Metabolic flux prediction via gene expression and metabolomics

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October, 2009

Metabolic stuff we've been doing recently

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Outline:

- Context-dependent Biomass Flux Balance Analysis (CB-FBA)
  - Predicting flux distributions by accounting for growth-associated demand for biomass production in a context-dependent manner
- RobustKnock: Predicting Metabolic Engineering Knockout Strategies for Chemical Production
  - Improving OptKnock by accounting for alternative pathways
- Predicting Metabolic Gene-Nutrient Interactions (GNIs) in yeast
  - Predicting constraints on nutrient availability in the growth media based on enzyme essentiality data
- Predicting Enzyme Sub-cellular Localization
  - Predicting enzymes' sub-cellular localization based on partial localization data for a subset of the enzymes in the network

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Metabolic Flux Balance Analysis with Context-dependant Biomass.
T. Benyamini, O. Folger, E. Ruppin, T. Shlomi,
RECOMB, Systems Biology, 2009 (to appear)

NOT PUBLISHED YET

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- Predicting Metabolic Engineering Knockout Strategies for Chemical Production: Accounting for Competing Pathways.
- N. Tepper, T. Shlomi (Submitted)

Predicting Metabolic Engineering Knockout Strategies for Chemical Production: Accounting for Competing Pathways.
N. Tepper, T. Shlomi (Submitted)

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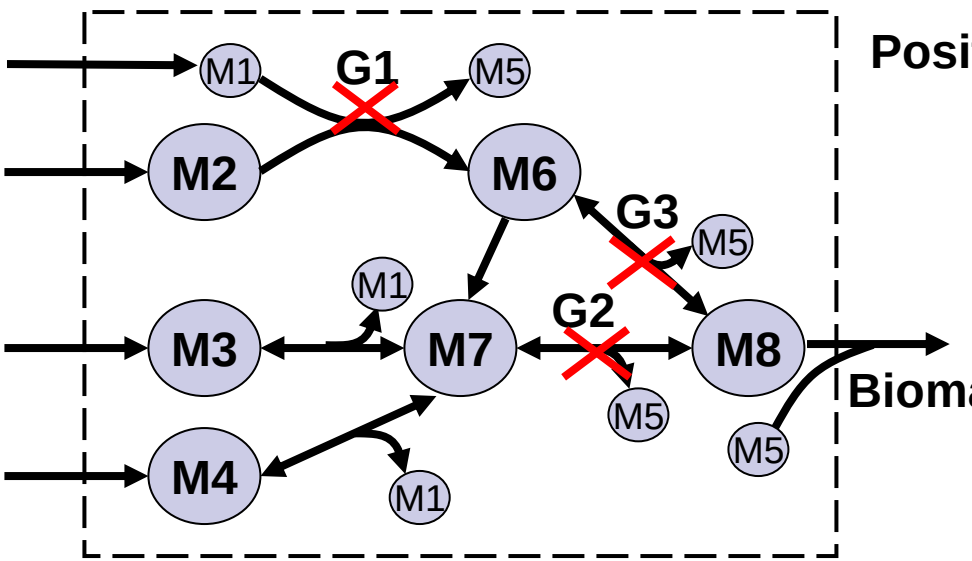
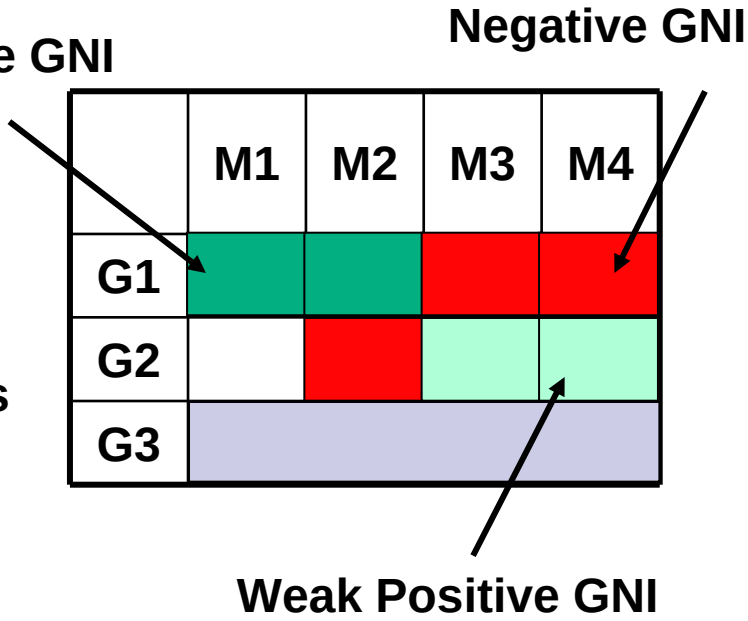
Metabolic Network-based Analysis of Yeast Gene-Nutrient Interactions.
I. Diamant, Y. Eldar, O. Rokhlenko, E. Ruppin, T. Shlomi.
Molecular BioSystems, DOI: 10.1039, 2009

Gene-Nutrient Interactions

- Under which growth media G1 is essential?

- A GNI represents a constraint on the presence/absence of a nutrient in the growth media under which a gene is essential
- A weak (vs. strong) GNI reflects a non-strict constraint





Diamant, et al., Molecular Biosystems, 2009

Predicting Gene-Nutrient Interactions

- Identified via a bi-level optimization problem
- Transformed into Mixed-Integer Linear Programming (MILP)

Maximize gene essentiality (wild-type vs. knockout growth-rate)
(over all growth media)

subject to

Maximize wild-type growth rate
(over fluxes)

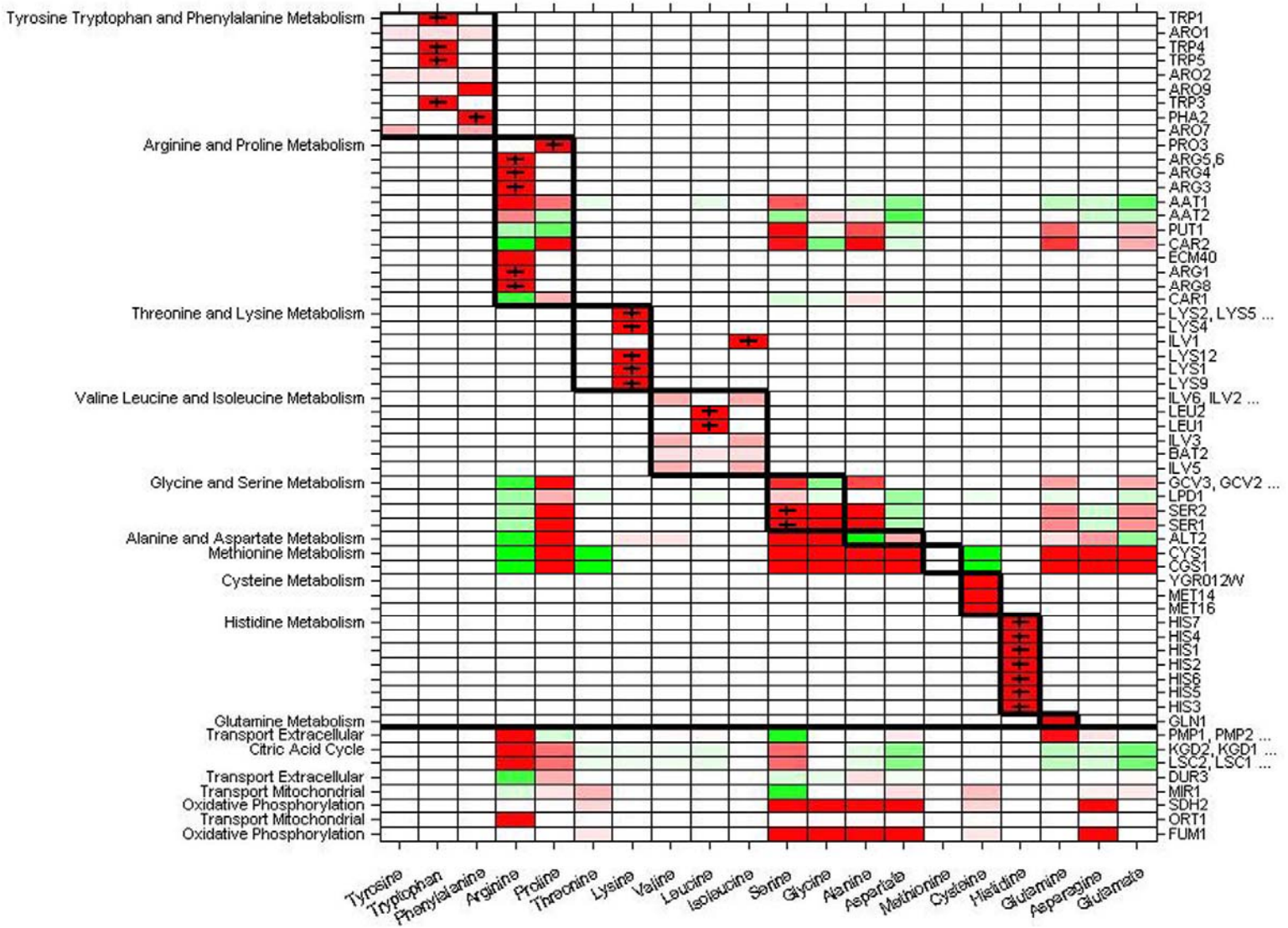
subject to
- uptake of media substrates
- network stoichiometry, thermodynamics and capacity constraints

Maximize knockout growth rate
(over fluxes)

subject to
- uptake of media substrates
- network stoichiometry, thermodynamics and capacity constraints
- Inactive knocked-out reactions

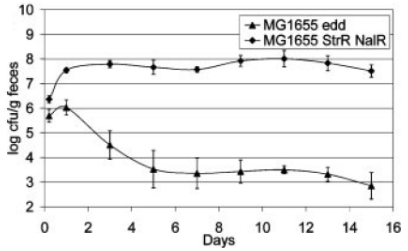
Diamant, et al., Molecular Biosystems, 2009

Gene-Nutrient Interactions in Yeast



GNI-based ‘Reverse Prediction’ of Growth Media Composition

- What is the natural growth environment of a pathogen within a host organism?
- Suppose we have in-vivo data on bacterial gene knockout essentiality

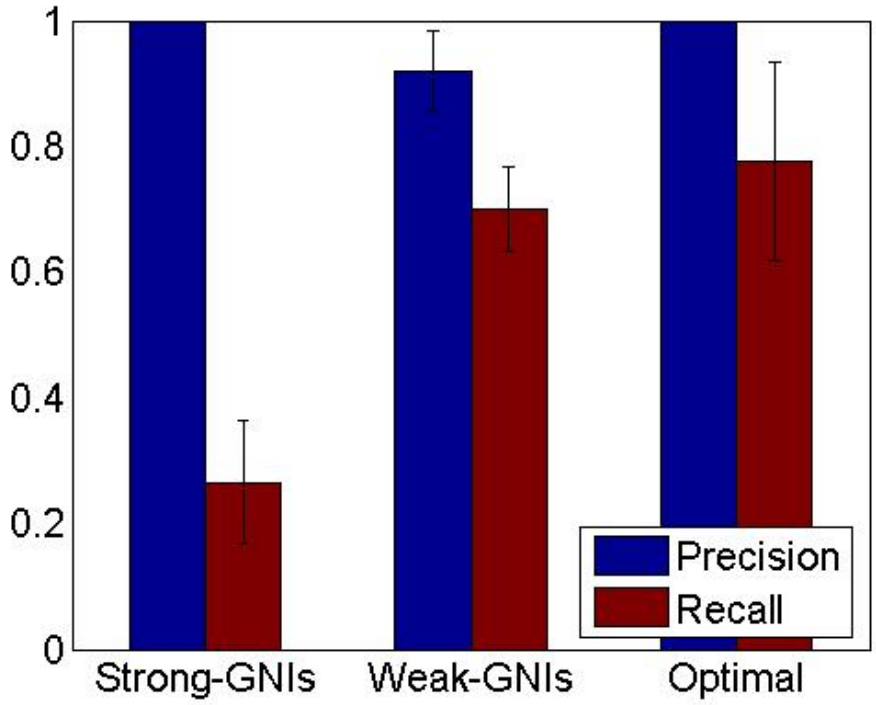


- Can we use the measured pattern of gene essentiality to predict constraints on the in-vivo growth environment of the bacteria?
- Unfortunately, we don’t have enough data of this kind. However…

Diamant, et al., Molecular Biosystems, 2009

GNI-based ‘Reverse Prediction’ of Growth Media Composition

- In simulations, GNI-based analysis provide accurate predictions of growth media composition based on gene essentiality data



Diamant, et al., Molecular Biosystems, 2009

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Network-based Prediction of Metabolic Enzymes' Subcellular Localization.
S. Mintz, A. Aharoni, E. Ruppin, T. Shlomi.
Bioinformatics, 25(12): 247-252, 2009 (ISMB'09)

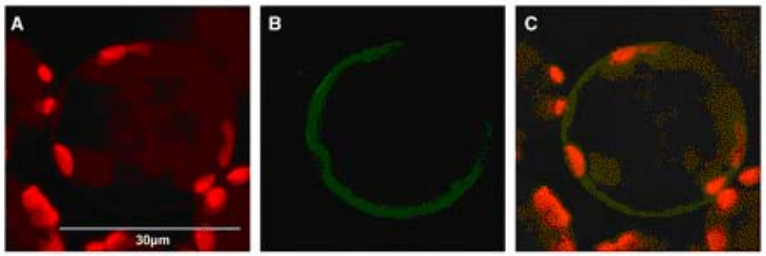
Derecting Protein Subcellular Localization

Experimental Methods:

- Green fluorescent protein (GFP) tagging
- Electron microscopy
- Subcellular fractionation + detection

Limitations:

- Costly
- Time-consuming



Wormit et.al, Plant Cell, 2006

Computational Methods:

- Sequence motifs
- Amino acid composition
- Homology
- PPI data

Limitations:

- Low number of compartments
- Performance varies across different organisms and compartments
- Relatively low availability of PPI networks

Mintz, et al., ISMB & Bioinformatics, 2009

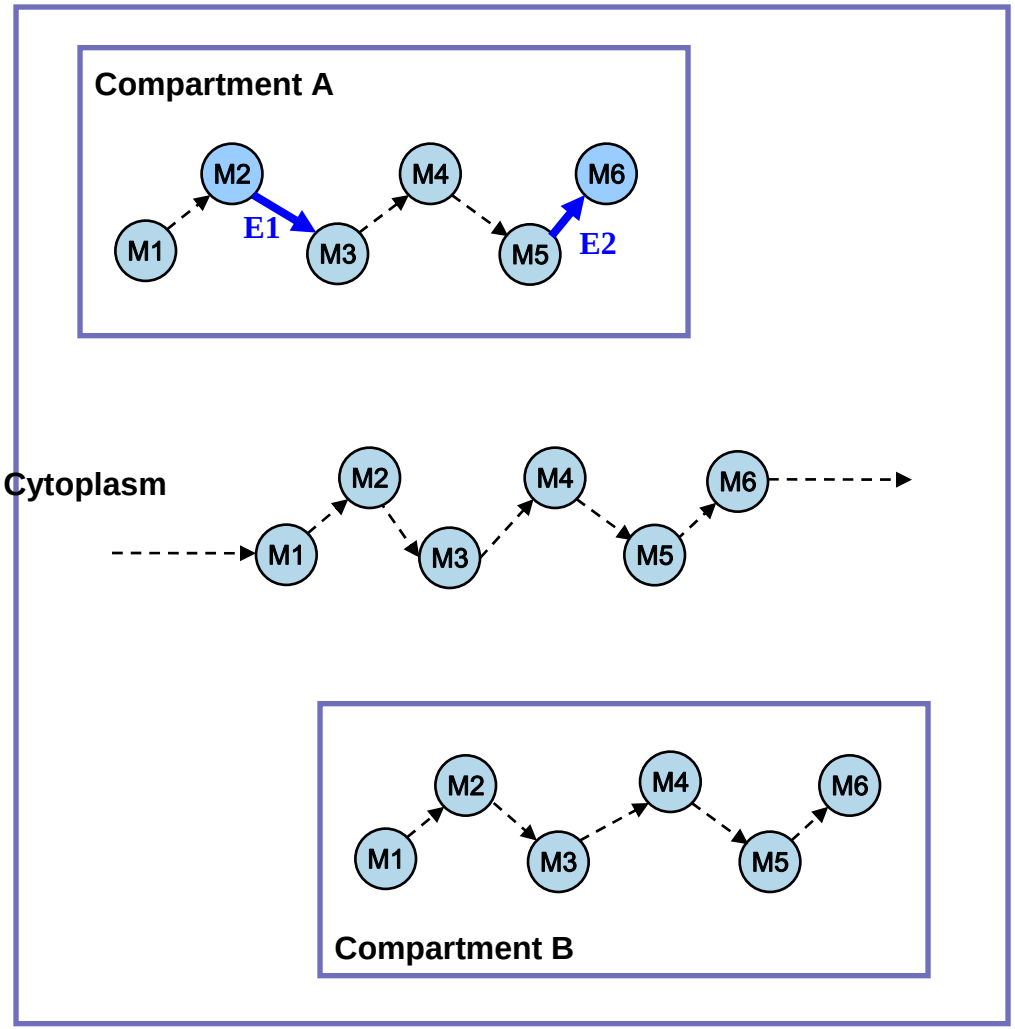
Research Objective

Predict metabolic enzymes' subcellular localization, based on:

- The organism's metabolic network
- Prior knowledge regarding localization of a subset of the enzymes
- Parsimonious assumption of minimal number of cross-membrane metabolite transports between compartments

Mintz, et al., ISMB & Bioinformatics, 2009

Minimal Metabolic Transport Assumption



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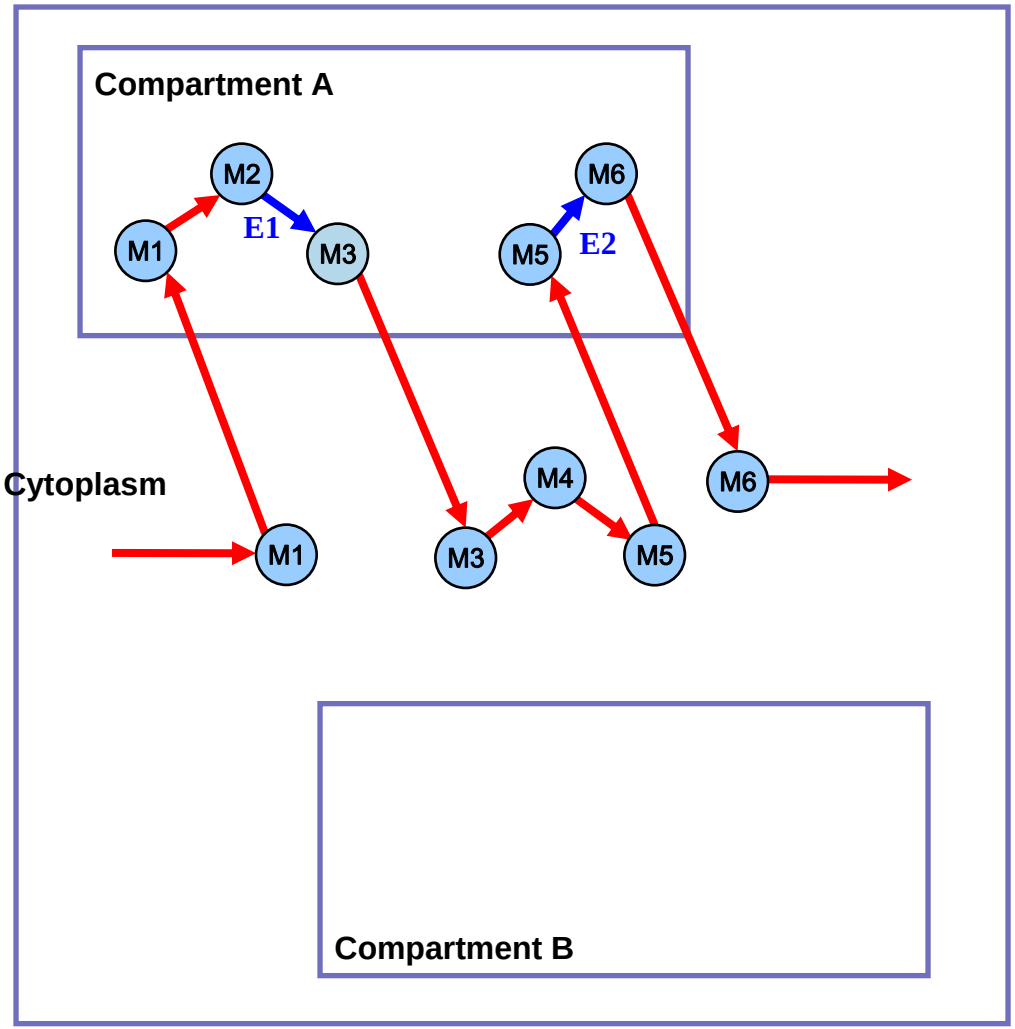

Mintz, et al., ISMB & Bioinformatics, 2009

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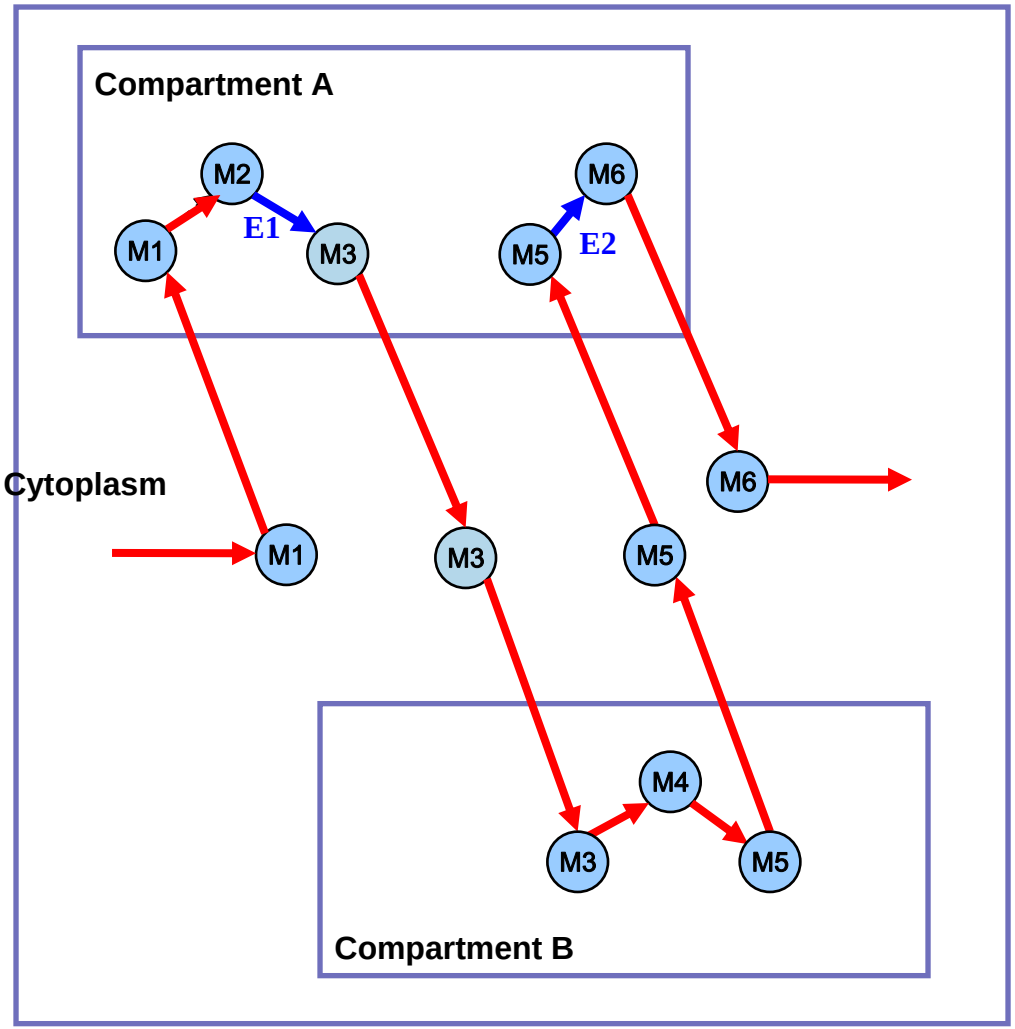
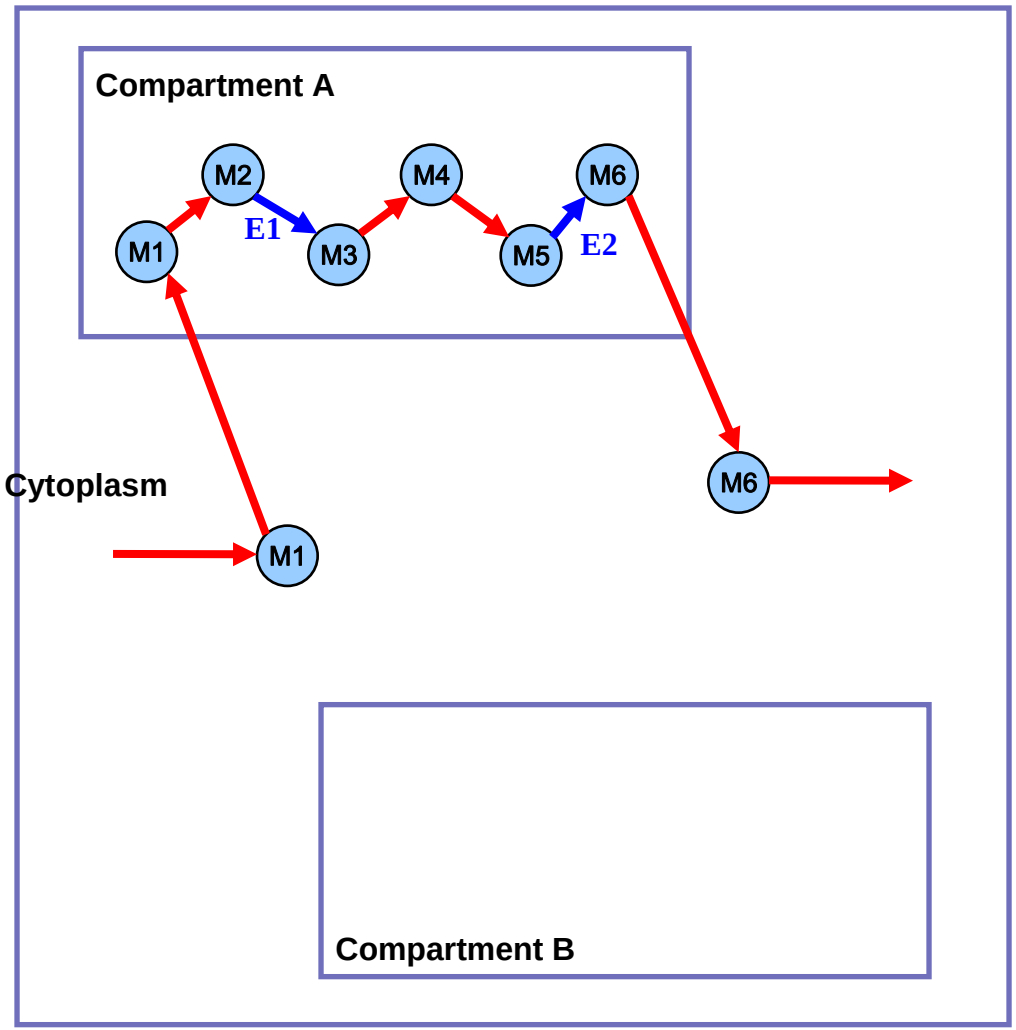


- Transport reactions depend on **transporter proteins**, imposing **energetic cost** or requiring the maintenance of a **membrane potential**

- **Minimize transport reactions**

Mintz, et al., ISMB & Bioinformatics, 2009

Minimal Metabolic Transport Assumption



- Match known localization data
- Assume minimal number of metabolite cross-membrane transports

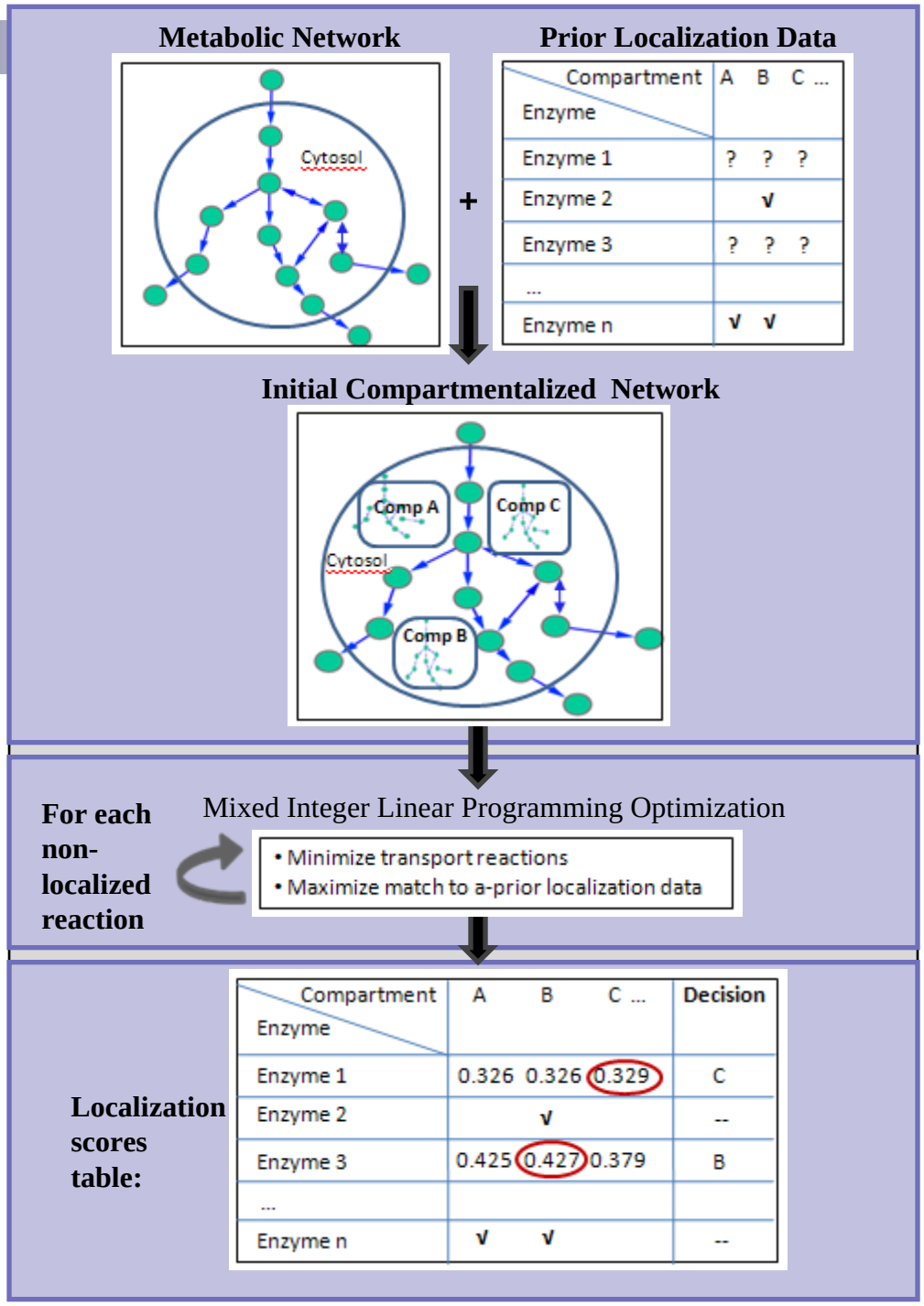
Mintz, et al., ISMB & Bioinformatics, 2009

CBM method for predicting localization:

Input:

Optimization process:

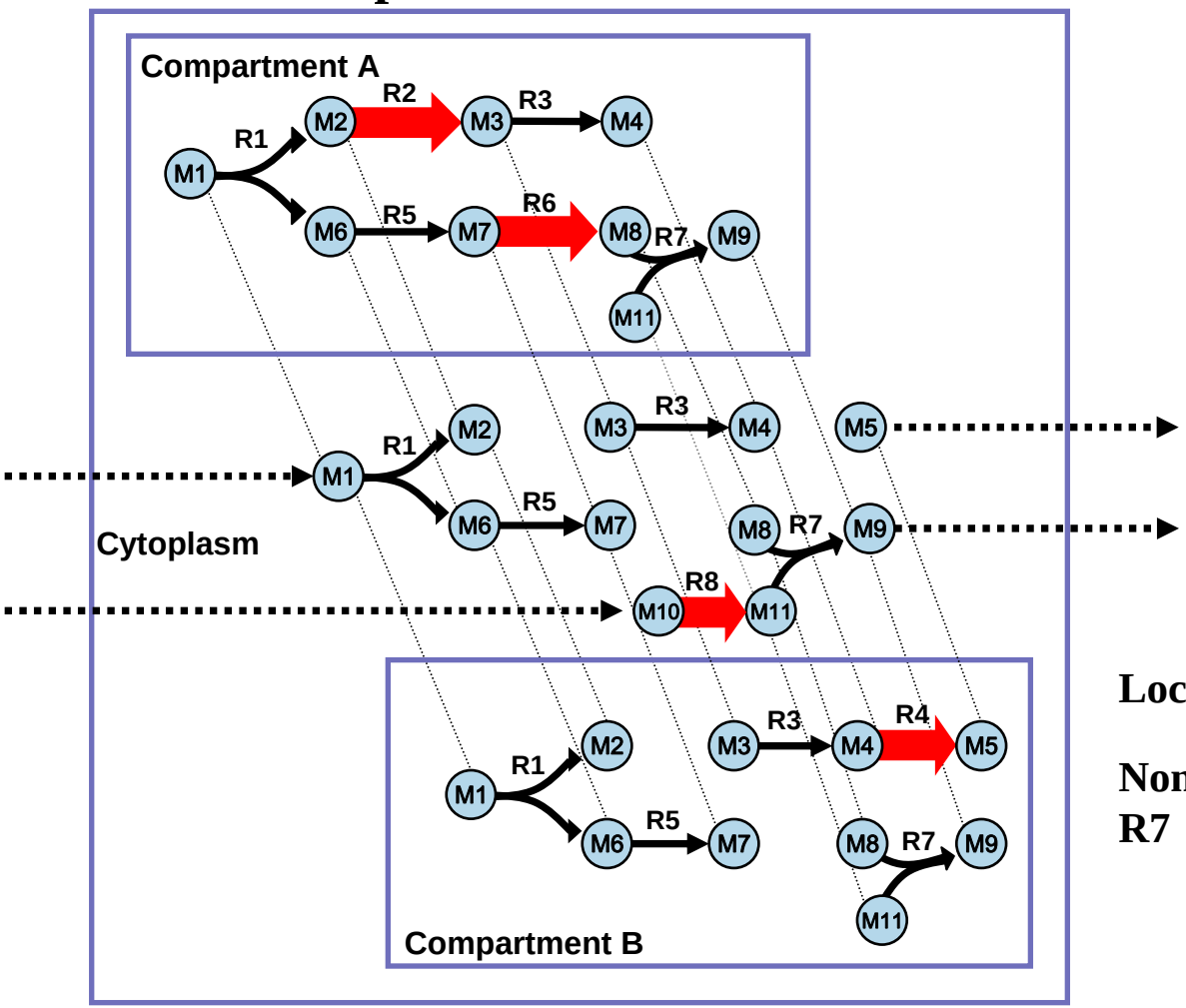
Output (prediction):



Mintz, et al., ISMB & Bioinformatics, 2009

Example

Initial compartmentalized network:



Localized reaction

Non-localized reaction

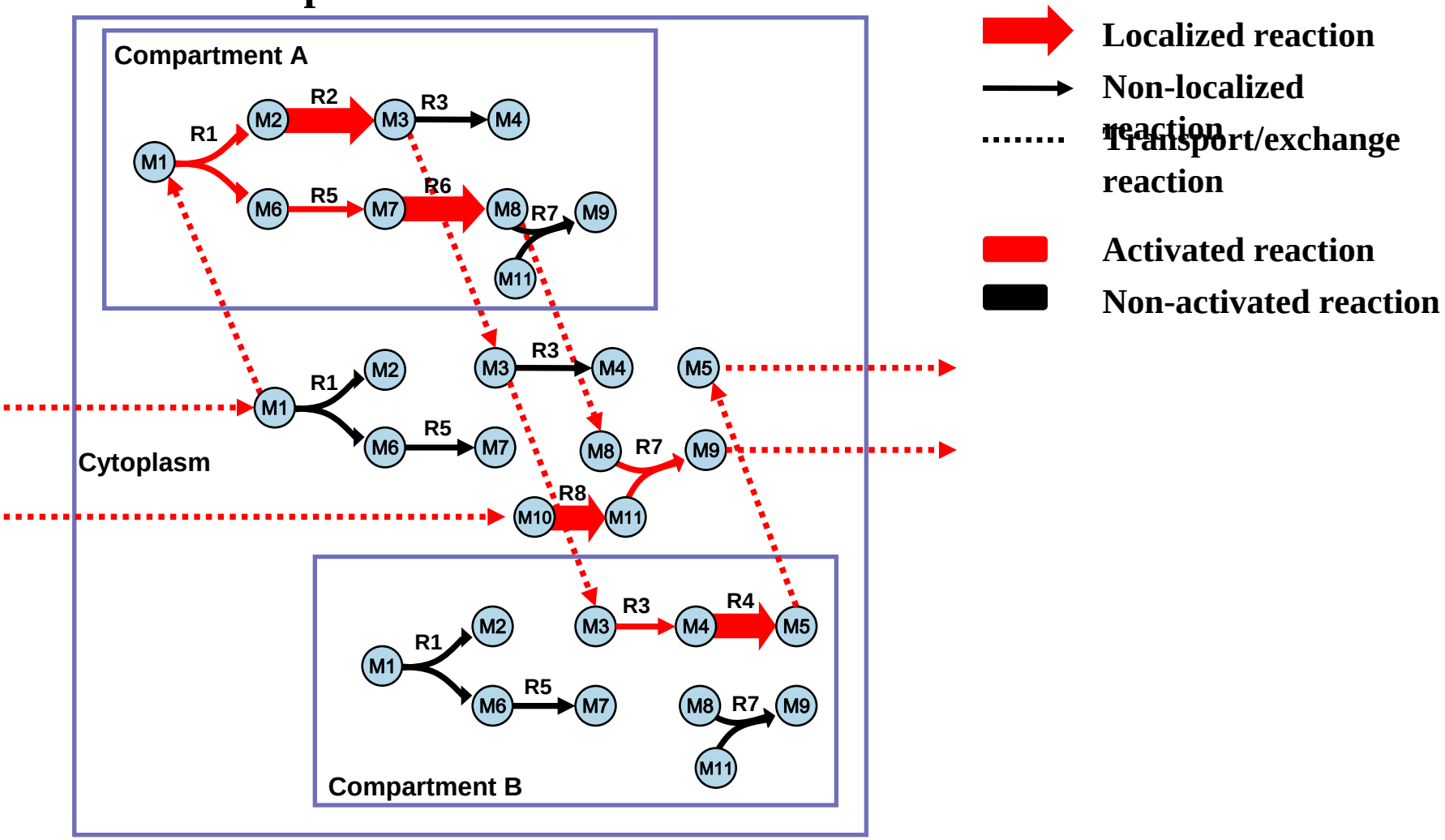
Transport/exchange reaction

Localized reactions – R2, R4, R6, R8

Non-localized reactions – R1, R3, R5, R7

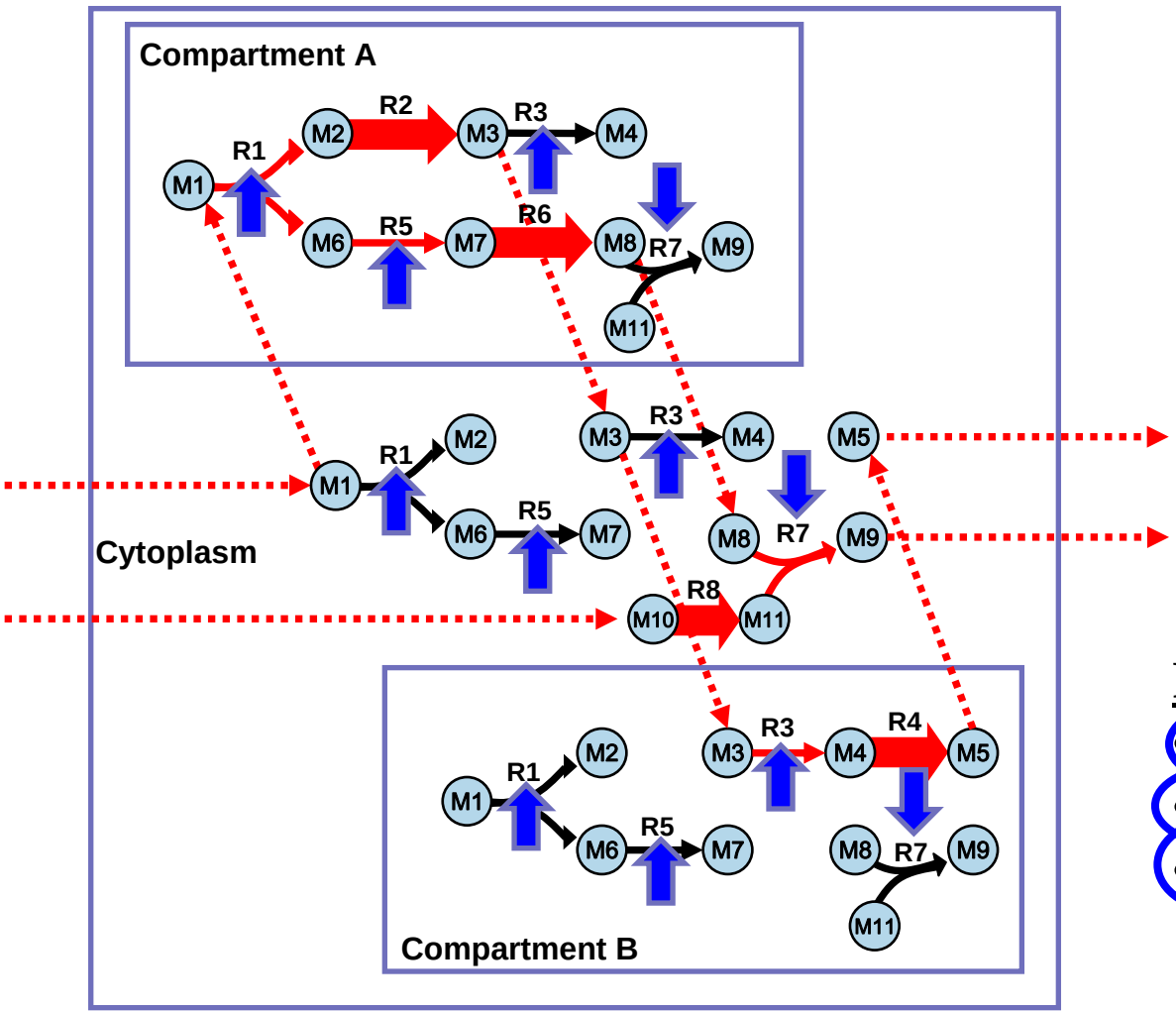
Example – Flux Distribution

Initial compartmentalized network:



Example – Results

Initial compartmentalized network:



Localized reaction

Non-localized reaction

Transport/exchange reaction

Activated reaction

Non-activated reaction

Predictions:

- R1, R5 - Compartment A
- R7 - Cytoplasm
- R3? - Compartment B

Validating Predictions via Metabolic Network of *S. cerevisiae*

- Genome-scale, fully compartmentalized metabolic network model of (Duarte et al, 2004)
- 1062 metabolites, 1149 reactions, 7 compartments

- To evaluate our method:
  1. Remove existing localization data
  2. **Cross validation test-** random localized vs. non-localized sets
  3. Apply our method
  4. Compute **- accuracy** (compared to experimental data)
     - coverage (portion of predictions with single predicted compartment)

Mintz, et al., ISMB & Bioinformatics, 2009

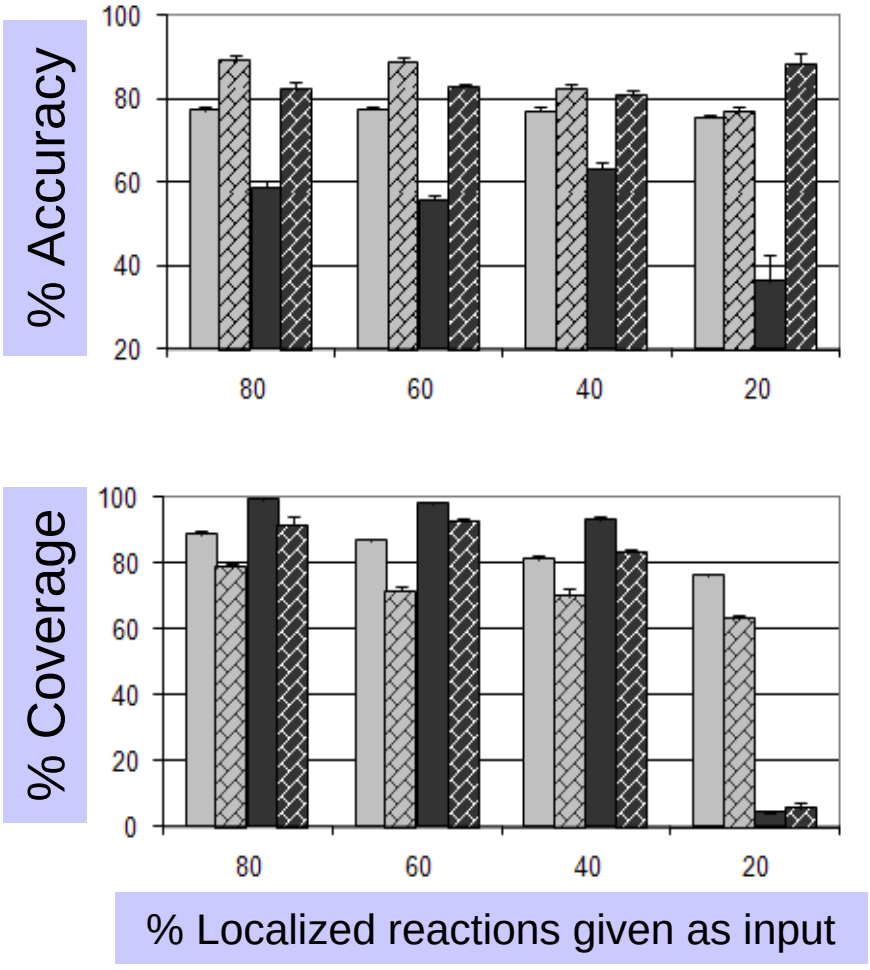
Comparison to Pathway Enrichment-Based Method

- Localization is determined based on the assignment of enzymes in pre-determined biochemical pathways
- For each pathway compute a set of hyper-geometric *p*-values reflecting the **pathway's enrichment for all compartments**, respectively
- Prediction based on compartment yielding the lowest *p*-value in the corresponding pathway

Mintz, et al., ISMB & Bioinformatics, 2009

Results

Accuracy and coverage for various fractions of localized



- **Robust accuracy**
- **Moderate coverage decline**

Collaborators

- My lab
  Naama Tepper
  Edward Vitkin
  Roi Adadi

- Eytan Ruppin's lab (Tel-Aviv)
  Tomer Benyamini
  Ori Folger
  Idit Diamant

- Asaph Aharoni's lab (Weizmann)
  Shira Mintz