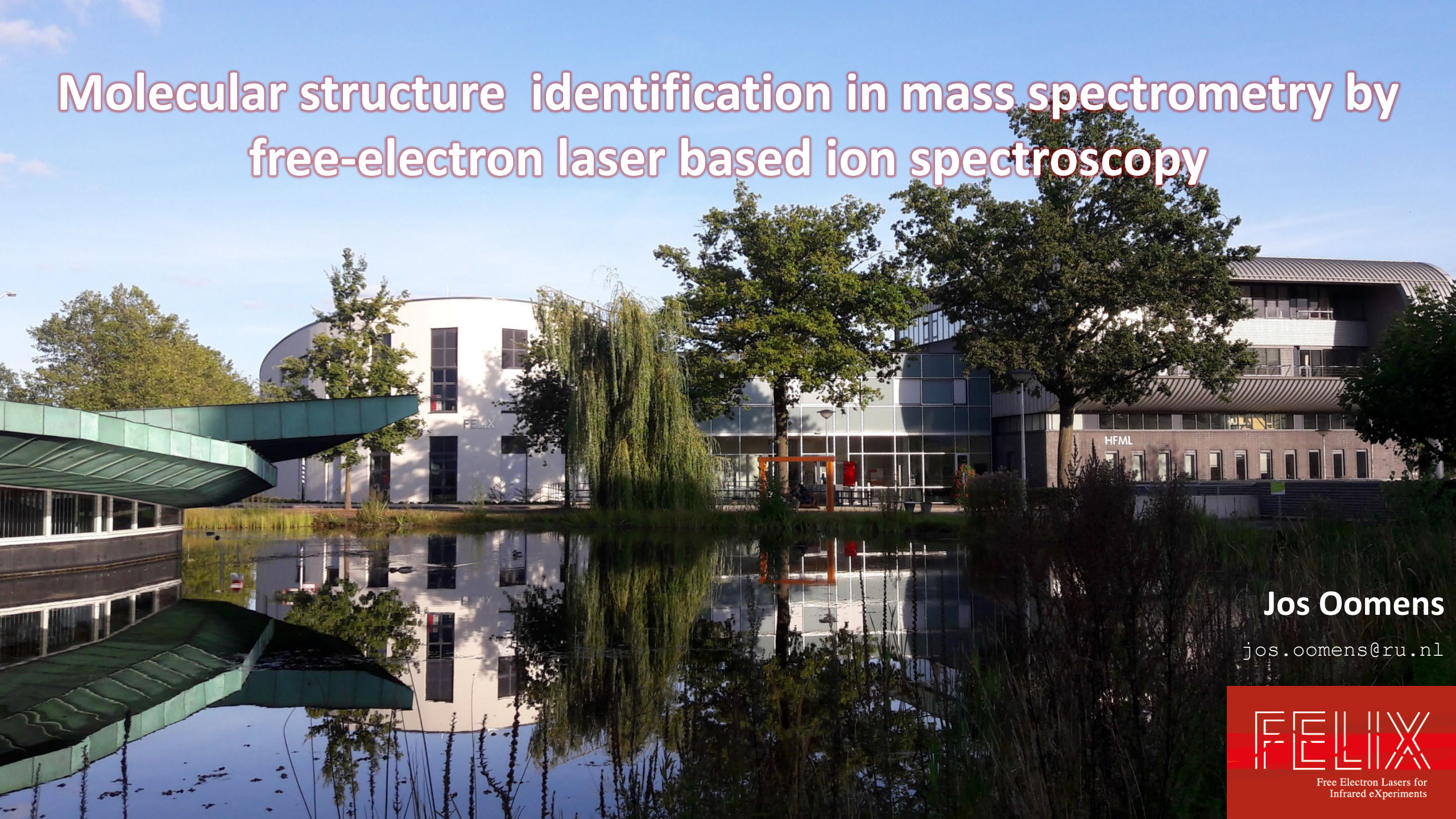
Molecular structure identification in mass spectrometry by free-electron laser based ion spectroscopy

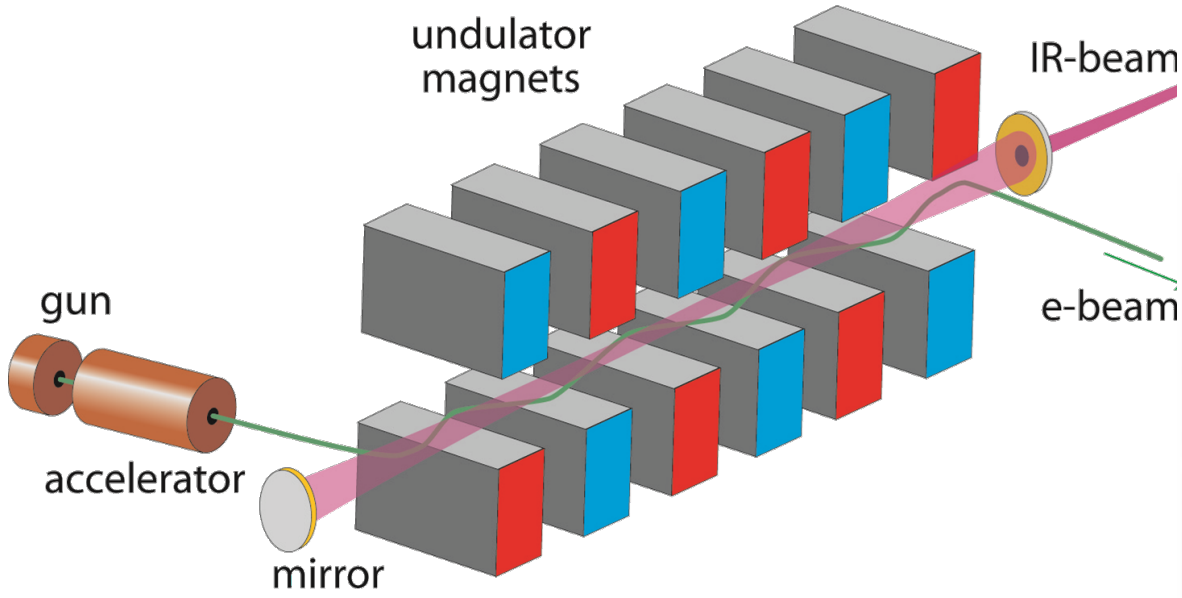


Jos Oomens

jos.oomens@ru.nl

FELIX
Free Electron Lasers for Infrared eXperiments

FELIX – FREE ELECTRON LASER FOR INFRARED EXPERIMENTS



Open access user facility

www.ru.nl/felix

User facility

Wavelength tunable 2.8 – 150 μm

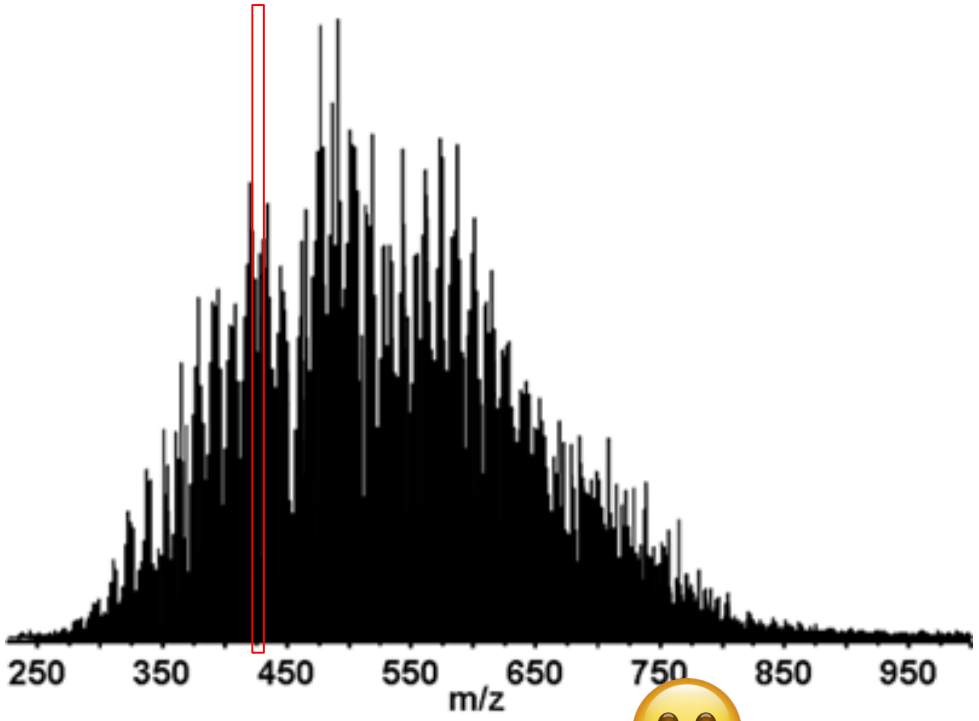
Pulse energy ~100 mJ per 5 μs pulse

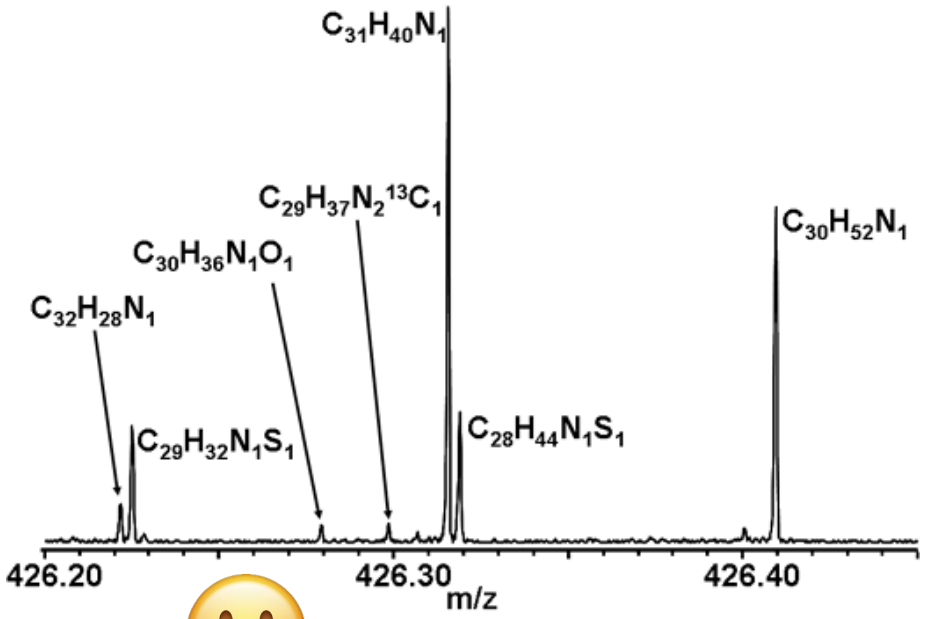
FWHM bandwidth >0.4% of λ

MASS SPECTROMETRY IN THE ANALYSIS OF COMPLEX MIXTURES

- High sensitivity
- High resolution

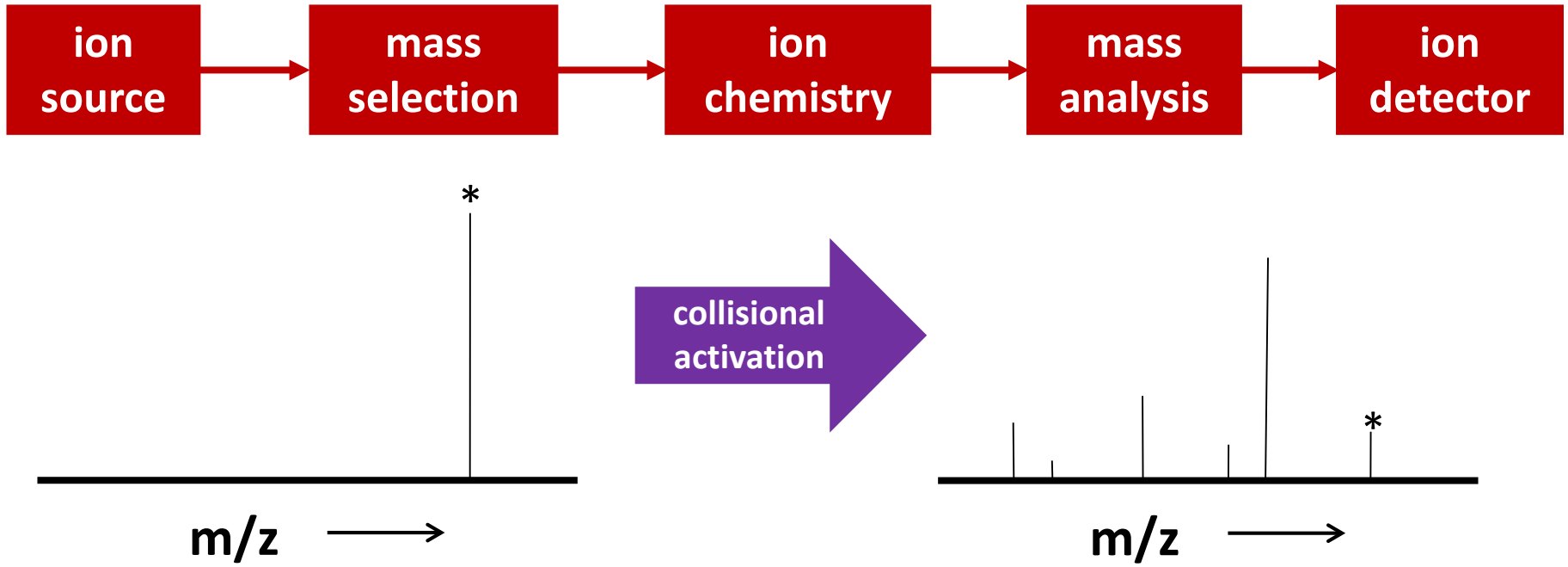
A.G. Marshall and coworkers, NHFML, Florida





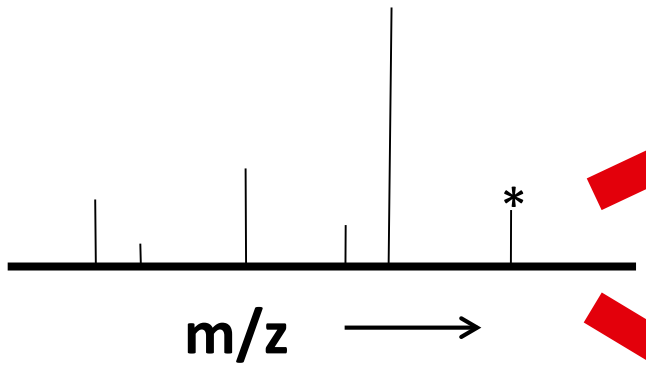
molecular weight → molecular formula → molecular structure

COLLISION-INDUCED DISSOCIATION MS/MS FOR SMALL MOLECULE IDENTIFICATION



- Fragment spectrum not predictable from *QM first principles*
- MS/MS library search
- Identify only *known unknowns*

MS/MS IDENTIFICATION WORKFLOW



MS/MS library

~ 100 000

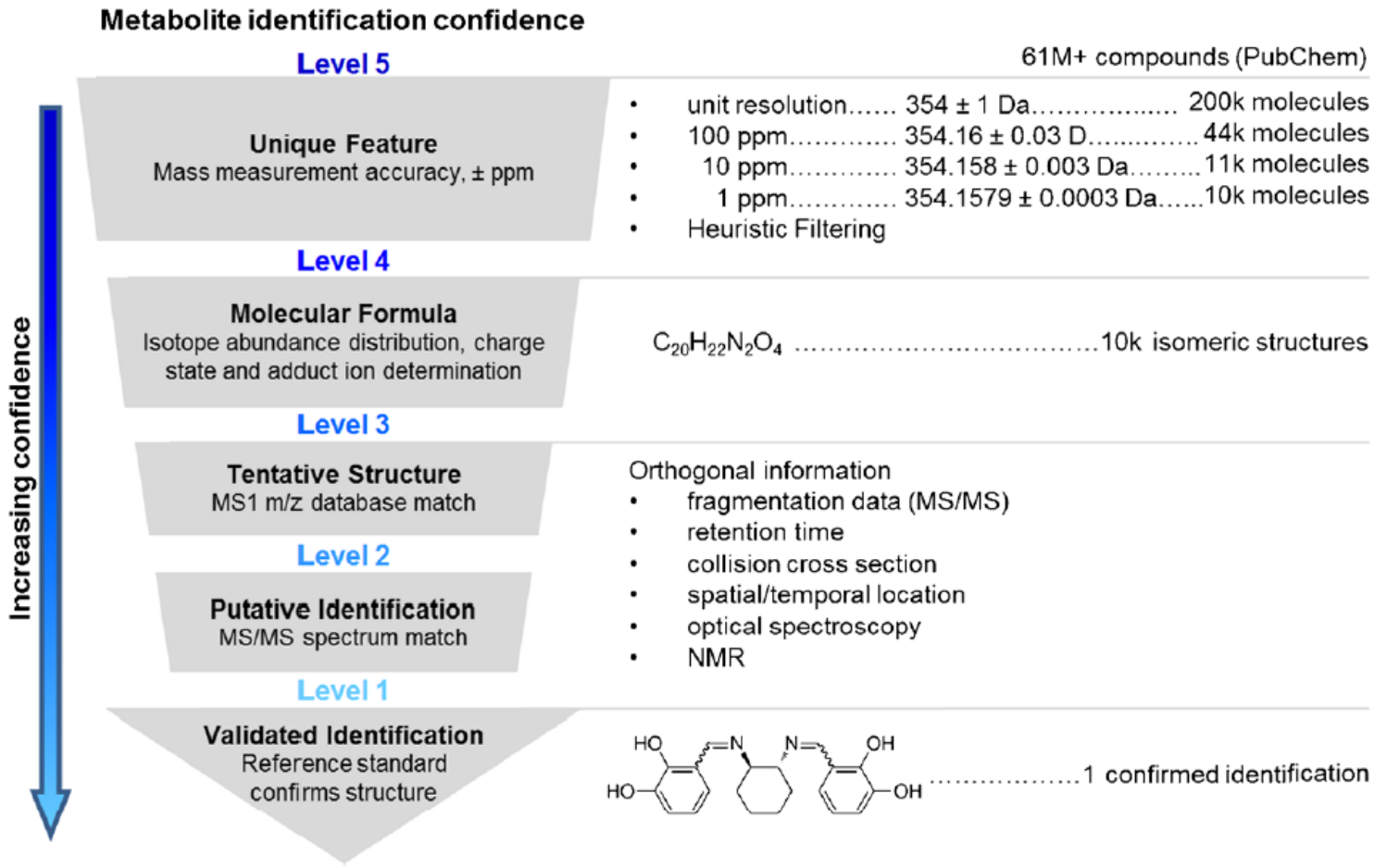
***in silico* MS/MS**

Heuristic and/or AI

PubChem

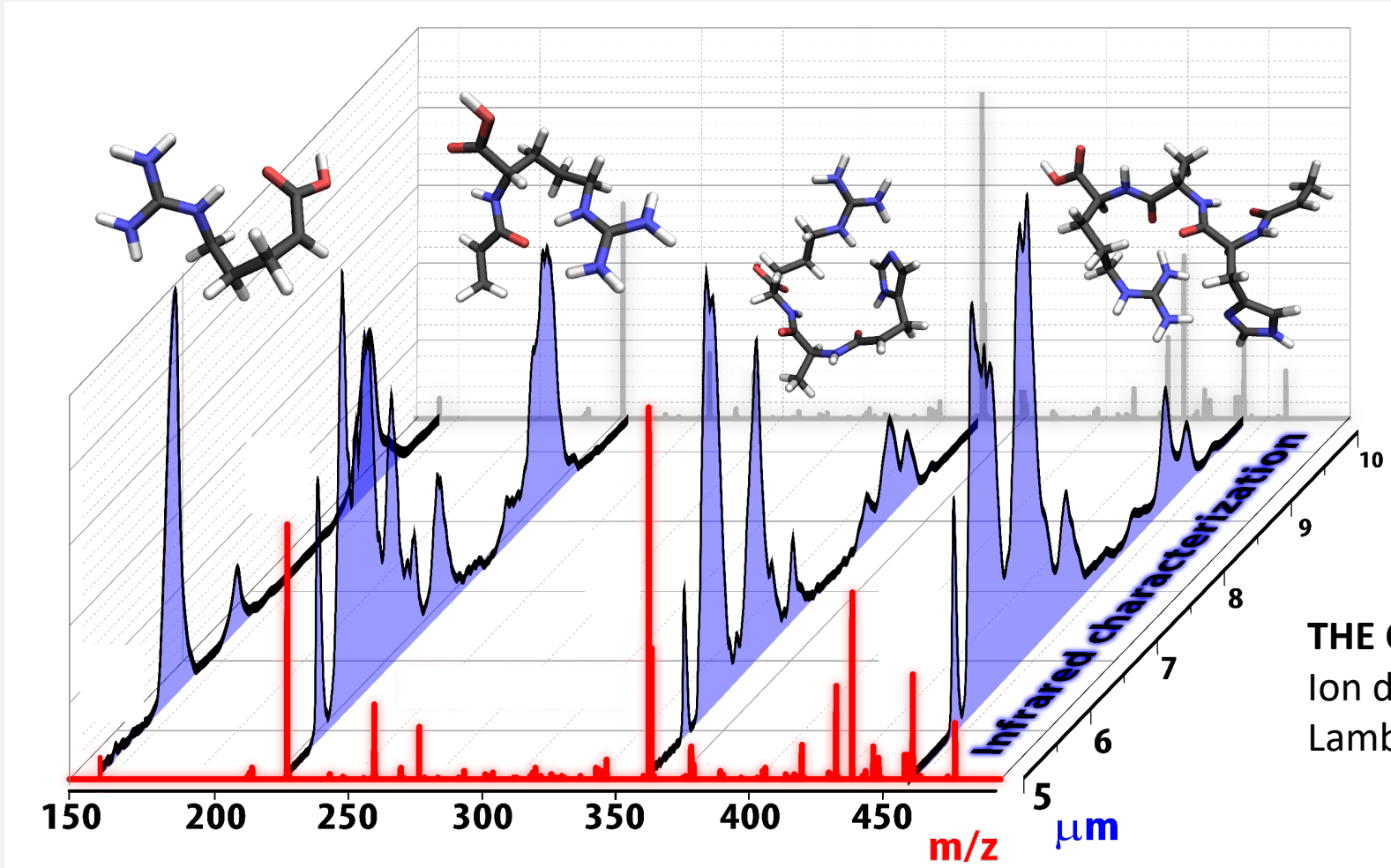
Collection	Live Count	Description
Periodic Table of Elements	118	Interactive periodic table wi
Compounds	115,056,553	Unique chemical structures

CONFIDENCE LEVELS IN MS-BASED MOLECULAR ID



Schrimpe-Rutledge, Codreanu, Sherrod, McLean, *JASMS* 2016, *27*, 1897

INTEGRATE IR SPECTROSCOPY WITH MASS SPECTROMETRY



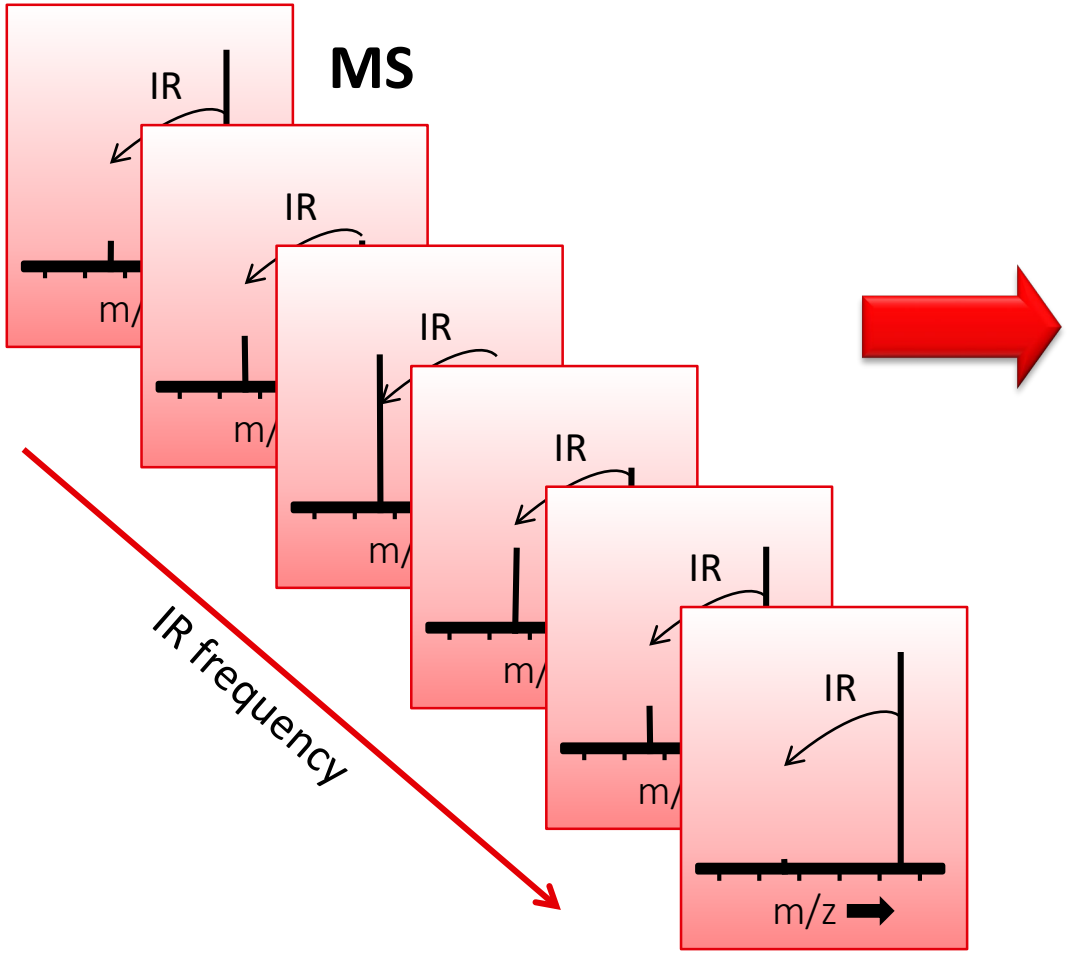
THE CHALLENGE

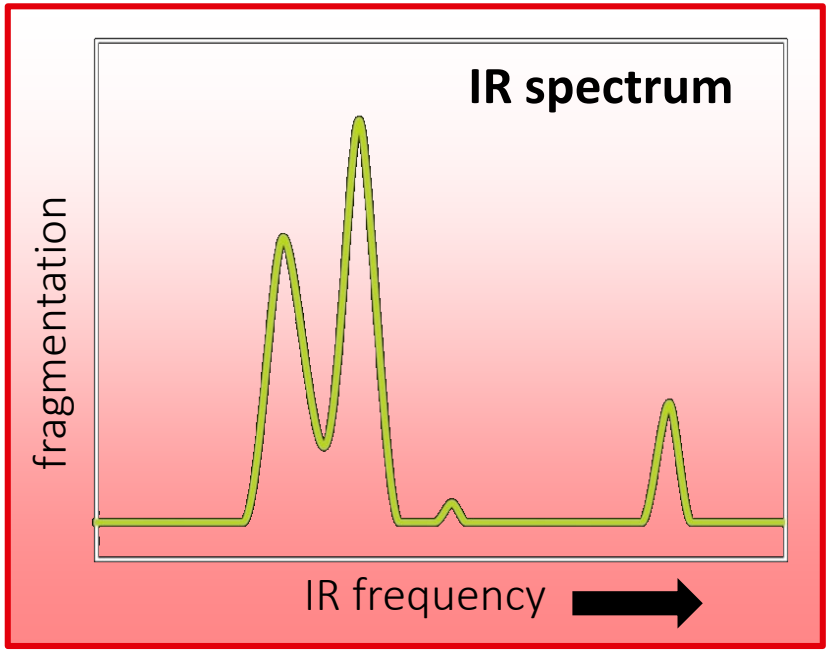
Ion density in MS ~10^4 cm^{-3}

Lambert-Beer requires ~10^{16} cm^{-3}

Martens, Grzetic, Berden, Oomens, *Nature Commun*. **2016**, *7*, 11754

INFRARED ION SPECTROSCOPY (IRIS)

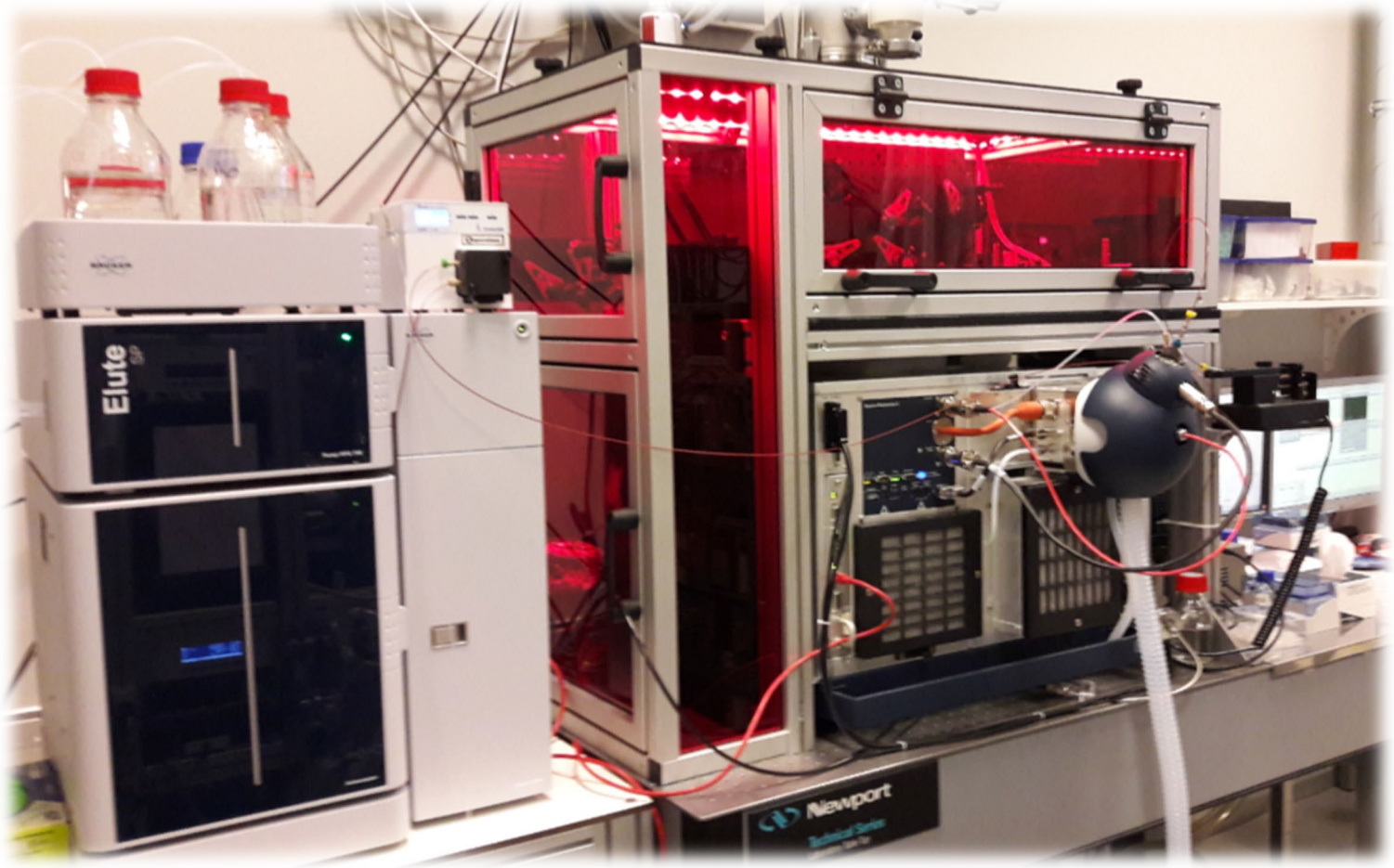


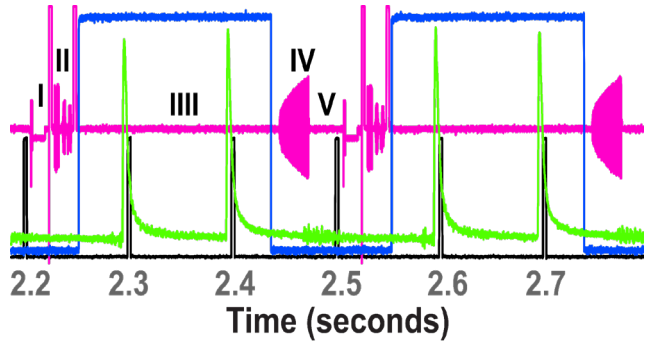


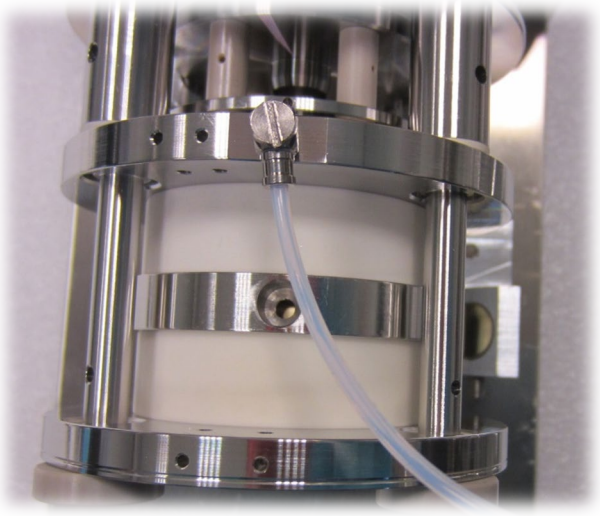
IR spectroscopy, but with

- selectivity of MS
- sensitivity of MS
- versatility of MS (LC, MS/MS, IMS)

EXPERIMENTAL: MODIFIED BRUKER *AMAZON* ION TRAP MASS SPECTROMETER







Martens, Berden, Gebhardt, Oomens, *Rev. Sci. Instrum.* **2016**, *87,* 103108

BIOMARKER DISCOVERY FOR METABOLIC DISEASES

Radboud University

TRANSLATIONAL METABOLIC LABORATORY AT RADBOUD UMC


Karlien Coene


Leo Kluijtmans

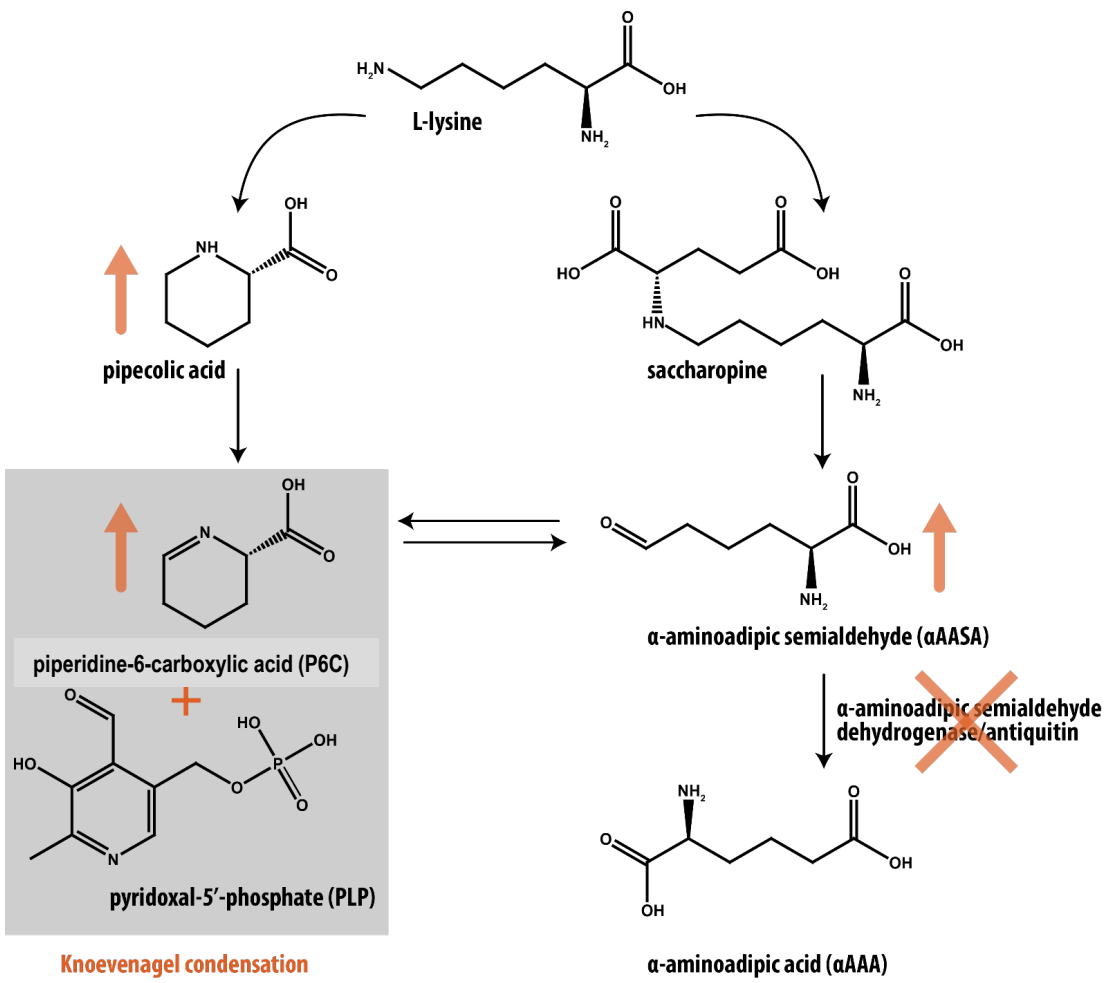

Udo Engelke

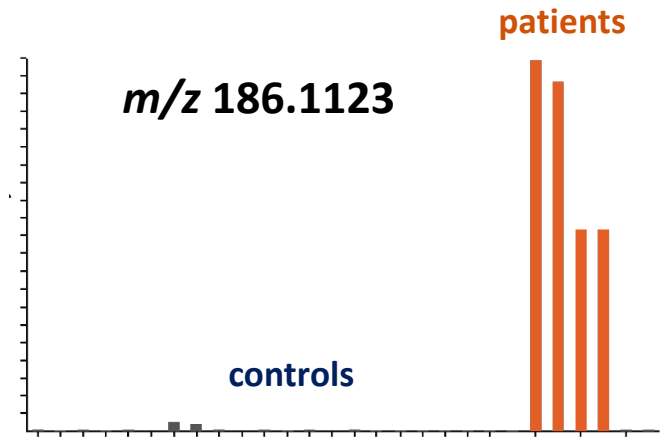

Ron Wevers

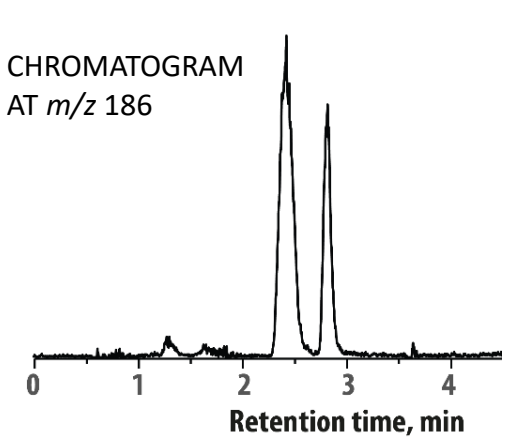

Dirk Lefeber

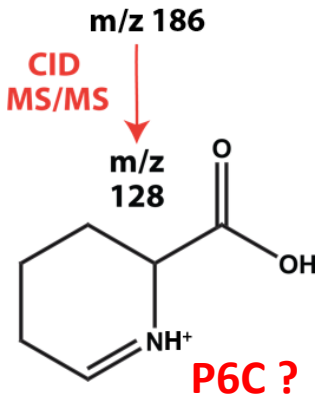
Radboudumc
university medical center

PYRIDOXINE DEPENDENT EPILEPSY (PDE)

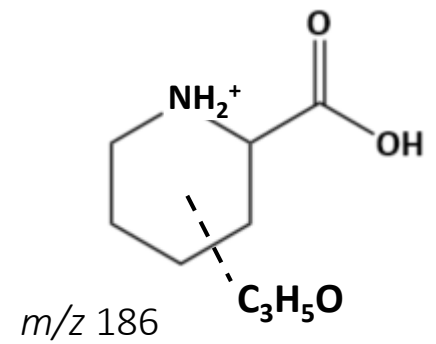






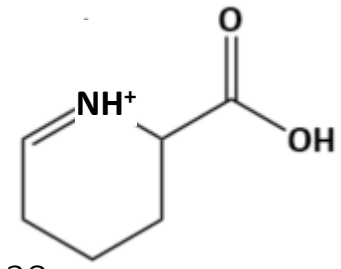


IRIS CONFIRMS THAT *M/Z* 128 CID FRAGMENT IS PROTONATED P6C



m/z 186

CID



m/z 128

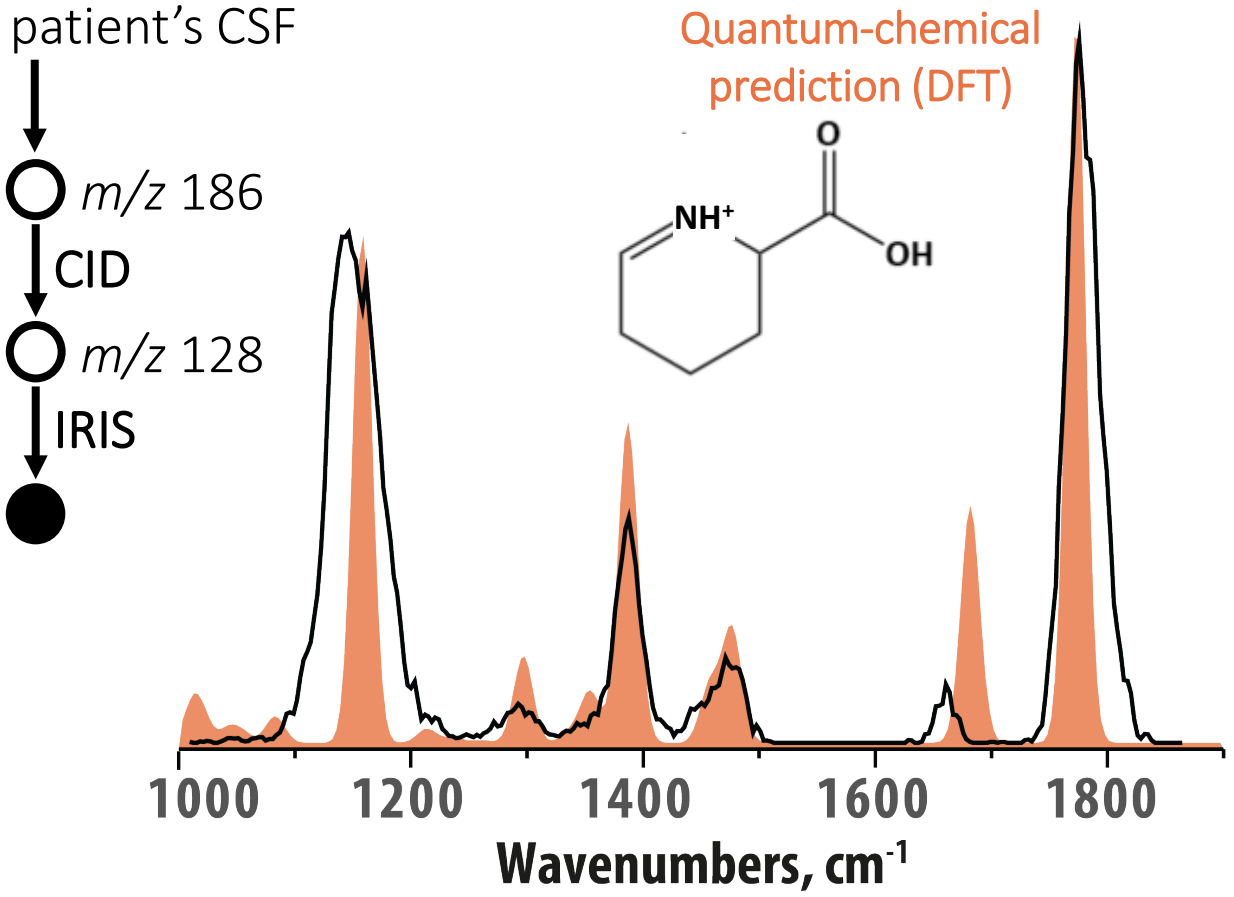
patient's CSF

m/z 186

CID

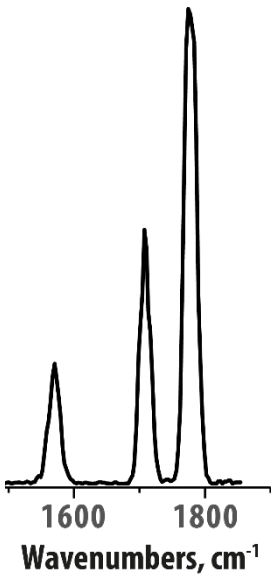
m/z 128

IRIS

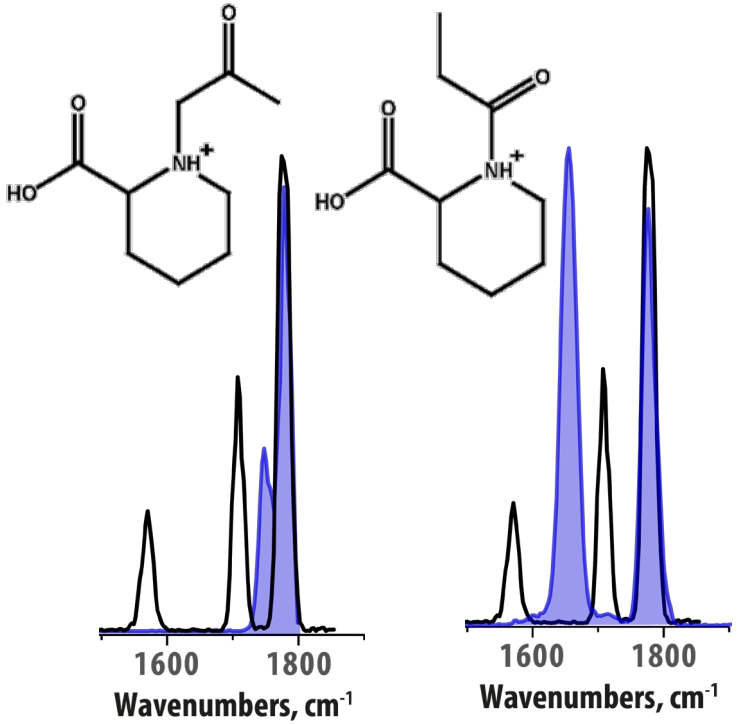


REFERENCE-FREE IDENTIFICATION OF *M/Z* 186 BIOMARKER

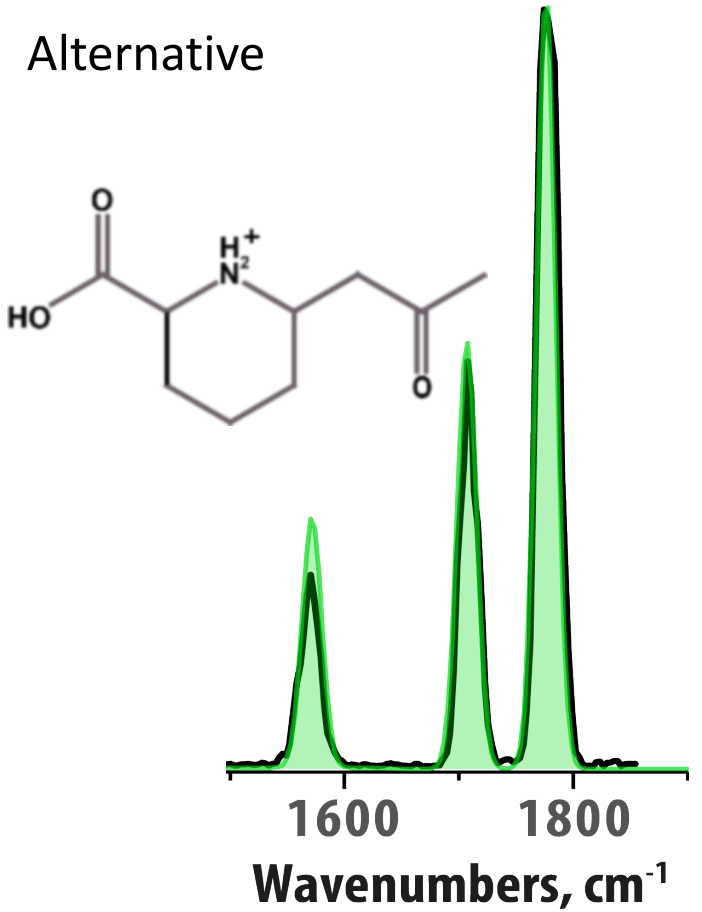
IR spectrum
unknown *m/z* 186



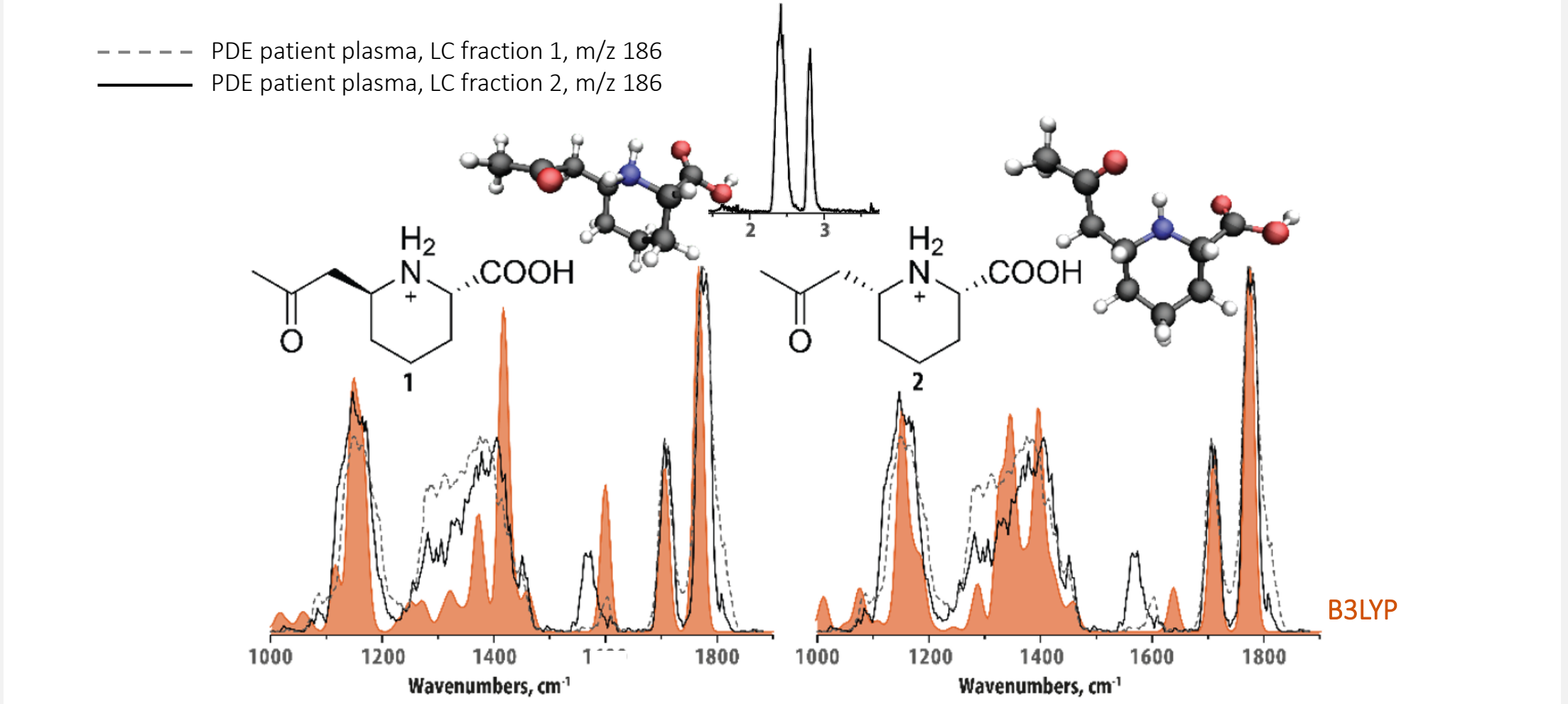
Two structures proposed



Alternative

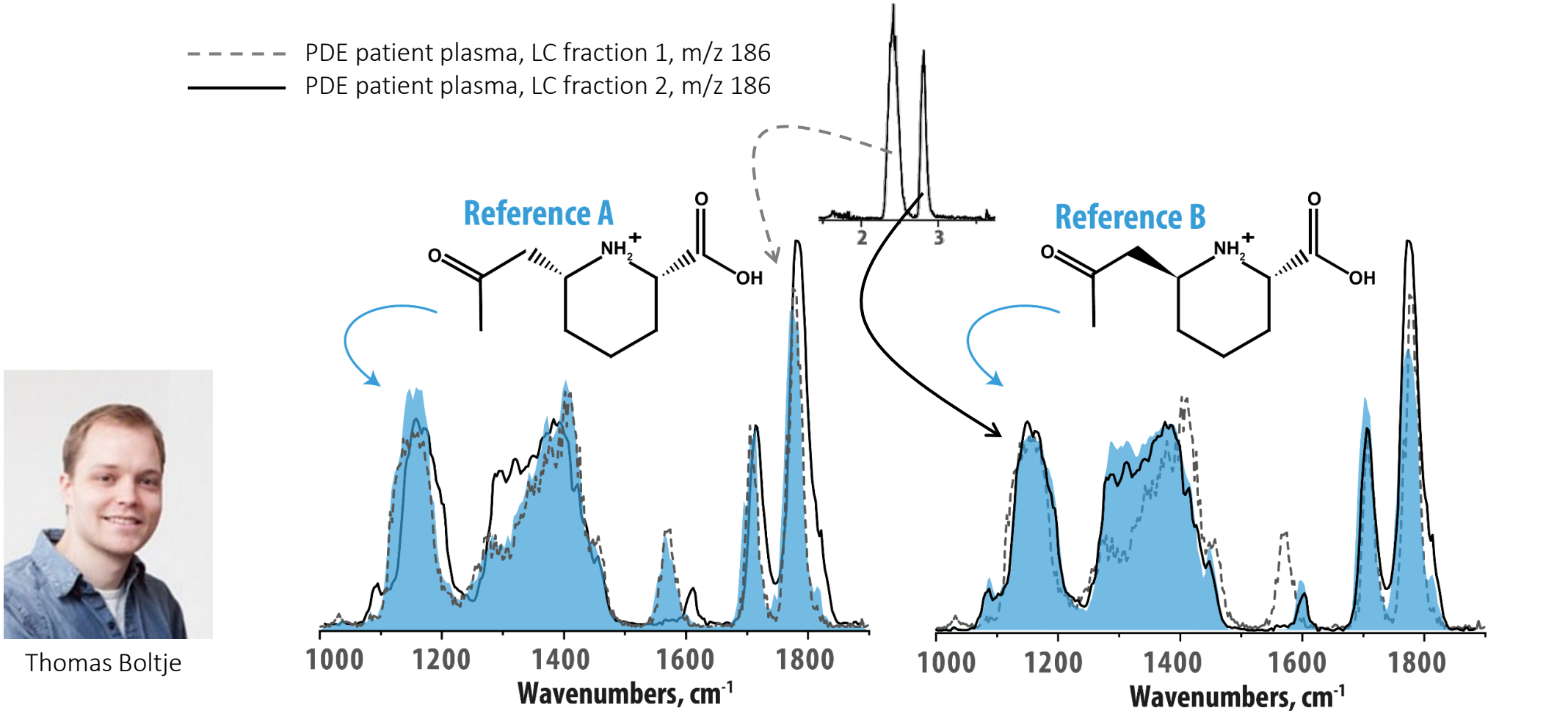


FULL IDENTIFICATION INCLUDING STEREOCHEMISTRY



Van Outersterp et al. *Anal. Chem.* **2021**, *93*, 15340
Engelke et al. *J. Clin. Invest.* **2021**, *131*, e148272

FULL IDENTIFICATION (LEVEL 1) INCLUDING STEREOCHEMISTRY USING SYNTHESIZED STANDARDS



Thomas Boltje

Radboud University

Van Outersterp et al. *Anal. Chem.* **2021**, *93*, 15340
Engelke et al. *J. Clin. Invest.* **2021**, *131*, e148272

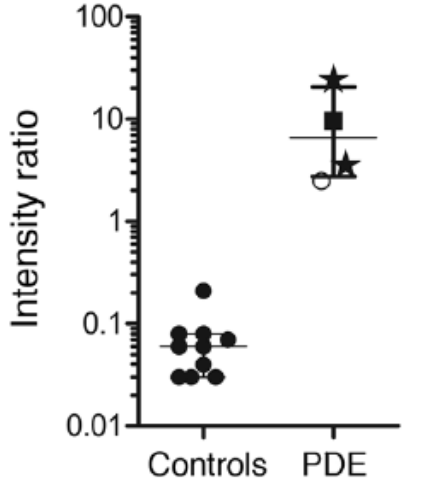
METABOLITE IDENTIFICATION

- Inclusion in newborn screening programs
- Pathophysiology of PDE
- Treatment

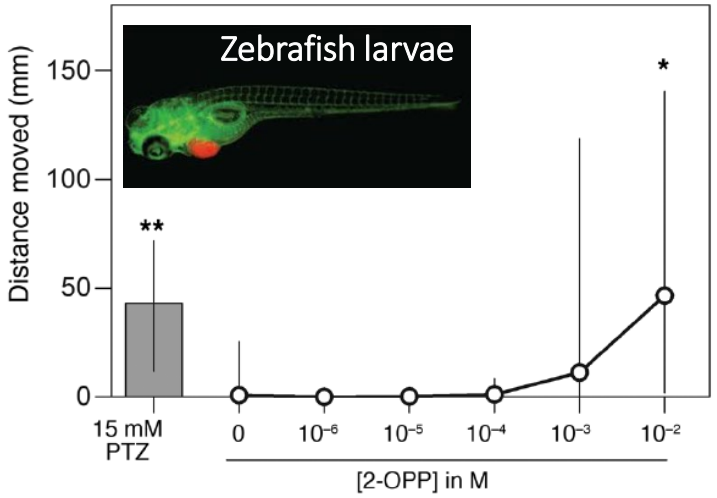
2-OPP is outstanding biomarker for PDE

Feature	*m/z*	RT (min)	Fold change,
Biomarker A [M + H]⁺	186.1123	2.33	200
Biomarker B [M + H]⁺	186.1123	2.55	145
Biomarker C [M + H]⁺	144.0652	3.05	45
α-AASA [M – H]⁻	144.0666	0.86	8
P6C [M + H]⁺	128.0706	0.86	9
Pipecolic acid [M + H]⁺	130.0861	1.04	3

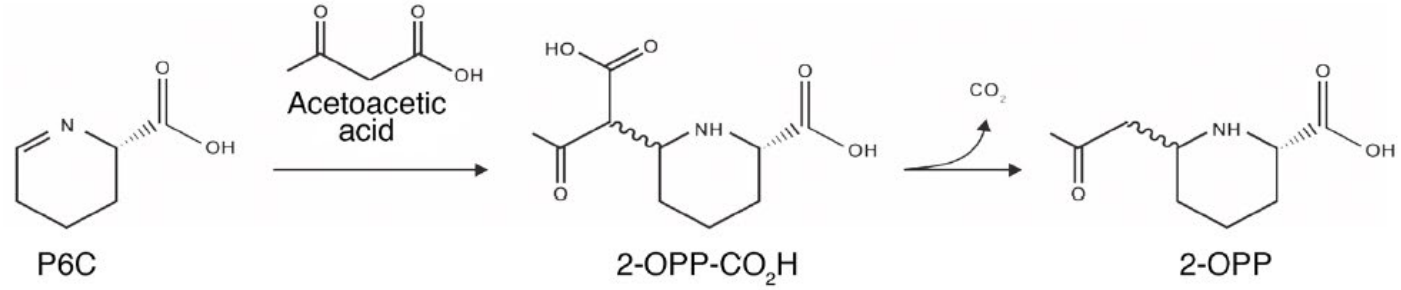
2-OPP in DBS



2-OPP induces epilepsy



2-OPP is formed from P6C and AcAc



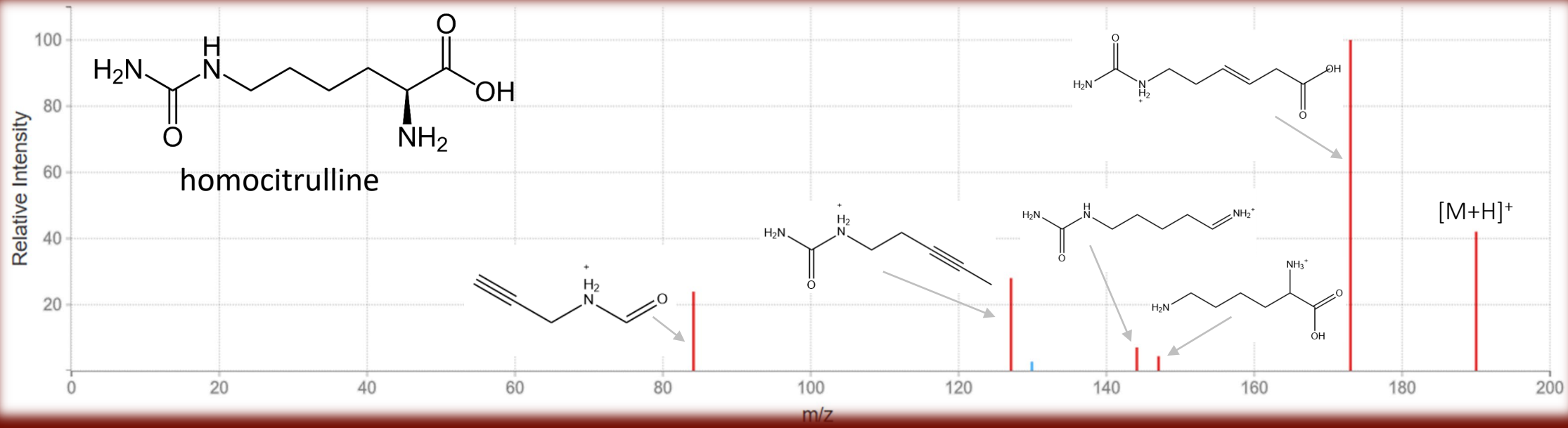
Engelke et al. *J. Clin. Invest.* 2021, *131*, e148272

MS/MS LIBRARY ANNOTATIONS

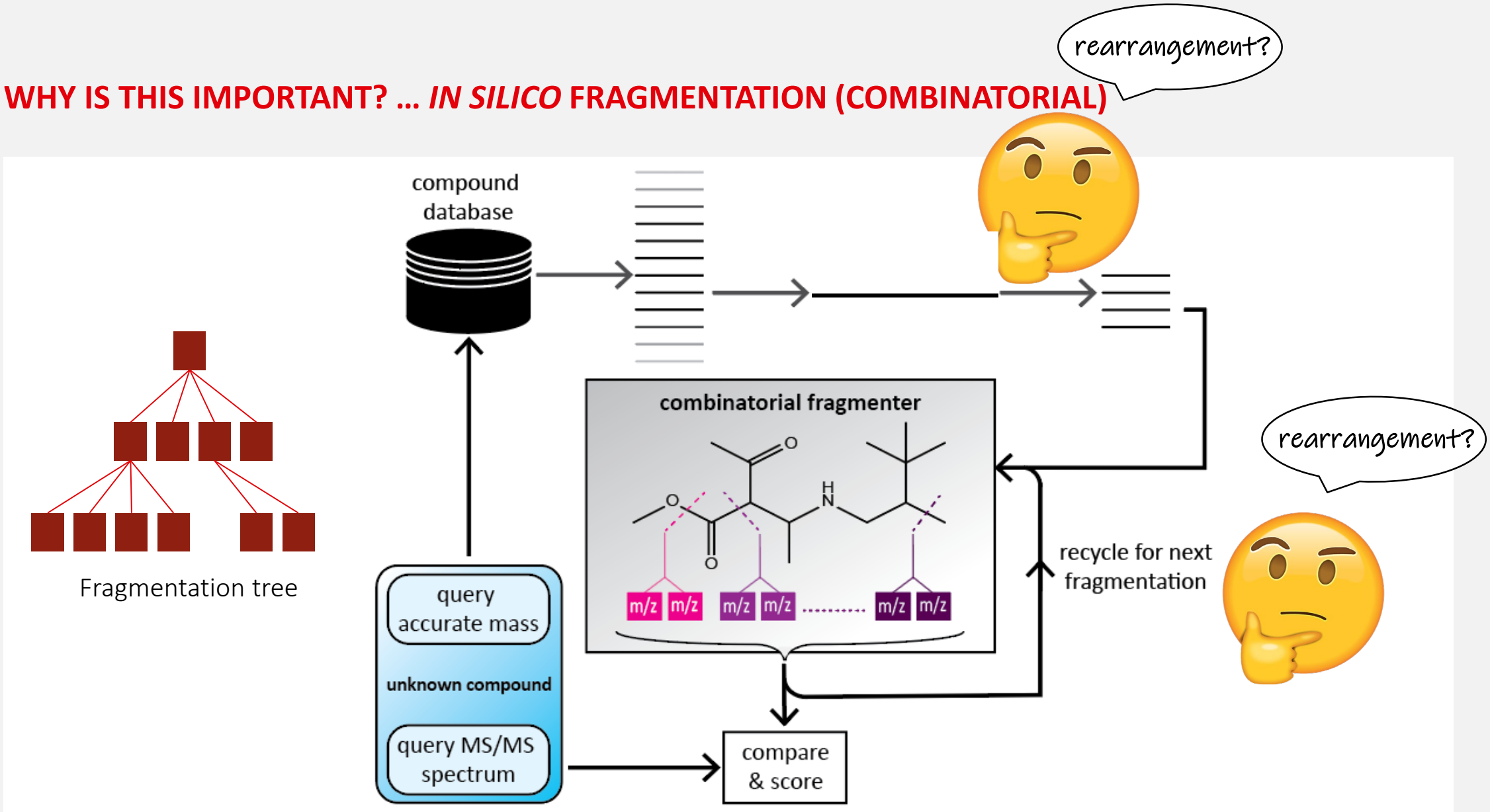
m/z CLOUD

hmdb
The Human Metabolome Database

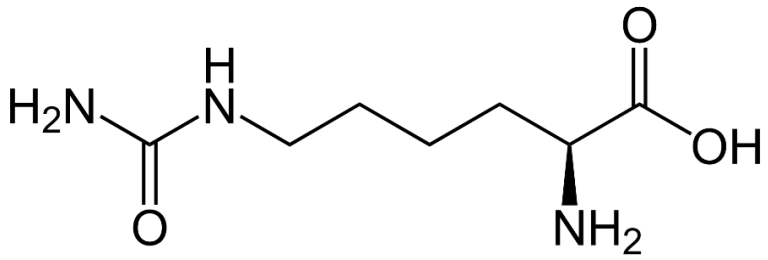
METLIN Gen2

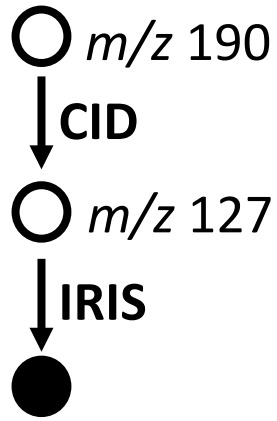


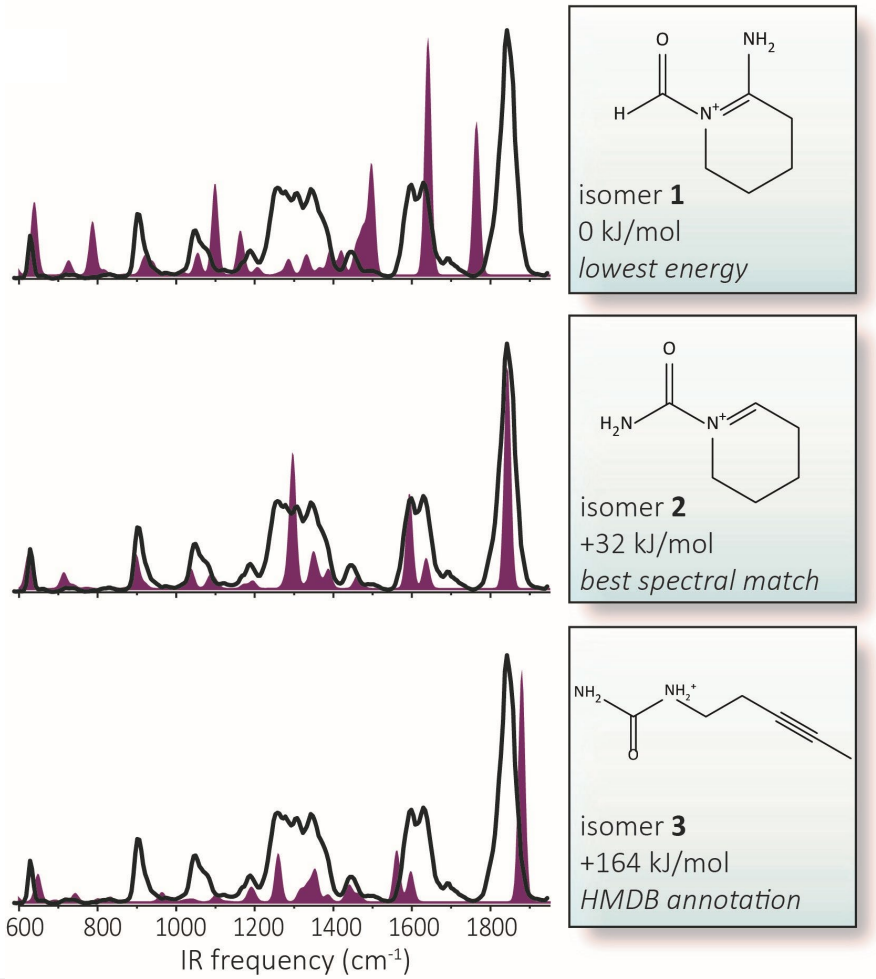
WHY IS THIS IMPORTANT? … *IN SILICO* FRAGMENTATION (COMBINATORIAL)



EXAMPLE: MS/MS FRAGMENT ION OF HOMOCITRULLINE AT *M/Z* 127







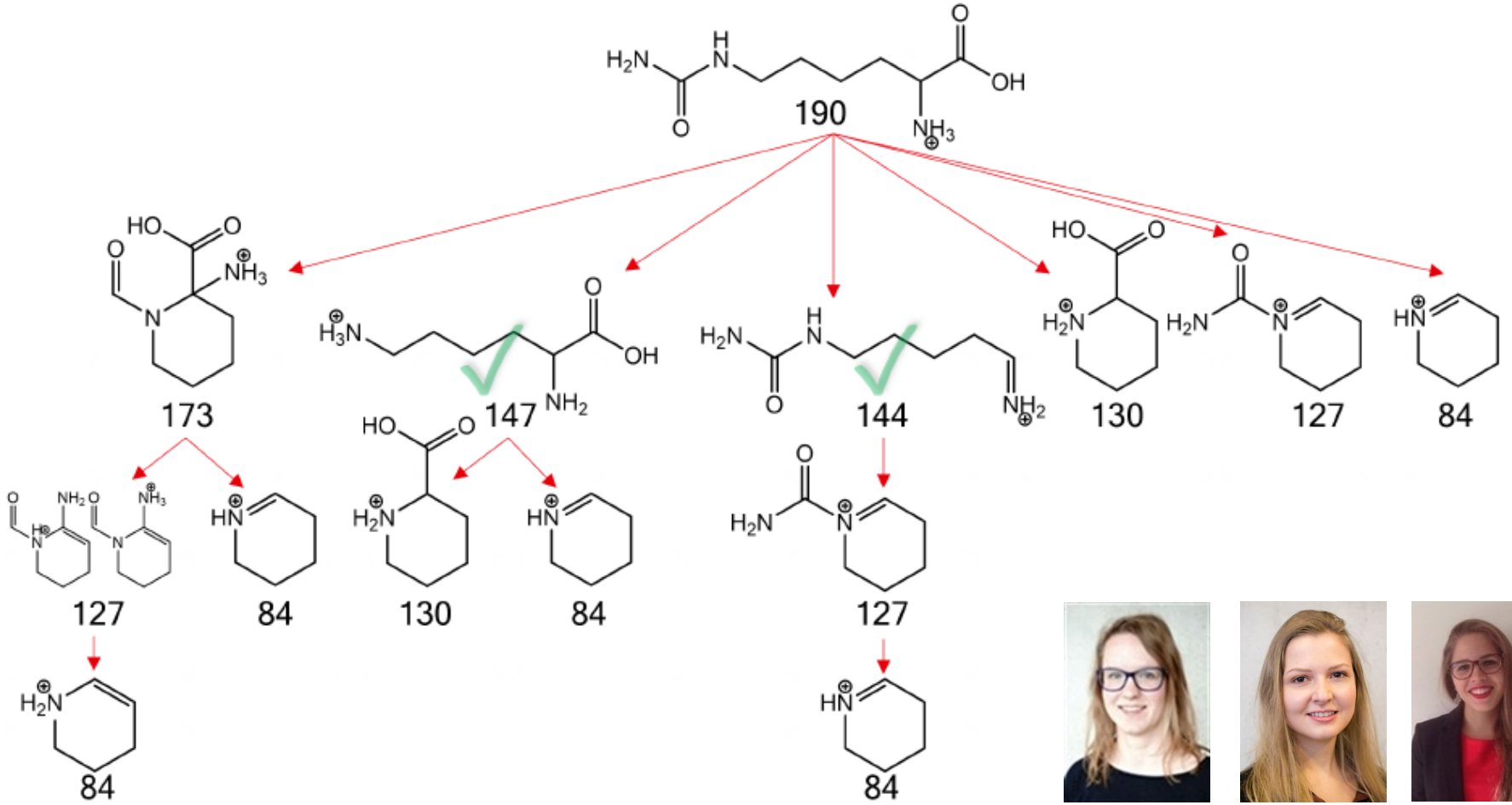
HOMOCITRULLINE FRAGMENTATION TREE

precursor

MS/MS

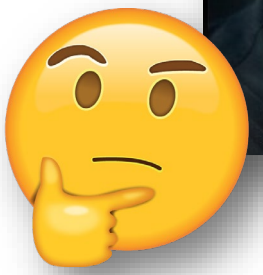
MS^3

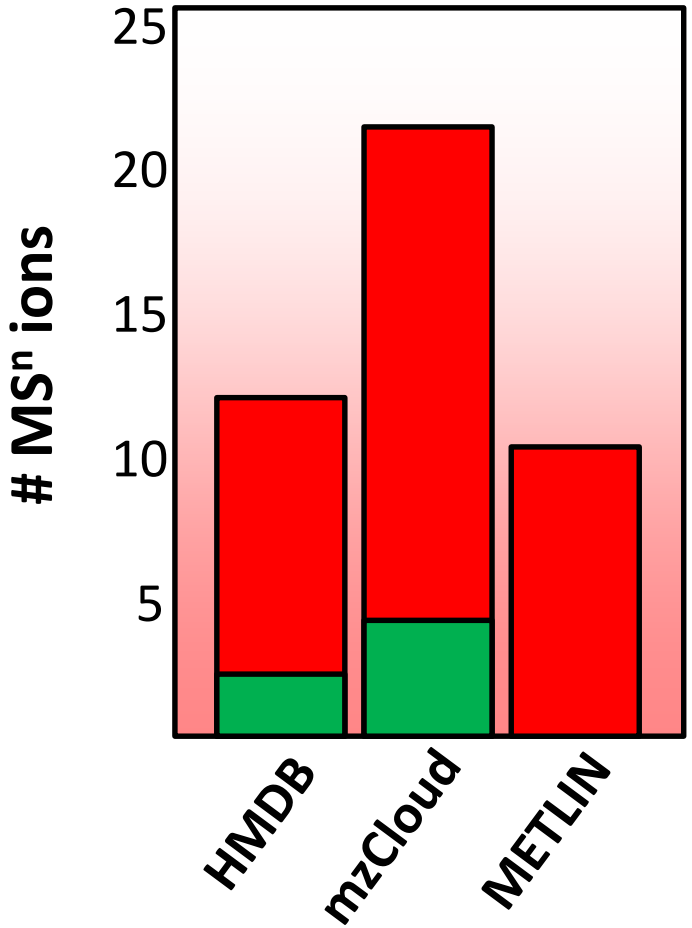
MS^4



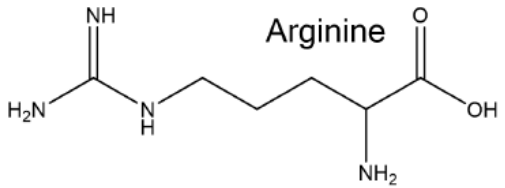
Lara van Tetering, Sylvia Spies, Quirine Wildeman

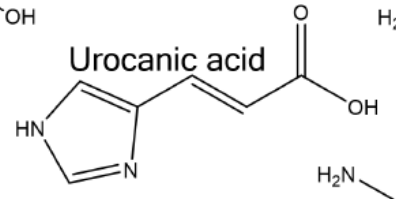
CONCLUSION: LIBRARY ANNOTATIONS ARE GENERALLY INCORREC

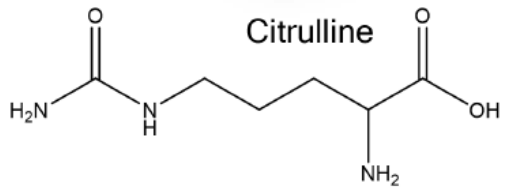


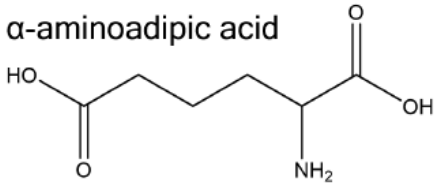


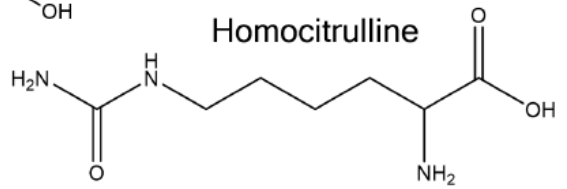
correct annotation
incorrect annotation











Lara van Tetering, Sylvia Spies, Quirine Wildeman

IRIS SPECTRA IN THE HMDB

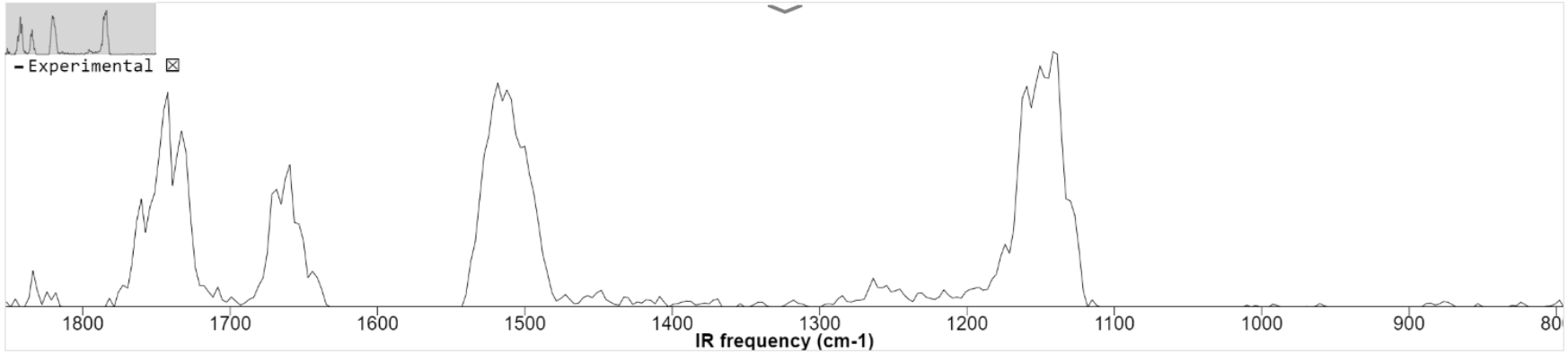
HMDB Browse Search Downloads About Contact Us

Spectrum Details

HMDB ID: HMDB0000860
Compound name: Phenylpropionylglycine
Spectrum type: IR Ion Spectrum
Adduct: [M+Na]+

Spectrum View

Spectra Viewer Instructions...

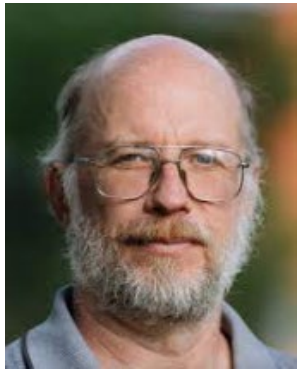


Experimental Conditions

HMDB ID: HMDB0000860
Spectrum type: IR Ion Spectrum
Adduct: [M+Na]+
Instrument Type: Bruker AmaZon Speed Quadrupole Ion Trap
Laser Power: 20-160 mJ
Number of Pulses: 1

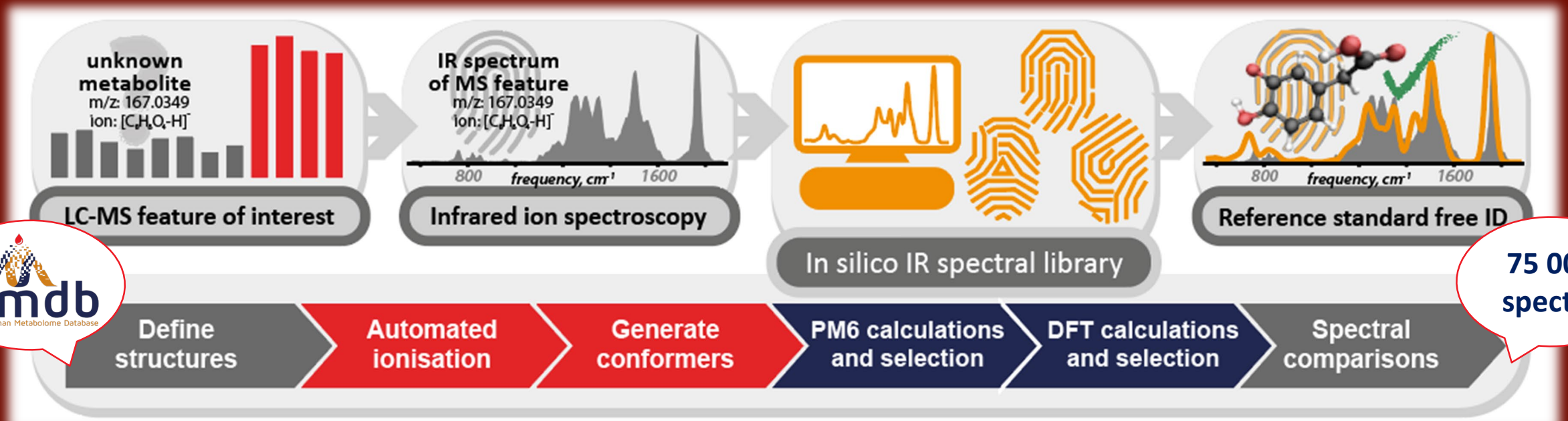
hmdb
The Human Metabolome Database

www.hmdb.ca

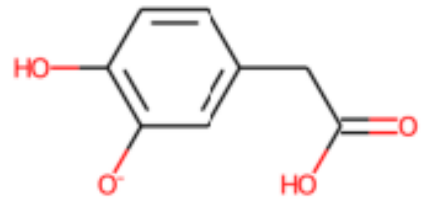


David Wishart, U of Alberta

AN AUTOMATED WORKFLOW FOR IRIS-BASED IDENTIFICATION

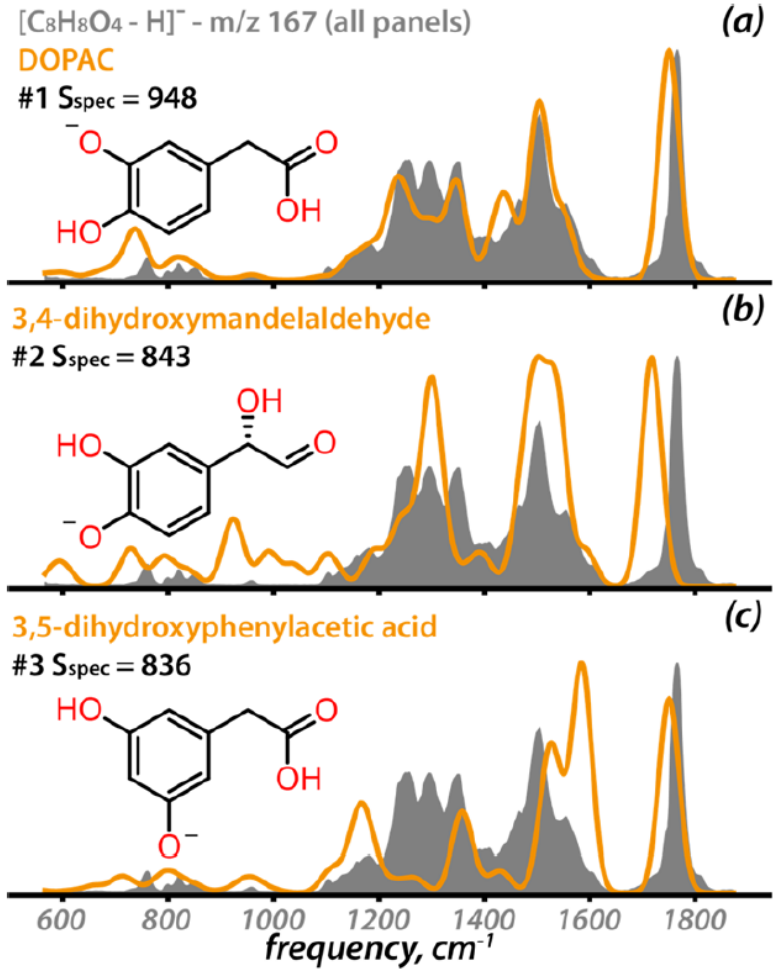


AUTOMATIC FINDING OF THE CORRECT ISOMER



DOPAC, $C_8H_9O_4$
m/z 167 (- ESI)
18 isomers in HMDB
16 deprotonatable

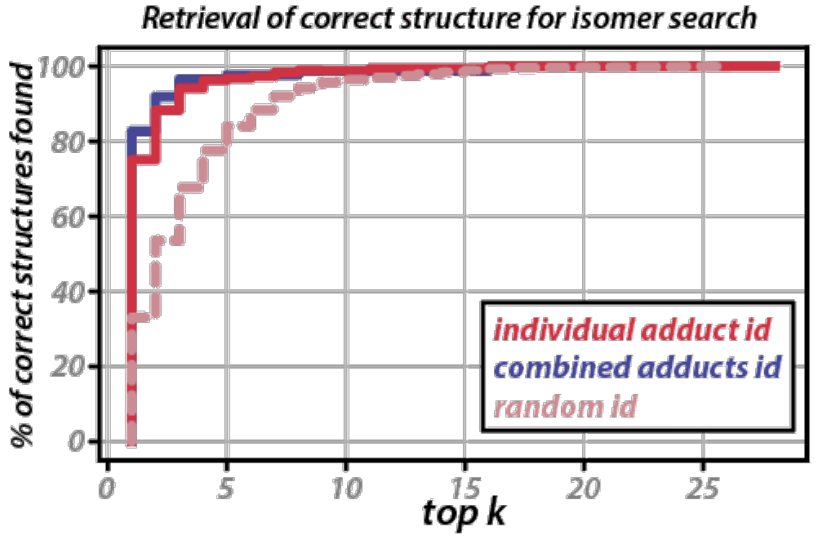
Result:
DOPAC returned as #1 match



Spectral similarity

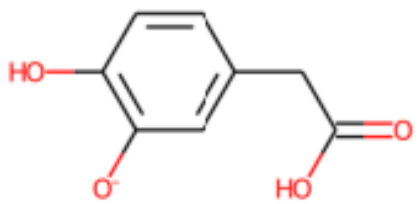
$$S_{spec} = 1000 \cdot \frac{\boldsymbol{a} \cdot \boldsymbol{b}}{|\boldsymbol{a}||\boldsymbol{b}|} = 1000 \cdot \frac{\sum_{i=1}^{n} a_i b_i}{\sqrt{\sum_{i=1}^{n} (a_i)^2}\sqrt{\sum_{i=1}^{n} (b_i)^2}}$$

For 189 IRIS spectra from 89 metabolites



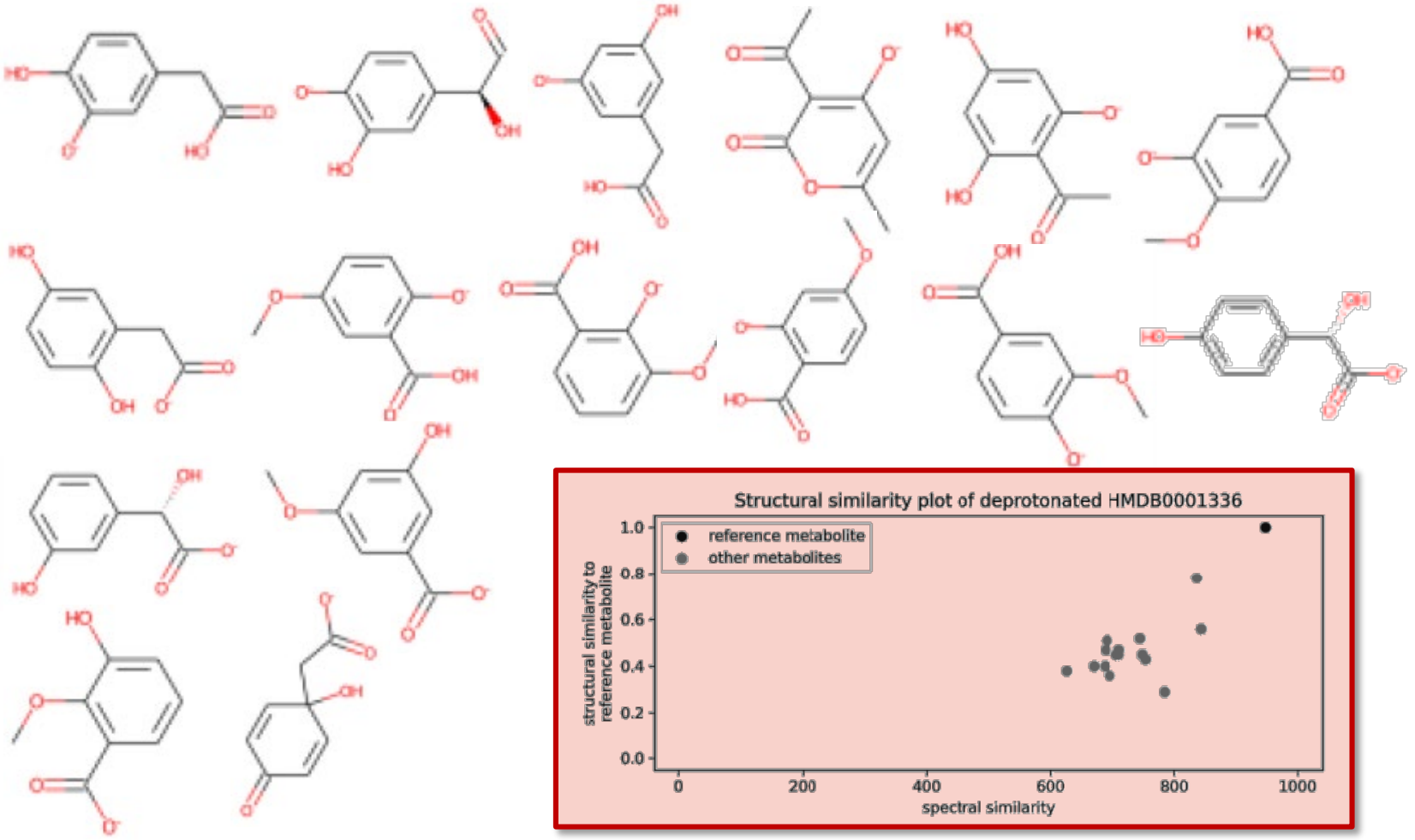
Houthuijs, Berden, Engelke, Gautam, Wishart, Wevers, Martens, Oomens, *Anal. Chem.* **2023**, *95*, 8998

AUTOMATIC FINDING OF THE CORRECT ISOMER



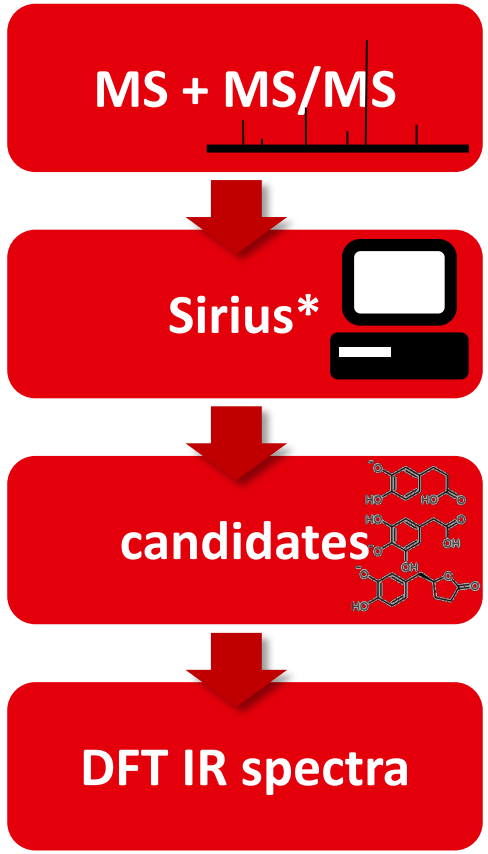
DOPAC, $C_8H_9O_4$
m/z 167 (- ESI)
18 isomers in HMDB
16 deprotonatable

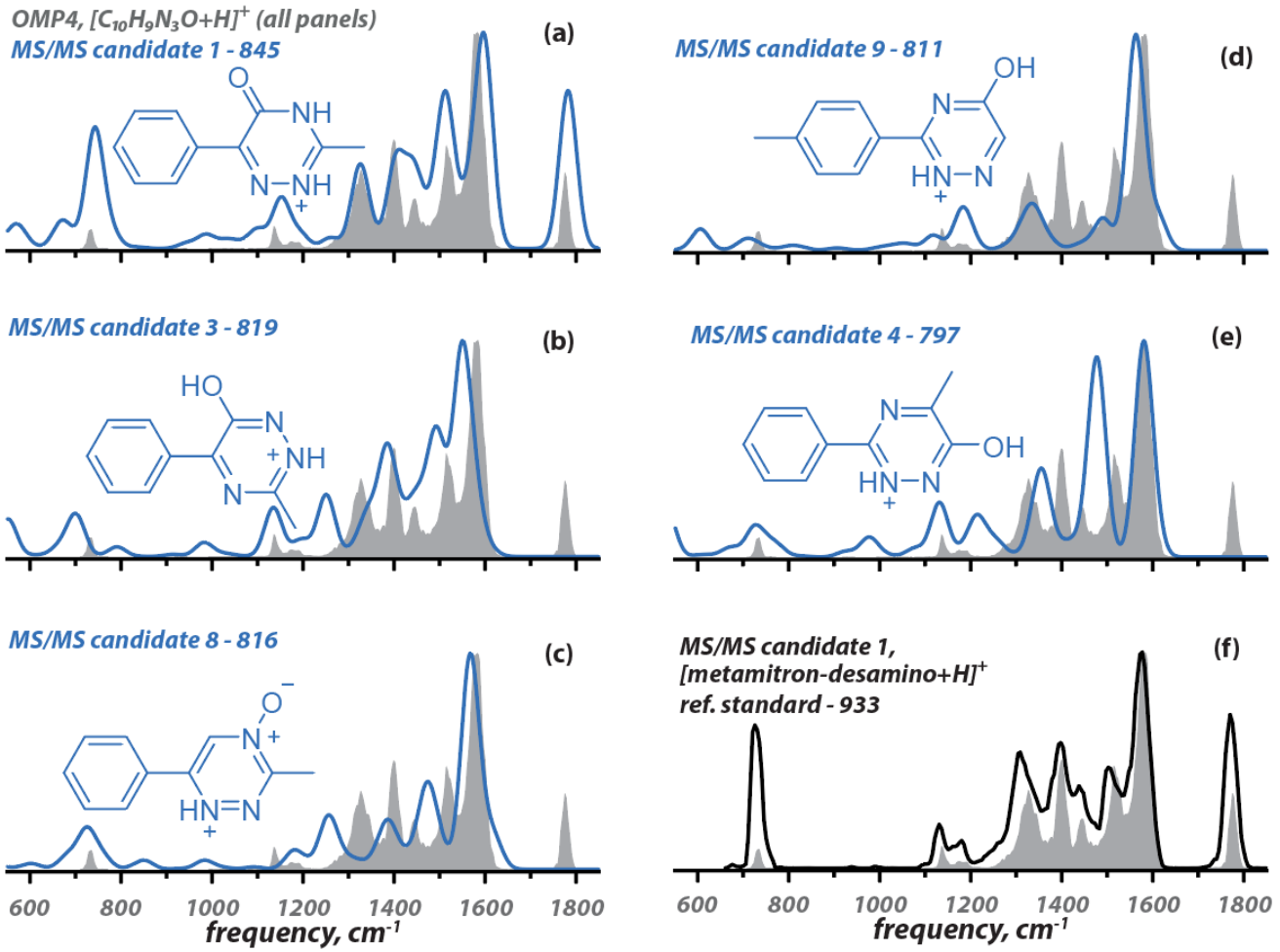
Result:
DOPAC returned as #1 match



Houthuijs, Berden, Engelke, Gautam, Wishart, Wevers, Martens, Oomens, *Anal. Chem.* **2023**, *95*, 8998

COMBINED MS/MS + IRIS IDENTIFICATION





* Dührkop, Fleischauer, Ludwig, Aksenov, Melnik, Meusel, Dorrestein, Rousu, Böcker, *Nature Meth.* 2019, *16*, 299

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CONCLUSION AND OUTLOOK

Combine with MS toolbox

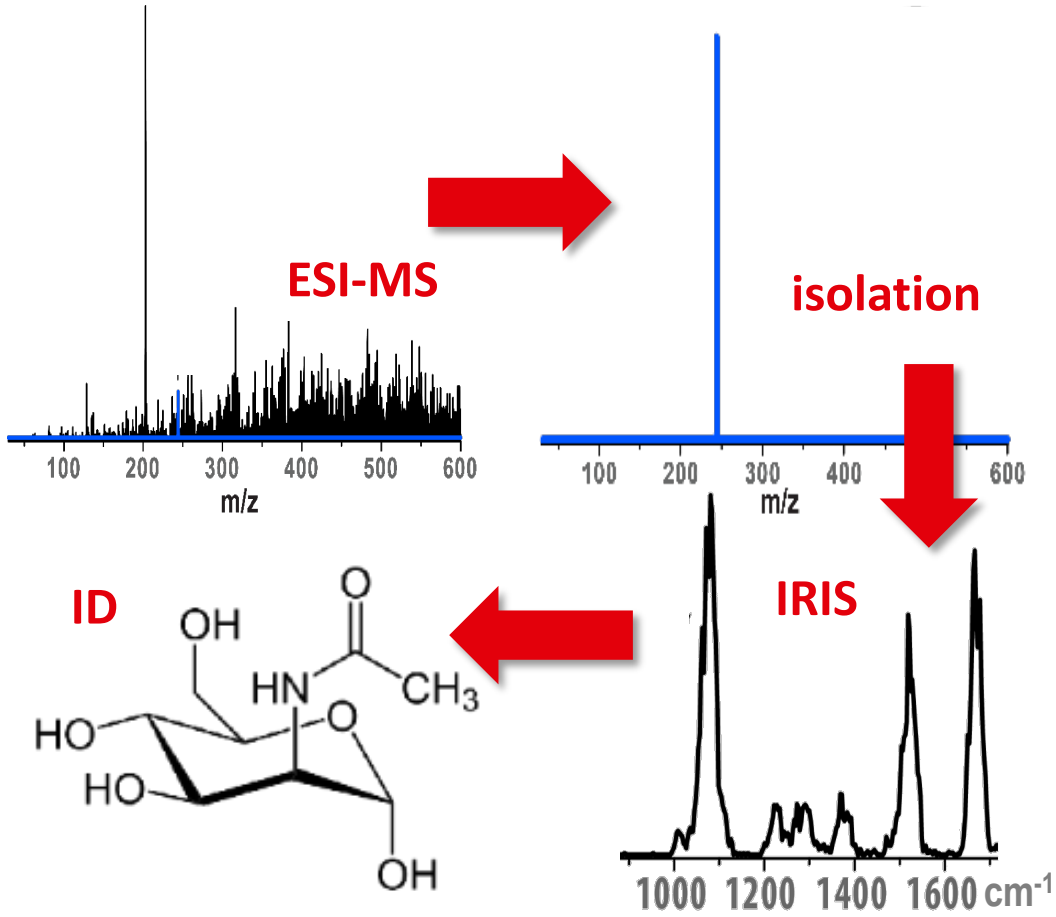
- Ion mobility & LC separation
- Accurate mass determination
- MALDI imaging

New analytical applications

- Environmental: water contaminants, air pollutants
- Forensics: novel psychoactive substances
- Drug metabolism
- Synthetic/organic chemistry

New methodologies

- 2-color
- Strategies for rapid identification
- Benchtop IRIS



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