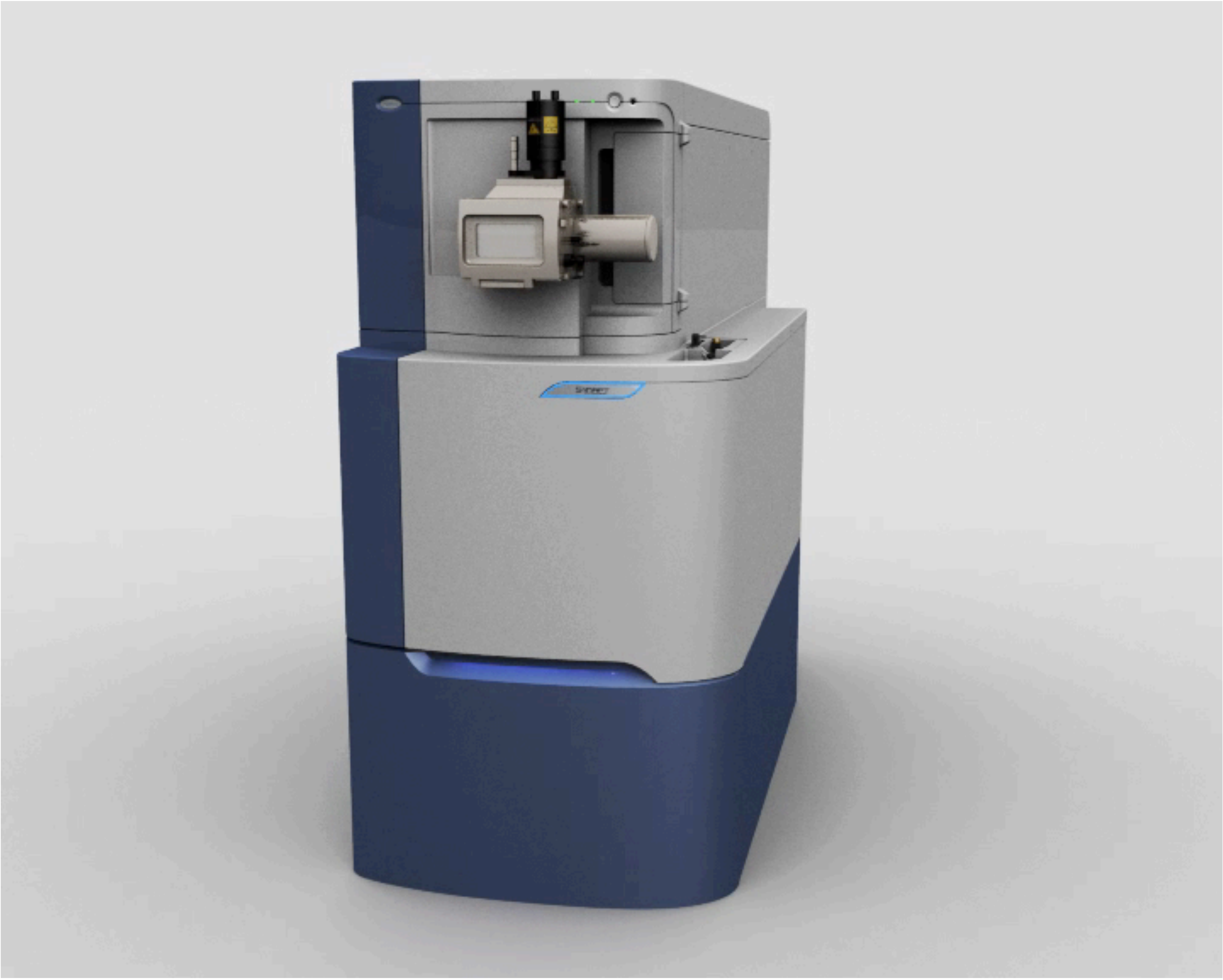
- Drift time is inversely proportional to charge
- Drift time is proportional to collision cross section (CCS, Ω)
- CCS depends on the radius of the gas, the ion, and their interaction

Some types of IM separation - Travelling wave

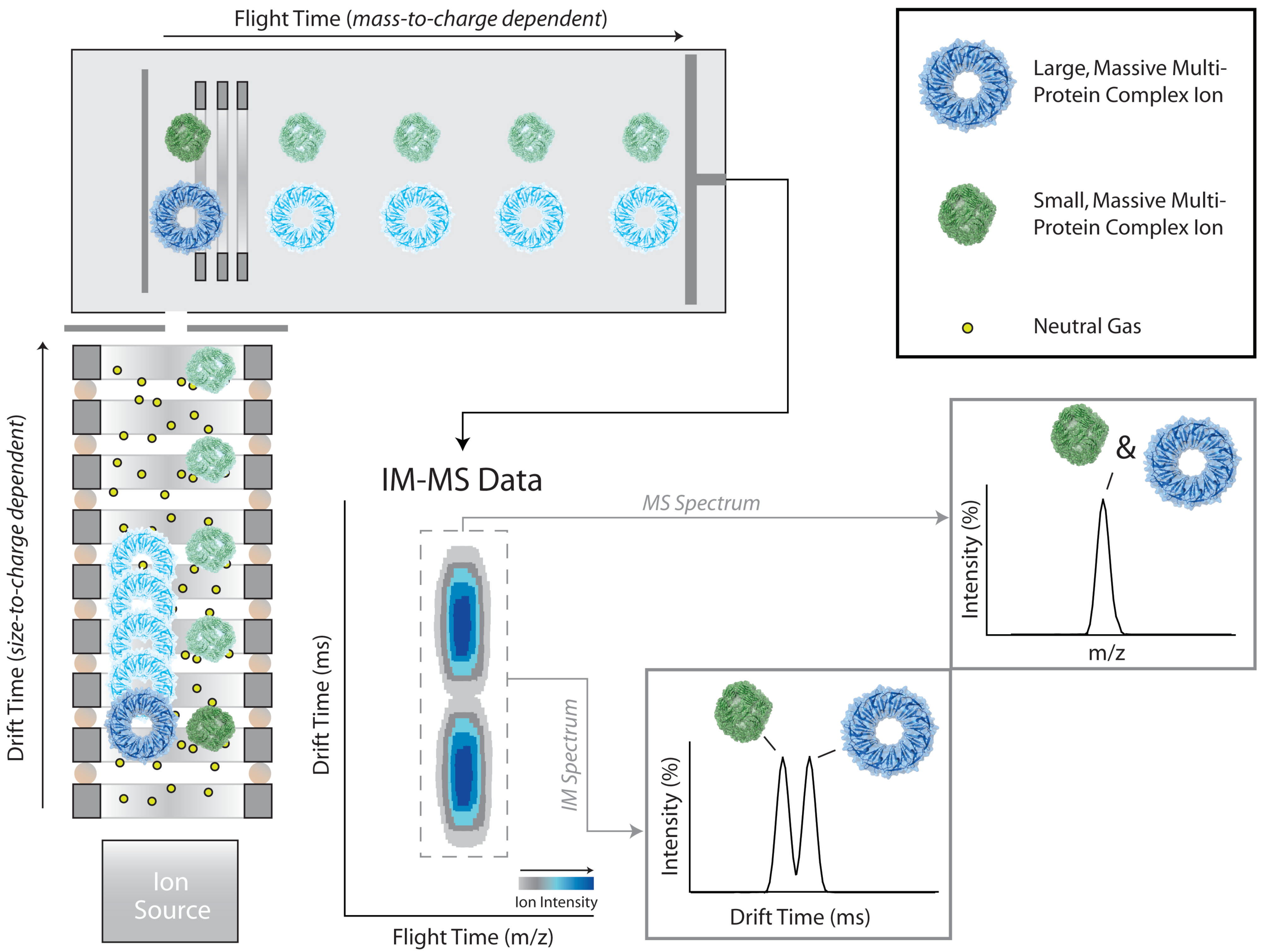




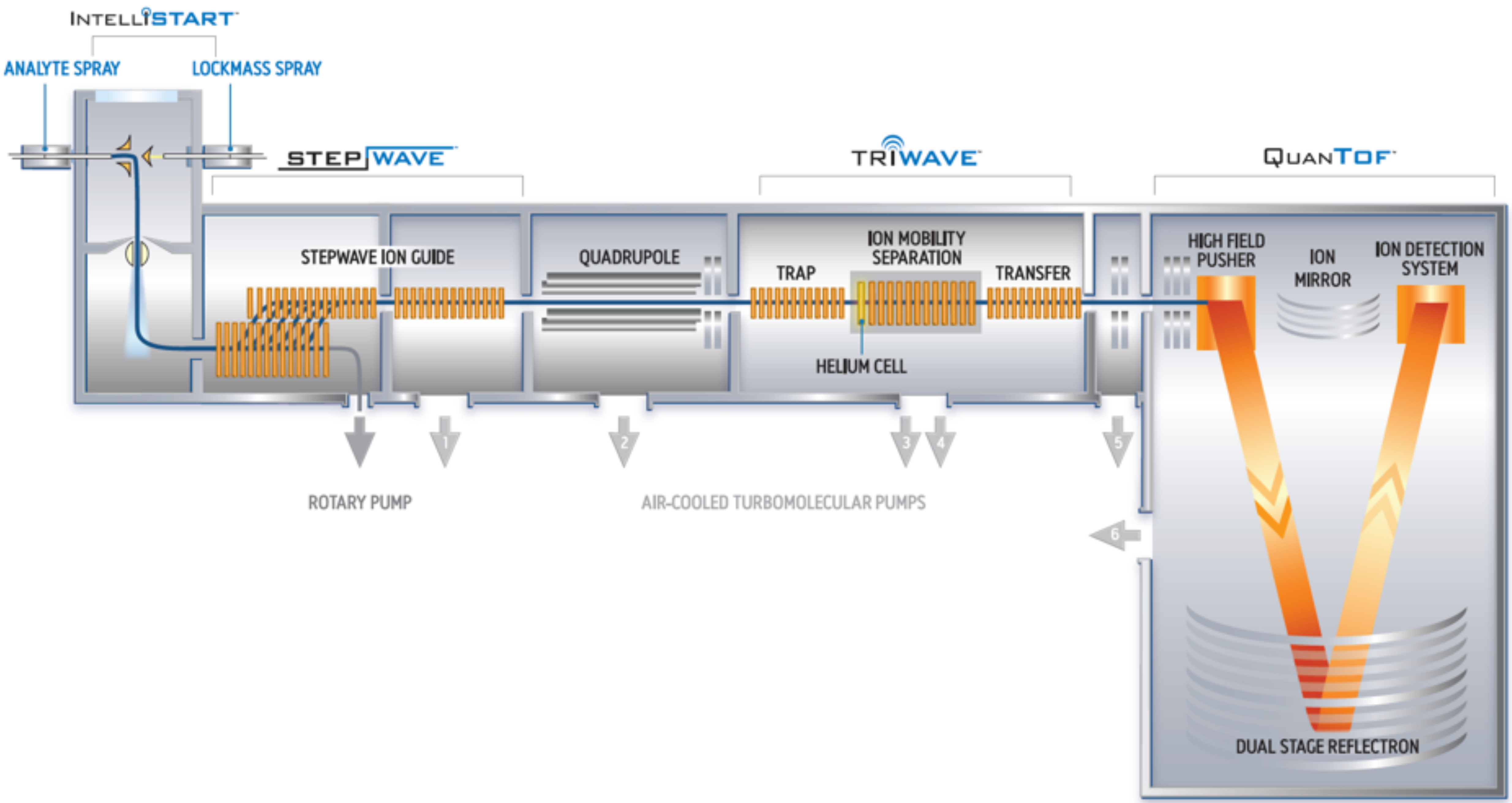
- DC waves travel down the cell
- Ions surf down the waves and “roll-over”
- RF confinement - transmission almost 100%



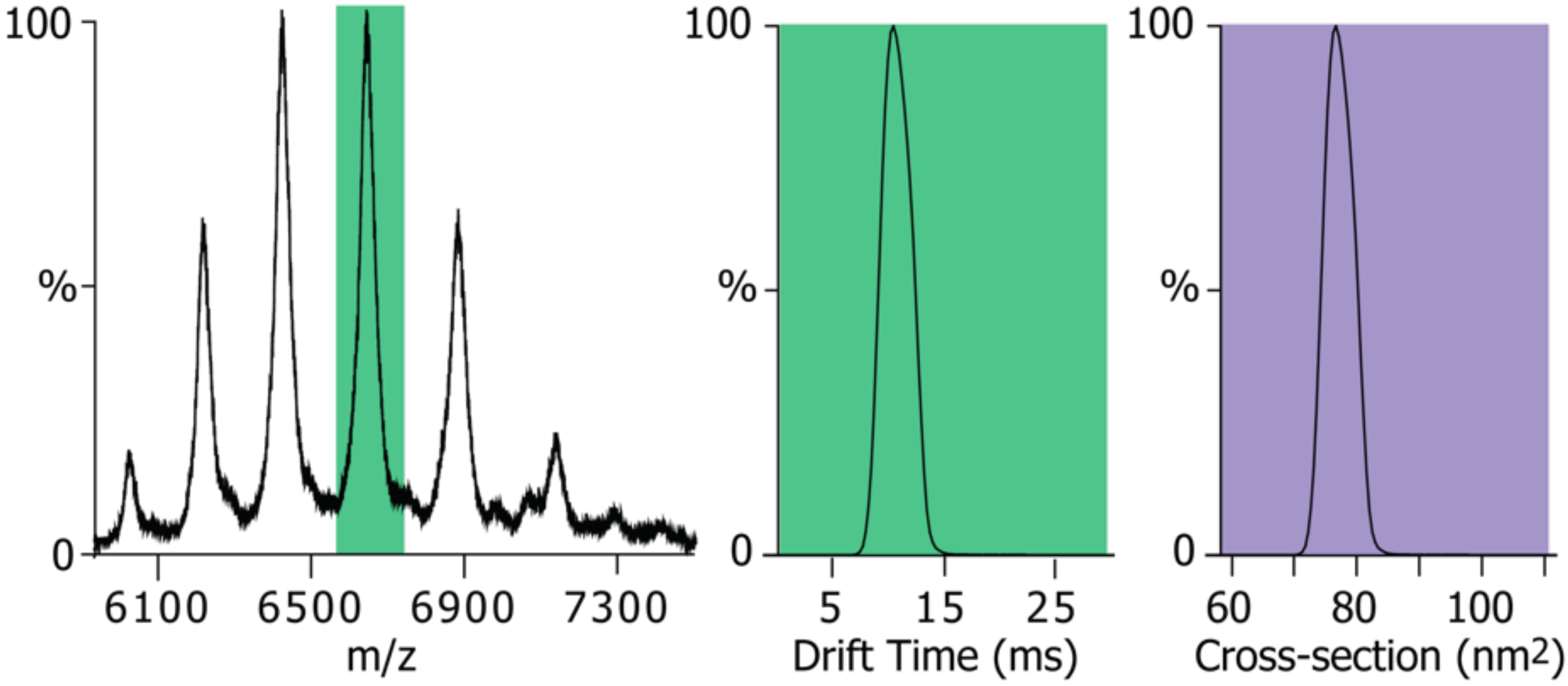
Ion mobility - mass spectrometry (IM-MS)



Ion mobility - integration into mass spectrometers



Obtaining an experimental CCS



- Every feature resolved in m/z has an associated drift time distribution
- Drift time is converted into CCS either directly or via calibration

IM resolution and peak widths

$$W_{\text{tot}} = W_{\text{diffusion}} + W_{\text{space-charge}} + W_{\text{pulse}} + W_{\text{reactions}} + W_{\text{conformations}}$$

- In our experimental set up the major contributor is the last term

Drift Tube

$$R_{DT} = \frac{1}{4}\left(\frac{ze}{k_B \ln 2}\right)^{1/2}\left(\frac{V}{T}\right)^{1/2}$$

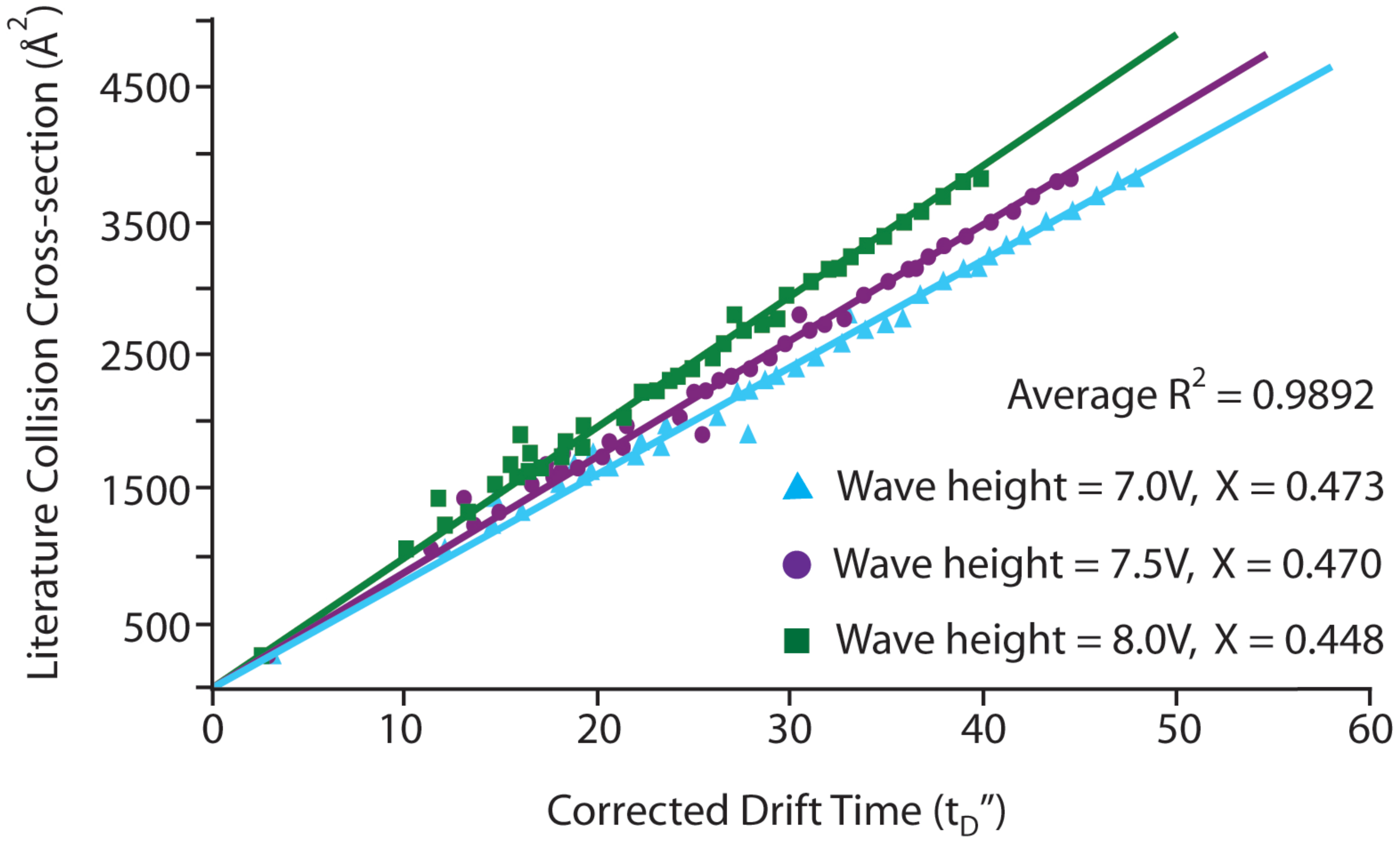
T-Wave

$$R_{TW} = \frac{1}{4}\left(\frac{2ze\ [WH]\ [WF]\ KE}{kT\ [WV]\ \ln 2}\right)^{\frac{1}{2}}$$

Resolution in T-wave is Defined Differently than in Drift Tubes

- Due to power term in conversion of time to CCS for T-Wave, CCS resolution is not equal to that in time (typically 2 or 3-fold higher)

Calibration procedure

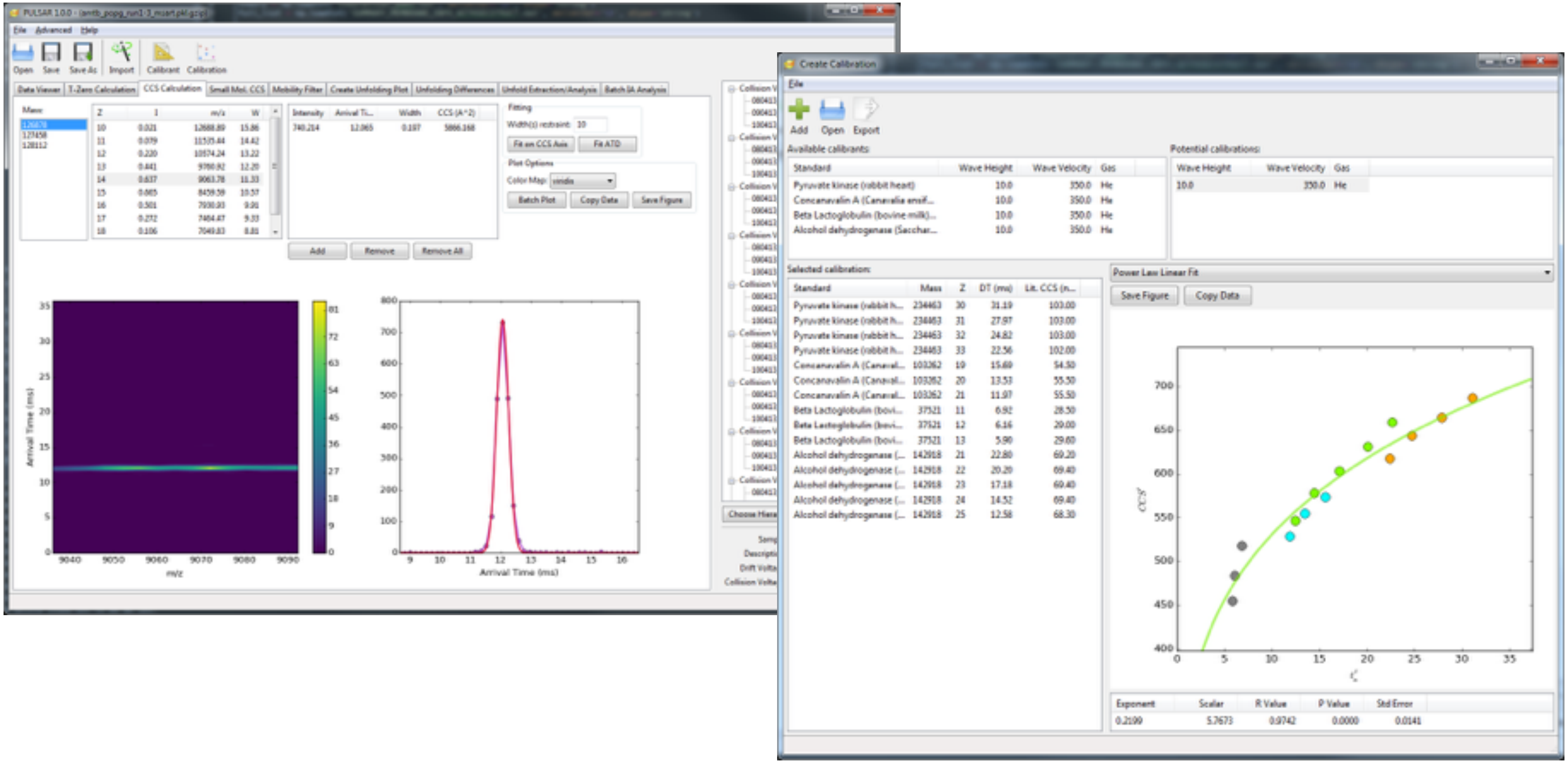


- Standard calibration approach
- Important to use appropriate calibrants

Ruotolo et al, Nat Protoc (2008), 3, 1139-52; Bush et al, Anal Chem (2010), 82, 9557-65

Measuring CCS - drift tube and travelling wave IMS

- Work through: http://www.fhi-berlin.mpg.de/mp/pagel/Pagel/Home.html
- Manufacturer’s software, or pulsar.chem.ox.ac.uk



Mass
126678
127458
128112

Z	I	m/z	W
10	0.021	12688.89	15.86
11	0.079	11535.44	14.42
12	0.220	10574.24	13.22
13	0.441	9760.92	12.20
14	0.637	9063.78	11.33
15	0.665	8459.59	10.57
16	0.501	7930.93	9.91
17	0.272	7464.47	9.33
18	0.106	7049.83	8.81

Intensity	Arrival Ti...	Width	CCS (A^2)
740.214	12.065	0.197	5866.168

Width(t) restraint: 10

Color Map: viridis

Available calibrants:

Standard	Wave Height	Wave Velocity	Gas
Pyruvate kinase (rabbit heart)	10.0	350.0	He
Concanavalin A (Canavalia ensif...	10.0	350.0	He
Beta Lactoglobulin (bovine milk)...	10.0	350.0	He
Alcohol dehydrogenase (Sacchar...	10.0	350.0	He

Potential calibrations:

Wave Height	Wave Velocity	Gas
10.0	350.0	He

Selected calibration:

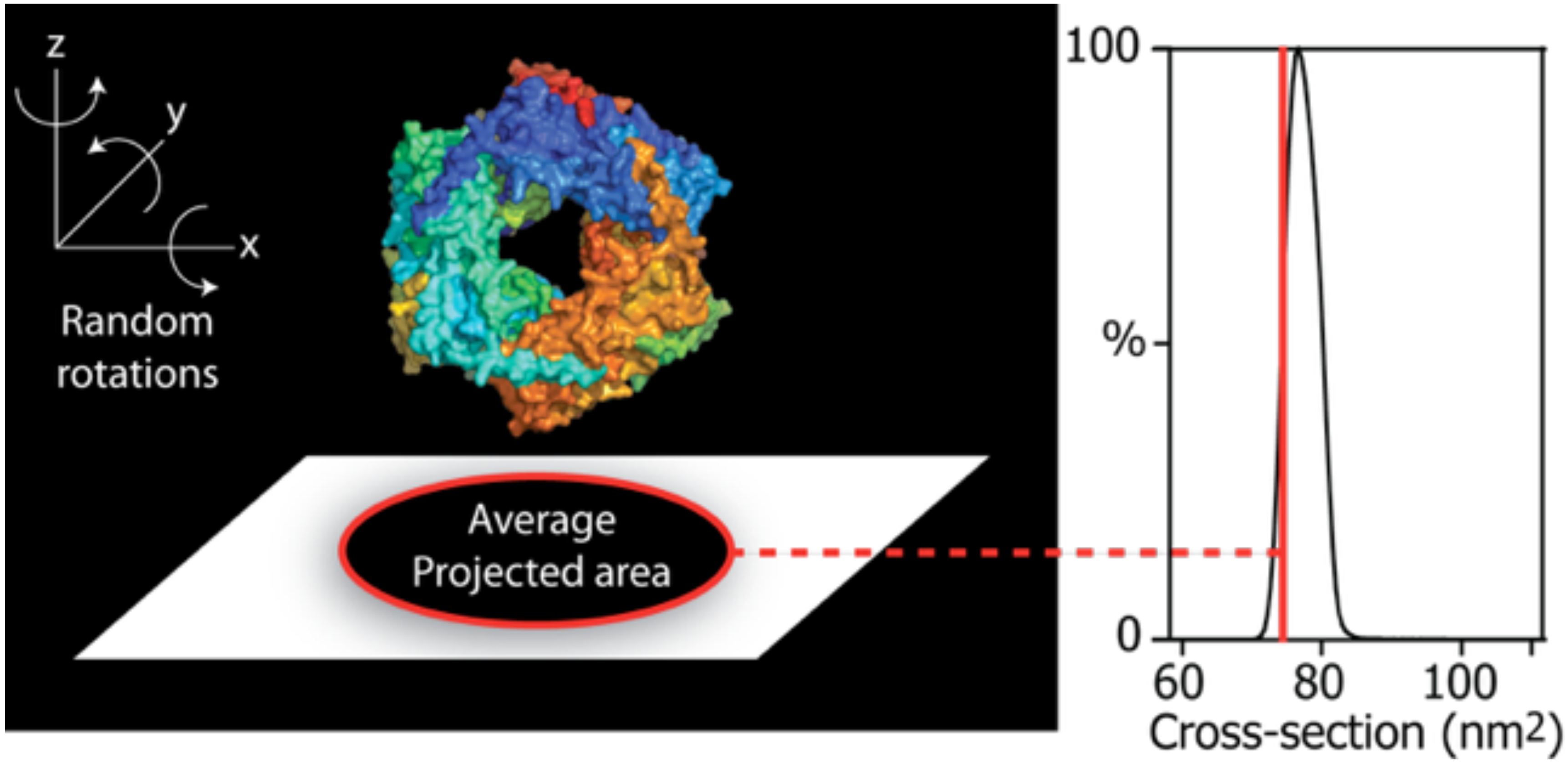
Standard	Mass	Z	DT (ms)	Lit. CCS (n...
Pyruvate kinase (rabbit h...	234463	30	31.19	103.00
Pyruvate kinase (rabbit h...	234463	31	27.97	103.00
Pyruvate kinase (rabbit h...	234463	32	24.82	103.00
Pyruvate kinase (rabbit h...	234463	33	22.56	102.00
Concanavalin A (Canaval...	103262	19	15.69	54.50
Concanavalin A (Canaval...	103262	20	13.53	55.50
Concanavalin A (Canaval...	103262	21	11.97	55.50
Beta Lactoglobulin (bovi...	37521	11	6.92	28.50
Beta Lactoglobulin (bovi...	37521	12	6.16	29.00
Beta Lactoglobulin (bovi...	37521	13	5.90	29.60
Alcohol dehydrogenase (...	142918	21	22.80	69.20
Alcohol dehydrogenase (...	142918	22	20.20	69.40
Alcohol dehydrogenase (...	142918	23	17.18	69.40
Alcohol dehydrogenase (...	142918	24	14.52	69.40
Alcohol dehydrogenase (...	142918	25	12.58	68.30

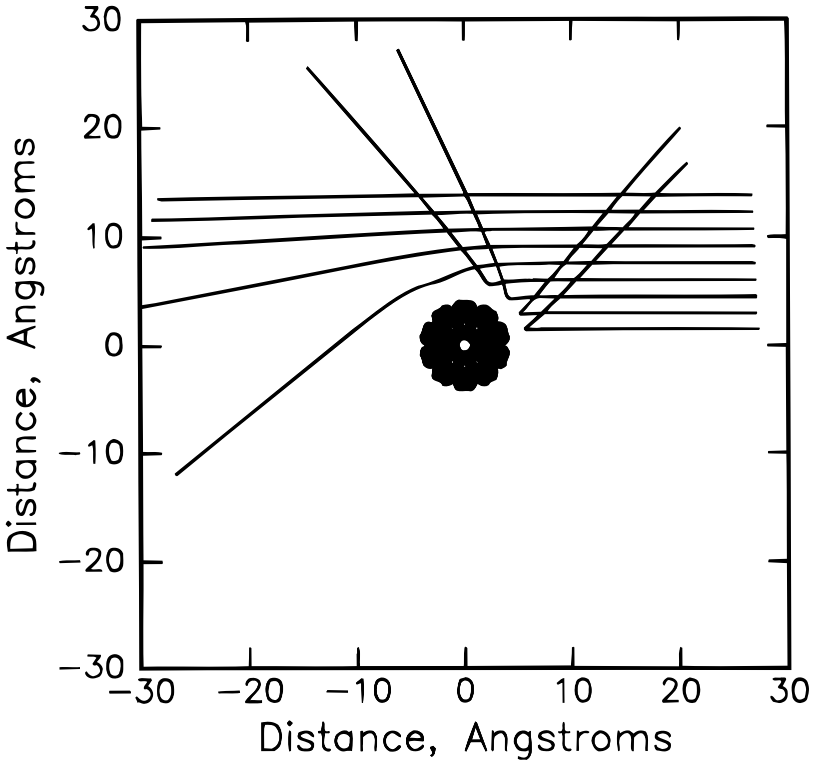
Power Law Linear Fit

Exponent	Scalar	R Value	P Value	Std Error
0.2199	5.7673	0.9742	0.0000	0.0141

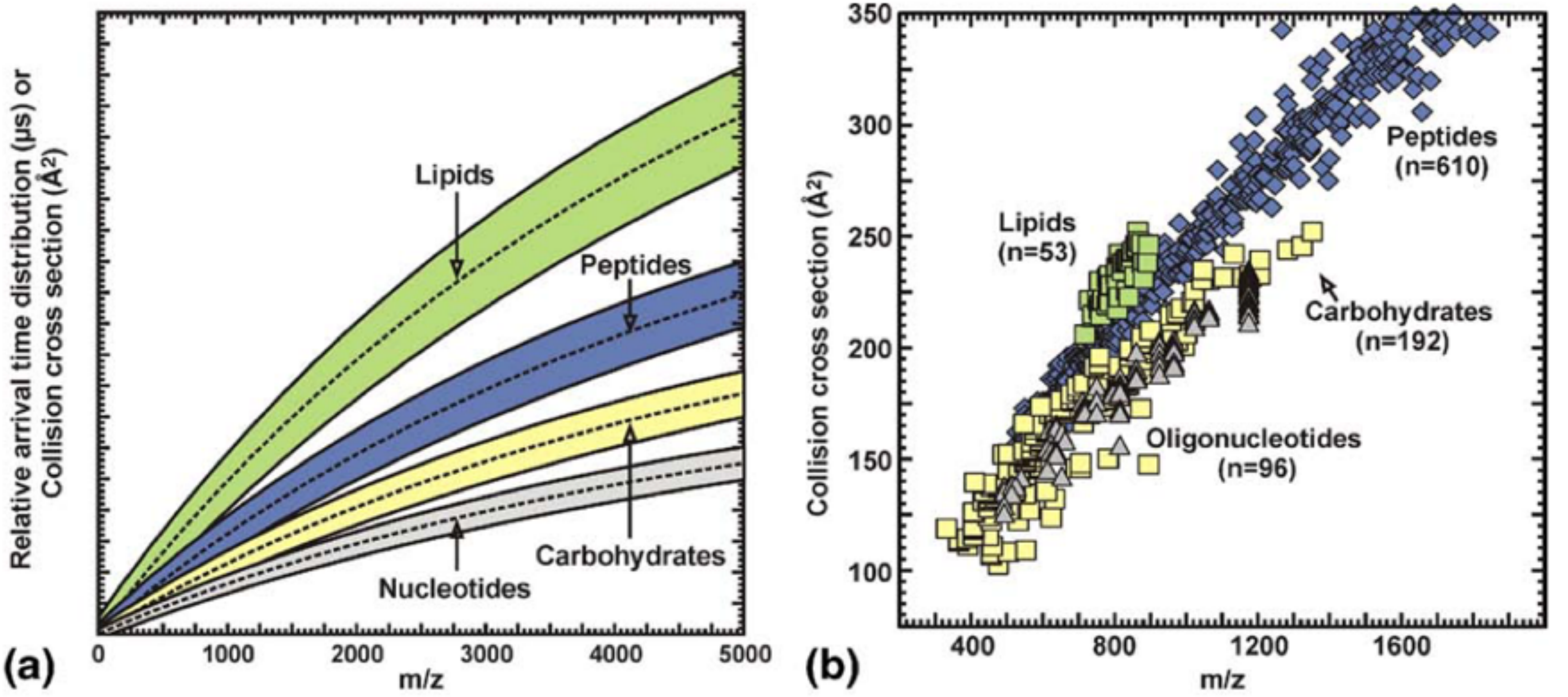
CCS calculation

- Different methods for calculating CCS from structures are available
- Fastest are based on a projection approximation (impact.chem.ox.ac.uk)
- For more detailed study the trajectory method is useful
- For electron densities EMnIM@chem.ox.ac.uk



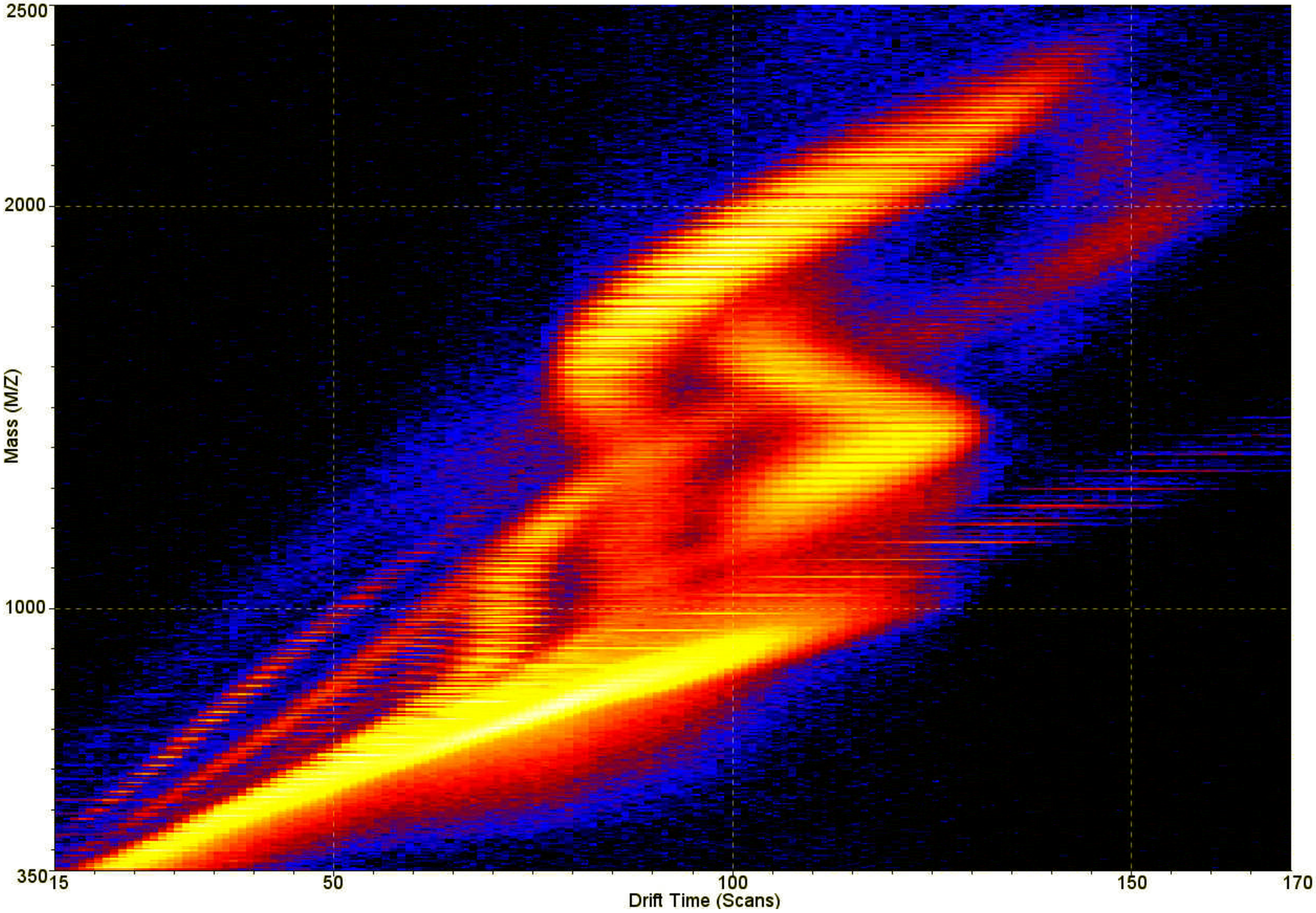


IM-MS “trendlines”



- Different bimolecular classes have different effective densities in vacuum

Heterogeneous molecules



- PEG sample - multiple different trends due to conformations, charging etc

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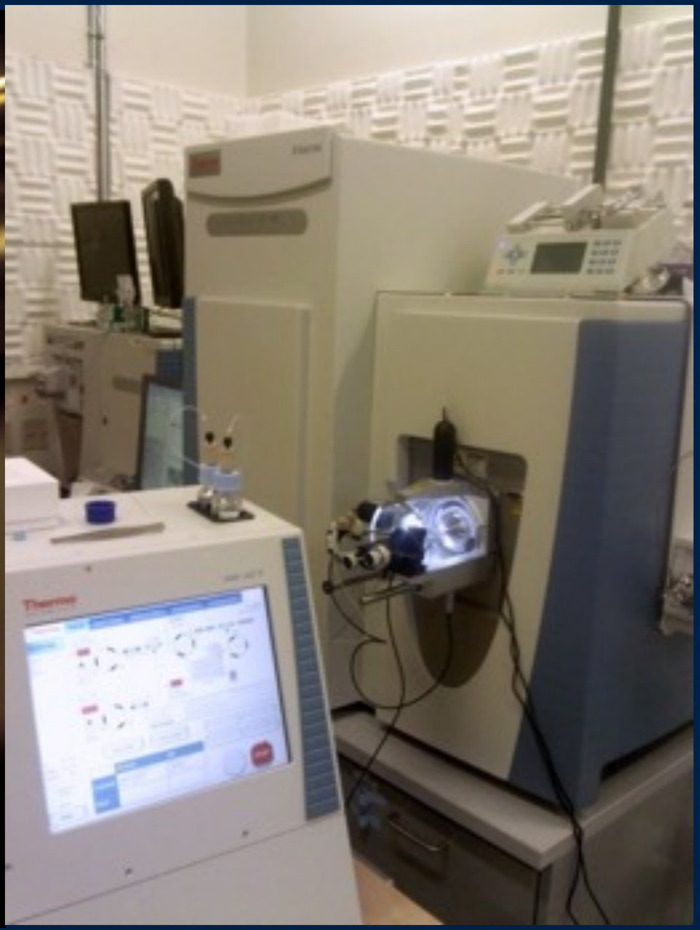
GRADUATE COURSE IN MASS SPECTROMETRY: LECTURE 3

Collisions in vacuum: cooling, activating, and sizing ions



Professor Justin Benesch, 27th October 2016