**Overview**

The goal of the model was to determine what parameters were pertinent to the speed and accuracy of our design. We wanted to our model to speed up the experimentation process in the lab and give us a good idea of the characteristics of our construct. We also wanted to determine the optimal protein to biomarker ratio to meet our time constraint of 5 mins, since our survey indicated users prefer a fast test.

**Reaction**

The reaction that we are trying to optimize is shown below. Each half of our split protein is attached to a part of horse radish peroxidase (HRP). The halves of our protein form a complex with the biomarker. This brings the two parts of split HRP (sHRP) closer together and primed to reconstitute. After sHRP is reconstituted, it is functional and oxidizes tetramethyl benzidine with hydrogen peroxide to form a blue product for our colorimetric output.

(insert reaction breakdown image)

The reaction between the two halves of the protein complex and the biomarker, tyrosol/farnesol, were modeled as following two-step mass action kinetics (the biomarker first binds with on half to form a complex and then the complex binds with the other half). We assumed that once both halves of the protein bound together and formed a complex, the reconstitution of the split-HRP was instantaneous. Or in other words, the formation of the protein complex was the rate limiting step. The reconstituted HRP then oxidizes TMB into a blue compound for our colorimetric response. The reconstituted HRP was modeled as following Michaelis Menten kinetics.

The initial values for the parameters are listed in the table below along with their relative sources.

(insert initial value table)

The dissociation constants from the FRB, FKBP, and rapamycin reaction were used for the split protein reconstitution kinetics in the absence of the actual dissociation constants for our product. This was done because our sHRP construct was based on study who linked their HRP halves to FRB and FKBP respectively [1]. These two proteins come together to form a complex in the presence of rapamycin. This is a similar process to what we hope to achieve with our construct; the split protein forms a complex in the presence of the biomarker, farnesol or tyrosol.

According to our sensitivity analysis below, the dissociation constants did not seem to alter our calculations very much unless they were outside of the plausible/useful range (k\_d > 1). For this reason, we used one value to characterize all the k\_d values for the reaction to simplify our analysis. The value used was the average of the k\_ds.

Shown below are two plots running the simulation with the real and averaged k\_ds to show that there is little difference in the output when using these two values.

(insert figure of real and averaged kd)

As you can see, there is little difference between the two curves, and the calculated standard square error (SSE) between them was around 1E-9. This led us to conclude that matlab cannot be as precise with such small dissociation constants and that the use of real or averaged k\_d didn’t matter that much.

**Protein Concentration**

In modeling methods we wanted to implement in the future to better characterize our product, we would need to determine certain reaction constants which relies on known reagent concentrations, including protein concentrations. Since poly-histidine tags were not included in our genetic construct and comparing the colorimetric output between our sHRP and wild type HRP was not viable (since the difference in output could be attributed to different concentrations or activity levels), we had to figure out a different way to determine our protein concentrations once we lysed the cell.

Our model offered us one solution. It was observed in simulations of the reaction at fixed protein concentrations but varying biomarker concentrations, that the concentration of reconstituted sHRP at equilibrium was the greatest (around 60% of initial split protein concentration, .166 M) when the protein halves and biomarker concentration were the same.

(insert graph here)

This makes sense intuitively. When there is a lower concentration of biomarker compounds than split protein, only as many protein halves can recombine as there are biomarker molecules. However, if there is a large concentration of biomarker molecules than split protein, they will bind to each half rapidly, creating many comp1 and comp2 which cannot bind to each other to form comp3 and reconstitute HRP.

This was an important observation to make because it allows us to “titrate” our lysate to determine the protein concentration.

**Sensitivity Analysis**

In our sensitivity analysis, we adjusted four parameters, the dissociation constant (averaged), the Michaelis Menten constants for split-HRP (k\_cat and k\_m), and the protein concentration. The purpose of the sensitivity analysis was to determine which of these parameters affected the time in which all the TMB was reacted the greatest. Each parameter except one was held constant at the initial values listed in the table above. The varied parameter was swept across 7 orders of magnitude in either direction.

(insert graph here)

We can see clearly that protein concentration and k\_cat affect the time that it takes TMB to fully react the greatest, changing it by around 15 and 10 orders of magnitude respectively. Each lower the time considerably as they increase. K\_m and k\_d on the other hand don’t change the time as much or only do once they wander out of the domain of possible values.

Analysis

Km: It makes sense that Km doesn’t affect the output until increased by many orders of magnitude since it is the half saturation constant in Michaelis Menten kinetics. That is, Km doesn’t have a large impact on the speed of the reaction until it about the same order of magnitude as the concentration of substrate. Since the amount of substrate, TMB, will most likely always be much larger than the amount of protein, Km can be dismissed as an important parameter.

K\_d: The dissociation constant has little impact on the output unless it is completely out of range of plausible values, i.e. greater than 1. We do not expect the reverse reaction to have a higher rate than the forward, otherwise our split protein construct would not be very useful.

k\_cat and protein concentration: These both cause a large change in the reaction time. This makes sense since they are the components of V\_max in the Michaelis Menten rate law. Both decrease the reaction time as they get larger because they increase the max rate the reaction is capable of.

In conclusion, according to our sensitivity analysis, it seems it would be crucial to determine our sHRP’s k\_cat value and adjust our protein concentrations in order to meet our time constraint.

**Future Modeling**

Now that we have determined the important parameters, we know what to focus on once our protein construct is synthesized. We can find k\_cat of our sHRP by producing a Lineweaver Burk plot. Then, we can run multiple simulations at different protein to biomarker concentrations (at both infected and healthy concentration levels) to determine the optimal ratio that performs under 5 mins. but still has a large enough difference in complete reaction time to distinguish between a positive and negative test.

**Receiver Operating Characteristic (ROC)**

The ROC is an important property of diagnostic tests in industry, plotting the true positive rate (sensitivity) over the false-positive rate (1 – specificity). The integral of this curve gives you the accuracy of your diagnostic test.

Whilst waiting for our wet lab crew to construct the actual protein construct, we were able to model its behavior and develop a plausible ROC for our product using the protein concentration estimation method described above.

If we were to assume that our paper test had a split protein concentration equal to the concentration of biomarker present in an infected user, then the amount of reconstituted HRP can be approximated according to (figure of protein concentration graph). We can model the function of our split proteins in this configuration using concentrations of wild type HRP that we would expect of reconstituted sHRP for a given amount of test concentration of biomarker according to the figure.

1. Martell, J. D., Yamagata, M., Deerinck, T. J., Phan, S., Kwa, C. G., Ellisman, M. H., et al. (2016). A split horseradish peroxidase for the detection of intercellular protein–protein interactions and sensitive visualization of synapses. Nature Biotechnology 64(2), p. 10-12.

**Equations**

reaction\_1 = kf1\*pqsR1\*farnesol-kr1\*pqsR\_far;

reaction\_2 = kf2\*pqsR2\*farnesol-kr2\*pqsR2\_far;

reaction\_3 = kf3\*pqsR\_far\*pqsR2-kr3\*pqsR\_far\_pqsR;

reaction\_4 = kf4\*pqsR2\_far\*pqsR1-kr4\*pqsR\_far\_pqsR;

reaction\_6 = (k\_cat\*pqsR\_far\_pqsR)\*TMB/(K\_m+TMB);

dydt(ipqsR1) = (-reaction\_1 - reaction\_4);

dydt(ipqsR2) = (-reaction\_2 - reaction\_3);

dydt(ifar) = (-reaction\_1 - reaction\_2);

dydt(ipqFar) = (reaction\_1 - reaction\_3);

dydt(ipq2Far) = (reaction\_2 - reaction\_4);

dydt(ipqFarpq) = (reaction\_3 + reaction\_4);

dydt(iTMB) = (-reaction\_6);

dydt(iblue) = (reaction\_6);

