Modelling Trafficking of Proteins within the Mammalian Cell Using Bio-PEPA

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Abstract. Bio-PEPA [2], a process algebra developed from PEPA [5] is used to model a process occurring in mammalian cells whereby the Src oncoprotein is trafficked between different parts of the cell [9]. Src is associated with cell movement and adhesion between cells which is linked to tumour formation [9]. A useful model of the protein's behaviour can provide predictions for new experimental hypotheses which may improve our understanding, and in time, lead to new therapies for cancer. The aim is to assess the suitability of Bio-PEPA for more detailed modelling of Src trafficking than that of a previous simpler Bio-PEPA model [4].

Bio-PEPA has the advantage that it provides many types of analysis, such as ordinary differential equations, stochastic simulation, continuous-time Markov chains, trace investigation and model-checking. These analyses are implemented in or accessible via the Bio-PEPA Eclipse Plug-in [3] (see `www.biopepa.org`).

The syntax of well-defined Bio-PEPA species components with locations [1] is given by $C \stackrel{def}{=} \sum_{i=1}^{n} (\alpha_i, \kappa_i)\ \mathtt{op}_i\ C$ such that $\alpha_i \neq \alpha_j$ for $i \neq j$. Here C is a constant of the form $A@loc$, each α_i is a reaction name, each κ_i is the stoichiometric coefficient for C in reaction α_i, and each $\mathtt{op}_i$ is a prefix operator describing the role of C is reaction α_i. The operator $\downarrow$ indicates a reactant, $\uparrow$ a product, $\oplus$ an activator, $\ominus$ an inhibitor and $\odot$ an arbitrary modifier. Hence a species component has a unique name and describes the reaction capabilities of that species. A Bio-PEPA model then has the syntax $P ::= P \underset{L}{\bowtie} P \mid C(x)$ where x represents the quantity of species C. Species cooperate over reactions in L, or $*$ can be used to denote all shared reactions. A species may only appear once in a well-defined model. A Bio-PEPA system is a tuple containing species components, model and additional information about locations, species, constants and rate functions.

The operational semantics consist of a capability transition relation where labels on the transitions record the reaction name and information about the species taking part in the reaction; and the stochastic relation where this information is transformed into a rate using a function specific to the reaction, giving a transition sytem labelled with reaction names and rates (as in PEPA [5]).

The protein under consideration here is the Src protein, a member of the Src family of proteins. It is a non-receptor protein tyrosine kinase which has two domains to which other molecules can bind [9]. In its inactive form, its conformation prevents access to these domains, whereas in its active form these domains

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are available, and Src can interact with other proteins. Research (described below) has shown that Src is trafficked around the cell in endosomes. These are membrane-bound compartments found in the cytoplasm of the cell. They merge with vesicles which engulf molecules on the inner side of the membrane, and their role is to sort molecules for recycling[1] and degradation. Endosomes move along microfilaments and/or microtubules, so movement is typically in one direction, often in towards the nucleus or out towards the cellular membrane. They tend to vary in contents rather than number or speed.

Usually, Src is found in two locations in the cell: a large amount of inactive Src is located around the nucleus, in the perinuclear region, and a much smaller amount of active Src is located on or near the membrane [7,8]. Sandilands, Frame and others have investigated how the addition of growth factor affects Src activity. After stimulation with fibroblast growth factor (FGF), Src is found in endosomes throughout the cytoplasm. Moreover, there is a gradient of inactive Src to active Src from perinuclear region to membrane and hence Src activation takes place in endosomes [8]. Furthermore, the persistence of active Src at the membrane is inversely related to the quantity of FGF added. When 1 nanogram of FGF is added, large quantities of active Src persist two hours after addition; in contrast, when 50 times this amount is added, the quantity of active Src is already reduced after 30 minutes and has returned to normal levels after 1 hour [7]. The goal for the model is to demonstrate this persistence behaviour.

The first challenge of the modelling is lack of experimental data. There is qualititative data: the gradient of active Src, and quantitative data: the timing of the persistence of active Src after growth factor addition. Additionally, the speed of endosomes can be determined because their movement is directional and has been measured. Research into endosomes has shown that there are both long and short recycling loops [6]. Hence from this, one can estimate how long it should take for an endosome to move through a long loop and a short loop. The second and longer term challenge is making the model concrete enough to be useful in prediction but abstract enough to be tractable.

An initial single combined long recycling loop model was developed but it did not demonstrate the required behaviour. After discussion with experimental biologists, a two loop model was built. In the shorter loop, which is always in operation, some of the active Src at the cell membrane is recycled in endosomes, and the hypothesis is that there will always be active Src about to be delivered to the membrane, ensuring the ongoing presence of active Src at the membrane. The longer recycling loop is only active on stimulation by FGF and involves trafficking of active Src bound to the FGF receptor (FGFR). When endosomes in this loop come close to the perinuclear region, they engulf inactive Src which is then activated during the movement of the endosome outwards. In both loops, it can happen that the endosome contents are degraded rather than recycled. Figure 1 illustrates these concepts. The Bio-PEPA model consists of seven species. Inactive Src in the perinuclear region is available in such large quantities that it can be treated implicitly. The model is moderately abstract;

[1] Here, recycling means to "return to the membrane for re-use".

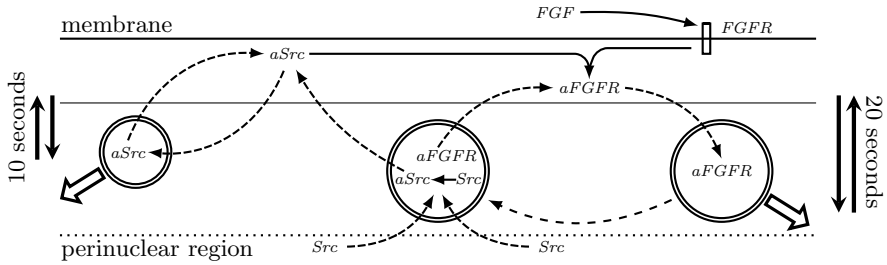

Fig. 1. The two loop model of Src trafficking: dashed arrows denote movement and solid arrows denote reactions. Double circles represent endosomes and the large outlined arrows indicate degradation of endosome contents. The short loop is on the left with the long loop on the right.

active Src at the membrane is a species, as are endosomes containing active Src. This is possible using the stoichiometry coefficient in modelling the interaction of species. Consider the following definition for active Src at the membrane.

$$\begin{aligned} aSrc@membrane \stackrel{def}{=} & (aSrc\_FGFR\_binding, 1) \downarrow aSrc@membrane \\ & + (into\_short\_loop\_endosome, 150) \downarrow aSrc@membrane \\ & + (outof\_short\_loop\_endosome, 150) \uparrow aSrc@membrane \\ & + (outof\_long\_loop\_endosome, 100) \uparrow aSrc@membrane \end{aligned}$$

The binding of active Src and FGFR to form active FGFR is an abstraction of a number of reactions whose detail is not necessary for the modelling but the stoichiometry of 1 indicates that one active Src molecule takes part in the binding (and FGFR has the same stoichiometry). However, for the other reactions which represent amounts of active Src either moving into endosomes or moving out of endosomes, the stoichiometric coefficient represents these quantities. The endosome species taking part in the reaction will have a stoichiometry of one to capture the notion that a single endosome contains multiple Src molecules. The stoichiometry for the *outof_short_loop_endosome* reaction can be made less than that for *into_short_loop_endosome* to describe the loss of active Src within the recycling loop, or alternatively this can be done by removal of endosomes.

Simulation demonstrates a short-lived peak of active Src at the membrane on addition of FGF as shown in Figure 2. This model does not demonstrate the behaviour for smaller amounts of FGF and this requires further exploration of the parameter space. It appears Bio-PEPA provides useful abstraction techniques.

Due to the limited data, this style of modelling can be described as quasi-quantitative and exploratory. However, the model has been useful for discussion with the biologists involved, both for the biological understanding of the modeller, and in raising interesting questions for the biologists about the underlying mechanisms. Ongoing discussions will develop the model further, and new techniques may provide more quantitative data. Recent research about Src in cancerous cells shows that when too much active Src at the membrane could lead to cell death, there is a mechanism to sequester this active Src and hence enable survival of the cell [10]. This shows that there may be multiple mechanisms by which Src is trafficked and suggests scope for a more complex model.

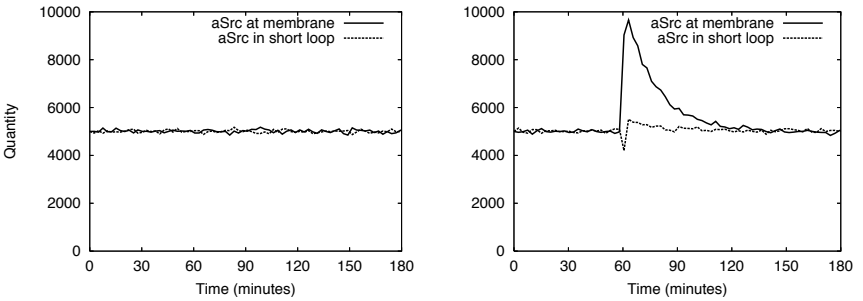

Fig. 2. Stochastic simulations without addition of growth factor (left) and with addition of growth factor at one hour (right). The graphs show the average of 10 simulations.

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References

1. Ciocchetta, F., Guerriero, M.L.: Modelling biological compartments in Bio-PEPA. Electronic Notes in Theoretical Computer Science 227, 77–95 (2009)
2. Ciocchetta, F., Hillston, J.: Bio-PEPA: a framework for the modelling and analysis of biological systems. Theoretical Computer Science 410(33-34), 3065–3084 (2009)
3. Duguid, A., Gilmore, S., Guerriero, M., Hillston, J., Loewe, L.: Design and development of software tools for Bio-PEPA. In: Dunkin, A., Ingalls, R., Yücesan, E., Rossetti, M., Hill, R., Johansson, B. (eds.) Proceedings of the 2009 Winter Simulation Conference, pp. 956–967 (2009)
4. He, X.: A Bio-PEPA Model of Endosomal Trafficking of Src Tyrosine Kinase. MSc dissertation, School of Informatics, University of Edinburgh
5. Hillston, J.: A compositional approach to performance modelling. Cambridge University Press (1996)
6. Jones, M., Caswell, P., Norman, J.: Endocytic recycling pathways: emerging regulators of cell migration. Current Opinion in Cell Biology 18, 549–557 (2006)
7. Sandilands, E., Akbarzadeh, S., Vecchione, A., McEwan, D., Frame, M., Heath, J.: Src kinase modulates the activation, transport and signalling dynamics of fibroblast growth factor receptors. EMBO Reports 8, 1162–1169 (2007)
8. Sandilands, E., Cans, C., Fincham, V., Brunton, V., Mellor, H., Prendergast, G., Norman, J., Superti-Furga, G., Frame, M.: Rhob and actin polymerization coordinate Src activation with endosome-mediated delivery to the membrane. Developmental Cell 7, 855–869 (2004)
9. Sandilands, E., Frame, M.: Endosomal trafficking of Src tyrosine kinase. Trends in Cell Biology 18, 322–329 (2008)
10. Sandilands, E., Serrels, B., McEwan, D., Morton, J., Macagno, J., McLeod, K., Stevens, C., Brunton, V., Langdon, W., Vidal, M., Sansom, O., Dikic, I., Wilkinson, S., Frame, M.: Autophagic targeting of Src promotes cancer cell survival following reduced FAK signalling. Nature Cell Biology 14, 51–60 (2011)