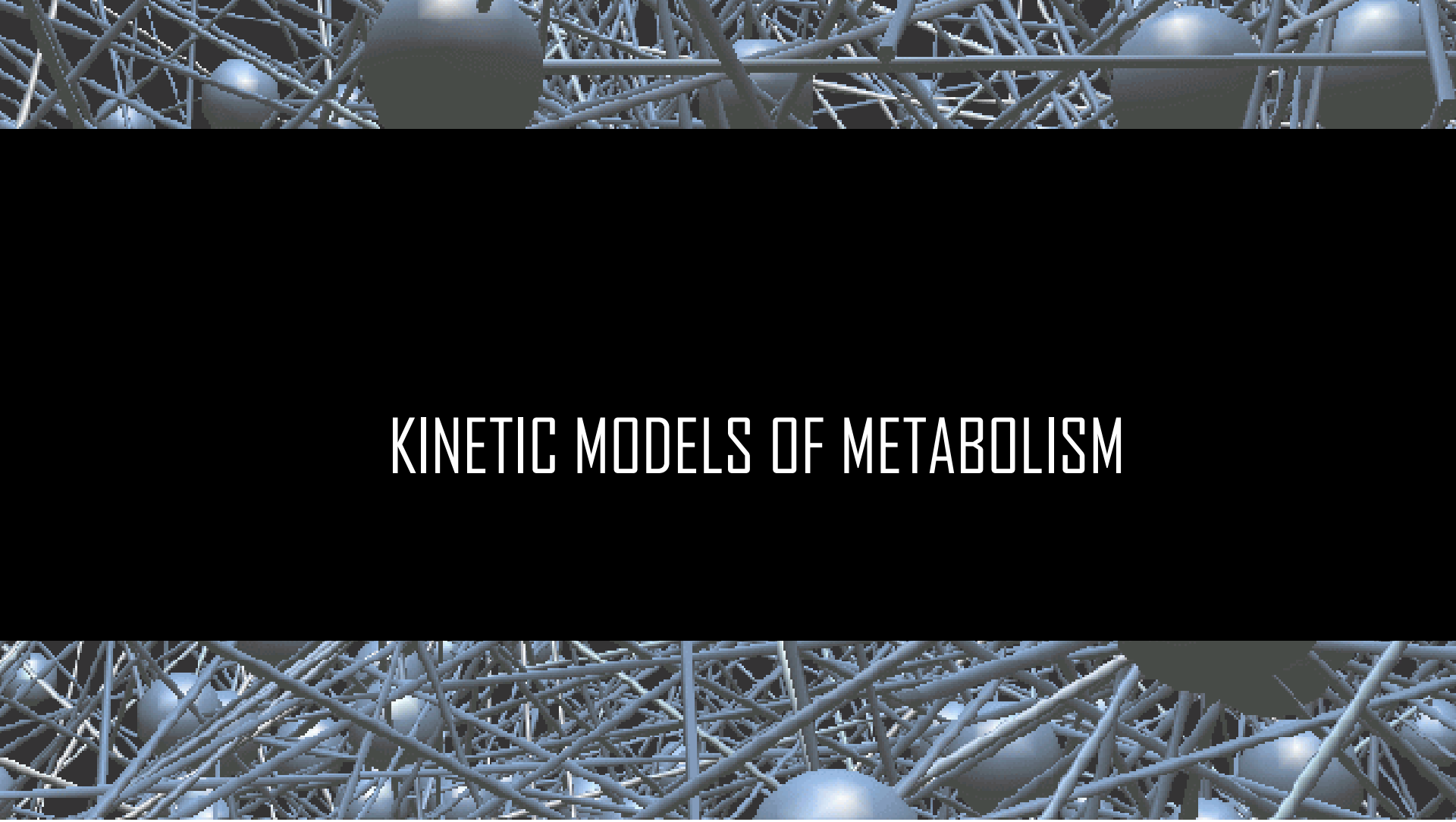


KINETIC MODELS OF METABOLISM

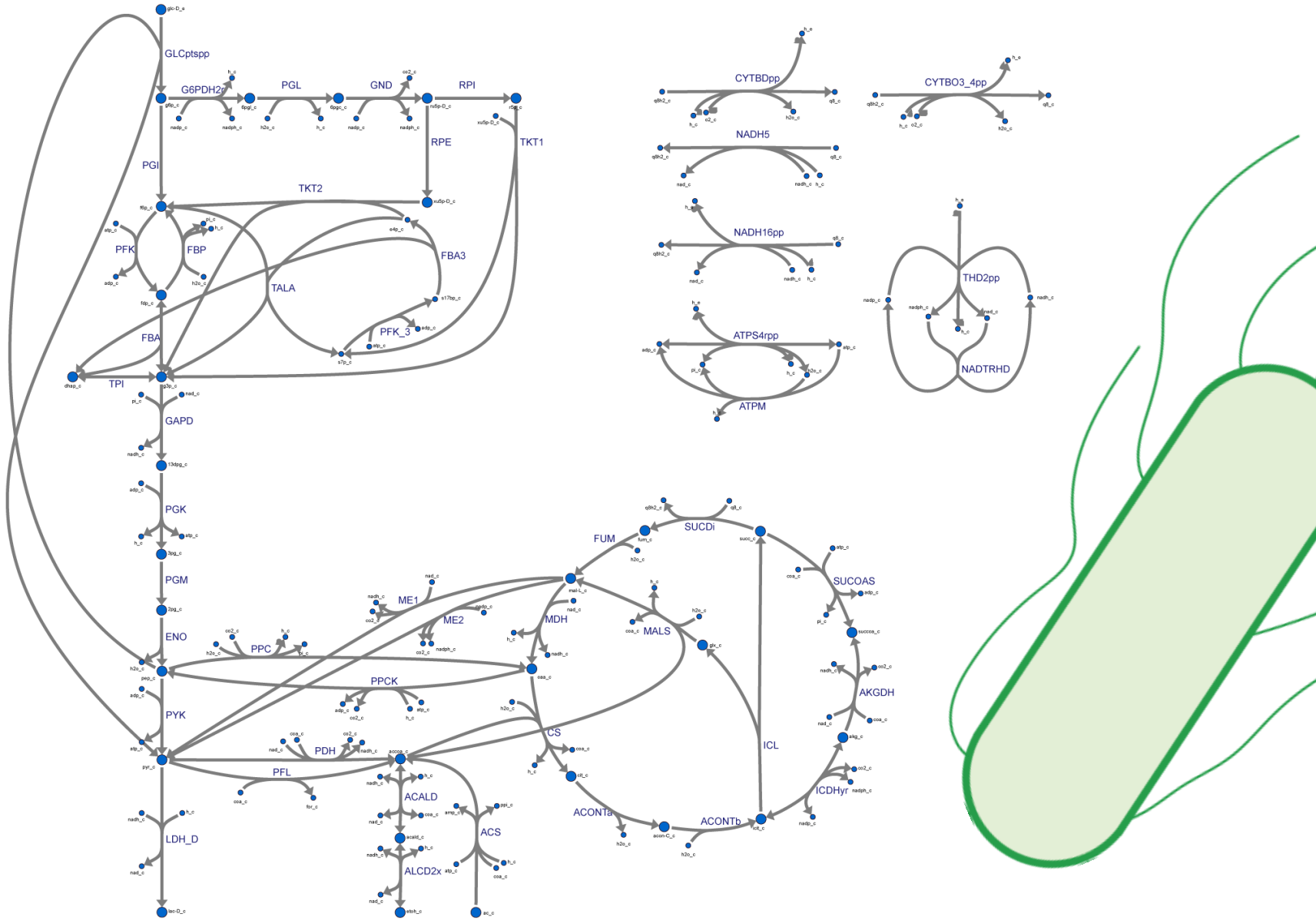
Principles and Applications of Systems Biology

EPFL

Vassily Hatzimanikatis

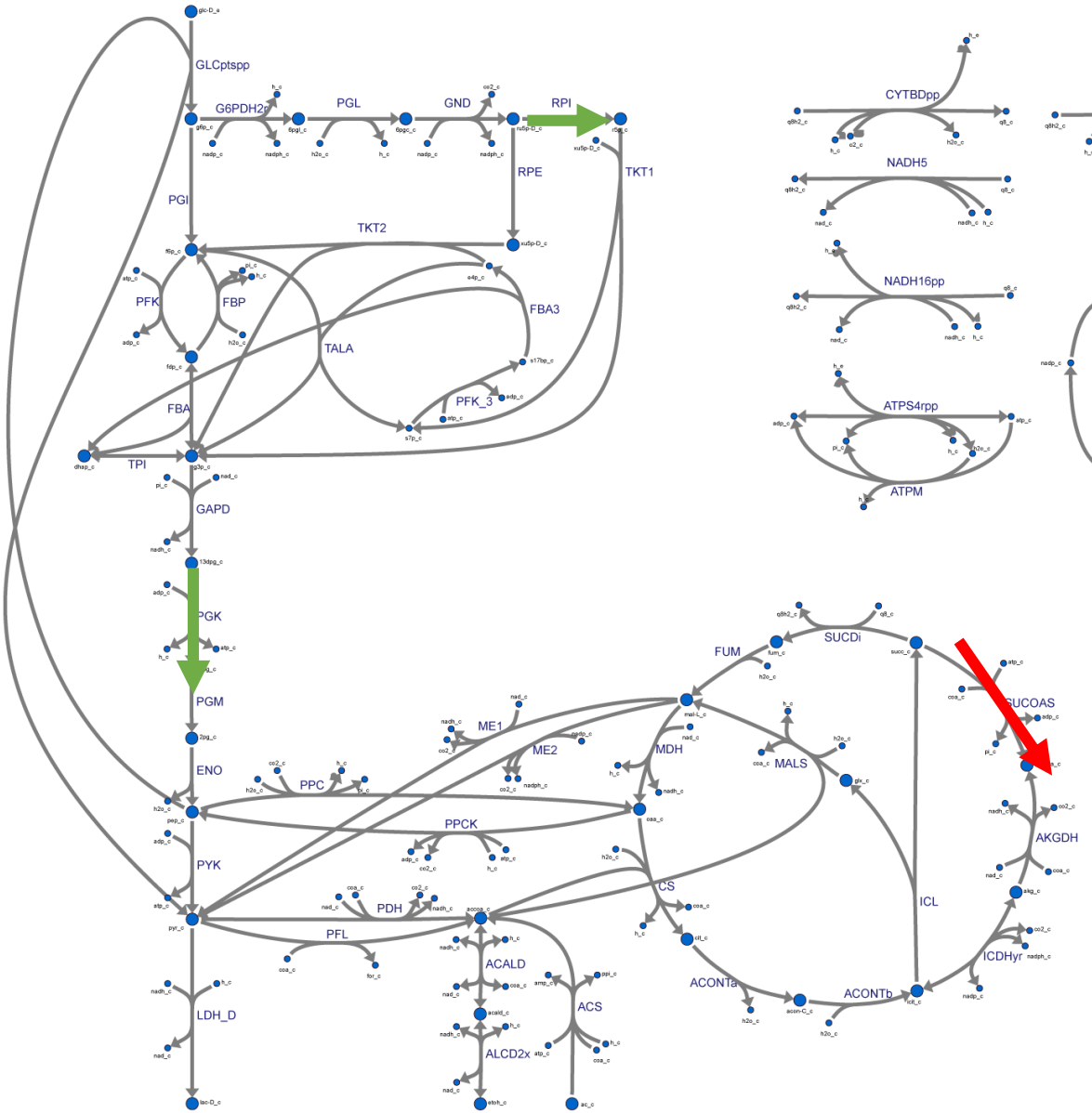
October 2025

Enzyme levels

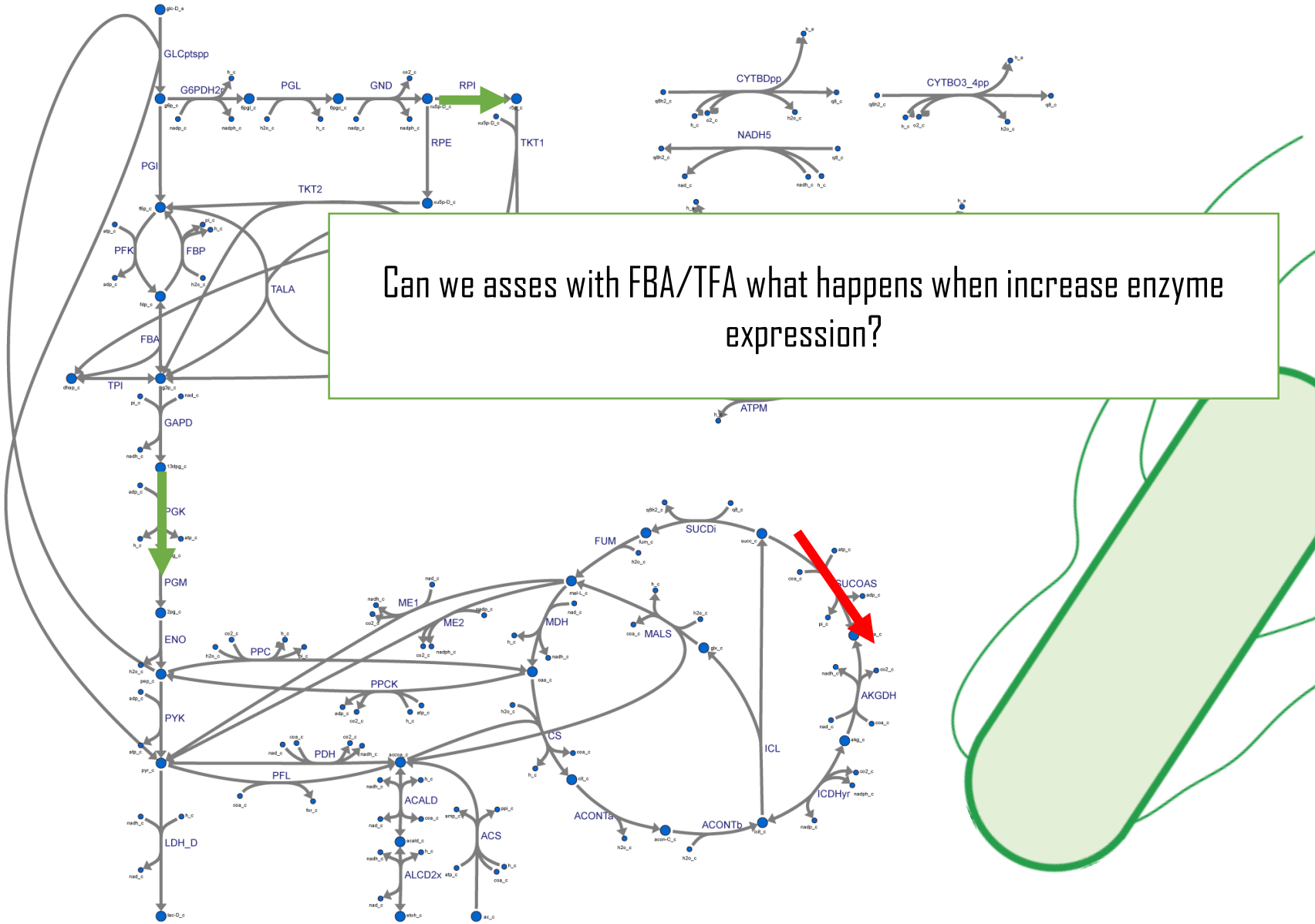


Enzyme levels

UP and DOWN regulation



Enzyme levels

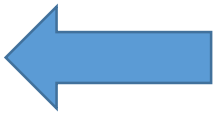


Can we asses with FBA/TFA what happens when increase enzyme expression?

Limitations

TFA/FBA:

$$0 = S\,v$$



Mass balance

$$\frac{\mathrm{d}x}{\mathrm{dt}} = S \cdot v(x,p)$$

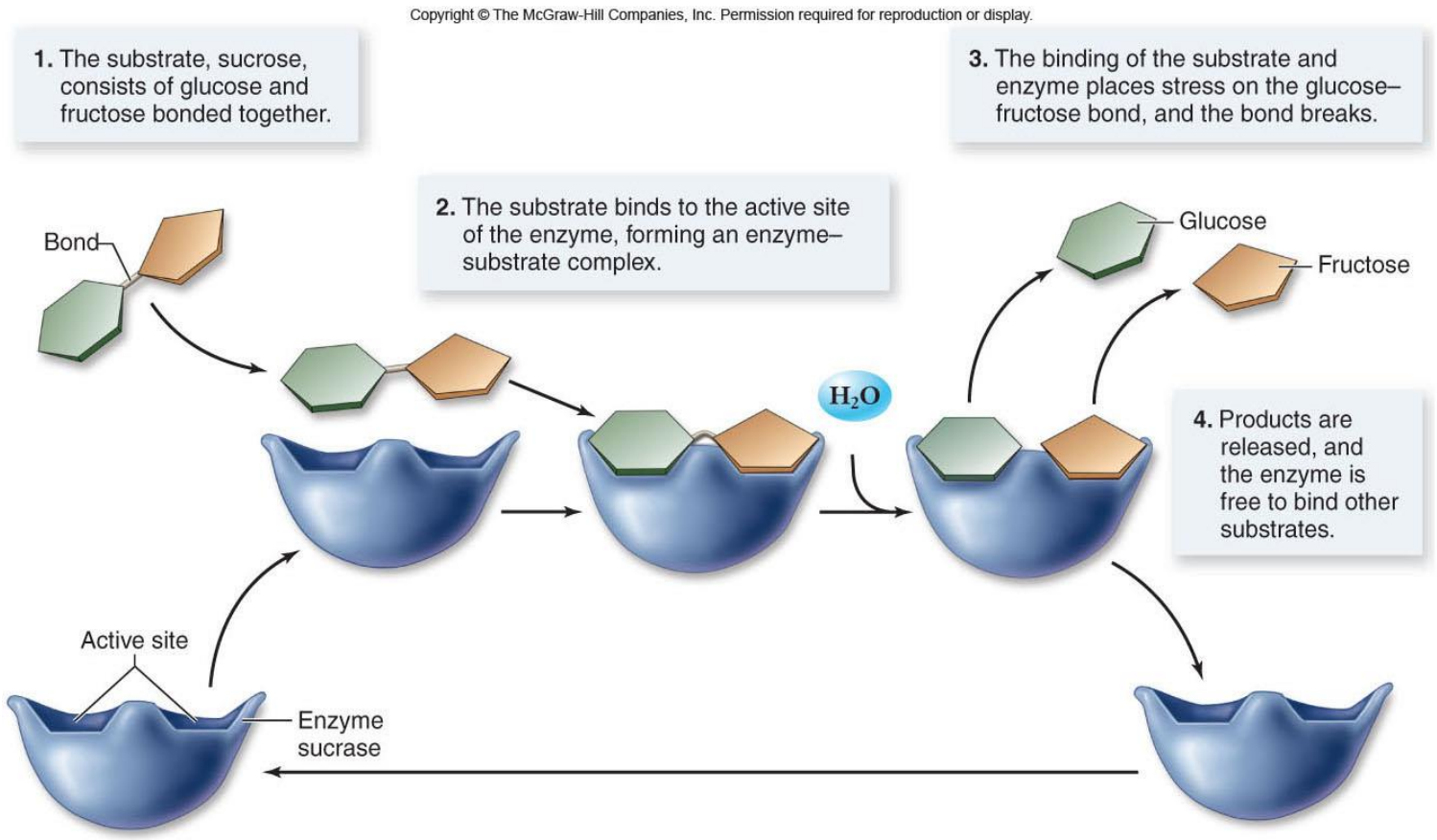
Steady state assumption

How can we describe the reaction rates as a function of the concentrations?

$$v(x,p)$$

Enzyme catalysis

Enzyme catalysis



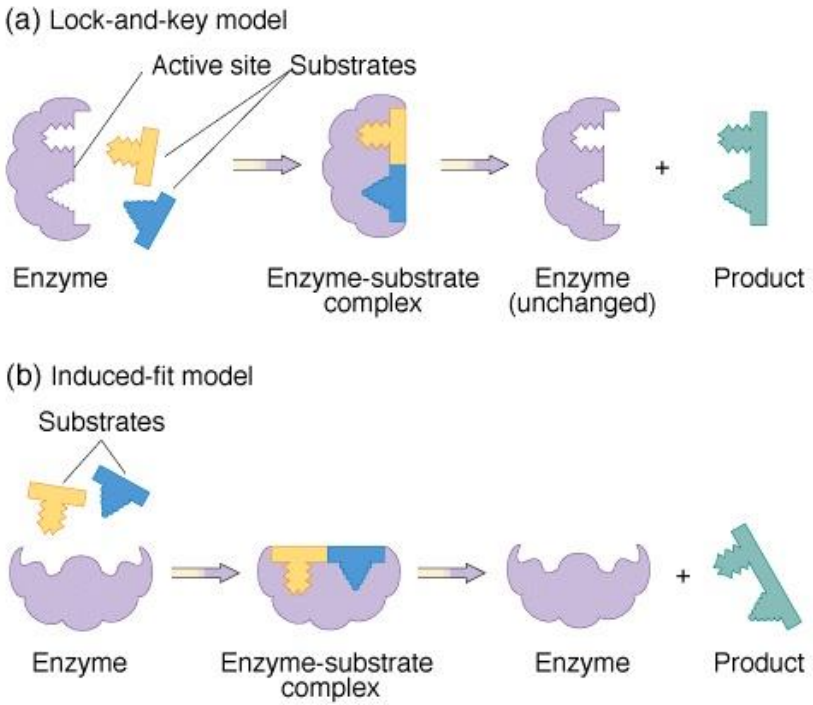
Enzyme catalysis

(a) Lock and key theory

- Binding site is a rigid entity
- Only one type of compound will fit.
- Analogous to how a lock allows only one key.

(b) Induced fit theory

- Binding site is flexible
- The conformation of the enzyme changes so that a stable binary complex forms.



The law of mass action

The law of mass-action

Rate of reaction is proportional to the product of the concentrations of the reactants to the power of their stoichiometric coefficients.

$$v_j = k_j \prod_i^N S^{n_{ij}}$$

The law of mass-action

Zeroth order reactions:

$$\longrightarrow s_1$$

generation of s_1 from some buffered (or external) source (S).

Rate:

$$v = k \text{ (constant)}$$

First order reactions:

$$s_1 \xrightarrow{k} s_2$$

isomerization, unassisted transport, degradation/dilution, or linearizat ion of other kinetics

Rate:

$$v = ks$$

Second order reactions:

$$s_1 + s_2 \xrightarrow{k} s_3$$

Binding/association events

Rate:

$$v = k\, s_1 s_2$$

The law of mass-action

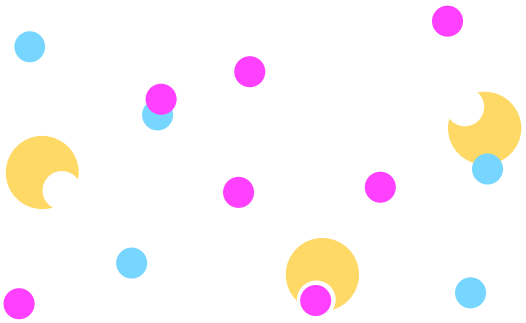
Rate of reaction is proportional to the product of the concentrations of the reactants to the power of their stoichiometric coefficients.

$$v_j = k_j \prod_i^N S^{n_{ij}}$$

Key assumptions:

- Well mixed environment
- Free diffusion of molecules
- Three dimensional collisions
- No interactions between molecules

Holds usually for
homogenous dilute solutions

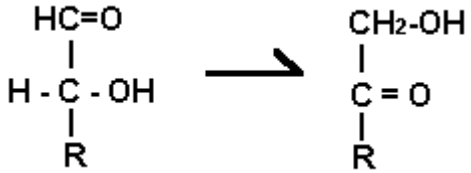


An example

Aldose-Ketose Isomerisation

- An aldose is a monosaccharide with an aldehyde group.
- Common ketoses have a ketone group at carbon 2.
- A hydrogen atom can be moved from one carbon atom to another converting an aldose into a ketose.

The rate v of the reaction A → B increases as the concentration of A increases.



A mathematical representation of the reaction rate using: mass action kinetics

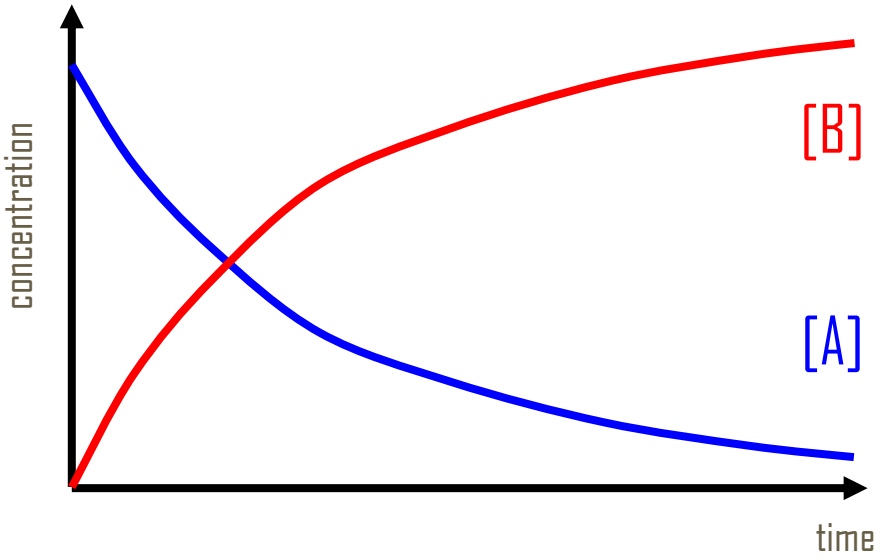
$$v = k_1[A]$$

Irreversible first order reaction

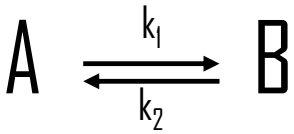
This quantitative description of the reaction rate can be used to characterize the rates of change of the bio-chemical species in the network:

$$\frac{d}{dt}[A](t) = -k_1[A](t)$$

$$\underbrace{\frac{d}{dt}[B](t)}_{\text{rate of change of concentration}} = \underbrace{k_1[A](t)}_{\text{+/- rate of reaction}}$$



Reversible reactions



$$\frac{d}{dt}[A](t) = -k_1[A](t) + k$$

$$\frac{d}{dt}[B](t) = k_1[A](t) - k$$

rate of change of concentration

+/- rate of forward reaction

+/- rate of reverse reaction

In Steady State:

$$0 = -k_1 A^{ss} + k_2 B^{ss}$$

$$0 = k_1 A^{ss} - k_2 B^{ss}$$

Solving results in:

$$\frac{B^{SS}}{A^{SS}} = \frac{k_1}{k_2} = K_{eq}$$

Michaelis-Menten Kinetics

Leonor Michaelis

Maud Menten

Google Scholar

die kinetik der invertinwirkung

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[PDF] Die **kinetik** der **invertinwirkung**

L Michaelis, ML Menten - Biochem. z, 1913 - chem.uwec.edu

The kinetics of enzyme3) action have often been studied using invertase, because the ease of measuring its activity means that this particular enzyme offers especially good prospects of achieving the final aim of kinetic research, namely to obtain knowledge on the nature of …

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Reaction mechanism

Michaelis & Menten (1913) investigated the kinetics of an enzymatic reaction mechanism, invertase, that catalyzes the hydrolysis of sucrose into glucose and fructose:

$$S + E \underset{k_r}{\overset{k_f}{\Leftrightarrow}} ES \overset{k_{cat}}{\Rightarrow} P + E$$

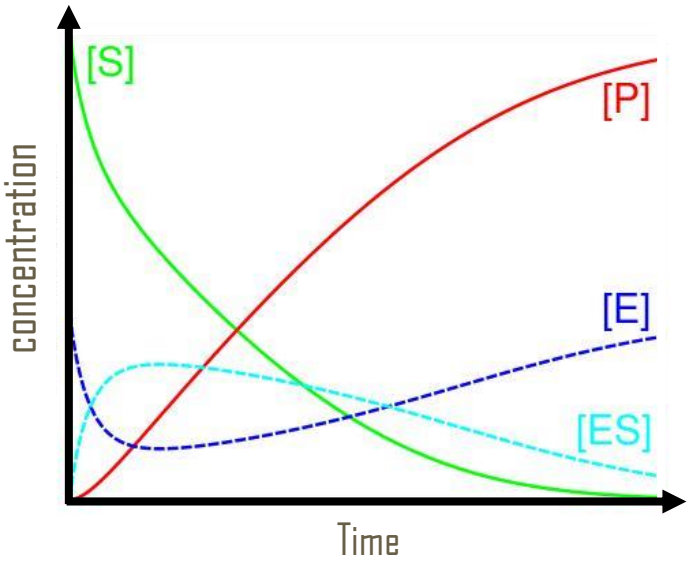
Formulate mass balances on elementary reactions:

$$\frac{d[S]}{dt} = -k_f[E][S] + k_r[ES]$$

$$\frac{d[E]}{dt} = -k_f[E][S] + k_r[ES] + k_{cat}[ES]$$

$$\frac{d[ES]}{dt} = +k_f[E][S] - k_r[ES] - k_{cat}[ES]$$

$$\frac{d[P]}{dt} = +k_{cat}[ES]$$



Simplification: QSSA

$$S + E \underset{k_r}{\overset{k_f}{\Leftrightarrow}} ES \overset{k_{cat}}{\Rightarrow} P + E$$

The Quasi-Steady-State Assumption (QSSA):

After a rapid transient phase the intermediate complex ES reaches a quasi-stationary phase where its concentration doesn't change in the observed time frame

$$\frac{d[ES]}{dt} = +k_f[E][S] - k_r[ES] - k_{cat}[ES] = 0$$

Thus:

$$\frac{k_r + k_{cat}}{k_f} = \frac{[E][S]}{[ES]} \quad \Rightarrow \quad \frac{k_r + k_{cat}}{k_f} = K_M$$

Michaelis-Menten constant

Using the conservation : $\mathrm{E} + \mathrm{ES} = \mathrm{E_{tot}} = \mathrm{const.}$

$$\frac{\mathrm{K_M[ES]}}{\mathrm{[S]}} + \mathrm{[ES]} = \mathrm{E_{tot}}$$

$$[ES] = [E_{tot}]\frac{[S]}{K_M + [S]}$$

Simplification: QSSA

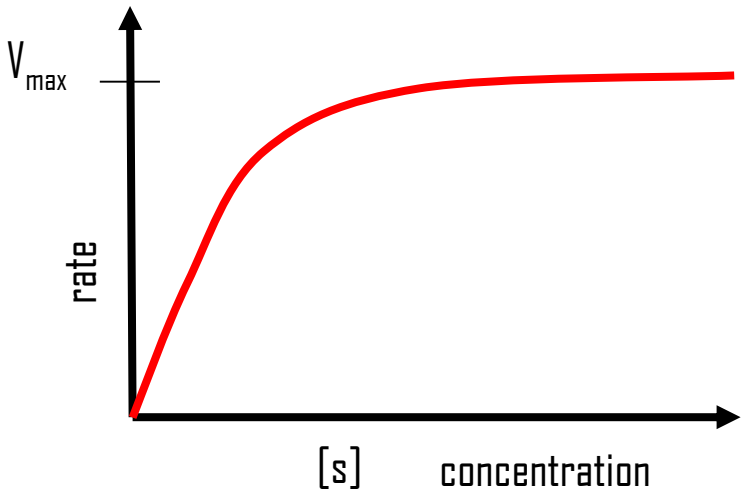
$$[ES] = [E_{tot}] \frac{[S]}{K_M + [S]}$$

$$S + E \underset{k_r}{\overset{k_f}{\Leftrightarrow}} ES \overset{k_{cat}}{\Rightarrow} P + E$$

The rate of production of [P]

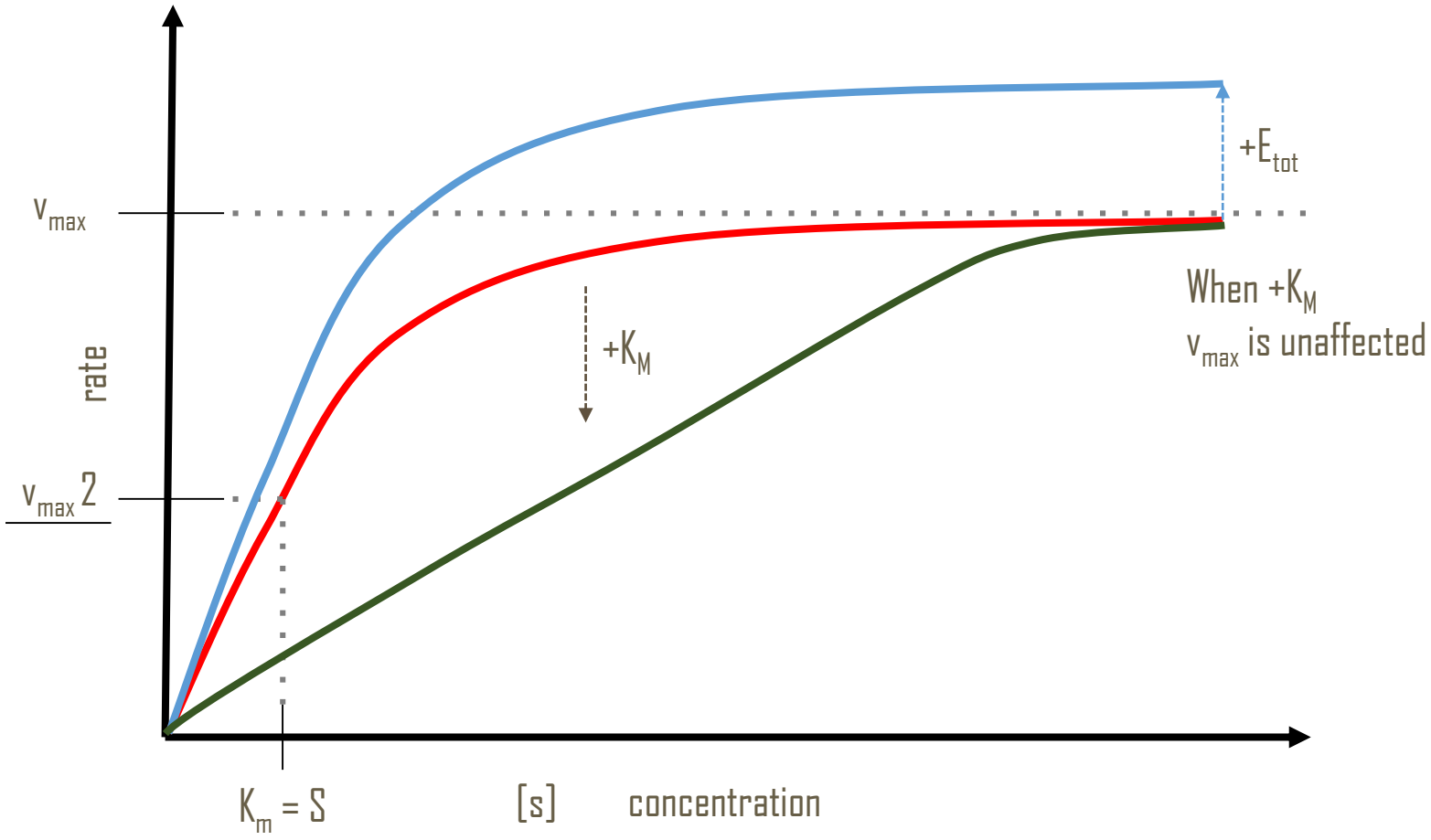
$$v_p = [ES] k_{cat}$$

$$v_f = [E_{tot}] k_{cat} \frac{[S]}{K_M + [S]}$$



QSSA Michaelis menten kinetics

$$S + E \underset{k_r}{\overset{k_f}{\Leftrightarrow}} ES \overset{k_{cat}}{\Rightarrow} P + E$$



Simplification: QEA

$$S + E \underset{k_r}{\overset{k_f}{\Leftrightarrow}} ES \overset{k_{cat}}{\Rightarrow} P + E$$

The Quasi-Equilibrium Assumption (QEA):

Binding of the substrate to the enzyme quickly reaches a quasi-equilibrium state.
Thus k_{cat} needs to be much slower than k_r

$$\frac{d[ES]}{dt} = +k_f[E][S] - k_r[ES] - k_{cat}[ES] = 0$$

Thus:

$$\frac{[E][S]}{[ES]} = \frac{[E_{tot} - ES][S]}{[ES]} = \frac{k_r}{k_f} = K_d$$ Dissociation constant

Using the conservation : $\mathrm{E} + \mathrm{ES} = \mathrm{E_{tot}} = \mathrm{const.}$

$$\frac{\mathrm{K_D}[\mathrm{ES}]}{[\mathrm{S}]} + [\mathrm{ES}] = \mathrm{E_{tot}} \Rightarrow [\mathrm{ES}] = \frac{\mathrm{E_{tot}}}{\frac{\mathrm{K_D}}{[\mathrm{S}]} + 1}$$

Simplification: QEA

$$S + E \underset{k_r}{\overset{k_f}{\Leftrightarrow}} ES \overset{k_{cat}}{\Rightarrow} P + E$$

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$$\frac{\mathrm{K_D[ES]}}{\mathrm{[S]}} + \mathrm{[ES]} = \mathrm{E_{tot}} \Rightarrow \mathrm{[ES]} = \frac{\mathrm{E_{tot}}}{\frac{\mathrm{K_D}}{\mathrm{[S]}} + 1} \quad \& \quad \mathrm{V = k_{cat}[ES]}$$

Simplification: QEA

$$S + E \underset{k_r}{\overset{k_f}{\Leftrightarrow}} ES \overset{k_{cat}}{\Rightarrow} P + E$$

The Quasi-Equilibrium Assumption (QEA):

Binding of the substrate to the enzyme quickly reaches a quasi-equilibrium state.
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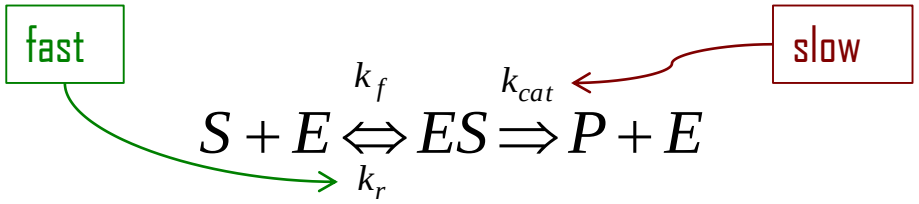
Using the conservation : $\mathrm{E} + \mathrm{ES} = \mathrm{E_{tot}} = \mathrm{const.}$

$$\frac{K_D[ES]}{[S]} + [ES] = E_{tot} \Rightarrow [ES] = \frac{E_{tot}}{\frac{K_D}{[S]} + 1} \quad \& \quad V = k_{cat}[ES] \Rightarrow$$

$$v_f = k_{cat}[E_{tot}]\frac{[S]}{K_D + [S]}$$

Validity of the QSSA and QEA

QEA hold if:



QSSA hold if:

$$\frac{k_r + k_{cat}}{k_f} = K_M$$

$E_{tot} << [K_m]$

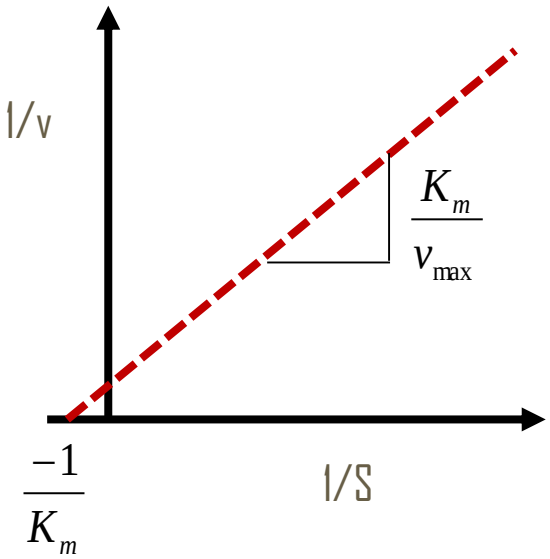
The total amount of Enzyme is very small relative to the MM-Parameter

$K_m << [S_{tot}]$

The total amount of substrate is very high relative to the MM-Parameter

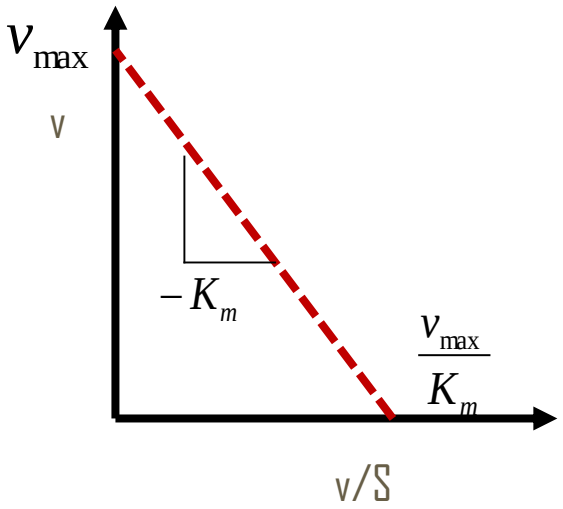
Estimation of Michaelis-Menten parameters

Lineweaver Burk Plot



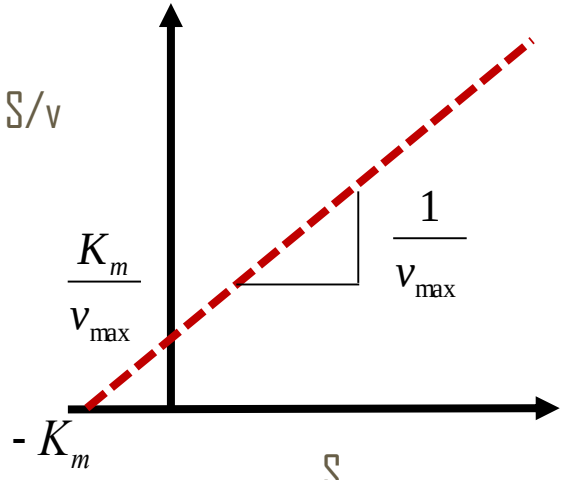
$$\frac{1}{v} = \frac{1}{v_{max}} + \frac{K_m}{v_{max}}\frac{1}{S}$$

Eadie Hofstee Plot



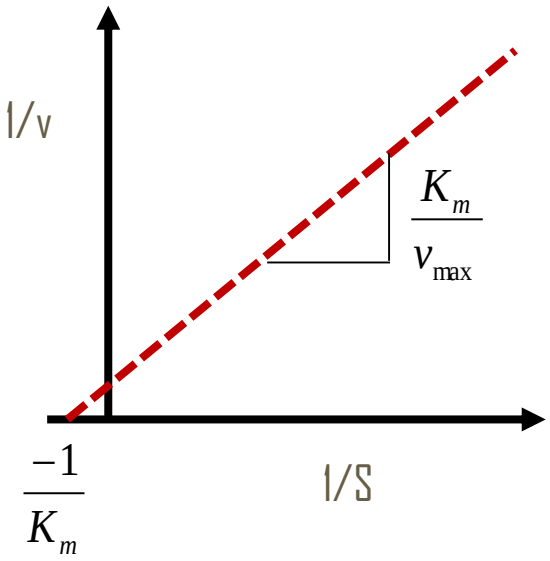
$$v = v_{max} - \frac{K_m v}{S}$$

Hanes Plot



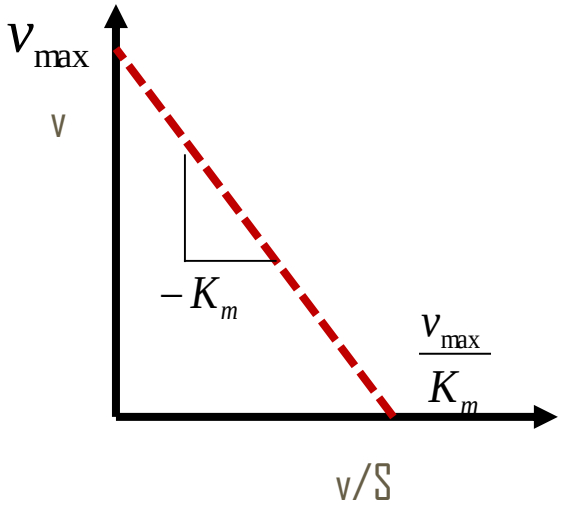
$$\frac{S}{v} = \frac{1}{v_{max}}S + \frac{K_m}{v_{max}}$$

Lineweaver Burk Plot



$$\frac{1}{v} = \frac{1}{v_{max}} + \frac{K_m}{v_{max}} \frac{1}{S}$$

Eadie Hofstee Plot

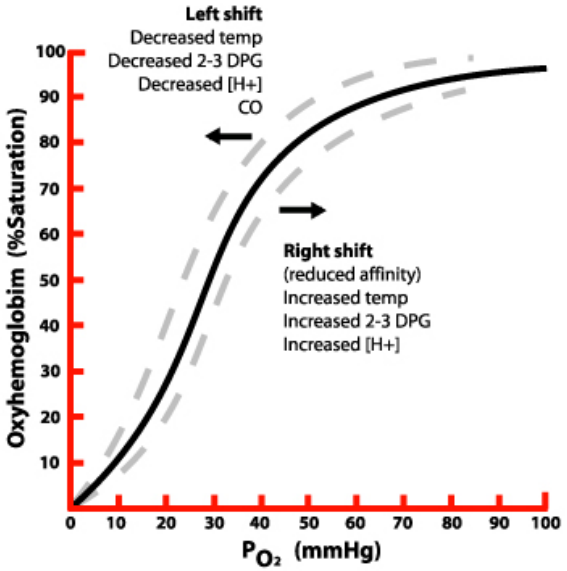


$$v = v_{max} - \frac{K_m v}{S}$$

Hill kinetics

Hill kinetics

It was originally formulated by Archibald Hill in 1910 to describe the sigmoidal O_2 binding curve of hemoglobin.



$$s_1 \xrightarrow{\mathrm{E}} s_2 \quad \text{or} \quad p_1 \xrightarrow{\mathrm{E}_1} p_2 \xrightarrow{\mathrm{E}_2} \cdots \xrightarrow{\mathrm{E}_n} p_n$$

Catalysis by cooperative enzyme or lumped description of multi-step process

Hill kinetics

A mechanistic explanation was not provided with the original formulation; a simplistic mechanistic model has been proposed for the derivation of the rate law

Assumptions:

- an oligomer with n subunits
- a binding site on each subunit for a ligand, S.
- when the first ligand binds, all the remaining ligands also bind simultaneously

$$E + nS \underset{k_r}{\overset{k_f}{\Leftrightarrow}} ES_n \Rightarrow$$

Applying the QSSA:

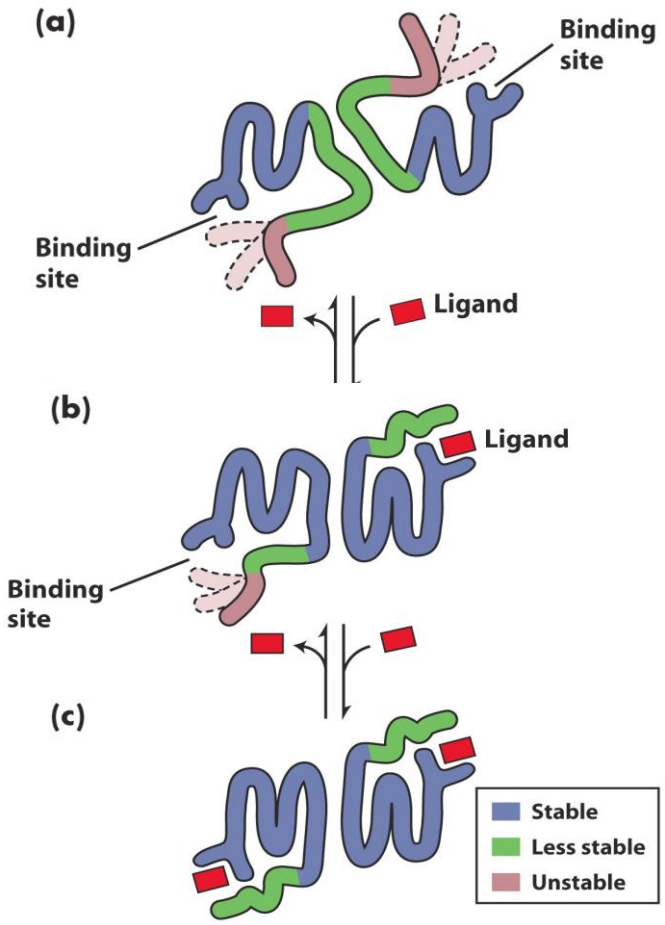
$$\frac{dS}{dt} = - v_f + v_r = 0 \qquad v_f = v_r \qquad \frac{[ES]}{[E][S]^n} = K_a$$

Using the conservation relationship:

$$\frac{[ES]}{[E_{tot}]} = \frac{[S]^n}{\frac{1}{K_a} + [S]^n} = \frac{[S]^n}{K_d + [S]^n} \qquad \text{with} \qquad \mathrm{K_a} = \frac{1}{\mathrm{K_d}}$$

Hill kinetics

- More than one binding sites
- Binding of ligand to one site alters the its affinity at other sites: cooperativity



Hill kinetics

The Hill rate law is usually met in literature in the form:

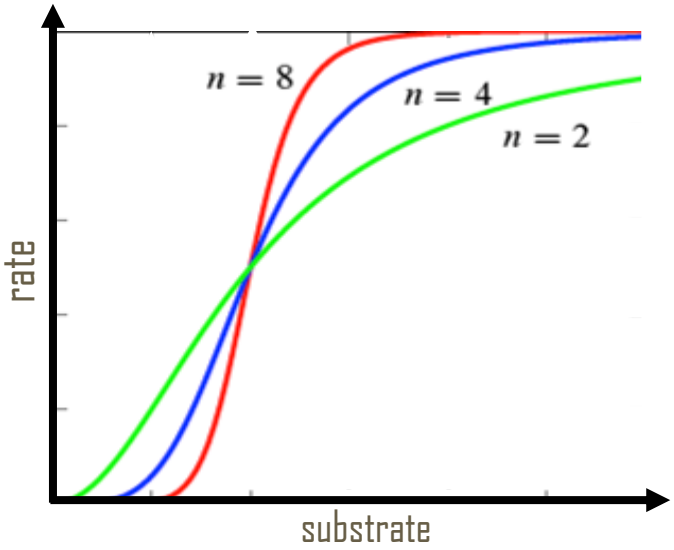
$$v = k_0 \frac{[S]^h}{K_H^h + [S]^h} \qquad K_H = \sqrt[h]{K_d}$$

The Hill coefficient (h):
Experimental determination of Hill coefficients often reveals fractional values indicating that the simple Hill model fails to adequately explain cooperativity!

Examples:

- Hemoglobin → 4 binding sites; h= 2.7
- PFK → 4 binding sites; h= 3.7

Although the Hill rate law is a poor representation of the underlying biology it has been widely used since it can capture sigmoidal behavior!



Generalized Mass action kinetics

Generalized mass action

A refinement to mass action:

Commonly known as generalized mass action kinetics is:

$$\frac{v}{v_0} = k\prod_{i=1}^{N}\left(\frac{S_i}{S_i^0}\right)^{g_i}$$

Where the subscript 0 refers to a reference state.

Captures non-linearity very well.

The exponents are real numbers, not restricted to integer values only.

Generalized mass action

Basic concept of mass action: Reaction velocity is proportional to the probability of collision of reactants which is proportional to their concentration

- We examined elementary reactions:

$$E + S \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES \qquad v_1 = k_1[E][S] \qquad v_{-1} = k_{-1}[ES]$$

- What happens for reactions of other types?

$$S + S \overset{k}{\rightarrow} S_2 \qquad aA + bB \overset{v}{\rightarrow} cC + dD$$

- Molecularity is taken into account as a means to describe the number of molecules required for the reaction to take place.

$$v = k[S]^2 \qquad v = k[A]^a[B]^b$$

- However such a formulation fails to capture saturation effects!

Convenience Kinetics

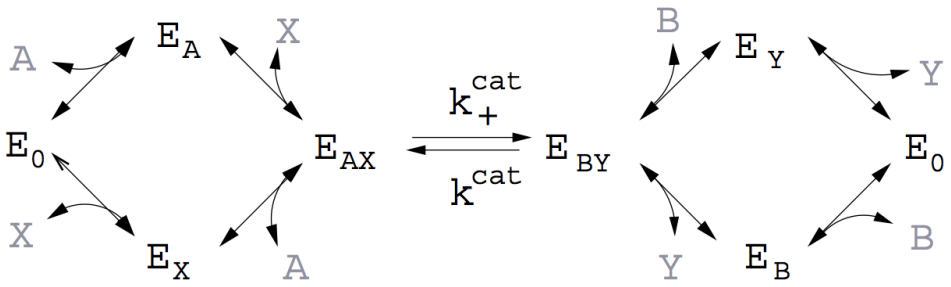
Convenience Kinetics

$$\alpha_1 A_1 + \alpha_2 A_2 + \ldots \leftrightarrow \beta_1 B_1 + \beta_2 B_2 + \ldots$$

$$v(\mathbf{a},\mathbf{b}) = E \frac{k_+^{cat} \prod_i \tilde{a}_i^{\alpha_i} - k_-^{cat} \prod_j \tilde{b}_j^{\beta_j}}{\prod_i (1 + \tilde{a}_i + \ldots + \tilde{a}_i^{\alpha_i}) + \prod_j (1 + \tilde{b}_j + \ldots + \tilde{b}_j^{\beta_j}) - 1}$$

$$\tilde{a}_i = a_i / k_{a_i}^M$$

$$\tilde{b}_j = b_j / k_{b_j}^M$$



Liebermeister &. Klipp: Bringing metabolic networks to life: convenience rate law and thermodynamic constraints, Theoretical Biology and Medical Modelling, 2006, 3:41

Reversible Hill Kinetics

Reversible Hill Kinetics

$$v = \frac{V_f \frac{S}{K_s} \left(1 - \frac{\Gamma}{K_{eq}}\right) \left(\frac{S}{K_s} + \frac{P}{K_p}\right)^{h-1}}{1 + \left(\frac{S}{K_s} + \frac{P}{K_p}\right)^{h}}$$

for h=1 it describes reversible Michaelis-Menten kinetics

$$v = \frac{V_f \frac{S}{K_s} \left(1 - \frac{\Gamma}{K_{eq}}\right) \left(\frac{S}{K_s} + \frac{P}{K_p}\right)^{h-1}}{\frac{\left(1 + \frac{M}{K_m}\right)^{h}}{1 + \alpha\left(\frac{M}{K_m}\right)^{h}} + \left(\frac{S}{K_s} + \frac{P}{K_s}\right)^{h}}$$

for $\alpha<1$ the modifier M acts as an inhibitor, otherwise it acts as an activator

Reversible Hill Kinetics

GRH equation: n Substrates $\rightleftharpoons$ n Products

$$v = V_{\mathrm{f}} \prod_{i=1}^{n_s} \alpha_i \left(1 - \frac{\Gamma}{K_{eq}}\right) \prod_{i=1}^{n_s} \left(\frac{(\alpha_i + \pi_i)^{h-1}}{1 + (\alpha_i + \pi_i)^h}\right)$$

Bi-uni reversible Hill equation: $\mathbf{A}_1 + \mathbf{A}_2 \rightleftharpoons \mathbf{P}$

$$v = \frac{V_{\mathrm{f}}\ \alpha_1\alpha_2 \left(1 - \frac{\Gamma}{K_{eq}}\right) (\alpha_1\alpha_2 + \pi)^{h-1}}{1 + (\alpha_1 + \pi)^h + (\alpha_2 + \pi)^h + (\alpha_1\alpha_2 + \pi)^h - 2\pi^h}$$

Bi-bi reversible Hill equation: $\mathbf{A}_1 + \mathbf{A}_2 \rightleftharpoons \mathbf{P}_1 + \mathbf{P}_2$

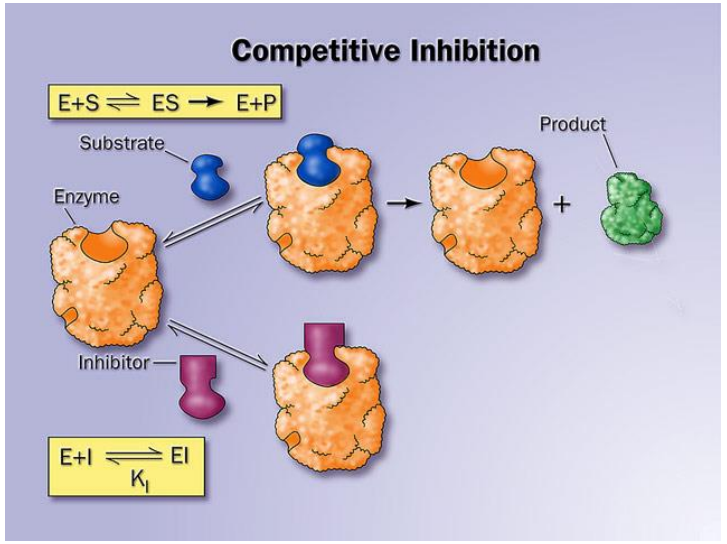
$$v = \frac{V_{\mathrm{f}}\alpha_1\alpha_2 \left(1 - \frac{\Gamma}{K_{eq}}\right) (\alpha_1 + \pi_1)^{h-1}(\alpha_2 + \pi_2)^{h-1}}{\prod_j^{n_m} \left[\frac{(1 + \mu_j^h)}{(1 + \sigma_j^{4h}\mu_j^h)}\right] + \prod_j^{n_m} \left[\frac{(1 + \sigma_j^{2h}\mu_j^h)}{(1 + \sigma_j^{4h}\mu_j^h)}\right] \left[(\alpha_1 + \pi_1)^h + (\alpha_2 + \pi_2)^h\right]^{n-1} + (\alpha_1 + \pi_1)^h(\alpha_2 + \pi_2)^h}$$

Enzyme regulation

Enzyme regulation - inhibition

Competitive Inhibitor

- The inhibitor can bind to the substrate binding site itself.
- The resulting complex is inactive.



$$v = \frac{v_{max} S}{K_S \left(1 + \frac{I}{K_I}\right) + S}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES \overset{k_2}{\Rightarrow} P + E$$

$$+$$

$$I$$

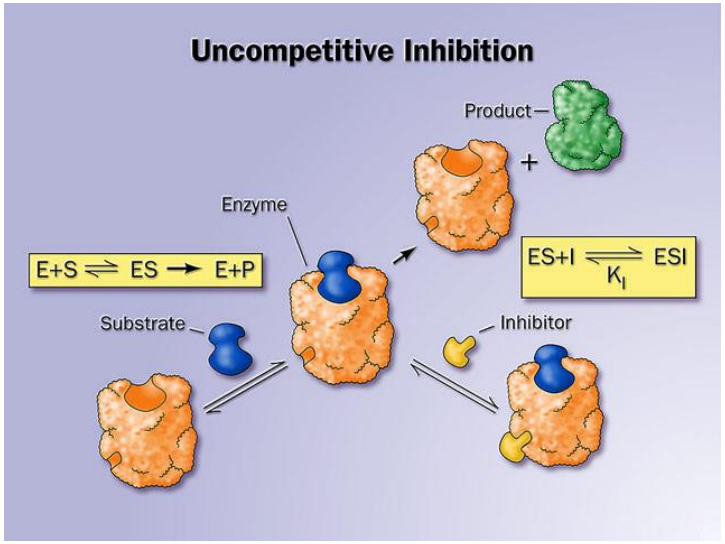
$$k_{-3} \qquad k_3$$

$$EI$$

Enzyme regulation - inhibition

Uncompetitive Inhibitor

- Binds and inhibits the [ES] complex.
- Requires the substrate to be already bound to the enzyme
- Commonly met in multiple substrate reactions
- The substrate can still bind on the enzyme, even when the inhibitor is present.



$$v = \frac{v_{\max} S}{K_M + S\left(1 + \frac{I}{K_I}\right)}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES \overset{k_2}{\Rightarrow} P + E$$

$$+$$

$$I$$

$$k_{-4} \qquad k_4$$

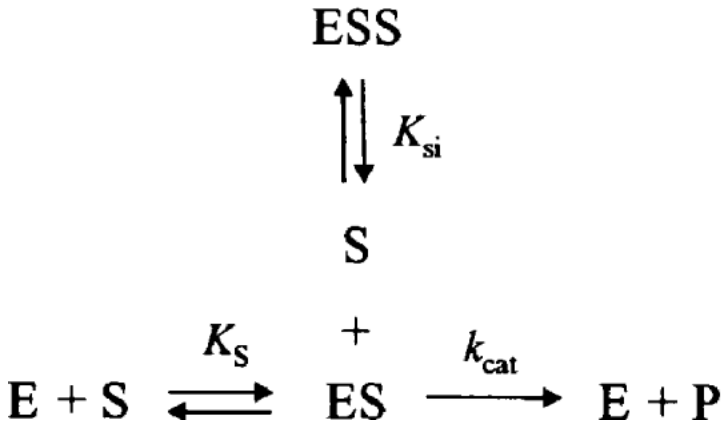
$$ESI$$

Enzyme regulation - inhibition

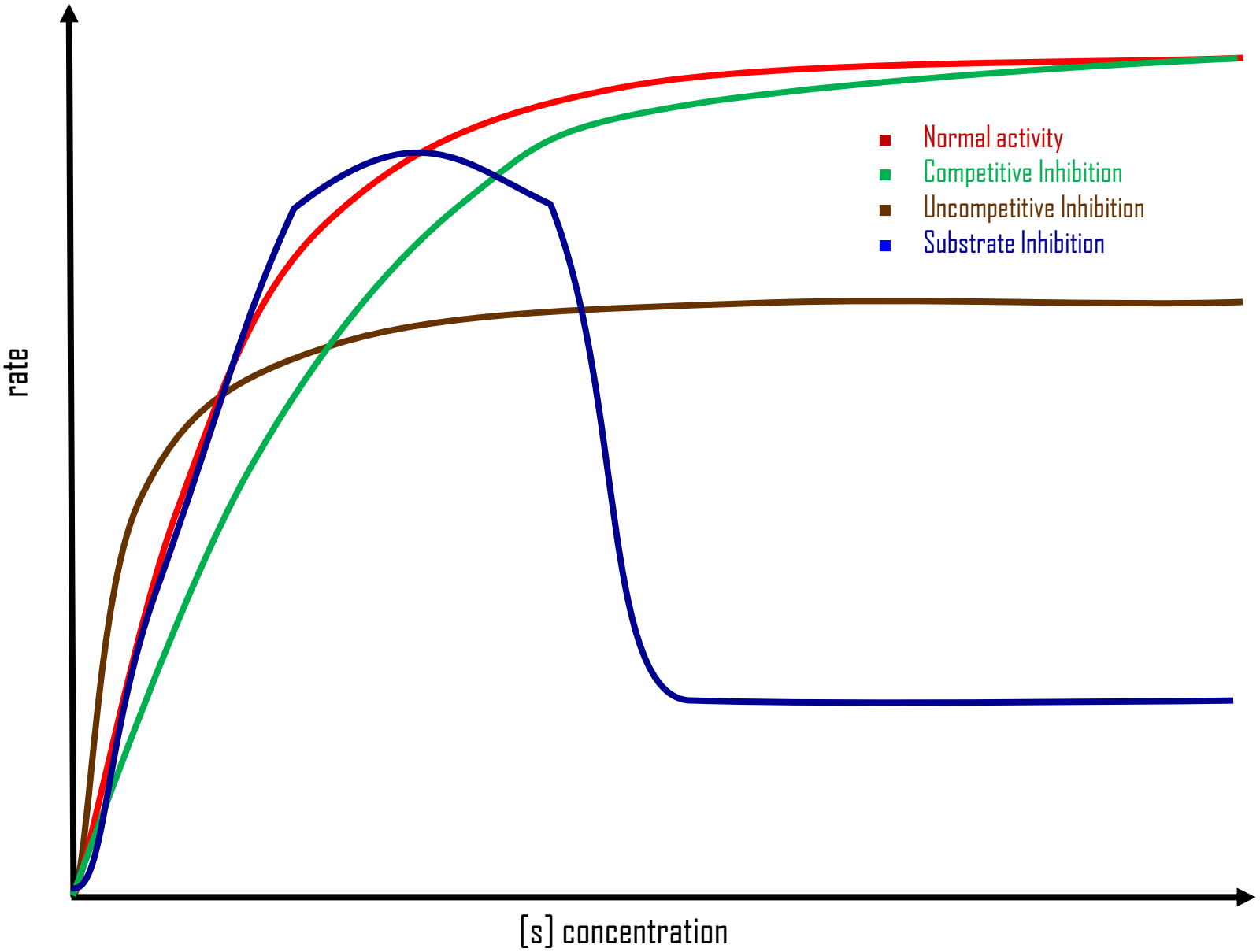
Substrate Inhibition

- At high concentrations, some substrates also inhibit the enzyme activity.
- Substrate inhibition occurs with about 20% of all known enzymes.
- The substrate is binding to a second, non-active site on the enzyme causing the inhibition.
- Analogous behavior to allosteric inhibition and product inhibition

$$v = \frac{v_{max} S}{K_M + S\left(1 + \frac{I}{K_I}\right)}$$



Enzyme regulation - inhibition



KINETIC MODELS OF THE WARBURG EFFECT

LCSB Master theses from Diane Bernhard-Bruls

Principles and Applications of Systems Biology

EPFL

Vassily Hatzimanikatis

October 2025

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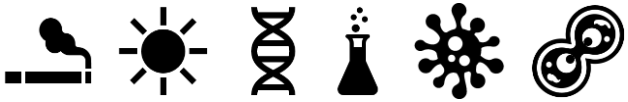
Dr. Daniel Weilandt
&
Dr. Maria Masid

Cancer

- 1 in 6 deaths caused by cancer
- 2nd most common cause of deaths
- Arises from genetic changes
- Induced by:

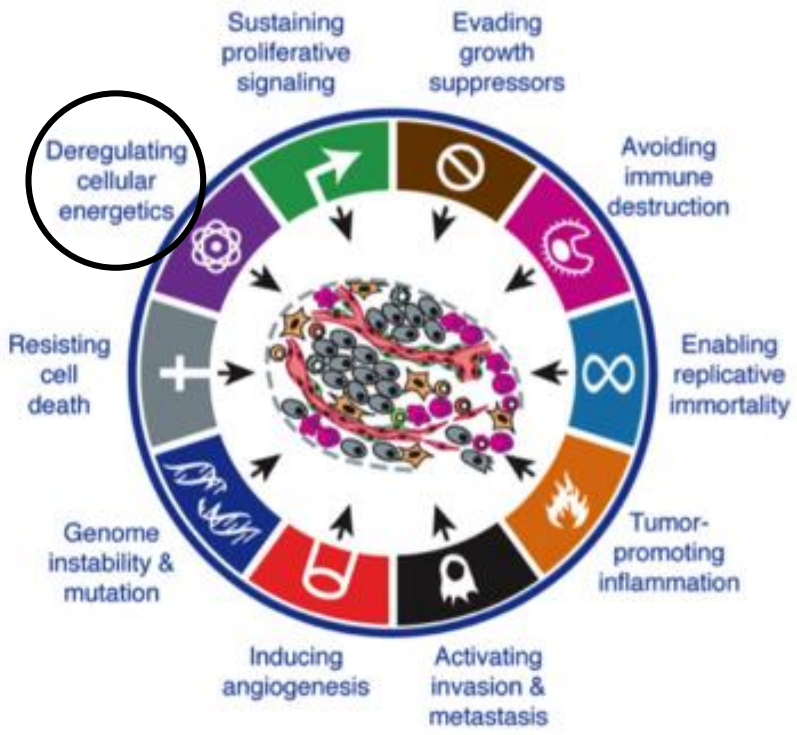


Cancer cases on the rise globally, but not equally, WHO report says

(CNN) — In the next two decades, the world could see a 60% increase in the number of cancer cases, according to a new report from the World Health Organization. In low- and middle-income countries, the increase could be as high as 81%.

Hallmarks of cancer

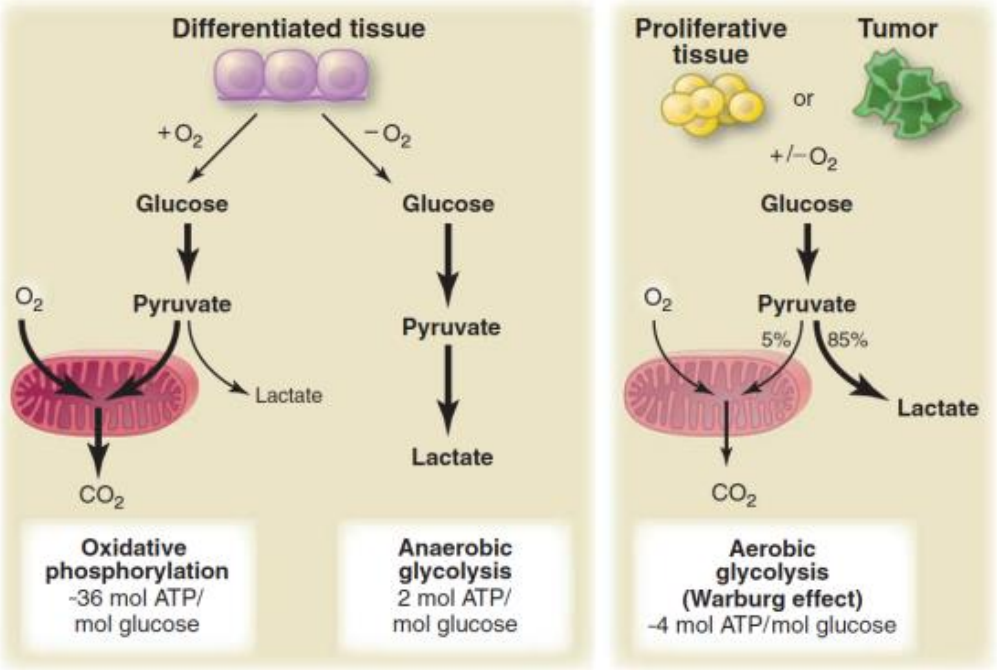
- Hanahan and Weinberg defined 8 hallmarks of cancer and 2 enabling characteristics:



Hanahan D. and Weinberg R.A. Hallmarks of cancer: The next generation. Cell, 144(5):646–674, 2011.

The Warburg Effect

- In the 1920s, O. Warburg: tumours were taking up huge amounts of glucose to produce lactate, instead of producing pyruvate:



Pictures from Vander Heiden M. G., Cantley L.C, and Thompson C.B. Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science, 334(5930):1029 – 1033, 2009

Computational studies on the Warburg Effect

- Over the last decade, extended research and work put in investigating the Warburg Effect in the cancer cells:

Genome-Scale Metabolic Modeling Elucidates the Role of Proliferative Adaptation in Causing the Warburg Effect

Tomer Shlomi[1]*, Tomer Benyamini[2], Eyal Gottlieb[3], Roded Sharan[2], Eytan Ruppin[2,4]*

PLoS Computational Biology | www.ploscompbiol.org 1 March 2011 | Volume 7 | Issue 3 | e1002018

A Flux Balance of Glucose Metabolism Clarifies the Requirements of the Warburg Effect

Ziwei Dai,[1,2] Alexander A. Shestov,[3] Luhua Lai,[2] and Jason W. Locasale[1,*]

1088 Biophysical Journal *111*, 1088–1100, September 6, 2016

A computational study of the Warburg effect identifies metabolic targets inhibiting cancer migration

Keren Yizhak[1,*,†], Sylvia E Le Dévédec[2,†], Vasiliki Maria Rogkoti[2], Franziska Baenke[3], Vincent C de Boer[4], Christian Frezza[5], Almut Schulze[3], Bob van de Water[2,‡] & Eytan Ruppin[1,6,‡,**]

Molecular Systems Biology **10**: 744 | 2014

Multi-scale computational study of the Warburg effect, reverse Warburg effect and glutamine addiction in solid tumors

Mengrou Shan[1]*, David Dai[1], Arunodai Vudem[1], Jeffrey D. Varner[1], Abraham D. Stroock[1,2]*

PLOS Computational Biology | https://doi.org/10.1371/journal.pcbi.1006584 December 7, 2018

Kinetic model optimization and its application to mitigating the Warburg effect through multiple enzyme alterations

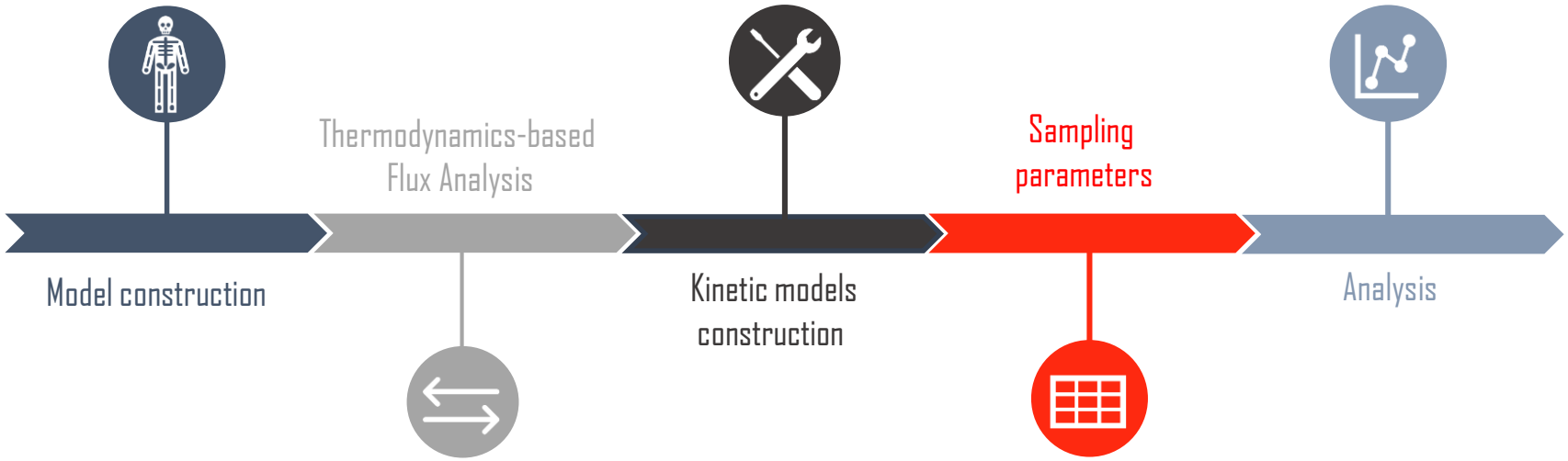
Conor O'Brien, Andrew Allman, Prodromos Daoutidis, Wei-Shou Hu*

C. O'Brien, et al. *Metabolic Engineering 56 (2019) 154–164*

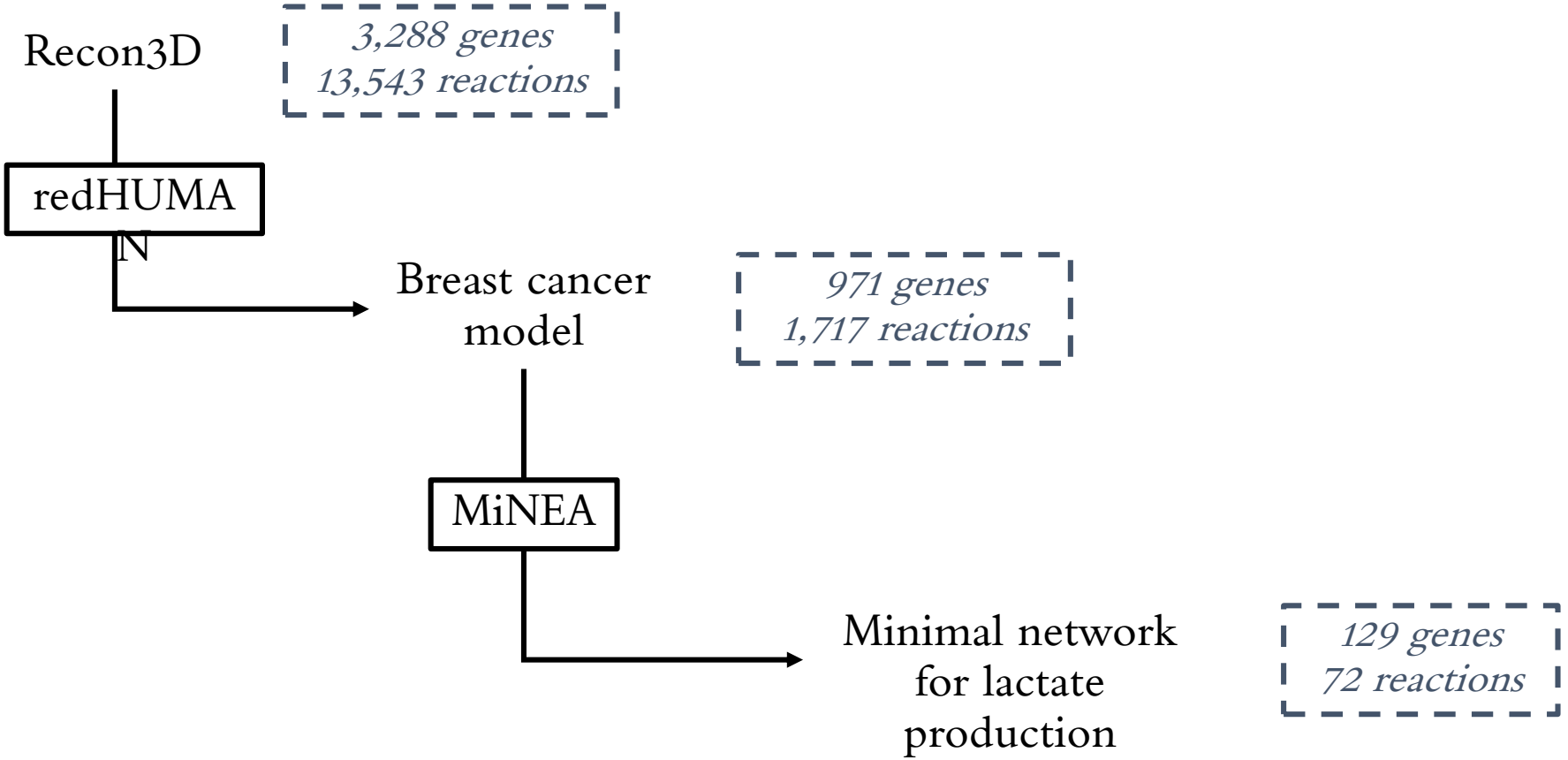
- However: few thermodynamic considerations (no TFA performed), models that do not represent cancer cells, kinetic models considering only reactions parameters from literature

Workflow overview

Aim of this work: build kinetic models that describe the Warburg Effect in breast cancer cells



Model construction



Model characteristics

- 72 reactions
  - 50 intracellular reactions
  - 14 transport reactions
  - 8 boundary reactions
- 7 bidirectional reactions
- 86 metabolites
- 22 moieties
- Exometabolomics and exofluxomics integrated from the NCI60 cell lines for breast cancer

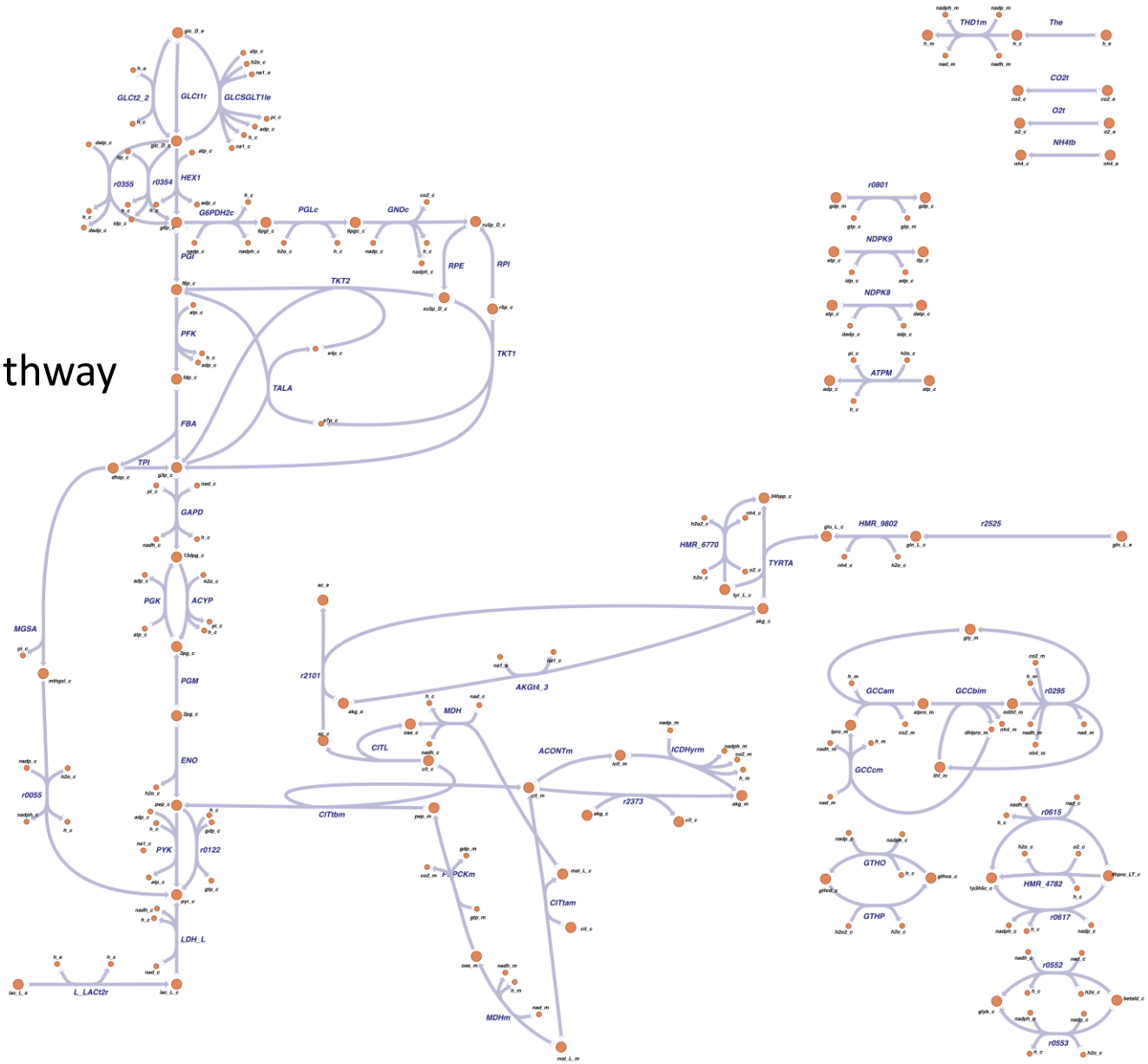
Reaction network model

Reactions from:

- Glycolysis pathway

- Pentose Phosphate pathway

- TCA cycle



Model characteristics

- Thermodynamically curated:

Category	Thermodynamic coverage
Intracellular reactions	88.0 %
Transport reactions	85.7 %
Metabolites	97.7 %

Kinetic Warburg model

Types of mechanisms present in the kinetic models:

Reaction molecularity	Mechanism	Number of reactions
Uni - Uni	Reversible Michaelis Menten	13
Uni - Bi	Uni - Bi reversible Hill	5
Bi - Bi	Generalized Reversible Hill	13
N - N	Generalized Reversible Hill	3
Other	Convenience	30

Kinetic models construction – Distribution of mechanisms

Mechanism	# reactions	Rxns ID
Reversible Michaelis Menten	13	ACONTm, GLCt1r, r2525, The, CO2t, ENO, O2t, PGI, PGM, RPE, RPI, TPI, NH4tb
Uni - Bi reversible Hill	5	MGSA, PGLc, FBA, CITL, HMR_9802
Bi –Bi Generalized Reversible Hill	13	CITtam, CITbm, r0801, r2101, r2373, L_LACt2r, NDPK8, NDPK9, PGK, TALA, TKT1, TKT2, TYRTA
Ter – Ter Generalized Reversible Hill	2	GLCt2_2, THD1m
Tetra – Tetra Generalized Reversible Hill	1	AKGt4_3
Uni – Ter Convenience	2	ATPM, ACYP
Bi – Ter Convenience	12	GCCbim, GCCcm, MDHm, PEPCKm, r0354, r0355, G6PDH2c, HEX1, LDH_L, MDH, PFK, HMR_6770
Bi – Tetra Convenience	2	ICDHyrm, GNDc
Bi – Penta Convenience	5	r0055, r0552, r0553, r0615, r0617
Ter – Uni Convenience	2	GTHP, HMR_4782
Ter – Bi Convenience	3	GCCam, r0122, PYK
Ter – Tetra Convenience	1	GAPD
Tetra – Ter Convenience	1	GTHO
Tetra – Hexa Convenience	1	GLCSGLT1le
Penta – Ter Convenience	1	r0295

Sampling parameters sets

For each TFA sample, 100 kinetic parameters sets sampled:

	$[K_M, V_{max}]_1$	$[K_M, V_{max}]_2$	$[K_M, V_{max}]_{100}$
$[C, V, \Delta_r G'^\circ]^1$	$[K_M, V_{max}]_1^1$	$[K_M, V_{max}]_2^1$	$[K_M, V_{max}]_{100}^1$
$[C, V, \Delta_r G'^\circ]^2$	$[K_M, V_{max}]_1^2$	$[K_M, V_{max}]_2^2$	$[K_M, V_{max}]_{100}^2$
$[C, V, \Delta_r G'^\circ]^{1000}$	$[K_M, V_{max}]_1^{1000}$	$[K_M, V_{max}]_2^{1000}$	$[K_M, V_{max}]_{100}^{1000}$

Parameters sets →

TFA Samples ↓

⇒ 100,000 kinetic parameters sets obtained from 1,000 TFA samples

Kinetic parameters sets selection

- **Objective**: retrieve only **physiologically relevant** parameter sets

fast dynamics

characteristic time of the models << characteristic time of breast cancer cells

- Growth rate for breast cancer cells: 0.03/hr, corresponding to a characteristic time of 33.3 hr

- Selection criteria: characteristic time has to be at least **10 times faster** than the characteristic time in breast cancer cells:

$$abs\left(\frac{1}{Re(\lambda_{k,max})}\right) < 3.33\ hr$$

Physiologically relevant representatives

For small perturbation:

$$\frac{d\boldsymbol{X}}{dt} = J\boldsymbol{X} + \boldsymbol{\mathcal{O}}(\boldsymbol{X^2})$$

- Slowest response
- Fastest response

$$\{\lambda_i, \boldsymbol{w}_i\} = eig(J)$$

$$y_i = \sum w_{ik}[X_k]$$

$$y_i(t) = y_{i,0} \exp\left(-\frac{t}{\tau_i} + i\omega_i t\right) \quad \tau_i = \text{abs}(1/\text{Re}(\lambda_i)$$

$$\omega_i = \text{Im}(\lambda_i)$$

Physiologically relevant representatives

For small perturbation:

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Exponential decay

Harmonic Oscillations

Physiologically relevant representatives

For small perturbation:

$$\frac{d\boldsymbol{X}}{dt} = J\boldsymbol{X} + \boldsymbol{\mathcal{O}}(\boldsymbol{X^2})$$

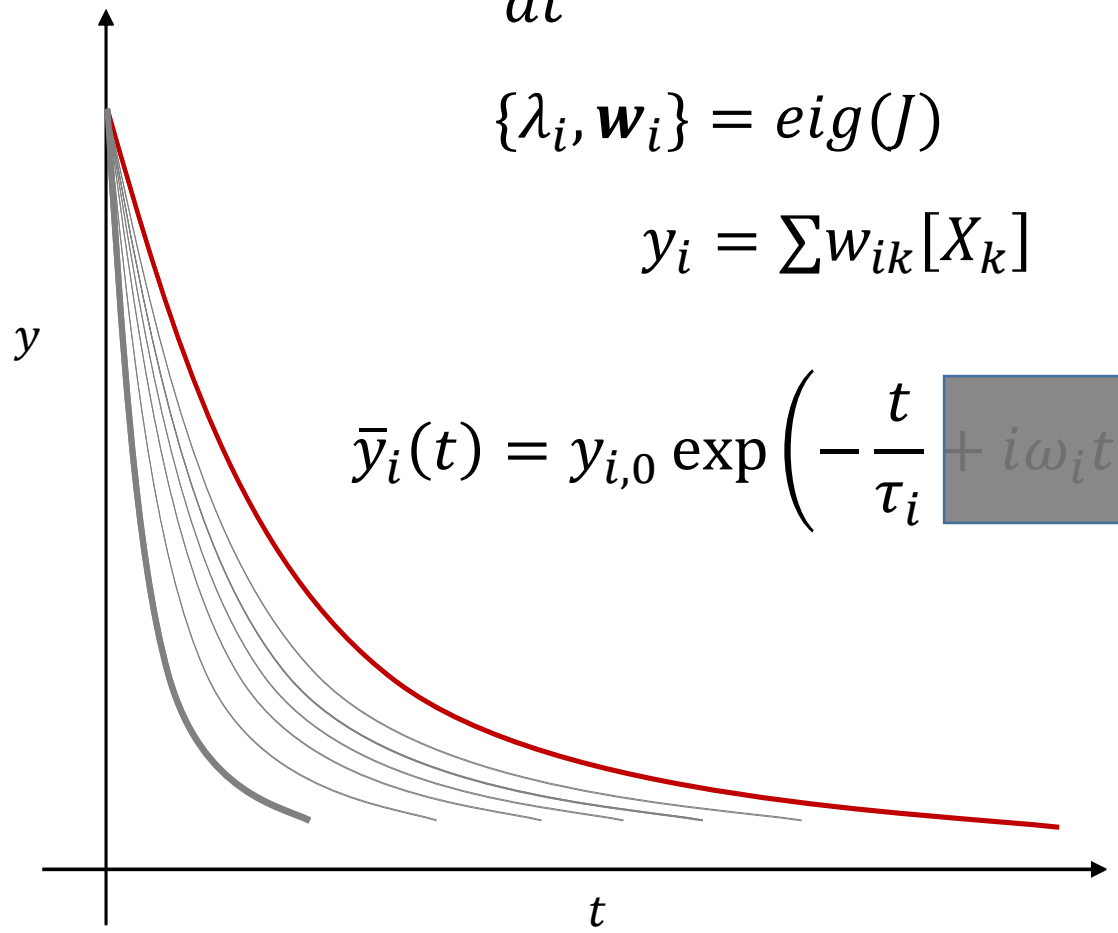
$$\{\lambda_i, \boldsymbol{w}_i\} = eig(J)$$

$$y_i = \sum w_{ik}[X_k]$$

$$\bar{y}_i(t) = y_{i,0} \exp\left(-\frac{t}{\tau_i} + i\omega_i t\right)$$

$$\tau_i = \text{abs}(1/\text{Re}(\lambda_i))$$

$$\omega_i = \text{Im}(\lambda_i)$$

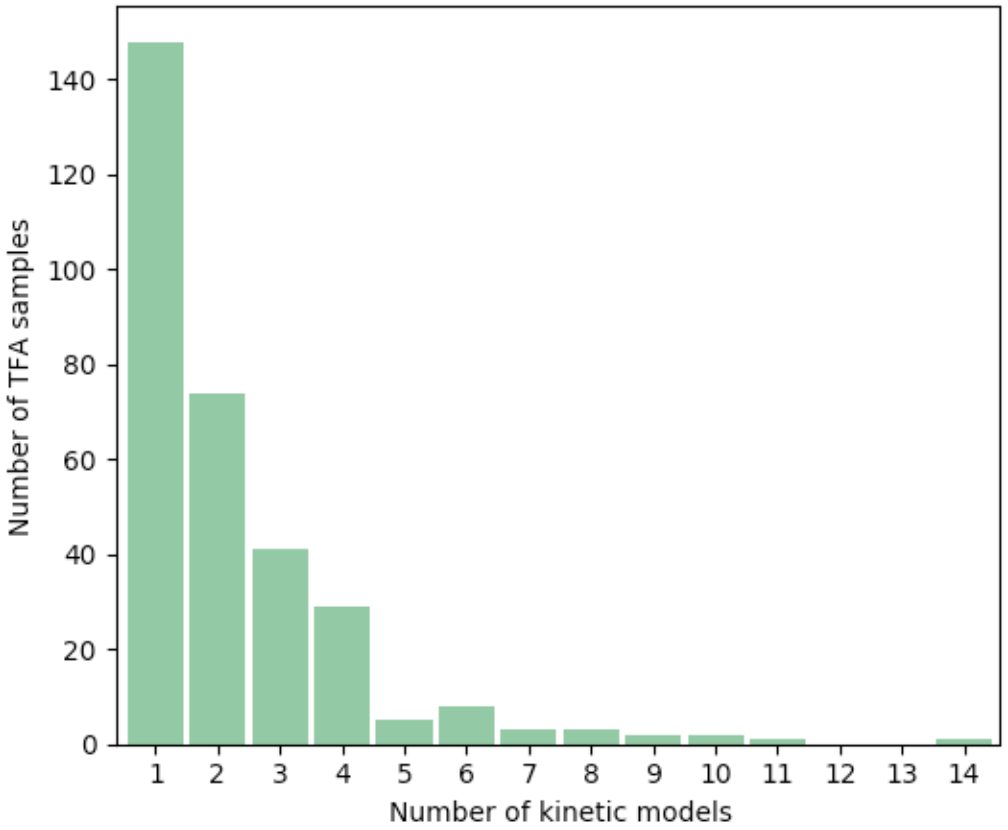


Slowest response

Fastest response

Kinetic parameters sets selection

- **100,000** kinetic parameters sets from 1,000 TFA samples
  - **317** TFA samples yielded to physiologically relevant parameters sets
  - **716** kinetic parameters sets retrieved

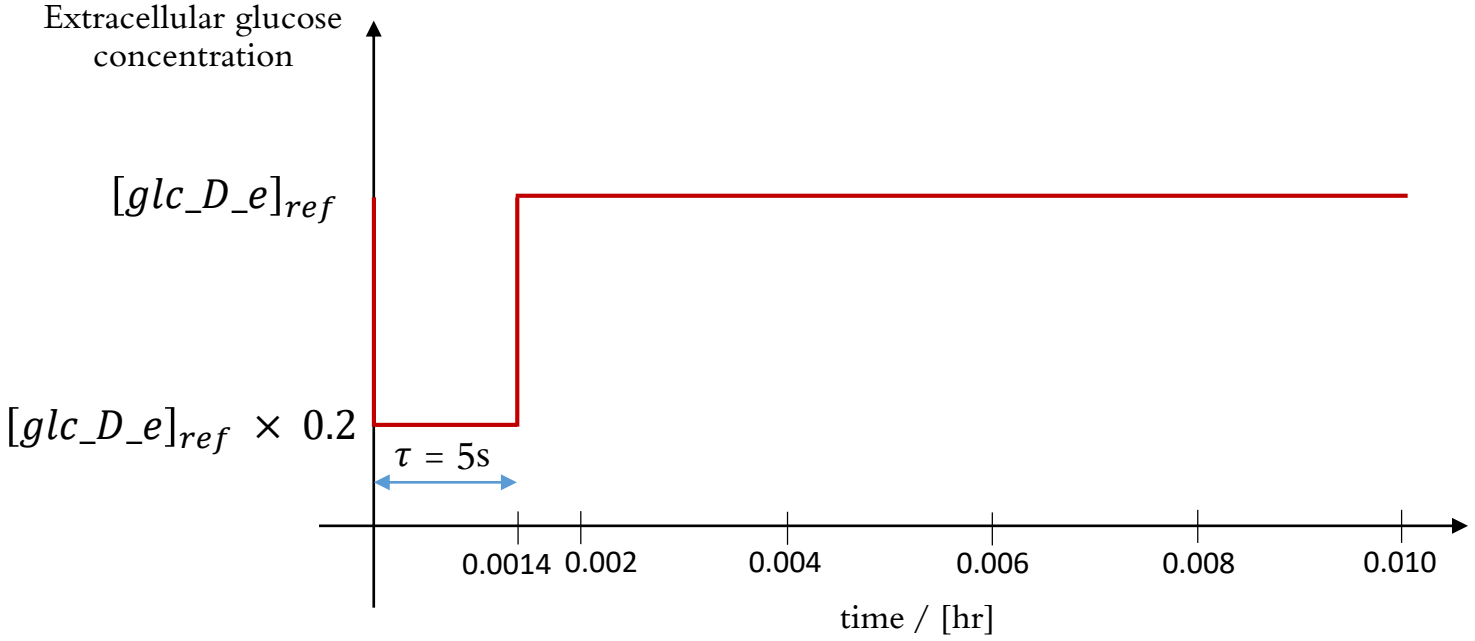


⇒ distribution of **physiological kinetic models** over the 317 TFA samples

Analysis

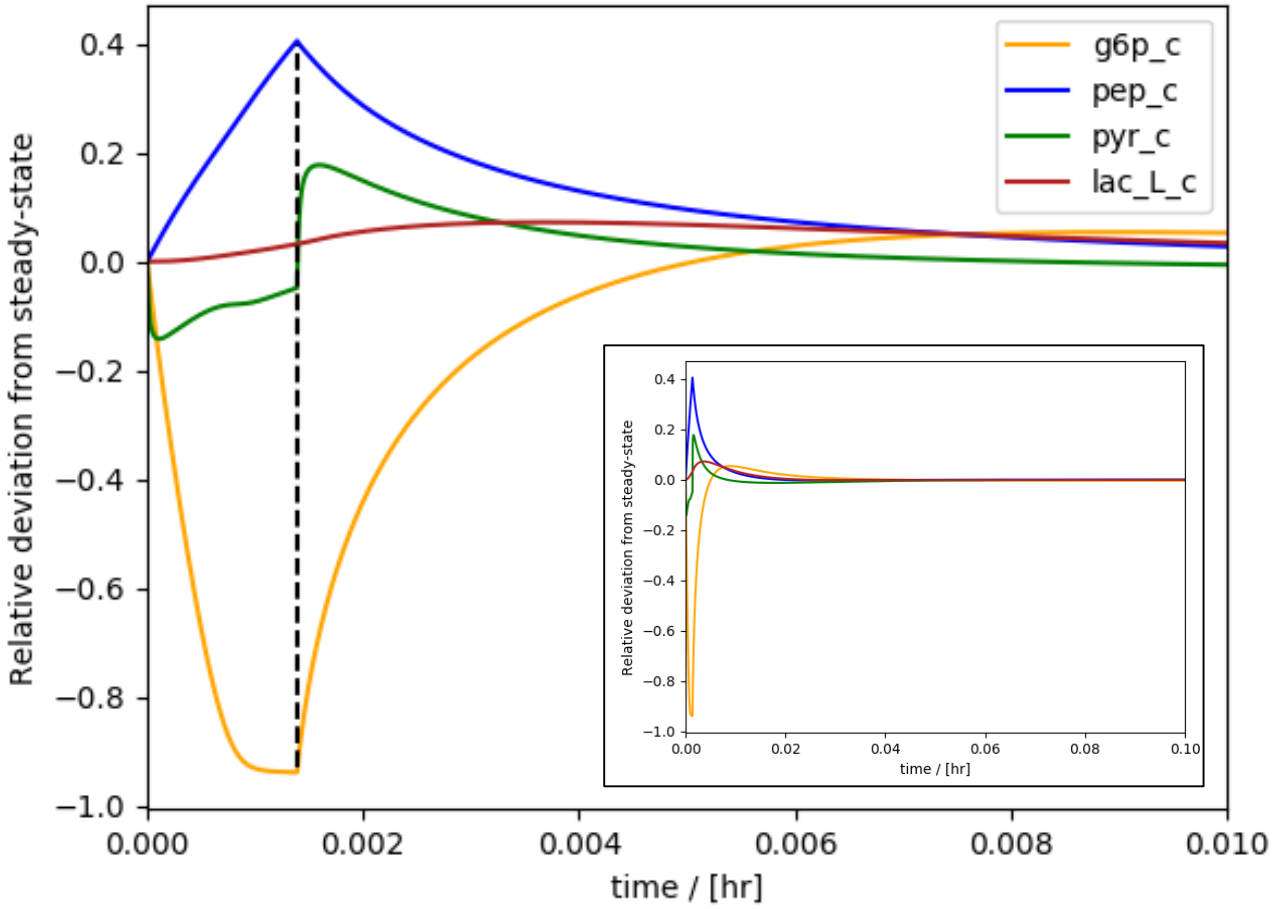
- **Objective:** perturb the model in order to assess the model behaviour

➢ Pulse in extracellular glucose concentration:



Glucose pulse simulation

Relative changes $\frac{[X_i] - [X_i]_{ref}}{[X_i]_{ref}}$ for **one** kinetic model:



Analysis

- **Objective:** perturb the model in order to assess the model behaviour

➢ Non-linear variable perturbation analysis

Sample **100 sets of initial conditions** from the **linear problem**:

$$0.8\,[X_i]_{ref} \leq [X_i] \leq 1.2\,[X_i]_{ref}$$

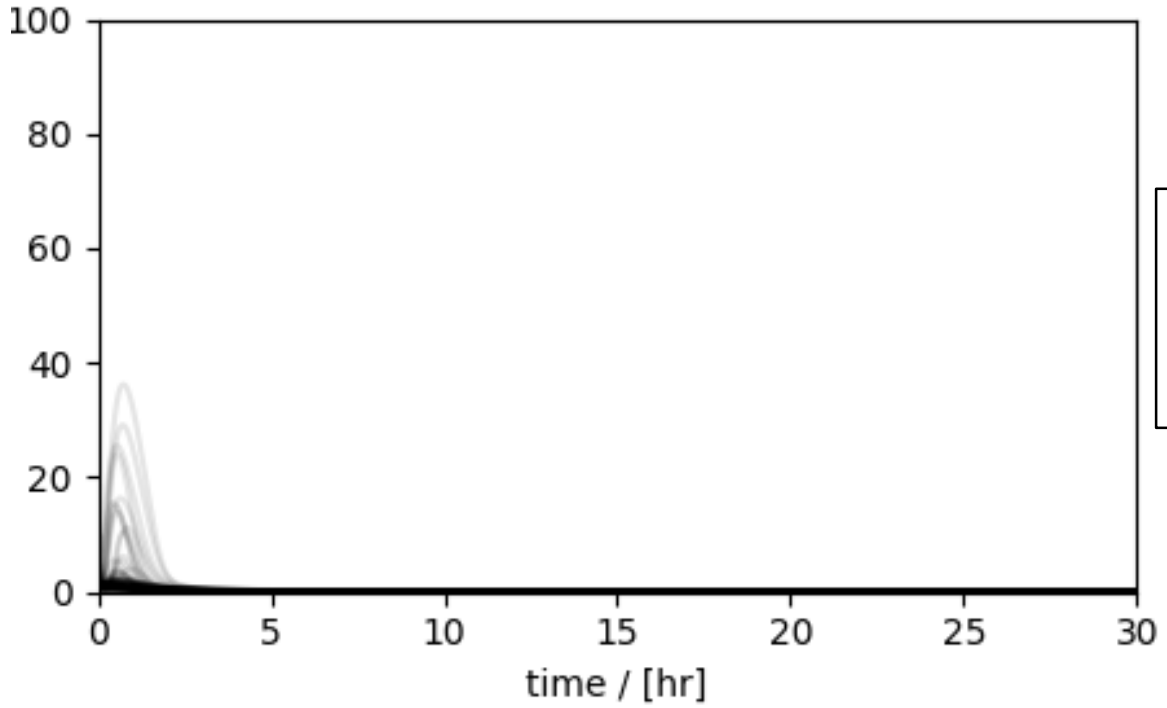
$$\sum_{i}^{N} L_{ij}[X_i] = [P_j]$$

L_{ij} coefficients of the conservation relationships
$[P_j]$ conserved pool concentration

Non-linear variable perturbation analysis

Metabolic responses of **kinetic model A** to 100 sets of sampled initial conditions

$$\left\| \frac{[X_i] - [X_i]_{ref}}{[X_i]_{ref}} \right\|_2$$



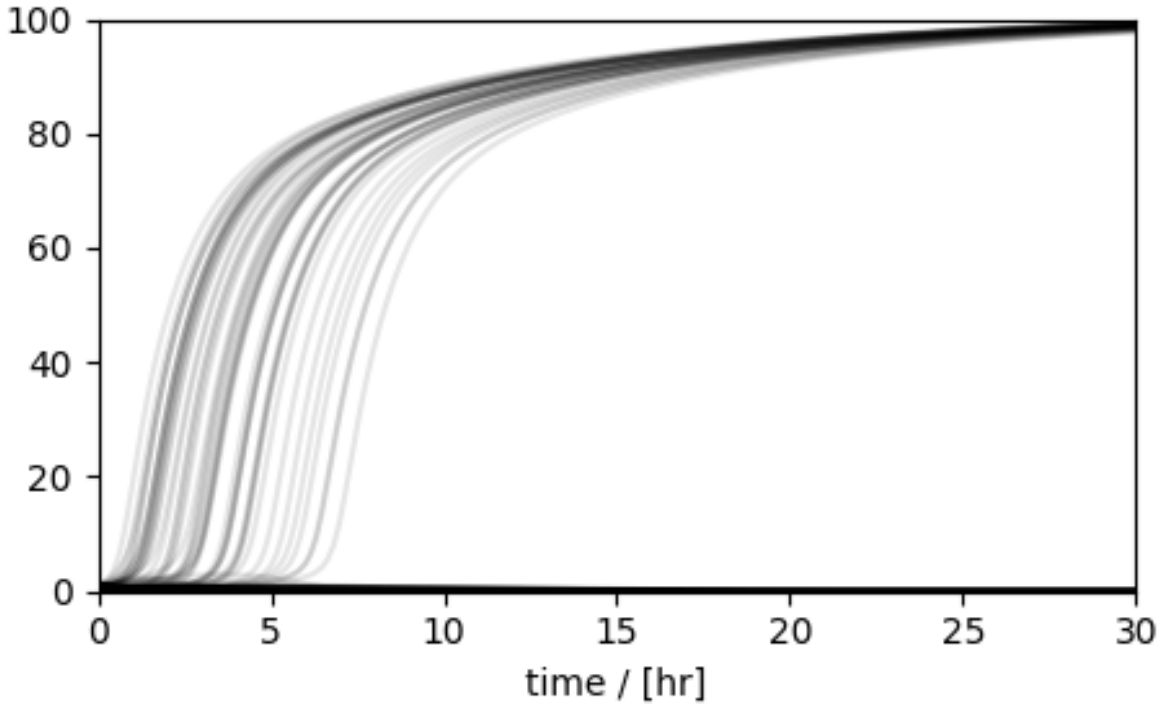
– : one metabolic response for one set of sampled initial conditions

For all the perturbations the model settles back in the reference steady-state

Non-linear variable perturbation analysis

Metabolic responses of **kinetic model B** to 100 sets of sampled initial conditions

$$\left\| \frac{[X_i] - [X_i]_{ref}}{[X_i]_{ref}} \right\|_2$$



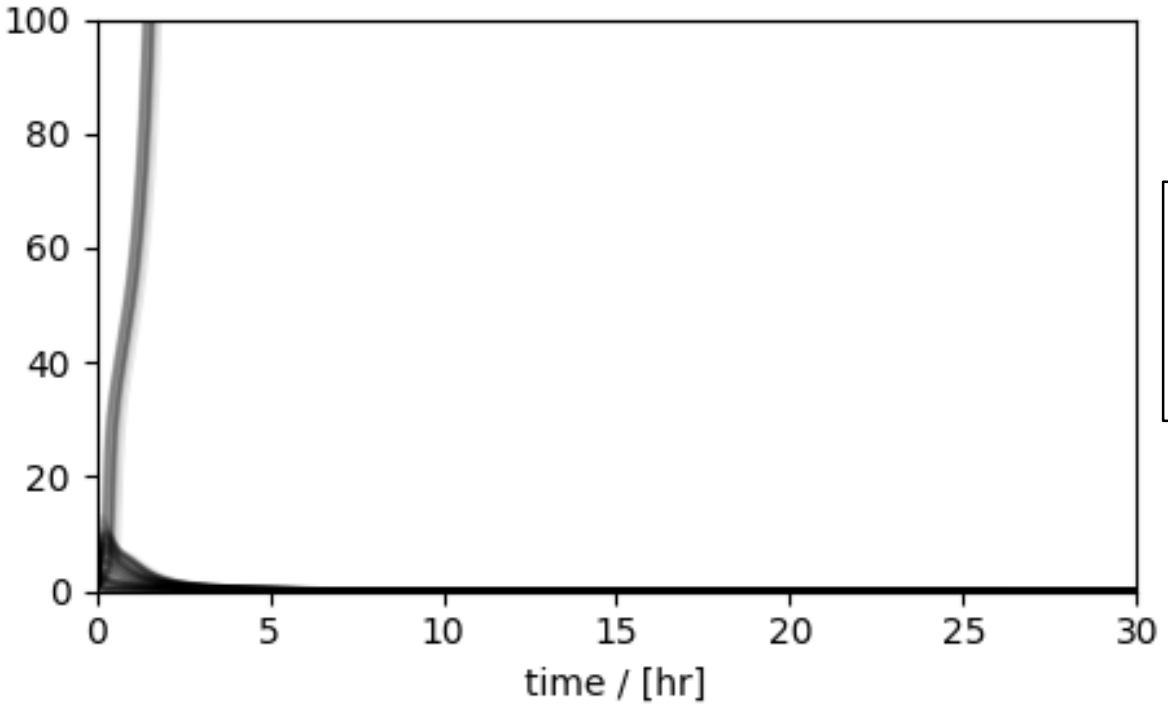
– : one metabolic response for one set of sampled initial conditions

For some perturbations the model settles in a different steady-state

Non-linear variable perturbation analysis

Metabolic responses of **kinetic model C** to 100 sets of sampled initial conditions

$$\left\| \frac{[X_i] - [X_i]_{ref}}{[X_i]_{ref}} \right\|_2$$



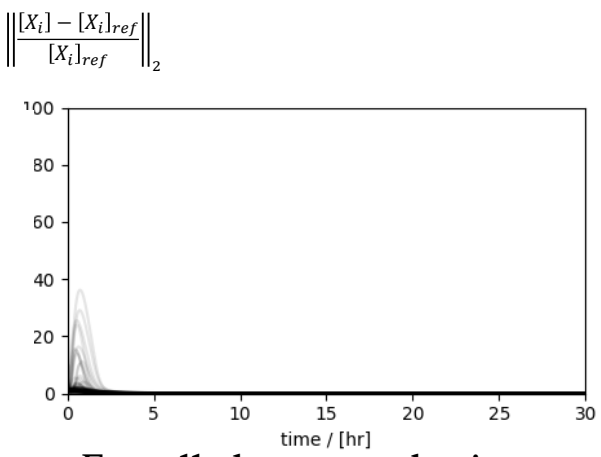
– : one metabolic response for one set of sampled initial conditions

For some perturbations the model does not settle

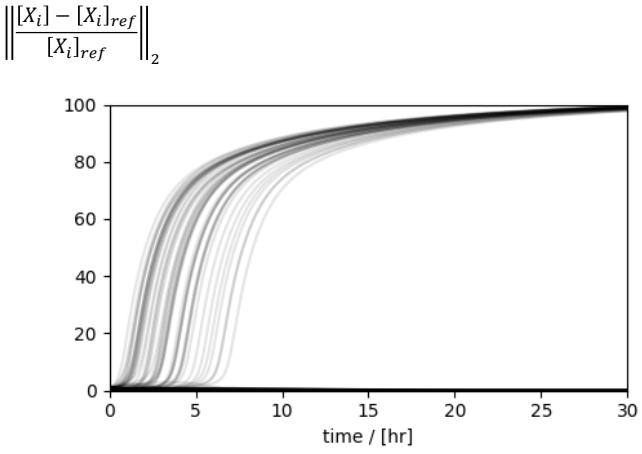
Non-linear variable perturbation analysis

Norm of the relative changes $\left\| \frac{[X_i] - [X_i]_{ref}}{[X_i]_{ref}} \right\|_2$

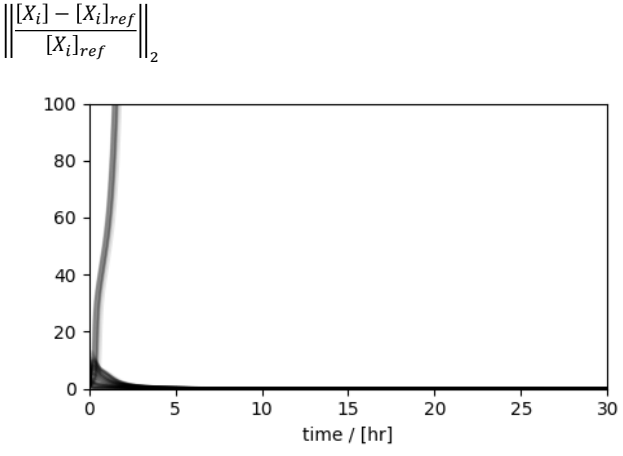
Observed 3 different behaviours in the models:



For all the perturbations the model settles back in the reference steady-state



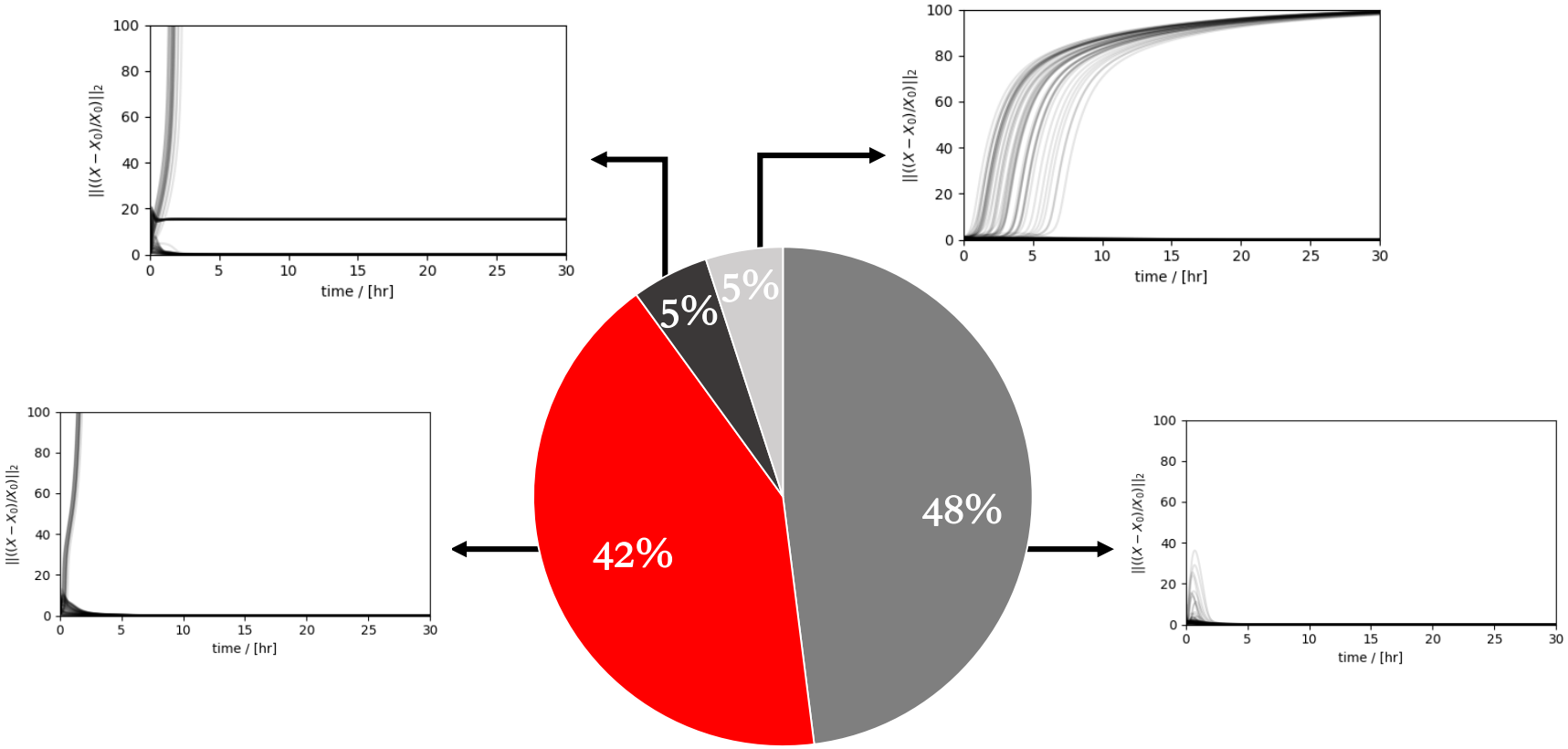
For some perturbations the model settles in a different steady-state



For some perturbations the model does not settle

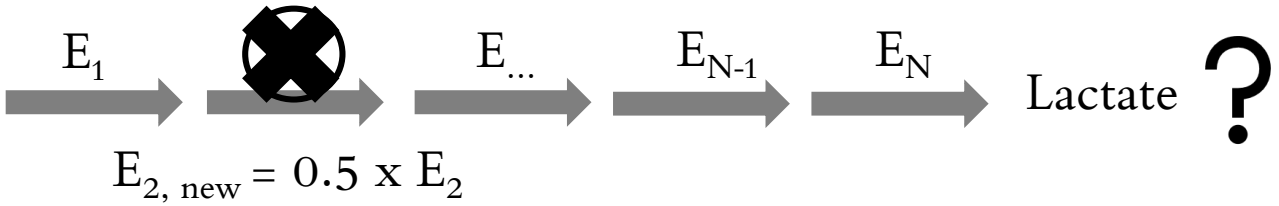
Non-linear variable perturbation analysis

Behaviour classification over the 716 kinetic parameters sets:



Metabolic Control Analysis

Objective: investigate the **local sensitivity** of the lactate production in the models and the effects of the **effective enzymes levels**



Metabolic Control Analysis

Fractional change of fluxes over the fractional change of the system parameters:

$$C_{P_j}^{v_i} = \frac{P_j}{v_i}\frac{dv_i}{dP_j} = \frac{dlnv_i}{dlnP_j} \cong \frac{P_j}{v_i}\frac{\Delta v_i}{\Delta P_j} \; or \; \frac{log_2\left(\frac{v_i}{v_{i,REF}}\right)}{log_2\left(\frac{P_j}{P_{j,REF}}\right)}$$

⇒ Compute the flux control coefficients for the lactate production over **all the kinetic models**

Metabolic Control Analysis

Fractional change of metabolite concentration and fluxes over the fractional change of the system parameters:

$$C_{P_j}^{X_i} = \frac{dln[X_i]}{dlnP_j} \qquad C_{P_j}^{v_i} = \frac{dlnv_i}{dlnP_j}$$

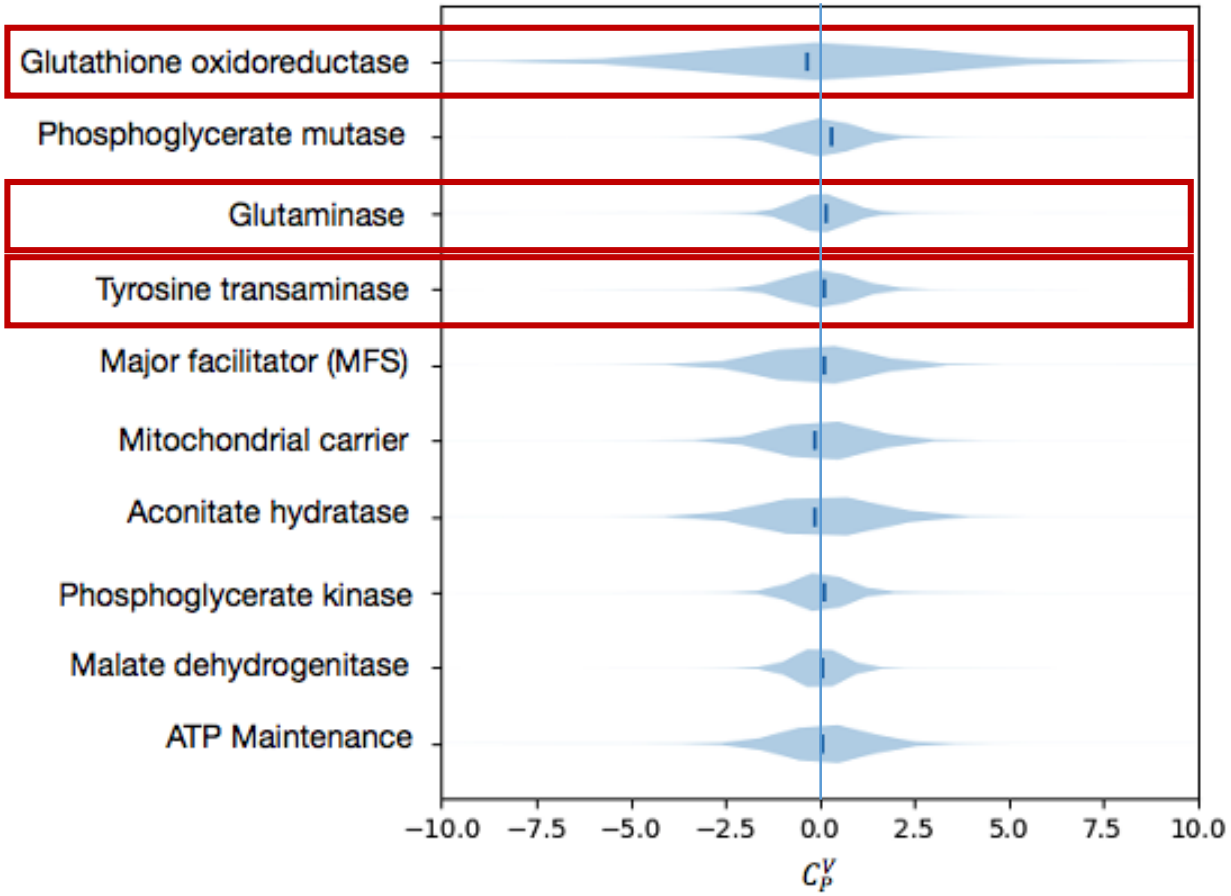
Given a metabolic model with dependent moieties:

$$\frac{d\boldsymbol{X_i}}{dt} = \boldsymbol{N_R v}(\boldsymbol{X_i}, \boldsymbol{X_d}\,(\boldsymbol{X_i}, \boldsymbol{p_m}), \boldsymbol{p_e}, \boldsymbol{p_s})$$

$\boldsymbol{N_R}$ reduced stoichiometric matrix
$\boldsymbol{X_i}$ independent metabolites concentration vector
$\boldsymbol{X_d}$ dependent metabolites concentration vector
$\boldsymbol{p_m}$ conserved moieties concentration vector
$\boldsymbol{p_e}$ enzymes parameters vector
$\boldsymbol{p_s}$ vector including all the other parameters

- Fell D., (1997) *Understanding the Control of Metabolism*, Portland Press.
- Heinrich R. and Schuster S. (1996) *The Regulation of Cellular Systems*, Chapman and Hall.

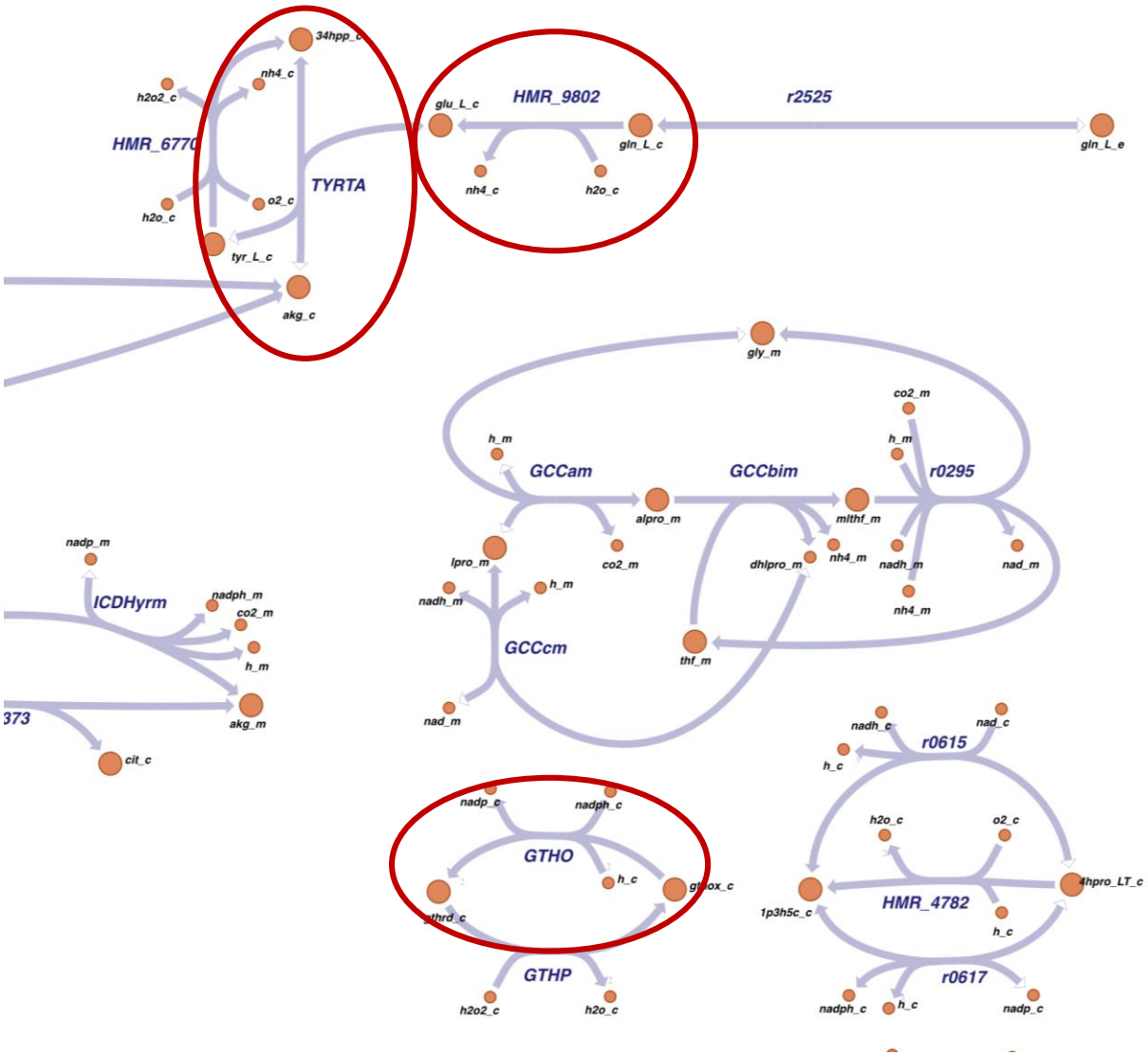
Metabolic Control Analysis



Reactions

- GTHO
  - glutathione disulphide + 2 H + NADPH → 2 glutathione + NADP
- TYRTA
  - L-Tyrosine + 2-Oxoglutarate → 3-(4-Hydroxyphenyl)pyruvate + L-Glutamate
- HMR_9802
  - L-glutamine + H_2O → L-Glutamate + NH_4

Metabolic Control Analysis



Parameter perturbations

Non-competitive inhibition simulation:

$$v_{max,r} = v_{max,r,ref} \times 0.5 = k_{cat} \boldsymbol{E_T}$$

(log)-linear fold changes obtained from the control coefficient:

$$\frac{p}{v}\frac{dv}{dp} = C_p^v$$

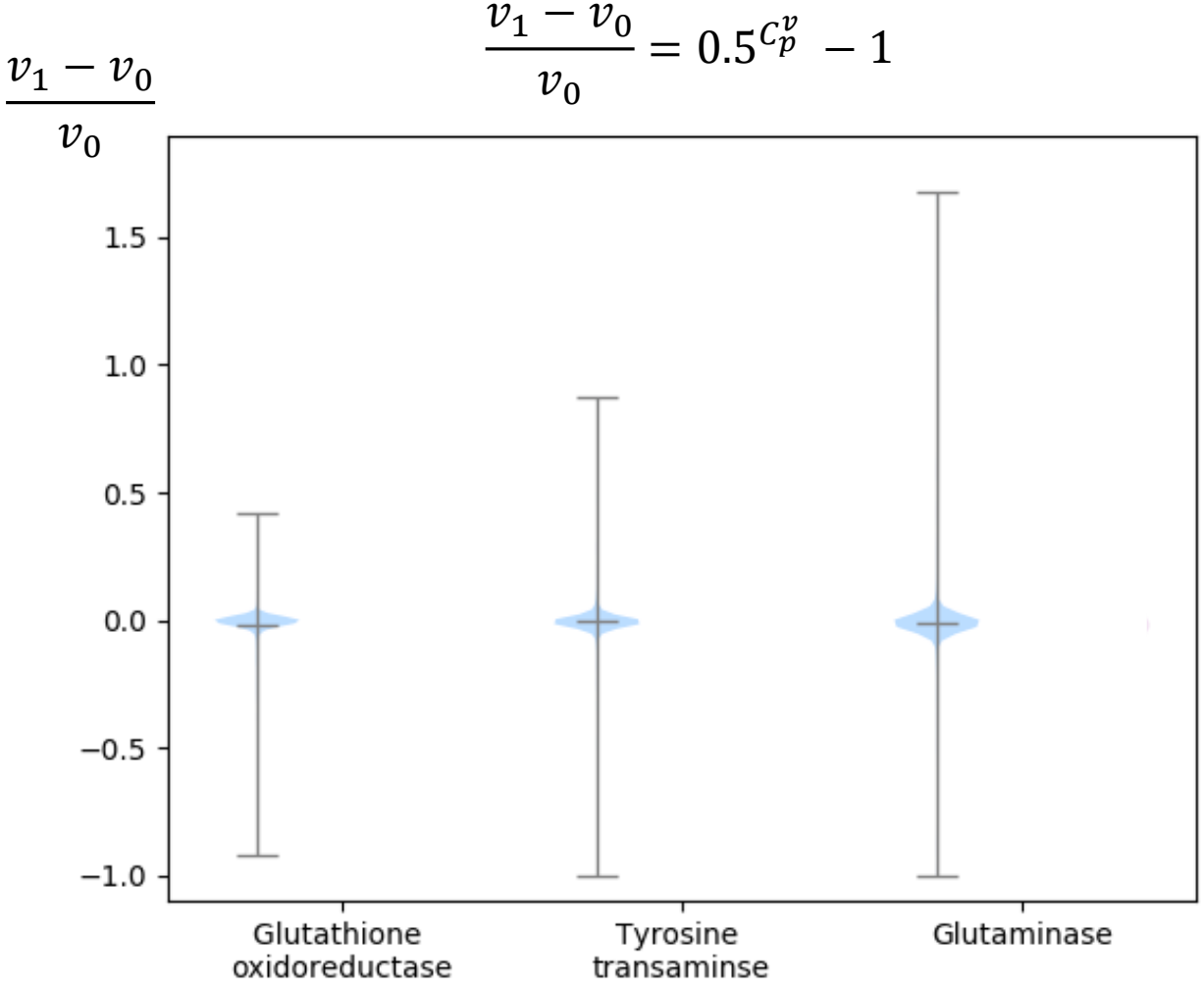
$$\int_{v_0}^{v_1} \frac{1}{v} dv = \int_{p_0}^{p_1} \frac{C_p^v}{p} dp$$

$$ln\left(\frac{v_1}{v_0}\right) = C_p^v \times ln(0.5)$$

$$\boxed{\frac{v_1 - v_0}{v_0} = exp\left(C_p^v \times ln(0.5)\right) - 1 = 0.5^{C_p^v} - 1}$$

Parameter perturbations

$$\frac{v_1 - v_0}{v_0} = 0.5^{C_p^v} - 1$$

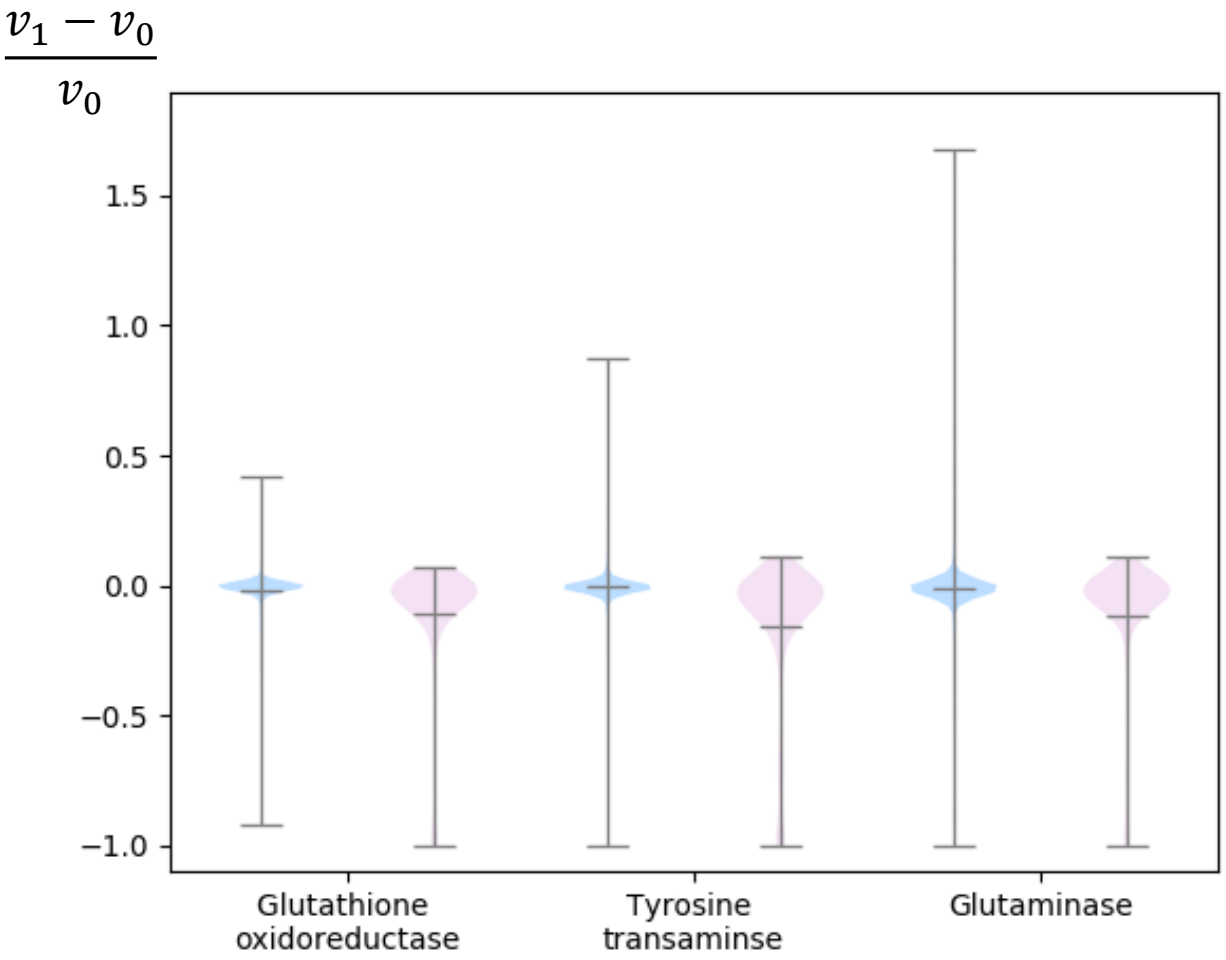


● : (log)-linear fold changes in lactate flux

Parameter perturbations

Non-competitive inhibition simulation: $v_{max,r} = v_{max,r,ref} \times 0.5$

$\Rightarrow$ solve the ODEs system

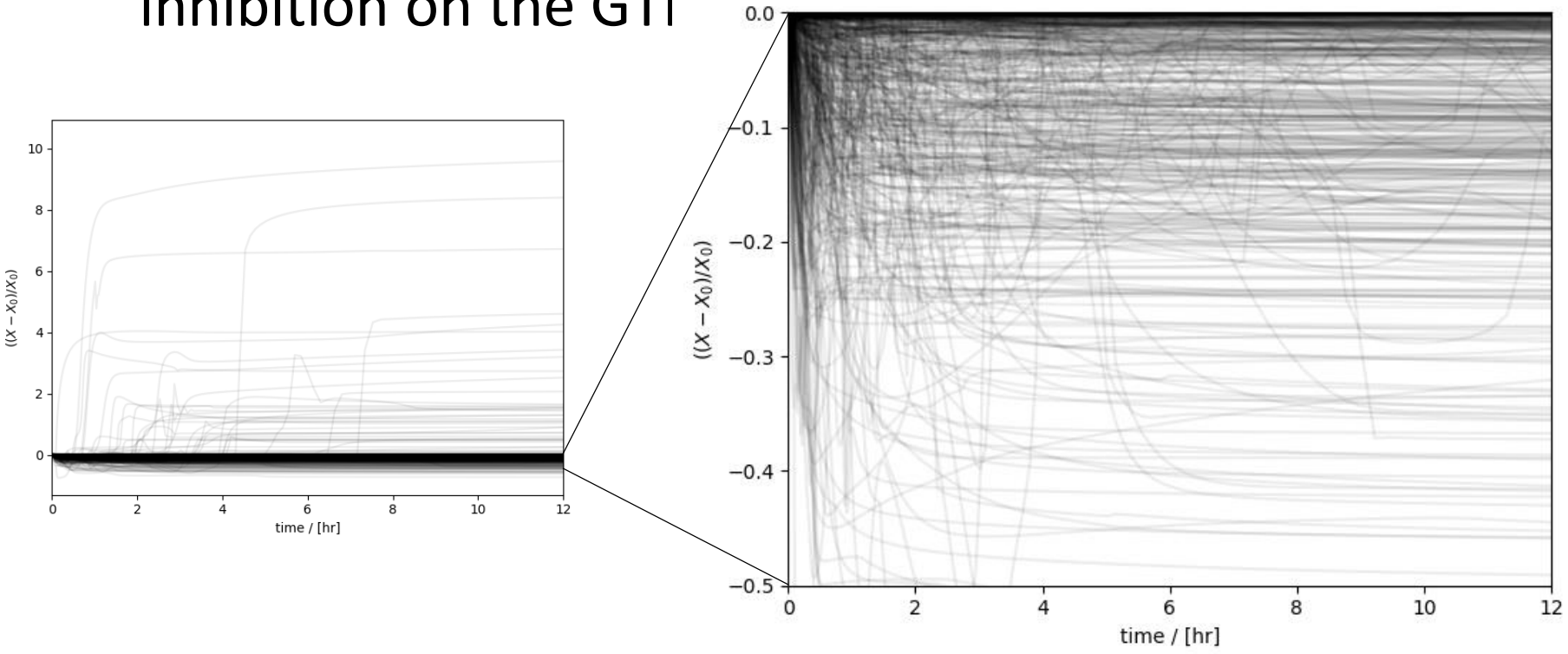


● : (log)-linear fold changes in lactate flux

● : non-linear fold changes in lactate flux

Parameter perturbations

Relative deviation from steady-state of lactate concentration for modelling non-competitive inhibition on the GTI



Conclusion

Aim of this work: build kinetic models that describe the Warburg Effect in breast cancer cells

- Successfully **built kinetic models** for a MiN describing the Warburg Effect
- Pruned to be both **stable** and **physiologically relevant**
- Included **different** reaction **mechanisms**: generalized reversible Hill, convenience etc.
- MCA results suggest link between the **Warburg effect** and the **glutamine uptake** and processing