Metabolomics for CHO-culture Production

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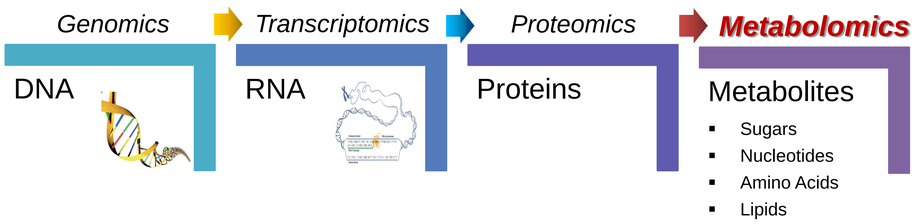
Abstract

Metabolomics study enables the examination and identification of small molecules that revealing information on the target metabolic pathways in a cell. Metabolites are involved either directly or indirectly with every aspect of cell function, and thus metabolomics is to be a reflection of the phenotype of cell. Metabolomics analyses have many potential applications due to their inherent advantages. An important application that has recently emerged is to characterize cell cultures expressing protein therapeutics. Cell metabolomics consists of four sequential steps: (1) sample preparation and extraction to measure the intracellular metabolites in CHO cells, (2) metabolic profiles of low-weight metabolites based on mass spectroscopy (MS) or nuclear magnetic resonance (NMR) spectroscopy, (3) metabolites identification and (4) data analysis. Metabolomics requires special attention to describe two key steps in metabolomics study such as the metabolite extraction and metabolite measurement in order to detect as many metabolites as possible in given cell. As a result, these sequential steps provide insight about the cellular biochemical processes.

Problem

Overview



Genomics and **proteomics** tell you what **might** happen, but **metabolomics** tells you what actually **did** happen!

Motivation

Metabolomics is important for several reasons:

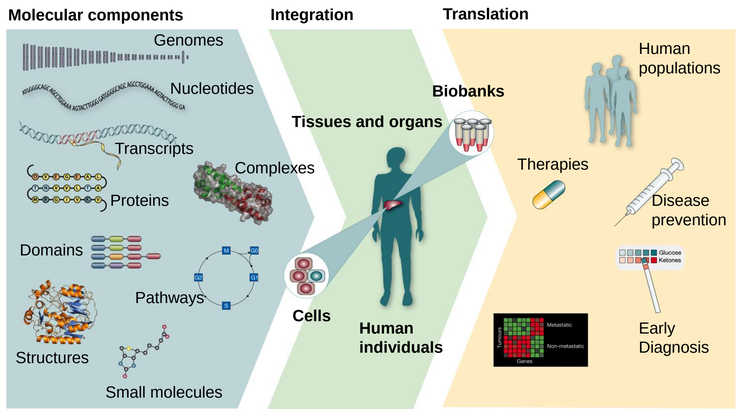
- Metabolome may correspond to groups of reactions that perform a common function
- Complex metabolic networks can be simplified based on their composition
- Insights about large-scale organization and evolutionary history can be achieved

Our approach is interesting because:

- Metabolomics is a post genomic technology which seeks to provide a comprehensive profile of all the metabolites present in cell-culture sample
- Determine how metabolite levels respond to genetic or environmental changes
- Execution is efficient and based on network flow computations

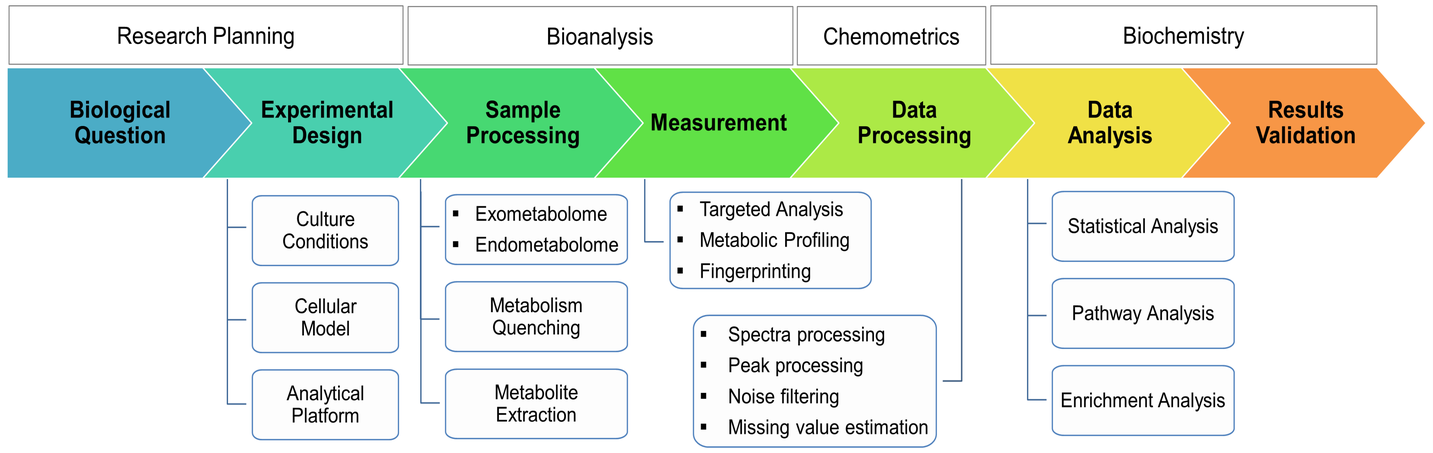
The Conceptual Approach In Metabolomics

From molecules to medicine:



Materials and Methods

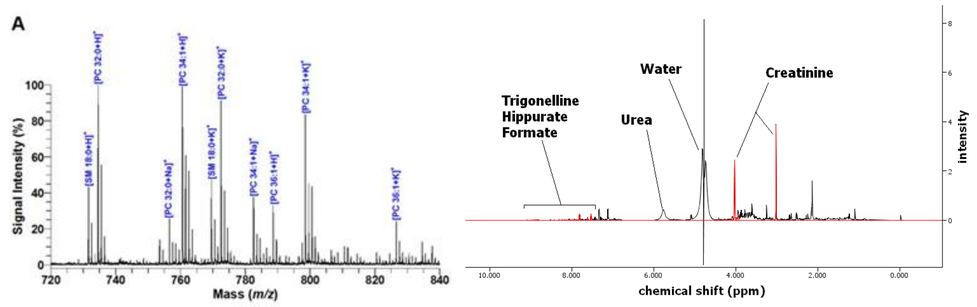
Metabolomics Workflow



Results

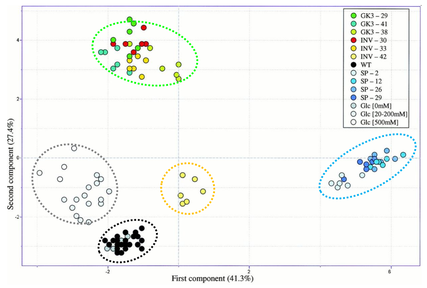
What Are The Data Like?

- Input is a complex mixture of metabolites
- Integrate across spectrum/identify specific compounds
- Examination of relative peak heights/integrals or compound levels
- So, quantitative in nature (more akin to gene expression than genotype data)



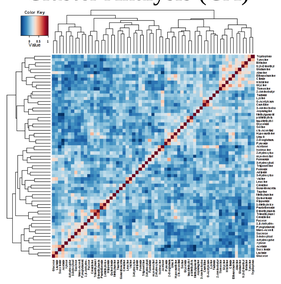
Data Analysis

- Principal Component Analysis (PCA)



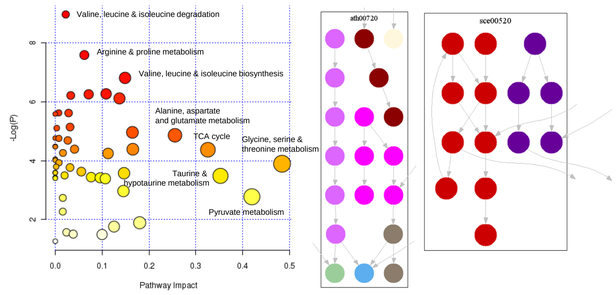
Identify and compare treatment classes.

- Cluster Analysis (CA)



Identify metabolite interactions.

- Metabolic reaction networks of CHO cells



Map to metabolic pathways.

Conclusion and Future Work

- The metabolomics enables the identification of the metabolome while revealing tremendous information about cellular function and the metabolic pathways of a cell. It is increasingly used in investigations of more subtle effects, such as the indication of pharmaceutical efficacy, and the probing of life-style changes, nutrition and the complex interconnection of metabolic processes.
- **Challenges in Metabolomics:** (1) metabolites have a wide range of molecular weights and ***large variations*** in concentration, (2) the metabolome is much ***more dynamic*** than proteome and genome, which makes the metabolome more ***time sensitive***, (3) metabolites can be either polar or nonpolar, as well as organic or inorganic molecules. This makes the ***chemical separation*** a key step in metabolomics, and (4) metabolites have ***chemical structures***, which makes the ***identification*** using MS an extreme challenge.
- **Future work we will explore:** additional effort is necessary for the development of metabolite identification and for the standardization of culture conditions, standardization of sample preparation protocols will ensure reproducibility and reliability, and development of new sample preparation methods will increase metabolite coverage in future manufacturing cell metabolomics studies.