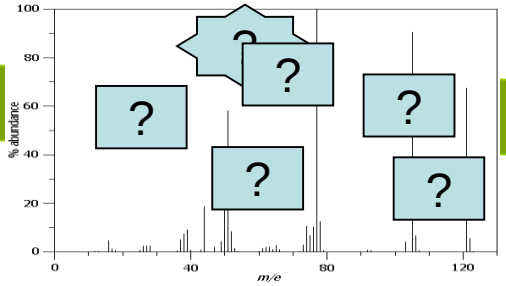
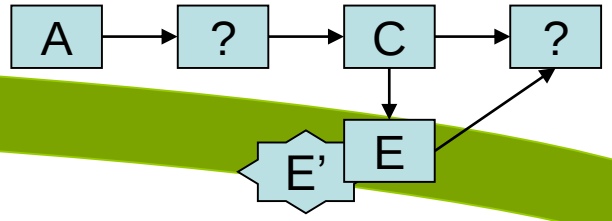
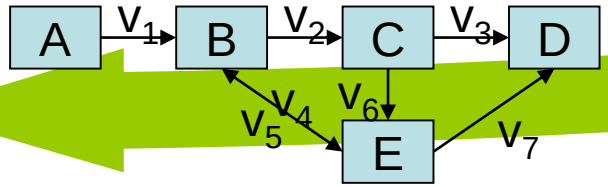
Bioinformatics for Metabolomics and Fluxomics

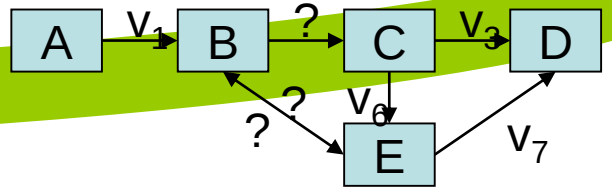
RL 1; metabolite identification





RL 1 & 2; pathway reconstruction

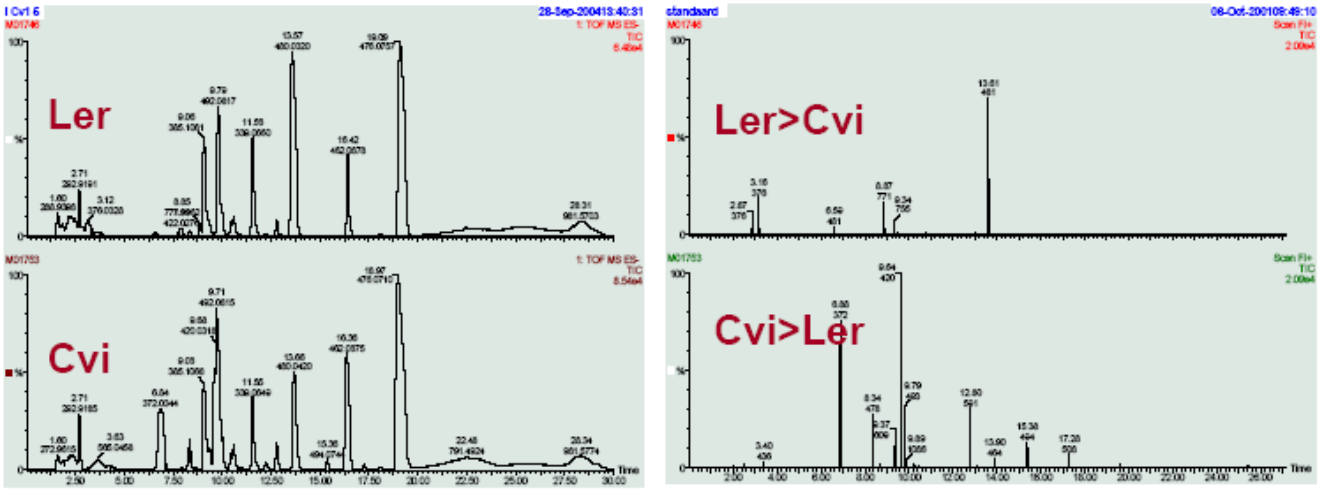




RL 2; metabolic flux analysis

Metabolites and Metabolic Fluxes Play Key Roles in Organisms

First Example Application Domain :
200,000 metabolites in plants



original LC-QTOF MS profiles

significantly different metabolites
(factor 2 or more, 99% conf., n=5)

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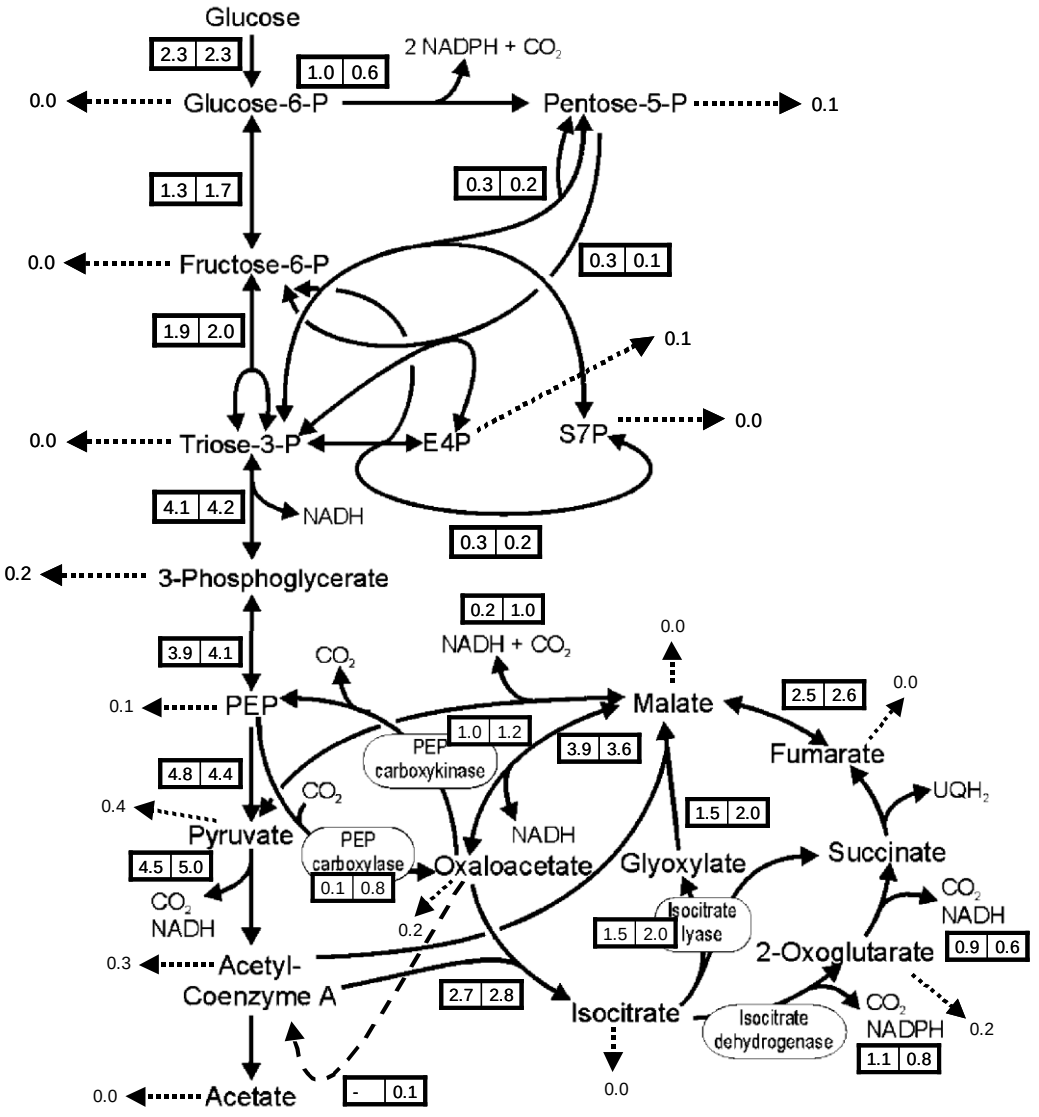
Metabolomics: (large scale) measurements of metabolites and their levels

Metabolites and Metabolic Fluxes Play Key Roles in Organisms

Second Example : metabolic flux analysis in micro-organisms

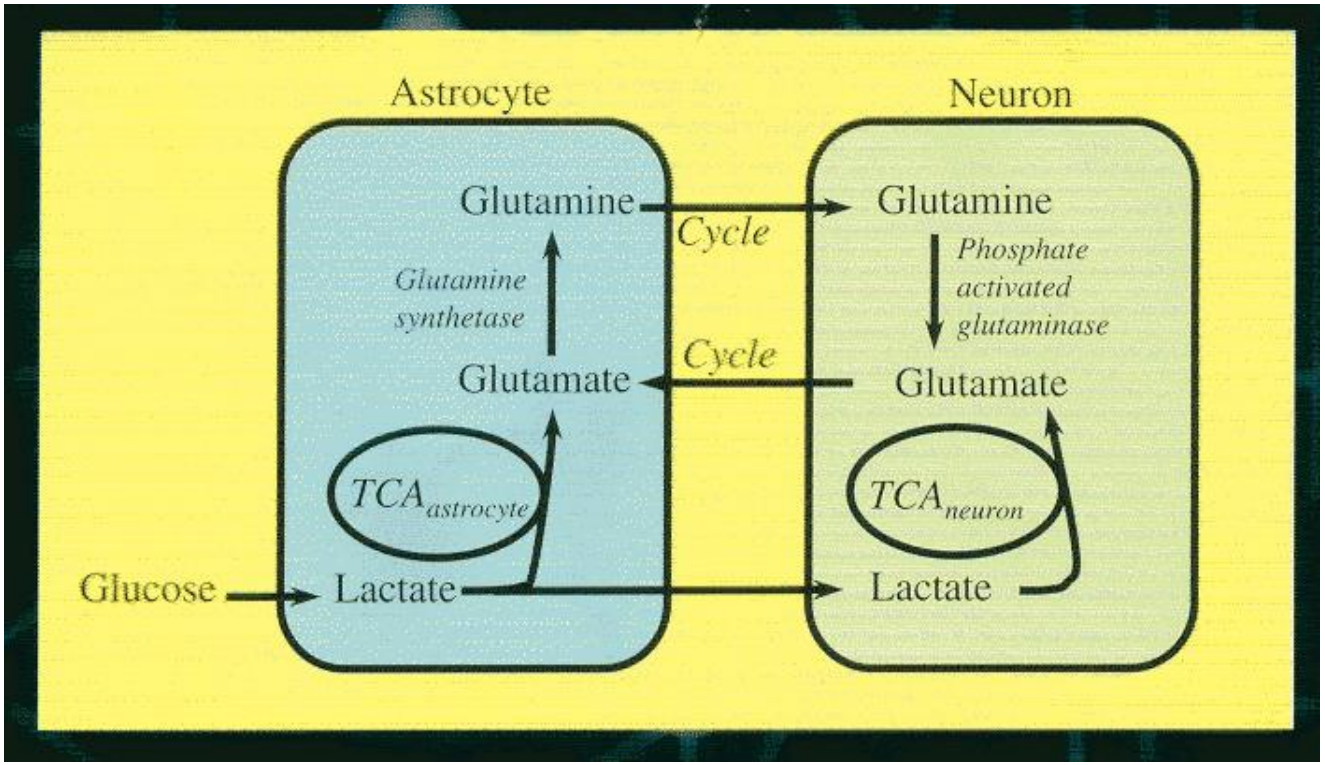
Metabolic flux analysis of *E. coli* strain grown in chemostat culture



Fluxomics: (large scale) measurements of metabolic fluxes

Metabolites and Metabolic Fluxes Play Key Roles in Organisms

Third Example : Human and Animal Brain Neurotransmitter Cycling



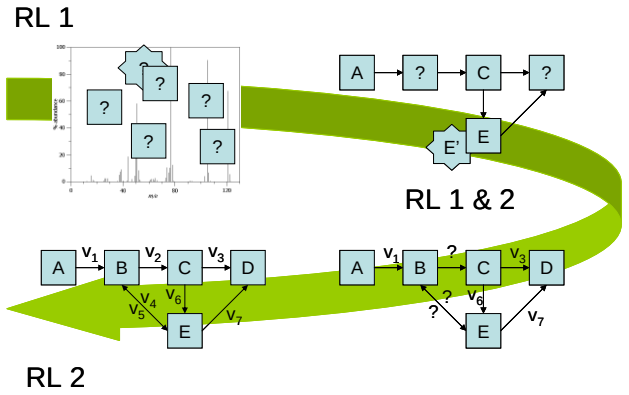
from: *Metabolic Engineering (2004)*

Fluxomics: (large scale) measurements of metabolic fluxes

Goals Project

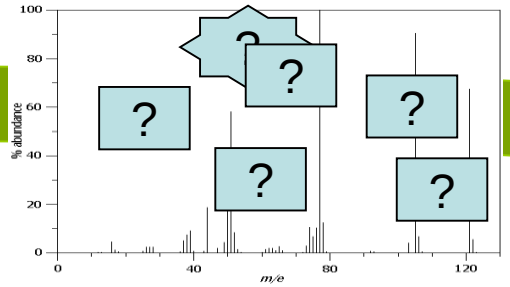
develop bioinformatics methods for

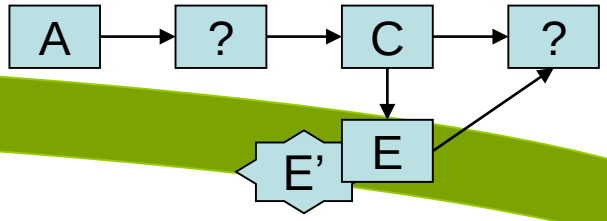
- metabolite and pathway identification
- quantification of metabolite levels and isotopic composition
- analysis of dynamic metabolic experiments
- quantification of metabolic fluxes



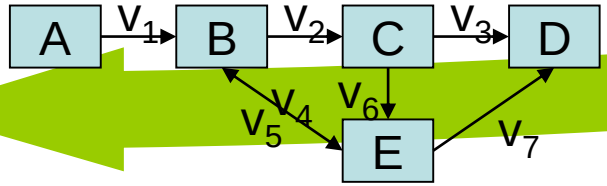
Two Connected Research Lines

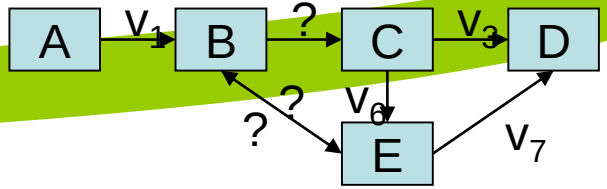
RL 1; metabolite identification





RL 1 & 2; pathway reconstruction





RL 2; metabolic flux analysis

Expertise in the Netherlands Bundled

Key Participants

Roeland C.H.J. van Ham, Raoul J. Bino,
Centre for BioSystems Genomics / Plant Research International, Wageningen

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Kluyver Centre / Delft University of Technology, Dept. of Biotechnology

Johannes H.G.M. van Beek,
Centre for Medical Systems Biology / VU University medical centre, Amsterdam

Ivo H.M. van Stokkum,
Centre for Medical Systems Biology / Applied Computer Science, Vrije Universiteit, Amsterdam

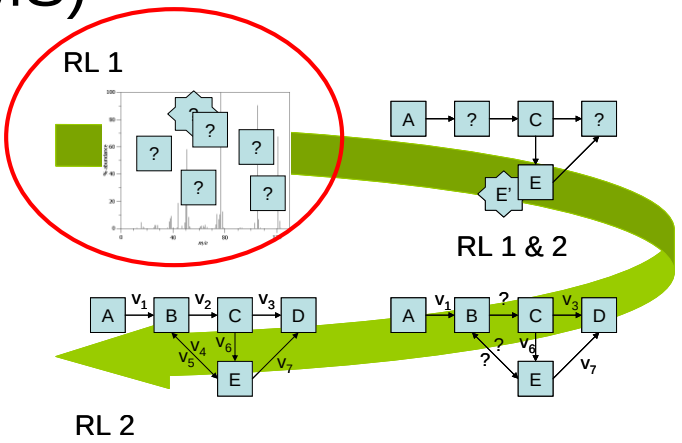
Further participants / consultants / collaborators :
on the one hand computer science/database (Bakker/Kok, Bal), signal analysis (Verheijen, Van Ormondt/De Beer) and bioinformatics (a.o. Heringa) expertise.

On the other hand many scientists with metabolic research expertise and interests.

RL1: Metabolite Identification

Develop platform for identification of metabolites from high-throughput metabolome data

- ❖ algorithms for compound identification from (LC-) mass spectrometry and NMR spectroscopy
- ❖ databases for raw and processed information; retrieving matching spectra of known chemical composition
- ❖ standardized and automated procedure for metabolite identification, in particular from LC-MS/MS (liquid chromatography coupled to tandem MS)



Metabolite Identification

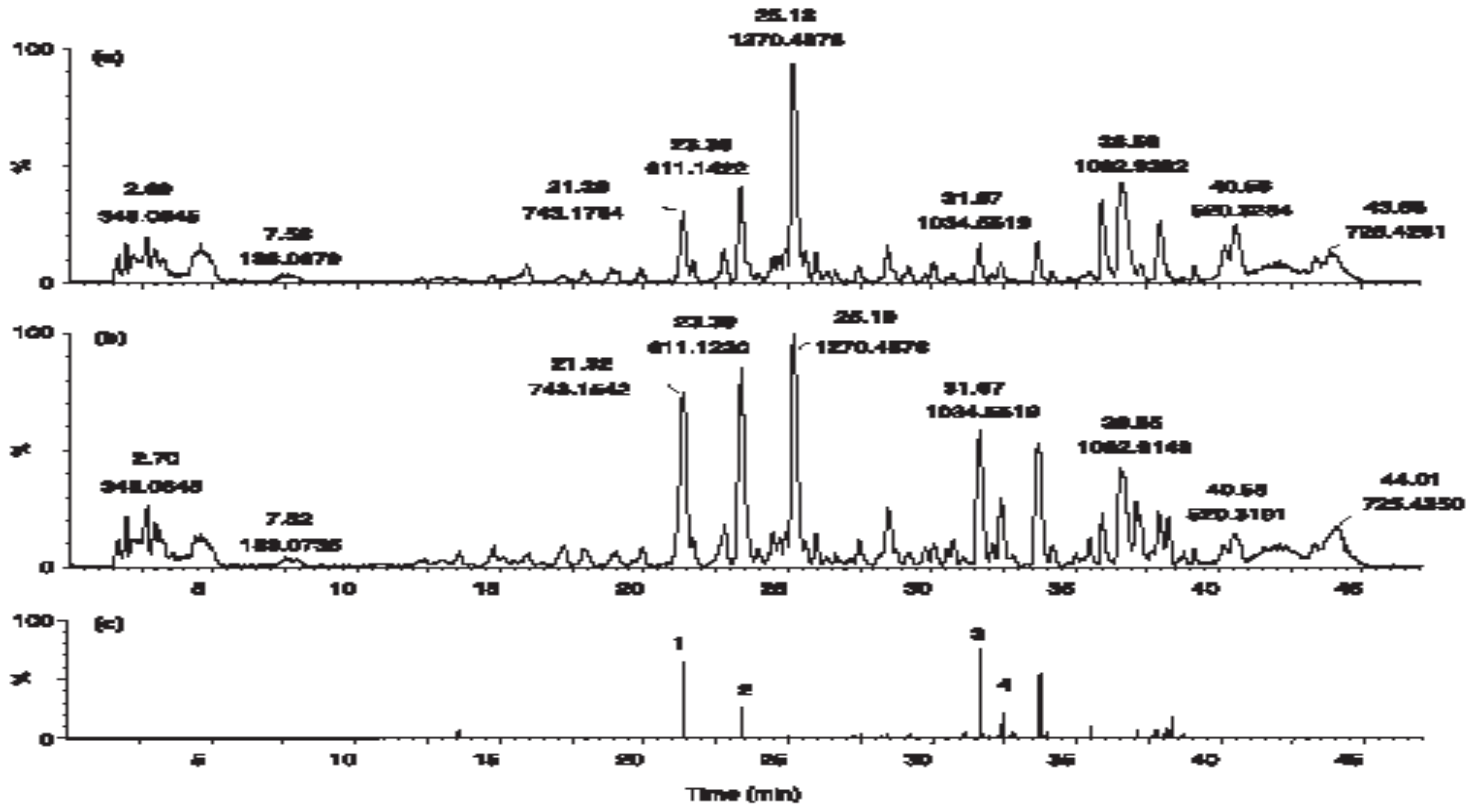
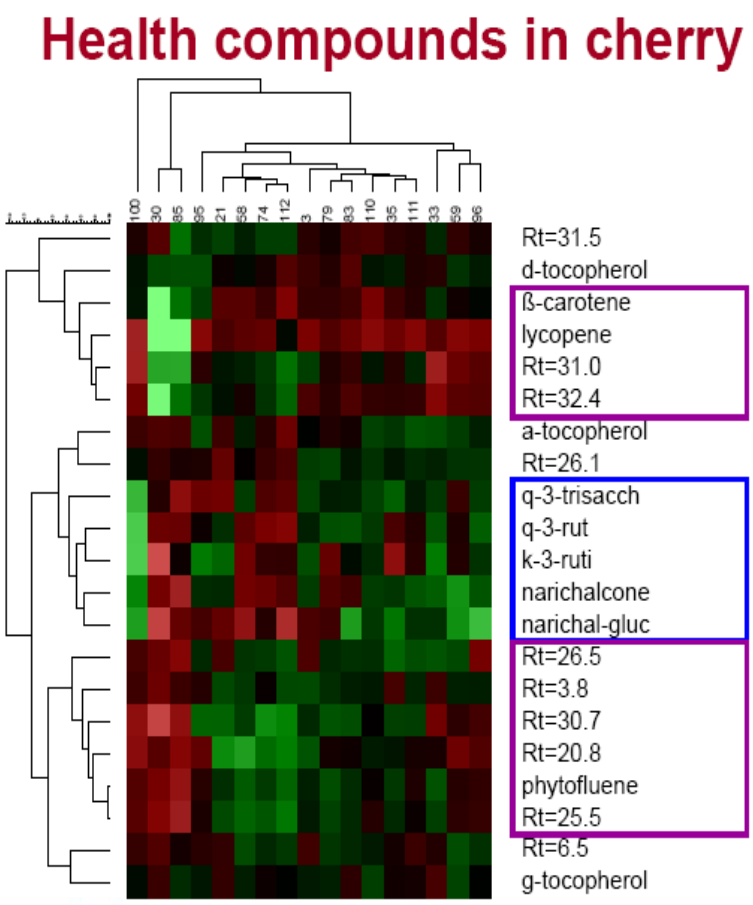


Fig. 2 Typical LC–QTOF–MS–ESI-positive chromatogram (given as base peak intensities) of aqueous methanol extracts from red fruits of control (a) and *hp-2*^{dg} (b) *Lycopersicon esculentum* (tomato) plants (numbers at peaks refer to retention time and mass), and mass peaks (c) that were significantly ($P < 0.05$) increased at least twofold in fruits of *hp-2*^{dg} plants compared with the control. All chromatographic data were processed and compared using METALIGN software. Peaks were identified based on mass fragmentation patterns and PDA absorbance: peak 1, quercetin-3-trisaccharide; 2, rutin; 3, tomatine; 4, naringenin-chalcone glucoside.

Bino et al. *New Phytologist* (2005) **166** : 427–438

Metabolite Identification



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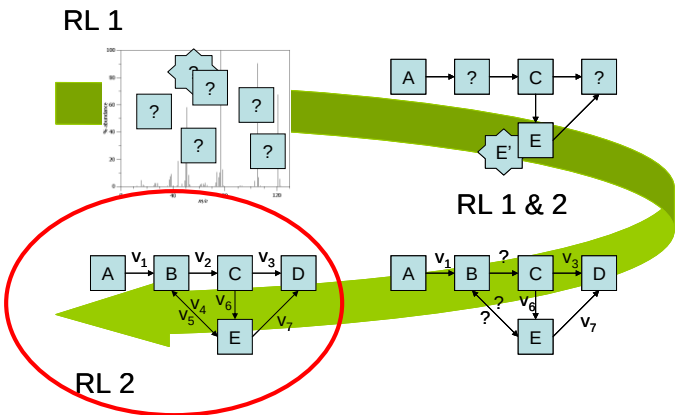
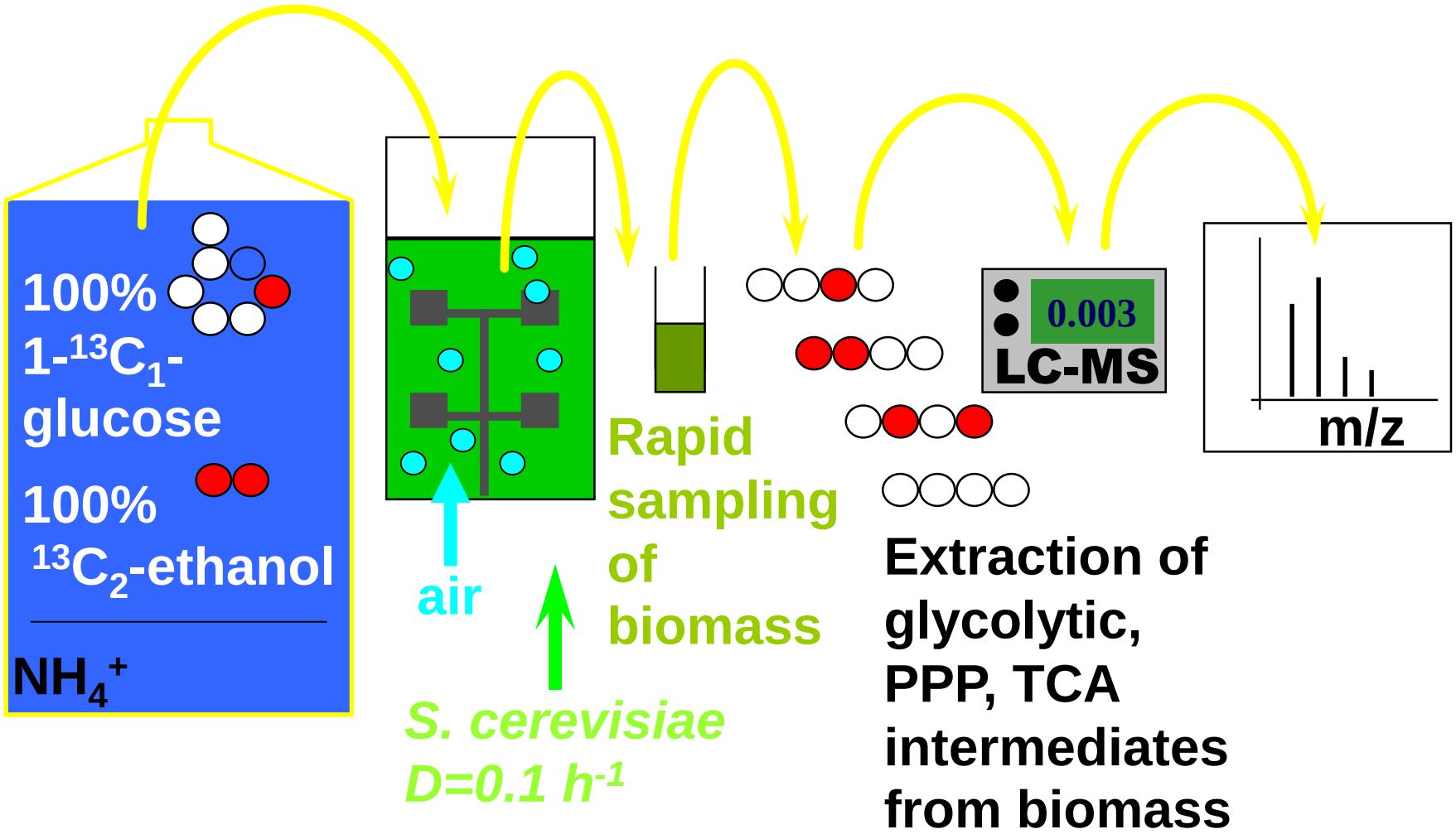
RL2: Metabolic Flux Analysis

Develop platform for flux analysis, derived from stable isotope incorporation measured with NMR and mass spectrometry

- ❖ a problem solving environment for simulation and analysis of metabolic flux models and experimental design
- ❖ optimization algorithms for flux quantification
- ❖ new metabolic pathway modules



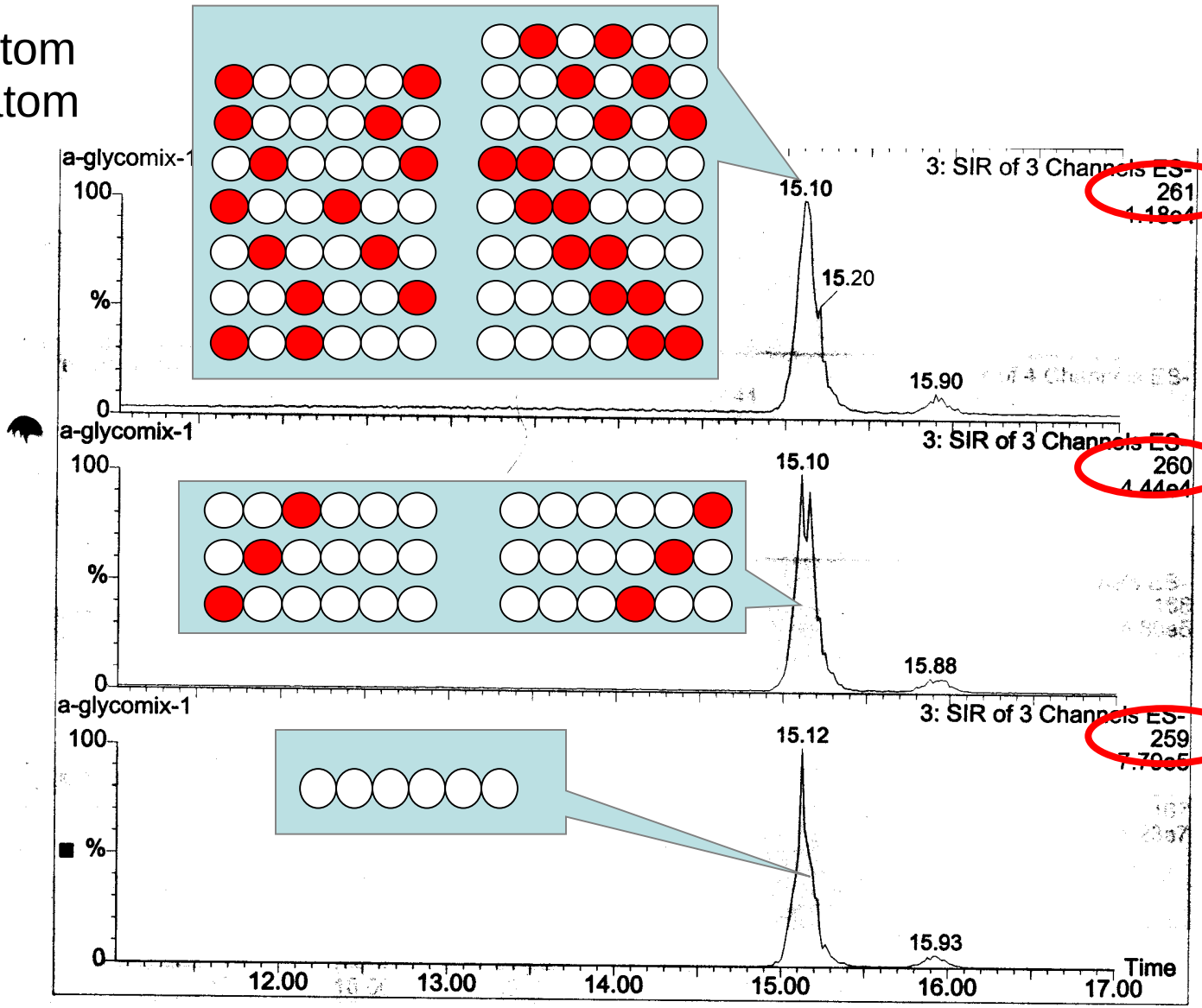
^{13}C-experiment for metabolic flux analysis in micro-organisms



Detection of mass isotopomer fractions of glucose-6-phosphate with LC-MS

○ = ^{12}C-atom

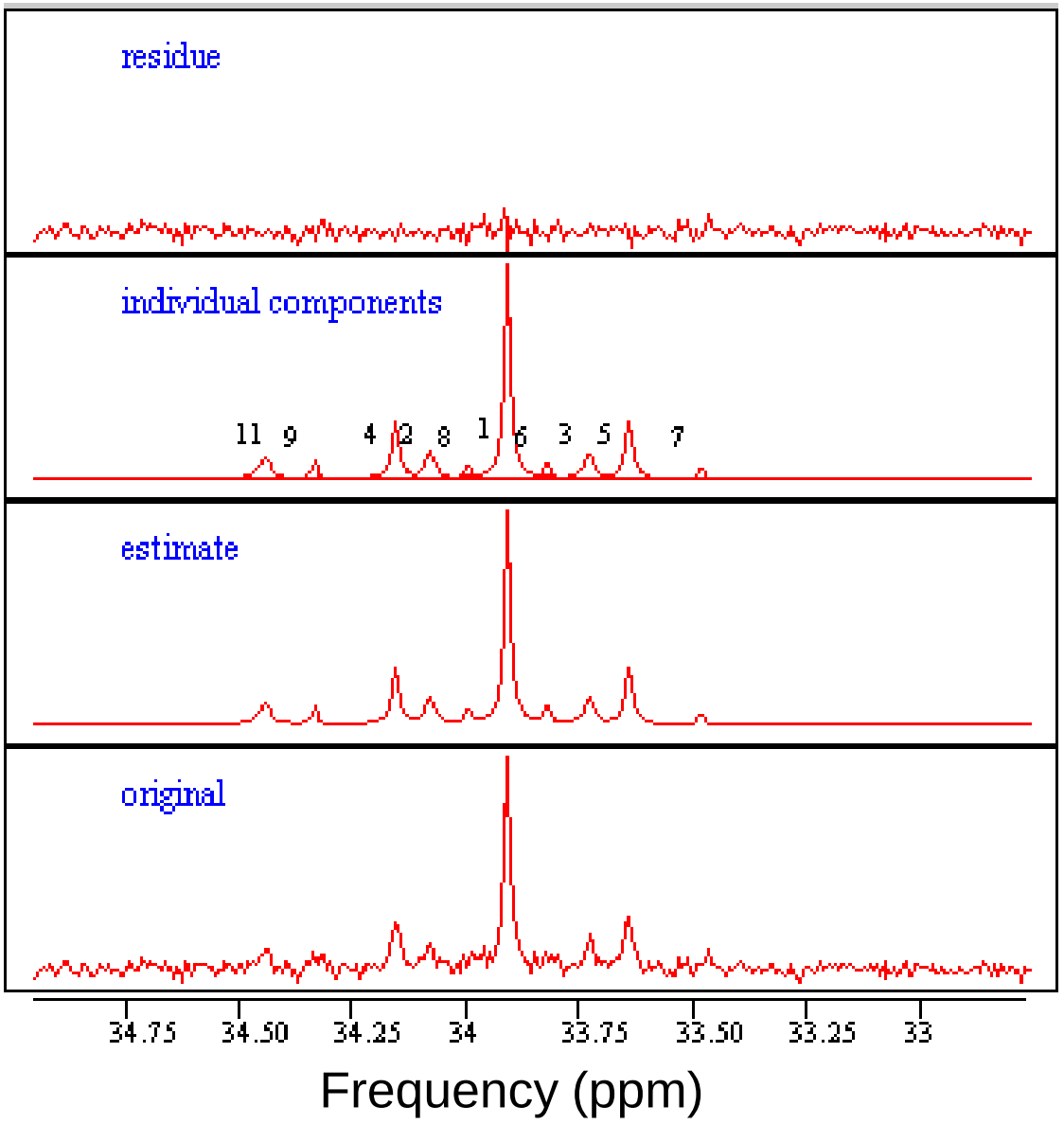
● = ^{13}C-atom



elution time →

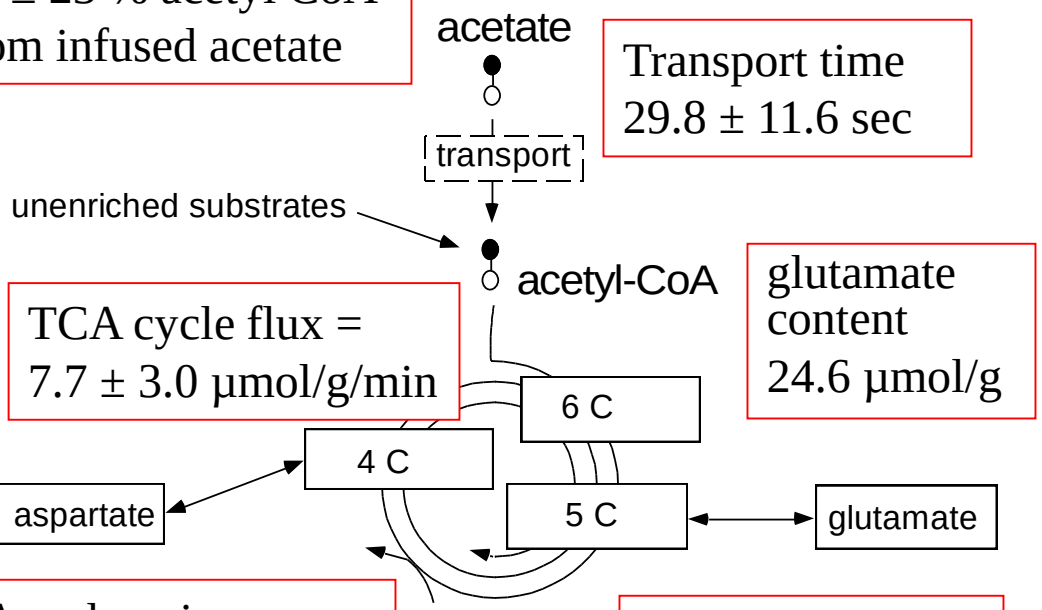
Flux Quantification *in vivo* Animal Experiment

Fit to NMR multiplets of the 4-carbon of glutamate from a biopsy from porcine heart



Flux Quantification *in Vivo* Animal Experiment

58 ± 23 % acetyl CoA
from infused acetate



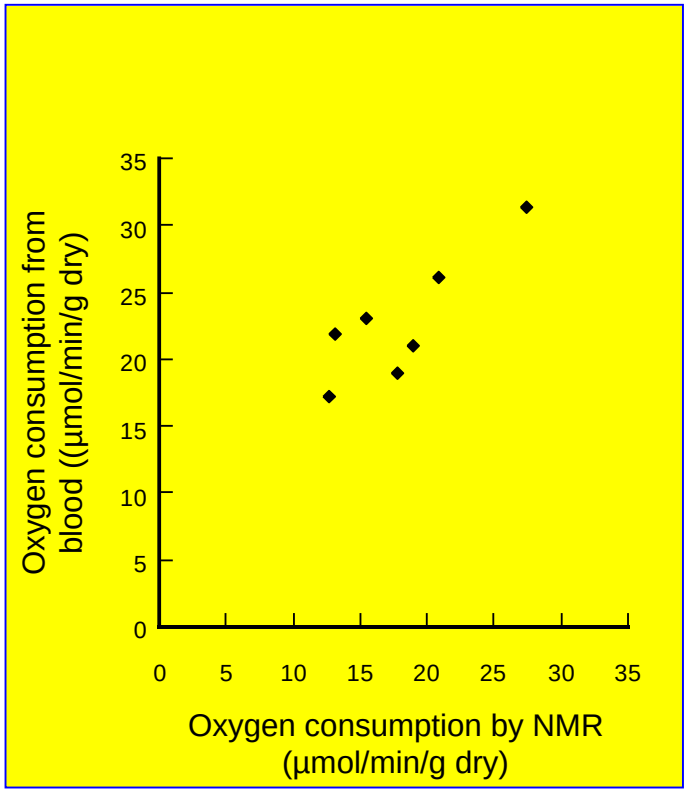
Transport time
29.8 ± 11.6 sec

TCA cycle flux =
7.7 ± 3.0 µmol/g/min

glutamate
content
24.6 µmol/g

Anaplerosis
16 ± 12 %
of TCA cycle flux

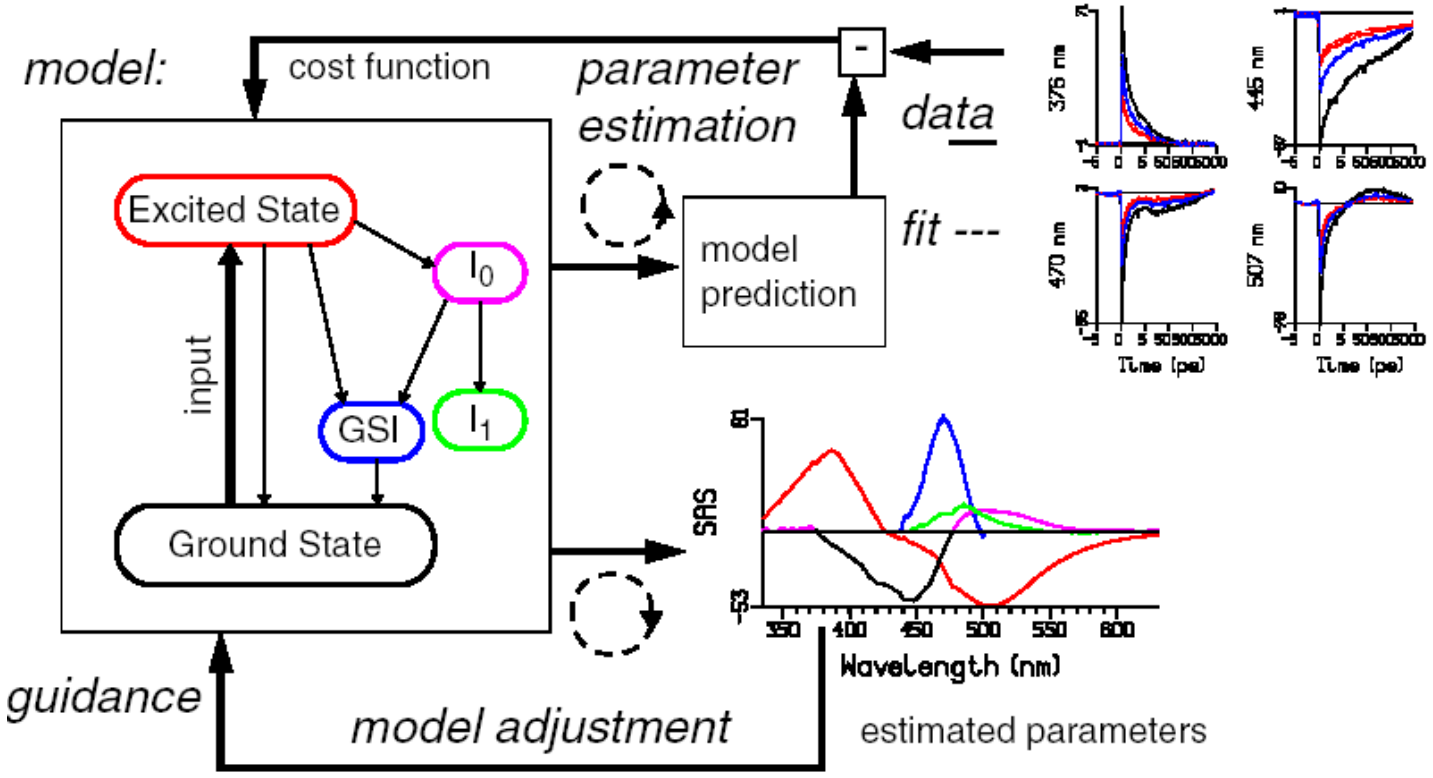
Transamination
17.4 ± 6.0
µmol/g/min



Myocardial Metabolism

Integrated Problem Solving Environment

Integrated PSE (Problem Solving Environment) for metabolic flux experiment analysis



TIMBeta

File Preprocess Weight Fix/Free IRF Compartmental model Spectral constraints Help

TIMBeta ψ

Starting values for decay rates:

Force positive rate constrants? No Yes

Number of iterations: 1

Plot linearly to time: 1

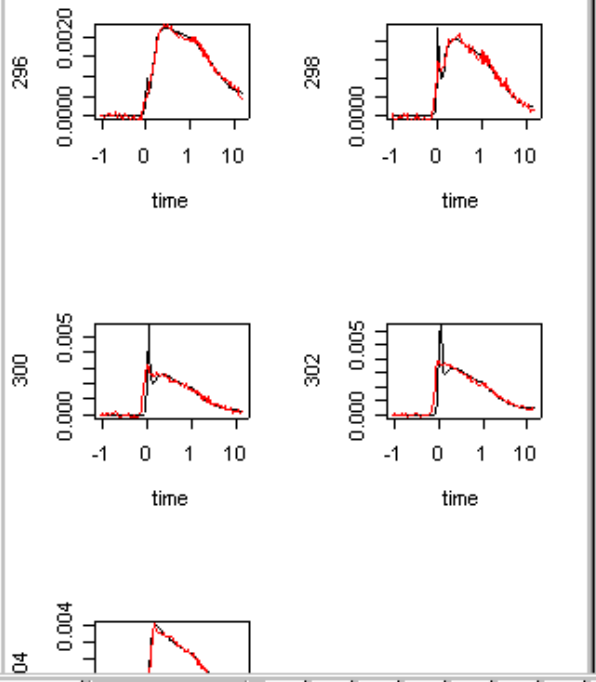
Summarize current dataset

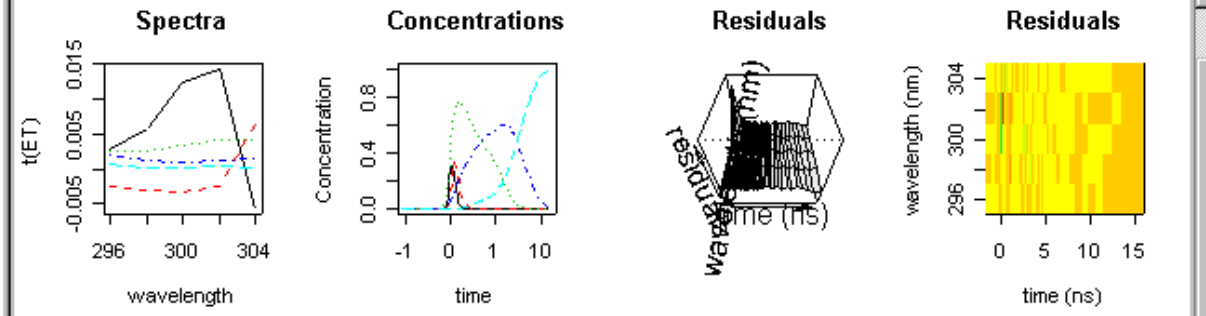
Summarize current model

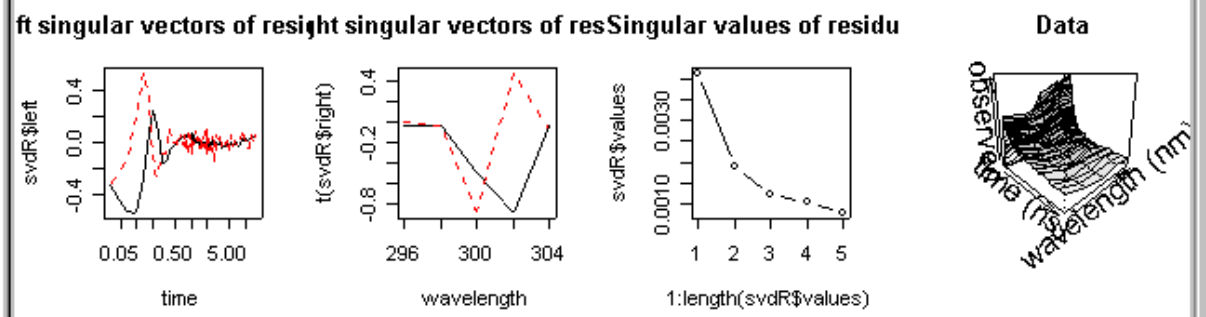
Register values

Fit current model

File History Resize Windows

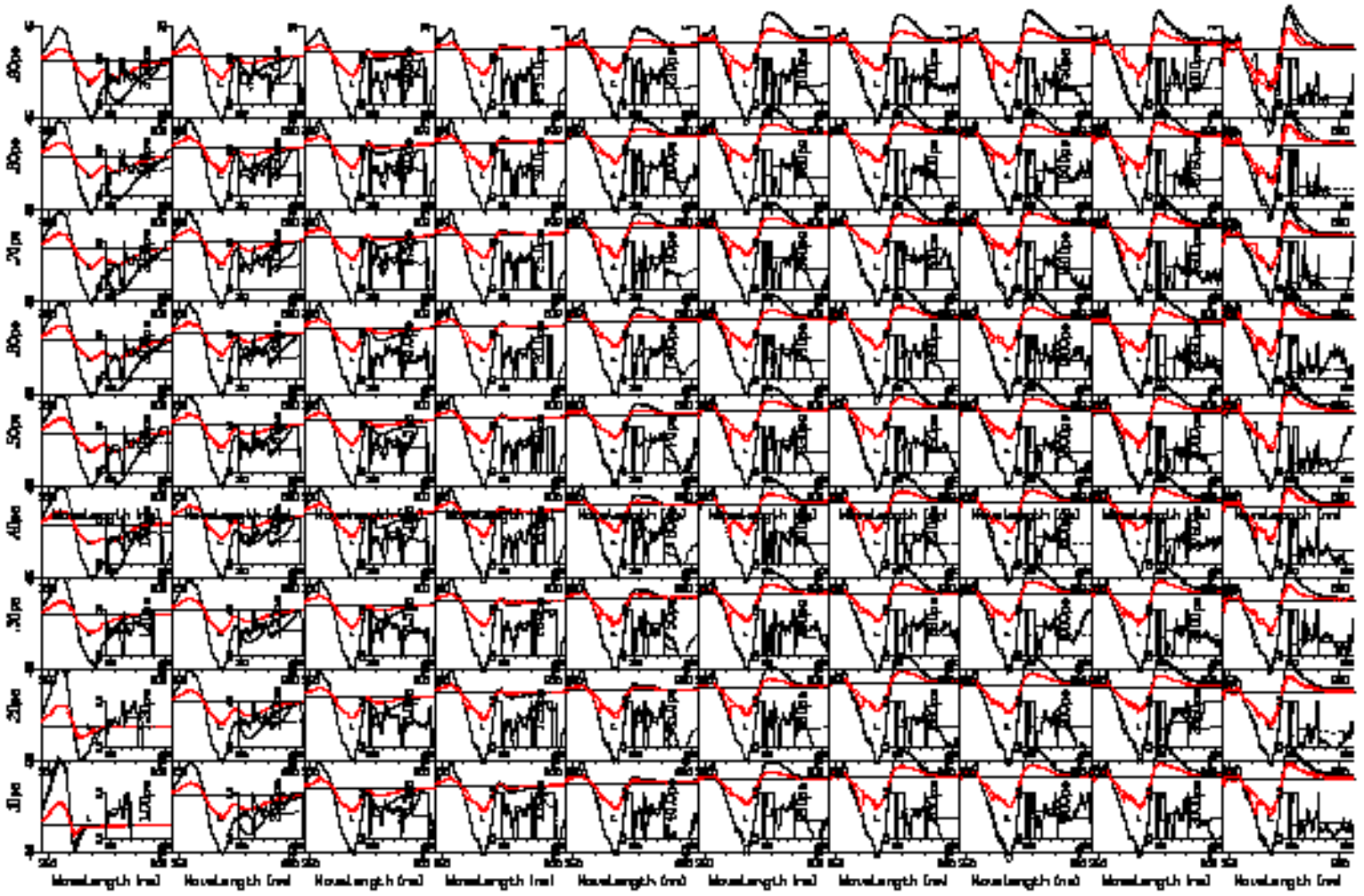






Start 5:46 PM

Large Data Sets Analysed



Summary

Bioinformatics tools and problem solving environments are developed for

- metabolite identification and quantification
- analysis of dynamic experiments and quantification of metabolic fluxes
- expertise in the Netherlands is bundled
- collaboration of bioinformaticians, computer scientists and domain experts