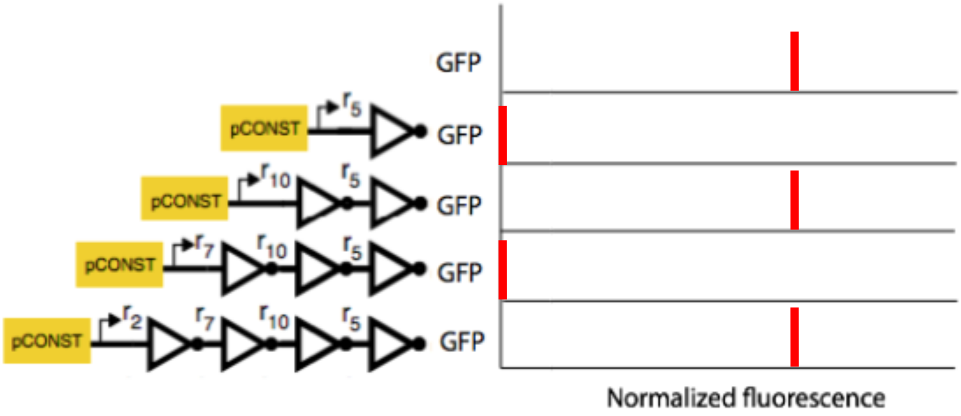
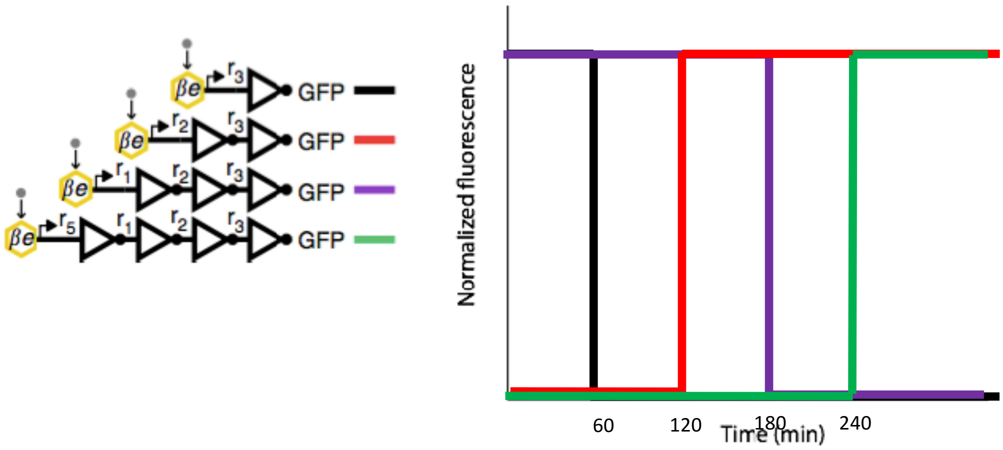
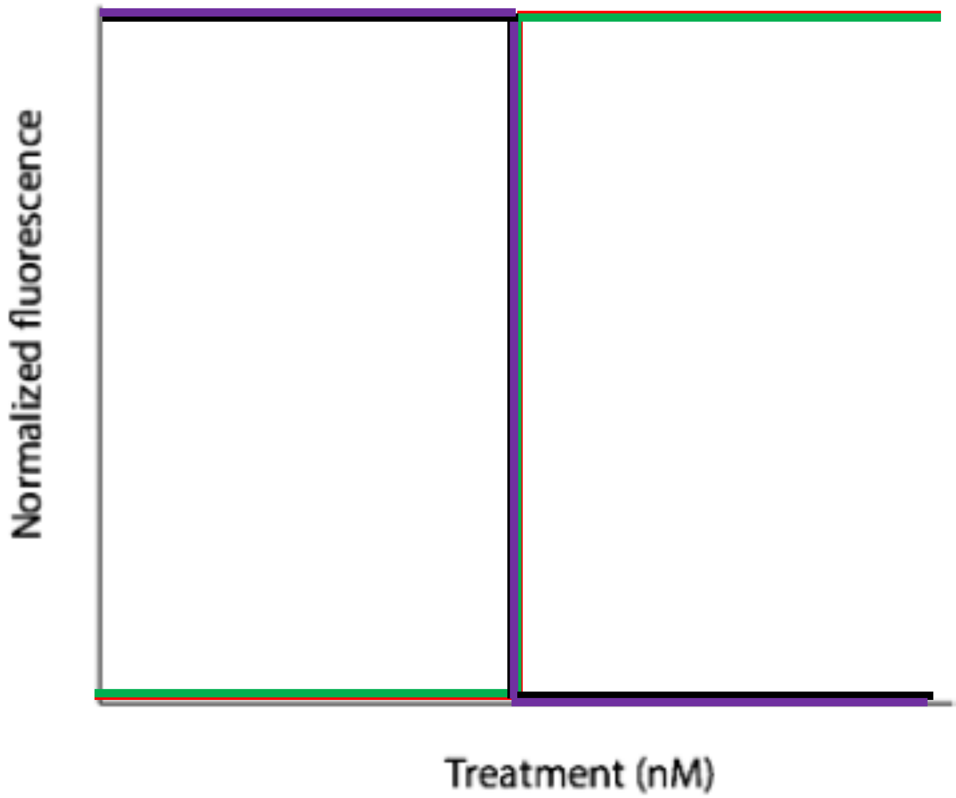
1.a.

My expected result is zero signal showed in the OFF state, but there's a intensity of fluorescence distributed from $10^{2.5}$-$10^{3.5}$, the possible explanations could be the autofluorecence from yeast, the leak signal. The result of ON state I assume should be a single sharp peak at a specific intensity, but it showed a distribution. The possible reasons are the difference between each cell, involving cell cycle, the physical condition, nutrition, etc. So that the expression of GFP would be affected and resulting an intensity distribution.

1.b.







The differences between the ideal result and experimental result, and the cause to the difference: 1) ideal plot has same level at ON state and zero at OFF state, and 2) steep slope (close to infinite) between two states. The ideal plot of concentration versus fluorescence in r3 and r1-r2-r3 are identical, and the plot of r2-r3 and r5-r1-r2-r3 are identical, too. But various line in experimental data.

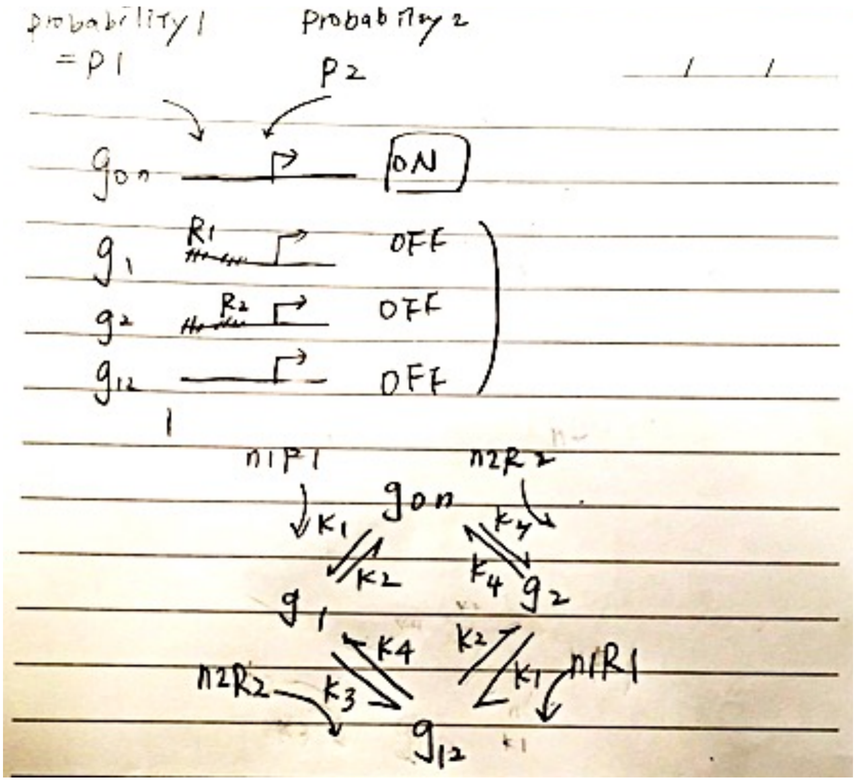
The non-idealized behaviors are caused by the accumulation of the leak at each repression and other unexpected regulation in biology system.

Bonus question: if I am the experimenter, I'll try to exclude the source of autofluorecence from the yeast and increase the intensity of ON signal, like finding higher quantum yield of fluorescence protein and try other cell line can express GFP better or less disturbance, try any possible to increase the span between ON and OFF. The other choice is increase the hill coefficient to make the slope steep, so the more factors involved in repression, the n can be larger, for example, dividing gRNA to guild RNA sequence and scaffold, which the n would become n+1.

2.a.

The region ranges from 10^{-4} to 10^{-2} (repressor concentration in x-axis) varied the most.

3.a



$$P1 \times P2 = P_{g_{ON}}$$

= R1 and R2 both unbound to g

$$P1 = \frac{k2/k1}{k2/k1 + [R1]^{n1}} = \frac{k2}{k2 + [R1]^{n1} \cdot k1}$$

$$P2 = \frac{k4/k3}{k4/k3 + [R_2]^{n2}} = \frac{k4}{k4 + [R2]^{n2} \cdot k3}$$

Both results calculated from probability P1*P2 and derived by CRN (calculated by Mathematica) are the same as below:

$$F_{ss} = F_{Max}\frac{k2k4}{(k2 + k1[R1]^{n1})(k4 + k3[R2]^{n2})} + F_0$$

3b.NOR gate