

# Reduction of nonlinear dynamic systems with an application to signal transduction pathways

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**Abstract:** Mathematical modelling of kinetic processes with different time scales allows a reduction of the governing equations using quasi-steady-state approximations (QSSA). A QSSA theorem is applied to a mathematical model of the influence that Raf kinase inhibitor protein (RKIP) has on the ERK signalling pathway. On the basis of previously published parameter values, the system of 11 ordinary differential equations is rewritten in a form suitable for model reduction. In accordance with the terminology of the QSSA theorem, it is established that four of the protein and protein-complex concentrations are ‘fast varying’, such that the corresponding kinetic equations form an attached system. Another concentration is ‘medium varying’ such that the corresponding equation is reduced with respect to the four fast ones. The other six concentrations are ‘slow varying’, which means the corresponding kinetic equations also present a reduced system with respect to the others. Analytical solutions, relating the steady-state values of the fast varying protein concentrations and the slow varying ones, are derived and interpreted as restrictions on the regulatory role of RKIP on ERK-pathway.

## 1 Introduction

In this paper, the term quasi-steady-state approximation (QSSA) is used in a sense explained in the work of Schneider and Wilhelm [1]. Model reduction based on this approximation has found various applications in systems biology, including studies related to cell proliferation, differentiation and the cell cycle [2]. In cell signalling, a pathway is understood as a network of recurrent biochemical reactions, partly connected by feedback loops. The present paper considers a previously published model for the interaction between Raf kinase inhibitor protein (RKIP) and the Ras/Raf/MEK/ERK pathway. The ubiquitously expressed Ras/Raf/MEK/ERK pathway controls fundamental processes and is often deregulated in human cancer [3]. In the work of Cho *et al.* [4], the RKIP influence is investigated through mathematical modelling and numerical simulation. A dynamical system of 11 nonlinear ordinary differential equations is used to represent biochemical reactions in the pathway. The proposed model allows the simulation of the sensitivity of the ERK pathway to variations in various parameters. A conclusion from that study was that RKIP modulates the final extent and duration of ERK activity. Of further interest is a possible restriction of this modulation dependent on initial conditions. Towards this end, we consider here a QSSA of the Ras/Raf/MEK/ERK pathway. We arrive at a reduction of the nonlinear dynamic system introduced in the work of Cho *et al.* [4] and derive analytical expressions to relate

the initial values of slow varying concentrations and steady-state values of fast varying ones.

A classical example of dimensionality reduction for nonlinear dynamic systems is the application to Michaelis–Menten type enzyme kinetics [5]. Examples of reversible enzyme catalytic reactions that are well described by reversible kinetic scheme can be found in the literature [6–8]. A recent contribution to the reversible case is presented in the work of Tzafriri and Edelman [9], where a QSSA for the reversible Michaelis–Menten equation is derived and its validity domain is delineated. Other publications, directly related to the reduction of pathway models, include the work of Millat *et al.* [10], Kholodenko *et al.* [11] and Kruger and Heinrich [12].

The present work presents a more general approach to a QSSA, based on corresponding theorem proved in the work of Tichonov [13]. Our aim is to demonstrate the application of the method in this new form to cell signalling pathways. In view of the fact that the model equations of the Ras/Raf/MEK/ERK pathway are rather typical for MAPK cascades, other applications of the results presented here can be foreseen. Some initial ideas of the present paper were introduced in the work of Fall *et al.* [14] and Petrov *et al.* [15]. In the following section, we introduce basic ideas of the QSSA approach.

## 2 QSSA theorem

Mathematical modelling of enzyme kinetics with different time scales leads, in general, to a dynamical system of the following form

$$\varepsilon \frac{d\vec{x}}{dt} = \vec{f}(\vec{x}, \vec{y}) \quad (1)$$

$$\frac{d\vec{y}}{dt} = \vec{g}(\vec{x}, \vec{y}) \quad (2)$$

where  $\vec{x} \in R^m$ ,  $\vec{y} \in R^n$ ,  $0 < \varepsilon \ll 1$ . Sensitivity analysis provides a powerful tool to obtain mathematical models of

enzyme kinetics in the form (1) and (2) [4, 15]. In the work of Cho *et al.* [4], the analysis of the system is used to determine which variables ( $\vec{x}$  in (1) and (2)) have the largest contribution to the signalling pathway. In the work of Petrov *et al.* [15], the QSSA theorem is applied and the parameter  $\varepsilon$  with a vanishing small value is introduced to rewrite the equation as a dynamical system of type (1) and (2). As it will be demonstrated below, if a system of type (1) and (2) can be obtained in a dimensional form, then the QSSA theorem does not require any non-dimensionalisation of the differential equations as it was required in the work of Petrov *et al.* [15]

In order to compare QSSA time-scale analysis with the QSSA theorem and further develop it, we summarise it here. In the work of Fall *et al.* [14], the following five steps of a QSSA were given:

- Analyse the parameters of the model to assess whether there are time scales that can be separated into ‘fast’ and ‘slow’.
- Define time constants for each time domain whose ratios define a small parameter  $\varepsilon$ .
- Select appropriate parameters in the model to non-dimensionalise the dependent variables.
- Non-dimensionalise the differential equations in each time domain and see which terms can be neglected as  $\varepsilon \rightarrow 0$ .
- Analyse the simplified equations, which represent the behaviour of the variables on the two scales.

Many problems of a mathematical nature could arise by following the procedure (a–e) in the context of complex systems. Some of which are considered in the work of Schneider and Wilhelm [1]. There, the work of Tichonov [13] is cited in connection with the necessity to define the very notion of QSSA. In the work of Kholodenko *et al.* [11], a more specific approach of a QSSA is applied. It considers equations for fast variables  $\vec{x}$ , contributing little to the dynamics of system of type (1) and (2). Yet they do interfere with the calculation of the system’s behaviour in terms of a numerical integration of the equations. Kholodenko *et al.* [11] present a way to solve this problem systematically for systems with time hierarchies. They identify the fast equations and fast variables, group them apart from the other (‘slow’) equations and variables, and then apply the appropriate QSSA to the fast subsystem. This then makes it possible to eliminate the fast equations. The analysis is suggested to be particularly applicable to biochemical reaction networks in biological cells, where a time hierarchy exists, the fastest being at the level of enzyme kinetics and the slowest at gene expression.

The present paper further investigates the QSSA approach considered in the work of Petrov *et al.* [15] and Kholodenko *et al.* [11]. On the other hand, we show that the QSSA theorem proved in the work of Tichonov [13] is not only very original and consistent from the pure mathematical point of view, but also effective in a computational sense, independently of the widespread time-scale procedure described above.

The QSSA theorem [13, 14] claims that the solution of the **complete** system (1) and (2) tends to the solution of the **reduced** system (2) at  $\varepsilon \rightarrow 0$ , if the following conditions are satisfied:

- There is an isolated equilibrium (steady state) solution of the **attached** system (1) (i.e. there is not other solution in its neighbourhood).

- The existing equilibrium solution of the attached system is stable for every value of the **slow** variables  $\vec{y}$ .
- The initial conditions (states) lie in a region of influence (a basin) of the equilibrium solution of the attached system.
- The solution of the complete system is single-valued and its right-hand sides are continuous.

It therefore follows that in every concrete case we can find the equilibrium solution of the attached system and to replace it in the reduced one. Moreover, we should demonstrate that all requirements of the formulated theorem are satisfied.

Considering the simplest example of two differential equations ( $m = 1, n = 1$ ) or  $\vec{x} \equiv x, \vec{y} \equiv y$ , the system (1) and (2) takes the form

$$\varepsilon \frac{dx}{dt} = f(x, y) \quad (3)$$

$$\frac{dy}{dt} = g(x, y) \quad (4)$$

The essence of the QSSA theorem claims that the character of the solution of (3) and (4) does not change when the small parameter  $\varepsilon$  converges to zero. Thus, we can assume  $\varepsilon = 0$  in (3) and instead of differential equations obtain algebraic ones for the steady-state value of fast variable  $x$

$$0 = f(x, y) \quad (5)$$

$$\frac{dy}{dt} = g(x, y) \quad (6)$$

From (5), the fast variable  $x$  can be expressed as a function of  $y$ , that is,  $x = \varphi(y)$  and substituted in (6). As a result, (6) becomes

$$\frac{dy}{dt} = g[\varphi(y), y] \quad (7)$$

In this way, the complete system of two equations (3) and (4) is reduced to the reduced system of one equation (7). Moreover, the stationary values of the fast variable  $x$  depend only on the current values of the slow variable  $y$ , but not on final stationary values. In this sense, the variable  $y$  plays role of a driver of the subordinated variable  $x$ .

The number of initial conditions of the reduced system (5) and (6) is smaller than that of the complete system (3) and (4). The initial condition of (3) is not used in (5) and (6). In accordance with the QSSA theorem, when the stationary solution of the attached system is isolated and stable, then the solution of the reduced system depends only on the initial values of the slow variables. Therefore, the presence of a small parameter is a necessary condition for dimensionality reduction of the complete system. The appropriate example for the introduction of a similar procedure for enzyme kinetic reactions is given in the work of Romanovskii *et al.* [16]. This leads to normalisation (or scaling) of the terms in the right-hand sides of the system equations. In this way, only  $\varepsilon$  is considered as a dimensionless parameter. We draw attention also to the fact that  $\varepsilon$  has no physical meaning (as for example concentration ratios of conserved quantities, conservation sums, steady-state values and so on, may have).

### 3 Dynamical model of the ERK signalling pathway regulated by RKIP

In this section, we introduce briefly the Ras/Raf/MEK/ERK signalling pathway. In view of the fact that it controls so important processes as cell differentiation and

proliferation, understanding the reaction mechanism of this module of cell signalling is of main scientific interest. Experimental investigations have described the role that RKIP plays on the behaviour of this pathway [3]. One hypothesis is that it inhibits activation of Raf and thus ‘downregulates’ the ERK pathway. This is based on evidence that RKIP inhibits the malignant transformation by Ras and Raf oncogenes in cell cultures and it is reduced in tumours. The graphical representation of the influence of RKIP on the ERK signalling pathway is shown in Fig. 1.

In fact, Fig. 1 illustrates only a part of the ERK pathway, that is, it considers the subset of the ERK pathway regulated by RKIP. It is constructed on the basis of enzyme kinetics reactions. Here, each node of the scheme is labelled and the corresponding protein is denoted. For example, Raf-1\* and RKIP are proteins, but Raf-1\*/RKIP is a complex built-up from the first two. The suffix -P and -PP denote phosphorylated and double phosphorylated proteins, respectively, say RKIP-P or ERK-PP. The concentration of each signalling component is denoted by  $m_i$  ( $i = 1, 2, \dots, 11$ ). Moreover, the forward and backward reactions are expressed by bi-directional and uni-directional arrows denote only dissociations. In addition, each reaction has a rate denoted by the rate constants  $k_i$  ( $i = 1, 2, \dots, 11$ ). In the work of Cho *et al.* [4], this biochemical diagram is represented mathematically by the following system of non-linear differential equations

$$\begin{aligned} \frac{dm_1}{dt} &= -k_1 m_1 m_2 + k_2 m_3 + k_5 m_4 \\ \frac{dm_2}{dt} &= -k_1 m_1 m_2 + k_2 m_3 + k_{11} m_{11} \\ \frac{dm_3}{dt} &= k_1 m_1 m_2 - k_2 m_3 - k_3 m_3 m_9 + k_4 m_4 \\ \frac{dm_4}{dt} &= k_3 m_3 m_9 - k_4 m_4 - k_5 m_4 \\ \frac{dm_5}{dt} &= k_5 m_4 - k_6 m_5 m_7 + k_7 m_8 \\ \frac{dm_6}{dt} &= k_5 m_4 - k_9 m_6 m_{10} + k_{10} m_{11} \end{aligned}$$

$$\begin{aligned} \frac{dm_7}{dt} &= -k_6 m_5 m_7 + k_7 m_8 + k_8 m_8 \\ \frac{dm_8}{dt} &= k_6 m_5 m_7 - k_7 m_8 - k_8 m_8 \\ \frac{dm_9}{dt} &= -k_3 m_3 m_9 + k_4 m_4 + k_8 m_8 \\ \frac{dm_{10}}{dt} &= -k_9 m_6 m_{10} + k_{10} m_{11} + k_{11} m_{11} \\ \frac{dm_{11}}{dt} &= k_9 m_6 m_{10} - k_{10} m_{11} - k_{11} m_{11} \end{aligned} \tag{8}$$

Here,  $m_i$  ( $i = 1, 2, \dots, 11$ ) are state variables representing concentrations of the proteins Raf-1\*, RKIP, Raf-1\*/RKIP, Raf-1\*/RKIP/ERK-PP, ERK-P, RKIP-P, MEK-PP, MEK-PP/ERK, ERK-PP, RP and RKIP-P/RP respectively, and  $k_i$  ( $i = 1, 2, \dots, 11$ ) are corresponding model coefficients (rate constants). In the work of Cho *et al.* [4], the numerical values of these coefficients are determined by parameter estimation (Table 1).

Moreover, in the same work of Cho *et al.* [4], time course data for parameter estimation are presented. From the work of Cho *et al.* [4] we select the values ( $\varepsilon = 0.01$ ); ( $\varepsilon^0 = 1$ );  $1/\varepsilon = 100$ ; to be characteristic dimensionless values of state variables ( $m_2, m_3, m_5, m_6$ ); ( $m_{11}$ ); ( $m_1, m_4, m_7, m_8, m_9, m_{10}$ ); respectively. This assumption is well grounded by the sequence of variable values, shown in Table 2.

The parameters and concentration values, shown in Table 1 and Table 2, respectively, are given here without units in view of the fact that we do not intend to compare them. What is of interest for us is not to compare parameters (some of them having different units) nor concentrations, but the terms in (8). In accordance with the scaling procedure, each term in the right-hand side of the system equations must have an order of one. Towards this end, we introduce scaling substitutions for the model variables as well as the model coefficients. The first ones are presented in Table 3.

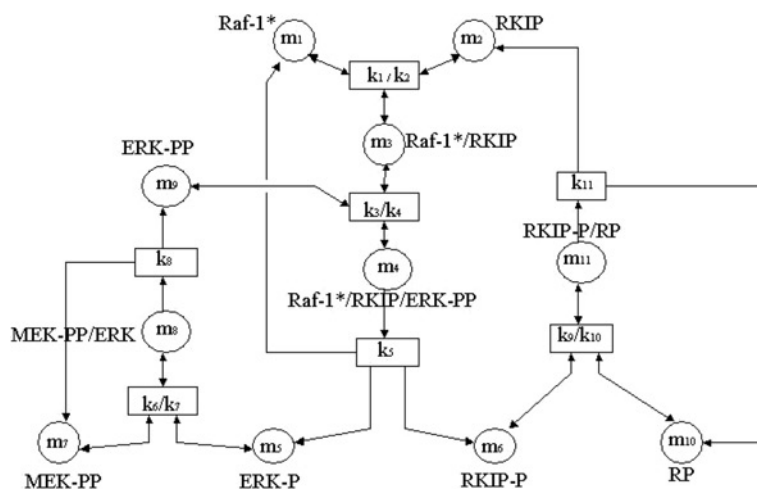


Fig. 1 Graphical representation of the ERK signalling pathway regulated by RKIP

Table 1: Summary of parameter values

Parameter	$k_1$	$k_2$	$k_3$	$k_4$	$k_5$	$k_6$	$k_7$	$k_8$	$k_9$	$k_{10}$	$k_{11}$
Estimated value	0.53	0.072	0.625	0.00245	0.0315	0.8	0.0075	0.071	0.92	0.00122	0.87

**Table 2: Stationary values of state variables**

State variable	$m_1$	$m_2$	$m_3$	$m_4$	$m_5$	$m_6$	$m_7$	$m_8$	$m_9$	$m_{10}$	$m_{11}$
Stationary value	66.7136	0.0501	0.0176	59.5687	0.0417	0.0152	63.5463	27.9537	173.5358	161.0518	2.1481

**Table 3: Substitutions for state variable of the system (8)**

State variable	$m_1$	$m_2$	$m_3$	$m_4$	$m_5$	$m_6$	$m_7$	$m_8$	$m_9$	$m_{10}$	$m_{11}$
Scaling form	$c_1/\varepsilon$	$\varepsilon c_2$	$\varepsilon c_3$	$c_4/\varepsilon$	$\varepsilon c_5$	$\varepsilon c_6$	$c_7/\varepsilon$	$c_8/\varepsilon$	$c_9/\varepsilon$	$c_{10}/\varepsilon$	$c_{11}$

Here, the new variables  $c_i$  ( $i = 1, 2, \dots, 11$ ) are not dimensionless. Nevertheless they have an order of one (i.e. they change in the interval between 0.1 and 10). The same approach is applied for scaling the model coefficients. The corresponding parameter substitutions are presented in Table 4.

Here, the new coefficients  $a_i$  ( $i = 1, 2, \dots, 11$ ) have also order of 1 conserving their physical dimensionality.

After replacing the variable and parameter transformation (given in Tables 3 and 4) in (8), we obtain the following system

$$\frac{1}{\varepsilon} \frac{dc_1}{dt} = -a_1 c_1 c_2 + \varepsilon^2 a_2 c_3 + a_5 c_4 \quad (9)$$

$$\varepsilon \frac{dc_2}{dt} = -a_1 c_1 c_2 + \varepsilon^2 a_2 c_3 + a_{11} c_{11} \quad (10)$$

$$\varepsilon \frac{dc_3}{dt} = a_1 c_1 c_2 - \varepsilon^2 a_2 c_3 - a_3 c_3 c_9 + a_4 c_4 \quad (11)$$

$$\frac{1}{\varepsilon} \frac{dc_4}{dt} = a_3 c_3 c_9 - a_4 c_4 - a_5 c_4 \quad (12)$$

$$\varepsilon \frac{dc_5}{dt} = a_5 c_4 - a_6 c_5 c_7 + a_7 c_8 \quad (13)$$

$$\varepsilon \frac{dc_6}{dt} = a_5 c_4 - a_9 c_6 c_{10} + \varepsilon a_{10} c_{11} \quad (14)$$

$$\frac{1}{\varepsilon} \frac{dc_7}{dt} = -a_6 c_5 c_7 + a_7 c_8 + a_8 c_8 \quad (15)$$

$$\frac{1}{\varepsilon} \frac{dc_8}{dt} = a_6 c_5 c_7 - a_7 c_8 - a_8 c_8 \quad (16)$$

$$\frac{1}{\varepsilon} \frac{dc_9}{dt} = -a_3 c_3 c_9 + a_4 c_4 + a_8 c_8 \quad (17)$$

$$\frac{1}{\varepsilon} \frac{dc_{10}}{dt} = -a_9 c_6 c_{10} + \varepsilon a_{10} c_{11} + a_{11} c_{11} \quad (18)$$

$$\frac{dc_{11}}{dt} = a_9 c_6 c_{10} - \varepsilon a_{10} c_{11} - a_{11} c_{11} \quad (19)$$

The presence of a small parameter  $\varepsilon$  in every term of this system determines its order. This means in accordance with the terminology of the QSSA theorem that the four equations (10), (11), (13) and (14) form an attached system, and the other seven form a reduced one. The set of both systems is referred to as the complete system. Moreover, (19) forms a medium varying system, in view of the fact that it is reduced with respect to (10), (11), (13) and (14), but an attached one with respect to (9), (12)

and (15)–(18). The QSSA theorem can be applied separately to every two groups of equations mentioned. It is worthy to note that the presence of epsilon and epsilon squared terms in the right-hand sides of (14), (18), (19) and (9)–(11), respectively, does not influence on the order of corresponding derivatives in the left-hand sides. This is in view of the circumstance that they can be just neglected in comparison of the other terms in the right-hand sides, when estimating the order of derivatives. These considerations are essential for an understanding and application of the QSSA theorem.

Next, we investigate some properties of the attached, medium varying, reduced and complete systems following from the QSSA theorem.

#### 4 Applying QSSA theorem to the scaled system

Consider the attached system of equations (10), (11), (13), (14) under condition that only the variables  $c_2, c_3, c_5, c_6$  are unknown functions of time. The system has a stationary (steady state) solution in the form

$$c_2^0 = \frac{a_{11} c_{11}}{a_1 c_1} + \frac{\varepsilon^2 a_2 (a_4 c_4 + a_{11} c_{11})}{a_1 a_3 c_1 c_9} \quad (20)$$

$$c_3^0 = \frac{a_4 c_4 + a_{11} c_{11}}{a_3 c_9} \quad (21)$$

$$c_5^0 = \frac{a_5 c_4 + a_7 c_8}{a_6 c_7} \quad (22)$$

$$c_6^0 = \frac{a_5 c_4 + \varepsilon a_{10} c_{11}}{a_9 c_{10}} \quad (23)$$

where  $c_1, c_4, c_7, c_8, c_9, c_{10}$  and  $c_{11}$  are slow variables leading the quasi-stationary (subordinated) variables  $c_2, c_3, c_5$  and  $c_6$ . Certainly the terms in (20) and (23) containing  $\varepsilon^2$  and  $\varepsilon$ , respectively, can be neglected.

In order to analyse the stability of the steady state (20)–(23), we introduce the substitutions

$$c_2 = c_2^0 + x \quad c_3 = c_3^0 + y \quad c_5 = c_5^0 + z \quad c_6 = c_6^0 + u \quad (24)$$

in the attached system of equations (10), (11), (13) and (14). As a result we obtain the variation equations

$$\frac{dx}{dt} = -\frac{a_1}{\varepsilon} c_1 x + \varepsilon a_2 y \quad (25)$$

**Table 4: Substitutions for the coefficients of the system (8)**

Parameter	$k_1$	$k_2$	$k_3$	$k_4$	$k_5$	$k_6$	$k_7$	$k_8$	$k_9$	$k_{10}$	$k_{11}$
Scaling form	$a_1$	$\varepsilon a_2$	$a_3$	$\varepsilon a_4$	$\varepsilon a_5$	$a_6$	$\varepsilon a_7$	$\varepsilon a_8$	$a_9$	$\varepsilon a_{10}$	$a_{11}$

$$\frac{dy}{dt} = \frac{a_1}{\varepsilon} c_1 x - \left( \varepsilon a_2 + \frac{a_3}{\varepsilon} c_9 \right) y \quad (26)$$

$$\frac{dz}{dt} = -\frac{a_6}{\varepsilon} c_7 z \quad (27)$$

$$\frac{du}{dt} = -\frac{a_9}{\varepsilon} c_{10} u \quad (28)$$

The variations  $z$  and  $u$  evidently tend asymptotically to zero. The variations  $x$  and  $y$  demonstrate the same behaviour in view of the fact that the corresponding Routh–Hurwitz coefficients [15] are positive

$$p = \frac{a_1 c_1}{\varepsilon} + \varepsilon a_2 + \frac{a_3 c_9}{\varepsilon} > 0 \quad q = \frac{a_1 a_3 c_1 c_9}{\varepsilon^2} > 0$$

Thus the steady state (20)–(23) is stable, which allows us to apply the QSSA theorem.

In accordance with the theorem, we replace the formulas (20)–(23) in the reduced system (9), (12) and (15)–(19). As a result, the following linear system of seven equations is obtained

$$\frac{dc_1}{dt} = \varepsilon a_5 c_4 - \varepsilon a_{11} c_{11} \quad (29)$$

$$\frac{dc_4}{dt} = \varepsilon a_{11} c_{11} - \varepsilon a_5 c_4 \quad (30)$$

$$\frac{dc_7}{dt} = -\varepsilon a_5 c_4 + \varepsilon a_8 c_8 \quad (31)$$

$$\frac{dc_8}{dt} = \varepsilon a_5 c_4 - \varepsilon a_8 c_8 \quad (32)$$

$$\frac{dc_9}{dt} = \varepsilon a_8 c_8 - \varepsilon a_{11} c_{11} \quad (33)$$

$$\frac{dc_{10}}{dt} = -\varepsilon a_5 c_4 + \varepsilon a_{11} c_{11} \quad (34)$$

$$\frac{dc_{11}}{dt} = a_5 c_4 - a_{11} c_{11} \quad (35)$$

Equation (35) can be considered in two ways: (i) as an attached system with respect to the reduced system (29)–(35); (ii) a reduced system with respect to the attached system (10), (11), (13) and (14). In this sense, (35) presents a medium varying system. Together with (30) it forms a two-dimensional linear dynamical system, which is independent from the other variables (concentrations) of the Ras/Raf/MEK/ERK signalling pathway. It is of interest to concretise the type of dynamical behaviour realised by both systems (30) and (35) for the actual (positive) values of the coefficients. For this purpose, we should calculate again the coefficients of the Routh–Hurwitz conditions

$$p = a_{11} + \varepsilon a_5 > 0 \quad q = \varepsilon a_5 a_{11} - \varepsilon a_5 a_{11} = 0$$

The driver is neither a ‘amplifier’ nor a ‘damper’. But for small fluctuations of the biochemical reaction rates of the pathway, its behaviour could change from stable towards unstable and vice versa. As a result, the behaviour of the whole pathway could change dramatically [15].

Further, we replace the substitutions from Tables 3 and 4 in (29)–(35) taking into account that the steady-state value  $c_{11}^0 = m_{11}^0$  can be substituted in (29), (30), (33) and (34). As

a result the reduced system assumes the form

$$\frac{dm_1}{dt} = k_5 m_4 - k_{11} m_{11}^0 \quad (36)$$

$$\frac{dm_4}{dt} = k_{11} m_{11}^0 - k_5 m_4 \quad (37)$$

$$\frac{dm_7}{dt} = -k_5 m_4 + k_8 m_8 \quad (38)$$

$$\frac{dm_8}{dt} = k_5 m_4 - k_8 m_8 \quad (39)$$

$$\frac{dm_9}{dt} = k_8 m_8 - k_{11} m_{11}^0 \quad (40)$$

$$\frac{dm_{10}}{dt} = -k_5 m_4 + k_{11} m_{11}^0 \quad (41)$$

$$\frac{dm_{11}}{dt} = k_5 m_4 - k_{11} m_{11} \quad (42)$$

Equation (42) can be solved analytically under the condition that the slow variable  $m_4$  is considered as a constant. Analytical solutions can also be obtained for the differential equations of the reduced system (36)–(41).

## 5 Analytical derivation of the reduced system solution

Here, we demonstrate how the dynamical driver properties can be treated in a more concrete manner under the QSSA.

From the ‘medium varying’ equation (42) of the driver, it follows that the steady-state value of  $m_{11}$  is determined by the formula

$$m_{11}^0 = \frac{k_5 m_4}{k_{11}} \quad (43)$$

where the second driver variable  $m_4$  is considered as a constant in view of the quasi-state-approximation (i.e. the QSSA theorem holds). By replacing (43) in equations (36) and (37), it is easy to obtain that

$$m_1(t) = \text{const.} = m_1^i \quad (44)$$

$$m_4(t) = \text{const.} = m_4^i \quad (45)$$

where  $m_1^i$  and  $m_4^i$  are initial values of  $m_1$  and  $m_4$ , respectively. Then we can substitute (45) in (43) and the obtained result, together with (45), can be replaced in (38)–(42). As a result the following equations are valid

$$\frac{dm_7}{dt} = -k_5 m_4^i + k_8 m_8 \quad (46)$$

$$\frac{dm_8}{dt} = k_5 m_4^i - k_8 m_8 \quad (47)$$

$$\frac{dm_9}{dt} = -k_5 m_4^i + k_8 m_8 \quad (48)$$

$$\frac{dm_{10}}{dt} = -k_5 m_4^i + k_5 m_4^i = 0 \quad (49)$$

$$\frac{dm_{11}}{dt} = k_5 m_4^i - k_{11} m_{11} \quad (50)$$

From equation (49), it follows that

$$m_{10}(t) = \text{const.} = m_{10}^i \quad (51)$$

where  $m_{10}^i$  is an initial value of the concentration  $m_{10}$ . The solution of (47) is

$$m_8(t) = \frac{k_5 m_4^i}{k_8} + \left( m_8^i - \frac{k_5 m_4^i}{k_8} \right) \exp(-k_8 t) \quad (52)$$

Here  $m_8^i$  is initial value of the concentration  $m_8$ . After replacing this solution in (46) and (48), and following integration, the following equations for  $m_7(t)$  and  $m_9(t)$  are obtained

$$m_7(t) = m_7^i + \left( m_8^i - \frac{k_5 m_4^i}{k_8} \right) (1 - \exp(-k_8 t)) \quad (53)$$

$$m_9(t) = m_9^i + \left( m_8^i - \frac{k_5 m_4^i}{k_8} \right) (1 - \exp(-k_8 t)) \quad (54)$$

where  $m_7^i$  and  $m_9^i$  are initial values of the concentrations  $m_7$  and  $m_9$ , respectively. The analytical solution of the medium equation (50) is

$$m_{11}(t) = \frac{k_5 m_4^i}{k_{11}} + \left( m_{11}^i - \frac{k_5 m_4^i}{k_{11}} \right) \exp(-k_{11} t) \quad (55)$$

From equations (43)–(54), the following relationships between initial and steady-state values of the concentrations  $m_1, m_4, m_7, m_8, m_9, m_{10}$  and  $m_{11}$  can be derived

$$\begin{aligned} m_1^0 &= m_1^i & m_4^0 &= m_4^i & m_7^0 &= m_7^i + m_8^i - \frac{k_5 m_4^i}{k_8} \\ m_8^0 &= \frac{k_5 m_4^i}{k_8} & m_9^0 &= m_9^i + m_8^i - \frac{k_5 m_4^i}{k_8} \\ m_{10}^0 &= m_{10}^i & m_{11}^0 &= \frac{k_5 m_4^i}{k_{11}} \end{aligned} \quad (56)$$

where the steady-state (stationary) values are denoted by zero upper indexes. It is seen that  $m_4^i$  is involved in the formulas for  $m_7^0, m_8^0, m_9^0$  and  $m_{11}^0$ . The other two steady state concentrations  $m_1^0$  and  $m_{10}^0$  are determined by their own initial values, which do not influence on the other stationary concentrations. Therefore, variable  $m_4$  plays the role of a driver with respect to the stationary concentrations  $m_7^0, m_8^0, m_9^0$  and  $m_{11}^0$ . The last values, however, take a role in the formulas for the steady-state values of the fast varying concentrations  $m_2, m_3, m_5$  and  $m_6$ . The corresponding formulas are easy to obtain from (20)–(23) by substituting there the corresponding relations from Tables 3 and 4. They are

$$\begin{aligned} m_2^0 &= \frac{k_{11} m_{11}^0}{k_1 m_1^0} & m_3^0 &= \frac{k_4 m_4^0 + k_{11} m_{11}^0}{k_3 m_9^0} \\ m_5^0 &= \frac{k_5 m_4^0 + k_7 m_8^0}{k_6 m_7^0} & m_6^0 &= \frac{k_5 m_4^0}{k_9 m_{10}^0} \end{aligned} \quad (57)$$

In view of the fact that formulas (56) express the stationary values, involved in the right-hand sides of (57), by the initial values of  $m_1, m_4, m_7, m_8, m_9, m_{10}$  and  $m_{11}$ , we can assert that  $m_4^i$  is a driver of the whole pathway steady state excepting  $m_1$  and  $m_{10}$ , which are always equal to their initial values. That means, by changing  $m_4^i$  we can essentially control (at least theoretically) the steady state of the quasi-stationary pathway in terms of input (initial values) and output (stationary values) relationships. The corresponding formulas relating the steady-state values of fast

variables and the initial values of slow variables have the form

$$\begin{aligned} m_2^0 &= \frac{k_5 m_4^i}{k_1 m_1^i} & m_3^0 &= \frac{(k_4 + k_5) m_4^i}{k_3 (m_9^i + m_8^i - k_5 m_4^i / k_8)} \\ m_5^0 &= \frac{k_5 (k_8 + k_7) m_4^i}{k_6 k_8 (m_7^i + m_8^i - k_5 m_4^i / k_8)} & m_6^0 &= \frac{k_5 m_4^i}{k_9 m_{10}^i} \end{aligned} \quad (58)$$

Namely, these relationships can be considered as abstract restrictions on the choice of initial concentration values of the fast varying concentrations of proteins and protein-complexes in the considered pathway. If they are near the corresponding steady-state values presented by (58), then the QSSA holds and RKIP ( $m_2$ ) does not modulate the final extent and duration of ERK ( $m_5$ ) activity, but on the contrary the very RKIP and ERK concentrations are determined by the initial values of the slow varying concentrations ( $m_1, m_4, m_7, m_8, m_9$  and  $m_{10}$ ). Equation (58) can be verified experimentally. In the last case, these relationships could be practically considered as restrictions on the regulatory role of RKIP on ERK pathway activity.

## 6 Computer realization of the complete and reduced system behaviours

In Fig. 2, the solution of fast varying components of the complete system (8) in time interval  $[0, 20]$  are shown at initial values of the concentrations

$$\begin{aligned} m_1 &= 67.95 & m_2 &= 0.372 & m_3 &= 0.091 \\ m_4 &= 58.25 & m_5 &= 0.088 & m_6 &= 0.228 \\ m_7 &= 66.33 & m_8 &= 25.17 & m_9 &= 176.41 \\ m_{10} &= 160.95 & m_{11} &= 2.244 \end{aligned} \quad (59)$$

It is obvious, that the fast variables  $m_2, m_3, m_5$  and  $m_6$  are indeed decaying rapidly to a very low and thereafter are essentially no longer varying over time. On the contrary, the slow variables  $m_1, m_4, m_5, m_6, m_7, m_8, m_9$  and  $m_{10}$  are converging to some steady-state values essentially different from zero, as shown in Fig. 3 (the slow varying concentrations are considered over a longer time interval than that of the fast variables). Moreover, the same figure shows that two of the variables ( $m_1$  and  $m_7$ ) are very similar. This fact allows us to understand a detail illustrated in Fig. 4, where the graphs of the six slow variables of both complete and reduced systems are plotted. Here, the four

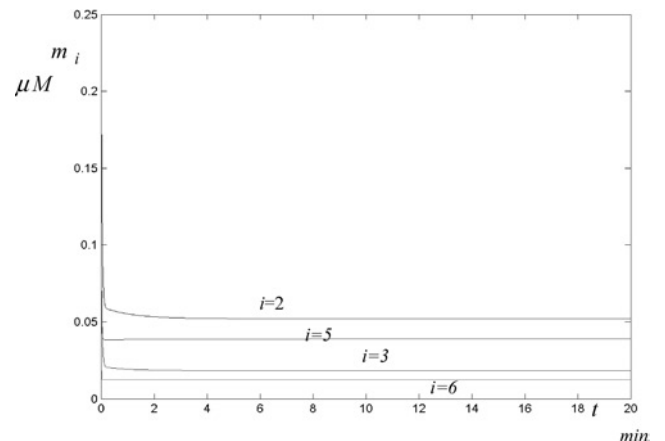
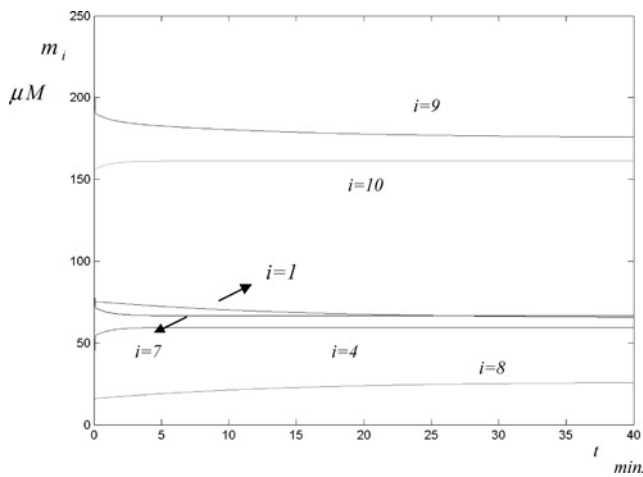


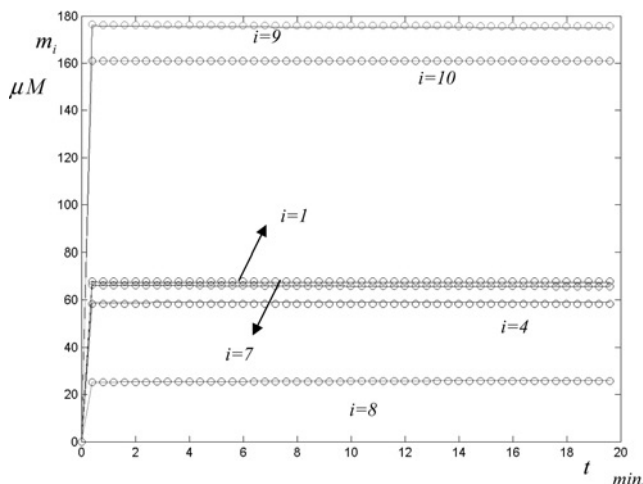
Fig. 2 Fast variables behaviour of the complete system



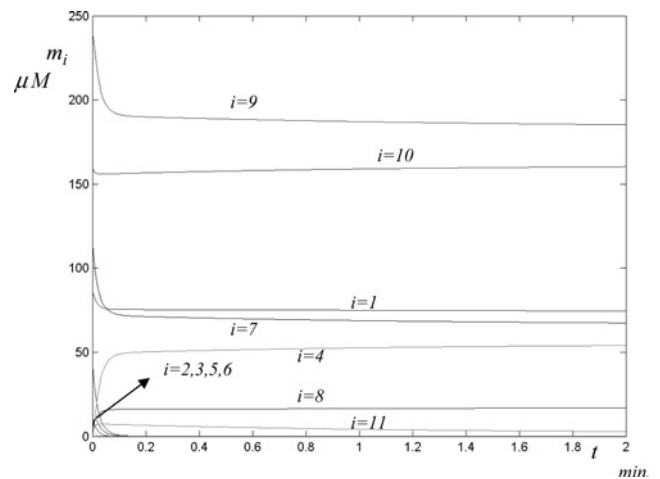
**Fig. 3** Slow variables behaviour of the complete system

graphs of the mentioned variables  $m_1$  and  $m_7$  (plotted by solid lines for the complete system, and dotted for the reduced one) almost coincide each other. Every two graphs (for complete and reduced system) for the other variables are very near too.

In Fig. 5, the graphs of all components of the solutions of complete system, for the initial values taken from a previous paper [4], are plotted in a ten times shorter interval [0, 2]. There, the steady-state values of the fast variables are very small and thus placed very near (invisible) to the axis of the coordinate system. The medium variable is a bit of higher and the slow varying ones are essentially higher. What is of interest here is the fact that the initial behaviour of the other variables is not under the influence of the initial value of  $m_4$  in the complete system. On the contrary,  $m_4$  is essentially influenced by the RKIP initial concentration ( $m_2$ ) as it is noted in the work of Cho *et al.* [4]. This is a paradoxical result in view of the fact that in the previous section we made a conclusion for predominant influence of the initial  $m_4$  on all other steady-state concentrations of the pathway except  $m_1^0$  and  $m_{10}^0$ . These results, however, allow us to draw the main inference that the later quasi-steady-state behaviour (determined by the reduced system) is essentially different from the initial one, demonstrated by the complete system far away from the stationary state. Therefore we can suppose that although the influence of RKIP on the ERK pathway is a



**Fig. 4** Coincidence of the graphs of complete and reduced system variables



**Fig. 5** Graphs of all components of the complete system solution

characteristic feature only for the complete system, the reduced system has also an inherent property, expressing the Raf-1\*/RKIP/ERK-PP influence on the quasi-steady-state behaviour of the pathway.

## 7 Conclusions

The main conclusion from the considerations made in this paper is that besides the influence of RKIP on the ERK signalling pathway, an influence of the post-initial value of the Raf-1\*/RKIP/ERK-PP protein complex, denoted as  $m_4^i$ , becomes an important factor when the system approaches its quasi-stationary state. The term post-initial is in sense of the above considerations of initial and later intervals of validity of the complete and reduced systems, respectively. Certainly, the complete system (8) holds any time, but the reduced system reveals a new property of the pathway near its steady state. It consists in the conclusion that the obtained relationships for the steady-state values of the fast variables can be considered as restrictions on concentrations of the considered pathway. The biological importance of this issue is that RKIP possibly plays a regulatory role of the ERK pathway only far away from the steady state of the pathway.

In a more general sense, it is also shown, that the considerations of time hierarchy in biochemical reactions allow us to reduce the number of differential equations of the Ras/Raf/MEK/ERK signalling pathway model, and to determine the driving reactions of the quasi-stationary transduction dynamics. For this purpose, the QSSA theorem for the quasi-stationary approximation (the expression of the equilibrium values of fast varying variables by the slow varying ones) is applied. As a result, the two-component reaction between Raf-1/RKIP/ERK-PP and RKIP-P/RP protein complexes is identified to be a driver of the dynamical behaviour of the signalling pathway, but in post-initial (or quasi-stationary) stage as is explained above.

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## 9 References

- 1 Schneider, K.R., and Wilhelm, Th.: 'Model reduction by extended quasi-steady-state approximation', *J. Math. Biol.*, 2000, **40**, pp. 443–450
- 2 Petrov, V., Nikolova, E., and Timmer, J.: 'Dynamical analysis of cell function models. A review', *J. Theor. Appl. Mech.*, 2004, **34**, (3), pp. 55–78
- 3 Kolch, W.: 'Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions', *Biochem. J.*, 2000, **351**, pp. 289–305
- 4 Cho, K.-H., Shin, S.-Y., Kim, H.-W., Wolkenhauer, O., McFerran, B., and Kolch, W.: 'Mathematical modeling of the influence of RKIP on the ERK signaling pathway', *CSMB 2003*, 2003, pp. 127–141
- 5 Michaelis, L., and Menten, M.L.: 'Die Kinetik der Invertinwirkung', *Biochem. Z.*, 1913, **49**, pp. 333–369
- 6 Alberty, R.A.: 'The rate equation for an enzymatic reaction' in Boyer, P., *et al.* (Eds): 'Kinetics, thermodynamics, mechanisms and basic properties. The enzymes' (Academic Press, New York, 1959), pp. 143–155
- 7 Sellin, S., and Mannervik, B.: 'Reversal of the reaction catalyzed by glyoxalase. I: Calculation of the equilibrium constant for the enzymatic reaction', *J. Biol. Chem.*, 1983, **258**, pp. 8872–8875
- 8 Duggleby, R.G.: 'Product inhibition of reversible enzyme-catalyzed reactions', *Biochim. Biophys. Acta*, 1994, **1209**, pp. 238–240
- 9 Tzafirri, A.R., and Edelman, E.R.: 'The total quasi-state-approximation is valid for reversible enzyme kinetics', *J. Theor. Biol.*, 2004, **226**, pp. 303–313
- 10 Millat, T., Bullinger, E., Rohwer, J., and Wolkenhauer, O.: 'Approximations and their consequences in dynamic modelling of signal transduction pathways', *Math. Biosci.*, accepted for publication
- 11 Kholodenko, B., Schuster, S., Garcia, J., Westerhoff, H.W., and Cascante, M.: 'Control analysis of metabolic systems involving quasi-equilibrium reactions', *Biochim. Biophys. Acta*, 1998, **1379**, pp. 337–352
- 12 Kruger, R., and Heinrich, R.: 'Model reduction and analysis of robustness for the *Wnt*/ $\beta$  catenin signal transduction pathway', *Genome Informatics*, 2004, **15**, (1), pp. 138–148
- 13 Tichonov, A.N.: 'Sistemy differentsialnyh uravneniy, soderzhashchie malye parametry pri proizvodnyh', *Matematicheskiiy sbornik*, 1952, **31**, (3), pp. 575–586 (in Russian)
- 14 Fall, C.P., Marland, E.S., Wagner, J.M., and Tyson, J.J.: 'Computational cell biology' (Springer-Verlag, New York, 2002)
- 15 Petrov, V., Nikolova, E., and Wolkenhauer, O.: 'A driver identification of the Ras/Raf/MEK/ERK signal transduction pathway', *Comptes Rendus de l'Acad. Bulg. Sci.*, 2005, **v.54** (6), pp. 639–644
- 16 Romanovskii, U.M., Stepanova, N.V., and Chernavskii, D.S.: 'Matematicheskoe modelirovanie v biofizike', 1975, Izd 'Nauka', Moskva (in Russian)