After completing the planning process, the next step was to design a system of differential equations that would account for the various pieces of the model. The first draft of this system is shown below, along with a brief description of what each equation represents, and what the various terms in each equation account for.

$$\frac{dP}{dt} = -binding + source + initial\,concentration + unbinding$$

This equation represents the pheromone concentration, (matA or matα) available to interact with the receptors of the cell. The equation was designed to account for the pheromones secreted by the yeast cells, the binding of the pheromone to the receptor, and the possible unbinding of the pheromone from the receptor. The secretion of the pheromone from one yeast cell was assumed to be independent of the changes exhibited by the other yeast cells. It was also assumed that this pheromone was equally distributed around the whole environment of the cells.

$$\frac{dB}{dt} = binding - unbinding - decay$$

$$\frac{dU}{dt} = -binding + unbinding$$

These two equations represent the two possible states of the pheromone receptor, (Ste2 or Ste3) bound to pheromone or unbound. Due to the relatively small time scale that these proteins significantly contribute to the behavior of the system, the production and decay terms were assumed to be negligible. Furthermore, this model also assumes that all unbound receptors are capable of being bound and that all bound receptors are capable of signal transduction.

$$\frac{dS}{dt} = f(B) - j(S)$$

$$\frac{dA}{dt} = h(S) + k(X, A)$$

These two equations represents a hypothetical signal that directly stimulated the production of the team's fluorescent hetero-transcription factor. The S equation signified the series of protein kinase interactions, caused by the production of bound pheromone receptor and a decay of the signal as the proteins are turned off. The A equation represented the total sum of the factors contributing to the synthesis of the construct, including the signal from the S equation, the auto-regulation of the construct, and the loss of the signal as the construct is synthesized.

$$\frac{dX}{dt} = X \cdot c \cdot - d \cdot X$$

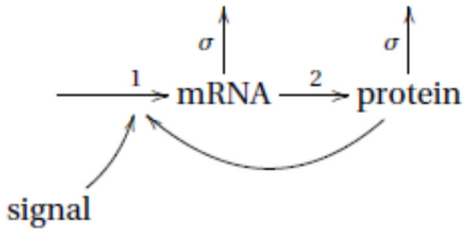
Finally this equation represented the concentration of the synthetic hetero-transcription factor that is produced by the signal, and is lost over time due to decay.

After creating this simple model, the team began doing research on the kinetic rate constants and binding affinities for the various parts of the model. During this research, the team came across a published model from Kofahl and Klipp's paper, *Modeling the dynamics of the yeast pheromone pathway*. Having read their paper, the team decided that a number of the interactions depicted in their model would be sufficient to be directly applied to the team's model. Furthermore, the existence of this model allowed for an approximation of the behavior of activated Ste12, and resulted in a revision of the direction of the model. Since the interactions of the pheromone and receptor were known and documented they were no longer necessary to be recreated as part of the model. Instead,

the team decided to do a simple model of the transcriptional and translational processes, according to the model presented in Nakakuki et. al.'s paper *Quantitative control of early transcription by erk and akt signal transduction*.

Despite these changes to the direction and emphasis of the model, the effect of mating factor on Ste12 activity remained important areas of interest. In order to better understand this relationship, two different routes were employed. First, the model from the paper was recreated and run under varying concentrations of mating factor. The results of those simulations indicated that there was a direct correlation between the amount of external pheromone and the amount of activated Ste12. The simulation also indicated that after activation, the relative level of active Ste12 remained fairly constant for the remainder of mating process. While there was significant evidence to believe that the results from these simulations were accurate representations of the system, the team also sent Kofahl and Klipp's model to the NTNU team for them to create a stochastic model of the system (to learn more about this see the Stochastic model section).

Nakakuki's model used a couple of very simple reactions to simulate the processes involved in transcription and translation. First there is some signal that is used to create mRNA. In the case of the team's model, the signal is directly proportional to the level of activated Ste12. Once produced, mRNA has two possible routes: either it decays and nothing comes of it, or it is translated and used to make protein. After the protein is made, it adds to the signal to generate more mRNA or it decays. The figure below presents the reaction diagram for this system.



A cell's level of activated Ste12 remains relatively constant, as previously established by the team's differential model that after a brief initiation period, once exposed to mating factor. This result was also confirmed with a stochastic model created by the NTNU team. Because of this apparent constant signal, the team simplified the signal to a constant. This simplification allowed the system for defining the system to a pair of differential equations:

$$\frac{dM}{dt} = v_1 \cdot \left(\frac{S+P}{k_1 + S + P} \right)^n - \sigma_1 \cdot M$$

$$\frac{dP}{dt} = k_2 \cdot M - \sigma_2 \cdot P$$

This small system acted to model the amount of mRNA, *M*, and the amount of protein, *P*. This model assumed that the signal and protein act identically with various enzymes, ie. the transcription apparatus, thus resulting in the Michaelis-Mentin form of the synthesis term of mRNA. While that assertion was not completely accurate, since the protein used a different enhancer element to promote the production of the protein, it is a similar enough system to be grouped into a single equation. This equation implied that there is some maximum rate to this reaction, *v1*, with some saturation concentrations of *S* and *P*, that allow the reaction to perform at one half of the maximum rate, *k1*. This model also assumed that there are *n* independent binding sites that must be bound before any mRNA is produced. The subtractive term of the mRNA represented the decay of the mRNA, with *σ1* representing a rate constant. The protein equation allowed for the production of protein based

upon some rate constant, *k2*, and the amount of mRNA, and the decay of protein based upon some decay rate, *σ2*.

In order to analyze this system, it was necessary to form a series of dimensionless scaled variables. The various concentration terms, *P* and *M*, were all scaled in terms of *k1*, creating new variables *p* and *m*. The time parameter was also scaled by a factor of an unknown value, represented by *t**, to form a new parameter τ. These scaling relationships are illustrated below:

$$p = \frac{P}{k_1} \qquad m = \frac{M}{k_1} \qquad \tau = \frac{t}{t^*}$$

Using the chain rule, substituting these new variables into the equation, and simplifying the expression the two new differential equations shown below were made.

$$\frac{dm}{d\tau} = \frac{t^* \cdot v_1}{k_1} \cdot \left(\frac{\frac{S}{k_1} + p}{1 + \frac{S}{k_1} + p} \right)^n - t^* \cdot \sigma_1 \cdot m$$

$$\frac{dp}{d\tau} = t^* \cdot k_2 \cdot m - t^* \cdot \sigma_2 \cdot p$$

For convenience, *t** was set equal to *k1/v1*, reducing the coefficient on the Michaelis-Mentin term to 1. Substituting that value of *t** into all instances in the equations, resulted in several groupings of variable becoming very apparent. These apparent groupings were grouped into dimensionless groups, shown below:

$$\alpha = \frac{S}{k_1} \qquad \beta = \frac{k_1 \cdot \sigma_1}{v_1} \qquad \gamma = \frac{k_1 \cdot k_2}{v_1} \qquad \delta = \frac{k_1 \cdot \sigma_2}{v_1}$$

Substituting these dimensionless variables into the differential equations results in the simplified equations depicted below:

$$\frac{dm}{d\tau} = \left(\frac{\alpha + p}{1 + \alpha + p} \right)^n - \beta \cdot m$$

$$\frac{dp}{d\tau} = \gamma \cdot m - \delta \cdot p$$

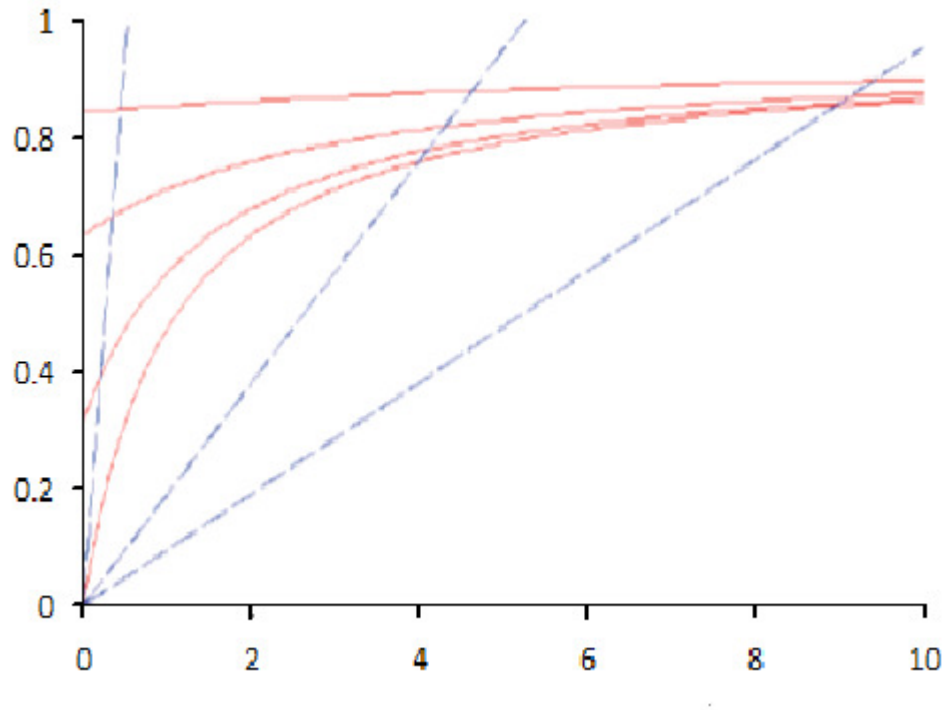
Once these simplifications had been made, the steady-state analysis of this system began. A system of differential equations approaches a steady state when the derivatives are set equal to zero. Solving the second equation for *m*, and substituting it into the first equation yielded the first equation, shown below. This equation was then solved for *p*, with a new unitless variable *B* introduced, which was the product of ß and δ divided by γ. This equation is also pictured below.

$$\left(\frac{\alpha + p}{1 + \alpha + p} \right)^n = \frac{\beta \cdot \delta}{\gamma} \cdot p$$

$$B = \frac{\beta \cdot \delta}{\gamma}$$

$$p = \frac{1 - (\alpha + 1)\cdot B \pm \sqrt{(1 - (\alpha + 1)\cdot B)^2 + 4\cdot\alpha\cdot B}}{2\cdot B}$$

Doing some algebraic analysis shows that as long as α is greater than zero, then both roots are real and opposite in signs. This means that there is only one significant non-zero state for this system one can't have a negative concentration. This can be understood qualitatively by considering the following graph:



This graph illustrates the intersection of the two functions shown below. The first equation is plotted in red, and the second equation is plotted in blue. By inspecting the graph, it can be seen that regardless of the value of *p*, there is only a single intersection of these functions. Therefore this is only one non-zero point where these equations are both satisfied, ie. only a single steady-state exists.

$$\frac{\alpha + p}{1 + \alpha + p}$$
$$\frac{\beta\cdot\delta}{\gamma}\cdot p$$

The other situation to consider is the zero solution, when there is no α, or signal. When this occurs then two possible steady states arise, shown below. The second of these steady states arises only when *B* is greater than one. In physical terms this relationship shows that the zero state is only possible if the product of the decay terms, *σ1* and *σ2* is greater than the product of *v1* and *k2*. Since there are now two different possible steady states, the stability of those steady states becomes an area of interest.

$$p_1^* = \frac{1 - B}{B} \qquad m_1^* = \left(\frac{\delta}{\gamma}\right)\cdot p_1^*$$

$$p_2^* = 0 \qquad\qquad m_2^* = 0$$

In order to evaluate the steady state of the system the Jacobian of the system was determined. The Jacobian of a system is a matrix consisting of the partial derivatives of each function in the system with respect to each variable. A sample of this is shown below:

$$\frac{dx}{dt} = f(x,y)$$
$$\frac{dy}{dt} = g(x,y)$$

$$\begin{bmatrix} \frac{\partial f}{\partial x} & \frac{\partial f}{\partial y} \\ \frac{\partial g}{\partial x} & \frac{\partial g}{\partial y} \end{bmatrix}$$

Applying this operation to the system of interest yields the following matrix of the Jacobian.

$$\begin{bmatrix} -\beta & \frac{1}{(1+\alpha+p^*)^2} \\ \gamma & -\delta \end{bmatrix}$$

For a steady state of the system to be asymptotically stable, the Jacobian must have a negative trace and a positive determinant. Since the trace is defined as the sum of the elements of the primary diagonal, ie. the diagonal running from the upper left to the lower right of the matrix, and both terms are negative, their sum must always be negative. Computing the determinant and setting it greater than zero results in the following relationship:

$$B - \frac{1}{(1+\alpha+p^*)^2} > 0$$

Combining all these bits of data the following set of conclusions were made about the project construct: if there is no input signal and *B* is greater than one, than only the zero steady state exists and it is stable. If there is no input signal and *B* is less than one then there are two stable steady states:

$$p^* = 0$$
$$p^* = \frac{1}{B} - 1$$

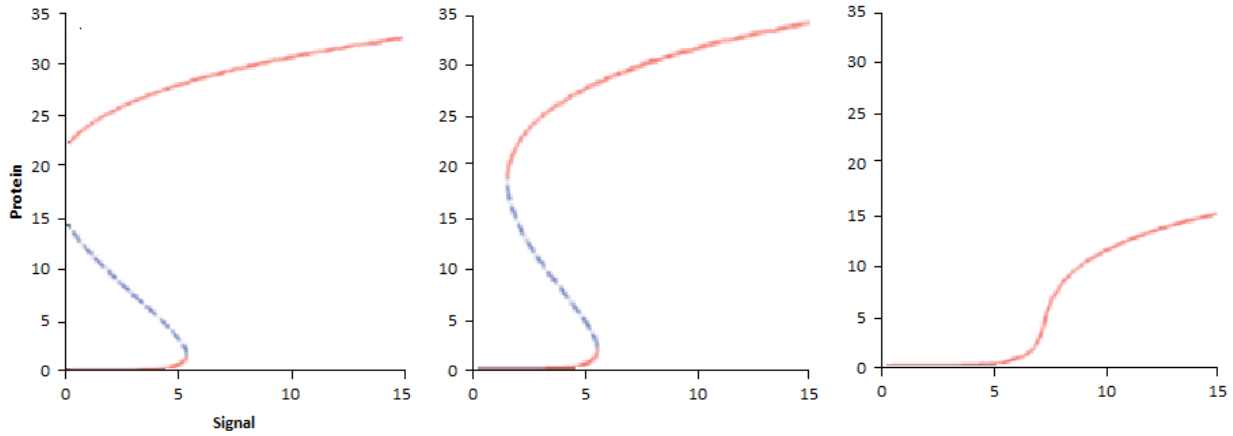
This same analysis was also performed for the case where there was an input signal. Performing some algebraic manipulation and a change of variables the following inequalities were made. Analyses of these inequalities lead to the conclusion that there is one significant steady state and it is stable.

$$B \cdot \left(1 - \frac{4 \cdot B}{\left(1 + (\alpha+1)\cdot B + \sqrt{(1+(\alpha+1)\cdot B)^2 - 4\cdot B}\right)^2} \right) > 0$$

$$1 > \frac{y}{\left(x+\sqrt{x^2-y}\right)^2}$$

$$\frac{y}{\left(x+\sqrt{x^2-y}\right)^2} < \frac{x^2}{\left(x+\sqrt{x^2-y}\right)^2} = \left(\frac{x}{x+\sqrt{x^2-y}}\right)^2 < 1$$

The figures below illustrate the three possible solutions predicted by the mathematical model. The x-axis is a measure of the external mating pheromone concentration, and the y-axis depicts the amount of protein. The first illustration depicts a system where once any signal or protein is present the circuit is turned on and continues to produce more protein up to a cap. The second depicts a system where there a particular threshold of mating pheromone required to bring about a stable level of protein, below this level, the protein production is transient and returns to zero. The third depicts a system similar to the second where there is a threshold, where the protein is created up to cap, however once the signal drops below that threshold, it again returns to zero.



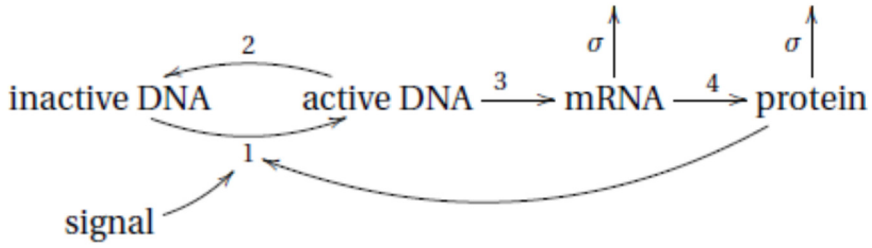
The same kind of analysis was performed on the following systems. The system shown below is exactly the same system, with the exception that it allowed for the perfect cooperatively between the various binding sites. That is to say that if one site is bound, it influences the binding of one or more of the other sites. This system was made because it was more likely to accurately to model the behavior of the construct, due to the cooperative behavior known to be expressed by Ste12. It was noteworthy that the constants *v1** and *k1** were similar in function to *v1* and *k1* from the original equations but not exactly the same values.

$$\frac{dM}{dt} = v_1^* \cdot \frac{(S+P)^n}{\left(k_1^*\right)^n + (S+P)^n} - \sigma_1 \cdot M$$

$$\frac{dP}{dt} = k_2 \cdot M - \sigma_2 \cdot P$$

The results of the analysis indicated that all the things that were true for the first system were also true for this system, so long as *n* was equal to one. When *n* was greater than one, the number of steady states increased from two to three. Analysis of these steady states indicated that like the previous model, the zero steady state and the largest steady state were stable, however, the middle steady state was unstable.

As a final portion of the mathematical modeling section of the project, the following more complex system was also considered. The illustration below illustrates a more realistic model of the processes of transcription and translation.



This more advanced model could be modeled using the following differential equations:

$$\frac{dA}{dt} = v_1 \cdot \frac{(S+P)\cdot I}{k_1 + I} - \frac{v_2 \cdot A}{k_2 + A} - k_3 \cdot A$$

$$\frac{dI}{dt} = v_2 \cdot \frac{A}{k_2 + A} - v_1 \cdot \frac{(S+P)\cdot I}{k_1 + I} + k_3 \cdot A$$

$$\frac{dM}{dt} = k_3 \cdot A - \sigma_1 \cdot M$$

$$\frac{dP}{dt} = k_4 \cdot M - \sigma_2 \cdot P$$

Because the DNA is not consumed during the conversion between active and inactive DNA, then it must be conserved. This means that those two differential equations, *A* and *I* could be combined into a single equation.

$$\frac{dA}{dt} = v_1 \cdot \frac{(S+P)\cdot(T-A)}{k_1 + T - A} - v_2 \cdot \frac{A}{k_2 + A} - k_3 \cdot A$$

$$\frac{dM}{dt} = k_3 \cdot A - \sigma_1 \cdot M$$

$$\frac{dP}{dt} = k_4 \cdot M - \sigma_2 \cdot P$$

The same analysis illustrated in the simple example was performed on this system of equations with the following results. For the dimensionless variable simplification, the following identities were used:

$$a = \frac{A}{k_1} \qquad p = \frac{P}{k_1} \qquad m = \frac{M}{k_1} \qquad \tau = \frac{t}{\frac{1}{v_1}}$$

$$\alpha = \frac{S}{k_1} \qquad \beta = \frac{\sigma_1}{v_1} \qquad \gamma = \frac{k_4}{v_1} \qquad \delta = \frac{\sigma_2}{v_1}$$

$$\varepsilon = \frac{k_3}{v_1} \qquad \theta = \frac{T}{k_1} \qquad \psi = \frac{v_2}{v_1 \cdot k_1} \qquad \phi = \frac{k_2}{k_1}$$

These variables resulted in the following series of differential equations:

$$\frac{da}{d\tau} = \frac{(\alpha + p)\cdot(\theta - a)}{1 + \theta - a} - \psi\cdot\frac{a}{\phi + a} - \varepsilon\cdot a$$

$$\frac{dm}{d\tau} = \varepsilon\cdot a - \beta\cdot m$$

$$\frac{dp}{d\tau} = \psi\cdot m - \delta\cdot p$$

The steady state identification of this system resulted in the following equations.

$$\eta = \frac{\delta}{\gamma}\cdot p$$

$$a = \frac{\beta\cdot\delta}{\varepsilon\cdot\gamma}\cdot p = \eta\cdot p$$

$$0 = \frac{(\alpha + p)\cdot(\theta - \eta\cdot p)}{1 + \theta - \eta\cdot p} - \frac{\psi\cdot\eta\cdot p}{\phi + \eta\cdot p} - \varepsilon\cdot\eta\cdot p$$

Analytical work beyond this point proved to be a very difficult task due to the number of unknown parameters. However, numerical investigations of this system showed that the one-way switch and the sigmoidal shape were still possible.