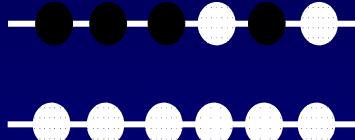


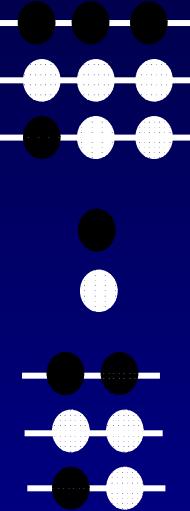
4. Development of experimental and computational tools to evaluate metabolic flux

Labeled Isotopes
e.g. C¹³ glucose

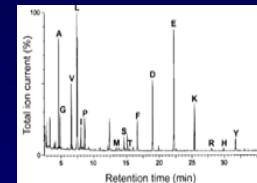


E.Coli cell

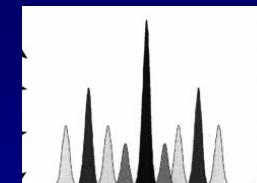
C¹³ destinations



Observations



GC-MS

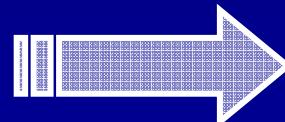


NMR

Publications

- Isotopomer analysis using GC/MS
(Christensen & Neilsen, 1999; Fischer & Sauer, 2003)
- Isotopomer analysis using NMR spectra
(Schmidt *et.al.*, 1999)
- Computational models for flux elucidation
(Zupke *et al.*, 1994; Wiechert & Graff, 1996;
Wiechert *et.al.* 1996; Mollney *et.al.* 1999)
- Optimization algorithms
(Phalakornkule *et.al.*, 2001 ; Ghosh *et.al.*, 2004;
Riascos *et.al.*, 2005, Zamboni *et al.*, 2005)

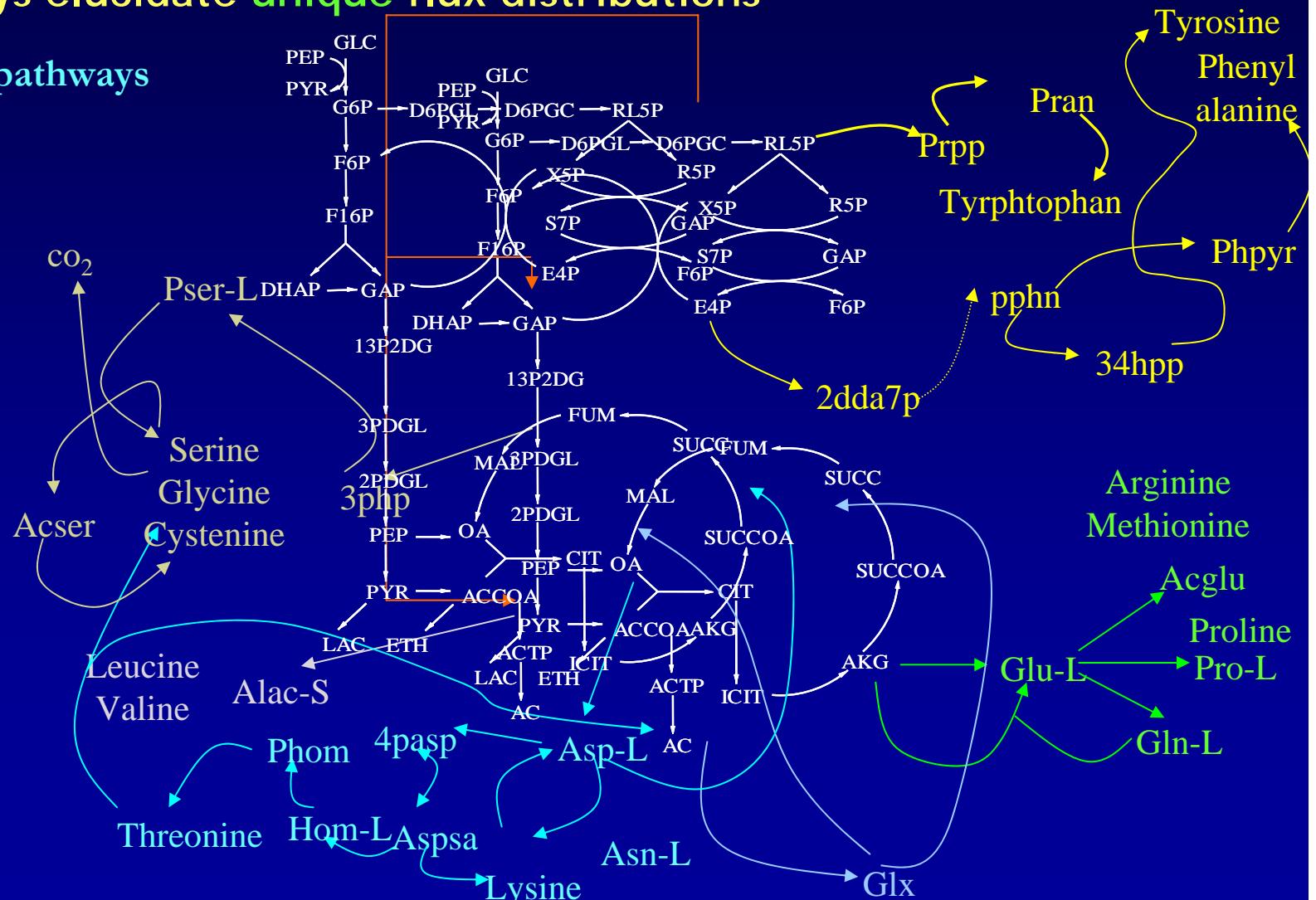
Flux Elucidation



Limitations

- Employed metabolic models are of **limited scope**
(i.e. 30-50 rxns)
- For genome-scale models (~1,000 rxns), measurables do not always elucidate **unique flux distributions**

Many relevant pathways
are absent...



Elucidating Fluxes in Genome-Scale Models using Isotopomer Labeling Experiments (Poster no: 92)

- Enable flux elucidation for genome-scale models

Isotopomer mapping matrices (IMM)

have been constructed
genome-scale models
of *E coli*

(Burgard & Van Dien)

Large-scale non-linear programming problem

Minimize

$$\sum_{i} \sum_{k} (I_{ik} - I_{ik}^{\text{exp}})^2$$

s.t.

$$\sum_{j|S_{ij}>0} S_{ij} v_j \left(\prod_{i|S_{ij}<0} \sum_k IMM_{jiiikk} I_{ik} \right) + \sum_{j|S_{ij}<0} S_{ij} v_j I_{ik} = 0 \quad \forall i, k$$

Isotopomer balance

$$\sum_j S_{ij} v_j = 0 \quad \forall i$$

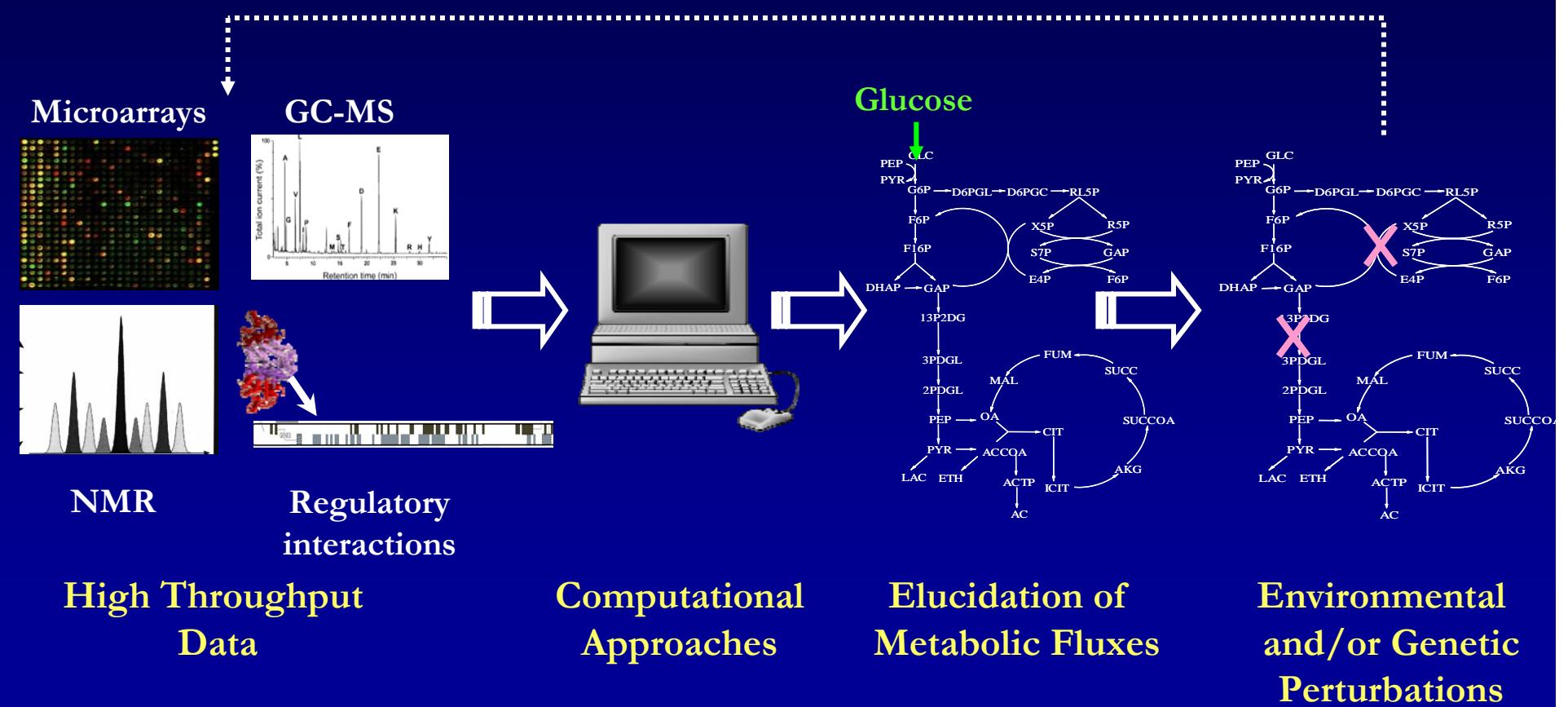
Mass balance

$$\sum_k I_{ik} = 1 \quad \forall i$$

Isotopomer fraction balance

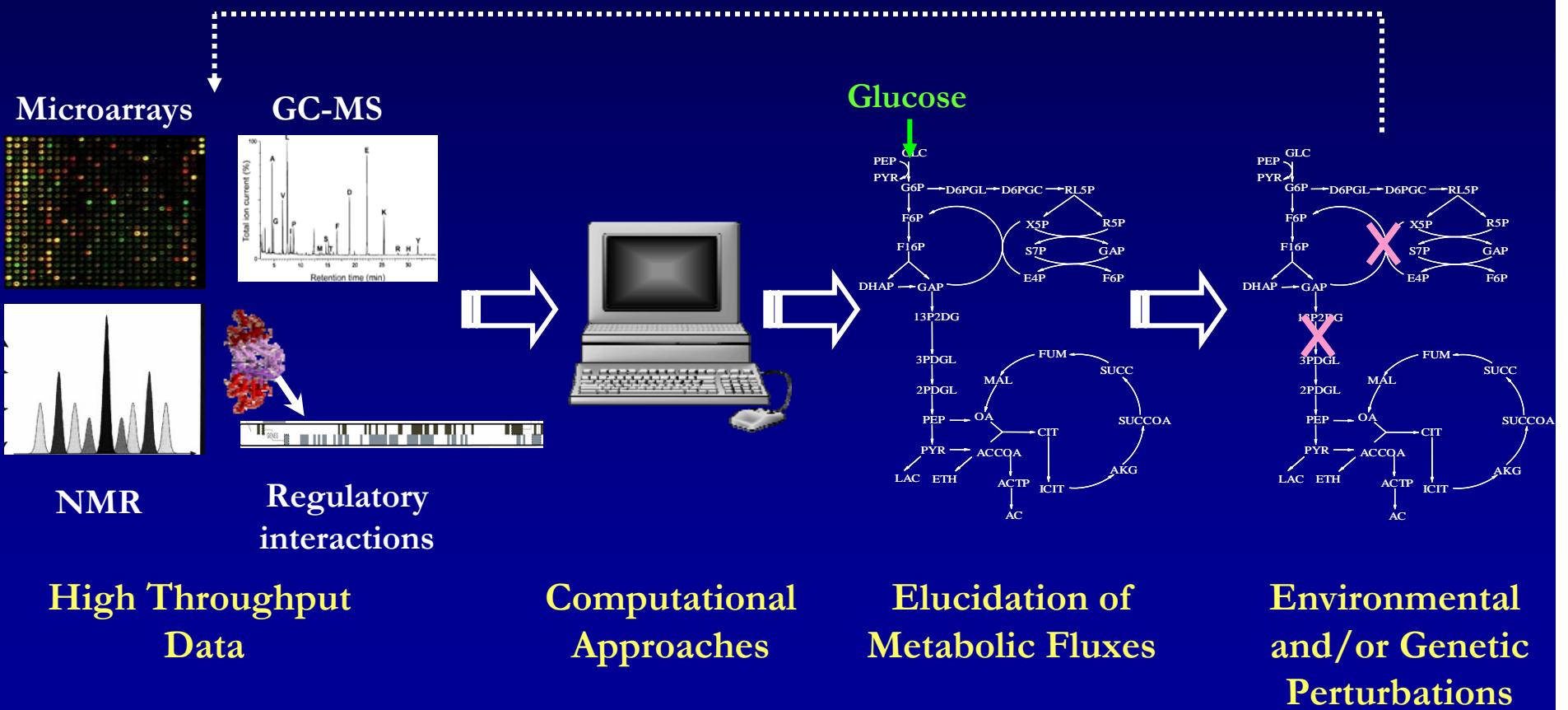
Question 1:

What are the bottlenecks in broadening the use of HT data for the quantitative estimation of metabolic fluxes ?



Question 2:

Given HT data, how can we use computations to reliably elucidate fluxes in metabolic networks ?



Question 3:

How can we anticipate the effect of environmental and/or genetic manipulations on metabolic fluxes ?

