

# Modeling stable metabolic interactions in microbial communities

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# Content

- ❑ Introduction to metabolic modeling of microbial communities
- ❑ Incorporation of steady-state composition
- ❑ Nash equilibrium in microbial community metabolism

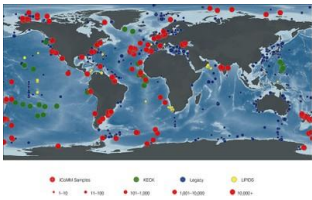
# Microbial communities

The most pervasive life form on the planet

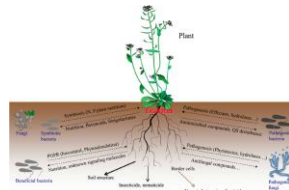
- $\sim 10^{30}$  prokaryotic cells on earth vs.  $\sim 10^{24}$  stars in the universe  
(Whitman *et al*, Proc Natl Acad Sci U S A, 1998)



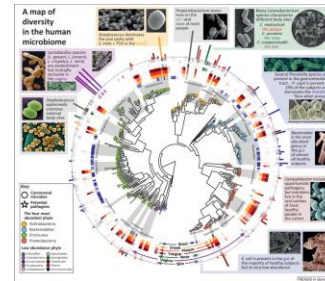
- Oceans, soil, plants, human body



Amaral-Zettler, 2010



Haichar *et al*, Soil Biol. Biochem., 2014



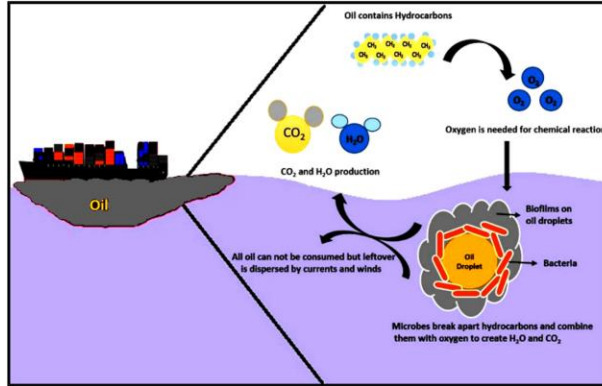
Morgan *et al*, Trends Genet., 2013

- Volcanoes, Great Salt Lake, hot springs, acid mine drainage



# Applications

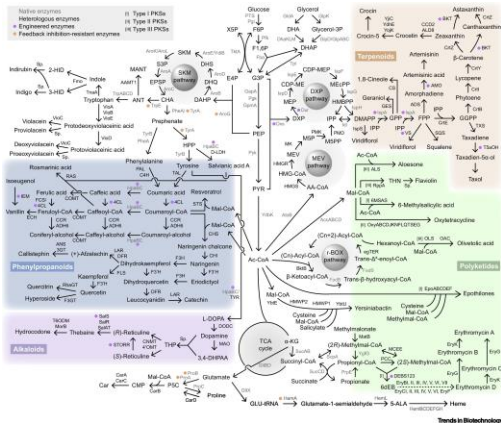
## □ Bioremediation



Oil spill bioremediation  
Balan et al., 2021, Archives of Microbiology

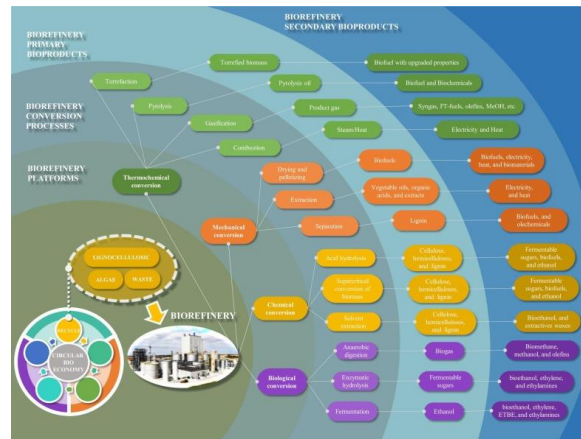
To name a few...

## □ Chemical production



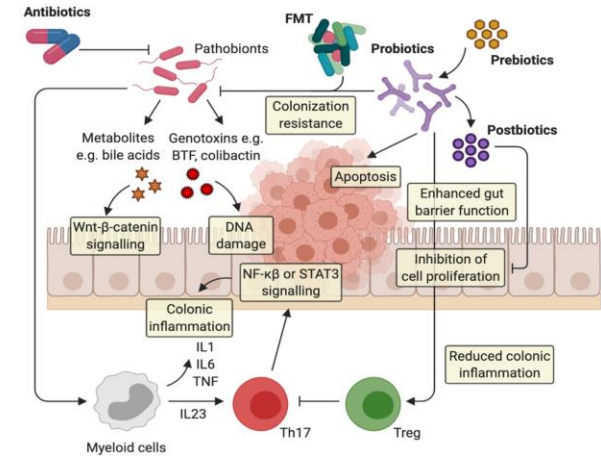
Natural products produced from *E. coli*  
Yang et al., 2020, Trends in Biotech.

## □ Biorefinery



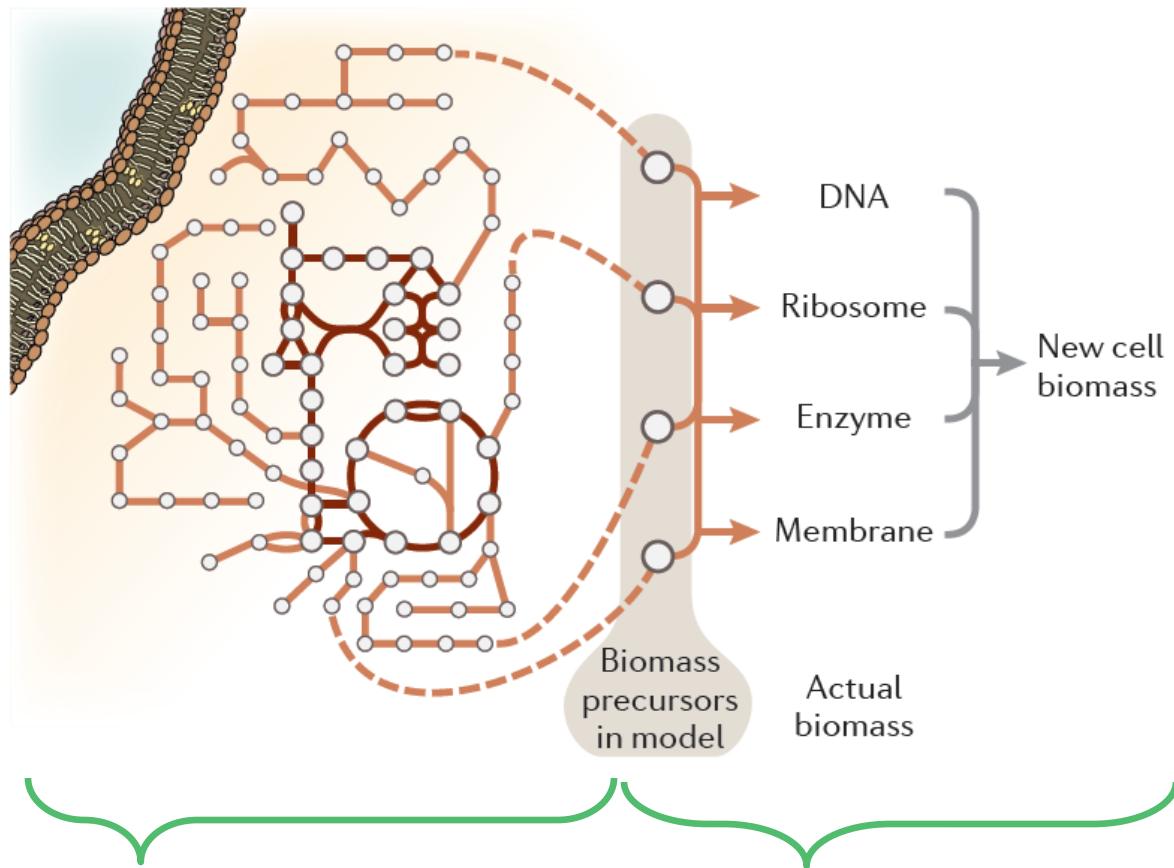
Ubando et al., 2020, Bioresource Tech.

## □ Biomedical



Gut Microbiota Manipulation for Colorectal Cancer Management  
Ubando et al., 2020, Bioresource Tech.

# Genome-scale metabolic Models (GEMs)



[Lewis et al., Nat Rev Microb, 2012](#)

## Metabolic reactions

identified from  
genome sequence



## Biomass composition

derived from experimental  
data in terms of  
macromolecules

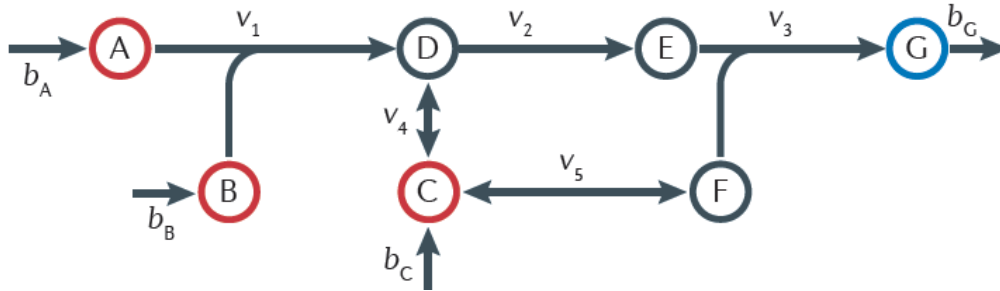


## Genome-scale metabolic model

that can describe  
cell growth

# Flux Balance Analysis (FBA)

a Reaction network



b Stoichiometric matrix

	$v_1$	$v_2$	$v_3$	$v_4$	$v_5$	$b_A$	$b_B$	$b_C$	$b_G$
A	-1	0	0	0	0	1	0	0	0
B	-1	0	0	0	0	0	1	0	0
C	0	0	0	-1	-1	0	0	1	0
D	1	-1	0	1	0	0	0	0	0
E	0	1	-1	0	0	0	0	0	0
F	0	0	-1	0	1	0	0	0	0
G	0	0	1	0	0	0	0	0	-1
Lower	0	0	0	$V_{m,r}$	$V_{m,r}$	0	0	0	0
Upper	$V_m$	$V_m$	$V_m$	$V_m$	$V_m$	1	$\infty$	$\infty$	$\infty$

Steady state assumption:

$$\frac{dx_i}{dt} = \sum_{\text{all reaction } j} S_{ij}v_j = 0$$

for all metabolite  $i$

Balance of D:

$$(1)v_1 + (-1)v_2 + (1)v_4 = 0$$

Reaction directionality:

$$v_{irreversible} \geq 0$$

Substrate available:

$$b_A \leq 1$$

[Lewis et al., Nat Rev Microb, 2012](#)

# Flux Balance Analysis (FBA)

Maximize  $v_{biomass}$

(Biological objective function)

subject to

$$\sum_{j=1}^M S_{ij} v_j = 0,$$

for all metabolite  $i$

(Steady state assumption)

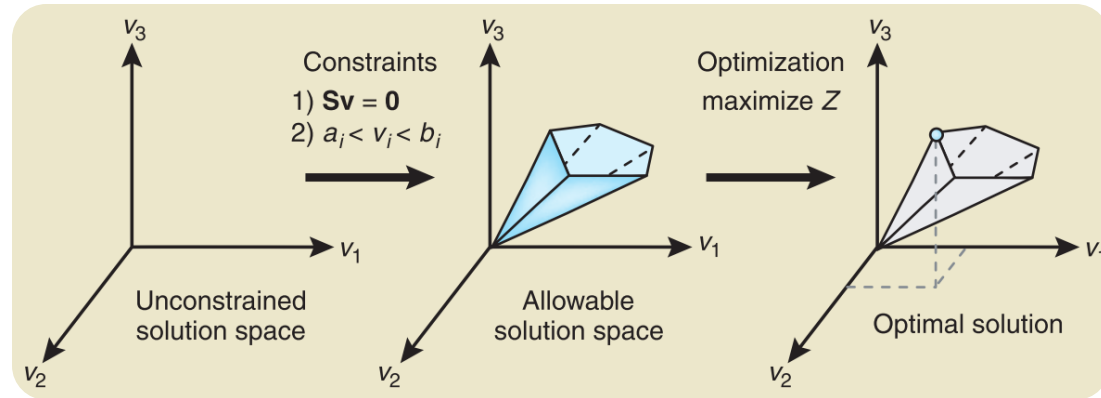
$$LB_j \leq v_j \leq UB_j$$

for all reaction  $j$

(Thermodynamic feasibility,  
nutrient availability)

Predict the max. biomass yield and metabolite productions using:

- Reaction stoichiometry and reversibility
- Macromolecular composition of the biomass

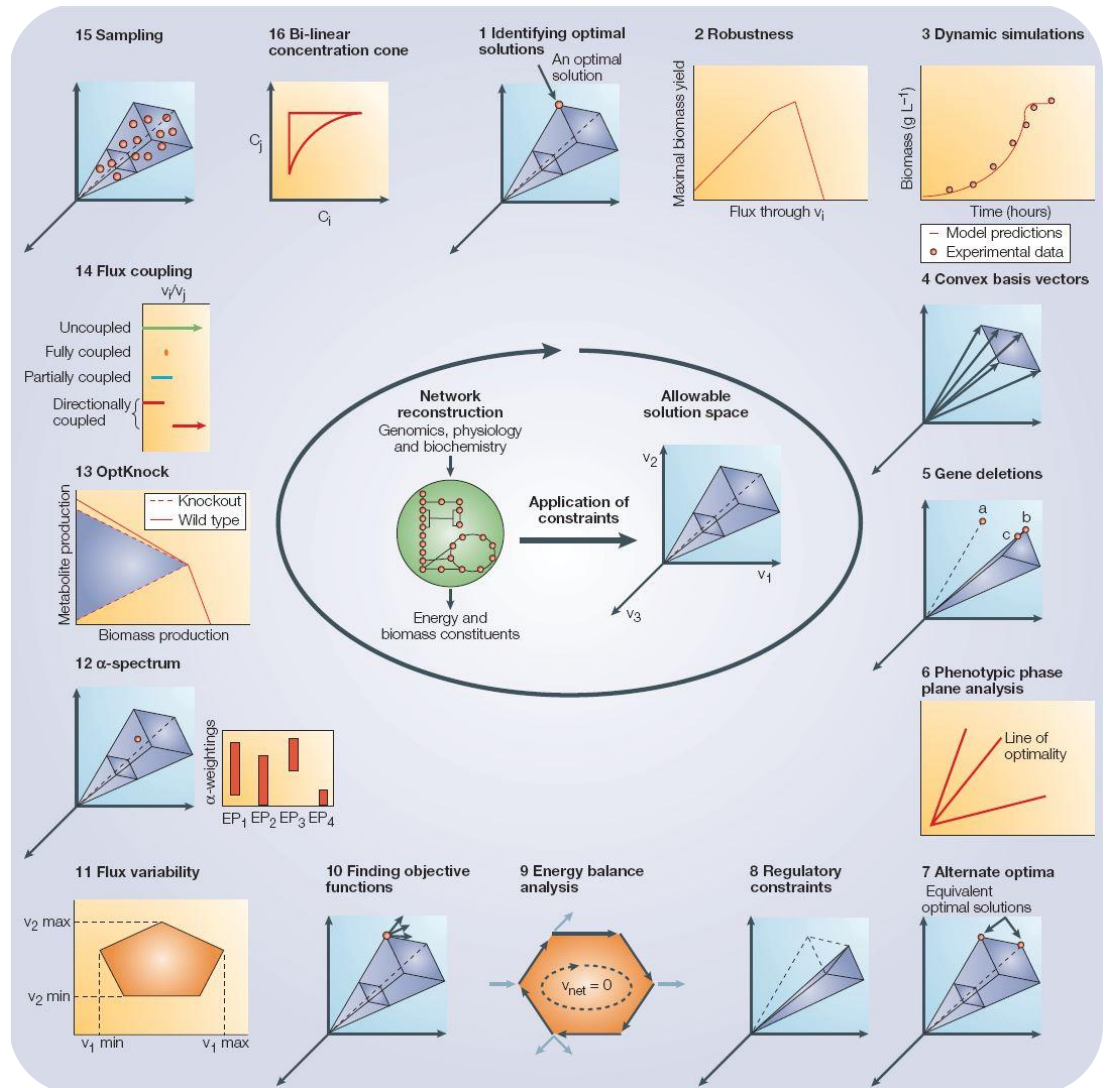


(Orth *et al.*, Nat. Biotechnol., 2010)



# Constraint-Based modeling techniques

FBA-based modeling techniques have been developed and become a toolbox for simulating cellular metabolism and predicting engineering strategies.



(Price *et al.*, Nat. Rev. Microb., 2004)



# Metabolic modeling of microbial communities

- Goal: describe and predict community metabolism and interactions using genome-scale metabolic models (GEMs)

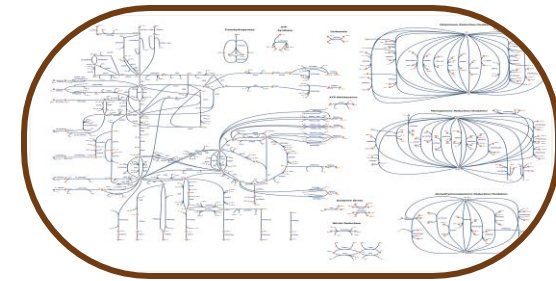
- **Supraorganism model:**

The microbiome as a single organism

Metagenome



MetaGEM



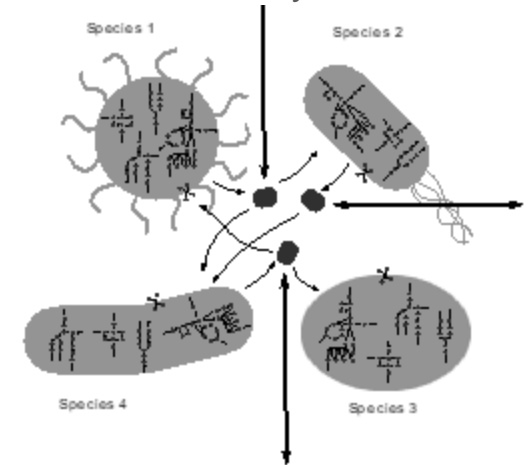
- **Multi-organism model:**

Model compartmentalized by taxa or representative species

Metagenome-assembled genomes/  
representative genomes



Community GEM



# Direct extension of FBA (Joint-FBA)

Intracellular steady state

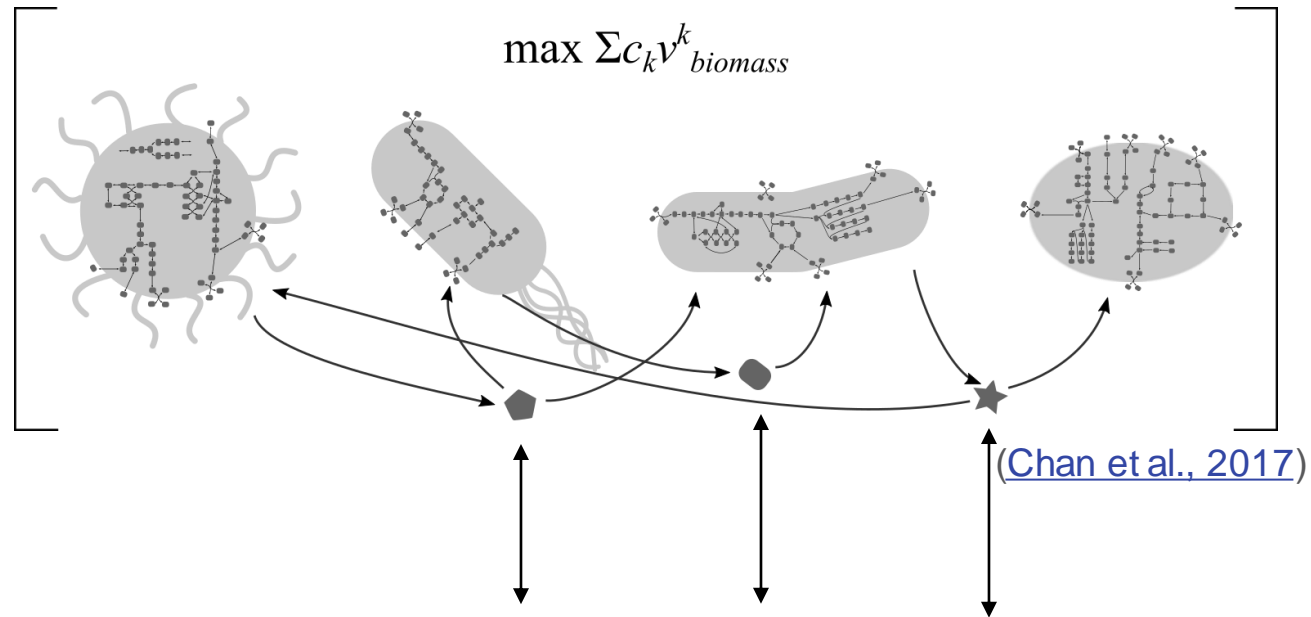
$$\sum_j S_{ij}^k v_j^k = 0$$

$$\forall i \in \mathbf{I}^k, k \in \mathbf{K}$$

Community exchange

$$\sum_k v_{ex,i}^k \geq lb_i^{uptake}$$

$$\forall i \in \mathbf{I}^{ex}$$



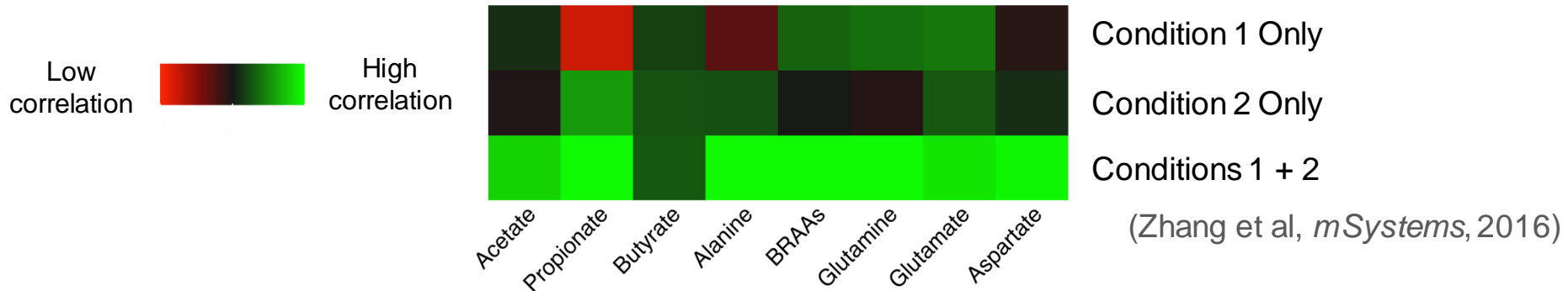
- ❑ FBA when applied to microbial communities simply maximizes the sum of all biomass production rate as one large compartmentalized model

# Testing hypotheses about microbiome metabolism

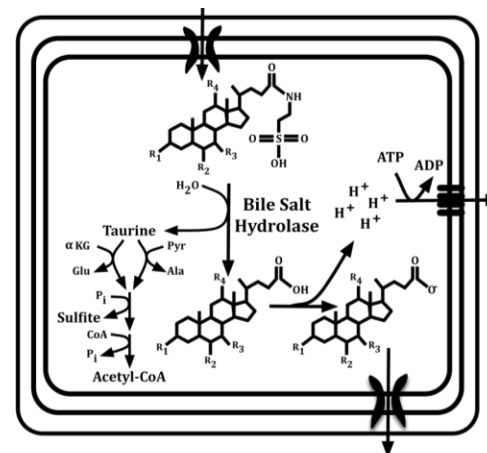
Test predicted production of short-chain fatty acids (SCFAs) and AAs if the following conditions are imposed in a ten-species gut community model:

1. **Bile salt hydrolase (BSH) activity** proportional to **Clostridia** and ***Lactobacillus***
2. **Community growth inhibited** in the presence of a drug (glycine- $\beta$ -muricholic acid )

Comparing Community Exported Metabolites to Experimental Measurements



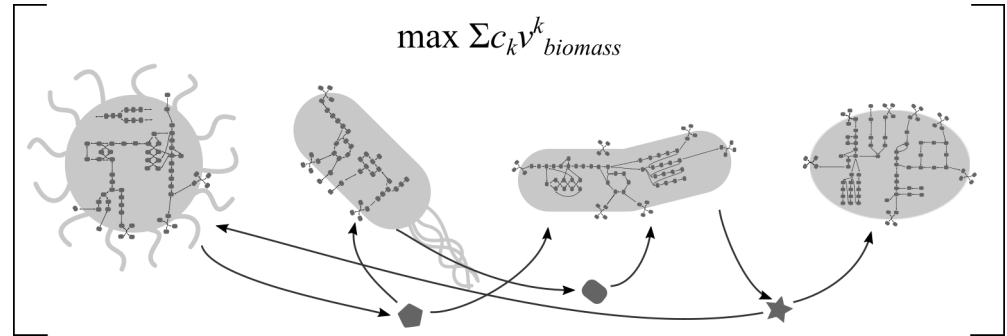
Taurine released by BSH activity becomes additional carbon and nitrogen sources for microbes



Sonomoto, et al., 2011

# Issues of Joint-FBA

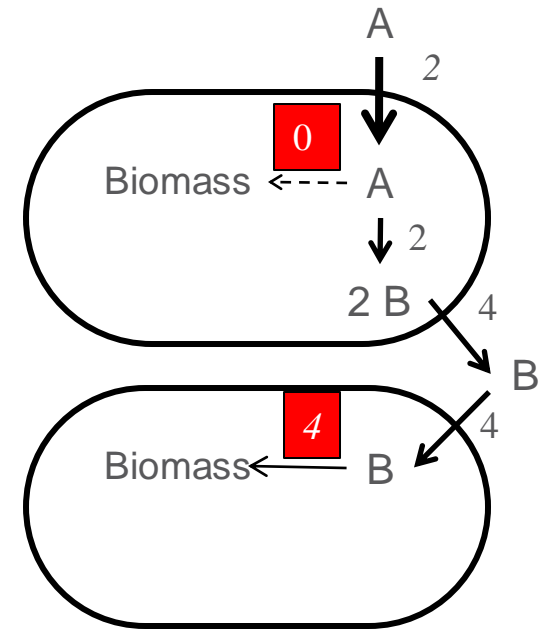
Joint-FBA: maximizes the sum of all biomass production rate



- ❑ **Forced altruism:** bias toward organisms with higher biomass yields through unrealistic cross feeding
- ❑ Partially mitigated by imposing an equal specific growth rate for all community members as a consequence of steady-state composition:

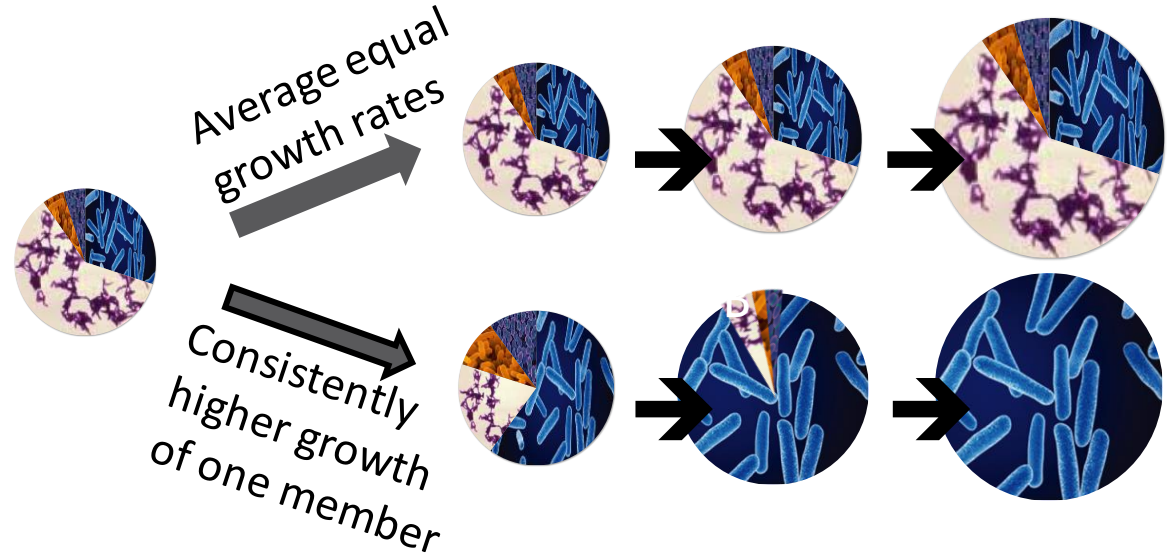
$$\frac{dX_k}{dt} = (\mu_k - D)X_k \quad x_k = \frac{X_k}{\sum_k X_k}$$

→ replicator equation:  $\frac{dx_k}{dt} = x_k \left( \mu_k - \sum_k \mu_k x_k \right)$

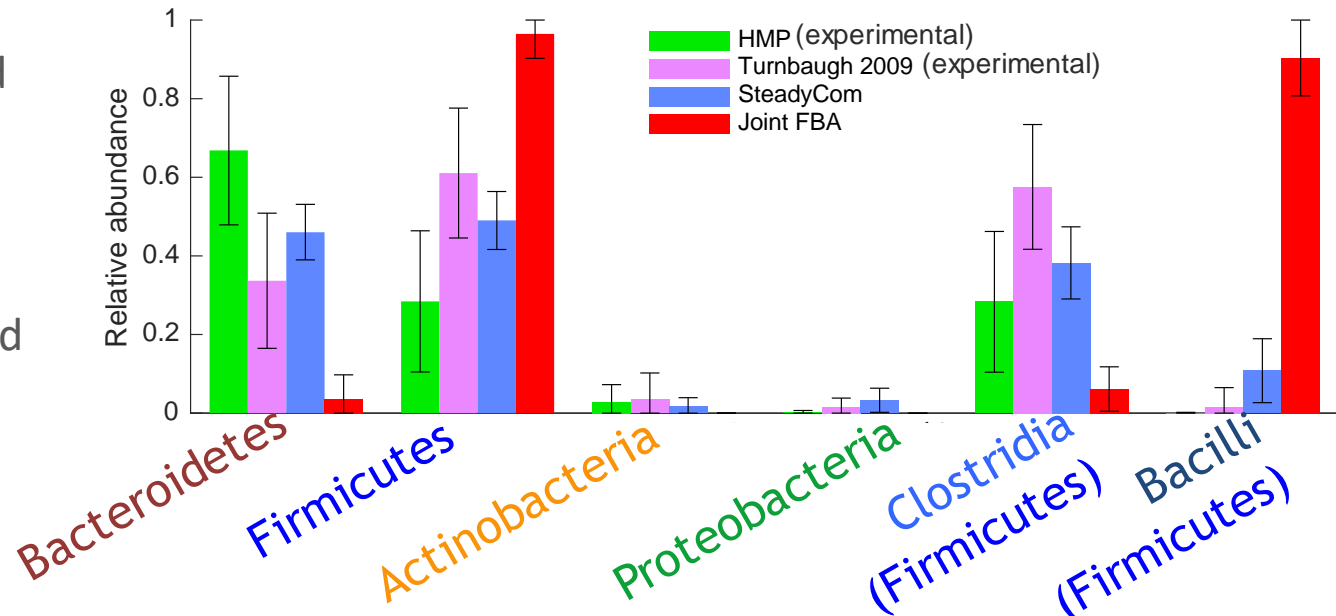


# Improvement: steady-state composition

- Community FBA (cFBA, Khandelwal, 2013)
- SteadyCom (Chan, 2017)



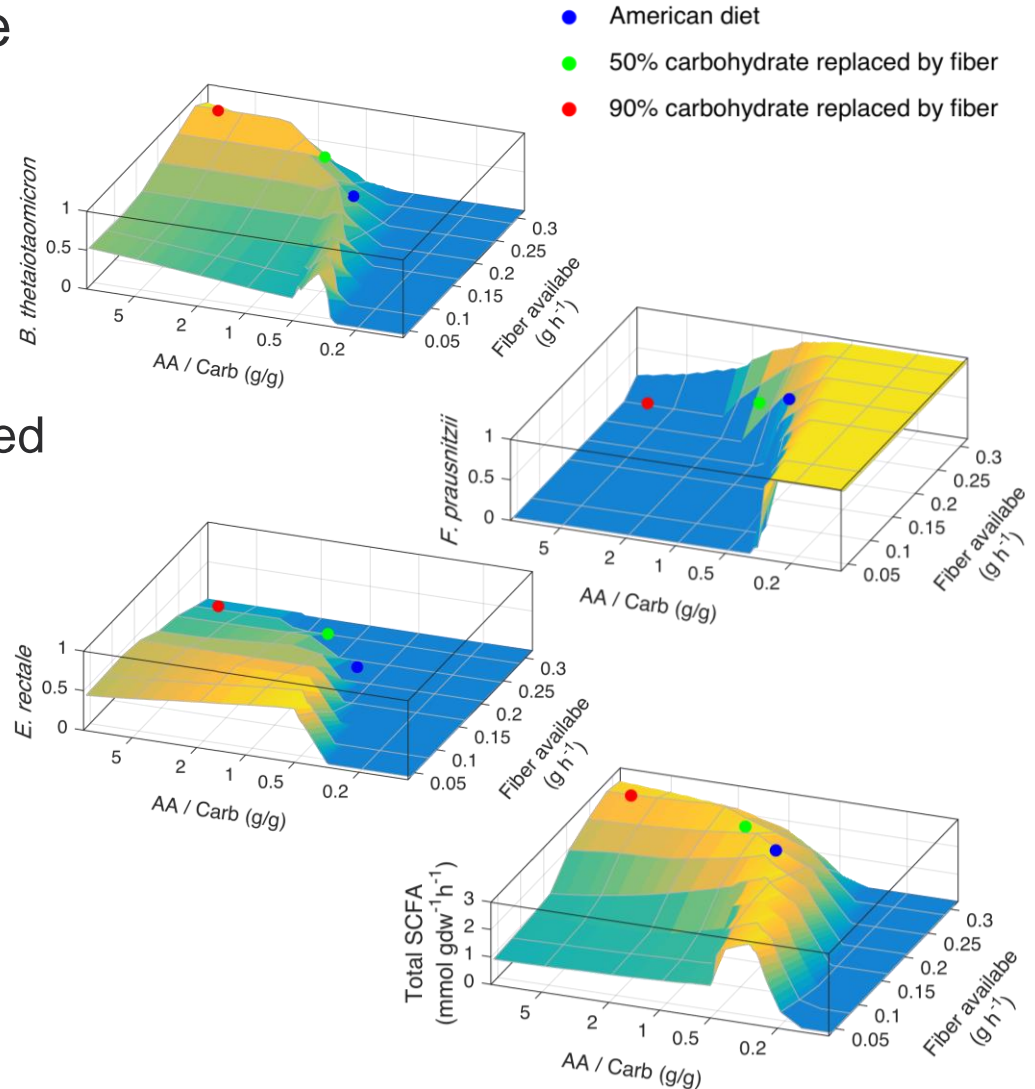
- Dominance by **Bacteroidetes** and **Firmicutes**
- Low but non-zero abundance **Actinobacteria** and **Proteobacteria**



# Assessing the impact of diet

Varying the nutrients available to the gut microbiome model:

- ❑ **Cross-feeding** (e.g., fiber-derived carbohydrates, acetate, H<sub>2</sub>S) and **Short-chain fatty acids (SCFAs)** are observed only when **Bacteroidetes** and **Clostridia** are interacting.
- ❑ **SCFA** productions generally **increase with fiber** consumption, consistent with experimental observations .  
(de Filippo *et al.*, PNAS, 2010, Shen *et al.*, Eur J Nutr, 2012)



# SteadyCom (Chan et al., PLOS Comput Biol, 2017)

$\mu$ : Community growth rate  
 $X^k$ : Biomass of organism k  
 $S_{ij}^k$ : Stoich. Coefficient of met i in rxn j for org. k  
 $LB_j^k$ : Lower bound  
 $UB_j^k$ : Upper bound  
 $r_{NGAM}^k$ : Non-growth associated maintenance

max  $\mu$

s.t.  $\sum_{j=1}^N S_{ij}^k V_j^k = 0$

$\forall i = 1, \dots, M$

$LB_j^k X^k \leq V_j^k \leq UB_j^k X^k$

$\forall j = 1, \dots, N$

$V_{NGAM}^k = r_{NGAM}^k X^k$  (proper scaling of fluxes)

$V_{biomass}^k = \mu X^k$  (steady-state composition)

$\forall k = 1, \dots, K$

$\sum_{k=1}^K V_{exchange(i)}^k + uptake_i - export_i = 0 \quad \forall i = 1, \dots, M^{exchange}$

$k = 1, \dots, K$  Organisms  
 $i = 1, \dots, M$  Metabolites  
 $j = 1, \dots, N$  Reactions

$\sum_{k=1}^K X^k = 1$  (relative to 1 gDW of community biomass)

## Units

$V_j^k$ : mmol hr<sup>-1</sup>

$V_{biomass}^k$ : gDW hr<sup>-1</sup>

$X^k$ : gDW

$\mu$ : hr<sup>-1</sup>

**Joint FBA**

max  $\sum_{k=1}^K v_{biomass}^k$

s.t.  $\sum_{j=1}^N S_{ij}^k v_j^k = 0 \quad \forall i = 1, \dots, M$

$LB_j^k \leq v_j^k \leq UB_j^k \quad \forall j = 1, \dots, N$

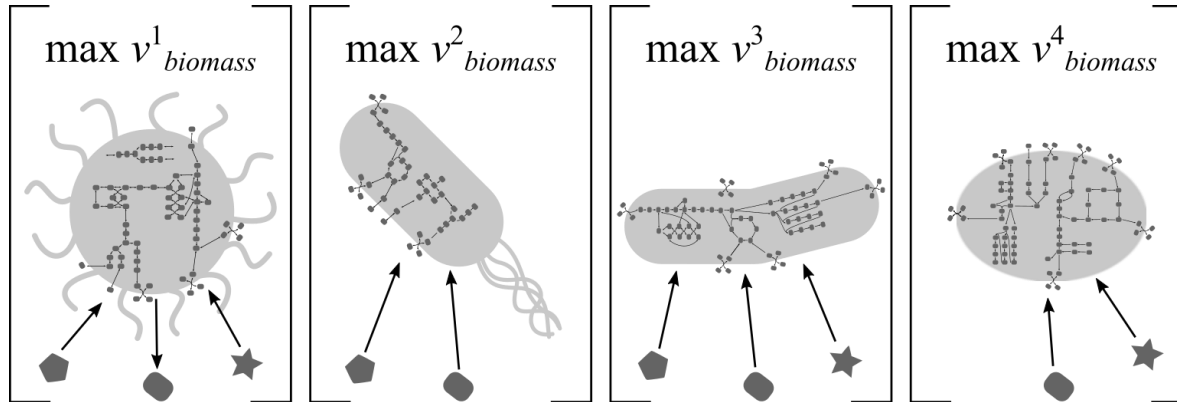
$v_{NGAM}^k = r_{NGAM}^k$

$\sum_{k=1}^K v_{exchange(i)}^k + uptake_i - export_i = 0 \quad \forall i = 1, \dots, M^{exchange}$

$\forall k = 1, \dots, K$



# Dynamic simulations - dynamic FBA (dFBA)



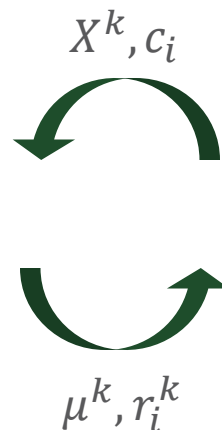
One optimization per organism

- ❑ dynamic extracellular environment
- ❑ Solve FBA-embedded diff. eqns. to update biomass and metabolite levels

At each time step  $t$ :

Predict growth rate and metabolite conversion rate given extracellular metabolite concentrations using FBA:

$$\mu^k, r_i^k = FBA \left( GEM_k, v_{i,uptake}^k (X^k, c_i) \right)$$



Update extracellular metabolite concentrations and biomasses using the differential population and metabolite balances:

$$\frac{dX^k}{dt} = \mu^k X^k$$

$$\frac{dc_i}{dt} = \sum_k X^k r_i^k$$

# Integrating steady-state and dynamic FBA

(Chan et al., 2019)

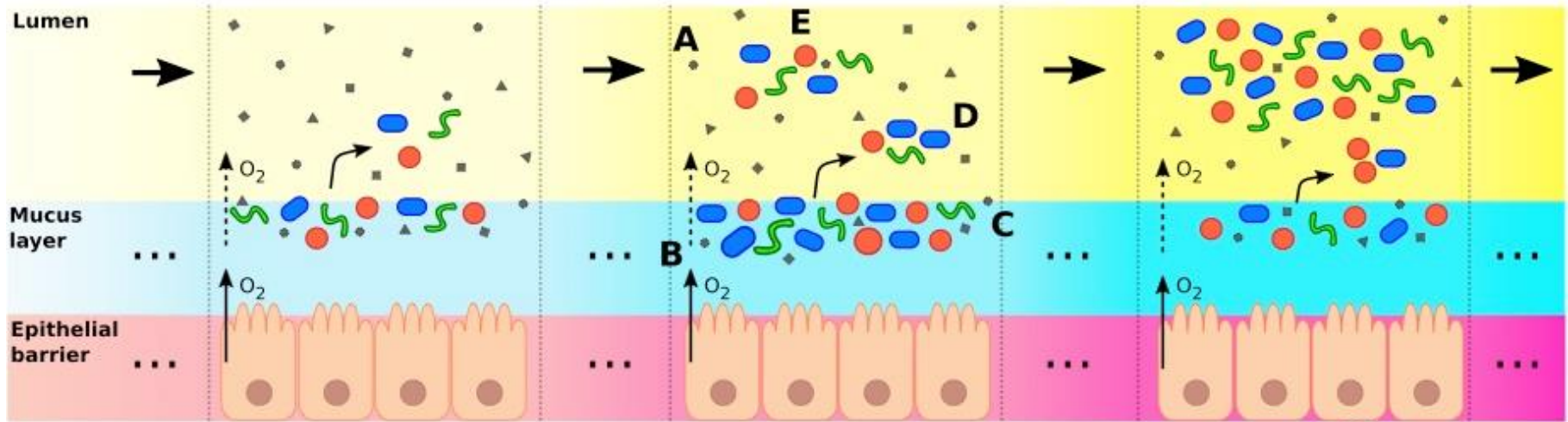
Section  $s - 1$   
in the intestines

Luminal contents from  
the previous section

Section  $s$   
in the intestines

Luminal contents flowing  
to the next section

Section  $s + 1$   
in the intestines



● ● ● microbes  
● ■ ▲ metabolites

$$dFBA(X_{lum}^{k,s}(t), c_i^s(t), r_{oxygen}^{total,s}, \alpha_{oxygen}^{lum,s})$$

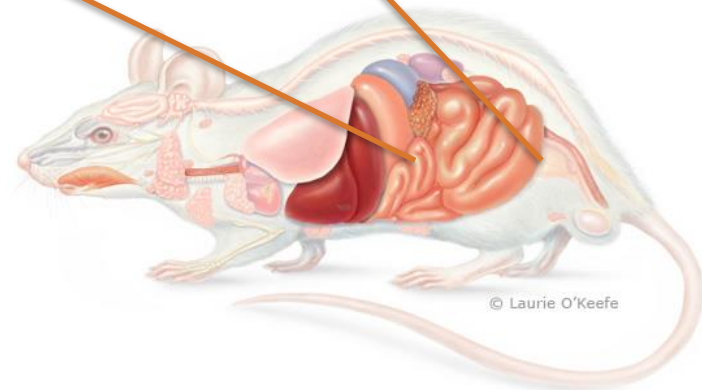
For  $t^s \in [0, T^s], s = 1, \dots, 7$

$$\begin{aligned} X_{lum}^{k,s}(0) &= X_{lum}^{k,s-1}(T^{s-1}) \\ c_i^s(0) &= c_i^{s-1}(T^{s-1}) \end{aligned}$$

$$\begin{aligned} \mu_{lum}^{k,s}, v_{ex}^{k(i)} \\ \mu_{muc}^{k,s}, v_{ex}^{k(i)} \end{aligned}$$

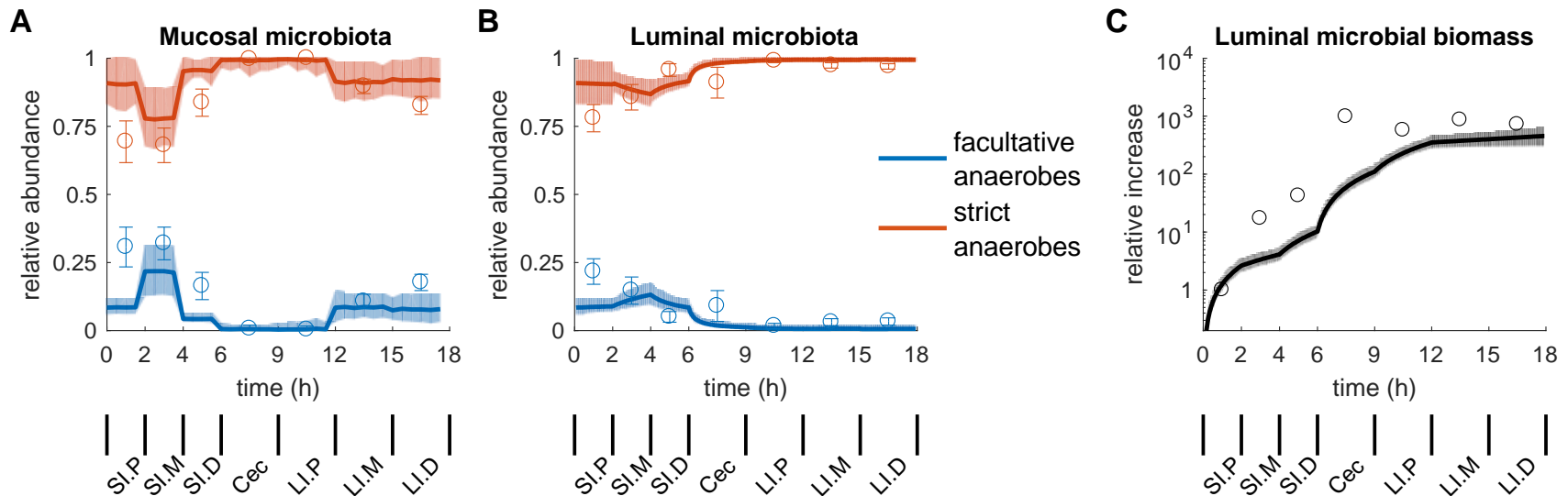
$$\begin{aligned} \frac{dX_{lum}^{k,s}}{dt^s} &= \mu_{muc}^{k,s} X_{muc}^{k,s} + \mu_{lum}^{k,s} X_{lum}^{k,s} \\ \frac{dc_i^s}{dt^s} &= \sum_{k \in K} (v_{ex}^{k(i)} + X_{lum}^{k,s} v_{ex}^{k(i)}) \end{aligned}$$

$$\text{SteadyCom}(X_{muc}^{total,s}, c_i^s(t), r_{oxygen}^{total,s}, \alpha_{oxygen}^{lum,s})$$



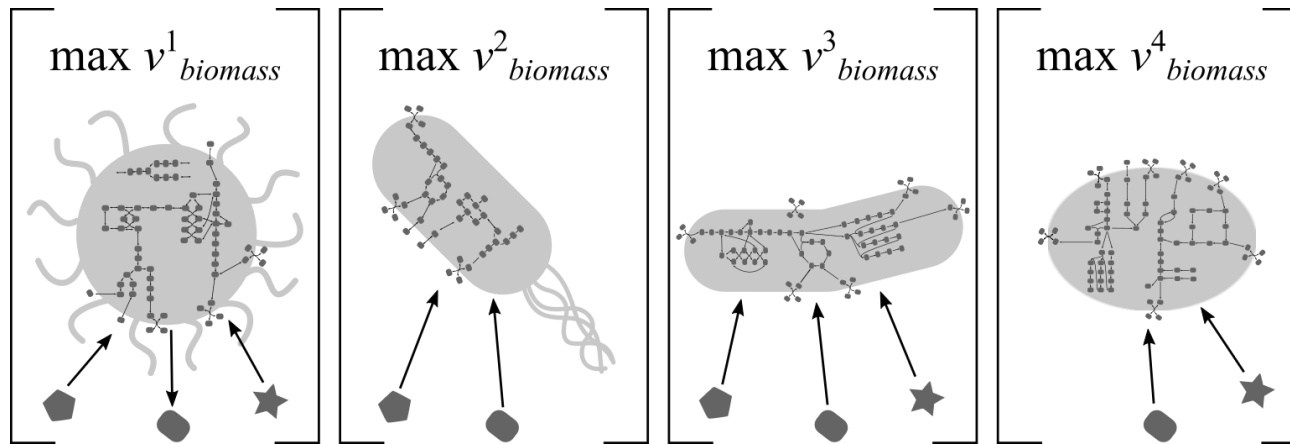
© Laurie O'Keefe

# Integrating steady-state and dynamic FBA



- ❑ Capture strict vs. facultative anaerobes and luminal biomass accumulation
- ❑ **Oxygen-to-biomass ratio** on the mucus layer appears to be an important force shaping the spatial organization of aerobes vs. anaerobes

# Issues of dynamic FBA

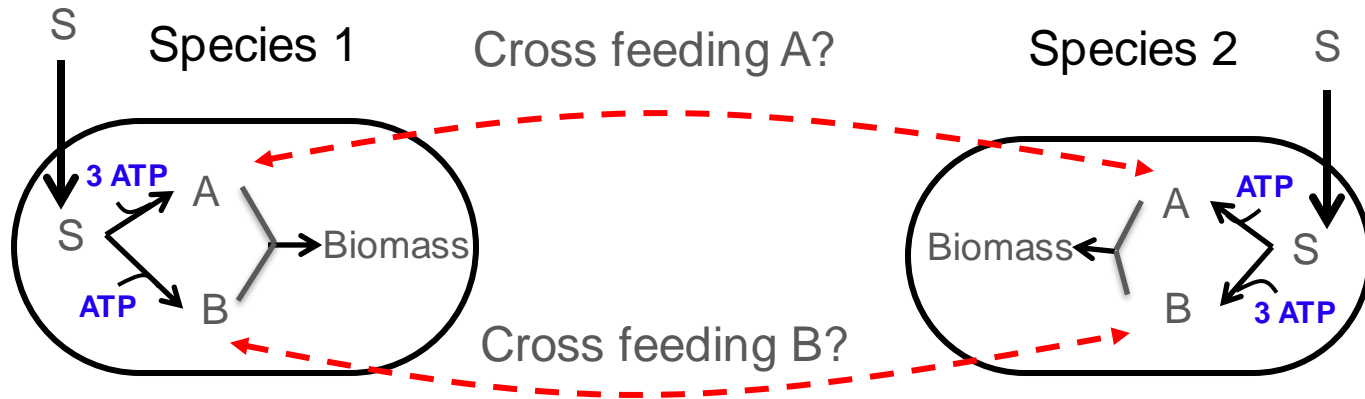


**One optimization  
per organism**

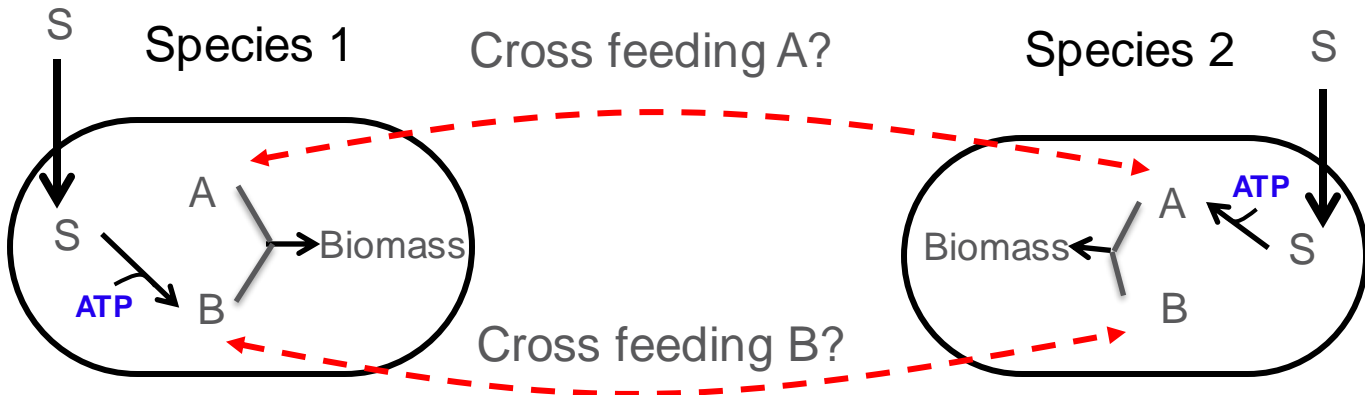
No way for each organism to 'sense and respond' to the metabolic potential of other organisms to, e.g., choose a mutualistic phenotype, which could be selected by adaptation

# Motivating examples

What will happen if two species, one more efficient in synthesizing one biomass precursor? Will they share resource to maximize yield?

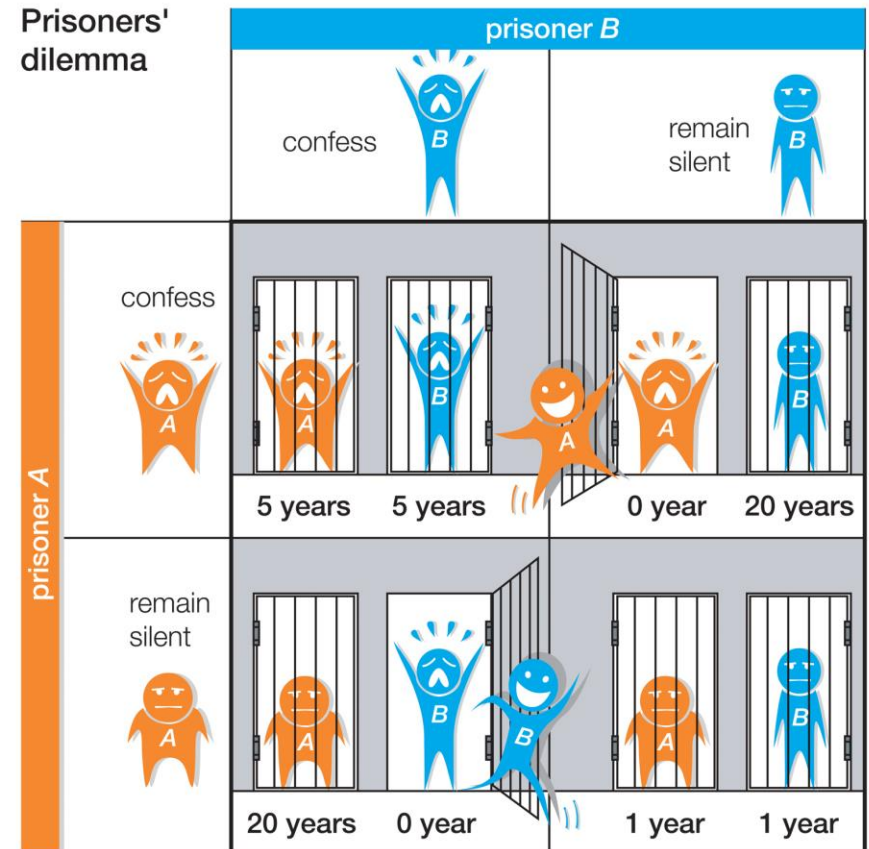


What will happen if each of them can synthesize only one? Will they share resource to maximize yield?



# Evolutionary Game Theory

- Game theory applied to ecology: natural selection determines what strategies will thrive
- Nash equilibrium (NE):** No player can do better by unilaterally changing its strategy
  - $E(s_1^*, s_2^*) \geq E(s_1^*, s)$  for any  $s$
  - $E(s_2^*, s_1^*) \geq E(s_2^*, s)$  for any  $s$
- Prisoners' dilemma:** Confession is the only (strict) NE



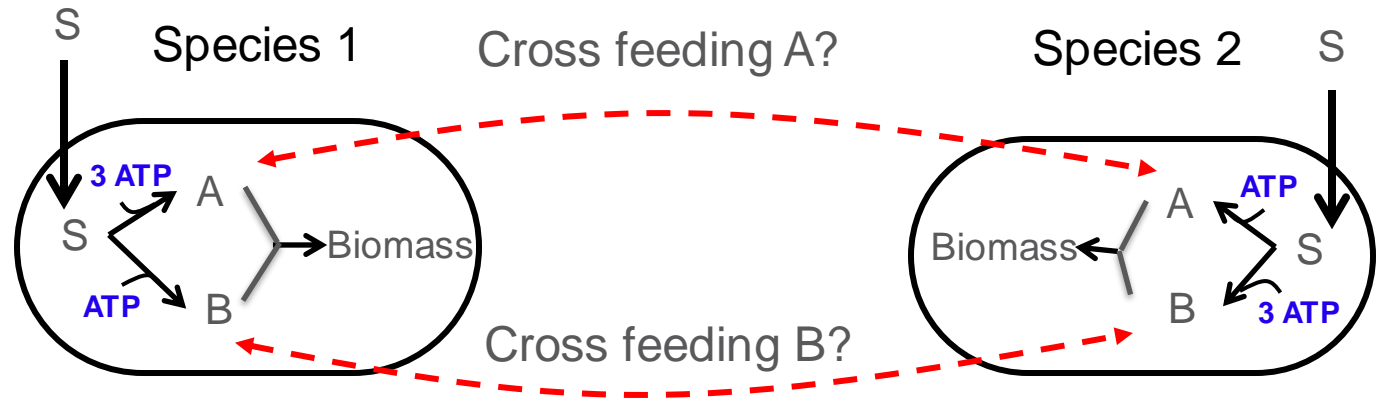
© 2010 Encyclopædia Britannica, Inc.

	$E(\text{confess, confess})$	$E(\text{confess, silent})$
	Confess	Silent
$E(\text{silent, confess})$	Confess	Silent
	Silent	
	$(-5, -5)$	$(0, -20)$
	$(-20, 0)$	$(-1, -1)$

# Motivating example 1

What will happen if two species, one more efficient in synthesizing one biomass precursor? Will they share resource to maximize yield?

□ Prisoners' dilemma!



Secretion rate of A by species 2

	0	1.39	2.78	4.17
0	(1.18, 1.18)	(1.42, 1.09)	(1.67, 1.01)	(1.91, 0.93)
1.39	(1.09, 1.42)	(1.34, 1.34)	(1.58, 1.26)	(1.83, 1.18)
2.78	(1.01, 1.67)	(1.26, 1.58)	(1.5, 1.5)	(1.75, 1.42)
4.17	(0.93, 1.91)	(1.18, 1.83)	(1.42, 1.75)	(1.67, 1.67)

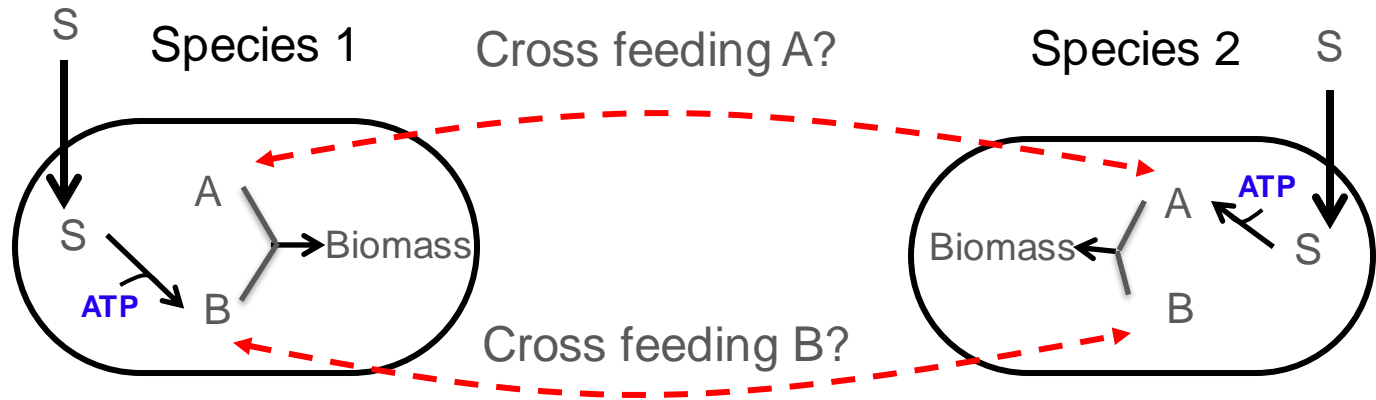
Secretion rate of B by species 1



# Motivating example 2

What will happen if each of them can synthesize only one? Will they share resource to maximize yield?

- ❑ All are (weak) NE!
- ❑ No incentive to cheat/cooperate
- ❑ But cooperating has a higher fitness



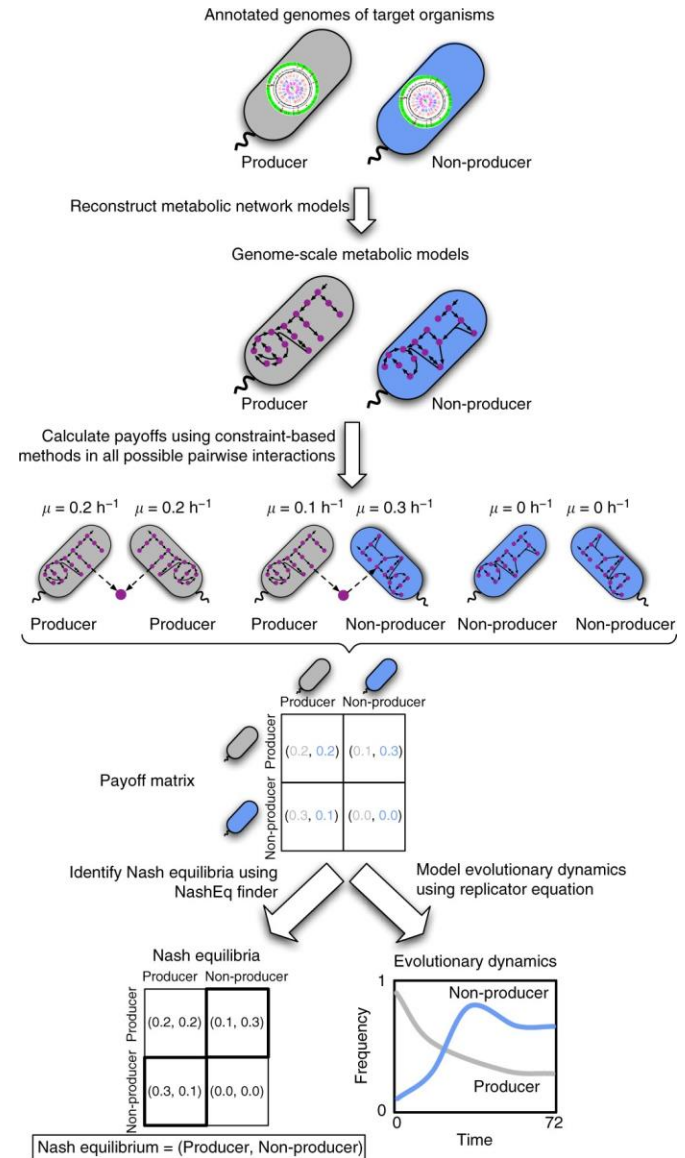
Secretion rate of A by species 2

	0	1.67	3.33	5
0	(0, 0)	(0.56, 0)	(1.11, 0)	(1.67, 0)
1.67	(0, 0.56)	(0.56, 0.56)	(1.11, 0.56)	(1.67, 0.56)
3.33	(0, 1.11)	(0.56, 1.11)	<b>(1.11, 1.11)</b>	(1.67, 1.11)
5	(0, 1.67)	(0.56, 1.67)	(1.11, 1.67)	<b>(1.67, 1.67)</b>

Secretion rate of B by species 1

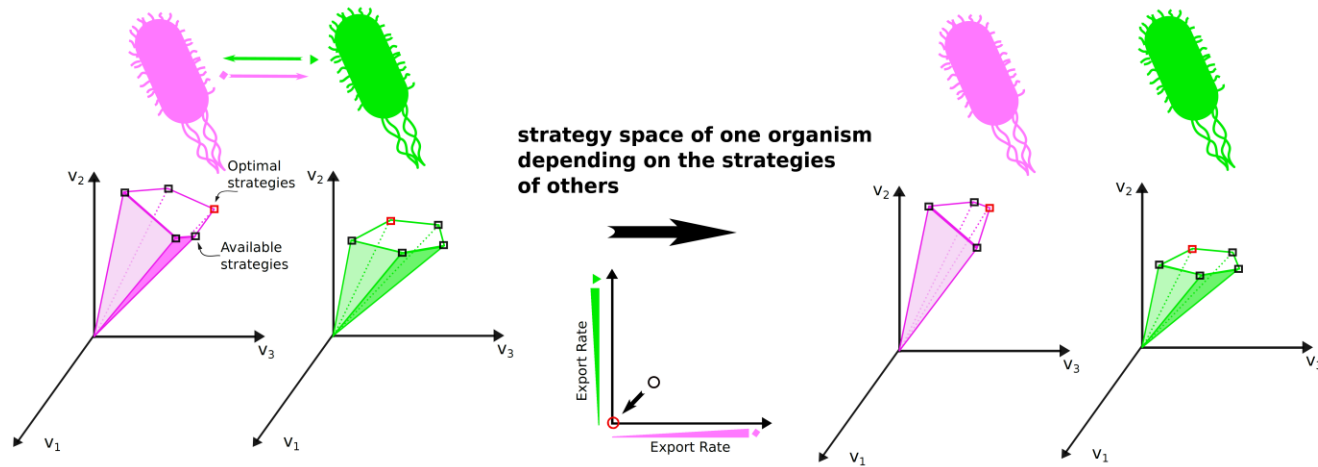
# How to find Nash Equilibria in general?

- ❑ Zomorodi and Segre 2017, Nature Comm.
  - ❑ Construct payoff matrices using FBA
  - ❑ Find NE
  - ❑ Simulate replicator dynamics
  
- ❑ Need to pre-define metabolic strategies



# Microbial metabolic interactions as games

A unique class of games where the continuous strategy space of one microbe depends on the strategies of other microbes



Not simple matrix games where strategies can be enumerated and have well established techniques to analyze.

# Bilevel optimization model

## NECom

Max: Community growth rate

(Optional,  
more likely to be stable)

s.t. [ Max: organism's growth rate ]

(Nash Equilibrium)

s.t. Intracellular mass balance

(Quasi-steady state)

Reaction directionalities

(Thermodynamic  
feasibility)

Limitation of uptake rates

based on crossfeeding availability

for each organism

Crossfeeding availability  
determine by metabolite export  
for each community metabolite

Ecological and Evolutionary  
Principles

Biochemical Principles

# Bilevel optimization model

$$\max \sum_k v_k^{biomass} \quad (\text{optional; find NE with higher overall growth})$$

subject to

$$\max v_k^{biomass} \quad (\text{max fitness constrained by the actions of other members; NE})$$

subject to

$$\sum_j S_{i,j,k} v_{j,k} = 0,$$

for each metabolite  $i$

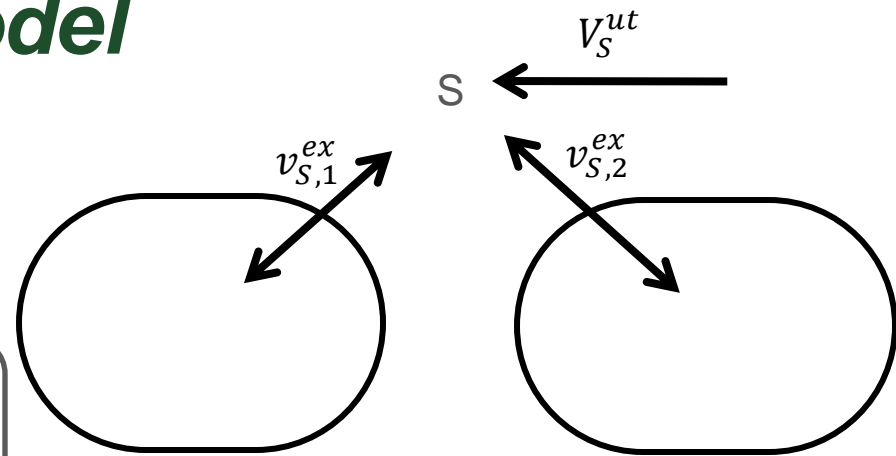
$$LB_{j,k} \leq v_{j,k} \leq UB_{j,k},$$

for each reaction  $j$

$$X_k v_{j,k}^{ex} + \max \left\{ \sum_{n,n \neq k} X_n v_{j,n}^{ex} + V_j^{ut}, 0 \right\} \geq 0$$

for each exchange reaction  $j$

$$v_{j,k}, v_{j,k}^{ex} \in \mathbb{R}$$



(intracellular mass balance)

(reaction directionality, uptake capacity)

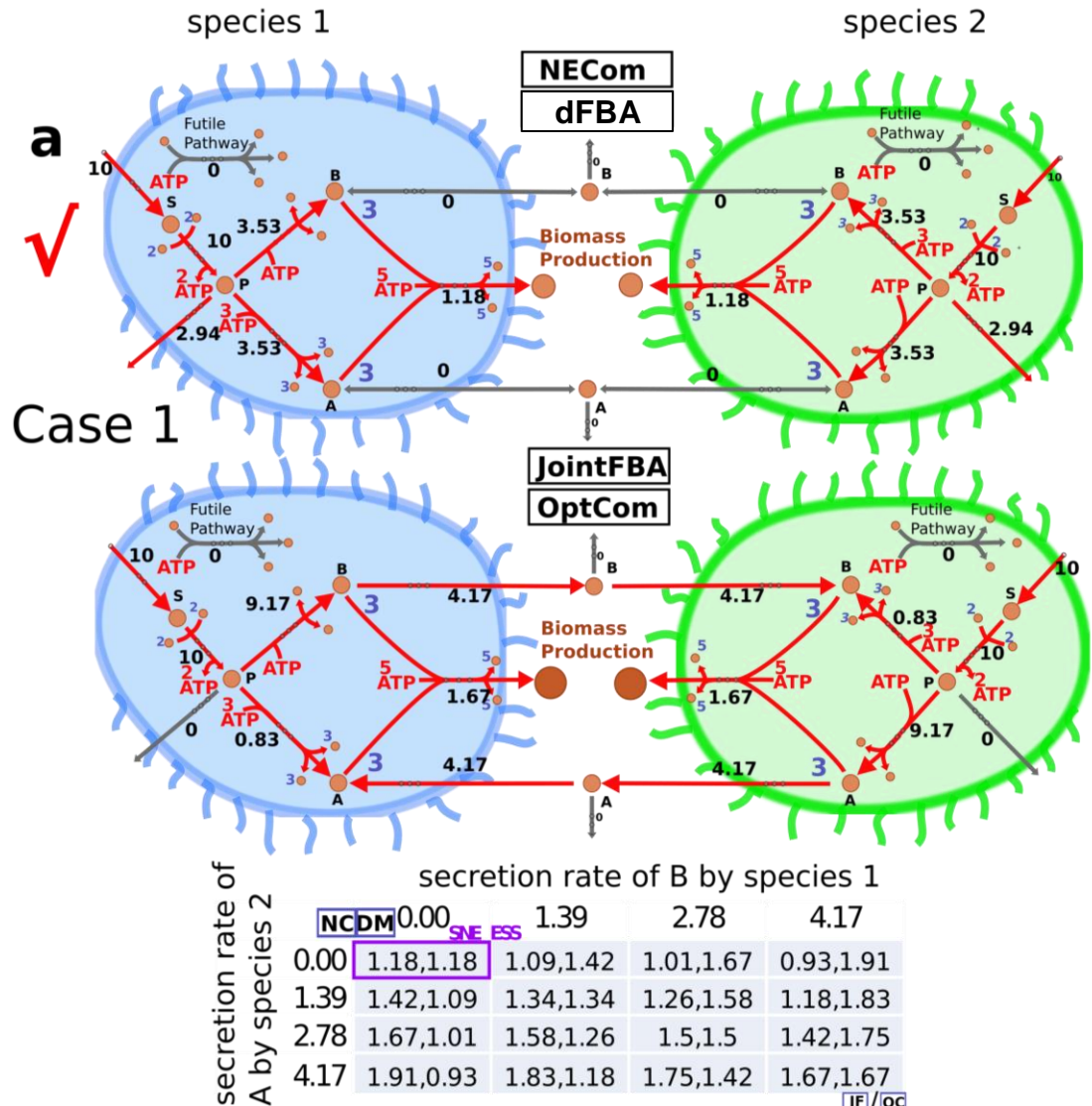
(constrain max. uptake only, avoid imposing min. production)

for all members  $k$

# Capturing Example 1

Two species, one more efficient in synthesizing one biomass precursor

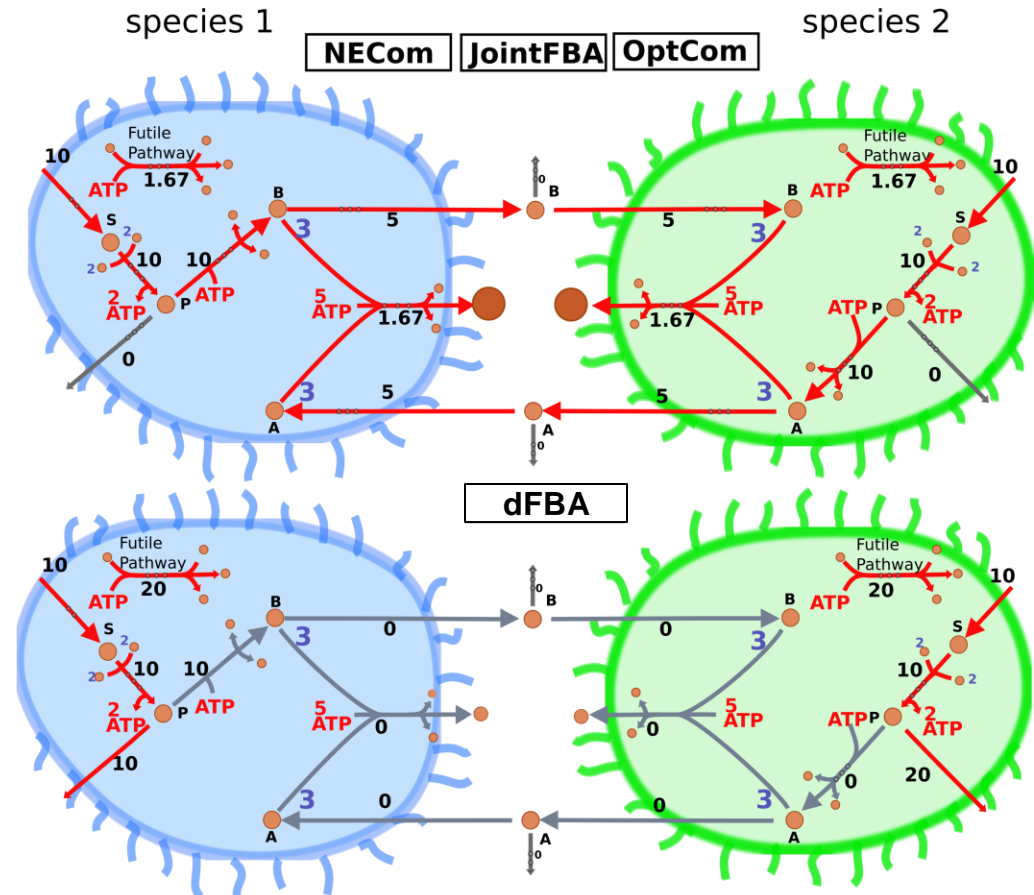
- ❑ dFBA and NECom predict the prisoners' dilemma correctly.
- ❑ Joint-FBA and OptCom predict mutualism



# Capturing Example 2

One only produces A,  
the other only B:

- ❑ NECom, Joint-FBA, and OptCom predict **mutualistic crossfeeding**.
- ❑ Non-cooperating is also predicted as a feasible solution
- ❑ dFBA predicts no growth (also tested on multiple auxotrophic *E. coli* mutants)
- ❑ Though export is still costly, there is no impact on fitness as growth is limited by the other precursor.



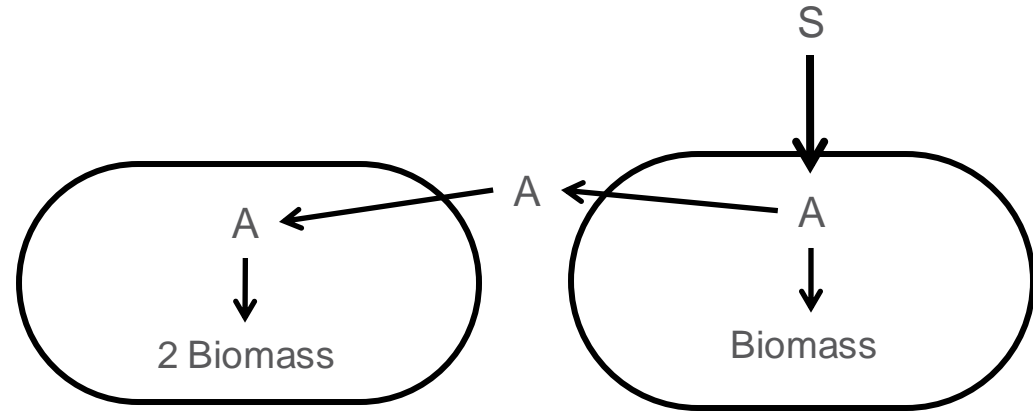
secretion rate of B by species 1

	0.00	1.67	3.33	5.00
secretion rate of A by species 2	0,0	0,0.56	0,1.11	0,1.67
1.67	0.56,0	0.56,0.56	0.56,1.11	0.56,1.67
3.33	1.11,0	1.11,0.56	1.11,1.11	1.11,1.67
5.00	1.67,0	1.67,0.56	1.67,1.11	1.67,1.67

NC/JF/OC



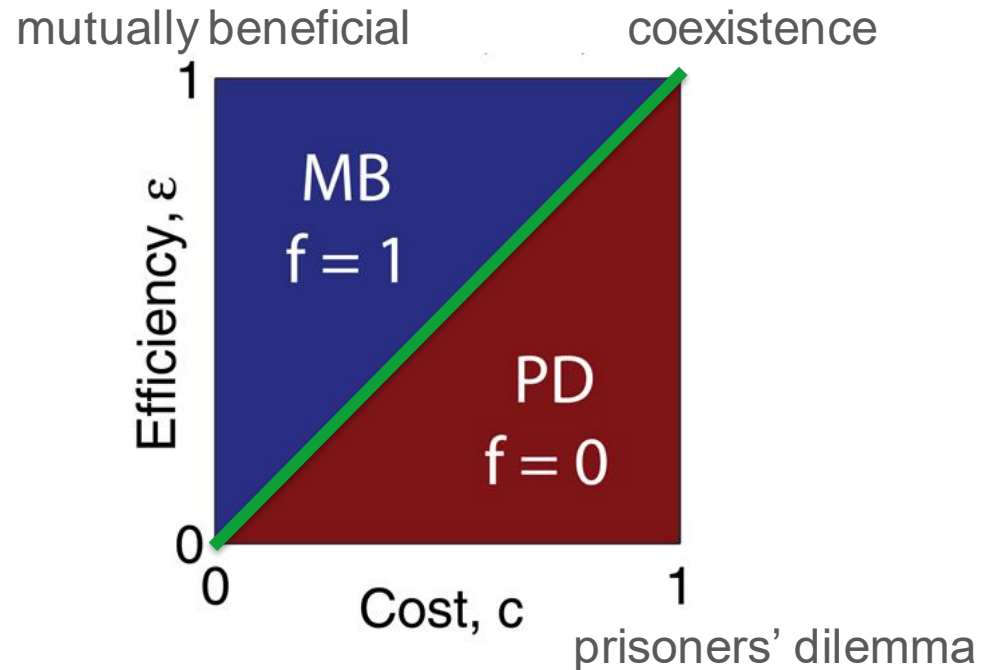
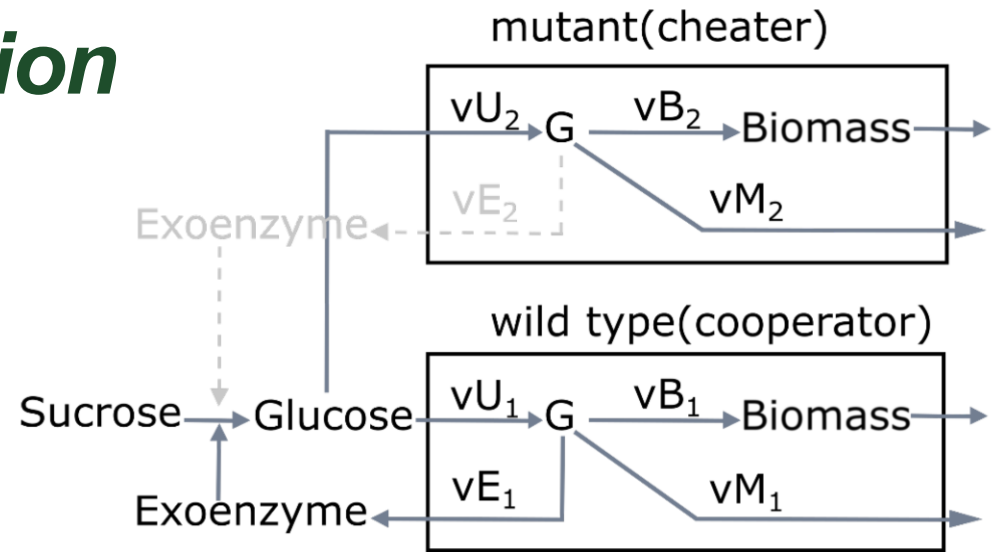
# Parasitism



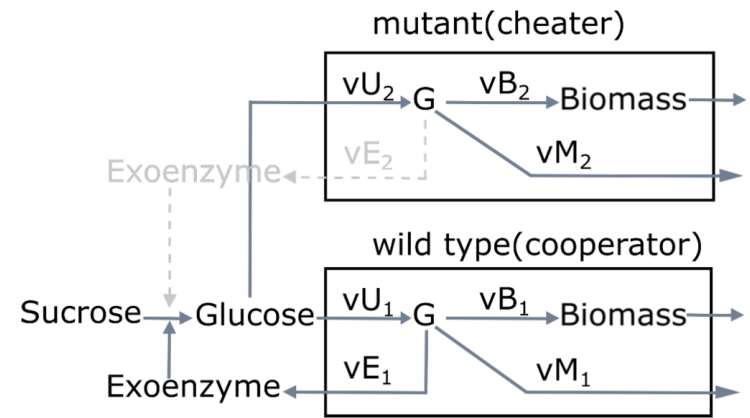
	Growth of sp1 (the beneficiary)	Growth of sp2 (the sacrificed)
NECom	0	0.83
Joint FBA	1.67	0.42
OptCom	1.67	0.42
Individual FBA	0	0.83

# Exoenzyme production

- Gore et al., 2009 initially analyzed this with cost of enzyme production  $c$  and capture efficiency of producer  $\varepsilon$  as parameters
- Reproduce the same trend when tested with 2 – 5 copies of the same member



# Exoenzyme production



Cost < benefit  
capture efficiency  $\varepsilon = 0$

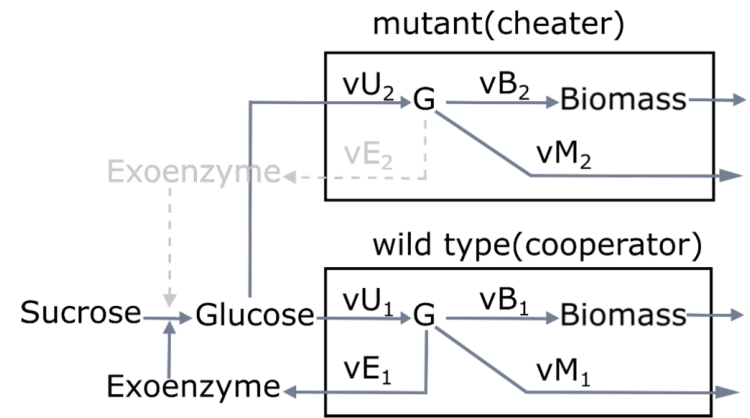
$(v_{B,1}, v_{B,2})$	0	0.01	0.02	0.03	0.04	$v_{E,2}$	0.05	0.06	0.07	0.08	0.09	0.1
0	(0.00,0.00)	(0.10,0.08)	(0.20,0.16)	(0.30,0.24)	(0.40,0.32)	(0.50,0.40)	(0.60,0.48)	(0.70,0.56)	(0.80,0.64)	(0.90,0.72)	<b>(1.00,0.80)</b>	
0.01	(0.08,0.10)	(0.18,0.18)	(0.28,0.26)	(0.38,0.34)	(0.48,0.42)	(0.58,0.50)	(0.68,0.58)	(0.78,0.66)	(0.88,0.74)	<b>(0.98,0.82)</b>	(0.98,0.80)	
0.02	(0.16,0.20)	(0.26,0.28)	(0.36,0.36)	(0.46,0.44)	(0.56,0.52)	(0.66,0.60)	(0.76,0.68)	(0.86,0.76)	<b>(0.96,0.84)</b>	(0.96,0.82)	(0.96,0.80)	
0.03	(0.24,0.30)	(0.34,0.38)	(0.44,0.46)	(0.54,0.54)	(0.64,0.62)	(0.74,0.70)	(0.84,0.78)	<b>(0.94,0.86)</b>	(0.94,0.84)	(0.94,0.82)	(0.94,0.80)	
0.04	(0.32,0.40)	(0.42,0.48)	(0.52,0.56)	(0.62,0.64)	(0.72,0.72)	(0.82,0.80)	<b>(0.92,0.88)</b>	(0.92,0.86)	(0.92,0.84)	(0.92,0.82)	(0.92,0.80)	
$v_{E,1}$ 0.05	(0.40,0.50)	(0.50,0.58)	(0.60,0.66)	(0.70,0.74)	(0.80,0.82)	<b>(0.90,0.90)</b>	(0.90,0.88)	(0.90,0.86)	(0.90,0.84)	(0.90,0.82)	(0.90,0.80)	
0.06	(0.48,0.60)	(0.58,0.68)	(0.68,0.76)	(0.78,0.84)	<b>(0.88,0.92)</b>	(0.88,0.90)	(0.88,0.88)	(0.88,0.86)	(0.88,0.84)	(0.88,0.82)	(0.88,0.80)	
0.07	(0.56,0.70)	(0.66,0.78)	(0.76,0.86)	<b>(0.86,0.94)</b>	(0.86,0.92)	(0.86,0.90)	(0.86,0.88)	(0.86,0.86)	(0.86,0.84)	(0.86,0.82)	(0.86,0.80)	
0.08	(0.64,0.80)	(0.74,0.88)	<b>(0.84,0.96)</b>	(0.84,0.94)	(0.84,0.92)	(0.84,0.90)	(0.84,0.88)	(0.84,0.86)	(0.84,0.84)	(0.84,0.82)	(0.84,0.80)	
0.09	(0.72,0.90)	<b>(0.82,0.98)</b>	(0.82,0.96)	(0.82,0.94)	(0.82,0.92)	(0.82,0.90)	(0.82,0.88)	(0.82,0.86)	(0.82,0.84)	(0.82,0.82)	(0.82,0.80)	
0.1	<b>(0.80,1.00)</b>	(0.80,0.98)	(0.80,0.96)	(0.80,0.94)	(0.80,0.92)	(0.80,0.90)	(0.80,0.88)	(0.80,0.86)	(0.80,0.84)	(0.80,0.82)	(0.80,0.80)	

Cost < benefit  
capture efficiency  $\varepsilon > 0$

$(v_{B,1}, v_{B,2})$	0	0.01	0.02	0.03	0.04	$v_{E,2}$	0.05	0.06	0.07	0.08	0.09	0.1
0	(0.00,0.00)	(0.09,0.09)	(0.18,0.18)	(0.27,0.27)	(0.36,0.36)	(0.45,0.45)	(0.54,0.54)	(0.63,0.63)	(0.72,0.72)	(0.81,0.81)	(0.90,0.80)	
0.01	(0.09,0.09)	(0.18,0.18)	(0.27,0.27)	(0.36,0.36)	(0.45,0.45)	(0.54,0.54)	(0.63,0.63)	(0.72,0.72)	(0.81,0.81)	(0.90,0.82)	(0.98,0.80)	
0.02	(0.18,0.18)	(0.27,0.27)	(0.36,0.36)	(0.45,0.45)	(0.54,0.54)	(0.63,0.63)	(0.72,0.72)	(0.81,0.81)	(0.90,0.84)	(0.96,0.82)	(0.96,0.80)	
0.03	(0.27,0.27)	(0.36,0.36)	(0.45,0.45)	(0.54,0.54)	(0.63,0.63)	(0.72,0.72)	(0.81,0.81)	(0.90,0.86)	(0.94,0.84)	(0.94,0.82)	(0.94,0.80)	
0.04	(0.36,0.36)	(0.45,0.45)	(0.54,0.54)	(0.63,0.63)	(0.72,0.72)	(0.81,0.81)	(0.90,0.88)	(0.92,0.86)	(0.92,0.84)	(0.92,0.82)	(0.92,0.80)	
$v_{E,1}$ 0.05	(0.45,0.45)	(0.54,0.54)	(0.63,0.63)	(0.72,0.72)	(0.81,0.81)	<b>(0.90,0.90)</b>	(0.90,0.88)	(0.90,0.86)	(0.90,0.84)	(0.90,0.82)	(0.90,0.80)	
0.06	(0.54,0.54)	(0.63,0.63)	(0.72,0.72)	(0.81,0.81)	(0.88,0.90)	(0.88,0.90)	(0.88,0.88)	(0.88,0.86)	(0.88,0.84)	(0.88,0.82)	(0.88,0.80)	
0.07	(0.63,0.63)	(0.72,0.72)	(0.81,0.81)	(0.86,0.90)	(0.86,0.92)	(0.86,0.90)	(0.86,0.88)	(0.86,0.86)	(0.86,0.84)	(0.86,0.82)	(0.86,0.80)	
0.08	(0.72,0.72)	(0.81,0.81)	(0.84,0.90)	(0.84,0.94)	(0.84,0.92)	(0.84,0.90)	(0.84,0.88)	(0.84,0.86)	(0.84,0.84)	(0.84,0.82)	(0.84,0.80)	
0.09	(0.81,0.81)	(0.82,0.90)	(0.82,0.96)	(0.82,0.94)	(0.82,0.92)	(0.82,0.90)	(0.82,0.88)	(0.82,0.86)	(0.82,0.84)	(0.82,0.82)	(0.82,0.80)	
0.1	(0.80,0.90)	(0.80,0.98)	(0.80,0.96)	(0.80,0.94)	(0.80,0.92)	(0.80,0.90)	(0.80,0.88)	(0.80,0.86)	(0.80,0.84)	(0.80,0.82)	(0.80,0.80)	

(mutually beneficial)

# Exoenzyme production



Cost = benefit  
 $\varepsilon \geq 0$

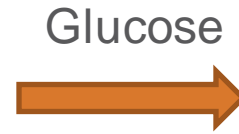
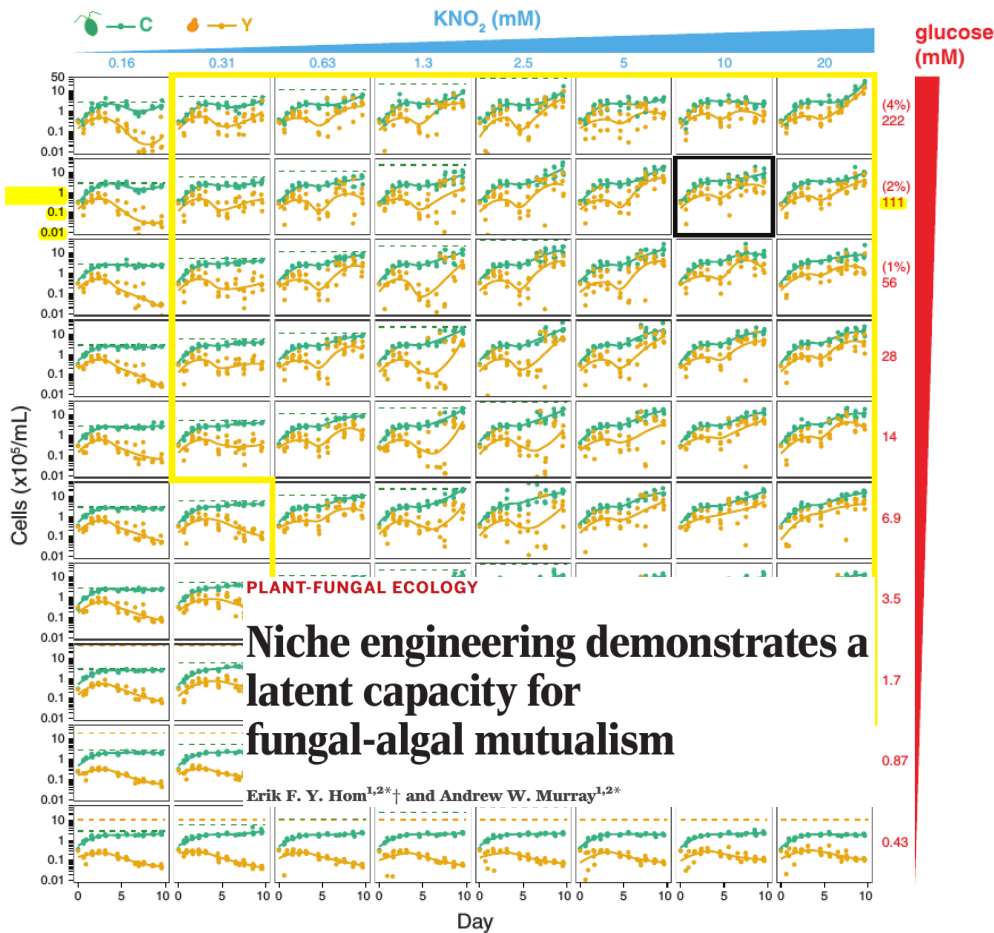
$(v_{B,1}, v_{B,2})$	$v_{E,2}$										
	0	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
0	(0.00,0.00)	(0.09,0.00)	(0.18,0.00)	(0.27,0.00)	(0.36,0.00)	(0.45,0.00)	(0.54,0.00)	(0.63,0.00)	(0.72,0.00)	(0.81,0.00)	(-inf,-inf)
0.01	(0.00,0.09)	(0.09,0.09)	(0.18,0.09)	(0.27,0.09)	(0.36,0.09)	(0.45,0.09)	(0.54,0.09)	(0.63,0.09)	(0.72,0.09)	(0.81,0.01)	(-inf,-inf)
0.02	(0.00,0.18)	(0.09,0.18)	(0.18,0.18)	(0.27,0.18)	(0.36,0.18)	(0.45,0.18)	(0.54,0.18)	(0.63,0.18)	(0.72,0.12)	(0.78,0.01)	(-inf,-inf)
0.03	(0.00,0.27)	(0.09,0.27)	(0.18,0.27)	(0.27,0.27)	(0.36,0.27)	(0.45,0.27)	(0.54,0.27)	(0.63,0.23)	(0.67,0.12)	(0.67,0.01)	(-inf,-inf)
0.04	(0.00,0.36)	(0.09,0.36)	(0.18,0.36)	(0.27,0.36)	(0.36,0.36)	(0.45,0.36)	(0.54,0.34)	(0.56,0.23)	(0.56,0.12)	(0.56,0.01)	(-inf,-inf)
$v_{E,1}$ 0.05	(0.00,0.45)	(0.09,0.45)	(0.18,0.45)	(0.27,0.45)	(0.36,0.45)	(0.45,0.45)	(0.45,0.34)	(0.45,0.23)	(0.45,0.12)	(0.45,0.01)	(-inf,-inf)
0.06	(0.00,0.54)	(0.09,0.54)	(0.18,0.54)	(0.27,0.54)	(0.34,0.54)	(0.34,0.45)	(0.34,0.34)	(0.34,0.23)	(0.34,0.12)	(0.34,0.01)	(-inf,-inf)
0.07	(0.00,0.63)	(0.09,0.63)	(0.18,0.63)	(0.23,0.63)	(0.23,0.56)	(0.23,0.45)	(0.23,0.34)	(0.23,0.23)	(0.23,0.12)	(0.23,0.01)	(-inf,-inf)
0.08	(0.00,0.72)	(0.09,0.72)	(0.12,0.72)	(0.12,0.67)	(0.12,0.56)	(0.12,0.45)	(0.12,0.34)	(0.12,0.23)	(0.12,0.12)	(0.12,0.01)	(-inf,-inf)
0.09	(0.00,0.81)	(0.01,0.81)	(0.01,0.78)	(0.01,0.67)	(0.01,0.56)	(0.01,0.45)	(0.01,0.34)	(0.01,0.23)	(0.01,0.12)	(0.01,0.01)	(-inf,-inf)
0.1	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)

Cost > benefit  
 $\varepsilon \geq 0$

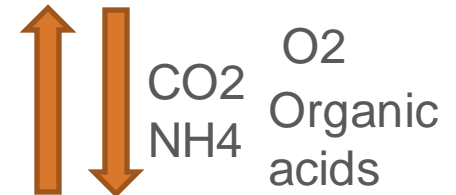
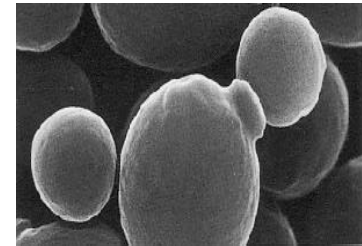
$(v_{B,1}, v_{B,2})$	$v_{E,2}$										
	0	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
0	(0.00,0.00)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)
0.01	(-inf,-inf)	(0.08,0.08)	(0.18,0.06)	(0.28,0.04)	(0.38,0.02)	(0.48,0.00)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)
0.02	(-inf,-inf)	(0.06,0.18)	(0.16,0.16)	(0.26,0.14)	(0.36,0.12)	(0.46,0.10)	(0.56,0.08)	(0.66,0.06)	(0.76,0.04)	(-inf,-inf)	(-inf,-inf)
0.03	(-inf,-inf)	(0.04,0.28)	(0.14,0.26)	(0.24,0.24)	(0.34,0.22)	(0.44,0.20)	(0.54,0.18)	(0.64,0.16)	(0.64,0.04)	(-inf,-inf)	(-inf,-inf)
0.04	(-inf,-inf)	(0.02,0.38)	(0.12,0.36)	(0.22,0.34)	(0.32,0.32)	(0.42,0.30)	(0.52,0.28)	(0.52,0.16)	(0.52,0.04)	(-inf,-inf)	(-inf,-inf)
$v_{E,1}$ 0.05	(-inf,-inf)	(0.00,0.48)	(0.10,0.46)	(0.20,0.44)	(0.30,0.42)	(0.40,0.40)	(0.40,0.28)	(0.40,0.16)	(0.40,0.04)	(-inf,-inf)	(-inf,-inf)
0.06	(-inf,-inf)	(-inf,-inf)	(0.08,0.56)	(0.18,0.54)	(0.28,0.52)	(0.28,0.40)	(0.28,0.28)	(0.28,0.16)	(0.28,0.04)	(-inf,-inf)	(-inf,-inf)
0.07	(-inf,-inf)	(-inf,-inf)	(0.06,0.66)	(0.16,0.64)	(0.16,0.52)	(0.16,0.40)	(0.16,0.28)	(0.16,0.16)	(0.16,0.04)	(-inf,-inf)	(-inf,-inf)
0.08	(-inf,-inf)	(-inf,-inf)	(0.04,0.76)	(0.04,0.64)	(0.04,0.52)	(0.04,0.40)	(0.04,0.28)	(0.04,0.16)	(0.04,0.04)	(-inf,-inf)	(-inf,-inf)
0.09	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)
0.1	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)	(-inf,-inf)

(coexistence)

# Application to algae-yeast coculture

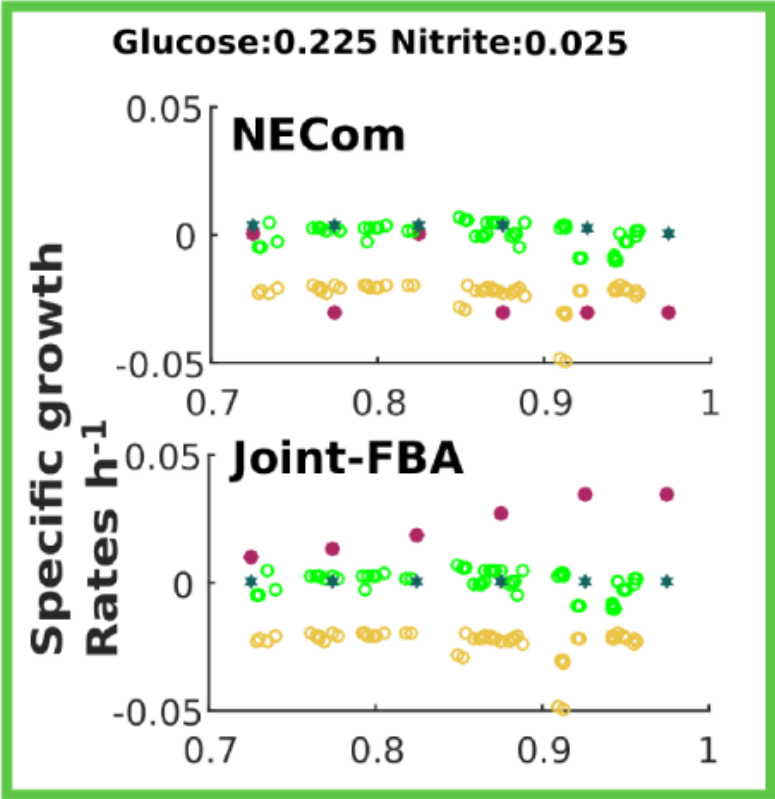
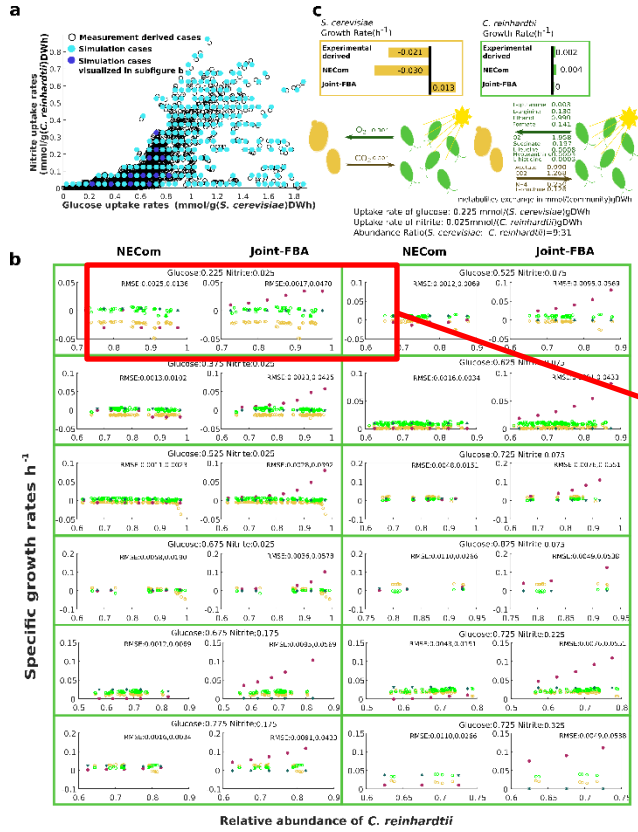


*Saccharomyces cerevisiae*



*Chlamydomonas reinhardtii*

# Analysis of 3,221 experimental data points



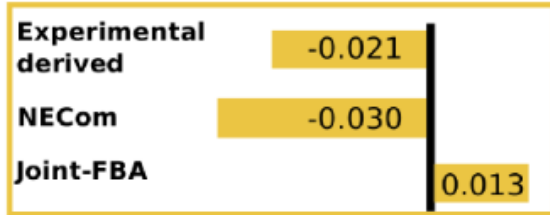
	Joint-FBA	NECOM	Reduction
<i>S. C</i>	0.0480	0.0088	▼ 81.7%
<i>C. R</i>	0.0085	0.0031	▼ 63.5%

Root mean squared error(RMSE) of predictions

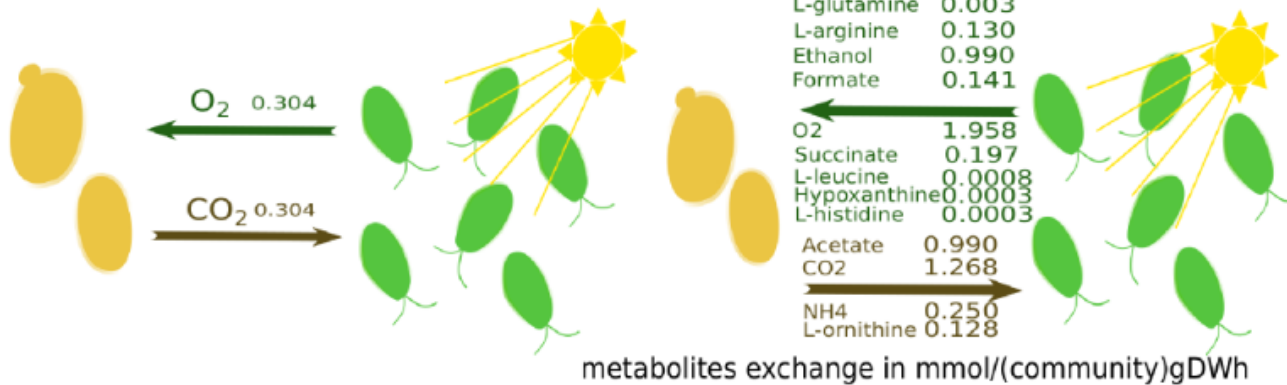
- Measurement derived data (*S. cerevisiae*)
- Simulation data *C. Reinhardtii*
- Measurement derived data (*C. reinhardtii*)
- ★ Simulation data *S. Cerevisiae*

# Analysis of 3,221 experimental data points

*S. cerevisiae*  
Growth Rate( $h^{-1}$ )



*C. reinhardtii*  
Growth Rate( $h^{-1}$ )



Uptake rate of glucose: 0.225 mmol/(*S. cerevisiae*)gDWh

Uptake rate of nitrite: 0.025mmol/(*C. reinhardtii*)gDWh

Abundance Ratio(*S. cerevisiae*: *C. reinhardtii*)=9:31

In order to maximize total biomass, direct FBA forces the algae to convert low energy metabolite (e.g CO<sub>2</sub>) to high energy metabolites (amino acids) to enhance growth of the yeast



# Summary

- ❑ Flux balance analysis (FBA) and dynamic flux balance analysis (dFBA) are important and fundamental frameworks for predicting microbial metabolism
- ❑ However, they have insufficiencies when directly applied to modeling microbial communities. Integrating new principles and new algorithms can improve metabolic modeling predictions.
- ❑ Artifact of FBA as a single-objective single-level optimization problem: forced altruism
  - Implement steady-state composition
  - Incorporate the concept of Nash equilibrium

# *Acknowledgement*

Graduate students:

Jingyi Cai

Parsa Ghadermazi

# Q & A