Institute for Molecular Medicine Finland
Nordic EMBL Partnership for Molecular Medicine

Mass Spectrometry in Metabolic flux profiling

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Outline

- ❑ Mass Spectrometry
  - Advantages of MS for MFA
  - GC-MS
- ❑ Metabolic Flux Analysis
  - Metabolic Engineering
  - ^{13}C based MFA
  - MFA_GC-MS
- ❑ Summary

Advantages of MS for metabolic flux profiling (1/2)

- Small amount of sample needed for analysis, which results in easy handling and provides the possibility to study metabolic fluxes in small reactors
- MS is much more sensitive than NMR analysis and can thus be applied to very low amounts and concentrations, which is especially useful for intracellular metabolites.
- Fast sampling techniques and analytical protocols have been recently developed that allow quantitative estimation of various intracellular metabolites
- Possible to investigate dynamic responses of the metabolism to defined changes of cultivation conditions.
- The measurement of selected diagnostic ions by single ion monitoring (SIM) may be used to further increase the sensitivity

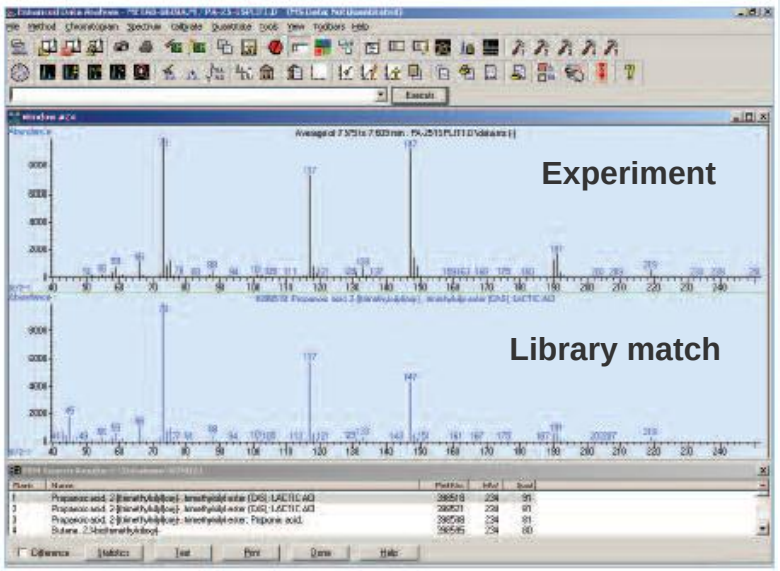
Advantages of MS for metabolic flux profiling (2/2)

- Mass spectrometry is sensible towards impurities
- By various derivatization methods, the substances of interest can be partially enriched and purified from cultivation broths.
- Moreover, combined techniques as GC–MS or HPLC–ESI–MS allow further separation.
- MS is the best available method for the determination of relative isotope intensities with an accuracy in the range of 0.1%
- Measurement of isotope distributions does not need quantitative isolation of the target molecules from the sample.

Features of GC/MS Metabolomics

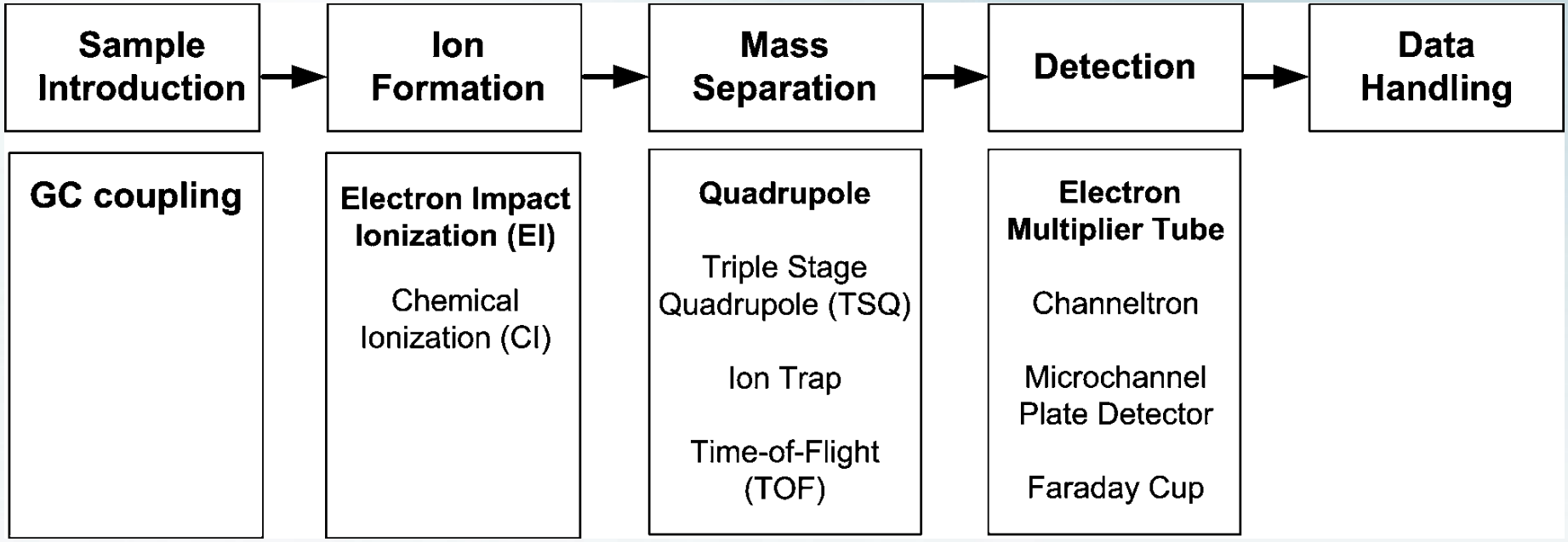
- Useful for volatiles or compounds that can be derivatized to volatile compounds (derivatization often required)
- Ideal for long chain compounds e.g. FFA, acyl carnitines, etc
- More stable and reproducible than LC/MS
- Most advanced metabolomics libraries
- Standards are typically required for positive identification
- Inexpensive technology

Agilent GC/MS systems provide high separation power and high sensitivity for metabolomic analyses. Library-searchable EI spectra greatly simplify identification.

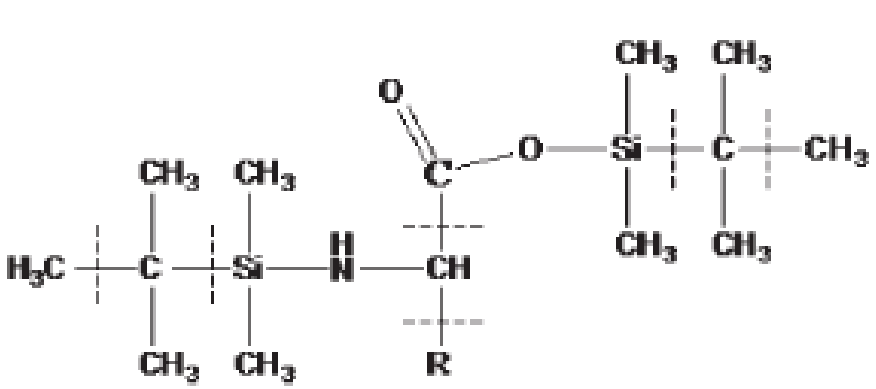


Overview of different GC-MS instrumentation types

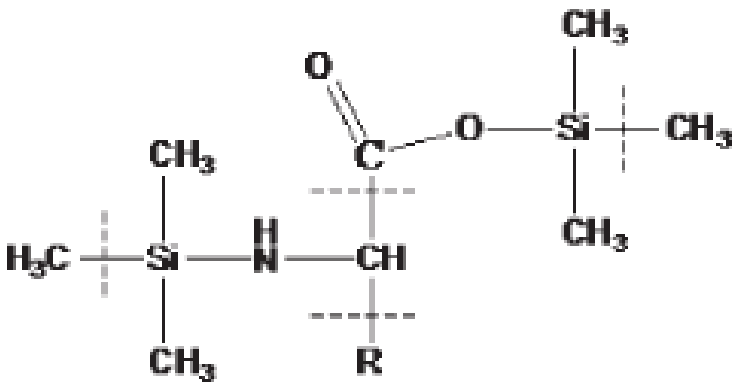
Gas Chromatography Mass Spectrometer



The molecular structure and the bond fragmentation positions between two silyation derivatized amino acids

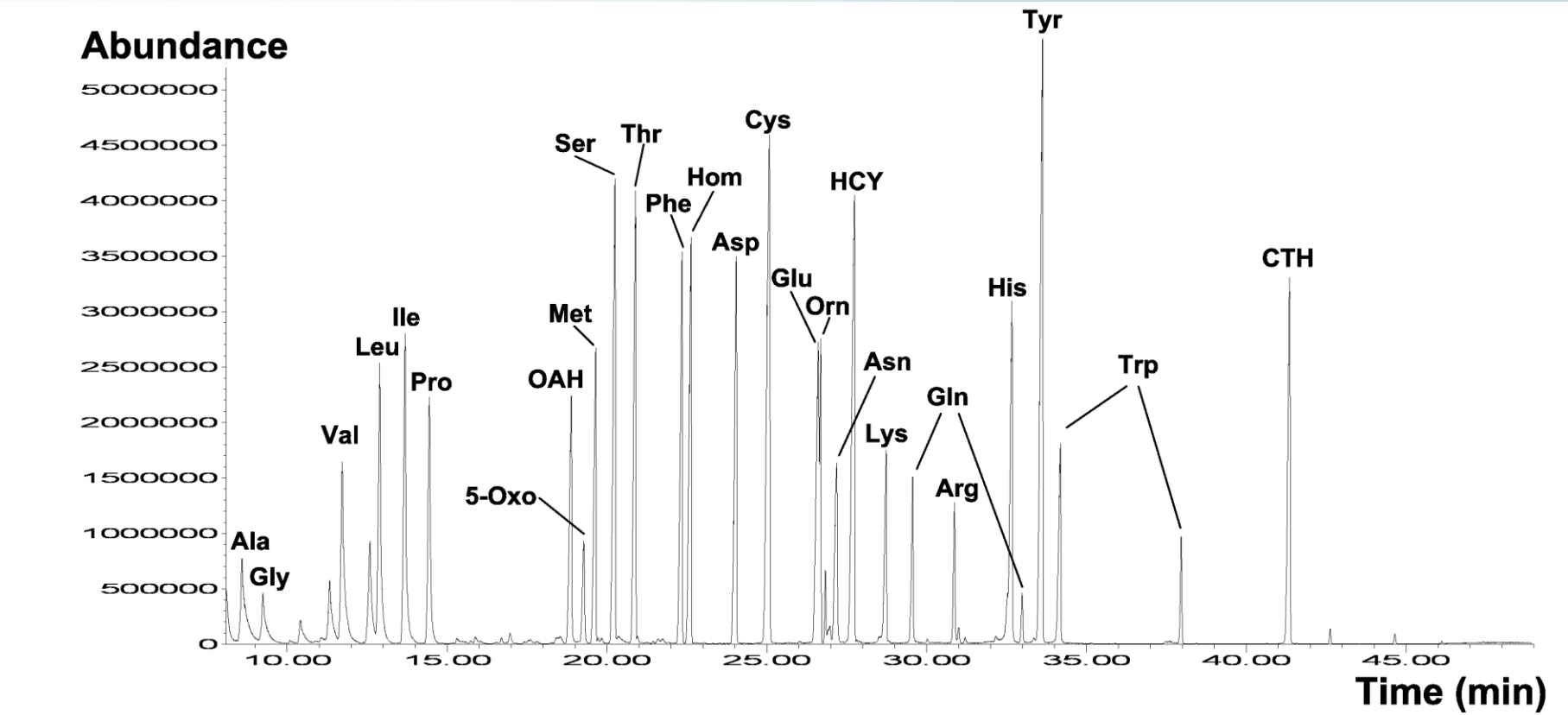


TBDMS-derivatized amino acids



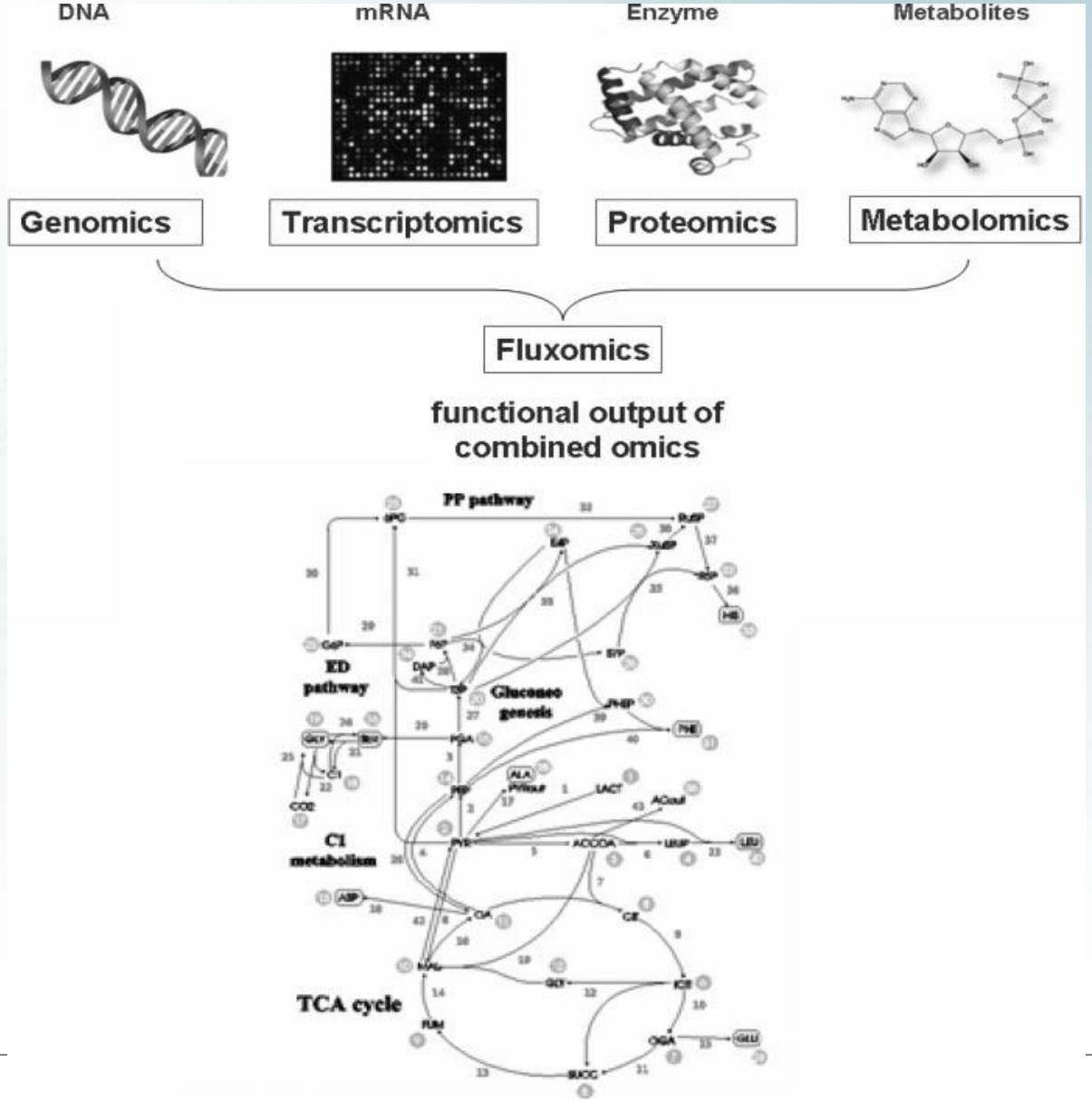
TMS-derivatized amino acids

Total ion current (TIC) spectrum of a sample with TBDMS-derivatized metabolites.



Metabolic Flux Analysis

Omics tools to investigate cellular metabolism



Wikimedia Commons, ChemDraw Ultra 8.0, Tang et al, 2009

Metabolic flux analysis (MFA)

- The system-wide quantification of intracellular fluxes in an organism

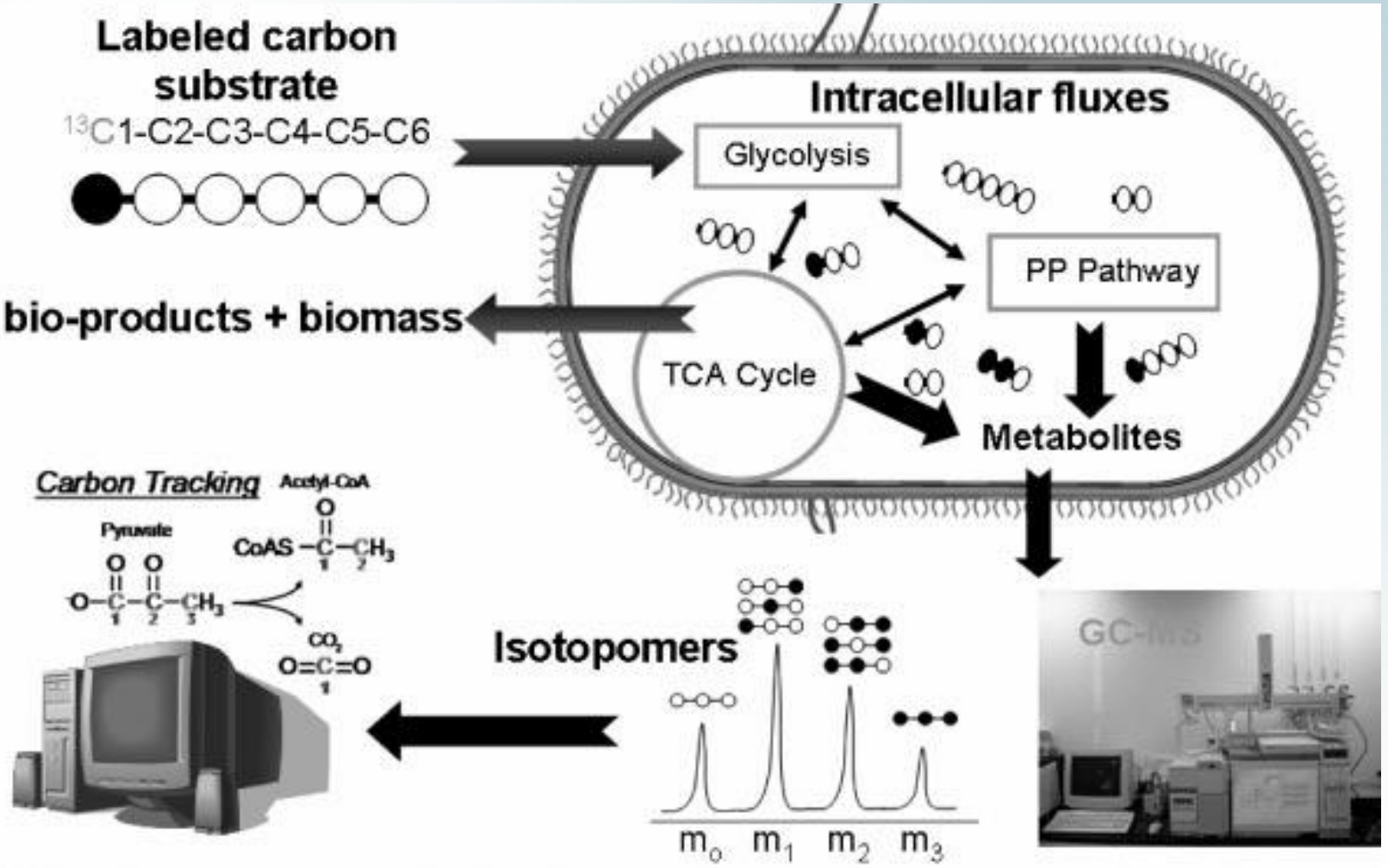
- Metabolic flux maps provide a quantitative depiction of carbon flow through competing metabolic pathways, thus providing

  - analysis of substrate utilization and product formation
  - flexibility or rigidity of carbon flow at network nodes
  - the rate of a given enzymatic reaction *in vivo*
  - and inferred availability of NADPH or ATP

^{13}C metabolic flux analysis

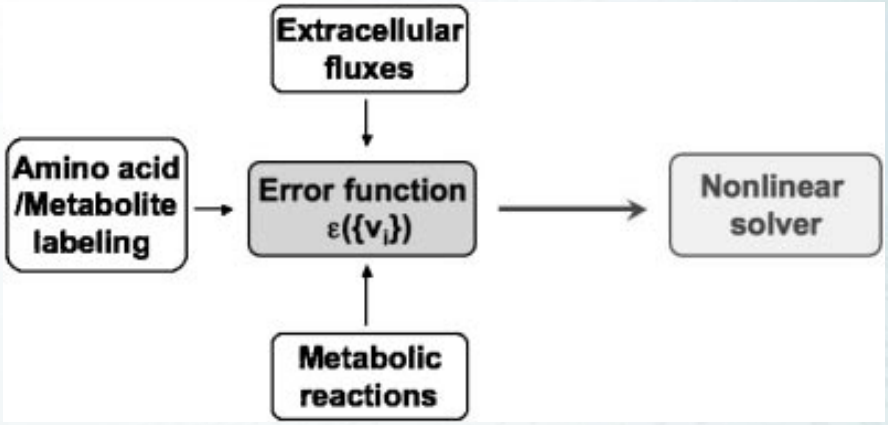
- Isotope detection via GC/MS or NMR of metabolites (e.g. amino acids from hydrolyzed protein)
- Quantifies intracellular metabolic fluxes for smaller reaction networks, where the fluxes are completely determined
- Isotopomers, which are isomers of a metabolite that differ in the labeling state (^{13}C or ^{12}C) of their individual carbon atoms, are a central concept in the analysis and mathematical modeling of ^{13}C MFA.

Protocol for ^{13}C-based flux analysis



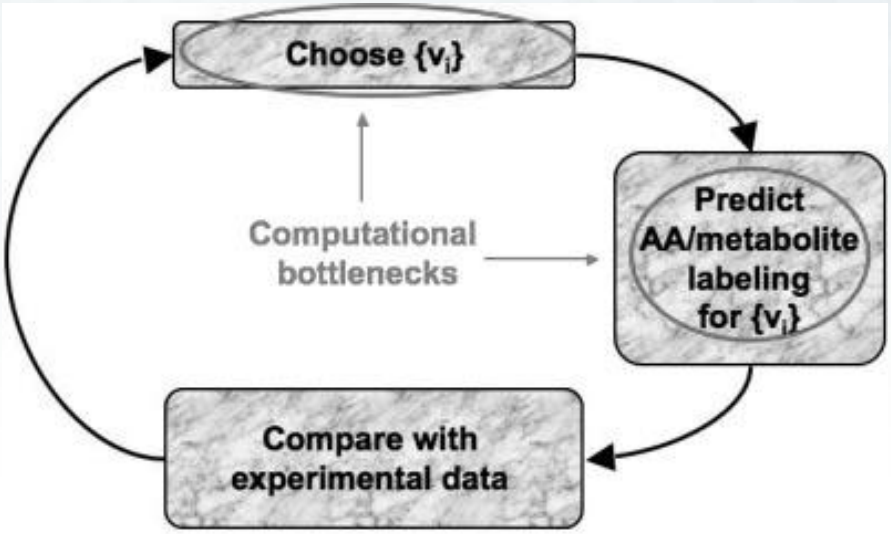
Tang et al, 2009

Inputs for metabolic flux analysis



The information on the metabolic reactions, amino acid/metabolite labeling and extracellular fluxes is combined to produce the error function e

Recursive procedure to obtain fluxes from amino acid/metabolite labeling information.

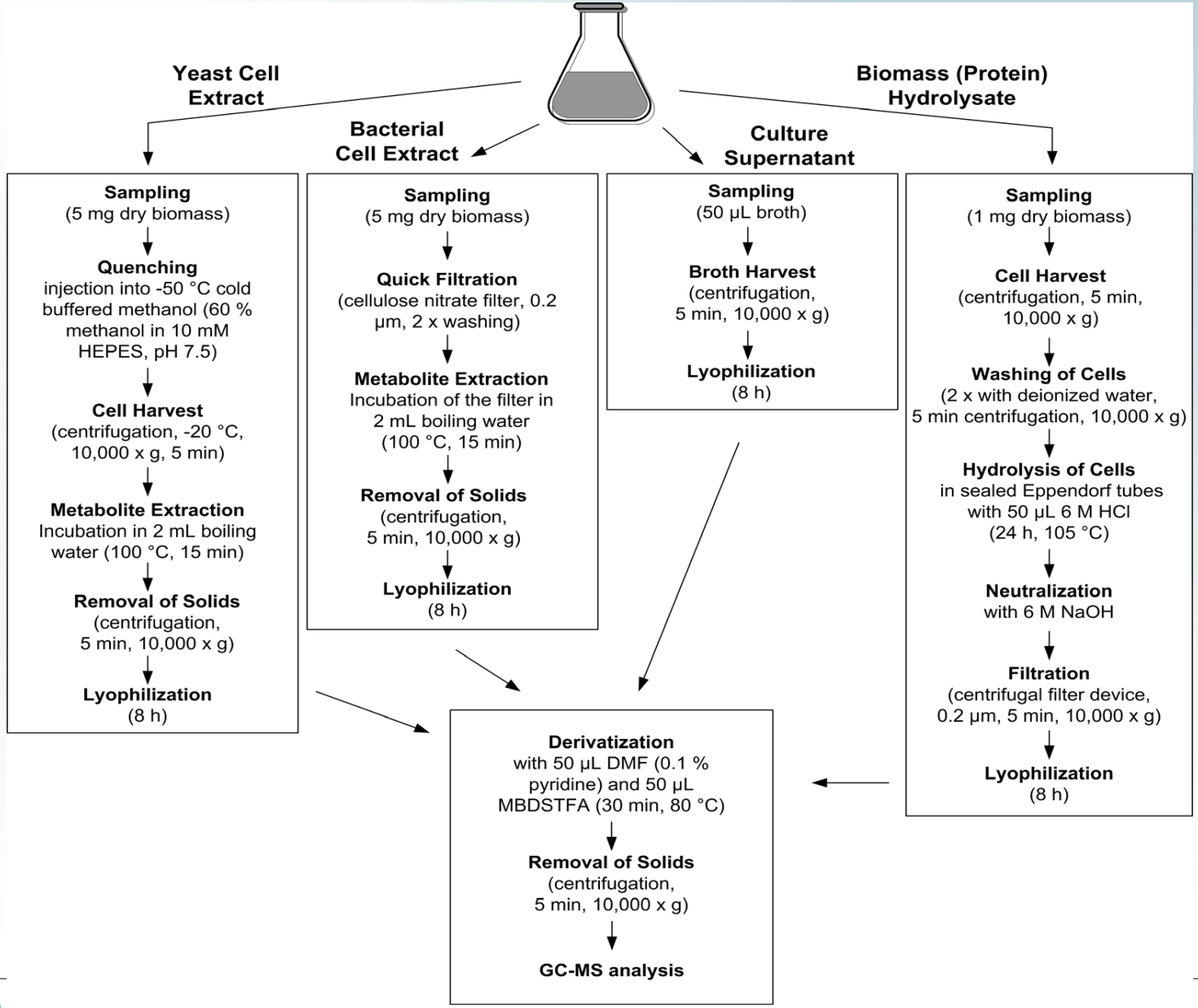


A set of fluxes {vi} is initially chosen and the expected aminoacid/metabolite labeling is calculated under the assumed fluxes {vi}.

Tang et al, 2009

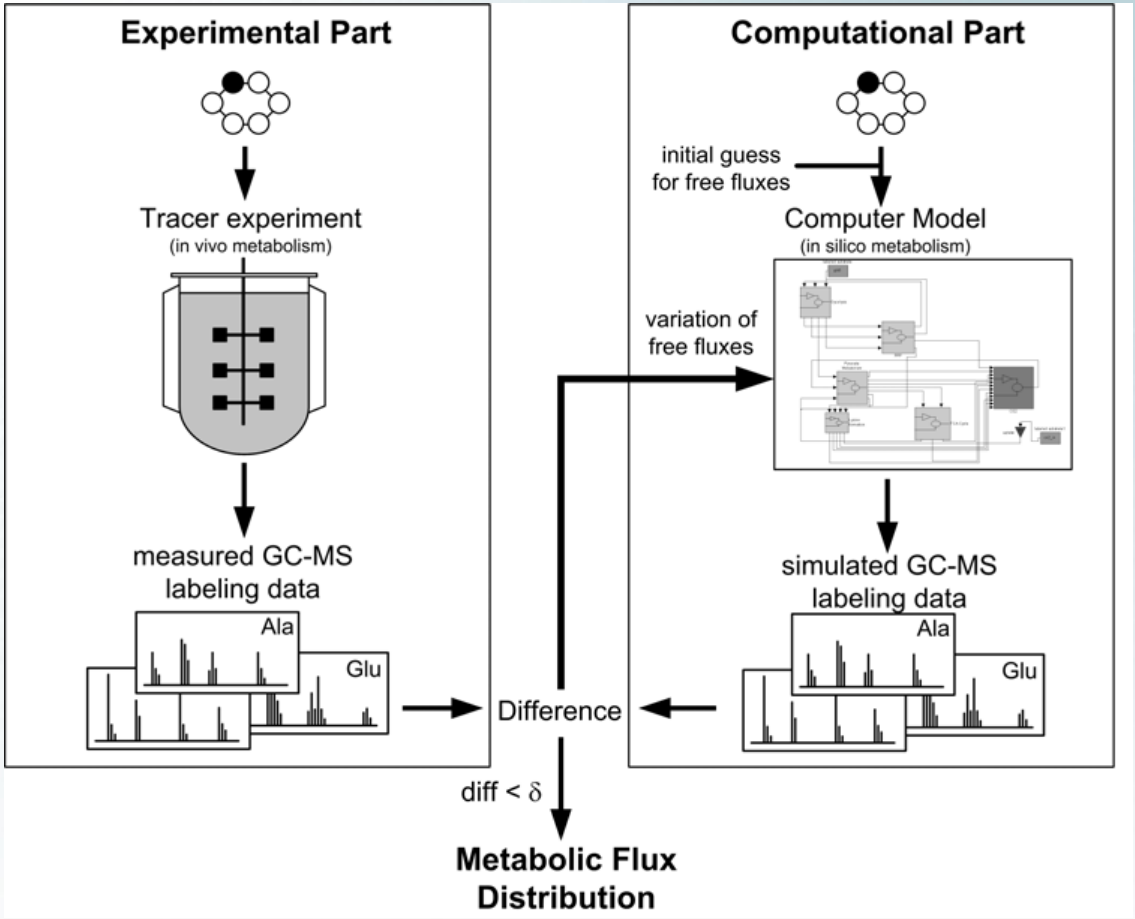
Metabolic flux profiling_GC-MS

Experimental protocols for sampling and processing of amino acids



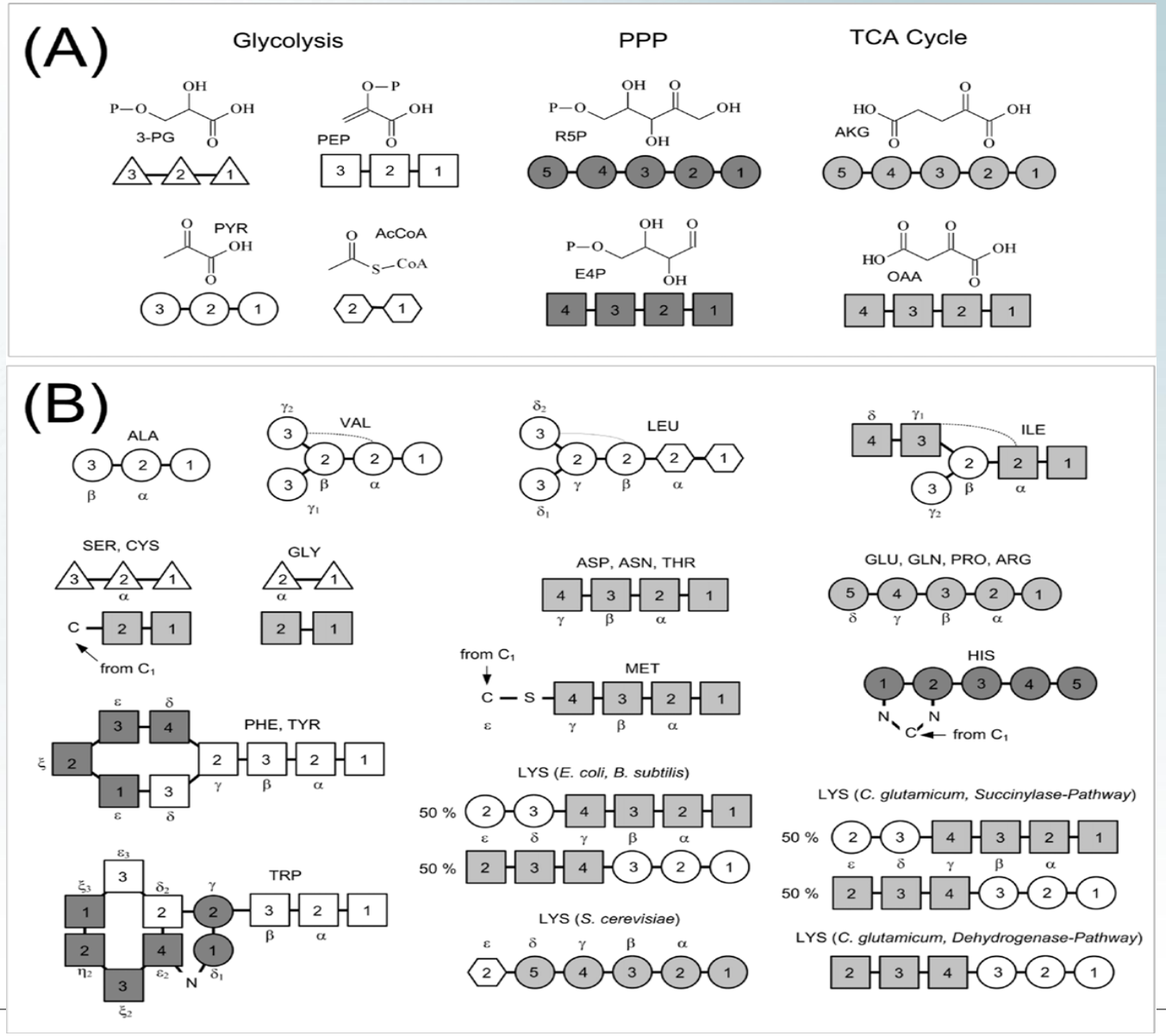
Wittmann, 2007

Strategy for ^{13}C metabolic flux analysis



Experimental part with the tracer study and the GC-MS labelling analysis and the computational part with the simulation of the labelling data via an isotopomer model representing the investigated metabolic network. The flux estimation is based on minimizing the deviation (δ) between the measured and the simulated labelling data.

Relationship between the carbon skeleton of amino acids and the carbon skeleton of their metabolic precursors for the anabolic pathways



Wittmann, 2007; Szyperski et al., 1998

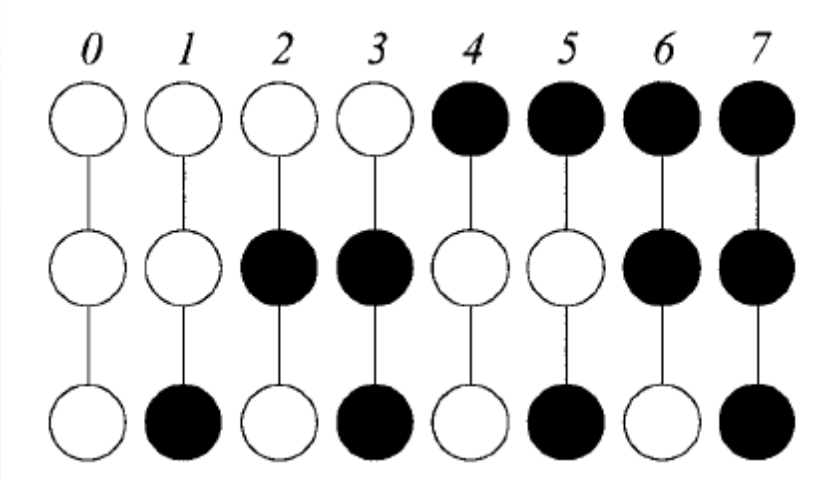
Isotopomer Analysis Using GC-MS

- Knowledge of the complete isotopomer distribution represents the ultimate amount of information on the labeling pattern of a metabolite.
- GC-MS offers a unique possibility of analyzing fragments larger than C3 and therefore able to give information which is complementary to the information that can be obtained from NMR spectroscopy.

Positional isotopomer and Mass isotopomers

- Specific number of ^{13}C atoms in specific positions in the molecule, uniquely determine the labeling pattern of a molecule
- The mass isotopomers only describe the number of ^{13}C atoms

The eight different positional isotopomers of a C3 molecule.



Isotopomer distribution vectors

$$\mathbf{IDV}_{\mathrm{Pyr}} = \begin{bmatrix} x_{\mathrm{Pyr}}(1) \\ x_{\mathrm{Pyr}}(2) \\ x_{\mathrm{Pyr}}(3) \\ x_{\mathrm{Pyr}}(4) \\ x_{\mathrm{Pyr}}(5) \\ x_{\mathrm{Pyr}}(6) \\ x_{\mathrm{Pyr}}(7) \\ x_{\mathrm{Pyr}}(8) \end{bmatrix} = \begin{bmatrix} x_{\mathrm{Pyr}}(000) \\ x_{\mathrm{Pyr}}(001) \\ x_{\mathrm{Pyr}}(010) \\ x_{\mathrm{Pyr}}(011) \\ x_{\mathrm{Pyr}}(100) \\ x_{\mathrm{Pyr}}(101) \\ x_{\mathrm{Pyr}}(110) \\ x_{\mathrm{Pyr}}(111) \end{bmatrix}$$

Mass distribution vector

$$\mathbf{MDV}_{\mathrm{Pyr}} = \begin{bmatrix} x_{\mathrm{Pyr}}(m) \\ x_{\mathrm{Pyr}}(m+1) \\ x_{\mathrm{Pyr}}(m+2) \\ x_{\mathrm{Pyr}}(m+3) \end{bmatrix}.$$

Fragment mass distribution vector

$$\mathbf{FMDV}_{\mathrm{Pyr}_{2,3}} = \begin{bmatrix} x_{\mathrm{Pyr}_{2,3}}(1) \\ x_{\mathrm{Pyr}_{2,3}}(2) \\ x_{\mathrm{Pyr}_{2,3}}(3) \end{bmatrix} = \begin{bmatrix} x_{\mathrm{Pyr}_{2,3}}(m) \\ x_{\mathrm{Pyr}_{2,3}}(m+1) \\ x_{\mathrm{Pyr}_{2,3}}(m+2) \end{bmatrix}.$$

Summed fractional labelling (SFL) analysis

- By calculating the average number of ^{13}C atoms in the fragments, which is equal to the sum of the fractional labelings of the carbon atoms in the fragment, the fractional labelings of the carbon positions in an amino acid can be calculated.

- The fractional labeling of each fragment, SFL, was calculated with the corrected intensities

$$\mathrm{SFL}(\mathrm{Ala}^{1-3}) = \frac{0 \cdot m_0^{1-3} + 1 \cdot m_1^{1-3} + 2 \cdot m_2^{1-3} + 3 \cdot m_3^{1-3}}{m_0^{1-3} + m_1^{1-3} + m_2^{1-3} + m_3^{1-3}}$$

superscripts - the carbon atoms present in the fragment
m_n - the corrected intensity of the mass isotopomer with m_n labeled carbon atoms

- The SFLs, as described above, were used as inputs to a mathematical routine that is used for quantifying the fluxes in the central carbon metabolism

Case: The sum of the fractional labelings of C1 and C2 in glycine, Gly(1,2), is calculated from the C1–C2 fragment of glycine

$$\mathrm{Gly}(1,2) = x(1) + x(2) = \frac{0 \cdot m_0^{1-2} + 1 \cdot m_1^{1-2} + 2 \cdot m_2^{1-2}}{m_0^{1-2} + m_1^{1-2} + m_2^{1-2}}$$

x(1) and x(2) are the fractional labelings of C1 and C2 of glycine, respectively

Fractional labeling of C2 in glycine, Gly(2) or *x(2), can be calculated*

$$\mathrm{Gly}(2) = x(2) = \frac{0 \cdot m_0^2 + 1 \cdot m_1^2}{m_0^2 + m_1^2}$$

The fractional labeling of C2 is explicitly given, and the fractional labeling of C1, *x(1),* can be calculated by

$$x(1) = \mathrm{Gly}(1,2) - \mathrm{Gly}(2)$$

Summary

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- Mass spectrometry is a powerful tool for metabolic flux profiling because of its higher sensitivity over other available tools, small amount of sample, etc.
- Tracer based techniques for metabolic flux profiling either single labelled or universal labelled.
- Tracer based studies coupled with mass spectrometry provide rich information on the metabolite flow in the biochemical network.
- Metabolic flux maps can be achieved either by advanced mathematical modelling or by using simple stoichiometric matrix based methods