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Modeling Complex Cellular Networks - robust switching in the cell cycle ensures a piecewise linear reduction of the regulatory network

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Abstract— **Cellular networks are inherently complex due to their large number of genes and proteins interacting through non-linear feedback loops. The identification of cellular networks from large-scale parallel measurements from the activity of genes and proteins is a tremendous challenge in the postgenomic era i. e. after the sequencing of the genome. Traditionally, this system identification problem has been viewed as a huge parameter estimation problem of an unknown non-linear system. Here we develop an approach where we approximate the complex system equations with a piecewise linear system, using a computational model of the cell cycle as a proof of principle. The modular and sparse structure of the cellular network makes it possible, to divide the model into subsystems, each having only a small number of inputs and outputs. As a rule, the subsystems operate as switches with or without delay. Since the cell cycle, as most biological systems, is robust against perturbations, the subsystems can be replaced by step functions and the main dynamical behaviour of the full complex cellular network can therefore be captured by the piecewise linear system. If other cellular networks are robust, sparse and modular, our approach of targeting a reduced system description instead of the full complex system, set the stage for not only a thorough characterization of the system dynamics but most importantly, it reduces the complexity of the parameter estimation problem.**

I. INTRODUCTION

As early as 1948, *Norbert Wiener*, the founding father of cybernetics, explicitly considered technical as well as biological systems as objects for the same scientific approach [18]. All attempts, until recently, at systems level understanding of biological systems suffered from inadequate data to enable such an approach [9]. Moreover, biological systems are inherently complex both in terms of the shear number of heterogeneous parts as well as to their non-linear interactions. Hence, the two main obstacles hampering us the last half century to fulfill the ambition of *Norbert Wiener* has been (i) how to deal with biological complexity and (ii) how to obtain sufficient amount of data. Thus, one aim of systems biology is to overcome the deficiencies of current models and to create comprehensive *dynamical models*.

However, recently new breakthroughs in large-scale parallel measurement techniques such as micro-arrays enable the monitoring of thousands of different genes in the same experiment [3], [10]. Moreover, an active field of research is on how to interpret and analyze these data. Several schemes have been developed with the objective of uncover the cellular pathways using gene expression data [9], [8], [12]. This can be described as either a bottom-up or as a top-down approach [9]. The former tries to compile independent experimental data into a conclusive representation of a gene regulatory network, the latter uses high-throughput data from DNA micro-array and other new measurement technologies to describe the full system at the same time.

Despite the advances in measurement technology, we are still left with the issue on how to handle the biological complexity both from a modeling perspective and also from a system identification point of view. In this paper, we suggest that a reduced model description is important to solve the system identification problem of revealing cellular networks from data.

Different approaches have been taken to identify biological systems. Tegnér *et al* [15] use perturbation around a fixed point to find the underlying connectivity of a dynamical systems. They assume that the system is in steady state and then use linear methods to analyze the system. If one wants to capture the global behavior of a nonlinear system such as oscillations or multiple fixed points this method is not applicable though.

One simplifying observation is that these systems are sparse and seem to have a modular structure [13]. Biological systems are also very robust against common fluctuations in the environment and within themselves [1]. In a dynamical model this can be reflected as a relative insensitivity of the qualitative behavior of the system towards changes in the parameters [5].

In this paper, we adapt the theory developed in systems and control engineering to approach the modeling of the cell cycle. We use these facts that biological systems are, apart from being large and complex also robust and sparse. Since this system is sparse the constituents, the proteins, can be divided into subsystems, having their own dynamical properties. Because of the robustness, on the other hand, the subsystems are not radically sensitive against changes in the parameters. Therefore these subsystems can be replaced

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with simpler descriptions, and thereby the stage is set for a mathematical analysis.

A nonlinear model of the cell cycle [11] is used as a representative computational model of a complex biological system. The cell cycle is a well studied system and the control system of the cell cycle, which determines whether cells will divide or die, is of clinical importance in the cancer field. The model system is divided into subsystems and we describe a reduced model of the system based on these subsystems. This method is akin to the system theoretical approach to model large engineering systems by tearing and zooming, see e.g. [19], [4]. By doing so we hope to have the possibility of performing a full mathematical analysis of the global dynamical behavior, such as the necessary and sufficient conditions for periodic oscillations to appear.

II. A MATHEMATICAL MODEL OF THE CELL CYCLE OF FISSION YEAST

The model system we have used is a dynamical model describing the cell cycle regulation of fission yeast [11]. During the cell cycle the cell replicates all its DNA, *S-phase* and divides into two, mitoses or *M-phase*. This is a complicated process where the order of events is very important. Between the M-phase and the S-phase there are also two so called gap-phases, *G1* and *G2*. This model describes the main parts of the regulatory machinery that drives this process in fission yeast.

The main goal of this regulatory system is to control the concentration of a molecule complex called MPF in the cell. MPF activity is required to start both DNA replication and mitosis. In fig. 1 (top) a numerical simulation of this model can be seen, the dashed line in this graph corresponds to the concentration of MPF. The proper execution of cell cycle events requires that MPF activity oscillates between low (G1 phase), intermediate (S and G2 phases) and high (M phase) levels [17]. For a more detailed description of the cell cycle physiology we refer to [11] and [17].

The differential equations of this nonlinear model can be seen in table I. The variables corresponds to protein or protein complex concentrations, either of the active form of a protein or the total concentration of that protein in the cell.

The system is built up from a set of intervened feedback loops, which all at some point passes the variable MPF. This makes it possible to view the system as a feedback system, see fig. 2, where the output MPF is fed back to the system.

There is also one external input to the system, the cell mass, M. In our simulations the cell is assumed to be in a nutrient rich medium and therefore the the mass increases exponentially during one cell cycle, see equation (4) table I. One cycle is ended when MPF decreases through a certain value, at that time the mass M is divided by 2.

TABLE I The differential and algebraic equations of the model system, from [11]. For a description of the equations we refer to [11]. The subdivision into subsystems are ours. When [MPF] decreases through 0.1, M is divided by two.

$$\underline{\underline{\text{Subsystem Prempf :}}}$$

$$\frac{d[\mathrm{Cdc13_T}]}{dt} = k_1\mathrm{M} - (k_2' + k_2''[\mathrm{Ste9}] + k_2'''[\mathrm{Slp1}])[\mathrm{Cdc13_T}] \quad (1)$$

$$\frac{d[\mathrm{preMPF}]}{dt} = k_{\mathrm{wee}}([\mathrm{Cdc13_T}] - [\mathrm{preMPF}]) - k_{25}[\mathrm{preMPF}] - (k_2' + k_2''[\mathrm{Ste9}] + k_2'''[\mathrm{Slp1}])[\mathrm{preMPF}] \quad (2)$$

$$\underline{\underline{\text{Subsystem Wee1 :}}}$$

$$k_{\mathrm{wee}} = k_{\mathrm{wee}}' + (k_{\mathrm{wee}}'' - k_{\mathrm{wee}}')G(V_{\mathrm{awee}}, V_{\mathrm{iwee}}[\mathrm{MPF}], J_{\mathrm{awee}}, J_{\mathrm{iwee}})$$

$$\underline{\underline{\text{Subsystem Cdc25 :}}}$$

$$k_{25} = k_{25}' + (k_{25}'' - k_{25}')G(V_{a25}[\mathrm{MPF}], V_{i25}, J_{a25}, J_{i25})$$

$$\underline{\underline{\text{Subsystem Ste9 :}}}$$

$$\frac{d[\mathrm{Ste9}]}{dt} = (k_3' + k_3''[\mathrm{Slp1}])\frac{1 - [\mathrm{Ste9}]}{J_3 + 1 - [\mathrm{Ste9}]} - (k_4'[\mathrm{SK}] + k_4[\mathrm{MPF}])\frac{[\mathrm{Ste9}]}{J_4 + [\mathrm{Ste9}]}$$

$$\underline{\underline{\text{Subsystem Slp1 :}}}$$

$$\frac{d[\mathrm{Slp1}]}{dt} = k_7[\mathrm{IEP}]\frac{[\mathrm{Slp1_T}] - [\mathrm{Slp1}]}{J_7 + [\mathrm{Slp1_T}] - [\mathrm{Slp1}]} - k_8\frac{[\mathrm{Slp1}]}{J_8 + [\mathrm{Slp1}]} - k_6[\mathrm{Slp1}]$$

$$\frac{d[\mathrm{Slp1_T}]}{dt} = k_5' + k_5''\frac{[\mathrm{MPF}]^4}{J_5^4 + [\mathrm{MPF}]^4} - k_6[\mathrm{Slp1_T}]$$

$$\frac{d[\mathrm{IEP}]}{dt} = k_9[\mathrm{MPF}]\frac{1 - [\mathrm{IEP}]}{J_9 + 1 - [\mathrm{IEP}]} - k_{10}\frac{[\mathrm{IEP}]}{J_{10} + [\mathrm{IEP}]}$$

$$\underline{\underline{\text{Subsystem Rum1}_\mathrm{T}\text{ :}}}$$

$$\frac{d[\mathrm{Rum1_T}]}{dt} = k_{11} - (k_{12} + k_{12}'[\mathrm{SK}] + k_{12}''[\mathrm{MPF}])[\mathrm{Rum1_T}]$$

$$\frac{d[\mathrm{SK}]}{dt} = k_{13}[\mathrm{TF}] - k_{14}[\mathrm{SK}]$$

$$[\mathrm{TF}] = G(k_{15}\mathrm{M}, k_{16}' + k_{16}''[\mathrm{MPF}], J_{15}, J_{16})$$

$$\underline{\underline{\text{MPF :}}}$$

$$[\mathrm{Trimer}] = \frac{2[\mathrm{Cdc13_T}][\mathrm{Rum1_T}]}{\sum + \sqrt{\sum^2 - 4[\mathrm{Cdc13_T}][\mathrm{Rum1_T}]}} \quad (3)$$

$$[\mathrm{MPF}] = \frac{([\mathrm{Cdc13_T}] - [\mathrm{preMPF}])([\mathrm{Cdc13_T}] - [\mathrm{Trimer}])}{[\mathrm{Cdc13_T}]}$$

$$\sum = [\mathrm{Cdc13_T}] + [\mathrm{Rum1_T}] + K_{\mathrm{diss}}$$

where :

$$G(a, b, c, d) = \frac{2ad}{b - a + bc + ad + \sqrt{(b - a + bc + ad)^2 - 4ad(b - a)}}$$

$$\underline{\underline{\text{Mass :}}}$$

$$\frac{d\mathrm{M}}{dt} = \mu M \quad (4)$$

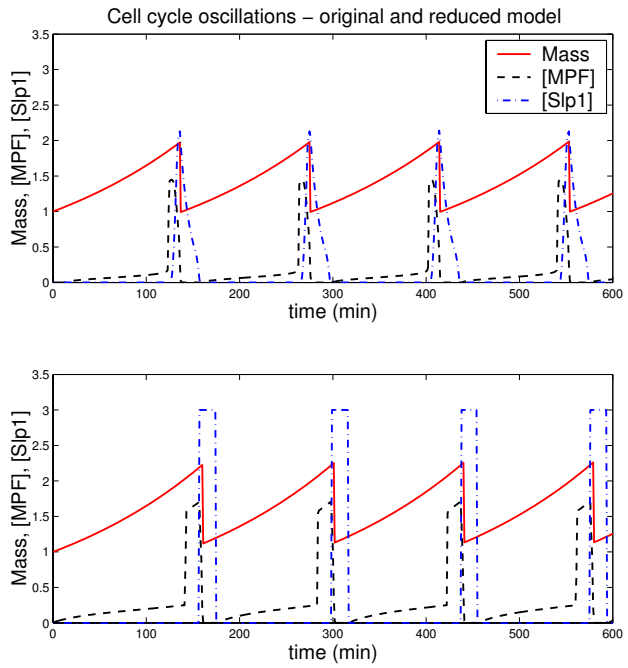

Fig. 1. Top: A simulation of the nonlinear dynamical model from table I and II. Initial values ($t = t_0$) where $[\mathrm{Cdc13_T}] = 0.1, [\mathrm{preMPF}] = 0, [\mathrm{Ste9}] = 0, [\mathrm{Slp1}] = 0, [\mathrm{Slp1_T}] = 1, [\mathrm{IEP}] = 0.1, [\mathrm{Rum1_T}] = 0.1, [\mathrm{SK}] = 0.1$ and $\mathrm{M} = 1$. Bottom: A simulation of the reduced piecewise linear model from table III and IV. Initial values where $[\mathrm{Cdc13_T}] = 0.1, [\mathrm{preMPF}] = 0, [\mathrm{Slp1}] = 0$ and $\mathrm{M} = 1$ and $[\mathrm{MPF}](t - t_{del}) = [\mathrm{MPF}](t_0)$ when $t < t_{del}$

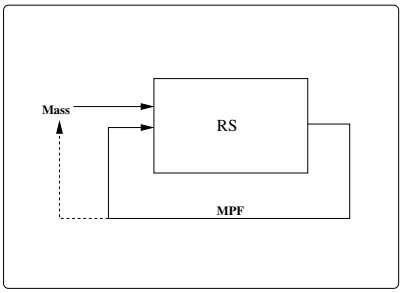

Fig. 2. A diagram of he MPF regulating system of the cell cycle. System RS regulates the concentration of MPF, depending on the mass, M, of the cell and the current MPF concentration which is fed back to the system. The mass is also divided by 2 every time MPF decreases through a certain value (the dashed line)

III. A REDUCED PIECEWISE LINEAR MODEL

The cell cycle model is divided into subsystems. These are replaced by simpler functions based on their dynamics, most often step functions. This makes it possible to view the system as a so called *piecewise linear* system and to describe the behavior of the system in two dimensions.

A. Subsystems

In fig. 3 we have drawn a directed graph of all interactions of the system. A node in this graph corresponds to a variable in the equations describing the system dynamics, see table I, and there is an edge from node i and j if i is on the right hand side of an equation defining j. For simplicity all edges that start and end at the same node have been left out.

This graph has been used to divide the system into subsystems, see table I, which are characterized by a steady state input/output graph, fig. 3, and a possible time delay. The input-output graph corresponds to a bifurcation diagram where the input to the subsystem are the bifurcation parameters and the output the bifurcation variable. The same model parameters as in [11] are used in these graphs, see table II. The time delay was determined from simulations, an example can be seen in fig. 4. In dividing this system we tried to get subsystems with few input and output variables and a simple input/output behaviour.

TABLE II Parameters in the original model

$k_1 = 0.03$	$k_2' = 0.03$	$k_2'' = 1$	$k_2''' = 0.1$
$k_3' = 1$	$k_3'' = 10$	$J_3 = 0.01$	$k_4' = 2$
$k_4 = 35$	$J_4 = 0.01$	$k_5' = 0.005$	$k_5'' = 0.3$
$k_6 = 0.1$	$J_5 = 0.3$	$k_7 = 1$	$k_8 = 0.25$
$J_7 = 0.001$	$J_8 = 0.001$	$k_9 = 0.1$	$k_{10} = 0.04$
$J_9 = 0.01$	$J_{10} = 0.01$	$k_{11} = 0.1$	$k_{12} = 0.01$
$k_{12}' = 1$	$k_{12}'' = 3$	$K_{diss} = 0.001$	$k_{13} = 0.1$
$k_{14} = 0.1$	$k_{15} = 1.5$	$k_{16}' = 1$	$k_{16}'' = 2$
$J_{15} = 0.01$	$J_{16} = 0.01$	$V_{awee} = 0.25$	$V_{iwee} = 1$
$J_{awee} = 0.01$	$J_{iwee} = 0.01$	$V_{a25} = 1$	$V_{i25} = 0.25$
$J_{a25} = 0.01$	$J_{i25} = 0.01$	$k_{wee}' = 0.15$	$k_{wee}'' = 1.3$
$k_{25}' = 0.05$	$k_{25}'' = 5$	$\mu = 0.005$	

B. The piecewise linear model

The subsystem input/output graphs and time delay have been used as templates to reduce the model. In simulations of the original model the mass oscillates between 1 and 2 and this is the regime of the external input where the reduced description of the system is made to work ($\mathrm{M} \geq 1$).

We make the assumption that the subsystems can be replaced by an action ("when x goes down y goes up" etc), corresponding to the steady-state input/output relationship described earlier, and possibly a time delay for this action. The input/output graphs have different shapes, but most of them have a steep sigmoid shape of the input/output curve, see fig. 3. We have replaced the equations of subsystem Ste9, Slp1, Wee1, Cdc25 and $\mathrm{Rum1_T}$ with step functions, a delayed step function or constant output, see table III. The parameters of the step functions were chosen so that the step function should resemble the input/output graph as well as possible.

Subsystem Prempf is harder to give a simple input/output graph description of since the input consists of five variables, $u_1 = (\mathrm{k_{25}}\ \mathrm{k_{wee}}\ [\mathrm{Ste9}]\ [\mathrm{Slp1}]\ \mathrm{M})'$, see table III, and we will treat this subsystem in more detail. The task of this subsystem can be seen as collecting the information from all other subsystems and making a decision. The differential equations of the subsystem describe the change of variables $[\mathrm{preMPF}]$ and $[\mathrm{Cdc13_T}]$, see equations (1) and (2), table I. Let these variables be the state of the subsystem $x = ([\mathrm{Cdc13_T}]\ [\mathrm{preMPF}])'$. Most variables on the right hand side of the equations (1) and (2), are discrete in the reduced model. If we group the discrete variables together

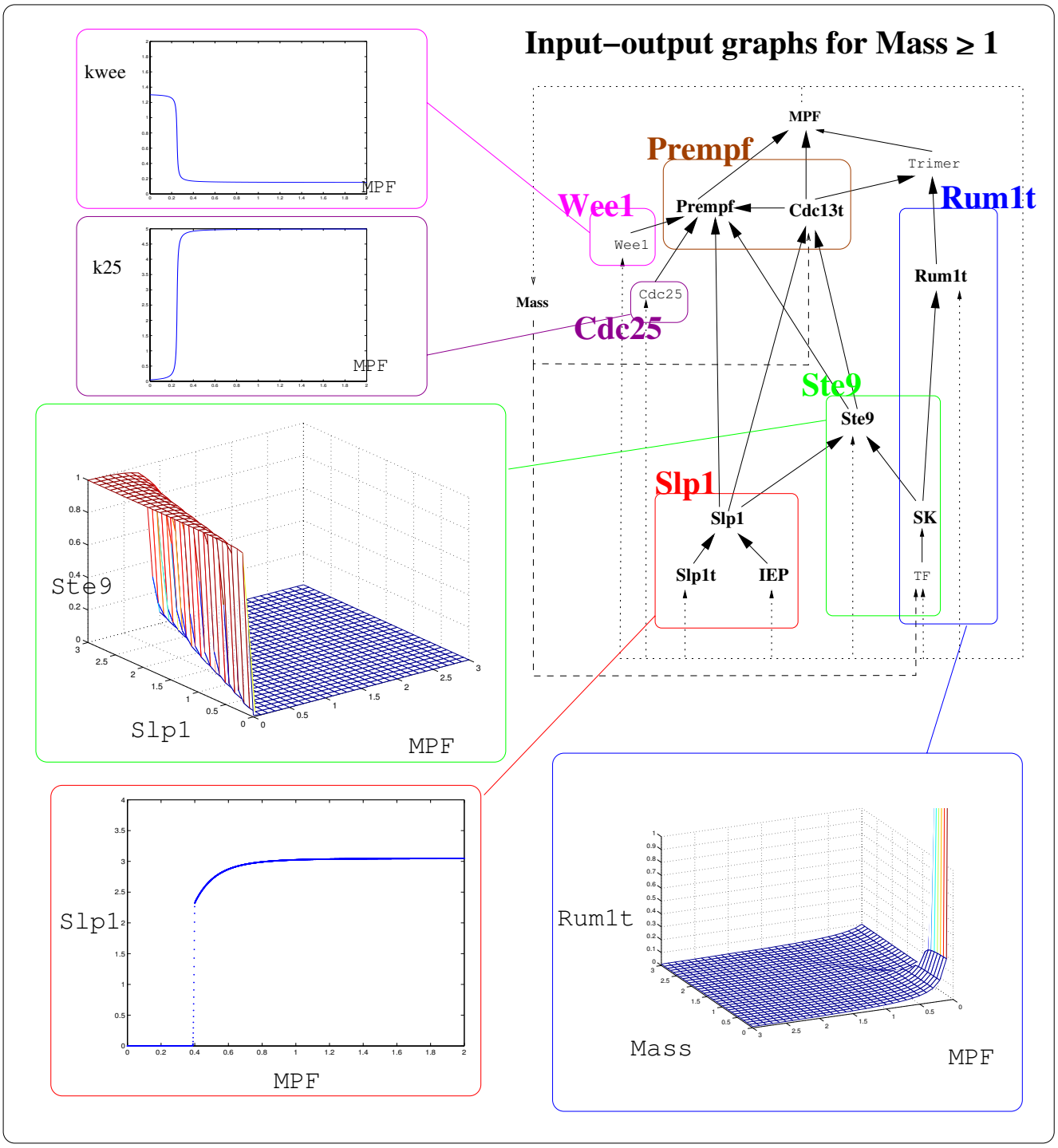

Fig. 3. Interaction network and subsystem input/output graphs of the cell cycle. A directed graph of all interactions of the dynamical model of the cell cycle, see table I. The nodes correspond to the variables of the model and there is an edge from node i to node j if variable i is on the right hand side of an equation defining variable j. This interaction network has been divided into subsystems and the steady state dynamics have been analyzed for each subsystem separately. This is illustrated by bifurcation diagrams where the input of the subsystem is used as the bifurcation parameter and the output as the bifurcation variable. Much in the same sense as in [16]. Only biologically possible stable fixed points has been drawn in these diagrams. The parameters of the original model have been used, see table II, and the diagrams have been calculated analytically when possible, as in [11], or numerically. Subsystem Ste9 also have mass, M, as an input variable, for this subsystem the input/output graph has approximately the same appearance for all tested $M \geq 1$, data not shown.

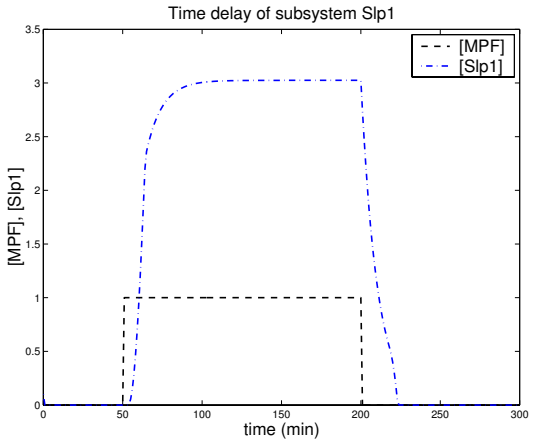

Fig. 4. The step response of the output $[Slp1]$ of subsystem Slp1 on the input $[MPF]$. The input to output delay of this subsystem is approximated from this graph, $t_{del} = 15$.

the system equations can be written

$$\dot{x} \quad = \quad A_i x + b u_1 \tag{7}$$

where $A_i = A_i(u_1)$ is a 2×2 matrix and the elements of A_i are linear functions of the discrete variables of the system

$$A_i = \begin{bmatrix} -(k_2'+k_2''[\text{Ste9}]+k_2'''[\text{Slp1}]) & 0 \\ \text{k}_{\text{wee}} & -(\text{k}_{\text{wee}}+\text{k}_{25}+k_2'+k_2''[\text{Ste9}]+k_2'''[\text{Slp1}]) \end{bmatrix}$$

and $bu_1 = (k_1\text{M} \;\; 0)'$, where M is the external input to the full system. The subscript i of A_i is there to indicate that the number of possible A_i matrices of course are finite, since the part of the input u_1 that affects A_i in the reduced model are discrete. A system like (7) is called a piecewise linear system, it comes together with a switching rule, which in our case depends on the input u_1 of the subsystem,

$$i(u_1) \tag{8}$$

see table III. This gives us a description of the subsystem as a *set* of linear systems. Depending on the input from the other subsystems, subsystem Prempf can be seen as alternating between different linear systems. The reduced model has, for tested initial values, qualitatively the same behaviour as the original one, see fig. 1.

C. Dynamics of the piecewise linear model

The task of the whole system is, as described earlier, to regulate the concentration of MPF. The piecewise linear system of subsystem Prempf decides the dynamics of $[\text{Cdc13}_\text{T}]$ and [preMPF], which in turn gives [MPF]. Therefore it is enough to know the dynamics of this subsystem to understand the full reduced system.

This subsystem is described by the system matrices A_i. The eigenvalues of A_i are

$$\lambda_1 = -(k_2' + k_2''[\text{Ste9}] + k'''[\text{Slp1}])$$
$$\lambda_2 = -(\text{k}_{\text{wee}} + \text{k}_{25} + k_2' + k_2''[\text{Ste9}] + k_2'''[\text{Slp1}])$$

TABLE III The reduced model

		Subsystem Prempf :
$u_1(t)$	=	$(\text{k}_{25}(t)\ \text{k}_{\text{wee}}(t)\ [\text{Ste9}](t)\ [\text{Slp1}](t)\ \text{M}(t))'$
$y_1(t)$	=	$([\text{Cdc13}_\text{T}](t)\ [\text{preMPF}](t))'$
$x(t)$	=	$([\text{Cdc13}_\text{T}](t)\ [\text{preMPF}](t))'$
$\dot{x}(t)$	=	$A_i x(t) + b u_1(t)$
i	=	S/G2 if $u_1(t) = (l_{25}\ h_{wee}\ l_{ste}\ l_{slp}\ \text{M} \geq 1)$
	=	M if $u_1(t) = (h_{25}\ l_{wee}\ l_{ste}\ l_{slp}\ \text{M} \geq 1)$
	=	EM_1 if $u_1(t) = (h_{25}\ l_{wee}\ l_{ste}\ h_{slp}\ \text{M} \geq 1)$
	=	EM_2 if $u_1(t) = (h_{25}\ l_{wee}\ h_{ste}\ h_{slp}\ \text{M} \geq 1)$
	=	G1 if $u_1(t) = (l_{25}\ h_{wee}\ h_{ste}\ h_{slp}\ \text{M} \geq 1)$
A_i	=	$A_i(u_1(t))$ see text
b	=	$\begin{bmatrix} 0 & 0 & 0 & 0 & k1 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}$
		Subsystem Cdc25 :
$u_2(t)$	=	$([\text{MPF}](t))$
$y_2(t)$	=	$(\text{k}_{25}(t))$
$y_2(t)$	=	$f(l_{25}, h_{25}, \theta_{25}, u_2(t)) = s_2(u_2(t))$
		Subsystem Wee1 :
$u_3(t)$	=	$([\text{MPF}](t))$
$y_3(t)$	=	$(\text{k}_{\text{wee}}(t))$
$y_3(t)$	=	$f(h_{\text{wee}}, l_{\text{wee}}, \theta_{\text{wee}}, u_3(t)) = s_3(u_3(t))$
		Subsystem Ste9 :
$u_4(t)$	=	$([\text{MPF}](t)\ [\text{Slp1}](t)\ \text{M}(t))'$
$y_4(t)$	=	$([\text{Ste9}](t))$
$y_4(t)$	=	$f(l_{\text{ste}}, h_{\text{ste}}, \theta_{\text{ste}}, \phi_{\text{ste}} u_4(t)) = s_4(u_4(t))$
ϕ_{ste}	=	$(\phi_{ste_1}\ \phi_{ste_2}\ 0)$
		Subsystem Slp1 :
$u_5(t)$	=	$[\text{MPF}](t)$
$y_5(t)$	=	$[\text{Slp1}](t)$
$y_5(t)$	=	$f(l_{\text{slp}}, h_{\text{slp}}, \theta_{\text{slp}}, u_5(t - t_{\text{del}}))$
		$= s_5(u_5(t - t_{\text{del}}))$
		Subsystem Rum1_T :
$u_6(t)$	=	$([\text{MPF}](t)\ \text{M}(t))'$
$y_6(t)$	=	$([\text{Rum1}_\text{T}](t))$
$y_6(t)$	=	h_{rum1t}
where :		
$f(b, a, \theta, x)$	=	$\begin{cases} b & \text{if } x \leq \theta \\ a & \text{if } x > \theta \end{cases} \quad a, b, x \in \mathbb{R}, x > 0$
		$\text{y}_{\text{system}}(\text{t}) = \text{MPF}$: (5)
[MPF]	=	$\dfrac{([\text{Cdc13}_\text{T}] - [\text{preMPF}])([\text{Cdc13}_\text{T}] - [\text{Trimer}])}{[\text{Cdc13}_\text{T}]}$
[Trimer]	=	$\dfrac{2[\text{Cdc13}_\text{T}][\text{Rum1}_\text{T}]}{\sum + \sqrt{\sum^2 - 4[\text{Cdc13}_\text{T}][\text{Rum1}_\text{T}]}}$
$\sum$	=	$[\text{Cdc13}_\text{T}] + [\text{Rum1}_\text{T}] + K_{\text{diss}}$
		Mass :
$\frac{d\text{M}}{dt}$	=	μM (6)
When	[MPF]	decreases through 0.1, M is divided by two.

TABLE IV Parameters reduced piecewise linear model

$k_1 = 0.03$	$k_2' = 0.03$	$k_2'' = 1$	$k_2''' = 0.1$
$h_{\text{wee}} = 1.3$	$l_{\text{wee}} = 0.2$	$\theta_{\text{wee}} = 0.25$	$h_{25} = 5$
$l_{25} = 0.2$	$\theta_{25} = 0.25$	$h_{\text{ste}} = 1$	$l_{\text{ste}} = 0$
$\theta_{\text{ste}} = 0.1$	$\phi_{\text{ste}_1} = -3.2$	$\phi_{\text{ste}_2} = 1$	$h_{\text{slp}} = 3$
$l_{\text{slp}} = 0$	$\theta_{\text{slp}} = 0.4$	$t_{\text{del}} = 15$	$h_{\text{rum1t}} = 0.1$
$K_{diss} = 0.001$			

and one can see that the fixed points of the subsystem Prempf is stable for all biologically possible solutions $([\text{Slp1}], [\text{Ste9}], \text{k}_{\text{wee}}, \text{k}_{25} \geq 0)$ if the parameters are positive and $k_2' > 0$. The fixed points can be written as

$$\begin{aligned} [\hat{\text{Cdc13}_{\text{T}}}] &= c_1\text{M} \\ [\hat{\text{preMPF}}] &= c_2\text{M}. \end{aligned} \tag{9}$$

where $\hat{x}$ denotes fixed point and $c_1 = k_1/(k_2' + k_2''[\text{Ste9}] + k_2'''[\text{Slp1}])$ and $c_2 = (\text{k}_{\text{wee}}k_1)/((k_2' + k_2''[\text{Ste9}] + k_2'''[\text{Slp1}])(\text{k}_{\text{wee}} + \text{k}_{25} + k_2' + k_2''[\text{Ste9}] + k_2'''[\text{Slp1}]))$. This means that each of the linear systems strive towards a protein concentration proportional to M, but with different proportionality constants. The mass is driving the system dynamics. Now, how many different matrices A_i do we have? A_i contains four discrete binary variables, so the theoretical possibility would be $2^4 = 16$. Are all of these used in the system? A_i depends on the input u_1 to subsystem Prempf, which in turn depends on the input from earlier subsystems, which finally all depend on [MPF]. We have one delay in the system t_{del}. This means that the input to subsystem Prempf can be written as functions depending only on $[\text{MPF}](t)$, $[\text{MPF}](t - t_{\text{del}}) = [\text{MPF}]_{\text{del}}(t)$ and the external input M

$$\begin{aligned} u_1 &= \left(\text{k}_{25}\ \text{k}_{\text{wee}}\ [\text{Ste9}]\ [\text{Slp1}]\ \text{M}\right)' \\ &= \Big(s_2([\text{MPF}])\ \ s_3([\text{MPF}]) \\ &\qquad s_4(([\text{MPF}]\ \ s_5([\text{MPF}]_{\text{del}})\ \ M))\ \ s_5([\text{MPF}]_{\text{del}})\ \ \text{M}\Big)' \end{aligned}$$

where s_2, s_3, s_4 and s_5 are step functions defined in table III. The matrix A_i depend on the four first elements of u_1 and M does not affect s_3 when $\text{M} \geq 1$. This means that [MPF] and $[\text{MPF}]_{\text{del}}$ together with the four step functions s_2, s_3, s_4 and s_5 decide the possible A_i matrices.

In fig. 5 a graph with [MPF] on the x-axis and $[\text{MPF}]_{\text{del}}$ on the y-axis has been drawn. In this graph the dashed lines correspond to the four step functions. One can see that only five of the 16 possible system matrices A_i are used, and the full reduced system can thus be described as five different linear systems, when $\text{M} \geq 1$. In this graph we have also drawn a trajectory of the system corresponding to the numerical simulation of the reduced model seen in fig. 1 (bottom).

The full system have input $u(t) = (\text{M}\ \ [\text{MPF}])'$ and output $y(t) = ([\text{MPF}])$, see fig. 2. Since A_i only depends on [MPF] and $[\text{MPF}]_{\text{del}}$ the full reduced system can be

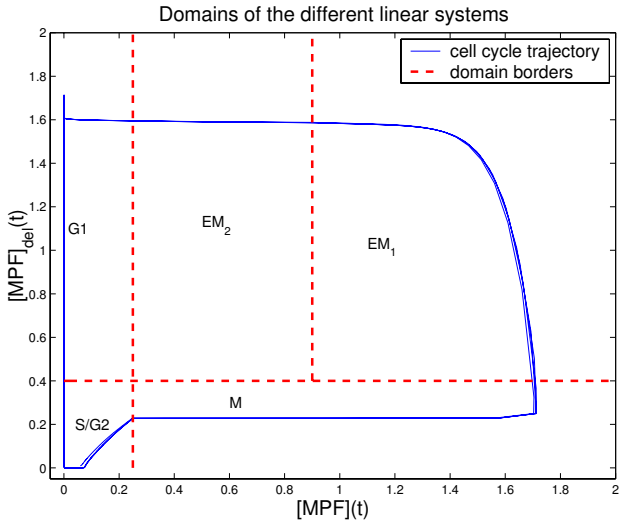

Fig. 5. The cell cycle can be described by five different linear systems ($M \geq 1$). The dashed lines corresponds to the step functions of table III. These functions all depend on $\text{MPF}(t)$ or $\text{MPF}(t - t_{del})$, which are on the x- and y-axis respectively. The trajectory of a simulation of the reduced system, table III and IV, are also shown. Initial values where the same as in fig. 1 (bottom). G1=G1-phase, S=S-phase, G2=G2-phase, M=Mitosis and EM=End Mitosis.

described as

$$\begin{aligned} \dot{x(t)} &= A_i x(t) + bu(t) \\ i &= i(u(t), u(t - t_{del})) \\ y(t) &= f(x(t)) \\ u(t) &= (M(t)\ \ y(t))' \\ i &\in \{\text{S/G2}, \text{M}, \text{EM}_1, \text{EM}_2, \text{G1}\} \end{aligned}$$

where x, u and A_i are described earlier $b = \left[\begin{smallmatrix} k_1 & 0 \\ 0 & 0 \end{smallmatrix}\right]$, M the external input to the system and $f(x)$ corresponds to equations (5) of table III. Finally in fig 5 we show a trajectory of the system in the two variables $y(t)$ and $y(t - t_{del})$ i. e. $[MPF]$ and $[MPF]_{del}$.

IV. Discussion

The idea of our approach to complex biological systems, worked through using the cell cycle as a test case, is to divide the system into subsystems of a more tractable size and then characterize and simplify these. The approach of subdividing systems and to look at the steady state dynamics have been used by others [2], [16]. With the process described here we reduced the number of state variables from 9 to 3 and halved the number of parameters, but more important a nonlinear system was approximated with a piecewise linear system. This makes a rigorous mathematical analysis possible. Although sophisticated computer simulations provide good insight to large complex systems, a theoretical based analysis is an important aspect in understanding complexity of biological systems. Currently, we are investigating necessary and sufficient conditions for stable limit cycles, i.e oscillations in this model. Here we utilize techniques used within mechanical and engineering

systems analysis e.g. [6], [7]. However, our system contains a certain delay and an immediate application of the technique to analyze stability of limit cycle [6] is therefore unlikely. But can be possible after special treatment of delay by introducing extra state variables, which will be further investigated.

Piecewise linear systems are a better approximation of nonlinearities than the linear ones. The latter can only be done in small regions around equilibrium and do not capture important properties of nonlinear systems like limit cycle oscillations.

The existence of a reduced piecewise linear system description also have implications for the problem of identifying a good model from large-scale parallel measurements of gene or protein activity. First, a minimal model clearly have fewer parameters and state equations, thus reducing the computational complexity of the parameter estimation problem. Secondly, if other biological system are similar to this, a piecewise linear description could perhaps be used as a mean to get models of these systems [14]. Whether these findings are exclusive for this regime of the cell cycle or if other systems can be treated in the same way is an open issue.

V. Conclusions

In this paper we reduced the Novak *et al*-cell cycle model, in the external regime $\mathrm{M} \geq 1$, by dividing the whole system into subsystems which were viewed as input/output systems. Based on the qualitative analysis of the input/output behavior of the subsystems at steady state we could replace the subsystems with simplified functions corresponding to this input/output behavior. Computer simulations showed that such a reduced system captures the behavior of the original model for the tested initial values. Most of the subsystems where replaced by step functions or step functions with a delay. Therefore the reduced system could be cast in the framework of piecewise linear systems.

We believe that such an representation will enable an analytical analysis of the system in terms of limit cycle and fixed point stability. It can also have implications on how to identify biological systems.

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