Modeling

Bacteria Aimed at Removing and Terminating Intestinal Invaders

The team decided to use the SimBiology application in MATLAB to model the three devices. The model will help the team understand the interactions in each devices and how devices would work in tandem. Each device model consists of sets of parameters that were found in the literature or through orders of magnitude approximation. The molecular interactions between species were modeled using the Law of Mass Action. Certain parameters from background literature searches depend on other kinetic equations such as Michaelis-Menten. These parameters were fit to follow law of Mass Action rate laws.

Since we used 96-well plates, the assumed working volume was 200 microliters.(2) The amount of bacteria was set to correlate with an optical density of 2.0. For E. coli, this amounts to 1.6E9 cells per milliliter. (1) The estimate for the amount of cells in each well was 3.2E8 cells. The team used BioBrick part pSB1C3, which is a high copy number plasmid. The average copy number for this plasmid is 200 per cell. (3) The promoter strengths were low, medium, and high. Using the RFP absorbance of three promoters (J23113, J23105, and J23100), a ratio was calculated with J23113 having the control absorbance (4).

The three device models developed were the Gliadin Degrader, Gliadin Cuff and Zonulin Cuff.

Gliadin Degrader Model

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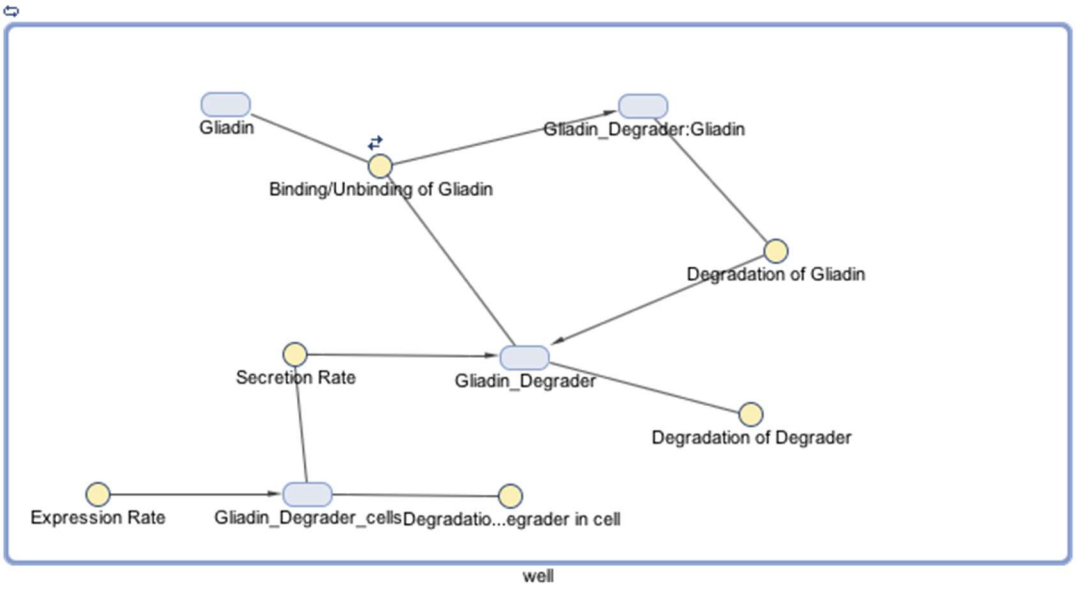


This diagram from SimBiology shows the molecular connections between each species. The governing equations for each species are:

$$\frac{d[GD]_{cells}}{dt} = K_E - K_s[GD]_{cells} - K_{DC}[GD]$$

$$\frac{d[GD]_{well}}{dt} = K_S[GD] - K_B[GD][G] - K_U[C] + K_{DG}[C] - K_D[GD]$$

$$\frac{d[G]}{dt} = -K_B[GD][G] + K_U[C]$$

$$\frac{d[C]}{dt} = k_B[GD][G] - K_U[C] - K_{DG}[C]$$

GD= Gliadin Degrader; G= Gliadin; C= gliadin degrader-gliadin complex

Kinetic Rate/Rate Constants	Significance
K_E	Expression
K_s	Secretion
K_{DC}	Degradation of Degrader in Cells
K_{DG}	Degradation of Gliadin
K_B	Association
K_U	Dissociation
K_D	Degradation of degrader in well

<u>Parameters of Gliadin Degrader Model</u>

The expression rate, K_E, was the product of the mRNA translation rate, the plasmids per cells number, number of cells and a promoter strength ratio. The average mRNA translation rate for E. coli cells is 15 amino acids per second. (5) The Gliadin Degrader had an average CDS of 2525 nucleotides per molecule. The mRNA translation rate for the degrader model is 1.06 degrader molecules per min per plasmid. This rate was multiplied by 200 plasmids per cell and 3.2E8 cells. Then, it was divided by Avogadro's number to yield 1.127E-13 moles per minute. The promoter strength was a ratio of the absorbance of desired promoter strength to the absorbance of the J23113 promoter.

The secretion rate constant, *Ks*, was less than 1, but the order of magnitude depended on what else was being expressed. Since it was just the Gliadin Degrader, the secretion rate constant was set to 0.01 min^{-1}.

The degradation of degrader in cell rate constant, $K_{DC,}$ was set to a low value, because the Gliadin Degrader was developed to have high resistance to degradation from other proteases. The rate constant was set to 1E-6 min^{-1}.

The degradation of gliadin constant, K_{DG}, was computed using Michaelis-Menten kinetics. In this model, k_{cat} is equal to K_{DG}. Using literature sources, the catalytic efficiency (k_{cat}/K_M) for the gliadin degrader was found to be 568.5 $M^{-1}s^{-1}$. (6) The gliadin degrader is similar to trypsin, so we made the assumption that it would have the same K_M as chymotrypsin. Chymotrypsin has a K_M of 1.5E-2 M. Using the catalytic efficiency provided, k_{cat} was 8.328 s^{-1}. An equation that relates K_M to k_{cat} is:

$$K_M = \frac{K_r + k_{cat}}{K_f}$$

In this model, we equate K_f to K_B and K_r to K_U, so the relationship between K_B and K_U is

$$K_B = \left(\frac{K_U}{k_{cat}} + 1\right) * \frac{k_{cat}}{K_M}$$

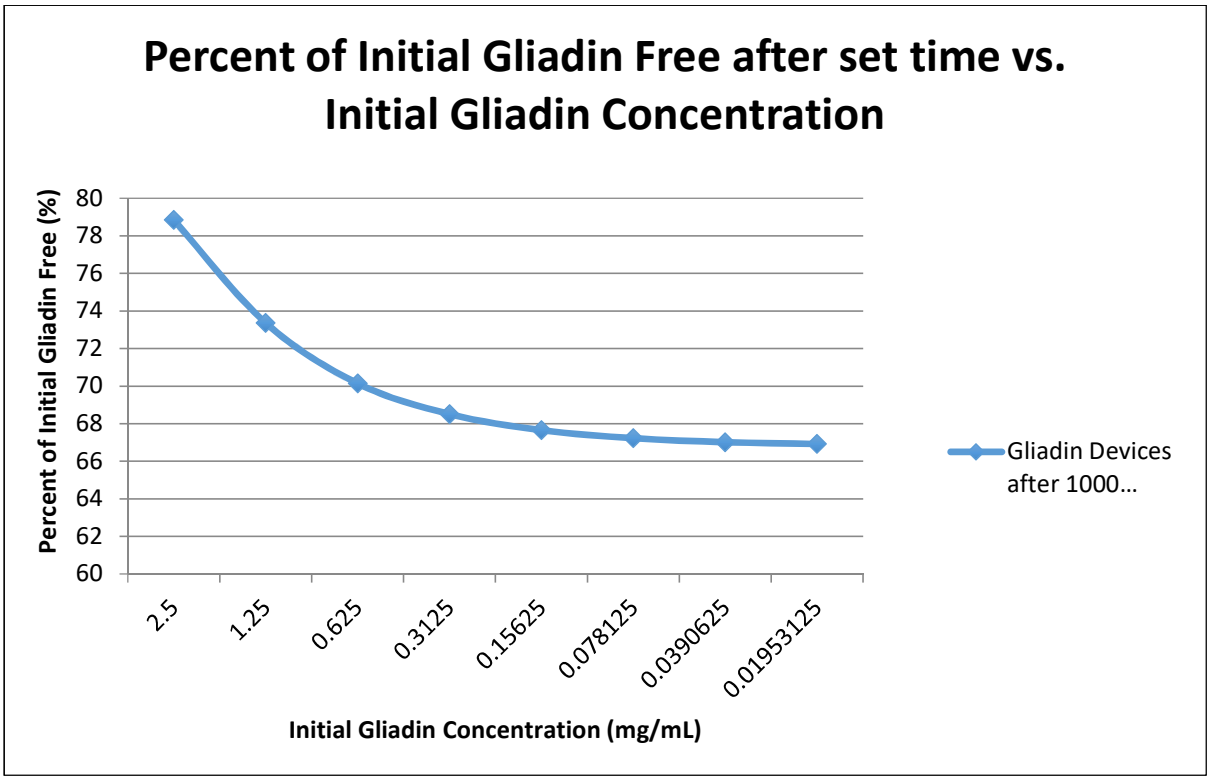
k_{cat} is 511.7 min^{-1}, k_{cat}/K_M was 34110 $M^{-1}min^{-1}$ and K_U was assumed to be 60 min^{-1}.

Therefore, the association constant, K_B, was 38100 M^{-1} min^{-1}.

The degradation of the degrader the well constant, K_D, was set to a low value in the same way that K_{DC} was. The well was supposed to simulate an intestinal environment and should be more reactive with the degrader than the protease concentration inside the cell. The constant was set to two orders of magnitude higher than K_{DC}, which is 1E-4 min^{-1}.

Gliadin Degrader – Gliadin Cuff Model

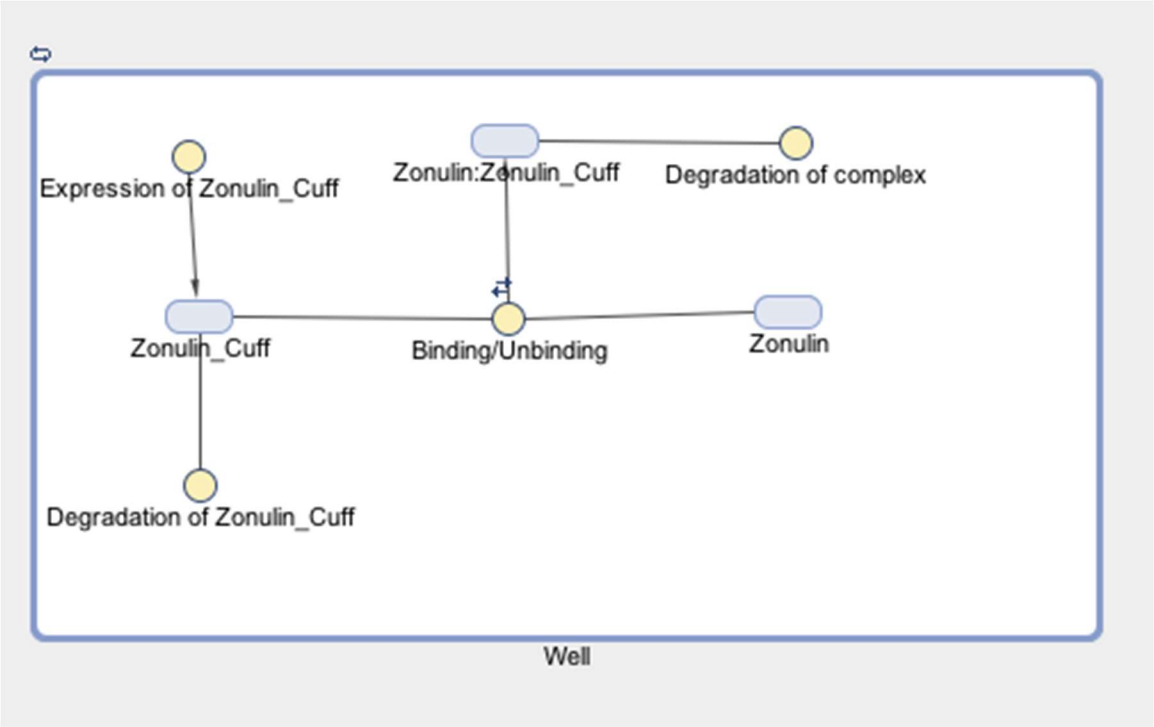
If the gliadin degrader and gliadin cuff were to be expressed in the same plasmid, it should be possible to reduce the percentage of gliadin unbounded, as there would be two mechanisms that are interacting with gliadin. It is assumed that the degraded and gliadin cuff do not interact. The expression rate would be dependent on the CDS for both devices. The rate of expression for both devices would be 1.001E-11 moles per minute. It was assumed that the expression rate for each would be half of the total expression rate. In addition to, the compartment of the cell is taken into account in relation to the gliadin cuff. The secretion rate was reduced to 0.001 min^{-1}. All of the other constants for each device were kept constant.



Graph 2. The relationship between the percent of the initial gliadin concentration unbounded by the Gliadin Cuff and the initial gliadin concentration for the gliadin devices.

Graph 2 illustrates another threshold where the percent of free gliadin does not decrease after a certain initial gliadin concentration. It seems to be around the same initial concentration that was observed in the previous graph. In order to increase the percentage of gliadin degraded and bound, the optical density would need to be increased to accommodate more cells that would increase the overall expression rate. Also, perhaps having a genetic switch that would enable the devices to have some lag time in between expression could reduce the effects that the molecules might have on each other.

Zonulin Cuff Model



The zonulin cuff model is similar to the gliadin cuff model. The zonulin cuff model has all the same parameters except for the initial concentration. Zonulin concentration in the body is on the ng/mL scale. Therefore, the zonulin cuff device need only have a large expression rate and the zonulin concentration will decrease quickly. The governing equations follow the same pattern as the gliadin cuff.

$$\frac{d[ZC]}{dt} = K_E - K_B[ZC][Z] + K_U[C] - K_D[C]$$

$$\frac{d[Z]}{dt} = -K_B[ZC][Z] + K_U[Z]$$

$$\frac{d[C]}{dt} = k_B[ZC][Z] - K_U[C] - K_C[C]$$

[Z] is the zonulin concentration. [ZC] is the zonulin cuff concentration.

As seen with the gliadin devices, halving of the expression rate might hinder the performance of each device. This wouldn't hinder the zonulin cuff because of the low concentration of zonulin it would be interacting with. Therefore, the gliadin degrader and zonulin cuff could be paired together in a plasmid with little drawback.

If the zonulin cuff and the gliadin cuff were to be expressed together, the cell compartment would have to be optimized to allow for an efficient amount of each receptor into the membrane. An increase in amount of cells would help alleviate the spatial limitation due to these two cuff devices.

References:

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