Appendix 3 - DSF

Gillian Forsyth, Aleksandra Plochocka, Robyn Shuttleworth

1 Introduction

In order to help analyse, construct and optimise the biochemical pathways in the Lung Ranger, we used a variety of mathematical tools to create algorithms and simulations. The derivation of the DSF model can be found in this appendix.

2 Chemical Reactions

$$\begin{aligned}
\mathrm{DSF} + \mathrm{RpfC} &\underset{k2}{\overset{k1}{\rightleftharpoons}} \mathrm{DSF}\cdot\mathrm{RpfC} \\
\mathrm{DSF}\cdot\mathrm{RpfC} &\xrightarrow{k3} \mathrm{DSF}\cdot\mathrm{RpfC_P} \\
\mathrm{DSF}\cdot\mathrm{RpfC_P} + \mathrm{RpfG} &\xrightarrow{k4} \mathrm{DSF}\cdot\mathrm{RpfC} + \mathrm{RpfG_P} \\
\mathrm{RpfG_P} + \mathrm{C{-}di{-}GMP} &\xrightarrow{k5} \mathrm{RpfG} \\
\mathrm{Clp} + \mathrm{C{-}di{-}GMP} &\underset{k7}{\overset{k6}{\rightleftharpoons}} \mathrm{Clp}\cdot\mathrm{C{-}di{-}GMP} \\
\mathrm{Clp} + \mathrm{PmanA} &\underset{k9}{\overset{k8}{\rightleftharpoons}} \mathrm{Clp}\cdot\mathrm{PmanA} \\
\xrightarrow{k10} \mathrm{Clp} &\xrightarrow{k11} \varnothing \\
\xrightarrow{k12} \mathrm{C{-}di{-}GMP} &\xrightarrow{k13} \varnothing \\
&\xrightarrow{K.Clp.PmanA} \mathrm{GFP}
\end{aligned}$$

First analysis of the system revealed that production of phosphorylated RpfG is dependent on DSF and so we rewrote the system as follows:

$$
\begin{aligned}
\mathrm{RpfG} &\xrightarrow{k1.DSF} \mathrm{RpfG_P} \\
\mathrm{RpfG_P} + \mathrm{C{-}di{-}GMP} &\xrightarrow{k2} \mathrm{RpfG} \\
\mathrm{Clp} + \mathrm{C{-}di{-}GMP} &\underset{k4}{\overset{k3}{\rightleftharpoons}} \mathrm{Clp \cdot C{-}di{-}GMP} \\
\mathrm{Clp} + \mathrm{PmanA} &\underset{k6}{\overset{k5}{\rightleftharpoons}} \mathrm{Clp \cdot PmanA} \\
&\xrightarrow{k7} \mathrm{Clp} \xrightarrow{k8} \varnothing \\
&\xrightarrow{k9} \mathrm{C{-}di{-}GMP} \xrightarrow{k10} \varnothing \\
&\xrightarrow{K.A} \mathrm{GFP}
\end{aligned}
$$

3 Differential Equations

The first step in the analysis of the system is to find a series of equations describing the kinetics. These equations are written in the form of differential equations to show the change in reactant concentrations over time.RpfG, R is phosphorylated at rate proportional to the concentration of DSF, D. Phosphorylated RpfG, R_P then degrades C-di-GMP, G at rate k_2.

$$\frac{dR}{dt} = k_2 G R_P - k_1 D R \tag{1}$$

$$\frac{dR_P}{dt} = k_1 D R - k_2 G R_P \tag{2}$$

C-di-GMP is produced at a rate k_9, degraded at rate k_{10} and binds to clp, C to form a complex, GC at rate k_3.

$$\frac{dG}{dt} = -k_2 G R_P - k_3 G.C + k_4 GC + k_9 - k_{10} G \tag{3}$$

$$\frac{dGC}{dt} = k_2 G R_P + k_3 G.C - k_4 GC \tag{4}$$

Free clp can bind to the manA promoter P to produce a promoter-bound complex, A which degrades as they dissociate. Clp is produced at rate k_7 and degraded at rate k_8.

$$\frac{dC}{dt} = k_3 G.C + k_4 GC - k_5 C.P + k_6 A + k_7 - k_8 C \tag{5}$$

$$\frac{dA}{dt} = k_5 C.P - k_6 A \tag{6}$$

Finally the synthesis of GFP, F, occurs at a rate proportional to A.

$$\frac{dF}{dt} = KA \tag{7}$$

4 Analysis

RpfG is either in a dephosphorylated,R, or a phosphorylated, R_P, state. Similarly the promoters are either in free-for P, or bound-form, A. The total number for each of these compounds can be written as:

$$R_o = R + R_P \tag{8}$$
$$P_o = P + A \tag{9}$$

Substituting into (2), (5) and (6) gives

$$\begin{aligned}
\frac{dR_P}{dt} &= k_1 D(R_o - R_P) - k_2 G R_P \\
\frac{dC}{dt} &= k_3 G.C + k_4 GC - k_5 C(P_o - A) + k_6 A + k_7 - k_8 C \\
\frac{dA}{dt} &= k_5 C(P_o - A) - k_6 A
\end{aligned}$$

5 Removing Signal

To try and gain an understanding of why our engineered $E.coli$ was expressing GFP in the absence of signal we removed signal from our model. Since the rate of phosphorylation of RpfG is proportional to the concentration of DSF, if there is no signal present there will be no phosphorylation. The system then becomes:

$$\frac{dG}{dt} = -k_3 G.C + k_4 GC + k_9 - k_{10} G$$
$$\frac{dGC}{dt} = k_3 G.C - k_4 GC \tag{10}$$
$$\frac{dC}{dt} = k_3 G.C + k_4 GC - k_5 C(P_o - A) + k_6 A + k_7 - k_8 C$$
$$\frac{dA}{dt} = k_5 C(P_o - A) - k_6 A \tag{11}$$

Since some reactions are faster compared to others the system can be simplified. It is known that the binding and dissociation of a complex occurs quicker than the synthesis of a protein and and so we can approximation the rate of change of the complex to be zero. This is also known as the quasi-steady state approximation. Setting (10) and (11) to be zero and rearranging gives:

$$GC = \frac{k3}{k4} G.C \qquad A = \frac{k_5 C P_o}{k_5 C + k_6}$$

We then find that

$$G = \frac{k_9}{k_{10}} \qquad C = \frac{k_7}{k_8}$$

This enables us to conclude that the rate of change of clp and C-di-GMP is independent of whether C-di-GMP is inhibiting clp.

6 Default Parameters

We set the parameters as follows:

Default Parameters	Value	Reference
Formation of RpfG[p] $(k_1)[s^{-1}]$	0.016	Set here
Degradation of c-di-GMP by RpfG[P] $(k_2)[s^{-1}]$	0.016	Set here
Clp and c-di-GMP association rate $(k_3)[M^{-1}s^{-1}]$	0.033	[3]
Clp and c-di-GMP dissociation rate $(k_4)[s^{-1}]$	0.117	[3]
Clp and P$manA$ association rate $(k_5)[M^{-1}s^{-1}]$	0.083	[3]
Clp and P$manA$ dissociation rate $(k_6)[s^{-1}]$	0.001	[3]
Production rate of Clp $(k_7)[s^{-1}]$	0.016	Set here
Degradation rate of Clp $(k_8)[s^{-1}]$	$1.6 * 10^{-5}$	Set here
Production rate of c-di-GMP $(k_9)[s^{-1}]$	$1.6 * 10^{-4}$	Set here
Degradation rate of c-di-GMP $(k_{10})[s^{-1}]$	$1.6 * 10^{-5}$	Set here
Maximal rate of GFP expression per promoter $(K)[s^{-1}]$	0.016	Set here
Concentration of promoters in the cell $(P)[\mu M]$	0.083	[1, 2]
Concentration of RpfG in the cell $(R)[\mu M]$	4.98	[1, 2]
Concentration of c-di-GMP in the cell $(G)[\mu M]$	2	Set here
Concentration of Clp in the cell $(C)[\mu M]$	2	Set here

References

[1] Leake, M.C. et al. *Variable stoichiometry of the TatA component of the twin-arginine protein transport system observed by in vivo single-molecule imaging*, Proc Natl Acad Sci USA, 40, 15376-15381 (2008).

[2] Twigg, A. et al. *Trans-complementable copy-number mutants of plasmid ColE1*, Nature, 283, 216-218 (1980).

[3] Chin, K.-H. et al. *The cAMP receptor-like protein $\{CLP\}$ is a novel c-di-GMP receptor linking cell–cell signaling to virulence gene expression in Xanthomonas campestris*, Journal of Molecular Biology, 396, 646 - 662 (2010).