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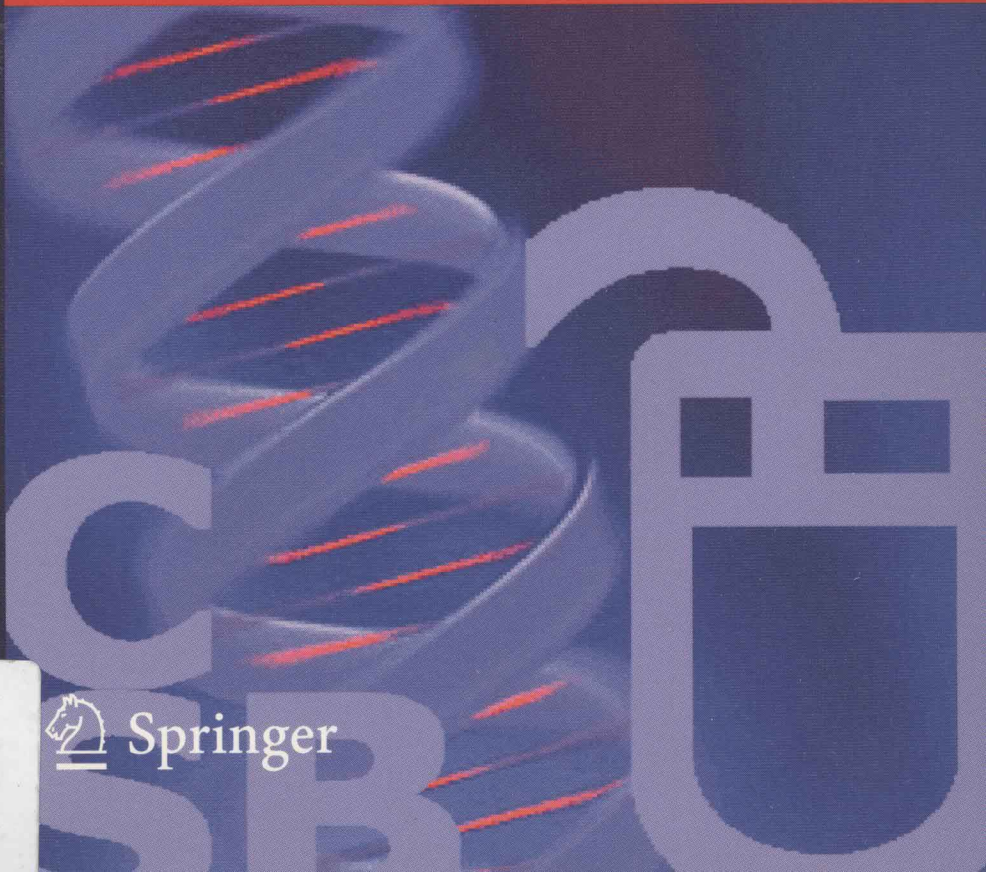

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Corrado Priami Anna Ingólfsdóttir
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Transactions on Computational Systems Biology VII

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Volume Editors

Anna Ingólfsdóttir
Aalborg University
Department of Computer Science
Fr. Bajersvej 7E, Aalborg, Denmark
E-mail: annai@cs.auc.dk

Bud Mishra
Cold Spring Harbor Laboratory
One Bungtown Road
Cold Spring Harbor, NY 11724, USA
E-mail: mishra@nyu.edu

Hanne Riis Nielson
Technical University of Denmark
Informatics and Mathematical Modelling
Richard Petersens Plads, 2800 Lyngby, Denmark
E-mail: riis@imm.dtu.dk

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Preface

This issue of the journal reports some selected contributions from the workshops *BioConcur 2004* chaired by Anna Ingolfsdottir and Hanne Riis Nielson and *BioConcur 2005* chaired by Bud Mishra and Corrado Priami.

There are three contributions from BioConcur 2004. The first one is by Calder, Gilmore and Hillston on the modelling of signalling pathways using the stochastic process algebra PEPA. The second contribution is by Kuttler and Niehren on gene regulation in π-calculus. The last contribution is by Remy, Ruet, Mendoza, Thieffry and Chsouiya on the relationships between logical regulator graphs and Petri nets.

There are five contributions from BioConcur 2005. The first contribution is by Eccher and Lecca on the automatic translation of SBML models to stochastic π-calculus. The second paper is by Blinov, Yang, Faeder and Hlavacek on the use of graph theory to model biological networks. The third contribution, by Jha and Shyamasundar, introduces biochemical Kripke structures for distributed model checking. The fourth paper is by Phillips, Cardelli and Castagna on a graphical notation for stochastic π-calculus. The last paper is by Remy and Ruet on differentiation and homeostatic behaviour of boolean dynamic systems.

The volume ends with a regular contribution by Margoninsky, Saffrey, Hetherington, Finkelstein and Warner that describes a specification language and a framework for the execution of composite models.

July 2006 Corrado Priami

LNCS Transactions on Computational Systems Biology – Editorial Board

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Modelling the Influence of RKIP on the ERK Signalling Pathway Using the Stochastic Process Algebra PEPA

Muffy Calder[1], Stephen Gilmore[2], and Jane Hillston[2]

[1] Department of Computing Science, The University of Glasgow, Glasgow, Scotland
muffy@dcs.gla.ac.uk
[2] Laboratory for Foundations of Computer Science, The University of Edinburgh, Scotland
stg@inf.ed.ac.uk, jeh@inf.ed.ac.uk

Abstract. This paper examines the influence of the Raf Kinase Inhibitor Protein (RKIP) on the Extracellular signal Regulated Kinase (ERK) signalling pathway [5] through modelling in a Markovian process algebra, PEPA [11]. Two models of the system are presented, a reagent-centric view and a pathway-centric view. The models capture functionality at the level of subpathway, rather than at a molecular level. Each model affords a different perspective of the pathway and analysis. We demonstrate the two models to be formally equivalent using the timing-aware bisimulation defined over PEPA models and discuss the biological significance.

1 Introduction

In recent years several authors have investigated the use of Petri nets and process algebras – techniques originating in theoretical computer science – for representing the biochemical pathways within and between cells [15,18,10]. Largely, the previous work has focussed on capturing the appropriate functionality at the molecular level and analysis is through simulation. In this paper we present a preliminary exploration of an alternative approach in which a more abstract approach is taken and the target mathematical representation is a continuous time Markov chain. This involves the analytical application of a process algebra to a biochemical pathway with feedback. Our goal is to develop more than one representation, suitable for different forms of analysis. We prove the two representations to be equivalent (i.e. bisimilar).

The process algebra which we use is Hillston's PEPA [11], a Markovian process algebra which incorporates stochastic durations and probabilistic choices. The system which we consider is the Ras/Raf-1/MEK/ERK signalling pathway, as presented in [5]. We believe that our modelling is novel because we are able to combine performance and different modelling viewpoints. Moreover we demonstrate the feasibility of using process algebra to model signalling pathways in a more abstract style than previously.

C. Priami et al. (Eds.): Trans. on Comput. Syst. Biol. VII, LNBI 4230, pp. 1–23, 2006.

We propose that process algebra models are appropriate in this domain for several reasons. First, an algebraic formulation of the model makes clear the interactions between the biochemical entities, or substrates. This is not always apparent in the classical, ordinary differential equation (ODE) models. Second, an algebraic approach permits comparison of high level descriptions. For example, when one is first building up a picture of a pathway from experimental evidence, it may be natural to describe the pathway in a fine-grained, distributed fashion, e.g. each substrate (in this case a protein) is described in terms of its interactions. That is, each (collection of a) protein is a process and all processes run in parallel, synchronising accordingly. But later, we may prefer a higher level view of a pathway which describes how a pathway is composed of (perhaps already well known) sub-pathways. Indeed we may wish to derive the latter from the former, or vice-versa. Third, a stochastic process approach allows reasoning about livelocks, deadlocks, and the performance of the behaviour of the pathway in the long-run.

This paper is an extended version of the earlier paper [2]. As previously, we concentrate primarily on alternative approaches to constructing a representation of a pathway. We show that two contrasting representations can indeed be identified. Moreover they can be formally shown to be equivalent. The novelty of this paper lies in the systematic transformation between the alternative representations which are presented in algorithmic form. The analysis of the model has also been somewhat extended.

In the next section we give a brief overview of cell signalling and the Ras/Raf-1/MEK/ERK pathway. In section 3 we give two different PEPA formulations of the pathway: the first is reagent-based (i.e. distributed) and the second is pathway-based. In section 4 we compare the two models and show them to be bisimilar. Section 5 contains some analysis of the underlying continuous time Markov model. Transformation between the two styles of representation is presented in section 6. There follows a discussion of further analysis, related work and our conclusions.

2 RKIP and the ERK Pathway

The most fundamental cellular processes are controlled by extracellular signalling [7]. This signalling, or communication between cells, is based upon the release of signalling molecules, which migrate to other cells and deliver stimuli to them (e.g. protein phosphorylation). Cell signalling is of special interest to cancer researchers because when cell signalling pathways operate abnormally, cells divide uncontrollably.

The Ras/Raf-1/MEK/ERK pathway (also called Ras/Raf, or ERK pathway) is a ubiquitous pathway that conveys mitogenic and differentiation signals from the cell membrane to the nucleus. Briefly, Ras is activated by an external stimulus, it then binds to and activates Raf-1 (to become Raf-1*, "activated" Raf) which in turn activates MEK and then ERK. This "cascade" of protein interaction controls cell differentiation, the effect being dependent upon the activity

of ERK. A current area of experimental scientific investigation is the role the kinase inhibitor protein RKIP plays in the behaviour of this pathway: the hypothesis is that it inhibits activation of Raf and thus can "dampen down" the ERK pathway. Certainly there is much evidence that RKIP inhibits the malignant transformation by Ras and Raf oncogenes in cell cultures and it is reduced in tumours. Thus good models of these pathways are required to understand the role of RKIP and develop new therapies. Moreover, an understanding of the functioning and structure of this pathway may lead to more general results applicable to other pathways.

Here, we consider how RKIP regulates the activity of the Raf-1/MEK/ERK module of the ERK pathway, as presented in [5]. This paper [5] presents a number of mathematical models in the form of nonlinear ODEs and difference equations representing the (enzyme) kinetic reactions, based on a graphical representation given in Figure 1. This figure is taken from [5], with some additions. Specifically, we have added MEK and an associated complex, following discussions with the authors[1].

We take Figure 1 as our starting point, and explain informally, its meaning. Each node is labelled by the protein (or substrate, we use the two interchangeably) it denotes. For example, Raf-1, RKIP and Raf-1*/RKIP are proteins, the last being a complex built up from the first two. It is important to note that Raf-1*/RKIP is simply a *name*, following biochemical convention; the / symbol is not an operator (in this context). A suffix -P or -PP denotes a phosyphorylated protein, for example MEK-PP and ERK-PP. Each protein has an associated concentration, denoted by $m1$, $m2$ etc. *Reactions* define how proteins are built up and broken down. We refer to the former as an association, or forward reaction, and the latter as a disassociation, or backward reaction. Associations are typically many to one, and disassociations one to many, relations. In the figure, bi-directional arrows denote both forward and backward reactions; unidirectional arrows denote disassociations. For example, Raf-1* and RKIP react (forwards) to form Raf-1*/RKIP, and Raf-1/RKIP disassociates (a backward reaction) into Raf-1* and RKIP. Reactions do not necessarily come in pairs; for example, Raf-1*/RKIP/ERK-PP disassociates into Raf-1*, ERK and RKIP-P. Each reaction has a rate denoted by the rate constants $k1$, $k2$, etc. These are given in the rectangles, with $kn/kn+1$ denoting that kn is the forward rate and $kn+1$ the backward rate. So for example, Raf-1* and RKIP react (forwards) with rate $k1$, and Raf-1/RKIP disassociates with rate $k2$.

Initially, all concentrations are unobservable, except for m_1, m_2, m_7, m_9, and m_{10} [5].

Figure 1 gives only a static, abstract view of the pathway; the dynamic behaviour is quite complex, particularly because some substrates are involved in more than one reaction. In the next section we develop two process algebraic models which capture that dynamic behaviour.

[1] Analysis of our original model(s) indicated a problem with MEK and prompted us to contact an author of [5] who confirmed that there was an omission.

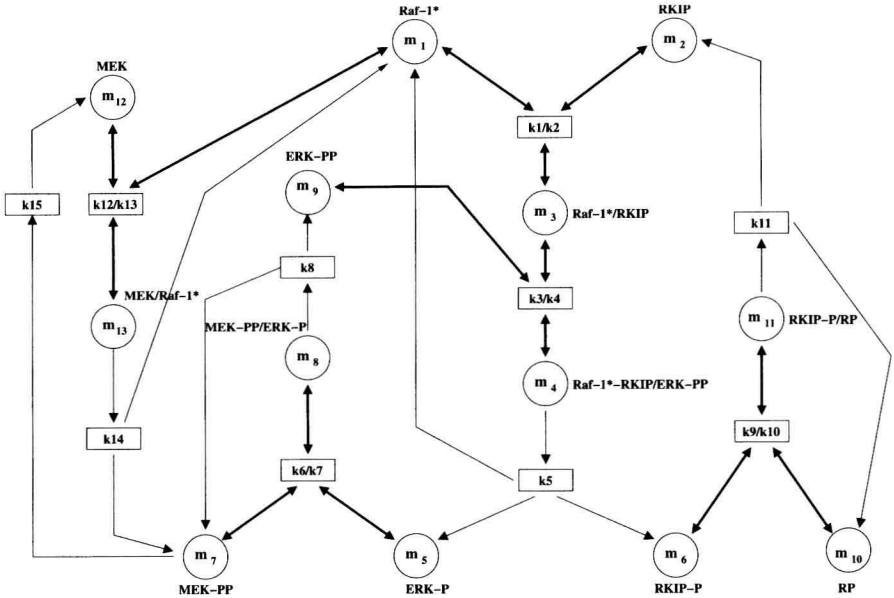

Fig. 1. RKIP inhibited ERK pathway

3 Modelling the ERK Signalling Pathway in PEPA

In this section we present two stochastic process algebra models of the ERK signalling pathway.

The two models presented here encode different views of the underlying biochemistry. The first is a reagent-centric view, focussing on the variations in concentrations of the reagents, fluctuating with phosphorylation and product formation, i.e. with association and disassociation reactions. This model provides a fine-grained, distributed view of the system. The second is a pathway-centric view, tracking the legitimate serialisations of activities. This model provides a coarser grained, more abstract view of the same system.

For some purposes in biological study the former view provides the right conceptual tools and powers the programme of analysis. For other purposes the pathway-centric view brings to the fore the dynamics of greatest interest. A major contribution of this paper is the unification of both views.

We express both models in the PEPA stochastic process algebra [11]. We assume some familiarity with this process algebra; a brief introduction to PEPA is contained in Appendix A. All activities in PEPA are timed. Specifically, their durations are quantified using exponentially-distributed random variables. The PEPA algebra supports multi-way cooperations between components: the result of synchronising on an activity α is thus another α, available for further synchronisation. The multi-way synchronisation of PEPA makes this process algebra ideally suited to this domain.

Each reaction in the pathway is represented by a multi-way synchronisation – on the reagents of the reaction[2]. We refer to reagents as *producers* and *consumers*, depending upon their role within the reaction. Table 1 gives the producers and consumers for reactions in the pathway. The first column names the reaction using the following convention. Reactions which are forward and backward are called *react*, with a prefix which is the associated rate constant. For example, *k1react* is the name of the reaction between Raf-1* and RKIP, to produce Raf-1*/RKIP. Thus *k1react* is a 3-way synchronisation. Reactions which are only disassociations are called *product* (because they produce *products*); again, the prefix denotes the associated rate constant. Table 1 gives only the forward reactions for the reactions which are both forward and backwards; to obtain the associated backward descriptions, replace Producer by Consumer and vice-versa.

Table 1. Reactions in the pathway

Reaction	Producer(s)	Consumer(s)
k1react	{ Raf-1*, RKIP }	{ Raf-1*/RKIP }
k3react	{ ERK-PP, Raf-1*/RKIP }	{ Raf-1*/RKIP/ERK-PP }
k6react	{ MEK-PP, ERK-P }	{ MEK-PP/ERK }
k9react	{ RKIP-P, RP }	{ RKIP-P/RP }
k12react	{ MEK, Raf-1* }	{ MEK/Raf-1* }
k5product	{ Raf-1*/RKIP/ERK-PP }	{ ERK-P, RKIP-P, Raf-1* }
k8product	{ MEK-PP/ERK }	{ MEK-PP, ERK-PP }
k11product	{ RKIP-P/RP }	{ RKIP, RP }
k14product	{ MEK/Raf-1* }	{ Raf-1*, MEK-PP }
k15product	{ MEK-PP }	{ MEK }

3.1 Modelling Centred on Reagents

The reagent-centred model is presented in Figures 2 and 3. In this view, we represent concentrations by a discrete number of abstract values. Here, we consider the coarsest possible discretisation: there are two values representing (continuous) concentrations; we refer to the two values as *high* and *low*. The former implies that a reagent *can* participate (as a producer) in a forward reaction; the latter implies that a reagent *can* participate (as a consumer) in a product, or (as a producer) in a backward reaction. Otherwise, the substrate is inert, with respect to a reaction. We discuss the effect of a finer granularity of abstract concentration on the model in Section 7.

We define the behaviour of each substrate in turn, for each concentration. Thus there are $2n$ equations, where n is the number of proteins. We adopt the naming convention that high concentrations have a H subscript and low concentrations have a L subscript.

Most equations involve a choice between alternative behaviours (notated by +). For example, even in one of the simplest cases, RKIP, where there is a simple

[2] We agree with the authors of [15] – reactions are fundamentally synchronous.

$$\begin{aligned}
\text{Raf-1}^*_{\text{H}} &\stackrel{def}{=} (k1react, k_1).\text{Raf-1}^*_{\text{L}} + (k12react, k_{12}).\text{Raf-1}^*_{\text{L}} \\
\text{Raf-1}^*_{\text{L}} &\stackrel{def}{=} (k5product, k_5).\text{Raf-1}^*_{\text{H}} + (k2react, k_2).\text{Raf-1}^*_{\text{H}} \\
&\quad + (k13react, k_{13}).\text{Raf-1}^*_{\text{H}} + (k14product, k_{14}).\text{Raf-1}^*_{\text{H}}
\end{aligned}$$

$$\begin{aligned}
\text{RKIP}_{\text{H}} &\stackrel{def}{=} (k1react, k_1).\text{RKIP}_{\text{L}} \\
\text{RKIP}_{\text{L}} &\stackrel{def}{=} (k11product, k_{11}).\text{RKIP}_{\text{H}} + (k2react, k_2).\text{RKIP}_{\text{H}}
\end{aligned}$$

$$\begin{aligned}
\text{MEK}_{\text{H}} &\stackrel{def}{=} (k12react, k_{12}).\text{MEK}_{\text{L}} \\
\text{MEK}_{\text{L}} &\stackrel{def}{=} (k13react, k_{13}).\text{MEK}_{\text{H}} + (k15product, k_{15}).\text{MEK}_{\text{H}}
\end{aligned}$$

$$\begin{aligned}
\text{MEK/Raf-1}^*_{\text{H}} &\stackrel{def}{=} (k14product, k_{14}).\text{MEK/Raf-1}^*_{\text{L}} + (k13react, k_{13}).\text{MEK/Raf-1}^*_{\text{L}} \\
\text{MEK/Raf-1}^*_{\text{L}} &\stackrel{def}{=} (k12react, k_{12}).\text{MEK/Raf-1}^*_{\text{H}}
\end{aligned}$$

$$\begin{aligned}
\text{MEK-PP}_{\text{H}} &\stackrel{def}{=} (k6react, k_6).\text{MEK-PP}_{\text{L}} + (k15product, k_{15}).\text{MEK-PP}_{\text{L}} \\
\text{MEK-PP}_{\text{L}} &\stackrel{def}{=} (k8product, k_8).\text{MEK-PP}_{\text{H}} + (k7react, k_7).\text{MEK-PP}_{\text{H}} \\
&\quad + (k14product, k_{14}).\text{MEK-PP}_{\text{H}}
\end{aligned}$$

$$\begin{aligned}
\text{ERK-PP}_{\text{H}} &\stackrel{def}{=} (k3react, k_3).\text{ERK-PP}_{\text{L}} \\
\text{ERK-PP}_{\text{L}} &\stackrel{def}{=} (k8product, k_8).\text{ERK-PP}_{\text{H}} + (k4react, k_4).\text{ERK-PP}_{\text{H}}
\end{aligned}$$

$$\begin{aligned}
\text{ERK-P}_{\text{H}} &\stackrel{def}{=} (k6react, k_6).\text{ERK-P}_{\text{L}} \\
\text{ERK-P}_{\text{L}} &\stackrel{def}{=} (k5product, k_5).\text{ERK-P}_{\text{H}} + (k7react, k_7).\text{ERK-P}_{\text{H}}
\end{aligned}$$

$$\begin{aligned}
\text{MEK-PP/ERK}_{\text{H}} &\stackrel{def}{=} (k8product, k_8).\text{MEK-PP/ERK}_{\text{L}} + (k7react, k_7).\text{MEK-PP/ERK}_{\text{L}} \\
\text{MEK-PP/ERK}_{\text{L}} &\stackrel{def}{=} (k6react, k_6).\text{MEK-PP/ERK}_{\text{H}}
\end{aligned}$$

$$\begin{aligned}
\text{Raf-1}^*\text{/RKIP}_{\text{H}} &\stackrel{def}{=} (k3react, k_3).\text{Raf-1}^*\text{/RKIP}_{\text{L}} + (k2react, k_2).\text{Raf-1}^*\text{/RKIP}_{\text{L}} \\
\text{Raf-1}^*\text{/RKIP}_{\text{L}} &\stackrel{def}{=} (k1react, k_1).\text{Raf-1}^*\text{/RKIP}_{\text{H}} + (k4react, k_4).\text{Raf-1}^*\text{/RKIP}_{\text{H}}
\end{aligned}$$

$$\begin{aligned}
\text{Raf-1}^*\text{/RKIP/ERK-PP}_{\text{H}} &\stackrel{def}{=} (k5product, k_5).\text{Raf-1}^*\text{/RKIP/ERK-PP}_{\text{L}} \\
&\quad + (k4react, k_4).\text{Raf-1}^*\text{/RKIP/ERK-PP}_{\text{L}} \\
\text{Raf-1}^*\text{/RKIP/ERK-PP}_{\text{L}} &\stackrel{def}{=} (k3react, k_3).\text{Raf-1}^*\text{/RKIP/ERK-PP}_{\text{H}}
\end{aligned}$$

$$\begin{aligned}
\text{RKIP-P}_{\text{H}} &\stackrel{def}{=} (k9react, k_9).\text{RKIP-P}_{\text{L}} \\
\text{RKIP-P}_{\text{L}} &\stackrel{def}{=} (k5product, k_5).\text{RKIP-P}_{\text{H}} + (k10react, k_{10}).\text{RKIP-P}_{\text{H}}
\end{aligned}$$

$$\begin{aligned}
\text{RP}_{\text{H}} &\stackrel{def}{=} (k9react, k_9).\text{RP}_{\text{L}} \\
\text{RP}_{\text{L}} &\stackrel{def}{=} (k11product, k_{11}).\text{RP}_{\text{H}} + (k10react, k_{10}).\text{RP}_{\text{H}}
\end{aligned}$$

$$\begin{aligned}
\text{RKIP-P/RP}_{\text{H}} &\stackrel{def}{=} (k11product, k_{11}).\text{RKIP-P/RP}_{\text{L}} + (k10react, k_{10}).\text{RKIP-P/RP}_{\text{L}} \\
\text{RKIP-P/RP}_{\text{L}} &\stackrel{def}{=} (k9react, k_9).\text{RKIP-P/RP}_{\text{H}}
\end{aligned}$$

Fig. 2. PEPA model definitions for the reagent-centric model

cycle between high and low concentrations, there is still a choice of how to return to a high concentration (by a backwards reaction, or through a product). Most behaviours are more complex.

The equations define the possible reactions within the pathway. All of the permissible interleavings of these reactions are obtained from the (synchronised) parallel composition of these components. Figure 3 shows how these are

$$
\begin{aligned}
&(\text{Raf-1}^*_{\text{H}} \underset{\{k1react,k2react,k12react,k13react,k5product,k14product\}}{\bowtie} \\
&\quad (\text{RKIP}_{\text{H}} \underset{\{k1react,k2react,k11product\}}{\bowtie} \\
&\quad\quad (\text{Raf-1}^*/\text{RKIP}_{\text{L}} \underset{\{k9react,k4react\}}{\bowtie} \\
&\quad\quad\quad (\text{Raf-1}^*/\text{RKIP/ERK-PP}_{\text{L}}) \underset{\{k9react,k4react,k5product\}}{\bowtie} \\
&\quad\quad\quad\quad (\text{ERK-P}_{\text{L}} \underset{\{k5product,k6react,k7react\}}{\bowtie} \\
&\quad\quad\quad\quad\quad (\text{RKIP-P}_{\text{L}} \underset{\{k9react,k10react\}}{\bowtie} \\
&\quad\quad\quad\quad\quad\quad (\text{RKIP-P/RP}_{\text{L}} \underset{\{k9react,k10react,k11product\}}{\bowtie} \\
&\quad\quad\quad\quad\quad\quad\quad (\text{RP}_{\text{H}} \parallel \\
&\quad\quad\quad\quad\quad\quad\quad\quad (\text{MEK}_{\text{L}} \underset{\{k12react,k13react,k15product\}}{\bowtie} \\
&\quad\quad\quad\quad\quad\quad\quad\quad\quad (\text{MEK/Raf-1}^*_{\text{L}} \underset{\{k14product\}}{\bowtie} \\
&\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad (\text{MEK-PP}_{\text{H}} \underset{\{k8product,k6react,k7react\}}{\bowtie} \\
&\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad (\text{MEK-PP/ERK}_{\text{L}} \underset{\{k8product\}}{\bowtie} \\
&\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad (\text{ERK-PP}_{\text{H}})))))))))))
\end{aligned}
$$

Fig. 3. PEPA model configuration for the reagent-centric model

composed in the PEPA algebra. The composition operator ($\bowtie$) is indexed by an activity set (i.e. the events whose participants must be synchronised). The left and right operands must cooperate on these activities, introducing a synchronisation point. The degenerate case of this composition operator (where the set is empty) provides the expected unrestricted parallel composition of the components, allowing all possible interleavings without synchronisation. This case is denoted by $\parallel$ (there is one occurrence).

The initial state of the model has high concentrations of some reagents and low concentrations of the others, as described in the previous section. Therefore, in Figure 3, proteins with an initial concentration are initially high; all others are low.

3.2 Modelling Centred on Pathways

A different view is afforded by the pathway-centric perspective. This de-emphasises reagents and emphasises sub-pathways within the signalling pathway. In this model, given in Figure 4, there are five (sub)pathways, one for each substrate with an initial concentration. Thus $Pathway_{10}$ corresponds to the pathway from RP (m_{10}), $Pathway_{20}$ to RKIP (m_2), $Pathway_{30}$ to ERK-PP (m_9), $Pathway_{40}$ to Raf-1* (m_1), and $Pathway_{50}$ to MEK-PP (m_7). Each (sub)pathway describes, in effect, how a substrate is consumed and then, eventually, replenished.

It is important to note that none of these (sub)pathways is *closed*, i.e. there are reactions with edges which are directed to/from outside of the (sub)pathway. Figure 6 gives a diagrammatic representation of the simplest pathway, $Pathway_{10}$.

$$Pathway_{10} \stackrel{def}{=} (k9react, k_9).Pathway_{11}$$
$$Pathway_{11} \stackrel{def}{=} (k11product, k_{11}).Pathway_{10} + (k10react, k_{10}).Pathway_{10}$$

$$Pathway_{20} \stackrel{def}{=} (k1react, k_1).Pathway_{21}$$
$$Pathway_{21} \stackrel{def}{=} (k3react, k_3).Pathway_{22} + (k2react, k_2).Pathway_{20}$$
$$Pathway_{22} \stackrel{def}{=} (k5product, k_5).Pathway_{23} + (k4react, k_4).Pathway_{21}$$
$$Pathway_{23} \stackrel{def}{=} (k9react, k_9).Pathway_{24}$$
$$Pathway_{24} \stackrel{def}{=} (k11product, k_{11}).Pathway_{20} + (k10react, k_{10}).Pathway_{23}$$

$$Pathway_{30} \stackrel{def}{=} (k3react, k_3).Pathway_{31}$$
$$Pathway_{31} \stackrel{def}{=} (k5product, k_5).Pathway_{32} + (k4react, k_4).Pathway_{30}$$
$$Pathway_{32} \stackrel{def}{=} (k6react, k_6).Pathway_{33}$$
$$Pathway_{33} \stackrel{def}{=} (k8product, k_8).Pathway_{30} + (k7react, k_7).Pathway_{32}$$

$$Pathway_{40} \stackrel{def}{=} (k1react, k_1).Pathway_{41} + (k12react, k_{12}).Pathway_{43}$$
$$Pathway_{41} \stackrel{def}{=} (k2react, k_2).Pathway_{40} + (k3react, k_3).Pathway_{42}$$
$$Pathway_{42} \stackrel{def}{=} (k5product, k_5).Pathway_{40} + (k4react, k_4).Pathway_{41}$$
$$Pathway_{43} \stackrel{def}{=} (k13react, k_{13}).Pathway_{40} + (k14product, k_{14}).Pathway_{40}$$

$$Pathway_{50} \stackrel{def}{=} (k15product, k_{15}).Pathway_{51} + (k6react, k_6).Pathway_{53}$$
$$Pathway_{51} \stackrel{def}{=} (k12react, k_{12}).Pathway_{52}$$
$$Pathway_{52} \stackrel{def}{=} (k13react, k_{13}).Pathway_{51} + (k14product, k_{14}).Pathway_{50}$$
$$Pathway_{53} \stackrel{def}{=} (k8product, k_8).Pathway_{50} + (k7react, k_7).Pathway_{50}$$

Fig. 4. PEPA model definitions for the pathway-centric model

In this case, the pathway is not closed because there are two missing edges associated with *k9react* and *k11product*.

This presentation facilitates the direct verification of simple properties of the model such as "the first observable activity is event X". For example, an initial syntactic inspection of this model would lead to the conclusion that the first activity is one of *k1react*, *k3react*, *k9react* or *k15product*. Processing the model with the PEPA Workbench [9] confirms that the initial model configuration allows only *k15product* and *k1react*, the others are not permitted because some necessary participants are not initially ready to engage in these reactions.

4 Comparison of Reagent and Pathway-Centric Models

The pathway-centric model captures longer chains of behaviour flow within the system, leading to a smaller number of component definitions. Differentiating fewer components in the pathways model leads to a simpler composition of model components, presented in Figure 5. This is not only a matter of presentation. A larger state vector representation occupies more memory so the