Non-targeted MS analyses in biomedical research

A potential approach for the selection of compounds to study in more detail

Svein-Ole Mikalsen

Faculty of Natural and Health Sciences
University of Faroe Islands

Red kite (Milvus milvus)

Northern shoveler (Anas clypeata)

Targeted vs non-targeted MS

	Targeted MS	Targeted MS/MS
Fully targeted (MRM)	+	+
Product ion scan	+	-
Precursor ion scanning	-	+
Neutral loss scan	-	Δm/z
Fully non-targeted	-	-

Some characterististics of targeted and non-targeted MS

	Targeted	Non-targeted
Specificity	+++	-
Absolute quant	+++	-
Relative quant	+++	-/+/++/+++
Dynamic range	+++	+
Reproducibility	+++	+/++
Unknowns	-	+++

Some characteristics of MS instruments (I)

	Q-q-Q	Ion Trap	Q-TOF	LIT-Orbitrap
Speed	+++	++	++	++/+
Accuracy	+	+	+++	+/++++
Resolution	+	+	+++	+/++++
Sensitivity	+++	++	+	++
Versatility	+++	+++	++	+++++
Dynamic range	+++	+	++	++
MS^n	-	+++	-	+++

Modified from Domon and Aebersold (2006) Science 312, 212-217

Some characteristics of MS instruments (II)

	Q-q-Q	Ion Trap	Q-TOF	LIT-Orbitrap
Speed	+++	++	++	++/+
Accuracy	+	+	+++	+/++++
Resolution	+	+	+++	+/++++
Sensitivity	+++	++	+	++
Versatility	+++	+++	++	+++++
Dynamic range	+++	+	++	++
MS^n	-	+++	-	+++
Unknowns	+	+/++	+++	++++

Modified from Domon and Aebersold (2006) Science 312, 212-217

Typical non-target questions in biomedical research

Typical approaches:

Non-targeted MS → non-targeted MS/MS → database search
Non-targeted MS → profile analysis → targeted MS/MS

- Which compounds are found in this sample?

- What distinguishes condition 1 from condition 2?

- Which modifications are present in protein A, and how do they change with treatment?

- Which bioactive compounds are present in this sample?

Typical non-target fields in biomedical research (I)

DNA → RNA → protein

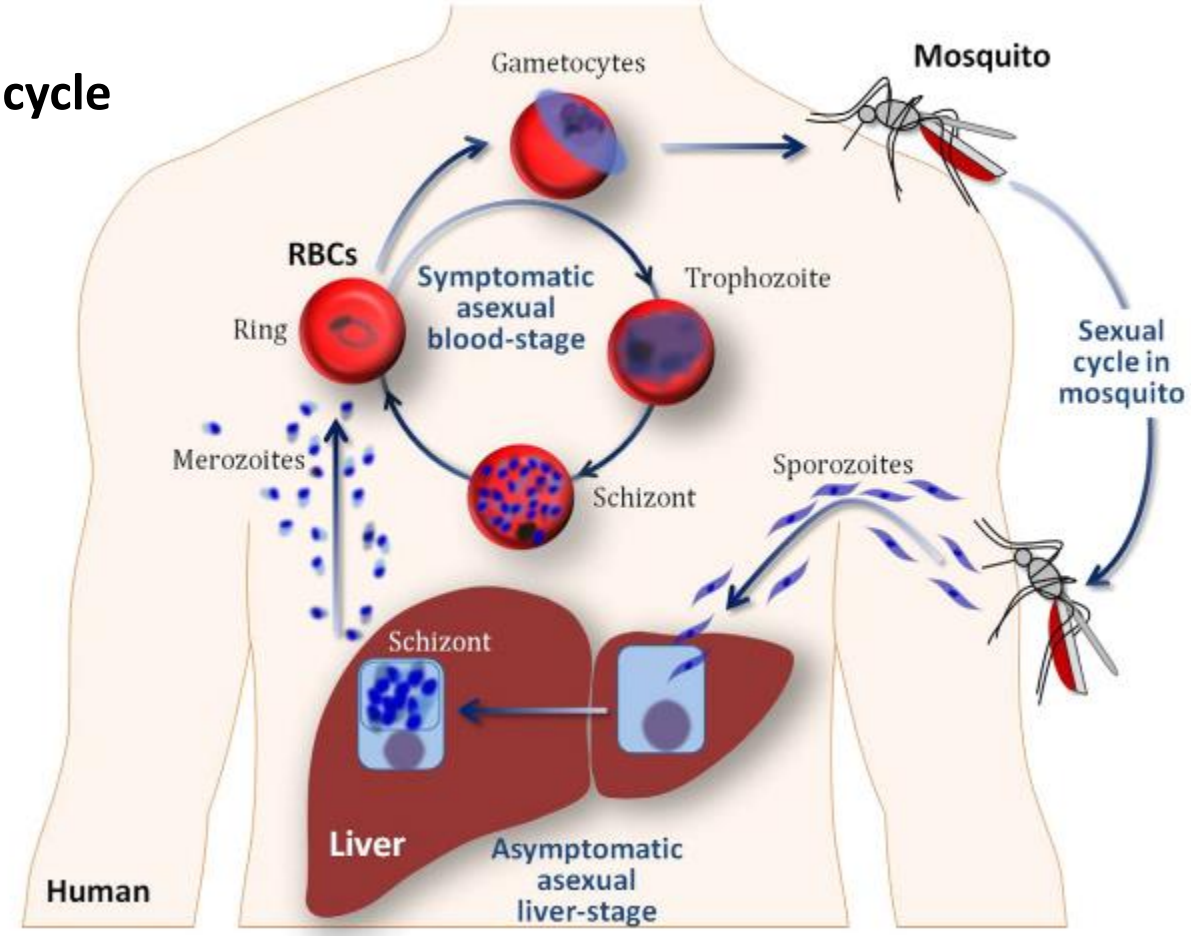
Genomics → "Functional genomics" → Proteomics

- Proteomics

- **Proteins are the workhorses of the organism**
- **Protein level may not correlate with mRNA level**
- **Post-translational modifications may change protein activity**
- **Protein interactions important for functions**

Proteomic example :
<u>The malaria parasite (I)</u>

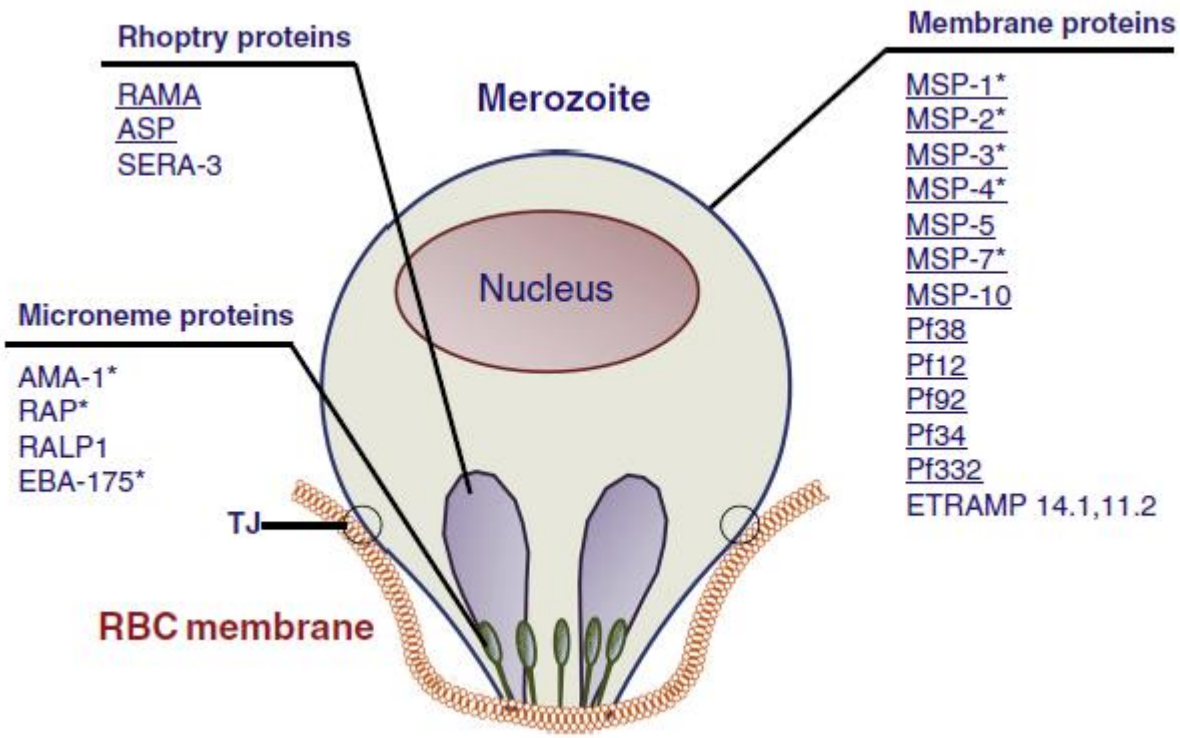
- Proteome during life cycle



Bautista et al (2014) J Proteomics 97, 107-125

Proteomic example:
The malaria parasite (II)

- Proteome during life cycle
- Proteome of subcellular structures
- Proteome during invasion



Bautista et al (2014) J Proteomics 97, 107-125

Proteomic example:
The malaria parasite (III)

- Proteome during life cycle
- Proteome of subcellular structures
- Proteome during invasion
- **Proteome of the infected erythrocyte**
- **Proteome of serum upon infection**
- **Proteome of the immune system**

Typical non-target fields in biomedical research (II)

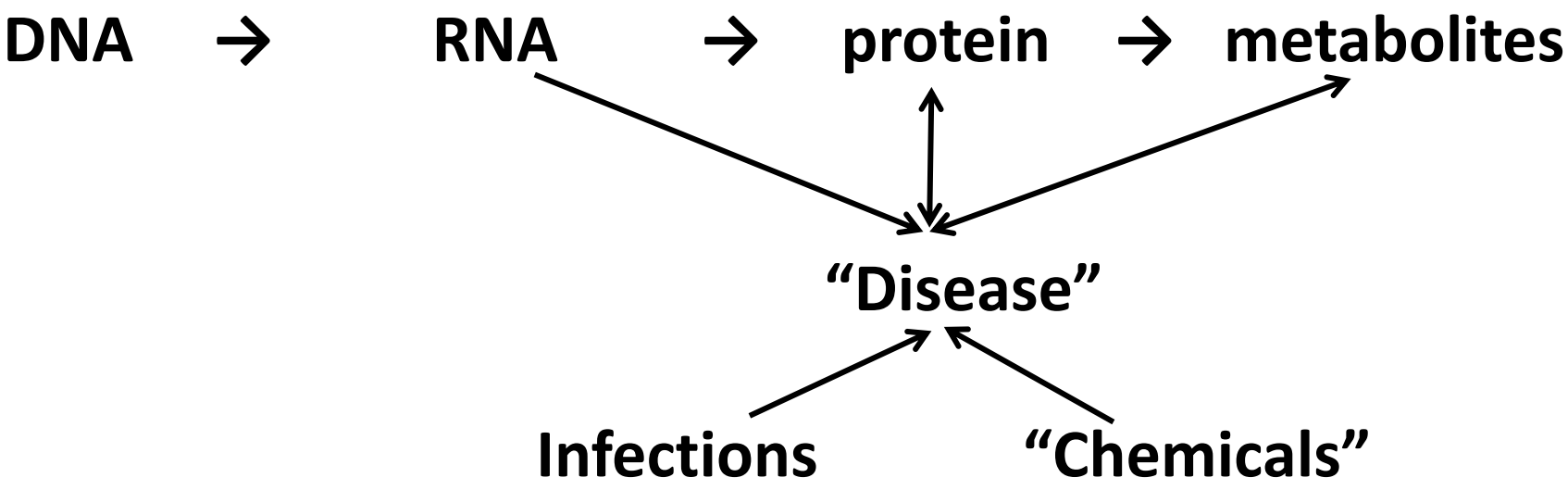
DNA → RNA → protein → metabolites

Genomics → "Functional genomics" → Proteomics → Metabolomics

- Metabolomics

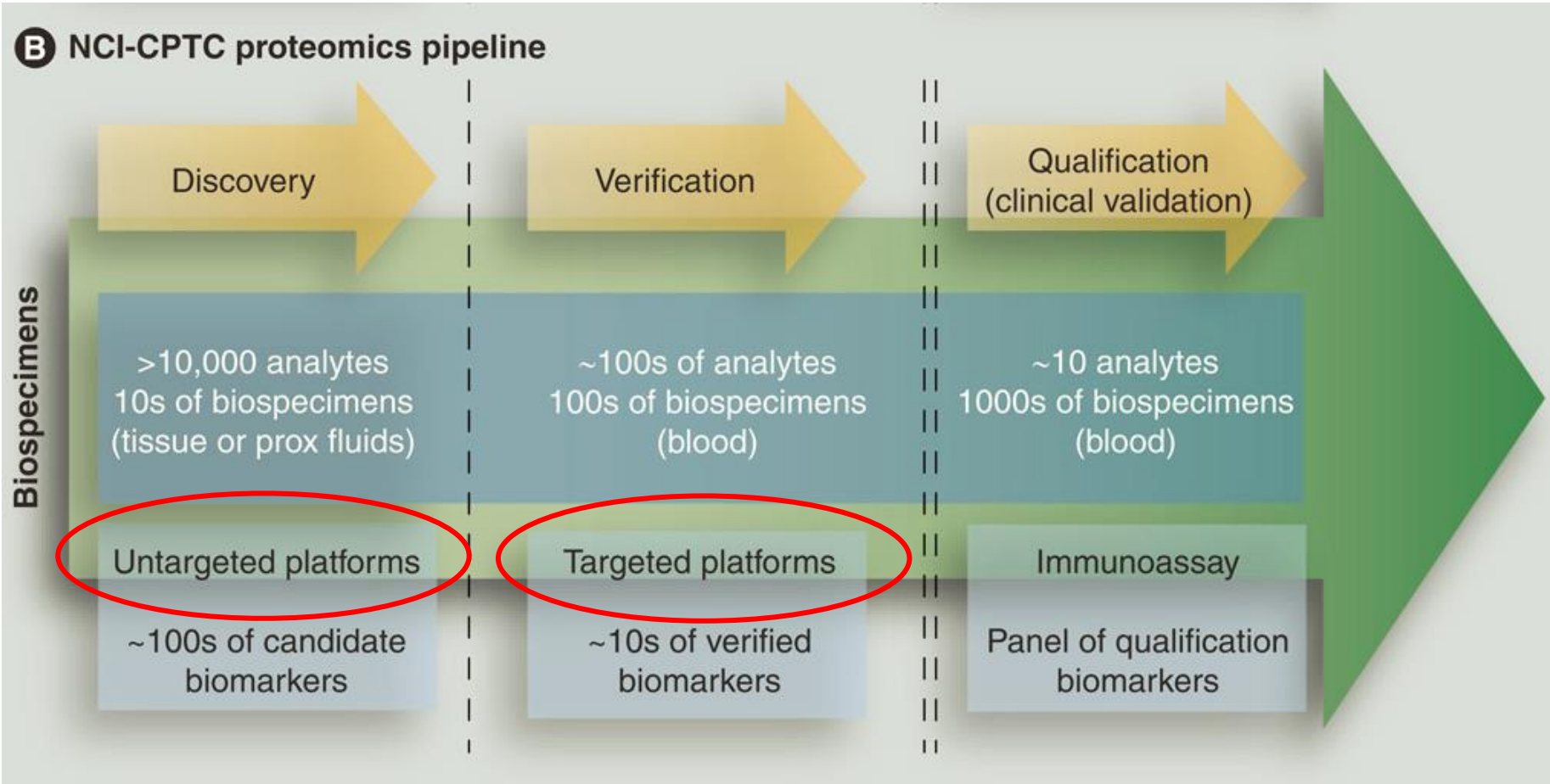
- Metabolites – direct signatures of biochemical activities
- Better correlation with phenotype?
- Blood, saliva, urine, cerebrospinal fluid, cytosol, etc.

Typical non-target fields in biomedical research (III)



- Biomarkers/markers (for disease, environmental impact, etc)
  - Diagnostic or prognostic markers
  - Sensitivity (LOD), specificity (false negatives, false positives)

Example:
Biomarkers: Pipeline from bench to bed



Boja et al (2011) Biomark Med 4, 799-803

Clinically Established Predictive Biomarkers for Targeted Therapies

- **Positive Predictive Biomarkers**
  - *Her2/c-ErbB2* amplification: Trastizumab, lapatinib in breast cancer
  - *EGFr* mutation: Gefitinib, erlotinib in non-small cell lung cancer
  - *c-Kit* mutation: Imatinib in GIST
  - *Alk* amplification/translocation: Crizotinib in lymphoma/lung cancer
  - *B-Raf* mutation: Vemurafenib in melanoma
  - *Bcr-Abl* translocation: Imantinib, dasatinib, nilotinib in CML/ALL
  - Oestrogen receptor expression: Anti-oestrogens in breast cancer
  - *RAR* translocation: All-*trans*-retinoic acid in PML
- **Negative Predictive Biomarkers**
  - *K-Ras/B-Raf* mutation: Cetuximab, panitumumab in colorectal cancer

Herbie Newell at http://www.ecmcnetwork.org.uk/prod_consump/groups/cr_common/@ecm/@gen/documents/generalcontent/cr_093646.pdf

Typical non-target fields in biomedical research (IV)

- Bioprospecting
    - Identifying compounds of fractions with bioactivities (blood pressure regulation, antioxidants, anti-microbial, anti-cancer, immune regulators...)

Example:

<u>Bioprospecting in salmon waste (I)</u>

Sample (salmon skin or other waste)

↓

Treat (homogenize, digest)

↓

Measure bioactivities (anti-oxidant, blood pressure regulation, anti-bacterial...)

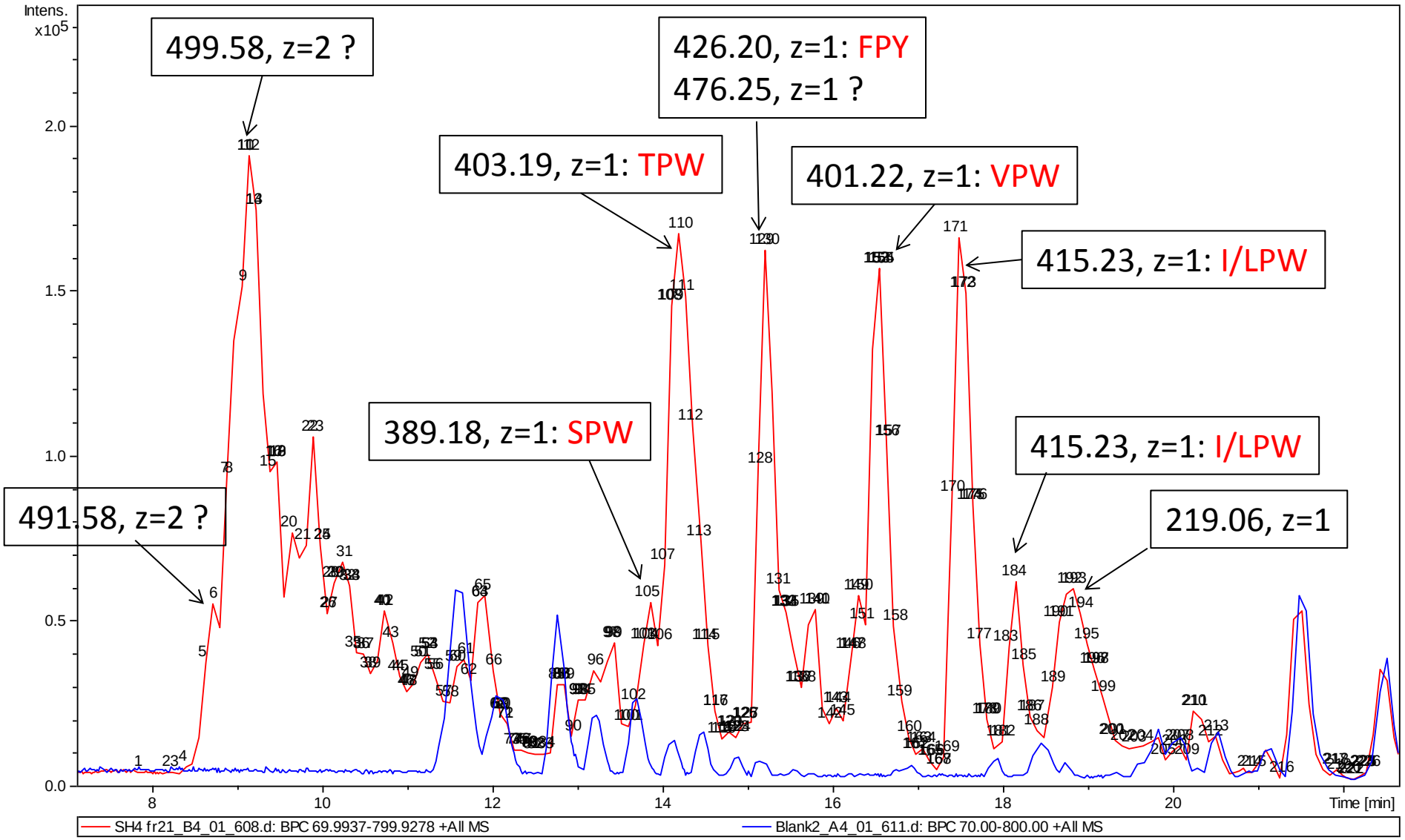
↕

Fractionate

↓

Characterize and identify compounds

Bioprospecting in salmon waste (II): A fraction from skin



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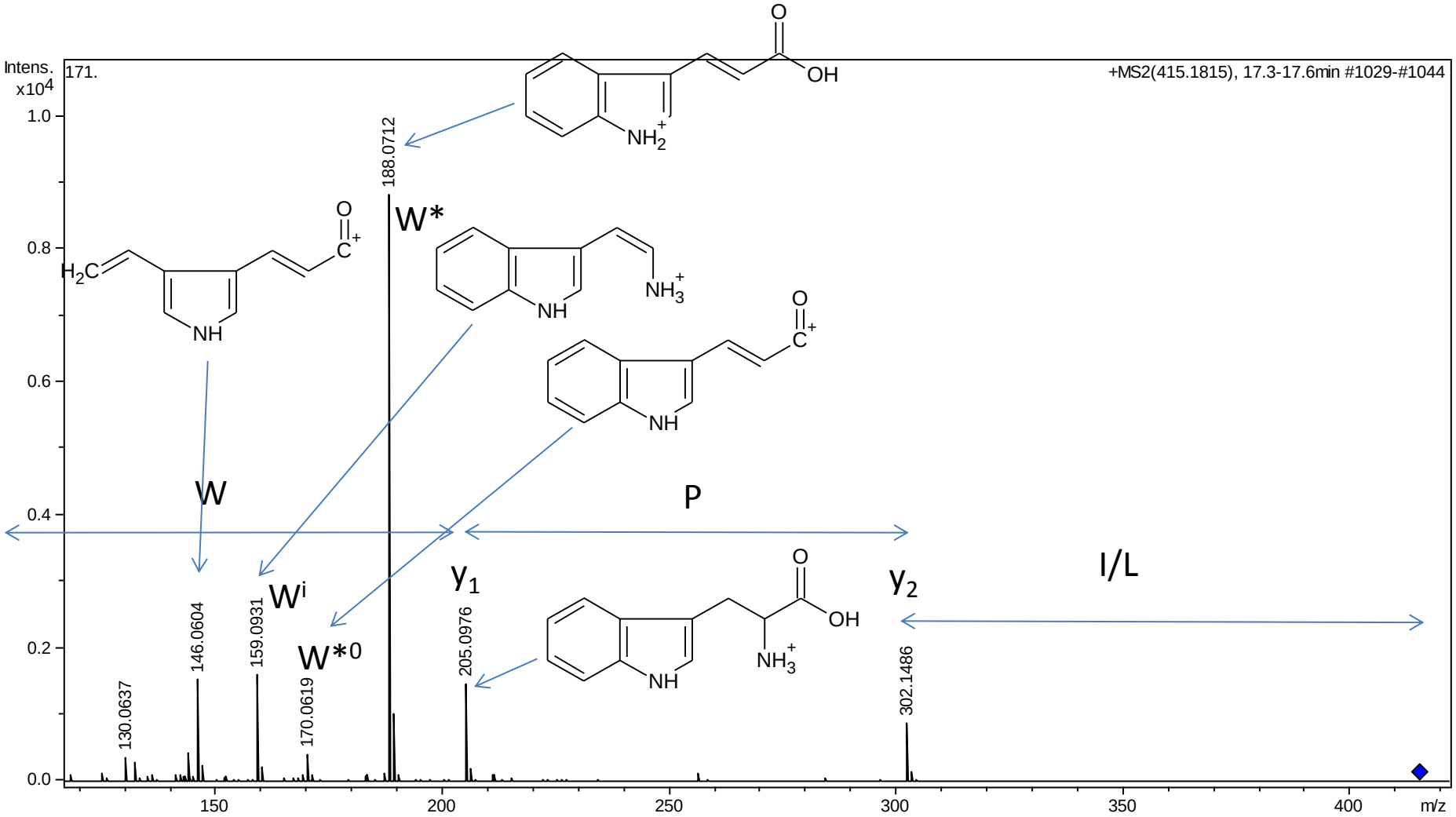
SS Falkenberg, HH Nielsen, SO Mikalsen, in prep.

MS/MS spectrum of (I/L)PW



SS Falkenberg, HH Nielsen, SO Mikalsen, in prep.

Non-targeted MS depends on databases (I)

Large scale data:

MS + MS/MS → Search motor + database → Statistically validated suggestions

	Search motor tool	Databases
Proteomics	Matrix Mascot	NCBI GenBank
	Protein Prospector	SwissProt
	Compomics	UniProt
Metabolomics	XCMS	Metlin
	MetaboSearch	KEGG
	MathDAMP	PubChem
	MetAlign	MassBank
	MZMine	Human Metabolome DataBase
	NIST	NIST
		Lipid Maps
		NORMAN

Non-targeted MS depends on databases (II)

Large scale data:

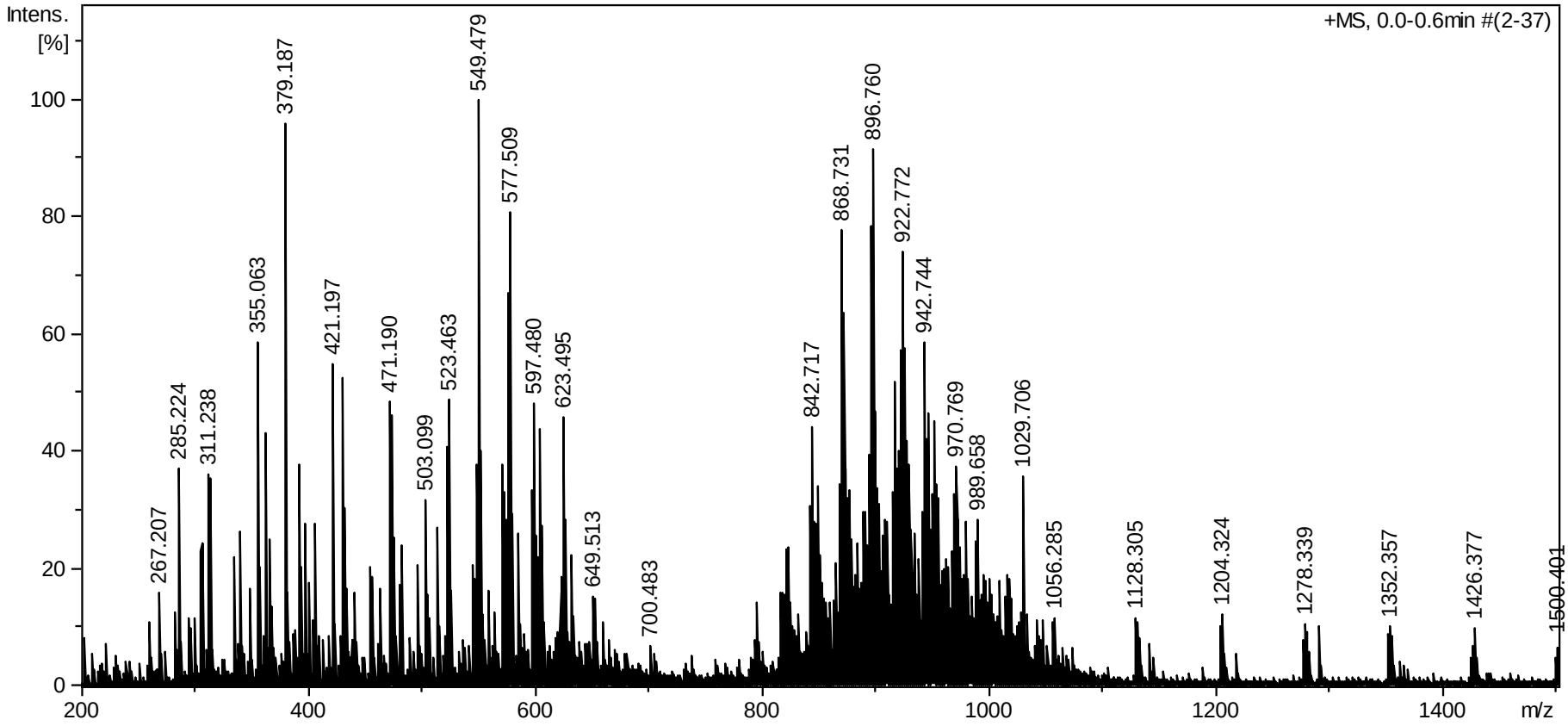
MS + MS/MS → Search motor + database → Statistically validated suggestions

Small-scale, search-motor inaccessible data:

MS + MS/MS → Manual interpretation → Confirm with compound

→ Manual database search → Confirm with compound

How to pick compounds of interest from a sample with unknown content?



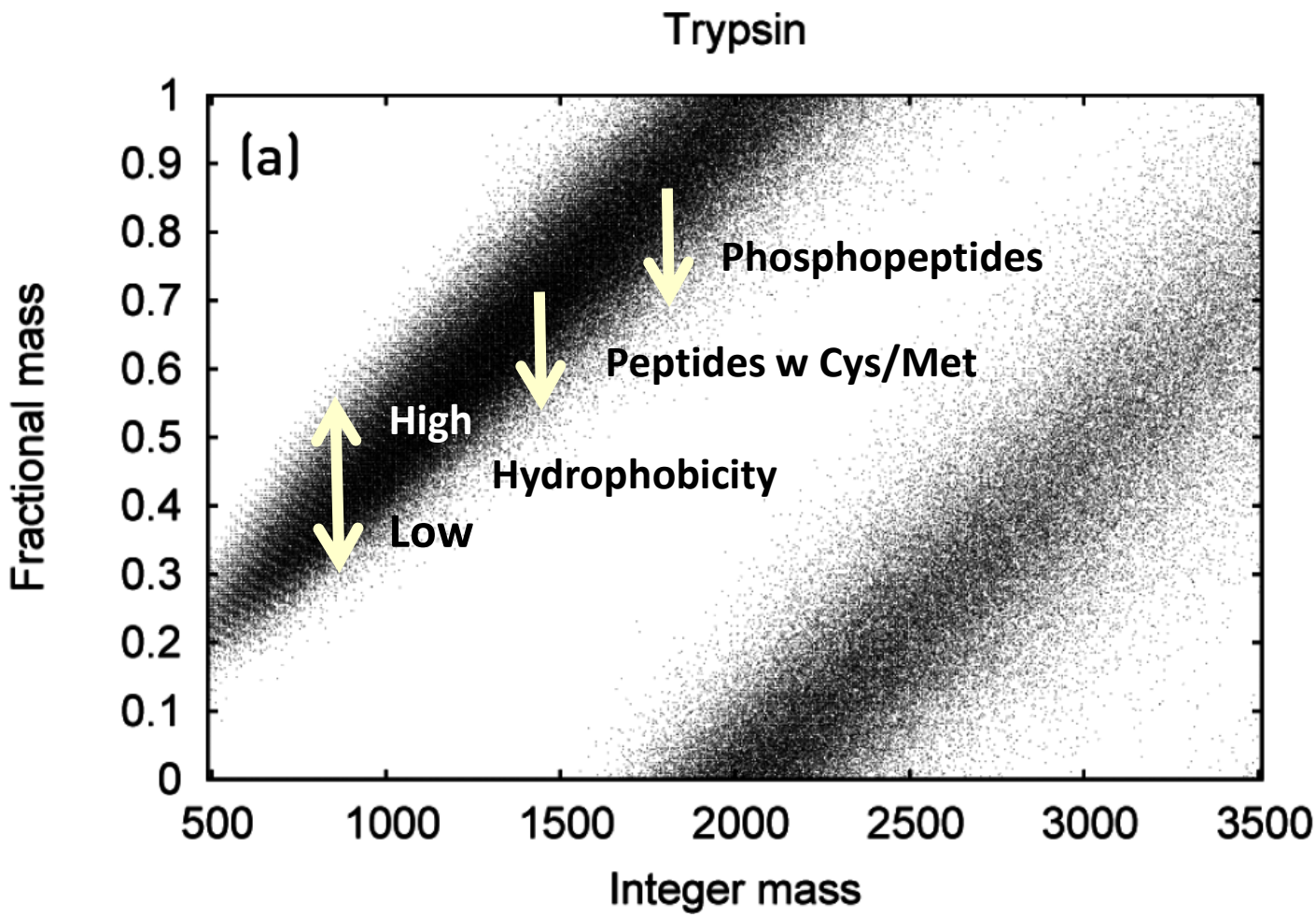
Isotopic distribution and exact mass give properties to the mass spectra of classes of compounds

- Isotopic peak distribution

- Fractional mass (mass defect, delta mass)

- Isotopic peak distance

(i) Peptides show distinct fractional mass distribution
(ii) Fractional mass indicates peptide properties

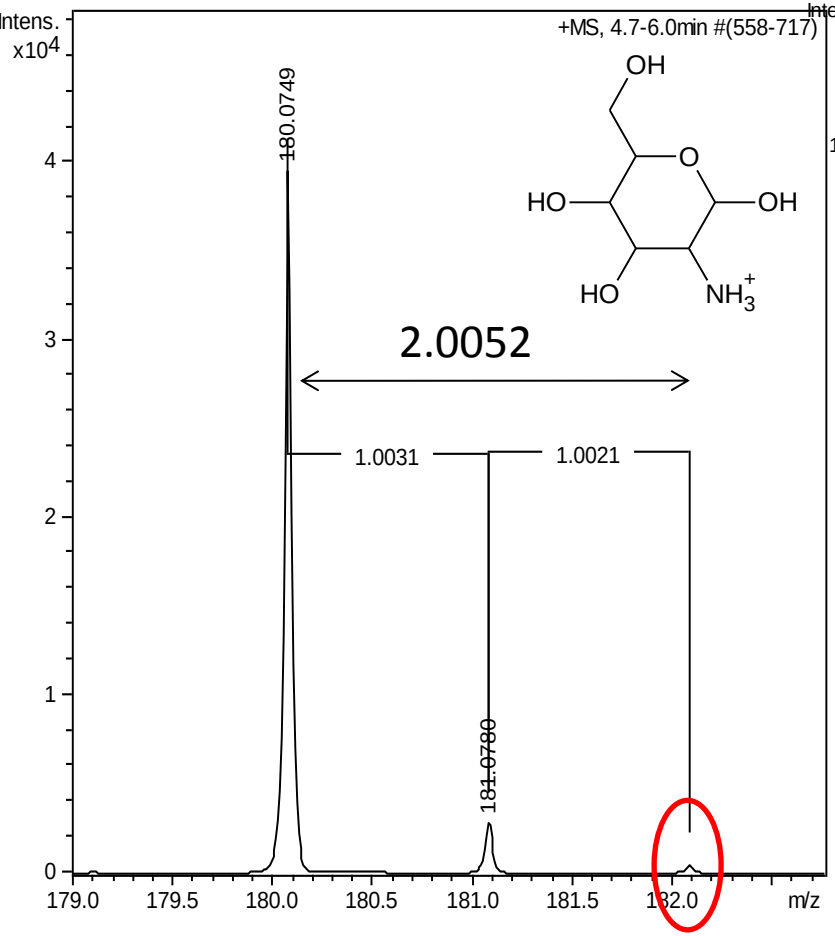


Barsnes et al (2008) Eur J Mass Spectrom 14, 311-317

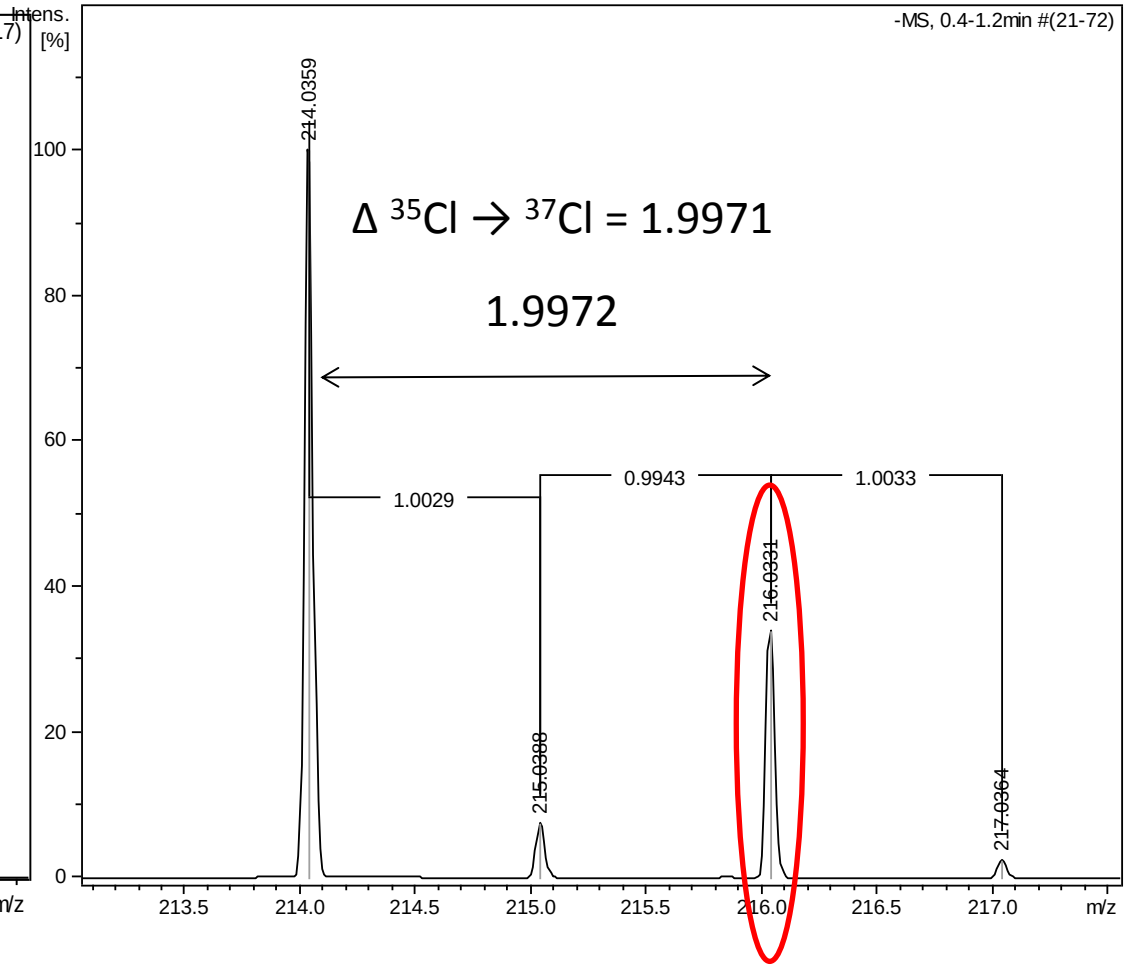
Isotope	Isotopic composition	Mass	Mass defect	Δ Isotope masses	
^{1}H	100	1.007825	+0.007825	1.006276	
^{2}H	0.0115	2.014101	+0.014101		
^{12}C	100	12.000000	0	1.003355	
^{13}C	1.08	13.003355	+0.003355		
^{14}N	100	14.003074	+0.003074	0.997035	
^{15}N	0.369	15.000109	+0.000109		
^{16}O	100	15.994915	-0.005085	1.004217	2.004201
^{17}O	0.038	16.999132	-0.000868	0.999984	
^{18}O	0.205	17.999116	-0.000884		
^{19}F	100	18.998403	-0.001597		
^{31}P	100	30.973762	-0.026238		
^{32}S	100	31.972071	-0.027929	0.999388	1.995796
^{33}S	0.80	32.971459	-0.028541	0.996408	
^{34}S	4.52	33.967867	-0.032133		
^{35}Cl	100	34.968853	-0.031147		1.997050
^{37}Cl	31.96	36.965903	-0.034097		
^{79}Br	100	78.918338	-0.081662		1.997953
^{81}Br	97.28	80.916291	-0.083709		

Elements influence peak intensities and peak distances

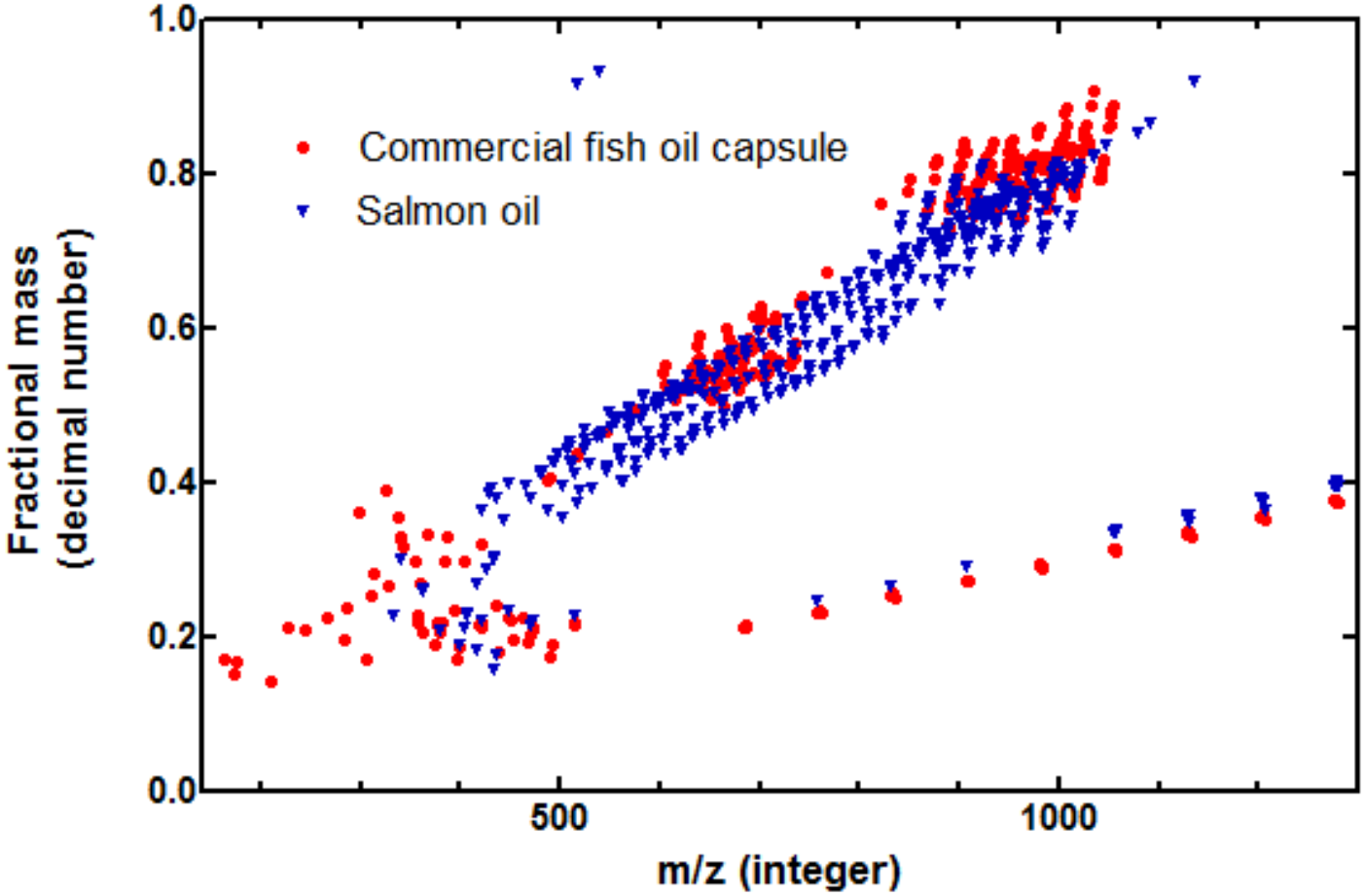
Glucosamine (positive mode)



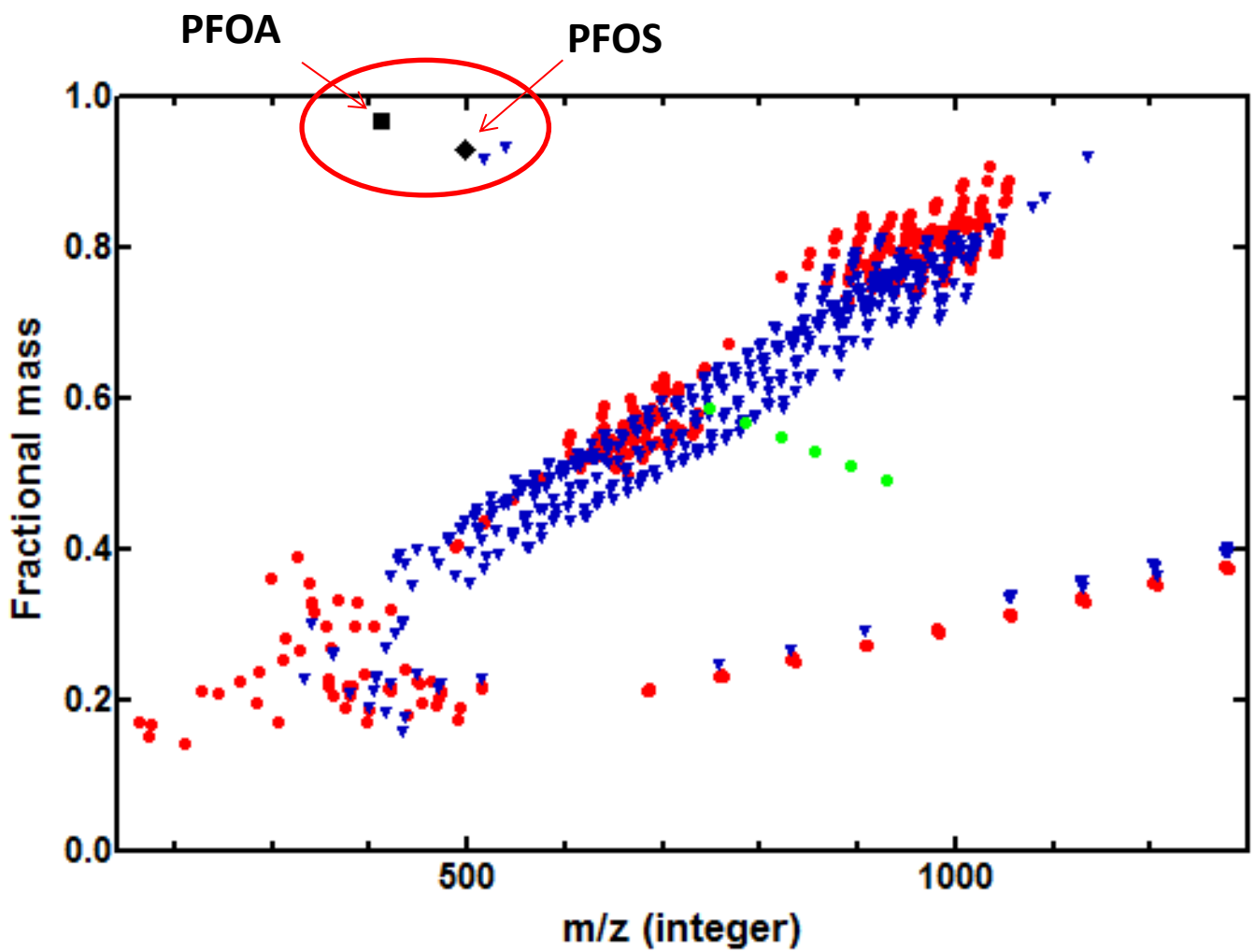
Glucosamine-Cl (negative mode)



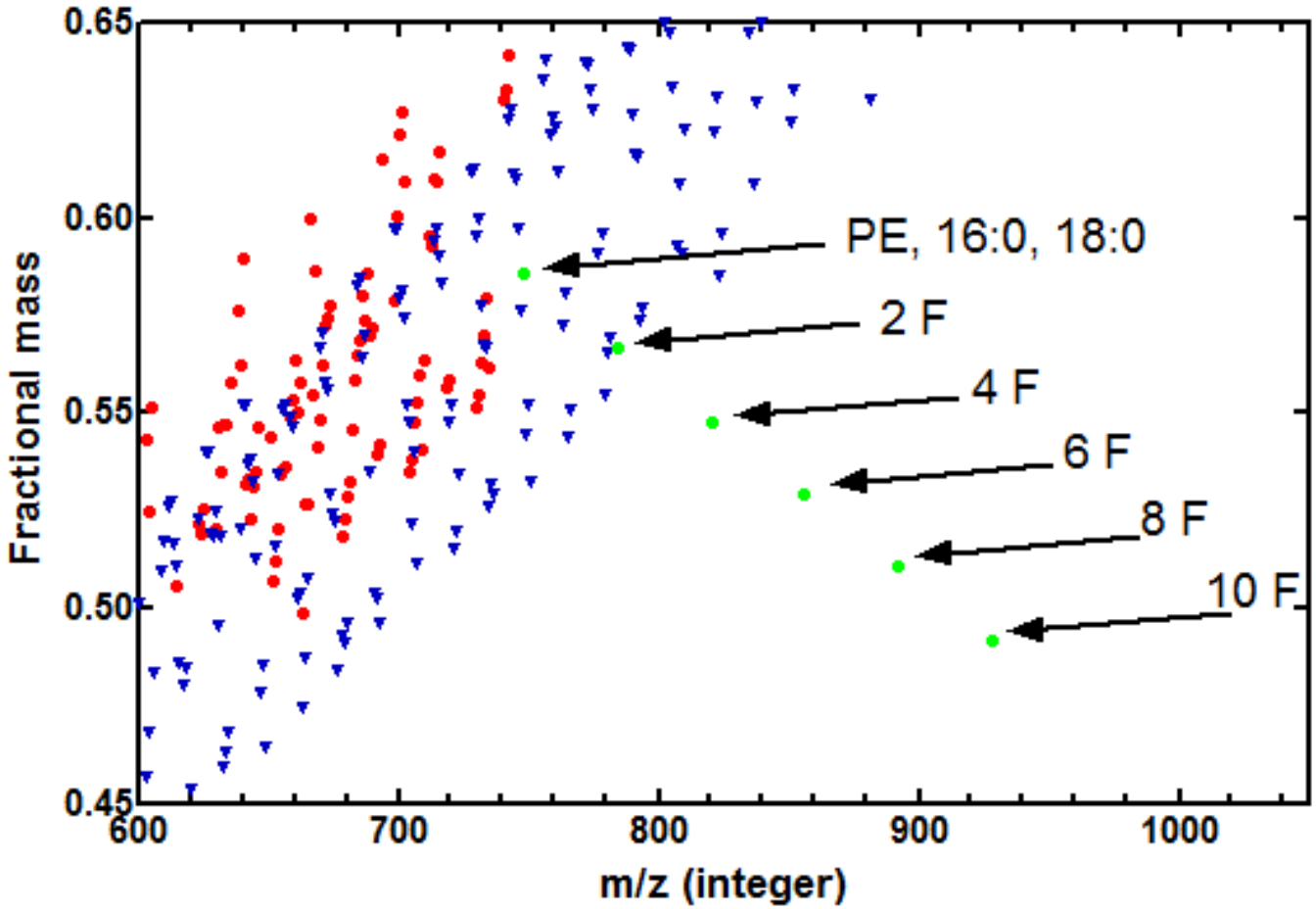
Lipids show distinct fractional mass distribution: Direct infusion of fish oils (MS)



Where are the fluorinated compounds?



Fluorines are affecting fractional mass

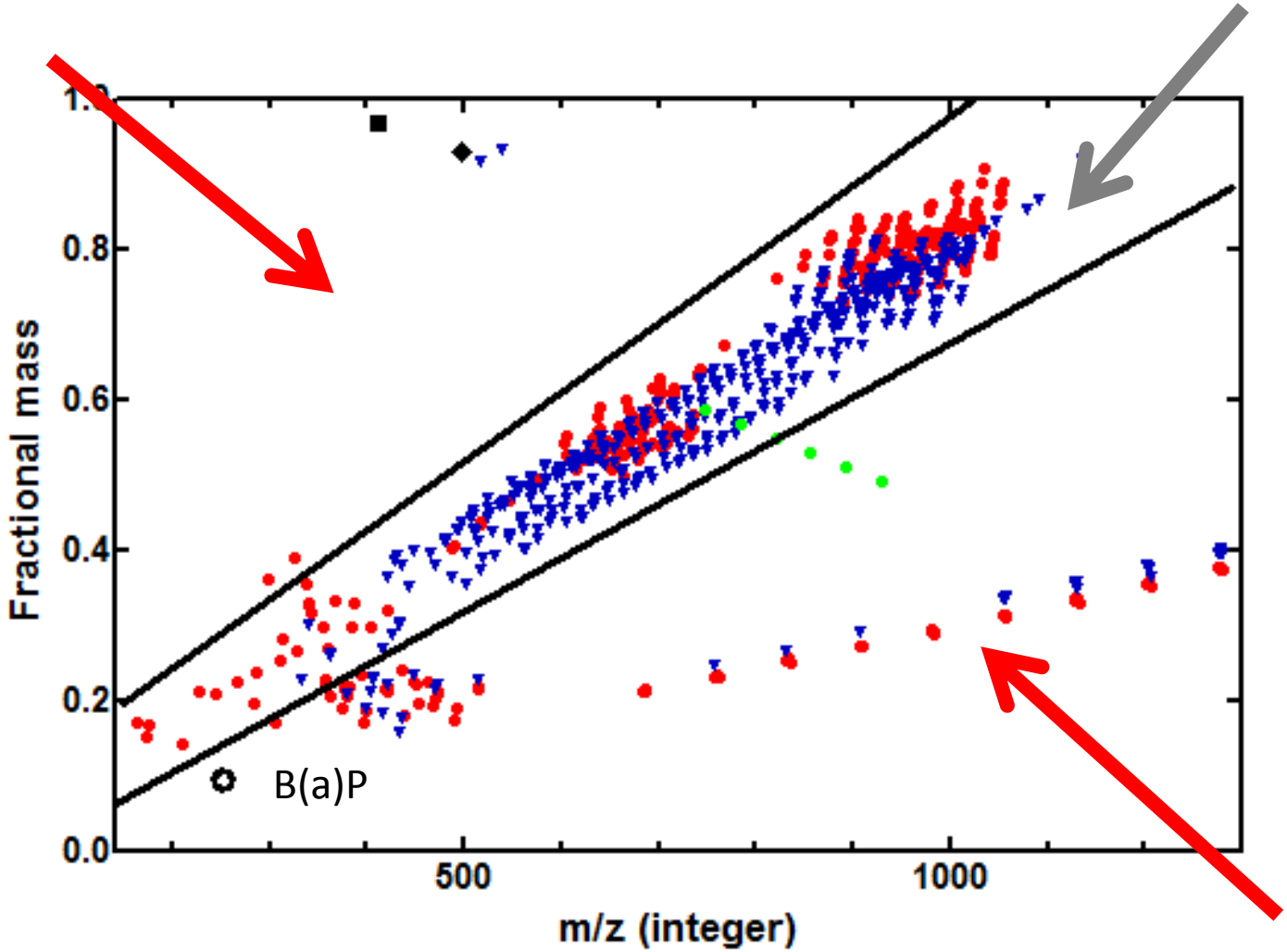


Property-specific targeted MS in non-targeted MS

	Peak distance	Isotope distribution	Fractional mass
Without halogens	-	+	+
F	-	++	++
Cl	+	+++	+++
Br	+	+++	+++

High-end suggestion (I)

MS-controlling software that can trigger MS-MS according to
- fractional mass

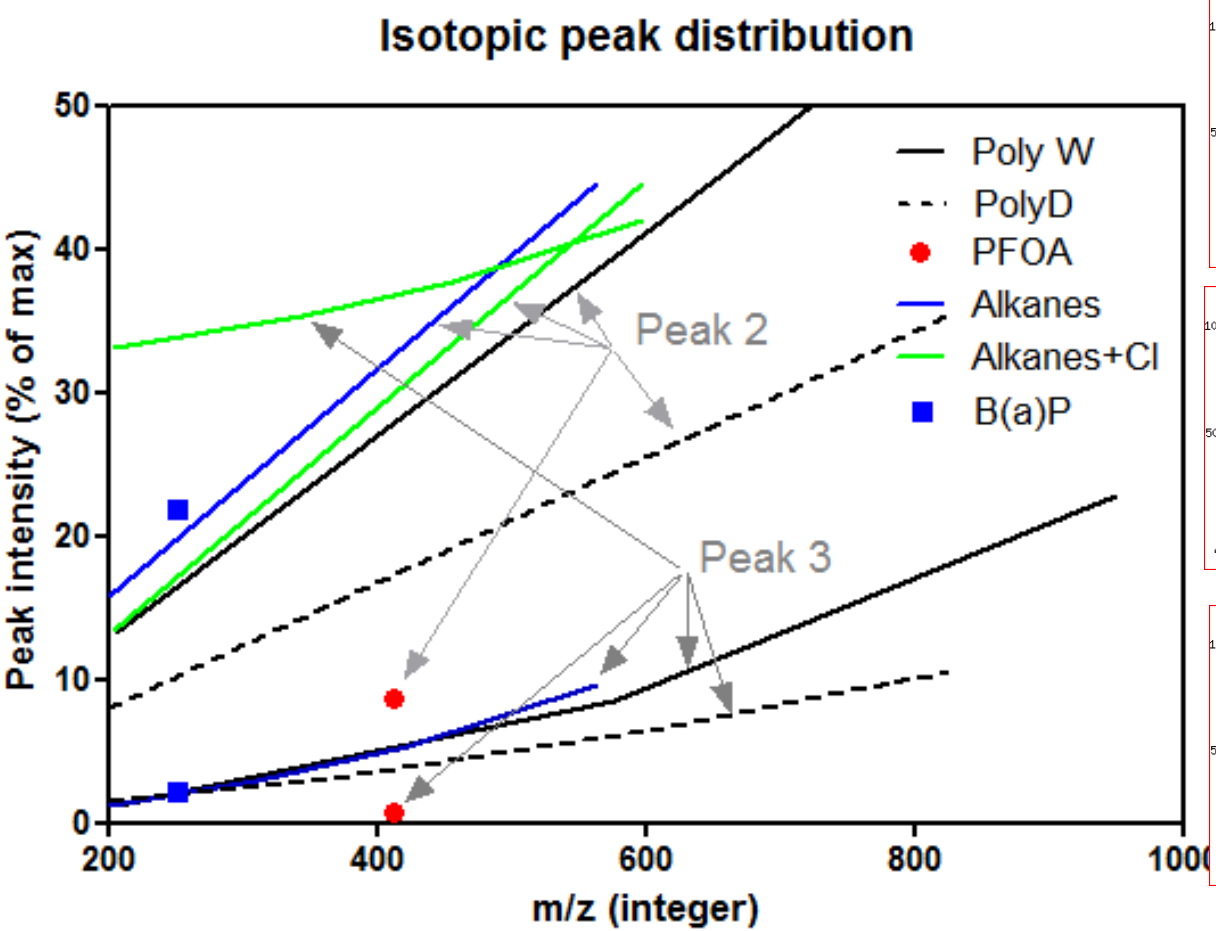


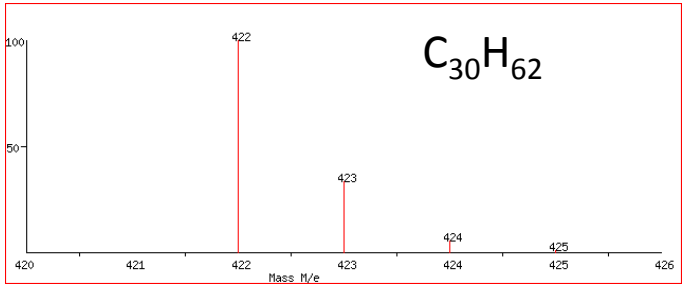
High-end suggestion (II)

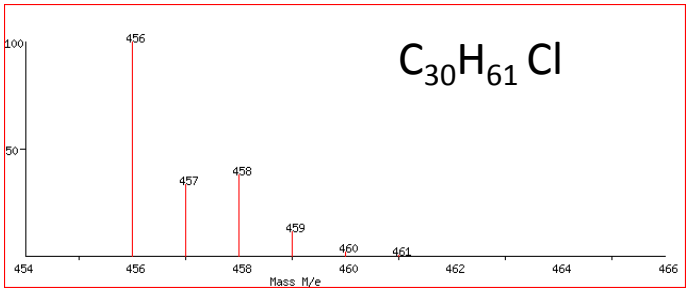
MS-controlling software that can trigger MS-MS according to

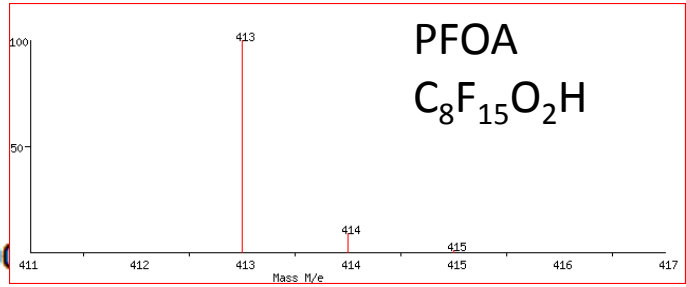
- fractional mass

- isotopic peak distribution









Low-end suggestion

Software tool extracting MS data from survey scans (MS scans), and analyze/plot them by

- fractional mass
- isotopic peak distribution
- isotopic peak distance

indicating compounds to target in next step.

University of Faroe Islands
Marjun Mortensen, technician
Tina Ziska, technician
Durita D. Djurhuus, PhD student

Aquaculture Research Station, Faroe Islands
Ása Jacobsen, PhD student

Environmental Agency, Faroe Islands
Maria Dam

National Food Institute, Technical University of Denmark
Susan Skanderup Falkenberg, PhD student
Henrik Heuch Nielsen, PhD, senior scientist

Our MS work is supported by:

granskingarráðið

Statoil

Atlantic Biotechnology