Hill Kinetics Meets P Systems

A Case Study on Gene Regulatory Networks as Computing Agents *in silico* and *in vivo*

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Eight Workshop on
Membrane Computing

ESIGNET

Outline

Hill Kinetics Meets P Systems

Introduction

- Research Project, Motivation, Intention
- Biological Principles of Gene Regulatory Networks (GRNs)
- Modelling Approaches, Transformation Strategies, Comparison

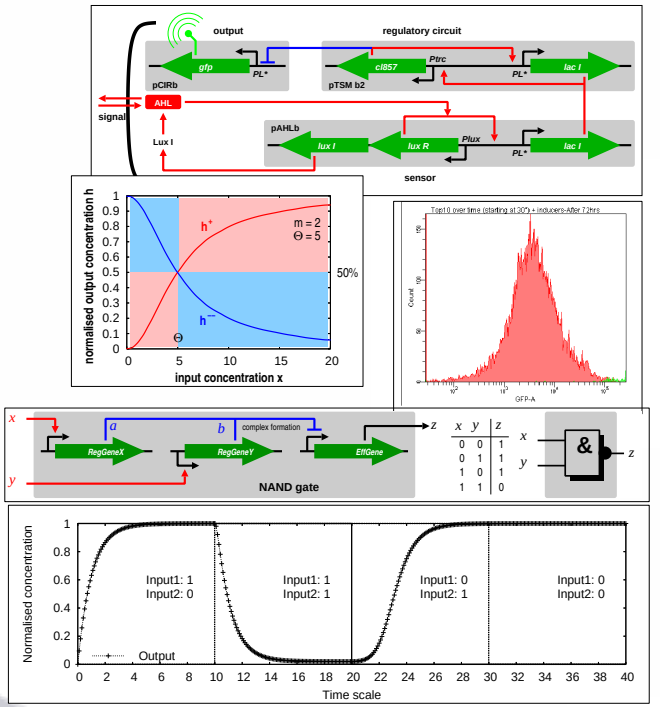
Hill Kinetics

- Definition and Discretisation
- P Systems Π_{Hill}
- Dynamical Behaviour
- Introductory Example

Case Study: Computational Units

- Inverter
- NAND Gate
- RS Flip-Flop and Its Validation *in vivo*

Discussion, Conclusion, Further Work



ESIGNET – Research Project

Evolving Cell Signalling Networks *in silico*

European interdisciplinary research project

- University of Birmingham (Computer Science)
- TU Eindhoven (Biomedical Engineering)
- Dublin City University (Artificial Life Lab)
- University of Jena (Bio Systems Analysis)

SIXTH FRAMEWORK PROGRAMME

Objectives

- Study the computational properties of GRNs
- Develop new ways to model and predict real GRNs
- Gain new theoretical perspectives on real GRNs

Computing Facilities

- Cluster of 33 workstations
  (two Dual Core AMD Opteron™ 270 processors)
- Use of Dresden BIOTEC laboratories for *in vivo* studies

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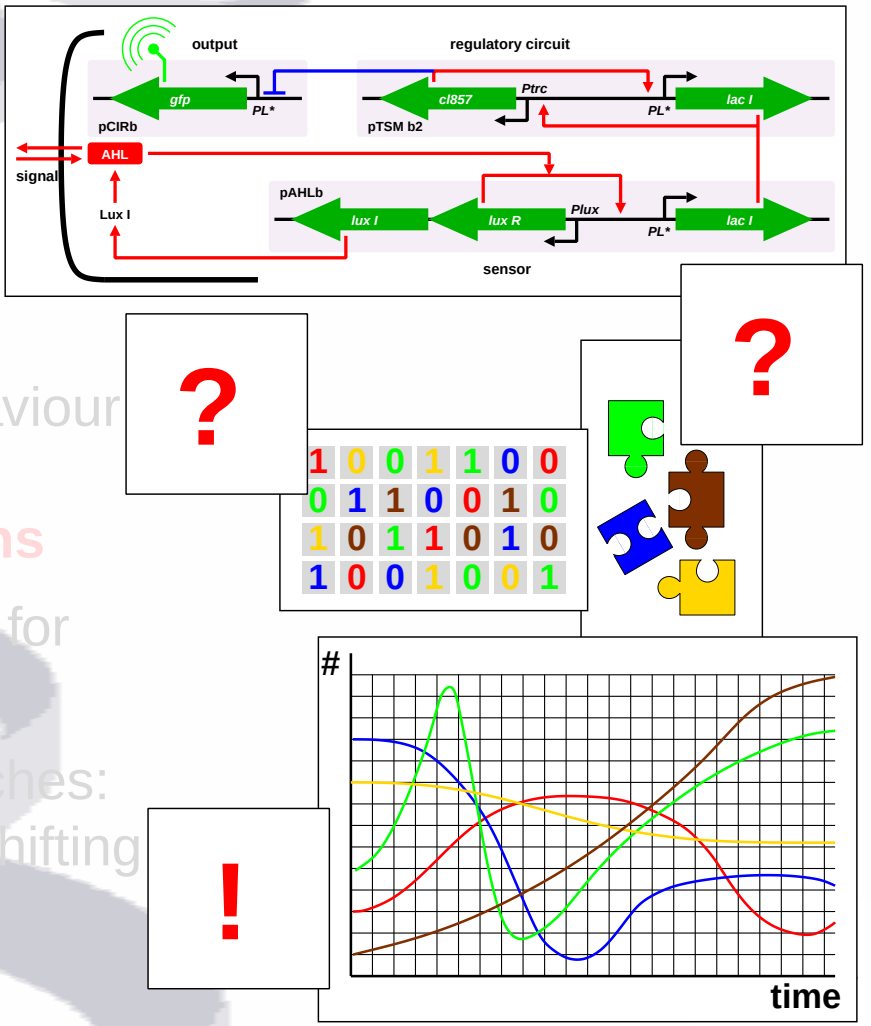
Motivation and Intention

Exploring Dynamical Behaviour of Gene Regulatory Networks

- Understanding biological reaction networks: essential task in **systems biology**
- Many coexisting approaches: **analytic, stochastic, algebraic**
- Each specifically emphasises certain modelling aspects
- Emulating dynamical system behaviour based on **reaction kinetics**
  $\Longrightarrow$ often key to **network functions**
- Reaction kinetics mostly specified for analytic models based on ODE
- Combining advantages of approaches: transformation strategies, model shifting
- **Example: Transformation of Hill Kinetics to P Systems**



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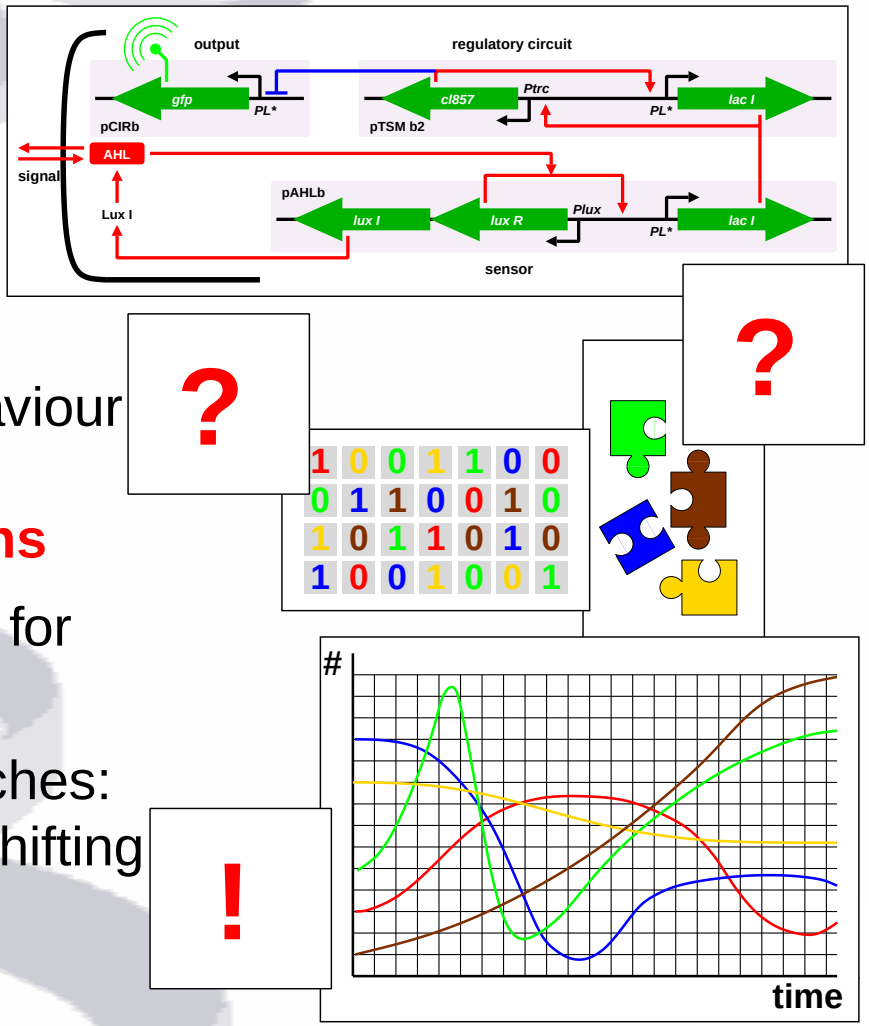
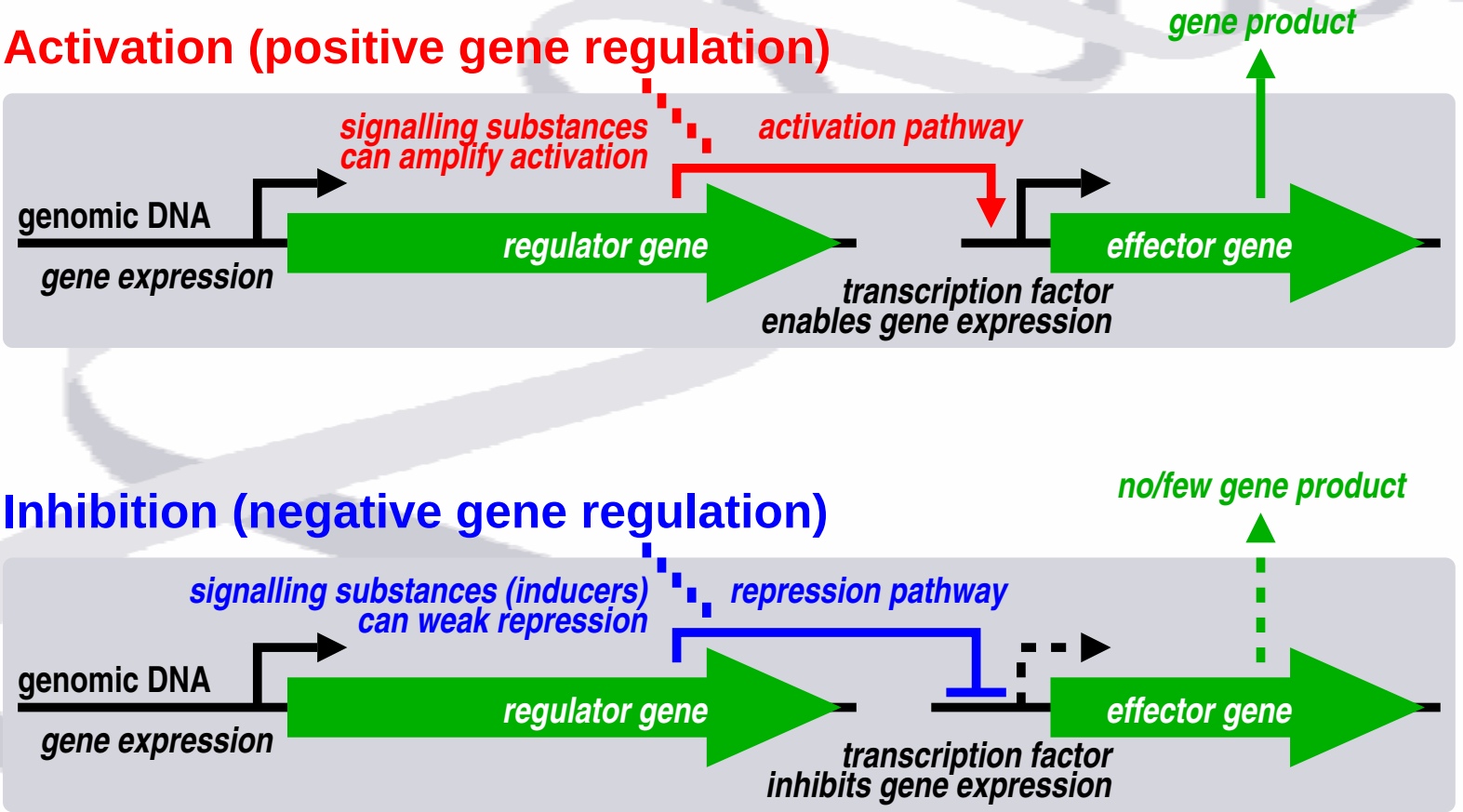


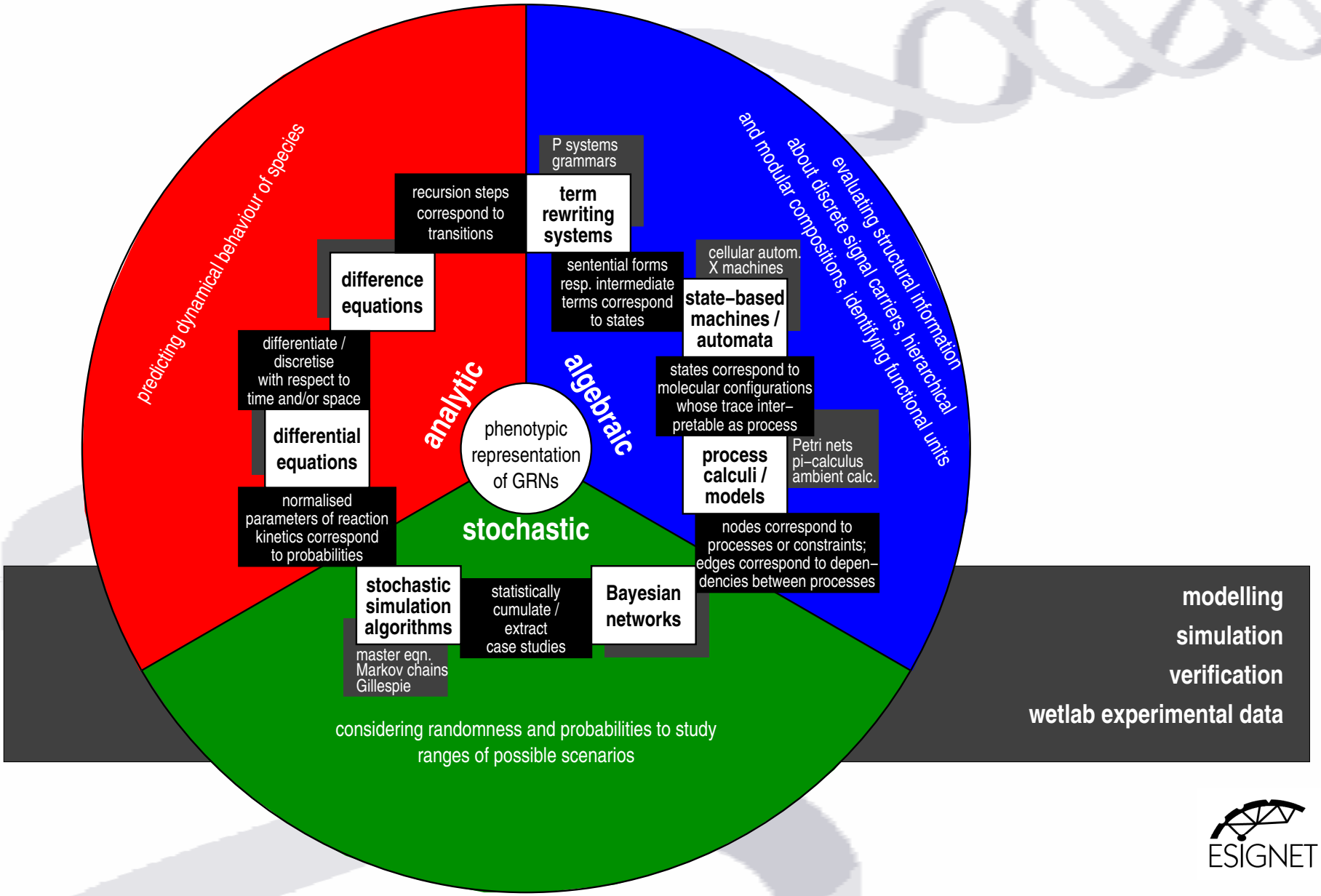
Biological Principles of Gene Regulation

Intercellular Information Processing of Spatial Globality within Organisms



Feedback loops: gene products can act as transcription factors and signalling substances forming gene regulatory networks

Modelling Approaches and Transformation Strategies



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Comparison of Approaches

Specific Advantages and Preferred Applications

Analytic approaches

- Primarily adopted from chemical reaction kinetics
- Macroscopic view on species concentrations
- Differential equations from generation and consumption rates
- Continuous average concentration gradients
- Deterministic **monitoring of temporal or spatial system behaviour**

Stochastic approaches

- Aspects of uncertainty: incorporating randomness and probabilities
- Ranges of possible scenarios and their statistical distribution
- Facilitating direct comparison with wetlab experimental data
- Statistics: **discovering correlations between network components**

Algebraic approaches

- Discrete principle of operation
- Embedding/evaluating structural information
- Modularisation, hierarchical graduation of provided system information
- Molecular tracing
- **Flexible instruments** regarding level of abstraction

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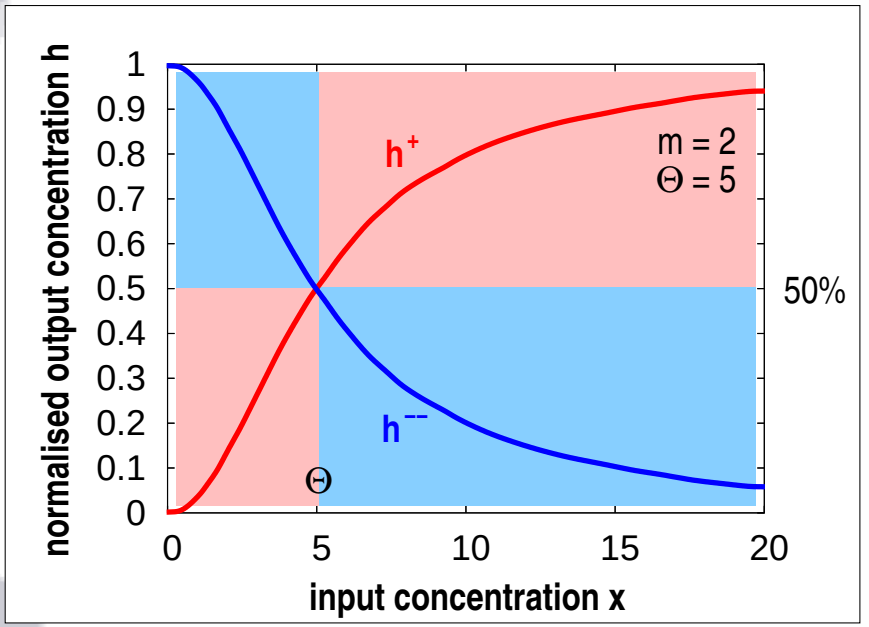
Hill Kinetics – Sigmoid-Shaped Threshold Functions

- Model cooperative and competitive aspects of interacting gene regulatory units dynamically and quantitatively
- Homogeneous and analytic
- Formulate relative intensity of gene regulations by **sigmoid-shaped threshold functions** $\mathrm{h}^+, \mathrm{h}^- : \mathbb{R} \times \mathbb{R} \times \mathbb{N} \to \mathbb{R}$
- $x \geq 0$: input concentration of **transcription factor** activating/ inhibiting gene expression
- $\Theta > 0$: threshold (50% level)
- $m \in \mathbb{N}_+$: degree of regulation



activation (upregulation) $\mathrm{h}^+(x, \Theta, m) = \frac{x^m}{x^m + \Theta^m}$

inhibition (downregulation) $\mathrm{h}^-(x, \Theta, m) = 1 - \mathrm{h}^-(x, \Theta, m)$

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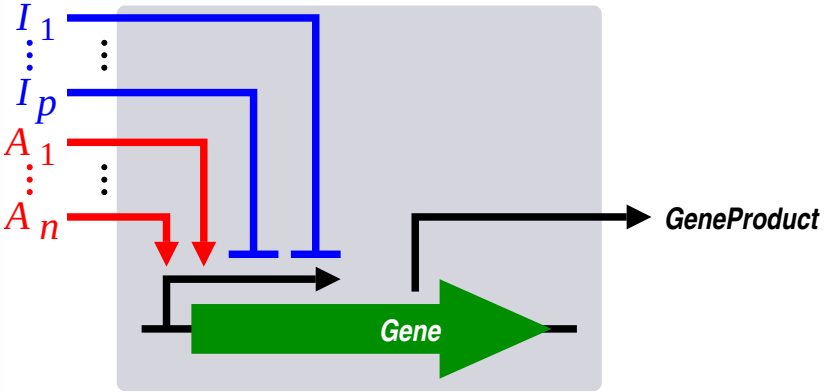
Hill Kinetics – Network Composition

- Several interacting (competing) transcription factors influence gene expression
- Activators A_i, inhibitors I_j and proportional factor $c_1 > 0$: determine **production rate** of a **gene product**
- Additional assumption of linear spontaneous **decay rate** $c_2 \cdot [GeneProduct]$ with $c_2 > 0$
- Differential equation for corresponding gene product:



$$\begin{aligned}\frac{\mathrm{d}\,[GeneProduct]}{\mathrm{d}t} &= ProductionRate - c_2[GeneProduct] \\ &= c_1 \cdot \mathrm{h}^+(A_1, \Theta_{A_1}, m) \cdot \ldots \cdot \mathrm{h}^+(A_n, \Theta_{A_n}, m) \cdot \\ &\quad (1 - \mathrm{h}^+(I_1, \Theta_{I_1}, m) \cdot \ldots \cdot \mathrm{h}^+(I_p, \Theta_{I_p}, m)) \\ &\quad - c_2 \cdot [GeneProduct]\end{aligned}$$

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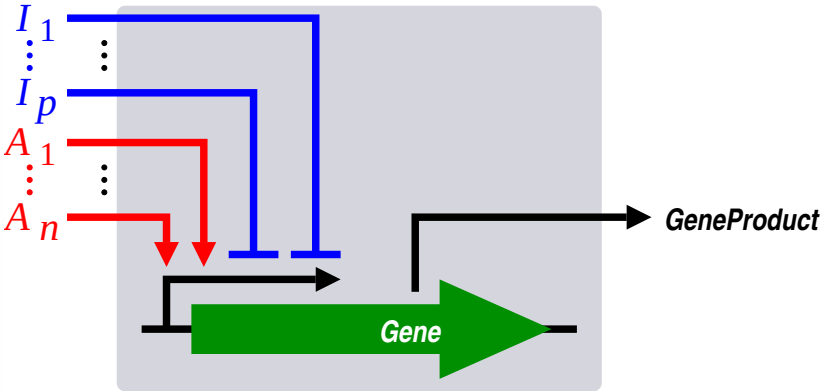
Hill Kinetics – Discretisation

- Discretisation with respect to **value** and **time** $\Longrightarrow$ homologous term rewriting mechanism
- Large but finite **pool of particles** (multiset)
- **Reaction:** remove number of reactant particles, simultaneously add all products
- Time-varying reaction rates $\Longrightarrow$ **variable stoichiometric factors**
- *Gene*: limiting resource
- Reaction conditions: presence of $A_1, \ldots, A_n$, absence ($\neg$) of $I_1, \ldots, I_p$
- $\Delta\tau \in \mathbb{R}_+$: step length between discrete time points t and $t+1$



$$s\ \mathit{Gene} \longrightarrow s\ \mathit{GeneProduct} + s\ \mathit{Gene} \Big|_{A_1,\ldots,A_n,\neg I_1,\ldots,\neg I_p} \quad \text{where}$$

$$s = \lfloor \Delta\tau \cdot c_1 \cdot [\mathit{Gene}] \cdot h^+(A_1, \Theta_{A_1}, m) \cdot \ldots \cdot h^+(A_n, \Theta_{A_n}, m) \cdot (1 - h^+(I_1, \Theta_{I_1}, m) \cdot \ldots \cdot h^+(I_p, \Theta_{I_p}, m)) \rfloor$$

$$u\ \mathit{GeneProduct} \longrightarrow \emptyset \quad \text{where} \quad u = \lfloor \Delta\tau \cdot c_2 \cdot [\mathit{GeneProduct}] \rfloor$$

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P Systems Π_{Hill} – Definition of Components

$$\Pi_{\text{Hill}} = (V_{\text{Genes}}, V_{\text{GeneProducts}}, \Sigma, [_1]_1, L_0, r_1, \ldots, r_k, \mathrm{f}_1, \ldots, \mathrm{f}_k, \Delta\tau, m)$$

- V_{Genes}: alphabet of genes, $V_{\text{Genes}} \cap V_{\text{GeneProducts}} = \emptyset$
- $V_{\text{GeneProducts}}$: alphabet of gene products. Let $V = V_{\text{Genes}} \cup V_{\text{GeneProducts}}$.
- $\Sigma \subseteq V_{\text{GeneProducts}}$: output alphabet
- $[_1]_1$: skin membrane as only membrane
- $L_0 \in \langle V \rangle$: initial configuration, multiset over V
- r_i: reaction rules with initial stoichiometric factors, $i = 1, \ldots, k$
  - $E_{i,0} \subseteq V \times \mathbb{N}$: multiset of r_i reactants at time point 0,
  - $P_{i,0} \subseteq V \times \mathbb{N}$: multiset of r_i products at time point 0,
  - $TF_i \in V_{\text{GeneProducts}}$: set of involved transcription factors
  - $r_i \in \langle E_{i,0} \rangle \times \langle P_{i,0} \rangle \times \mathcal{P}(TF_i)$ whereas
    $\langle A \rangle$: all multisets over A, $\mathcal{P}(A)$: power set over A
- f_i: corresponding function for updating stoichiometric factors
  - $\mathrm{f}_i : \mathbb{R}_+ \times \langle V \rangle \times \mathbb{N}_+ \to \mathbb{N}$ with $(\Delta\tau, L_t, m) \mapsto s$
- $\Delta\tau \in \mathbb{R}_+$: time step between discrete time points t and $t+1$
- $m \in \mathbb{N}_+$: degree of all sigmoid-shaped functions h^+ and h^- used in f_i

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P Systems Π_{Hill} – Dynamical Behaviour

Iteration scheme

- Updating system configuration L_t and
- Stoichiometric factors of reaction rules $r_i \in \langle E_{i,t} \rangle \times \langle P_{i,t} \rangle \times \mathcal{P}(TF_i)$
- Starting from initial configuration L_0

$$L_{t+1} = L_t \ominus Reactants_t \uplus Products_t \quad \text{with}$$

$$Reactants_t = \biguplus_{i=1}^{k} (E_{i,t+1} \cap L_t)$$

$$Products_t = \biguplus_{i=1}^{k} (P_{i,t+1} \cap L_t)$$

$$E_{i,t+1} = \{(e, a') \mid (e, a) \in E_{i,t} \ \wedge a' = \mathrm{f}_i(\Delta\tau, L_t, m)\}$$

$$P_{i,t+1} = \{(q, b') \mid (q, b) \in P_{i,t} \ \wedge b' = \mathrm{f}_i(\Delta\tau, L_t, m)\}$$

Informally:

- Specification of $E_{i,t+1}$ and $P_{i,t+1}$: all reactants e and products q remain unchanged over time, just their stoichiometric factors updated from a to a' (reactants) and from b to b' (products) according to functions f_i

Computational output

- Function $\mathrm{output} : \mathbb{N} \longrightarrow \mathbb{N}$ with $\mathrm{output}(t) = |L_t \cap \{(w, \infty) \mid w \in \Sigma\}|$

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P Systems Π_{Hill} – Introductory Example

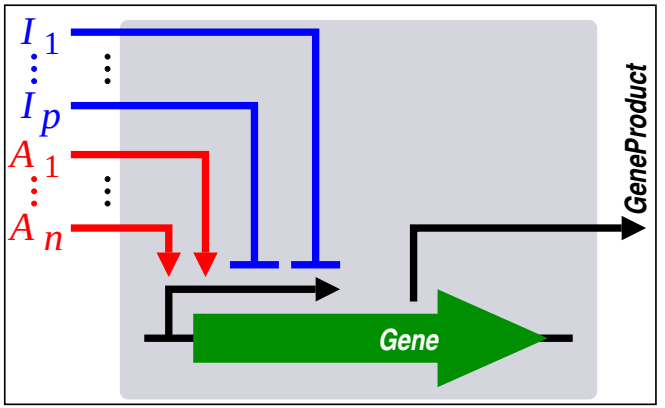
$$\Pi_{\text{Hill,GRNunit}} = (V_{\text{Genes}}, V_{\text{GeneProducts}}, \Sigma, [\,]_1, L_0, r_1, r_2, \mathrm{f}_1, \mathrm{f}_2, \Delta\tau, m)$$

$$V_{\text{GeneProducts}} = \{A_1, \ldots, A_n, \neg I_1, \ldots, \neg I_p, \mathit{GeneProduct}\}$$

$$V_{\text{Genes}} = \{\mathit{Gene}\}$$

$$\Sigma = \{\mathit{GeneProduct}\}$$

$$L_0 = \{(\mathit{Gene}, g), (A_1, a_1), \ldots, (A_n, a_n), (\neg I_1, i_1), \ldots, (\neg I_p, i_p)\}$$



$$r_1 : s_1\ \mathit{Gene} \longrightarrow s_1\ \mathit{GeneProduct} + s_1\ \mathit{Gene}\Big|_{A_1,\ldots,A_n,\neg I_1,\ldots,\neg I_p}$$

$$r_2 : s_2\ \mathit{GeneProduct} \longrightarrow \emptyset$$

$$\mathrm{f}_1(\Delta\tau, L_t, m) = \lfloor \Delta\tau \cdot |L_t \cap \{(\mathit{Gene}, \infty)\}| \cdot \frac{|L_t \cap \{(A_1, \infty)\}|^m}{|L_t \cap \{(A_1, \infty)\}|^m + \Theta_{A_1}^m} \cdot \ldots \cdot \frac{|L_t \cap \{(A_n, \infty)\}|^m}{|L_t \cap \{(A_n, \infty)\}|^m + \Theta_{A_n}^m} \cdot \left(1 - \frac{|L_t \cap \{(\neg I_1, \infty)\}|^m}{|L_t \cap \{(\neg I_1, \infty)\}|^m + \Theta_{\neg I_1}^m} \cdot \ldots \cdot \frac{|L_t \cap \{(\neg I_p, \infty)\}|^m}{|L_t \cap \{(\neg I_p, \infty)\}|^m + \Theta_{\neg I_p}^m}\right) \rfloor$$

$$\mathrm{f}_2(\Delta\tau, L_t, m) = \lfloor \Delta\tau \cdot |L_t \cap \{(\mathit{GeneProduct}, \infty)\}| \rfloor,\ \Delta\tau \in \mathbb{R}_+,\ m \in \mathbb{N}_+$$

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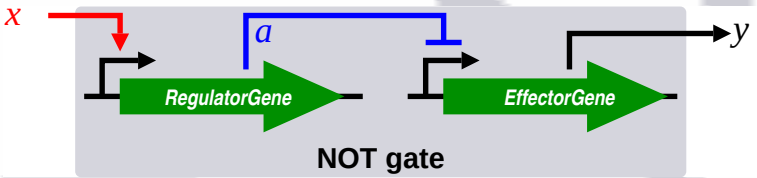
Case Study: Inverter

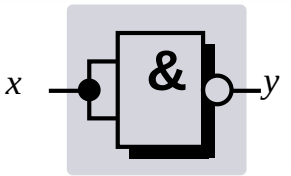
Gene Regulatory Networks as Computational Units

Input: concentration levels of transcription factor x

Output: concentration level of gene product y

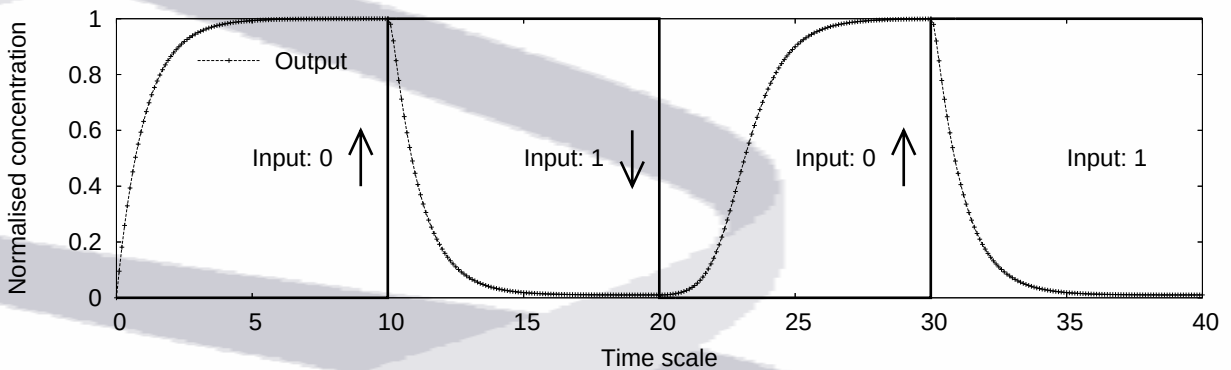


x	y
0	1
1	0



Dynamical behaviour depicted for $m = 2$, $\Theta_j = 0.1$, $j \in \{x, a\}$,

$$a(0) = 0, y(0) = 0, x(t) = \begin{cases} 0 & \text{for } 0 \leq t < 10;\ 20 \leq t < 30 \\ 1 & \text{for } 10 \leq t < 20;\ 30 \leq t < 40 \end{cases}$$



$$\dot{a} = \mathrm{h}^{+}(x, \Theta_x, m) - a$$

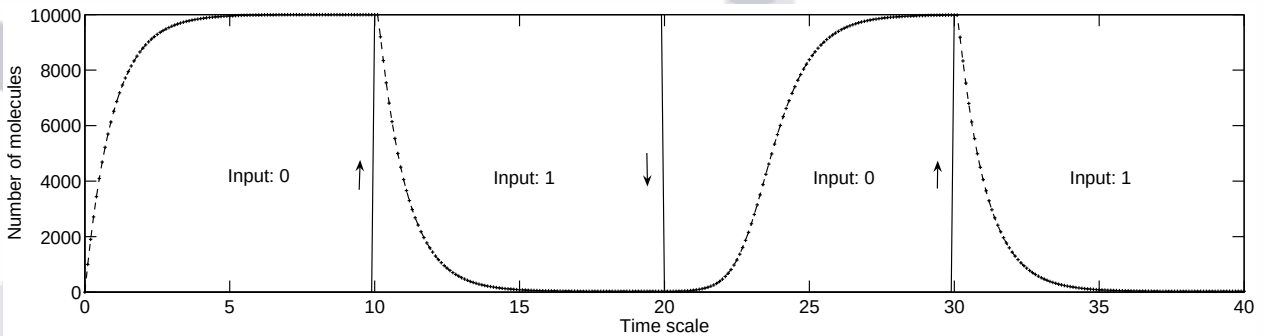
$$\dot{y} = \mathrm{h}^{-}(a, \Theta_a, m) - y$$

Case Study: Inverter

Definition and Simulation of Corresponding P System $\Pi_{\text{Hill,GRNinv.}}$

$$
\begin{aligned}
\Pi_{\text{Hill,GRNinv.}} &= (V_{\text{Genes}}, V_{\text{GeneProducts}}, \Sigma, [\,]_1, L_0, r_1, \ldots, r_4, \mathrm{f}_1, \ldots, \mathrm{f}_4, \Delta\tau, m)\\
V_{\text{Genes}} &= \{\textit{RegulatorGene}, \textit{EffectorGene}\}\\
V_{\text{GeneProducts}} &= \{x, y, \neg a\}\\
\Sigma &= \{y\}\\
L_0 &= \{(\textit{RegulatorGene},rg), (\textit{EffectorGene},eg), (x,x_0), (y,y_0), (\neg a,a_0)\}\\
r_1 &: s_1\ \textit{RegulatorGene} \longrightarrow s_1\ \neg a + s_1\ \textit{RegulatorGene}\ \big|_x\\
r_2 &: s_2\ \neg a \longrightarrow \emptyset\\
r_3 &: s_3\ \textit{EffectorGene} \longrightarrow s_3\ y + s_3\ \textit{EffectorGene}\ \big|_{\neg a}\\
r_4 &: s_4\ y \longrightarrow \emptyset\\
\mathrm{f}_1(\Delta\tau, L_t, m) &= \left\lfloor \Delta\tau \cdot |L_t \cap \{(\textit{RegulatorGene}, \infty)\}| \cdot \frac{|L_t \cap \{(x,\infty)\}|^m}{|L_t \cap \{(x,\infty)\}|^m + \Theta_x^m} \right\rfloor\\
\mathrm{f}_2(\Delta\tau, L_t, m) &= \lfloor \Delta\tau \cdot |L_t \cap \{(\neg a, \infty)\}| \rfloor\\
\mathrm{f}_3(\Delta\tau, L_t, m) &= \left\lfloor \Delta\tau \cdot |L_t \cap \{(\textit{EffectorGene}, \infty)\}| \cdot \left(1 - \frac{|L_t \cap \{(\neg a,\infty)\}|^m}{|L_t \cap \{(\neg a,\infty)\}|^m + \Theta_{\neg a}^m}\right) \right\rfloor\\
\mathrm{f}_4(\Delta\tau, L_t, m) &= \lfloor \Delta\tau \cdot |L_t \cap \{(y, \infty)\}| \rfloor
\end{aligned}
$$

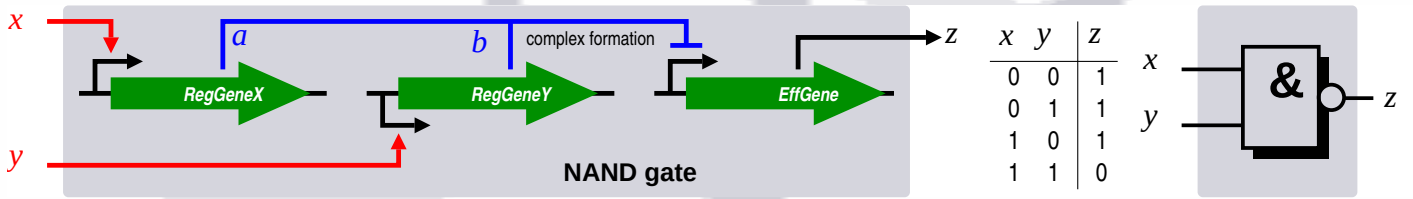
- Dynamical behaviour depicted for $m = 2$, $\Delta\tau = 0.1$, $\Theta_j = 500$, $j \in \{x, \neg a\}$
- $rg = 10,000$, $eg = 10,000$, $x_0 = 0$, $y_0 = 0$, $a_0 = 0$ (MATLAB, P system iteration scheme)



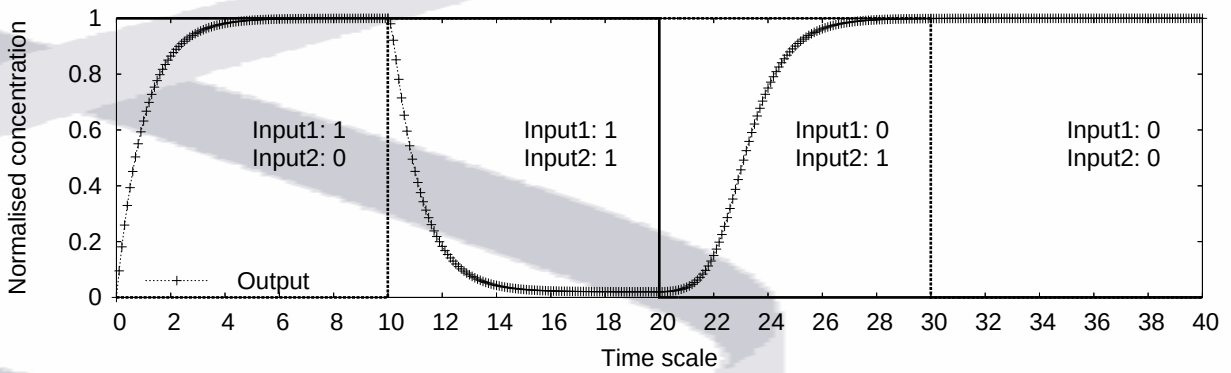
Case Study: NAND Gate

Input: concentration levels of transcription factors x (inp.1), y (inp.2)

Output: concentration level of gene product z



Dynamical behaviour depicted for $m = 2$, $\Theta_j = 0.1$, $j \in \{x, y, a, b\}$



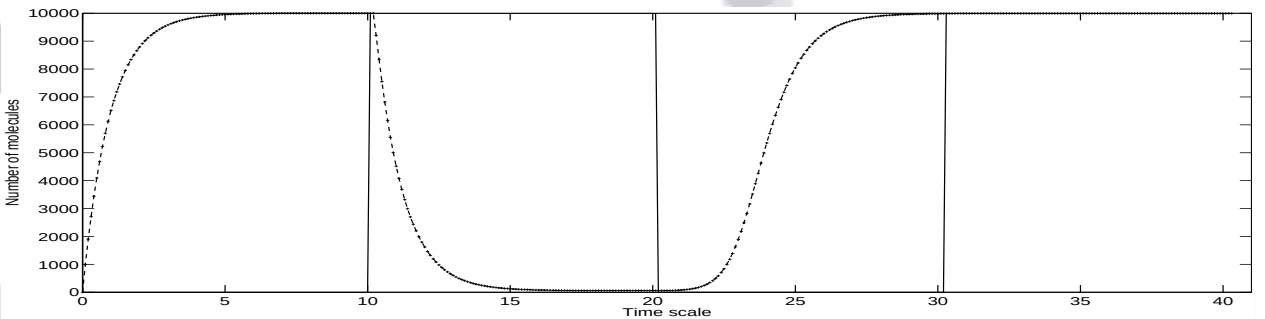
$$\dot{a} = \mathrm{h}^+(x, \Theta_x, m) - a$$
$$\dot{b} = \mathrm{h}^+(y, \Theta_y, m) - b$$
$$\dot{z} = 1 - \mathrm{h}^+(a, \Theta_a, m) \cdot \mathrm{h}^+(b, \Theta_b, m) - z$$

Case Study: NAND Gate

Definition and Simulation of P System $\Pi_{\text{Hill,GRNnand}}$

$$
\begin{aligned}
\Pi_{\text{Hill,GRNnand}} &= (V_{\text{Genes}}, V_{\text{GeneProducts}}, \Sigma, [\,]_1, L_0, r_1, \ldots, r_6, \mathrm{f}_1, \ldots, \mathrm{f}_6, \Delta\tau, m) \\
V_{\text{Genes}} &= \{\mathit{RegGeneX}, \mathit{RegGeneY}, \mathit{EffGene}\} \\
V_{\text{GeneProducts}} &= \{x, y, z, \neg a, \neg b\} \\
\Sigma &= \{z\} \\
L_0 &= \{(\mathit{RegGeneX}, \mathit{rgx}), (\mathit{RegGeneY}, \mathit{rgy}), (\mathit{EffGene}, \mathit{eg}), \\
&\quad (x, x_0), (y, y_0), (z, z_0), (\neg a, a_0), (\neg b, b_0)\} \\
r_1 &: s_1\ \mathit{RegGeneX} \longrightarrow s_1\ \neg a + s_1\ \mathit{RegGeneX}\ \big|_x \\
r_2 &: s_2\ \neg a \longrightarrow \emptyset \\
r_3 &: s_3\ \mathit{RegGeneY} \longrightarrow s_3\ \neg b + s_3\ \mathit{RegGeneY}\ \big|_y \\
r_4 &: s_4\ \neg b \longrightarrow \emptyset \\
r_5 &: s_5\ \mathit{EffGene} \longrightarrow s_5\ z + s_5\ \mathit{EffGene}\ \big|_{\neg a, \neg b} \\
r_6 &: s_6\ z \longrightarrow \emptyset \\
&\vdots
\end{aligned}
$$

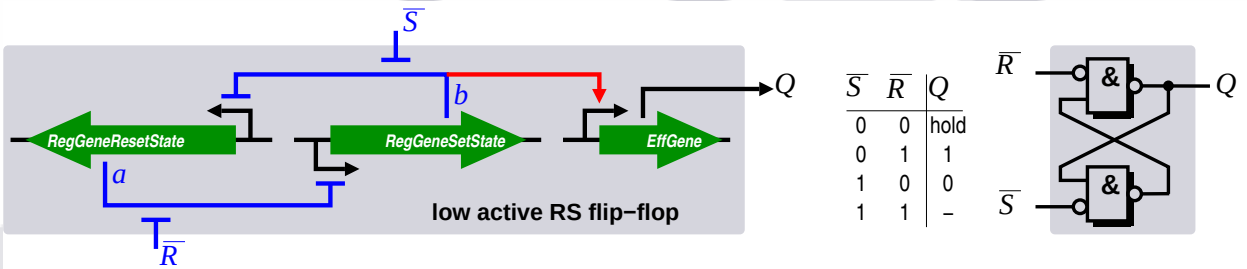
- **Simulation result** (MATLAB, P system iteration scheme)
- Dynamical behaviour depicted for $m = 2$, $\Delta\tau = 0.1$, $\Theta_j = 500$, $j \in \{x, y, \neg a, \neg b\}$
- $\mathit{rgx} = 10,000$, $\mathit{rgy} = 10,000$, $\mathit{eg} = 10,000$, $x_0 = 0$, $y_0 = 0$, $z_0 = 0$, $a_0 = 0$, $b_0 = 0$



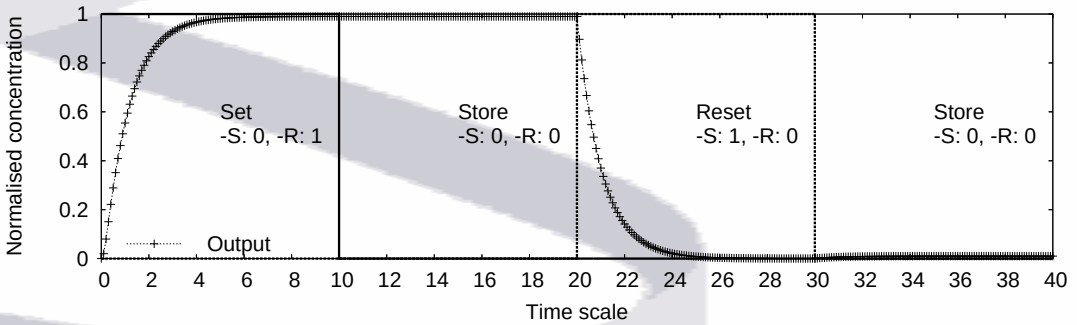
Case Study: RS Flip-Flop

Input: concentration levels of transcription factors $\overline{S}, \overline{R}$

Output: concentration level of gene product Q



Dynamical behaviour depicted for $m = 2$, $\Theta_j = 0.1$, $j \in \{a, b, \overline{R}, \overline{S}\}$



$$\dot{a} = 1 - \mathrm{h}^+(b, \Theta_b, m) \cdot \mathrm{h}^-(\overline{S}, \Theta_{\overline{S}}, m) - a$$

$$\dot{b} = 1 - \mathrm{h}^+(a, \Theta_a, m) \cdot \mathrm{h}^-(\overline{R}, \Theta_{\overline{R}}, m) - b$$

$$\dot{Q} = \mathrm{h}^+(b, \Theta_b, m) \cdot \mathrm{h}^-(\overline{S}, \Theta_{\overline{S}}, m) - Q$$

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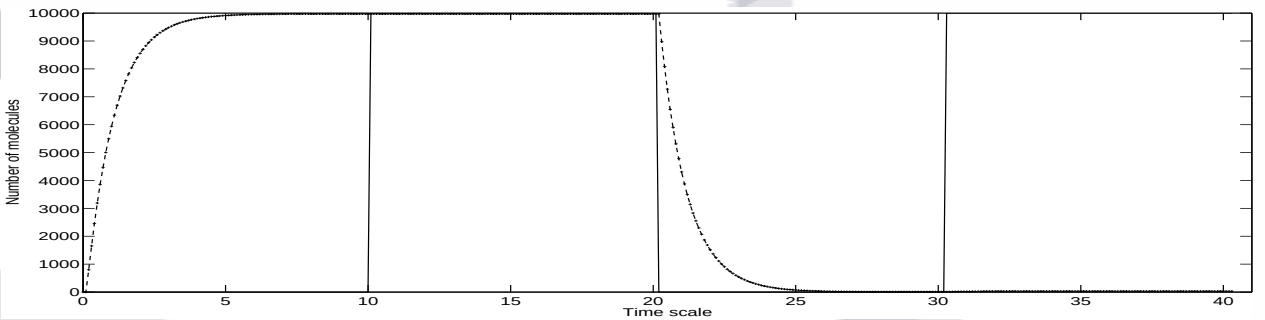
Case Study: RS Flip-Flop

Definition and Simulation of P System $\Pi_{\text{Hill,GRNrsff}}$

$$
\begin{aligned}
\Pi_{\text{Hill,GRNrsff}} &= (V_{\text{Genes}}, V_{\text{GeneProducts}}, \Sigma, [_1]_1, L_0, r_1, \ldots, r_6, \mathrm{f}_1, \ldots, \mathrm{f}_6, \Delta\tau, m) \\
V_{\text{Genes}} &= \{\mathit{RegGeneResetState}, \mathit{RegGeneSetState}, \mathit{EffGene}\} \\
V_{\text{GeneProducts}} &= \{Q, \neg\overline{S}, \neg\overline{R}, \neg a, \neg b\} \\
\Sigma &= \{Q\} \\
L_0 &= \{(\mathit{RegGeneResetState}, rgr), (\mathit{RegGeneSetState}, rgs), \\
&\quad (\mathit{EffGene}, eg), (Q, q_0), (\overline{S}, ss_0), (\overline{R}, rs_0), (\neg a, a_0), (\neg b, b_0)\} \\
r_1 &: s_1\ \mathit{RegGeneResetState} \longrightarrow s_1\ \neg a + s_1\ \mathit{RegGeneResetState}\big|_{\neg\overline{S},\neg b} \\
r_2 &: s_2\ \neg a \longrightarrow \emptyset \\
r_3 &: s_3\ \mathit{RegGeneSetState} \longrightarrow s_3\ \neg b + s_3\ \mathit{RegGeneSetState}\ \big|_{\neg\overline{R},\neg a} \\
r_4 &: s_4\ \neg b \longrightarrow \emptyset \\
r_5 &: s_5\ \mathit{EffGene} \longrightarrow s_5\ Q + s_5\ \mathit{EffGene}\ \big|_{\neg\overline{S},\neg b} \\
r_6 &: s_6\ Q \longrightarrow \emptyset \\
&\vdots
\end{aligned}
$$

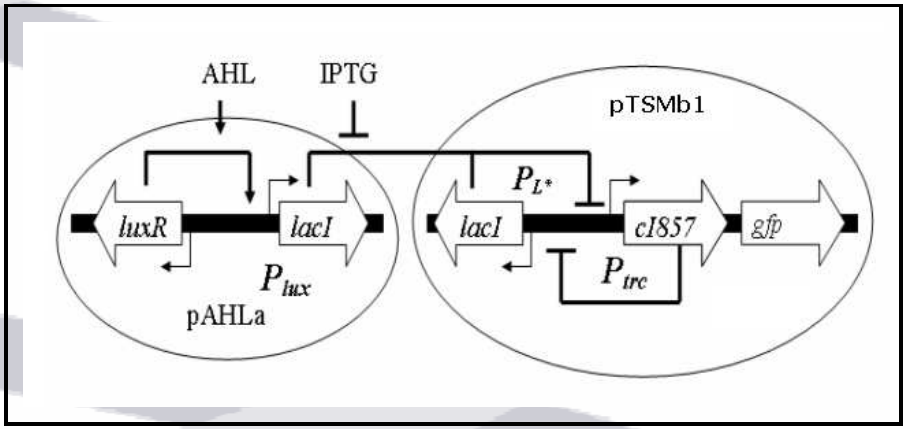
- **Simulation Result** (MATLAB, P system iteration scheme)
- Dynamical behaviour depicted for $m = 2$, $\Delta\tau = 0.1$, $\Theta_j = 500$, $j \in \{\neg a, \neg b, \neg\overline{R}, \neg\overline{S}\}$
- $rgr = 10,000$, $rgs = 10,000$, $eg = 10,000$, $q_0 = 0$, $ss_0 = 0$, $rs_0 = 0$, $a_0 = 0$, $b_0 = 0$



Wetlab Implementation of GRN-Based RS Flip-Flop

Experimental Setup

- *in vivo* system (bistable toggle switch in *Vibrio fischeri*) mimics RS flip-flop
- Encoding of all genes using two constructed plasmids
- Quantification of its performance using flow cytometry
- Presence or absence of inducers AHL and IPTG acts as input signals, green fluorescent protein (*gfp*) as output



Collaboration with S. Hayat, at this time Dresden University of Technology, BIOTEC laboratories.
Thanks to J.J. Collins, W. Pompe, G. Rödel, K. Ostermann, L. Brusch for their support.

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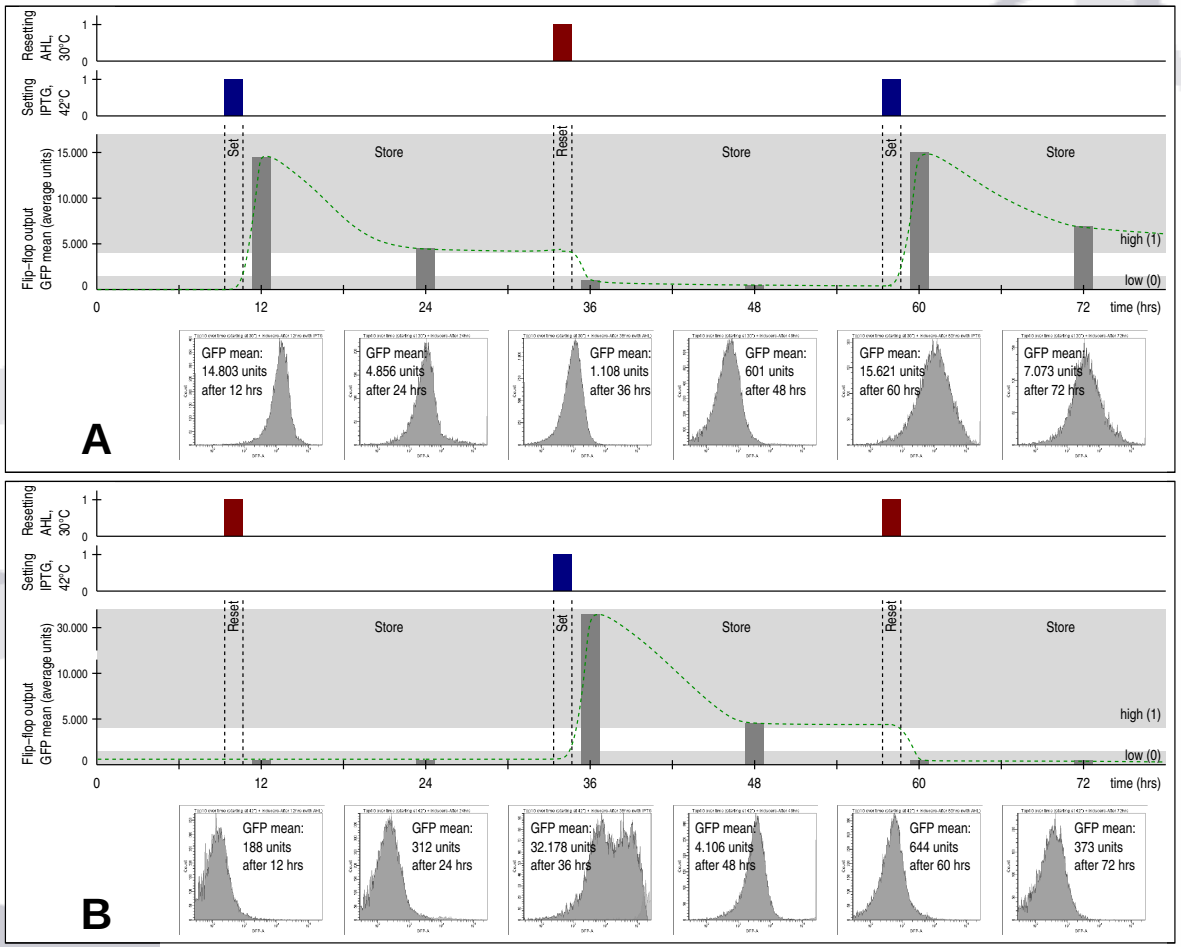
Wetlab Experimental Results



Repeated activation and deactivation of the toggle switch based on inducers and temperature. Temperature was switched every 24 hours. Cells were incubated with inducers for 12 hours, followed by growth for 12 hours without inducers, initially kept at 30°C (**A**) and 42°C (**B**). The cells successfully switched states thrice.

Collaboration with S. Hayat, at this time Dresden University of Technology, BIOTEC laboratories.
Thanks to J.J. Collins, W. Pompe, G. Rödel, K. Ostermann, L. Brusch for their support.

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Discussion and Conclusion

Discussion

- Gap between idealised models and observed wetlab data
- Consider GRN of the **whole** microorganism rather than isolated part
- Granularity of simulation
- Undersatisfy problem: avoid conflicts between reactions at low reactant amounts

Conclusion

- P systems framework for applications in systems biology:
- Map reaction kinetics to P systems with dynamical behaviour
- Exemplified by transformation of Hill kinetics for GRNs
- Computability issues addressed by logic gates as simple GRNs

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Further Work

Theory

- Integrate structural information into Π_{Hill}, extend symbol objects to string objects
- Introduce matching strategies based on string objects
- Unify Π_{Hill} with Π_{CSN} (presented at WMC7)

Computational Applications

- Design GRNs for solution to NP-complete problems and for emulating behaviour of different automata (first results)
- Emerge artificial GRNs by evolutionary computation

Wetlab

- Coupling of several GRN-based computational units *in vivo*
- Coping with side effects (signal weakening)
- Grant application in preparation.
  Interested in participation? hinze@minet.uni-jena.de

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