FIMM
Institute for Molecular Medicine Finland
Nordic EMBL Partnership for Molecular Medicine

Introduction to Clinical Metabolomics

TransMed Course: Basics in Clinical Proteomics and Metabolomics. Oct 10-19, 2012

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10/10/12

dd.mm.yyyy

FIMM
Institute for Molecular Medicine Finland
Nordic EMBL Partnership for Molecular Medicine

FIMM Technology Centre
Metabolomics Unit Core Facility
Biomedicum 2U, 2nd Floor

Services: Targeted quantitative high-throughput metabolomics Analyses for medical applications

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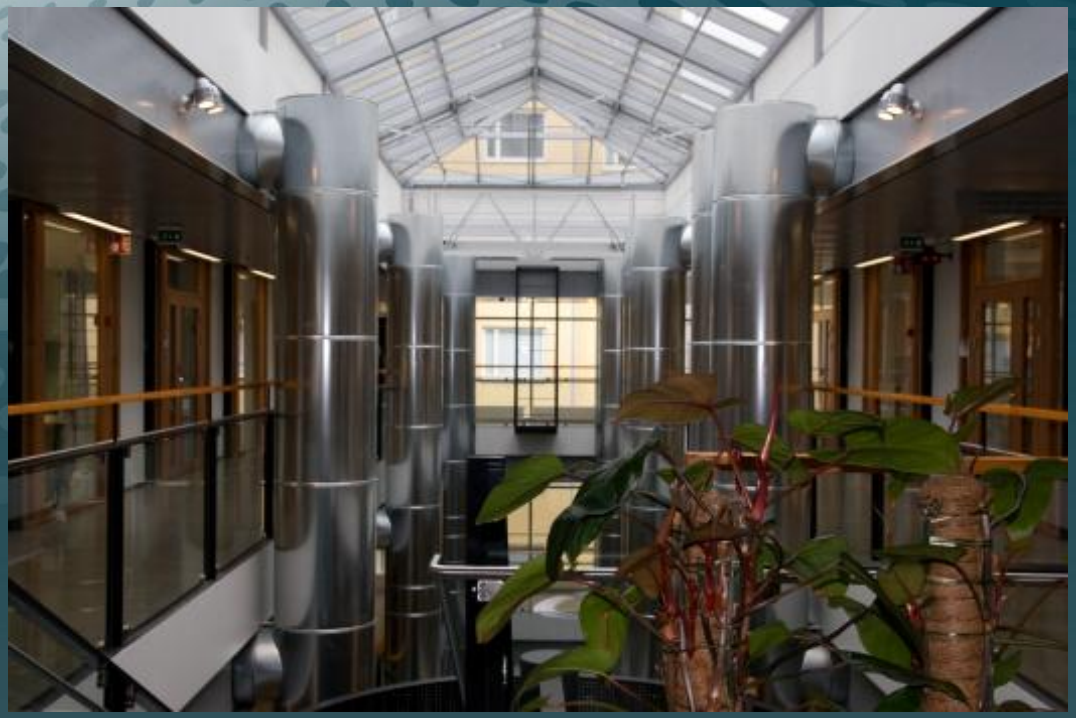

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Metabolomics Unit

Launched in 2011

Waters XEVO-TQS triple quadrupole

Hamilton star liquid handling system

Facility up and running in 2012

Outline

- ❑ **What is metabolomics?**
- ❑ **Why metabolomics?**
- ❑ **Challenges in metabolomics**
- ❑ **Metabolomics platforms**
- ❑ **Metabolomics work flow**
- ❑ **Applications of metabolomics**
- ❑ **Small test**

What is Metabolomics?

- **Metabolites** are bio-active small molecules involved in the biochemical network
- **Metabolome** represents the collection of all metabolites in a given biological sample
- **Metabolomics** is the systematic identification and quantitation of the metabolites
- **Clinical Applications** include studies of chronic diseases, pharmacology, pre-clinical drug trials, toxicology, transplant monitoring, newborn screening and clinical chemistry.

Brief historical note

1500-2000BC China
•Ants used to detect patients with diabetes

1940s-1970s
•Advances in analytics
•Pattern recognition
→ Metabolic profiling

21st century
•Advances in analytics
•Biostatistics & Bioinformatics
→ Modern era of metabolomics and systems biology

Metabolomics is not new

Proc. Nat. Acad. Sci. USA
Vol. 68, No. 10, pp. 2374–2376, October 1971

Quantitative Analysis of Urine Vapor and Breath by Gas–Liquid Partition Chromatography

(orthomolecular medicine/vitamins/controlled diet)

LINUS PAULING*, ARTHUR B. ROBINSON*, ROY TERANISHI†, AND PAUL CARY*

* Department of Chemistry, Stanford University, Stanford, California 94305; and † Western Regional Laboratory, U.S. Department of Agriculture

Contributed by Linus Pauling, July 29, 1971

ABSTRACT When a human being is placed for several days on a completely defined diet, consisting almost entirely of small molecules that are absorbed from the stomach into the blood, intestinal flora disappear because of lack of nutrition. By this technique, the composition of body fluids can be made constant (standard deviation about 10%) after a few days, permitting significant quantitative analyses to be performed. A method of temperature-programmed gas–liquid partition chromatography has been developed for this purpose. It permits the quantitative determination of about 250 substances in a sample of breath, and of about 280 substances in a sample of urine vapor. The technique should be useful in the application of the principles of orthomolecular medicine.

that take place in his body, usually catalyzed by enzymes, could be obtained by the thorough quantitative analysis of body fluids. Moreover, the thorough quantitative analysis of body fluids might permit differential diagnosis of many diseases in a more effective way than is possible at the present time.

During the past 3 years we have been engaged in developing instrumental techniques for this purpose. The problem is to make a quantitative determination of the amounts of each of several hundred substances present in a sample of urine, blood, spinal fluid, breath, saliva, or tissue. In order that the results of the analysis be significantly representative of the

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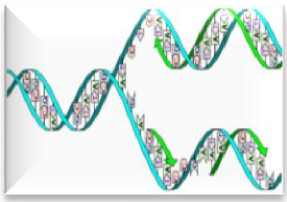
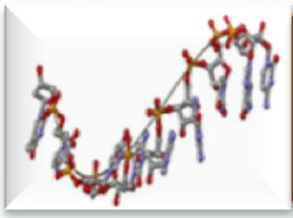
Why Metabolomics ?



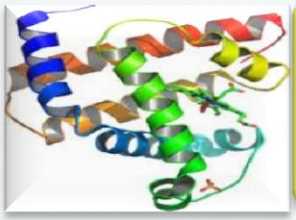
Genomics
- Genes
- ~25,000

- End point of cellular regulation



Transcriptomics
- mRNA
- ~100,000

- Dynamic & time sensitive
- Sensitivity

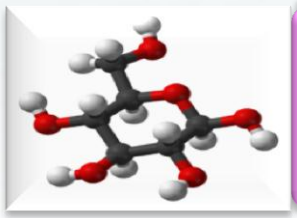


Proteomics

Proteins

~1,000,000

- High throughput
- Suitable platform for sys bio



Metabolomics
- Metabolites
- ~3000

- Integrated studies
  (Metabolomics + GWAS,
  Metabolomics + transcriptomics)

Phenotype → **Functional readout**

Metabotype → **Gate to personalised medicine**

Why is metabolomics necessary?

- The proteome cannot be completely predicted from the transcriptome due to some differences in regulatory mechanisms.
- The metabolome is further down the line from gene function and so reflects more closely the activities of a cell at the functional level.
- A metabolite may come from more than one metabolic pathway and it is only when you conduct a study on the metabolome as a whole that you can identify which pathways are involved in it's metabolism.
- Metabolomics can be viewed as complementary to transcriptomics and proteomics.

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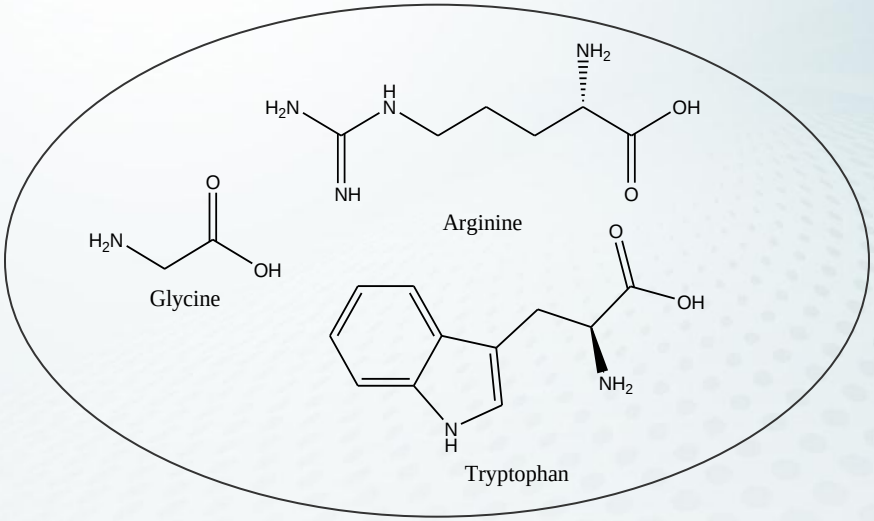
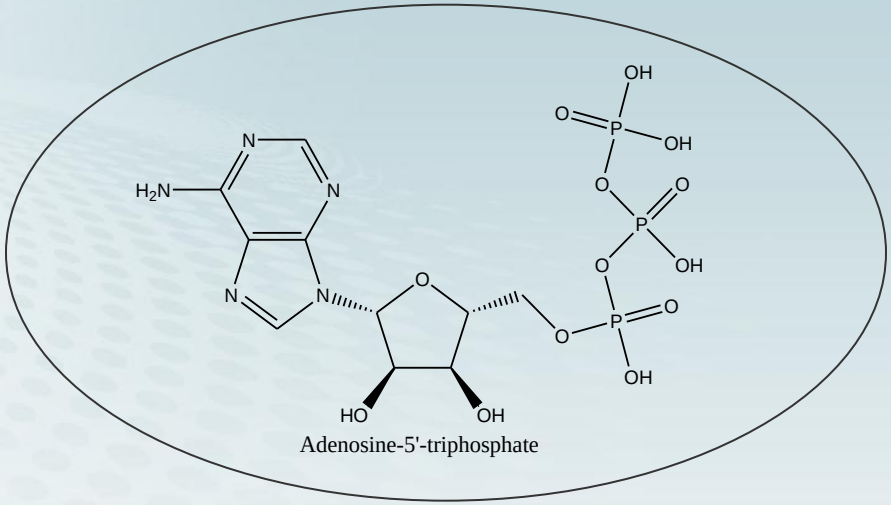
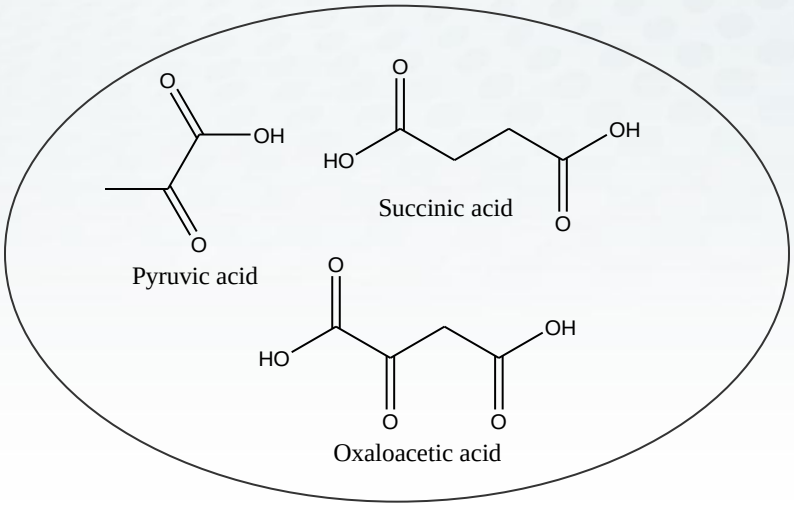
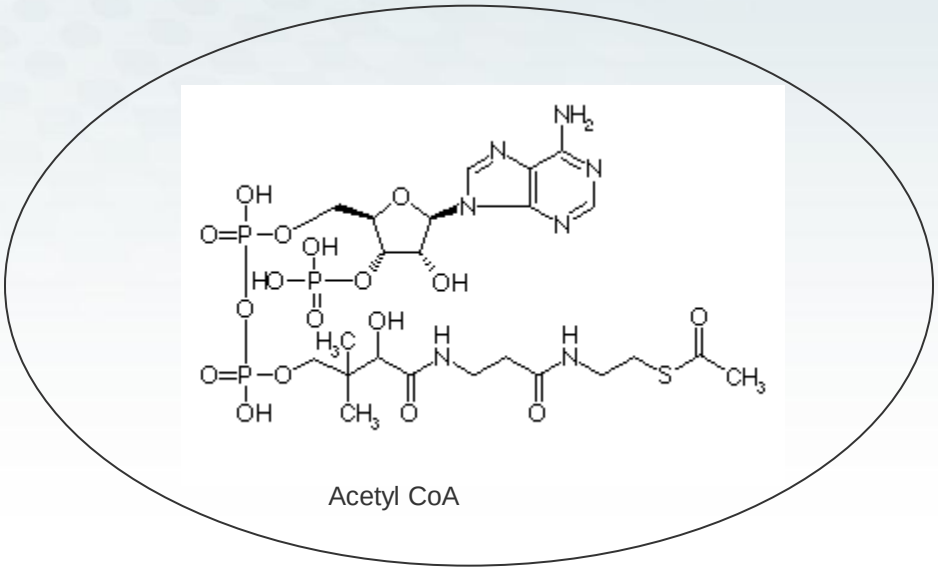
Structural Diversity of Metabolites







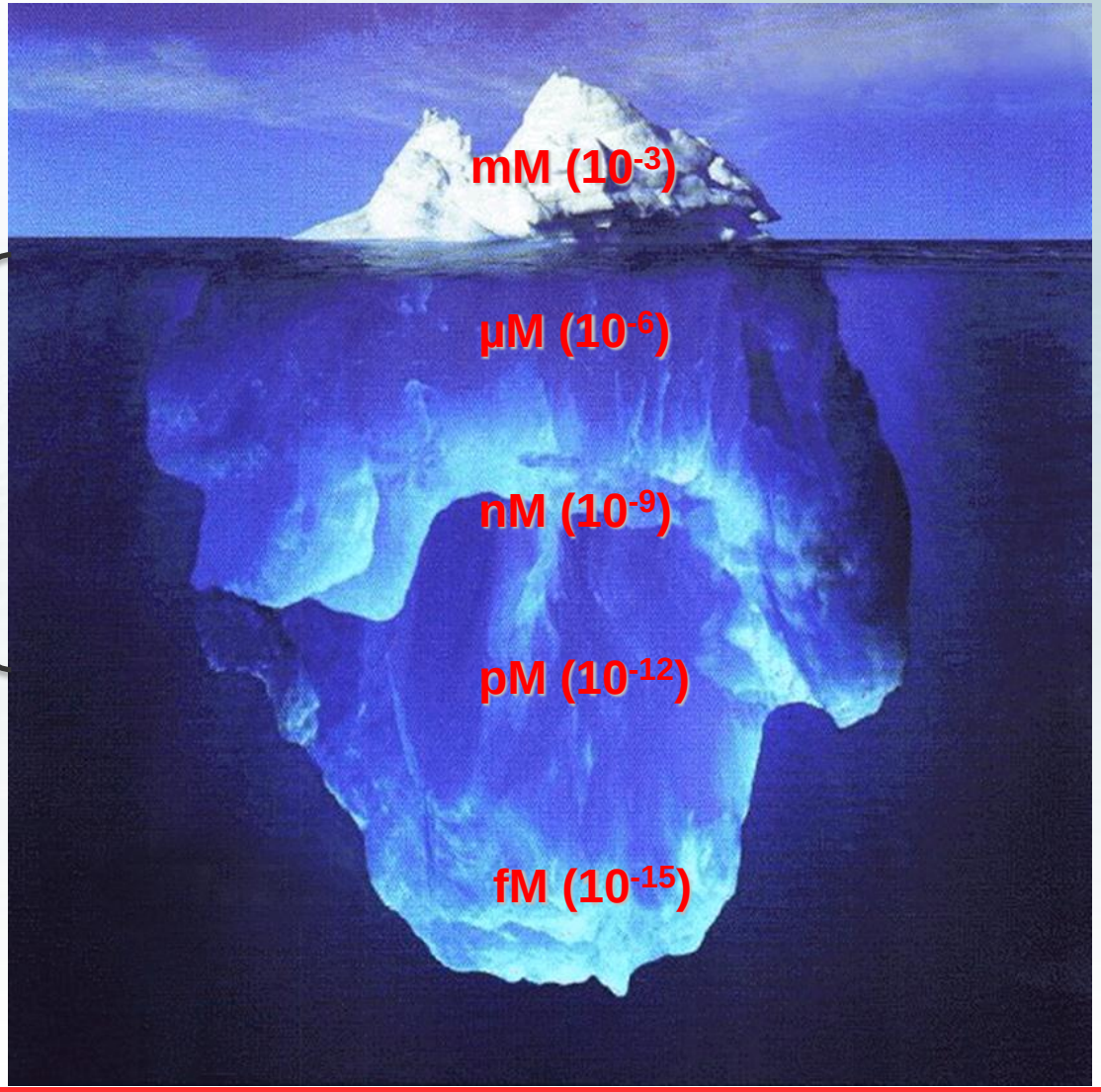


Metabolome

NMR

Mass Spectrometry

CUSTOM



Homeostasis ‘Housekeeping’

Organic Acids

Lipids

Amino Acids

Nucleotides

Steroids

Eicosanoids

Neurotransmitters

Peptides

Trace elements

Biofluid metabolic profile = Phenotype

Adapted from Sumner, LW, et al., Phytochem, 62, 817,2003

Challenges in Metabolomics

- **Successful extraction of metabolites from cellular matrices is dependent upon the type extraction procedure.**
- **When you have a structural diverse group of compounds, it becomes difficult to develop methods that can separate them all.**
- **Ionization/Visualization of all metabolites is also difficult to achieve, as each compound will vary in its affinity for a detector.**

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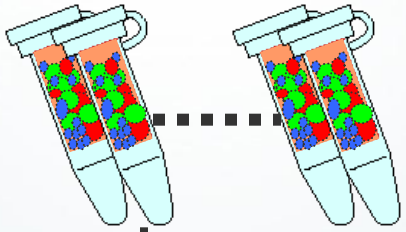
Metabolomics platform



Global profiling

- High Concentration NMR profile
- (GCx)GCMS-profile
- LCMS derivatized

Class-targeted profiling

- Glycerolipids, Sphingomyelins, Ceramides & Cerebrosides LCMS
- Bile acids, steroids, fatty acids, oxylipids LC-FTMS
- Nucleosides, Acyl CoA, phosphorylated LCMS
- peptides LCMS
- fatty acids GCMS
- Endocannabinoids LCMS

Target Biomarker Assays

- (Multiplex) Immunoassays GC, HPLC

→ Metabolic Profile

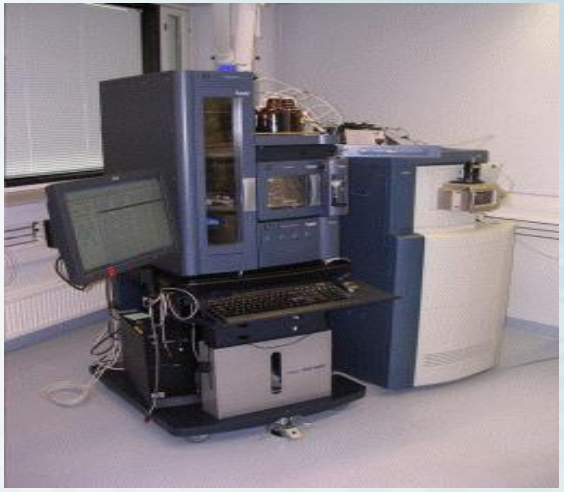
Metabolomics Analyses

- **Global analysis**
  - An analysis of the total detectable content of the sample (e.g. MS/NMR spectrum of serum/urine)
  - Primarily used for the detection of novel entities
  - Q-ToF/MS
  - GCxGC-ToF/MS
- **Targeted analysis**
  - An analysis focused onto a specific molecule or molecules (e.g. measurement of a specific *m/z*)
  - Used for the measurement of known variables for a model
  - Triple quadrupole MS

Common mass spectrometers

LC-ion trap

UPLC-Q-TOF



LC-ion trap–FT-ICR

Triple quadrupole

GCxGC-TOF

GC-TOF

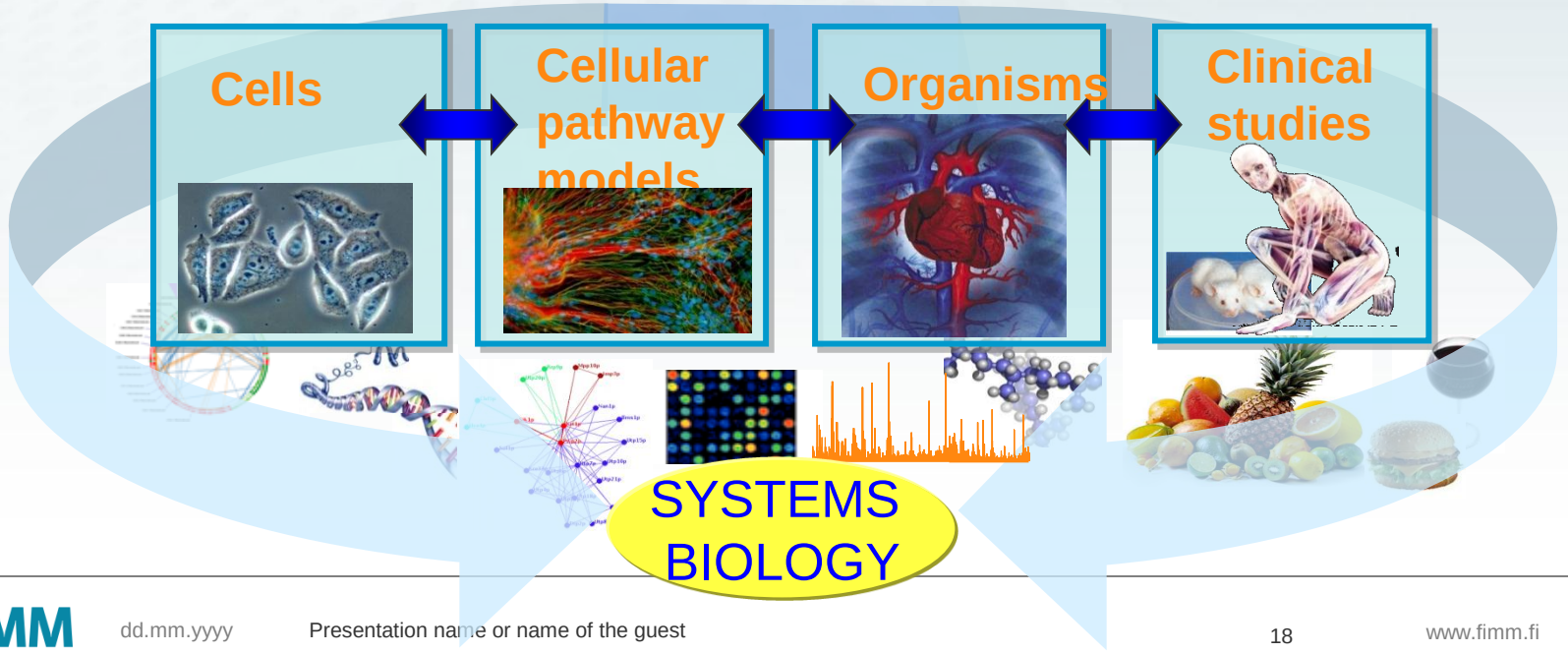
Metabolomics_systems biology

❑ Sensitivity

Small changes in activities of individual enzymes lead to small changes in metabolic fluxes, but can lead to large changes in metabolite concentrations

❑ Throughput

The metabolomics platforms afford higher throughput than current transcriptomics or proteomics technologies



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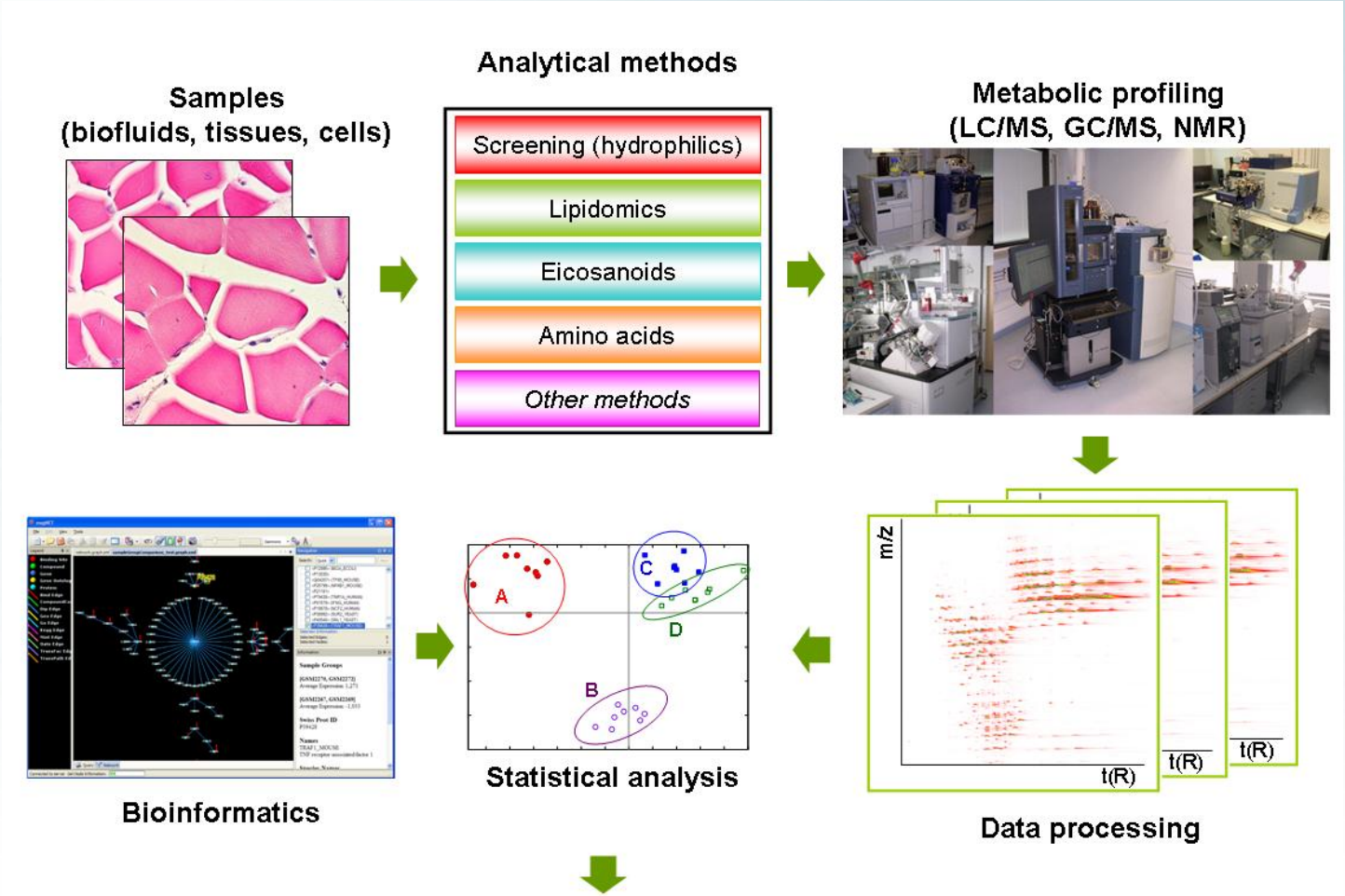
Metabolomics work flow

Experiment design + Analytical chemistry + Chemometrics + Bioinformatics



Metabolite extraction

Extraction protocols differ based on the type of sample

- **Serum/plasma**
- **Tissues**
- **Cells**
- **Urine**
- **CSF**
- **Saliva**
- **Dried blood spots**

And the type of analysis

- **Polar metabolites**
- **Non-polar metabolites**

There is NO single extraction method that could extract all the metabolites

Metabolite Separation

Different techniques are available based on the type of analysis

- **Liquid chromatography**
  Suitable for compounds that are non-volatile in nature
  - HPLC
  - UPLC
  - Nano-LC
  - 2D-LC

- **Gas chromatography**
  Suitable for compounds that are volatile in nature
  - GC
  - 2D-GC

Metabolite analysis

Ionization | **Mass Sorting (filtering)** | **Detection**

Ion Source → **Mass Analyzer** → **Ion Detector**

Form ions (charged molecules)

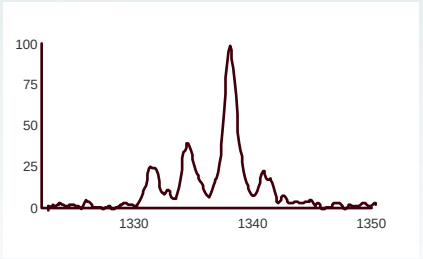
Sort Ions by Mass (m/z)

Detect ions

Acquiring a Mass Spectrum

Inlet

- **Liquid**
- **Vapor**



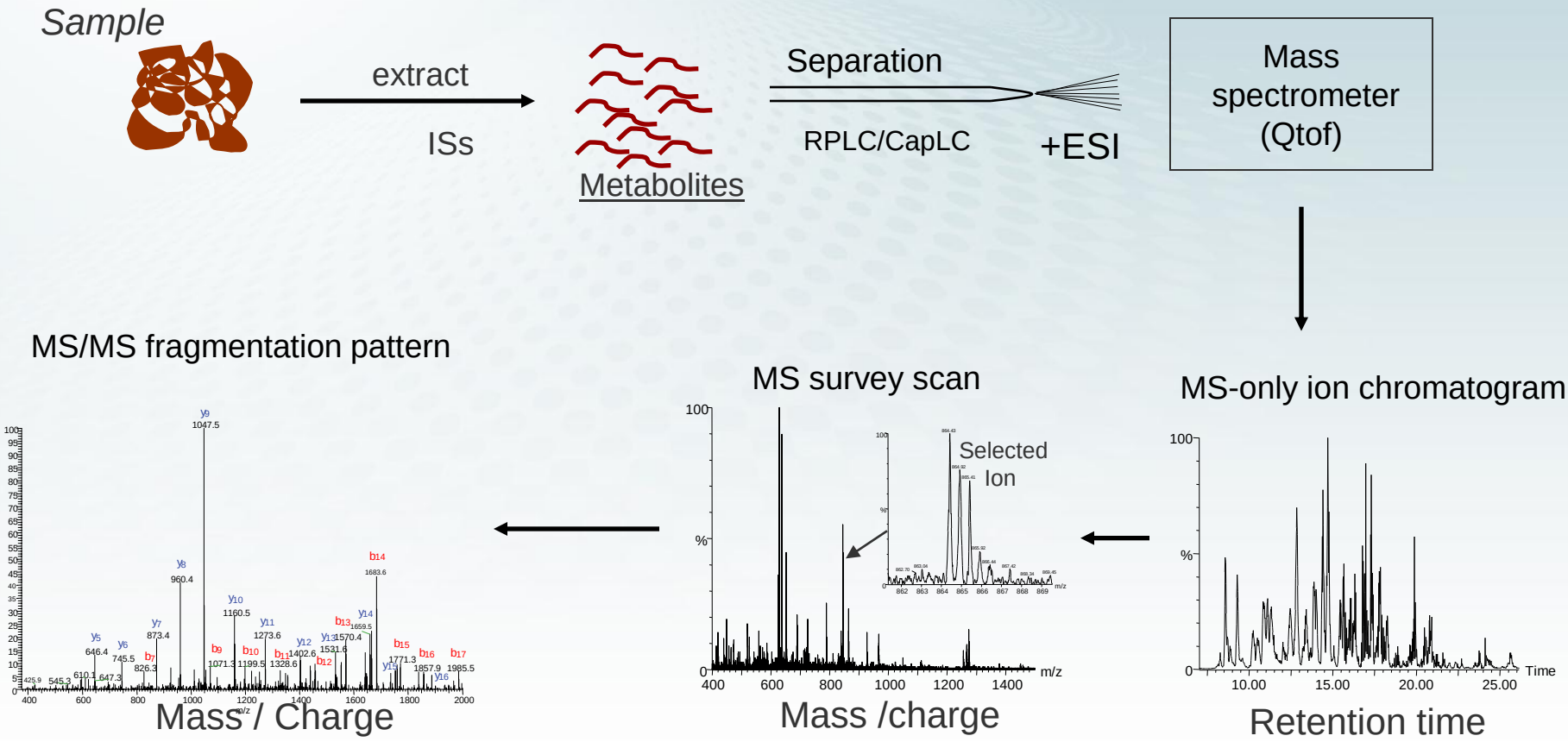
Mass Spectrum

The separation of ions based on their mass / charge ratio'

MS types: quadrupole, triple quad, TOF, quad-TOF, ion trap, ICR

All compounds must be ionized prior to MS detection!

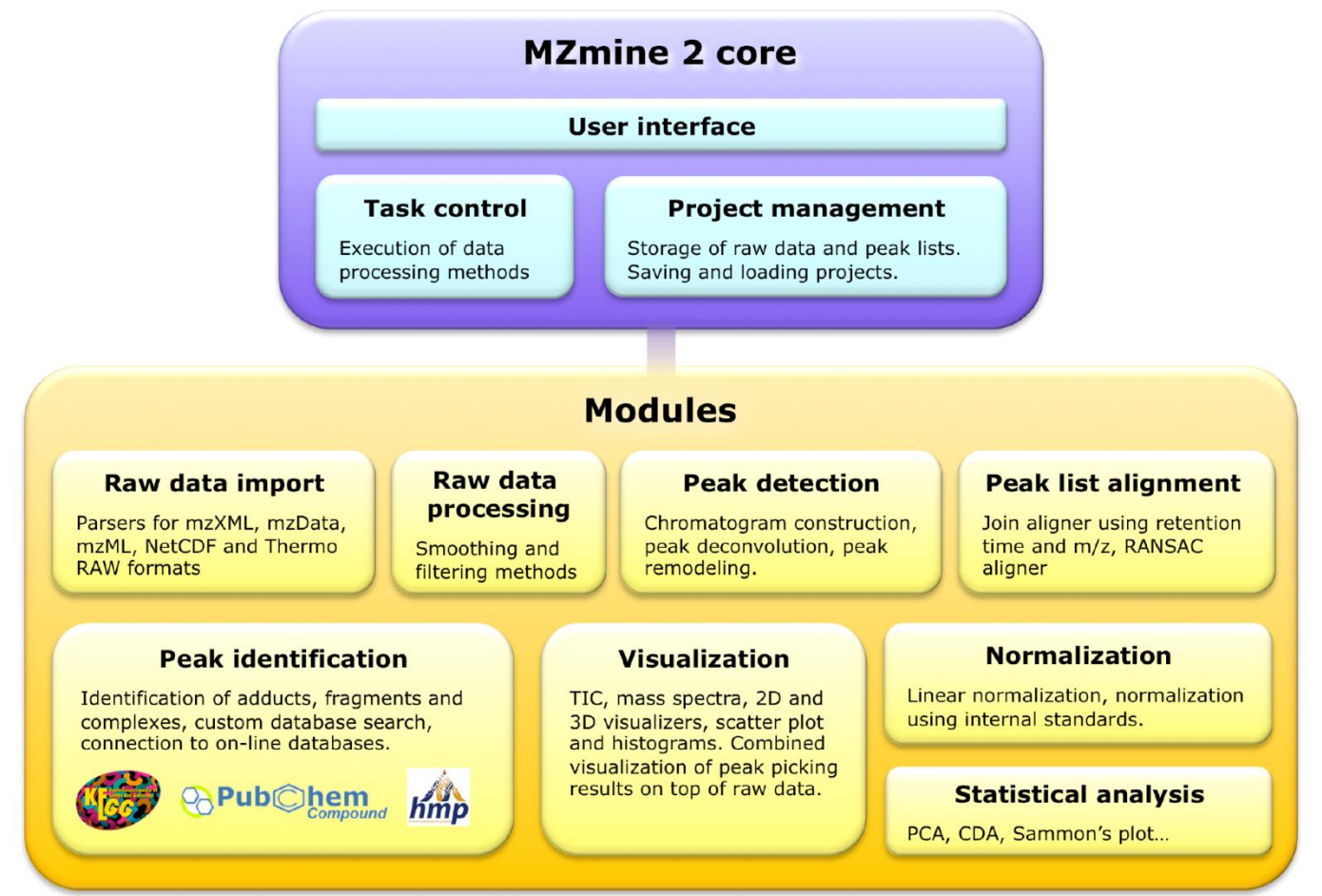
LC/MS metabolomics platform



Data processing

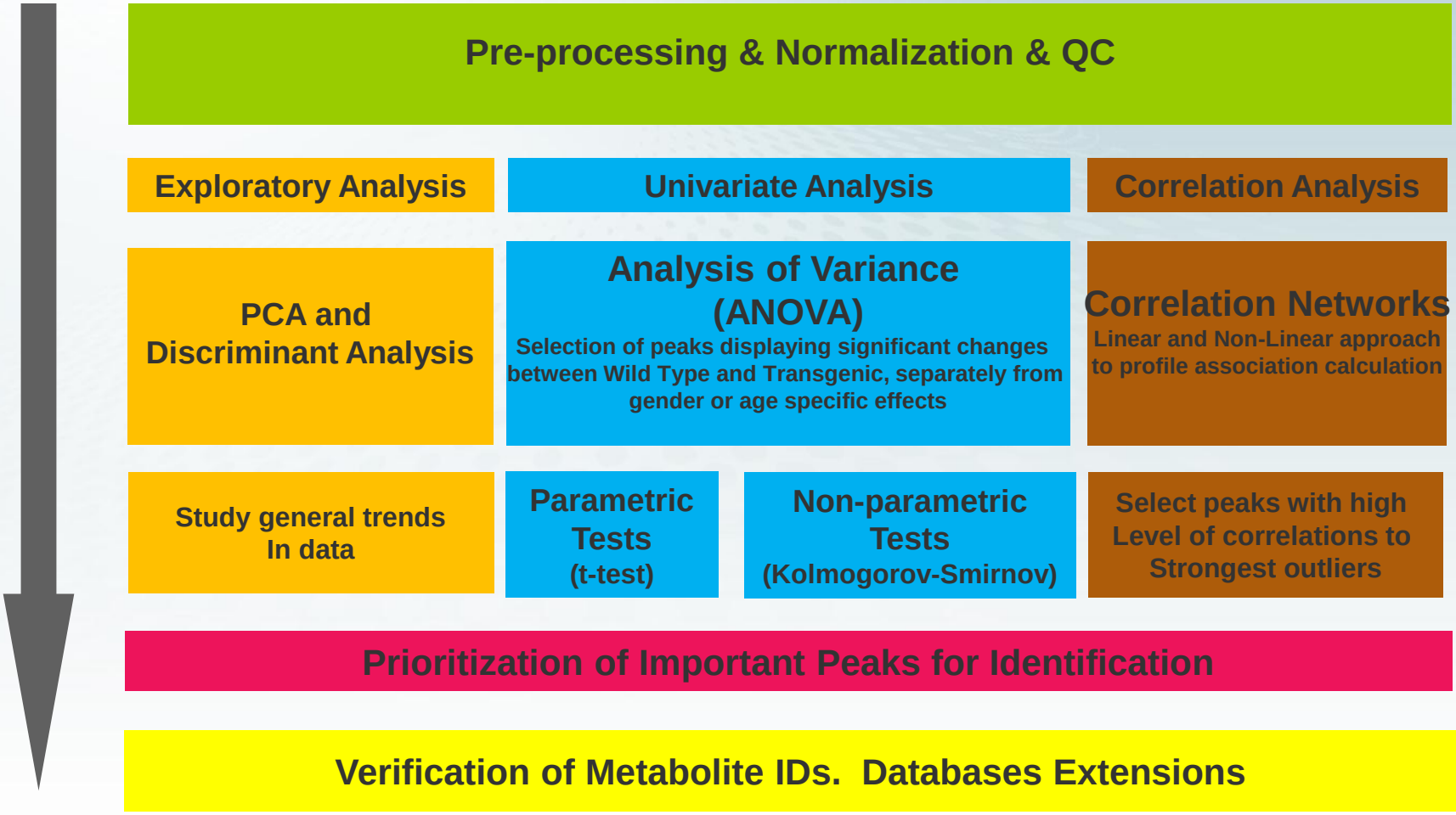
MZmine 2

1. **Platform independent** (e.g. Windows, Linux, Mac)
2. **Modular software design** (easy to join development effort)



(http://mzmine.sourceforge.net/)

Data Analysis



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Clinical Metabolomics Applications

nature news

nature news home | news archive | specials | opinion | features | news blog

Published online 14 December 2009 | Nature | doi:10.1038/news.2009.1128

News

Surgeons get real-time tissue profiling

Nuclear magnetic resonance technology could reduce time spent under the knife.

Ananyo Bhattacharya

Chemical fingerprints of tissue samples taken during operations could soon help surgeons to decide where to make their incisions. Two groups — one based in the United Kingdom, the other in France — are leading efforts to use nuclear magnetic resonance (NMR) spectroscopy to

Could tomorrow's surgeons be guided by nuclear magnetic resonance?

nature Vol 457|12 February 2009|doi:10.1038/nature07762

LETTERS

Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression

Arun Sreekumar[1,2,3,4], Laila M. Poisson[5]*, Thekkelnaycke M. Rajendiran[1,3]*, Amjad P. Khan[1,3]*, Qi Cao[1,3], Jindan Yu[1,3], Bharathi Laxman[1,3], Rohit Mehra[1,3], Robert J. Lonigro[1,4], Yong Li[1,3], Mukesh K. Nyati[4,6], Aarif Ahsan[6], Shanker Kalyana-Sundaram[1,3], Bo Han[1,3], Xuhong Cao[1,3], Jaeman Byun[7], Gilbert S. Omenn[2,7,8], Debashis Ghosh[4,5,11], Subramaniam Pennathur[2,4,7], Danny C. Alexander[12], Alvin Berger[12], Jeffrey R. Shuster[12], John T. Wei[4,9], Sooryanarayana Varambally[1,3,4], Christopher Beecher[1,2,3] & Arul M. Chinnaiyan[1,2,3,4,9,10]

nature chemical biology

ARTICLE

PUBLISHED ONLINE: 2 MAY 2010 | DOI: 10.1038/NCHEMBIO.364

Metabolic oxidation regulates embryonic stem cell differentiation

Oscar Yanes[1], Julie Clark[2], Diana M Wong[1], Gary J Patti[1], Antonio Sánchez-Ruiz[2], H Paul Benton[1], Sunia A Trauger[1], Caroline Desponts[2], Sheng Ding[2]* & Gary Siuzdak[1]*

nature medicine

published online 20 March 2011; doi:10.1038/nm.2307

Metabolite profiles and the risk of developing diabetes

Thomas J Wang[1-3], Martin G Larson[3,4], Ramachandran S Vasan[3,5], Susan Cheng[2,3,6], Eugene P Rhee[1,7,8], Elizabeth McCabe[2,3], Gregory D Lewis[1,2,8], Caroline S Fox[3,9,10], Paul F Jacques[11], Céline Fernandez[12], Christopher J O'Donnell[2,3,8], Stephen A Carr[8], Vamsi K Mootha[8,13,14], Jose C Florez[8,13], Amanda Souza[8], Olle Melander[15], Clary B Clish[8] & Robert E Gerszten[1,2,8]

Clinical Metabolomics Applications

- > 95% of all diagnostic clinical assays look for small molecules
- 89% of known drugs are small molecules
- 50% of all drugs are derived from pre-existing metabolites
- 30% of identified genetic disorders involve diseases of small molecule metabolism
- Monitor/measure metabolite flux, consequences from gene KOs, and enzyme or pathway kinetics

Goldsmith, P., et al., J Surg Res, 2010. 160(1), 122-32.

Clinical Metabolomics Applications

- Generate metabolic “signatures” for disease states or host responses
- Obtain a more “holistic” view of metabolism (and treatment)
- Accelerate assessment & diagnosis
- More rapidly and accurately (and cheaply) assess/identify disease phenotypes and functions of unknown genes
- Monitor gene/environment interactions
- Rapidly track effects from toxins/drugs/surgery

Metabolomics : Future?

nature Vol 463|7 January 2010

OPINION

2020 visions

For the first issue of the new decade, *Nature* asked a selection of leading researchers and policy-makers where their fields will be ten years from now. We invited them to identify the key questions their disciplines face, the major roadblocks and the pressing next steps. Visit go.nature.com/htW8uM to respond and to add your vision.

Metabolomics

Jeremy K. Nicholson
Head, Department of Surgery and Cancer, Imperial College London

The analysis of the chemical fingerprints left by metabolic processes has already started to play a crucial part in personalized medicine, particularly cancer therapy. This stems from the understanding that humans are metabolic superorganisms carrying the genomes of many symbiotic organisms, all of which can affect an individual's physiology. Human metabolism is heavily influenced by interactions between our own genes and the activities of gut microbes, as well as by diet and environmental stressors.

- **Metabolomics** is <u>one out of 14 fields</u>
- Other fields in Nature's 2020 vision in which **metabolomics** plays/can play a major role

'Personalized Medicine',

'Microbiome',

'Drug Discovery',

'Mental Health'.

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Small assignment

1. What is metabolomics?
2. What is the biggest challenge in metabolomics?
3. What are different metabolomics platforms?
4. What is the typical metabolomics work flow?
5. What are the clinical applications of metabolomics?