Solution to Assignment 4

1 Q1

The BioBrick parts for cI gene and CI protein regulated promoter are found in

- `http://partsregistry.org/wiki/index.php?title=Part:BBa_R0051`
- `http://partsregistry.org/Part:BBa_R0051`

Note that there exists many variations of the promoter. The one shown above (Part # BBa_ R0051) has two operator sites (DNA binding sites) for the repressor CI. These operator sites can be modified to create a promoter with different binding affinity for the repressor.

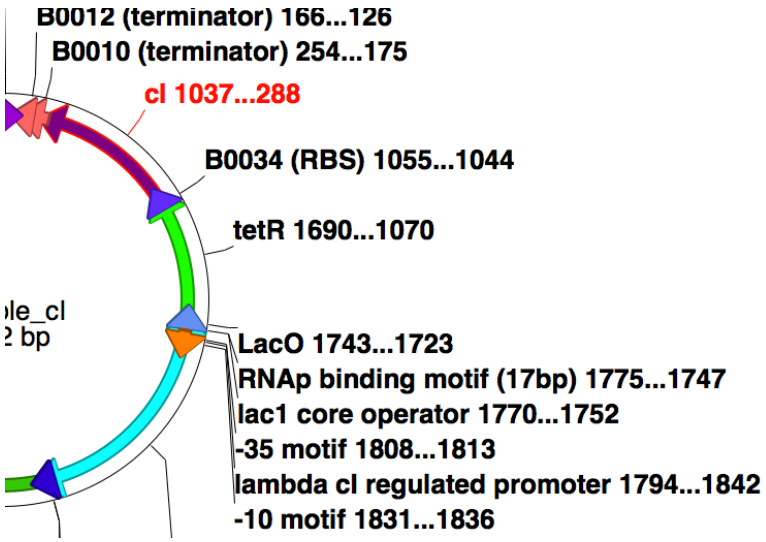

Fig. 1: Plasmid map of the modified bistable circuit. The original tetR gene is replaced with cI gene (highlighted in orange) and the pTet promoter is replaced with the promoter regulated by CI protein.

2 Q2

To solve for the dynamics of the first order cumulant (i.e. first-order moment, or the mean) of B, let $\psi = \langle B \rangle$.

$$\frac{d}{dt}\langle B\rangle = \left\langle \frac{s}{s+k_1}\left(\psi(B+1)-\psi(B)\right)+k_2 B\left(\psi(B-1)-\psi(B)\right)\right\rangle \tag{1}$$

$$= \left\langle \frac{s}{s+k_1}\left(\langle B\rangle+1-\langle B\rangle\right)+k_2 B\left(\langle B\rangle-1-\langle B\rangle\right)\right\rangle \tag{2}$$

$$= \frac{s}{s+k_1}-k_2\langle B\rangle \tag{3}$$

For the second order cumulant (i.e. second-order centered-moment, or the variance) of B, write the time derivative of the variance as follows,

$$\frac{d}{dt}\left(\langle B^2\rangle-\langle B\rangle^2\right) = \frac{d}{dt}\langle B^2\rangle - 2\langle B\rangle\frac{d}{dt}\langle B\rangle \tag{4}$$

The expression for the second term on the right-hand side is already given in Eq (3). For the time derivative of $\langle B^2 \rangle$, let the test function, ψ be $\langle B^2 \rangle$.

$$\begin{aligned}
\frac{d}{dt}\langle B^2\rangle &= \left\langle \frac{s}{s+k_1}\left(\psi(B+1)-\psi(B)\right)+k_2 B\left(\psi(B-1)-\psi(B)\right)\right\rangle && (5)\\
&= \left\langle \frac{s}{s+k_1}\left(\langle B^2+2B+1\rangle-\langle B^2\rangle\right)+k_2 B\left(\langle B^2-2B+1\rangle-\langle B^2\rangle\right)\right\rangle && (6)\\
&= \left\langle \frac{s}{s+k_1}\left(\langle B^2\rangle+2\langle B\rangle+1-\langle B^2\rangle\right)+k_2 B\left(\langle B^2\rangle-2\langle B\rangle+1-\langle B^2\rangle\right)\right\rangle && (7)\\
&= \left\langle \frac{s}{s+k_1}\left(2\langle B\rangle+1\right)+k_2 B\left(-2\langle B\rangle+1\right)\right\rangle && (8)\\
&= \frac{s}{s+k_1}+\frac{2s}{s+k_1}\langle B\rangle-2k_2\langle B^2\rangle+k_2\langle B\rangle && (9)
\end{aligned}$$

Substituting the expression into Eq (4), we get the following equation for the time derivative of the variance,

$$\begin{aligned}
\frac{d}{dt}Var(B) &= \frac{s}{s+k_1}+\frac{2s}{s+k_1}\langle B\rangle-2k_2\langle B\rangle^2+k_2\langle B\rangle-2\langle B\rangle\left(\frac{s}{s+k_1}-k_2\langle B\rangle\right) && (10)\\
&= \frac{s}{s+k_1}-2k_2\left(\langle B^2\rangle-\langle B\rangle^2\right)+k_2\langle B\rangle && (11)\\
&= \frac{s}{s+k_1}-2k_2 Var(B)+k_2\langle B\rangle && (12)
\end{aligned}$$

The two coupled ODEs can be solved for the steady-state, giving the following values

$$\begin{aligned}
\langle B\rangle_{ss} &= \frac{s}{(k_1+s)k_2} && (13)\\
Var(b)_{ss} &= \frac{s}{(k_1+s)k_2} && (14)
\end{aligned}$$

The steady-state Coefficient of Variation is then, $\left(\frac{s}{k_2(k_1+s)}\right)^{-1/2}$. Figure 4 shows the relationship between the steady-state value of B and the coefficient of variation of B as s increases (k_1 and k_2 are fixed).

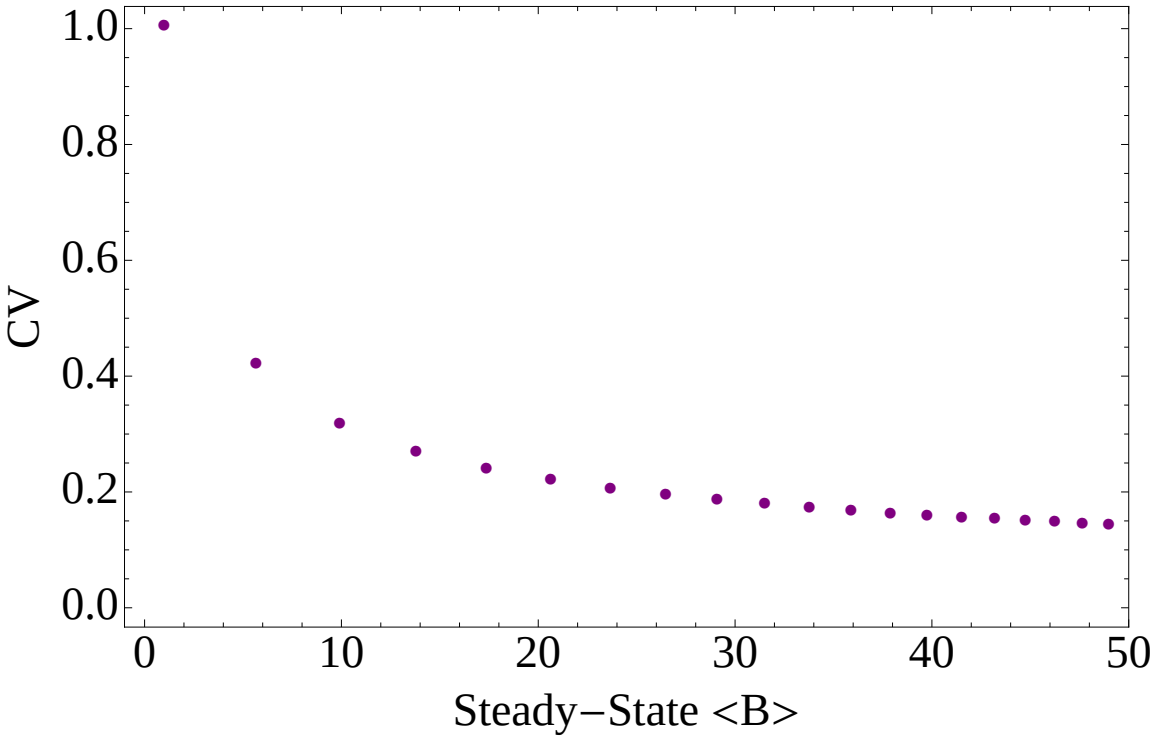

Fig. 2: Sensitivity plot of steady-state $\langle B\rangle$ vs Coefficient of Variation at $k_1 = 0.1, k_2 = 0.01$ and s is varied from 0.001 to 0.1.

3 Q3

$$s + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R^* \tag{15}$$

$$g_{on} + R^n \underset{k_4}{\overset{k_3}{\rightleftharpoons}} g_{off} \tag{16}$$

At equilibrium, Eq 15 and 16 yield the following relationship between the active and inactive signaling molecule.

$$k_1 s\, R_{ss} = k_2 R^*_{ss} \tag{17}$$

$$k_3 g_{on}\, R^n = k_4\, g_{off} \tag{18}$$

Solving Eq 17 for R_{ss} and using the conservation of mass $R_{tot} = R + R^*$, the steady-steady value of active repressor is $\frac{k_2\, R_{tot}}{k_2 + k_1\, s}$. Substituting the expression into Eq 18, and solving for the fraction of g_{on},

$$\frac{g_{on,ss}}{g_{on,ss} + g_{off,ss}} = \frac{g_{on,ss}}{g_{on,ss} + \frac{k_3}{k_4} g_{on,ss} R^n_{ss}} \tag{19}$$

$$= \frac{g_{on,ss}}{g_{on,ss} + \frac{k_3}{k_4} g_{on,ss} \left(\frac{k_2\, R_{tot}}{k_2 + k_1\, s}\right)^n} \tag{20}$$

$$= \frac{1}{1 + \frac{k_3}{k_4} \left(\frac{k_2\, R_{tot}}{k_2 + k_1\, s}\right)^n} \tag{21}$$

Figure 5 shows the fraction of g_{on} at steady-state as a function of the signaling molecule. The sensitivity of the g_{on} fraction increases as cooperatively coefficient increases. As n approaches ∞, the response approaches a step-response.

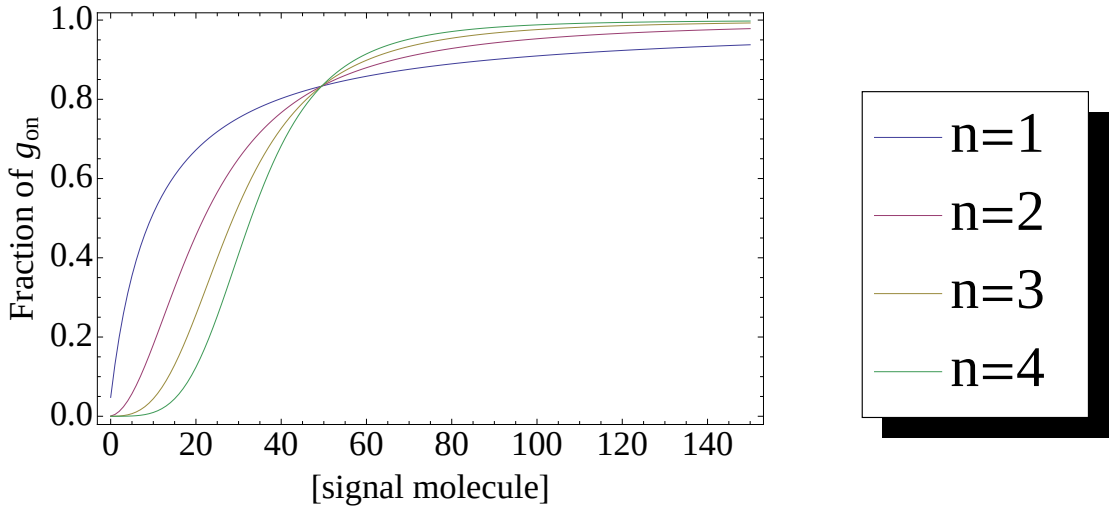

Fig. 3: Fraction of g_{on} as function of signal molecule concentration and cooperative coefficient, n.

4 Q4

The gro code in the next page shows a realization of bistable switch. The simulation assumes that rfp and gfp act as both reporters and transcriptional repressors. The transcription rate of each gene are set as the sum of leaky expression and hill function of its repressor. In Figure 6 the histogram of gfp and rfp count numbers are shown. Notice that because the repression rate of both RFP and GFP are set quite high, majority of the cells have low copy number of RFP and GFP. This trend is more obviously highlighted in the scatter plot (Figure **??**). Notice that there aren't any cells that have both high GFP and RFP count numbers. The cell can be in either of "High GFP" or "High RFP" state.

```
include gro
```

```
set ( "growth_rate", 0.1 );
set ( "throttle", 0 );
set ( "dt", 0.02 );
//set ( "gfp_saturation_max", 200 );
//set ( "gfp_saturation_min", 20 );
//set ( "rfp_saturation_max", 200 );

fun act x v k . v*x^2 / ( 1 + k*x^2 );
fun rep x v k . v / ( 1 + (k*x)^2 );

srand(-1);

// lacI parameters
arL := 0.1 * 69.4 / 2.35;          // mRNA / min / fL
brL := - log ( 0.5 ) / 3.69; // 1/min
apL := 30.0;                 // protein / min / fL / RNA
bpL := 0.01;                 // 1 / min
tetBind := 10;               // tetR repression efficiency
tetLeak := 0.004;             // lac promoter leakiness

// tetR parameters
arT := 0.1 * 69.4 / 2.35;          // mRNA / min / fL
brT := - log ( 0.5 ) / 3.69; // 1/min
apT := 30.0;                  // protein / min / fL / RNA
bpT := 0.01;                 // 1 / min
lacBind := 10;               // lacI repression efficiency
lacLeak := 0.004;              // lac promoter leakiness

mode := 0;
sim := 10;
n := 0;
t := 0;
Ts := 0;
numCells := 0;

program bss() := {

lMax := 500;
tMax := 500;

rL := 0;
gfp := rand(10);
rT := 0;
rfp := rand(10);


rate ( ( tetLeak + rep rfp arL tetBind ) * volume ) : { rL := rL + 1 };
rate ( brL * rL ) :     { rL := rL - 1 };
rate ( apL * rL ) :     { gfp := gfp + 1 };
rate ( bpL * gfp ) :    { gfp := gfp - 1 };

rate ( ( lacLeak + rep gfp arT lacBind ) * volume ) : { rT := rT + 1 };
rate ( brT * rT ) :     { rT := rT - 1 };
rate ( apT * rT ) :     { rfp := rfp + 1 };
rate ( bpT * rfp ) :    { rfp := rfp - 1 };

gfp/volume > lMax   :    { set( "growth_rate", 0.1 - (gfp/3000) ) };

gfp/volume > lMax*2   :    { die() };
```

```
rfp/volume > tMax   :    {
          set( "growth_rate", 0.1 - rfp/2000 )
};

rfp/volume > tMax*2   : { die() };

  daughter : { numCells := numCells + 1 };
  mode = 2 : { print( n, ", ", gfp, ", ", rfp, "\n" ) };

};

program report() := {

  needs gfp, rfp, rL, rT;

  selected : { message ( 1,
          "cell "  <> tostring(id)
     <> ": rLac="  <> tostring(rL)
     <> ", [GFP]="   <> tostring(floor(gfp/volume))
     <> ": rTet="  <> tostring(rT)
     <> ", [RFP]="   <> tostring(floor( rfp/volume )) ) }
};

program main() := {

  mode = 0 & n < sim : {
    srand(-1);
    ecoli ( [], program bss() + report() sharing rL, rT, gfp, rfp );
    mode := 1;
  };

  true : { t := t + dt };

  mode = 1 & numCells > 990 : {
    mode := 2;
    Ts := t;
  };

  mode = 2 & Ts < t & n < sim : {
    n := n + 1;
    mode := 0;
    t := 0;
    Ts := 0;
    numCells := 0;
    reset();
  };

  n = sim : { exit(); };

};
```

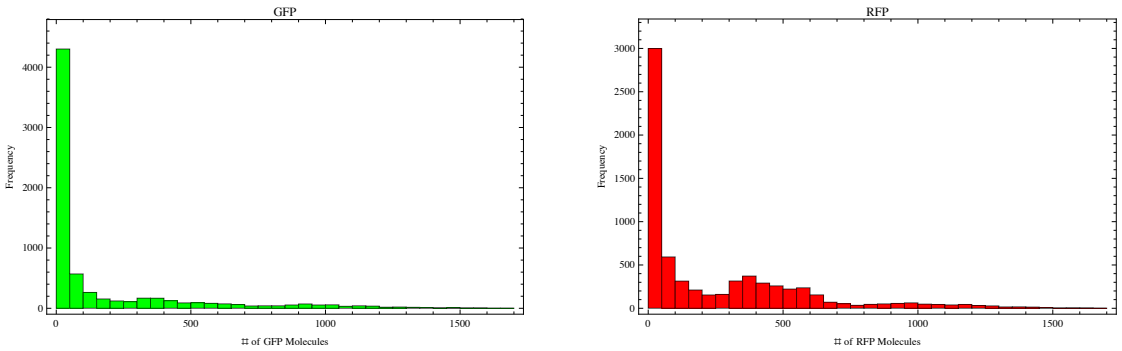

Fig. 4: Histogram of GFP and RFP molecule counts simulated in gro.

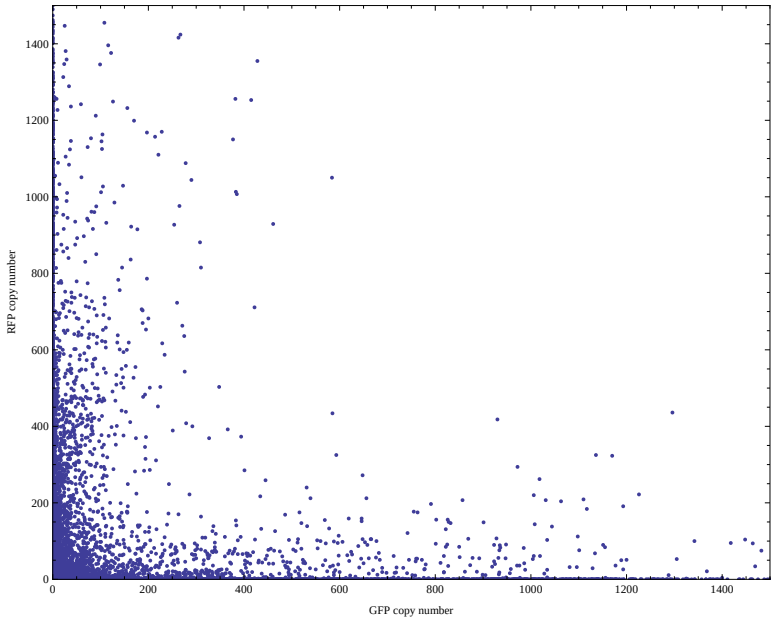

Fig. 5: Scatter plot of GFP and RFP molecule counts simulated in gro.

5 Grade Metric

1. (2.5pts) (Grad : 2pts)
   - 50% each for correct sequence for cI gene and the promoter regulated by CI protein
2. (2.5pts) (Grad : 2pts)
   - 25% each for correct derivation of moment and variance dynamics
   - 50% for correct sensitivity plot between the steady-state B vs CV B
3. (2.5pts) (Grad : 2pts)
   - 25% each for correct steady state expression for active repressor and fraction of ON gene
   - 50% for correct plot of signaling molecule vs ON gene fraction over varied cooperativity coefficient
4. (2.5pts) (Grad : 2pts)
   - 50% for correctly modeling the bistable switch dynamics (mutual repression)
   - 50% for the histogram
5. (Grad : 2pts)
   - Project ideas
6. (2pts)

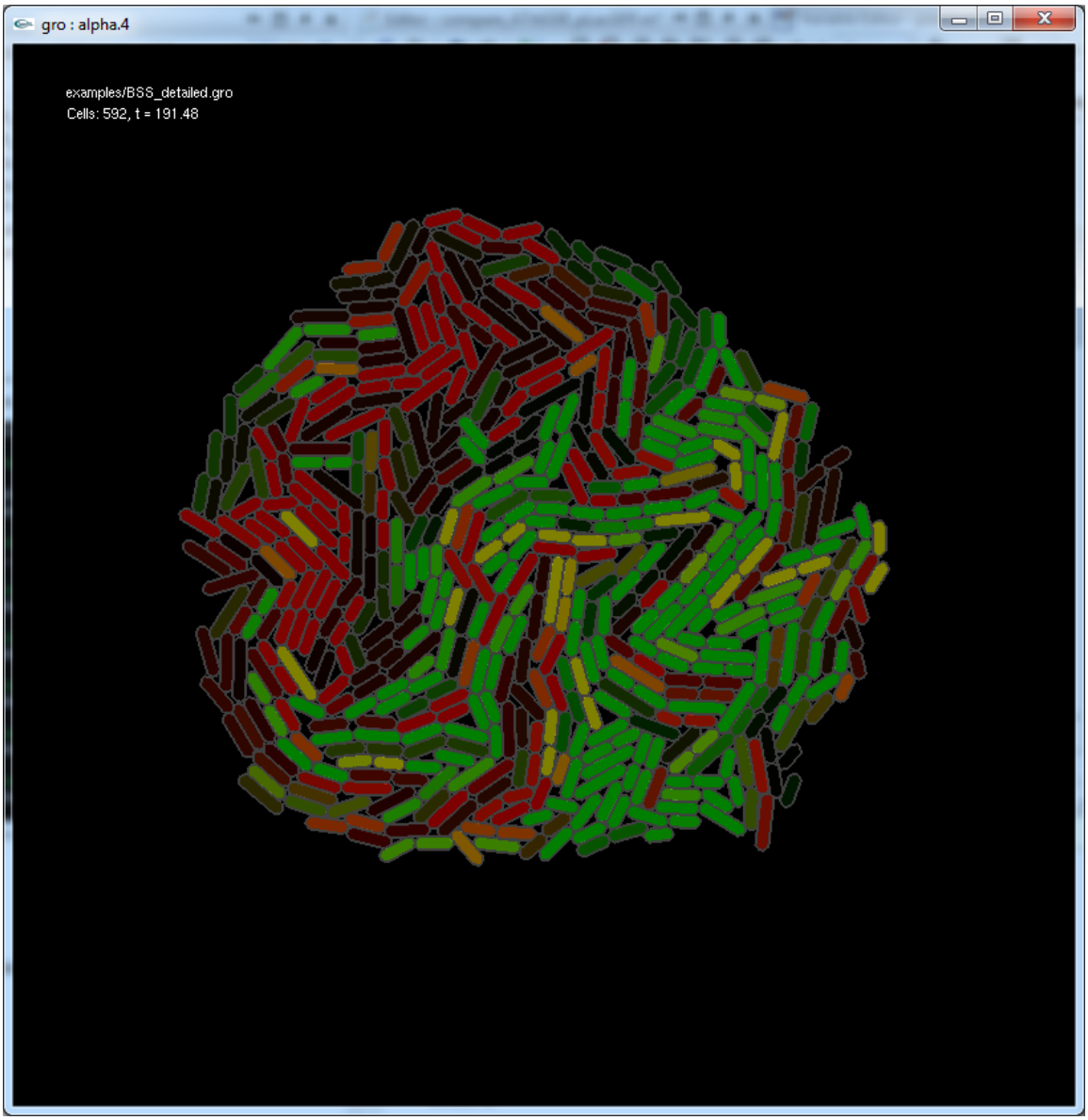

Fig. 6: Screen shot of gro simulation (BSS_detailed.gro). Notice that many cells are neither red nor green.

– Extra credit