Symposium EU-Russia: Prospects for Cooperation in Biotechnology in the Seventh Framework Programme

An Analytical Platform for Environmental, Plant and Microbial Metabolomics Based on Ultrahigh Resolution Mass Spectrometry (FTMS)

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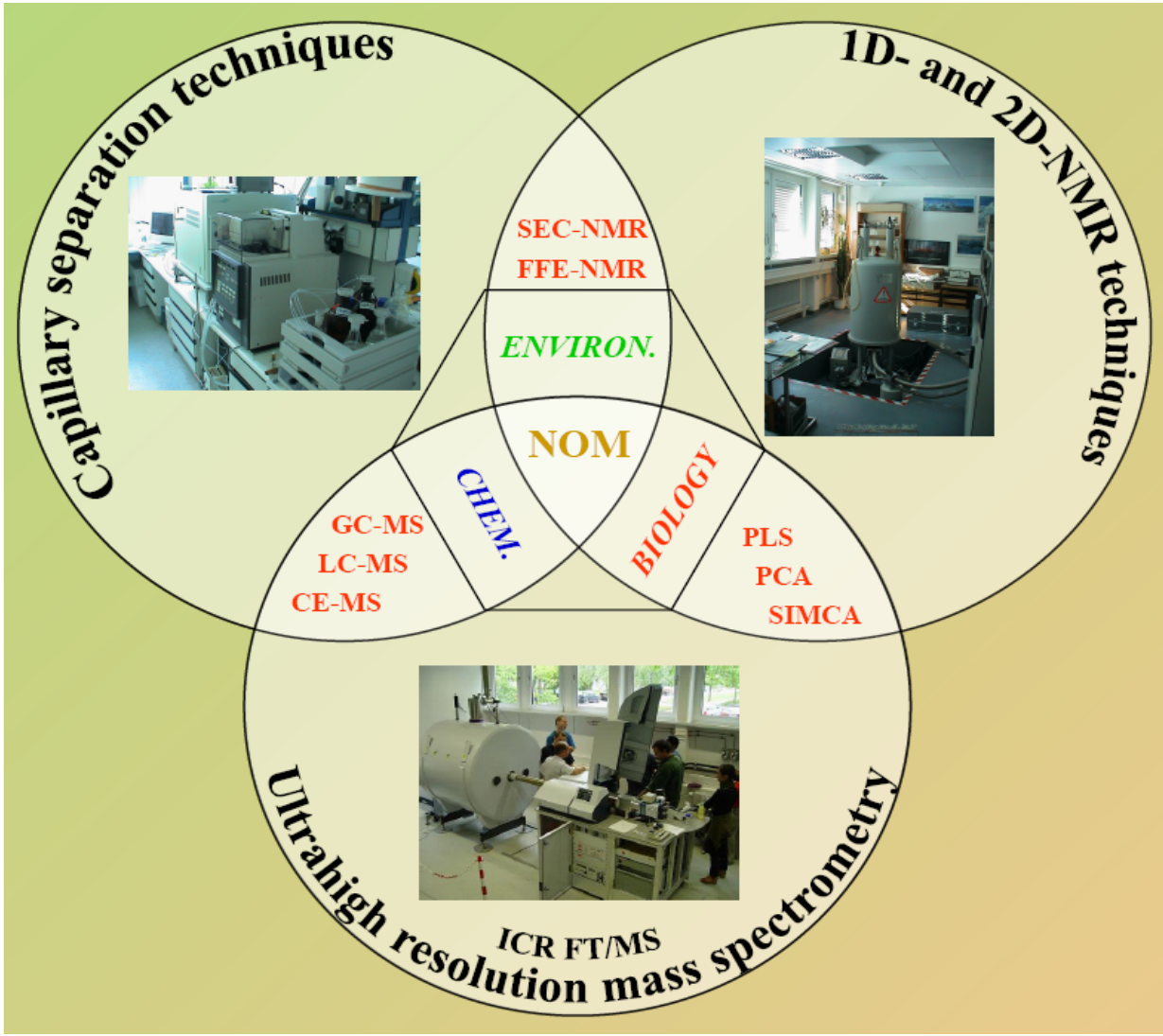
Molecular BioGeoAnalysis

Munich, Germany

Analytical platform



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FTICR Principles

Analytical approaches

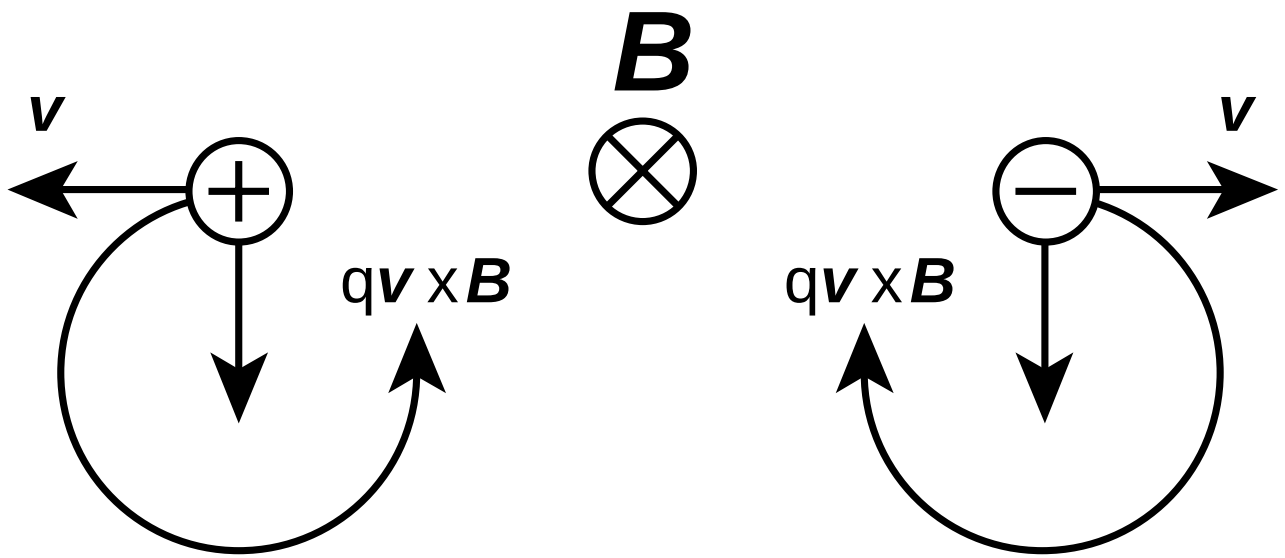
Analysis of interactions in the Rhizosphere:

***N*-Acylhomoserine lactones and Quorum Sensing**

Mechanism of PGPR activity in soil Actinobacteria

Other Projects

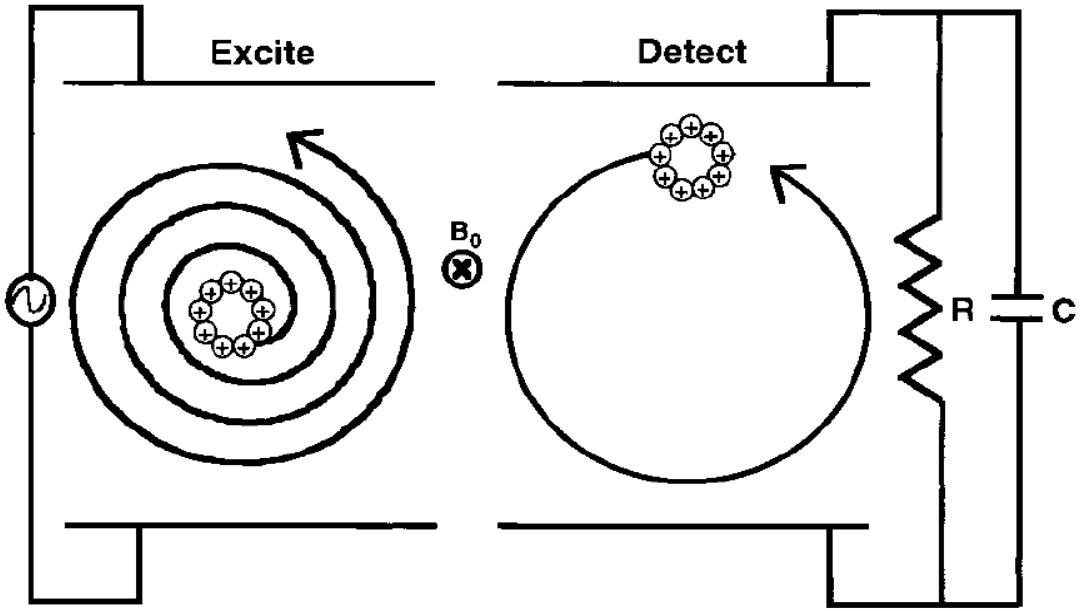
FTMS basics



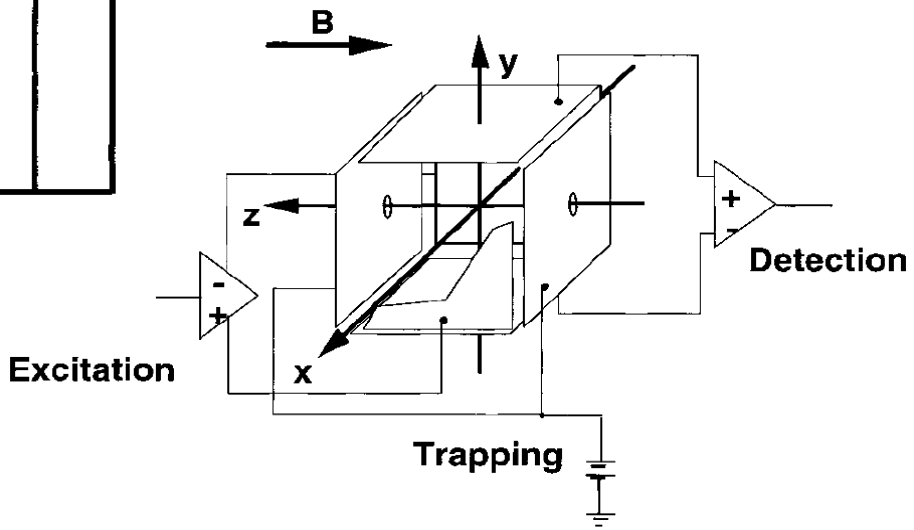
$$\omega_c = \frac{qB_0}{m}$$

Marshall et al., *Mass Spectrom. Rev.*, 1998, **17**, 1

FTMS basics

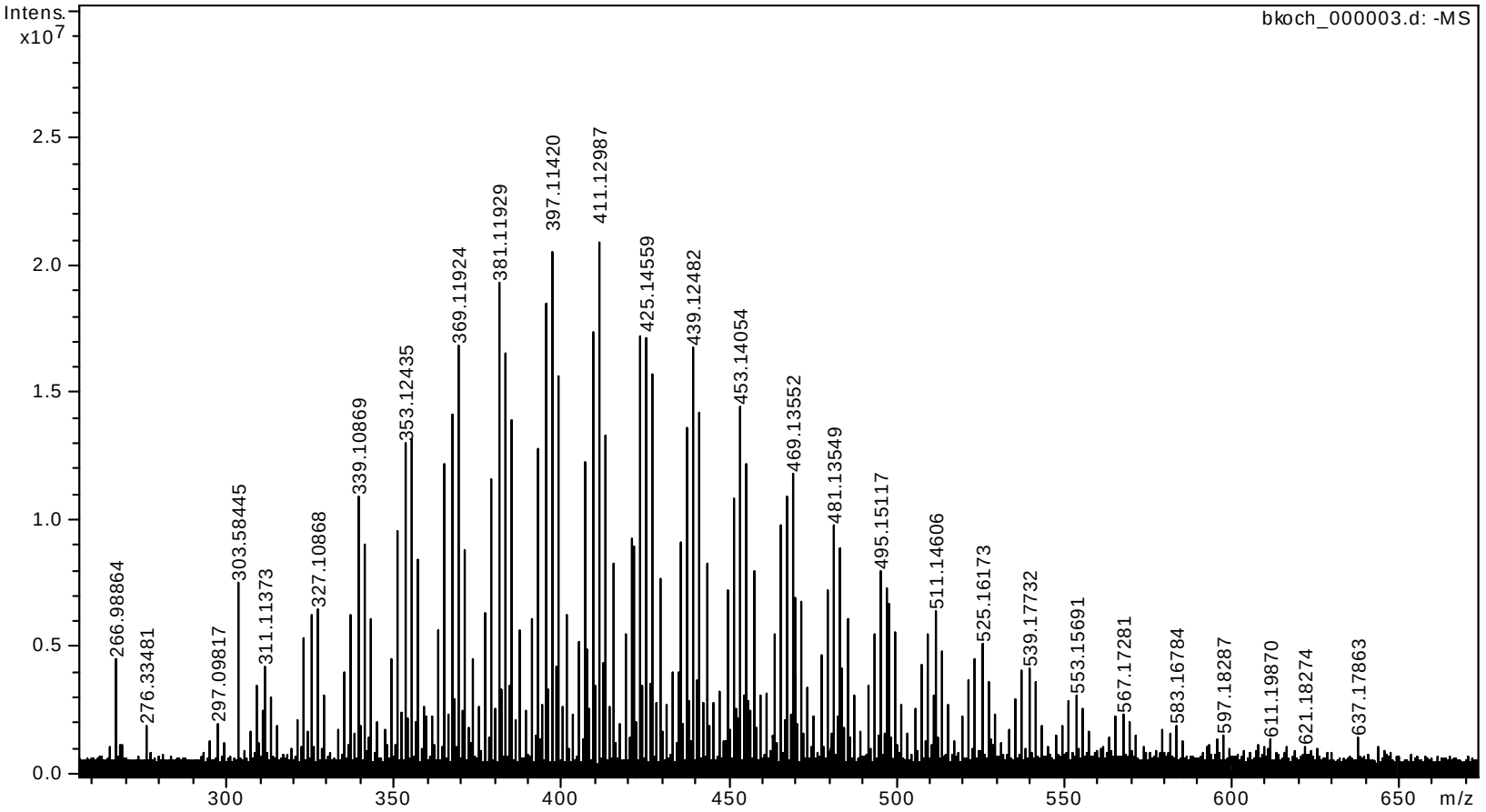


$$\omega_c = \frac{qB_0}{m}$$



Marshall et al., *Mass Spectrom. Rev.*, 1998, **17**, 1

Ultrahigh resolution



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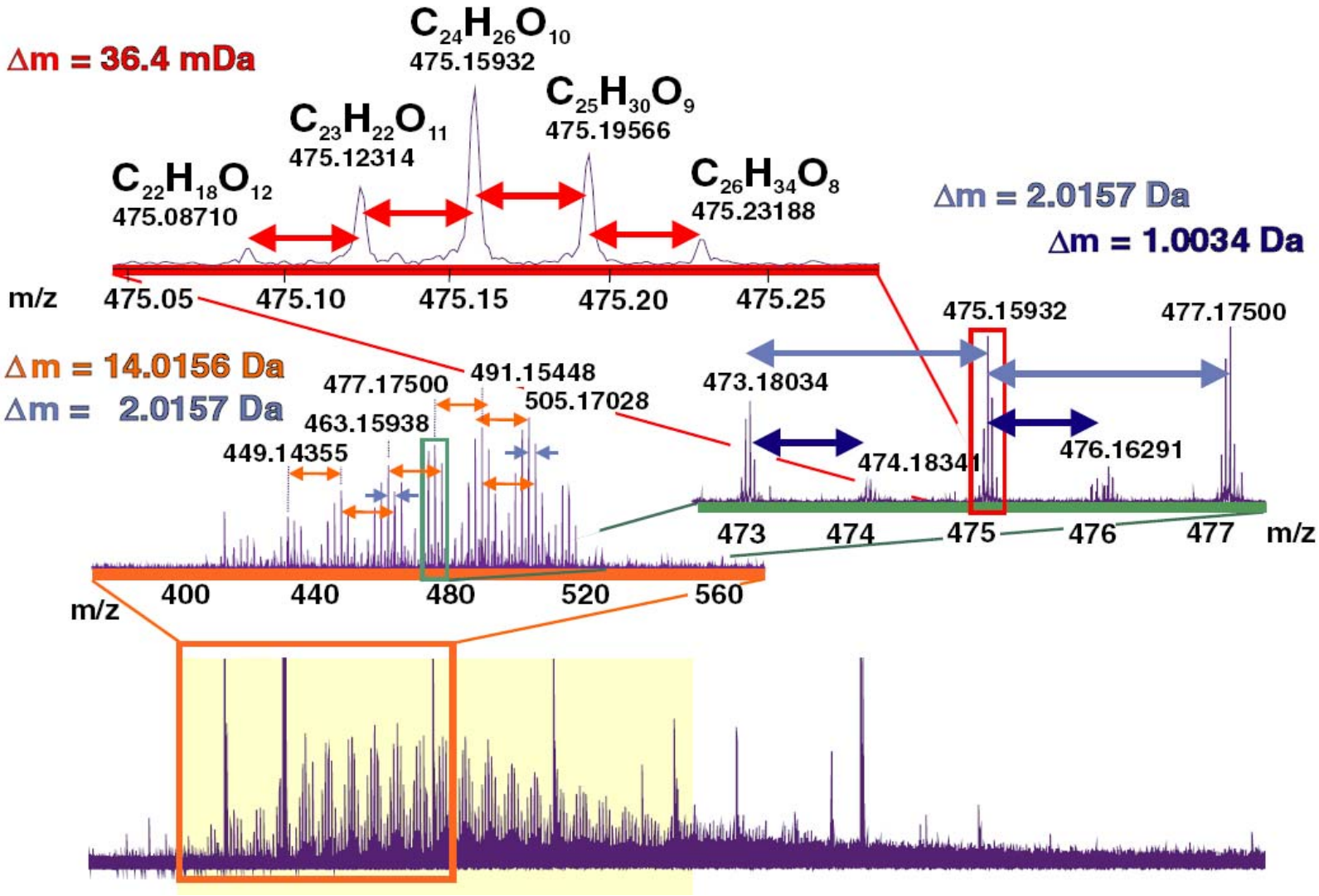

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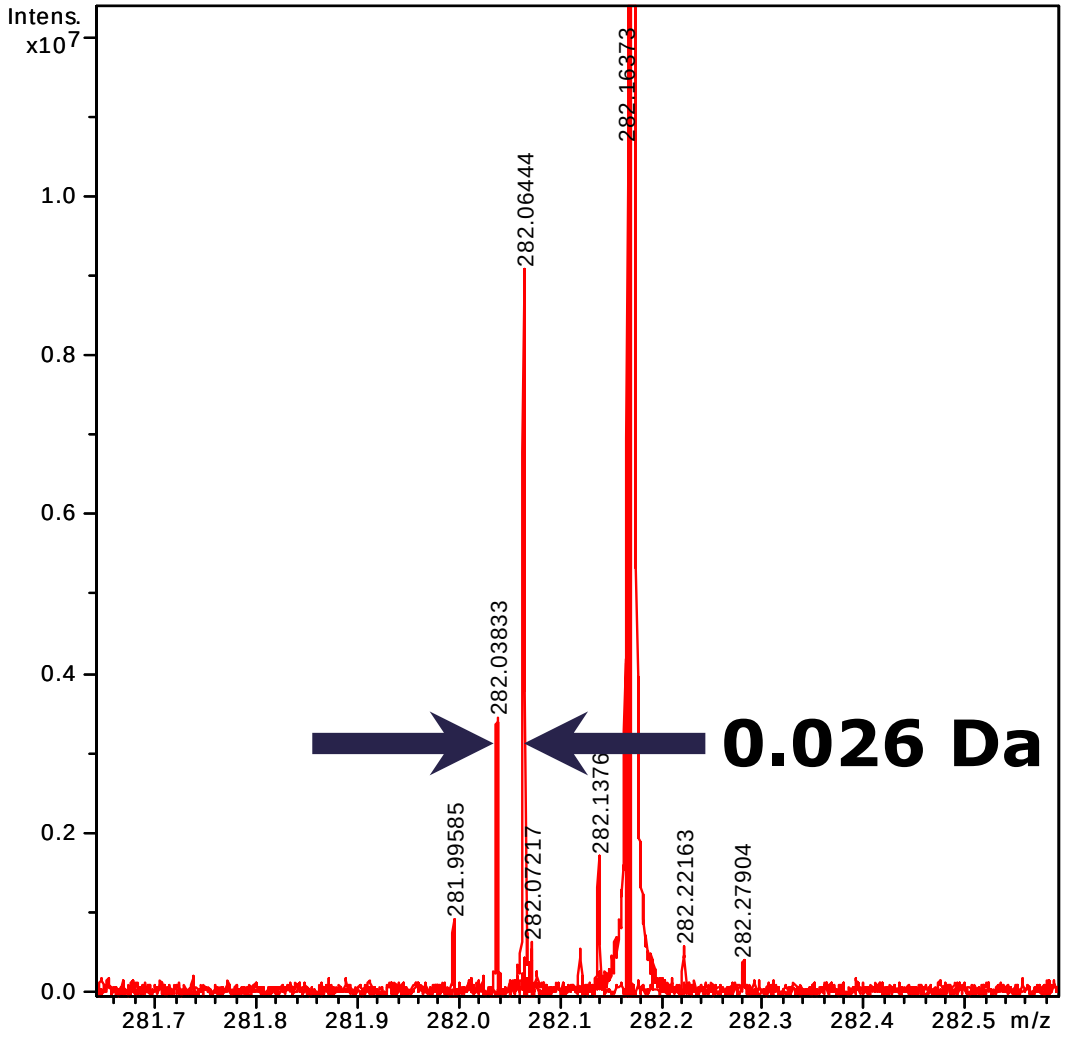


Ultrahigh mass precision

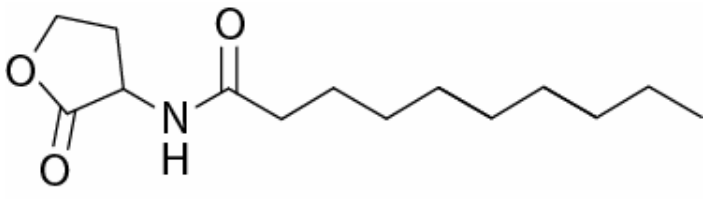
	Exact mass [Da]	Error [μDa]
^{1}H	1.00782503214	0.00035
^{2}H	2.01410177799	0.00036
^{3}H	3.0160492675	0.0011
^{3}He	3.01602930970	0.00086
^{4}He	4.0026032497	0.0010
^{13}C	13.0033548378	0.0010
^{12}C	12.0000000000	0.0010
^{14}C	14.0032419884	0.0040
^{14}N	14.00307400524	0.00086
^{15}N	15.00010889844	0.00092
^{16}O	15.9949146221	0.0015
^{17}O	16.999131501	0.22
^{18}O	17.999160419	0.9
^{20}Ne	19.9924401759	0.0020
^{23}Na	22.989769675	0.23
^{28}Si	27.9769265327	0.0020
^{31}P	30.973761512	0.20
^{32}S	31.972070690	0.12
^{34}S	33.967866831	0.11
^{39}K	38.963706861	0.3
^{40}Ar	39.9623831232	0.0030

Audi, G. & Wapstra, A. H. *Nucl. Phys. A,* 1995, **595**, 409

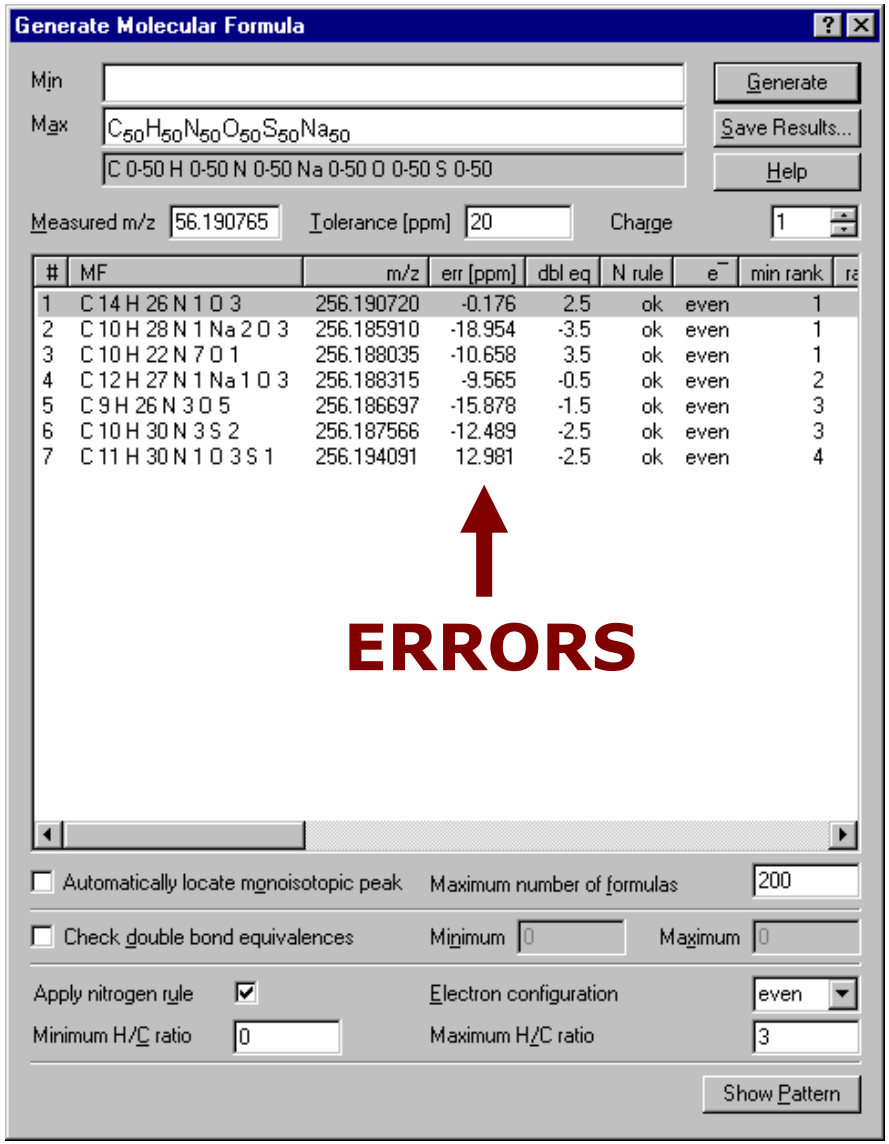
Ultrahigh mass precision

Only limited number of elemental combinations *fits* into an exact molecular mass

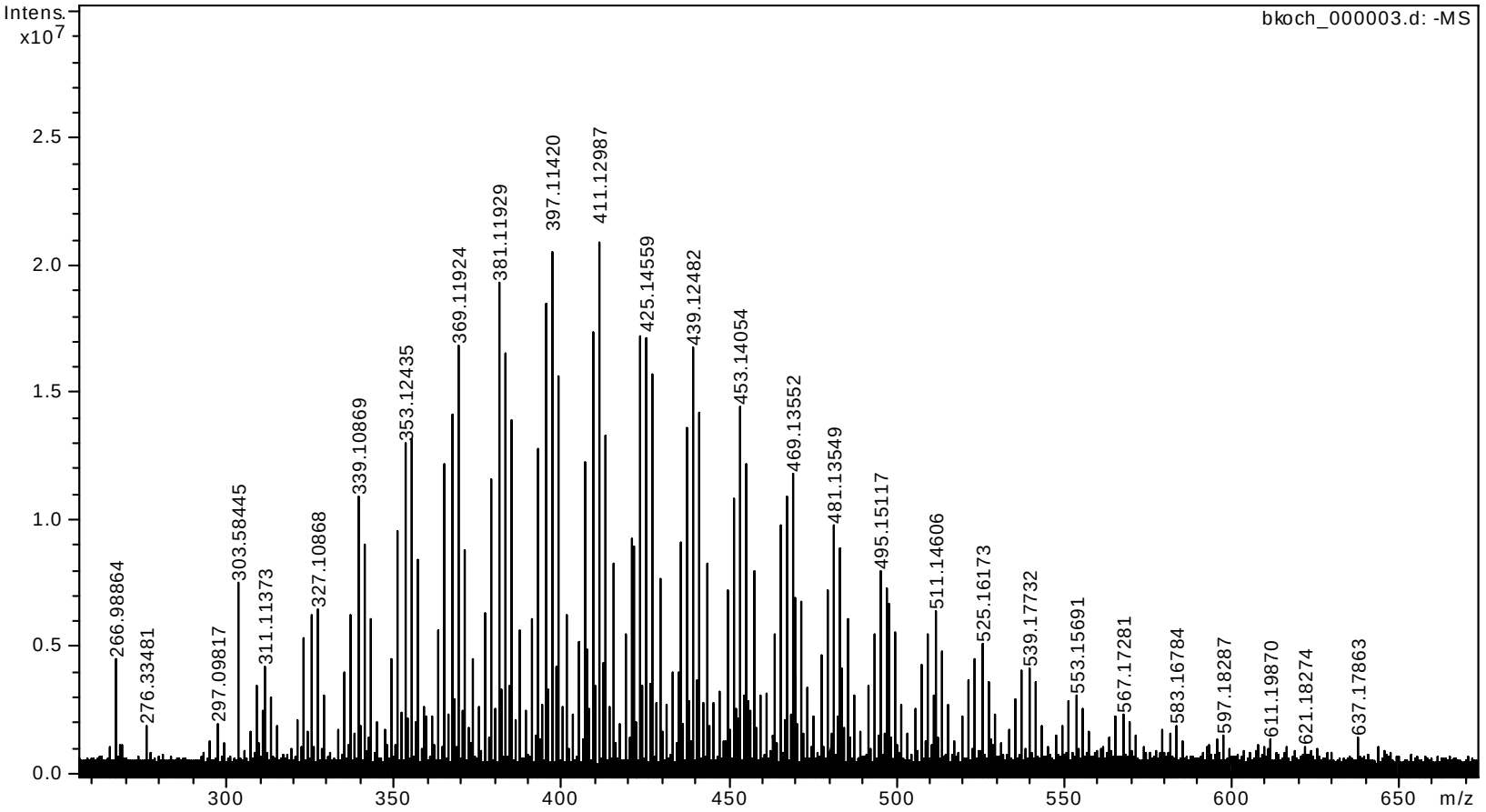
$C_{14}H_{26}NO_3$ →



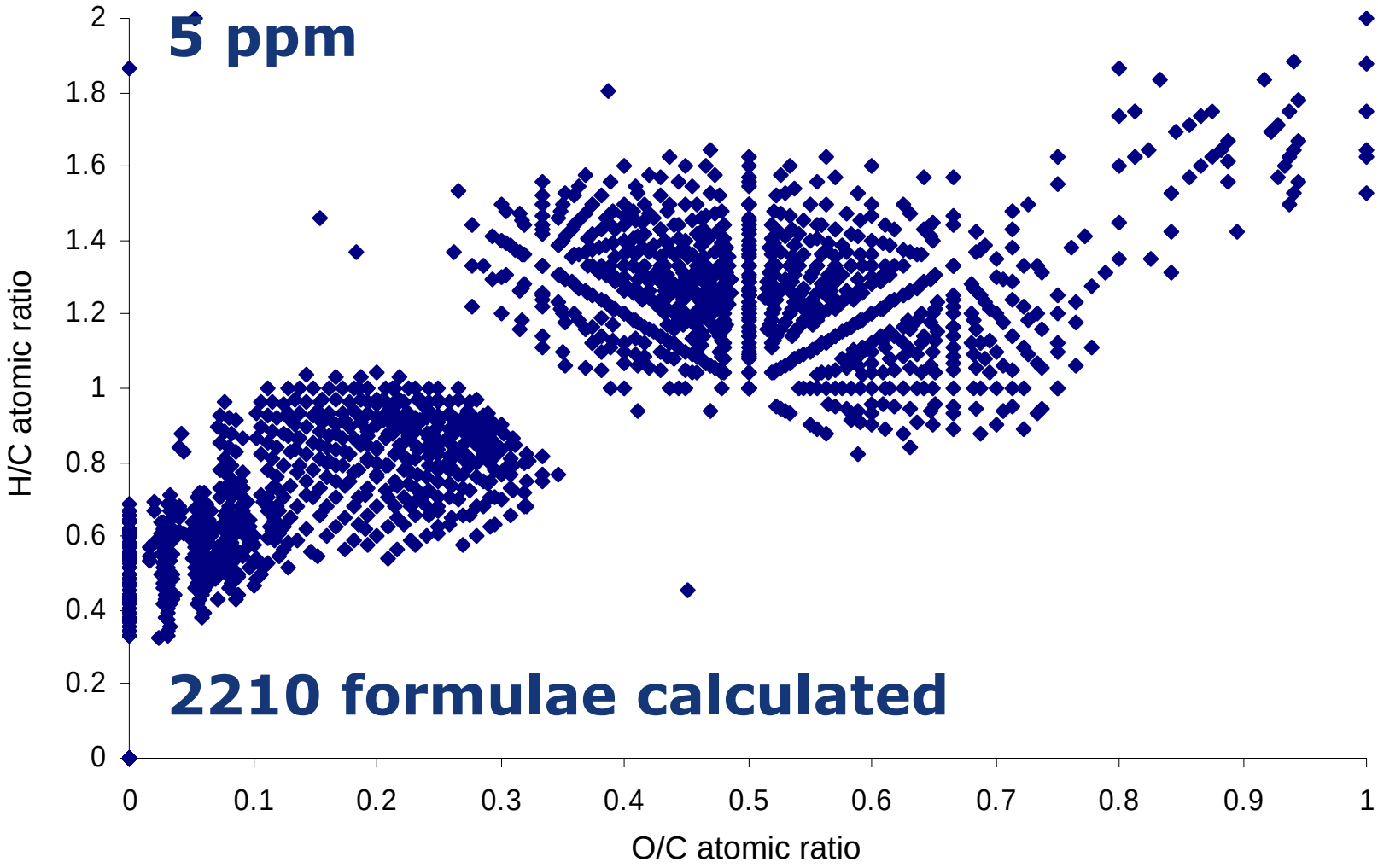
$C_{14}H_{25}NO_3 + H^+$



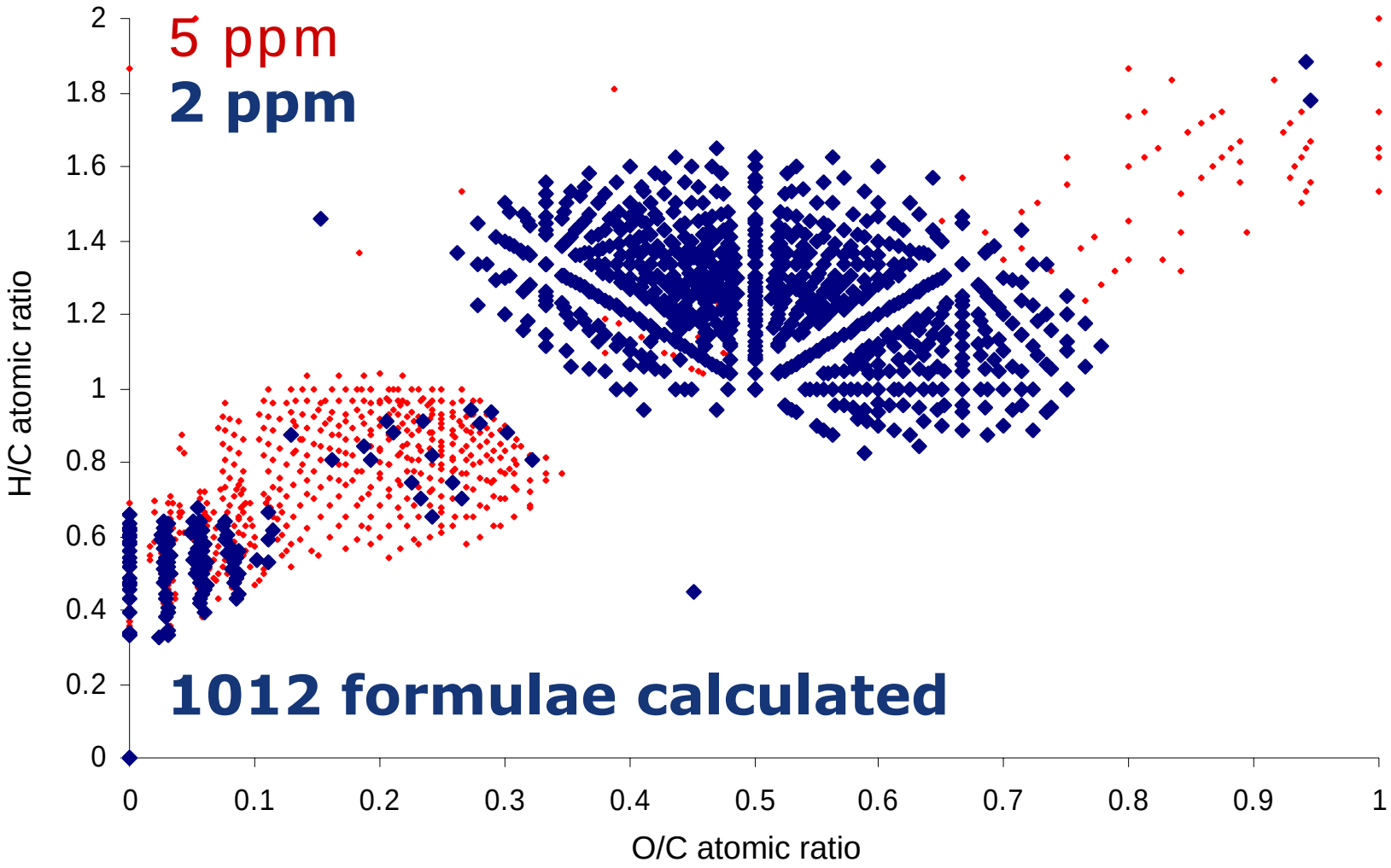
Ultrahigh resolution



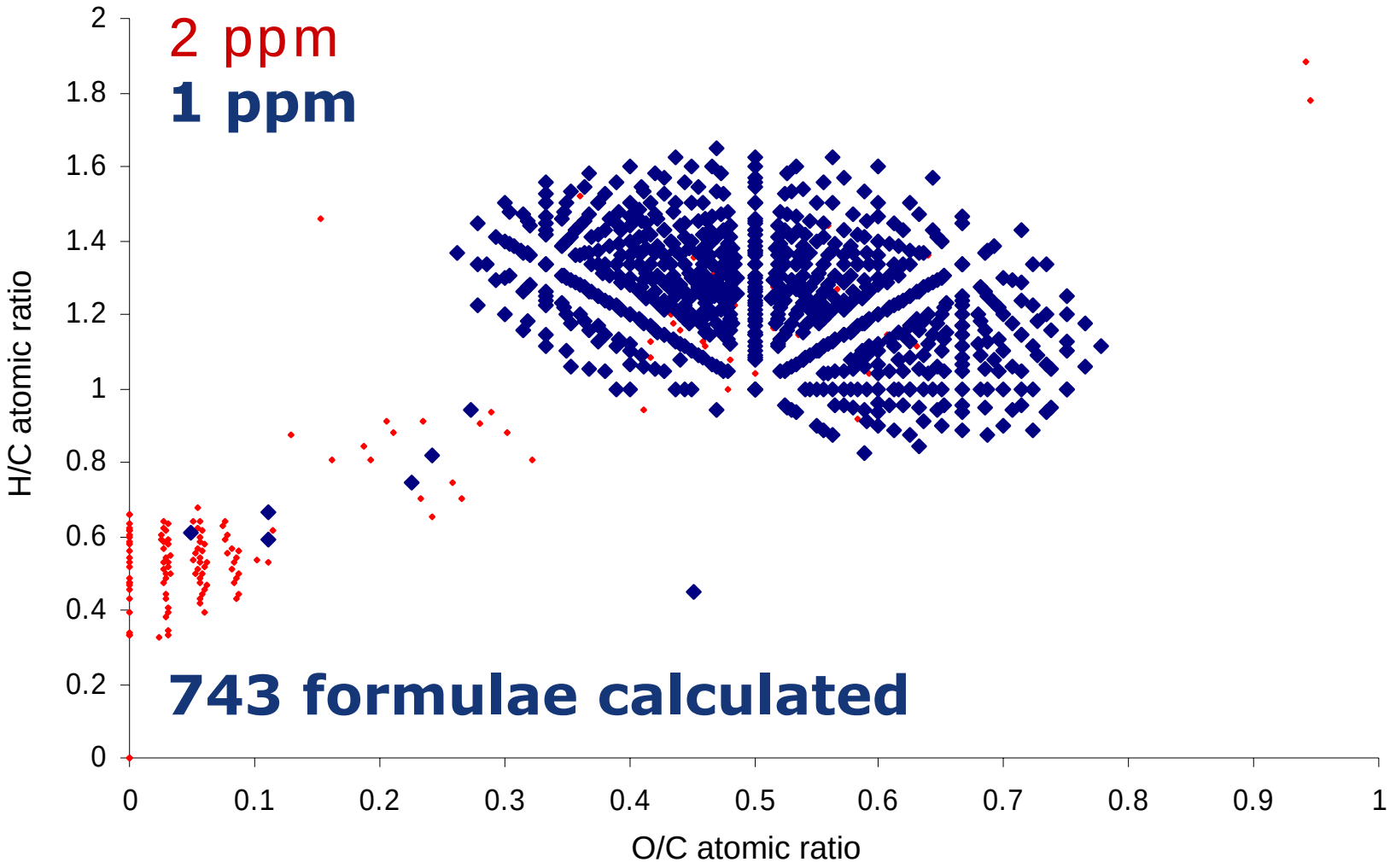
Bias elemination by mass precision



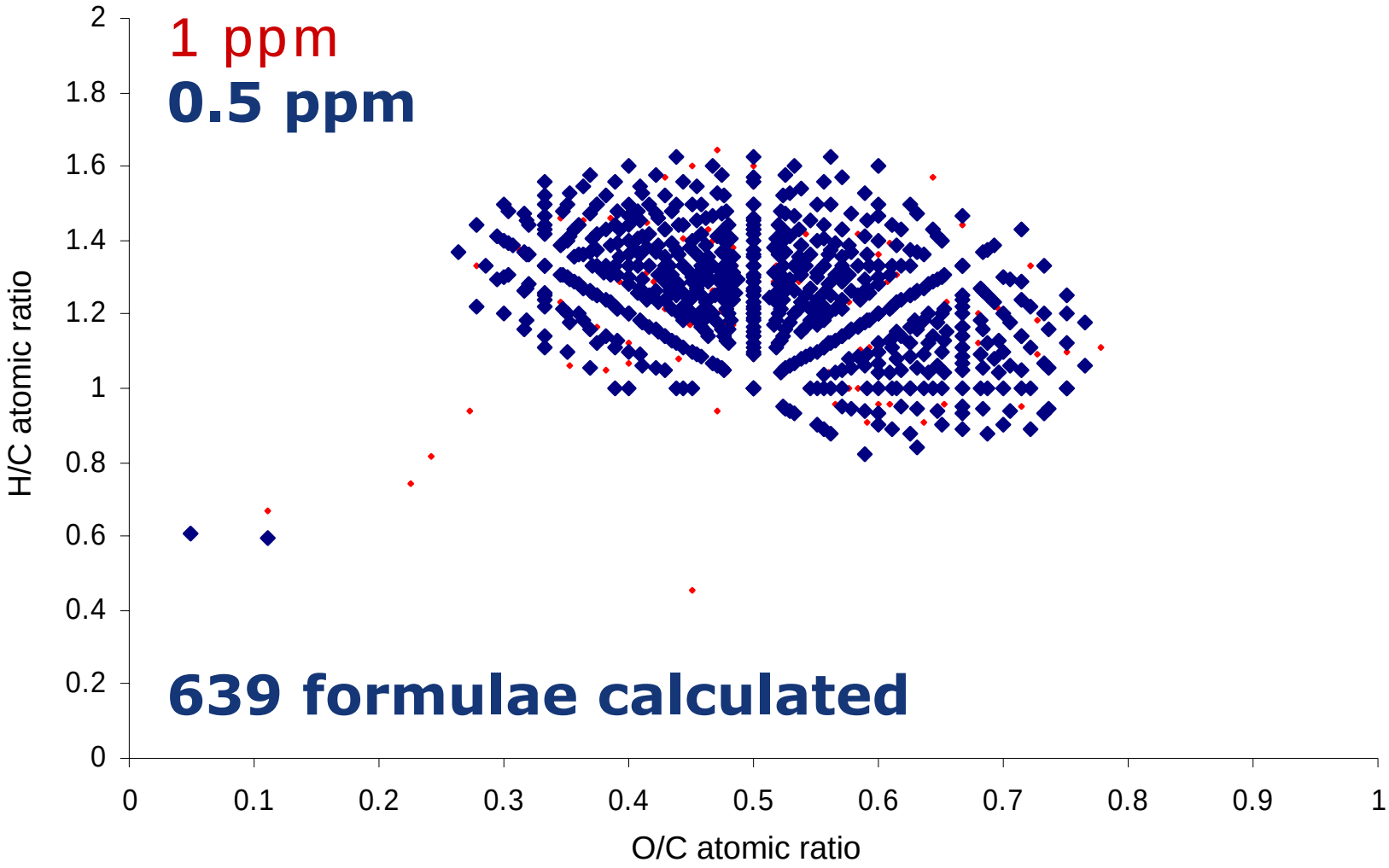
Bias elemination by mass precision



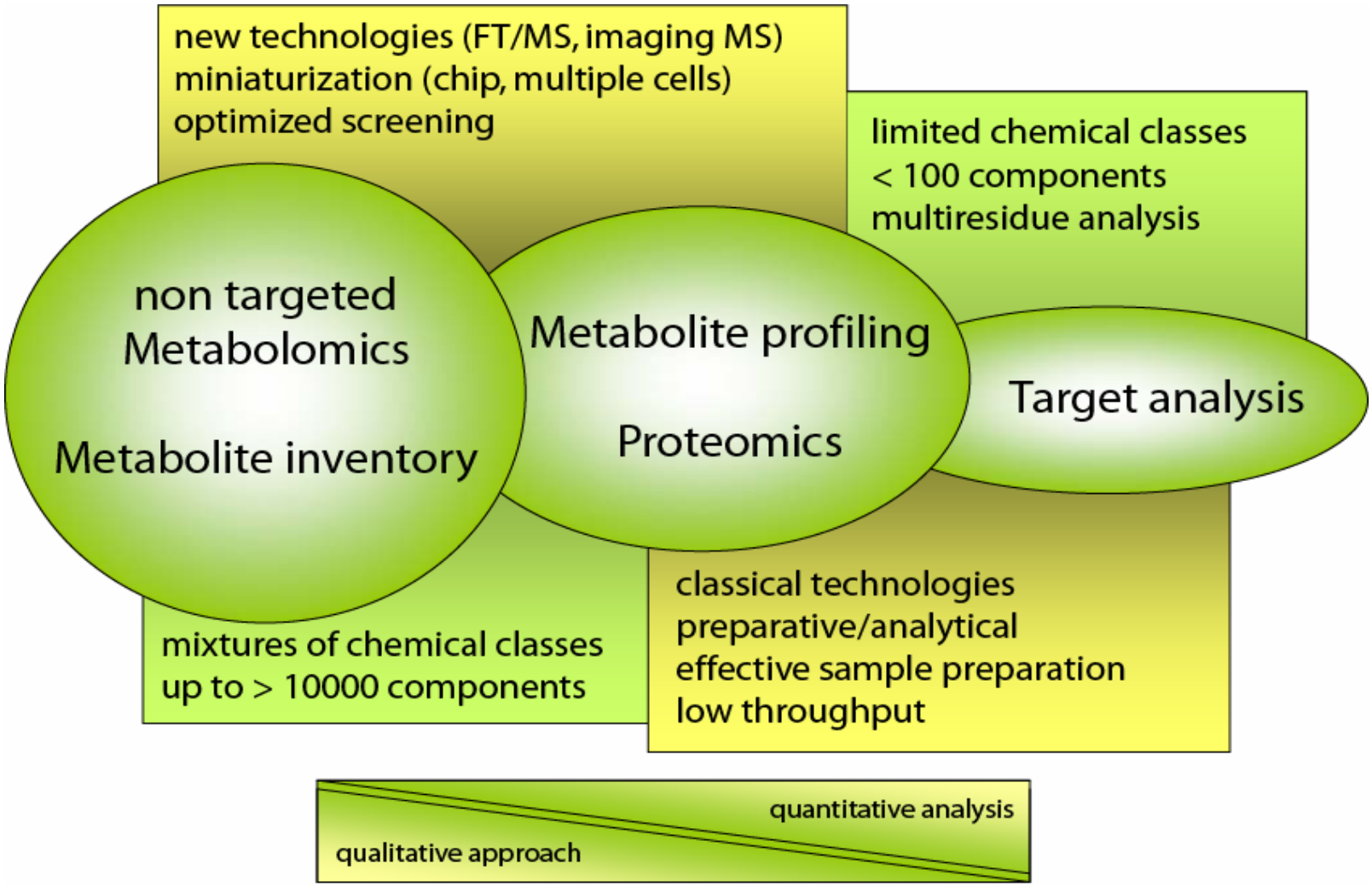
Bias elemination by mass precision



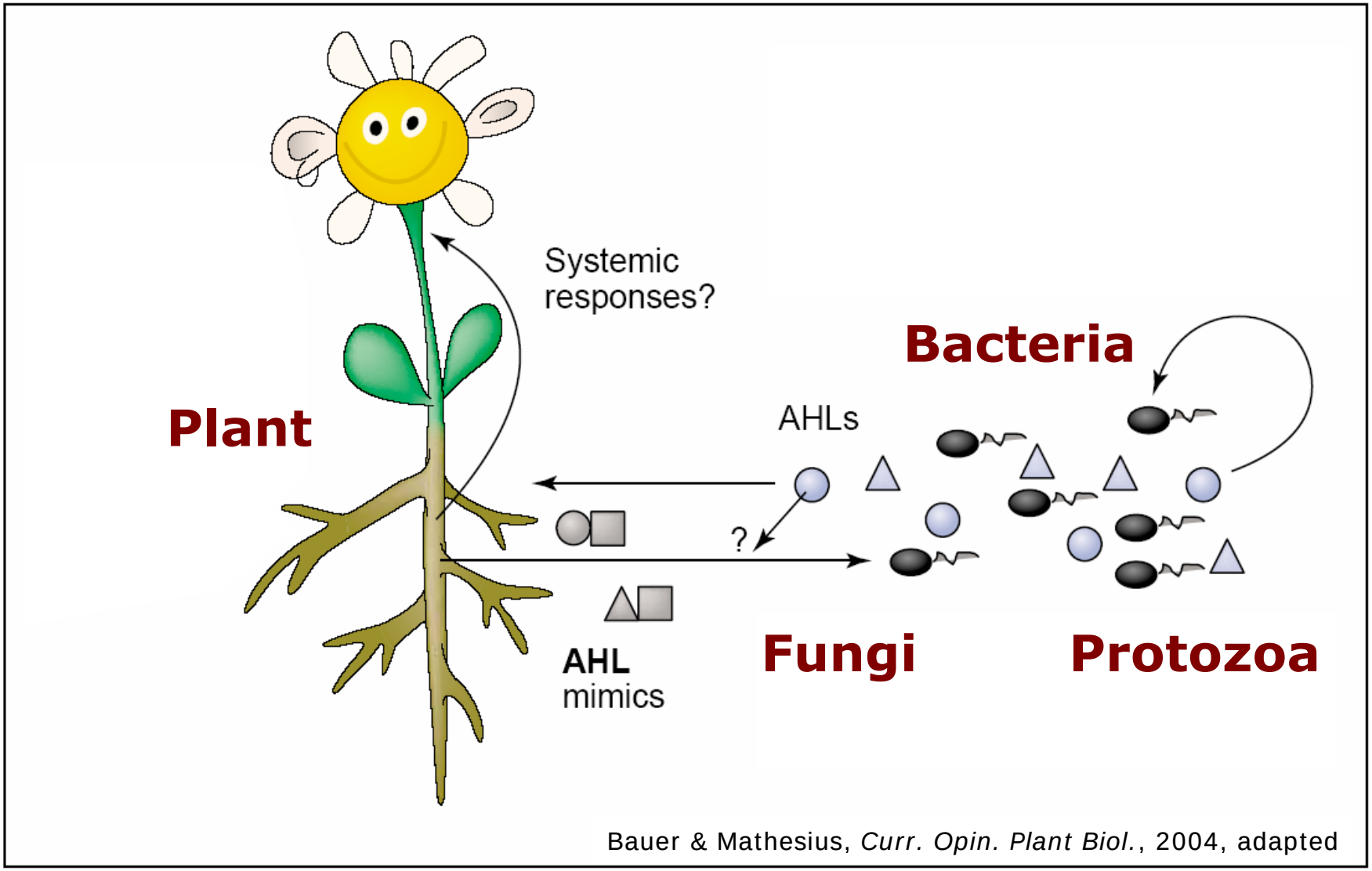
Bias elemination by mass precision



Fields of interest

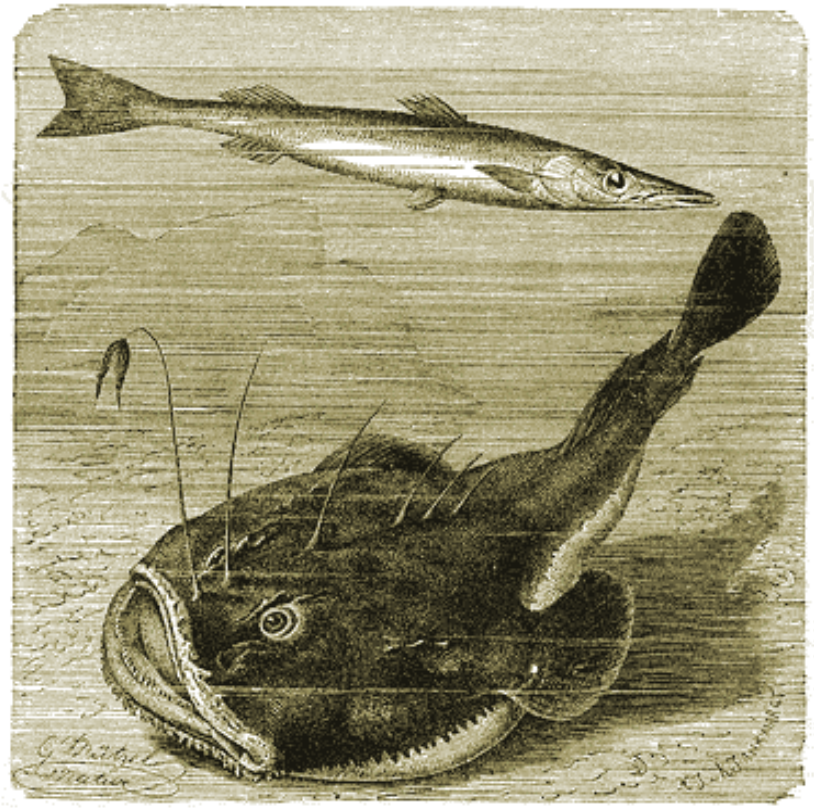


Rhizosphere interactions



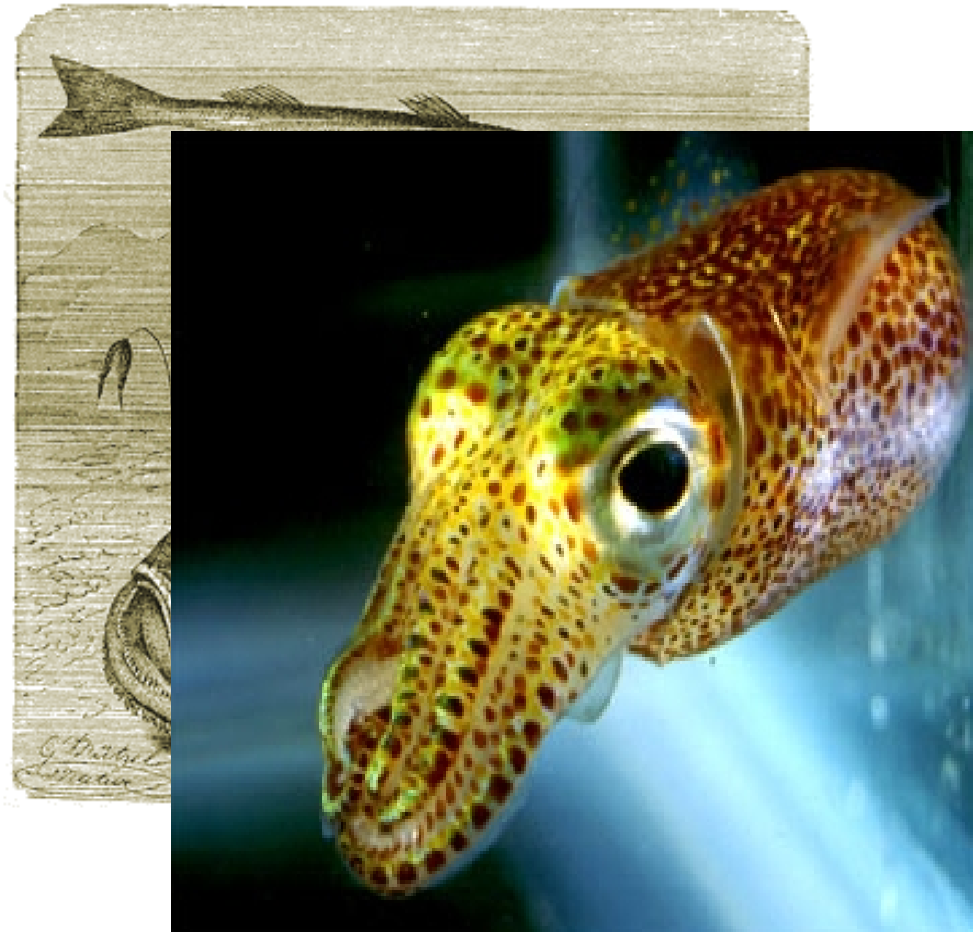
Bauer & Mathesius, *Curr. Opin. Plant Biol.*, 2004, adapted

Quorum Sensing



Fuqua & Greenberg, *Nat. Rev. Mol. Cell Biol.,* 2002, **3**, 4111

Quorum Sensing



Finding: luminescent bacteria from marine symbioses regulate light production in dependance of their cell density.

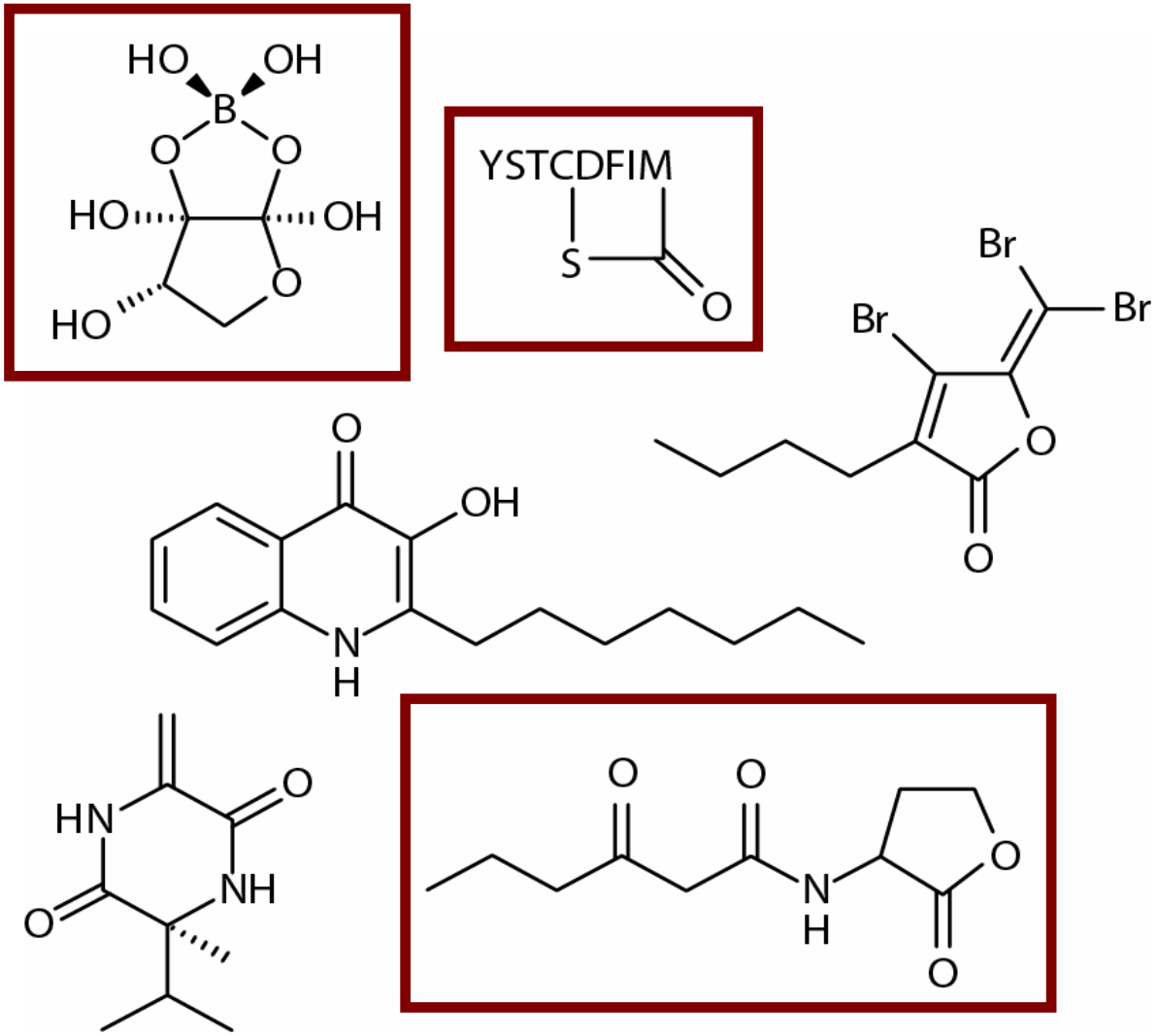
Explanation: changes in the observed “physiological behavior” are a consequence of production, accumulation of, and reaction to, specific signalling molecules.

Conclusion: bacteria are able to actively communicate, not only to react to.

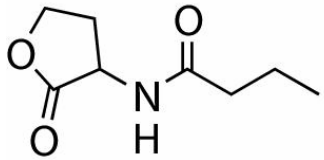
➔ First step to multicellularity?

Fuqua & Greenberg, *Nat. Rev. Mol. Cell Biol.,* 2002, **3**, 4111

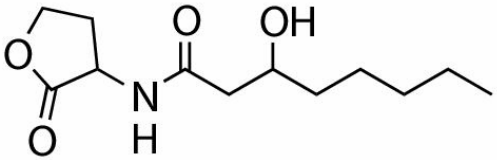
Signalling molecules



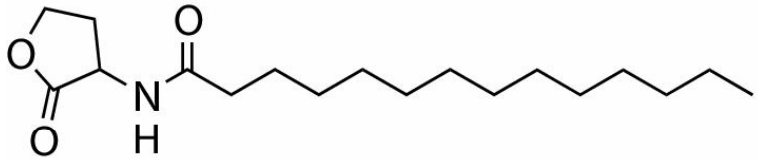
N-Acylhomoserine Lactones



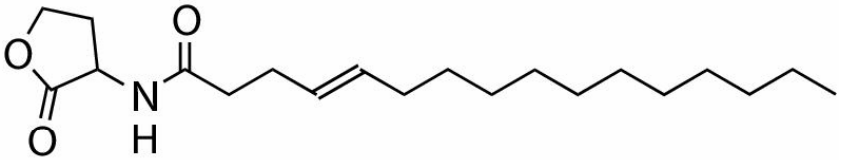
N-Butanoyl-D/L-Homoserinlacton
C4-HSL



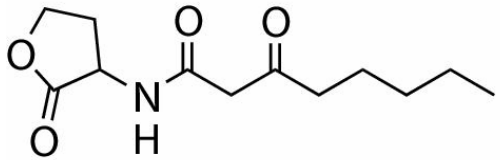
3-Hydroxy-*N*-Octanoyl-D/L-Homoserinlacton
OH-C8-HSL



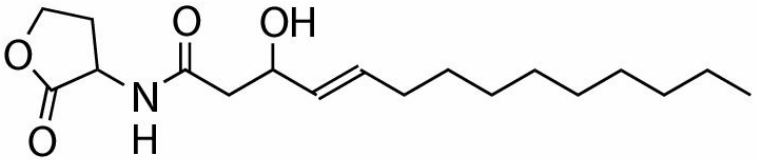
N-Tetradecanoyl-D/L-Homoserinlacton
C14-HSL



N-Hexadecenoyl-D/L-Homoserinlacton
C16:1-HSL

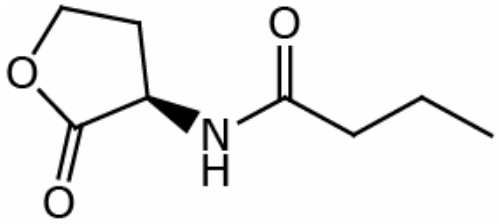


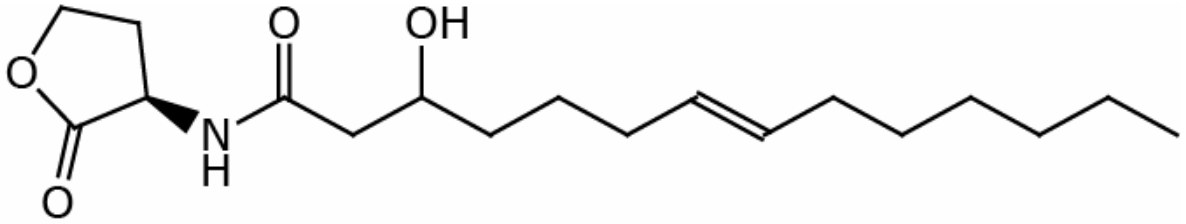
3-oxo-*N*-Octanoyl-D/L-Homoserinlacton
O-C8-HSL



3-Hydroxy-*N*-Tetradecenoyl-D/L-Homoserinlacton
OH-C14:1-HSL

Environment and Health

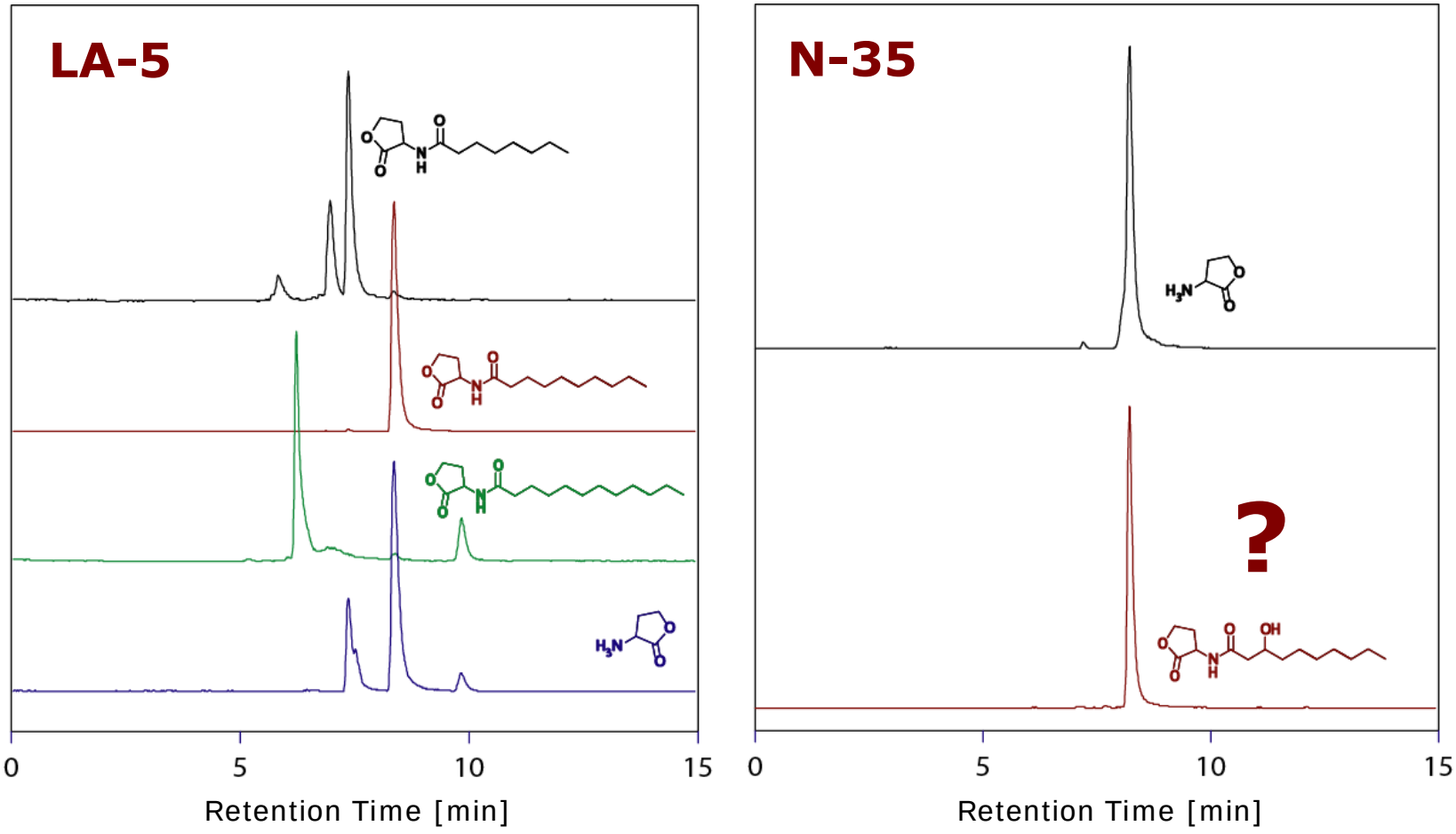




C4-HSL	**Aeromonas sp.**	**C8-HSL**	**Rhizobium leguminosarum**	**O-C6-HSL**	Rahnella aquatilis
	Pseudomonas sp.		**Serratia marcescens**		**Rhizobium leguminosarum**
	Serratia liquefaciens		**Sinorhizobium meliloti**	▶	**Serratia sp.**
	Vibrio harveyi		Vibrio fischeri		Vibrio fischeri
C6-HSL	**Aeromonas sp.**		**Yersinia pseudotuberculosis**		**Yersinia sp.**
▶	**Burkholderia sp.**	**C10-HSL**	▶ **Burkholderia sp.**	**O-C8-HSL**	**Enterobacter agglomerans**
	Chromobacterium violaceum		▶ **Pseudomonas fluorescens**		**Pseudomonas syringae**
	Escherichia coli	**C12-HSL**	**Brucella melitensis**	**O-C10-HSL**	Vibrio anguillarum
▶	**Pseudomonas sp.**		**Burkholderia vietnamensis**	**O-C12-HSL**	▶ **Pseudomonas aeruginosa**
	Ralstonia solanacearum	**C14-HSL**	**Burkholderia vietnamensis**	**O-C14-HSL**	**Sinorhizobium meliloti**
	Rhizobium leguminosarum	**C16-HSL**	Rhodobacter capsulatus	**O-C16-HSL**	**Sinorhizobium meliloti**
	Serratia sp.	**C18-HSL**	**Sinorhizobium meliloti**	**OH-C6-HSL**	**Pseudomonas fluorescens**
	Vibrio sp.	**O-C6-HSL**	**Aeromonas hydrophila**		**Vibrio anguillarum**
	Yersinia sp.		**Aeromonas salmonicida**	**OH-C8-HSL**	**Pseudomonas fluorescens**
C7-HSL	**Rhizobium leguminosarum**		**Enterobacter agglomerans**		**Rhizobium leguminosarum**
	Serratia marcescens		**Erwinia sp.**	**OH-C10-HSL**	**Pseudomonas fluorescens**
C8-HSL	▶ **Burkholderia sp.**		**Hafnia alvei**	**OH-C14:1-HSL**	**Pseudomonas fluorescens**
	Pseudomonas chlororaphis		Nitrosomonas europaea		**Rhizobium leguminosarum**
	Ralstonia solanacearum		**Pseudomonas sp.**	**C16:1-HSL**	**Sinorhizobium meliloti**

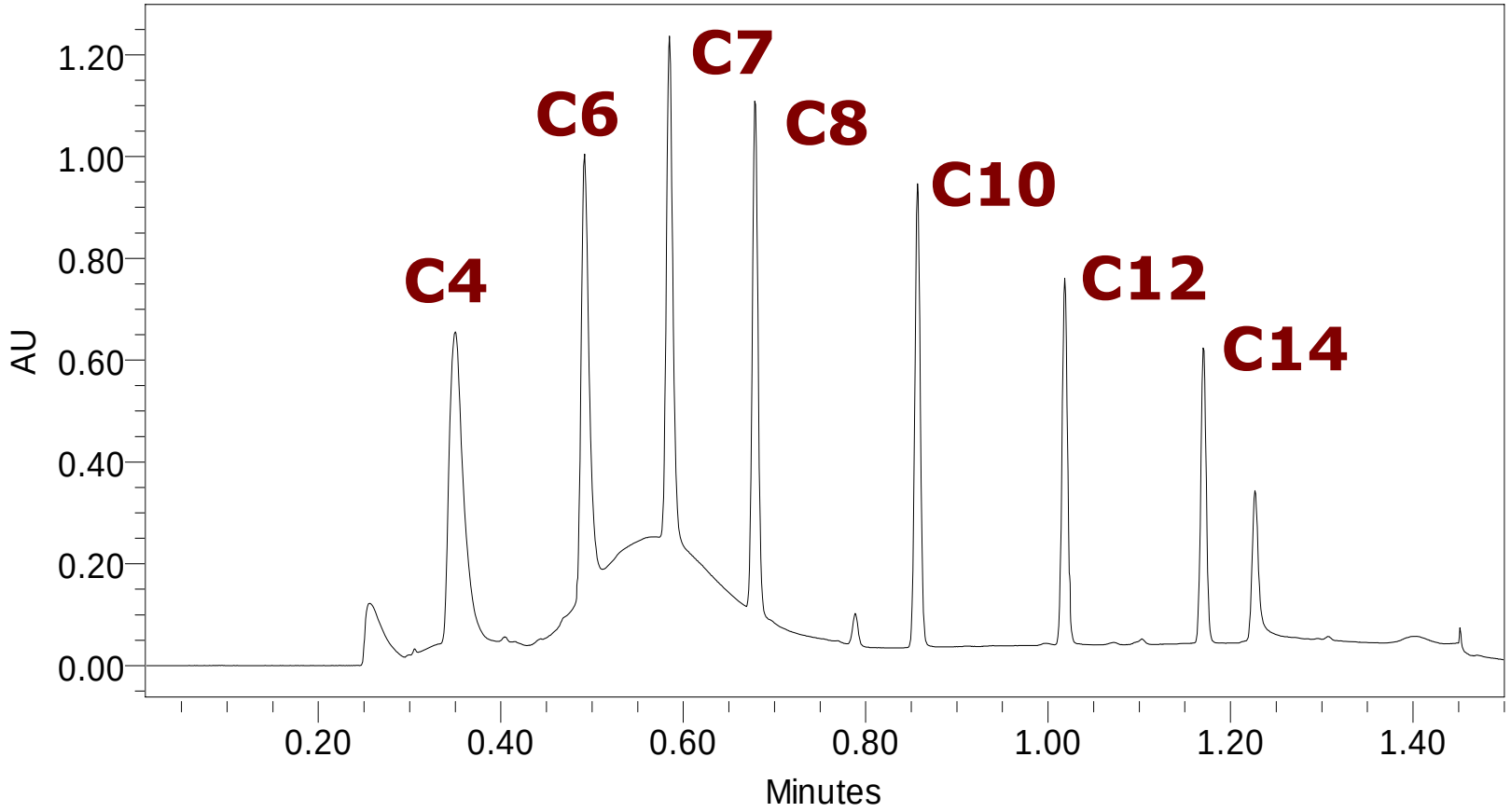
Targeting rhizosphere bacteria

Nano-LC/Ion Trap MS



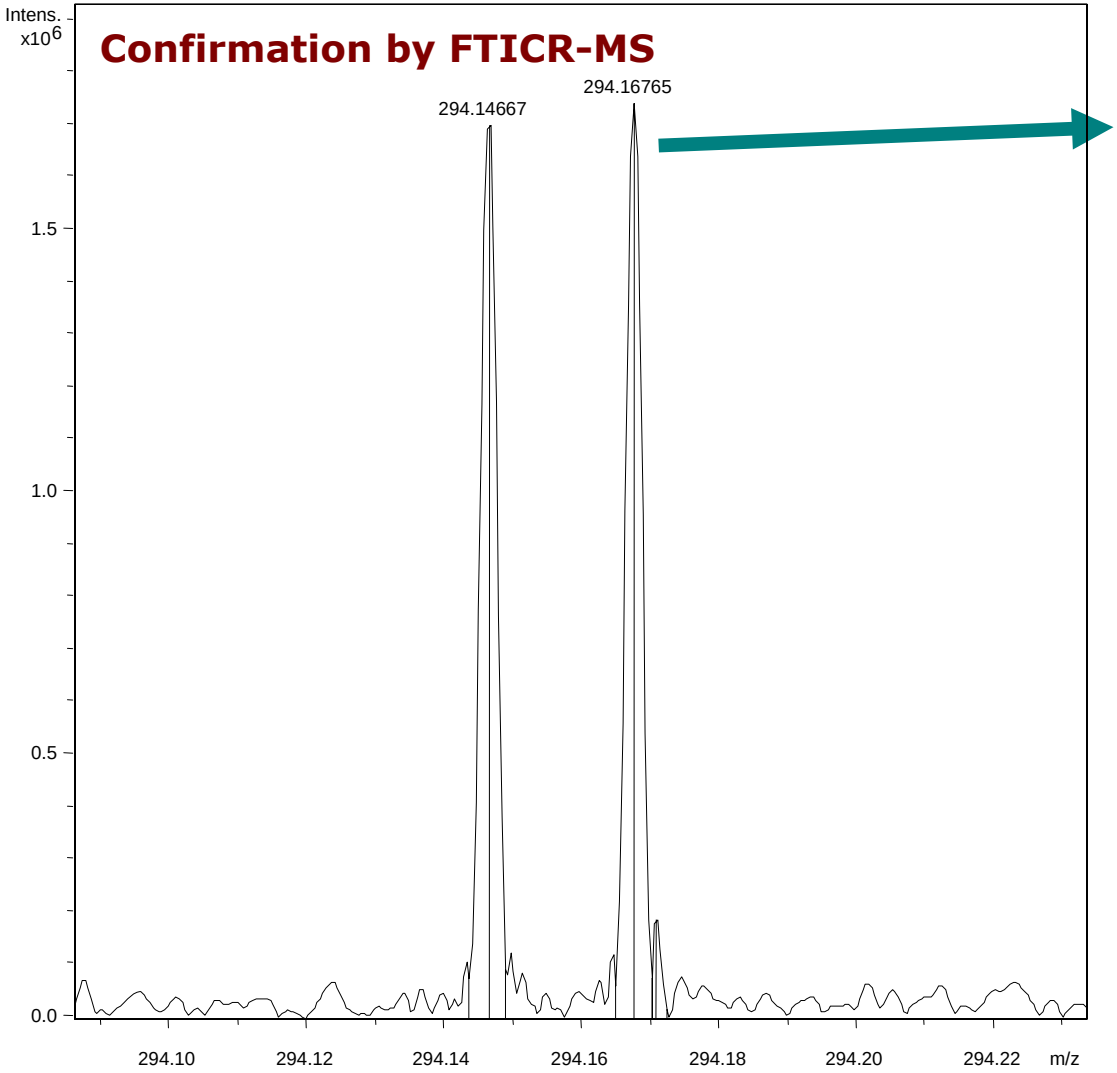
Frommberger et al., *Anal. Bioanal. Chem*., 2004, **378**, 1014

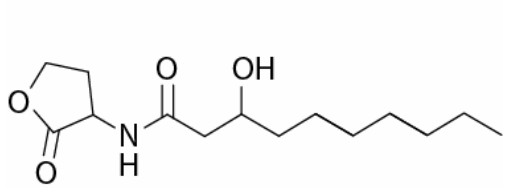
UPLC



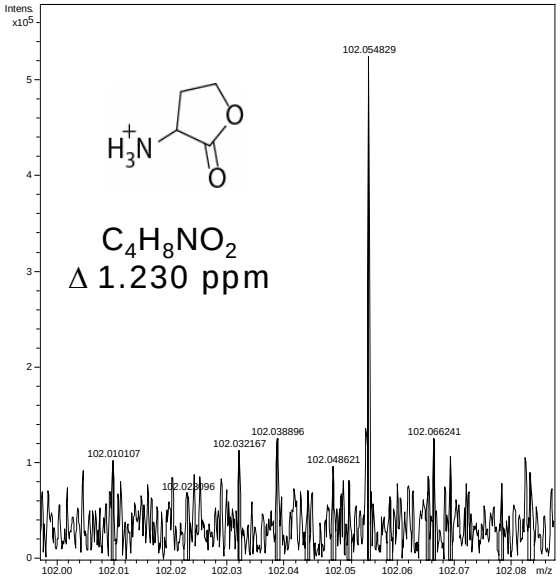
Fekete et al., *Electrophoresis*, submitted.

Target analysis





$[C_{14}H_{25}NO_4+Na]^+$
Theory: 294,16757 amu
Exp.: 297,16766 amu
Error: -0,291 ppm



Competition in the rhizosphere

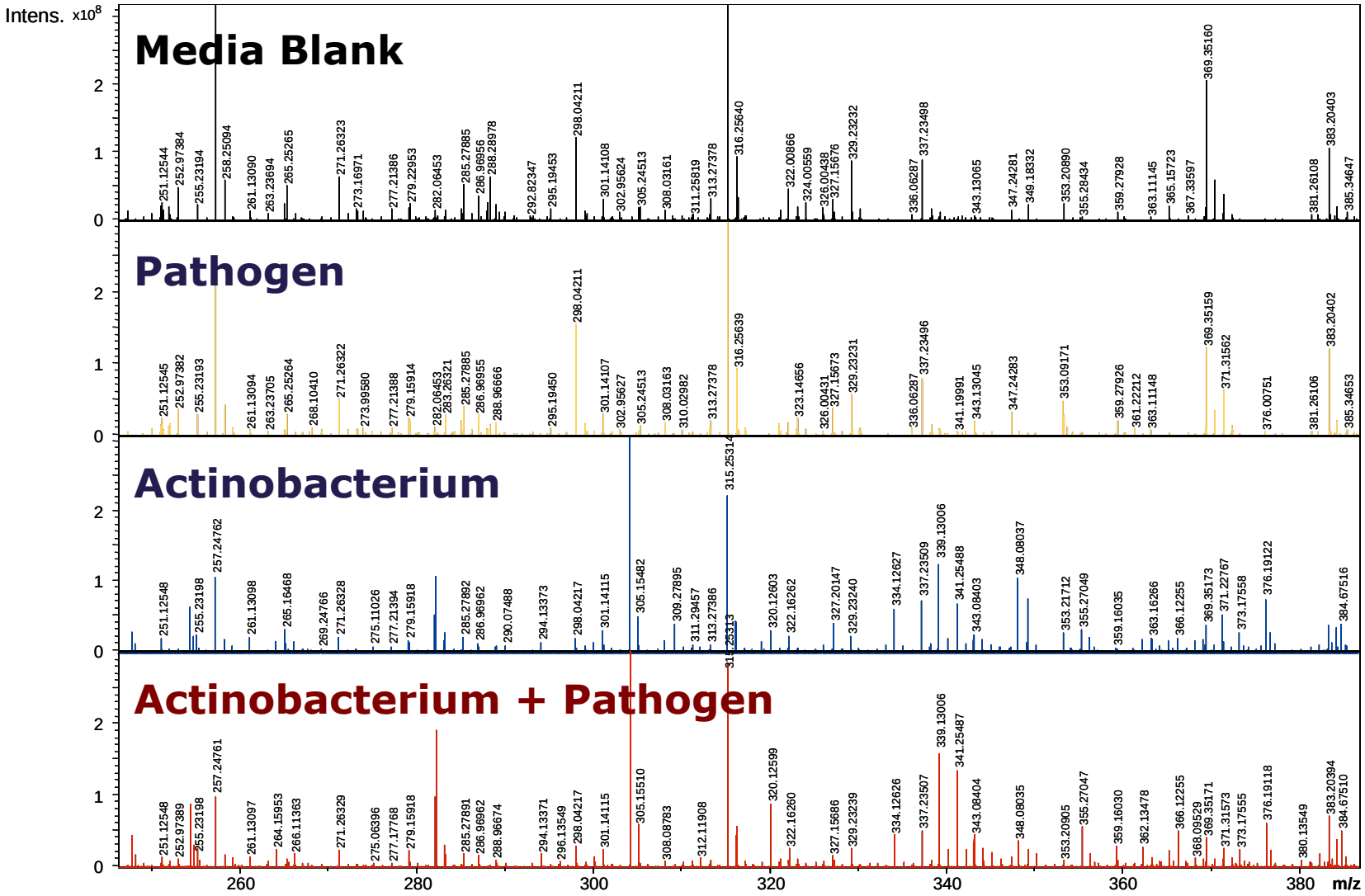
Mechanisms of PGPR activity

Plant pathogen (P)

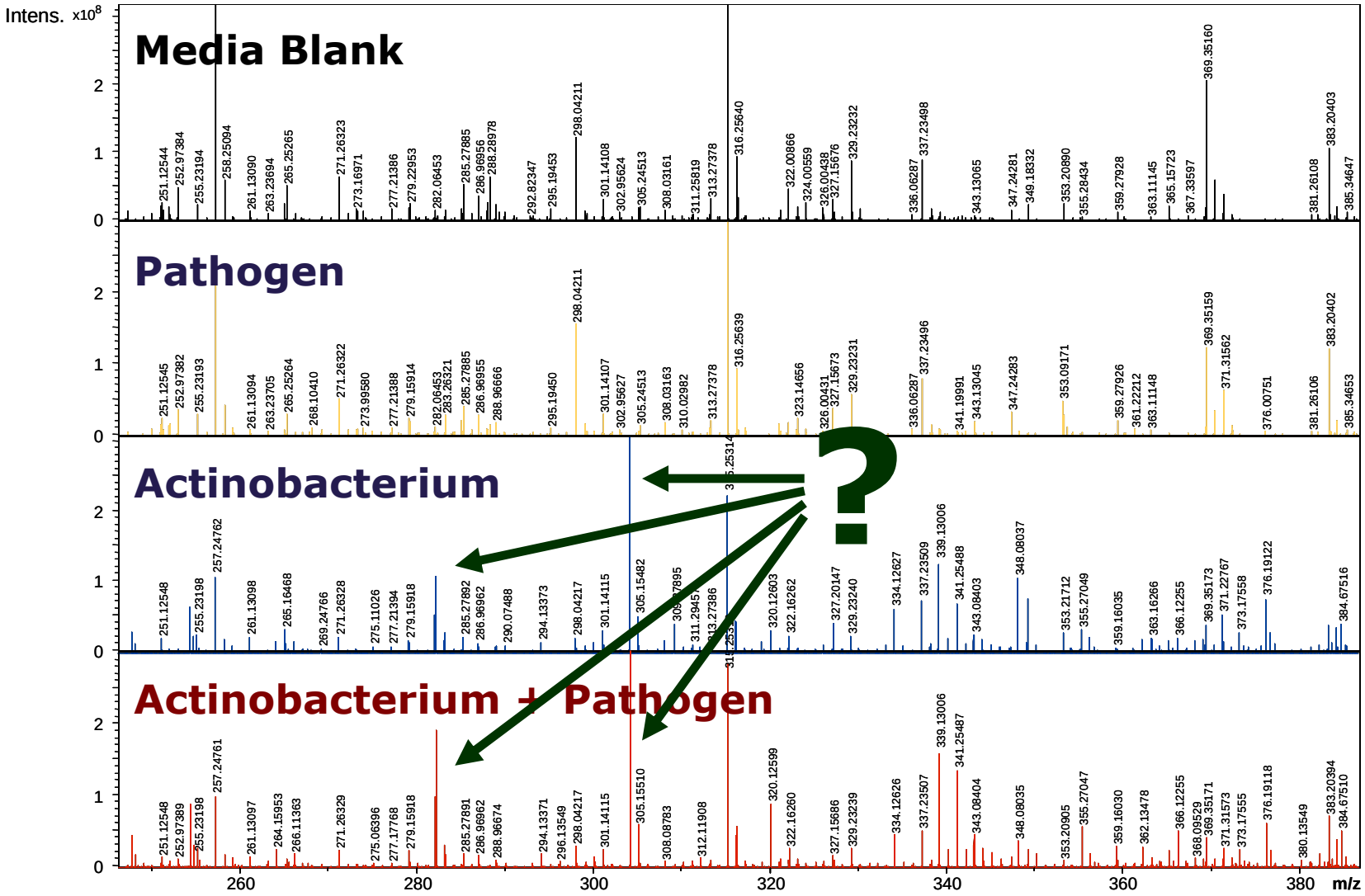
Actinobacterium (A)

Cooperation at GSF: M. Schloter, F. Häsler

Cocultivation in liquid medium



Cocultivation in liquid medium



Analytical approach

Cultivation of Actinomycete in synthetic medium
(reduces complexity)

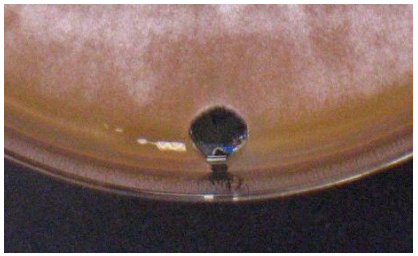
Activity assay of supernatant
(V8 agar, together with P)

Chromatographic fractionation of supernatant
(C18 SPE)

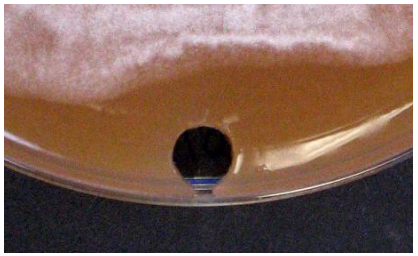
Activity assays of SPE fractions
(V8 agar, together with P)

Substance identification by FTICR-MS
(hopefully!)

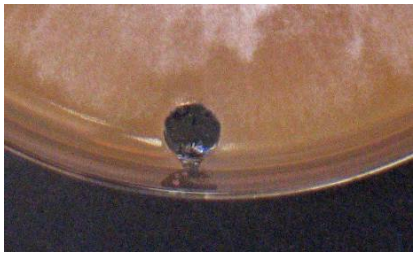
Chromatographic fractionation



Elution with 30% MeOH



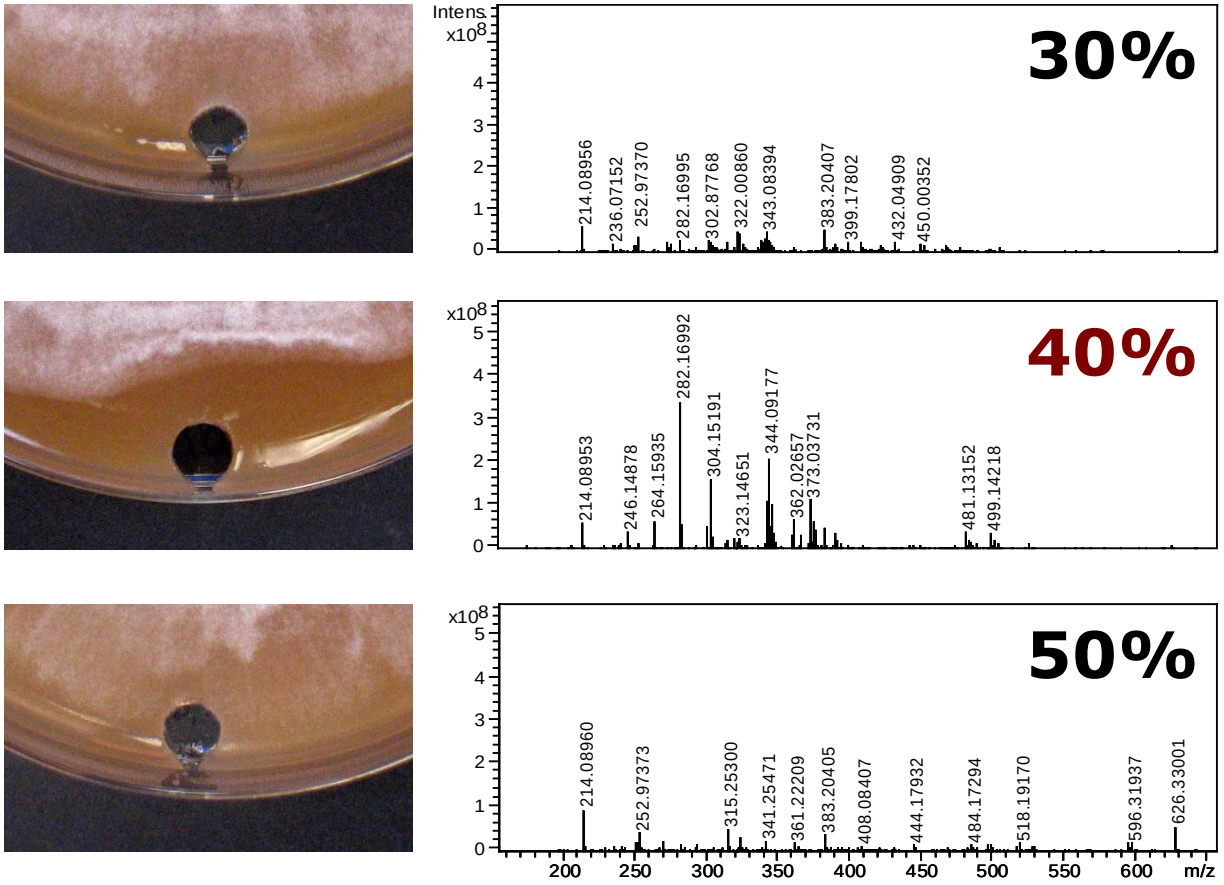
Elution with 40% MeOH



Elution with 50% MeOH

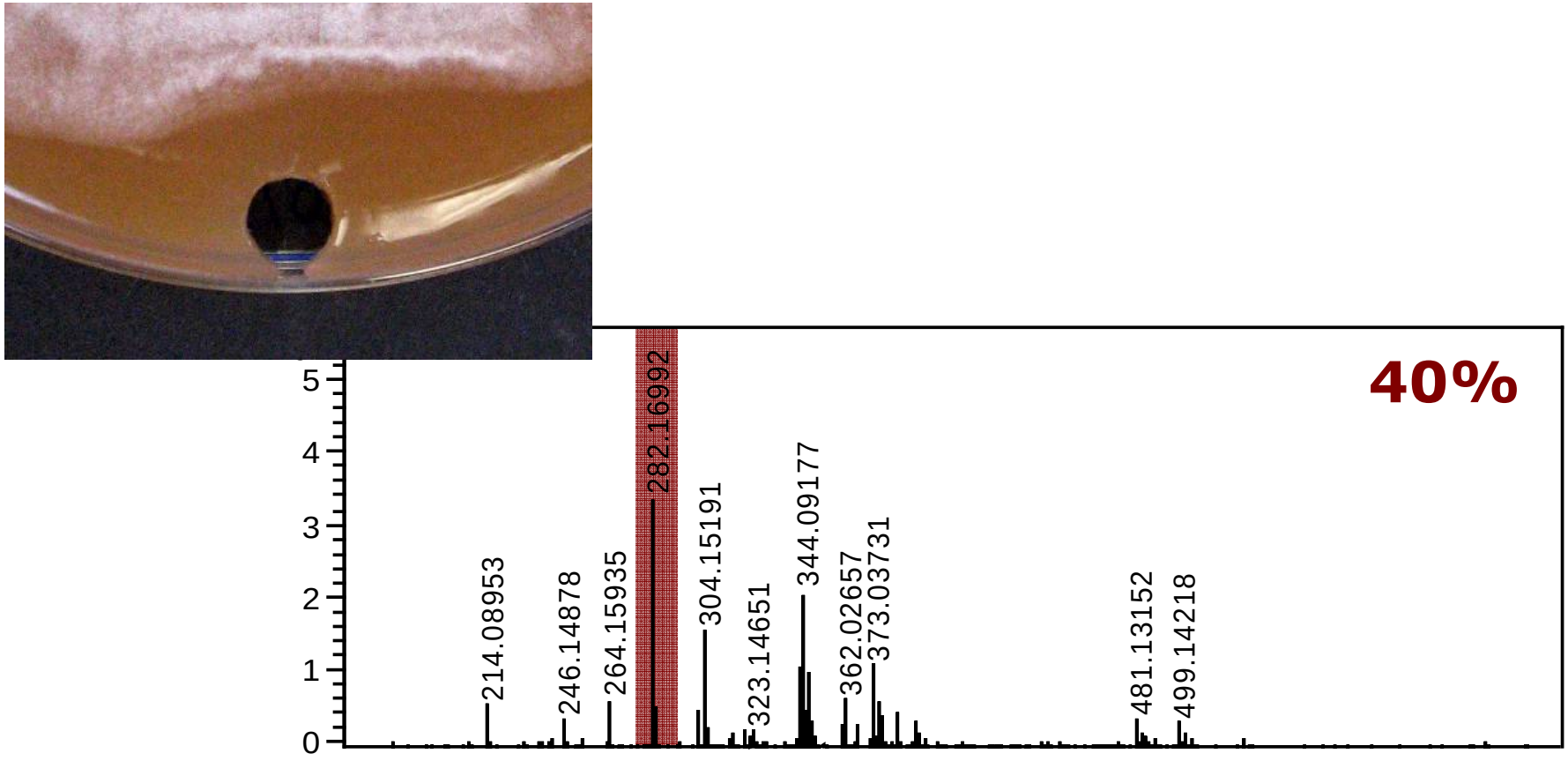
Cooperation at GSF: M. Schloter, F. Häsler

Identification by FTMS



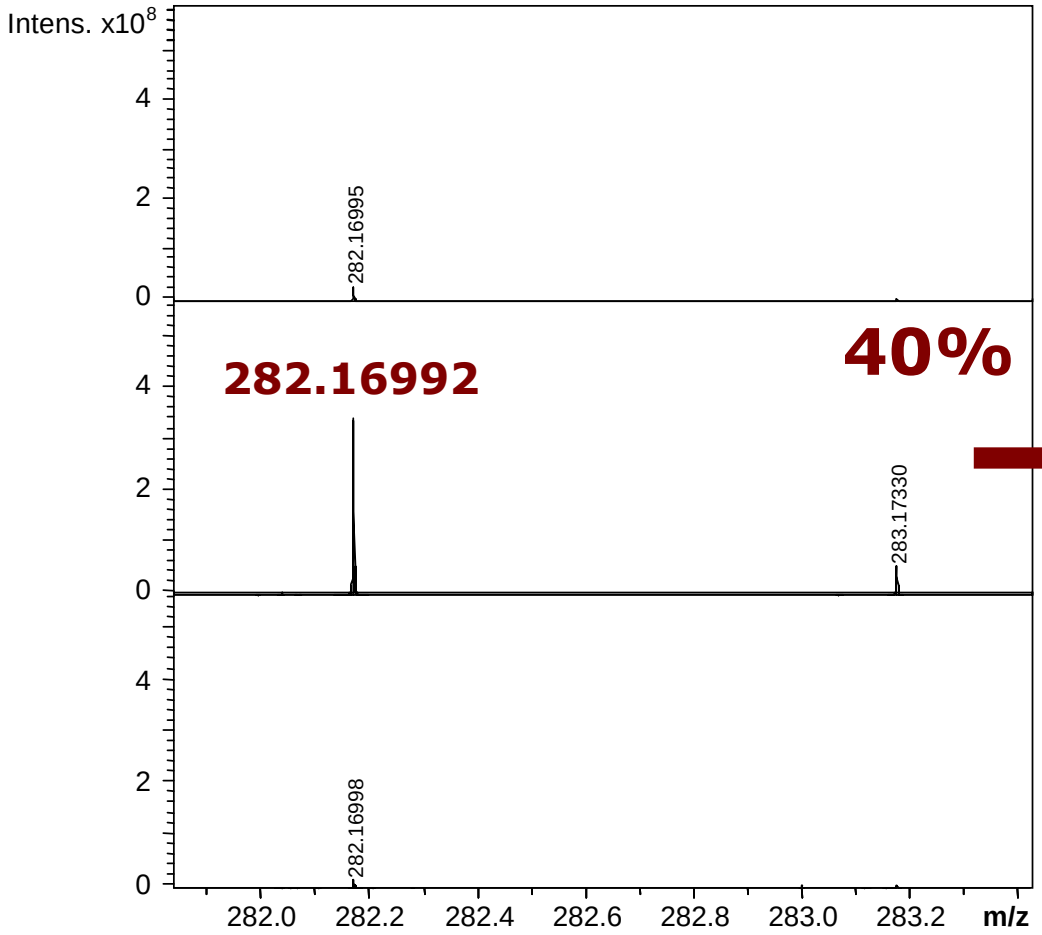
Cooperation at GSF: M. Schloter, F. Häsler

Identification by FTMS

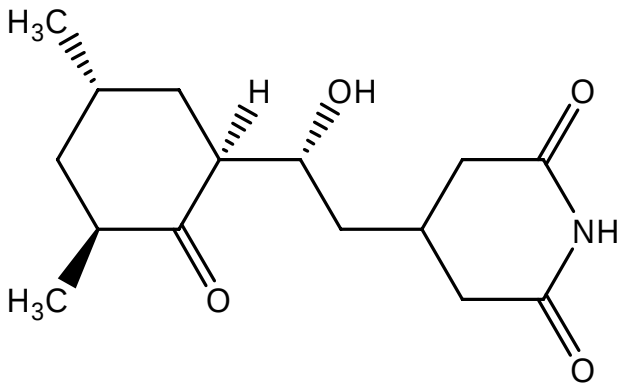


Cooperation at GSF: M. Schloter, F. Häsler

Identification by FTMS



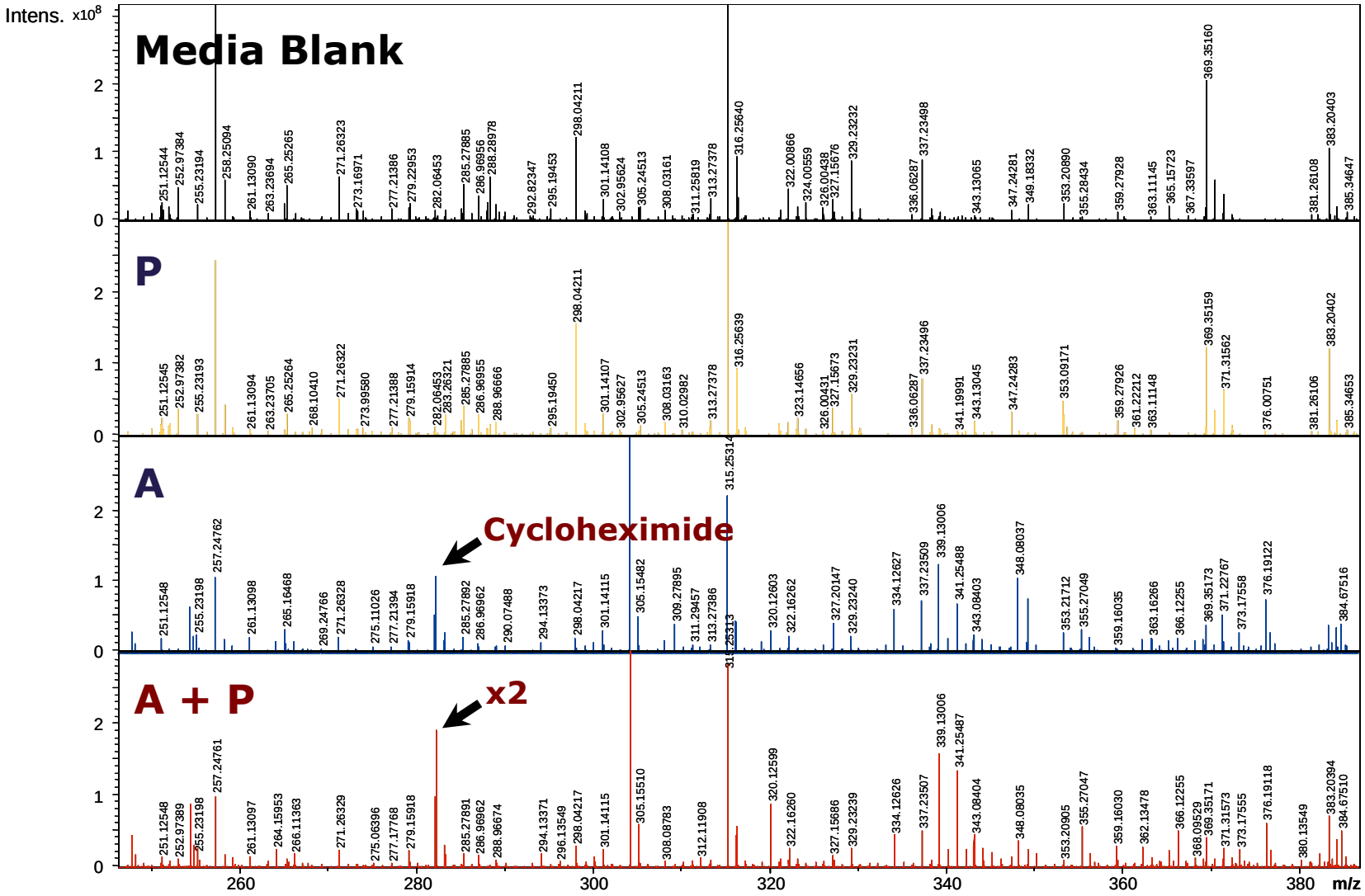
$C_{15}H_{24}NO_4$
(error 0.213 ppm)



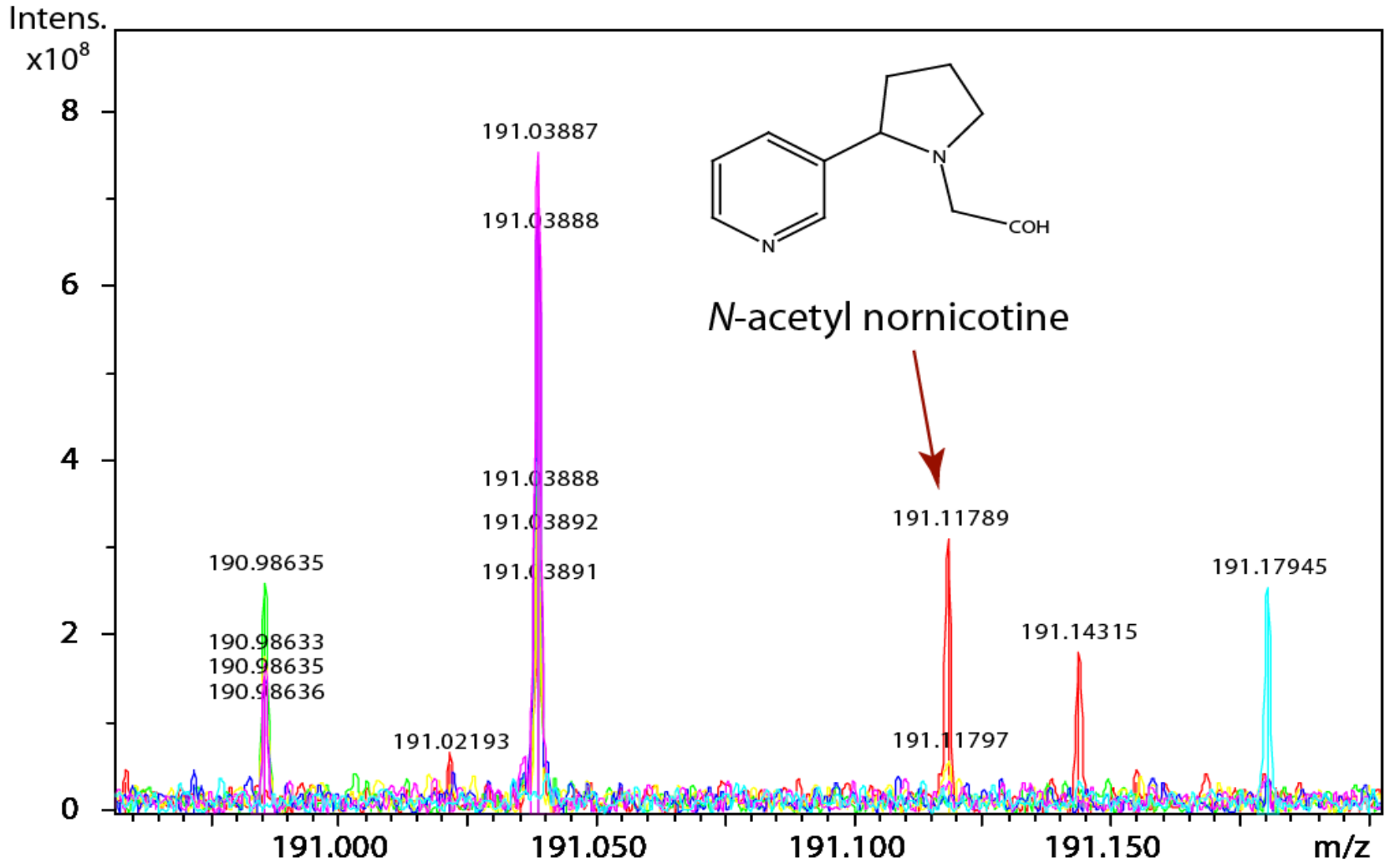
Cycloheximide

Cooperation at GSF: M. Schloter, F. Häsler

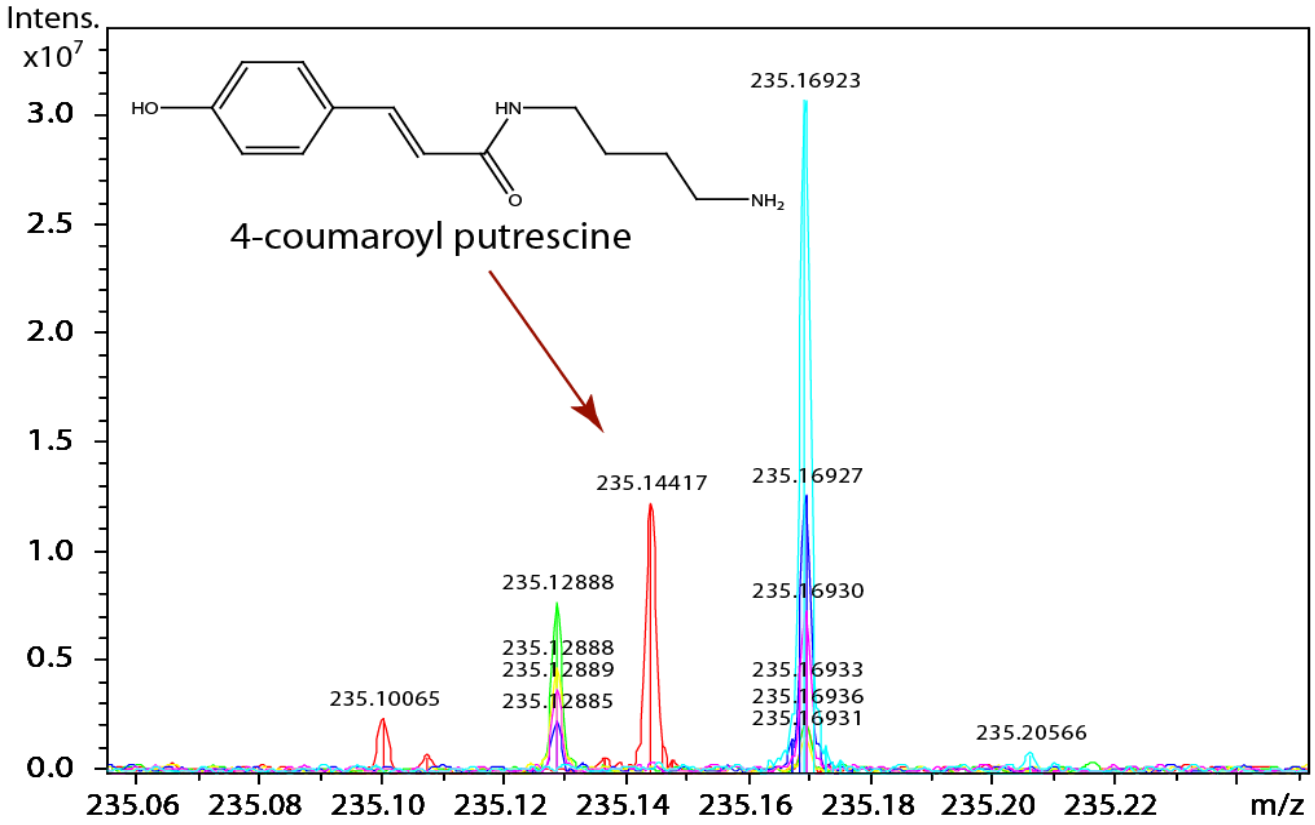
Constitutive or induced?



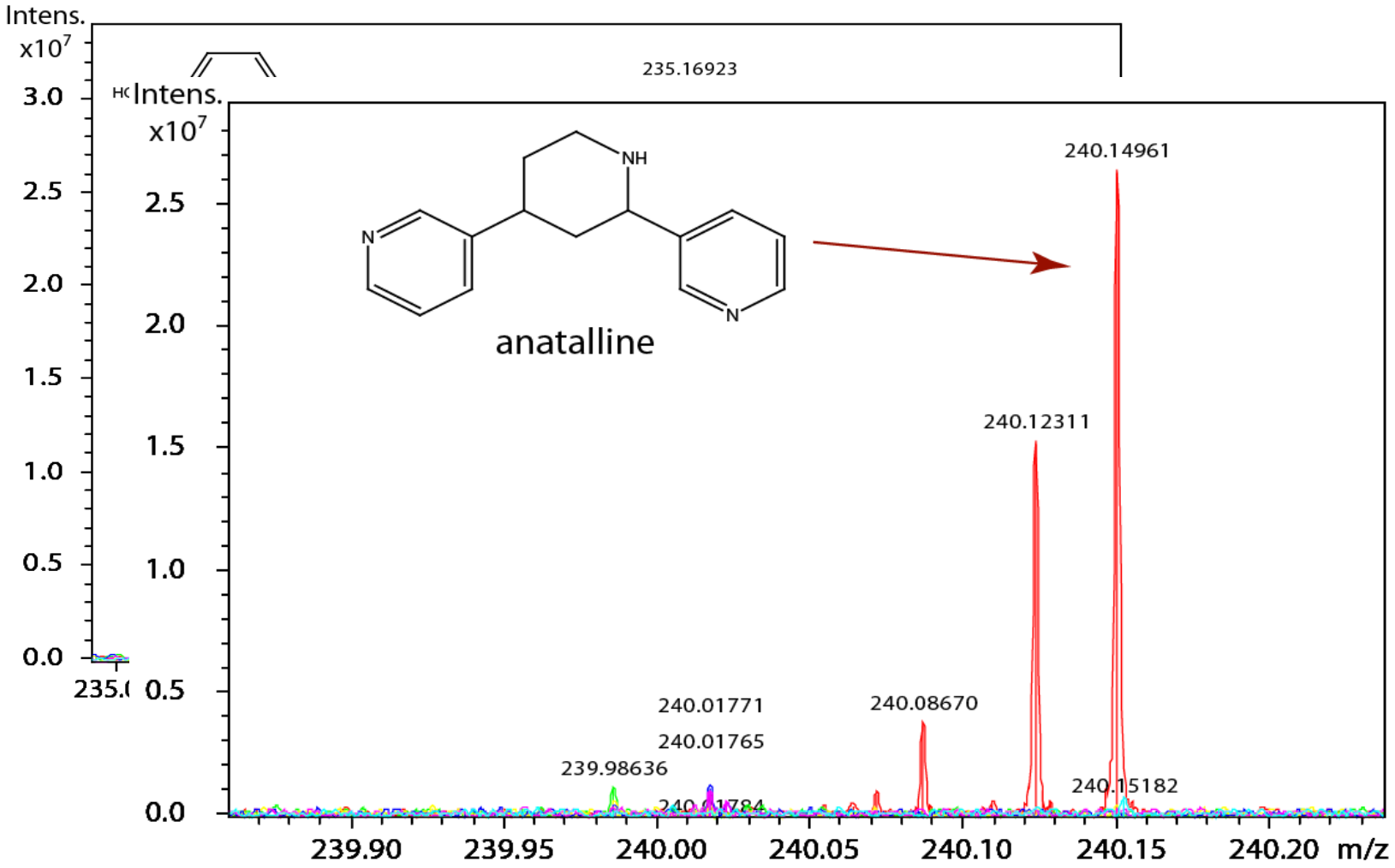
Tobacco metabolites



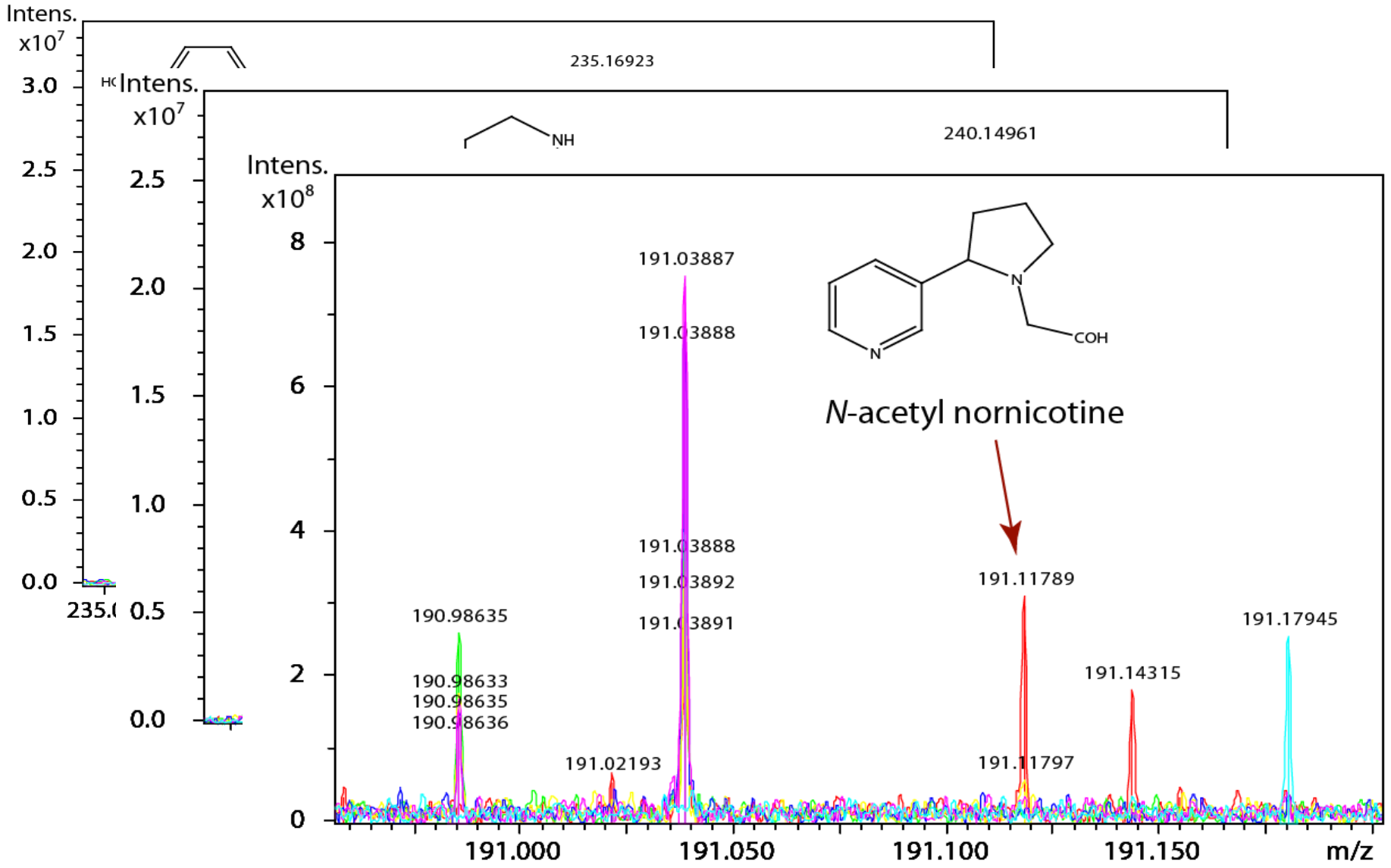
Tobacco metabolites



Tobacco metabolites



Tobacco metabolites



Much more (… and still not complete …)

Peptide biomarkers for cancer and kidney diseases
H. Mischak, P. Zürbig, Mosaiques AG, Hannover

Chemical Taxonomy of bacteria ecotypes
R. Rossello-Mora, IMEDEA, Mallorca

***Arabidopsis* mutant analysis**
T. Schäffner, B. Messner, BIOP, GSF

Tobacco metabolomics
K. Wieland, U. Schurr, FZ Jülich

Chemical Ecology of caterpillars
W. Boland H. Meischak, MPI of Chemical Ecology, Jena

UV/ozone induced metabolites of different plants
W. Heller, BIOP, GSF

Different questions in groundwater ecology
U. Kunapuli, R. Meckenstock, IGOE, GSF

Geochemistry, NOM, DOM, DOC, Aerosols, Wine …
➔ Philippe Schmitt-Kopplin

Much more here …

From molecular understanding to knowledge-based design and innovative uses of humic materials in biotechnology (DESIGNER-BIOHUMICS)

Molecular-level analysis of natural organic matter and humic substances

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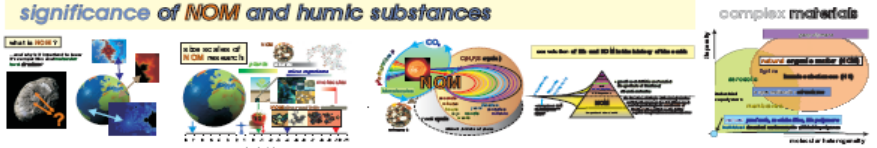
[A]GSF Research Center for Environment and Health, Institute of Ecological Chemistry, Ingolstaedter Landstrasse 1, D-85764 Neuherberg, Germany;
[B]Department of Chemistry, Lomonosov Moscow State University, Leninskie Gory 1-3, Moscow 119992, Russia.

significance of NOM and humic substances



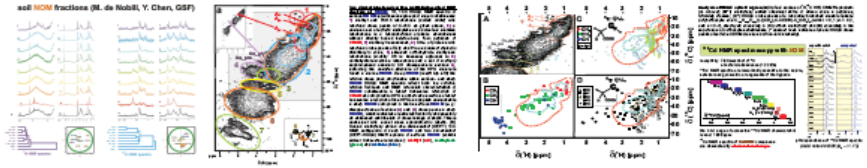
Natural organic matter (NOM) on earth is abundant in marine, humic and terrestrial ecosystems; in all these environments, NOM is comprised of an extremely complex array of chemical structures and interactions across a large range of time- and size- scales. Common to all these environments are the fundamental molecular aspects of life, and an availability of extended mineral surfaces for interaction and binding of NOM. The dynamic equilibrium of NOM generation and decomposition spans time scales across many orders of magnitude (microseconds to hundred-thousands of years) and it results from a combination of biotic and abiotic reactions. Photochemical degradation, one of the most significant abiotic reactions of NOM, often results in small molecules like CO_2 which are mobile and are easily distributed within various ecosystems.

molecular-level analytical characterization of **NOM** establishes quantitative **quality criteria**, leading to prognosis of properties and **structure-activity relationships**; **NOM** materials can be **designed** according to desired properties

NMR characterization of NOM and humic substances

unsurpassed, isotope-specific and nearly quantitative information about short range molecular order

relative insensitivity with regard to other analytical methods

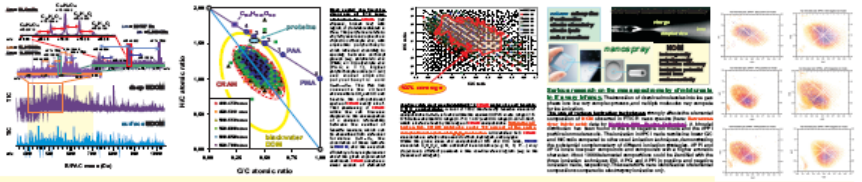


1D NMR for classification and quantification of coarse **NOM** chemical environments; **higher dimensional NMR** for reliable NMR resonance assignment; **mathematical analysis** for assessment and in-depth **molecular-level analysis**

FTICR mass spectral analysis of NOM and humic substances

unsurpassed combination of (ultrahigh) resolution and sensitivity

molecular-level information of ionizable molecules ONLY; differentiation of isomers is not trivial



Integrated analysis of NOM molecular level structure

In-depth **molecular level analysis** of **NOM** contributes to the **yet missing** establishment of **quality criteria** of **NOM** and will ultimately lead to a **quantitative description** of their **composition and properties**

Большое спасибо!