به نام خدا

A Systems Approach to Modeling Cell-Specific Metabolic Networks

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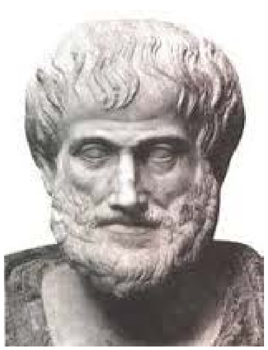
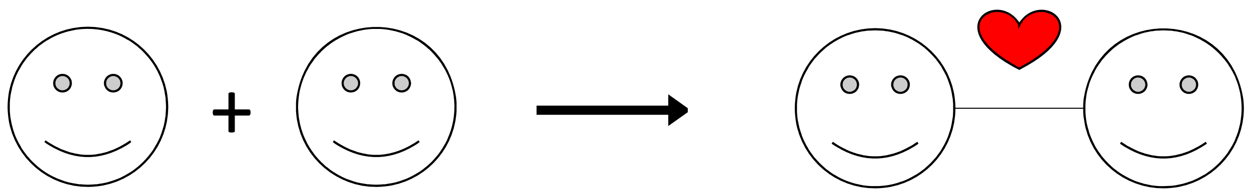
Systems Biology

An Introduction

The whole is greater than the sum of the parts.

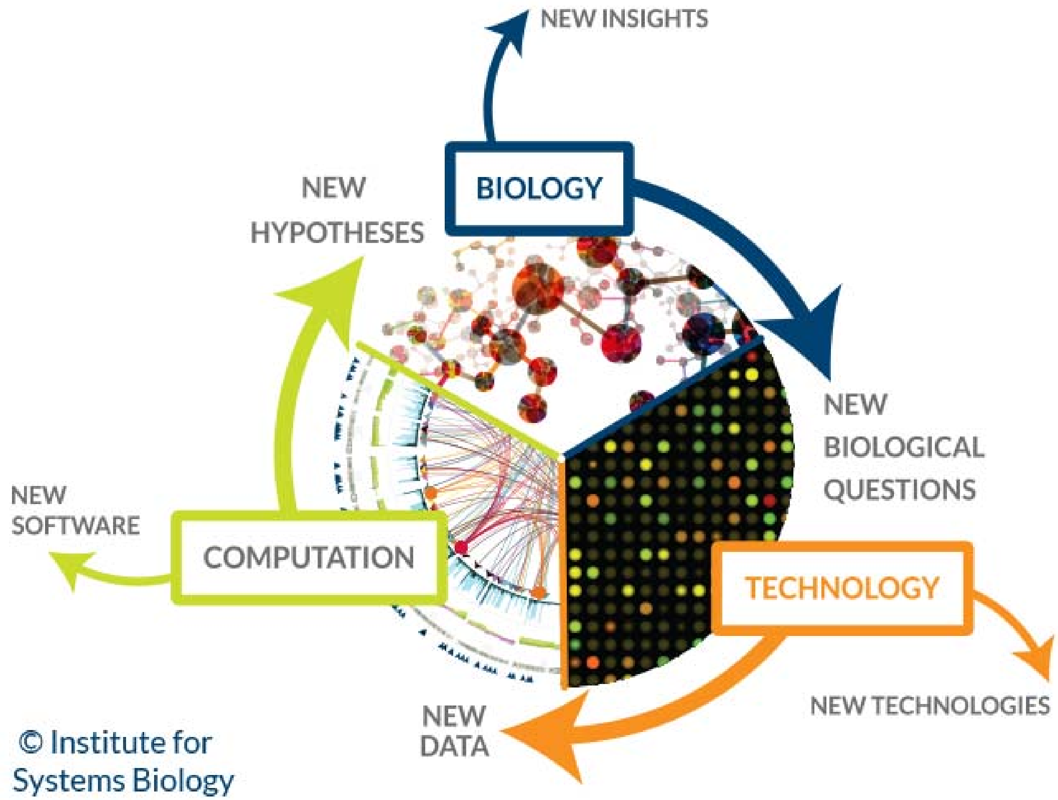


Aristotle



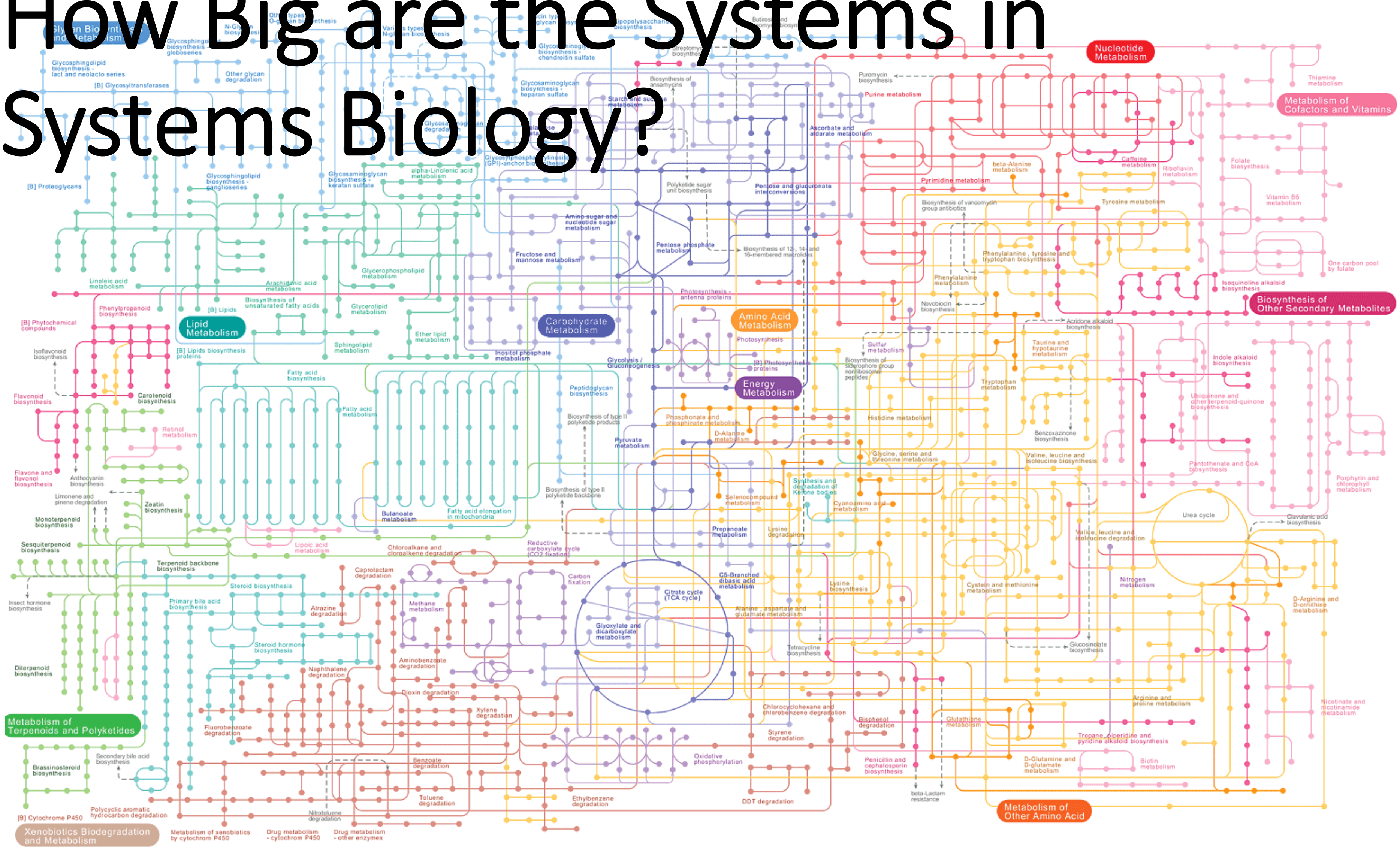
Systems Science

What is Systems Biology?



How Big are the Systems in Systems Biology?

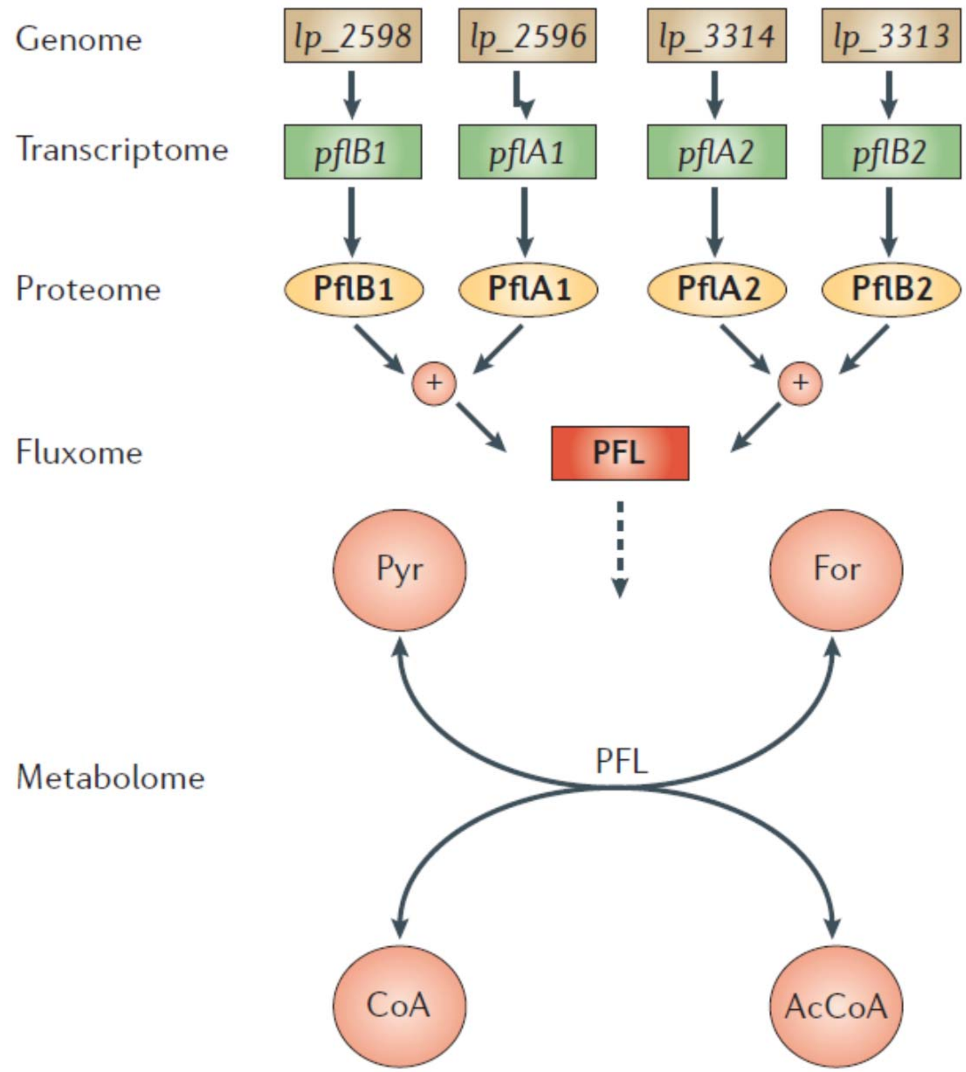
How Big are the Systems in Systems Biology?



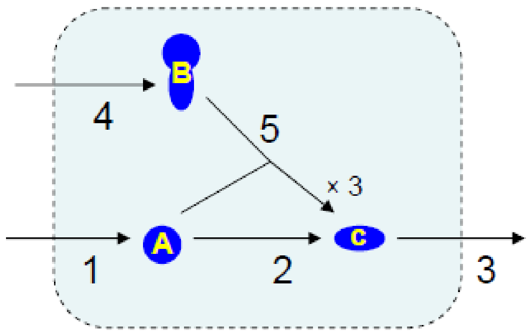
Analysis of Metabolic Networks

A Constraint-Based Approach

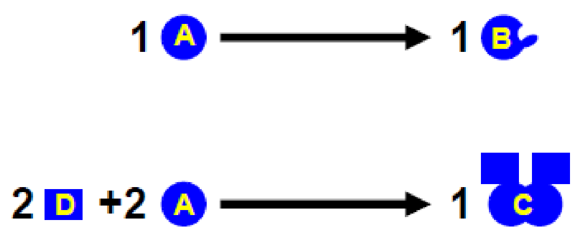
Central Dogma



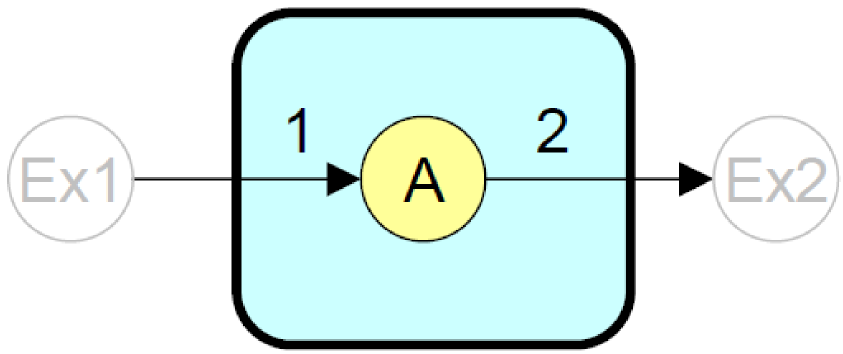
A toy metabolic network



Stoichiometric Coefficients

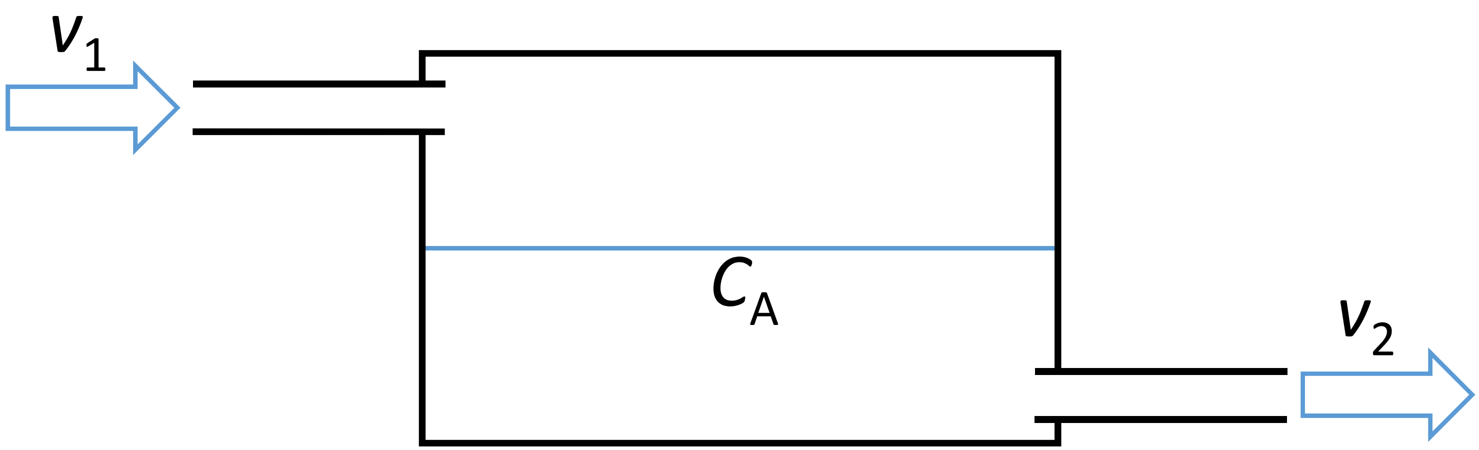


Reaction fluxes at steady state conditions

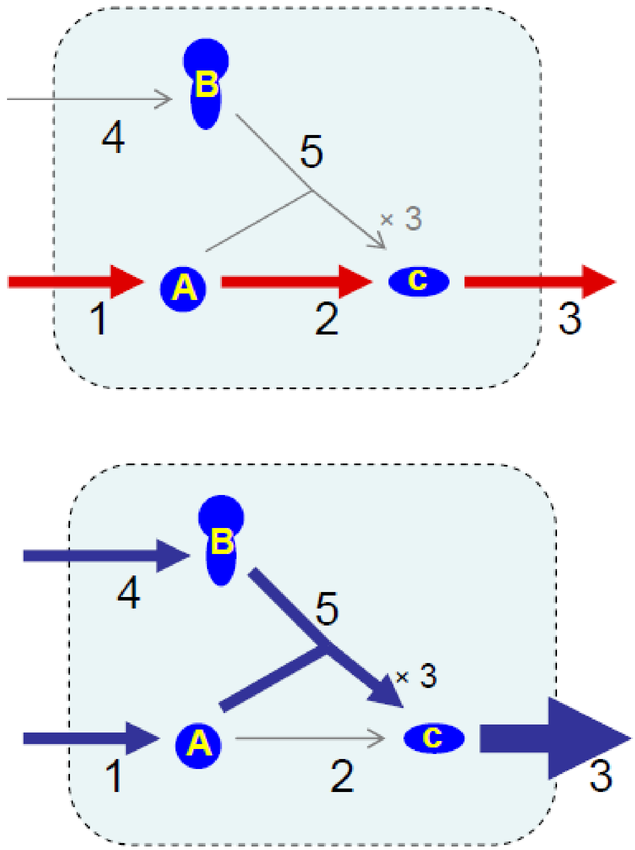


$$v_1 = v_2 \rightsquigarrow \frac{dC_A}{dt} = 0$$

A metaphor!

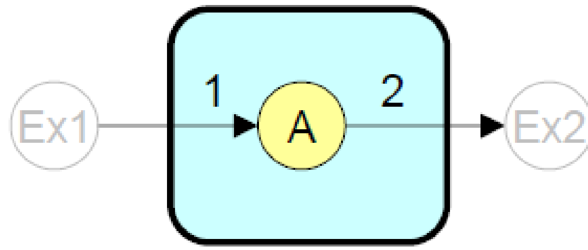


Flux distribution (= Flux vector)



$$\begin{array}{ccl} & & \underline{\;\;1\;\;\;2\;\;\;3\;\;\;4\;\;\;5\;\;} \\ \color{red}{V^1} & \color{red}{=} & \color{red}{(1,1,1,0,0)} \\ \color{blue}{V^2} & \color{blue}{=} & \color{blue}{(1,0,3,1,1)} \end{array}$$

Flux Balance



The rate of increase in C_A can be computed as: $\frac{dC_A}{dt} = v_1 - v_2$

This network has the following stoichiometric matrix: $S = (+1 \; -1)$

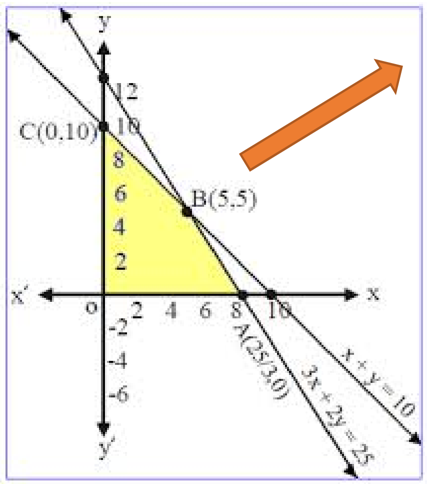
Here, any flux distribution is a 2-dimentional vector: $\vec{v} = \binom{v_1}{v_2}$.

Therefore, $\frac{dC_A}{dt} = S \cdot \vec{v}$. This equation is true for all networks.

Linear Constraints on Fluxes

- Typically, it is assumed that the internal metabolites are not produced or consumed when the cells grow.
- Constant concentration of each metabolite means that the system is in **steady-state** conditions.
- This can be written as the following set of "linear" constraints: $\frac{d\vec{C}}{dt} = S \cdot \vec{v} = \vec{0}$. These are the "**stoichiometric constraints**".
- Additionally, the set of irreversible reactions, Irr, is known. This implies that we have a set of linear "**thermodynamic constraints**": $v_i \geq 0$ **for all** $i \in Irr$.

Basic Concepts: Linear Programming



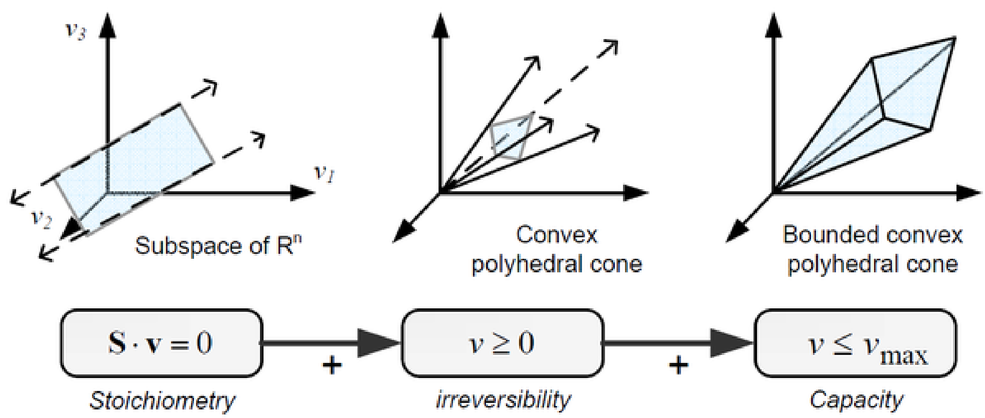
$$\textbf{max} \quad 1.1x + y$$

$$\textbf{subject to:} \; 3x + 2y \leq 25$$

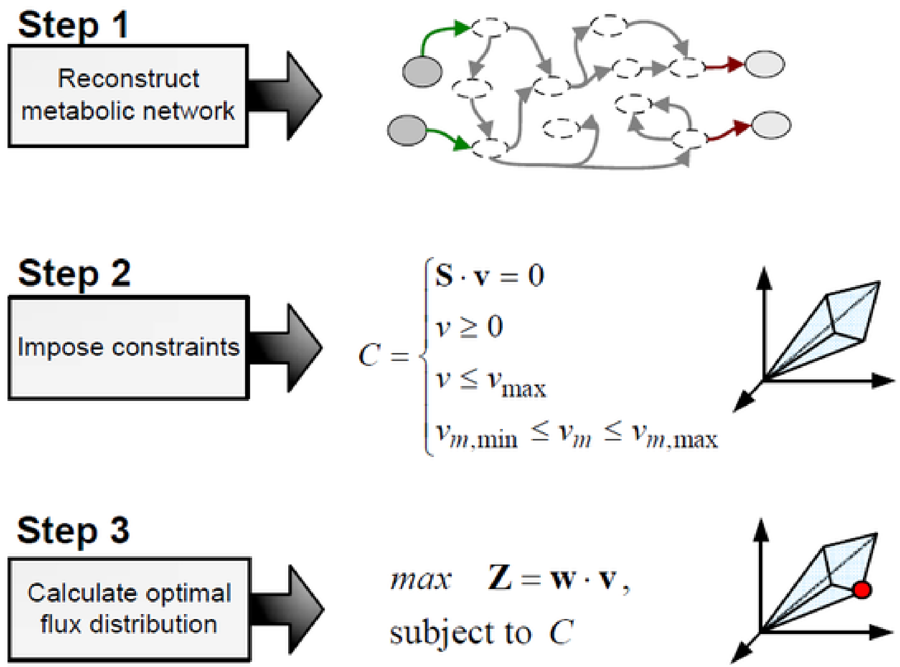
$$x + y \leq 10$$

$$x, y \geq 0$$

Basic Concepts: Linear Programming

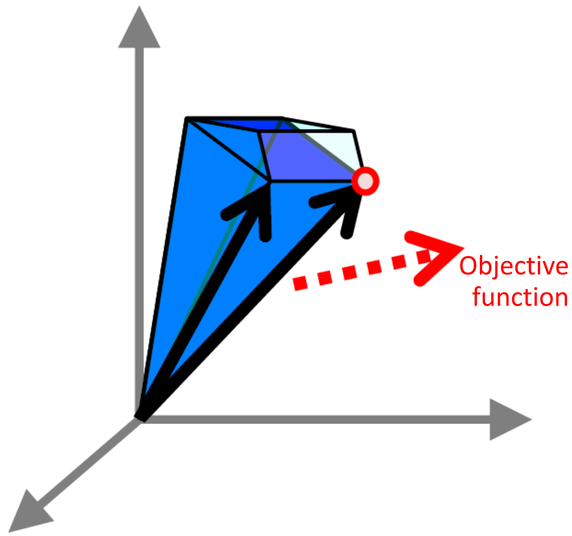


Basic Concepts: Flux Balance Analysis (FBA)



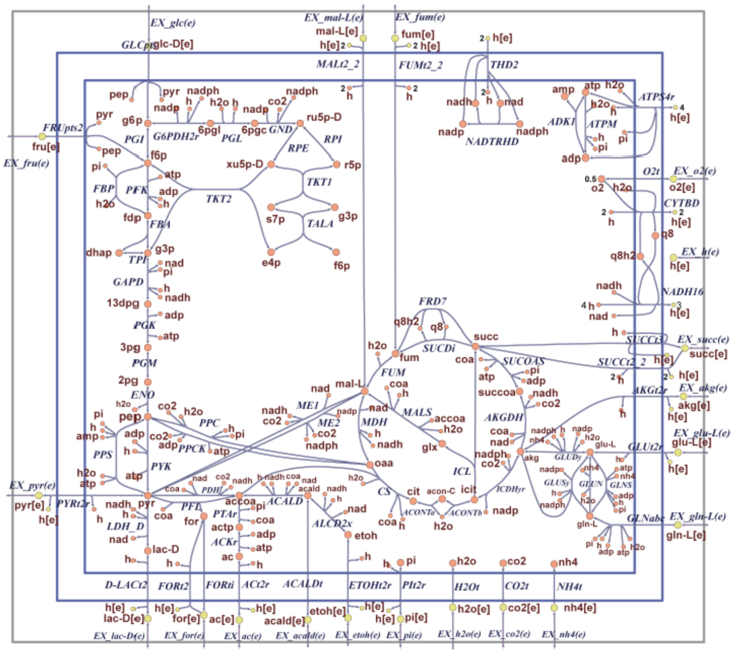
FBA ➡ **max:** objective function
subject to: constraints ➡ a feasible flux distribution

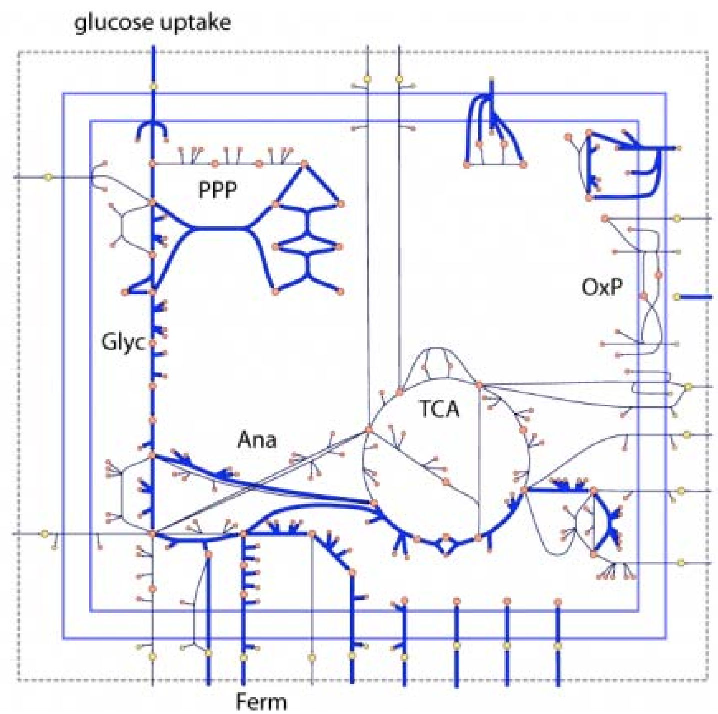
Flux Balance Analysis (FBA)



Max	$\mathbf{c}^T\mathbf{v}$	
Subject to:	$\mathbf{S}.\mathbf{v} = \mathbf{0}$	
	$0 \leq v_i$	for any irreversible reaction i
	$\alpha_i \leq v_i \leq \beta_i$	for i=1, …, n

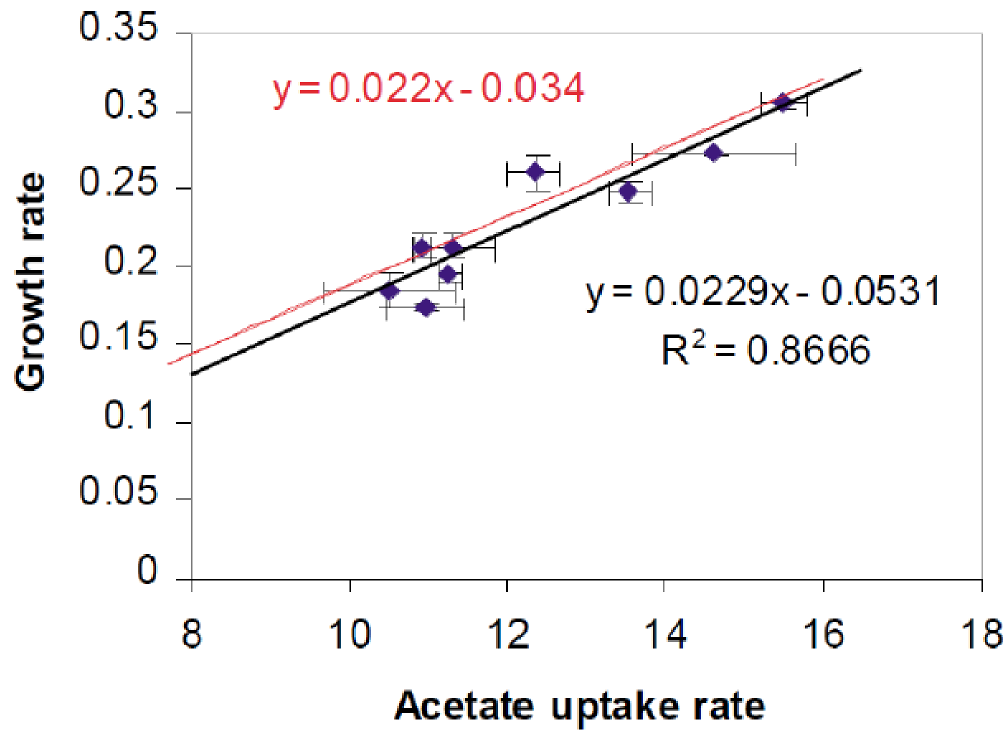
Flux Balance Analysis (FBA)





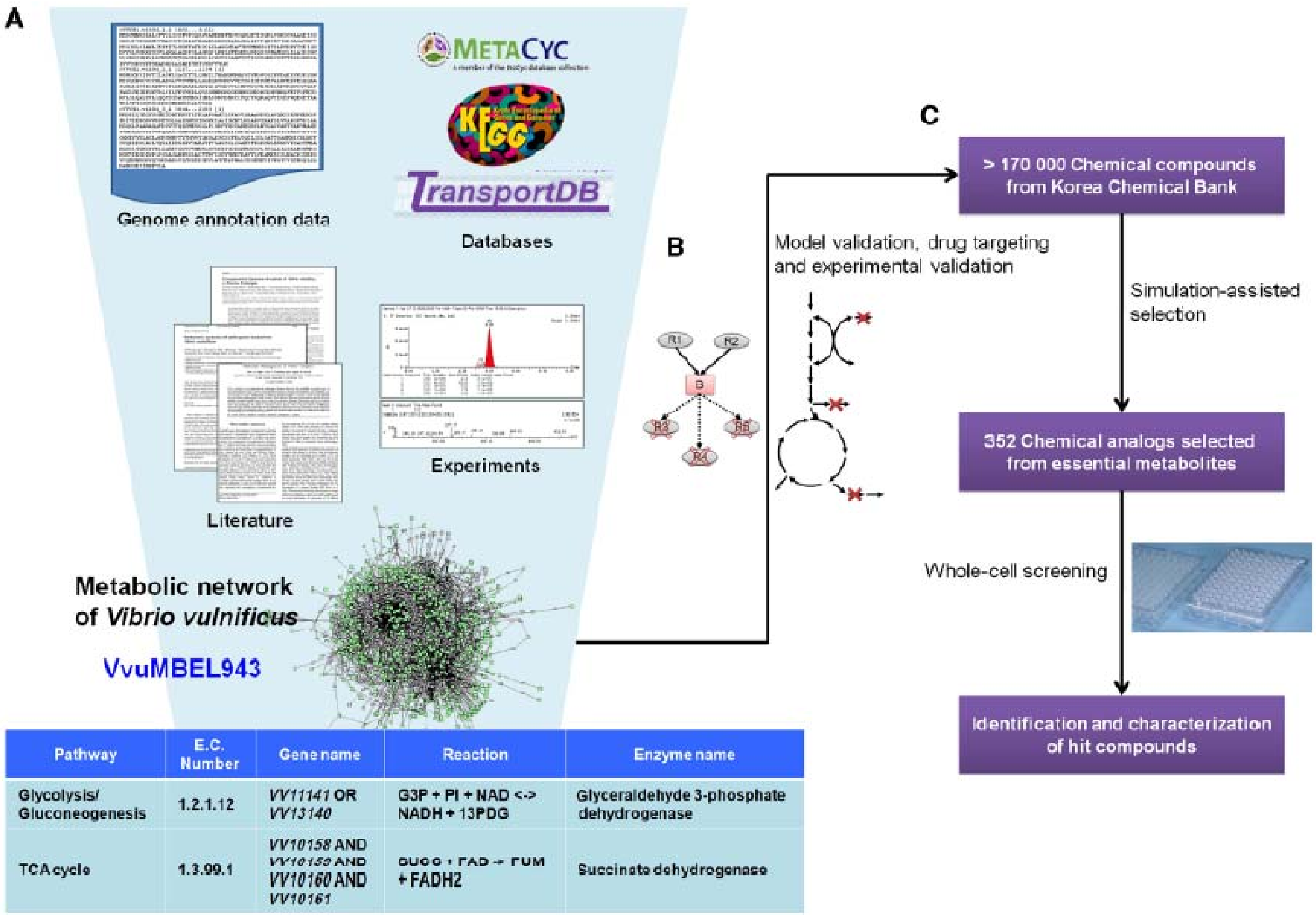
FBA ➔ **max:** objective function
subject to: constraints ➔ a feasible flux distribution

Example: Modeling *E. coli* growth



Edwards et al., 2001, Nature Biotechnology, 19:125-130.

Application: Finding Drug Targets



Pathway	E.C. Number	Gene name	Reaction	Enzyme name
Glycolysis/ Gluconeogenesis	1.2.1.12	*VV11141* OR *VV13140*	G3P + PI + NAD <-> NADH + 13PDG	Glyceraldehyde 3-phosphate dehydrogenase
TCA cycle	1.3.99.1	*VV10158* AND *VV10159* AND *VV10160* AND *VV10161*	SUCC + FAD → FUM + FADH2	Succinate dehydrogenase

Kim et al., 2011, Molecular Systems Biology, 7:460.

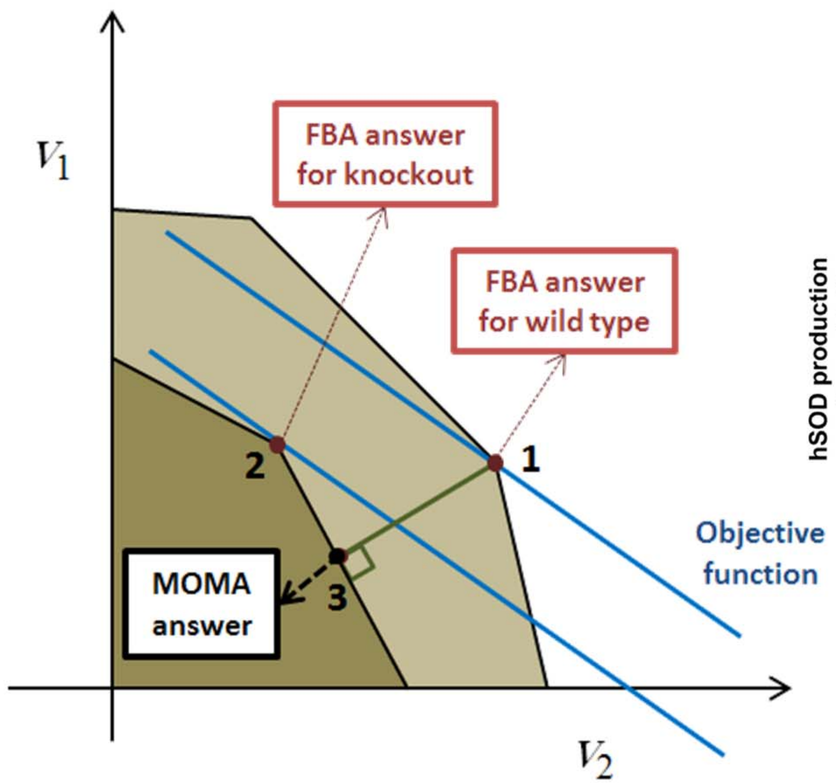
Recombinant Protein Production

How metabolic modeling can help

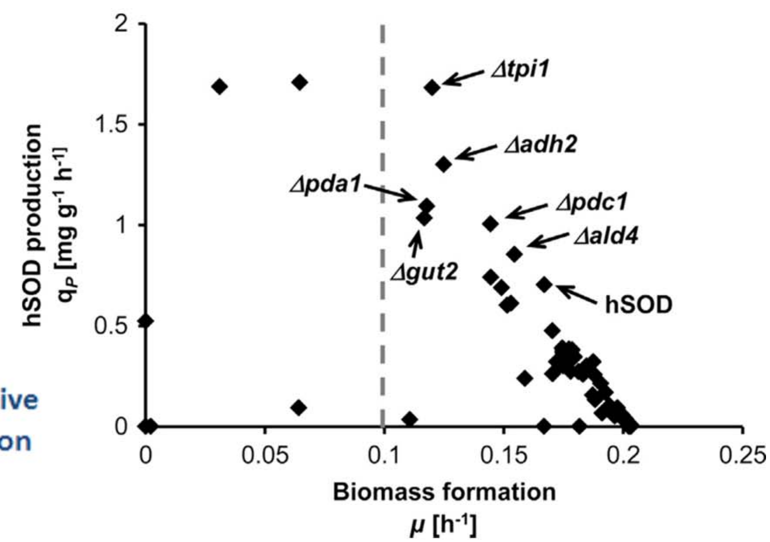
In silico prediction of gene manipulations

- "MOMA" for knock-out (Segrè *et al.*, Proc. Natl. Acad. Sci., 2002)
- "FSEOF" for over expression (Choi *et al*., Appl. Environ. Microbiol., 2010)

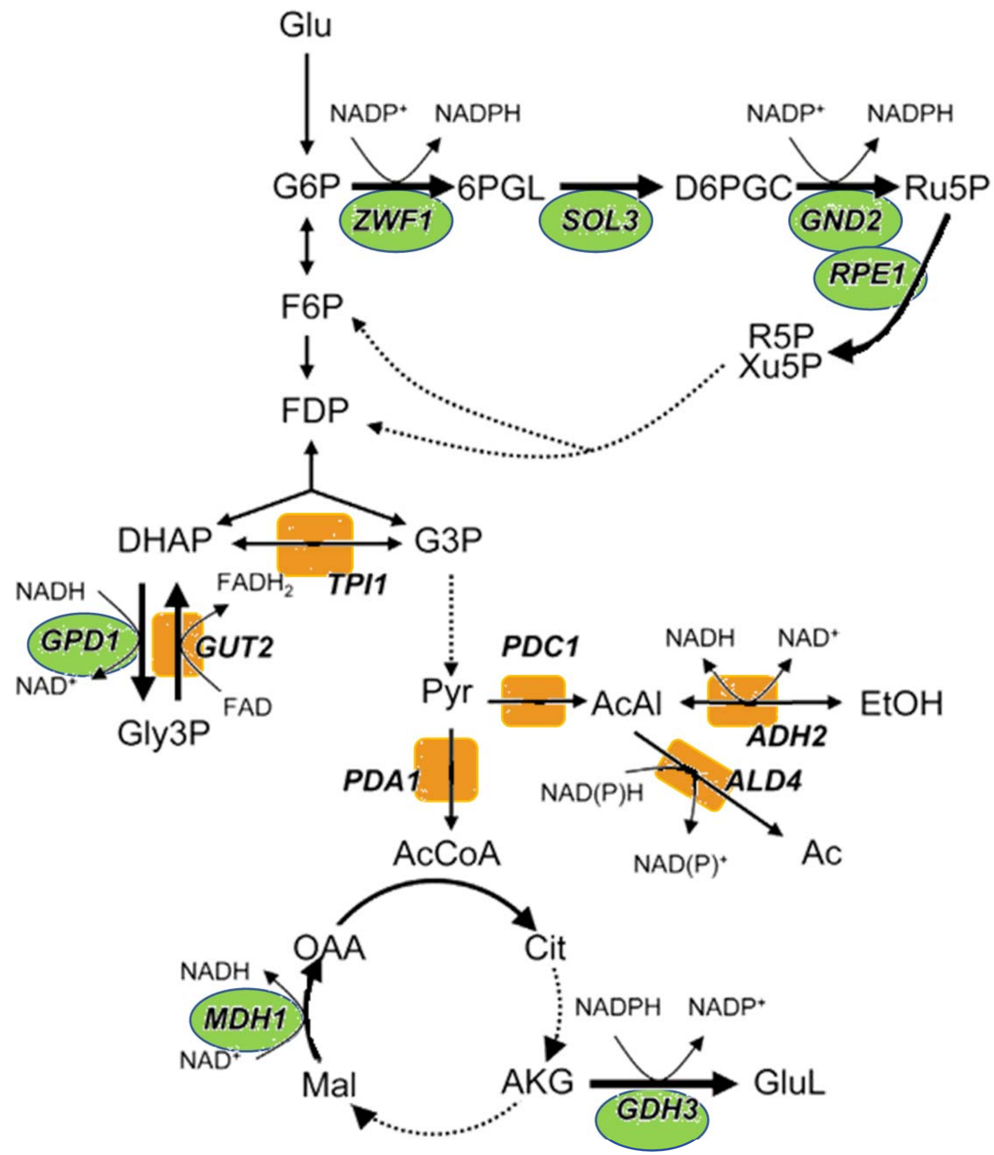
MOMA



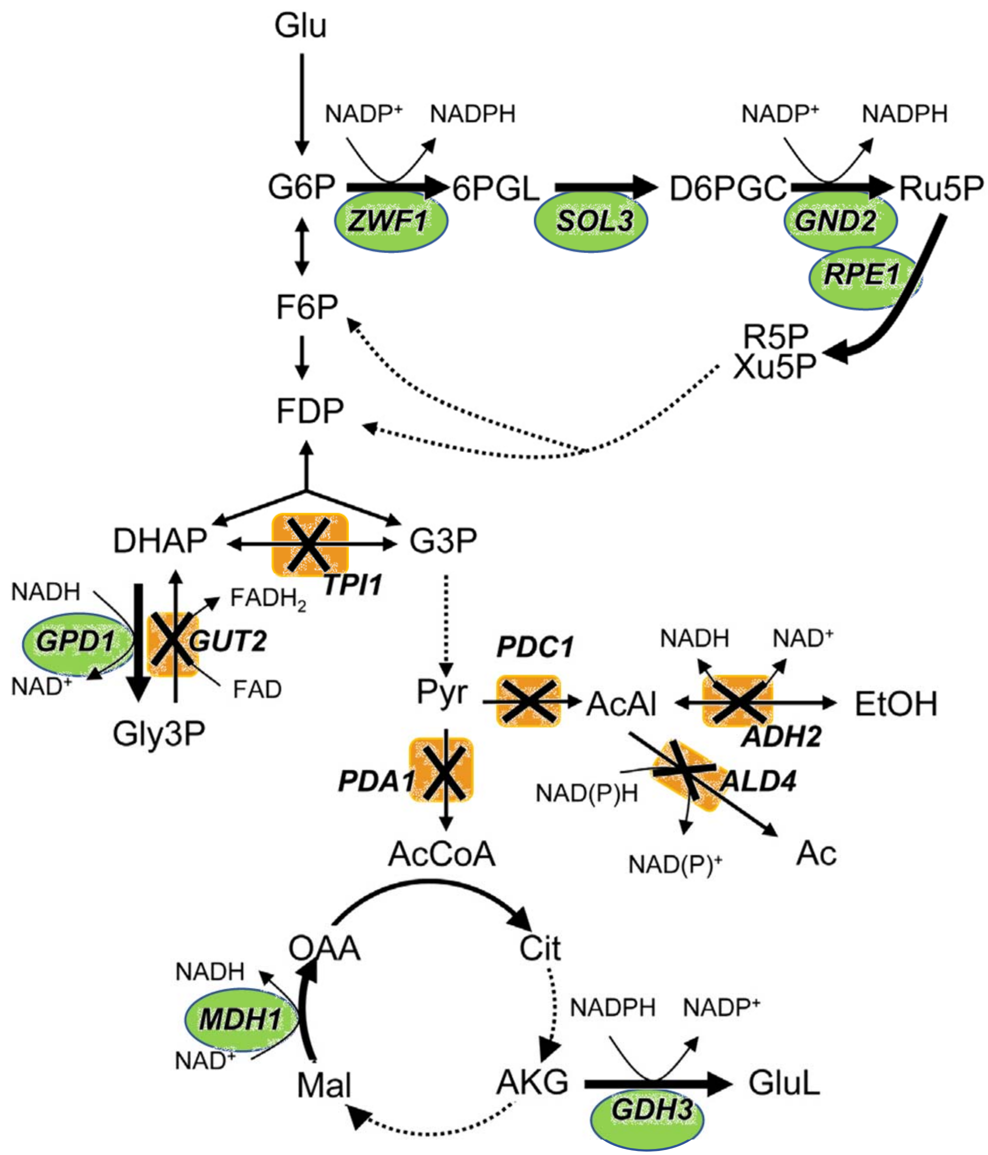
V_1
FBA answer for knockout
FBA answer for wild type
1
2
3
MOMA answer
Objective function
V_2

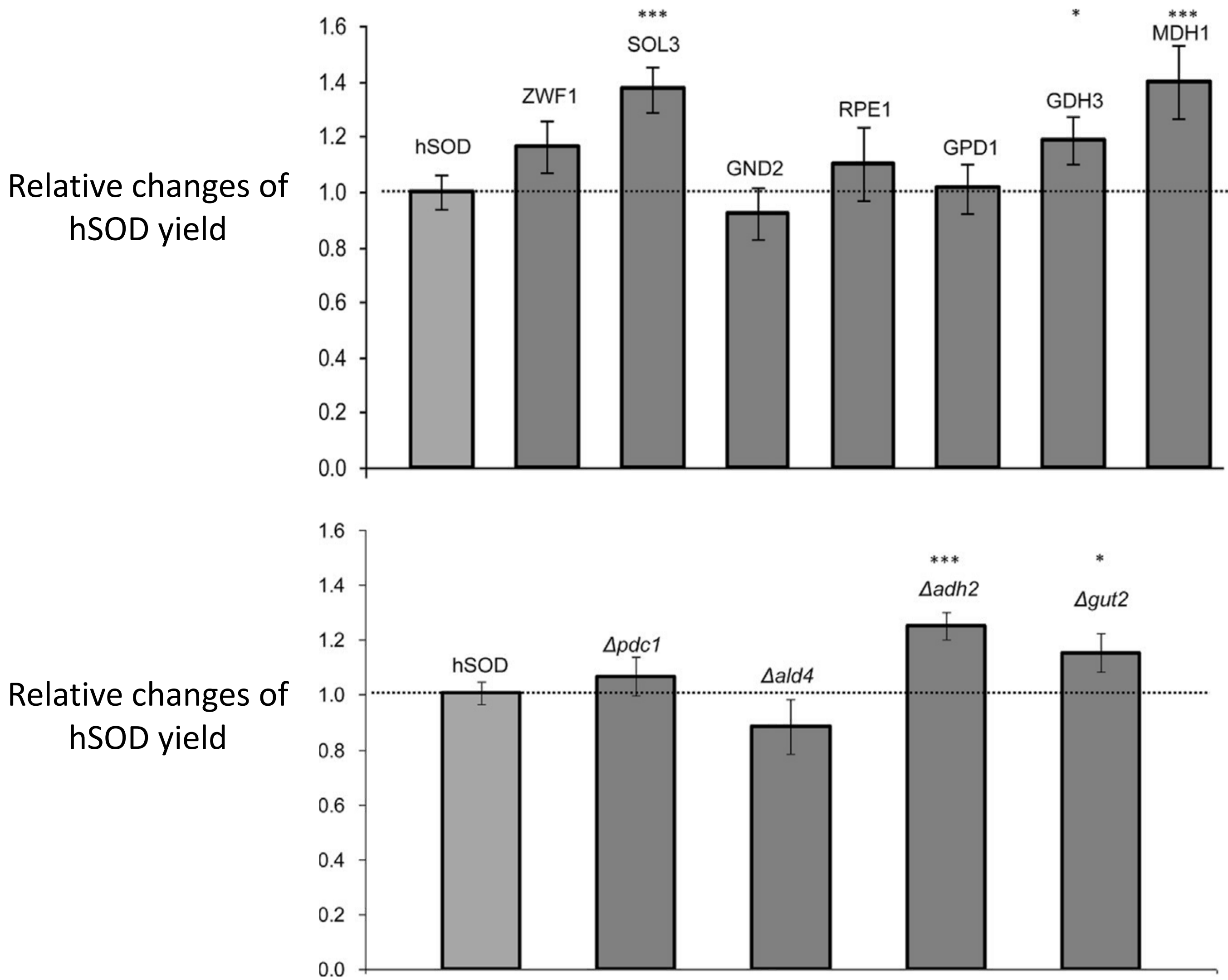


hSOD production
q_P [mg g^{-1} h^{-1}]
Biomass formation
μ [h^{-1}]
Δtpi1
Δadh2
Δpda1
Δpdc1
Δald4
Δgut2
hSOD
0
0.5
1
1.5
2
0
0.05
0.1
0.15
0.2
0.25



Glu
NADP+
NADPH
G6P
ZWF1
6PGL
SOL3
D6PGC
NADP+
NADPH
GND2
Ru5P
RPE1
F6P
R5P
Xu5P
FDP
DHAP
TPI1
G3P
NADH
FADH2
GPD1
GUT2
NAD+
FAD
Gly3P
PDC1
Pyr
AcAl
NADH
NAD+
ADH2
EtOH
PDA1
ALD4
NAD(P)H
NAD(P)+
Ac
AcCoA
OAA
Cit
NADH
MDH1
NAD+
Mal
AKG
NADPH
NADP+
GDH3
GluL

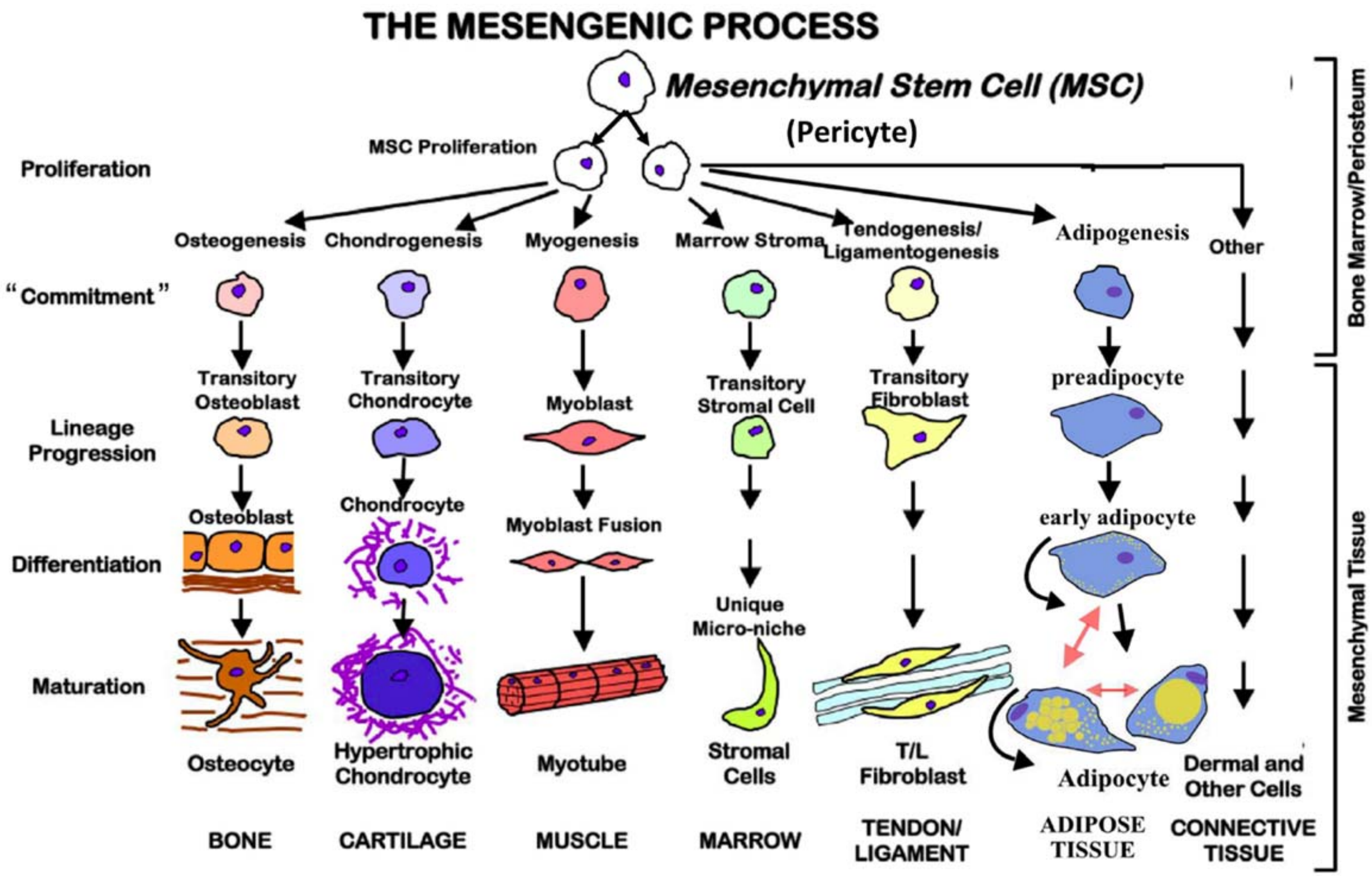

Glu
NADP+
NADPH
G6P
6PGL
D6PGC
Ru5P
ZWF1
SOL3
GND2
RPE1
F6P
R5P
Xu5P
FDP
DHAP
G3P
TPI1
NADH
FADH2
GPD1
GUT2
NAD+
FAD
Gly3P
PDC1
Pyr
AcAl
ADH2
EtOH
ALD4
PDA1
NAD(P)H
NAD(P)+
AcCoA
Ac
OAA
Cit
MDH1
NADPH
NADP+
Mal
AKG
GDH3
GluL


Relative changes of hSOD yield
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
hSOD
ZWF1
***
SOL3
GND2
RPE1
GPD1
*
GDH3
***
MDH1
Relative changes of hSOD yield
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
hSOD
Δpdc1
Δald4
***
Δadh2
*
Δgut2

Modeling Stem Cell Metabolism

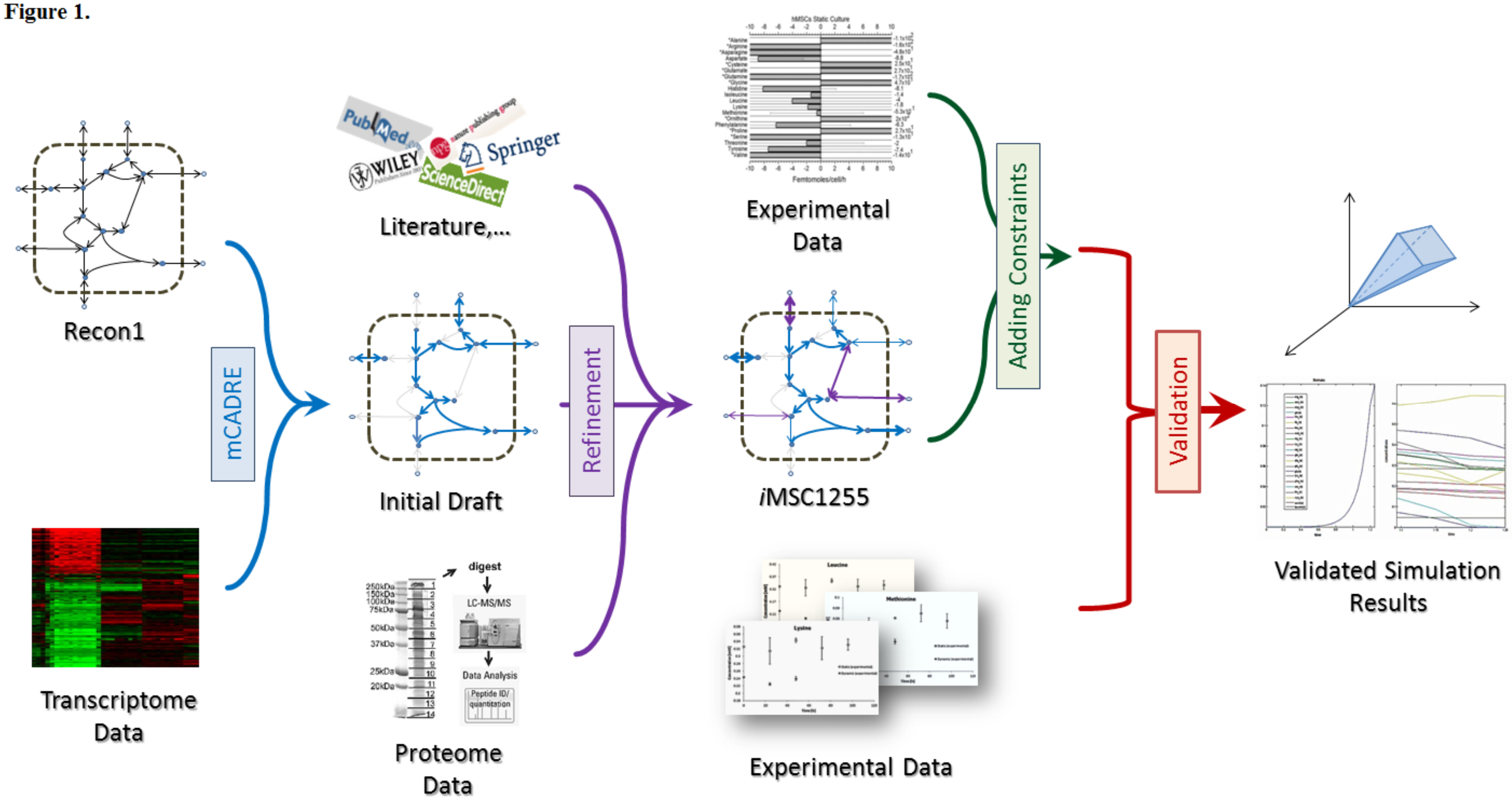
Application to MSCs

Mesenchymal Stem Cells

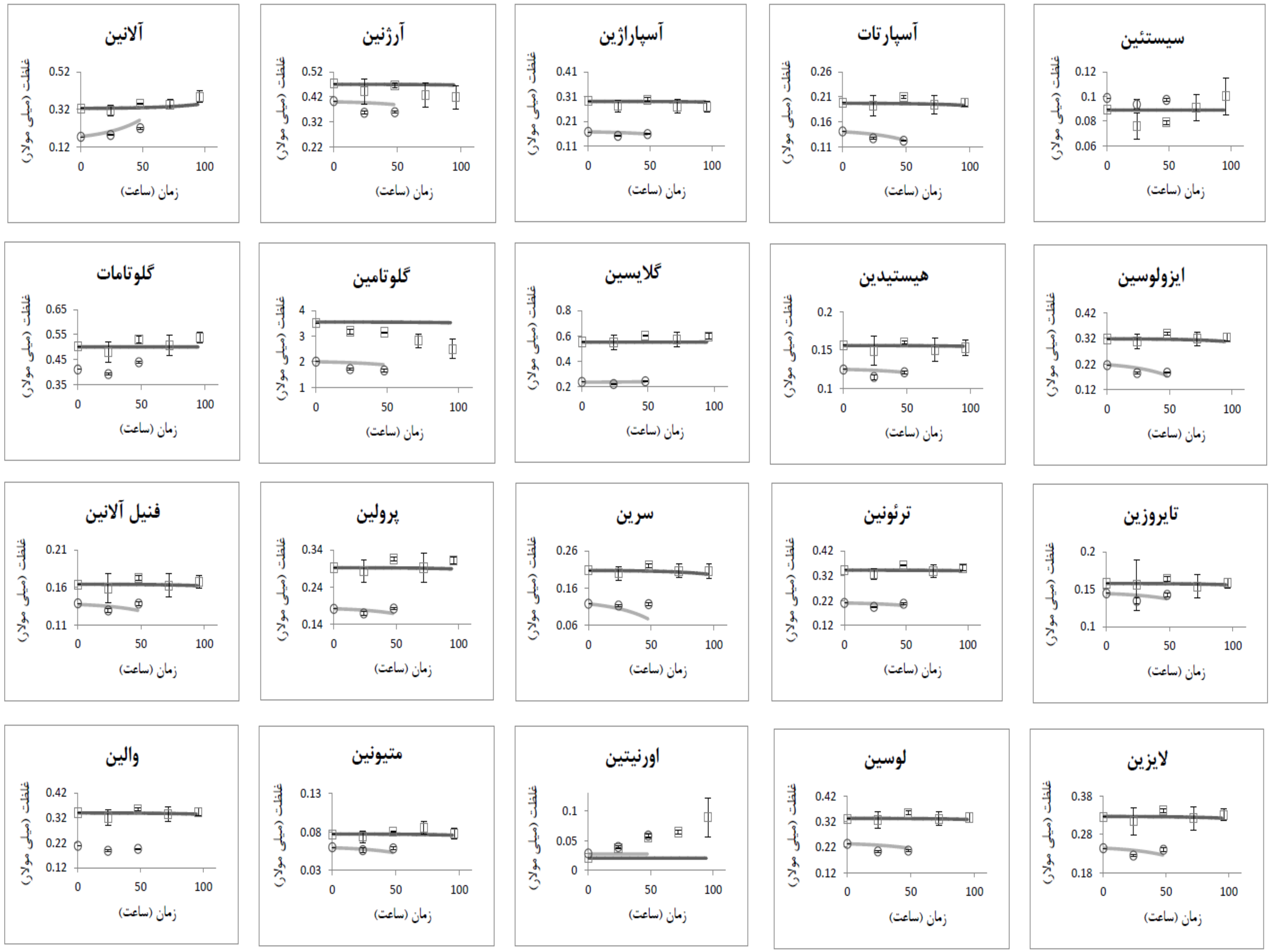


Stem Cell Metabolic Network

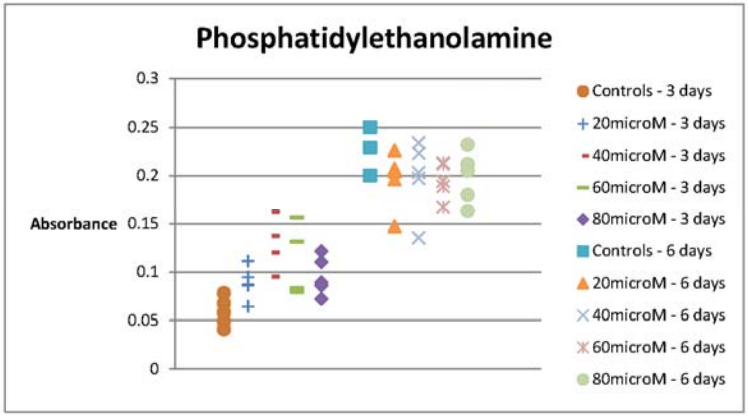
Figure 1.

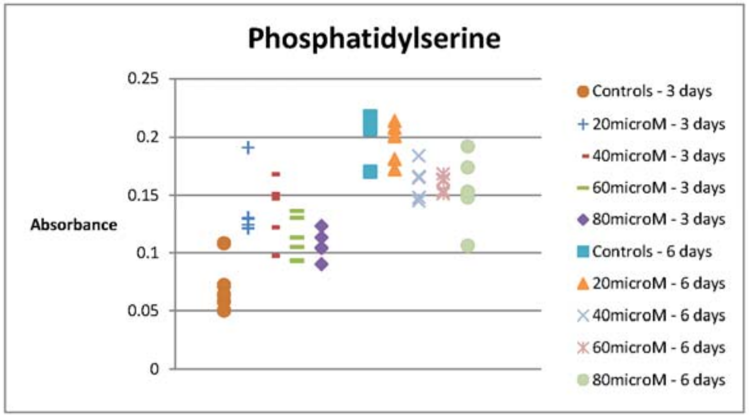


Fouladiha et al., 2015, Cell Proliferation, 48:475-485.


سیستئین
آسپارتات
آسپاراژین
آرژنین
آلانین
ایزولوسین
هیستیدین
گلایسین
گلوتامین
گلوتامات
تایروزین
ترئونین
سرین
پرولین
فنیل آلانین
لایزین
لوسین
اورنیتین
متیونین
والین
غلظت (میلی مولار)
زمان (ساعت)

Speed up cell division

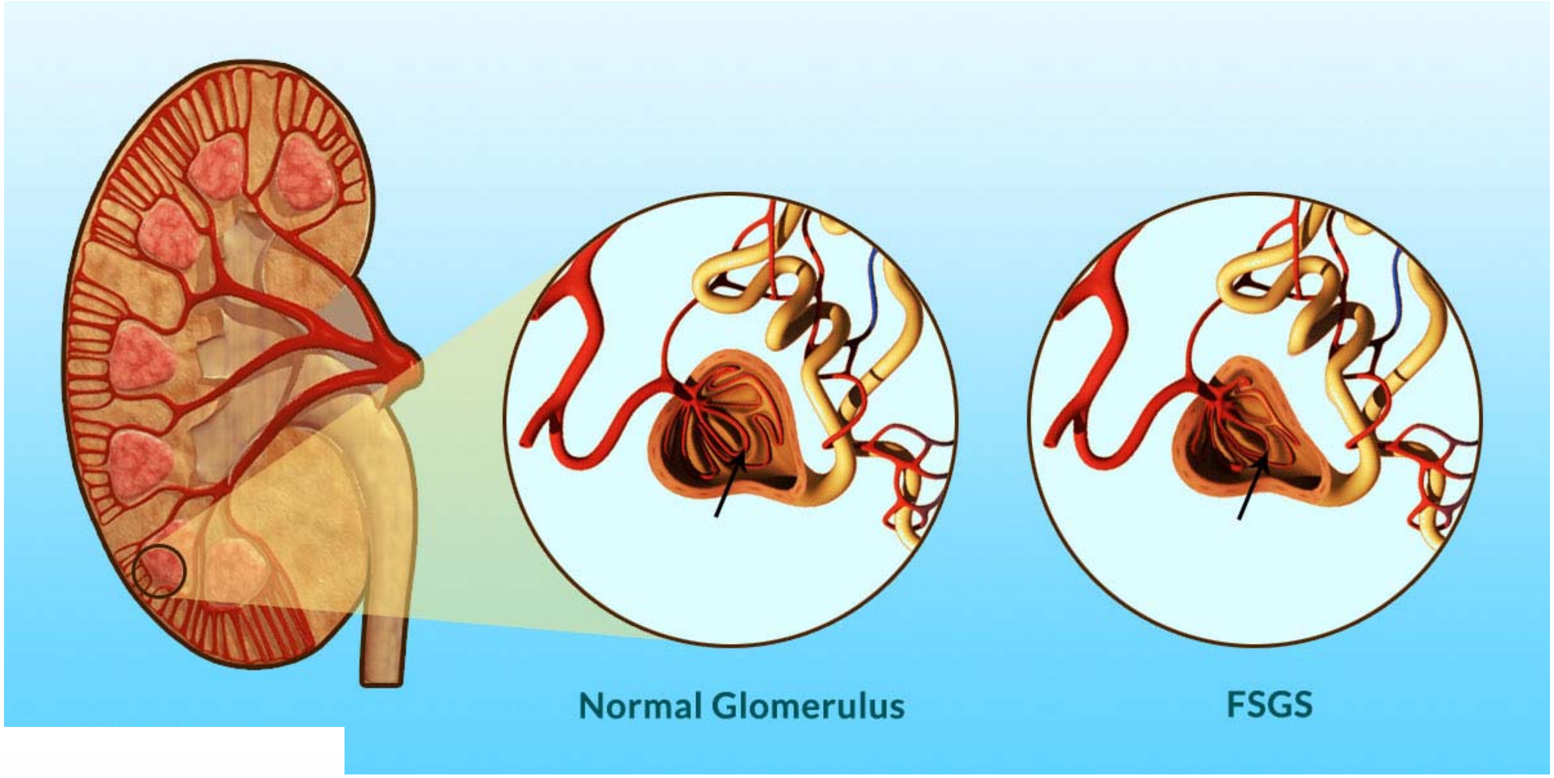


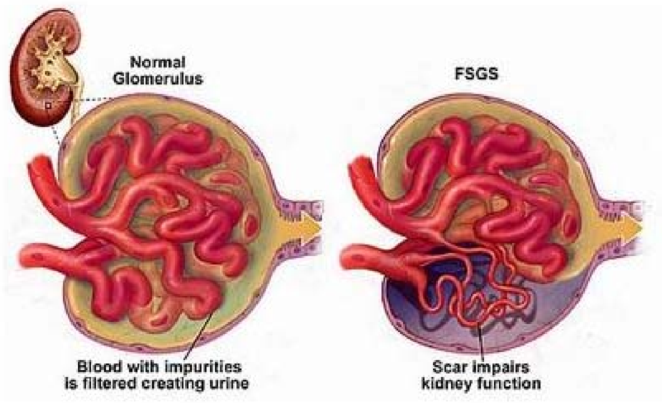


Modeling Kidney Metabolism

Application to FSGS

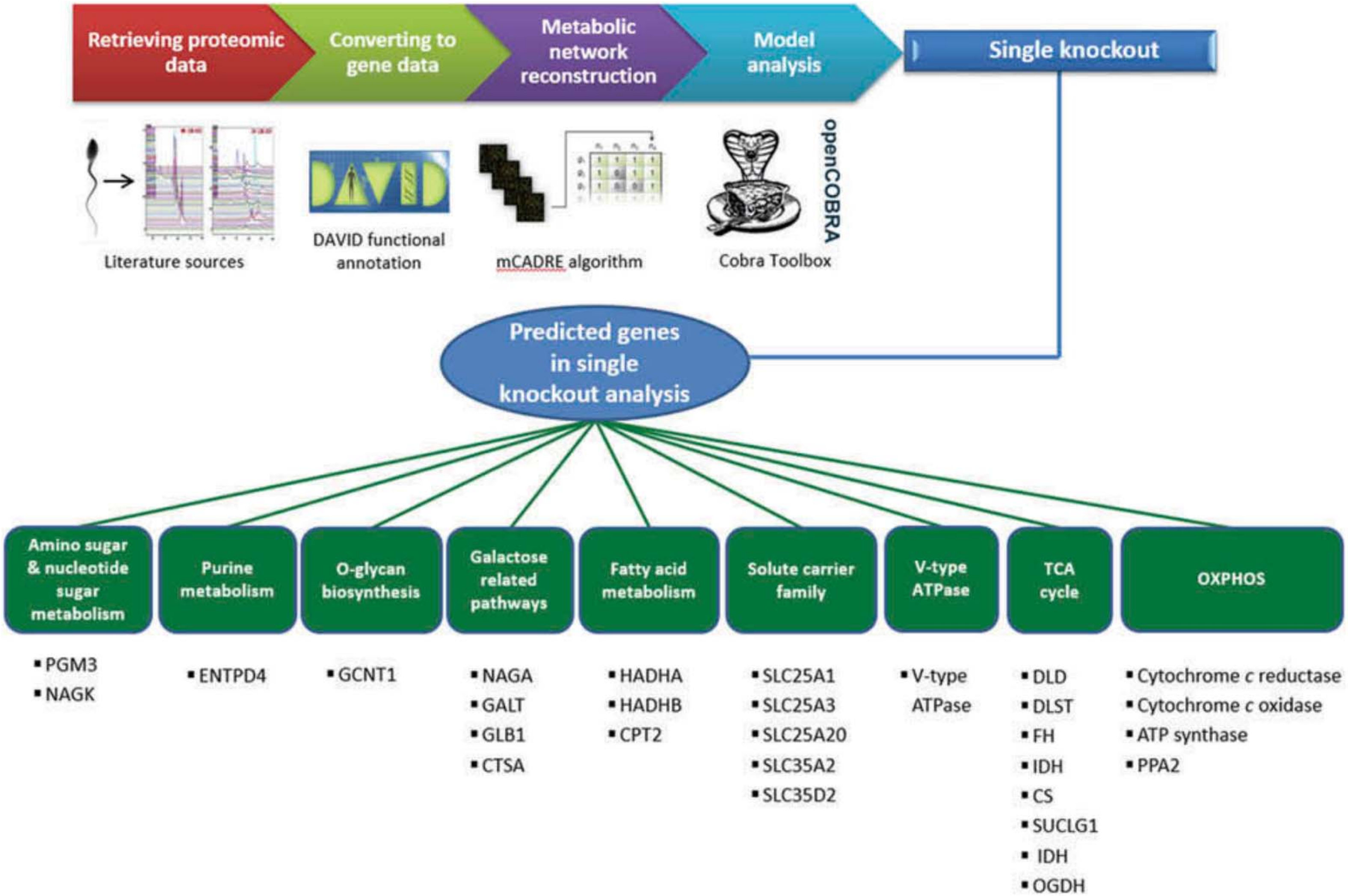
FSGS


Normal Glomerulus
FSGS


Normal
Glomerulus
FSGS
Blood with impurities
is filtered creating urine
Scar impairs
kidney function

Modeling Infertility Metabolism

Sperm metabolism



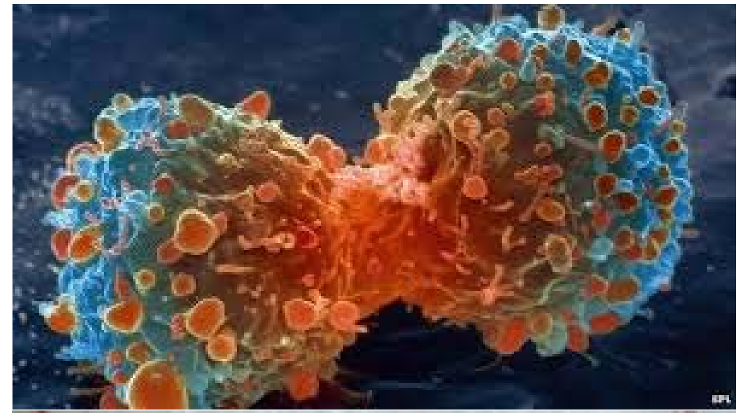
Asghari et al. (2017) *Syst. Biol.* Reproduct. Med. 63: 1.

Modeling Cancer Metabolism

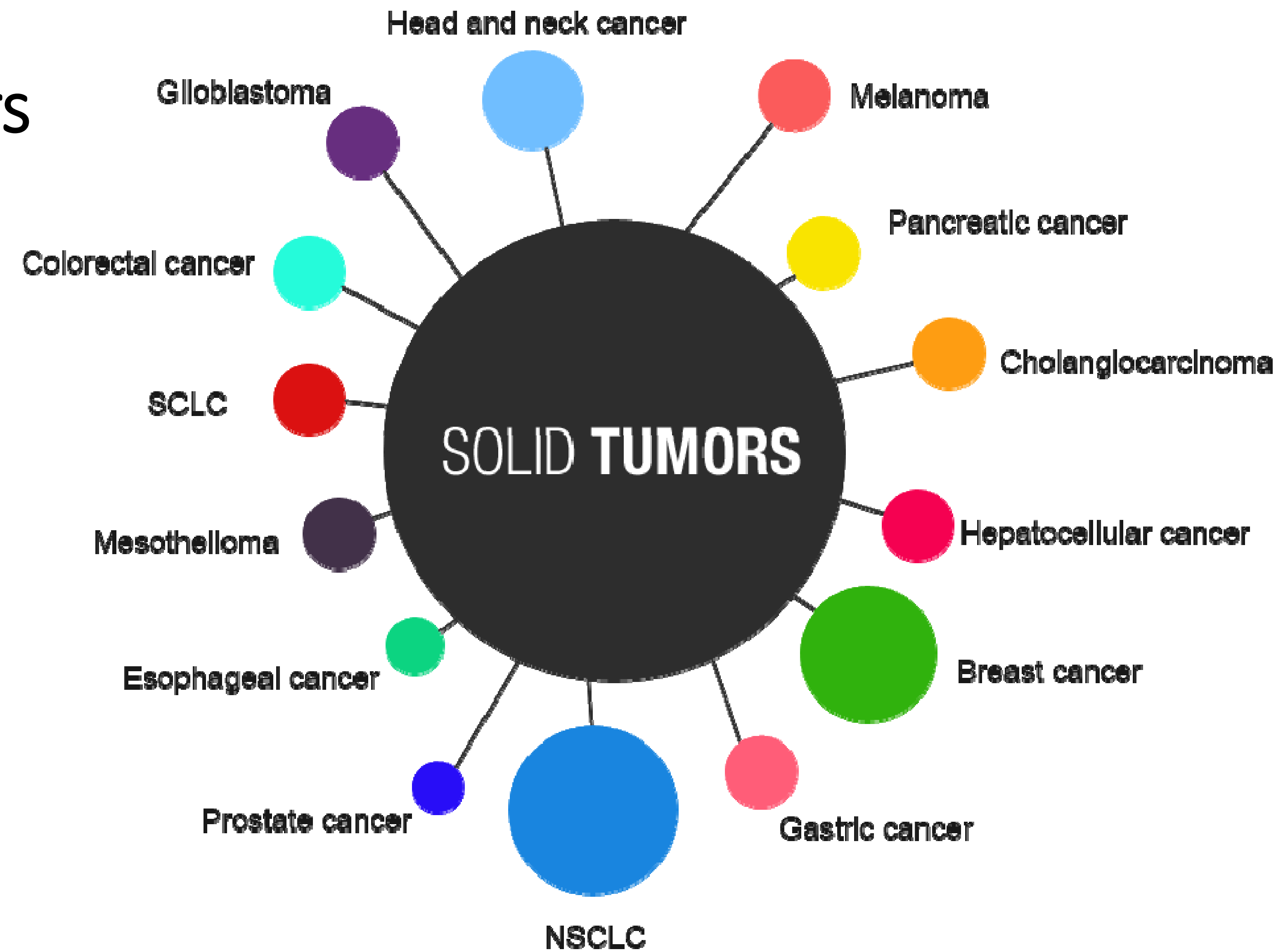
Introduction: Importance of Cancer

- Cancer is the uncontrolled growth and spread of cells.

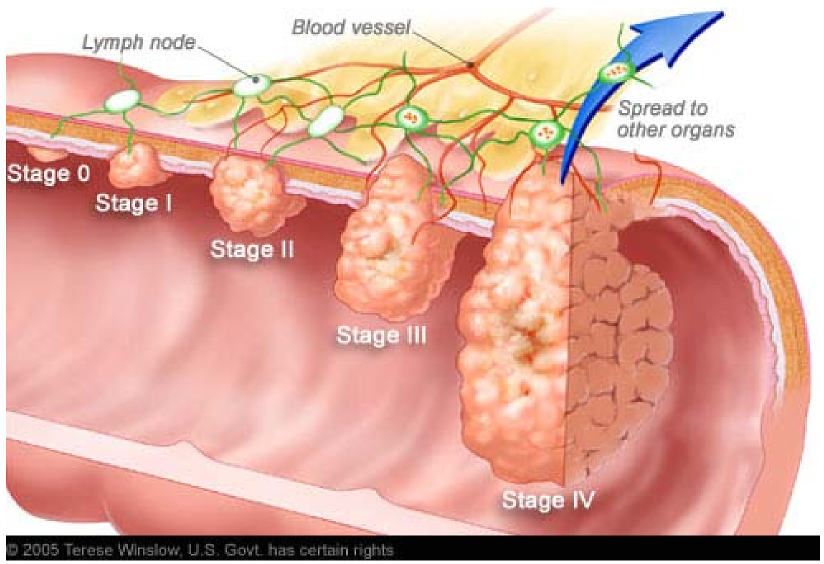
- Its global burden has risen to **14.1** million new cases and **8.2** million deaths in per year.



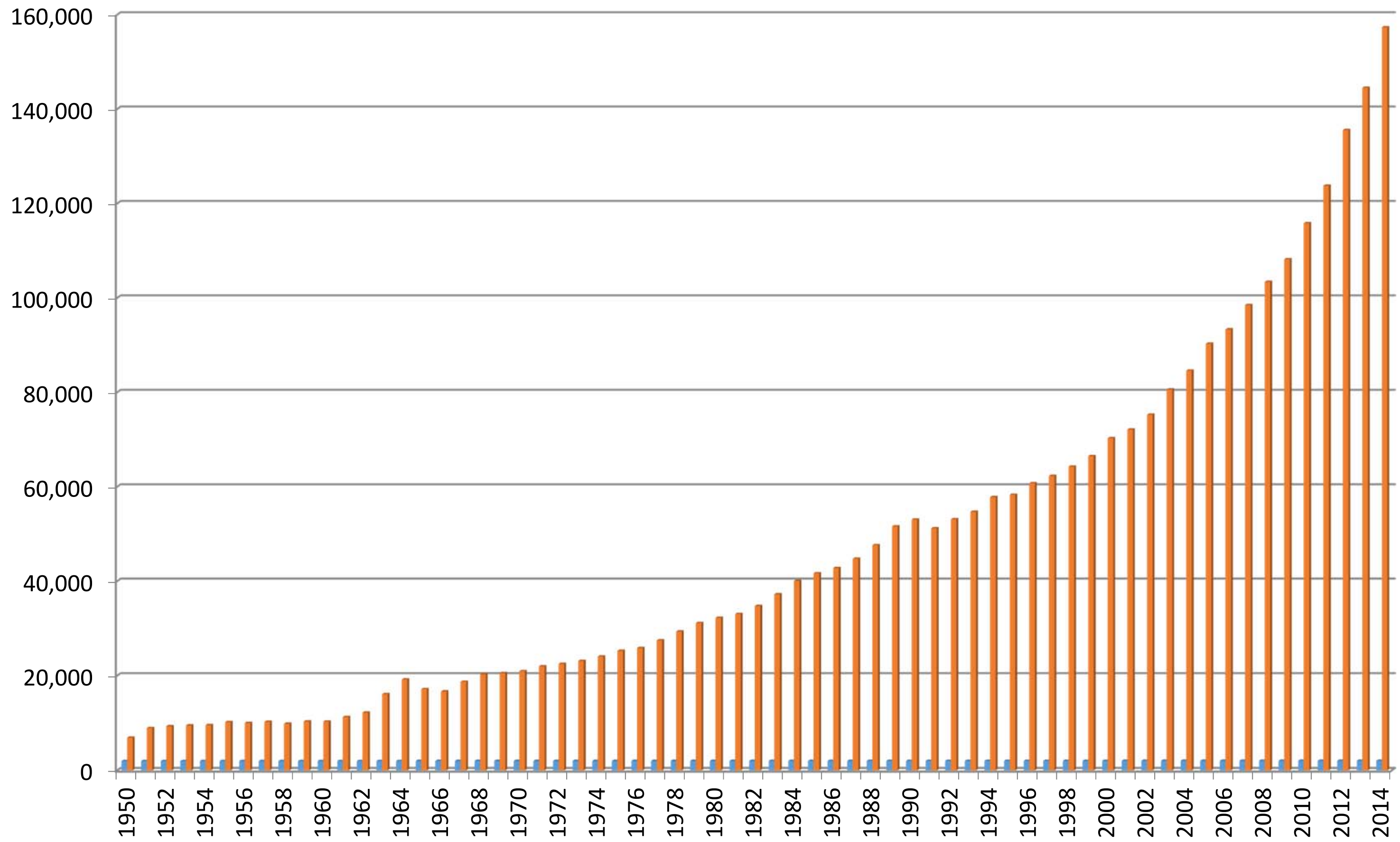
Solid Tumors



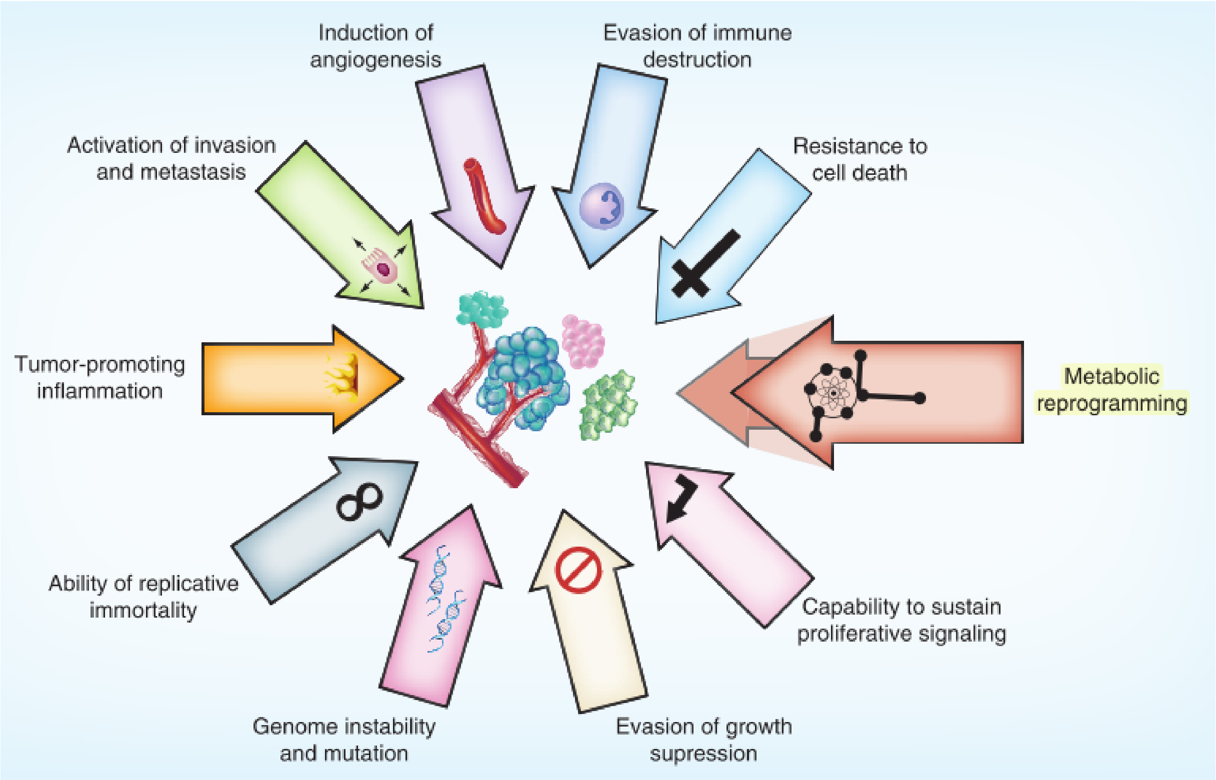
Solid Tumor Growth



Cancer-related publications based on PubMed



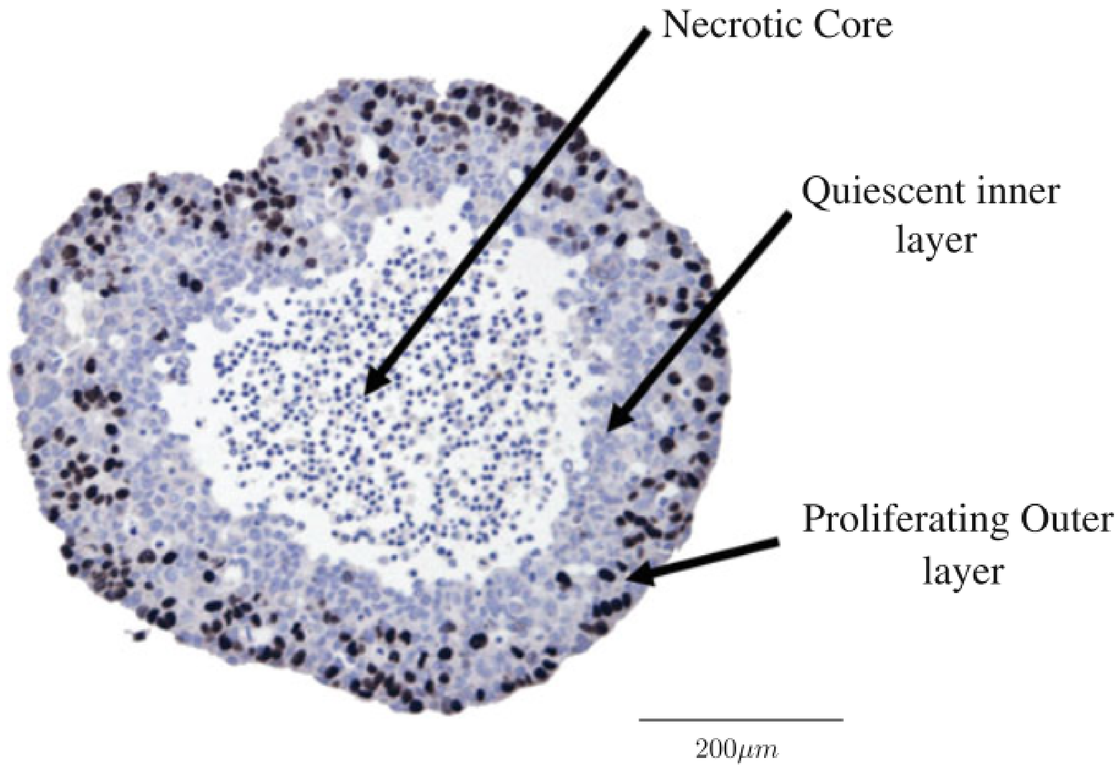
Hallmarks of Cancer



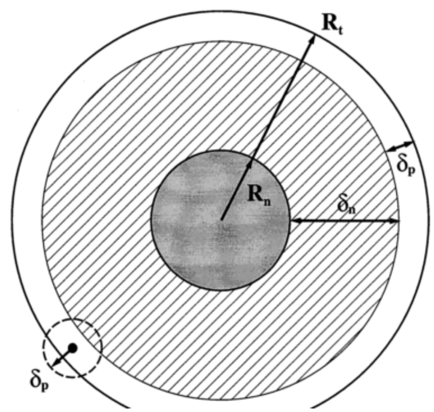
*Hanahan and Weinberg (2011) *Cell* 144: 646–674.

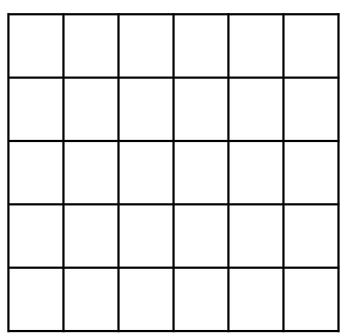
Modeling Solid Tumors

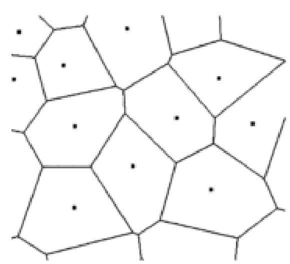
- The goal of modeling is to:
  - model tumor growth rate
  - model composition of tumor
  - predict the effect of drugs
  - (predict other phenotypes)

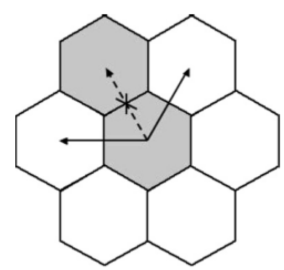


Continuous vs. Discrete Models



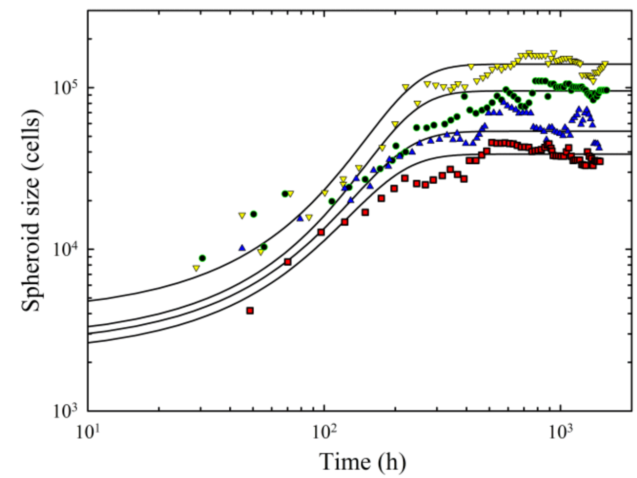






Continuous Models

- Describe the numerical changes of the variables that represent the system
- Give information about the overall tumor morphology
- Neglect the influences of individual cells in the environment
- Employ classical differential equations-based models
- Shortcoming in simulating emergent behavior
- Assume homogeneity of system components



Exponential-linear

$$\begin{cases} \frac{dV}{dt} = a_0 V, & t \leq \tau \\ \frac{dV}{dt} = a_1, & t > \tau \\ V(t=0) = V_0 \end{cases}$$

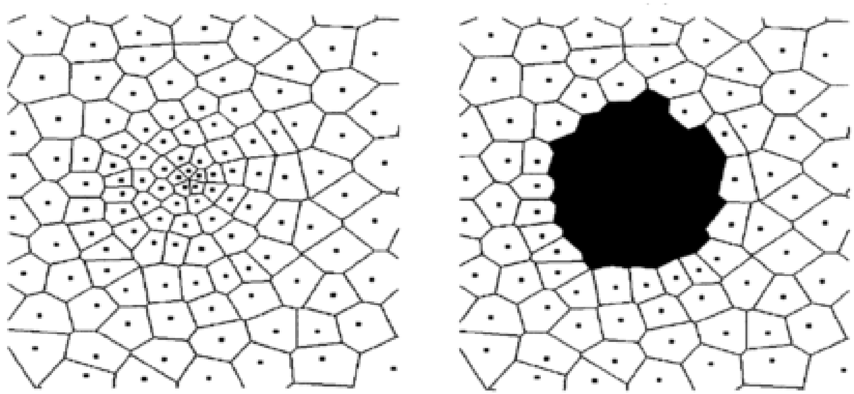
Generalized logistic

$$\begin{cases} \frac{dV}{dt} = aV\left(1 - \left(\frac{V}{K}\right)^{\alpha}\right) \\ V(t=0) = 1\,mm^3 \end{cases} \longrightarrow \quad V(t) = \frac{V_0 K}{(V_0^{\alpha} + (K^{\alpha} - V_0^{\alpha})e^{-a\alpha t})^{\frac{1}{\alpha}}}$$

Gompertz model

$$\begin{cases} \frac{dV}{dt} = ae^{-\beta t}V \\ V(t=0) = 1\,mm^3 \end{cases} \longrightarrow \quad V(t) = e^{\frac{a}{\beta}(1-e^{-\beta t})}$$

Example of Discrete models: Adaptive Lattice



Kansal, A., et al. (2000) *Journal of Theoretical Biology,* 203:367-382.

Example of Hybrid models: Inclusion of Metabolism

- Intracellular and extracellular pH (H^+ production) and lactate concentration and their effects on cells

- Incorporation of NHE and MCT transporters

$$\frac{dH_I(\mathbf{x},t)}{dt} = \frac{2\Phi_G \mathrm{J}(V^g - V(\mathbf{x},t))}{H_I(\mathbf{x},t)+b} + d_1 + \phi(\mathbf{x},t)$$

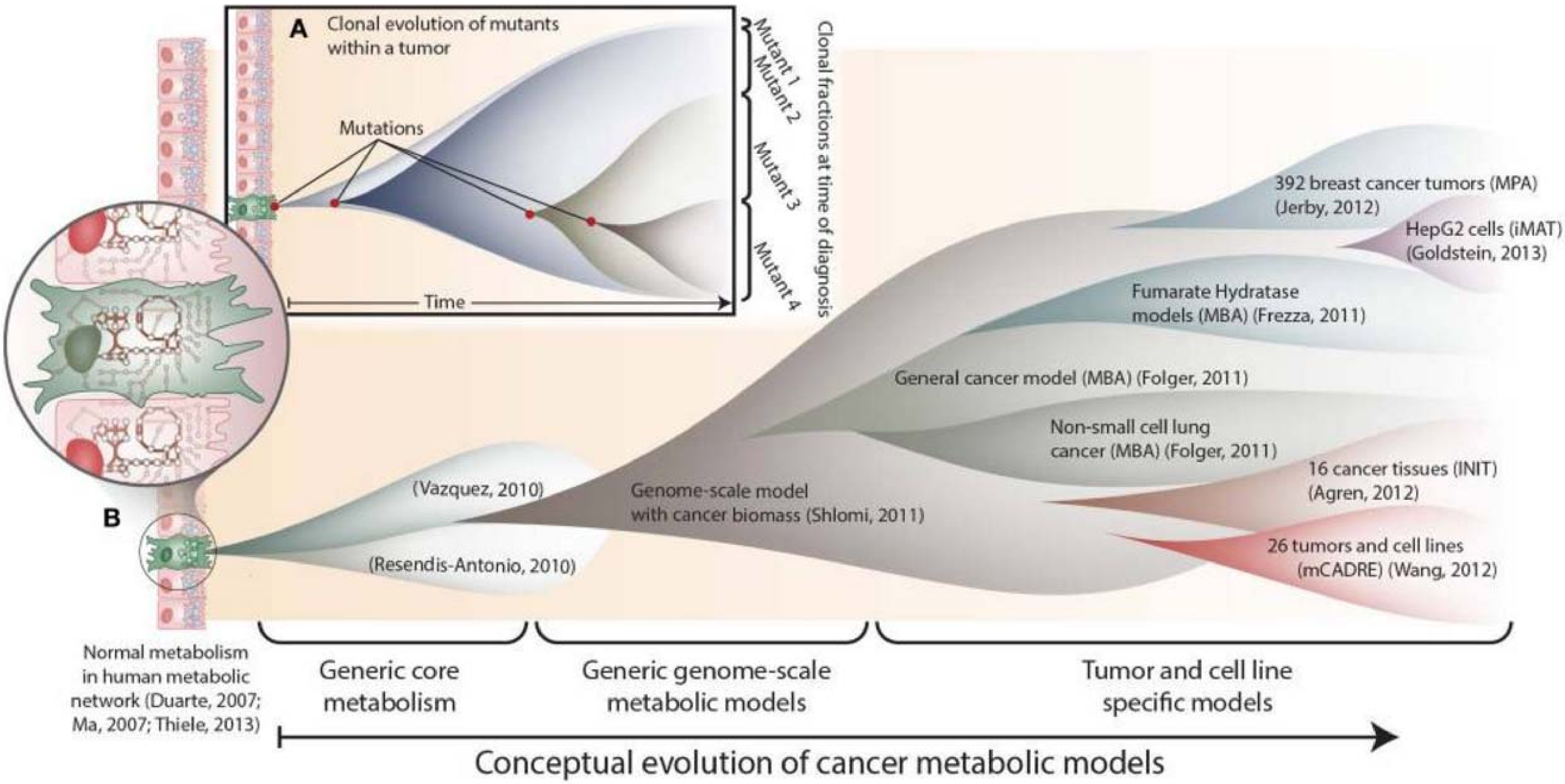
$$\frac{\partial H_E(\mathbf{x},t)}{\partial t} = D_H \nabla^2 H_E(\mathbf{x},t) - \phi(\mathbf{x},t)$$

$$\frac{dL_I(\mathbf{x},t)}{dt} = \frac{2\Phi_G \mathrm{J}(V^g - V(\mathbf{x},t))}{H_I(\mathbf{x},t)+b} + d_4 - \alpha_4 L_I(\mathbf{x},t) - \theta(\mathbf{x},t)$$

$$\frac{\partial L_E(\mathbf{x},t)}{\partial t} = D_L \nabla^2 L_E(\mathbf{x},\mathbf{t}) + \theta(\mathbf{x},t)$$

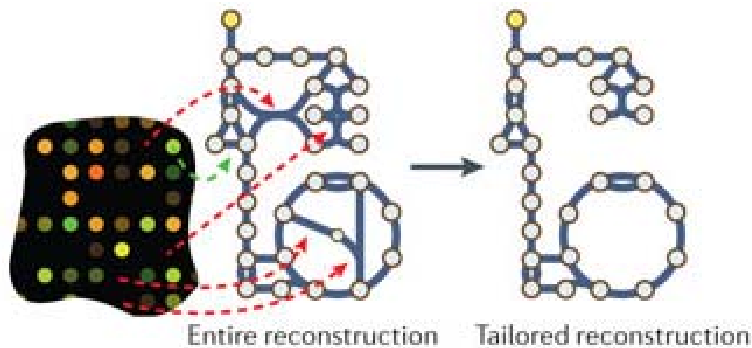
Al-Husari et al. (2014) *Journal of Mathematical Biology* 69: 839-873.

Metabolic Network Models of Cancer



Lewis and Abdel-Haleem (2013) *Front Physiol* 4: 237.

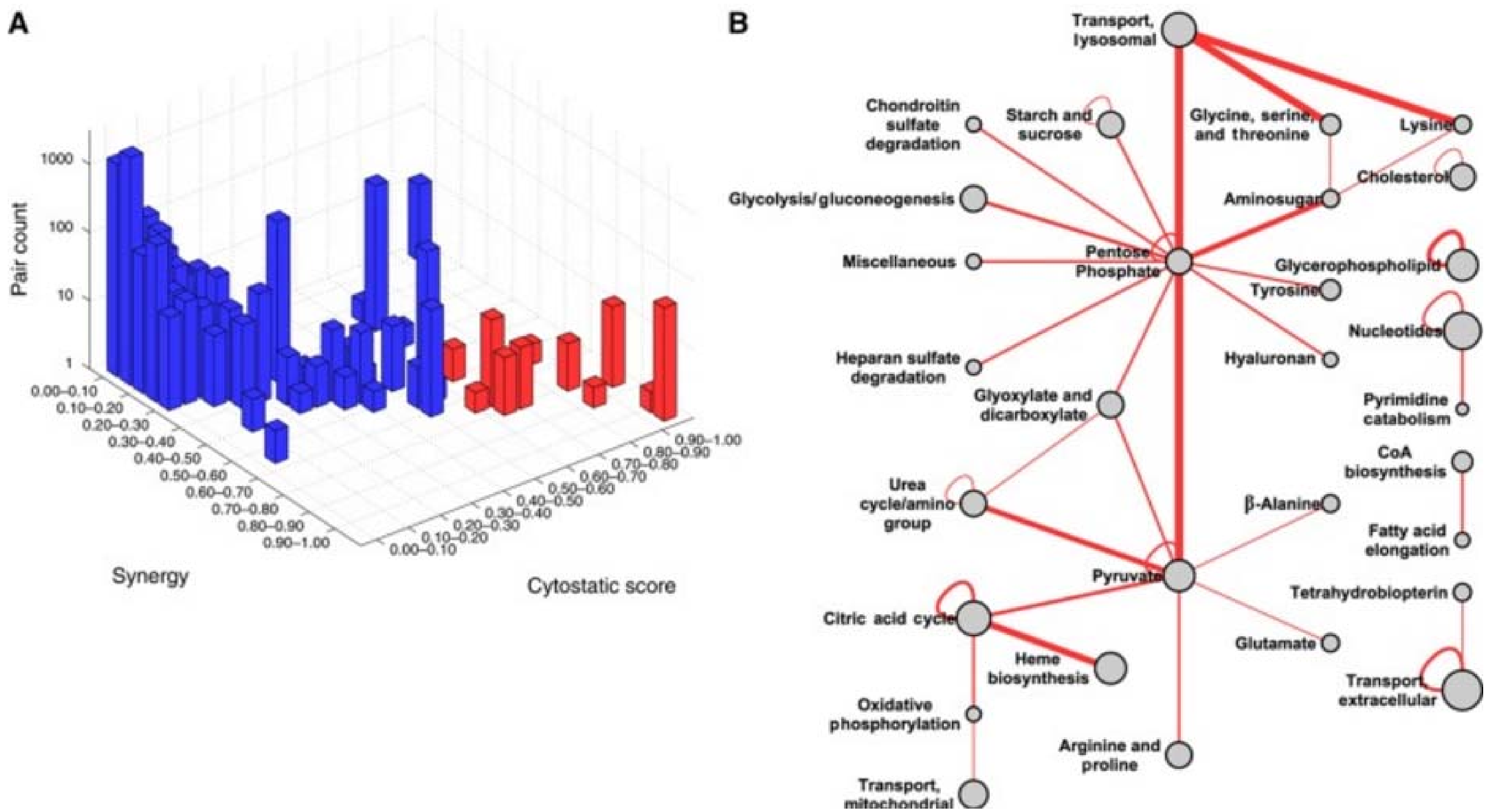
Generic Cancer Metabolic Model



MBA Algorithm [Jebry et al., *Mol. Syst. Biol.* 6 (2010) 401]

Folger et al. (2011) *Mol. Syst. Biol.* 7: 501.

Predictions of the Generic Cancer Metabolic Model



Another Generic Cancer Metabolic model

- Developed by blockade of tumor suppressor genes of Recon1
- Includes 3788 reactions, 2766 metabolites, 404 uptake reactions

Molecular BioSystems

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
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Cite this: *Mol. BioSyst.*, 2014, 10, 3014

Reconstruction of a generic metabolic network model of cancer cells†

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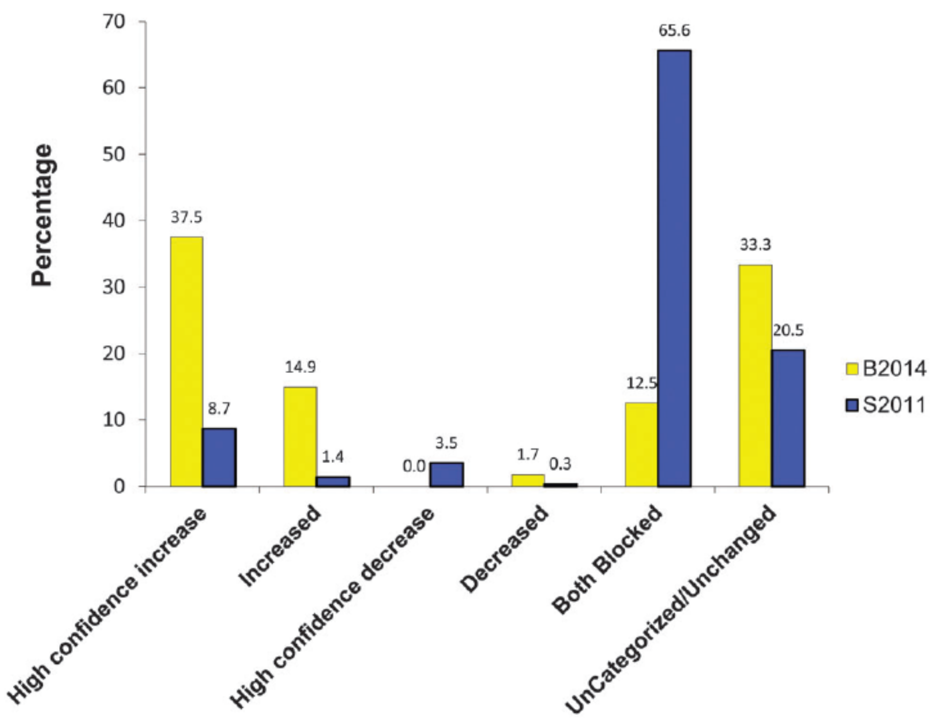
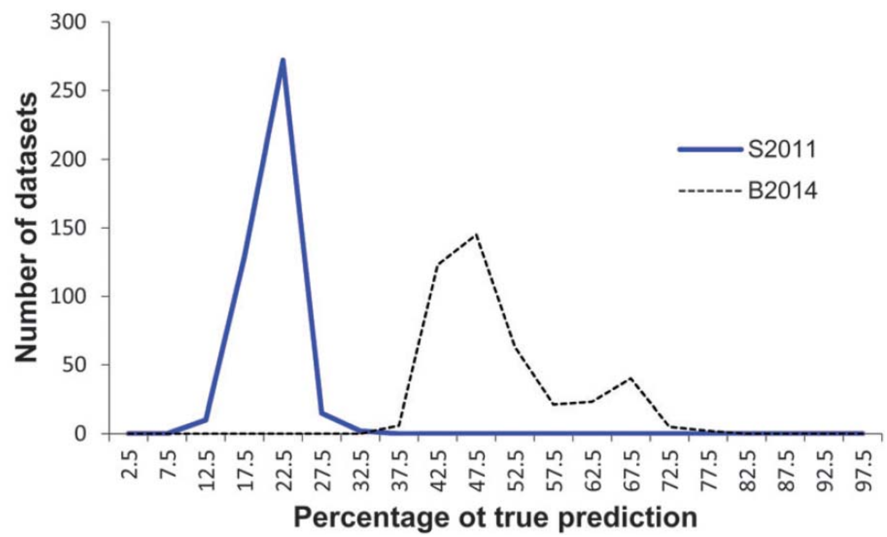
Hadi and Marashi (2014) *Molecular BioSystems* 10: 3014-3021.

Comparing the Two Generic Models



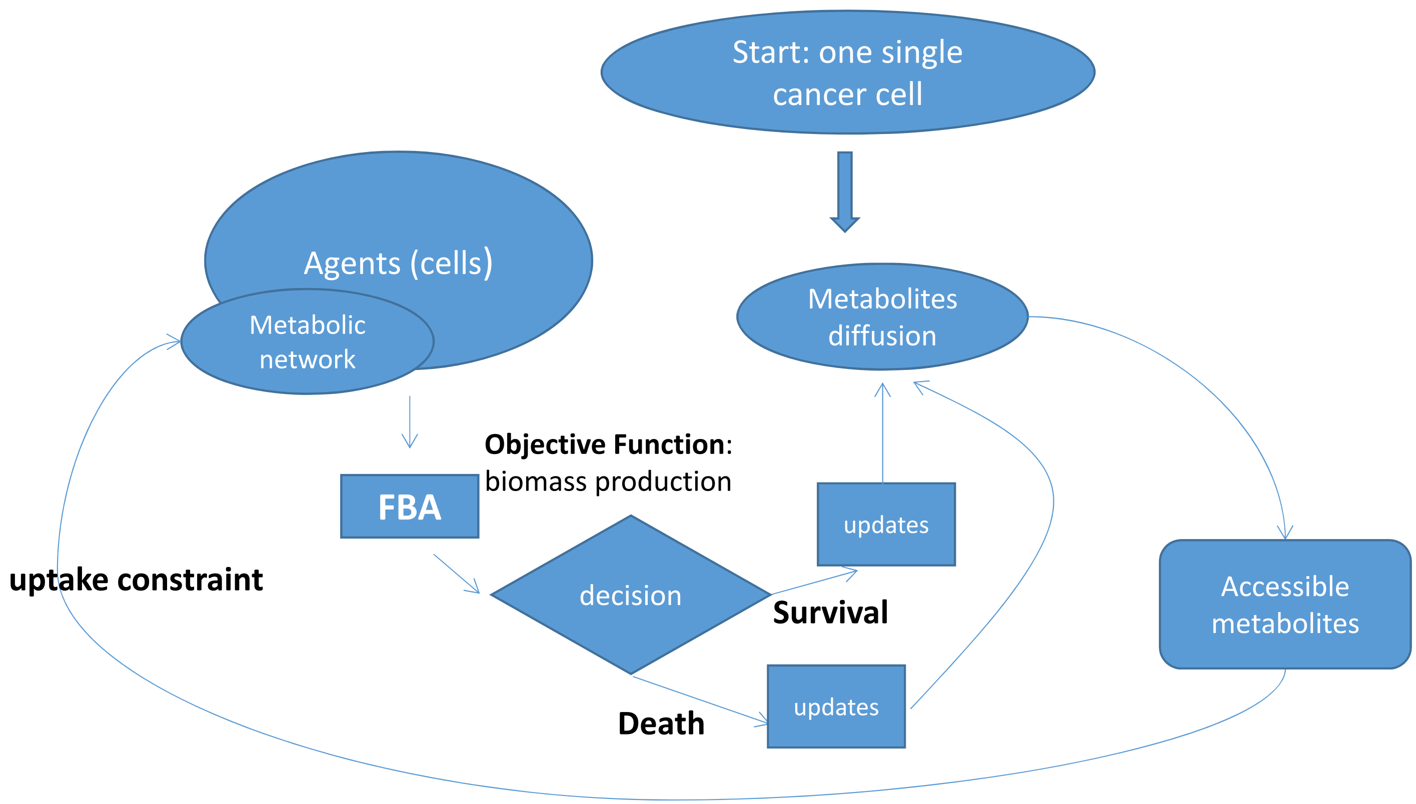


Hadi and Marashi (2014) *Molecular BioSystems* 10: 3014-3021.

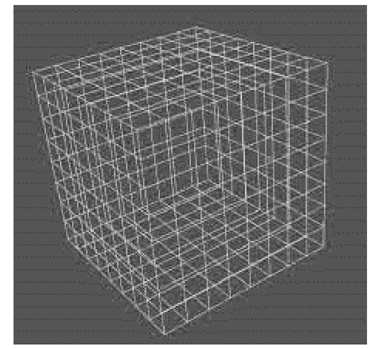
Our New Approach for Modeling Solid Tumors

Constraint-based model of cancer integrated in cellular automata model

The Hybrid Model for Simulating Tumor



Modeling Characteristics



- 3D lattice
- Compartment attributes:
  - Occupation state
  - Necrosis state
  - Metabolites concentrations
- Agents (cancer cells) attributes:
  - Biomass value
  - Position
  - State (proliferation vs. quiescence)

Growth Medium in Flux Balance Analysis

- Major metabolites: **Oxygen and D-glucose**
- all uptake reactions blocked **except**:

The only 17 open uptake reactions		
vitamin D3	adenine	L-lysine *
L-arginine	phosphate	**L-valine ***
L-histidine *	**L-tryptophan ***	**L-methionine ***
L-leucine *	**L-threonine ***	**O_2**
D-glucose	L-aspartate	**Sphinganine 1-p**
L-phenylalanine *	**L-isoleucine ***	

(***** = essential amino acid) (**bold =** critical for biomass production by FBA)

The Link between Boundary Flux and External Concentration

- Inward flux constraints (lower/upper bounds):
  - for **O_2** and **glucose**:
    1) Maximum uptake capability
    2) Extracellular metabolite concentration

Relating metabolite concentration to uptake flux constraint

$$Q_{Gluc,transmembrane} = \frac{Q_{Gluc,max} c_{Gluc}}{K_{m,Gluc} + c_{Gluc}}$$

$$Q_{Ox,transmembrane} = \frac{Q_{Ox,max} c_{Ox}}{K_{m,Ox} + c_{Ox}}$$

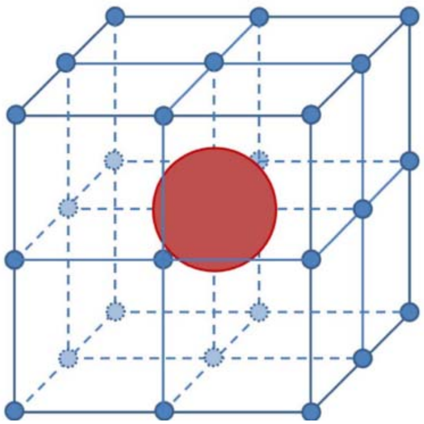
- for other nutrients:
  - A bound reasonable according to their blood concentration and cellular uptake capacity

Venkatasubramanian et al. (2006) *Journal of Theoretical Biology* 242: 440-453.

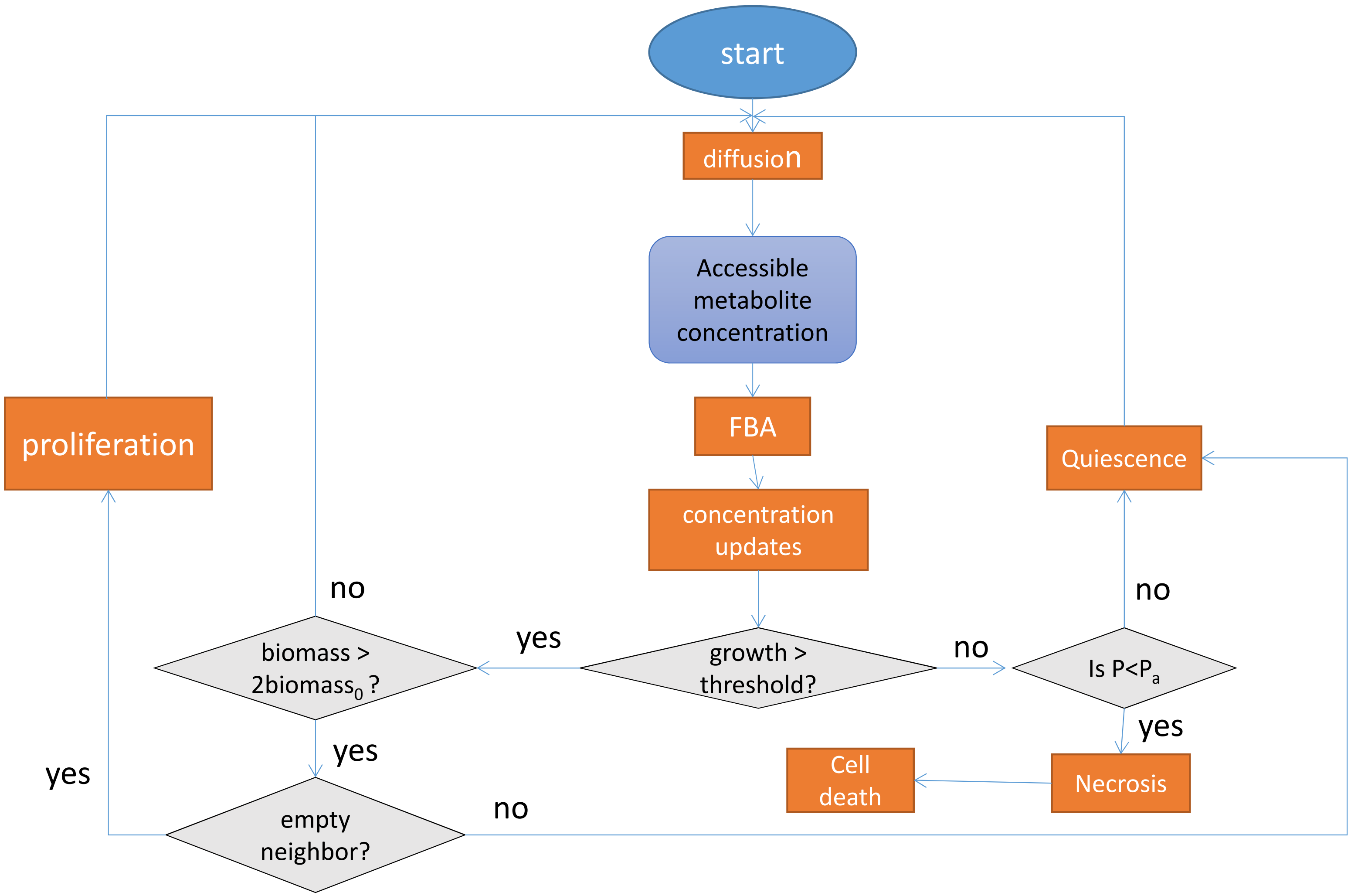
Diffusion / Metabolic fluxes

- **Boundary condition**: constant O_2 and glucose concentration
- PDE solving for diffusion: **Fick's equation**

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}\left(D_e \frac{\partial C}{\partial x}\right) + \frac{\partial}{\partial y}\left(D_e \frac{\partial C}{\partial y}\right) + \frac{\partial}{\partial z}\left(D_e \frac{\partial C}{\partial z}\right)$$

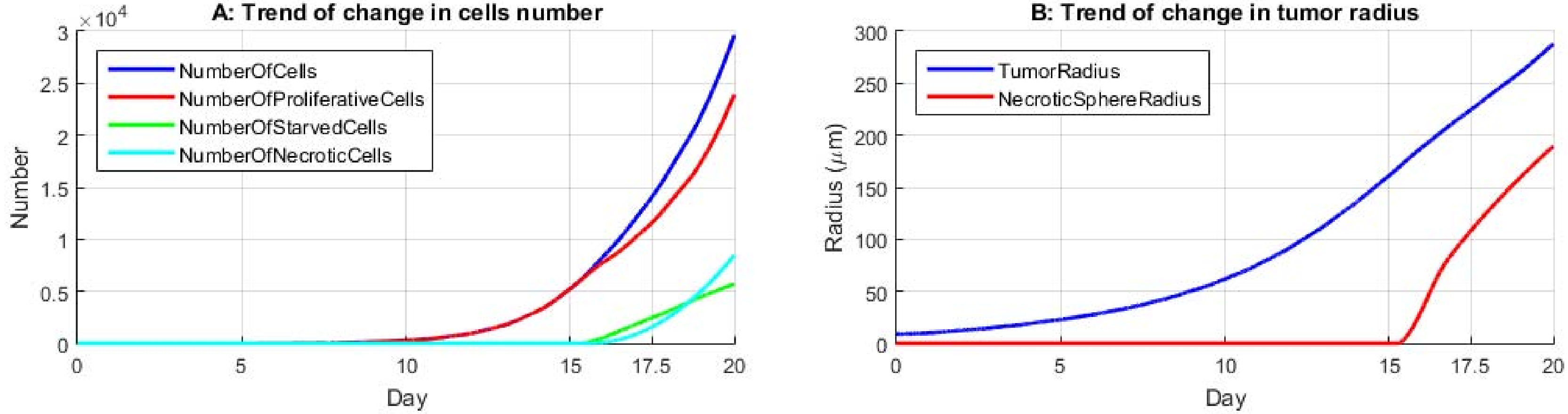


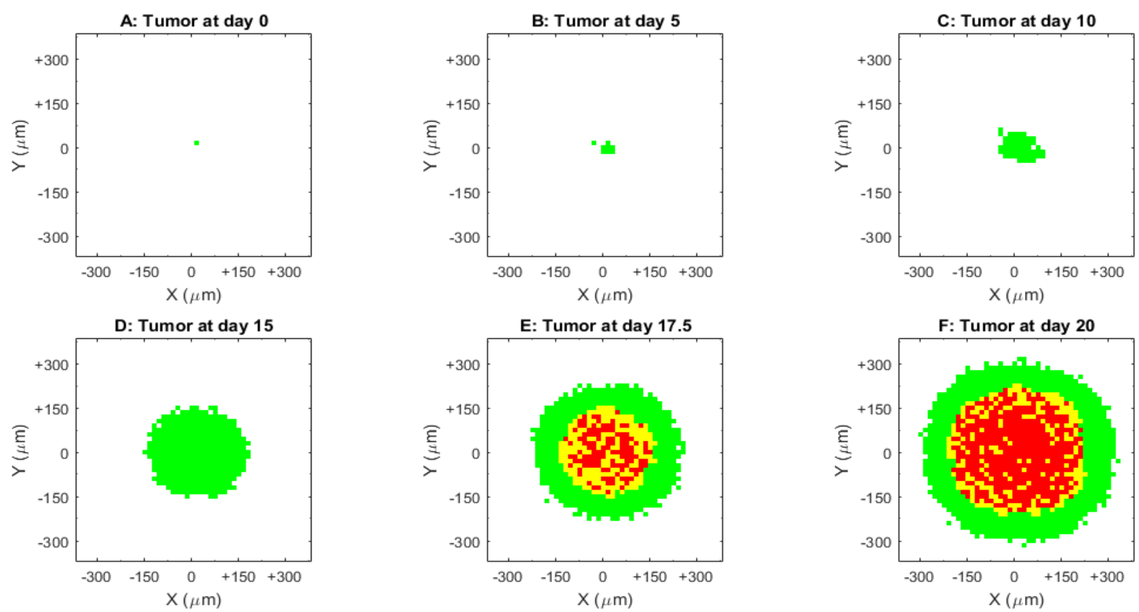
Cell 'fate' decision



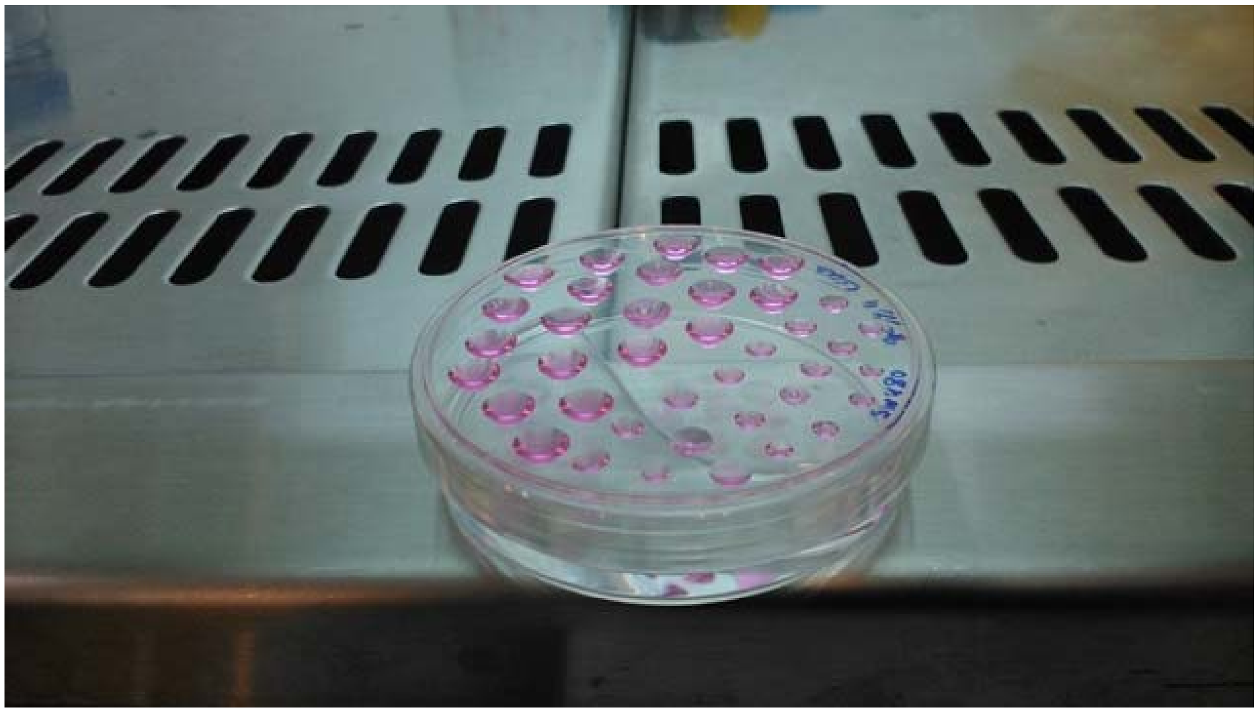
Results: *In silico* and *In vitro*

Modeling tumor growth for 20 days


A: Trend of change in cells number
B: Trend of change in tumor radius
NumberOfCells
NumberOfProliferativeCells
NumberOfStarvedCells
NumberOfNecroticCells
TumorRadius
NecroticSphereRadius
Number
Radius (μm)
Day

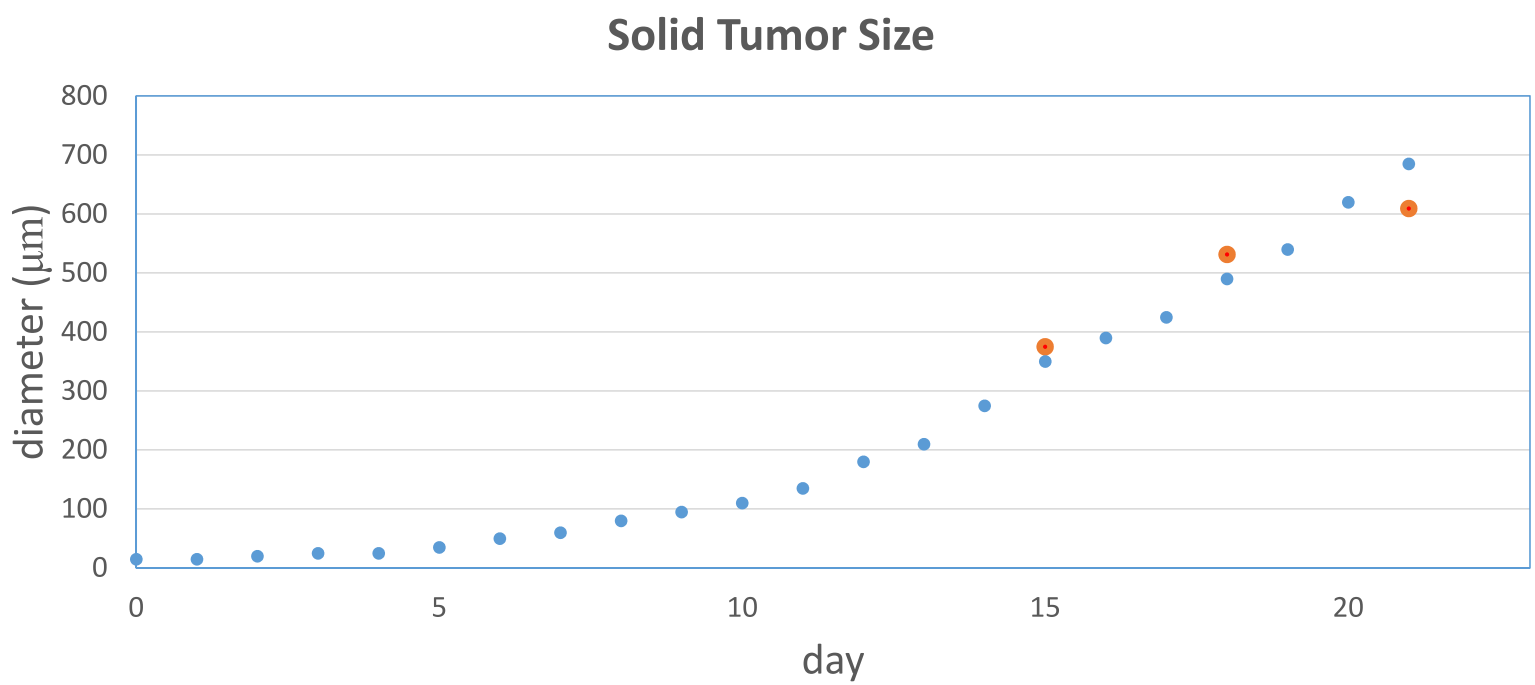

A: Tumor at day 0
B: Tumor at day 5
C: Tumor at day 10
D: Tumor at day 15
E: Tumor at day 17.5
F: Tumor at day 20
X (μm)
Y (μm)

Development of Spheroids by Hanging Drop Method

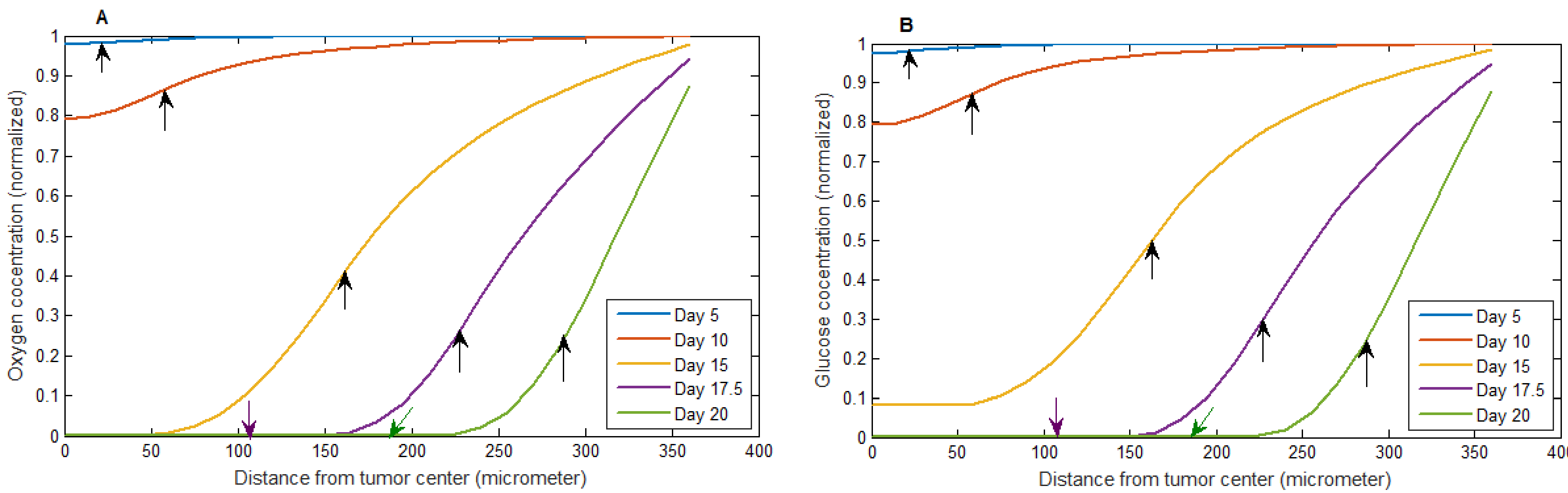


In vitro (Wet-Lab) Results

Agreement between *in silico* and *in vitro* results



Advantage of our Model: Metabolites profiles



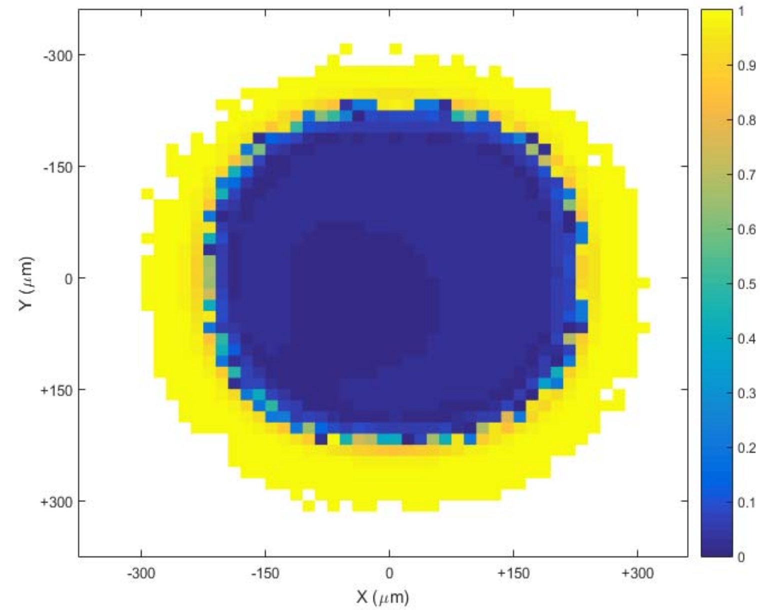
Advantage of our Model: Gene Expression Profiles

- 78 reactions were found:

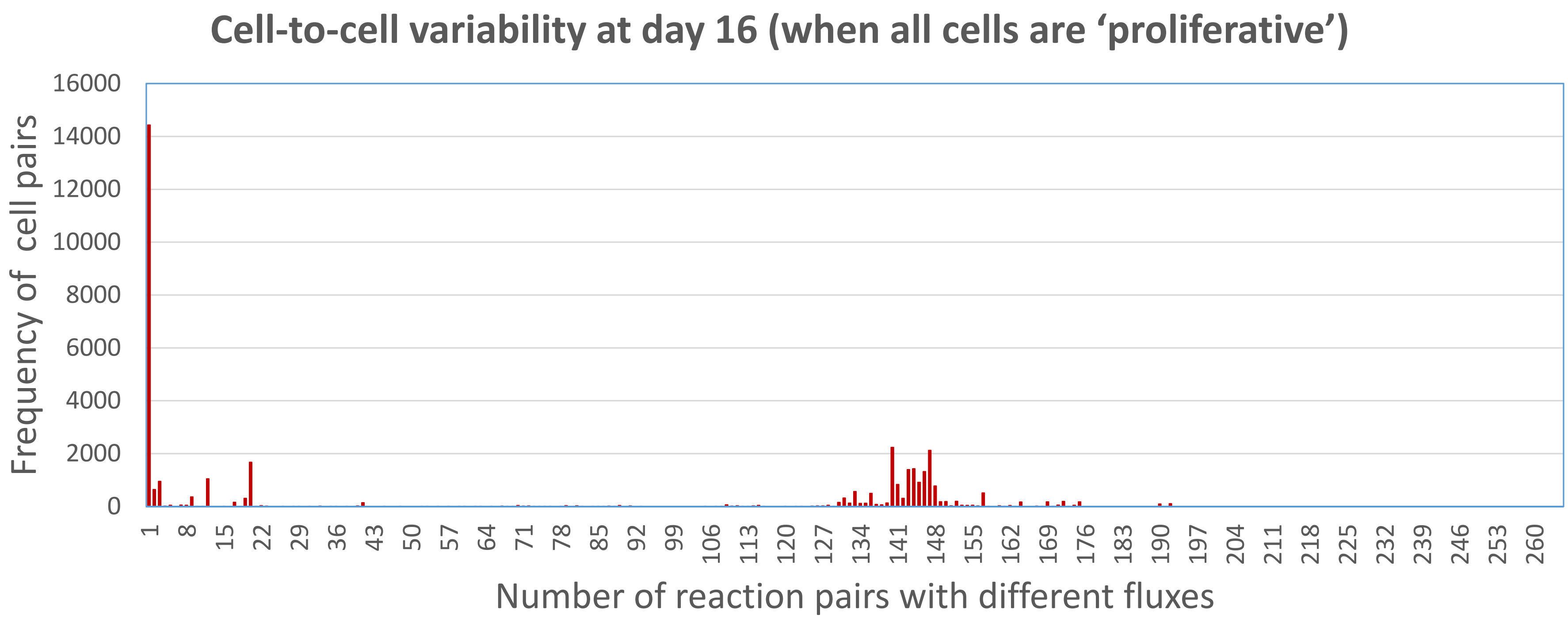
Flux in necrotic core = 0

Flux in surface cells > 0

Example: phosphoglycerate dehydrogenase



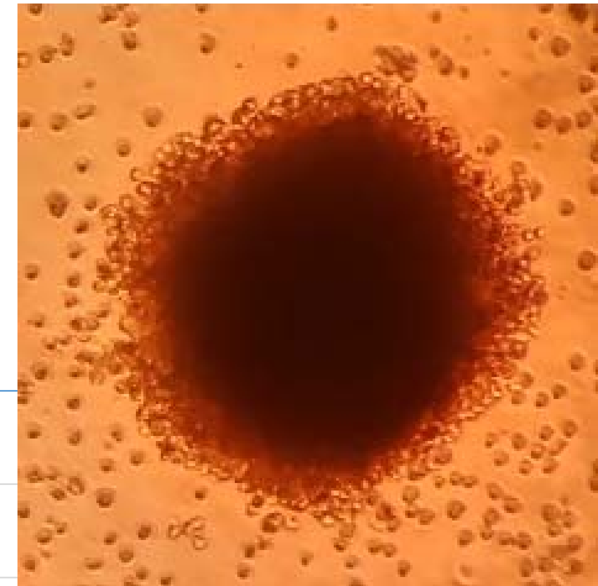
Advantage of our Model: Cell-to-Cell Variability

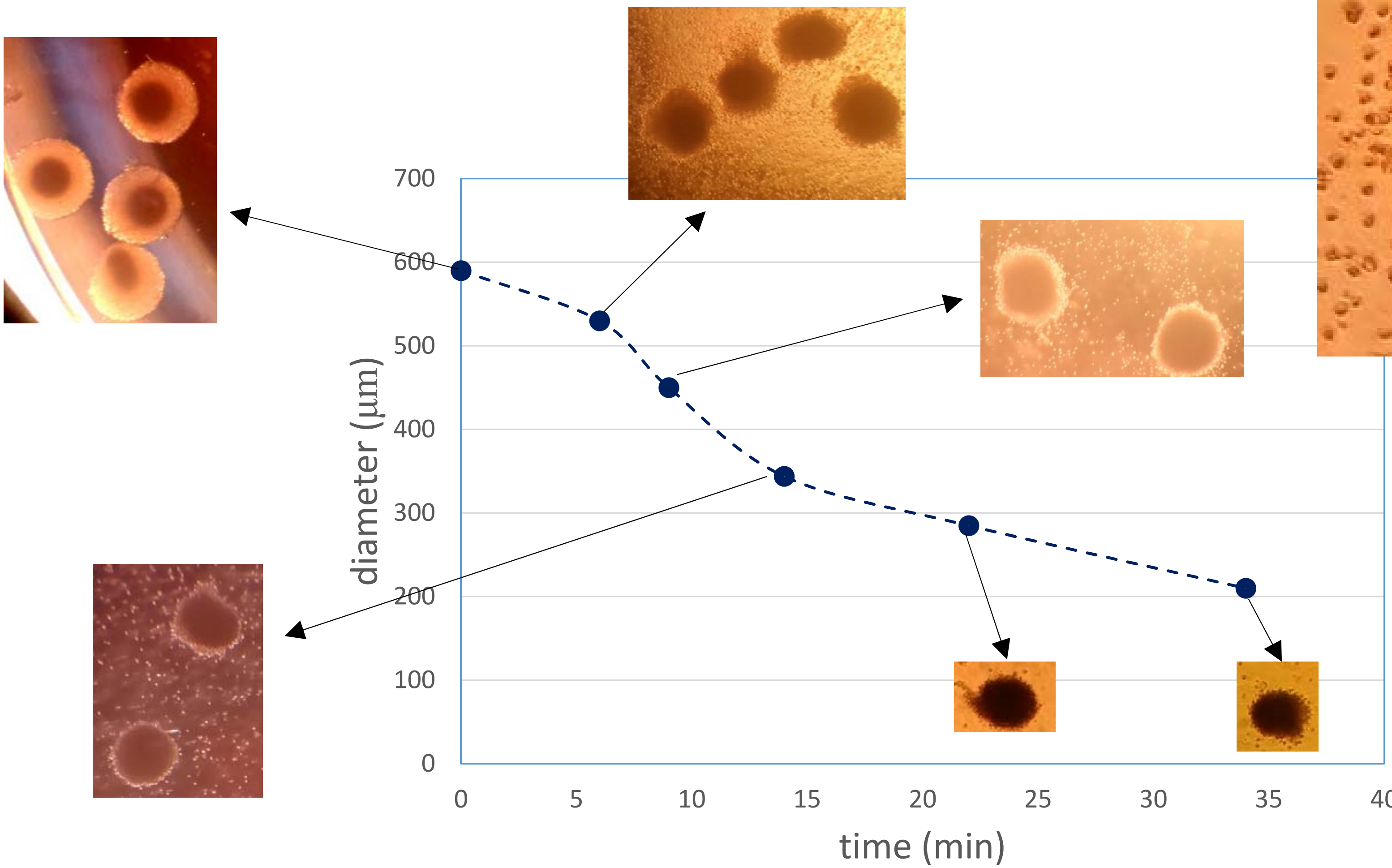


Next Steps

- Validating tumor growth rate
- Validating the number of proliferative, quiescent and necrotic cells
- Validating gene expression profiles of important oncogenes
- Investigating the source of cell-to-cell variability

Partial Digestion of Spheroids

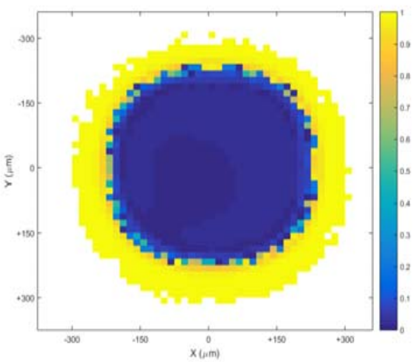




Validation of Gene Expression Profiles

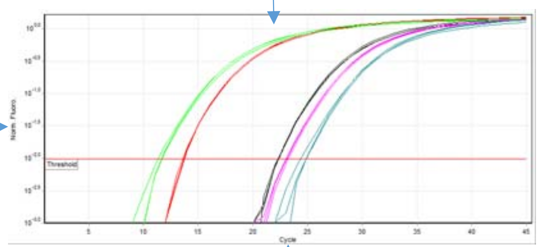
Gene selection

Primer design



In silico results

validation



Real-time PCR

Selective dissociation

Partial digestion

Thanks for your attention!