

MODELING THE CITRIC ACID CYCLE, ELECTRON TRANSPORT CHAIN, MALATE-ASPARTATE AND CITRATE-PYRUVATE SHUTTLES

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01/06/2018

SZÉCHENYI  2020



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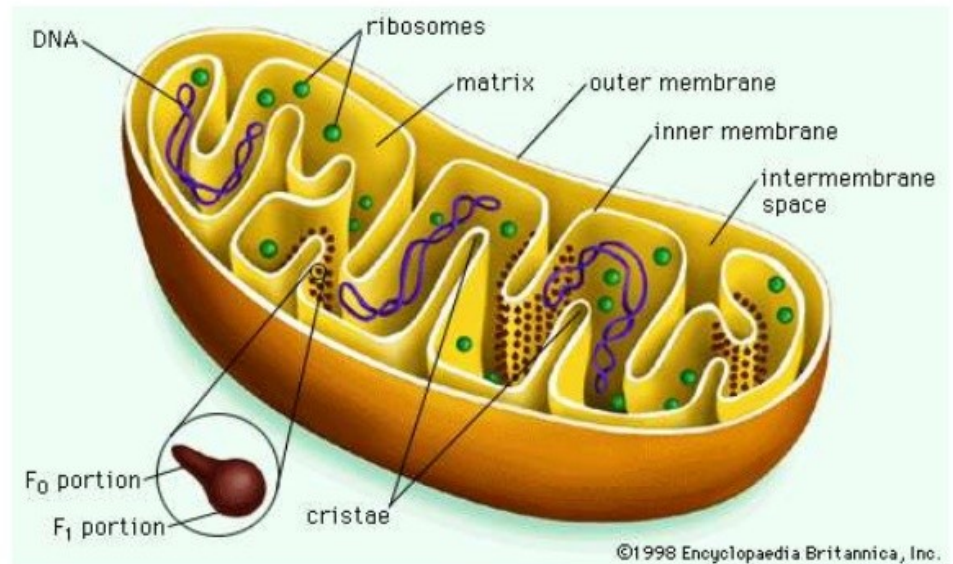
Detailed task description

- Construct a suitable quantitative model which describes the temporal changes in the quantities of CAC molecules
- Describe qualitatively the changed operation of the catalysing enzymes as well as the modified operation of the intermediate molecules
- Predict mitochondria's ability to adapt to this new metabolic state by modifying other metabolic pathways and predict when will be the metabolism pathological

Morphology and functions

- Main role in metabolism
- Bioenergetics
- Heat regulation
- Ca^{2+} homeostasis
- Apoptosis

Structure of Mitochondria.



Description of the modell

- 3 individual  **moduls**: citric acid cycle, malate-aspartate shuttle, citrate-pyruvate shuttle
- 39 differential variables: concentrations of each substrates
- Reaction rate: Michaelis-Menten kinetic:
 - irreversible reactions: elementary reaction
 - reversible reactions: two elementary reaction steps
- Transports: via concentration gradients
- Parameters: K_m and V_{max} values (15 enzymes, 5 transporters)

Michaelis-Menten kinetic equation

$$V_S = \frac{V_{max}[S]}{[S] + K_M}$$

- V_S : temporary reaction rate [$\mu\text{M}/\text{min}$]
- V_{max} : maximum reaction rate of enzyme [$\mu\text{M}/\text{min}$]
- K_m : Michaelis-Menten constant [mol/l]
- $[S]$: Substrate concentration [mol/l]

Reaction rates of the first order reactions

- Irreversible reactions:

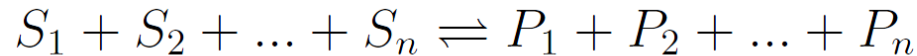
$$V_{S \rightarrow P} = \frac{V_{max}(E)[S]}{[S] + K_M(S, E)}$$

- Reversible reactions:

$$V_{S_1 \rightarrow S_2} = \frac{V_{max}(E)[S_1]}{[S_1] + K_M(S_1, E)} \quad V_{S_2 \rightarrow S_1} = \frac{V_{max}(E)[S_2]}{[S_2] + K_M(S_2, E)}$$

$$\begin{aligned} V_{S_1 \rightleftharpoons S_2} &= \frac{V_{max}(E)[S_1]}{[S_1] + K_M(S_1, E)} - \frac{V_{max}(E)[S_2]}{[S_2] + K_M(S_2, E)} = \\ &= V_{max}(E) \cdot \left(\frac{[S_1]}{[S_1] + K_M(S_1, E)} - \frac{[S_2]}{[S_2] + K_M(S_2, E)} \right) \end{aligned}$$

Reaction rates of the higher order reactions



$$V_{S_1, S_2, \dots, S_n} = V_{max}(E) \cdot \left(\frac{[S_1]}{[S_1] + K_M(S_1, E)} \right) \cdot \left(\frac{[S_2]}{[S_2] + K_M(S_2, E)} \right) \cdot \dots \cdot \left(\frac{[S_n]}{[S_n] + K_M(S_n, E)} \right)$$

$$V_{P_1, P_2, \dots, P_n} = V_{max}(E) \cdot \left(\frac{[P_1]}{[P_1] + K_M(P_1, E)} \right) \cdot \left(\frac{[P_2]}{[P_2] + K_M(P_2, E)} \right) \cdot \dots \cdot \left(\frac{[P_n]}{[P_n] + K_M(P_n, E)} \right)$$

$$\begin{aligned} V_{S \rightleftharpoons P} &= V_{S_1, S_2, \dots, S_n} - V_{P_1, P_2, \dots, P_n} = \\ &= V_{max}(E) \cdot \left(\left(\frac{[S_1]}{[S_1] + K_M(S_1, E)} \right) \cdot \left(\frac{[S_2]}{[S_2] + K_M(S_2, E)} \right) \cdot \dots \cdot \left(\frac{[S_n]}{[S_n] + K_M(S_n, E)} \right) - \right. \\ &\quad \left. - \left(\frac{[P_1]}{[P_1] + K_M(P_1, E)} \right) \cdot \left(\frac{[P_2]}{[P_2] + K_M(P_2, E)} \right) \cdot \dots \cdot \left(\frac{[P_n]}{[P_n] + K_M(P_n, E)} \right) \right) \end{aligned}$$

Parameters

- K_m and V_{max} values:
 - Own measurements:
 - α -ketoglutarate dehydrogenase
 - succinate dehydrogenase
 - malate dehydrogenase
 - **mailc** enzyme
 - From literature (15+5):
 - citric acid cycle (8)
 - malate-aspartate shuttle: enzymes (4) and transporters (2)
 - citrate-pyruvate shuttle: enzymes (7) and transporters (3)

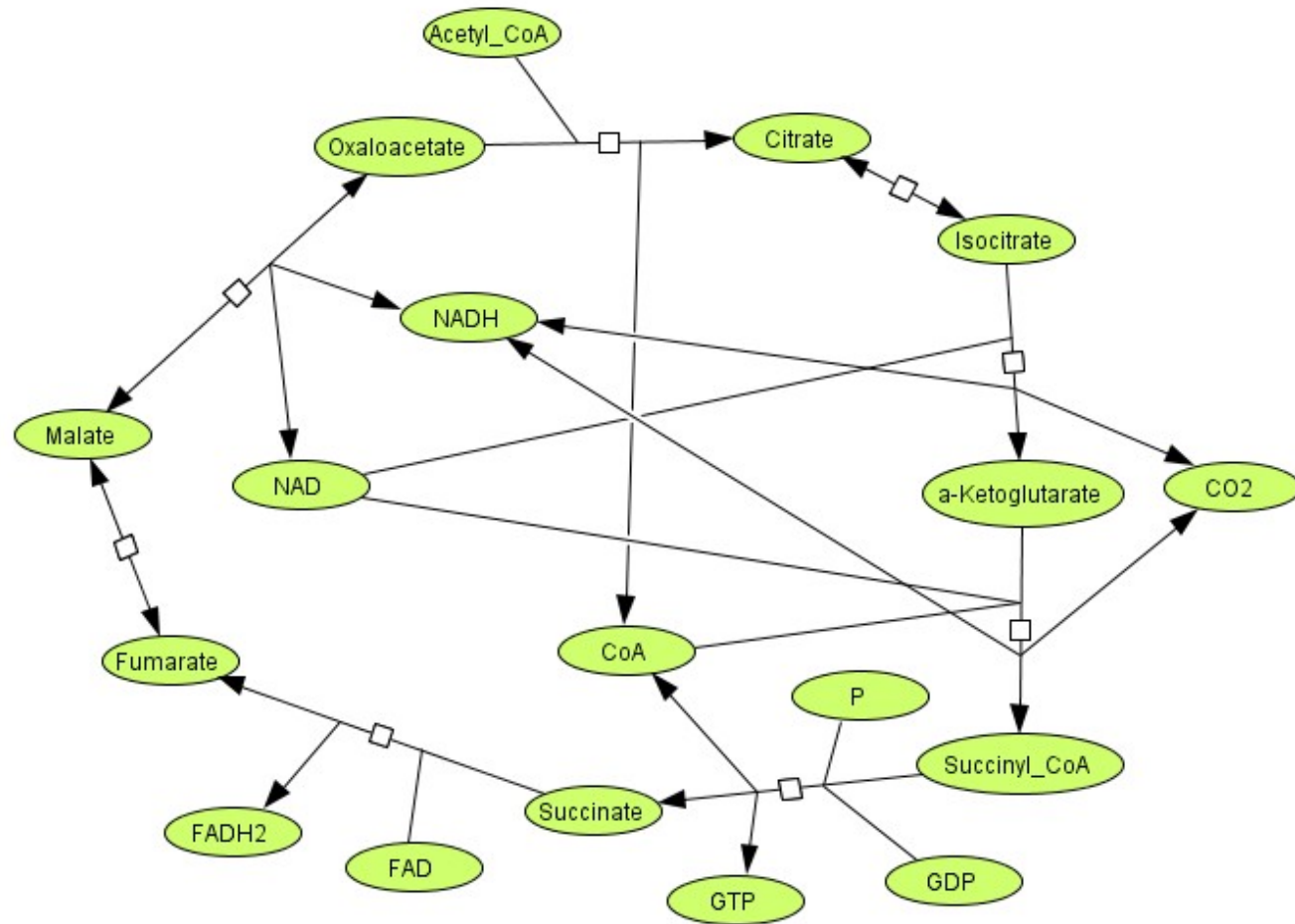
Simulations: MATLAB platform

1. Input: initial values (concentrations of substrates) and parameters of the enzymes
2. Calculates the dynamic changes of the substrate concentrations
3. Creates plots
4. Users can change:
 - Initial values → simulate recovery dynamic from acute changes
 - Parameters → simulate chronic state, mutations of enzymes

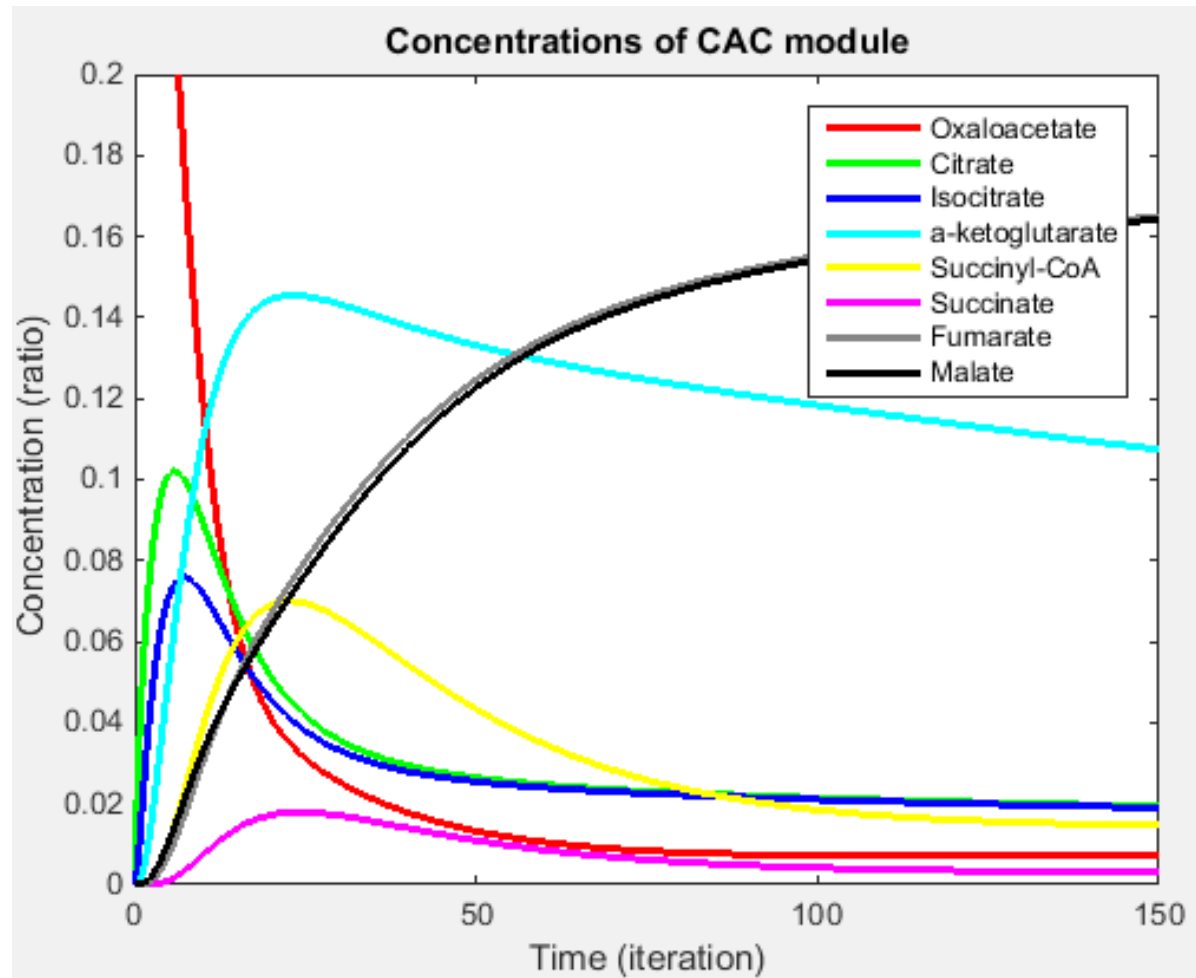
First modul: citric acid cycle

- 5 irreversible and 3 reversible reactions (11 differential equations)
- 18 differential variables: $[AcCoA_m]$, $[CoA_m]$, $[Cit_m]$, $[ICit]$, $[\alpha KG]$, $[SucCoA]$, $[Suc]$, $[Fum]$, $[Mal_m]$, $[OA_m]$, $[NAD^+_m]$, $[NADH_m]$, $[FAD]$, $[FADH_2]$, $[GDP]$, $[GTP]$, $[P_m]$, $[CO_{2,m}]$
- Inputs: $[AcCoA_m]$, $[NAD^+_m]$ (3x), $[FAD]$, $[GDP]$, $[P_m]$,
- Outputs: $[CoA_m]$, $[NADH_m]$ (3x), $[FADH_2]$, $[GTP]$, $[CO_{2,m}]$ (2x)

First modul: citric acid cycle



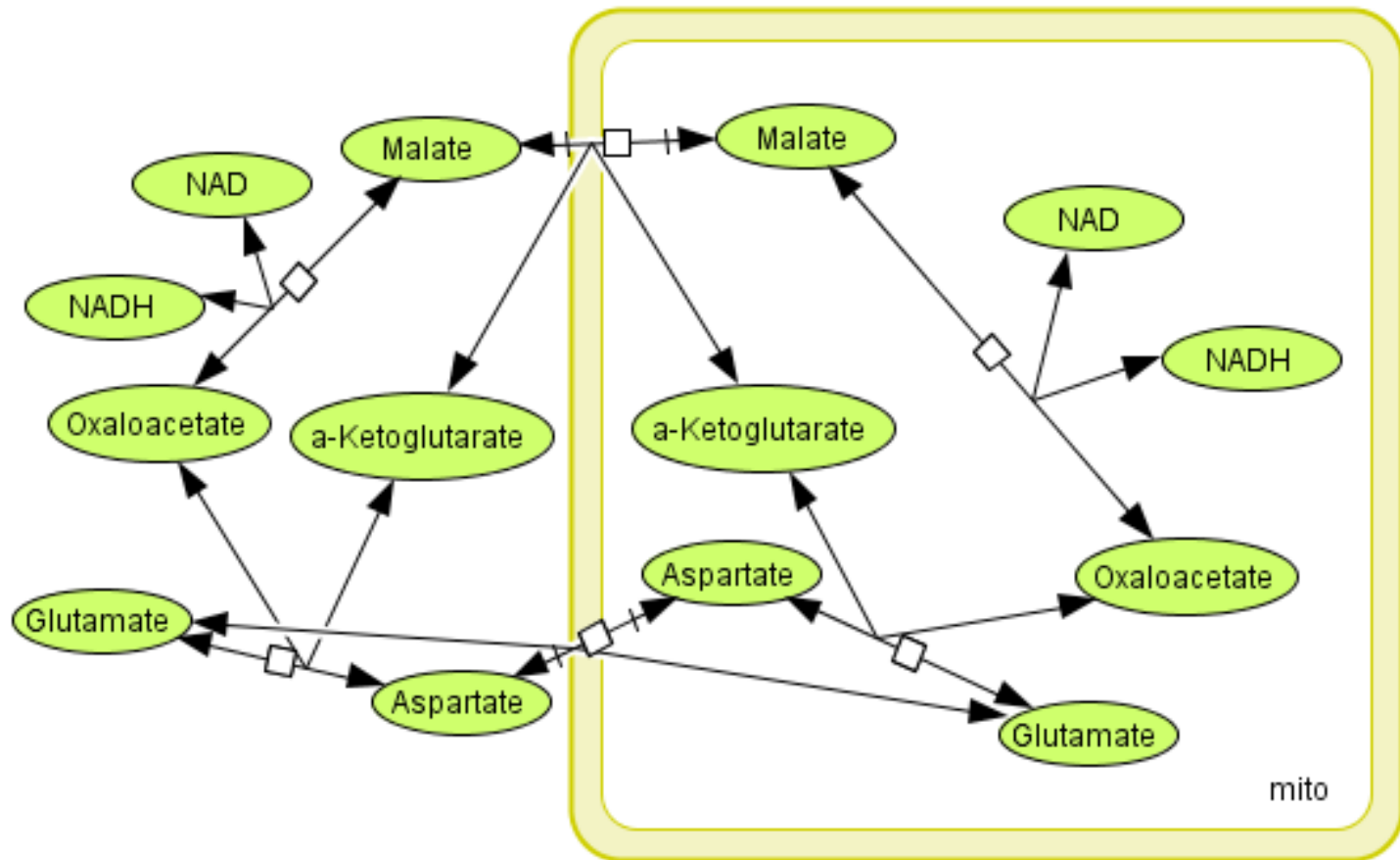
First modul: citric acid cycle



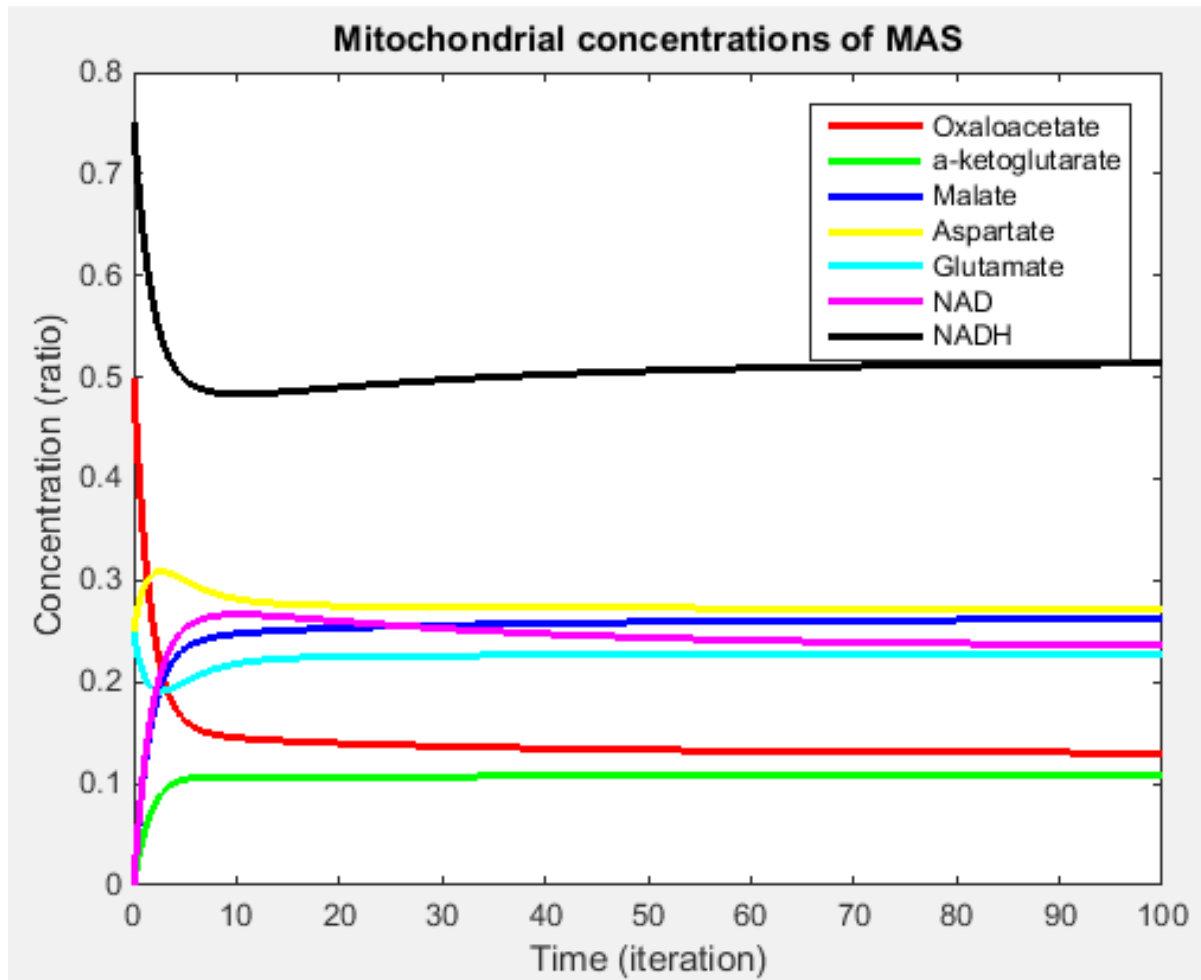
Second modul: malate-aspartate shuttle

- 4 reversible reactions and 2 transporters (12 differential equations)
- 14 differential variables: $[\alpha\text{KG}_m]$, $[\text{Mal}_m]$, $[\text{OA}_m]$, $[\text{Asp}_m]$, $[\text{Glu}_m]$, $[\text{NAD}^+_m]$, $[\text{NADH}_m]$, $[\alpha\text{KG}_c]$, $[\text{Mal}_c]$, $[\text{OA}_c]$, $[\text{Asp}_c]$, $[\text{Glu}_c]$, $[\text{NAD}^+_c]$, $[\text{NADH}_c]$
- Inputs: $[\text{NADH}_c]$, $[\text{NAD}^+_m]$
- Outputs: $[\text{NADH}_m]$, $[\text{NAD}^+_c]$

Second modul: malate-aspartate shuttle



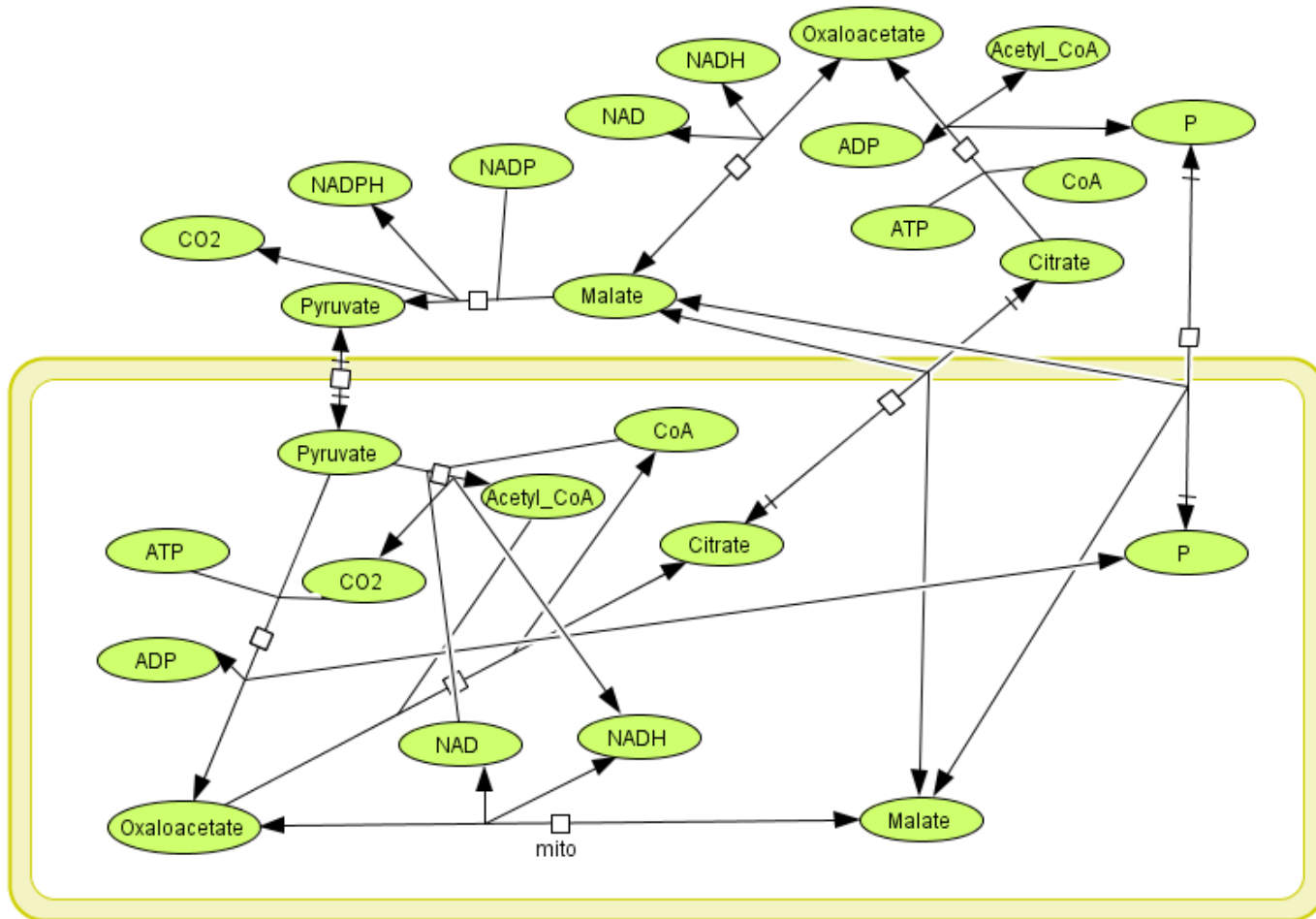
Second modul: malate-aspartate shuttle



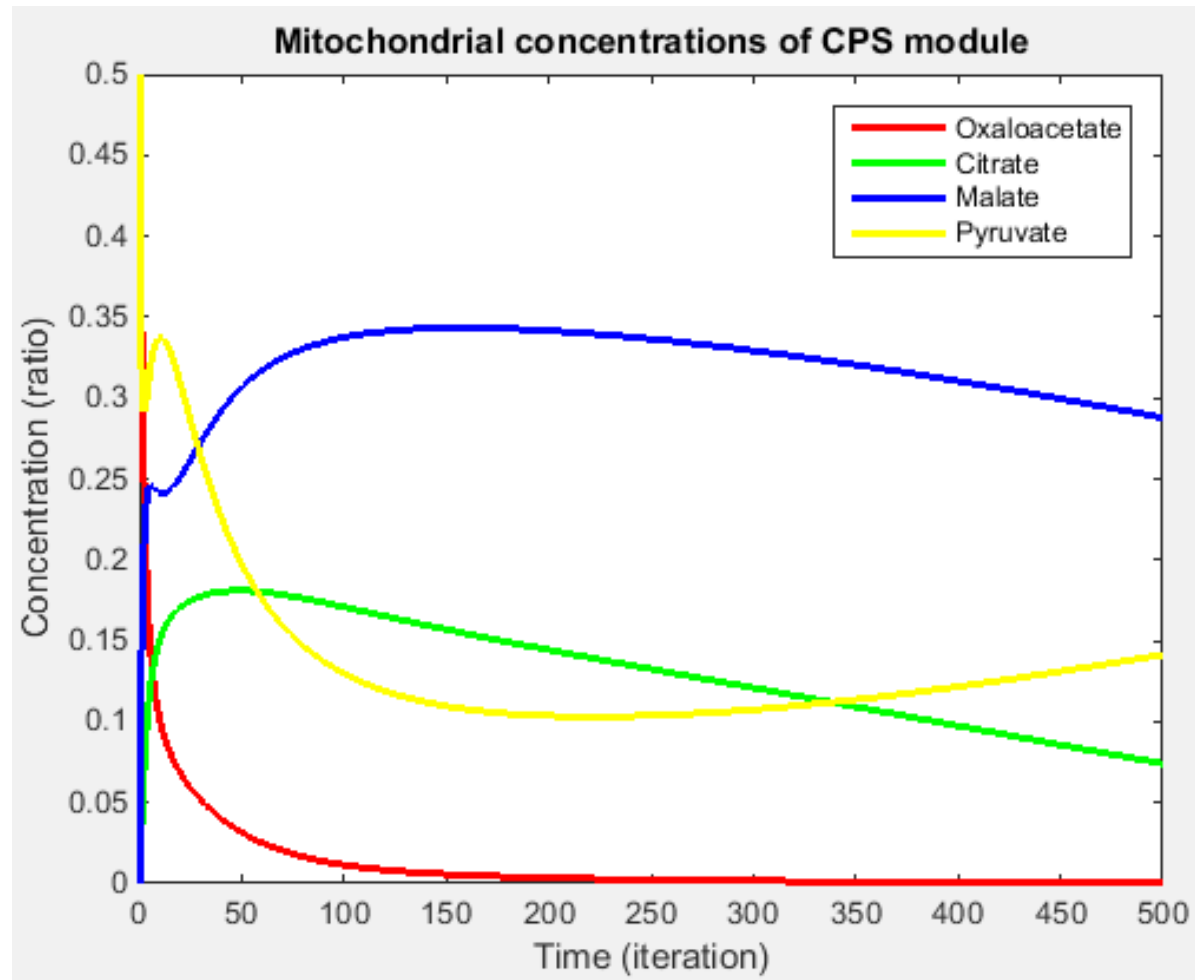
Third modul: citrate-pyruvate shuttle

- 5 irreversible and 2 reversible reactions (9 differential equations)
- 3 transporters (6 differential equations)
- 26 differential variables: $[AcCoA_m]$, $[CoA_m]$, $[Cit_m]$, $[Mal_m]$, $[OA_m]$, $[Pyr_m]$, $[NAD^+_m]$, $[NADH_m]$, $[ADP_m]$, $[ATP_m]$, $[P_m]$, $[CO_{2,m}]$, $[AcCoA_c]$, $[CoA_c]$, $[Cit_c]$, $[Mal_c]$, $[OA_c]$, $[Pyr_c]$, $[NAD^+_c]$, $[NADH_c]$, $[NADP^+_c]$, $[NADPH_c]$, $[ADP_c]$, $[ATP_c]$, $[P_c]$, $[CO_{2,c}]$
- Inputs: $[ATP_m]$, $[CoA_c]$, $[NADH_c]$, $[NADP^+_c]$, $[ATP_c]$
- Outputs: $[ADP_m]$, $[P_m]$, $[AcCoA_c]$, $[NAD^+_c]$, $[NADPH_c]$, $[ADP_c]$, $[P_c]$, $[CO_{2,c}]$

Third modul: citrate-pyruvate shuttle



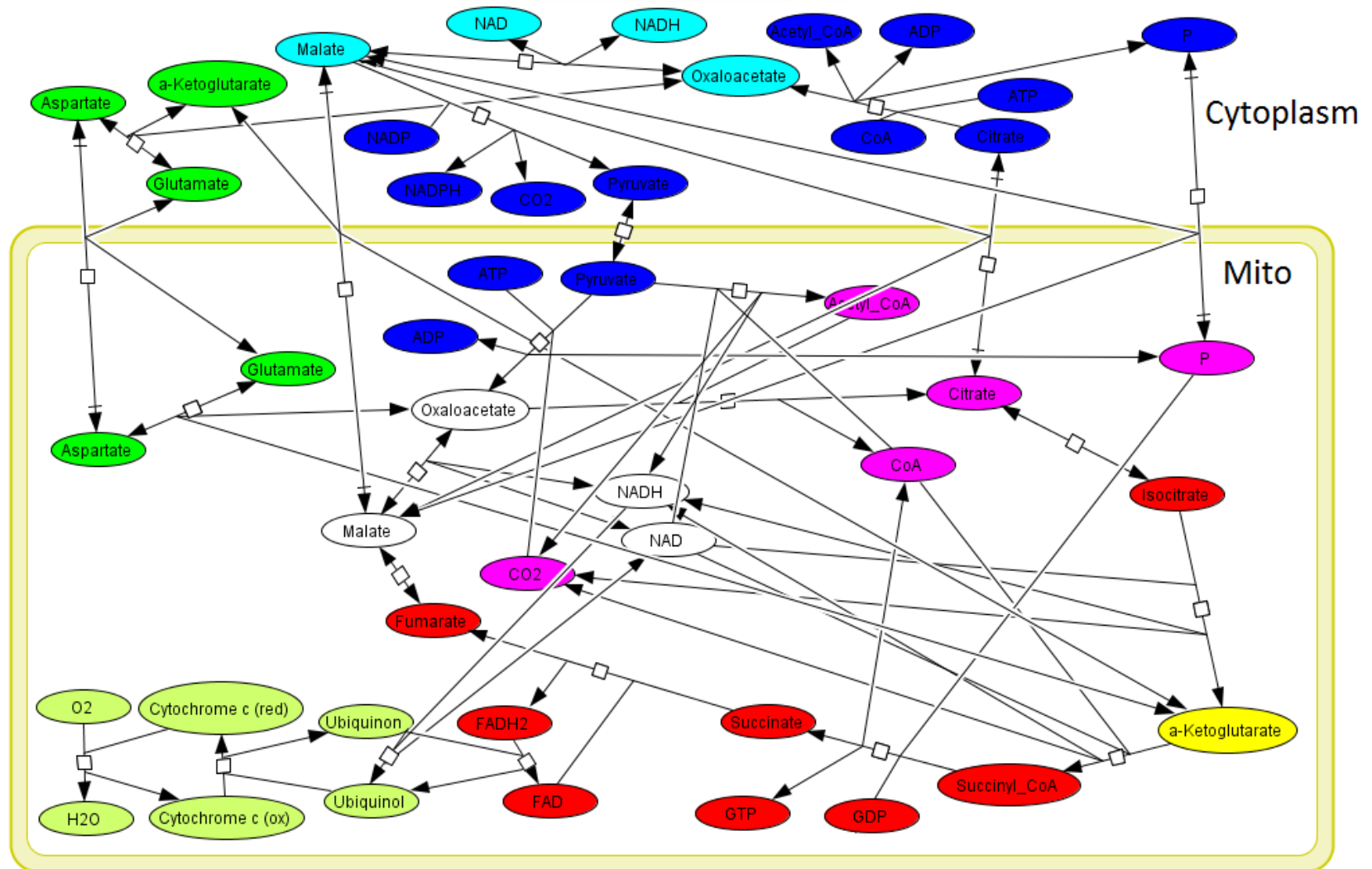
Third modul: citrate-pyruvate shuttle



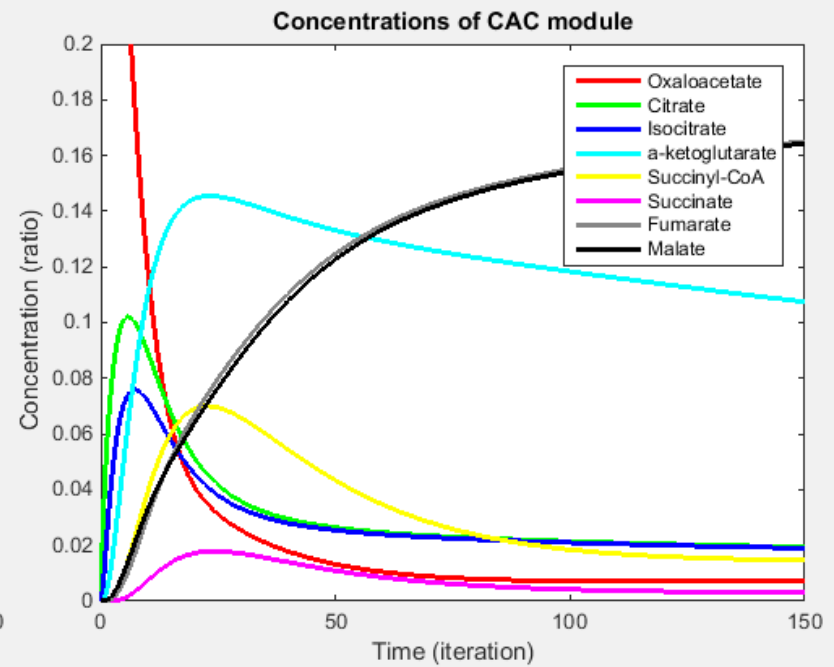
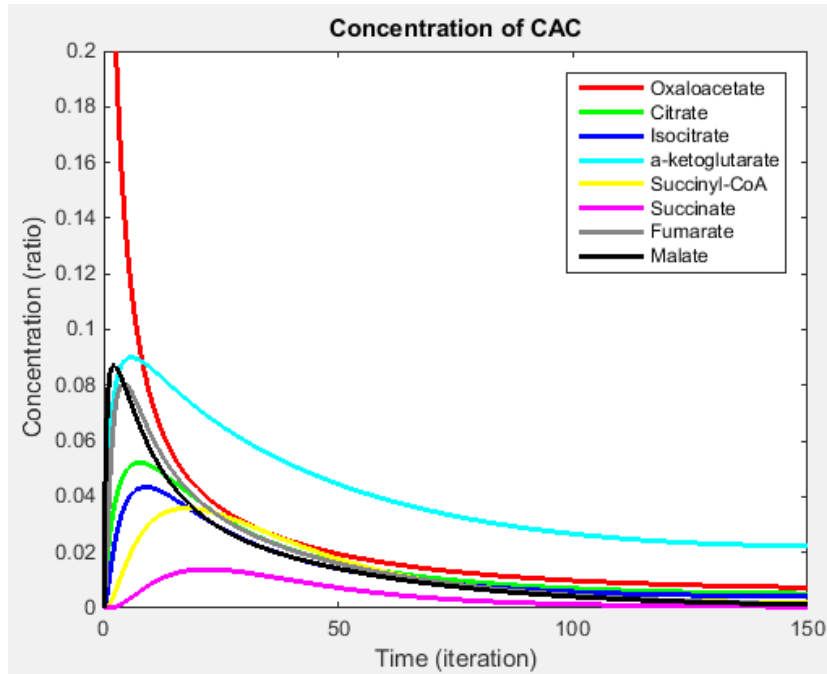
Integrated model

- 9 irreversible and 6 reversible reactions
- 5 transporters
- 39 differential variables: union of variables from individual modules
- Inputs: $[GDP]$, $[ATP_m]$, $[CoA_c]$, $[NADH_c]$, $[NADP^+_c]$, $[ATP_c]$
- Outputs: $[GTP]$, $[ADP_m]$, $[AcCoA_c]$, $[NAD^+_c]$, $[NADPH_c]$, $[ADP_c]$, $[CO_{2,c}]$

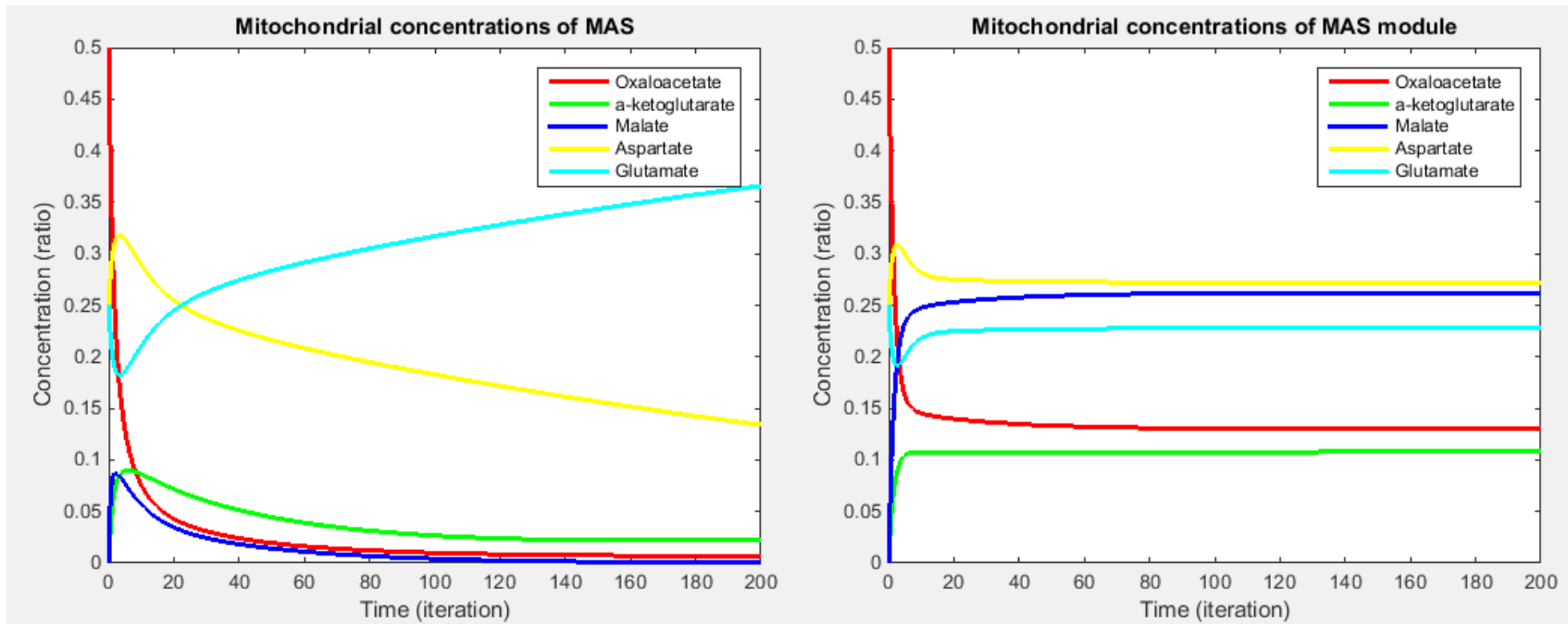
Integrated model reactions



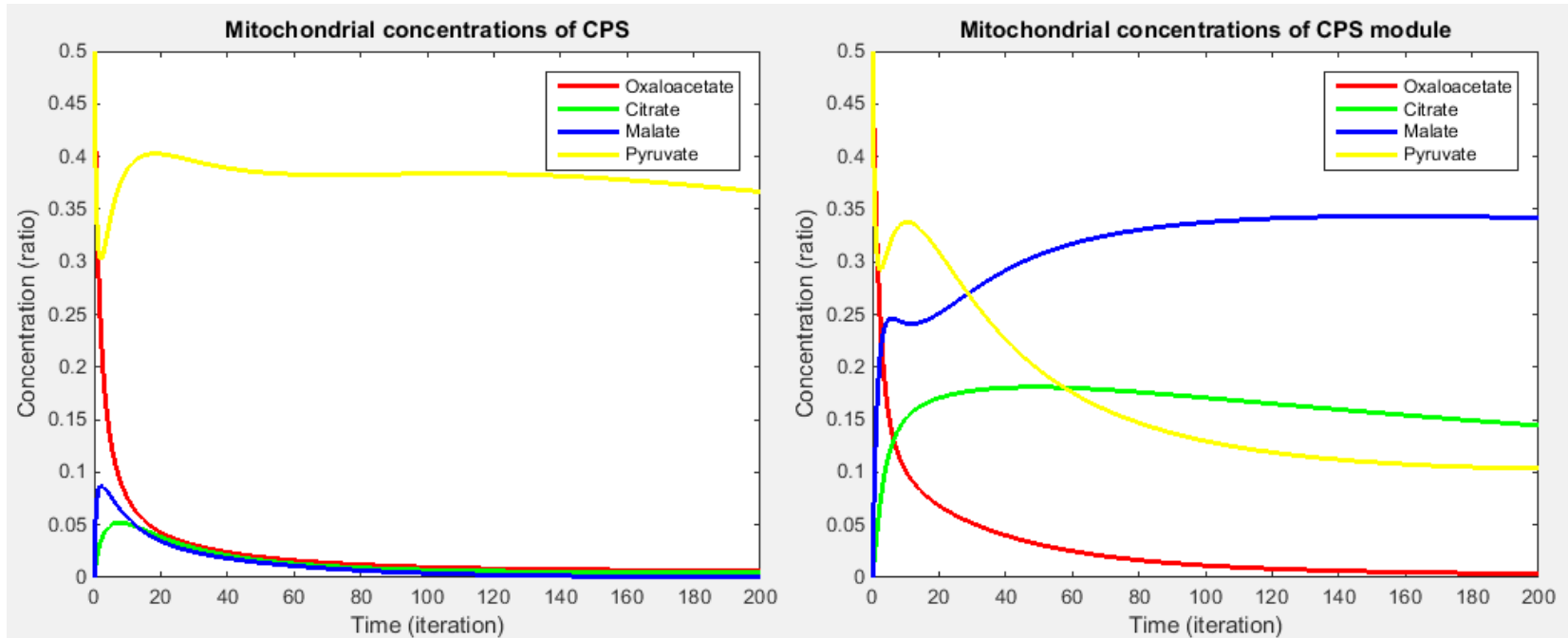
Comparison I: Citric acid cycle



Comparison II: Malate-aspartate shuttle



Comparison III: Citrate-pyruvate shuttle



Simulation results

- Individual **moduls**:
 - Huge inaccuracy were experienced, because we **observe** that isolated systems
 - We can control these systems easily via input molecules
- Integrated model:
 - We can observe the dynamic of full **metabilic** system
 - **Moduls controls** each other via their inputs and outputs
 - In this simplified state the model gives qualitatively acceptable results

Further plans

- Involving more transporters which can make our system more physiological, because the modeling of the connection between the mitochondrion and its environment
- Some substances are usable as inhibitors for enzymes and transporters (K_i), it would be practical to build to the model
- New metabolic pathways that are connected to CAC or its anaplerotic reactions to make our results more accurate
- Measure parameter values of mitochondrial enzymes at similar conditions (K_m and V_{max}), because in the literature we did not find coherent **datas**
- Simulate modified functions and equilibrium states of CAC which are caused by one or more mutant enzymes or transporters. If we change the parameters of enzymes or transporters with these parameters we can study mitochondria which have enzyme or transporter mutations

Acknowledgement

Dr. László Tretter, professor
Semmelweis University,
Department of Medical Biochemistry

Dr. Gábor Szederkényi, professor
Pázmány Péter Catholic University
Faculty of Information Technology and Bionics

THANK YOU FOR YOUR ATTENTION!

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Other models of citric acid cycles I.

- Wu F et al (2007), Computer modeling of mitochondrial tricarboxylic acid cycle, oxidative phosphorylation, metabolite transport, and electrophysiology
- Nazaret C et al (2009), Mitochondrial energetic metabolism: a simplified model of TCA cycle with ATP production

Other models of citric acid cycles II.

- Korla K (2013), Modelling the Krebs cycle and oxidative phosphorylation
- Korla K (2015), Kinetic simulation of malate-aspartate and citrate-pyruvate shuttles in association with Krebs cycle

PRESENTATION HEADING

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Modeling the citric acid cycle, malate-aspartate and citrate-pyruvate shuttles

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19/04/2018

SLIDE HEADING

- **Bullet Point**

Lorem ipsum dolor sit amet,
consectetur adipiscing elit.
Curabitur nec nisi vestibulum,
interdum leo vitae, consequat
ligula. Mauris ultrices elit vitae
metus pellentesque, sit amet
vulputate nisl commodo.

- **Bullet Point 2**

*Quat ligula. Mauris ultrices elit
vitae metus pellentesque*

- **Bullet Point 3**

QUAT LIGULA. MAURIS
ULTRICES ELIT VITAE
METUS PELLENTESQUE
Mauris ultrices elit vitae metus
pellentesque, sit amet