Appendix 1 - PQS

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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

$$\begin{aligned}
\mathrm{PQS}_{\mathrm{external}} &\xrightarrow{k} \mathrm{PQS}_{\mathrm{internal}} \\
2\,\mathrm{PQS}_{\mathrm{internal}} + \mathrm{PQSR}_2 &\underset{k2}{\overset{k1}{\rightleftharpoons}} \mathrm{PQSR}_2 \cdot \mathrm{PQS}_2 \\
\mathrm{PQSR}_2 \cdot \mathrm{PQS}_2 + \mathrm{PpqsA}_{\mathrm{free}} &\underset{k4}{\overset{k3}{\rightleftharpoons}} \mathrm{PQSR}_2 \cdot \mathrm{PQS}_2 \cdot \mathrm{PpqsA} \\
&\xrightarrow{PQSR2.PQS2.PpqsA} \mathrm{mCherry} \\
\mathrm{PQS}_{\mathrm{internal}} &\xrightarrow{d} \varnothing
\end{aligned}$$

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. 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:

$$
\begin{aligned}
\frac{dS_i}{dt} &= kS_e - \underbrace{2k_1{S_i}^2R + 2k_2C}_{=0} - dS_i \qquad (8)\\
\frac{dC}{dt} &= \underbrace{k_1{S_i}^2R - k_2C}_{=0} + \underbrace{k_3C(P_o - A) - k_4A}_{=0}\\
\frac{dA}{dt} &= \underbrace{k_4A - k_3C(P_o - A)}_{=0}\\
\frac{dM}{dt} &= KA
\end{aligned}
$$

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}

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

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

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

Now then

$$
\begin{aligned}
\frac{dmCherry}{dt} &= KA\\
&= K\frac{k_3CP_o}{k_4 + k_3C}\\
&= K\frac{k_3\frac{k_1}{k_2}R{S_i}^2P_o}{k_4 + k_3\frac{k_1}{k_2}R{S_i}^2}\\
&= K\frac{k_3\frac{k_1}{k_2}R(\frac{k}{d}S_e)^2P_o}{k_4 + k_3\frac{k_1}{k_2}R(\frac{k}{d}S_e)^2}
\end{aligned}
$$

$$\frac{d[mCherry]}{dt} = \frac{KP_o[S_e]^2}{\frac{k_2k_4d^2}{k_1k_3k^2R} + [S_e]^2} \tag{9}$$

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:

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]

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:

$$\begin{aligned} K_D &= \frac{k_{dissociation}}{k_{association}} \\ K_{D_2} &= \frac{k_4}{k_3} \end{aligned}$$

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

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[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.

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