THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Knowledge that will change your world

Introduction to metabolomics research

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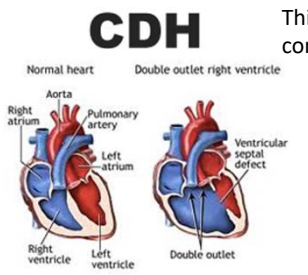
Targeted Metabolomics & Proteomics Laboratory

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

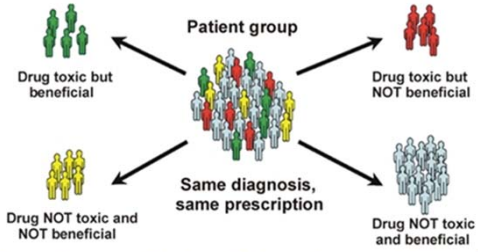


This model works for congenital diseases

Biliary atresia

https://loveyabeckett.files.wordpress.com

This has evolved into precision medicine and optimization of therapy



http://personalizedmedicineproject.weebly.com/

NIH Precision Medicine Initiative – All of Us

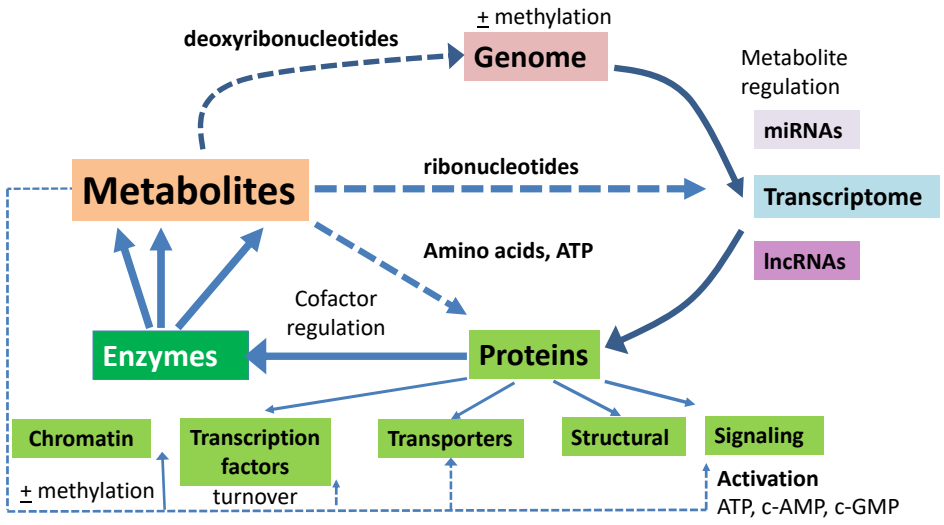
What is "Metabolomics"?

- 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.

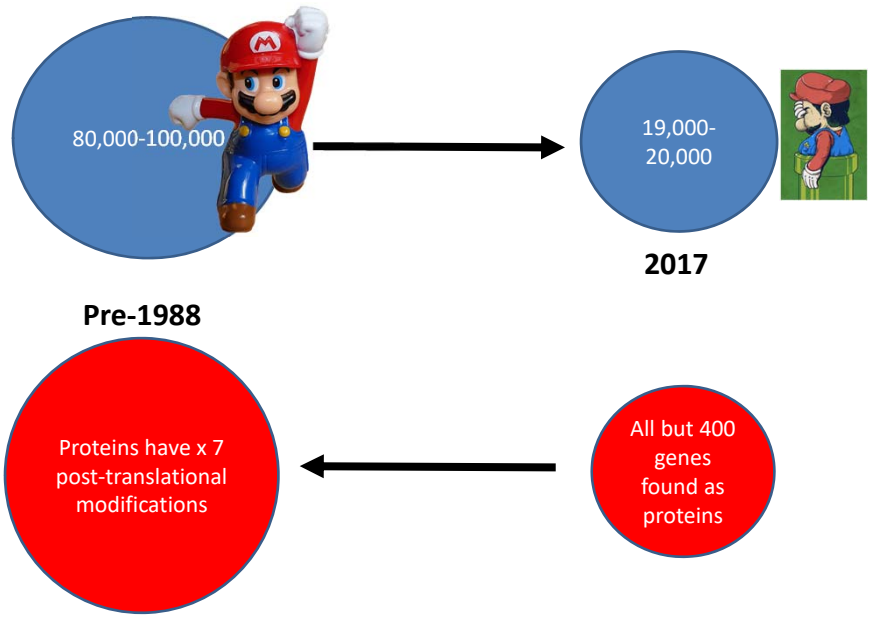
Synopsis

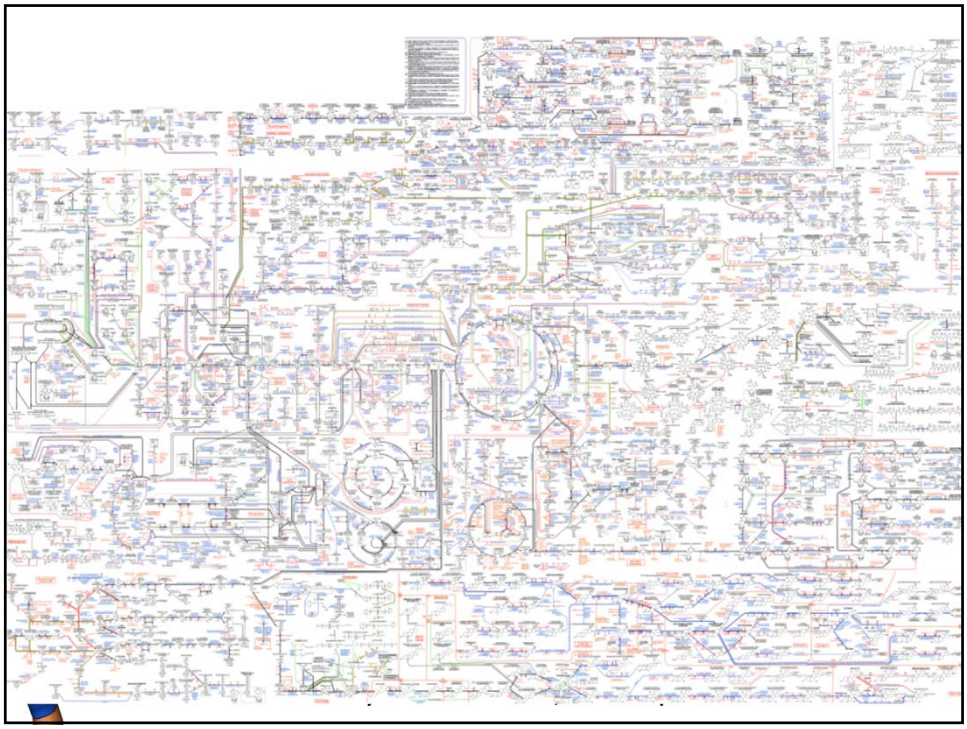
- Why has the metabolome (and metabolomics) become so important?
- What is the metabolome?
- How do I do a metabolomics experiment?
- What platform can I use?
- How do I analyze the data?
- Can I integrate metabolomics data with other –omics data? See Karan Uppal in 2017 Workshop
- Future for metabolomics in clinical and precision medicine

Metabolites are associated with every aspect of cellular events



Genes failed to meet expectations





The metabolome is very complex!

Smart and philosophic hunter-gatherers

Whoever invented me, sold me short, so I have to be a hunter-gatherer to get the missing pieces

Something's just not right – our air is clean, our water is pure, we all get plenty of exercise, everything we eat is organic and free-range, and yet nobody lives past thirty!

https://www.pinterest.com/pin/283867582737972541/

Meat eater

Berry eater

Amino acids

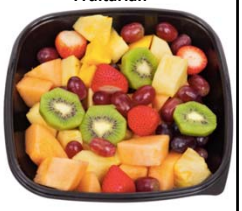
Essential	Non-essential
Arg*	Ala
His	Asn
Ile	Asp
Leu	Cys
Lys	Gln
Met	Glu
Phe	Gly
Thr	Pro
Trp	Ser
Val	Tyr

Have to eat foods rich in these

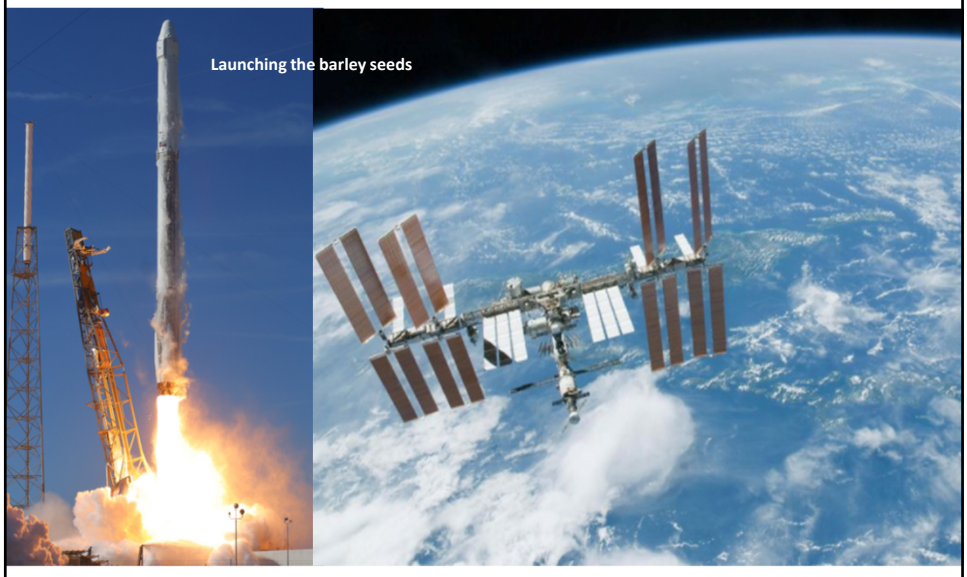
Amino acids made by human genes

Vegetarian

Fruitarian

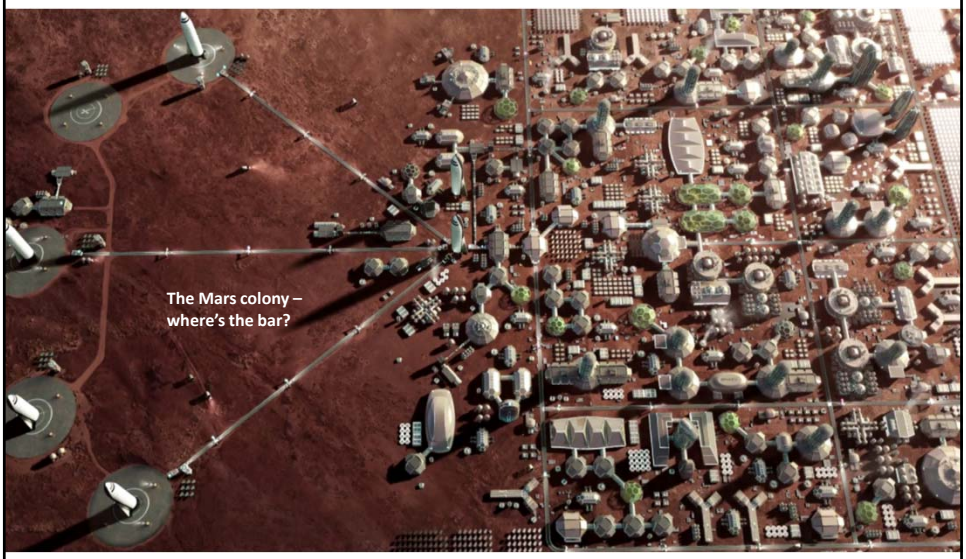


Fermented ales have been important historically



The International Space Station is no exception

Elon Musk's Mars colony



More fermentation in our diets

Dark beer – B vitamins

Rich in polyphenols

Yoghurt +polyphenols

Tempeh - soy

Kimchi - cabbage

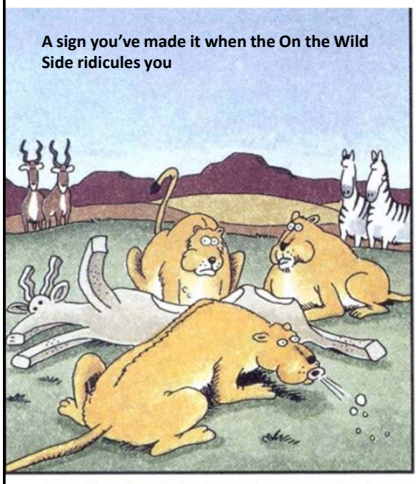
Bitter beer – male hop UK

Alcohol/calories

Plain, but fermented

Ogi + hibiscus flower syrup

A sign you've made it when the On the Wild Side ridicules you



In sudden disgust, the three lionesses realized they had killed a tofudebeest—one of the Serengeti's obnoxious health antelopes.

Be kind to your "cat"

Vet. Pathol. 25:48-57 (1988)

Veno-occlusive Disease of the Liver in Captive Cheetah

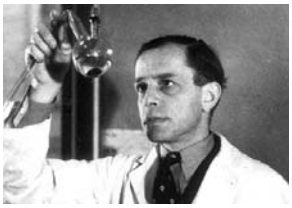
The main hepatic lesion was seen in 60% of the sexually mature cheetah (out of 126 captive animals). Observed in 1 year olds, but got worse with age and led to liver failure. Came from supplementation of the horsemeat diet with soy protein and the phytoestrogens therein.

Cats are exquisitely sensitive to aspirin and tylenol

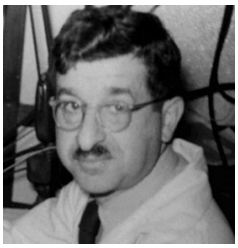
- **The defect is in UGT1A6 which has become a pseudogene – the WT form glucuronidates phenols (a mechanism to excrete them)**
  - **Cats are hypercarnivores**
    - **Not exposed to modern drugs or plants in which there are substantial amounts of phenols**
  - **Victims of "Use it or lose it"**
  - **Diet-driven evolution**
- **Mutations in exon 1**
  - **Stop codons at bp 274-276 and 379-381 (>10 MYA)**
- **UGT1A1 that glucuronidates bilirubin is unaffected**

Where did metabolomics came from?

Transition of mass spectrometry to biology

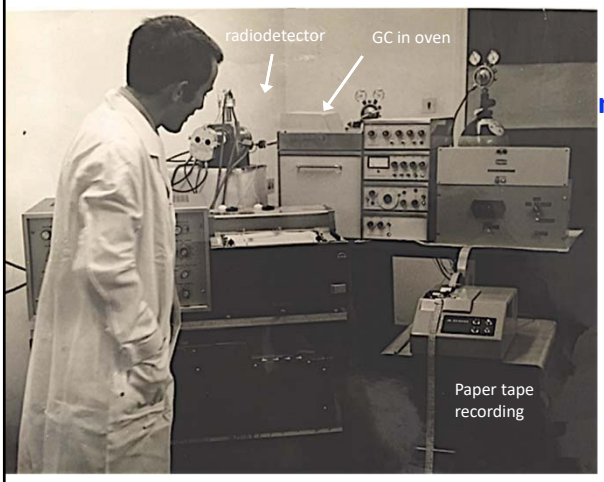


Ralf Schoenheimer



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**



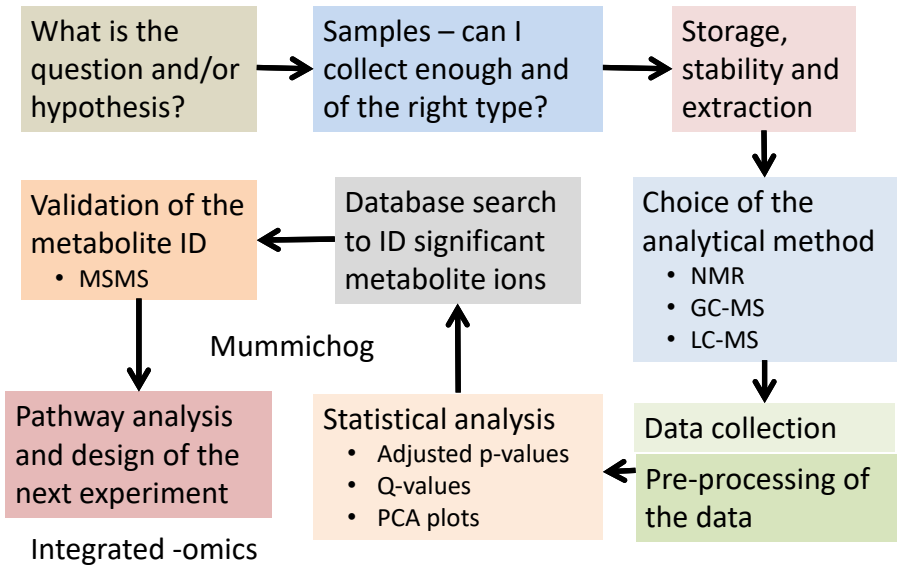
Radio-GC analysis

metabolomics in its infancy

Radio gas-liquid chromatography with digitization of collected data

Developed this for my PhD work (1967-1970) to study glucose metabolism in acellular slime mold, *Physarum polycephalum*

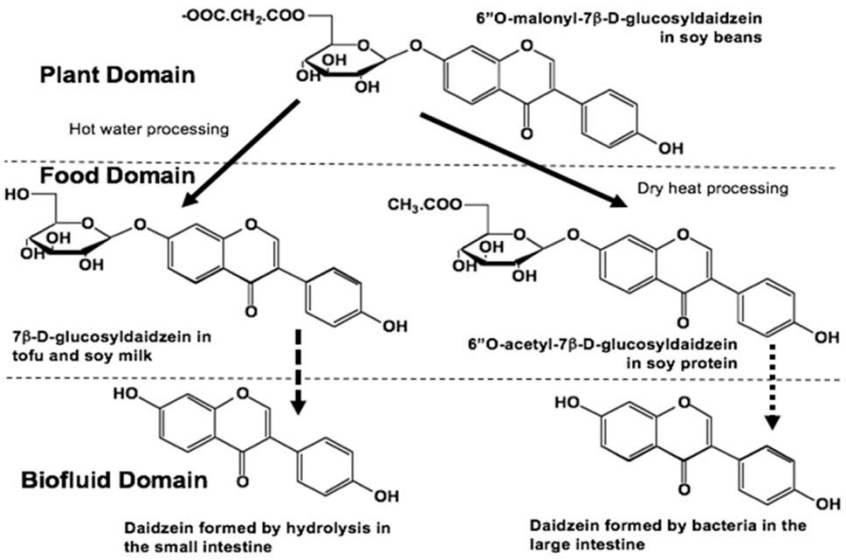
Metabolomics workflow



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

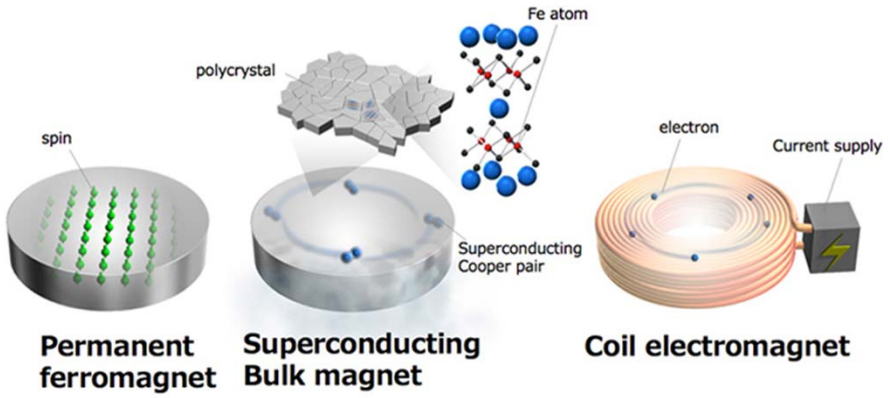


Types of LC-MS analysis

Single quadrupole LC-MS analysis	Triple quadrupole LC-MS analysis
LC-time-of-flight (TOF)-MS	Multiple reaction monitoring (MRM)
FT-ICR MS	Q-TOF
Orbi-trap	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.

Today in Computing

On my desk in 2018

- **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**
- **Cost ~$2,000 – same my first Apple II+ with 48 k of memory**

IBM Blue-Gene

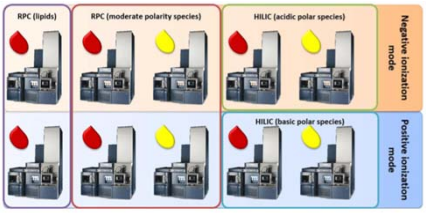
- **Massive parallel computing**
- **Replaced by Dell EMC cluster operating at 110 Tflops**
- **In its current configuration it has 78 compute nodes, each with 24-core processors (1,872 cores total)**
- **Operates with 10 and 40 GB ethernet**

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: https://transforming-science.com/tag/eastern-regional-comprehensive-metabolomics-research-core/
  - 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

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

Great challenges in metabolomics

- **The extent of the metabolome**
  - From gaseous hydrogen to earwax
- **Having complete databases**
  - METLIN has 1 million+ metabolite records, but your problem always creates a need to have more
  - Improvement in the size of a MSMS database (100,000+)
- **Storing and processing TBs of data**
- **Standards and standard operating procedures**
- **Being able to do the analyses in real time**

Questions?