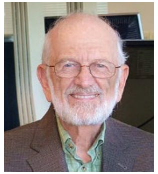

The 70th ASMS Conference, Minneapolis, MN

Extremely Low Ion Counting Capabilities with Orbitrap FTMS

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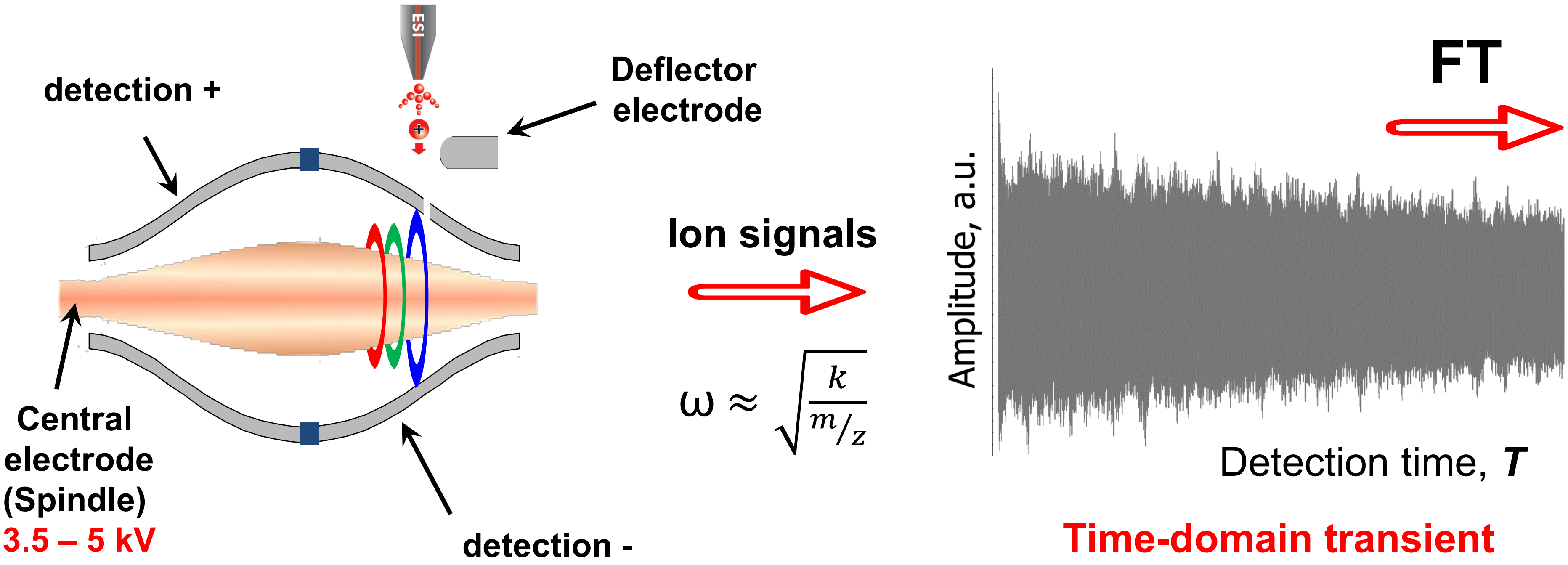
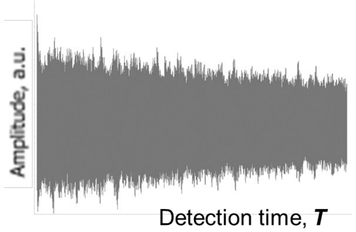
Thursday, June 9, 2022: ThOE am
Fundamentals: Unconventional Approaches in MS (Honoring R. Graham Cooks)

Ion Detection in Orbitrap FTMS

- Ion identity (*m/z*) is encoded as a frequency of ion oscillations in an ion trap
- Frequencies of ion oscillations are measured as time-domain signals (**transients**)
- Fourier transform (FT) decodes transients to reveal frequencies (*m/z*) values



Properties of Time-Domain Transients



$R \sim T$

- Resolution increases linearly with transient length, *T*
  - Longer transients provide higher resolution

$S/N \sim z$

- Sensitivity increases linearly with ion charge state, *z*
  - Multiply charged ions provide more abundant peaks in mass spectra

$S/N \sim \sqrt{T}$

- Sensitivity increases as a square root of transient length, *T*
  - Longer transients provide more abundant peaks in mass spectra

$S/N \sim \sqrt{N}$

- Sensitivity increases as a sqrt of a number *N* of averaged transients
  - Scan averaging (microscans) provide better quality mass spectra

Time-domain transients represent the truly unreduced (full) data

Consequences of Time-Domain Transients Properties

- Charge detection mass spectrometry (CDMS): single transient analysis
  - Individual multiply (highly) charged ions provide sufficiently strong ion signals
  - Increased length transients allow to detect even low charge state single ions

    Exploris: 4+ peptide ions, SNR = 3.5 with 1 s transients *Makarov et al., IJMS, 2021, 116607*
  - That enables Individual Ion Counting, I2MS, and related CDMS methods (e.g. DMT)

    Williams, Makarov, Kelleher, Heck, Chait, …

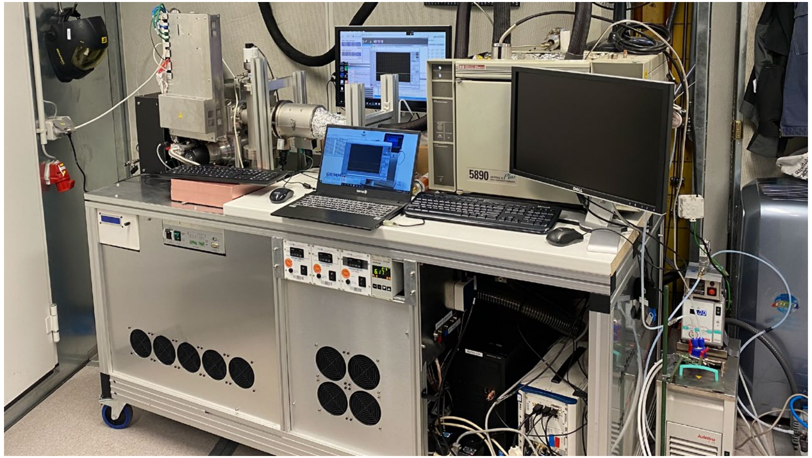
- Many applications benefit from averaging (many) transients and/or spectra
  - Complex mixture analysis, e.g., petroleomics *Rogers, Marshall, …*
  - Top-down mass spectrometry for protein analysis, including mAbs
  - Intact mass measurements of large proteins, viruses

Conventionally, 100 – 1 000 transients are averaged

Unconventionally, what can be achieved when more transients are averaged?!

Low Ion Counting in MS Applications

Rare singly (or multiply) charged ions can enter a mass spectrometer once every 10-100 seconds as isolated species or in a matrix of other, vastly more abundant, ions.



Ship diesel engine analysis, TP172

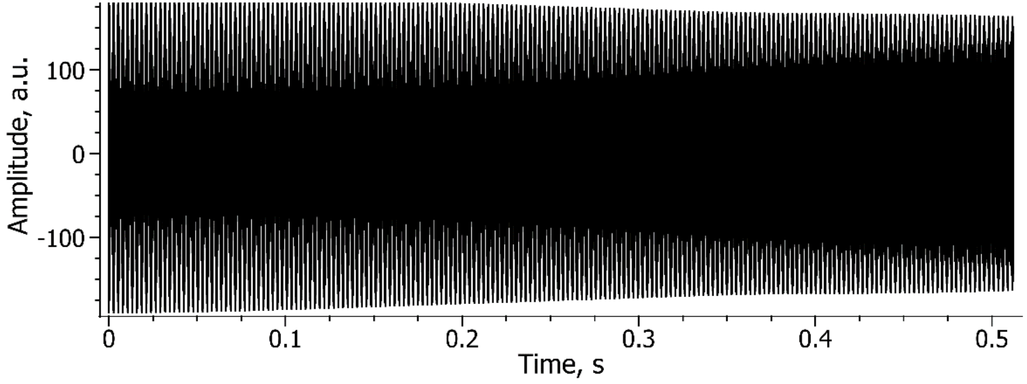
- Aerosol chemistry research
- Ambient air quality monitoring
- Complex mixture analysis
- Isotopic ratio analysis of elements
- Breath analysis

- Orbitraps offer high and ultra-high resolution capabilities
- Certain measurements can be done over an extended time – long data averaging

How low ion counting capabilities can Orbitraps offer?

Fullerene (C_{60}) – The Reference Molecule

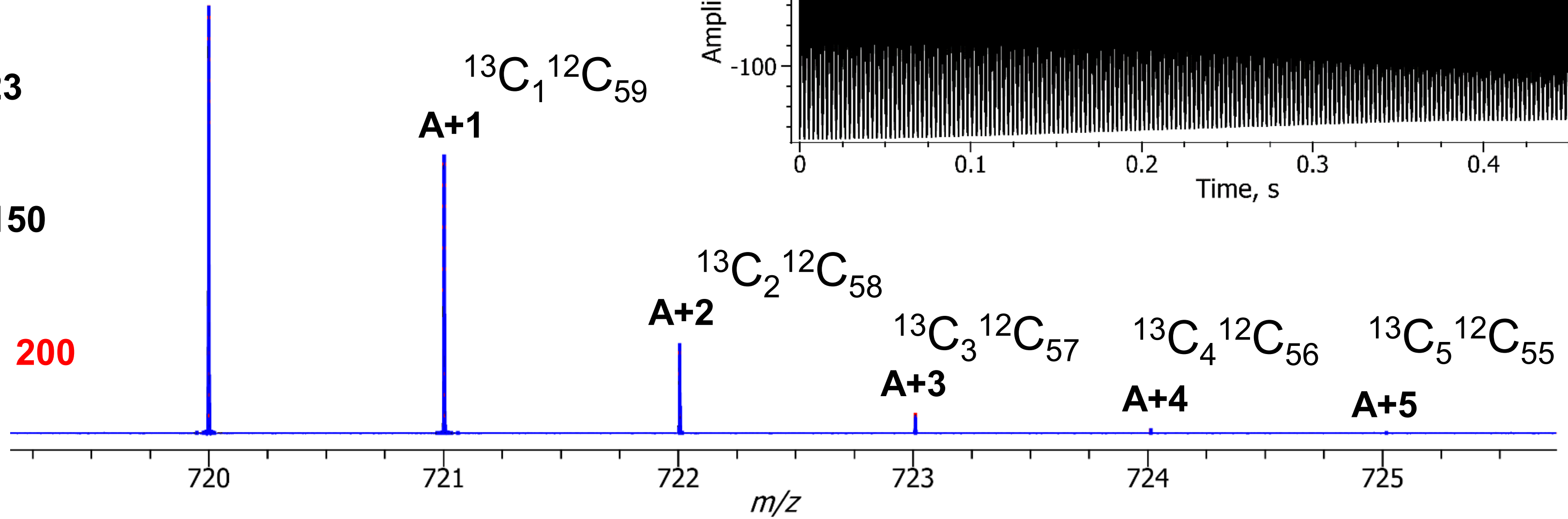
In-silico analysis of $\mathbf{C_{60}}$ ($M^{+\cdot}$)
Q Exactive HF Orbitrap
Tacq = 512 ms



$\frac{A}{A+3}$ = **23**

$\frac{A}{A+4}$ = **150**

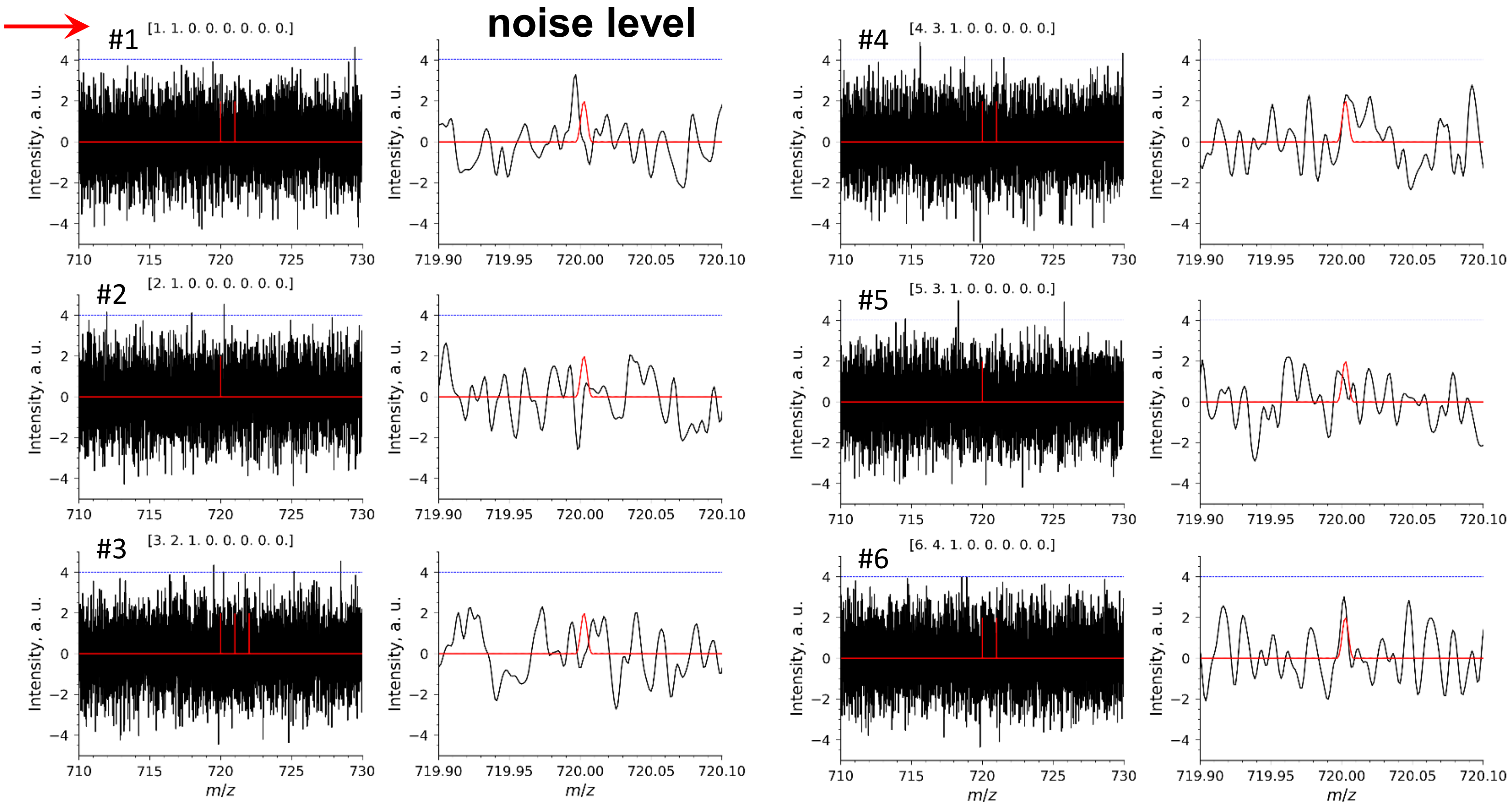
$\frac{A}{A+5}$ = **1 200**



	A	A+1	A+2	A+3	A+4	A+5
m/z	719.9994	721.00278	722.00614	723.00948	724.01289	725.01621
Abundance rel.	1.00000	0.65060	0.20765	0.04335	0.006682	0.00082

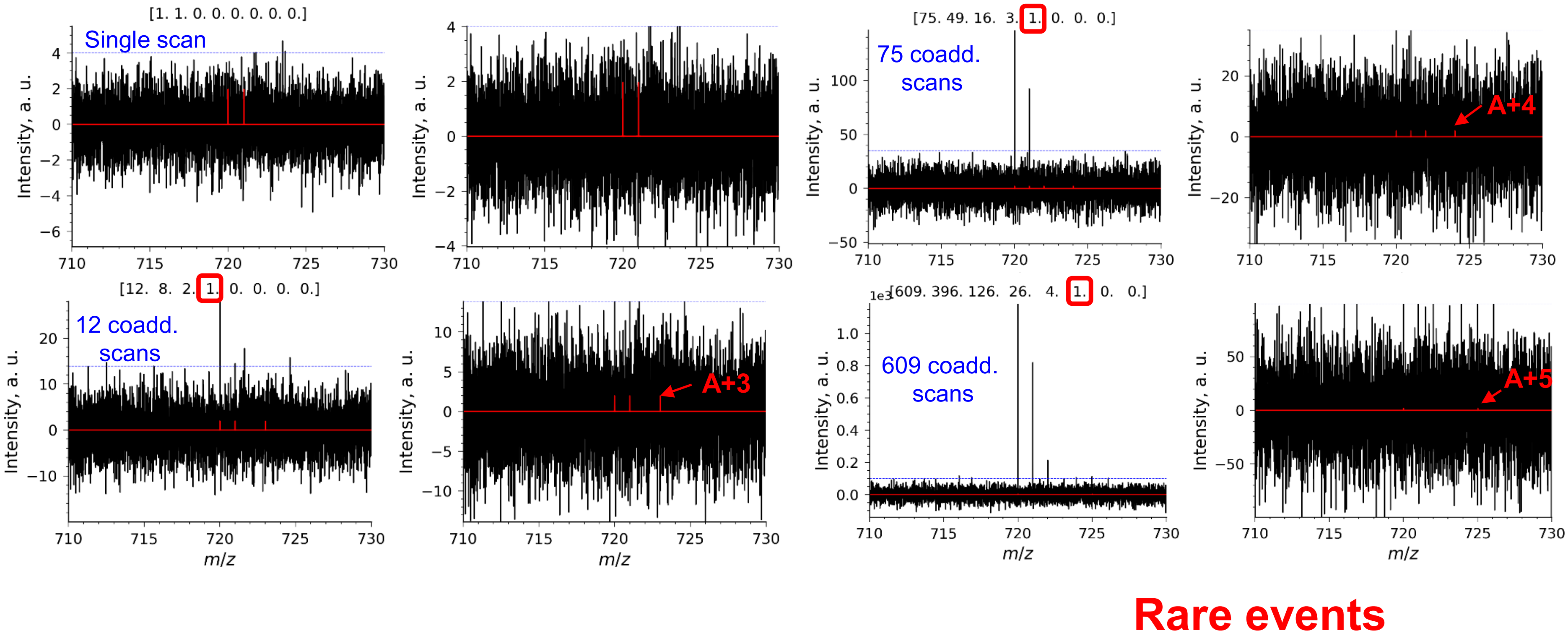
Fullerene (C_{60}) Low Ion Counting – Single Scan Data

	/ single scan
A events	1
A+1 events	1
A+2 events	0
A+3 events	0
A+4 events	0
A+5 events	0



Simulations: Q Exactive HF, C_{60}, Tacq = 512 ms, single scan

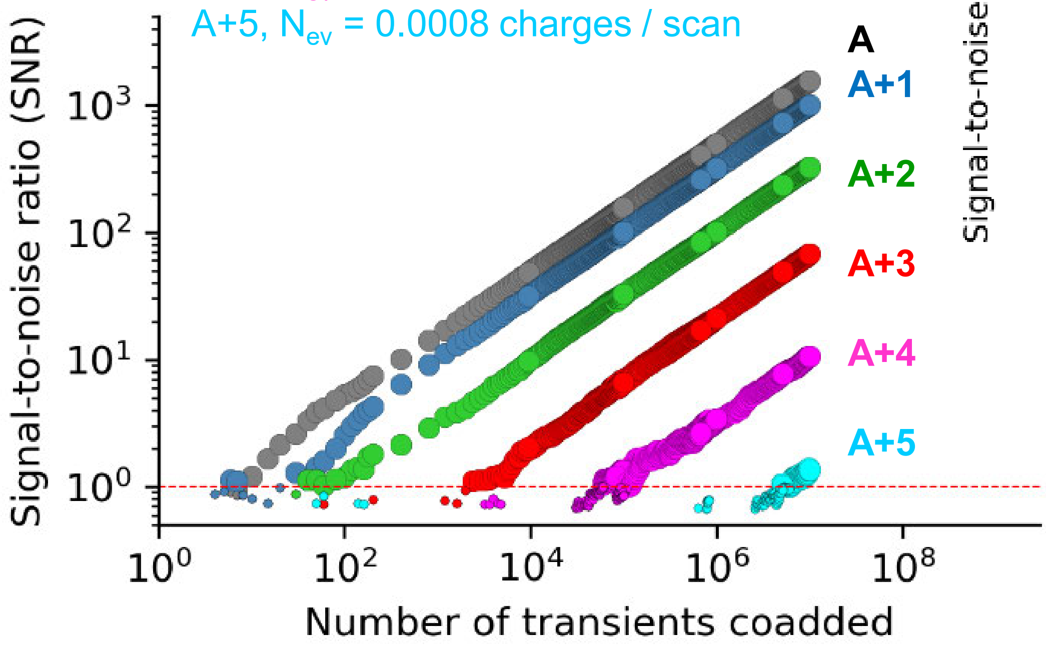
Fullerene (C_{60}) Analysis – Transient Summation

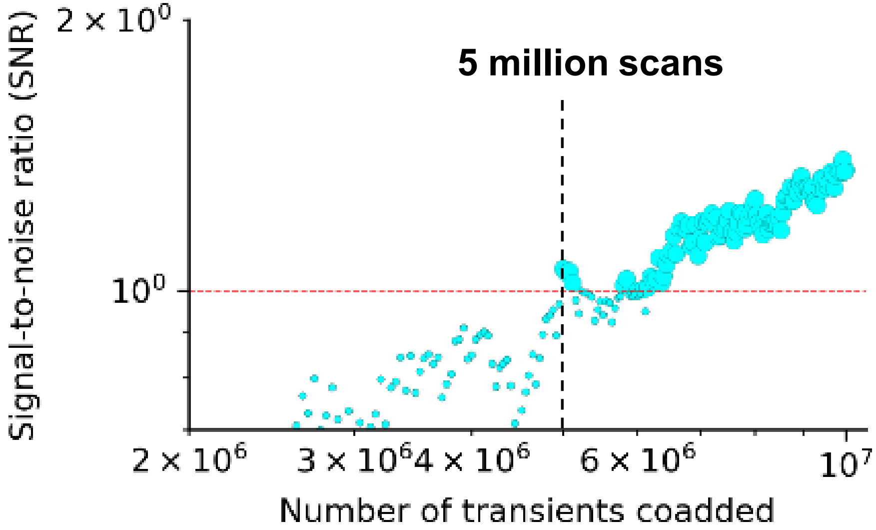


Simulations: Q Exactive HF, C_{60}, Tacq = 512 ms, multiple scans

Low Ion Counting – Transient Summation

A, N_{ev} = 1 charge / scan
A+1, N_{ev} = 0.650 charges / scan
A+2, N_{ev} = 0.208 charges / scan
A+3, N_{ev} = 0.043 charges / scan
A+4, N_{ev} = 0.006 charges / scan
A+5, N_{ev} = 0.0008 charges / scan





SNR = 1 = 6*σ = 2 charges
@ Tacq = 512 ms

SNR into Charges per Second Calibration

Detection period, s	SNR in 1 scan for z=+20	MSNR (Makarov SNR) for z=+1
0.192	1.78	0.089
0.384	2.57	0.1285
0.768	**3.7**	**0.185**
1.536	5.3	0.265
3.072	7.6	0.38

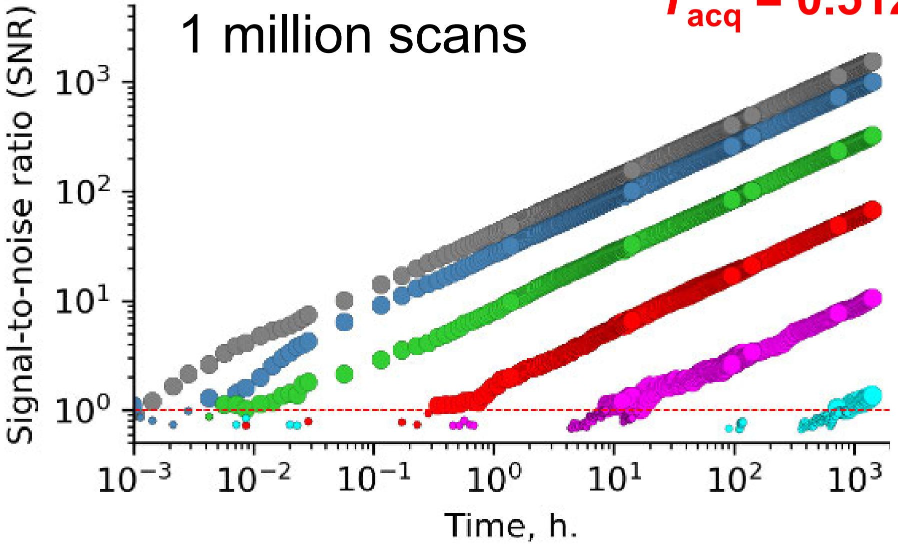
Denisov & Makarov, JASMS, 2009, 1486–1495

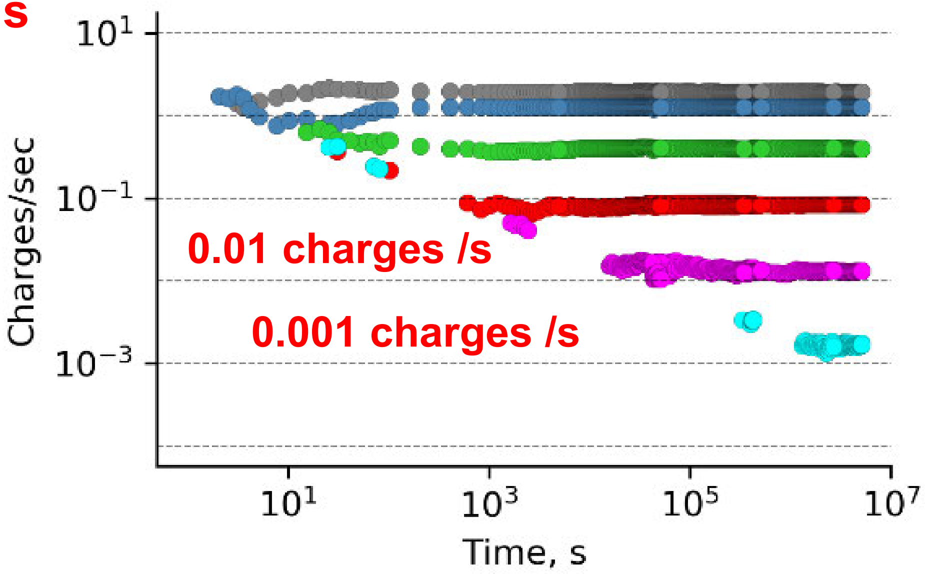
$$\boldsymbol{Charges/s} = \frac{\boldsymbol{SNR}}{\sqrt{\boldsymbol{N}_{\boldsymbol{scans}}} \cdot \boldsymbol{MSNR}} / \boldsymbol{T}_{\boldsymbol{acq}}$$

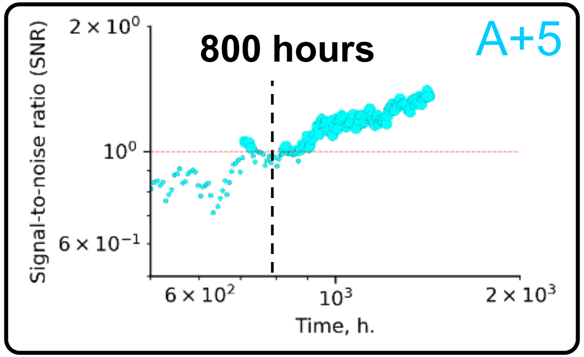
$\boldsymbol{N_{scans}}$ A necessary number of averaged scans to reach a given SNR

SNR Conversion into Time and Charges per Second

T_{acq} = 0.512 s







A, N_{ev} = 1 ch. / scan
A+1, N_{ev} = 0.650 ch. / scan
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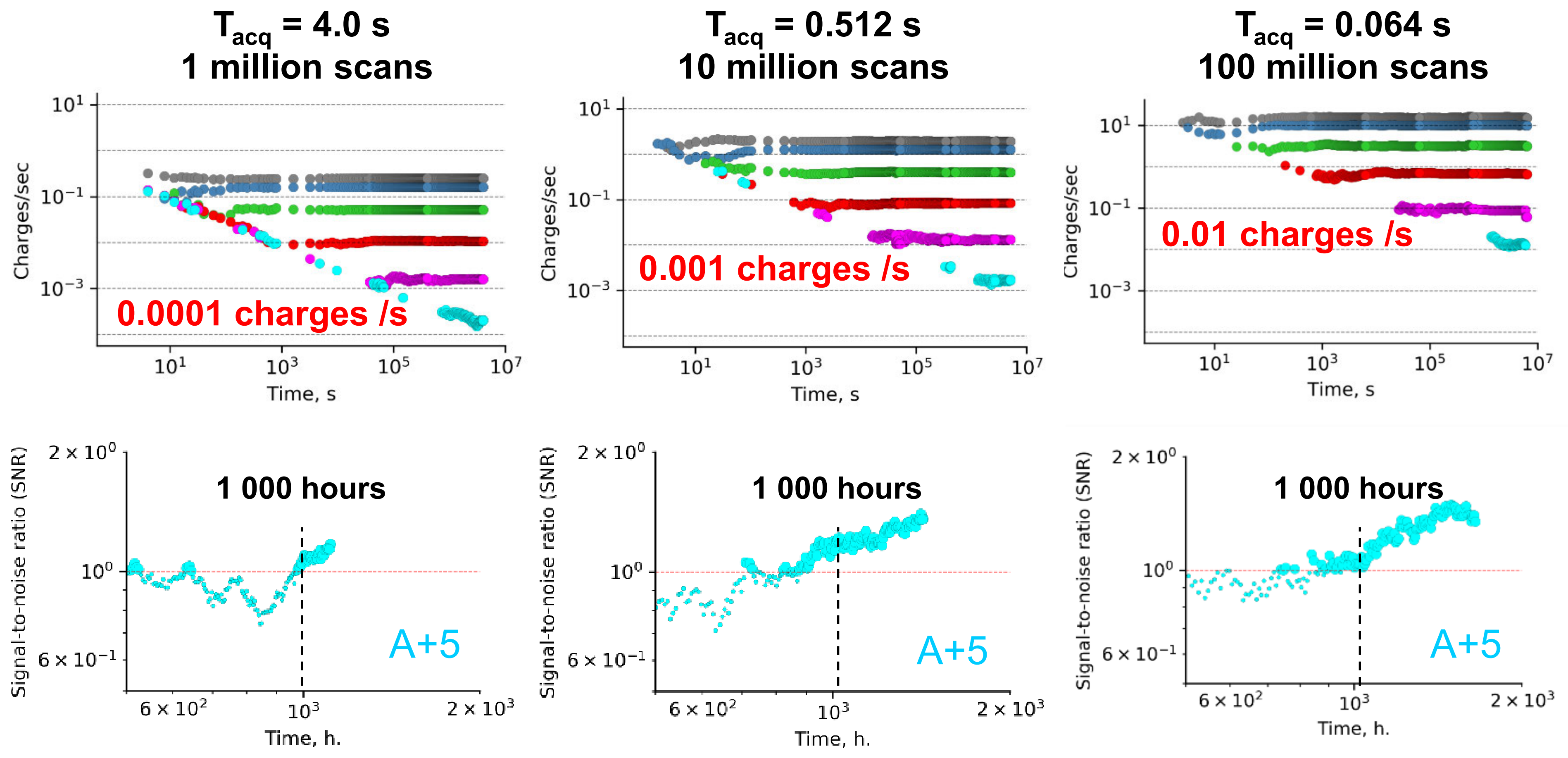
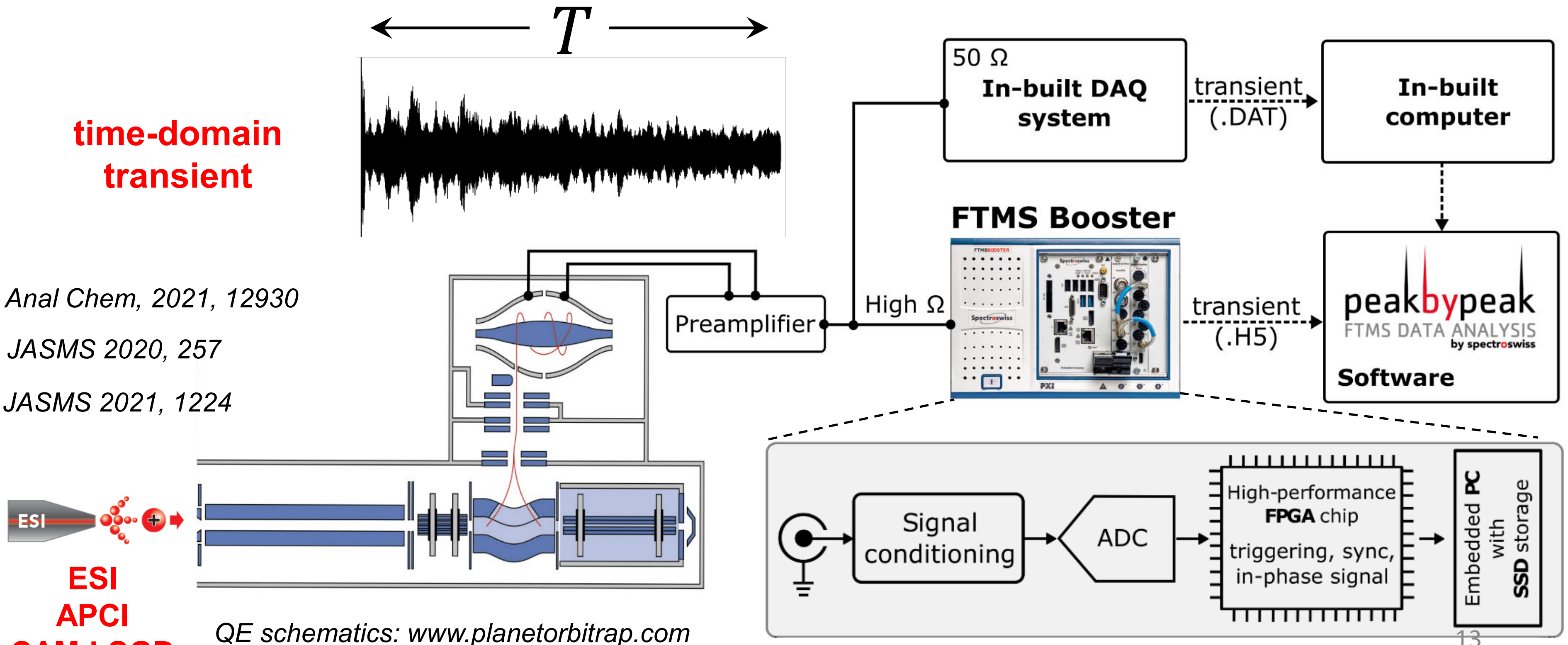
A+3, N_{ev} = 0.043 ch. / scan
A+4, N_{ev} = 0.006 ch. / scan
A+5, N_{ev} = 0.0008 ch. / scan

The Low Limit: How Many Charges / Second?



Experimental Set-Up

- Extended duration measurements performed on a Q Exactive HF Orbitrap FTMS
- Time-domain transients acquisition with an add-on DAQ system (FTMS Booster)



Anal Chem, 2021, 12930

JASMS 2020, 257

JASMS 2021, 1224

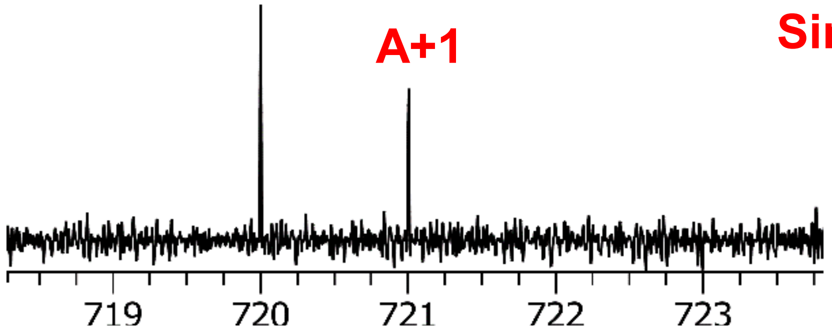
ESI
APCI
CAM LSGD

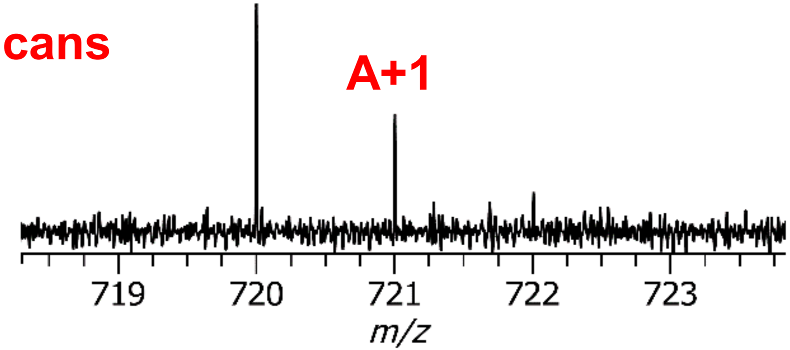
QE schematics: www.planetorbitrap.com

Experiment: C_{60} Analysis with a Q Exactive HF

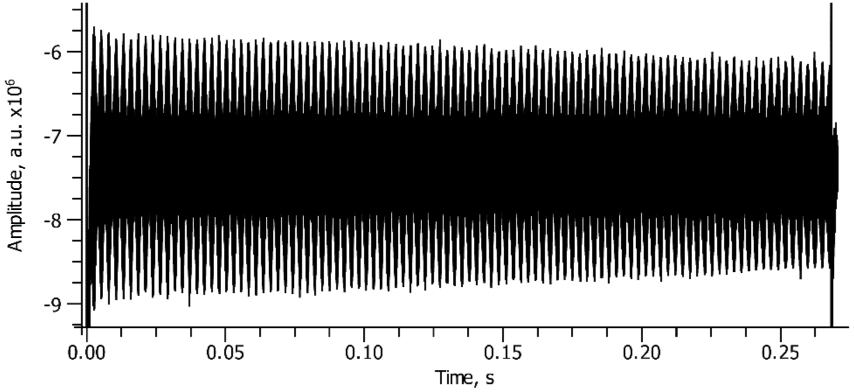
APCI, SIM = 50 *m/z*, R = 120k, T_{acq} = 256 ms, AGC = 2e5, IT_{max} = 1ms
total experimental time: 440 min

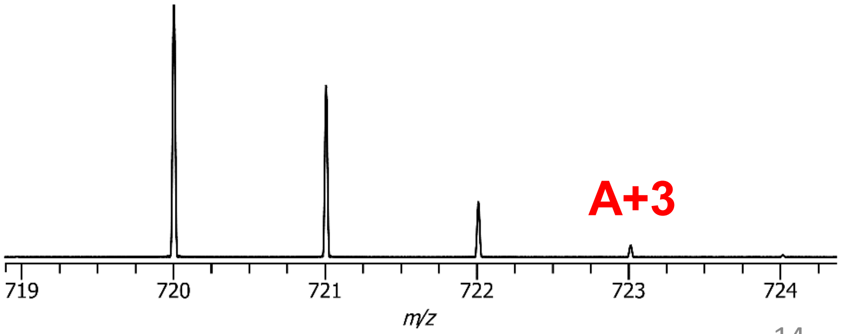
Single scans





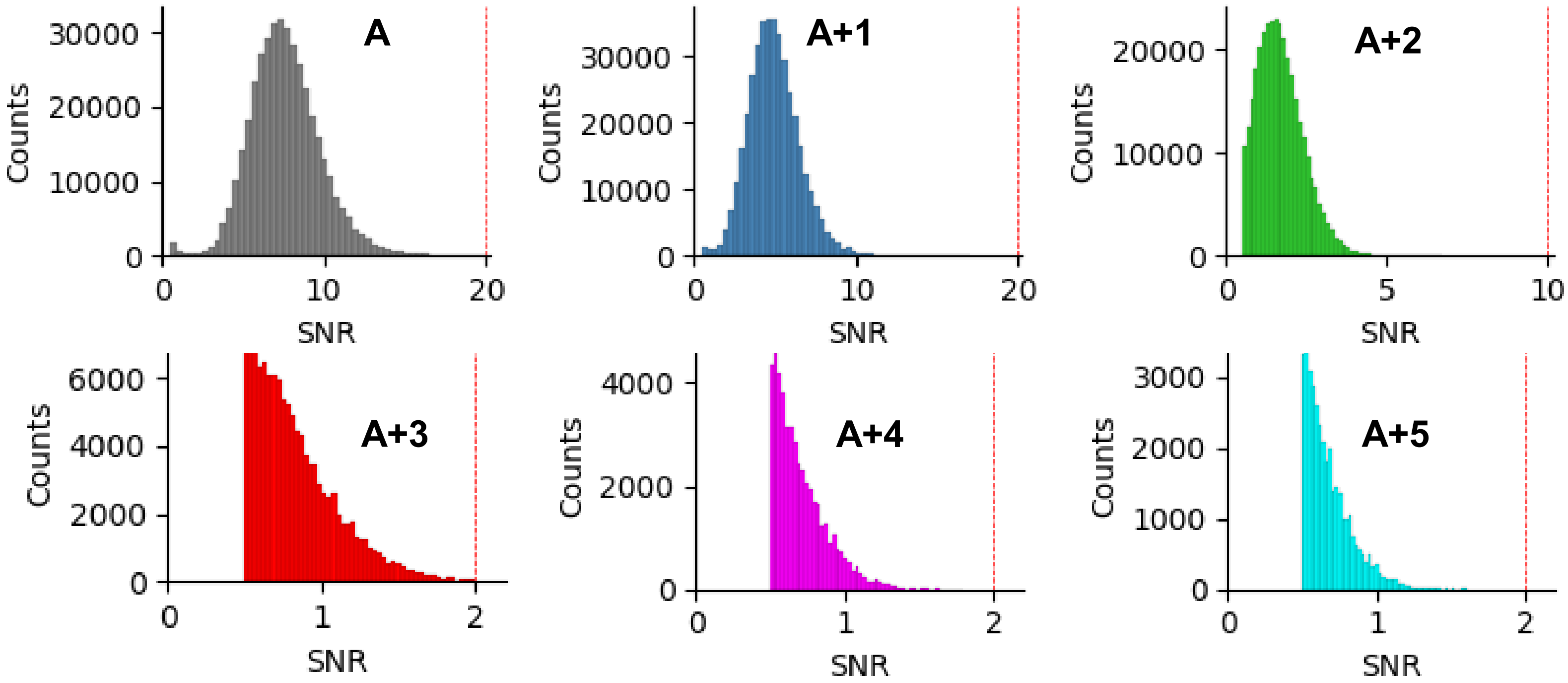
averaged, 100k scans



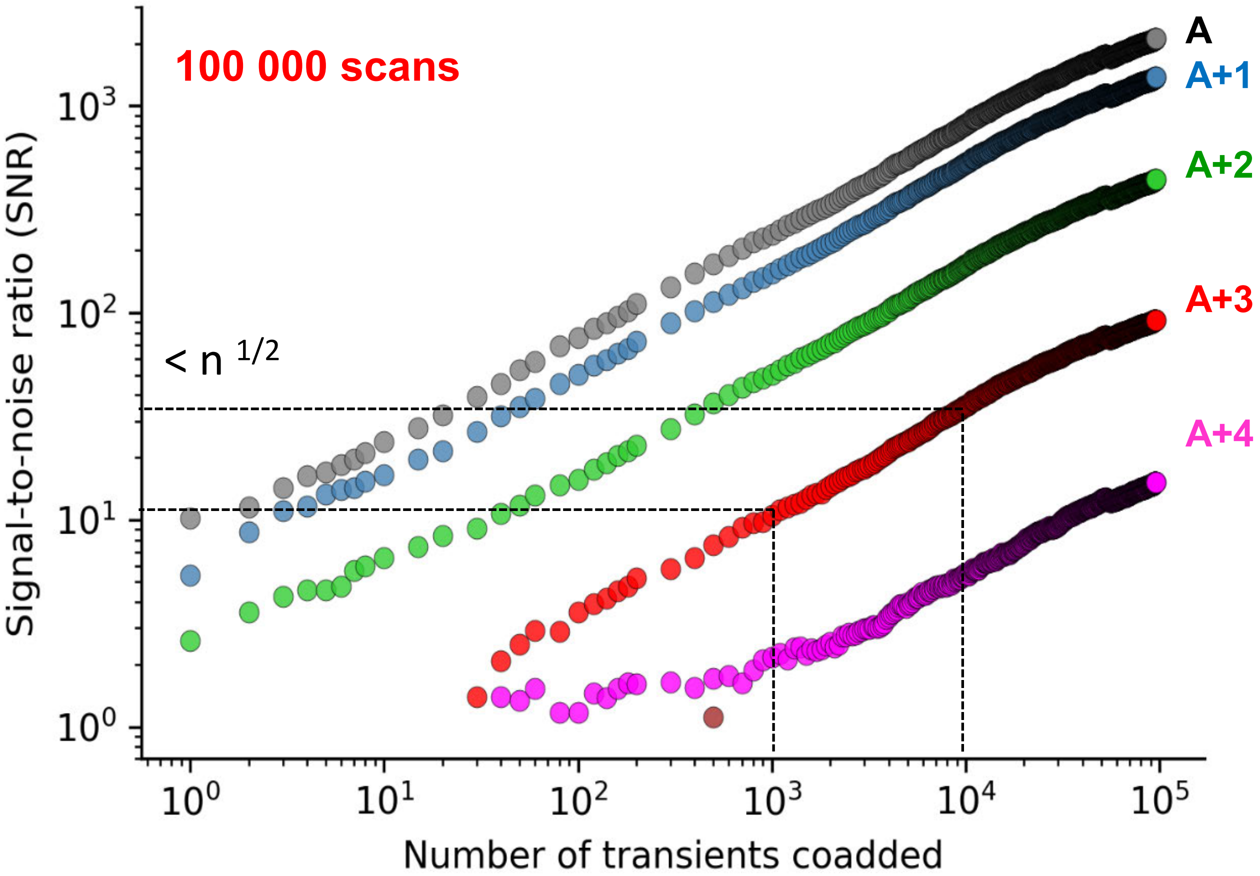


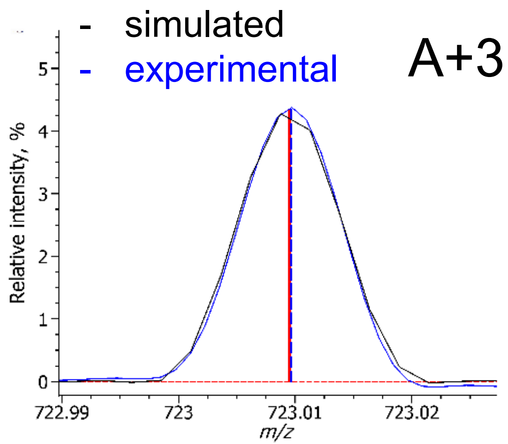
Ion Statistics

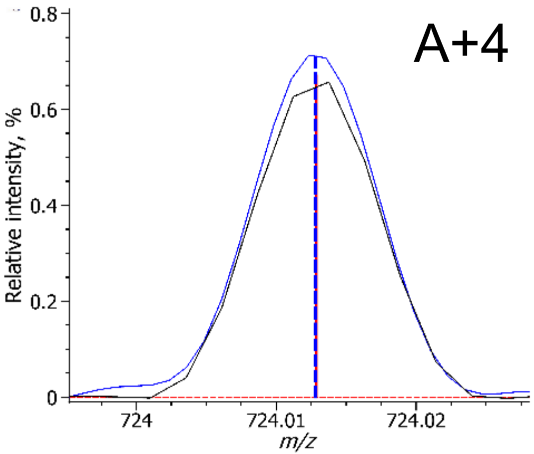
APCI, SIM = 50 *m/z*, R = 120k, T_{acq} = 256 ms, AGC = 2e5, IT_{max} = 1ms
total experimental time: 440 min



Experiment: C_{60} Analysis with a Q Exactive HF



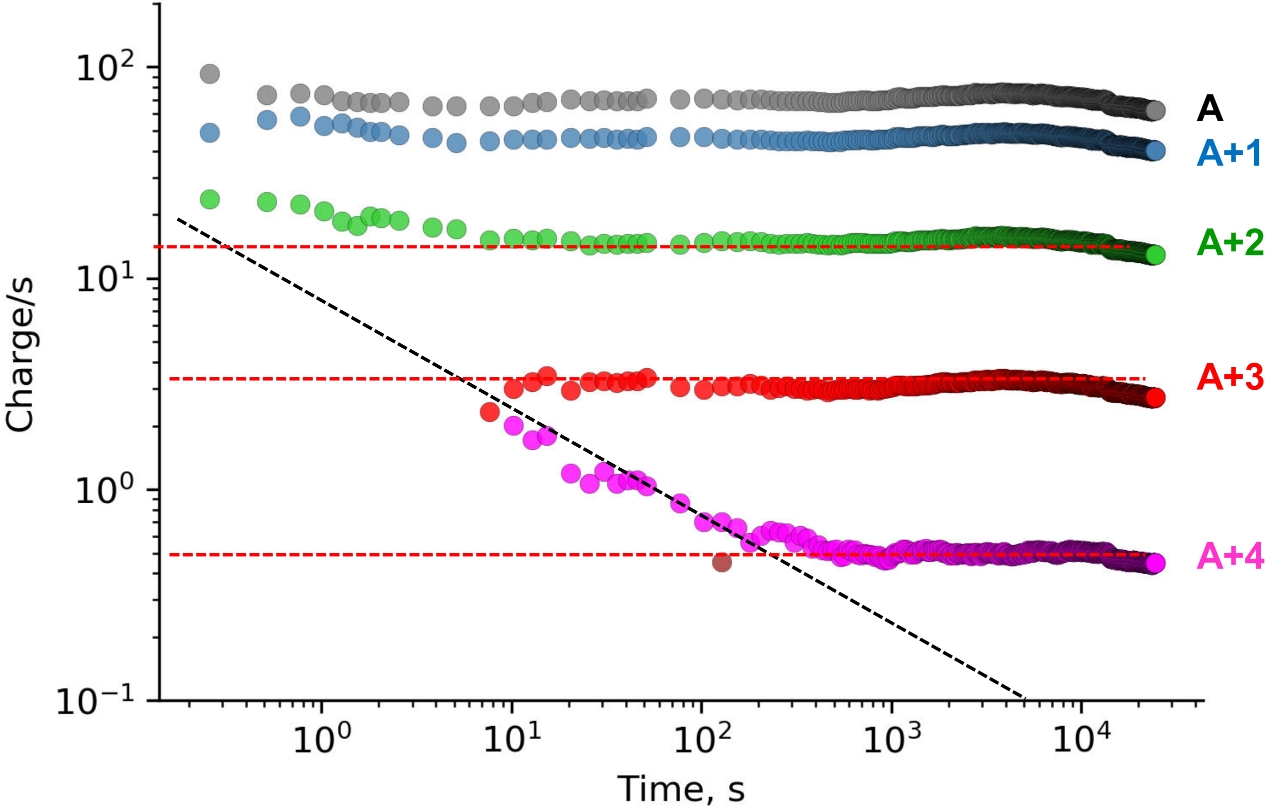




How many charges per second of a measurement time?

APCI, SIM = 50 *m/z*, R = 120k, T_{acq} = 256 ms, AGC = 2e5, IT_{max} = 1ms
total experimental time: 440 min

MSNR?
T_{acq}?
N_{scans}?

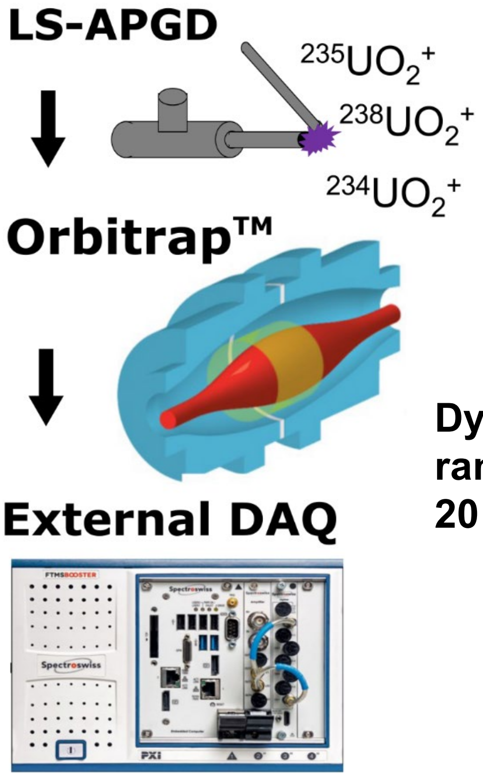


~ 10 charges / s

~ 3 charges / s

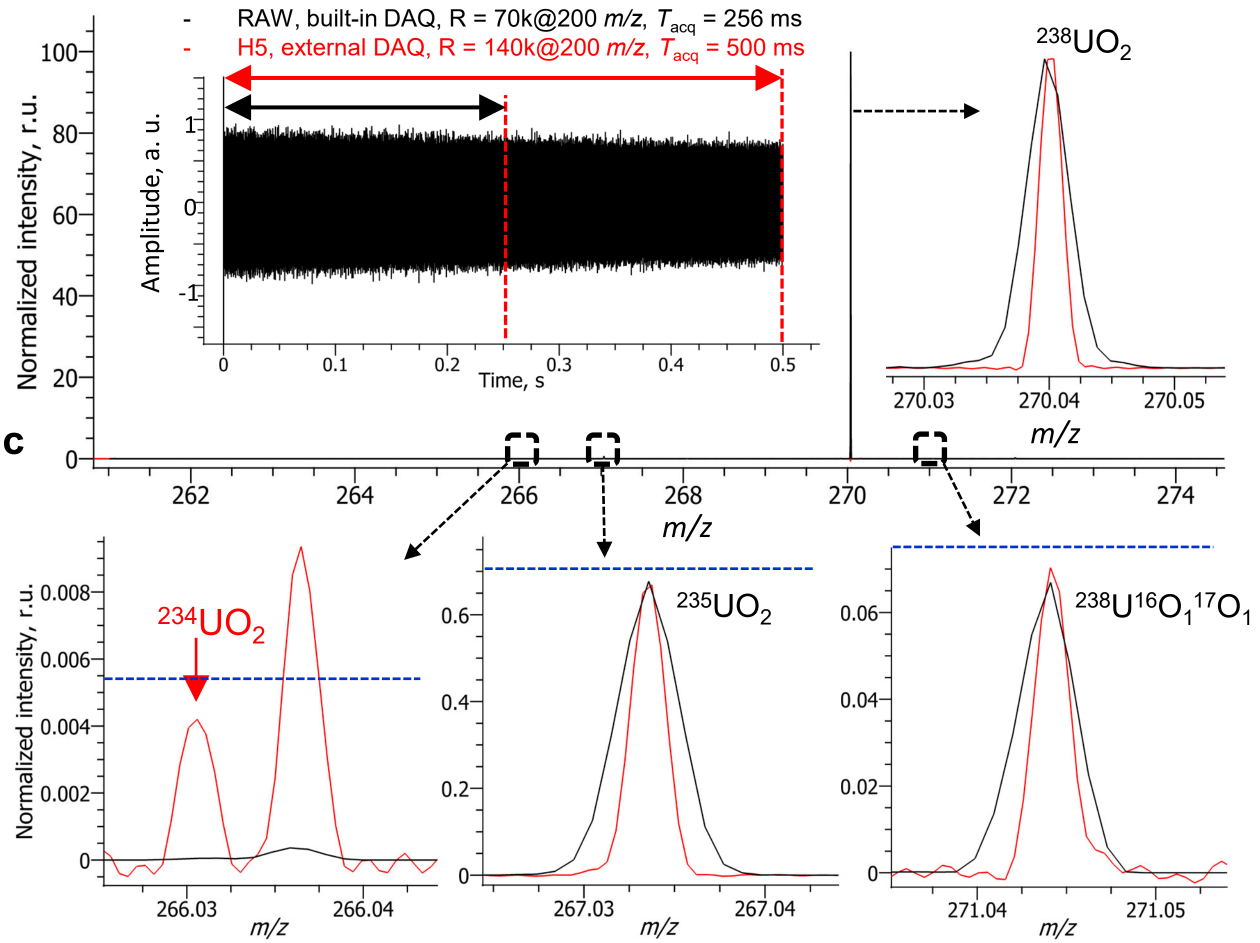
~ 0.5 charges / s

Uranium Isotopic Ratio Analysis: Extreme Dynamic Range



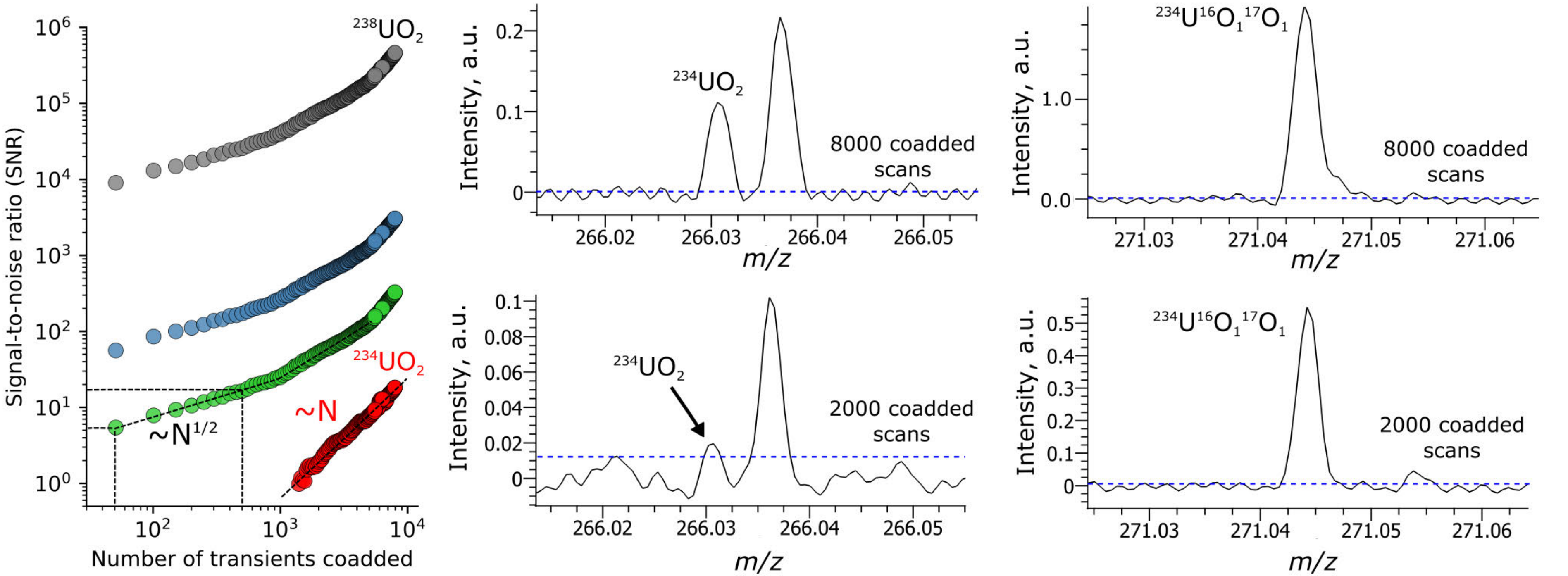
8 000 transients

Dynamic range 20 000!



Markus et al., JASMS, 2021, 1224

Detection of Rare Events (Singly Charged Ions)



No signal!

~ 0.1 charges / s

Ken Markus et al., JASMS, 2021, 32, 5, 1224–1236

Enabling isotopic ratio analysis for extremely challenging elements

Conclusions

Time-domain transient analysis enables ion detection over extended periods with only one charge injected into Orbitrap per 10 (…100) seconds measurement time

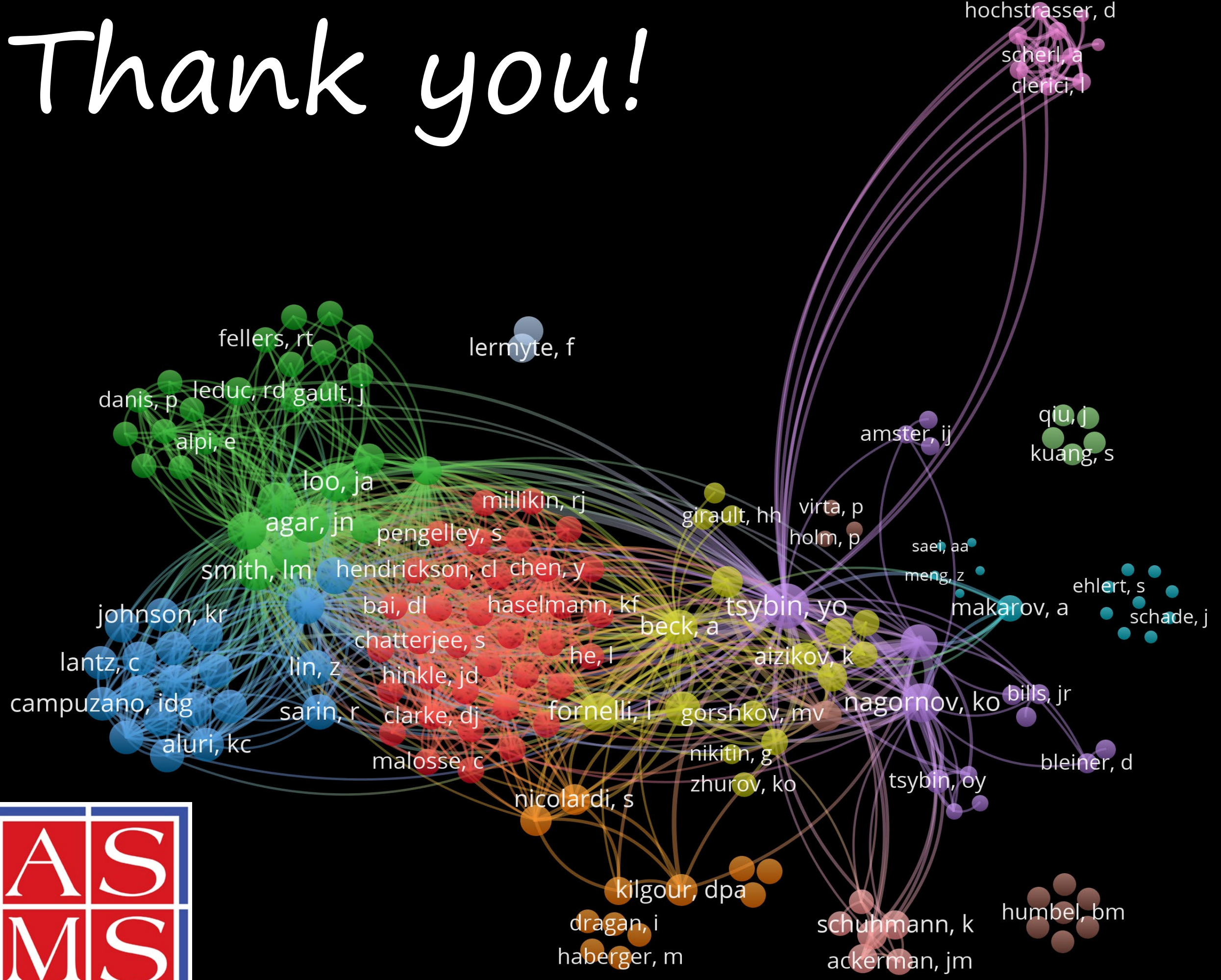
To detect ion signals with SNR > 4 and 512 ms time-domain transients:

- 1 charge per scan will require ~100 scans
- **1 charge per 10 scans - ~5 000 - 10 000 scans**
- 1 charge per 100 scans - up to 1 million scans (suggested *in-silico*, to demonstrate!)

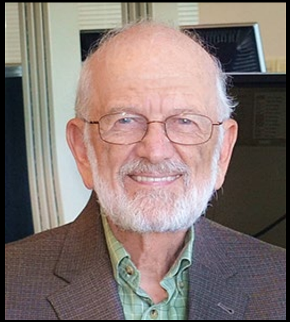
- The induced current detection realized in Orbitraps demonstrate its exceptional power
- Extended experiments reveal the depth of information contained in the time-domain transients
- Absorption mode FT mass spectra (unreduced) are readily available and provide equal performance to time-domain transients, even for the extremely low ion counting applications
- Understanding of these capabilities may benefit particular applications, including elemental analysis (isotopes), clinical, e.g., breath analysis, and environmental monitoring (matrix effects?)

The power and full capabilities of the Orbitraps (and ICRs!) are yet to be realized!

Thank you!



Richard Knochenmuss
RKResearch LLC



R. Graham Cooks