



VI Bioanalytical School - INCTBio

Mass spectrometry-based omic sciences in the search of biomarkers for psychiatric diseases and alternative treatments

Prof. Dr. Alessandra Sussulini

University of Campinas

Department of Analytical Chemistry

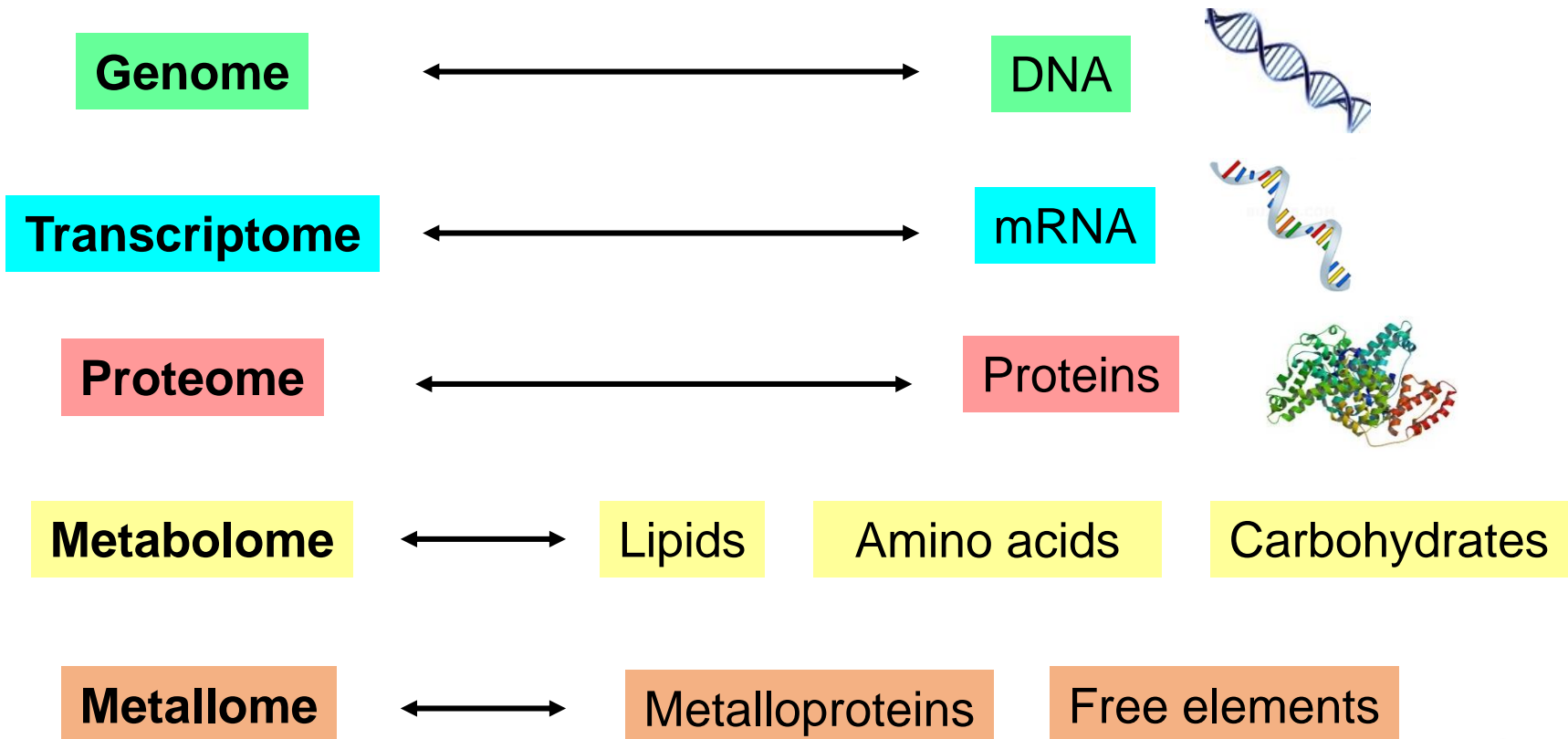
LaBIOmics – Laboratory of Bioanalytics and Integrated Omics

E-mail: sussulini@iqm.unicamp.br

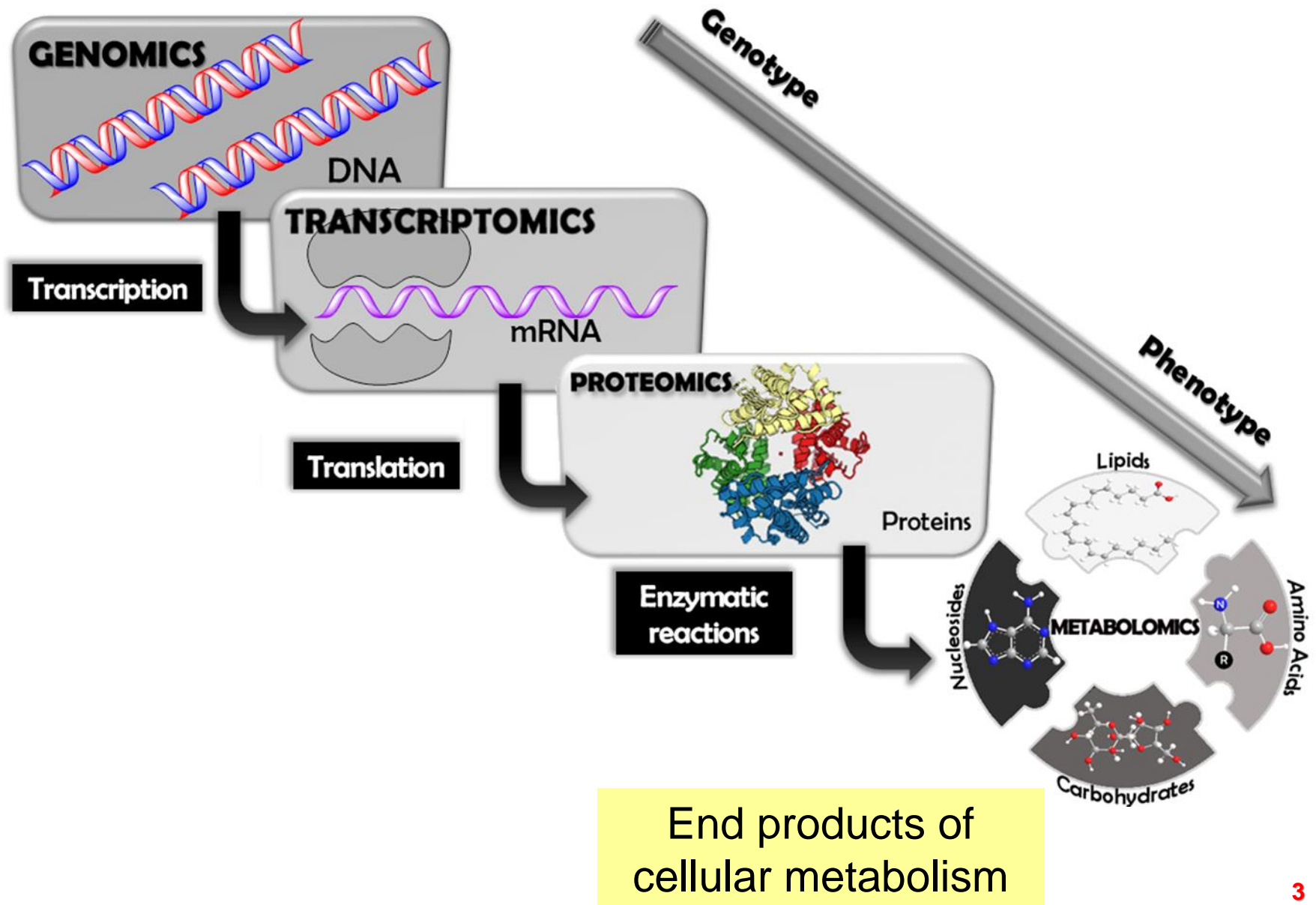
Londrina, November 29th 2018

Omic sciences

Identification, characterization and quantification of **biomolecules** and **elements** involved in the structure, function and dynamics of biological systems

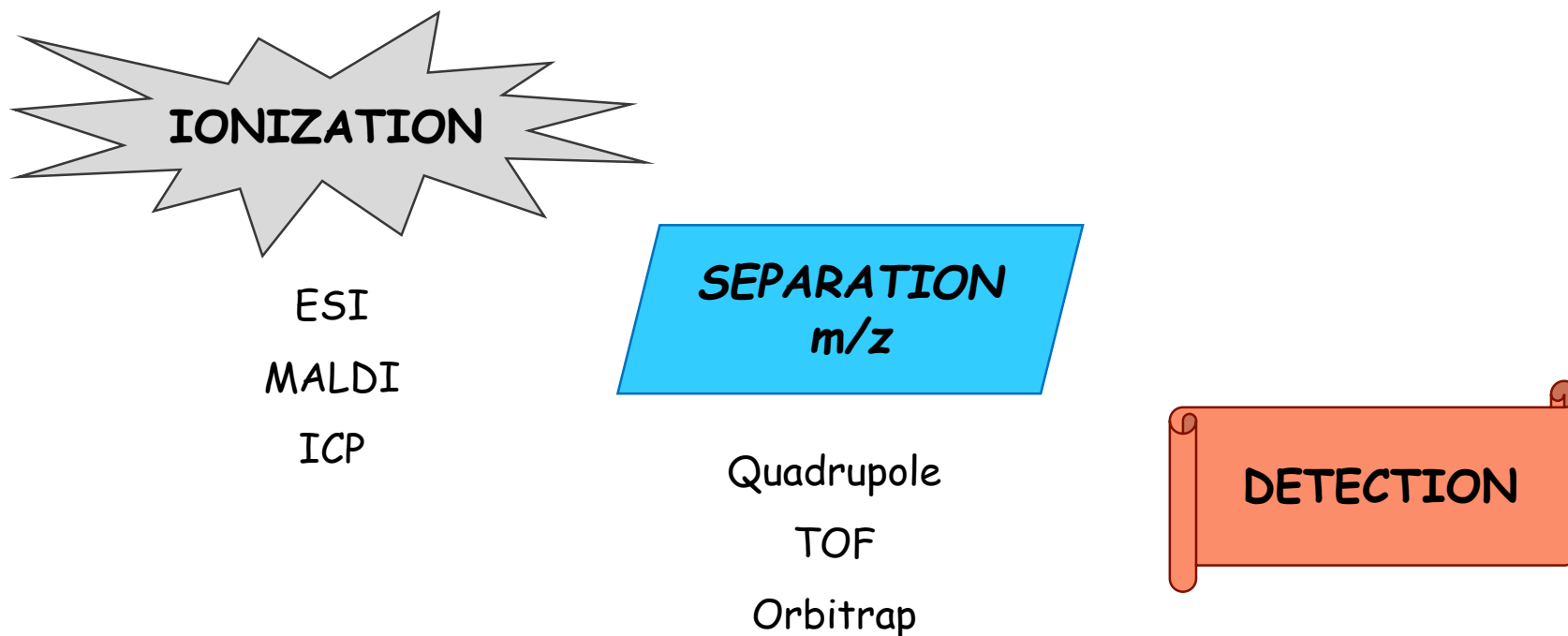


Omic sciences



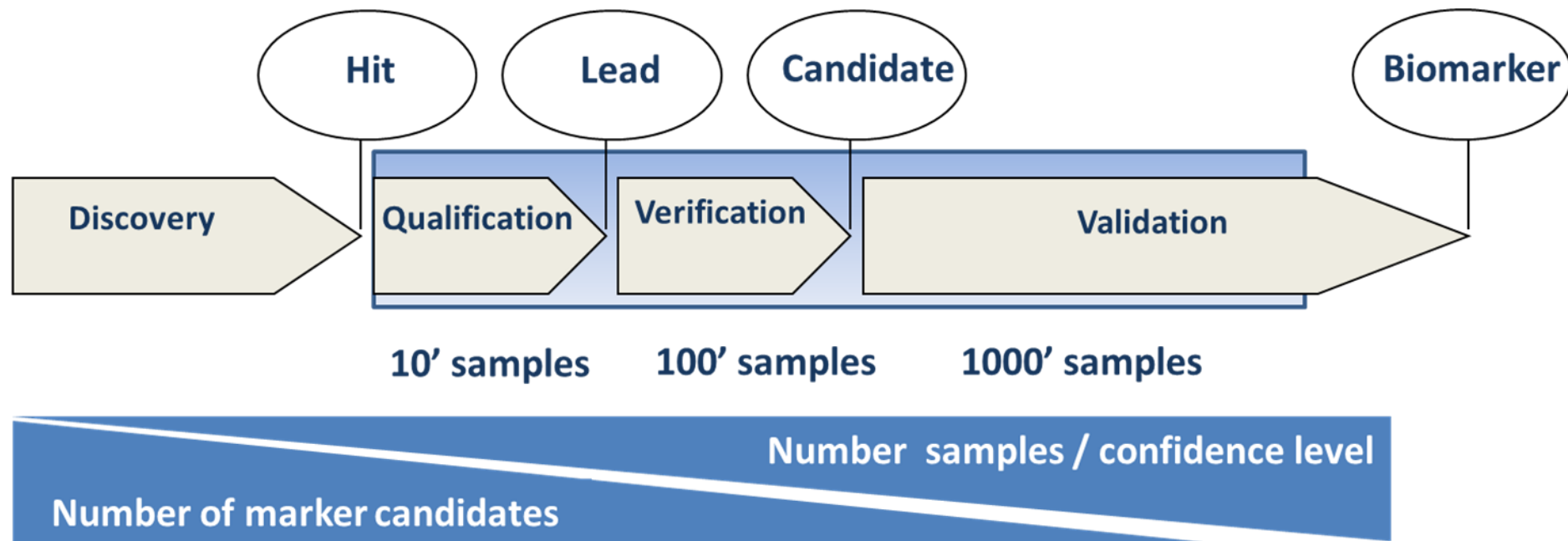
Mass spectrometry (MS)

- Measurement of atomic or molecular masses of charged species in the gas phase → **mass-to-charge ratio (m/z)**
- Qualitative, quantitative, and structural analyses



Biomarkers

Biological characteristics that can be measured and evaluated as indicators of **normal or pathogenic processes** or **therapeutic interventions**



Biomarkers

Improvement of
diagnostics

Evaluation of
treatment efficacy

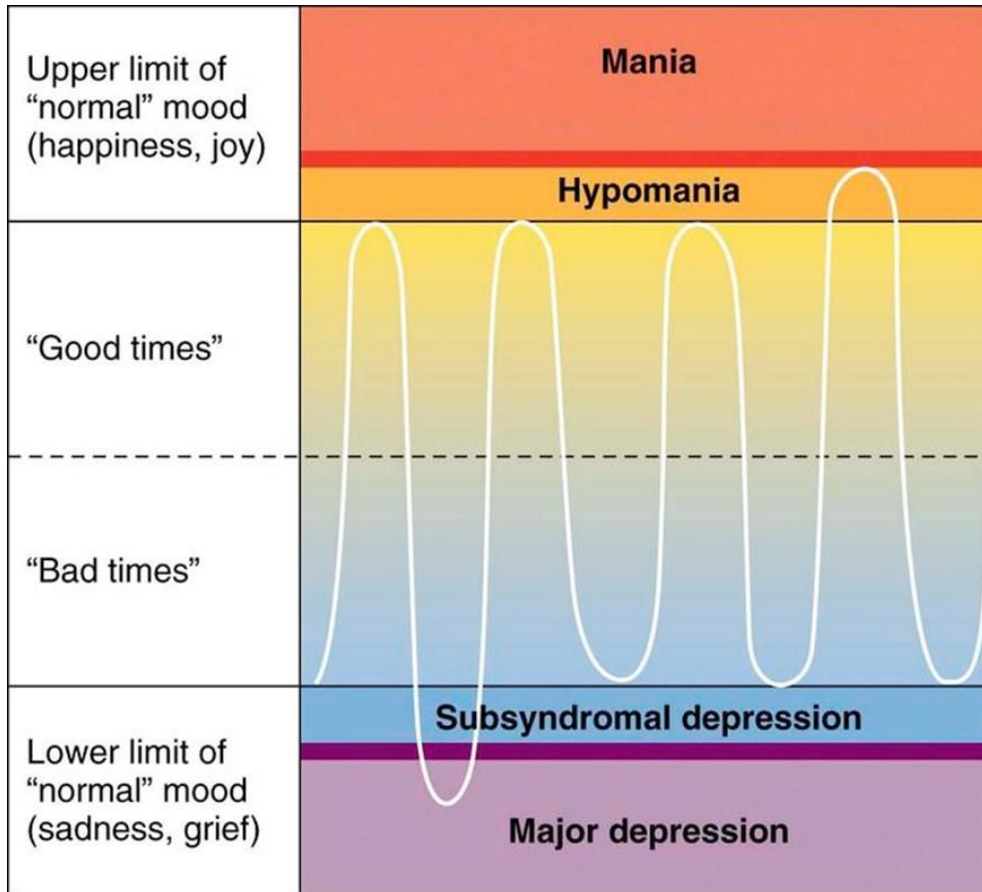
**Importance of
biomarkers in
Medicine**

Understanding of
molecular
mechanisms

Development of
new drugs

Bipolar disorder

Characterized by mood oscillations: **mania** x **depression**



Treatment:

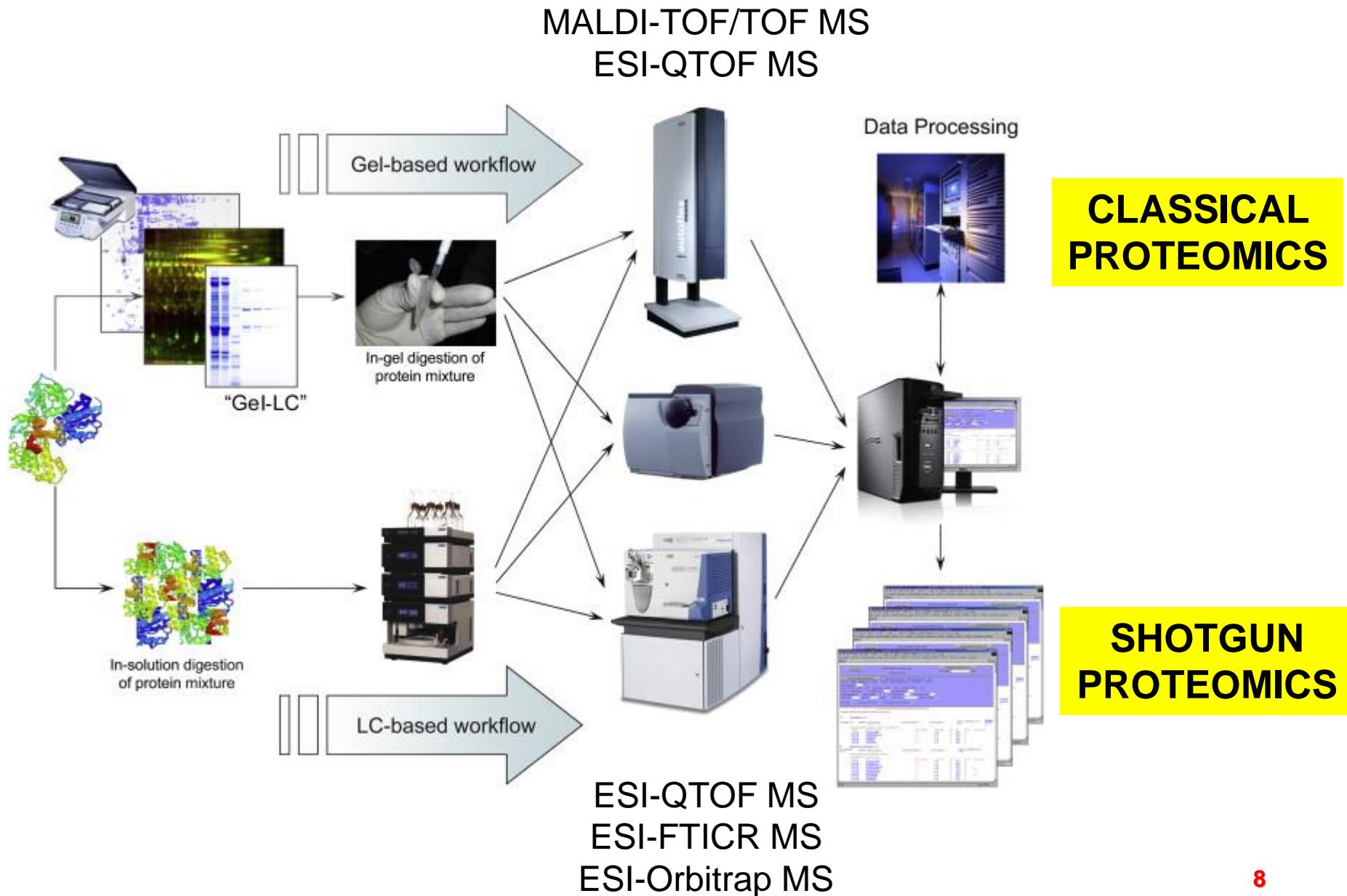
Mood stabilizers
Antipsychotics

Causes:

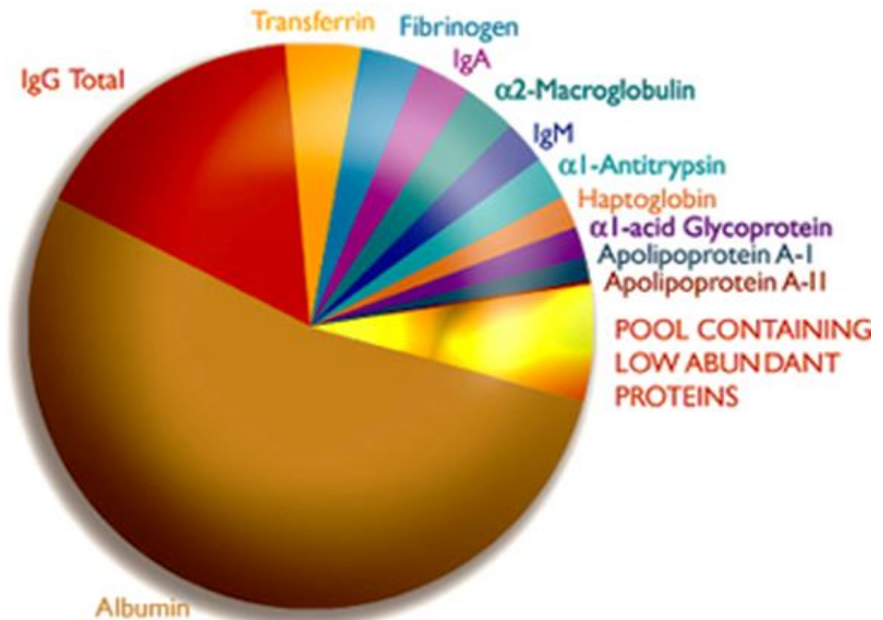
Still unknown
(genetics, neurological,
environmental factors)

Diagnosis:

Only clinical



Blood serum

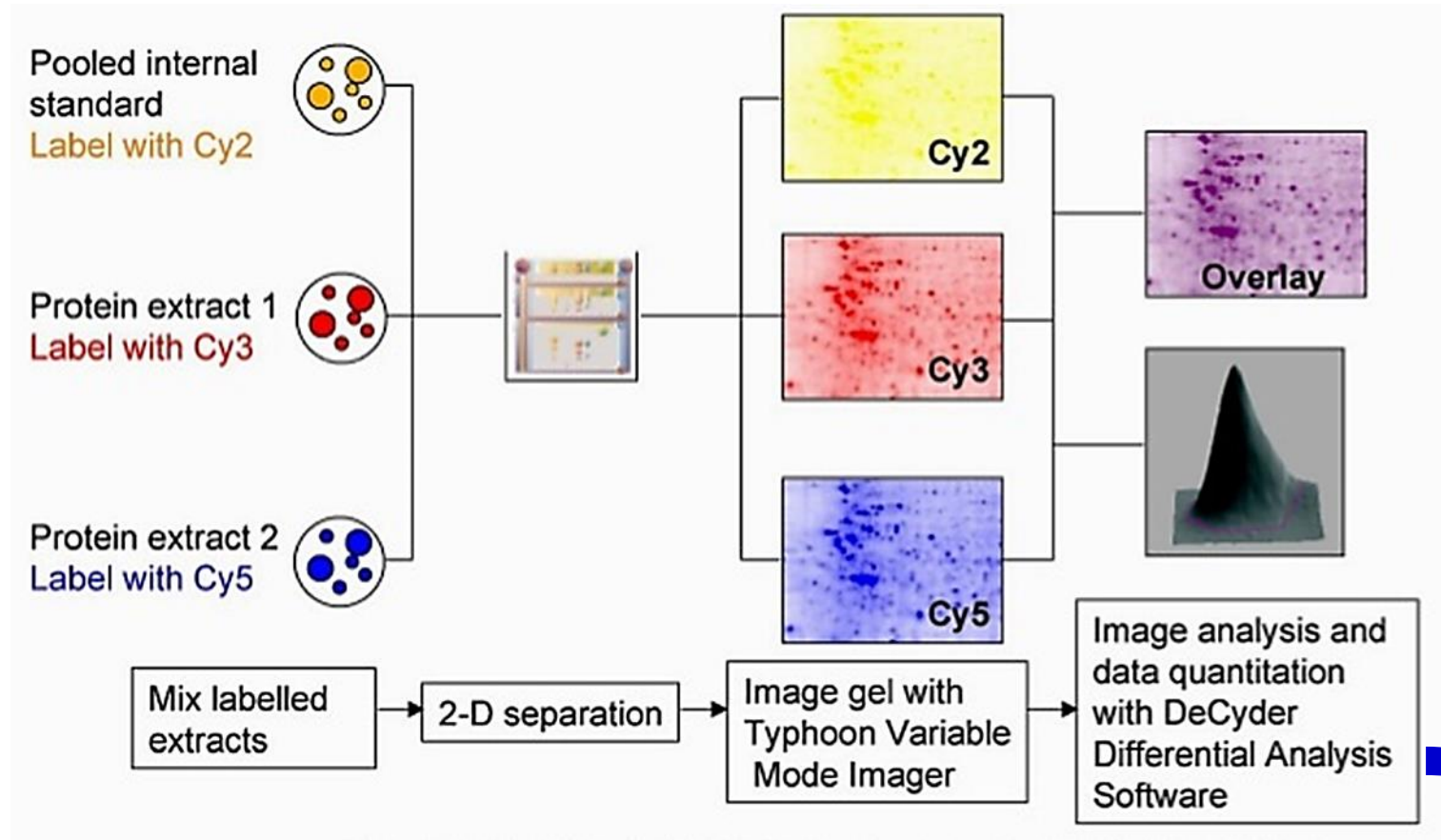


Sample preparation:

- ✓ Depletion (removal of the major proteins)
- ✓ Equalization (enrichment of minor proteins)

CLASSICAL PROTEOMICS

Separation and relative quantification: 2-D DIGE (differential in-gel electrophoresis)



Statistical analysis: selection of differential proteins

Proteomics

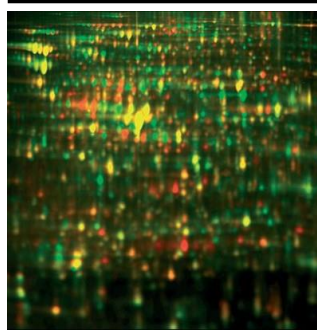


Serum samples:
Protein extraction
and equalization



MM

pI



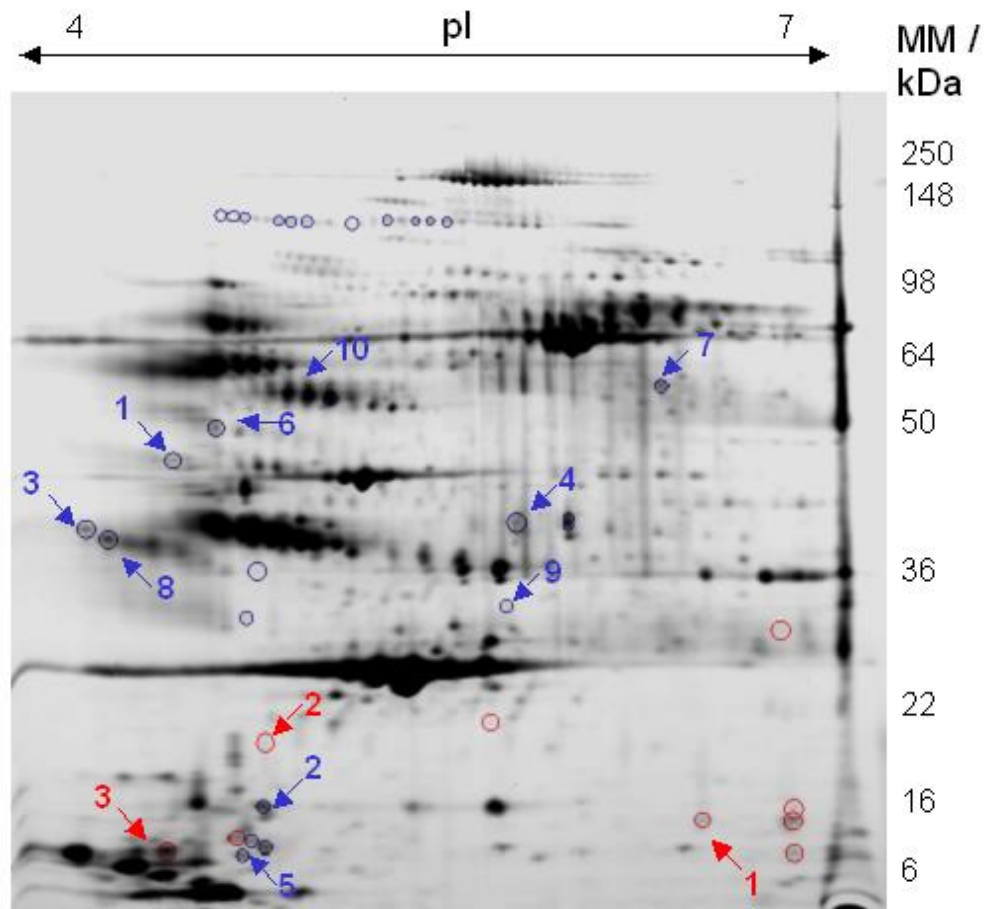
**Separation and
quantification:**
2-D DIGE



Protein identification:
MALDI-TOF/TOF MS



Bipolar + Li
Internal standard (mix)
Bipolar - Li



- 3 **upregulated** proteins in Bipolar + Li (apolipoprotein A-I)
- 10 **downregulated** proteins in Bipolar + Li

SHOTGUN PROTEOMICS



Depletion



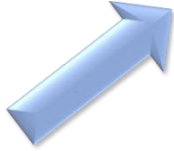
FPLC ÄKTA Start
(GE Healthcare™)

Columns:

1. HiTrap™ Blue HP
2. HiTrap™ Desalting

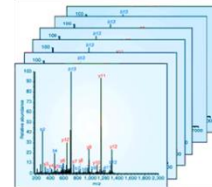


Trypsin
digestion



nLC LTQ Orbitrap
Velos - MS
(Thermo Fisher™)

Data
analysis



Control (HC) x Bipolar (BD) x Schizophrenia (SCZ)

#Acc.	Protein ID	p-value	Presence
P01011	Alpha-I-antichymotrypsin	4,03 10 ⁻⁰⁴	HC – BD – ↑SCZ
O95445	Apolipoprotein M	1,75 10 ⁻⁰³	HC – ↑BD – SCZ
Q8TF39	Zinc finger protein 483	8,08 10 ⁻⁰³	HC – ↓BD; BD – ↑SCZ
P02753	Retinol-binding protein 4	1,98 10 ⁻⁰²	HC – ↑SCZ
G3V5H5	Tryptophan--tRNA ligase, cytoplasmic	3,70 10 ⁻⁰²	HC – ↓BD
P69905	Hemoglobin subunit alpha	4,41 10 ⁻⁰²	HC – ↑BD
P02763	Alpha-I-acid glycoprotein I	0,06	HC – ↑BD
P02750	Leucine-rich alpha-2-glycoprotein	0,07	HC – ↑SCZ
O14791	Apolipoprotein L1	0,13	↑BD – SCZ
P02768	Serum albumin	0,21	HC – ↑BD
P01042	Kininogen-I	0,22	HC – ↓BD
P01024	Complement C3	0,25	↑BD – SCZ
P08185	Corticosteroid-binding globulin	0,34	HC – ↓SCZ
P02760	Protein AMBP	0.47	HC – ↑BD

73 % of the differential proteins
are associated with **HDL**

Metabolomics



Endogenous and exogenous metabolites

Lipids

Carbohydrates

Amino acids

Nucleotides

Analytical techniques:

NMR



GC-MS



LC-MS



Metabolomics

Experimental data



Matrix X	Matrix Y
Data (retention time, m/z)	Response (intensities)

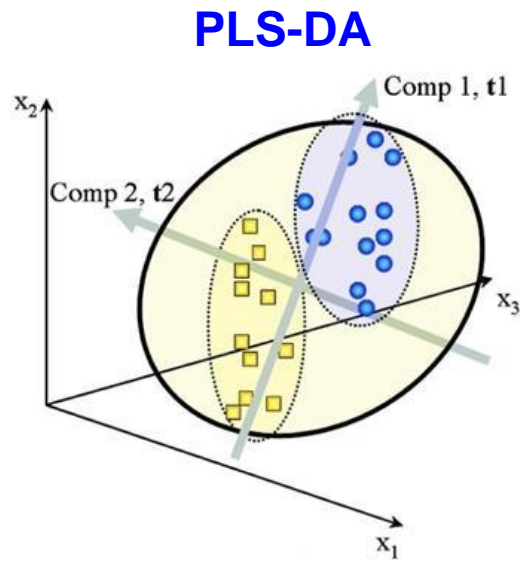
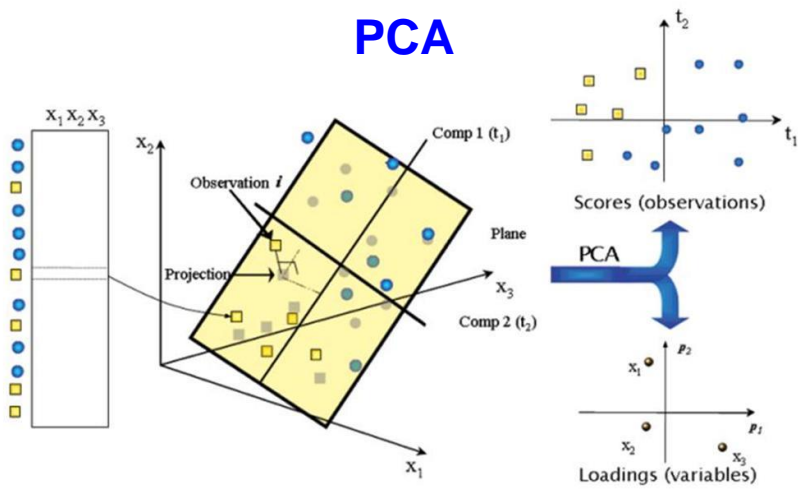


Pretreatment:

Correction of the matrix by removing information not related to the target variables



Multivariate statistical analysis



Metabolomics: Preliminary study

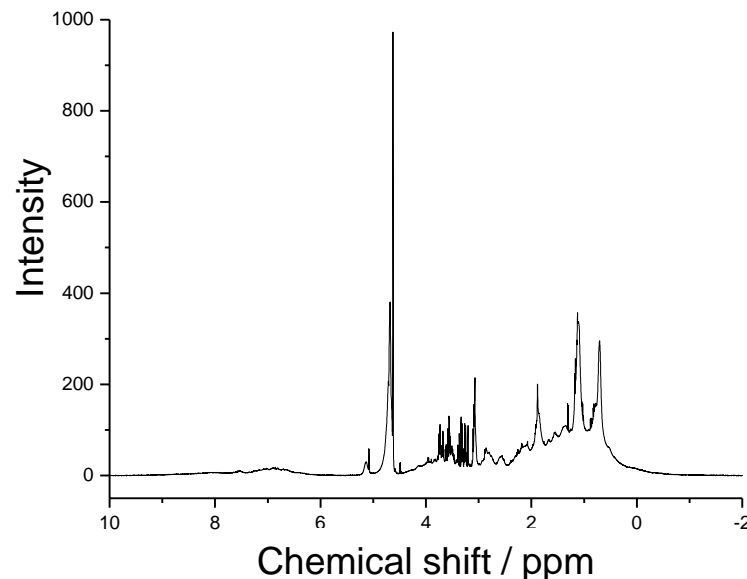


Sample preparation:
250 μL serum +
350 μL D_2O



Varian INOVA-500
 $B_0 = 11.7 \text{ T}$
499.89 MHz

^1H NMR (500 MHz) spectra for
each sample

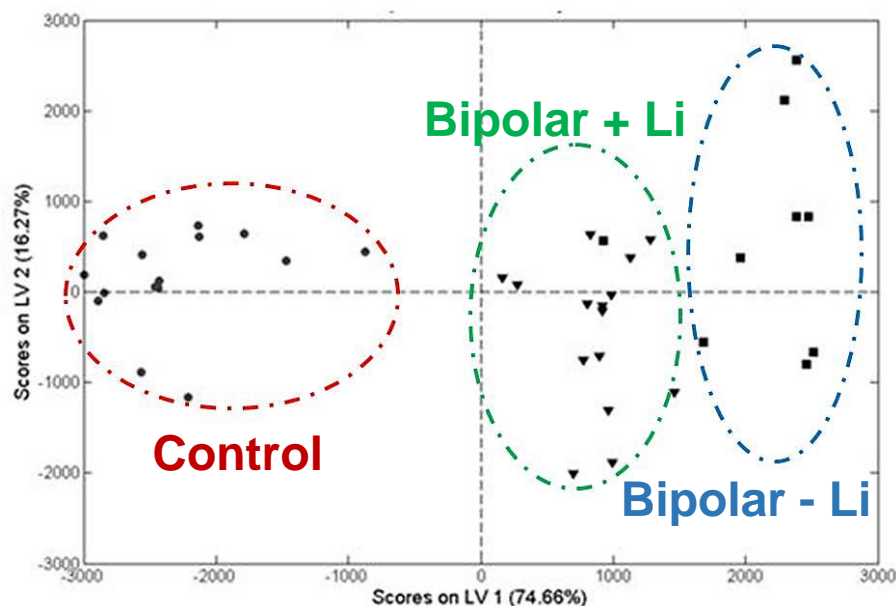


Pretreatment (**OSC**)



Multivariate statistical analysis
(**PLS-DA**)

Metabolomics: Preliminary study



Chemometrics:
Groups
differentiated
according to their
metabolic profiles

Differential metabolites:

- **Lipids** (glycoprotein, mono and poly unsaturated fatty acids)
- **Lipid metabolism-related molecules** (acetate, glutamate, choline and myo-inositol)
- **Amino acids** (glutamate, glutamine)

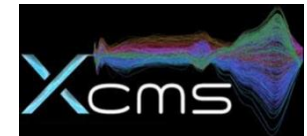
Lipidomics

Sample preparation:

Lipid extraction
(Bligh & Dyer)



Data analysis



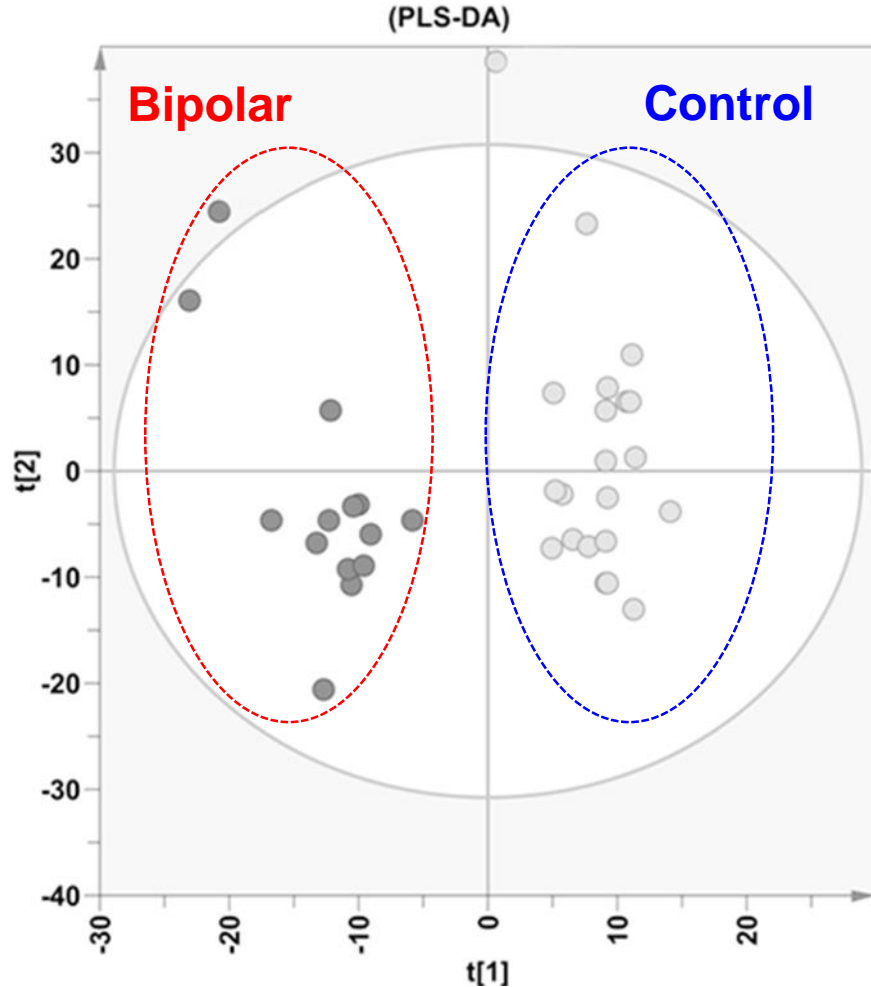
UHPLC-MS/MS

(Agilent **UHPLC**1290
ESI-QTOF 6550)

SIMCA



Lipidomics: Global analysis



Scores plot PLS-DA
($R^2_Y=0.976$ and $Q^2_Y=0.910$)

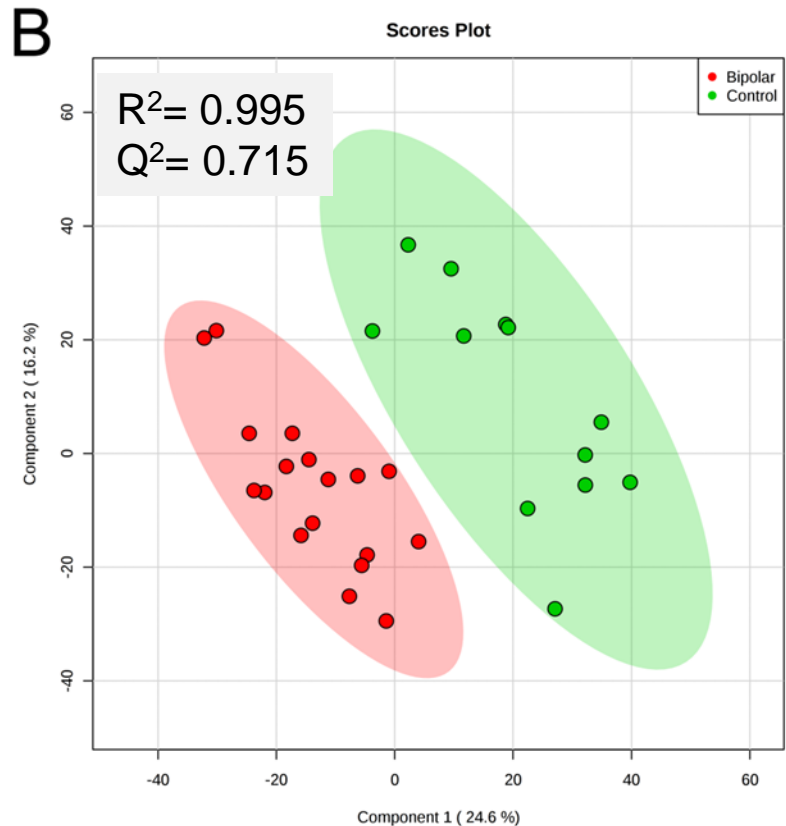
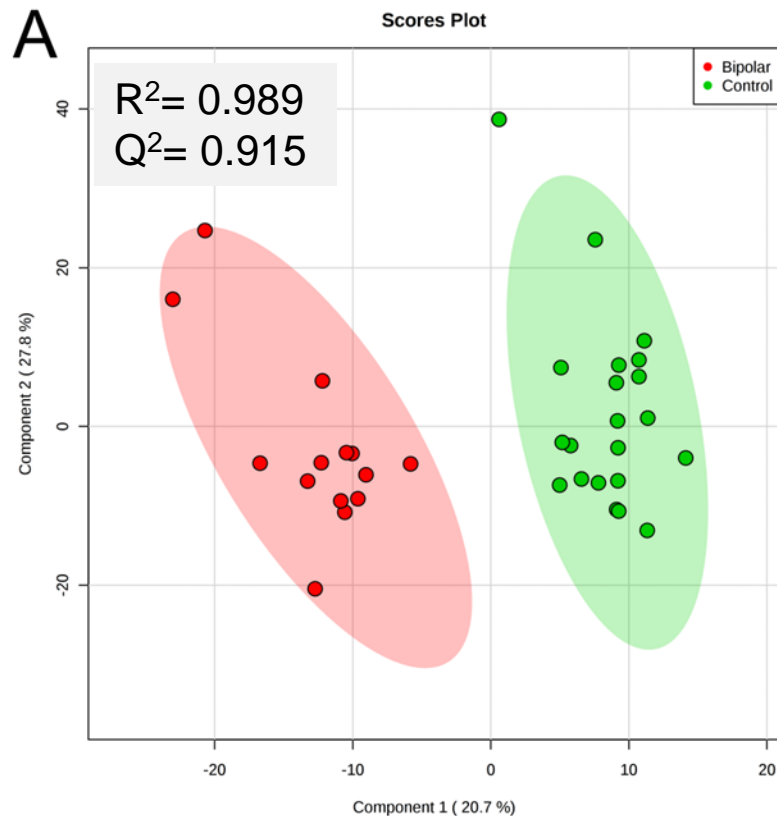
Chemometrics:

Groups
differentiated
according to their
lipid profiles

Main differential lipid classes:

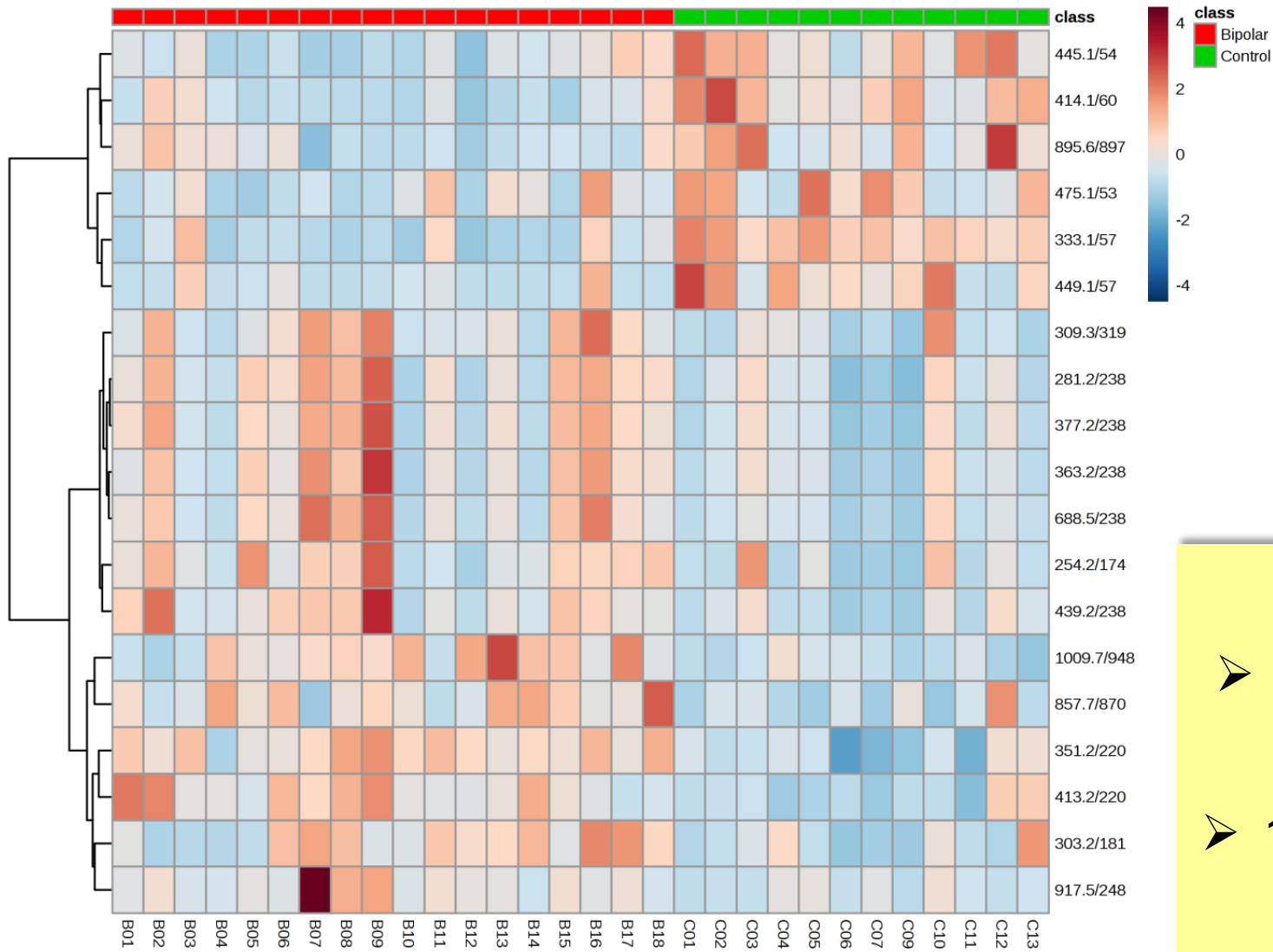
- Fatty acids
- Glycerophospholipids

Lipidomics: Biological validation



Independent data set:
separation between control
and bipolar groups

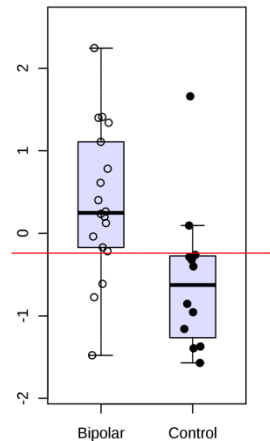
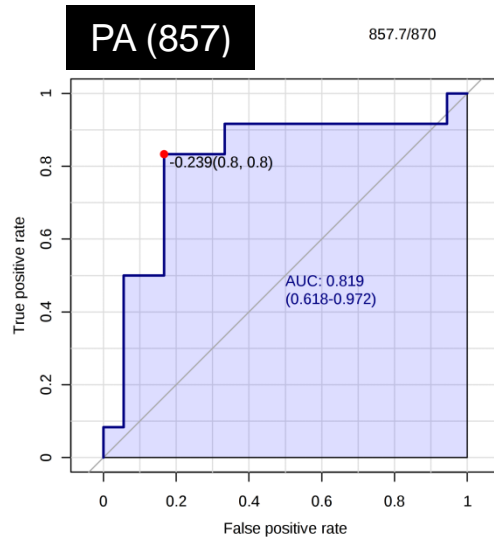
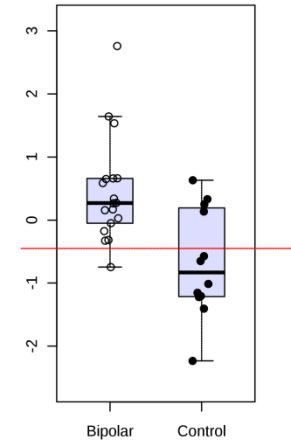
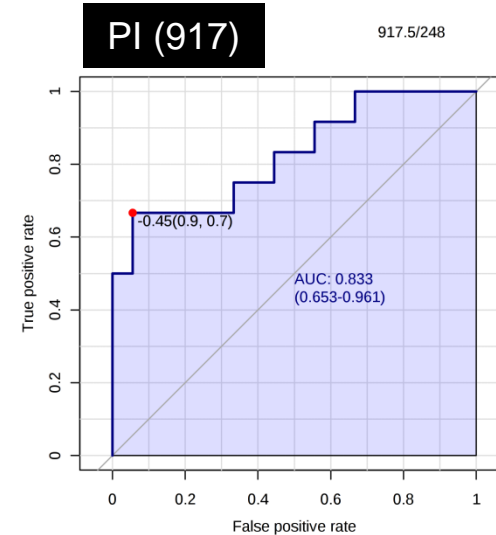
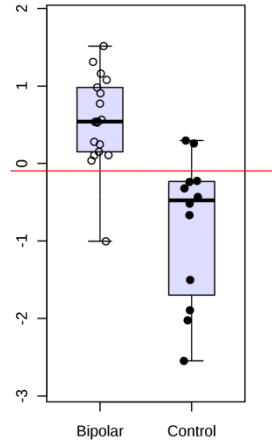
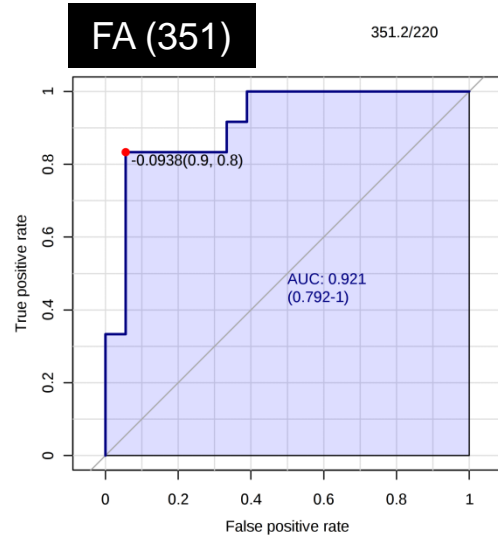
Lipidomics: Biological validation



ESI (-):

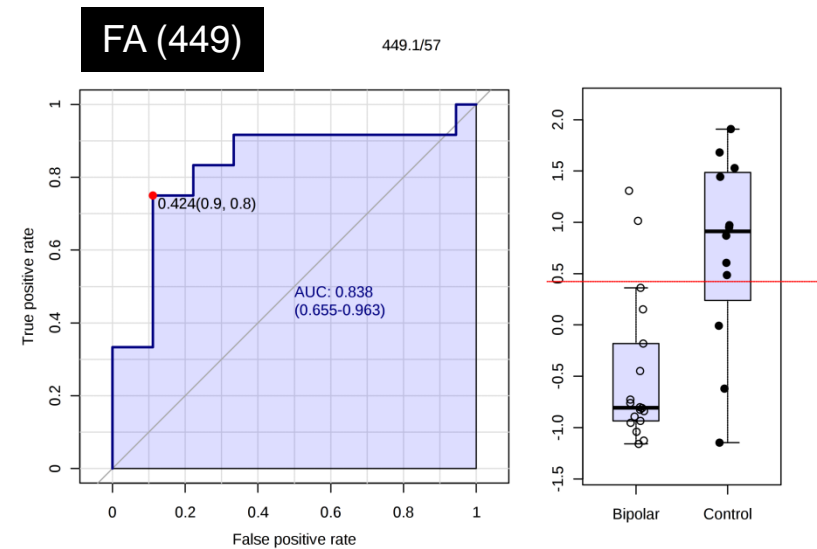
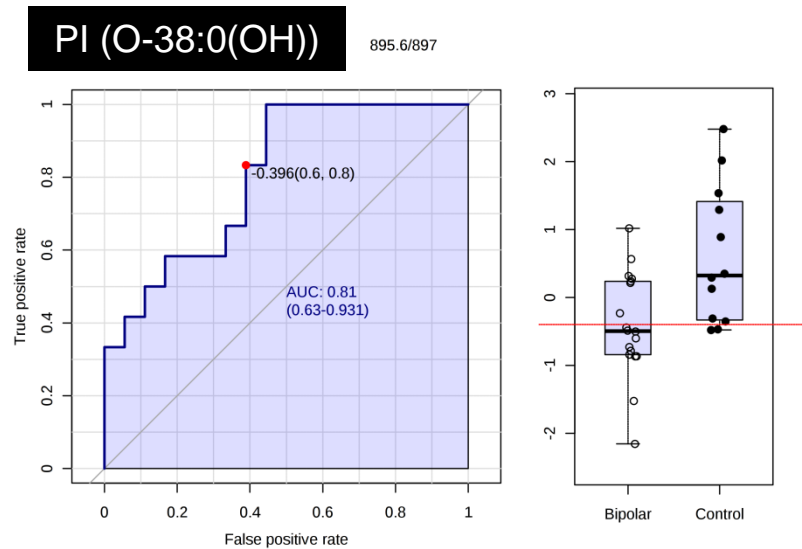
- 6 enhanced in **control group**
- 13 enhanced in **bipolar group**

Lipidomics: Potential biomarkers



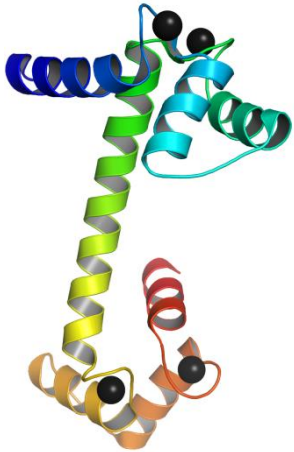
Higher concentration
level in **bipolar** group

Lipidomics: Potential biomarkers



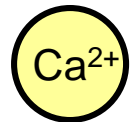
Higher concentration
level in **control group**

Metalloproteomics

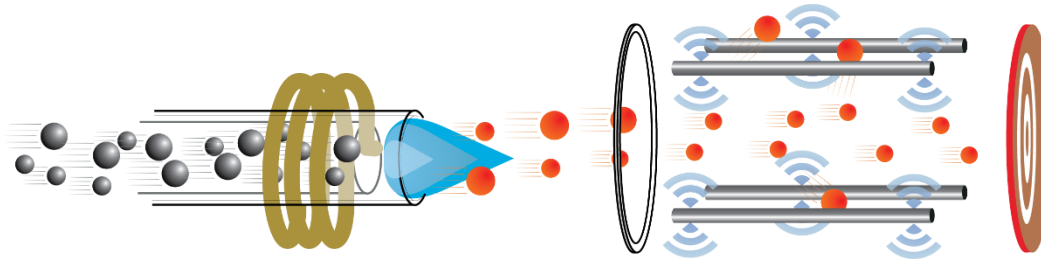


Metallomics

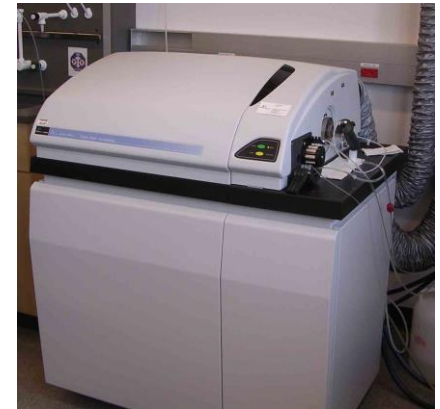
Ionomics



ICP MS: (Semi) quantitative elemental analysis



Sample preparation:
dilution



**Determination of the
elements: ICP MS**

Statistical analysis:
ANOVA



14 differential ions

(ANOVA, 95 % confidence)

+: higher level among
the 3 groups

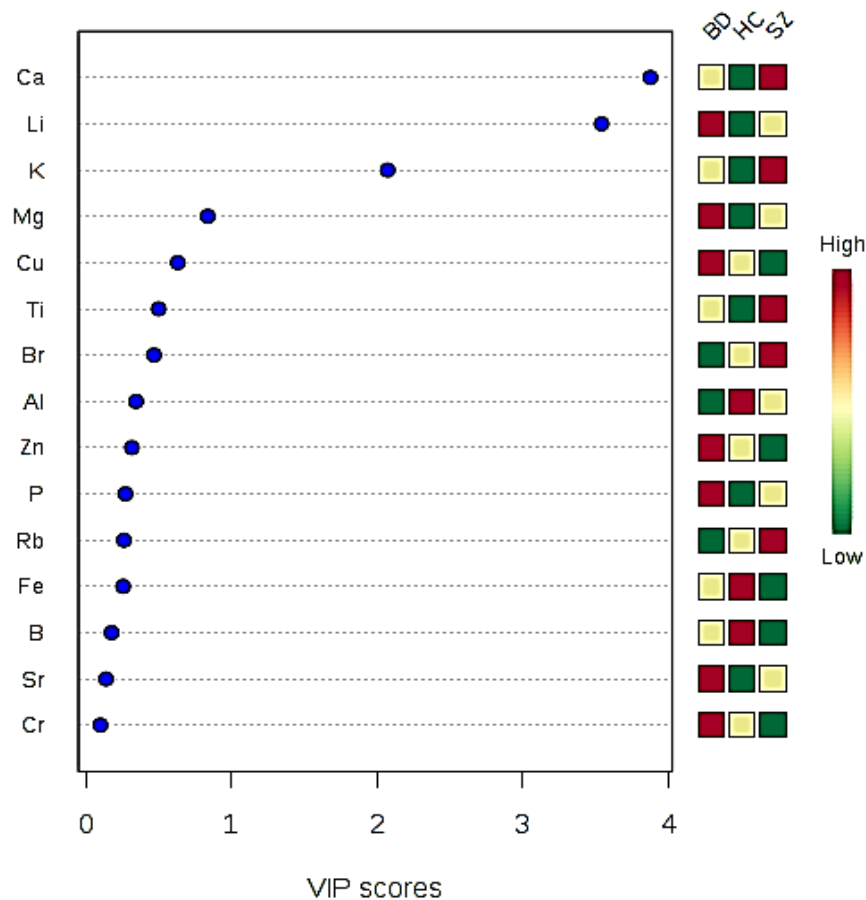
++: higher level between
bipolar patients

Zn: higher level in
Bipolar + Li

Depression: Zn
levels reduced

Ion	Control	Bipolar + Li	Bipolar - Li
Li		++	+
Si			++
K			++
Cr			++
Fe			++
Se			++
Zn		++	+
B	+		
Mg			+
As			+
S			+
Cl			+
P			+
Sr			+

Control (HC) x Bipolar (BD) x Schizophrenia (SCZ)



Main differential ions:

Ca: ↑ SZ

K: ↑ SZ

Li: ↑ BD

Zn: ↑ BD

Mg: ↑ BD

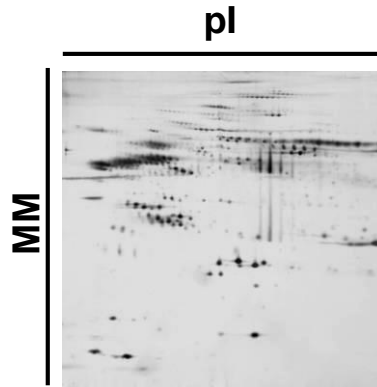
Cr: ↑ BD

P: ↑ BD

Metalloproteomics



Serum samples:
Protein extraction
and depletion



Protein separation:
2-D PAGE

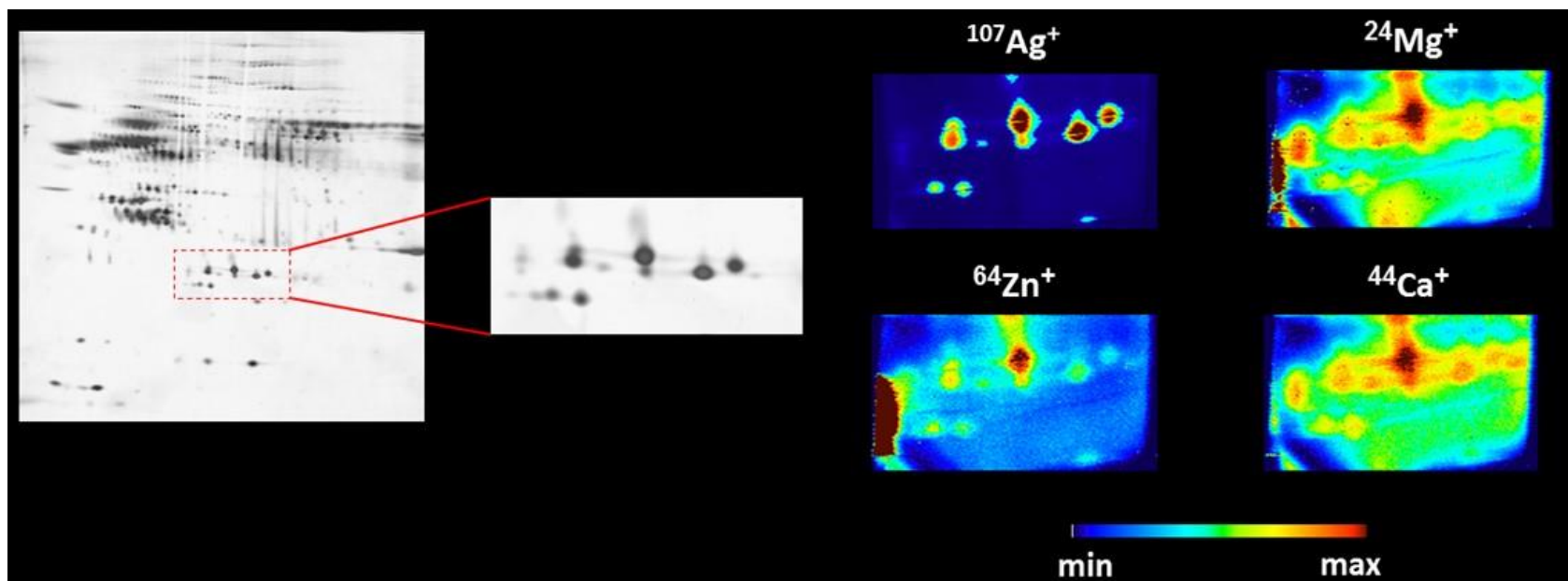


**Mapping of the
elements bound to
proteins:**
LA-ICP MS



Protein identification:
MALDI-TOF/TOF MS

Metalloproteomics



Differences in the metalloproteomic profiles:

- **Apolipoprotein A-I bound to Ca (Control)**
- **Apolipoprotein A-I bound to Ca (Bipolar + Li)**
- **Apolipoprotein A-I not bound to Ca (Bipolar - Li)**

Depression

Characterized by **sadness** and/or **loss of interest** in activities once enjoyed, thoughts of suicide, sleep disorders



- ✓ Only 50.4 % of the patients respond to the treatment
- ✓ 32 % respond partially
- ✓ 45 % do not respond: **treatment-resistant depression**

Treatment:

Antidepressives,
psychotherapy

Causes:

Genetic,
environmental,
personality factors

Diagnosis:

Only clinical (blood test
discards thyroid
problems)

Alternative treatment for depression

Ayahuasca:



+



=



Banisteriopsis caapi

Psychotria viridis

Ayahuasca

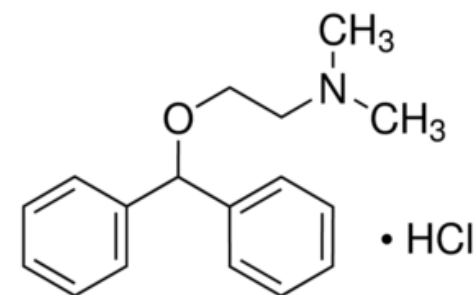
- Metabolomics and metallomics evaluation of Ayahuasca aiming at possible therapeutical applications

Ayahuasca characterization

Targeted metabolomics:

Quantification of active principles by **UHPLC-ESI-QqQ MS**

Analyte	MM (g mol ⁻¹)	[M+H] ⁺ (<i>m/z</i>)	Monitored transitions
DMT	188	189	189 > 58
			189 > 144
THH	216	217	217 > 188
			217 > 200
HME	212	213	213 > 170
			213 > 198
HML	214	215	215 > 174
			215 > 200
IS	255	256	256 > 152
			256 > 167

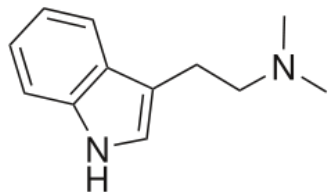


Internal standard (IS)

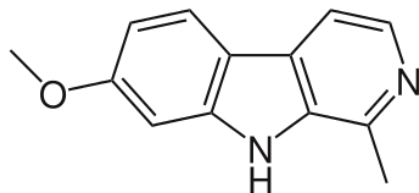
2 monitored
transitions:

- ☐ Quantification
- ☐ Confirmation

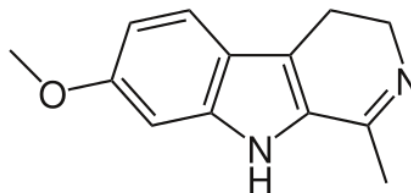
Ayahuasca characterization



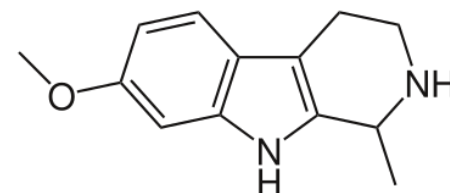
***N,N*-
dimethyltryptamine
(DMT)**



Harmine (HME)



Harmaline (HML)



**Tetrahydroharmine
(THH)**

**Determined concentrations in ayahuasca
samples from São Paulo state (n = 38):**

	Concentration (mg/L)			
	DMT	THH	HME	HML
Minimum	62	403	414	44
Maximum	340	3088	1816	392
Mean	232	1947	1322	240

Ayahuasca characterization

Ionomics:

Total elemental concentration determined by **ICP OES** and **ICP MS**

Macroelements determined in 19 ayahuasca samples from São Paulo state by ICP OES

Total concentration, mg/L		
	Minimum	Maximum
Ca	102 ± 7	664 ± 23
Mg	313 ± 6	1542 ± 26
P	47 ± 2	616 ± 23
K	2017 ± 58	7263 ± 100
Sr	2.0 ± 0.2	11.4 ± 0.5
Rb	3.9 ± 0.2	37 ± 4

Ayahuasca characterization

Microelements determined in 19 ayahuasca samples from São Paulo state by ICP MS

	Concentração total, µg/L	
	Mínima	Máxima
Li	4.5 ± 0.2	76.3 ± 0.7
Al	< LQ	9709 ± 205
Mn	4848 ± 274	93519 ± 4385
Fe	1753 ± 0	6902 ± 143
Cu	< LQ	169 ± 40
Co	< LQ	170 ± 6
Zn	616 ± 94	19264 ± 520
As	< LQ	
Cd	3.8 ± 0.5	26.9 ± 0.7
Ba	185 ± 10	3813 ± 43
Hg	< LQ	
Tl	3.8 ± 0.2	31.6 ± 0.4
Pb	< LQ	351 ± 9

Next steps

Serum samples from patients with **treatment-resistant depression**
(Collaboration with UFRN, Natal, Brazil)

- ✓ Treated with ayahuasca
- ✓ Treated with placebo



- Samples collected before and after 48 h of the treatment

Metabolomics and Lipidomics: evaluation of the efficacy of ayahuasca in depression treatment (alternative therapy)

Conclusions

Mass spectrometry: huge amount of information →
guide **biomarkers discovery**

Biomarkers: improving the **diagnosis** and
evaluating **treatment efficacy**

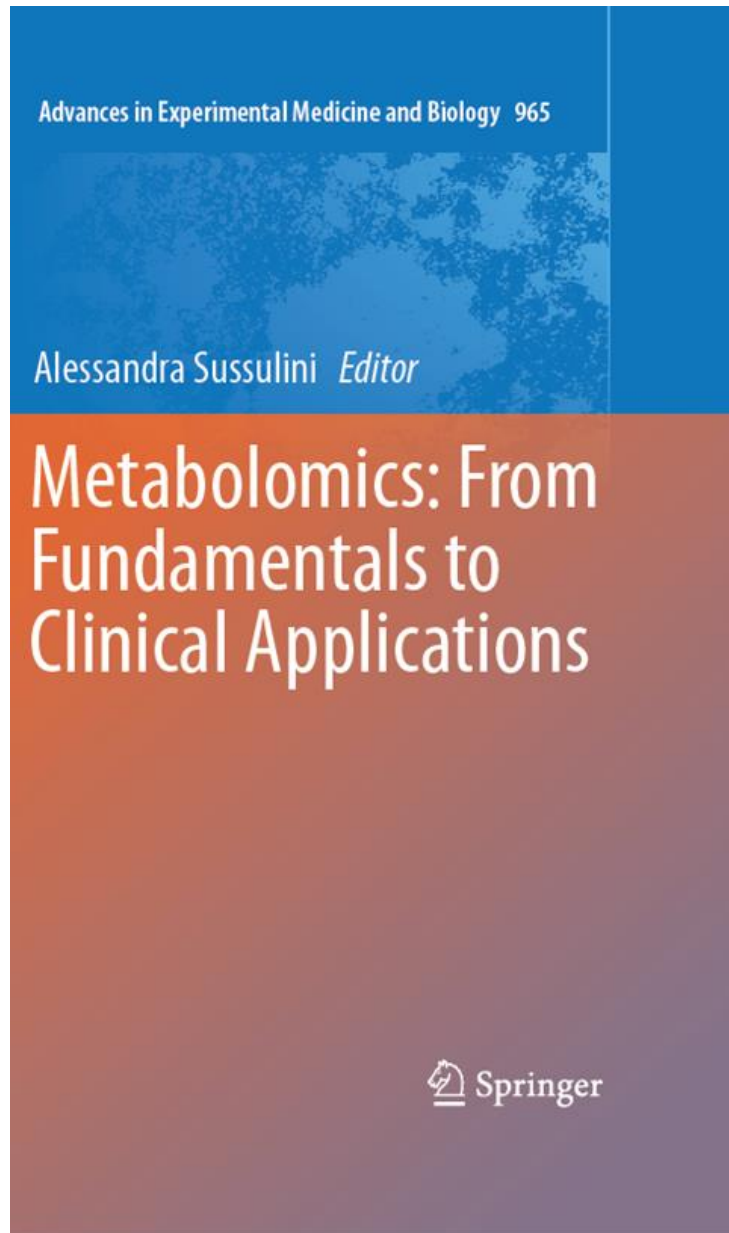
Multi-omics: employed within the context of
personalized medicine

Laboratory of Bioanalytics and Integrated Omics



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More about Metabolomics



Released in
2017

Acknowledgements



LaBIOmics Team

Prof. Dr. Luís Tófoli (FCM)

Prof. Dr. Dráulio Araújo (UFRN)

Prof. Dr. Nicole Coelho (UFRN)

Prof. Dr. Marcos Eberlin (IQ)

Prof. Dr. Marco Arruda (IQ)

Helle Kaasik (Uni. Tartu, Estônia)

