SIP goes Proteomics – Elucidation of structure and function of microbial communities

PD Dr. Martin von Bergen

Keystone 2010

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ENVIRONMENTAL
RESEARCH – UFZ


Microbial Life is complex
➔ Who eats what, where and when?
Courtesy of Matthias Kästner, UFZ
12C-Toluol + Nitrat, Aussenseite, 11.06.07, 1kV, 5mm
2 µm

History and features of SIP approaches in microbiology

1998: Labelling of phospolipids (Boschker et al.)

- Direct linking of microbial populations to specific biogeochemical processes by 13C-labelling of biomarkers
- Acetate consumption rather by SRB similar to Desulfotomaculum acetoxidans than by Desulfobacter spp.

+ Combined assessment of species and metabolic function

- Limited taxonomic resolution

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2000: DNA-SIP (Radajewski et al.)

- Labelled DNA can be purified, amplified and sequenced yielding wide taxonomic information coupled with information on metabolic activity
- Example of different methylotrophic species obtained by feeding $^{13}CHOH$

+ Combination of genetic methods allows even culture independent assessment of species
- Information on the potential but not the actual function
- Density gradient centrifugation enables only low resolution, results in high minimal labelling

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2002: RNA-SIP (Manefield et al.)

• Labelled RNA can be purified, amplified and sequenced yielding wide taxonomic information coupled with information on functional aspects
• Example of phenol degradation in aerobic bioreactor and identification of a Thauera spp. as a keyplayer

+ Combination of genetic methods allows even culture independent assessment of species
+ Detection of transcription of functional genes
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2006: Nano-SIMS (Lechene et al.)

- High spatial resolution in combination with measurement of secondary ions
- Analysis of microbial nitrogen fixation by detection of 15N incorporation

+ Single cell analysis
+ high dynamic range
+ Direct correlation between metabolomic activity and species identification in combination with FISH
- Due to tedious sample preparation only consortia of limited complexity can be analysed
- relatively costly instruments

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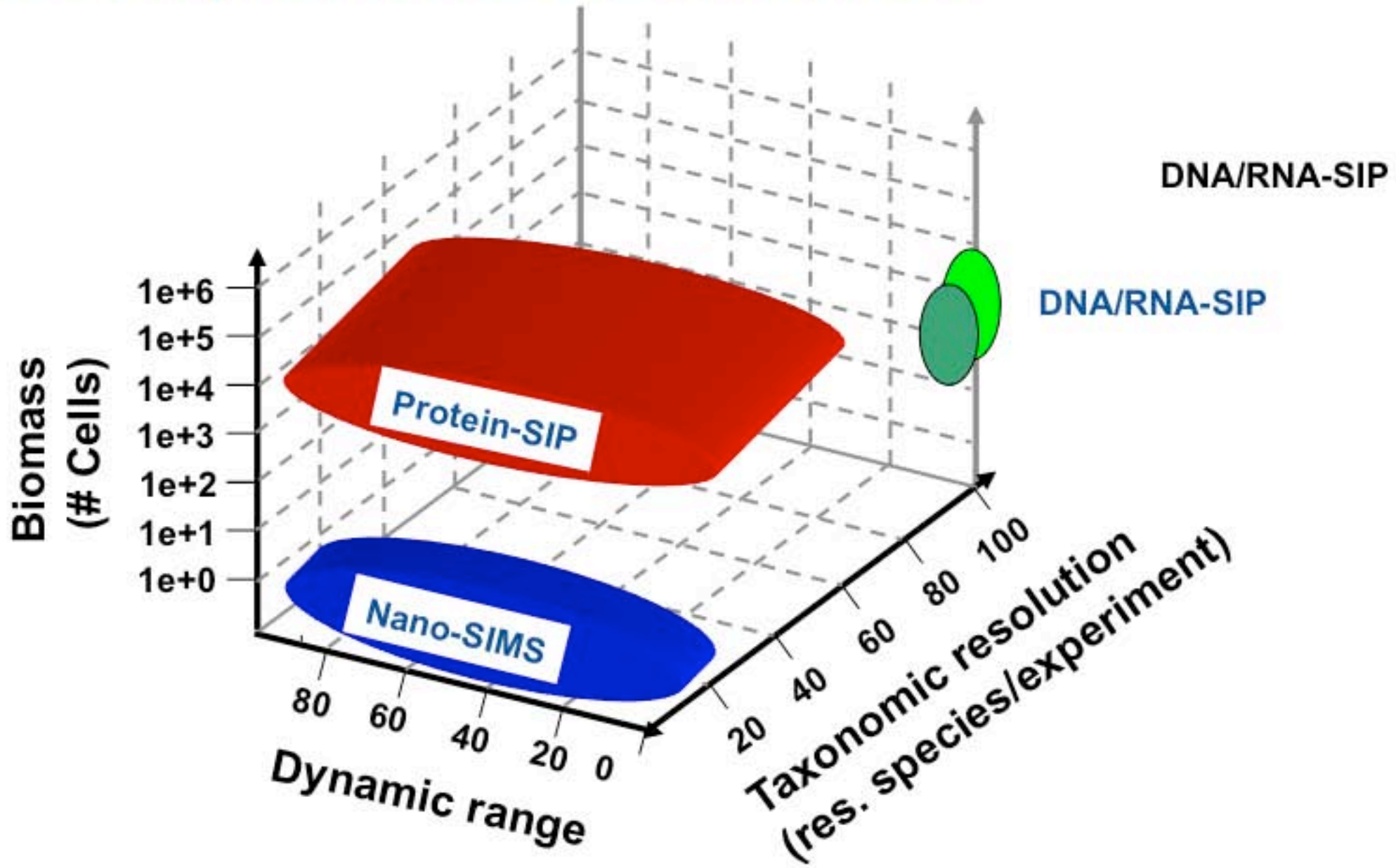
2008: Protein-SIP (Jehmlich et al.)

- Labelled Protein can be extracted and analysed by gel-based or LC-MS-based approaches, mass spectrometry yields identification of protein (and species) and determination of metabolic activity
- Example of toluene degradation anoxic mixed culture

+ Mass spectrometry of peptides enables high sensitivity and dynamic range for incorporation
+ Direct correlation between function and metabolic activity
- Accuracy of annotation of proteins to a species depends on genomic information

SIP-approaches in terms of taxonomic resolution, dynamic range and needed biomass



Further criteria can be : availability/cost of instrumentation
time needed
proof of functionality

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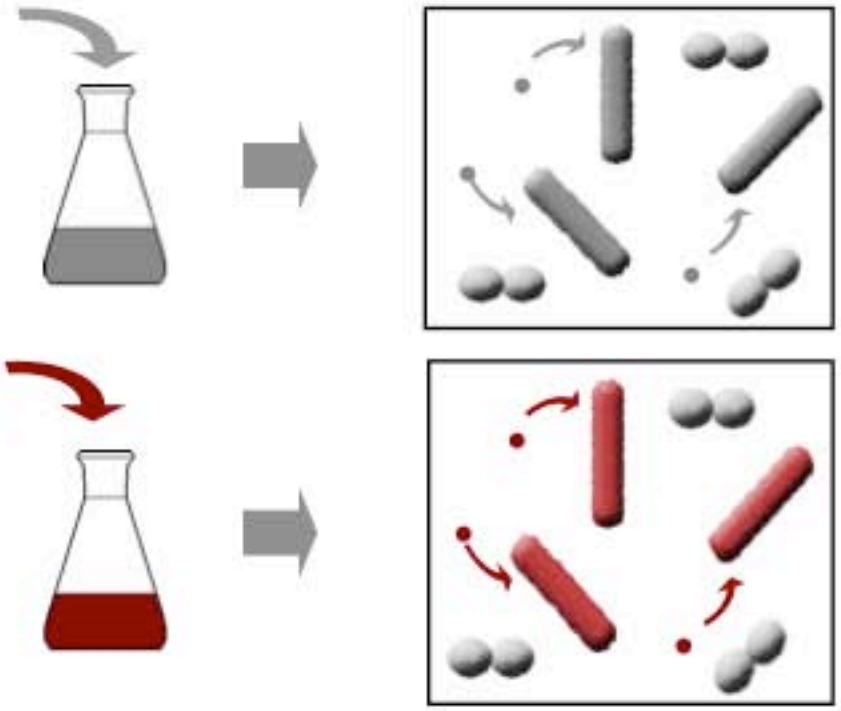
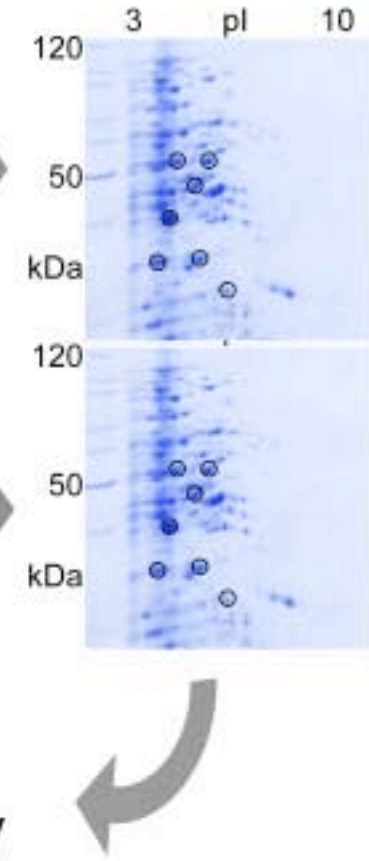
Workflow: Protein-based stable isotope probing (Protein-SIP)

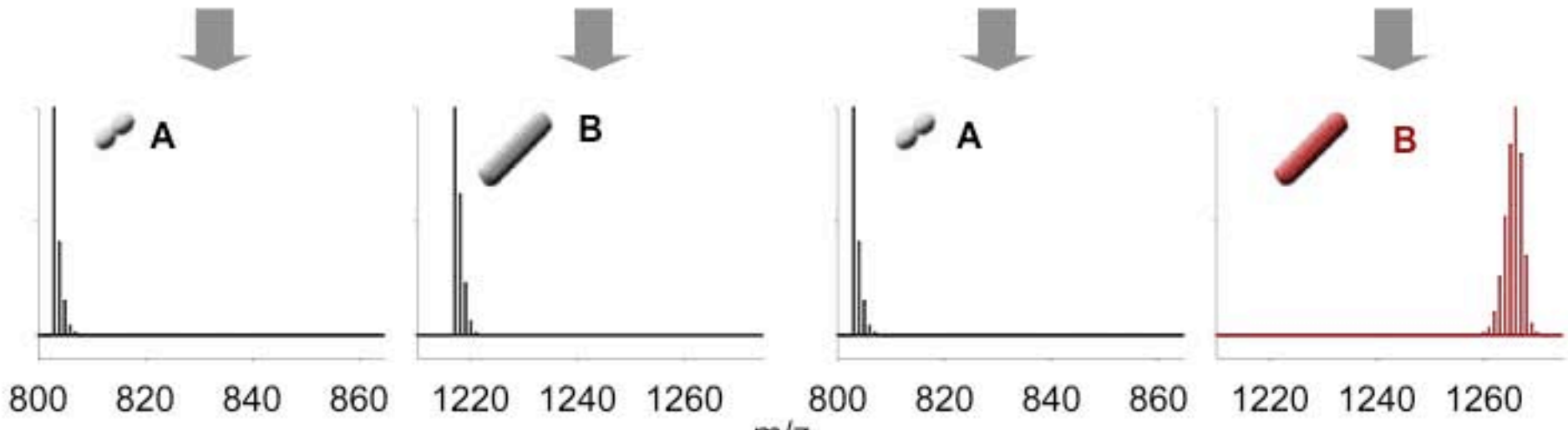
- „light“ substrate
  e.g $^{12}C_6$ benzene
- „heavy“ substrate
  e.g $^{13}C_6$ benzene



Protein extraction

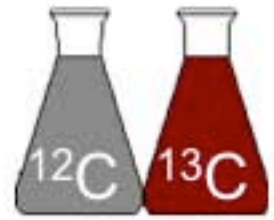


tryptic digestion
mass spectrometry



mass spectra

Toluene degrading anoxic mixed culture for proof of principle



⇢ anoxic degradation of $^{13}C_7$ labeled toluene by *Azoarcus* EbN1

In an artificial community with UFZ-1 culture (that does not grow on toluene but on gluconate)

⇣

Determination of ^{13}C incorporation levels to link

functionality and identity in the experimentally designed consortium

Jehmlich et al, *ISME* (2008)

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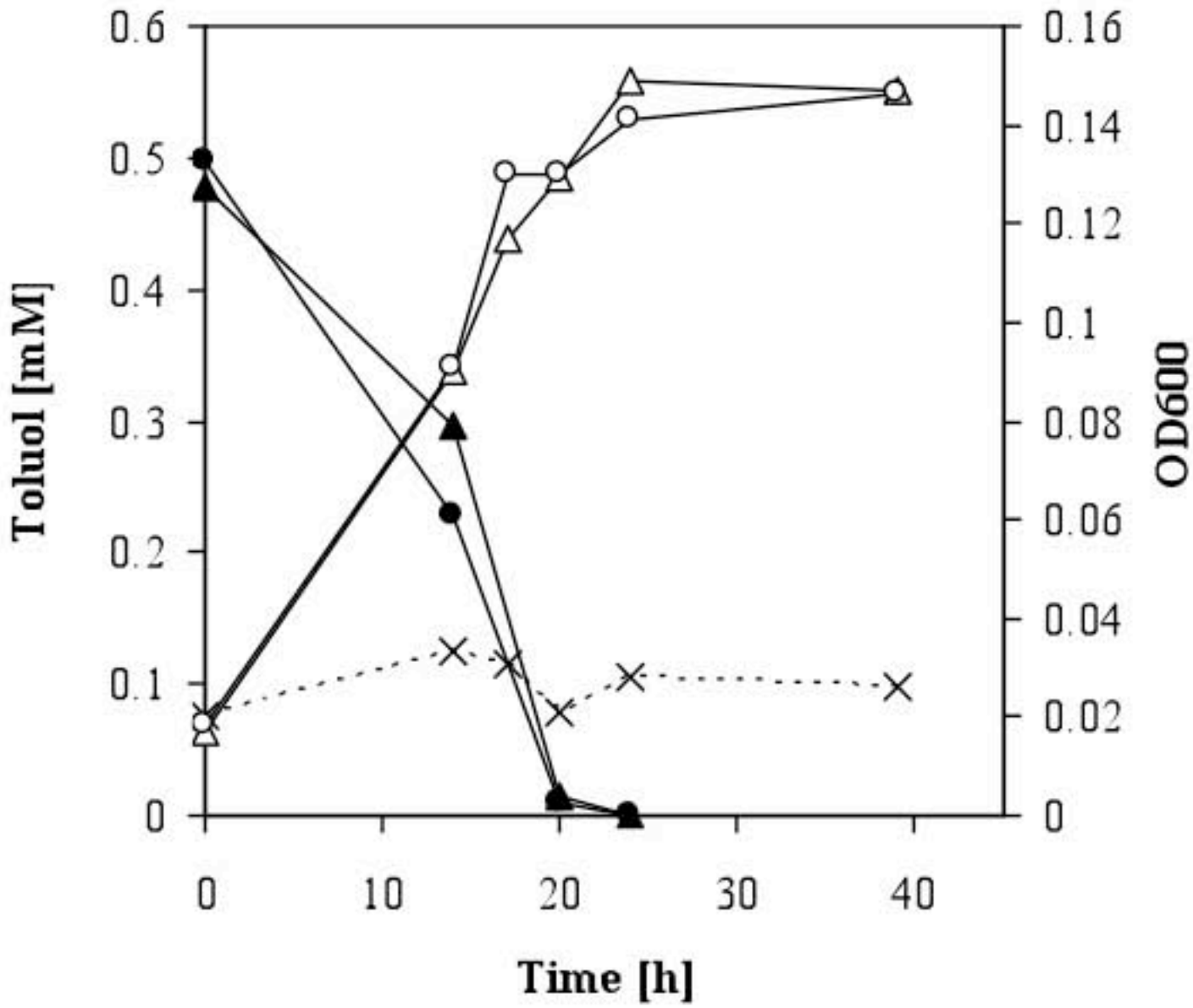
EbN1 does not distinguish between ^{12}C and ^{13}C toluene



$^{13}C_7$ toluene: circles
$^{12}C_7$ toluene: triangles no substrate: dashed line

Jehmlich et al, *ISME* (2008)

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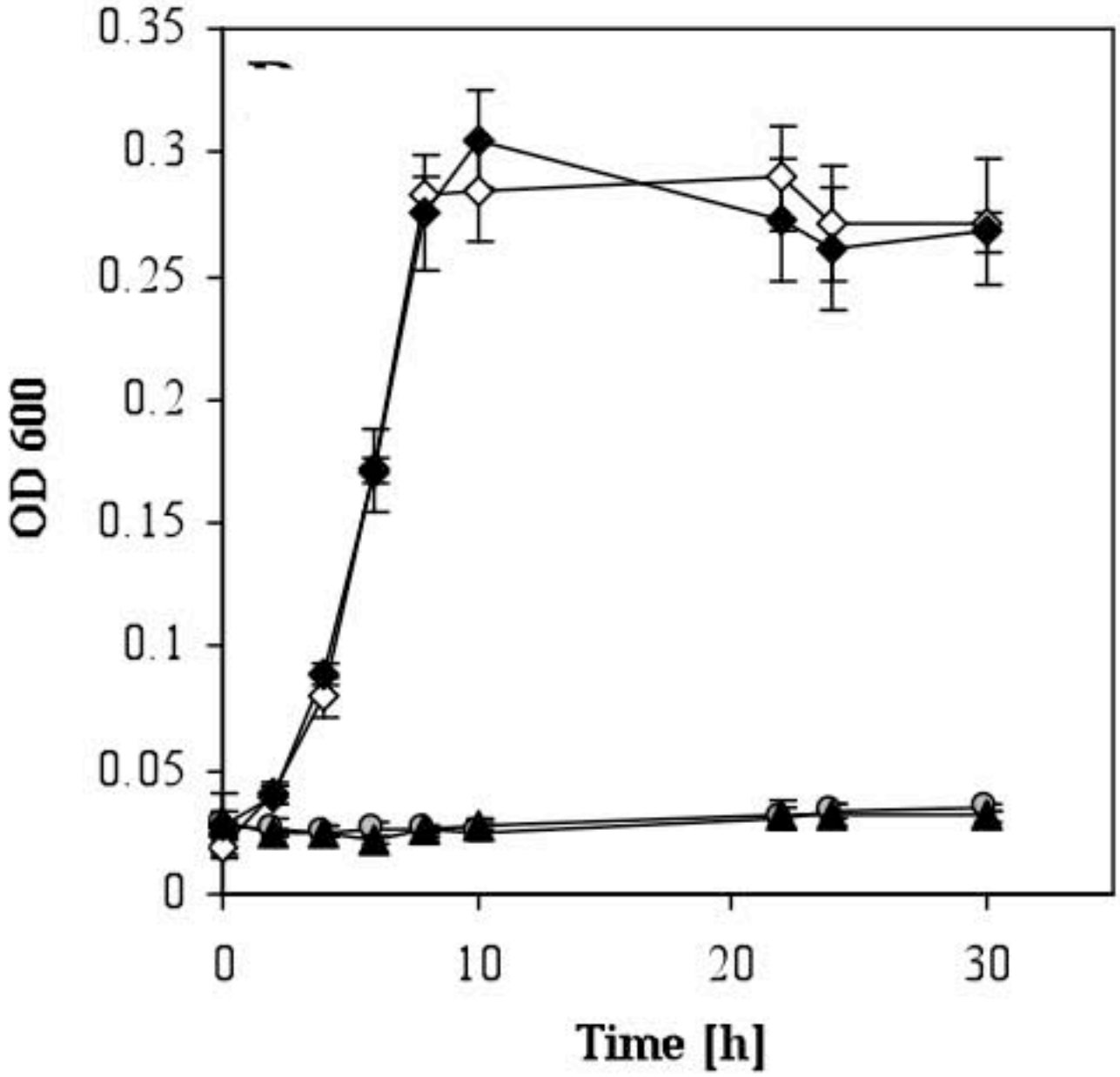
UFZ-1 does not grow on toluene

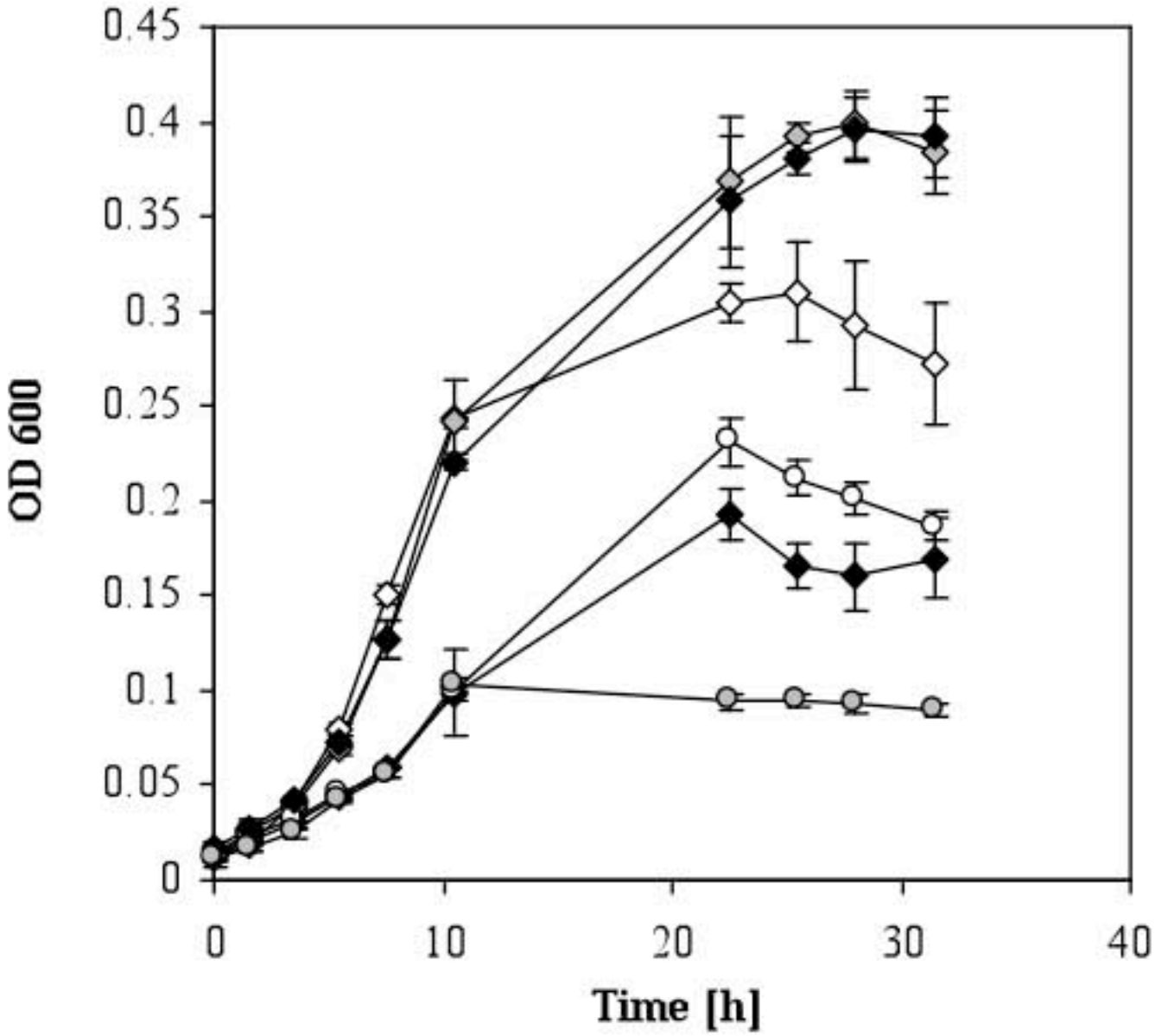


gluconate: white diamonds
toluene: black triangles

gluconate + toluene: black diamonds.

Jehmlich et al, *ISME* (2008)

Mixed culture of EbN1 and UFZ exhibits stable growth



$^{13}C_7$ toluene: white circles

$^{13}C_7$ toluene: black circles

gluconate: white diamonds

gluconate + ^{12}C toluene: black diamonds

gluconate + $^{13}C_7$- toluene: grey triangles

No substrate: grey circles

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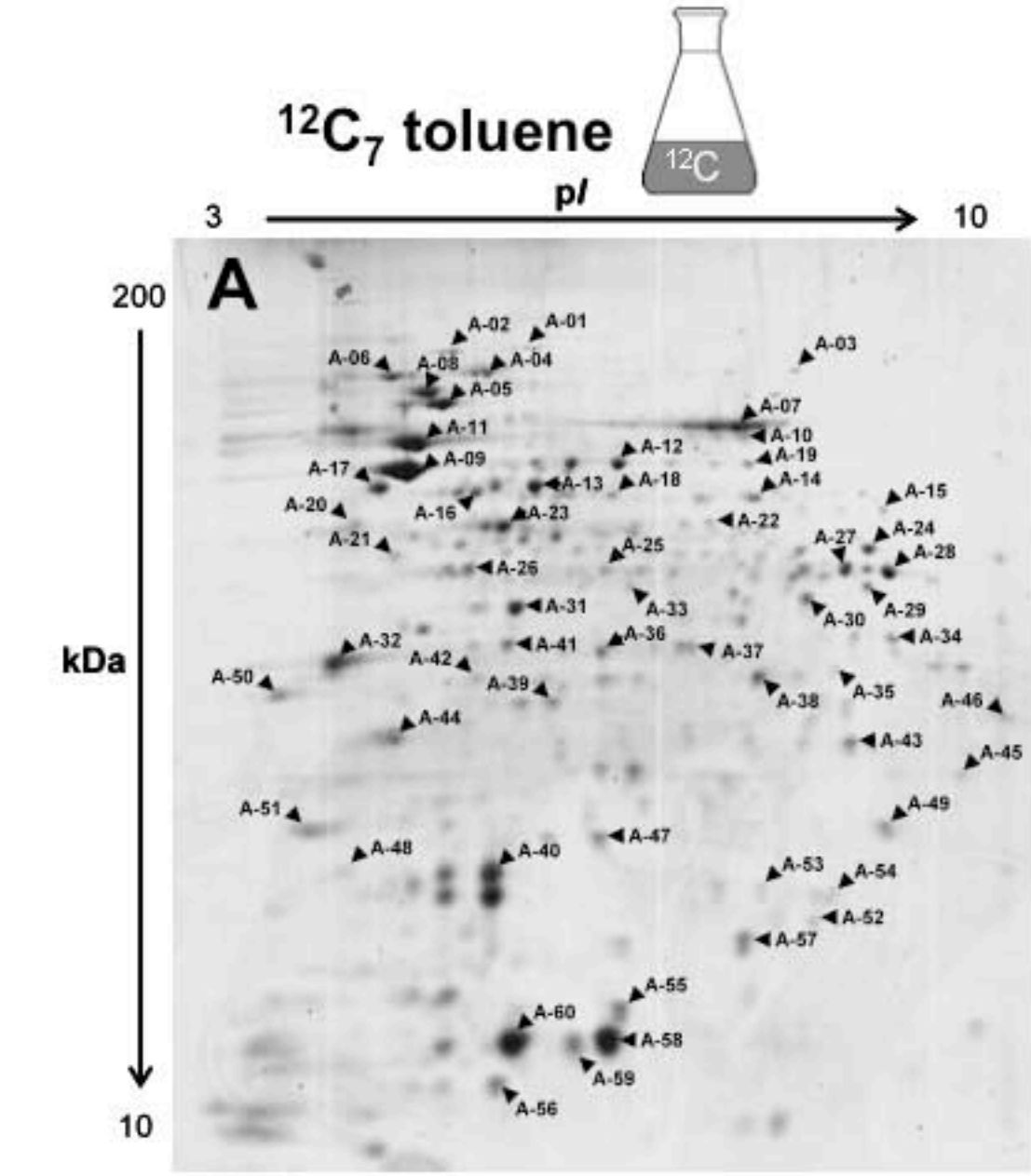
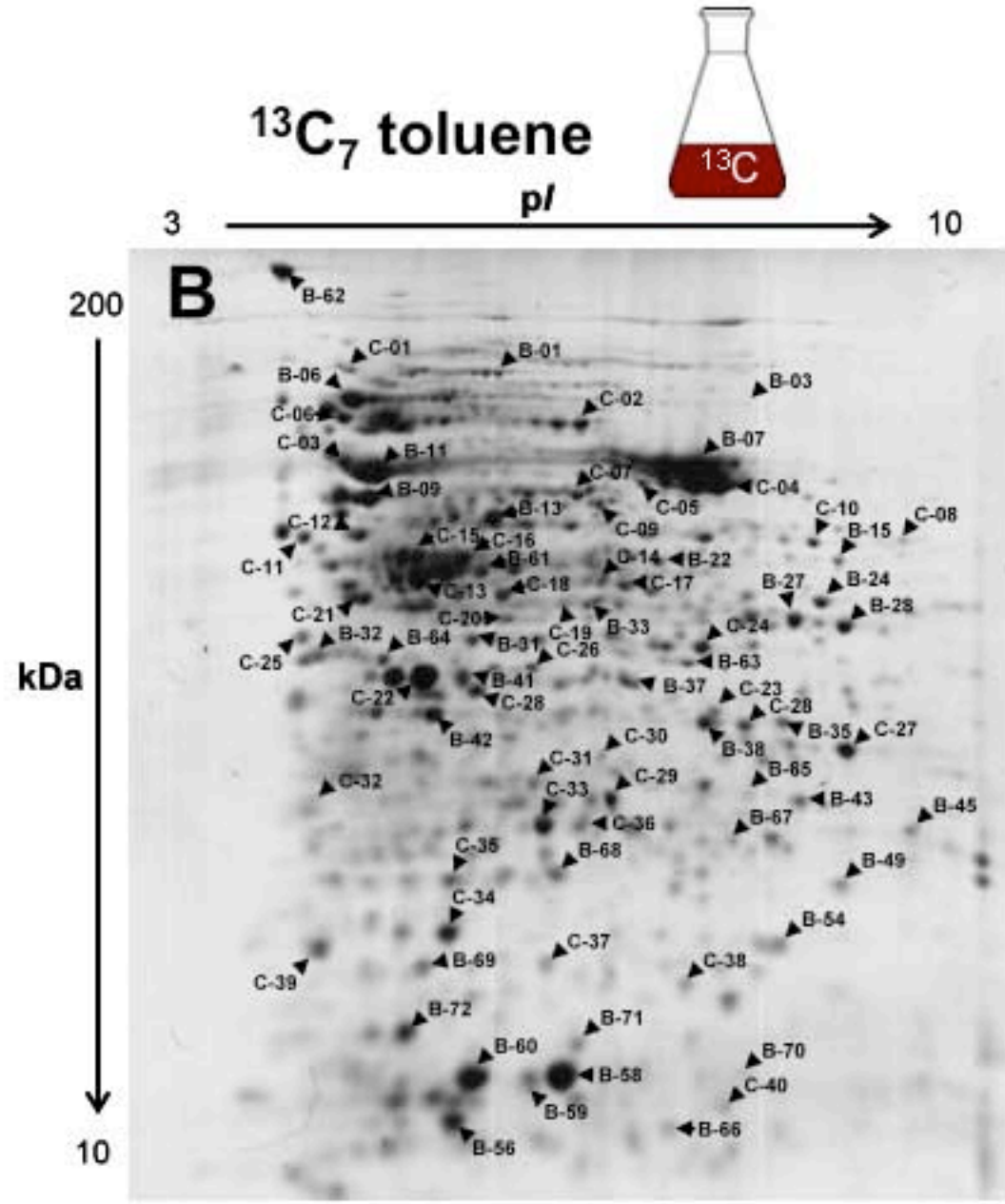
Jehmlich et al, *ISME* (2008)

2DE for pre-separation





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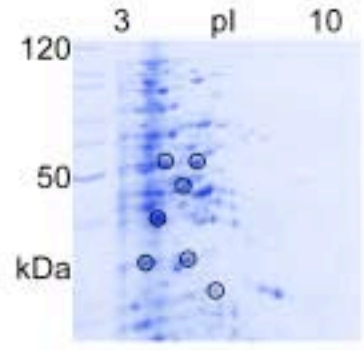
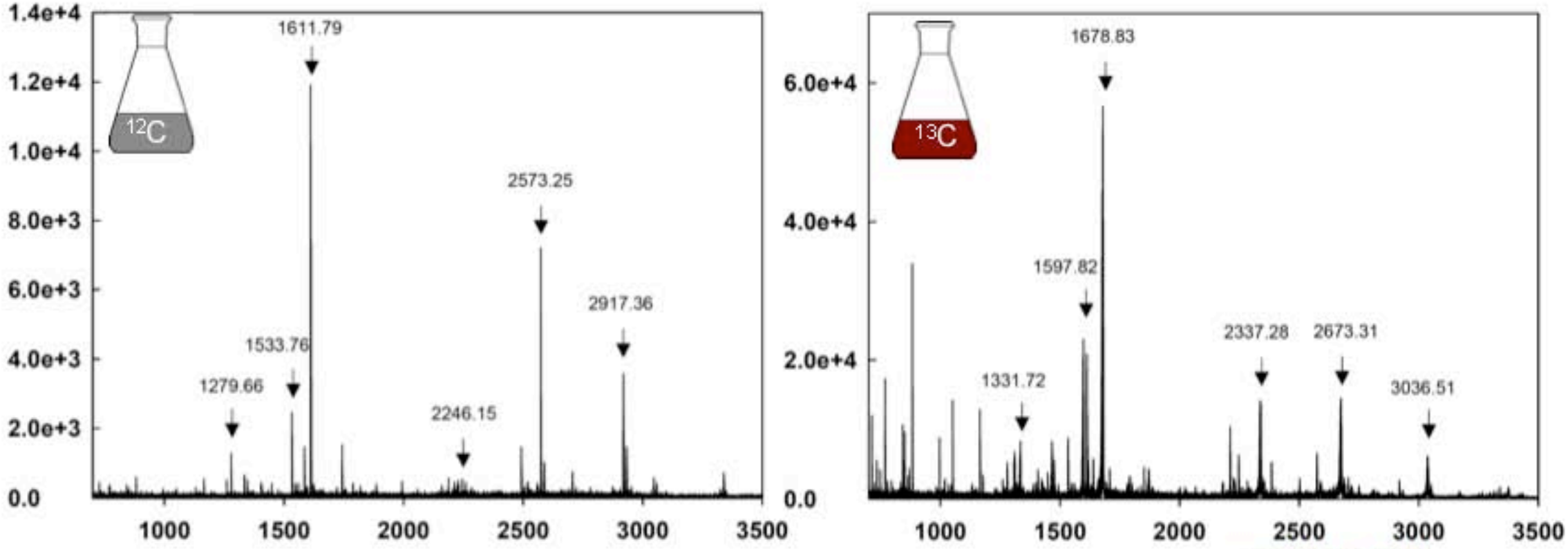
Jehmlich et al, *ISME* (2008)

Molecular details in peptide mass fingerprints



Cut out spots → Reduction Alkylation → Tryptic digestion → MALDI-MS/MS



Jehmlich et al, *ISME* (2008)

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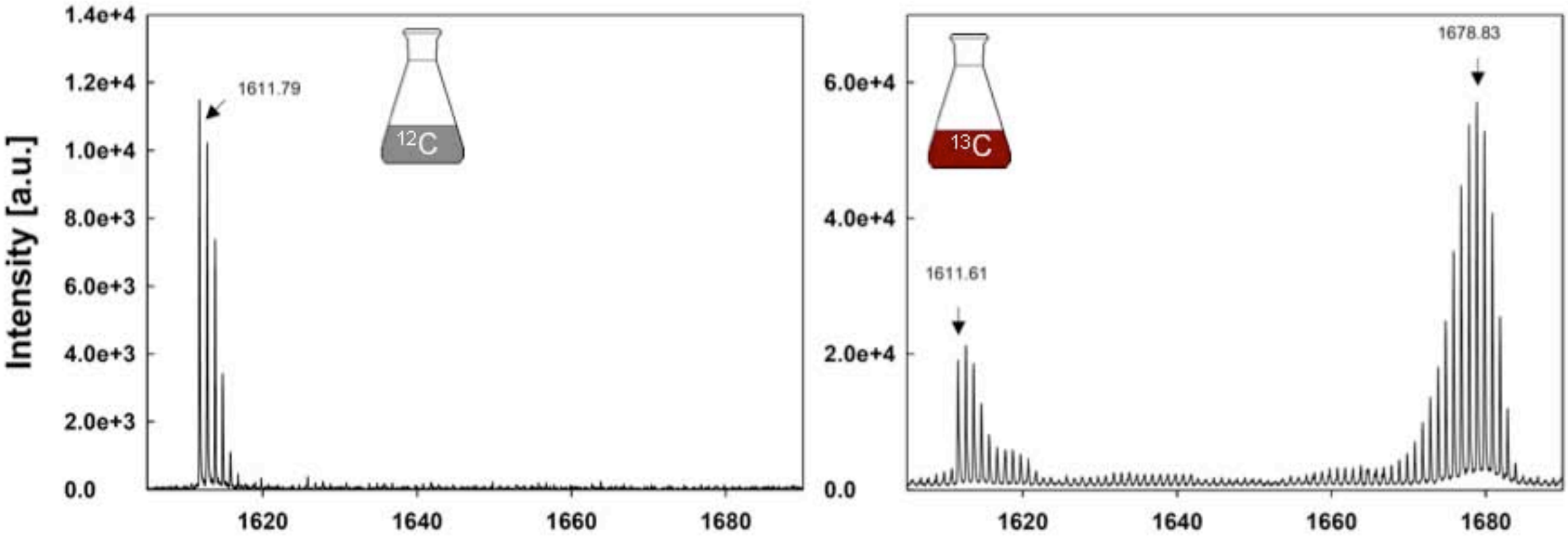
Zoom in reveals altered isotopic envelopes



Jehmlich et al, *ISME* (2008)

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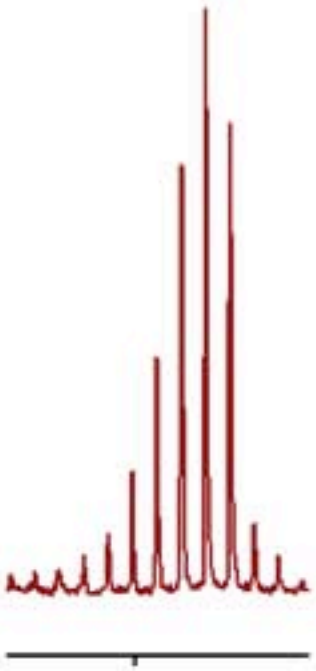
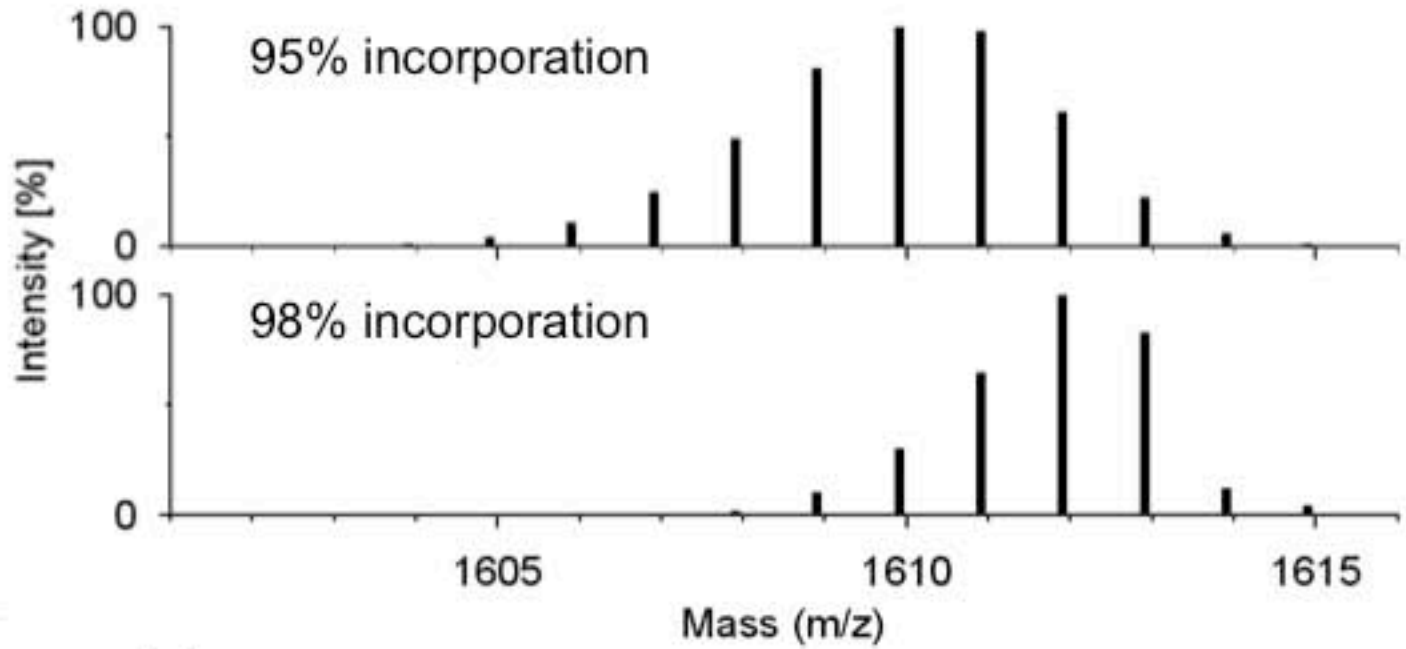
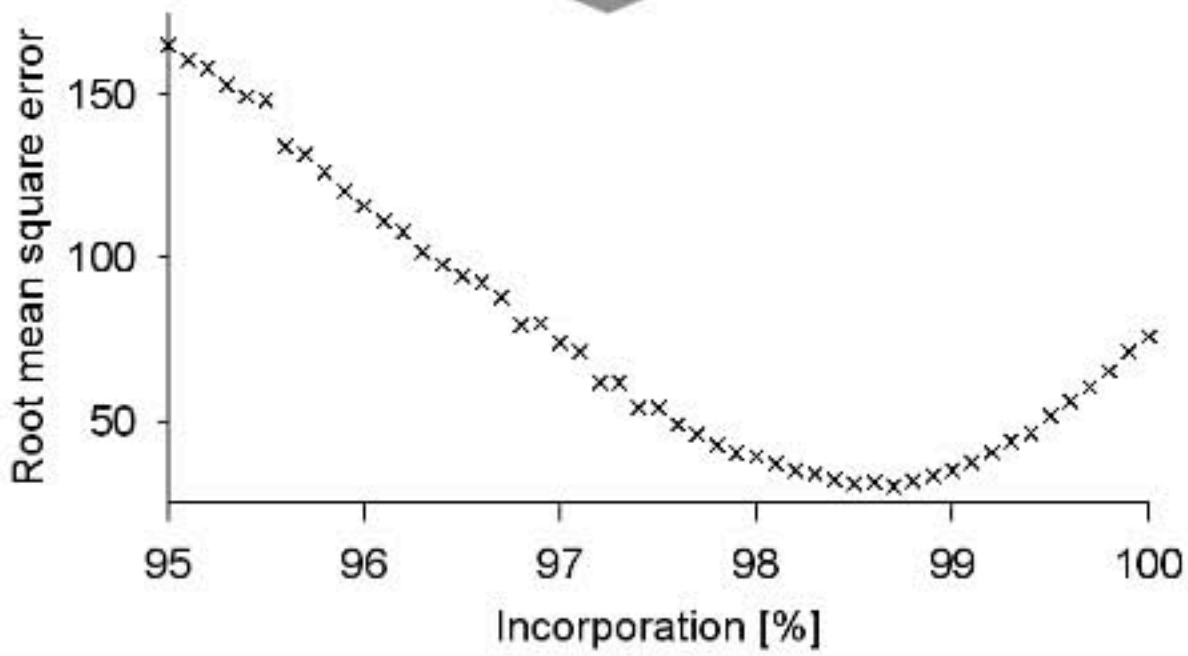
Calculation of incorporation

Measurement



Computational simulation





98.7% incorporation

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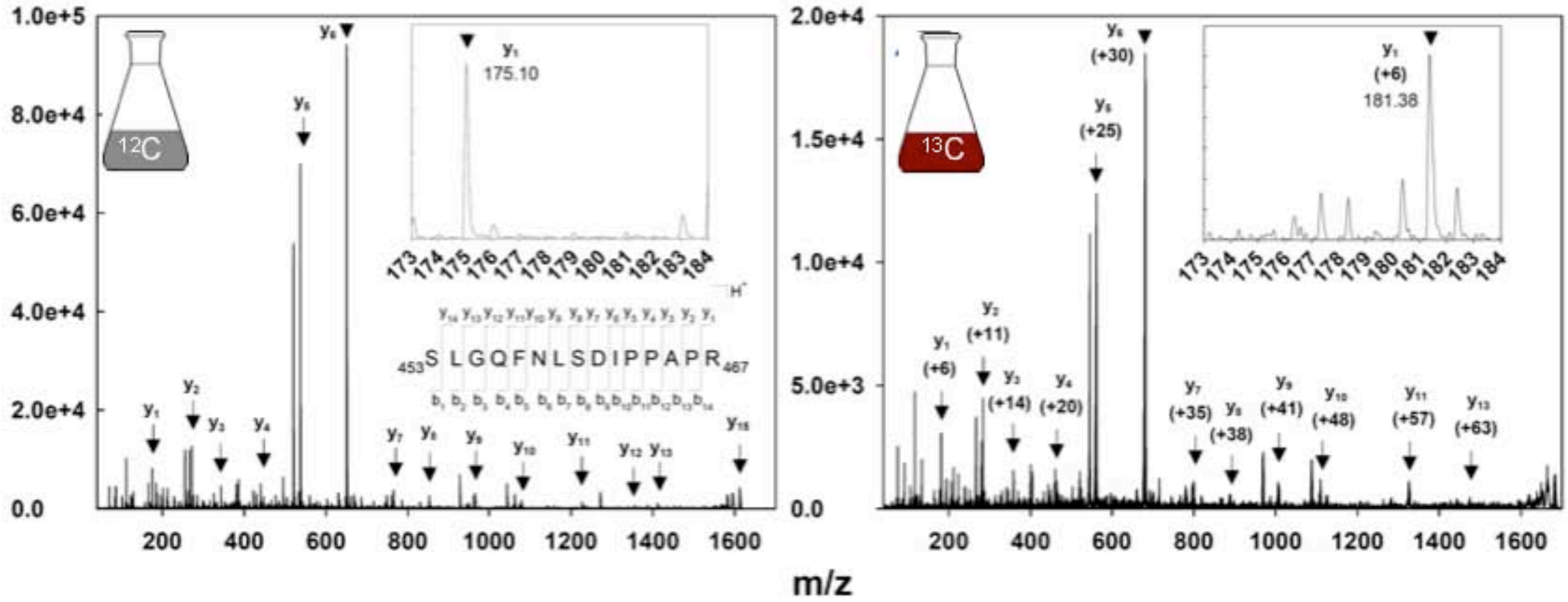
Molecular details on the MS/MS level



Jehmlich et al, *ISME* (2008)

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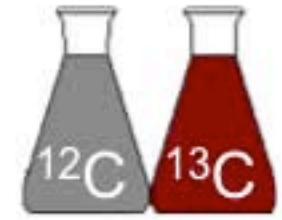
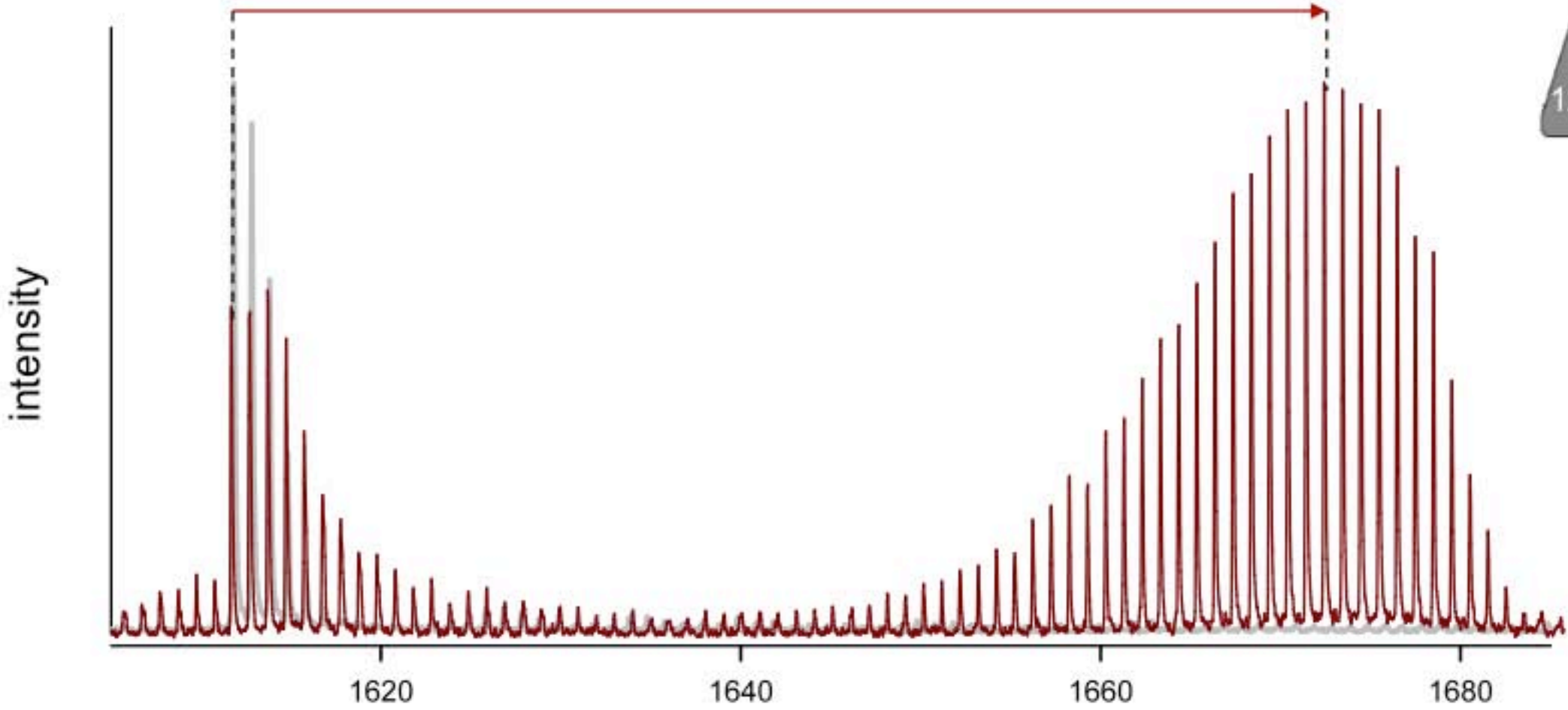
^{13}C incorporation takes place only into EbN1 and ...





detail view of
one peptide

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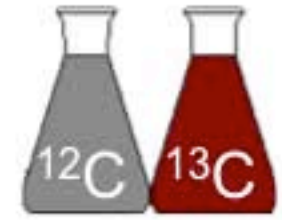
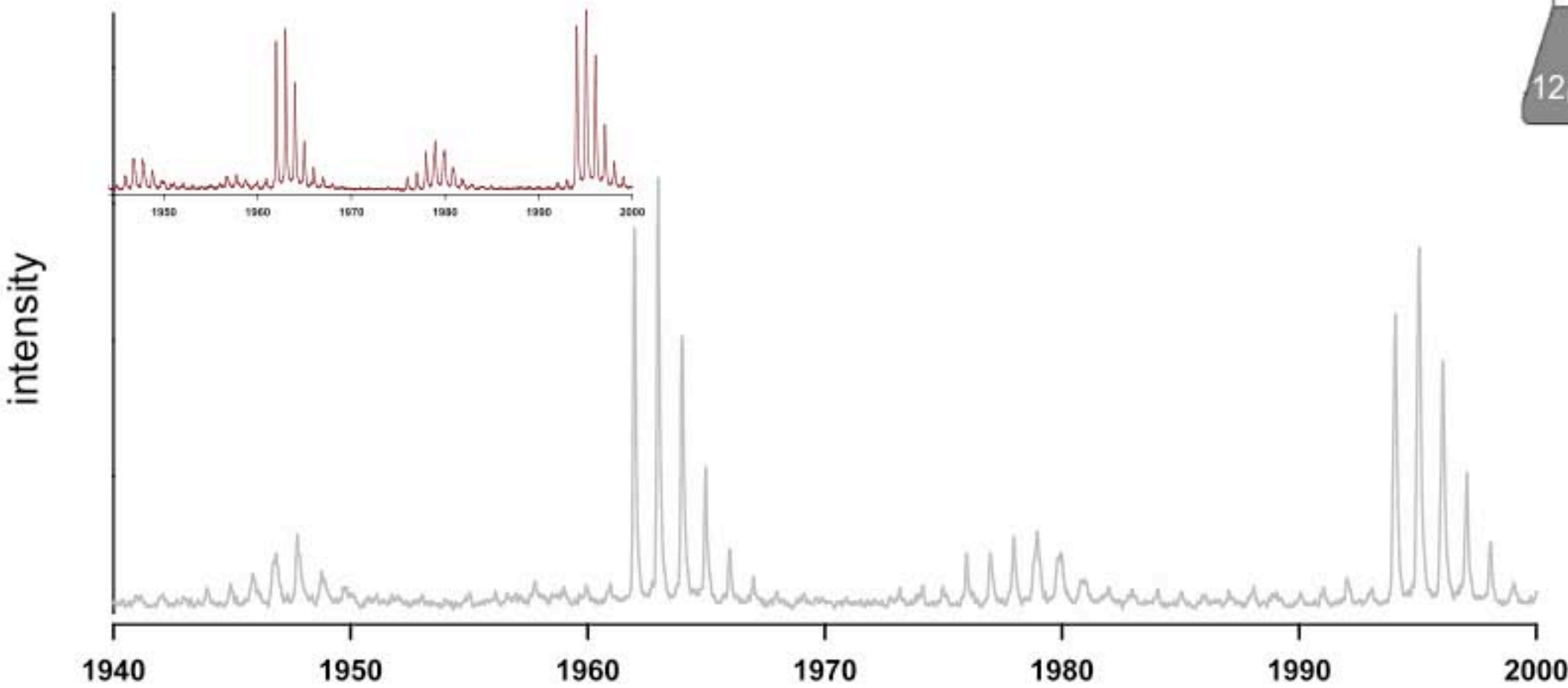
... not in UFZ-1 proteins





detail view of
one peptide

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What about sensitivity and dynamic range of SIP approaches?

⇢ **Sensitivity in respect to the amount of biomass**

- **so far most experiments are performed "off situ"**
- **DNA/RNA-SIP have the potential to be used "in situ" using bactraps with loaded substrates**
- **Protein-SIP might be possible with bactraps**

⇢ **Sensitivity in terms of detectable incorporation**

- **DNA/RNA-SIP are hampered by low resolution of density gradient centrifugation**
- **High resolution MS enables Protein-SIP to detect 2% incorporation of ^{13}C**

⇢ **Dynamic range**

- **DNA/RNA-SIP are mostly on/off**
- **Protein-SIP allows 2-100% range**

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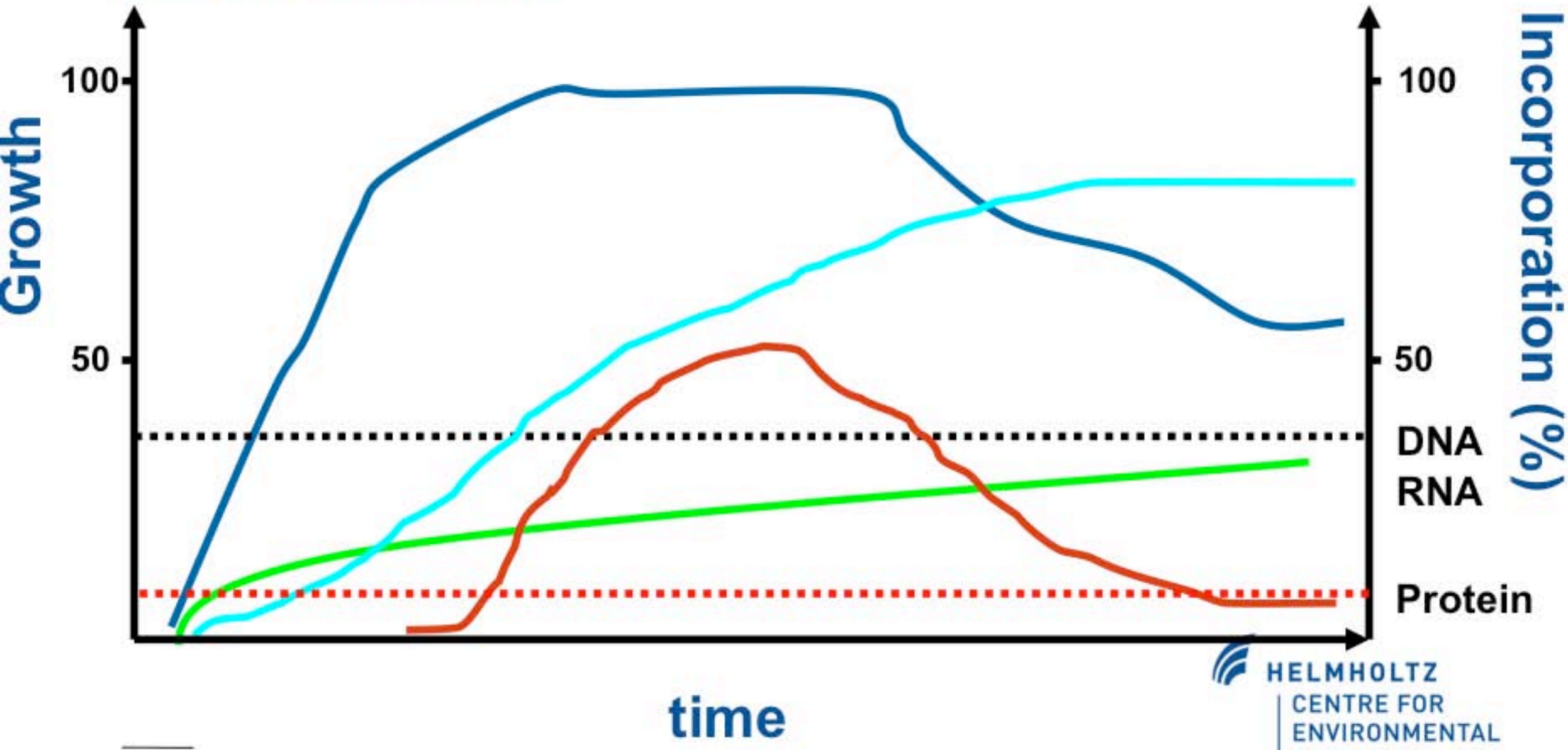
Composition of communities must be considered variable and dynamic

➔ **Who eats what, where and when?**

➔ **Sensitivity and dynamic range must cope with variability of microbial communities**



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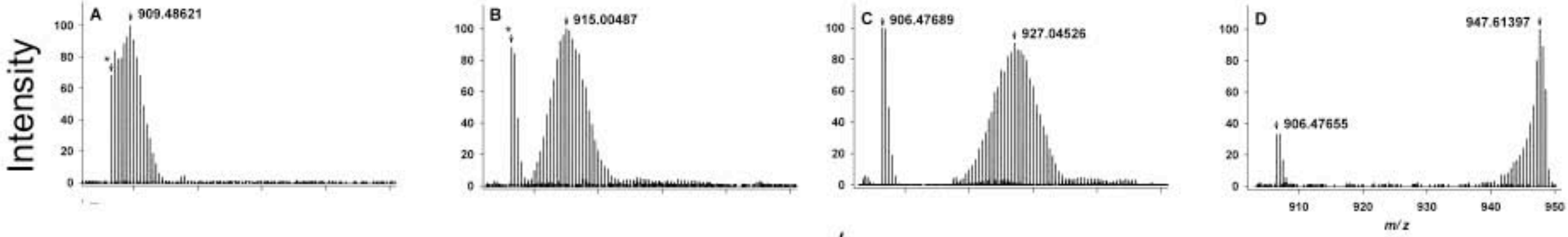
Sensitivity and dynamic range of Protein-SIP

Aerobic growth of *P. putida* on $^{13}C_6$ benzene with defined ^{13}C content



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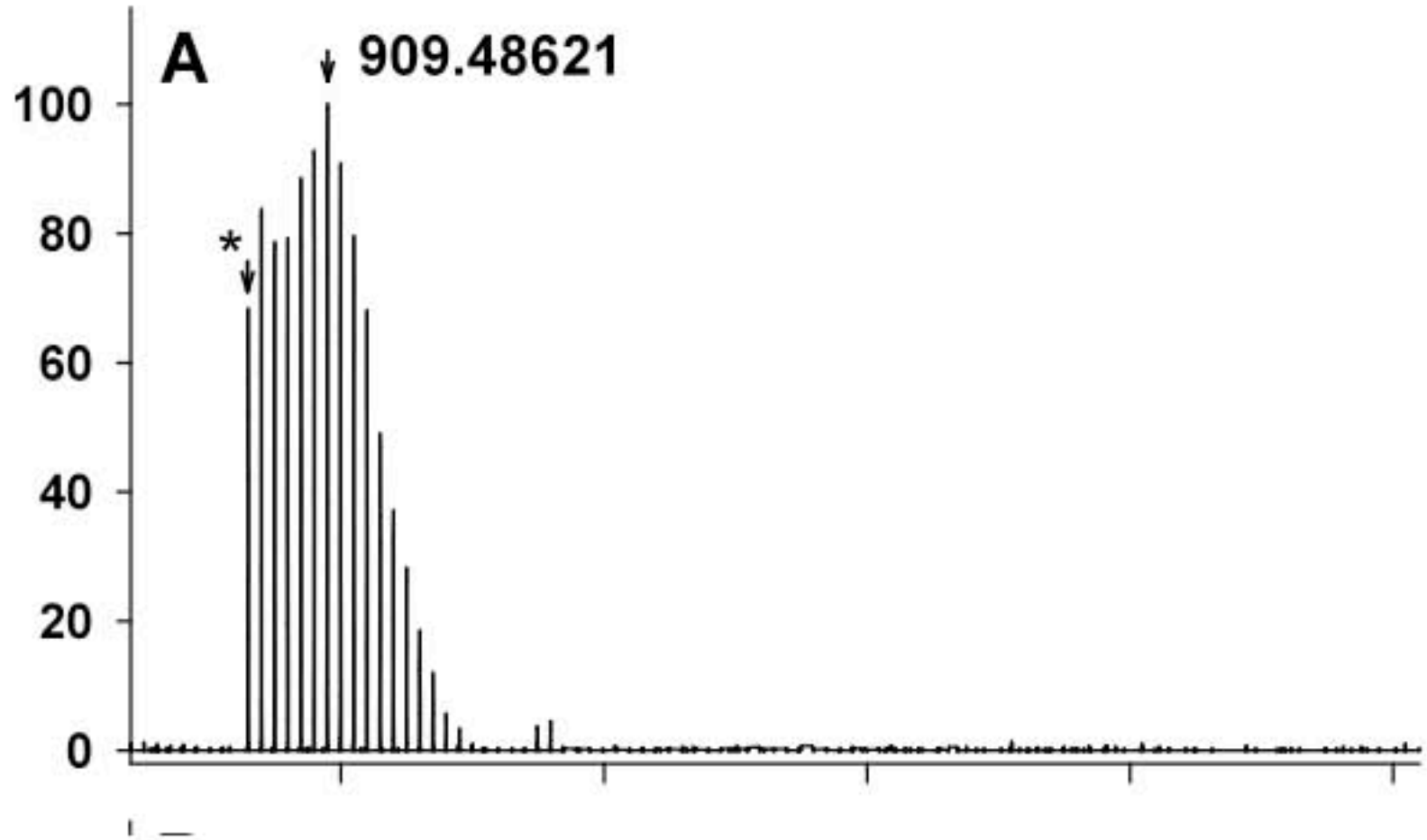
High resolution MS allows sensitivity down to 2% incorporation

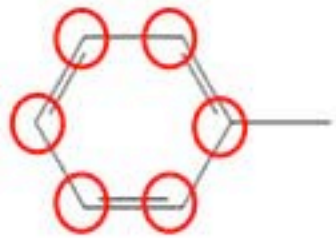


With 10% incorporation the highest peak shifted 6 Da, indicating a sensitivity down to 2%

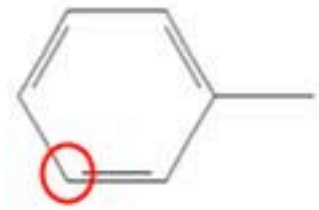
Jehmlich et al, *Mol. & Cell. Proteom.* (2009)

Why do we need high sensitivity and dynamic range?

- Analysis of carbon flux in microbial communities
- Usage of incomplete labelled substrates reduces costs

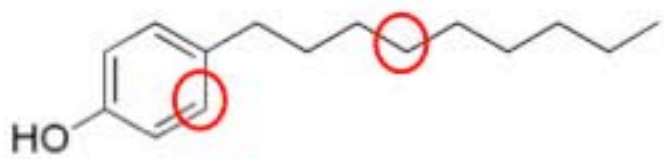


1 mg Toluene $^{13}C_6$= 435€

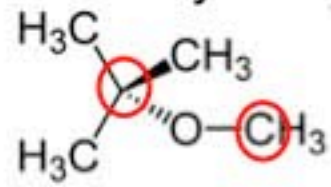


1 mg Toluene $^{13}C_1$= 12-45€

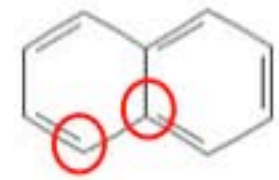
- High sensitivity allows usage of specifically labelled positions



nonylphenol



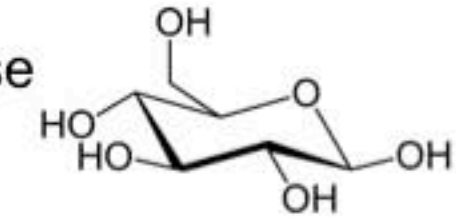
MTBE

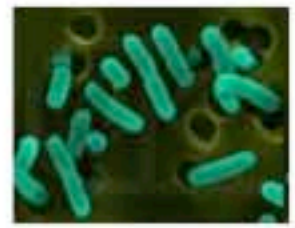


naphthalene

- Analysis of systems with high intrinsic dilution of substrate e.g. trophic networks

D-glucose





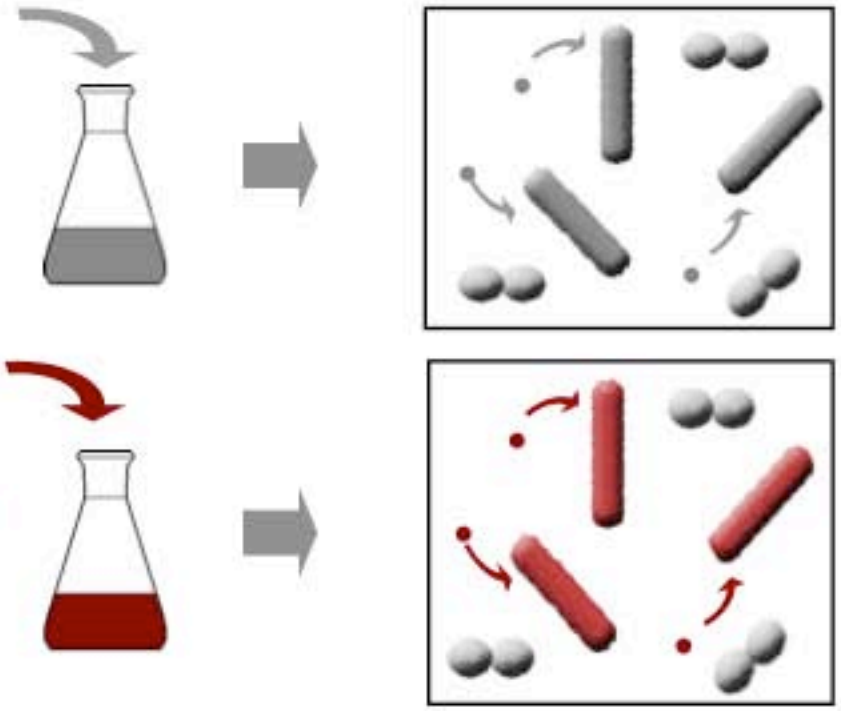
Uptake by bacteria

Uptake by protists

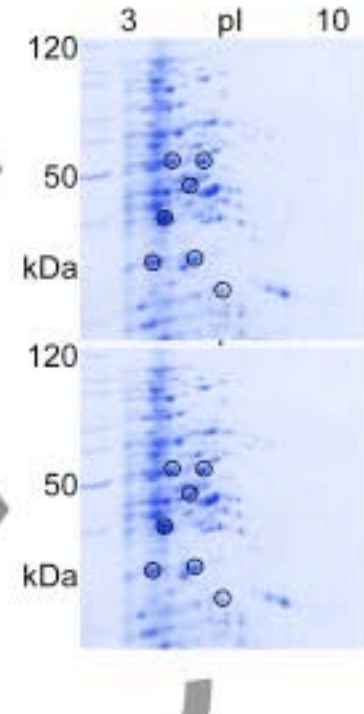
?

Workflow: Protein-based stable isotope probing (Protein-SIP)

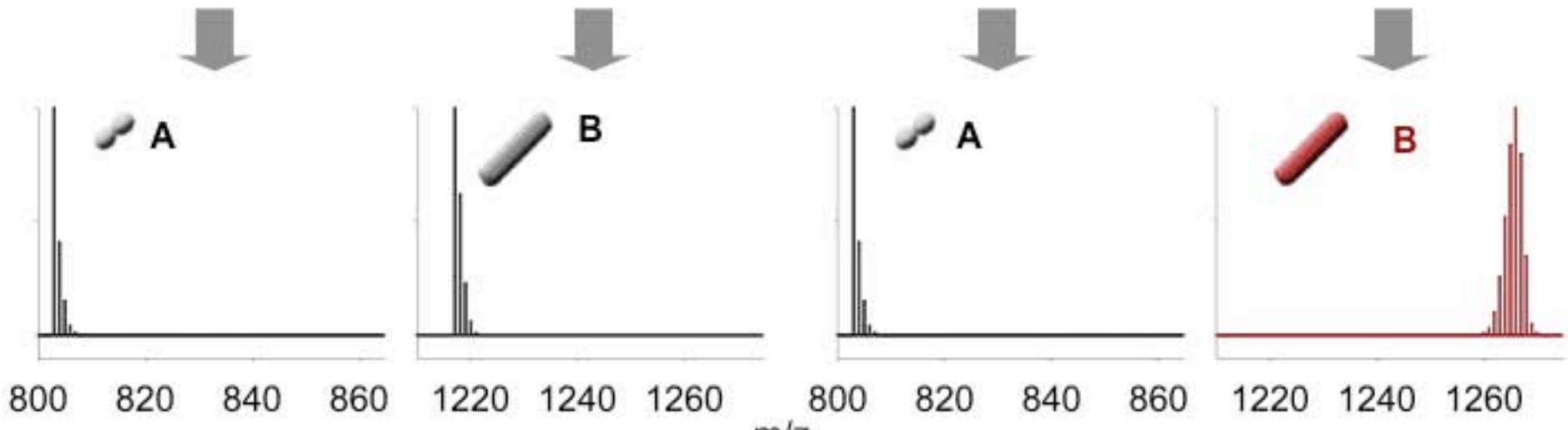
- „light“ substrate
  e.g $^{12}C_6$ benzene
- „heavy“ substrate
  e.g $^{13}C_6$ benzene



Protein extraction



tryptic digestion
mass spectrometry



mass spectra

Half decimal place rule provides a comparison independe calculation of incorporation

- half decimal place rule (HDPR):

mass values of tryptic peptides:

1,420.7 → 14 : 2 = 7

1,630.8 → 16 : 2 = 8

2,058.0 → 20 : 2 = 10

2,389.2 → 24 : 2 = 12

- ^{12}C / ^{13}C isotope mass differ by exactly 1.003355 amu

e.g.	1 C-atom	+0.003355 amu
	10 C-atoms	+0.03355 amu
	100 C-atoms	+0.3355 amu

- accurate mass spectrometer is required

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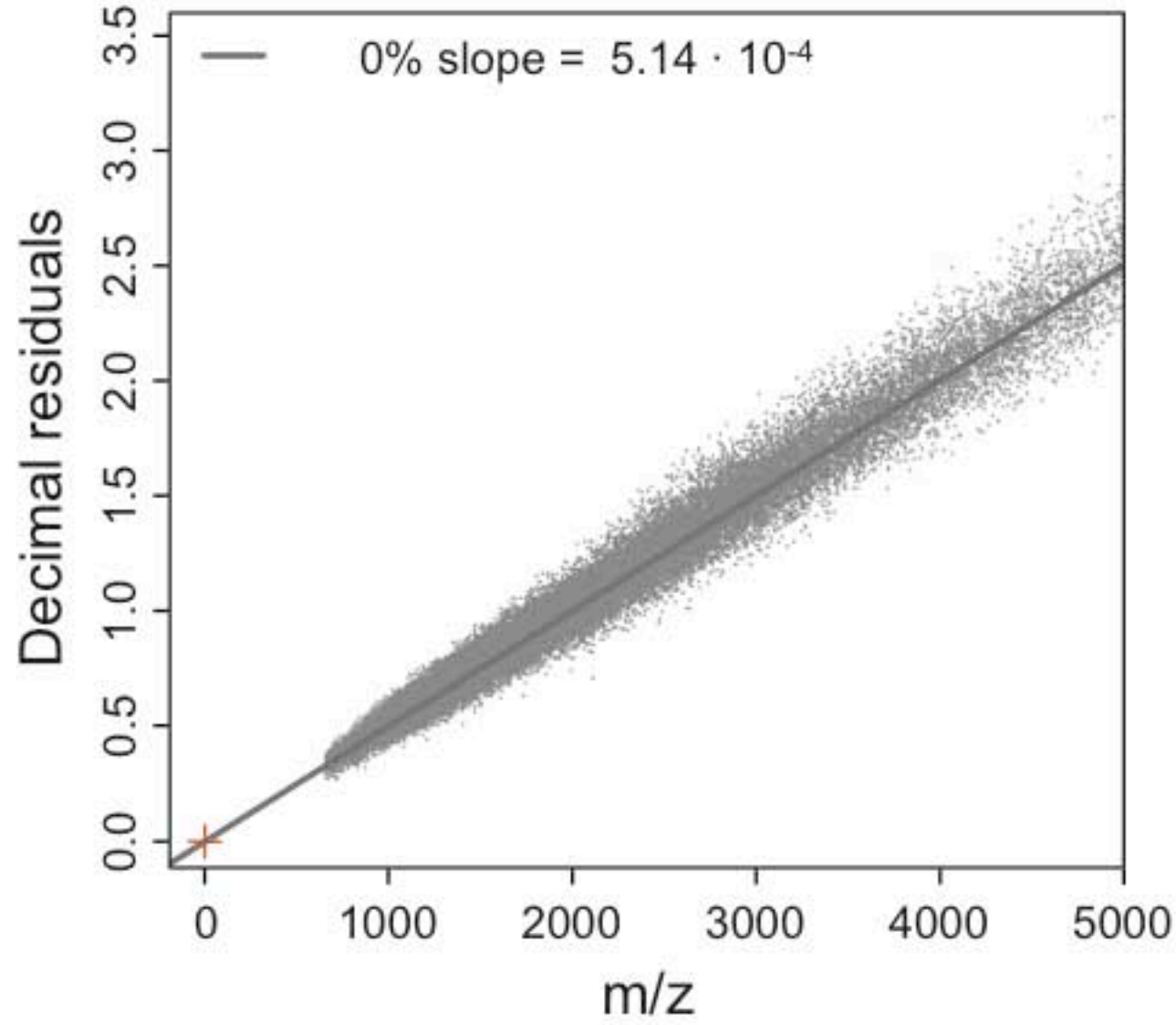
Decimal slope shows regular distribution

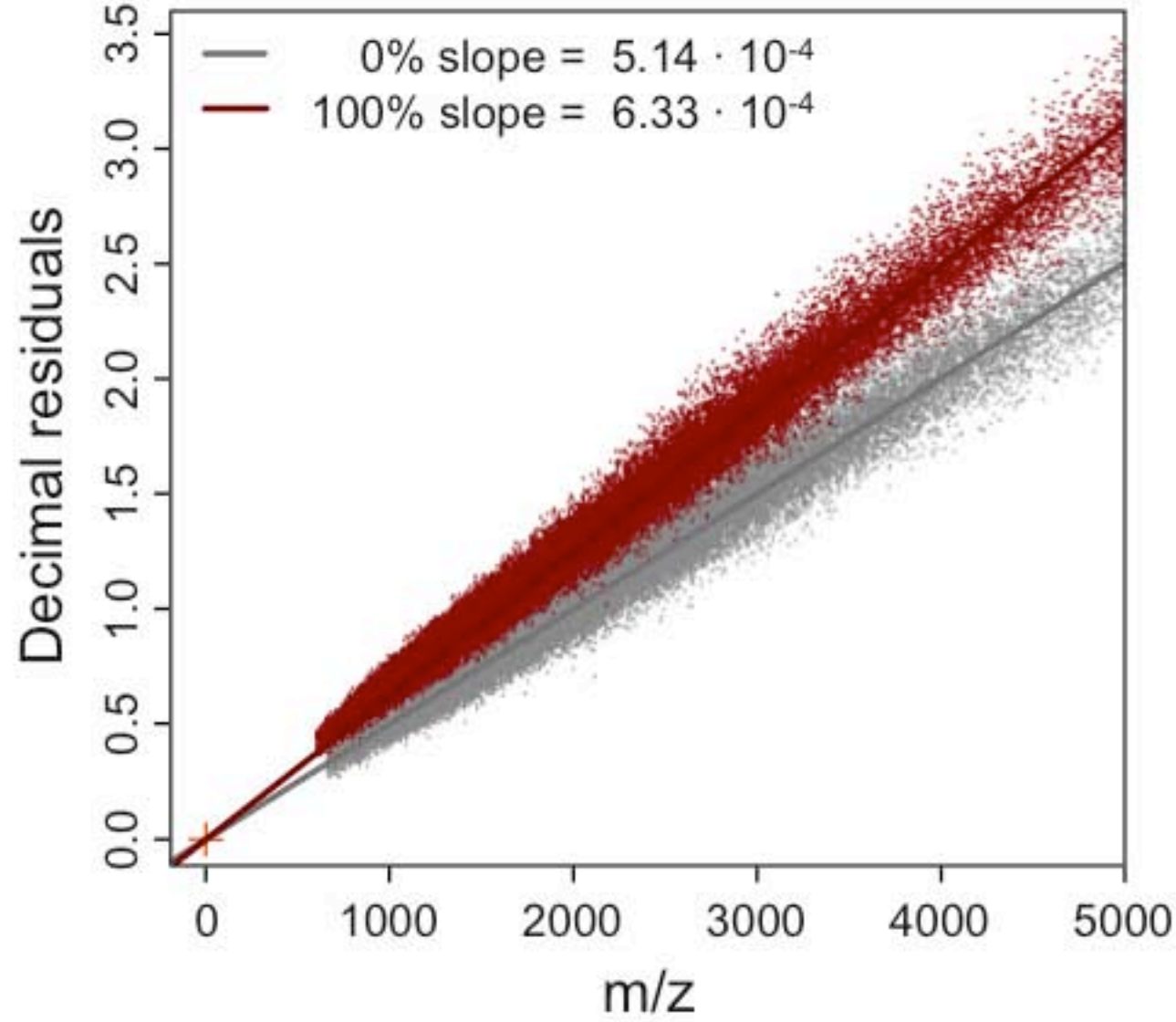
- Helicobacter pylori
  tryptic peptides
  app. 80,000 data points
  each point: tryptic peptides
  fragments lengths varied
  between 2 and
  40 amino acids



Fetzer et al, (2009) in preparation

Simulation points to a probable differentiation of ^{12}C and ^{13}C peptides

- each ^{12}C atom mass was substituted by their ^{13}C atom mass to simulate a theoretical 100 atom % incorporation level



Fetzer et al, (2009) in preparation

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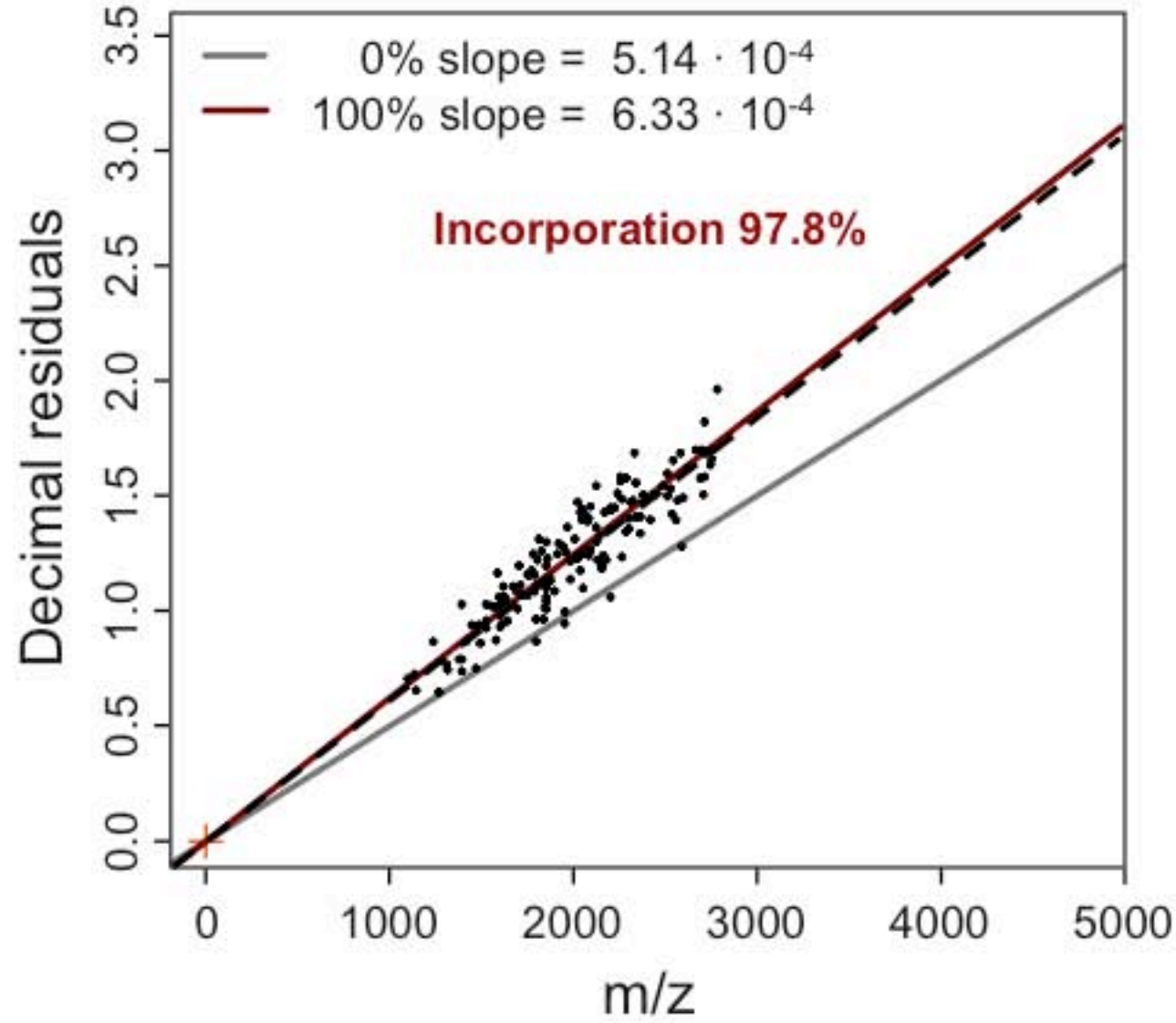
Practical application using fully labeled ^{13}C *P. putida* peptides

- calculated incorporation depends on the number of used peptide masses and the accuracy of MS measurement



$$^{13}C\% = \frac{\text{slope(exp)} - \text{slope}(^{12}C)}{\text{slope}(^{13}C) - \text{slope}(^{12}C)} \cdot 100\%$$

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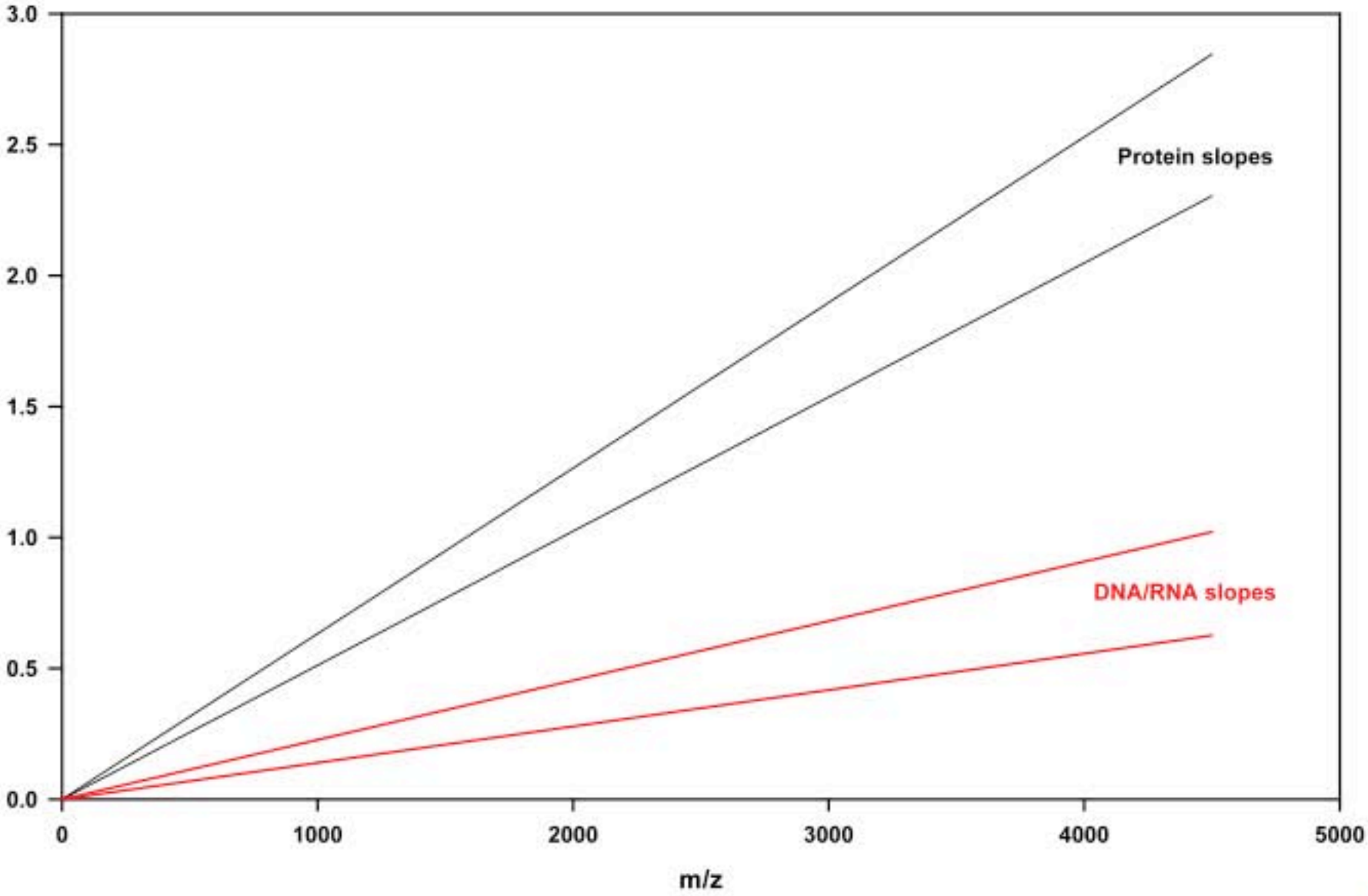
The half decimal rule applies also to DNA/RNA



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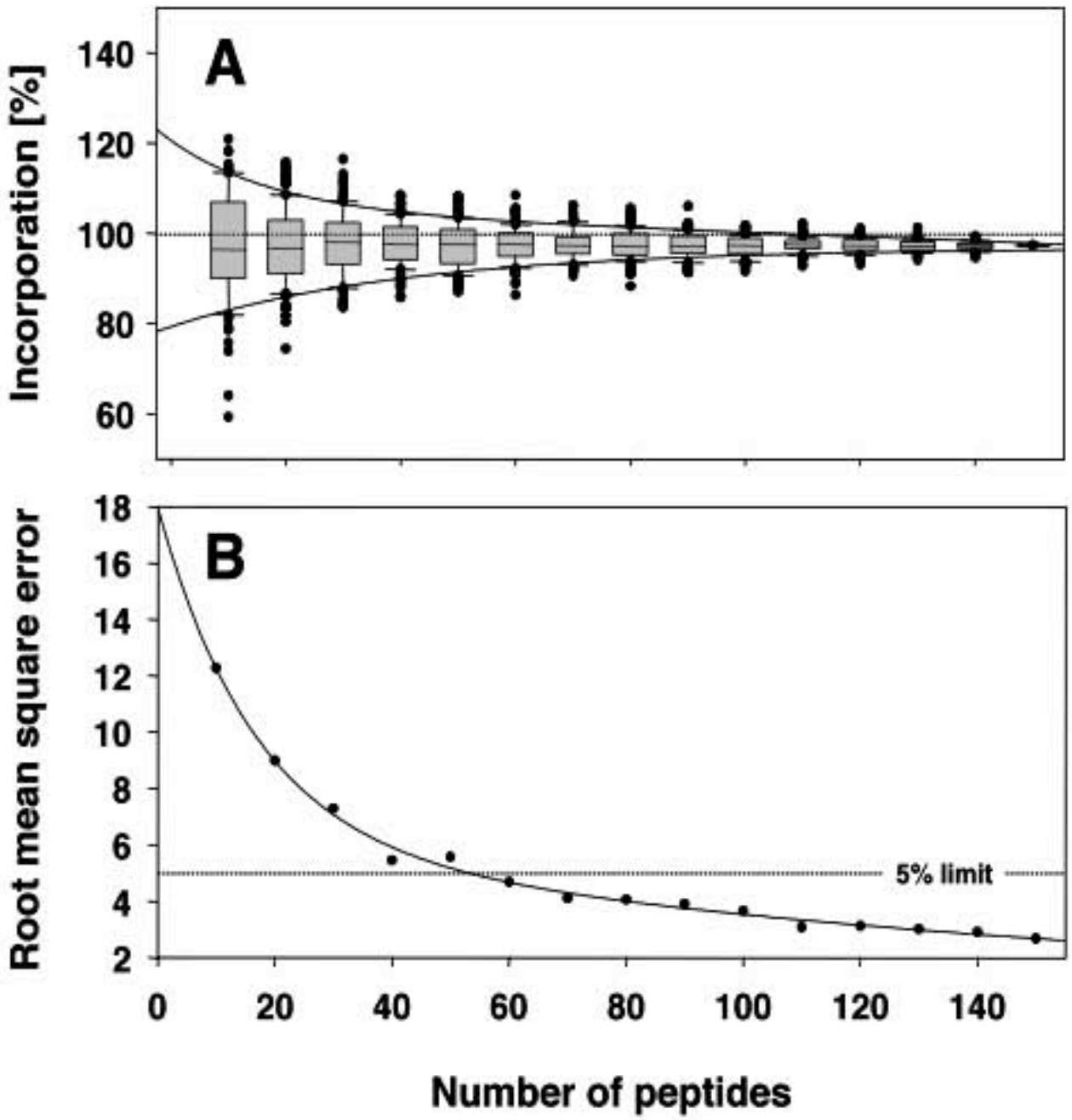
50 peptide masses are required for deviation < 5%



calculated incorporation depends on the number of used peptide masses and the accuracy of MS measurement

Fetzer et al, (2009) in revision

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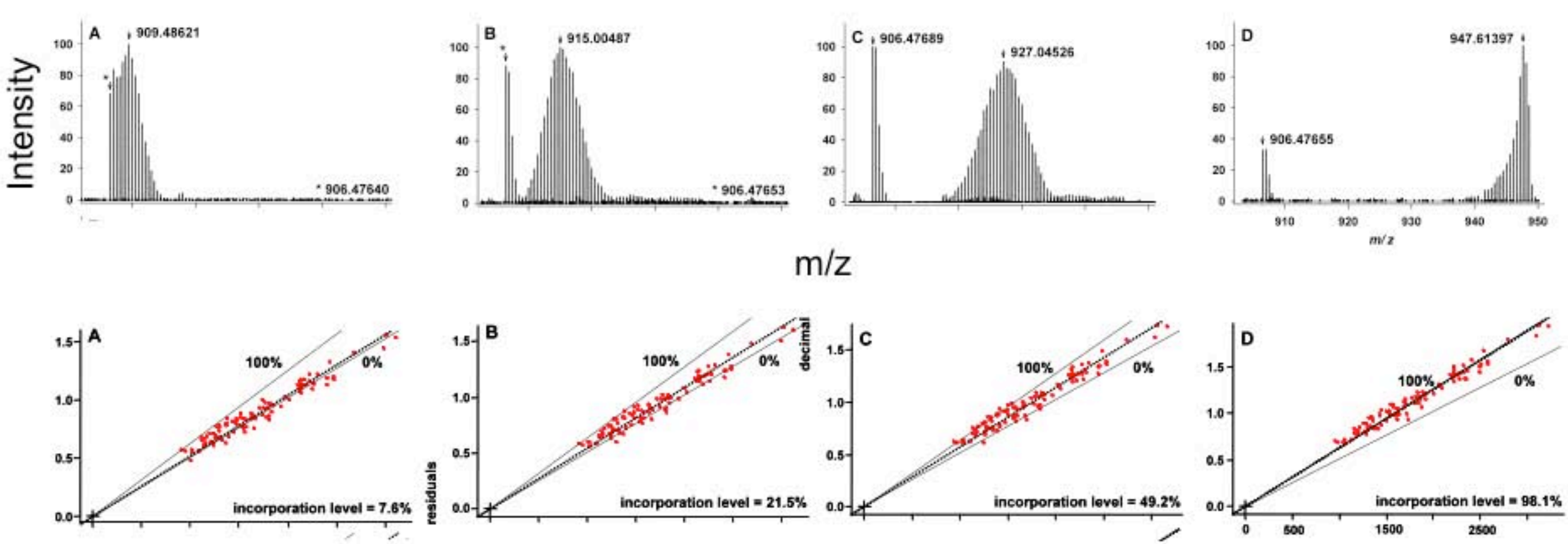
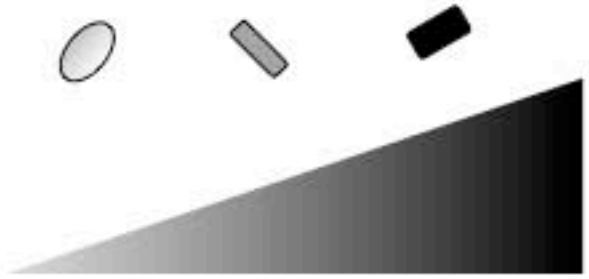
HDPR yields comparison independent determination of incorporation

Aerobic growth of *P. putida* on $^{13}C_6$ benzene with defined ^{13}C content

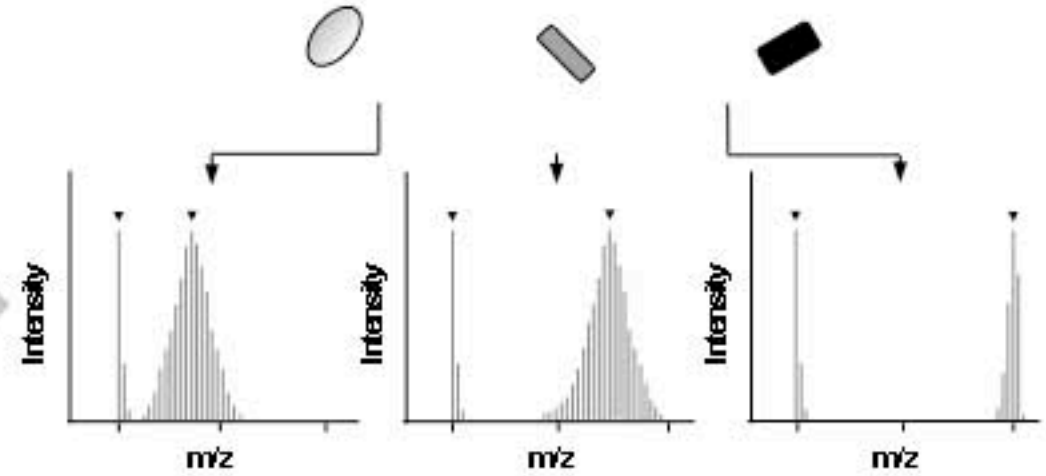


Workflow of Protein-SIP

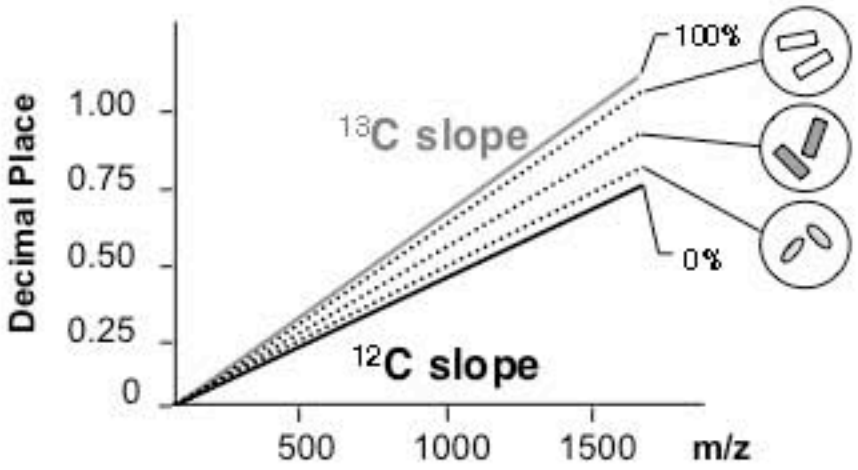
Assimilation



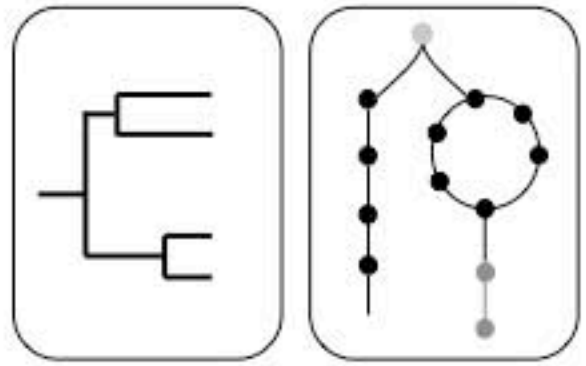
Level of incorporation



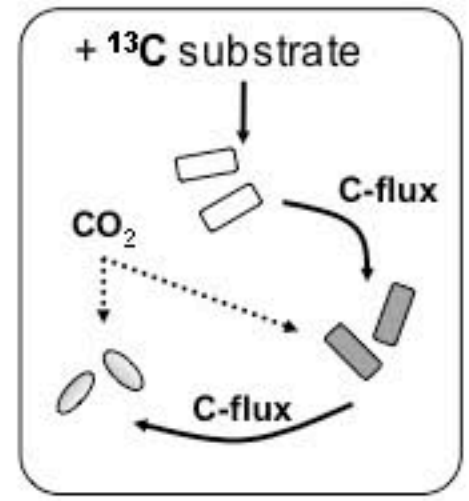
MS-Analysis of peptides



Calc. of incorporation

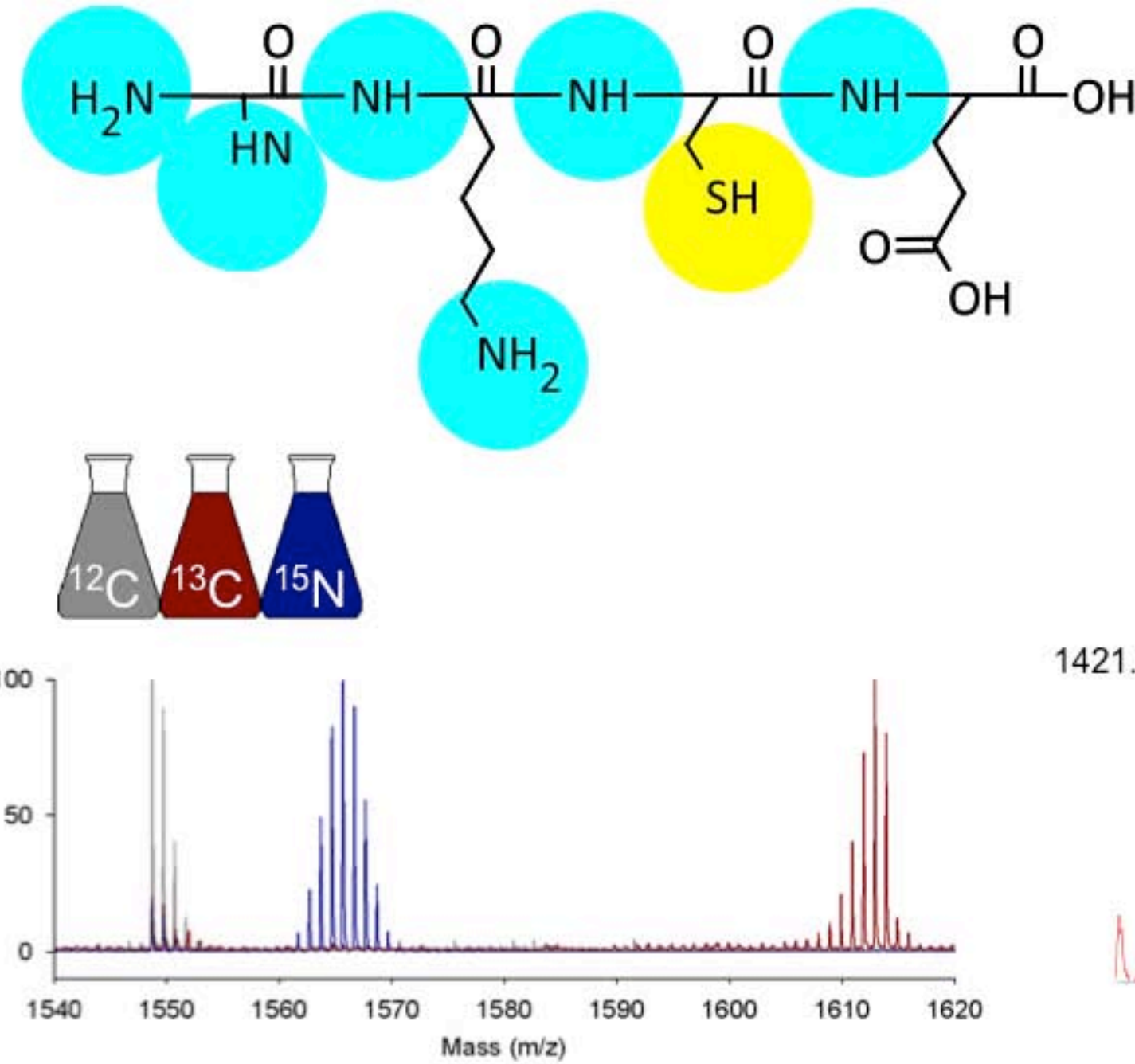


Phylogeny and Physiology



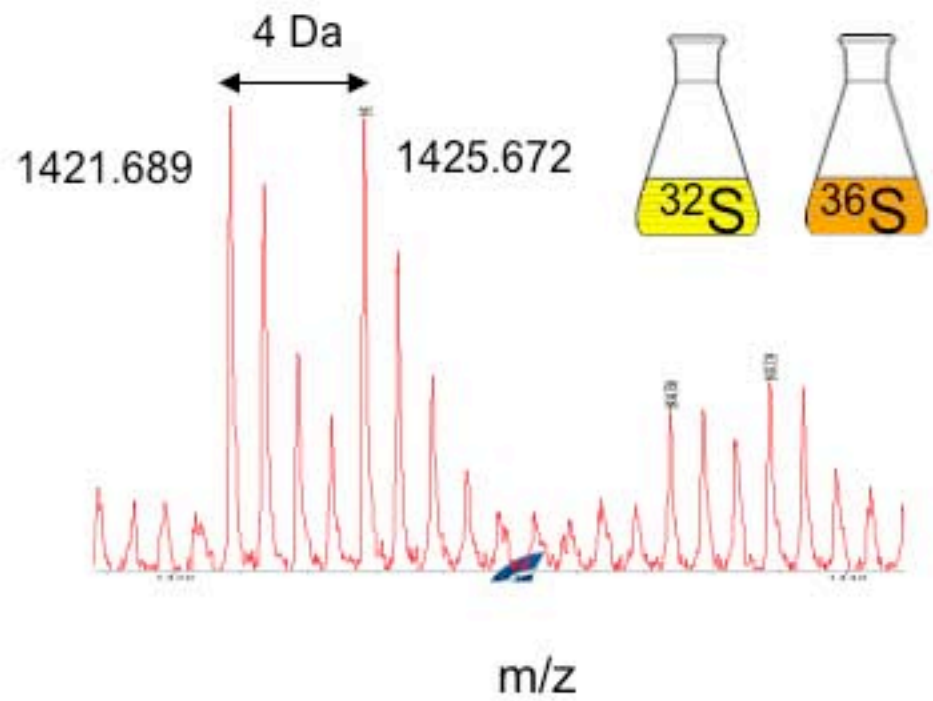
Reconstruction of food web

Protein-SIP can be extended to N and S



1-2 N/average amino acid

5% of amino acids contain sulfur



Jehmlich et al, RCMS, 2008, Jehmlich et al., RCMS,2009, Germam Patent application

Conclusions

- Protein-SIP provides concomitantly information about functional proteins and metabolic activity of species
- Sensitivity in terms of biomass fits well with „*off situ*“ experiments, „*in situ*“ might be possible as well
- Protein-SIP, in contrast to DNA/RNA-SIP, features a dynamic range (2-100%) of incorporation of ^{13}C
- High resolution mass spectrometry and the Half decimal place rule allow a comparison independent and solely LC-MS based approach
- Protein-SIP can be extended to other isotopes (^{15}N, ^{36}S)
- Time resolved analysis of carbon flux in microbial communities becomes possible

Example for importance of genomic information

Cooperation with Prof. Suflita on Proteomics of alkane degraders

Desulfoglaeba alkanexedens

Anaerobic sulfate reducer, degrades C_6-C_{12} *n*-alkanes completely to CO_2

Isolated from a oily-wastewater storage facility

Cells were grown on butyrate and decane, both under sulfate-reducing conditions

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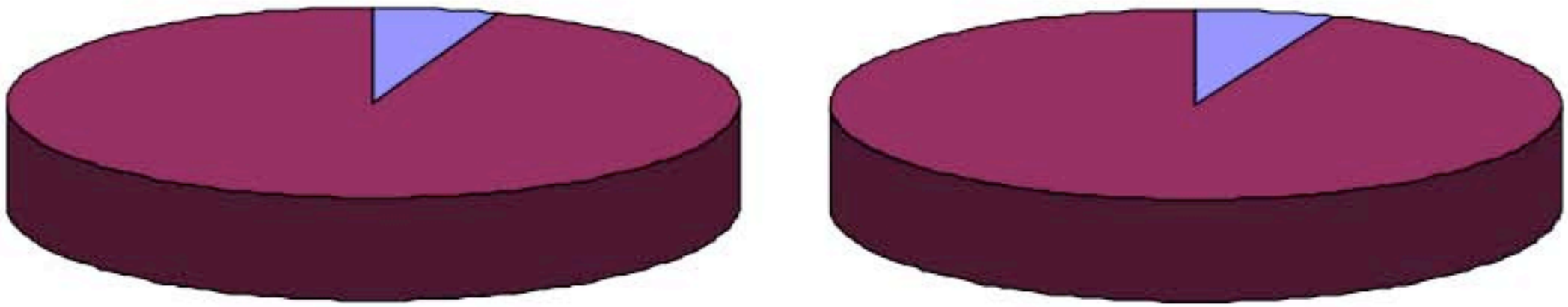
NCBI is not sufficient for environmentally important species



Growth with alkane:
17 without and
287 identifications
with genome

Growth with butyrate:
21 without and
324 identifications
with genome

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Further developments in Environmental Proteomics

- Improvement of isolation procedures
- Steady increase in genomic information of relevant species and communities
- Usage of proteins as Biomarkers for physiolgical properties in ecosystems

- Need for scientific exchange
- We plan to organize a meeting on Environmental Proteomics in 2011 in Leipzig to continue this event

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SPP 1319

Hope to see you 2011 in Leipzig…

12C-Toluol + Nitrat, Aussenseite, 11.06.07, 1kV, 5mm

2 µm