Modelling EGFR signalling network using continuous membrane systems

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Abstract. The complexity of networks of biological signalling pathways is such that the development of simplifying models is essential in trying to understand the wide-ranging cellular responses they can generate. In this paper a continuous variant of membrane systems is introduced and used to model the epidermal growth factor receptor signalling network which is known to play a key role in tumour cell proliferation, angiogenesis and metastasis.

Keywords: membrane computing, EGFR signalling network, signal transduction

1 Introduction

Membrane Computing is an emergent branch of Natural Computing introduced by G. Păun in [7]. Since then it has received an important attention from the scientific community. In fact, Membrane Computing has been selected by the Institute for Scientific Information, USA, as a fast *Emerging Research Front* in Computer Science, and [6] was mentioned in [11] as a highly cited paper in October 2003.

This new non-deterministic model of computation starts from the assumption that the processes taking place in the compartmental structure of a living cell can be interpreted as computations. The devices of this model are called *P systems*. Roughly speaking, a P system consists of a cell-like membrane structure, in the compartments of which one places multisets of objects which evolve according to given rules in a synchronous non-deterministic maximally parallel manner.

Most variants of membrane systems have been proved to be computationally complete, that is equivalent in power to Turing machines, and computationally efficient, that is being able to solve computationally hard problems in polynomial time. P systems as a discrete model of computation have also been used to model biological phenomena (see the volume in [1]); and as a continuous model in [5]. A first formalization of non-discrete P system and a way to approximate them was introduced in [2].

In this paper we introduce a continuous variant of P systems different from that in [5] and we use it to model the epidermal growth factor receptor (EGFR) signalling network. Up to now the usual mathematical formalization of biochemical signalling networks has been done using differential equations. This paper introduces a novel formalization of this phenomena in a computational framework.

The epidermal growth factor receptor (EGFR) belongs to the tyrosine kinase family of receptors. Binding of the epidermal growth factor (EGF) to the extracellular domain of EGFR induces receptor dimerization and autophosphorylation of intracellular domains. Then a multitude of signaling proteins are recruited starting a complex signalling cascade that transfers the activation signal from the receptor to the nucleus. Dysregulated EGFR expression, ligand production and signalling have been proved to have a strong association with tumourgenesis. As a result of this, EGFR has been identified as a key biological target for the development of novel anticancer therapies.

The paper is organised as follows. Continuous P systems are introduced in the next section. In section 3 we discuss how continuous P systems can be approximated by discrete systems in order to implement them in computers; a description of the EGF signalling network is given in section 4. In section 5 the model of the EGF signalling network is presented. Some results are exposed in the next section. Finally, conclusions and future work are given in the last section.

2 Continuous P Systems

Usual variants of P systems are discrete models of computation where in every step the rules are applied in a maximal way an integer number of times. Here we introduce a variant whose systems can evolve in every instant applying a maximal set of rules a positive real number of times determined by a function $\mathcal{K}$. This variant is inspired by the fact that in vitro chemical reactions evolve in a continuous way following a *rate* that depends on the concentration of the reactants.

Roughly speaking, a continuous P system consists in a membrane structure, a hierarchically arranged set of membranes. More formally, a membrane structure is a rooted tree, where the nodes are called membranes, the root is called skin, and the leaves are called elementary membranes. Informally we can represent a membrane structure using Venn diagrams.

In the membrane structure one places multisets of objects; usual P systems deal with discrete multisets but here we work with continuous multisets. A *continuous multiset* over an alphabet Σ is a mapping from Σ to $\mathbf{R}^+$ where $\mathbf{R}^+ = \{x \in \mathbf{R} \,:\, x \geq 0\}$.

Next we give a formal definition of continuous P systems. A *continuous* P *system* is a construct, $\mathbf{\Pi} = (\Sigma, \mu, w_{1,0}, \ldots, w_{n,0}, \mathcal{R}, \mathcal{K})$, where:

1. $n \geq 1$ is the degree of the system (number of membranes);
2. $\Sigma = \{c_1, \ldots, c_m\}$ is the alphabet of *objects*;

3. μ is a *membrane structure* consisting of n membranes labelled with $1, \ldots, n$ (usually, we identify the membranes with labels from a finite set H).
4. $w_{1,0}, \ldots, w_{n,0}$ are continuous multisets associated with each membrane of the membrane structure μ
5. $\mathcal{R}$ is a finite set of *rules* of the form $r \equiv (u, v, u', v', i)$ where $u, v, u', v' \in \Sigma^*$, and $1 \leq i \leq n$. We represent a rule r as follows:

$$u\ [\ v\ ]_i\ \rightarrow\ u'[\ v'\ ]_i$$

   Notation: $(r)_1 = u$; $(r)_2 = v$; $(r)_3 = u'$; $(r)_4 = v'$ and $(r)_5 = i$.
6. $\mathcal{K}$ is the rate of application function which associates with each rule and multiplicity of the objects in μ the rate of application of the rule:

$$\mathcal{K} : \mathcal{R} \times \mathcal{M}_{n\times m}(\mathbf{R}^+) \rightarrow \mathbf{R}^+$$

   where $\mathcal{M}_{n\times m}(\mathbf{R}^+)$ is the set of matrixes of order $n \times m$ over $\mathbf{R}^+$.

For usual P systems we talk about computations but for continuous P systems we prefer to think of evolutions. An *evolution* of a continuous P system is a mapping from $\mathbf{R}^+$ to $\mathcal{M}_{n\times m}(\mathbf{R}^+)$.

A *configuration* of a continuous P system $\mathbf{\Pi}$ at an instant $t \in \mathbf{R}^+$ is a matrix of order $n \times m$ over $\mathbf{R}^+$. We interpret the configurations as assignments of continuous multisets to the membranes of the system.

Thus, an evolution E of a continuous P system associates with each $t \in \mathbf{R}^+$ a configuration $E(t)$ of the system at the instant t.

$$E(t) = (a_{i,j}(t))_{\substack{1 \leq i \leq n \\ 1 \leq j \leq m}}$$

For each i, $1 \leq i \leq n$, we denote by $w_i(t)$ the continuous multisets over $\Sigma = \{c_1, \ldots, c_m\}$ defined as follows: $(w_i(t))(c_j) = a_{ij}(t)$ for $1 \leq j \leq m$.

In order to describe how to determine the evolution of a P system we need to define the *relevant rules to a membrane*.

Given a continuous P system, $\mathbf{\Pi} = (\Sigma, \mu, w_{1,0}, \ldots, w_{n,0}, \mathcal{R}, \mathcal{K})$, and a membrane i $(1 \leq i \leq n)$ we denote:

$$\begin{aligned} \mathcal{R}_i &= \{r\ :\ (r)_5 = i\}, \\ \mathcal{R}_i^* &= \{r\ :\ f((r)_5) = i\}, \end{aligned}$$

where $f((r)_5)$ is the father of the membrane $(r)_5$ in the membrane structure μ. We say that the rules in $\mathcal{R}_i \cup \mathcal{R}_i^*$ are the *relevant rules* to the membrane i.

The way a continuous P system, $\mathbf{\Pi} = (\Sigma, \mu, w_{1,0}, \ldots, w_{n,0}, \mathcal{R}, \mathcal{K})$, evolves is determined by the initial multisets $w_{1,0}, \ldots, w_{n,0}$ and the rate of application function $\mathcal{K}$. We define the *initial configuration* of $\mathbf{\Pi}$ as the assignment of the continuous multisets $w_{1,0}, \ldots, w_{n,0}$ to the membranes $1, \ldots, n$ of the system.

The rules are applied during the evolution of the system according to $\mathcal{K}$ following the next criterion. At an instant $t \in \mathbf{R}^+$, a rule $r \in \mathcal{R}$ is applied $\mathcal{K}(r, E(t))$ *times*; that is $\mathcal{K}(r, E(t))$ units of the reactants are consumed and

$\mathcal{K}(r, E(t))$ units of the products are produced. In this sense we can say that the rules are applied in a $\mathcal{K}$-maximal way.

More precisely, given an object $c_j \in \Sigma$, $1 \leq j \leq m$, and a membrane i, $1 \leq i \leq n$, the real number $(w_i(c_j))(t)$, denoted by $|c_j|_i(t)$, is determined by the next formula:

$$|c_j|_i(t) = |c_j|_i(0) + \sum_{r \in R_i \wedge c_j \in alph((r)_4)} \int_0^t \mathcal{K}(r, \mathbf{\Pi}(s))\, ds + \quad (1)$$

$$+ \sum_{r \in R_i^* \wedge c_j \in alph((r)_3)} \int_0^t \mathcal{K}(r, \mathbf{\Pi}(s))\, ds - \quad (2)$$

$$- \sum_{r \in R_i \wedge c_j \in alph((r)_2)} \int_0^t \mathcal{K}(r, \mathbf{\Pi}(s))\, ds - \quad (3)$$

$$- \sum_{r \in R_i^* \wedge c_j \in alph((r)_1)} \int_0^t \mathcal{K}(r, \mathbf{\Pi}(s))\, ds \quad (4)$$

Observe that on the one hand the effect of the application of the rules in (1) and (2) increases $|c_j|_i(t)$ because c_j appears in the right-hand side of the rules (c_j is a product) but on the other hand (3) and (4) decrease $|c_j|_i(t)$ because c_j appears in the left-hand side of the rules (c_j is a reactant).

3 Approximating Continuous P Systems

In computers real numbers are represented by a finite set of rational numbers. Therefore like in most continuous models we need to develop approximations in order to simulate evolutions of continuous P systems in computers.

As shown in the previous section in order to determine the configuration of a system at given instant t we only need to compute an integral of the rate of application function $\mathcal{K}$. In consequence to approximate continuous P systems in a finite set of instants $t_0, \cdots, t_q$ we can use any suitable known numerical method to approximate integrals. Here for simplicity we use the rectangle rule; that is, we suppose $t_{l+1} - t_l = p$ is *small enough* to assume that $\mathcal{K}$ remains constant and equal to $\mathcal{K}(r, E(t_l))$ in the interval $[t_l, t_{l+1}]$ for $l = 0, \ldots, q-1$. With this assumption we design the next method which gives $E(t_0), \ldots, E(t_q)$.

$|c_j|_i(0) = w_{i,0}(c_j)$

$$|c_j|_i(t_{l+1}) = |c_j|_i(t_l) + \sum_{r \in R_i \wedge c_j \in alph((r)_4)} p\, \mathcal{K}(r, \mathbf{\Pi}(t_l)) +$$

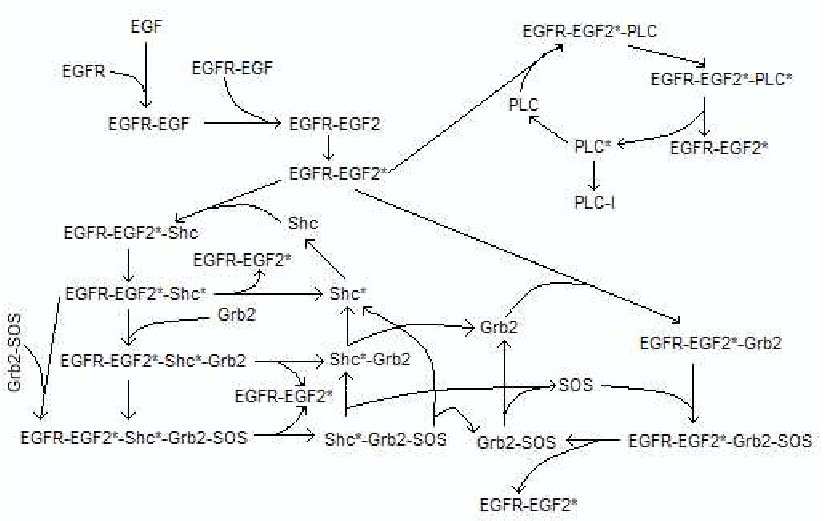
$$+ \sum_{r \in R_i^* \wedge c_j \in alph((r)_3)} p\, \mathcal{K}(r, \mathbf{\Pi}(t_l)) -$$

$$- \sum_{r \in R_i \wedge c_j \in alph((r)_2)} p\, \mathcal{K}(r, \mathbf{\Pi}(t_l)) -$$

$$- \sum_{r \in R_i^* \wedge c_j \in alph((r)_1)} p\, \mathcal{K}(r, \mathbf{\Pi}(t_l))$$

4 EGFR Signalling Network

In this section we describe the part of the $EGFR$ signalling network depicted below which only considers three coupled cycles of interactions between the phosphotyrosine residues of the $EGFR$ and three cytoplasmic proteins, namely $Grb2$, Shc and PLC_γ.



The epidermal growth factor receptor ($EGFR$) belongs to the family of protein-tyrosine kinase receptors, which regulate cell growth, survival, proliferation and differentiation. $EGFR$ is activated when epidermal growth factor (EGF) (or another EGF family factor like transforming growth factor–α, $TGF{-}\alpha$) binds to its extracellular domain forming the complex $EGFR{-}EGF$. Binding of the ligand to the receptor induces receptor dimerisation (association of two receptor monomers) yielding a complex we will denote as $EGFR{-}EGF_2$. Then autophosphorylation of tyrosine residues on the cytoplasmic tail takes place producing the phosphorylated receptor, $EGFR{-}EGF_2^*$. Tyrosine phosphorylation triggers the binding of several cytoplasmic proteins to the receptor. As mentioned before we only consider here three proteins as an initial core model.

One of these proteins is phospholipase C-γ (PLC_γ). This first cycle starts when PLC_γ binds to the phosphorylated receptor forming the complex $EGFR{-}EGF_2^* - PLC_\gamma$ which is phosphorylated yielding $EGFR - EGF_2^* - PLC_\gamma^*$. This cycle is completed when the last complex dissociates into $EGFR - EGF_2^*$ and phosphorylated phospholipase C-γ (PLC_γ^*) which in turn can either be dephosphorylated or translocate to the cell membrane.

Another cycle starts with the binding of growth factor receptor-binding protein 2 ($Grb2$) to a receptor phosphotyrosine producing the complex $EGFR - EGF_2^* - Grb2$. This complex is a branch point in the network but we will only follow the link to the Ras signalling pathway. The binding of the Son of Sevenless homolog protein (SOS) produces the ternary complex $EGFR - EGF_2^* - Grb2 -$

SOS which subsequently dissociates into the phosphorylated receptor and the complex $Grb2 - SOS$, which further dissociates into $Grb2$ and SOS.

Src homology and collagen domain protein (Shc) plays a key role in the last cycle. Shc binds to the receptor producing the complex $EGFR - EGF_2^* - Shc$ which is phosphorylated to yield $EGFR - EGF_2^* - Shc^*$ which can either dissociate into $EGFR - EGF_2^*$ and Shc^* or form with $Grb2$ the ternary complex $EGFR - EGF_2^* - Shc^* - Grb2$. Then this complex may dissociate to yield the phosphorylated receptor and the complex $Shc^* - Grb2$. Alternately SOS can also bind to the ternary complex to produce the four protein complex $EGFR - EGF_2^* - Shc^* - Grb2 - SOS$ which can also be formed by the binding of the complex $Grb2 - SOS$ to $EGFR - EGF_2^* - Shc^*$. Subsequently the four protein complex dissociates into $EGFR - EGF_2^*$ and $Shc^* - Grb2 - SOS$ which further dissociates into Shc^* and $Grb2 - SOS$. Finally $Grb2 - SOS$ yields $Grb2$ and SOS and Shc^* is dephosphorylated by phosphatases to produce Shc.

Observe that there exists a *cross-talk* between the last two cycles meanwhile the first one is quite independent from the others. Moreover all the chemicals reactions described here are reversible.

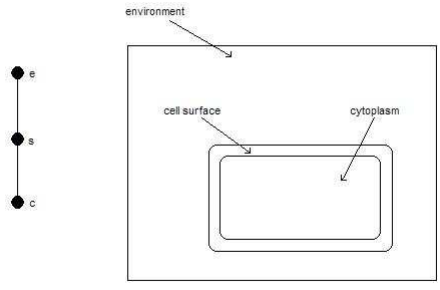
5 Modelling EGFR Signalling Network by P Systems

In this section we use a continuous P system, $\mathbf{\Pi} = (\Sigma, \mu, w_{e,0}, w_{s,0}, w_{c,0}, \mathcal{R}, \mathcal{K})$, to model the part of the EGFR signalling network described in the previous section. The system $\mathbf{\Pi}$ is defined as follows:

• **Alphabet:** In the alphabet Σ we collect all the proteins and complexes of proteins that take part in the signalling cascade.

$\Sigma = \{EGF, EGFR, PLC_\gamma, PLC_\gamma^*, PLC_\gamma\text{–}I, Shc, Shc^*, Grb2, SOS, EGFR\text{–}EGF\}$
$\cup \{EGFR\text{–}EGF_2, EGFR\text{–}EGF_2^*, EGFR\text{–}EGF_2^*\text{–}PLC_\gamma, EGFR\text{–}EGF_2^*\text{–}Shc\}$
$\cup \{EGFR\text{–}EGF_2^*\text{–}Shc^*, EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2, EGFR\text{–}EGF_2^*\text{–}Grb2\}$
$\cup \{EGFR\text{–}EGF_2^*\text{–}Grb2\text{–}SOS, EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\text{–}SOS\}$
$\cup \{Grb2\text{–}SOS, Shc^*\text{–}Grb2\text{–}SOS, Shc^*\text{–}Grb2\}$

• **Membrane Structure:** In the part of the EGFR signalling network that we are modelling there are three relevant regions, namely the *environment*, the *cell surface* and the *cytoplasm*. We represent them in the membrane structure as the membranes labelled with: e for the environment, s for the cell surface and c for the cytoplasm.



• **Initial Multisets:** In the initial multisets we represent the initial concentrations of the chemical substances in the environment, the cell surface and the cytoplasm. These concentration has been obtained from the references to simulate over-saturation of the environment with EGF (see [3]).

$$w_{e,0}(p) = \begin{cases} 200 & \text{if } p = EGF \\ 0 & \text{otherwise} \end{cases}$$

$$w_{s,0}(p) = \begin{cases} 100 & \text{if } p = EGFR \\ 0 & \text{otherwise} \end{cases}$$

$$w_{c,0}(p) = \begin{cases} 10 & \text{if } p = PLC_\gamma,\, Grb2,\, SOS \\ 0 & \text{otherwise} \end{cases}$$

• **Rules and Rate of application function:** In the rules we model the chemical reactions described in the previous section. To model the reactions we use the *Law of Mass Action* which says that the rate of a reaction is proportional to the product of the concentrations of the reactants. That is, if we have a reaction of the form:

$$r_1 \ + \cdots + \ r_n \ \rightarrow \ p_1 + \cdots + p_m,$$

then the rate of this reaction is $k|r_1| \cdots |r_n|$, where k is called *kinetic constant*.

We also use the *Michaelis law* that states that in a reaction that takes place in presence of a catalyst and where the concentration of the substrate is present in large excess over the concentration of the catalyst the rate of application of the reaction is:

$$\frac{k|S|}{K + |S|},$$

where $|S|$ is the concentration of the substrate and k, K are called *Michaelis constants*.

The kinetic and Michaelis constants are taken from the literature, see the references [3],[10] and their bibliography.

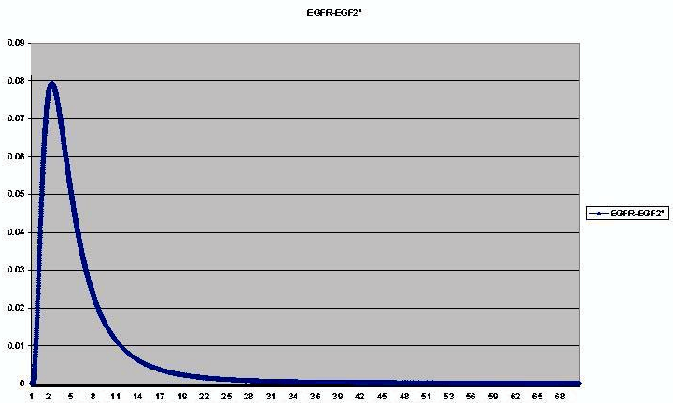
RULES	**RATE**
$EGF\,[\,EGFR\,]_s \ \rightarrow \ [\,EGFR\text{–}EGF\,]_s$	$0.003\|EGF\|_e\|EGFR\|_s$
$[\,EGFR\text{–}EGF\,]_s \ \rightarrow \ EGF\,[\,EGFR\,]_s$	$0.06\|EGFR\text{–}EGF\|_s$
$[\,EGFR\text{–}EGF,\, EGFR\text{–}EGF\,]_s \ \rightarrow \ [\,EGFR\text{–}EGF_2\,]_s$	$0.01\|EGFR\text{–}EGF\|_s^2$
$[\,EGFR\text{–}EGF_2\,]_s \ \rightarrow \ [\,EGFR\text{–}EGF,\, EGFR\text{–}EGF\,]_s$	$0.1\|EGFR\text{–}EGF_2\|_s$
$[\,EGFR\text{–}EGF_2\,]_s \ \rightarrow \ [\,EGFR\text{–}EGF_2^*\,]_s$	$\|EGFR\text{–}EGF_2\|_s$
$[\,EGFR\text{–}EGF_2^*\,]_s \ \rightarrow \ [\,EGFR\text{–}EGF_2\,]_s$	$0.01\|EGFR\text{–}EGF_2^*\|_s$
$EGFR\text{–}EGF_2^*\,[\,PLC_\gamma\,]_c \ \rightarrow \ EGFR\text{–}EGF_2^*\text{–}PLC_\gamma\,[\,]_c$	$0.06\|EGFR\text{–}EGF_2^*\|_s\|PLC_\gamma\|_c$

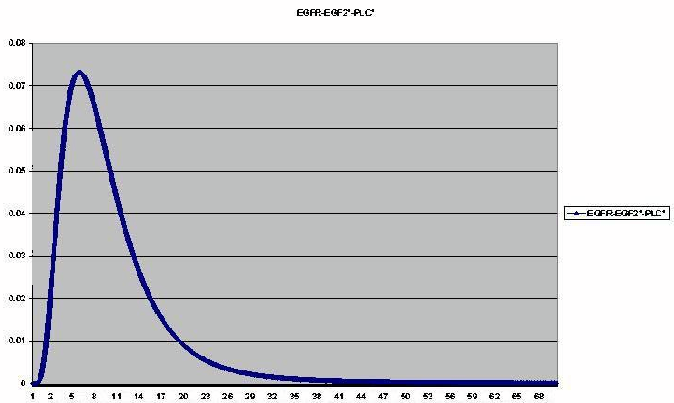
RULES	**RATE**
$EGFR\text{–}EGF_2^*\text{–}PLC_\gamma\,[\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\,[\,PLC_\gamma\,]_c$	$0.2\lvert EGFR\text{–}EGF_2^*\text{–}PLC_\gamma\rvert_s$
$[\,EGFR\text{–}EGF_2^*\text{–}PLC_\gamma\,]_s \;\rightarrow\; [\,EGFR\text{–}EGF_2^*\text{–}PLC_\gamma^*\,]_s$	$\lvert EGFR\text{–}EGF_2^*\text{–}PLC_\gamma\rvert_s$
$[\,EGFR\text{–}EGF_2^*\text{–}PLC_\gamma^*\,]_s \;\rightarrow\; [\,EGFR\text{–}EGF_2^*\text{–}PLC_\gamma\,]_s$	$0.05\lvert EGFR\text{–}EGF_2^*\text{–}PLC_\gamma^*\rvert_s$
$EGFR\text{–}EGF_2^*\text{–}PLC_\gamma^*\,[\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\,[\,PLC_\gamma^*\,]_c$	$0.3\lvert EGFR\text{–}EGF_2^*\text{–}PLC_\gamma^*\rvert_s$
$EGFR\text{–}EGF_2^*\,[\,PLC_\gamma^*\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\text{–}PLC_\gamma^*\,[\,]_c$	$0.006\lvert EGFR\text{–}EGF_2^*\rvert_s\lvert PLC_\gamma^*\rvert_c$
$[\,PLC_\gamma^*\,]_c \;\rightarrow\; [\,PLC_\gamma\,]_c$	$\dfrac{\lvert PLC_\gamma^*\rvert_c}{100+\lvert PLC_\gamma^*\rvert_c}$
$PLC_\gamma^*\,[\,]_s \;\rightarrow\; [\,PLC_\gamma\text{–}I\,]_s$	$\lvert PLC_\gamma^*\rvert_c$
$[\,PLC_\gamma\text{–}I\,]_s \;\rightarrow\; PLC_\gamma^*\,[\,]_s$	$0.03\lvert PLC_\gamma^*\text{–}I\rvert_s$
$EGFR\text{–}EGF_2^*\,[\,Grb2\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\text{–}Grb2\,[\,]_c$	$0.003\lvert EGFR\text{–}EGF_2^*\rvert_s\lvert Grb2\rvert_c$
$EGFR\text{–}EGF_2^*\text{–}Grb2\,[\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\,[\,Grb2\,]_c$	$0.05\lvert EGFR\text{–}EGF_2^*\text{–}Grb2\rvert_s$
$EGFR\text{–}EGF_2^*\text{–}Grb2\,[\,SOS\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\text{–}Grb2\text{–}SOS\,[\,]_c$	$0.01\lvert EGFR\text{–}EGF_2^*\text{–}Grb2\rvert_s\lvert SOS\rvert_c$
$EGFR\text{–}EGF_2^*\text{–}Grb2\text{–}SOS\,[\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\text{–}Grb2\,[\,SOS\,]_c$	$0.06\lvert EGFR\text{–}EGF_2^*\text{–}Grb2\text{–}SOS\rvert_s$
$EGFR\text{–}EGF_2^*\text{–}Grb2\text{–}SOS\,[\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\,[\,Grb2\text{–}SOS\,]_c$	$0.03\lvert EGFR\text{–}EGF_2^*\text{–}Grb2\text{–}SOS\rvert_s$
$EGFR\text{–}EGF_2^*\,[\,Grb2\text{–}SOS\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\text{–}Grb2\text{–}SOS\,[\,]_c$	$0.0045\lvert EGFR\text{–}EGF_2^*\rvert_s\lvert Grb2\text{–}SOS\rvert_s$
$[\,Grb2\text{–}SOS\,]_c \;\rightarrow\; [\,Grb2, SOS\,]_c$	$0.0015\lvert Grb2\text{–}SOS\rvert_c$
$[\,Grb2, SOS\,]_c \;\rightarrow\; [\,Grb2\text{–}SOS\,]_c$	$0.0001\lvert Grb2\rvert_c\lvert SOS\rvert_c$
$EGFR\text{–}EGF_2^*\,[\,Shc\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\text{–}Shc[\,]_c$	$0.09\lvert EGFR\text{–}EGF_2^*\rvert_s\lvert Shc\rvert_c$
$EGFR\text{–}EGF_2^*\text{–}Shc[\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\,[\,Shc\,]_c$	$0.6\lvert EGFR\text{–}EGF_2^*\text{–}Shc\rvert_s$
$[\,EGFR\text{–}EGF_2^*\text{–}Shc\,]_s \;\rightarrow\; [\,EGFR\text{–}EGF_2^*\text{–}Shc^*\,]_s$	$6\lvert EGFR\text{–}EGF_2^*\text{–}Shc\rvert_s$
$[\,EGFR\text{–}EGF_2^*\text{–}Shc^*\,]_s \;\rightarrow\; [\,EGFR\text{–}EGF_2^*\text{–}Shc\,]_s$ $EGFR\text{–}EGF_2^*\text{–}Shc^*[\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\,[\,Shc^*\,]_c$	$0.06\lvert EGFR\text{–}EGF_2^*\text{–}Shc\rvert_s$ $0.3\lvert EGFR\text{–}EGF_2^*\text{–}Shc^*\rvert_s$
$EGFR\text{–}EGF_2^*\,[\,Shc^*\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\text{–}Shc^*[\,]_c$	$0.0009\lvert EGFR\text{–}EGF_2^*\text{–}Shc^*\rvert_s$
$[\,Shc^*\,]_c \;\rightarrow\; [\,Shc\,]_c$	$\dfrac{1.7\lvert Shc^*\rvert_c}{340+\lvert Shc^*\rvert_c}$
$EGFR\text{–}EGF_2^*\text{–}Shc^*[\,Grb2\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\,[\,]_c$	$0.003\lvert EGFR\text{–}EGF_2^*\text{–}Shc^*\rvert_s\lvert Grb2\rvert_c$
$EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\,[\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\text{–}Shc^*[\,Grb2\,]_c$	$0.1\lvert EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\rvert_s$
$EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\,[\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\,[\,Shc^*\text{–}Grb2\,]_c$	$0.3\lvert EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\rvert_s$
$EGFR\text{–}EGF_2^*\,[\,Shc^*\text{–}Grb2\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\,[\,]_c$	$0.0009\lvert EGFR\text{–}EGF_2^*\rvert_s\lvert Shc^*\text{–}Grb2\rvert_c$
$EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\,[\,SOS\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\text{–}SOS[\,]_c$	$0.01\lvert EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\rvert_s\lvert SOS\rvert_c$
$EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\text{–}SOS[\,]_c \;\rightarrow\; EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\,[\,SOS\,]_c$	$0.0214\lvert EGFR\text{–}EGF_2^*\text{–}Shc^*\text{–}Grb2\text{–}SOS\rvert_s$

RULES	RATE
$EGFR–EGF_2^*–Shc^*–Grb2–SOS[\,]_c \;\rightarrow\; EGFR–EGF_2^*\,[Shc^*–Grb2–SOS]_c$	$0.12\|EGFR–EGF_2^*–Shc^*–Grb2–SOS\|_s$
$EGFR–EGF_2^*\,[Shc^*–Grb2–SOS]_c \;\rightarrow\; EGFR–EGF_2^*–Shc^*–Grb2–SOS[\,]_c$	$0.00024\|EGFR–EGF_2^*\|_s\|Shc^*–Grb2–SOS\|_c$
$[Shc^*, Grb2]_c \;\rightarrow\; [Shc^*–Grb2]_c$	$0.003\|Shc^*\|_c\|Grb2\|_c$
$[Shc^*–Grb2]_c \;\rightarrow\; [Shc^*, Grb2]_c$	$0.1\|Shc^*–Grb2\|_c$
$[Shc^*–Grb2, SOS]_c \;\rightarrow\; [Shc^*–Grb2–SOS]_c$	$0.03\|Shc^*–Grb2\|_c\|SOS\|_c$
$[Shc^*–Grb2–SOS]_c \;\rightarrow\; [Shc^*–Grb2, SOS]_c$	$0.064\|Shc^*–Grb2–SOS\|_c$
$[Shc^*–Grb2, SOS]_c \;\rightarrow\; [Shc^*–Grb2–SOS]_c$	$0.021\|Shc^*–Grb2\|_c\|SOS\|_c$
$[Shc^*–Grb2–SOS]_c \;\rightarrow\; [Shc^*–Grb2, SOS]_c$	$0.1\|Shc^*–Grb2–SOS\|_c$
$EGFR–EGF_2^*–Shc^*[Grb2–SOS]_c \;\rightarrow\; EGFR–EGF_2^*–Shc^*–Grb2–SOS[\,]_c$	$0.009\|EGFR–EGF_2^*–Shc^*\|_s\|Grb2–SOS\|_c$
$EGFR–EGF_2^*–Shc^*–Grb2–SOS[\,]_c \;\rightarrow\; EGFR–EGF_2^*–Shc^*[Grb2–SOS]_c$	$0.0429\|EGFR–EGF_2^*–Shc^*–Grb2–SOS\|_s$

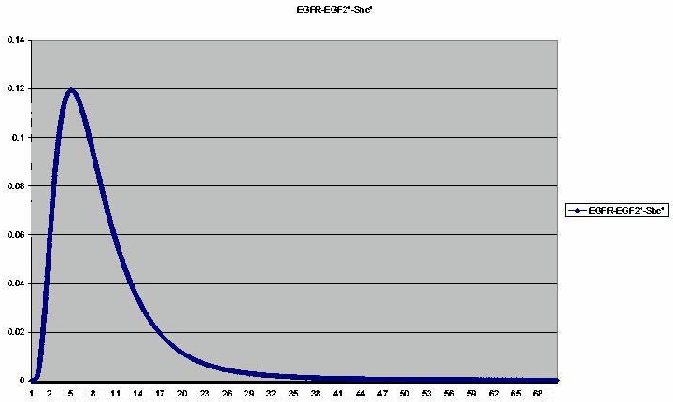
6 Results

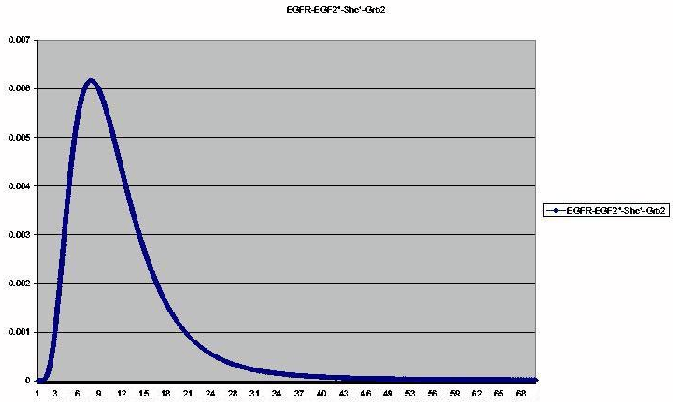
In this section we present the evolution of concentration of a number of key complexes in the simulation of the continuous P system introduced in the previous section. In order to approximate the evolution of the P system we have used the method introduced in section 3.

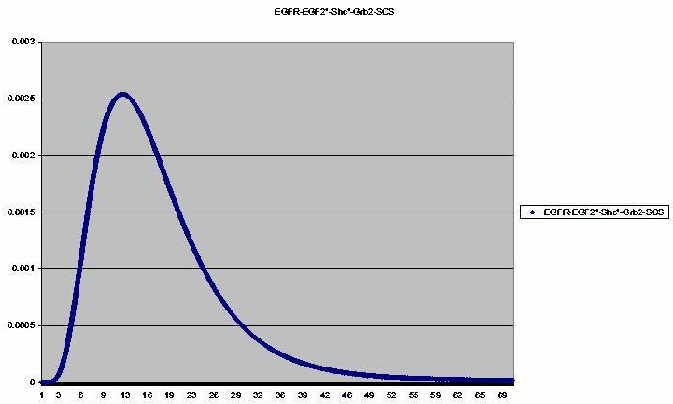


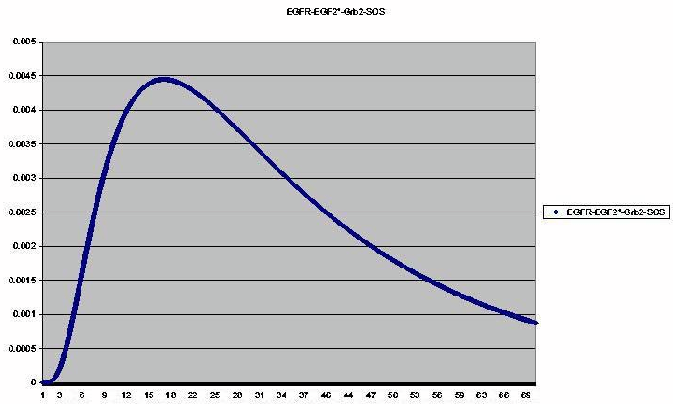


The two figures shown in the previous page depict the evolution of the concentration of the phosphorylated receptor $EGFR - EGF_2^*$ and the complex $EGFR - EGF_2^* - PLC^*$ during 70 seconds. Both graphics show an early response to EGF reaching pronounced maximum within the first 5 seconds and then the concentrations descend to low levels. These results agree quite well with biological data (see [3] and [10]).



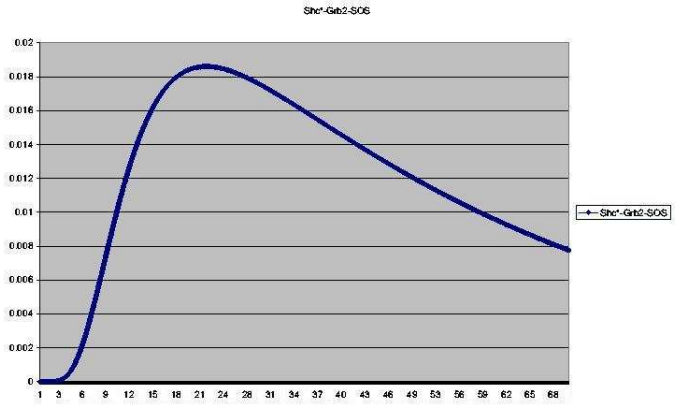






The previous graphics depict the evolution during 70 seconds of the concentration of the complexes $EGFR-EGF_2^*-Shc^*$, $EGFR-EGF_2^*-Shc^*-Grb2$, $EGFR-EGF_2^*-Shc^*-Grb2-SOS$ and $EGFR-EGF_2^*-Grb2-SOS$. They also present the same pattern discussed for the first two figures. Nevertheless

the next two graphics, which depict the evolution in time of the concentration of $Shc^* - Grb2$ and $Shc^* - Grb2 - SOS$, present more sustained responses to EGF stimulation which is also in accordance with biological data (see [3] and [10]).



7 Conclusions and Future Work

In this paper we have introduced continuous P systems, a variant of membrane systems, and we have used them to develop a biochemically plausible model of part of the EGFR signalling network. The results obtained are in accordance with biological data showing that continuous P systems and membrane computing, in general, are a reliable framework for simulating biological phenomena.

Nevertheless here we have only modelled a part of the $EGFR$ signalling network; currently we are expanding the model to consider receptor internalization and degradation, activation of $Ras - GTP$, the MAP kinase cascade and the expression of the target gene, $c - fos$.

About the way of approximating continuous P systems it is interesting to bound the error in the approximation that we make when using different numerical methods to compute integrals. So we can design better approximations than the one used in this paper.

As mentioned in the introduction $EGFR$ is a target for the development of novel anticancer therapies. In future work we intend to use this model to investigate the effect of various therapies, like kinase inhibition and radiation therapy, on the signalling network.

Finally in order to make this model more attractive to biologist and biomolecular researchers we are developing software with a friendly interface using CLIPS and JAVA.

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