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The 3rd International Conference and Exhibition on Metabolomics & Systems Biology March 24-26, 2014 San Antonio, USA

"Integration of omics into metabolic flux distribution by complementary elementary mode analysis for large-scale metabolic networks"

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Kyushu Institute of Technology

Contents

1. Background

2. Problems and objectives

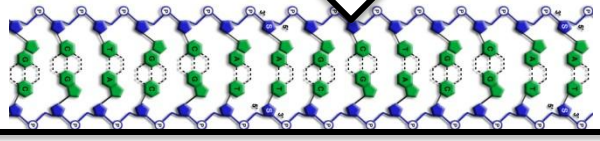
3. Method

4. Results and discussion

5. Concluding remark

Introduction

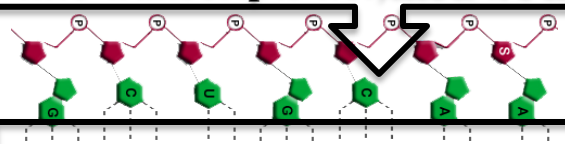
Entirety of all genes under the given studies
i.e., **DNA sequence of an organism**



DNA

Genomics – 40,000 Genes

Entirety of all genes that are converted into transcripts i.e.,**mRNA molecules**



RNA

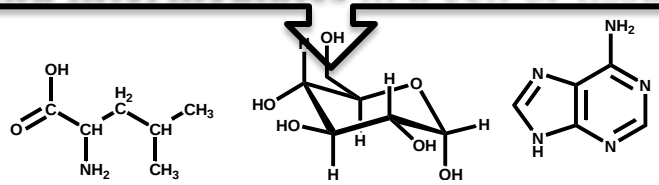
Transcriptomics – 1,00000 Transcripts

Entirety of **all proteins** found in a given cell or tissue

Protein

Proteomics – 10,00,000 Proteins

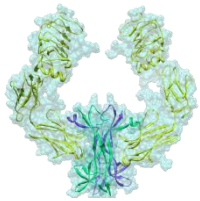
Entirety of **all metabolism products and intermediates** in a cell or tissue



Biochemicals (Metabolites)

Metabolomics – 2,400 Compounds

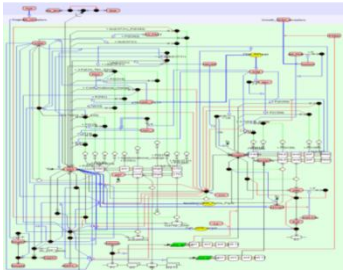
Research Concept



Genome
Omics
Molecular Biology
Medical data

Bioinformatics (Top-down): Parameter values are estimated to reproduce biological behaviors

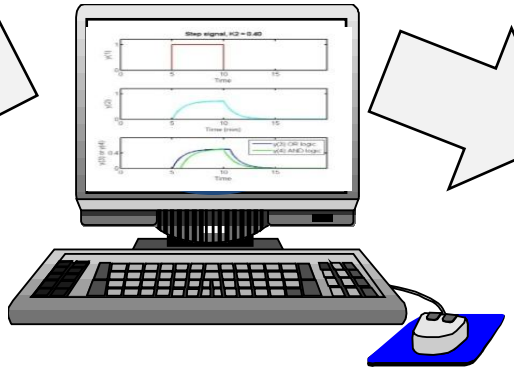
Systems Biology (Bottom-up): Mechanistic model construction based on molecular architecture



$$\frac{dy(1)}{dt} = 0$$

$$\frac{dy(2)}{dt} = km(2) \cdot \frac{y(1)}{K(2) + y(1)} - kd(1) \cdot y(2)$$

$$\frac{dy(3)}{dt} = km(3) \cdot OR(y(1), y(2), K(1), K(2)) - kd(1) \cdot y(3)$$



$$\begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \\ v_7 \end{pmatrix} = \begin{pmatrix} 1 & 1 & 1 & 1 & 0 \\ 1 & 0 & 0 & 1 & 0 \\ 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 1 \\ 1 & 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 & 1 \end{pmatrix} \begin{pmatrix} c_1 \\ c_2 \\ c_3 \\ c_4 \\ c_5 \end{pmatrix}$$

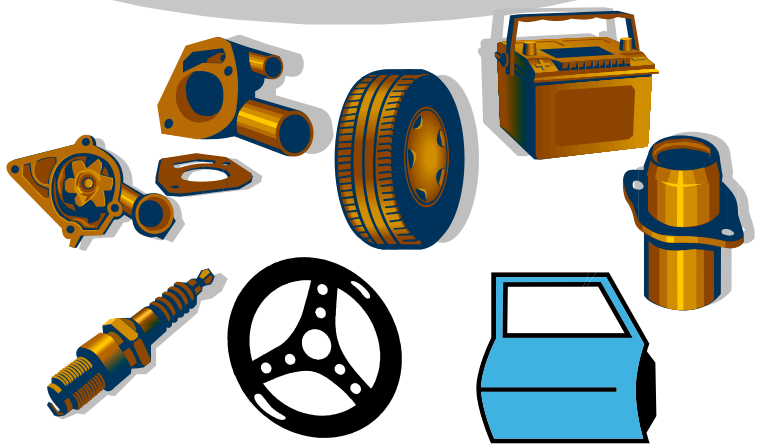
Computer-aided drug design/development; disease diagnosis, prevention, and treatment; and patient care/welfare

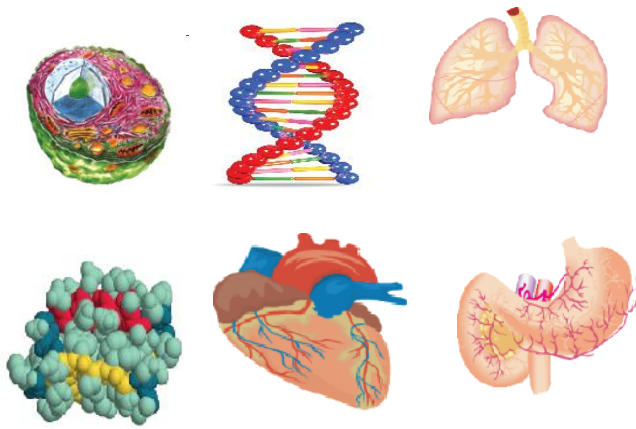
Seeds

Needs from Medical school, Hospitals, and Companies

Bioengineering (Problem-based Research)

Compare two systems





- We want to understand the system, we want to repair when some parts are failing; we want to improve the performance more.
- For example in human body, How genes and proteins that are working in the organism has been accumulated, such that to understand as a system of complex biological reaction is the systems biology.

Background

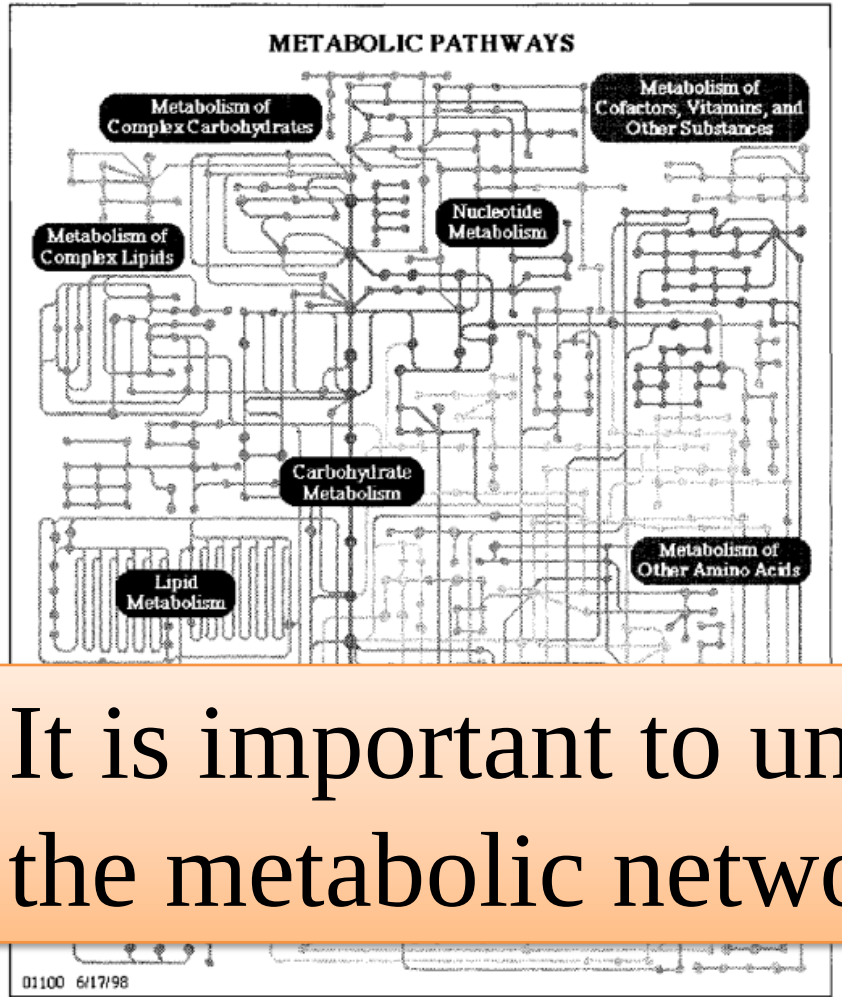


Figure 1:Metabolic pathway MAP

Metabolism is an important **biological processes**

…But

These are complex and highly interconnected

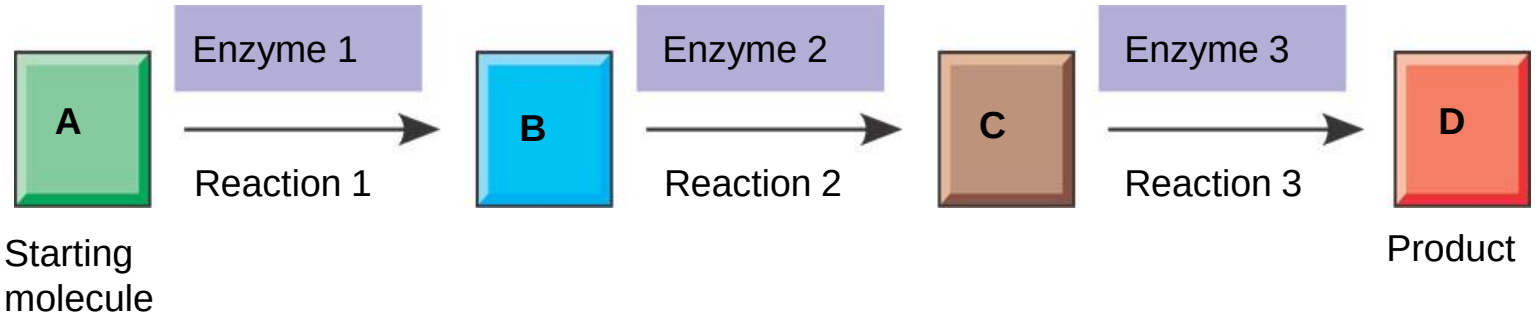
Metabolic disorder is related to a disease

- Diabetes
- high blood pressure
- Cancer …etc

It is important to understand properly of the metabolic networks using EM.

Metabolic Pathways

- Biochemical pathways are the organizational units of metabolism.
- Metabolism is the total of all chemical reactions carried out by an organism.
- A metabolic pathway has many steps that begin with a specific molecule and end with a product, each catalyzed by a specific enzyme.



Elementary mode analysis

Network-based metabolic pathway analysis:

- ❖ Elementary mode (EM) analysis
- ❖ Extreme pathways (ExP) analysis

- ❑ EM is the minimal set of enzymes that can operate at steady state, while the set of extreme pathways is the systemically independent subset of the EMs.

- ❑ The EM coefficients (EMCs) indicate the quantitative contribution of their associated EMs and can be estimated by the maximizing the general objective function.

Problems

- Ordinary EM analysis is that the number of EMs suffers from a combinatorial explosion.
- EMs can be described by many scalar products of each EM, the predicted fluxes must be independent of them.
- Many organisms still do not provide any specific objective biological function.

Objectives

To overcome the existing problems, we proposed a several times faster and efficient EM algorithm named the complementary EMs (cEM) analysis.

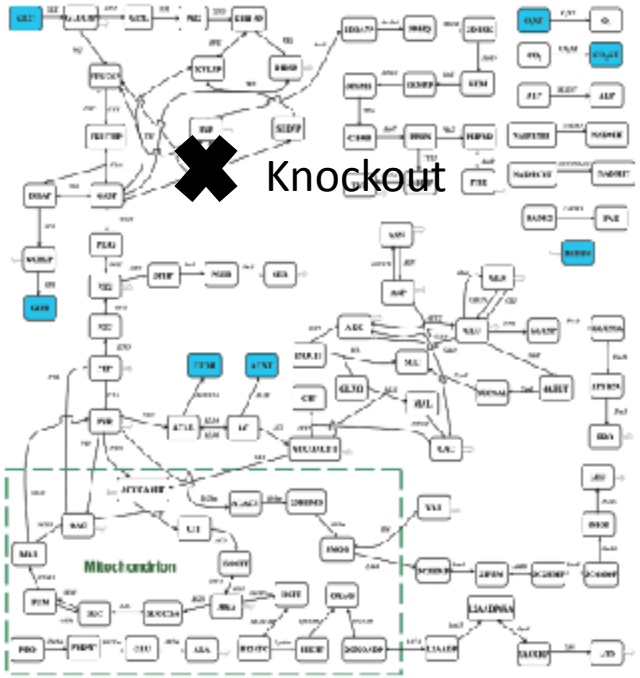
❖EM decomposition [1] which generates the major EMs.

❖Alpha-spectrum [2] can be computed even when the flux is partially unknown.

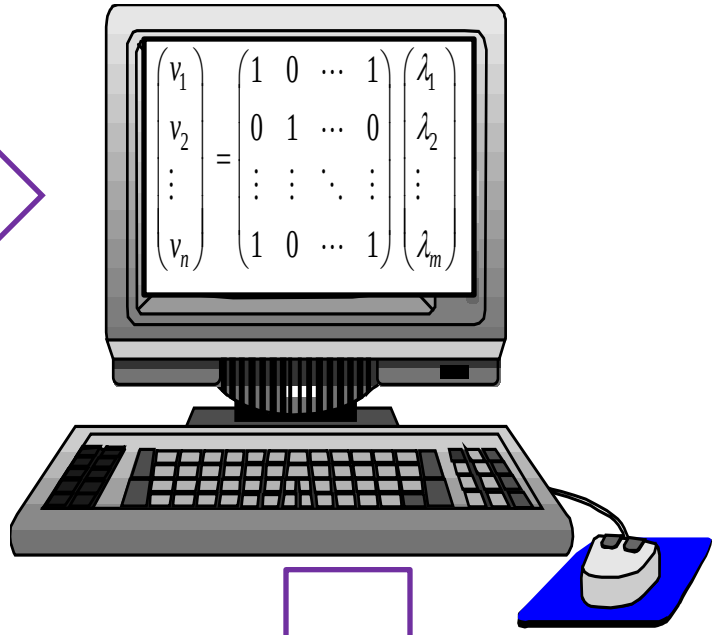
❖MEP [3] to optimize the EMCs

General process

Genetically modified mutant

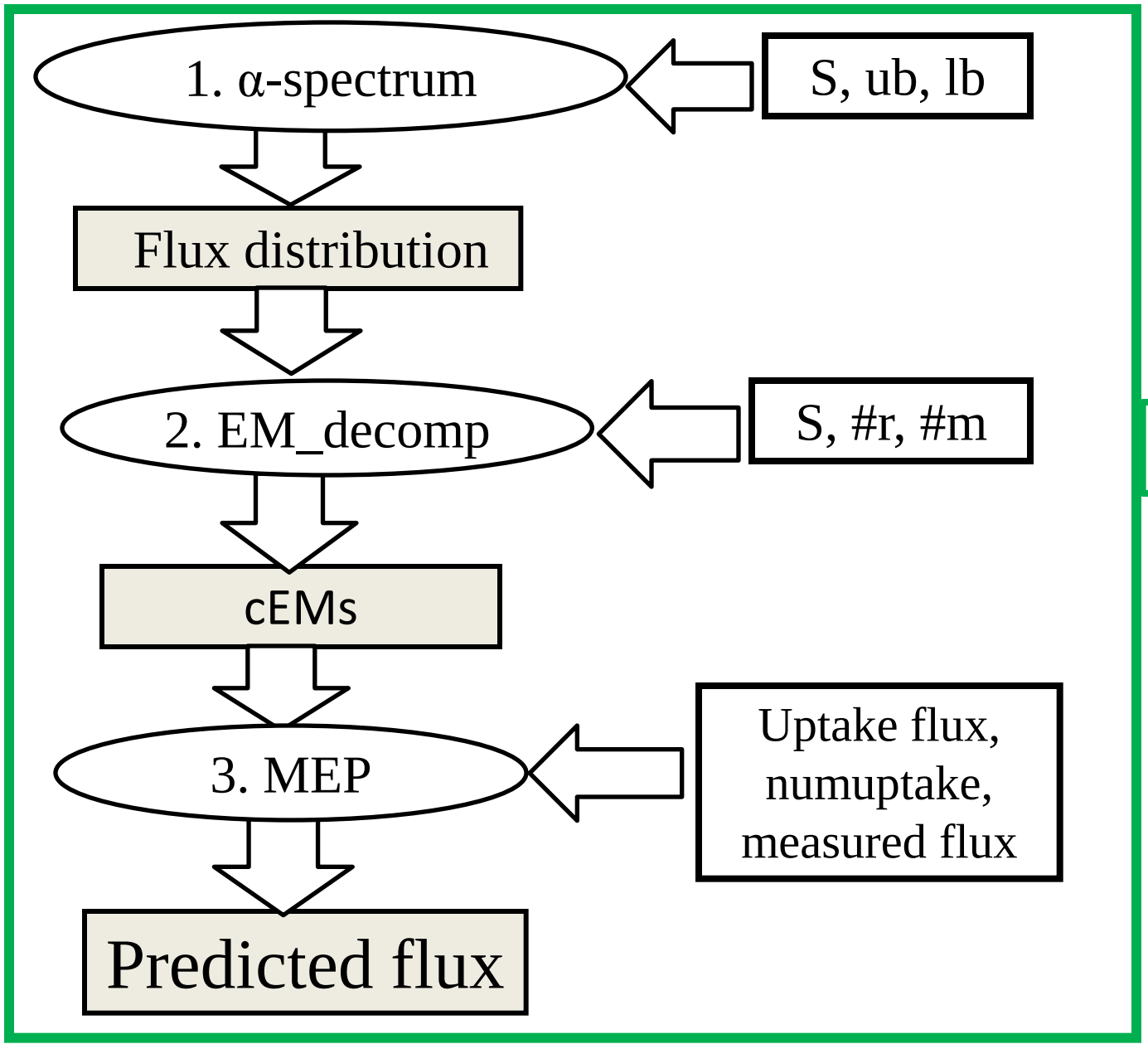


cEM analysis



Prediction of flux distribution

A flow chart of the cEM analysis



The white and grey square boxes are the data and ovals are the algorithms.

Alpha spectrum method [2]

Alpha spectrum method is applied to optimize the flux distribution. It is defined by:

$$\text{Max/Min} \quad \mathbf{v}_i = (v_1, v_2, ..., v_n)^t$$

$$\text{where, i} = 1, 2, 3, \ \ldots, \ n \ ; \qquad \begin{cases} -1000 < v_i^{rev} < 1000 \\ 1e-8 < v_i^{irrev} < 1000 \end{cases}$$

$$\text{Subject to: } \mathbf{S} \cdot \mathbf{v} = 0$$

Input: Stoichiometric matrix, ub, lb

Output: V_{max}, V_{min}

EM decomposition method [1]

- EM decomposition method is applied to generate the major EMs responsible for flux distribution.
- Mixed integer linear programming (MILP)
- It is an iterative method.

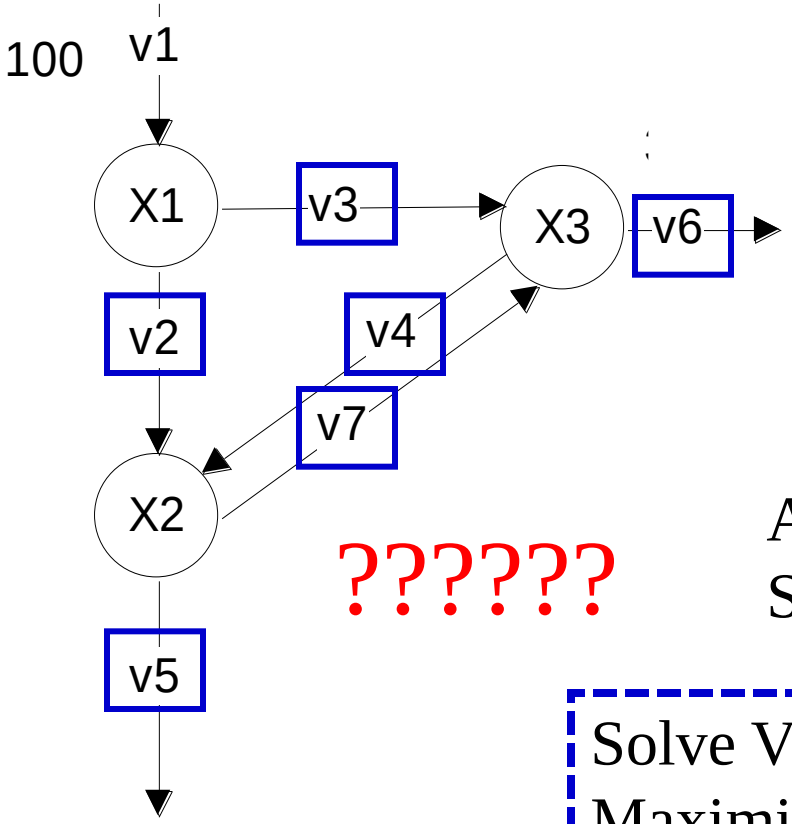
Input: Flux distribution, S, #reaction, #metabolites

Output: EMs

Different objective functions

Methods	Objective Function	Advantage/Disadvantage
LP	$\max \; v_{biomass} = \sum_{i=1}^{ne} p_{biomass,i} \cdot \lambda_i$	Max biomass or ATP production are can vary between different organisms and physiological conditions. Thus the objective function are not good choice.
QP	$\min \sum_{i=1}^{ne} \lambda_i^2$	Suitable when objective function cannot be defined in biological terms. Restricted for small or medium scale and depends on scalar product of EM.
ECFLP	$Max / Min \; \lambda_i \;\; (i = 1,2,\ldots,ne)$	ECFLP is not theoretical but empirical.
MEP	$\max \; -\sum_{i=1}^{ne} \rho_i \ln \rho_i$ s.t. $\sum_{i=1}^{ne} \rho_i = 1; \; \sum_{i=1}^{ne} \rho_i x_{d,i} = v_d$	Straight forward formula and theoretically sound background. No need additional biological hypothesis or objective function and not depends on scalar product of EM

Elementary mode analysis



Example model

Reaction V= (v_1,v_2,v_3,v_4,v_5,v_6,v_7)

Metabolites = X1, X2, X3

$$S = \begin{pmatrix} 1 & -1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 1 & -1 & 0 & -1 \\ 0 & 0 & 1 & -1 & 0 & -1 & 1 \end{pmatrix}$$

At steady-state mass balance equation as $S \cdot V = 0$

Solve V:

Maximize or minimize V_i , ($V_i >= 0$, i=2…7)

v_1=100; subject to $S \cdot V = 0$

12 sets of flux distributions will be solved.

$$\mathrm{EM}(\mathbf{P}) = \begin{pmatrix} \mathrm{EM1} & \mathrm{EM2} & \mathrm{EM3} & \mathrm{EM4} & \mathrm{EM5} \\ 0.5774 & 0.5774 & 0.5 & 0.5 & 0 \\ 0.5774 & 0 & 0 & 0.5 & 0 \\ 0 & 0.5774 & 0.5 & 0 & 0 \\ 0 & 0 & 0.5 & 0 & 0.7071 \\ 0.5774 & 0 & 0.5 & 0 & 0 \\ 0 & 0.5774 & 0 & 0.5 & 0 \\ 0 & 0 & 0 & 0.5 & 0.7071 \end{pmatrix}_{7\times5}$$

MEP

EMC (λ)
43.30
43.30
50
50
0

Quantitative contributions $=\lambda_i \cdot P(\text{numuptake}, i)$; where, i=number of EM and numuptake =the row in EM matrix P for uptake / input flux.

The 4 cEM and flux distributions by cEM analysis are as follows:

$$\mathrm{cEM} = \begin{pmatrix} \mathrm{EM1} & \mathrm{EM2} & \mathrm{EM3} & \mathrm{EM4} \\ 0.5774 & 0.5774 & 0.5 & 0.5 \\ 0.5774 & 0 & 0 & 0.5 \\ 0 & 0.5774 & 0.5 & 0 \\ 0 & 0 & 0.5 & 0 \\ 0.5774 & 0 & 0.5 & 0 \\ 0 & 0.5774 & 0 & 0.5 \\ 0 & 0 & 0 & 0.5 \end{pmatrix}_{7\times4}$$

Flux distributions
100
50
50
25
50
50
25

Application in large-scale metabolic model

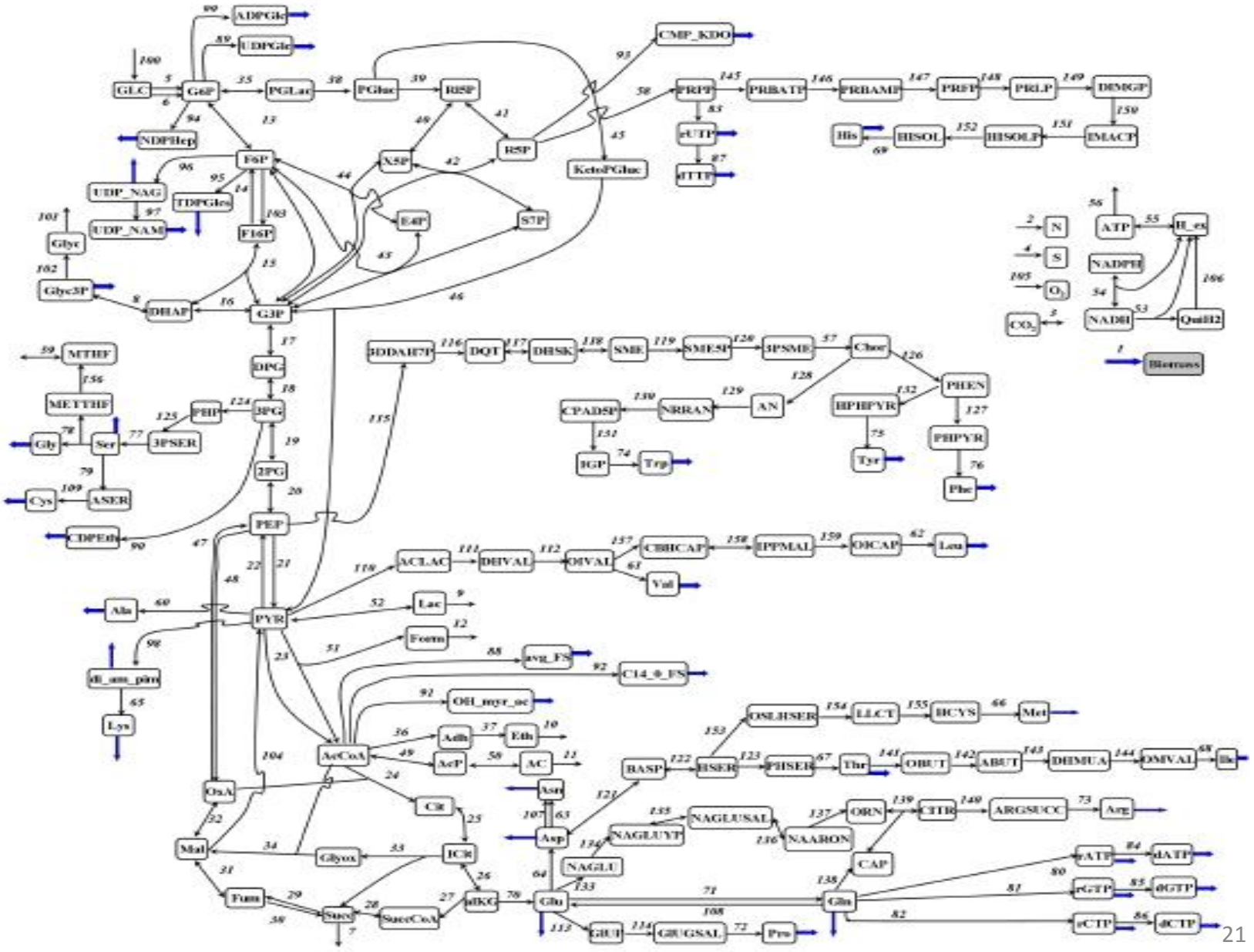
Model-I

The model-I was involved 140 metabolites and 156 reactions for the *E. coli pta-pfkA* gene knockout mutant undergoing adaptive evolution of 30 and 60 days under anaerobic condition.

Model-II

The model-II was involved 140 metabolites and 157 reactions for the *E. coli pta-adhE-pfkA-glk* gene knockout mutant undergoing adaptive evolution of 30 and 60 days under anaerobic condition.

Metabolic network map for *E. coli*



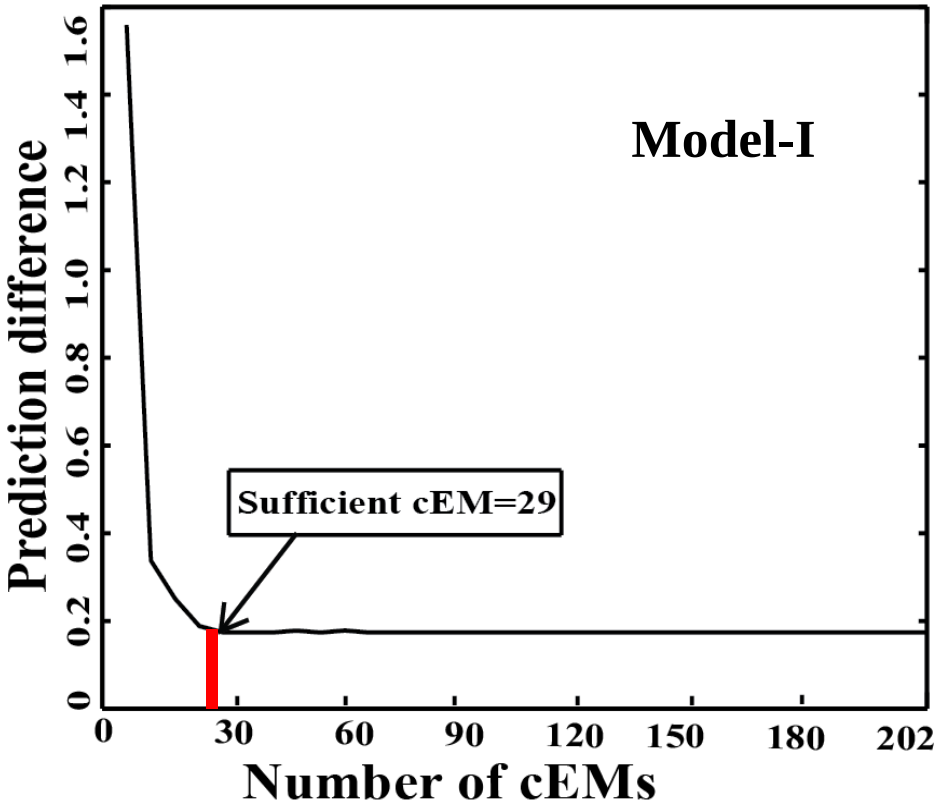
Quantitative contributions for input flux

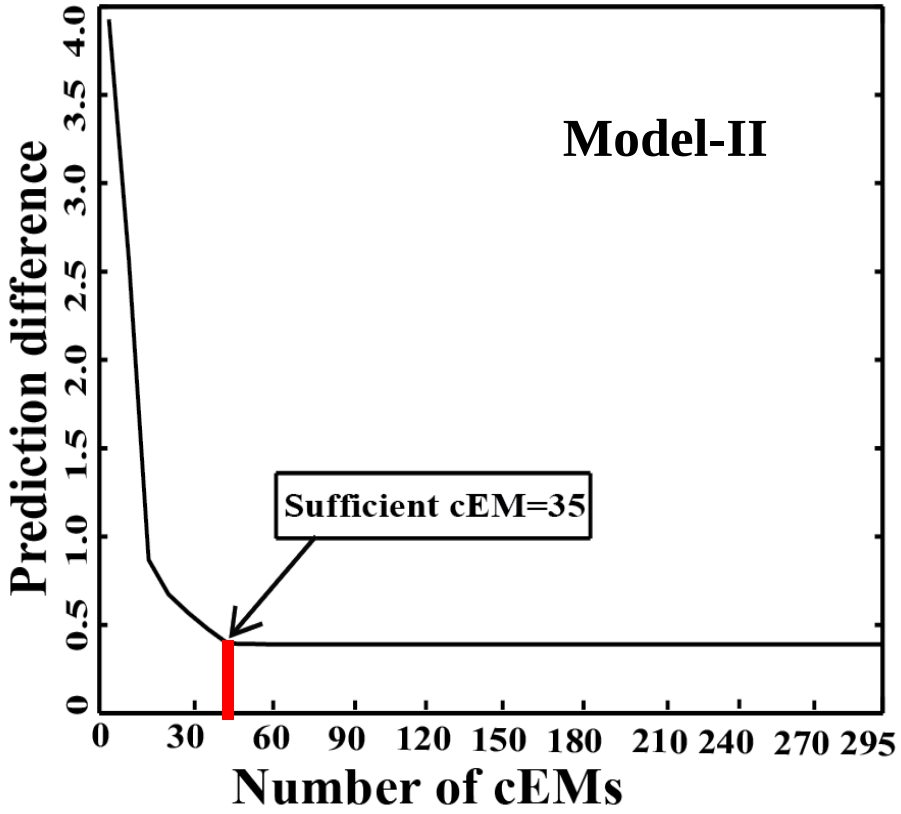
Model	Methods	EM matrix (P)	EMC(λ)
Model-I	Ordinary EM	156 × 122126	122126 × 1
	cEM	156 × 202	202 × 1
Model-II	Ordinary EM	157 × 321416	321416 × 1
	cEM	157 × 295	295 × 1

Quantitative contributions $=\lambda_i \cdot P(\text{numuptake}, i)$ ……(*)

where, i=number of EM and numuptake =the row in EM matrix P for uptake / input flux.

Necessary and sufficient cEM

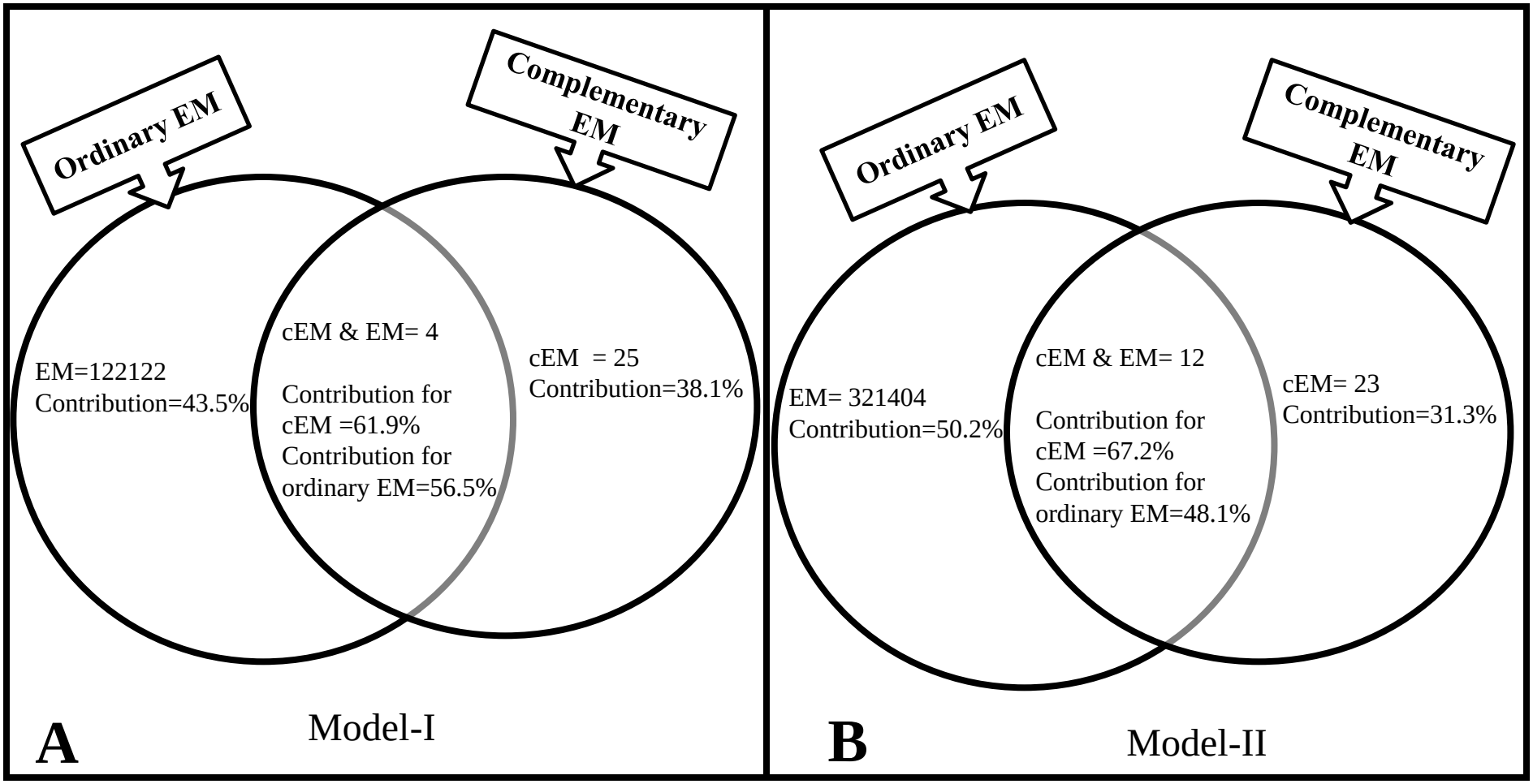




$$\text{Predection difference} = \sqrt{\frac{1}{u}\sum_{t=1}^{u}\left(v_{t,cEM} - v_{t,\text{ordinary EM}}\right)^2}$$

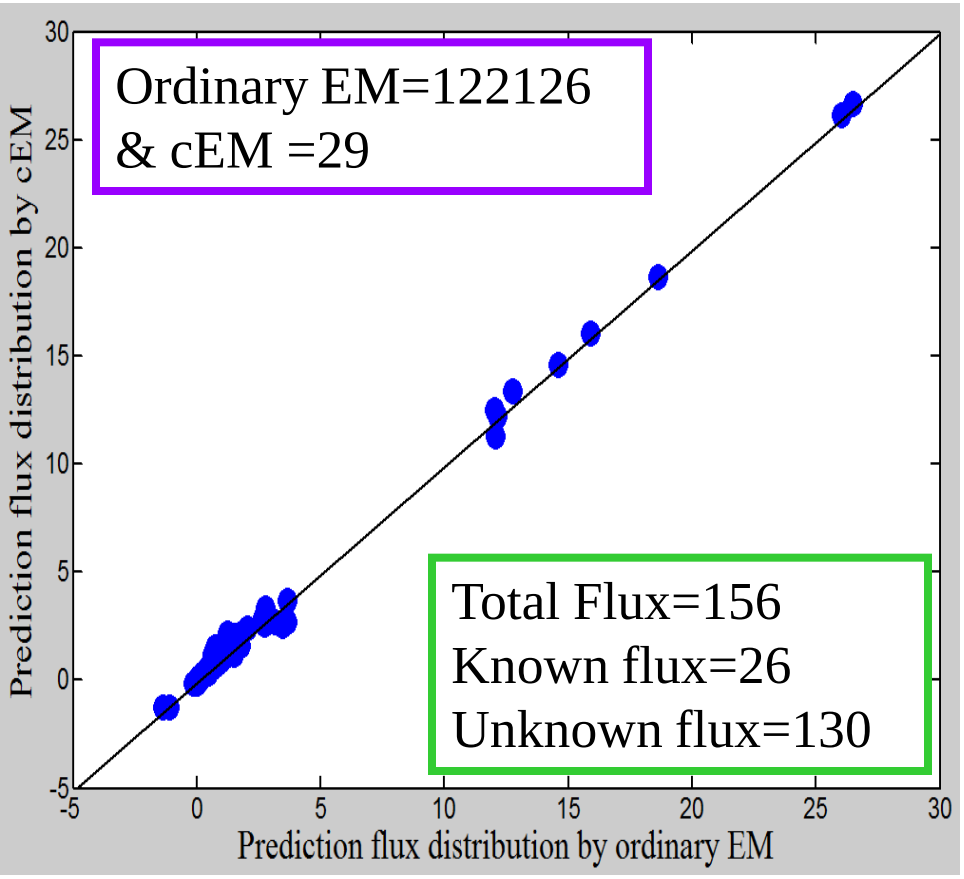
where $v_{t,cEM}$ and $v_{t,\text{ordinary EM}}$ are the predicted fluxes for the *t*th reaction by the cEM and ordinary EM analyses, respectively; u is the number of the unmeasured fluxes.

The employed cEMs and EMs and their quantitative contributions to input flux

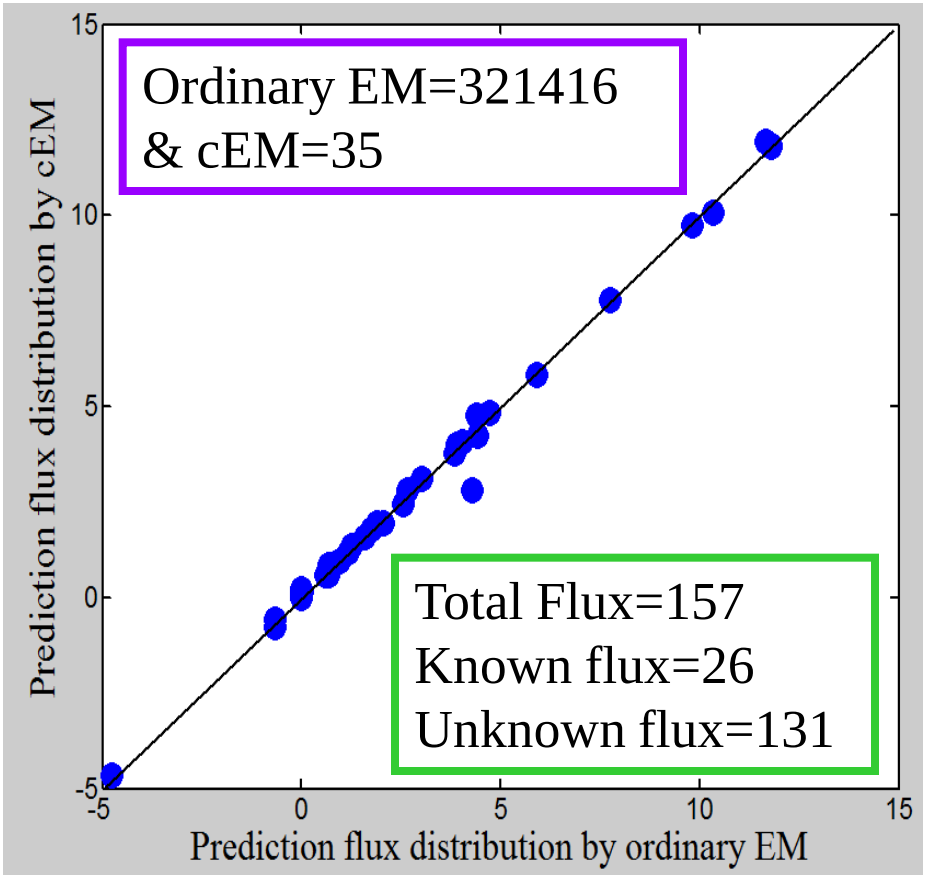


Compare Results (Cont.)

Model-I



Model-II



Genetic Modification of Flux (GMF) [4]

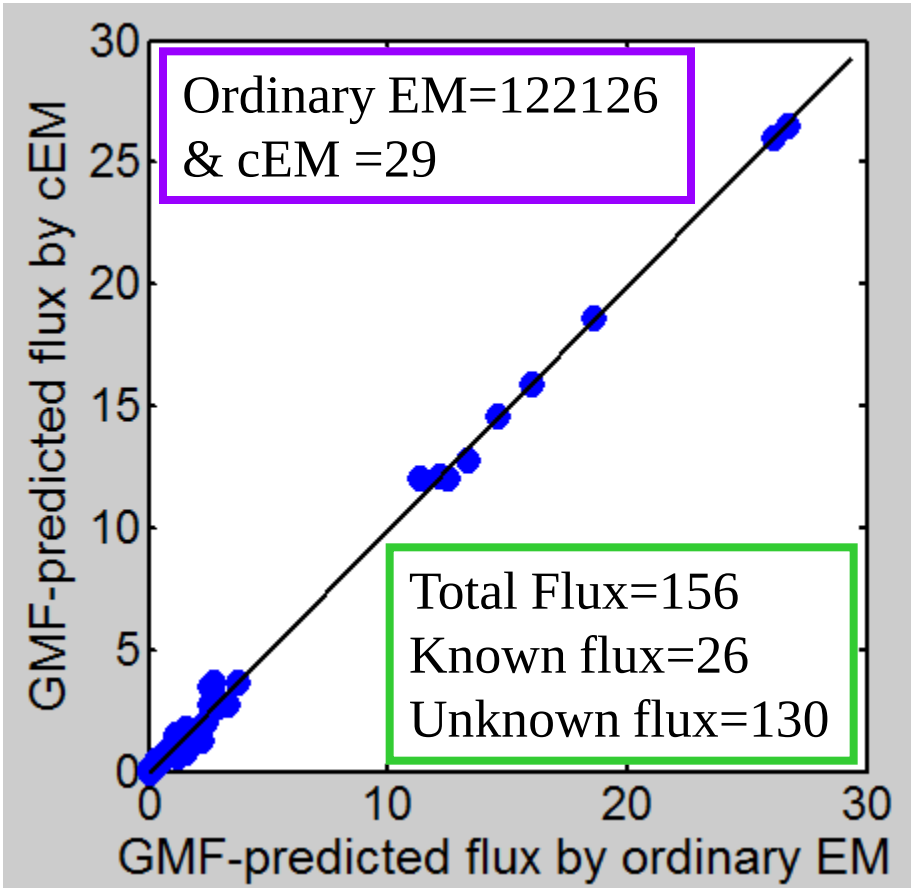
- GMF is an EM-based algorithm that couples with modified control effective flux (mCEF) and enzyme control flux (ECF).

- mCEF was proposed based on CEF to estimate the gene expression patterns in genetically modified mutants in terms of specific biological function.

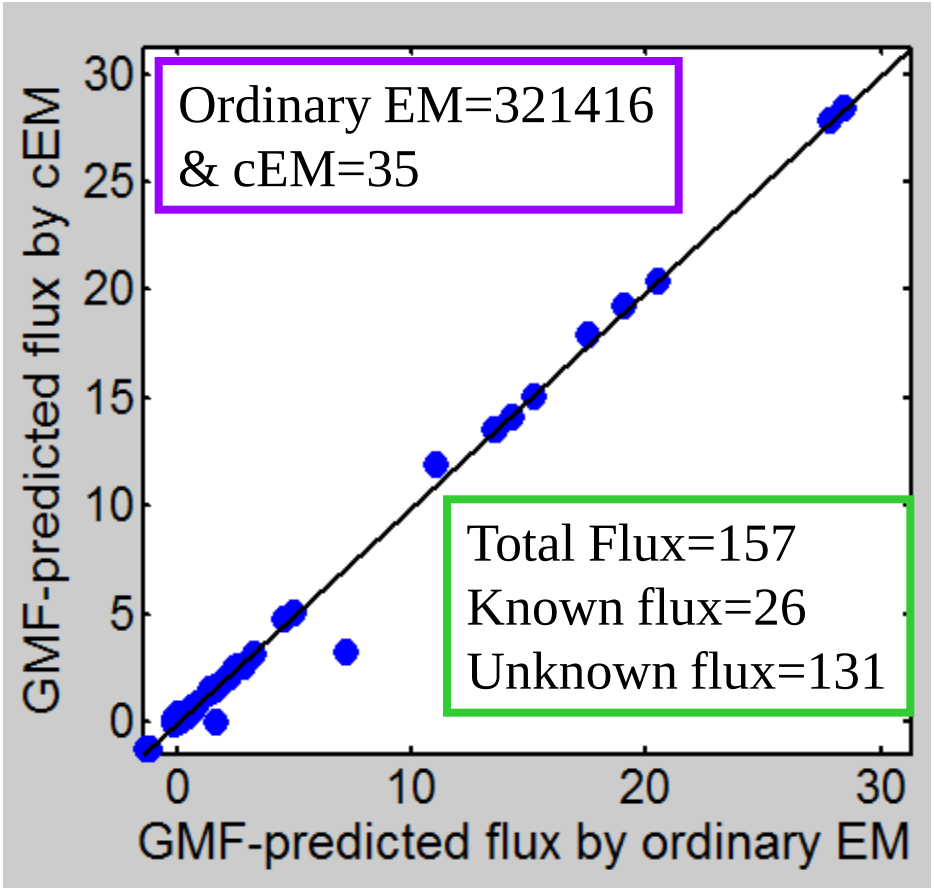
- ECF predicts how change in enzyme profile affects the flux distribution.

Compare Results (Cont.)

Model-I



Model-II



Pearson's correlation coefficient (r) and coefficients of determination (R^2) between the experimental flux and predicted flux.

Model	Condition	Method	Pearson's Correlation (r)	Coefficients of determination (R^2)
Model-I	Wild type	Ordinary EM	0.9980	0.9960
		cEM	0.9982	0.9964
	Mutant type	Ordinary EM	0.9973	0.9947
		cEM	0.9975	0.9950
Model-II	Wild type	Ordinary EM	0.9639	0.9291
		cEM	0.9634	0.9281
	Mutant type	Ordinary EM	0.9989	0.9978
		cEM	0.9989	0.9978

r range between 0.9634 and 0.9989, R^2 ranging from 0.9281 to 0.9978. These statistical analyses demonstrate the r and R^2 remarkably high, and provide statistically significant correlation between the experimental flux and predicted flux by cEM and ordinary EM analyses.

Pearson's correlation coefficient (r) and coefficients of determination (R^2) between the experimental flux and GMF-predicted flux.

Model	Adaptive evolution	Method	Pearson's Correlation (r)	Coefficients of determination (R^2)
Model-I	30 days	Ordinary EM	0.9696	0.9401
		cEM	0.9680	0.9370
	60 days	Ordinary EM	0.9756	0.9519
		cEM	0.9743	0.9492
Model-II	30 days	Ordinary EM	0.8723	0.7610
		cEM	0.9873	0.9747
	60 days	Ordinary EM	0.9908	0.9817
		cEM	0.9841	0.9684

r range between 0.8723 and 0.9908, R^2 ranging from 0.7610 to 0.9817. These statistical analyses demonstrate the r and R^2 remarkably high, and provide statistically significant correlation between the experimental flux and GMF-predicted flux by proposed and ordinary method.

Calculation speed and accuracy

Model	Method		# EM	Total running time(s)	Prediction error
Model-I	Ordinary EM	CNA	122126	600+770.871=1370.871[a]	0.0233
		efmtool	122126	100+780.871=980.871[a]	0.0233
	cEM		202	10+34+1.561=45.561[b]	0.0268
Model-II	Ordinary EM	CNA	321416	6000+1050.56=7050.56[a]	0.0813
		efmtool	321416	200+1000.56=1200.56[a]	0.0233
	cEM		295	12+38+1.805=51.805[b]	0.0475

$$\text{Predection error} = \sqrt{\frac{1}{m}\sum_{i=1}^{m}\left(v_{i,\text{prediction}} - v_{i,\text{exp}}\right)^2}$$

[a] The ordinary EM analysis consists of two steps (EM extraction and flux prediction by MEP) to predict the flux distributions. [b] The cEM analysis consists of three steps (α-spectrum, cEM extraction, and flux prediction by MEP).

Concluding Remarks

- We found 29 and 35 cEMs for model-I and model-II are enough to estimate the flux distributions, at which the prediction difference almost converges.

- The predicted (MEP & GMF) flux distribution by cEMs was very consistent with that by the ordinary EM analyses.

- The cEM method, where neither requires the initial generation of a full set of EMs nor any objective biological function, which is often computationally demanding, memory improvements and reduced the cost.

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Acknowledgement

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