

# Quantitative Assessment of Thermodynamics on the Solution Space of Genome-Scale Metabolic Models

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- 1 Introduction and Background
- 2 Incorporating Thermodynamics into Flux-Balance Analysis
- 3 Quantitative Assessment of Thermodynamics on the Solution Space
- 4 Conclusions



## Why models?

- Understanding cellular behavior
- Contextualize high-throughput data

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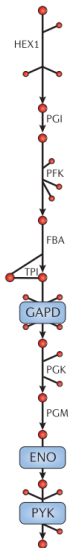
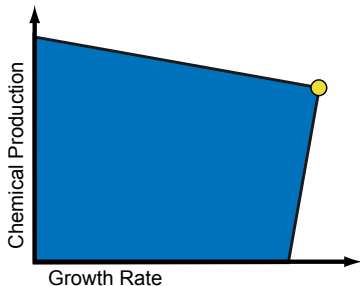
## Flux distribution subject to

- Steady-state mass balance constraints
- Limits on fluxes

## And want to

- Maximize growth

## Flux Balance Analysis (FBA)

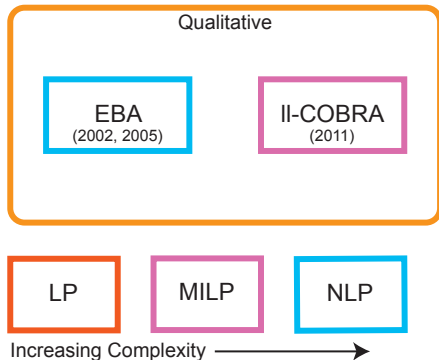


## Tighter Constraints on the Flux Space

- Elimination of thermodynamically infeasible loops:  $A \rightarrow B \rightarrow C \rightarrow A$

## Insight into Cellular Metabolism

- Thermodynamic feasibility of cellular pathways
- Quantitative information on intracellular concentrations

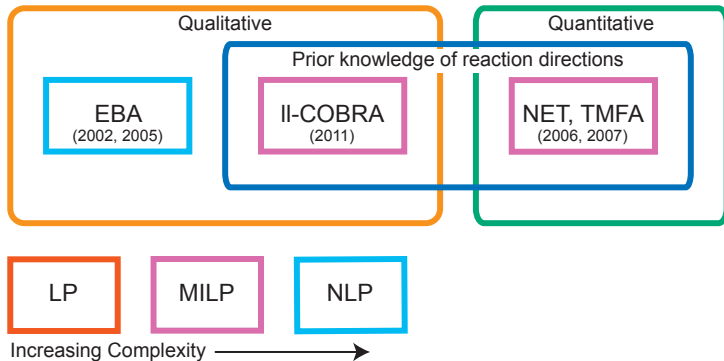


**EBA:** Beard, et al, *Biophys J* 2002.

**II-COBRA:** Schellenberger, et al, *Biophys J* 2011.

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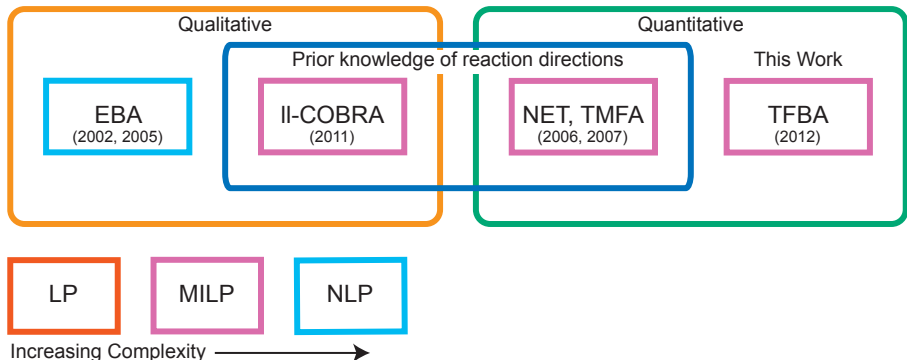
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# Incorporating Thermodynamics



Thermodynamic flux-balance analysis (TFBA) enables a *quantitative approach* to thermodynamics *without relying on prior knowledge of reaction directions*



**TFBA:** Hamilton et al, *under review*.





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## TFBA Formulation

**max** cellular growth

**s.t.** mass balance

all reactions bidirectional

$$\Delta_r G' \cdot v \leq 0$$

$\Delta_r G'$  calculation

$\Delta_r G'^0$  definition

global concentration bounds

**Transport:** Henry, et al, *Biophys J* 2007.

**GC Method:** Jankowski et al, *Biophys J* 2008.

**TFBA:** Hamilton, et al, *under review*.

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## MIP Formulation of $\Delta G_j \cdot v < 0$

$$v > 0 \implies \delta_{fwd} = 1$$

$$\Delta_r G' < 0 \implies \delta_{fwd} = 1$$

$$v < 0 \implies \delta_{rev} = 1$$

$$\Delta_r G' > 0 \implies \delta_{rev} = 1$$

$$\delta_{fwd} + \delta_{rev} \leq 1$$

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## Calculating $\Delta_r G'$

$$\Delta_r G' = \Delta_r G'^0 + RT \sum S \ln C + \Delta_t G'^0$$

## $\Delta_r G'^0$ by Group Contribution (GC)

$$\Delta_r G'^0 = \sum S \Delta_f G'^0$$

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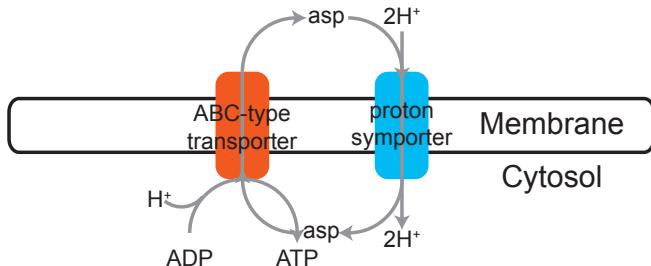


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# Elimination of ATP-Generating Cycles



- TFBA predicts ATP-generating cycles

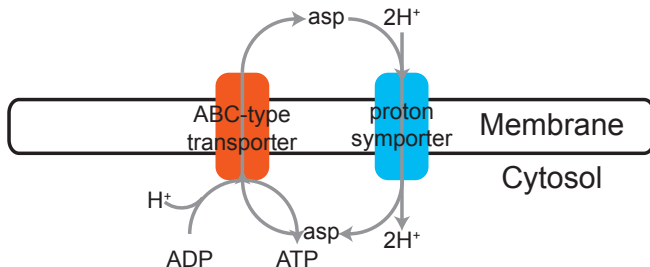


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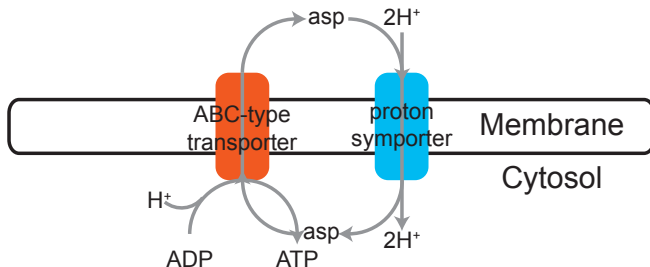
TFBA	$[x]_{min}$ (mM)	$[x]_{max}$ (mM)
ADP	0.095	20
ATP	0.01	2.1

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# Elimination of ATP-Generating Cycles



- TFBA predicts ATP-generating cycles
- Adding concentration constraints eliminates cycles



TFBA	$[x]_{\min}$ (mM)	$[x]_{\max}$ (mM)	$[x]_{\text{meas}}$ (mM)
ADP	0.095	20	0.56
ATP	0.01	2.1	<b>9.6</b>

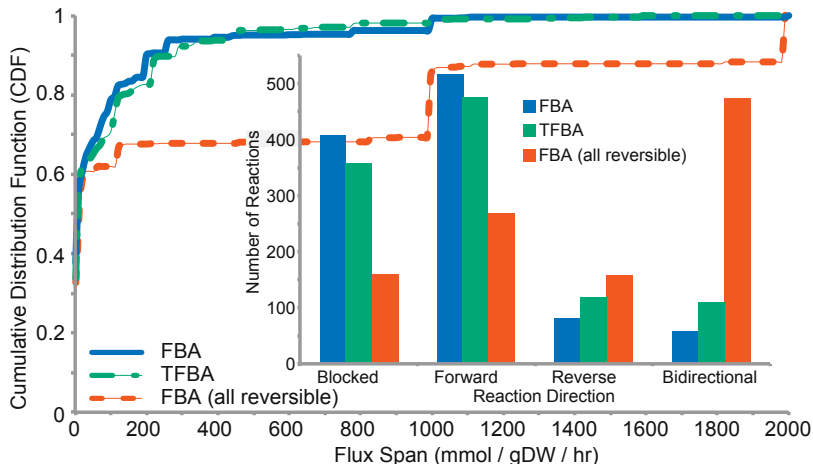
**TFBA:** Hamilton et al, *under review*.



# Effect of Thermodynamics on the Flux Space



- TFBA assumes all reactions can be bidirectional
- Thermodynamic interactions shrink the flux space



**TFBA:** Hamilton et al, *under review*.



- CONGA identifies single-gene deletions for which TFBA and FBA give different predictions (**G**rowth vs. **N**o **G**rowth)
- Better prediction in 7 cases: TFBA predicts a reversible reaction which FBA does not
- Worse prediction in 12 cases: TFBA predicts a reversible reaction which is physiologically irreversible
- Synthetic Lethal (SL) Finder identifies the reaction responsible for the phenotype difference

**CONGA:** Hamilton and Reed, *PLoS ONE* 2011.

**TFBA:** Hamilton, et al, *under review*.

**Exp. Data:** Baba, et al, *Mol Syst Biol* 2006.

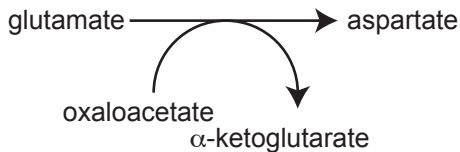
**SL Finder:** Suthers, et al, *Mol Syst Biol* 2009.

# Analyzing Better Predictions



- $\Delta aspC$  predicted to be rescued by *aspA*
- Constructed  $\Delta aspC \Delta aspA$  mutant

*aspC*: aspartate aminotransferase



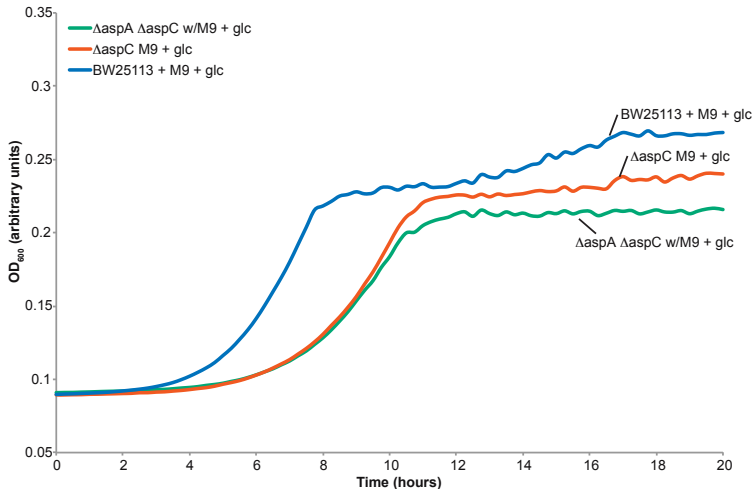
*aspA*: aspartate-ammonia lyase



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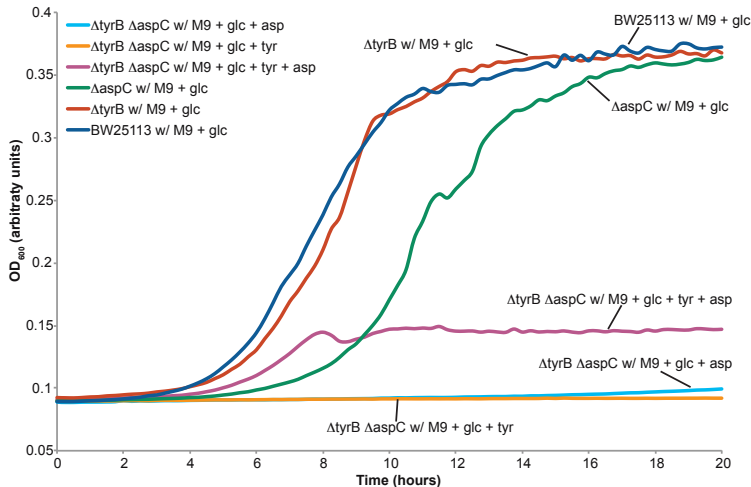
- $\Delta aspC \Delta aspA$  mutant proved viable
- Sequence similarity suggested *tyrB* as an isoform for *aspC*



# Analyzing Better Predictions



- $\Delta aspC \Delta tyrB$  mutant proved nonviable - *tyrB* is an isoform for *aspC*
- Constrain the direction of *aspC*





## Batch Culture

	Uncons.	Cons.	Total
Overlap	46	33	79
No Over.	9	19	28
No Data	409	96	505
Total	474	138	612

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# Using TFBA to Predict Metabolite Concentrations

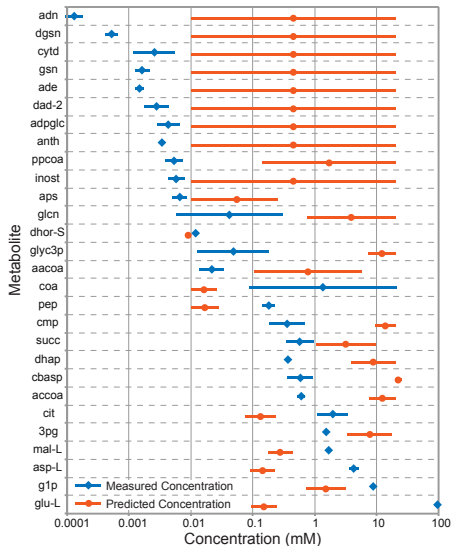


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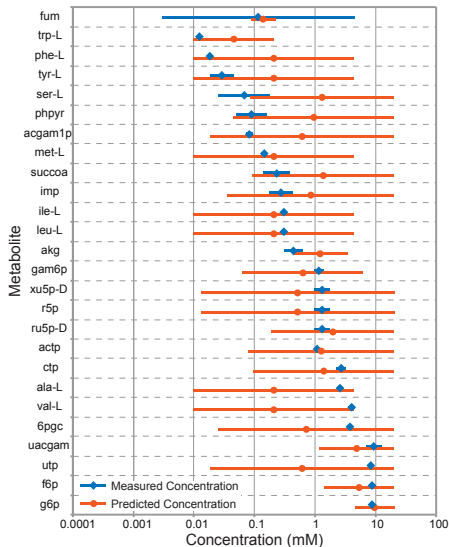


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## Prof. Jennifer Reed

- Dr. Dave Baumler
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- Wai Kit Ong
- Chris Tervo
- Trang Vu
- Xiaolin Zhang
- Undergraduate Students
  - **Vivek Dwivedi**
  - Mink Arunrattanamook
  - John de Friel



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