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Modeling and Control of the Protein Synthesis process in Eukaryotic cells

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***Abstract*— Protein synthesis is an essential process of cell cycle and growth in eukaryotic cells. The initiation stage of the translation process is known to be the most crucial in regulation of gene expression. This paper presents a reduced model of the initiation process using the eukaryotic initiation factor (eIF)-2 unit and proposes methods to control it.**

Linearization of the model is presented as a measure to simplify the analysis and the control applications. The properties of the linear model were investigated and compared to the nonlinear model. It was shown that the linear model is (marginally) stable and a linear controller was introduced to regulate the production level of protein.

A nonlinear state feedback control was also applied in order to increase production of protein in a controlled manner. In both linear and nonlinear models, the rate of protein synthesis can be regulated using a specific factor as an intracellular input, and by means of measurement techniques available today. The density of the ribosomes on the mRNA can then be set to a desired level. If this strategy can be implemented *de facto*, then a genuine control on protein synthesis process can be obtained.

I. Introduction

Protein synthesis is a central process in every eukaryotic and prokaryotic cell. Proteins are the building blocks in every cell, each with its own specific amino acid sequence and structure. The protein synthesis process is regulated mainly in its initiation phase [1] with several regulation layers. Eukaryotic initiation factor (eIF)-4 regulates the loading site on the mRNA, preparing the ground for translation whereas eIF-2, perhaps the most important of all the regulatory mechanisms, regulates translation based on the availability of the amino acids for elongation. Controlling the initiation phase, particularly eIF-2, will therefore permit to set the concentrations of the proteins in the cell to the desired levels. The economical benefits of an increase in protein production are obvious from a biotechnology perspective, from pharmaceutics to applications such as synthetic alginate production and the food industry.

The binding of eIF-2 to GTP is a prerequisite for the initiation process. Most of the regulation mechanisms of translational control are affected by reversible modifications of translational factors, chiefly through phosphorylation [2]. The end product of the last stage of the initiation phase, eIF2·GDP, needs to be recycled to eIF2·GTP in order to maintain it concentrations (Figure 1). Phosphorylation converts eIF-2 from an exchange substrate to a competitive inhibitor of another important factor, eIF2B [1]. Since the levels of eIF2B in the cell are two- to five-fold lower than that of eIF-2, only partial phosphorylation of eIF-2 is sufficient to inhibit all the eIF2B and to prevent recycling of eIF-2 [3]. This paper investigates strategies which can be applied to a translational model given by [4] in order to actively control the initiation process and thus determine the translation rate. Several studies have demonstrated how overexpression of eIF-2 in yeast cells increases translation rates [5], while GCN2 inhibits translation [6]. Since translation rate is promoted when eIF-2 levels are induced, one might be able to apply control theory to compute the concentrations of eIF-2 needed to obtain a specific (desired) level of translation.

Yeast, our eukaryotic model, is perhaps the most well-studied organism in molecular biology. Molecular genetic techniques, such as gene manipulation, gene deletion and transformation, are well established in these microorganism. Due to these facts we are proposing an approach to test our control ideas in a yeast cell.

The initiation control process can be presented by the following state space representation,

$$\begin{aligned} \dot{x} &= f(t; x, u, \xi) \quad x \in \mathbb{R}^n \\ y &= h(t; x), \quad y \in \mathbb{R}^q \end{aligned} \tag{1}$$

where $x(t)$ is the state vector, $u(t)$ represents the input signals, ξ is the noise term, $y(t)$ is the output signal of the model and f and h are nonlinear functions of the process and the output, respectively. Ribosome subunits 40S, 60S and 80S will be denoted here as r_{40}, r_{60} and r_{80}, respectively.

II. Model formulation

TABLE I
Variables in the eIF-2 control model

Variable	Description
e_2	eIF-2
e_B	eIF2B
e_D	eIF2·GDP complex
e_{pD}	$eIF2_p$·GDP complex (phosphorylated e_D)
e_T	eIF2·GTP complex
D	inactive complex $eIF2_p$·GDP·eIF2B
e_{com}	eIF2·GDP·eIF2B complex
G_t	GCN2·tRNA
sc	48S initiation complex

The process of initiation control via eIF-2 subunits can be described by a set of nonlinear differential equations in a

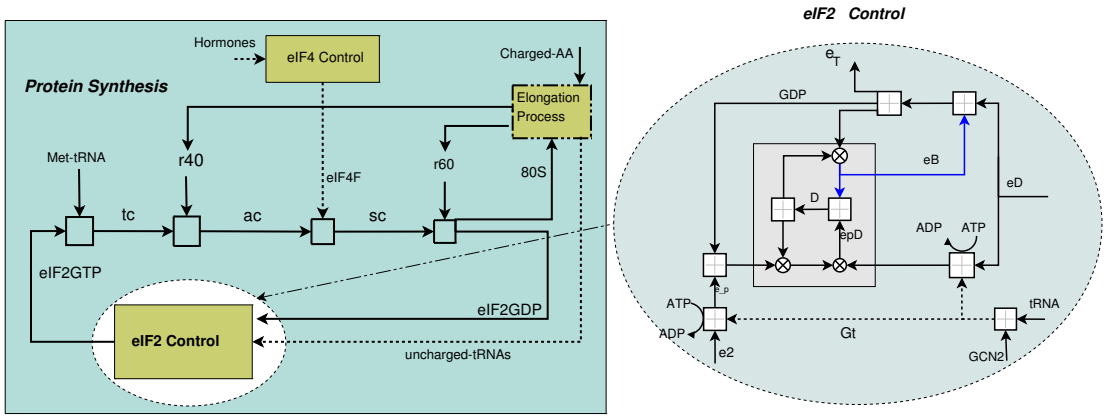

Fig. 1. The initiation process (left) and the eIF-2 control unit (right).

similar manner to [4], illustrated in Figure 1. The model is given by the next set

$$\dot{e}_B = -k_{71}e_Be_D - k_{21}e_Be_{pD} + k_{22}D + k_{72}e_{com} \quad (2a)$$
$$\dot{e}_D = -k_{71}e_De_B + \rho_u^{-1}k_6sc + k_{92}\ e_{pD} - k_{91}e_DG_t \quad (2b)$$
$$\dot{e}_{pD} = -k_{21}e_{pD}e_B + k_{22}D - k_{92}e_{pD} + k_{91}e_DG_t \quad (2c)$$
$$\dot{e}_T = -k_4e_T + k_{72}e_{com} + u(t) \quad (2d)$$
$$\dot{e}_{com} = k_{71}e_Be_D - k_{72}e_{com} \quad (2e)$$
$$\dot{D} = k_{21}\ e_B\ e_{pD} - k_{22}D \quad (2f)$$
$$\dot{sc} = k_4e_T - \rho_u^{-1}k_6sc \quad (2g)$$

with the input signal $u(t)$. A list with description of the variables is given by Table I. G_t is modeled as a disturbance element. The state space model above can be rewritten using the following matrix notation:

$$\dot{x}(t) = \Phi(x,t) + Bu(t) + F(x)\xi, \qquad x(t) \in \mathbb{R}^7 \quad (3)$$
$$y(t) = sc(t)$$

$u(t)$ is the input of intracellular eIF-2, $F(x)\xi(t)$ is the noise term, $x(t) = [e_B\ e_D\ e_{pD}\ e_T\ e_{com}\ D\ sc]^T$ is the vector of variables, $B = (0\ 0\ 0\ 1\ 0\ 0\ 0)^T$ is the input matrix and $F(x) = (0\ \ -k_{91}e_D\ k_{91}e_D\ 0\ 0\ 0\ 0)^T$ is the process noise matrix. Under normal feeding conditions (i.e. unlimited supply of amino acids), the noise term (G_t) is negligible [4].

III. Linear model

A quick observation of (3) reveals that the nonlinearity lies in the multiplication of $e_B(t)$. Linearization is feasible under the assumption of a constant feeding modus: either a starvation state, where the concentration of GCN2 is high (thus $G_t = \bar{G}$) or under normal amino acids supply conditions, where $G_t \to 0$. Local stability properties of (3) can be found by elimination of $\dot{e}_B(t)$, assuming a constant value $\bar{e}_B$. We define a new state vector $x_L = (e_D\ \ e_{pD}\ e_T\ e_{com}\ D\ sc)^T$. System (3) then takes the form

$$\dot{x}_L = \Phi_L(t, x_L, u) \quad (4)$$

and the discrepancy between the nonlinear and the linear systems is defined as

$$\epsilon(t) = \|x(t) - x_L(t)\|_\infty \quad (5)$$

Following this linearization method, (4) can be rewritten as a linear system:

$$\dot{x}_L = Ax_L + Bu + F\xi \quad (6)$$

where A matrix is

$$A = \begin{pmatrix} (-k_{71}\bar{e}_B - k_{91}\bar{G}) & k_{92} & 0 & 0 & 0 & \rho_u^{-1}k_6 \\ k_{91}\bar{G} & (-k_{21}\bar{e}_B - k_{92}) & 0 & 0 & k_{22} & 0 \\ 0 & 0 & -k_4 & k_{72} & 0 & 0 \\ k_{71}\bar{e}_B & 0 & 0 & -k_{72} & 0 & 0 \\ 0 & k_{21}\bar{e}_B & 0 & 0 & -k_{22} & 0 \\ 0 & 0 & k_4 & 0 & 0 & -\rho_u^{-1}k_6 \end{pmatrix}$$

The values of k_{ij} and $\bar{e}_B$ are given by Table II. The pole of

TABLE II
VALUES OF THE RATE CONSTANTS k_{ij} OF THE SYSTEM (6), TAKEN FROM [4].

Parameter	Value
k_{21}, k_{22}	1, 0.2 s^{-1}
k_4	0.9 s^{-1}
k_{71}, k_{72}	0.1, 0.95 s^{-1}
k_{91}, k_{92}	0.08, 0.5 s^{-1}
$\rho^{-1}k_6$	0.05 s^{-1}
$\bar{e}_B$	0.01-1 $nmol\ g^{-1}$

the system matrix A corresponding to the state e_D is zero when $\bar{e}_B = 0$ whereas the remaining poles lie within $\mathbb{R}e < 0$, thus for $\bar{e}_B = 0$ all the states but e_D converge asymptotically to zero. For increasing $\bar{e}_B > 0$, the pole corresponding to sc approaches zero whereas the remaining poles have finite values in $\mathbb{R}e < 0$. The discrepancy between the linear and the nonlinear systems in the elongating ribosomes sc is zero for $e_B(0) = 0$ as expected (Figure 3). For $e_B(0) > 0$ the error is positively correlated with $e_B(0)$ with a peak of 0.6% discrepancy at $e_B(0) = 0.37$. This low discrepancy suggests that the nonlinear system can be replaced by the linear one when supply of amino acids is sufficient (i.e. $G_t \approx 0$). It is possible to apply a feedback control law to (6) that will drive $sc(t)$ to a desired value sc_d by manipulating the input $e_T(t)$. This can be performed using linear systems theory, for instance by calculating a gain vector K (see for example [7] for review on linear control methods) or alternatively, by solving an optimal control problem using

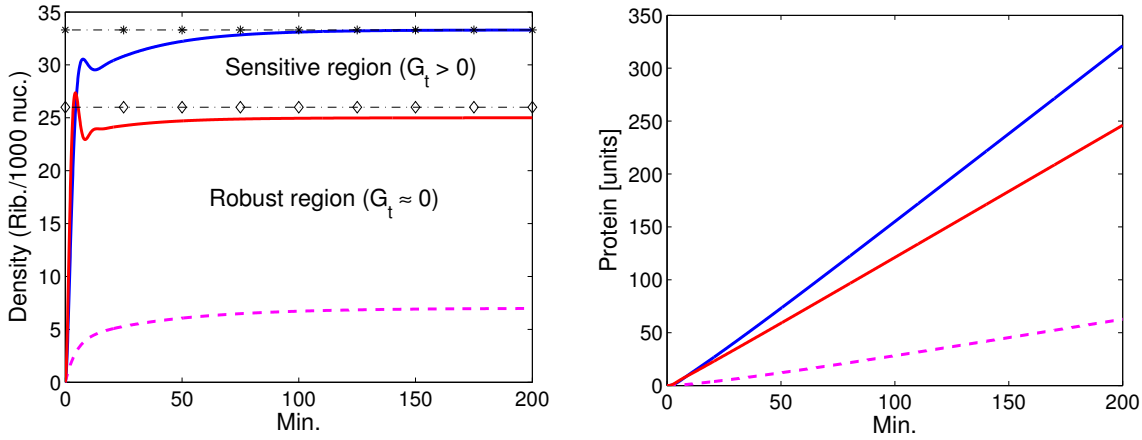

Fig. 4. Simulations of the linear system (6) using the values $\bar{e}_B = 0$ with (solid) and without (dashed) control law. The top most horizontal line represent maximum ribosome density (Rib /1000 nucleotides) whereas the bottom line represent the border between the sensitive and robust regions. Right: Protein production of the unforced system ($u(t) = 0$), of the maximum density (top) and of the case with set-point $sc_d = 23$ ribosomes.

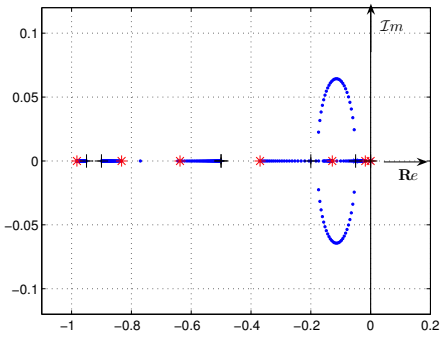

Fig. 2. The trajectories of the poles of A for increasing values $\bar{e}_B$. All the poles lie within $\mathbb{R}e \leq 0$.

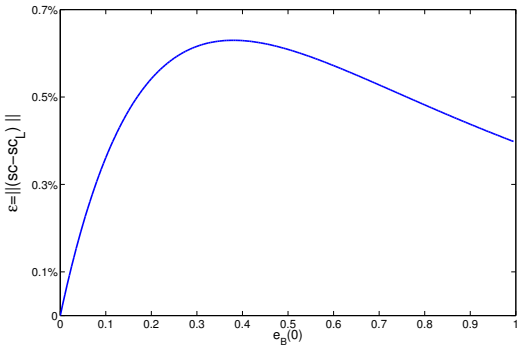

Fig. 3. The error given by eq. (5) as a function of $e_B(0)$. The linear system is relatively accurate (maximum error of $\approx 0.5\%$ and the inaccuracy is sharply decreasing for initial values $e_B < 0.4$

dynamic programming in order to guarantee positivity of the states $\forall t$ [8]. Results of the simulations are presented in Figure 4. The values $\bar{e}_B = 0.5$ was chosen to simulate a worst case (peak discrepancy). Applying a control law (solid) increases the number of 80S ribosomes loaded on the mRNA to the desired value (middle curve with a set value of 24 ribosomes in this case). The top most horizontal line represents the potential capacity of the density (ribosomes / 1000 nucleotides). The uncontrolled density response lies at about 25% of the maximum density, well inside the robust region (see below).

IV. Robustness

Upon amino acids deficiency, GCN2 binding to tRNA (denoted G_t) increases several fold, inhibiting loading of ribosomes on the mRNA [4]. This behavior can represent two different phases of this model. Phase I occurs under the conditions of sufficient concentrations of amino acids for elongation whereas phase II occurs when one or more amino acids are depleted, which increases the concentrations of free tRNAs, and consequently elevating the level of G_t. Increase in the latter inhibits ribosome loading and consequently reducing the ribosome density. However, if the density of the ribosomes on the mRNA is large, consumption rate of the amino acids becomes too large to cover their uptake through the membranes and stall of ribosome on the mRNA is imminent.

Previous experiments in yeast cells [9] showed that the density of the ribosome on the mRNA is much lower than its potential (Table III). Measurements of the 80S ribosome subunit density for the genes CMD1 and TDH3 (under normal conditions and sufficient amino acids supply) indicated that density reaches only 27% for CMD1 and 21% for TDH3 of its potential [4]. Considering these measurements (and others, see [9]), it seems remarkable that the function of ribosome loading on the mRNA apparently is inefficient. However, considering the concept of robustness in cells [10], these results are well inside the robust region, where consumption of amino acids is relatively slow, preventing rapid depletion and thus maintaining G_t near zero (Figure 4). Active control can increase the amount of ribosomes loaded

on the mRNA, but this might risk rapid depletion of amino acids and fast increase in G_t. This transition between the two phases usually introduce fluctuations in the elongation rate [4]. Furthermore, in the event of complete depletion of the limiting amino acid, the ribosomes that are under elongation process stall on the mRNA, unable to complete the translation process (see "Discussion").

TABLE III
THE MAXIMUM (POTENTIAL) DENSITY (IN RIBOSOMES/1000 NUCLEOTIDES AND RIBOSOMES/MRNA) AND THE MEASURED DENSITY OF TWO TRANSCRIPTS CMD1 AND TDH3 [4].

Gene	# nucl.	max. (Rib./1000 nucl.)	max. (Rib./ mRNA)	measured (Rib./1000 nucl.)	measured (Rib./ mRNA)
CMD1	441	33.3	14.7	9	4
TDH3	999	33.3	33.2	7	7

V. APPLYING FEEDBACK CONTROL TO THE NONLINEAR SYSTEM

Recruiting ribosome 80S is a process which its prerequisite is formation of 48S complex, joined with 60S subunit [11]. The last stage in this process can be written as [4]

$$sc(t) + r_{60}(t) \rightarrow r_{80}(t) + e_D(t)$$

such that each complex sc is transformed to one r_{80} subunit. Assuming that the concentrations of 60S and 40S subunits are not rate limiting, increase in sc will yield increase in the elongating ribosomes (r_{80}). As in the linear case, the control objective is to calculate the input e_T to a value which achieves the desired number of ribosomes on the mRNA strand (within the elongation capacity). It was demonstrated that the eIF-2 initiation process is Lyapunov stable [4] and $\mathcal{L}_2$ stable [12]. This implies that the input eIF-2 does not risk instability of the system. One strategy is to apply a nonlinear state feedback control to the input $u(t)$, given by the following

$$\dot{x}(t) = \Phi(x,t) + Bu(t) \tag{7a}$$
$$y(t) = Cx(t) \tag{7b}$$

with $\Phi(x,t)$ given by (3) and the vectors $B = (0,\ 0,\ 0,\ 1,\ 0,\ 0,\ 0)^T$, $C = (0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 1)$ and the measured output $y(t) = sc(t)$, as illustrated in Figure 5. By using feedback information from the states e_T and e_{com},

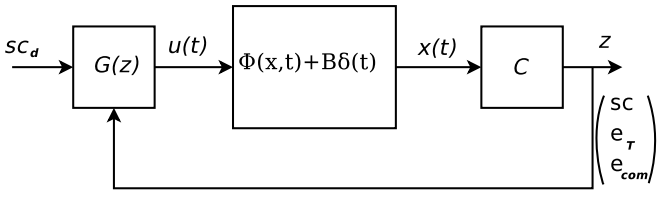

Fig. 5. State feedback control scheme for system model (3).

a control law is calculated such that $r_{80}(t)$ is driven to a desired value r_{80d}. Define $z = (sc,\ e_T,\ e_{com})^T$ to be the measurement vector of the states. By applying a standard procedure (see for example [13]), the output $y = r_{80}(t)$ is time-derived twice and the control law is calculated to be

$$u(t) = -k_{72}e_{com} - \frac{k_6^2}{k_4}sc + k_4 e_T^2 + k_6 e_T + \delta \tag{8}$$

where δ is chosen to force the internal dynamic asymptotic stable

$$\delta = \ddot{r}_{80d} - \delta_1 \dot{e} - \delta_2 e, \quad \delta_{1,2} > 0 \tag{9}$$

$e = r_{80}(t) - r_{80d}$ is the control error, δ_1 and δ_2 are found by pole-placement method. Thus by applying $u(t)$ the control error is reduced asymptotically to zero.

Example 5.1: We applied the control law (8) to the system model (3) with the desired values $r_{80d} = 22$, $\dot{r}_{80d} = 0$ and $\ddot{r}_{80d} = 0$. The values $\delta_1 = 6$ and $\delta_2 = 11$ were calculated by pole placement method. r_{80} is driven to the desired value $r_{80d} = 22$ after 10 minutes (Figure 6 upper right). For comparison, the results of the unforced system ($u(t) = 0$) are presented by the dashed line. By controlling e_T (the input signal), the amount of 80S ribosomes is significantly higher under feedback control.

VI. DISCUSSION

The natural behavior of the cell seems somewhat peculiar, as it operates with a low ribosome density on the mRNA relatively to the loading capacity (see [9] and Table III). Measurements of both CMD1 and TDH3 transcripts (as well as many other genes; see for instance [9]) where found to operate on 27% and 21% of their maximum density (Figure 4), although these genes are not known to have rare codon sequences or any significant presence of secondary structures in the 5'UTR. One hypothesis is that the eukaryotic cells strive to maintain robustness, operating only in phase I whenever possible, the robust region. This is achieved by avoiding ribosome loading on the mRNAs to their potential capacity. As was mentioned previously, maximum loading implies high consumption of amino acids, the resource for the translation process. Due to the relatively slow rate of amino acids uptake through the cell outer membrane comparing with the high rate of translation, transcripts that contain large number of ribosomes on the open reading frames (ORFs) are most likely to experience a translation stall (entering phase II, where ribosomes will not advance beyond a codon in the absence of the associated charged-tRNA). Large number of stalled ribosomes on the mRNA (for instance density $>$ 20 ribosomes/1000 nucleotides) would prove wasteful, particularly if the mRNA is synthesized in large quantities. Long time delays with absence of the limiting amino acids may theoretically cause the mRNA and all its ribosomes to deteriorate and the peptide chain to degrade, without producing a single protein. The loss of energy and resources is then obvious, considering the size of the ribosomes (machinery with about 3 mill. nucleotides), the cost of mRNA transcription and the cost of translation (reduction of about one GTP to GDP for each amino acid in the sequence). This may even prove devastating under certain conditions, for instance in low energy state or stress

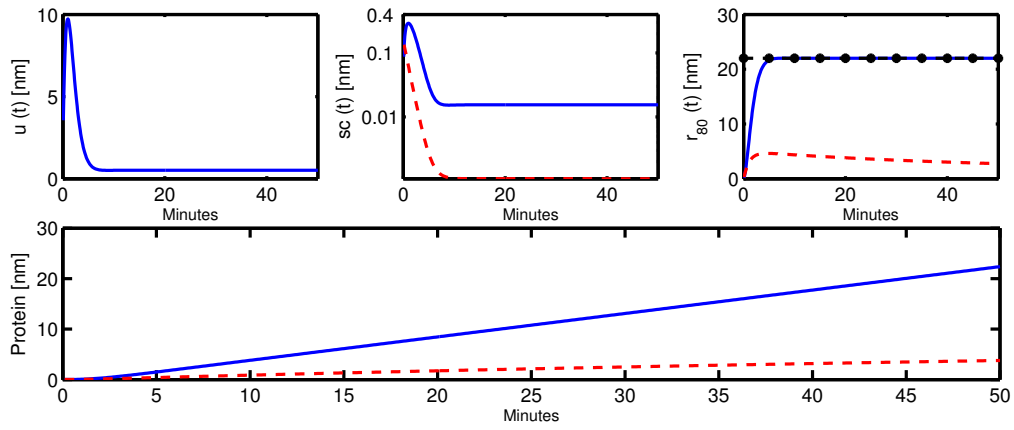

Fig. 6. Comparison from simulation of the unforced system (3) (dashed) to the case of applying state feedback control $u(t)$ from (8) to the model (solid). The line $(-*-)$ in the middle figure denotes the desire value sc_d.

due to viral attack. In the light of the mentioned above, we can argue that cells will tend to become conservative with ribosome density. Approaching the border line where the amino acids uptake can no longer keep up with the amino acids consumption will risk significant waste of resources (due to ribosome stall). This will cause the cell to enter the sensitive region where translation is not smooth (Figure 4). In this sensitive region, stall can still occur despite the counter effect of G_t to dampen the ribosome loading rate.

It was shown that by controlling the levels of the 48S initiation complex (sc), it is possible to determine the amount of 80S subunits loaded onto the mRNA, actively controlling the rate of the protein synthesis process. Applying control over the initiation process can be performed by using either the linear model or a state feedback control applied to the nonlinear model. Using these technique, it is feasible to drive the output 48S initiation complex (sc) to a desired level by inducing the intracellular input eIF-2 in a calculated and controlled manner. In fact, the control law in (8) implies explicitly that the only states which need to be measured are the concentrations of eIF2·GTP (e_T), the complex eIF2·GDP·eIF2B (e_{com}) and 48S initiation complex (the output sc). These were all measured for eukaryotic cells; Time series measurements of eIF-2 and the eIF2·GDP·eIF2B complexes were taken in extracts of HeLa cells [5]. Several studies demonstrated measurements of the 48S complex (sc) and the 80S ribosomes [14], [15]. Furthermore, it is feasible to use purified eIF-2 in a process to form eIF2·GTP and to induce it in a controlled manner to the cell extract *in vitro* [5] or by other methods *in vivo*. The time series measurements and the potential use of eIF-2 suggest that the feedback control structure as described in equation (7) is applicable *in vitro*. Consequently, a direct control on the density of 80S subunits on the mRNA can be achieved, possibly increasing the density to a high desired value. Moreover, it was already proved that the nonlinear system is $\mathcal{L}_2$ stable [4], implies that overexpression of eIF-2 will not risk instability of the process, consistent with experimental results [5].

Applying control over the initiation phase is theoretically plausible both *in vitro* and *in vivo* for several reasons: 1) The states that are required to be measured in order to apply control are indeed available for measurement as described above, and 2) from a bioengineering perspective, control on the exact amount of protein synthesis is feasible by tuning solely the levels of eIF2·GTP, without the need to induce any other factors or introduce gene manipulation in the host cell (at least *in vitro*). Many experiments on yeast cells [9] indicated that the cell operate with low density of ribosomes on the mRNA, i.e. protein synthesis is far from its potential. Control will permit production increase of many fold (Figure 4).

In order to study the rate of translation *in-vivo*, based on the model suggested here, it is desirable to have a system where the expression of eIF-2 can be controlled. A common method for fine-tuning gene expression in molecular biology is by driving the gene with a regulatable promoter of different strengths and characteristics. Tight control of the eIF-2 expression can be achieved by exchanging the native promoter of the eIF-2 gene on the yeast genome with a regulatable promoter via promoter replacement method, by the use of the homologous recombination [16]. Having a regulatable promoter can be advantageous as by varying the inducer concentration desired level of eIF-2 expression can be achieved over a time span. In the decision of which regulatable promoter system to use, there are several options to consider, e.g., p*DAN1*, in which the expression can be regulated by oxygen concentration [17], p*ICL1*, where expression can be activated by addition of alkanes and acids and repressed by glucose and glycerol [18] and p*tetO7*, in which the repression is controlled by addition of doxycycline [19]. Among these p*DAN1* can be appealing as it has been stated that there is no basal level expression under aerobic conditions [17]. When a regulatable promoter is utilized to drive the expression of any gene it is important to define

the upper and the lower limits. Both extremes may have detrimental effect on the cell since eIF-2 is necessary for cell function. It is known that overexpression of eIF-2 may cause a problem to the cell as abundance of eIF-2 may lead to sequestration of other initiation complexes. Causing the eIF-2 expression to drop below a critical level may impair essential processes and intracellular growth and thus the control strategy should include at least upper and lower input constraints.

Channeling the overexpressed eIF-2 into the protein production of one desired gene may be a challenging task both *in vitro* and *in vivo*. We propose an approach to overcome this issue by creating a condition where attraction of ribosome to the desired gene could be increased. $5'$-UTR has a major role in transcript stability and in the initiation step of translation. This region's several feature i.e, length, GC content, and structure known to affect the rate of translational initiation [20]. Since the yeast genome has been sequenced, and transcriptional regulation of genes to some extend have been reported, with the help of computational biology naturally high expressed genes $5'$-UTR's can be identified from public databases. Recently [21], the median length of $5'$-UTR was found to be 50 bp in the yeast genome. It has been already stated that translation of transcripts with short $5'$-UTR is more efficient [20]. Therefore tailoring methods like directed evolution or random mutagenesis can easily be applied on generating large $5'$-UTR libraries based on the identified UTR sequences. By screening these libraries different $5'$-UTR mutants can be identified which may have exhibit a high level of translation. By this approach the total pool of eIF-2 in the cell may have larger impact on the desired gene.

Finally, maintaining all the elements of the state vector $x(t)$ positive can be achieved by including state constrains to the optimal controller. It was shown (section III) that the error between the linear model and the nonlinear is relatively small so optimal control from linear theory can be applied. It is also important to mention that it may be technically challenging to fine-tune the intracellular input u to the desired level (Figure 6). Several solution currently exist but they are all limited by the rate of sampling and its accuracy.

VII. Conclusions

A reduced model of the initiation phase of protein synthesis is presented. The manner applying state feedback control using measurements from three of the states was shown to be feasible. It was demonstrated that wild type cells operate on low ribosome density on the mRNA, whereas feedback control can increase this density 3-5 fold. This active intracellular control can have great impact on the production of proteins *in vitro*, but also theoretically *in vivo* (by regulating the intracellular eIF-2 levels).

VIII. Acknowledgment

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