

Flux Balance Analysis

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Flux Balance Analysis (FBA)

- ❖ Flux balance analysis (FBA), an optimality-based method for flux prediction, is one of the most popular modeling approaches for metabolic systems.
- ❖ Flux optimization methods do not describe *how* a certain flux distribution is realized (by kinetics or enzyme regulation), but *which* flux distribution is optimal for the cell; e.g., providing the highest rate of biomass production at a limited inflow of external nutrients.
- ❖ This allows us to predict flux distributions without the need for a kinetic description.

Flux Balance Analysis (FBA)

- * FBA investigates the theoretical capabilities and modes of metabolism by imposing a number of constraints on the metabolic flux distributions:
 - * The assumption of a steady state: $\mathbf{S} \times \mathbf{v} = \mathbf{0}$.
 - * Thermodynamics constraints: $a_i \leq v_i \leq b_i$.
 - * An optimality assumption: the flux distribution has to maximize (or, minimize) an objective function $f(v)$

$$f(v) = \sum_{i=1}^r c_i v_i$$

Geometric Interpretation of FBA

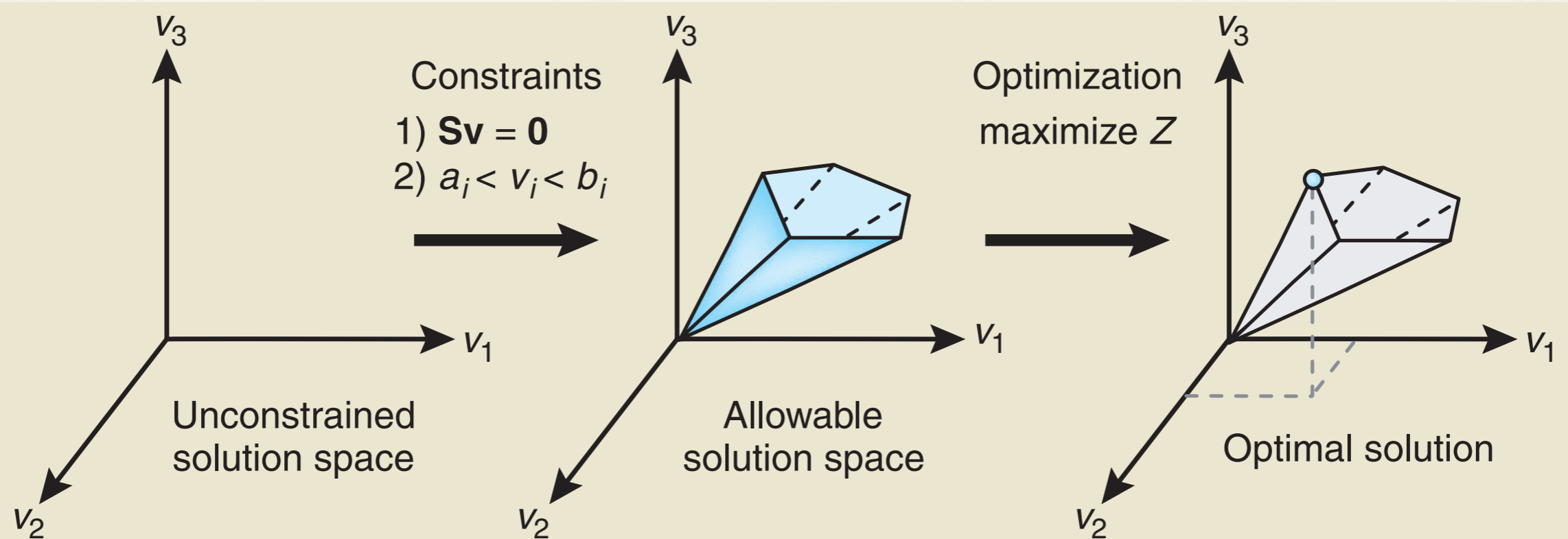
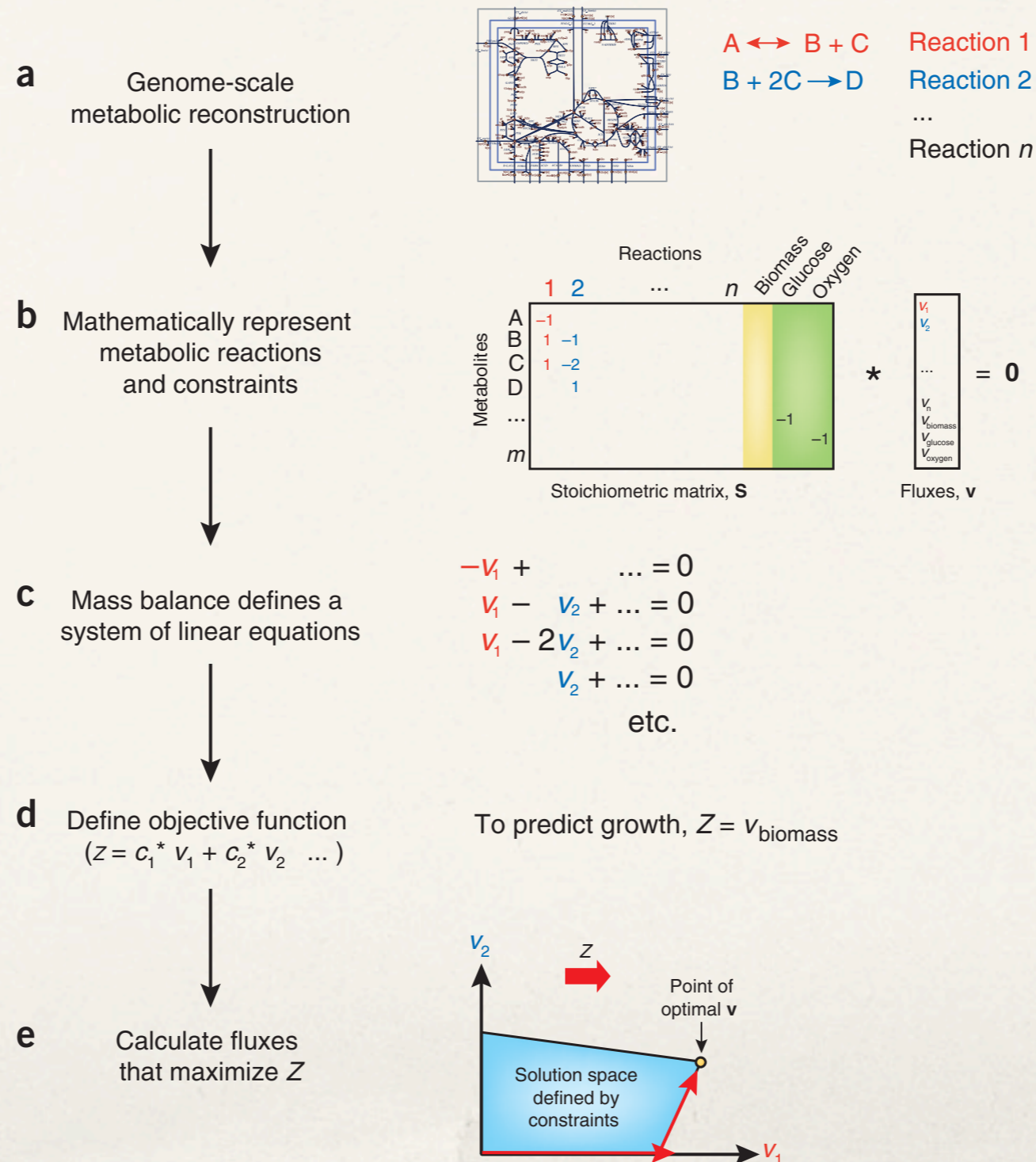


Figure 1 The conceptual basis of constraint-based modeling. With no constraints, the flux distribution of a biological network may lie at any point in a solution space. When mass balance constraints imposed by the stoichiometric matrix \mathbf{S} (labeled 1) and capacity constraints imposed by the lower and upper bounds (a_i and b_i) (labeled 2) are applied to a network, it defines an allowable solution space. The network may acquire any flux distribution within this space, but points outside this space are denied by the constraints. Through optimization of an objective function, FBA can identify a single optimal flux distribution that lies on the edge of the allowable solution space.

[Source: "What is flux balance analysis?", Nat Biotech.]

Formulation of an FBA Problem



What to Optimize?

- ❖ Minimize ATP production: the most energy-efficient state
- ❖ Minimize nutrient intake: the fittest state under nutrient shortage
- ❖ Maximize metabolite production: the biochemical production capabilities of certain desirable metabolites such as lysine, phenylalanine, etc.
- ❖ Maximize biomass formation: maximal growth rate
- ❖ ...

Producing Biomass

- ❖ Growth can be defined in terms of the biosynthetic requirements to make a cell.
- ❖ These requirements are based on literature values of experimentally determined biomass composition.
- ❖ Thus, biomass generation is defined as a linked set of reaction fluxes draining intermediate metabolites in the appropriate ratios and represented as an objective function Z .

Biomass Formation in *E. coli*

- ❖ The requirements for making 1g of *E. coli* biomass from key cofactors and biosynthetic precursors have been documented.
- ❖ This means that for *E. coli* to grow, all these components must be provided in the appropriate relative amounts.
- ❖ Key biosynthetic precursors are used to make all the constituents of *E. coli* biomass. Their relative requirements to make 1g of *E. coli* biomass are:

$$\begin{aligned} Z_{\text{precursors}} = & +0.205V_{\text{g6P}} + 0.071V_{\text{F6P}} + 0.898V_{\text{R5P}} \\ & + 0.361V_{\text{E4P}} + 0.129V_{\text{T3P}} + 1.496V_{\text{3PG}} \\ & + 0.519V_{\text{PEP}} + 2.833V_{\text{PYR}} + 3.748V_{\text{AcCoA}} \\ & + 1.787V_{\text{OAA}} + 1.079V_{\alpha\text{KG}} \end{aligned}$$

Biomass Formation in E. coli

- ❖ In addition to precursors, cofactors are needed to drive the process.
- ❖ The cofactors requirement to synthesize the monomers from the precursors (amino acids, fatty acids, nucleic acids) and to polymerize them into macromolecules is

$$Z_{\text{cofactors}} = 42.703V_{\text{ATP}} - 3.547V_{\text{NADH}} + 18.22V_{\text{NADPH}}$$

Biomass Formation in *E. coli*

- * The mass and cofactor requirements to generate *E. coli* biomass are:

$$Z_{\text{biomass}} = Z_{\text{precursors}} + Z_{\text{cofactors}}$$

Resources for FBA

- ❖ The BIGG database: <http://bigg.ucsd.edu/>
- ❖ The COBRA toolbox: <http://opencobra.sourceforge.net/>
- ❖ FASIMU: <http://www.bioinformatics.org/fasimu/>

Other Applications of FBA

Modular Epistasis in Yeast Metabolism

- ❖ Genes can be classified by categories related to functions of the cell (e.g., translation, energy metabolism, mitosis, etc.) based on textbook knowledge.
- ❖ Can we infer functional associations directly from deletion experiments?
- ❖ If two gene products can compensate for each other's loss, then deleting both of them will have a much stronger impact on cell fitness than one would expect from their single deletions.

Modular Epistasis in Yeast Metabolism

- ❖ On the other hand, if two gene products are essential parts of the same pathway, a single deletion would already shut down the pathway and a double deletion would not have any further effect.
- ❖ Accordingly, we may try to infer functional relationships among the gene products by comparing the fitness losses caused by combined gene deletions.

Modular Epistasis in Yeast Metabolism

- ❖ *Epistasis* describes how the fitness loss due to a gene mutation depends on the presence of other genes.
- ❖ It can be quantified by comparing the fitness of a wild type organism, e.g., the growth rate of a bacteria culture, to the fitness of single and double deletion mutants.
- ❖ A single gene deletion (for gene i) will decrease the fitness (e.g., the growth rate) from a value f_{wt} to a value f_i , leading to a growth defect $w_i = f_i / f_{wt} (\leq 1)$.

Modular Epistasis in Yeast Metabolism

- ❖ For a double deletion of unrelated genes i and j , we may expect a multiplicative effect $w_{ij}=w_iw_j$ (no epistasis).
- ❖ If the double deletion is more severe ($w_{ij}<w_iw_j$), we call the epistasis *aggravating*.
- ❖ If the double deletion is less severe ($w_{ij}>w_iw_j$), we call the epistasis *buffering*.
- ❖ Both cases of aggravating and buffering epistasis indicate functional associations between the genes in question.

Modular Epistasis in Yeast Metabolism

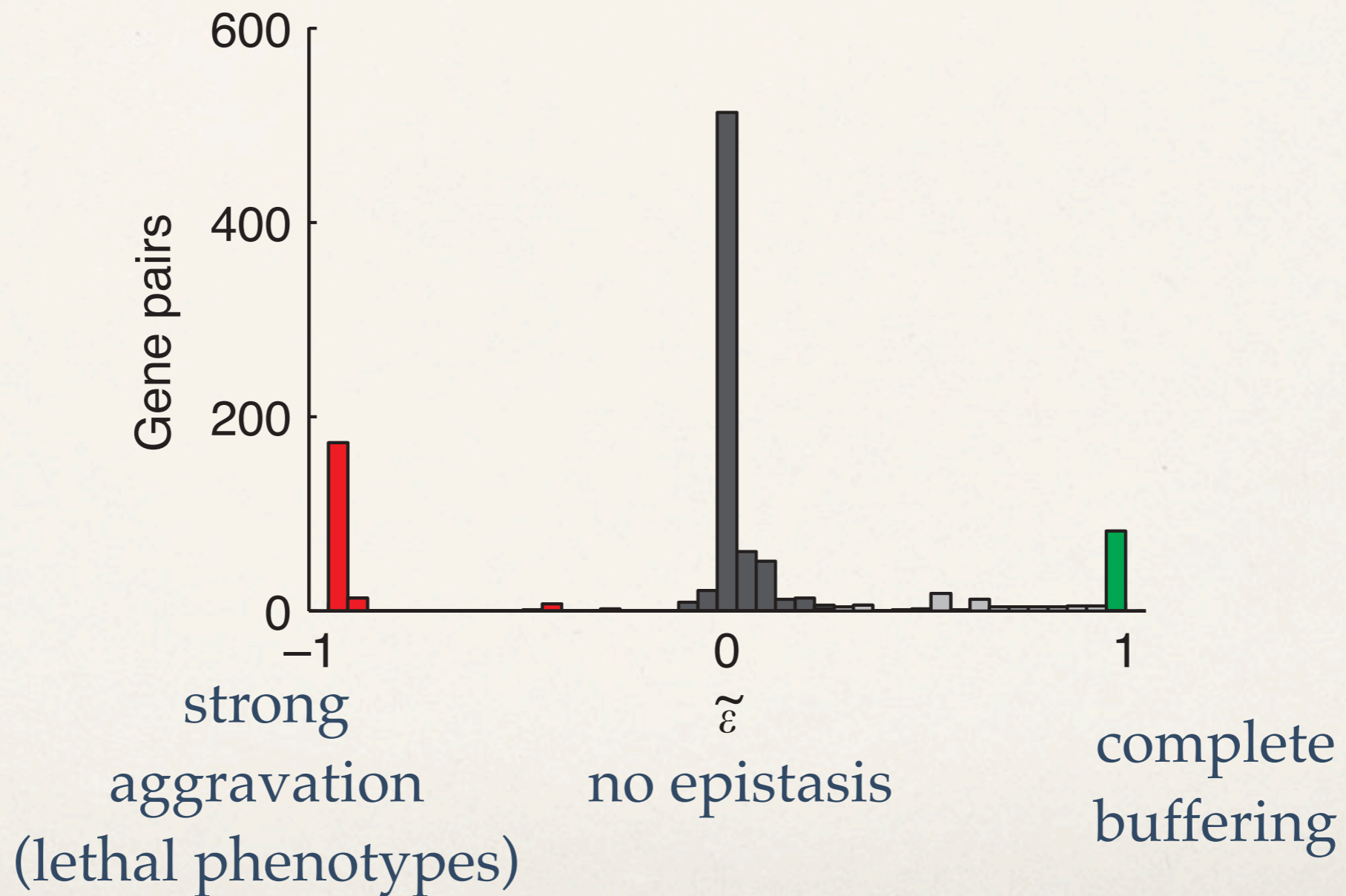
- ❖ Segre *et al.* (Nature Genetics, 37(1):77-83, 2005) recently used FBA to predict growth rates of the yeast *S. cerevisiae* and to calculate the epistatic effects between all metabolic genes.
- ❖ The model predicted relative growth defects of all single and double deletion mutants, from which they computed an epistasis measure for each pair of genes:

$$\hat{\epsilon}_{ij} = \frac{w_{ij} - w_i w_j}{|\hat{w}_{ij} - w_i w_j|}$$

extreme buffering extreme aggravation

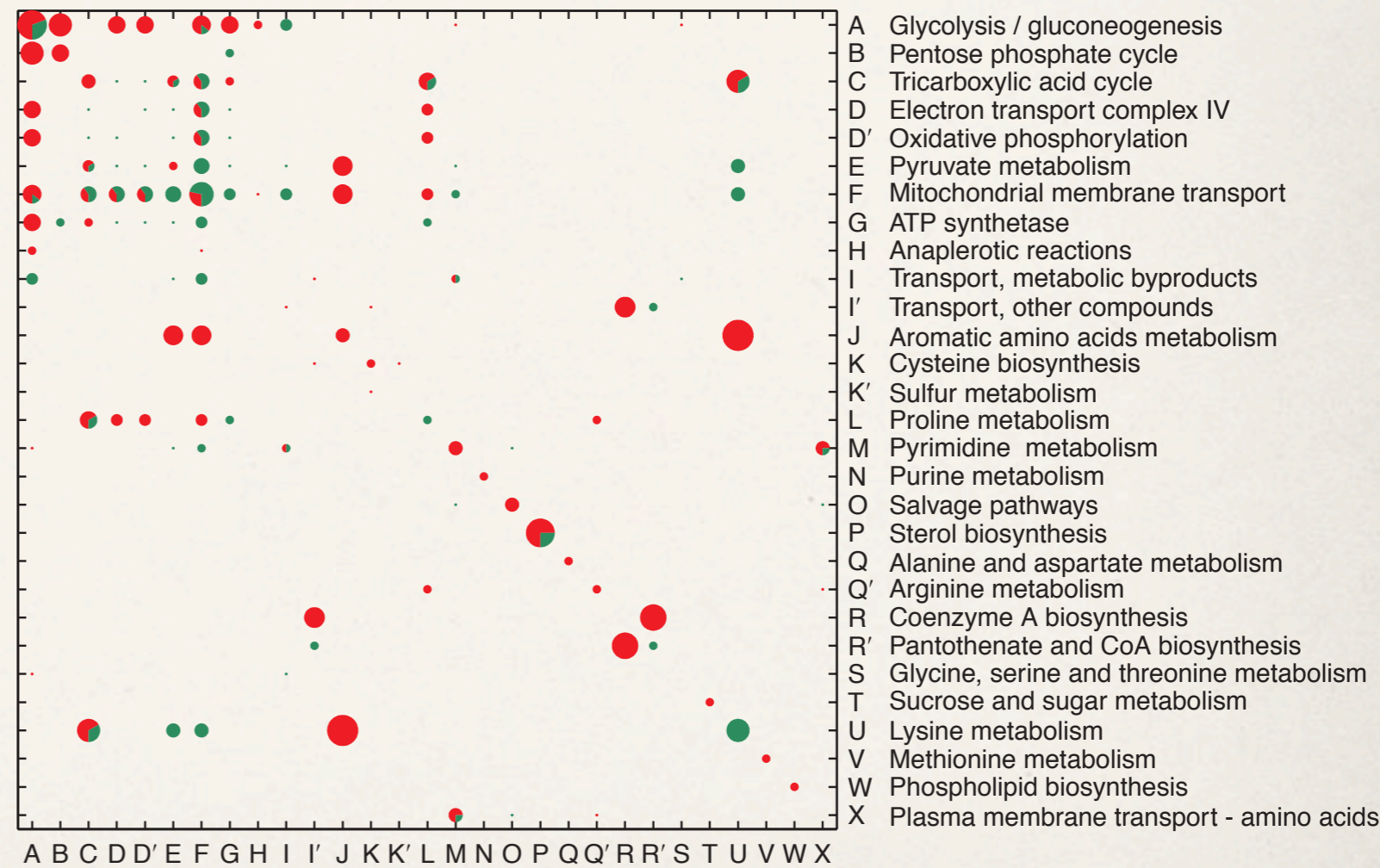
$\hat{w}_{ij} = \min\{w_i, w_j\}$ $\hat{w}_{ij} = 0$

Modular Epistasis in Yeast Metabolism



Modular Epistasis in Yeast Metabolism

Figure 2 Epistatic interactions between genes classified by functional annotation groups tend to be of a single sign (*i.e.*, monochromatic). (a) Representation of the number of buffering and aggravating interactions within and between groups of genes defined by common preassigned annotation from the FBA model. The radii of the pies represent the total number of interactions (ranging logarithmically from 1 in the smallest pies to 35 in the largest). The red and green pie slices reflect the numbers of aggravating and buffering interactions, respectively. Monochromatic interactions, represented by whole green or red pies, are much more common than would be expected by chance. (b) Sensitivity



The Interplay Between Metabolism and Gene Regulation

- ❖ Recently, Shlomi *et al.* (MSB 3:101) conducted a computational analysis of the interplay between metabolism and transcriptional regulation in *E. coli*.
- ❖ To enable such an analysis, the authors proposed a new method, *steady-state regulatory flux balance analysis* (SR-FBA), for predicting gene expression and metabolic fluxes in a large-scale integrated metabolic-regulatory model.

An Integrated Network

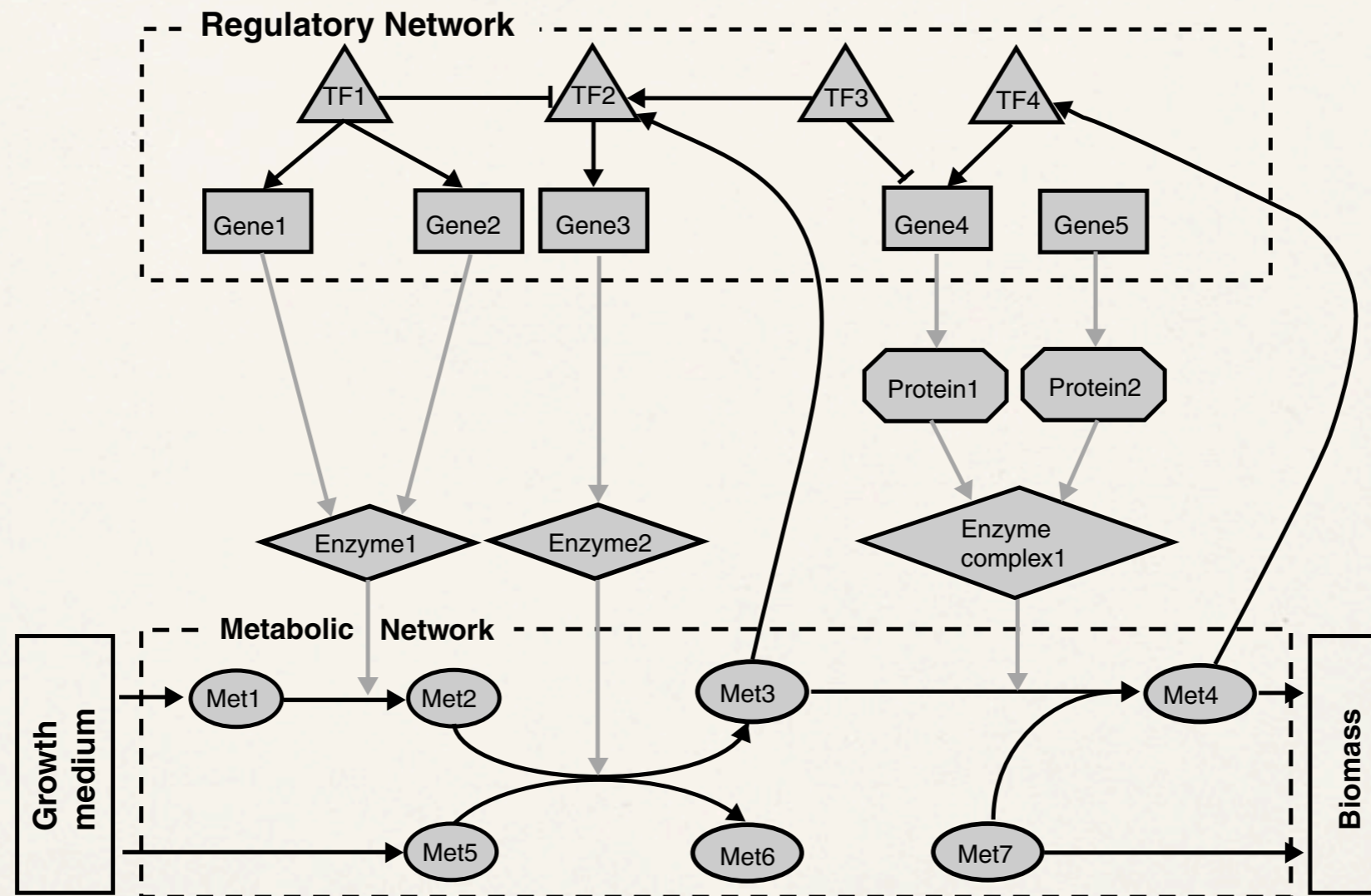


Figure 1 A schematic representation of an integrated metabolic and regulatory network. The regulatory network component consists of a set of interactions between TFs and other TFs and genes. The metabolic network component consists of a set of biochemical reactions between metabolites, with metabolites available from growth medium as input, and a pseudo-metabolite representing biomass production as output. The regulatory component affects the metabolic component through the expression of proteins that catalyze the biochemical reactions (downward pointing arrows). The metabolic component affects the regulatory component via the activation or inhibition of TF expression via the presence of specific metabolites (upwards arrows).

The SR-FBA Method

- * In addition to the metabolic constraints, there are
 - * *Regulatory constraints*: e.g., ' $G1 = \text{NOT}(\text{TF1}) \text{ AND } \text{TF2}$ ' (gene G1 is expressed if and only if TF1 is not expressed and TF2 is expressed).
 - * *Genes-to-reactions mapping constraints*: e.g., ' $R1 = (\text{P1 AND P2}) \text{ OR } (\text{P3 AND P4})$ ' (reaction R1 is catalyzed by either the enzyme complex P1-P2 or by the enzyme complex P3-P4).
 - * *Reaction enzyme state constraints*: The absence of a catalyzing enzyme for a specific reaction should constrain the flux through this reaction to zero.
 - * *Reaction predicates constraints*: The reaction predicate b_i represents a rule in the form ' $\text{FLUX}(j) > c$ ', where $c \in \mathbf{R}$.

The Interplay Between the Two Networks

- ❖ The combined functional state of the entire system in a given constant environment, referred to as *metabolic-regulatory steady state* (MRS), is described by a pair of consistent metabolic and regulatory steady states, which satisfy both the metabolic and regulatory constraints.
- ❖ The SR-FBA method identifies an MRS for the integrated metabolic-regulatory model.

The Interplay Between the Two Networks

- ❖ Each transcription factor (TF) and TF-regulated gene (i.e., genes associated with a regulatory role in the model) can be either in an *expressed* or *non-expressed state*, if it is expressed or non-expressed, respectively, in all alternative MRS solutions attainable within a given growth medium.
- ❖ In both cases, the genes are considered to have a *determined expression state*.
- ❖ Alternatively, the gene is considered to have an *undetermined expression state* if it is expressed in some of the alternative MRS solutions but non-expressed in others in the same medium.

The Interplay Between the Two Networks

- ❖ In parallel, each gene is characterized by its *flux activity state*, which reflects the existence of non-zero flux through one of the metabolic enzymatic reactions that it encodes.
- ❖ It can have a *determined* or *undetermined activity state*.
- ❖ Obviously, the expression and activity states are inter-dependent as a gene cannot be metabolically active if it is not expressed.

The Interplay Between the Two Networks

- ❖ Using the SR-FBA method, the author quantified the effect of transcriptional regulation on metabolism by measuring the fraction of genes whose flux activity is determined by the integrated model but not by the metabolic component alone.

The Interplay Between the Two Networks

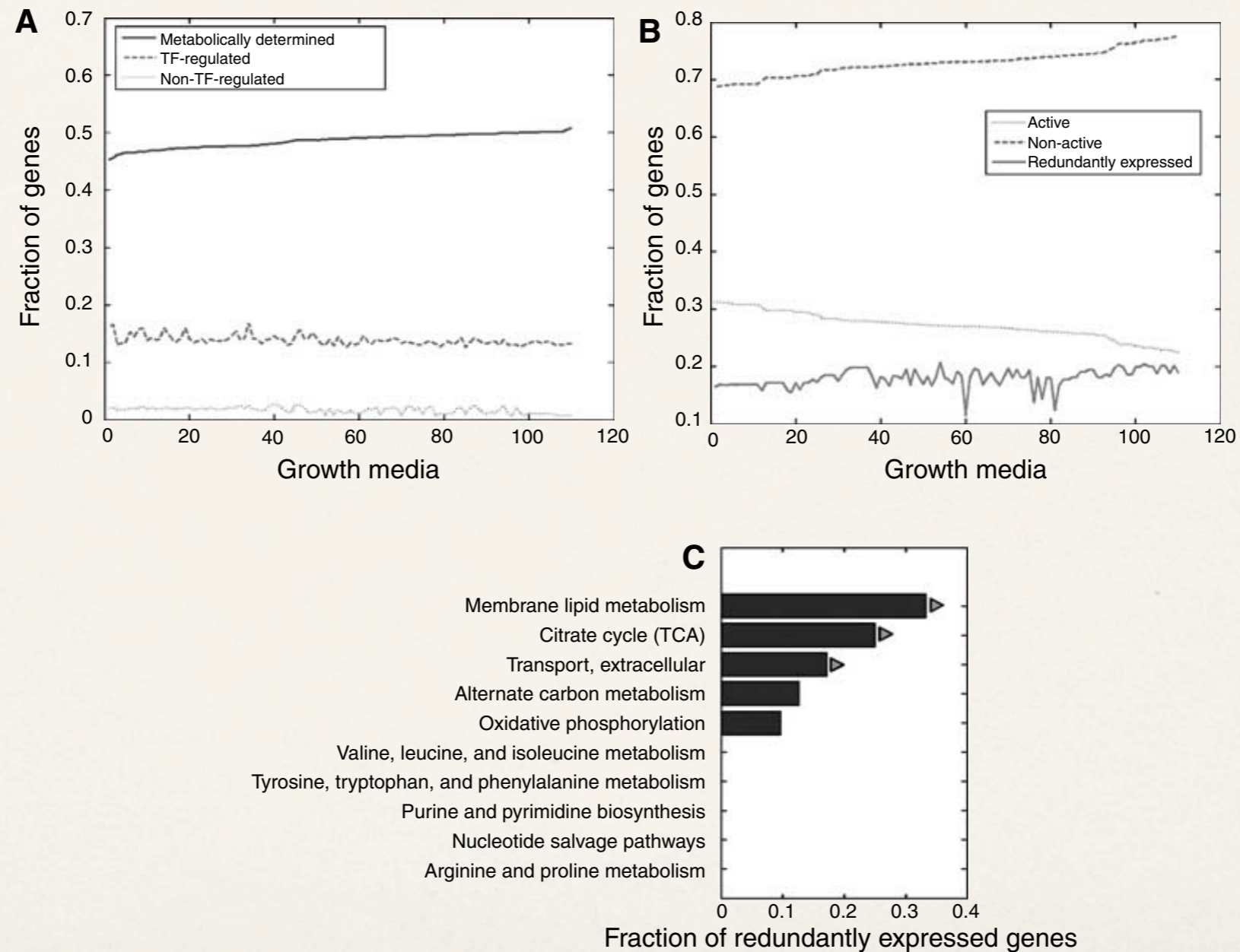


Figure 2 (A) The fraction of metabolic-determined genes and the fraction of regulatory-determined genes across different growth media. For the latter, we show the fraction of genes that are TF-regulated and the fraction of non-TF-regulated genes. (B) The fraction of genes that are metabolically determined to be active, inactive and redundantly expressed, from the set of metabolically determined genes. (C) The distribution of redundantly expressed genes within various functional metabolic categories. Triangles represent a statistically significant enrichment.

The Interplay Among Networks as Revealed by FBA

- ❖ Klipp et al., “Integrative model of the response of yeast to osmotic shock.” *Nature Biotechnology*, 23(8):975-982, 2005.
- ❖ Covert et al., “Integrating metabolic, transcriptional regulatory and signal transduction models in *Escherichia coli*.” *Bioinformatics*, 24(18): 2044-2050, 2008.
- ❖ Lee et al., “Dynamic analysis of integrated signaling, metabolic, and regulatory networks.” *PLoS Computational Biology*, 4(5), 2008.