Course 2851 Principles of Metabolism
Metabolism and endocrinology programme, Karolinska Institutet

Lecture 13

Measuring metabolites

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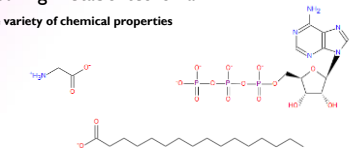
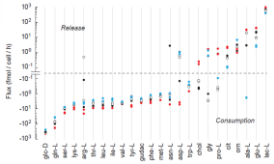
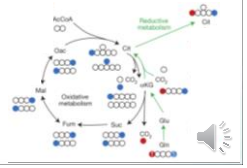
Measuring metabolites is hard

Wide variety of chemical properties



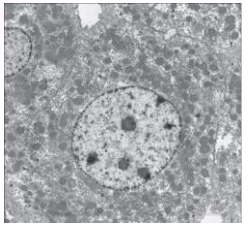
Accurate measurements are fundamental

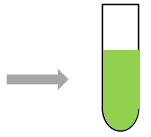




Step 1: get the metabolites!

Metabolite extraction





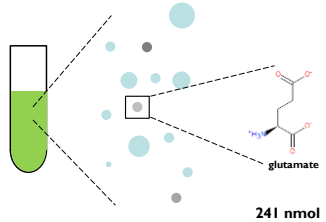
- Correct solvent?
- Could metabolite degrade?
- Inactivate enzymes

Basic problems of analytical chemistry

Separation

Identification

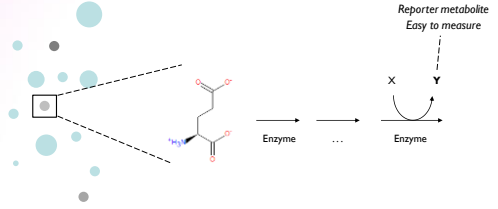
Quantification



241 nmol

Enzymatic assays (”kits”)

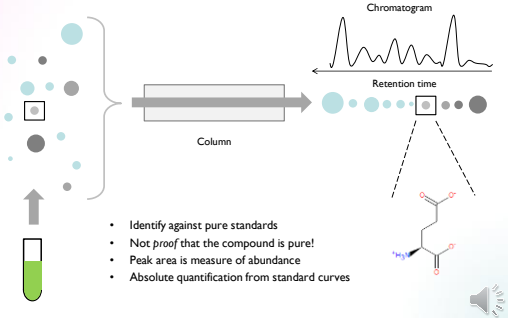
Use enzyme specificity to identify metabolites



- Pure enzymes, added in excess
- Complete reaction, quantify reporter metabolite
- Absolute quantification from standard curves

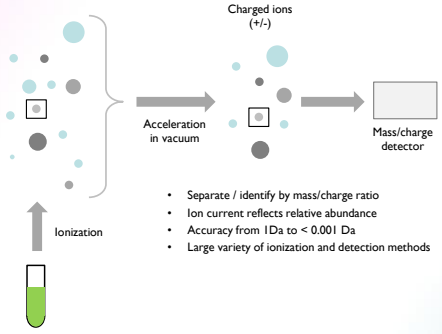
Chromatography

Separation by chemical properties



- Identify against pure standards
- Not *proof* that the compound is pure!
- Peak area is measure of abundance
- Absolute quantification from standard curves

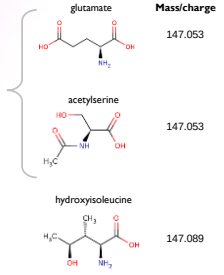
Mass spectrometry



- Separate / identify by mass/charge ratio
- Ion current reflects relative abundance
- Accuracy from 1Da to < 0.001 Da
- Large variety of ionization and detection methods

Benefits of high mass accuracy

Different molecules can be *very* close in mass



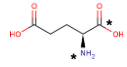
Mass/charge

147.053

147.053

147.089

Isotopes can be separated

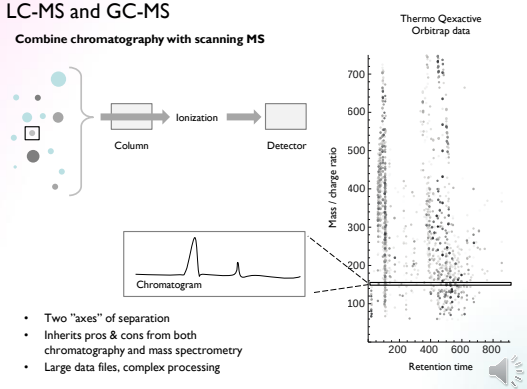


13C – 12C = 1.003

15N – 14N = 0.997

LC-MS and GC-MS

Combine chromatography with scanning MS

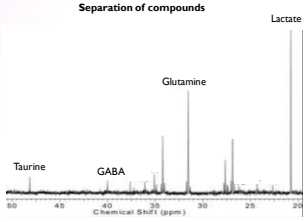


- Two "axes" of separation
- Inherits pros & cons from both chromatography and mass spectrometry
- Large data files, complex processing

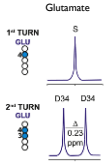
Nuclear magnetic resonance (NMR) spectroscopy

Separation by chemical shift of atom nuclei

Separation of compounds



Separation of isotopomers (13C NMR)



- Measure sample directly, quantitative
- Low sensitivity

- Some positional information
- Does not detect unlabeled atoms!

Marin-Valencia et al, Cell Metabolism 15:827--836, 2012