P systems–based Modelling of Cellular Signalling Pathways

Mario J. Pérez-Jiménez

Research Group on Natural Computing
Dpt. of Computer Science and Artificial Intelligence, University of Sevilla
Avda. Reina Mercedes s/n, 41012 Sevilla, Spain
Email: marper@us.es

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Summary
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- Modelling biological processes.
- P systems to simulate biosignalling cascades.
- Strategies for the evolution.
- Modelling EGFR signalling.
- Modelling FAS–apoptosis.
- Conclusions.
Modelling biological processes
Model: abstraction of the real world onto a mathematical/computational domain

- Relevance
- Computability
- Understandability
- Extensibility

P systems satisfy the above properties.
Intracellular signalling pathways are vital to the control and regulation of cell behaviour.

- The EGFR is the best-understood receptor system (target of successful therapies against cancer).
- The FAS–induced apoptotic signalling pathway is a relevant process for combating cancer, AIDS and neurodegenerative diseases.
- Ordinary differential equations have been used to model kinetics of conventional *macroscopic* chemical reactions.
- Different agent–based approaches are being used to model biological systems.
- *Microscopic* approach.
- *Mesoscopic* approach.
P system–based model

- A syntactic framework.
- A strategy
Some strategies

- Deterministic waiting times.
- Multi–compartmental Gillespie’s algorithm.
- Continuous application of the rules.
- P metabolic algorithm.
- Dynamical probabilistic algorithm.
- ....
The syntactic framework

Π = (O, L, µ, M₁, M₂, ..., Mₙ, R₁, ..., Rₙ)

(a) Transformation, complex formation and dissociation rules:

[α] → [β]
[α, β] → [γ]
[α] → [β, γ]

(b) Diffusing in and out:

[α] → α
α[ ] → [α]
The syntactic framework

\[ \Pi = (O, L, \mu, M_1, M_2, \ldots, M_n, R_1, \ldots, R_n) \]

(a) Transformation, complex formation and dissociation rules:

\[ [a]_I \rightarrow [b]_I \]
\[ [a, b]_I \rightarrow [c]_I \]
\[ [a]_I \rightarrow [b, c]_I \]

(b) Diffusing in and out:

\[ [a]_I \rightarrow a [ ]_I \]
\[ a [ ]_I \rightarrow [a]_I \]
(c) \textit{Binding and debinding rules:}
\[
\begin{align*}
a \ [ \ b \ ]_l & \rightarrow \ [ \ c \ ]_l \\
[ \ a \ ]_l & \rightarrow b \ [ \ c \ ]_l
\end{align*}
\]

(d) \textit{Recruitment and releasing rules:}
\[
\begin{align*}
a \ [ \ b \ ]_l & \rightarrow c \ [ \ ]_l \\
c \ [ \ ]_l & \rightarrow a \ [ \ b \ ]_l
\end{align*}
\]
P Systems using DWT Algorithm

- Deterministic approach
- Deterministic waiting times algorithm
  - In vivo chemical reactions take place in parallel in an asynchronous manner
How to compute mesoscopic rate constants from the macroscopic ones used in differential equations.

- Our rules model reactions of the form: \( A + B \xrightleftharpoons[k_d]{k_a} C \)
- The equilibrium constant:

\[
K_{eq} = \frac{[C]}{[A] \cdot [B]} \quad (1)
\]

\[
K_{eq} = \frac{k_a}{k_d} \quad (2)
\]

- Gibbs free energy is related to the equilibrium constant \( K_{eq} \):

\[
K_{eq} = \exp\left(\frac{-\Delta G}{R \cdot T}\right) \quad (3)
\]

where \( R = 1.9872 \text{ cal mol}^{-1} \text{ Kelvin}^{-1} \) is the universal gas constant and \( T \) is the absolute temperature.
Our approach uses mesoscopic rate constants: they are determined from their macroscopic counterparts:

\[ c_a = \frac{k_a}{A \cdot V}; \quad c_d = k_d \]

where \( A = 6.023 \cdot 10^{23} \) is Avogadro’s number and \( V \) is the cell volume.

Each rule \( r \) has associated a velocity, \( v_r \), by multiplying \( c_r \) by the multiplicities of the reactants.

The waiting time for the first execution of the rule \( r \) is \( \tau_r = \frac{1}{v_r} \).
Deterministic waiting times algorithm

1. Calculate the WT for all the rules in all the membranes
2. Sort the rules according their WT.
3. Select the membrane which has associated the rule with minimal WT.
4. Apply that rule only once.
5. Recalculate the WT only for those rules which are in the compartments affected by the applied rule.
6. For each such rule compare the new WT with existing WT and keep the smallest one among the two.
7. Repeat steps 2 to 6 until the time of the simulation $t$ reaches or exceeds a prefixed time.
Modelling EGFR Signalling

- The epidermal growth factor receptor (EGFR) belongs to the tyrosine kinase family of receptors.
- Binding of the EGF induces receptor dimerisation and autophosphorylation of intracellular domains.
- A multitude of proteins are recruited starting a complex signalling cascade.
- The receptor follows a process of internalisation, ubiquitination and degradation in endosomes.
Two marginal pathways:

- First: C-\(\gamma\) (PLC\(\gamma\)).
- Second: PI3K.

Two principal pathways (leading to activation of Ras-GTP):

- First: It is a cycle where Grb2 and SOS bind to the phosphorylated receptor. Later, the complex Grb2-SOS is released in the cytoplasm.
- Second: Shc binds to the receptor and it is phosphorylated. Then either Shc* is released in the cytoplasm or the proteins Grb2 and SOS binds to the receptor yielding a protein complex (EGFR-EGF2*-Shc*-Grb2-SOS).
★ Ras-GTP is activated by these two pathways and in turn it stimulates the Mitogen Activated Protein (MAP) kinase.
★ Phosphorylated ERK regulates several cellular proteins and nuclear transcription factors.
★ There exist cross-talks.
The model

\[ \Pi_{\text{EGF}} = (O, \{ e, s, c \}, \mu, (w_1, e), (w_2, s), (w_3, c), R_e, R_s, R_c) \]

- Alphabet:

<table>
<thead>
<tr>
<th>Object</th>
<th>Protein or Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF</td>
<td>Epidermal Growth Factor</td>
</tr>
<tr>
<td>EGFR</td>
<td>EGF Receptor</td>
</tr>
<tr>
<td>EGFR-EGF \textsubscript{2}</td>
<td>Dimerazated Receptor</td>
</tr>
<tr>
<td>EGFR-EGF \textsubscript{2}*-Shc</td>
<td>EGFR-EGF \textsubscript{2}* and Shc complex</td>
</tr>
<tr>
<td>MEK</td>
<td>Mitogenic external regulated kinase</td>
</tr>
<tr>
<td>ERK</td>
<td>External regulated Kinase</td>
</tr>
</tbody>
</table>
• **Membrane Structure:** the *environment*, the *cell surface* and the *cytoplasm*.
• **Initial Multisets:** number of molecules of the chemical substances in the regions (Moehren, Schoeberl et al. 2002).

\[
w_1 = \{ \text{EGF}^{200} \} \\
w_2 = \{ \text{EGFR}^{250}, \text{Ras-GDP}^{200} \} \\
w_3 = \{ \text{Shc}^{250}, \text{PLC}_{\gamma}^{150}, \text{PI3K}^{50}, \text{SOS}^{40}, \text{Grb2}^{80}, \text{TP}_1^{100}, \text{TP}_2^{450}, \text{TP}_3^{450}, \text{TP}_4^{125}, \text{Raf}^{80}, \text{MEK}^{400}, \text{ERK}^{400}, \text{P}_1^{80}, \text{P}_2^{80}, \text{P}_3^{300} \} \]
• **Rules:** Two examples.

  The set of rules associated with the environment consists only of one rule \( r \):

  \[
  EGF \ [\ EGFR \]_s \rightarrow \ [\ EGF-EGFR \]_s , \quad k = 0.003 \ nM^{-1} s^{-1}
  \]

  The waiting time associated to \( r \) is:

  \[
  \tau_r = \frac{1}{0.003 \cdot |EGF| \cdot |EGFR|}
  \]
One example from the set of rules associated to the cell surface:

\[
[\text{EGFR}, \text{EGFR}]_s \rightarrow [\text{EGFR}_2]_s, \quad k = 0.011 \text{ nM}^{-1}\text{s}^{-1}
\]

The waiting time associated to \( r' \) is:

\[
\tau_{r'} = \frac{1}{0.011 \cdot |\text{EGFR}|^2}
\]
Results and discussions

Evolution of the number of autophosphorylated receptors.
Evolution of the number of doubly phosphorylated MEK.
Overexpression of EGF in the environment and EGFR on the cell surface of cancerous cells.

We investigate the effect of different EGF concentrations and number of receptors on the signalling cascade.
The receptor autophosphorylation is clearly concentration dependent.

It is to expect different cell responses to different EGF concentrations.
The number of doubly phosphorylated MEK does not depend on the number of signals in the environment.
Evolution of the number of doubly phosphorylated MEK when there is 100 nM and 1000 nM of receptors on the cell surface.
Modelling FAS-apoptosis

Apoptosis, programmed cell death (Kerr, Willie, Currie, 1972) Apoptosis is a cellular response to a cellular insult. It is mediated by a family of proteases called caspases.

- caspase 8, caspase 9 and caspase 10: initiators
- caspase 3, caspase 6 and caspase 7: effectors

Other effector molecule in apoptosis is Apaf-1.
The other regulators of apoptosis are the Bcl2 family members.

- Bcl2 and Bcl-xL, have an anti-apoptotic function.
- Bax, Bak, Bid and Bad, are pro-apoptotic molecules.

Two pathways activated by FAS have been identified (Scaffidi et al. 1998):

- Type I: *death receptor pathway*.
- Type II: *(mitochondrial pathway)*.
The model

\[ \Pi_{FAS} = (O, \{e, s, c, m\}, \mu, (w_1, e), (w_2, s), (w_3, c), (w_4, m), R_e, R_s, R_c, R_m) \]

- **Alphabet:**

<table>
<thead>
<tr>
<th>Object</th>
<th>Protein or Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAS</td>
<td>Fas protein, member of the Tumor Necrosis Factor family</td>
</tr>
<tr>
<td>FASL</td>
<td>Fas Ligand</td>
</tr>
<tr>
<td>FADD</td>
<td>Fas–associating protein with death domain</td>
</tr>
<tr>
<td>:</td>
<td>:</td>
</tr>
<tr>
<td>Apaf</td>
<td>Apoptotic protease activating factor</td>
</tr>
<tr>
<td>Smac</td>
<td>Second mitochondria–derived activator of caspase</td>
</tr>
<tr>
<td>XIAP</td>
<td>X–linked inhibitor of apoptosis protein</td>
</tr>
</tbody>
</table>
• Membrane Structure: the environment, the cell surface, the cytoplasm and the mitochondria.

• Initial Multisets: number of molecules of the chemical substances in regions (Hua et al. 2005).

\[ w_1 = \{ FASL^{12500} \} \]
\[ w_2 = \{ FAS^{6023} \} \]
\[ w_3 = \{ FADD^{10040}, CASP8^{20074}, FLIP^{48786}, CASP3^{120460}, Bid^{15057}, Bax^{50189}, XIAP^{18069}, Apaf^{60230}, CASP9^{12046} \} \]
\[ w_4 = \{ Smac^{60230}, Cyto.c^{60230}, Bcl2^{45172} \} \]
**Rules:** An example.
The set of rules associated with the environment consists only of one rule \( r_1 \).

\[
FASL [ FAS ]_s \rightarrow [ FASC ]_s, \quad k_1 = 9.09E - 05 \text{ nM}^{-1}\text{s}^{-1}
\]

The waiting time associated to \( r_1 \) is

\[
\tau_{r_1} = \frac{1}{9.09E - 05 \cdot |FASL| \cdot |FAS|}
\]
Results and discussions

- We implemented in Java a simulator for the P system.
- It accepts as input an SBML file containing the rules and initial concentrations for the molecules in the system.
- We compared our results with both the experimental data and with the ODEs simulation data reported in Hua et al.

(Effects of Bcl-2 levels on FAS signalling-induced caspase-3 activation, *The Journal of Immunology*, 175, 2 (2005), 985-995).
Caspase 3 was compared to the experimental data.
There are cells which are not sensitive to Bcl2 overexpression: in these cells caspase 8 directly activates caspase 3.

Scaffidi et al. (1998) has suggested that the type of pathway is chosen based on the concentration of caspase 8 generated in active form following FASL binding.

We check this hypothesis.
Bcl2 is known to block the mitochondrial pathway; however, it is not clear the mechanism through which Bcl2 can block the pathway of type II.

We analyze the caspase 3 activation kinetics in this pathway by considering different mechanisms to block the mitochondrial pathway: Bcl2 might bind with (a) Bax, (b) Bid, (c) tBid, or (d) bind to both Bax and tBid.
We design four different P systems having the rules:

- $r_1, \ldots, r_{95}, r_{96}, r_{97}$ for the case (a).
- $r_1, \ldots, r_{95}, r'_{96}, r'_{97}$ for the case (b).
- $r_1, \ldots, r_{95}, r''_{96}, r''_{97}$ for the case (c).
- $r_1, \ldots, r_{97}, r_{98}, r_{99}$ for the case (d).

All the other rules remain the same for all the cases.
Bcl2 binding to both Bax and tBid is the most efficient mechanism for the pathway in comparison with the results obtained for the cases (a), (b) or (c).
Conclusions

- P systems as a new computational modelling tool for the dynamic behaviour of integrated signalling systems.
- P systems are also general specification of the biological phenomena that can be evolved using different strategies.
- The strategy DTW algorithm has been introduced, and illustrated with the simulation of two relevant biological phenomena.
- Guide to combining models and experiments to understand complex biological processes as integrated systems.
- Our results show good correlation with the experimental data reported in the literature and with simulators based on ODEs.
Thank You!