Ion-electron and ion-atom reactions of gas-phase proteins in omnitrap

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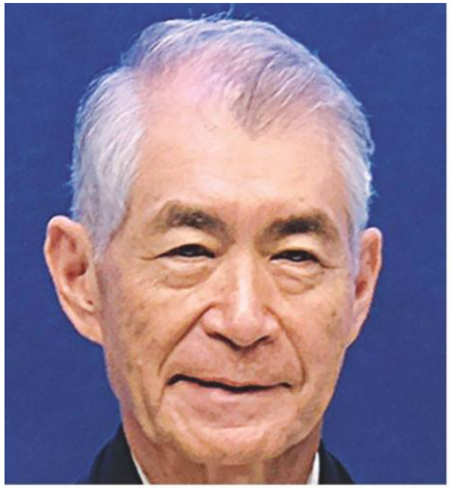
Nobel prize in Medicine or Physiology 2018



Tasuku Honjo

James Allison

What for?...

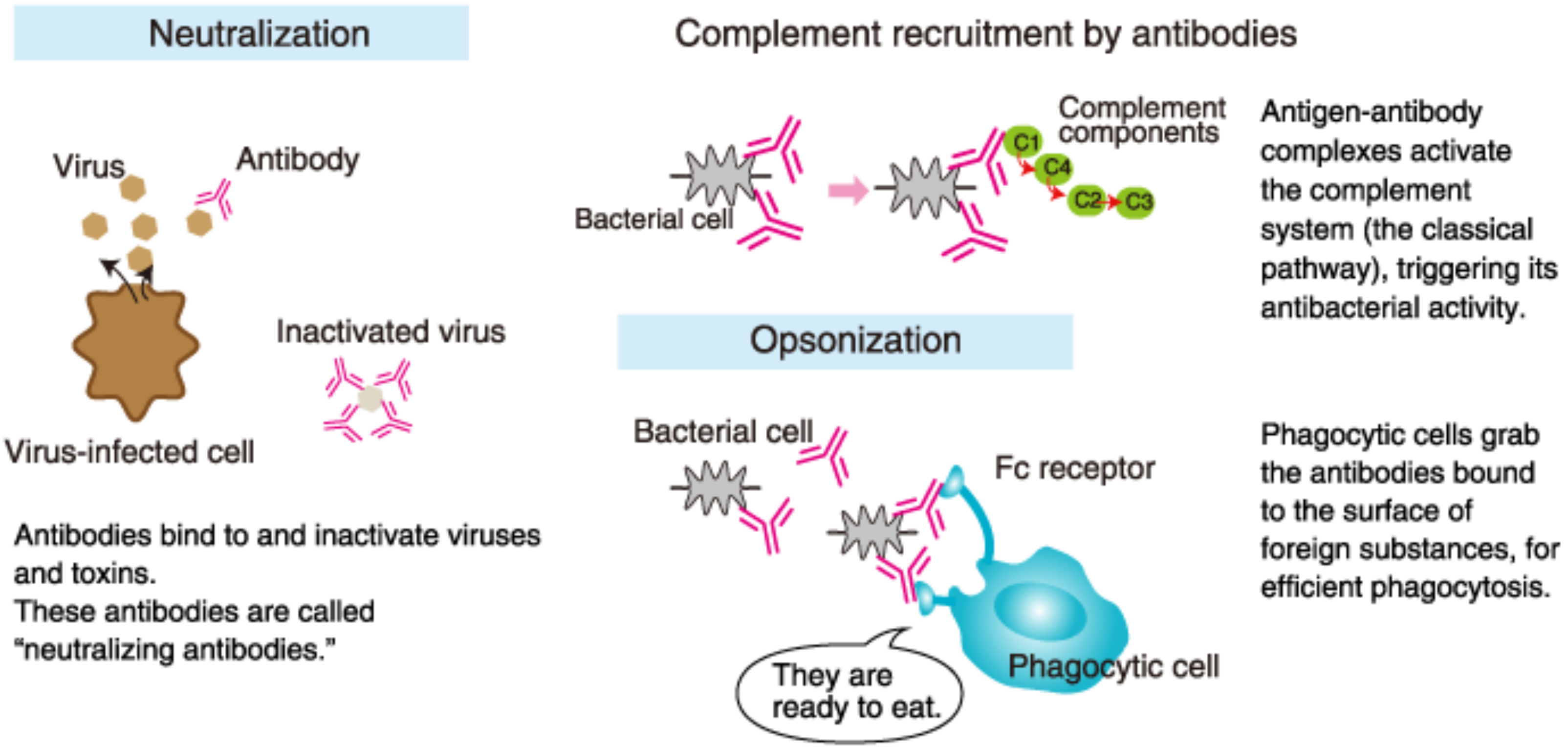
Unlike more traditional forms of cancer treatment that directly target cancer cells, **Allison and Honjo figured out how to help the patient's own immune system tackle the cancer more quickly**.

Imagine the world

where all human diseases are cured by body's own immune system…

But what is immune system?

The main function of the humoral **immune system** is the production of **antibodies**.
An **antibody** (Ab), or immunoglobulin (Ig), is a large, Y-shaped protein
produced mainly by plasma cells that is used by the immune system to neutralize
pathogens such as pathogenic bacteria and viruses.



Antibodies bind to and inactivate viruses and toxins.
These antibodies are called "neutralizing antibodies."

Antigen-antibody complexes activate the complement system (the classical pathway), triggering its antibacterial activity.

Phagocytic cells grab the antibodies bound to the surface of foreign substances, for efficient phagocytosis.

Ig structure and polyclonal complexity

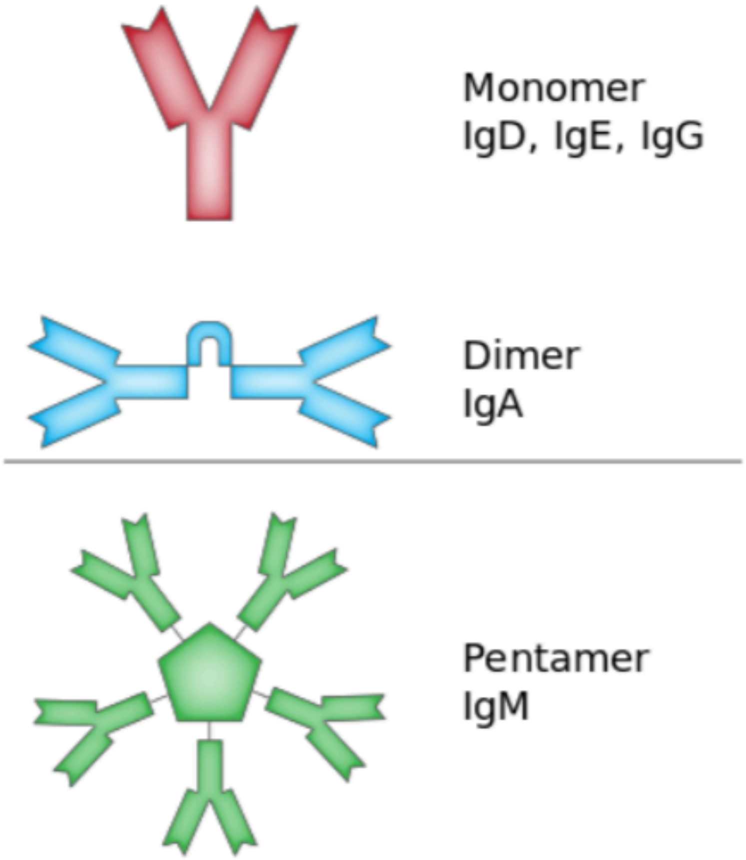
Ig concentration in blood: 7 -15 g/L (15-25% of all protein content)

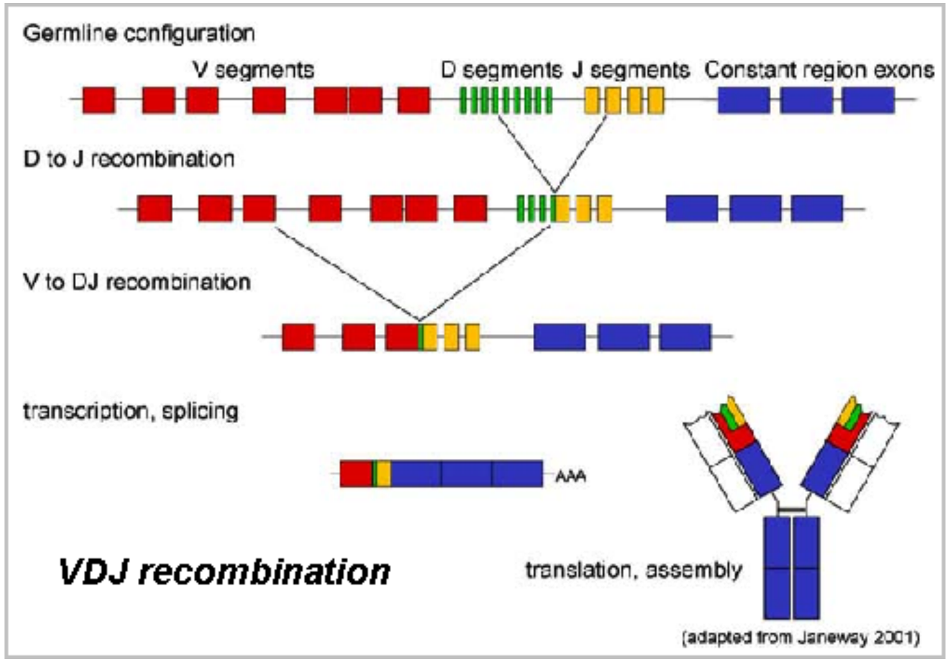
Theoretically 10^{15} different antigen binding sites (but more likely 10^{13})

C= conserved
V=variable <40
D=diversity <23
J=Joining <6

V(D)J-recombination

Heavy chain (VDJ) / Light chains - κ / λ , (VJ)





Why is the polyclonal antibody repertoire (“Ig[G]ome”) of interest?

Old paradigm

“Antigen specificity is determined by a completely random process which will result in unique antigen binding complementary determining regions (CDRs) in antibodies (of similar target) in different individuals”

Emerging paradigm

“Ig with particular antigen specificity will show a level of inter-individual sequence homology”

To know how immune system works, one needs to sequence the Ig-ome

References

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Zubarev lab: Lundström SL et al. PLOS-ONE, 2014

TOP SPEC

GRANT: H2020- *FETOPEN* - *Future and Emerging Technologies (FET) – Open research and innovation actions*

The TopSpec EU project (2019-21) will develop a novel TOP-down tandem mass SPECtrometry (MS/MS) platform for Ig analysis.

Partners:

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FasmaTech
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biomotif *Capturing the essence of life*
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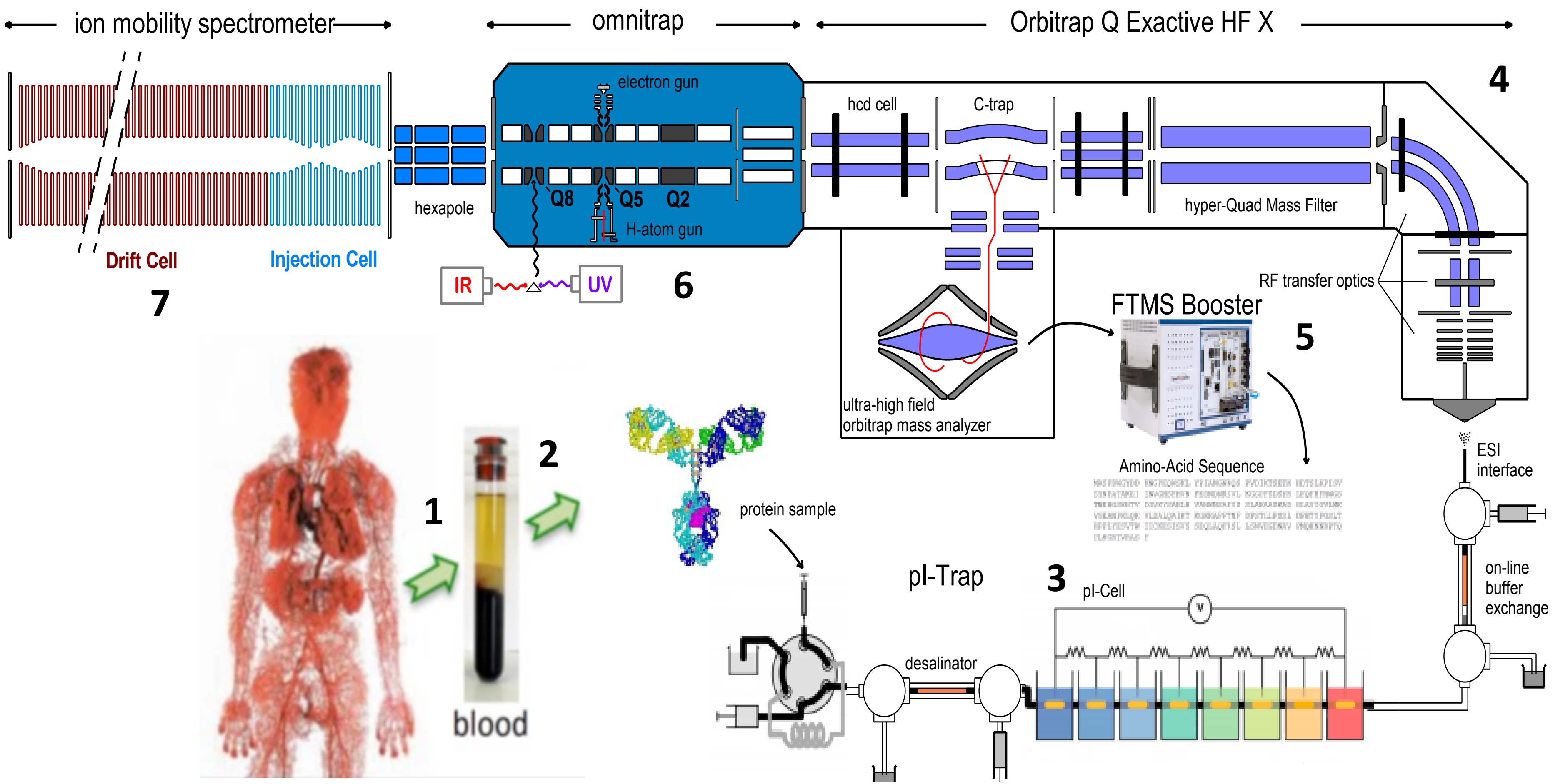
Spectroswiss
Yury Tsybin
Anton Kozhinov
Konstantin Nagornov

NOTTINGHAM TRENT UNIVERSITY
David Kilgour

TopSpec envisioned



1) Clinical sample; 2) IgG (protein) enrichment

3) pI-Trap online pI fractionator; /desalinator/buffer exchanger

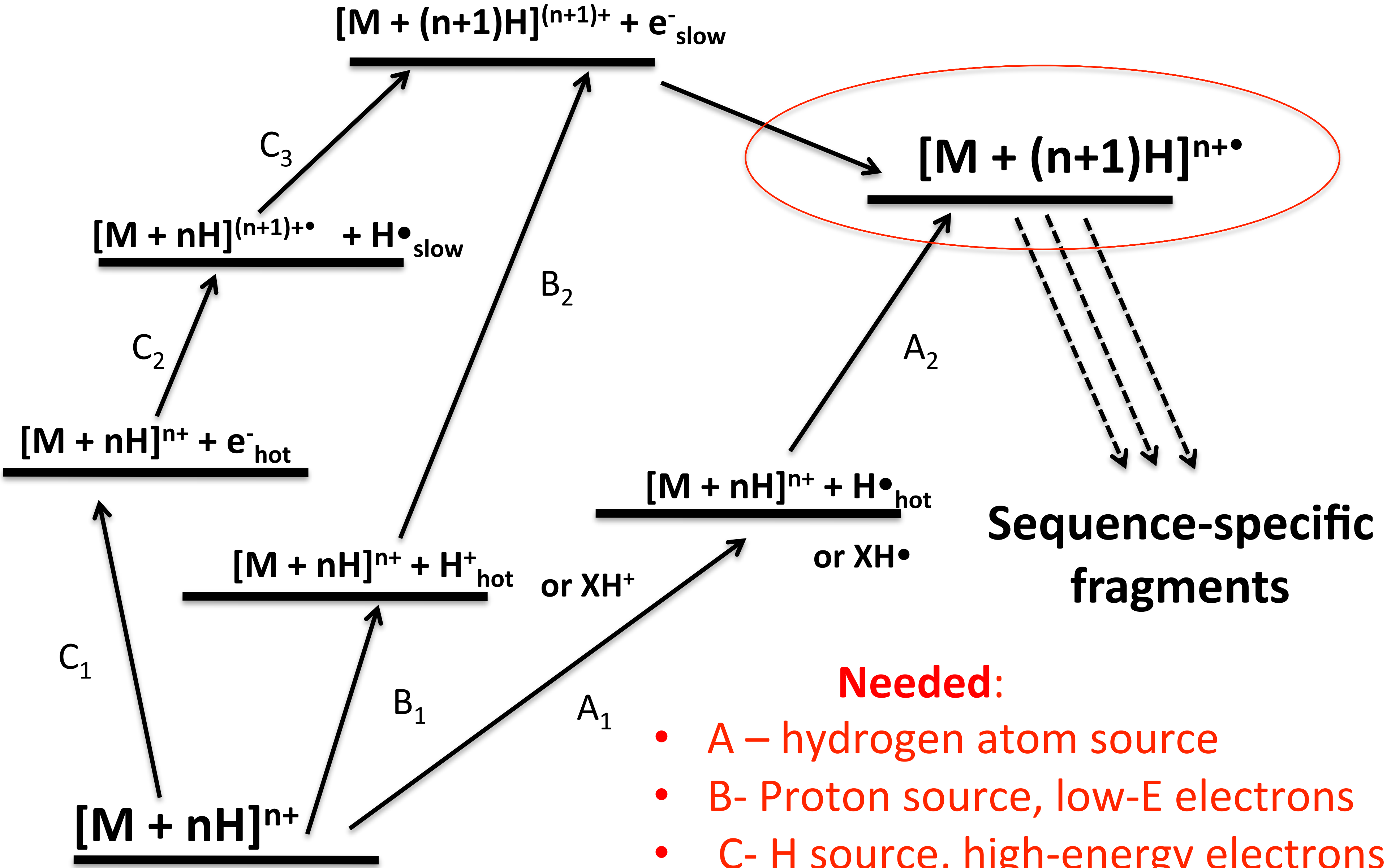
4) Modified Q-Exactive HF X ultra-high-resolution FT mass spectrometer

5) FTMS Booster – high-performance data acquisition and real-time big data processing system

6) Omnitrap – all-inclusive MS/MS device for protein sequencing; 7) ion mobility device

Aim: *Hydrogen-abundant radical cations*

without charge reduction



$[M + (n+1)H]^{(n+1)+} + e^{-}_{slow}$

C_3

$[M + nH]^{(n+1)+\bullet}$ $+ H\bullet_{slow}$

C_2

$[M + nH]^{n+} + e^{-}_{hot}$

C_1

B_2

$[M + nH]^{n+} + H^{+}_{hot}$ or XH^{+}

B_1

$[M + (n+1)H]^{n+\bullet}$

A_2

$[M + nH]^{n+} + H\bullet_{hot}$

or $XH\bullet$

A_1

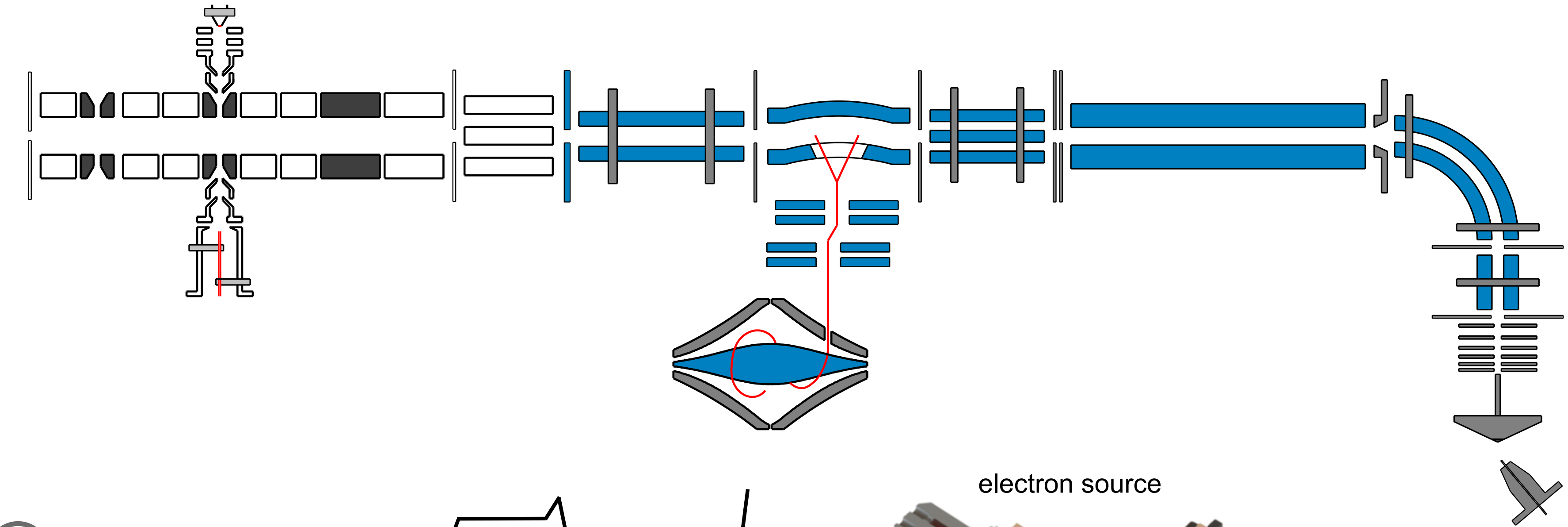
$[M + nH]^{n+}$

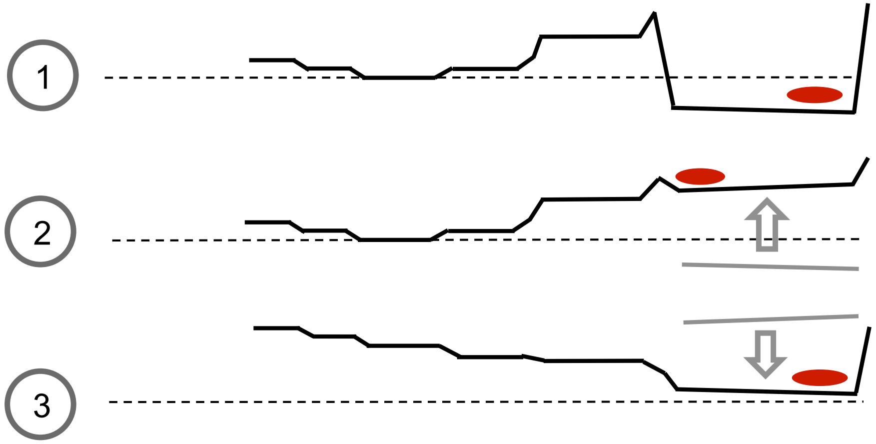
Sequence-specific fragments

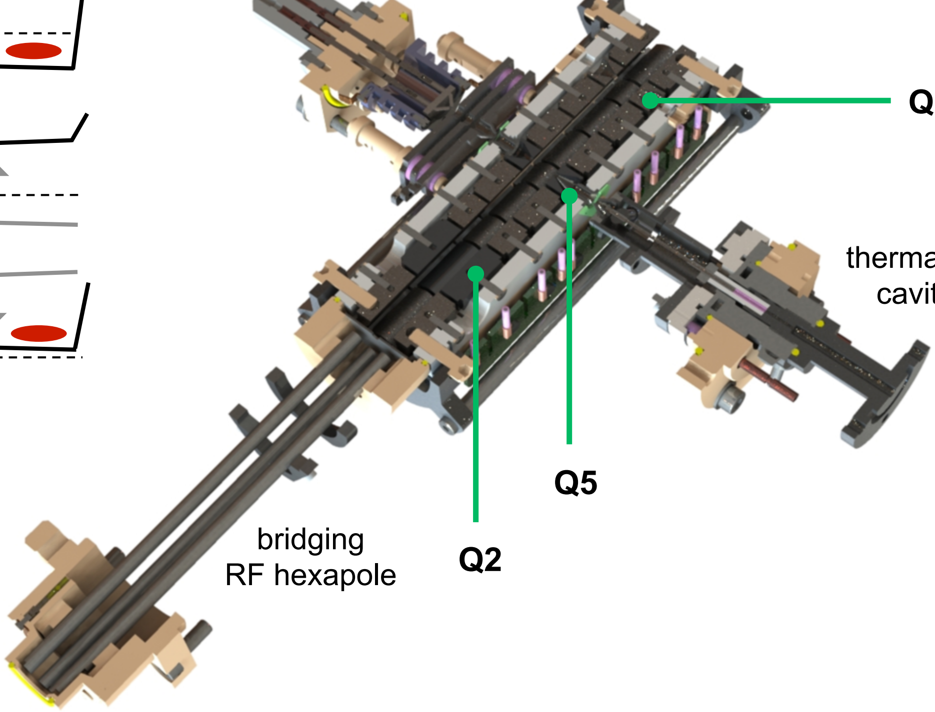
Needed:

- A – hydrogen atom source
- B- Proton source, low-E electrons
- C- H source, high-energy electrons

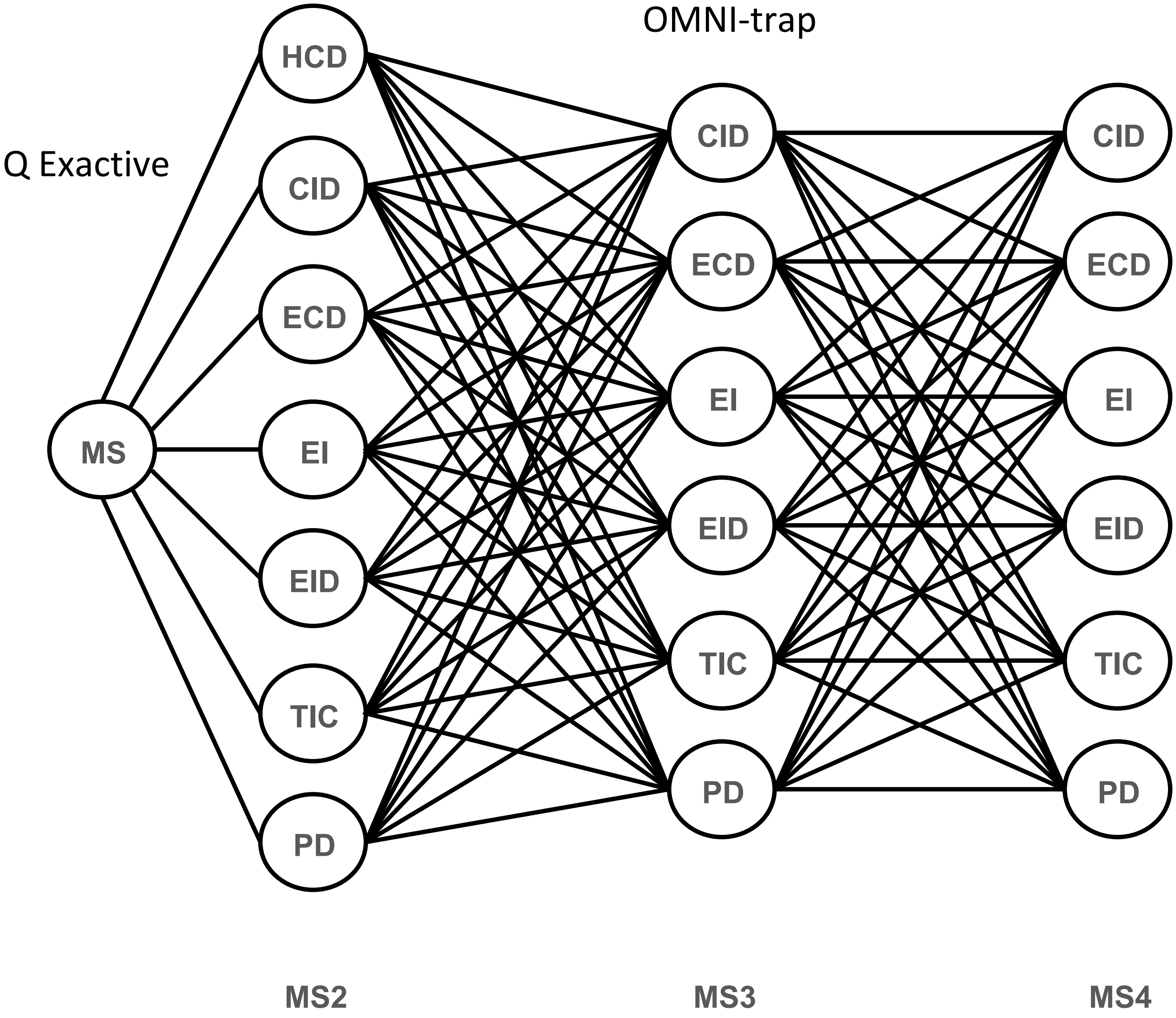
Omnitrap – Q Exactive Combination





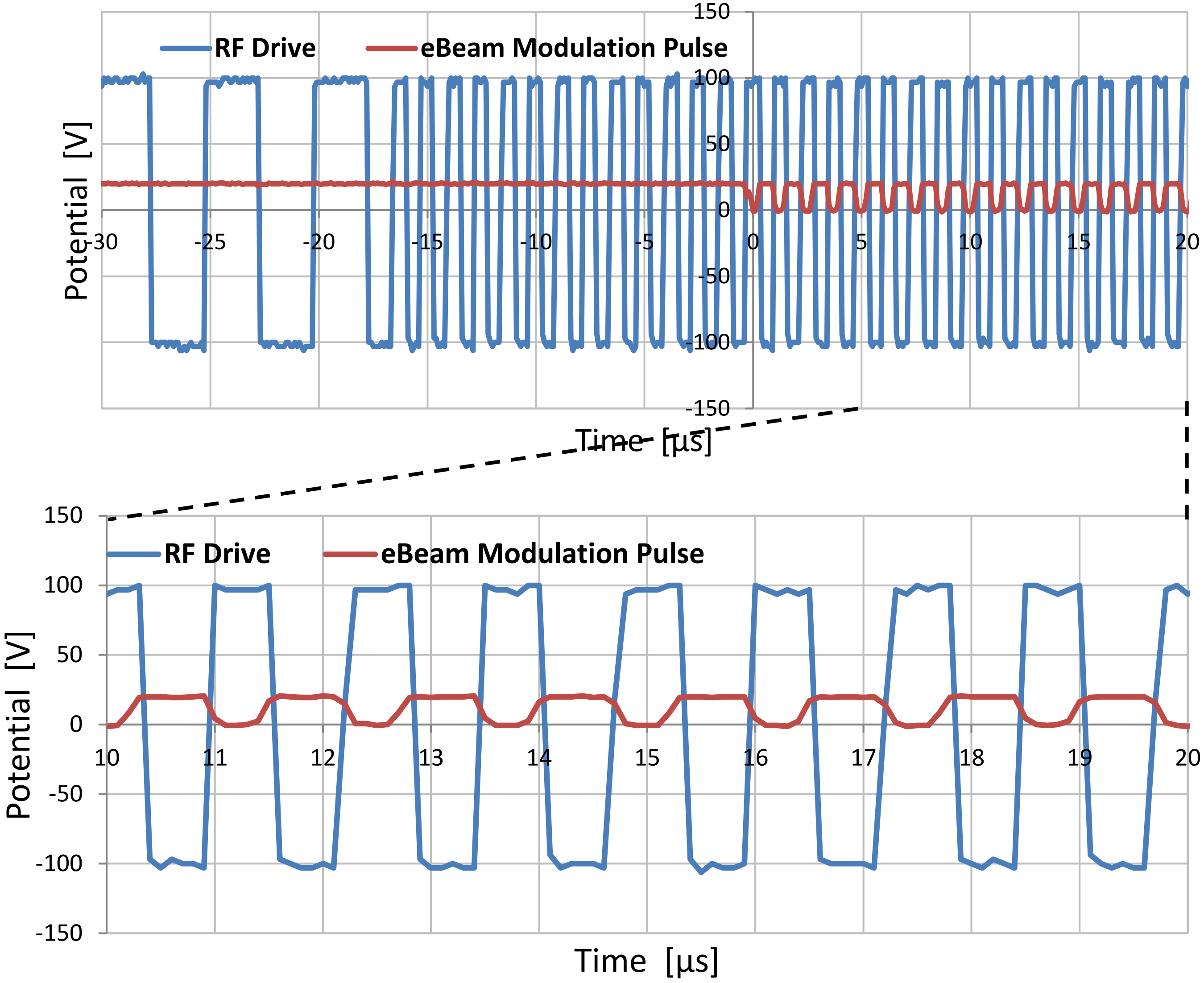


Omnitrap-Orbitrap activation network



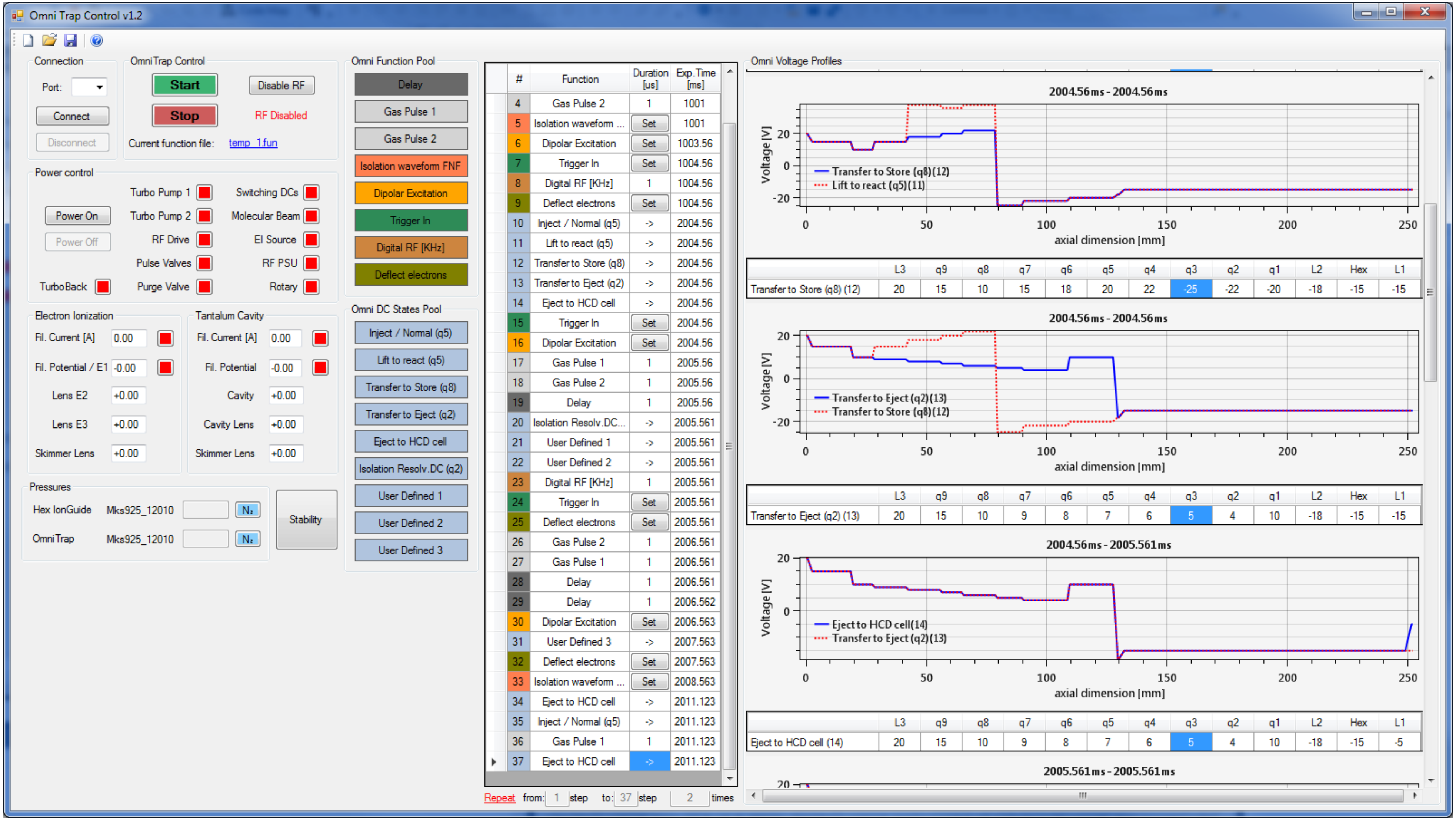
Omnitrap is fully digital

All potentials are well defined most of the time



oscilloscope traces

User Interface



RF Disabled

Current function file: temp_1.fun

Power control

Turbo Pump 1	🟥	Switching DCs	🟥
Turbo Pump 2	🟥	Molecular Beam	🟥
RF Drive	🟥	EI Source	🟥
Pulse Valves	🟥	RF PSU	🟥
Purge Valve	🟥	Rotary	🟥
TurboBack	🟥		

Electron Ionization

Fil. Current [A]	0.00	🟥
Fil. Potential / E1	-0.00	🟥
Lens E2	+0.00	
Lens E3	+0.00	
Skimmer Lens	+0.00	

Tantalum Cavity

Fil. Current [A]	0.00	🟥
Fil. Potential	-0.00	🟥
Cavity	+0.00	
Cavity Lens	+0.00	
Skimmer Lens	+0.00	

Pressures

Hex IonGuide	Mks925_12010		N₂
OmniTrap	Mks925_12010		N₂

#	Function	Duration [us]	Exp.Time [ms]
4	Gas Pulse 2	1	1001
5	Isolation waveform ...	Set	1001
6	Dipolar Excitation	Set	1003.56
7	Trigger In	Set	1004.56
8	Digital RF [KHz]	1	1004.56
9	Deflect electrons	Set	1004.56
10	Inject / Normal (q5)	->	2004.56
11	Lift to react (q5)	->	2004.56
12	Transfer to Store (q8)	->	2004.56
13	Transfer to Eject (q2)	->	2004.56
14	Eject to HCD cell	->	2004.56
15	Trigger In	Set	2004.56
16	Dipolar Excitation	Set	2004.56
17	Gas Pulse 1	1	2005.56
18	Gas Pulse 2	1	2005.56
19	Delay	1	2005.56
20	Isolation Resolv.DC...	->	2005.561
21	User Defined 1	->	2005.561
22	User Defined 2	->	2005.561
23	Digital RF [KHz]	1	2005.561
24	Trigger In	Set	2005.561
25	Deflect electrons	Set	2005.561
26	Gas Pulse 2	1	2006.561
27	Gas Pulse 1	1	2006.561
28	Delay	1	2006.561
29	Delay	1	2006.562
30	Dipolar Excitation	Set	2006.563
31	User Defined 3	->	2007.563
32	Deflect electrons	Set	2007.563
33	Isolation waveform ...	Set	2008.563
34	Eject to HCD cell	->	2011.123
35	Inject / Normal (q5)	->	2011.123
36	Gas Pulse 1	1	2011.123
37	Eject to HCD cell	->	2011.123

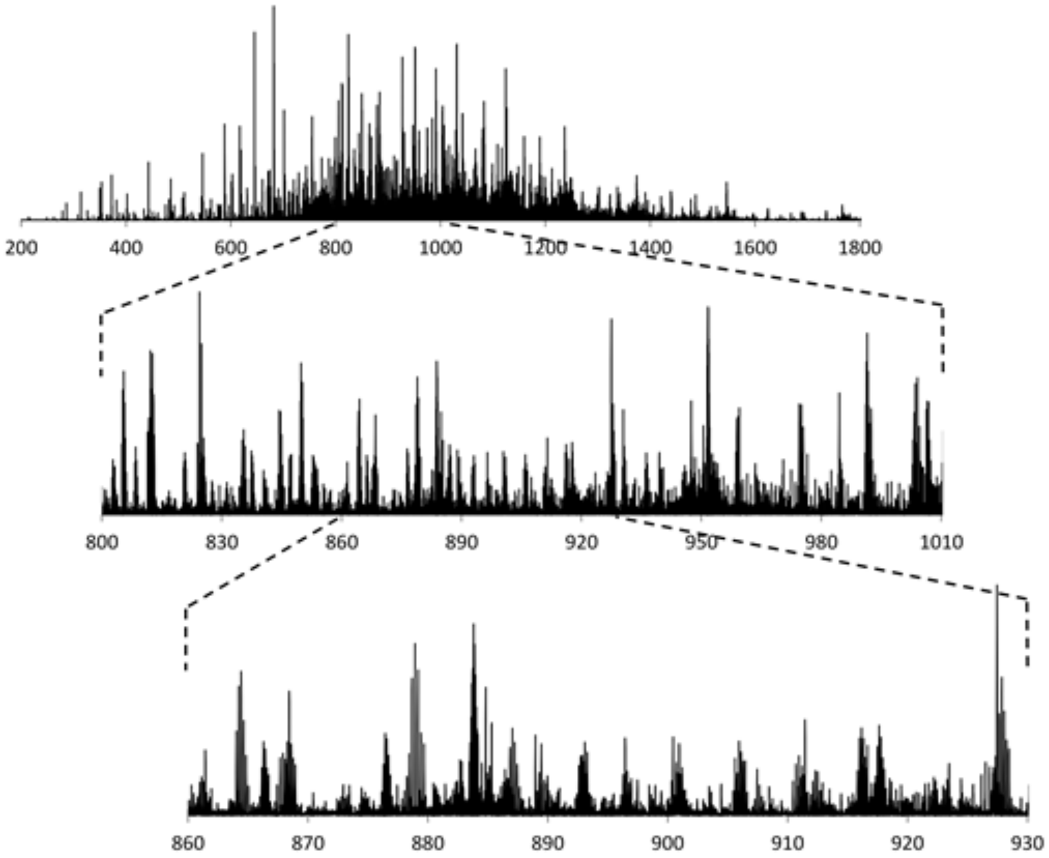
Repeat from: 1 step to: 37 step 2 times

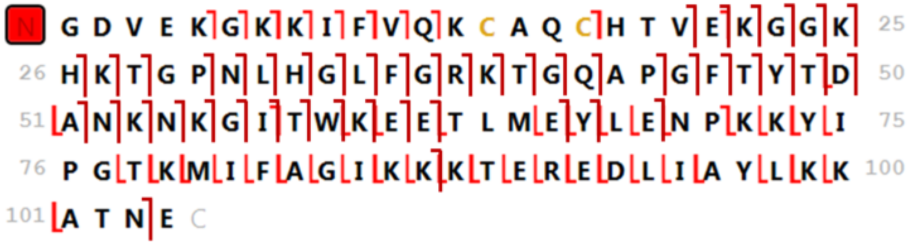
	L3	q9	q8	q7	q6	q5	q4	q3	q2	q1	L2	Hex	L1
Transfer to Store (q8) (12)	20	15	10	15	18	20	22	-25	-22	-20	-18	-15	-15

	L3	q9	q8	q7	q6	q5	q4	q3	q2	q1	L2	Hex	L1
Transfer to Eject (q2) (13)	20	15	10	9	8	7	6	5	4	10	-18	-15	-15

	L3	q9	q8	q7	q6	q5	q4	q3	q2	q1	L2	Hex	L1
Eject to HCD cell (14)	20	15	10	9	8	7	6	5	4	10	-18	-15	-5

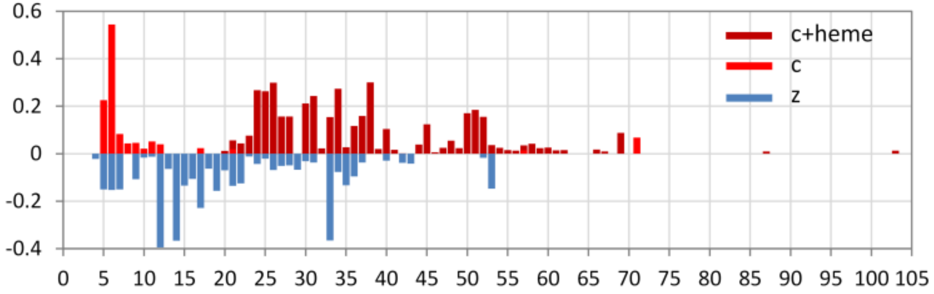
Electron Capture Dissociation – cytochrome c



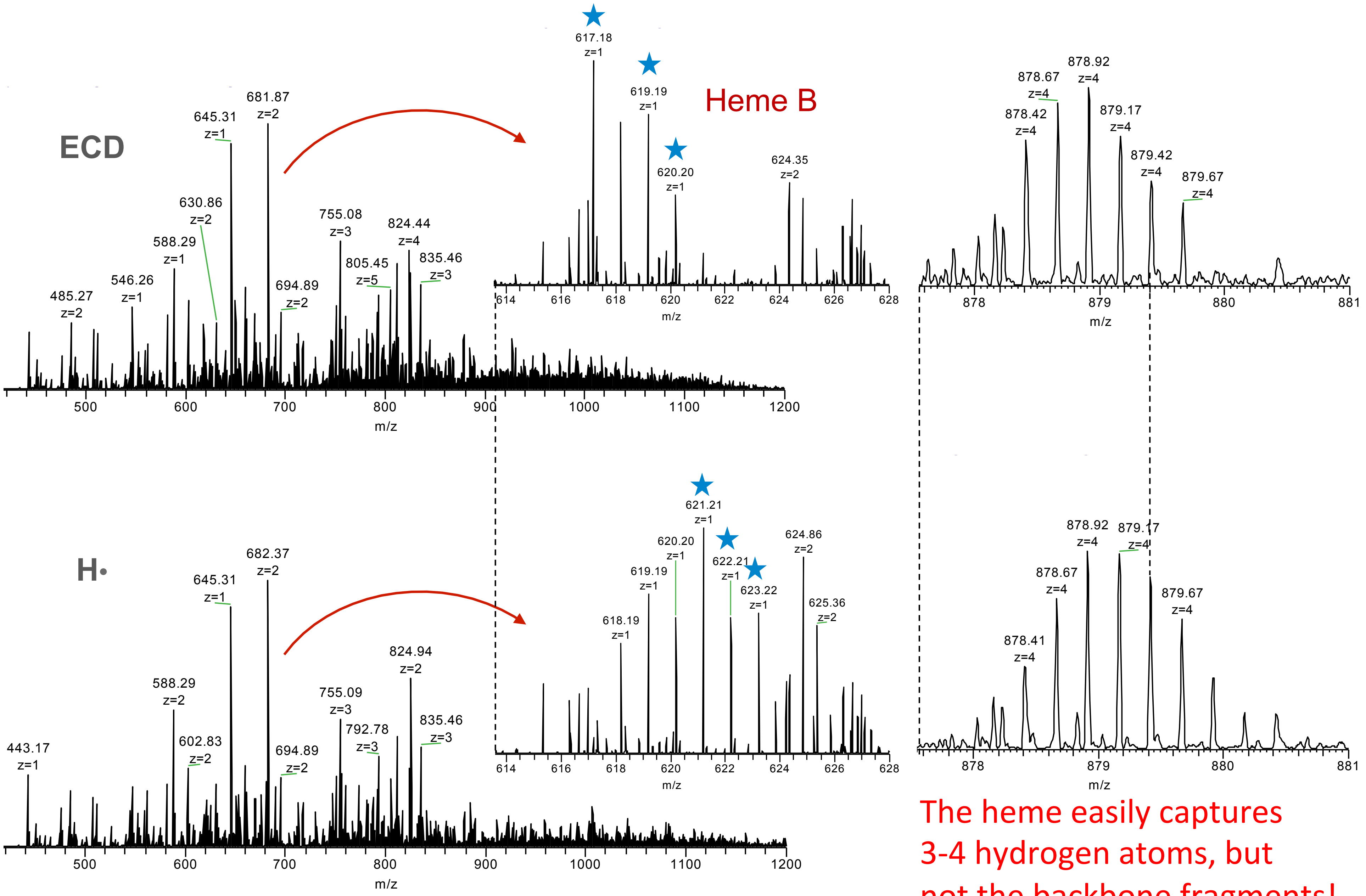


Cytochrome C sequence highlighting c,z fragments identified in ProSight Lite.

>80% sequence coverage. Only <15% of monoisotopic peaks are processed!



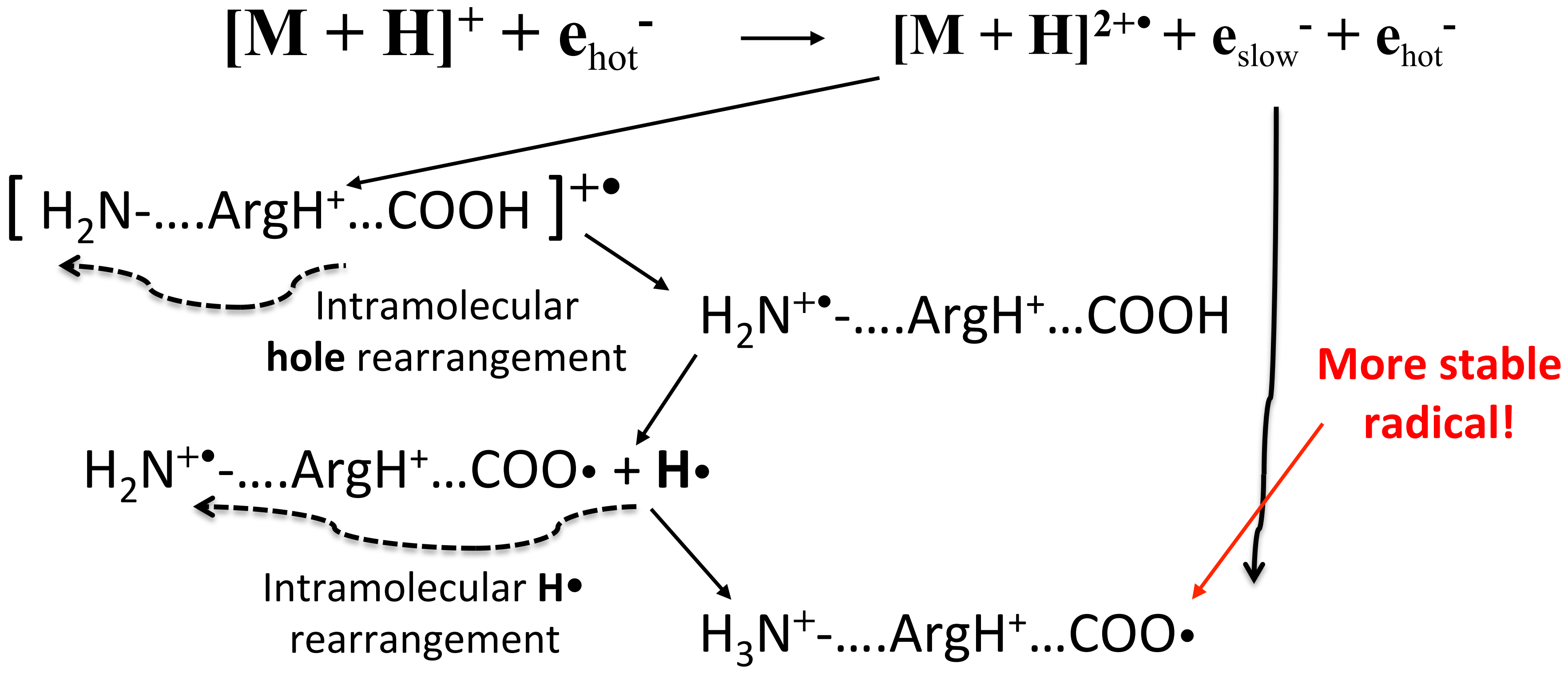
MS2 – cytochrome c ECD → HAB



The heme easily captures 3-4 hydrogen atoms, but not the backbone fragments!

Intramolecular radical rearrangement

M.L. Nielsen et al. / Chemical Physics Letters 330 (2000) 558–562



$$[M + H]^+ + e_{hot}^- \longrightarrow [M + H]^{2+\bullet} + e_{slow}^- + e_{hot}^-$$

$[\ H_2N\text{-....ArgH}^+\text{...COOH}\ ]^{+\bullet}$

Intramolecular
hole rearrangement

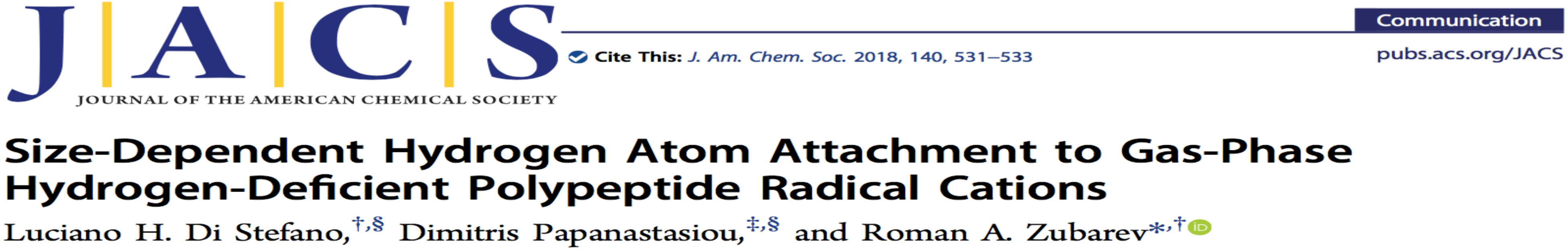
$H_2N^{+\bullet}\text{-....ArgH}^+\text{...COOH}$

More stable radical!

$H_2N^{+\bullet}\text{-....ArgH}^+\text{...COO}\bullet$ + **H•**

Intramolecular **H•**
rearrangement

$H_3N^+\text{-....ArgH}^+\text{...COO}\bullet$



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Communication

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Size-Dependent Hydrogen Atom Attachment to Gas-Phase Hydrogen-Deficient Polypeptide Radical Cations

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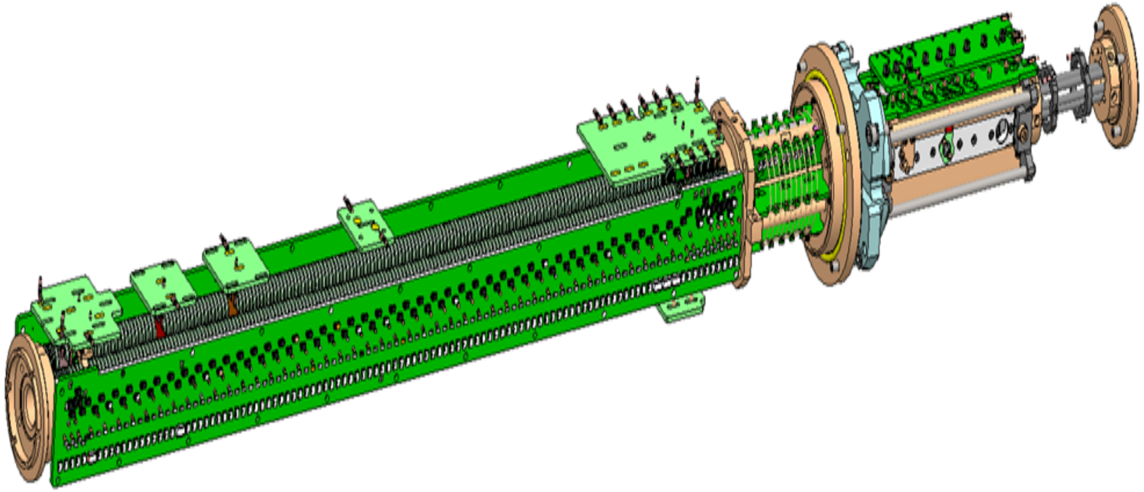
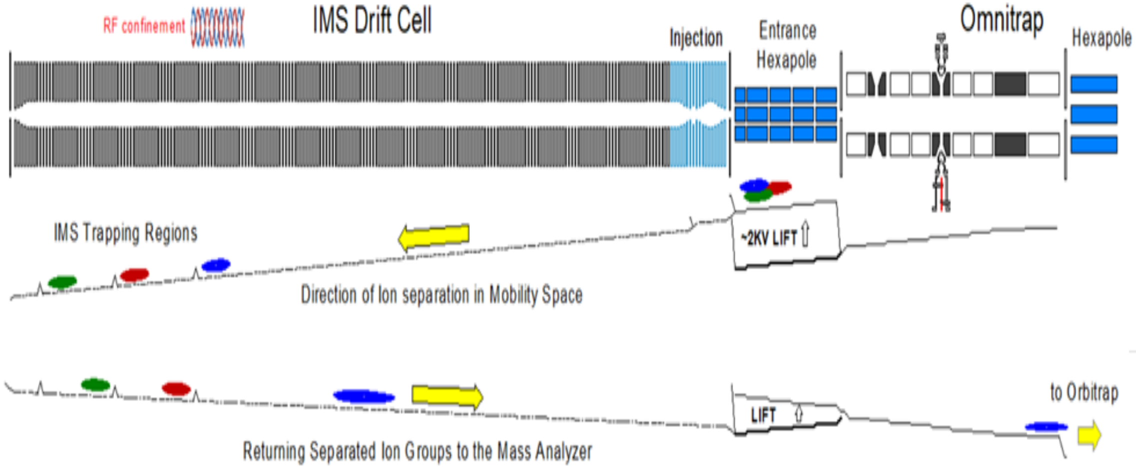
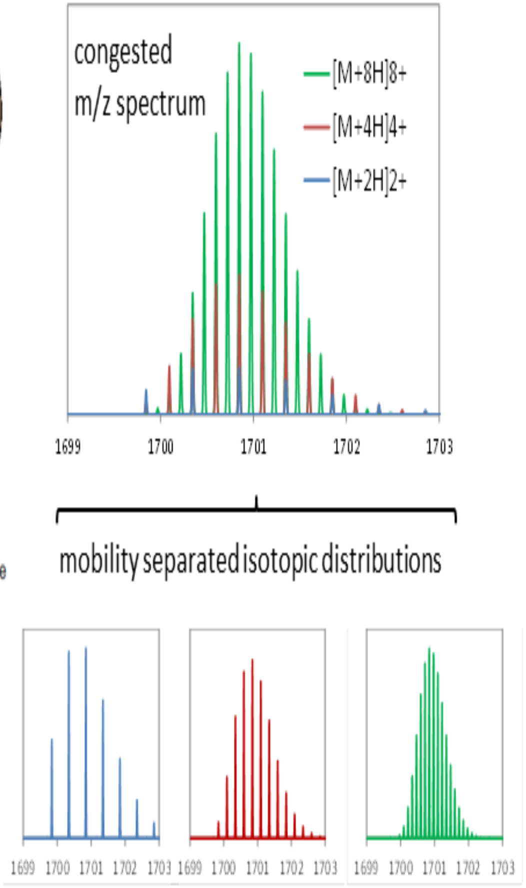
MSn – IMSn Configuration

Top-Down Spectra Complexity Reduction







Hydrogen Atom Dissociation (HAD)

A″

$$[M + nH]^{n+} + \underset{>0.1eV}{H^{\cdot}} \longrightarrow \underset{hypervalent\ +}{[M + (n+1)H]^{n+\cdot}} \longrightarrow \text{fragments} \qquad (1)$$

Electron Ionization & Hydrogen Attachment & ECD

B″

$$[M + nH]^{n+} + \underset{10\text{-}100eV}{e^{-}} \longrightarrow [M + nH]^{(n+1)+\cdot} + 2e^{-} \qquad (2a)$$

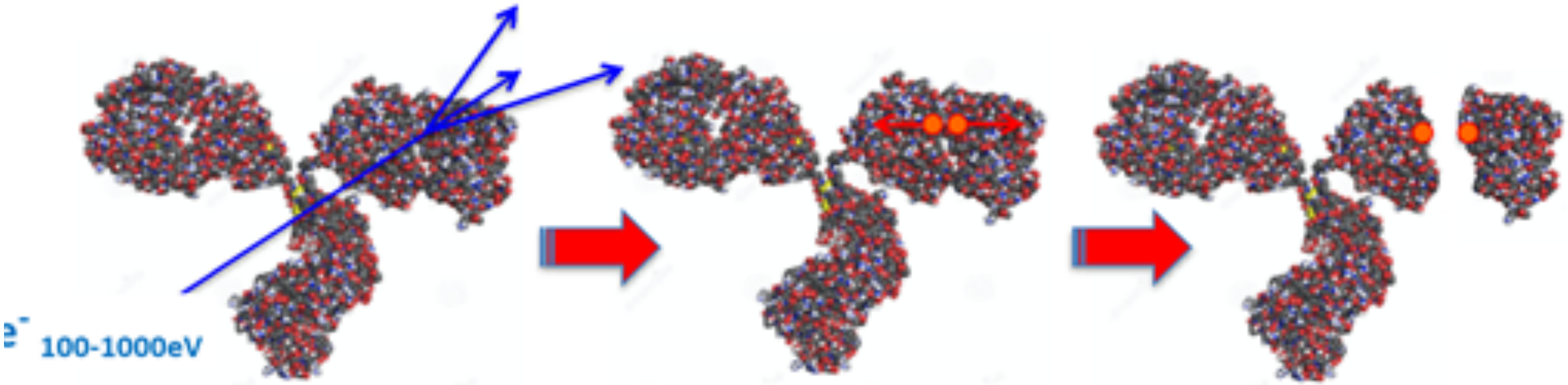
$$[M + nH]^{(n+1)+\cdot} + \underset{0.1\text{-}1eV}{H^{\cdot}} \longrightarrow [M + (n+1)H]^{(n+1)+} \qquad (2b)$$

$$[M + (n+1)H]^{(n+1)+} + \underset{0.1\text{-}1eV}{e^{-}} \longrightarrow \underset{hypervalent\ +}{[M + (n+1)H\ ]^{n+\cdot *}} \longrightarrow \text{fragments} \qquad (2c)$$

Coulomb Explosion Dissociation (CEM)

C″

$$[M + nH]^{n+} + \underset{10\text{-}100eV}{e^{-}} \longrightarrow [M + nH\ ]^{(n+>2)+\cdot *} + >3e^{-} \qquad (3)$$



Top-down spectra deconvolution