1a.

1a) Convert $\langle n \rangle$ to $B = \langle m_c \rangle \hat{N}_c V$

sample size = 1 mL

OD = 0.1

Given that OD 0.1 corresponds to 1×10^8 $\frac{\text{cells}}{\text{mL}}$

$\langle m_c \rangle$ has units $\frac{\text{gDW}}{\text{cell}}$

$\hat{N}_c \rightarrow \frac{\#\text{ of cells}}{\text{mL}}$

$V \rightarrow$ mL

$V = 1$ mL

$\hat{N}_c = 1 \times 10^8$ $\frac{\text{cells}}{\text{mL}}$ due to OD of 0.1

To find $\langle m_c \rangle$ find percentage of mRNA that lacZ mRNA ($\langle n \rangle$) makes up. Find mRNA dry cell mass.

* Using BioNumbers Reference 100064 taken from Neidhart, F.C. (1996) a table containing data for E. Coli B/r cells with a doubling time of 40 min. is given. This matches description of problem well enough to use data.

* Assumption: Neidhart's data matches closely enough to use

Given is the information

1,380 mRNA molecules are in a cell

mRNA is 2.3×10^{-15} $\frac{\text{gDW}}{\text{cell}}$

$\langle m_c \rangle = \frac{\langle n \rangle}{\text{mRNA copies}} \cdot \text{mRNA} \frac{\text{gDW}}{\text{cell}}$

$\langle m_c \rangle = \frac{\langle n \rangle}{1{,}380} \cdot (2.3 \cdot 10^{-15})$

(Table 1)

IPTG	$\langle n \rangle$	B (gDW)
0.0	19	3.167×10^{-9}
5e-4	21	3.5×10^{-9}
0.005	41	6.83×10^{-9}
0.012	67	1.12×10^{-8}
0.053	86	1.43×10^{-8}
0.216	93	1.55×10^{-8}
1.0	93	1.55×10^{-8}

1b.

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

@ psuedo steady state $\dot{m} = 0$ $\quad m_i = m^*$

rearrange to get

$$m^* = \frac{r_{x,i}\,\bar{u}_i}{\mu+\theta}$$

From Lecture 2 Deterministic Mathematical Models of ... given a simple reaction scheme to solve for r_x

Assume that transcription can be modeled by

$$G_j + R_x \rightleftharpoons (G_j : R_x)_c$$
$$(G_j : R_x)_c \longrightarrow (G_j : R_x)_o$$
$$(G_j : R_x)_o \longrightarrow R_x + G_j$$
$$(G_j : R_x)_o \longrightarrow m_j + R_x + G_j$$

let $r_x = k_E^x (G_j : R_x)_o$

and $R_{x,T} = R_x + (G_j : R_x)_c + (G_j : R_x)_o$

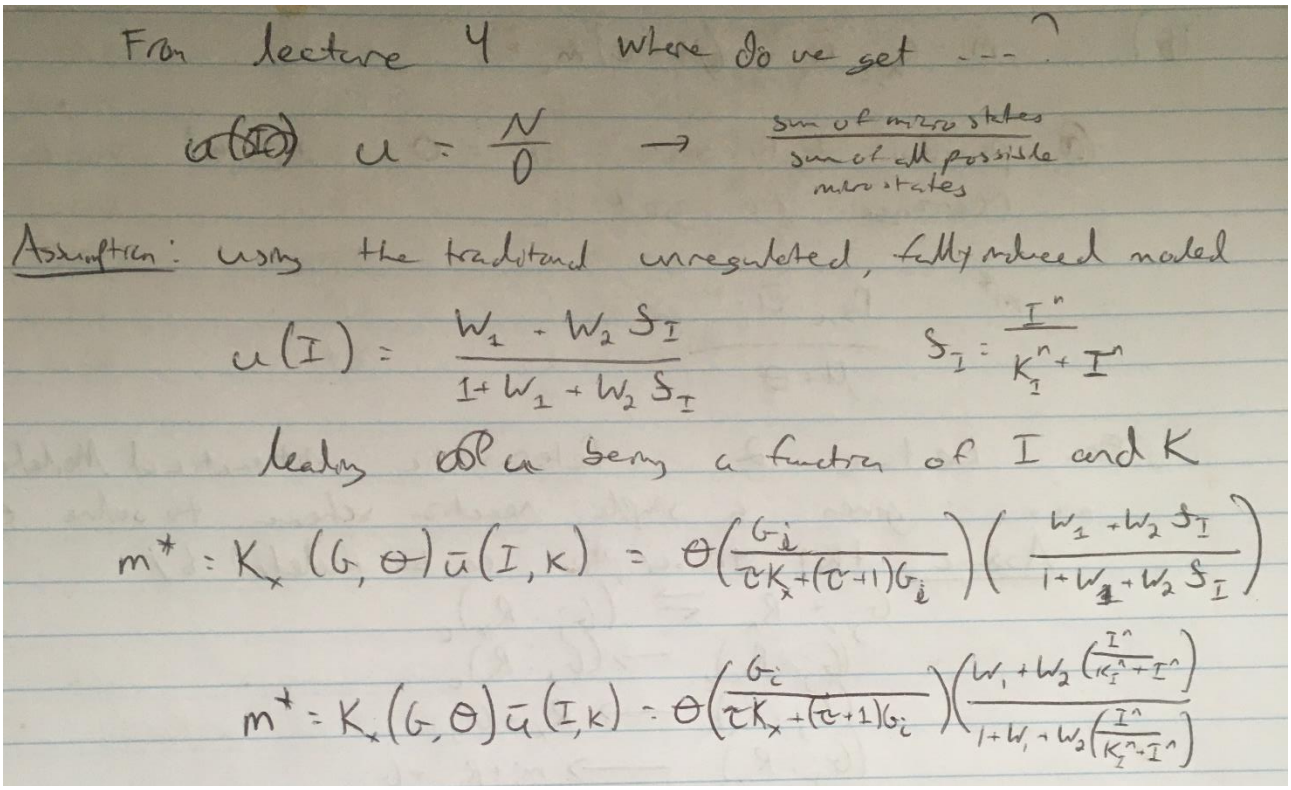
Assuming steady state and solving all rate equations with rate kinetic constants results in

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

let $K_x = \frac{r_{x,i}}{\mu+\theta}$

with $\theta = \frac{k_{E,i}^x \cdot R_{x,T}}{\mu+\theta}$

$$K_x = \theta \left(\frac{G_j}{\tau_{x,j} K_{x,j} + (\tau_{x,j}+1) G_j} \right)$$



1d.

Parameter	Value	Source
ke	39 nuc/sec	Garamella et al ACS Synth Biol 5:344-55 2016
L (length)	1000 nuc	Given
ke_star	ke/L = .039*3600 = 1,404 1/hr	Calculated using Formula from Lecture 2 packet
R_polymerase (amount of polymerase in E.coli)	5000	Bionumbers ID: 101440 Click the link to the table
mass_polymerase	4.8e5 Da	Bionumbers ID: 104925
R_T [gDW-1e8cells]	R_polymerase*mass_polymerase* 1.66054e-24 * 1e8 = 3.99e-7	Calculated
Gene_copy_number	2 copies/cell	Given
G [uMol of gene]	Gene_copy_number/Avogadro's # * 1e6/(4.7e-16)	Dimensional analysis
Initiation_time	22.0 s	Taken from CellFree.jsol. PS2 solution. (I know it's cell

		free but couldn't find anything better)
k_I [1/hr]	1/Initiation_time*3600 = 163.6	Used formula in Kinetics.jl of PS2
tau []	ke_star/k_I = 8.58	Assumed that ka was negligible and calculated tau as shown in Lecture 2 packet
Transcription_Saturation	0.0136 [uMol]	Taken from CellFree.jsol. PS2 solution. (I know it's cell free but couldn't find anything better)
half_life	5 minutes	Given
deg (degradation constant)	1/half_life/60*log(2)= 8.32 (1/hr)	Used formula in Kinetics.jl of PS2
Double_time	40 minutes	Given
Dilution	Log(2)/double_time/60 = 1.04 (1/hr)	Used formula in Kinetics.jl of PS2
W1 (first scalar of inhibitor, leaky expression)	0.0018616516387985468	Solved for W1 when I = 0
W2 (second scalar of inhibitor, inhibitor present)	0.0073171957725891405	Solved for W2 when I = 1000 uM so can assume all inhibitor was bound
n (hill coefficient)	2.0	Kuhlman T, Zhang Z, Saier MH Jr, Hwa T. Combinatorial transcriptional control of the lactose operon of Escherichia coli. *Proc Natl Acad Sci U S A*. 2007;104(14):6043-6048. doi:10.1073/pnas.0606717104
I [uM]	0.0000001, 0.5, 5.0, 12.0, 53.0, 216.0, 1000.0	Given and converted into uM. 0.0000001 used instead of 0 so that it could be plotted on semilog
z (copies of lac Z)	19,21,41,67,86,93,93	Given
Mc	3.166666666666669e-9, 3.500000000000003e-9, 6.833333333333339e-9, 1.1166666666666676e-8, 1.4333333333333344e-8, 1.5500000000000013e-8, 1.5500000000000013e-8	Conversion of z to B. Shown in part 1a with citation.
K_inhibitor [uM]	8	Found by plotting the calculated values against the actual values and modifying

		till best visual fit was achieved. (ie. A guess)

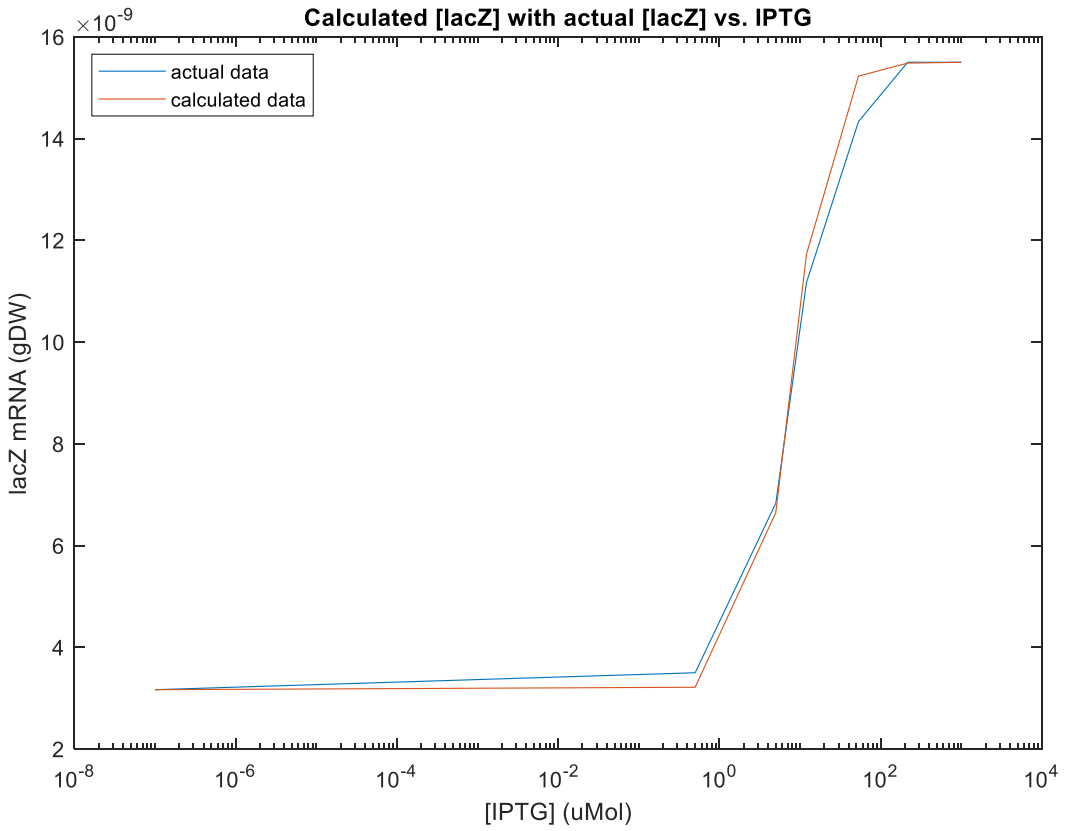
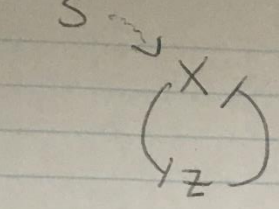


Figure 1: Plot of actual data compared to the calculated data in this model. A semilogx graph is utilized. The actual data and calculated data are very similar.

The model does fit and has the correct shape. To have an even better fit the hill coefficient and the inhibitor saturation constant can both be tweaked. 1d.jl and one_d.m were utilized to produce the results above.

2a.

2a)



This system is similar to that in Gardner et al., 2000, with the dimensionless form

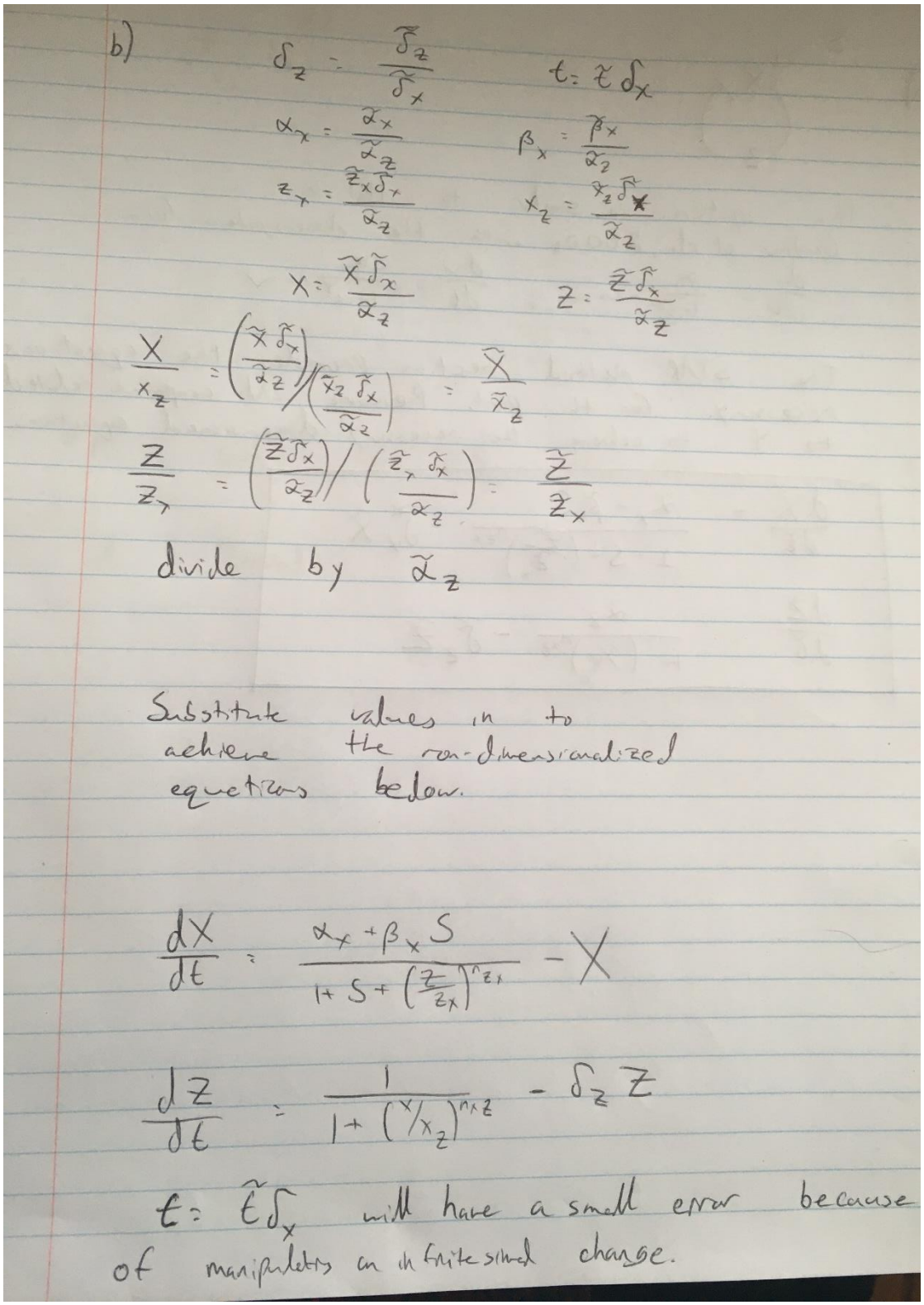
$$\frac{du}{dt} = \frac{\alpha_1}{1+v^{\beta}} - u \qquad \frac{dv}{dt} = \frac{\alpha_2}{1+u^{\gamma}} - v$$

The STAR Method section provides the equations necessary for this part. Remove all components related to Y to achieve the necessary dimensioned equations

$$\frac{d\tilde{X}}{d\tilde{t}} = \frac{\tilde{\alpha}_X + \tilde{\beta}_X S}{1 + S + \left(\frac{\tilde{Z}}{\tilde{Z}_X}\right)^{n_{ZX}}} - \tilde{\delta}_X \tilde{X}$$

$$\frac{d\tilde{Z}}{d\tilde{t}} = \frac{\tilde{\alpha}_Z}{1 + \left(\frac{\tilde{X}}{\tilde{X}_Z}\right)^{n_{XZ}}} - \tilde{\delta}_Z \tilde{Z}$$

2b.


b)
$\delta_z = \frac{\tilde{\delta}_z}{\tilde{\delta}_x}$
$t = \tilde{t}\,\delta_x$
$\alpha_x = \frac{\tilde{\alpha}_x}{\tilde{\alpha}_z}$
$\beta_x = \frac{\tilde{\beta}_x}{\tilde{\alpha}_z}$
$z_x = \frac{\tilde{z}_x \tilde{\delta}_x}{\tilde{\alpha}_z}$
$x_z = \frac{\tilde{x}_z \tilde{\delta}_x}{\tilde{\alpha}_z}$
$X = \frac{\tilde{X}\tilde{\delta}_x}{\tilde{\alpha}_z}$
$Z = \frac{\tilde{Z}\tilde{\delta}_x}{\tilde{\alpha}_z}$
$\frac{X}{x_z} = \left(\frac{\tilde{X}\tilde{\delta}_x}{\tilde{\alpha}_z}\right) \Big/ \left(\frac{\tilde{x}_z \tilde{\delta}_x}{\tilde{\alpha}_z}\right) = \frac{\tilde{X}}{\tilde{x}_z}$
$\frac{Z}{z_x} = \left(\frac{\tilde{Z}\tilde{\delta}_x}{\tilde{\alpha}_z}\right) \Big/ \left(\frac{\tilde{z}_x \tilde{\delta}_x}{\tilde{\alpha}_z}\right) = \frac{\tilde{Z}}{\tilde{z}_x}$
divide by $\tilde{\alpha}_z$
Substitute values in to achieve the non-dimensionalized equations below.
$\frac{dX}{dt} = \frac{\alpha_x + \beta_x S}{1 + S + \left(\frac{Z}{z_x}\right)^{n_{zx}}} - X$
$\frac{dZ}{dt} = \frac{1}{1 + \left(X/x_z\right)^{n_{xz}}} - \delta_z Z$
$t = \tilde{t}\,\delta_x$ will have a small error because of manipulations on infinitesimal change.

2c.

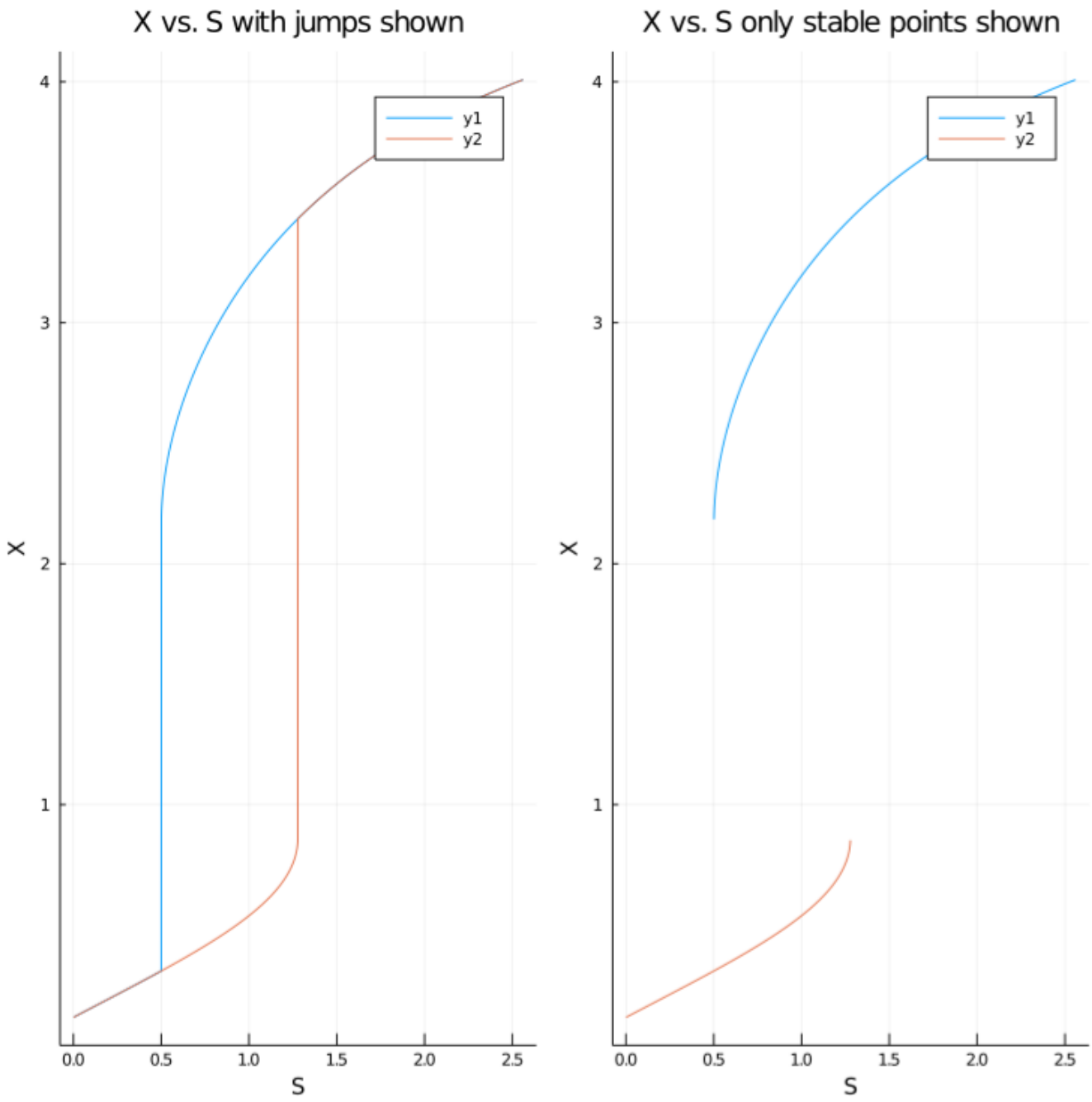


Figure 2: Left: Plot with initial conditions of (8.0, 0.0) and (0.0, 8.0) capturing the edge values that produce a three fixed-point system. Indicates that values between S = 0.5 and S = 1.28 contain an unstable fixed point. **Right**: A plot of all stable points.

The figure shown in 1b can be somewhat reproduced. There is a clear region where there is an unstable fixed point, and 2 stable fixed points, flanked by regions with only 1 stable fixed point (Figure 2, left). The differences between the two are when the instability occurs and the length of the instability. My graphs show an instability that occurs at a low S value and lasts only for a short duration, from S = 0.5 to S = about 1.28 (Figure 2, right). The figures in the paper show the instability occur at a seemingly much higher S value and last for a longer duration. However, these differences could be due to the x-axis scale used.

2d.

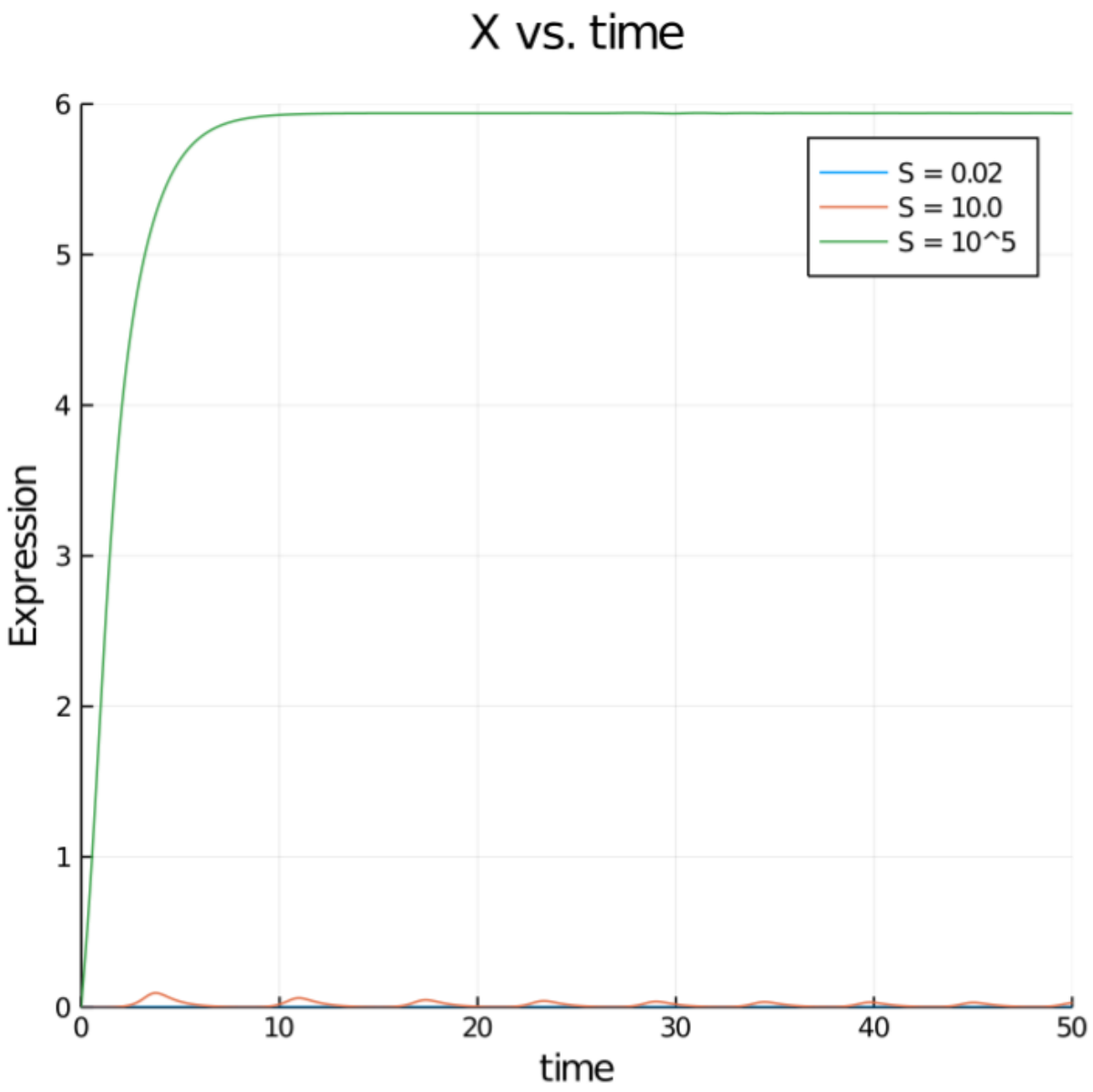


Figure 3: Plot of X-values of the AC-DC circuit wiht varying values of S. This variation influences whetehr there is a steady state or an oscillatory state.

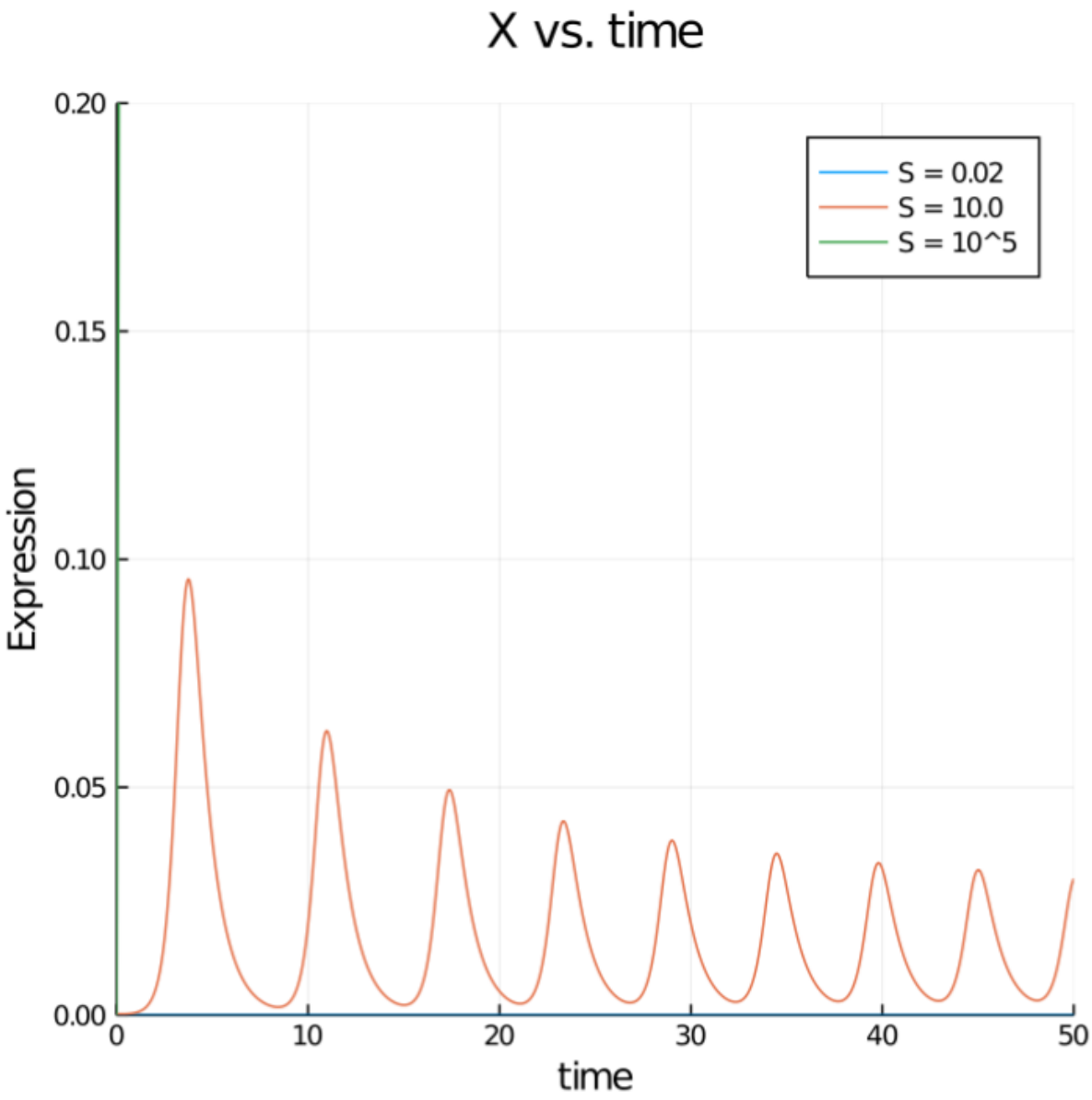


Figure 4: Zoomed in figure of Figure 3 to better show the oscillations occurring to X at S = 10.0

2e.

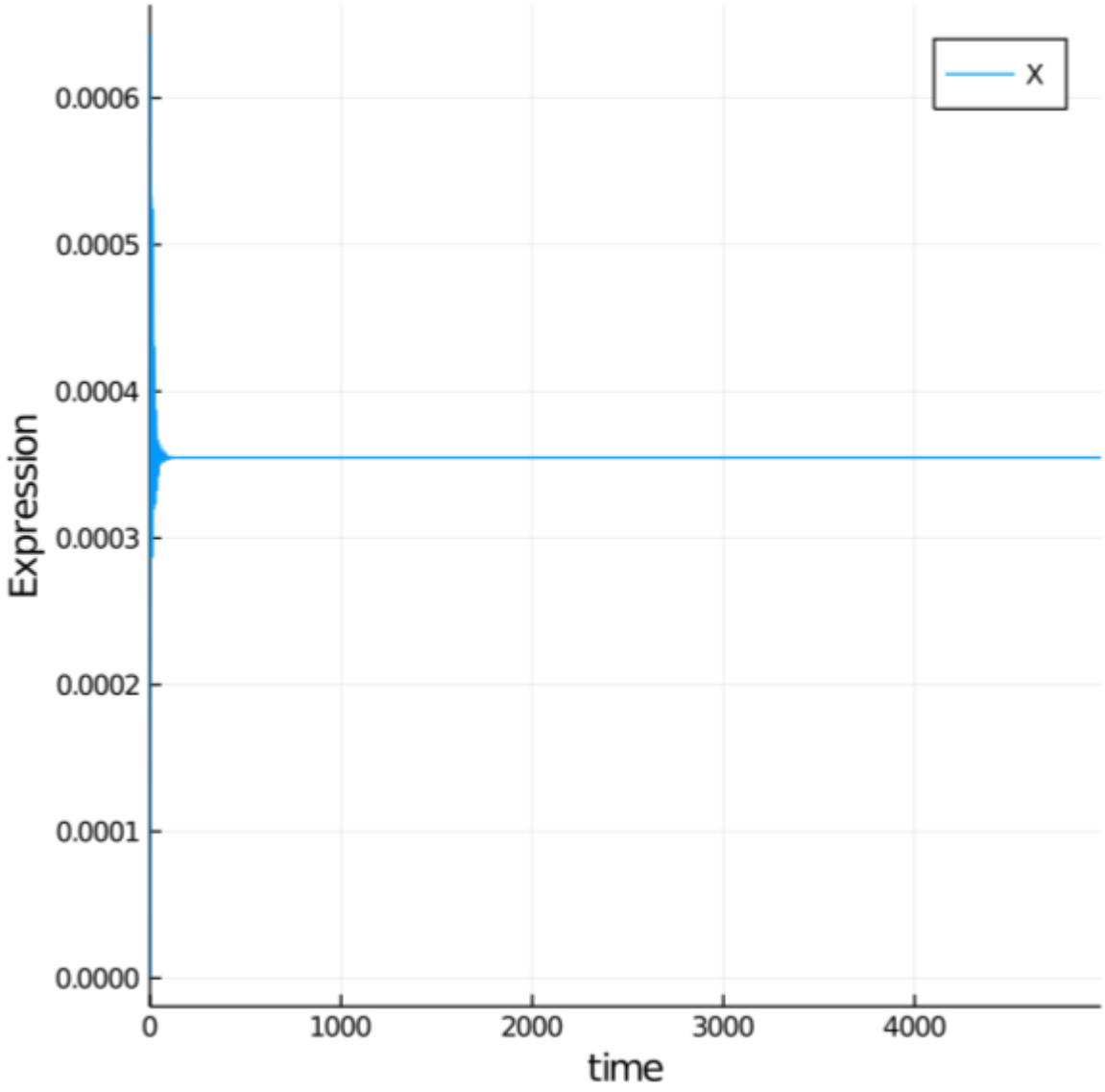


Figure 5: Plot of X over time with S = 0.5. This is near the Hopf bifurcation point, but still stable as the line converges and oscillations cease.

For S = 0.6 it is difficult to see oscillations in the plot but if you look at outputted data there are clearly oscillations occurring.

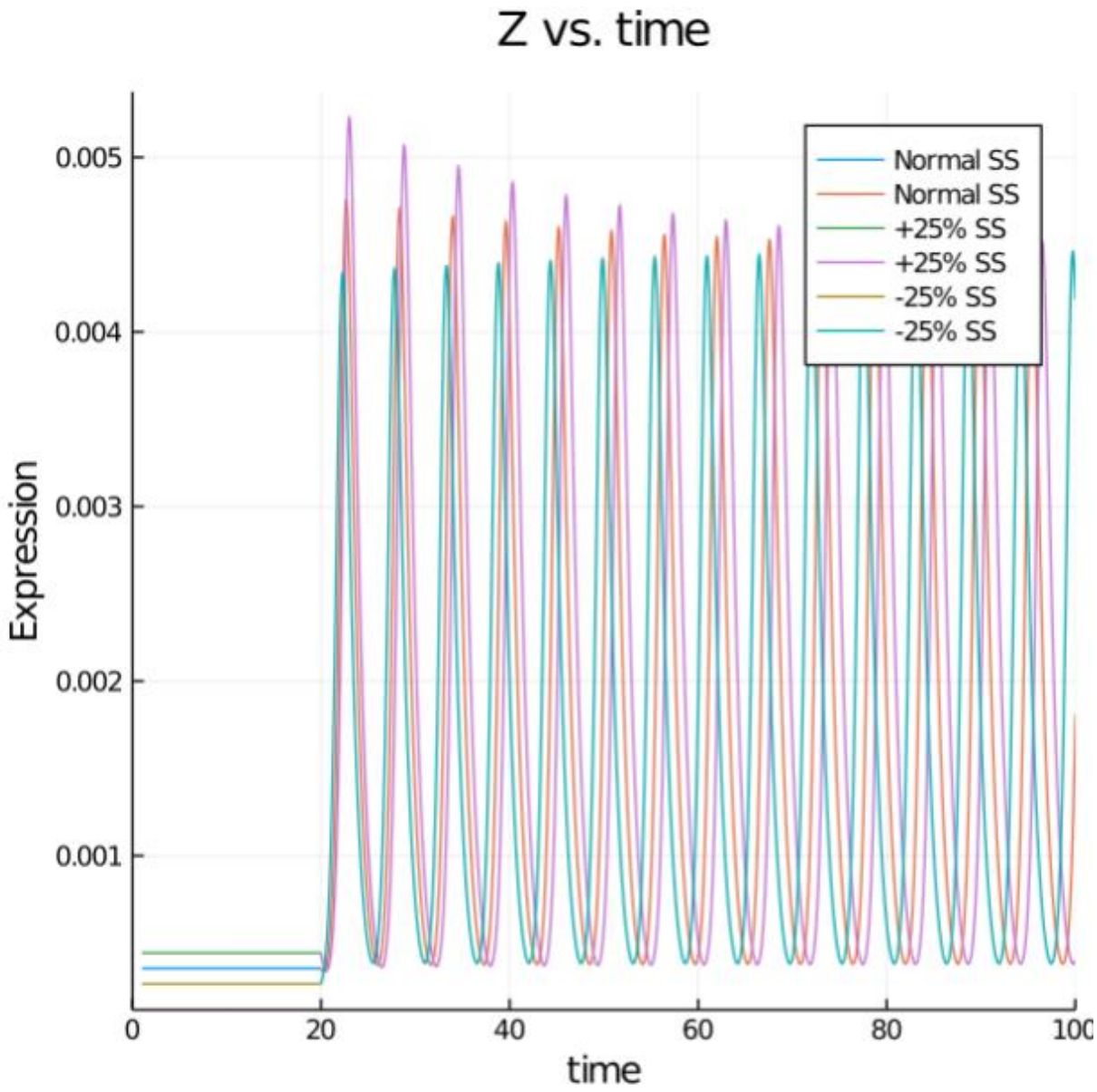


Figure 6: Plot of X over time with S = 0.5 initially and then changing to S =100. Passing through the Hopf bifurcation point leads to incoherent oscillations.

An initial S value of 0.5 was used with steady state value of X,Y,Z = [0.001437877053527658, 0.5724866675720363, 0.00035486318379280115]. The oscillations exhibit some incoherence as they do drift slowly out of phase with one another. This can best be seen if you look at the valleys of the oscillations. You can observe that the distance between the lowest points are slowly increasing.

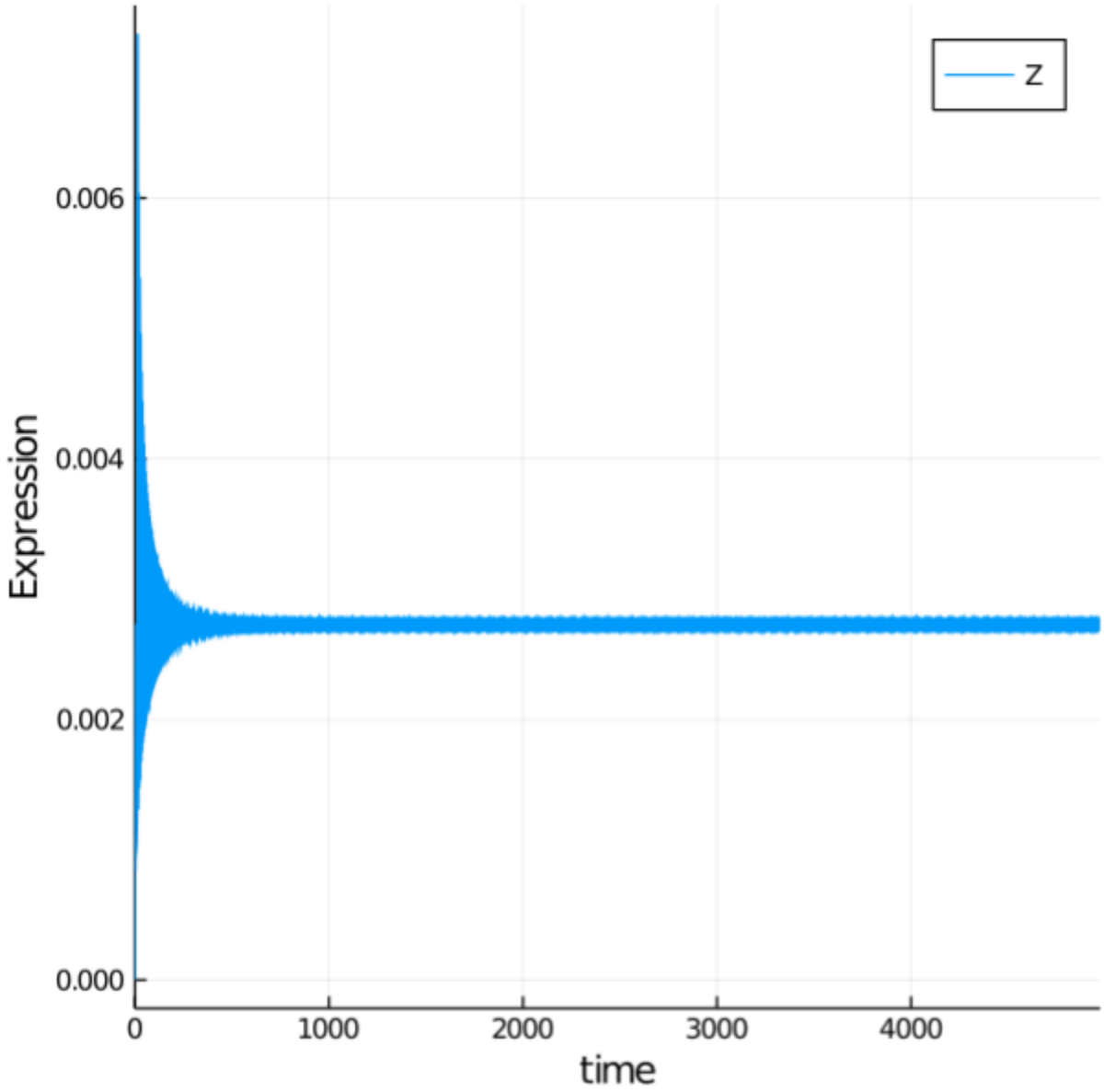


Figure 7: Plot of X over time with S = 35,000. This is near the saddle point point, producing an oscillatory state.

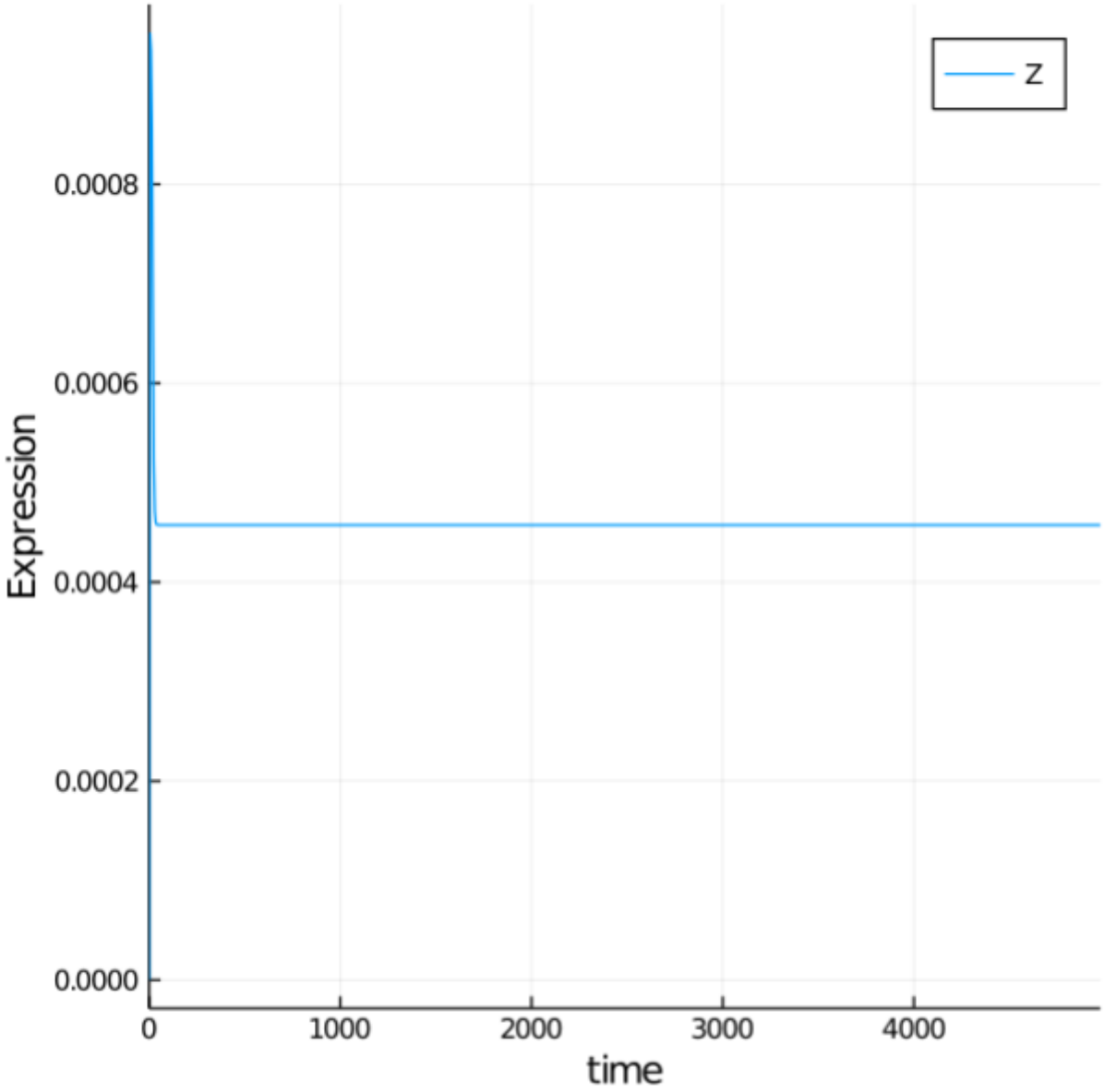


Figure 8: Plot of X over time with S = 35,500. This is near the saddle point bifurcation point, but still stable as the line converges and oscillations cease.

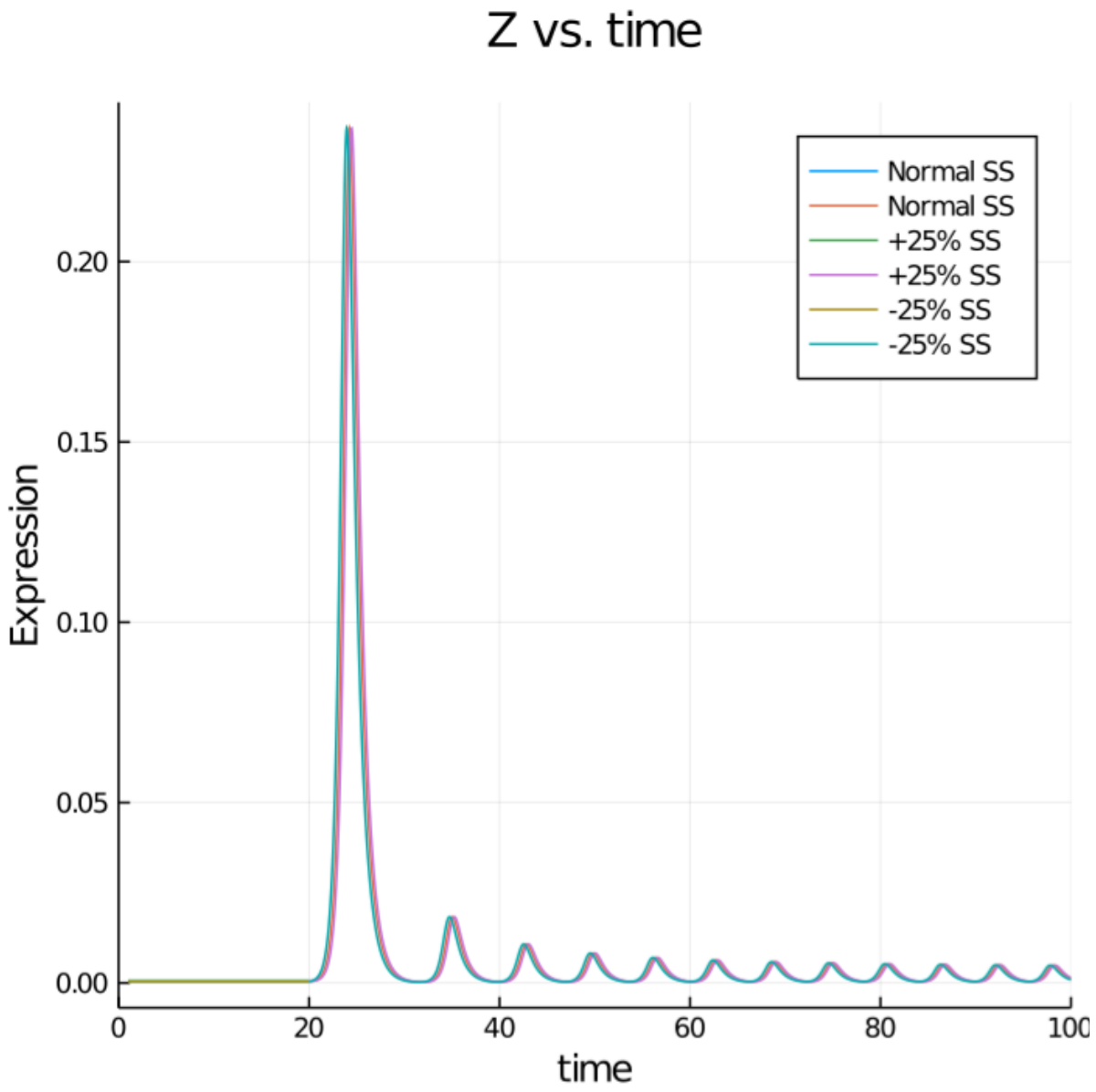


Figure 9: Plot of X over time with S = 35,500 initially and then changing to S =100. Passing through the saddle point bifurcation point leads to coherent oscillations.

An initial S of 35,500 was used with steady state values of X,Y,Z = [5.500447083746545, 0.003972411934522704, 0.00045740359285327027]. The oscillations are coherent because as cells pass through the saddle-node bifurcation they are doing so at values far from those in the oscillatory state. Due to this each cell has a very similar initial phase and trajectory leading as it enters this oscillatory state resulting in coherent oscillations.

2f. S = 105 is clearly within a region of oscillation associated with the Hopf bifurcation spiral. If cells begin at this state after time they will have divergent values of Z. If they are then shifted to S = 100 many cells will be out of phase from each other and moving to a region that has even greater oscillations according to Figure 2, but are not at expression levels too far from those that can occur at S=100. This increasing oscillation with only a small difference between values should lead to incoherence as trajectories increasingly diverge.