1.Overview

Modeling is a powerful tool in synthetic biology and engineering. Mmodeling has provided us with an important engineering approach to characterize our pathways and predict their performance, thus helped us with modifying and testing our designing.

Basically, the models built by us can be divided into two parts.

On kinetic model parts, we hope to gain insight of the gene expression dynamics of our whole circuit. And also we tried to better characterize our parts, analyze our experimental data,and protein transport and concentration changes throughout the whole process. Several tools including ODEs and interpolation are employed.

2.Kinetic model

2.1 analysis of the problem

2.1.1.expression

Plasmids are transcribed and translated to produce mitoCas9,and the same plasmid are transcriber to produce sgRNA.

$$plasmid \xrightarrow{k_1} mitoCas9$$

$$plasmid \xrightarrow{k_2} sgRNA$$

2.1.2. bonding

The mitoCas9 and sgRNA are bound together in the external solution out of the mitochondria.

$$mitoCas9 + sgRNA \xrightarrow{k_3} mitoCas9/sgRNA_F$$

2.1.3. entering mitochondria:

There is a small model for the process of mitoCas9 protein entering mitochondria nested in it. The reaction equations for the process is under the leadership of MTS presequences, which can bind to the outer membrane of mitochondria.This model includes an initial, rapidly established partitioning between presequence free in the external solution ($[P_F]$) and presequence bound externally to the outer membrane of the mitochondria ($[P_B]$).

$$P_F \rightleftharpoons P_B$$

This binding remains in equilibrium during the import and is described by the partition coefficient, r (liter/ m^2 refers to the ratio between the concentration of bound presequence (with respect to the surface of the mitochondria) and the concentration of free presequence.

$$r = \frac{[P_B]_S}{[P_F]}$$

After the presequence binds to the mitochondrial surface, its subsequent translocation can be considered to be analogous to a unireactant enzymatically catalyzed process, except that all the steps take place within the confines of the mitochondrial membranes.

$$P_B + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E * P_B \xrightarrow{k_2} P_I + E$$

The concentrations of bound presequences or of intrinsic, membranebound proteins that are defined relative to the area of the external mitochondrial surface will be noted by a subscript or superscript S (for example, $[P_B l_S)$.The value C_M is the concentration of mitochondria (g/liter), and K_s (m2/g) is a proportionality factor that relates the surface area of the outer membrane of the mitochondria to the amount of mitochondrial protein. So that:

$$[P_B]_S = \frac{[P_B]}{K_S C_M} \tag{1}$$

According to the Briggs-Haldane steady-state assumption, which assumes that the concentration of $E * P_B$ is in a steady state and its rate of consumption is equal to the rate of formation, then we can get the following expression

$$K_M^S = \frac{k_1 + k_2}{k_1} = \frac{[E]_S [P_B]_S}{[E * P_B]_S} \tag{2}$$

According to the expression (1), we can write the following expression

$$\frac{[E]_S}{[E * P_B]_S} = \frac{[E]}{[E * P_B]} \tag{3}$$

Uni the expression (2) and the expression (3), then

$$[E] = \frac{K_M^S [E * P_B]}{[P_B]_S} \tag{4}$$

$[E_T]$ is the total bulk molar concentration of the translocator, and from the law of conservation of mass, we can get the following expression

$$[E_T] = [E] + [E * P_B] \tag{5}$$

Unit the expression (4) and the expression (5), then

$$[E * P_B] = \frac{[E]_T [P_B]_S}{K_M^S + [P_B]_S} \tag{6}$$

The bulk molar concentration of the translocator, $[E_T]$, is related to the bulk concentration of mitochondria by an unknown constant of proportionality, K_E (mol of translocator per g of mitochondrial protein), such that

$$E_T = K_E C_M \tag{7}$$

Unit the expression (6) and expression (7), then

$$[E * P_B] = \frac{K_E C_M [P_B]}{K_M^S C_M K_S + [P_B]} \tag{8}$$

According the law of conservation of mass, we can get the rate equation of P_F and P_B, and add them up

$$\frac{d([P_F] + [P_B])}{dt} = -k_2 [E * P_B] \tag{9}$$

From the binding experiments, the relationship between $[P_F]$ and $[P_B]$ is known:

$$[P_F] + [P_B] = [P_B](\frac{C_M^{50}}{C_M} + 1) \tag{10}$$

Unit the expression (8) ,(9) and (10), then

$$C_M K_M^S K_S \frac{d[P_B]}{[P_B]} + d[P_B] = -k_2 K_E (C_M)^2 (C_M^{50} + C_M)^{-1} dt \tag{11}$$

Integration of this expression over the range $[P_B]_0$ to $[P_B]$ yields:

$$C_M K_M^S K_S \ln \frac{[P_B]_0}{[P_B]} + ([P_B]_0 - [P_B]) = \frac{k_2 K_E (C_M)^2 t}{(C_M^{50} + C_M)} \tag{12}$$

However, no unique convergent fit could be obtained. Then we applied the same kinetic data to a simplified version of the integrated rate equation that was appropriate for subsaturating P_B. In that case, $[E] \approx [E]_T$, and $[E * P_B]$ could be neglected in the conservation-of-mass relationship. Then:

$$K_M^S K_S \ln \frac{[P_B]_0}{[P_B]} = k_2 K_E C_M t (C_M^{50} + C_M) \tag{13}$$

It should be noted that Eq.(13) is the limit of Eq.(12) when $C_M K_S K_s ln([P_B l_0/[P_B]) >> ([P_B]_0 - [P_B l)$. The data from the presequence imports gave a good fit to this equation with $k_2 K_E (K_s K_s)^{-1} = 0.19 min^{-1}$.

The symbol declaration is:

P_F: presequence free in the external solution
P_B: presequence bound externally to the outer membrane of the mitochondria
E : a translocator that transfers presequence from the outer membrane into the mitochondria
C_M: the concentration of mitochondria (g/liter)
K_s: a proportionality factor that relates the surface area of the outer membrane of the mitochondria to the amount of mitochondrial protein (m2/g)
E_T: the total bulk molar concentration of the translocator
K_E: mol of translocator per g of mitochondrial protein

2.1.4.incising:
After the mitoCas9/sgRNA entering the mitochondria, it can specifically bind the targets (mitochondria gene) and incise them.

$$mitoCas9/sgRNA_I + target \longrightarrow mitoCas9/sgRNA/target \xrightarrow{k_4} mitoCas9/sgRNA_I + detarget$$

In this expression, $[mitoCas9/sgRNA_I]$ is $[mitoCas9/sgRNA_B]_0 - [mitoCas9/sgRNA_B]$

2.2 solutions and implication

$$\frac{d[plasmid]}{dt} = -k_1[plasmid] - k_2[plasmid] \tag{1}$$

$$\frac{d[mitoCas9]}{dt} = k_1[plasmid] - k_3[mitoCas9][sgRNA] \tag{2}$$

$$\frac{d[sgRNA]}{dt} = k_2[plasmid] - k_3[mitoCas9][sgRNA] \tag{3}$$

$$\frac{d[mitoCas9/sgRNA_F]}{dt} = k_3[mitoCas9][sgRNA] \tag{4}$$

$$r = \frac{[mitoCas9/sgRNA_B]}{[mitoCas9/sgRNA_F]K_S C_M} \tag{5}$$

$$K_M^S K_S \ln \frac{[mitoCas9/sgRNA_B]_0}{[mitoCas9/sgRNA_B]} = k_2 K_E C_M t(C_M^{50} + C_M) \tag{6}$$

$$[mitoCas9/sgRNA_I] = [mitoCas9/sgRNA_B]_0 - [mitoCas9/sgRNA_B] \tag{7}$$

$$\frac{d[target]}{dt} = -\frac{[mitoCas9/sgRNA_I][target]}{k_4 + [target]} \tag{8}$$

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