1.a. Gram dry weight of an E.coli cell = 280×10^{-15} gDW/cell (BN100008) = $\langle m_c \rangle$

Number of cells/mL at OD600 = 10^8 cells/mL = N_c

Sample volume = 1mL = V

$$B = \langle m_c \rangle N_c V$$
$$= 280 \times 10^{-15} \times 10^8 \times 1$$
$$= 280 \times 10^{-7} \text{ gDW}$$

1. $$\langle n \rangle = 19 \frac{\text{mRNA}}{\text{cell}} = \frac{19}{6.022 \times 10^{23}} \frac{\text{mol mRNA}}{\text{cell}}$$

$$= \frac{19 \times 10^9}{6.022 \times 10^{23}} \frac{\text{nmol mRNA}}{\text{cell}} \times 10^8 \frac{\text{cell}}{\text{mL}} \times 1\text{mL}$$

$$= 3.155 \times 10^{-6} \text{ nmol mRNA}$$

$$\langle n \rangle = \frac{3.155 \times 10^{-6}}{280 \times 10^{-7}} \frac{\text{nmol mRNA}}{\text{gDW}} = 0.1126 \text{ nmol/gDW}$$

Using the same procedure all other values were converted.

$\langle n \rangle$ (mRNA/cell)	$\langle n \rangle$ (nmol/gDW)
19	0.112
21	0.124
41	0.243
67	0.397
86	0.510
93	0.551
93	0.551

1.(b) $\dot{m}_i = r_{x,i}\bar{u}_i - (\mu + \theta_{m,i})m_i$

At pseudo steady state $m_i = m_i^*$, $\dot{m}_i = 0$

$\Rightarrow -m_i^*(\mu + \theta_{m,i}) + r_{x,i}\bar{u}_i = 0$

$$\Rightarrow \boxed{m_i^* = \frac{r_{x,i}\bar{u}_i}{(\mu + \theta_{m,i})}} \quad -(1)$$

For a gene G_j, the kinetic limit is given by $r_{x,j}$

$$r_{x,j} = k_{E,j} R_{x,T}\left(\frac{G_j}{\tau_{x,j}K_{x,j} + (\tau_{x,j}+1)G_j}\right)$$ (From Lecture notes)

and $\bar{u}_i = \dfrac{W_1 + W_2 f_1}{1 + W_1 + W_2 f_1}$ where $f_1 = \dfrac{I^n}{K^n + I^n}$ (From lecture notes)

Substituting above eq^ns in (1) we get

$$\therefore m_j^* = \underbrace{\boxed{\frac{k_{E,j}R_{x,T}}{(\mu+\theta_{m,j})}\left(\frac{G_j}{\tau_{x,j}K_{x,j} + (\tau_{x,j}+1)G_j}\right)}}_{\text{Gain fn } K_x} \underbrace{\boxed{\left[\frac{W_1 + W_2\left(\frac{I^n}{K^n+I^n}\right)}{1 + W_1 + W_2\left(\frac{I^n}{K^n+I^n}\right)}\right]}}_{\bar{u}}$$

~~where~~

$$\Rightarrow \boxed{m_j^* = K_x(\theta, G_j)\,\bar{u}(I, K)}$$

where $K_x(\theta, G_j)$ is the gain function

$\bar{u}$ is the promoter function,

$\tau_{x,j} = \dfrac{k_{E,j}}{k_I}$ is the time constant, $K_{x,j} = \dfrac{k_-}{k_+}$ is the saturation constant

μ is the dilution/growth rate (h^{-1})

θ is the degradation constant. (h^{-1})

I is the inducer concentration (mM)

W_1, W_2 are weight factors.

W_1, W_2, n, K are predicted from the model to match experimental data.

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1.(d) When plotted it was observed that the model did not have the correct fit and shape initially.

The in vivo concentration of RNAP was used to calculate the gain function. However, to get close to the experimental data, the RNAP concentration needed to be increased from 30nM to 126 nM (in vitro).

This shows that higher mRNAP concentration in vitro leads to higher transcription rates.

Also n, W1, W2 and K influenced the shape of the curve.

Predicted values of $n = 1.5$, $W_1 = 0.26$, $W_2 = 190$, $K = 0.24\,mM$ resulted in a close fit to the graph.

2(a)

The dimensional form for the model is given by:

$$\boxed{\begin{aligned} \frac{d\tilde{X}}{d\tilde{t}} &= \frac{\tilde{\alpha}_x + \tilde{\beta}_x S}{1 + S + \left(\tilde{Z}/\tilde{Z}_x\right)^{n_{zx}}} - \tilde{\delta}_x \tilde{X} \\ \frac{d\tilde{Z}}{d\tilde{t}} &= \frac{\tilde{\alpha}_z}{1 + \left(\tilde{X}/\tilde{X}_z\right)^{n_{xz}}} - \tilde{\delta}_z \tilde{Z} \end{aligned}}$$

2.(b) Using the non-dimensional quantitities:

$$\delta_z = \frac{\tilde{\delta}_z}{\tilde{\delta}_x}, \quad \tilde{t} = \tilde{t}\tilde{\delta}_x, \quad \Rightarrow \quad d\tilde{t} = \tilde{\delta}_x dt$$

$$\alpha_x = \frac{\tilde{\alpha}_x}{\tilde{\alpha}_z}, \quad \beta_x = \frac{\tilde{\beta}_x}{\tilde{\alpha}_z}, \quad \beta$$

$$Z_x = \frac{\tilde{Z}_x \tilde{\delta}_x}{\tilde{\alpha}_z}, \quad X_z = \frac{\tilde{X}_z \tilde{\delta}_x}{\tilde{\alpha}_z},$$

$$X = \frac{\tilde{X}\tilde{\delta}_x}{\tilde{\alpha}_z}, \quad Z = \frac{\tilde{Z}\tilde{\delta}_x}{\tilde{\alpha}_z} \Rightarrow dX = d\tilde{X}\frac{\tilde{\delta}_x}{\tilde{\alpha}_z}, \; dZ = d\tilde{Z}\frac{\tilde{\delta}_x}{\tilde{\alpha}_z}$$

we get the non dimensional equations:

$$\frac{\tilde{\delta}_x \tilde{\alpha}_z \cancel{\tilde{\delta}_x}}{\cancel{\tilde{\delta}_x}\cancel{\tilde{\alpha}_z}\cancel{\tilde{\alpha}_z}}\frac{dX}{dt} = \frac{\tilde{\alpha}_x + \tilde{\beta}_x S}{1 + S + \left(\frac{Z}{Z_x}\right)^{n_{zx}}} - \tilde{X}\tilde{\delta}_x$$

$$\Rightarrow \frac{dX}{dt} = \frac{1}{\tilde{\alpha}_z}\left[\frac{\tilde{\alpha}_x + \tilde{\beta}_x S}{1 + S + \left(Z/Z_x\right)^{n_{zx}}}\right] - \frac{\tilde{X}\tilde{\delta}_x}{\tilde{\alpha}_z}$$

$$\boxed{\frac{dX}{dt} = \frac{\alpha_x + \beta_x S}{1 + S + \left(Z/Z_x\right)^{n_{zx}}} - X}$$

Non dimensional

$||^{ly}$ for $$\frac{d\tilde{Z}}{d\tilde{t}} = \frac{\tilde{\alpha}_z}{1 + \left(X/X_z\right)^{n_{xz}}} - \tilde{\delta}_z \tilde{Z}$$

$$\Rightarrow \frac{\tilde{\alpha}_z \tilde{\delta}_x}{\tilde{\delta}_x}\frac{dZ}{dt} = \frac{\tilde{\alpha}_z}{1 + \left(X/X_z\right)^{n_{xz}}} - \tilde{\delta}_z \tilde{Z}$$

$$\Rightarrow \frac{dZ}{dt} = \frac{1}{\tilde{\alpha}_z}\left[\frac{\tilde{\alpha}_z}{1 + \left(X/X_z\right)^{n_{xz}}}\right] - \frac{\tilde{\delta}_z \tilde{\delta}_x \tilde{Z}}{\tilde{\alpha}_z \tilde{\delta}_x}$$

$$\Rightarrow \boxed{\frac{dZ}{dt} = \frac{1}{1 + \left(X/X_z\right)^{n_{xz}}} - \delta_z Z}$$

Non-dimensional

The small error is in eq^n (3). The RHS of ~~$t = \tilde{t}\delta_y$~~ $t = \tilde{t}\tilde{\delta_x}$ was missing a tilde over δ_x.

$\boxed{2.(c)}$ The oscillations are ~~coherent~~ incoherent for point selected below the hopf bifurcation point at $S = 0.4$. Steady state values at this point are

$X = 0.0012$
$Y = 0.6$
$Z = 0.0004$.

For the point above the saddle node bifurcation, the oscillations are coherent at $S = 35500$. Steady state values obtained were

$X = 5.5$
$Y = 0.01$
$Z = 0.00049$.

It is observed that the oscillations when moving from a region below the hopf point into a region above the hopf point are incoherent. This is the result of difference in initial gene expression. The oscillations originating from the hopf bifurcation start close to an unstable spiral center. As the oscillations pass the hopf bifurcation small differences in the oscillations (initial) are amplified by the unstable spiral centre and final oscillations result in lack of coherence. This instability can be observed in the X, Y, Z vs t diagrams where as S is increased, the oscillations become unsteady.

Below the saddle bifurcation node the oscillations pass into the limit cycle but are not associated with the attractive oscillatory regime i.e. the oscillations do not enter the limit cycle undergoing a large change in phase.

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This leads to coherent oscillations below the saddle node bifurcation. Moreover, it can be noticed from X, Y, Z vs t diagrams just below the saddle point, that the oscillations have constant amplitude, decrease which leads to coherent oscillations below saddle node.

Both these observations can be observed in the X vs t graphs.

2(f) This isn't possible from the parameter values mentioned in Table S.1 because, both 100 and 105 lie in a region b/w the hopf bifurcation and saddle node bifurcation. At both these values, oscillations are observed with varying amplitude which indicates they lie more towards the hopf bifurcation point. Moreover, from the discussion in the paper, a homoclinic bifurcation exists ~~in~~ this region which leads to unstable oscillations. Both these factors lead to incoherent oscillations when S is changed from 105 to 100 and a saddle bifurcation node is not present at S=105 for the given parameters.

2(c) The curves in figure 1b are qualitatively reproducible. It was observed that ~~at a~~ at higher values of S the X values approach 5.

When unstable steady states were plotted, it was observed that multiple points were present at similar values of S, corroborating presence of bistability.