Measurement uncertainty in quantitative metabolomics

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Outline

- Biological and industrial relevance
- Metabolomics and measurements
- Concentrations and fluxes
- Alternative metabolic pathways
- Analytical approach
- Uncertainty of measurement and correction
Biochemical Studies of Living Cells

- Bacteria, fungi, mammalian, plant
- Understanding metabolism
- Understanding the interactions between compartments in cells
- Understanding the effect of extraneous chemicals (drugs, poisons) on the cell
- Producing chemicals, proteins and drugs from cell cultures
- Enhancing these production pathways

Cell compartments and interactions

White arrows …. flow of information
Black arrows …. regulations

S. Klein and E. Heinzle, 2012
Metabolomics

- Provides absolute or relative metabolite levels (intracellular or extracellular)
- BUT: Concentration changes cannot unambiguously be interpreted as changes of metabolic rates (fluxes)
- Increase in concentration can be from:
  - Increased activity of producing enzymes
  - Increased pool (reservoir) within cell
  - Decreased activity of consuming enzymes

The measurement problem

- Small quantities of cell mass (mg)
- Numerous small molecules involved in metabolic pathway
- Most of the molecules are highly reactive
- Temporary development of concentration profiles is relevant
- Difficult to „freeze“ the temporary state
- Temporary state inferred from degree of labelling of molecules via $^{13}$C glucose
Analytical solution

42 analytes in *Pichia pastoris* broth for production of human superoxide dismutase

- Quenching of metabolism: sampling of cell broth into 60% MeOH at -30°C
- Filtered and stored at -80°C
- Extracted with 4 ml EtOH (75%) for 3 min at 85°C
- Automated ethoximation and trimethylsilylation
- GC-CI-TOFMS

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T. Mairinger et al., 2015

Human Cu-Zn superoxide dismutase mutant G93A

http://www.rcsb.org/pdb/explore/jmol.do?structureId=2ZKY&bionumber=5&view=symmetry
GC-run

- Amino acids
- Sugars
- Phosphates
- Intermediates

Mass spectrometric pattern from isotopologues

- 13-C labelling for a C-3 molecule
- Random statistical frequency 1:3:3:1

- The bigger the molecule the more isotopologues
One of 42 analytes: Sedoheptulose-7-phosphate

- 7 Trimethylsilyl and 1 Ethoximate
- 29 C atoms and 7 Si atoms

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Glucose-6-phosphat

Phase 1 (oxidativ)

Ribulose-5-phosphat

Ribose-5-phosphat (C5)

Xylulose-5-phosphat (C5)

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Stryer Biochemie, 2013
Components of uncertainty

- From analytical protocol:
  - Ionization and transmission efficiency
  - Poisson statistics
  - Background subtraction
  - Integration

- From naturally occurring isotopes:
  - $^{13}\text{C}$ in backbone and derivatization reagent
  - $^{28}\text{Si}$, $^{29}\text{Si}$, $^{30}\text{Si}$ in derivatization reagent

Excel solution for analytical protocol
Excel solution for naturally occurring isotopes

Data from:

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Monte Carlo Simulation

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### Results for Sedoheptulose Phosphate

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### Conclusions

- The analytical protocol can be considered fairly robust
- The corrections lead to significantly different „true“ isotopic signature from labelling
- The corrections for natural isotopic abundances cause an increase of uncertainty of about a factor of 3 - 4
- The significance of these corrections on the flux modelling is not yet established

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