# Modelling Scaffold-mediated Crosstalk between the cAMP and the Raf-1/MEK/ERK Pathways

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**Abstract.** We study the biochemical processes involved in scaffold-mediated crosstalk between the cAMP and the Raf-1/MEK/ERK pathways. We model the interactions by a continuous time Markov chain with levels and analyse properties using Continuous Stochastic Logic and the symbolic probabilistic model checker PRISM. We consider a number of biologically relevant properties of the model, including sequentially dependent events and pulsating behaviour. In order to handle these kinds of properties, we extend the model with signs of first order derivatives.

## 1 Introduction

In the context of intracellular signalling pathways, a scaffold is a protein whose main role is to anchor particular proteins in the proper locations for receiving signals or transmitting them. In addition, under certain circumstances, a scaffold can increase the output of a signalling cascade or decrease the response time for a faster output.

In this paper we present a model of scaffold-mediated crosstalk between the cyclic adenosine monophosphate (cAMP) and the Raf-1/MEK/ERK pathways, an interaction that has an important role in the regulation of cell proliferation, transformation and survival. We use continuous time Markov chains with levels (CTMC with levels) [CDHC09] and the PRISM model checker [KNP07] to develop and analyse a number of predictive models. Our aim is to provide new quantitative analysis and new ways to model and reason about behaviour that is not yet completely understood or quantified.

We express properties in Continuous Stochastic Logic (CSL) [BHHK03], in particular some restrictive case of sequentiality properties and pulsations. In order to analyse these kinds of properties, for each variable of interest we add a new variable in the model representing the sign of its derivative.

The paper is organised as follows. In the next section we introduce the basics about scaffolds and the role of an AKAP scaffold in particular. In Section 3 we give an overview of the PRISM model. Section 4 contains a description of some properties of interest and the results of analysis. Conclusion and directions for future work follow. We note that the behaviour of scaffold proteins modelled here is the topic of current wet-lab investigation. Our models have been informed by discussions with a number of experimentalists: some aspects of behaviour are still unknown and the subject of conjecture.

## 2 Scaffold proteins and the AKAP

In intracellular signal transduction pathways, scaffolds are proteins playing an organisational role rather than a signalling role [JEF00]. Scaffolds have a anchoring function

by placing particular proteins in the proper intracellular locations for receiving signals or transmitting them. Experiments have shown that under certain circumstances, a kinase scaffold can increase the output of a signalling cascade or decrease the response time for a faster output. Therefore scaffolds may also exhibit a catalytic function. Another property of the scaffolds is combinatorial inhibition: if there are too many or too few of either scaffolds or kinases, the output of the pathway decreases.

We are interested in particular in the behaviour of the *A-kinase anchoring protein* (AKAP for short) in the context of crosstalk between cyclic AMP and the Raf-1/MEK/ERK pathway. The species we are interested in are the following: (i) cyclic adenosine monophosphate (cAMP); (ii) protein kinase A (PKA), the main cAMP effector; (iii) Raf-1 with two phosphorylation sites of interest, Serine 338 (S338) and Serine 259 (S259); (iv) phosphodiesterase 8 (PDE8A1), (v) phosphatase PP.

If the concentration level of cAMP goes above the basal one, cAMP activates PKA by binding to its regulatory subunits. When PKA becomes activate, its catalytic subunits catalyse the transfer of ATP terminal phosphates to the phosphorylation site S259 of Raf-1. The site S338 of Raf-1 is inhibited when S259 is phosphorylated. Only when S338 gets phosphorylated, the pathway Raf-1/MEK/ERK is activated and the signalling cascade begins.

The catalytic function of PKA would sometimes couple with the AKAP, by binding PKA together with phosphodiesterase PDE8A1 on the scaffold to form a complex that functions as a signal module. Under these conditions, as the cell is stimulated, cAMP activates PKA, and then PKA is responsible for the activation of PDE8A1 (by phosphorylation). PDE8A1 converts cAMP to AMP by hydrolysis. If phosphorylated, PDE8A1 degrades more cAMP, hence rapidly reducing the amount of cAMP that can activate PKA hence leading to a feedback mechanism for downregulating PKA.

The inhibition of Raf-1 at S338 is correlated with a high activity of PKA. At the beginning, cAMP synthesis is induced, causing a rise of PKA's activity that continues in the inhibition of Raf-1.

We illustrate the AKAP scaffold and the regulatory mechanism described above in Figure 1. We use three different types of arrow to distinguish between different types of interactions:

- A activates or phosphorylates B : $A \longrightarrow\!\!\!\!\succ B$
- A dephosphorylates B : $A - - \succ B$
- A degrades B : $A \longrightarrow\!\!\!\!\dashv B$

The arrow with no source and with target cAMP represents a diffusion of cAMP from the environment.

The AKAP scaffold has three positions to be filled by PKA, Raf-1 and PDE8A1 respectively. Hereafter we use a binary representation of the states of each position: 1 for activation or phosphorylation and 0 otherwise. The second position concerns the state of the site S259 of Raf-1. If the scaffold position for PDE8A1 is not filled, we only represent the first two positions of the scaffold. For instance $S100$ stands for a filled scaffold with active PKA and unphosphorylated PDE8A1 and site S259, whereas $S01$ for an unfilled scaffolded with inactive PKA and phosphorylated S259.

In Figure 2 we describe the biochemical reactions of the model. Each reaction is given in pseudo-chemical notation, with explicit reference to the scaffold positions (the underlying reactions have mass action kinetics). We associate reaction rate constants (from $r_1$ to $r_{26}$) with each biochemical reaction.

Currently, we do not have good experimental data concerning rates for the reactions. However, we have some information on the ratio between the rate of PKA

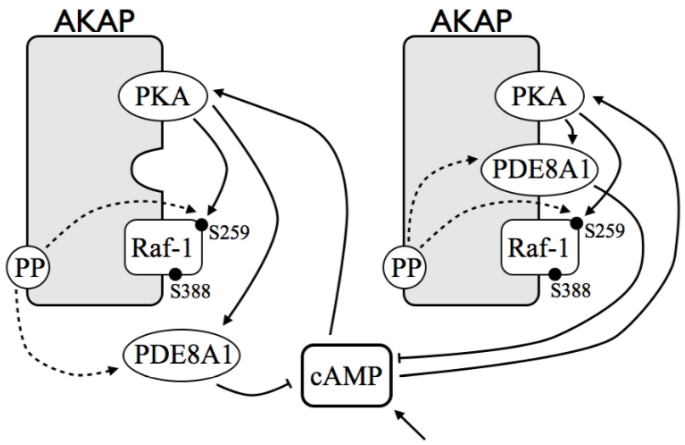


**Fig. 1.** Interactions between cAMP, filled AKAP scaffold, unfilled scaffold and free PDE8A1

**(cAMP diffusion)**
$\_ \rightarrow_{r_1} cAMP$

**(PKA activation)**
$S000 + cAMP \rightarrow_{r_2} S100$
$S00 + cAMP \rightarrow_{r_3} S10$

**(S259 phosphorylation)**
$S100 \rightarrow_{r_4} S110$
$S101 \rightarrow_{r_5} S111$
$S10 \rightarrow_{r_6} S11$

**(S259 dephosphorylation)**
$PP + S010 \rightarrow_{r_7} PP + S000$
$PP + S011 \rightarrow_{r_8} PP + S001$
$PP + S01 \rightarrow_{r_9} PP + S00$

**(cAMP release)**
$S111 \rightarrow_{r_{17}} S011 + cAMP$
$S11 \rightarrow_{r_{18}} S01 + cAMP$

**(PDE8A1 phosphorylation)**
$S100 \rightarrow_{r_{10}} S101$
$S110 \rightarrow_{r_{11}} S111$
$S10 + PDE8A1 \rightarrow_{r_{12}} S10 + pPDE8A1$
$S11 + PDE8A1 \rightarrow_{r_{13}} S11 + pPDE8A1$

**(PDE8A1 dephosphorylation)**
$PP + S001 \rightarrow_{r_{14}} PP + S000$
$PP + S011 \rightarrow_{r_{15}} PP + S010$
$PP + pPDE8A1 \rightarrow_{r_{16}} PP + PDE8A1$

**(cAMP degradation)**
$S011 + cAMP \rightarrow_{r_{19}} S011$
$S001 + cAMP \rightarrow_{r_{20}} S001$
$S100 + cAMP \rightarrow_{r_{21}} S100$
$S110 + cAMP \rightarrow_{r_{22}} S110$
$S010 + cAMP \rightarrow_{r_{23}} S010$
$S000 + cAMP \rightarrow_{r_{24}} S000$
$pPDE8A1 + cAMP \rightarrow_{r_{25}} pPDE8A1$
$PDE8A1 + cAMP \rightarrow_{r_{26}} PDE8A1$

**Fig. 2.** Biochemical reactions occurring during scaffold-mediated crosstalk between the cAMP and the Raf-1/MEK/ERK pathway

phosphorylating Raf-1 at S259 and PDE8A1 (either on the scaffold or not). On unfilled scaffolds, PKA phosphorylates two or three times less unscaffolded PDE8A1 than Raf-1 at S259 from the same scaffold. On filled scaffolds, PKA phosphorylates at the same rate Raf-1 at S259 and PDE8A1. Consequently the relation between constant rates of the reactions involving PKA phosphorylating either PDE8A1 or Raf-1 is: $r_4 = r_5 = r_6 = r_{10} = r_{11} = 3 * r_{12} = 3 * r_{13}$. In addition, phosphorylated PDE8A1 degrades about three times more cAMP than PDE8A1 does, hence the following ratios between the constants rates of the reactions where PDE8A1 degrades cAMP: $r_{19} = r_{20} = r_{21} = r_{22} = r_{23} = r_{24} = 3 * r_{25} = 9 * r_{26}$.

Finally, when PKA and PDE8A1 form a complex on the scaffold, PKA's activity becomes more efficient.

Our discussions with experimentalists have revealed the following expectations, or conjectures, about AKAP behaviour.

*Question 1.* Increasing the amount of phosphorylated PDE8A1 leads to a cascade of changes in the concentration levels of the other reactants: decreasing amounts of cAMP and active PKA, and an increase in the activity of Raf-1 due to lower levels of phosphorylated Raf-1 at site S259. Informally, we express this behaviour by the following implication, where $\uparrow$ ( resp. $\downarrow$) denotes increase (resp. decrease):

$$\uparrow \text{pPDE8A1} \Rightarrow \downarrow \text{cAMP} \Rightarrow \downarrow \text{active PKA} \Rightarrow \downarrow \text{pRaf-1}_{\text{S259}} \equiv \uparrow \text{active Raf-1}$$

*Question 2.* Interactions between cAMP and Raf-1 and other molecules are not considered in the current model. However, the system is not closed, we include a kind of exogenous interaction concerning the diffusion of cAMP. We conjecture this leads to a fluctuation of the concentrations of some reactants. Namely, the system should exhibit a pulsating behaviour corresponding to the feedback mechanism for the downregulation of PKA coupled with the diffusion of cAMP. Time courses from laboratory experiments suggest the presence of a pulsating behaviour. Pulsation ensures that the state of the Raf-1 pathway alternates such that it is not always (or for a very long period of time) either active or inactive (which may increase the risk of disease). Note that we call such a behaviour pulsating, not oscillating. This is because oscillation assumes fluctuation around a given value; current data does not provide us with such a value, hence our choice of pulsating rather than oscillating behaviour.

## 3 Modelling AKAP with CTMCs with Levels

Following the style adopted in [CVGO06], we define a PRISM model for a CTMC with levels as follows. There are modules for cAMP, scaffold, PDE8A1 and PP, each with corresponding variables representing levels of concentrations. In particular, the module for the scaffold has a variable for each possible combination of positions (S000, S100, S101, S110, S011, S010, S001, S111, S00, S10, S01, S11). Commands in the modules correspond to reactions, which are synchronised on each participating module (i.e. consumers and producers in the chemical reaction). Additionally, we defined diffusion of cAMP from time to time.

As an example, consider the reaction $r_2$ where cAMP activates PKA when the level of cAMP is above the basal level. Then in the module describing cAMP we add the command:

```
[activate_PKA] (cAMP > basal_camp) -> (cAMP) : (cAMP' = cAMP-1);
```

while in the module describing the scaffold we have the coupling command:

```
[activate_PKA] (S000 > 0) & (S100 < scaffold_max) ->
               (r2*S000) : (S100' = S100+1) & (S000' = S000-1);
```

In the definition of the PRISM model, we used the convention that the rate constant goes in the command corresponding to the consumer reactant, in this case PKA. By synchronisation on the common label, the reaction rate will then be the product of the constant rate $r_2$ and the concentration levels of cAMP and PKA.

Unless otherwise stated, we assume the number of levels $N = 2$. We consider maximum $N$ levels of filled/unfilled scaffolds, $2 * N$ levels of phosphatase PP, and around $N/2$ levels of unscaffolded PDE8A1. Whereas for cAMP, since it is diffused in the system, we allow a greater concentration of cAMP, maximum $10 * N$. Full details of the model are available from the authors.

## 4 Analysis

We use rewards based properties and CSL properties to formalise the questions posed in Section 2 and PRISM to verify their satisfaction (or not).

First, we consider Question 1, using rewards to compute the expected level of concentration at a particular time.

Second, we consider transient properties concerning the sequentiality of events and pulsating behaviour. We extend the states of the CTMC model to include (signs of) derivatives, for some variables of interest.

**Reward-based Properties.** For each species of interest: phosphorylated PDE8A1, free cAMP (not bound to some PKA), active PKA and phosphorylated Raf-1 at site S259, we add a reward construct to compute the expected level of concentration at a particular time. For example, the reward associated with phosphorylated PDE8A1 computes the sum of the level of unscaffolded and phosphorylated PDE8A1 and the levels of scaffolded and phosphorylated PDE8A1, at each time instant.

```
rewards "phosphopde8"
    true : pPDE8A1 + S101 + S111 + S001 + S011;
endrewards
```

In Figure 3 we plot the expected levels of concentration for each species. Note a delayed pulsation of all variable values. However, this does not prove that the properties expressed in Section 2 are satisfied; for this, we formulate temporal properties.

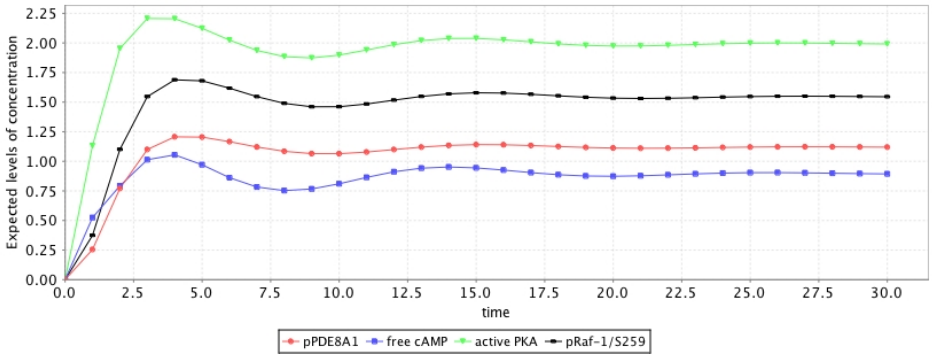


**Fig. 3.** Expected levels of concentrations of phosphorylated PDE8A1, free cAMP, active PKA, and phosphorylated Raf-1 at site S259 after 30 time-units

**Derivative-based Transient Properties.** We extend the CTMC with levels model such that for each species in the biochemical system, we add a new variable representing the sign of the value of the derivative. Then, each PRISM command associated with a transition updates not only the values of some variables, but also the signs of their derivative as well. For efficiency reasons we include a derivative only for variables occurring in the properties of interest. In our particular model, we add derivatives for cAMP, active PKA and scaffolded phosphorylated PDE8A1.

We illustrate here how the signs of the derivatives are updated in the scaffold module for the reaction where cAMP activates PKA:

```
[activate_PKA] (S000 > 0) & (S100 < scaffold_max) ->
                (r2*S000) : (S100' = S100+1) & (S000' = S000-1) &
                (drv_PKA_A' = 1) & (drv_S259_P' = 0) & (drv_PDE8_P' = 0);
```

Since the level of active PKA is affected (increased) by this command, the derivative of PKA becomes positive, whereas the rest of derivatives become 0 since there is no change in the variables values.

We now formalise the temporal properties. In the following, for $x$ a variable, we denote by $\downarrow x$ a negative derivative of $x$ and by $\uparrow x$ a positive derivative of x.

*Necessarily Preceded.* We express the property in Question 1 as a temporal query using the *necessarily preceded* or *requirement* pattern [MRM+08]. This pattern represents an ordering relation between two events, the occurrence of the later being conditioned by the occurrence of the former: *a state $\phi$ is reachable and is necessary preceded all the time by a state $\psi$*. The associated CTL formula of this pattern is:

$$EF\phi \wedge (AG((\neg\psi) \Rightarrow AG(\neg\phi)))$$

Assume the following two state formula:

$$\phi = \downarrow \text{cAMP} \wedge \downarrow \text{active PKA} \qquad \psi = \uparrow \text{pPDE8A1}$$

with $\phi$ corresponding to a state where the levels of cAMP and active PKA are decreasing, and $\psi$ to a state where the level of phosphorylated PDE8A1 is increasing. Employing basic propositions equivalences, we translate the requirement pattern into CSL to obtain the following formula which was checked as **true** for our PRISM model:

$$P_{>0}[F\phi] \wedge P_{\leq 0}[F(\neg((\neg\psi) \Rightarrow P_{\geq 1}[F(\neg\phi)]))]$$

*Pulsating behaviour.* An oscillating behaviour concerns fluctuation around a given value $k$. Oscillation and its expression as temporal formulas in CTL and PCTL have been studied in [BMM09] and informally described as *always in the future, the variable $x$ departs from and reaches the values $k$ infinitely often.* The corresponding CTL formula is:

$$AG(((x = k) \Rightarrow EF(x \neq k)) \wedge ((x \neq k) \Rightarrow EF(x = k)))$$

However, as discussed earlier, we are interested in pulsating behaviour, i.e. no fixed $k$. We therefore consider oscillations (around 0) of the values of the first derivatives of some variables. We refer to this approximate oscillating behaviour as pulsation. Note that we can observe a pattern corresponding to a pulsation in Figure 3: we repeatedly have the situation where the level of phosphorylated PDE8A1 increased whereas the levels of cAMP and active PKA decreased, and then the level of phosphorylated

PDE8A1 decreased whereas the levels of cAMP and active PKA increased. Assume the following two state formulas:

$$\phi = \uparrow \text{pPDE8A1} \wedge \downarrow \text{cAMP} \wedge \downarrow \text{active PKA}$$
$$\psi = \downarrow \text{pPDE8A1} \wedge \uparrow \text{cAMP} \wedge \uparrow \text{active PKA}$$

We translate the CTL temporal formula describing an oscillation to a CSL formula for a pulsation involving the two state formulas above and we obtain the following formula checked as **true** for our model using PRISM:

$$P_{\leq 0}[F(\neg(\phi \Rightarrow P_{>0}[F\psi]) \vee \neg(\psi \Rightarrow P_{>0}[F\phi]))]$$

## 5 Related work

Derivatives have been considered previously in the context of model checking. For example in BIOCHAM [Fag05,RBFS08], the query language associated is LTL with constraints over real numbers, evaluated using a symbolic model checker written in Prolog. Again, in the context of BIOCHAM [CRCFS04], a weaker form of oscillation properties expressed in CTL are used with the symbolic model checker NuSMV; the oscillating behaviour is approximated by the necessary but not sufficient formula $EG((EF\neg\varphi) \wedge (EF\varphi))$.

## 6 Conclusion and Future Work

We have developed a stochastic model of the behaviour of the AKAP scaffold and scaffold-mediated crosstalk between the cAMP and the Raf-1/MEK/ERK pathways. The model is a CTMC with levels and is implemented in the PRISM language.

We have considered questions and conjectures concerning system behaviour posed by experimentalists; these include sequentially dependent events and pulsating behaviour. In the context of imprecise and incomplete data, pulsation seems more appropriate than oscillation. We have used rewards and CSL to express the properties, and checked them with the PRISM model checker. In order to express pulsation, we have added a representation of signs of first derivatives to the stochastic model. Preliminary discussions with experimentalists confirm their interest and validation of the model and analysis.

Future work includes adding explicit derivatives, so that we can reason about the amplitude of the pulsation. We will refine the model with data on the rates, as the data becomes available, and add more detail such as it requires 4 molecules of cAMPs to activate one PKA. We will also investigate the value of adding second derivatives to identify local minima and maxima.

We have investigated further hypotheses, such as whether the way PDE8A1 decreases the PKA phosphorylation of Raf-1 at S259 can be counterbalanced by the addition of a PDE8A1 inhibitor such as the a drug Dipyridamole. This is the topic of a further paper.

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