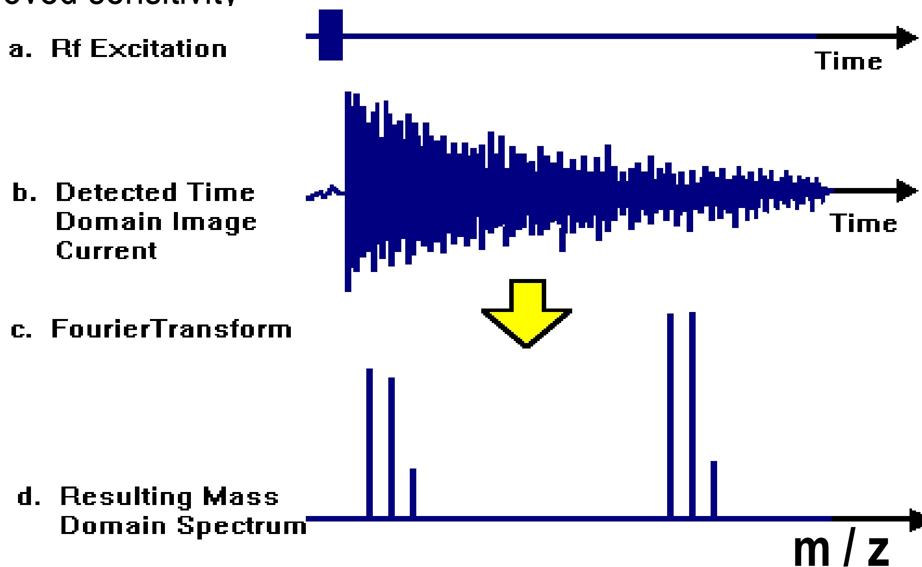
BIOC4004 - Industrial Biochemistry
Lecture 21 - Mon Mar 22, 04

Topics for the Day:

- Proteomics
  - FTICR-MS
- Metabolomics
- Comparison of platforms and systems biology

Down the pipeline: FTICR-MS (Part II)

- Conventional MS: destructive detection of ions in a "race towards the detector"
  - detect collision between ion and electrode
- FTICR detects the current generated by ion (ie.non destructive), signal can be amplified
  - improved sensitivity



- As the electrical signal decays, a spectrum as a function of *m* or *m/z* can be obtained using "math". Cyclotron frequencies can be measured with very high precision
  - high accuracy mass measurements (ie. ultra-high resolving power)

<u>FTICR (Fourier Transform Ion Cyclotron Resonance) MS has:</u>

- insane sensitivity (100 X more than current tandem MS)
- insane resolution (can get down to less than 1 ppm error)
- insane speed (10-100 times faster analysis)

- We can start using MS to analyze real proteomes, not single proteins spots from gels or single LC peaks
- Near single-cell sensitivity
- Aug 2002: identified 1,900 proteins from *Deinococcus radiodurans*
  - broadest proteomics analysis to date

<u>The catch:</u>

- $1,5 Million USD
- no one makes them commercially (ok, they do now, but very pricey !!!)
- huge amounts of data generated…require huge informatics infrastructure

Metabolomics

• same as proteomics, except you are looking at small molecules, not proteins:

> The term '**metabolome**' refers to the entire complement of all the small molecular weight metabolites inside a cell suspension (or other sample) of interest.

Why ???

• It is likely that the measurement of the metabolome in different physiological states will be much more discriminating for the purposes of functional genomics.
  - changes in individual enzyme concentrations can have substantial effects on metabolite concentrations (small molecule inventory)
  - these changes reflect anabolic and catabolic processes inside the cell
  - changes that may not be obvious at the transcriptome or at the proteome level may still affect the metabolome
  - besides, we already know that metabolic disturbances affect the intracellular pool of metabolites (eg. many medical tests)

• The only difference between that and metabolomics is the scale of things
  - ideally we could look at
    - very small samples
    - very complex mixtures (like intracellular contents)
    - flux in the small molecule inventory of a cell (time series)
      - drug response: metabonomics

Metabolomics

The Problems

• using conventional mass spec, mass accuracies are a big problem because the margin of error could give ambiguous identifications:

eg. M/z = 16 $\pm$ 1 is it heavy water or just regular water ???

• also:
- conventional MS would require **lots** of starting material
- for comprehensive metabolomics, need much more resolving power than conventional MS can deliver……..
  - you still only have m/z data to infer the molecular structure of the molecule in question
  - many (un)related compounds could have “similar” m/z
  - need very high mass accuracy
  - otherwise data could fit different possibilities

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- insane sensitivity (100 X more than current tandem MS)
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• We can start using MS to analyze **METABOLOMES**

• Near single-cell sensitivity

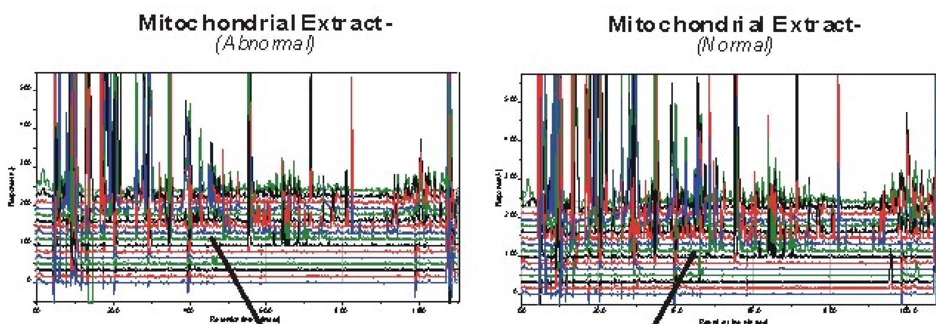
The catch:
- you already know what the catch is !!!!

Metabolomics…other things to consider

• From a practical point of view, even with FTICR-MS there are limitations to the types of questions that you want to be answering with metabolomics.

• Get Selective (collecting data is easy, analysis is the hard part):

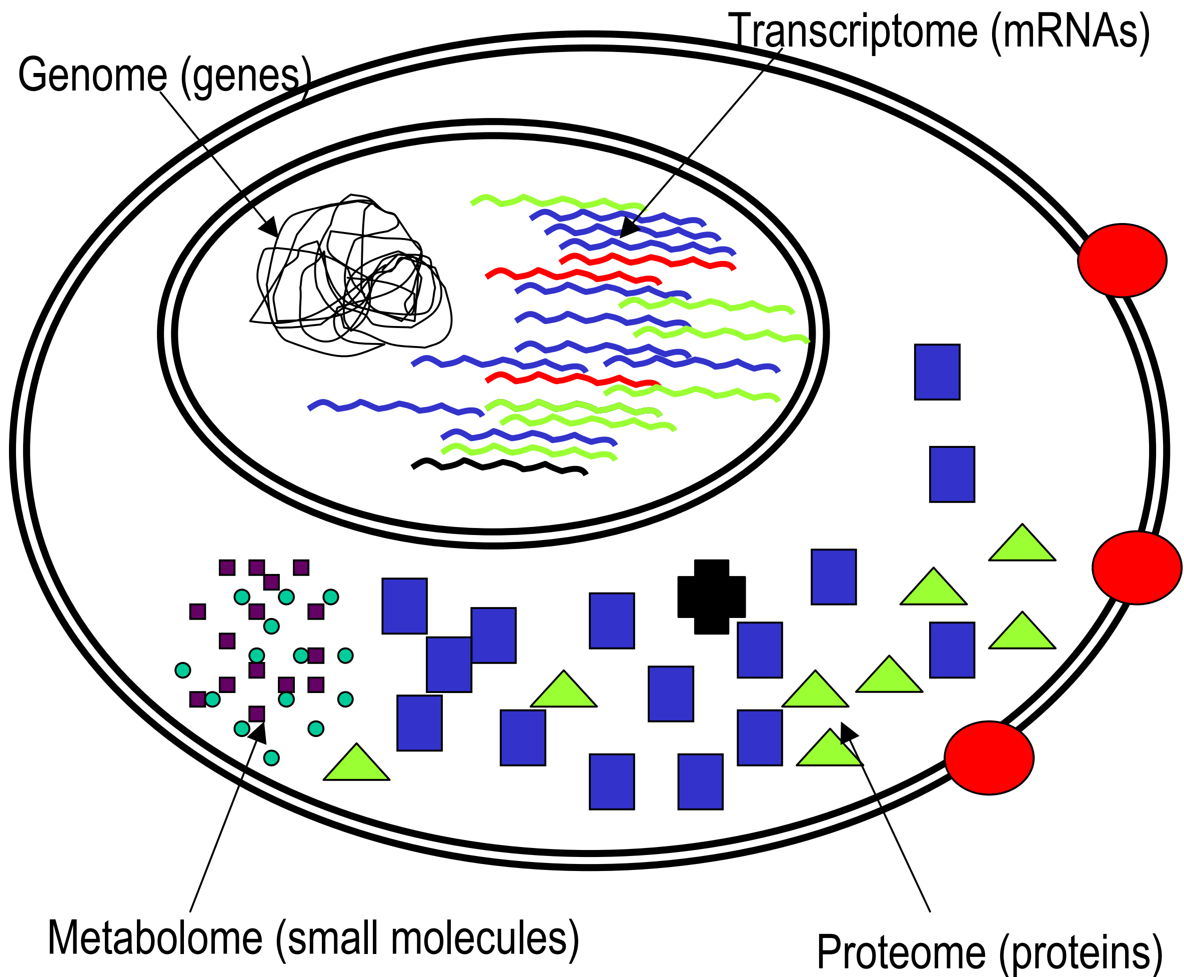
- stick to looking at "small" numbers of relevant compounds
- look at compounds that are relatively unique
  - otherwise the complexity of the data becomes crippling
  - limitless variety of carbohydrates, amino acids, lipids, and other small molecules.
- if a metabolite is affected by many different cellular pathways how could you tell **which** pathway is affected ?
- need sophisticated bioinformatics to tease out relevant changes in a metabolomic profile
- look for correlations between changes and phenotype



Try to find the peaks that matter !!!

- **but you still may not know what the peaks correspond to**
- **need a database of compounds and their spectra**
  - **molecular formula prediction**

Transcriptome vs Proteome vs Metabolome (Pt I)



- We're always interested in comparing a test sample to a control sample
- We're interested in determining if there are differences in the repertoire and relative abundance of different analytes in different types of samples

DNA, RNA, Protein and Small Molecules are all a part of life processes, but which level of study is more relevant ?

- DNA is the most indirect because it measures "potential" for gene expression
- RNA is a direct measurement of gene expression but an indirect measure of life processes
- Protein and metabolite profiling are more direct measures of life processes because they measure the "effectors"

DNA < RNA < proteins < metabolites ???

Transcriptome vs Proteome vs Metabolome (Pt II)

"Biological Relevance"

**DNA < RNA < Proteins
Metabolites**

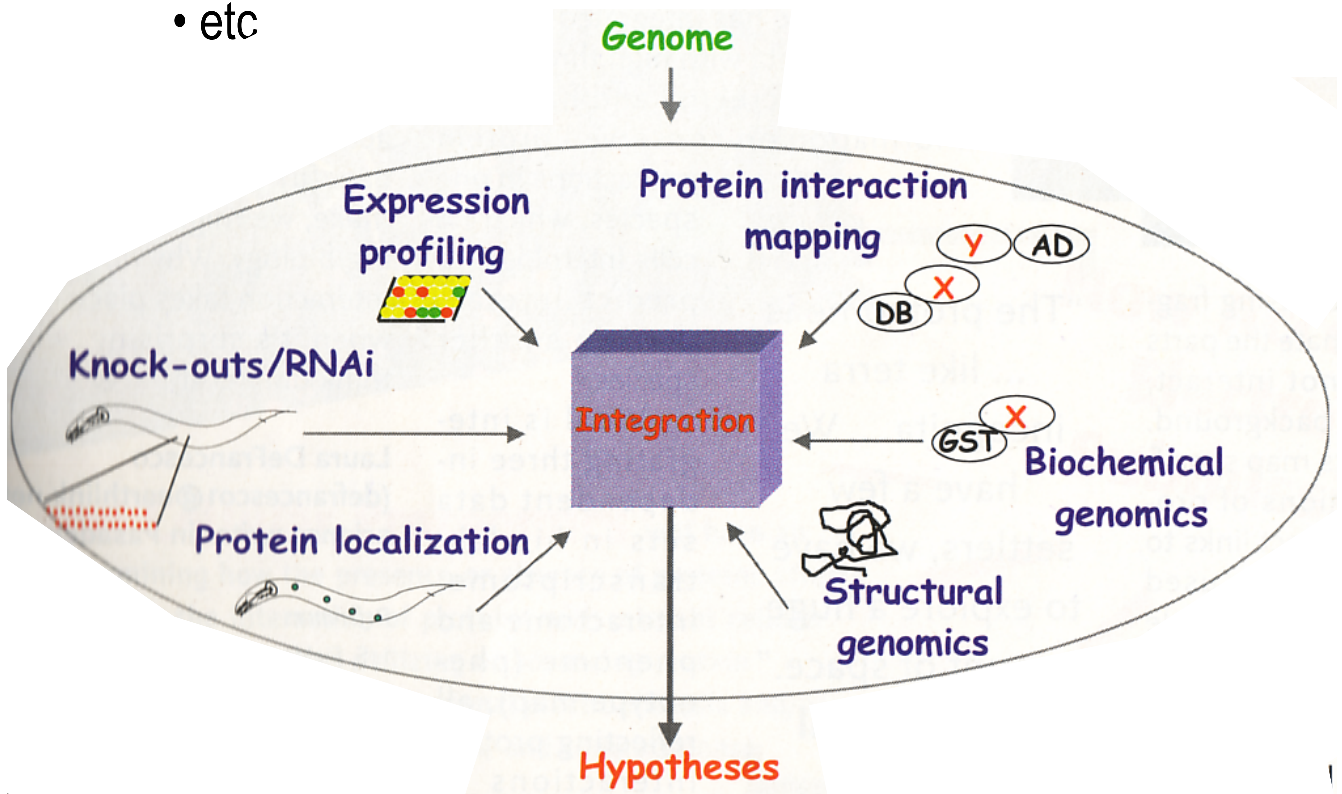
"Accessibility"

DNA > RNA > proteins > metabolites

	The GOOD	The BAD
Genome	• determines total potential of organism • "easy" to obtain (bioinformatics, automation • a finite problem • "stable"	• only determines "potential" • unknown genes • nothing about what's going on "right now"
Transcriptome	• determines gene activity • gene activity ~ phenotype • data is "ordered" (ie. arrays) • every data point tested is known • "all RNA behaves similarly"	• gene activity ~ phenotype • RNA vs protein levels do not always correlate • post-transcriptional events ? • Limit of detection ?
Proteome	• determines what proteins are present • good proteome/phenotype correlation • can see post-transcriptional events • can see post-translational events • protein localization	• proteome is very variable • many proteins are never detected • complexity (modifications, splicing…) • expensive equipment • proteins behave differently
Metabolome	• determines metabolic processes going on • metabolites "drive life" • small changes in RNA/protein can give large differences in metabolites • every phenotypic difference due to metabolic changes	• requires super expensive equipment and infrastructure • exceedingly complex bioinformatics • interconnected pathways could make interpretation difficult

Transcriptome vs Proteome vs Metabolome (Pt III)

- FTICR is going to remain out of the reach of most researchers
  - metabolomics is still far from being adopted in many labs
  - high resolution proteomics suffers from the same
- the best results will be obtained combining approaches
  - SYSTEMS BIOLOGY
  - complementing technological strengths and weaknesses
  - eg. Use expression profiling in combination with proteomics
- study of gene function still requires "functional assays"
  - knock-outs
  - protein interaction maps
  - cellular localization maps
  - etc…
  - etc



Testing and Validation