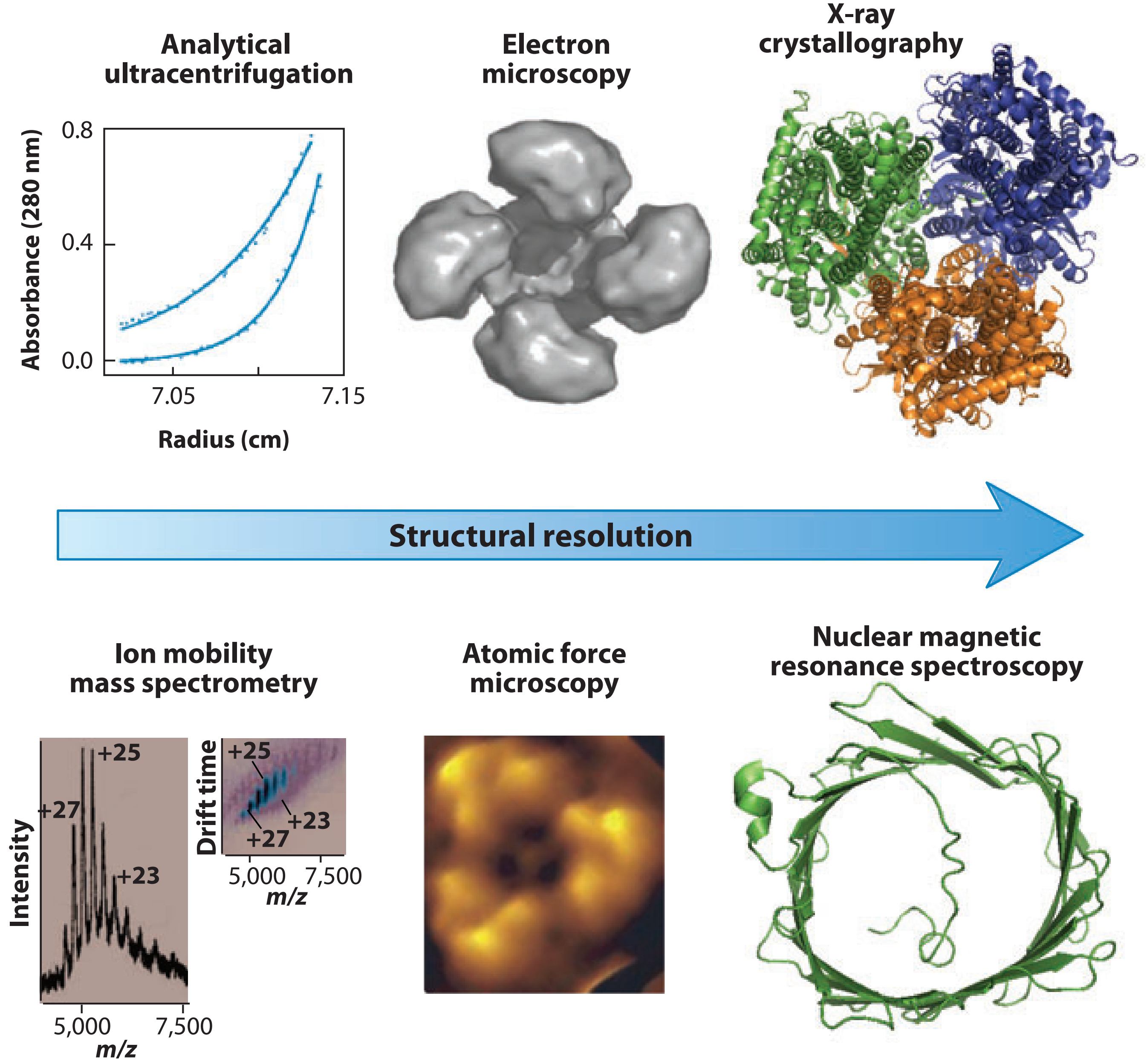
Mass Spectrometry

from observation to structural information

Marc-André Delsuc
Renafobis - 2018

cnrs
Inserm
UNIVERSITÉ DE STRASBOURG
CASC4DE
igbmc



Emerging mass spectrometry (MS) methods for studying membrane protein complexes and their relationship to other biophysical techniques

from N.Barrera, C.Robinson
Annu. Rev. Biochem. 2011. 80:247–71

PLAN

- Principles of Mass Spectrometry
  - measuring m/z
  - ionisation methods
  - shape of a MS spectrum of a protein
  - fragmentation methods

- Examples of use in Structural Biology
  - Large multicomponent Complexes
  - Ligand binding
  - H-D exchange and other chemical labelling
  - Cross-Linking
  - Ionic Mobility

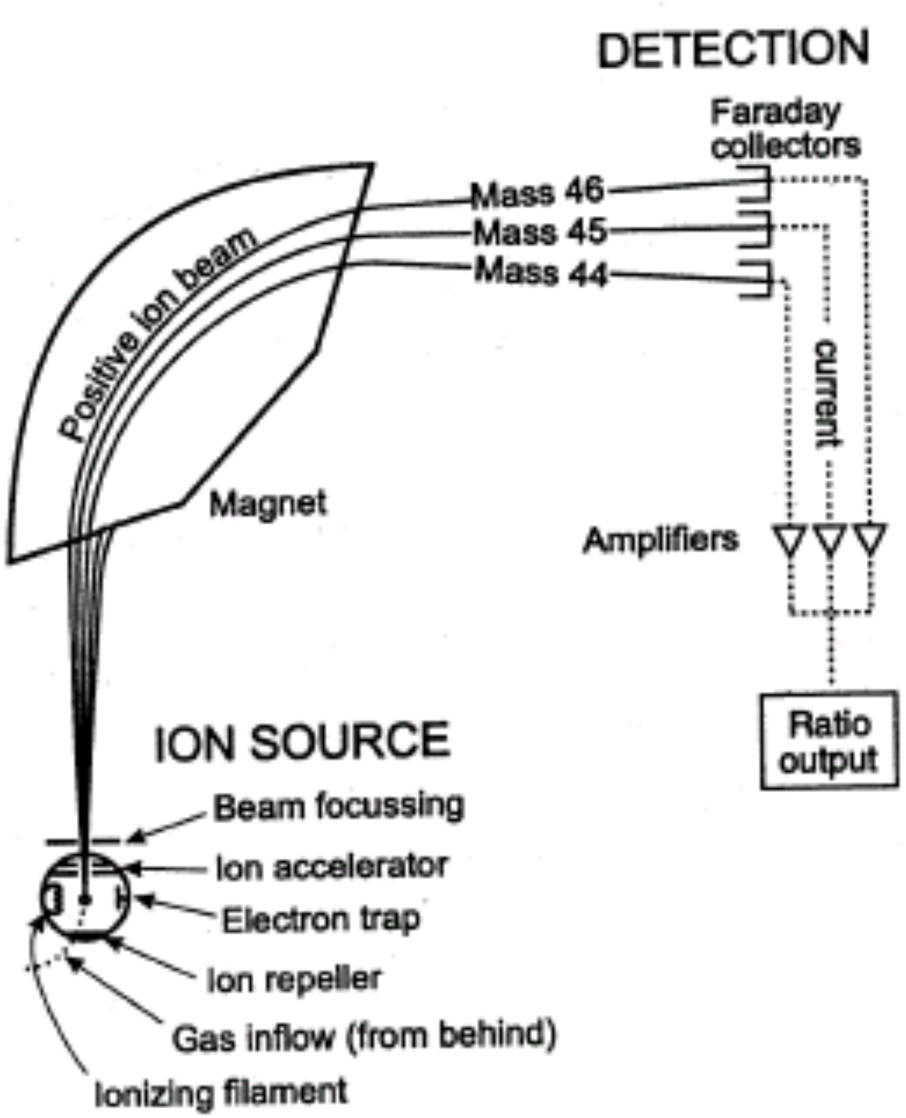
- 2D MS
  - … teasing you ….

Principles of Mass Spectrometry

- a charged molecule
- a fly in the vacuum
- the trajectory in inflected

$$\vec{B} \quad \vec{E}$$

- a detector senses the ion
- the sensing allows measuring the molecular mass



Basis

- We need ions in vacuum

- 3 fundamental steps
  - ionisation / separation / detection

- Electrostatic/electrodynamic interactions
  => we measure ONLY *m/z*
  - not just *m*

- m unit = 1 Dalton : 1atom-gram
  - definition 1/12 mass of ^{12}C atom
  - 1 Da = 1.66 10^{-27} kg

- m/z unit : 1 Thomson = 1Da / $1e^-$

Detection

- There is a large range of approaches for separating ions, in all cases:
  - the ion in vacuum flies in E and B fields
    - homogeneous or varying in space
    - static or varying in time
  - E and B field apply forces to the ion, - proportionnal to the charge : z
  - the ion follows Newton law - depend on the mass m
  - the displacement is dependent on the mass and the charge
  - only m/z can be measured

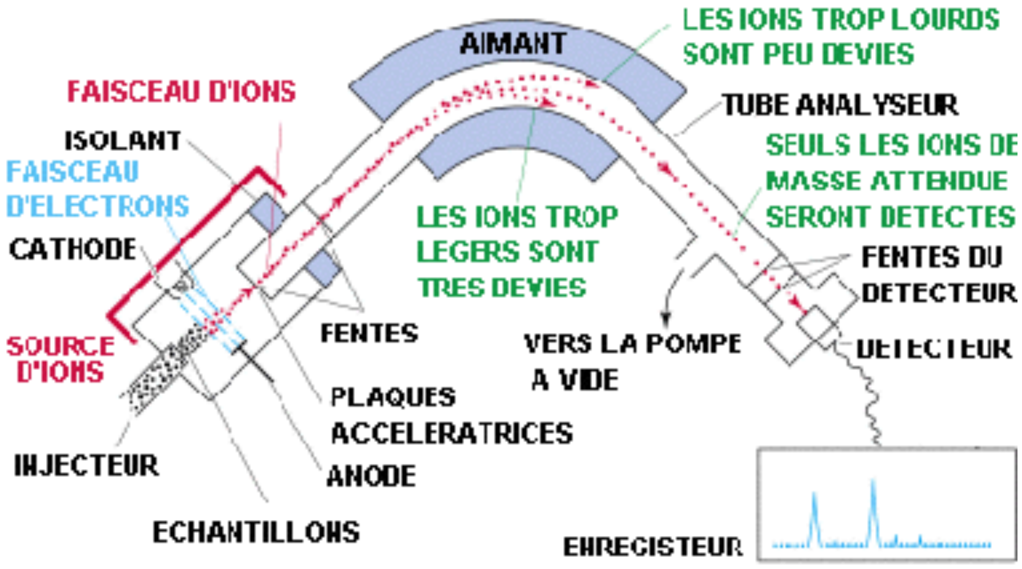
- different measurement methods
  - sector instruments
  - Time Of Flight
  - Quadrupole
  - Ion Trap
  - Orbitrap
  - Ionic Cyclotronic Resonance

Equations of motion

- Electrostatic force $\vec{F}_e = q\vec{E}$
- Lorentz force $\vec{F}_L = q\vec{v} \wedge \vec{B}$
- Ponderomotive force $\vec{F}_p = -\frac{q^2}{4m\omega^2}\nabla E^2$
- Newton law $\vec{F} = m\vec{\gamma}$

sector instrument

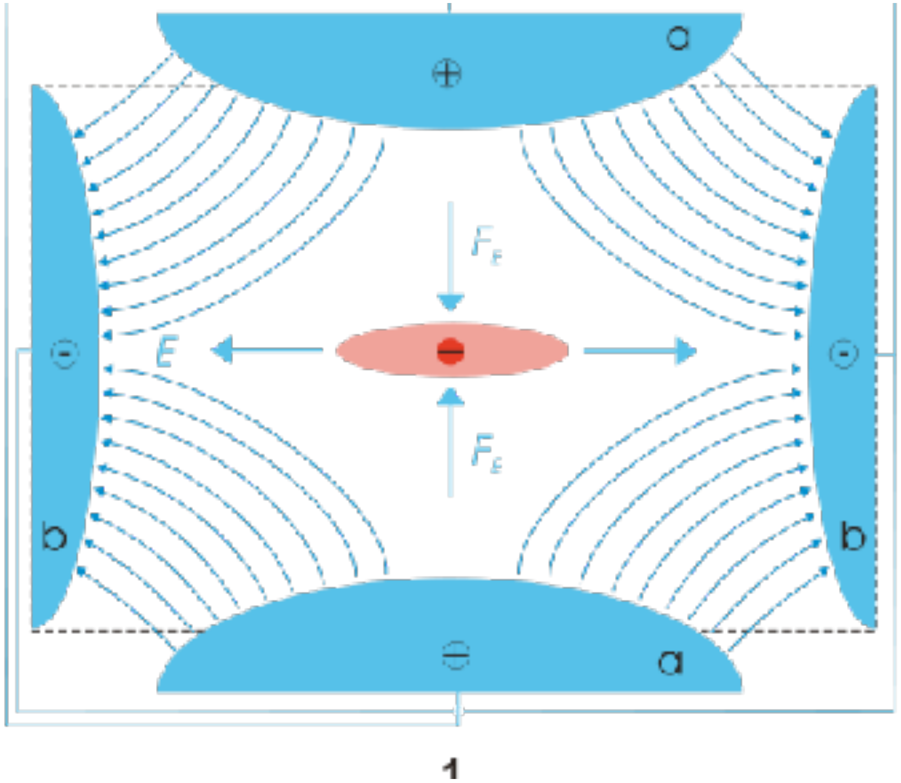
- Principle
  - simplest
    - deflection by a homogeneous, static E or B field
    - m/z is measured by the position of the impact
  - improved
    - deflection by a homogeneous, time varying E or B field
    - m/z is measured by the time of the impact at a given point
  - both field can be used to improve resolution
  - not really used any more



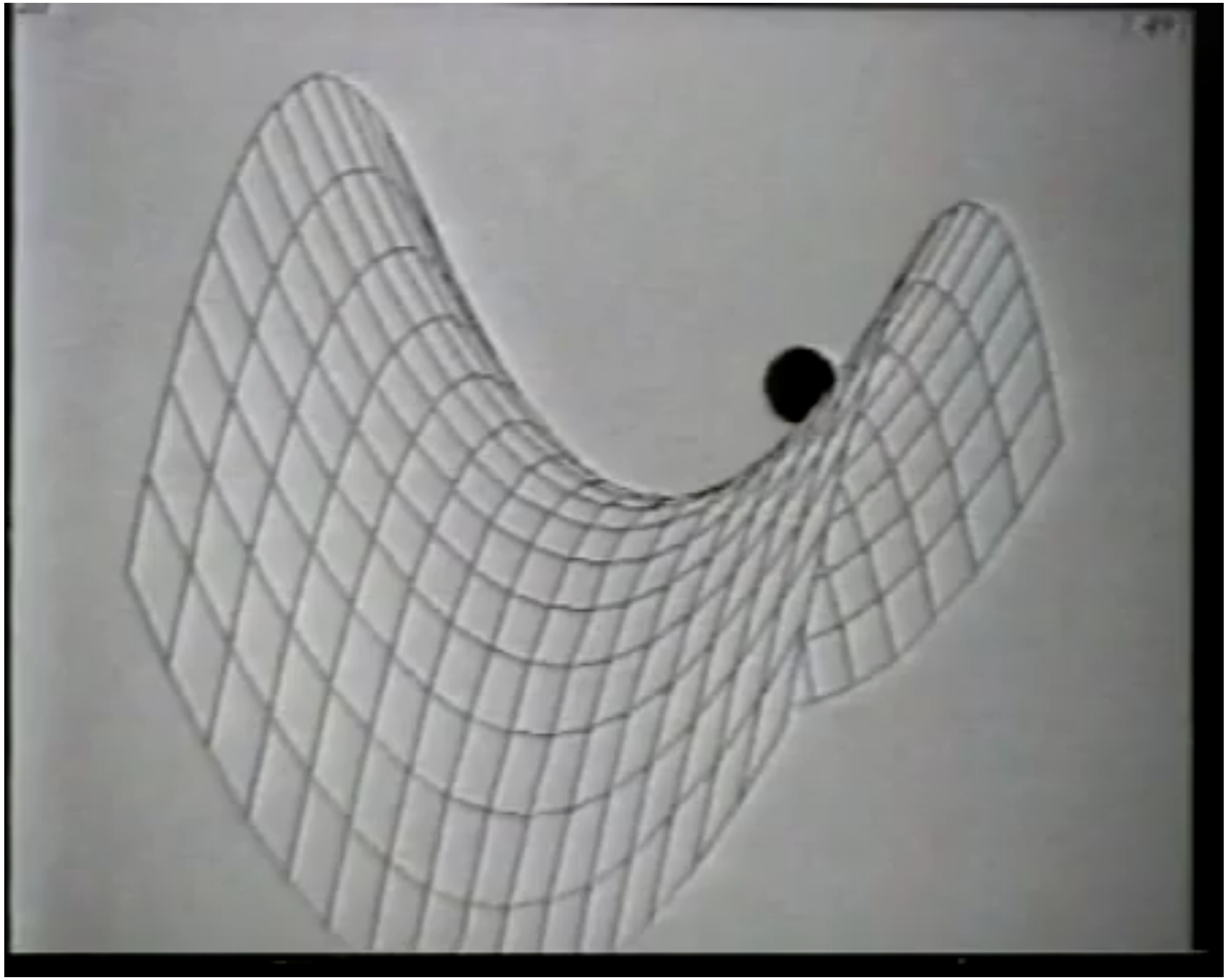
Paul trap

$$\phi_o = U + V\cos\Omega t$$

$$\phi_{r,z} = \frac{\phi_o}{r_o^2}(r^2 - 2z^2)$$



Wolfgang Paul - 1989 Physics Nobel Prize

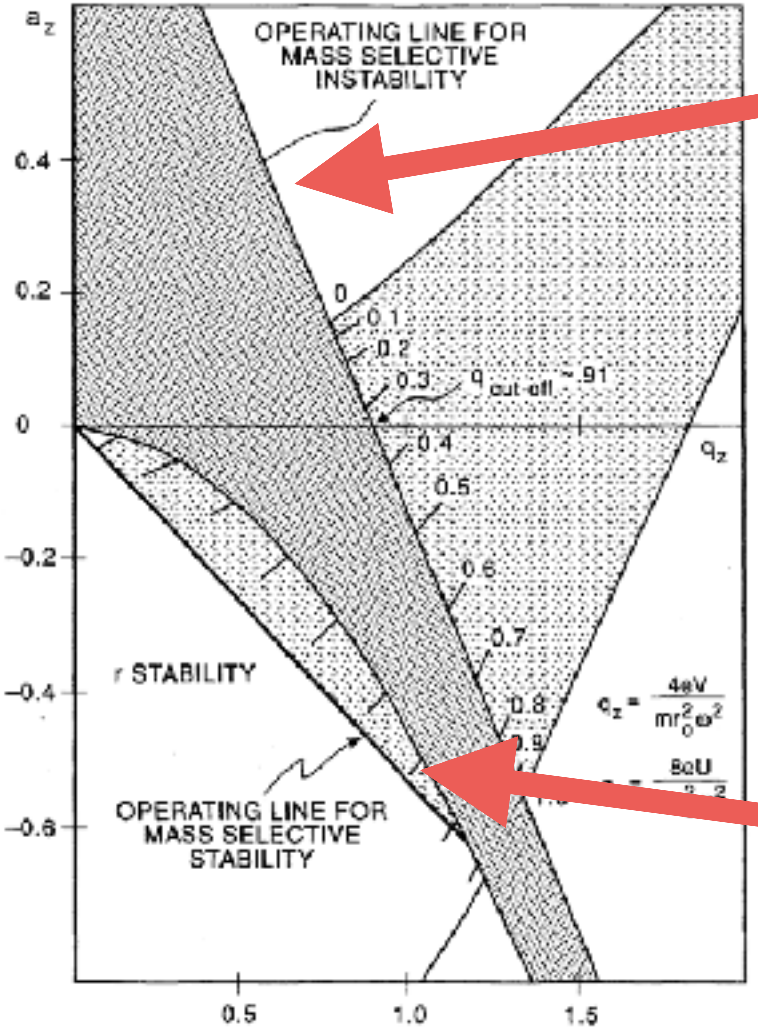


stability zones

$$\phi_o = U + V\cos\Omega t$$

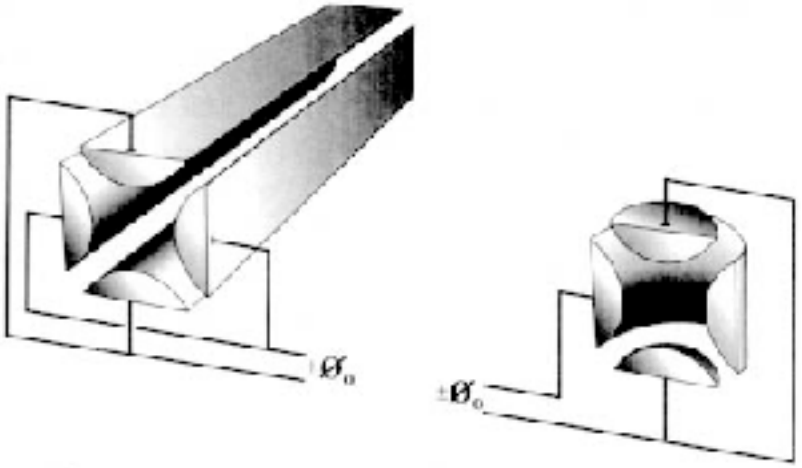
$$a = \frac{8qU}{mr_o^2\Omega^2}$$

$$b = -\frac{4qV}{mr_o^2\Omega^2}$$



A Trap

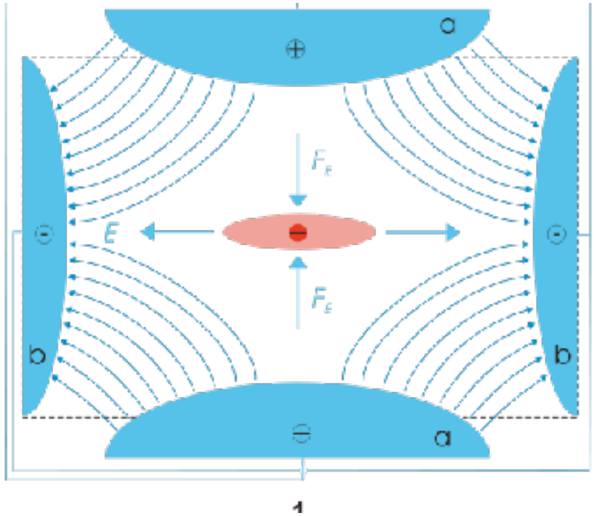
A Quadrupole

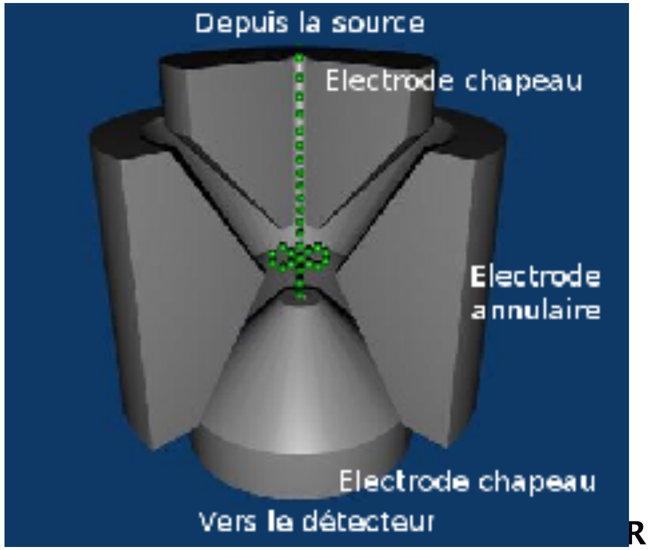


Ion trap

- unstable trajectory in a fluctuating E field
  - stable trajectories are trapped into a cell
  - some trajectories are **unstable** (depending on m/z)
  - sweeping the frequency ejects ion relative to m/z value

- in practical
  - Allows storing ions for some time
  - resolution is not very high

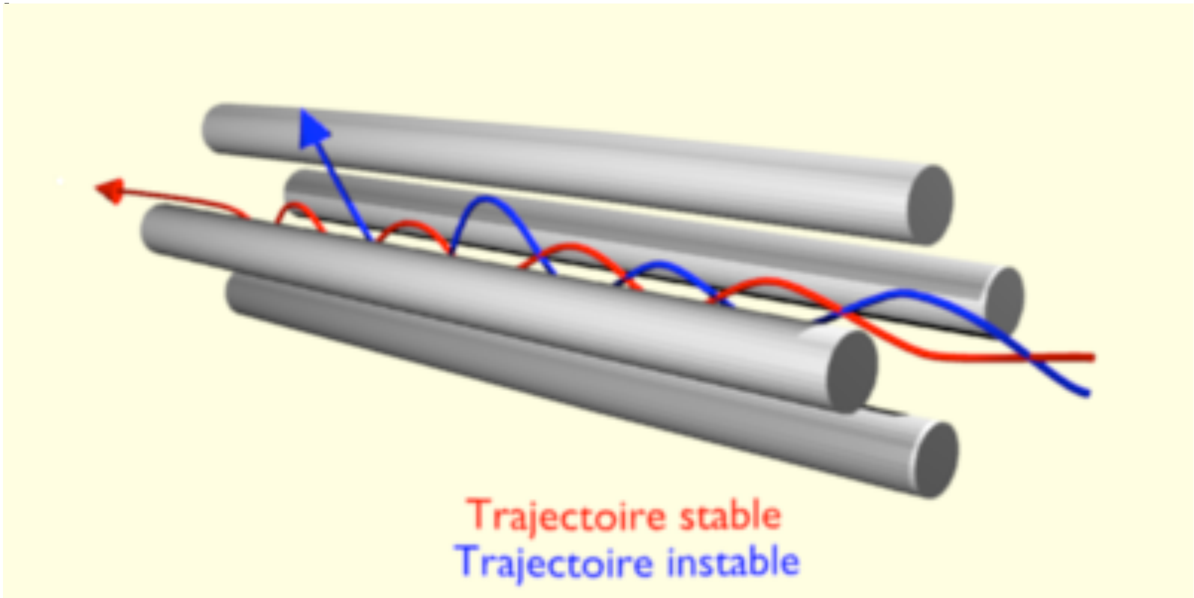




Quadrupole

- resonant trajectory in a fluctuating E field
    - some trajectories are **stable** (depending on m/z)
    - the frequency of the E determines m/z of the stable trajectory
    - sweeping the frequency

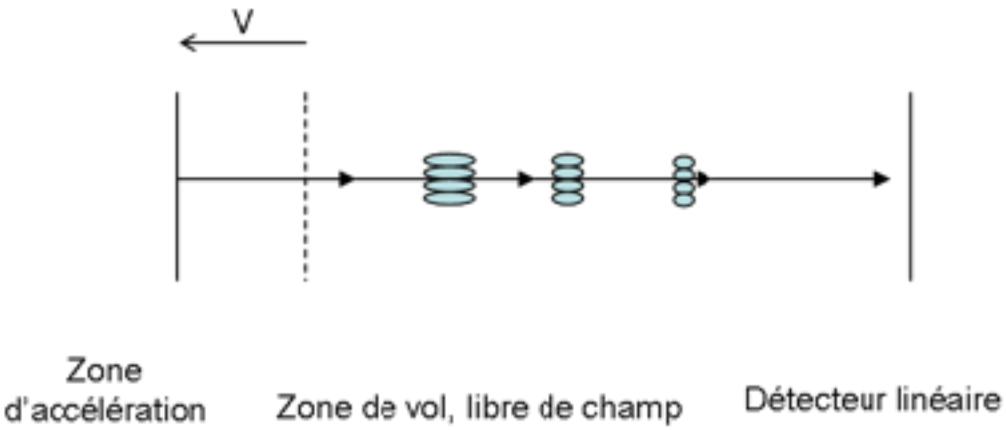
- improvement
    - longer quadrupo
    - higher tensions
    - hexapoles



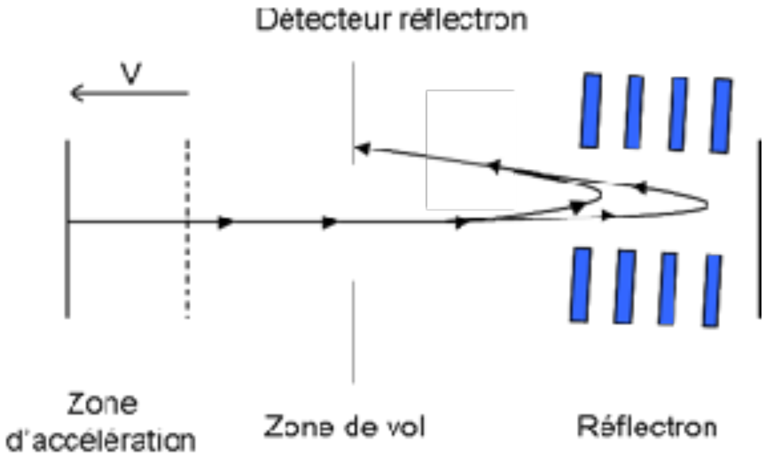
Time Of Flight

$$E_c = qU = \frac{1}{2}mv^2$$

- Principle
  - ions are accelerated by E field
  - ions are injected at a given time (MALDI)
  - m/z is measured by the time it takes to reach the detector



- improvements
  - The longer the path, the higher the resolution
  - refocalisation of different energies
    - Use of reflection chamber



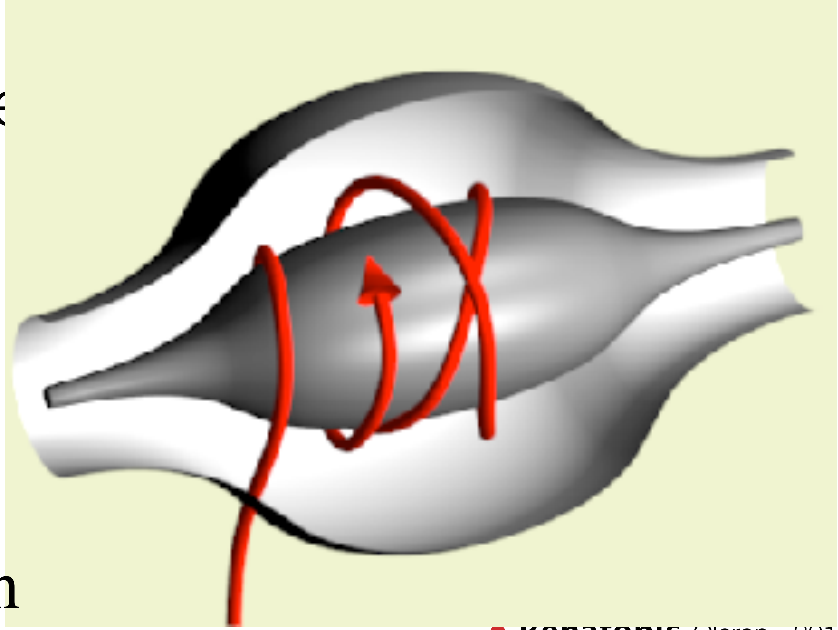
Orbitrap

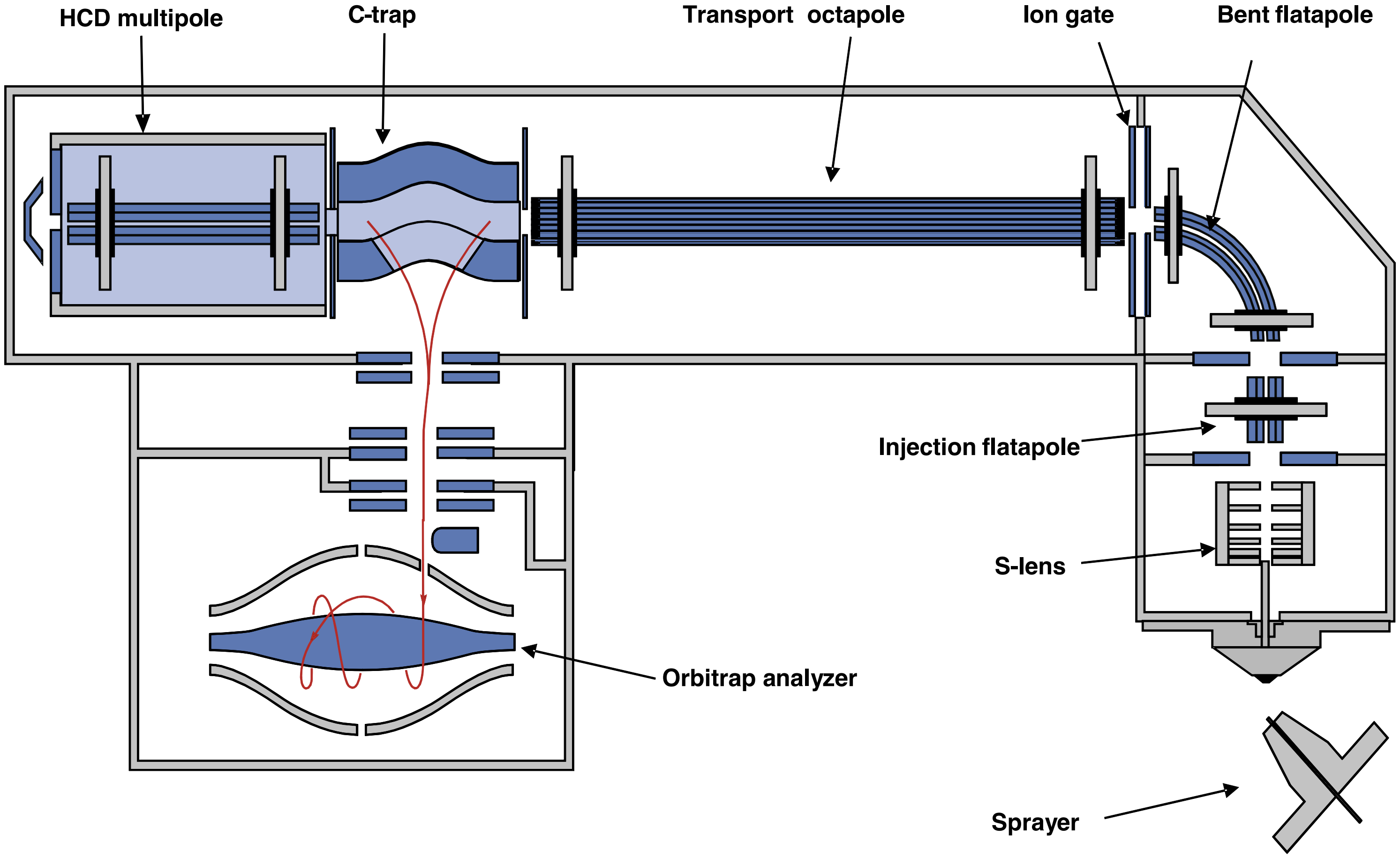
- Stable ion orbits into a Electrostatic cell
  - static Eo
  - all ions are measured at the same time
  - orbit frequency depends on m/z
  - Fourier Transform gives frequency, thus m/z

- In practical
  - very high resolution and sensitivity
  - speed, sensitivity and resolution de
  - requires very high vacuum
  - patented by ThermoFisher

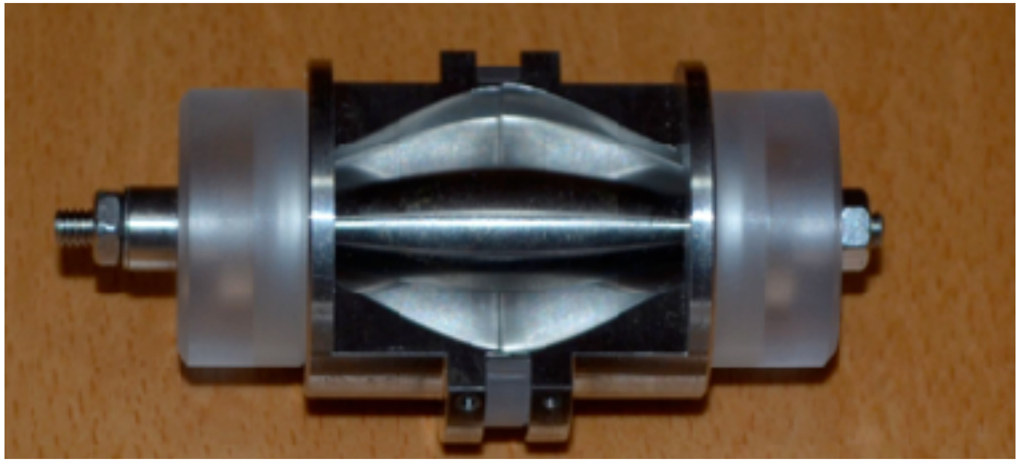
$$\frac{m}{z} \propto \sqrt{\frac{1}{f}}$$

Spectra obtained by Fourier Transform

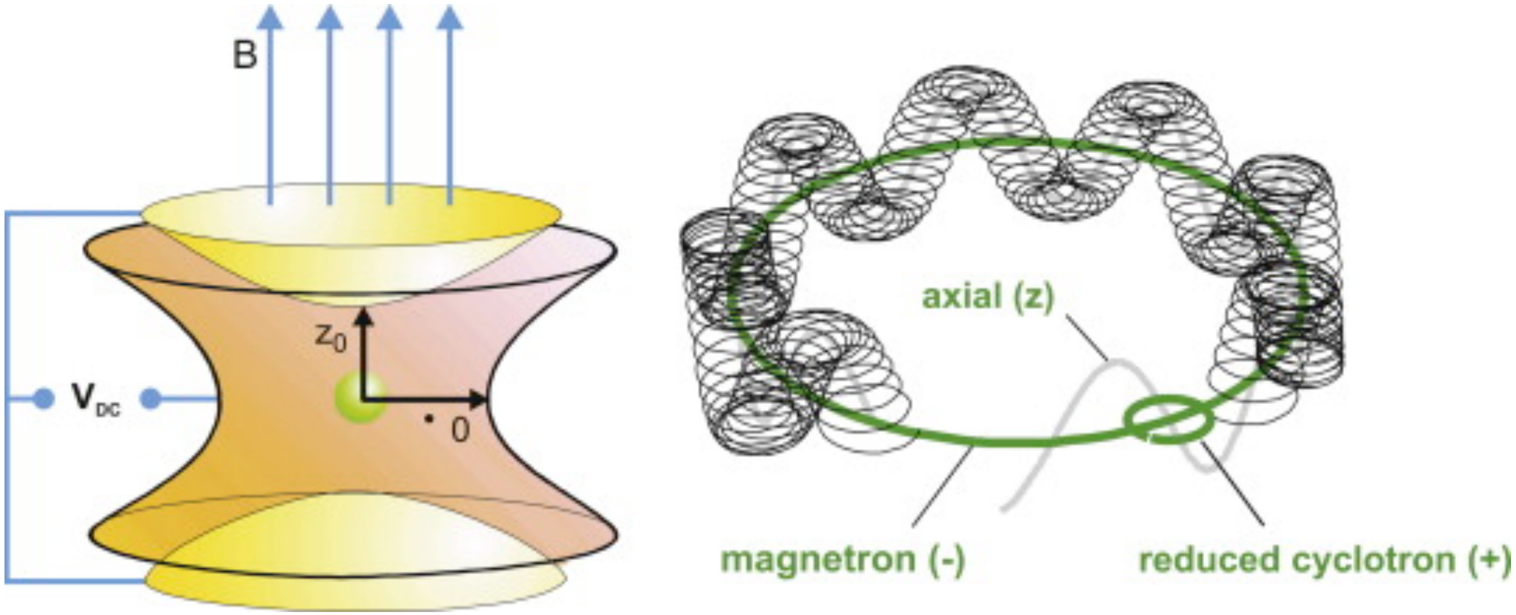




Supplementary Figure 1. Schematic of the modified Exactive Plus instrument (ThermoFisher Scientific, Bremen, Germany) with HCD option.



Pening Trap



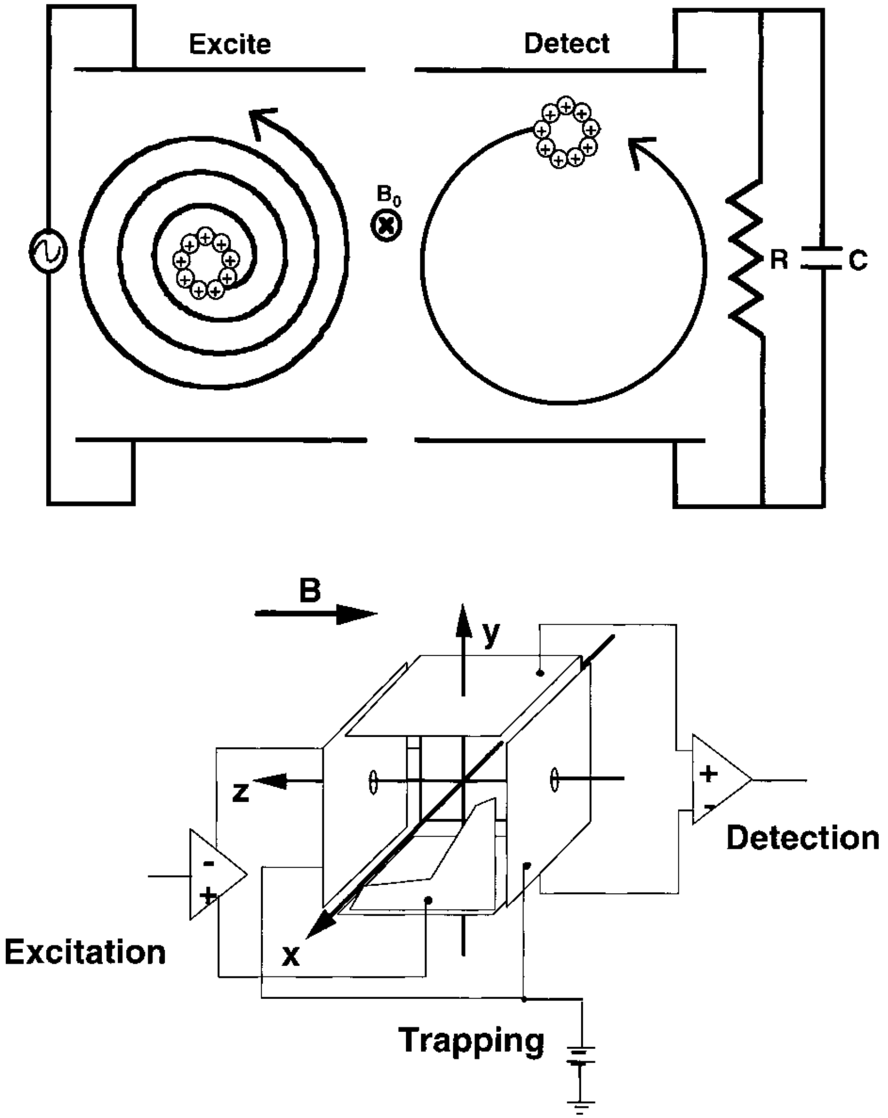


FIGURE 7. Incoherent ion cyclotron orbital motion (top left) is converted to coherent (and, therefore, detectable) motion (top right) by the application of a rotating electric field, which rotates in the same sense and at the ICR frequency of the ions of a given *m*/*z* value. The electronic circuitry is shown in the bottom diagram.

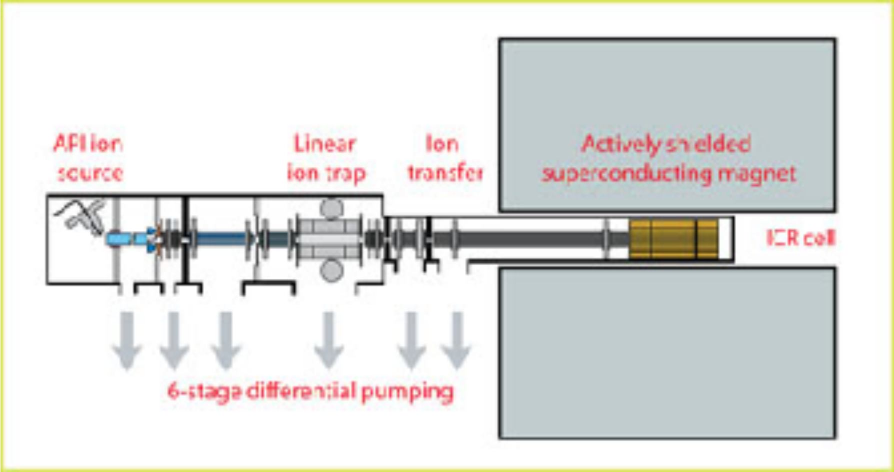
FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY: A PRIMER

Alan G. Marshall,*† Christopher L. Hendrickson, and George S. Jackson†
Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory, Florida State University, 1800 East Paul Dirac Dr., Tallahassee, FL 32310

Received 7 January 1998; revised 4 May 1998; accepted 6 May 1998

FT-ICR

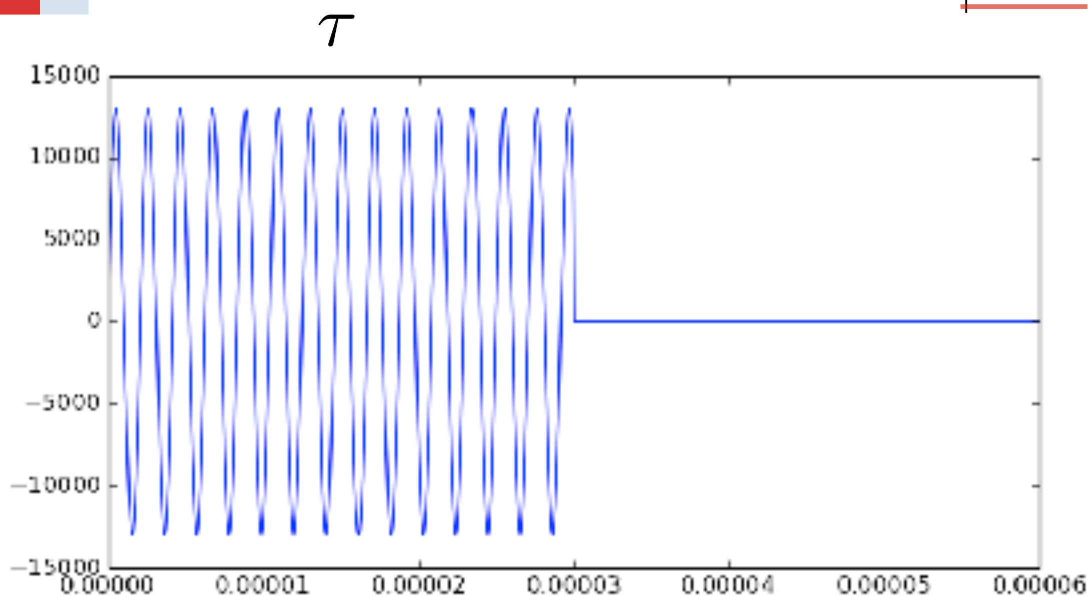
- Stable ion orbits into a magnetic field
  - static homogeneous Bo
  - all ions are measured at the same time
  - orbit frequency depends on m/z
  - Fourier Transform gives frequency, thus m/z
- In practical
  - very high resolution and sensitivity
  - speed, sensitivity and resolution depend on value of Bo
  - requires very high vacuum
  - high Bo requires cryomagnet

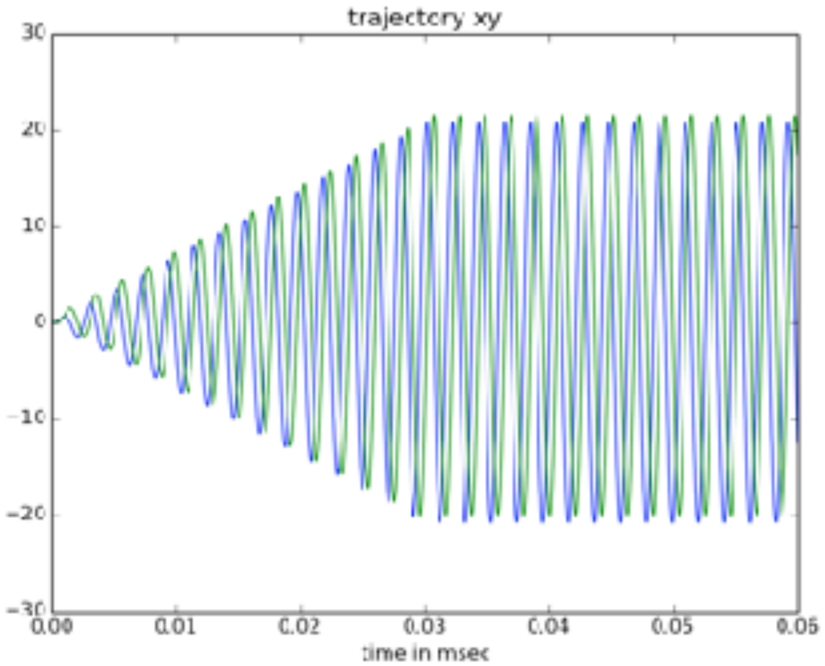


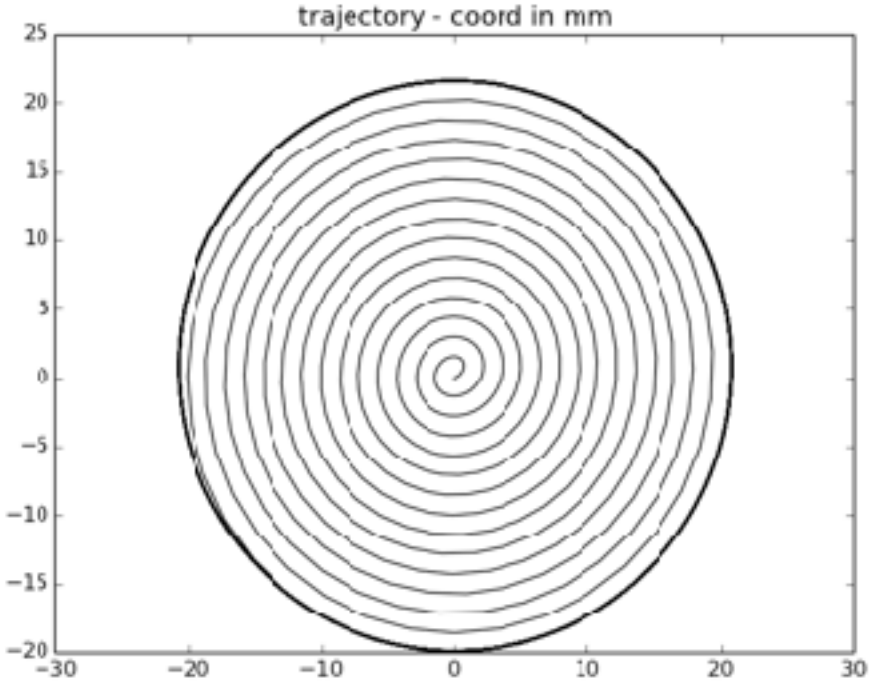
$$\frac{m}{z} \propto \frac{1}{f}$$

Spectra obtained by Fourier Transform

pulse



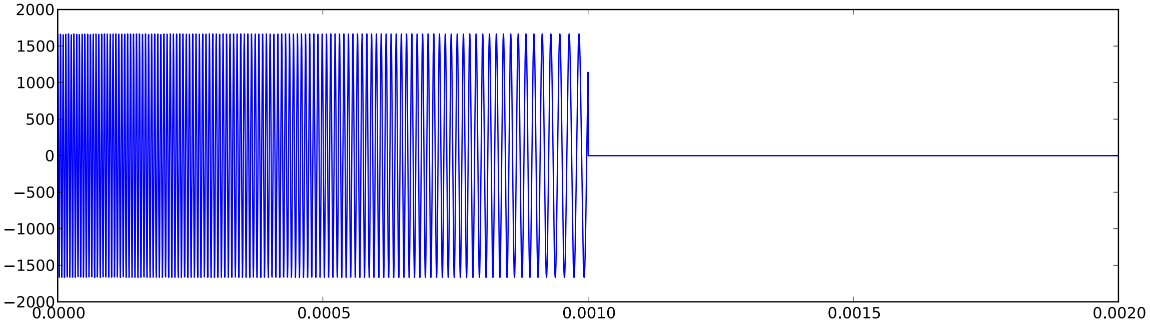


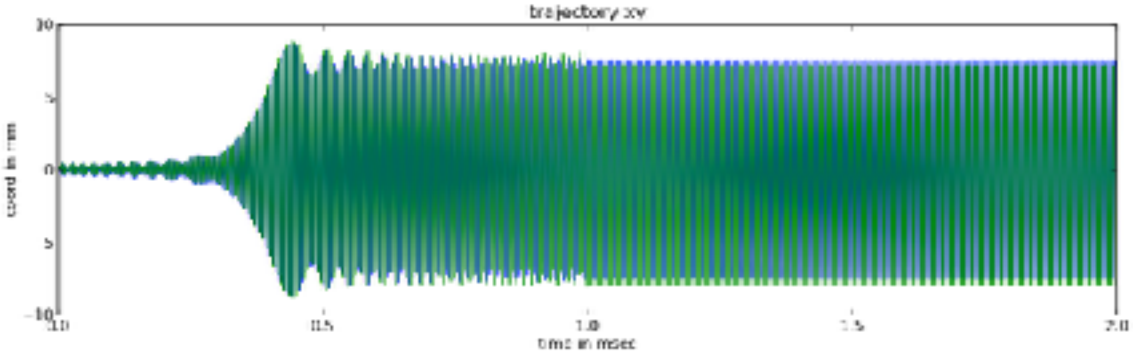


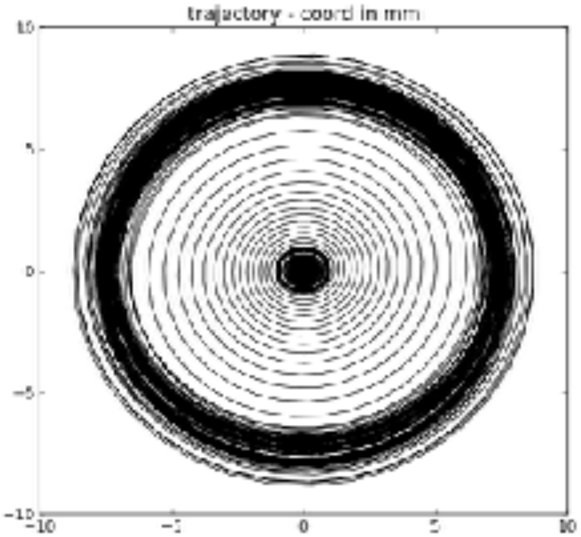
$$R = \frac{U\tau}{2B_o}$$

- radius is independent on m/z
- detected signal is proportional to R

Chirp pulses



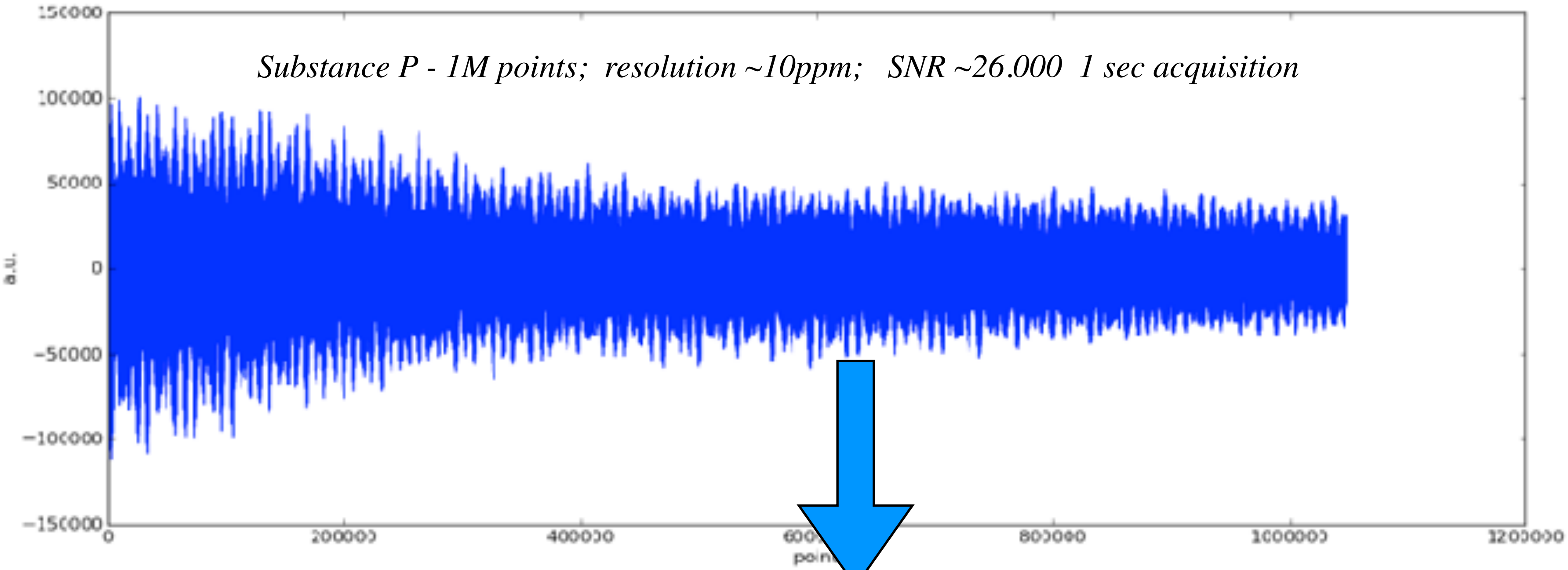




- broad-band spectra
  - 50kHz - 1MHz
- Direct detection
  - no carrier
- Chirp pulses
  - t=0 not easy to defined
  - complex phase dependence
- Simulation needed
  - Lorentz + Newton

Evolution simulator

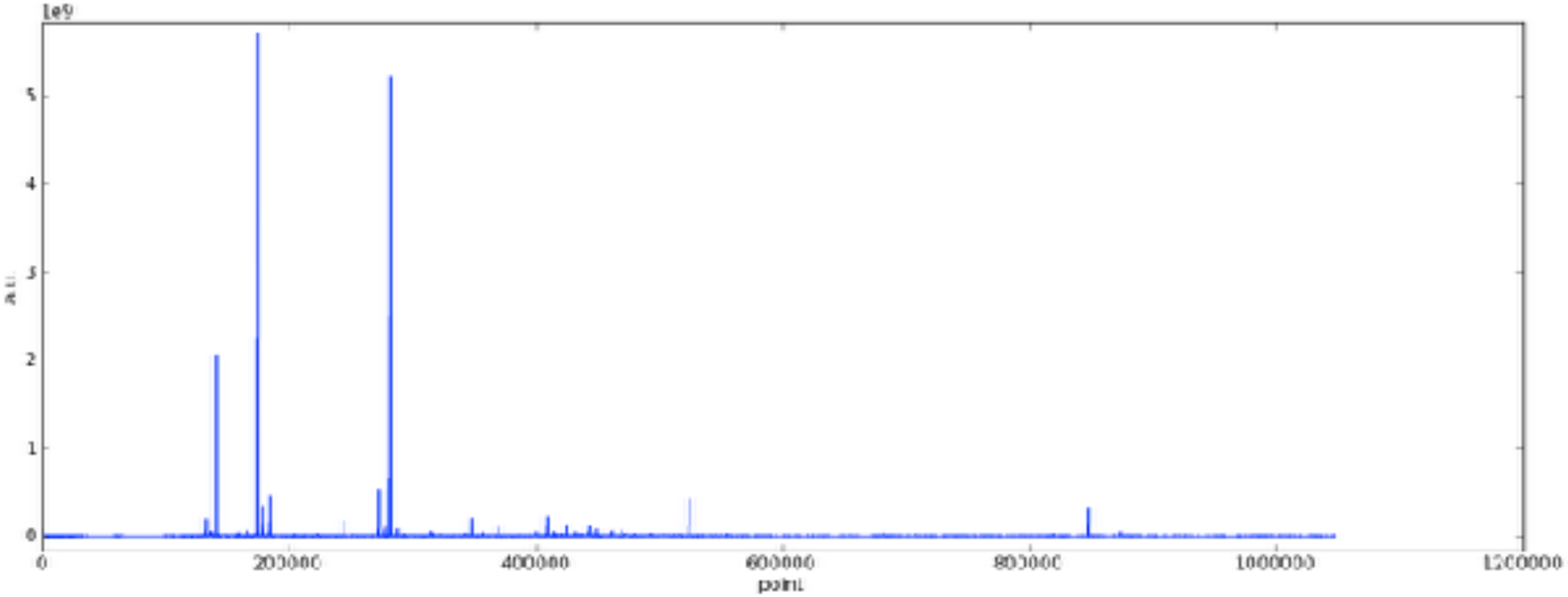
```
Swept pulse
Frequency : 200.000-50.000 kHz
sweep width : 150.000 kHz    sweep steps : 1000
duration : 1.000 msec
Epp : 1666.67 V/m
approx excitation radius : 11.82 mm

resonant frequency 144151.41 Hz
final radius : 7.56 mm
```



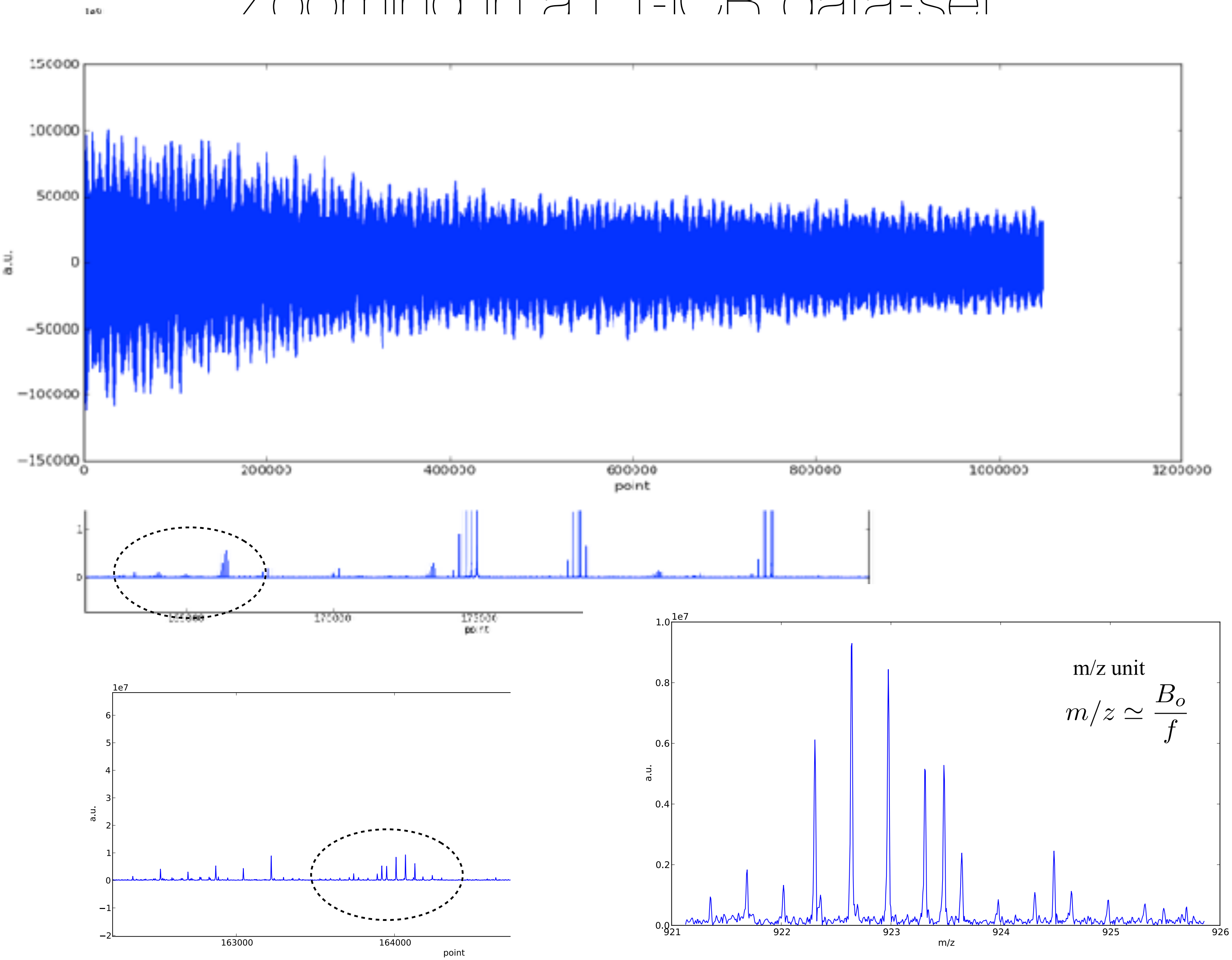
Substance P - 1M points; resolution ~10ppm; SNR ~26.000 1 sec acquisition

Fourier Transform
Modulus

```
import Apex as A
d = A.Import_1D("/DATA/YSPTS_MS_000004.d")
d.chsize(2*1024*1024).rfft().modulus()
```



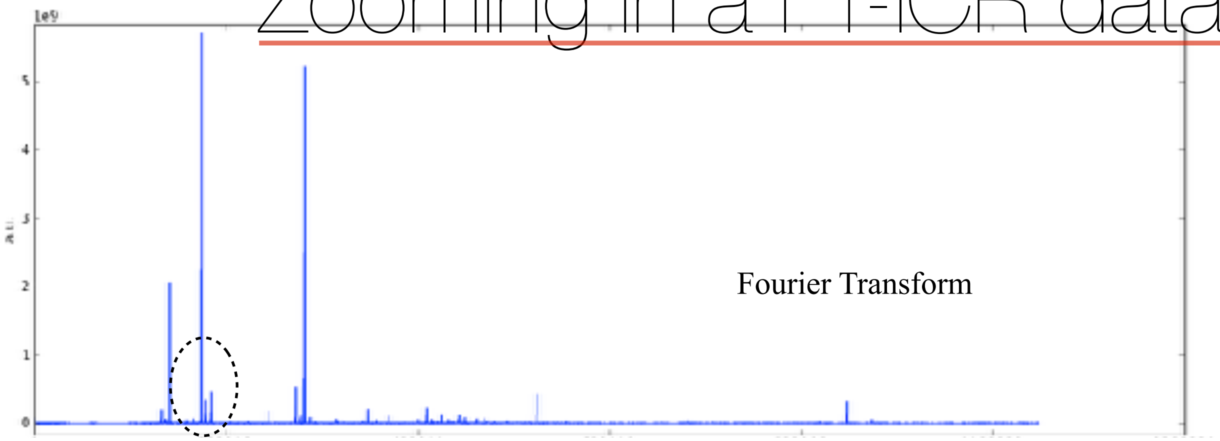
Zooming in a FT-ICR data-set



m/z unit

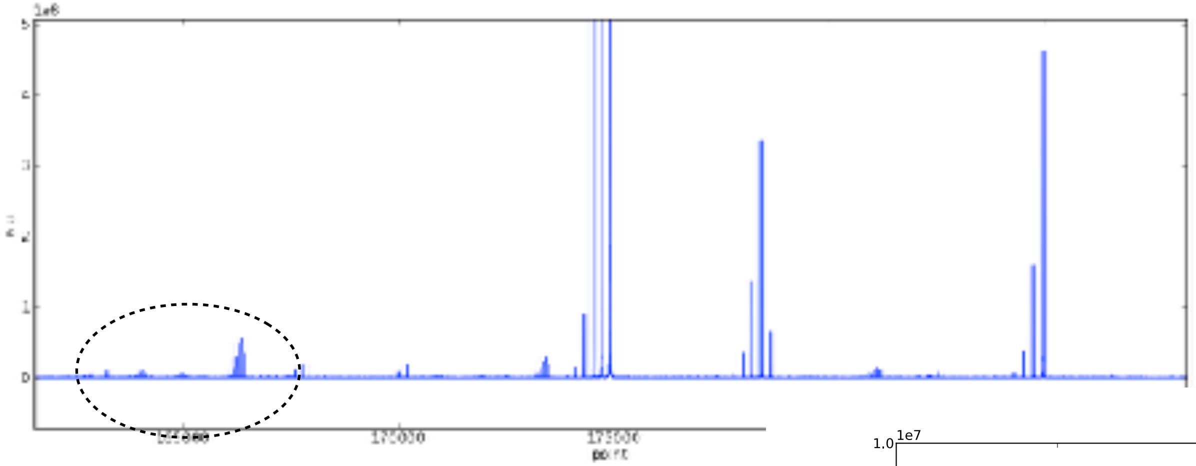
$$m/z \simeq \frac{B_o}{f}$$

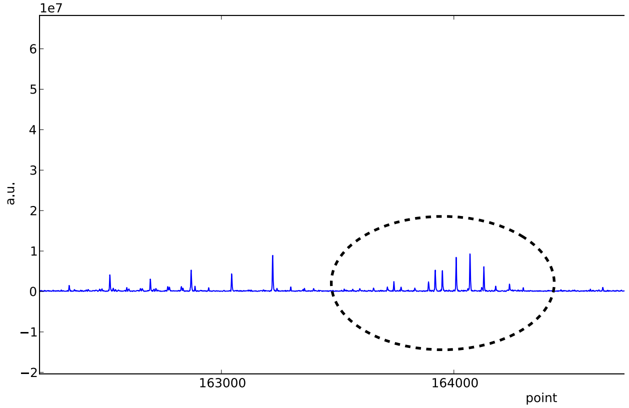
Zooming in a FT-ICR data-set

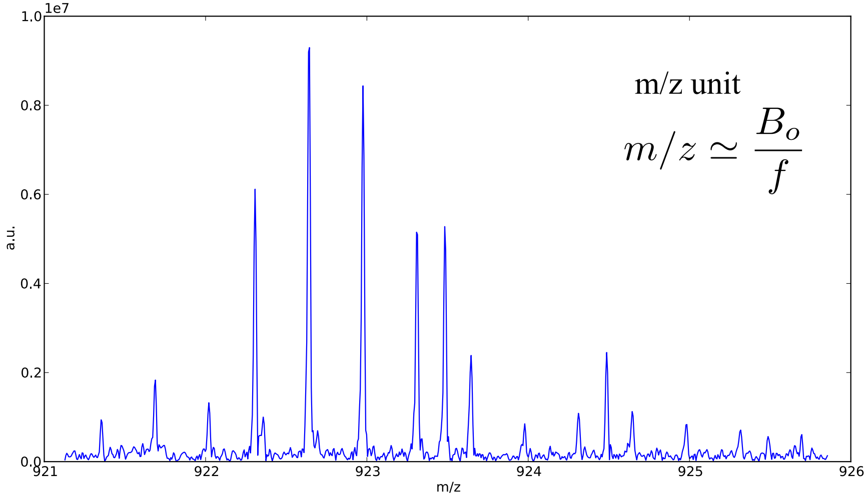


Fourier Transform

Substance P
1M points;
resolution ~10ppm
SNR ~26.000
1 sec acquisition





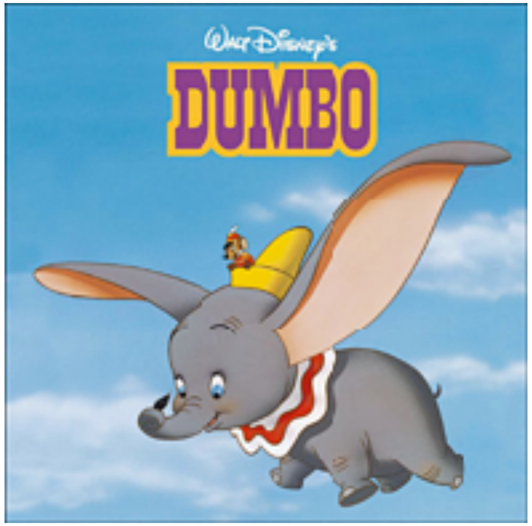


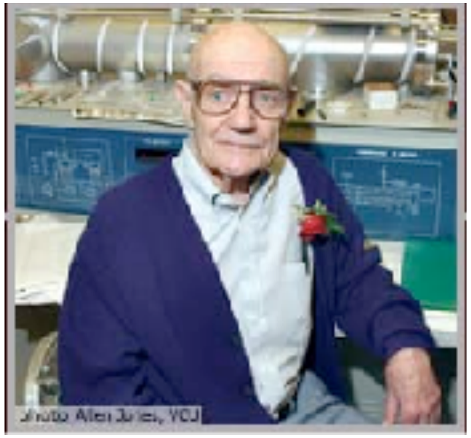
m/z unit

$$m/z \simeq \frac{B_o}{f}$$

Ionisation methods

- of course elephants can fly





John Fenn

Koichi Tanaka

Kurt Wüthrich

Nobel prize in Chemistry 2002

MALDI : Matrix Assisted Laser Desorption/Ionisation

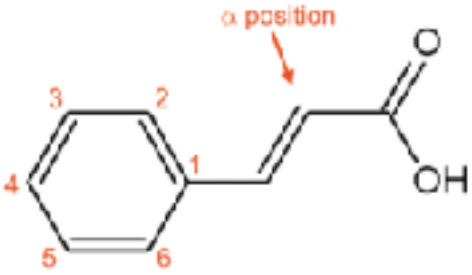
- Matrix

  absorbs light energy (UV laser)

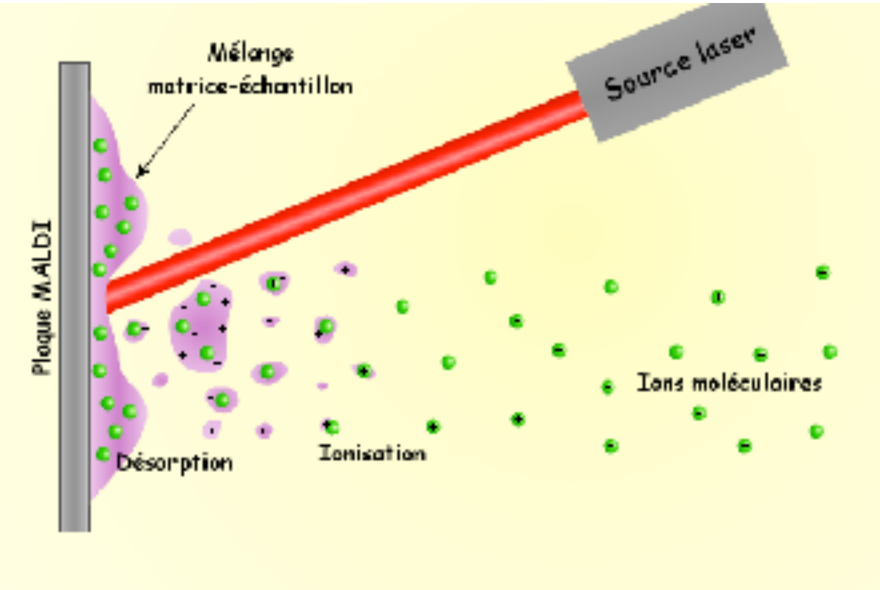
  ionizes the molecule without breaking it

- Typically
  - cinnamic acid
  - but also

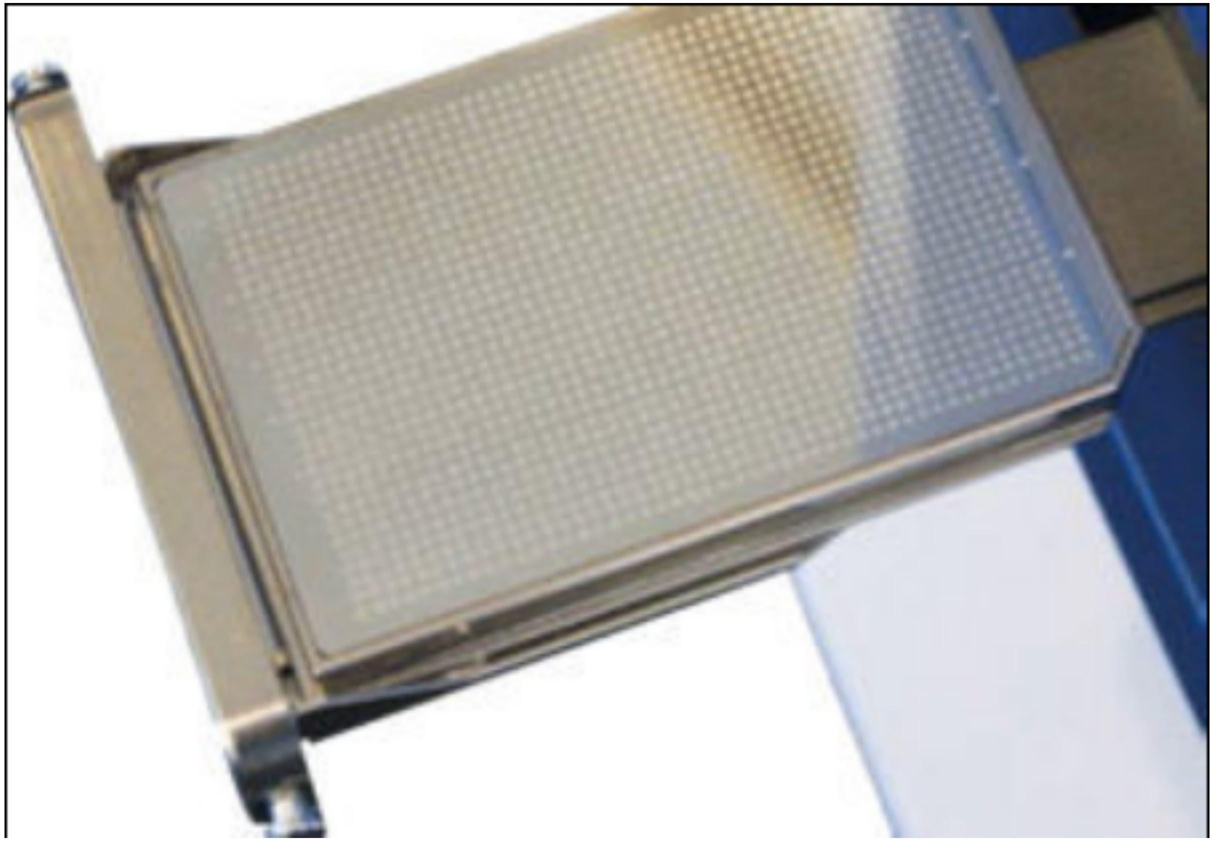
  Ferrulic acid / Sinapic acid / DiHydroxy Benzoic acid / etc..



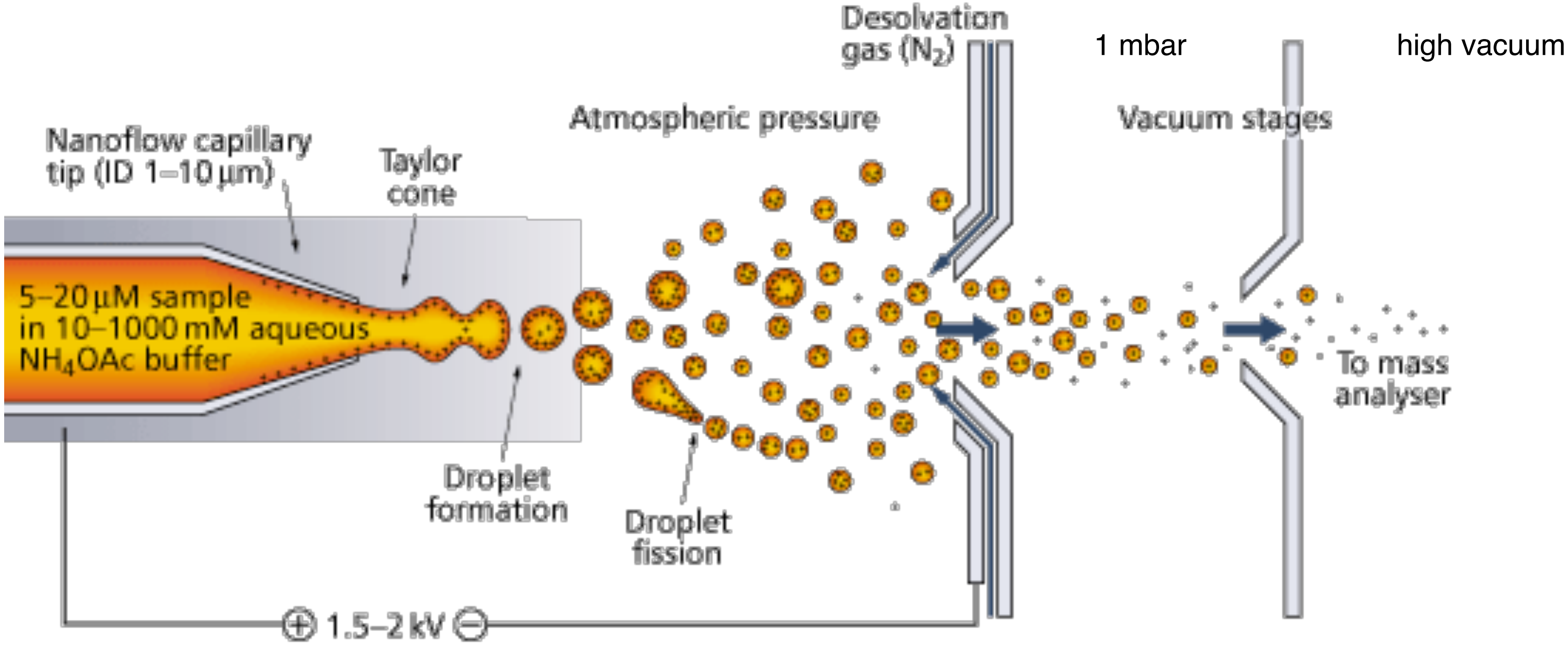
cinnamic acid

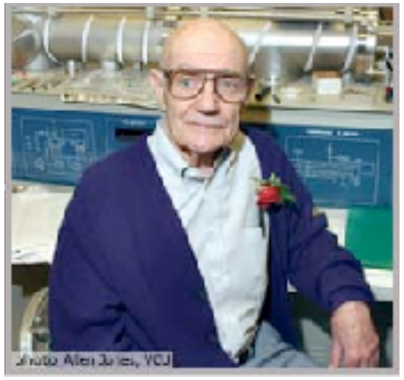


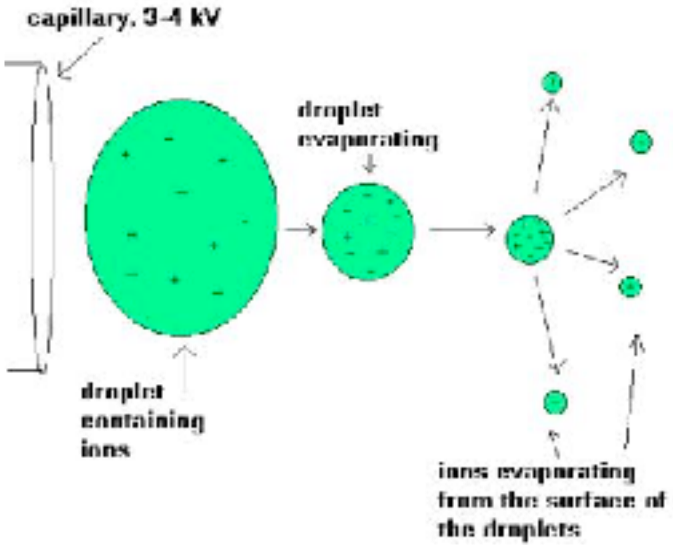

1
3
5
7
9
11
13
15
17
19
21
23
A
B
C
D
E
F
G
H
CAL1
CAL2
CAL5
CAL6
AB



ESI : Electro Spray Ionization

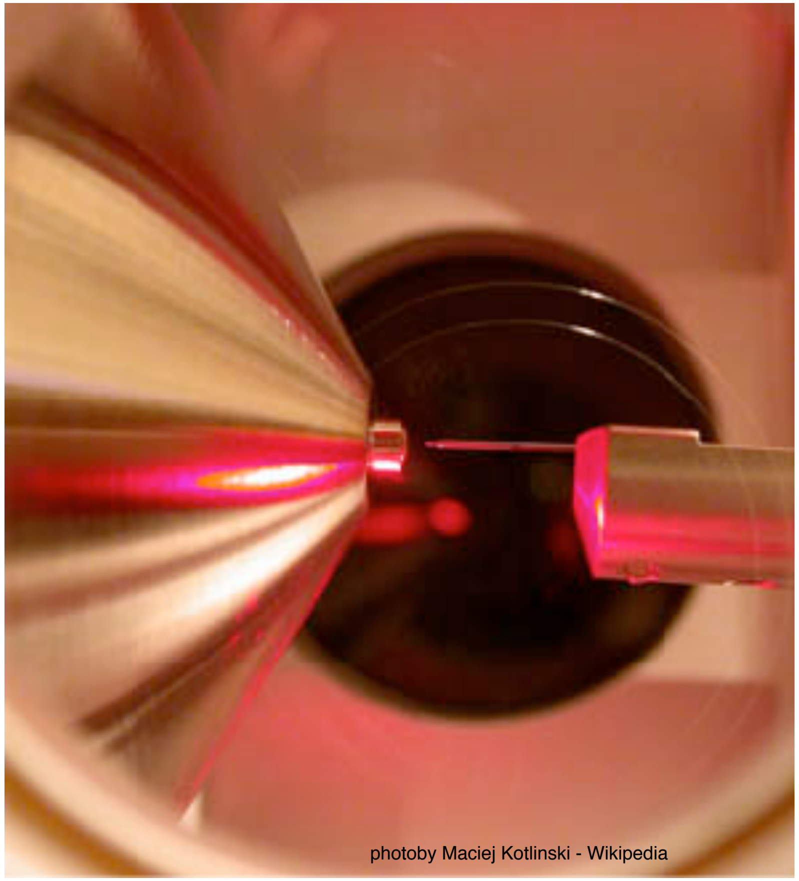






ESI : Electro Spray Ionization

photo by Robert White - Wikipedia



photoby Maciej Kotlinski - Wikipedia

ESI requirements

- volatile buffers

  so that no salt remains on the molecule of interest

  - positively charged

    ammonium

  - negatively charged

    carbonate

    formiate

    acetate

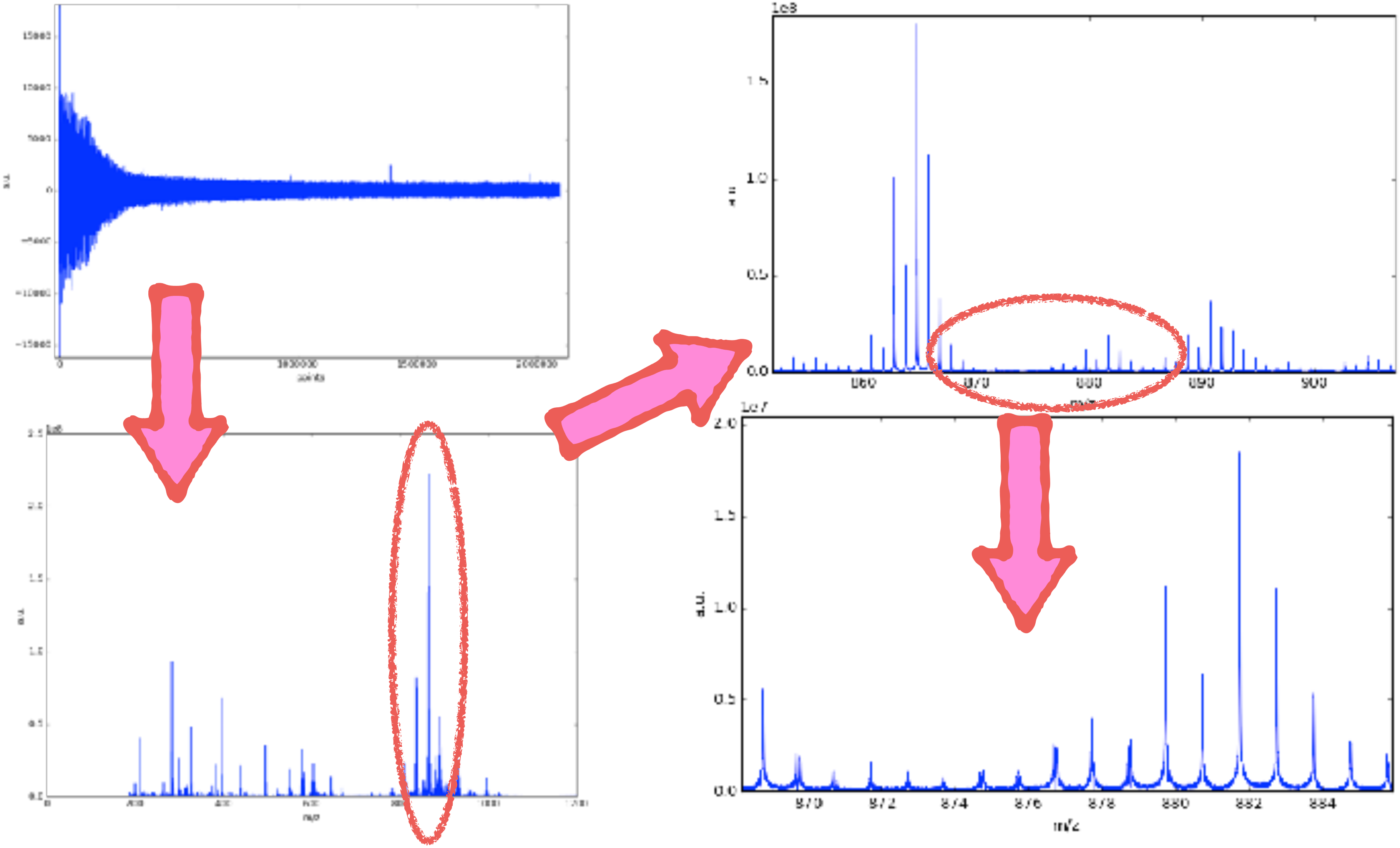
  - *and that’s about it* !

- see for instance
  - http://www.rsc.org/suppdata/an/c1/c1an15123a/c1an15123a.pdf

all together : a MS spectrometer

- Combine :
  - Usually some Chromatography
    - GC
    - HPLC
    - nanoLC
    - capillary electrophoresis
    - nothing => infusion
  - one source
    - ESI
    - MALDI
    - etc..
  - one measurement
    - trap / quadrupole
    - TOF
    - Orbitrap
- Hence
  - ESI-Orbitrap
  - Maldi-TOF
  - MALDI-LTQ
  - GC-TOF

One example



- Whisky sample

Resolution

- Resolution in MS
  - depends on several aspects
  - measured by the ratio R

$$R = \frac{m/z}{\Delta m/z}$$

- Detection techniques
  - Ion Trap < Quadrupole < TOF < Orbitrap < FT-ICR
    1.000 5k 20k 50k 300k 1M - 5M

From NIST web-site

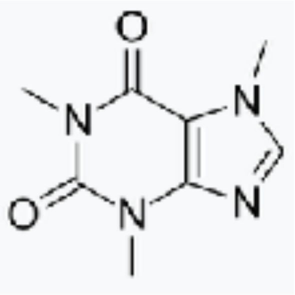
Atomic Weights and Isotopic Compositions for All Elements

Isotope			Relative Atomic Mass	Isotopic Composition	Standard Atomic Weight	Notes
1	H	1	1.00782503207(10)	0.999885(70)	1.00794(7)	g,m,r,b,w
		2	2.0141017778(4)	0.000115(70)		
		3	3.0160492777(25)			
2	He	3	3.0160293191(26)	0.00000134(3)	4.002602(2)	g,r,a
		4	4.00260325415(6)	0.99999866(3)		
3	Li	6	6.015122795(16)	0.0759(4)	6.941(2)	g,m,r,c,i
		7	7.01600455(8)	0.9241(4)		
4	Be	9	9.0121822(4)	1.0000	9.012182(3)	
5	B	10	10.0129370(4)	0.199(7)	10.811(7)	g,m,r
		11	11.0093054(4)	0.801(7)		
6	C	12	12.0000000(0)	0.9893(8)	12.0107(8)	g,r
		13	13.0033548378(10)	0.0107(8)		
		14	14.003241989(4)			
7	N	14	14.0030740048(6)	0.99636(20)	14.0067(2)	g,r,a,d
		15	15.0001088982(7)	0.00364(20)		
8	O	16	15.99491461956(16)	0.99757(16)	15.9994(3)	g,r,e,w
		17	16.99913170(12)	0.00038(1)		
		18	17.9991610(7)	0.00205(14)		

Exact Mass

- small molecules

$$R = \frac{m/z}{\Delta m/z}$$



```
C_8 H_10 N_4 O_2
194.08037557901997
```

Isotopic pattern

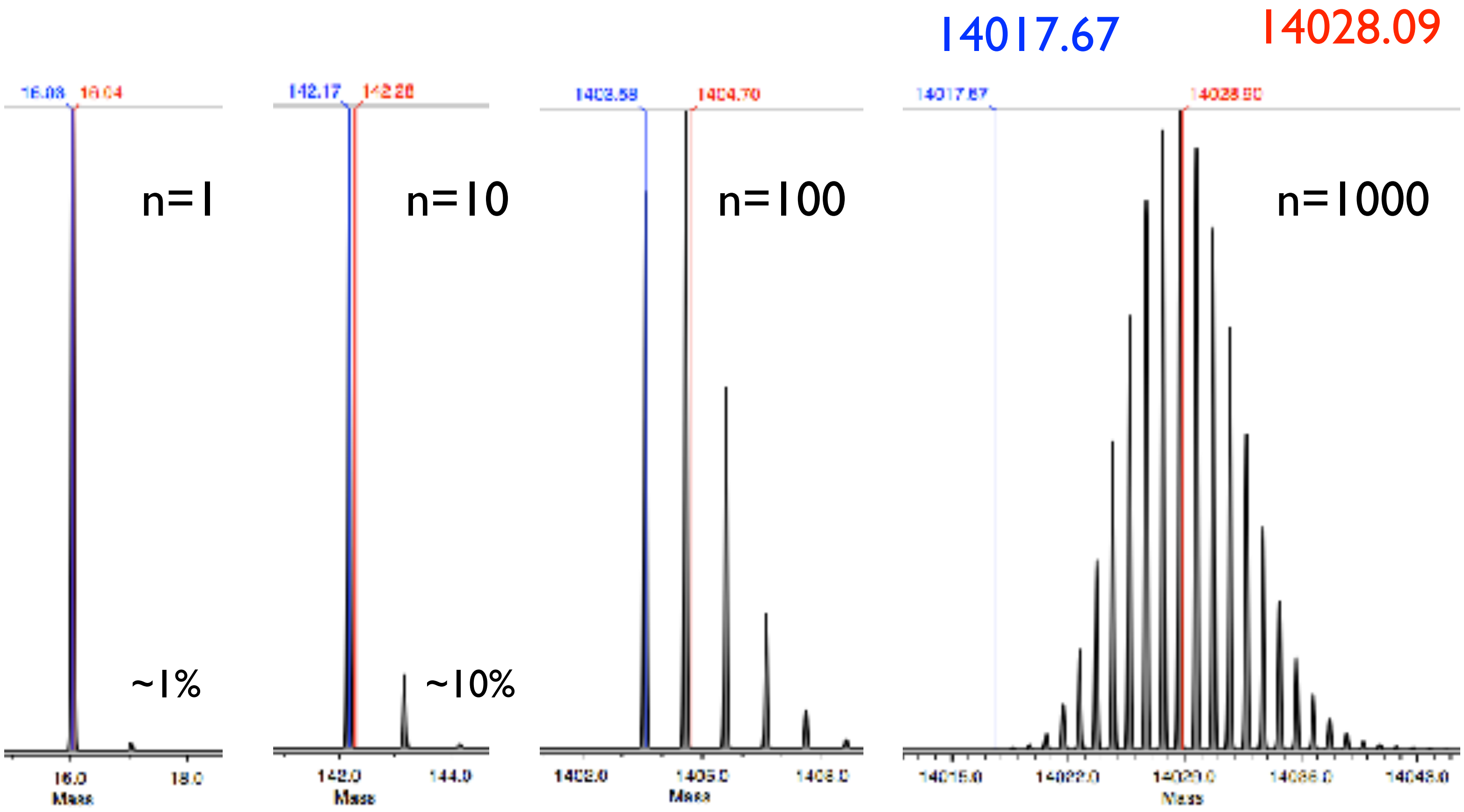
- Each atom type displays an isotopic profile
  - list of isotope natural abundance for common atoms

isotopic ratio		+1	+2
H	99.99 %	0.015 %	
C	98.9 %	1.1 %	
N	99.63 %	0.37 %	
O	99.76 %	0.038 %	0.2 %
P	100 %		
S	95.02 %	0.75 %	4.21 %

http://www.sisweb.com/referenc/source/exactmaa.htm
http://www.sisweb.com/mstools/isotope.htm

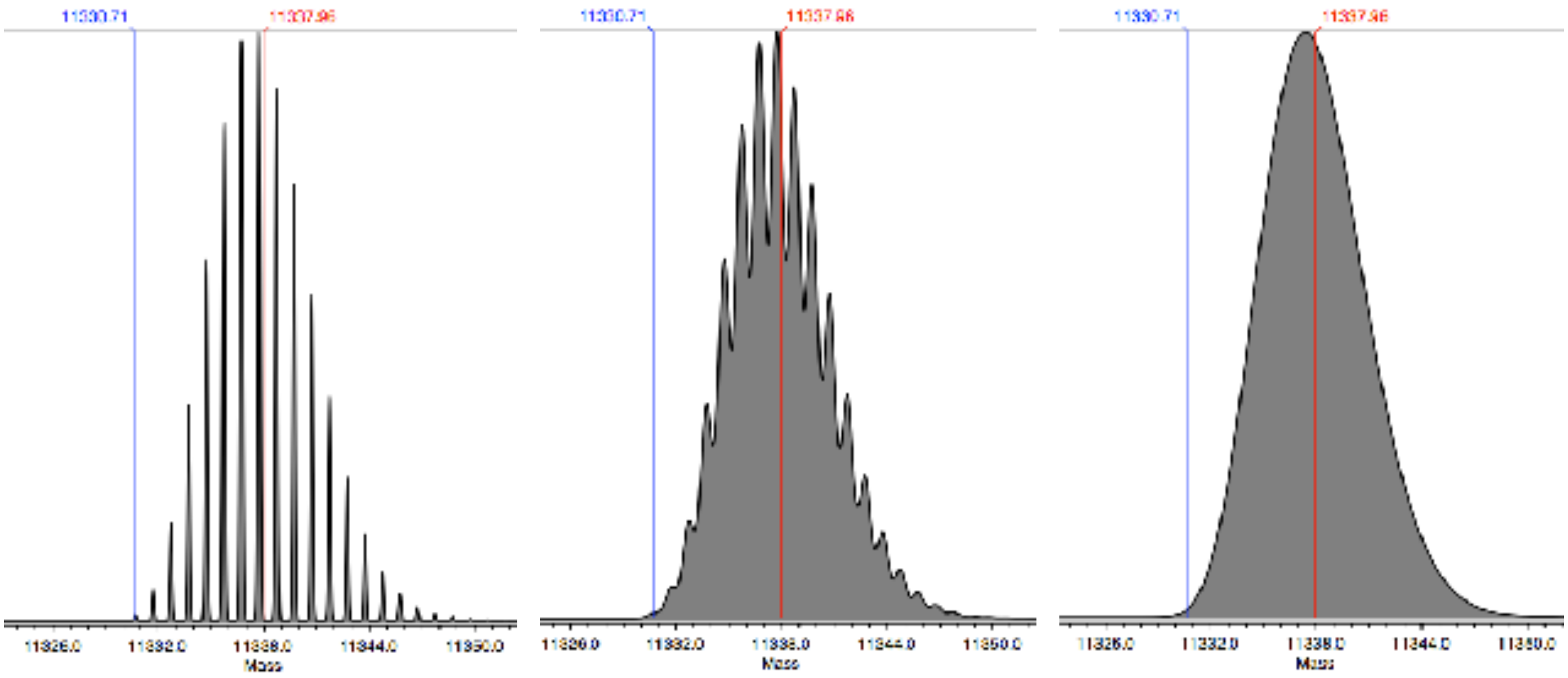
Molecular Mass definition - Isotopic pattern

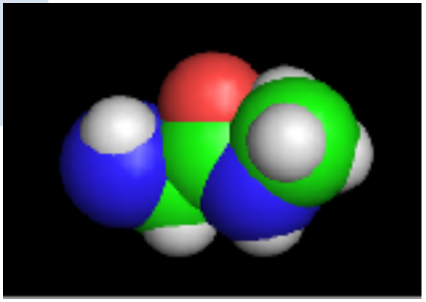
- For Cn peak at 12n+1 is proportionnal to n
- example here for linear alcanes C_nH_{2n+2}
  - monoisotopic mass / average mass

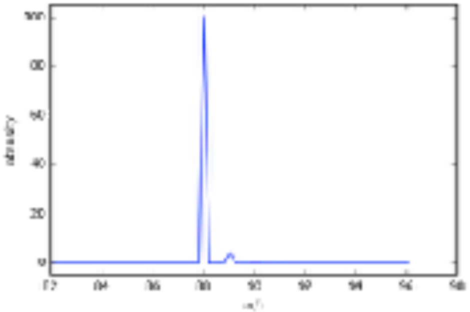


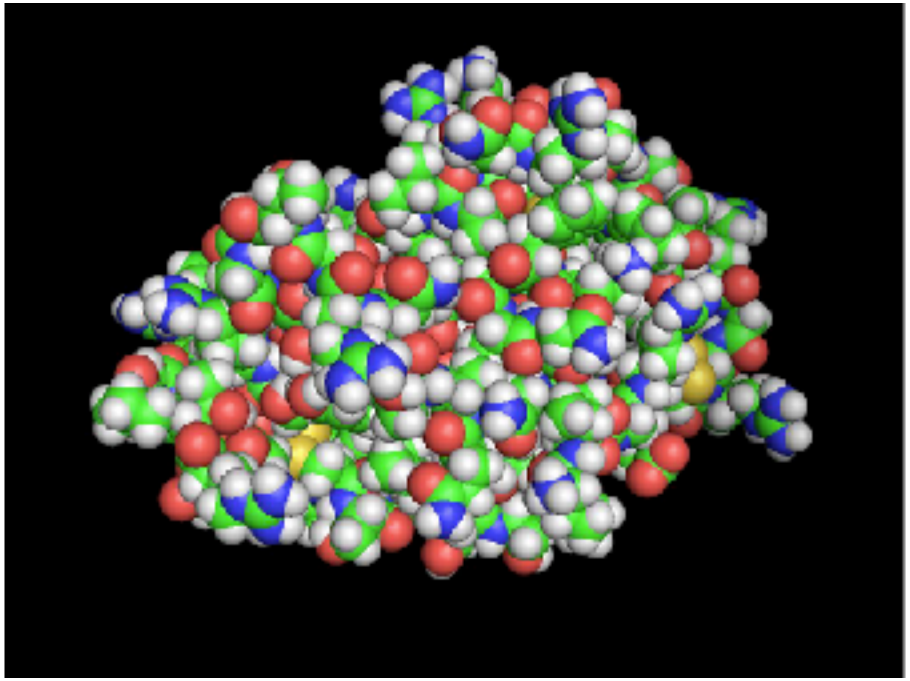
Isotopic pattern

- Protein empirical formula
  - eg : $C_1 H_{1.59} N_{0.27} O_{0.31} S_{0.01}$ *(different expressions exist)*
  - The aspect of the pattern depends on the resolution (and on the charge state)
  - here simulated for a 11kD protein
  - note how *monoisotopic mass ≠ average mass ≠ top of the*





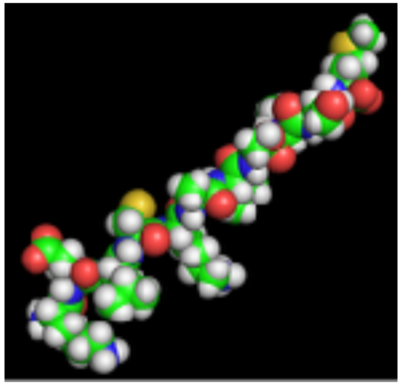


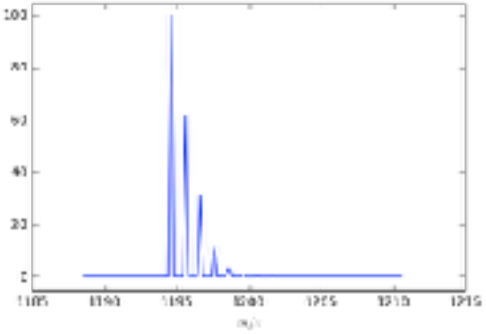


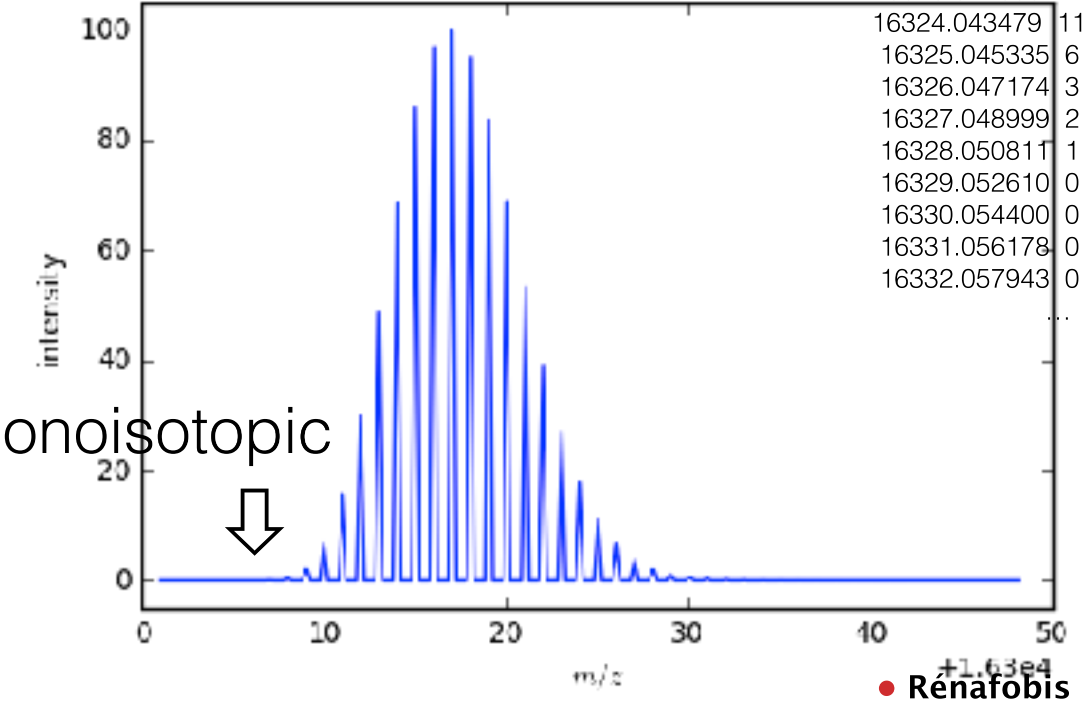
C_725 H_1122 N_194 O_215 S_10

16306.003396	0.0544783371
16307.006259	0.4815908078
16308.008995	2.1746869097
16309.011617	6.6792852462
16310.014141	15.6785827113
16311.016578	29.9699795375
16312.018937	48.5483260540
16313.021227	68.4889810809
16314.023455	85.8283420627
16315.025629	96.9892347469
16316.027753	100.0000000000
16317.029832	94.9628506636
16318.031872	83.7024337398
16319.033877	68.9202526525
16320.035849	53.3017111235
16321.037793	38.8992040048
16322.039711	26.8962383101
16323.041605	17.6814074296
16324.043479	11.0855202359
16325.045335	6.6465388834
16326.047174	3.8202539089
16327.048999	2.1095643109
16328.050811	1.1213685311
16329.052610	0.5748121676
16330.054400	0.2845871917
16331.056178	0.1362794827
16332.057943	0.0631972378

...

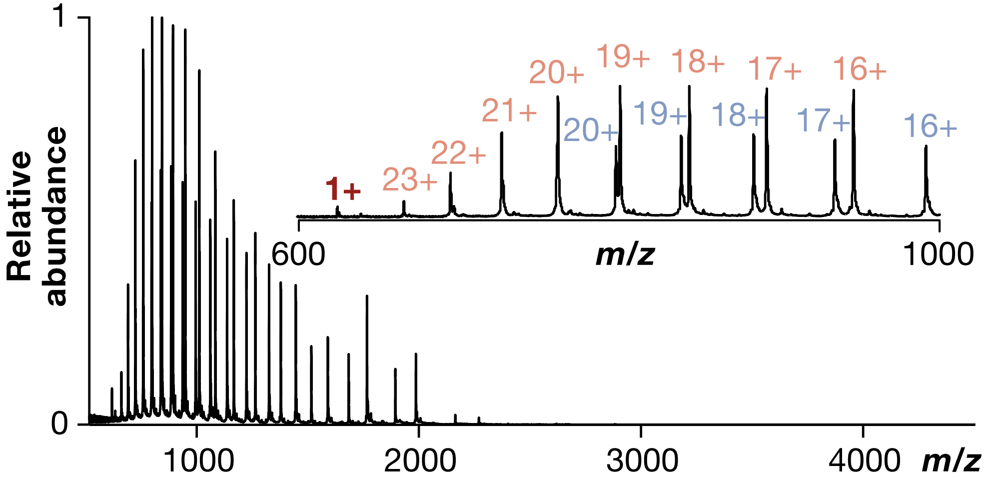




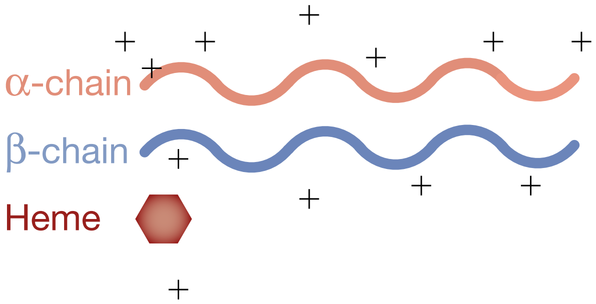


charge state

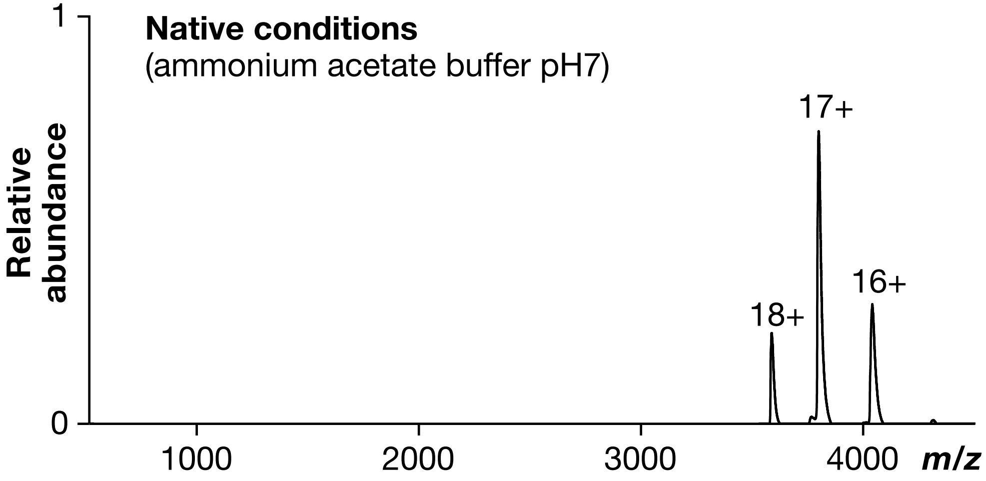
Denaturing conditions
(acidified aqueous solution)

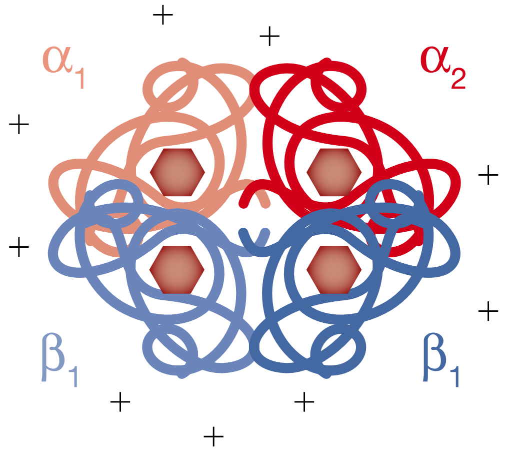


Prop to length ⇒ to MW



Native conditions
(ammonium acetate buffer pH7)





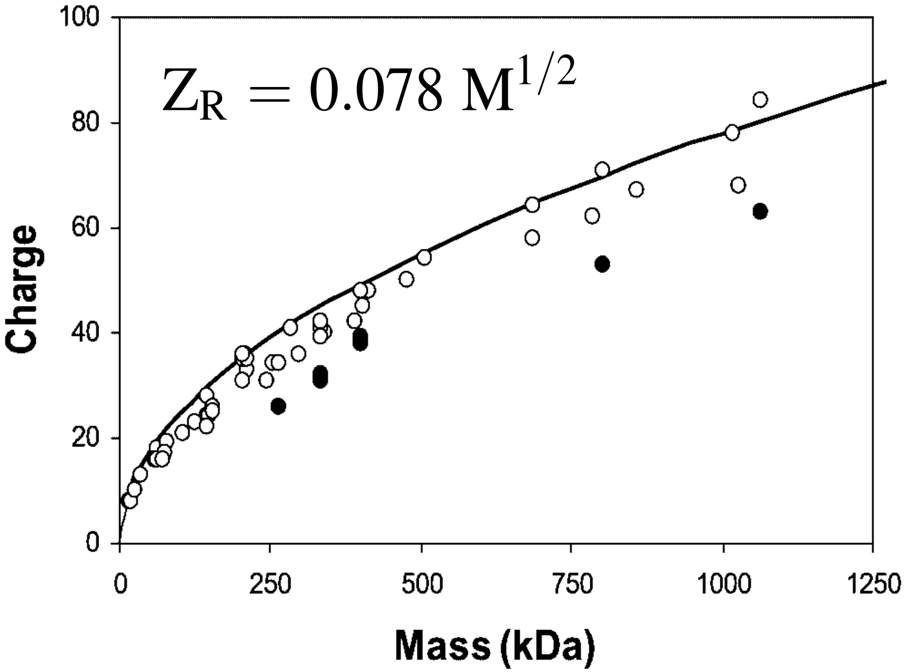
Prop to surface ⇒ to MW$^{2/3}$

P. Lössl, M. van de Waterbeemd & A. JR Heck

The EMBO Journal (2016) 35: 2634–2657

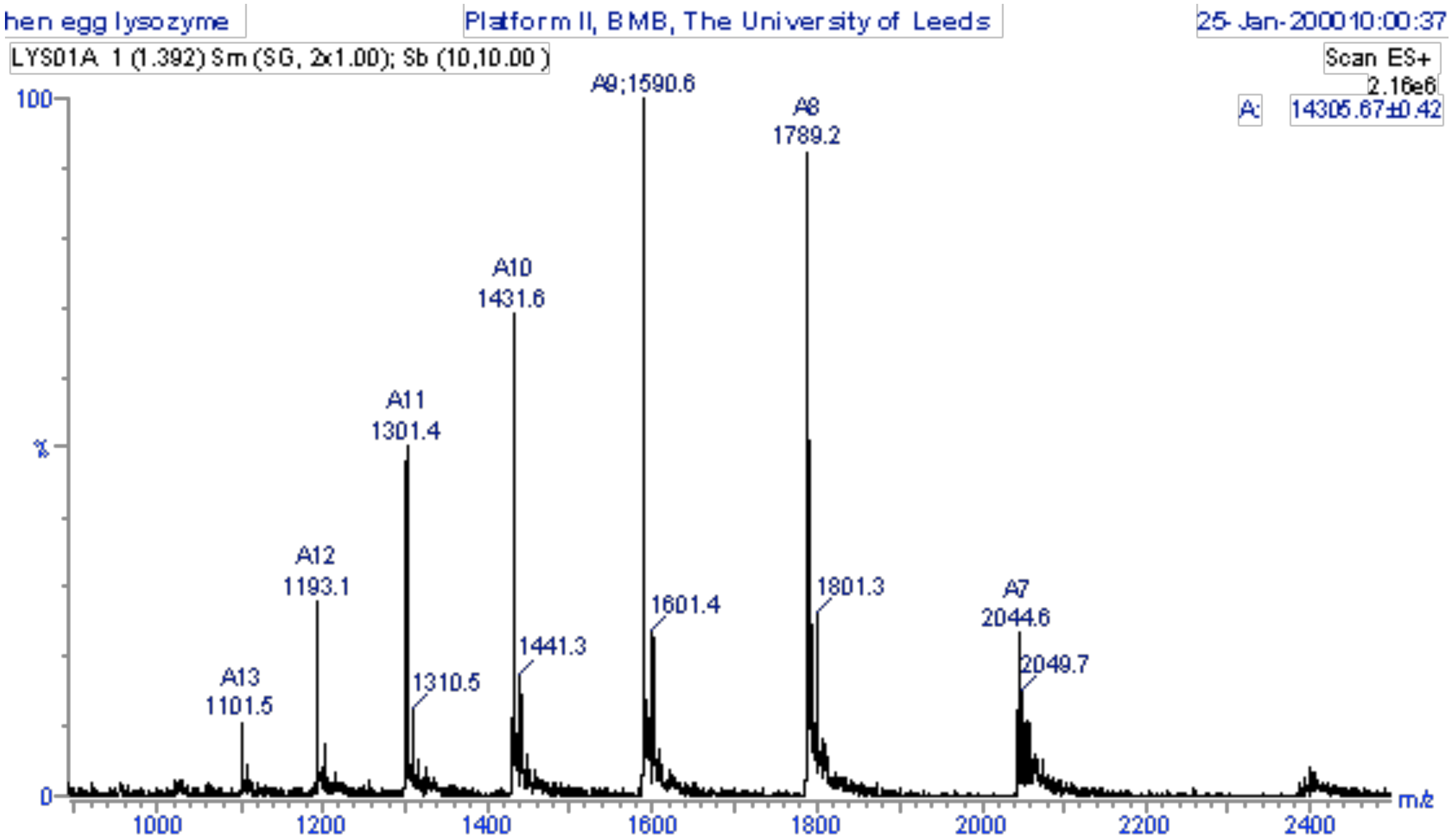
Charge/Mass dependence

- unfolded proteins
  - charge depends only on primary sequence and pH
- folded proteins
  - charge is only on the surface
  - Rayleigh model Z_R
    - charges are in the droplet when sprayed
    - droplet evaporate by coulombic fission
    - protein is assumes folded ad spherical, charges on the protein are at the limit of the maximum coulombic density.



Number of observed mean charges of a number of globular proteins and protein complexes compared with the Raleigh limit model predicted charge. The number of observed charges is very close to the Raleigh limit on water droplets of the same size as the protein. All proteins were sprayed from 50 mM ammonium acetate at neutral pH. (o) Represents positive ion mode and (•) negative ion mode.

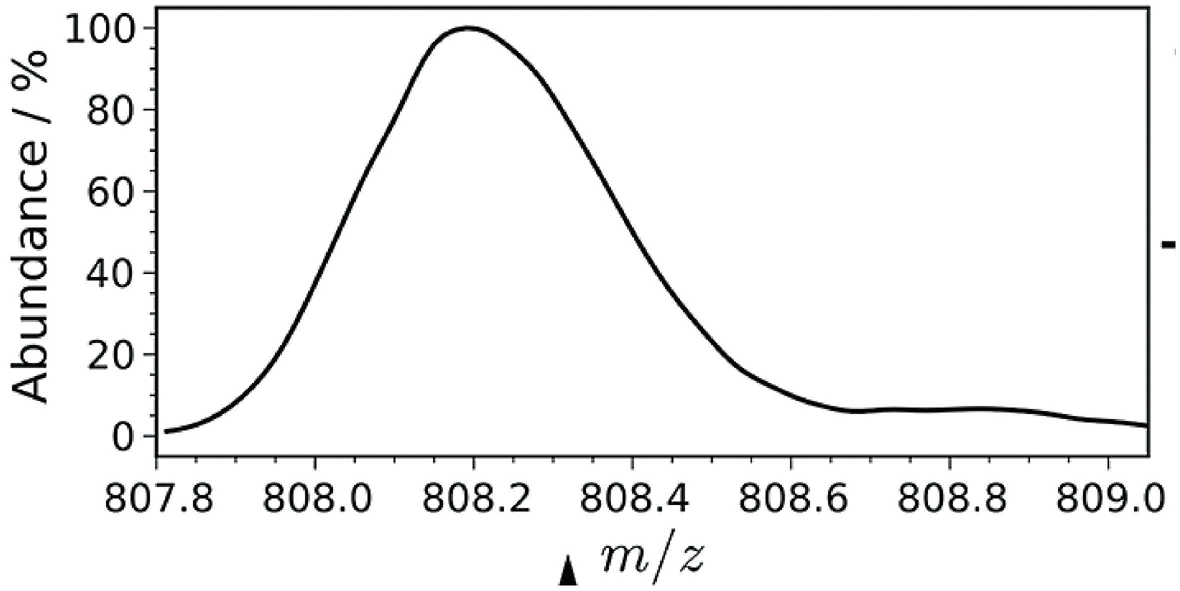
10kD $\Rightarrow Z \sim 9+$
100kD $\Rightarrow Z \sim 28+$
1MD $\Rightarrow Z \sim 90+$

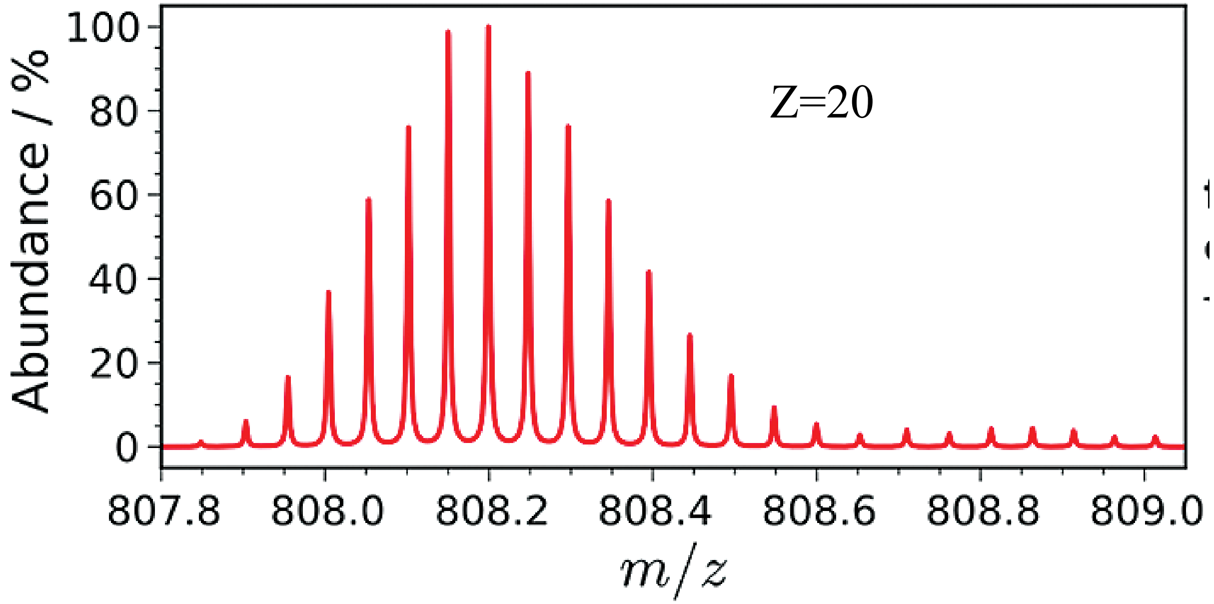


Lysozyme ESI-TOF spectrum

Myoglobin

$H_{1212}C_{769}N_{210}O_{218}S_2$





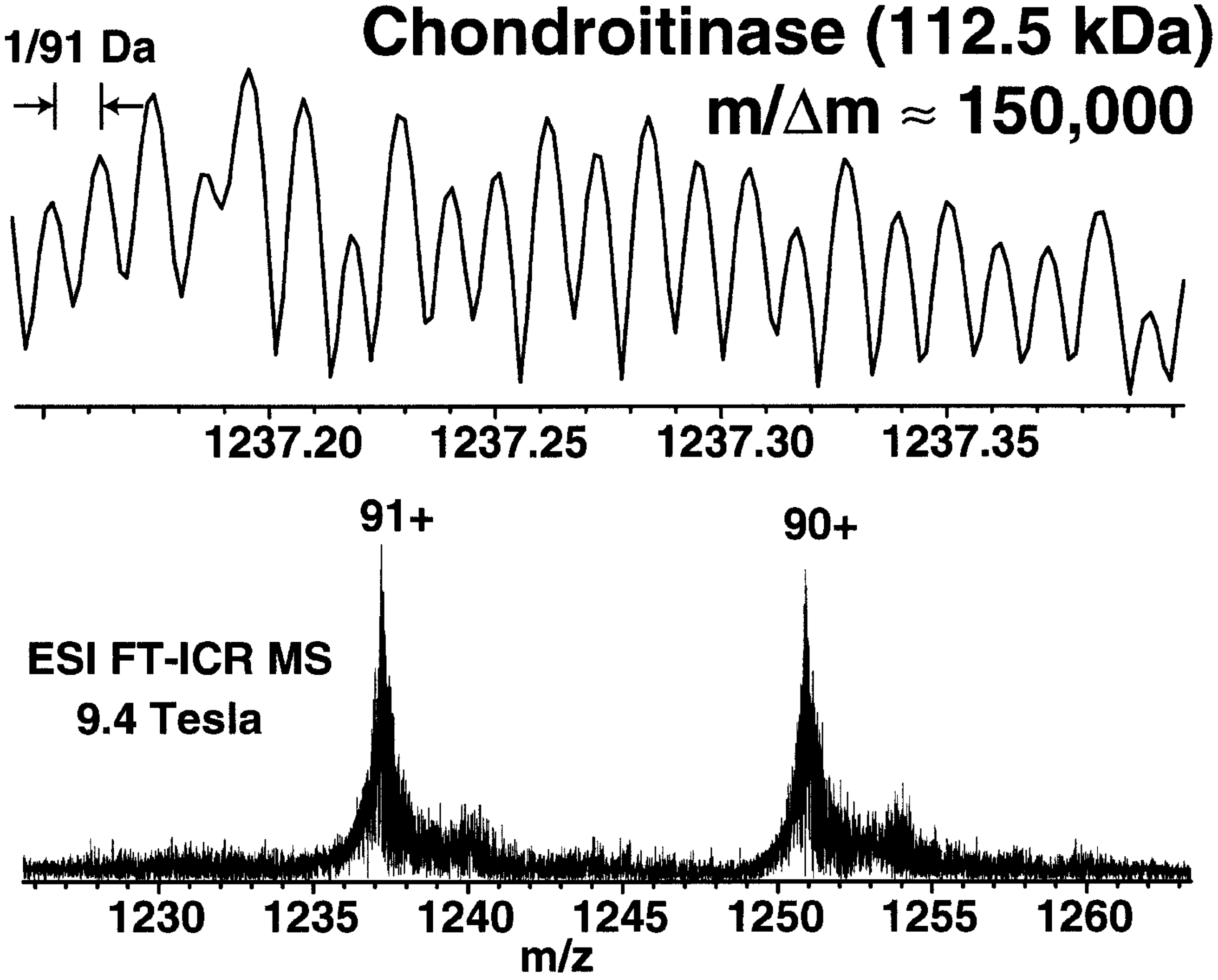
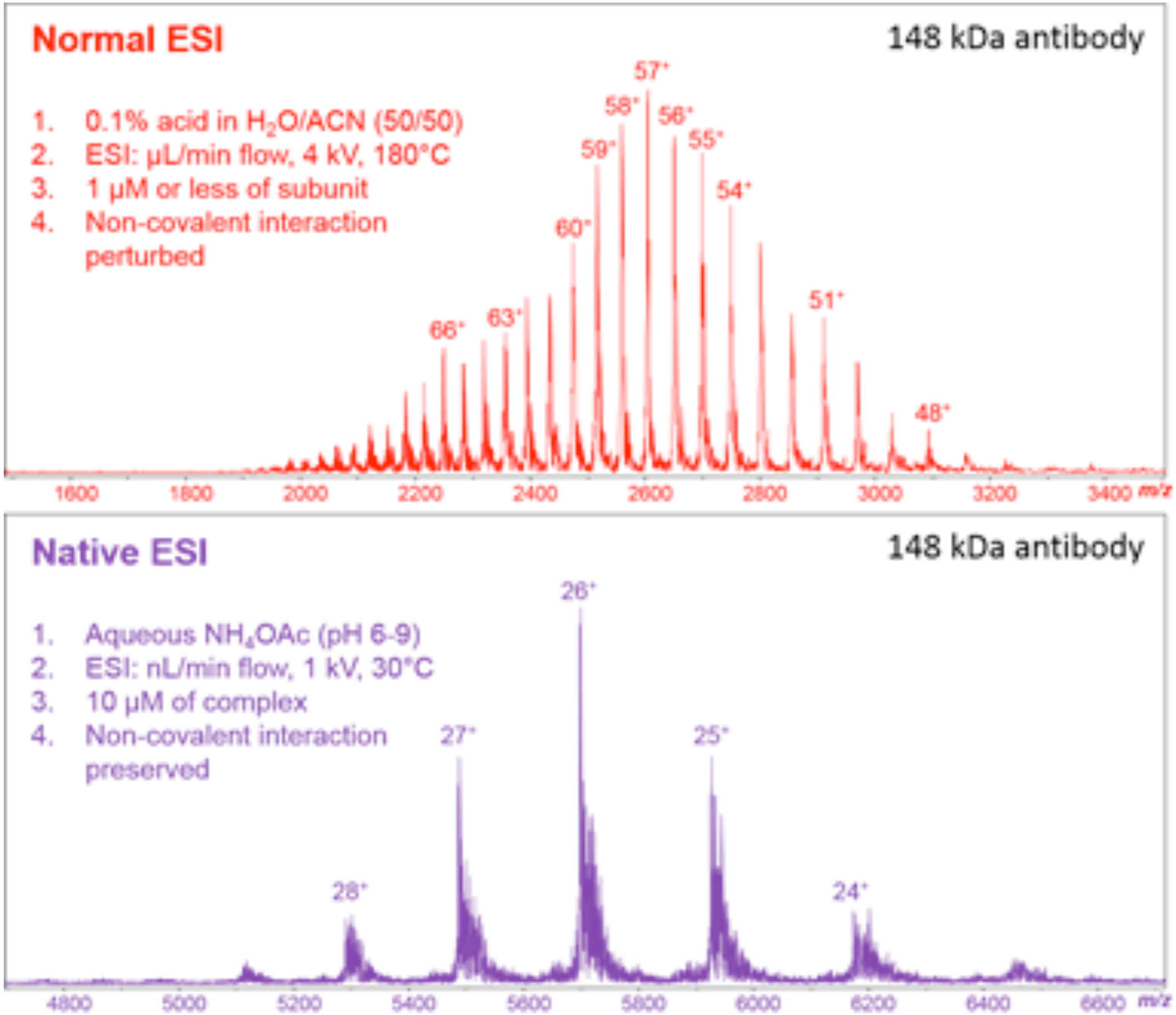


FIGURE 22. ESI FT-ICR mass spectra of chondroitinase I. Bottom: Heterodyne data for SWIFT-isolated ions, $1226 < m/z < 1273$, with external ion accumulation (Senko et al., 1997), from 10 co-added time-domain signals; the peaks at m/z 1240 and 1254 correspond to an unidentified adduct of ~260 Da. Top: Mass scale-expansion showing unit mass resolution of the isotopic distribution of the $z = 91$ charge state. Data kindly provided by N. Kelleher and described in detail elsewhere (Kelleher et al., 1997).

Charge depend on protein state



Normal ESI

148 kDa antibody

1. 0.1% acid in H_2O/ACN (50/50)
2. ESI: µL/min flow, 4 kV, 180°C
3. 1 µM or less of subunit
4. Non-covalent interaction perturbed

Native ESI

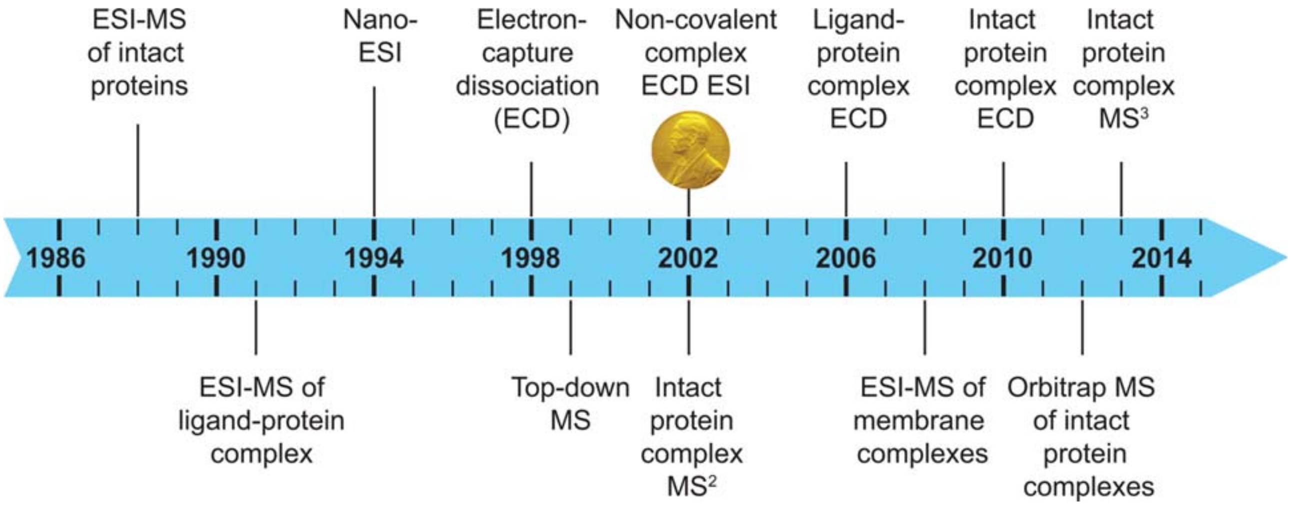
148 kDa antibody

1. Aqueous NH_4OAc (pH 6-9)
2. ESI: nL/min flow, 1 kV, 30°C
3. 10 µM of complex
4. Non-covalent interaction preserved

http://msr.dom.wustl.edu/tutorial-native-mass-spectrometry/

Examples of use in Structural Biology

- Not proteomics!
- Structural Information
  - non covalent molecular interactions
    - Large multicomponent Complexes
    - Ligand binding
  - H-D exchange and other chemical labelling
  - Cross-Linking
  - Ionic Mobility
  - fragmentation



1986
1990
1994
1998
2002
2006
2010
2014

ESI-MS of intact proteins
ESI-MS of ligand-protein complex
Nano-ESI
Electron-capture dissociation (ECD)
Top-down MS
Non-covalent complex ECD ESI
Intact protein complex MS^2
Ligand-protein complex ECD
ESI-MS of membrane complexes
Intact protein complex ECD
Orbitrap MS of intact protein complexes
Intact protein complex MS^3

from E. Boeri Erba C. Petosa

PROTEIN SCIENCE 2015 VOL 24:1176—1192

NATURE COMMUNICATIONS | (2018)9:328 | DOI: 10.1038/s41467-017-02443-x

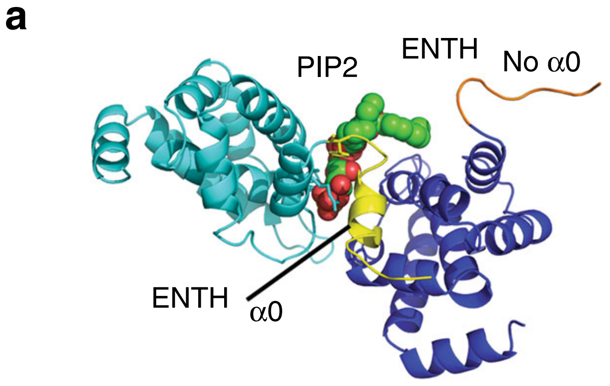
ARTICLE

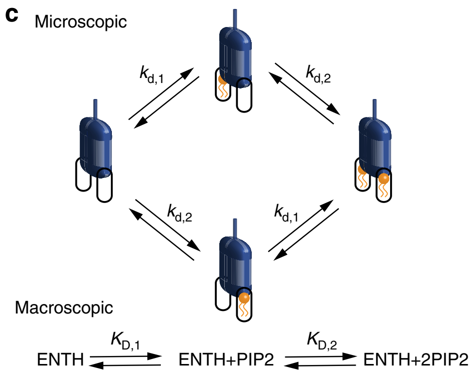
DOI: 10.1038/s41467-017-02443-x OPEN

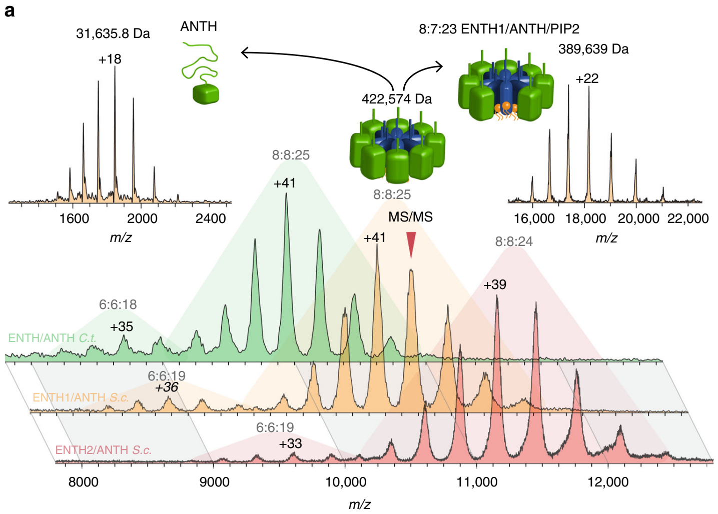
Epsin and Sla2 form assemblies through phospholipid interfaces

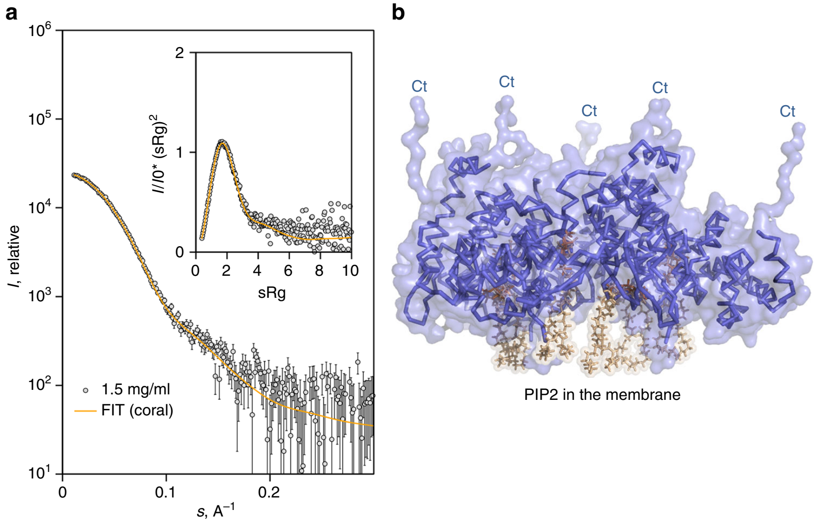
Maria M. Garcia-Alai[1], Johannes Heidemann[2], Michal Skruzny[3], Anna Gieras[1,4], Haydyn D.T. Mertens[1], Dmitri I. Svergun[1], Marko Kaksonen[5], Charlotte Uetrecht [2,6] & Rob Meijers [1]

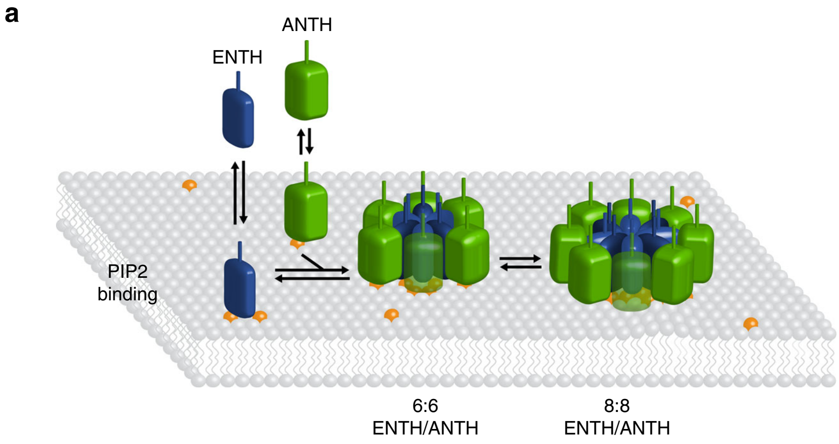
In clathrin-mediated endocytosis, adapter proteins assemble together with clathrin through interactions with specific lipids on the plasma membrane … Here, we show that the membrane–proximal domains ENTH of epsin and ANTH of Sla2 form complexes through phosphatidylinositol 4,5-bisphosphate (PIP2) lipid interfaces. Native mass spectrometry reveals how ENTH and ANTH domains form assemblies by sharing PIP2 molecules ….

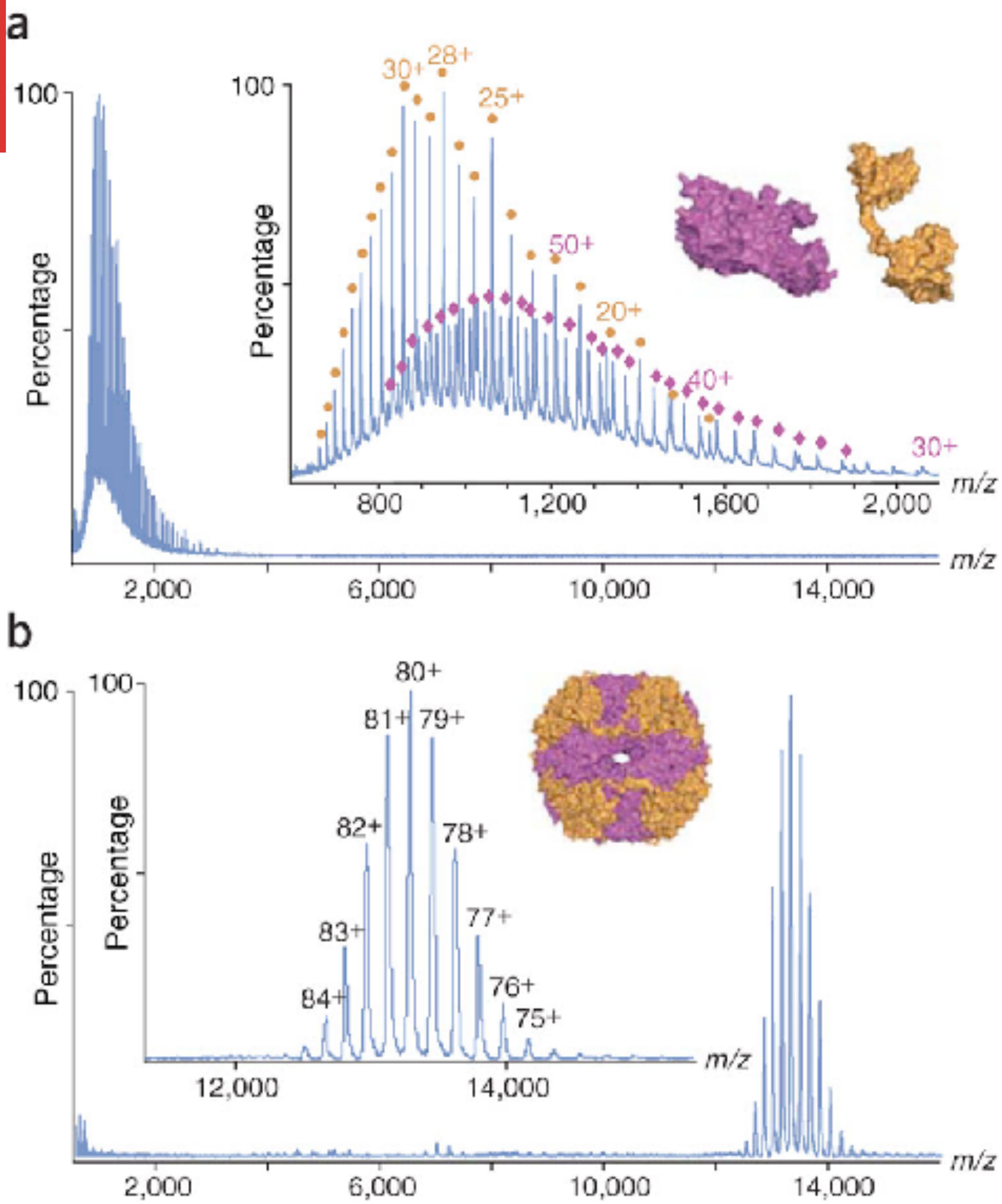












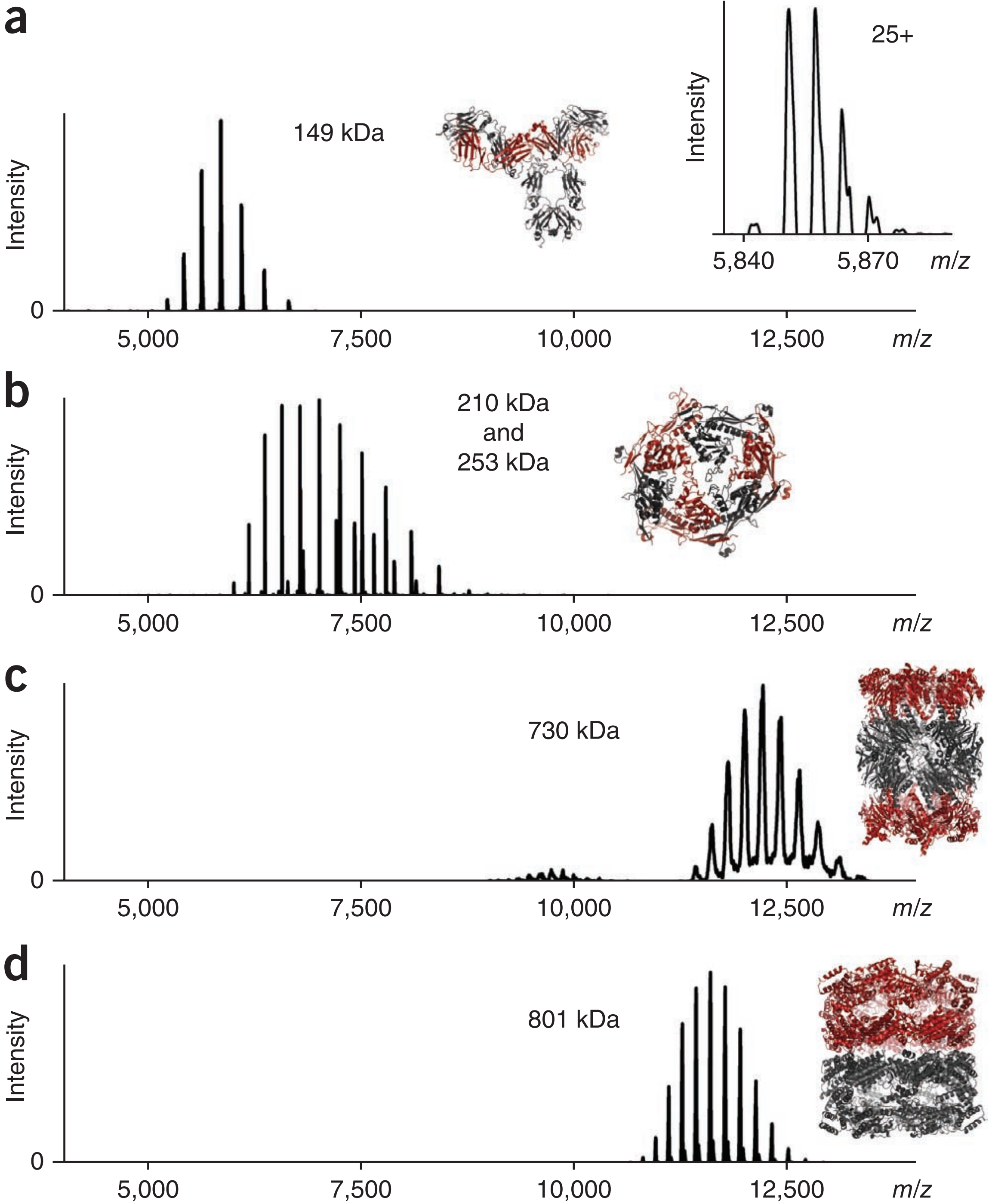
Denatured and native mass spectra of *H. pylori* urease.

(**a**) Denatured urease was electrosprayed from an aqueous 50% (vol/vol) acetonitrile containing 0.1 % (vol/vol) formic acid solution revealing individual charge distributions from the multiply charged α (26.6 kDa, orange) and β (61.7 kDa, magenta) monomers of urease. (**b**) A mass spectrum of native urease electrosprayed from an aqueous ammonium acetate solution (bottom) displaying multiple ion signals that originate from multiple charged species of the $\alpha_{12}\beta_{12}$ intact urease machinery with a measured mass of 1,063.4 ± 1.0 kDa. Insets are close-ups of the indicated regions. The cartoons are adapted from the X-ray structure of the intact $\alpha_{12}\beta_{12}$ urease.

Albert J R Heck
Nature Methods 5, 927 - 933 (2008)
doi:10.1038/nmeth.1265

Native mass spectrometry: a bridge between interactomics and structural biology

Large Complexes by MS /with Orbitrap

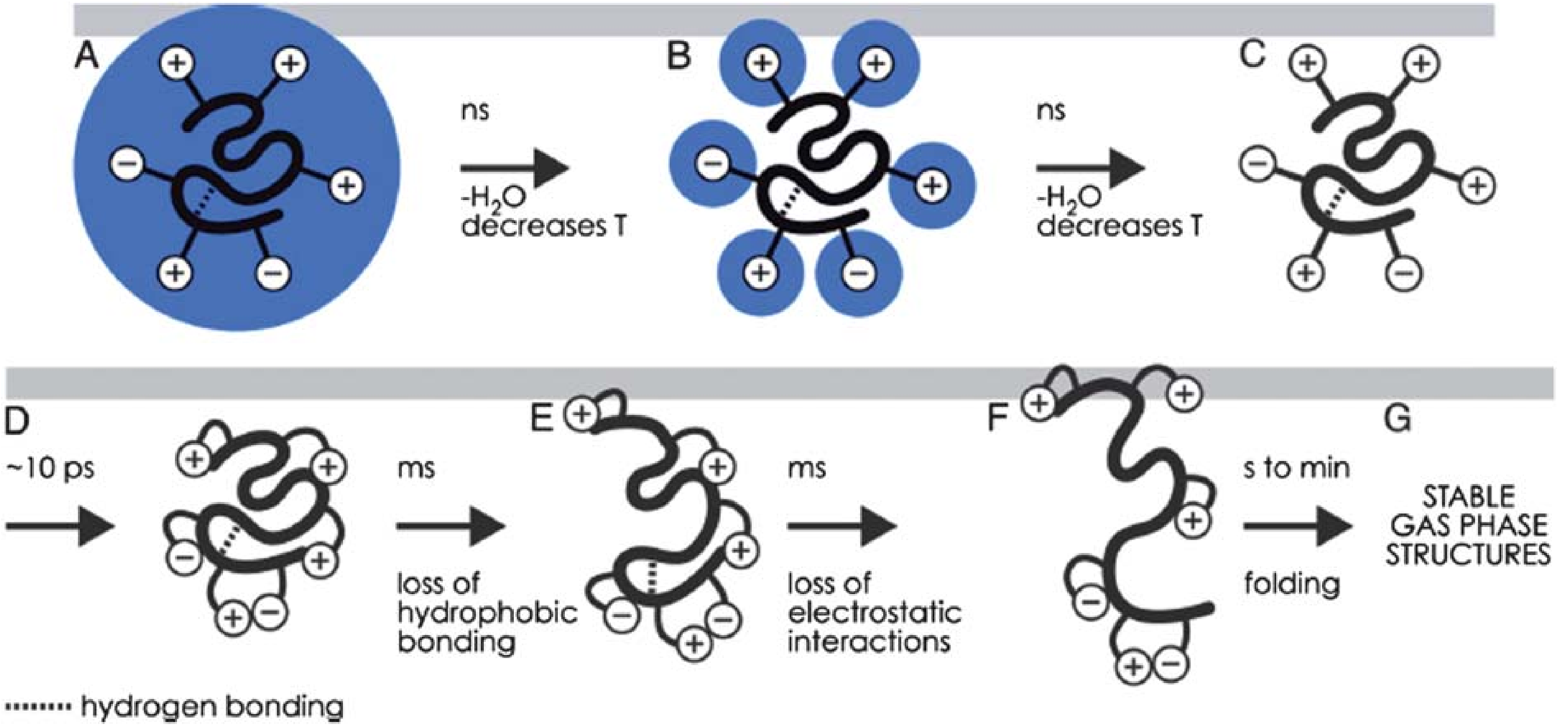


Orbitrap-based mass spectra of intact proteins and protein assemblies. (a–d) Native mass spectra of IgG antibody (a), bacteriophage HK97 capsid pentamers and hexamers (b), yeast 20S proteasome (c) and *E. coli* GroEL (d). Illustrative crystal structures are shown for each protein. Inset in a shows an enlargement of the 25+ charge state of IgG1.

Rose, R. J., Damoc, E., Denisov, E., Makarov, A. & Heck, A. J. R. High-sensitivity Orbitrap mass analysis of intact macromolecular assemblies. *Nat Meth* **9**, 1084–1086 (2012).

ESI & interactions in the vacuum

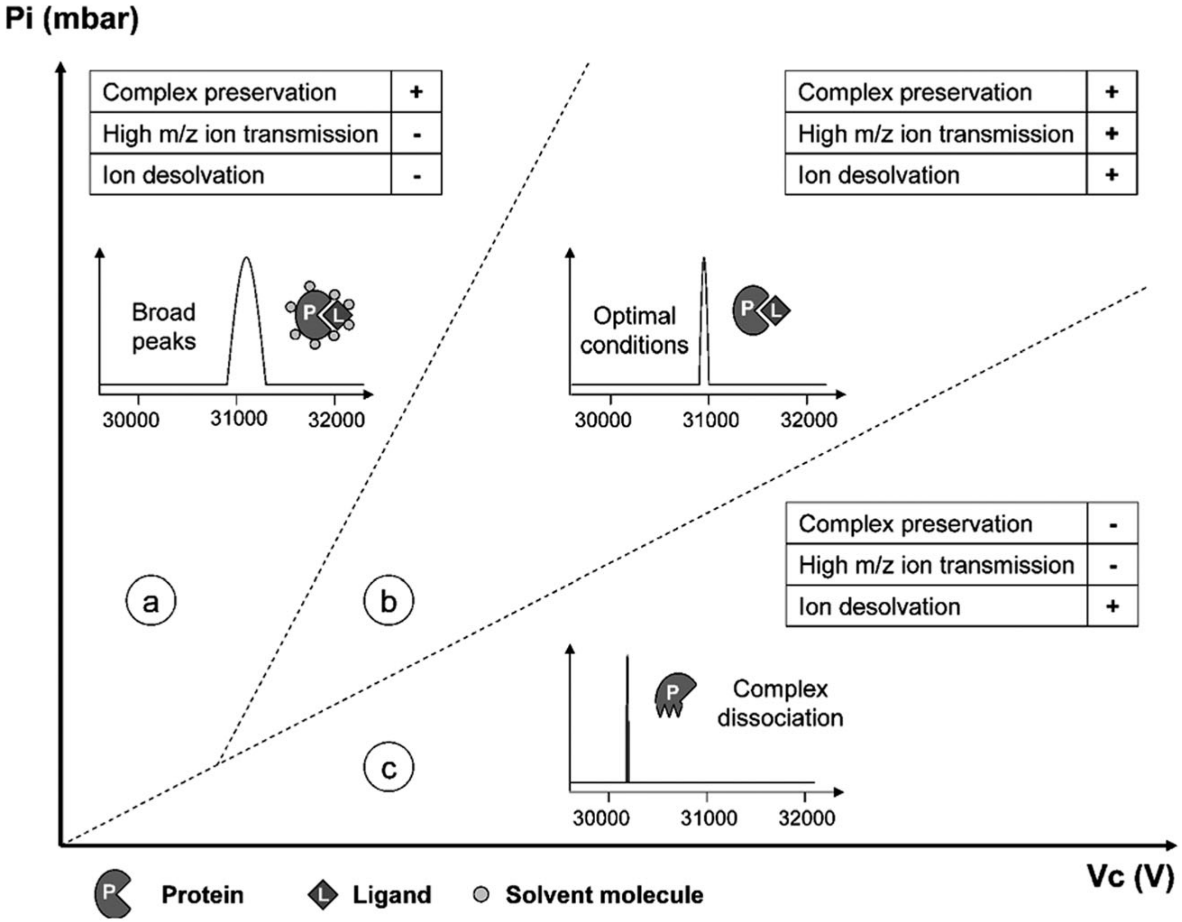
- sequentially
  - loss of water molecules
  - loss of hydrophobic interactions
  - loss of Van der Walls interactions
  - weakening of electrostatic interactions



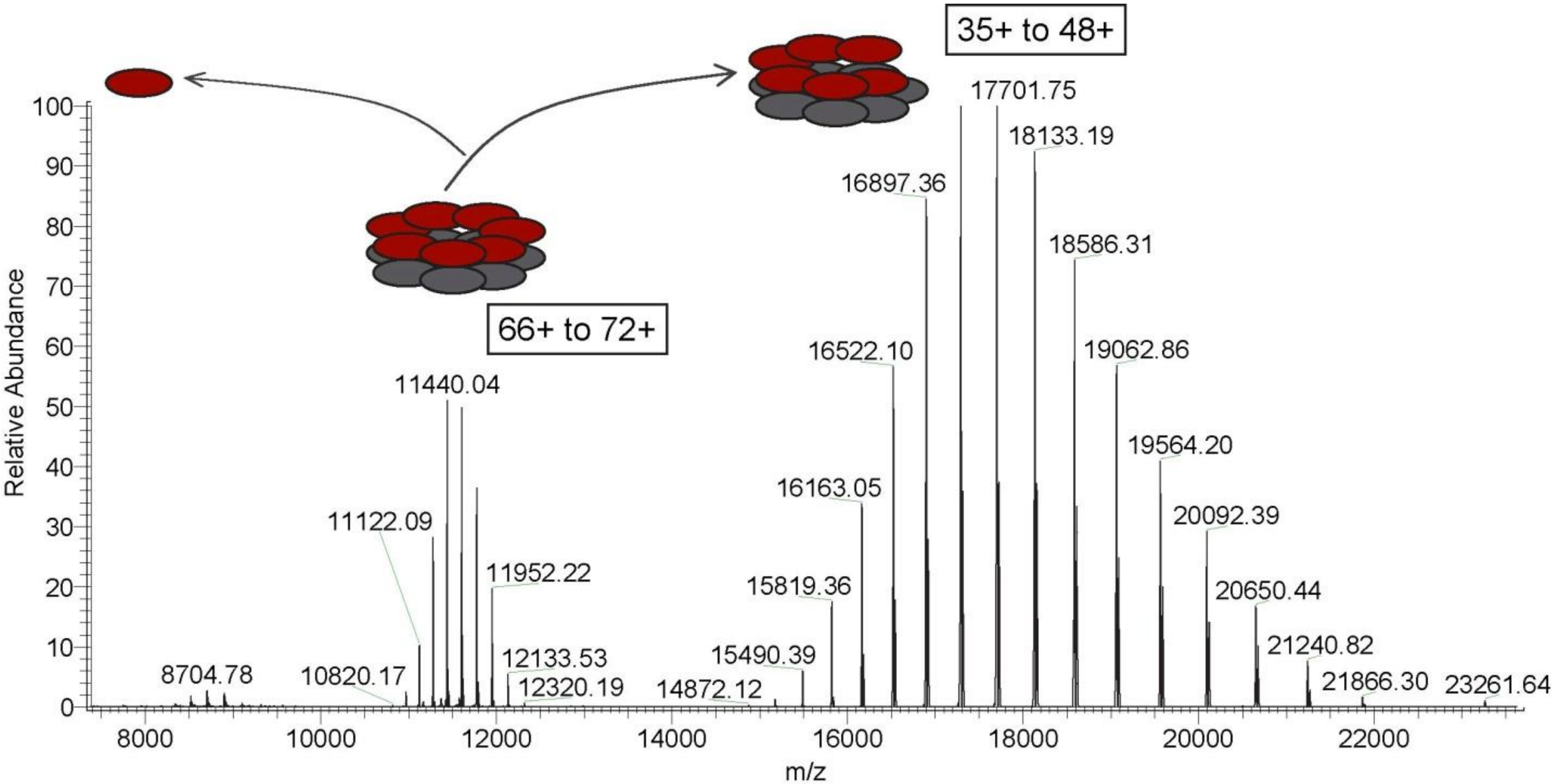
K. Breuker and F. W. McLafferty, Proc. Natl. Acad. Sci. U. S. A., 2008, 105, 18145–18152

Large Complexes

- stabiliy
- collisions in source



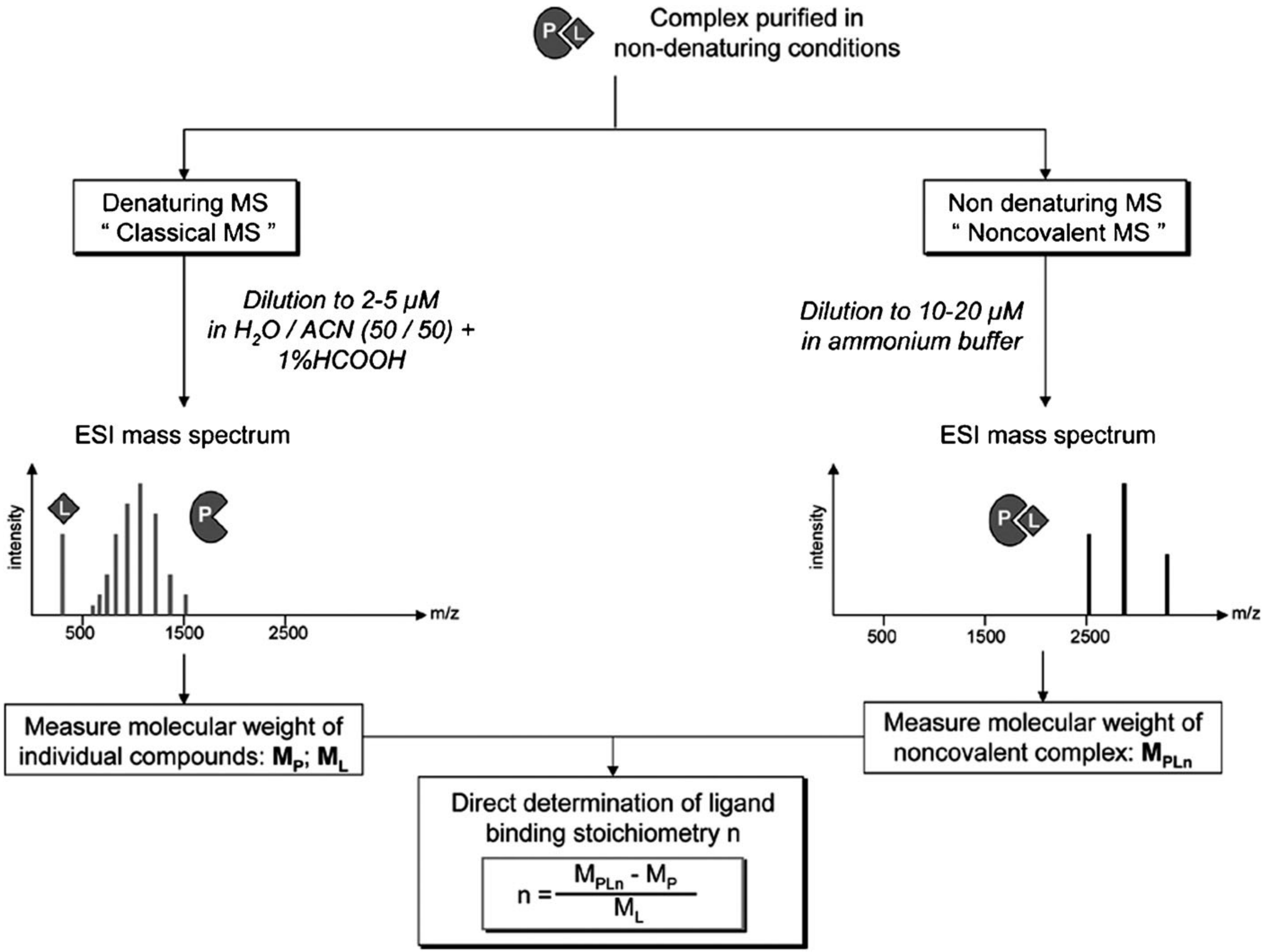
Pacholarz et al *Chem. Soc. Rev.*, 2012, 41, 4335–4355



Supplementary Figure 8. Tandem mass spectrum using HCD activation of the 14-subunit GroEL precursor ions. This asymmetric charge/subunit dissociation pathway, as shown in the cartoon, is typical for gas-phase dissociation of non-covalently bound protein complexes by collisional activation. 13-subunit GroEL fragment ions are detected at *m/z* values up to and above 20,000 Th.

Protein-Ligand

- studying protein-ligand interactions in native mode



Complex purified in non-denaturing conditions

Denaturing MS
" Classical MS "

Dilution to 2-5 µM in H_2O / ACN (50 / 50) + 1%HCOOH

ESI mass spectrum

Measure molecular weight of individual compounds: $\mathbf{M_P}$; $\mathbf{M_L}$

Non denaturing MS
" Noncovalent MS "

Dilution to 10-20 µM in ammonium buffer

ESI mass spectrum

Measure molecular weight of noncovalent complex: $\mathbf{M_{PLn}}$

Direct determination of ligand binding stoichiometry n

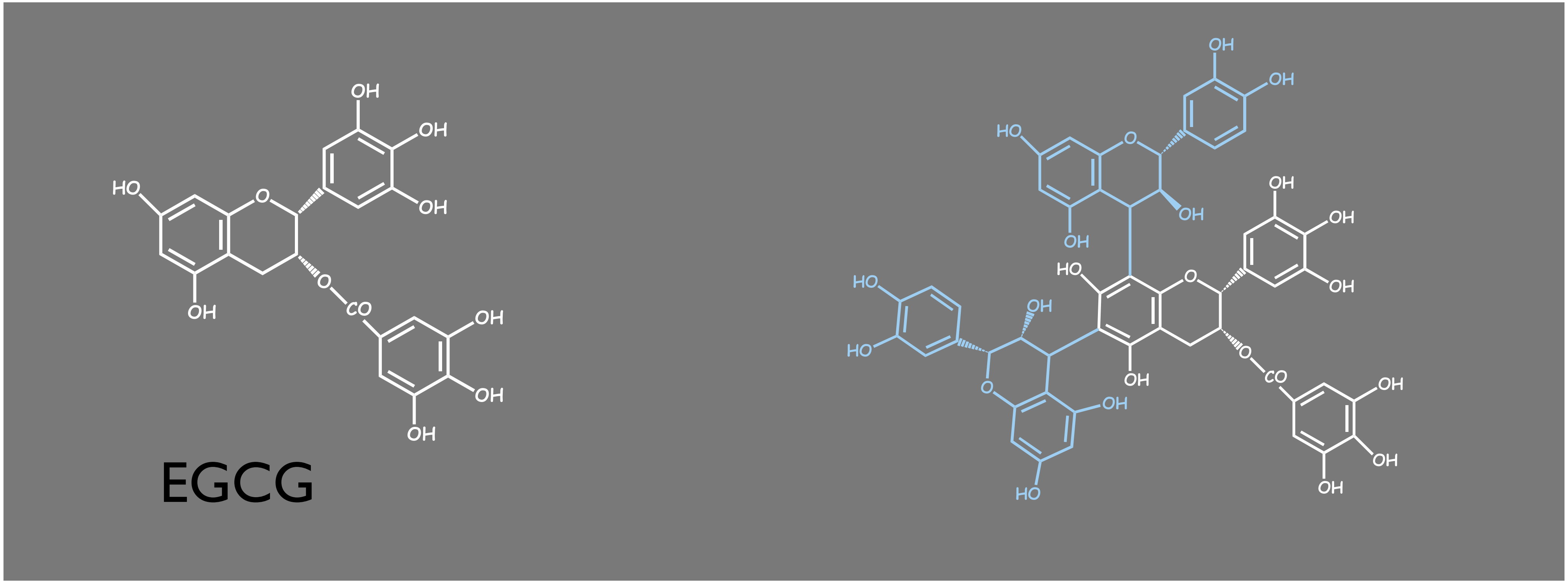
$$n = \frac{M_{PLn} - M_P}{M_L}$$

IB5 - salivary protein

- IDP - low complexity

SPPG**KPQGPP**QQEGN**KPQGPP**PPG
KPQGPPPAGGNPQQPQAPPAG
KPQGPPPP**PQG**GRPPRPAQGQQPPQ

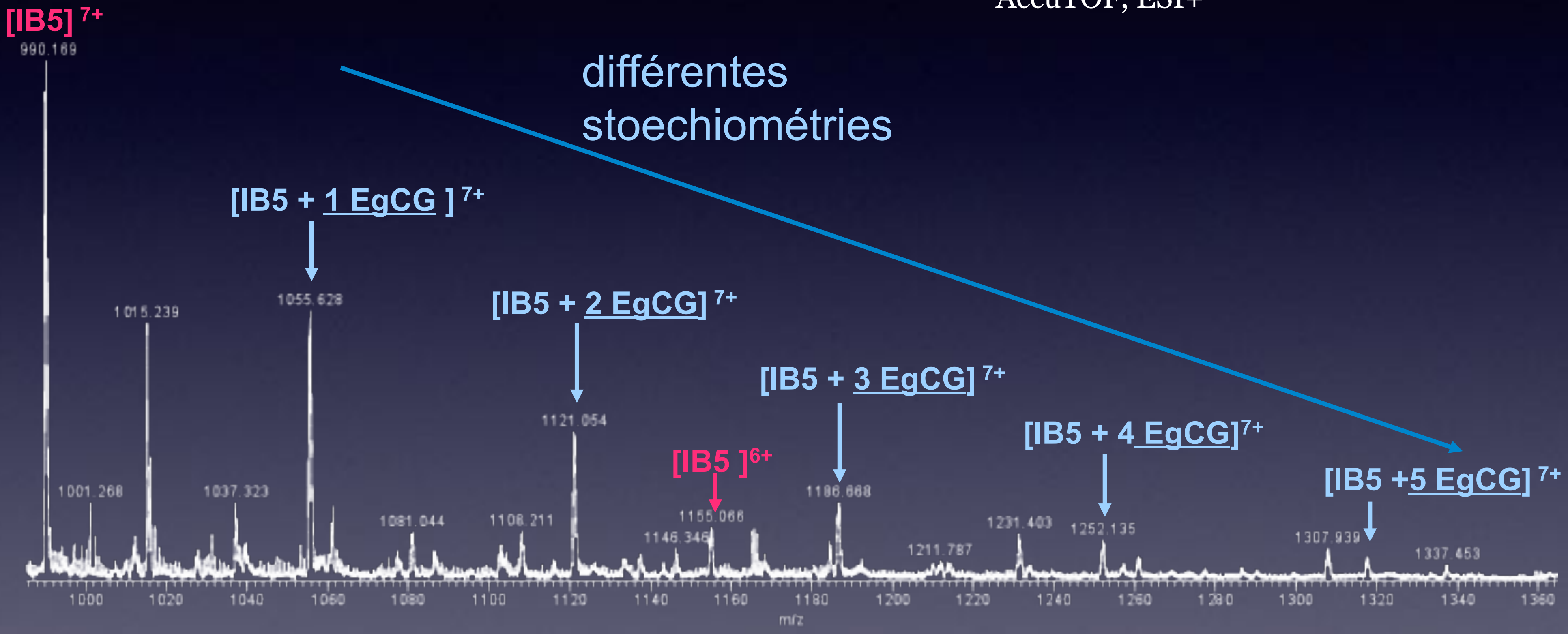
- Interact selectively with polyphenols (proanthocyanidines)


EGCG

IB5 / TANNINS

Interaction IB5-EgCG

milieu: H_2O/EtOH (88/12) pH=3,2 (CH_3COOH)
Rapport 1:10 IB5:EgCG (IB5 5µM ; EgCG 50µM)
AccuTOF, ESI+



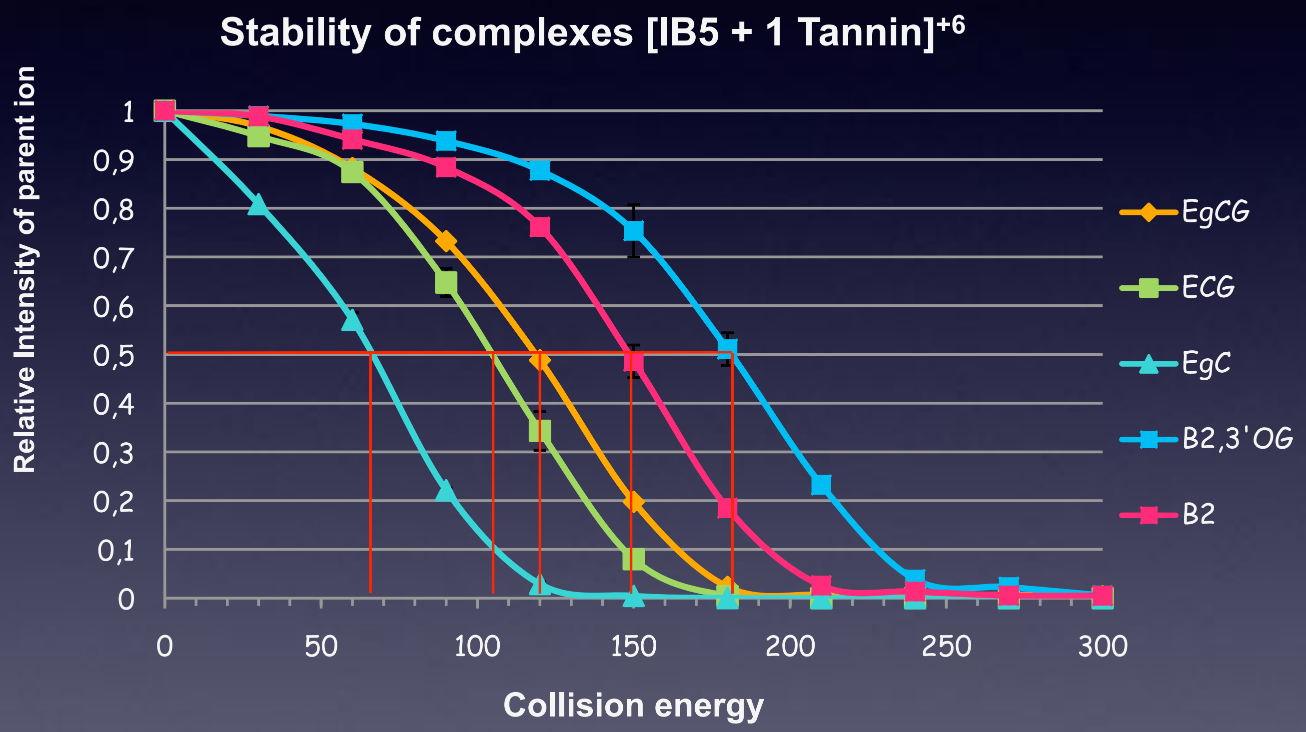
➜ Formation de complexes IB5:EgCG avec différentes stoechiométries (de 1:1 à 1:5)

Francis Canon / Véronique Cheynier

IB5 / TANNINS

Impact de la structure des tanins sur l’interaction

collision contre un gaz neutre   depend de la vitesse d’accélération



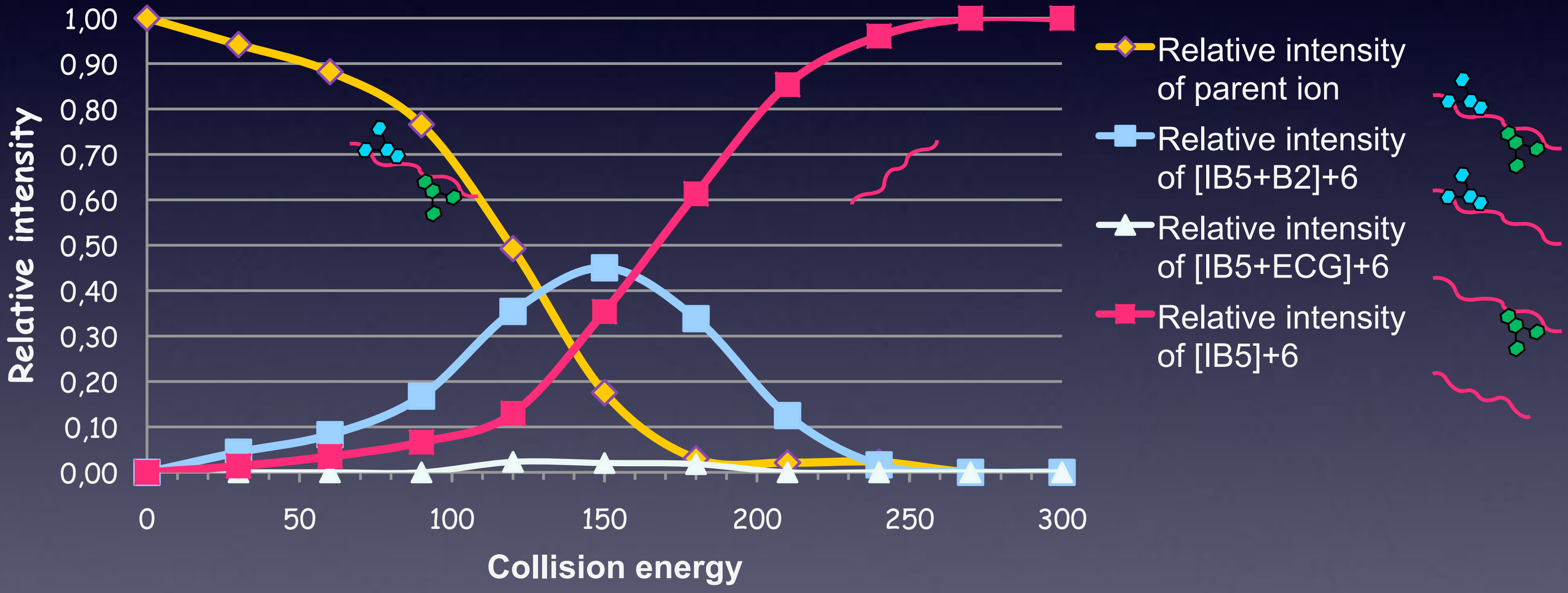
	IB5-EgC	IB5-ECG	IB5-EgCG	IB5-B2	IB5-B2,3’OG
E_{50} (eV)	67.5	105	120	150	180
E_{cm} (eV)	0.26	0.4	0.45	0.55	0.65

Francis Canon / Véronique Cheynier

IB5 / TANNINS

Expériences de MS/MS: cas des complexes IB5:tanin 1:2

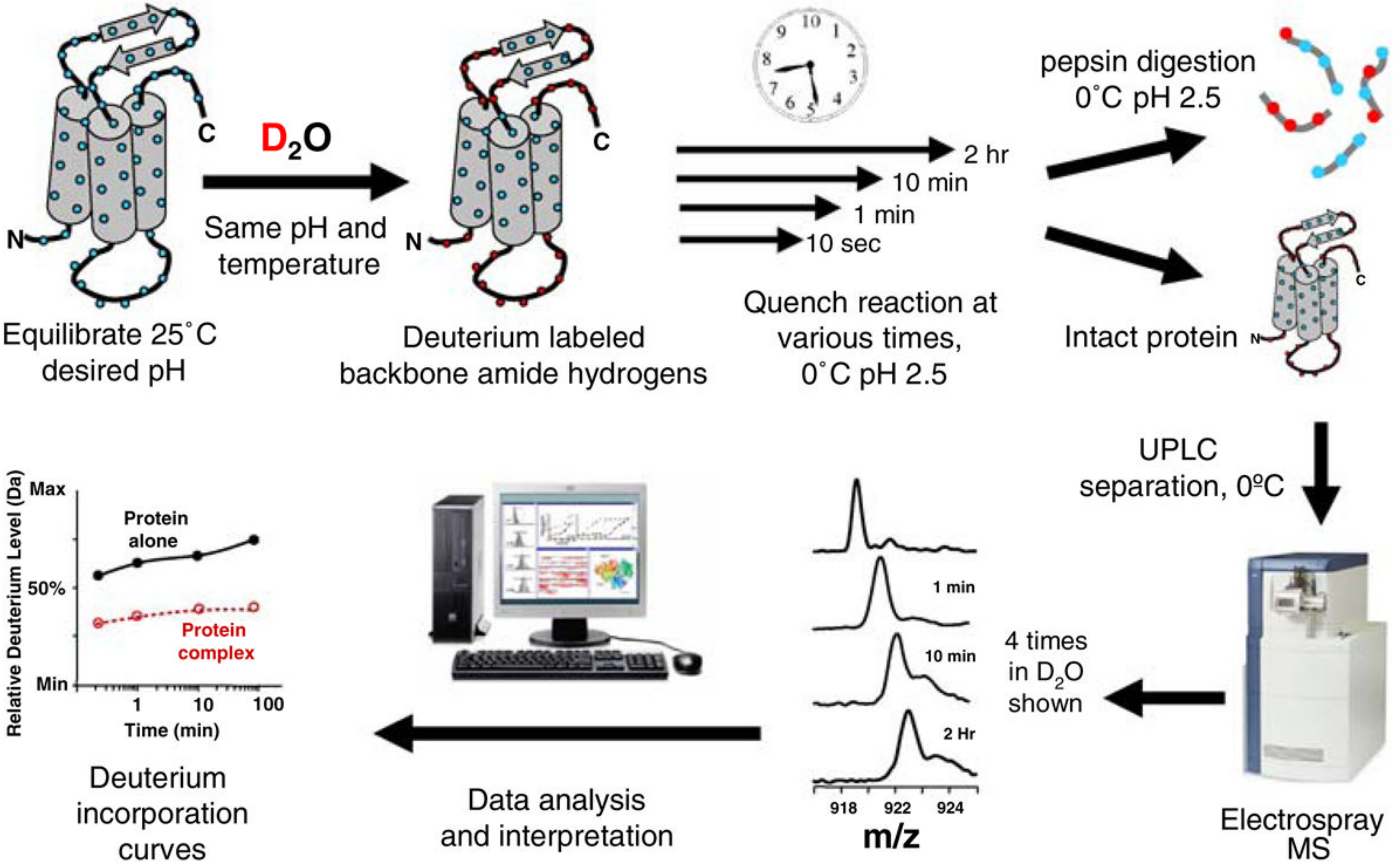
Exemple de l'hétérocomplexe IB5:(B2/ECG)



➜ **Les deux tanins ne sont pas libérés en même temps**

Francis Canon / Véronique Cheynier

H / D exchange



S. R. Marcsisin and J. R. Engen *Anal Bioanal Chem*. 2010 June ; 397(3): 967–972. doi:10.1007/s00216-010-3556-4.

H / D exchange

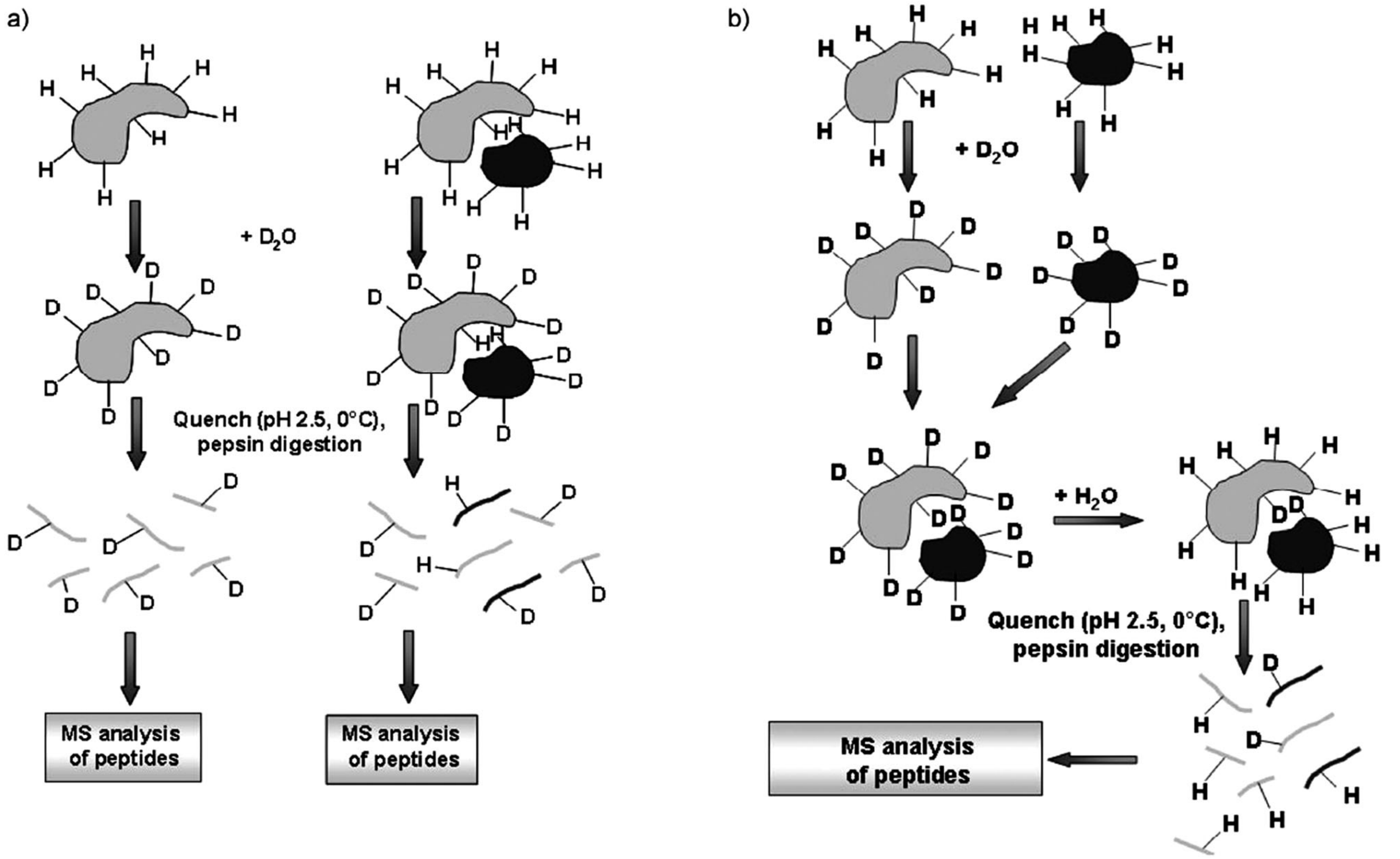
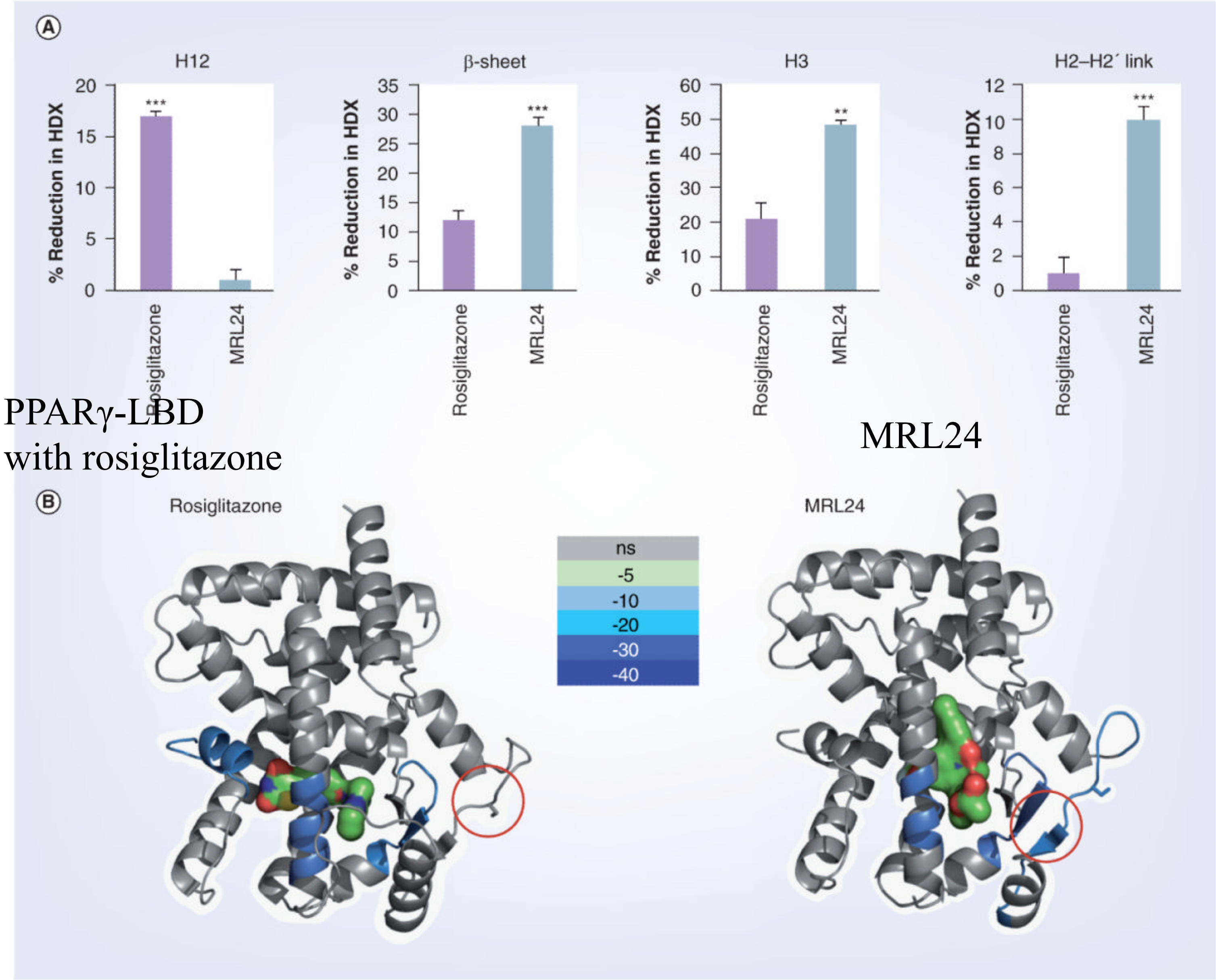


Fig. 9 Scheme of on- and off-exchange approaches used in HDX experiments. Reproduced from A. Sinz, 2007[5] with permission from John Wiley and Sons.

H / D exchange



PPARγ-LBD
with rosiglitazone

MRL24

Bruning JB, Chalmers MJ, Prasad S, et al.. *Structure*. 2007; 15(10):1258–1271.

Cross-Link

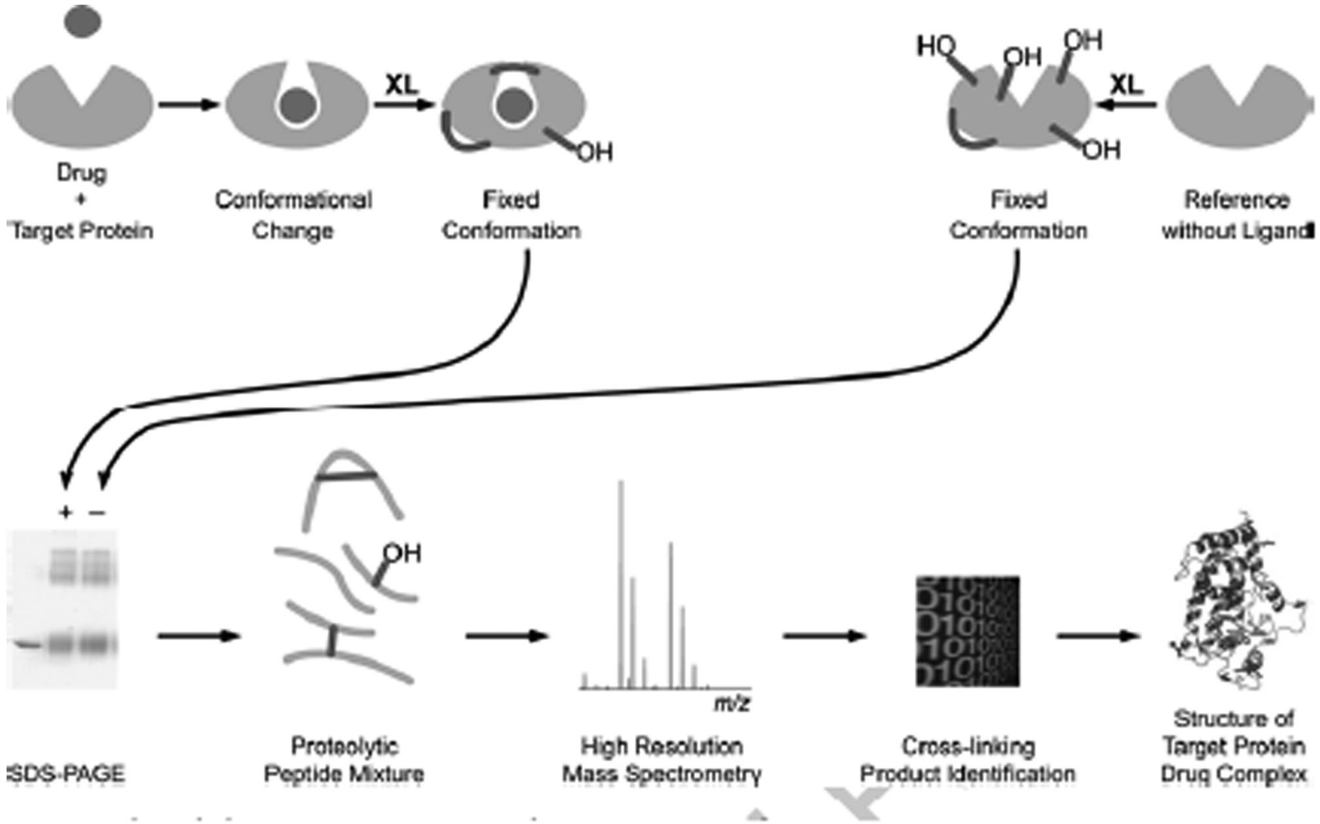
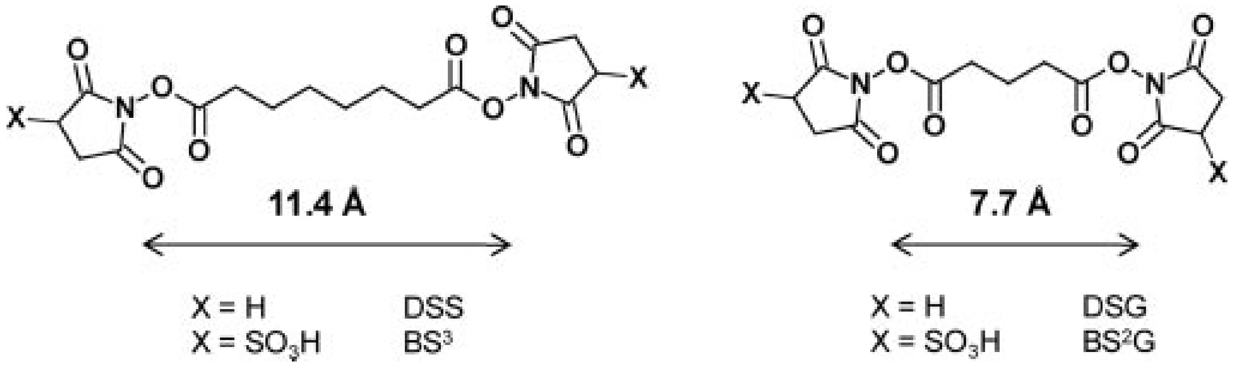


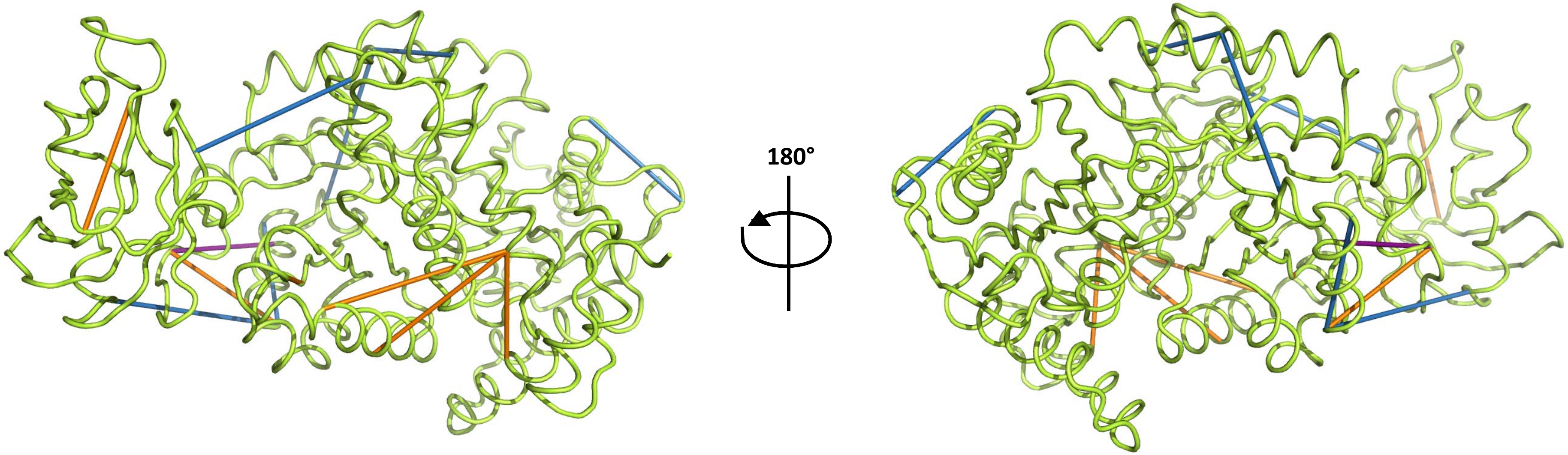
Fig. 12 Analytical strategy for analysing conformational changes in protein upon ligand binding by chemical cross linking and high resolution mass spectrometry. Reproduced from Muller and Sinz[180] with permission from Springer.



one example

Pyruvate kinase (KPYM_RABIT, P11974), PDB structure 2G50

DSS **PDH** **ZL**



A. Leitner, L.A.Joachimiak, P. Unverdorben, T.Walzthoeni, J.Frydman, F.Förster, and R.Aebersold

PNAS **111** 26 9455–9460

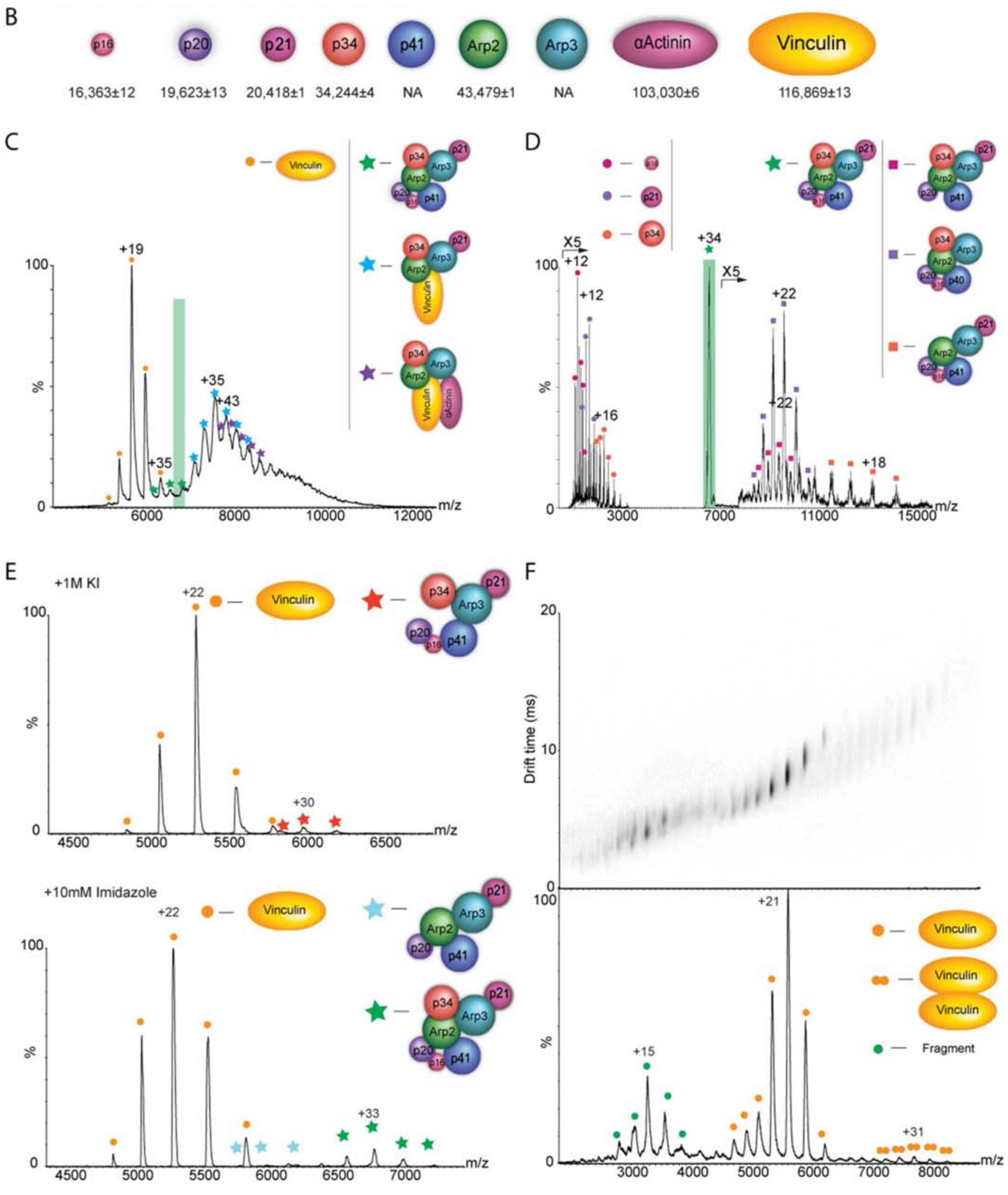
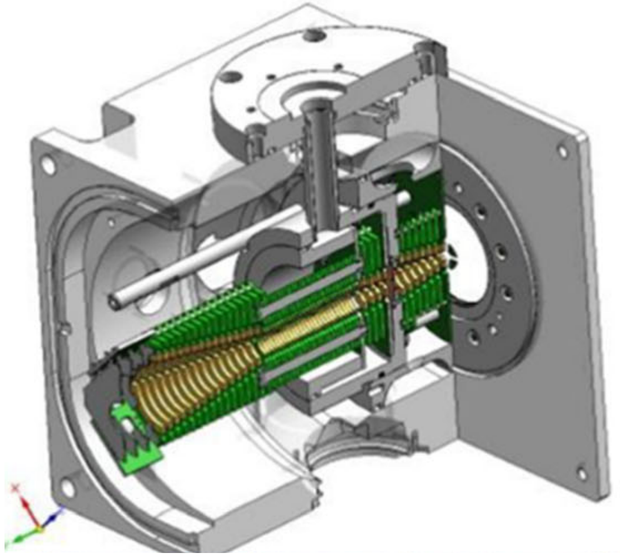


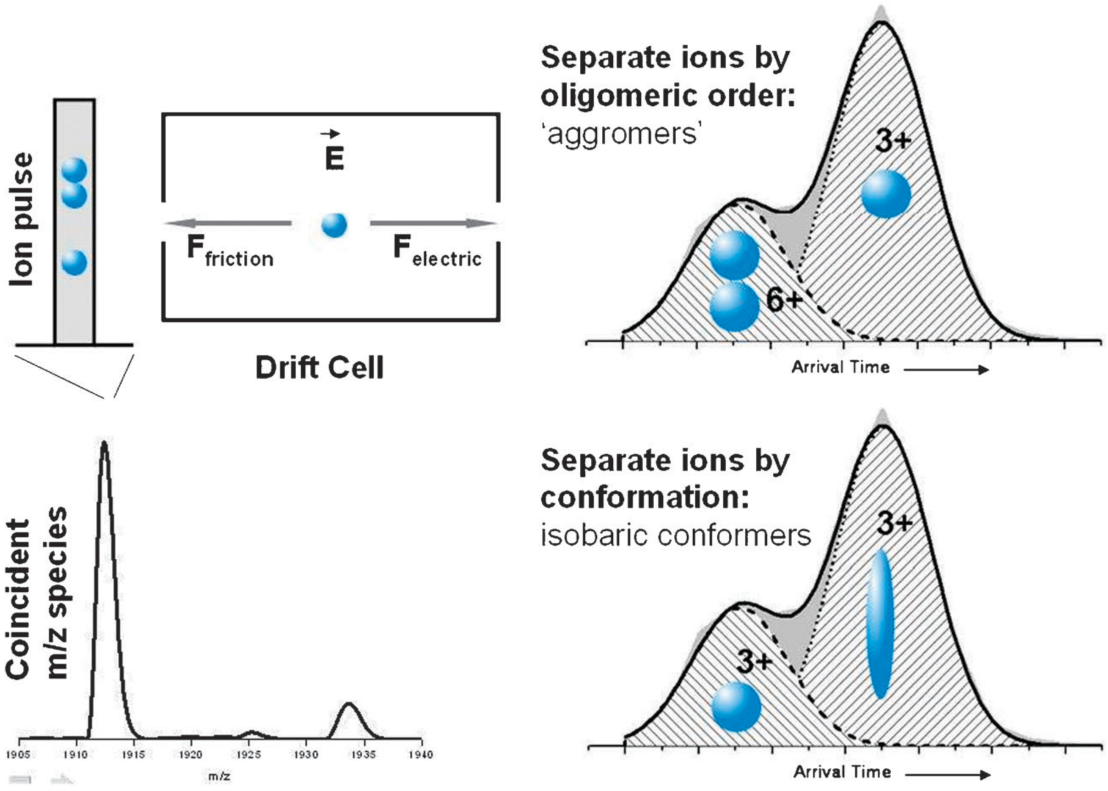
Figure 3.

vinculin forms hybrid complexes with components of the Arp2/3 actin polymerization complex

Chorev DS, et al., *Nat Commun*, 2014, 5, 3758

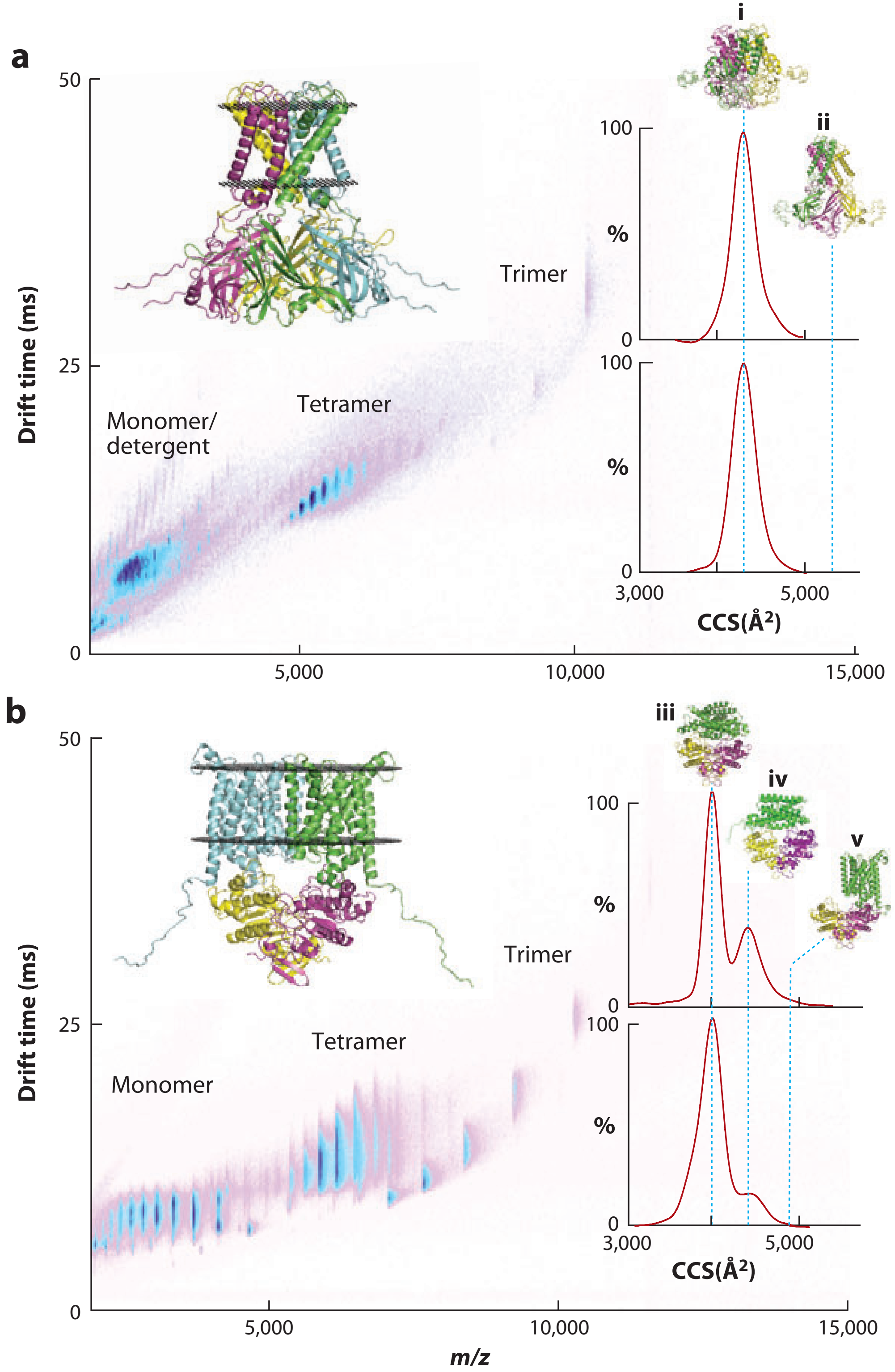
Ionic Mobility





Separate ions by oligomeric order:
'aggromers'

Separate ions by conformation:
isobaric conformers



from N.Barrera, C.Robinson
Annu. Rev. Biochem. 2011. 80:247–71

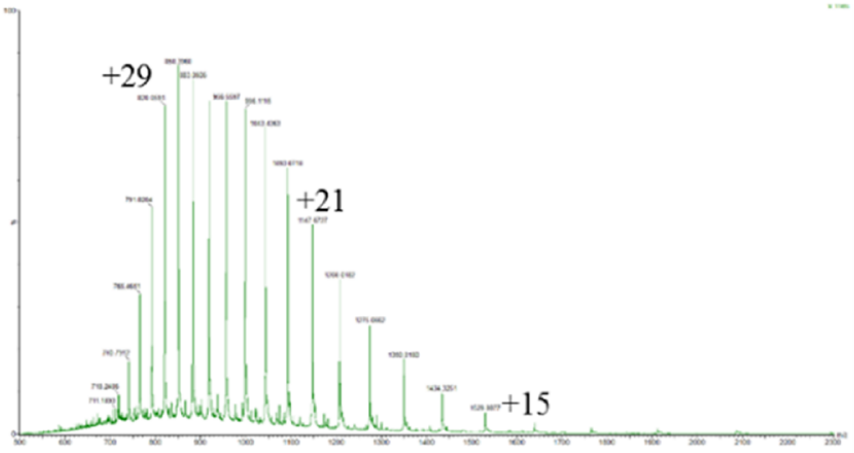
Another example

Monitoring Conformational Landscape of Ovine Prion Protein Monomer Using Ion Mobility Coupled to Mass Spectrometry

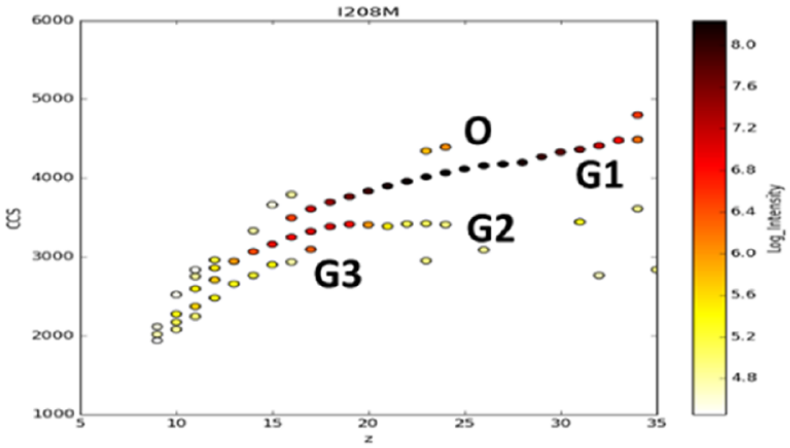
J. Am. Soc. Mass Spectrom. (2017) 28:303–314
DOI: 10.1007/s13361-016-1522-x

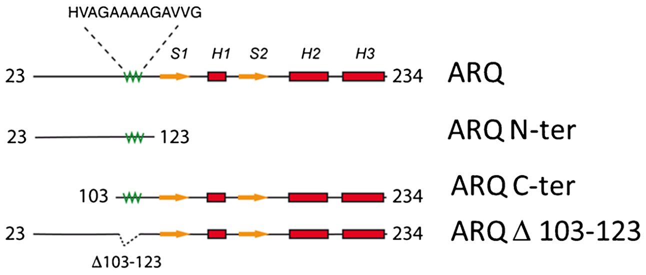
Guillaume Van der Rest,[1] Human Rezaei,[2] Frédéric Halgand[1]

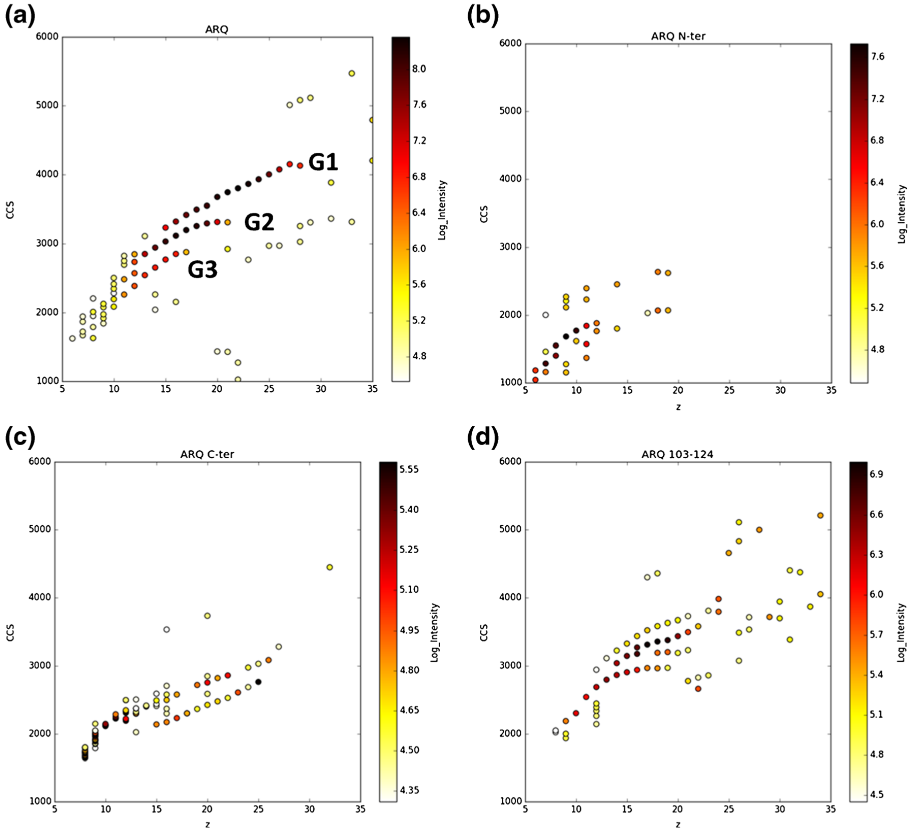
(a)



(b)

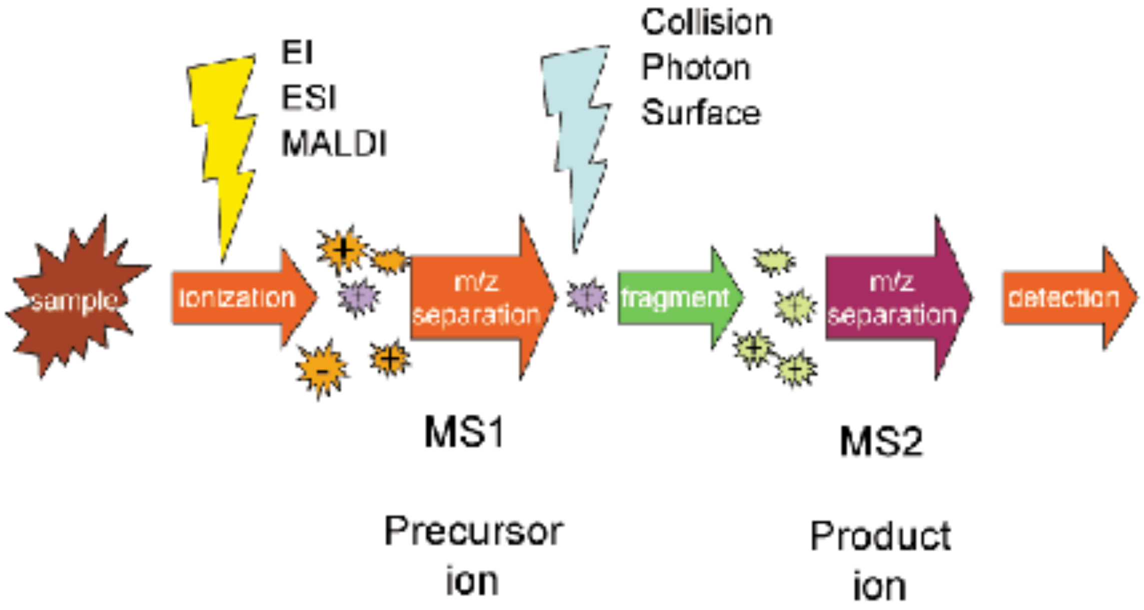






Tandem MS : MS-MS

- Tandem MS : coupling
  - the first MS select one m
  - a fragmentation is applie
  - the mass spectrum of the



- Several fragmentation techniques available

  Mostly :

  - CID : Collision Induced Dissociation

    collision with a neutral gaz : eg Argon
  - IRMPD : IR Multiple Photon Dissociation

    irradiation with a IR laser
  - ECD : Electron Capture Dissociation

    bombardment with e-
  - ETD : Electron Transfert Dissociation

    transfert of e- by collision with charged molecules

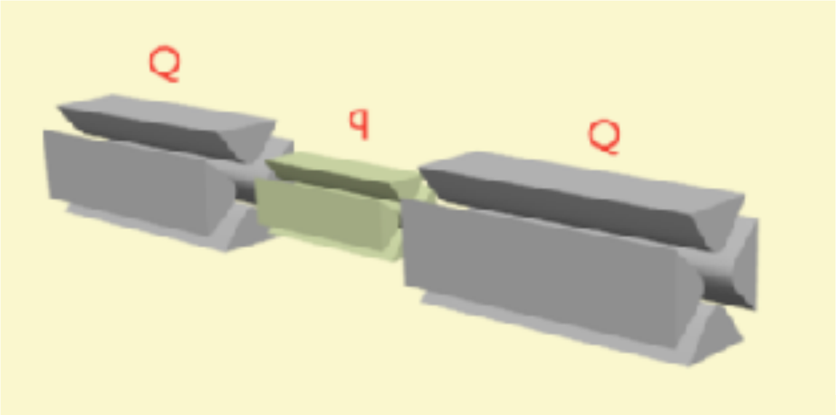
Tandem MS : MS-MS

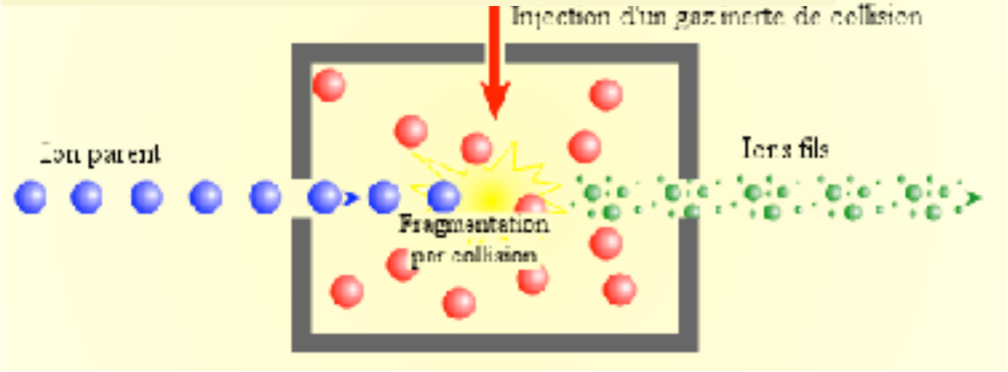
- Tandem MS : coupling two MS measure in series.
  - the first MS select one m/z
  - a fragmentation is applied to the parent peak
  - the mass spectrum of the gradients is determined

- Several fragementation technique available
    - Mostly :
  - CID : Collision Induced Dissociation
    - collision with a neutral gaz : eg Argon
    - SID - HCD
  - ETD : Electron Transfer Dissociation
    - bombardment with e-
    - EDD - ECD
  - IRMPD : IR Multiple Photon Dissociation
    - irradiation with a IR laser
    - BIRD

MS-MS : several possible geometry

- coupling 2 MS plus a dissociation chamber
    - QqQ (Q3)





- but also
    - Q-TOF
    - Q-Orbitrap
    - TOF-TOF

proteomics

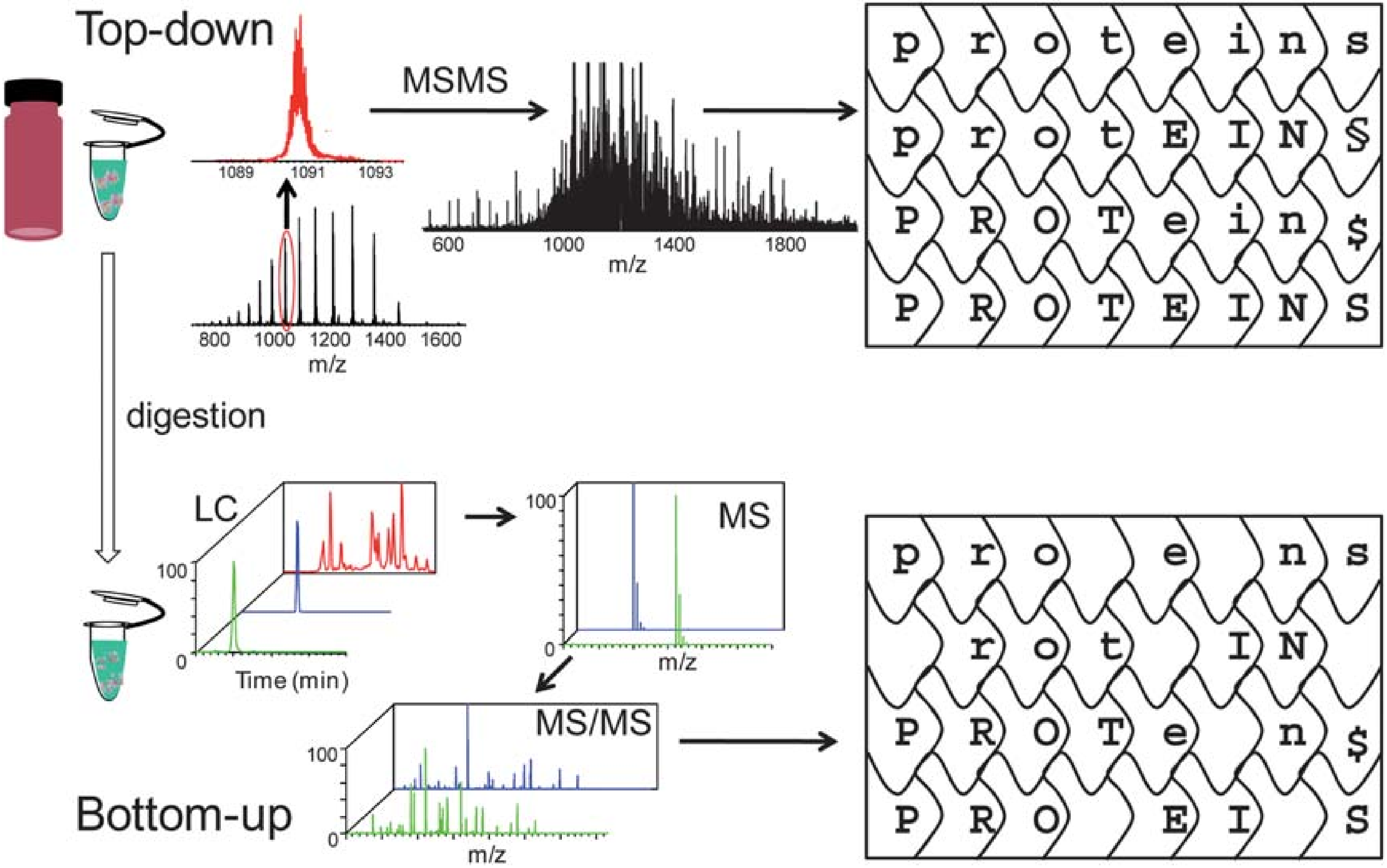
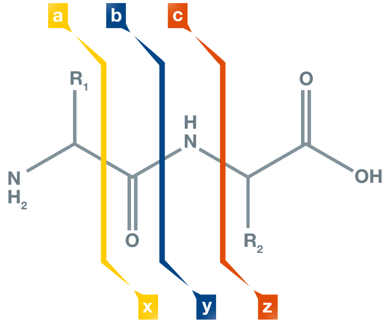
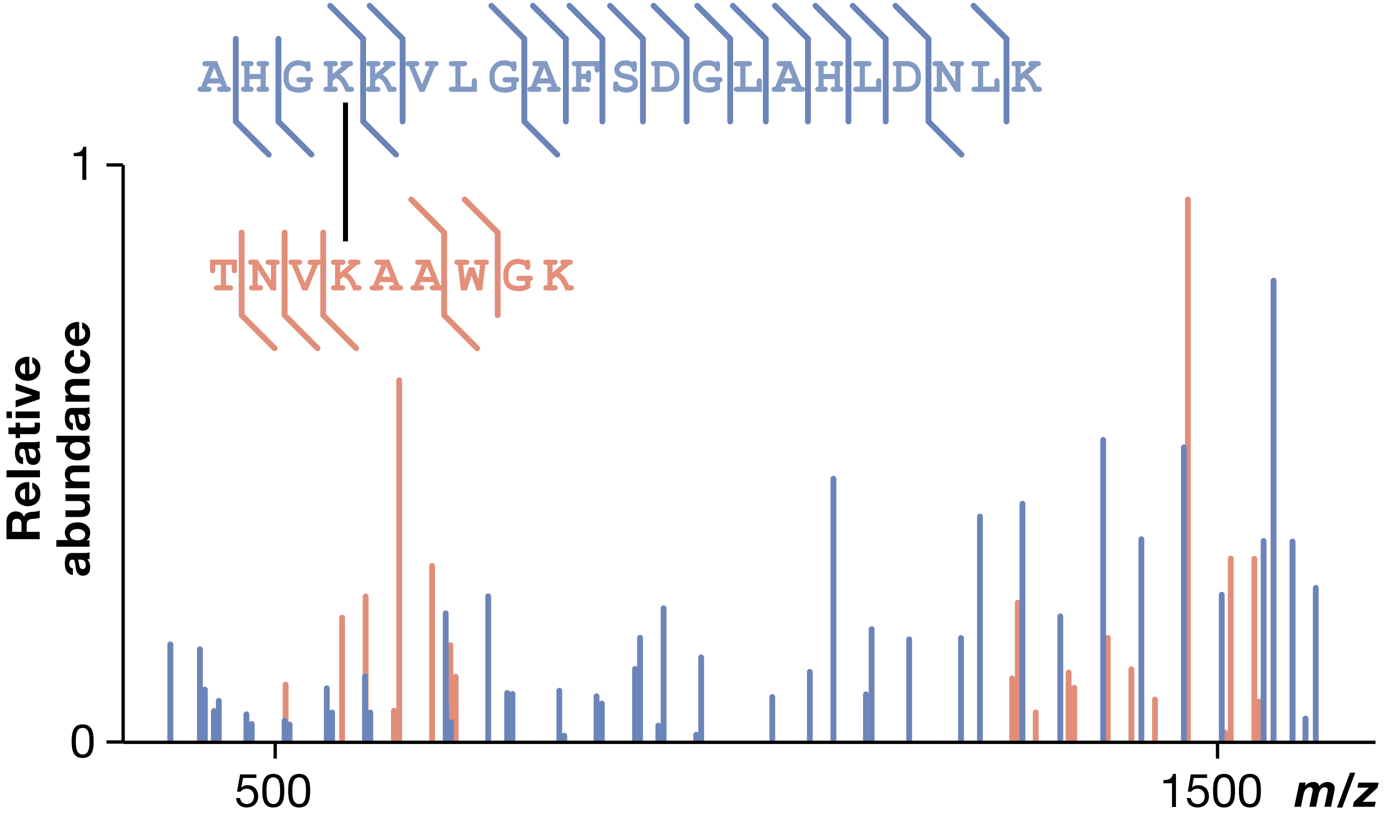


Fig. 1 Comparison of top-down and bottom-up workflows.

Fragmentation

- fragmentation and identification from databases





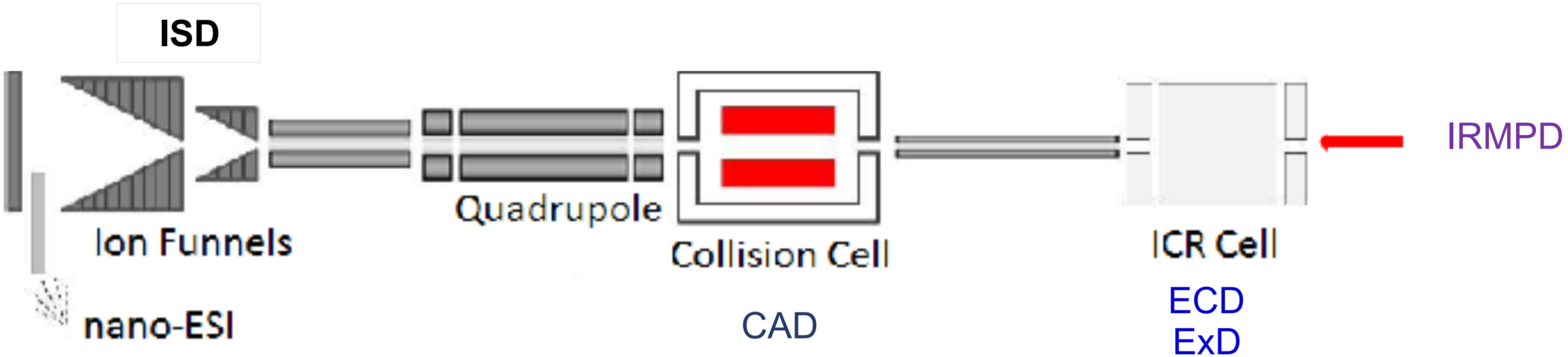
P. Lössl, M. van de Waterbeemd & A. JR Heck

The EMBO Journal (2016) 35: 2634–2657

Directly Obtaining Both Proteomics and Structural Information by Native Top-Down MS?

- ➢ Native MS for macromolecular complexes
- ➢ Top-down capability
  - High resolution
  - High mass accuracy
  - Multiple fragmentation techniques

solarix 15-Tesla



ISD

IRMPD

CAD

ETD

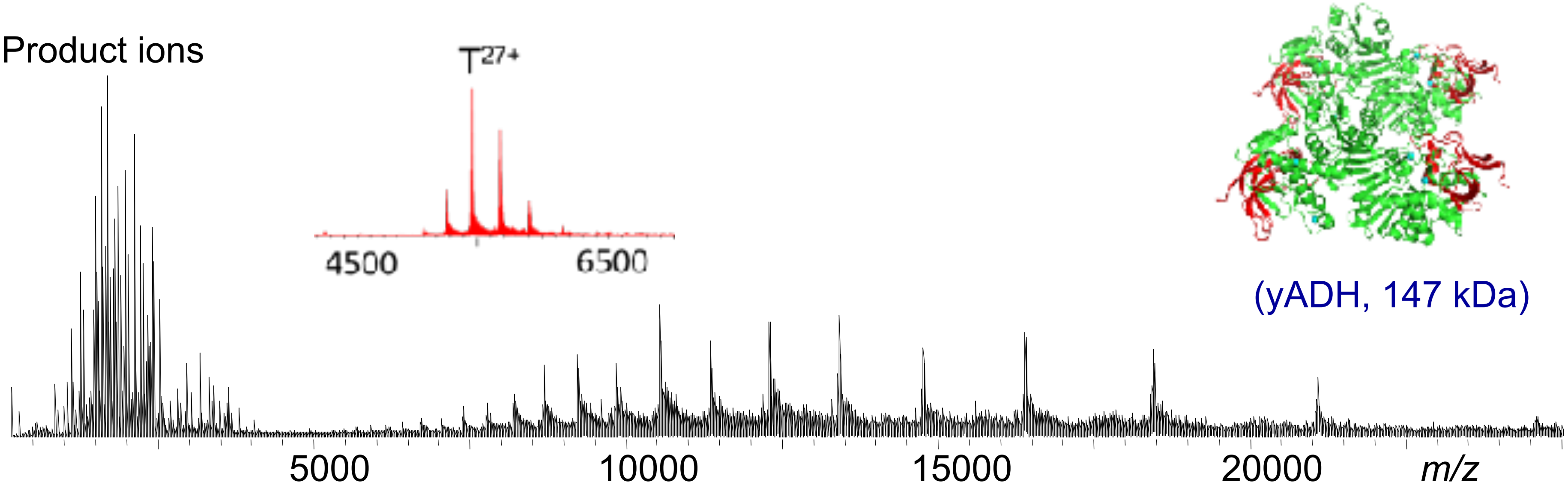
ECD
ExD

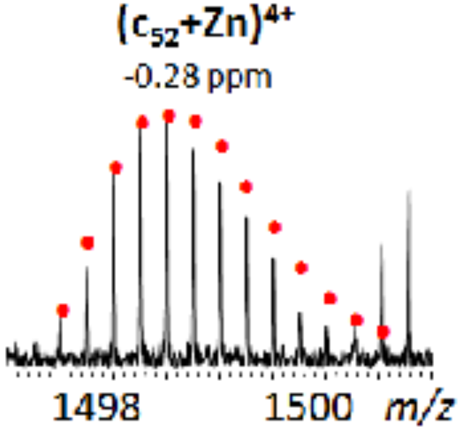
1~2 μL, 3~20 μM
in ~200 mM NH_4OAc

From Huilin Li

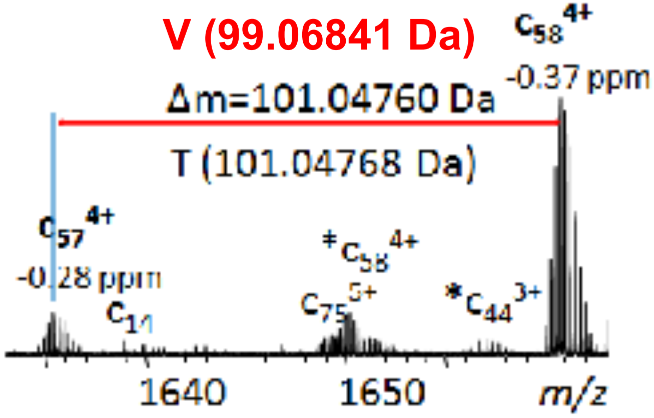
Native Top-Down MS

– Harvest the synergy between proteomics and native MS

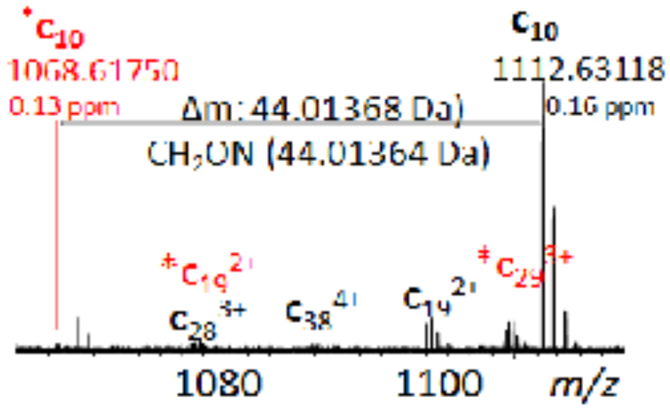




zinc-binding



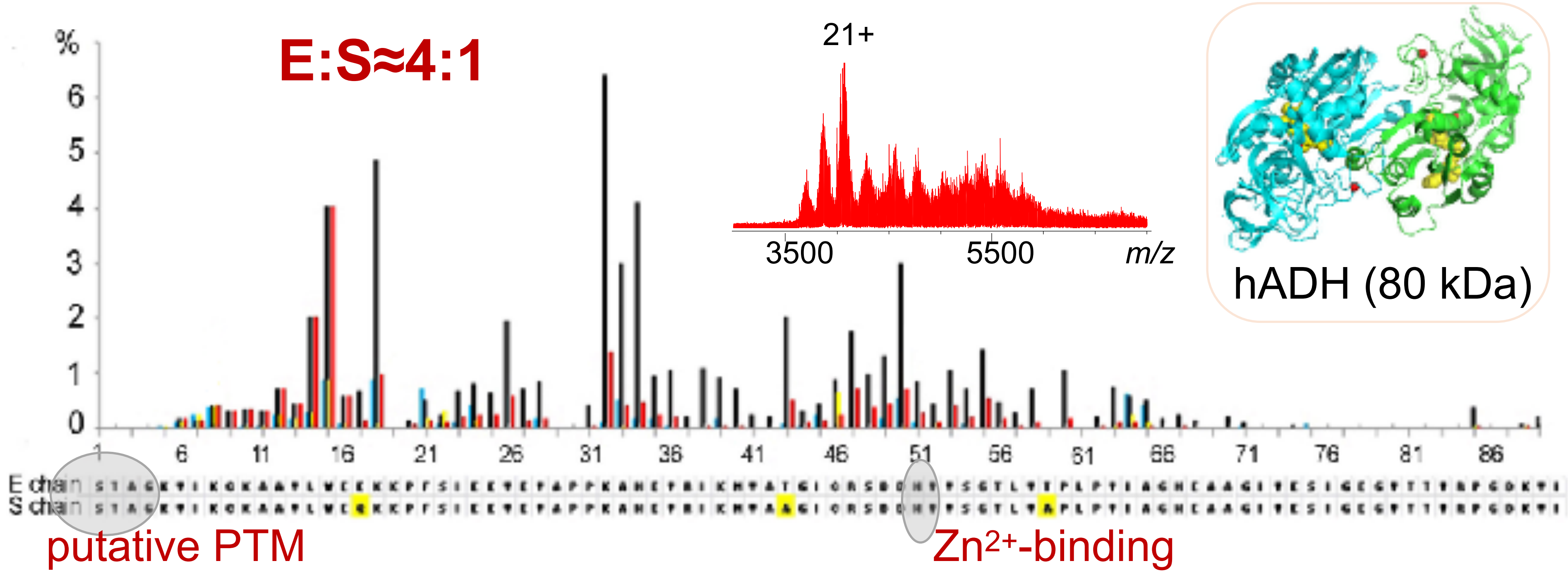
site mutation (V58T)

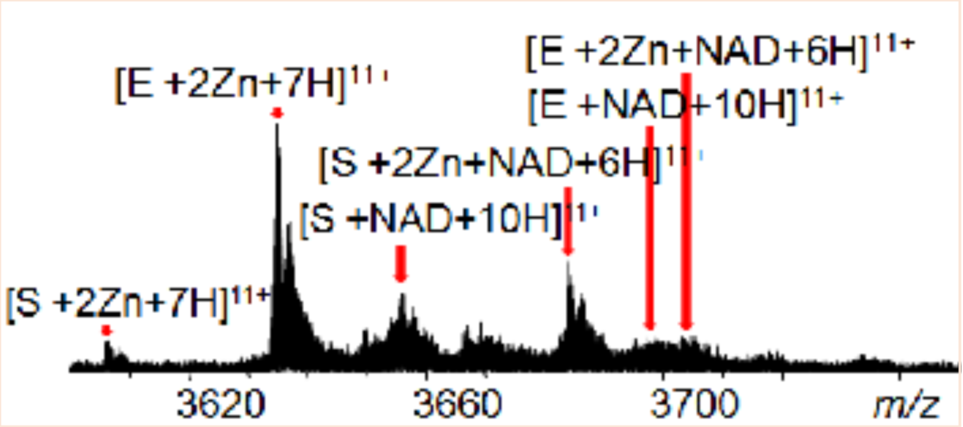


putative PTM

Li, H. et al. *J. Am. Soc. Mass Spectrom.* **2014**. 25, 2060-2068.

All Information in One Experiment

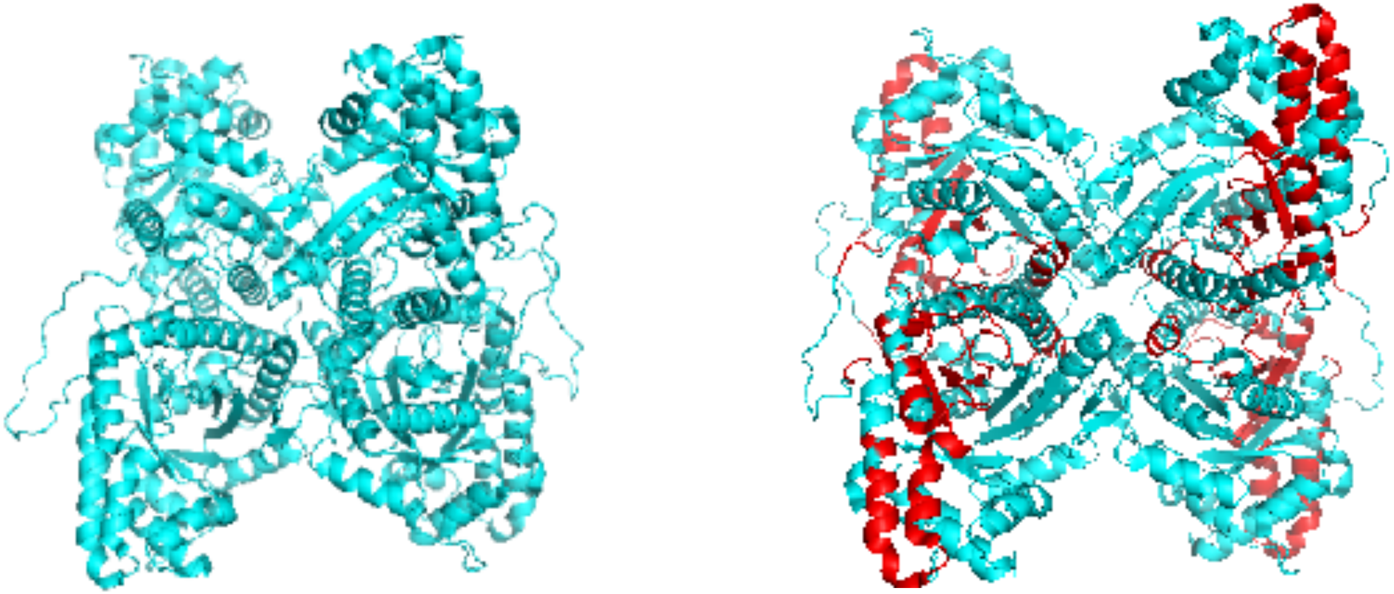


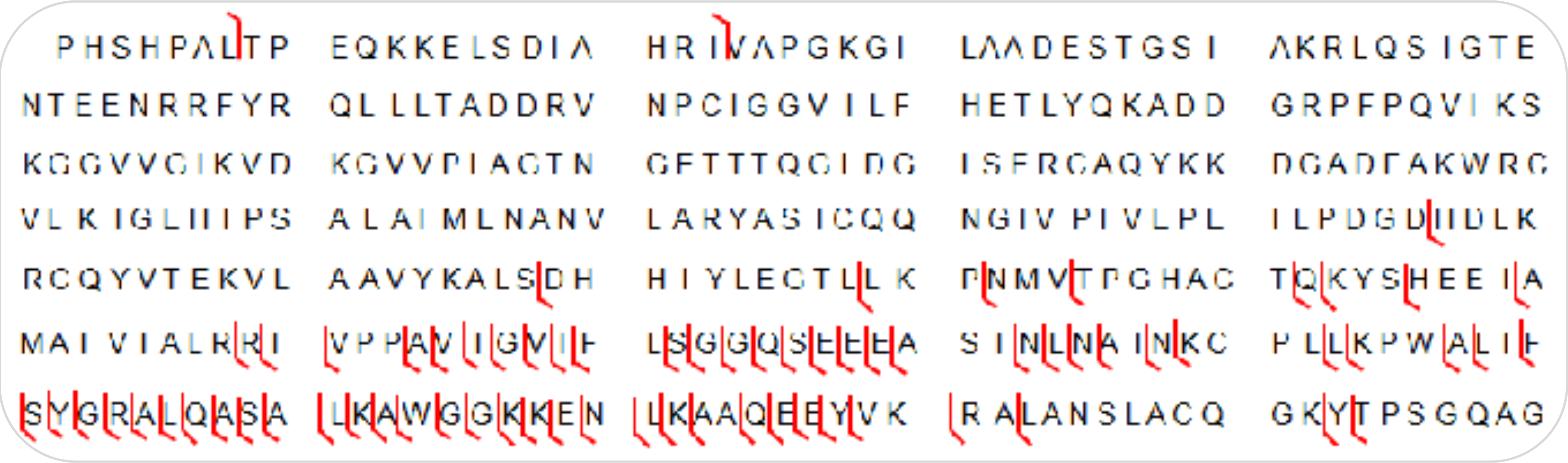


Overall, 40% sequence coverage

Li, H. et al. *J. Am. Soc. Mass Spectrom.* **2014**. 25, 2060-2068.

ECD Reveals Outer Surface Residues of Aldolase Tetramer (158 kDa)

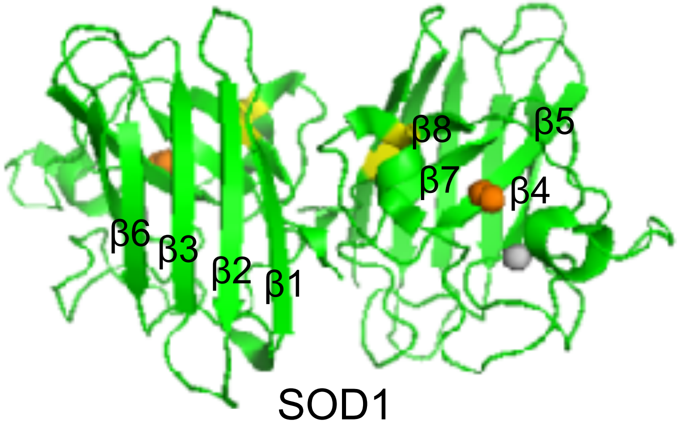




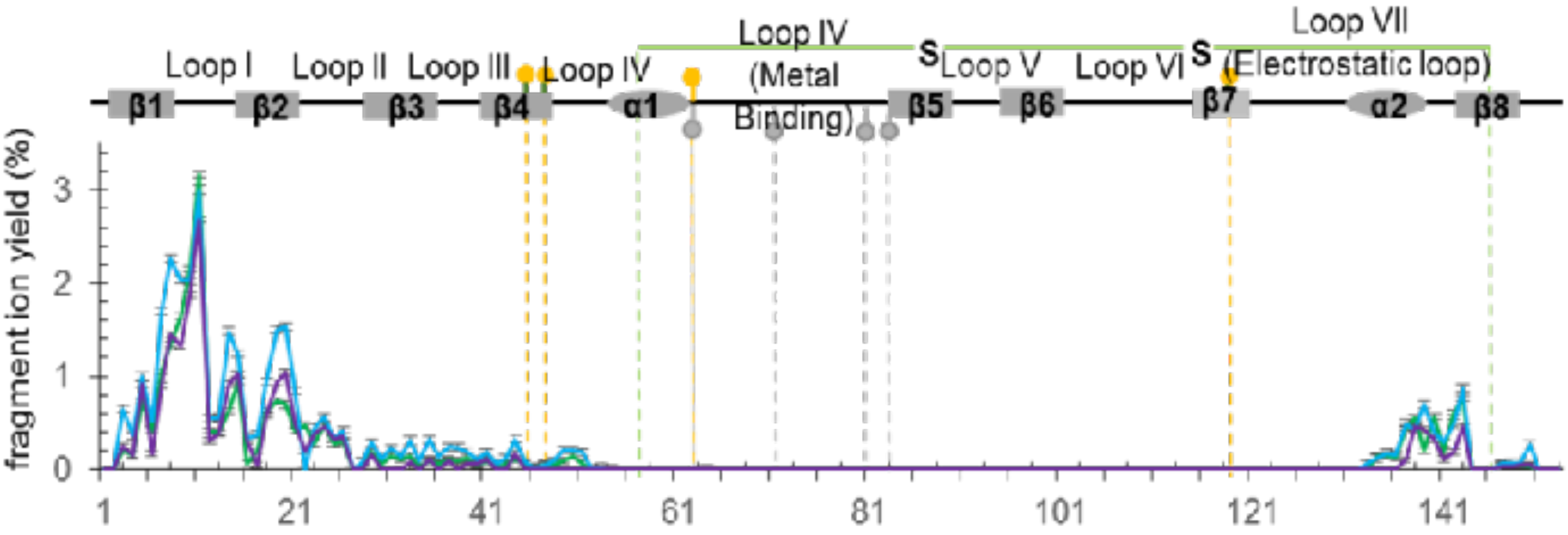
PHSHPALTP EQKKELSDIA HRIVAPGKGI LAADESTGSI AKRLQSIGTE
NTEENRRFYR QLLLTADDRV NPCIGGVILF HETLYQKADD GRPFPQVIKS
KGGVVGIKVD KGVVPLAGTN GETTTQGLDG LSERCAQYKK DGADFAKWRC
VLKIGEHTPS ALAIMENANV LARYASICQQ NGIVPIVEPE ILPDGDHDLK
RCQYVTEKVL AAVYKALSDH HIYLEGTLLK PNMVTPGHAC TQKYSHEEIA
MATVTALRRT VPPAVTGVTF LSGGQSEEEA SINLNAINKC PLLKPWALTF
SYGRALQASA LKAWGGKKEN LKATQEEYVK RALANSLACQ GKYTPSGQAG

Li, H. et al. *Anal. Chem.* **2014.** 86, 317-320

Revealing Structural Similarity upon Metal Binding



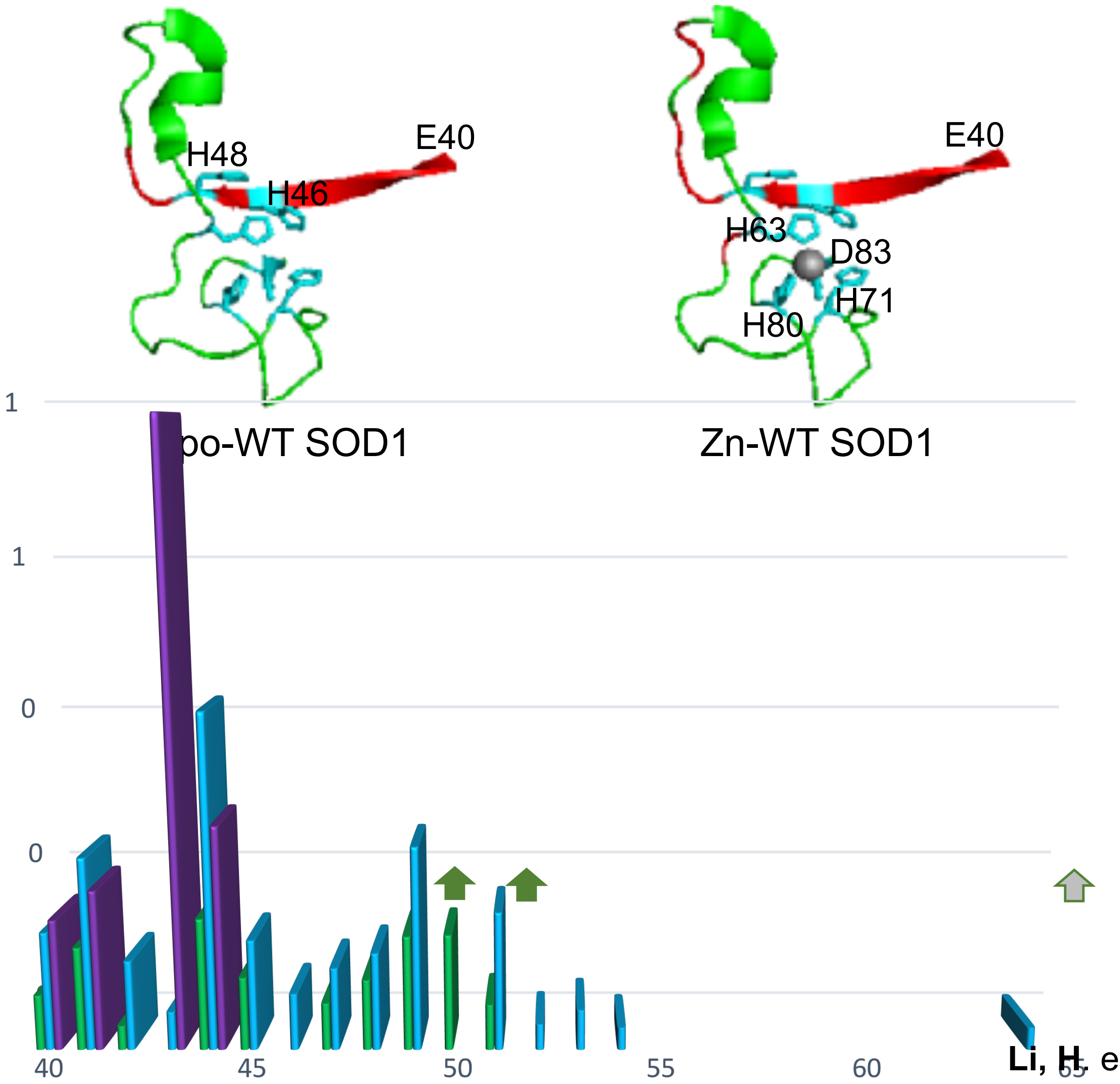
- Cu (His 46, 48, 63 and 120)
- Zn (His 63, 71, 80, and Asp 83)
- Disulfide bond (Cys57 and Cys146)



Green: Apo-WT SOD1; Cyan: Zn-SOD1; Purple: Cu,Zn-SOD1

Li, H. et al. *Anal. Chem*. **2017.** 89, 2731-2738

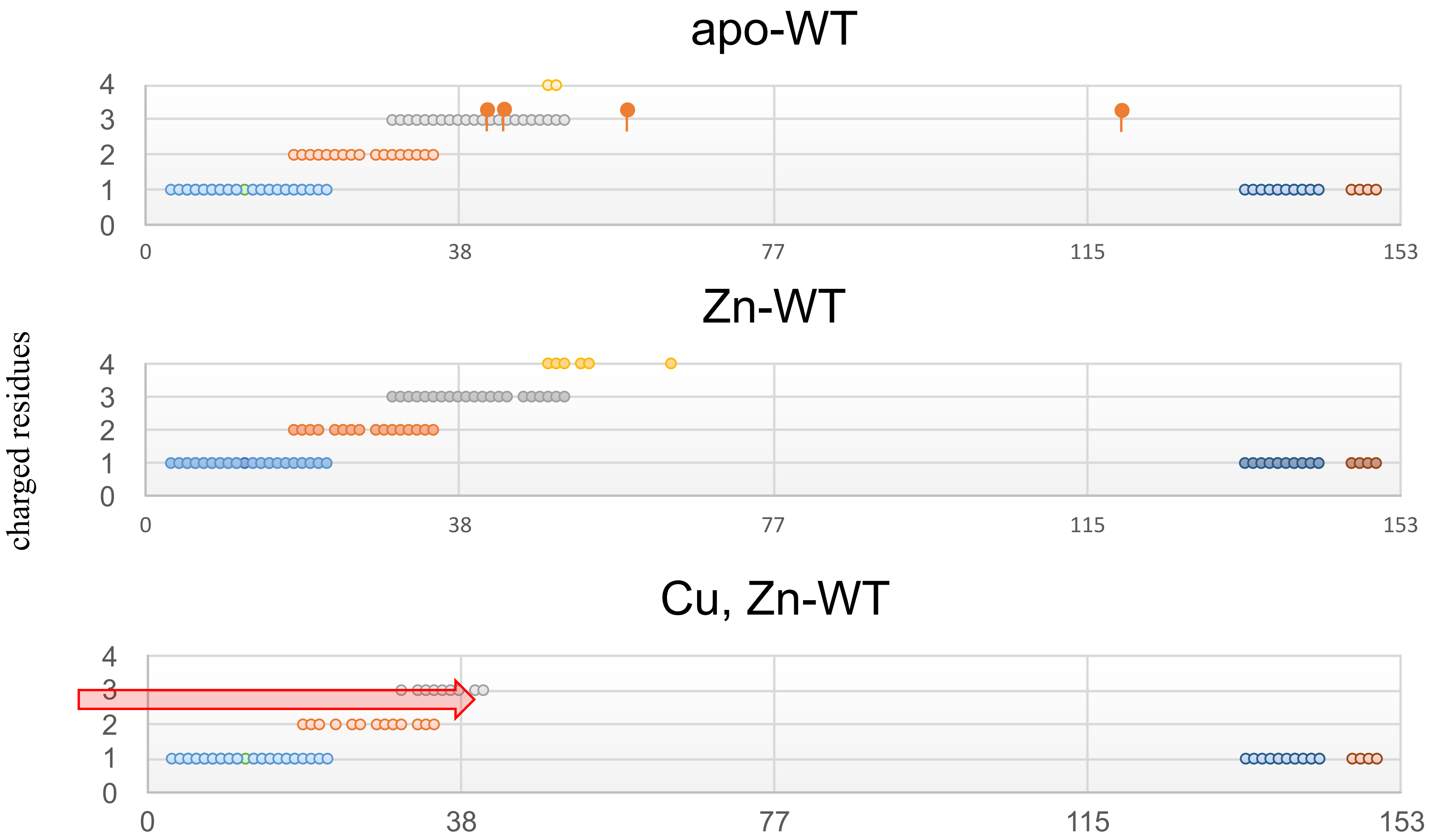
Revealing Structural Difference upon Metal Binding



Green: Apo-WT SOD1 Cyan: Zn-SOD1
Purple: Cu,Zn-SOD1

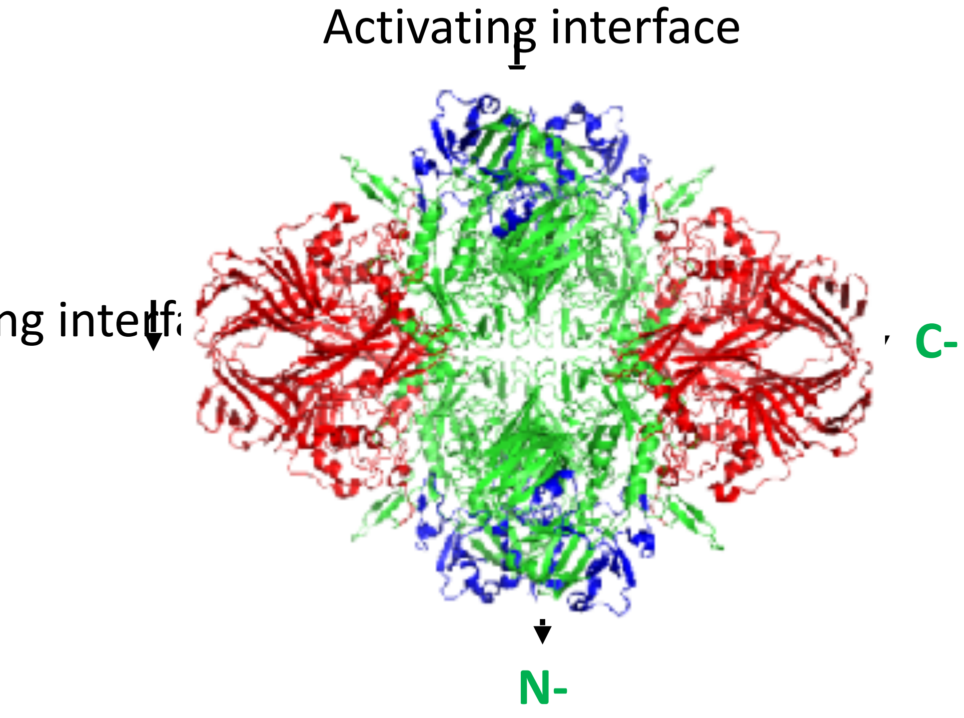
Li, H. et al. *Anal. Chem*. **2017.** 89, 2731-2738

Cu Binding Shields the Charged Residues (His46, His48)



Li, H. et al. *Anal. Chem.* **2017.** 89, 2731-2738

Native Top-Down MS of β-Galactosidase Tetramer (465 kDa)

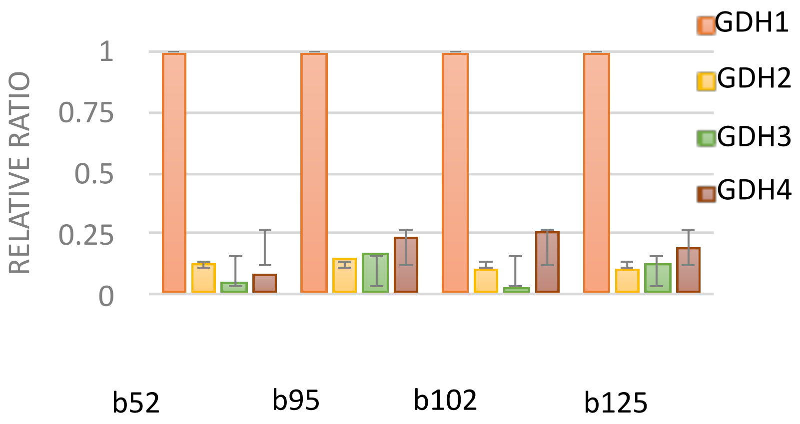


ECD: no c/z• ions (N- an C-termini are involved in interfaces)

IRMPD: 42% sequence coverage from the C-terminal, no PTMs observed

CAD: 12% sequence coverage from the N-Terminal; N-terminal is highly modified; at least four proteoforms

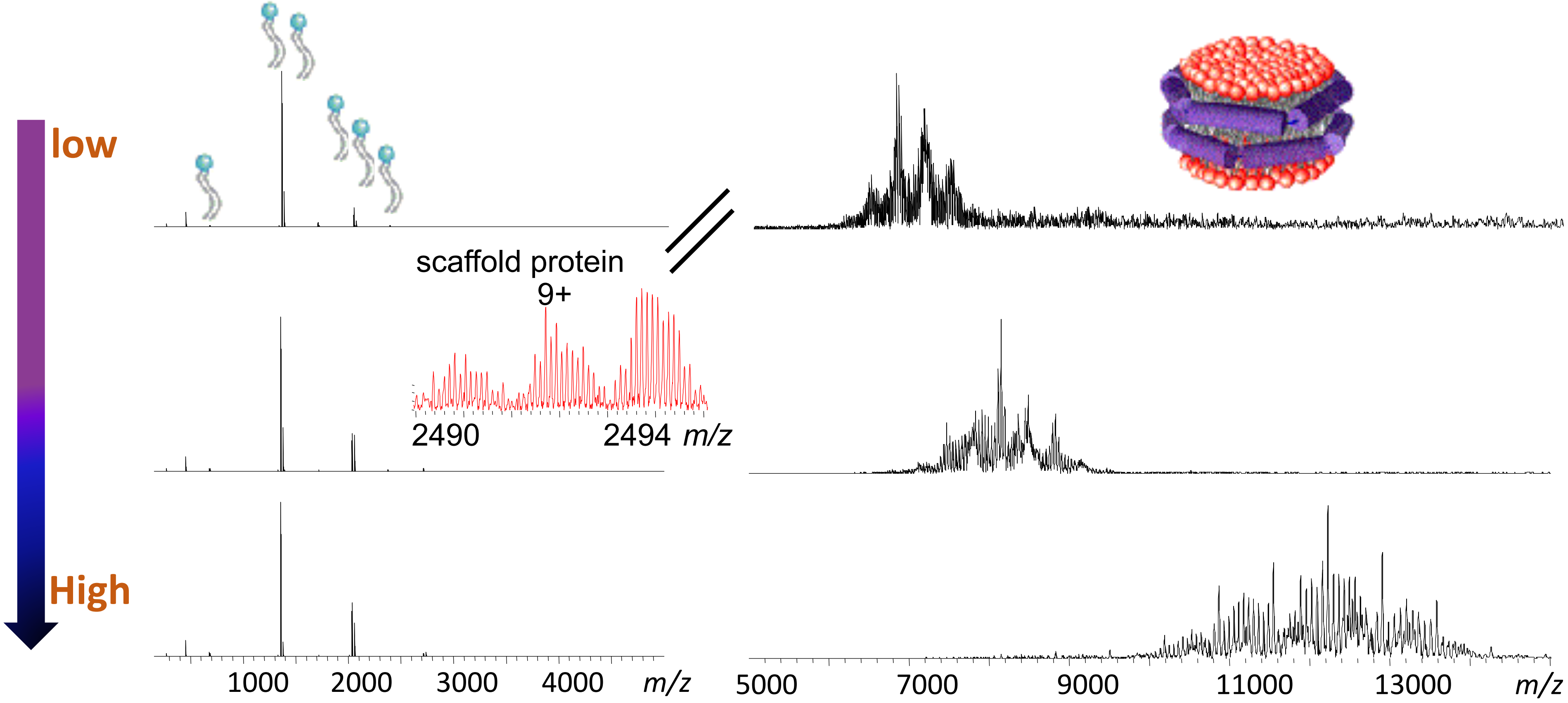
N-terminal tail in cyan



Li, H. et al. *Nat. Chem.* 2017.

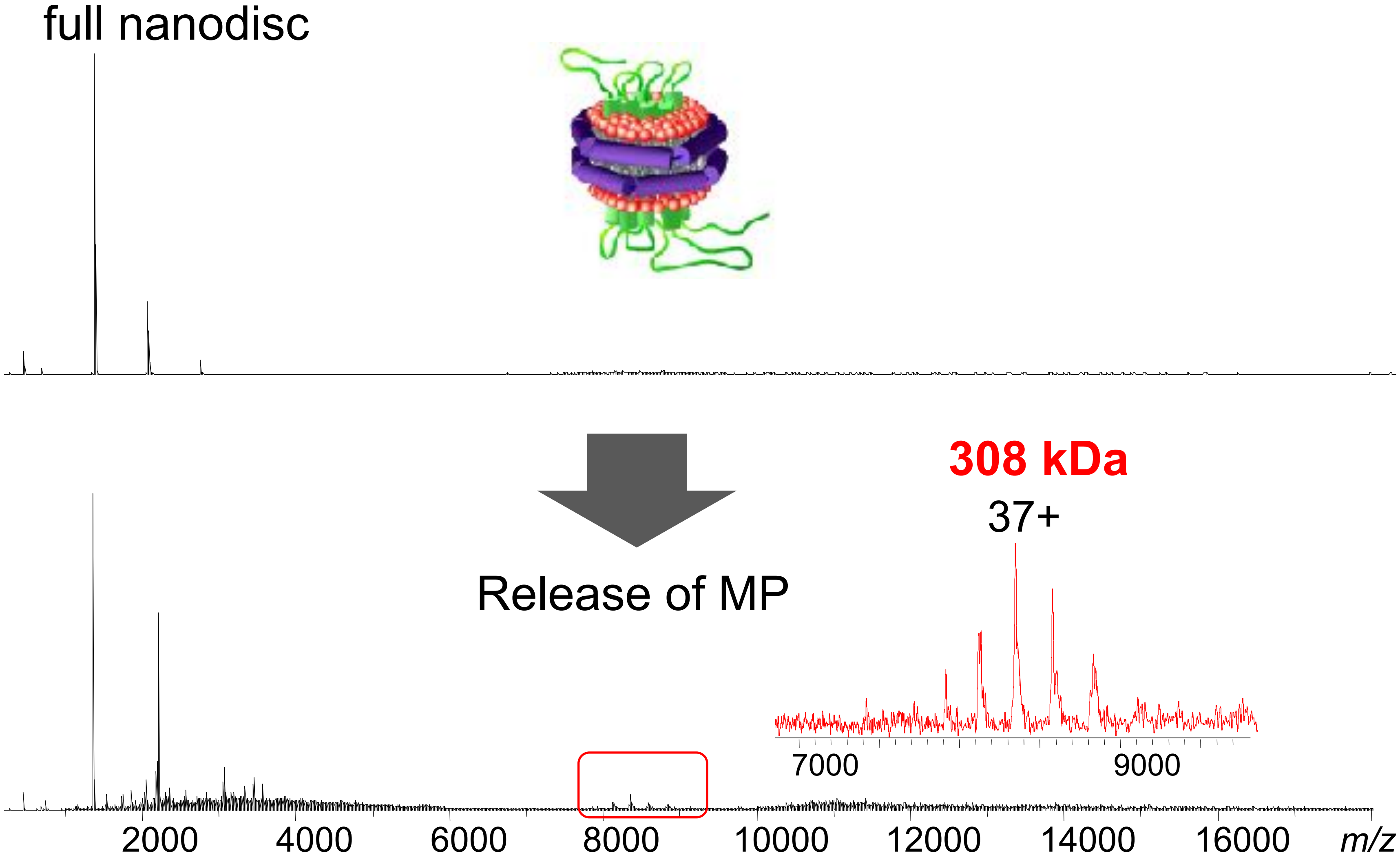
Reveals Empty Nanodisc Composition

- ➢ Empty nanodisc is about 130~190 kDa
- ➢ 2 scaffold proteins (22.45 kDa)
- ➢ 125~214 DMPCs (677.5 Da)

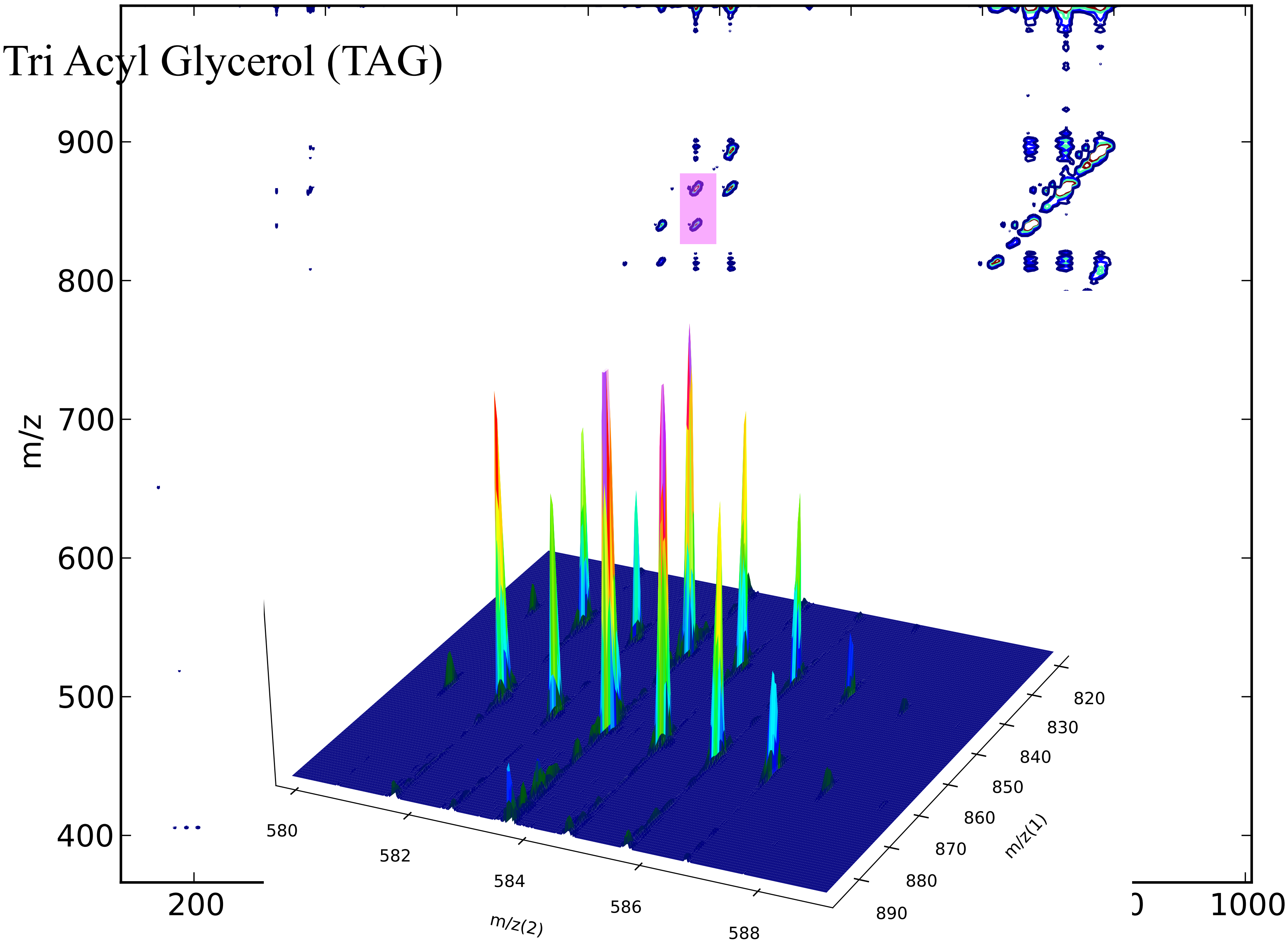


I Campuzano, **H Li**, et al. *Anal. Chem*. **2016.** 88, 12427-12436.

Release Membrane Protein Complex from Nanodisc



2D-MS



Tri Acyl Glycerol (TAG)

NMR - MS what's in common ?

- A Magnet
- Fourier transform

- Fourier transform

FT-ICR MS

NMR

Fourier Transform

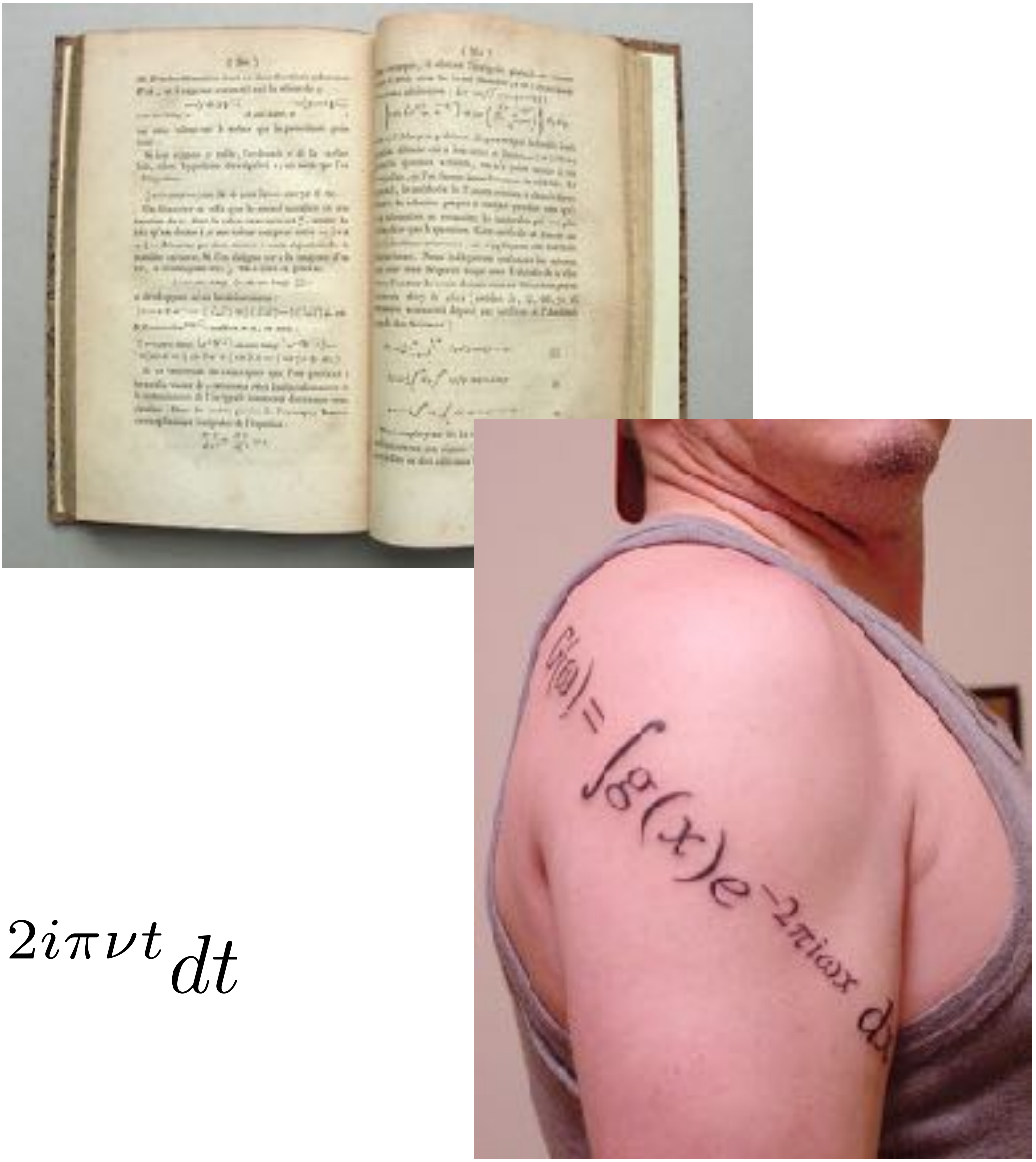
Jean Baptiste Joseph Fourier
(21 Mars 1768 – 16 Mai 1830)

$$f(t) \ : \ \mathbb{R} \rightarrow \mathbb{C}$$

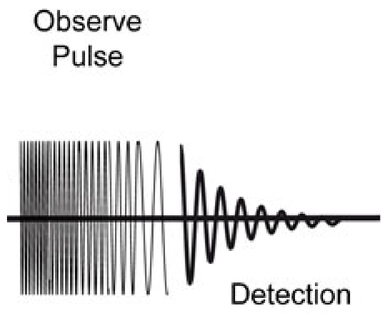
$$F(\nu) \ : \ \mathbb{R} \rightarrow \mathbb{C}$$

$$\mathcal{F} : f(t) \overset{\mathcal{F}}{\mapsto} F(\nu)$$

$$F(\nu) = \int_{-\infty}^{\infty} f(t) e^{-2i\pi\nu t} dt$$



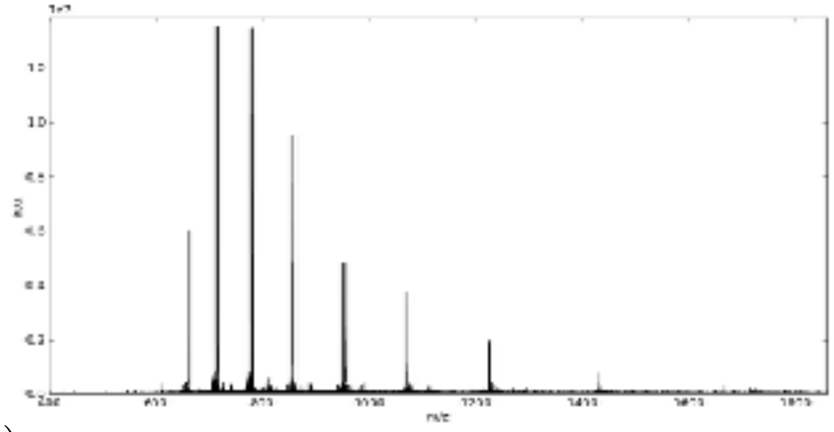
1D FT-ICR



$d(t)$

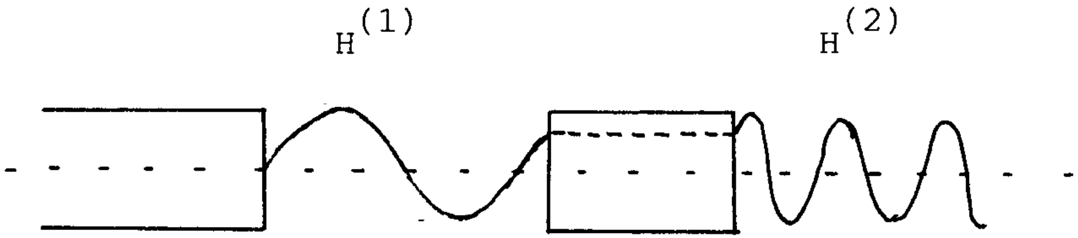
1D Fourier Transform

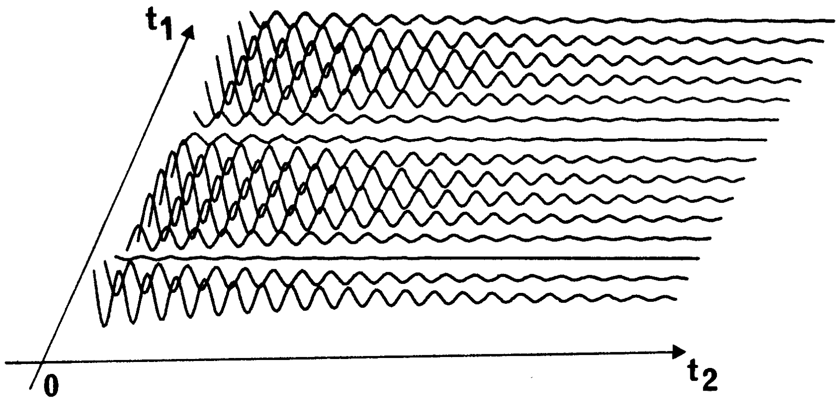
$D(\omega)$

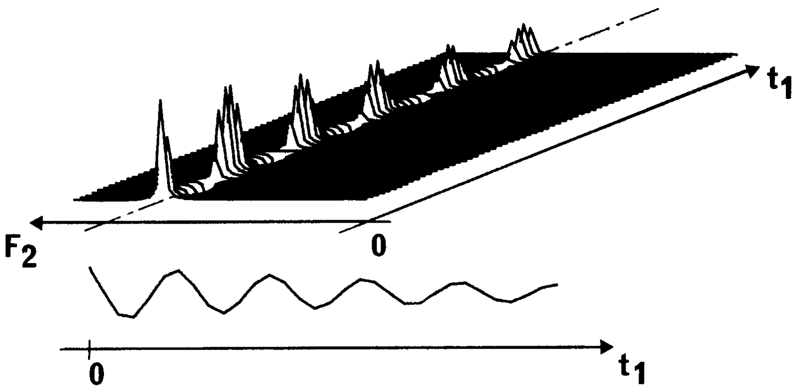


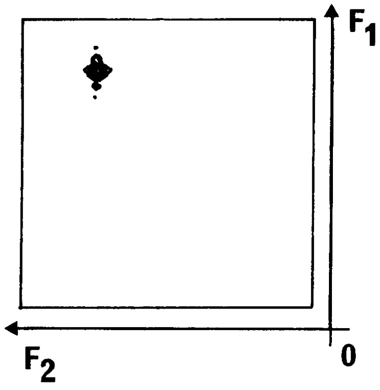
$$\xrightarrow{m/z \,\propto\, 1/\omega} S(m/z)$$

2D NMR









- H1 et H2 : deux phénomènes liés
  différents battements entre 2 oscillate
- possible car retour à la situation initial
- t1 : durée / t2 : date
- peu de contraintes sur le choix
  des valeurs de t1

M-A Delsuc Thèse d'État
1985 - Univ. Paris XI

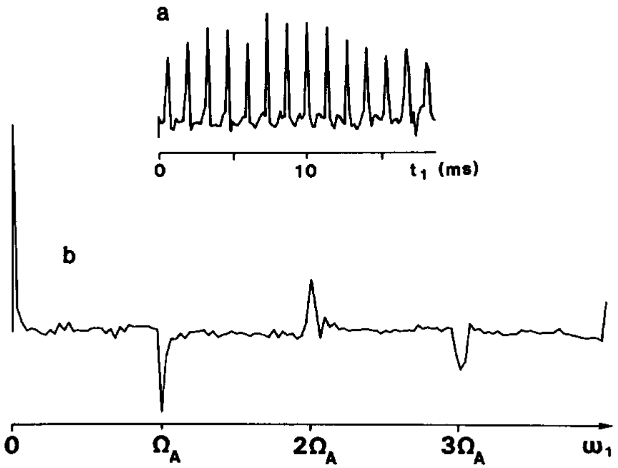
Volume 138, number 2,3 CHEMICAL PHYSICS LETTERS

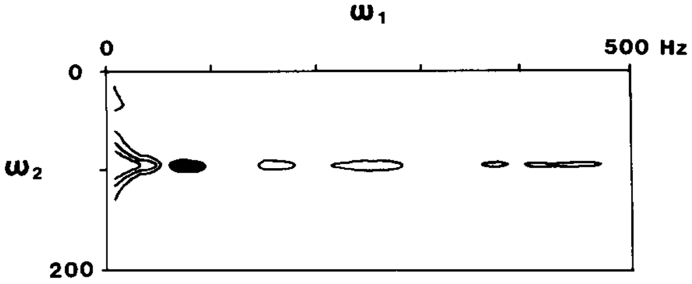
1987

TWO-DIMENSIONAL FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

Peter PFÄNDLER, Geoffrey BODENHAUSEN
Institut de Chimie Organique, Université de Lausanne, Rue de la Barre 2, CH-1005 Lausanne, Switzerland

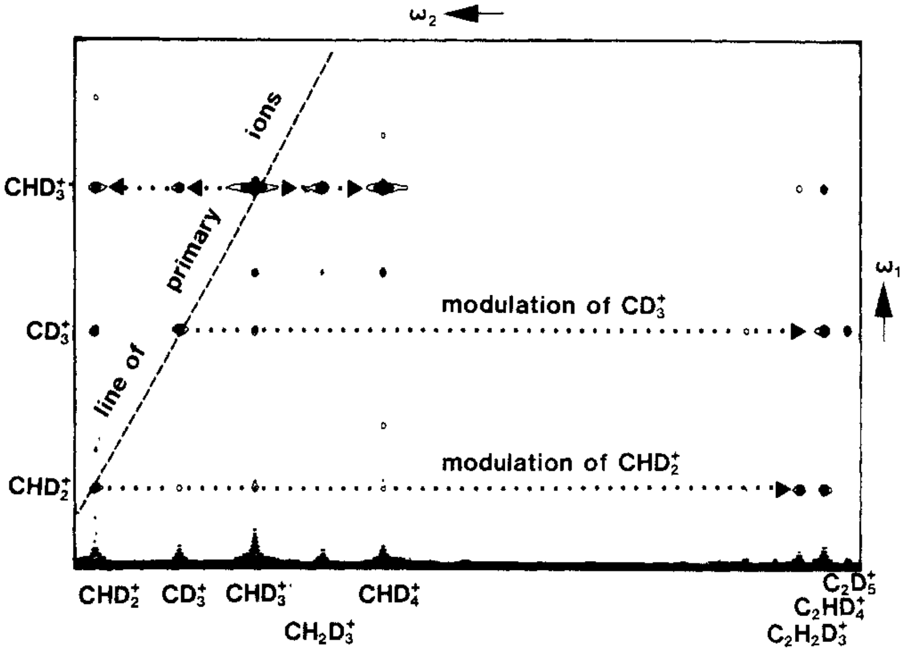
Jacques RAPIN, Raymond HOURIET and Tino GÄUMANN
Institut de Chimie Physique, Ecole Polytechnique Fédérale, CH-1015 Lausanne, Switzerland





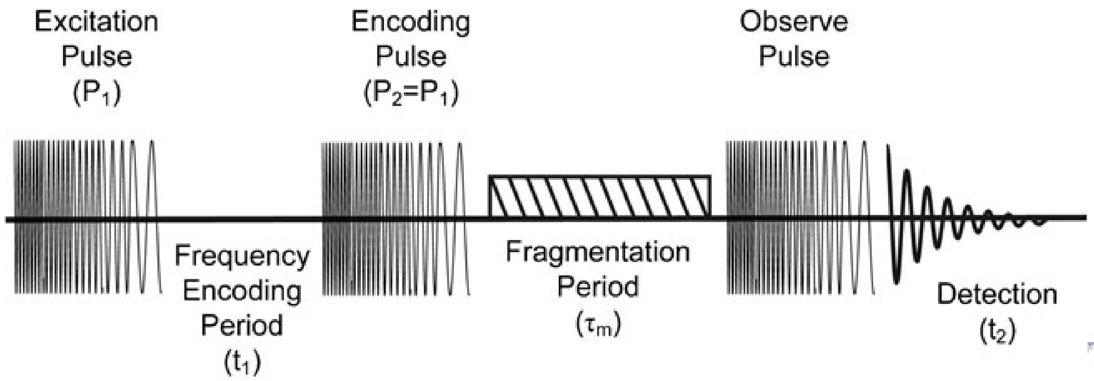
1988

P Pfaendler, G Bodenhausen, J Rapin, M Walser, T Gaümann
Broad-band two-dimensional Fourier transform ion cyclotron resonance.
J Am Chem Soc (1988) vol. 110 (17) 5625-5628



2D FT-ICR

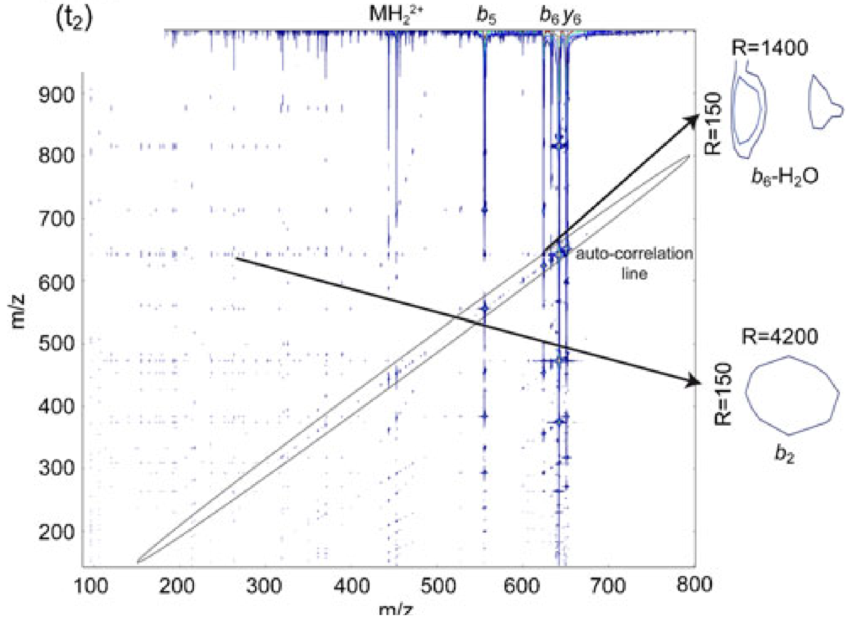
G.Bodenhausen
ENS Paris
C.Rolando
Univ. Lille



2D Fourier Transform

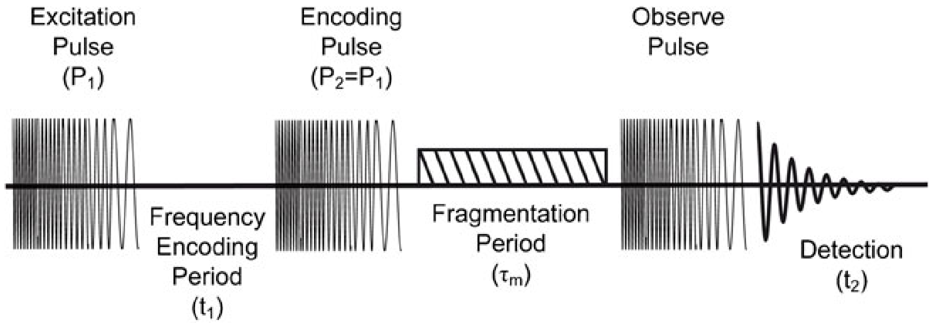
$$d(t_1, t_2) \xrightarrow{FT(t_1,t_2)} D(\omega_1, \omega_2)$$

$$\xrightarrow{m/z \propto 1/\omega} S(m_1/z_1,\, m_2/z_2)$$

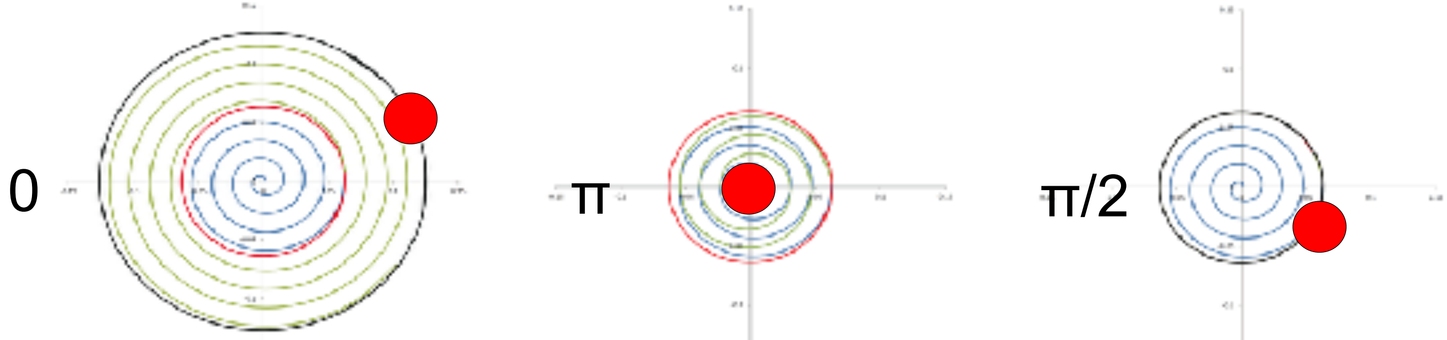


Pfaendler, P., Bodenhausen, G., Rapin, J., Walser, M. E., & Gäumann, T. (1988). *J.Am.Chem.Soc.*, ***110***, 5625-5628.
van Agthoven, M. A., Delsuc, M.-A., Bodenhausen, G. & Rolando, C. (2013) *Anal Bioanal Chem* **405,** 51–61.

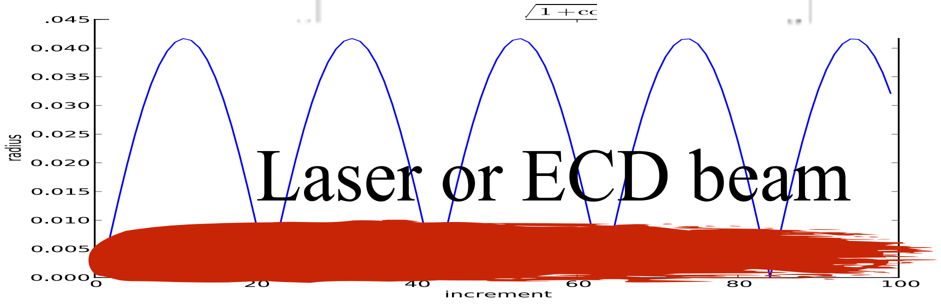
Principle of 2D FT-ICR



Role of Excitation and Encoding Pulses



$$\sqrt{1 + cos(\omega t_1)}$$

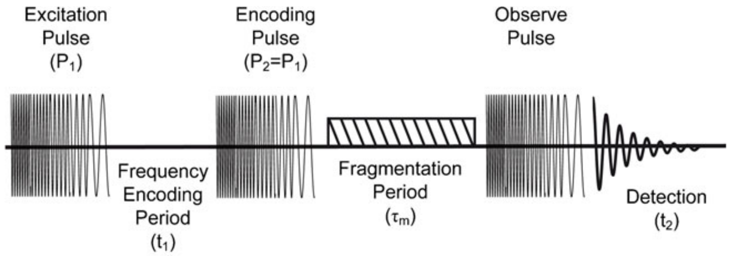


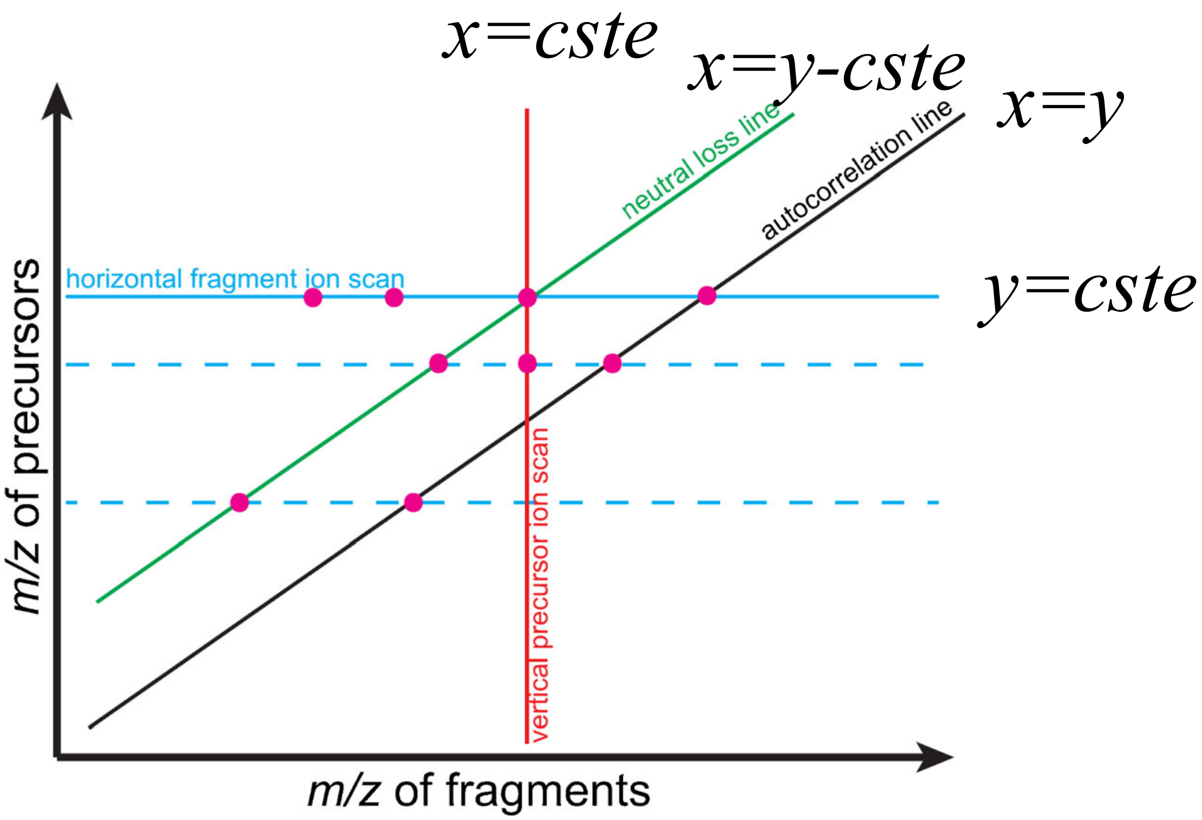
Laser or ECD beam

$$d(t_1, t_2)$$

exploration of the 2D map

- following alignments

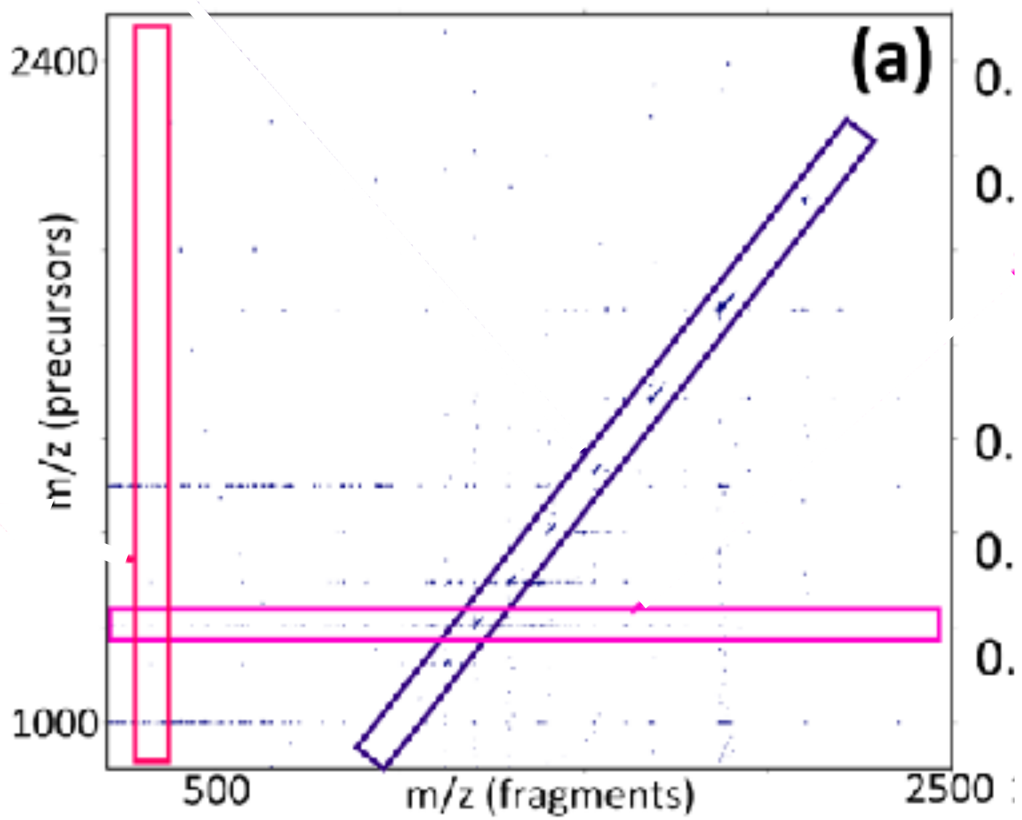




Top-down proteomics

- Calmoduline

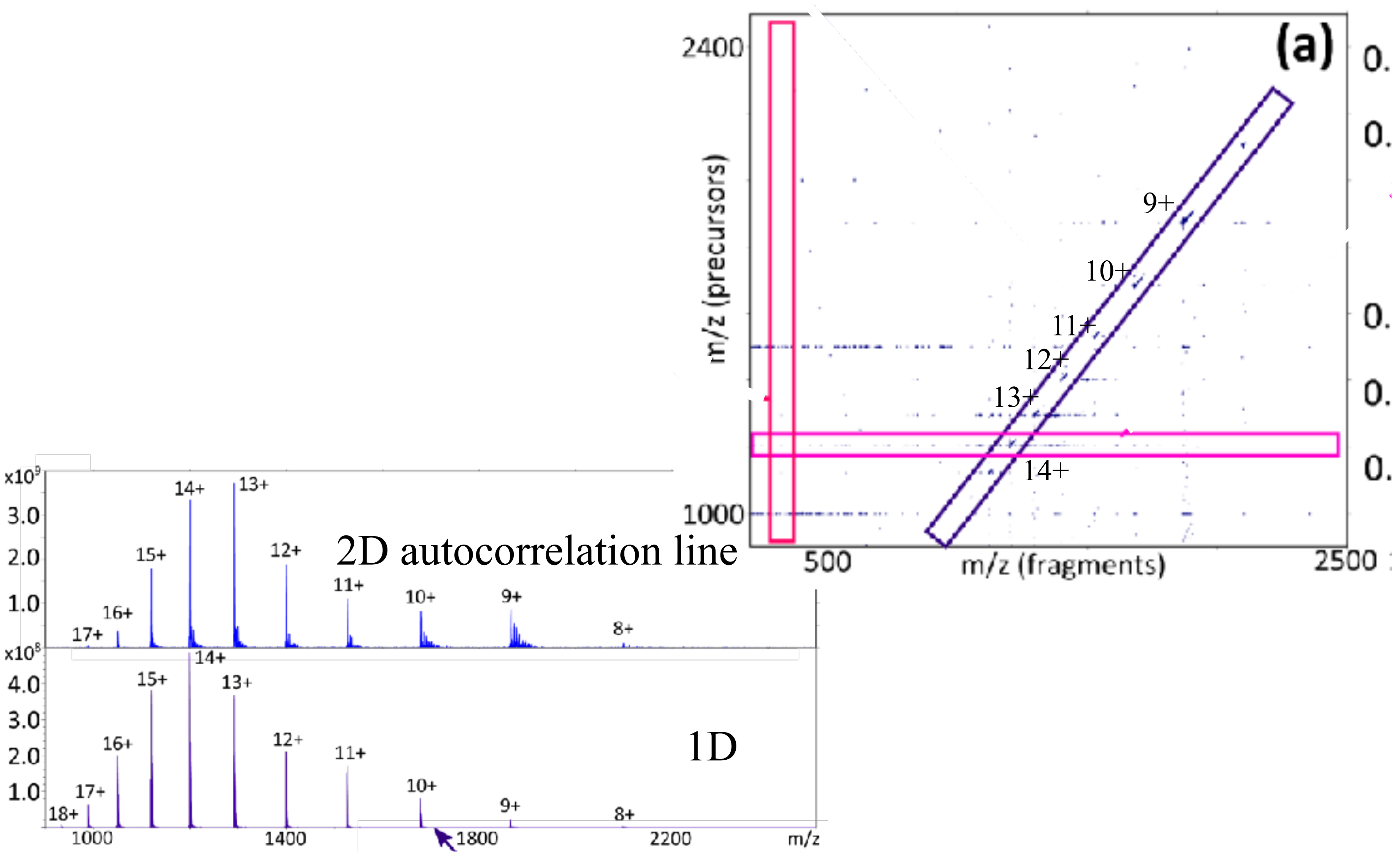
  - 2D FT-ICR IRMPD
    - 512 x 4M = 2 Gpoints
    - 20 min. acquisition
    - **R1 ~ 180**
    - **R2 ~ 420.000**



Floris, F., van Agthoven, M. A., Chiron, L., Soulby, A., Wootton, C. A., Lam, P. Y., Barrow, M.P., Delsuc, M-A., O'Connor, P. (2016). *J. Am. Soc. MS, 27*(9), 1531–1538

Top-down proteomics

- Calmoduline



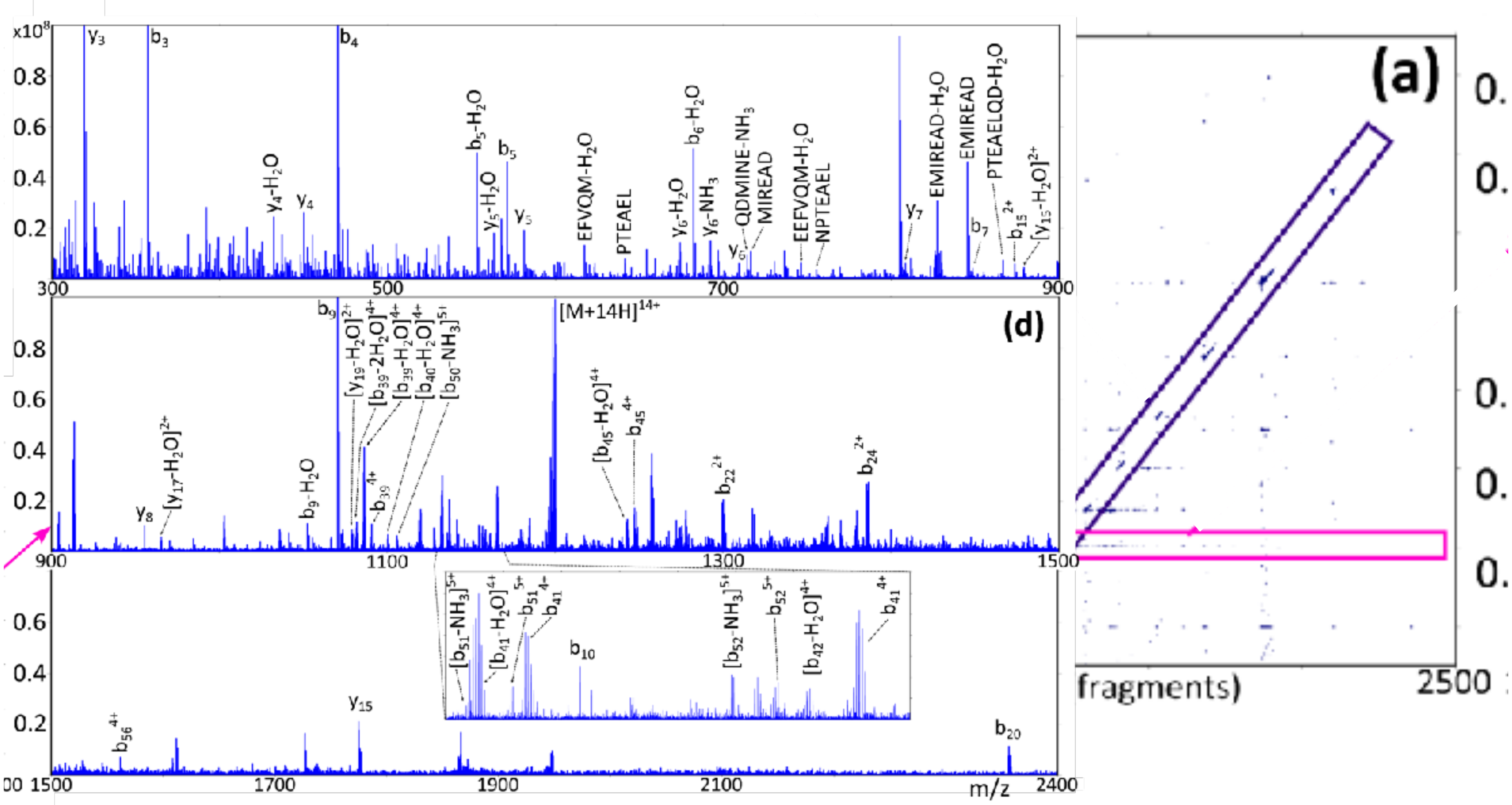
2D autocorrelation line

1D

Top-down proteomics

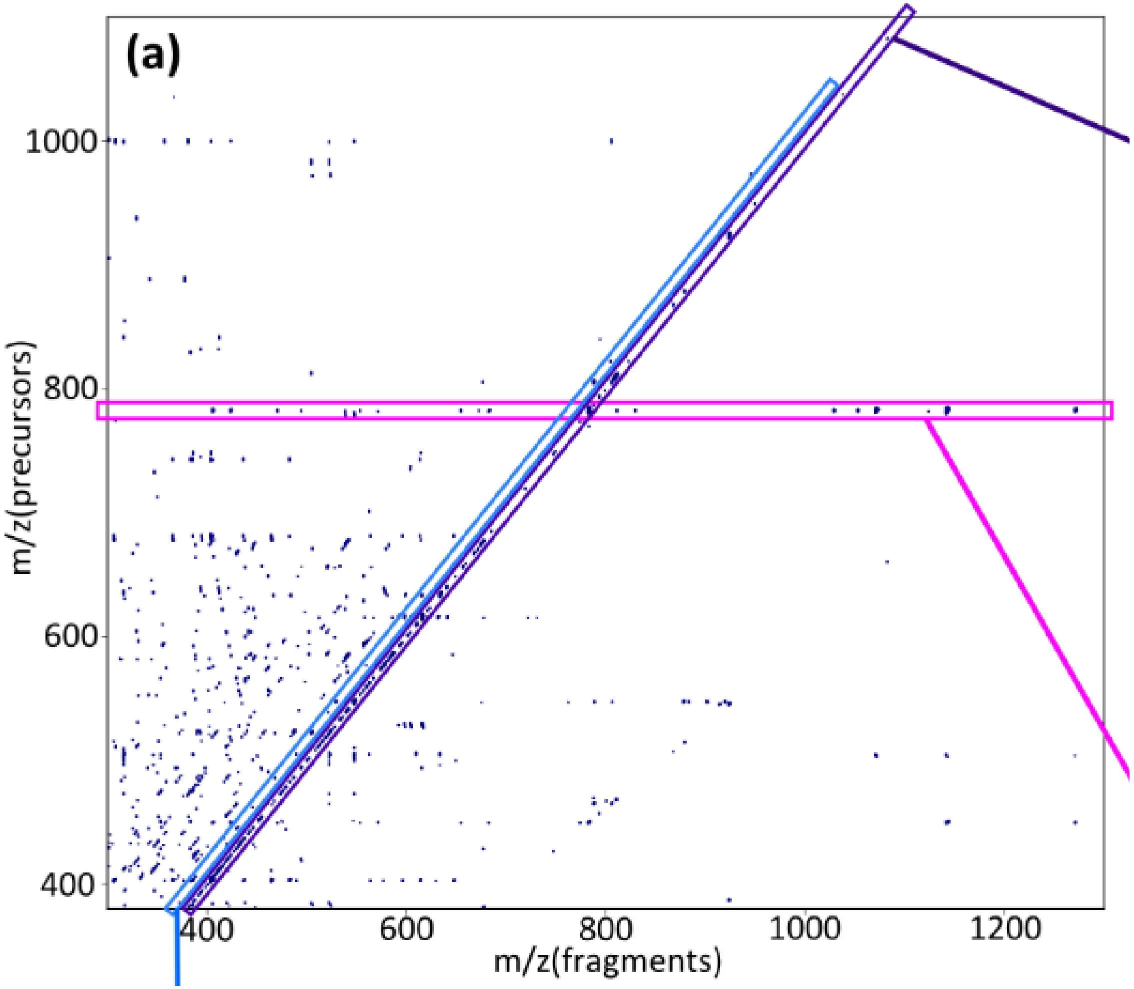
- Calmoduline

  2D fragment ion scan at 14+



Bottom-up proteomics

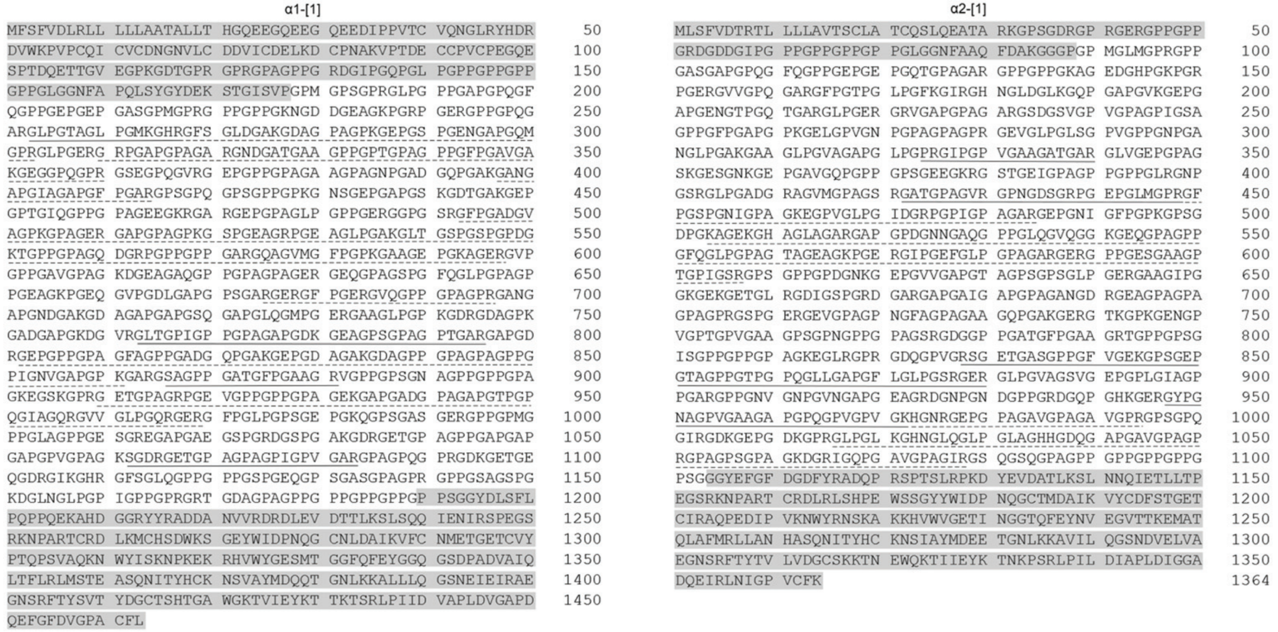
- Calmoduline
  - 2D FT-ICR IRMPD
    - 4096 x 512k = 2 Gpoint
    - 50 min. acquisition
    - **R1 ~ 1200**
    - **R2 ~ 60.000**



Bottom-up proteomics

- Collagen protein
  - complex protein
    - heavyly transformed: hydroxy-proline / hydroxy-lysine
    - packed helices   and dense Hbound network
    - Bovin Collagen Type1: α1 and α2 chains (~2x 1400 aa)
    - tryptic digest

Blank regions are low complexity, with repetiti
of   X-Y-Gly   pattern



α1-[1]

```
MFSFVDLRLL LLLAATALLT HGQEEGQEEG QEEDIPPVTC VQNGLRYHDR    50
DVWKPVPCQI CVCDNGNVLC DDVICDELKD CPNAKVPTDE CCPVCPEGQE   100
SPTDQETTGV EGPKGDTGPR GPRGPAGPPG RDGIPGQPGL PGPPGPPGPP   150
GPPGLGGNFA PQLSYGYDEK STGISVPGPM GPSGPRGLPG PPGAPGPQGF   200
QGPPGEPGEP GASGPMGPRG PPGPPGKNGD DGEAGKPGRP GERGPPGPQG   250
ARGLPGTAGL PGMKGHRGFS GLDGAKGDAG PAGPKGEPGS PGENGAPGQM   300
GPRGLPGERG RPGAPGPAGA RGNDGATGAA GPPGPTGPAG PPGFPGAVGA   350
KGEGGPQGPR GSEGPQGVRG EPGPPGPAGA AGPAGNPGAD GQPGAKGANG   400
APGIAGAPGF PGARGPSGPQ GPSGPPGPKG NSGEPGAPGS KGDTGAKGEP   450
GPTGIQGPPG PAGEEGKRGA RGEPGPAGLP GPPGERGGPG SRGFPGADGV   500
AGPKGPAGER GAPGPAGPKG SPGEAGRPGE AGLPGAKGLT GSPGSPGPDG   550
KTGPPGPAGQ DGRPGPPGPP GARGQAGVMG FPGPKGAAGE PGKAGERGVP   600
GPPGAVGPAG KDGEAGAQGP PGPAGPAGER GEQGPAGSPG FQGLPGPAGP   650
PGEAGKPGEQ GVPGDLGAPG PSGARGERGF PGERGVQGPP GPAGPRGANG   700
APGNDGAKGD AGAPGAPGSQ GAPGLQGMPG ERGAAGLPGP KGDRGDAGPK   750
GADGAPGKDG VRGLTGPIGP PGPAGAPGDK GEAGPSGPAG PTGARGAPGD   800
RGEPGPPGPA GFAGPPGADG QPGAKGEPGD AGAKGDAGPP GPAGPAGPPG   850
PIGNVGAPGP KGARGSAGPP GATGFPGAAG RVGPPGPSGN AGPPGPPGPA   900
GKEGSKGPRG ETGPAGRPGE VGPPGPPGPA GEKGAPGADG PAGAPGTPGP   950
QGIAGQRGVV GLPGQRGERG FPGLPGPSGE PGKQGPSGAS GERGPPGPMG  1000
PPGLAGPPGE SGREGAPGAE GSPGRDGSPG AKGDRGETGP AGPPGAPGAP  1050
GAPGPVGPAG KSGDRGETGP AGPAGPIGPV GARGPAGPQG PRGDKGETGE  1100
QGDRGIKGHR GFSGLQGPPG PPGSPGEQGP SGASGPAGPR GPPGSAGSPG  1150
KDGLNGLPGP IGPPGPRGRT GDAGPAGPPG PPGPPGPPGP PSGGYDLSFL  1200
PQPPQEKAHD GGRYYRADDA NVVRDRDLEV DTTLKSLSQQ IENIRSPEGS  1250
RKNPARTCRD LKMCHSDWKS GEYWIDPNQG CNLDAIKVFC NMETGETCVY  1300
PTQPSVAQKN WYISKNPKEK RHVWYGESMT GGFQFEYGGQ GSDPADVAIQ  1350
LTFLRLMSTE ASQNITYHCK NSVAYMDQQT GNLKKALLLQ GSNEIEIRAE  1400
GNSRFTYSVT YDGCTSHTGA WGKTVIEYKT TKTSRLPIID VAPLDVGAPD  1450
QEFGFDVGPA CFL
```

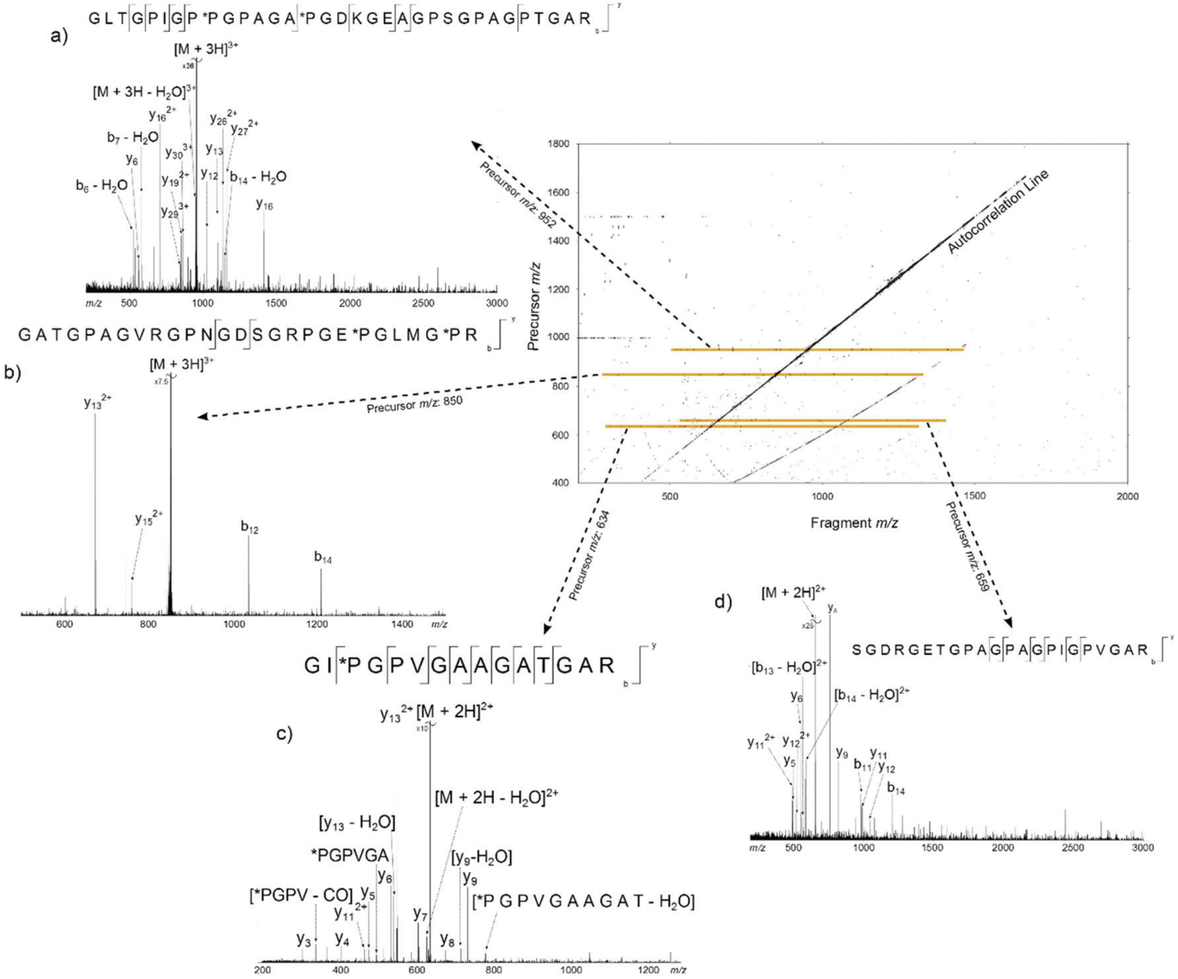
α2-[1]

```
MLSFVDTRTL LLLAVTSCLA TCQSLQEATA RKGPSGDRGP RGERGPPGPP    50
GRDGDDGIPG PPGPPGPPGP PGLGGNFAAQ FDAKGGGPGP MGLMGPRGPP   100
GASGAPGPQG FQGPPGEPGE PGQTGPAGAR GPPGPPGKAG EDGHPGKPGR   150
PGERGVVGPQ GARGFPGTPG LPGFKGIRGH NGLDGLKGQP GAPGVKGEPG   200
APGENGTPGQ TGARGLPGER GRVGAPGPAG ARGSDGSVGP VGPAGPIGSA   250
GPPGFPGAPG PKGELGPVGN PGPAGPAGPR GEVGLPGLSG PVGPPGNPGA   300
NGLPGAKGAA GLPGVAGAPG LPGPRGIPGP VGAAGATGAR GLVGEPGPAG   350
SKGESGNKGE PGAVGQPGPP GPSGEEGKRG STGEIGPAGP PGPPGLRGNP   400
GSRGLPGADG RAGVMGPAGS RGATGPAGVR GPNGDSGRPG EPGLMGPRGF   450
PGSPGNIGPA GKEGPVGLPG IDGRPGPIGP AGARGEPGNI GFPGPKGPSG   500
DPGKAGEKGH AGLAGARGAP GPDGNNGAQG PPGLQGVQGG KGEQGPAGPP   550
GFQGLPGPAG TAGEAGKPGE RGIPGEFGLP GPAGARGERG PPGESGAAGP   600
TGPIGSRGPS GPPGPDGNKG EPGVVGAPGT AGPSGPSGLP GERGAAGIPG   650
GKGEKGETGL RGDIGSPGRD GARGAPGAIG APGPAGANGD RGEAGPAGPA   700
GPAGPRGSPG ERGEVGPAGP NGFAGPAGAA GQPGAKGERG TKGPKGENGP   750
VGPTGPVGAA GPSGPNGPPG PAGSRGDGGP PGATGFPGAA GRTGPPGPSG   800
ISGPPGPPGP AGKEGLRGPR GDQGPVGRSG ETGASGPPGF VGEKGPSGEP   850
GTAGPPGTPG PQGLLGAPGF LGLPGSRGER GLPGVAGSVG EPGPLGIAGP   900
PGARGPPGNV GNPGVNGAPG EAGRDGNPGN DGPPGRDGQP GHKGERGYPG   950
NAGPVGAAGA PGPQGPVGPV GKHGNRGEPG PAGAVGPAGA VGPRGPSGPQ  1000
GIRGDKGEPG DKGPRGLPGL KGHNGLQGLP GLAGHHGDQG APGAVGPAGP  1050
RGPAGPSGPA GKDGRIGQPG AVGPAGIRGS QGSQGPAGPP GPPGPPGPPG  1100
PSGGGYEFGF DGDFYRADQP RSPTSLRPKD YEVDATLKSL NNQIETLLTP  1150
EGSRKNPART CRDLRLSHPE WSSGYYWIDP NQGCTMDAIK VYCDFSTGET  1200
CIRAQPEDIP VKNWYRNSKA KKHVWVGETI NGGTQFEYNV EGVTTKEMAT  1250
QLAFMRLLAN HASQNITYHC KNSIAYMDEE TGNLKKAVIL QGSNDVELVA  1300
EGNSRFTYTV LVDGCSKKTN EWQKTIIEYK TNKPSRLPIL DIAPLDIGGA  1350
DQEIRLNIGP VCFK                                         1364
```

Simon, H., van Agthoven, M., Lam, P. Y., Floris, F., Chiron, L., Delsuc, M.-A., Rolando, C., Barrow, M., O'Connor, P. (2016). *Analyst*, **141**, 157–165

Bottom-up proteomics

- Collagen protein

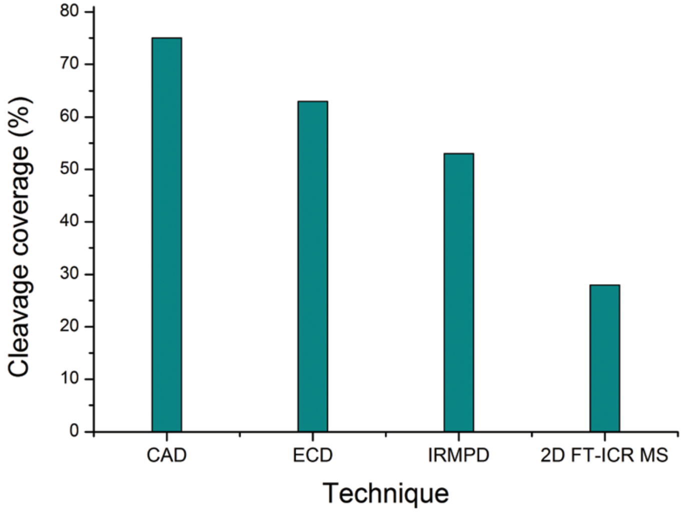


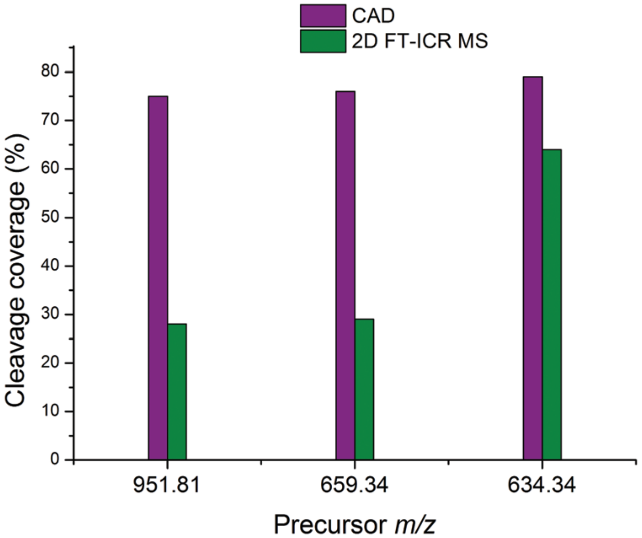
- 2D FT-ICR IRMPD
  - 4k x 256k = 1 Gpoints
  - 60 min. acquisition
  - **R1 ~ 630**
  - **R2 ~ 13.000** (*m/z*=850)

Simon, H., van Agthoven, M., Lam, P. Y., Floris, F., Chiron, L., Delsuc, M.-A., Rolando, C., Barrow, M., O’Connor, P. (2016). *Analyst*, **141**, 157–165

Bottom-up proteomics

- Collagen protein
  - comparing CAD, ECD, IRMPD, 2D IRMPD





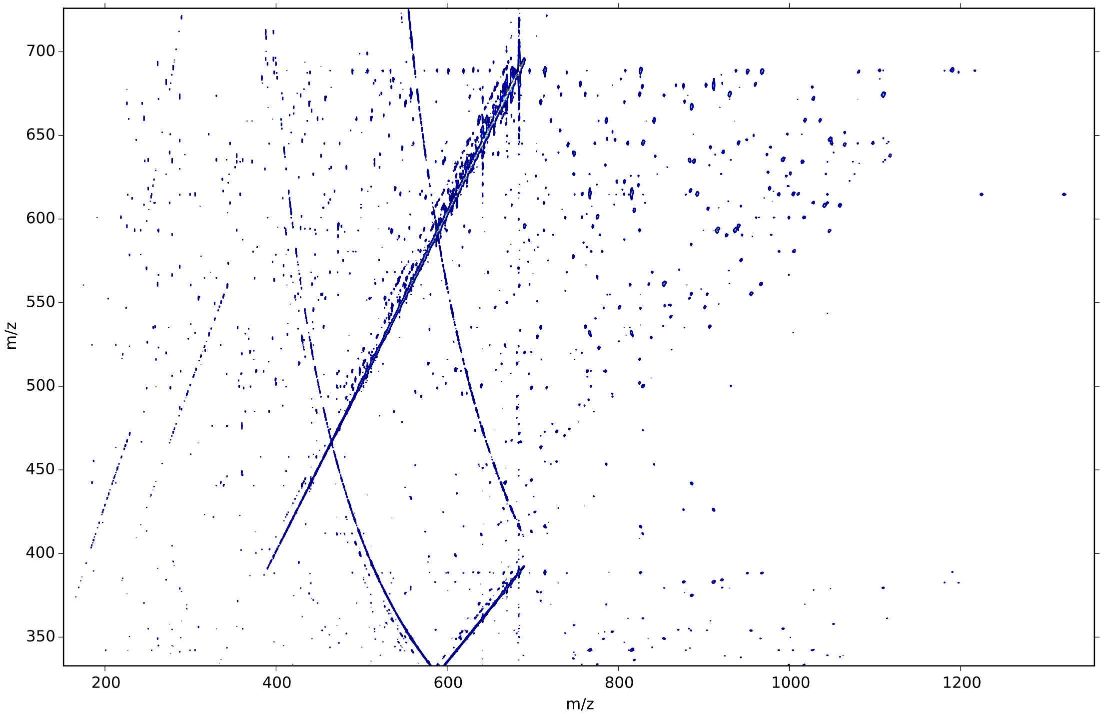
Comparing coverage

not bad for a technique only a few years old!

Simon, H., van Agthoven, M., Lam, P. Y., Floris, F., Chiron, L., Delsuc, M.-A., Rolando, C., Barrow, M., O'Connor, P. (2016). *Analyst*, **141**, 157–165

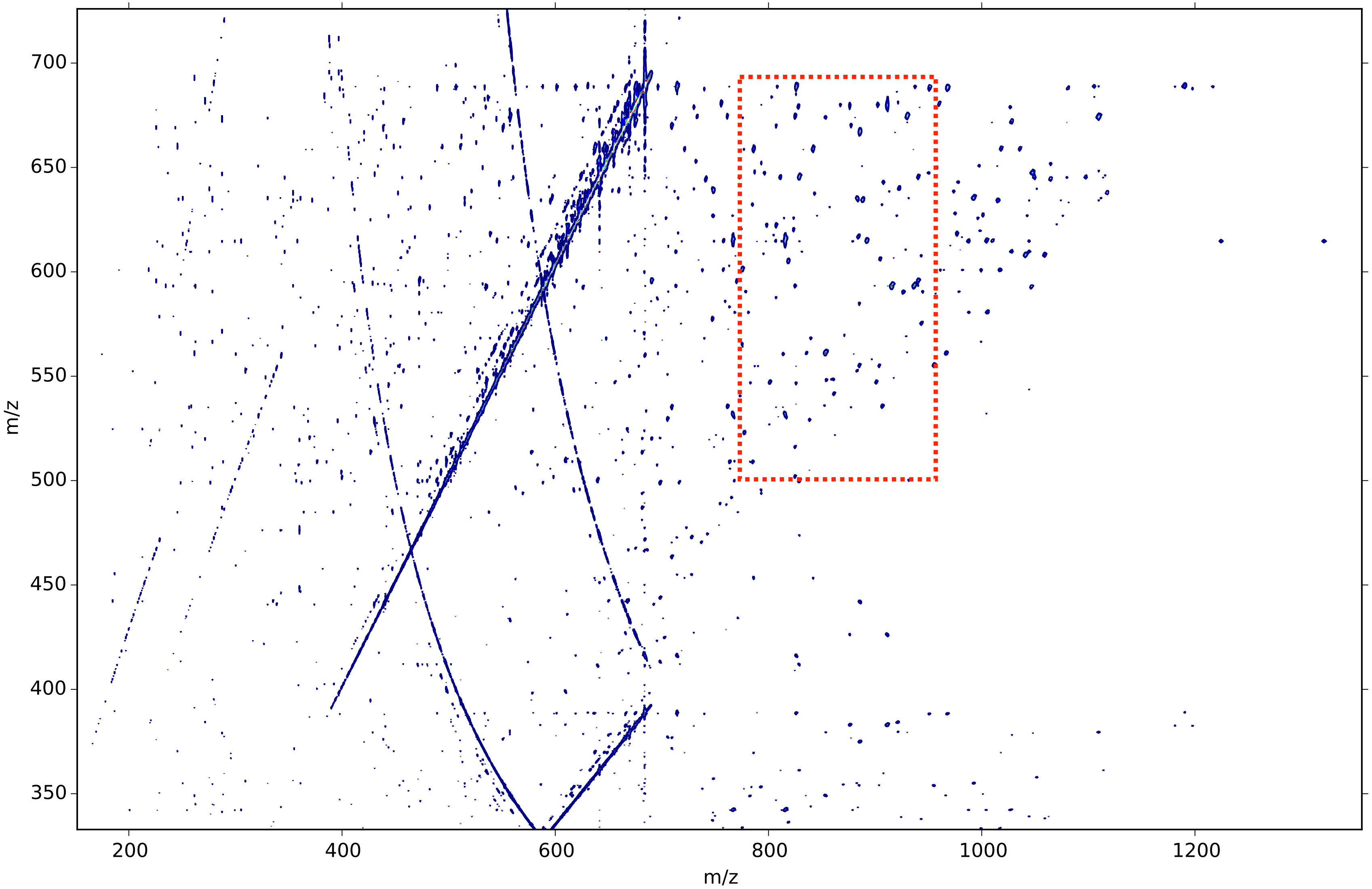
Bottom-up proteomics

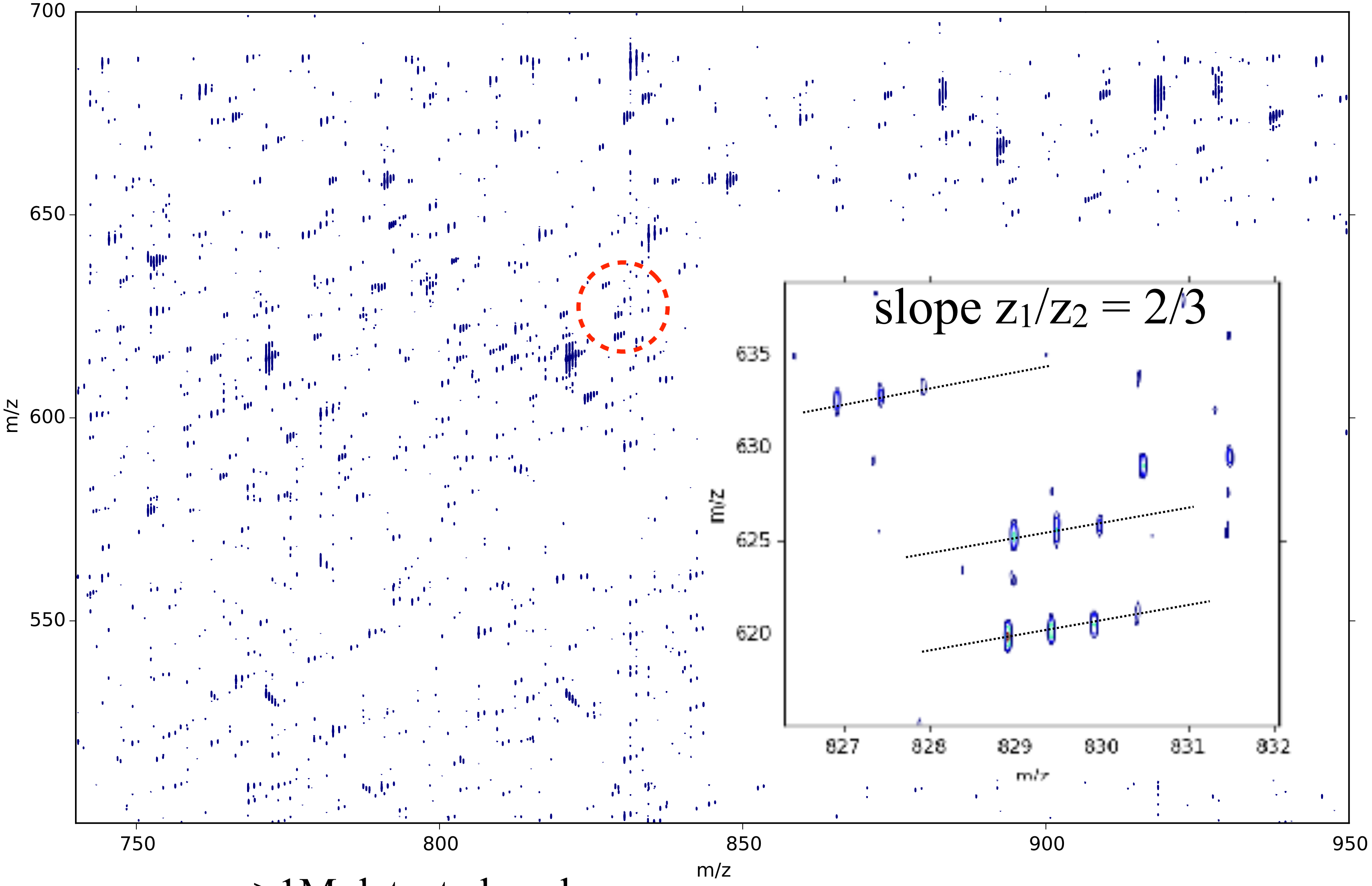
- yeast cells
  - cell extract
  - tryptic digest
  - no separation



- 2D FT-ICR IRMPD
  - 4096 x 256k = 1 Gpoint
  - processed to 4k x 1M = 4 Gpoints
  - 50 min. acquisition
  - **R1 ~ 1500**
  - **R2 ~ 60.000**

Parent Excitation limited to
m/z = 330 .. 700



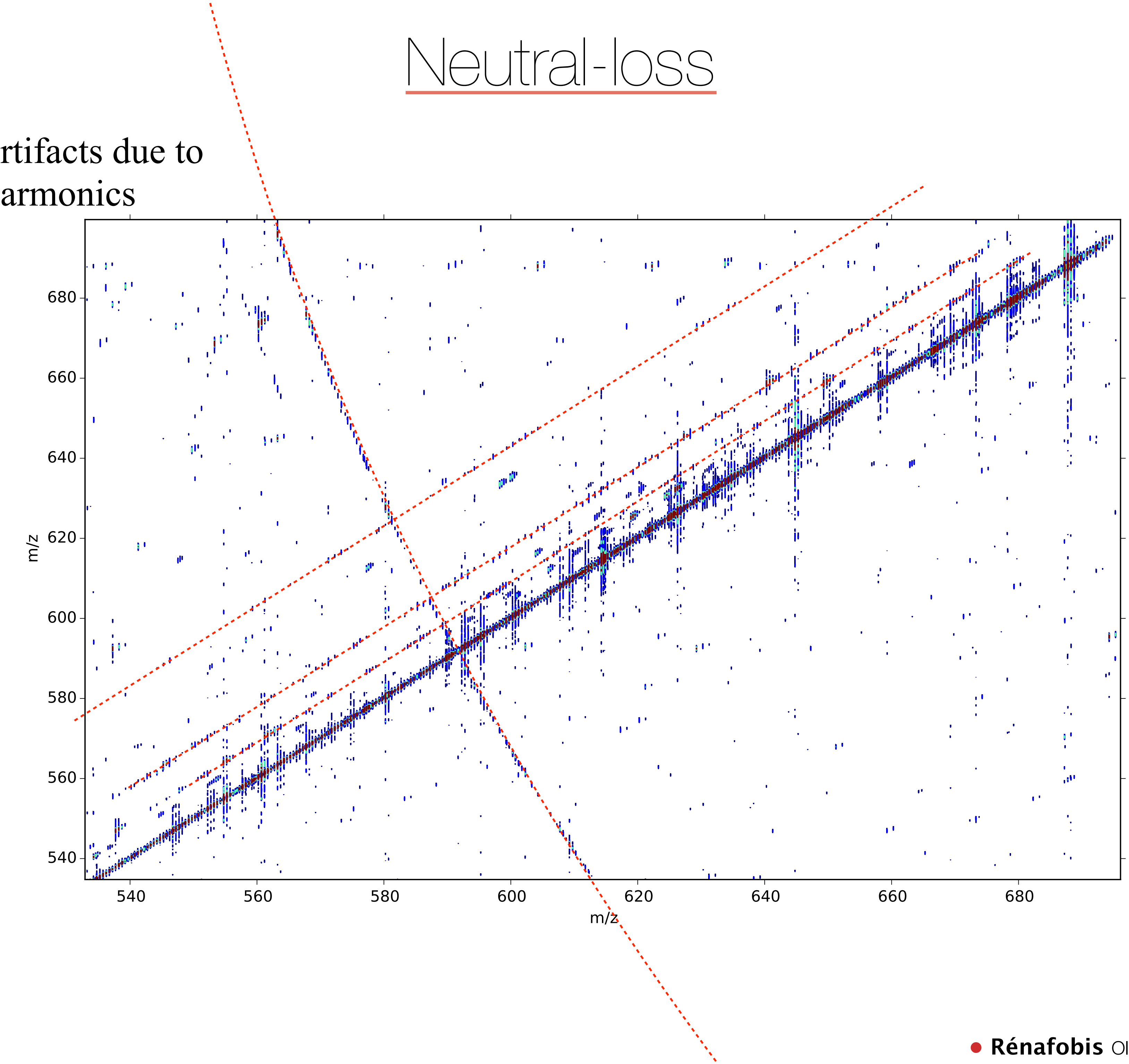


>1M detected peaks

>100k? potential PSM

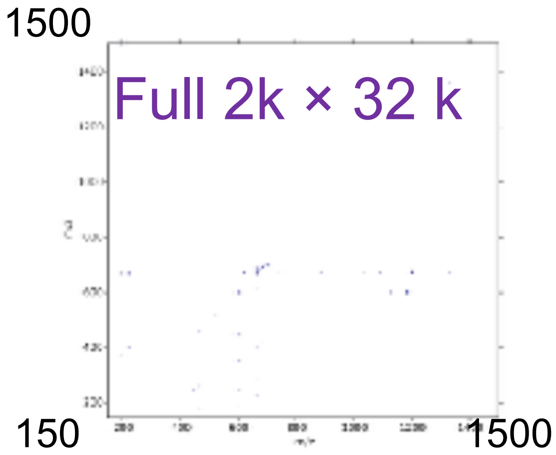
Neutral-loss

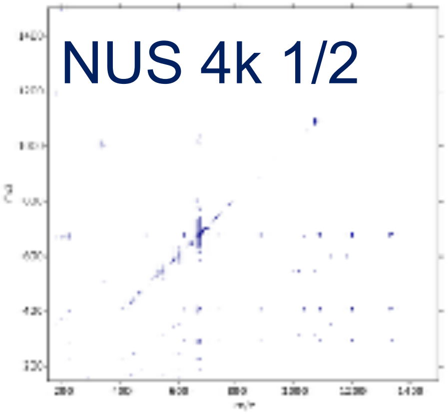
Artifacts due to Harmonics

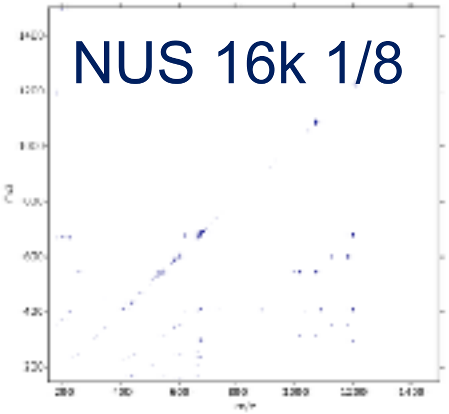


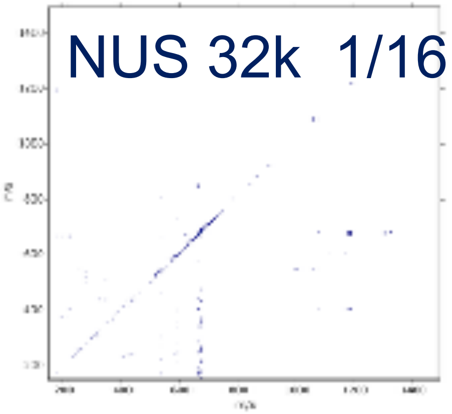
NUS: substance P, 2D spectrum overview

- Non-Uniform Sampling / non-Fourier Analysis









Substance P, 1 pmol.µL , nanoESI, ECD, 45 min

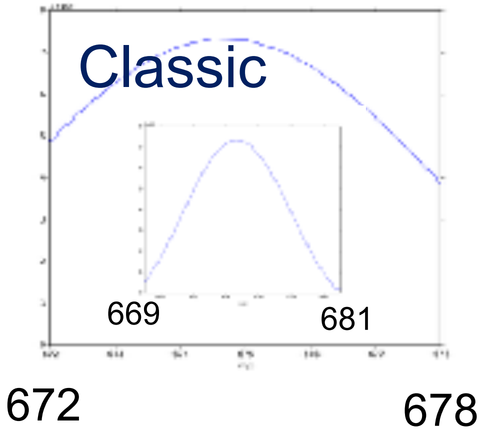
Solarix 7 Tesla, Paracell®,

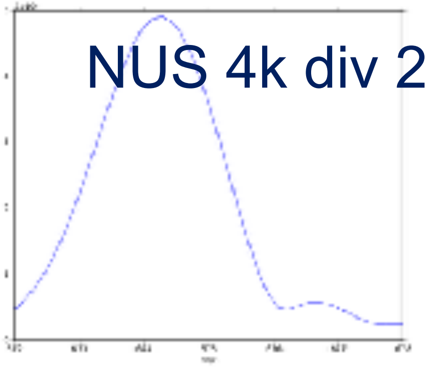
all experiments 2k × 128k

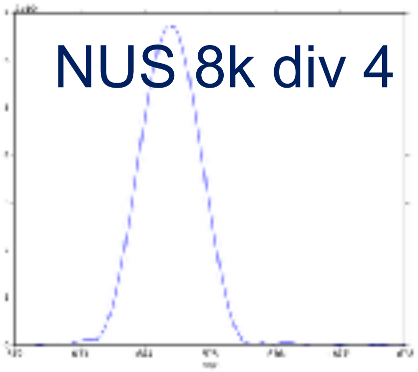
but convering

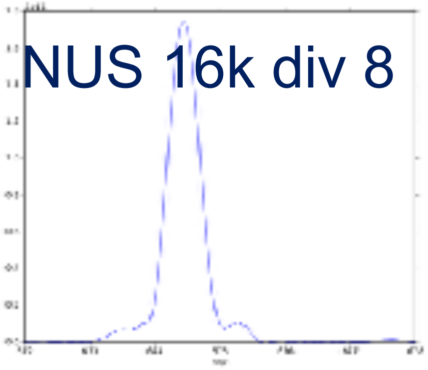
4k / 16k / 32k x 128k

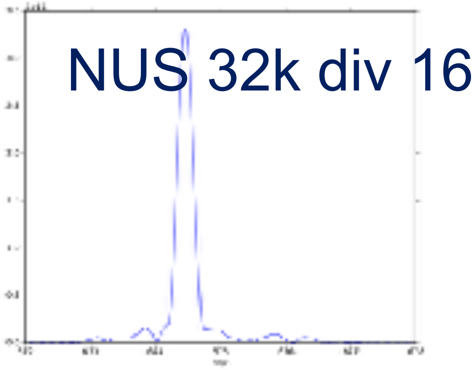
The overall aspect
of the 2D spectrum is preserved.

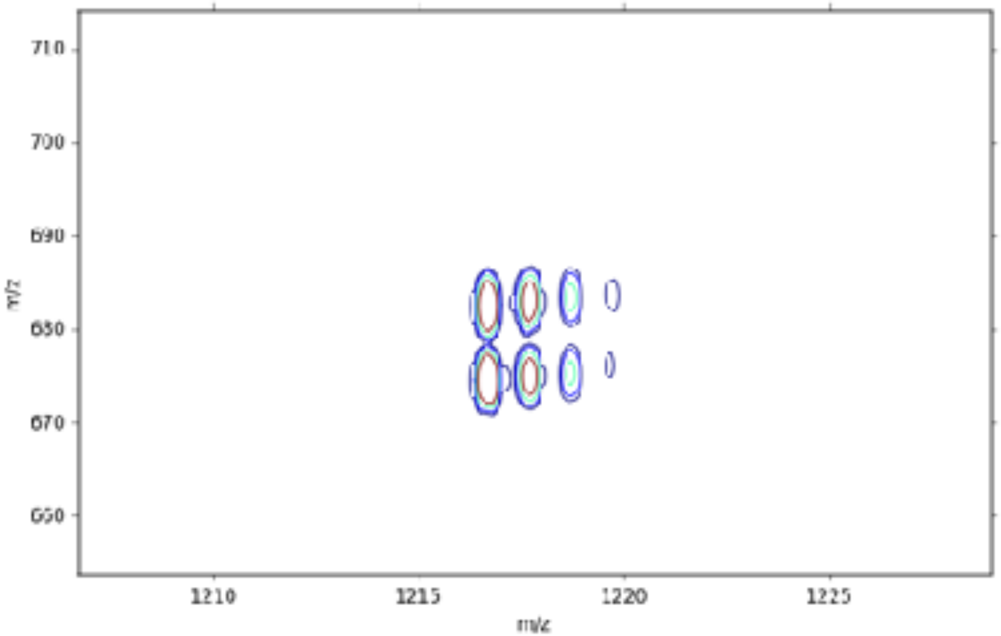

Classic
669
681
672
678


NUS 4k div 2

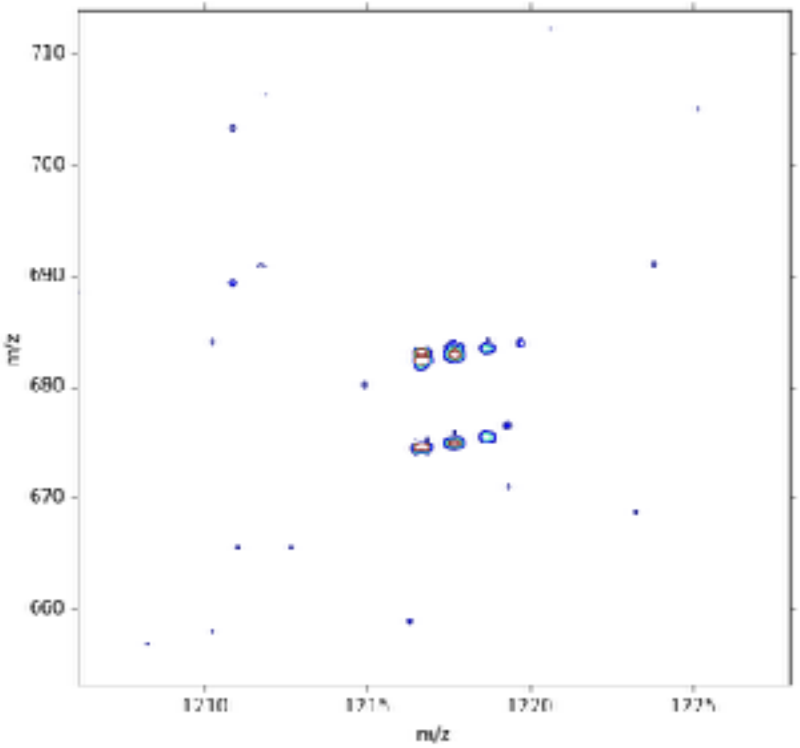

NUS 8k div 4


NUS 16k div 8


NUS 32k div 16



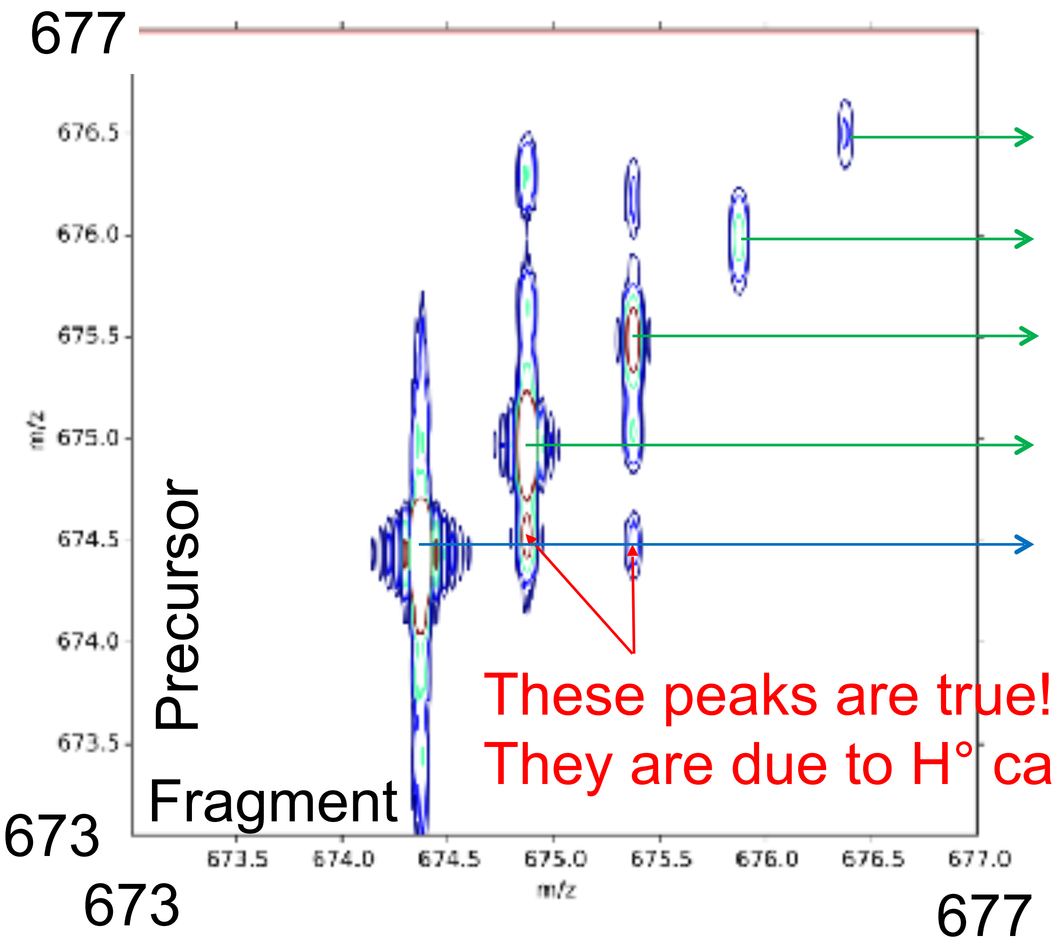
Classic



NUS 32k div 16

NUS (32k, 1/16) 2D zoom on doubly charged

- R1 ~ 14.000
- R2 ~ 150.000



4th isotope

3rd isotope

2nd isotope

1st isotope

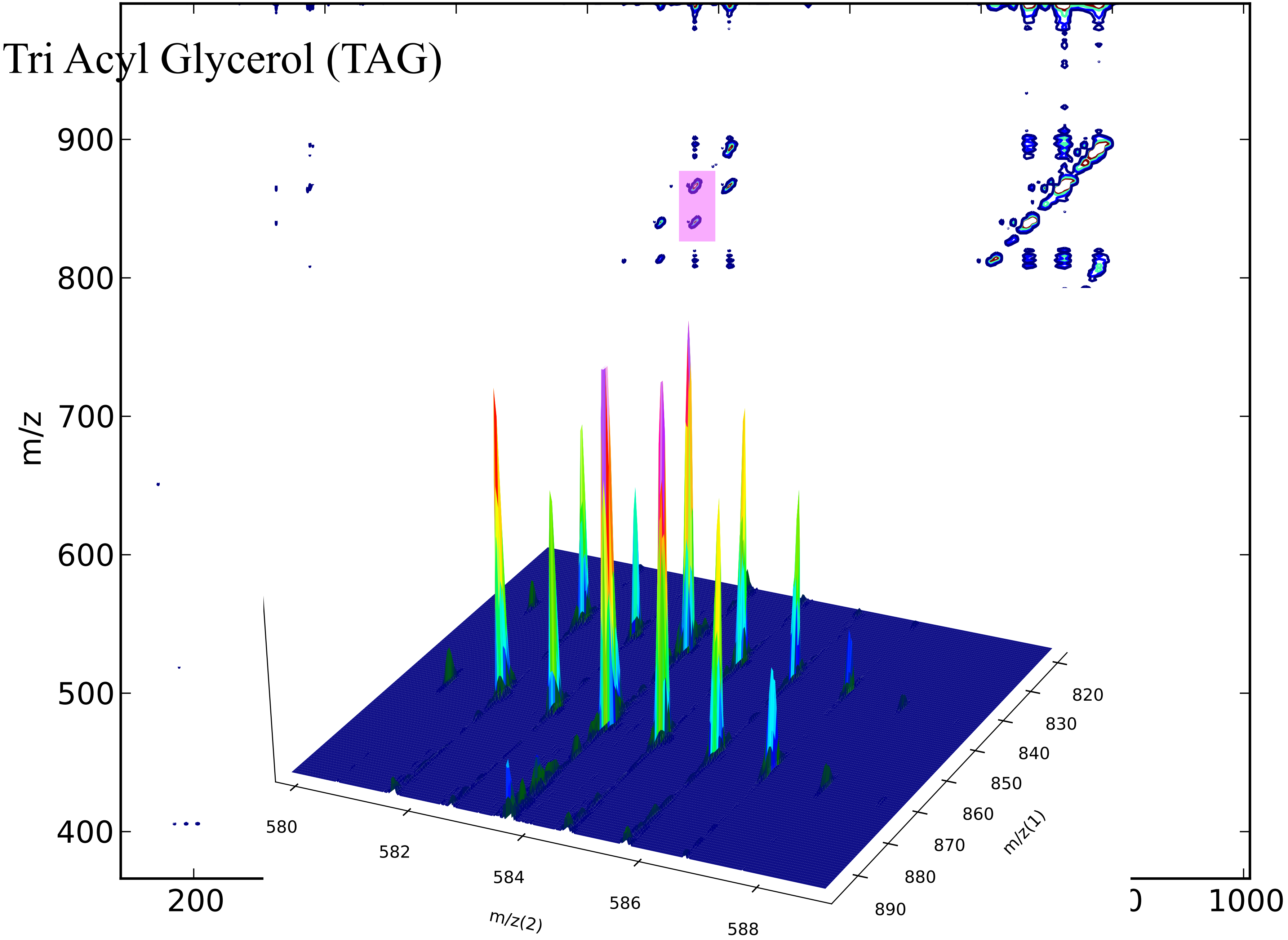
Monoisotopic peak

These peaks are true!
They are due to H° capture.

File = 140Gb
compressed to 30Gb

Bray, F., Bouclon, J., Chiron, L., Witt, M., Delsuc, M.-A., & Rolando, C. (2017). Nonuniform Sampling Acquisition of 2D FT-ICR MS for Increased Mass Resolution of Tandem Mass Spectrometry Precursor Ions. *Anal Chem,* 89(17), 8589–8593. http://doi.org/10.1021/acs.analchem.7b01850

On a triglyceride mixture



Tri Acyl Glycerol (TAG)

1. ligand binding
a. Recent developments in protein–ligand affinity mass spectrometry (http://europepmc.org/articles/PMC3043251)
b. Mass spectrometry-based approaches to protein–ligand interactions (http://www.tandfonline.com/doi/abs/10.1586/14789450.2.4.475?src=recsys&journalCode=ieru20)
c. Mass spectrometry based tools to investigate protein–ligand interactions for drug discovery (http://pubs.rsc.org/en/Content/ArticleLanding/2012/CS/C2CS35035A#!divAbstract)

2. Kd measurements - with all the caveat on ESI, etc..
a. Converting Solution Macromolecular Thermodynamic Properties into Gas-Phase Mass Spectrometry Observations (http://www.cell.com/cell-chemical-biology/fulltext/S1074-5521(02)00221-1)
b. Sizing Up Protein–Ligand Complexes: The Rise of Structural Mass Spectrometry Approaches in the Pharmaceutical Sciences (http://www.annualreviews.org/doi/full/10.1146/annurev-anchem-061516-045414#f1)
c. A General Mass Spectrometry-Based Assay for the Quantitation of Protein–Ligand Binding Interactions in Solution (http://pubs.acs.org/doi/abs/10.1021/ja026574g)

3. multi molecular complexes
a. The diverse and expanding role of mass spectrometry in structural and molecular biology (http://onlinelibrary.wiley.com/doi/10.15252/embj.201694818/full)
b. Advances in the Mass Spectrometry of Membrane Proteins: From Individual Proteins to Intact Complexes (http://www.annualreviews.org/doi/abs/10.1146/annurev-biochem-062309-093307?journalCode=biochem)
c. The emerging role of native mass spectrometry in characterizing the structure and dynamics of macromolecular complexes (http://onlinelibrary.wiley.com/doi/10.1002/pro.2661/pdf)
d. Mass spectrometry guided structural biology (http://www.sciencedirect.com/science/article/pii/S0959440X16301440)

4. HDX
a. Hydrogen exchange mass spectrometry for studying protein structure and dynamics (http://pubs.rsc.org/en/content/articlelanding/2011/cs/c0cs00113a#!divAbstract)
b. Differential hydrogen/deuterium exchange mass spectrometry analysis of protein–ligand interactions (http://europepmc.org/articles/PMC3113475)
c. Hydrogen exchange mass spectrometry: what is it and what can it tell us? (https://link.springer.com/article/10.1007/s00216-010-3556-4?no-access=true)

5. cross-linking
a. Chemical cross-linking and mass spectrometry to map three-dimensional protein structures and protein–protein interactions (http://onlinelibrary.wiley.com/doi/10.1002/mas.20082/abstract)
b. Probing Native Protein Structures by Chemical Cross-linking, Mass Spectrometry, and Bioinformatics (http://www.mcponline.org/content/9/8/1634.full)
c. Chemical cross-linking/mass spectrometry targeting acidic residues in proteins and protein complexes (http://www.pnas.org/content/111/26/9455.long)
d. Chemical cross-linking and native mass spectrometry: A fruitful combination for structural biology (http://onlinelibrary.wiley.com/doi/10.1002/pro.2696/full#references)

6. fragmentation
a. Top-down mass spectrometry: Recent developments, applications and perspectives (http://pubs.rsc.org/en/content/articlelanding/2011/an/c1an15286f#!divAbstract)
b. Native mass spectrometry of photosynthetic pigment–protein complexes (http://www.sciencedirect.com/science/article/pii/S0014579313000197)
c. 193 nm Ultraviolet Photodissociation Mass Spectrometry of Tetrameric Protein Complexes Provides Insight into Quaternary and Secondary Protein Topology (http://pubs.acs.org/doi/abs/10.1021/jacs.6b03905)

acknowledgment to Huilin Li

Thank you