Targeted Metabolomics & Proteomics Laboratory

Wonderful World of Technology
March 22, 2018

Metabolomics, a rapidly evolving contributor to precision medicine, and how to do it

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UAB

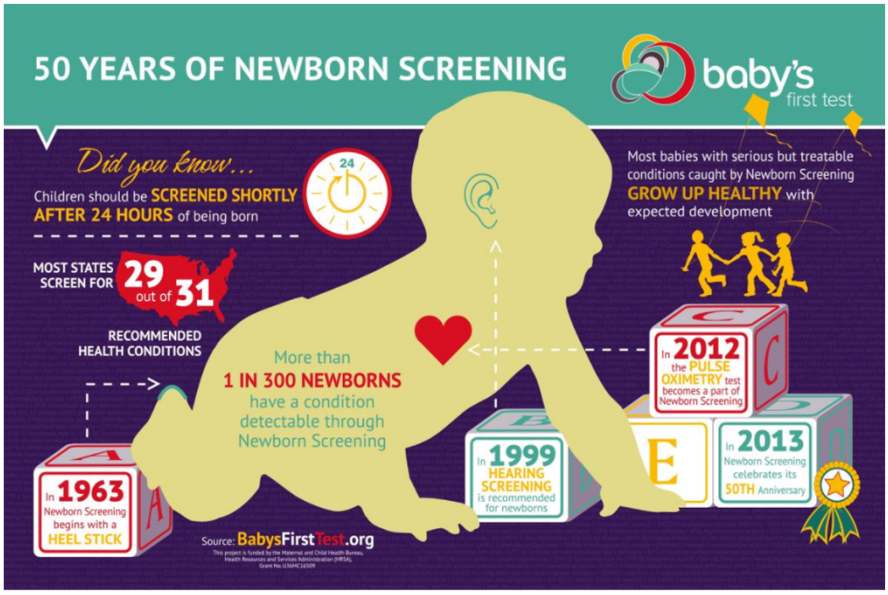
To be posted at http://tmpl.uab.edu

See also http://www.uab.edu/proteomics/massspec/classes/schedule.php

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?

Targeted metabolomics is not new



Metabolomics and NIH Research 1948-2018

Microbiome

Metabolomics

2014 –"deep" proteomics reveals the presence of 400+ intergenic proteins

2012 Human genome ENCODE project reveals the extent of DNA expression and roles for "junk" DNA, as well as intergenic proteins

2006 First ENCODE project on 1% of the human genome reveals RNAs coming from more than one gene

2004 Tiling arrays reveal that most of the genome is expressed

1988-2000 Sequencing of the human genome – *period of non-orthogonal research* – where did all the genes go? junk DNA?

1980-1988 Sequencing of genes – cDNA libraries – *orthogonal research*

1950s-early 1980s Identification and purification of proteins

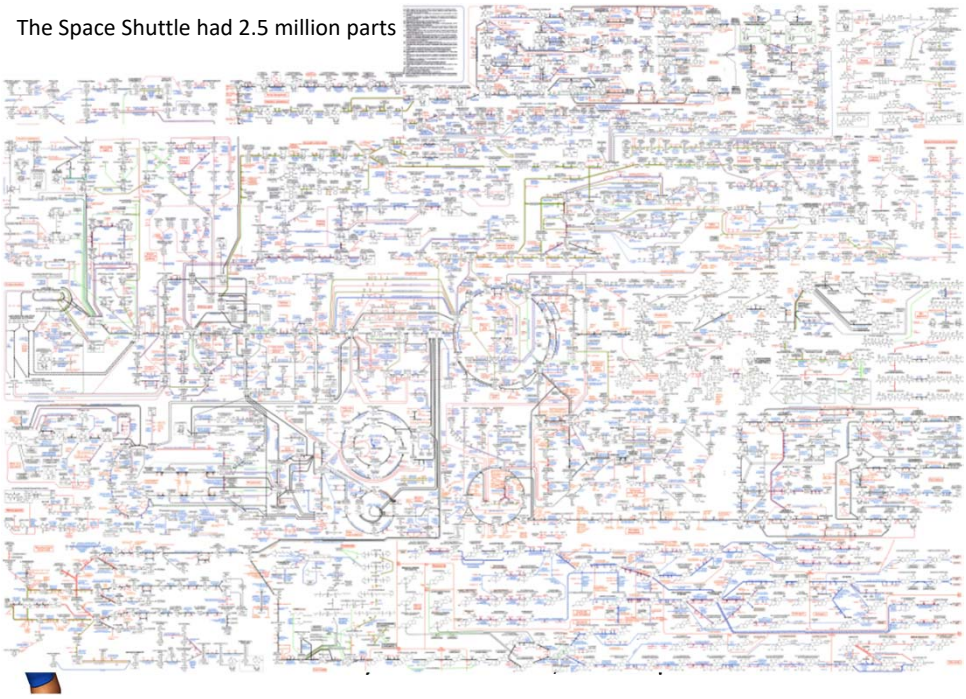
1950s-60s emphasis on determining metabolic pathways – 20+ Nobel prizes

Bloch Lynen Krebs

So, what is the metabolome?

The metabolome is very complex!

The Space Shuttle had 2.5 million parts



Meat eater

Berry eater

Amino acids

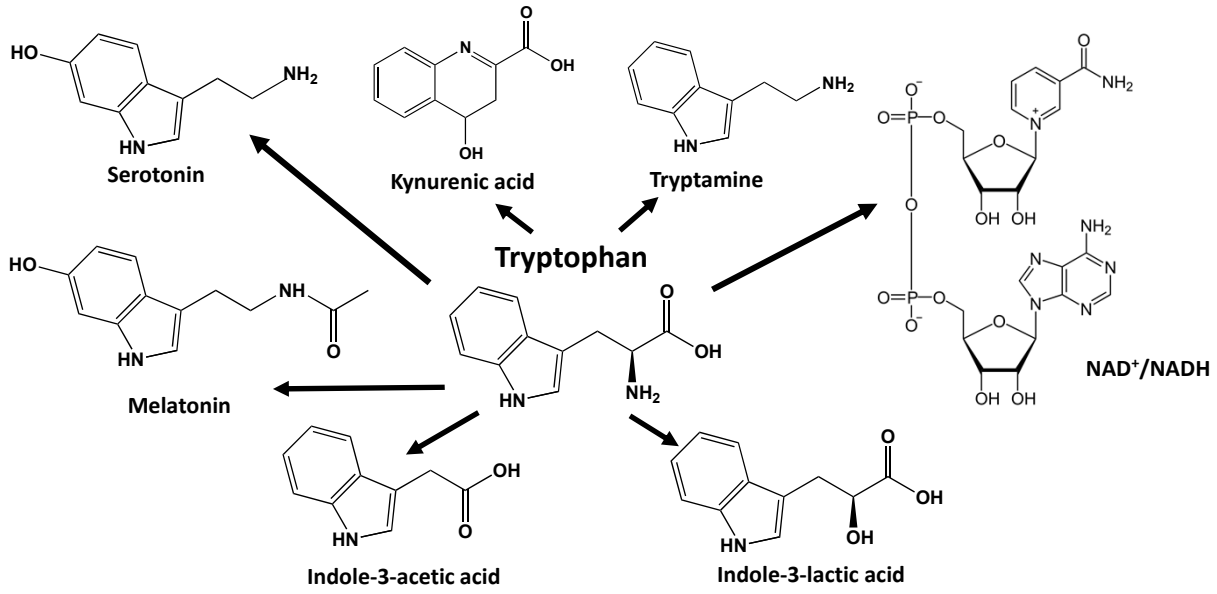
Essential	Non-essential
Arg*	Ala
His	Asn
Ile	Asp
Leu	Cys
Lys	Gln
Met	Glu
Phe	Gly
Thr	Pro
Trp	Ser
Val	Tyr

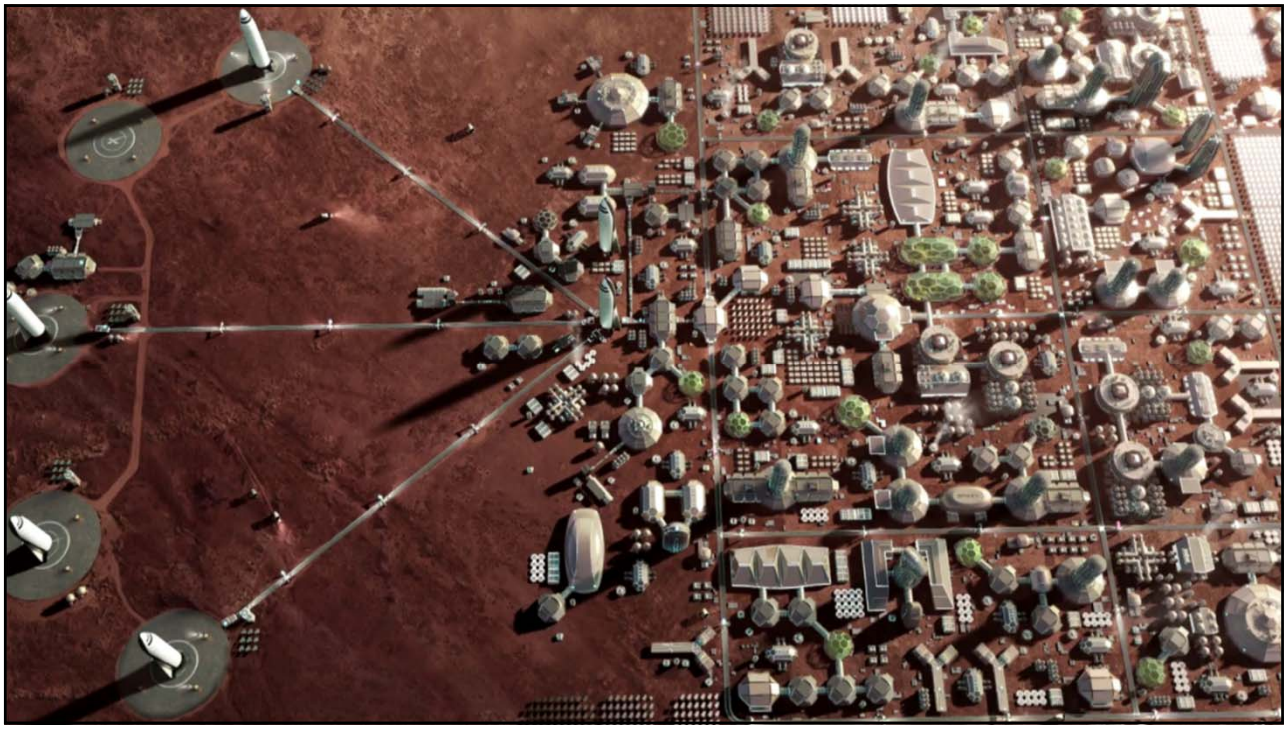
Have to eat foods rich in these

Vegetarian

Fruitarian

Amino acids are not just used for making proteins





Dark beer – B vitamins

Rich in polyphenols

Yoghurt +polyphenols

Tempeh - soy

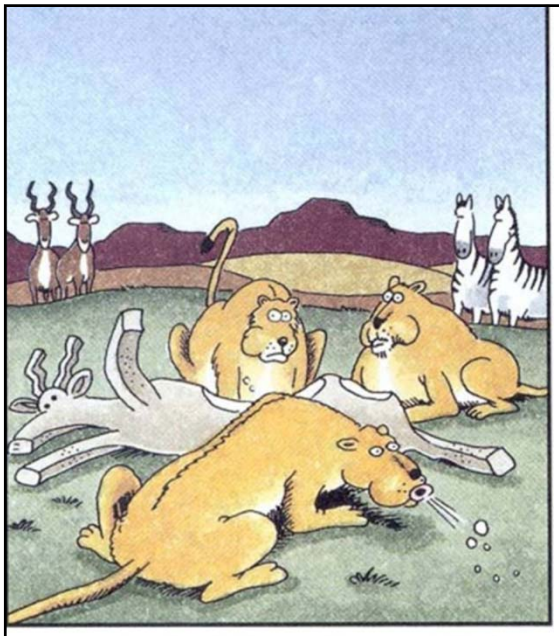
Kimchi - cabbage

Bitter beer – male hop UK

Alcohol/calories

Plain, but fermented

Ogi + hibiscus flower syrup



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)

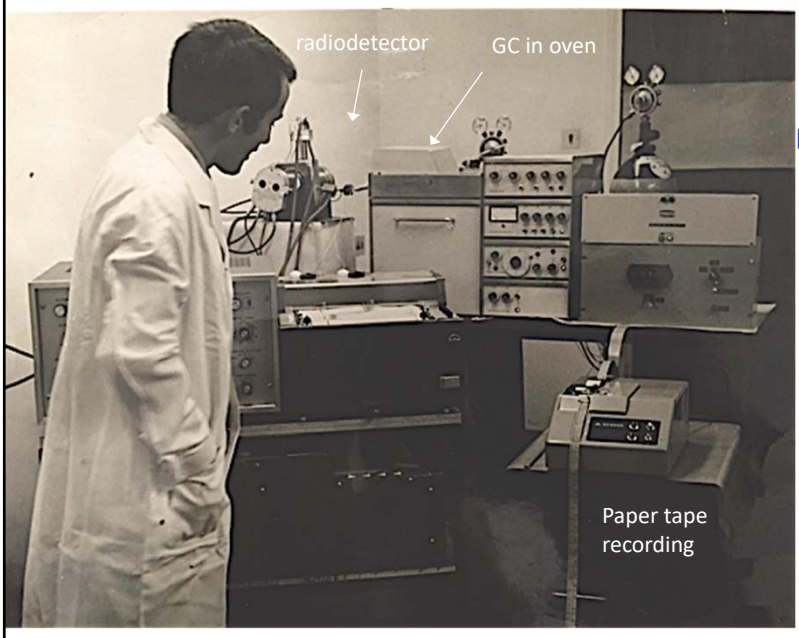
Veno-occlusive Disease of the Liver in Captive Cheetah

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 diet with soy protein.

Cats are exquisitely sensitive to aspirin and tylenol

- **The defect is in UGT1A6 which has become a pseudogene – the WT form glucuronidates phenols**
  - **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**

Measuring the metabolome



Radio-GC analysis

metabolomics in its infancy

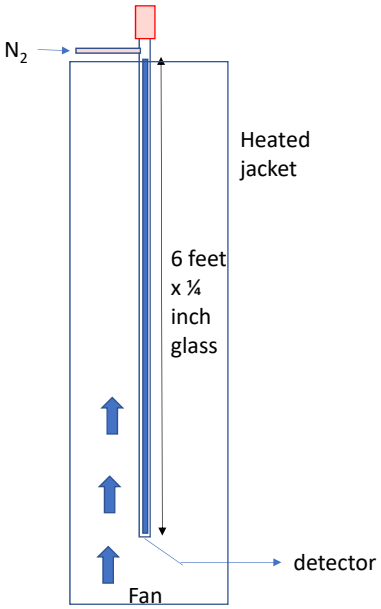
Radio gas-liquid chromatography with digitization of collected data

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

Modern metabolomics

BS1

Evolution of GC columns

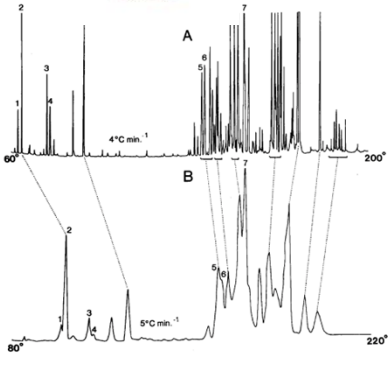


Coiled or U-shaped columns

Back pressure was a problem due to gas compressibility

Open tubular capillary (Golay) was patented in 1956. Not developed until the 1970s.

Quartz capillary
GC column

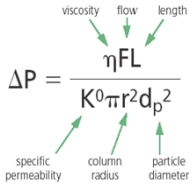


Slide 16

BS1 Barnes, Stephen, 8/21/2017

HPLC

- **Its principle**
  - Martin and Synge (1941).. "*the smallest HETP (height equivalent to a theoretical plate) should be obtainable by using very small particles and a high pressure difference across the length of the column*."
- **It has several advantages over GC**
  - Not necessary for the biochemical to go into the gas phase prior to separation
  - The stationary phase can be modified to many different chemistries
  - **The mobile phase (a liquid) is essentially non-compressible**
    - Linear flow velocity is the same at the top and bottom of the column
- **One big disadvantage**
  - Smaller particles => smaller HETP & better efficiency, but => greater back pressure



UPLC operates at 15,000 psi

- **Open tubular nanoLC?**
  - Can engineering coat the walls of an extended nano-fluidics network (reproducibly!!)

Mass analyzer of choice for untargeted metabolomics

- **Quadrupole-orthogonal time-of-flight (Q-TOF)**

Agilent 6500

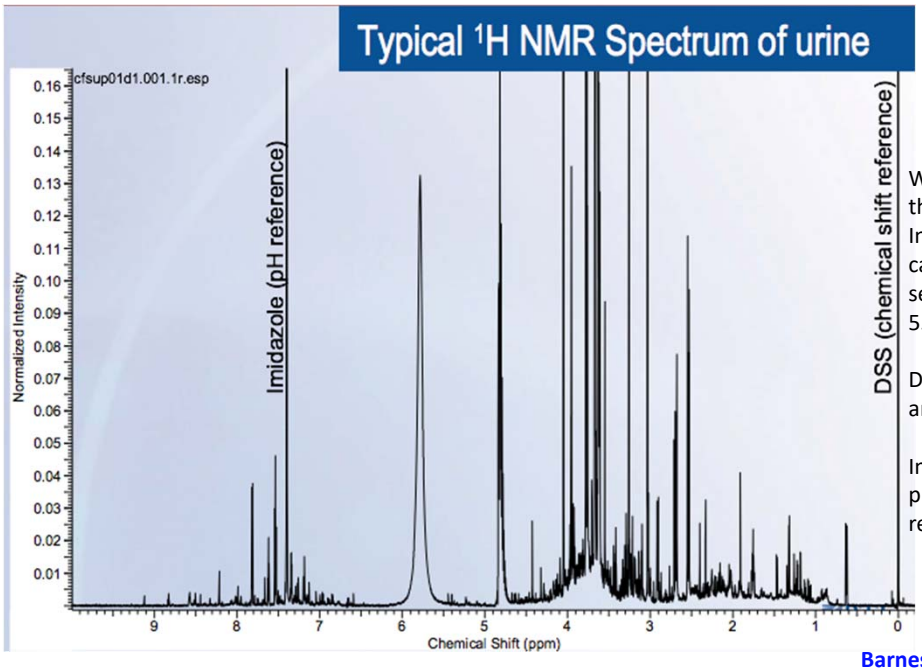
Waters Synapt G2/HMDS

Bruker

Sciex TripleTOF 6600

Current models have 40-80,000 mass resolution and 1-3 ppm mass accuracy

Nuclear Magnetic Resonance

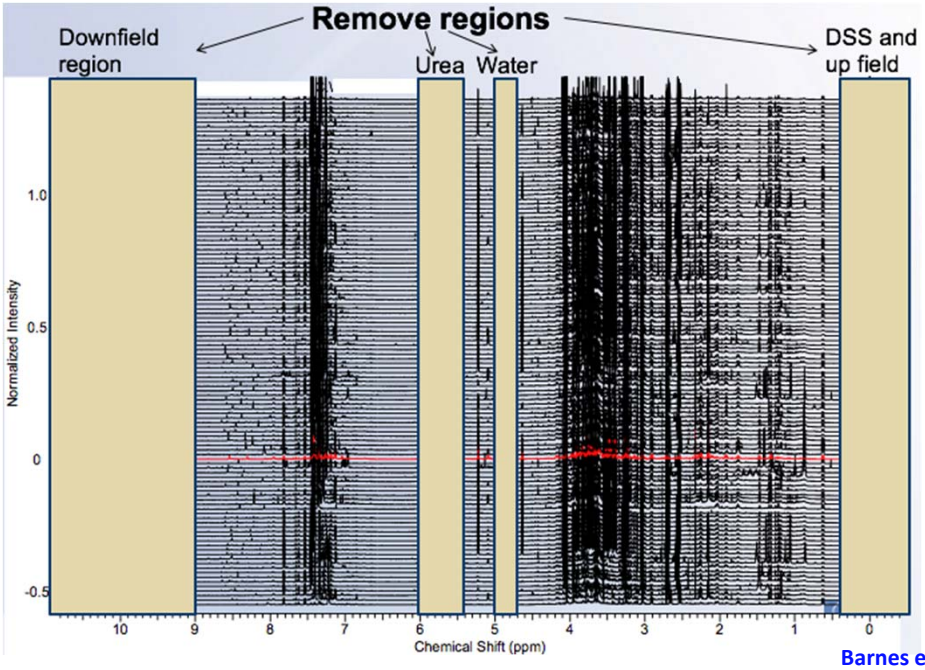


Whereas I had to to get rid of H_2O, these days it is not necessary. Instead, D_2O is added (10%) to calibrate the instrument. Pulse sequences are used to suppress the 55 M H_2O signal.

DSS is used as the reference signal and is set to 0.00 ppm.

Imidazole is used to determine the pH. This allows correction of proton resonances that are pH-sensitive.

Barnes et al. J Mass Spec 51:461-475



NMR is **highly reproducible** and **quantitative**, but has limited sensitivity. Measurements made at one institution or machine can be reproduced (after proper calibration) at another another institution or machine type.

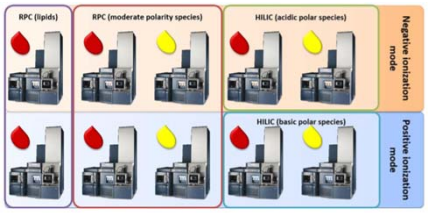
The NMR signal is spectroscopic and does not change.

Barnes et al. J Mass Spec 51:535-548

MRC-NIHR National Phenome Centre

600 MHz NMR instruments in surgical suite

This is Next-GEN precise medicine for everyone



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

Iknife - revolutionizing surgery

The UK National Phenome Center, LC-MS labs

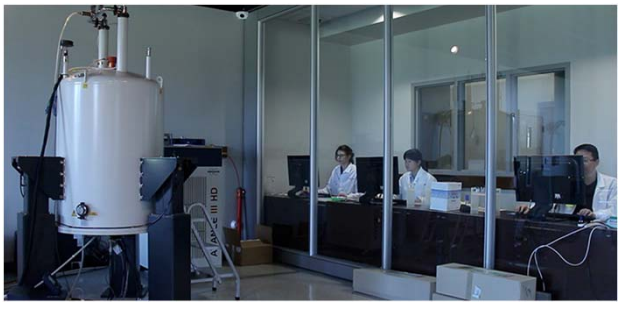
UAB capabilities in metabolomics

SCIEX 5600 TripleTOF
with Eksigent nanoLC
Research

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

SCIEX 6500 Qtrap with SelexION

Agilent 6530 QTOF???
Clinical



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

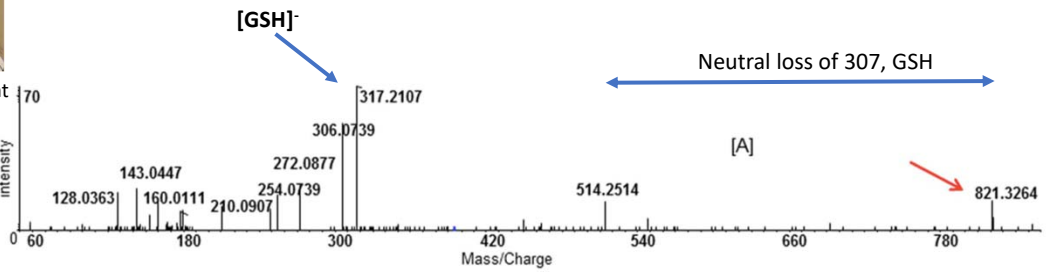
NIH Common Fund Metabolomics Program

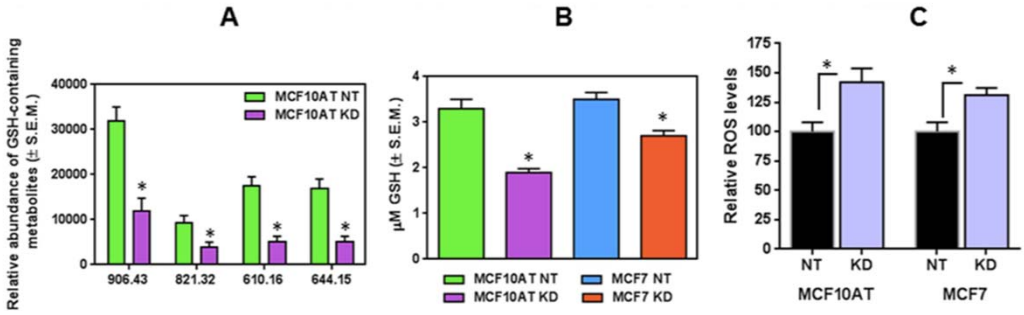
http://www.metabolomicsworkbench.org/

Examples of metabolomics applications

Lalita Shevde-Samant

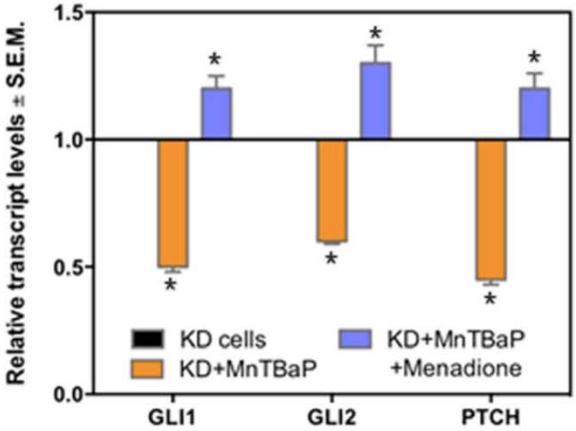
Effect of loss of Merlin in MCF10 cells



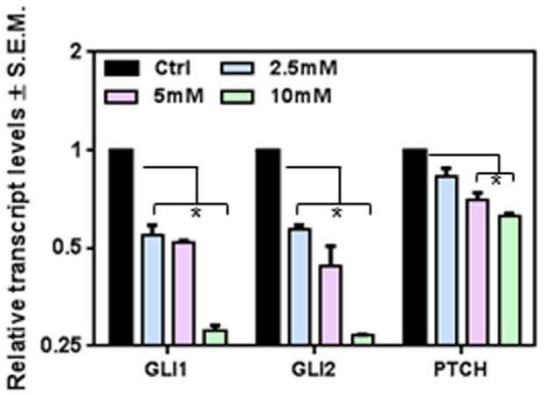


Das et al., Scientific Reports 2017

Loss of GSH leads to activation of sonic hedgehog signaling



Menadione induces ROS

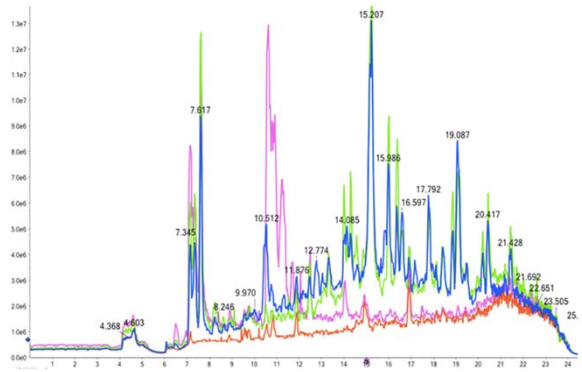


GSH restores the suppression of Hgh signaling

Das et al., Scientific Reports 2017

Fecal metabolites (!) in children with spondyloarthritis ± immunosuppression

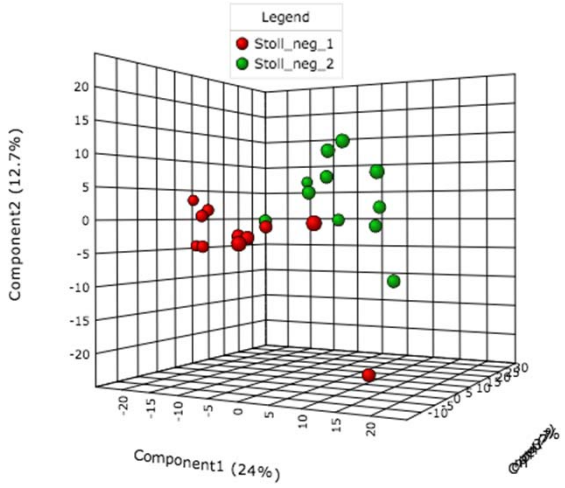
- **Challenge**
  - Transfering metabolites in feces in 20% glycerol to a microfluidic network ending in a 5 μm diameter spray needle
- **Method**
  - Disperse 100 mg feces in 0.1% formic acid
  - Centrifuge at 15,000 g to get "fecal water"
  - Extract the fecal water with ethyl acetate



- Purple trace – 0.1% formic acid
- Green trace – neutral pH (water)
- Blue trace – 150 mM NaOH
- Red trace - Blank

It worked!!

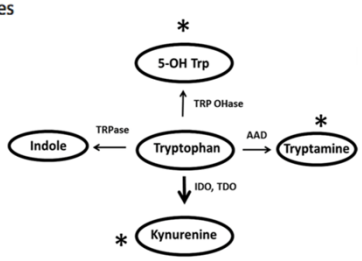
Results of the study



3D-Partial Least Squares Discriminant Analysis

Table 2. Pathways under-represented among patients in cohort 1

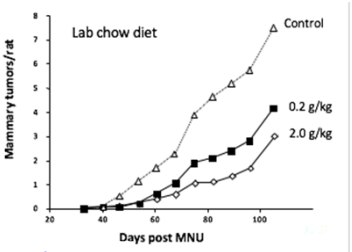
Pathway	*Overlap size*	*Pathway size*	*P-value*
Negatively charged ions			
Glycosphingolipid biosynthesis—ganglioseries	5	7	0.00091
Tryptophan metabolism	13	46	0.00106
Glycosphingolipid biosynthesis—globoseries	3	3	0.00122
Glycosph			0.00125
Positively ch			
Tryptoph			0.0038
Xenobioti			0.00544



Tryptophan is the source of ligands for the aryl hydrocarbon receptor

Stoll et al. Genes and Immunity (2016)

Rat model of mammary carcinogenesis – originally animals fed **chow** diets – tumors induced by a single injection of MNU (Clinton J. Grubbs).
Chemopreventive agent evaluation – pre-1985 to now. ***Very reproducible***. Barnes & Grubbs (1990) showed that removal of soy from the diet substantially increased tumors. Genistein added to the diet reversed tumor induction, but only if rats received soy early in life.



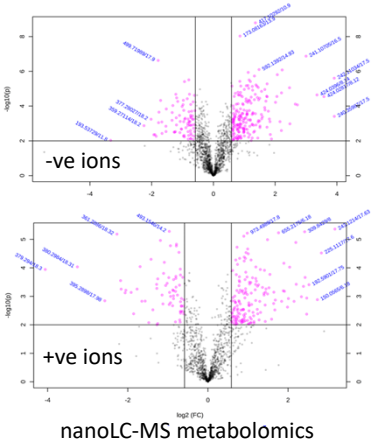
2013 – the diet (NIH-7001) was treated to control for microbial growth with Co-60 γ-radiation. **Unexpectedly, tumor number was reduced by 40%!!**

Experiment repeated with one batch of NIH-7001 that was split into two, one of which was exposed to the Co-60 γ-radiation. **Again, tumor number was reduced (by 38%).**

Questions:

1. **Is the fecal microbiome altered? YES**
2. **Is the urinary metabolome altered? YES**

More fundamental, did the Co-60 γ-radiation alter the diet?
ANSWER, yes, big time



ssible identity *
oxo-2-nonenal
droxy-oxo-nonenal
:2-oxo
:2-monoOH (HODE)
:2-diOH
:0-diOH ester of 8:1-diOH
:1-diOH ester of 8:1-diOH-oxo
:1 ester of 18:2-diOH

ined by MSMS

nanoLC-MS metabolomics

)17 Aug;52(8):507-516

Future: the metabolome of a patient

- **Metabolomics on urine/plasma/serum can assess:**
  - From the pattern of human (and perhaps microbial) metabolites, how does the patient's metabolome change during different stages of their disease process (acute and recovery)
- **Medications**
  - Are they taking their medication and is it the correct medication?
  - What other medications are they taking (prescribed, antibiotics, OTC, *other*)?
  - What beverage did they drink last?
  - Are they consuming unusual foods/dietary supplements?
- **What is the subject's metabolic age?**

Advanced metabolomics

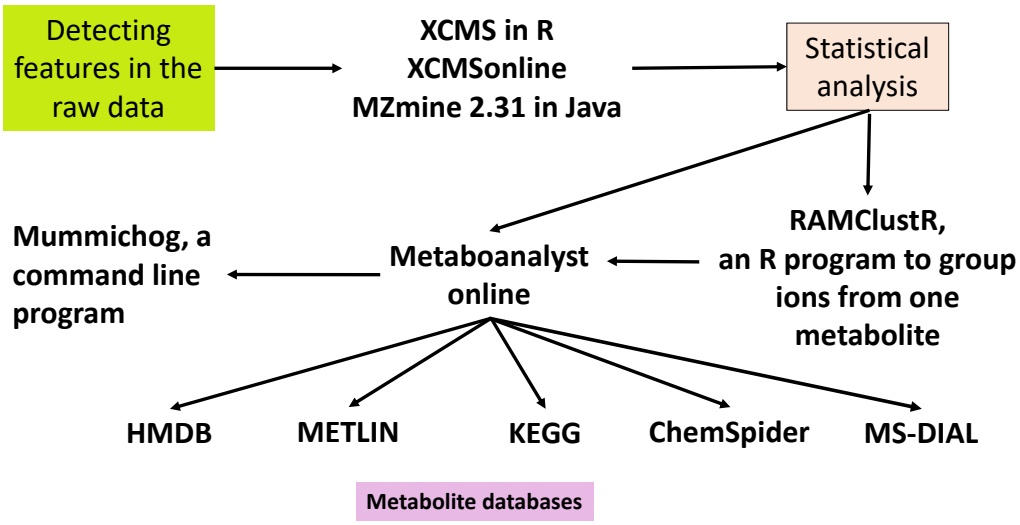
Single cell analysis – Peter Nemes, PhD (U. Maryland)
http://www.uab.edu/proteomics/metabolomics/workshop/2017/videos/nemes1_day2.html

The iKnife – precision surgery (metabolomics) on the operating table – Mr. James Kinross, PhD, FRCS (Imperial College, London)

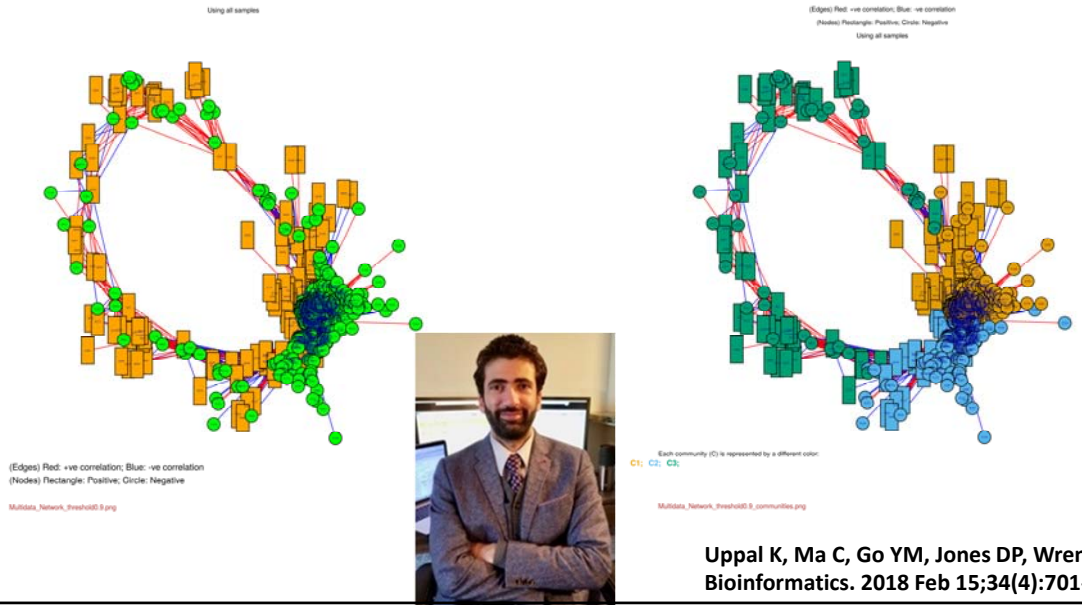
http://www.uab.edu/proteomics/metabolomics/workshop/2016/videos/kinross_day2.html

Tissue imaging metabolomics – Janusz Kabarowski, PhD (UAB)
http://www.uab.edu/proteomics/metabolomics/workshop/2017/videos/kabarowski_day4.html

Data analysis

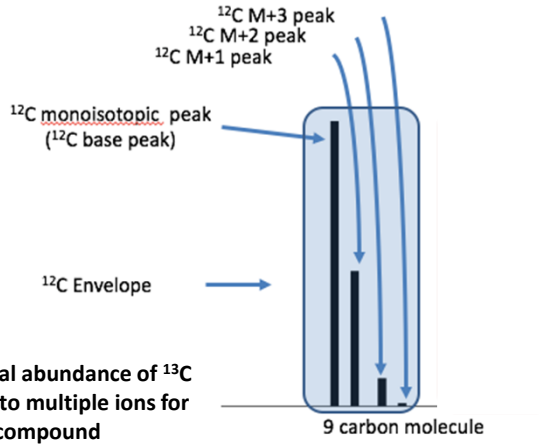


Network Analysis using xMWAS



Uppal K, Ma C, Go YM, Jones DP, Wren J. Bioinformatics. 2018 Feb 15;34(4):701-702.

Isotope ratio outlier analysis to facilitate identification and reproducibility in metabolomics



Natural abundance of ^{13}C leads to multiple ions for each compound

This reagent is added to all samples – allows high quality QA/QC

Growing yeast on 95% ^{13}C-glucose leads to all metabolites with this distinct isotope signature and no ^{12}C signal

Key issues in metabolomics

- **Design the experiment well**
  - **Discuss with a statistician before starting**
- **Collect samples as best you can – better fresh**
  - **Ideally, have no hemolysis in blood samples**
  - **Tissue samples should be frozen at -80^{o}C ASAP**
    - **In animal experiments, flush the tissue to be excised with ice-cold PBS, then freeze clamp**
  - **For cells, decant medium, flush dish with ice-cold PBS to remove extracellular components (10 s), and then add methanol cooled in dry ice**
- **Some samples will have already been collected**
  - **Learn as much as you can about how the sample was handled and stored**

Key issues in metabolomics-2

- **Numbers of samples per group** (to develop hypotheses)
  - Cells 3-5
  - Mice/rats 10-12
  - Patients 20-25 (controlled study)
  - Patients 100-500 (Epidemiologic or uncontrolled study)
- **The numbers needed to test hypotheses depend on the variance observed in the preliminary study** (work with a statistician to evaluate this)
  - Stan Hazen, discovering trimethylamine N-oxide in patients with adverse cardiovascular risk, chose a wide range of risk and carefully matched the risk patients with healthy controls (avoid antibiotics and other medications)
  - His initial study had **50** patients and controls – a validation study had **25** per group

Wang et al. Nature 472: 57-63 (2011)

Key issues in metabolomics-3

- **How much will it cost?**
  - A standard approach is to (1) extract the biological material, (2) carry out nanoLC-MSMS (negative and positive ions) and (3) process the data

Extraction

$12.50 per sample

Nano-LC-MSMS

$200 per sample

Data analysis

$400 per study

Examples

- 2 groups of cells (n=5)
  - 2x5x$12.50 + 10x$200 + $400 = $2,525
- 2 groups of animals (n=10)
  - 2x10x$12.50 + 20x$200 +$400 = $4,650
- 2 groups of patients (n=25)
  - 2x20x$12.50 + 50x$200 + $400 = $10,525

Summary

- **Metabolomics (integrated metabolism and chemistry of living cells) has had a long history and depends on the ability to separate, recognize and quantify individual components**
  - Its development has depended on engineering and micro/nano system innovations as well as computational development
- **Metabolomics is an important aspect of the overall research on the functions that control life (along with other –omics research) and is an important adjunct to current precision medicine**
- **UAB is building a considerable experience in metabolomics – analytical resources have moderate capacity – need to be expanded**

Acknowledgements

- **AT (Tony) James**
- **Sir Ernst Boris Chain**
- **Keith RL Mansford, PhD**
- **Alan Hofmann, MD**

- **Clinton J Grubbs, PhD**
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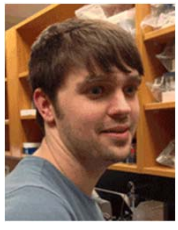
Taylor Berryhill


Mikako Kawai

Thank you - Questions?