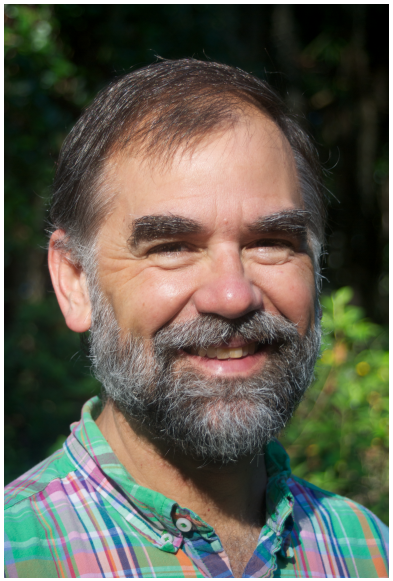
The University of Georgia

Introduction to Metabolomics (1)



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Lecture 1 Outline:
Overall Introduction to Metabolomics

- **Students should learn:**
  - **What is Metabolomics?**
  - **What is Systems Biology and how does Metabolomics relate?**
  - **Short history of Metabolomics**
  - **Basic steps needed for a Metabolomics study**
- **Students should already know:**
  - **Basics of organic chemistry and introduction to biochemistry.**

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Recommended Reading

- Nicholson, J. K., and Lindon, J. C. (2008) "Systems biology: Metabonomics", *Nature 455, 1054-1056. Short overview article by one of the leading groups.*
- Dunn, W. B., Broadhurst, D. I., Atherton, H. J., Goodacre, R., and Griffin, J. L. (2011) "Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy", *Chem. Soc. Rev., 40,* 387-426. *Fairly comprehensive review of the field. A bit dated now.*
- Barnes, S. et al., Training in metabolomics research. I. Designing the experiment, collecting and extracting samples and generating metabolomics data. *J Mass Spectrom* **2016,** *51* (7), 461-75. *Good recent review of metabolomics, with an emphasis on practical aspects.*
- Barnes, S. et al., Training in metabolomics research. II. Processing and statistical analysis of metabolomics data, metabolite identification, pathway analysis, applications of metabolomics and its future. *Journal of Mass Spectrometry* **2016,** *51* (8), 535-548. *Part II of the Barnes review*

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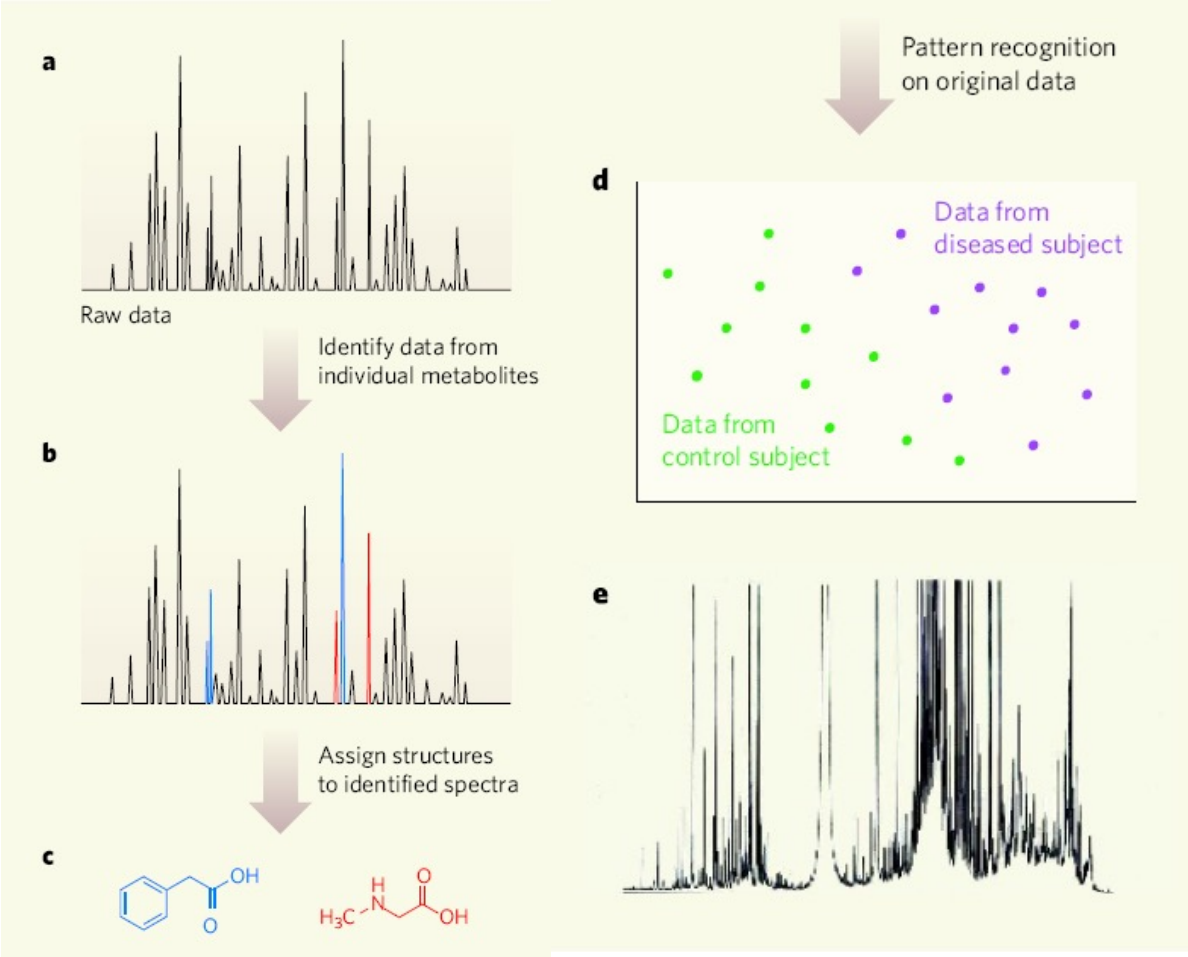
Metabolomics is "Big Data"

Statistical Analysis is a major component



Nicholson and Lindon, 2008

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What is Metabolomics?

- We will use the definition "Metabolomics is the comprehensive study of all metabolites present in a biological system." (Dunn et al., 2011; Fiehn, 2002)
- For the purposes of these lectures, metabolomics is equivalent to metabonomics

Dunn, W. B.; Broadhurst, D. I.; Atherton, H. J.; Goodacre, R.; Griffin, J. L., Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chemical Society Reviews* **2011,** *40* (1), 387-426.
Fiehn, O., Metabolomics - the link between genotypes and phenotypes. *Plant Molecular Biology* **2002,** *48* (1-2), 155-171.

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How do we define a metabolite?

- Small biological molecule with a molecular weight generally less than 1000 Da (or slightly larger…).
- The boundary between small peptides, small RNAs, and general metabolites is blurry.
- Metabolites have a much greater chemical variability than genes or proteins.
- Structural identification of metabolites is more variable than for protein or DNA sequencing.

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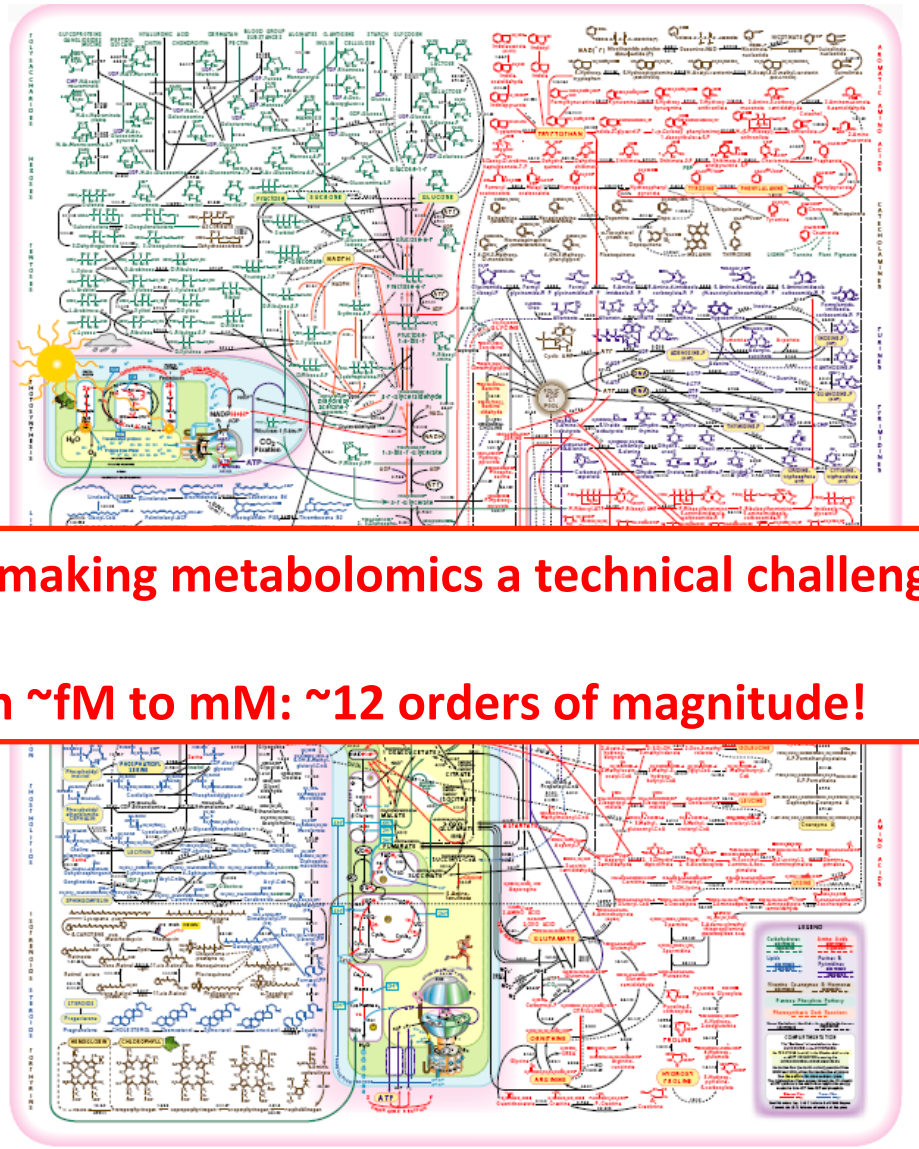
What is a Metabolite?

Any organic molecule detectable
in the body with a MW < 1000 Da

Peptides
Oligonucleotides
Sugars
Nucleosides
Organic acids



These groups are chemically diverse, making metabolomics a technical challenge

They also range in concentration from ~fM to mM: ~12 orders of magnitude!

Lipids
Steroids
Alkaloids
Drugs (xenobiotics)

Adapted from Workshops with Jean-Luc Wolfender

Origins of Metabolites

- Metabolites can originate from the "main organism" (e.g. human, animal, plant)
- OR from exposures and diet (exposome)
- OR from associated microbes (e.g. gut micro flora in animals or endophytic fungi in plants)
- OR from biosynthetic pathways involving **both** the host and microbes

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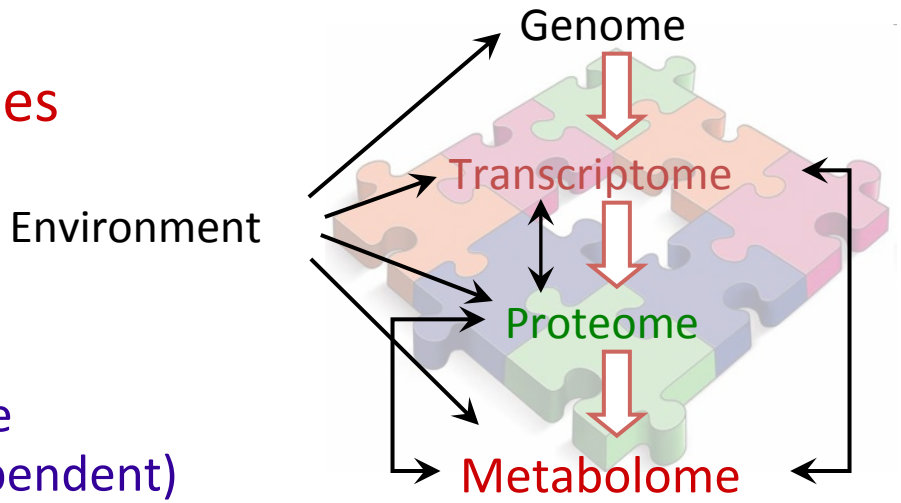
Systems Biology

Systems Biology is the inter-disciplinary study of complex interactions occurring in biological systems.

It is based on Omics technologies and mathematical models



Genomics

Study of genes (the only -ome which is not context dependent)

Transcriptomics

All the mRNA in a cell/tissue/organism

Proteomics

All the proteins in a cell/tissue/organism

Metabonomics/Metabolomics

All the metabolites in a cell/tissue/organism

Adapted from Wolfender

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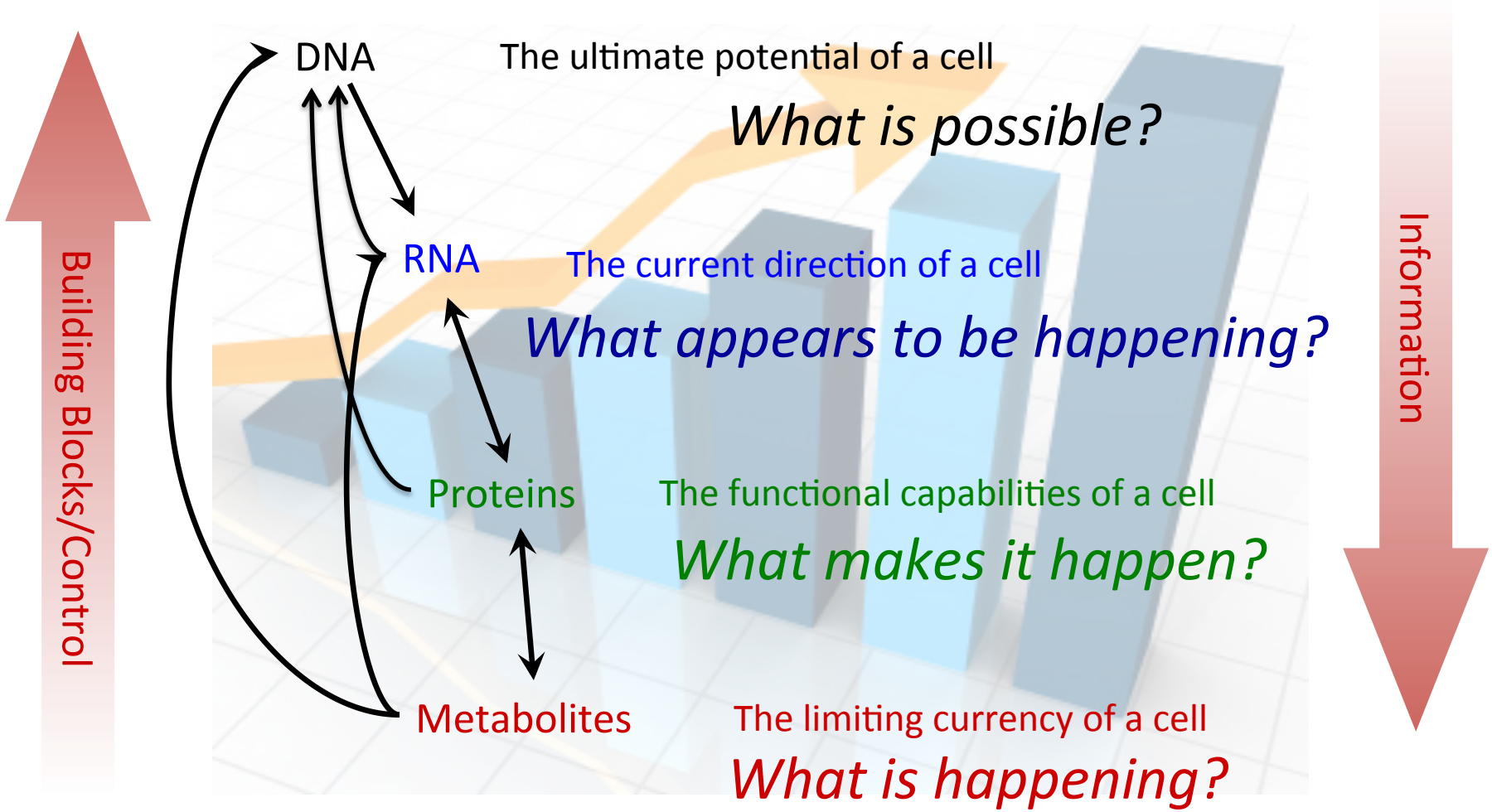
Interactions of Networks in the Cell: Systems Biology



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The Metabolome is Sensitive to

- Genotype
- Changes in mRNA
- Changes in protein
- Associated microbes
- External environment:
  - Diet
  - Disease
  - Treatment
  - Exposure to toxins, drugs, etc.

This sensitivity is both an advantage and disadvantage in metabolomics.

It can be difficult to identify direct associations between cause and response.

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A (brief) History of Metabolomics

Figure 1 | Metabonomics of yore. This urine wheel was published in 1506 by Ullrich Pinder, in his book *Epiphanie Medicorum*. It describes the possible colours, smells and tastes of urine, and uses them to diagnose disease.

NATURE|Vol 455|23 October 2008

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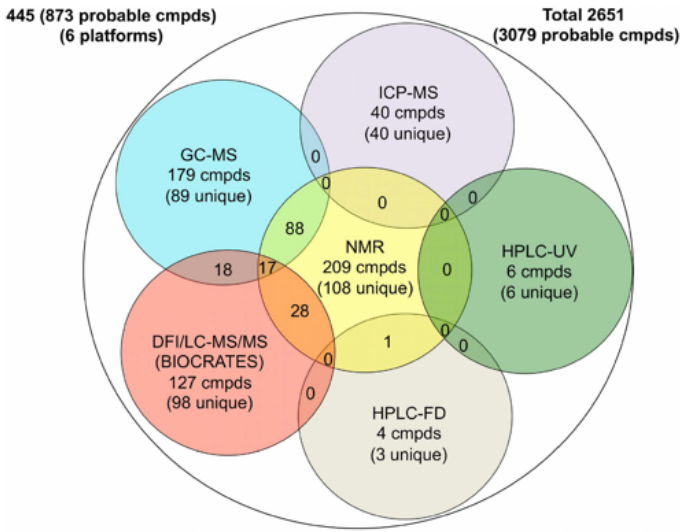
Human Urine



Bouatra, S.; et al., Wishart, D. S., The human urine metabolome. *PLoS One* **2013,** *8* (9), e73076.

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For example, have you wondered about the smell of urine after eating asparagus?

(If you haven't ever wondered, you may not have the gene necessary to smell it!)

The volatile organic compounds responsible for the smell are identified as:
methanethiol,
dimethyl sulfide,
dimethyl disulfide,
bis(methylthio)methane,
dimethyl sulfoxide, and
dimethyl sulfone.

French novelist Marcel Proust famously wrote in 1913 that asparagus "transforms my chamber-pot into a flask of perfume." And one British men's club is said to have put up a sign reading, "During the asparagus season, members are requested not to relieve themselves in the hat stand."

Wikipedia and
Waring RH, Mitchell SC and Fenwick GR (1987). "The chemical nature of the urinary odour produced by man after asparagus ingestion". *Xenobiotica* **17** (11): 1363–1371.

Mitchell, S.C. (2001). "Food idiosyncrasies: beetroot and asparagus". *Drug Metabolism and Disposition*. **29** (4): 539–543.

http://www.webmd.com/food-recipes/features/why-pee-smells-funny-eat-asparagus

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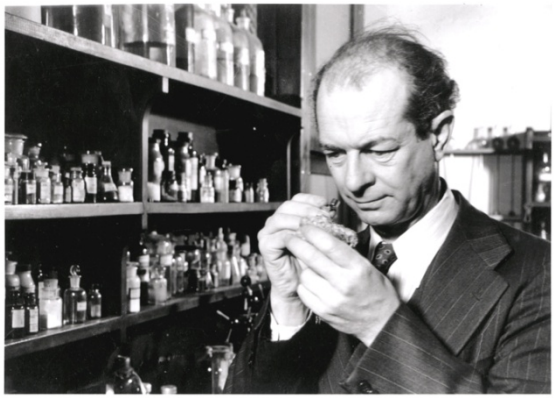
A (brief) History of Metabolomics

Linus Pauling hypothesised on the predictive capacity of chromatographic profiling of body fluids for detection and diagnosis of human disease.



Chromatographic separation techniques were developed in the late 1960's.

Robinson and Pauling published "Quantitative Analysis of Urine Vapor and Breath by Gas-Liquid Partition Chromatography" in 1971.

The Metabolome and Metabolomics concept were proposed in the 1990s. (Holistic approach—in contrast to reductionist)

In January 2007, the Human Metabolome Project, completed the first draft of the human metabolome, consisting of 2,500 metabolites, 1,200 drugs and 3,500 food components. The current release of the HMDB contains over 42,000 metabolite entries. (http://hmdb.ca/).

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Direct GC Analysis

280 substances separated and quantified.

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

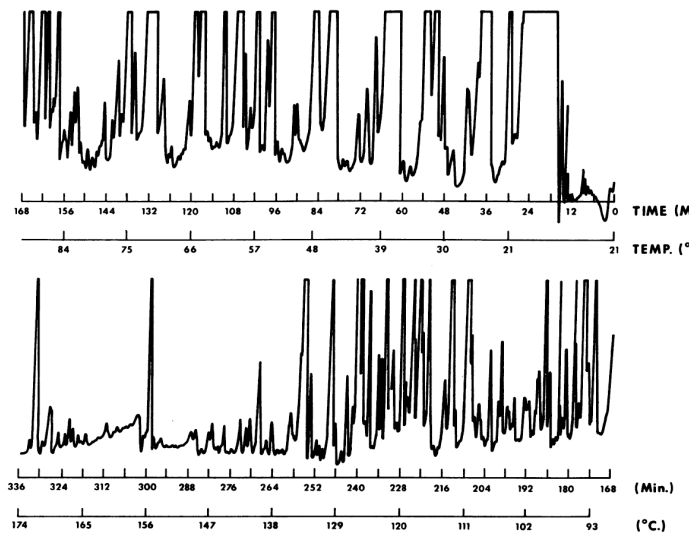

FIG. 1. Chromatogram of urine vapor.

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

(orthomolecular medicine/vitamins/controlled diet)

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

Contributed by Linus Pauling, July 29, 1971

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Comprehensive metabolic profiling

It is estimated that the metabolome extends over 12 magnitudes of concentration (fmol-mmol), and the number of metabolites in the plants is estimated to exceed 200,000 and only about 10,000 are known.

There are certainly 100s of thousands of metabolites in humans if we include the exposome, diet, microbiome, etc.

It is not currently possible to analyse the entire range of metabolites by a single analytical method.

Different separation and detection methods should be used either individually or in combination to detect and quantify hundreds or perhaps thousands of metabolites.

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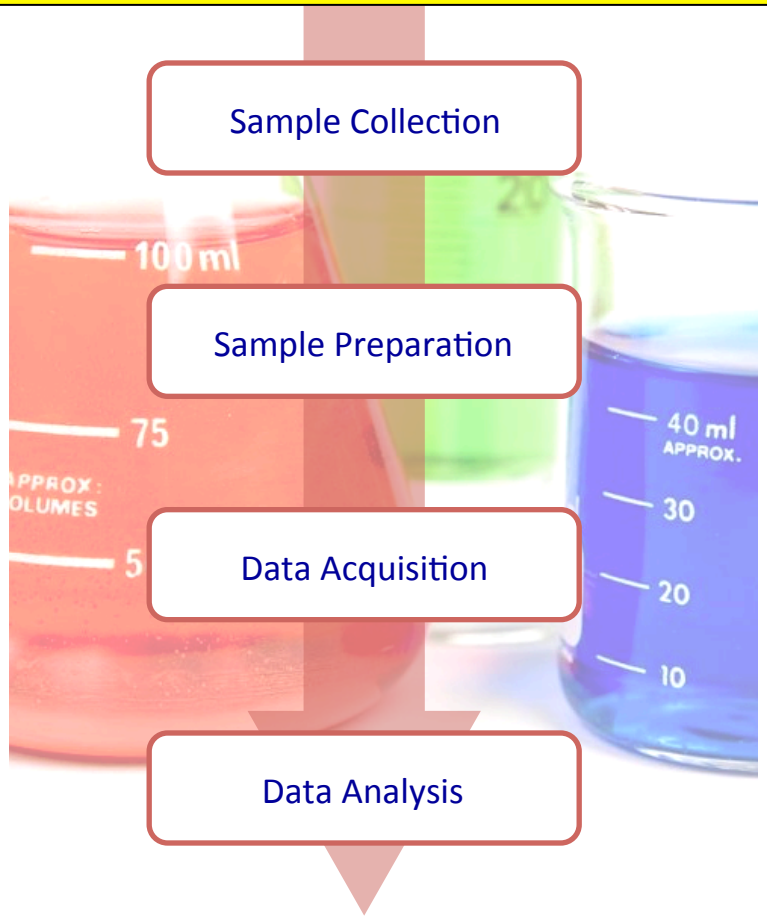
General Procedure For Metabolomics

Every study needs to begin with a ***study design*** that clearly defines the goals of the study, the necessary controls, the numbers of samples that will be needed, and plans for analysis. This should be in consultation with an expert statistician ***before*** data are collected.



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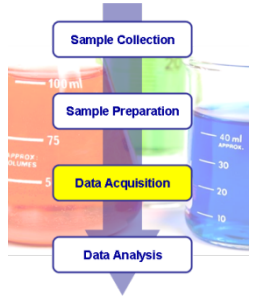
Technologies For Data Acquisition

Multiple analytical platforms are required to cover the huge chemical diversity and dynamic range of the metabolome.



Two major technologies are well-established today:

Mass Spectrometry (MS)

Nuclear Magnetic Resonance (NMR)

Hyphenated platforms allow deeper insights into the metabolic complexity

Gas Chromatography/MS
Liquid Chromatography/MS
Capillary Electrophoresis/MS

Liquid Chromatography/NMR

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Next Lecture

Introduction to Metabolomics (2)

- Strengths and weaknesses of NMR
- Strengths and weaknesses of Mass Spec
- Why you need both!

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