CALIFORNIA INSTITUTE OF TECHNOLOGY
Biology and Biological Engineering (BBE)

BE 150

M. Elowitz and R. M. Murray
Winter 2013

Problem Set #5

Issued: 8 Feb 2013
Due: 20 Feb 2013

1. For this problem, we return to our standard model of transcription and translation process.

$$0 \underset{\delta_m}{\overset{k_1}{\rightleftharpoons}} \mathrm{M}$$
$$\mathrm{M} \xrightarrow{\gamma} M + \mathrm{P}$$
$$\mathrm{P} \xrightarrow{\delta_p} 0$$

   where M is the mRNA concentration, P is the protein concentration.

   (a) Simulate the stochastic system above until time $T = 100$ using Simbiology. Use the following parameters: $k_1 = 1, \delta_m = 0.5\gamma = 5, \delta_p = 0.1$. Plot the resulting number of mRNAs and protein over time.

   (b) Now assume that the proteins are degraded much more slowly than mRNA and the rate of protein degradation is 0.05. To maintain similar protein levels, the translation rate is now 0.5. Simulate this system as above. What difference do you see in protein level? Comment on the effect of protein degradation rates on noise.

   (c) Take the final value of the protein concentration in parts (a) and (b) and create a histogram showing the distribution of expression levels. Fit this to a gamma distribution and compute the mean and variance of the resulting gamma distribution.

2. Consider a population of cells that express a flourescent protein. Flourescent protein expression level is controlled by one of types of two promoters. While the mean expression level of the promoters is roughly the same, their noise level is different. This problem explores the effect of different selection stringencies on the noise in a population of cells under selective pressure.

   (a) To create a population of cells, start with 100 cells where each cell has a genotype (μ_i, σ_i). Draw each μ_i from a normal distribution with a mean of 100 and a standard deviation of 5. To simulate the "high noise" and "low noise" populations, draw σ_i randomly from one of two normal distributions (mean = 5, standard deviation = 0.5 OR mean = 15, standard deviation = 1.5).

   "Grow" the population of cells from this starter population by generating 100 different phenotypes for each genotype using a normal distribution with mean, μ_i and standard deviation, σ_i. Let each of these values correspond to the FP expression level of a cell single cell. Make sure you keep track of the genotype of each of the descendant cells. Plot a histogram of FP levels for your population of cells.

   (b) Perform "selections" of varying stringency on your cell population by taking the cells with the top 1%, 5% and 25% of FP levels. "Regrow" these cells in an unbiased manner until

you reach your initial population level (i.e. pick a parent from your selected population at random then use it's genotype to generate a new cell phenotype, you can assume that the genotype stays the same).

(c) What is the mean expression level for each of your new populations? What is $< \sigma_i >$ for each of them? Plot a histogram of σ_i to compare the differences in noise between selection conditions. What is effect does selection stringency have on noise levels? (You might want to run this a few times to confirm trends)

(d) Explain how this might affect how you do selections in a directed evolution experiment.

3. Consider a population of normal and persister bacterial cells that are able to switch to the other type spontaneously. The dynamics that describe their evolution in the presence of two possible environments, corresponding to the presence of antibiotic (stress condition) or absence of antibiotic (growth condition) are the following:

$$\dot{n}(t) = \mu_n n(t) - an(t) + bp(t)$$
$$\dot{p}(t) = \mu_p p(t) - bp(t) + an(t)$$

μ_n and μ_p, are growth rates for normal and persister cells, respectively. μ_n is positive under growth conditions and negative under antibiotic conditions, while μ_p is small in both environments. a and b are the rates of switching from normal to persister cell and from persister to normal cell, respectively. a and b are constant over time - this is shown experimentally in (Balaban et al. 2004).

(a) Consider the evolution of a wild type and hipQ mutant strain of *E. coli*. The hipQ strain exhibits a 1000-fold higher rate of switching from normal type to persister cells. Run a deterministic simulation of the dynamics above for 200 hours, given that there is a cycle of a 20.5 hour growth condition followed by a 2.5 hour stress condition. To maintain constant population conditions, rescale the population at every time step according to the following specification: If the total population size, N_{tot}, which counts all cells of both strains, exceeds some upper limit, $N_{max} = 110000$, the population is rescaled to a smaller size $N_i = 100000$. The rescaling is done by assigning to $n(t)$ and $p(t)$ a random value sampled from a Poisson distribution having mean $\frac{n(t)N_i}{N_{tot}}$ and $\frac{p(t)N_i}{N_{tot}}$ respectively. Start with 50,000 wild type cells and 50,000 hipQ cells. The parameters for wild type and hipQ *E. coli* strains are defined in table 1. Plot all states.

Table 1: Model parameters

Strain	Conditions	μ_n	μ_p	a	b
Wild Type	Growth	2	0	1.2×10^{-6}	0.1
Wild Type	Antiobiotic	-4	-0.4	1.2×10^{-6}	0.1
hipQ	Growth	2	0.2	0.001	10^{-6}
hipQ	Antiobiotic	-4	-0.4	0.001	10^{-6}

(b) Show analytically that the wild type will outgrow the mutant for the system above.

(c) (Optional) Develop a stochastic simulation with parameters from part a). Note that a Gillespie SSA implementation in Matlab will be too slow.

(d) With results from either the simulation you develop, or the stochastic simulation results from the paper explain the differences between the deterministic model and the stochastic model.

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Bi 250b

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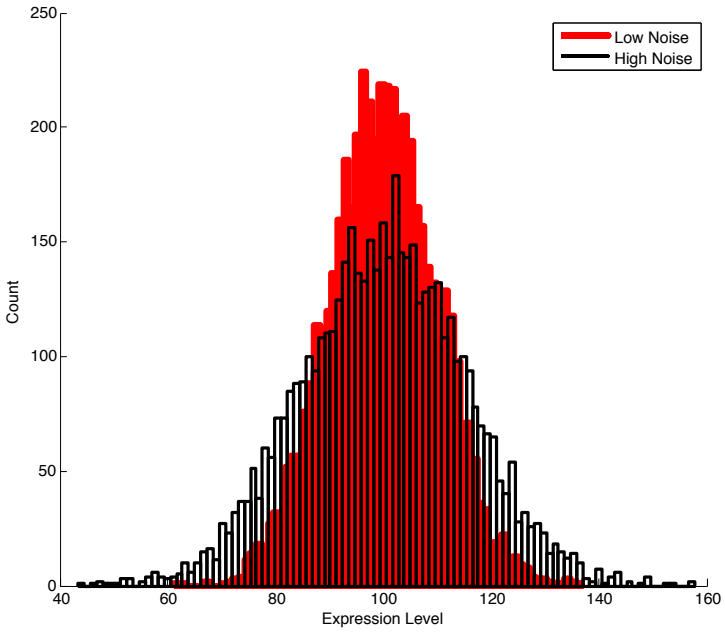
   (a) Say you perform a selection by picking out the top $n\%$ brightest cells and regrowing the population. How would you expect the tightness of your selection (i.e. picking the top 5% brightest cells vs. picking the top 25% brightest cells) to affect your mean fluoresence levels if you grow each population to the same level as your initial population? How would this affect the expression noise level of each population?

   To get a better idea of the numbers involved you can estimate the number of cells that will be selected for a certain percentile using the following command in MATLAB:

```
5000*(1-normcdf(X,mu,sigma))
```

   where X is the fluorescent protein expression cutoff you want, mu=100 , and sigma=10 for the low noise population and 15 for the high noise population. What is the ratio between high-noise cells and low-noise cells if your protein expression level cutoff is 110 units? 130 units?



(b) Explain how this might affect how you do selections in a directed evolution experiment.