Electron Activated Dissociation in a Q-TOF Mass Spectrometer empowered by Zeno Trap Pulsing

Takashi Baba, Pavel Ryumin, Eva Duchoslav, Anjali Chelur, Bill Loyd, Igor Chernushevich, Jason Causon, and Jose Castro-Perez

ThOA am 08:50 Nov 04 / 2021 ASMS, Philadelphia

SCIEX
The Power of Precision

Introduction: Benefit of Time-of-Flight Mass Spectrometers

- Resolution: Quadrupole < TOF (m/Δm ~ 30,000-50,000) < Fourier transform
- ion transmission enhanced by **Zeno trap pulsing**
- Advantage: High sensitivity & precursor consumption (ion statistics)

- Applications using high sensitivity performance:

  Detection of weak or rare **Electron Activated Dissociation (EAD)** products
  - Top-down protein sequencing (this work: top-down *de novo* sequencing)
  - Structural elucidation of lipids & small molecules (this work: phospholipids with negative charge)

Electron Activated Dissociation (EAD)

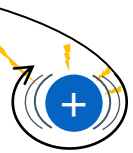
ref. T. Baba et al., Anal. Chem. 2014

free electron

e-

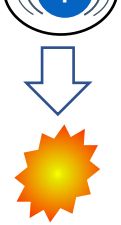
any energy

eg. ECD (0 eV)

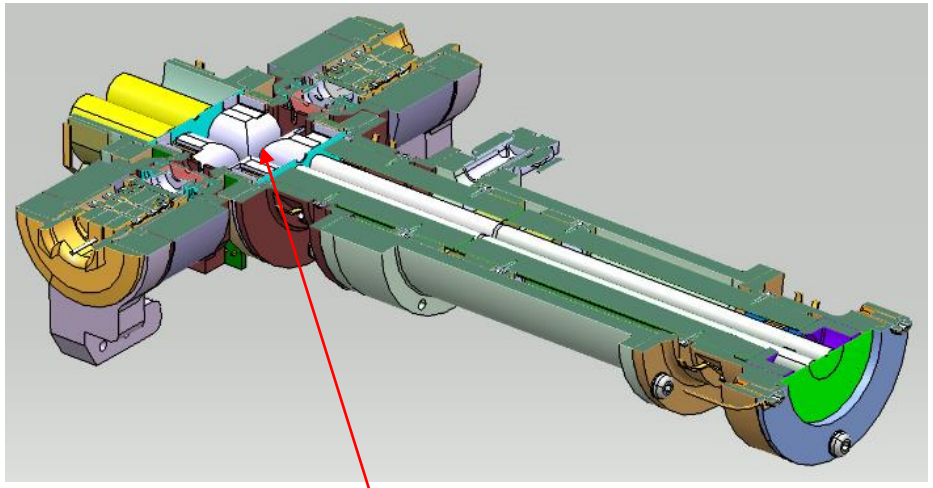


radical state

fragments

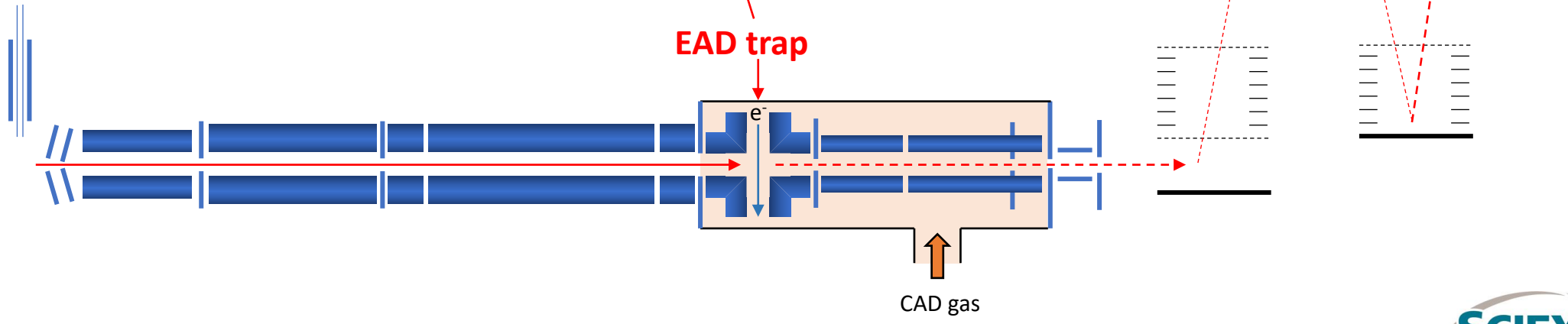


Q2 unit for EAD-CAD-Zeno



MCP detector

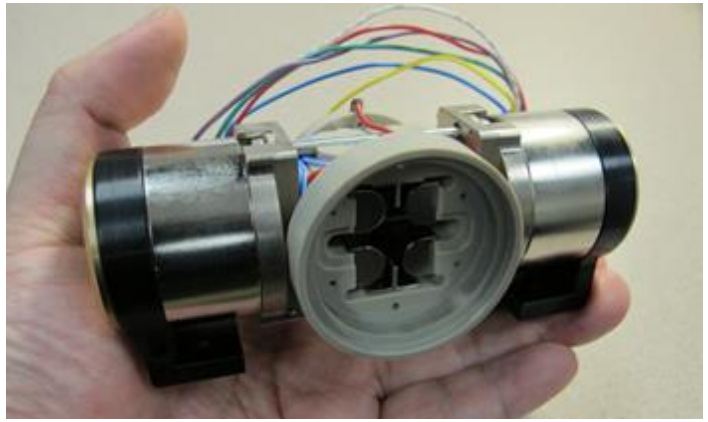
N-TOF

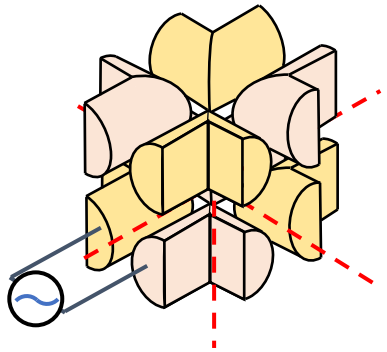


SCIEX
The Power of Precision

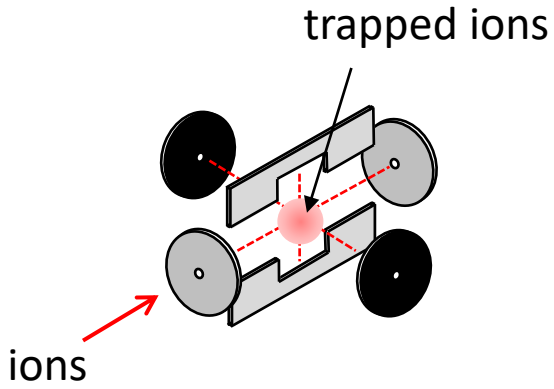
EAD device using a branched RF ion trap

ref. T. Baba et al., Anal. Chem. 2014
T. Baba et al., ASMS 2014, Oral

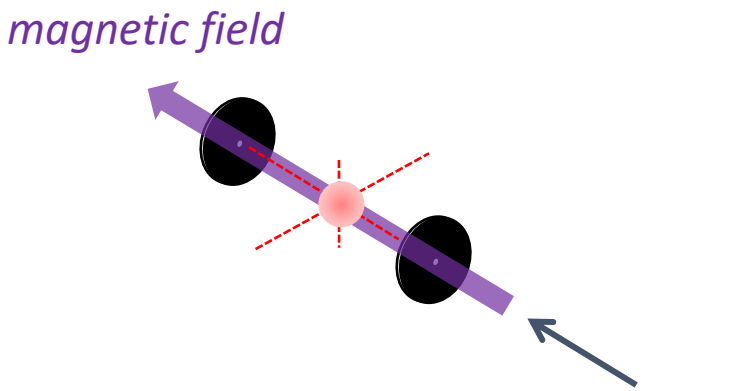




RF ion trap potential



electrostatic lenses

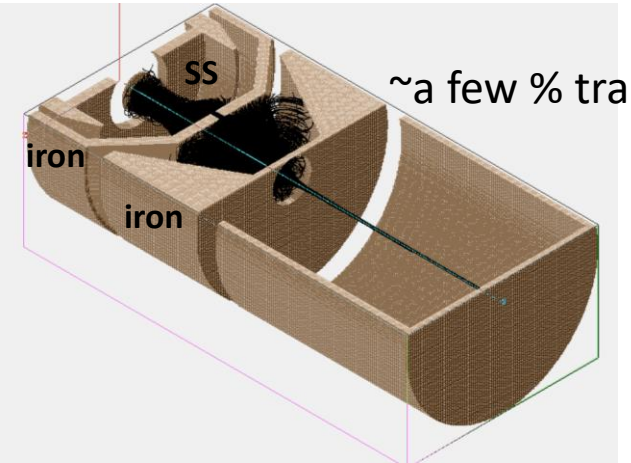


electron beam

SCIEX
The Power of Precision

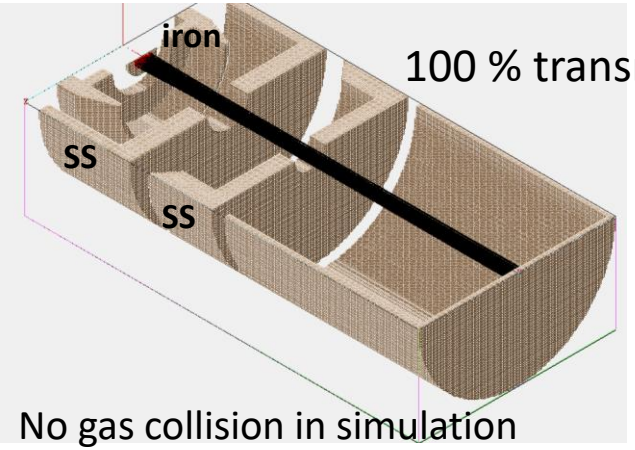
Improvement of electron beam transmission

ref. K. Chen et al. ASMS 2019
T. Baba et al. JASMS 2021



~a few % transmission (2014-)

redesign of the magnetic circuit

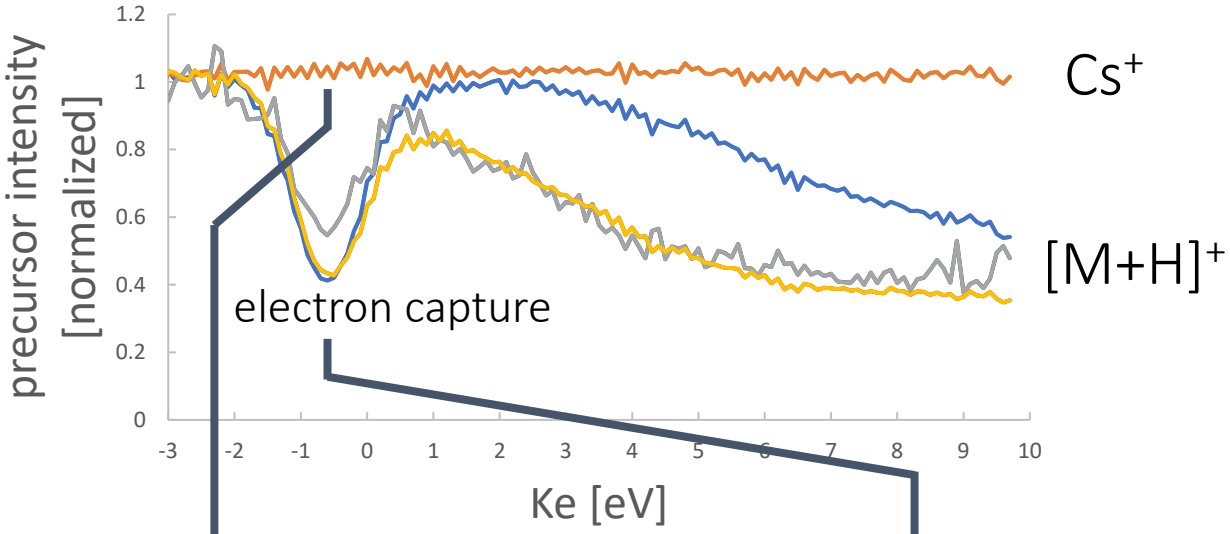


100 % transmission (2019)

No gas collision in simulation

electron capture by singly charged ions

electron irradiation time : 10 ms



e-

atomic ions

no excitation states

e-

molecular ions

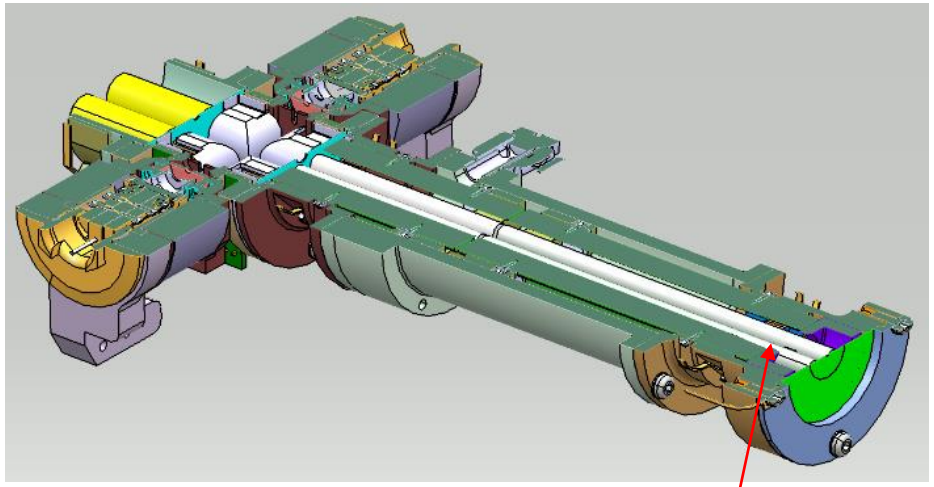
The electron excites vibrational modes during flight, then captured.

SCIEX
The Power of Precision

Zeno trap pulsing for ion transmission enhancement

ref. I. Chernushevich et al. JASMS2009

Q2 unit for EAD-CAD-Zeno

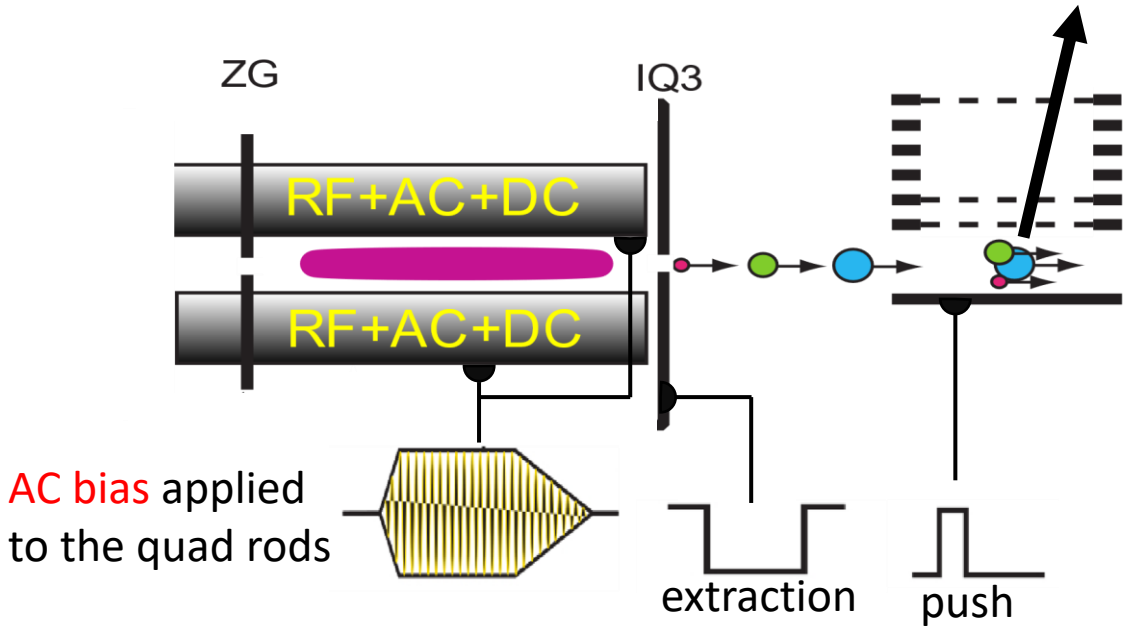


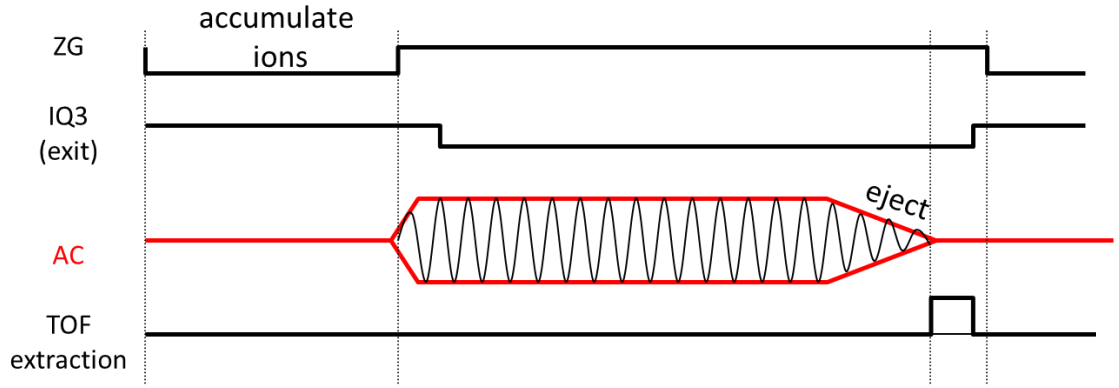
SCIEX
The Power of Precision

Zeno trap pulsing principle

ref. I. Chernushevich et al. JASMS2009

m/z ordered ion ejection from IQ3





(1) Axial trapping by pseudo potential by the AC bias
*AC "bias": the same AC phase on all four rods

(2) **m/z ordered ion ejection** by combination of the lamping AC and the extraction field on IQ3

Spatial focusing of wide range of m/z in the TOF accelerator

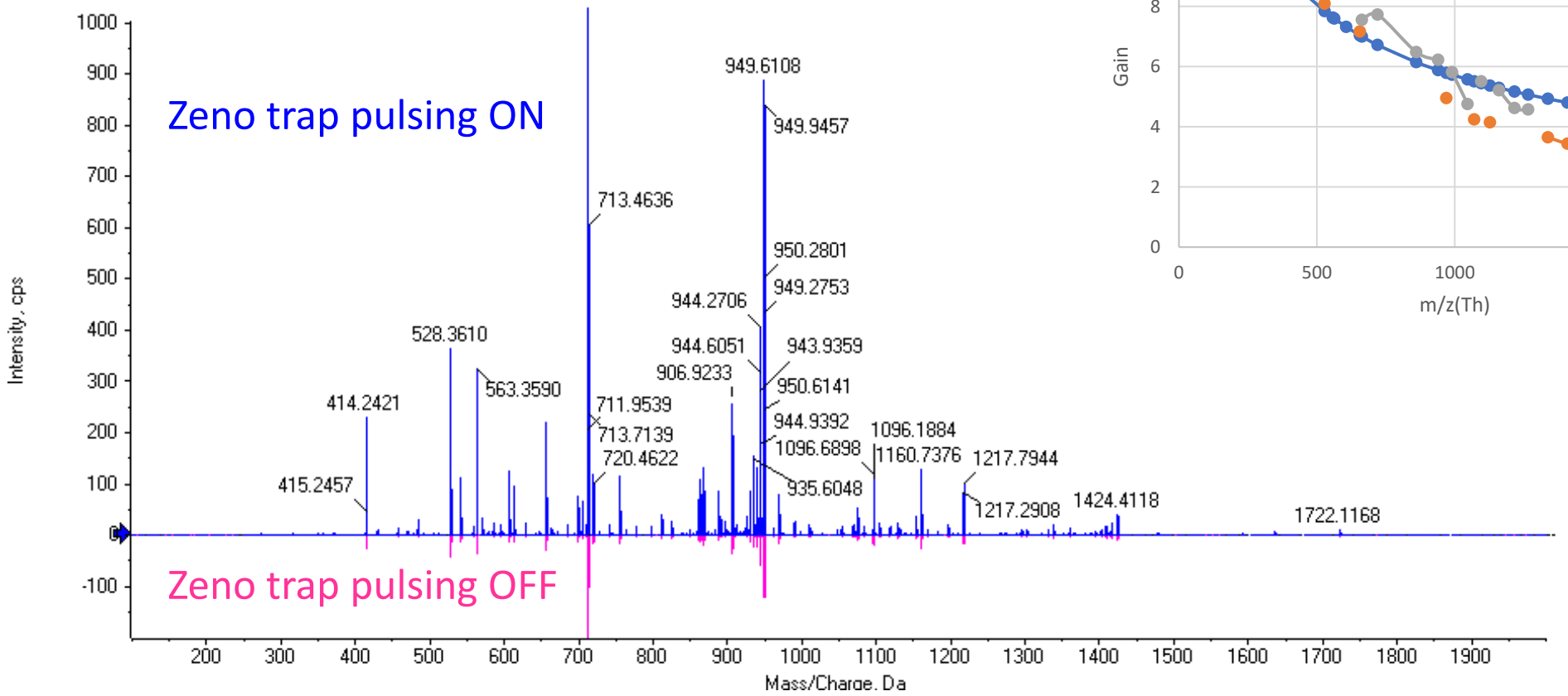
(3) Acceleration of the focused ions

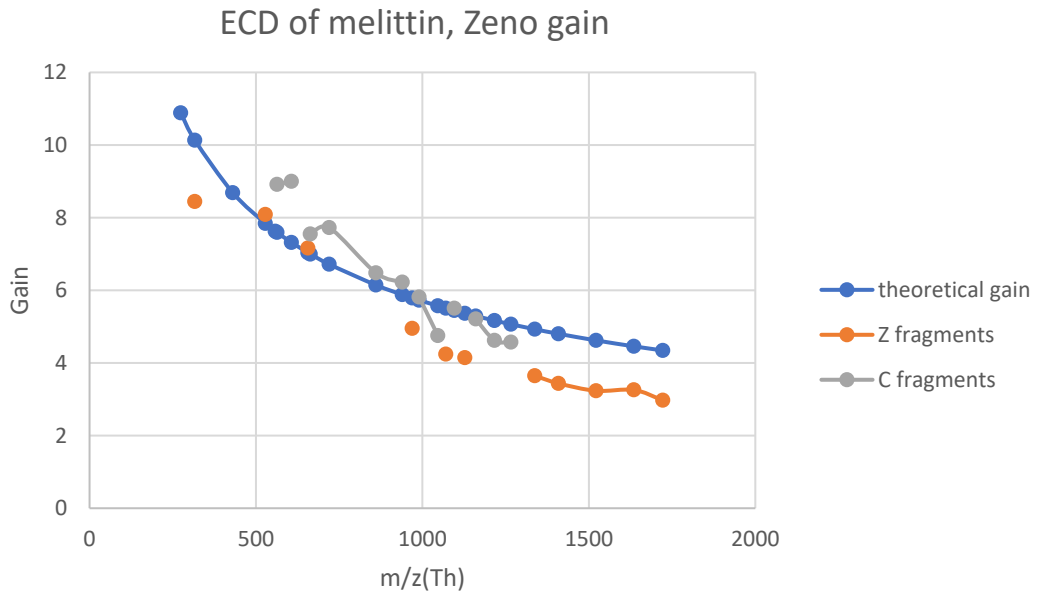
SCIEX
The Power of Precision

Enhancement of ion transmission 4-10x

ref. P. Ryumin et al. ASMS 2018

ECD on melittin (medium size peptide)





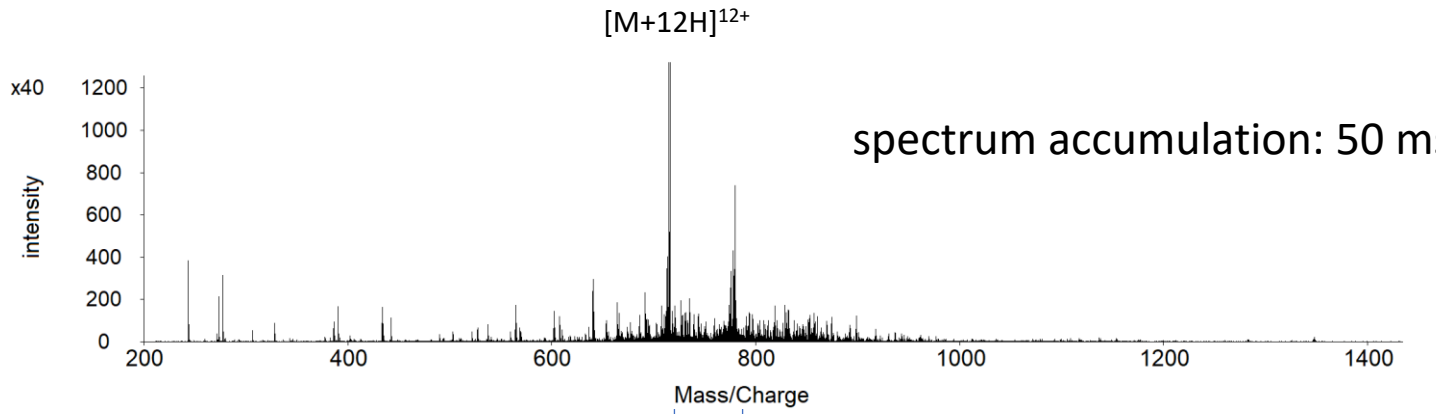
SCIEX
The Power of Precision

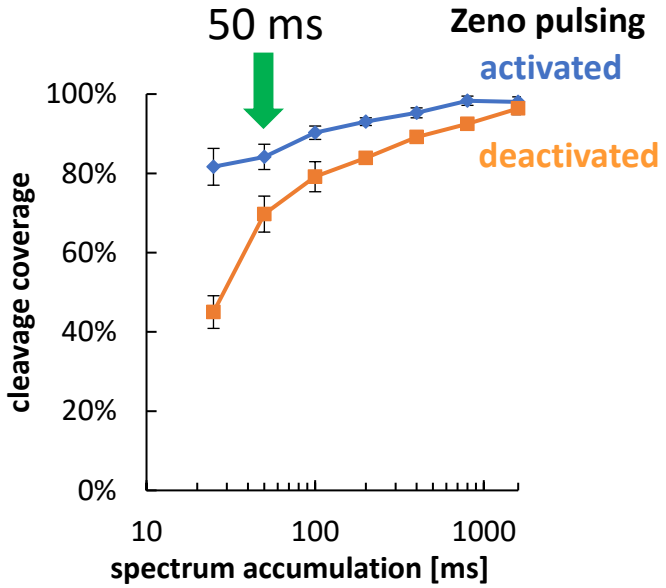
ref. T. Baba et al. JASMS 2021

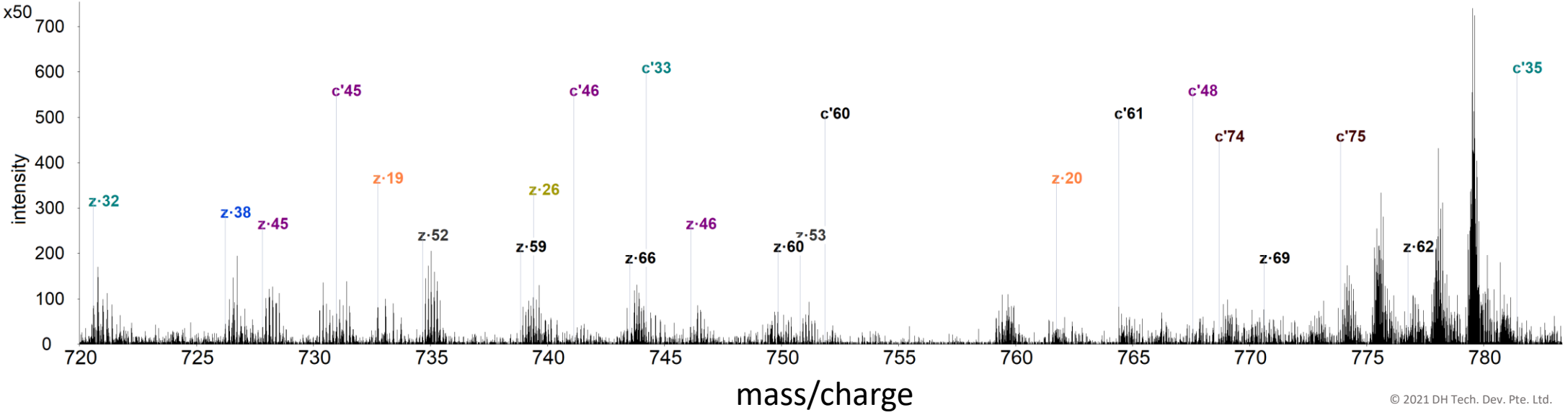
EAD and Zeno trap pulsing are combined. Ubiquitin 12+

Spectrum accumulation: 50 ms → cleavage coverage = 84%

Top-down LC-MSMS of small proteins is possible. **MOB pm 03:30**







SCIEX The Power of Precision

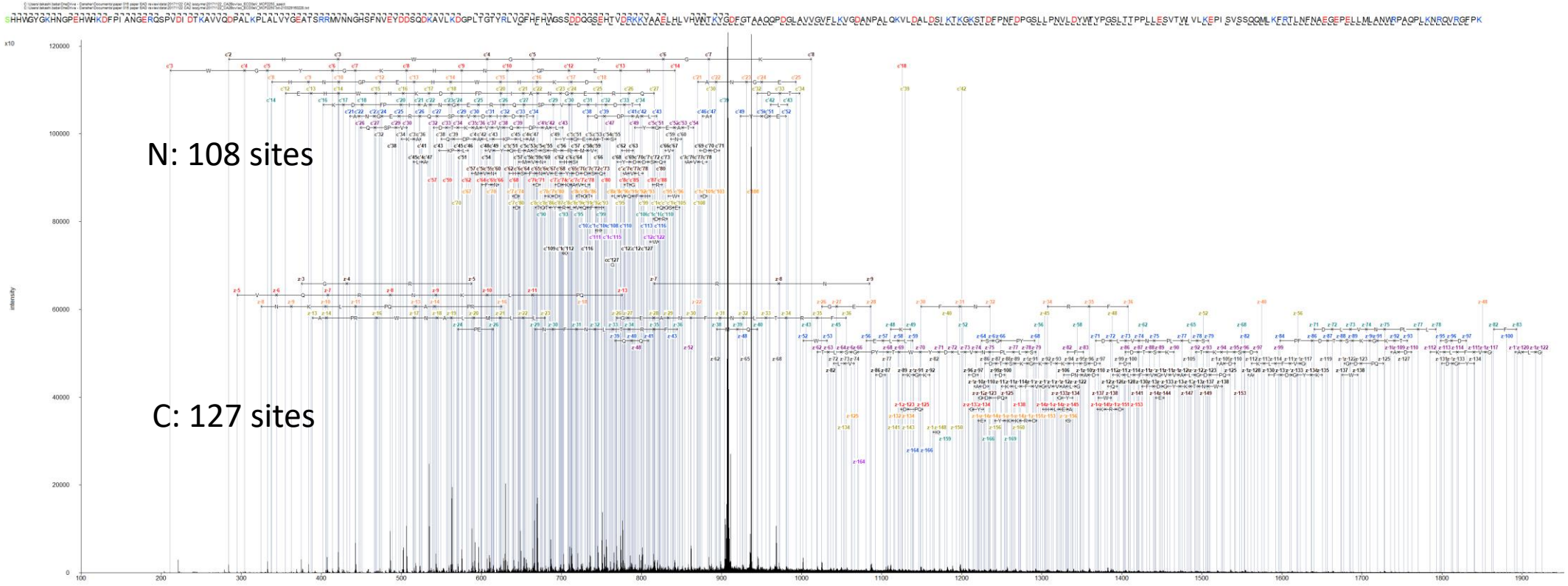
Top-down sequencing of medium size protein carbonic anhydrase 2 (CA2)

ref. T. Baba et al. JASMS 2021

Accumulation for 4 min → Cleavage coverage: 90.2%

c', z^dot

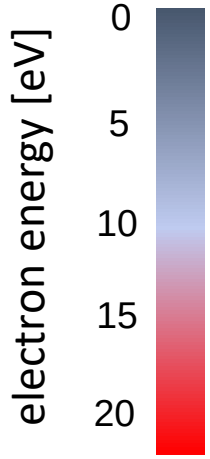
SHHWGYGKHN GPEHWHKDFP IANGERQSPV DIDTKAVVQD PALKPLALVY
GEATSRRMVN NGHSFNVEYD DSQDKAVLKD GPLTGTYRLV QFHFHWGSSD
DQGSEHTVDR KKYAAELHLV HWNTKYGDFG TAAQQPDGLA VVGVFLKVGD
ANPALQKVLD ALDSIKTKGK STDFPNFDPG SLLPNVLDYW TYPGSLTTPP
LLESVTWIVL KEPISVSSQQ MLKFRTLNFN AEGEPELLML ANWRPAQPLK
NRQVRGFPK



SCIEX
The Power of Precision

Electron Activated Dissociation (EAD) family

positive precursors



electron capture dissociation (ECD) → **proteomics**

hot electron capture dissociation (hot ECD) → **glycoproteomics**
Leu / Ile, disulfides

electron impact excitation of ions from organics (EIEIO) → **structural lipidomics**

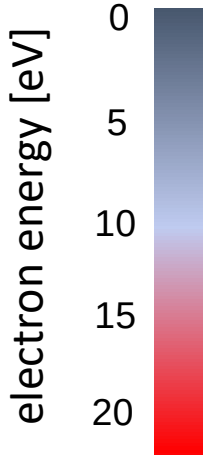
electron ionization dissociation (EID)

electron induced dissociation (EID)

bottom up
middle down
top down

5 other orals and 16 posters in this ASMS.

negative precursors



negative ion ECD (niECD)

electron detachment dissociation (EDD)

ASMS 2020

electron induced dissociation (EID) →

This presentation

Top-down & de novo sequencing

structural identification of acidic phospholipids

SCIEX
The Power of Precision

difficulty of top-down & *de novo* sequencing

many possibility of isomeric aa combinations

N=GG, 114.042928	ES=DT, 216.074623
Q=AG, 128.058578	PQ=AGP, 225.111342
GT=AS, 158.069143	QV=LN=GGL=AGV, 227.126992
GL=AV, 170.105528	NN=GGN=GGGG, 228.085856
GN=GGG, 171.064392	EV=DL, 228.111008
GQ=AN=AGG, 185.080042	DN=DGG, 229.069872
EG=AD, 186.064058	QT=AGT=AAS, 229.106257
AQ=AAG, 199.095692	AY=FS, 234.100443
LS=TV, 200.116093	LQ=AGL=AAV, 241.142642
NS=GGS, 201.074957	NQ=GGQ=AGN=AGGG, 242.101506
NP=GGP, 211.095692	KN=GGK, 242.137891
NV=GGV=AAA, 213.111342	EN=DQ=EGG=ADG, 243.085522
QS=NT=GGT=AGS, 215.090607	……

One miss cleavage can increase sequence uncertainty
→
ECD is a less AA preferential dissociation than CID.

ECD vs. CID:
one cleavage by an electron
→ less internal fragmentation than CID.

Still need to avoid over electron irradiation
to reduce further dissociation of fragments.

ECD vs. hot ECD:
ECD produces less a• and y contamination

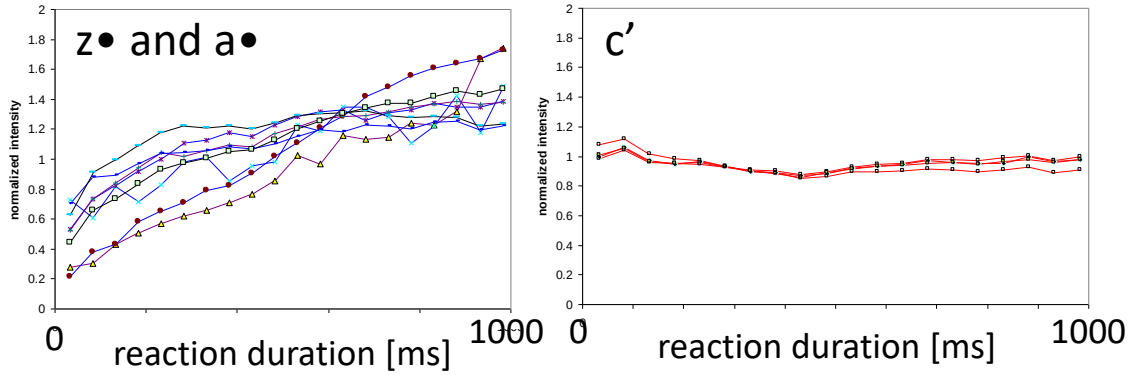
z• separation from c’ for confident sequencing

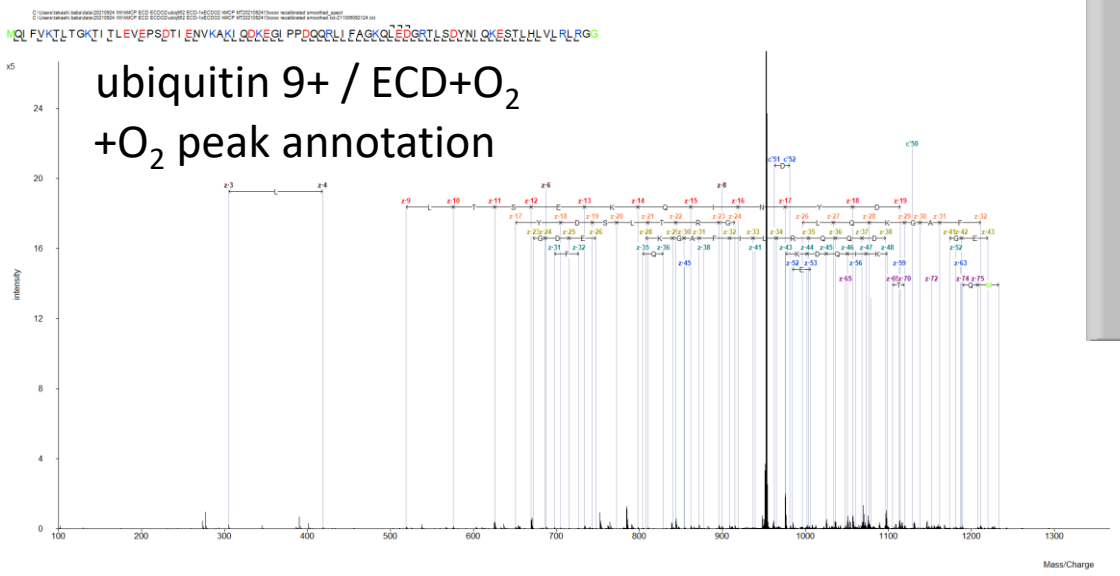
ref. Y. Xia et al. JACS2006
T. Baba et al. ASMS2009

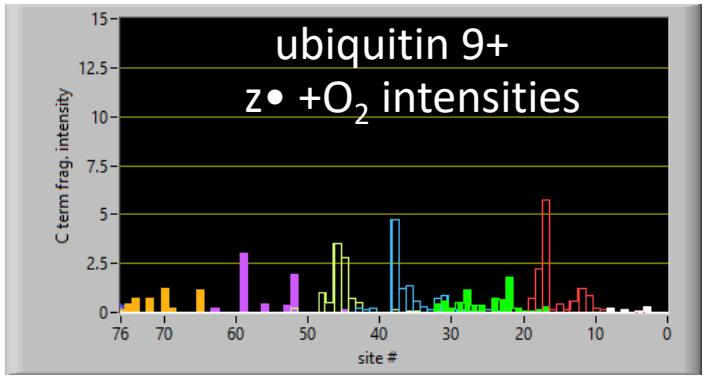
z• fragments can react with O_2
ref. Y. Xia et al. JACS2006
ref. T. Baba et al. ASMS2009
exception: Lys (K). No z• + O_2, but z-H

→ C terminal fragments (z•) can be separated from the total spectrum

Previous works: peptides
This work: +O_2 events appeared across the entire protein backbone.







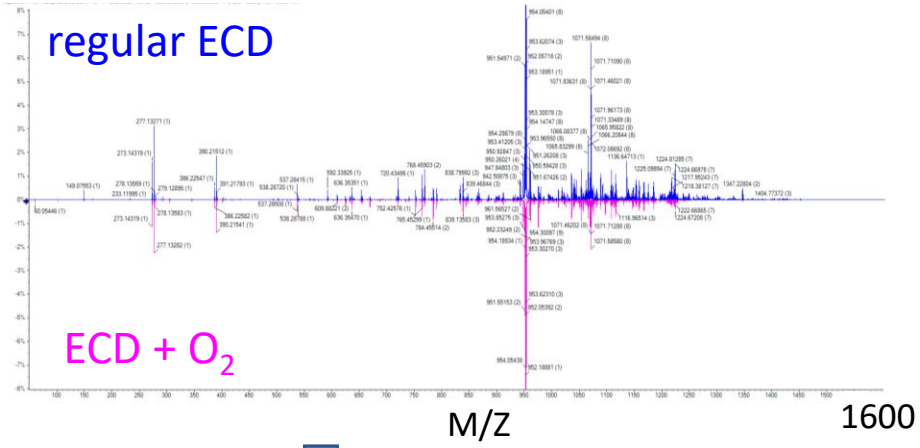
N terminus C terminus
coloring: fragment charge states

de novo sequencing of ubiquitin 9+

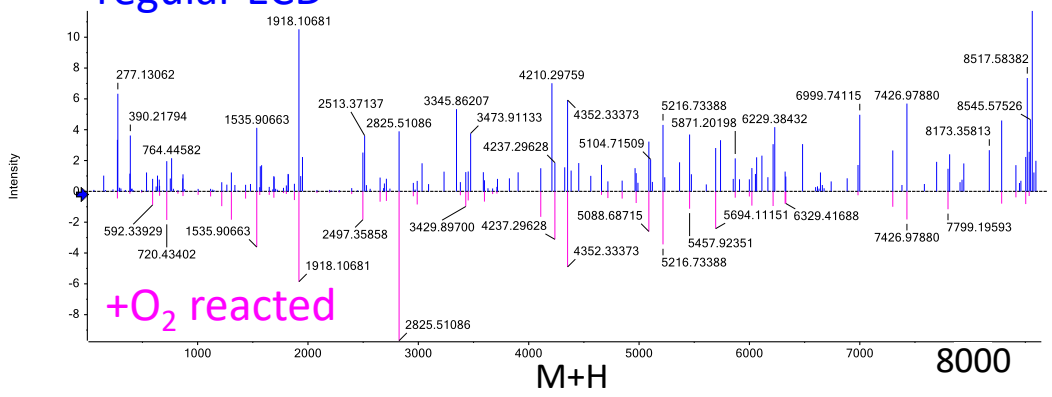
ref. T. Baba et al. ASMS2009

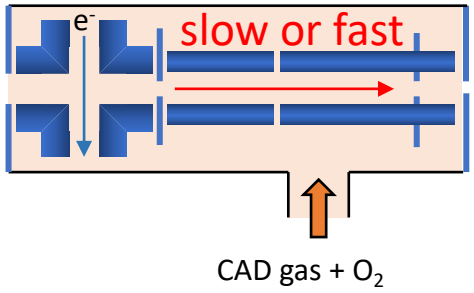
Regular operation: Q2Linac bias >> Q2 ROD bias

$+O_2$ operation: Q2Linac bias = Q2 ROD bias



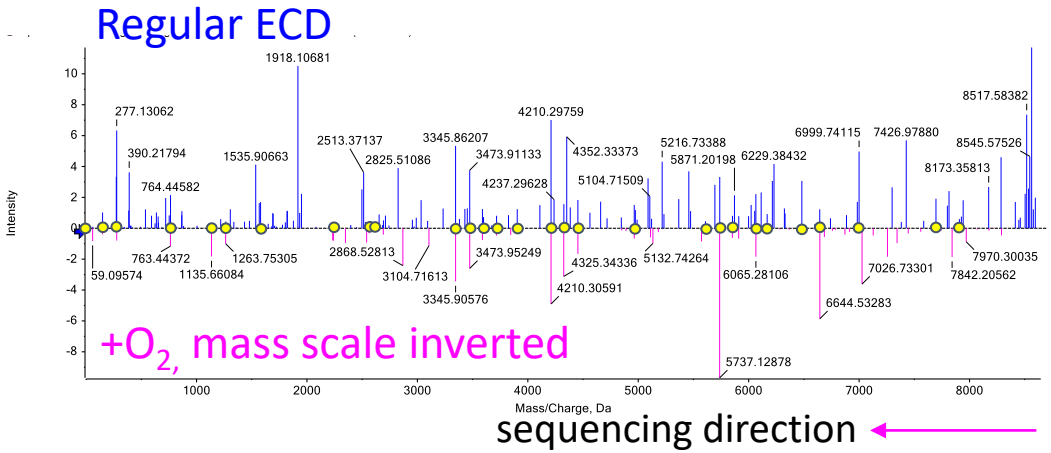
single charge conversion &
mono isotopic selection





(1) de novo sequencing between two golden pairs

sequencing direction



(2) de novo sequencing between two O_2 events

*Additional purification was applied: removing hot ECD contamination: a• and y by [a• = c'-CH_2NO and y=z• -NH_2] and z+O2 and a+O2

SCIEX The Power of Precision

ubiquitin(UBB): *de novo* sequencing result

M=Q=L-F-V-K=T-L=TG=K-LT=T-L=E=VEPS-D-T-L-E-NVK-A-K-L=Q=D=K=E-G-LPP=
D=Q=Q-R-LL=F=AG-K-QL-E=D=G=R=T-LS-D-Y-NL-QK-E-S-TL-H=LV=LR-L-RG-G

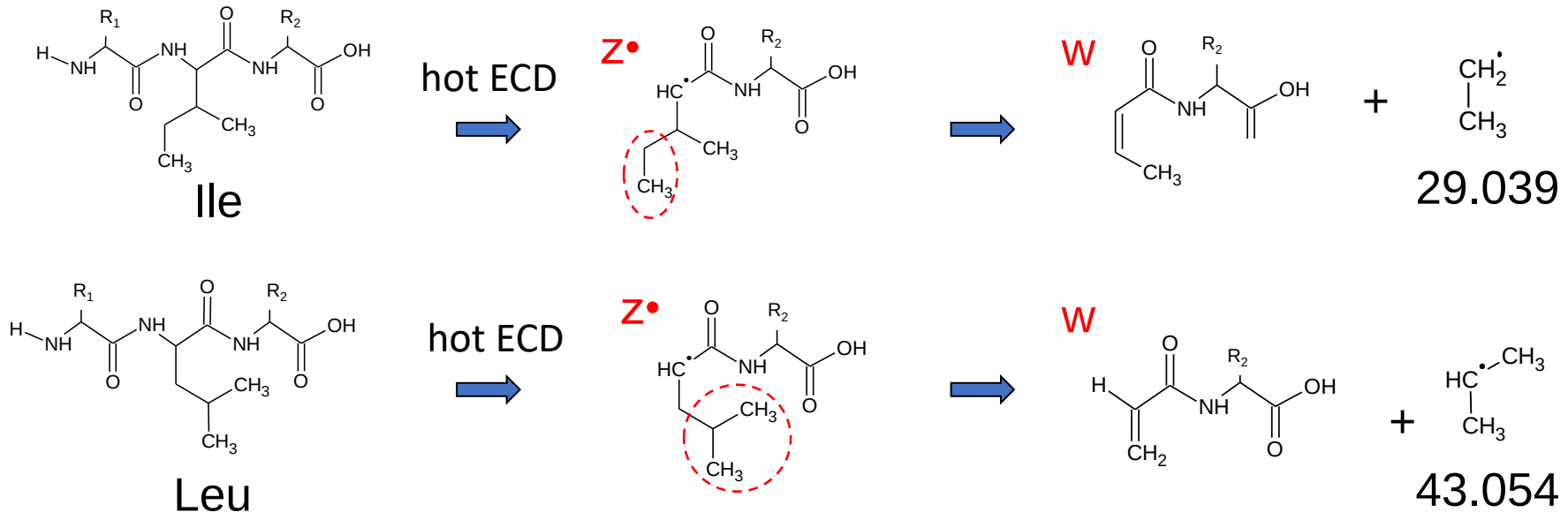
green - : Golden pairs are used, sequencing from the N terminus
red - : z• candidates are used, sequencing from the C terminus
violet = : both green & blue

Red AA: unique & correct answer. (except L/I)
Black AA: correct answer but not unique
= permutable, isomeric (e.g. Q=AG)

Can be solved by conventional top-down sequencing

Distinguishing Leu / Ile by secondary dissociation in hot ECD (15eV)

ref. F. Kjeldsen et al. Chem. Phys. Lett 2002
K. Rajabi et al. ASMS2020



hot ECD (15eV) applied to ubiquitin 9+

	z•75	z•70	z•65	z•63	z•55	z•48	z•42	z•35	z•34	z•28	z•22	z•17	z•11	z•9	z•7	z•5
standard aa	**Ile**	**Leu**	**Ile**	**Ile**	**Ile**	**Ile**	**Ile**	**Leu**	**Ile**	**Leu**	**Leu**	**Ile**	**Leu**	**Leu**	**Leu**	**Leu**
z• -Leu intensity	5.7	0.0	3.2	7.4	0.0	4.1	9.9	5.5	0.0	4.3	5.0	1.5	1.5	0.6	0.4	1.8
z• -Ile intensity	0.0	0.0	0.0	3.4	2.3	1.7	4.4	4.1	4.6	7.1	4.7	1.4	0.2	0.0	0.0	0.0
Hot ECD told	Leu		Leu	Leu	Ile	Leu	Leu	Leu	Ile	Ile	Leu	Leu	Leu	Leu	Leu	Leu

Low success rate when fragment charge is large.
Difficult to find w ions from overlapped other products.
opportunity for improvement: higher mass resolution should solve this problem.

SCIEX The Power of Precision

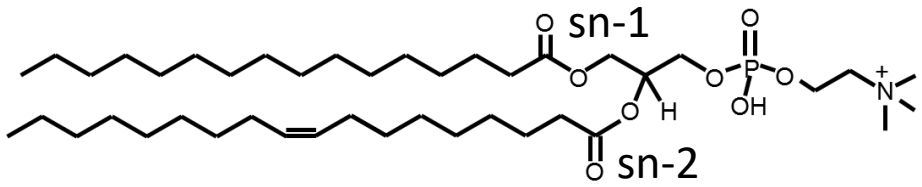
EID on positive phospholipid: complete structurral analysis

ThOE am 09:50, very soon

ref. L. Campbell et al. Anal. Chem. 2015
T. Baba et al. JLR 2018, etc.
T. Baba et al. ASMS 2016, oral

Electron beam energy 10 eV to avoid neutralization by electron capture

Zeno trap pulsing ON → spectrum accumulation of 0.1 s



head group = PC

backbone = glycerol

~~sphingosine~~

regioisomer diagnostic peak

sn-1/sn-2 = 16:0/18:1

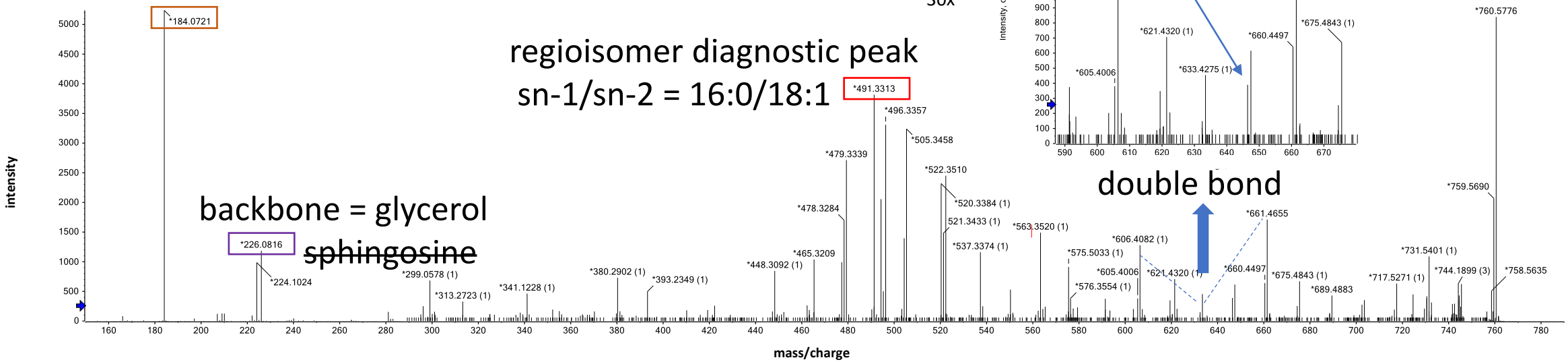
double bond

cis/~~trans~~

[M+H]+

30x

30x



EID (EIEIO) worked on all types of complex lipids, but inefficient ionization of acidic lipids was the problem.

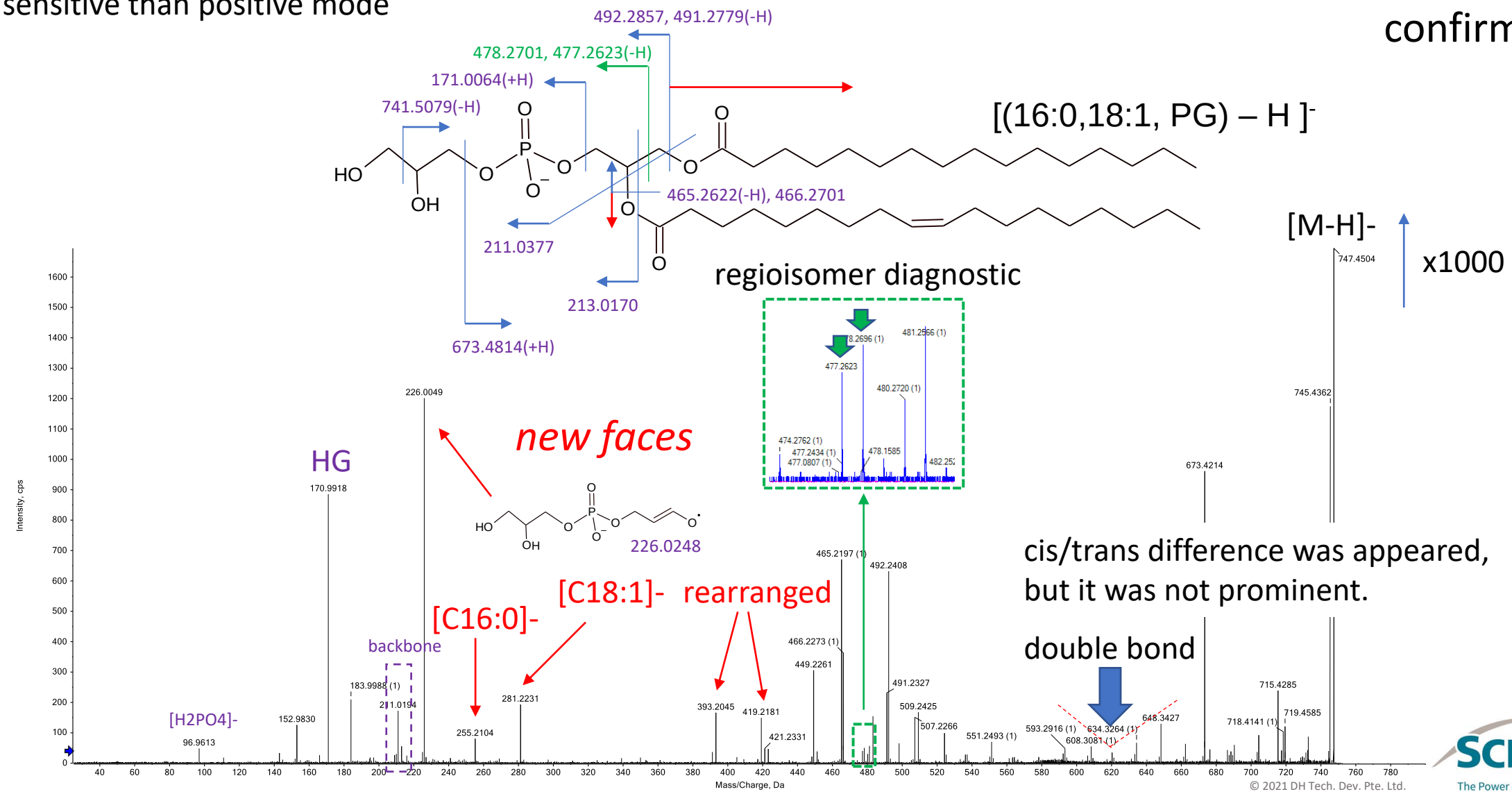
SCIEX
The Power of Precision

Negative EID (35eV) on acidic phospholipids

Main process is electron detachment loss (neutralization)

30x less sensitive than positive mode

PI, PA, PG, PS: confirmed.



SCIEX The Power of Precision

Summary

(1) High sensitivity of Zeno trap enabled- TOF MS is ideal for top-down sequencing, and other molecular identification by low abundant products.

(2) Efficient electron activated dissociation allows various dissociation mode with high speed: ECD, hot ECD, EID, etc. including negatively charged species.

These features are available in ZenoTOF 7600 (Sciex)*

*Ke = 35eV and oxygen introduction are research features, not available in the product.

The latest publication (T. Baba et al. 2021, JASMS)
doi.org/10.1021/jasms.0c00425

SCIEX
The Power of Precision

Trademarks / Licensing

The SCIEX clinical diagnostic portfolio is For In Vitro Diagnostic Use. Rx Only. Product(s) not available in all countries. For information on availability, please contact your local sales representative or refer to https://sciex.com/diagnostics. All other products are For Research Use Only. Not for use in Diagnostic Procedures.

RUO-MKT-11-13994-A