



UNIVERSITÀ DI PARMA

Metodologie omiche untarget per
la caratterizzazione degli alimenti

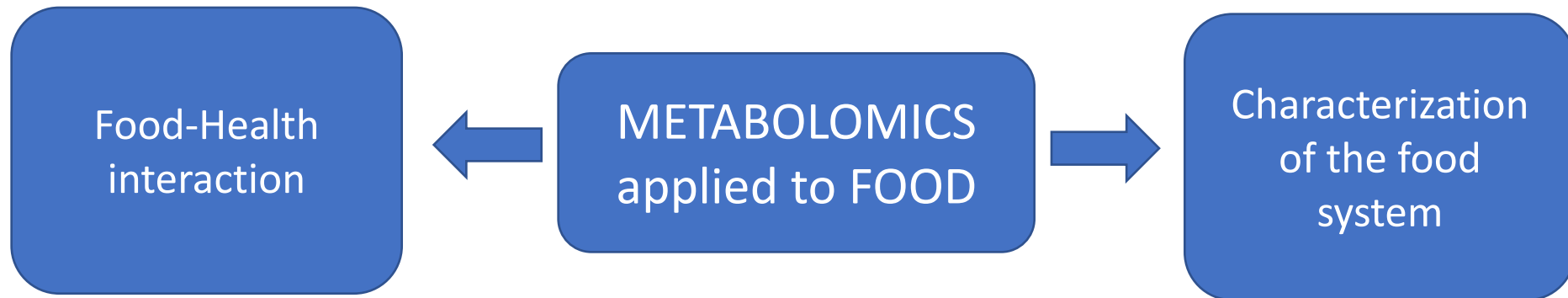
Chiara Dall'Asta

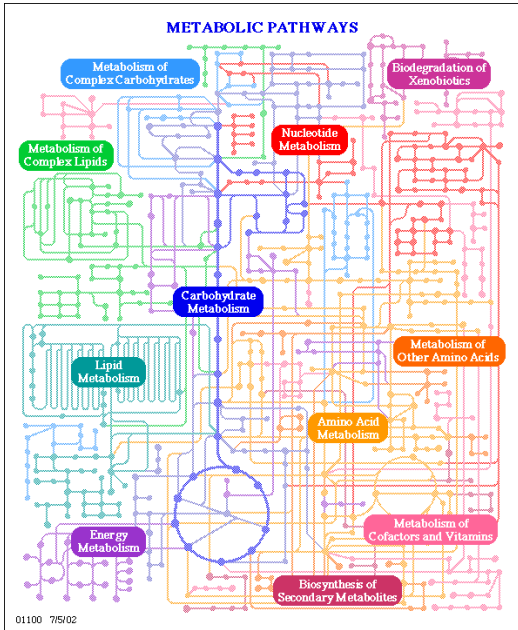
Metabolome: definition

Collection of all the small endogenous and exogenous molecules that can be found in a cell, organ, or organism

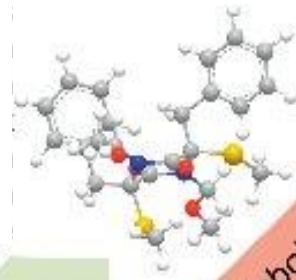
Primary metabolism → life-cycle related compounds

Secondary metabolites → response of the living organism to the external conditions



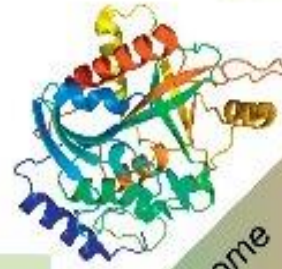


Metabolomics



Metabolome

Proteomics



Proteome

PHENOTYPE

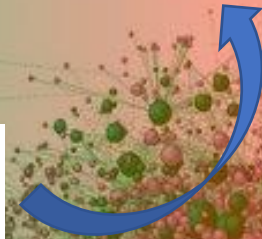


Transcriptomics



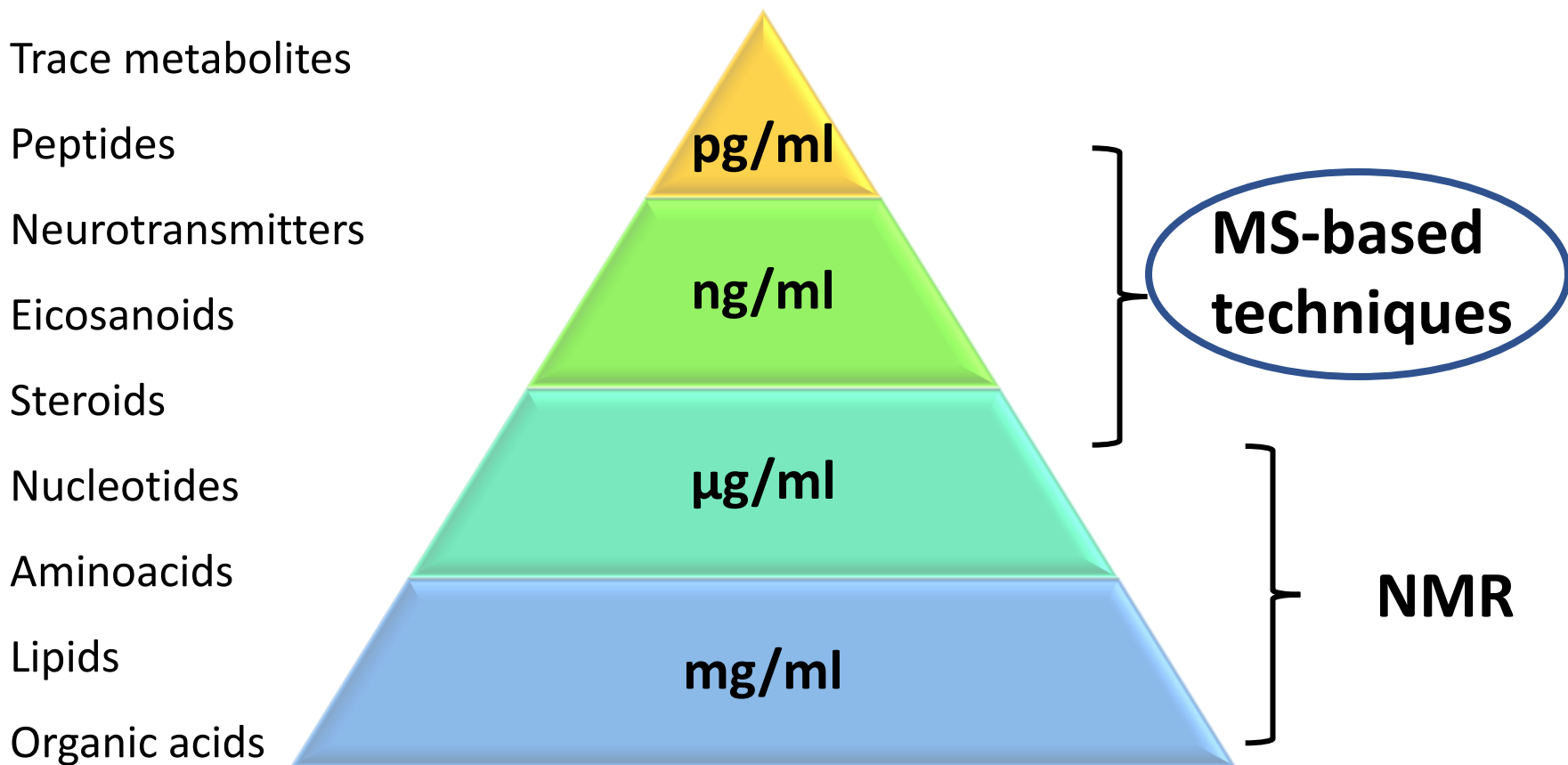
Epigenomics

Genomics

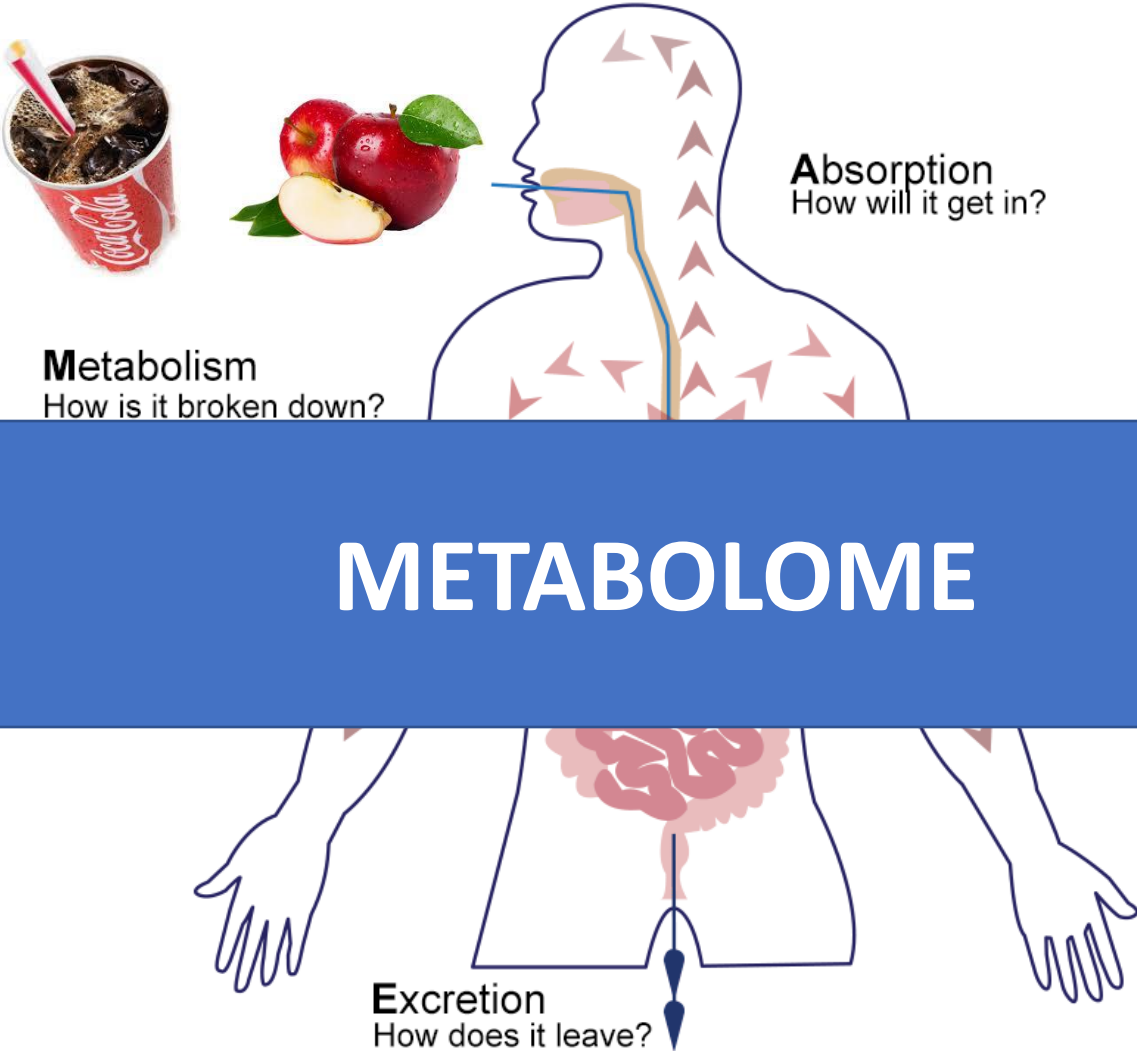


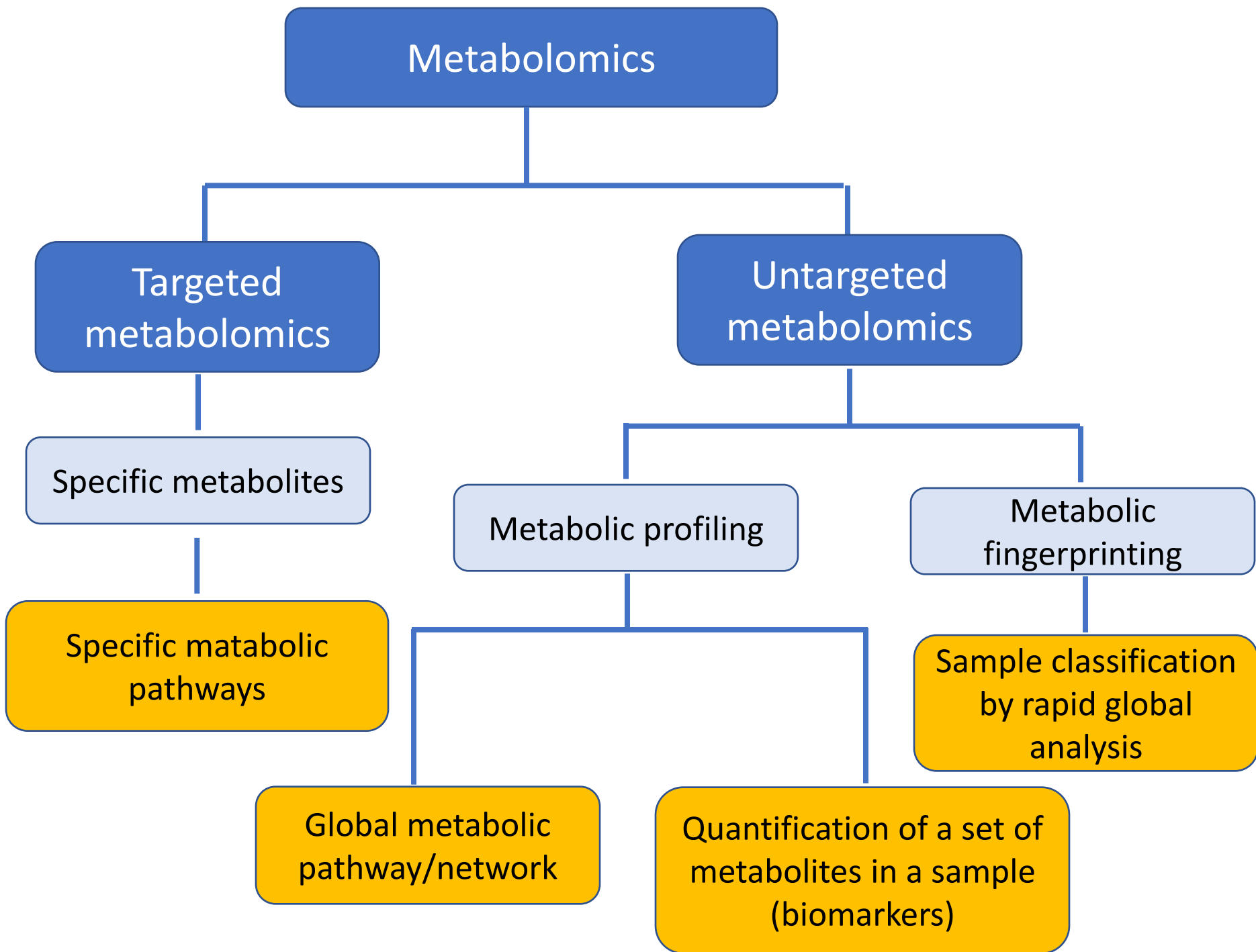
Complexity →

Which technique?



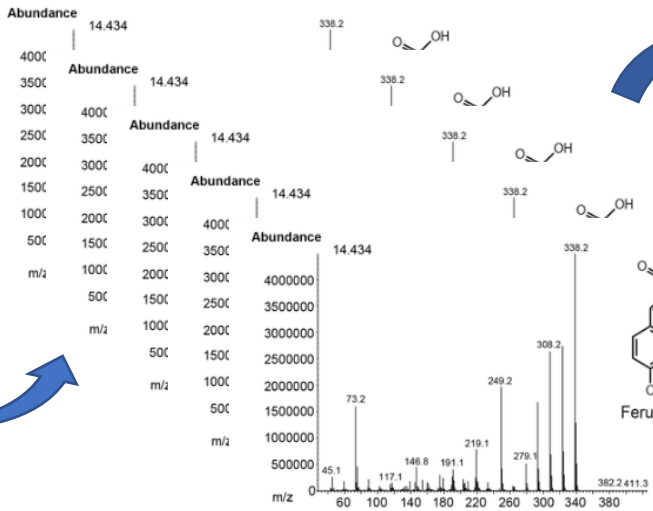
Metabolomics for Food & Health



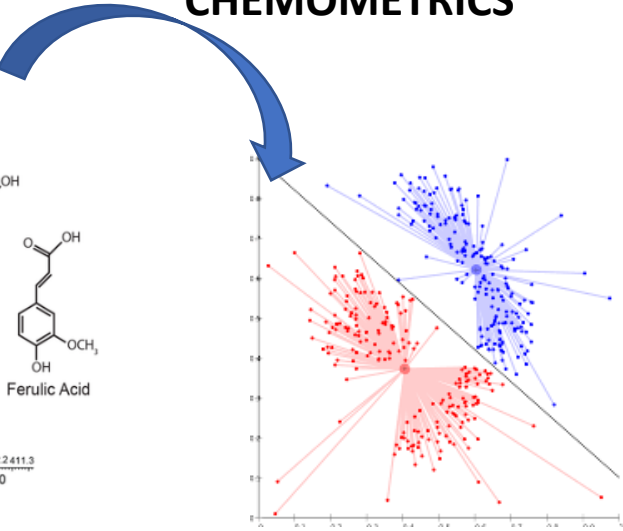


Untargeted metabolomics: fingerprinting

Sample classification by rapid global analysis of the metabolic fingerprint
YOU DON'T KNOW WHAT YOU'RE MEASURING
(and you basically don't care)



CHEMOMETRICS



MODEL VALIDATION



Untargeted metabolomics workflow

EXPERIMENTAL
DESIGN

SAMPLE
PREPARATION

SEPARATION and
DETECTION

UHPLC-HRMS analysis



DATA TREATMENT
DATA PRE-TREATMENT

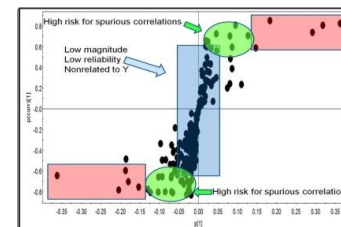
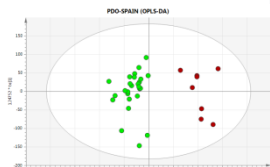
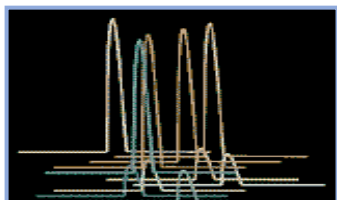
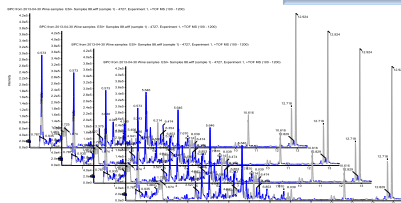
SIMCA
BY UMETRICS

VALIDATION

SELECTION of
STATISTICAL VARIABLES

MARKERS
IDENTIFICATION

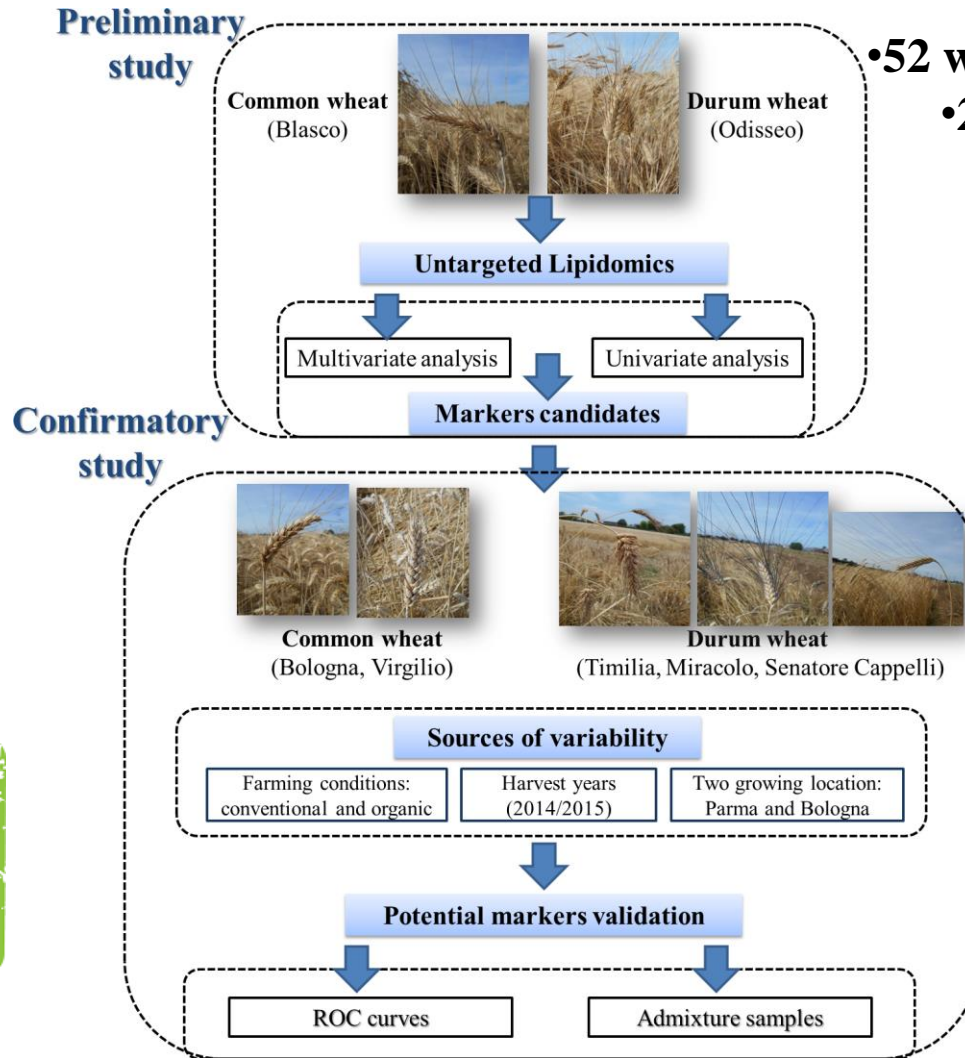
BIOLOGICAL
INTERPRETATION



Untargeted metabolomics: Fingerprinting for food authentication



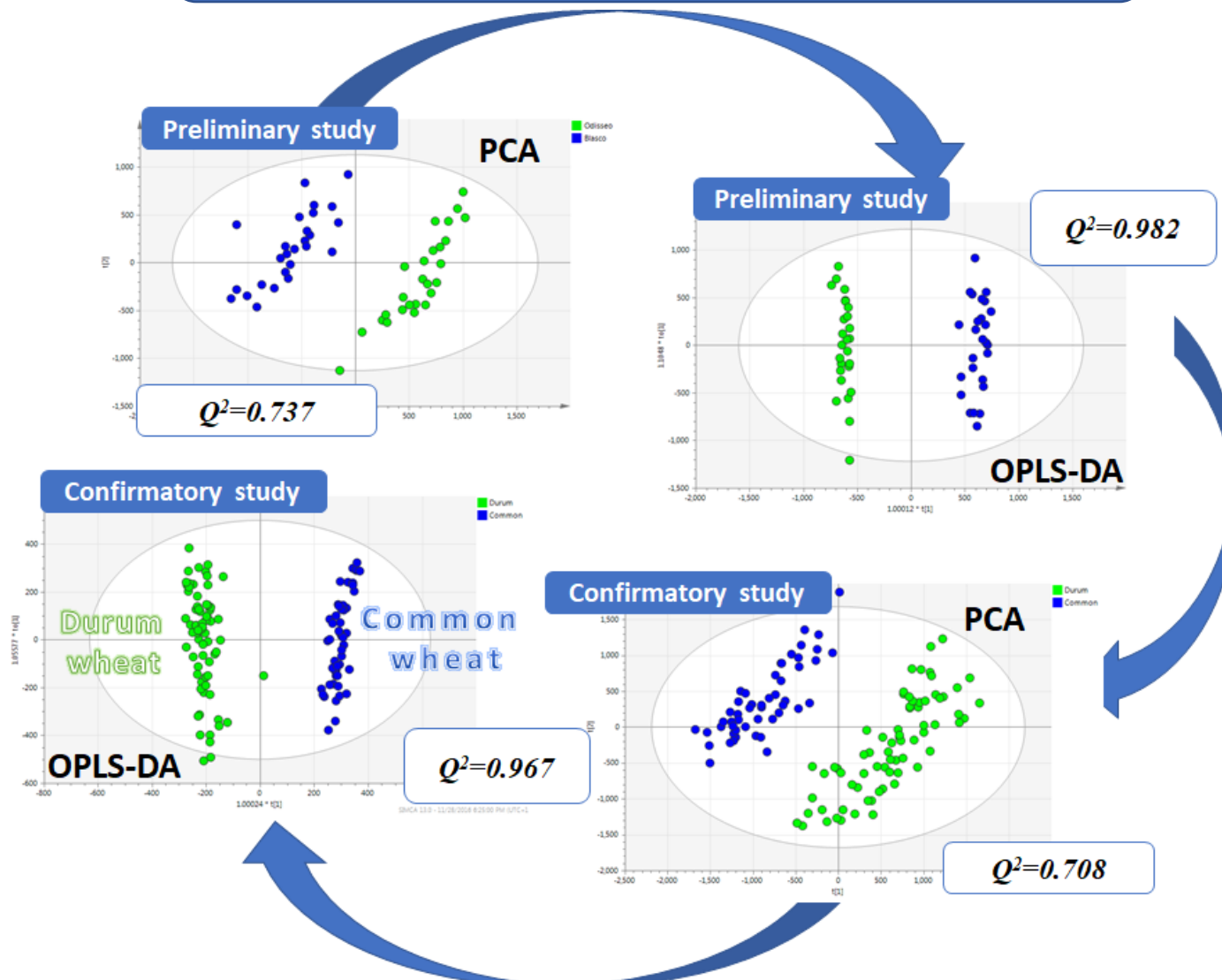
INSTITUTE OF
CHEMICAL TECHNOLOGY
PRAGUE



•52 wheat samples
•2 varieties

•171 wheat samples
•7 varieties
•2 farming conditions
•2 harvest years

Untargeted metabolomics: Fingerprinting for food authentication



Untargeted metabolomics: Fingerprinting for food authentication

- There's a need of standardized methods and workflows
- Bottleneck: authentic sample collection!



GUIDELINES FOR UNTARGETED
MS-BASED METHODS FOR
FOOD FINGERPRINT



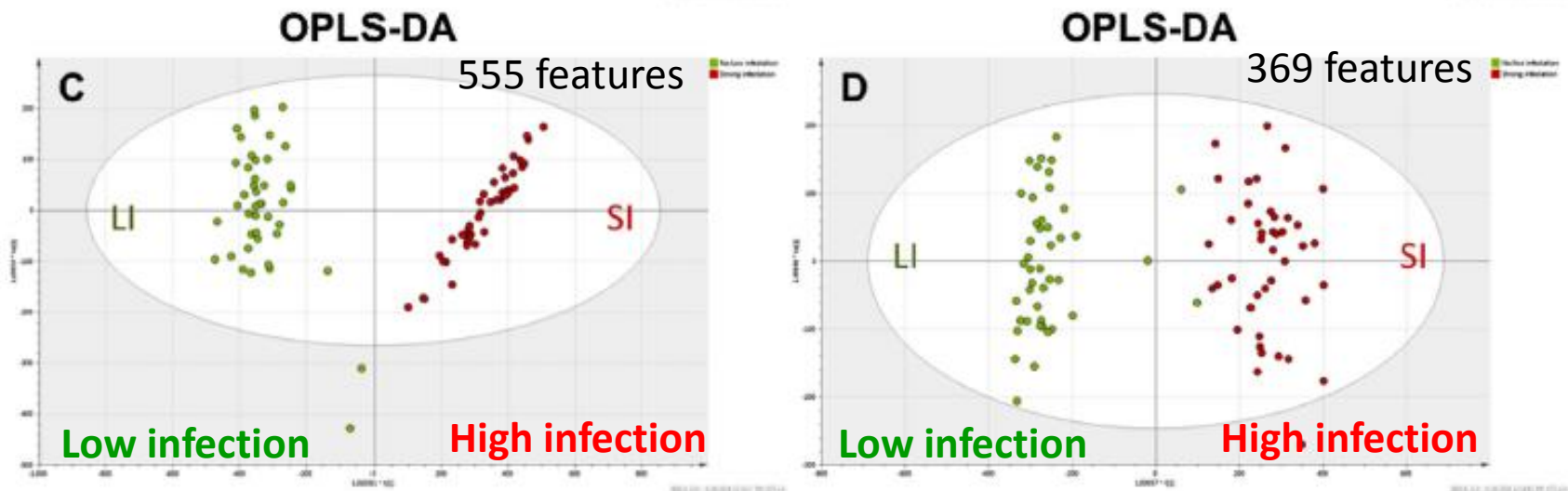
Untargeted metabolomics: Fingerprinting for biomarker elucidation

Sample classification by rapid global analysis of the metabolic fingerprint
YOU DON'T KNOW WHAT YOU'RE MEASURING
(but you care!!)



**86 wheat samples harvested
in Czech Republic naturally
contaminated by different
mycotoxins**

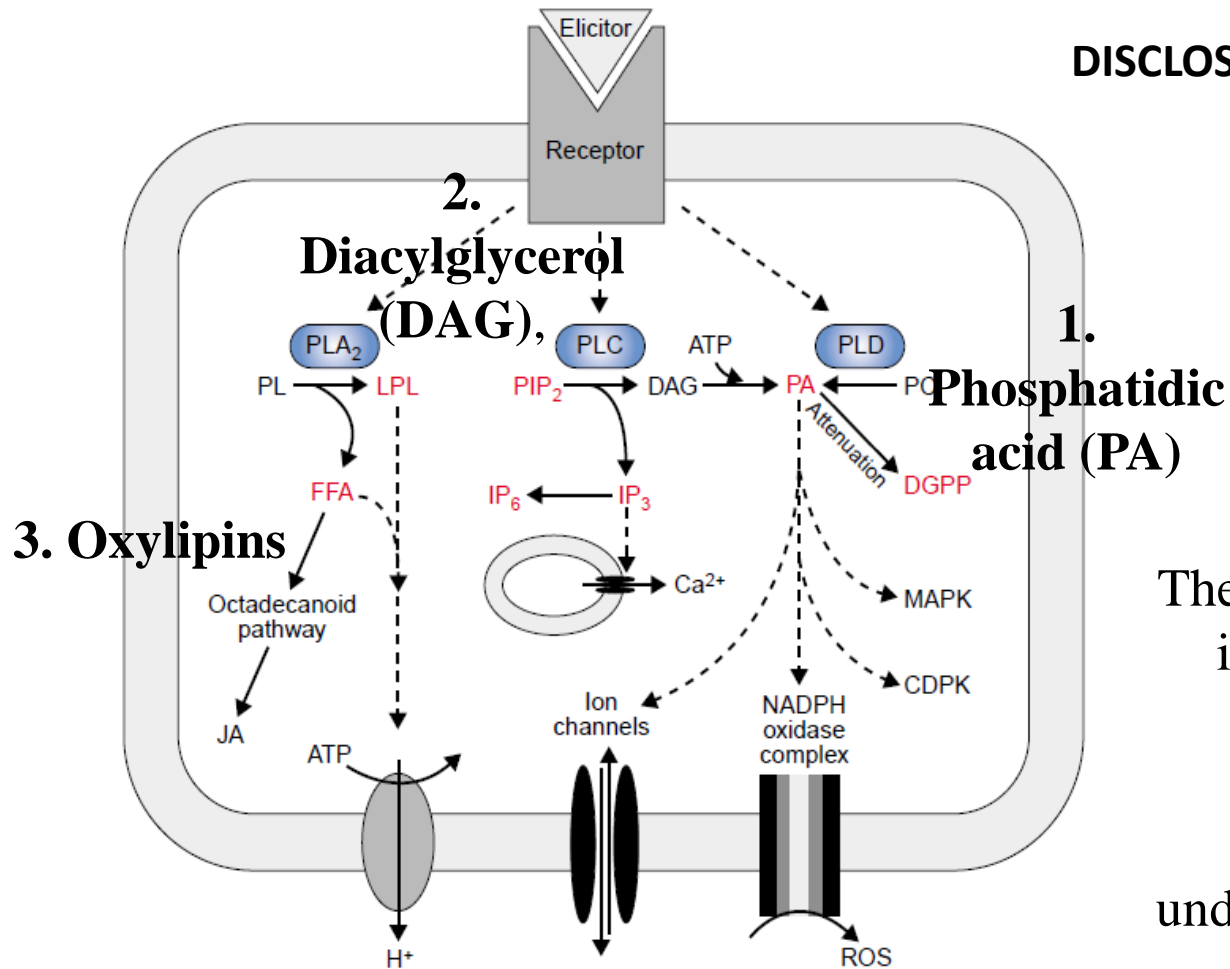
Untargeted metabolomics: Fingerprinting for biomarker elucidation



Tentative identification	Pseudomolecular ion	m/z	RT (min)	Molecular formula	Mass error (Δ ppm)	p-value
13-Keto octadecadienoic acid	[M-H] ⁻	293.2122	3.56	C ₁₈ H ₃₀ O ₃	0.0	3.7E-281,2
13-Hydroxy octadecadienoic acid	[M-H] ⁻	295.2122	3.57	C ₁₈ H ₃₂ O ₃	1.0	1.1E-281,2
12,13-Di-Hydroxy octadecadienoic acid	[M-H] ⁻	313.2122	2.69	C ₁₈ H ₃₂ O ₄	0.3	1.0E-221,2
12,13-Di-hydroxy octadecenoic	[M-H] ⁻	313.2385	2.93	C ₁₈ H ₃₄ O ₄	1.7	3.0E-221,2
5-Nonadecanoylresorcinol (C19:0)	[M-H] ⁻	375.3286	6.66	C ₂₉ H ₄₆ O ₂	4.5	4.5E-211,2
5-Heneicosylresorcinol (C21:0)	[M-H] ⁻	3601	7.20	C ₂₇ H ₄₆ O ₂	4.7	1.6E-201,2
5-Tricosylresorcinol (C23:0)	[M-H] ⁻	3888	7.75	C ₂₉ H ₅₂ O ₂	1.6	1.3E-181,2
Diacylglycerol (C15:1/C18:2)	[M+H] ⁺	577.4825	6.59	C ₃₃ H ₆₄ O ₂	0.3	1.9E-191,2
Phosphatidic acid (C18:2/C18:2)	[M+NH ₄] ⁺	4.5091	7.23	C ₃₆ H ₆₈ O ₈ P	1.6	1.2E-191,2
Phosphatidic acid (C16:0/C18:2)	[M+NH ₄] ⁺	0.5060	7.46	C ₃₇ H ₆₈ O ₈ P	1.5	6.3E-201,2

Untargeted metabolomics: Fingerprinting for biomarker elucidation

DISCLOSING THE LIPID SIGNATURE

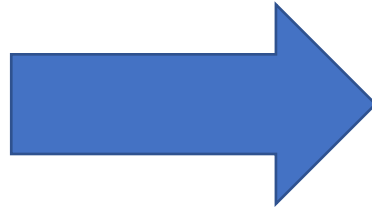


The lipid biosynthetic pathway is involved in the plant defence responses.

Biomarkers are under/overexpressed according to the fungal infection

Untargeted metabolomics: Data collection and compound identification

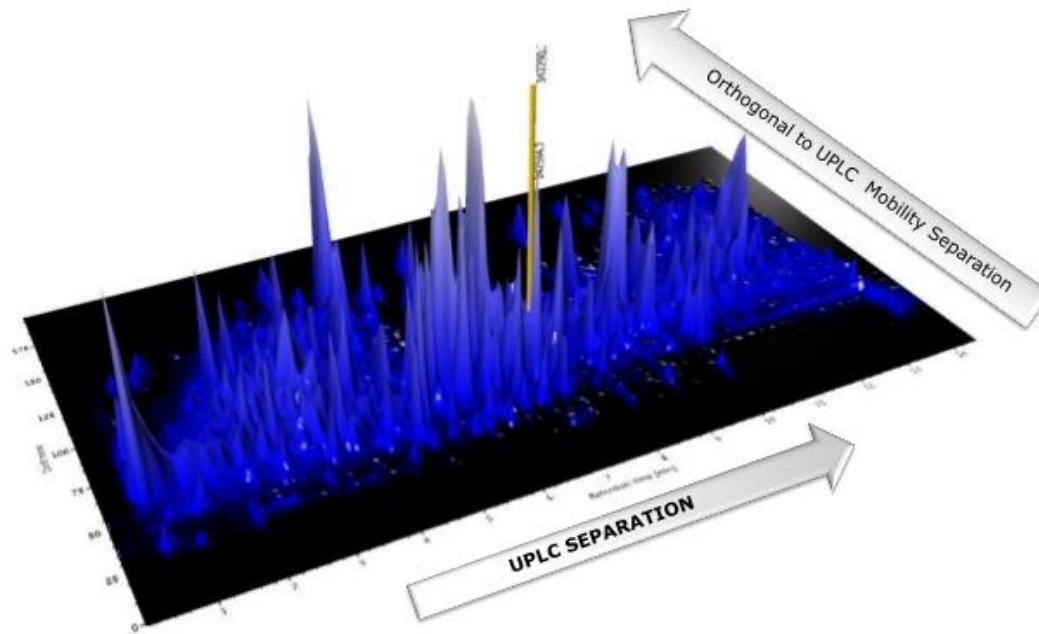
LC-HRMS



LC-HR-IMS

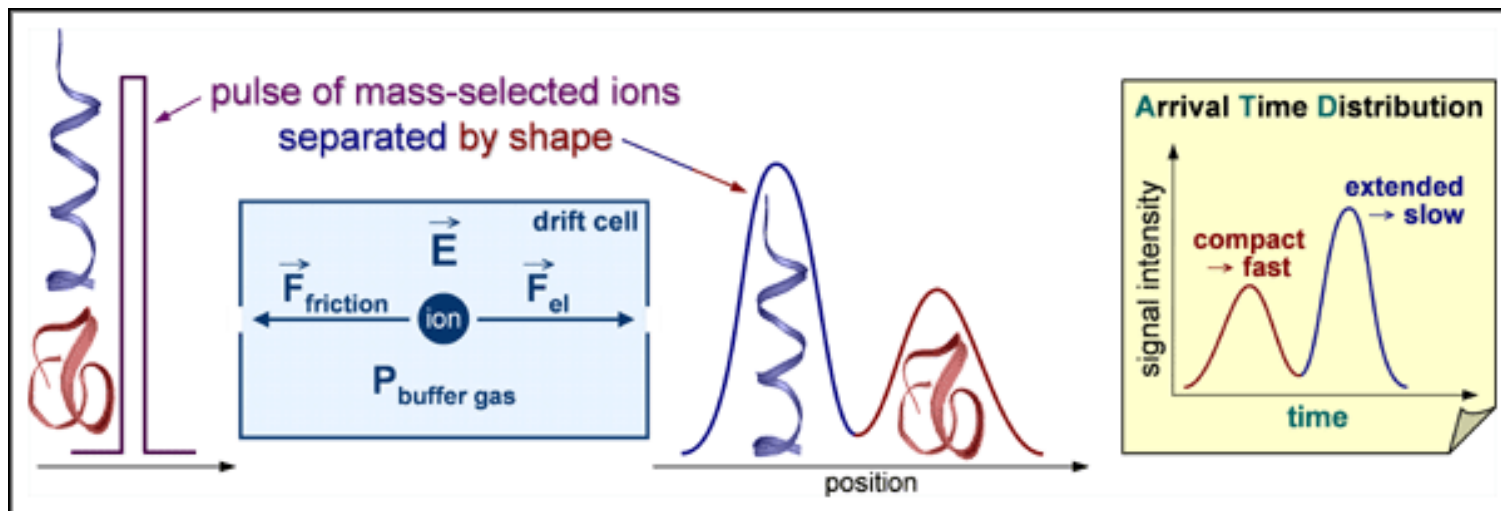
- Untargeted screening
- Unlimited number of monitored ions
- Retrospective data analysis

- Isomer/Isobar compounds separation
- Reduced and filtered background
- Enhanced compounds identification

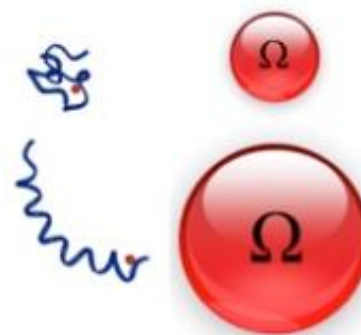


Untargeted metabolomics: Data collection and compound identification

IM-MS allows the separation of ionized molecules based on their structural properties such as size and shape, in addition to their mass-to-charge ratio.

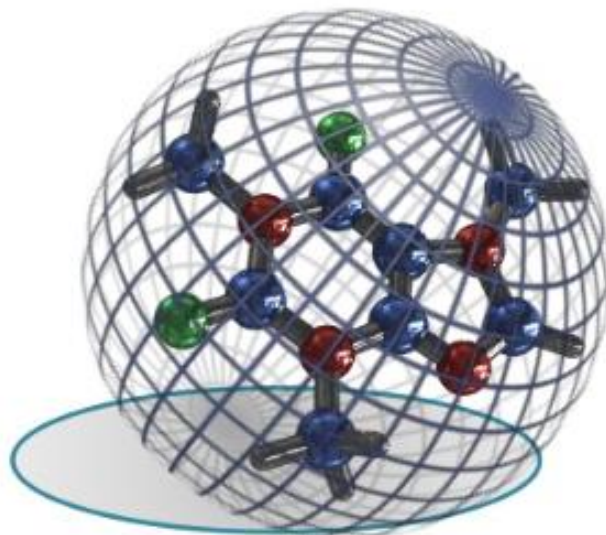


The time it takes for the charged species to transverse the drift tube can then be converted into a collision cross section value, which is representative of a rotationally averaged surface area.



Untargeted metabolomics: calculation of CCS values

- CCS is an important distinguishing characteristic of an ion which is related to:
 - chemical structure
 - 3-dimensional conformation
- CCS is a robust and precise physicochemical property of an ion.



CCS as a highly specific
molecular descriptor for
identification

A DATABASE OF CCS VALUES FOR PHYTOCOMPOUNDS IDENTIFICATION

- To overcome matrix effect
- To overcome the lack of analytical standards
- To allow identification of co-eluting isobars

Mass Spectrometry



"Okay—who put my lunch through the mass spectrometer..?"

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