Modelling and dynamic behaviour of eIF2 dependent regulatory system with disturbances

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***Abstract*—Eukaryotic initiation factor 2 (eIF2) is a central controller of the eukaryotic translational machinery. To sustain the on-going translation activity, eIF2 cycles between its GTP and GDP bound states. However, in response to cellular stresses, the phosphorylation of eIF2 takes place, which acts as an inhibitor of the guanine nucleotide exchange factor eIF2B and switches the translation activity on physiological timescales. The main objective of this work is to investigate the stability of the regulatory system under nominal conditions, parametric fluctuations and structural damages. In this paper, a mathematical model of eIF2 dependent regulatory system is used to identify the stability-conferring features within the system with the help of direct and indirect methods of Lyapunov stability theory. To investigate the impact of intrinsic fluctuations and structural damages on the stability of regulatory system, the mathematical model has been linearised around feasible equilibrium point and the variation of system poles have been observed. The investigations have revealed that the regulatory model is stable and able to tolerate the intrinsic stressors but becomes unstable when particular complex is targeted to override the undesirable interaction. Our analyses indicate that, the stability is a collective property and damage in the structure of the system changes the stability of the system.**

***Index Terms*—Protein synthesis, mathematical modelling, structural stability, targeting undesirable interaction, species elimination.**

I. Introduction

Protein synthesis is itself a highly robust and complex process consisting of mainly three phases, namely initiation, elongation and termination [1]. Among these phases, initiation is the very first step responsible for protein synthesis and predominantly targeted for control of gene expression levels [2]. Translation initiation is a dominant locus of control, which depends hugely on the initiation factors (eIFs) [3]. One of the main role of initiation factors is to ensure that the translation activity is sustainable.

To maintain the on-going translation activity, the role of eIF2 is to cycle between its active (GTP-bound) and inactive (GDP-bound) states. The primary role of eIF2 in translation initiation is to carry methionyl tRNA to the 40S ribosomal subunit [3]. To participate in upcoming round of translation, eIF2 stimulates its active state with the help of guanine nucleotide exchange factor (GEF) termed eIF2B, by releasing GDP from eIF2:GDP and forming eIF2:GTP, and further regulated by a guanidine dissociation inhibitor (GDI) function of eIF5 [4]. The eIF2:GDP is released from translation initiation process as eIF2:GDP:eIF5 complex [5].

During amino-acid starvation, uncharged tRNA (u-tRNA) influences eIF2 by phosphorylating it with the help of highly conserved single-substrate kinase namely general control non-derepressible 2 (GCN2). Investigation has revealed that, u-tRNA and charged tRNA are in competition with each other for binding with GCN2, but GCN2 holds comparatively lesser affinity for aminoacylated tRNA than u-tRNA [6]. Although GCN2 can bind to both aminoacylated tRNA and u-tRNA, it only activates by binding to u-tRNA [7], [8]. Phosphorylation converts eIF2 to a competitive inhibitor of eIF2B, and binds with eIF2B to form a tight complex that disrupts nominal translation activity [9]. Compared to eIF2, GEF eIF2B is usually present in substoichiometric amounts [10] and even partial phosphorylation of eIF2 can quantitatively block translation activity.

From computation point of view, the dynamic aspects of regulatory pathways involving eIF2 can be studied in the context of overall translation initiation pathway or specific reactions focussing on the core reaction [11]–[14]. In this paper, the latter technique is adopted for investigating the stability of the regulatory system that involves the core eIF2:GDP complexes required for phosphorylation of eIF2:GDP and formation of tight complex eIF2-P:eIF2B.

There are several reasons to investigate the stability of the eIF2 dependent regulatory system, such as, in drug design targeting particular complex to override the undesirable interaction while avoiding unintended targets is one of the attractive possibilities [15], [16]. Although, aiming particular target to override the undesirable interaction is currently a huge challenge, in many cases this objective has been attained through trial-and-error and rational design approaches [15]. Another example includes, transient protein complex that holds suitable level of stability, which can be broken by external factor such as phosphorylation. Hence, stability can be specified as one of the important features that should be observed in formation of protein complexes [17]. Hence, the objective of this paper is to investigate the impact of reaction elimination on the stability of the eIF2 dependent regulatory system with the help of control theory.

The remainder of the paper is organized as follows. In Section II, a mathematical model for eIF2 dependent regulatory system is developed using mass action kinetics. Section III presents the detailed analyses of the dynamical behaviour of eIF2 dependent regulatory system and the switching behaviour of eIF2 dependent regulatory system. In the same section the stability of the biochemical network is discussed for

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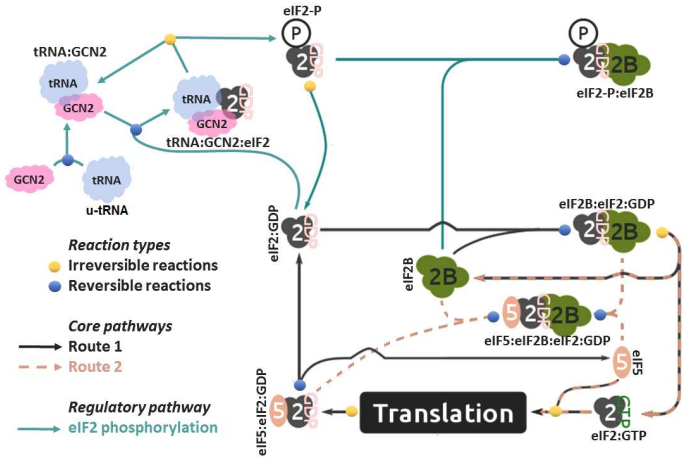

Fig. 1. The eIF2 dependent regulatory model demonstrates the role of u-tRNA in activating GCN2 and halting the ongoing translation activity. To sustain ongoing translation activity, regeneration of eIF2:GDP is required by forming eIF2B:eIF2:GDP GEF complex. The GEF complex can form either by release of eIF5 prior to recruitment of eIF2B (route 1) or through eIF5:eIF2B:eIF2:GDP intermediate complex (route 2). Phosphorylated eIF2 (eIF2-P) binds with eIF2B and form a tight complex, resulting into quantitative blockage of translation activity. The starting point of eIF2 phosphorylation is activation of kinase GCN2 after binding to u-tRNA.

perturbed system, and the impact of structural damage on general behaviour (in frequency domain) and stability of the system is also presented. Finally, the paper is concluded in Section IV.

II. Materials and Methods

In the present study, translational control via phosphorylation of eIF2 is considered as a molecular switch, which changes the translation activity on physiological timescales. To demonstrate the role of highly conserved kinase GCN2 on the general translation activity, a computational biology approach is adopted. In this study, the mass-action kinetic modelling approach [18] is used to develop the deterministic eIF2 model and tools from control theory are used to investigate the stability of the model. Note that, the deterministic models are amenable to analysis methods from the domain of linear control theory, which is not possible from stochastic models.

Fig. 1 demonstrates the role of inactive and active kinase GCN2 on translation activity. It is well known that, GCN2 activates after binding to u-tRNA during amino-acid deprivation, which results into down-regulation of translation activity. The mathematical model is designed to mimic two established states of the eIF2 cycle. The first state is referred as the normal state (non-stress state) that corresponds availability of plenty of amino-acids. While another state is the stress state, when GCN2 activates by combining to u-tRNA during amino-acid starvation.

As stated earlier, both u-tRNA and charged tRNAs compete with each other for binding with GCN2 [6], but GCN2 only activates after binding to u-tRNA. So changing concentration of this activated complex can be modelled either by displacing charged tRNA with u-tRNA from GCN2 or by increasing the concentration of u-tRNA binding to GCN2. The model illustrated in Fig. 1, includes the pathway (termed as route 2) for the regeneration of eIF2:GDP due to intermediate complex constituting factors eIF2B and eIF5 [5]. The formation of eIF2:GDP:eIF2B GEF complex can either occur via release of eIF5 prior to the recruitment of eIF2B (termed as route 1), or through eIF2:GDP:eIF5:eIF2B intermediate complex [19], [20]. In the recent study, it has been found that eIF2B functions as an activator of eIF5 dissociation from eIF2:GDP:eIF5 complex, indicates that out of the two routes, latter route is preferred for the regeneration of eIF2:GDP [19], [20].

The biomolecular reactions and differential equation involved in the eIF2 pathway are given in supplementary file S1 and S2 respectively. Note that, the mathematical model is implemented in Matlab and solved using a modified rosenbrock solver (ode23s) [21]. To parametrise the proposed mathematical model, the Levenberg-Marquardt (LM) algorithm [22] is used with target translation flux of 13,000 proteins per cell per second (haploid) as an algorithm constraint [23]. The total cellular concentrations reported in the literature are as follows: eIF2 = 18 μM [23], [24], eIF2B = 1.8 μM [23], [24], eIF5 = 18 μM [24] and GCN2 = 0.03 μM [23].

It is worthy to note that, most of the parameters are not known from experiments, hence estimating remaining parameters using LM algorithm will result into large parameter space that is able to fit the target translation flux of 13,000 proteins per cell per second. To narrow down the large parameter space to single precise set of rate constants, it is necessary to include the robustness property of the translation activity against mutation induced changes in rate constants [25], [26].

The robustness of mathematical model against mutation induced changes can be tested by obtaining the minimal change in translation flux against parametric perturbations through reaction containing reaction rates C_{11}/C_1. The processes of parameter estimation and narrowing large parameter space to single parameter set is discussed below.

The LM algorithm is initiated with random initial conditions following aforementioned total cellular concentrations of eIF2, eIF2B, eIF5 and GCN2. Note that, LM algorithm might fall into local minima during the parameterisation process that may result into uncertain combination of parameter values, which may not follow experimental observations. Therefore to overcome this limitation, the fitting process is repeated 10^5 times and the fit is validated using (1), which guarantees that the algorithm is not falling into local minima [27].

$$\xi_1 = |Y_{1D} - Y_1(Y_i(0), C_j, t)| \tag{1}$$

where, ξ_1 is an absolute error between $in\ vitro$ and simulated experimental values, Y_{1D} is the $in\ vitro$ experimental data value for haploid yeast cell, C_j is the set of rate constant and $Y_1(Y_i(0), C_j, t)$ is the simulated experimental value of protein obtained by solving ODEs for $Y_i(0)$ and C_j initial conditions, and $j \in [1, 20]$. Note that, out of 10^5 random experiments, the percentage of parameter combinations giving $\xi_1 \equiv 0$ is 1.684%. The percentage value of parameter combinations defines the exact estimation of the experimental translation flux because in that case $Y_{1D} \simeq Y_1(Y_i(0), C_j, t)$. The percentage value suggests large combinations of rate constants are accu-

rately fitting the target translation flux of 13,000 proteins per cell per second for the current mathematical model.

To narrow down the large parameter space to single parameter set, every individual combination of parameter is perturbed to $\pm 50\%$ from its original value and the normalised average error ξ_2 is recorded, which can be determined from (2).

$$\xi_2 = \frac{1}{T}\int_0^T \frac{|Y_1(Y_i(0), C_m, t) - Y_1(Y_i(0), \Delta C_m, t)|}{\max(Y_1(Y_i(0), C_m, t), Y_1(Y_i(0), \Delta C_m, t))}\, dt \tag{2}$$

where, T is the evaluation time and ΔC_m is equal to $\pm 50\%$ of original C_m value and m belongs to $[2, 20] \ni [11]$. The purpose of perturbing the rate constants is to analyse the robustness of translation activity against mutation induced changes, which is illustrated in supplementary file S6. Lower value of ξ_2 defines high robustness against parametric changes [28]. Hence, the parameter combination giving lowest value of ξ_2 is considered in this study. The estimated rate constants are given in supplementary file S5.

III. Results

In this section, the dynamic control properties of regulatory systems involving the eIF2 pathway is to be investigated with the help of simulation results. To simulate the impact of amino-acid starvation on translation activity, the value of u-tRNA is varied from 0 M (non-stress case) to 1.05×10^{-5} M (stress case). A zero cellular concentration of u-tRNA implies its absence in the system due to availability of amino-acids in abundance. While increasing the value of u-tRNA from zero acts as a stress signal implying accumulation of u-tRNA in the amino-acid starved cell, and its role as GCN2 activator which impacts on the translation activity. The switching property of the model is discussed in the sub-section.

A. Switching behaviour of eIF2 dependent regulation

It is well known that, eIF2 is a G-protein acting as a molecular switch inside the cells, which sets translational activity to different levels in physiological timescales, which depends on activation and interaction of kinase GCN2 with u-tRNA. To observe this scenario, the initial concentration of u-tRNA is varied from zero onwards. Activation of GCN2 has a reciprocal effect on changes to the translation activity which is evident from Figs. 2 and 3.

Fig. 2 shows slow ramping of translation activity from 13,000 proteins per cell per sec. (100%) to starvation in response to u-tRNAs and activated GCN2. The transition of state occurs when the existing pool of tRNA becomes uncharged without modifying the total pool of tRNA. The increasing pool of u-tRNA into the system has activated GCN2 that forms tRNA:GCN2 complex, which eventually phosphorylates eIF2α. In return eIF2-P acts as an inhibitor for GEF eIF2B, resulting in the reduction in the eIF2:GTP complex and down-regulation of the overall translation activity. Observing the stress behaviour of the mathematical model, it can be asserted that severe activation of GCN2 is leading to cessation of levels of translation that could sustain cell metabolism, which is consistent with the actual biological behaviour [7]. The

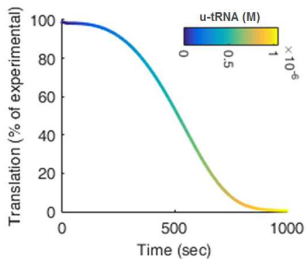

Fig. 2. Role of slow ramping of u-tRNA in response to starvation. The kinase activity of GCN2 is activated by binding to u-tRNA, resulting in downregulation of translation activity. The variation of colour from blue to yellow represents increasing strength of u-tRNA.

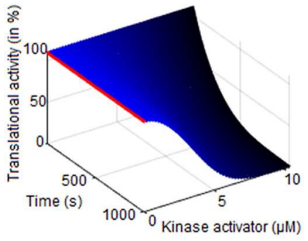

Fig. 3. Activation of GCN2 changes translation activity on physiological timescale. The red line represents uninterrupted translation activity when there are plenty of amino-acids, while faded blue to dark blue surface represents the impact of increasing strength of kinase activator (u-tRNA) on translation activity. The translational activity declines quickly with increase in the concentration level of activator.

initial conditions used to mimic this scenario are given in supplementary files S3 and S5.

To further assess the impact of varying strength of u-tRNA (Y_{13}) on translation activity, the concentration of kinase activator is varied from 0 M to 1.05×10^{-5} M, and the output translation values are plotted against increasing values of kinase activator, which is illustrated in Fig. 3. Fig. 3 shows that, the higher the concentration of kinase activator, the steeper is the ramp of cessation of translation levels.

B. Stability of the eIF2 dependent regulatory system

As stated earlier, the biological systems are often unaffected by the variations or fluctuations in the dynamical parameters [25], [26], [29]. The main objective of this paper is to investigate the stability of the eIF2 dependent regulatory system against parametric fluctuations and structural damage. Prior investigating the stability of the system (in Lyapunov sense), the eIF2 dependent regulatory system is to be represented in the generalised state space form, that can be defined below:

$$\dot{\boldsymbol{Y}} = f(\boldsymbol{Y}, t) \tag{3}$$

where, vector $\boldsymbol{Y}$ is the non-negative concentration of the species and t is the time.

According to the direct Lyapunov stability theory, an equilibrium point of a non-linear system is said to be stable locally if all the solutions starting at nearby points stay nearby; otherwise, it is said to be unstable. On the other hand the system is locally asymptotically stable if all the solutions

starting at nearby points approaches towards equilibrium point as time $t \to \infty$.

Theorem 1: If there exists a Lyapunov function $V(\boldsymbol{Y}, t) \geq 0$ for a system $\boldsymbol{Y}(t+\delta) - \boldsymbol{Y}(t) = f(\boldsymbol{Y}, t)$ satisfying:
$\frac{\partial V(\boldsymbol{Y},t)}{\partial \boldsymbol{Y}}\Big(f(\boldsymbol{Y},t)\Big) \leq 0$
then the equilibrium point of the system is stable locally.

The equilibrium point of the model is mentioned in supplementary file S4. Note that, the steady states obtained from LM algorithm are used as initial concentrations for non-stress case, which are close to the equilibrium point. The reason behind such selection is to demonstrate the flow of model from nominal state to stress state through slow ramping of u-tRNAs and activated GCN2 in response to starvation. To investigate the Lyapunov stability of the non-linear eIF2 system around the equilibrium point, consider a positive definite candidate Lyapunov function $V(\boldsymbol{Y}, t)$ for the system defined in supplementary file S2:

$$\begin{aligned} V(\boldsymbol{Y},t) =& 2 \times \text{Protein} + 2 \times \text{eIF5:eIF2:GDP} \\ &+ \text{eIF2B} + 3 \times \text{eIF5:eIF2B:eIF2:GDP} \\ &+ \text{eIF5} + 2 \times \text{eIF2B:eIF2:GDP} + \text{eIF2:GDP} \\ &+ \text{eIF2:GTP} + \text{GCN2} + 2 \times \text{tRNA:GCN2} \\ &+ \text{eIF2-P} + 2 \times \text{eIF2-P:eIF2B} + \text{u-tRNA} \\ &+ 3 \times \text{tRNA:GCN2:eIF2} \end{aligned} \tag{4}$$

where $V(0,t) = 0$ and $V(\boldsymbol{Y},t) > 0 \ \forall \boldsymbol{Y} \neq 0$ because the concentration of all species is either greater than or equal to zero. Note that, there is no general rule to obtain the Lyapunov function of the system, but the best way is to initiate with a linear summation function that includes all the species/variables of the system and modify the function analytically until Theorem 1 is satisfied.

According to the Lyapunov theorem the system is stable if derivative of $V(\boldsymbol{Y}, t)$ with respect to time t is negative definite or negative semidefinite. The derivative of $V(\boldsymbol{Y}, t)$ in (4) with respect to time is given by

$$\dot{V}(\boldsymbol{Y},t) = -C_{20} \times \text{u-tRNA} \tag{5}$$

Since C_{20} is non-negative, from eqs. (4) and (5) it is evident that $\dot{V}(\boldsymbol{Y},t) \leq 0$. Therefore, the given system is stable. The existence of Lyapunov function constituting weighted summation of states indicate that the stability of eIF2 regulatory system depends on its structure. Time evolution of Lyapunov function and its temporal derivative for stress and non-stress case is illustrated in Fig. 4. To improve the visualisation of Fig. 4, the individual plots for V and dV/dt are also illustrated in supplementary file S7.

To further strengthen our result, the Lyapunov indirect method is used. In indirect method, the proposed model is linearised around the equilibrium point and the behaviour of system based on its poles are observed after introducing intrinsic and structural disturbances. The poles of Jacobian matrix of perturbed model are given in supplementary file S8. Note that, poles of the Jacobian matrix of a non-linear system or a linearised system are the frequencies at which the value of the denominator of transfer function is zero [30].

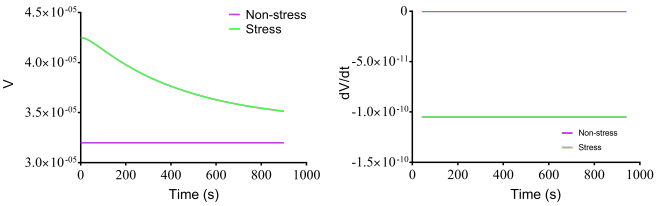

Fig. 4. Time evolution of Lyapunov function and its temporal derivative for stress and non-stress cases: (a) V, and (b) dV/dt.

C. Stability analysis of structurally damaged eIF2 regulatory system

As stated earlier, the dynamic aspects of the eIF2 dependent model can be studied either by considering overall translation initiation pathway or by focusing on specific reactions [11]–[14]. In the former case, model might contain large number of ODEs and parameter sets, which usually result into huge computational burden. To reduce the computational complexity and prevent wrong simplifications of the models, various techniques have been suggested in the literature [31]–[33]. While the latter case is relatively free from such computational complexities. In the literature, various techniques have been reported, which reduces the complex system to a simplified version to avoid the computational burden [30], [34], [35]. However in biomolecular systems, model reduction techniques should not result in lose of their physical meaning and should not change the interaction matrices that mimics the behaviour of the original system [36].

In control theory, model reduction techniques are helpful in determining the important states within the system that are responsible for achieving nominal behaviour. Among various model reduction techniques, matched DC gain method is the popular one [30]. The purpose of opting matched DC gain method is that, the behaviour of the modified system should not vary significantly with respect to the original system in the frequency domain using Bode plot. The Bode plot is able to estimate the extent at which the overall system behaviour changes in frequency domain, when particular species (one state) or group of species (multiple states) have been targeted. The Bode plot estimates the frequency behaviour of the model by evaluating the transfer function of the linear system in its state-space model using the Laplace transform [30].

After species or variable elimination, the matched DC gain method re-computes the state-space matrices, which is discussed in supplementary file S10. The matched DC gain method is applicable to both oscillatory and non-oscillatory systems. The only difference is that, in non-oscillatory system, the Bode magnitude plot is enough to observe the system behaviour. While in oscillatory system, the change in oscillations after state elimination can be represented with the help of Bode phase plot.

Prior to investigating the impact of dimensionality reduction on behaviour of eIF2 regulatory system, it is beneficial to eliminate the dynamics of u-tRNA from the original non-linear model, because investigation has revealed that u-tRNA lies

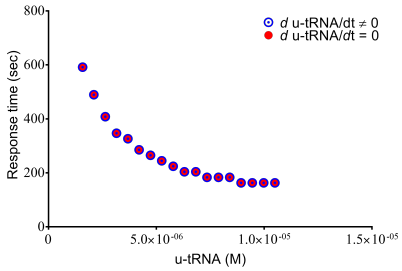

Fig. 5. The response time of translational activity (time after which translation activity has dropped by 99%) as a function of u-tRNA (Y_{13}) concentration. Equating the dynamics of u-tRNA to zero, is not impacting the response time of translation activity.

on fast time-scale and its concentration is not changed with time. Therefore the dynamics of this particular species (state) can be recognised as fast dynamics compared to the other species. Fig. 5 illustrates the time to reach a 99% reduction in translational activity as a function of u-tRNA concentration. If the mathematical model is allowed to run at the steady-state and then the concentration of u-tRNA is raised from zero onwards, then in response to starvation, the translation activity will drop to zero over time; which implies that the model can achieve response times on physiological time scales. The rate of response is concentration dependent, with a lower threshold for the response time (arbitrarily defined here as the time after which translation has dropped by 99%) between two and three minutes. From Fig. 5 it is evident that, substituting ${}^{d\,\text{u-tRNA}}/_{dt} = 0$ is not impacting the translational activity. Note that, substituting ${}^{d\,\text{u-tRNA}}/_{dt} = 0$ to zero gives freedom to consider u-tRNA as input $u = {}^{(C_{13} \times \text{tRNA:GCN2})}/_{(C_{12} \times \text{GCN2} + C_{20})}$, and hence control tool such as matched DC gain model reduction technique [30] can be used for system analysis.

According to Lyapunov indirect method, the non-linear model can be linearised around equilibrium point to investigate its dynamic properties such as stability of the model. The stability of the structurally damaged system can be easily analysed with the help of the Lyapunov indirect method just by observing the poles of the system, which is not easily possible by the Lyapunov direct method that requires new candidate Lyapunov function after each species/complex elimination. The state space representation of modified non-linear model with single input single output (SISO) can be defined as follows:

$$\dot{\boldsymbol{Y}}(t) = \boldsymbol{\mathcal{Y}}(\boldsymbol{Y}, t) + \boldsymbol{\mathcal{B}}(\boldsymbol{Y}(t))u \tag{6a}$$

$$Z(t) = Y_1(t) \tag{6b}$$

where, $\boldsymbol{\mathcal{B}}(\boldsymbol{Y}(t)) = [0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ -C_{12}Y_9\ C_{12}Y_9\ 0\ 0\ 0]^T$, $Z(t)$ is the output signal or translation, and Y_9 =GCN2. The state space representation of approximate SISO linear model of a modified non-linear system can be described in the form:

$$\dot{\boldsymbol{Y}} = \mathcal{A}\boldsymbol{Y} + \boldsymbol{B}u \tag{7a}$$

$$Z = \boldsymbol{D}\boldsymbol{Y} \tag{7b}$$

where $\mathcal{A}$ is a Jacobian matrix given in supplementary file S10, $\boldsymbol{Y} = [Y_1\ Y_2\ \cdots\ Y_{12}\ Y_{14}]^T$, $\boldsymbol{B}$ and $\boldsymbol{D}$ are the constant input and output matrices given as:

$$\boldsymbol{B} = [0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ -4.331 \times 10^{-11}\ 4.331 \times 10^{-11}\ 0\ 0\ 0]^T$$

$$\boldsymbol{D} = [1\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0]$$

TABLE I
DIAGONAL ENTRIES OF JOINT GRAMIAN (g_{diag})

Species	Protein	eIF5:eIF2:GDP	eIF2B	eIF5:eIF2B:eIF2:GDP
g_{diag}	∞	∞	6.53×10^{-7}	3.27×10^{-9}
Species	eIF5	eIF2B:eIF2:GDP	eIF2:GDP	eIF2:GTP
g_{diag}	2.22×10^{-9}	3.42×10^{-12}	1.07×10^{-13}	6.17×10^{-16}
Species	GCN2	tRNA:GCN2	eIF2-P	eIF2-P:eIF2B
g_{diag}	1.52×10^{-17}	1.60×10^{-19}	6.60×10^{-22}	6.38×10^{-25}
Species	u-tRNA	tRNA:GCN2:eIF2		
g_{diag}	-	1.34×10^{-26}		

Note that, vector $\boldsymbol{Y}$ includes all species except u-tRNA (Y_{13}), which is considered as input. It is worthy noting that, after obtaining the desirable mathematical model, the concept of linearisation around the equilibrium point has been introduced for analysing the stability of the system. The size of the Jacobian matrix $\mathcal{A}$ is 13×13 (supplementary file S10). The elements of matrices $\mathcal{A}$, $\boldsymbol{B}$ and $\boldsymbol{D}$ have their own significance. The diagonal elements of $\mathcal{A}$ represent emerging (or disappearing) effect of the species due to reversible (or irreversible) interaction whereas off-diagonal elements represent interaction between species. It is clear that the poles of matrix $\mathcal{A}$ is important in understanding the stability of the model against intrinsic disturbances and structural damages.

In this section joint Gramian (g) based model truncation concept [37] is adopted to eliminate the species using matched DC gain method. The diagonal entries of g (g_{diag}) reflect the combined controllability and observability of the balanced model. In other words, g_{diag} measures the contribution of each species to the input/output behavior. The value of g_{diag} reflects the relative importance of species in maintaining input-output characteristics of the system. Hence, the species comprising small value of g_{diag} can be eliminated from the system because it might not affect the balanced model. Further, the frequency response and poles are employed to investigate the impact of species elimination having low g_{diag} value on the stability of the system. If truncating single species or group of species having low g_{diag} value is not showing deviation from the SISO linear system in frequency domain, then it implies that particular species or group of species can be targeted without compromising nominal behaviour of the system. The diagonal entries of the joint gramian g_{diag} are given in Table I.

Note that, the offset value opted for evaluating joint Gramian is 10^{-8}, that is, if the magnitude of a pole is less than $|10^{-8}|$ in $s-$plane, then the pole is very close to zero and it will be considered as unstable mode and represented as ∞ in Table I. The truncation process is to be initiated from lowest g_{diag} that is tRNA:GCN2:eIF2. The purpose of opting lowest g_{diag} value is to observe the extent to which the model is reducible without showing frequency deviation with respect to the linear SISO model. Fig. 6 shows the comparative frequency

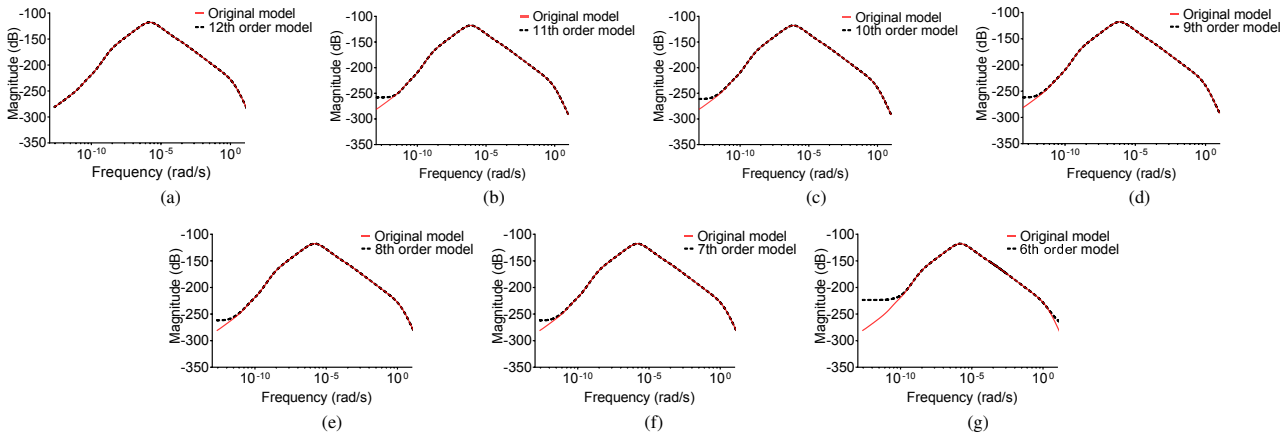

Fig. 6. Comparison of Bode magnitude plots after dimension reduction. Overlapping of the original and truncated plots across the frequency range represent similarity in the behaviour of the models. Larger the difference in the plots between two models, higher the difference in the behaviour. Red solid line represents the linear SISO model while green dotted line represents the reduced model after eliminating: (a) tRNA:GCN2:eIF2, (b) tRNA:GCN2:eIF2 and eIF2-P:eIF2B, (c) tRNA:GCN2:eIF2, eIF2-P:eIF2B and eIF2-P, (d) tRNA:GCN2:eIF2, eIF2-P:eIF2B – tRNA:GCN2, (e) tRNA:GCN2:eIF2, eIF2-P:eIF2B – GCN2, (f) tRNA:GCN2:eIF2, eIF2-P:eIF2B – eIF2:GTP, and (g) tRNA:GCN2:eIF2, eIF2-P:eIF2B – eIF2:GDP

TABLE II
COMPARISON OF POLES OF LINEAR SISO AND REDUCED MODELS

	SISO model	12th order model	11th order model
Poles	-100.5×10^3	-100.5×10^3	-100.5×10^3
	-606.6	-711.1	-208.2
	-213.5	-208.2	-7.5
	-129.1	-7.5	-1.8
	-7.5	-1.8	-1.3
	-1.8	-1.3	-0.5
	-1.3	-0.5	-99.9×10^{-6}
	-0.5	-99.9×10^{-6}	-7.8×10^{-7}
	-100.0×10^{-6}	-7.8×10^{-7}	7.4×10^{-7}
	$10.2 \times 10^{-9} + 1.7 \times 10^{-6}i$	7.4×10^{-7}	5.3×10^{-11}
	$10.2 \times 10^{-9} - 1.7 \times 10^{-6}i$	5.3×10^{-11}	2.1×10^{-12}
	-6.3×10^{-12}	2.1×10^{-12}	–
	1.0×10^{-12}	–	–

	10th order model	9th order model	8th order model
Poles	-208.2	-7.2	-1.9
	-7.5	-1.8	-1.4
	-1.8	-1.3	-0.5
	-1.3	-0.5	-99.9×10^{-6}
	-0.5	-99.9×10^{-6}	-7.8×10^{-7}
	-99.9×10^{-6}	-7.8×10^{-7}	7.4×10^{-7}
	-7.8×10^{-7}	7.4×10^{-7}	5.3×10^{-11}
	7.4×10^{-7}	5.3×10^{-11}	2.1×10^{-12}
	5.3×10^{-11}	2.1×10^{-12}	–
	2.1×10^{-12}	–	–

	7th order model	6th order model
Poles	-1.6	-0.4
	-0.5	-7.8×10^{-7}
	-99.9×10^{-6}	-99.9×10^{-6}
	-7.8×10^{-7}	7.4×10^{-7}
	7.4×10^{-7}	5.3×10^{-11}
	5.3×10^{-11}	2.1×10^{-12}
	2.1×10^{-12}	–

responses prior and after eliminating the set of species from the system. Note that, the order of the system is defined as the total number of species or variables available in the mathematical model that has non-zero dynamics.

From the Bode magnitude plot in Fig. 6(a), it can be stated that eliminating species tRNA:GCN2:eIF2 is not showing any deviation from the linear SISO system. Therefore it can be concluded that the reduced system is nicely capturing the behaviour of the SISO linear system. Now considering another species eIF2-P:eIF2B having second lowest g_{diag} value for elimination along with tRNA:GCN2:eIF2. Eliminating both species is showing deviation from the SISO linear system (refer Fig. 6(b)). The deviation in Bode plot after reducing the model to the 11th order suggests that eliminating two species together has slightly changed the general behaviour of the model and these species is important in maintaining the general behaviour of the system. Again Figs. 6(c)-(g) show very similar behaviour as that of Fig. 6(b). Hence, it can be stated that eliminating set of species has impacted the performance of the SISO linear system. Therefore these species (except tRNA:GCN2:eIF2) are critically important for the system and targeting these species may result into excessive change in model's behaviour.

The analysis above shows that the structure of the defined system is not damageable without compromising its behaviour, and robustness determining features are generally distributed unevenly between the species. Further, this is the same case when stability of the system is under observation. Table II shows the placement of poles after model reduction or structural damage. In Table II, some of the poles lie between the range $[-10^{-8}, 10^{-8}]$, which is the offset value opted for evaluating joint Gramian. Hence, any pole that lies within the offset value should be considered as zero. Note that, the magnitude of real part of a pole is important for understanding the local stability of the system, that is, if any pole of the model has positive magnitude of real part greater than offset value, then it represents instability. So, the focus of Table II is the real part of the pole's magnitude that is greater than 10^{-8}. A comparison of poles of the SISO model with reduced models in Table II reveals that, damaging the structure of the model or removal of species/complex has shifted one of the poles towards the right half of $s-$plane that is 7.4×10^{-7}. Hence, due to one positive pole, it can be asserted that the reduced systems are unstable around the given equilibrium point. In other words, damage in the structure of the system has pushed the system towards instability and stability of the system can be considered as a collective property. It is worth noting that, the novelty of the work presented in this paper lies in exploring the structural stability of the system with the help of tools from control theory. The proposed approach

is applicable to any mathematical model which satisfies two conditions: existence of Lyapunov function and input-output relation of species.

IV. Conclusion

In this paper the dynamic control properties of regulatory systems involving eIF2 has been addressed with the help of the mathematical model. The investigation has revealed that the stability of proposed mathematical eIF2 dependent regulatory model has an ability to tolerate the intrinsic stressors or parametric fluctuations, which is the core property of the biochemical pathway. The key objective of the paper is to investigate the stability of the eIF2 regulatory model using Lyapunov indirect method of stability under system's structural damage. This paper has shown that the stability of the eIF2 dependent regulatory system is a collective property and damaging the structure of the defined system usually causes instability, which is in consistence with the reality.

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