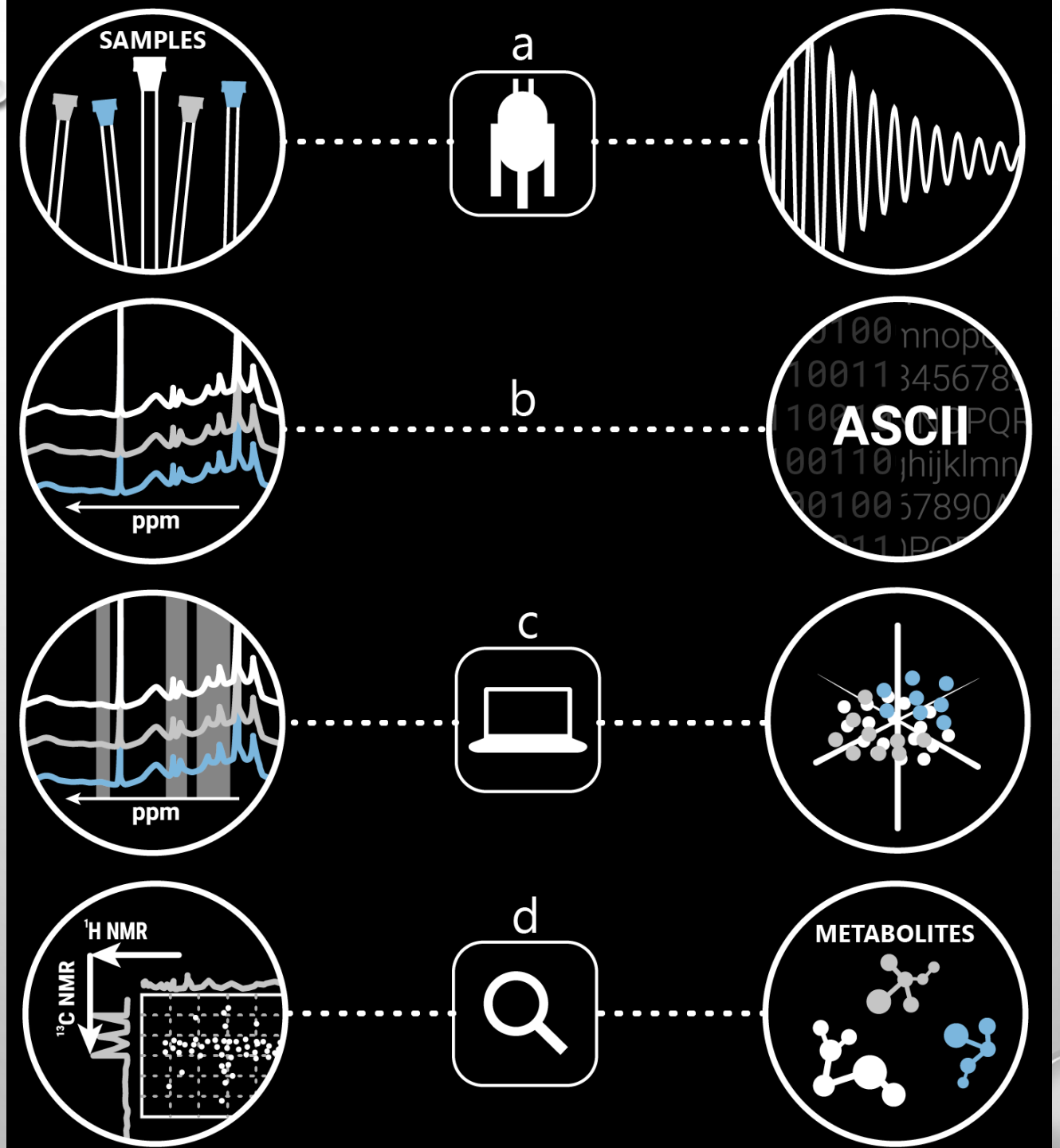
6th Bioanalytical School – INCT Bioanalítica

November 28-30, 2018. Londrina, PR, Brazil

LJUBICA TASIC

APPLICATION OF NMR SPECTROSCOPY IN STUDIES OF HUMAN, ANIMAL AND PLANT DISEASES

Anal. Methods **2017**, *9*, 1078-1096.

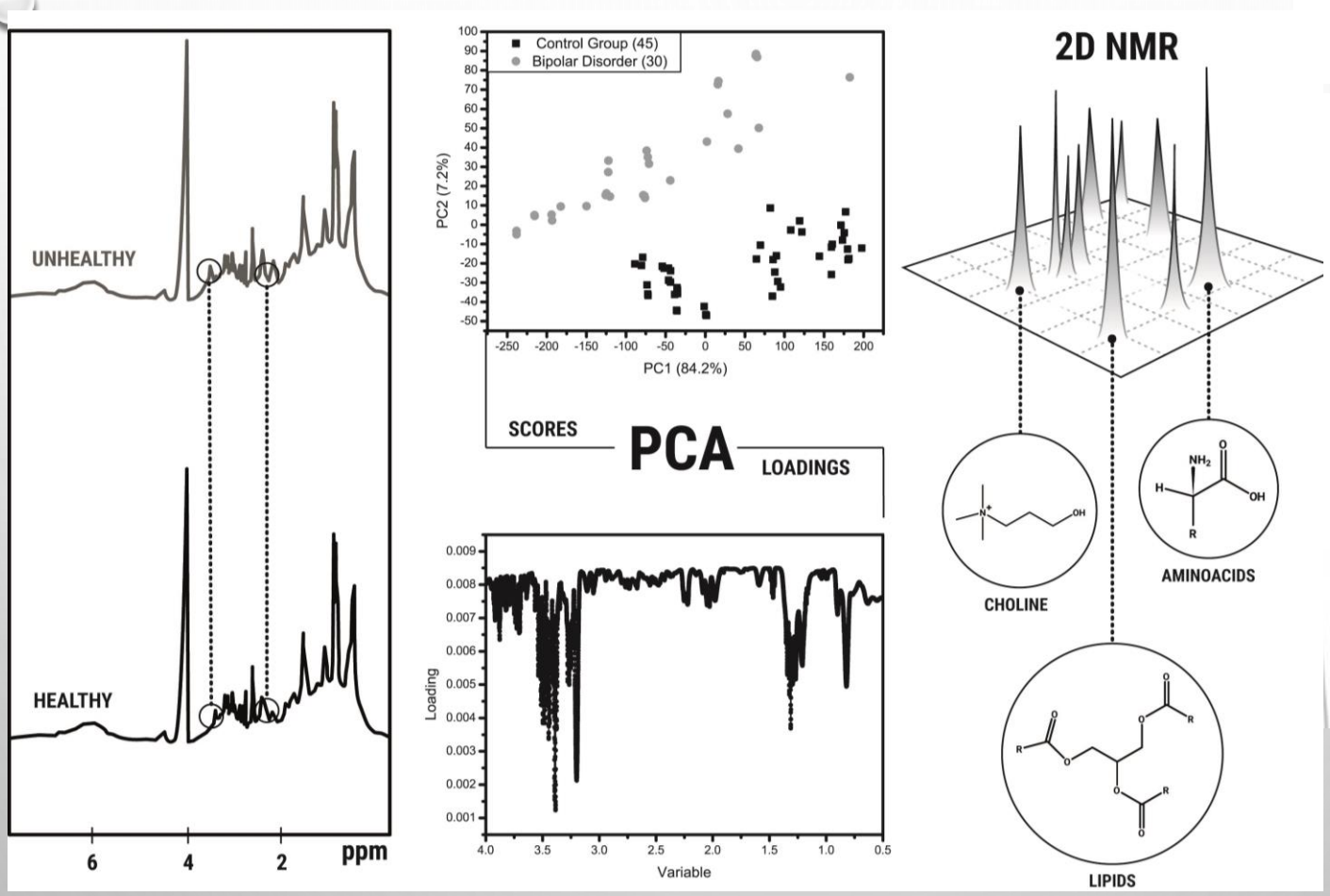

SAMPLES
a
b
ppm
ASCII
c
ppm
d
^{1}H NMR
^{13}C NMR
ML
METABOLITES

METABOLITES

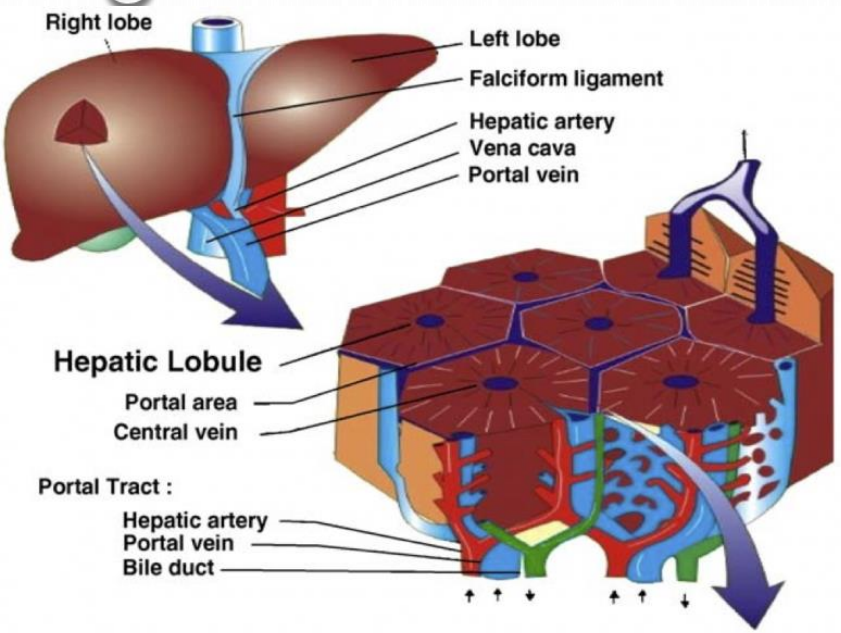
- PLANTS
- ANIMALS
- HUMAN DISEASES
  - PSYCHIATRIC PROBLEMS: MENTAL DISORDERS
    - SCZ, BIPOLAR, CRACK-COCAINE ADDICTION
  - CANCER RESEARCH – YOUNG CHILDREN CANCER, INFANCY
    - OSTEOSARCOMA
    - NEPHROBLASTOMA – WILMS TUMOR (KYDNEYS)
    - HEPATOBLASTOMA - LIVER CANCER – CHILDREN YOUNGER THAN 3 YEARS OLD
    - GLIOBLASTOMA

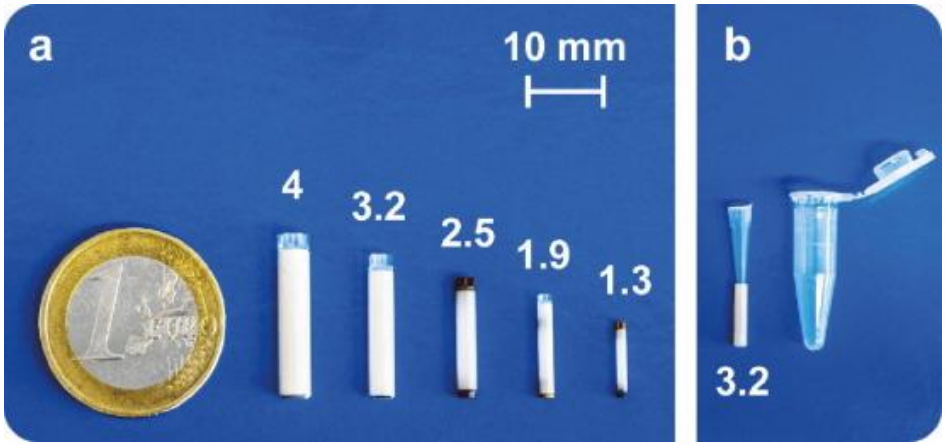
Anal. Methods **2017**, *9*, 1078-1096.

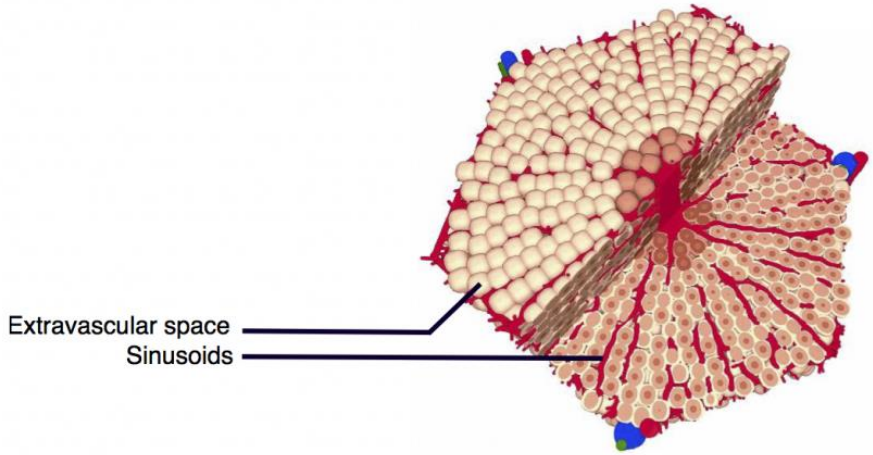
Biomarkers – NMR liquid state



BIOMARKERS – SEMISSOLID NMR







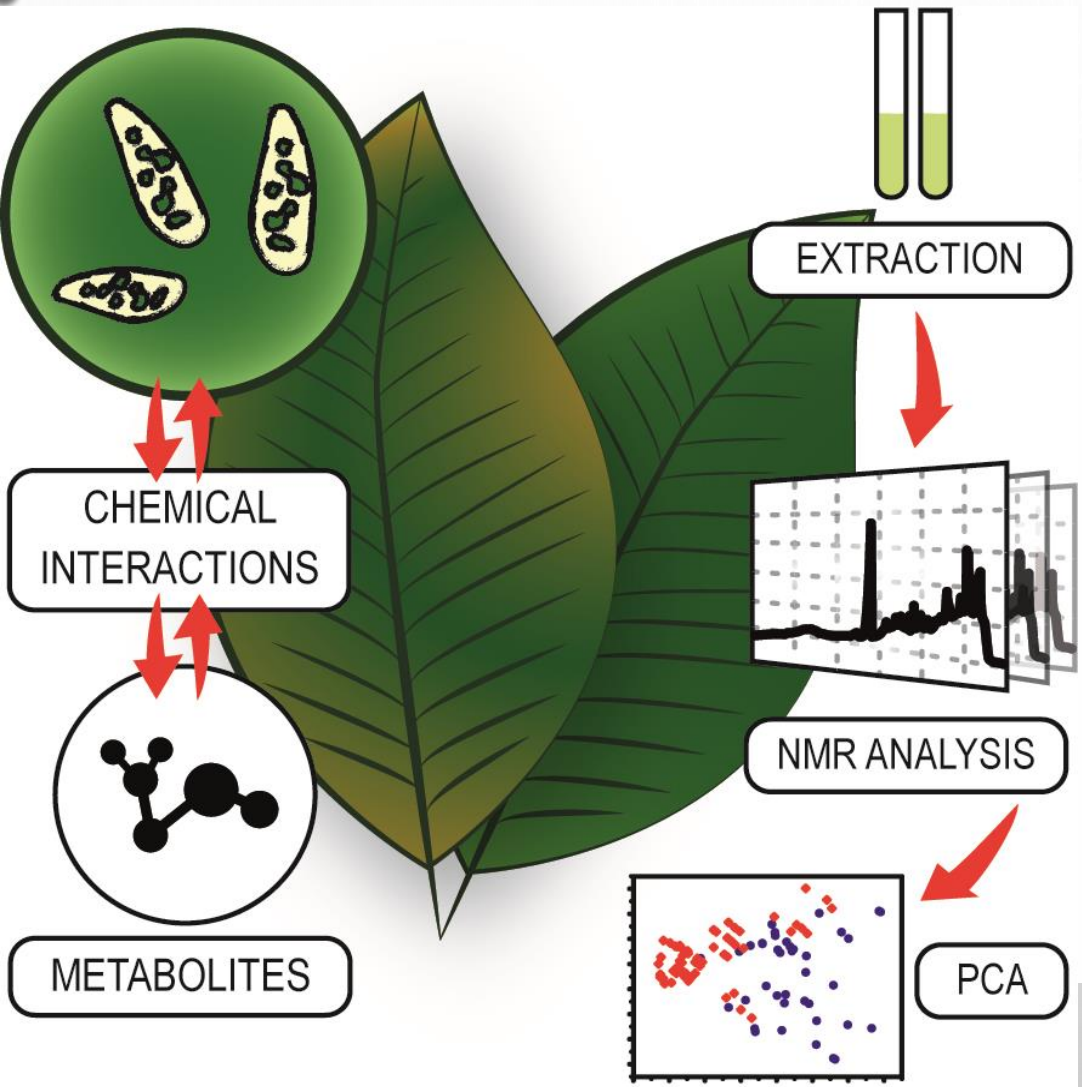
PLANT DESEASES – ORANGES

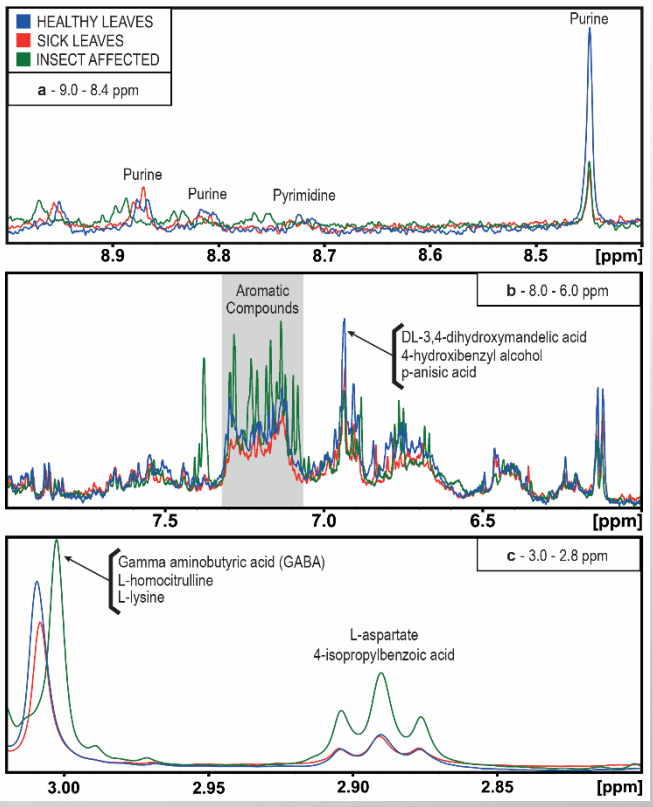
Xanthomonas axonopodis pv. *citri*

Xyllela fastidiosa

Liberibacter Candidatus *Asiaticus* (Clas)

Liberibacter Candidatus *Asiaticus*

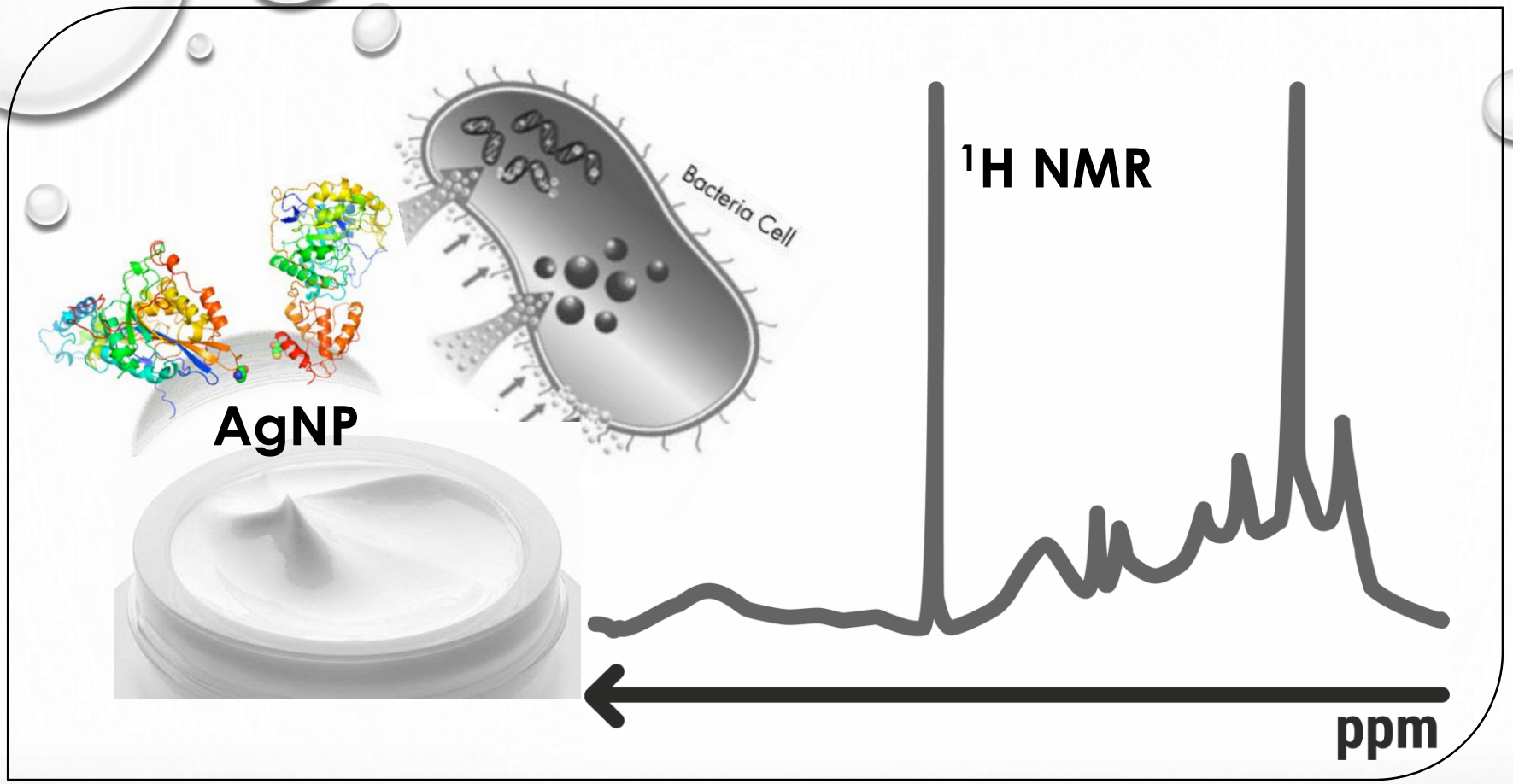




Pontes, J. G. M. *et al.*, Metabolomics by NMR Spectroscopy in Plant Disease diagnostic: Huanglongbing as a Case Study. *ChemistrySelect* **2016**, *6*, 1176 – 1178.

ANIMAL DISEASES

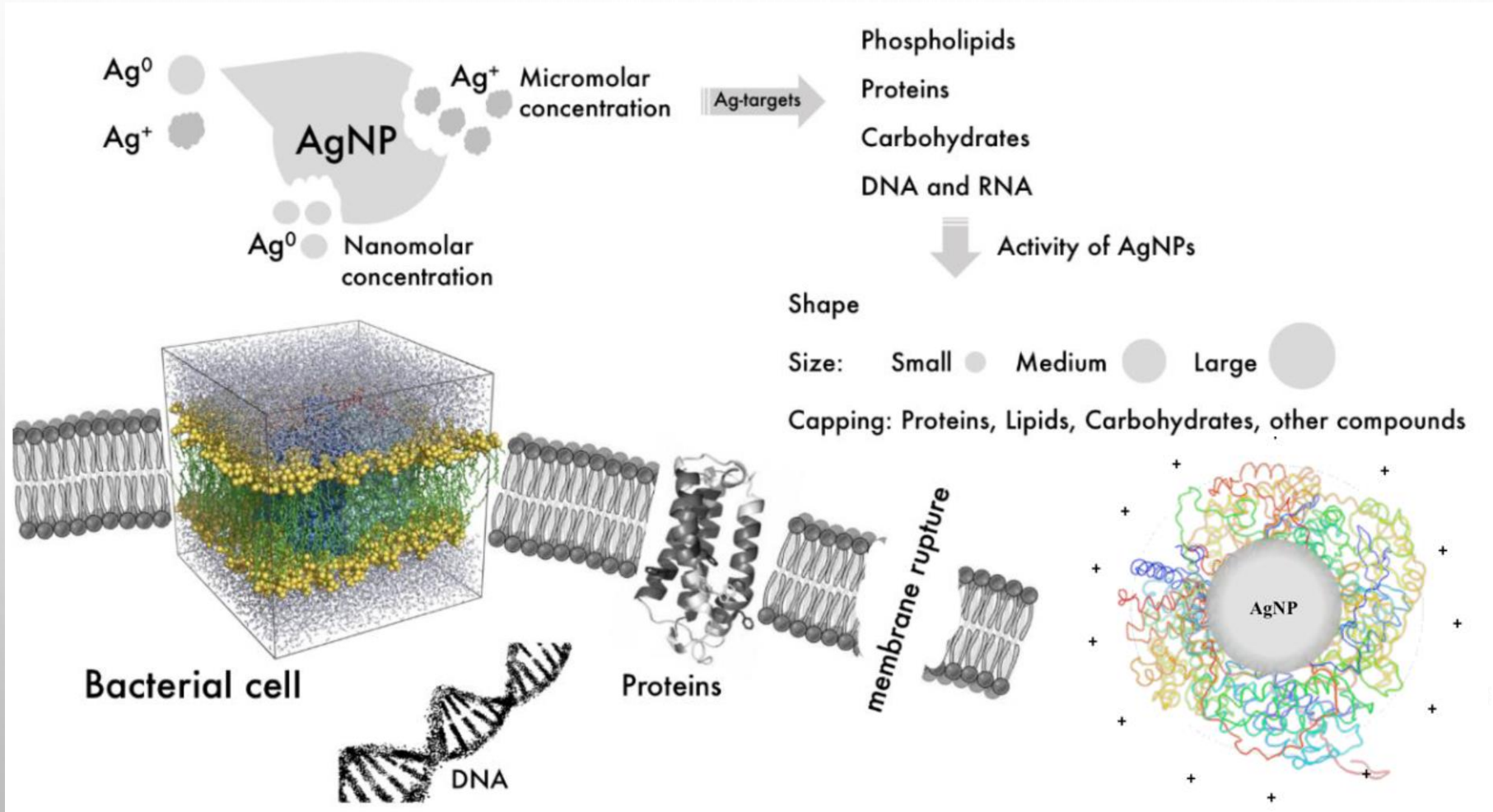
Corynebacterium pseudotuberculosis
(*Corynebacterium ovis*)



Biogenic nanosilver in a pharmaceutical cream for wound healing in animal and human healthcare.

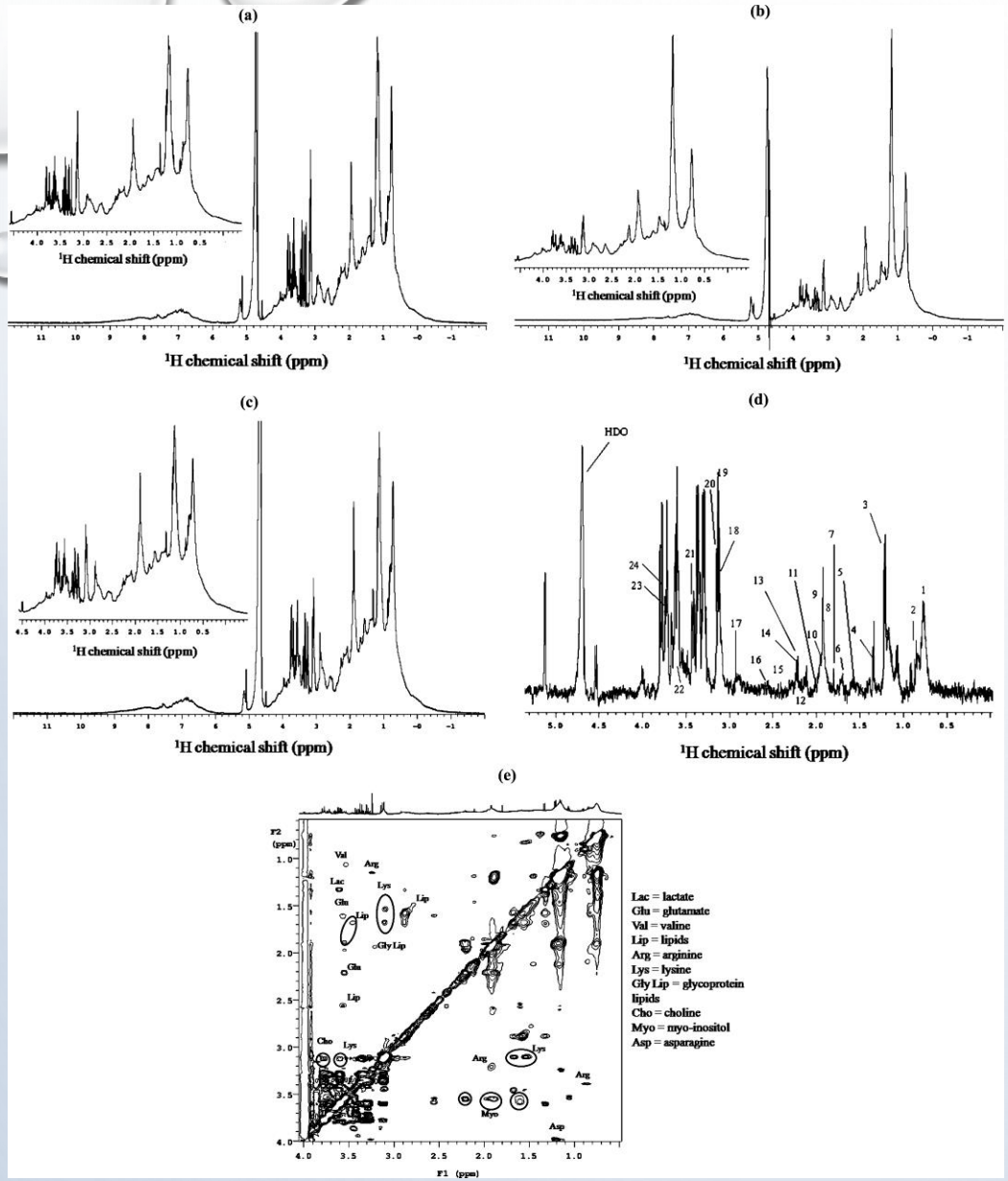
Pontes, J. *et al.*, *Metabolomics* (Los Angel) **2017**, *7*, 2.

Stanisic *et al.*, *RSC Advances* **2018**, just accepted


Ag0
Ag+
AgNP
Ag+ Micromolar concentration
Ag0 Nanomolar concentration
Ag-targets
Phospholipids
Proteins
Carbohydrates
DNA and RNA
Activity of AgNPs
Shape
Size: Small Medium Large
Capping: Proteins, Lipids, Carbohydrates, other compounds
Bacterial cell
DNA
Proteins
membrane rupture
AgNP

C. pseudotuberculosis

MENTAL DISORDERS



^{1}H NMR spectra for a blood serum sample of (a) a control subject, (b) a bipolar disorder patient treated with lithium, and (c) a bipolar disorder patient not treated with lithium. The expanded −0.5 to +4.4 ppm region used in chemometrics is shown in the upper left corners (a−c). The peak assignments described in Table 3 are shown in (d) on the *T*2-edited spectrum of a control sample. (e) ^{1}H−^{1}H TOCSY NMR spectrum of human blood serum recorded (control sample) using MLEV-17 as a spin-locking scheme with a mixing time of 90 ms. Some important metabolites are indicated.

Published in: Alessandra Sussulini; Alessandra Prando; Danilo Althmann Maretto; Ronei Jesus Poppi; Ljubica Tasic; Cláudio Eduardo Muller Banzato; Marco Aurélio Zezzi Arruda; *Anal. Chem.* 2009, 81, 9755-9763.
DOI: 10.1021/ac901502j
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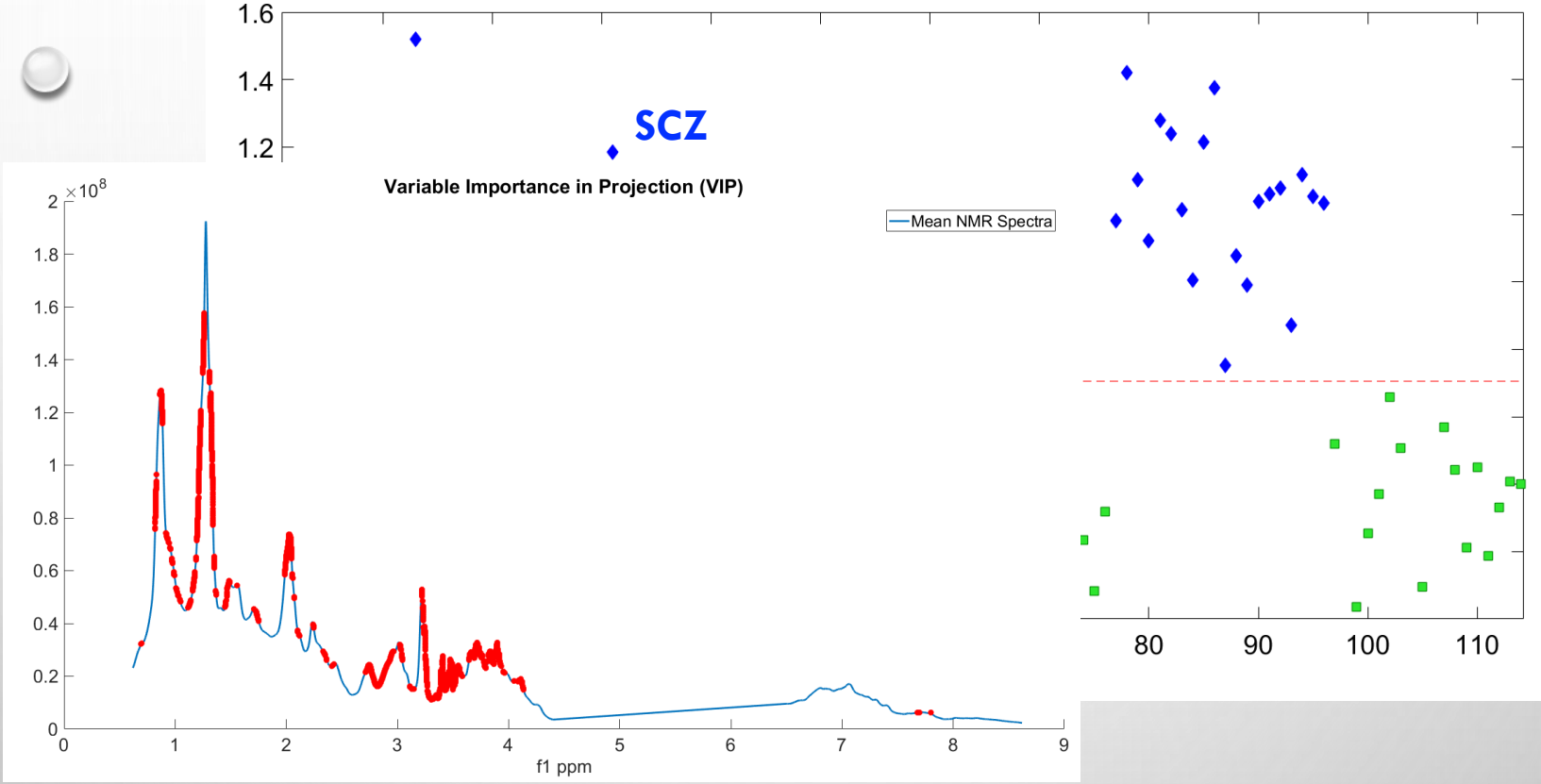
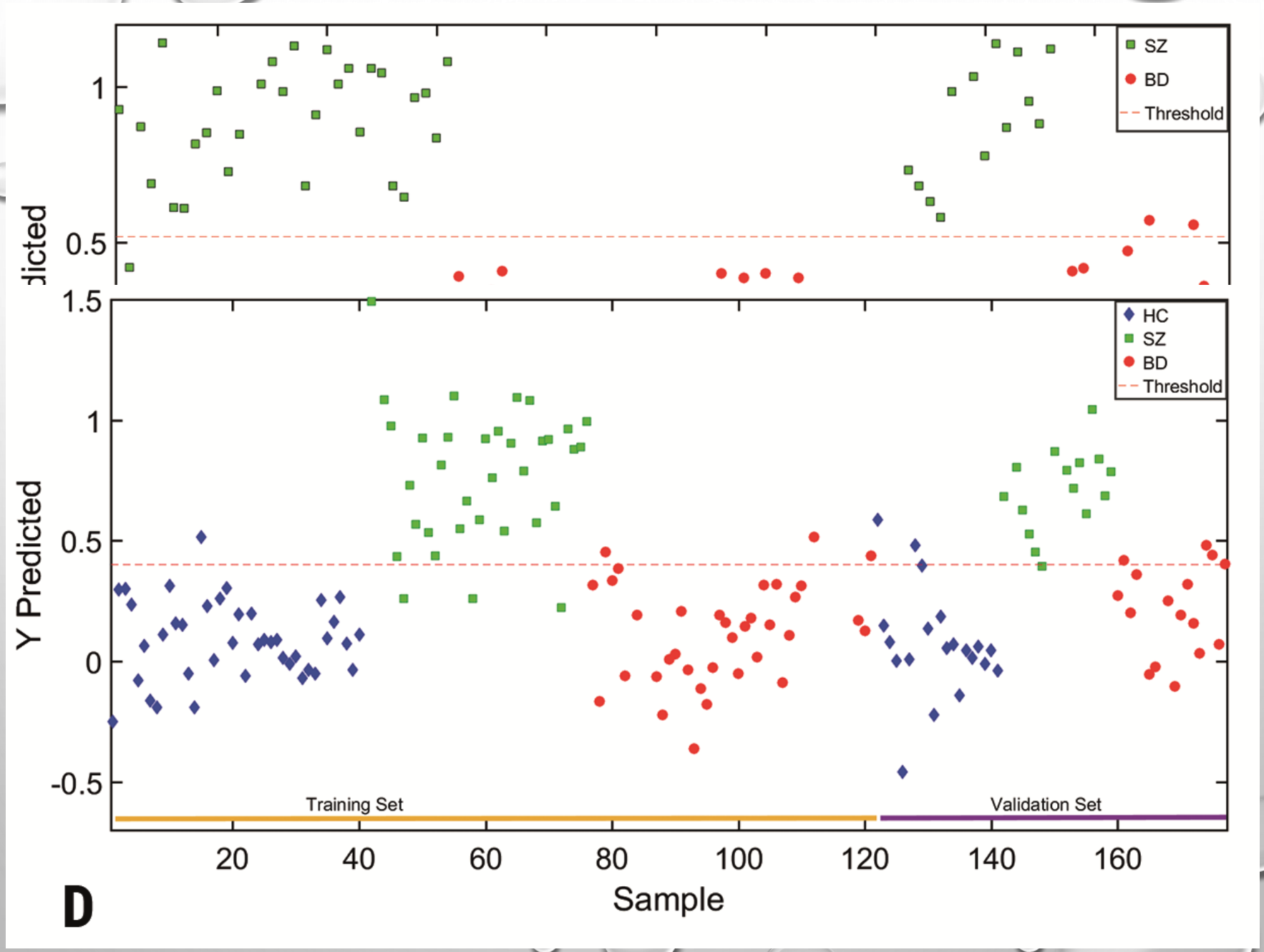
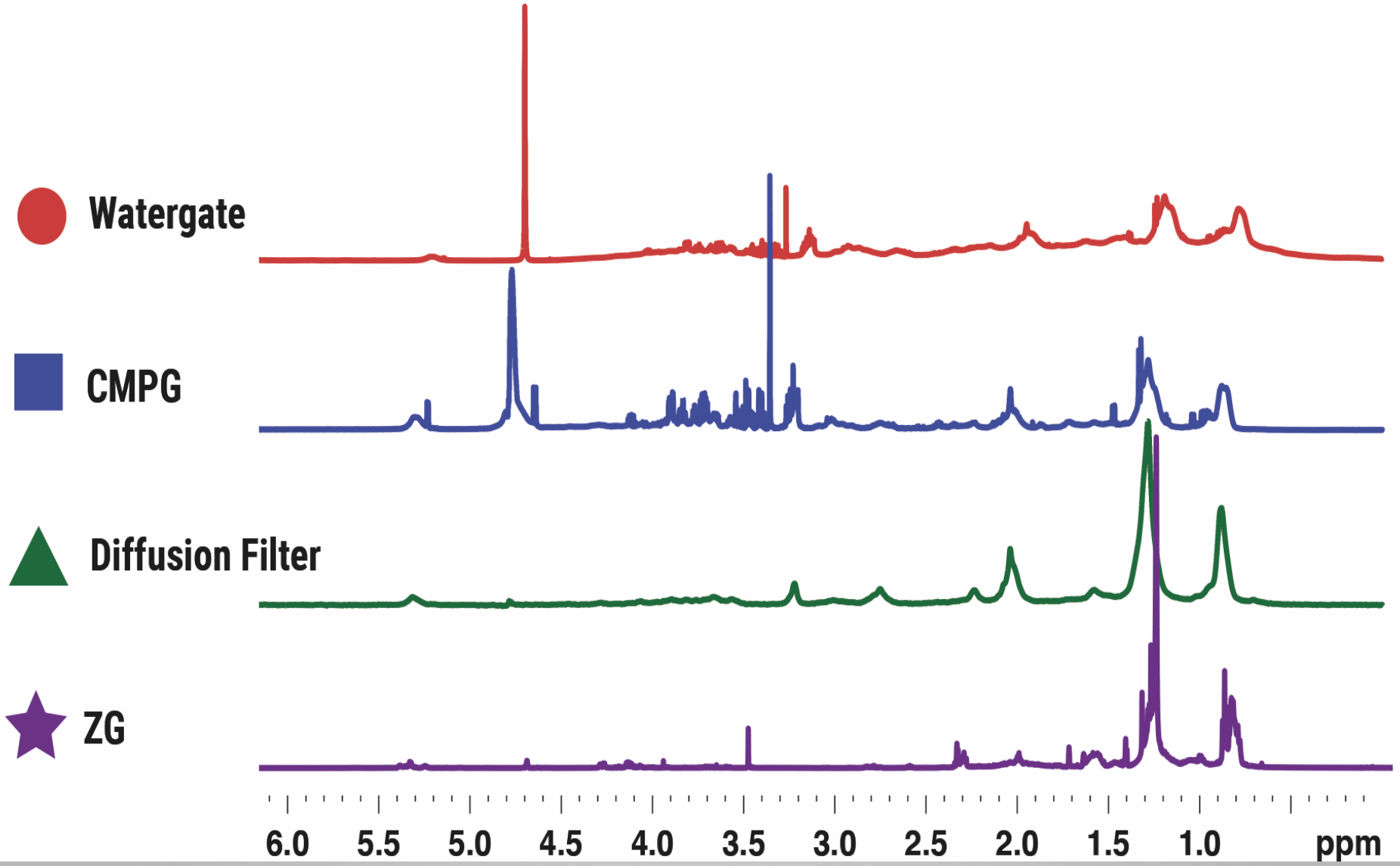
PLS-DA



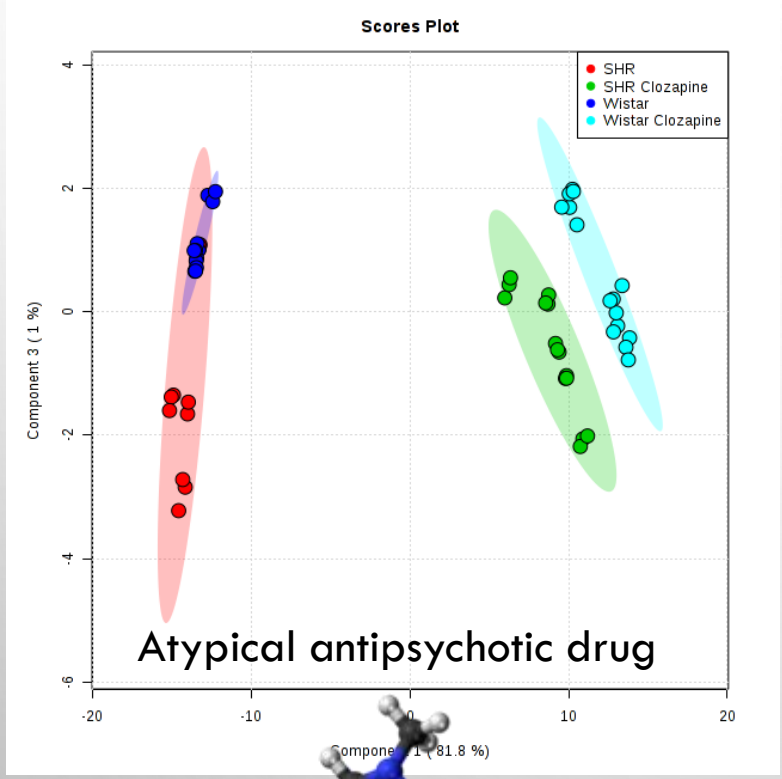
Tasic *et al.*, *Chemistry Select* **2017**, 2, 2927-2930. DOI: 10.1002/slct.201700009
Tasic *et al.*, *Schizophrenia Research* **2016**, DOI: 10.1016/j.schres.2016.12.024
Tasic *et al.*, **BR 10 2015 031930**, data de depósito: **18/12/2015**, INPI - Instituto Nacional da Propriedade Industrial.

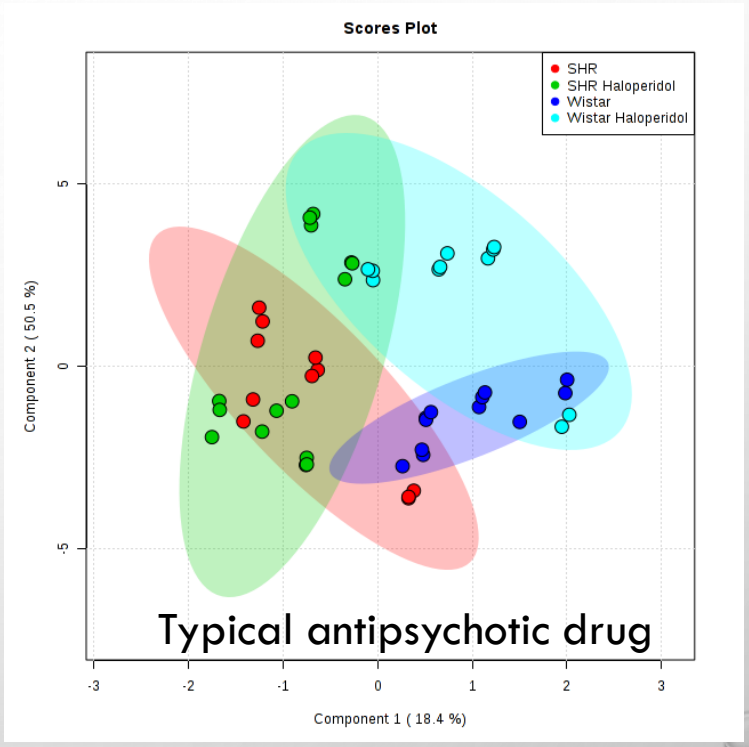

dicted
1
0.5
SZ
BD
Threshold
Y Predicted
1.5
1
0.5
0
-0.5
HC
SZ
BD
Threshold
Training Set
Validation Set
20
40
60
80
100
120
140
160
Sample
D

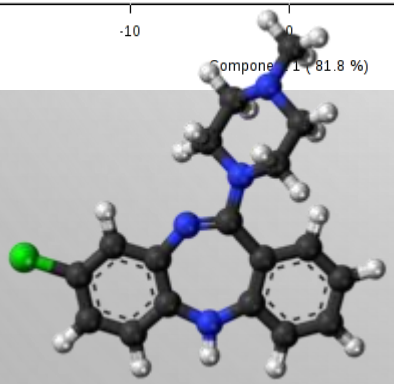
LIPIDS, METABOLITES & NMR

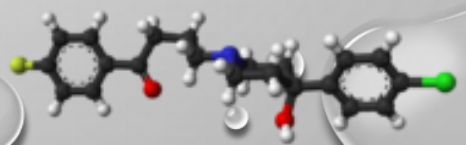


SCZ LIPIDOMICS



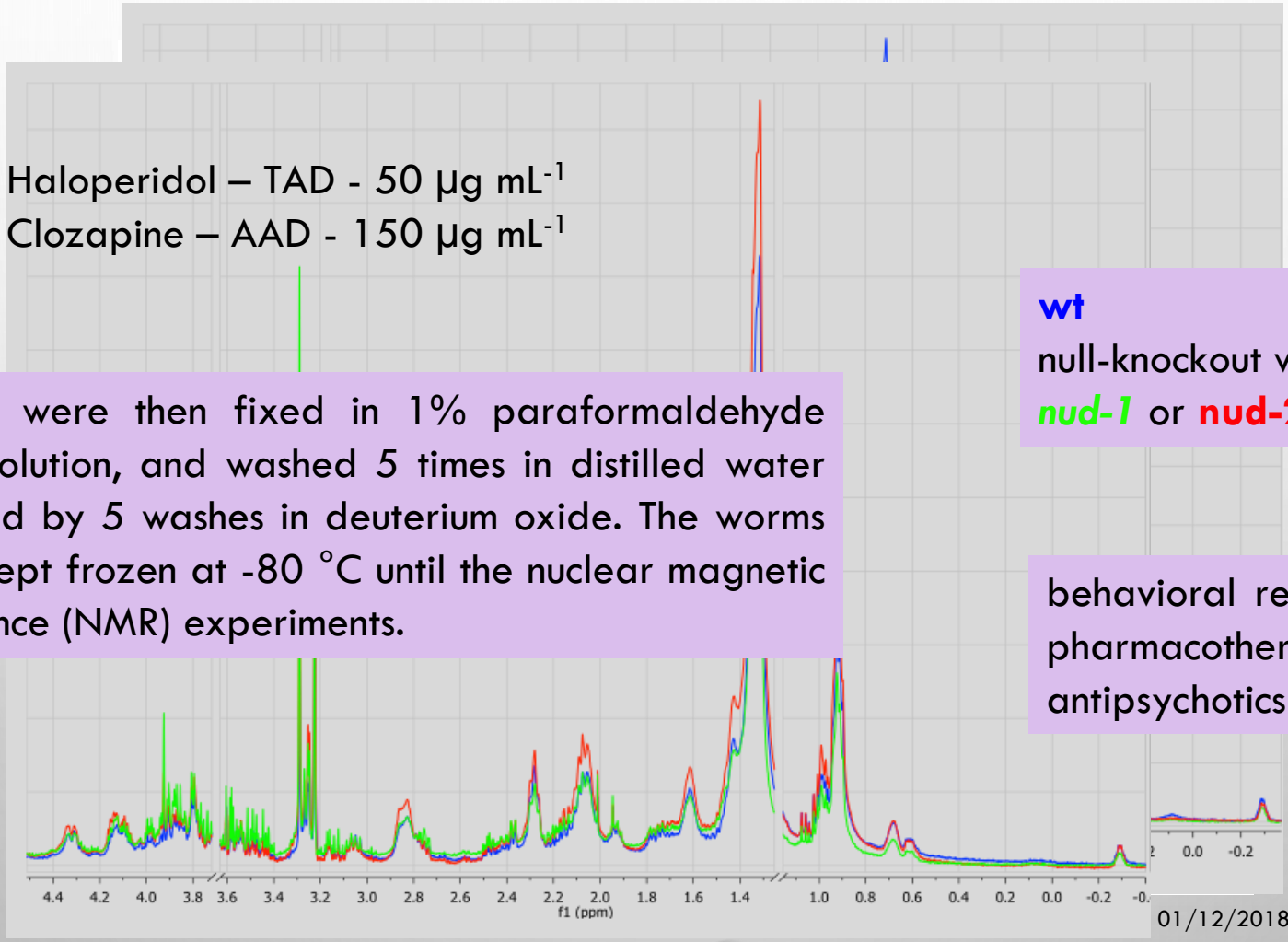






ANIMAL MODELS

Haloperidol – TAD - 50 µg mL^{-1}
Clozapine – AAD - 150 µg mL^{-1}



Worms were then fixed in 1% paraformaldehyde (PFA) solution, and washed 5 times in distilled water followed by 5 washes in deuterium oxide. The worms were kept frozen at -80 °C until the nuclear magnetic resonance (NMR) experiments.

wt
null-knockout worms for
nud-1 or **nud-2**

behavioral response to the pharmacotherapy with antipsychotics

nematode *Caenorhabditis elegans*

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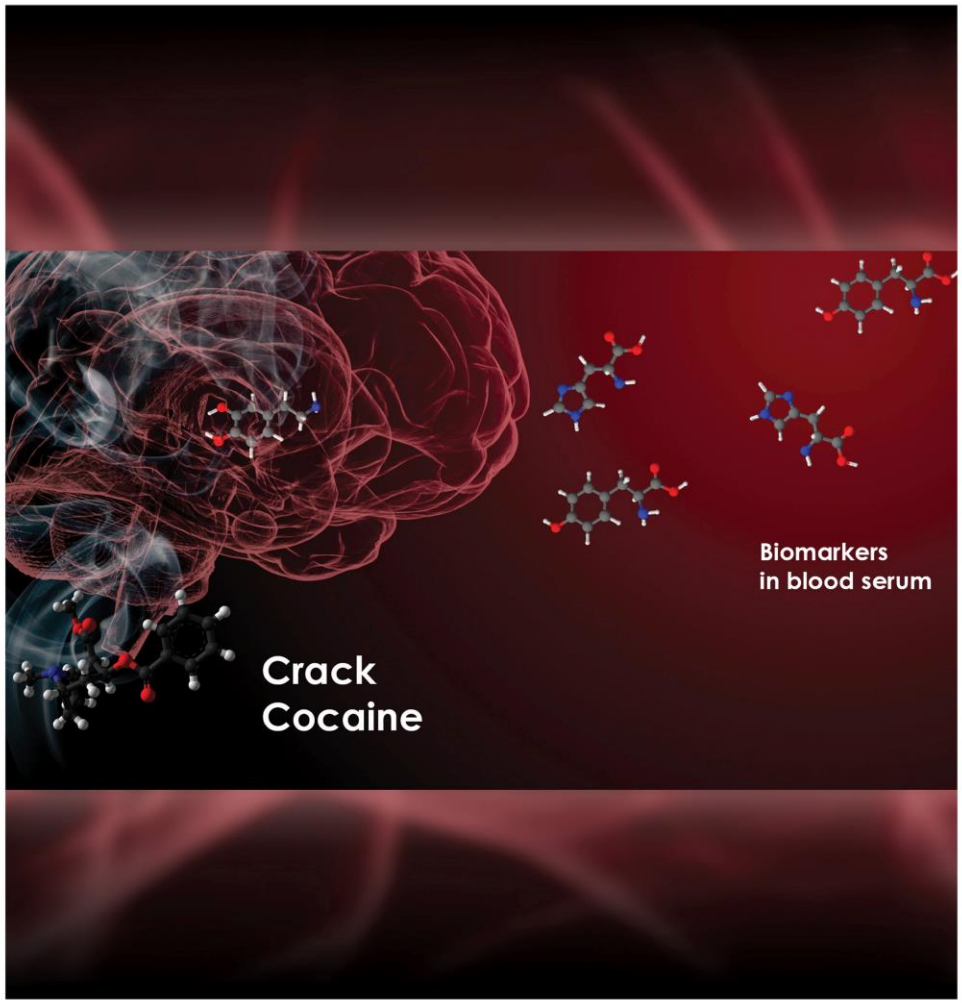
Journal of proteome research

Volume XX Number XX pubs.acs.org/jpr



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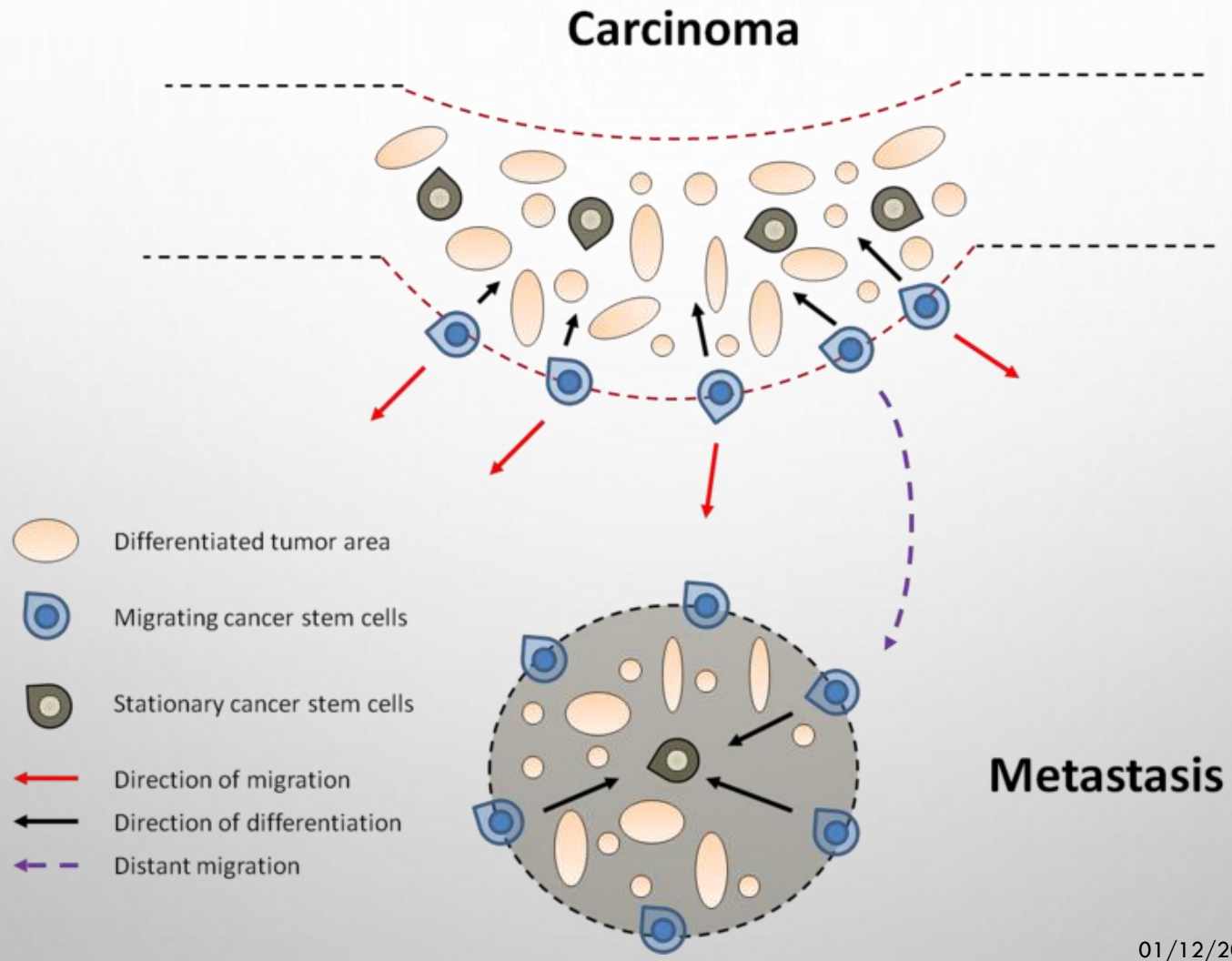
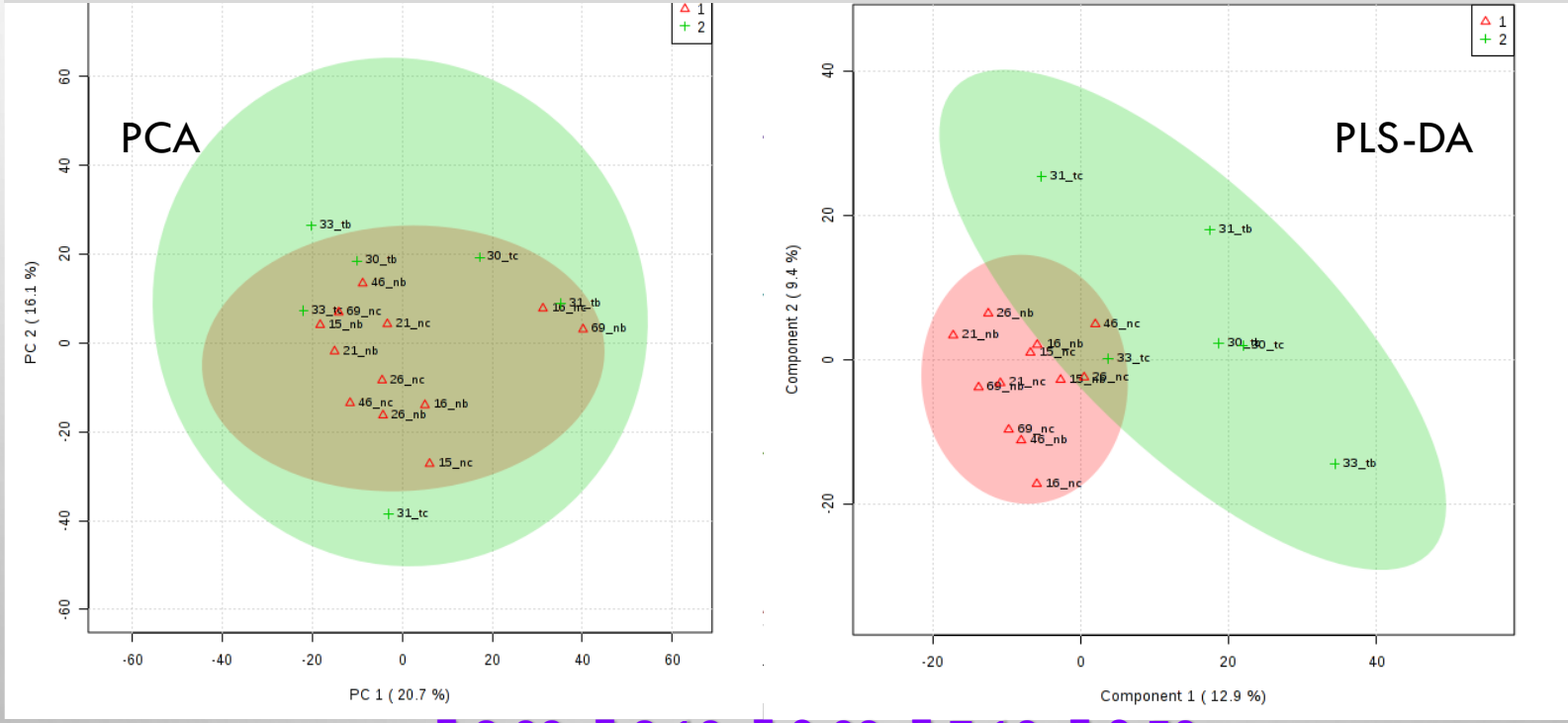
CANCER RESEARCH



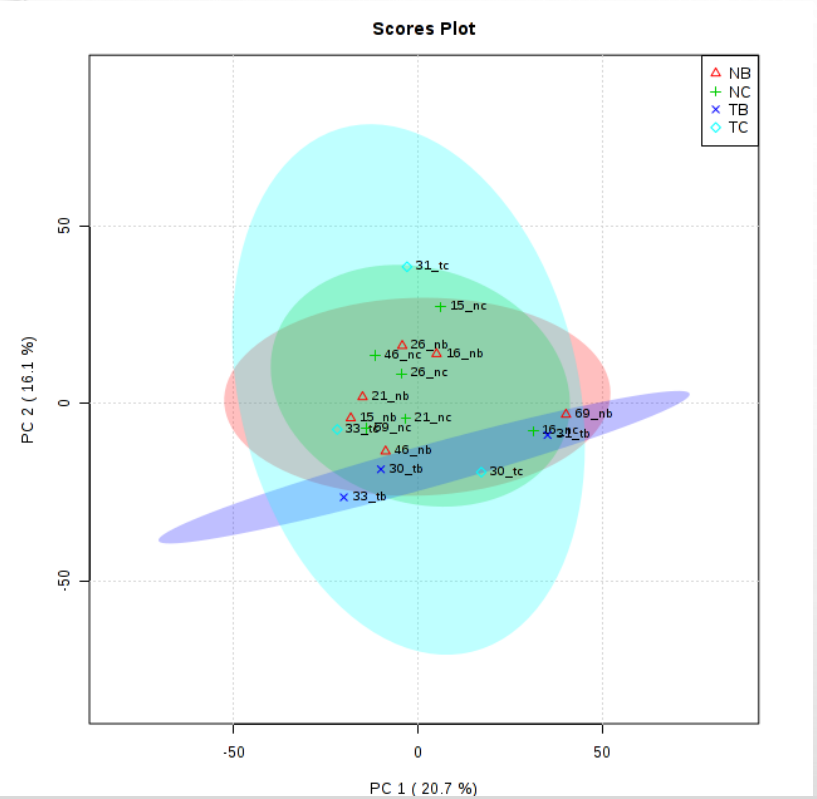
HEPATOBLASTOMA TISSUE METABOLOMIC PROFILING BY HR-MAS NMR

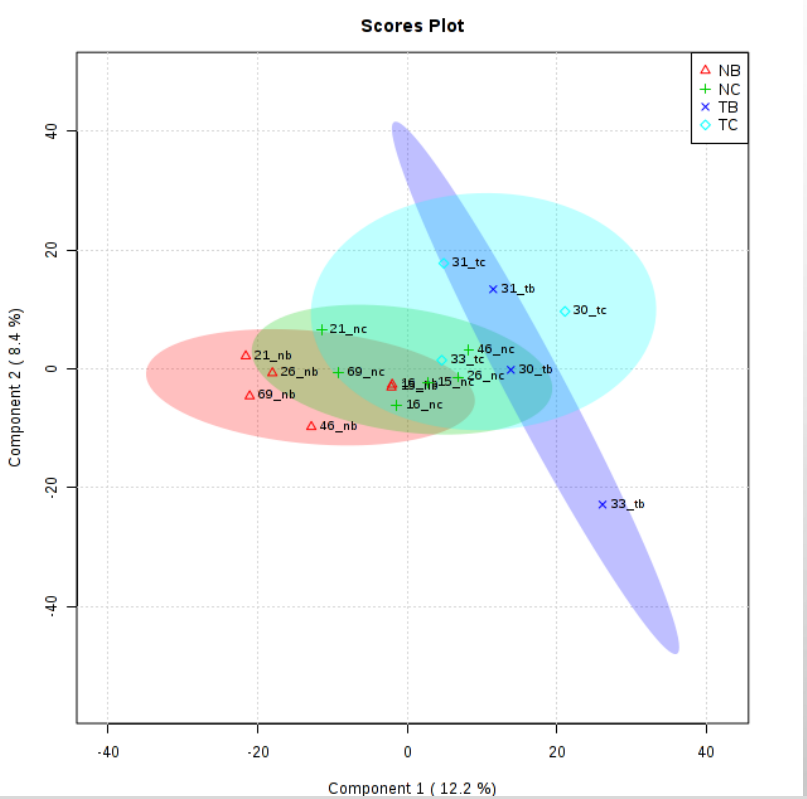
T_2-eddited ^1H NMR (CPMG) data of healthy (1, red) and tumor affected liver tissues (2, green).



δ 2.09, δ 2.10, δ 3.60, δ 7.10, δ 8.50

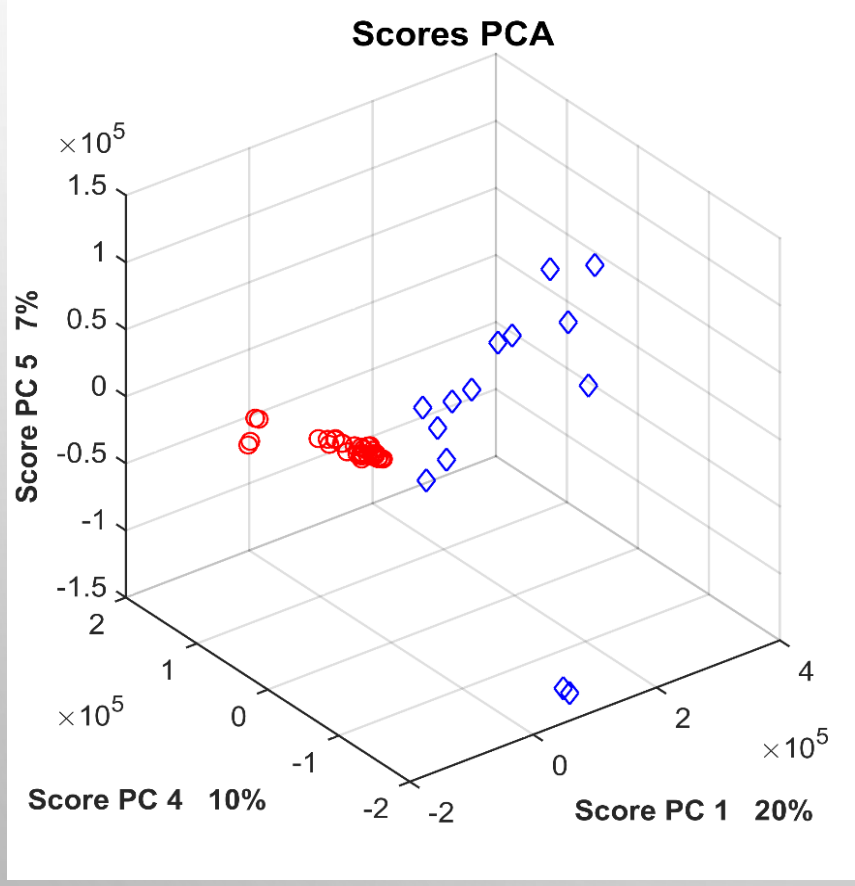
HEPATOBLASTOMA





Score-graphs of principal components analysis (PCA - left) and partial least squares discriminant analysis (PLS-DA - right) performed with the T_2-eddited ^1H NMR (CPMG) data of liver samples. Tumor Border (TB), Tumor Center (TC), Healthy Liver Border (NB), Healthy Liver Sample (NC).

OSTEOSARCOMA



Classification parameters obtained for PLS-DA model after LOOCV.

CPMG	WATERGATE
Sensitivity	Sensitivity
100.00	95.00
Specificity	Specificity
92.00	100.00
Accuracy	Accuracy
98.00	96.22

OSTEOSARCOMA

18 cases (9 females and 9 males) with samples from tumor and peripheral blood prior to any systemic treatment (chemotherapy or radiotherapy). Pediatric patients aged between 11 and 20 years (n = 15) and three patients were adults.

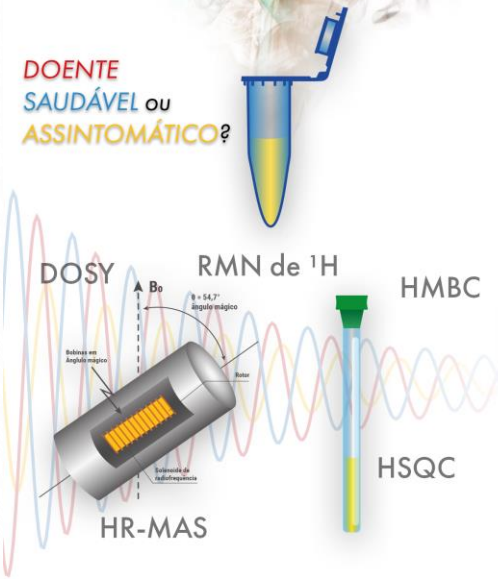
Alterations in the metabolism of methyl branched-chain fatty acids in VLDL and LDL are associated to CH_3 and CH_2 and lipids =CH- and -CH_2, as well as with some aromatic amino acids (Tyr, His, Phe).

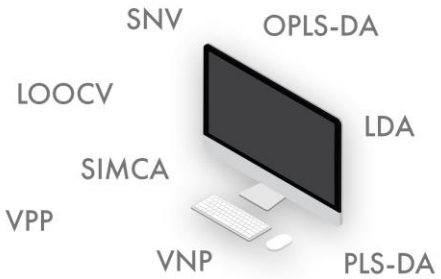
Lipids are not having just a passive lipid storage depot but could have a dynamic role in energy balance of the cells.

Somos metabólitos

Como a metabolômica e a espectroscopia de ressonância magnética nuclear podem ajudar a revolucionar o modo como doenças são diagnosticadas e monitoradas

DOENTE
SAUDÁVEL ou
ASSINTOMÁTICO?





Amostragem

Amostras biológicas de humanos, animais, bactérias ou plantas são coletadas e preparadas para posterior análise

Análise

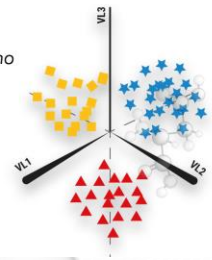
Técnicas de RMN são utilizadas para analisar as amostras

Bases de dados online

Os espectros obtidos são interpretados e associados a metabólitos por comparação com bases de dados

Quimiometria

Análises quimiométricas detectam diferenças ínfimas de concentrações de metabólitos chave, também conhecidos como biomarcadores, e separam as amostras dentre organismos doentes, saudáveis e assintomáticos



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Thank you!