IBEHS 2P03: Health Solutions Design Projects II

M4: Detecting Heavy Metals and Contaminants in Drinking Water

Group 21

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Description of Model and System

The model represents a solution to our problem of testing water for heavy metal contaminants. The system demonstrated by Figure 1, is designed to give a visual alert when lead (Pb), mercury (Hg), or both are detected. If mercury is present, green fluorescent protein (GFP) will be expressed. On the other hand, if lead is present, yellow fluorescent protein (YPF) will be expressed. If both mercury and lead are present, TetR, CI, and blue fluorescent protein (BFP) are expressed. TetR is produced in this mechanism to inhibit the production of YFP if both mercury and lead are present. Similarly, CI is produced in this mechanism to inhibit the production of GFP if both mercury and lead are present. The overall mechanism contains an AND gate which is demonstrated by the outputs expressed when both lead and mercury are present. Table 1 portrays a truth table that represents this mechanism.

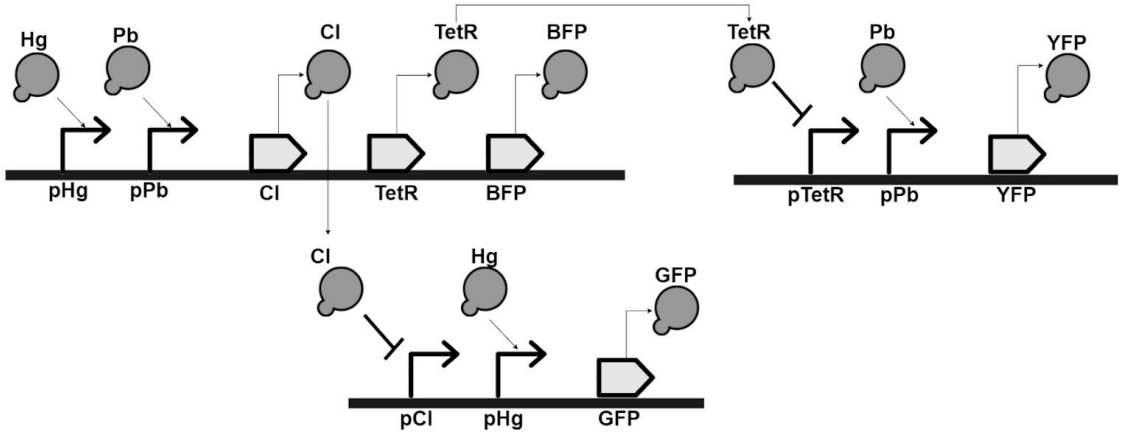

Figure 1: SBOL schematic of the lead and mercury detecting system for when one or both contaminants are present.

Table 1: A truth table that represents the overall mechanism in which Hg and Pb are the inputs and GFP, BFP, and YFP are the outputs.

Hg	Pb	GFP	BFP	YFP
0	0	0	0	0
0	1	0	0	1
1	0	0	1	0
1	1	1	0	0

Assumptions

There were several assumptions that were made when designing the system. To begin with, the promoters in series act similarly to non-competitive inhibition, but without the rapid equilibrium assumption that the product is formed. Secondly, the equations are based on GFP production, but used for all the proteins produced. Majority of the proteins and mRNAs in the system are very similar to GFP and would have had similar equations. When determining the removal of the proteins and mRNA, a zero rate assumption was made. Based on the values found in literature, the dilution of protein and mRNA can be neglected as degradation has a larger impact [1],[2]. Next, the constant rate assumption was made for the degradation of both mRNA and protein. A mass action rate assumption was also made allowing the rate of mRNA and protein decay to be proportional to the mRNA and protein concentrations, respectfully. Lastly, a steady state assumption was made as mRNA levels reach a stable state faster than proteins.

Noncompetitive inhibition

Noncompetitive inhibitors can bind to an enzyme and change the conformation of an enzyme as well as its active site, the efficiency will decrease because the substrate is unable to bind to the enzyme. As the inhibitor binds to the enzyme and the enzyme-substrate complex, it reduces the concentration of enzyme available for proper catalysis. In scheme 1, where E is enzyme, I is inhibitor, ES is enzyme-substrate complex, ESI is the molecule after the inhibitor is bound to the enzyme-substrate complex. TetR and Cl is the noncompetitive inhibitor to inhibit the production of GFP and YFP.

Simulation Plots

The graphs produced from SimBiology produced unexpected results, including the production of negative concentrations, an impractical result (Figure 2). What was expected was that when Hg is present, only GFP is produced and when only Pb is present, only YFP is produced. When Hg and Pb are present, only BFP is produced, as Hg and Pb cause the expression of TetR and CI which will inhibit the production of YFP and GFP, respectively. When there is no Hg or Pb, present, nothing is produced.

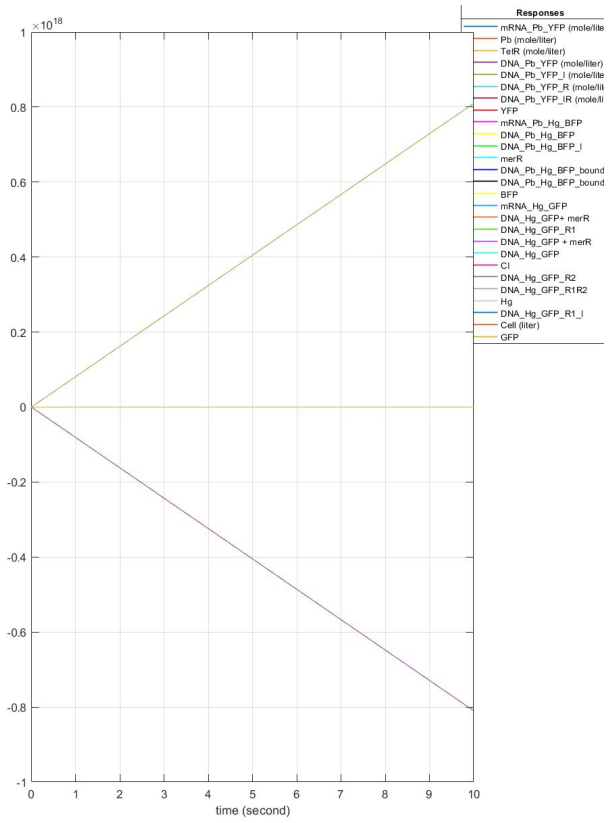

Figure 2. Simulation output for concentration of species (M) v time (s). Results were unexpected, as no species should reach a negative concentration.

Equations and Constants

The system acts similarly to a noncompetitive system as seen in Scheme 1. The following equations for the system were derived from it as well, following mass action kinetics.

Scheme 1

(Km')		(Km')
E + S ⇌ ES	E = P (promoter)	P + I ⇌ PI
+ +	S = I (inducer)	+ +
I I	I = R (repressor)	R R
⇌ ⇌		(KI) ⇌ ⇌(KI)
EI+ S ⇌ ESI		PR + I ⇌ PIR
(Km')		

Pb sensor, Hg-independent

$$\frac{d[PI]}{dt} = K_1[P][I] - K_{-1}[PI] + K_{-2}[PIR] - K_2[PI][R]$$

$$\frac{d[PR]}{dt} = K_3[P][R] - K_{-3}[PR] + K_{-4}[PIR] - K_4[PR][I]$$

$$\frac{d[PIR]}{dt} = K_4[PR][I] - K_{-4}[PIR] + K_2[PI][R] - K_{-2}[PIR]$$

$$\frac{d[P]}{dt} = -K_1[P][I] + K_{-1}[PI] - K_3[P][R] + K_{-3}[PR]$$

$$\frac{d[mRNA]}{dt} = K_{trsc}[PI] - d_m[mRNA]$$

Hg sensor, Pb-independent

$$\frac{d[PR_1]}{dt} = K_1[P][R_1] - K_{-1}[PR_1] + K_{-2}[PR_1R_2] - K_2[PR_1][R_2]$$

$$\frac{d[PR_2]}{dt} = K_3[P][R_2] - K_{-3}[PR_2] + K_{-4}[PR_1R_2] - K_4[PR_2][R_1]$$

$$\frac{d[PR_1R_2]}{dt} = K_4[PR_2][R_1] - K_{-4}[PR_1R_2] + K_2[PR_1][R_2] - K_{-2}[PR_1R_2]$$

$$\frac{d[P]}{dt} = -K_1[P][R_1] + K_{-1}[PR_1] - K_3[P][R_2] + K_{-3}[PR_2]$$

$$\frac{d[PR_1]}{dt} = K_1[P][R_1] - K_{-1}[PR_1] - K_5[PR_1][I] + K_{-5}[PR_1I] - K_2[PR_1][R_2] + K_{-2}[PR_1R_2]$$

$$\frac{d[mRNA]}{dt} = K_{trsc}[PI] - d_m[mRNA]$$

$$P = mRNA$$

$$I = Hg$$

$$R_1 = merR$$

$$R_2 = CI$$

Hg and Pb sensors, Hg- and Pb-dependent

$$\frac{d[PI]}{dt} = K_1[P][I] - K_{-1}[PI] + K_{-2}[PI_{bound}] - k_2[PI][protein]$$

$$\frac{d[P_{bound}]}{dt} = K_3[P][R] - K_{-3}[P_{bound}] + K_{-4}[PI_{bound}] - K_4[P_{bound}][I]$$

$$\frac{d[PI_{bound}]}{dt} = K_4[P_{bound}][I] - K_{-4}[PI_{bound}] + K_2[PI][protein] - K_{-2}[P_{bound}I]$$

$$\frac{d[P]}{dt} = -K_1[P][I] + K_{-1}[PI] - K_3[P][protein] + K_{-3}[P_{bound}]$$

$$\frac{d[mRNA]}{dt} = K_{trsl}[PI] - d_m[mRNA]$$

Translation Systems

$$\frac{d[protein]}{dt} = K_{trsl}[mRNA] - K_{dm}[protein]$$

The equations used in the system had various constants, which are listed in Table 2 below. The constants were obtained from eukaryotic systems that produced GFP. This was a reasonable assumption as the majority of the proteins produced in the system are similar to GFP.

Table 2: Constants used.

mRNA degradation [3]	0.25 h^{-1}
Protein degradation [4]	0.2 h^{-1}
K_1 [5]	4.5×10^4 $mM^{-1}s^{-1}$
K_{-1} [5]	2.3×10^4 s^{-1}
K_2 [5]	1.2×10^2 $mM^{-1}s^{-1}$
K_{-2} [5]	1.0×10^{-2} s^{-1}
K_3 [6]	2.4×10^4 $mM^{-1}s^{-1}$
K_{-3} [6]	9.4×10^{-4} s^{-1}
K_4 [6]	7.3×10^3 $mM^{-1}s^{-1}$
K_{-4} [6]	2.5×10^{-4} s^{-1}
K_tl [7]	2.78×10^{-15} nm/h

References

[1] I. Golding, J. Paulsson, S. M. Zawilski, and E. C. Cox, “Real-Time Kinetics of Gene Activity in Individual Bacteria.”

[2] E. Eden et al., “Proteome half-life dynamics in living human cells,” Science (80-.)., vol. 331, no. 6018, pp. 764–768, Feb. 2011.

[3] S. Subramanian and F. Srienc, “Quantitative analysis of transient gene expression in mammalian cells using the green fluorescent protein,” 1996.

[4] I. G. Darvey, R. Shracer, and L. D. Kohn, “Integrated Steady State Rate Equations and the Determination of Individual Rate Constants,” 1975.

[5] V. Chandra Sekhar and B. V Plapp, “Rate Constants for a Mechanism Including Intermediates in the Interconversion of Ternary Complexes by Horse Liver Alcohol Dehydrogenase1",” 1990.

[6] A. Takai, Y. Ohno, T. Yasumoto, and G. Mieskes, “Estimation of the rate constants associated with the inhibitory effect of okadaic acid on type 2A protein phosphatase by time-course analysis,” Biochem. J., vol. 287, pp. 101–106, 1992.

[8] B. Lewin, *Genes VIII*. 2003.