First Steps in Modeling Human Metabolism on a Genomic Scale

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Why Study Human Metabolism?

- Metabolic diseases (obesity, diabetics) are major sources of morbidity and mortality.

- Many common disorders (such as neurodegenerative disorders and cancer) exhibits significant metabolic alterations

- Metabolic enzymes and their regulators are gradually becoming viable drug targets

- In born errors of metabolism cause acute symptoms and even death in early age

- In-vivo studies of tissue-specific metabolic functions are limited in scope

- Because its there..

Previous computational studies of Human Metabolism

- Dynamic modeling of individual pathways
  - Detailed description of the genes, reactions, enzymes
  - No connections between pathways
- Specific cell-types and organelles

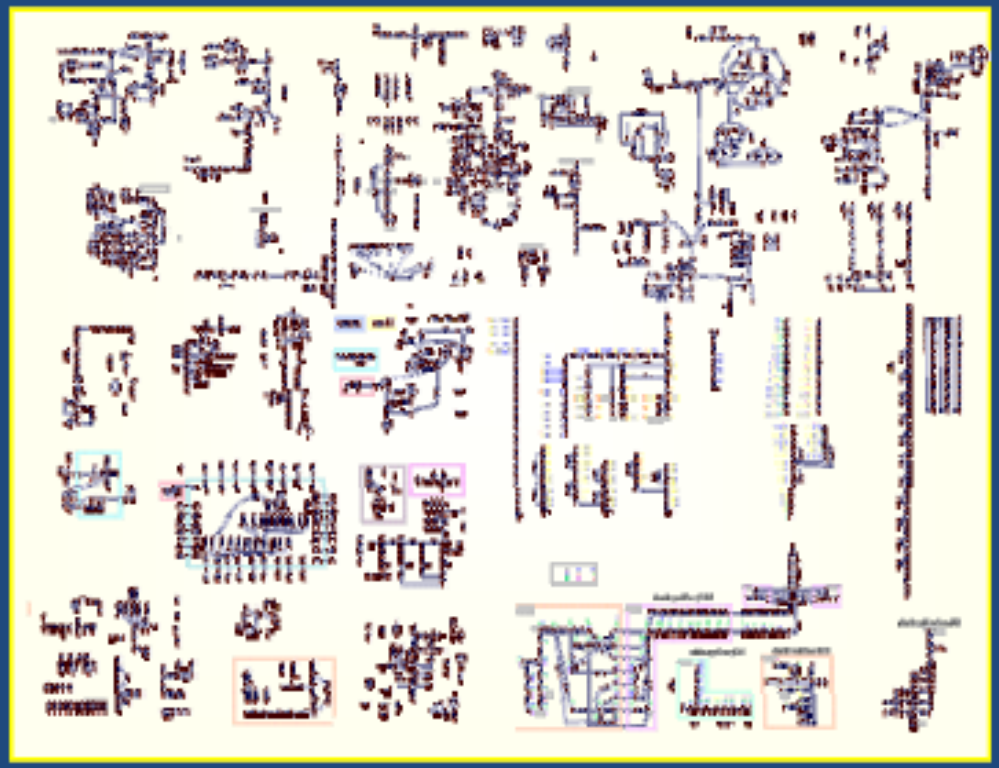
  Red blood cell : Brumen M, Heinrich R, 1984, Schuster R, Holzhütter HG, 1995, Wiback et al. 2002

  Mitochondria: Vo et al. 2004
- It all changed in 2007…

Recon 1: A human metabolic network

Duarte *et al*. PNAS, 104(6):1777-82 (2007)

Global Metabolic Map
Comprehensively represents known reactions in human cells

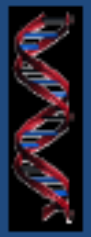


2,712 metabolites

3,311 reactions

7 compartments

1,496 genes total



Genome annotation-based reconstruction

1,134 genes

Gap filling and literature-based reconstruction

362 genes

http://bigg.ucsd.edu

UCSD Bioengineering

Systems Biology Research Group
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Reconstruction Timeline

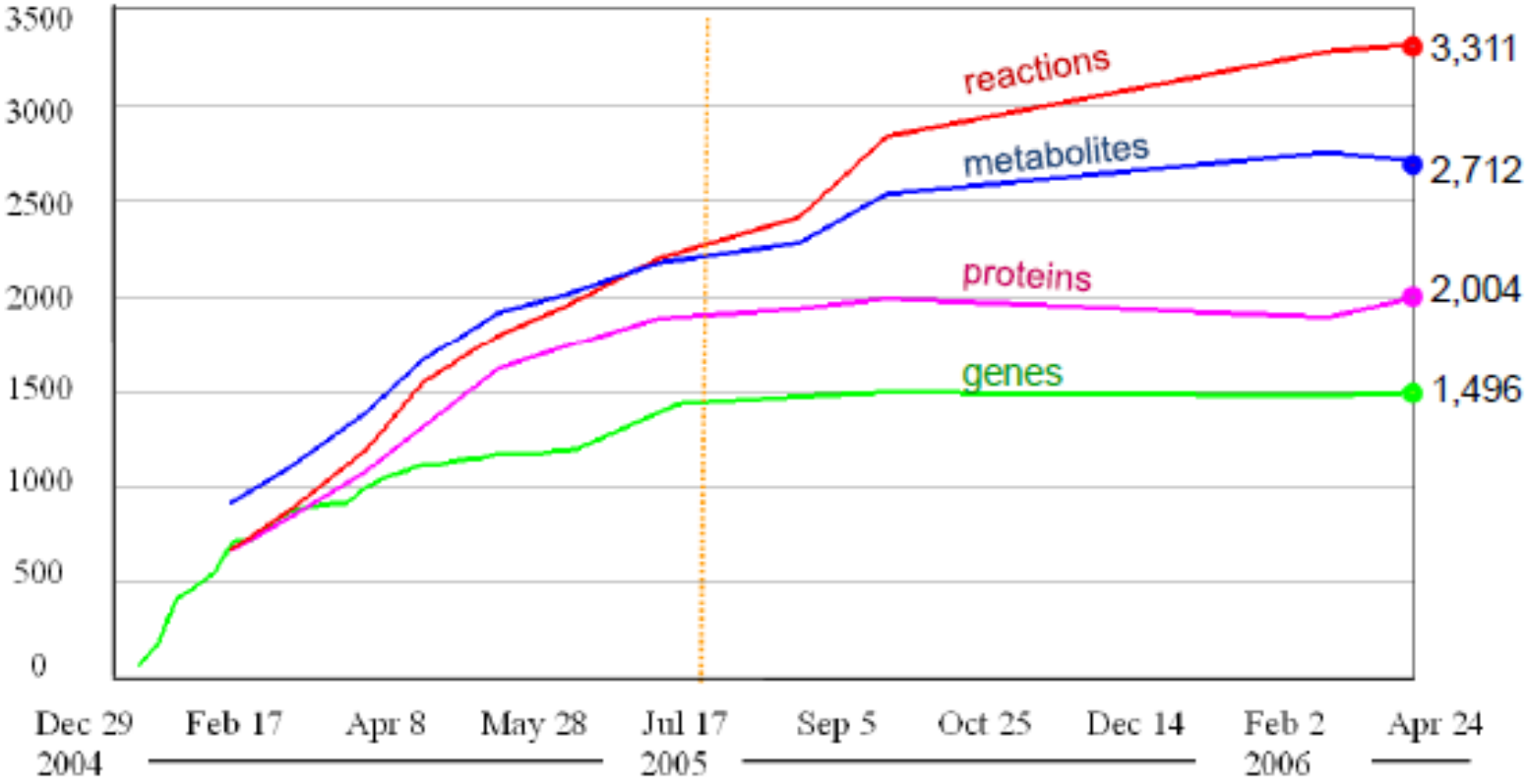
Annotation-based reconstruction

+ 1,134 genes

(- 731 automated mapped genes)

Gap filling and literature-based reconstruction

+ 362 genes



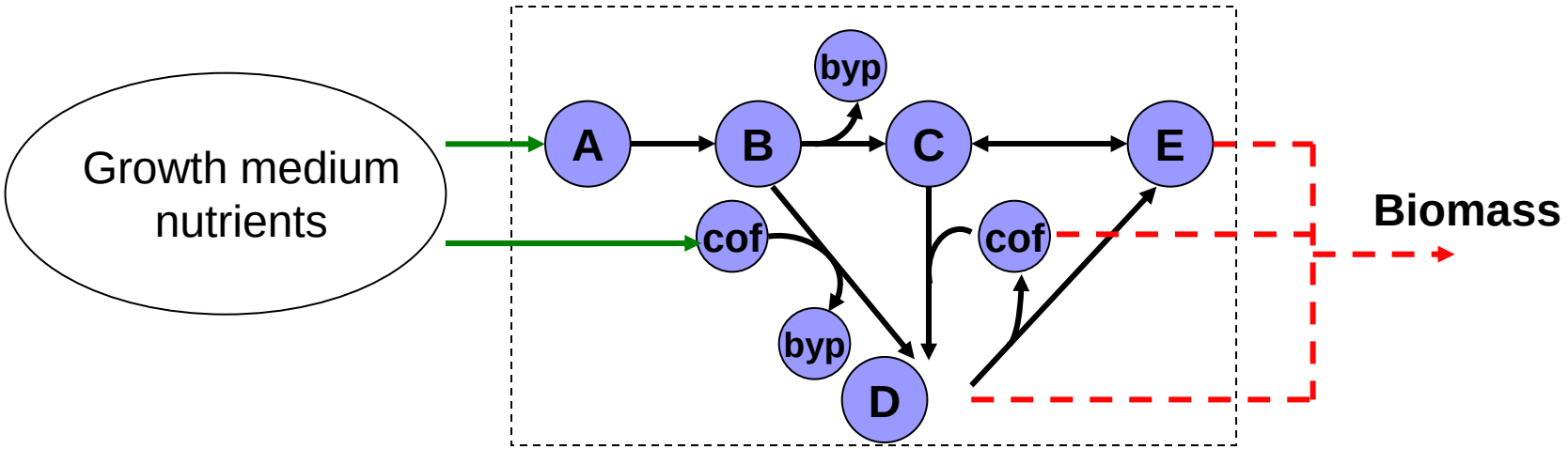
Our work on modeling Human Metabolism – topics covered today:

- 1. A method for *integrating expression data* for generating tissue specific metabolic descriptions
- 2. *Biomarker identification* in Inborn Errors of Metabolism
- 3. **A generic method for the construction of tissue-specific models and its application to *build and test a liver model***

1. Tissue-specific modeling [T. Shlomi, M. Cabili, M. Herggard, B. Palsson, & E. Ruppin; Nat. Biotech. 2008]

- CBM: Predict metabolic reaction rates under steady-state constraints:
  - Mass balance: equal metabolite production and consumption rates
  - Thermodynamic: irreversibility of reactions
  - Enzymatic capacity: bounds on enzyme rates

- **Requires a specification of the *growth media* and (in the FBA-like variants) of an *objective function that should be maximized*, both which are unknown re. specific human tissues!?**



Modeling human tissue metabolism via CBM is hence problematic

•Va
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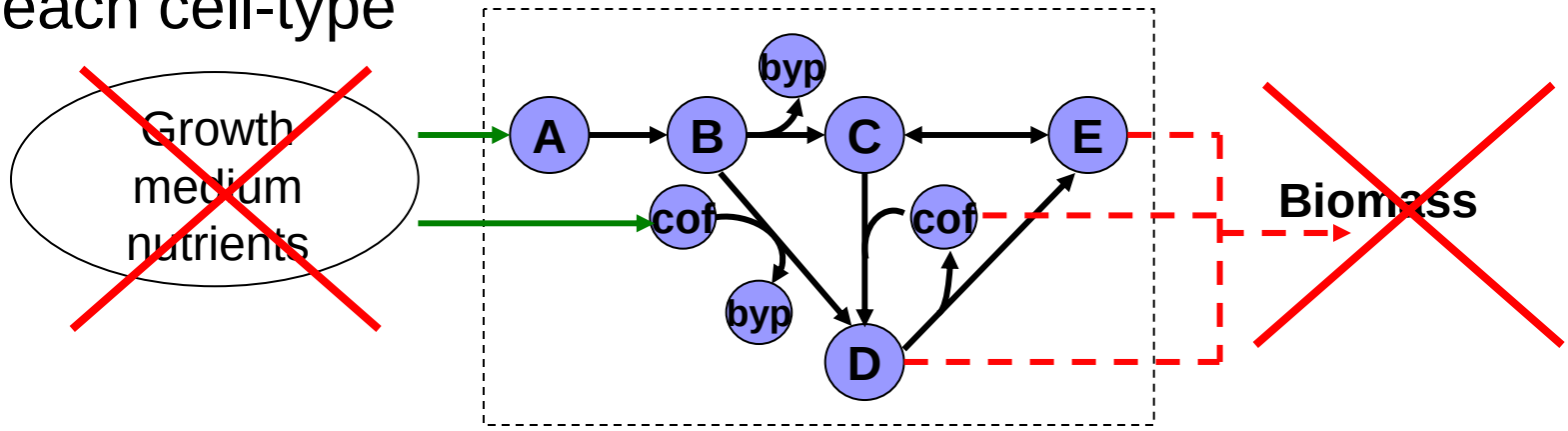
(like biomass maximization for microbial species)

Can we use constraint-based modeling to systematically predict tissue-specific metabolic behavior? ?

•Unknown uptake and secretion reactions of each cell-type



[T. Shlomi, M. Cabili, M. Herggard, B. Palsson, & E. Ruppin; Nat. Biotech. 2008]

- Develop a general approach for predicting tissue-specific metabolic states
- Provide the first large-scale description of the metabolism of various human tissues

- Our solution is based on model integration with tissue-specific gene and protein expression data
- Motivated by the assertion that highly expressed genes are expected to carry metabolic flux and vice versa

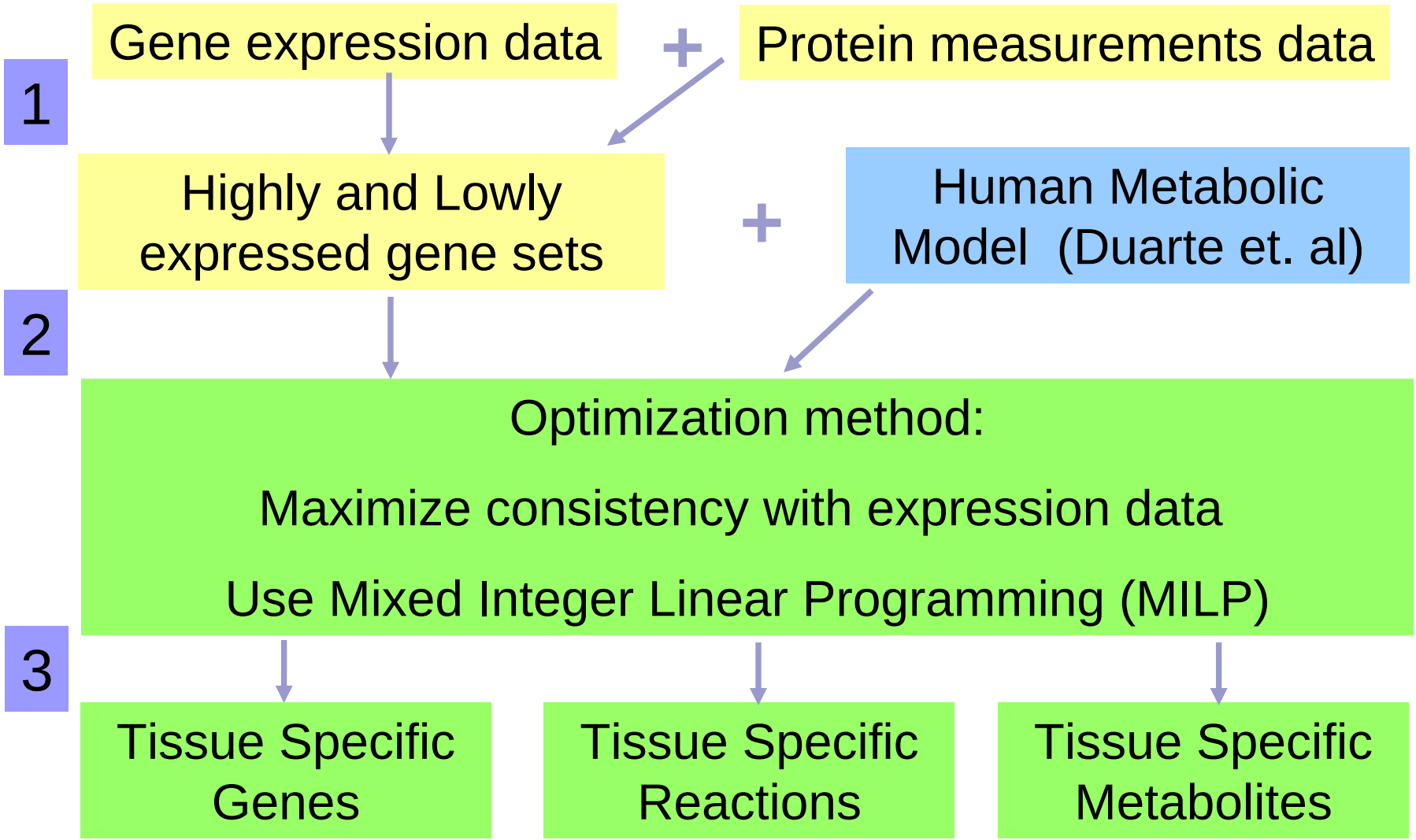
Enzyme expression level vs. metabolic flux level

- Changes in gene expression levels significantly correlate, but not absolutely, with changes in measured and predicted fluxes
  - Schuster, et al, 2002, Famili, et al. 2003, Daran-Lapujade et al. 2004, Bilu, et al. 2006

- Gene expression lead to the characterization of different tissue-specific metabolic functions
  - Levine et al. 2006, Yanai et al.2005, Son et al. 2005

Thus, metabolic reactions can be *transcriptionally (expression) regulated* and/or *post-transcriptionally regulated*

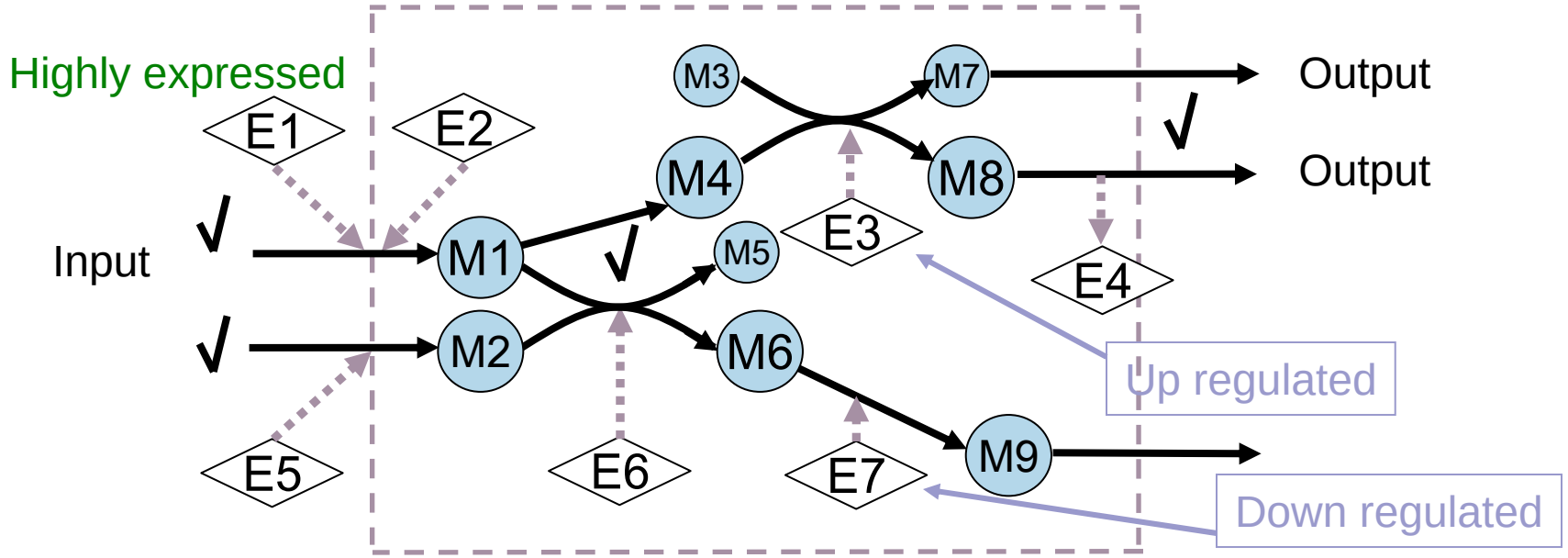
Our Method


Gene expression data
+
Protein measurements data
1
Highly and Lowly expressed gene sets
+
Human Metabolic Model (Duarte et. al)
2
Optimization method:
Maximize consistency with expression data
Use Mixed Integer Linear Programming (MILP)
3
Tissue Specific Genes
Tissue Specific Reactions
Tissue Specific Metabolites

Network Integration with Tissue-Specific Expression Data

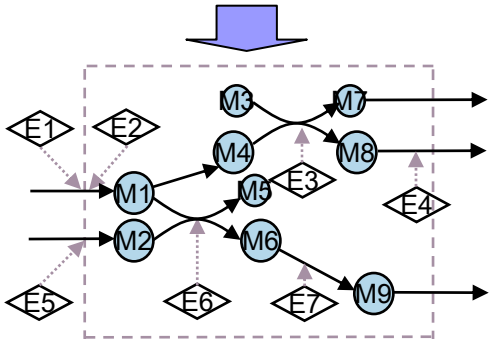
- ***Gene's flux activity states*** -reflect the absence/existence of non-zero flux thro…code
- Comparison …***xpression state*** will teach us on ***post transcription regulation***

4 out of 5 reactions were consistent with the expression state

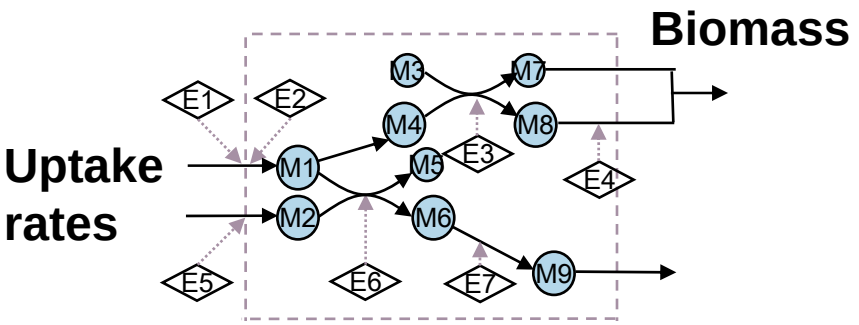


Validating the Method in Predicting Yeast Metabolism

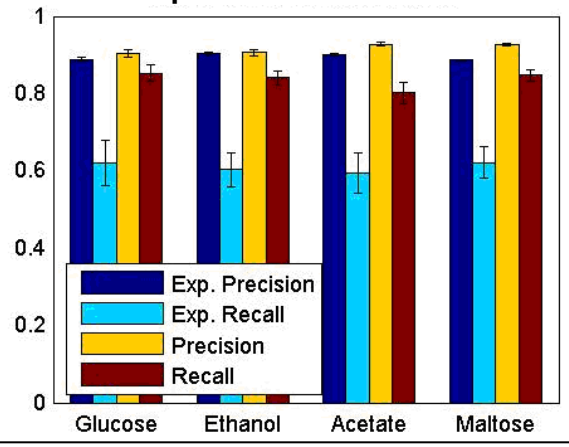
Expression data under various media



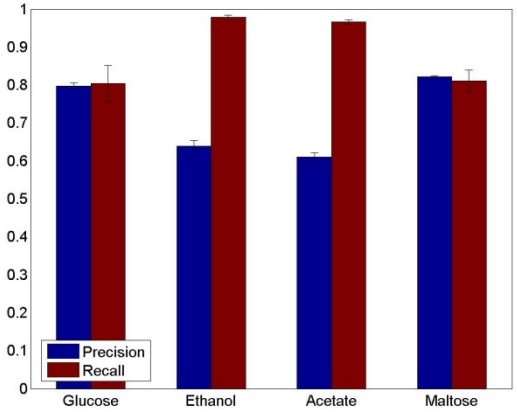
Flux Balance Analysis (FBA) growth maximization



Comparison with FBA



Comparison with measured fluxes



(Daran-Lapujade et al' 04)

Precision 71%
Recall 89%

Applying the Method to the Human model

- Employing the model of Duarte et al.
- Gene and protein expression from GeneNote (Shmueli et al. 2003) and HPRD (Mishra et al. 2006)

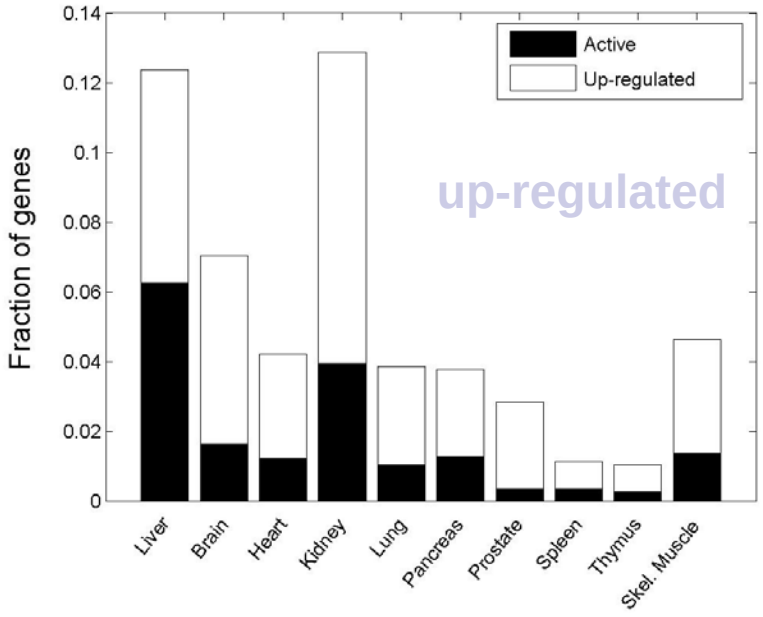
- 10 tissues:

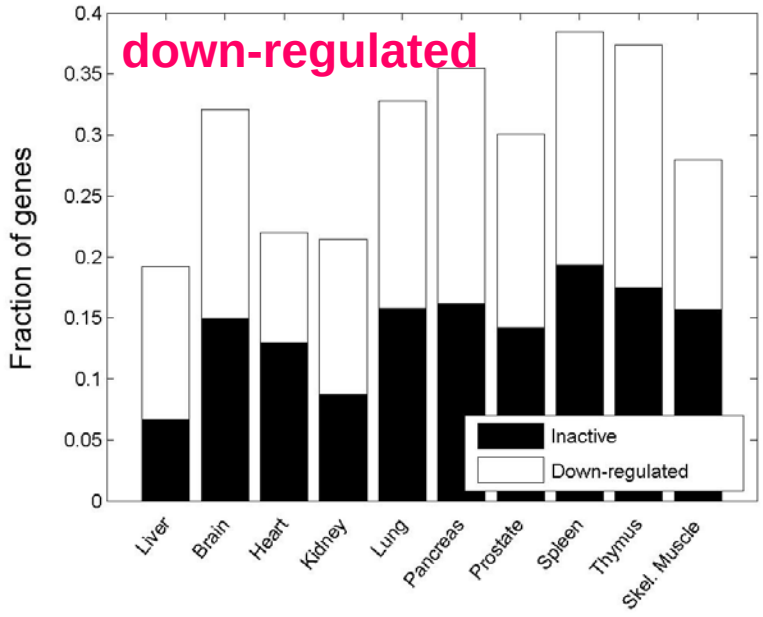
  brain, heart, kidney, liver, lung, pancreas, prostate, spleen, skeletal muscle and thymus.

- The activity state of 644 genes was uniquely determined in at least one tissue, with an average of 408 genes per tissue (our method provides confidence estimates on the predicted gene activities).

Post-transcriptional Regulation of Metabolic Genes

- 20% of the metabolic genes are predicted to be post-transcriptionally regulated across tissues
- average of **42 (3.6%)** genes post-transcriptionally **up-regulated** and **180 (15.4%)** post-transcriptionally **down-regulated** in each tissue





Large Scale Validation

Large-Scale Mining of Tissue-Specificity Data

- Tissue-specificity of genes, reactions, and metabolites is significantly correlated with all data sources

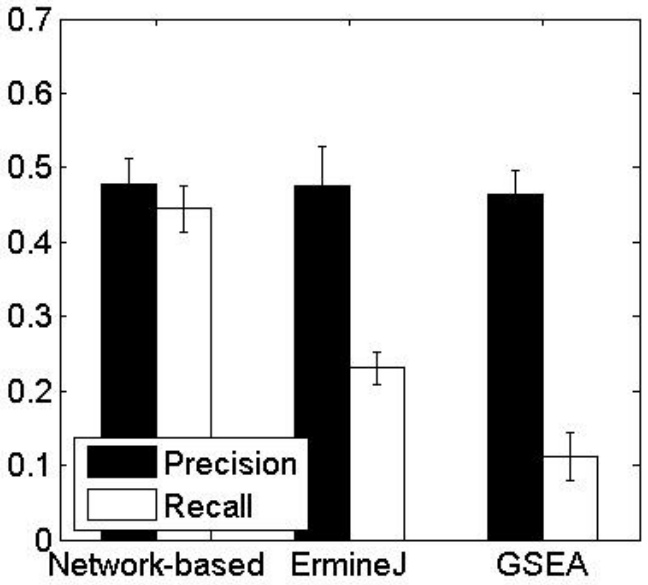
- Tissue specificity of post-transcriptional **up regulated** elements is **significantly high**

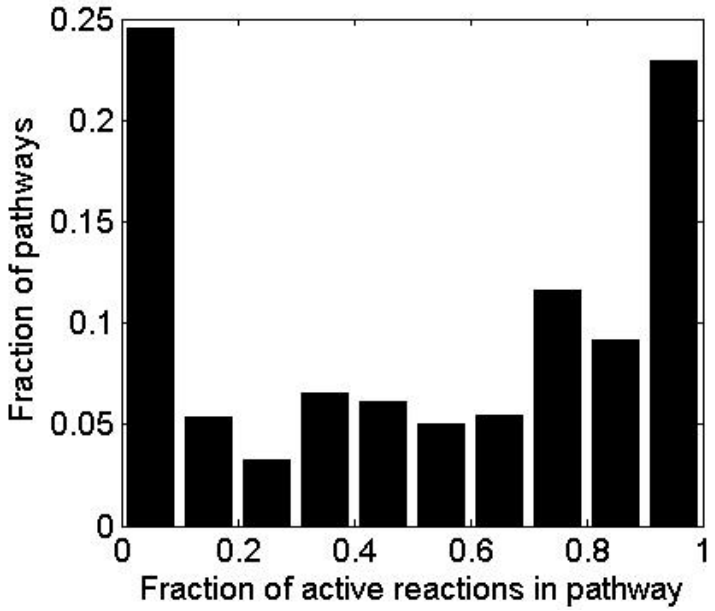
- Tissue specificity of post-transcriptional **down regulated** elements is **significantly low**

Category	Validation data source	Global accuracy			Up regulation accuracy			Down regulation accuracy		
		Pre.	Rec.	*p*-value	Pre.	Rec.	*p*-value	Pre.	Rec.	*p*-value
All genes	WEB	0.37	0.37	$2.6 \cdot 10^{-9}$	0.33	0.18	$1.6 \cdot 10^{-8}$	0.82	0.24	$2.2 \cdot 10^{-3}$
Disease genes	OMIM[37]	0.49	0.55	$<10^{-300}$	0.47	0.50	$<10^{-300}$	0.85	0.22	$3.6 \cdot 10^{-4}$
Transporter Genes	HMTD[34], TCDB[35]	0.57	0.41	$6.1 \cdot 10^{-7}$	0.64	0.21	0.06	0.8	0.25	0.03
Enzymatic Reactions	BRENDA[31]	0.7	0.42	$4. \cdot 10^{-12}$	0.55	0.21	$2.6 \cdot 10^{-12}$	0.68	0.25	$3.7 \cdot 10^{-25}$
All metabolites	HMDB[30]	0.36	0.47	$<10^{-300}$	0.32	0.33	$7.4 \cdot 10^{-8}$	0.81	0.21	$4 \cdot 2^{-7}$
Exchange metabolites	HMDB[30]	0.36	0.38	$3.2 \cdot 10^{-3}$	0.33	0.25	0.06	0.8	0.2	0.01

Comparison with Pathway Enrichment-Based Methods

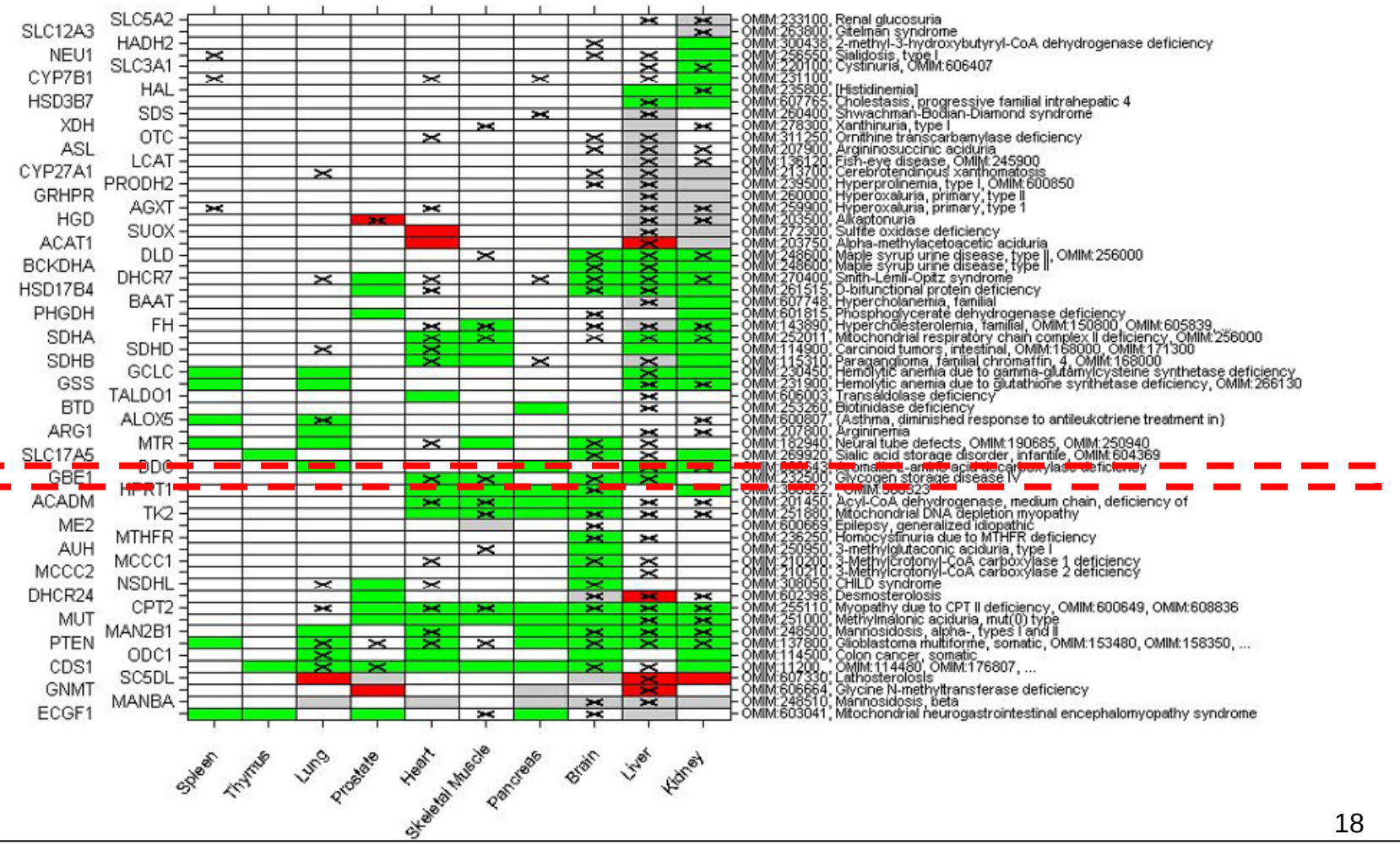
- Gene activity can also be predicted based on the membership of a gene in a pathway whose other genes are highly expressed
- State-of-the-art pathway enrichment methods (ErmineJ Lee et al. 2005
- and GSEA Subramanian et al. 2005) provide lower recall
- **The classical partition of the network to metabolic pathways is problematic as many of the pathways are only partially activated across tissues**





Metabolic Disease-Causing Genes

- Many disease genes (OMIM) are predicted to be post-transcriptional up-regulated specifically in tissues affected by the disease



iMET – Integrative Metabolic Expression Tool (Hadas Tzur, N. Yehudai, E.R, Tomer Shlomi)

- A web server for integrating context-specific expression data with a metabolic network model:

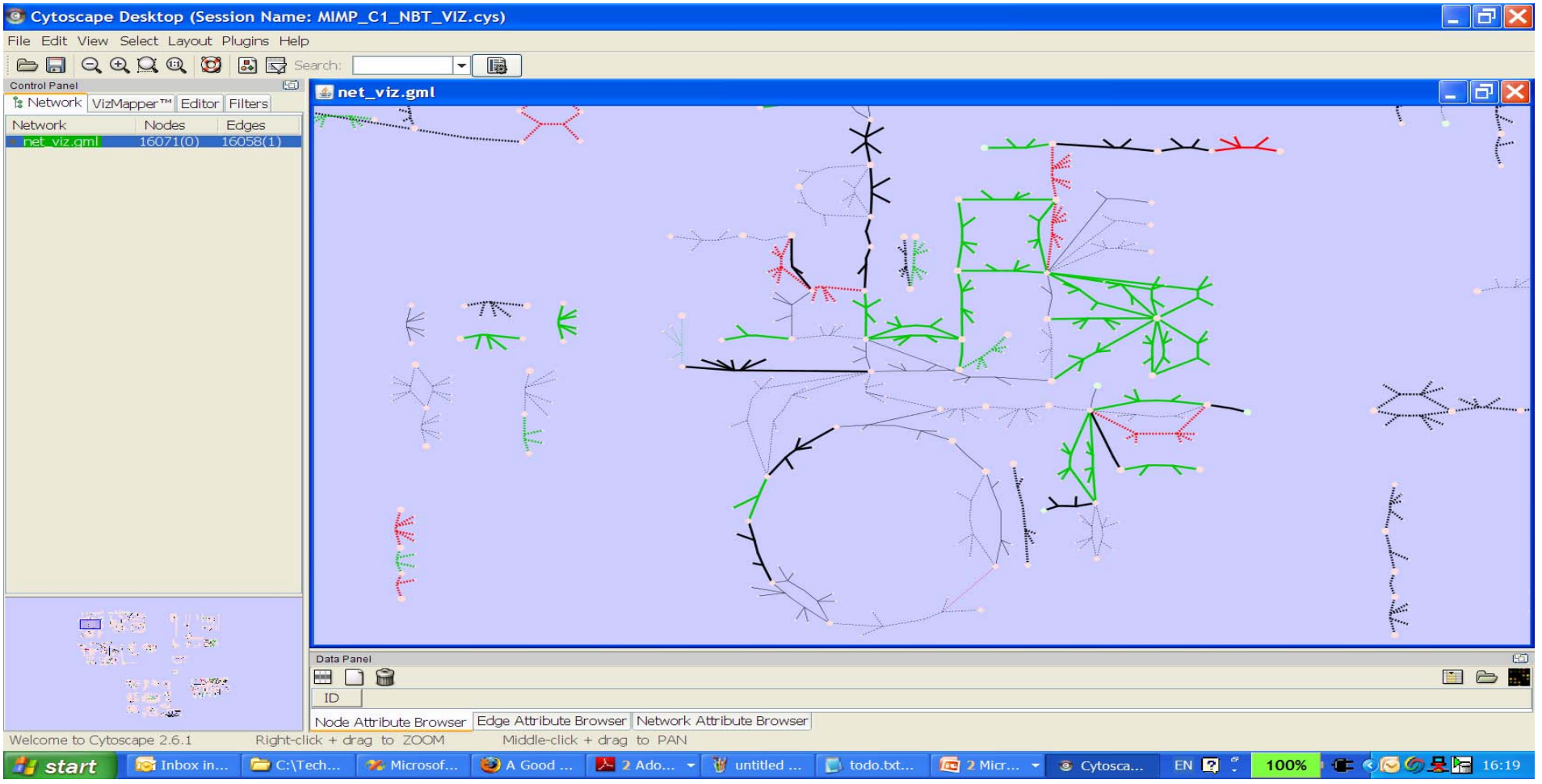
  http://www.cs.technion.ac.il/~tomersh/tools

- Supports integration with the human network as well as various microbes (***S****. cerevisiae, E. coli* ,etc)

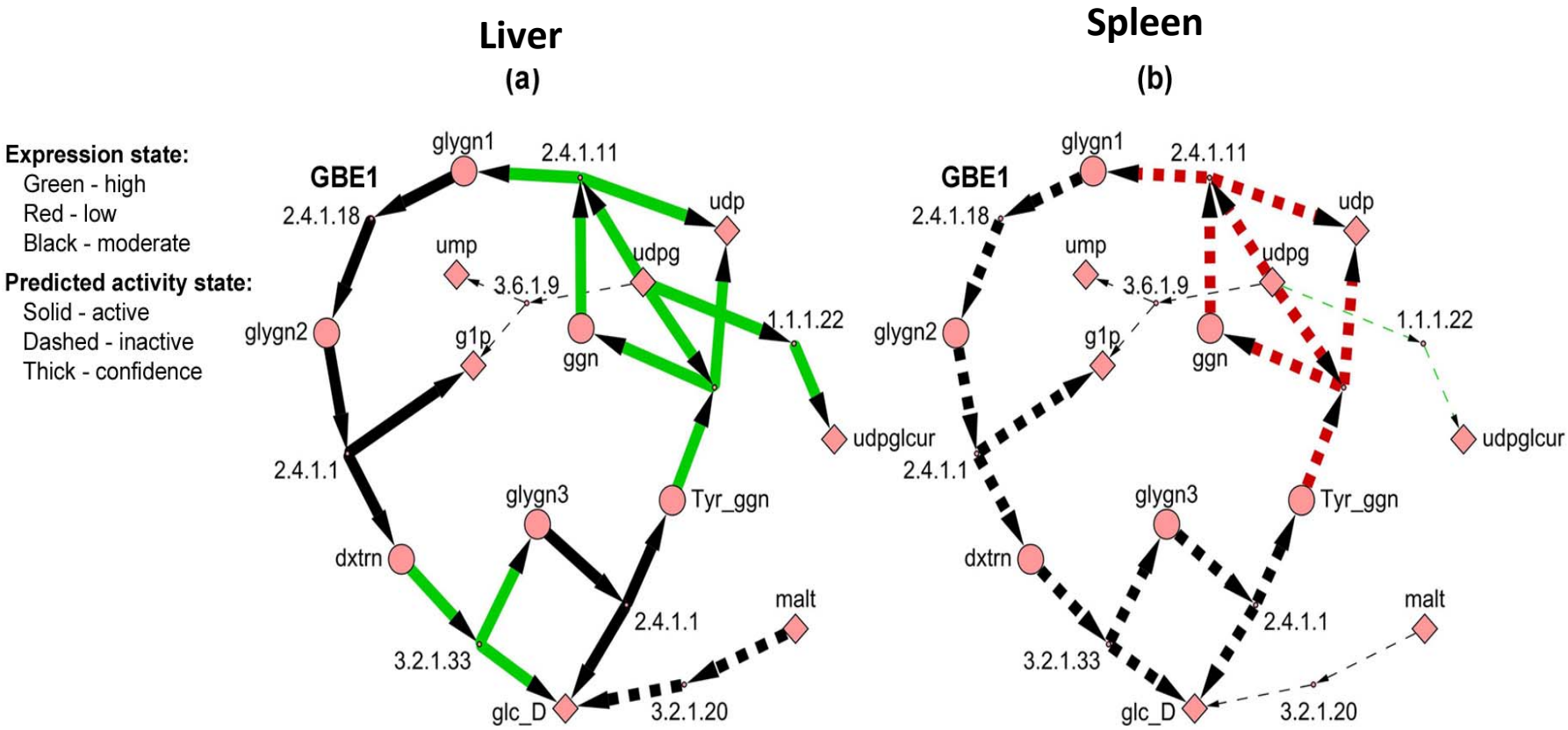
- Input: Expression levels of metabolic genes
- Output: Network visualization (Cytoscape) of predicted flux activity

iMET
Visualizing Metabolic flux prediction

Output: Network visualization (Cytoscape) of predicted flux activity



iMET
What can iMET do for you?



GBE1 (glycogen storage disease) predicted to be post-transcriptionally up-regulated in the liver

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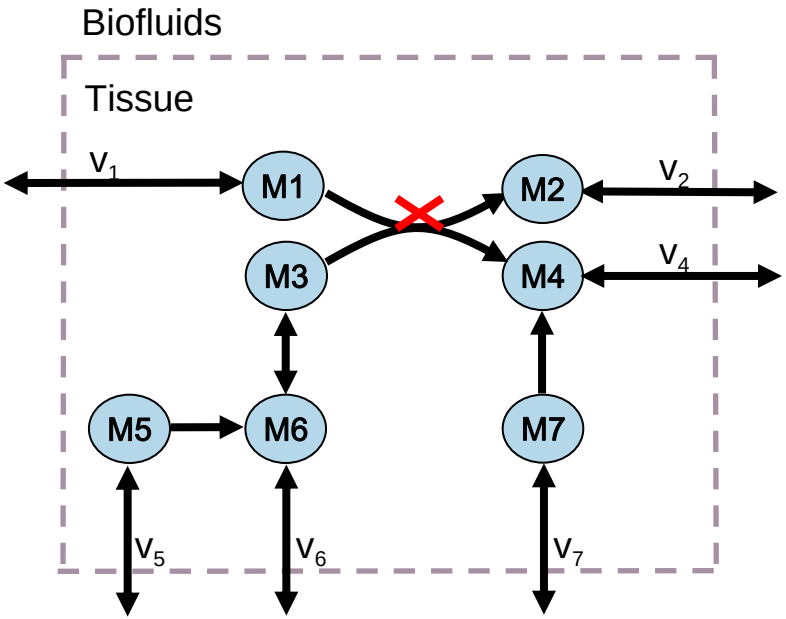
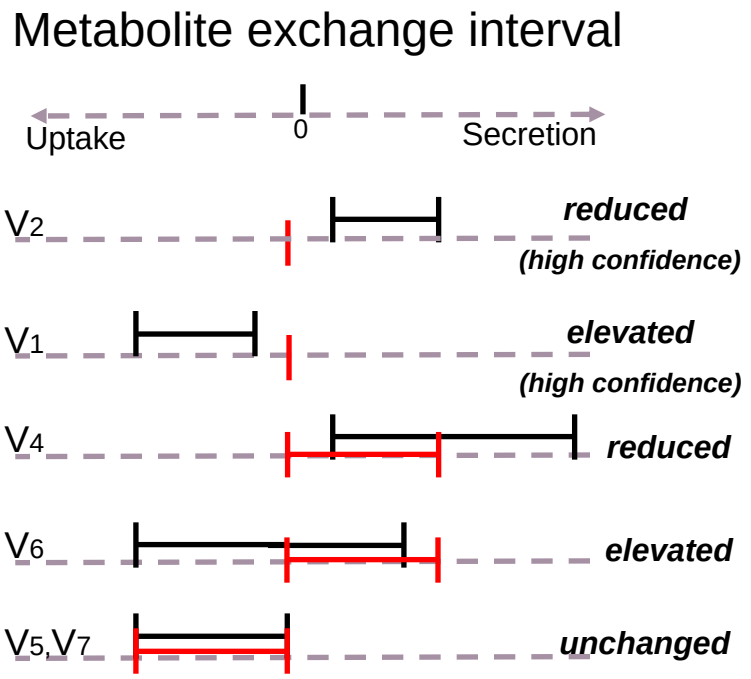
2. A method for predicting metabolic biomarkers (T. Shlomi, M. Cabili & E. Ruppin, MSB 2009)

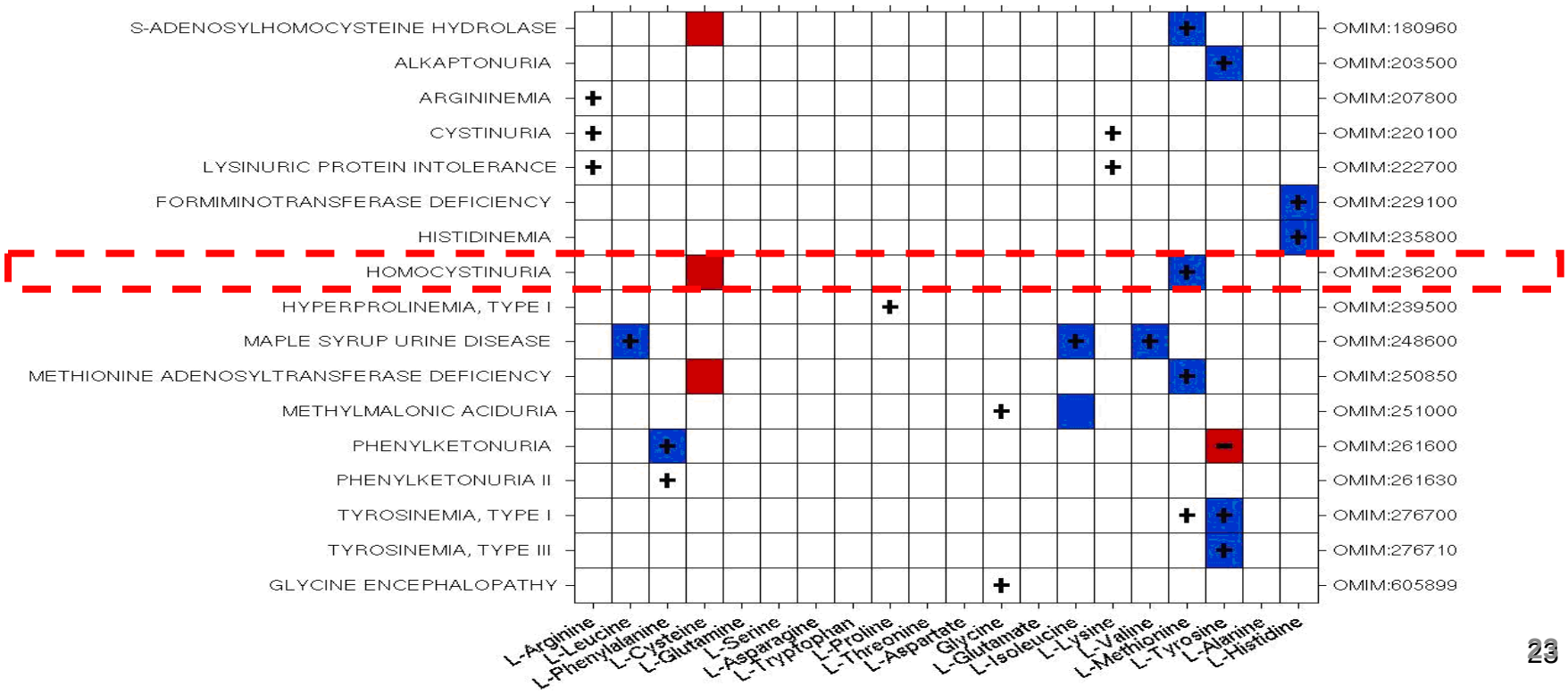
- In-born errors of metabolism are commonly diagnosed via biofluid metabolomics, identifying metabolites with altered concentrations
- Perform systematic biomarker prediction for all known genetic metabolic disorders via a genome-scale model





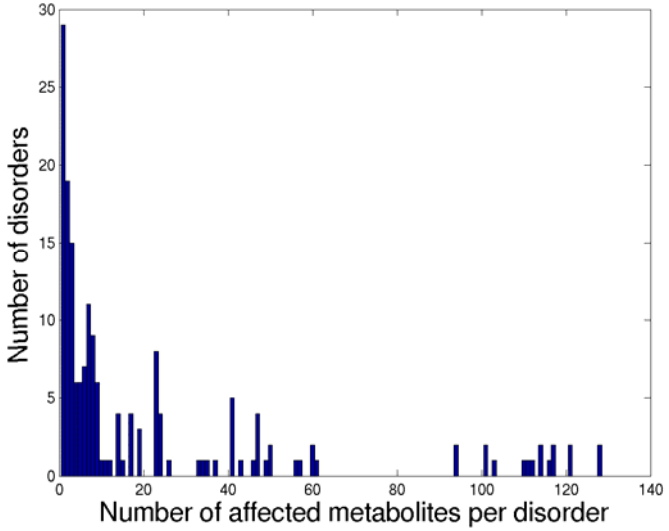
Validating predicted biomarkers

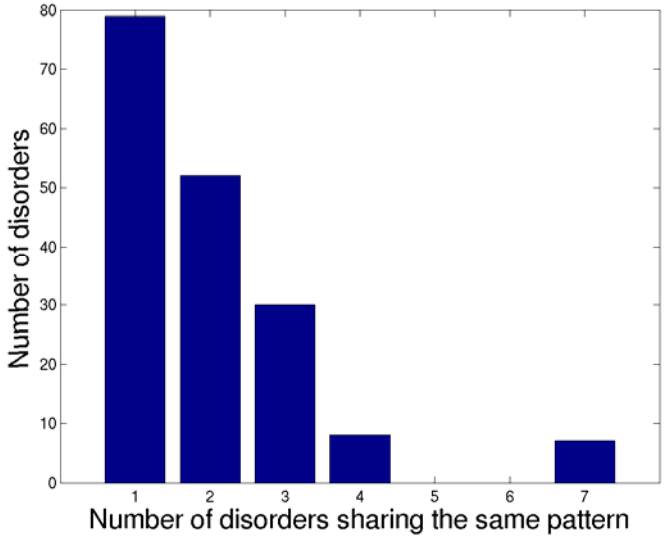
- Extracting data on known biomarkers for in-born errors of amino-acid metabolism
- The predictions are significantly correlated with the known biomarkers (*p*-value=4·10-13) – precision = 0.76, recall = 0.56



Predicting biomarkers for an array of in-born errors of metabolism

- The concentration of 223 metabolites is predicted to change as a result of 176 possible dysfunctional enzymes
- A high fraction of the disorders (42%) are predicted to have very few biomarker changes (less than 6)
- Many of the disorders (45%) have a unique set of biomarker alterations - these predictions may be used for the unique diagnosis of metabolic disorders via biofluids metabolomics





Interim Summary (1,2)

- A general computational method for predicting metabolic behavior based on gene expression data
  - Does not require an objective function definition
  - Does not require data on metabolite uptake rates
- Characterize tissue-specific metabolic behavior of 10 human tissues, showing the significant role of post-transcriptional regulation.
- Predict tissue-specificity of disease genes
- Predict tissue-specific metabolite exchange with biofluids
- Predict metabolic biomarkers (changes in metabolite concentrations) for known inborn errors of metabolism

All the credit is due to our Met-Lab members:

- Tomer Benyamini, Ori Folger, Livnat Jerby, Adi Shabi, Hadas Tzur, Naor Yehudai, Keren Yizchak, Raphy Zarecky.
- To my close collaborator and friend, Tomer Shlomi.

Thank you!