CONTROL THEORY APPROACH TO STUDY MULTIPLE FEEDBACKS IN LAC-OPERON SYSTEM

Introduction:

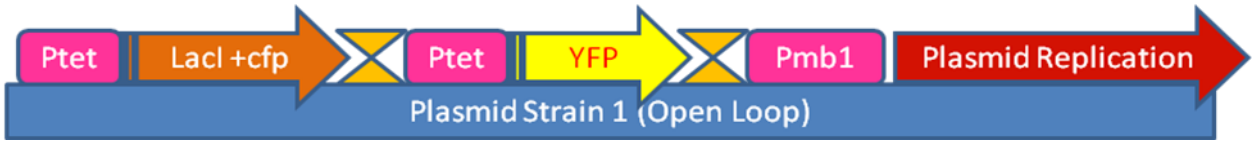
Biological systems have an ability to adapt to the changes in their environment, so that they can either insulate themselves from the adversities of the external environment or capitalize on the available resources. This capability of biological systems has been attributed to the presence of complex structures of feedback loops in the regulatory networks (Khammash and El-Samad, 2004). Therefore, feedback control loops are ubiquitous in nature. Various processes in biological systems are modular in nature, with each module carrying out a particular part of the process (Hartwell et al., 1999). Thus, in many biological systems, regulation of each sub-process takes place through multiple feedback loop configurations. Thus, every sub-process is independently regulated, to maintain a desired level of the product. This is in contrast with the conventional notion of feedback control, wherein the overall process is regulated by regulating the sub-process at the very beginning of the process system.

We wish to demonstrate the utility of a multiple feedback control system as compared to a single feedback control system. This utility is characterized in terms of faster response with reduced noise.

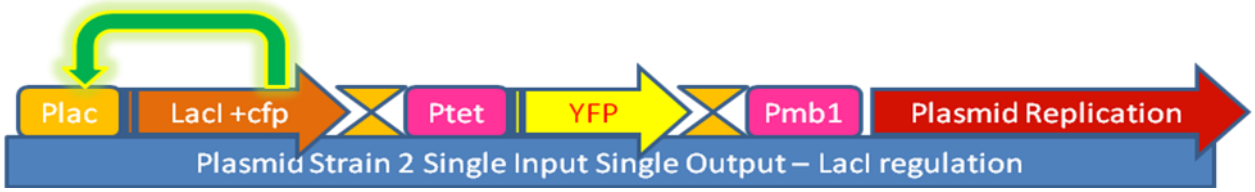
We would be using the lac operon system in an E. coli cell for this purpose. The lac operon system is a complex mechanism used for the digestion of lactose. The lac operon system when expressed results in the formation of the protein lacI. We have introduced a modified plasmid in the E. coli cell. The lacI produced from the operon within this plasmid has a cfp(cyan fluorescent protein) attached to it, whose fluorescence is an indicator of the amount of lacI present in the system. Further, the plasmid number also keeps growing with a separate mechanism. The plasmid is attached with a yfp(yellow fluorescent protein) so that the fluorescence of the protein acts as an indicator of its number. The promoter of the lacI system(ptet) can further be modified(to plac) such that lacI can itself bind to the promoter and repress its expression. This forms one level of feedback where the process output is being regulated by the output itself. Further the replication of plasmid number can be modified in such a way that the lacI produced inhibits the process. Thus we have a unique structure where the process output can regulate not only itself, but also the number of processes.

Thus we have 2 control levels. By combination, we have 4 different control loops or structures possible, expressed in 4 different strains. They are as follows:-

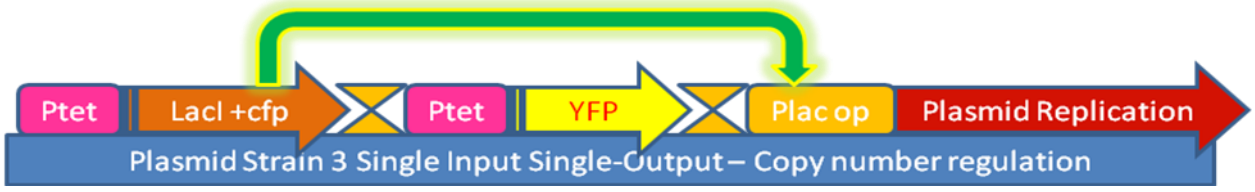
Strain 1 (Open loop):- The promoter of both lacI and plasmid number replication is unaffected by lacI. This forms the open loop or the simplest of structures which is unregulated.



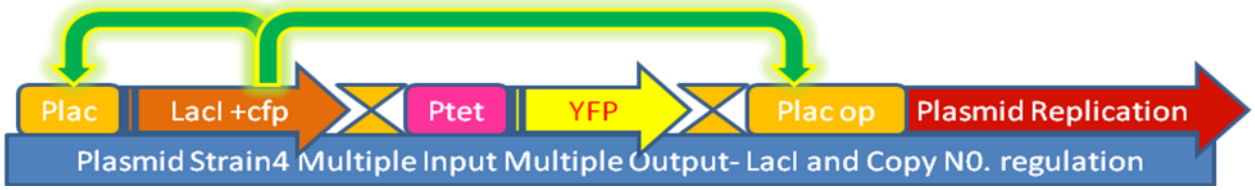
Strain 2 (Single Input Single Output with LacI regulation [SISO_LacI]):- The promoter for the lac system is changed to plac. Thus lacI can bind to its promoter site and prevent its expression. This forms one level of regulation. The copy number is unregulated.



Strain 3(Single Input Single Output with Copy number regulation [SISO_CN]):- The promoter for replication of plasmid is modified such that lacI can bind to its site and prevent replication. Thus the copy number is regulated. LacI expression is unregulated.



***Strain 4 (Multiple Input Multiple Output with regulation both on LacI and copy number [MIMO])*:-** Both the promoters are modified so that lacI produced can regulate the expression of lacI as well as the replication of plasmids. This forms the highest level of control.



Effect of IPTG on system

Further, an interesting study can be conducted by addition of IPTG in the system. IPTG(isopropyl-beta-D-thiogalactopyranoside), is an inducer for the lac system. It binds with LacI. Thus in any of the regulated strains IPTG can potentially bind to any of the lacI molecules which are attached to the promoter sites, releasing them and thus causing expression of the system. Thus a regulated system with a high amount of IPTG should resemble with the open loop system in its behaviour. We wish to characterize this effect too by control analysis where the benefits of feedback loops must decrease because of the resemblance to open loop behaviour. We try and examine this too.

We try and linearize the system of equations for strain 4 and introduce controllers in place of the feedback terms. Then we try to do further analysis for the system using bode plots for the linearized system and magnitude bode plot for the sensitivity function. The equations for strain 4 are as given below:

$$\frac{d(C)}{dt} = \eta \frac{C}{K + C^2} \frac{{K_1}^2}{{K_1}^2 + LacI^2} - (\mu + \beta_1)C$$

$$\frac{dLacI}{dt} = k_1 C \frac{{K_2}^2}{{K_2}^2 + LacI^2} - (\mu + \beta_2)LacI - k_4 \left(\frac{IPTG}{K_c + IPTG}\right)^2 LacI$$

Where C: Plasmid Copy umber

In conventional man-made industrial processes, the plant and the controller can be analysed separately.. On the other hand, in biological systems, the controls are intertwined in the process models and their analysis becomes difficult. In our system, we have distributed feedback system (DFS) with controls at the levels of cell plasmid regulation and direct regulation of LacI production in each plasmid. The corresponding engineering control design would be control only on the number of plasmids. We try to use elements of control theory to determine whether such a distributed feedback system (DFS) would be much more robust than conventional feedback system (CFS).

Further we try to examine whether multiple feedback loops are better than open-loop systems for attenuation of noise around the steady-state in presence of external uncertainties. We try to analyse the standard-errors for LacI for strain 4 and strain 1. We try to determine the effects of external uncertainties on production of plasmid copy number, production of LacI and both on production of plasmid copy number and production of LacI together. The normalized standard-deviation for strain 4 are expected to be lower as compared to that of strain 1 which will demonstrate the better noise attenuation of external uncertainties in multiple feedback systems.

Solution Strategy:

In our engineered system, we try to determine whether DFS gives a better, faster and robust performance than CFS. The model equations are linearized around a setpoint. The linear equation model can be represented as given in the figures below. The controller on the plasmid copy number is given by $C_1(s)$ while control on the production of LacI is given by the control block $C_2(s)$. In CFS, $C_2(s)$ would be absent and the effect of single regulator $C_1(s)$ is taken into consideration. The controllers are designed as conventional Proportional-Integral (PI) controllers such that the response of the linearized system closely matches the original nonlinear system. To understand the role of multiple feedbacks, we take help of the frequency response tools from control systems' theory. Frequency response methods allow us to obtain a pictorial view of the performance of the system across a range of operating conditions and uncertainties. We make use of the Bode plots for both, the conventional feedback system and the distributed feedback system to compare their performance. Further we try and do frequency response analysis for the system with high IPTG and compare the performance of distributed feedback system and conventional feedback systems.

The linearized system of equations is as given below:

$$\Delta C(s) = \frac{\Delta C_o}{s + \mu + \beta_1 - \frac{K_1^{\,2}}{K_1^{\,2} + LacIs^2}(K - C_s^{\,2}) \Big/ (K + C_s^{\,2})^2} +$$

$$\frac{\eta \frac{C_s}{K + C_s^{\,2}} C1(s) \Delta LacI}{s + \mu + \beta_1 - \frac{K_1^{\,2}}{K_1^{\,2} + LacIs^2}(K - C_s^{\,2}) \eta / (K + C_s^{\,2})^2}$$

$$\Delta LacI(s) = \frac{k1\, C_s\, C2(s)}{s + \mu + \beta_2 - k_4\left(\frac{IPTG}{K_c + IPTG}\right)^2} \Delta LacI + \frac{k1 \frac{K_2^{\,2}}{K_2^{\,2} + LacI^2}}{s + \mu + \beta_2 - k_4\left(\frac{IPTG}{K_c + IPTG}\right)^2} \Delta C(s)$$

Where C : Plasmid Copy Number.

The steady state and dynamic data were obtained from experiments. Under normal conditions, the plasmid copy number reaches a steady state value of 100. The process and controller parameters for the DFS were tuned in a manner as to obtain steady state and dynamic characteristics that closely match with experimental data. The linearization is done around this set-point. Since linear systems are analysed using deviation variables, necessary changes are made in the system equations to reflect the use of deviation variables. The original nonlinear controllers are replaced with PI controllers and those are tuned accordingly so as to closely match the response of the nonlinear controllers. The resultant block is then used for analysing magnitude and phase Bode plots and for performing sensitivity analysis of the system.

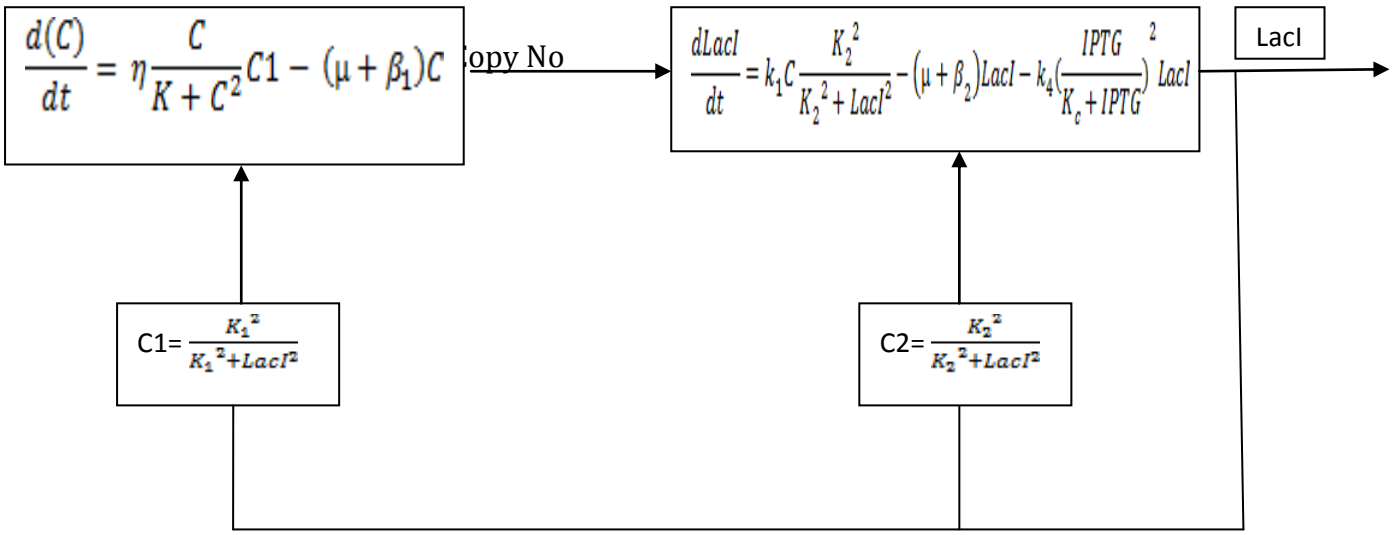


Fig 1: Block Diagram Representation of Dynamic Model for strain 4 of Lac operon. The LacI is given as feedback to both production of plasmids and LacI production. In a DFS, we have such distributed feedback. In a conventional feedback system, we have feedback only on the regulation of copy-number,η=445min^{-1},k1=50min^{-1},k4=25min^{-1},K=5000,K1=4500,K2=1000,K_c=200μM,μ=0.4/60min^{-1},β_1=0.001/60 min^{-1}, β_2=0.1/60 min^{-1}.

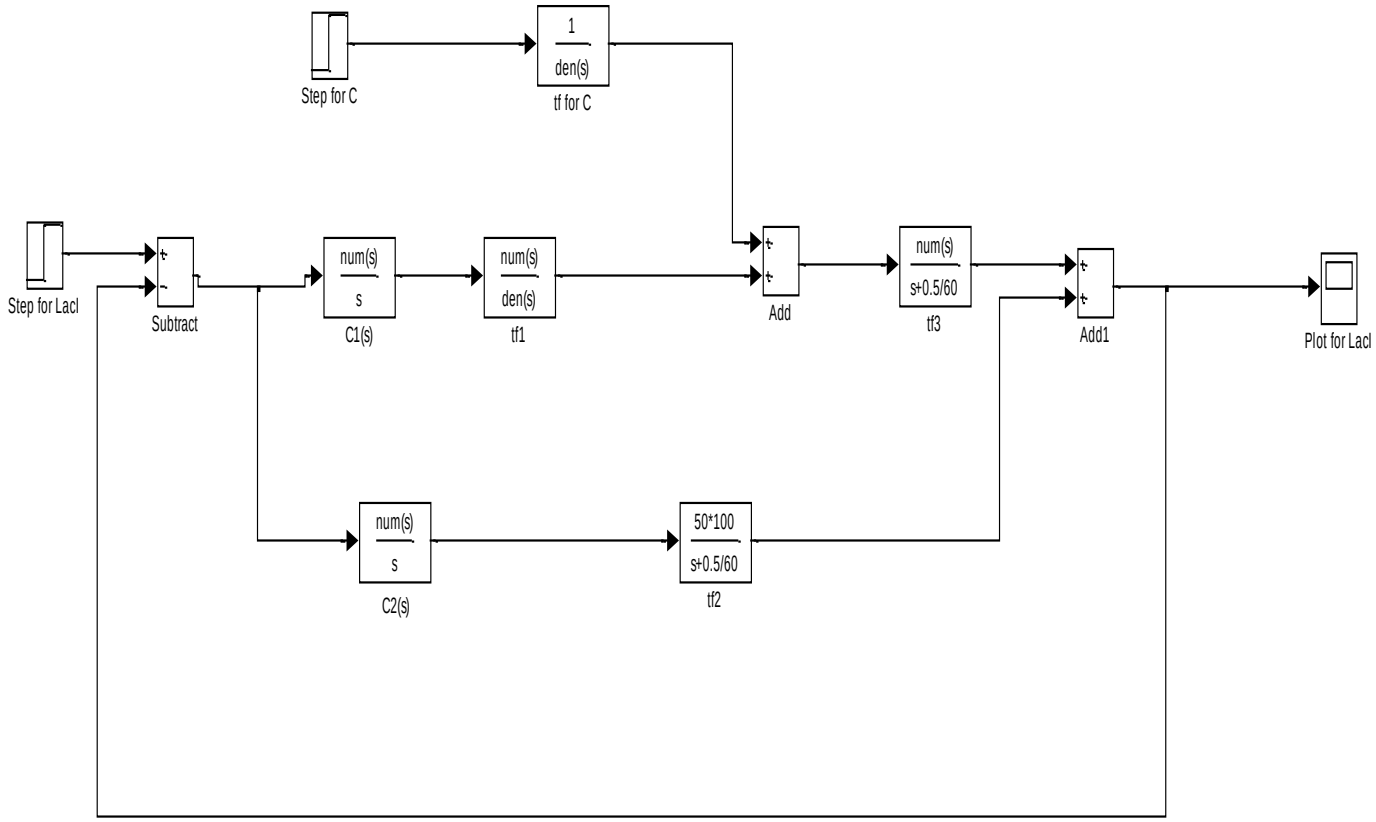


Fig2: Block representation of linearized LacI system. The system was linearized around the set-point (C_s=100, $LacI_s$=8380) using Taylor Series expansion. The system is then converted to Laplace domain and represented as shown above. The two controllers are of proportional-integral (PI) type and in the s-domain. We design the controllers by putting parameters as $K_i + I_i/s$. Ki = { 0.000035, 0.0000052}, Ii= { 0.00000028, 0.000000023}

We add noise in the system such that standard-deviation/steady-state value for both multiple-feedback system and open–loop system is the same. This characterizes same level of noise in both the systems. The noise at the LacI steady-state is characterized by standard-deviation/mean value of the LacI level at the output. For noise in replication of plasmid copy number, mean is 0, and variance is 10 for multiple feedback and 62.5 for open-loop systems respectively. For noise in production of plasmid copy number, mean is 0, and variance is 10 for multiple feedback and 18779 for open-loop systems respectively. The Simulink block diagram for external noise is as given below:

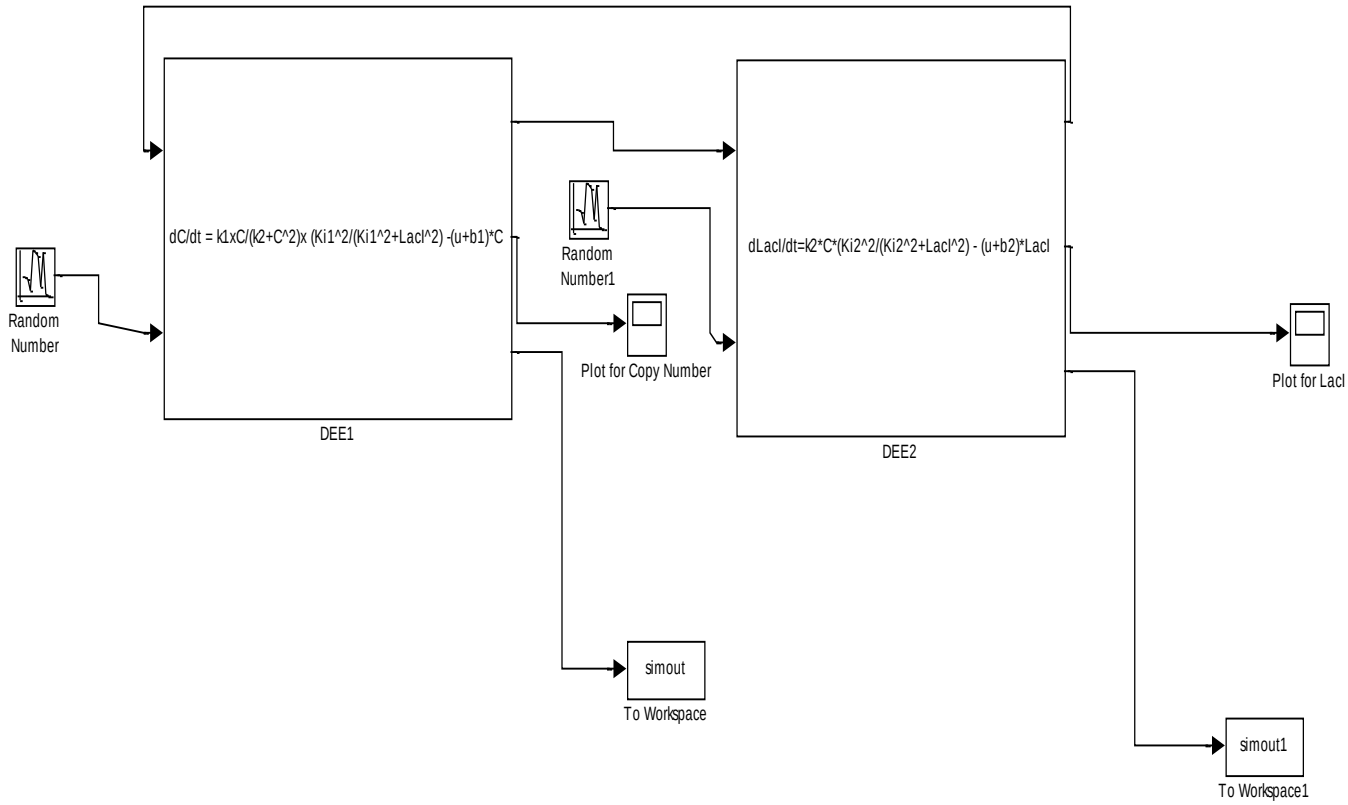
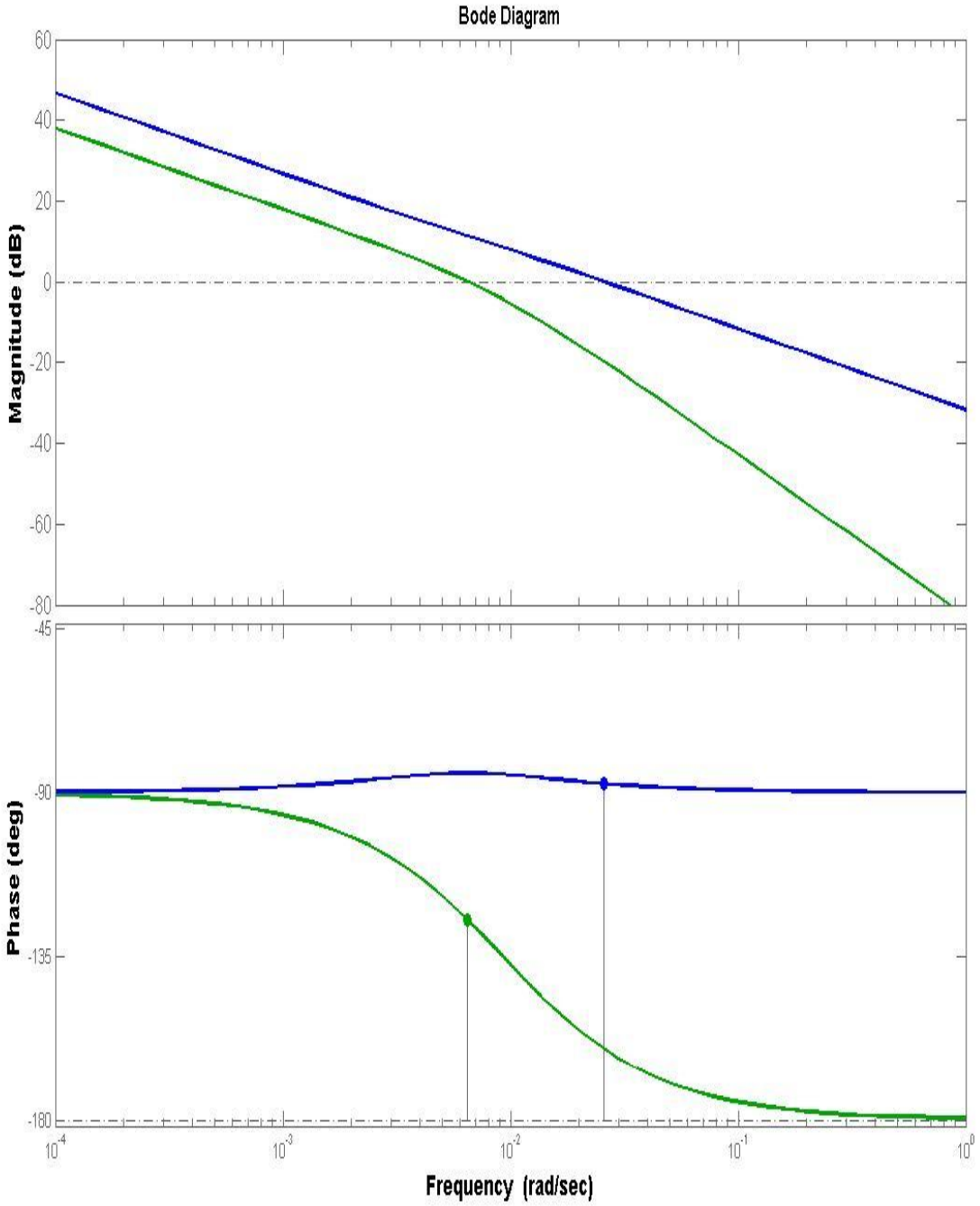


Fig 3: Simulink block model for LacI system with external noise. For noise in replication of plasmid copy number, mean is 0, and variance is 10 for multiple feedback and 62.5 for open-loop systems respectively. For noise in production of plasmid copy number, mean is 0, and variance is 10 for multiple feedback and 18779 for open-loop systems respectively.The standard-deviation/mean value of the LacI is used to characterize the noise at the output.

Results:

We plot the magnitude and phase Bode plots for the DFS and CFS for the system with no IPTG and see the effects of distributed, multiple feedbacks on the gain and phase margins.



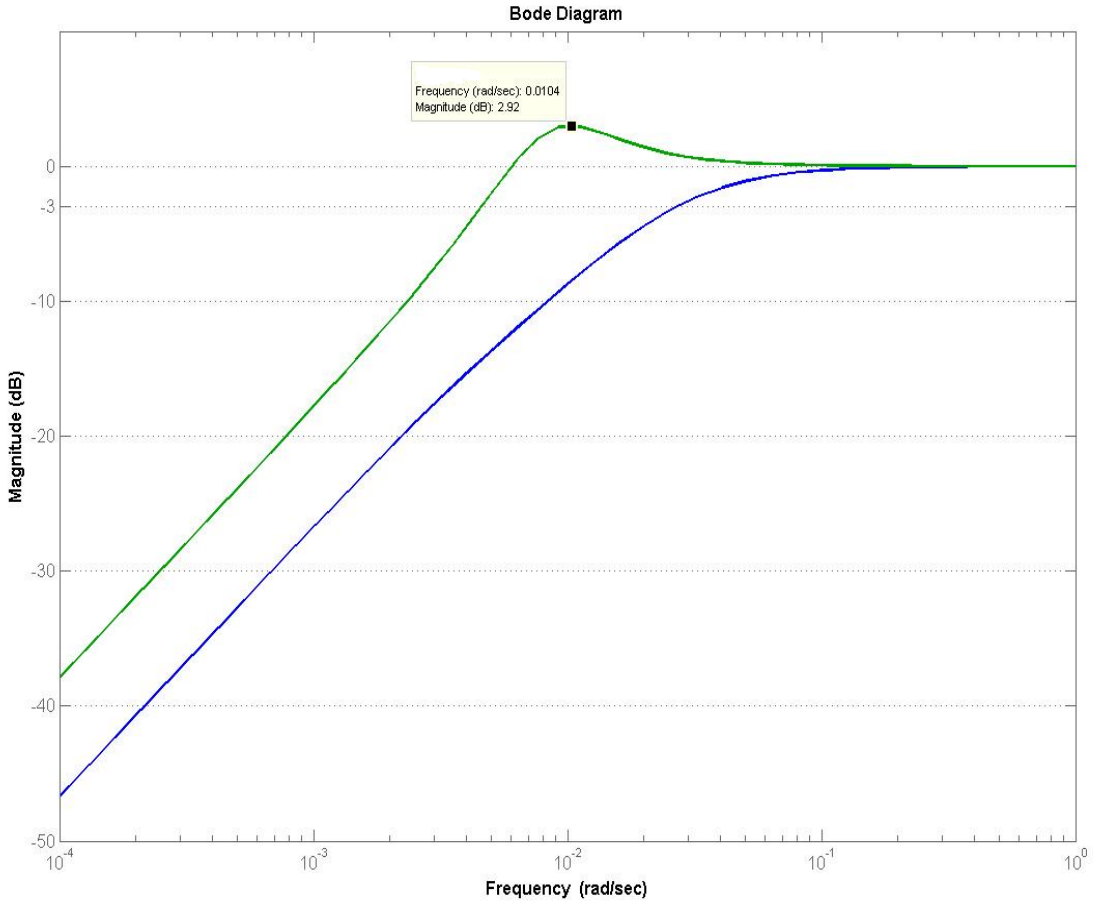
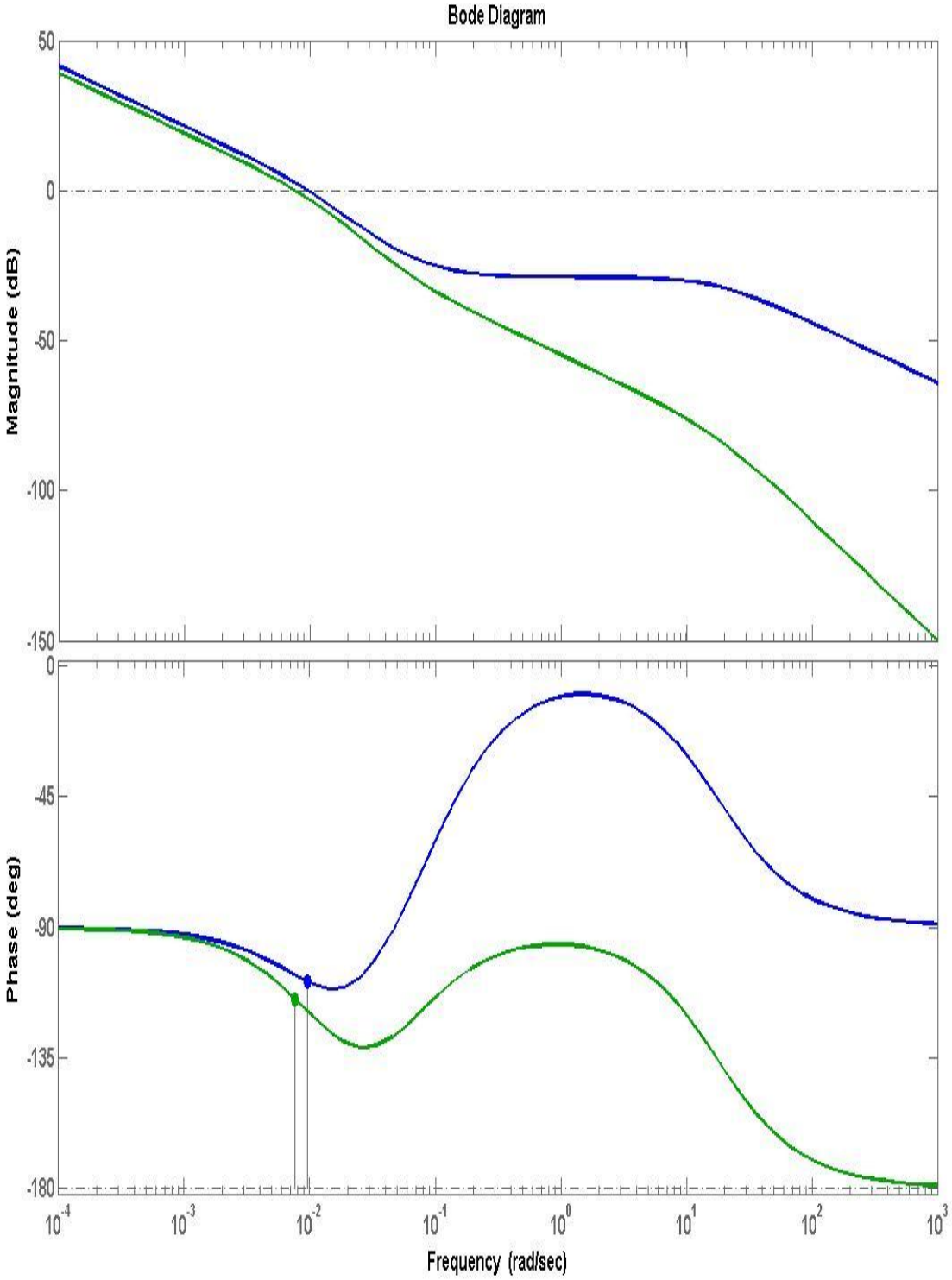


Fig 4: Magnitude, phase and sensitivity bode plots for LacI system given in linear model. The green line represents CFS with only $C_1(s)$, while blue line represents DFS with both $C_1(s)$ and $C_2(s)$. The gain margin for both CFS and DFS is ∞. The phase margin is 92.2° for DFS and 56° for CFS. The increased bandwidth from 0.00428 rad/min to 0.0255 rad/min indicates faster response and improved noise rejection. The CFS has higher peak of 2.92 dB while DFS has no peak, again indicating better noise-attentuation.


Bode Diagram
Magnitude (dB)
50
0
-50
-100
-150
Phase (deg)
0
-45
-90
-135
-180
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
Frequency (rad/sec)

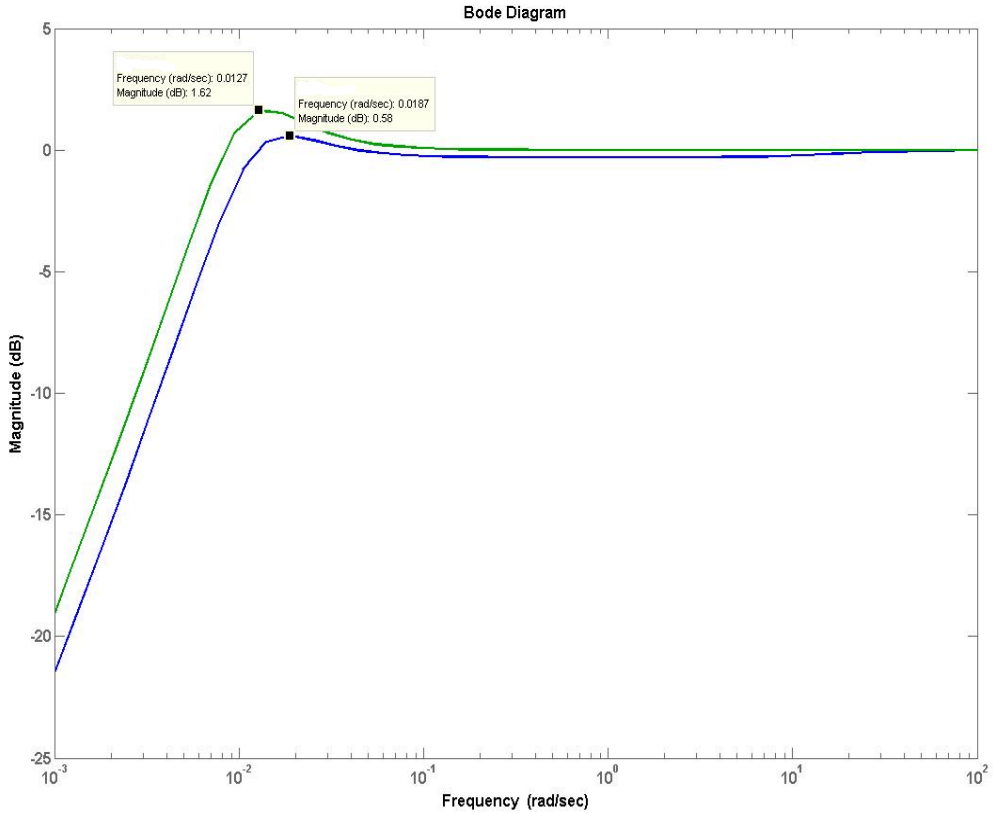


Fig 5: Magnitude, phase and sensitivity bode plots for LacI system with 1000 µM IPTG for linear model given in Fig 2. The green line represents CFS with only $C_1(s)$, while blue line represents DFS with both $C_1(s)$ and $C_2(s)$. The gain margin for both CFS and DFS is ∞. The phase margin is 70° for DFS and 64° for CFS. The bandwidth increase is not significant for DFS from 0.0061 rad/min to 0.0078 rad/min indicates hardly any difference in noise rejection. The CFS has higher peak of 1.62 dB while DFS has a peak at 0.58 dB indicating a lower peak and a slight better performance in noise attentuation.

As we can see the phase margin for the DFS is 92.2° when compared to 56° in the case of CFS. This indicates that the DFS can take care of delays in production LacI directly and by virtue of production of multiple plasmid copies better than the CFS which has regulation only on the plasmid copy number.

The sensitivity magnitude plot shows the sensitivity of the system for noise. The CFS has a peak at 2.92 dB or 1.4. The sensitivity over 0dB indicates noise makes the response of the system unstable. Sensitivity below 0dB indicates noise attenuation. DFS has its sensitivity below 0 dB indicating robust response and better noise rejection. The increased bandwidth from 0.00428 rad/min to 0.0255 rad/min indicates a faster response for DFS as compared to CFS. The increased bandwidth also shows better noise rejection over a wide range of frequencies and hence a far robust response as compared to CFS.

For high concentration of IPTG, 1000μM in the cell, we plot the magnitude and phase Bode plots for the DFS and CFS for the system with no IPTG and see the effects of distributed, multiple feedbacks on the gain and phase margins. As we can see the phase margin for DFS is 70^{o} and for CFS is 64^{o}. The difference in the phase margins for the DFS and CFS has reduced considerably indicating less advantage of DFS over CFS.

The CFS has a peak at 1.62dB while the DFS has a peak at 0.58 dB. The bandwidth for the CFS and DFS are 0.0061rad/min and 0.0078rad/min respectively. This is very less as compared to the DFS with no IPTG indicating more resemblance to open loop structure and less advantage of DFS.

Effect of External Noise on Multiple-Feedback System (strain 4) as compared to Open Loop System (strain 1):

Noise only in Replication of Copy Number:

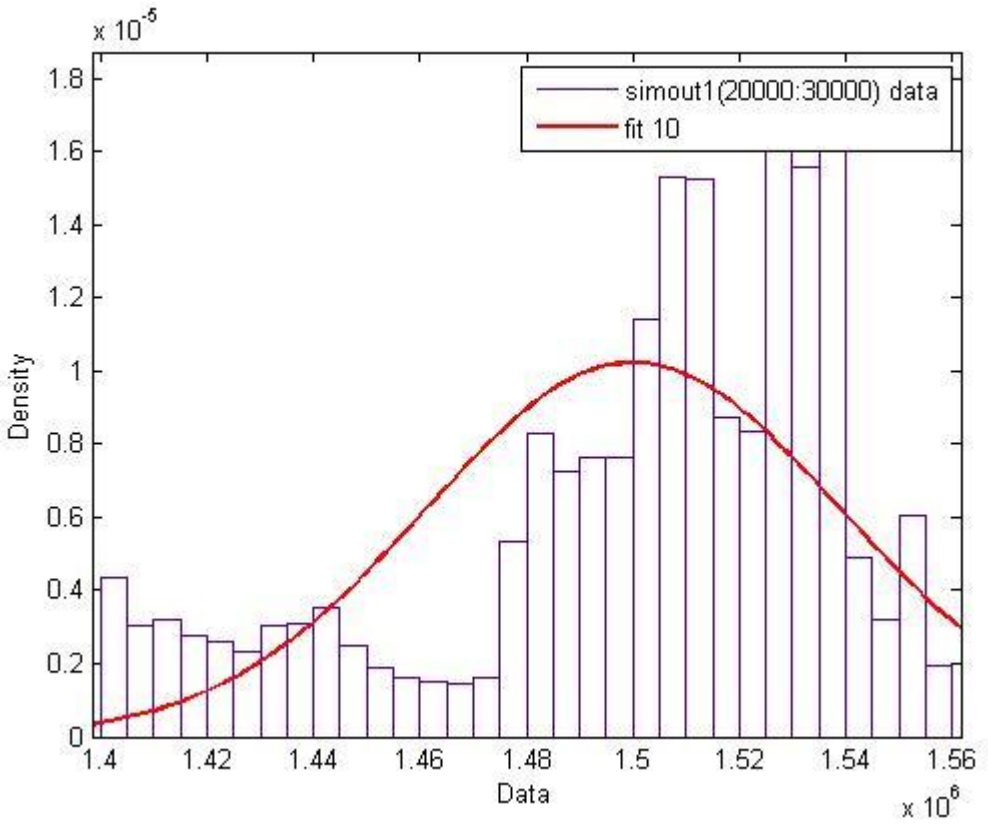


Fig 6: Fit of a normal distribution around steady-state for noise in replication of copy number for open loop system. The noise given is such that standard deviation/steady-state value for plasmid copy number is equal for open-loop and multiple-feedback systems. The noise given had a variance of 62.5. The normalized standard deviation is 0.0260 and the mean is 1.500e+08.

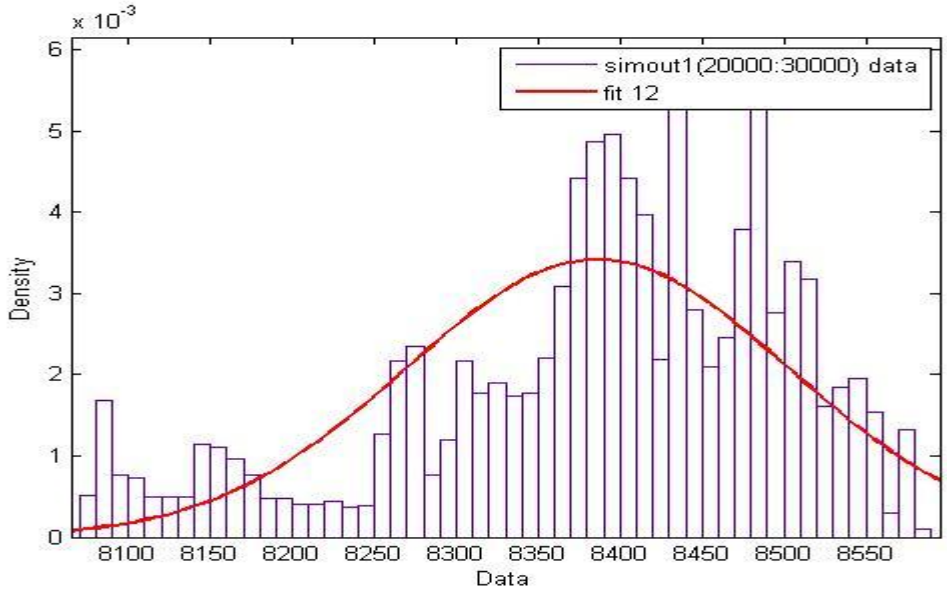


Fig 7: Fit of a normal distribution around steady-state for noise in replication of copy number for multiple-feedback system. The noise given is such that standard deviation/steady-state value for plasmid copy number is equal for open-loop and multiple-feedback systems. The noise given had a variance of 10. The normalized standard deviation is 0.0138 and the mean is 8386.16.

Noise only in Production of LacI:

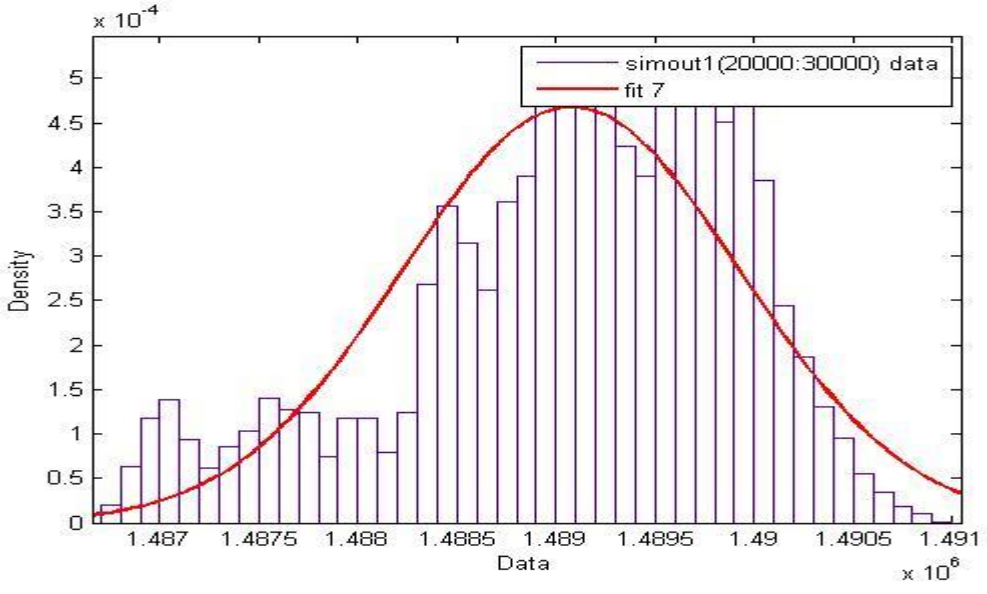


Fig 8: Fit of a normal distribution around steady-state for noise in production of LacI for open-loop system. The noise given is such that standard deviation/steady-state value for LacI production is equal for open-loop and multiple-feedback systems. The noise given had a variance of 10. The normalized standard deviation is 5.7262e-004 and the mean is 1.489e+08.

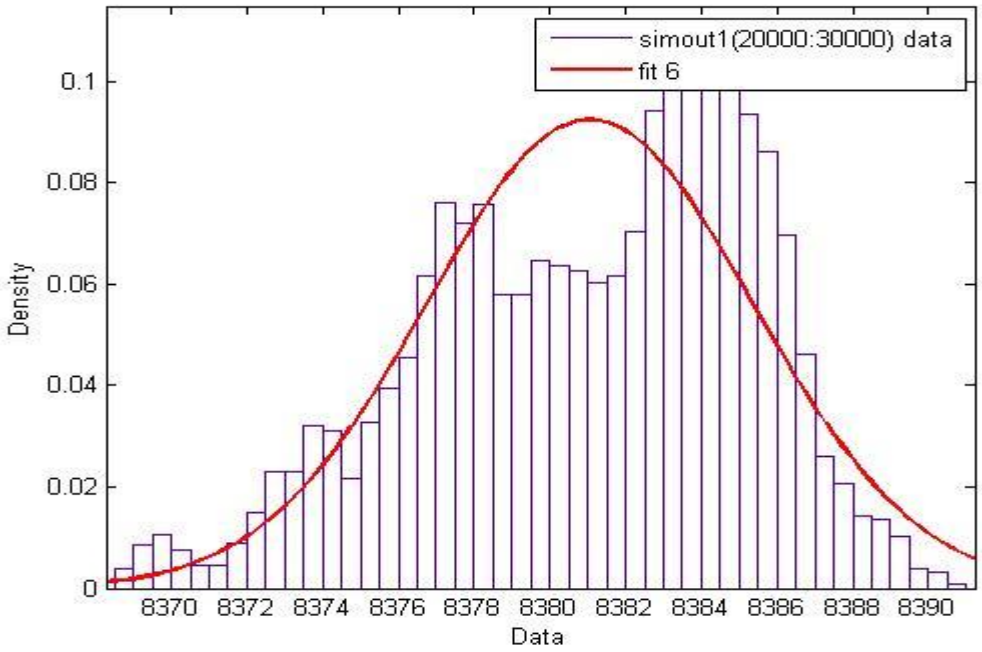


Fig 9: Fit of a normal distribution around steady-state for noise in production of LacI for multiple-feedback system. The noise given is such that standard deviation/steady-state value for LacI production is equal for open-loop and multiple-feedback systems. The noise given had a variance of 18779. The normalized standard deviation is 5.1499e-004and the mean is 8381.06.

Noise in Production of LacI and Replication of Copy Number:

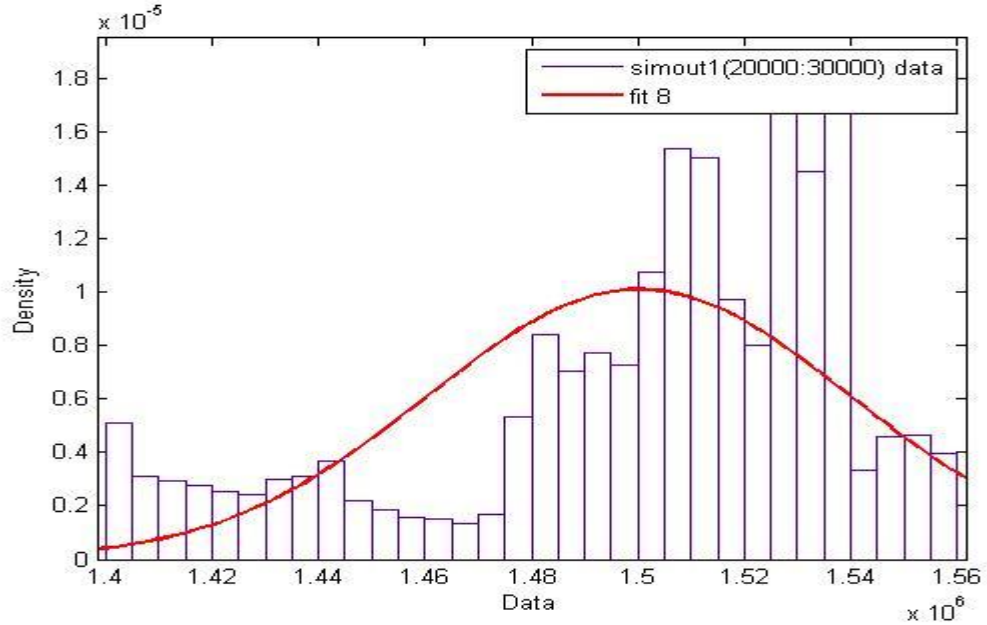


Fig 10: Fit of a normal distribution around steady-state for noise in production of LacI and replication of copy number for open-loop system. The noise given is such that standard deviation/steady-state value for LacI production is equal for open-loop and multiple-feedback systems. The noise given had a variance of 18779 for LacI production and 62.5 for copy number. The normalized standard deviation is 0.0263 and the mean is 1.50019e+08.

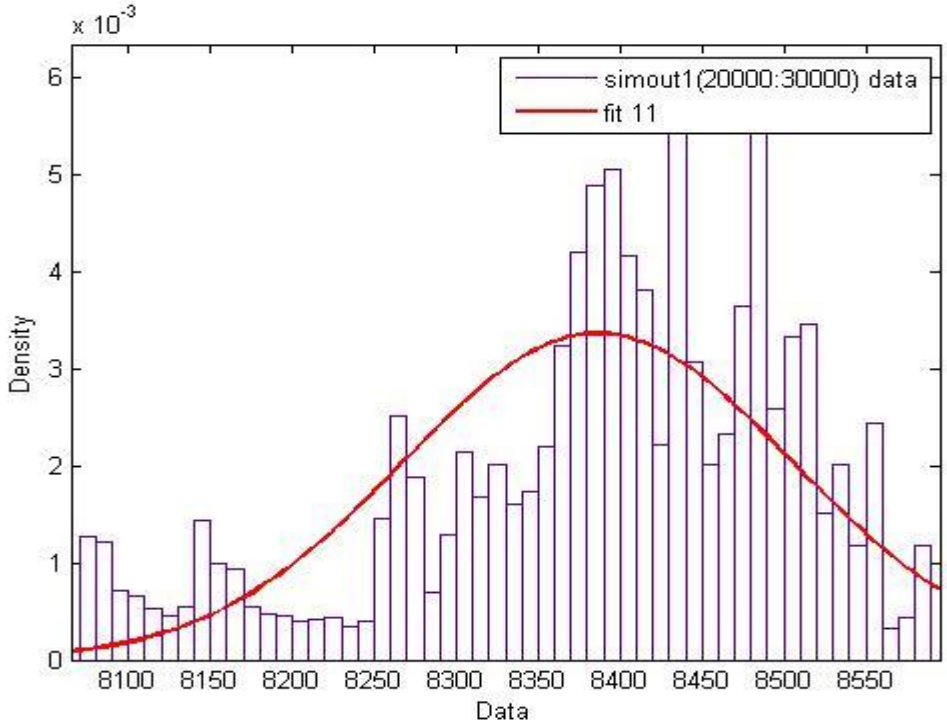


Fig 11: Fit of a normal distribution around steady-state for noise in production of LacI and replication of copy number for multiple-feedback system. The noise given is such that standard deviation/steady-state value for LacI production is equal for open-loop and multiple-feedback systems. The noise given had a variance of 10 for LacI production and 10 for copy number. The normalized standard deviation is 0.0141and the mean is 8386.45.

Conclusion:

Cellular functions require a cell to respond rapidly to changes in environment in and around the cell. Cells need to maintain concentrations of substances at certain level within the cell. Due to stochasticity in the processes, uncertain cellular environment and mutation, cells need a robust design, that comes through formation of regulatory networks. In nature, biological systems have been able to do so by developing multiple and distributed regulatory pathways with regulation at various levels.

In our engineered system, we try to analyse the robustness and speed of response of multiple and distributed feedback structure over single, conventional feedback structure.

In the phase Bode plots, we obtain greater phase margin for DFS, indicating better ability to handle time delays in the system. The bandwidth increase of around 6 times in the sensitivity plots directly translate to faster response and better noise rejection for the DFS as compared to CFS.

For the system with high IPTG concentration of 1000μM, the phase margin for the DFS reduces to 70^{o}. The bandwidth reduces to 0.0078 rad/min indicating less noise rejection capacity. Thus, the benefit of DFS over CFS has reduced for the system if more IPTG is added to the system.

In presence of external uncertainties, multiple feedback systems have more noise attenuation as compared to open-loop systems.

Thus, in our engineered system, we have been able to demonstrate by control theory approach that multiple, distributed feedback implies that the cell can respond rapidly to external or internal changes, and at the same time, is robust to uncertainties affecting the system due to internal or external reasons. The system responds better in presence of external uncertainties if there are multiple-distributed feedback in the system.

References

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3. Venkatesh, K.V., Bhartiya, S. And Ruhela, A. (2004). Multiple feedback loops are key to a robust dynamic performance of tryptophan regulation in *Escherichia coli*. *FEBS Letters 563 (2004) 234-240*.