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Metabolomics & Lipidomics

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Metabol(n)omics – What's in name

- Metabolome: the entire set of metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes
- Metabonomics: "the quantitative measurement of the dynamic multiparametric metabolic responses of multicellular systems to pathophysiological stimuli or genetic modification" Nicholson et al. Xenobiotica, 29:1181-1189, 1999
- Metabolomics: "measurement of metabolite concentrations and fluxes in cells, cell complexes and cytologically defined tissues".
- No real distinction on analytical workflows
- Both approaches used as a phenotypic module for system understanding (functional genomics and systems biology)
- Metabonomics extends system phenotyping from endobiotics to the all exposome including xenobiotics (drugs), nutrients (diet), microbiota (extended genome) and environmental factors.

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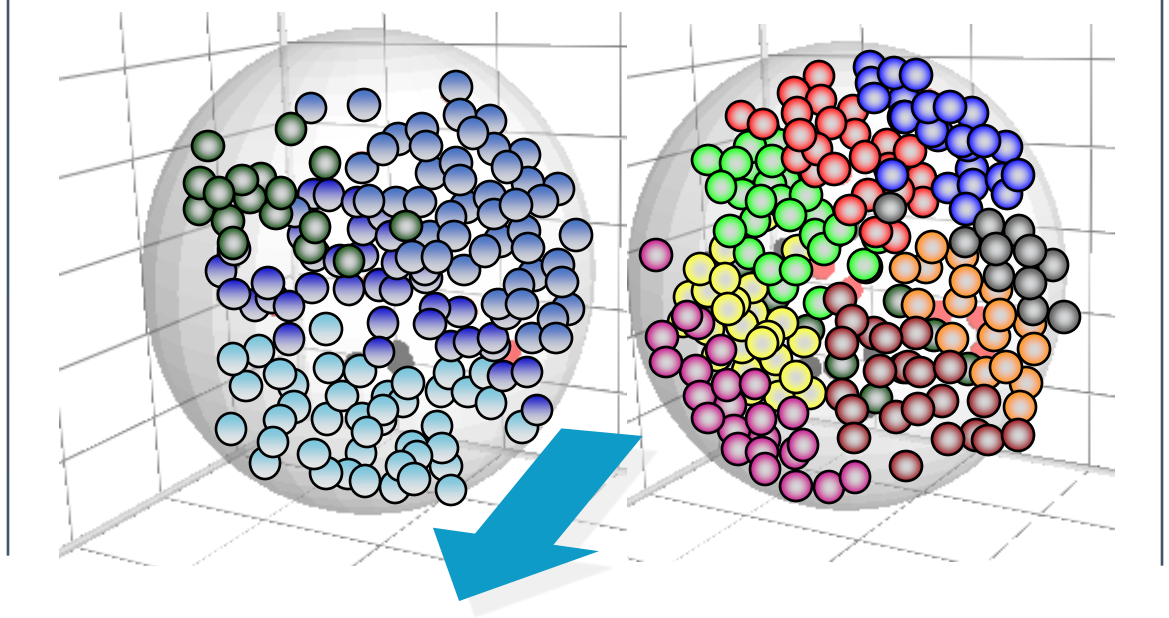
The Metabolome

Intrinsic factors

- Body composition
- Tissue turnover
- Resting metabolic rate
- Age
- Genotype
- Health status
- Reproductive status
- Diurnal cycle



Extrinsic factors

- Diet
- Drugs
- Physical activity
- Microbiota
- Stress
- Lifestyle

The Metabolome

The sum of all endogenous and exogenous metabolites

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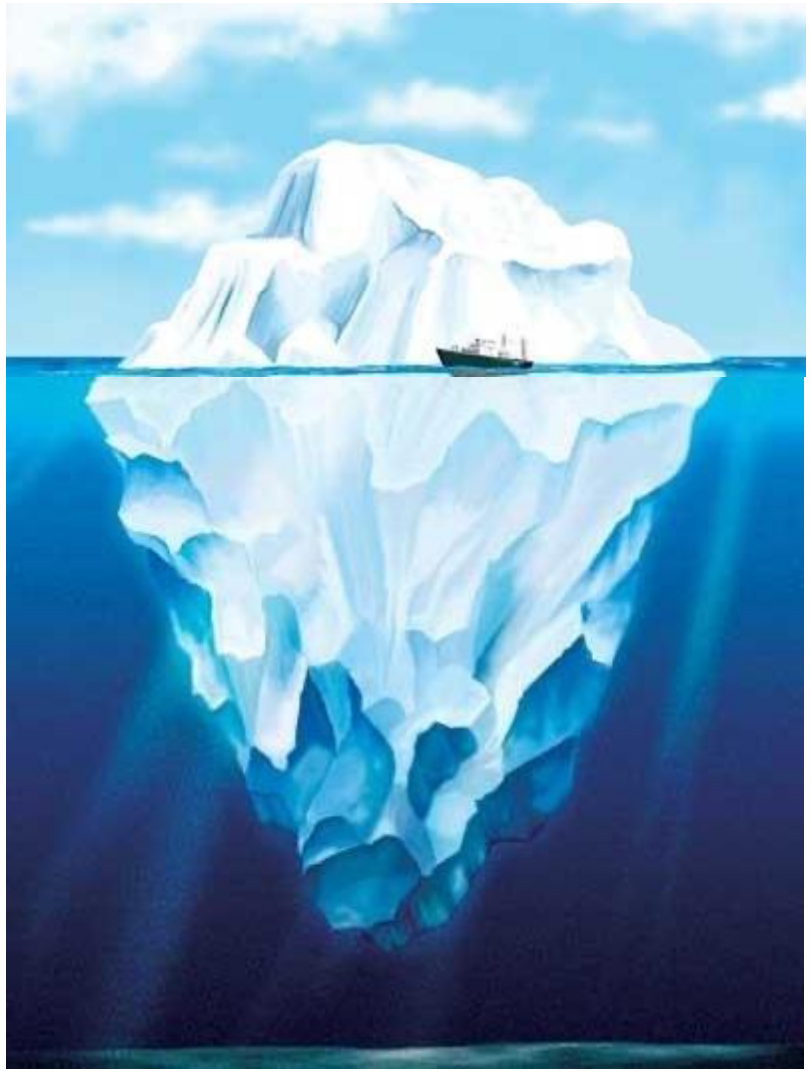
Analytical strategies for metabolic profiling

The Metabolome

Holistic

Targeted



System level
Holistic profile
- *Homeostatic status*
- *Surrogate biomarkers*

Semi-holistic & targeted quantitative profiles
- **Lipidomics**

Sub-pathway level
Tracer analysis
Fluxes

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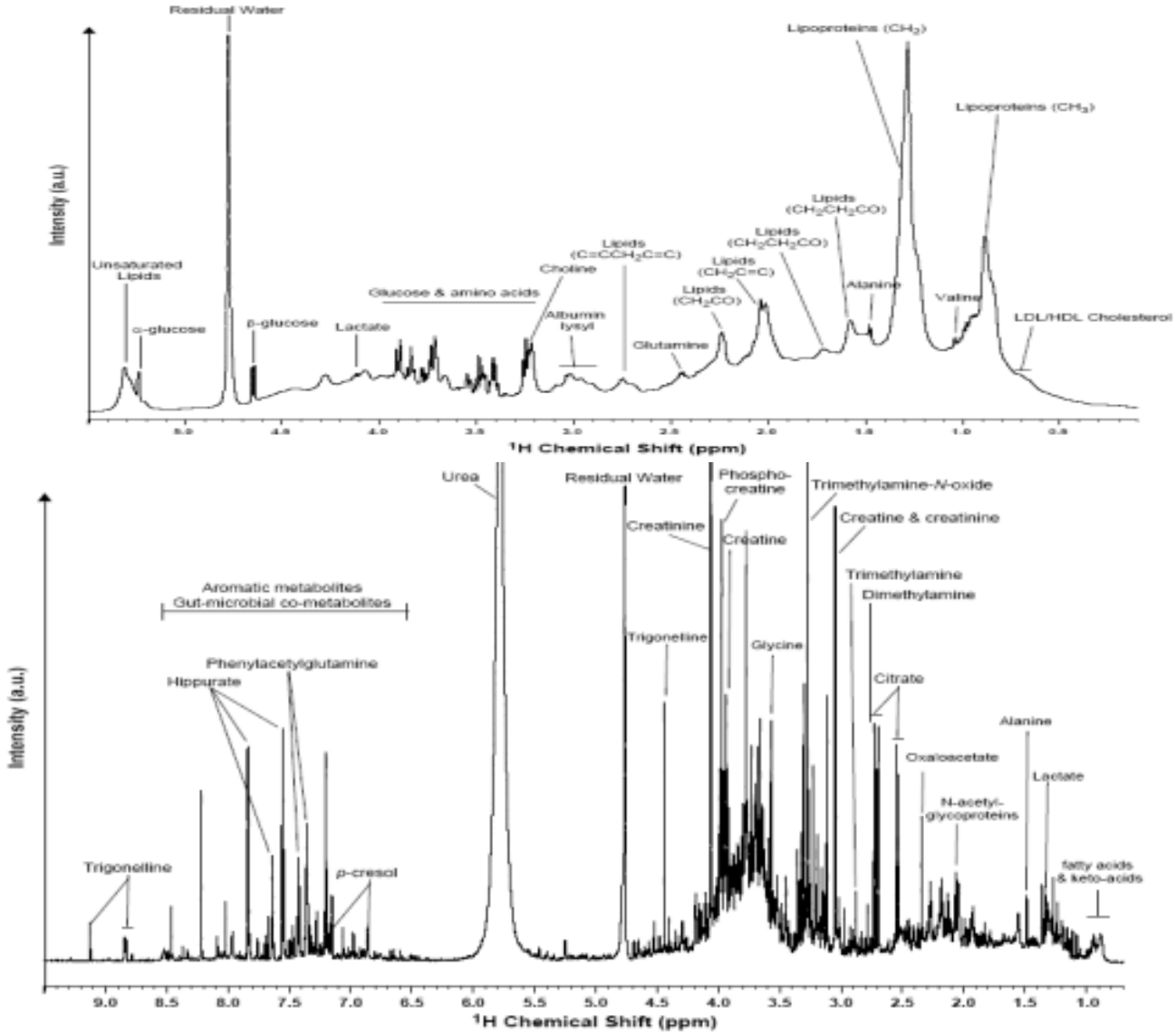
Holistic metabolic profiling by ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy

Key features

- high throughput
- highly robust
- provides information on:
  - Central energy metabolism
  - Amino acids
  - Lipids (lipoproteins)
  - Glucose
  - Microbial metabolites
- Efficient for hypothesis generation and functional genomics (GWAS)
- Provides guidance for subsequent targeted analyses



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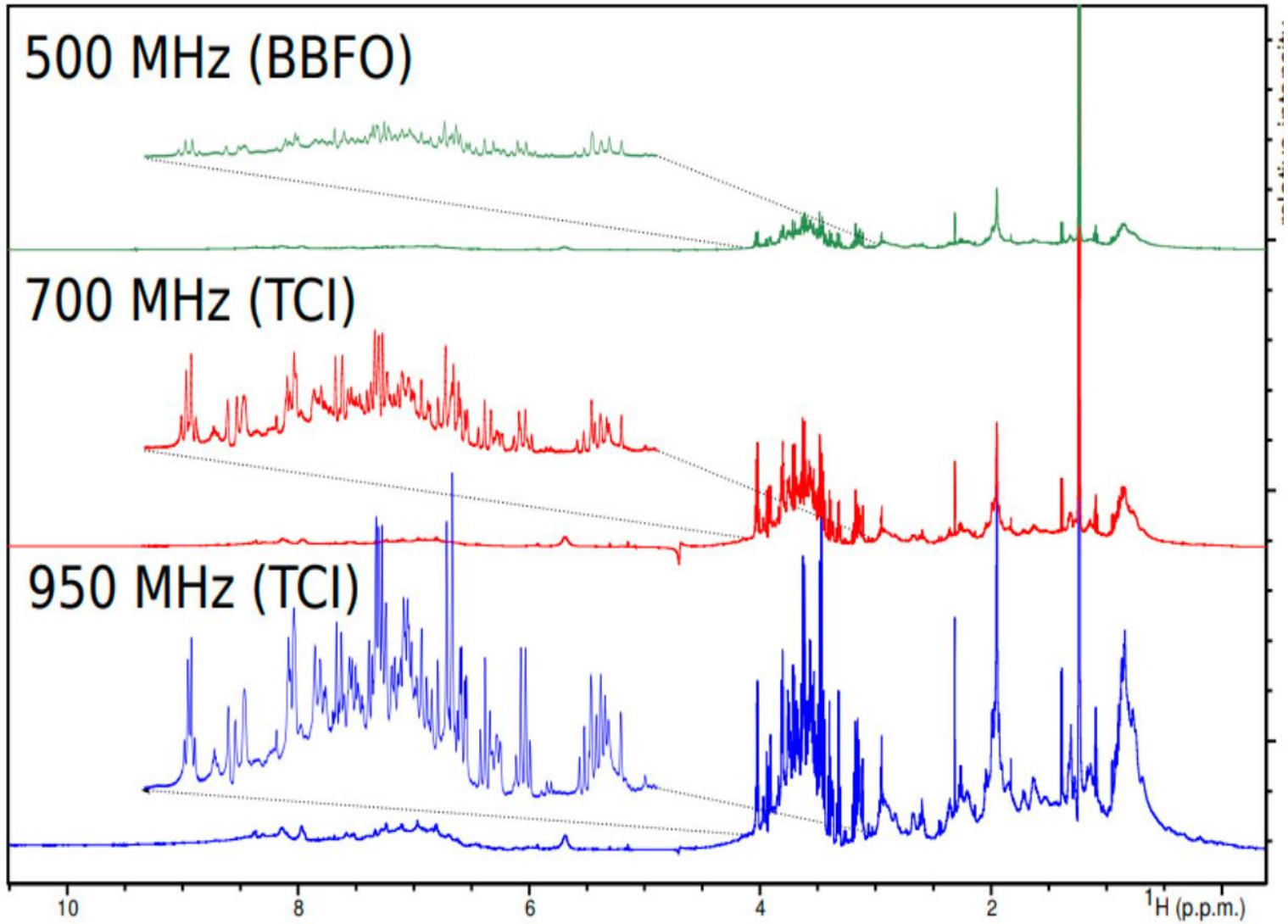
Resolution gain by increasing frequency of NMR magnets (^{1}H NMR of bovine serum)



Emwas et al. Metabolites 2019, 9, 123

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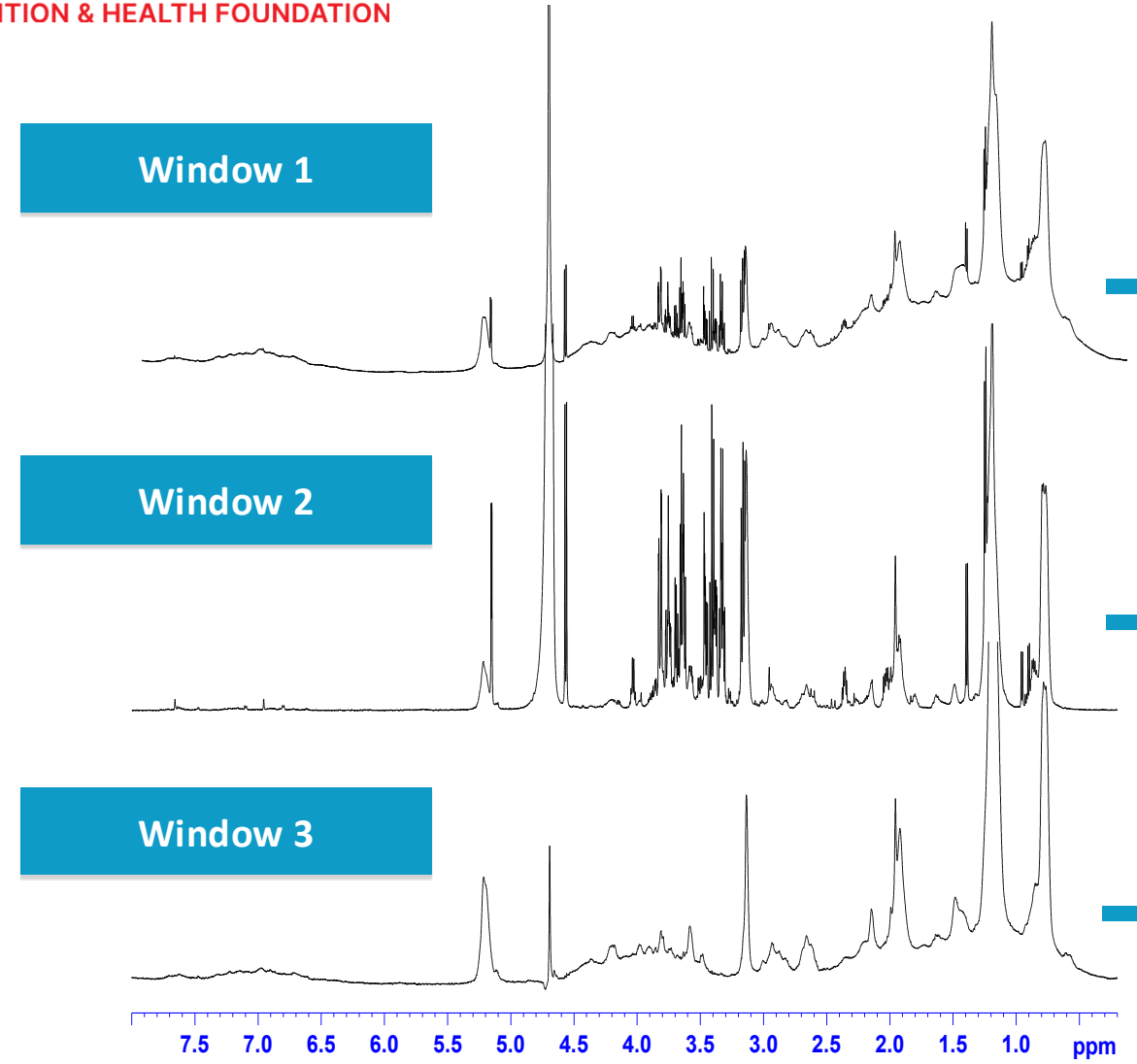
^{1}H NMR-based metabolic profiling of blood plasma
1 sample for multiple "metabolic windows



No sample preparation
3 min/spectrum , 15 min/sample

Standard Noesy spectrum
Macromolecular species (lipoproteins)
+ several small molecules (amino acids, glucose,..)

Standard CPMG spectrum
Focus on low molecular weight metabolites
(organic acids, amino acids, osmolytes,…)

Standard Diffusion-edited spectrum
Focus on high molecular weight metabolites
(lipoproteins, proteins and glycoproteins…)

Spectra registered at 600 MHz

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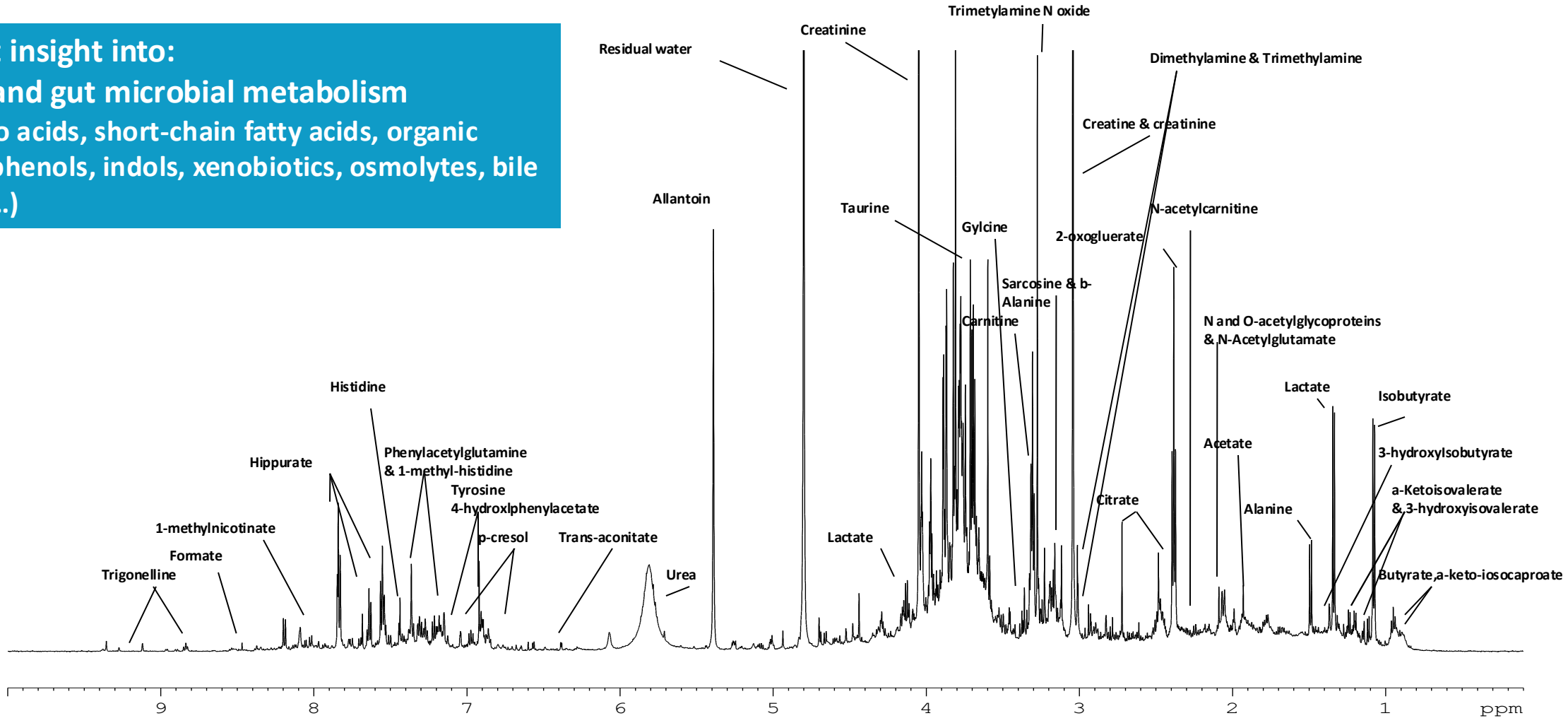
^{1}H NMR-based metabolic profiling of urine

Direct insight into:
Host and gut microbial metabolism
(Amino acids, short-chain fatty acids, organic acids,phenols, indols, xenobiotics, osmolytes, bile acids,…)



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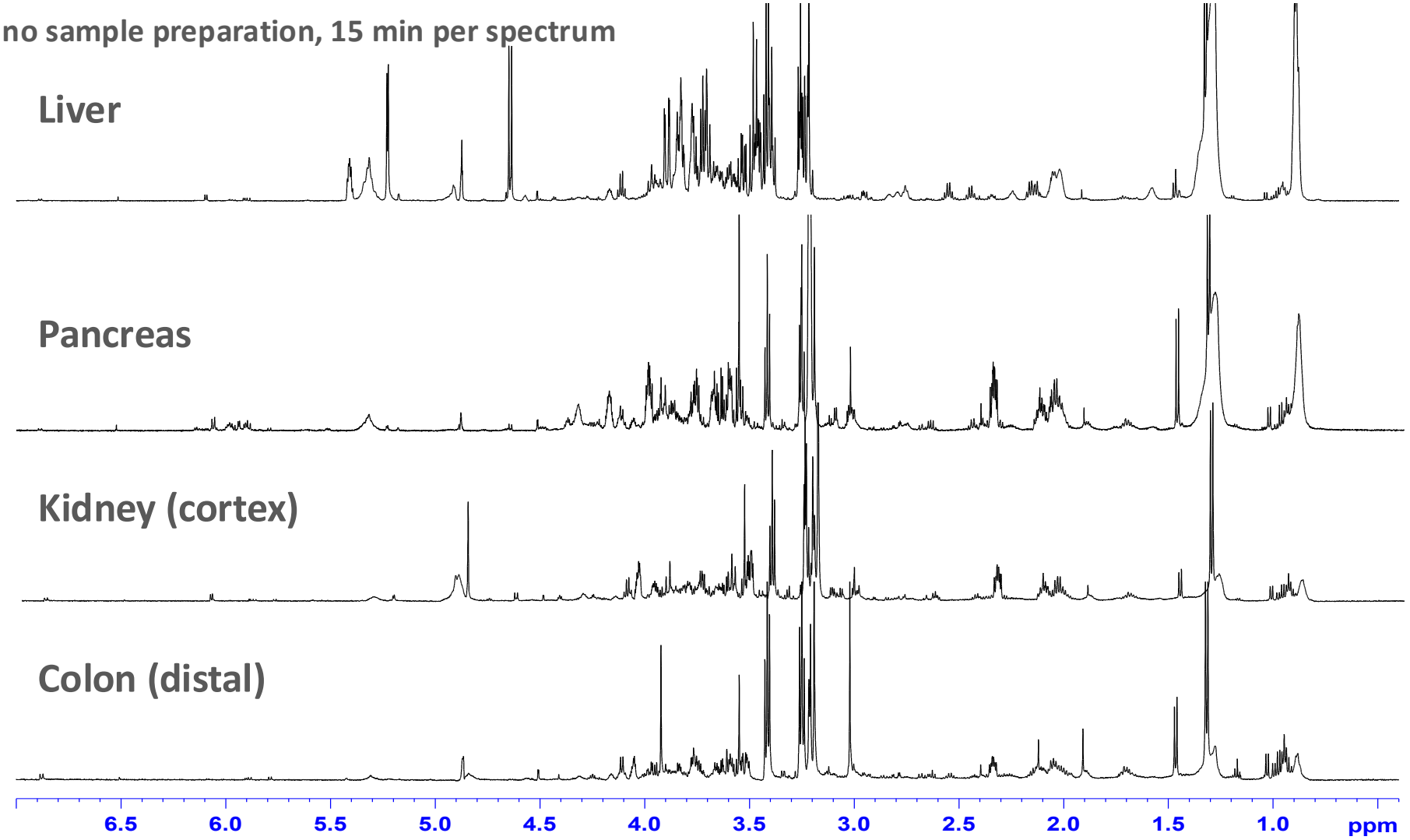
^{1}H NMR-based metabolic profiling of intact tissues
High resolution magic angle spinning NMR

Mouse tissues, no sample preparation, 15 min per spectrum



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Mass spectrometry based metabolic profiling

Untargeted approach

Used for biomarker discovery (scope: cover the broader possible range of metabolites)
Techniques:

- High resolution mass spectrometry (ex: Time Of Flight, Orbitrap technologies)
- Different chromatographic hyphenation techniques available
- Limitations: relative quantitation, can generate artefactual signals (ion adducts)

Targeted approach

Used when *a priori* metabolic knowledge is available on the investigated question (scope: obtain quantitative information)
Techniques:

- Tandem mass spectrometry
- Different chromatographic hyphenation techniques available
- Limitations: reduced panel of analytes or necessity to cumulate several analytical panels thus several analytical methods, sample preparation needs to be optimized for specific analyses

Other approaches

Stable-isotope assisted metabolomics (metabolic fluxes)
Molecular imaging techniques

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The metabol(n)omics data work flow
A multidisciplinary approach, from information to knowledge

Data acquisition

✓ Metabolic profiling using analytical chemistry techniques
✓ Signal processing steps to generate metabolic readouts
✓ Metabolite identification/ quantitation
✓ Main techniques: Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS)

Data mining

✓ Chemometrics: pattern recognition techniques (unsupervised, supervised)

✓ Identification/quantitation of metabolites of interest (differentiating groups)

Data interpretation

✓ Pathway analysis

✓ Data integration

✓ Biological interpretation (biomarkers, mechanism of action…)

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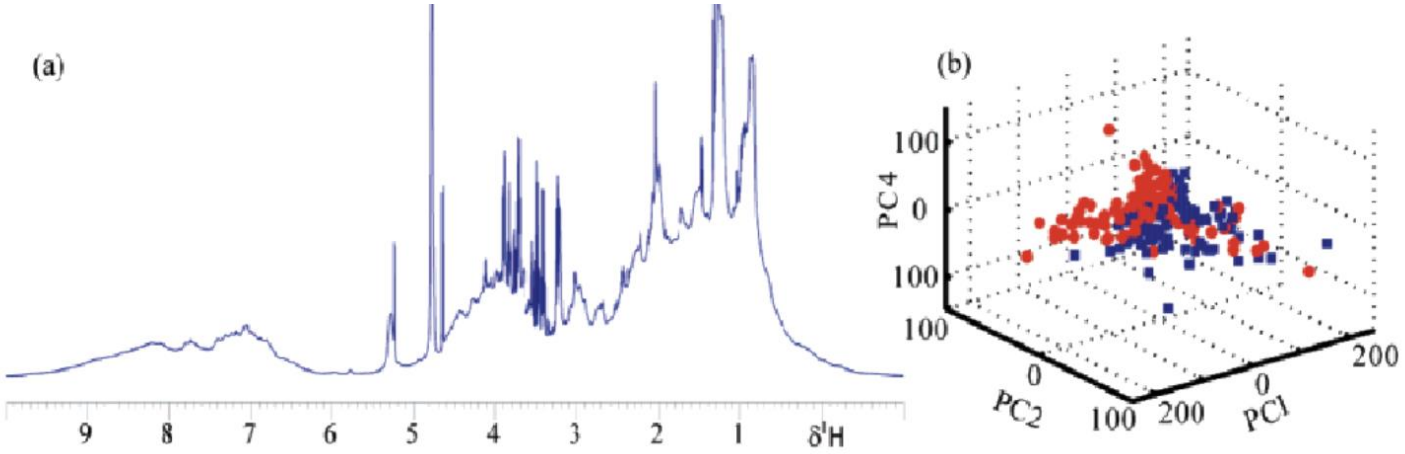
Examples of commonly used pattern recognition techniques

Unsupervised techniques

- No a priori knowledge (e.g. unknown sample class/assignment)
- Aim: to visualize the overall structure of the data (variance)
- Principal Component Analysis (PCA)
  - Reduces the multidimensional data space into its low-dimensional representation
  - Projects the data variance on space defines by principal components
  - Each PC (PC1, PC2…) express maximum amount of variance, which was not accounted for by previous PC
  - Allows to identify metabolic signals explaining the observed metabolic variance
- Others: robust PCA, Hierarchical cluster analysis, K-means



From: Rezzi, S., et al. (2007). Human metabolic phenotypes link directly to specific dietary preferences in healthy individuals. Journal of proteome research, 6 11, 4469-77 .

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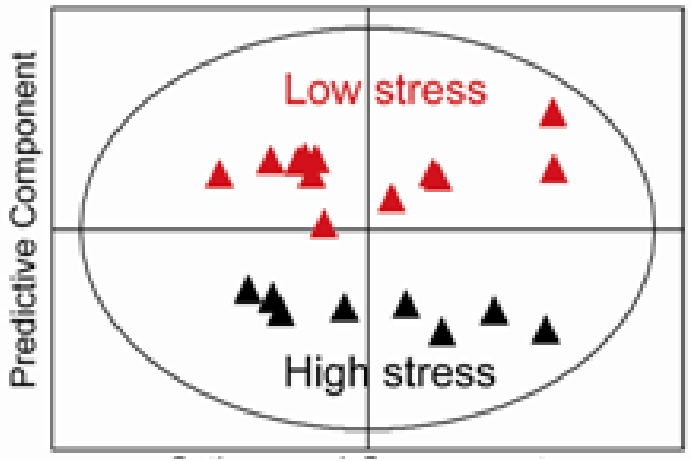
Examples of commonly used pattern recognition techniques

Supervised techniques

- *a priori* knowledge (e.g. known sample class/assignment)
- Aims: classification of samples or prediction of specific parameters (continuous variable)
- Regression techniques (partial least square), partial Least Squares Discriminant Analysis
  - Reduces the multidimensional data space into its low-dimensional representation
  - Projects the data on space defines by latent variables (e,g, linear combinations of original variables or metabolic signals)
  - Allows to identify metabolic signals showing correlations and explaining classification or prediction
- Others: Orthogonal PLS-DA, linear discriminant analysis
- Risk to build false classification models due to high dimensionality of the data→model validation is needed



From: Martin FP., S. Rezzi et al. J Proteome Res. 2009 Dec;8(12):5568-79.

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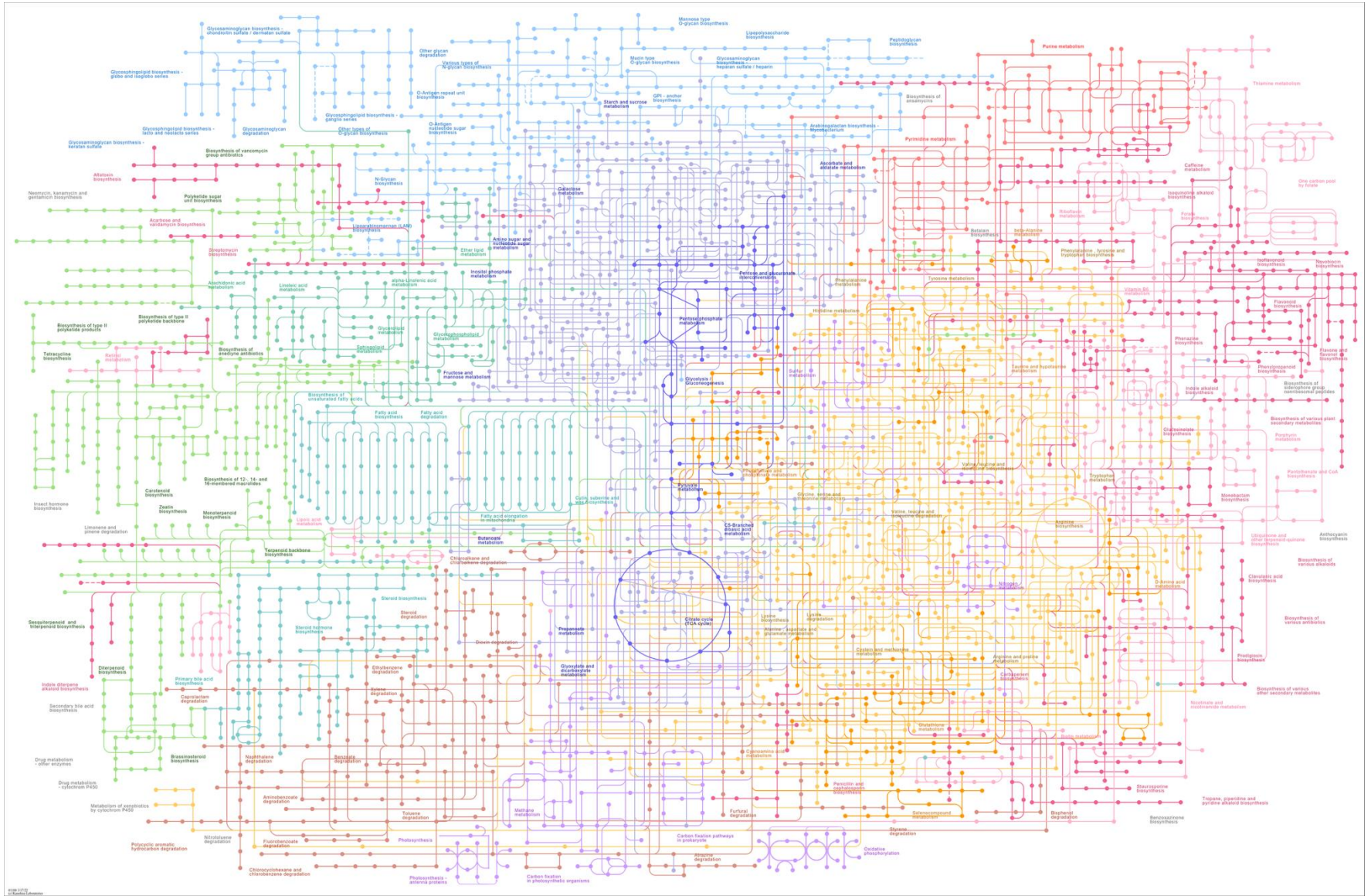
Data analysis and interpretation

Need to know how to read and understand metabolic pathways



Source
https://www.genome.jp/pathway/map01100+M00009

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Data analysis and interpretation

*

Tool	MetaboAnalyst	MeltDB	metaP-Server	SIMCA-P	SAS
Software type	Web-based	Web-based	Web-based	Stand-alone	Stand-alone
License	Free	Free (registry required)	Free	Commercial	Commercial
Data input	Data table, NMR, MS, GC-MS data, compound/peak lists	Raw mass spectral files	Data table	Data table	Data table
Graphical interface	+++	++	++	+++	+/−
Normalization	+++	+	+	++	++
Univariate analysis	+++	++	+++		+++
Multivariate analysis	+++	++	+	+++	+++
Clustering	+++	++			++
Classification	++				++
Enrichment analysis	++				
Pathway analysis	+++		+		
Pathway visualization	++				
Integration with other omics data		+			
Peak annotation	++	+++			

The level of support for a particular feature is rated by the number of '+', with '+++' as the highest.

* from: J. Xia & D.S. Wishart, Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst, Nature protocols, 6, 2011, 743

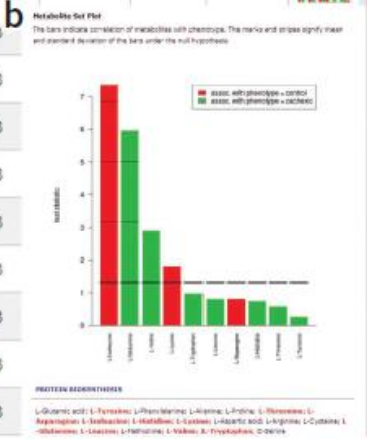
*

Example of results from metabolite set enrichment analysis

a

Metabolite Set	Total	Hit	Statistic	Expected	P Value	Holm P	FDR	Details
PYRIMIDINE METABOLISM	36	3	5.9815	1.3158	0.0026037	0.11977	0.065958	
BETA-ALANINE METABOLISM	13	2	7.5707	1.3158	0.0028677	0.12905	0.065958	
KETONE BODY METABOLISM	10	1	9.089	1.3158	0.0077101	0.33925	0.10832	
PURINE METABOLISM	45	2	5.9459	1.3158	0.0094194	0.40503	0.10832	
GLUTAMATE METABOLISM	18	3	4.2634	1.3158	0.019852	0.8338	0.18264	
VALINE, LEUCINE AND ISOLEUCINE DEGRADATION	36	3	3.6972	1.3158	0.060012	1.0	0.4159	
INOSITOL METABOLISM	19	1	4.5238	1.3158	0.063289	1.0	0.4159	
MITOCHONDRIAL ELECTRON TRANSPORT CHAIN	15	2	3.3111	1.3158	0.062012	1.0	0.42415	
PROTEIN BIOSYNTHESIS	19	10	2.2262	1.3158	0.093859	1.0	0.42415	
BETAINE METABOLISM	10	2	2.9882	1.3158				
CITRIC ACID CYCLE	23	6	2.4262	1.3158				
PANTOTHENATE AND COA BIOSYNTHESIS	10	1	3.1919	1.3158				
GALACTOSE METABOLISM	25	3	2.4909	1.3158				
PROPANOATE METABOLISM	18	1	2.9087	1.3158				
TYROSINE METABOLISM	38	3	2.3462	1.3158				
PHOSPHOLIPID BIOSYNTHESIS	19	1	2.7762	1.3158				
UREA CYCLE	20	4	2.1039	1.3158				
METHIONINE METABOLISM	24	4	1.8857	1.3158				

b



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Metabolomics application domains

- Pharmaceutical research (drug toxicity, efficacy, drug response prediction)
- Environment research (physical & chemical exposures, biofuel production)
- Molecular epidemiology (biomarker identification & validation, gene-metabolome associations, developmental biology)
- Nutrition (Nutritional Metabolomics)
  - Dietary pattern footprints
  - Dietary intake biomarkers
  - Gut-host metabolic cross talk
  - Metabolic effects of nutritional intervention

Phenotyping stratification for preventive medicine and medical care: Phenome Center concept

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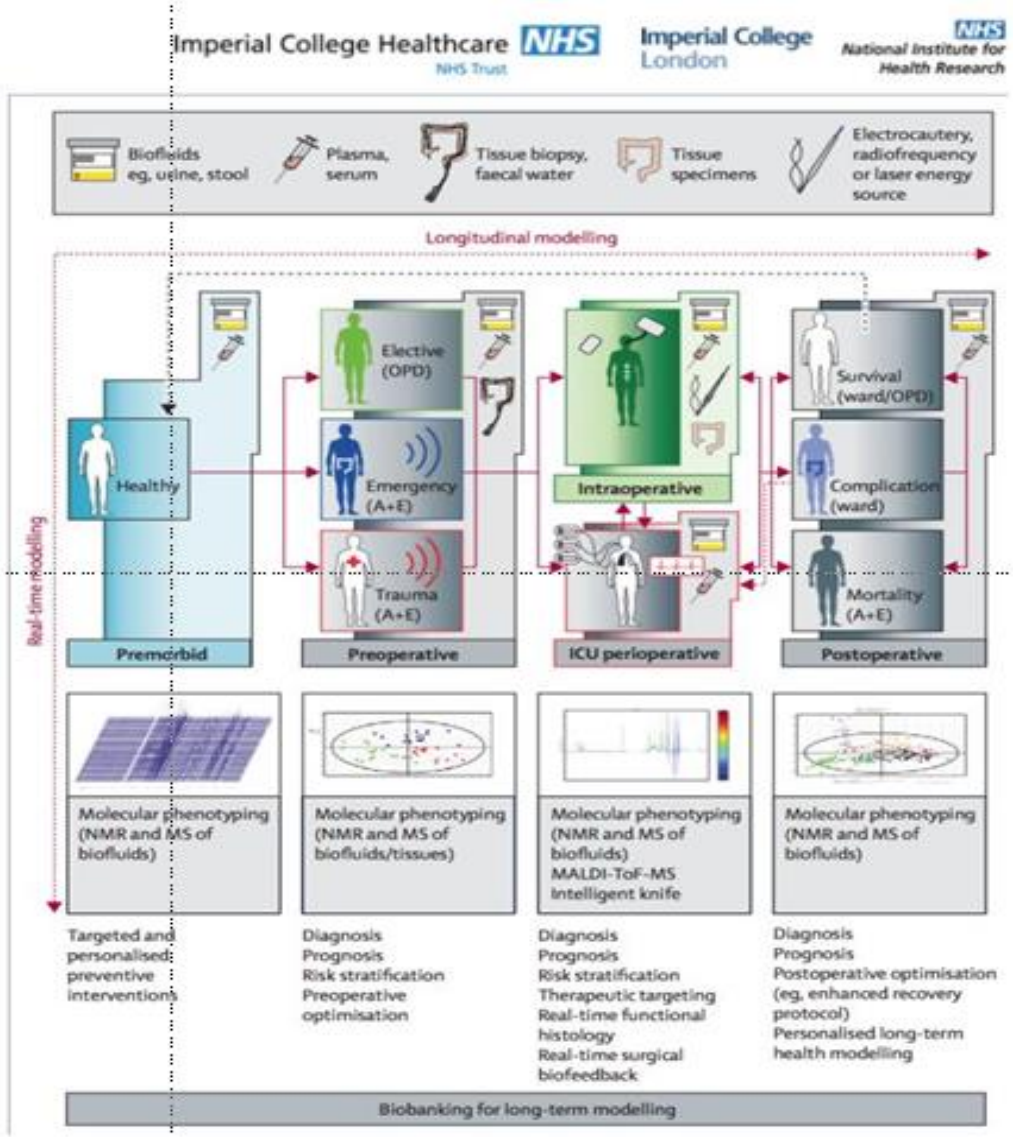
Imperial Comprehensive Biomedical Research Centre
Translating research into patient benefits

'Phenotyping The Patient Journey'

THE LANCET

Kinross, J. Holmes, E. Darzi, A. and Nicholson JK: Metabolic Monitoring of Surgical Patients. (May 28th 2011, Vol 377 1817-1819)



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Pharmaco-Metabonomics to enable prognostic biomarker discovery for patient stratification

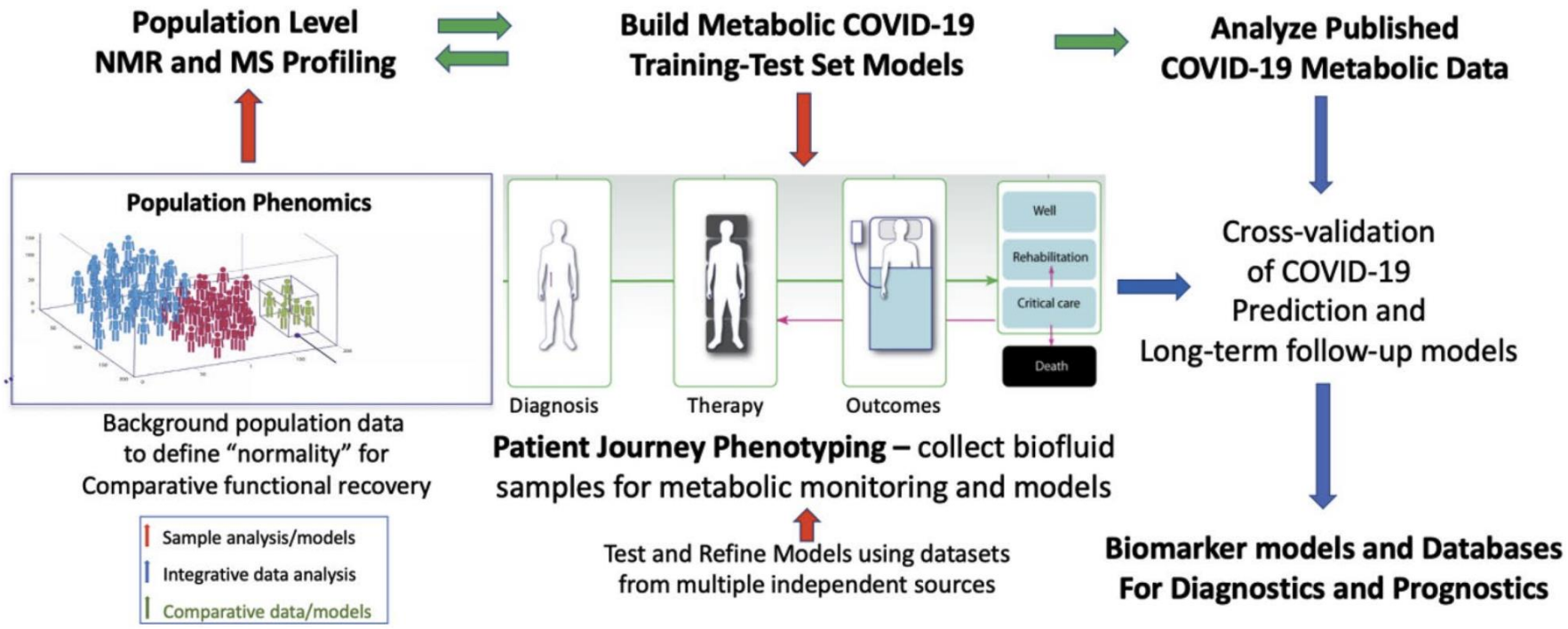

Fig. 1 A framework for understanding the natural history of an emergent zoonotic disease using metabotyping: schematic illustration of the collective COVID-19 patient journey from health to disease using a metabolic systems framework to assess disease progression and recovery in relation to associated studies that enable model cross-validation through published literature and sequential analysis of multiple disease cohort samples. The population phenomics box illustrates the collection of different metabolic signatures from population subgroups some of which may have different disease risks

Source: Nicholson JK. Molecular Phenomic Approaches to Deconvolving the Systemic Effects of SARS-CoV-2 Infection and Post-acute COVID-19 Syndrome. Phenomics. 2021 Jul 22:1-8.

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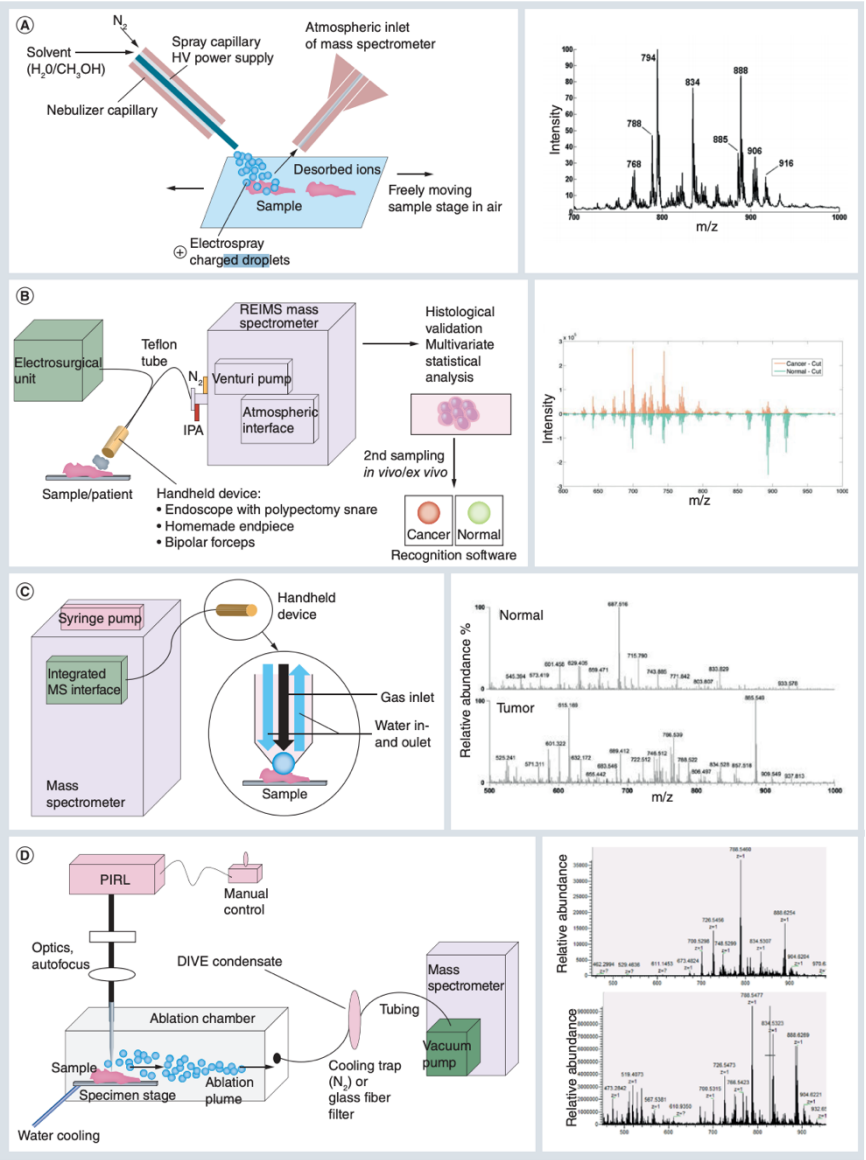
Mass spectrometry-based intraoperative tumor diagnostics



Schematic figure of mass spectrometry based methods for intraoperative cancer diagnosis (left) with corresponding examples of mass spectra (right)

iKnife. Rapid evaporative mass spectrometry coupled to eligible surgical devices

MasSpec pen.

Picosecond infrared laser

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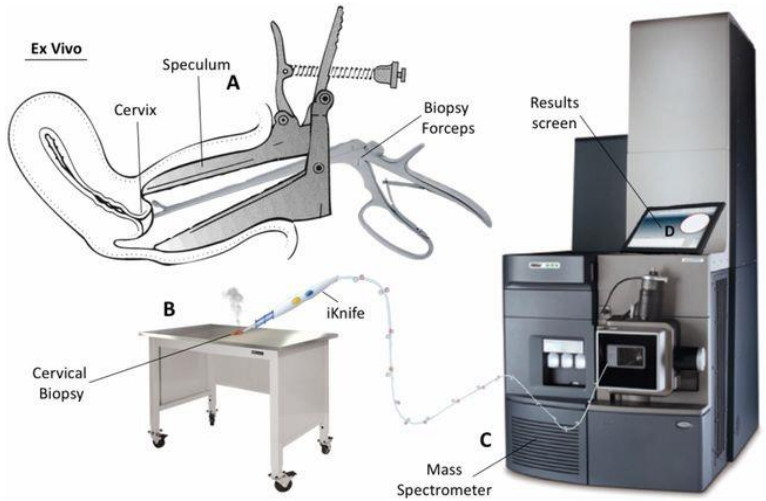
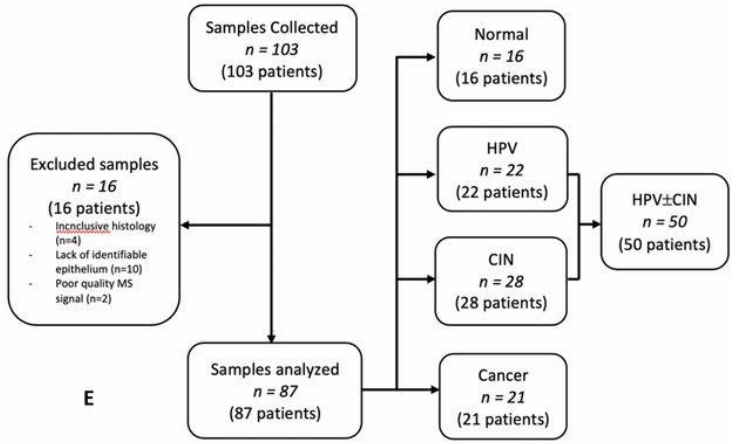
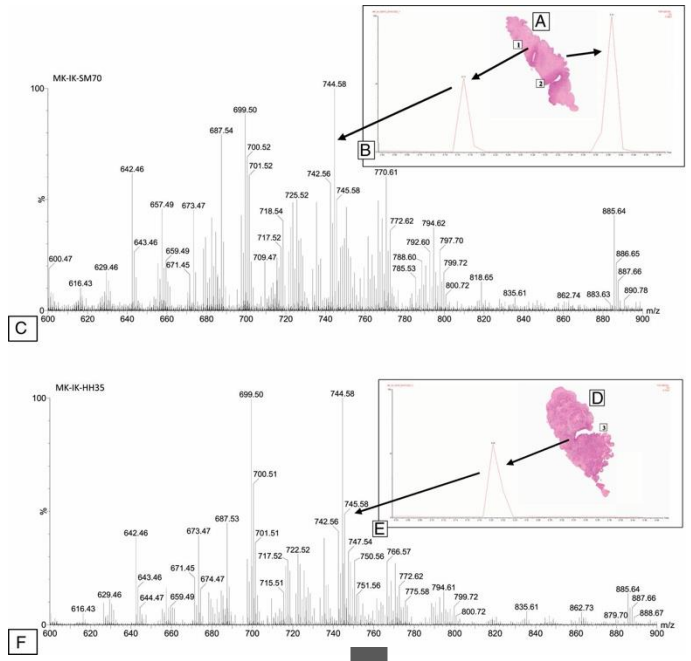
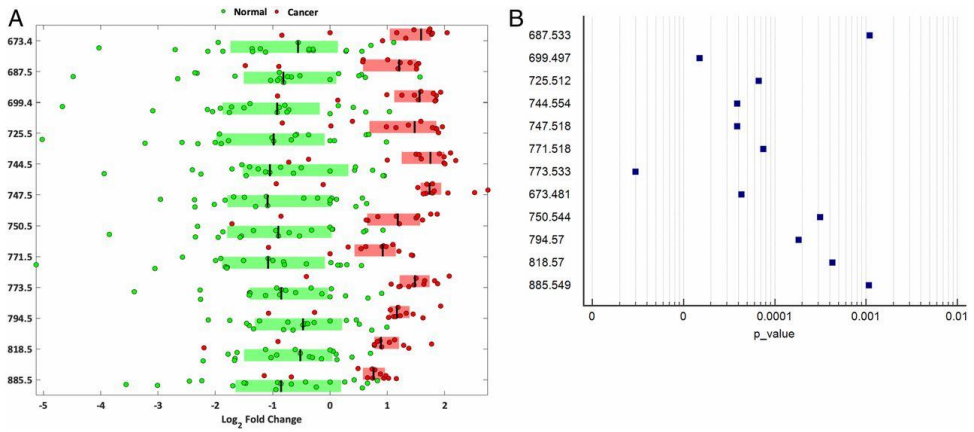
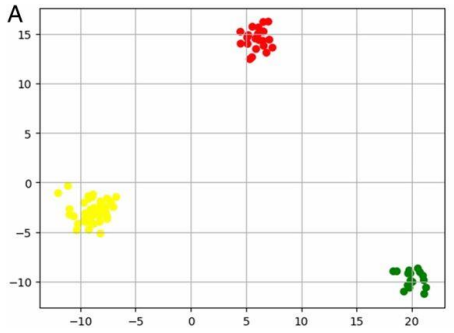
The intelligent knife (iKnife) and its intraoperative diagnostic advantage for the treatment of cervical disease











B

Proportion (%) of classification when correlating iKnife diagnosis to gold standard histology				
Predicted iKnife diagnosis	Histopathological Diagnosis			Cross Validation
	Normal (n=16)	HPV ± CIN (n=50)	Cancer (n=21)	
Normal	100% (16/16)	0% (0/50)	0% (0/21)	100%
HPV±CIN	0% (0/16)	100% (50/50)	0% (0/21)	
Cancer	0% (0/16)	0% (0/50)	100% (21/21)	

Normal Cervical Tissue
HPV ± CIN
Cancerous Cervical Tissue

Tzafetas M et al. Proc Natl Acad Sci U S A. 2020 31;117(13):7338-7346

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Advantages and limitations of NMR and MS 1/3

	NMR	Mass Spectrometry
Reproducibility	High reproducibility is one of the fundamental advantages of NMR spectroscopy.	Compared to NMR spectroscopy, MS data are less reproducible.
Sensitivity	Intrinsically low but can be improved with multiple scans (time), higher magnet field strength, cryo-cooled and microprobes, and hyperpolarization methods.	High sensitivity is a major advantage of MS; metabolites with nanomolar concentrations can be readily detected
Selectivity	NMR is generally used for nonselective analysis. Peak overlaps from multiple detected metabolites pose major challenges.	MS is selective. However, in combination with chromatography (such as liquid and gas phase separation), it is a superior tool for targeted analysis.
Sample measurement	Enables relatively fast measurement using 1D ^{1}H-NMR spectroscopy, where all metabolites at a detectable concentration level can be observed in one measurement.	Different ionization methods are required to maximize the number of detected metabolites.

Emwas et al. Metabolites 2019, 9, 123

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Advantages and limitations of NMR and MS 2/3

	NMR	Mass Spectrometry
Sample preparation	Involves minimal sample preparation, usually transferring the sample to an NMR tube and adding deuterated locking solvent. Can be automated.	More demanding; requires chromatography; requires sample derivatization for gas chromatography (GC)-MS.
Sample recovery	NMR is nondestructive and, hence, several analyses can be carried out on the same sample. Additionally, the sample can be recovered and stored for a long time.	MS is destructive technique; therefore, the sample cannot be recovered. However, it needs only a small amount of sample.
Quantitative analysis	NMR is inherently quantitative as the signal intensity is directly proportional to the metabolite concentrations and number of nuclei in the molecule.	The intensity of the MS line is often not correlated with metabolite concentrations as the ionization efficiency is also a determining factor.

Emwas et al. Metabolites 2019, 9, 123

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Advantages and limitations of NMR and MS 3/3

	NMR	Mass Spectrometry
Fluxomics Analysis	NMR permits both in vitro and in vivo metabolic flux analyses. Its inherently quantitative nature also enables precise quantification of precursors and products. Mapping of stable isotope locations and incorporating points in molecules is very easy via NMR.	MS can be used for fluxomics analysis; however, the destructive nature of MS-based methods means it is somewhat more limited than NMR-based fluxomics. In vivo fluxomics is not possible with MS, and isotope mapping is more difficult.
Tissue samples	Using high-resolution magic-angle sample spinning (HRMAS) NMR, it is possible to detect metabolites in tissue samples.	Although some MALDI-TOF approaches can be used to detect metabolites in tissue samples, these approaches are still far from being routine.
Number of detectable metabolites	Depending on spectral resolution, usually less than 200 metabolites can be unambiguously detected and identified in one measurement.	Using different MS techniques, it is possible to detect thousands of different metabolites and identify several hundred.
Targeted analysis	NMR spectroscopy can be used for both targeted and untargeted analyses, but it is not commonly used for targeted analyses.	Both GC-MS and liquid chromatography (LC)-MS are superior for targeted analyses
In vivo studies	Using magnetic resonance spectroscopy (MRS), in vivo investigation can be carried out most often using nuclei such as ^{1}H and ^{31}P.	Although desorption electrospray ionization (DESI) may be a useful way to analyze tissue samples during surgery, MS is not used for in vivo metabolomics studies.

Emwas et al. Metabolites 2019, 9, 123

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Lipidomics

The large-scale study of pathways and networks of cellular lipids in biological systems.

Lipidome: comprehensive set of lipids at different levels of the biological organization (cell, tissue, organism)

Subset of the "metabolome"

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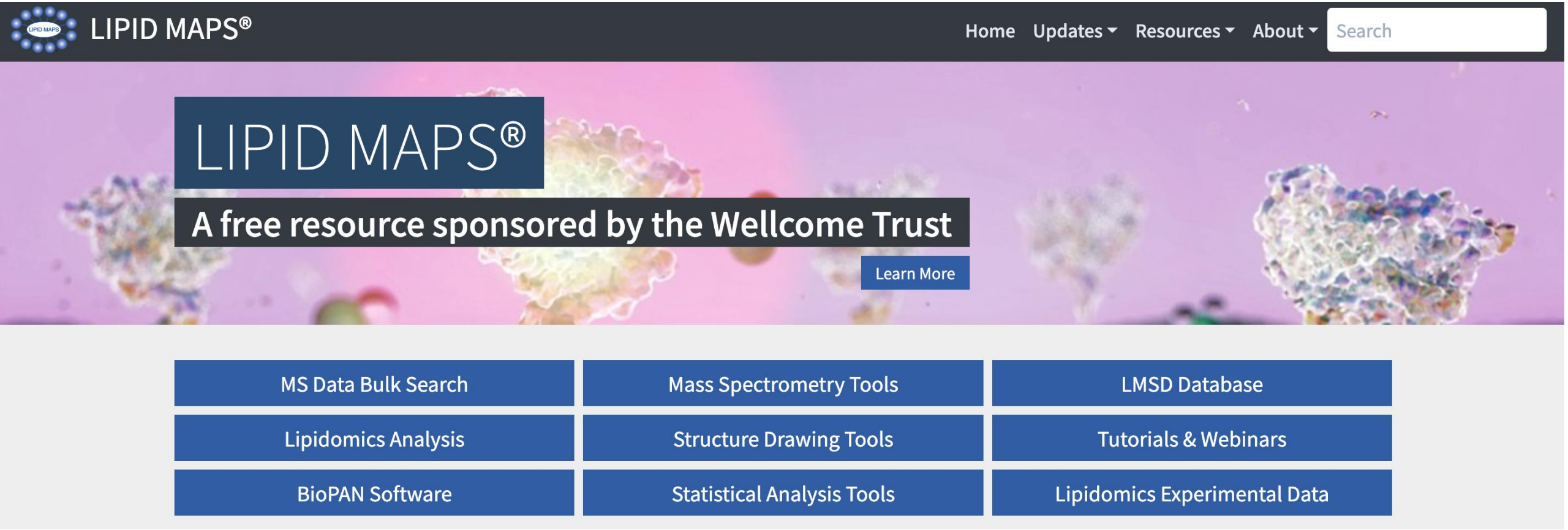
Lipid Maps
A lipid specialized knowledge base



https://www.lipidmaps.org/

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Lipid nomenclature

Lipids may be broadly defined as hydrophobic or amphiphilic small molecules that originate entirely or in part from two distinct types of biochemical subunits or "building blocks": ketoacyl and isoprene groups.

The lipid classification scheme is chemically based and driven by the distinct hydrophobic and hydrophilic elements that compose the lipid

Lipids may be divided into eight categories:

Fatty acyls	Saccharolipids
Glycerolipids	Polyketides
Glycerophospholipids	Sterol lipids
Sphingolipids	Prenol lipids

* Fahy,E. *et al*, Journal of Lipid Research. 2005 Vol. 46, 839-862

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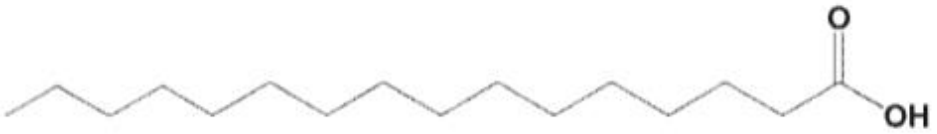
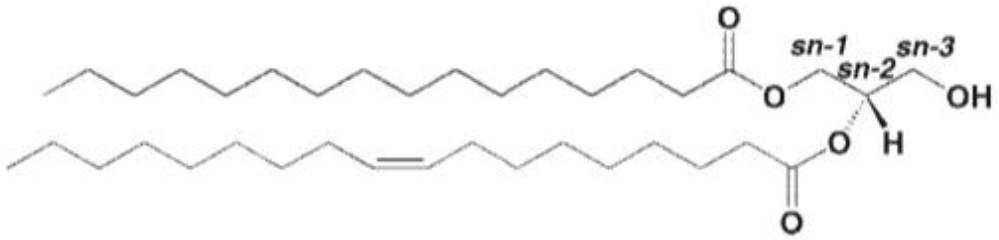
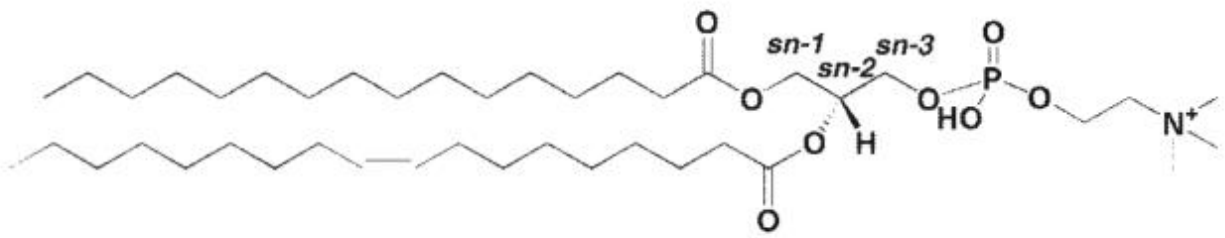
Representative structures 1/2



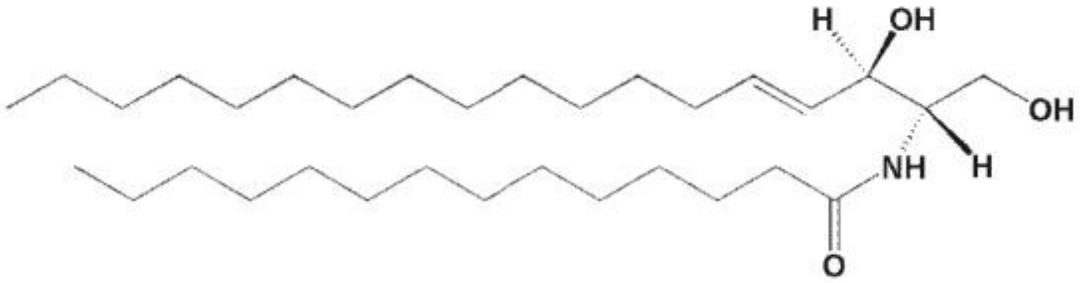
(a) Fatty Acyls: hexadecanoic acid



(b) Glycerolipids: 1-hexadecanoyl-2-(9Z-octadecenoyl)-*sn*-glycerol



(c) Glycerophospholipids: 1-hexadecanoyl-2-(9Z-octadecenoyl)-*sn*-glycero-3-phosphocholine



(d) Sphingolipids: N-(tetradecanoyl)-sphing-4-enine

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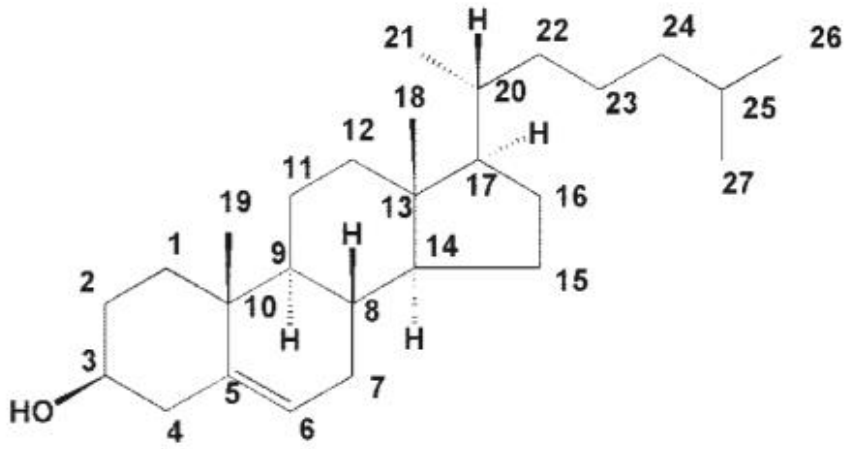
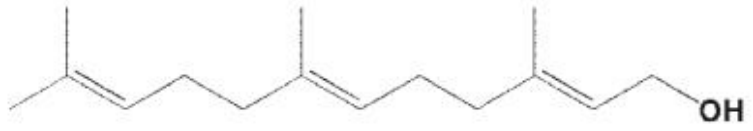
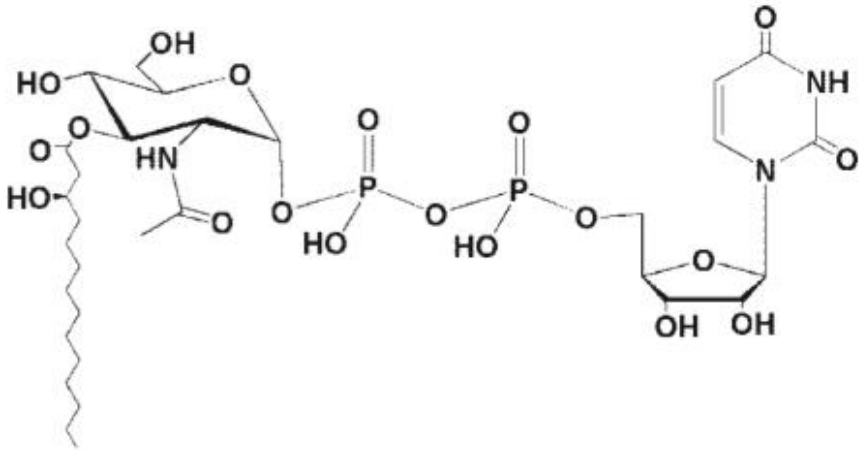
Representative structures 2/2



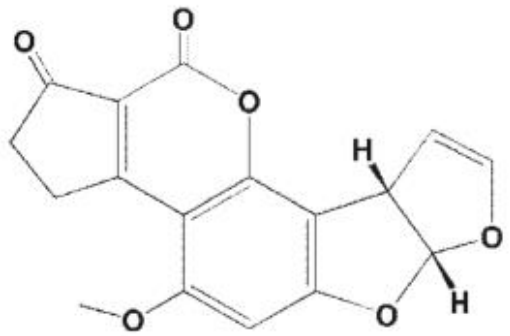
(e) Sterol Lipids: cholest-5-en-3β-ol



(f) Prenol Lipids: 2E,6E-farnesol



(g) Saccharolipids: UDP-3-O-(3R-hydroxy -tetradecanoyl)-αD-N-acetylglucosamine



(h) Polyketides: aflatoxin B1

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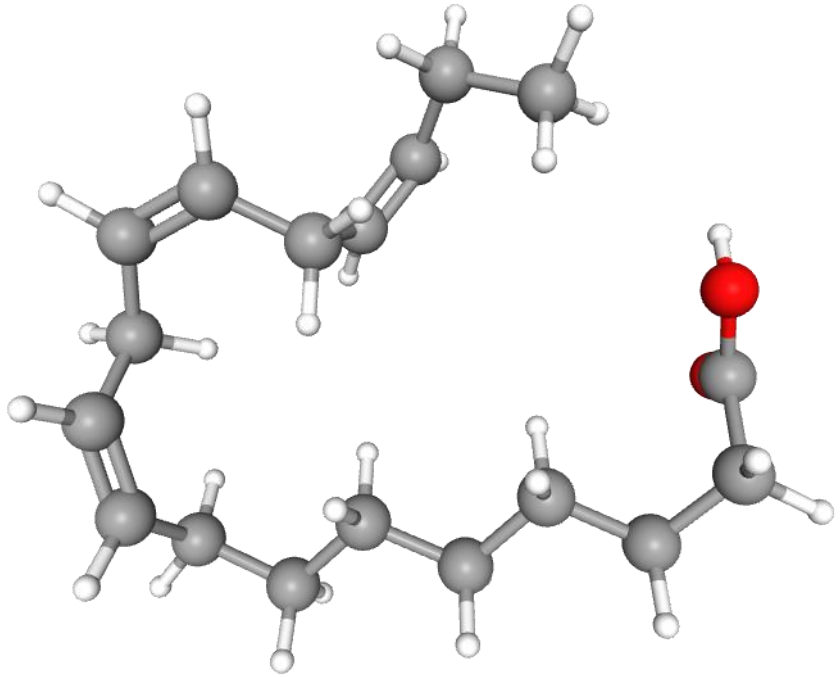
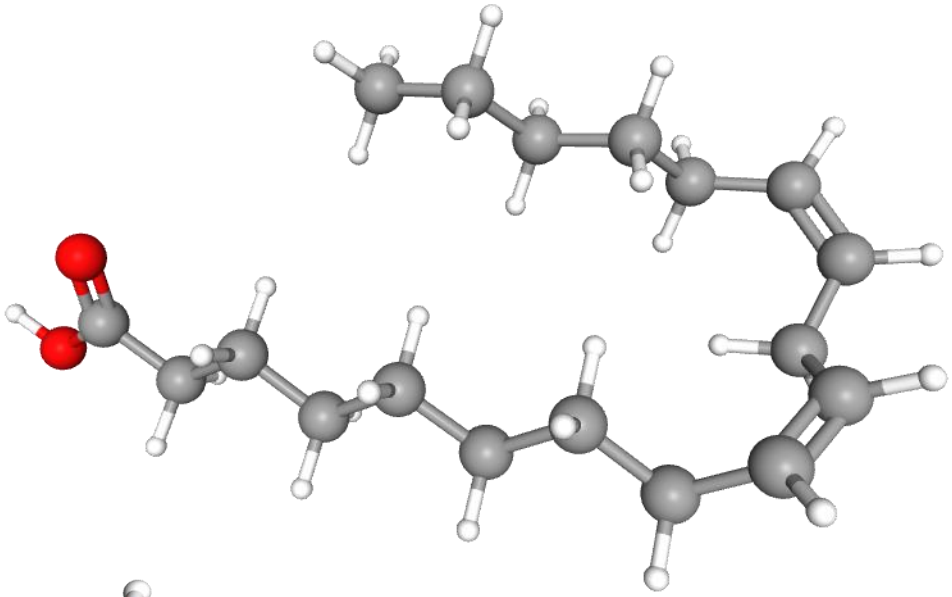
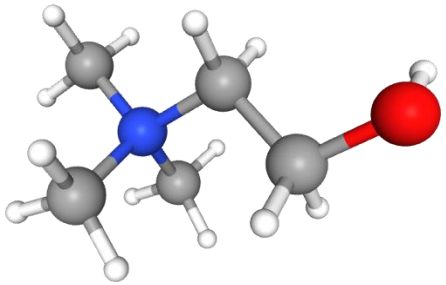
Essential lipids and lipid components for humans

alpha-linolenic acid (omega-3 fatty acid)



linoleic acid (omega-6 fatty acid)





choline

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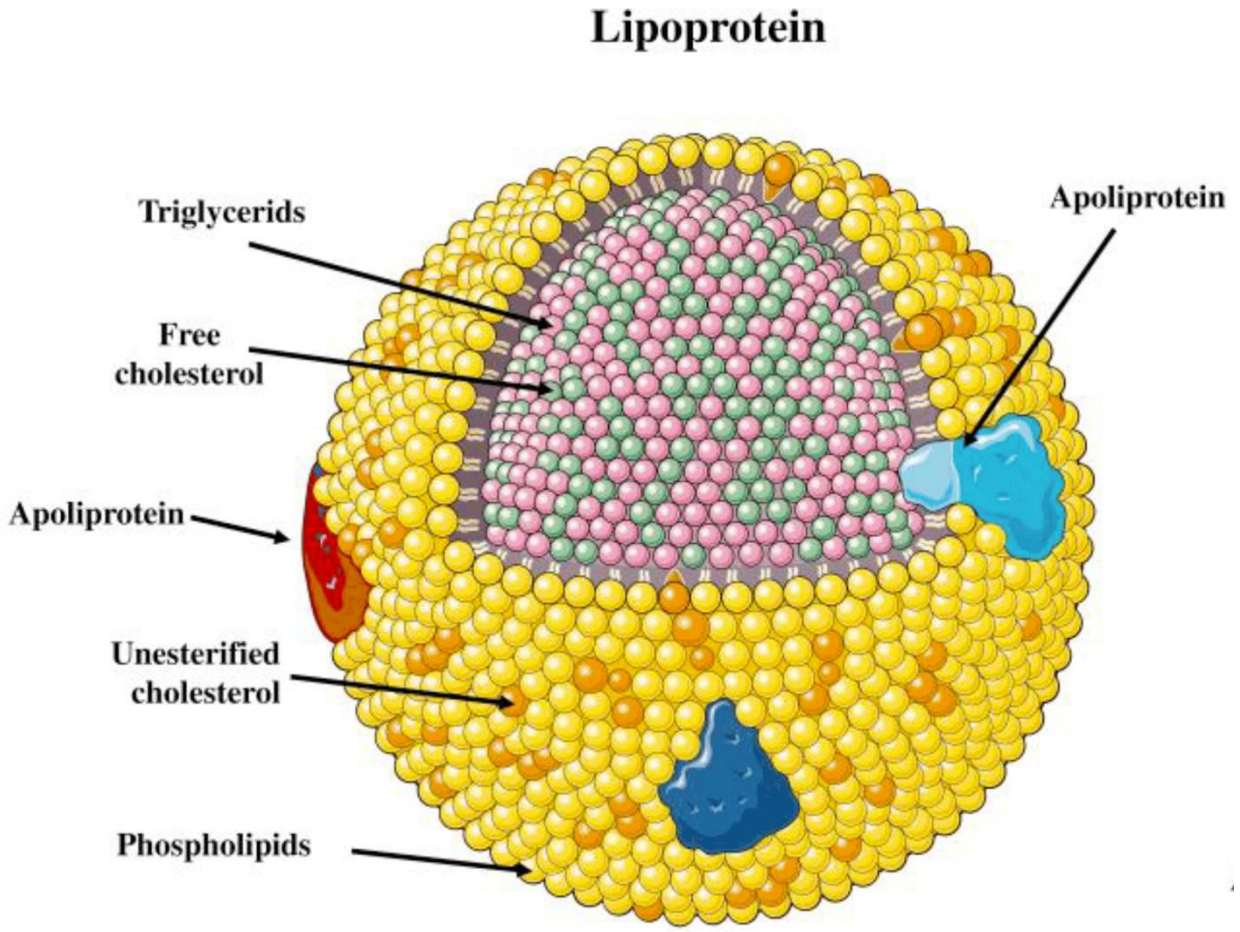
Transport of lipids via lipoproteins

Lipoproteins are complex particles that have a central hydrophobic core of non-polar lipids, primarily cholesterol esters and triglycerides.

The hydrophobic core is surrounded by a hydrophilic membrane consisting of phospholipids, free cholesterol, and apolipoproteins.

Lipoproteins are important for transporting cholesterol, triglycerides and liposoluble nutrients (vitamins) throughout the body.



Source: Lampsas S et al. Lipoprotein(a) in Atherosclerotic Diseases: From Pathophysiology to Diagnosis and Treatment. Molecules. 2023; 28(3):969. https://doi.org/10.3390/molecules28030969

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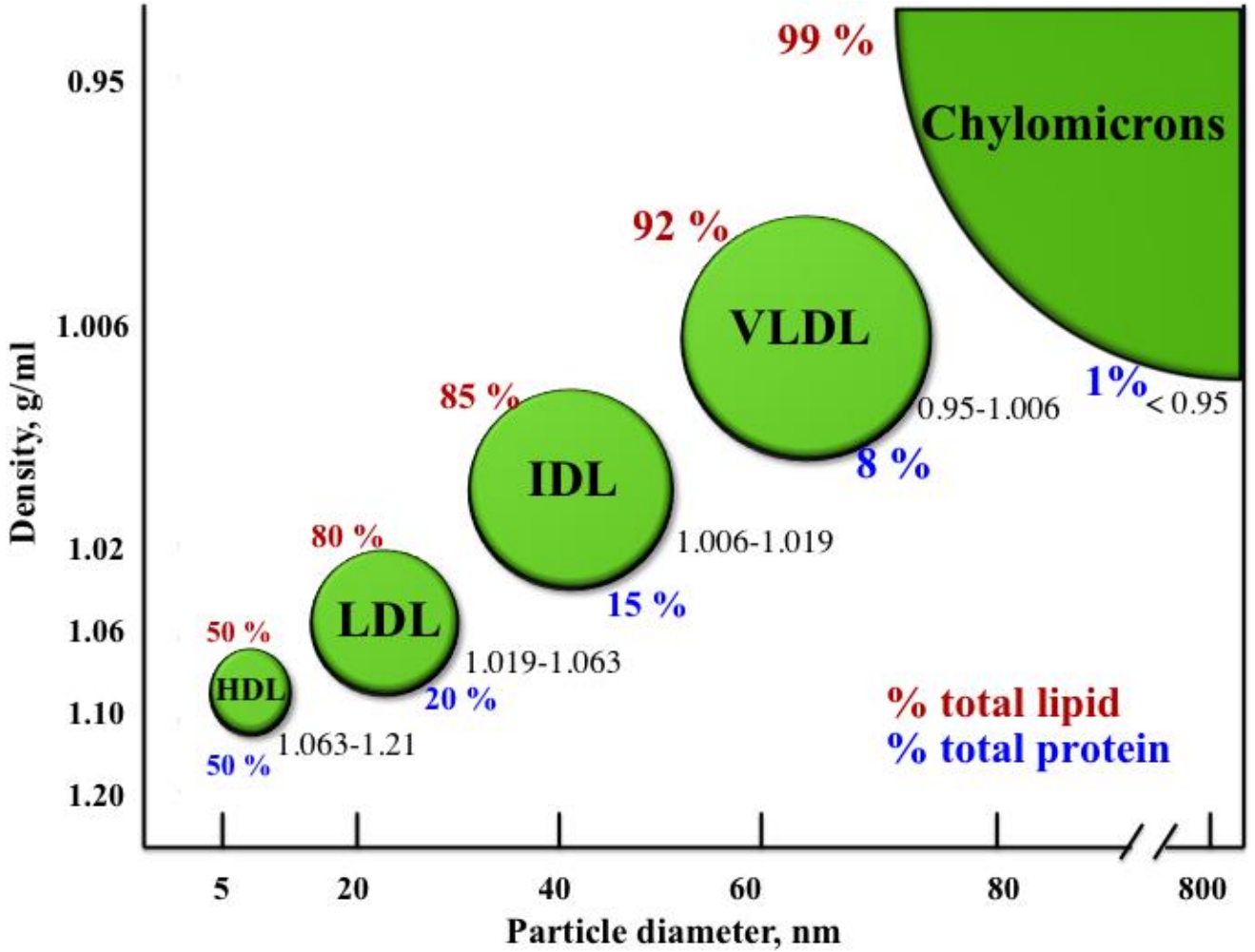
Transport of lipids via lipoproteins

Very low-density lipoproteins (VLDLs) (0.96-1.006 g/cm³)
- transport triglycerides from the liver to various tissues in the body for energy or storage.

Intermediate-density lipoproteins (IDLs) (1.007-1.019 g/cm³)
- transport a variety of fats and cholesterol left from VLDLs from the tissues to the liver

Low-density lipoproteins (LDLs) (1.020-1.062 g/cm³)
- transport cholesterol and other lipids from the liver to tissues.

High-density lipoproteins (HDLs) (1.063-1.210 g/cm³)
- transport cholesterol out of the bloodstream and into the liver, where it is either reused or removed from the body with bile.



The classification of the major types of lipoproteins are based on their densities. Density range is shown as well as lipid (red) and protein (blue) content.

Source:
https://chem.libretexts.org/Bookshelves/Introductory_Chemistry/Map%3A_Fundamentals_of_General_Organic_and_Biological_Chemistry_%28McMurry_et_al.%29/24%3A_Lipid_Metabolism/24.02%3A_Lipoproteins_for_Lipid_Transport

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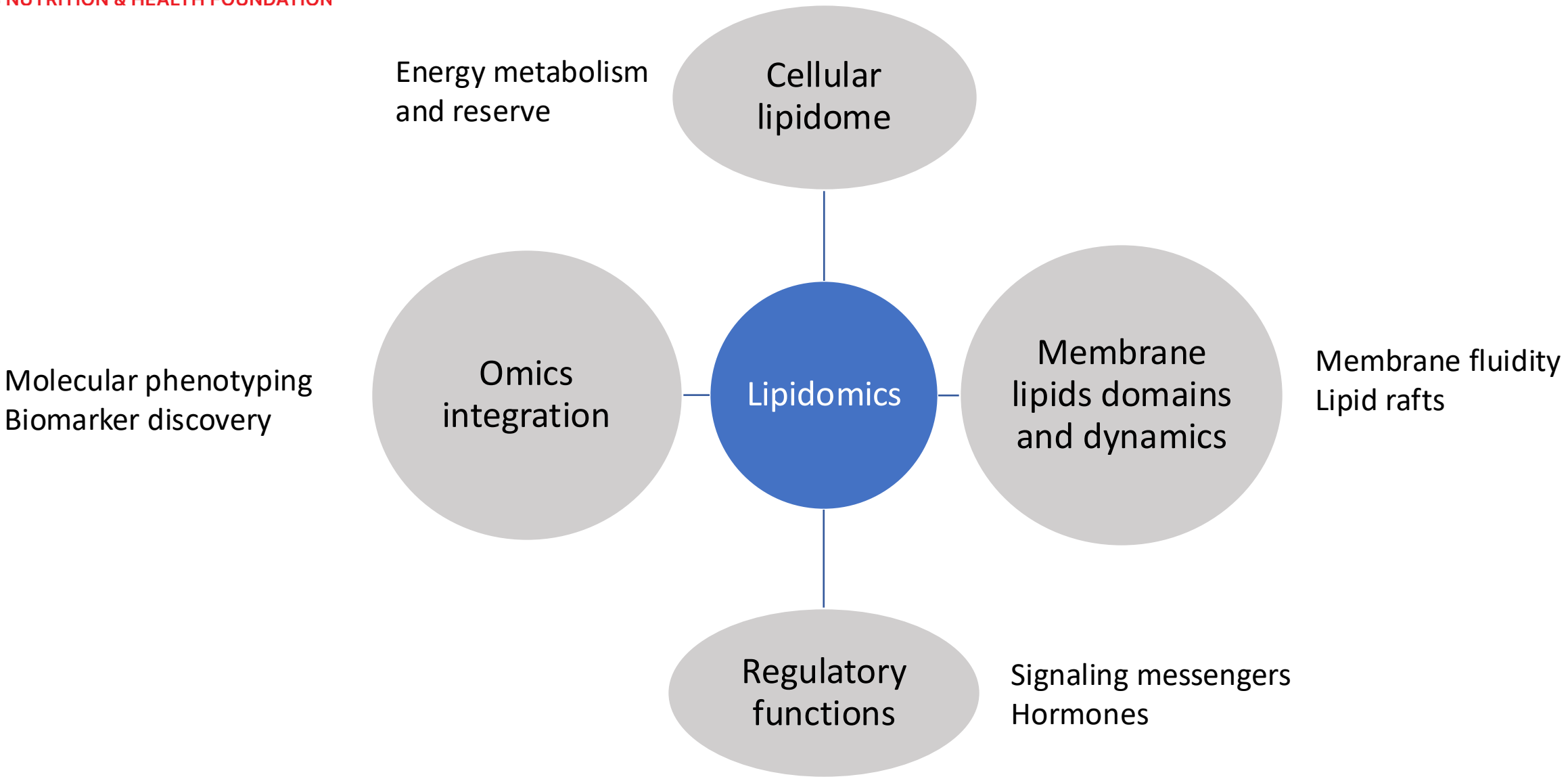
Lipidomics main application fields



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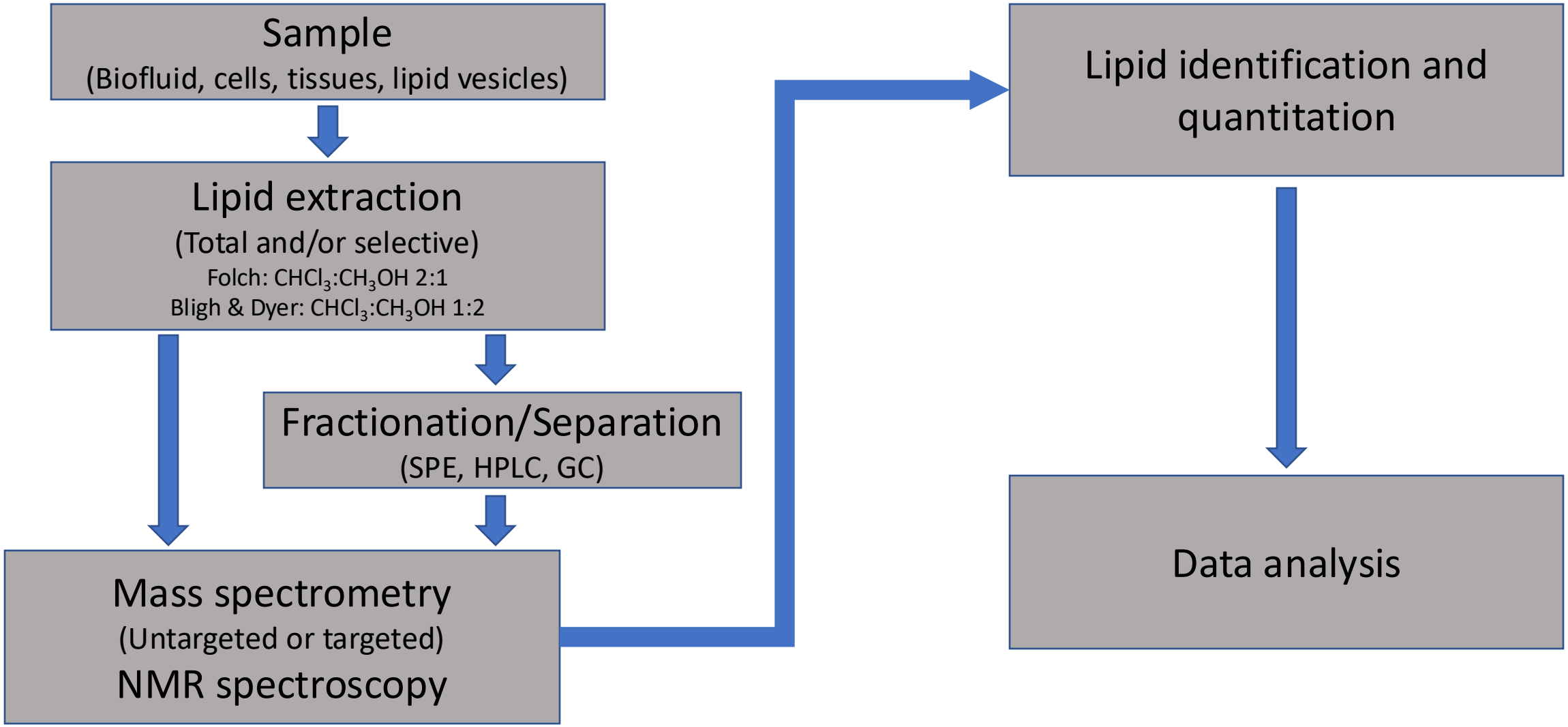
Lipidomics workflow for MS and NMR



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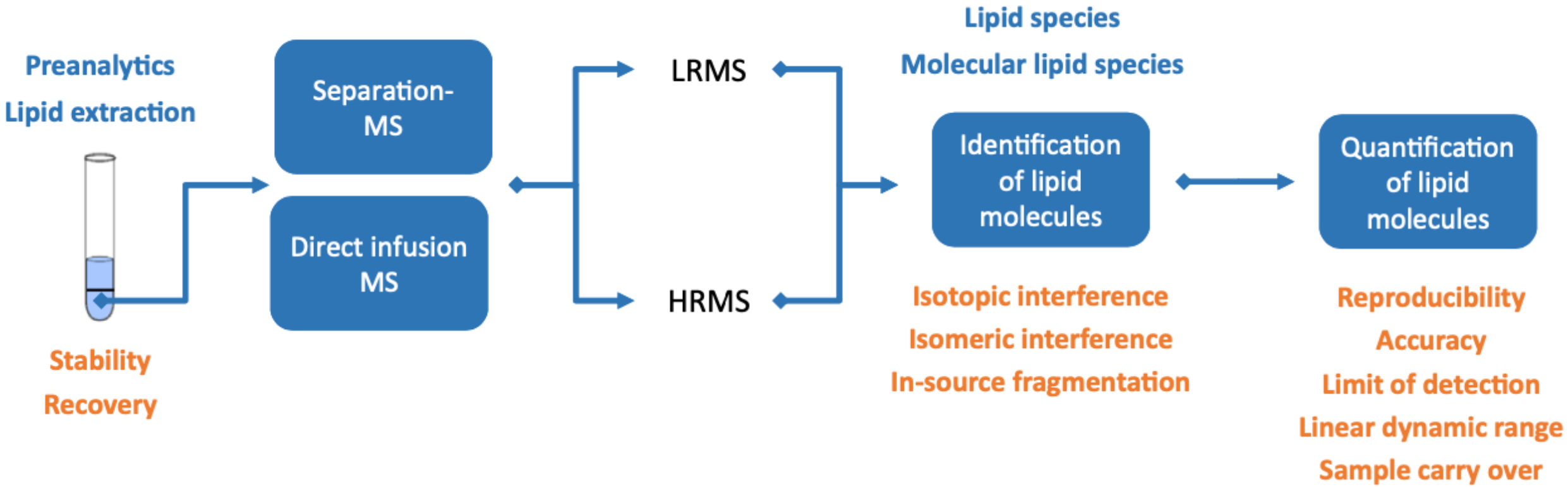
Lipidomics workflow for MS



Köfeler et al. J. Lipid Res. (2021) 62 100138

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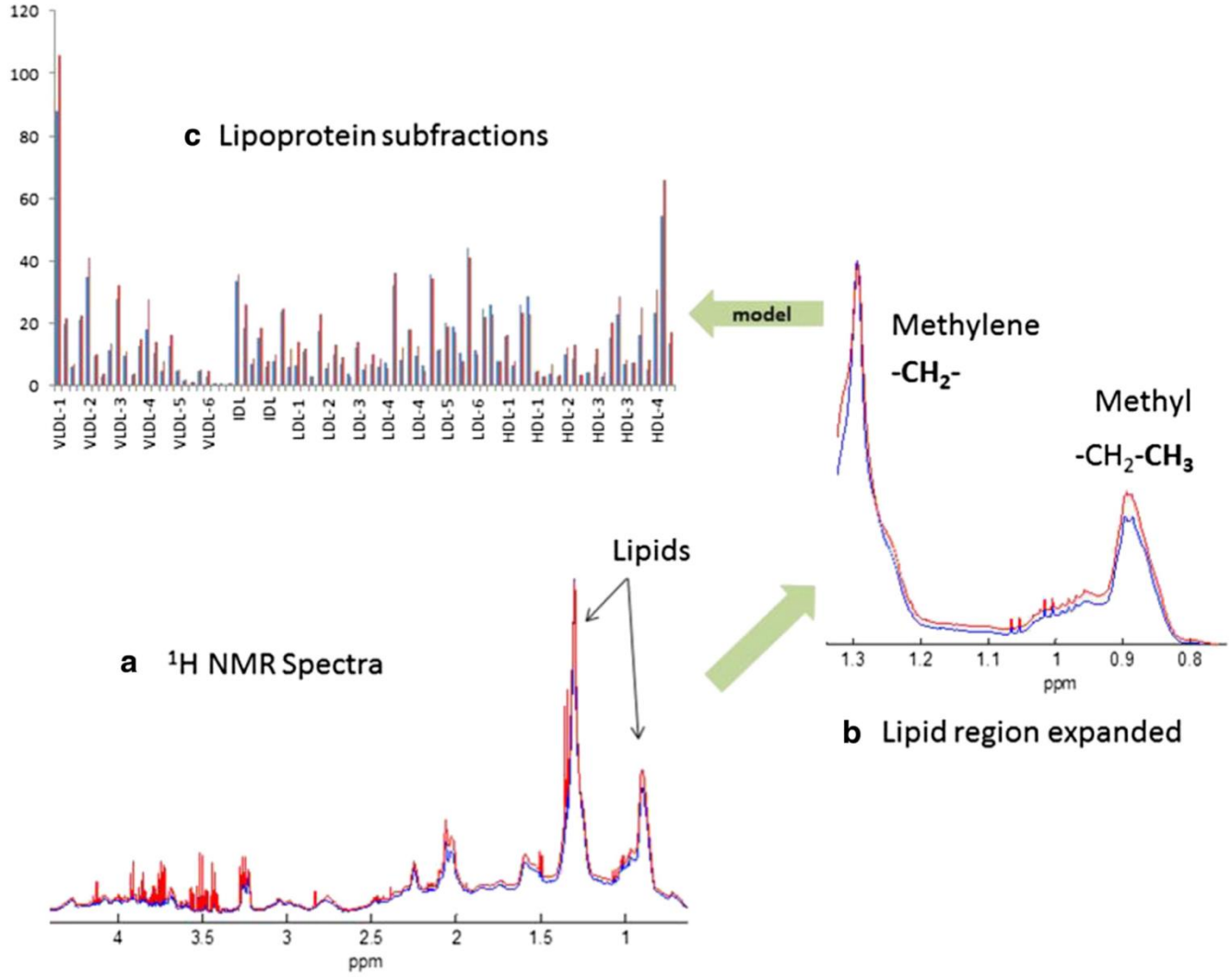
NMR-based lipidomics

Measure of lipoprotein fractions and subfractions



VLDL

- VLDL 1
- VLDL 2
- VLDL 3
- VLDL 4
- VLDL 5
- VLDL 6

LDL

- LDL 1
- LDL 2
- LDL 3
- LDL 4
- LDL 5
- LDL 6

HDL

- HDL 1
- HDL 2
- HDL 3
- HDL 4

IDL

Flote et al. Lipids in Health and Disease (2016) 15:56

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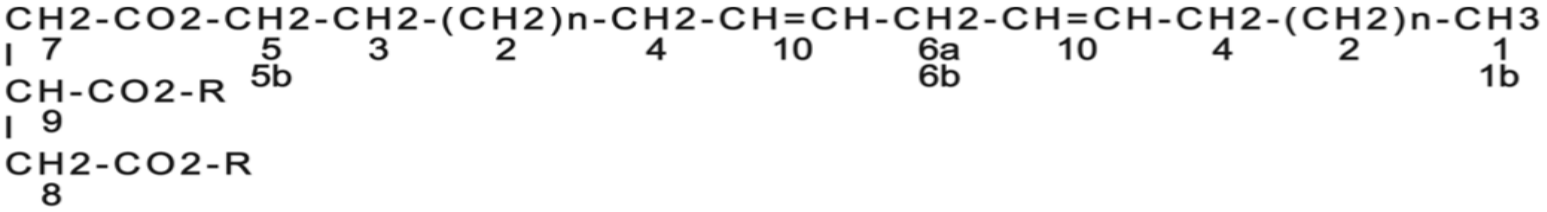
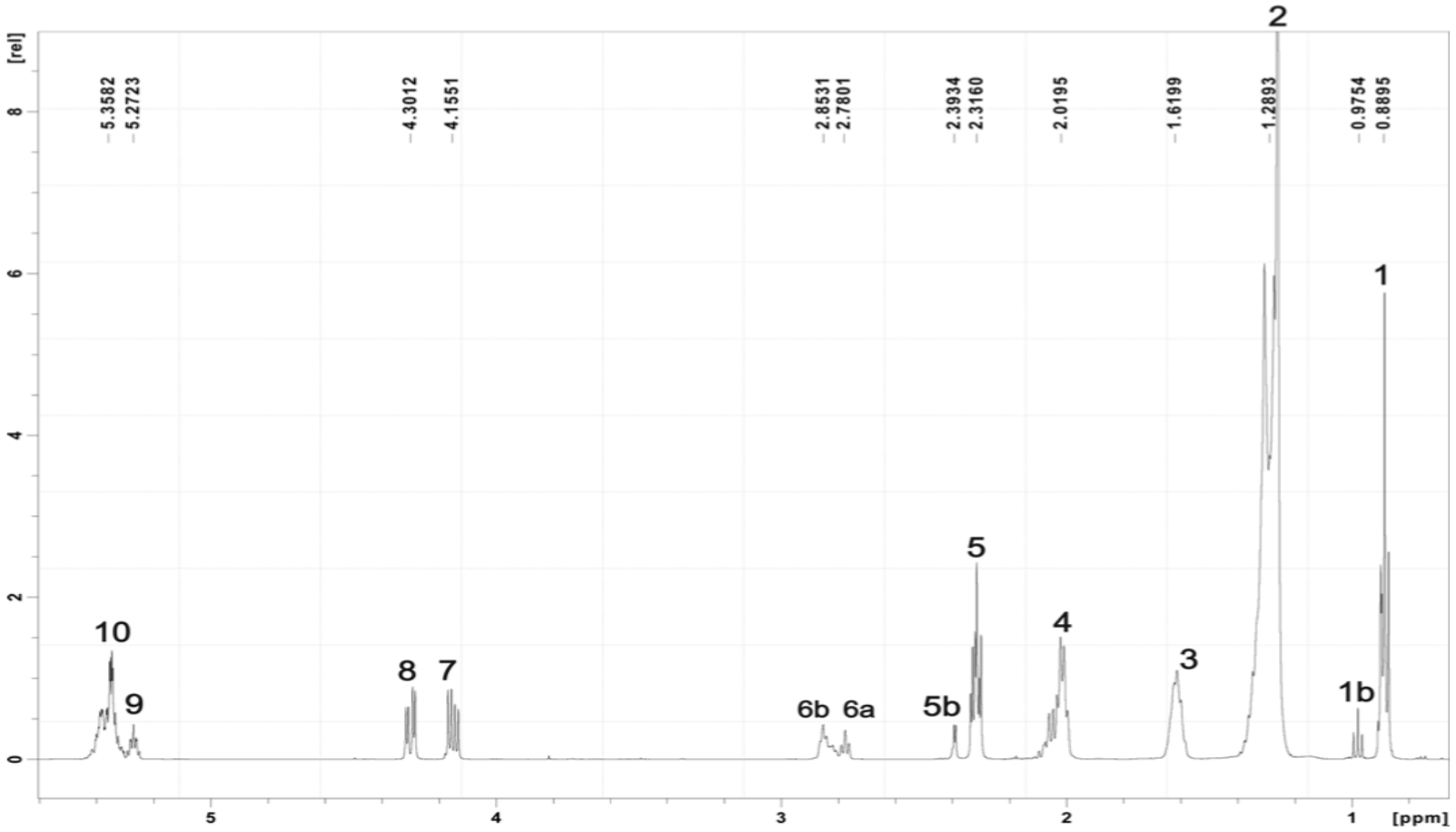
NMR-based lipidomics

Measure of lipid extracts





Lipid extract of rat adipose tissue:
- chloroform/methanol (2/1)

^{1}H NMR spectrum at 11.7 Tesla (500 MHz)

Proteome Res. 2016 Mar 4;15(3):868-78.