VERIFICATION OF ROBUSTNESS PROPERTY IN CHEMICAL REACTION NETWORKS

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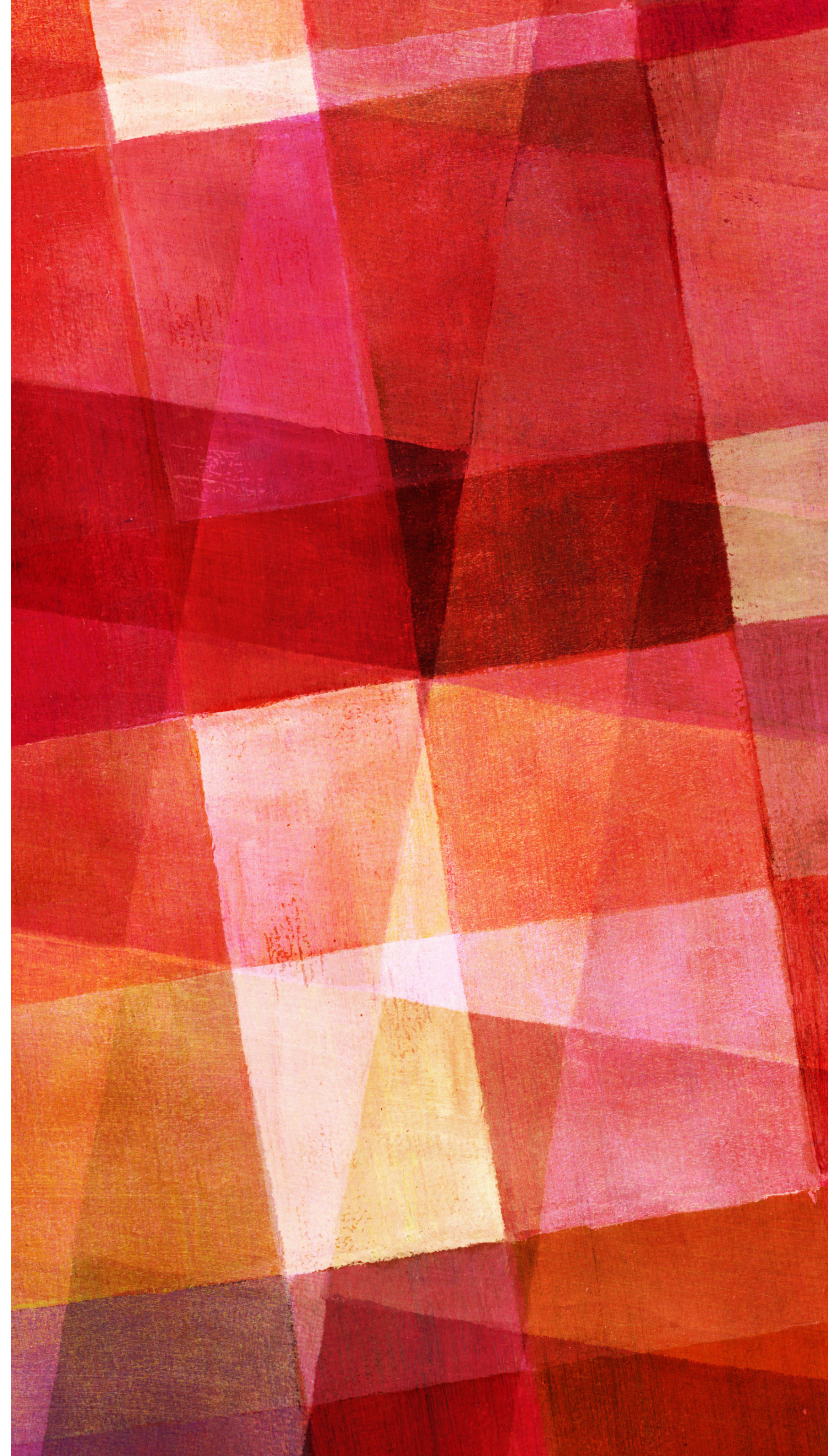


HELLO!

- Nov 2016 — Started PhD
- Nov 2018 — INRIA
- Mar 2019 — MPI
- Oct 2019 — PhD thesis submission

Modelling, Simulation and Verification of Biological Systems Group

SUPERVISED BY: ROBERTA GORI AND PAOLO MILAZZO

PUBLICATIONS

Publications presented in the thesis:

- R. Gori, P. Milazzo and L. Nasti *Towards an Efficient Verification Method for Monotonicity Properties of Chemical Reaction Networks.* (BIOINFORMATICS 2019).
- L. Nasti, R. Gori and P. Milazzo, *Formalizing a Notion of Concentration Robustness for Biochemical Networks*. STAF Workshops 2018: 81-97
- R. Gori, P. Milazzo and L. Nasti and F. Poloni, *Efficient analysis of Chemical Reaction Networks Dynamics based on Input-Output monotonicity* (Submitted).
- L. Nasti and C. Zechner. *Verification and analysis of Robustness in Becker-Döring equations* (in preparation).

Other publications:

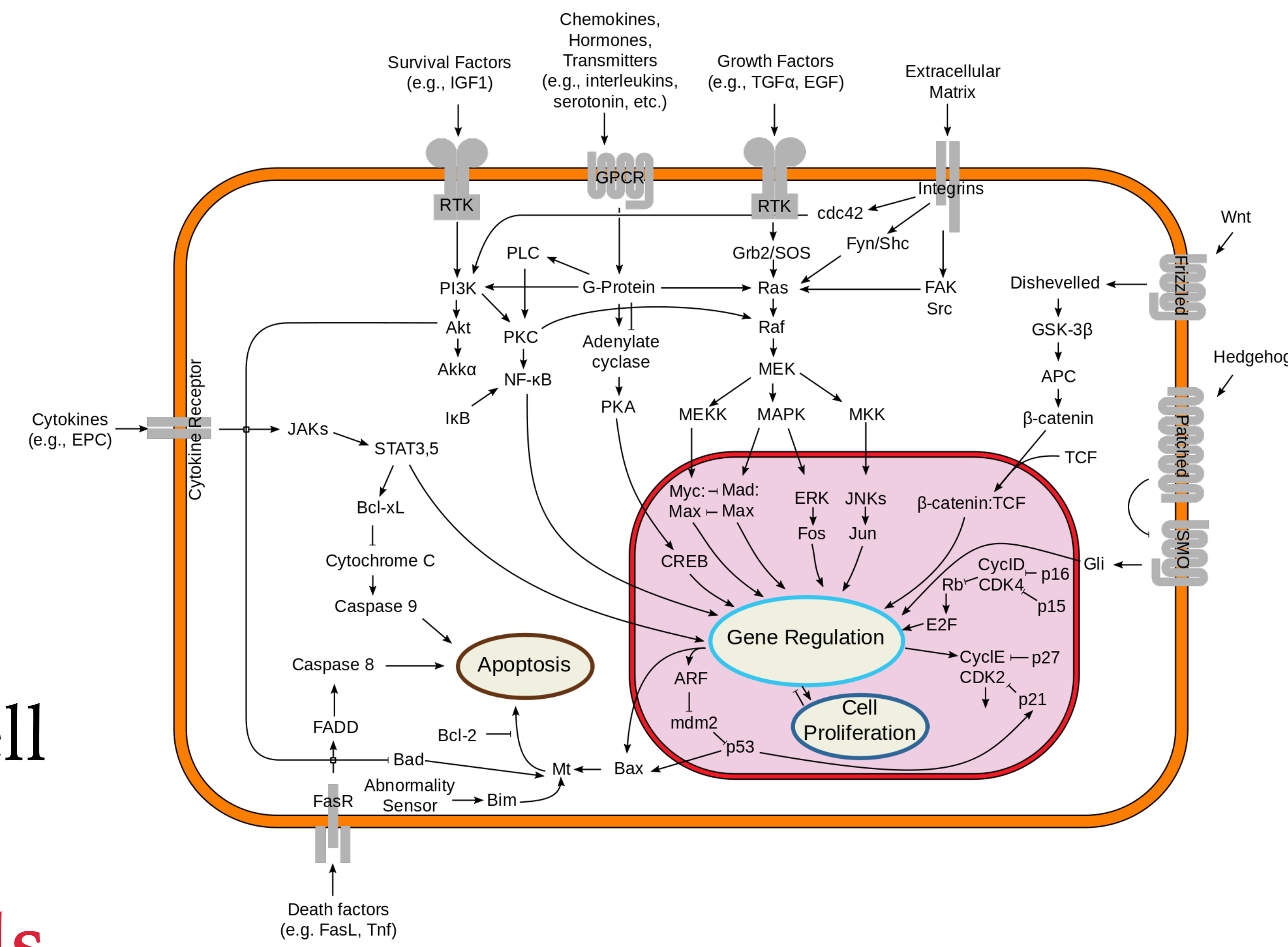
- L. Nasti and P. Milazzo, *A computational model of internet addiction phenomena in social networks*. International Conference on Software Engineering and Formal Methods, 86-100.
- L. Nasti and P. Milazzo, *A Hybrid Automata model of social networking addiction.* Journal of Logical and Algebraic Methods in Programming. Volume 100, November 2018, Pages 215-229.
- R. Gori, P. Milazzo and L. Nasti, *A survey of gene regulatory networks modelling methods: from ODEs, to Boolean and bio-inspired models* (Submitted).

OUTLINE

- What is **robustness**?
- Formalisation: **CRN** and **Petri Nets**
- Why and how to study **monotonicity** in CRN?
- Results: **Sufficient conditions** and **Tools**
- Applications: **Becker-Döring equations**
- Future work

BACKGROUND

- A cell is a **very complex system**
- Chemical reaction networks (***pathways***) govern the basic cell's activities
- To examine the structure of the cell as a whole, we can design **multiscale and predictive models**



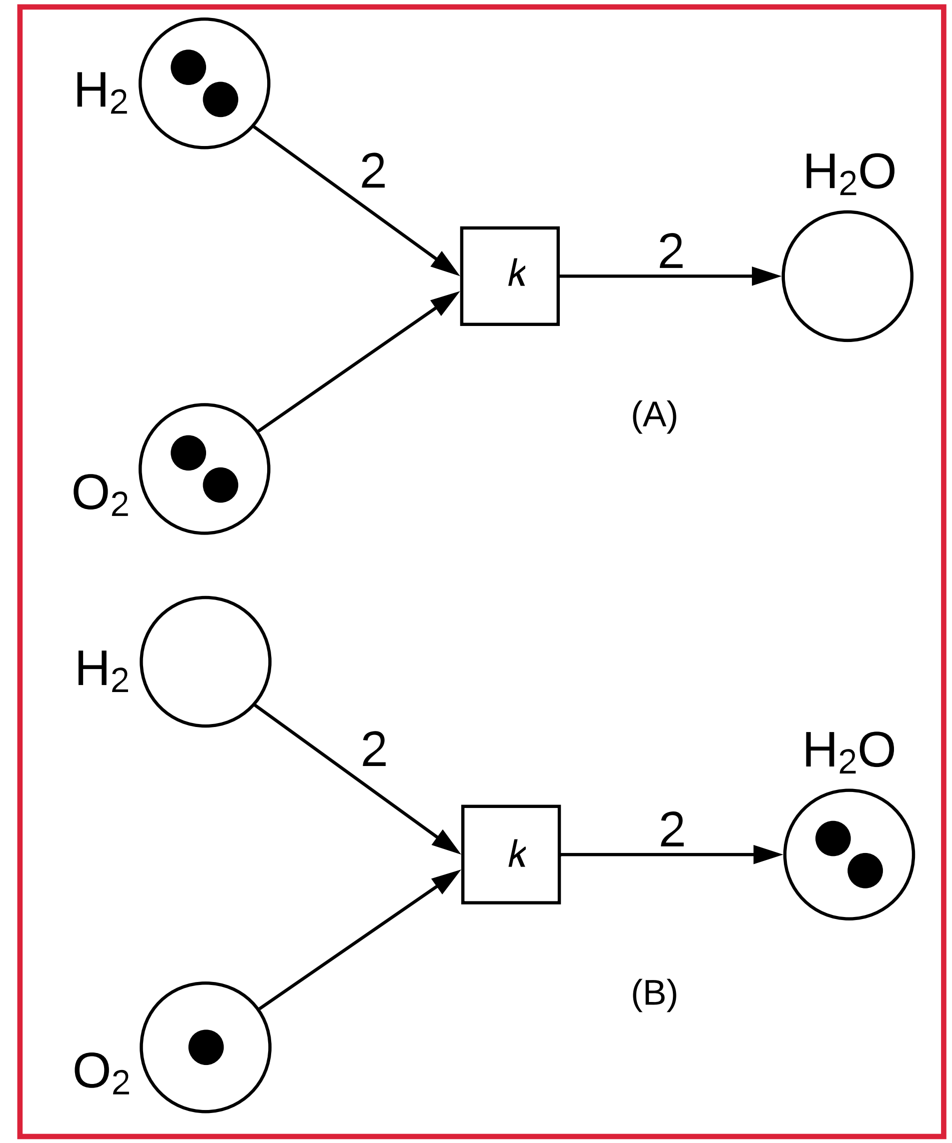
CHEMICAL REACTIONS

Stoichiometric coefficient

$$aA + bB \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} cC + dD$$

Reactants — Rate — Products

- **Kinetic rate:** rate of a reaction
- **Reactant:** chemical species that is consumed
- **Product:** chemical species that is created
- **Stoichiometric coefficient:** the number of species involved in the reaction
- **Concentrations:** [A], [B], [C], [D]



$$2\,H_2 + O_2 \xrightarrow{k} 2\,H_2O$$

CHEMICAL KINETICS

$$aA + bB \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} cC + dD$$

➤ **Law of mass action:** reaction rate is proportional to the reactants product

$$r_1 = k_1 [A]^a [B]^b$$

$$r_2 = k_{-1} [C]^c [D]^d$$

$$\frac{d[A]}{dt} = \overbrace{-ak_1[A]^a[B]^b}^{\text{direct reaction term}} \overbrace{+ak_{-1}[C]^c[D]^d}^{\text{inverse reaction term}}$$

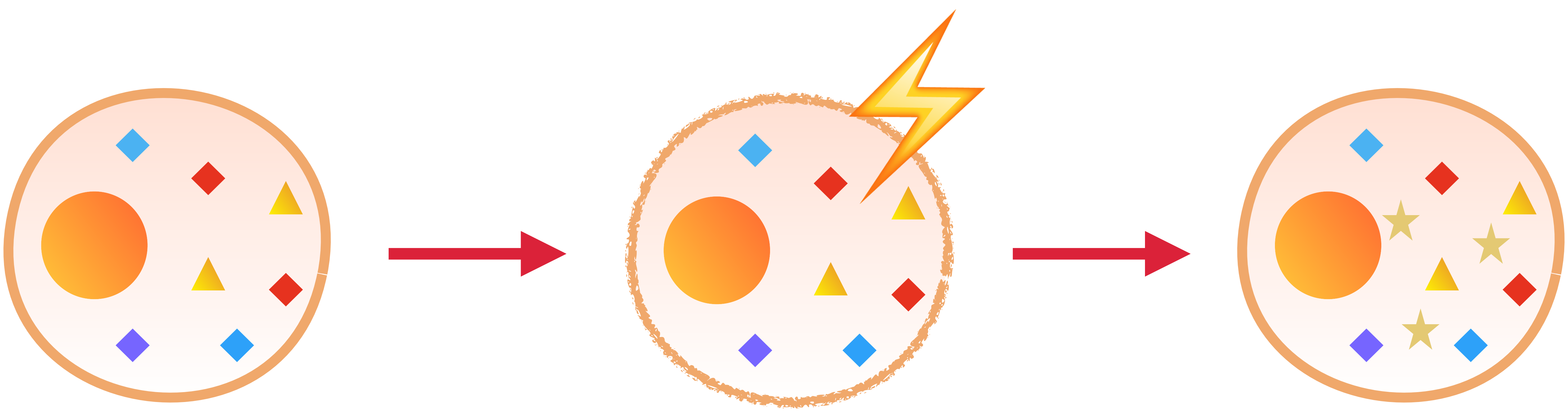
$$\frac{d[B]}{dt} = -bk_1[A]^a[B]^b + bk_{-1}[C]^c[D]^d$$

$$\frac{d[C]}{dt} = +ck_1[A]^a[B]^b - ck_{-1}[C]^c[D]^d$$

$$\frac{d[D]}{dt} = +dk_1[A]^a[B]^b - dk_{-1}[C]^c[D]^d.$$

ROBUSTNESS PROPERTY

- **Robustness:** A fundamental feature of complex evolving systems, for which the behaviour of the system remains essentially constant, despite the presence of internal and external perturbations.



ROBUSTNESS IN LITERATURE

- In [Kitano, 2007]:

Robustness is the ability of a system to maintain specific functionalities against perturbations.

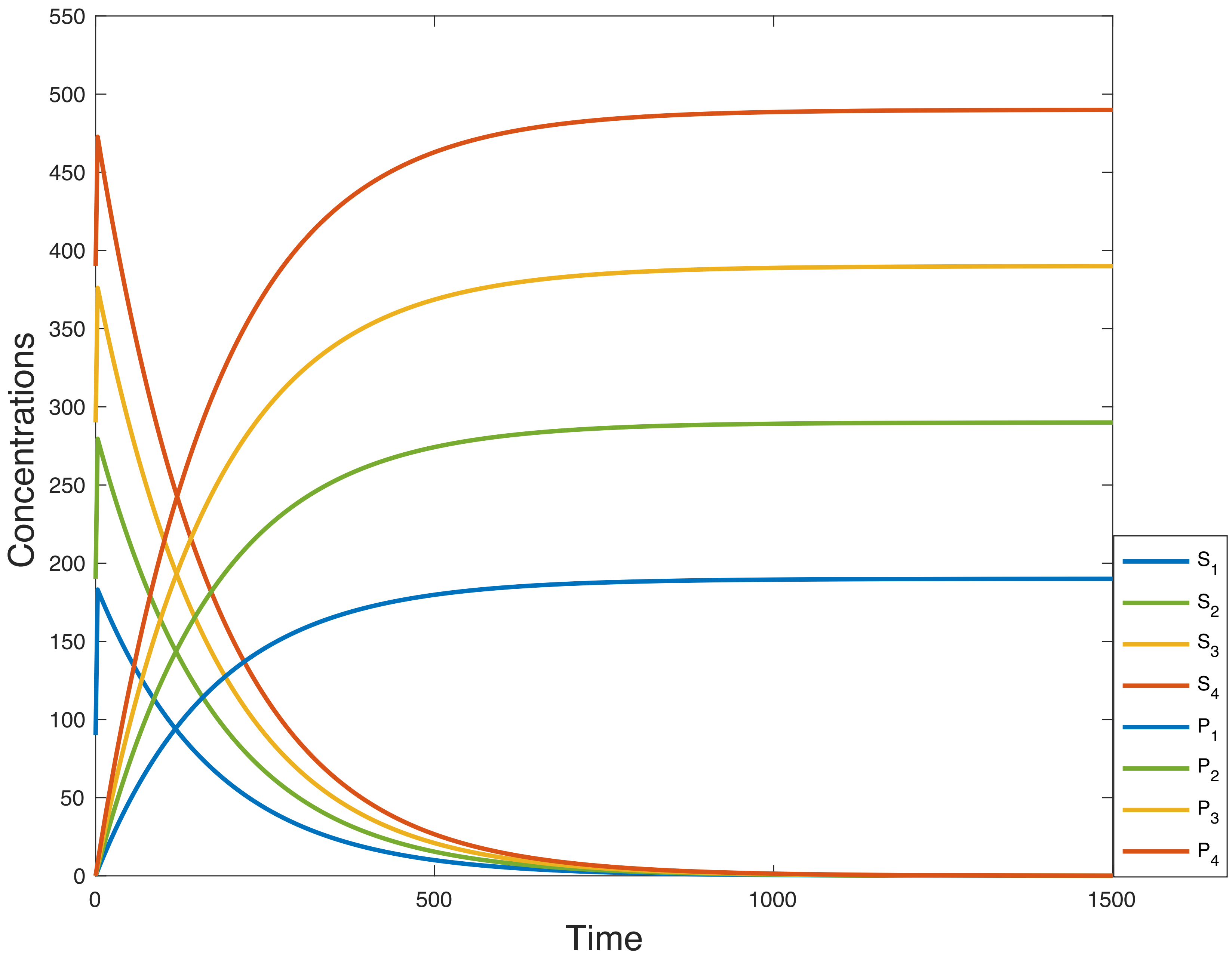
- In [Rizk *et al.*, 2008]:

The robustness of a system is measured as the *distance* of the system behaviour under perturbations from its reference behaviour expressed as temporal logic formula.

OUR PROPOSED WORK

- **New formal definition** of robustness, namely initial concentration robustness
- Our new definition is able to analyse **all the chemical species** involved in the CRN
- Our new robustness notion can be proved by performing **simulations**

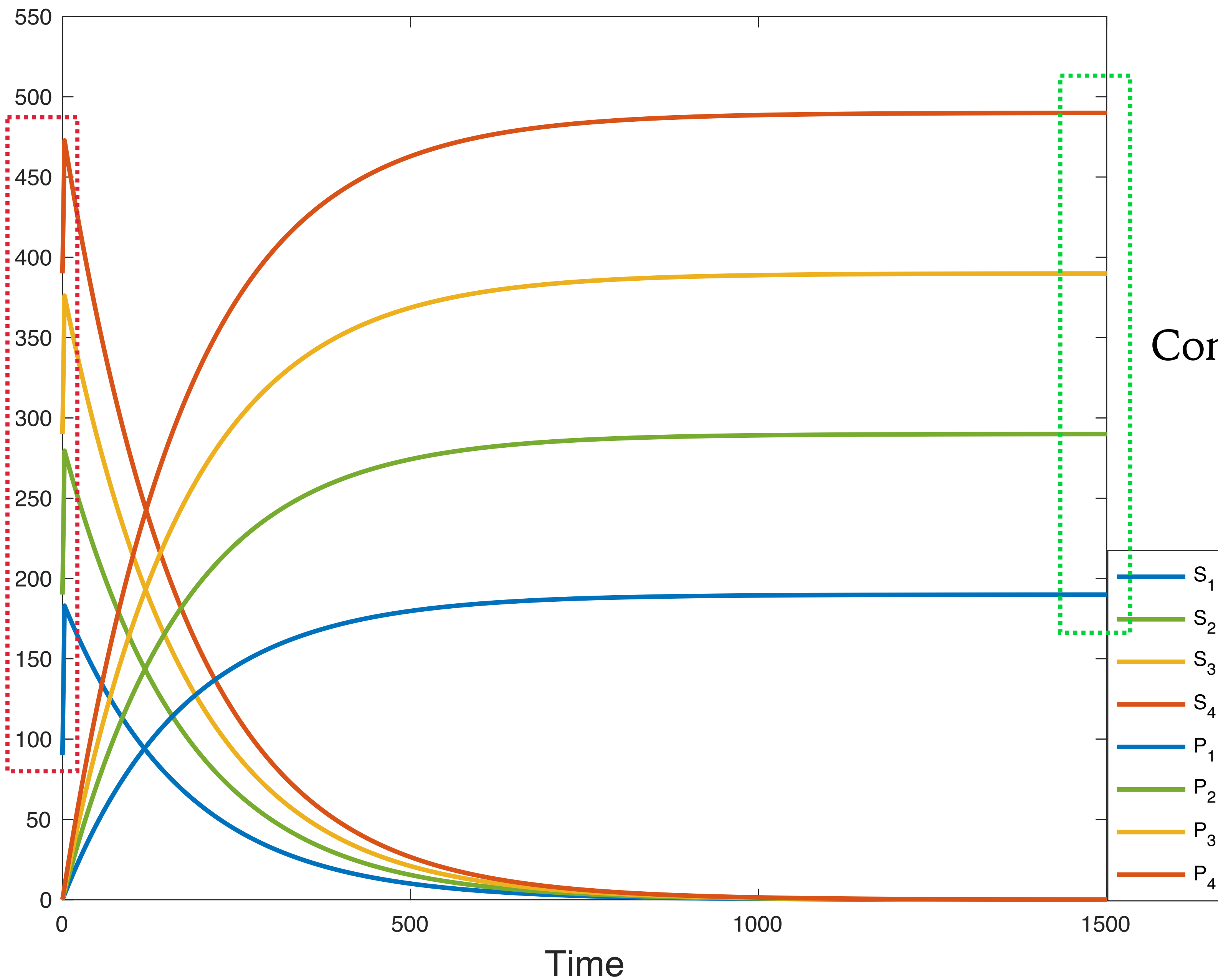
INITIAL CONCENTRATION ROBUSTNESS



$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

INITIAL CONCENTRATION ROBUSTNESS

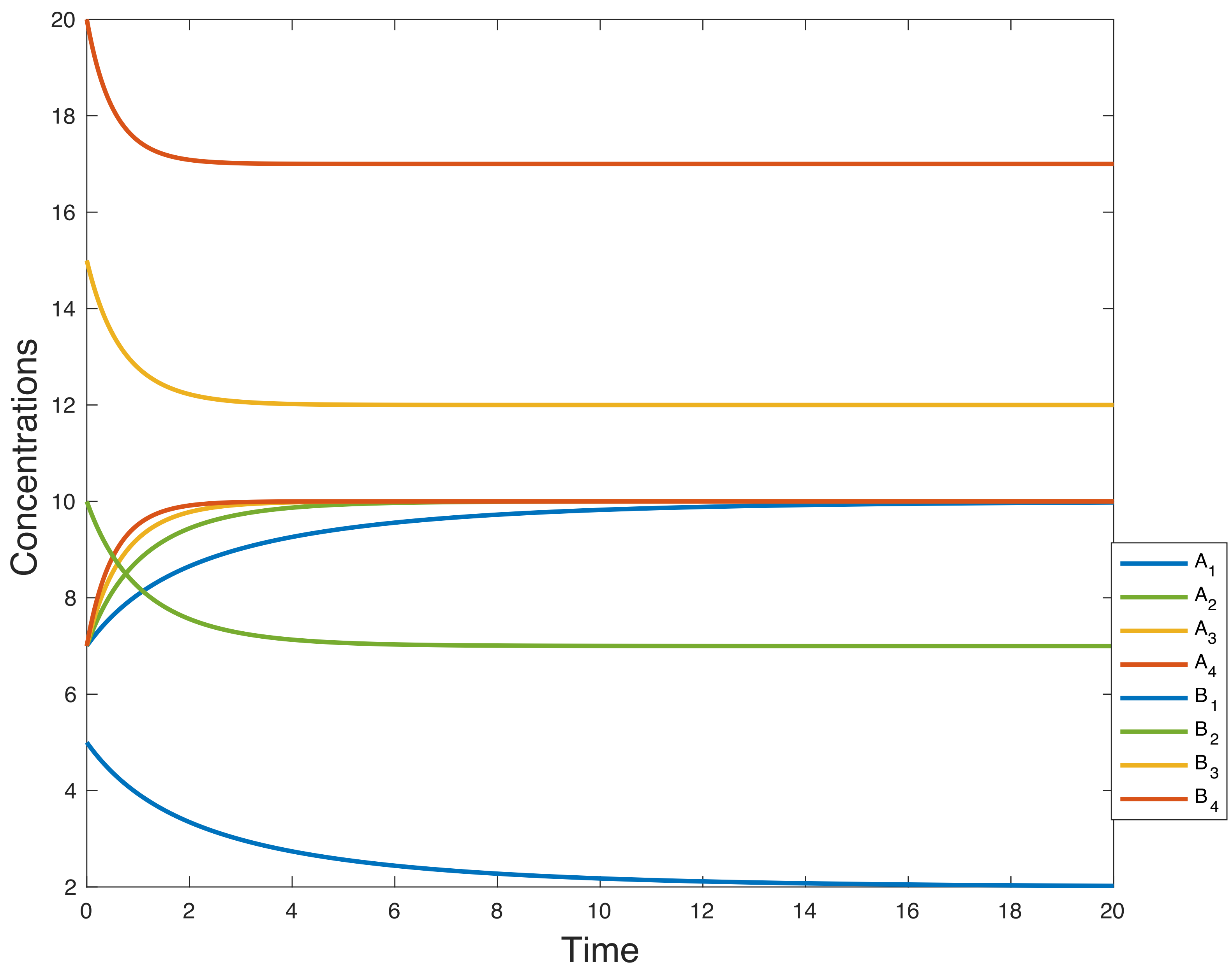
Initial concentrations [S]



Concentrations of [P] at steady state

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

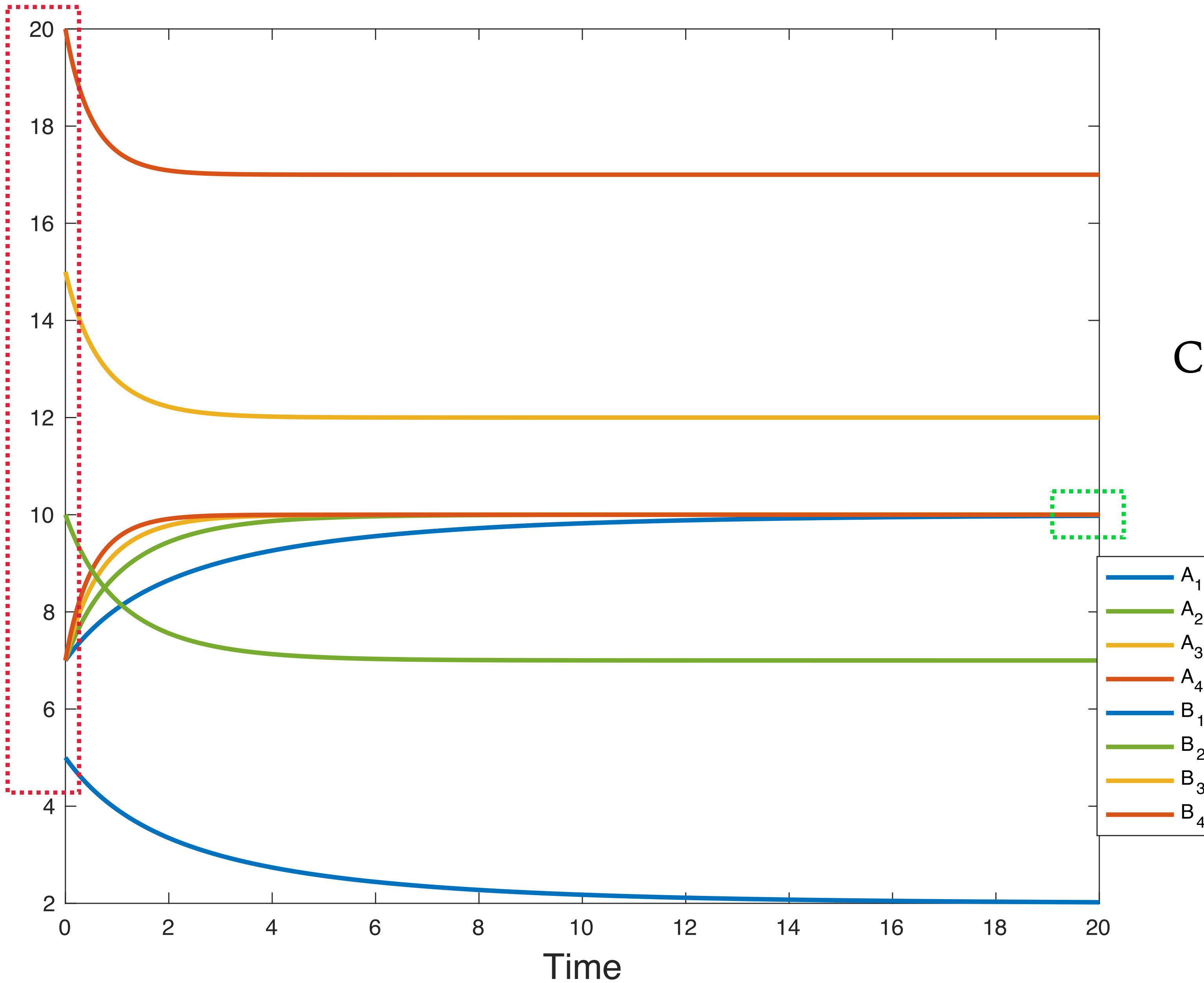
INITIAL CONCENTRATION ROBUSTNESS



$$A + B \xrightarrow{k_1} 2B \quad B \xrightarrow{k_2} A$$

INITIAL CONCENTRATION ROBUSTNESS

Initial concentrations [B]



Concentrations of [A] at steady state

$$A + \boxed{B} \xrightarrow{k_1} 2B \quad B \xrightarrow{k_2} \boxed{A}$$

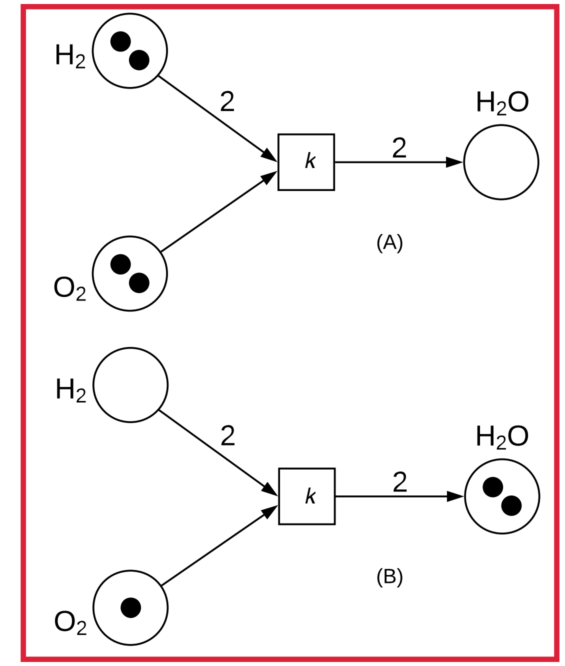
CONTINUOUS PETRI NETS FORMALISM DEFINITION

A continuous Petri net N is a quintuple:

$$N = \langle P, T, F, C, m_0 \rangle$$

where:

- P is the set of continuous *places*, conceptually species
- T is the set of continuous *transitions*, that consume and produce species
- $F \subseteq (P \times T) \cup (T \times P) \rightarrow \mathbb{R}_{\geq 0}$ represents the set of arcs in terms of a function giving the weight of the arc as result: a weight equal to 0 means that the arc is not present
- C : $F \rightarrow \mathbb{R}_{\geq 0}$ is a function, which associates each transition with a *rate*
- m_0 is the *initial marking*, that is the initial distribution of *tokens* (representing resource instances) among places. A marking is defined formally as $m : P \rightarrow \mathbb{R}_{\geq 0}$



$$2\,H_2 + O_2 \xrightarrow{k} 2\,H_2O$$

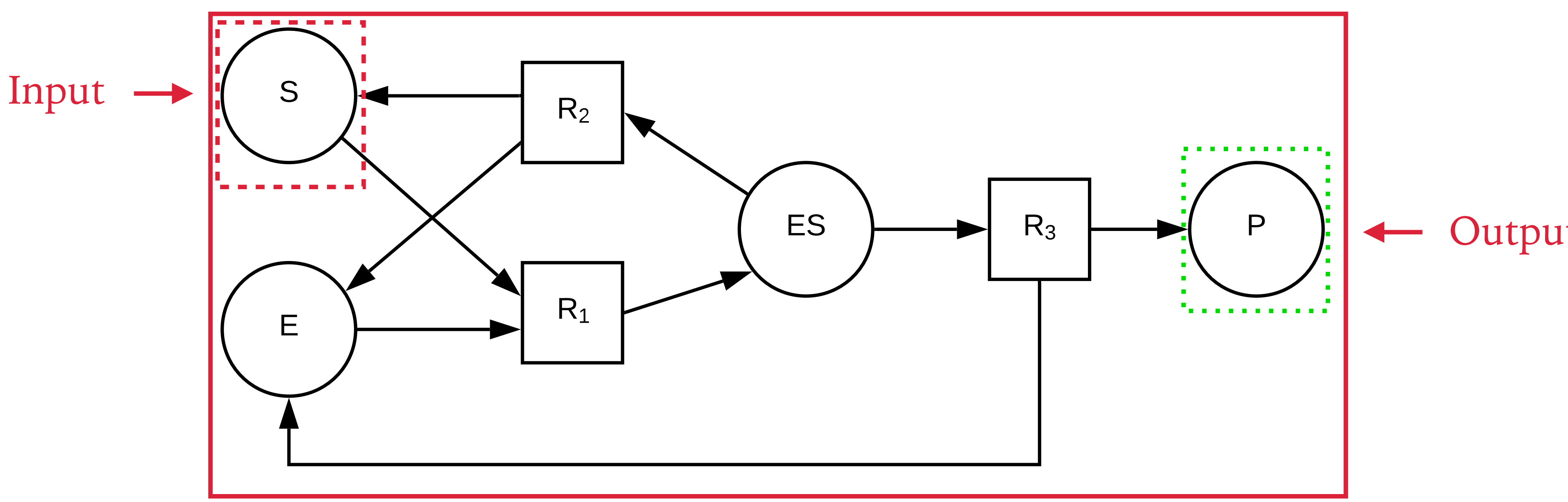
FORMAL DEFINITION OF ROBUSTNESS: AUXILIARIES CONCEPTS

- **Definition 1 (Intervals)**. We define the interval domain

$$\mathcal{I} = \{[n, m] \mid n, m \in \mathbb{R}_{\geq 0} \cup \{+\infty\} \text{ and } n \leq m\}$$

Moreover we say that $x \in [n, m] \quad iff \quad n \leq x \leq m.$

- **Definition 2 (Interval marking).** An interval marking is a function $m_{[\,]} : P \to I$. We call $M_{[\,]}$ the domain of all interval markings.



$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

MY NEW FORMAL DEFINITION OF ABSOLUTE ROBUSTNESS

- **Definition 3 (α-Robustness)**. A Petri net PN with output place *O* is defined as *a-robust* with respect to a given marking $m_{[]}$ iff $\exists\, k \in \mathbb{R}$ such that $\forall\, m \in m_{[]}$, the marking m'_{ss} corresponding to the steady state reachable from *m*, is such that

$$m'_{ss}(O) \in \left[k - \frac{\alpha}{2}, k + \frac{\alpha}{2},\right]$$

Observations:

- the wider are the intervals of the initial interval marking, the more robust is the network
- the smaller is the value of α, the more robust is the network

EXAMPLE OF APPLICATION OF ABSOLUTE ROBUSTNESS: TOY MODEL

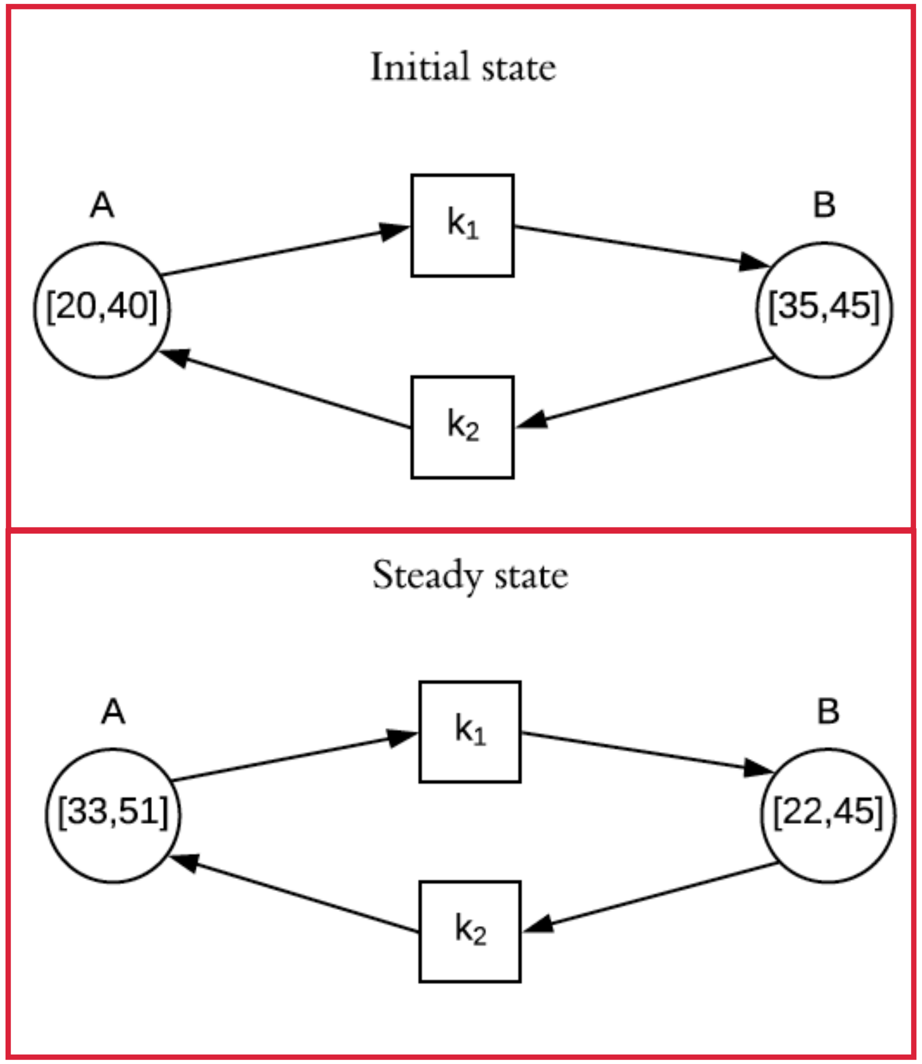
Given a set of chemical reactions:

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$$

Applying our definition:

- with A as output we obtain:
  m'(A) = [33, 51] → α=18

- with B as output we obtain:
  m'(B) = [22, 45] → α=23



FORMAL DEFINITION OF RELATIVE ROBUSTNESS

- **Definition 4** **(β-Robustness)**. Given a Petri net PN, with an input place I and output place O. The relative initial concentration robustness is defined as:

$$\frac{n_O}{n_I}$$

where n_O and n_I are respectively the normalized α-robustness and the normalized interval marking of I.

- **Normalized α-robustness:** $\frac{\alpha}{k}$

- **Normalized Interval Marking:** $\frac{max - min}{k}$

EXAMPLE OF APPLICATION OF RELATIVE ROBUSTNESS : TOY MODEL

Considering A as input and B as output.

Normalized α-robustness:

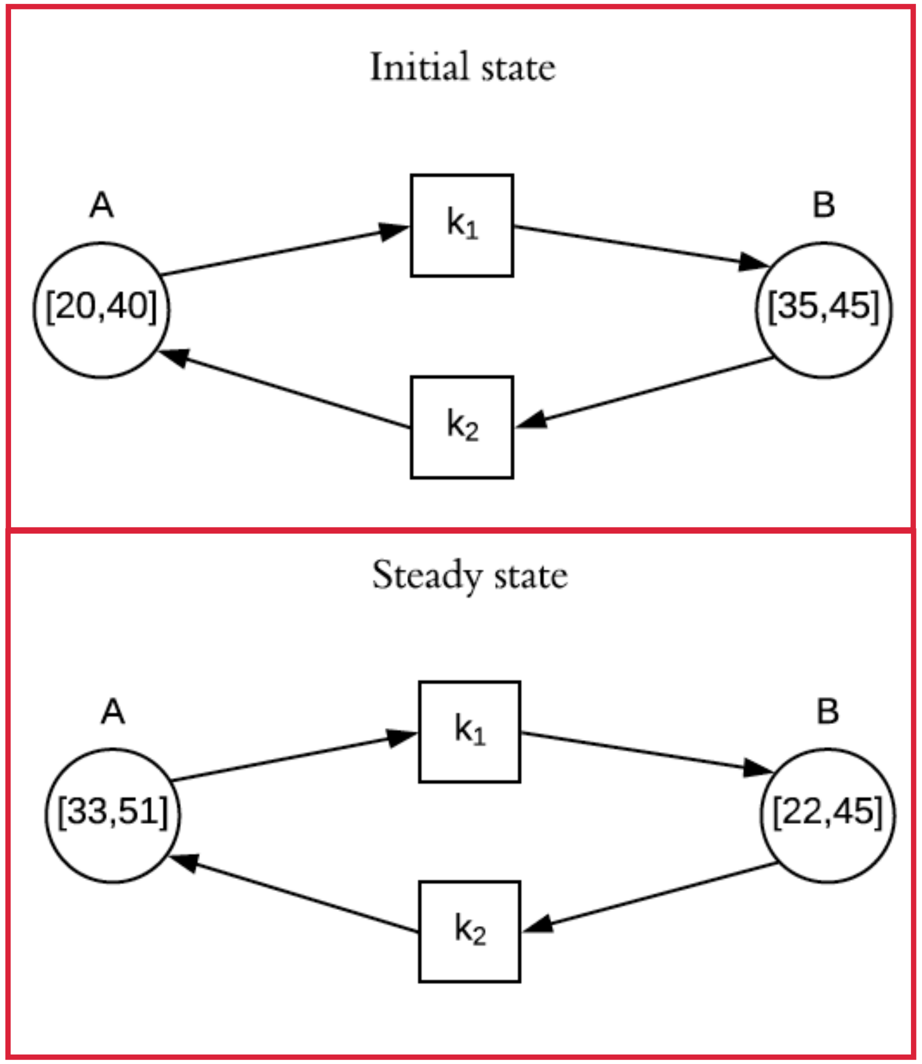
$$n_O = \frac{\alpha}{k} = \frac{23}{33.5} = 0.68$$

Normalized Interval Marking:

$$n_I = \frac{m_I}{k} = \frac{20}{30} = 0.66$$

Relative β-robustness:

$$\beta - robustness = \frac{n_O}{n_I} = \frac{0.68}{0.66} = 1.03$$

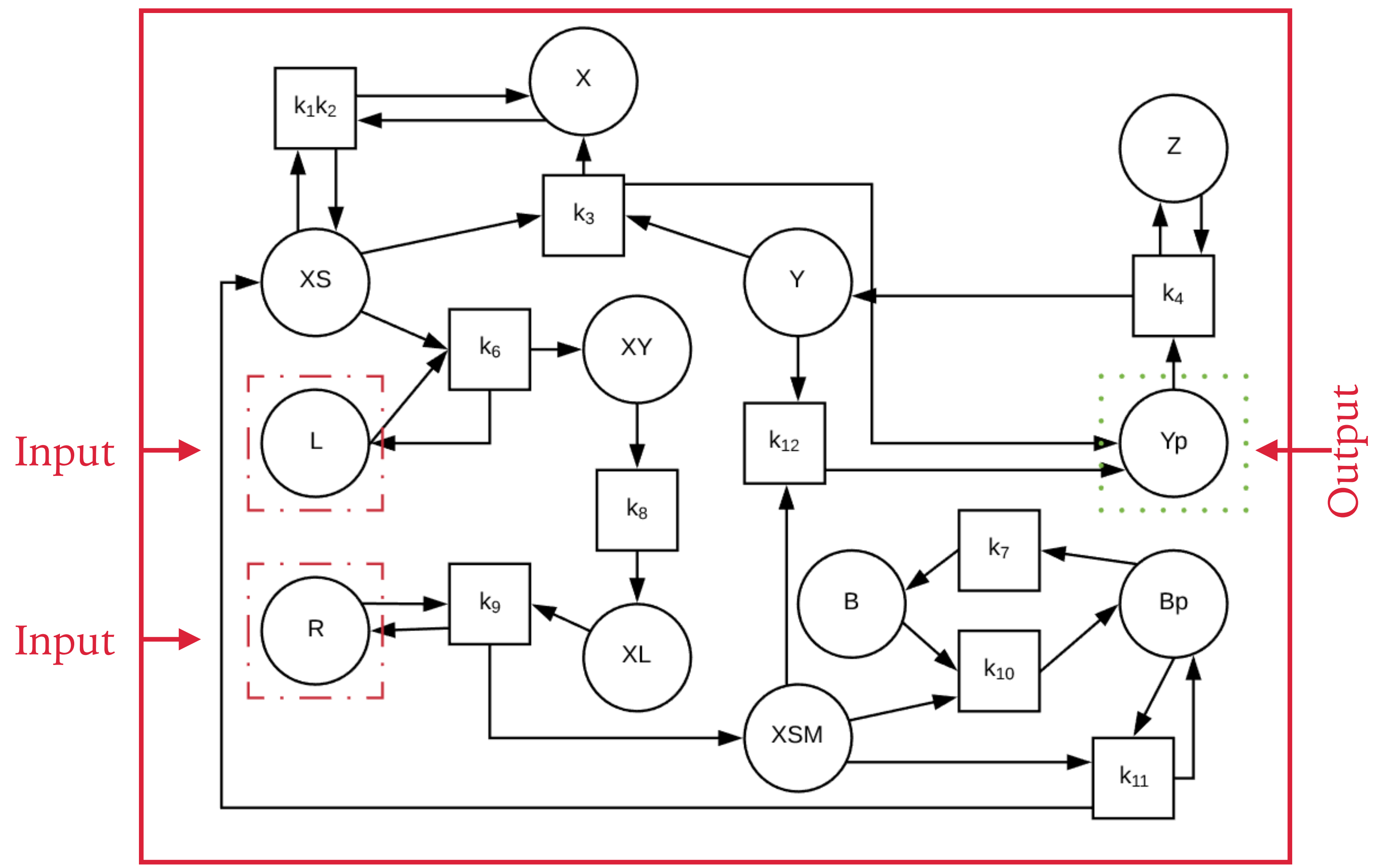


EXAMPLE OF APPLICATION OF OUR DEFINITION : CHEMOTAXIS OF E. COLI

- Given a set of reactions:

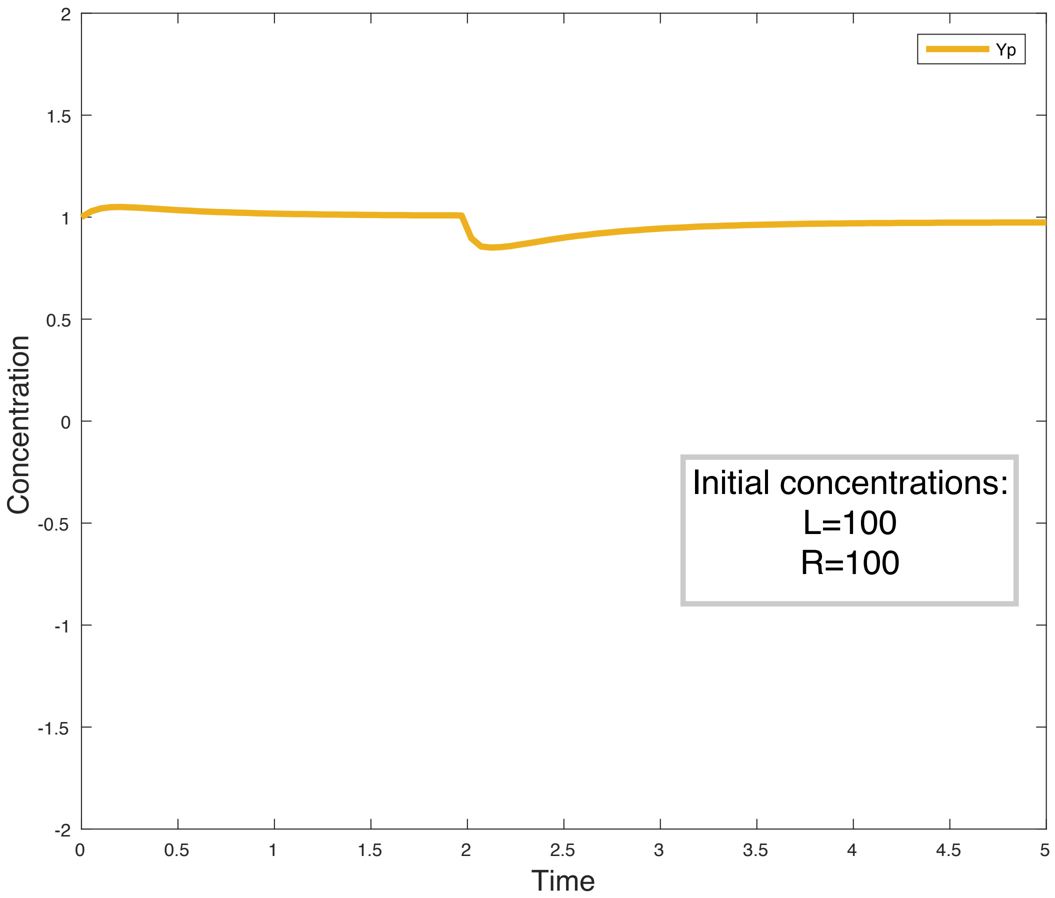
$$X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} XS$$
$$XS + Y \xrightarrow{k_3} Yp + X$$
$$Yp + Z \xrightarrow{k_4} Y + Z$$
$$Yp \xrightarrow{k_5} Y$$
$$L + XS \xrightarrow{k_6} L + XY$$
$$BP \xrightarrow{k_7} B$$
$$XY \xrightarrow{k_8} XL$$
$$R + XL \xrightarrow{k_9} XSM + R$$
$$XSM + B \xrightarrow{k_{11}} Bp + XSM$$
$$XSM + Bp \xrightarrow{k_{12}} XS + Bp$$
$$XSM + Y \xrightarrow{k_{13}} Yp + X$$

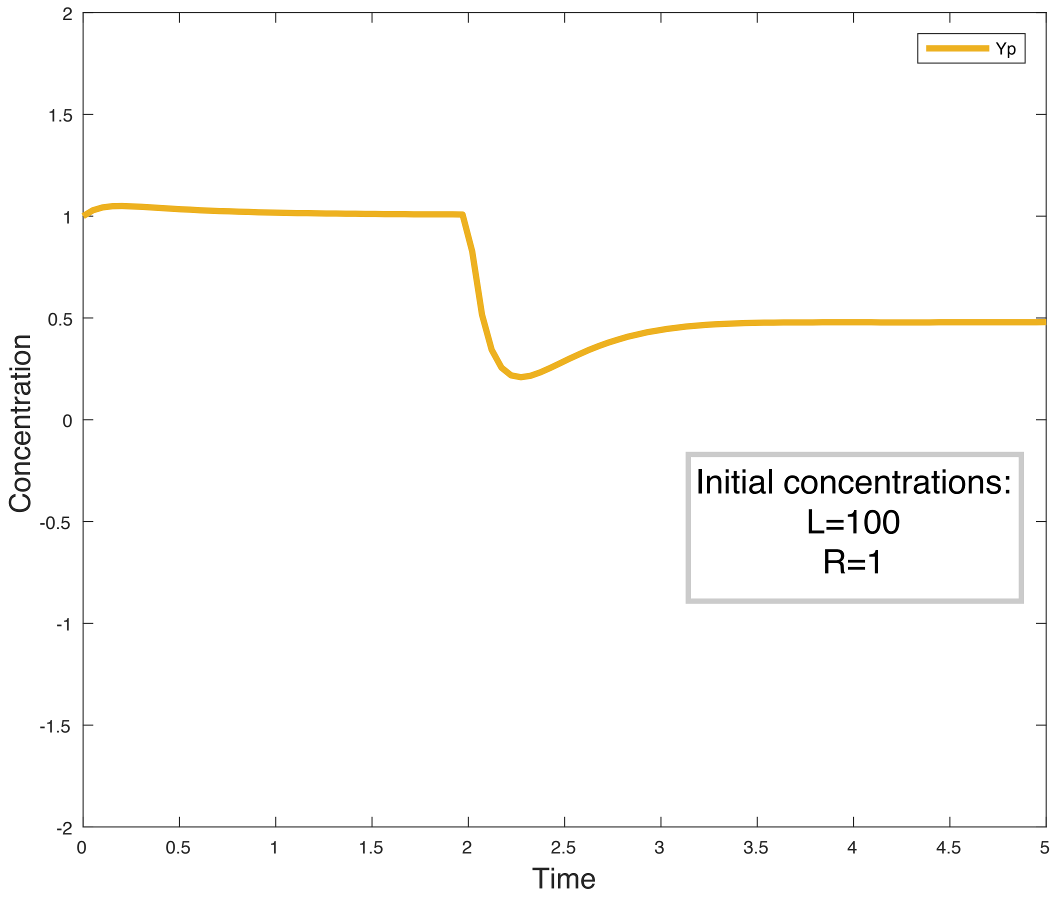
- We build the Petri net:



CHEMOTAXIS OF E.COLI: SIMULATION RESULTS

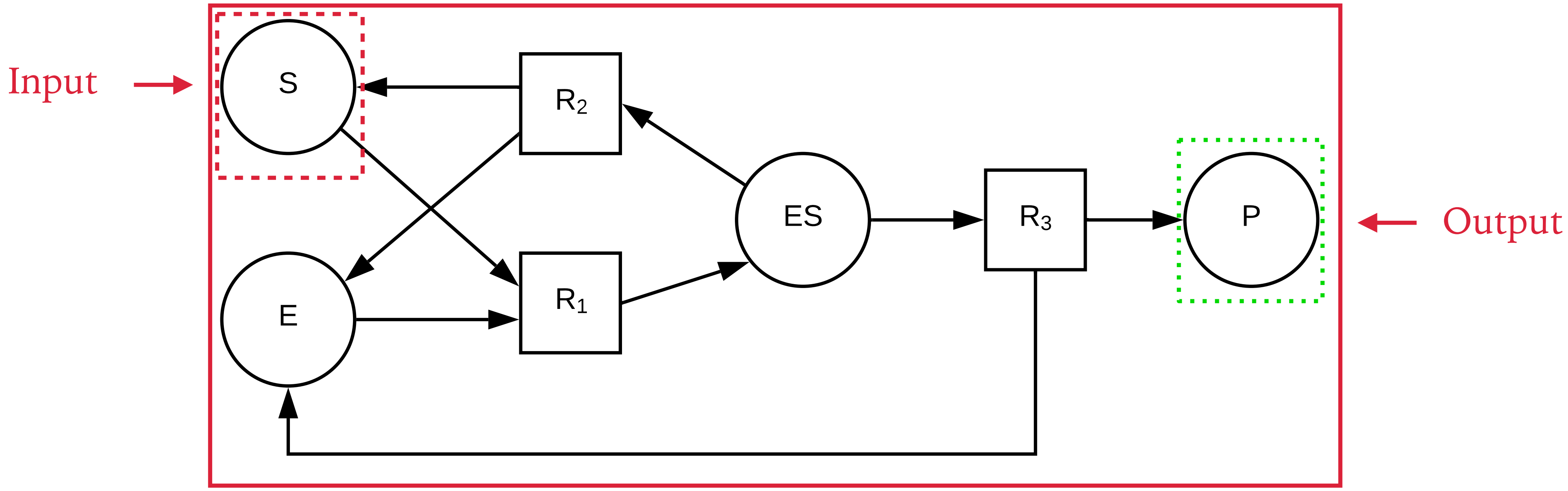
We vary the **initial concentration of the inputs** ([R]) and we obtain these concentrations for the species [Yp]. Hence, we obtain α=0.5 and β=0.35.





TO VERIFY OUR DEFINITION

- Our goal: to verify our definition
- How: experiments by **simulations**
- Example:

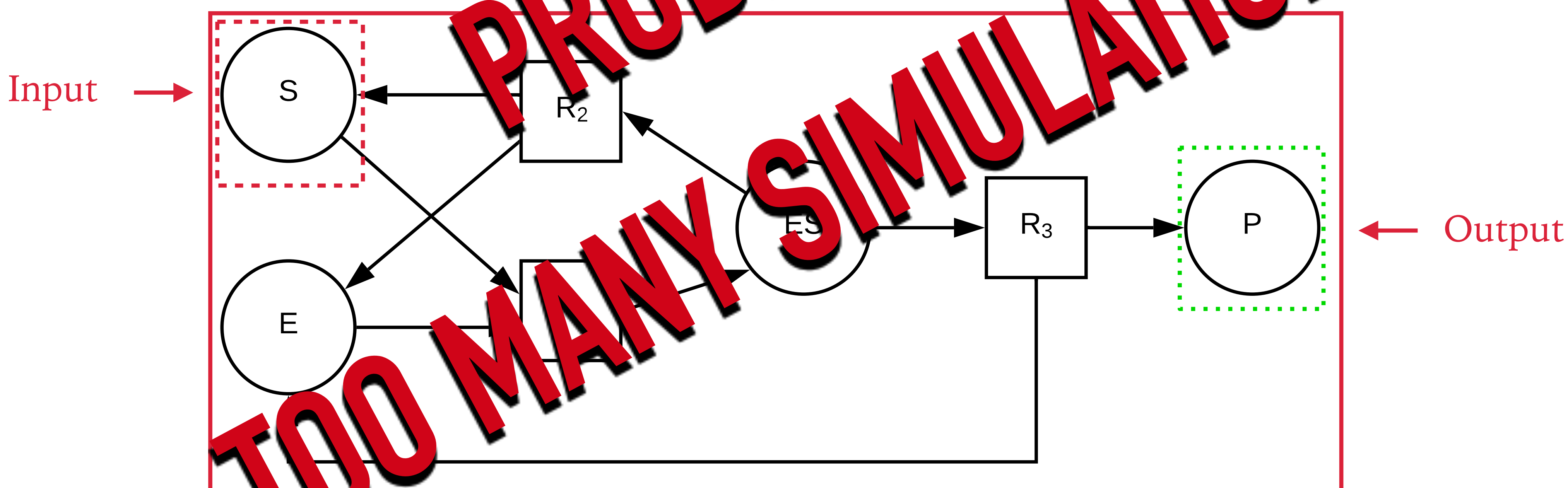


$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

TO VERIFY OUR DEFINITION

- Our goal: to verify our definition
- How: experiments by **simulations**
- Example:

PROBLEM: TOO MANY SIMULATIONS!



$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

HOW TO LIMIT THE COMPUTATIONAL EFFORT OF SIMULATIONS?

HOW TO LIMIT THE COMPUTATIONAL EFFORT OF SIMULATIONS?

MONOTONICITY

MONOTONICITY IN CRN

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

➤ In [Angeli *et al.*, 2008]:

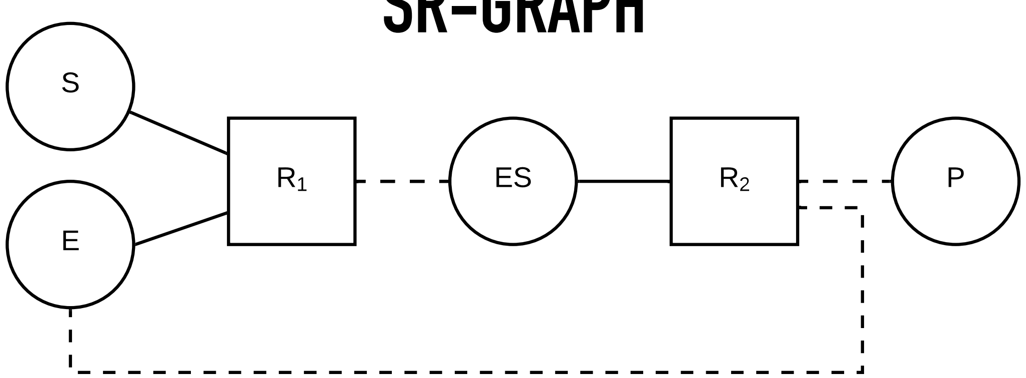
1. Very **strong notion of monotonicity**: each species have to increase or decrease continually

2. This notion of monotonicity work on **particular chemical reaction networks**

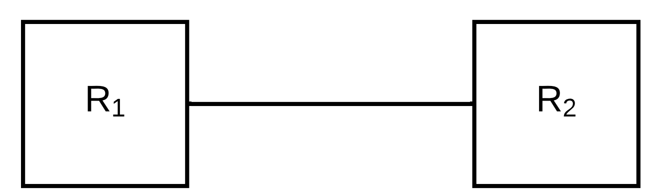
3. To provide graphical conditions to check **global monotonicity:**

The system is **orthant-monotone** if the associated R-graph is **sign consistent**, hence when any loop has an even number of negative edges.

SR-GRAPH



R-GRAPH



MONOTONICITY IN CRN

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

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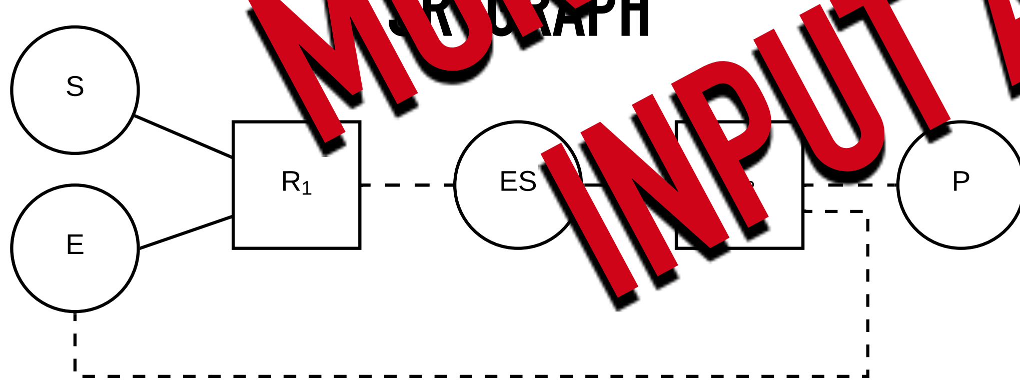
1. Very **strong notion of monotonicity**: each species have to increase or decrease continually
2. This notion of monotonicity work on **particular chemical reaction networks**
3. To provide graphical conditions to check **global monotonicity:**

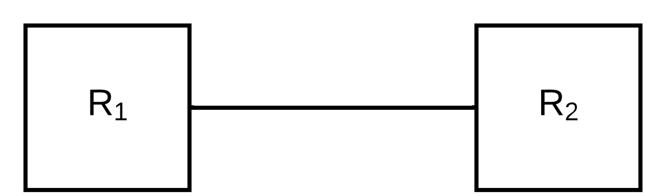
The system is **orthant-monotone** if the associated R-graph is **sign consistent**, hence when any loop has an even number of negative edges.

BUT WE NEED MONOTONICITY BETWEEN INPUT AND OUTPUT!

SR-GRAPH



R-GRAPH



INPUT-OUTPUT MONOTONICITY

- **Positive Input-Output Monotonicity.** Given a set of reactions R, species O is positively monotonic w.r.t $I \in R$ iff, $\forall\ \bar{I} \geq I$, $\bar{O} \geq O$, for every time $t \in \mathbb{R}_{\geq 0}$.

- **Negative Input-Output Monotonicity.** Given a set of reactions R, species O is negatively monotonic w.r.t $I \in R$ iff, $\forall\ \bar{I} \geq I$, $\bar{O} \leq O$, for every time $t \in \mathbb{R}_{\geq 0}$.

INPUT-OUTPUT MONOTONICITY

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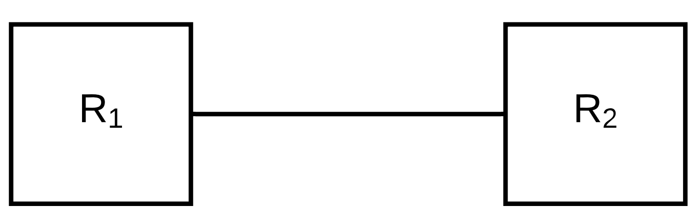
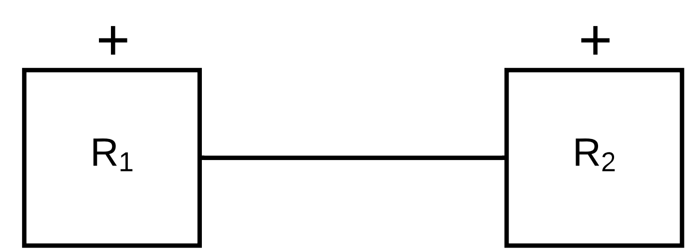
- A **consistent labelling of a signed graph** (V_R, E_+, E_-) is a labelling $s: V \rightarrow \{+,-\}$ in which vertices $R_i, R_j \in V_R$ have the same label if $R_i, R_j \in E_+$, and opposite labels if $R_i, R_j \in E_-$

R-GRAPH



LR-GRAPH



OUR RESULT: INPUT-OUTPUT MONOTONICITY THEOREM

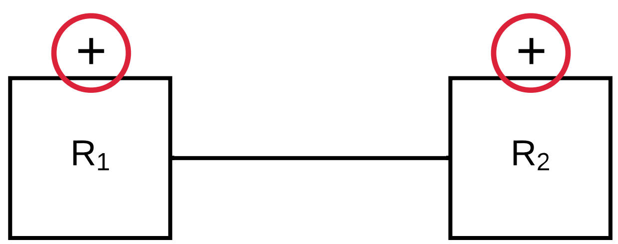
- **Theorem.** Let a set of chemical reactions G be given, with I and O as input and output species. If the following three conditions hold:

    1. the R-graph of G has the **positive loop property** and hence admits a **consistent labelling s;**
    2. The species I participates in **only one reaction** R_I;
    3. The species O participates in **only one reaction** R_O.

INPUT-OUTPUT MONOTONICITY: MICHAELIS MENTEN KINETICS

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

LR-GRAPH



STOICHIOMETRIC MATRIX

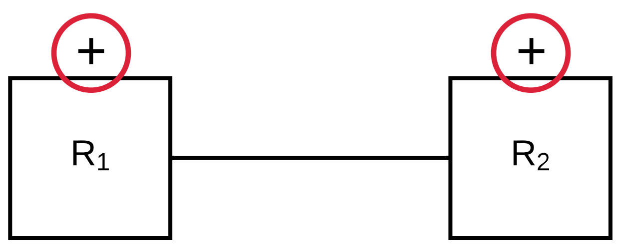
$$\Gamma = \begin{array}{c} \\ E \\ S \\ ES \\ P \end{array} \begin{array}{c} \begin{array}{cc} R_1 & R_2 \end{array} \\ \begin{pmatrix} -1 & +1 \\ -1 & 0 \\ +1 & -1 \\ 0 & +1 \end{pmatrix} \end{array}$$

- P is **positively monotonic** w.r.t S

INPUT-OUTPUT MONOTONICITY: MICHAELIS MENTEN KINETICS

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

LR-GRAPH



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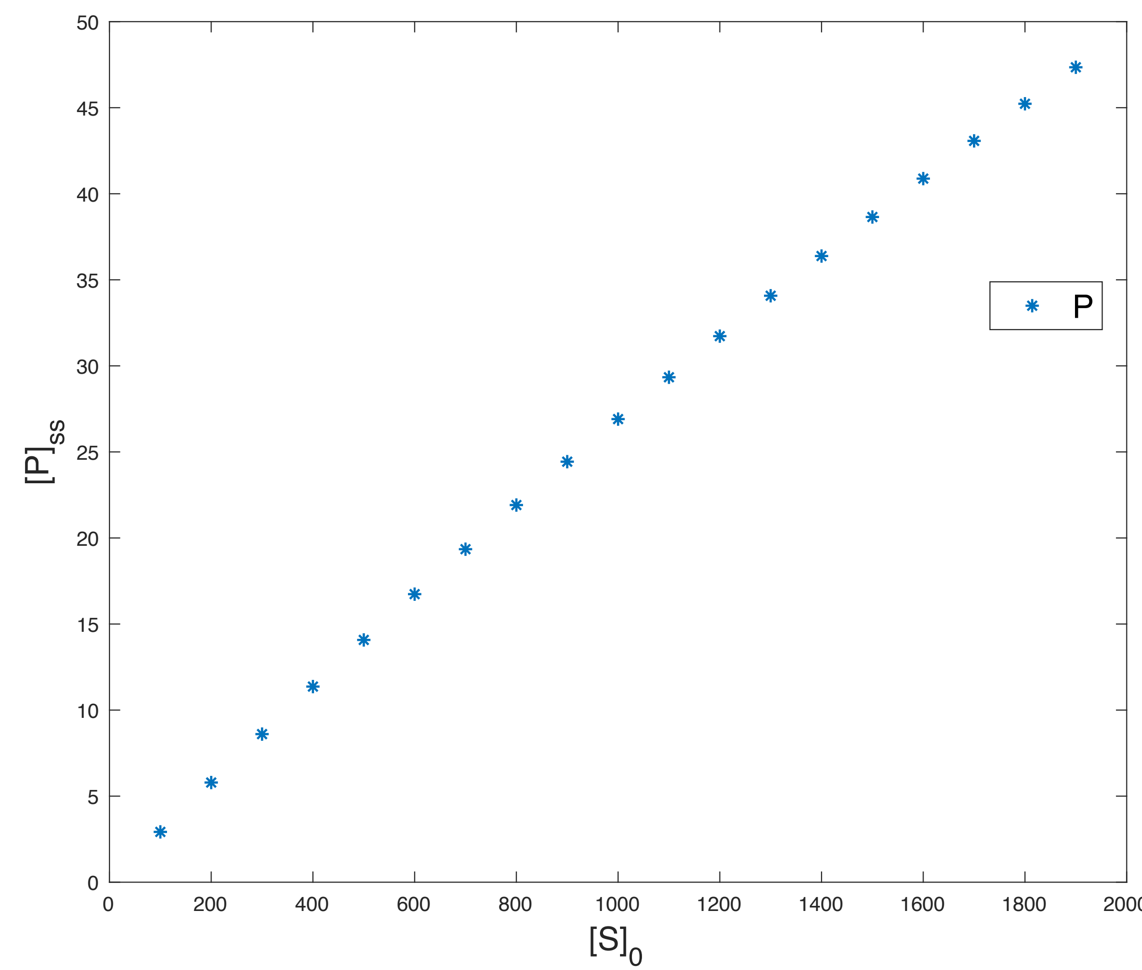
SIMULATION RESULT

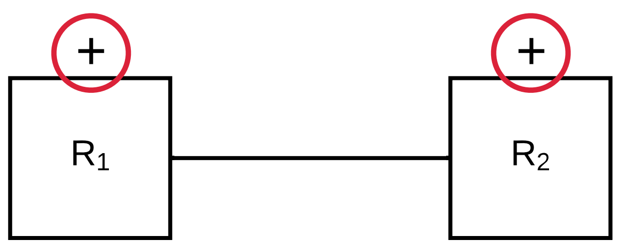


- P is positively monotonic w.r.t S

INPUT-OUTPUT MONOTONICITY: MICHAELIS MENTEN KINETICS

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

LR-GRAPH



STOICHIOMETRIC MATRIX

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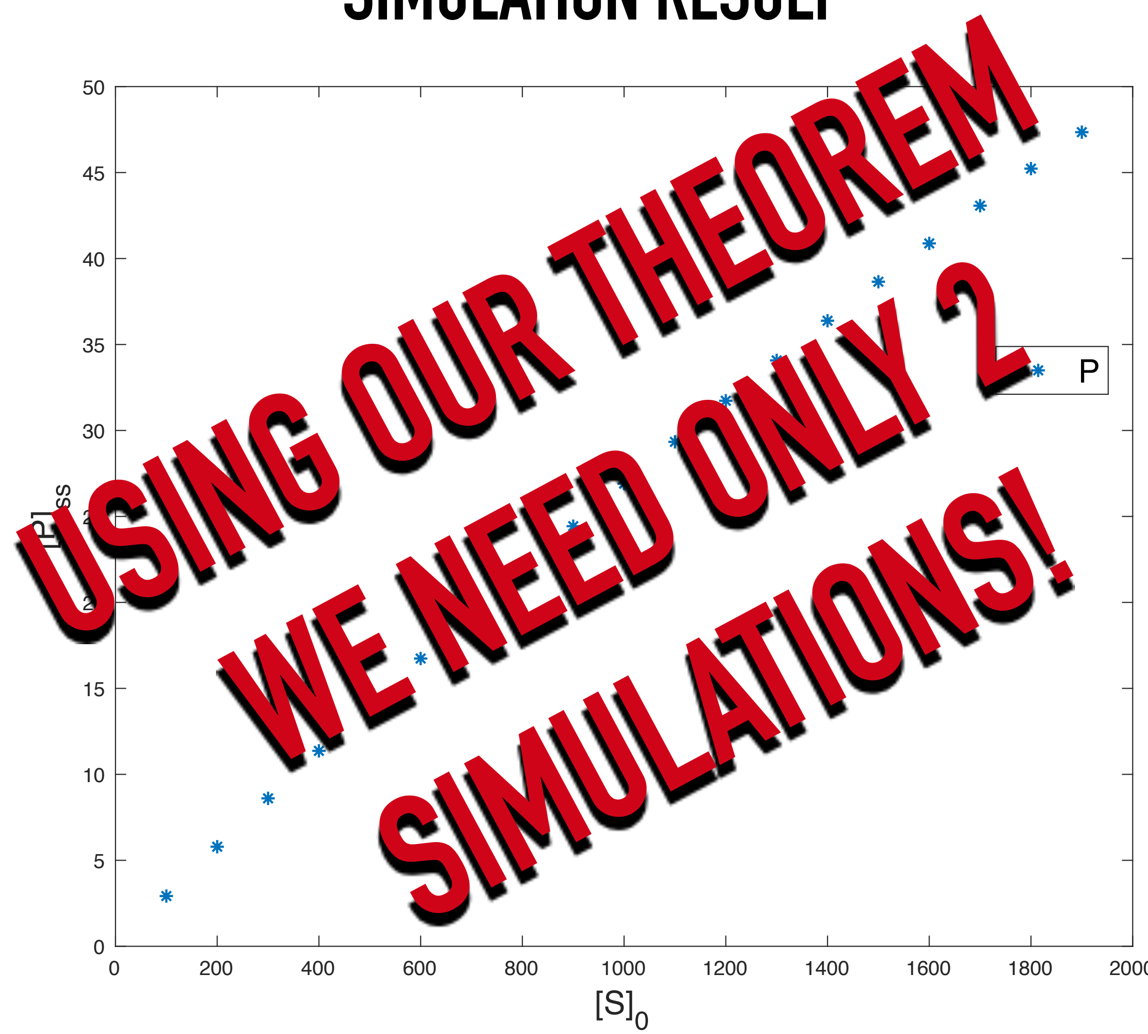
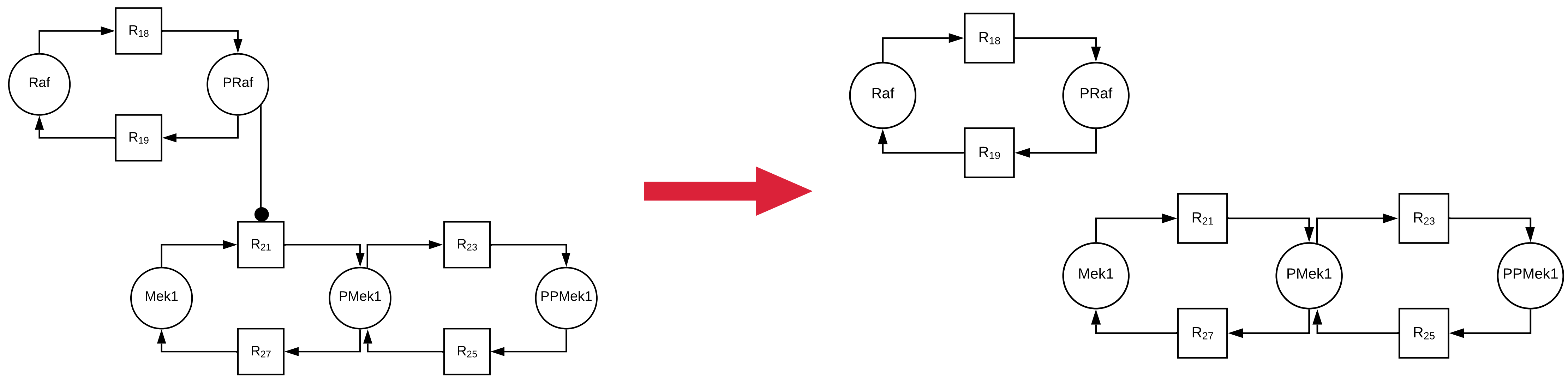
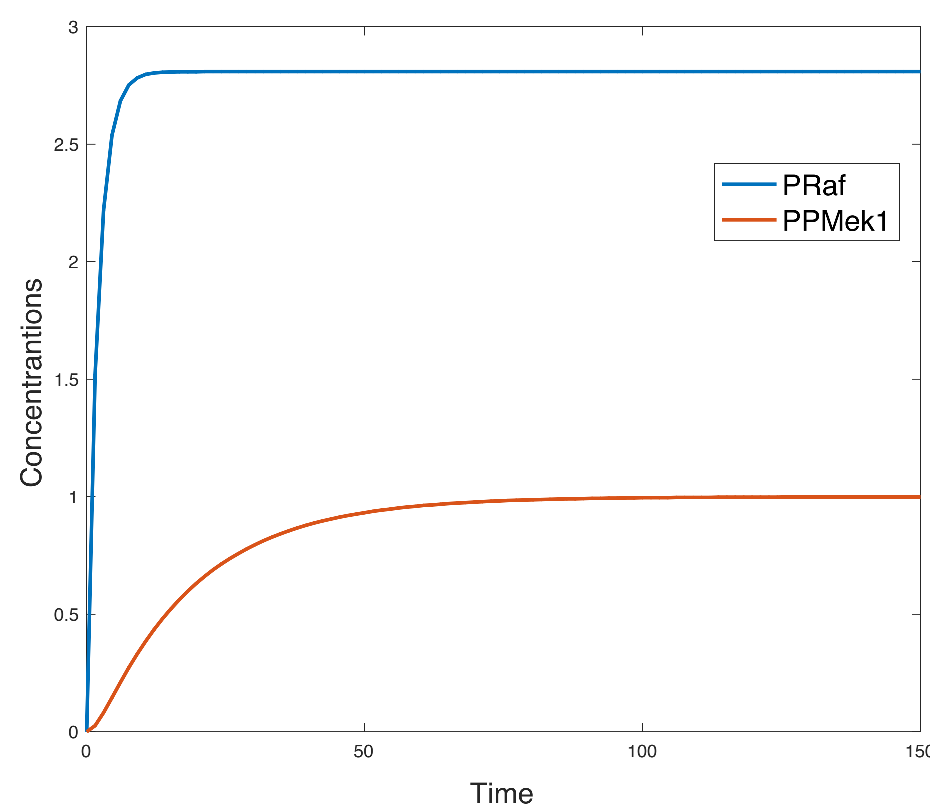
SIMULATION RESULT



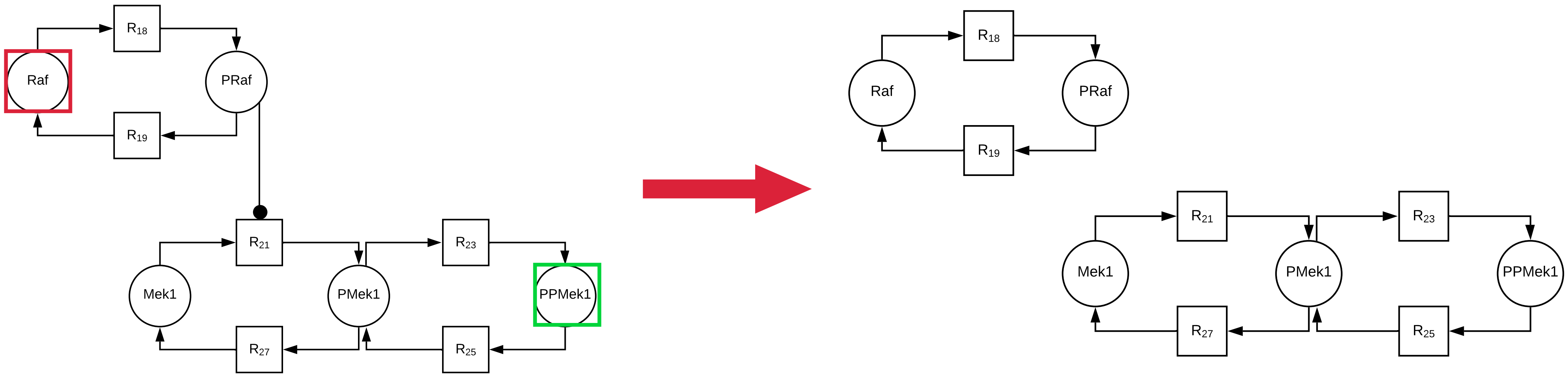
- P is positively monotonic w.r.t S

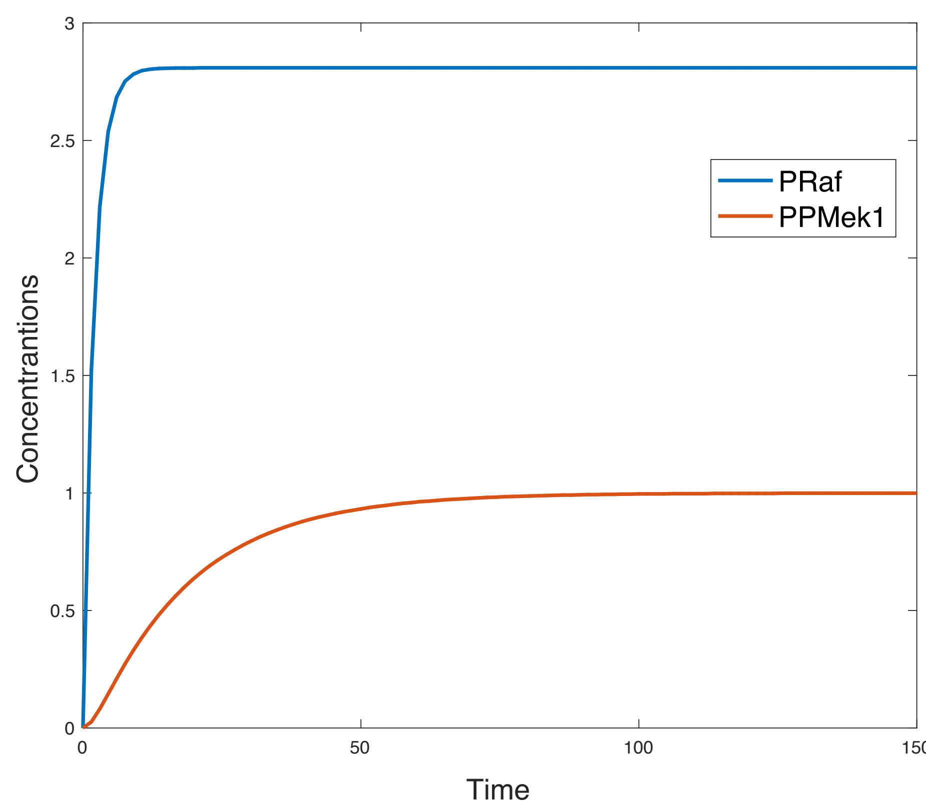
APPLY OUR RESULT TO A MORE COMPLEX SYSTEM: ERK SIGNALLING PATHWAY





APPLY OUR RESULT TO A MORE COMPLEX SYSTEM: ERK SIGNALLING PATHWAY



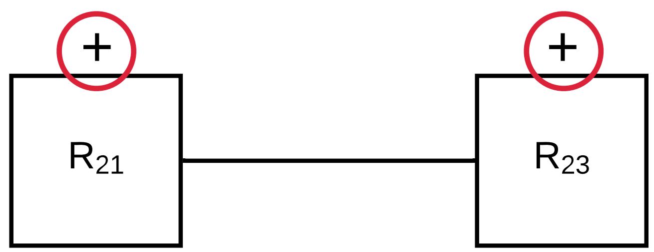


INPUT-OUTPUT MONOTONICITY: ERK SIGNALLING PATHWAY

$$\text{Mek1} \underset{R_{27}}{\overset{R_{21}}{\rightleftharpoons}} \text{PMek1}$$

$$\text{PMek1} \underset{R_{25}}{\overset{R_{23}}{\rightleftharpoons}} \text{PPMek1}$$

LR-GRAPH



STOICHIOMETRIC MATRIX

$$\Gamma = \begin{array}{c} \\ \text{Mek1} \\ \text{PPMek1} \end{array} \begin{array}{c} \begin{array}{cc} R_1 & R_2 \end{array} \\ \begin{pmatrix} -1 & 0 \\ 0 & +1 \end{pmatrix} \end{array}$$

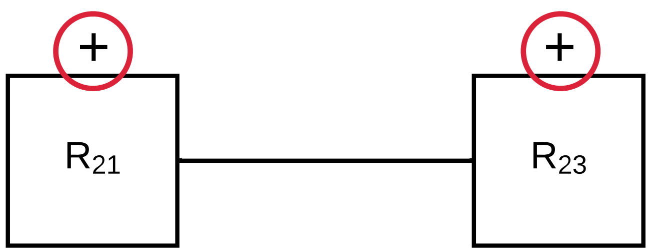
- PPMek1 is **positively monotonic** w.r.t Raf

INPUT-OUTPUT MONOTONICITY: ERK SIGNALLING PATHWAY

$$\text{Mek1} \underset{R_{27}}{\overset{R_{21}}{\rightleftharpoons}} \text{PMek1}$$

$$\text{PMek1} \underset{R_{25}}{\overset{R_{23}}{\rightleftharpoons}} \text{PPMek1}$$

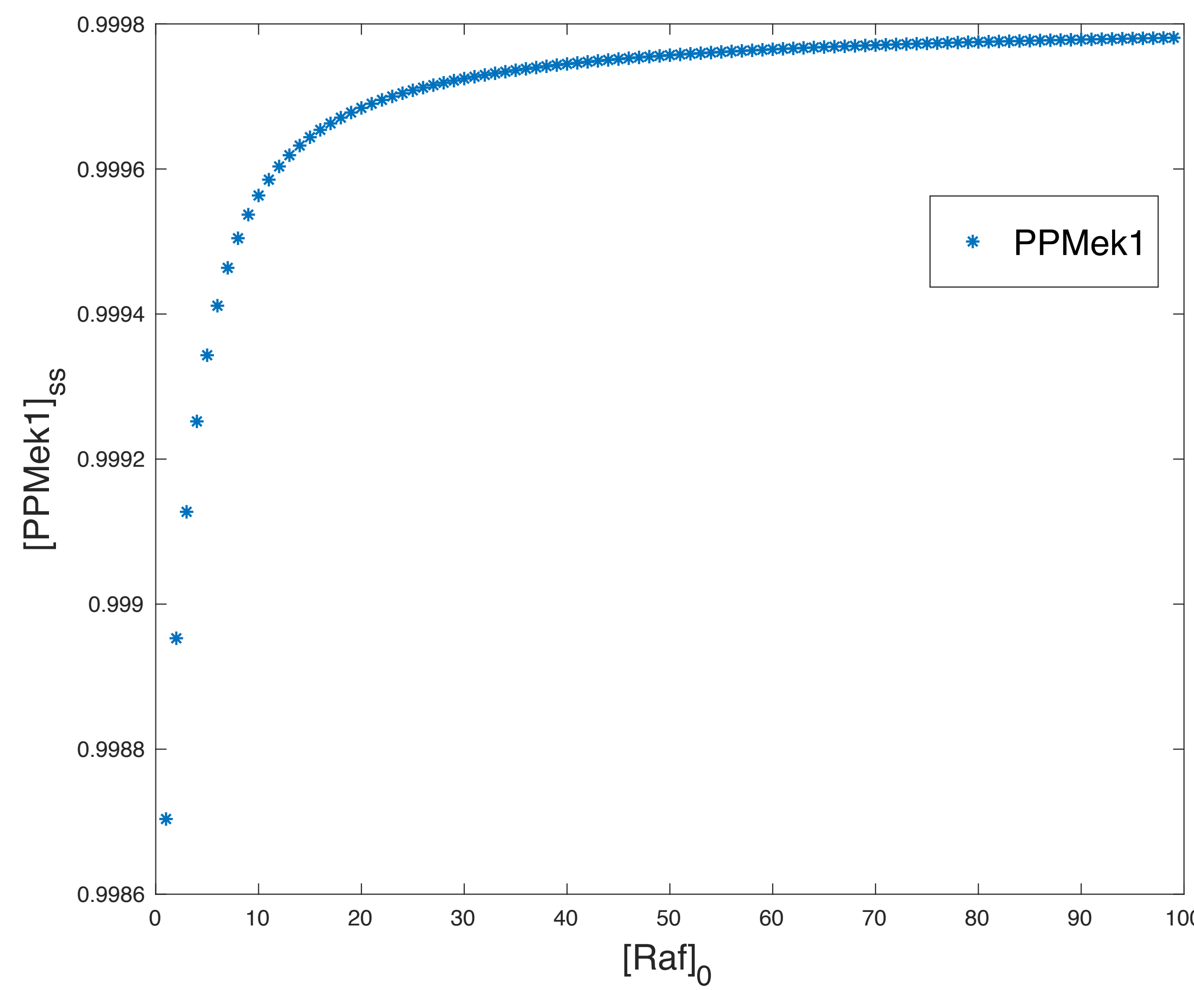
LR-GRAPH



STOICHIOMETRIC MATRIX

$$\Gamma = \begin{matrix} & R_1 & R_2 \\ \text{Mek1} & -1 & 0 \\ \text{PPMek1} & 0 & +1 \end{matrix}$$

SIMULATION RESULT



➤ PPMek1 is positively monotonic w.r.t Raf

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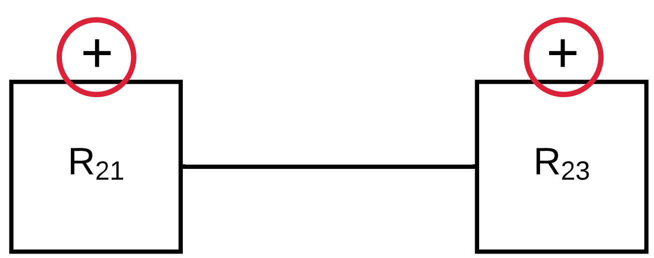
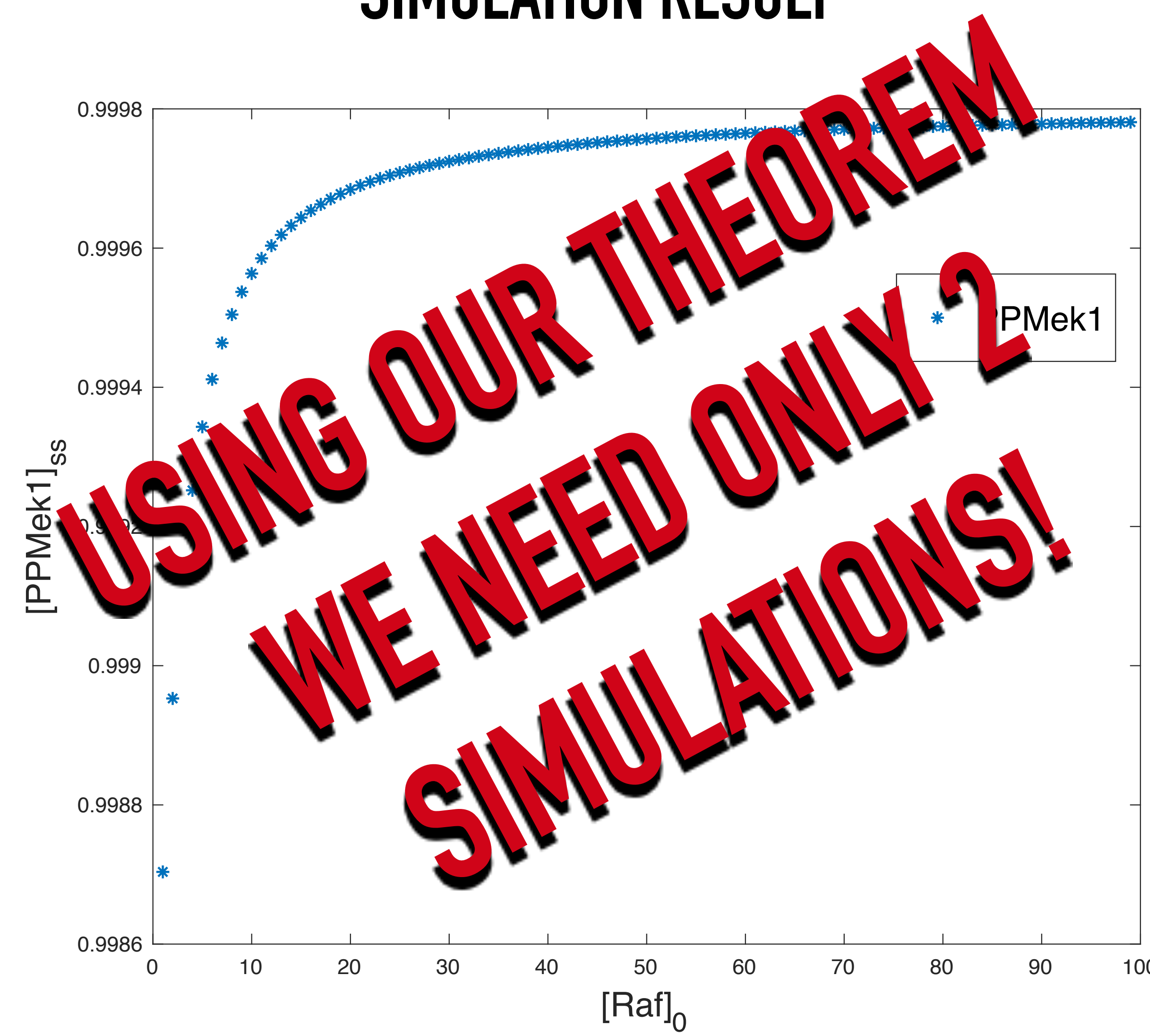


STOICHIOMETRIC MATRIX

$$\Gamma = \begin{array}{c} \\ \text{Mek1} \\ \text{PPMek1} \end{array} \begin{array}{c} R_1 \quad R_2 \\ \begin{pmatrix} -1 & 0 \\ 0 & +1 \end{pmatrix} \end{array}$$

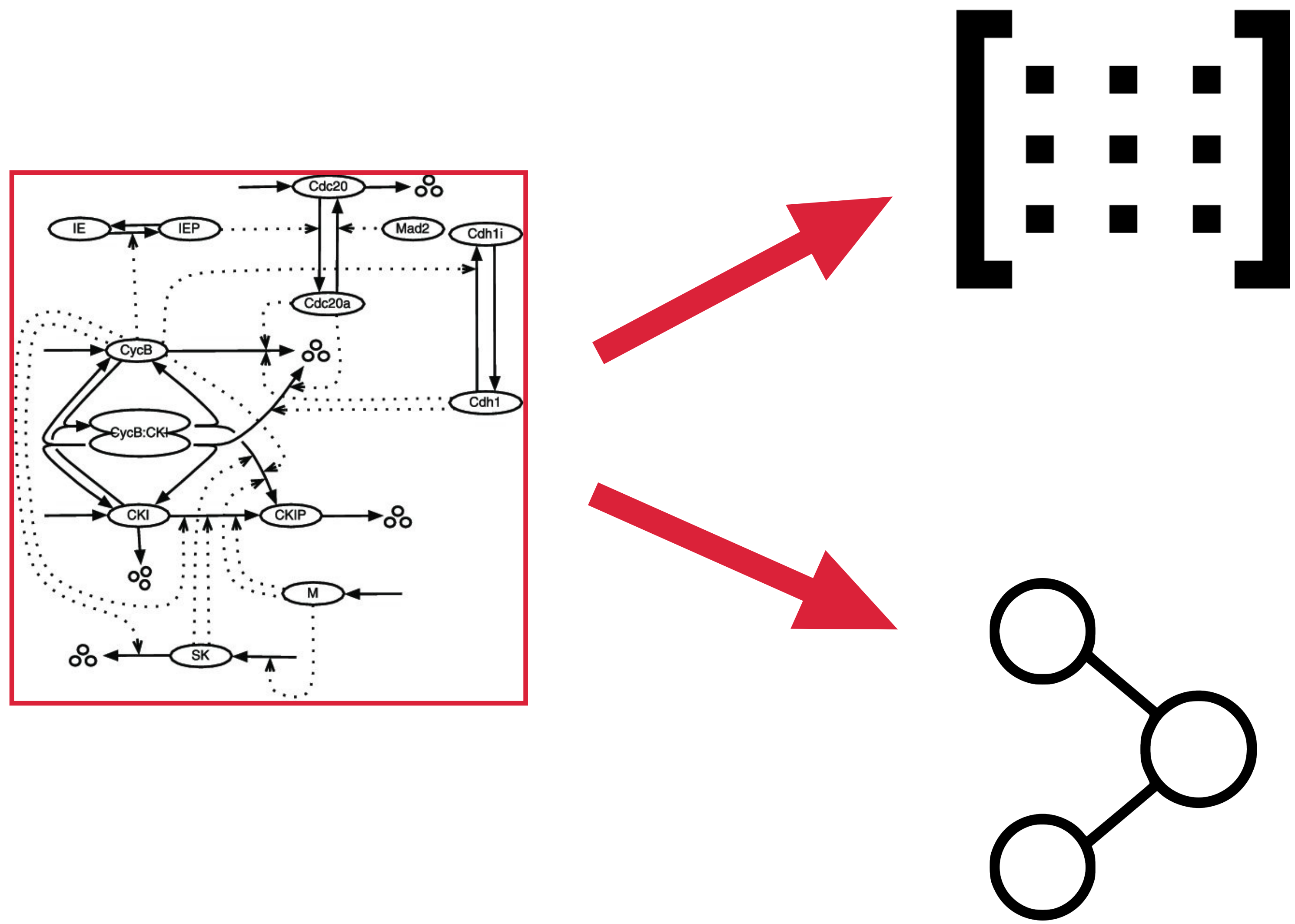
SIMULATION RESULT



- PPMek1 is positively monotonic w.r.t Raf

INPUT-OUTPUT GRAPHTOOL

- Tool (in Python) to verify our sufficient conditions on big graphs



BEYOND OUR PREVIOUS APPROACH:

STUDYING ROBUSTNESS IN BECKER–DÖRING EQUATIONS

BECKER-DÖRING MODEL

- It is a model that describes **condensations phenomena** at different pressures

- The clusters give rise to two types of reactions:

$$C_1 + C_i \underset{b_{i+1}}{\overset{a_i}{\rightleftharpoons}} C_{i+1}$$

where:

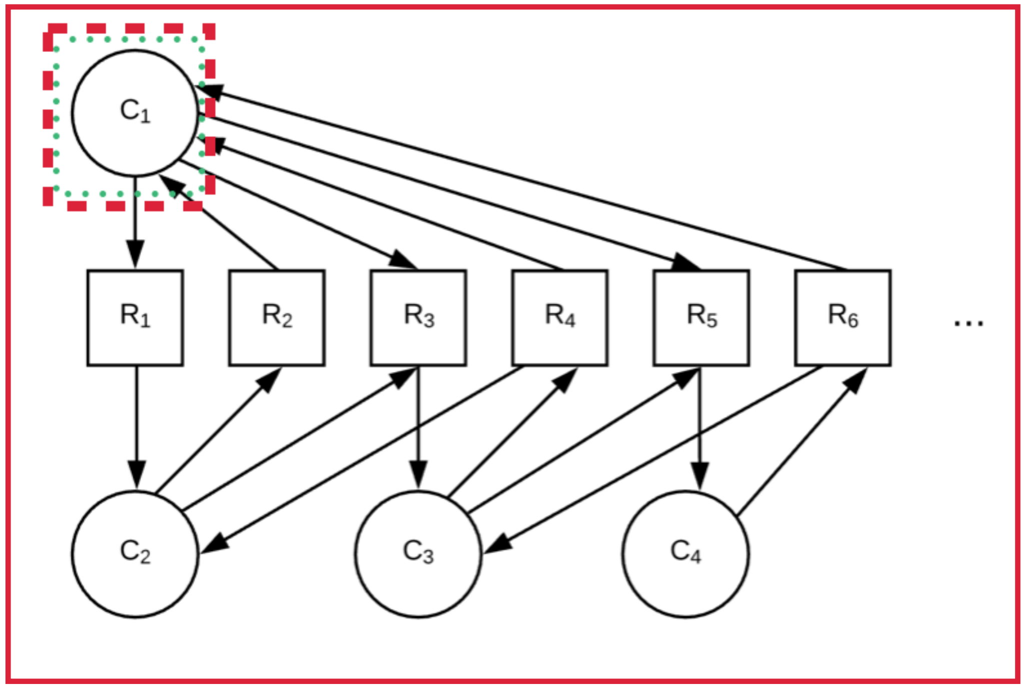
- C_i denotes clusters consisting of i particles
- Coefficients a_i and b_{i+1} stand, respectively, for the rate of **aggregation** and **fragmentation**
- Rates may depend on the size of clusters involved in the reactions
- The mass is **constant** and it depends on the initial condition of the system

WHY TO STUDY ROBUSTNESS IN BD MODEL

- In [Saunders *et al.*, 2015]:

Development of a theoretical model, based on the Becker-Döring equations, that is robust for particular conditions.

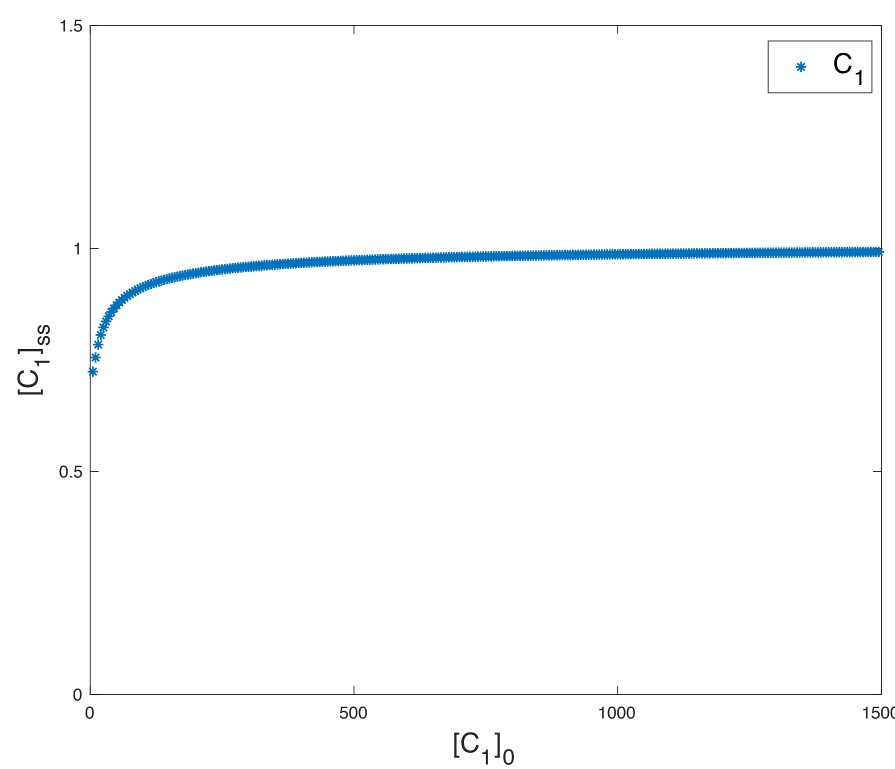
- The Petri net is potentially infinite.
- We cannot apply Input-Output Monotonicity Theorem.



STEADY STATE ANALYSIS

- **Theorem.** Let a and b be the coefficient rates of coagulation and fragmentation process in the Becker-Döring system, ρ the mass of the system and $[C_1]_{ss}$ the concentration of monomers at the steady state. Then, as $\rho \to \infty$, $[C_1]_{ss} \to \frac{b}{a}$.

- With rates a=b, changing the initial concentration of C_1, the monomer concentration at the steady state tends to 1



CONCLUSIONS

- **Formal definition** of absolute and relative concentration robustness
- **Sufficient conditions** to study monotonicity between Input and Output species
- Implementation of **Input-Output GraphTool**
- Verification of Robustness of **Becker-Döring equations**

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FUTURE WORK

- **Stochasticity**
- Investigation of other **topological features**
- Applicability to **new specific problems**
- Analysis of Becker-Döring equations with **real rates**

QUESTIONS?

thank you!