



Metabolomics & Lipidomics

October 15 2024

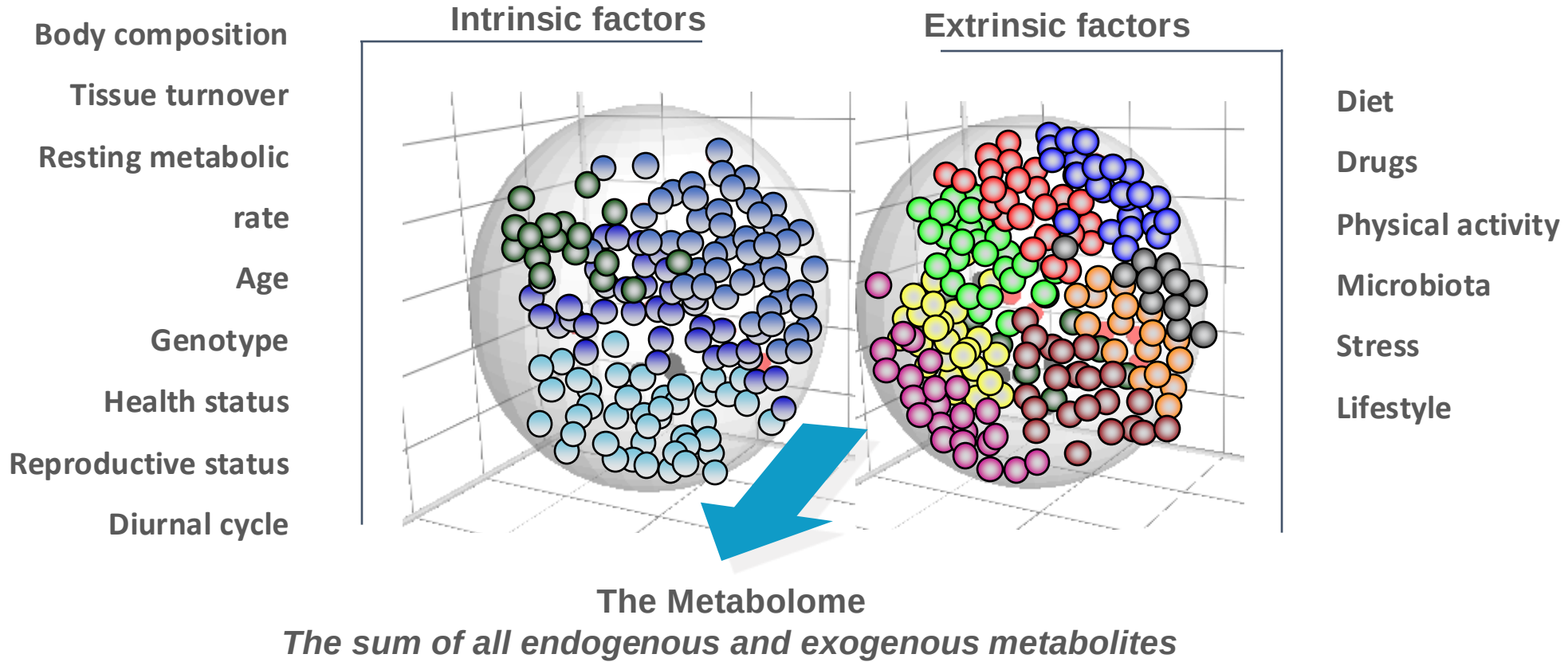
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Metabol(n)omics – What's in name

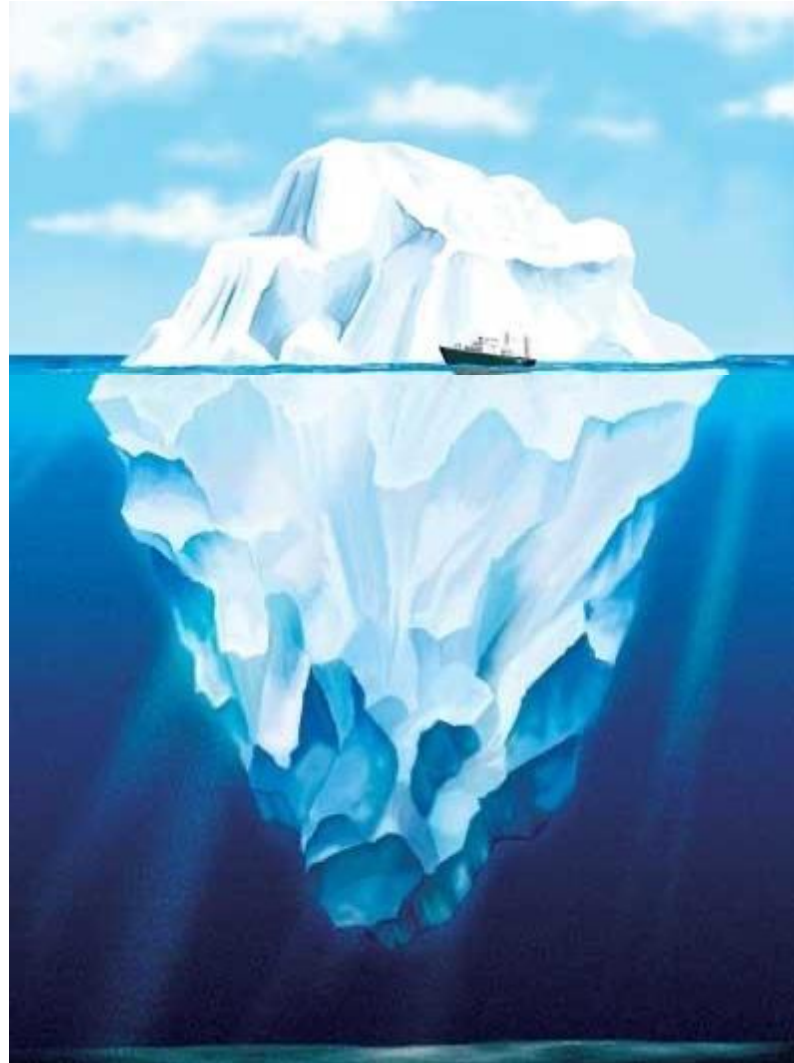
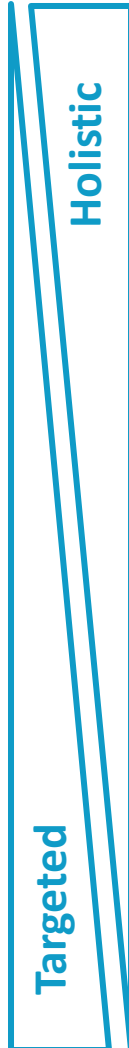
- Metabolome: the entire set of metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes
- Metabonomics: “the quantitative measurement of the dynamic multiparametric metabolic responses of multicellular systems to pathophysiological stimuli or genetic 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.

The Metabolome



Analytical strategies for metabolic profiling

The Metabolome



System level

Holistic profile

- Homeostatic status
- Surrogate biomarkers

Semi-holistic & targeted quantitative profiles

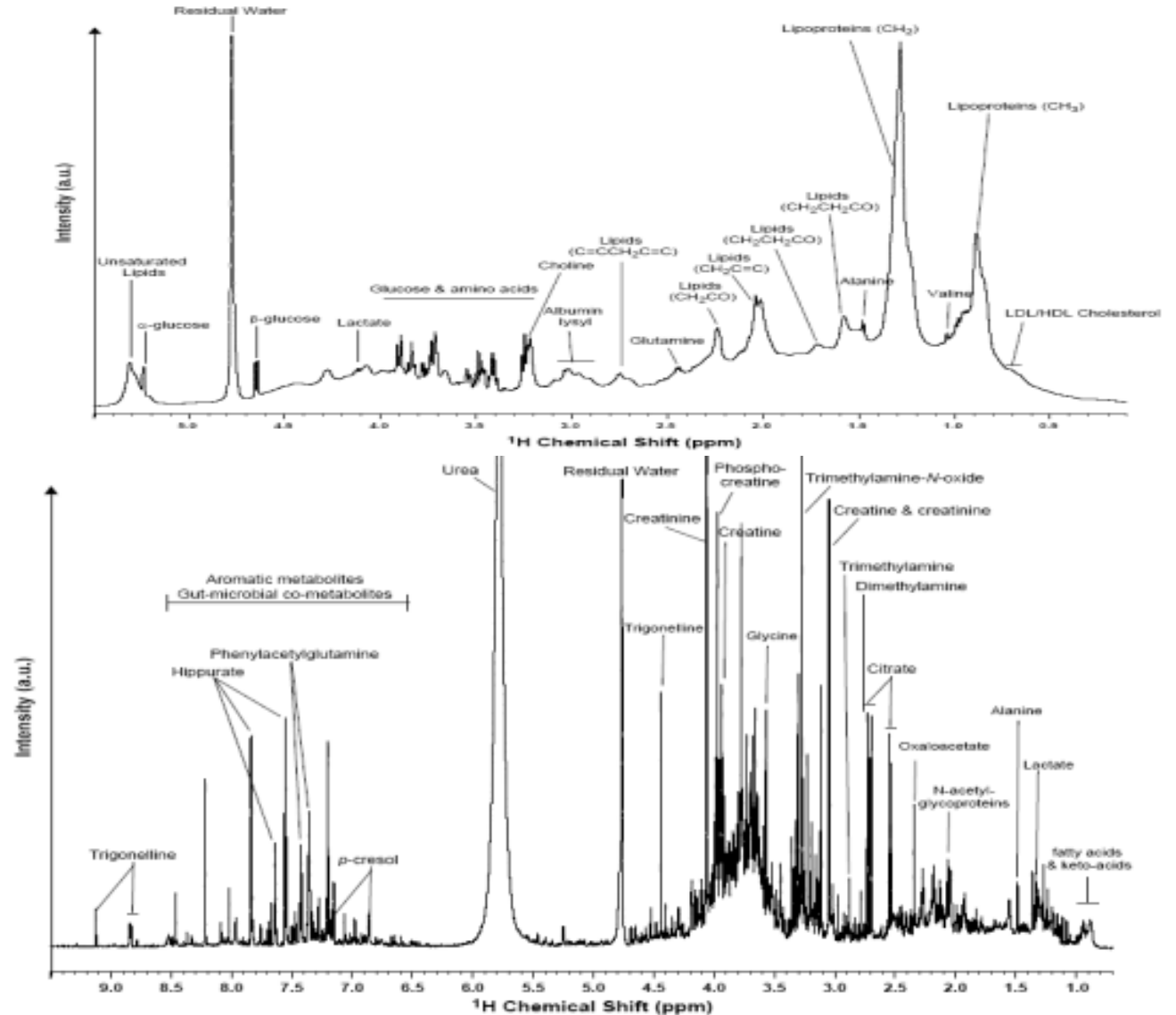
- Lipidomics

Sub-pathway level Tracer analysis Fluxes

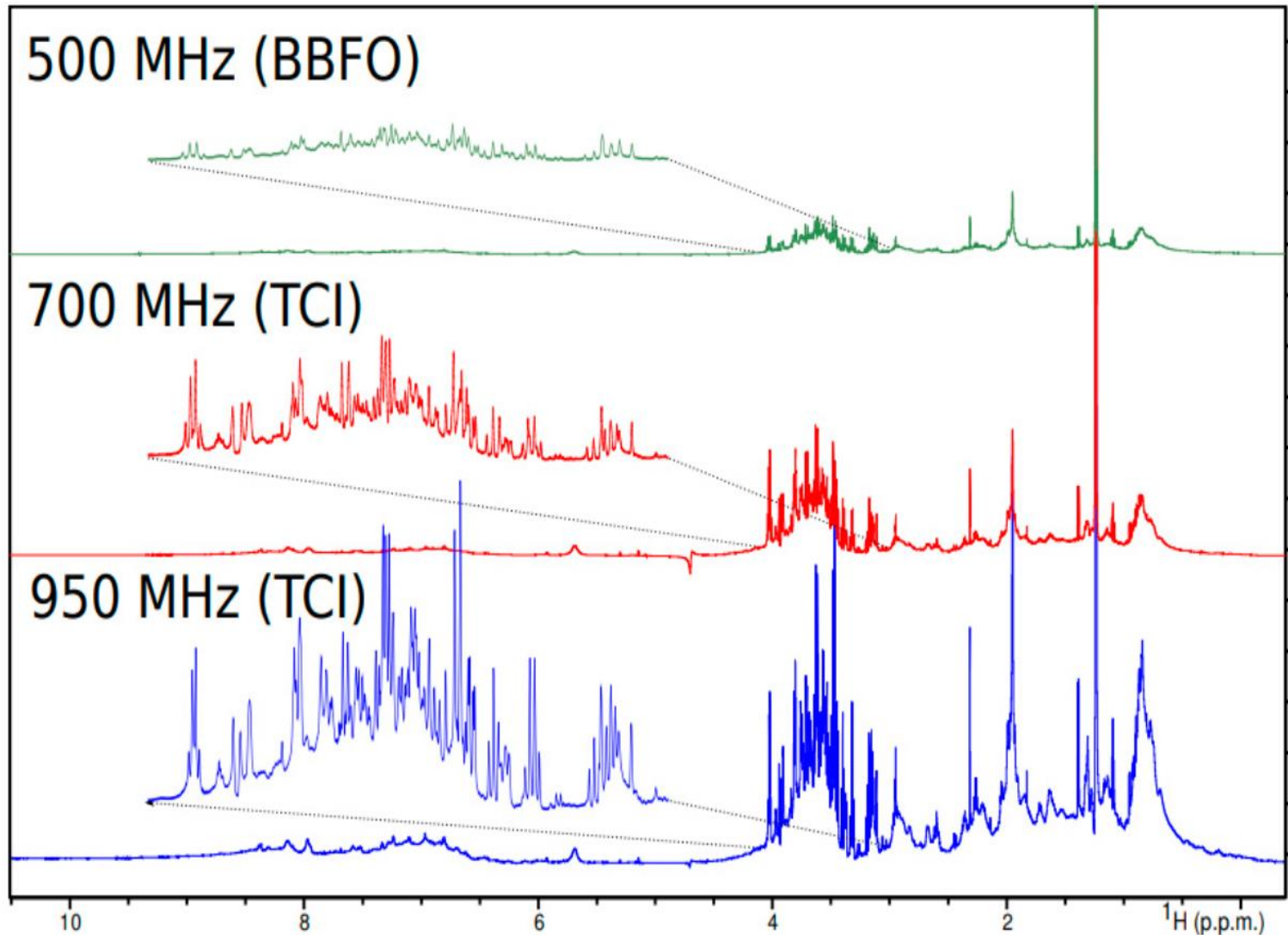
Holistic metabolic profiling by ^1H Nuclear Magnetic Resonance (^1H NMR) spectroscopy

Key features

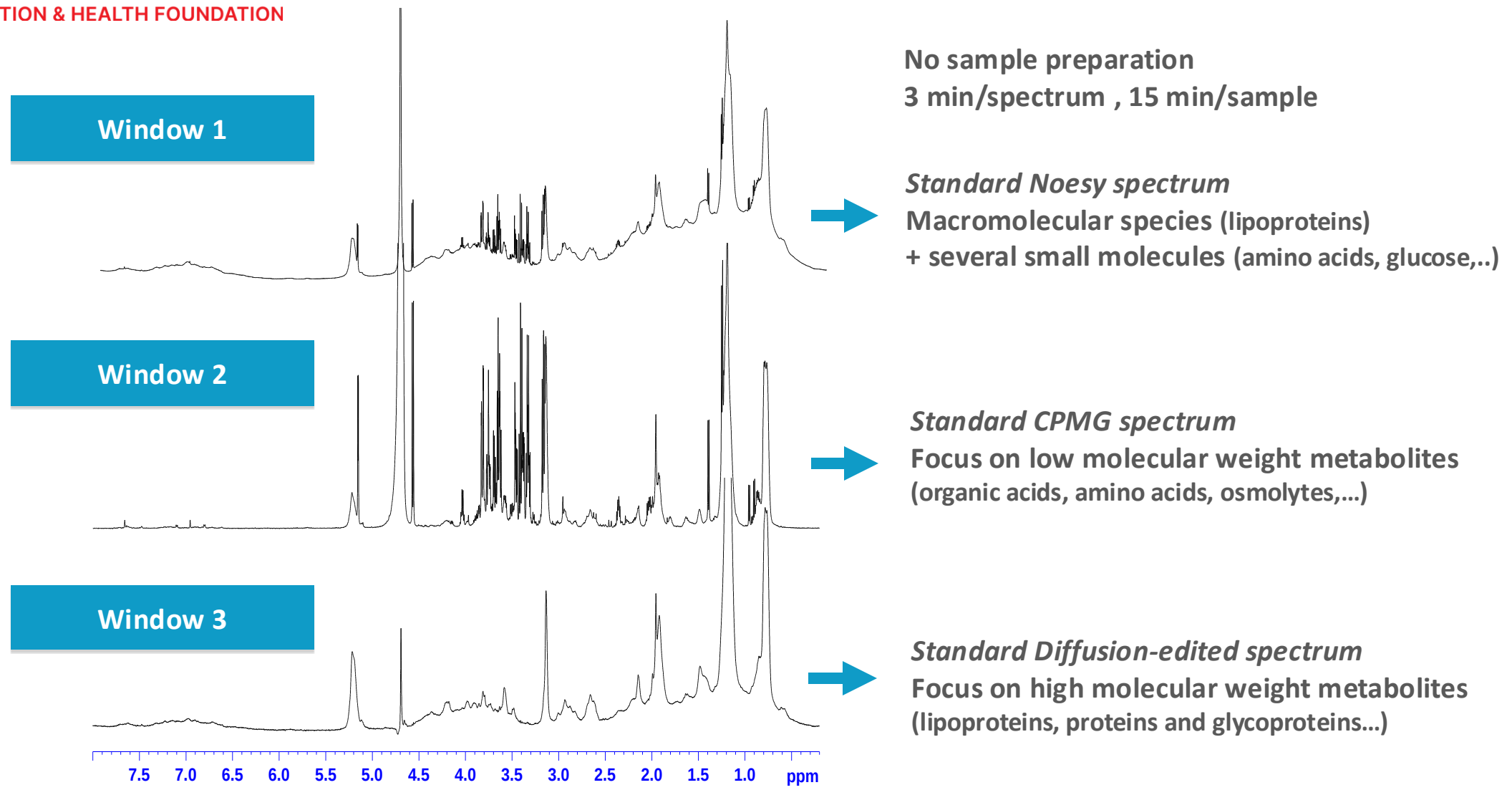
- high throughput
- highly robust
- provides information on:
 - Central energy metabolism
 - Amino acids
 - Lipids (lipoproteins)
 - Glucose
 - Microbial metabolites
- Efficient for hypothesis generation and functional genomics (GWAS)
- Provides guidance for subsequent targeted analyses



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

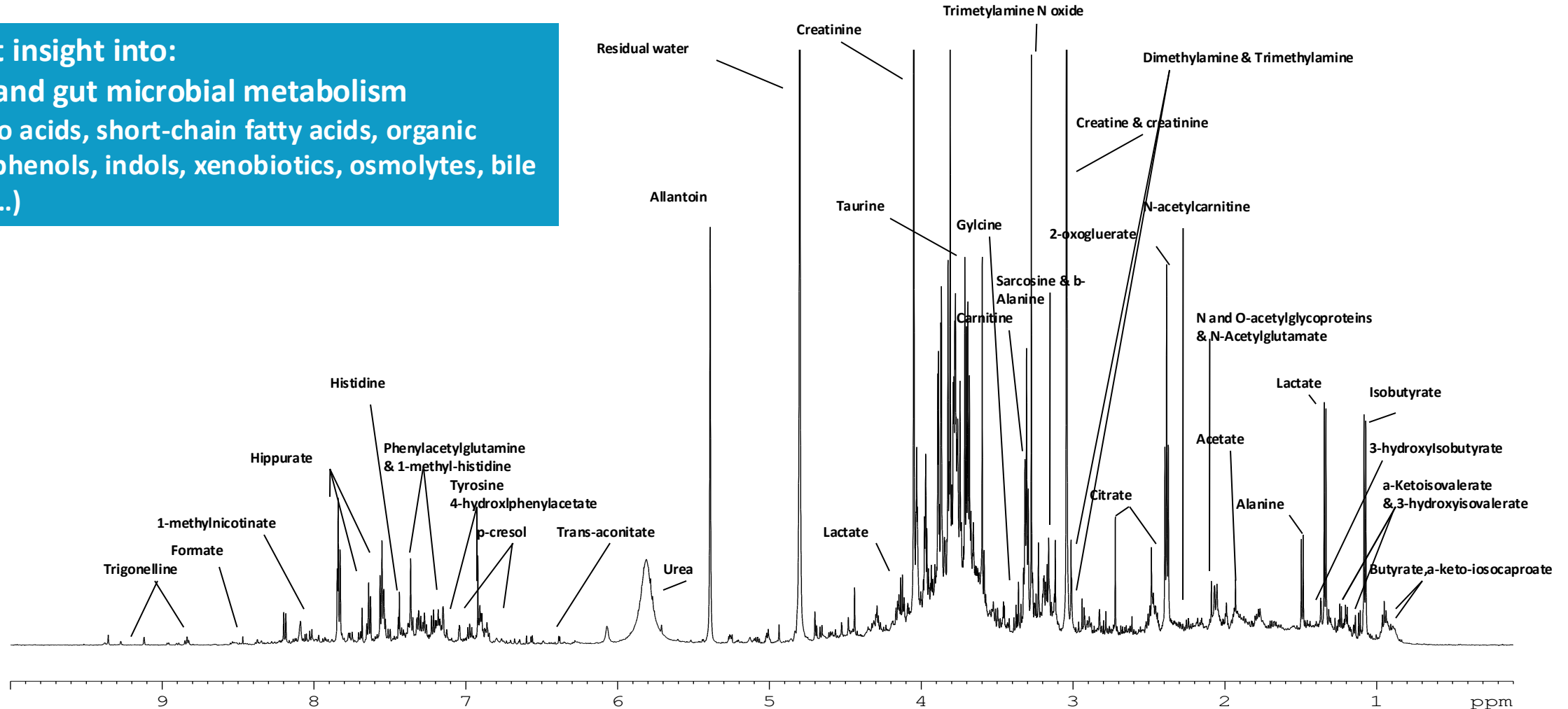


^1H NMR-based metabolic profiling of blood plasma 1 sample for multiple “metabolic windows”



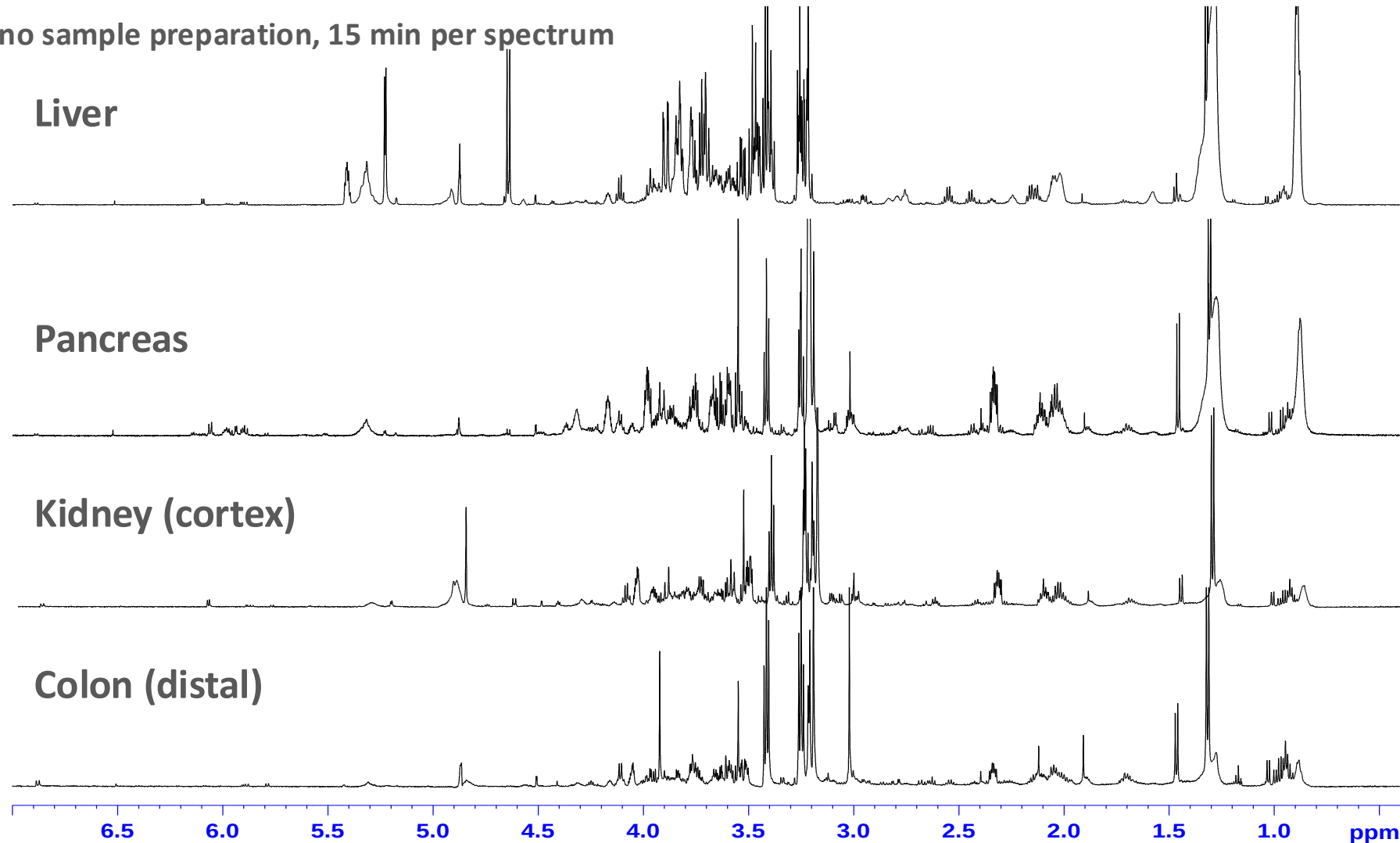
^1H NMR-based metabolic profiling of urine

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



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

Mouse tissues, no sample preparation, 15 min per spectrum



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)



Other approaches

Stable-isotope assisted metabolomics
(metabolic fluxes)

Molecular imaging techniques

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



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)

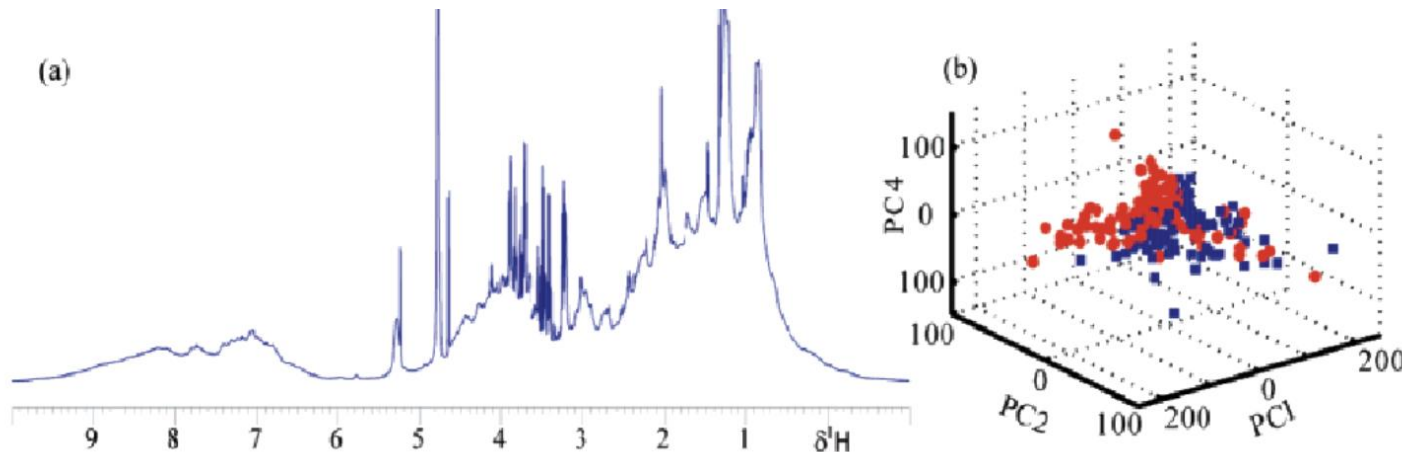
Data interpretation

- ✓ Pathway analysis
- ✓ Data integration
- ✓ Biological interpretation (biomarkers, mechanism of action...)

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

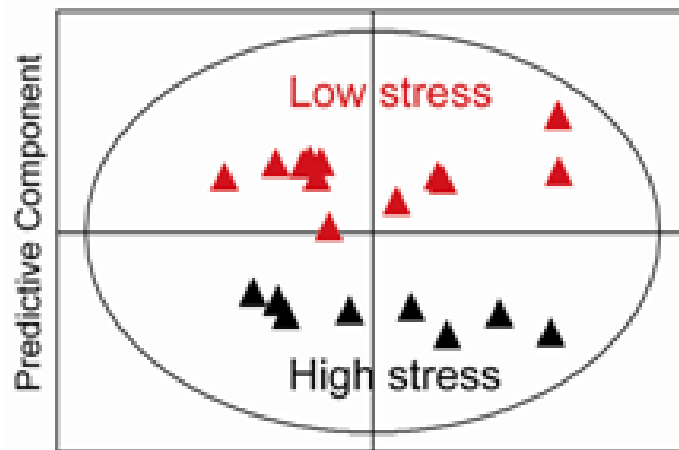


From: Rezzi, S., et al. (2007). Human metabolic phenotypes link directly to specific dietary preferences in healthy individuals. Journal of proteome research, 6 11, 4469-77 .

Examples of commonly used pattern recognition techniques

Supervised techniques

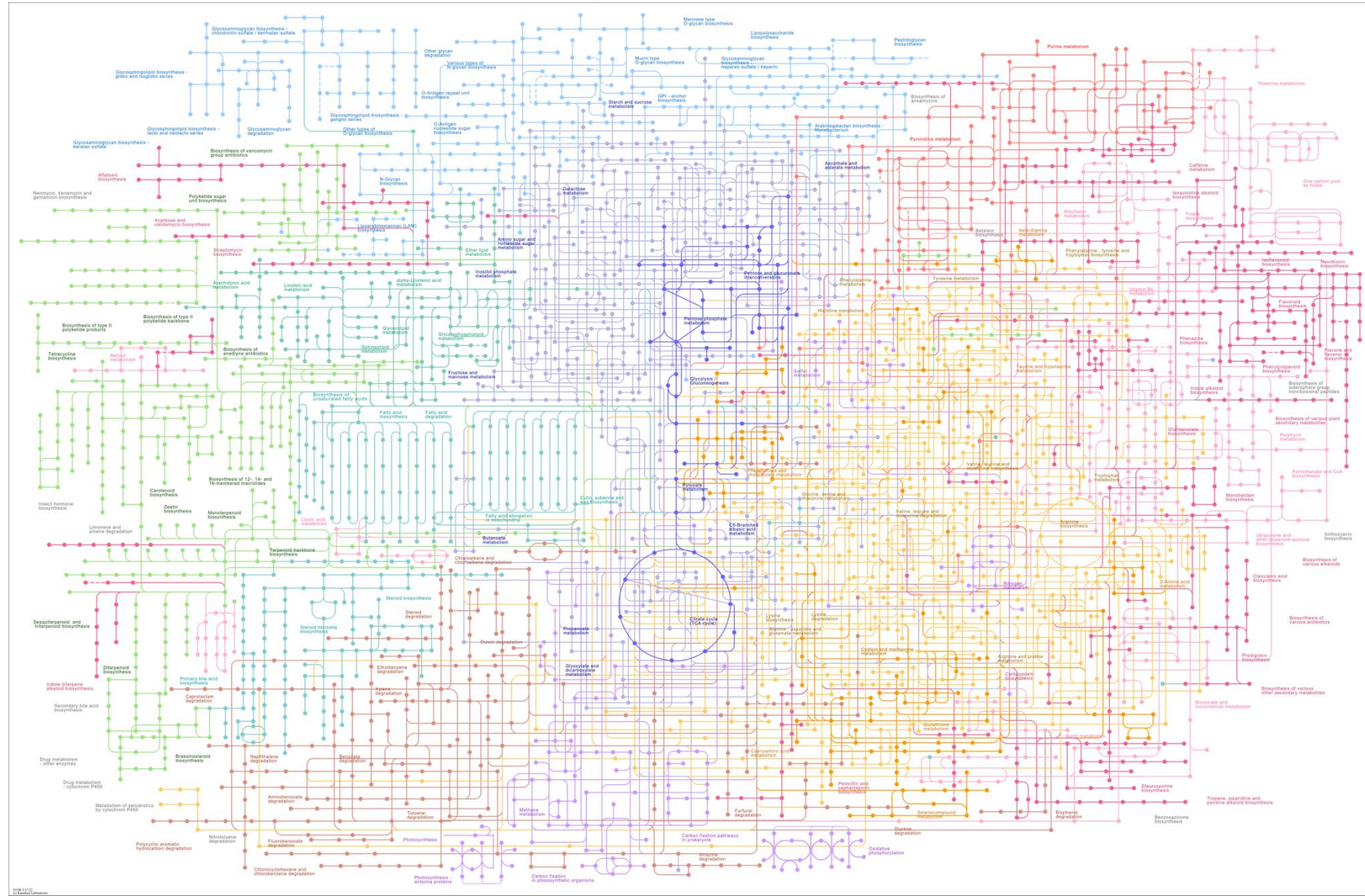
- *a priori* knowledge (e.g. known sample class/assignment)
- Aims: classification of samples or prediction of specific parameters (continuous variable)
- Regression techniques (partial least square), partial Least Squares Discriminant Analysis
 - Reduces the multidimensional data space into its low-dimensional 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.

Data analysis and interpretation

Need to know how to read and understand metabolic pathways



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

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	++	+++			

* 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

[illegible]

- 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

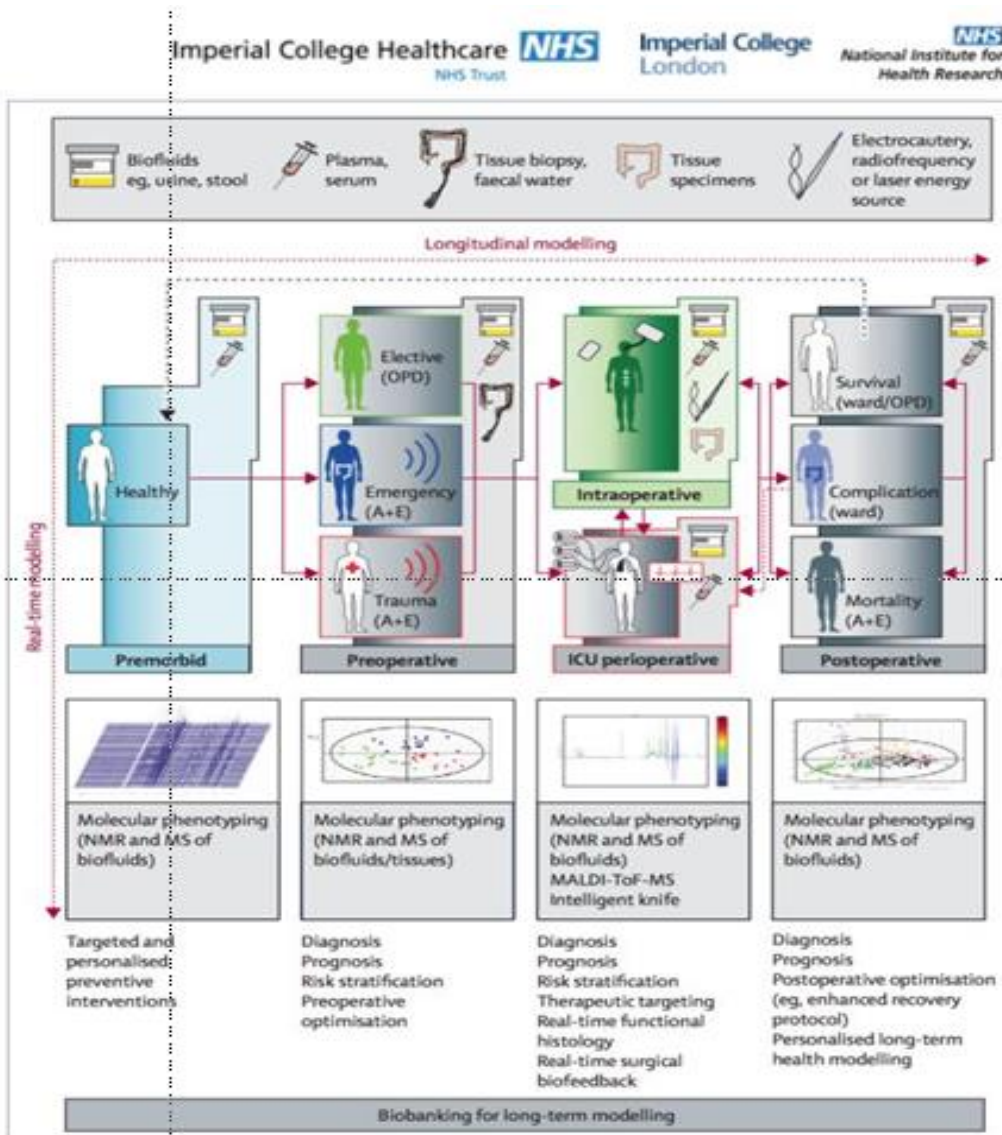
Phenotyping stratification for preventive medicine and medical care: Phenome Center concept

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)



Pharmaco-Metabonomics to enable prognostic biomarker discovery for patient stratification

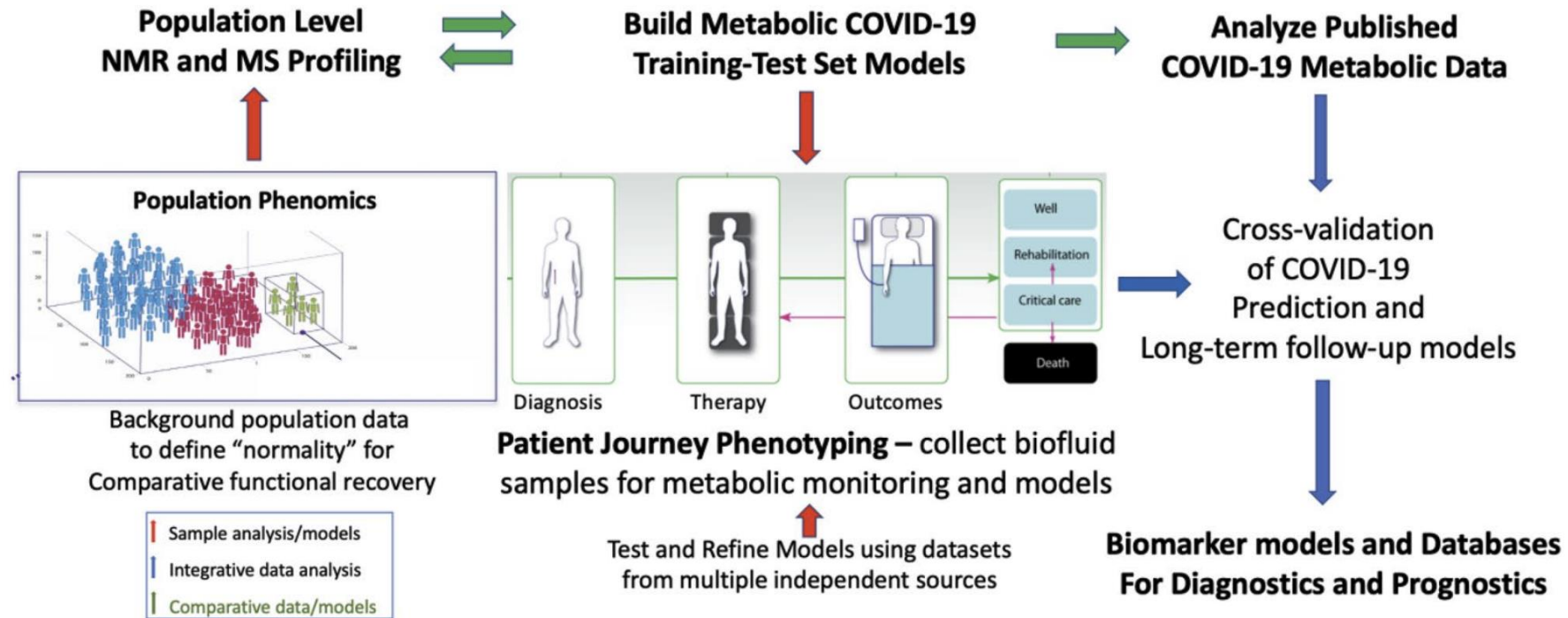
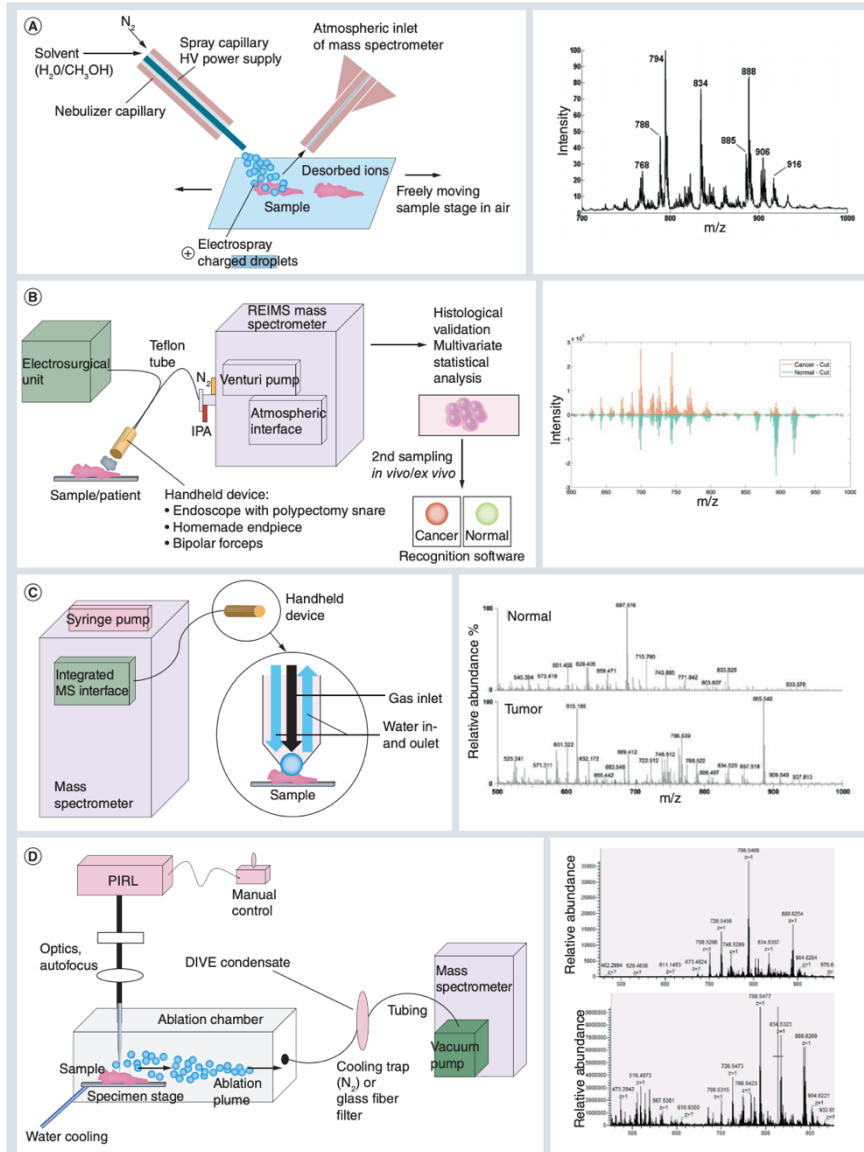


Fig. 1 A framework for understanding the natural history of an emergent zoonotic disease using metabotyping: schematic illustration of the collective COVID-19 patient journey from health to disease using a metabolic systems framework to assess disease progression and recovery in relation to associated studies that enable model cross-val-

idation 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

Mass spectrometry-based intraoperative tumor diagnostics



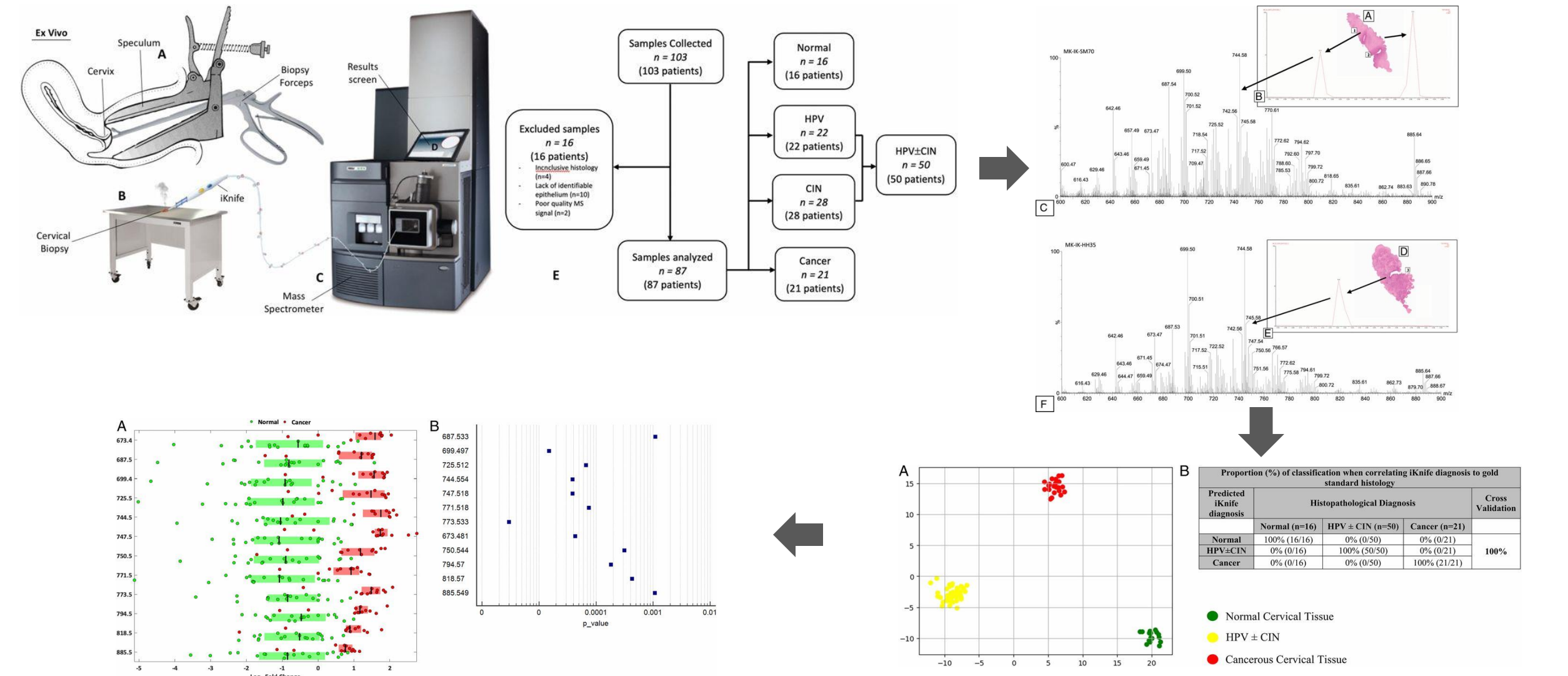
Schematic figure of mass spectrometry based methods for intraoperative cancer diagnosis (left) with corresponding examples of mass spectra (right)

iKnife. Rapid evaporative mass spectrometry coupled to eligible surgical devices

MasSpec pen.

Picosecond infrared laser

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



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.

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.

	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 ¹H and ³¹P.	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.

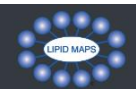
The large-scale study of pathways and networks of cellular lipids in biological systems.

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

Subset of the "metabolome"

Lipid Maps

A lipid specialized knowledge base



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<https://www.lipidmaps.org/>

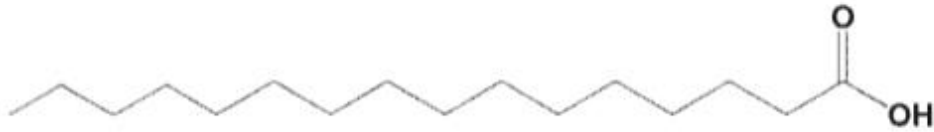
Lipids may be broadly defined as hydrophobic or amphiphilic small molecules that originate entirely or in part from two distinct types of biochemical subunits or "building blocks": ketoacyl and isoprene groups.

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

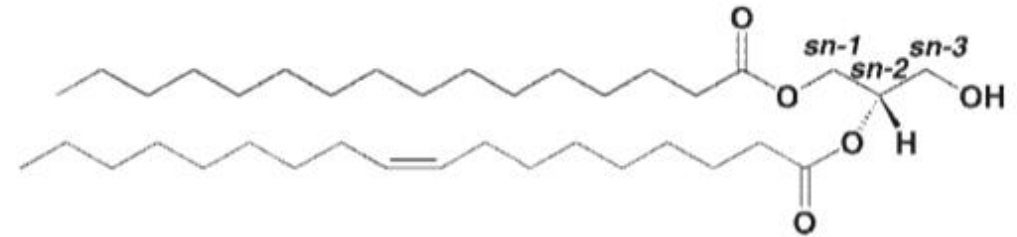
Lipids may be divided into eight categories:

Fatty acyls	Saccharolipids
Glycerolipids	Polyketides
Glycerophospholipids	Sterol lipids
Sphingolipids	Prenol lipids

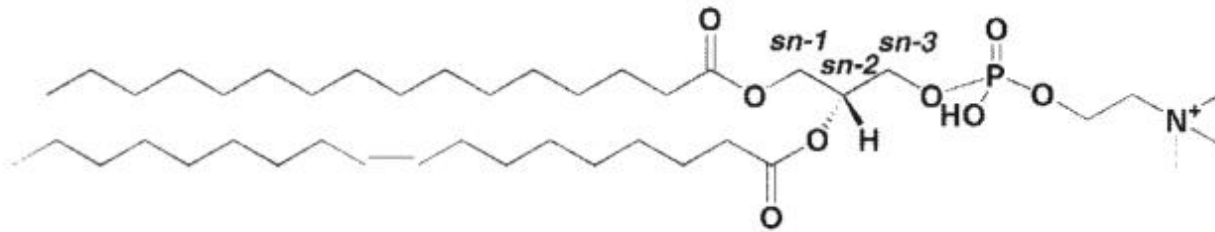
Representative structures 1/2



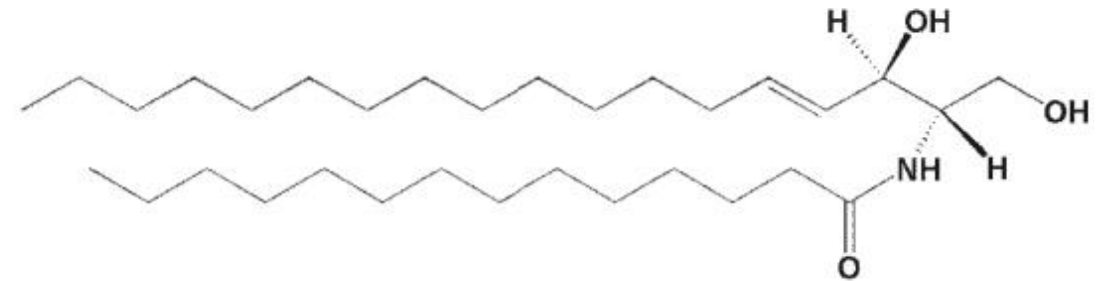
(a) Fatty Acyls: hexadecanoic acid



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

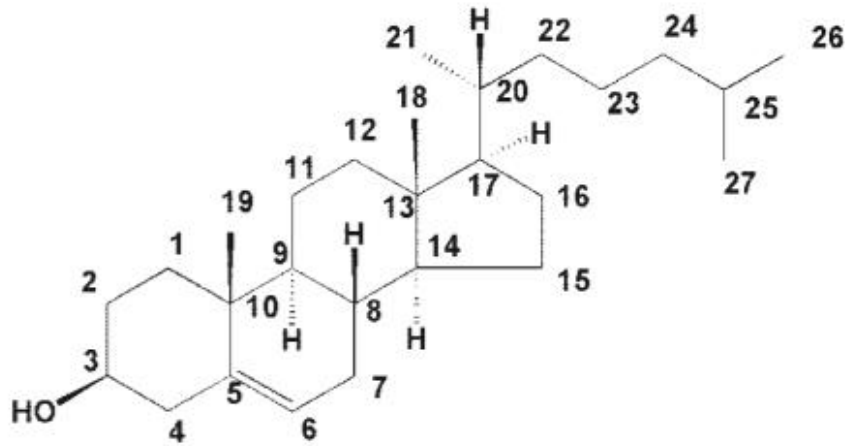


(c) Glycerophospholipids: 1-hexadecanoyl-2-(9Z-octadecenoyl)-*sn*-glycero-3-phosphocholine

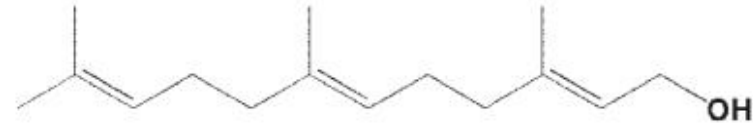


(d) Sphingolipids: N-(tetradecanoyl)-sphing-4-enine

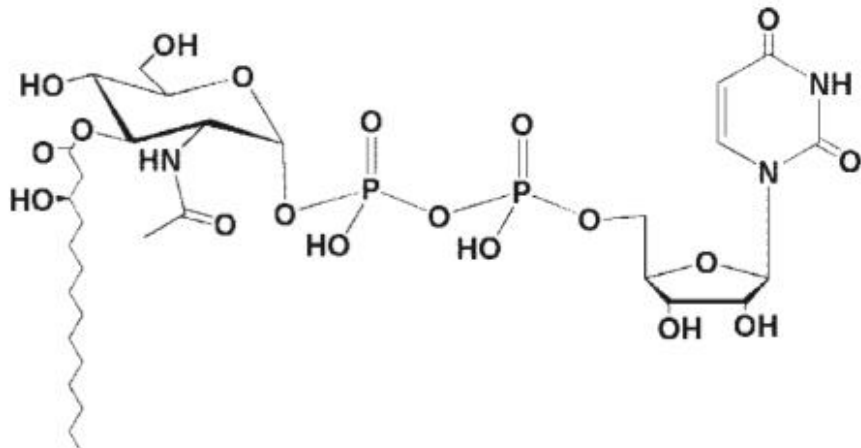
Representative structures 2/2



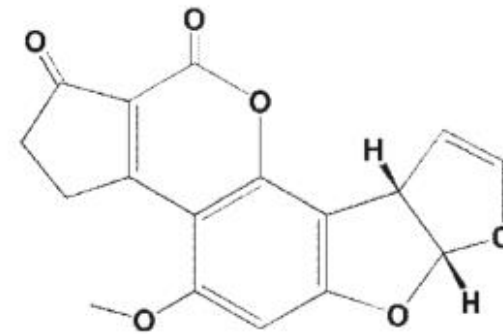
(e) Sterol Lipids: cholest-5-en-3β-ol



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



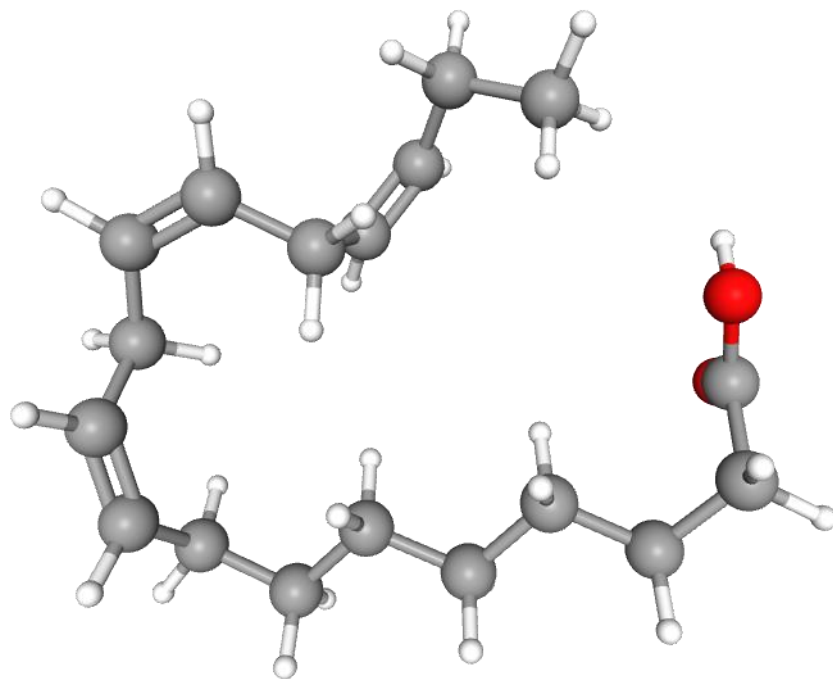
(g) Saccharolipids: UDP-3-O-(3R-hydroxy-tetradecanoyl)-αD-N-acetylglucosamine



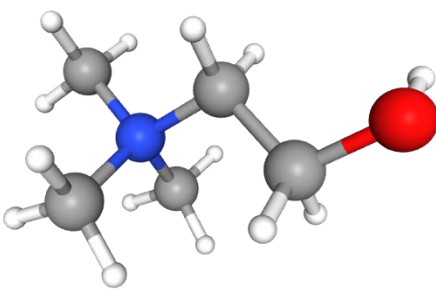
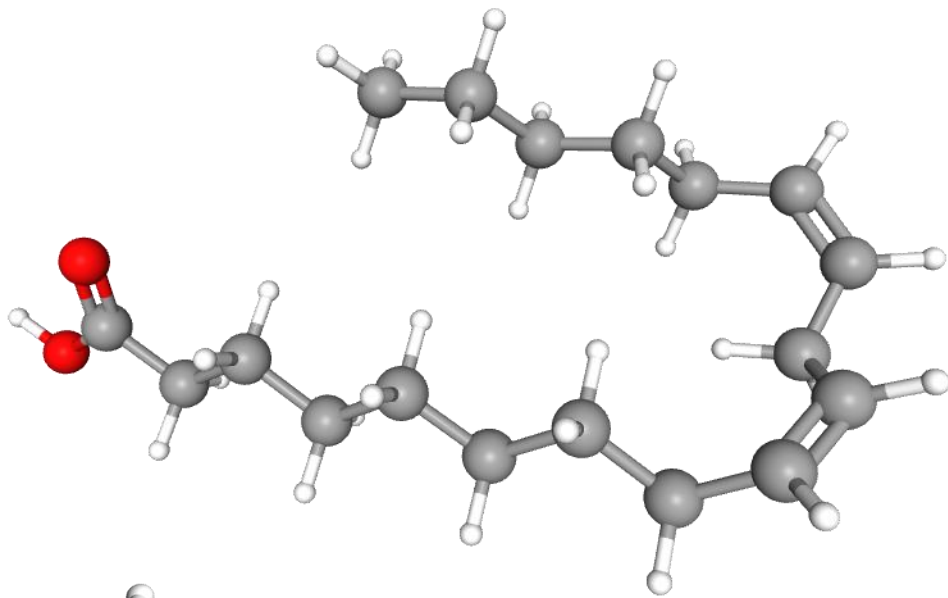
(h) Polyketides: aflatoxin B1

Essential lipids and lipid components for humans

alpha-linolenic acid (omega-3 fatty acid)



linoleic acid (omega-6 fatty acid)



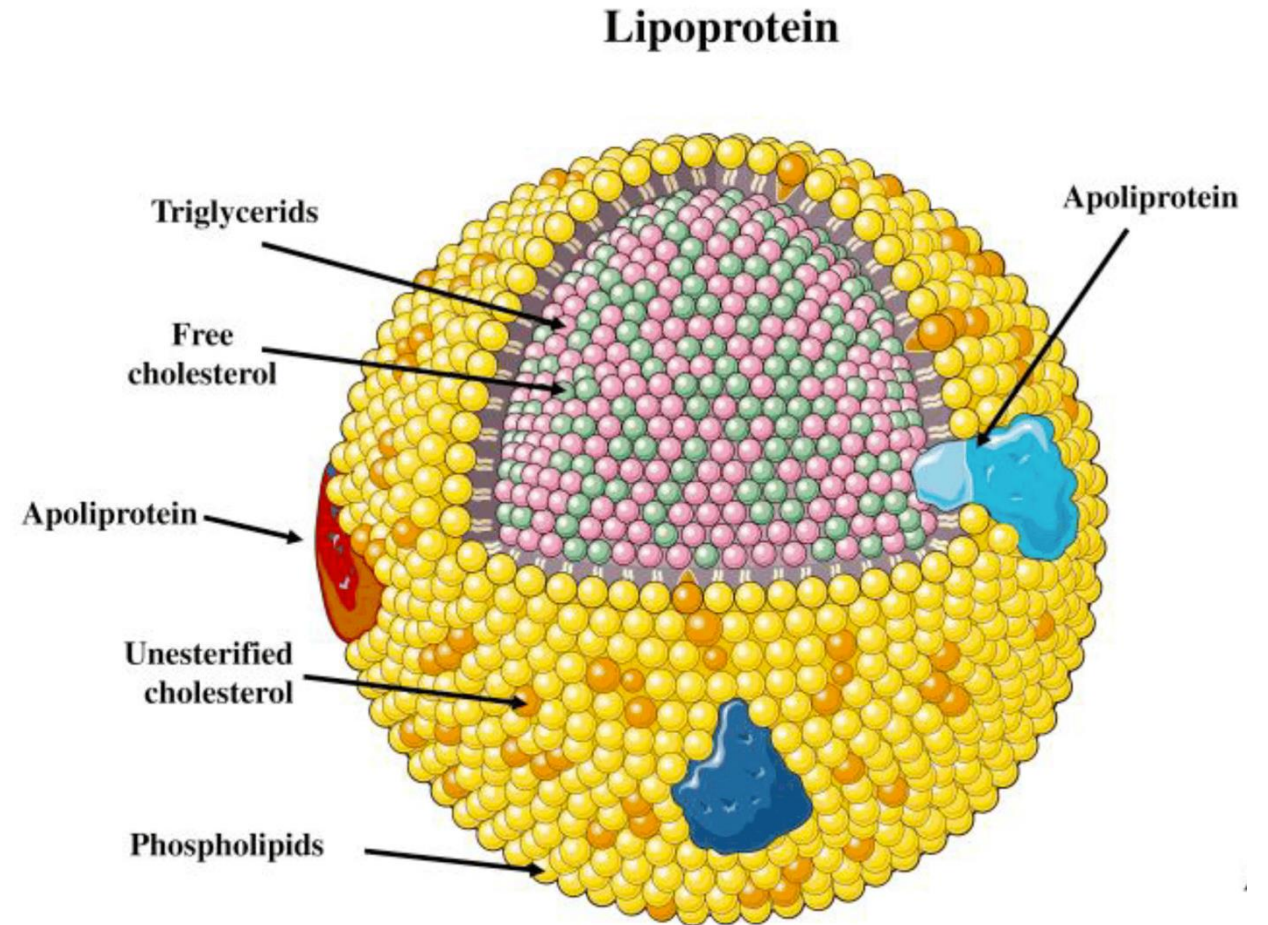
choline

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.



Transport of lipids via lipoproteins

Very low-density lipoproteins (VLDLs) ($0.96\text{-}1.006\text{ g/cm}^3$)

- transport triglycerides from the liver to various tissues in the body for energy or storage.

Intermediate-density lipoproteins (IDLs) ($1.007\text{-}1.019\text{ g/cm}^3$)

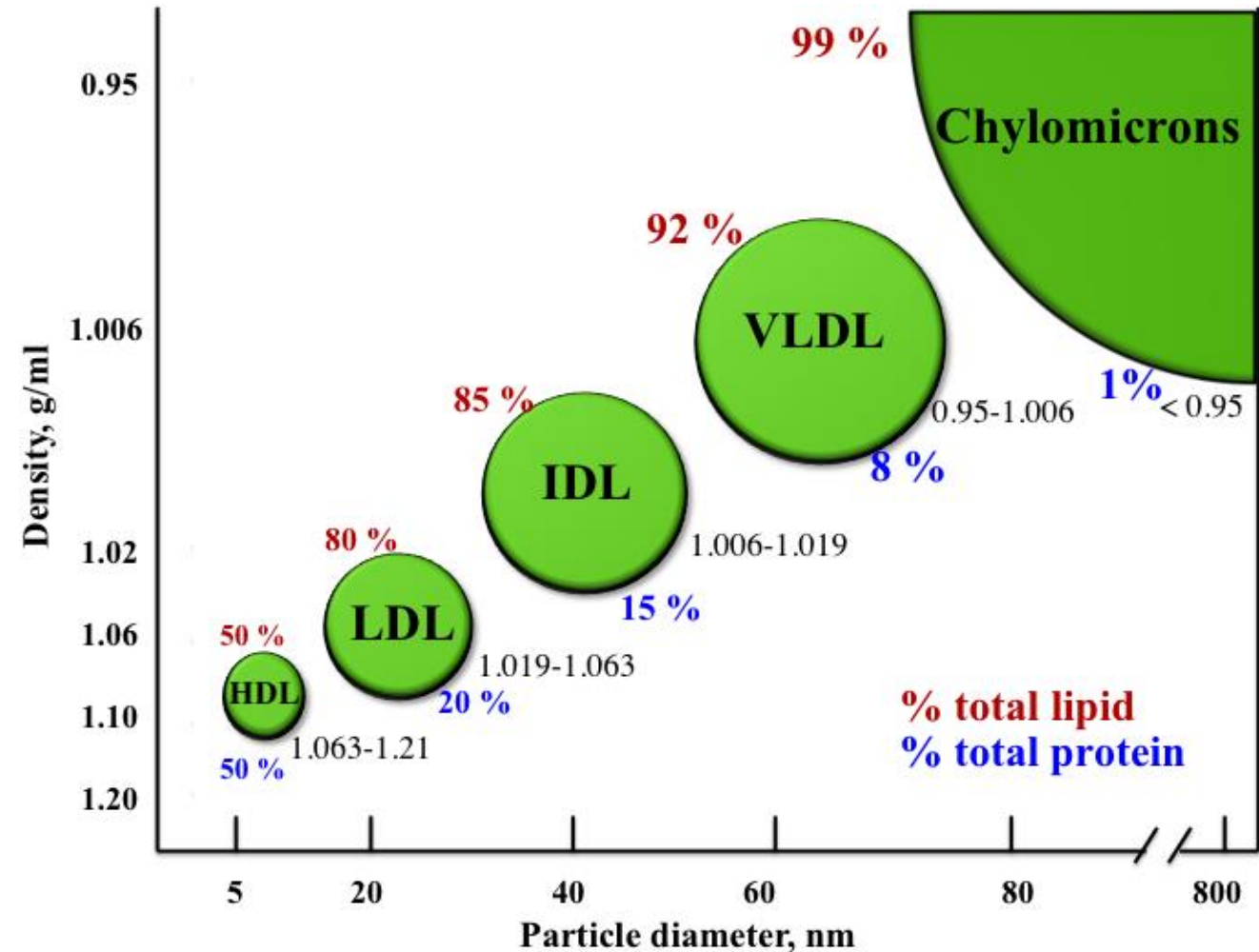
- transport a variety of fats and cholesterol left from VLDLs from the tissues to the liver

Low-density lipoproteins (LDLs) ($1.020\text{-}1.062\text{ g/cm}^3$)

- transport cholesterol and other lipids from the liver to tissues.

High-density lipoproteins (HDLs) ($1.063\text{-}1.210\text{ g/cm}^3$)

- transport cholesterol out of the bloodstream and into the liver, where it is either reused or removed from the body with bile.

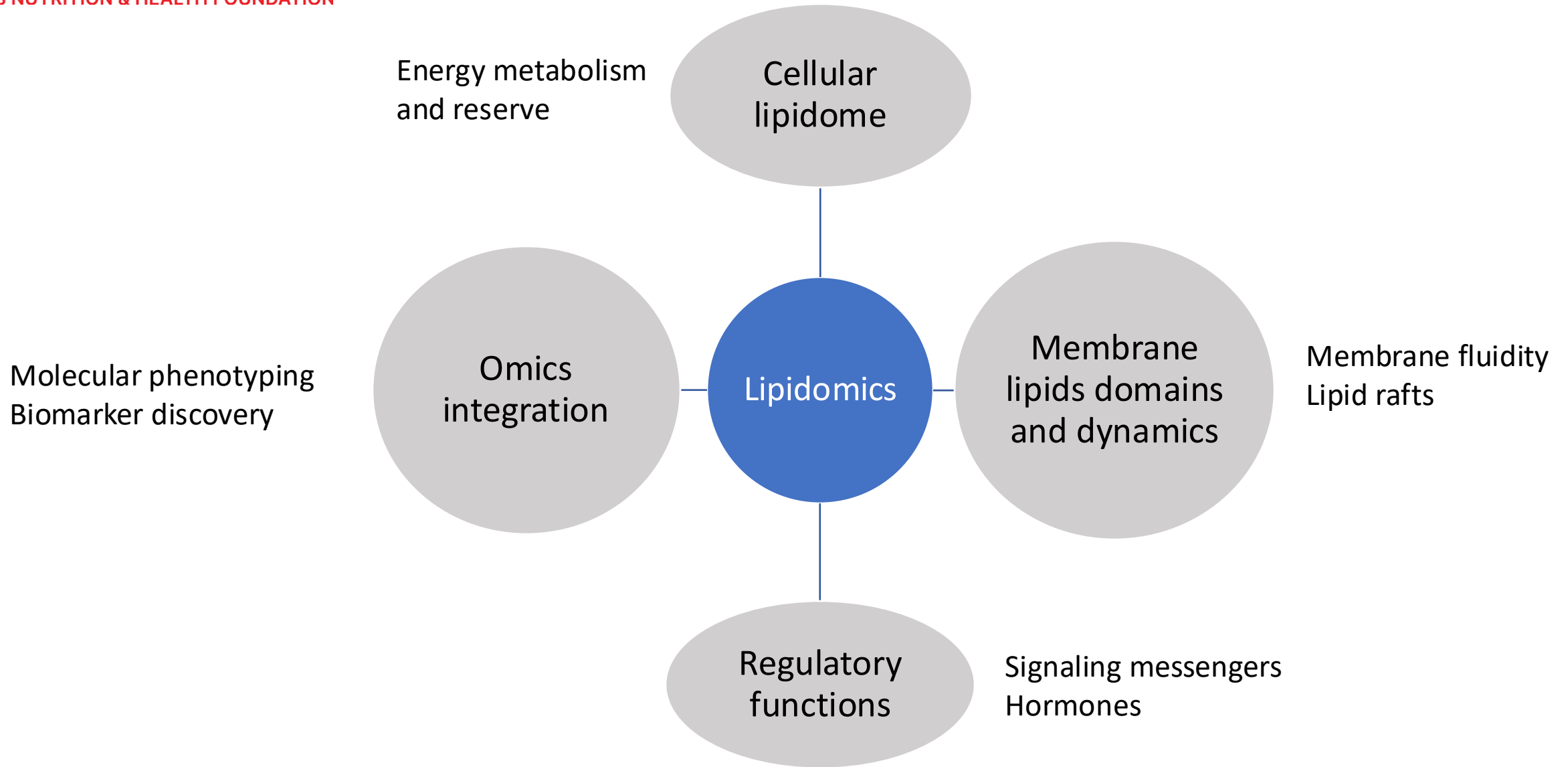


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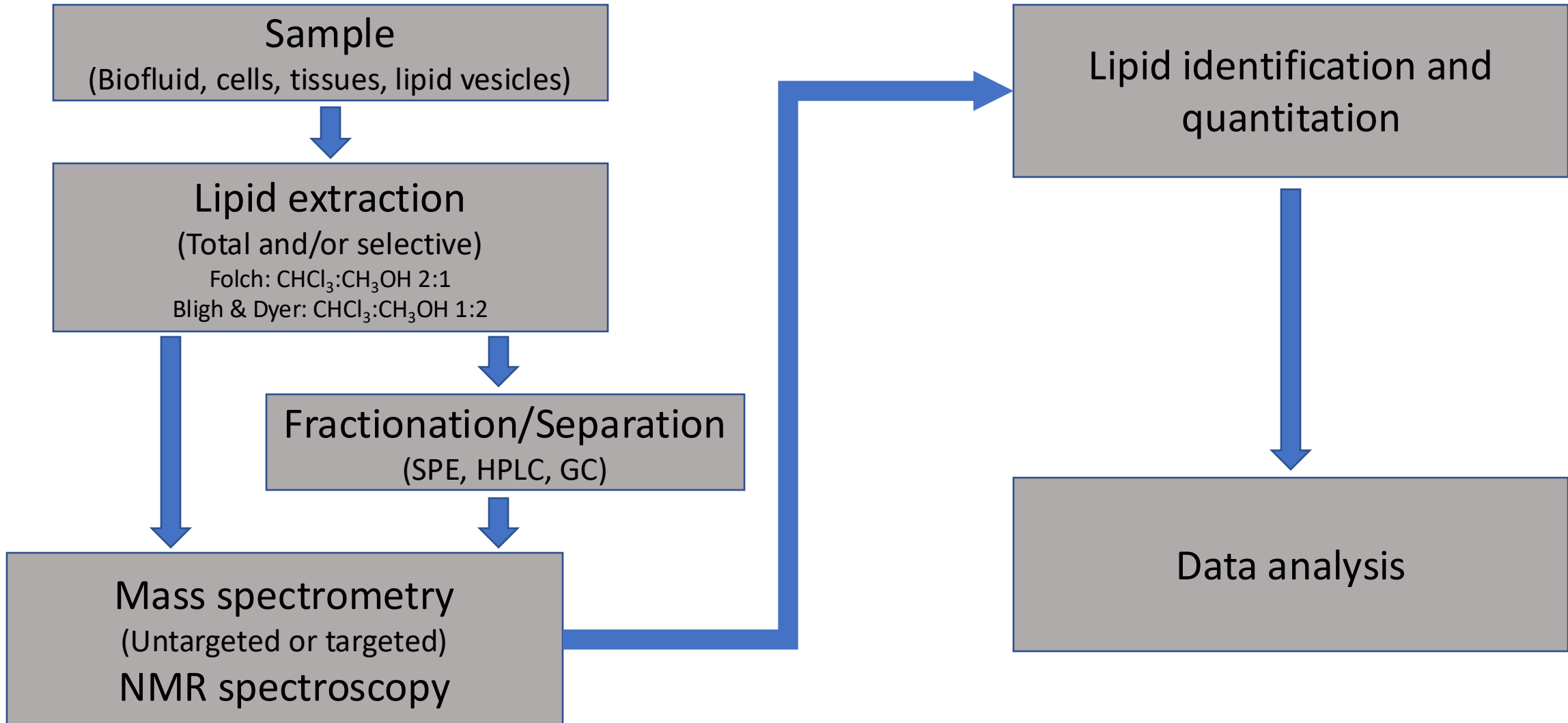
https://chem.libretexts.org/Bookshelves/Introductory_Chemistry/Map%3A_Fundamentals_of_General_Organic_and_Biological_Chemistry_%28McMurry_et_al.%29/24%3A_Lipid_Metabolism/24.02%3A_Lipoproteins_for_Lipid_Transport

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.

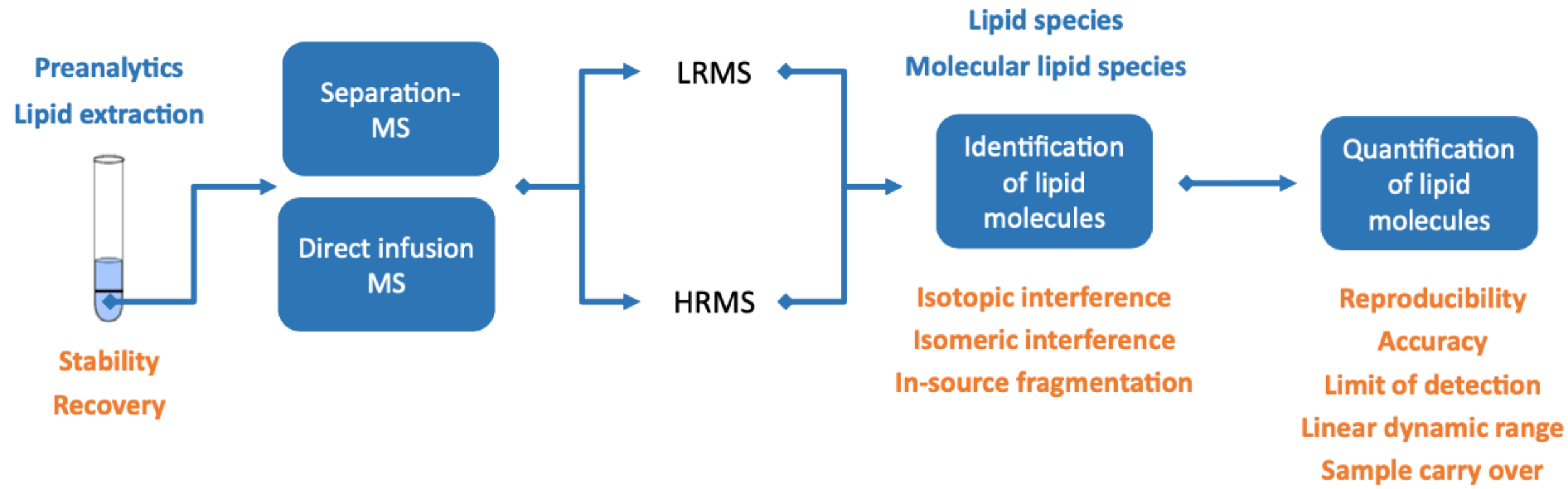
Lipidomics main application fields



Lipidomics workflow for MS and NMR

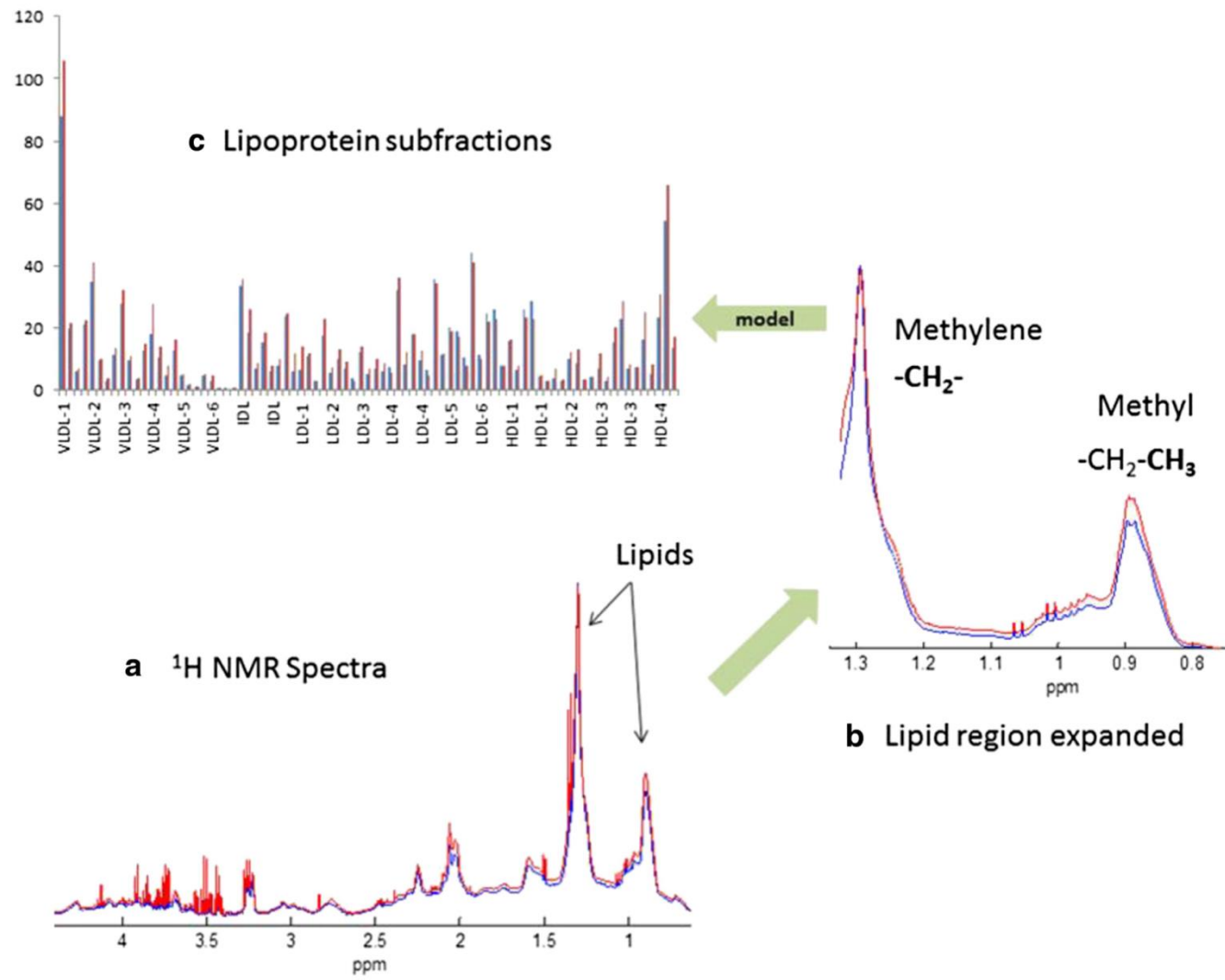


Lipidomics workflow for MS



NMR-based lipidomics

Measure of lipoprotein fractions and subfractions



VLDL	LDL
VLDL 1	LDL 1
VLDL 2	LDL 2
VLDL 3	LDL 3
VLDL 4	LDL 4
VLDL 5	LDL 5
VLDL 6	LDL 6

HDL	IDL
HDL 1	
HDL 2	
HDL 3	
HDL 4	

