Theoretical Modeling of a Biosensor Expressing Nitrate Concentration in 3-Tiered Color Scale

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ABSTRACT

In recent years, methods like spectroscopy have emerged as a viable option to accurately measure underwater concentrations of nitrate. Excessive nitrate levels in bodies of water cause environmental concerns, such as eutrophication. As such, the role of accurate and responsive sensors became significant in artificially maintaining the optimal nitrate level. Conventional techniques are costly, so monitoring a wide region like a lake is impractical. To address the aforementioned issue, we propose a cost-effective nitrate biosensor utilizing synthetic biology. The sensor expresses nitrate concentrations in a 3-tiered color scale through fluorescent protein. This paper discusses the mathematical approach to modeling this nitrate biosensor prior to the experiments handling living cells. The ultimate goal is to transition from green to red fluorescence as nitrate gets more concentrated. The mechanism for this involves various processes that happen simultaneously, effectively divided into three groups that each represent a component of the biosensor. We present a set of differential equations with relevant variables that model the behaviors of components in response to a spike in nitrate level. The ideal values for respective variables are determined by initializing them with arbitrary values and fine-tuning them by plotting them in Matlab. In conclusion, the model demonstrates the potential of the biosensor to detect underwater nitrate concentrations robustly.

1. INTRODUCTION

Nitrate (NO_3^-) is a critical component of the ecosystem and is essential for plant growth. Low nitrate levels in the soil will result in poor plant growth, which is detrimental to farming. While low nitrate levels have a huge negative impact on plants, plants are also susceptible to high nitrate levels, which can cause soil acidification. High levels of nitrate in local ecosystems such as lakes are also the main cause of eutrophication and poor water quality, damaging aquatic life. Therefore, it is extremely important to maintain an optimal level of nitrate in both farming and the ecosystem.

The key to artificially maintaining the optimal nitrate level is accurate, fast, and real-time detection of the nitrate levels so that the levels can be adjusted as soon as too low or high nitrate levels are detected. Fortunately, it has become possible to accurately detect nitrate levels in water through the development of nitrate sensors that utilize UV absorption as a readout, similar to widely used spectroscopy methods. The main issue with these sensors, however, is the cost – these sensors can be as low as several hundred dollars, while the price of high-end, sensitive sensors can range up to tens of thousands of dollars (not including the cost of repair and maintenance). Such high costs make the purchase of these sensors impractical.

To solve such impracticality, we aimed to develop a nitrate sensor that is much less costly and uses a different method of nitrate detection. To this end, we utilized a synthetic biology approach to incorporate machinery into living cells (bacteria) that can continuously and sensitively detect nitrate levels in water (Seo et al., 2019). Our approach uses a previously established nitrate-responsive promoter system, where the presence of nitrate allows the expression of the fluorescent protein of choice (Valdez-Cruz et al., 2010). The fluorescence intensity generated within the cells can then easily be read through any camera, which converts RGB values within the cells to estimate the current nitrate level. In our design, the cells will glow green when the nitrate level is low and turn red when the nitrate level is too high (Fig. 1). Our machinery also incorporates a second set of promoter-controlled regions which allows rapid degradation of green fluorescent protein in the presence of high nitrate concentration, allowing the color transition to be fast (Cameron et al. 2014).

Our method has a few inherent advantages compared to traditionally used methods. One, the sensing is real-time, whereas most traditional spectroscopy-based sensors would have to collect samples at different time points for absorption measurements (Ozdemir, 2018). Second, the material cost of our sensor is extremely low. Also, while using software to measure exact RGB values is highly recommended, it is possible to tell just by eye whether the nitrate level is too high without systematic measurement.

While having some significant advantages, our sensor doesn't come without its own disadvantages. Our primary concern during the development of our sensor has been its unpredictability and tunability since all biological systems possess inherent heterogeneity due to cell-cell variabilities. To address this issue, we tried to model the behavior of our sensor to determine how robust our sensor is, and how sensitive it is to changing some of the components within the sensor in a case where we would want to tune its sensitivity. To achieve this, we attempted to model the system behavior of our biological system to see how our sensor will respond to various changes.

2. RESULTS

2.1 Division of the system and its components into different sub-processes

Our sensor consists of three promoters (one constitutive promoter and two responsive promoters, PyeaR and PL). More important than the promoters themselves are the seven different processes that occur concurrently to produce the desired response, namely emission of green fluorescence at low nitrate levels that transitions into red at the presence of high nitrate levels.

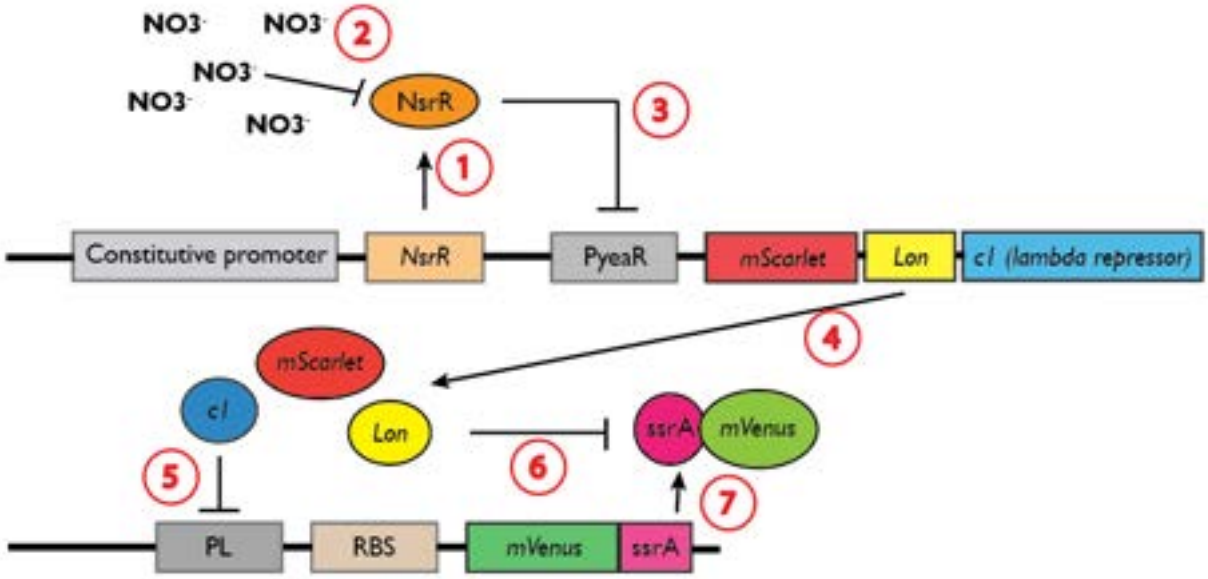

Figure 1. Diagram of the components in our sensor-system. The sensor has three promoters that act with or against each other to produce robust and rapid response following changes in the nitrate concentration.

As noted by the circled numbers in Figure 1, there are mainly 7 different processes that are simultaneously happening:

1. Expression of NsrR protein from the constitutive promoter.
2. Inhibition of NsrR protein from NO3-.
3. Inhibition of PyeaR promoter by NsrR protein.
4. Expression of mScarlet, Lon, and c1 lambda repressor from PyeaR promoter.
5. Inhibition of PL promoter by c1 lambda repressor.
6. Degradation of mVenus-ssrA through bacterial degron Lon.
7. Expression of mVenus-ssrA from promoter PL

Some of the processes directly affect others, while some indirectly do so. Therefore, we have broken up the processes into three grouped processes, where each “grouped” processes will

directly affect the response of the other group. These groups are largely based on each promoter-connected segments, which are explained in detail below.

2.2 Modeling the activity of NsrR in response to nitrate levels (Processes 1 and 2)

We first tried to model processes 1 and 2 together to see how NsrR protein responds to changes in nitrogen level:

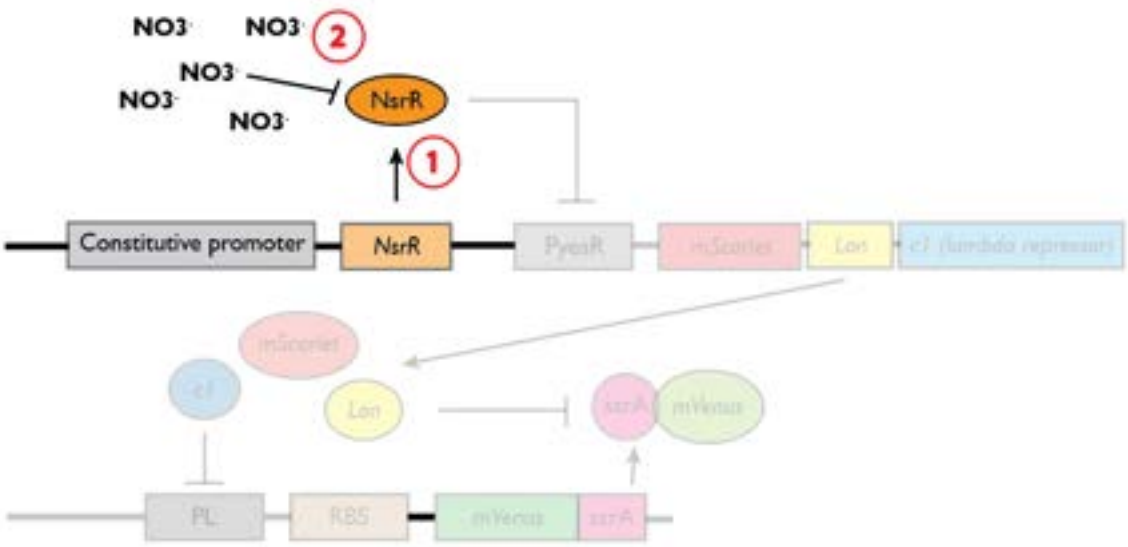

Figure 2. Diagram of the components in our sensor-system when NsrR is expressed.

To start off, we make one critical assumption which will not only apply to NsrR, but will also apply to the expression of all other proteins in the system:

Assumption 1: The rate of protein expression from a promoter is always positive (or 0 at minimum), but is inversely proportional to the total amount of that particular protein (i.e protein level saturates at a certain level).

One important thing to note here is that in the case of NsrR, the system starts off (at t=0) at steady state where there is maximal amount of NsrR and mVenus and no NO3. We will make another assumption here to simplify the system a little bit further. We assume that the expression of NsrR from the constitutive promoter is much stronger than the spontaneous degradation of NsrR, so the concentration of NsrR stays constant and at maximal level starting t=0. Therefore:

Assumption 2: $at\ t \geq 0,\ [NsrR] = [NsrRmax]$

Where $[NsrR]$ denotes the concentration of total NsrR in the system (unit: M) and $[NsrRmax]$ refers to the saturation concentration of NsrR. It is important to note that while the concentration

of NsrR remains constant in our model, the concentration of *active* NsrR will change depending on the input. This allows us to make another assumption:

Assumption 3: The activity decrease in NsrR protein is proportional to the amount of NO3 present.

With the above three assumptions, we propose the following equation to explain the total concentration of **active** NsrR over time in response to NO3:

$$\frac{d[NsrR]_{active}}{dt} = \frac{k_1[NsrRmax]}{[NsrRmax]+k_{sat}[NsrR]_{active}} - k_{NO3}[NsrR]_{active}{}^{a}[NO3^-]^b \quad \cdots(1)$$

Where $[NsrR]_{active}$ is the concentration of active NsrR protein on a given time (M), constant k_1 explaining the maximal recovery rate of active NsrR following the removal of NO3 ($M \cdot sec^{-1}$), constant k_{sat} being a dimensionless constant that explains how sensitive is active NsrR to saturation, constant a being how sensitive is NO3 inhibition to NsrR concentration, and constants k_{NO3} ($M \cdot sec^{-1}$) and b both denoting the fundamental inhibition efficiency of NO3 on NsrR .

Writing the behavior of active NsrR over time the above way has the following consequences and merits:

1. As $[NsrR]_{active}$ increases and gets closer to the saturation point of total NsrR concentration, the rate of increase in $[NsrR]_{active}$ is dampened (saturates) as it will be true in the real world. The corollary to this is: $If\ [NsrR]_{active} \approx [NsrRmax]$, $\frac{d[NsrR]}{dt} \approx 0$ (given that $k_{sat} \gg k_1$). Since this corollary equation has a condition that $k_{sat} \gg k_1$, we will state this assumption explicitly:

Assumption 4: $k_{sat} \gg k_1$.

2. When $[NsrR]_{active}$ = 0, $\frac{d[NsrR]}{dt} = k_1$, the maximal recovery rate of active NsrR.
3. Per *Assumption 3*, the rate of decrease in active NsrR is proportional, although not directly proportional, to the total amount of NO3 present.
4. Finally, when $[NsrR]_{active} = 0$, the term $k_{NO3}[NsrR]_{active}[NO3^-]^a$ is also 0, meaning that active NsrR will never become negative. More importantly, as $[NsrR]_{active}$ decreases, the inhibition of active NsrR by NO3 also decreases, alluding to how the inhibition by NO3 also saturates as there are less and less active NsrR.

We used MATLAB to simulate The changes in active NsrR concentration in response to various changes in nitrogen concentration. The source code is provided in the appendix. Here, we came up with some arbitrary values for the constants to qualitatively assess the response. For instance, when:

$$[Nsrmax] = 0.1,\ k_1 = 0.00001,\ a = 0.1,\ k_{sat} = 50,\ k_{NO3} = 0.0001,\ b = 0.8$$

We can see the following response when we spike in (increase) nitrate for a certain time and then decrease it back to zero:

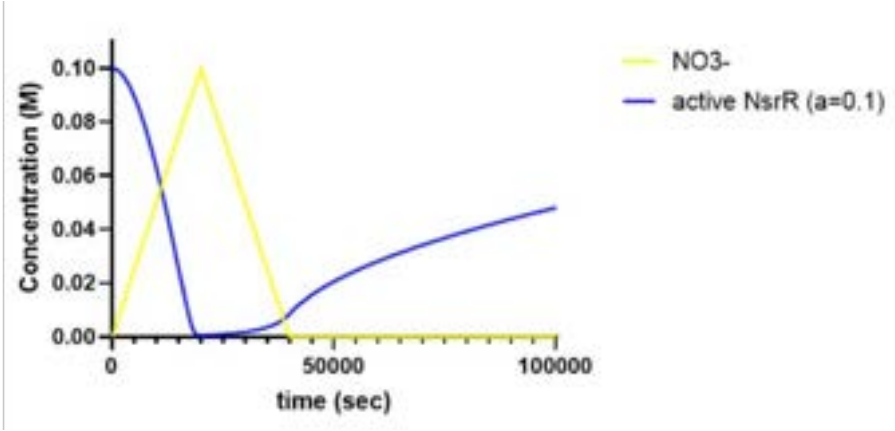

Figure 3. The concentration changes of NO_3- and NsrR over time

We can clearly see that active NsrR rapidly decreases as NO3 levels increase, and starts to slowly recover as the NO3 level goes down. Again, ***Assumption 4:*** $k_{sat} \gg k_1$ is very critical to this behavior – the saturation behavior of NsrR changes dramatically as the ratio of k_{sat} and k_1 becomes smaller and smaller. The following graph demonstrates this point very well:

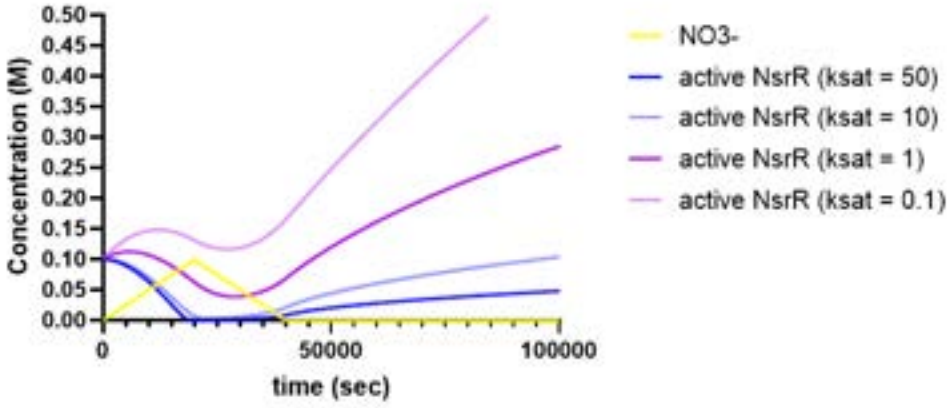

Figure 4. The concentration changes of active NsrR over time with regard to various Ksat values

In this case, increasing k_{sat} above 1 causes the system to diverge very quickly over the saturation limit since it starts to violate ***Assumption 4***.

We can also fix the ratio of k_{sat} and k_1 as it was and try to play around with other constants. For instance, we can fix everything and try changing k_{NO3}:

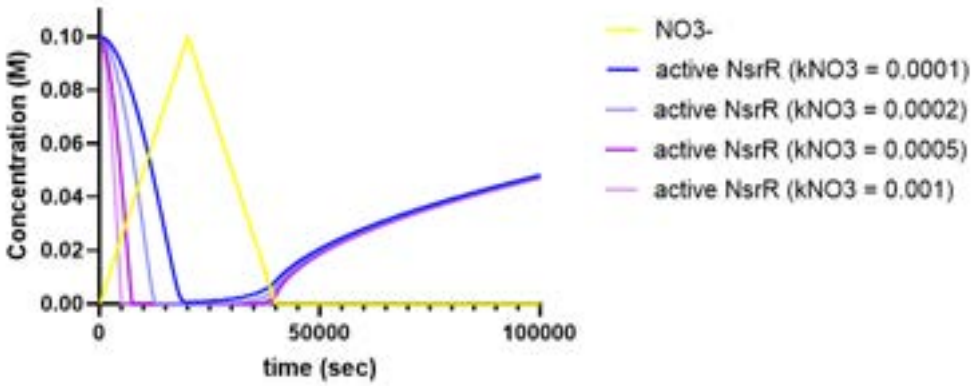

Figure 5. The concentration changes of active NsrR when the NO3 inhibition efficiency is increased.

Just like we would expect, increasing the NO3 inhibition efficiency causes active NsrR to decrease more rapidly. Changing constant *b* would have a similar effect on the system.

We can also try changing the constant *a* change the dependency of NO3 inhibition on NsrR concentration:

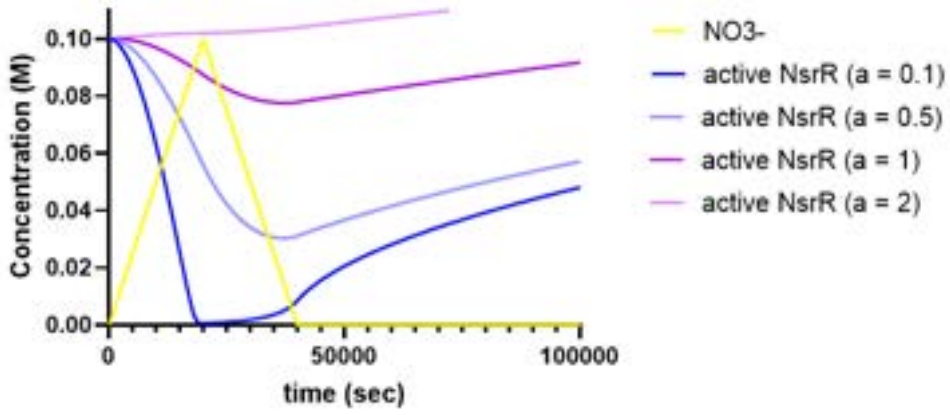

Figure 6. The concentration changes of active NsrR over time when a > 1.

We can quickly see that the system spirals out of control as we increase constant *a* to be larger than 1.

So, in summary, there is a range of constants we can choose from depending on the range of nitrogen concentration, and the system doesn't behave like what we would expect it to if the constants go over or below the range. For certain constants such as k_{sat} and k_1, their ratios matter more than their actual values.

2.3 Modeling the production of mScarlet in response to active NsrR concentration (Processes 3 and 4)

Now that we more or less explained how NsrR will behave in response to nitrogen concentration, it is time to model arguably the more important components of the system: mScarlet and mVenus in response to NsrR/Nitrogen. We will start off by modeling behavior of mScarlet, since NsrR directly affects expression of mScarlet (as well as Lon and c1 lambda repressor):

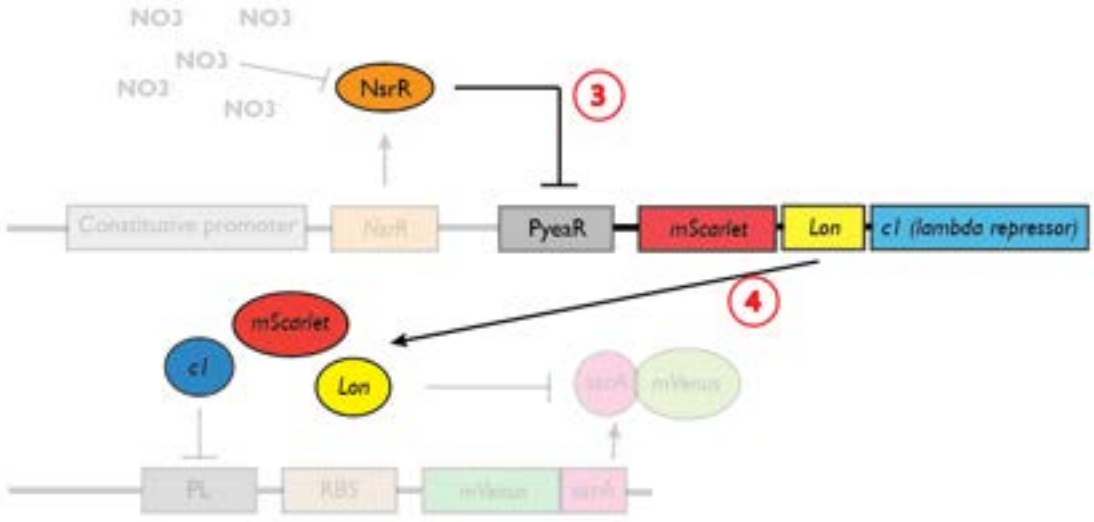

Figure 7. Diagram of the components in our sensor-system that demonstrates the expression of mScarlet by NsrR.

Fortunately, we have already established some basic assumptions that apply to all systems while modeling NsrR behavior above. Using similar logic used to generate these assumtpions, we can write down the differential equation for RFP, Lon, and c1 lambda repressor with three additional assumptions:

Assumption 5: All proteins in the system go through spontaneous degradation, the rate of which is proportional to the total amount of that particular protein present in the system.

Assumption 6: The expression rate of mScarlet, Lon, and c1 are the same since they are under the same promoter.

Assumption 7: Although probably not true in practice, we assume the spontaneous degradation rate of mScarlet, Lon, and c1 to be equal for simplicity.

With these additional assumptions, we can write:

$$\frac{d[mScarlet]}{dt} = \frac{d[Lon]}{dt} = \frac{d[c1]}{dt} = \frac{k_{PmScarlet}[mScarletmax]}{[mScarletmax]+k_{mScarletsat}[mScarlet]} - k_{mScarletdeg}[mScarlet]^{c} \quad \cdots(2)$$

Where $k_{PmScarlet}$ denotes the rate of expression of mScarlet under this promoter system and constants $k_{mScarletdeg}$ and c refer to the degradation rate and the dependence of degradation rate on the concentration of mScarlet, respectively. Naturally, the next step is to explain how the expression of mScarlet is affected by active NsrR. To do so, we make the following assumption:

Assumption 8: the expression rate of PyeaR promoter is inversely proportional to the amount of active NsrR in the system.

With this assumption, we propose the following equation:

$$k_{PmScarlet} = \frac{k_{PyeaR}}{([NsrR]+1)^{d}} \quad \cdots(3)$$

Where constants "k_{PyeaR}" ($M^{d+1} \cdot sec^{-1}$) promoter strength of PyeaR and constant d denotes the dependency of mScarlet expression on NsrR concentration. This equation also incorporates the following assumption:

Assumption 9: When [NsrR] is close or equal to 0, the expression rate of mScarlet is the basal rate of PyeaR promoter expression, k_{PyeaR}.

This assumption transforms equation (2) into:

$$\frac{d[mScarlet]}{dt} = \frac{\frac{k_{PyeaR}}{([NsrR]+1)^{d}}[mScarletmax]}{[mScarletmax]+k_{mScarletsat}[mScarlet]} - k_{mScarletdeg}[mScarlet]^{c} \quad \cdots(4)$$

$$\frac{d[mScarlet]}{dt} = \frac{d[Lon]}{dt} = \frac{d[c1]}{dt}$$

Now, we can look at how changes in nitrogen levels will affect mScarlet level over time. We kept the parameters for NsrR equation the same ($[Nsrmax] = 0.1,\ k_1 = 0.00001,\ a = 0.1,\ k_{sat} = 50,\ k_{NO3} = 0.0001,\ b = 0.8$) while we arbitrarily used the following parameters for equation (4) so that mScarlet behaves reasonably as we expect the system to behave:

$k_{PyeaR} = 0.00002,\ [mScarletmax] = 0.1,\ k_{mScarletsat} = 50,\ k_{mScarletdeg} = 0.000001,\ c = 1,\ d = 50$

Under these conditions, the change in mScarlet over time looks as follows:

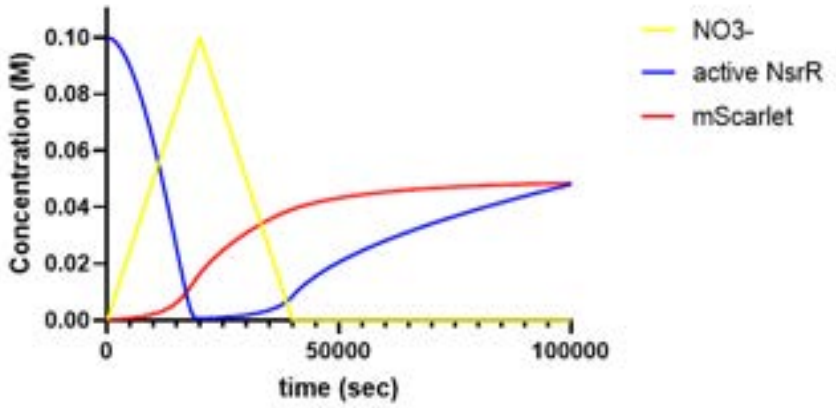

Figure 8. The relationship among the concentrations of NO_3-, active NsrR, and mScarlet

As we did in when modeling the behavior of NsrR, what happens if we change the constants? Let's first try to play around with the promoter strength with everything else fixed:

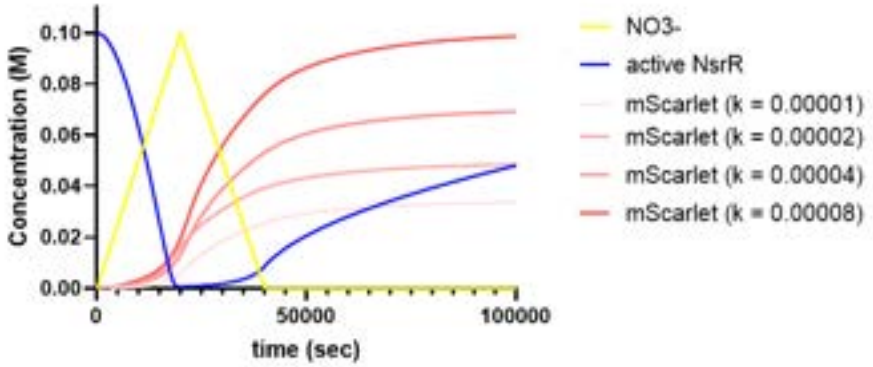

Figure 9. The concentration changes of mScarlet over time with regard to various promoter strengths

Not surprisingly, increasing the promoter strength definitely increases the rate at which mScarlet increases in response to decrease in NsrR levels. Similarly, we can change constant d:

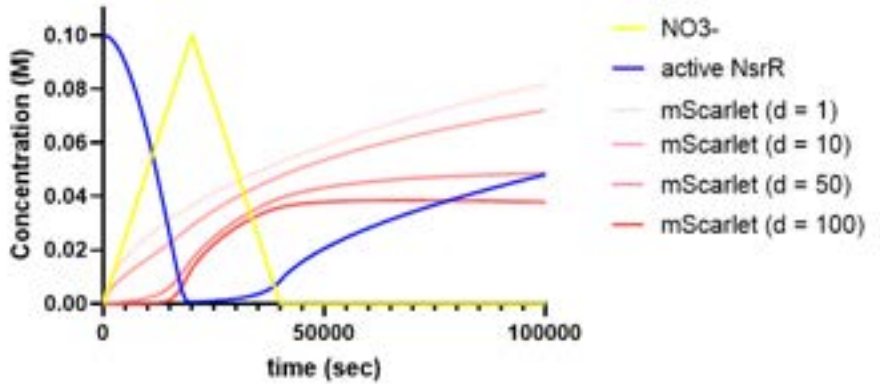

Figure 10. The concentration changes of mScarlet over time with regard to various d values

Again, not surprisingly, as d increases (i.e mScarlet is more sensitive to NsrR concentration), the graph bends downward. However, it is interesting to note that d has to be above certain range (in this case, 50) for the system to properly come to saturation.

Similarly, we can try to change the degradation rate, $k_{mScarletdeg}$:

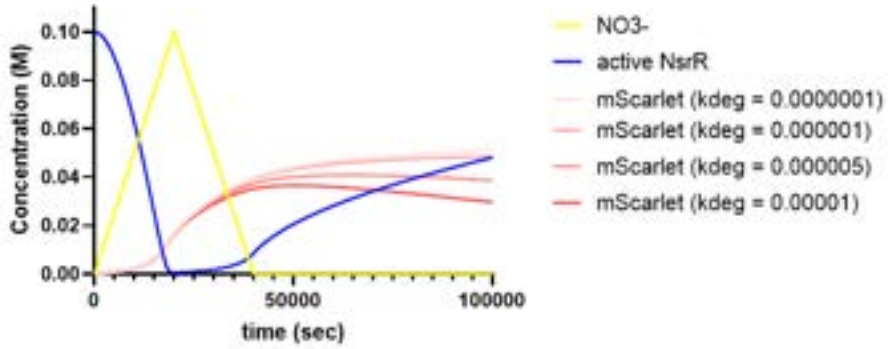

Figure 11. The concentration changes of mScarlet over time with regard to various degradation rates

It is interesting to note that changing the degradation constant does not change the increasing behavior of mScarlet initially, while it makes more significant contribution as NsrR recovers after taking out NO3 from the system. Of course, this behavior will change if we, for instance, change constant c to make the system more sensitive to degradation.

Last but not least, since the changes in mScarlet over time is a function of NsrR concentration, changes in NsrR behavior will also cause changes in how mScarlet behaves. For instance, we will try changing constant a like we did in the previous section and see how mScarlet changes over time:

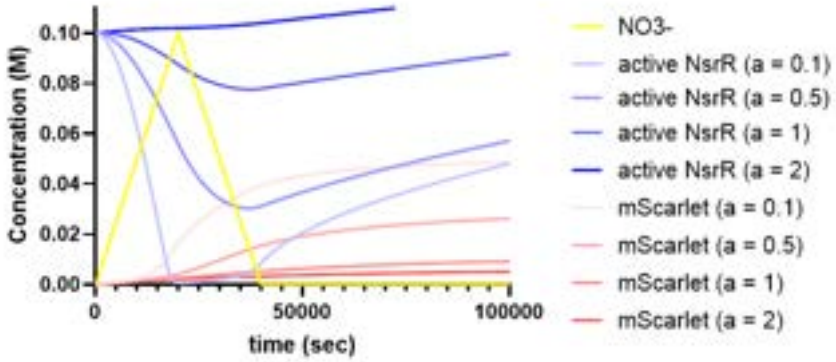

Figure 12. The concentration changes of mScarlet with regard to varying NsrR behaviors.

2.4 Modeling the change in mVenus levels in response to nitrogen (Processes 5, 6, and 7)

Finally, we will model the last bit of the system, which is change in mVenus levels over time.

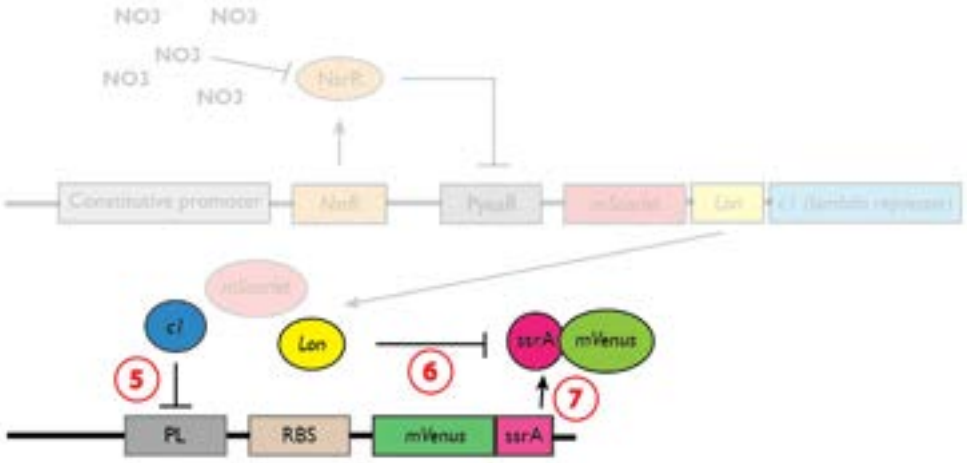

Figure 13. Diagram of the components in our sensor-system.It demonstrates the mechanism involving mVenus.

mVenus is quite identical to how we modeled mScarlet with one twist: mVenus has *double* inhibition since not only c1 repressor represses promoter PL, but also Lon actively degrades mVenus-ssrA. To simplify things, we make another assumption here:

Assumption 10: degradation rate of mVenus-ssrA by Lon is proportional to the level of mVenus present in the system.

It's fair to say this particular assumption is quite redundant with *Assumption 5*, but we've laid it out once again here since we wanted to distinguish the spontaneous degradation of mVenus from active degradation by Lon.

Therefore, we propose the following equation for the change in rate of mVenus:

$$\frac{d[mVenus]}{dt} = \frac{\frac{k_{PL}}{([c1]+1)^e}[mVenusmax]}{[mVenusmax]+k_{mVenussat}[mVenus]} - k_{mVenusdeg}[mVenus]^f \quad \cdots(5)$$

Where k_{PL} is the promoter strength of PL promoter and constant c is the inhibition efficiency of c1 on the PL promoter. Thanks to *Assumption 5* and *Assumption 10*, the degradation by Lon as well as the spontaneous degradation of mVenus were lumped into a single linear term, $k_{mVenusdeg}[mVenus]^f$.

We make one final critical assumption to explain mVenus in terms of mScarlet:

Assumption 11: *at t=0, the system has maximal amount of mVenus-ssrA and no mScarlet, Lon, or c1 expressing.*

This assumption is not too far of a stretch since our bacteria starts off glowing full-green and only turns red in response to the presence of nitrogen.

Due to *Assumption 11* along with *Assumption 6*, we can make the following claim:

$$Since\ \frac{d[mScarlet]}{dt} = \frac{d[Lon]}{dt} = \frac{d[c1]}{dt}$$

$$and\ [mScarlet] = [Lon] = [c1] = 0\ \ at\ t = 0,$$

$$[mScarlet] = [Lon] = [c1]\ at\ any\ given\ time.$$

Since this is the case, we can write the set of differential equations for mVenus in terms of mScarlet:

$$\frac{d[mVenus]}{dt} = \frac{\frac{k_{PL}}{([mScarlet]+1)^e}[mVenusmax]}{[mVenusmax]+k_{mVenussat}[mVenus]} - k_{mVenusdeg}[mVenus]^f \quad \cdots(6)$$

Similar to what we did before, we'll get some arbitrary constants in as a default to see how mVenus will behave. Here is our initial setting:

$$k_{PL} = 0.00001,\ [mVenusmax] = 0.1,\ k_{mVenussat} = 50,\ k_{mVenusdeg} = 0.001,\ e = 50,\ f = 2$$

And this is how mVenus level changes over time under this condition:

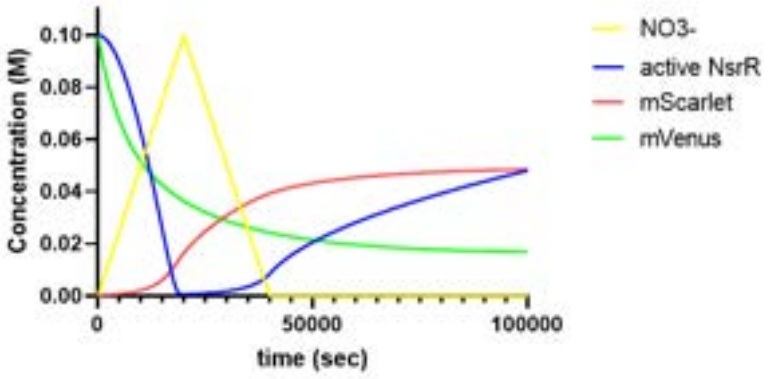

Figure 14. The relationship among the concentrations of NO_3-, active NsrR, mScarlet, and mVenus.

Like we did before, we can change some constants which will most definitely change how mVenus change over time. For instance, we can change how mScarlet behaves by changing the promoter strength of kPyeaR:

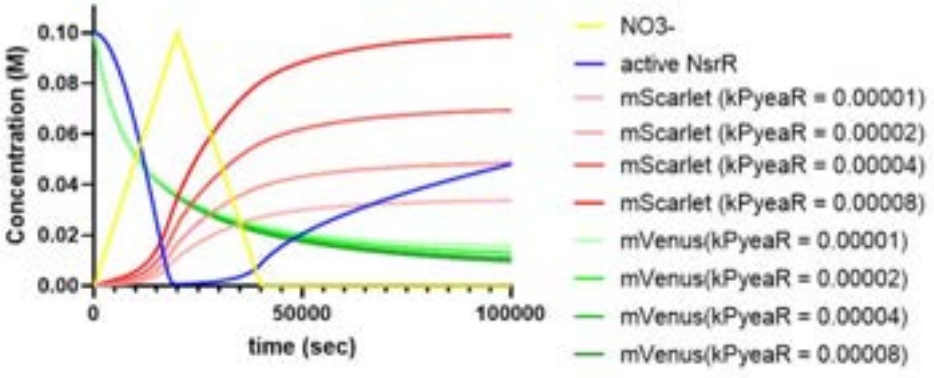

Figure 15. The concentration changes of mVenus over time with regard to varying mScarlet behaviors due to changes in the promoter strength

One thing that strikes out is that while mScarlet changes dramatically, mVenus level seems to be affected only minimally by mScarlet levels. Similar things when we change mScarlet level profile in a different way:

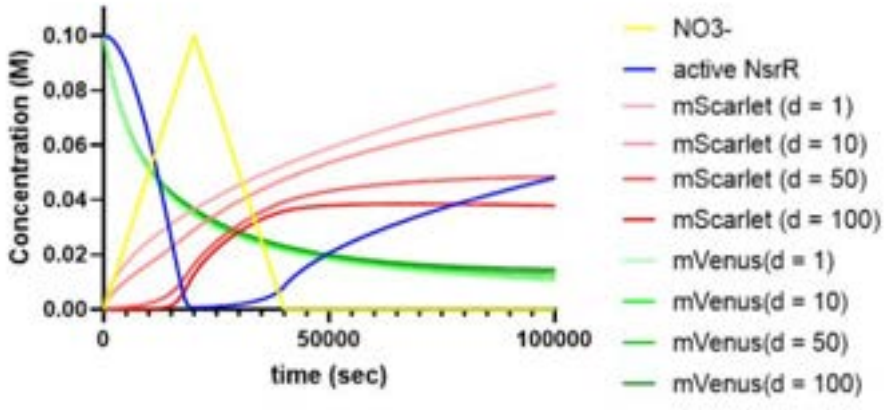

Figure 16. The concentration changes of mVenus over time with regard to varying mScarlet behaviors due to changes in d value

This phenomenon probably comes from the fact that for mVenus, the degradation term is dominant. For instance, if we change the degradation rates:

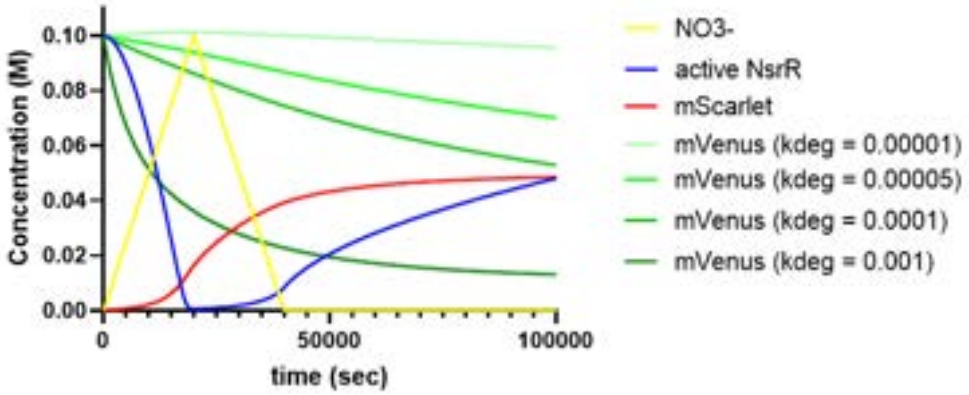

Figure 17. The concentration changes of mVenus over time with regard to various degradation rates.

We can see that even a five-fold change in the degradation term causes the curve to shift dramatically.

3. DISCUSSION

Throughout the results section, we've given a detailed explanation of how we've tried to model our system and how changing various constants changes the behavior of the expression profile

of the proteins over time. However, it is easy to overlook the fact that we've actually chosen the right values for our 18 variables/constants to make it along the way. We have indicated in the previous sections that the variables were chosen arbitrarily, but there is more to it. It is true that the *absolute values* of the constants do not matter that much, but we've realized after many iterations that the *ratios between the values of the constants* actually do matter a lot. Here were some considerations when choosing the values for our variables:

- The ratio between the concentration of nitrogen and the promoter strengths matter. In this case, the promoter strength should be 10,000 times lower than the maximal NO3 level.
- The promoter strength of the constitutive promoter and promoter PL have to be lower than the strength of PyeaR promoter for us to observe rapid decrease in mVenus and rapid increase in mScarlet level over time.
- The degradation term has to be at least 10-fold lower than the promoter strength for mScarlet to express properly. (Which makes a lot of sense in the world of Biology). Similarly, the degradation term of mVenus has to be sufficiently larger than the spontaneous degradation rate of mScarlet to appropriately represent our plasmid design.
- Per *Assumption 4*, the saturation constants have to be significantly higher than the promoter strength. To be more precise, we've determined that the saturation constant has to be *at least* 16,667-fold higher than the promoter strength for the system to behave properly.
- Constants *a* and *b* have to be fractions while constants *c, d, e,* and *f* have to be larger than 1.

There are some more minor requirements in addition to these, but the 5 bullet points above summarize the most important factors that come into consideration for the system to behave well. The second point of discussion is, "how robust it this system?". If the ratios that we've described above are met, the system is fairly robust, not diverging out in a crazy manner.

One last important question to address is "*What components can we change to optimize the system?"* This is actually fairly easy to answer, mostly because we don't have much power over which components we can change. For instance, we cannot change how well NO3 inhibits NsrR, how well NsrR inhibits PyeaR, how well Lon degrades mVenus-ssrA, nor how fast do all these proteins degrade spontaneously, etc. The *only* thing we can change is the promoter strength. In short, we can only change three variables from all 18 variables – $k_{constitituve}$, k_{PyeaR}, and k_{PL}. The silver lining is that changing these constants actually can change the behavior of protein expression significantly (as we've seen in how changing k_{PyeaR} dramatically changes the mScarlet expression over time).

In conclusion, we can confidently say that the system has a great potential to work robustly, given the right environment. While the system requires a lot of fine tuning to behave well, we can try to change the promoters to modulate the system response – which is not impossible at all (in fact, we actually did it in our experiments).

4. APPENDIX

A. Source code for simulation in MATLAB

```
clear;
clc;
close all;

%k1 = recovery rate of active NsrR
k1 = 0.00001;
%NsrRmax = saturation level of NsrR
NsrRmax = 0.1;
%a = NO3 inhibition dependency on active NsrR concentration
a = 0.1;
%ksat = saturation dependency of NsrR
ksat = 50;
%kNO = NO3 inhibition strength 1
kNO = 0.0001;
%b = NO3 inhibition strength 2
b = 0.8;
%kPyeaR = basal promoter expression rate of PyeaR promoter
kPyeaR = 0.00002;
%RFPmax = saturation concentration of RFP
RFPmax = 0.1;
%kRFPsat = saturation constant for mScarlet/RFP
kRFPsat = 50;
%kRFPdeg = degradation rate of mScarlet/RFP
kRFPdeg = 0.000001;
%c = dependency of RFP degradation on RFP concentration
c = 1;
%d = dependency of promoter kPyeaR strength on NsrR concentration
d = 50;
%GFPmax = saturation concentration of GFP
GFPmax = 0.1;
%kPL = PL promoter strength
kPL = 0.00001;
%kGFPsat = GFP saturation constant
kGFPsat = 50;
%e = dependency of promoter kPL strength on c1 concentration
e = 50;
%kGFPdeg = degradation constant for GFP
kGFPdeg = 0.001;
%f = dependency of GFP degradation on GFP concentration
f = 2;

%constructing time
t = [];
for i = 1:100000
    t = [t i];
end
```

```
%%
%increasing and then decreasing NO3
NO3mat = [];
NO3 = 0;
dNO3 = 0.000005;

for i = 1:length(t)/5
    NO3 = NO3 + dNO3;
    NO3mat = [NO3mat NO3];
end

for i = 1:length(t)/5
    NO3 = NO3 - dNO3;
    NO3mat = [NO3mat NO3];
end

for i = 1:(3*length(t)/5) + 1
    NO3mat = [NO3mat 0];
end


%%
%constructing NsrR over time
dNsrR = 0;
NsrRinit = NsrRmax;
NsrRmat = [NsrRinit];
for i = 1:length(t) - 1
    dNsrR = k1*NsrRmax/(NsrRmax + ksat*NsrRmat(i)) - kNO*NsrRmat(i)^a*NO3mat(i)^b;
    if NsrRmat(i)+dNsrR < 0
        NsrRmat = [NsrRmat 0];
    else
        NsrRmat = [NsrRmat NsrRmat(i)+dNsrR];
    end
end

%constructing RFP over time
dRFP = 0;
RFPinit = 0;
RFPmat = [0];
for i = 1:length(t) - 1
      dRFP = (((kPyeaR/(NsrRmat(i) + 1)^d)*RFPmax)/(RFPmax +
kRFPsat*RFPmat(i)))-kRFPdeg*RFPmat(i)^c;
      RFPmat = [RFPmat RFPmat(i)+dRFP];
end
```

```
%constructing GFP over time
GFPmat = [GFPmax];
for i = 1:length(t) - 1
    dGFP =
(kPL*GFPmax/(RFPmat(i)+1)^e)/(GFPmax+kGFPsat*GFPmat(i))-kGFPdeg*GFPmat(i)^f;
    if GFPmat(i)+dGFP < 0
        GFPmat = [GFPmat 0];
    else
        GFPmat = [GFPmat GFPmat(i)+dGFP];
    end
end

%plotting GFP & RFP over time
plot(t, GFPmat, 'Color','green')
hold on
plot(t, NO3mat, 'Color', 'yellow')
plot(t, NsrRmat, 'Color', 'blue')
plot(t, RFPmat, 'Color', 'red')
legend('GFP','NO3-','NsrR', 'RFP')
```

B. Summary of all the constants that were used

Constant	Physical meaning	Default Value	Unit
k_1	recovery rate of active NsrR	0.00001	$M \cdot sec^{-1}$
$[NsrRmax]$	saturation level of NsrR	0.1	M
k_{sat}	saturation dependency of NsrR	50	-
k_{NO3}	NO3 inhibition strength 1	0.0001	$M^{-(a+b)} \cdot sec^{-1}$
a	NO3 inhibition dependency on active NsrR concentration	0.1	-
b	NO3 inhibition strength 2	0.8	-
k_{PyeaR}	basal promoter expression rate of PyeaR promoter	0.00002	$M^{d+1} \cdot sec^{-1}$
d	dependency of promoter kPyeaR strength on NsrR concentration	50	-
$[mScarletmax]$	saturation concentration of RFP	0.1	M
$k_{mScarletsat}$	saturation constant for mScarlet	50	-
$k_{mScarletdeg}$	degradation rate of mScarlet	0.000001	$M^{1-c} \cdot sec^{-1}$
c	dependency of mScarlet degradation on mScarlet concentration	1	-
k_{PL}	PL promoter strength	0.00001	$M^{e+1} \cdot sec^{-1}$

$[mVenusmax]$	saturation concentration of mVenus	0.1	M
$k_{mVenusdeg}$	degradation constant for mVenus	0.001	$M^{1-f} \cdot sec^{-1}$
e	dependency of promoter kPL strength on c1 concentration	50	-
f	dependency of GFP degradation on mVenus concentration	2	-

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