UNIVERSITÀ DI PISA

FORMALIZING ROBUSTNESS OF BIOCHEMICAL NETWORKS

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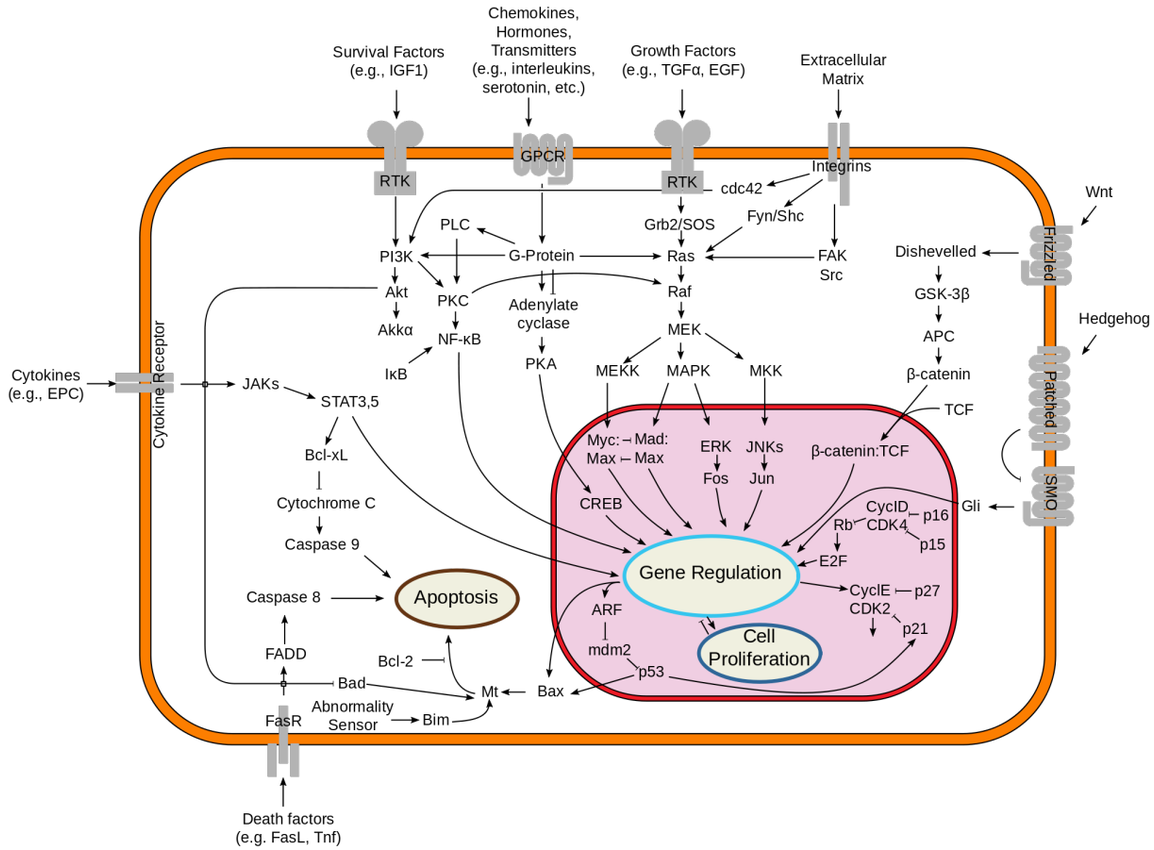
BACKGROUND

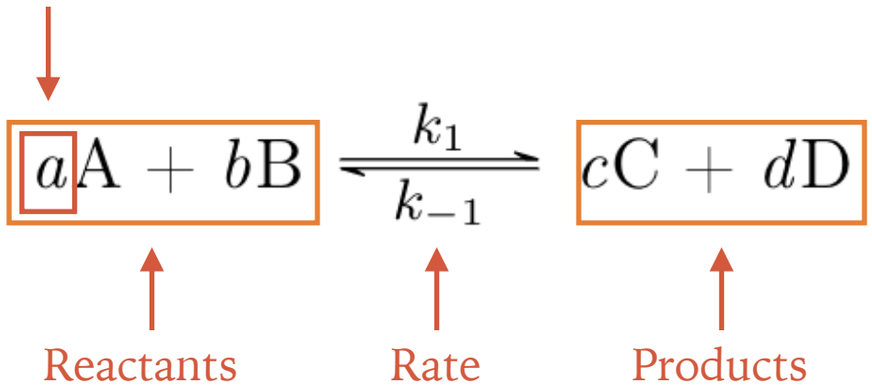
- A cell is a **very complex system**
- Chemical reaction networks (***pathways***) govern the basic cell's activities
- To examine the structure of the cell as a whole, we can design **multiscale and predictive models**



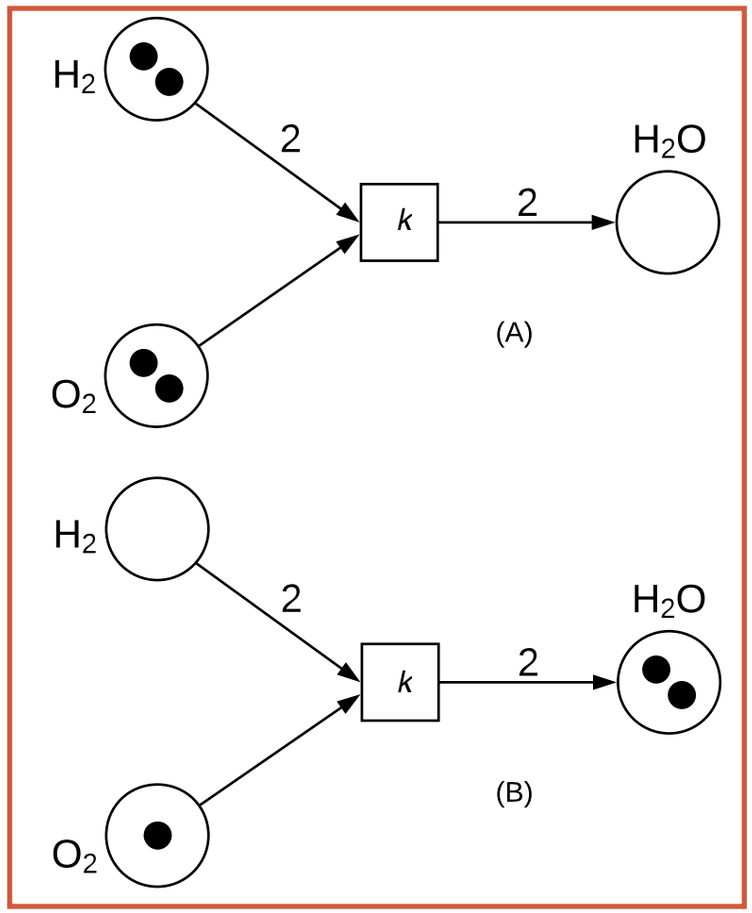
Experiments *in vitro* ⟷ Experiments *in silico*

CHEMICAL REACTIONS

Stoichiometric coefficient



- **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

$$r \propto [reactants] \longrightarrow r = k_1[A]^a[B]^b$$

$$r_{direct} = r_{inverse}$$

$$\begin{aligned}
\frac{d[A]}{dt} &= \overbrace{-ak_1[A]^a[B]^b}^{\text{direct reaction term}} \overbrace{+ak_{-1}[C]^c[D]^d}^{\text{inverse reaction term}} \\
\frac{d[B]}{dt} &= -bk_1[A]^a[B]^b + bk_{-1}[C]^c[D]^d \\
\frac{d[C]}{dt} &= +ck_1[A]^a[B]^b - ck_{-1}[C]^c[D]^d \\
\frac{d[D]}{dt} &= +dk_1[A]^a[B]^b - dk_{-1}[C]^c[D]^d
\end{aligned}$$

ROBUSTNESS PROPERTY

- Our goal: to define **robustness** property

- **Robustness** allows the system to preserve its functions despite internal and external perturbations

In nature, there are **different mechanisms** ensuring this property:

- Modularity
- System control
- Redundancy
- Structural stability

ROBUSTNESS IN LITERATURE

- Many definitions, which find *sufficient conditions* for **particular biochemical networks**
- In [Shinar-Feinberg]:

The **sufficient condition** states that a mass action system can be considered robust if it admits a positive steady state, the underlying reaction network has a **deficiency=1** and there are distinct non-terminal complexes that differ only in a single species.

$$deficiency = number\ of\ nodes\ -\ linkage\ classes\ -\ rank.$$

$$\boxed{A + B} \xrightarrow{\alpha} 2B$$
$$\boxed{B} \xrightarrow{\beta} A$$

- In [Barkai-Leibler]:

A system is robust only if its operation does not depend on initial concentration of chemical species involved. Introduction of **degree** in robustness

OUR PROPOSAL

- Formal definition of robustness property
- Analyse systems with deficiency > 1
- Execution of the system by simulations
- Analyse robustness degree

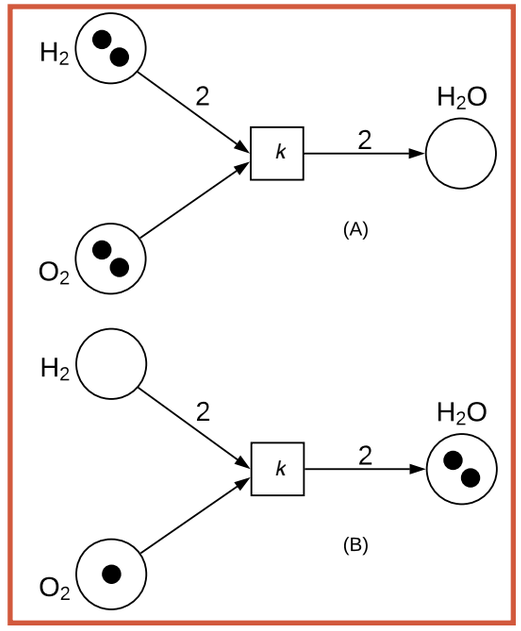
CONTINUOUS PETRI NETS FORMALISM DEFINITION

A continuous Petri net N is a quintuple:

$$N=<P, T, F, W, m_0>$$

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
- $W : F \rightarrow \mathbb{R}_{\geq 0}$ 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 $m : P \rightarrow \mathbb{R}_{\geq 0}$



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

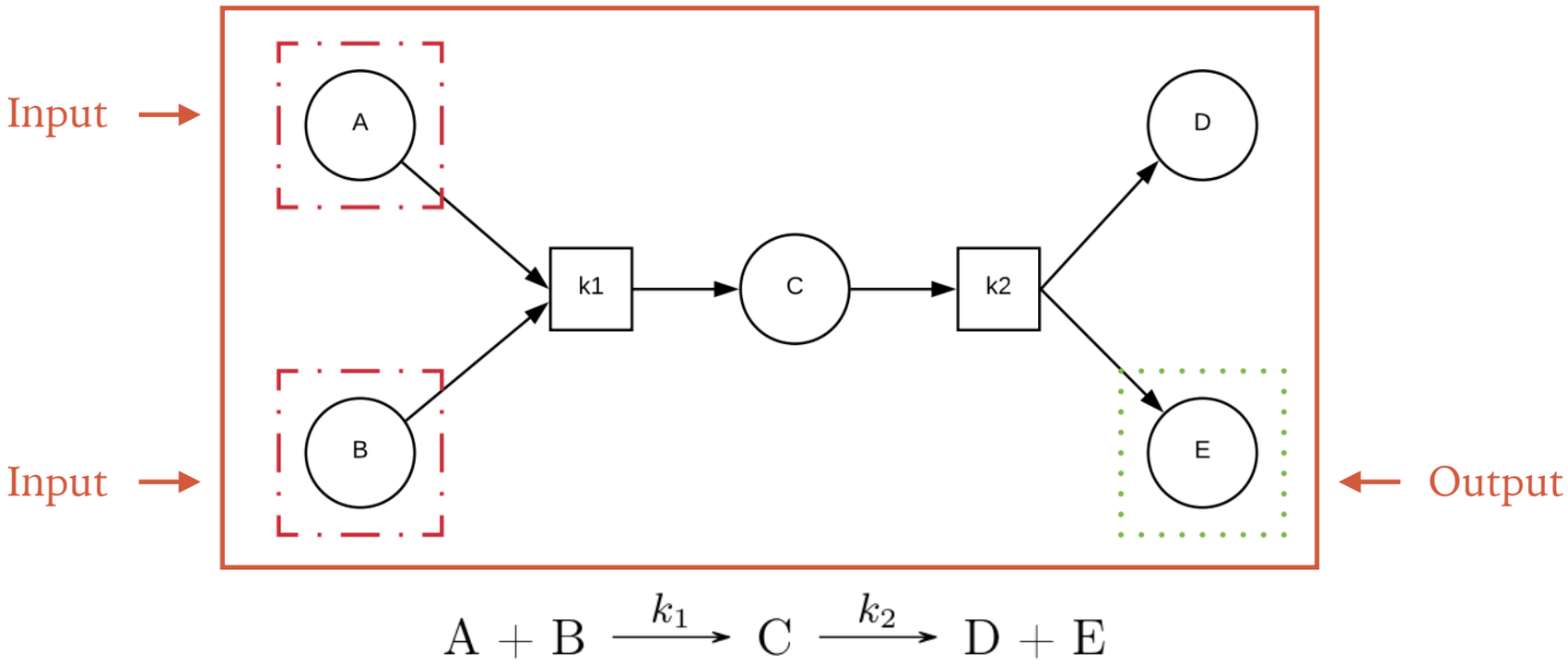
FORMAL DEFINITION OF ROBUSTNESS: AUXILIARIES CONCEPTS

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

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

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

- **Definition 2 (Interval marking).** An interval marking is a function $\mathrm{m}_{[\,]}: \mathrm{P} \rightarrow \mathrm{I}$. We call $\mathrm{M}_{[\,]}$ the domain of all interval markings.



FORMAL DEFINITION OF ROBUSTNESS

- **Definition 3 (*a*-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'* corresponding to the steady state reachable from *m*, is such that

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

Observations:

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

EXAMPLE OF APPLICATION OF OUR DEFINITION : TOY MODEL

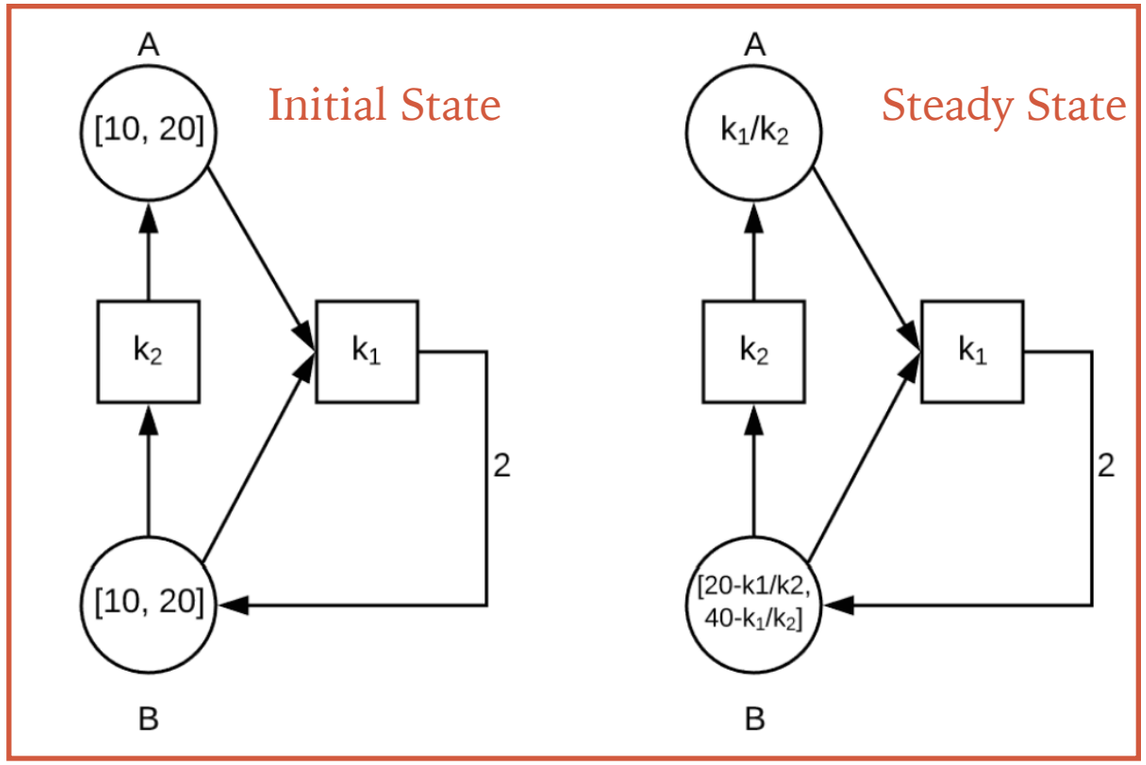
Given a set of chemical reactions:

$$\mathrm{A} + \mathrm{B} \xrightarrow{k_1} 2\,\mathrm{B} \qquad \mathrm{B} \xrightarrow{k_2} \mathrm{A}$$

We calculate the concentration of chemical species **at the steady state:**

$$A = \frac{k_2}{k_1} \qquad B = \theta - \frac{k_2}{k_1}$$

where θ is the sum of initial concentrations of A and B.



We apply the definition: for A we obtain a=0. For B we obtain a=20, because:

$$B = [20 - \frac{k_2}{k_1}, 40 + \frac{k_2}{k_1}]$$

EXAMPLE OF APPLICATION OF OUR DEFINITION : IDHKP-IDH

- Given a set of reactions:

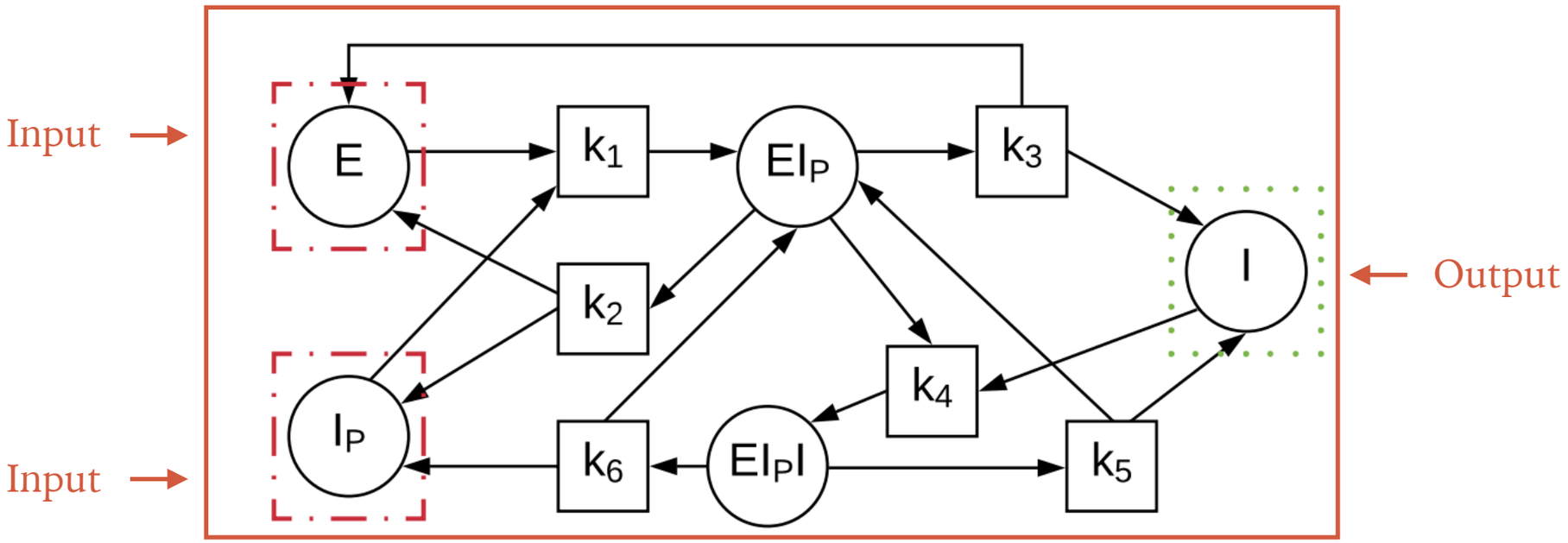
$$\mathrm{E} + \mathrm{I_P} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{EI_P}$$

$$\mathrm{EI_P} \xrightarrow{k_3} \mathrm{E} + \mathrm{I}$$

$$\mathrm{EI_P} + \mathrm{I} \underset{k_5}{\overset{k_4}{\rightleftharpoons}} \mathrm{EI_PI}$$

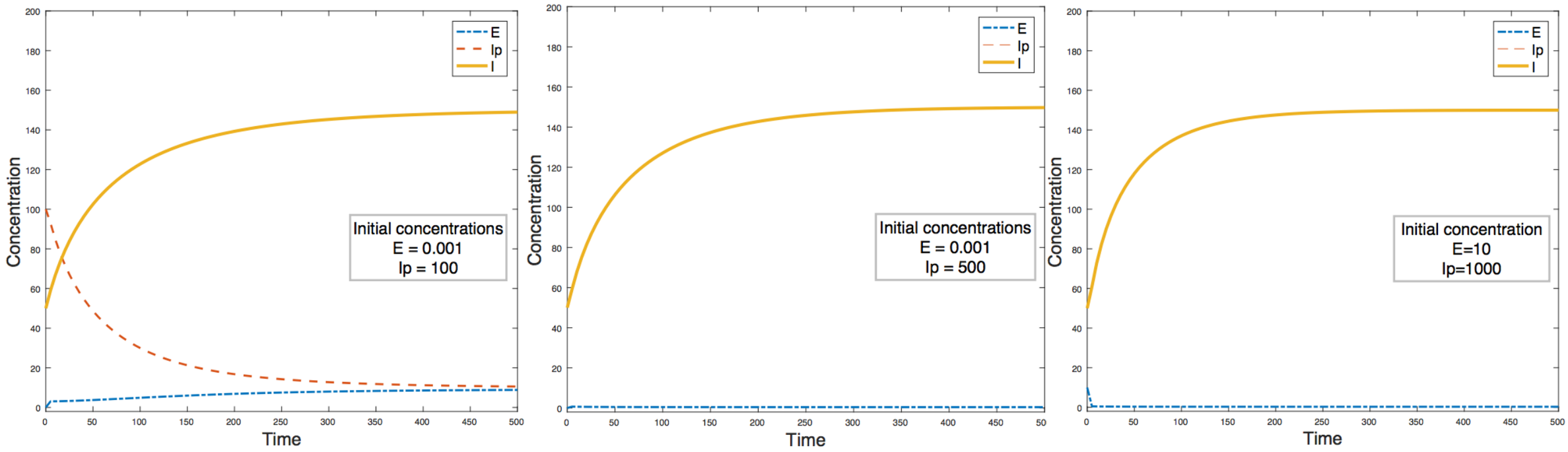
$$\mathrm{EI_PI} \xrightarrow{k_6} \mathrm{EI_P} + \mathrm{I_P}$$

- We build the Petri nets and choose input and output



IDHKP-IDH: SIMULATION RESULTS

We vary the **initial concentration of the inputs** ([Ip] and [E]) and we obtain exactly the same concentration value for [I]. Hence, $a=0$.

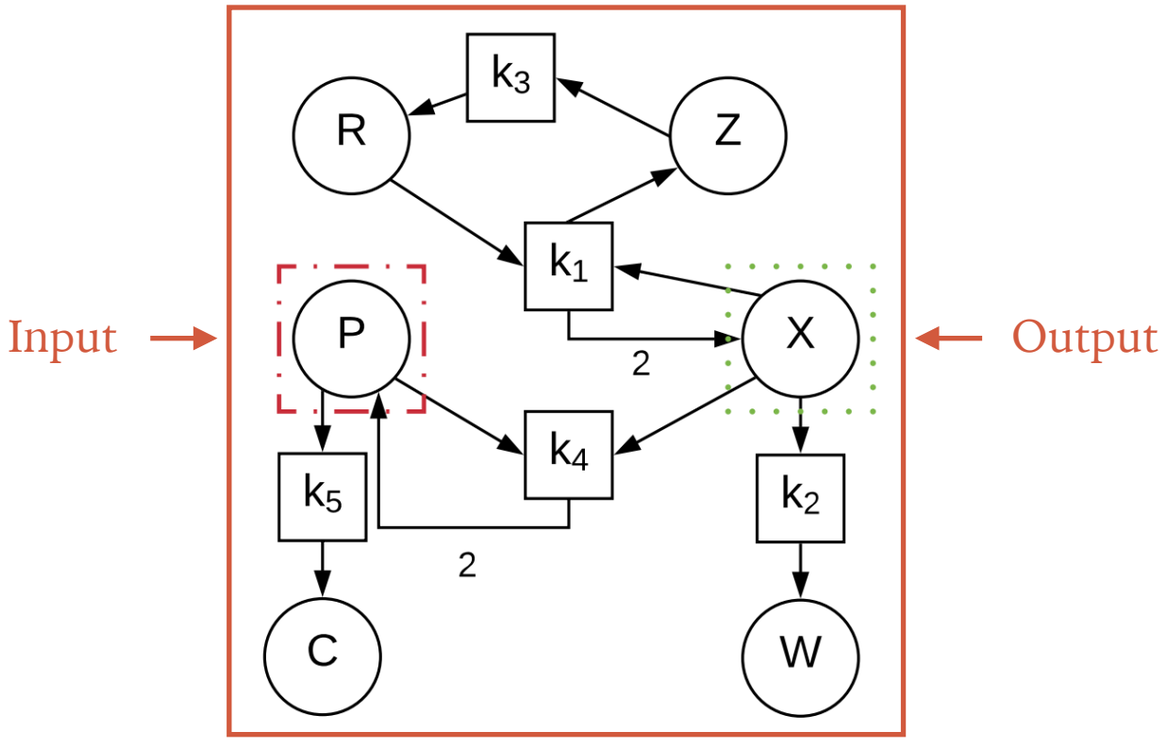


EXAMPLE OF APPLICATION OF OUR DEFINITION : ENZYME ACTIVITY

- Given a set of reactions:

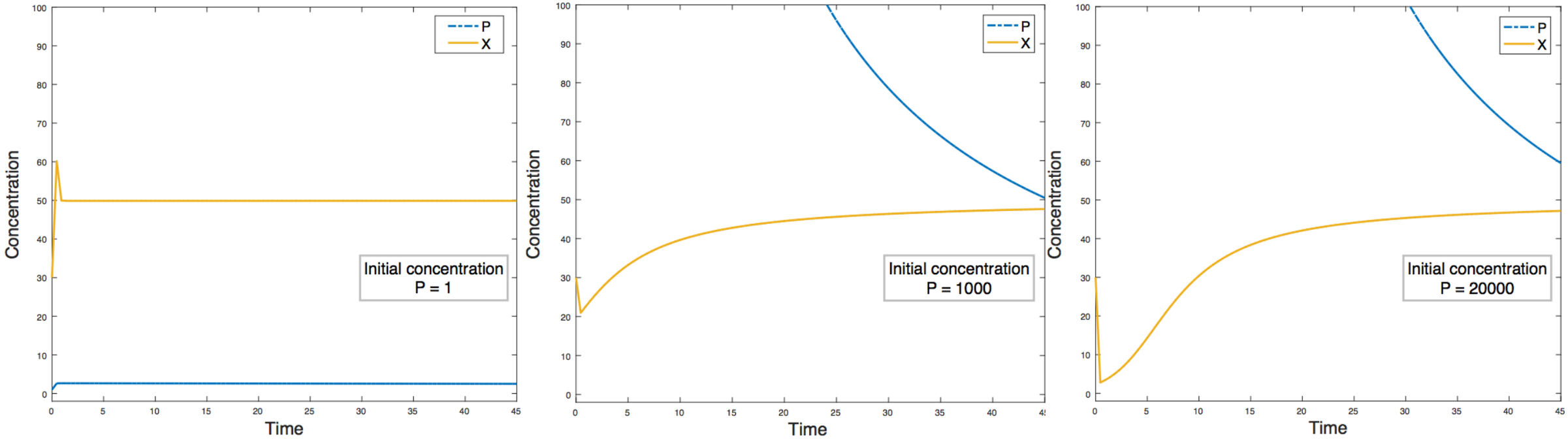
$$\mathrm{R} + \mathrm{X} \xrightarrow{k_1} \mathrm{X} + \mathrm{X} + \mathrm{Z}$$
$$\mathrm{X} \xrightarrow{k_2} \mathrm{W}$$
$$\mathrm{Z} \xrightarrow{k_3} \mathrm{R}$$
$$\mathrm{X} + \mathrm{P} \xrightarrow{k_4} \mathrm{P} + \mathrm{P}$$
$$\mathrm{P} \xrightarrow{k_5} \mathrm{C}$$

- We build the Petri nets and choose input and output



ENZYME ACTIVITY AT SATURATION: SIMULATION RESULTS

We vary the **initial concentration of the inputs** ([P]) and we obtain these concentrations for the species [X]. Hence, we obtain $a=3$.

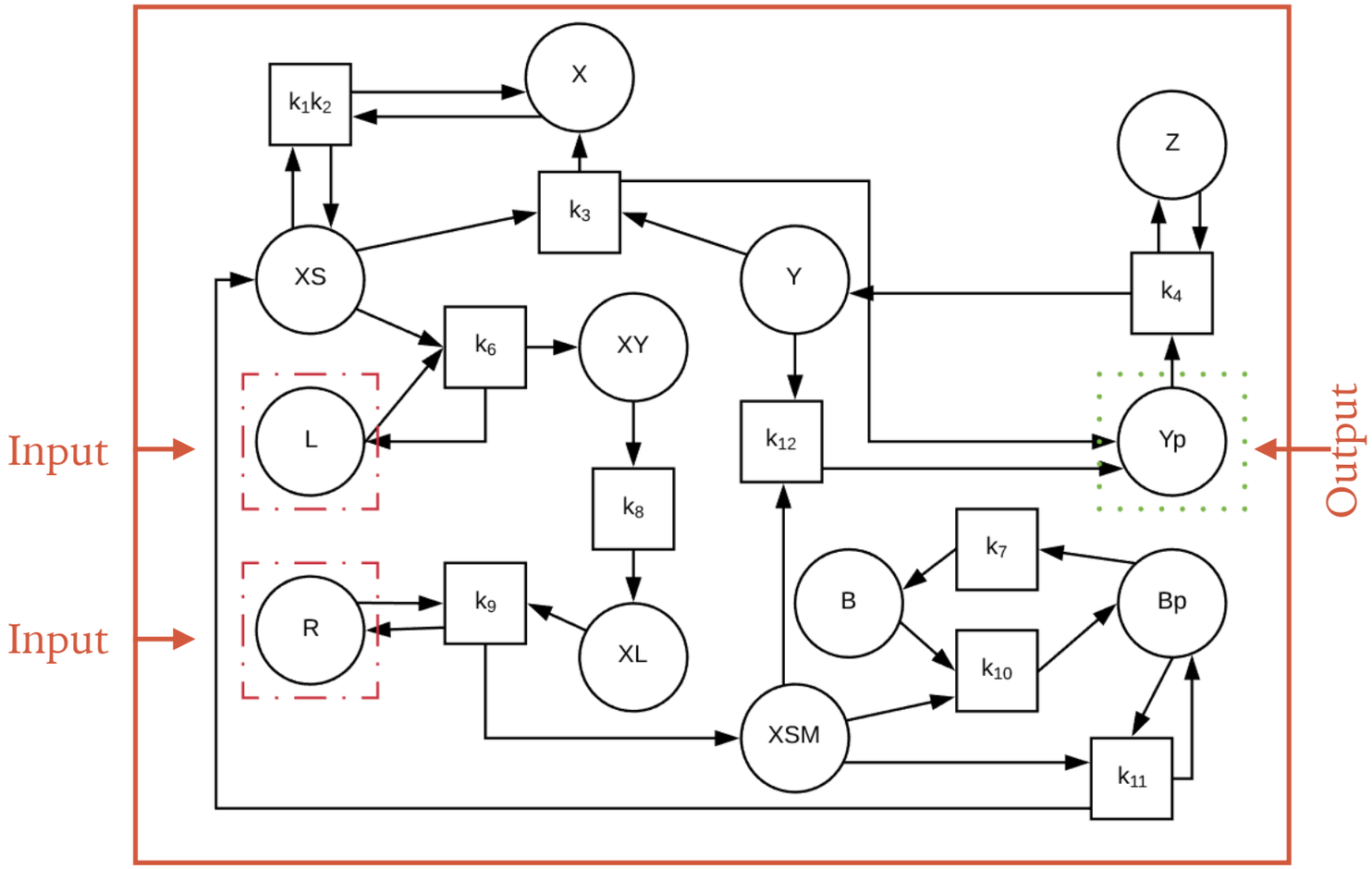


EXAMPLE OF APPLICATION OF OUR DEFINITION : CHEMOTAXIS OF E. COLI

- Given a set of reactions:

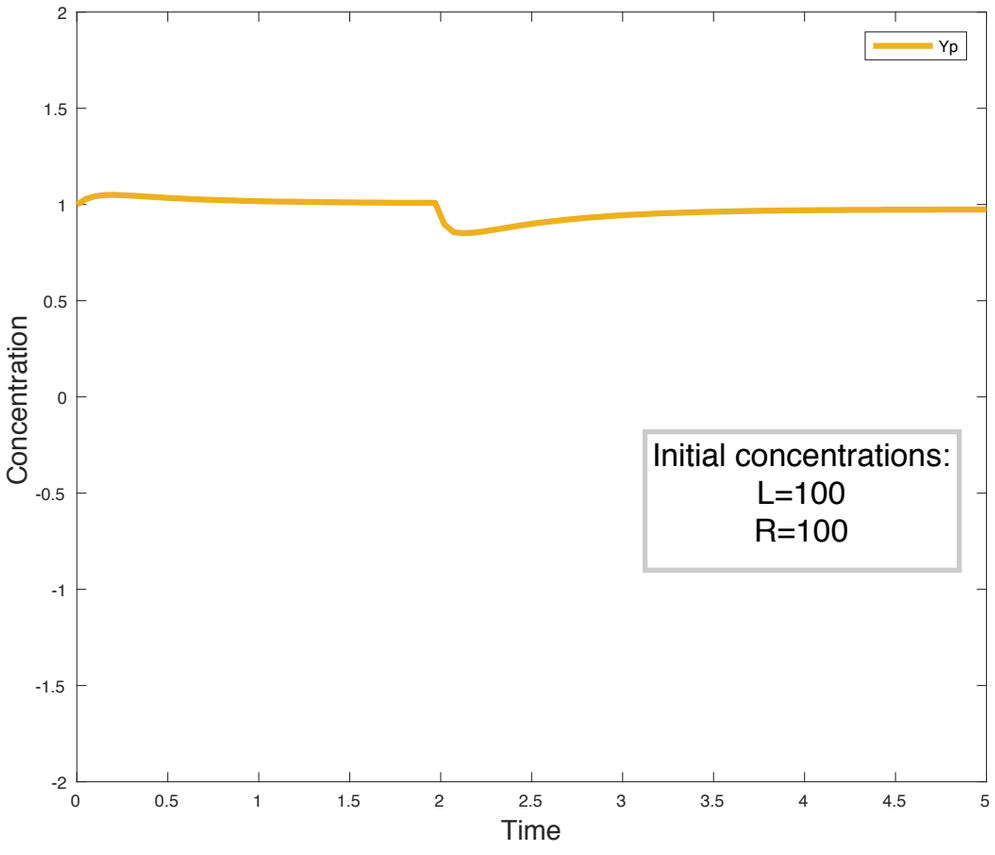
$$\mathrm{X} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{XS}$$
$$\mathrm{XS} + \mathrm{Y} \xrightarrow{k_3} \mathrm{Yp} + \mathrm{X}$$
$$\mathrm{Yp} + \mathrm{Z} \xrightarrow{k_4} \mathrm{Y} + \mathrm{Z}$$
$$\mathrm{Yp} \xrightarrow{k_5} \mathrm{Y}$$
$$\mathrm{L} + \mathrm{XS} \xrightarrow{k_6}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

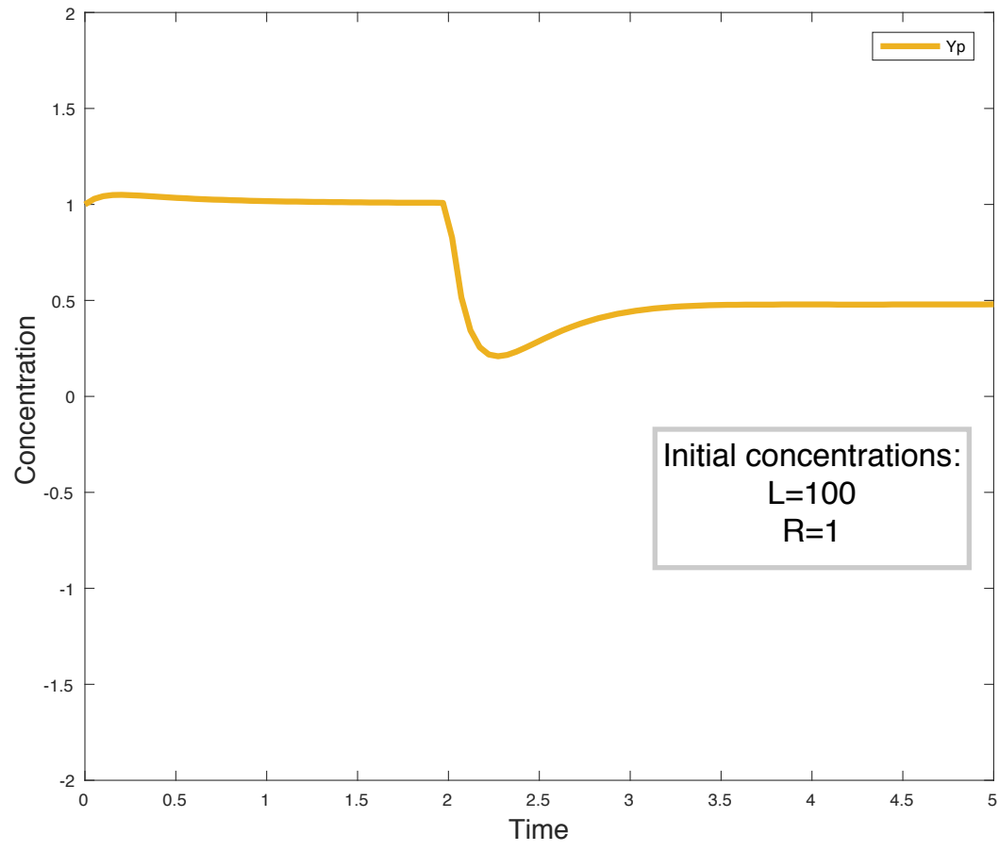
- We build the Petri net:



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 $a=0.5$





CONCLUSIONS

- Formal definition of robustness property
- Definition able to capture different kind of robustness
- Analysis of the system by simulations

Future work:

- Analyse **monotonicity** of the system
- Find a **sufficient condition** limiting the computational effort

QUESTIONS?

thank you!