# Methods

## Determining Initial Parameters

The system is known to have negative feedback, but the specifics are unclear. The paper began with six different simple models to determine which parameters are dominant and to see if they can determine any consensus parameters. The researchers gathered data about expression levels of Msm2 and p53 by taking single cell images over time. They then adjusted parameters for each model to match the data. The researchers sought to match the observed undamped oscillations in the system over multiple days. The parameters they determined for models IV, V, and VI appeared to have consensus in production and degradation rates. We chose to use model VI because it required a time delay in the system.

## Modeling the System

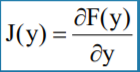
We went about modeling the system with the same parameters and initial conditions as determined in the paper. Using the MATLAB function dde23, we were able to incorporate the time delay in our set of differential equations and solve for a number of time points. This allowed us to produce plots showing the concentrations of the species and signal over any given amount of time. The system of time-delay differential equations is defined in modelVI.m. No noise was introduced at this time to allow us to explore the stability and tendencies of the system.

## Stability Analysis

We analyzed the stability of the system first by observation of the concentrations over a long period of time (figure 1?). This was most useful because we clearly had an oscillating non-linear system with a time-delay, making mathematical analysis difficult.

Additionally, we analyzed the system when the time delay is equal to 0, which allows the system to reach steady-state. In such a scenario scenario, Mdm2 has no maturation period and is immediately able to act on p53, allowing the species to reach an equilibrium rather than oscillate. Without a time delay, the development of the system from given initial conditions is determined by MATLAB’s ode45 function and visualized in figure 2.

The mathematical analysis first involved finding the steady state solution of the system using MATLAB’s fsolve function. The system is then linearized using Taylor’s theorem, allowing us to analyze stability using the Jacobian matrix.



*Top*: Taylor’s theorem. DF(yss) represents the first partial derivative of F(y), evaluated at steady state.

R(y, yss) includes all higher order terms, which are negligible near the steady state solution.

*Bottom*: The Jacobian matrix, which is equivalent to DF(yss).

After evaluating the Jacobian at the steady state solution, MATLAB’s eig function can be applied to find all eigenvalues.

General form of eigenvalues, solutions of the characteristic equation.

The real parts of the eigenvalues are taken to indicate stability, with all values required to be negative to assume that the system is stable at the steady state solution. If any eigenvalue is positive, the solution is unstable. In the case that an eigenvalue has no real part, the linearized analysis is not sufficient in making decisions about the stability of the system. Furthermore, if there are any complex components, we can expect the system to oscillate towards or away from the solution. This method was only applied to systems that had steady states. Results of these analyses were confirmed with comparison to concentration plots over time.

## Exploring Parameters

We chose parameters that could have disease implications as well as explored how adding a drug element could affect the system. First we looked at the simple case of changing signal conditions by changing Bs, the constant signal production rate. Furthermore, we can look at dynamic conditions where the signal is turned off and on at varying intervals.

Next, the degradation rate of Mdm2 was altered to create a system that has dampened oscillations. This system was plotted and the stability was analyzed as described previously. Lastly, another species was introduced: a drug that increases the degradation of Mdm2. This system was also plotted and the stability was analyzed.

Note: the time delay no longer affects the tumor condition. Neither does drug affect the eventual steady state stability.