Diagnosis of Alzheimer's Disease Through Beta-Amyloid Detector

SimBiology Model of Biological Circuit

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SBOL Schematic

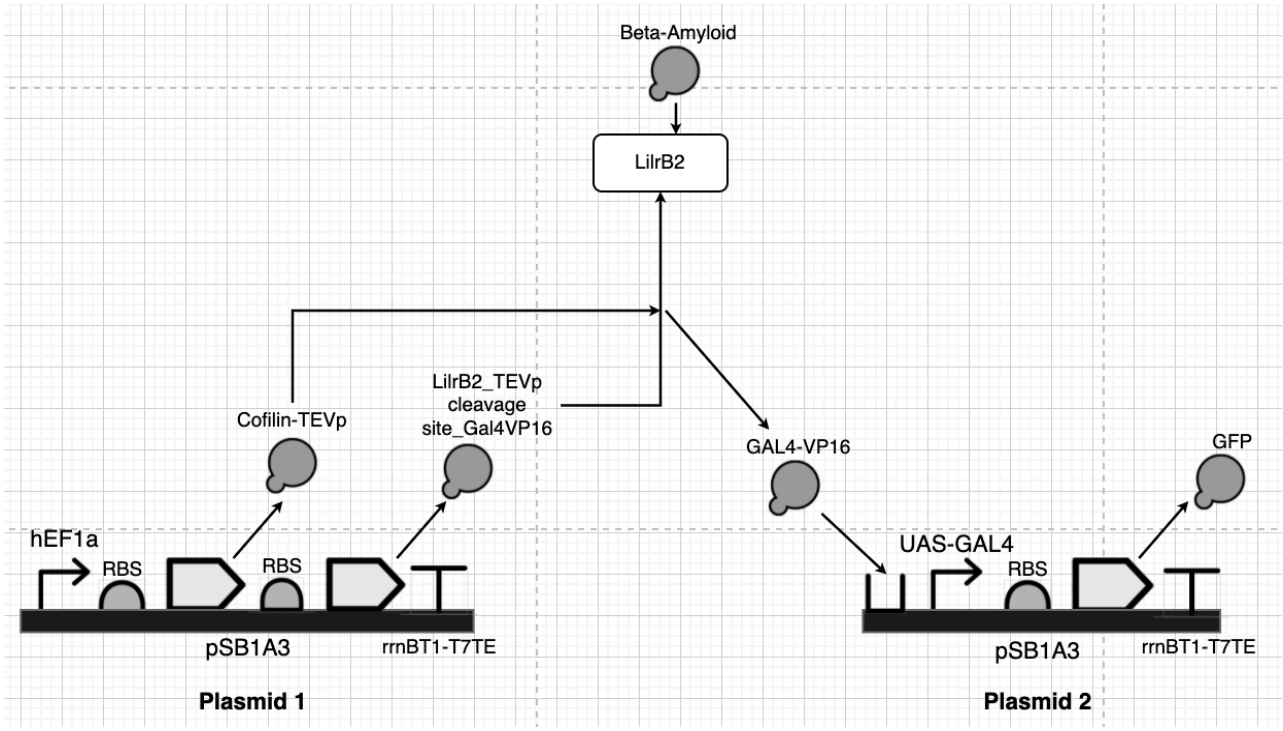


Figure 1: SBOL Schematic of the proposed circuit. Cofilin-TEVp and LilrB2_TEVp cleavage site_GAL4VP16 are constitutively promoted by hEF1a. Once Beta-Amyloid binds to the LilrB2 receptor, Cofilin-TEVp binds to the TEVp cleavage site and GAL4-VP16 is released. As a result, GAL4-VP16 positively induces the UAS-GAL4 promoter to produce GFP. Overall, the production of GFP should indicate the presence of Beta-Amyloid in the surrounding environment of the E. Coli host.

Methodology

The circuit, as depicted in Figure 1, was recreated in SimBiology. Cofilin-TEVp and the LilrB2_TEVp cleavage site_GAL4VP16 are under the regulation of the constitutive promoter hEF1a. As a result, to model the transcription rate of these two molecules, the constant rate assumption is made, which states that $rate_{transcription} = k_{trsc}$, where k_{trsc} is the constant of transcription of the hEF1a promoter. Pedone et. al. [1] experimentally found various parameters for constitutive promoter, including the k_{trsc} of hEF1a, and as a result our k_{trsc} was derived from there. The translation of the mRNA for either protein was defined as $rate_{translation} = \frac{[translation\ efficiency]}{[average\ mRNA\ life\ time]}$, where translation efficiency is 20 proteins/mRNA and the mRNA half-lives for both the Cofilin-TEV protein and LilrB2-TEV protein cleavage site is 2 minutes [2]. The latter was used in the calculation of the average mRNA lifetime based on the equation: $average\ mRNA\ life\ time = \frac{[mRNA\ half\ life]}{log(2)}$.The value for the translation efficiency was assumed to be the same as the translation efficiency value used by Elowitz in his article

regarding transcriptional regulators [2]. This article uses the steady state assumption to determine the translation efficiency and mRNA half-lives. The model in the article also uses E. Coli as the host cell, which corresponds to our circuit as well. Due to these similarities, we can assume that the values used in the article are similar to ours.

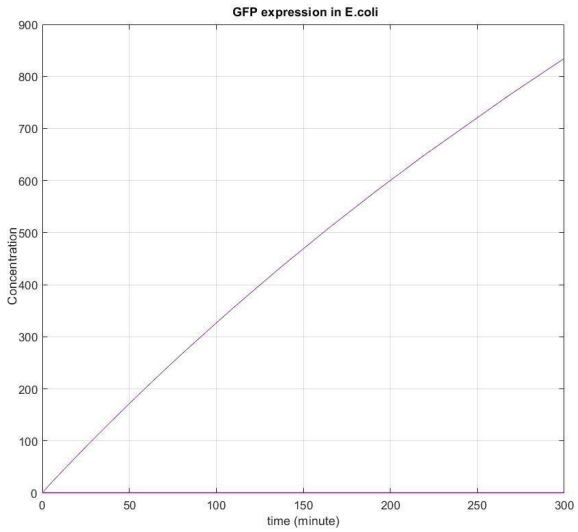
The complexation of the two proteins, Cofilin-TEVp and LilrB2-TEVp as seen in Figure 1, occur to release GAL4-VP16. The association of this complex is needed to establish the production of GFP. The association speed (*aps*) and dissociation speed (*dps*) values were assumed to be the same as the *aps* and *dps* values of the CAP and cAMP complexation reaction in an E.Coli cell [3]. Due to the specificity of the Cofilin-TEVp and LilrB2-TEVp complexation reaction for our circuit, it was necessary to assume these values to move forward with the modelling. The reason the CAP and cAMP complexation reaction values satisfy this need is attributed to CAP and cAMP both being proteins. Many experiments study the strength between a protein and some sequence of DNA for example (such as a promoter or operator). Furthermore, the CAP and cAMP complexation occurs in the same cell type as our model, E.Coli [3]. As a result, it is safe to assume the complexation seen in our circuit will have similar values.

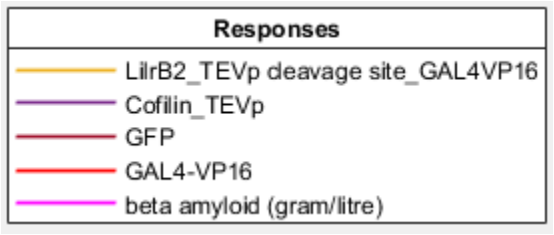
Beta-Amyloid then binds to the LilrB2 surface receptor and thereby initiates the release of GAL4-VP16. In the corresponding SimBiology model, this beta amyloid protein is seen at the very top of the cell attached to a protein degradation reaction ($rate_{protein\ degradation} = kd_{prot} \times [protein]$) where the kd_prot variable is defined by the equation: $kd_{prot} = \frac{log(2)}{[Protein\ half\ life]}$, using the mass action rate assumption and GFP's half life of 26 hours [4]. As this release is dependent on the concentration of Beta-Amyloid, it's reaction rate is defined by: $rate_{GAL4VP16\ release} = kf \times Complex$, where $kf = [k_{trsc\ of\ hEF1a}] \times (\frac{[beta\ amyloid]}{K} \div (n + \frac{[beta\ amyloid]}{K}))$ and n=1.

The transcription of GFP occurs under the promoter UAS-GAL4, which is positively induced by GAL4-VP16. As a result, in order to model the rate of transcription of GFP the mass action rate assumption is made. Therefore, the rate of transcription for GFP was found to be as follows, $rate_{GFP\ transcription} = [k_{trsc\ of\ UAS-GAL4}] \times (\frac{[GAL4-VP16]}{K} \div (n + \frac{[GAL4-VP16]}{K}))$, where n=1. The k_{trsc} of the UAS-GAL4 promoter was estimated using the Key Numbers for Cell Biologists [5], which stated that max transcription by RNA polymerase is 100 bases/s. By using this number and the known length of the UAS-GAL4 promoter we were able to convert k_{trsc} from bases/s to nM/s. For the translation of GFP, the base formula ($rate_{translation} = \frac{[translation\ efficiency]}{[average\ mRNA\ half\ life]}$) was used with substitutions made to account for the half life for GFP's mRNA which is estimated to be 7 hours [6]. It can be noted that this value was derived from the half life of wild

GFP's mRNA rather than lab GFP, however it was assumed that the half lives of either subspecies would be numerically similar.

Results and Discussion





Note that larger images of the graph are placed in Appendix A if needed.

Figure 2a: Expression of GFP when the presence of Beta-Amyloid is set to zero.

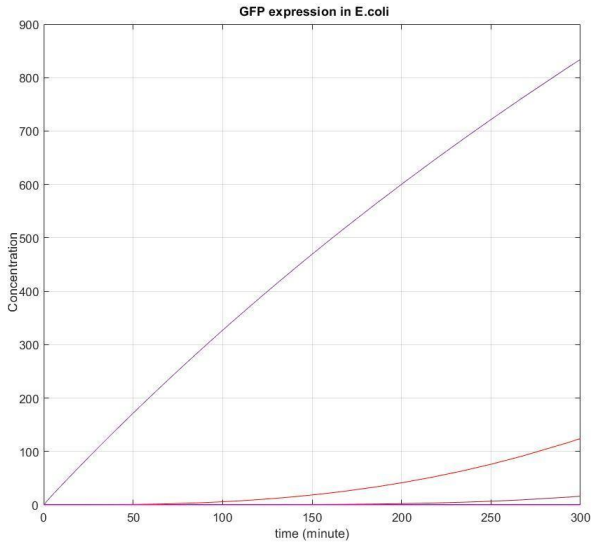


Figure 2b: Expression of GFP when Beta-Amyloid presence is set to 1.3 x 10^{-6} grams/litre.

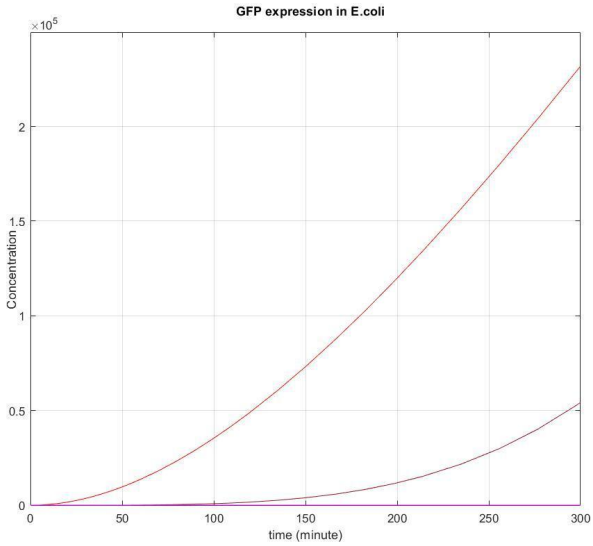


Figure 2c: Expression of GFP when Beta-Amyloid presence is set to 50 gram/litre.

As shown in Figure 2a, when Beta-Amyloid levels are at zero, GAL4-VP16 is not being released due to a lack of the complexation and cleavage reaction between Cofilin-TEVp and LilrB2-TEVp cleavage site. As a result of low GAL4-VP16 level, it cannot positively induce the UAS-GAL4 promoter to produce GFP. Therefore, both GAL4-VP16 and GFP concentrations remain at zero. In Figure 2b, Beta-Amyloid levels are set to 1.3 e-6 g/L, the experimental value of Beta-Amyloid present in the CSF of Alzheimer's disease patients [7]. At this level, Beta-Amyloid is able to bind to the LilrB2 receptor and release UAS-GAL4, positively inducing the UAS-GAL4 promoter to produce GFR as a result. Therefore, both UAS-GAL4 and GFP are being produced. Finally, in Figure 2c, there is a larger concentration of Beta-Amyloid. As more Beta-Amyloid is binding to the LilrB2 receptor, more GAL4-VP16 is being released which results in more GFP being produced. Due to the figures and explanations provided above, we can confirm that GFP indicates the presence of Beta-Amyloid in the surrounding environment of the E.Coli host as expected.

Appendix A

Larger images of Figures 2a - 2c.

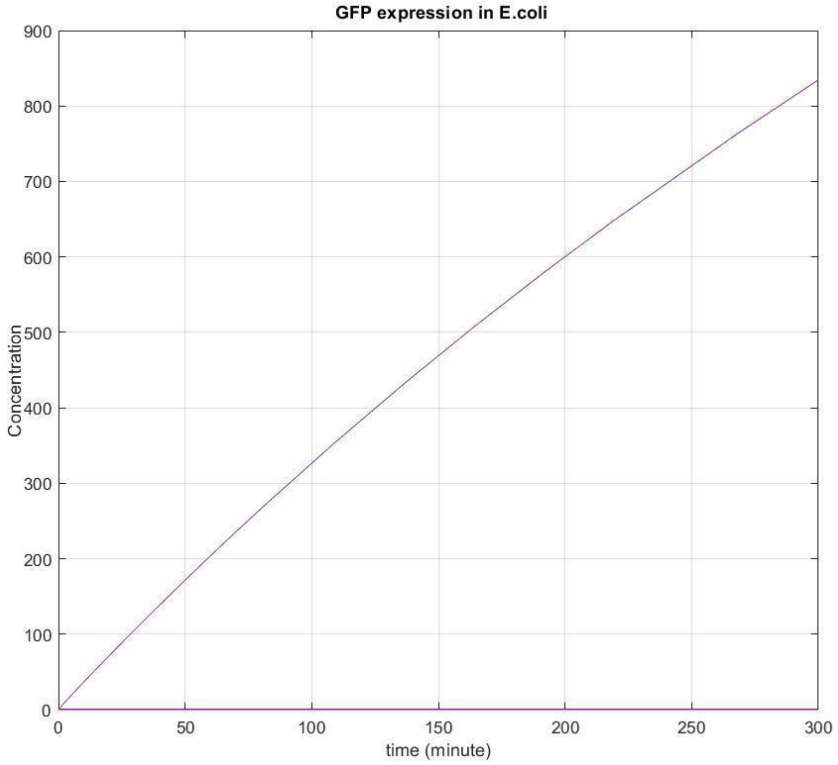


Figure A: Please refer to Figure 2a in the *Results and Discussion* section.

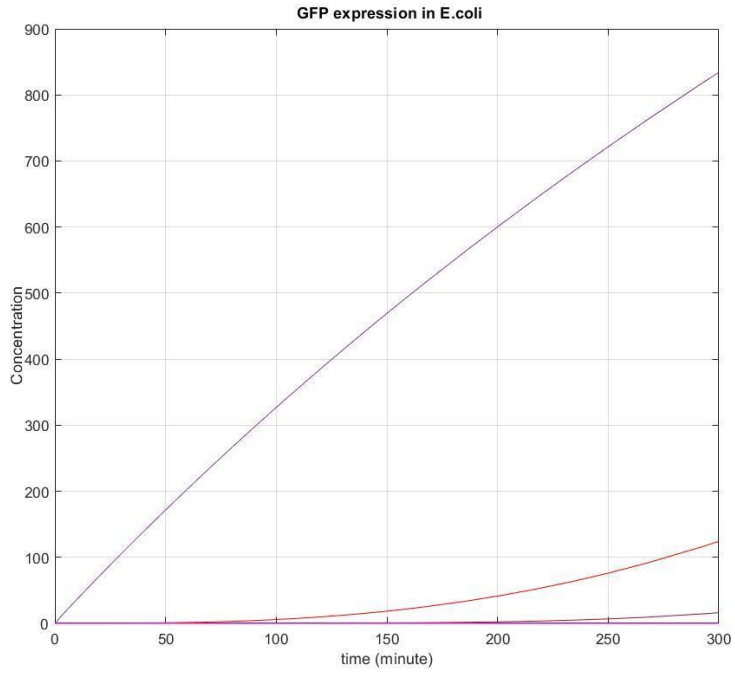


Figure B: Please refer to Figure 2b in the *Results and Discussion* section.

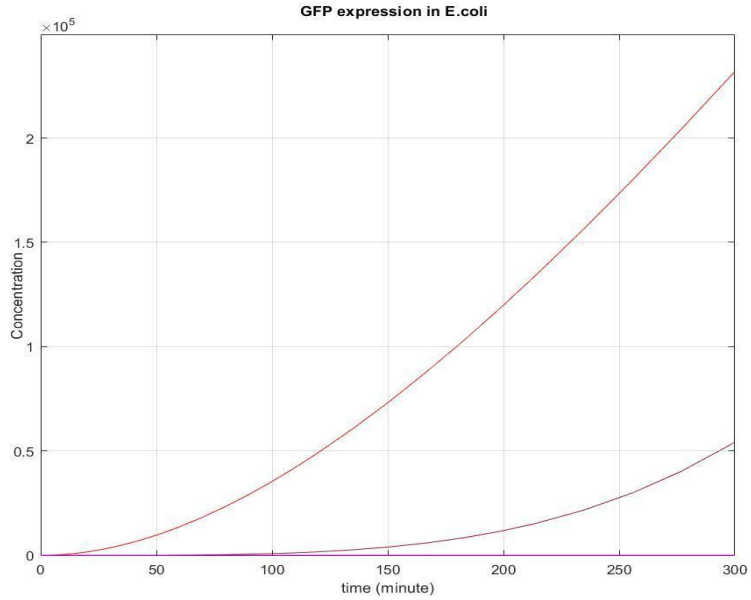


Figure C: Please refer to Figure 2c in the *Results and Discussion* section.

References

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