THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Knowledge that will change your world

Introduction to metabolomics research

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Targeted Metabolomics & Proteomics Laboratory

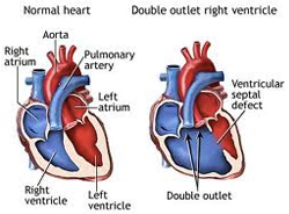
What is "Metabolomics"?

- Metabolomics is like other types of –omics analysis (microarray, RNA-Seq, proteomics, etc.)
  - Offers a "comprehensive" view of all detectable chemicals (not just metabolites)
  - Can be applied to body fluids
    - Plasma/sera, urine, saliva, tears, fecal water, etc.
  - Also to tissues
    - Liver, lung, heart, kidney, brain, eyes, etc.
  - And to single cells
    - Human, rodent, yeast, bacteria, etc.

Defining who we are chemically

- **Are we "*Living in the Promisedland*" as per Willie Nelson's song?**
- **Does an understanding of the functions of human genes define the chemical make up of our body fluids and tissues?**
- **How does metabolomics provide information on the circulating chemicals?**
- **Are the detected chemicals metabolites produced by human enzymes?**
- **So, what are we really exposed to? And does it make a difference?**

A great deal of emphasis is being placed on the importance of DNA sequencing

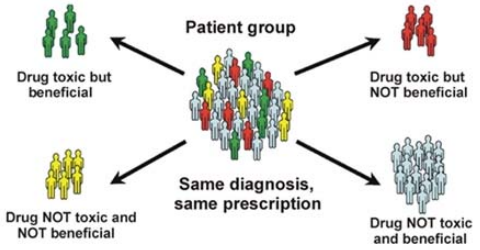


This model works for congenital diseases

Biliary atresia

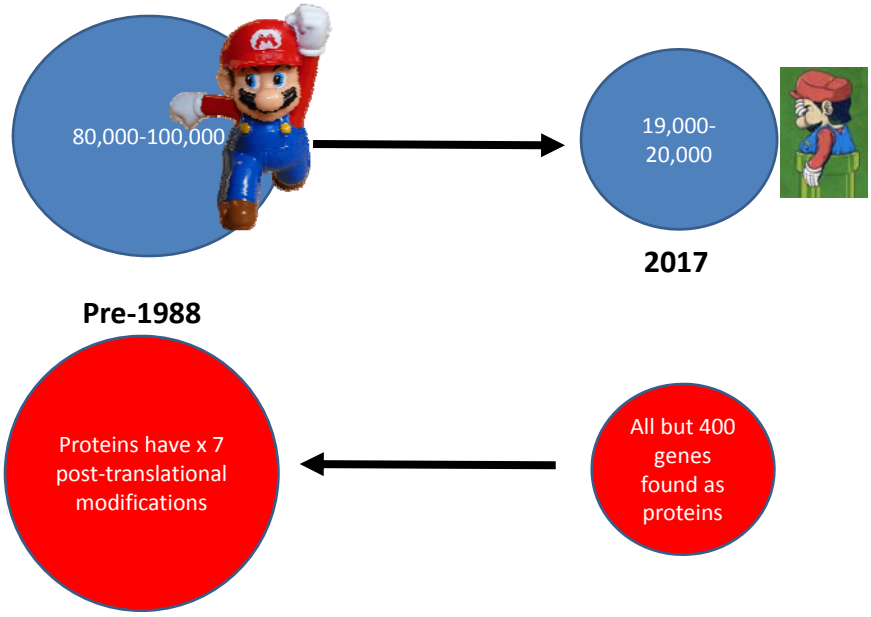
https://loveyabeckett.files.wordpress.com

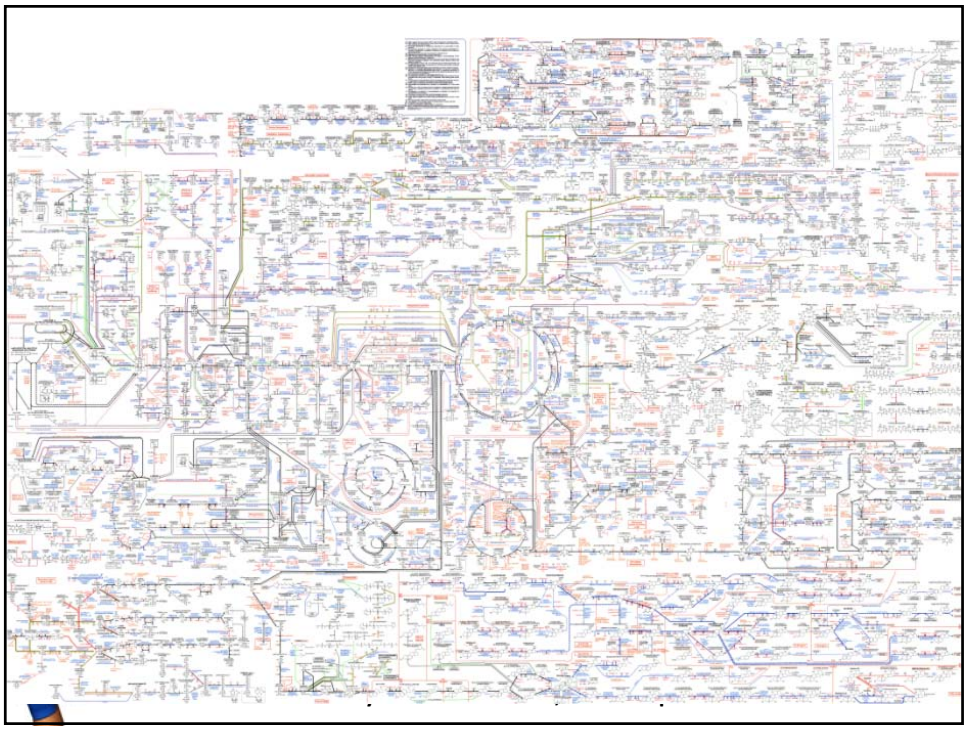
This has evolved into precision medicine and optimization of therapy



http://personalizedmedicineproject.weebly.com/

Genes failed to meet expectations





Where does the metabolome come from?

- **It starts with what fixes CO_2 and N_2**

Trees convert CO_2 to organic compounds

Field of soybeans – they fix N_2 because of nitrogen-fixing bacteria in their root nodules

Plants have more genes than humans

- **Why? Plants can't run away!!**
- **Instead, they have to practice chemical warfare to prevent attack by aphids and microorganisms**
- **Many plants are poisonous to us**
- **Understanding which plants were safe to eat, or were so if cooked, represented the rise of agriculture and civilization**

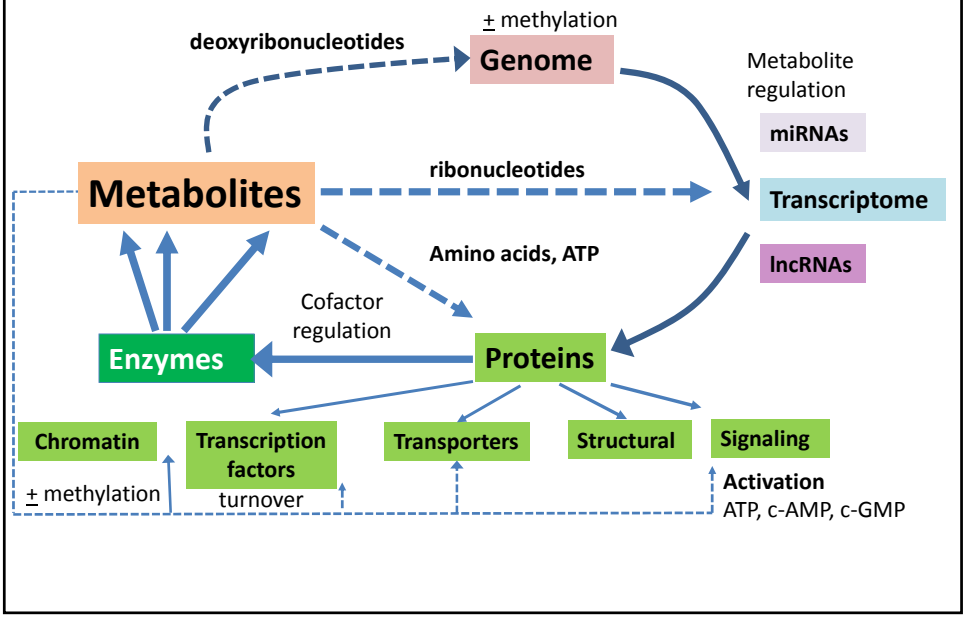
Compounds in plants and fruits

- Carotenoids
- Many vitamins
- Polyphenols and anthocyanins
- Not made by human cells

Other sources of body chemicals

- **The microbiomes**
  - **Humans are not single organisms**
  - **Instead, we are super-organisms**
  - **The gut microbiome has 10 times the number of cells found in the rest of the (human) body**
  - **It makes novel compounds that are absorbed, enter the blood stream and tissues**
- **Chemicals from the environment**
  - **industrial contaminants, therapeutics, supplements**
- **Interactions between the xenobiotics and the human enzyme systems**

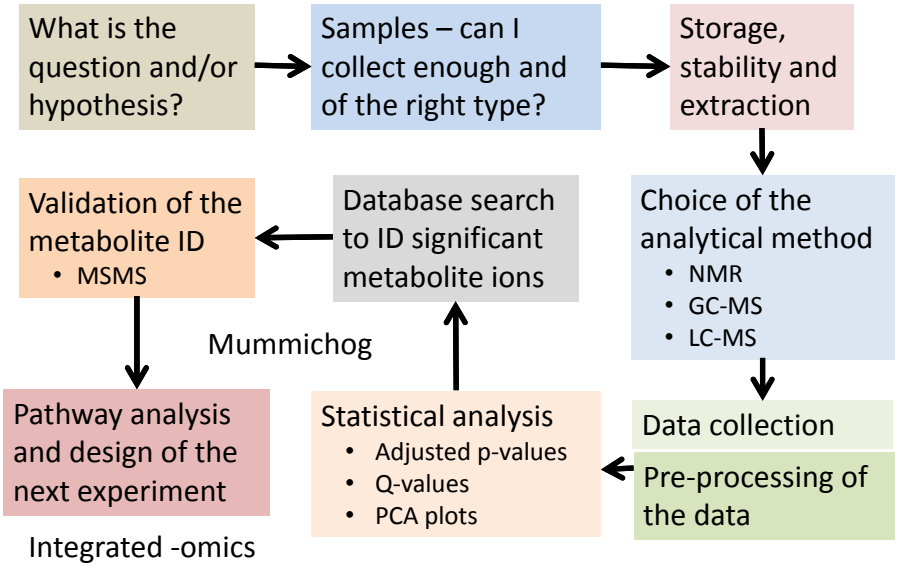
Metabolites are associated with every aspect of cellular events



World without gas!

The metabolome is very complex!

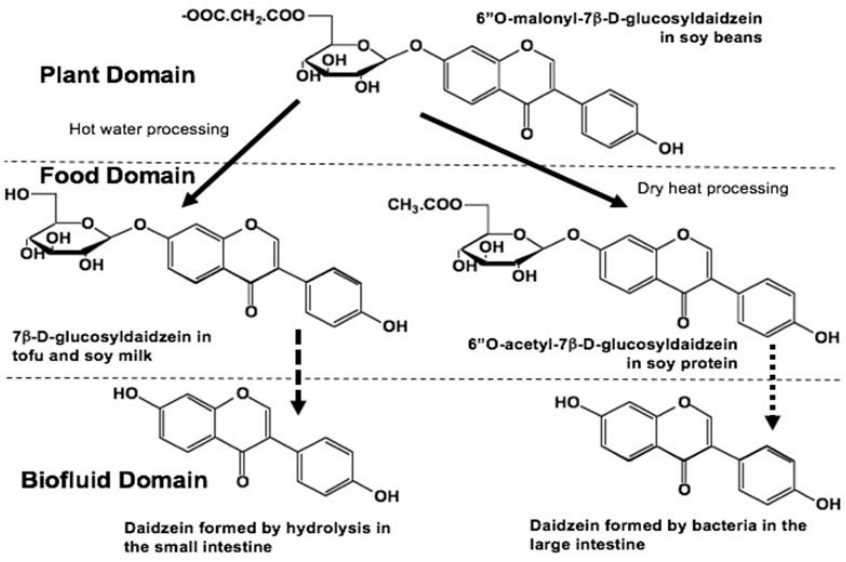
Metabolomics workflow



Course goals

- To understand
  - The vital roles of metabolites
  - The origins of metabolites
  - That metabolomics is high dimensional
  - The best methods for extracting metabolites
  - How to select the analytical approach
  - Qualitative and statistical analysis of the data
  - How to identify the "interesting" metabolites
  - How to map to (or define) pathways
  - The value of stable isotopes

Complexity in metabolism beyond cell culture
Inter-genome events



Where did metabolomics came from?

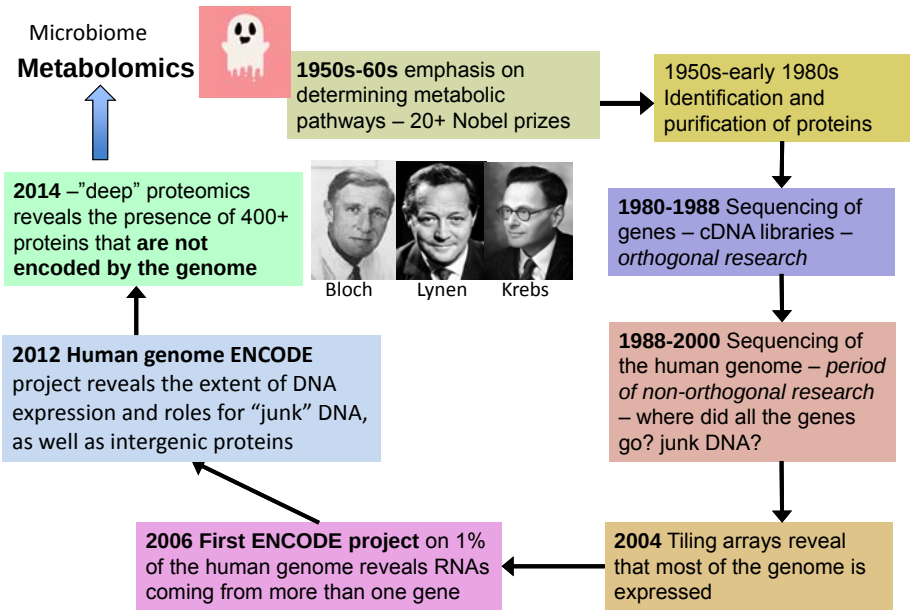
Transition of mass spectrometry to biology

Ralf Schoenheimer

David Rittenberg

- **While the politicians, tyrants, dictators and despots were salivating at the thought of developing nuclear weapons from unstable isotopes in the early part of the 20th Century, two scientists began the pursuit of the peaceful use of stable isotopes, initially deuterium (^{2}H), and later carbon (^{13}C) and nitrogen (^{15}N), to study biochemical pathways**
- **Understanding the pathways of metabolism was born**

Metabolomics and NIH Research 1948-2016



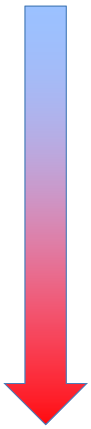
Progress in LC-MS

- **Commercial HPLC appeared in the early 1970s to separate thermally stable and unstable molecules**
- **The challenge remained to find a way to get the unstable compounds into the gas phase**
  - **Applied to macromolecules (peptides, proteins) as well as metabolites**
- **Thermospray had some initial success**
- **Electrospray ionization and chemical ionization radically changed analysis, allowing compounds to go into the gas phase at atmospheric pressure and room temperature**

LC-MS

- Suddenly, there were what appeared to be no limits (or very few) to what could be analyzed
- Unheard of, robust mass spectrometers came into play
  - "A reliable mass spectrometer" was considered in 1990 to be an oxymoron

Types of LC-MS analysis



Single quadrupole LC-MS analysis

LC-time-of-flight (TOF)-MS

FT-ICR MS

Orbi-trap

Triple quadrupole LC-MS analysis

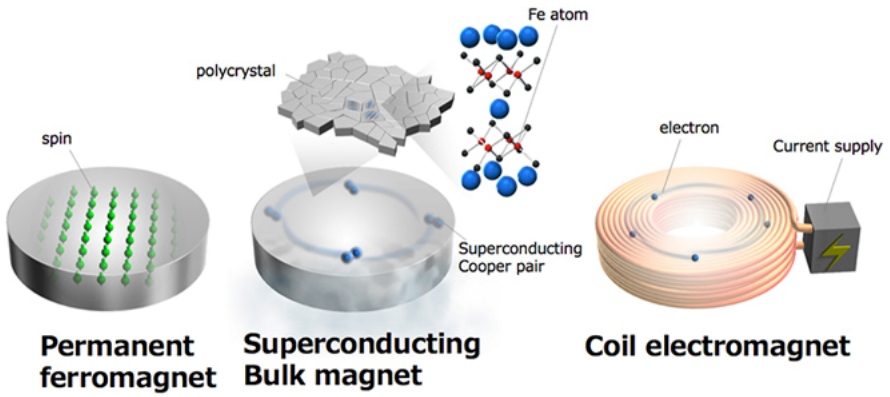
Multiple reaction monitoring (MRM)

Q-TOF

TripleTOF

Ion Mobility

NMR spectroscopy and metabolomics

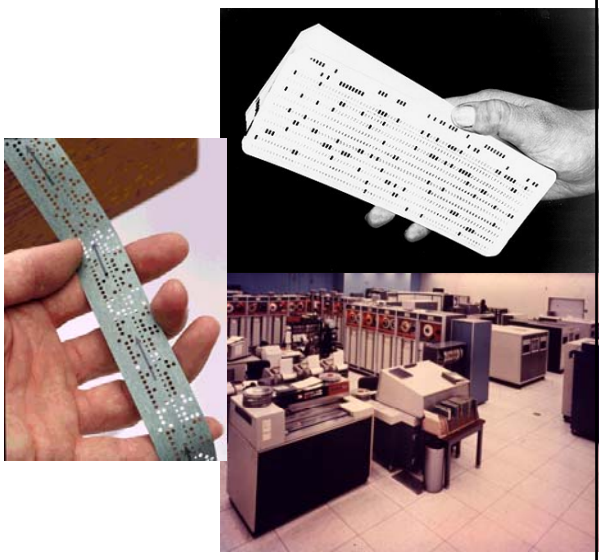


https://nationalmaglab.org/images/news_events/news/2015/october/pnictide_magnetism_1oct2015.jpg

NMR has had several critical development steps – Fourier Transform analysis of collected data, increase in field strength with superconducting magnets, micro-coil, cryogenic analysis, hyperpolarization.

Changing times in Computing

- **1950 The Cambridge colleagues of Watson and Crick calculated the structure of DNA by putting data onto punched cards and taking them by train to London for analysis – and to the fog – the "cloud" in 1950s**
- **1964 Seymour Cray develops the CDC 6600 (1 Mflops)**
- **1967 I used paper tape to collect data from a radio gas chromatograph and then submitted them via a terminal reader to the CDC 6600 at the University of London**



Today in Computing

On my desk in 2017

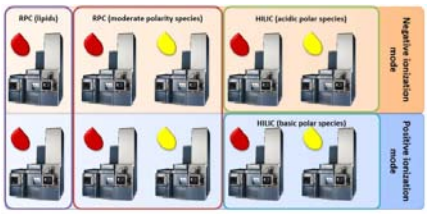
- The Apple MacBook Air with 2 quad core Intel i7 processors
  - Operates at 2.0 GHz
  - Memory of 8 GB
    - Access 1.333 GHz
  - 512 GB Flash memory storage
  - 10 Gbs Thunderbolt I/O
- Also cost ~$2,000

IBM Blue-Gene

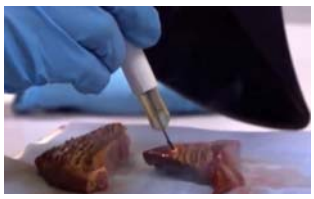
- Parallel processing with 2,048 700 MHz computers operating at 4.733 Tflops
- Replaced by Cheaha, in its current configuration it has 48 compute nodes with two 2.66GHz 6-core Intel CPUs per node (576 cores total)
- It operates at 6.125 Tflops

MRC-NIHR National Phenome Centre

600 MHz NMR instruments in surgical suite



Mass spectrometers (10 Q-TOFs) each dedicated to one assay format

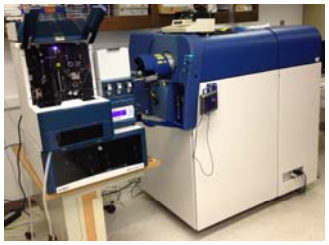


Iknife - revolutionizing surgery

This is Next-GEN precise medicine

The UK National Phenome Center, LC-MS labs

UAB capabilities in metabolomics



SCIEX 5600 TripleTOF
with Eksigent nanoLC

TMPL mass spec lab
MCLM 459/427
Stephen Barnes, Director
205-934-7117/3462

SCIEX 6500 Qtrap with SelexION

Central Alabama NMR facility
Chemistry Bdg
William Placzek, Director
205-934-2465

Great challenges in metabolomics

- **The extent of the metabolome**
  - **From gaseous hydrogen to earwax**
- **Having complete databases**
  - **METLIN has 220,000+ metabolite records, but your problem always creates a need to have more**
  - **Improvement in the size of a MSMS database**
- **Storing and processing TBs of data**
- **Standards and standard operating procedures**
- **Being able to do the analyses in real time**

NIH Common Fund Metabolomics Program

- **Metabolomics Workbench**: http://www.metabolomicsworkbench.org/
- **Regional Comprehensive Metabolomics Research Centers**
  - University of Michigan: http://mrc2.umich.edu/index.php
  - UC Davis Metabolomics Center: http://metabolomics.ucdavis.edu/
  - UNC-CH: http://www.uncnri.org/wp-content/uploads/2016/12/NIHERCMRC.pdf
  - SE Center for Integrated Metabolomics: http://secim.ufl.edu/
  - Resource Center for Stable Isotope Metabolomics: http://bioinformatics.cesb.uky.edu/bin/view/RCSIRM/
  - Mayo Clinic Metabolomics Resource: http://www.mayo.edu/research/core-resources/metabolomics-resource-core/overview