Appendix 1 - PQS

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 PQS model can be found in this appendix.

2 Chemical Reactions

$$\mathrm{PQS_{external}} \xrightarrow{k} \mathrm{PQS_{internal}}$$
$$2\,\mathrm{PQS_{internal}} + \mathrm{PQSR_2} \underset{k2}{\overset{k1}{\rightleftharpoons}} \mathrm{PQSR_2} \cdot \mathrm{PQS_2}$$
$$\mathrm{PQSR_2} \cdot \mathrm{PQS_2} + \mathrm{PpqsA_{free}} \underset{k4}{\overset{k3}{\rightleftharpoons}} \mathrm{PQSR_2} \cdot \mathrm{PQS_2} \cdot \mathrm{PpqsA}$$
$$\xrightarrow{PQSR2.PQS2.PpqsA} \mathrm{mCherry}$$
$$\mathrm{PQS_{internal}} \xrightarrow{d} \varnothing$$

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. External PQS, S_e, moves into the cell at rate k forming internal PQS, S_i which degrades at rate d. Two S_i bind to the receptor at rate k_1 and dissociate at rate k_2.

$$\frac{dS_i}{dt} = kS_e - 2k_1{S_i}^2R + 2k_2C - dS_i \tag{1}$$

The signal-receptor tetramer, C is formed and degraded as S_i binds and dissociates from the receptors. C binds to the promoter, P_F, at rate k_3 and dissociates at rate k_4.

$$\frac{dC}{dt} = k_1{S_i}^2R - k_2C - k_3CP_F + k_4A \tag{2}$$

Therefore the tetramer-promoter complex, A, is produced when C and P_F bind and degrades as they dissociate.

$$\frac{dA}{dt} = k_3 C P_F - k_4 A \tag{3}$$

Finally the synthesis of mCherry, M, occurs at a rate proportional to A.

$$\frac{dM}{dt} = KA \tag{4}$$

4 Analysis

The pqsA promoters are in either free-from, P_F, or bound-form, A, and so the total number of promoters is equal to:

$$P_o = P_F + A \tag{5}$$

Applying (5) to (2) and (3)

$$\frac{dC}{dt} = k_1 {S_i}^2 R - k_2 C + k_3 C(P_o - A) - k_4 A \tag{6}$$

$$\frac{dA}{dt} = k_4 A - k_3 C(P_o - A) \tag{7}$$

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 (7) to be zero and rearranging gives:

$$A = \frac{k_3 C P_o}{k_4 + k_3 C}$$

This value for A can substituted into the other equations. C can also be assumed to be in quasi-steady state and after setting (6) to be zero and rearranging gives:

$$C = \frac{k_1}{k_2} R {S_i}^2$$

Our system then becomes:

$$\begin{aligned}
\frac{dS_i}{dt} &= kS_e - \underbrace{2k_1 {S_i}^2 R + 2k_2 C}_{=0} - dS_i \qquad (8)\\
\frac{dC}{dt} &= \underbrace{k_1 {S_i}^2 R - k_2 C}_{=0} + \underbrace{k_3 C(P_o - A) - k_4 A}_{=0}\\
\frac{dA}{dt} &= \underbrace{k_4 A - k_3 C(P_o - A)}_{=0}\\
\frac{dM}{dt} &= KA
\end{aligned}$$

Since (8) is a linear differential equation of the form $x' + px = q$, it can be solved using the integrating factor method where the integrating factor is e^{dt}

$$\begin{aligned}
\frac{d}{dt}(S_i e^{dt}) &= e^{dt} k S_e \\
S_i e^{dt} &= \frac{k}{d} S_e e^{dt} + const \\
&= \frac{k}{d} S_e e^{dt} - \frac{k}{d} S_e \\
&= \frac{k}{d} S_e (e^{dt} - 1) \\
S_i &= \frac{k}{d} S_e e^{-dt} (e^{dt} - 1) \\
&= \frac{k}{d} S_e (1 - e^{-dt})
\end{aligned}$$

but $e^{-dt} \rightarrow 0$

$$S_i \approx \frac{k}{d} S_e$$

Now then

$$\begin{aligned}
\frac{dmCherry}{dt} &= KA \\
&= K \frac{k_3 C P_o}{k_4 + k_3 C} \\
&= K \frac{k_3 \frac{k_1}{k_2} R {S_i}^2 P_o}{k_4 + k_3 \frac{k_1}{k_2} R {S_i}^2} \\
&= K \frac{k_3 \frac{k_1}{k_2} R (\frac{k}{d} S_e)^2 P_o}{k_4 + k_3 \frac{k_1}{k_2} R (\frac{k}{d} S_e)^2}
\end{aligned}$$

$$\frac{d[mCherry]}{dt} = \frac{K P_o [S_e]^2}{\frac{k_2 k_4 d^2}{k_1 k_3 k^2 R} + [S_e]^2} \tag{9}$$

Equation (9) portrays that the expression of mCherry is dependent on the concentration of PQS present in the sputum sample.

5 Default Parameters

We used the following parameters:

The values for k_3 and k_4 were derived from an EC_{50} value [1] . This EC_{50} value can be used to approximate K_D [4]and then:

Default Parameters	Value	Reference
PQS and PQSR association rate, $(k_1)[M^{-1}s^{-1}]$	0.0793	[5]
PQS and PQSR dissociation rate, $(k_2)[s^{-1}]$	0.016	[5]
PQS_2PQSR and P$pqsA$ association rate, $(k_3)[M^{-1}s^{-1}]$	0.016	[1]
PQS_2PQSR and P$pqsA$ dissociation rate, $(k_4)[s^{-1}]$	0.117	[1]
Rate of PQS movement into the cell, $(k)[s^{-1}]$	$1.6 * 10^{-4}$	Set here
Rate of PQS movement out of the cell, $(d)[s^{-1}]$	$1.6 * 10^{-4}$	Set here
Maximal rate of mCherry expression per promoter $(K)[s^{-1}]$	0.016	Set here
Concentration of promoters in the cell $(P)[\mu M]$	0.083	[2, 3]
Concentration of receptors in the cell $(R)[\mu M]$	4.98	[2, 3]

$$K_D = \frac{k_{dissociation}}{k_{association}}$$
$$K_{D_2} = \frac{k_4}{k_3}$$

It is worth noting that K_{D_1} is 10-fold lower than K_{D_2}. This implies that PQSR has a higher binding affinity for PQS than the promoter. (The lower the K_D the higher the binding affinity)

References

[1] Ilangovan, A. et al. *Structural basis for native agonist and synthetic inhibitor recognition by the Pseudomonas aeruginosa quorum sensing regulator PqsR (MvfR)*, JPLoS Pathog, 9, e1003508 (2013).

[2] 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).

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

[4] Wu, G *Assay Development: Fundamentals and Practices*, Wiley-Blackwell, 2010.

[5] Zender, M. et al. *Discovery and biophysical characterization of 2-Amino-oxadiazoles as novel antagonists of PqsR, an important regulator of Pseudomonas aeruginosa virulence*, J Med Chem, 56, 6761-6774 (2013).