

Introduction to metabolomics research

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Targeted
Metabolomics &
Proteomics
Laboratory

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MANA Microbiome group

- Organized by Dr. Robert Quinn (Michigan St)
- January 9, 2023 at 10 am
- Zoom:
 - <https://us06web.zoom.us/j/86838414835?pwd=N0lPbmU3Sk85MVBpUTluWkdud05HZz09>
- Meeting ID: 868 3841 4835
- Passcode: 022336
- Speakers: Alex Smith and Dr. Joe Zackular, U Penn
- Paper: <https://www.nature.com/articles/s41586-022-05438-x>
- Title: “Enterococci enhance *Clostridioides difficile* pathogenesis”

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Course goals

1. To understand the **vital** roles of small molecules/metabolites

To provide energy for the chemical and enzymatic processes of life

To provide the building blocks for the macromolecules (DNA, RNA, proteins, carbohydrates, lipids)

As co-factors

As signaling molecules

As biomarkers for disease

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Course goals

2. To understand the **origins** of metabolites

- Produced by (human) cells
- Produced by **the things that we eat (the food-ome)**
 - Plants (wheat, corn)
 - Fruits (apples, oranges, strawberries)
 - Vegetables (rice, potatoes, broccoli, peas)
 - Dairy products, including fermented forms
 - Meat from other animals
 - Xenobiotics
- Produced by **microorganisms** in our bodies
- Synthetic: Therapeutics, smoking, household chemicals

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Course goals

3. To understand that a metabolomics experiment is **high dimensional**
- i.e., it compares the intensities of hundreds, if not thousands, of distinct species
 - Very important statistical consequences
 - Cannot afford to do a robust experiment that fully satisfies theoretical statistical principles
 - Very important to sit down with a statistician prior to executing an experiment

Dr. Hemant Tiwari

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Course goals

4. To select the appropriate method for extracting/recovering metabolites
- **Metabolites encompass an enormous range of chemistries**
 - Gaseous (H_2 , H_2S)
 - Volatile (butyric acid, acetone, skatole)
 - Hydrophilic (glucose)
 - Charged-positive/negative (amino acids, nucleotides, organic acids, amines)
 - Hydrophobic (lipids, steroids, hydrocarbons)
 - **No single method suitable for all metabolites**

Dr. Prasain

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Course goals

5. Selecting the analytical approach

– *In situ* analysis

- Laser ablation of frozen tissue
- Other desorption methods
- Magic angle spinning NMR
- Other spectroscopic methods such as Raman CAR

– Extracted samples

- NMR
- GC-MS (1- and 2D chromatography and MSMS)
- LC-MS (1- and 2D chromatography and MSMS)
- CE-MS

– Targeted vs untargeted analysis

Drs. Placzek, Edison (UGA) and Barnes

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Course goals

6. Analysis of the data

– Data alignment

- NMR methods
- LC-MS and GC-MS methods (XCMS; ADAP; MS-DIAL)

– Statistical evaluation

- Univariate and multivariate analysis (MetaboAnalyst)
- XCMSonline
- Peaks to Pathways (Metaboanalyst)

– Data visualization

- XCMSonline
- MZmine

Dr. Barnes

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Course goals

7. Identifying metabolites

- Use of MS (absolute mass)
 - MS-DIAL
 - METLIN
 - Peaks to Pathways
 - ChemSpider
- MSMS (fragmentation spectra)
 - METLIN
 - MS-DIAL
- Metabolite standards (IROA kit)
- Importance of retention time
 - Multiple column conditions

Dr. Barnes and Prasain

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Course goals

8. Pathways and applications

- Peaks to Pathways/Metaboanalyst
- KEGG pathway mapping
- Applications to:
 - Adverse cardiovascular risk
 - Diabetes
 - Lens and kidney diseases including COVID-19
 - Cancer
- Integration with other –Omics (Dr. Hu – Emory U)
- Machine learning/Artificial Intelligence (Dr. Marquez-Lago)

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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.

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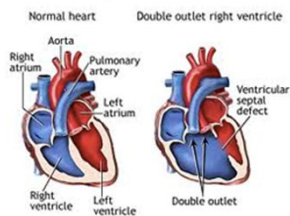
Defining who we are chemically

- 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?

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A great deal of emphasis has been placed on the importance of DNA sequencing

CDH



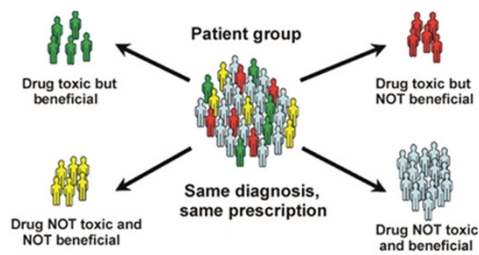
This model works for congenital diseases

Biliary atresia



<https://lovevabeckett.files.wordpress.com>

This has evolved into precision medicine and optimization of therapy



<http://personalizedmedicineproject.weebly.com/>

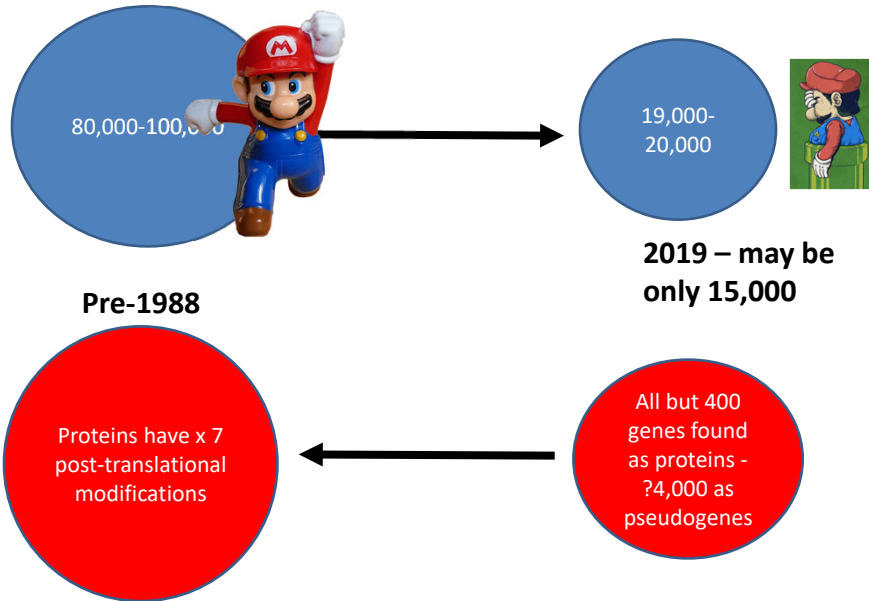
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Metabolomics in the newborn

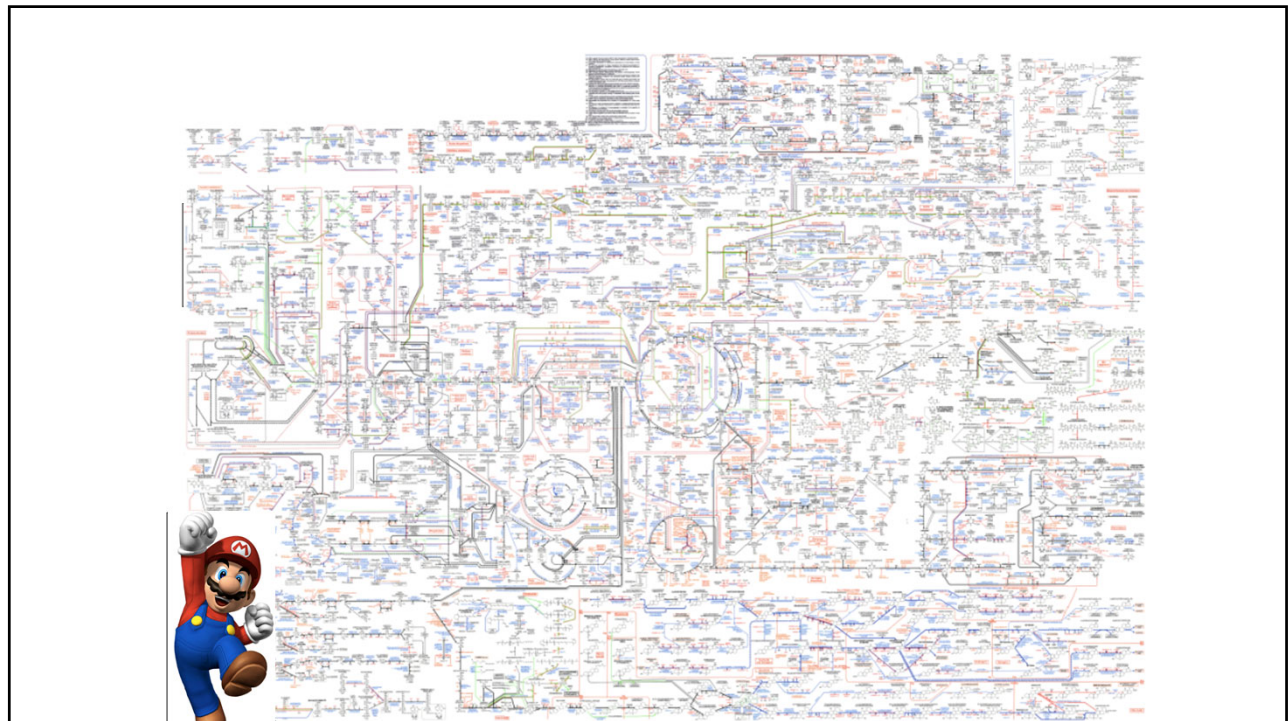
Dr. Dan Sharer

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However, genes failed to meet full expectations



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Where does the metabolome come from?

- It starts with what fixes CO₂ and N₂



Trees and plants convert CO₂ to organic compounds - **Rubisco**



Field of soybeans – they fix N₂ because of nitrogen-fixing bacteria in their root nodules

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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**



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Compounds in plants and fruits

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



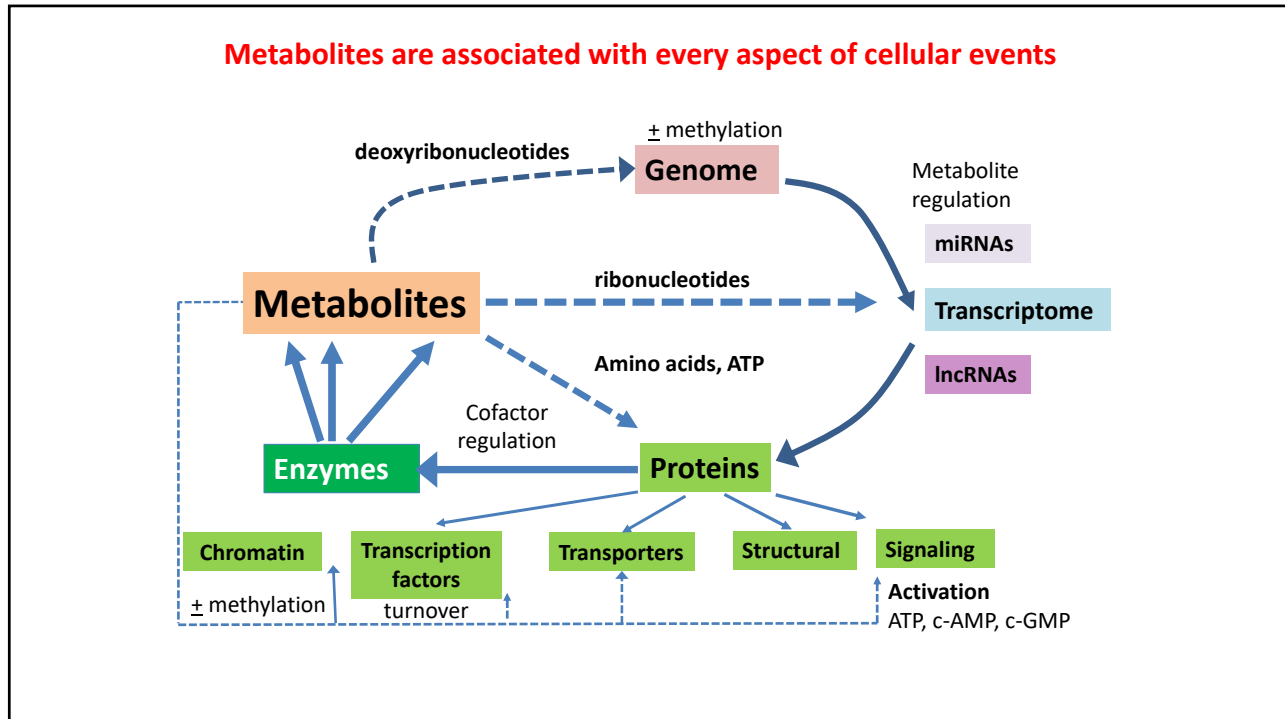
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Other sources of body chemicals

- **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**



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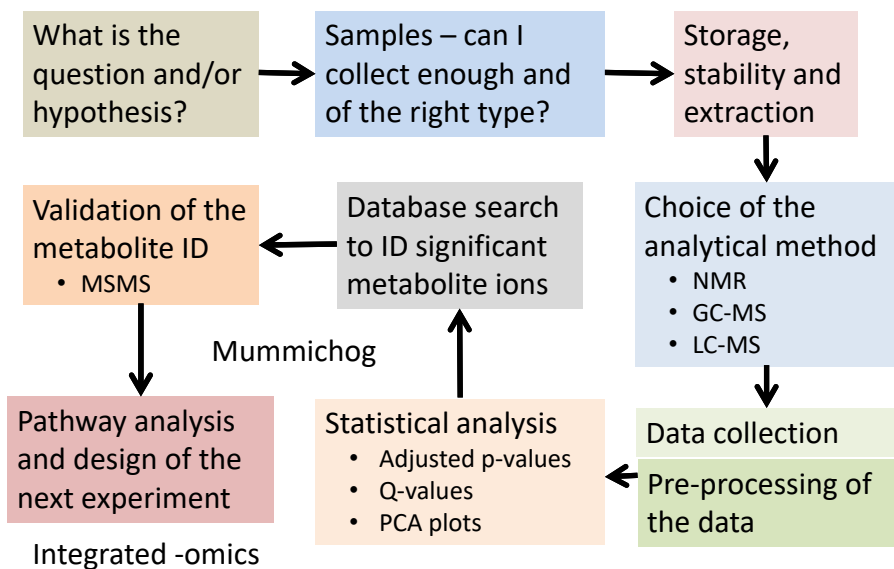
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The metabolome is very complex!

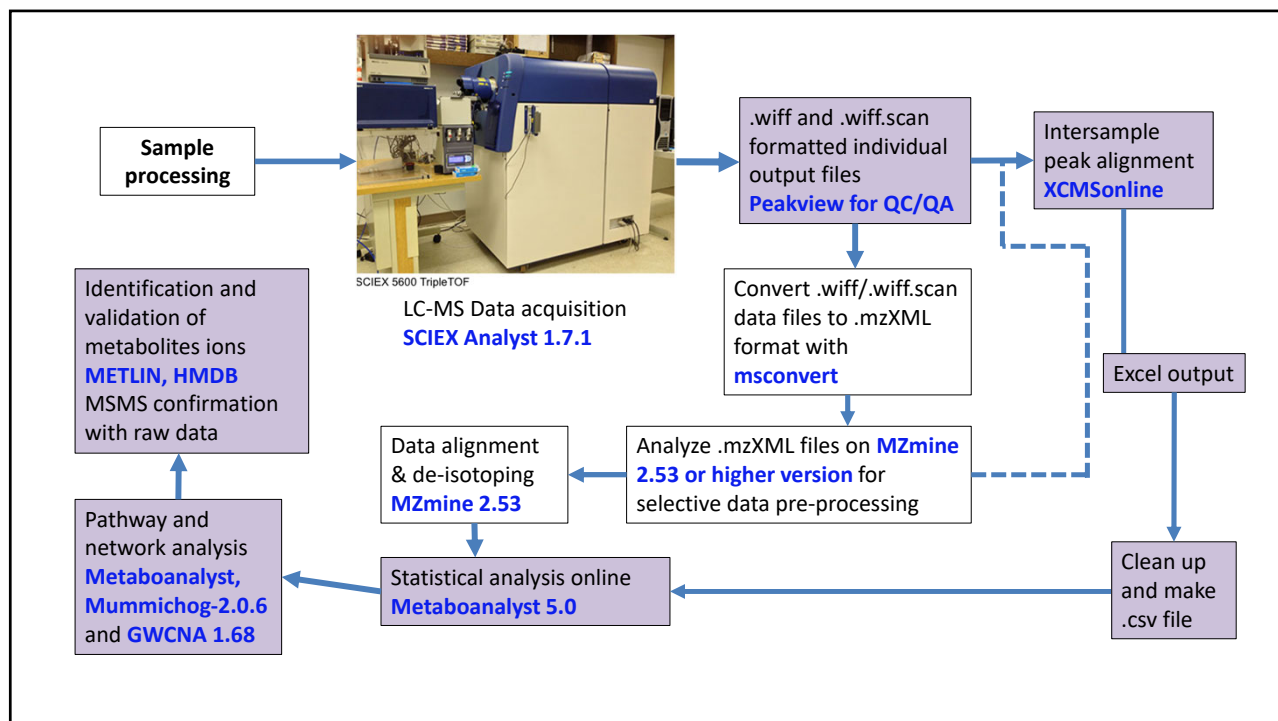


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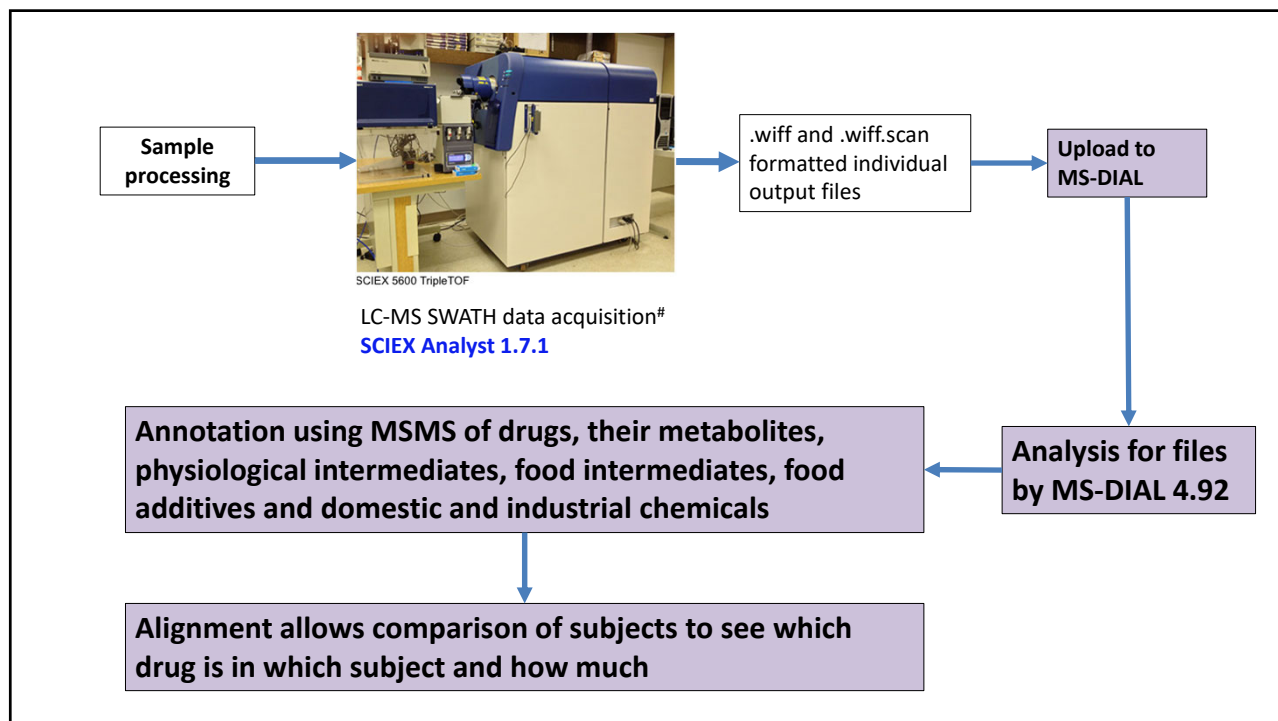
Metabolomics workflow



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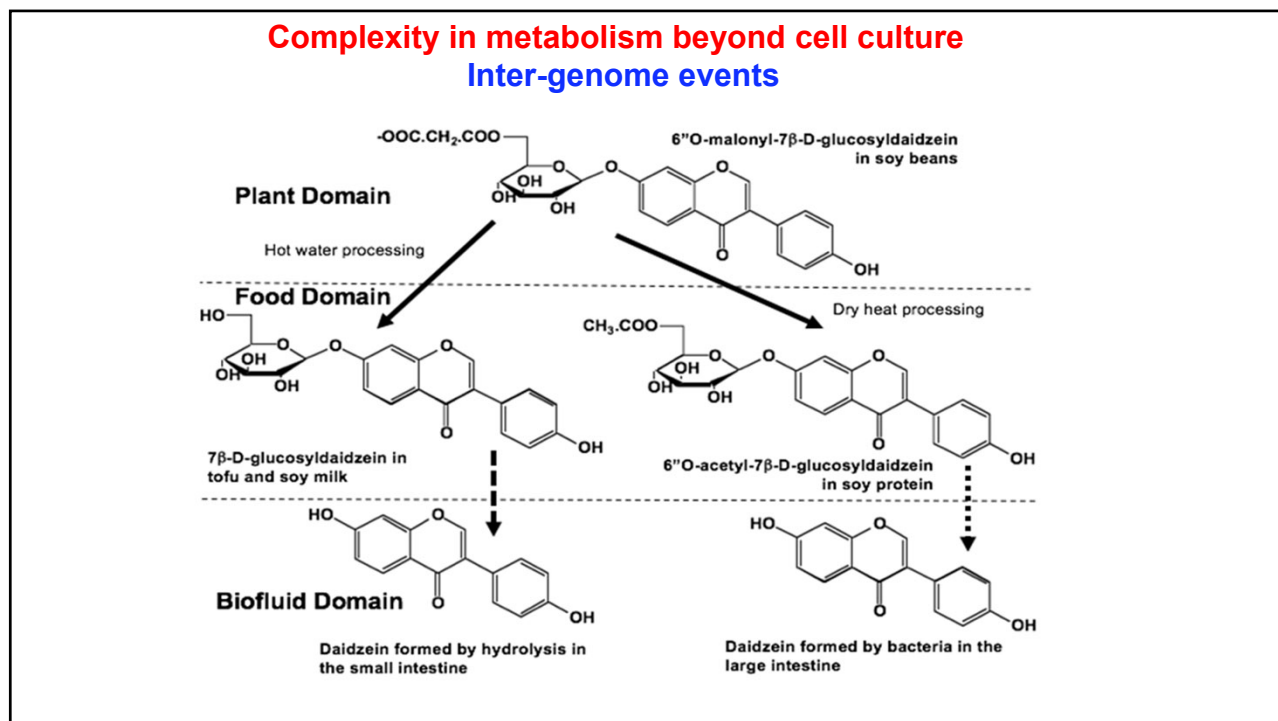


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Complexity in metabolism beyond cell culture Inter-genome events

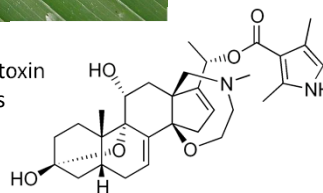


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The Amazonian poison dart frog



Their skin contains molecules like batrachotoxin which irreversibly poisons the Na⁺-channels



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Two questions

Why isn't the batrachotoxin a poison to the frog?

ANSWER: The frog has mutations of three residues in the Na⁺-channel protein that prevent binding of the batrachotoxin

Does the frog synthesize the toxin?

ANSWER: It doesn't, it gets the toxin from what it eats – ants, beetles, etc.

So, it all depends on what you eat.

Dart frogs bred in captivity and fed a non-insect diet don't make batrachotoxin

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Where did metabolomics come from?

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Transition of mass spectrometry to biology



Ralf Schoenheimer

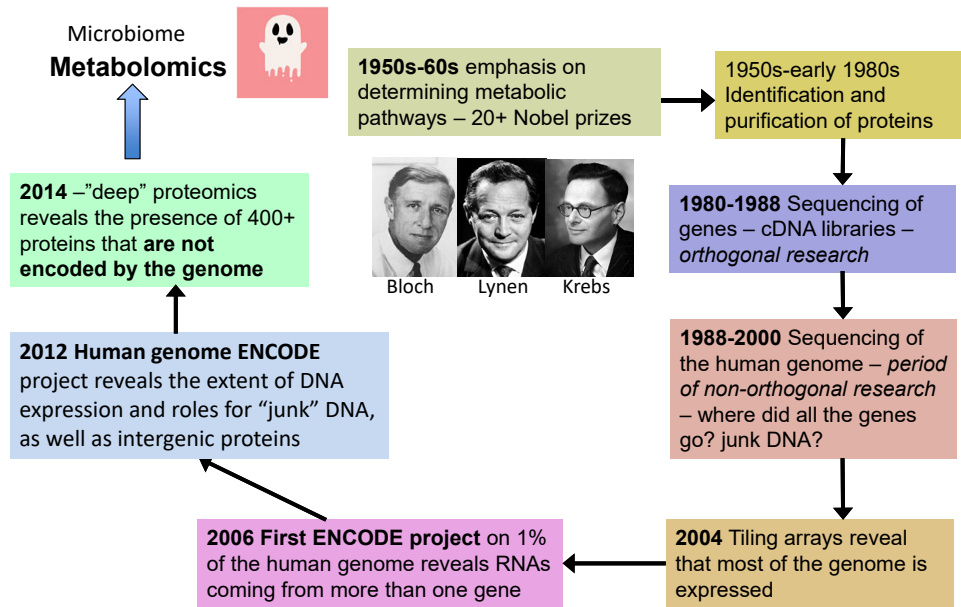


David Rittenberg

- 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 (²H), and later carbon (¹³C) and nitrogen (¹⁵N), to study biochemical pathways
- Understanding the pathways of metabolism was born

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Metabolomics and NIH Research 1948-2023



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Structure of NIH Research

Individual Research institutes (27)
NIDDK, NHLBI, NIAID, etc.

National Centers
CIT, CSR, FIC, NCCIH, NCRR, NIHCC

Office of the Director

2005 Review by Congress

Division of Program Coordination, Planning, and Strategic Initiatives, DPCPSI

NIH Common Fund

NIH Council of Councils

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NIH National Institutes of Health
Office of Nutrition Research

<https://dpcpsi.nih.gov/onr>

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COVID-19 is an emerging, rapidly evolving situation.

- Get the latest public health information from CDC »
- Get the latest research information from NIH »
- NIH staff guidance on coronavirus (NIH Only) »

ONR
Office of
Nutrition
Research

DPCPSI > ONR

Office of Nutrition Research (ONR)

Advancing nutrition science to promote health and reduce the burden of diet-related diseases

The Office of Nutrition Research:

- Advises the National Institutes of Health (NIH) Director, Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI) Director, and other key officials on matters relating to research on nutrition
- Coordinates implementation of the Strategic Plan for NIH Nutrition Research
- Coordinates research projects in nutrition science conducted or supported by the NIH Institutes and Centers (IC)
- Identifies research projects that deserve expanded effort and support by the ICs
- Develops, leads, and manages trans-NIH nutrition research projects in cooperation with the ICs
- Represents the NIH on intradepartmental or interagency committees on nutrition research and related policy issues

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Technologies in Metabolomics

- **Gas-chromatography-mass spectrometry (GC-MS)**
- **Liquid chromatography-mass spectrometry (LC-MS)**
- **Capillary electrophoresis-mass spectrometry (CE-MS)**
- **Nuclear magnetic resonance (NMR)**

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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

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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

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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)

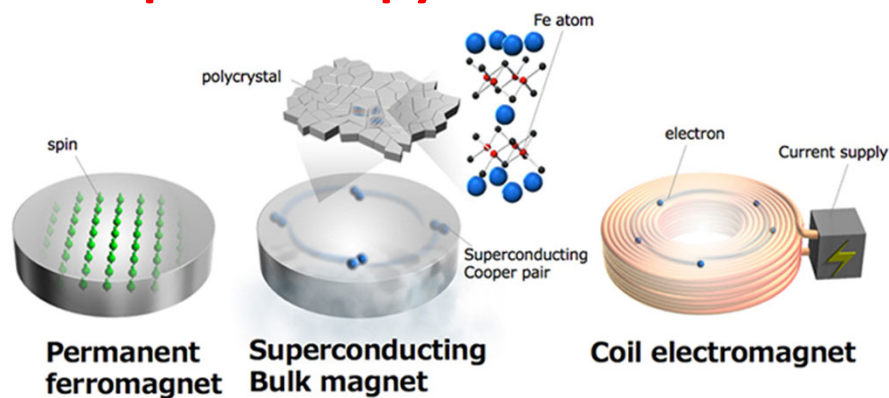
Q-TOF

TripleTOF

Ion Mobility

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NMR spectroscopy and metabolomics



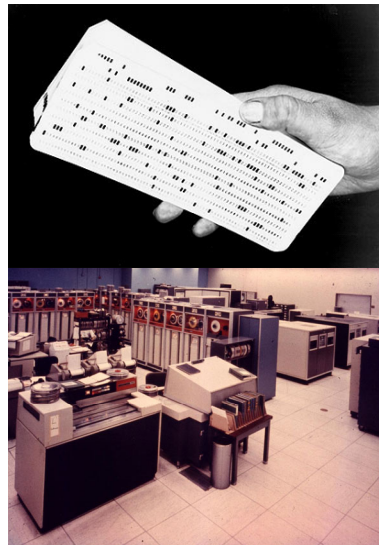
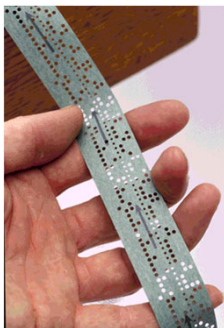
https://nationalmaglab.org/images/news_events/news/2015/october/pnictide_magnetism_1oct2015.jpg

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

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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



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My first computers



At Imperial College (1968)

Digital PDP-9 computer with 8 k of memory
 Data points were collected at 1 Hz on paper tape
 Fed into the computer with an optical reader
 The program to digitize collected GC data was 6.5 k
 The model I had had a screen to display the data



At UAB (1979)

Apple II+ computer with 48 k of memory
 Programs and collected data were uploaded from magnetic tape
 (disk drives came a little later)
 With a monitor, the cost (1979 dollars) was \$1,999
 1980 – “hacked” into the UAB IBM 370 mainframe which had a memory of 12 MB

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Today in Computing



On my desk in 2023

- The Apple MacBook Pro with 10 core M1 pro processors and 16-core GPUs
 - Operates at >3.2 GHz
 - Random access memory 16 GB
 - Access 200 GB/s
 - 1 TB Flash memory storage
 - 40 GB/s Thunderbolt I/O
- Also cost ~\$2,000 (2022 dollars)



Cheaha high-performance computing

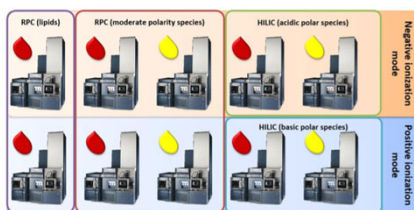
- UAB's first supercomputer (IBM Blue-Gene) operated at 4.733 Tflop/s
- Replaced by Cheaha - in its current configuration it has 8192 conventional CPU cores and 6.6 PB raw data storage
- It can operate at 619 Tflop/s (max)

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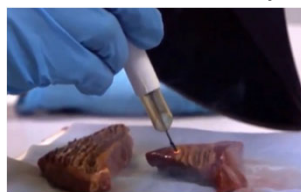
MRC-NIHR National Phenome Centre



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

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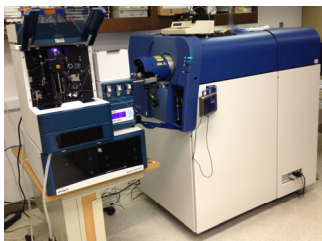
The UK National Phenome Center, LC-MS labs

<https://www.imperial.ac.uk/phenome-centre/>



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UAB capabilities in metabolomics

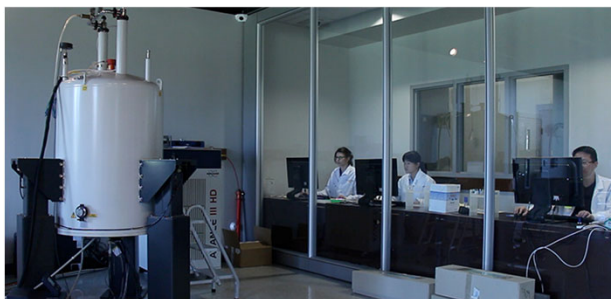


SCIEX 5600 TripleTOF
with Eksigent nanoLC

TMPL mass spec lab
BBRB 709/715
Stephen Barnes, Director
205-934-3462



SCIEX 6500 Qtrap with SelexION



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

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Great challenges in metabolomics

- **The extent of the metabolome**
 - From gaseous hydrogen to earwax
- **Having complete databases**
 - METLIN has over 1 million 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**

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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>
- **Other resources**
 - See this [link](#)

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Metabolomics Societies

- **[The Metabolomics Society](#)**
 - This is the international society of metabolomics
 - It has an annual meeting held in late June each year
 - In 2023 it will be at Niagara Falls, Canada, June 18-22
- **[The Metabolomics Association of North America \(MANA\)](#)**
 - MANA is for investigators from the US, Canada and Mexico
 - Has an online journal club organized by Art Edison (UGA)
 - Other online groups include the Microbiome Group, Precision Medicine/Nutrition and SODA (data analysis)