Concepts of metabolomics

- Dr. Cheng Zhang
- Senior Researcher
- Royal Institute of Technology, KTH

Learning Objectives

You will learn about

Metabolomics (targeted and untargeted)

Analytical technologies

Metabolomics applications & limits

Main processing pipelines

Tips, pitfalls and traps

Outline

What is metabolomics?

Experimental Design

Data Generation / Analytical technologies

Applications and Limits

Preprocessing

Data cleaning / analysis

Outline

What is metabolomics?

Experimental Design

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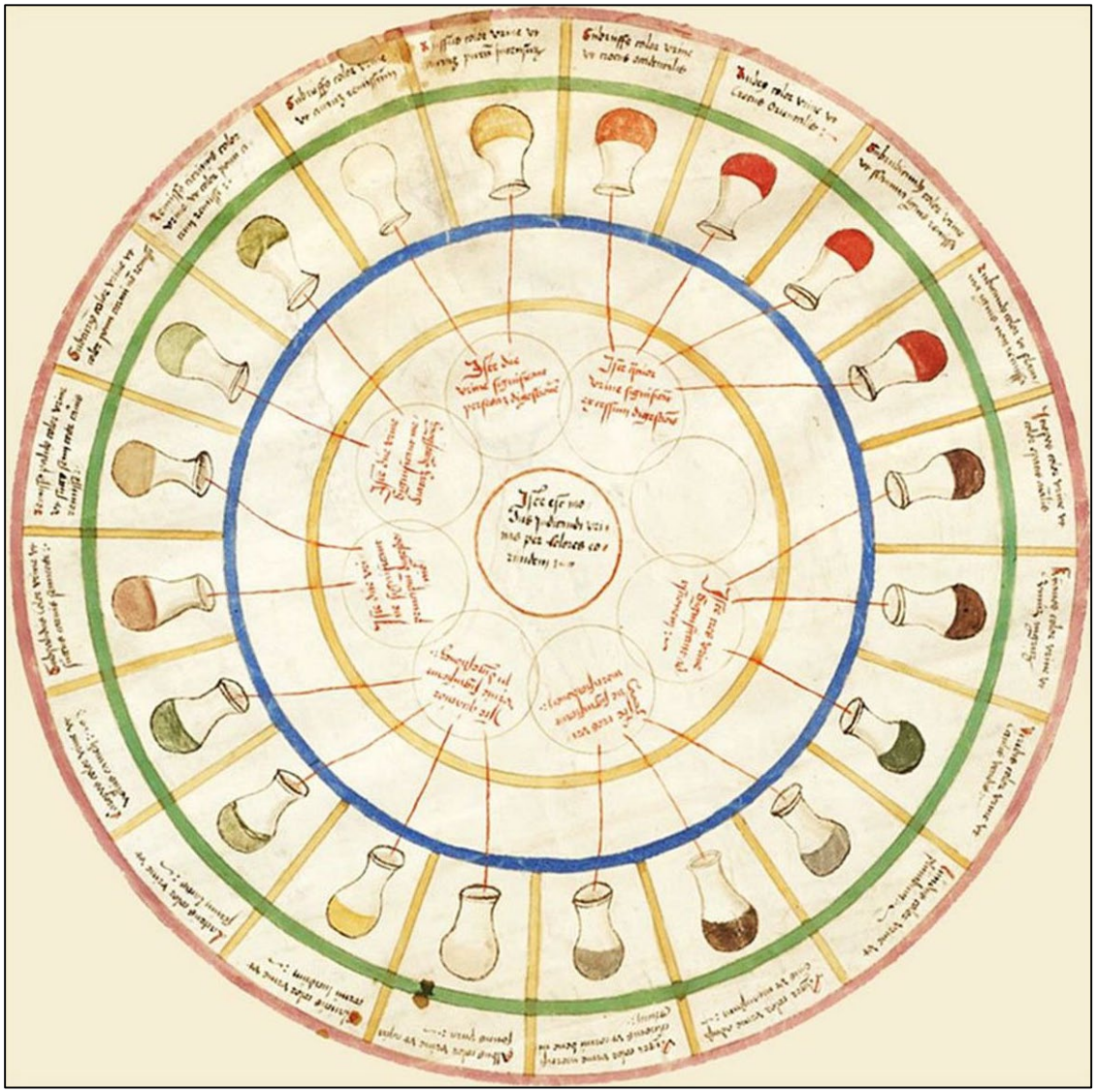
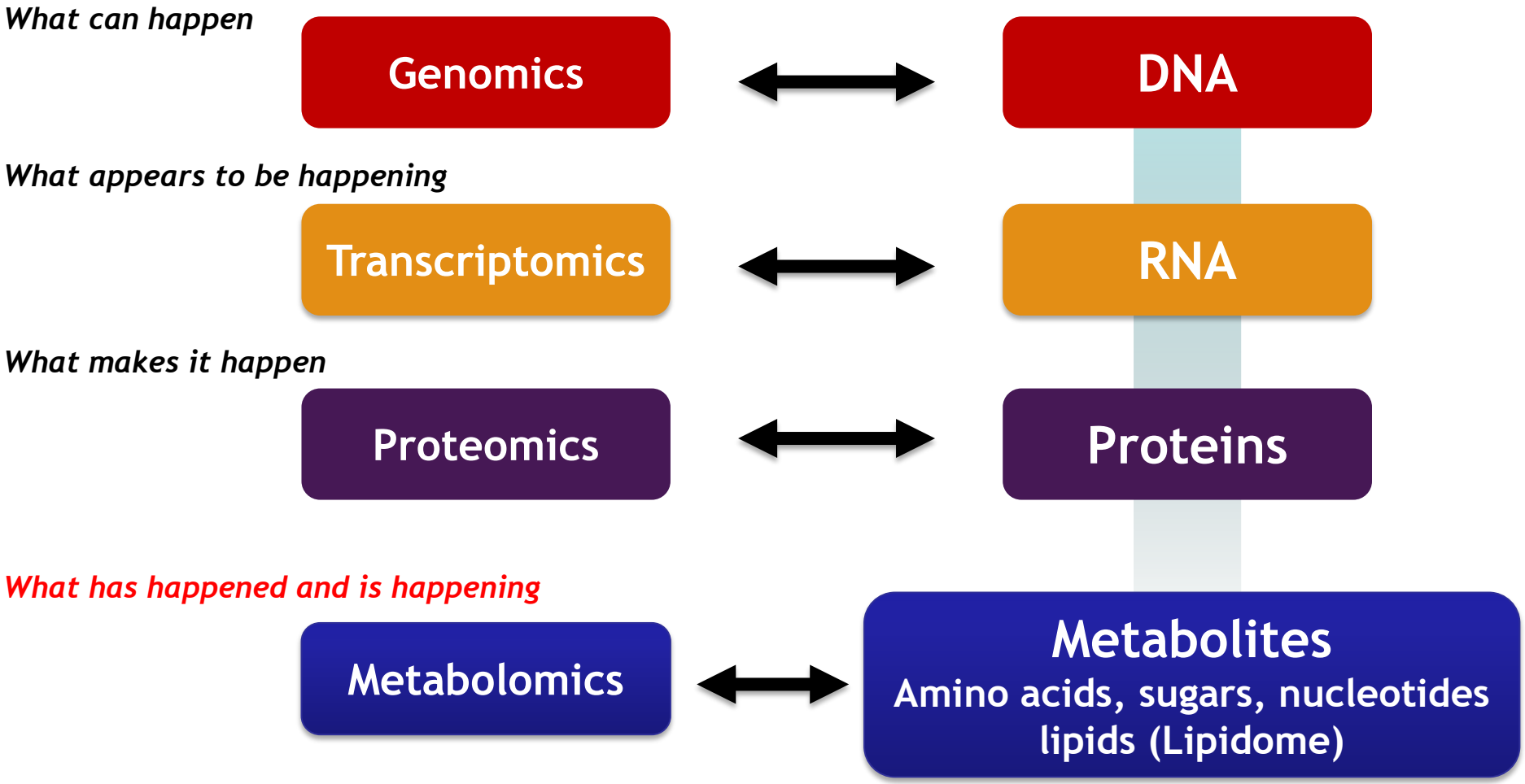
Data cleaning / analysis

The urine wheel



Metabolomics



What is a metabolite?

Metabolites:

- **Small molecules (<1500 Da)**
- **Ultimate support of the biological information**
- **Includes human & microbial products**
- **Endogenous metabolites: produced by the host organism**
- **Exogenous metabolites: not produced by the host organism**

Metabolome:

- **refers to the complete set of metabolites in a biological sample**

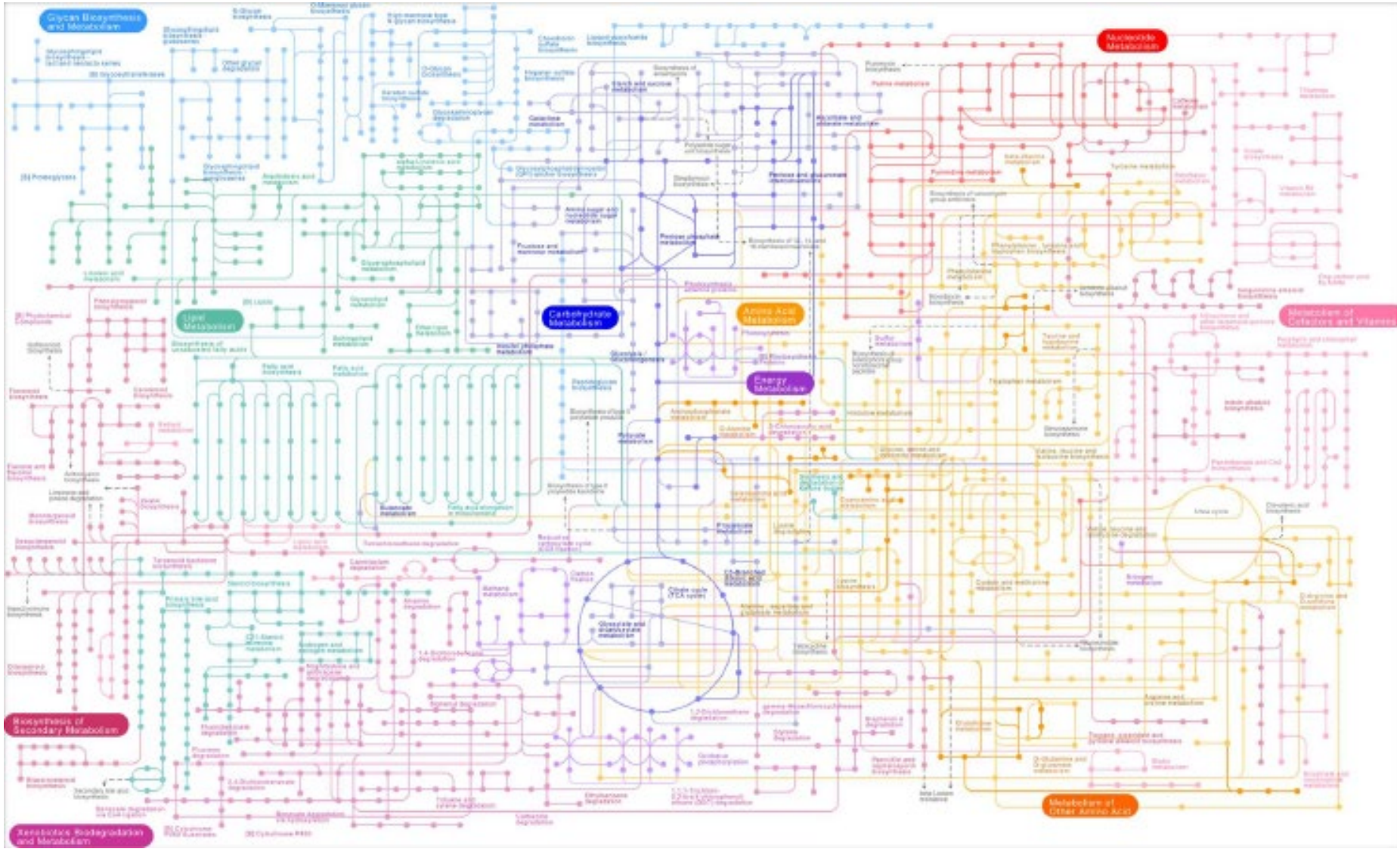
MetaboLomics / MetaboNomics:

➔ **Metabolic Profiling / Metabotyping**

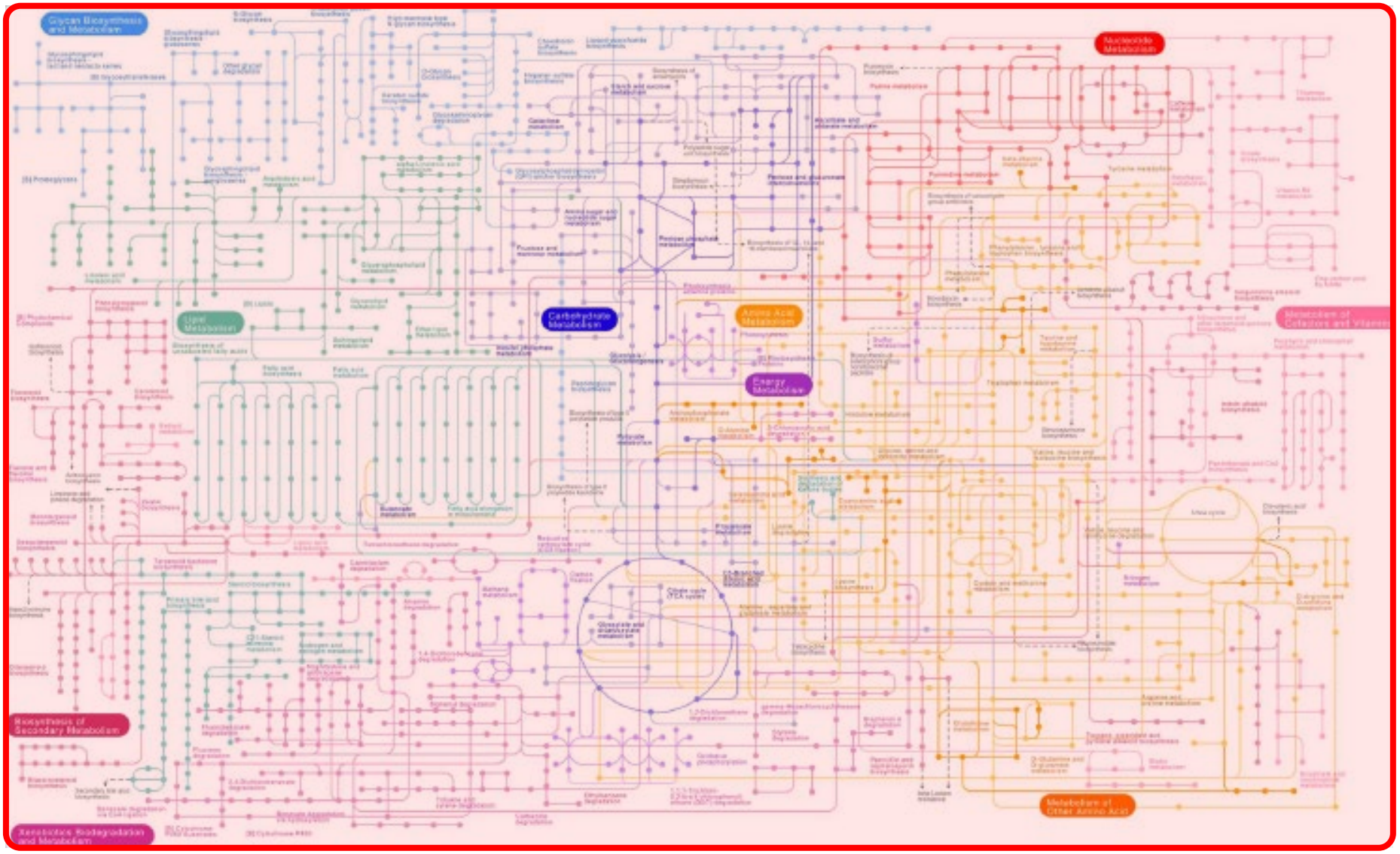
"the quantitative measurement of the metabolic ***responses*** of ***complex systems*** to a pathophysiological ***stimulus*** or genetic modification".

(Nicholson, J. K., et al 1999, Xenobiotica, 29, 1181-89.)

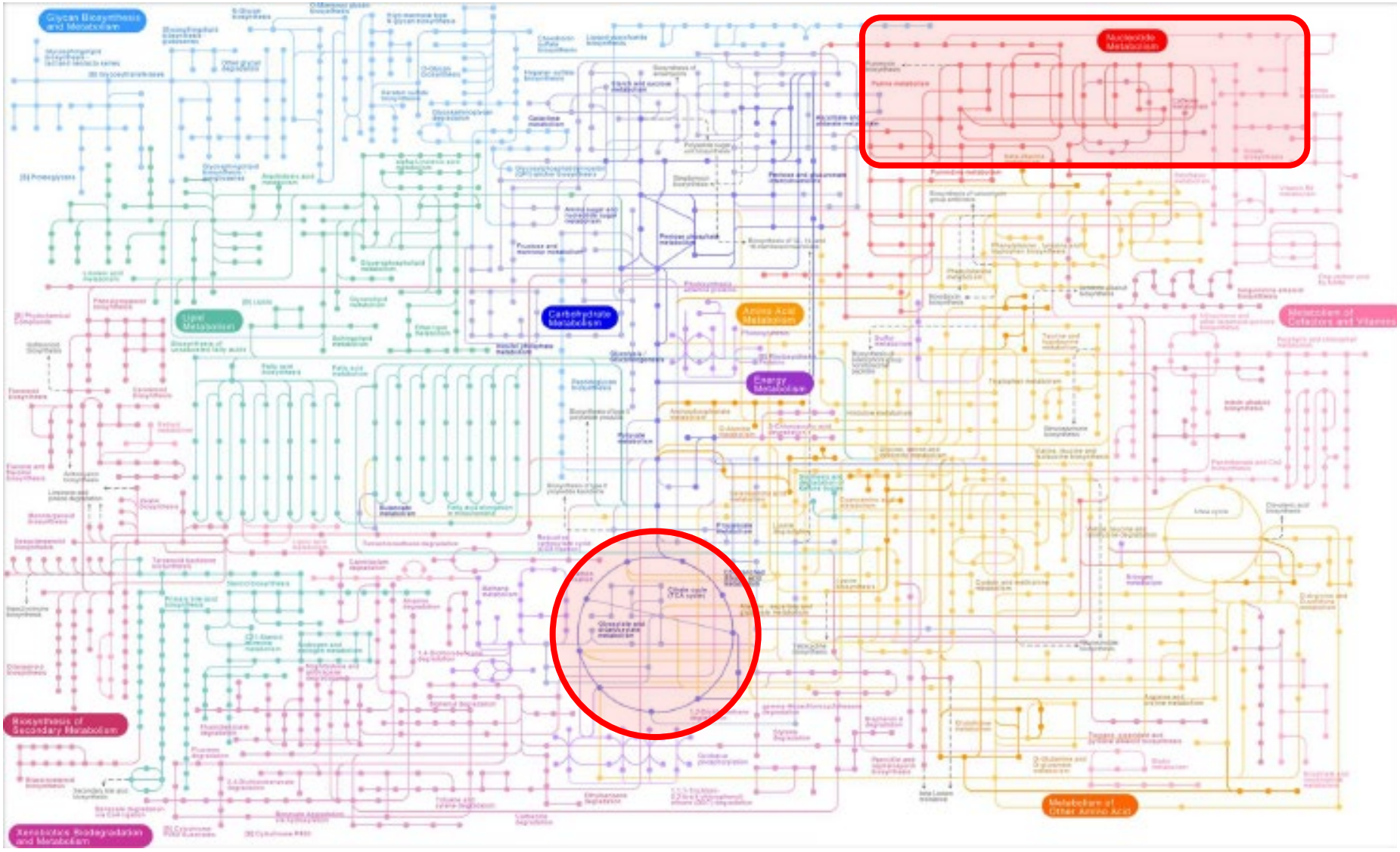
Metabolomics and metabolism



Untargeted metabolomics



Targeted metabolomics



Outline

What is metabolomics?

Experimental Design

Data Generation / Analytical technologies

Applications and Limits

Preprocessing

Data cleaning / analysis

Biological Information Generation

Experimental Design

"To consult the statistician after an experiment is finished is often merely to ask him to conduct a post-mortem examination. He can perhaps say what the experiment died of."

Sir Ronald Fisher (1938)

Experimental Design may help

1. Clear and precise study objective
2. Sample type and size
3. Sampling and sample preparation strategy
4. Number of samples / Biological - Analytical replicates
5. Analytical technology(ies)
6. Collection of meta-data (Categorical, continuous, ordinal…)
7. Confounding factors
8. Randomization
9. Data analysis strategies (univariate *vs*. multivariate)
10. Biological Interpretation and insights
11. Validation (Biological/Analytical)
12. …Name it

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What is metabolomics?

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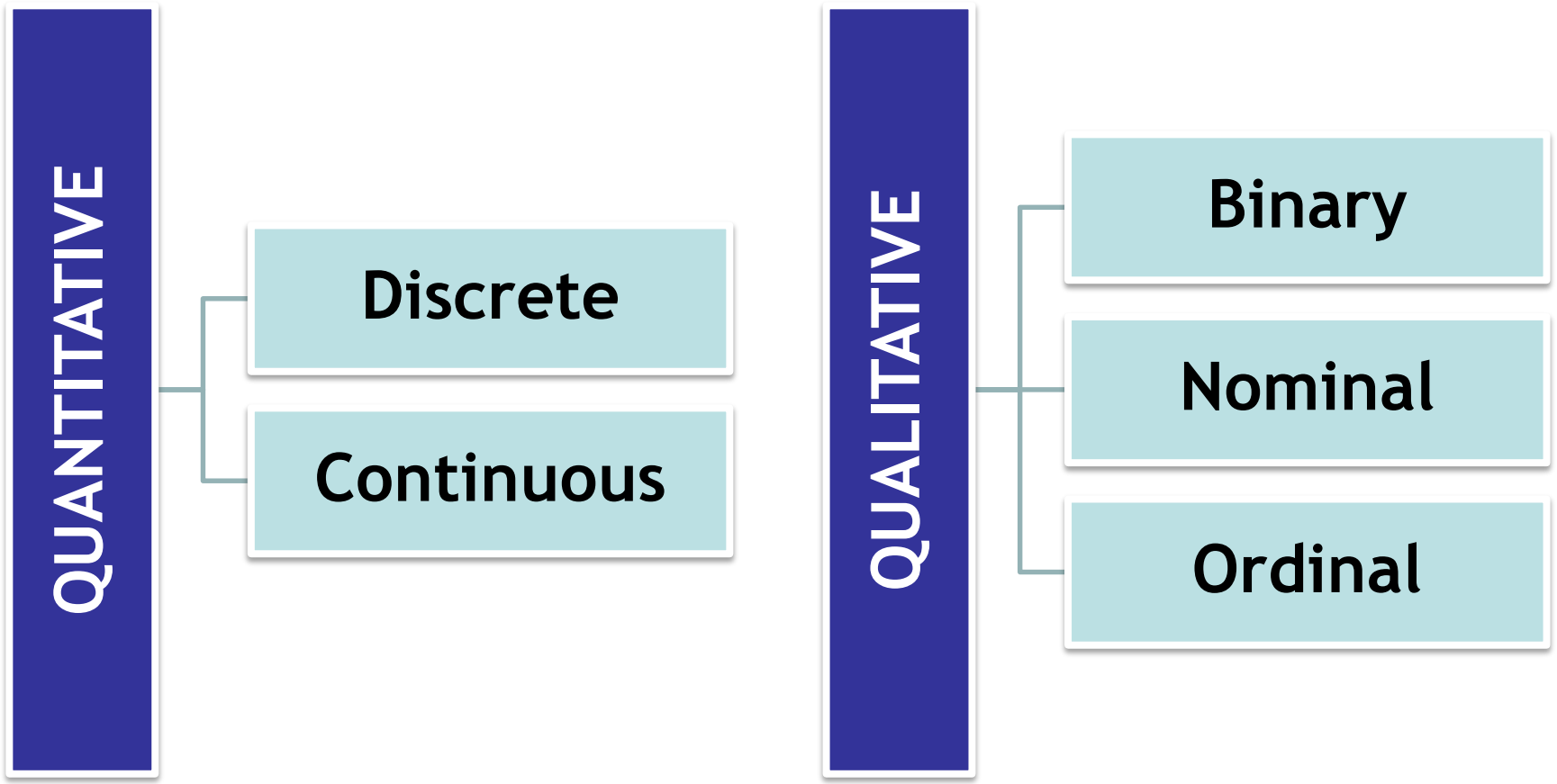
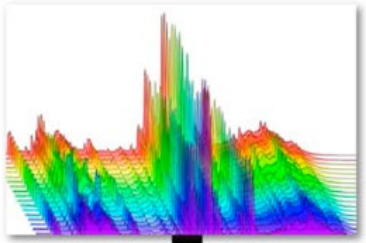
Know your data



Metabolomics



Quantitative approach

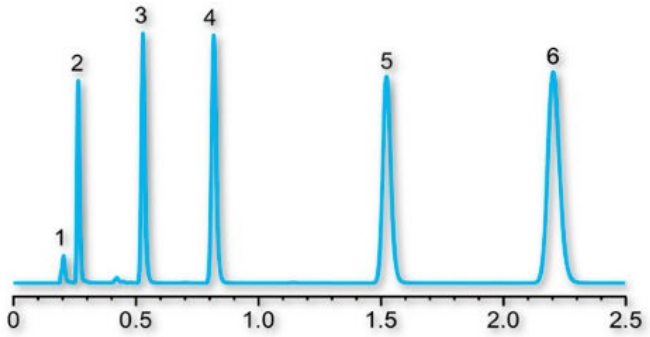
Targeted approach

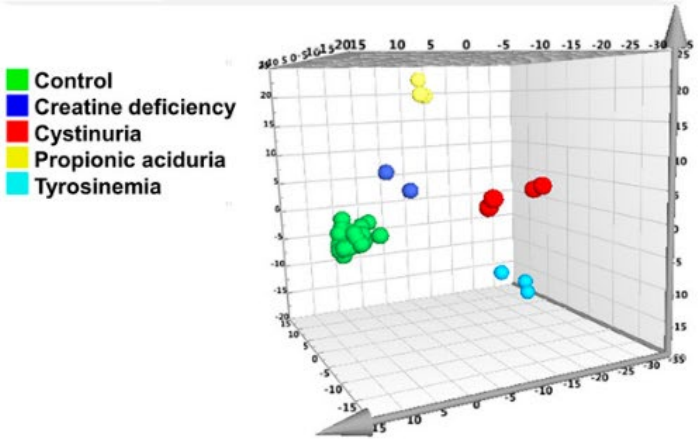
(Set of Biomarkers Quantitation)

Chemometric approach

Untargeted approach

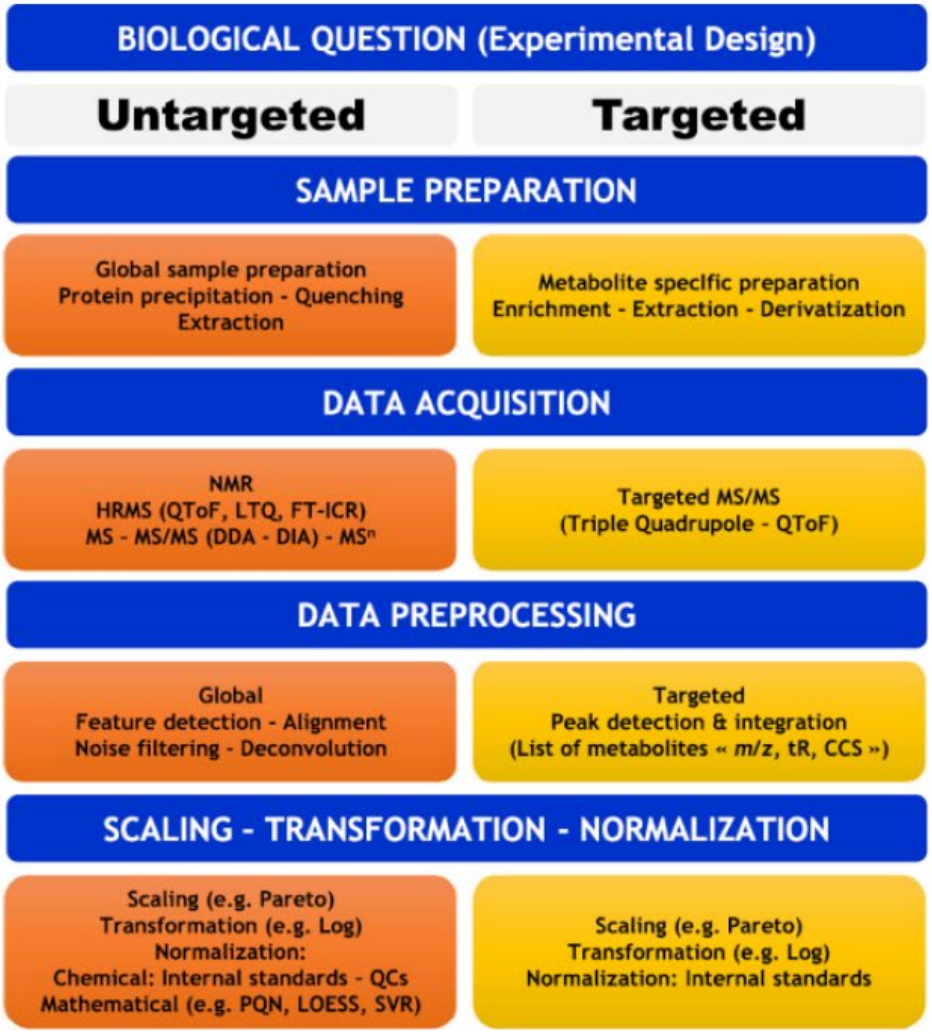
(Global Metabolic Fingerprints)

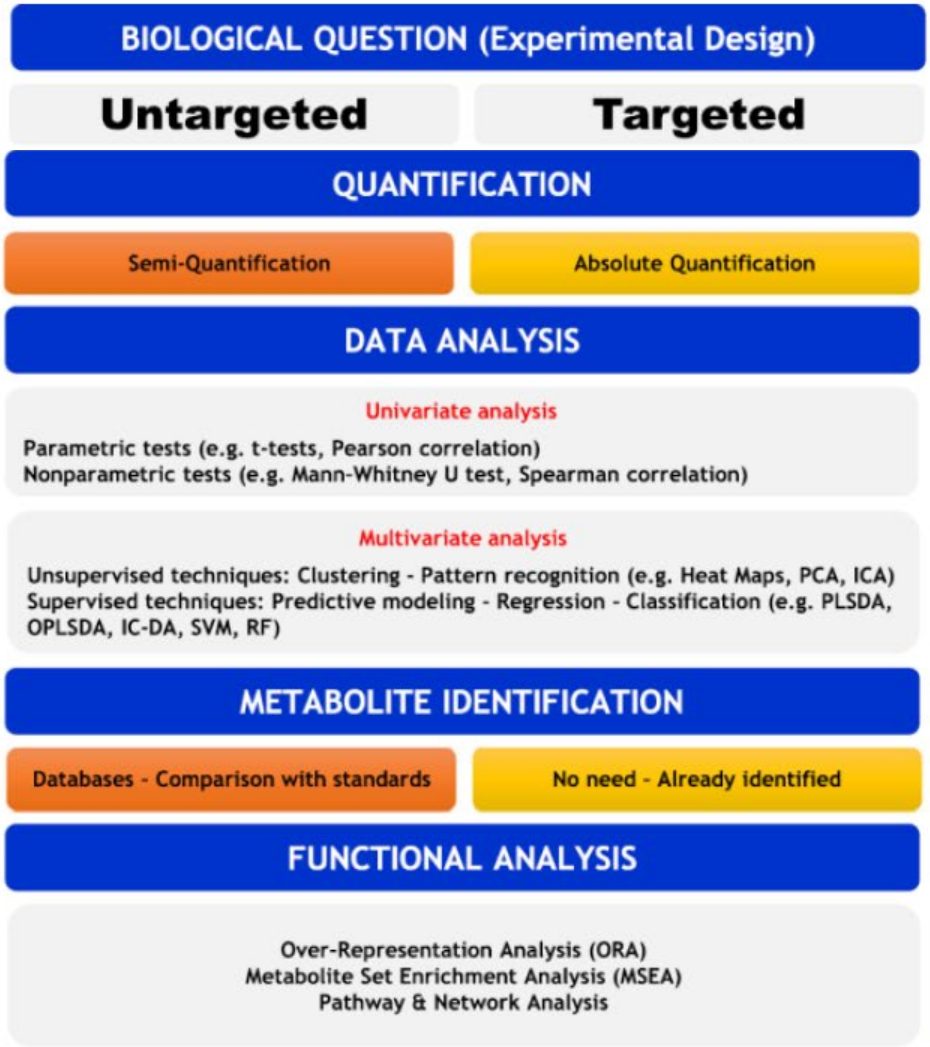




Tebani et al. IJMS. 2016

Experimental Design





Tebani et al. IJMS. 2017

Metabolomics worflow

1

Question « Biology »

Experimental design

Sampling

Sample preparation

2

Instrumentation

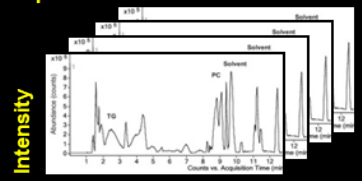
Data Acquisition

Data preprocessing

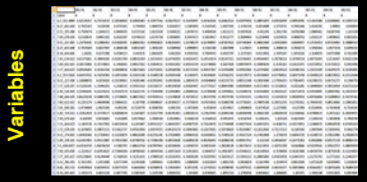
Data Analysis (Modeling)

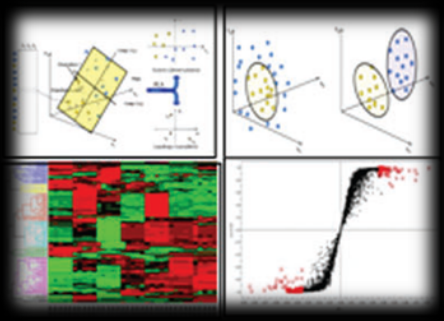
MS / NMR

Samples



(Filtration, detection, alignement, normalization)





3

Discriminant variables annotation

Metabolite Identification

Biological Insights

Putative identification

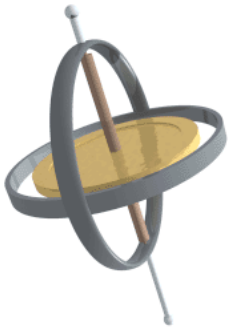
Databases

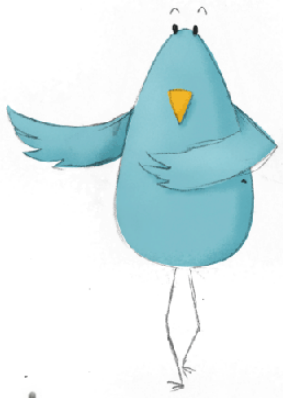
Comparaison with standards

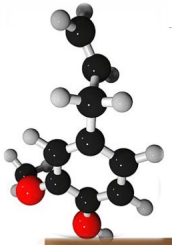
Pathway analysis
Network analysis

Tebani, A. *IJMS.* 2016, *17*, 1167

Biological Information Extraction







NMR spectroscopy

Mass spectrometry

Biological Information Extraction

NMR spectroscopy

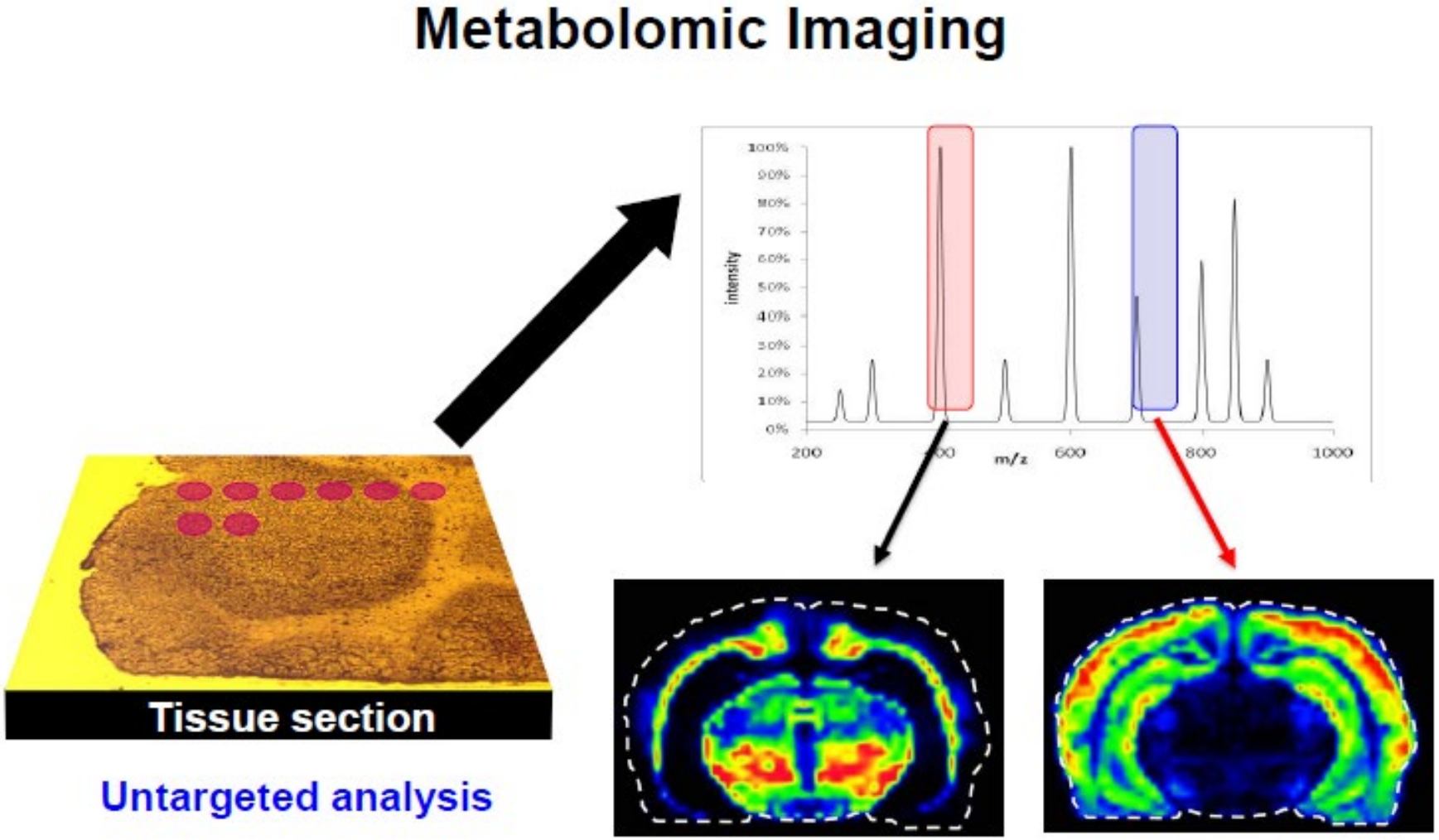
- Tissues, biofluids and extracts
- Interaction of spin active nuclei (^{1}H, ^{13}C, ^{31}P) with electromagnetic fields gives molecular information
- Non-destructive
  Cross-instrument robustness

Mass spectrometry

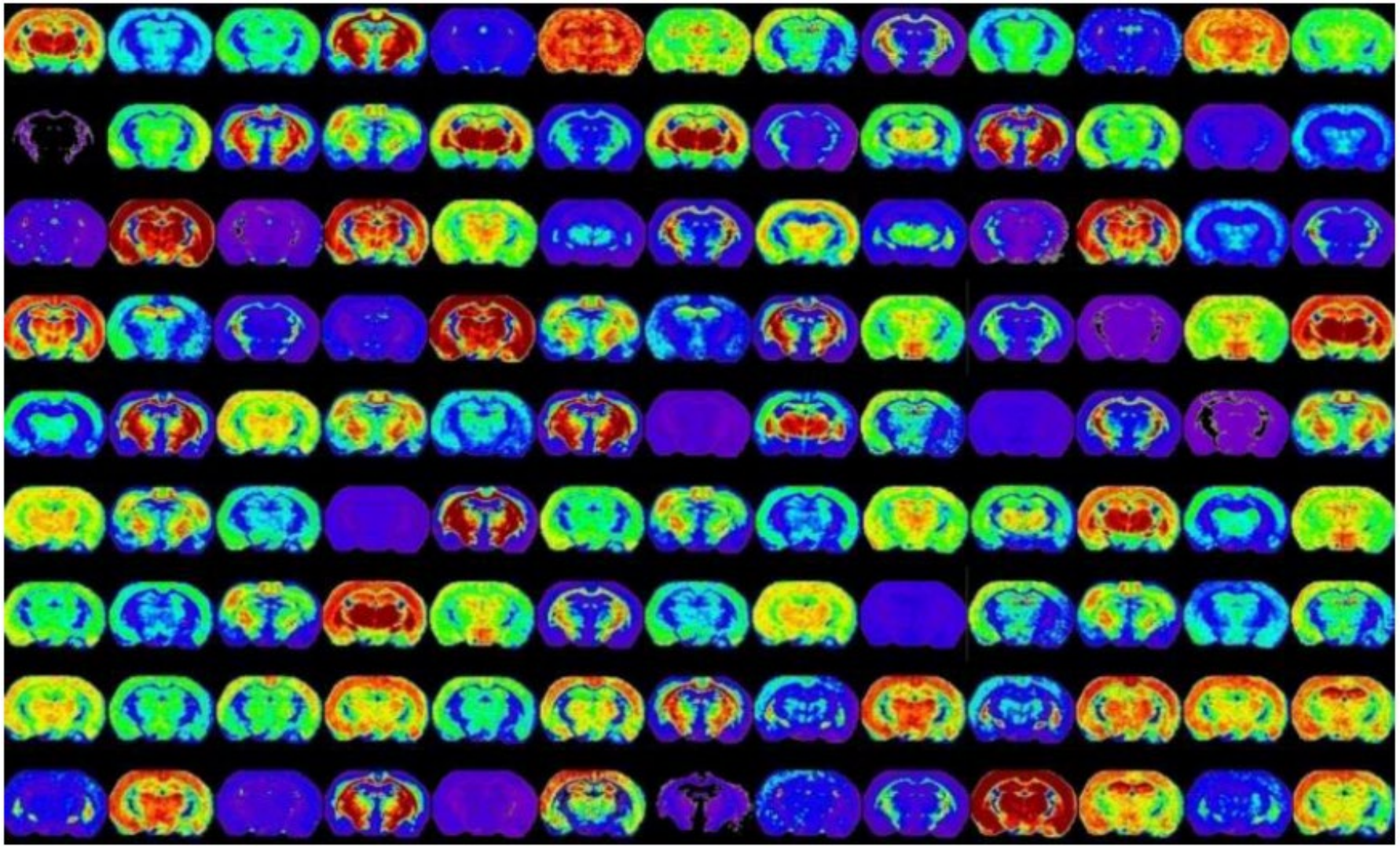
- Tissues, biofluids and extracts
- Mass to charge ratio *(m/z)*
- Sensitivity
  Higher metabolome coverage

Tebani et al. JIMD. 2016

Metabolomics-based imaging



Metabolomics-based imaging



Journal of Mass Spectrometry
Volume 46, Issue 2, pages 209-222, 24 JAN 2011 DOI: 10.1002/jms.1876
http://onlinelibrary.wiley.com/doi/10.1002/jms.1876/full#fig1

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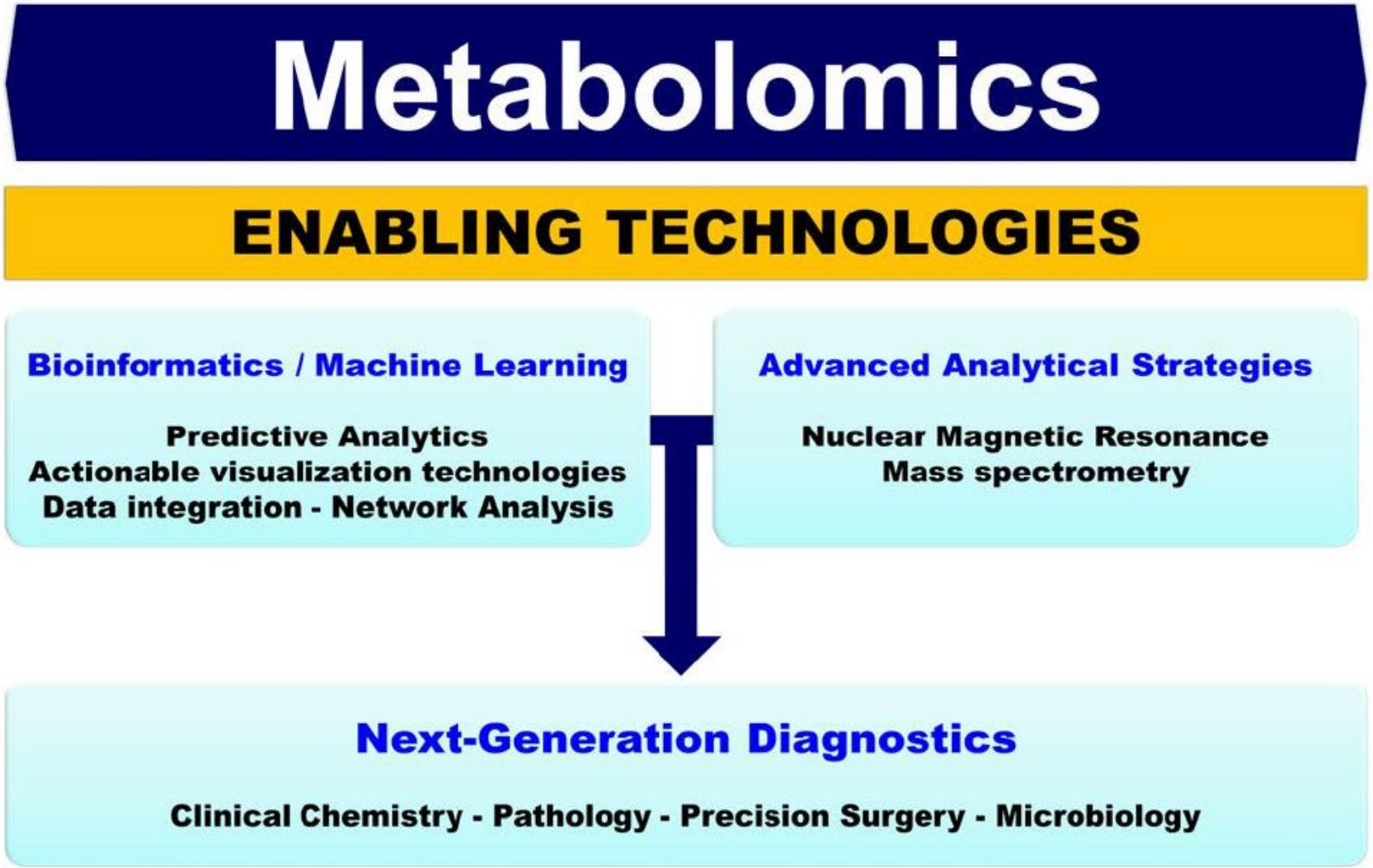
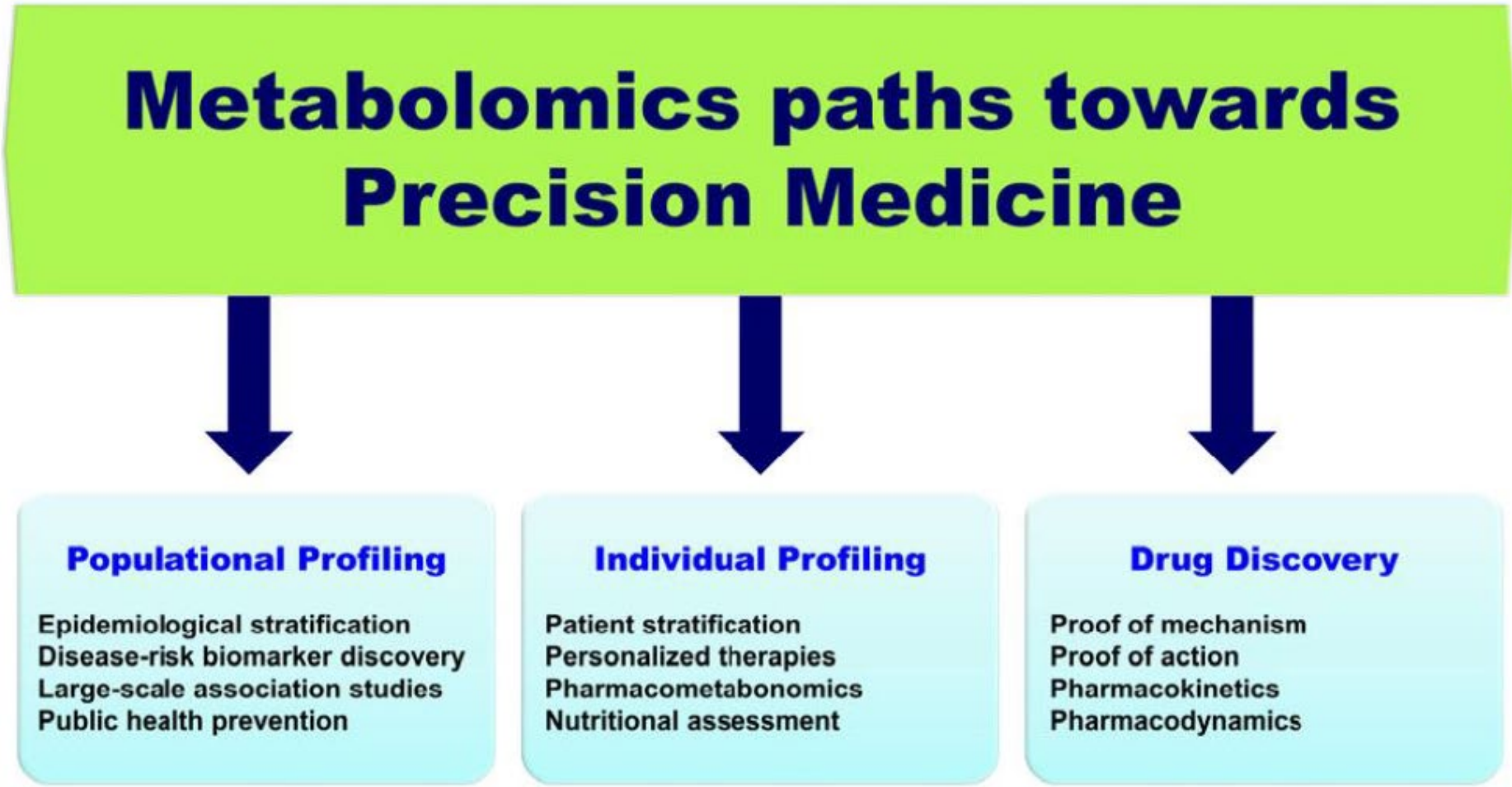
Applications



Bekri S, *Expert Review of Precision Medicine and Drug Development* 1.6 (2016): 517-532
https://doi.org/10.1080/23808993.2016.1273067

Applications



Bekri S, *Expert Review of Precision Medicine and Drug Development* 1.6 (2016): 517-532
https://doi.org/10.1080/23808993.2016.1273067

Applications

Article

molecular systems biology

The gut microbiota modulates host amino acid and glutathione metabolism in mice

Adil Mardinoglu[1,2,†,*], Saeed Shoaie[1,†], Mattias Bergentall[3,4], Pouyan Ghaffari[3], Cheng Zhang[2], Erik Larsson[3,4], Fredrik Bäckhed[3,4] & Jens Nielsen[1,2]

Host-Microbiota intecations

Pharmacometabonomic Investigation of Dynamic Metabolic Phenotypes Associated with Variability in Response to Galactosamine Hepatotoxicity

Muireann Coen,*[,†] Françoise Goldfain-Blanc,[‡] Gaëlle Rolland-Valognes,[§] Bernard Walther,[||] Donald G. Robertson,[⊥] Elaine Holmes,[†] John C. Lindon,[†] and Jeremy K. Nicholson*[,†]

Responder Non-responder Prediction

Int. J. Mol. Sci. 2016, *17*(7), 1167; doi:10.3390/ijms17071167 Open Access

Review

Clinical Metabolomics: The New Metabolic Window for Inborn Errors of Metabolism Investigations in the Post-Genomic Era

Abdellah Tebani [1,2,3] ✉, Lenaig Abily-Donval [2,4] ✉, Carlos Afonso [3] ✉, Stéphane Marret [2,4] ✉ and Soumeya Bekri [1,2,*] ✉

Inherited Metabolic Diseases

An Integrative Approach for Identifying a Metabolic Phenotype Predictive of Individualized Pharmacokinetics of Tacrolimus

PB Phapale[1,2], S-D Kim[2], HW Lee[1,2], M Lim[1,2], DD Kale[1,2], Y-L Kim[2,3], J-H Cho[4], D Hwang[4] and Y-R Yoon[1,2]

Human Drug Pharmacokinetics

RESEARCH ARTICLE

CANCER DIAGNOSTICS

Intraoperative Tissue Identification Using Rapid Evaporative Ionization Mass Spectrometry

Júlia Balog,[1*] László Sasi-Szabó,[2*] James Kinross,[3,4] Matthew R. Lewis,[3] Laura J. Muirhead,[3,4] Kirill Veselkov,[3] Reza Mirnezami,[4] Balázs Dezső,[5] László Damjanovich,[2] Ara Darzi,[4] Jeremy K. Nicholson,[3†] Zoltán Takáts[3†]

Pathology and Cancer

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-019-08936-1 OPEN

Assessing the causal association of glycine with risk of cardio-metabolic diseases

Laura B.L. Wittemans[1], Luca A. Lotta[1], Clare Oliver-Williams[2,3], Isobel D. Stewart[1], Praveen Surendran[2], Savita Karthikeyan[2], Felix R. Day[1], Albert Koulman[1,4], Fumiaki Imamura[1], Lingyao Zeng[5,6], Jeanette Erdmann[7,8,9], Heribert Schunkert[5,6], Kay-Tee Khaw[10], Julian L. Griffin[11], Nita G. Forouhi[1], Robert A. Scott[1], Angela M. Wood[2], Stephen Burgess[2,12], Joanna M.M. Howson[2], John Danesh[2,13], Nicholas J. Wareham[1], Adam S. Butterworth[2] & Claudia Langenberg[1]

Large-scale epidemiological profiling

Metabolomics-based imaging

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Science News ... from universities, journals, and other resea

'Intelligent Knife' Tells Surgeon Which Tissue Is Cancerous

July 17, 2013 — Scientists have developed an "intelligent knife" that can tell surgeons immediately whether the tissue they are cutting is cancerous or

Related Topics

Health & Medicine Article

RESEARCH ARTICLE

CANCER DIAGNOSTICS

Intraoperative Tissue Identification Using Rapid Evaporative Ionization Mass Spectrometry

Júlia Balog,[1]* László Sasi-Szabó,[2]* James Kinross,[3,4] Matthew R. Lewis,[3] Laura J. Muirhead,[3,4] Kirill Veselkov,[3] Reza Mirnezami,[4] Balázs Dezső,[5] László Damjanovich,[2] Ara Darzi,[4] Jeremy K. Nicholson,[3†] Zoltán Takáts[3†]

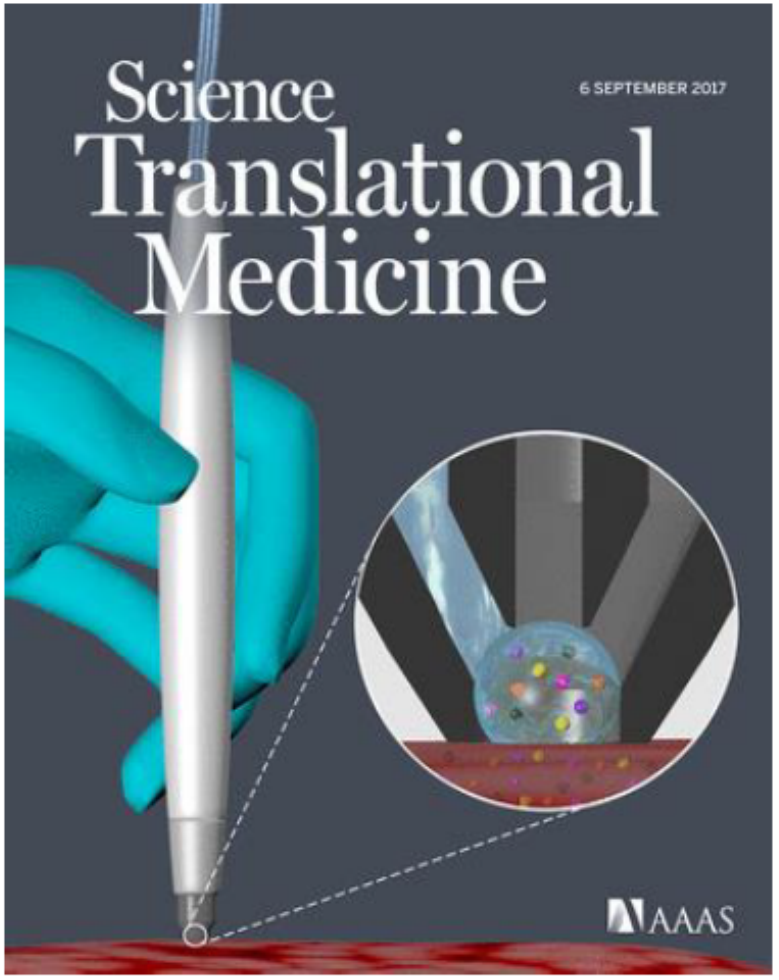
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER DIAGNOSTICS

Nondestructive tissue analysis for ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system

Jialing Zhang,[1] John Rector,[1,2] John Q. Lin,[1] Jonathan H. Young,[1] Marta Sans,[1] Nitesh Katta,[2] Noah Giese,[1] Wendong Yu,[3] Chandandeep Nagi,[3] James Suliburk,[4] Jinsong Liu,[5] Alena Bensussan,[1] Rachel J. DeHoog,[1] Kyana Y. Garza,[1] Benjamin Ludolph,[1] Anna G. Sorace,[6] Anum Syed,[2] Aydin Zahedivash,[2] Thomas E. Milner,[2] Livia S. Eberlin[1]*

Real time Metabolomics: Precision Surgery

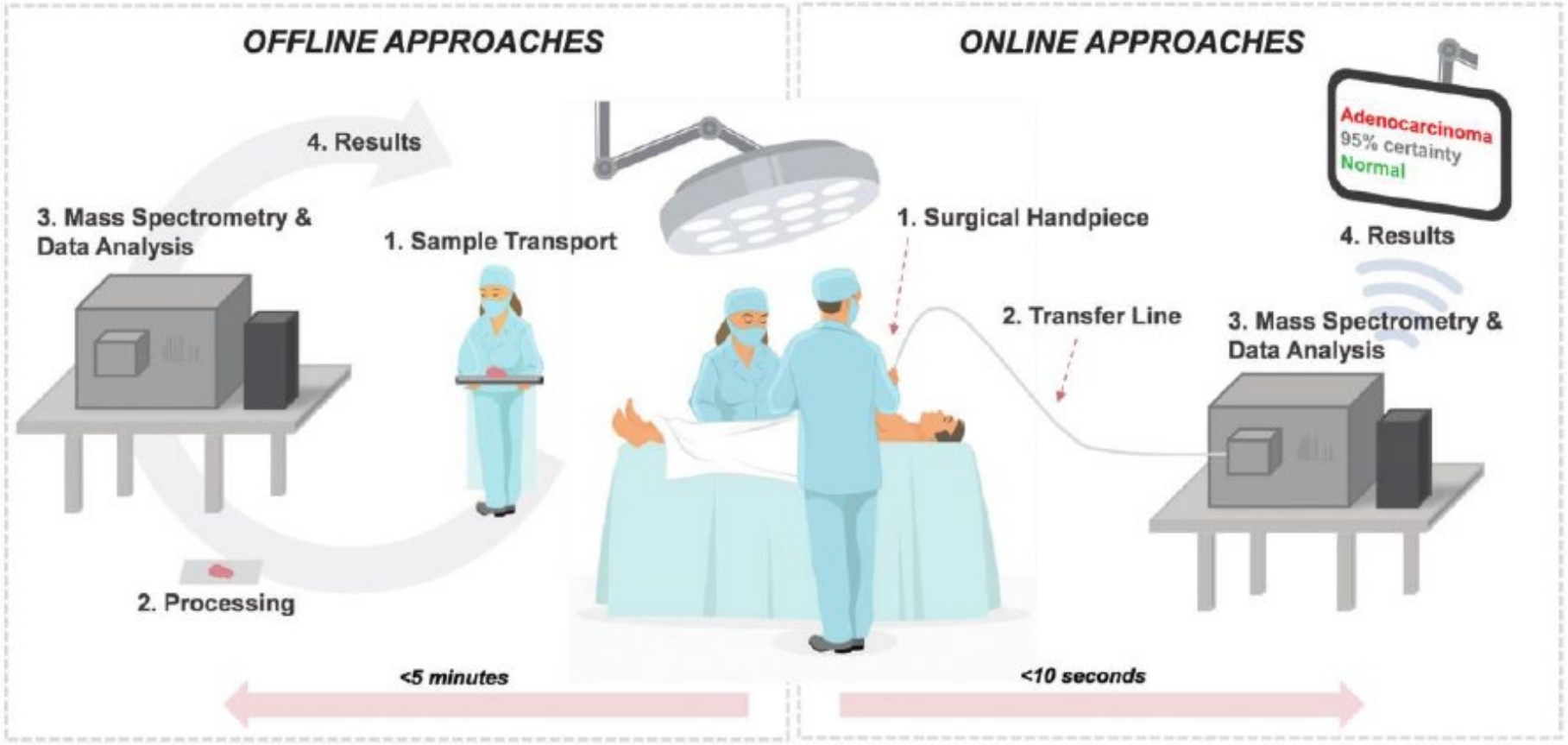


Metabolomics-based imaging

Sci Transl Med 17 July 2013: Vol. 5, Issue 194, p. 194ra93

Metabolomics-based imaging

Real time Metabolomics: Precision Surgery



Demian R. Ifa *et al*. Clin Chem 62:1 (2016)

Sci Transl Med 17 July 2013: Vol. 5, Issue 194, p. 194ra93

Limits

Metabolite Identification are the main bottlenecks of metabolomics for large adoption in both translational and clinical context.

Lack of standardized **annotation** of the metabolome is important for functional analysis and integration with other omics through GEMs

More **absolute quantification** of metabolites is needed (targeted and untargeted) to achieve reliability and robustness

Standardization and Harmonization is a prerequisite for large adoption

Miniaturization will enhance high-throuput

Automation, Data Visualization and Clinical Actionability at different stages, instrument-, pre- and post-analytic levels including data processing, integration and interpretation are very important issues for large clinical adoption of any diagnostic innovation

Outline

What is metabolomics?

Experimental Design

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Applications and Limits

Preprocessing

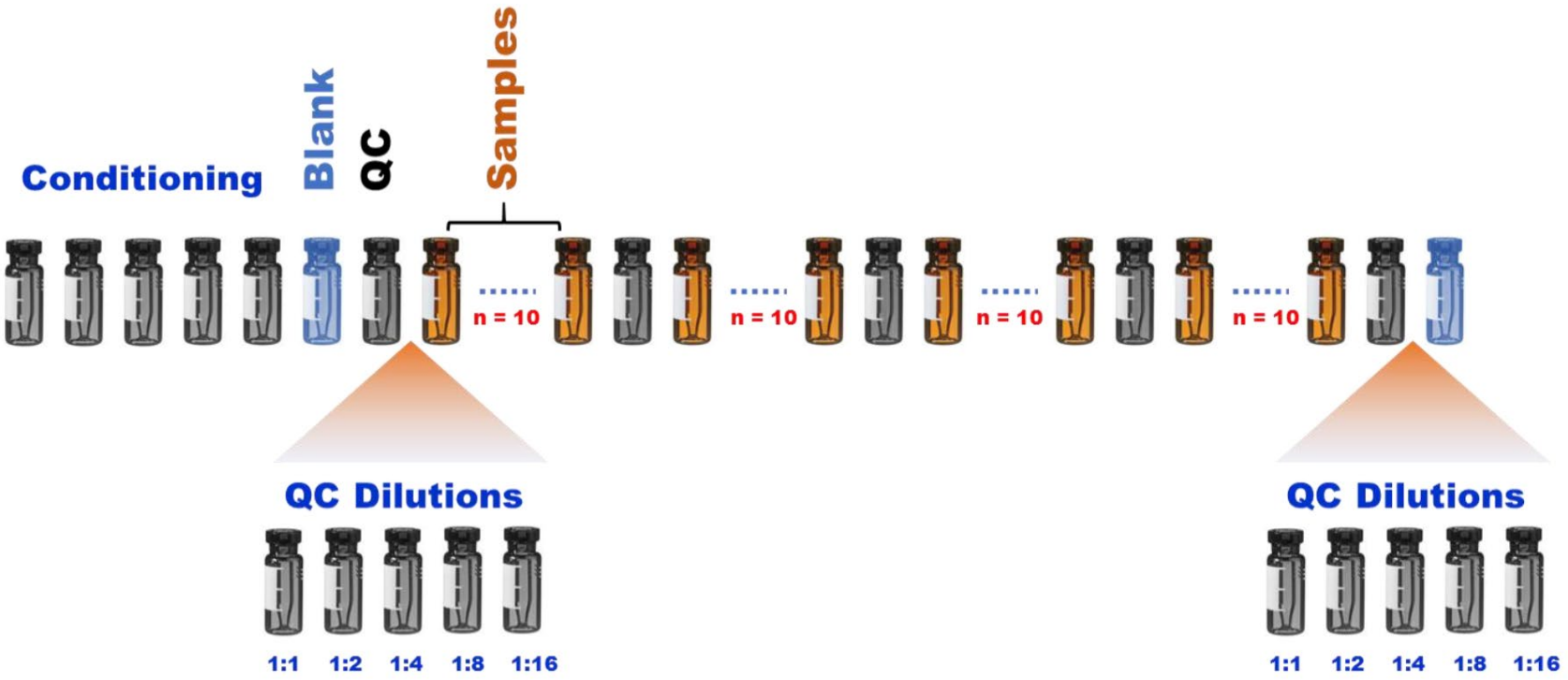
Data cleaning / analysis

Data preprocessing

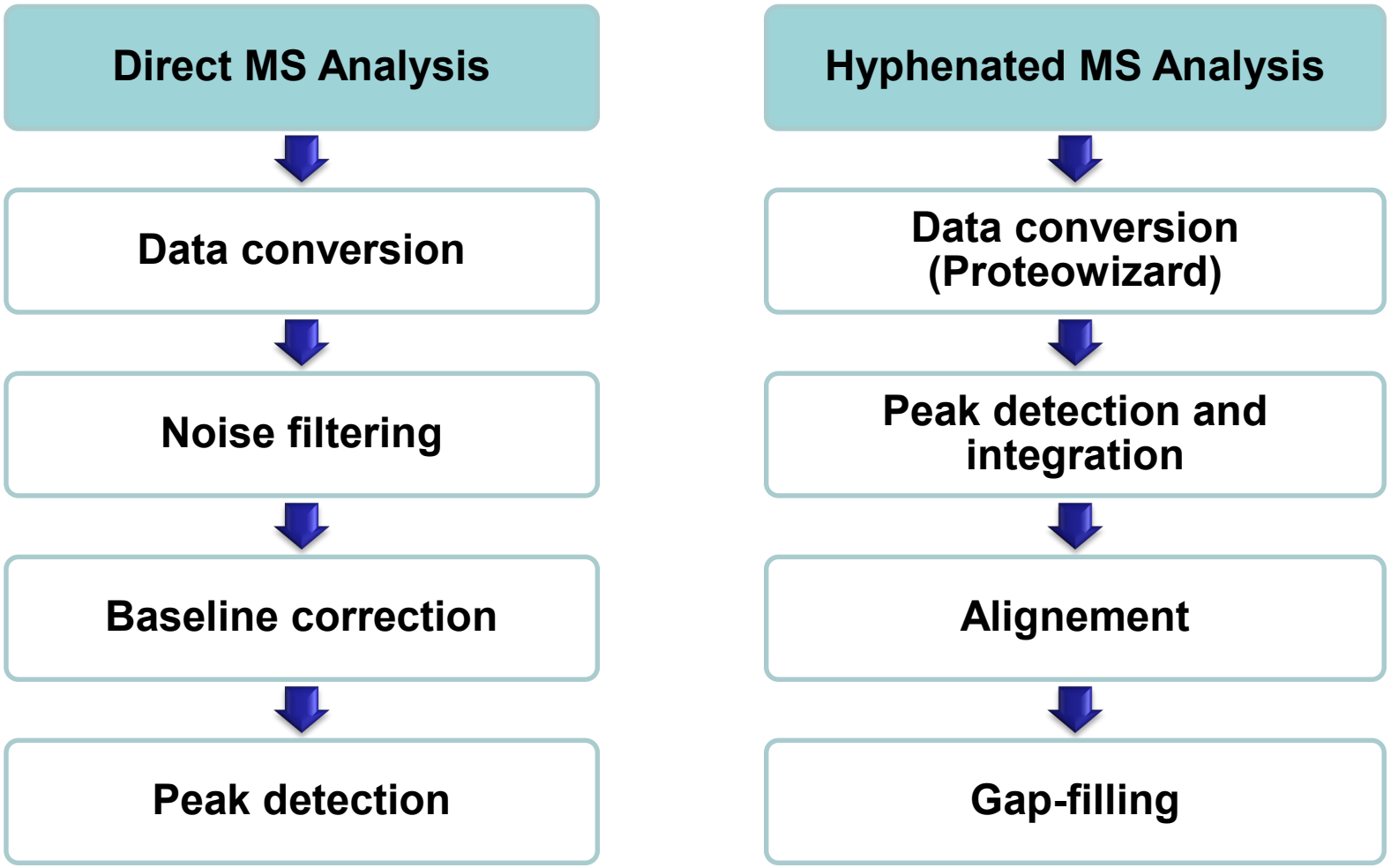
Signal → **Data**

Samples	Variables									
	6	0	4	8	0	2	2	7	2	2
	0	1	8	0	8	0	0	3	4	9
	4	4	0	4	0	1	4	0	1	8
	8	0	2	8	2	4	8	4		0
	0	4	6	0	5	2	0	8	0	2
	2	8	9	5	5	5	2	0	4	6
	4	9	5	6	5	4	5	2	8	9
	1	8	0	8	5	0	1	8	0	8
	4	0	4	0	0	4	2	0	4	0
	0	2	8	2	5	8	0	2	8	2

Data preprocessing



Data processing



Data processing softwares

Table 1 Software tools commonly used for the preprocessing of metabolomics data

Tool	Instrument data type	Software type	Website	References
XCMS	LC–MS, GC–MS	R Package	http://bioconductor.org/packages/release/bioc/html/xcms.html	Smith et al. (2006)
OpenMS—FeatureFinderMetabo	LC–MS	GUI	http://ftp.mi.fu-berlin.de/pub/OpenMS/release-documentation/html/TOPP_FeatureFinderMetabo.html	Bertsch et al. (2010)
MetAlign	LC–MS	Windows GUI	http://www.wageningenur.nl/en/show/MetAlign-1.htm	Lommen & Kools (2012)
MS-DIAL	LC–MS	Windows GUI	http://prime.psc.riken.jp/Metabolomics_Software/MS-DIAL/index.html	Tsugawa et al. (2015)
mzMatch	LC–MS	R Package	http://mzmatch.sourceforge.net/index.php	Scheltema et al. (2011)
IDEOM	LC–MS	Excel Template	http://mzmatch.sourceforge.net/ideom.php	Creek et al. (2012)
AMDIS	GC–MS	Windows GUI	http://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:amdis	Meyer et al. (2010)
MetaboliteDetector	GC–MS	CLI, GUI	http://md.tu-bs.de	Hiller et al. (2009)
MET-IDEA	GC–MS	Windows CLI	http://bioinfo.noble.org/download	Broeckling et al. (2006)
MeltDB	LC–MS, GC–MS	Web App	https://meltdb.cebitec.uni-bielefeld.de/cgi-bin/login.cgi	Kessler et al. (2013)
metaMS	GC–MS	R Package	http://bioconductor.org/packages/release/bioc/html/metaMS.html	Wehrens et al. (2014)
MSeasy	GC–MS	R Package	https://cran.r-project.org/web/packages/MSeasy/index.html	Nicolè et al. (2012)
SpectConnect	GC–MS	Web App	http://spectconnect.mit.edu	Styczynski et al. (2007)
rNMR	NMR	R Package	http://rnmr.nmrfam.wisc.edu	Lewis et al. (2009)

CLI command line interface, *GUI* graphical user interface

Spicer, R., Salek, R.M., Moreno, P. et al. Metabolomics (2017) 13: 106

Data processing softwares

Electrophoresis 2019, *40*, 227–246

Biswapriya B. Misra[1]
Subhashree Mohapatra[2]

[1]Department of Internal Medicine, Section of Molecular Medicine, Medical Center Boulevard, Winston-Salem, NC, USA
[2]Independent Researcher, 151 Edgeway Drive, Winston-Salem, NC, USA

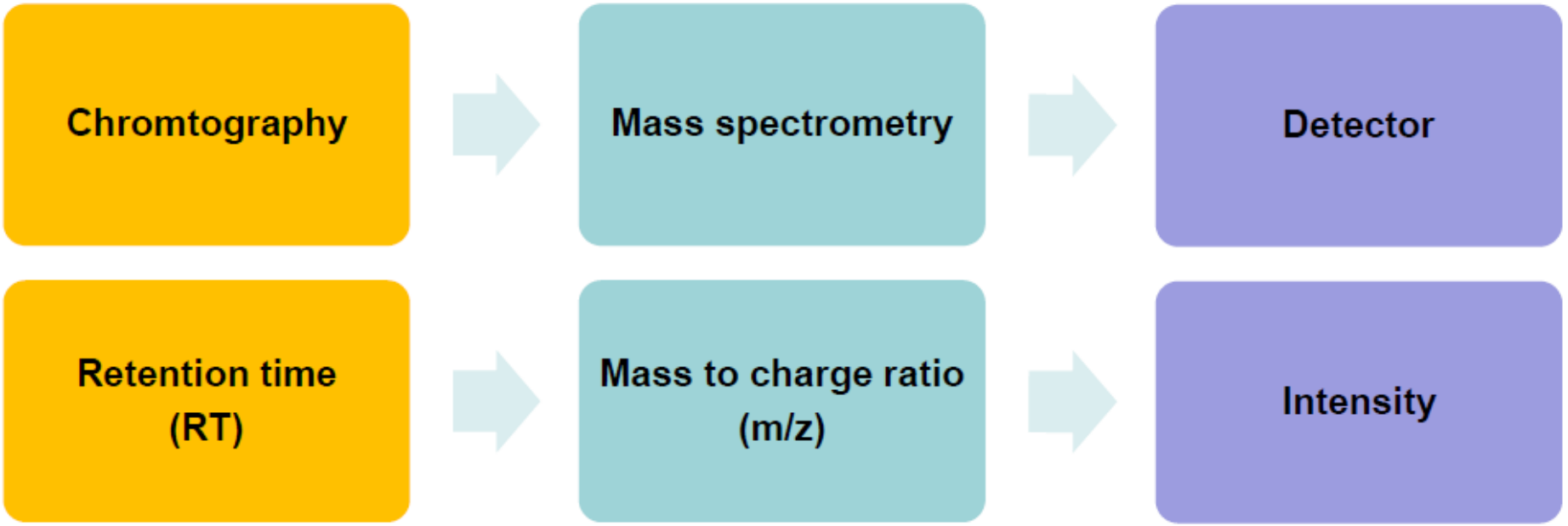
Received October 11, 2018
Revised November 9, 2018
Accepted November 9, 2018

Review

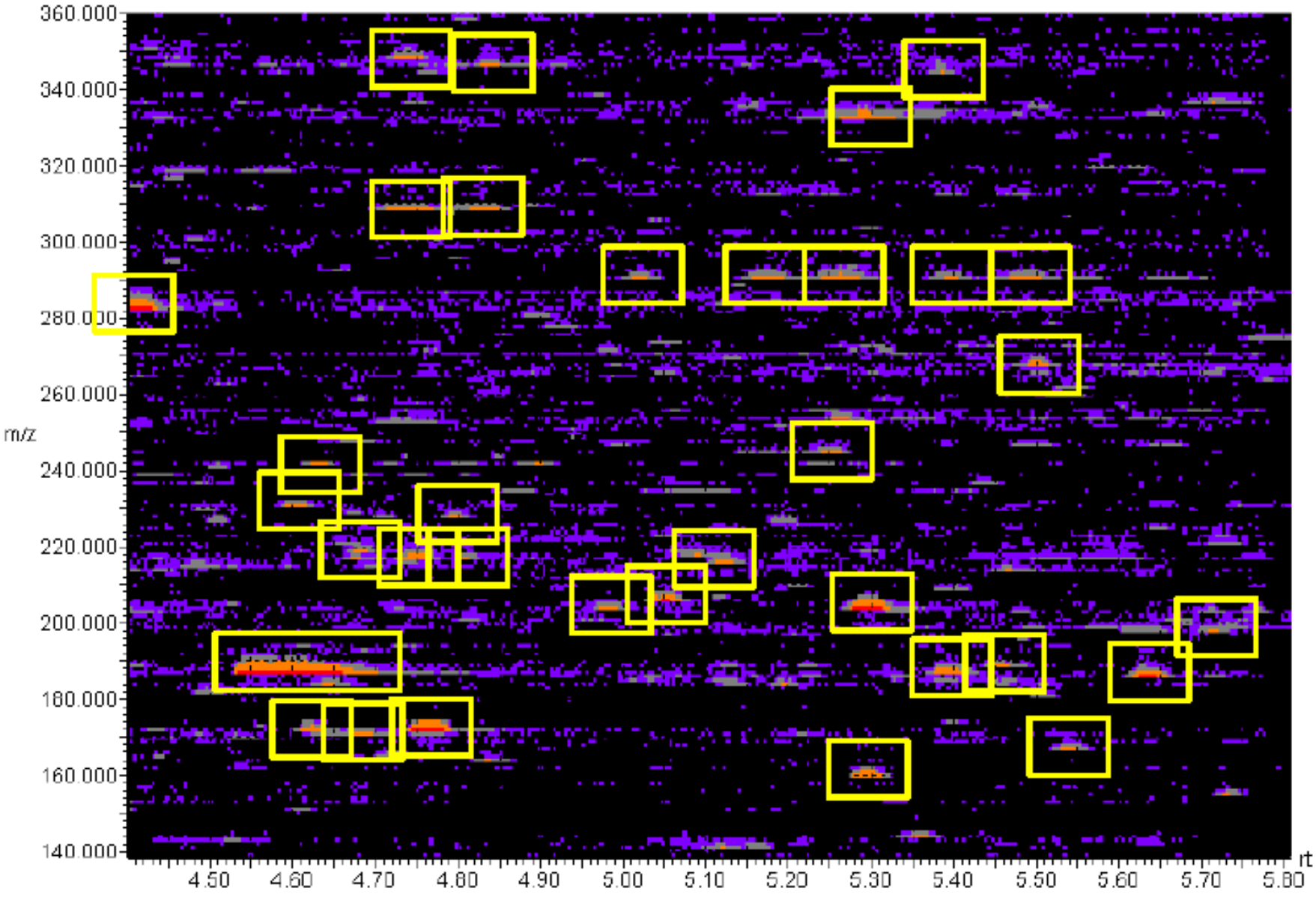
Tools and resources for metabolomics research community: A 2017–2018 update

The scale at which MS- and NMR-based platforms generate metabolomics datasets for both research, core, and clinical facilities to address challenges in the various sciences—ranging from biomedical to agricultural—is underappreciated. Thus, metabolomics efforts spanning microbe, environment, plant, animal, and human systems have led to continual and concomitant growth of in silico resources for analysis and interpretation of these datasets. These software tools, resources, and databases drive the field forward to help keep pace with the amount of data being generated and the sophisticated and diverse analytical platforms that are being used to generate these metabolomics datasets. To address challenges in data preprocessing, metabolite annotation, statistical interrogation, visualization, interpretation, and integration, the metabolomics and informatics research community comes up with hundreds of tools every year. The purpose of the present review is to provide a brief and useful summary of more than 95 metabolomics tools, software, and databases that were either developed or significantly improved during 2017–2018. We hope to see this review help readers, developers, and researchers to obtain informed access to these thorough lists of resources for further improvisation, implementation, and application in due course of time.

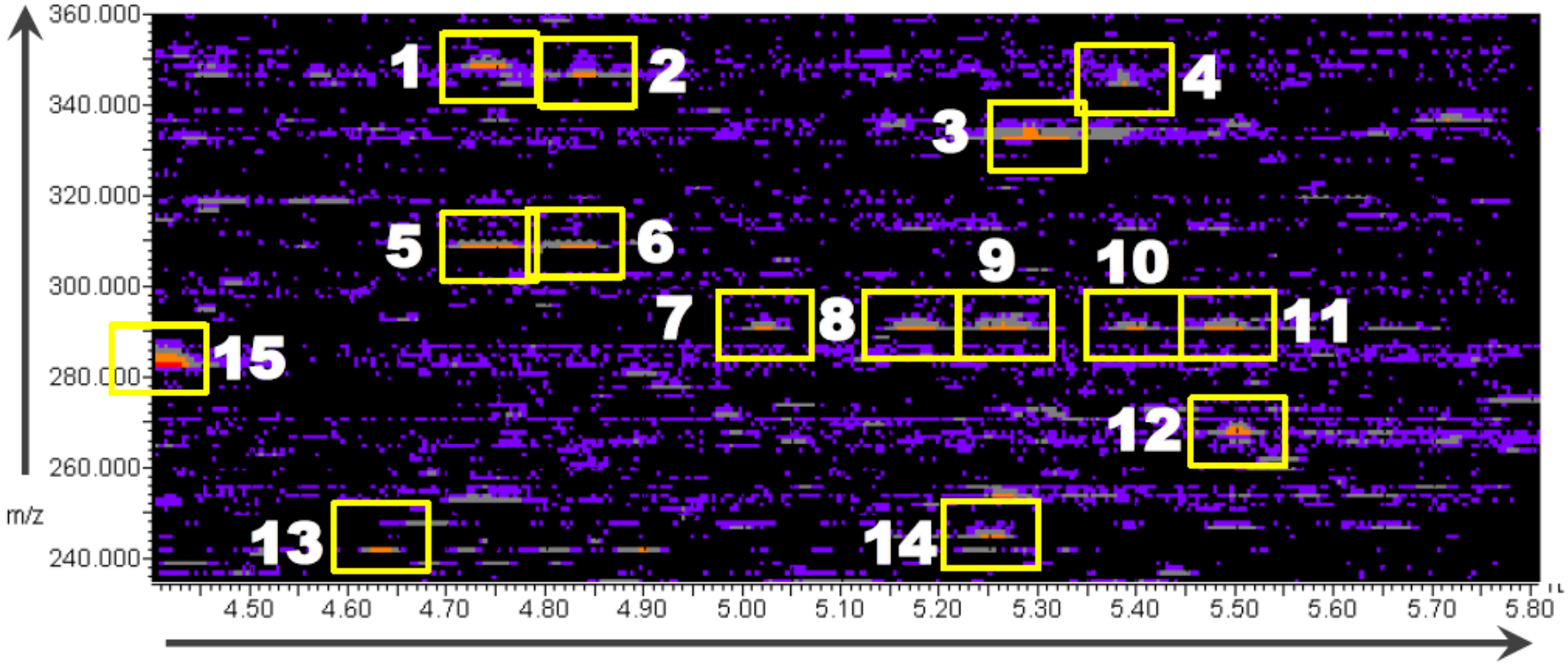
Output data structure



Feature detection in 3-dimensional data



Feature detection in 3-dimensional data



Feature detection in 3 dimensions

Concatenated to single term representing each feature

Feature = RT_mz

1. Mass (m/z)
2. Chromatographic retention time (RT)
3. Intensity ("counts")

Output data structure

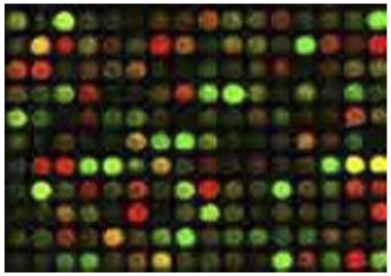
Feature Identifier

Sample identifier

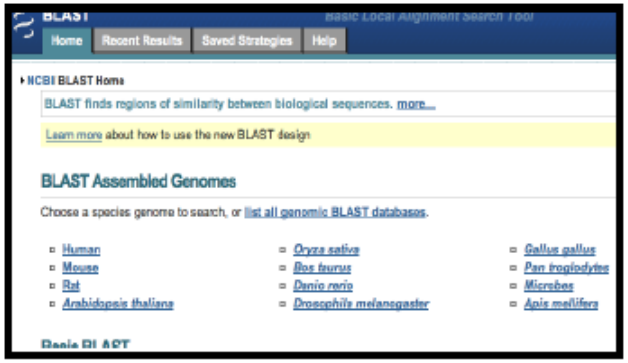
Feature.name	mz	rt	QC.nor.rsd	R2	X180501_1805235_T_NEG	X180501_1805236_F_NEG	X180501_1805237_T_NEG	X180501_1805238_F_NEG	X180501_1805239_QC_NEG
Label	mz	rt	QC.nor.rsd	R3	T	F	T	F	QC
13.22_855.6905m/z	855,6905	13,22	2,78	0,90	1,595756608	1,127168446	0,90987049	1,502157626	1,5242749
12.78_1175.8173m/z	1175,8173	12,78	3,75	0,89	1,012068895	1,924264871	0,941052344	1,24659619	0,700269515
13.11_832.6373m/z	832,6373	13,11	2,58	0,90	1,895778753	0,878647757	0,668815029	3,531989245	1,739529775
13.84_857.7065m/z	857,7065	13,84	2,50	0,91	1,446563144	1,459917821	0,95356938	1,72439594	1,702321205
5.97_339.2102m/z	339,2102	5,97	2,61	0,96	0,462711866	0,319314738	0,528005243	0,457088002	0,340008931
13.20_829.6733m/z	829,6733	13,20	3,62	0,86	1,694567338	1,470567656	0,934753971	1,746620672	1,462169722
12.41_899.6548n	898,6100	12,41	5,13	0,93	1,651790124	0,927875874	0,937857223	1,247611732	1,462013507
9.87_745.5764m/z	745,5764	9,87	4,30	0,93	1,218828015	1,276430502	1,070145633	1,561077878	1,428686119
11.62_828.6054m/z	828,6054	11,62	2,83	0,94	1,503987724	0,941322885	0,941501372	1,483197405	1,458004305
11.49_807.5658n	852,6062	11,49	5,46	0,91	1,031303648	0,798662614	0,683650659	2,074864895	1,016034483
10.74_747.5927m/z	747,5927	10,74	1,41	0,91	1,426032378	0,958717603	1,165625673	0,822211366	1,333198095
10.54_824.5737m/z	824,5737	10,54	2,97	0,92	1,02955304	0,68418959	0,920950584	1,168736087	2,084530777
12.31_854.6227m/z	854,6227	12,31	1,90	0,91	0,922779377	0,893270646	0,801607214	2,58524926	0,979421296
13.84_925.6952m/z	925,6952	13,84	2,97	0,91	1,541006208	1,549550309	0,964287935	1,703201186	1,722283028
11.21_826.5906m/z	826,5906	11,21	2,25	0,91	0,883019997	1,045390523	0,81326039	1,939078305	1,314143816
11.30_802.5890m/z	802,5890	11,30	1,88	0,93	1,3456936	1,026355676	1,006204223	0,940871989	1,513826994
11.30_870.5778m/z	870,5778	11,30	3,85	0,91	1,311794457	0,989558753	1,212741308	0,797155748	1,214987377
13.02_803.6564m/z	803,6564	13,02	5,33	0,91	1,430486792	1,911681286	1,338784094	1,130707907	1,540004947
13.12_900.6262m/z	900,6262	13,12	5,84	0,86	2,015477313	0,820250179	0,604150649	3,738009603	1,8536893
7.16_303.2418m/z	303,2418	7,16	4,66	0,91	1,379045696	0,893287758	1,514480954	1,358690786	2,126265644
11.64_934.6489n	915,6310	11,64	5,34	0,75	2,603452294	1,936584452	2,406736471	1,606282757	1,385685385
9.47_915.6317m/z	915,6317	9,47	7,79	0,88	2,289866624	1,380027238	1,588154586	0,952194138	1,786313204
13.86_993.6850m/z	993,6850	13,86	5,55	0,77	1,390865724	1,187803446	0,929504794	1,249722929	1,466552135
12.06_804.6054m/z	804,6055	12,06	2,78	0,92	1,709644369	0,933713733	0,696277652	1,561432257	1,782679331
6.03_566.3656m/z	566,3656	6,03	2,90	0,86	1,165464794	0,949195488	0,790945442	1,089173428	1,673271311
12.72_856.6375m/z	856,6375	12,72	3,32	0,92	1,420020977	0,930475089	0,884645361	2,959682093	0,998135122

Feature intensity

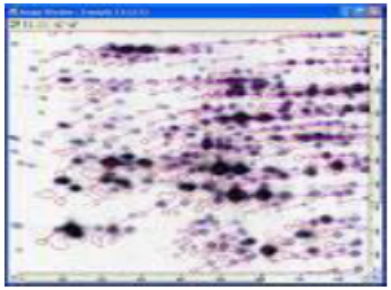
The biggest challenge is Annotation



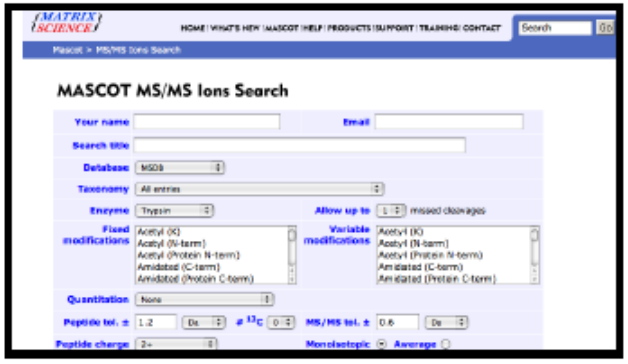
DNA/RNA



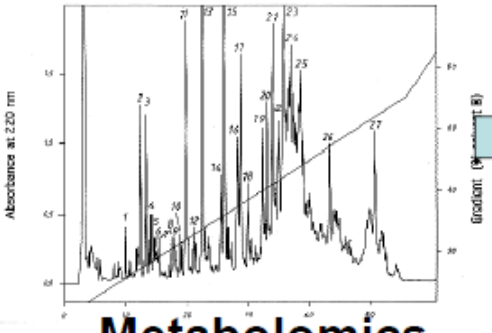
Gene IDs +
Transcript
Abundance



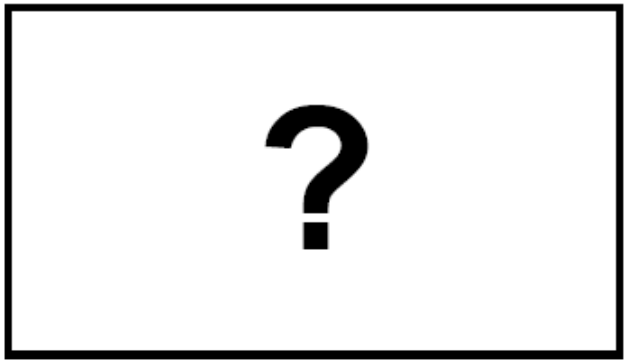
Proteomics



Protein IDs +
Concentrations



Metabolomics



Metabolite IDs +
Concentrations

Metabolome Databases

http://metabolomicssociety.org/resources/metabolomics-databases

www.hmdb.ca

www.drugbank.ca

www.ymdb.ca


www.phenol-explorer.eu

www.ecmdb.ca

www.foodb.ca

www.cowmetdb.ca


www.t3db.ca

www.smpdb.ca

www.csfmetabolome.ca

www.serummetabolome.ca

www.urinemetabolome.ca

The biggest challenge is Annotation

Levels of Metabolite Identification in MS

1. **Positively identified compounds**
   Confirmed by match to known standard
2. **Putatively identified compounds**
   Match to MS + RT or MS/MS + RT
3. **Compounds putatively identified in a compound class**
4. **Unknown compounds**

Processing tools

Commercial tools

- Agilent MassHunter Profinder
- Bruker's ProfileAnalysis
- Thermo SIEVE™
- Waters' Progenesis QI
- SkyLine

Free options

- XCMS Online
- MZmine

Processing tools

XCMS

The first open source tool for spectra processing

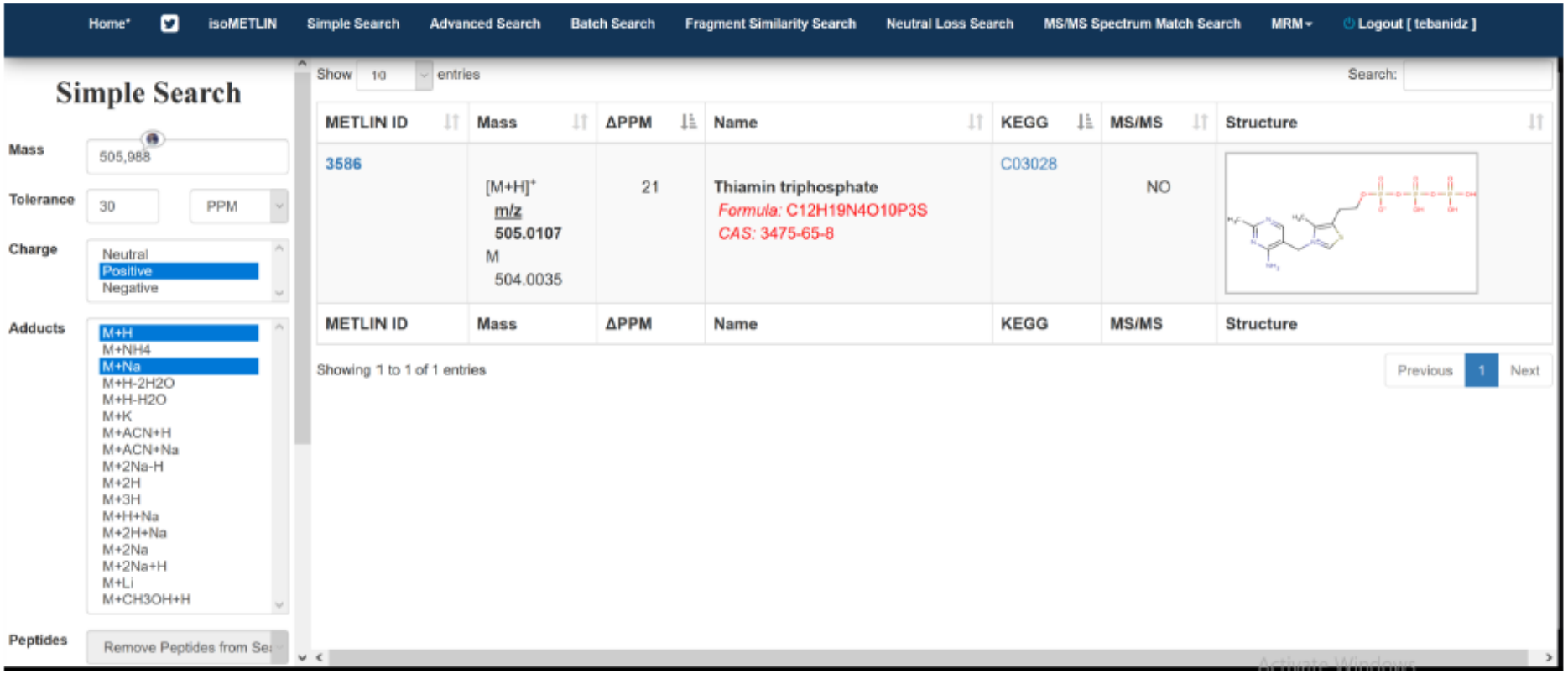
Does peak picking, peak matching and retention time alignment

Available as a program and a server

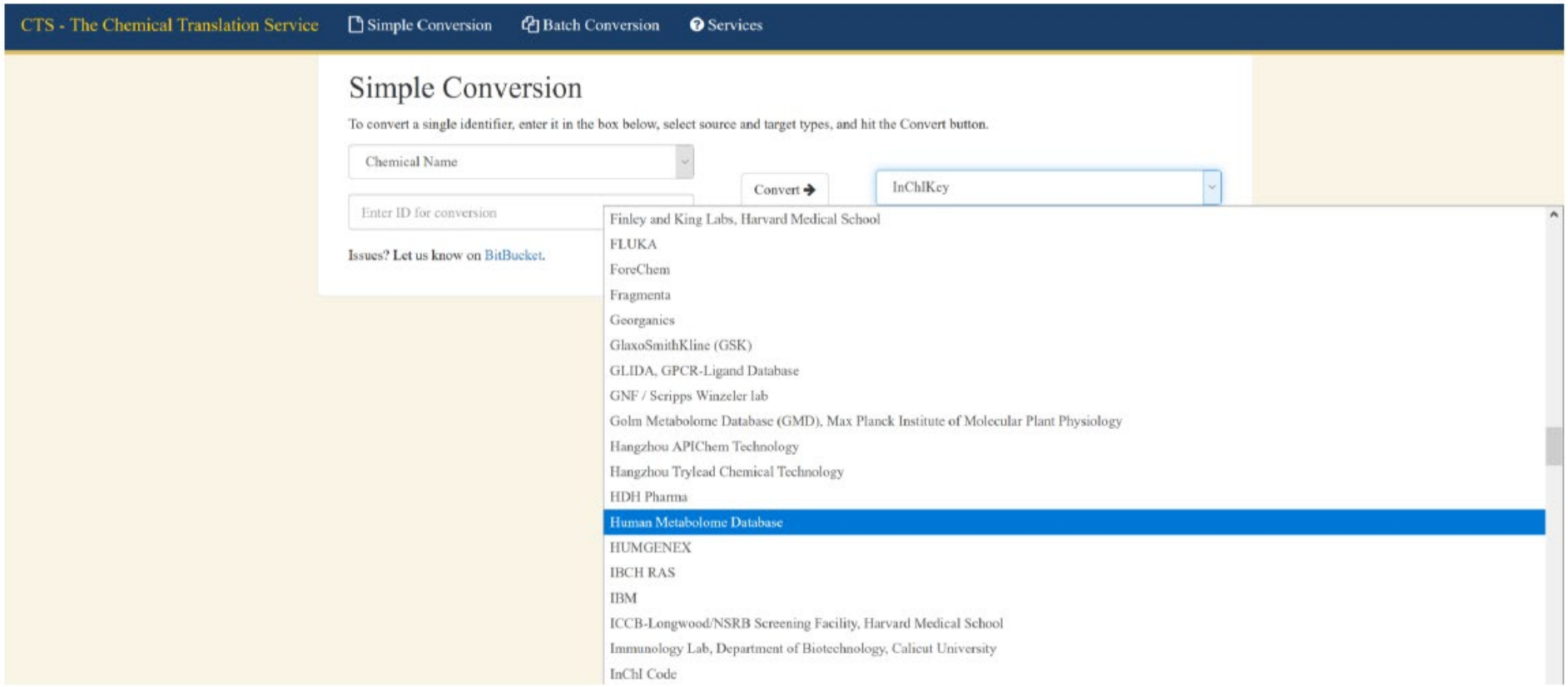
Accepts multiple formats: mzXML, mzData, .cdf (NetCDF), .d folders (Agilent; Bruker), .wiff files (AB SCIEX)

Metabolite identification is not the focus in XCMS (linked to Metlin)

METLIN


Home*
isoMETLIN
Simple Search
Advanced Search
Batch Search
Fragment Similarity Search
Neutral Loss Search
MS/MS Spectrum Match Search
MRM
Logout [tebanidz]
Simple Search
Mass
505,988
Tolerance
30
PPM
Charge
Neutral
Positive
Negative
Adducts
M+H
M+NH4
M+Na
M+H-2H2O
M+H-H2O
M+K
M+ACN+H
M+ACN+Na
M+2Na-H
M+2H
M+3H
M+H+Na
M+2H+Na
M+2Na
M+2Na+H
M+Li
M+CH3OH+H
Peptides
Remove Peptides from Se
Show 10 entries
Search:
METLIN ID
Mass
ΔPPM
Name
KEGG
MS/MS
Structure
3586
[M+H]+
m/z
505.0107
M
504.0035
21
Thiamin triphosphate
Formula: C12H19N4O10P3S
CAS: 3475-65-8
C03028
NO
Showing 1 to 1 of 1 entries
Previous
1
Next

Annotation Conversion



Outline

What is metabolomics?

Experimental Design

Data Generation / Analytical technologies

Applications and Limits

Preprocessing

Data cleaning / analysis

Data cleaning and analysis

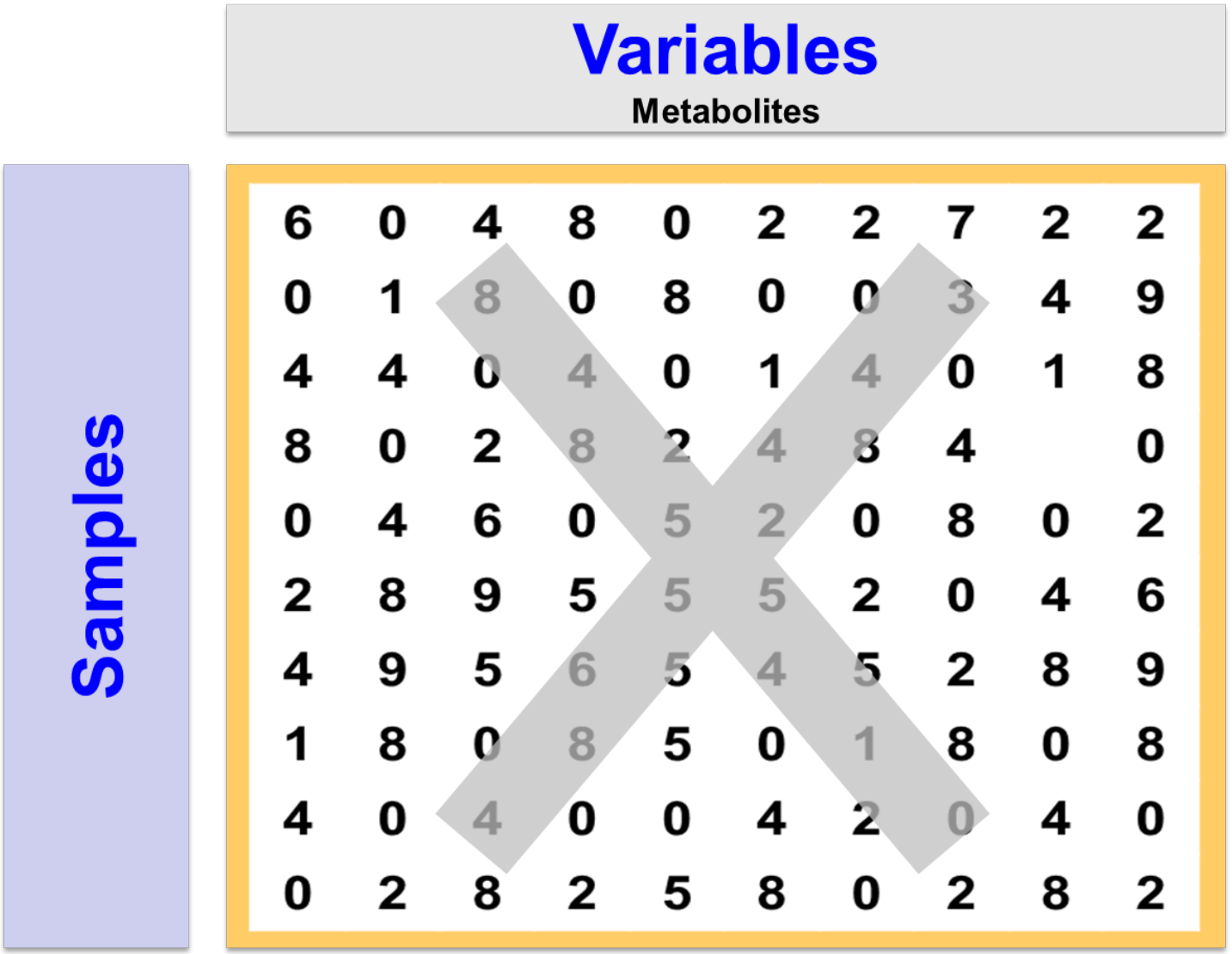
Input

- A matrix containing numerical values
  - Concentrations (Targeted)
  - Peak intensities (Untargeted)
- Meta-data
  - Class labels, experimental factors

Output

- Discriminant features
- Clustering patterns
- Biological Inference
- Biomarkers
- Predictive models

Data cleaning and analysis



Data cleaning and analysis

Variables

Metabolites

Samples

Information

+

Noise

Data Analysis / Data cleaning

Remove as much as possible noise

Extract as much as possible information

Data Analysis / Data cleaning

Data cleaning

- Missing values imputation
- Filtering (Min, IQR, RSD, CV, R2 …)
- Normalization

Data Analysis / Data cleaning

Cut-off

Feature.name	mz	rt	QC.nor.rsd	R2	X180501_1805235_T_NEG	X180501_1805236_F_NEG	X180501_1805237_T_NEG	X180501_1805238_F_NEG	X180501_1805239_T_NEG
Label	mz	rt	QC.nor.rsd	R3	T	F	T	F	T
13.22_855.6905m/z	855,6905	13,22	2,78	0,90	1,595756608	1,127168446	0,90987049	1,502157626	1,5242749
12.78_1175.8173m/z	1175,8173	12,78	3,75	0,89	1,012068895	1,924264871	0,941052344	1,24659619	0,700269515
13.11_832.6373m/z	832,6373	13,11	2,58	0,90	1,895778753	0,878647757	0,668815029	3,531989245	1,739529775
13.84_857.7065m/z	857,7065	13,84	2,50	0,91	1,446563144	1,459917821	0,95356938	1,72439594	1,702321205
5.97_339.2102m/z	339,2102	5,97	2,61	0,96	0,462711866	0,319314738	0,528005243	0,457088002	0,340008931
13.20_829.6733m/z	829,6733	13,20	3,62	0,86	1,694567338	1,470567656	0,934753971	1,746620672	1,462169722
12.41_899.6548n	898,6100	12,41	5,13	0,93	1,651790124	0,927875874	0,937857223	1,247611732	1,462013507
9.87_745.5764m/z	745,5764	9,87	4,30	0,93	1,218828015	1,276430502	1,070145633	1,561077878	1,428686119
11.62_828.6054m/z	828,6054	11,62	2,83	0,94	1,503987724	0,941322885	0,941501372	1,483197405	1,458004305
11.49_807.5658n	852,6062	11,49	5,46	0,91	1,031303648	0,798662614	0,683650659	2,074864895	1,016034483
10.74_747.5927m/z	747,5927	10,74	1,41	0,91	1,426032378	0,958717603	1,165625673	0,822211366	1,333198095
10.54_824.5737m/z	824,5737	10,54	2,97	0,92	1,02955304	0,68418959	0,920950584	1,168736087	2,084530777
12.31_854.6227m/z	854,6227	12,31	1,90	0,91	0,922779377	0,893270646	0,801607214	2,58524926	0,979421296
13.84_925.6952m/z	925,6952	13,84	2,97	0,91	1,541006208	1,549550309	0,964287935	1,703201186	1,722283028
11.21_826.5906m/z	826,5906	11,21	2,25	0,91	0,883019997	1,045390523	0,81326039	1,939078305	1,314143816
11.30_802.5890m/z	802,5890	11,30	1,88	0,93	1,3456936	1,026355676	1,006204223	0,940871989	1,513826994
11.30_870.5778m/z	870,5778	11,30	3,85	0,91	1,311794457	0,989558753	1,212741308	0,797155748	1,214987377
13.02_803.6564m/z	803,6564	13,02	5,33	0,91	1,430486792	1,911681286	1,338784094	1,130707907	1,540004947
13.12_900.6262m/z	900,6262	13,12	5,84	0,86	2,015477313	0,820250179	0,604150649	3,738009603	1,8536893
7.16_303.2418m/z	303,2418	7,16	4,66	0,91	1,379045696	0,893287758	1,514480954	1,358690786	2,126265644
11.64_934.6489n	915,6310	11,64	5,34	0,75	2,603452294	1,936584452	2,406736471	1,606282757	1,385685385
9.47_915.6317m/z	915,6317	9,47	7,79	0,88	2,289866624	1,380027238	1,588154586	0,952194138	1,786313204
13.86_993.6850m/z	993,6850	13,86	5,55	0,77	1,390865724	1,187803446	0,929504794	1,249722929	1,466552135
12.06_804.6054m/z	804,6055	12,06	2,78	0,92	1,709644369	0,933713733	0,696277652	1,561432257	1,782679331
6.03_566.3656m/z	566,3656	6,03	2,90	0,86	1,165464794	0,949195488	0,790945442	1,089173428	1,673271311
12.72_856.6375m/z	856,6375	12,72	3,32	0,92	1,420020977	0,930475089	0,884645361	2,959682093	0,998135122

Data Analysis / Data cleaning

Missing values imputation

Replace by
- mean
- median
- min
- …

k-nearest neighbour (KNN)
PCA based methods
…

Wolfram et al. *Bioinformatics*, Volume 23, Issue 9, 1 May 2007, Pages 1164–1167

Data Analysis / Normalization

Sample normalization (row-wise)

To remove systematic variation between experimental conditions **unrelated** to the biological differences (i.e. dilutions, mass)

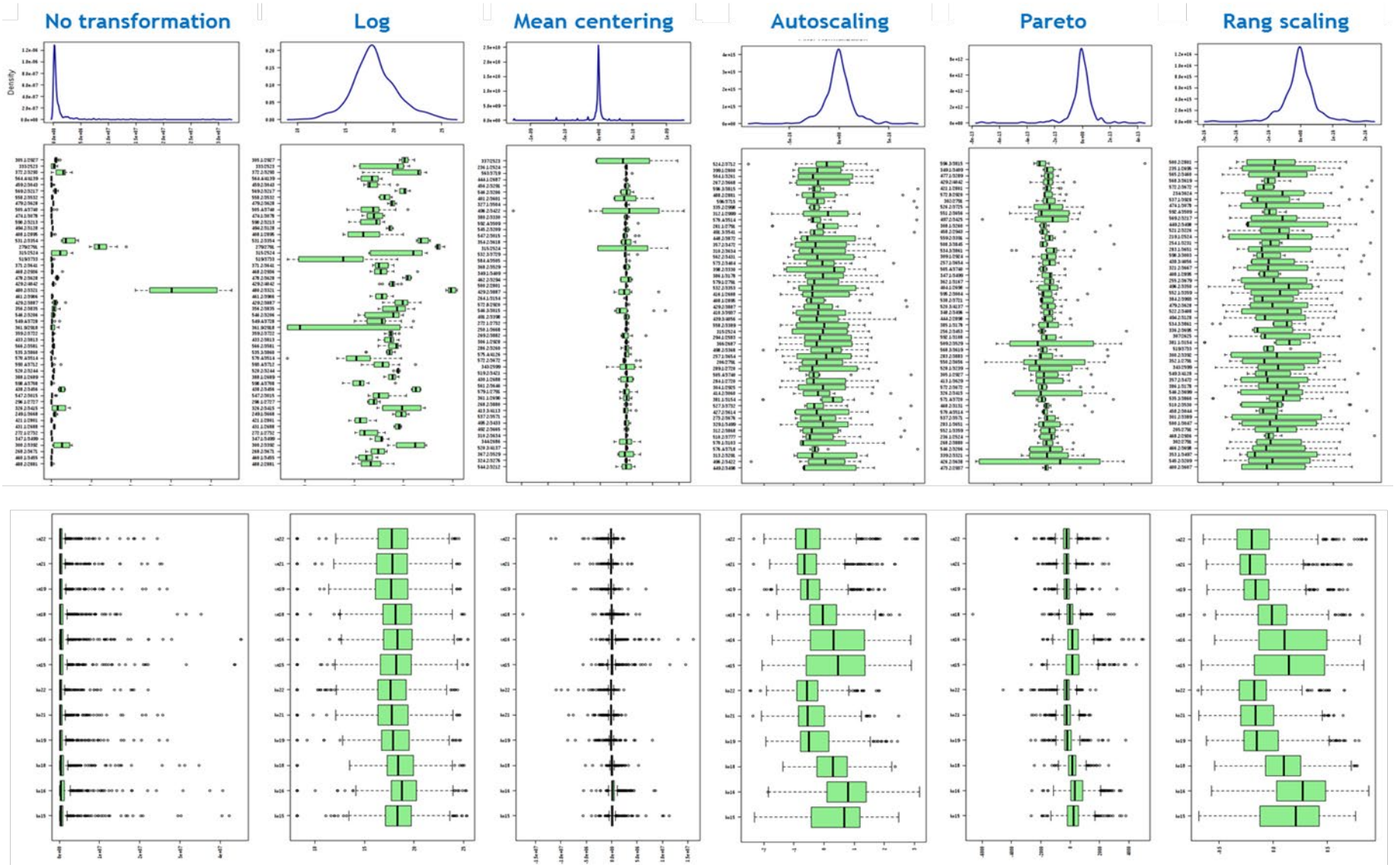
- Total signal, sum of signals
- Reference compound: Internal standards, endogenous metabolites
- Reference sample: QCs, Controls

Feature normalization (column-wise)

To bring variances of all features close to equal

- Log transformation
- Scaling

Data Analysis / Normalization



Data Analysis / Normalization

W162–W170 *Nucleic Acids Research, 2017, Vol. 45, Web Server issue* *Published online 19 May 2017*
doi: 10.1093/nar/gkx449

NOREVA: normalization and evaluation of MS-based metabolomics data

Bo Li[1,†], Jing Tang[1,†], Qingxia Yang[1,2,†], Shuang Li[1], Xuejiao Cui[1], Yinghong Li[1], Yuzong Chen[3], Weiwei Xue[1], Xiaofeng Li[1] and Feng Zhu[1,2,*]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 July 2018 doi:10.20944/preprints201807.0059.v1

Peer-reviewed version available at *Metabolites* **2018**, *8*, 47; doi:10.3390/metabo8030047

Review

Data Normalization in NMR-based Metabolomics

Helena U. Zacharias[1], Michael Altenbuchinger[2] and Wolfram Gronwald[3]*

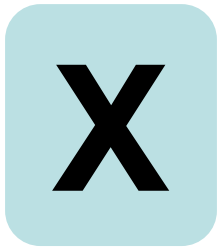
Data Analysis

Tow main objectives

Descriptive data analysis (Unsupervised learning)

Mining massive datasets to discover hidden
- *data structures*
- *hidden relationships*
- *patterns, trends and clusters*
- *outliers*

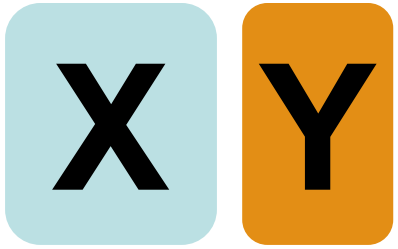
Dimension reduction



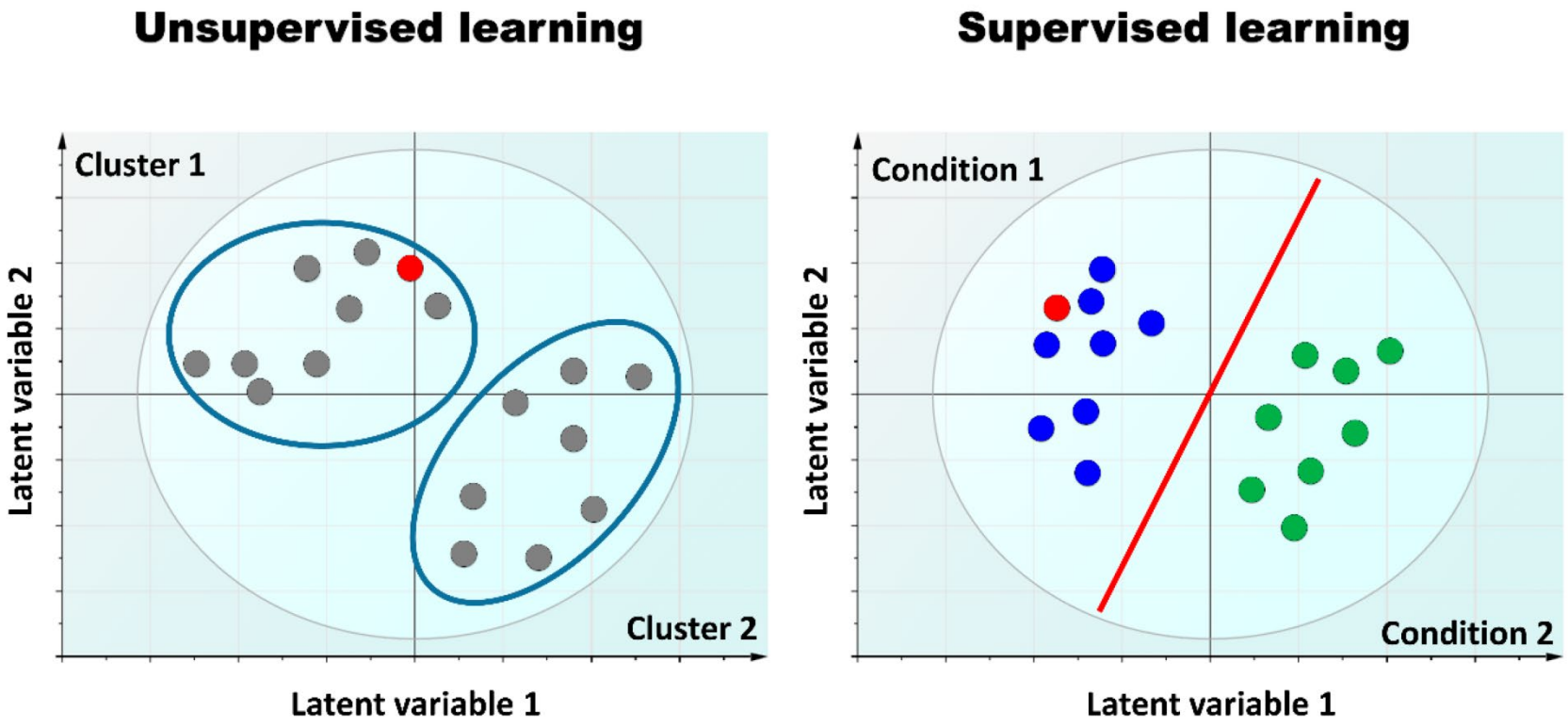
Predictive data analysis (Supervised learning)

Building models for specific tasks using training datasets
- *regression*
- *classification,*
- *pattern recognition*
- *machine learning tasks*

Assessing the predictive accuracy of the models using new datasets



Data Analysis



Tebani A *et.al*. Int J Mol Sci. 2016 Sep; 17(9): 1.

Data Analysis / Unsupervised learning

Clustering

Organize the 1000s of variables into blocks

Variables in each block are more homogenous

Key parameter: similarities (Distance, Spearman, Pearson …)

Similarity between samples - Similarity between clusters

Visualization using Heatmaps

- *K-means*
- *Hierarchical Methods*

Dimension reduction

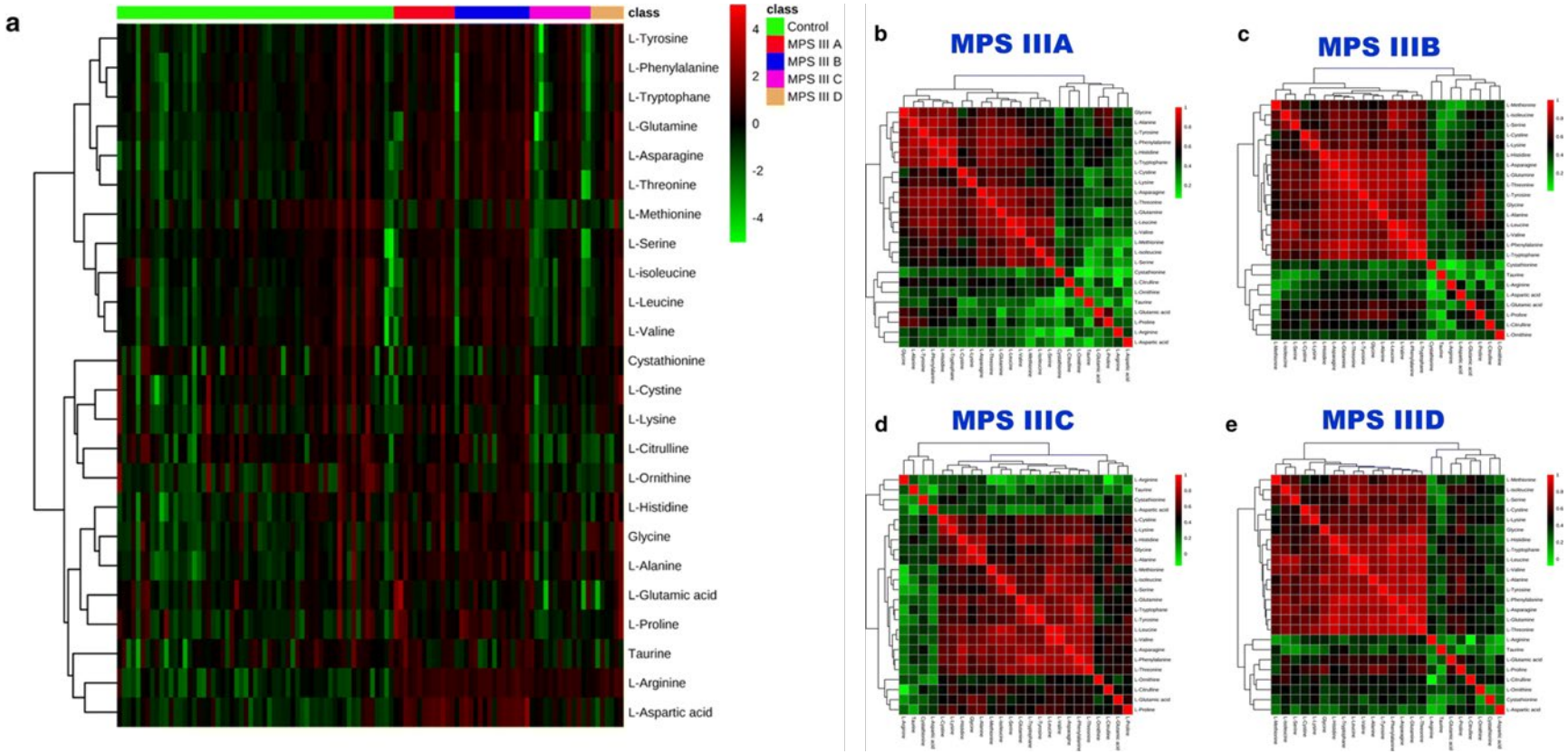
Reduce the high-dimensional data

1000s into low-dimensions (Latent variables)

- *Principal component analysis (PCA)*

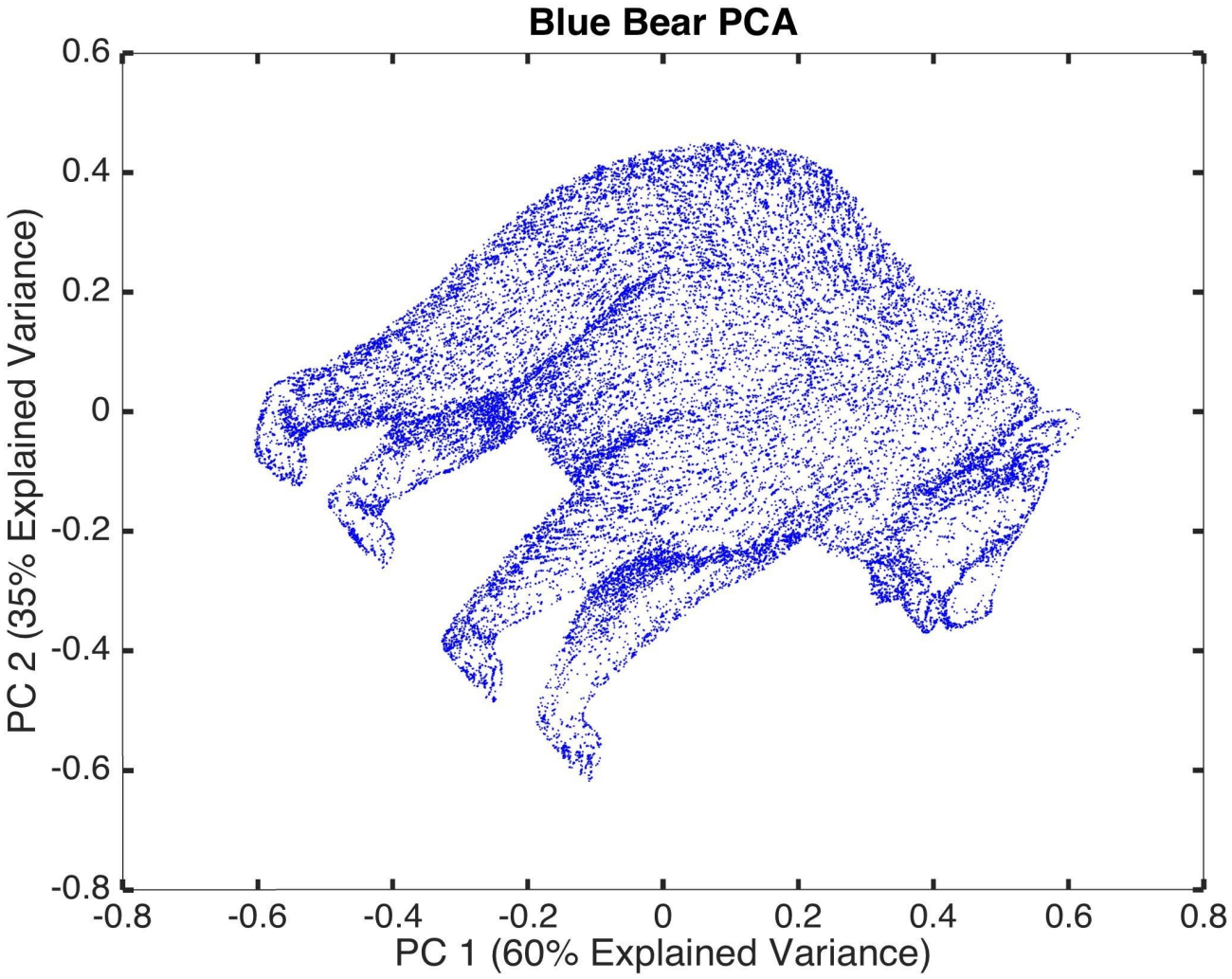
Data Analysis / Unsupervised learning

Correlation Heatmap



Tebani A *et.al*. JTRM 2018

Data Analysis / Unsupervised learning (PCA)



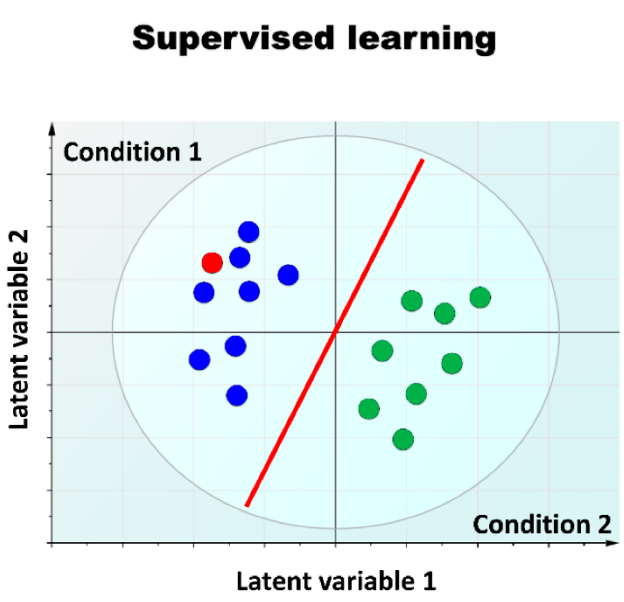
Data Analysis / Supervised learning

Linear Discriminant Analysis
Partial Least Squares
k-Nearest Neighbors
Random Forest
Support Vector Machines
Bayesian networks
Neural Networks

… name it

https://jawbone.com/blog/classifying-steps-machine-learning/

Data Analysis / Supervised learning



f(x) = y

Supervised = algorithm does know true answer

Attempt to find rule which predicts output for given input

Two cases

Classification - output is discrete (Class label)

Regression - output is continuous

Model validation

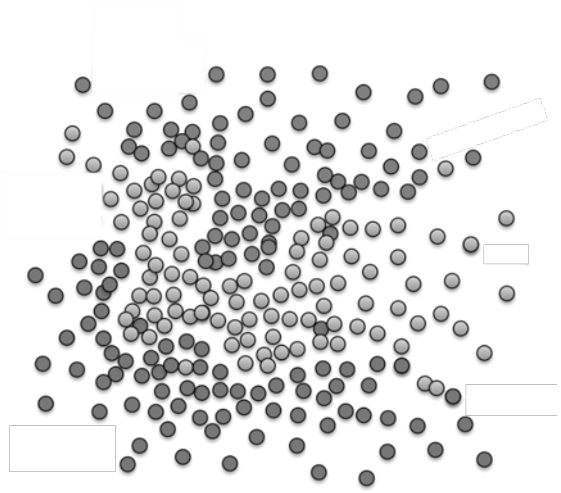
A *statistically valid* model

- Has good fit to the data
- Is *predictive* of new data

Validation methods

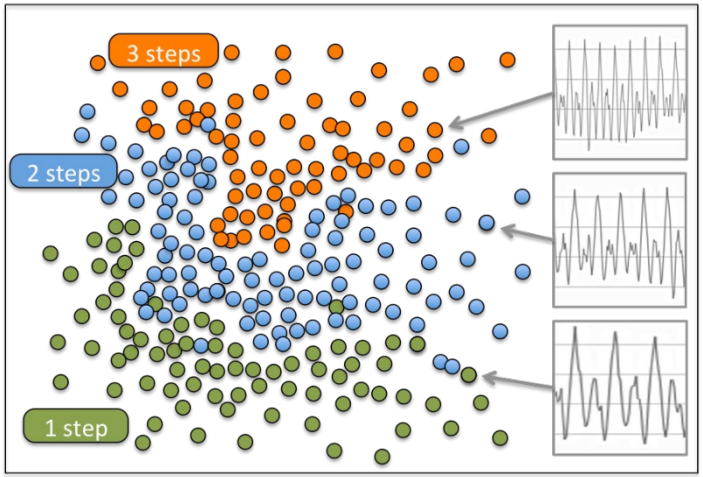
- Training set / Testing set
- Cross-validation
- Permutation test

Data Analysis / Supervised learning



Data Analysis / Supervised learning

1



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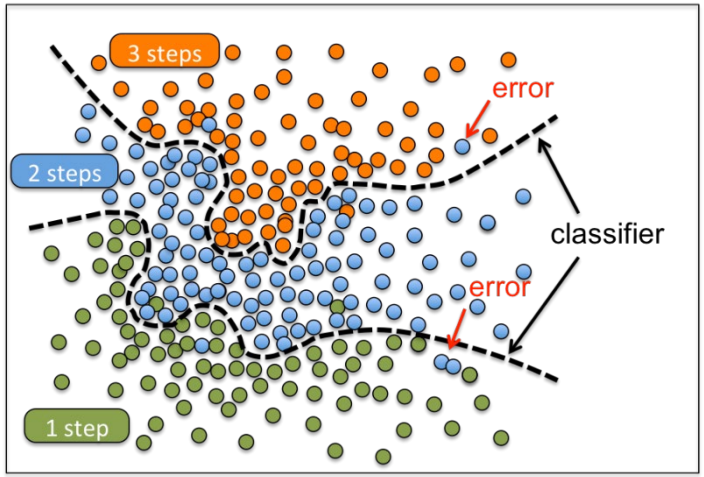

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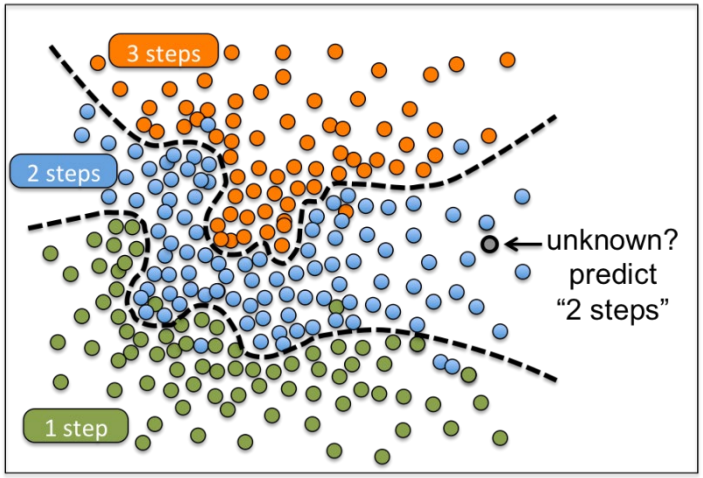

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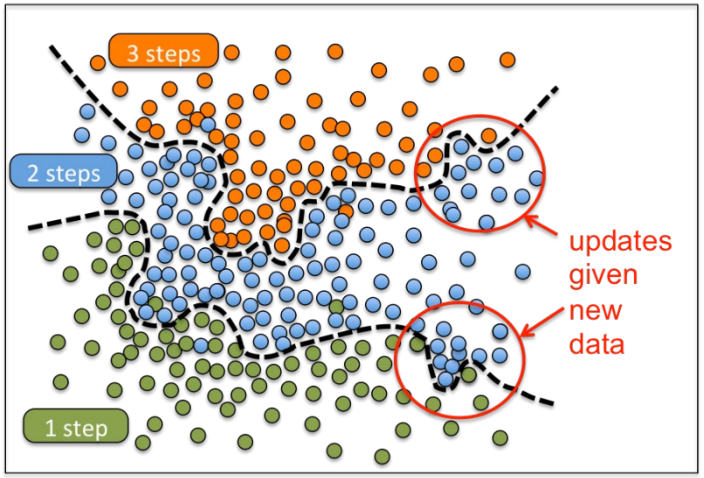

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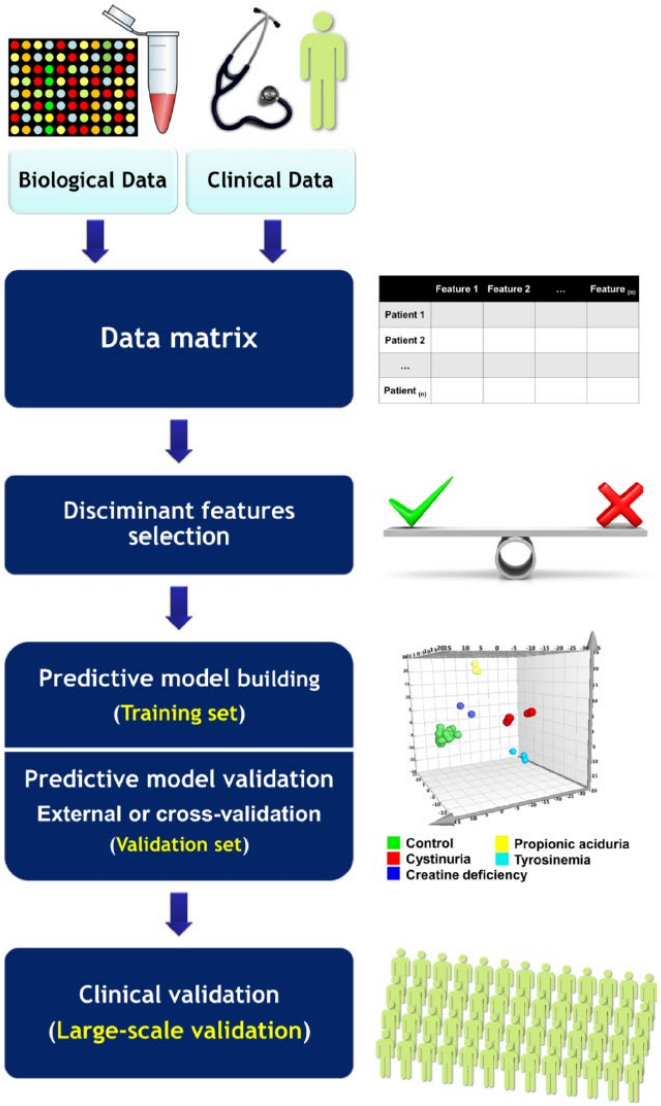
Data Analysis / Supervised learning



Tebani *et al.* Int J Mol Sci. 2016 Sep; 17(9): 1.

Training dataset: a set of examples used to build the predictive model

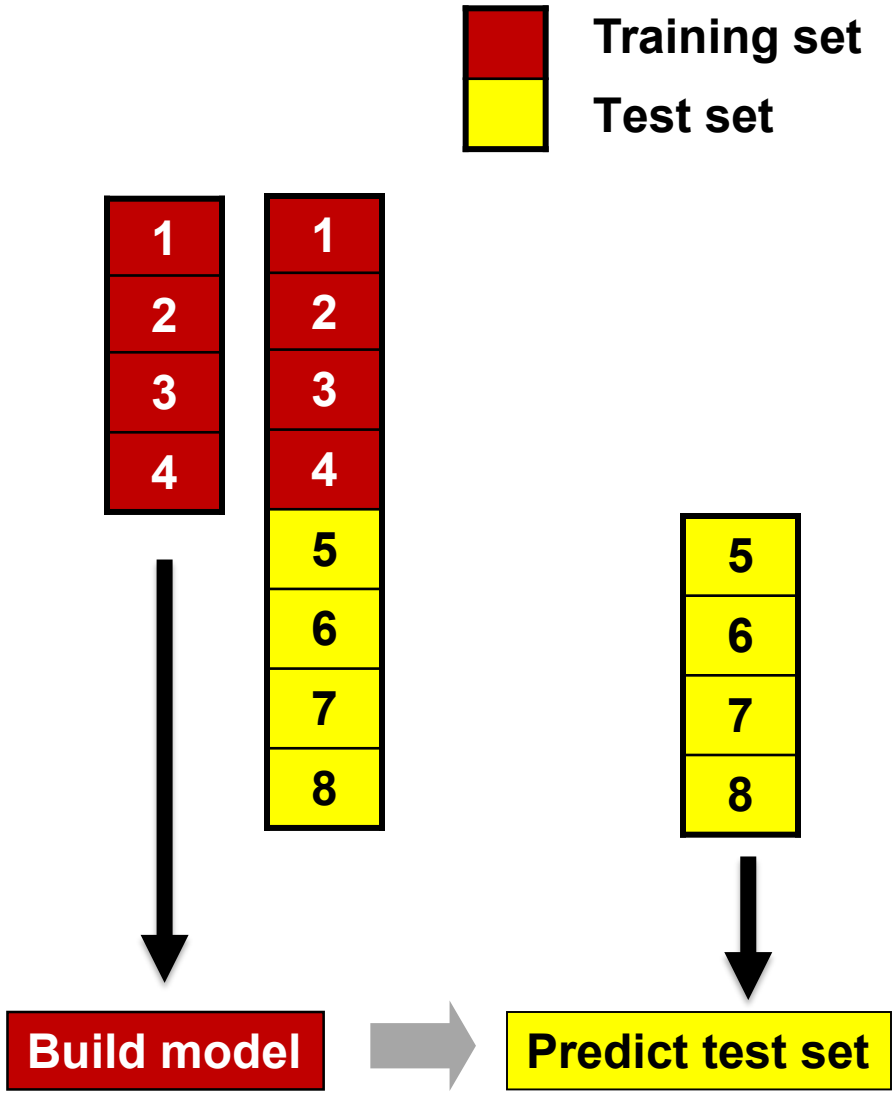
Validation dataset: a set of examples used to refine the model parameters and estimate the error.

Test dataset: used only to evaluate the predictive performance of the model. They are never used during the learning or testing process.

Data Analysis / Supervised learning

***Validation: split* the data**

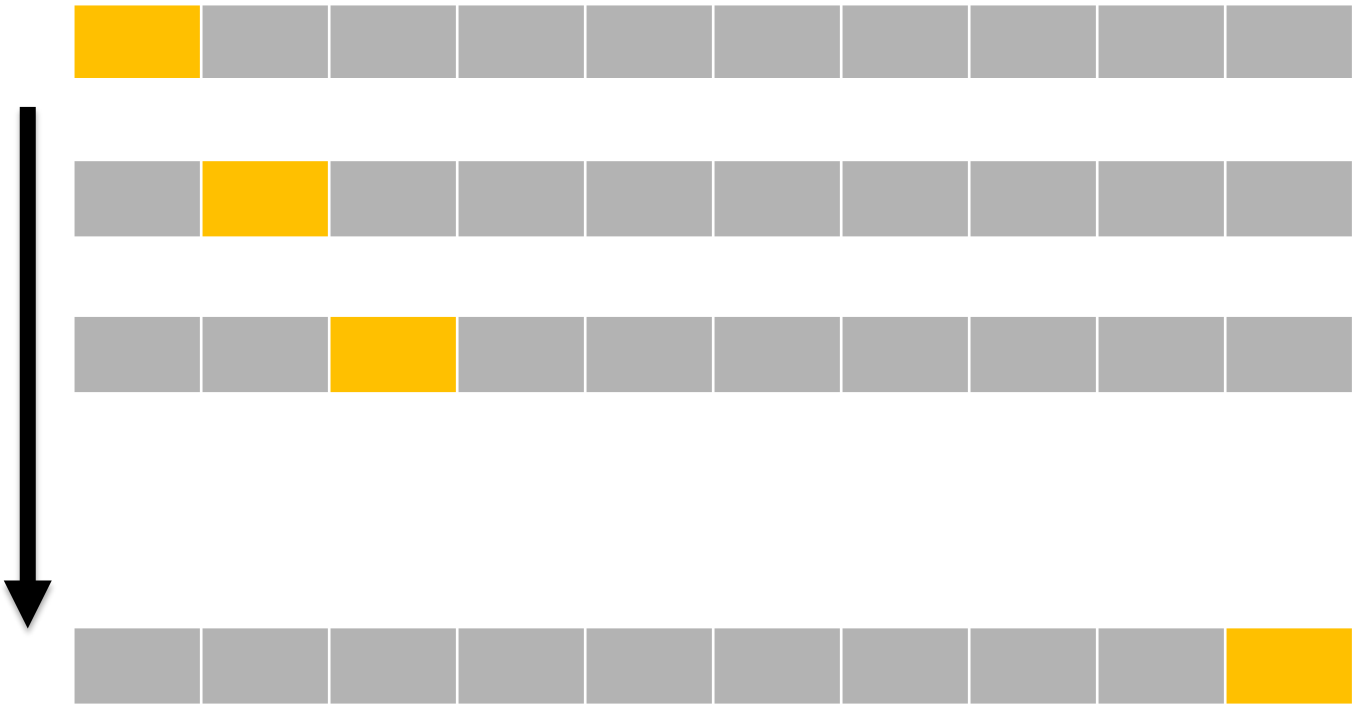
- Training set – to build the model
- Test set – to validate the model



Data Analysis / Supervised learning

Cross-Validation

- Divide the dataset into k parts
- Use k-1 for training and 1 for testing
- Determine an optimization metric (RMSE, Accuracy, ROC, …)
- Iterate



Take home message

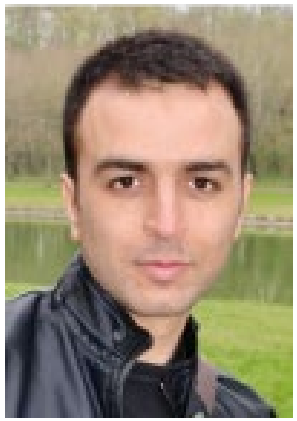
Experimental design +++++++

Know you data

Main technologies are MS and NMR

Annotation is challenging in untargeted metabolomics

Acknowledge & Questions



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