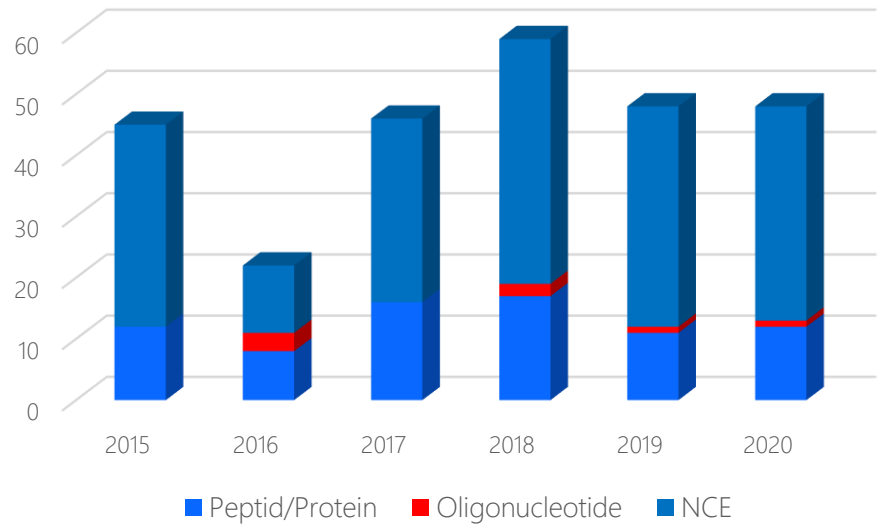
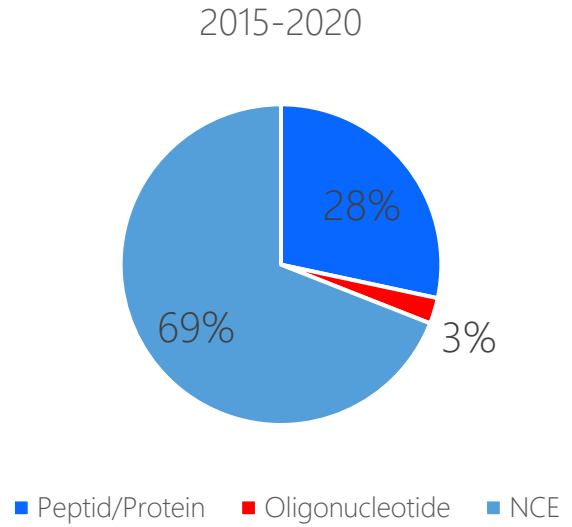
# Extending capabilities: NativeMS and optical spectroscopy using modified mass spec instruments

MSVision

Dedicated to Mass Spectrometry

# Why dedicated high mass MS instrumention?



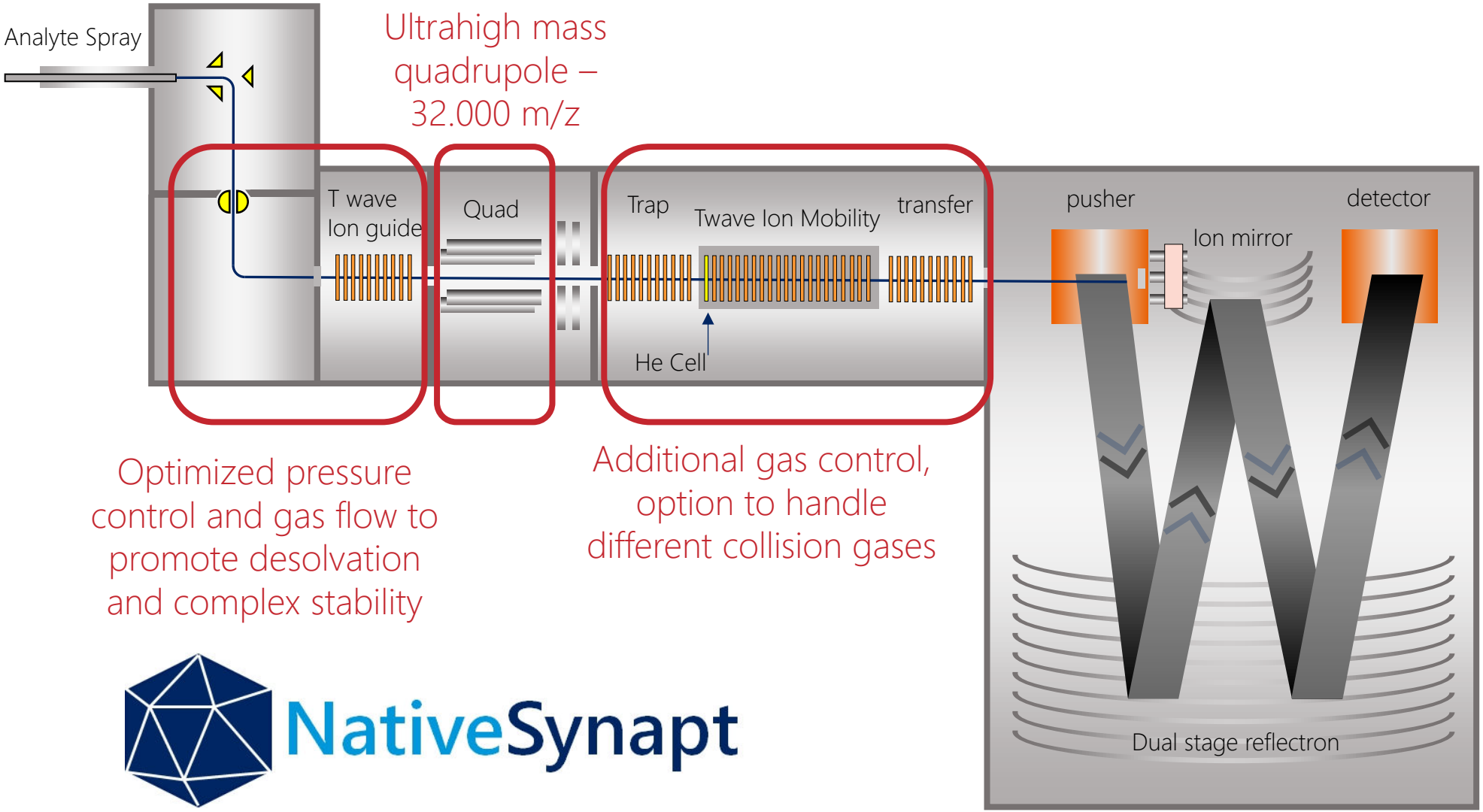

- About 1/3rd of the FDA approved drugs are biologics, the vast majority of them peptides or proteins
- Biologics have different requirements regarding QC/QA, ADME and metabolism studies and release analytics than small molecules
- Typical biologics such as monoclonal antibodies have masses in the range of 150 kDa, are heterogeneous and can undergo agglomerisation. Detection is sometimes difficult for standard instruments.

# Components of a dedicated high mass system

- When doing high mass MS of hybrid systems (e.g. QExactive or QTOF), one needs to look at the mass ranges of the individual components separately.
- A high TOF mass range is useless if the quadrupole upfront cannot effectively transfer the ions. The same holds true for an Orbitrap detector.

- Therefore a dedicated high mass mass spectrometer consists out of several, equally important components:
  - An optimized ion source and transfer to properly desolvate and transfer large ions into the instrument
  - A quadrupole which is capable of handling large m/z values. At least 10.000, the more the better for optimal transmission. m/z 4.000 is rather useless when looking at m/z of 20.000 and beyond, sensitivity will be poor.
  - Optimized fragmentation to effectively analyze the individual components of a larger complex
  - Optimized mass detector in terms of mass range, necessary resolution, pressure regimes and detection efficiency

# The NativeSynapt.

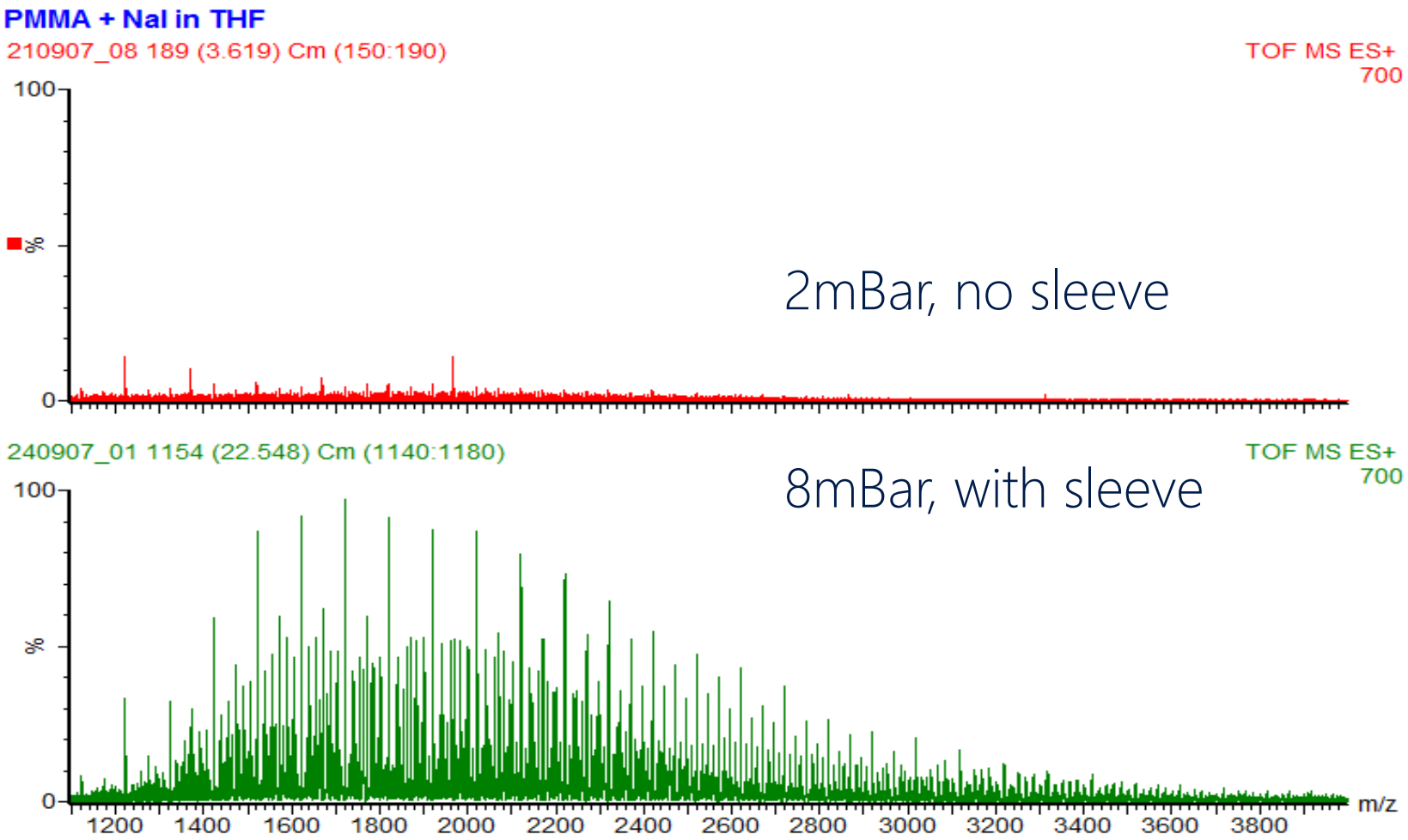



Optimized pressure control and gas flow to promote desolvation and complex stability

Additional gas control, option to handle different collision gases

NativeSynapt

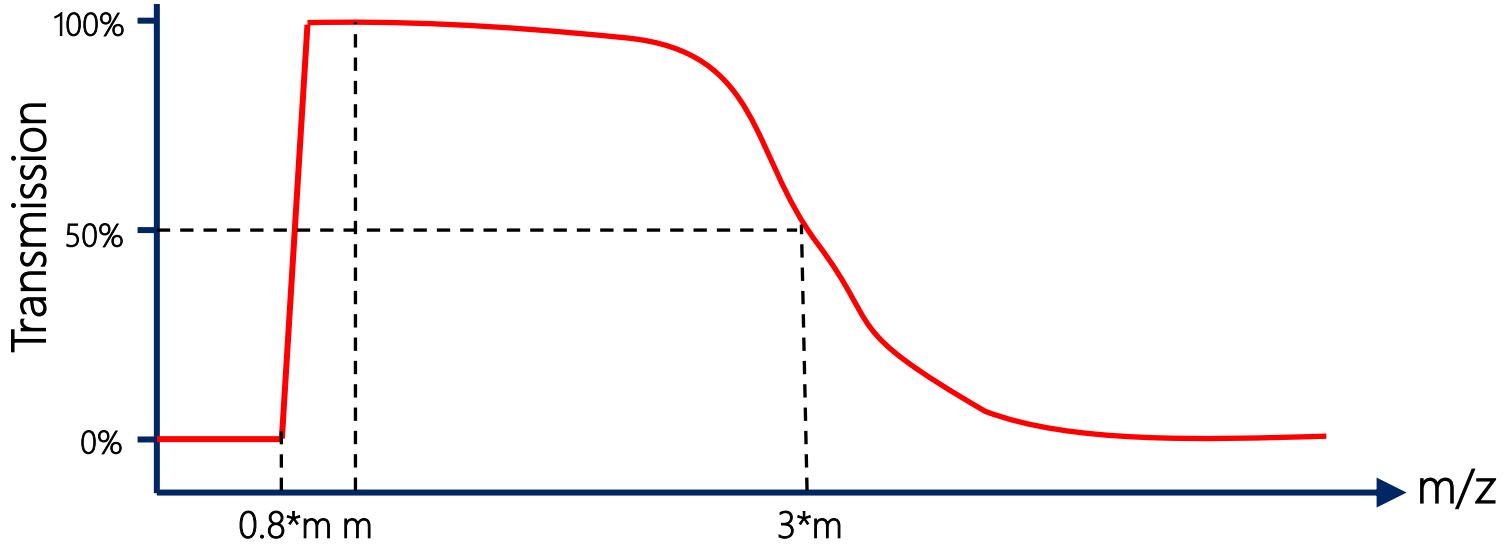
# Modifying the inlet system for optimized vacuum




PMMA synthetic polymer, average molecular weight 10,000 Da

# Mass dependance of quadrupole transmission

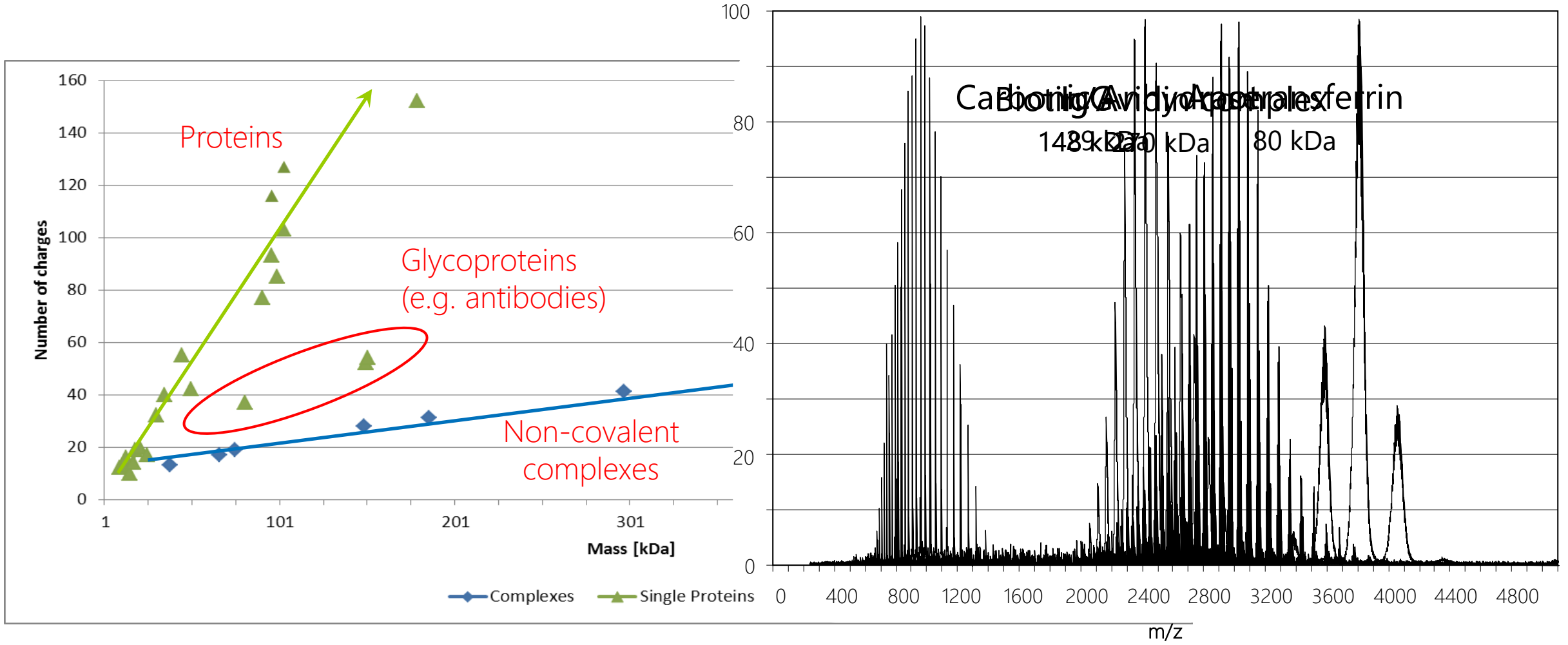
Quadrupoles show reduced transmission of ions with m/z > ~3x the set quad mass. The maximum possible quad mass setting is hardware dependant (upper isolation mass limit) and defined by maximum potential and applied RF frequencies.




- At MS Vision we know how to build high mass quadrupoles since 20 years
- Currently quadrupoles with upper mass limit for isolation of >30 kDa available
- Ongoing research to push the limits of the technology to 100 kDa upper isolation limit, current record is at 47 kDa for isolation

# Why a dedicated high mass MS instrument?

- The larger the molecule gets, the higher the mass. Mass-to-charge-ratio can grow even faster depending on physical properties such as glycosylation or complex formation



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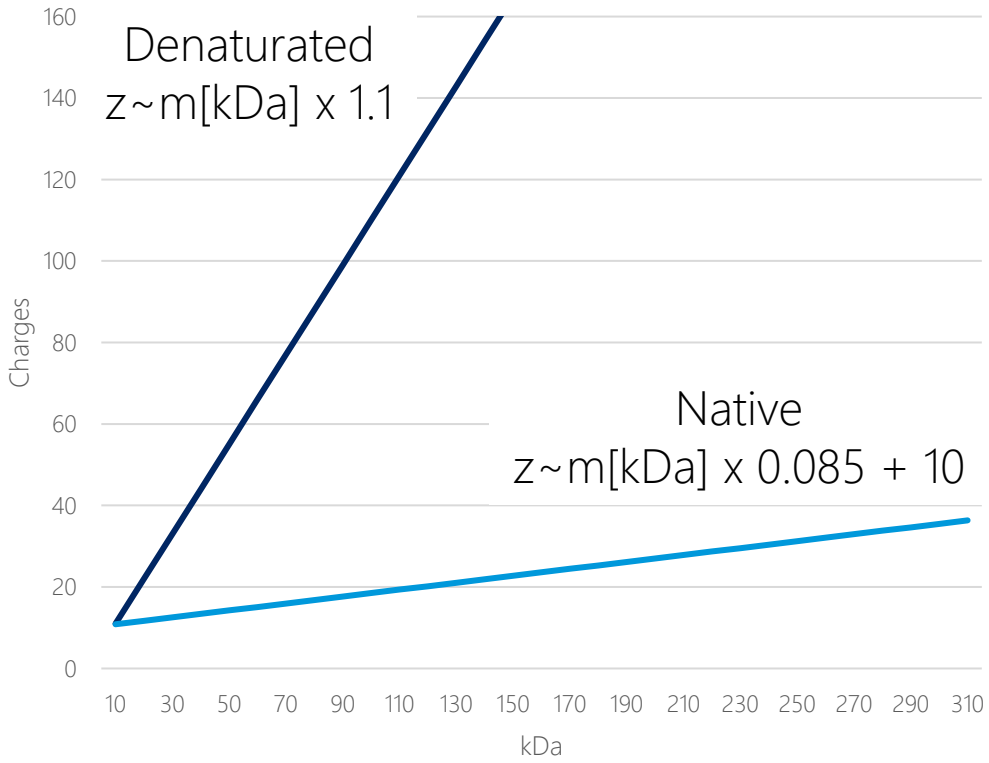
# How does m/z behave for denaturated vs. native ESI?



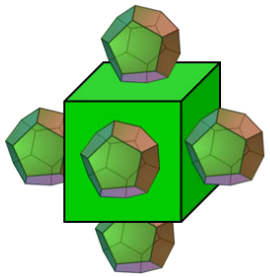

Formulas are just approximations to describe the linear curves!

- Calculation roughly describes the observed curves
- Charge for denaturated ESI increases ~13x faster than for native ESI
- m/z increases MUCH faster for native ESI than for denaturated ESI
- Does this make sense? If we use these formulas
  - a mass of 464 kDa would have ~49 charges under native ESI and show m/z of ~9.500 Da
  - 800 kDa would result in ~70 charges and m/z of 11.500

  → we will come back to that later!

# Why does m/z increase faster in native MS?

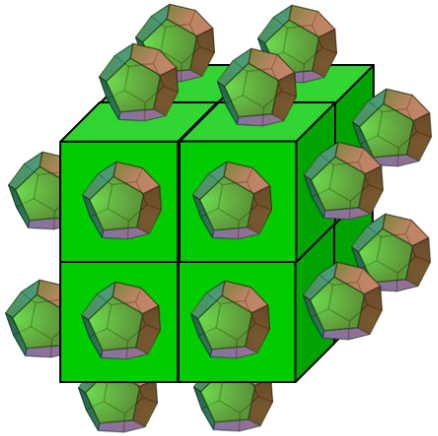
- The larger the molecule gets, the higher the mass. Mass-to-charge-ratio detected by MS can grow even faster depending on physical properties such as glycosylation or complex formation



m = 12000 Da

z = 6

m/z = 12000 Da/6 = 2000

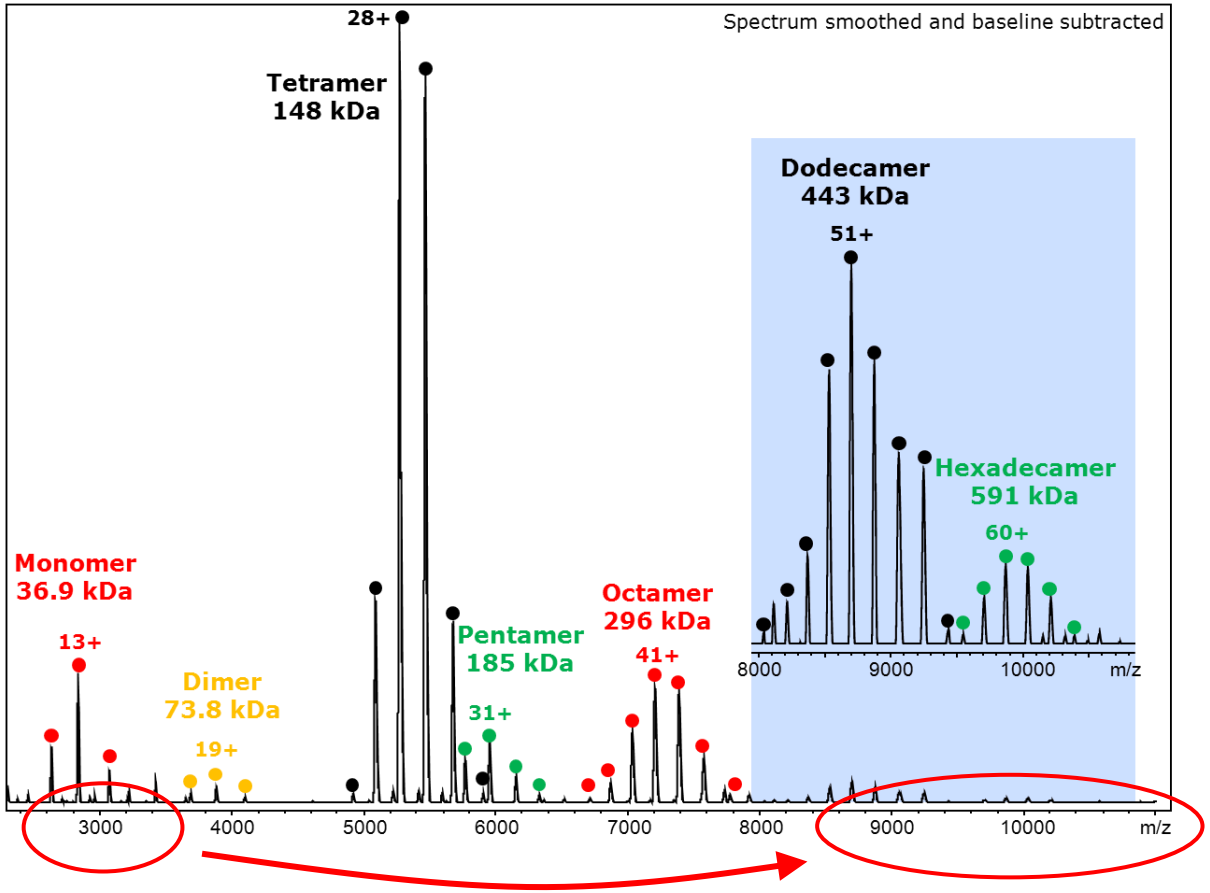


m = 8*12000 Da = 96000 Da

z = 6*4=24

m/z = 96000 Da /24 = 4000

m/z shifted by a factor of 2



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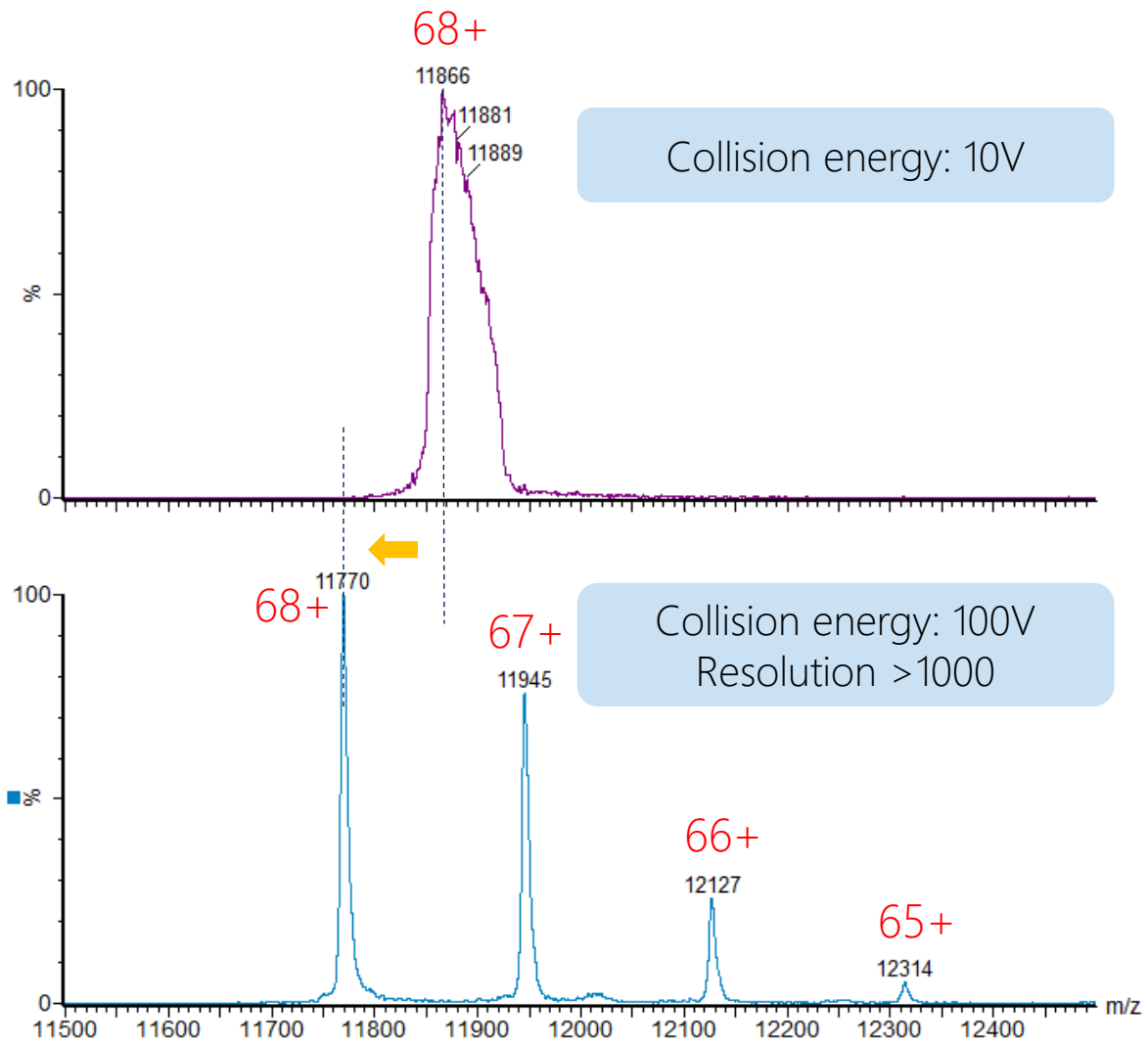
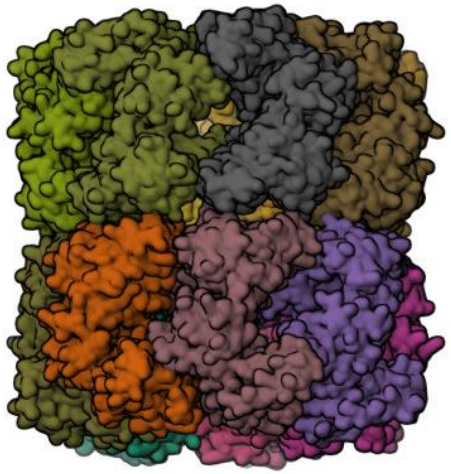
# Improvements due to optimized collision energies



GroEL
801 kDa 14mer

| | |
|---|---|
| Difference in m/z: | 110 |
| Charge state: | 68 |
| Mass loss: | 7,500 Da |

Losses: solvent molecules!

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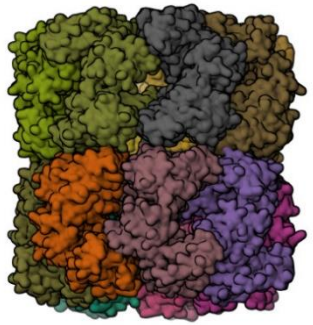
# Optimized high mass ion fragmentation (precursor m/z $11770^{68+}$)

Collision energy: 150V
Pressure: 0.020mBar

## GroEL

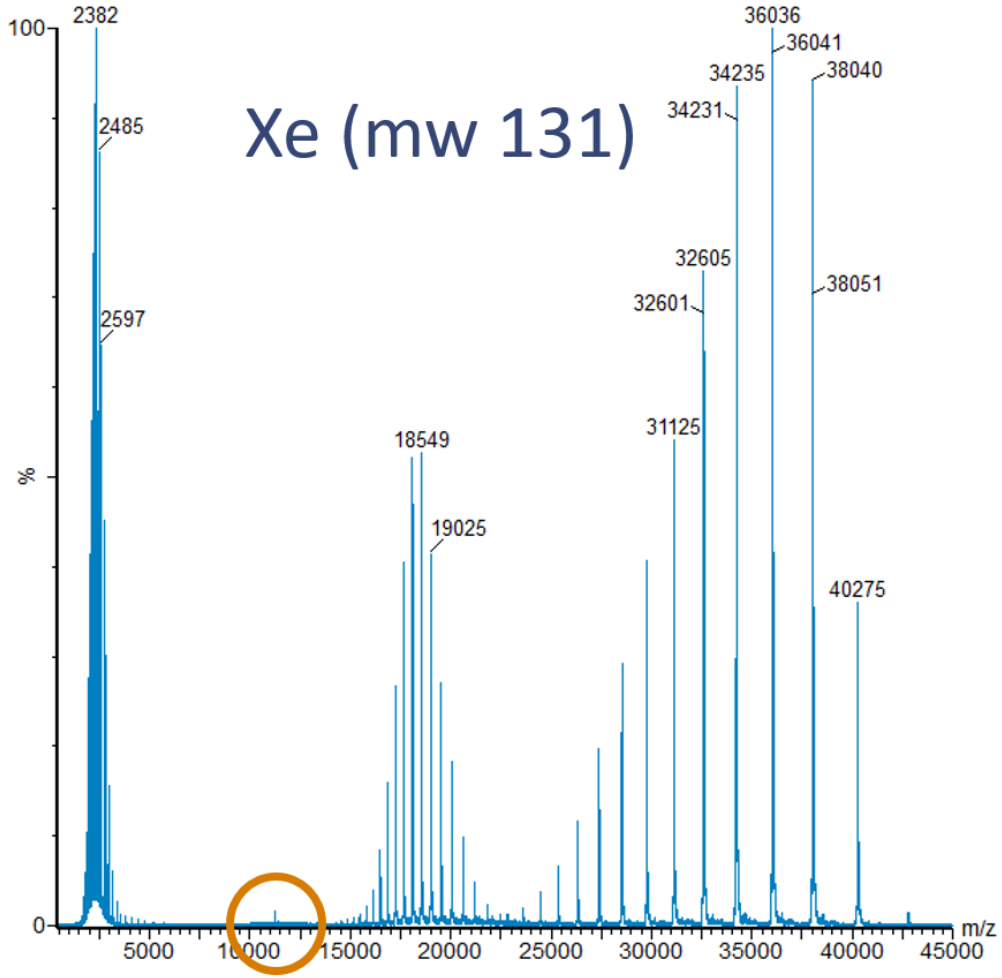
### Ar (mw 40)



801 kDa 14mer




### Xe (mw 131)

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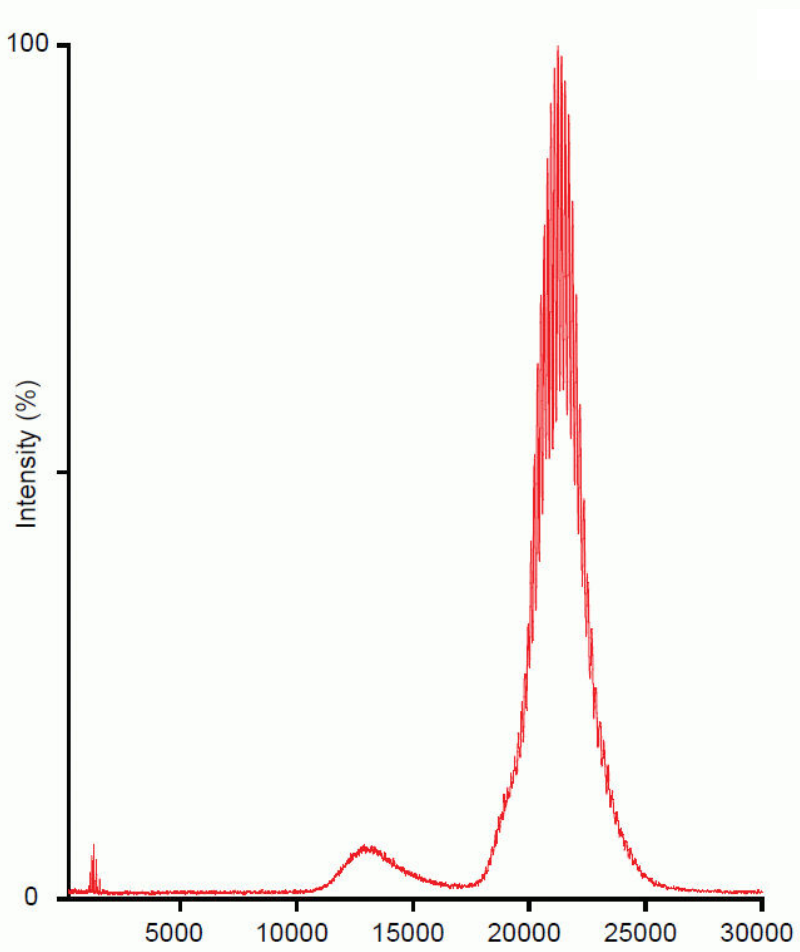
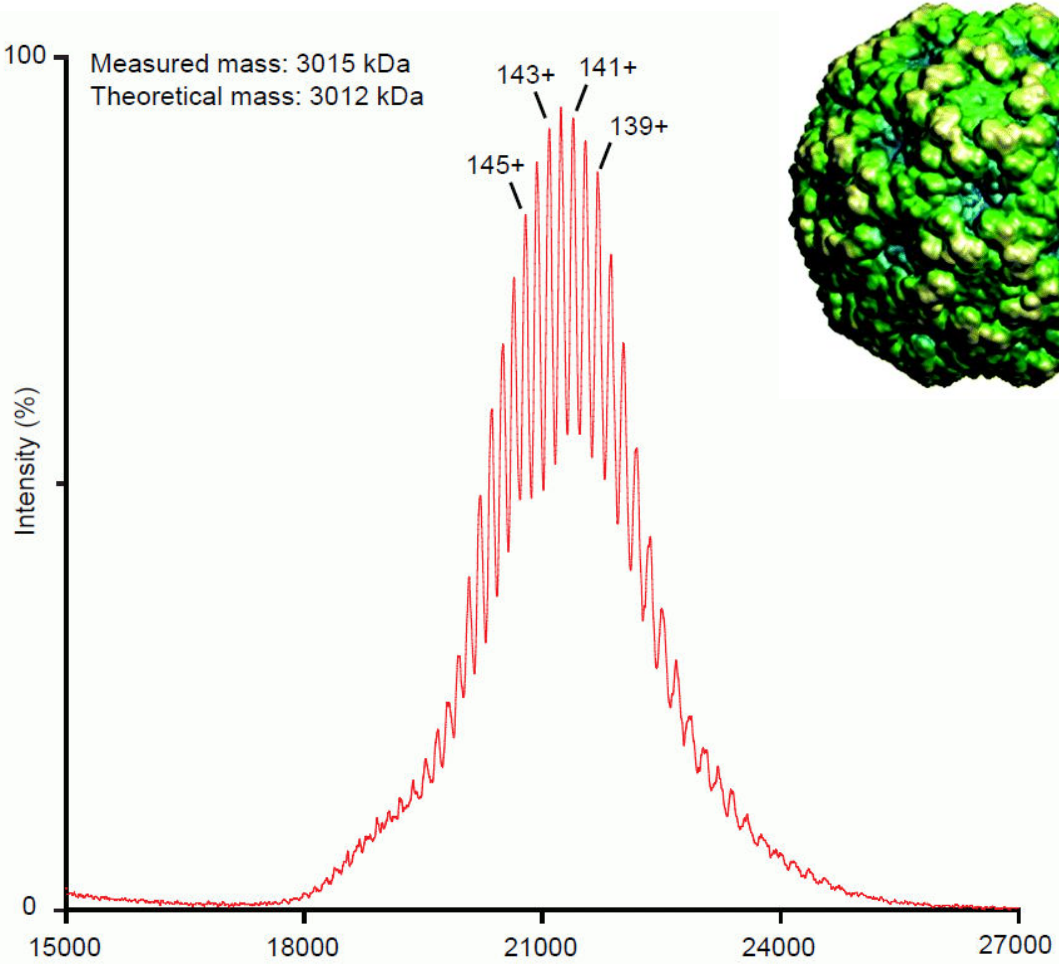
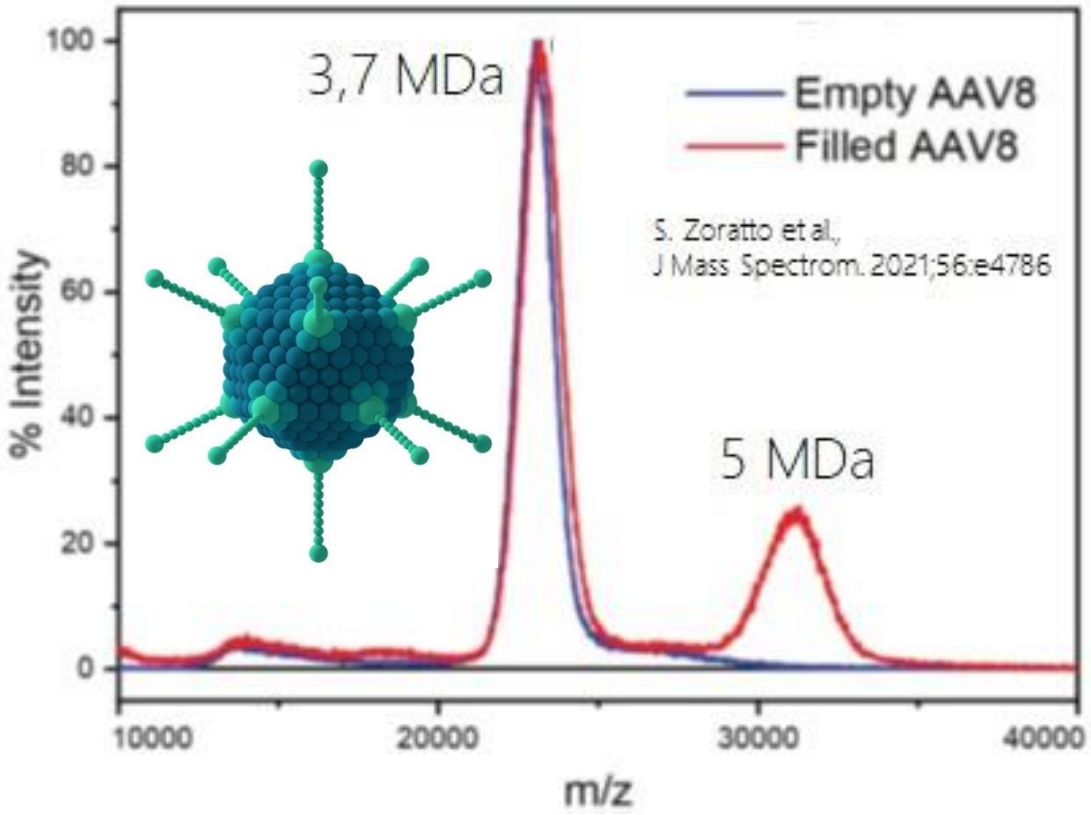
# Virus capsid 3,000,000 Da

Collision energy: 200V
Pressure: 0.020mBar Xe



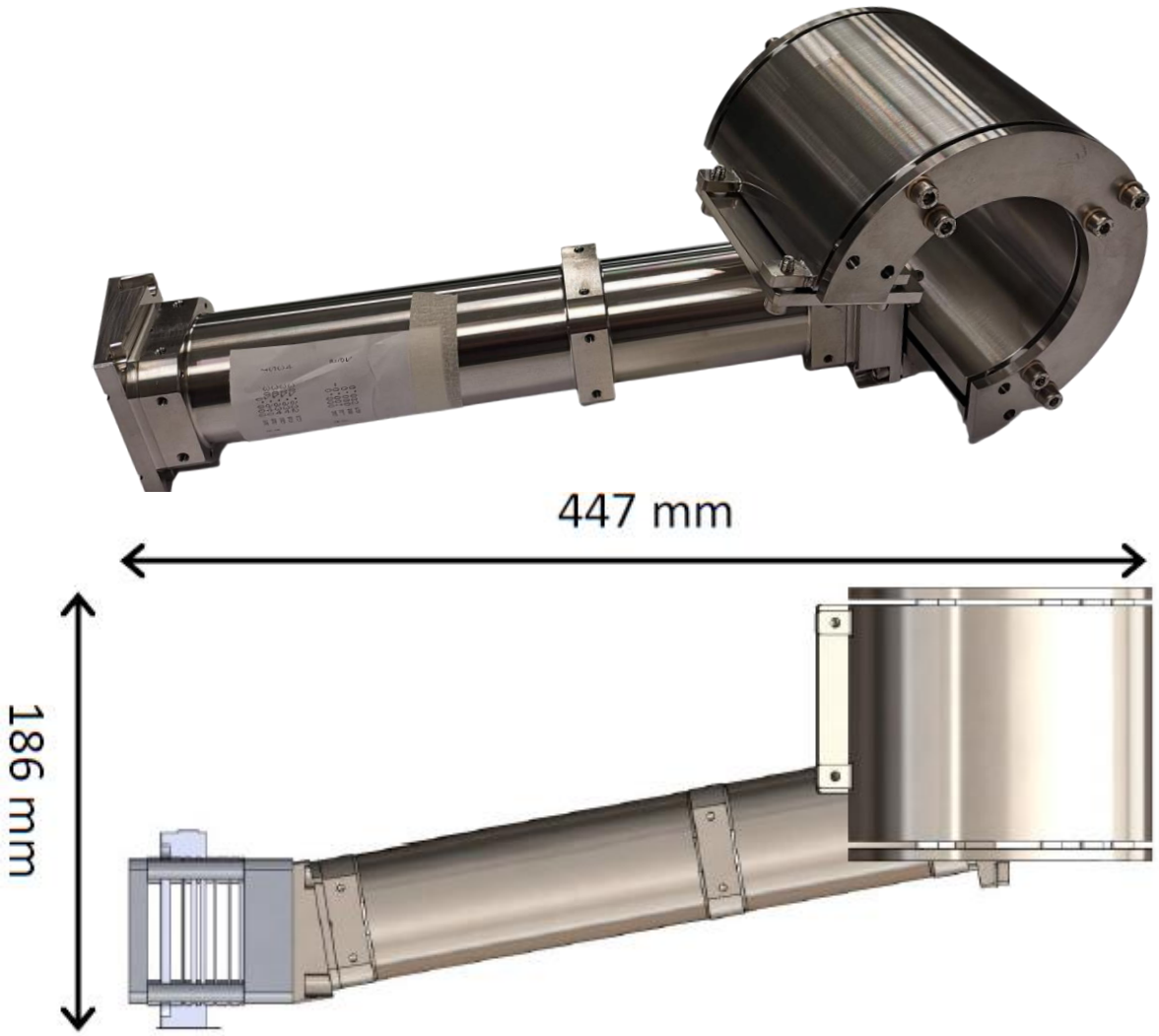
# Adeno-associated virus particles (AAV)




Adeno-associated virus particles (AAV) or virus-like particles (VLP) are of major interest as transport vehicles in vaccines (e.g. for the AZ Covid vaccine).

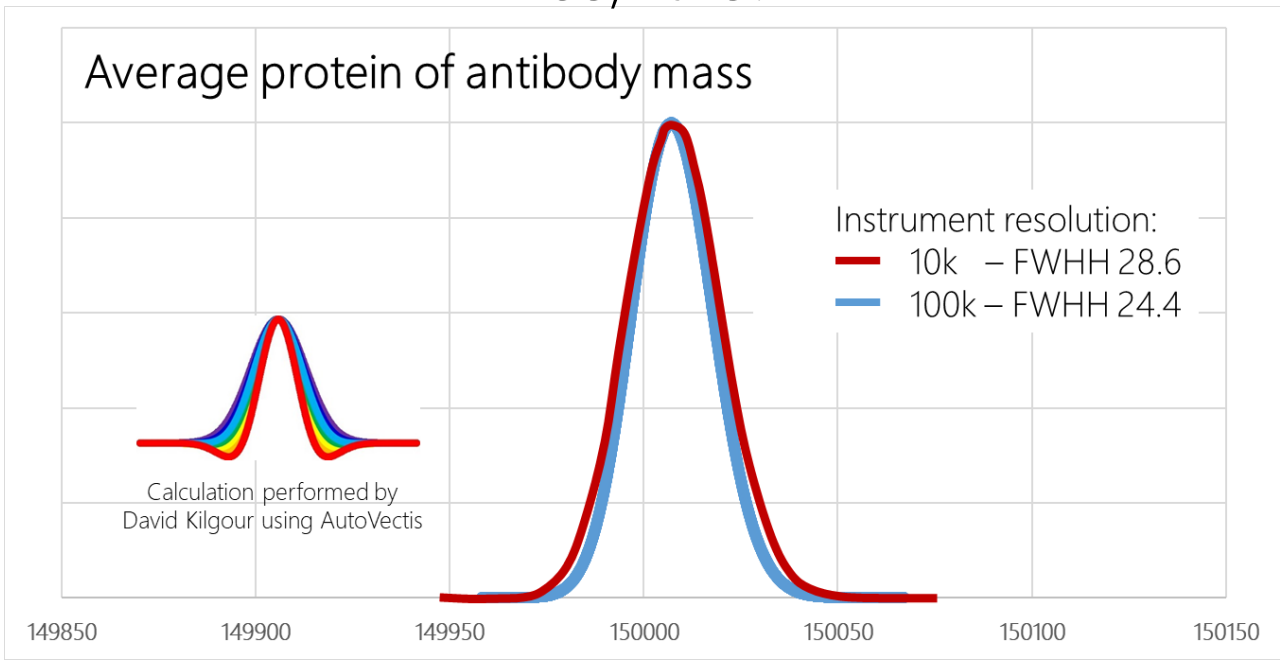
Native MS allows for the direct determination of the load of AAV's. Unlike conventional methods, it does not monitor the shape (which undergoes only minor changes upon loading) or absorbance ratios (which can be influenced by matrix components) it monitors the mass change. The ratio between loaded and unloaded particles can be determined unambiguously.

# A new TOF detector principle – Electrostatic Analyzer (ESA)




- Resolution ~8-10.000
- Inherent metastable filtering

But is 10k resolution enough?
Yes, it is!



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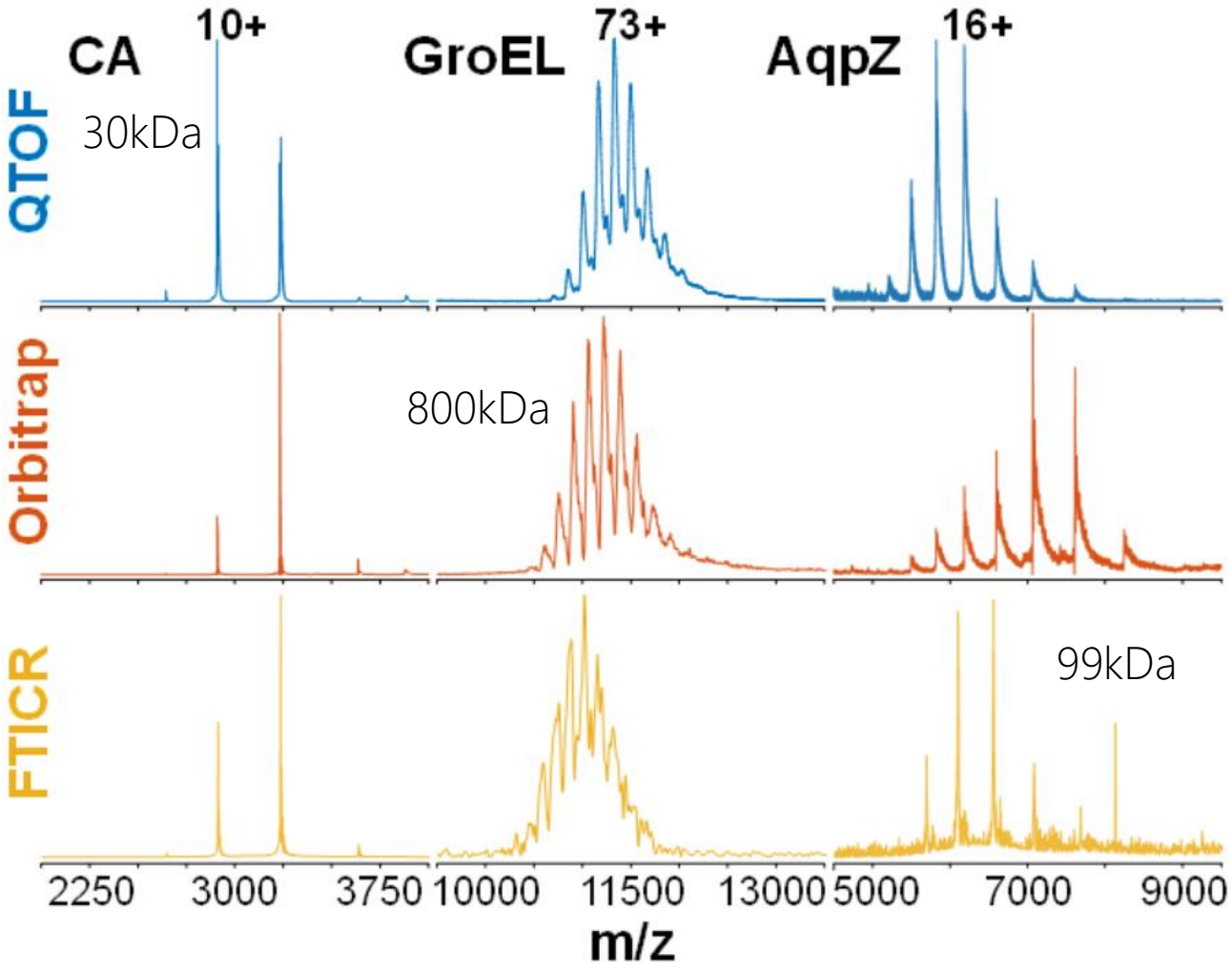
# TOF vs. OT vs. FT-ICR in practice



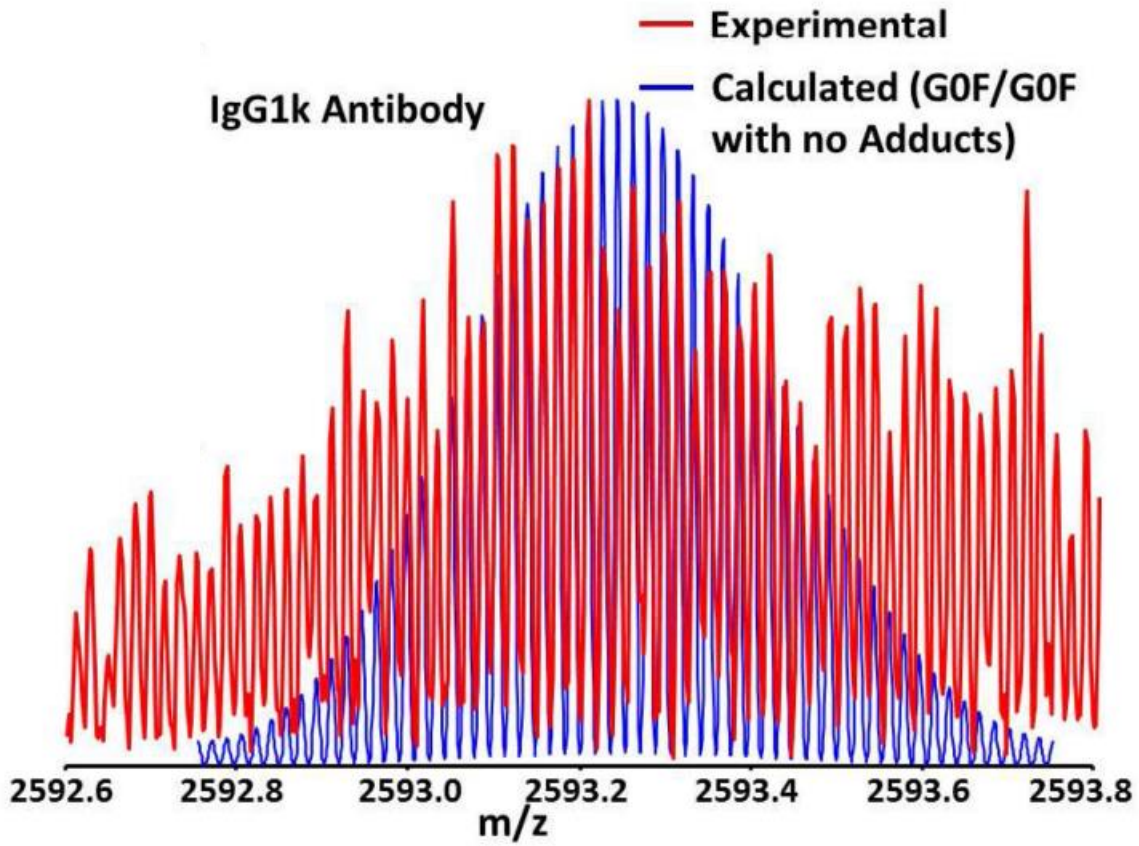

There is no significant advantage in using high resolution when analysing very big molecules!

(but depending on the actual instrument the border where „big" starts varies…)

**Top-down mass spectrometry of native proteoforms and their complexes: a community study**
Tanja Habeck *et al.*
Nature Methods, 2024 May 14;
doi: 10.1038/s41592-024-02279-6

# Will ultrahigh resolution provide better results?

Likely not, as due to limited number of ions for very large proteins the ion statistics is rather poor:

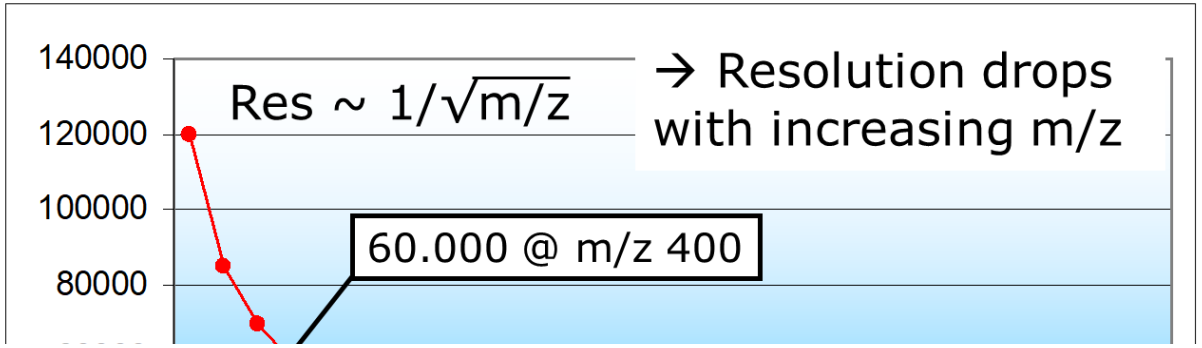



The resulting isotopic pattern representation is often so poor that fitting the theoretical isotopic pattern is largely random. Often results based on average masses are comparable or even better!

Unit Mass Baseline Resolution for an Intact 148 kDa Therapeutic Monoclonal Antibody by FT-ICR Mass Spectrometry
Santosh G. Valeja et al., Anal Chem. 2011 November 15; 83(22): 8391–8395. doi:10.1021/ac202429c

# Why not using Orbitrap for higher resolution?

- There are mainly three reasons why we just recently started to modify Orbitraps for NativeMS
  - The technical capabilities / know how
  - As discussed, the high resolution of Orbitraps don't give a real benefit for intact proteins
  - The physics – Orbitraps are inherently not very good at high masses with resolution and detection!




Journal of the American Society for Mass Spectrometry

pubs.acs.org/jasms Research Article

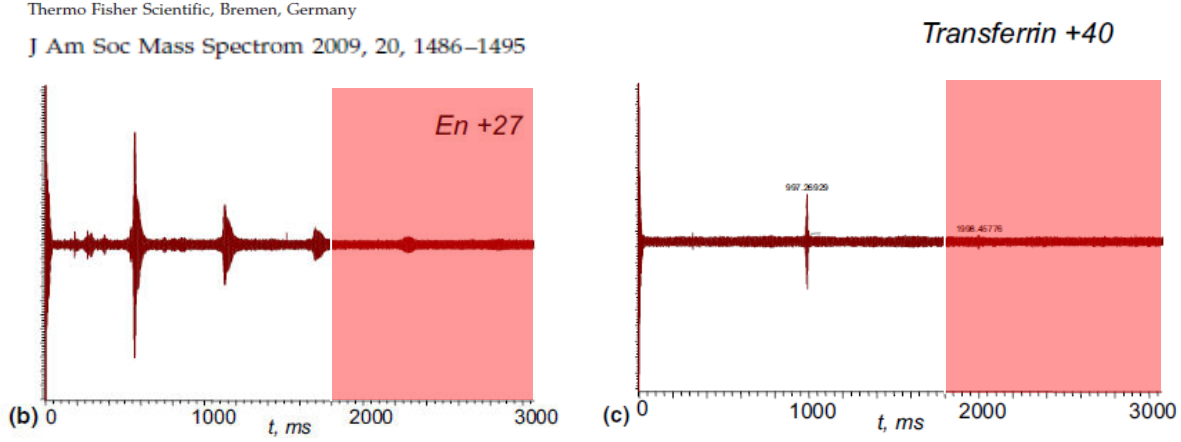
**Characterization of the Time-Domain Isotopic Beat Patterns of Monoclonal Antibodies in Fourier Transform Mass Spectrometry**

Konstantin O. Nagornov, Anton N. Kozhinov, Natalia Gasilova, Laure Menin, and Yury O. Tsybin*

**Dynamics of Ions of Intact Proteins in the Orbitrap Mass Analyzer**

Alexander Makarov and Eduard Denisov
Thermo Fisher Scientific, Bremen, Germany

J Am Soc Mass Spectrom 2009, 20, 1486–1495



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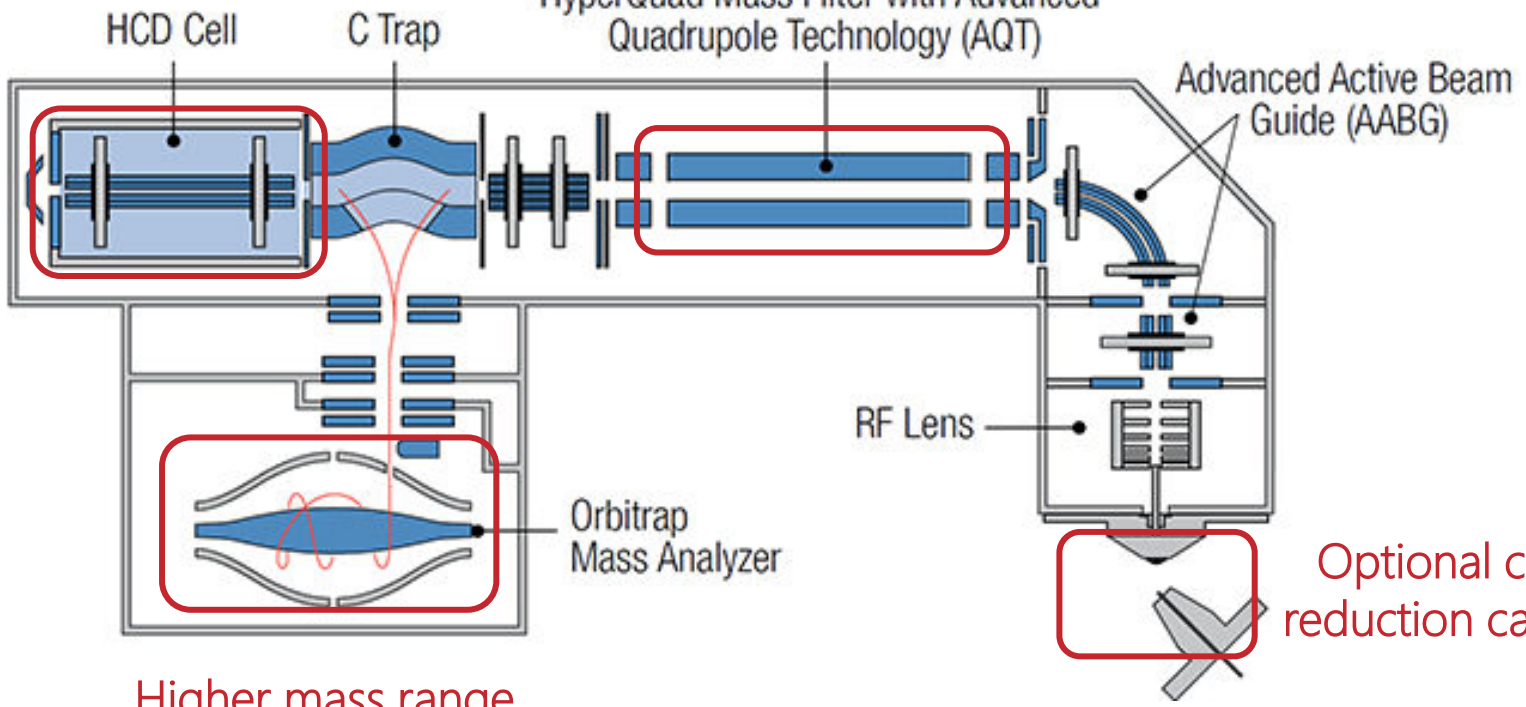
# How does the NativeQE differ from the original QExactive?

Pulsed gas valve for for improved HCD fragmentation

Improved mass range for Q1 – m/z 10.000

NativeQE
Helps you keeping an eye on biologics

Prepared for UVPD fragmentation




Optional charge reduction capability

Higher mass range for detection



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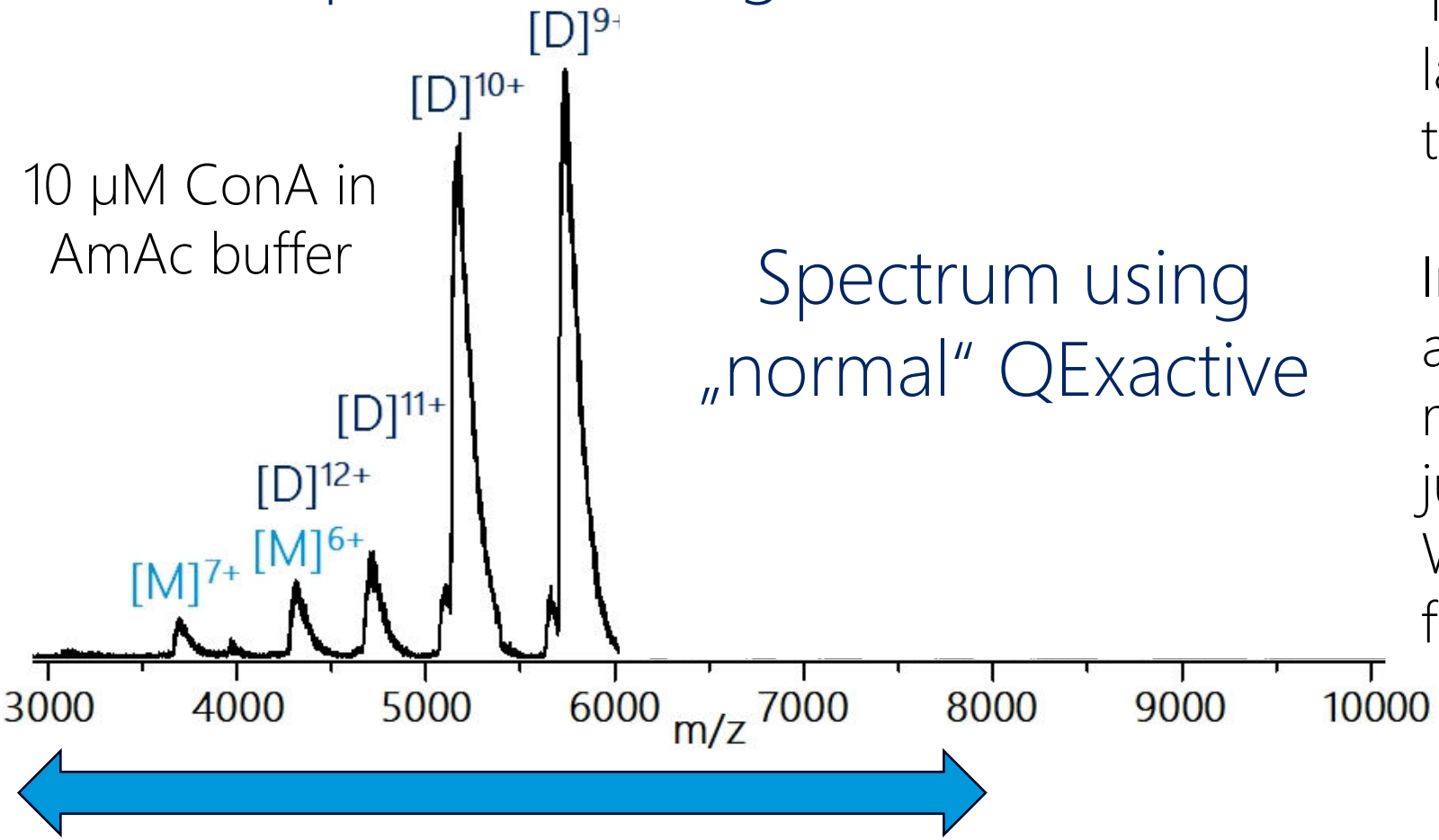
# NativeQE – enhanced detection mass range and nativeMS

## Spectrum using NativeQE




10 µM ConA in AmAc buffer

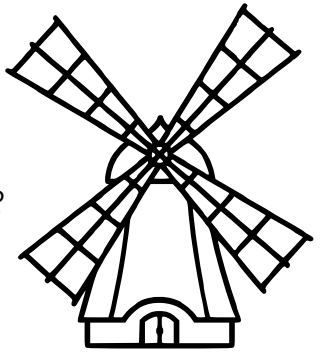
Spectrum using „normal“ QExactive

typical mAb detection mass range

The NativeQE has a significantly larger detection mass range than the regular QExactive.

In native MS, monoclonal antibodies have their charge maximum at around m/z 6.000, just at the limit of the QExactive. With NativeQE you will get the full picture!

*The Netherlands. Creators of space since the 16th century.*





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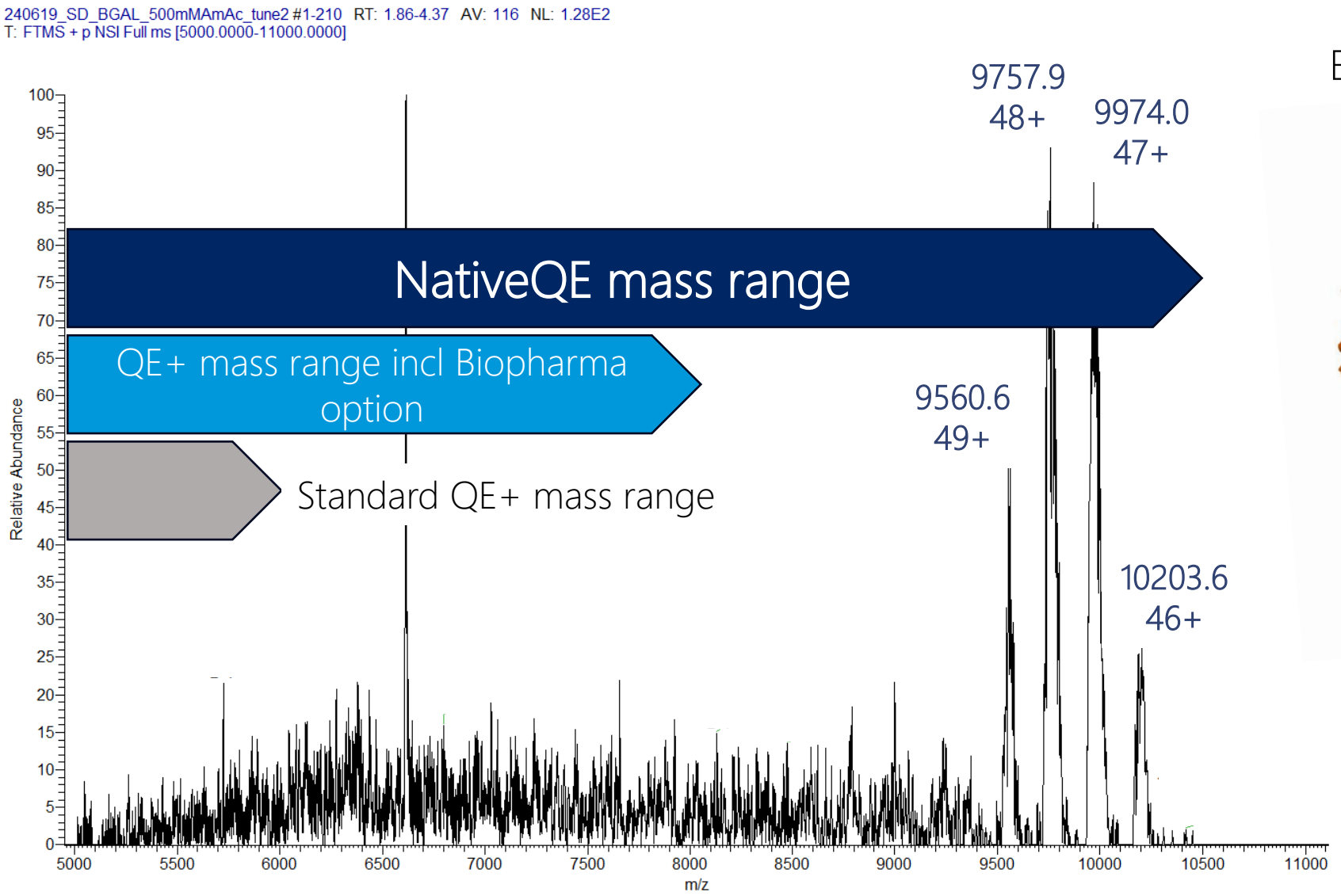
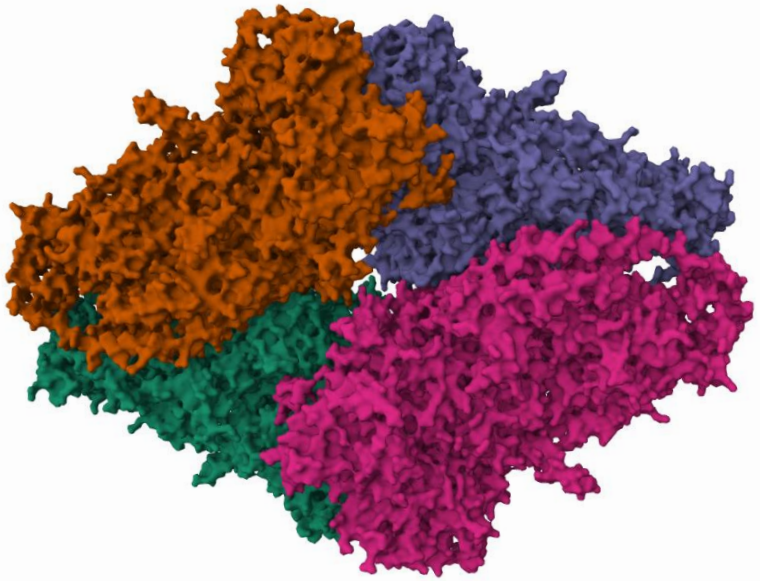
# How far can we go in mass?




E. Coli β-Galactosidase tetramer



464.477 Da



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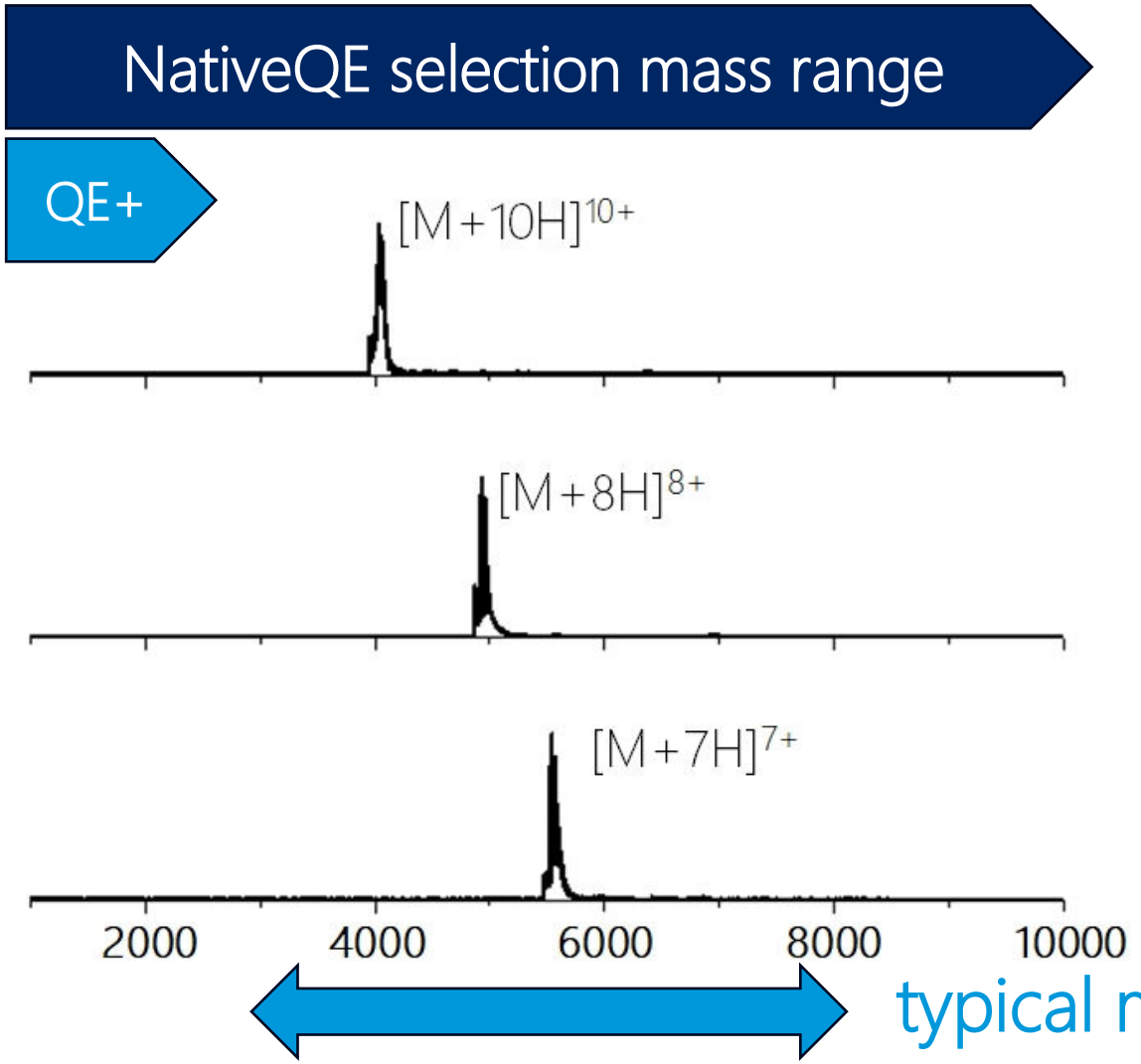
# High mass isolation – making QExactive fit for biologics



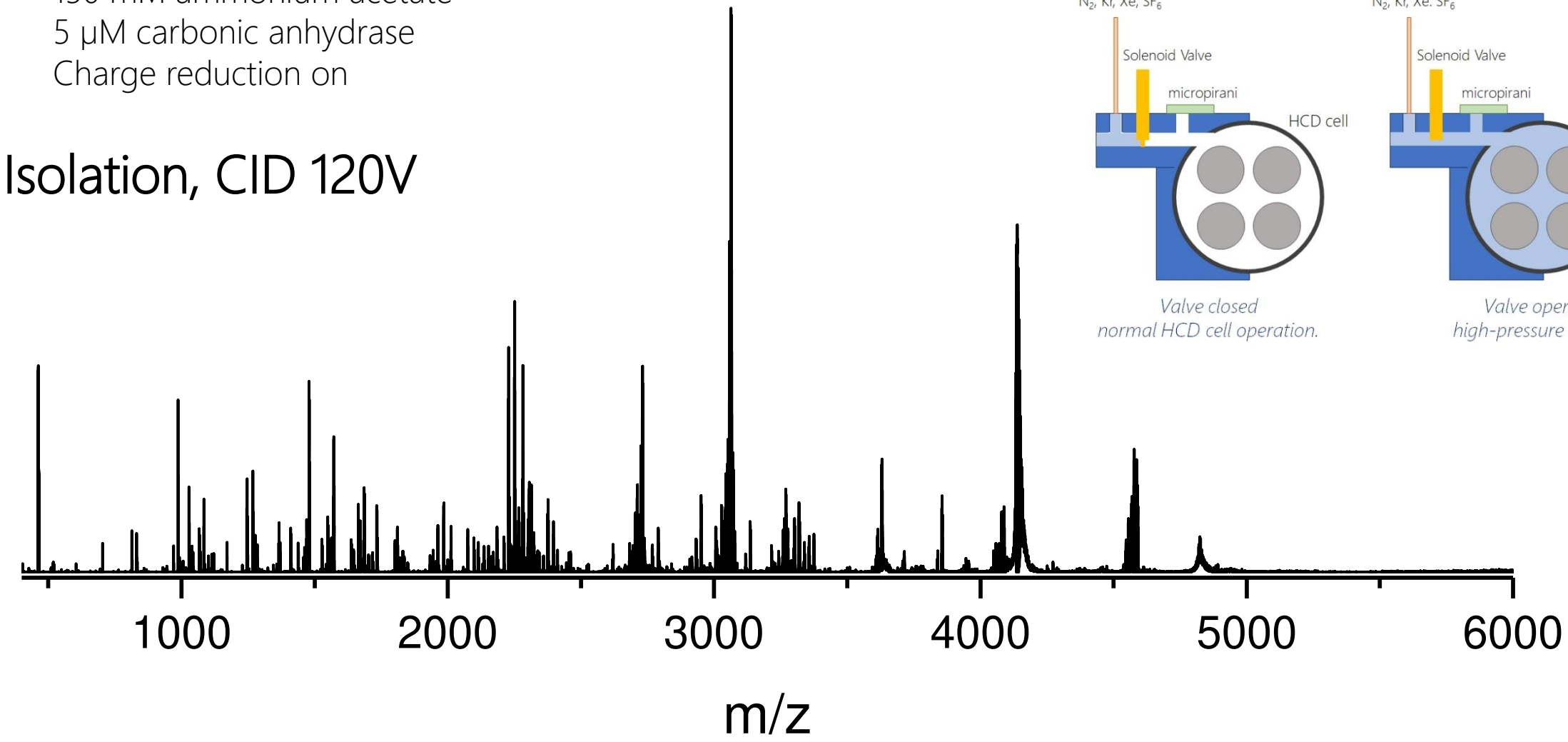

Isolation of different charge states of ovalbumin (~40 kDa) produced using nanoESI and charge reduction.

The high mass quadrupole isolates the individual charge states up to m/z 10.000

# Top down fragmentation - Carbonic Anhydrase (29kDa)

150 mM ammonium acetate
5 µM carbonic anhydrase
Charge reduction on

Isolation, CID 120V









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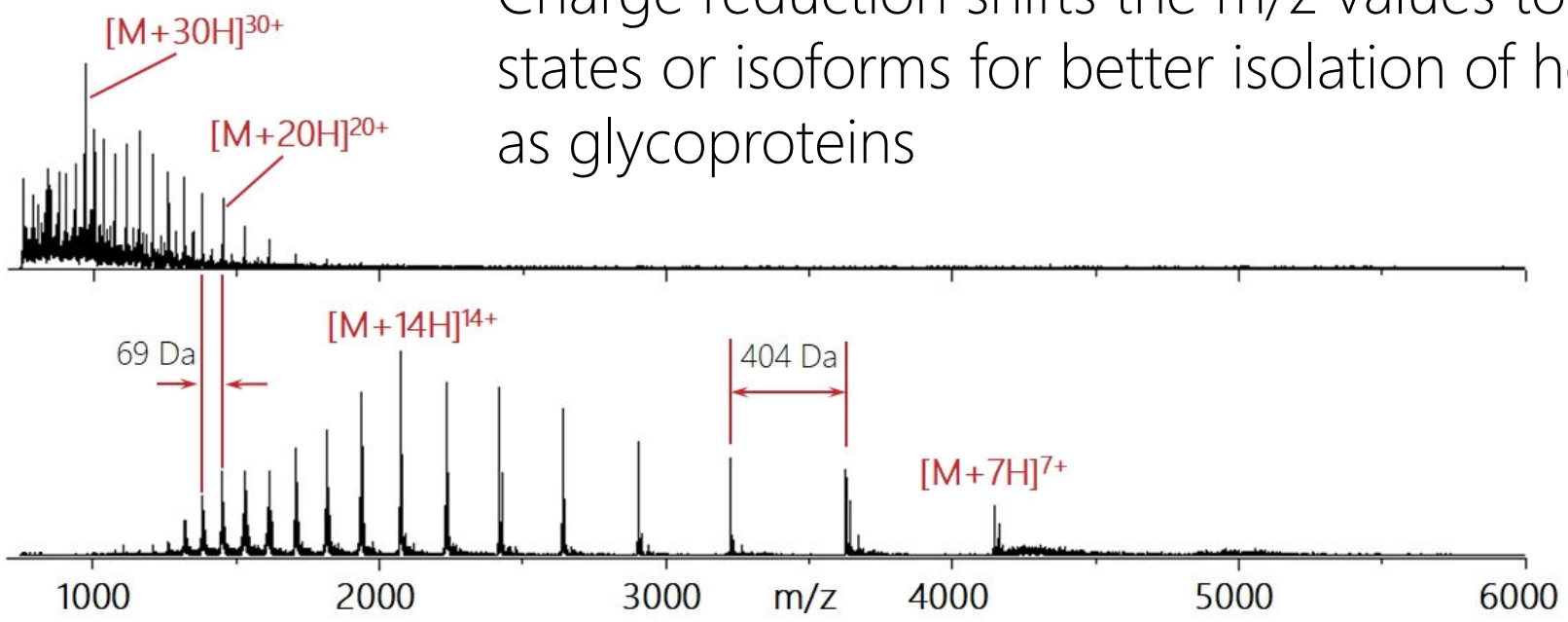
# Optional charge reduction – creating space for ions

The optional charge reduction offers two main advantages:

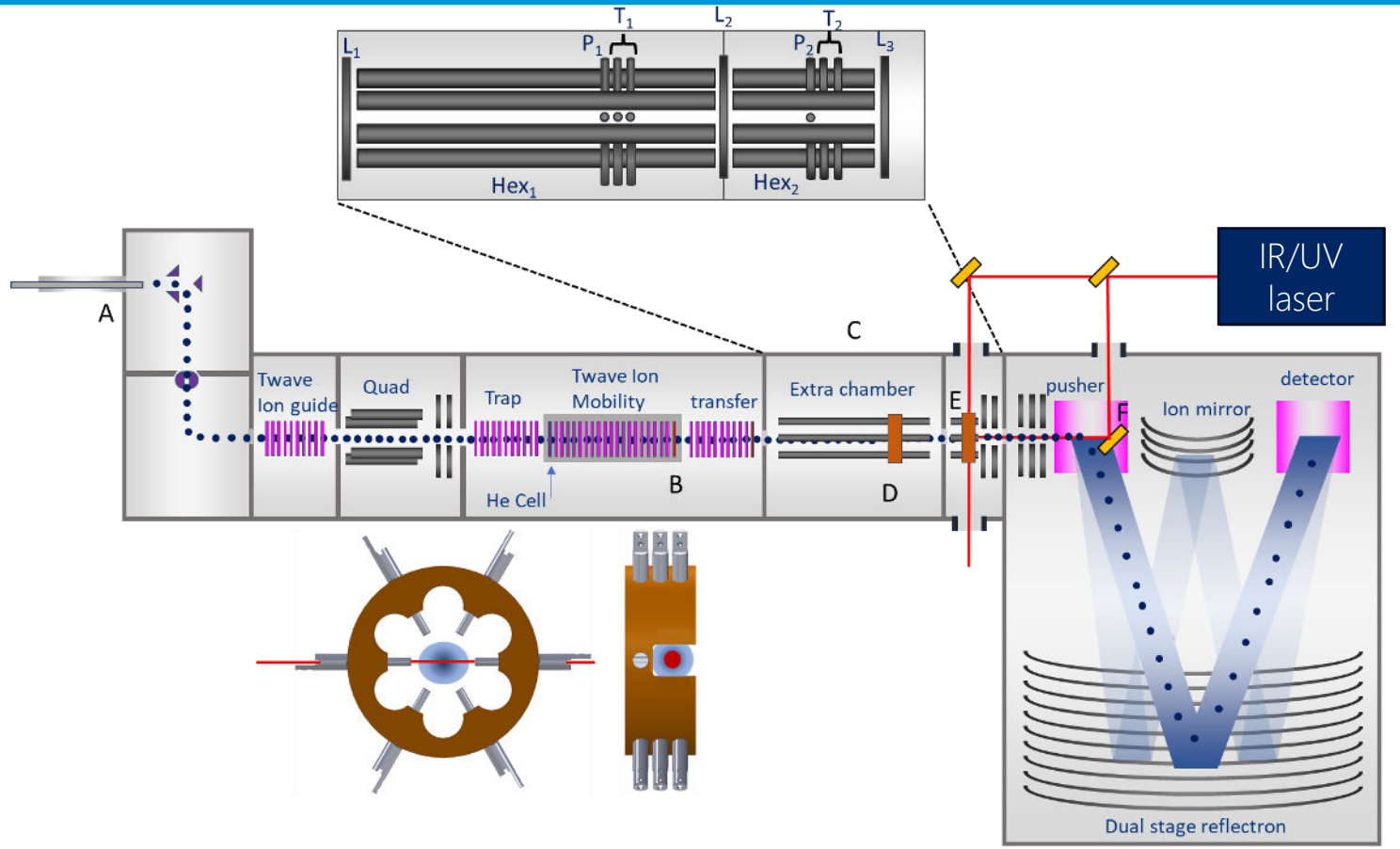
- ionization in acidic buffers is more efficient than under native MS conditions. Charge reduction preserves the ionization efficiency while reducing the charge state.
- Charge reduction shifts the m/z values to improve separation of charge states or isoforms for better isolation of heterogeneous molecules such as glycoproteins




*The Netherlands. Creators of space since the 16th century.*

# How to combine optical spectroscopy with mass spectrometry?

# The Photosynapt

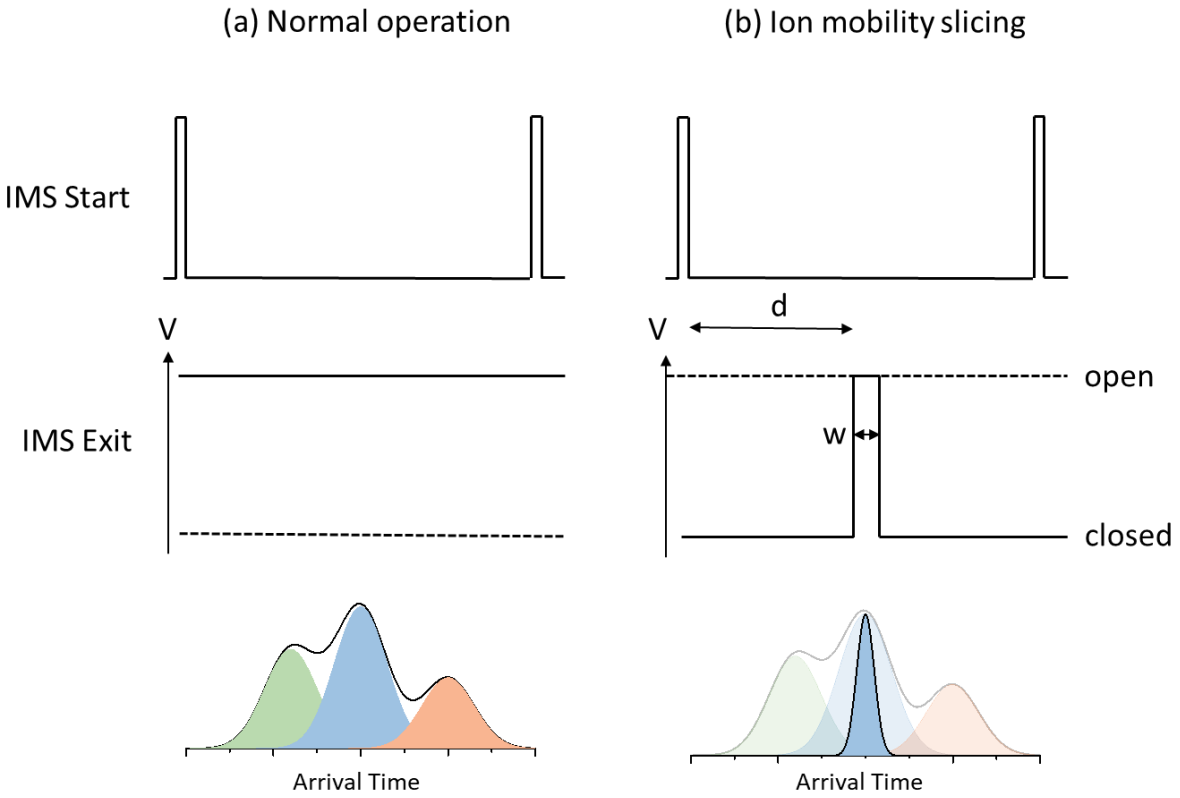



- Ion mobility slicing
- Dual ion traps for enhanced duty cycle
- Perpendicular irradiation (IR/UV) and on axis irradiation (IR or UV)

PhotoSynapt

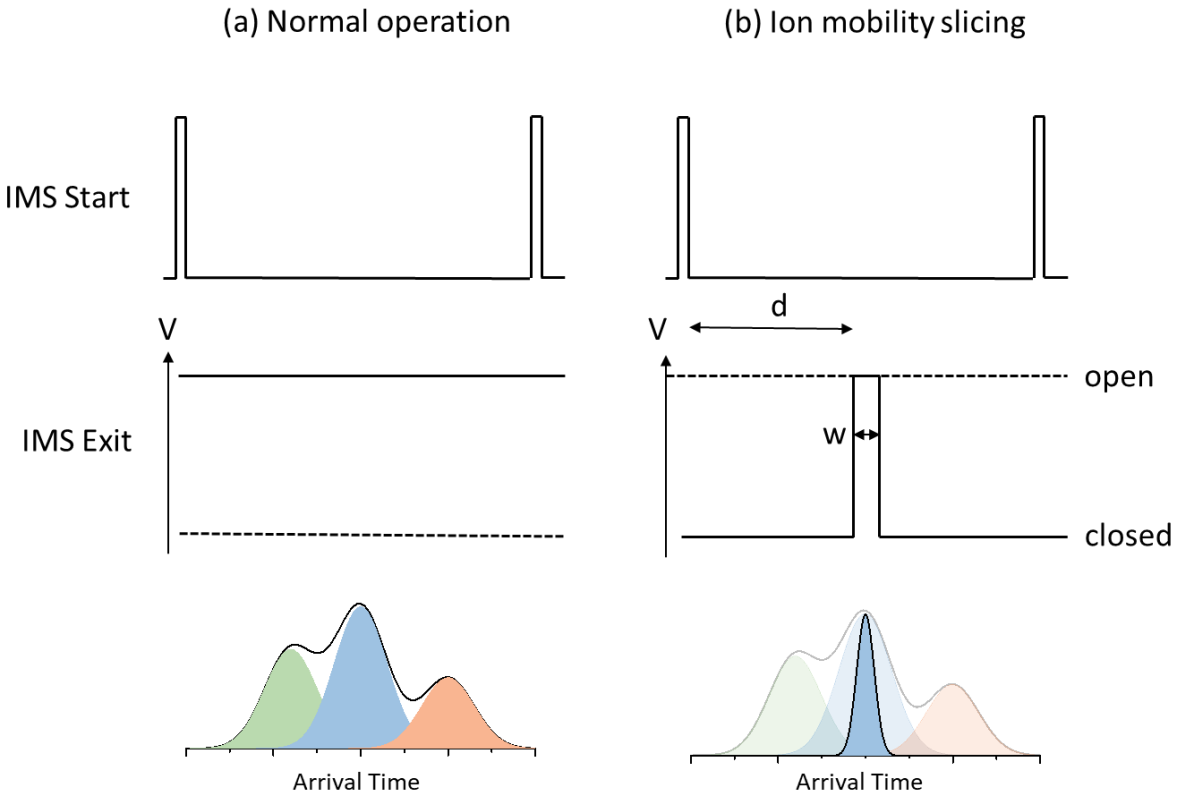
A. Pressure Sleeve.
B. Ion Mobility Slicing
C. Additional Vacuum Chamber
D. First Hexapole and pin trap
E. Second hexapole and pin trap
F. IR/UV Optical Access
G. IR Optical Access and Gold Mirror

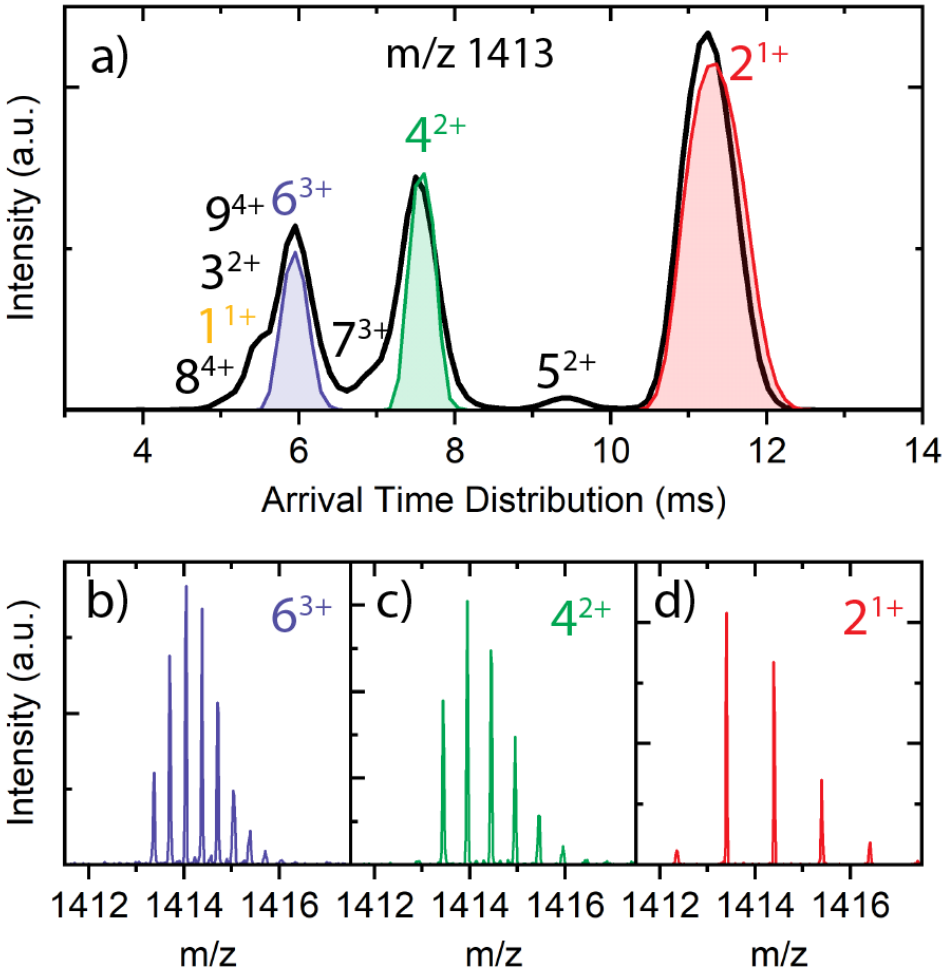
# Ion Mobility Slicing




- Normally, in IMS mode you always acquire a mobilogram (intensity versus drift time)
- Ion mobility slicing allows you to cut and accumulate specific ion species at distinct migration times (similar to fraction collection in LC or heart-cutting in GC)
- These selected ion species can subsequently be analysed by optical spectroscopy and/or mass spectrometry
- This allows to separate sterical isomers, protein conformations, etc.

# Ion Mobility Slicing in action – VEALYL oligomers

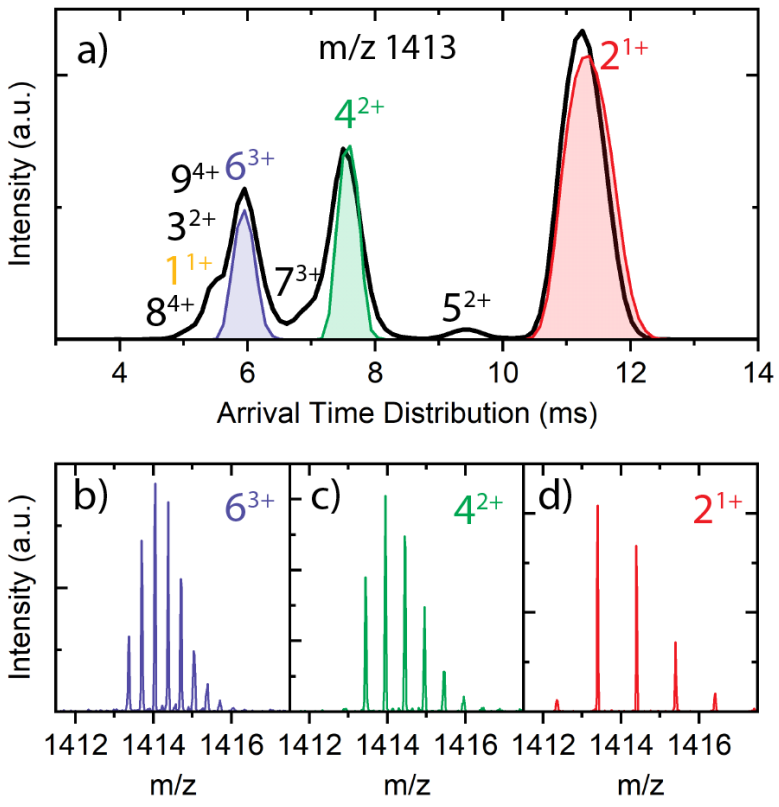


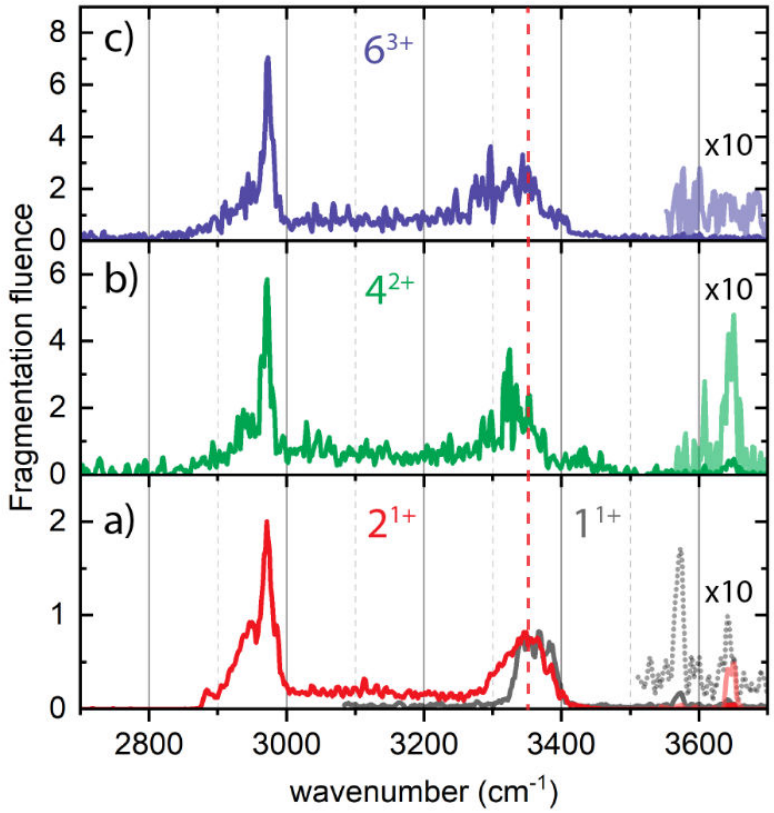

Data courtesy of Anouk Rijs

# Mobility resolved IR ion spectroscopy – VEALYL oligomers

Ion mobility




IRMPD




Data courtesy of Anouk Rijs

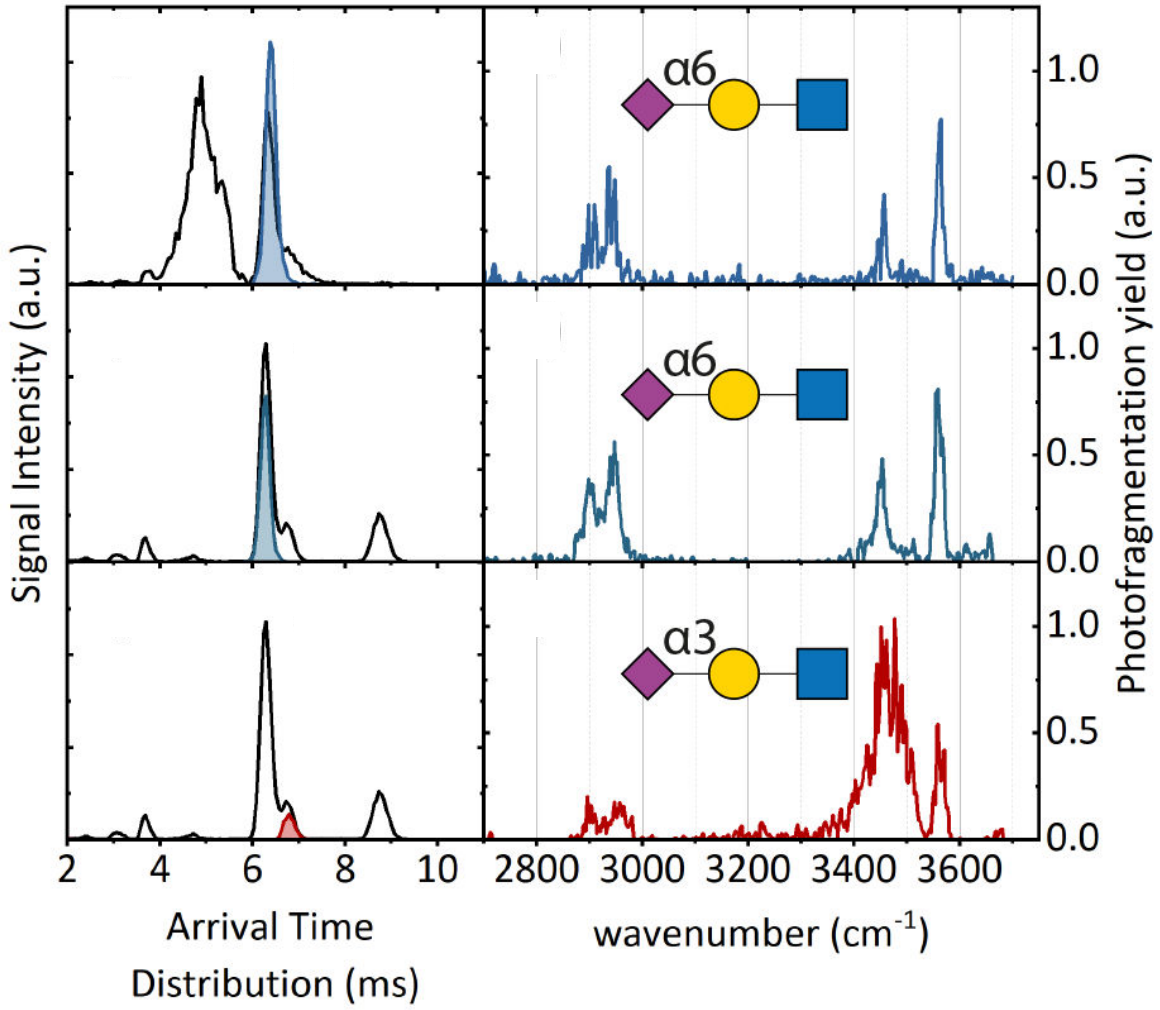
# Photosynapt Results: Distinguishing isomeric glycan structures

Glycans cleaved from: m/z 657

Transferrin
(95% α6)

hAGP
52:48 α6: α3

hAGP
52:48 α6: α3




B3 of glycan from the two proteins generated by in source dissociation.

Mass selected in quadrupole

Mobility selected using IMS slicing

Probed using IRMPD to record IR spectra.

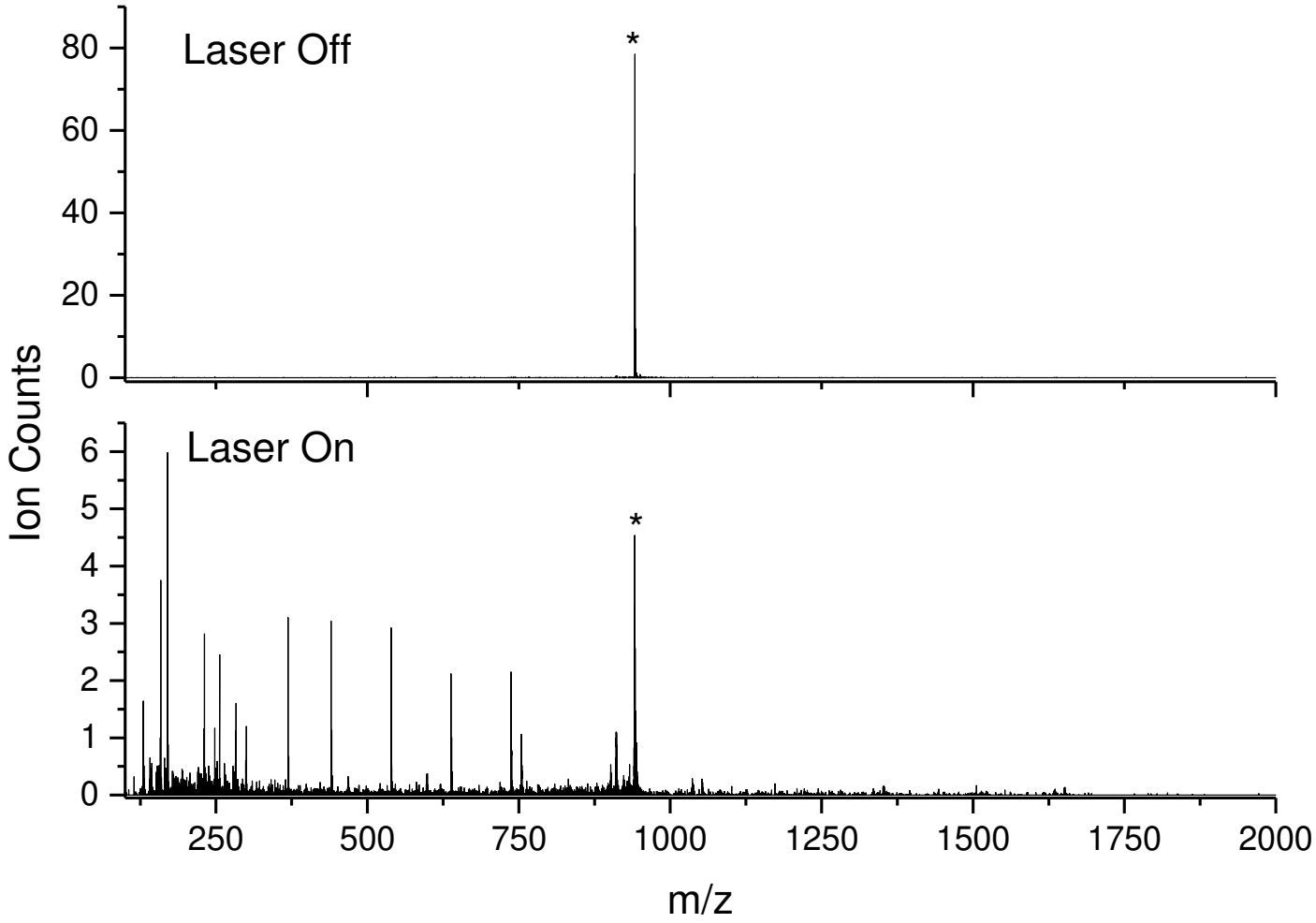
Clear differences observed in the spectra of α6 and α3 linked sialic acid – particularly in H bonding region.

Sjors Bakels,Melissa Bärenfänger, Anouk Rijs

# Results – UVPD @ 213 nm

No mobility selection
1 second trapping time

Gramicidin 2+






MSVision
Dedicated to Mass Spectrometry

# Going from pin traps to cryotraps

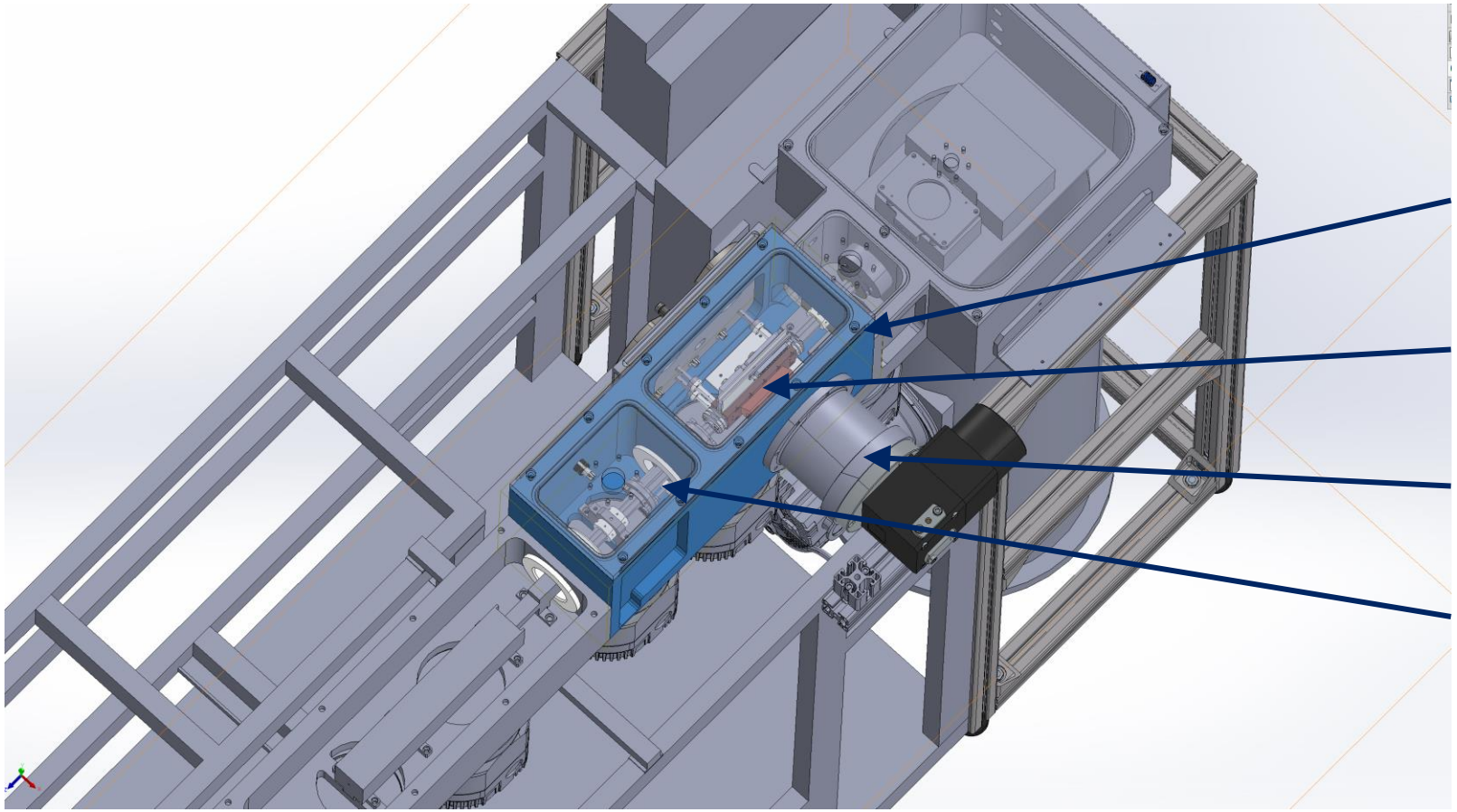
- Ion mobility and time slicing as before
- Ions are tagged with nitrogen, and loss of nitrogen due to absorption of photons used as action for spectroscopy rather than fragmentation.
- Detection of a mass loss of 28 Da caused by vibrational excitation much easier to observe than fragmentation as less photons are required. But: cryogenic temperatures needed (~40K)

→ Gain in sensitivity

→ Used for cryogenic IR action spectroscopy

*Instrument almost completely tested in the lab in Almere*

# How to get from regular traps to cryo-traps?



Additional vacuum chamber

IsoSpec Cryotrap

Cryo cooler

Transfer optics

# Be an MS Visionary!