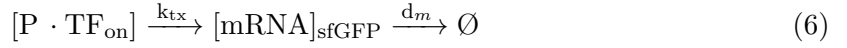


Optogenetic Induction of sfGFP protein

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1 Chemical reactions involved in the system

- Reactions in the system



- Colony growth and dilution

Before we begin, we have to consider the dilution of the colony, considering a 2D area growth since we are obtaining data from whole colonies:

$$\frac{dA(t)}{dt} = \mu A(t)$$

$$\mu(t) = \frac{1}{A(t)} \frac{dA(t)}{dt}$$

Taking the logistic area model as done in the FluoPi paper (Nuñez, I., 2017), the following is derived:

$$A(t) = \frac{A_{max}}{1 + e^{-\mu_{max}(t-t_{50})}}$$

$$\mu(t) = \frac{\mu_{max}}{1 + e^{\mu_{max}(t-t_{50})}}$$

Where the dilution is dependant on time ($\mu(t)$).

- Dynamics of the transcription factor in the system - Reactions (1) through (4)

For the equations we have:

$$\frac{d[\text{TF}_{\text{off}}]}{dt} = \frac{k_{tx}k_{tr}}{d_m} - d_p[\text{TF}_{\text{off}}] - \text{I}_{\text{green}}k_{\text{on}}[\text{TF}_{\text{off}}] + \text{I}_{\text{red}}k_{\text{off}}[\text{TF}_{\text{on}}] + k_{\text{off}_{\text{base}}}[\text{TF}_{\text{on}}] - \mu(t)[\text{TF}_{\text{off}}] \quad (8)$$

$$\frac{d[\text{TF}_{\text{on}}]}{dt} = \text{I}_{\text{green}}k_{\text{on}}[\text{TF}_{\text{off}}] - \text{I}_{\text{red}}k_{\text{off}}[\text{TF}_{\text{on}}] - d_p[\text{TF}_{\text{on}}] - k_{\text{off}_{\text{base}}}[\text{TF}_{\text{on}}] - \mu(t)[\text{TF}_{\text{on}}] \quad (9)$$

From the mass conservation principle we have that $[\text{TF}_{\text{tot}}] = [\text{TF}_{\text{off}}] + [\text{TF}_{\text{on}}] = C$ where C is a constant, we can replace $[\text{TF}_{\text{off}}] = [\text{TF}_{\text{tot}}] - [\text{TF}_{\text{on}}]$, and the equations are modified as:

$$\frac{d[\text{TF}_{\text{tot}}]}{dt} = \frac{k_{tx}k_{tr}}{d_m} - (d_p + \mu(t))[\text{TF}_{\text{tot}}] \quad (10)$$

$$\frac{d[\text{TF}_{\text{on}}]}{dt} = \text{I}_{\text{green}}k_{\text{on}}([\text{TF}_{\text{tot}}] - [\text{TF}_{\text{on}}]) - \text{I}_{\text{red}}k_{\text{off}}[\text{TF}_{\text{on}}] - k_{\text{off}_{\text{base}}}[\text{TF}_{\text{on}}] - d_p[\text{TF}_{\text{on}}] \quad (11)$$

Also assuming that the total amount of TF reaches and maintains a steady state, we can obtain an expression for TF_{tot} :

$$[\text{TF}_{\text{tot}}](\mu) = \frac{k_{tx}k_{tr}}{d_m(d_p + \mu(t))} \quad (12)$$

Assuming that the TF_{on} rapidly reaches equilibrium with the total transcription factor, we can simplify the equations as follows:

$$\frac{d[\text{TF}_{\text{on}}]}{dt} = I_{\text{green}}k_{\text{on}}([\text{TF}_{\text{tot}}] - [\text{TF}_{\text{on}}]) - I_{\text{red}}k_{\text{off}}[\text{TF}_{\text{on}}] - k_{\text{off}_{\text{base}}}[\text{TF}_{\text{on}}] - d_p[\text{TF}_{\text{on}}] \approx 0 \quad (13)$$

$$[\text{TF}_{\text{on}}]_{ss} = \frac{I_{\text{green}}k_{\text{on}}}{I_{\text{green}}k_{\text{on}} + I_{\text{red}}k_{\text{off}} + k_{\text{off}_{\text{base}}} + d_p} \cdot [\text{TF}_{\text{tot}}](\mu) \quad (14)$$

Using this final expression for the quasi-steady state of the activated transcription factor concentration (14), we can replace it once we derive the promoter activity functions in the following section.

- Promoter activity functions - Reaction (5)

The promoter used in the synthetic construct can be found in one of two possible states, either bound to the respective transcription factor, or unbound. Thus, we can represent the total probability of activity (between 0 and 1) as:

$$P_{\text{bound}} = \frac{[P \cdot \text{TF}_{\text{on}}]}{[P] + [P \cdot \text{TF}_{\text{on}}]} \quad (15)$$

We can define an equilibrium constant for the binding of the TF to the DNA (from reaction (5)), $K_1 = \frac{k_{f1}}{k_{r1}} = \frac{[P \cdot \text{TF}_{\text{on}}]}{[P][\text{TF}_{\text{on}}]}$, from which we can derive that $[P \cdot \text{TF}_{\text{on}}] = K_1[P][\text{TF}_{\text{on}}]$, therefore:

$$P_{\text{bound}} = \frac{K_1[P][\text{TF}_{\text{on}}]}{[P] + K_1[P][\text{TF}_{\text{on}}]} = \frac{K_1[\text{TF}_{\text{on}}]}{1 + K_1[\text{TF}_{\text{on}}]} \quad (16)$$

We can also replace $K_1 = 1/(K_d)$, and dividing the expression by K_1 gives us:

$$P_{\text{bound}} = \frac{[\text{TF}_{\text{on}}]}{K_d + [\text{TF}_{\text{on}}]}$$

Which is the familiar Hill function for activation.

- Dynamics of the reporter protein (superfolder GFP)

The following ODEs describe the transcription and translation of the mRNA and sfGFP:

$$\frac{d[\text{mRNA}_{\text{sfGFP}}]}{dt} = \beta_0 + k_{tx} \left(\frac{[\text{TF}_{\text{on}}]}{K_d + [\text{TF}_{\text{on}}]} \right) - d_m[\text{mRNA}_{\text{sfGFP}}] \quad (17)$$

$$\frac{d[\text{sfGFP}]}{dt} = k_{tr}[\text{mRNA}_{\text{sfGFP}}] - d_p[\text{sfGFP}] - \mu(t)[\text{sfGFP}] \quad (18)$$

Under the quasi-steady-state assumption for the sfGFP mRNA, we set the rate of change of mRNA concentration to zero, which implies that the production and degradation of mRNA rapidly reach a balance. This assumption simplifies the ordinary differential equation (ODE) for

mRNA dynamics to an algebraic equation, allowing us to solve for the steady-state concentration of mRNA, denoted as $[\text{mRNA}_{ss}]$.

Given the ODE for mRNA dynamics in (6):

$$\frac{d[\text{mRNA}_{sfGFP}]}{dt} = \beta_0 + k_{tx} \left(\frac{[\text{TF}_{on}]}{K_d + [\text{TF}_{on}]} \right) - d_m[\text{mRNA}_{sfGFP}] \quad (19)$$

Setting the rate of change to zero for the steady state:

$$0 = \beta_0 + k_{tx} \left(\frac{[\text{TF}_{on}]}{K_d + [\text{TF}_{on}]} \right) - d_m[\text{mRNA}_{ss}] \quad (20)$$

Solving for $[\text{mRNA}_{ss}]$:

$$[\text{mRNA}_{ss}] = \frac{\beta_0 + k_{tx} \left(\frac{[\text{TF}_{on}]}{K_d + [\text{TF}_{on}]} \right)}{d_m} \quad (21)$$

This expression represents the steady-state concentration of sfGFP mRNA as a function of the transcription factor concentration $[\text{TF}_{on}]$, the basal transcription rate β_0 , the transcription rate constant k_{tx} , the mRNA degradation rate d_m , and the dissociation constant K_d . We assume that the mRNA dynamics occur on a timescale sufficiently small enough to neglect dilution effects.

Replacing the steady-state mRNA expression in the sfGFP ODE:

$$\frac{d[\text{sfGFP}]}{dt} = k_{tr} \frac{\beta_0 + k_{tx} \left(\frac{[\text{TF}_{on}]}{K_d + [\text{TF}_{on}]} \right)}{d_m} - d_p[\text{sfGFP}] - \mu(t)[\text{sfGFP}] \quad (22)$$

And from the simplification performed on equation (14) we can replace on (22):

$$\frac{d[\text{sfGFP}]}{dt} = \hat{\beta}_0 + \hat{\beta}_1 \cdot \left(\frac{\left(\frac{I_{green} k_{on}}{I_{green} k_{on} + I_{red} k_{off} + k_{off_{base}} + d_p} \cdot [\text{TF}_{tot_{ss}}](\mu) \right)}{K_D + \left(\frac{I_{green} k_{on}}{I_{green} k_{on} + I_{red} k_{off} + k_{off_{base}} + d_p} \cdot [\text{TF}_{tot_{ss}}](\mu) \right)} \right) - d_p[\text{sfGFP}] - \mu(t)[\text{sfGFP}] \quad (23)$$

We can group certain terms and convert it to a format compatible with the fluorescence per pixel we obtain from the experimental data:

$$\frac{d(I/A)}{dt} = \underbrace{\frac{\alpha_{fl}}{\frac{\text{fluorescence}}{\text{unit of [sfGFP]}}}}_{\text{unit of [sfGFP]}} \cdot \underbrace{\left(\frac{\alpha_{prod}}{\frac{\text{unit of [sfGFP]}}{\text{time}}} - \frac{d_p[\text{sfGFP}]}{\frac{\text{unit of [sfGFP]}}{\text{time}}} - \frac{\mu(t)[\text{sfGFP}]}{\frac{\text{unit of [sfGFP]}}{\text{time}}} \right)}_{d[\text{sfGFP}]/dt} \quad (24)$$

$$\frac{d(I/A)}{dt} = \alpha_{fl} \cdot \alpha_{prod} - d_p \left(\frac{I}{A} \right) - \mu(t) \left(\frac{I}{A} \right) \quad (25)$$

$$\frac{d(I/A)}{dt} = \tilde{\beta}_0 + \tilde{\beta}_1 \cdot \left(\frac{\left(\frac{I_{green} k_{on}}{I_{green} k_{on} + I_{red} k_{off} + k_{off_{base}} + d_p} \right) [\text{TF}_{tot_{ss}}](\mu)}{K_D + \left(\frac{I_{green} k_{on}}{I_{green} k_{on} + I_{red} k_{off} + k_{off_{base}} + d_p} \right) [\text{TF}_{tot_{ss}}](\mu)} \right) - d_p \left(\frac{I}{A} \right) - \mu(t) \left(\frac{I}{A} \right) \quad (26)$$

Where:

$$\tilde{\beta}_0 = \alpha_{fl} \cdot \hat{\beta}_0 = \alpha_{fl} k_{tr} \beta_0 / d_m \quad (27)$$

$$\tilde{\beta}_1 = \alpha_{fl} \cdot \hat{\beta}_1 = \alpha_{fl} k_{tr} k_{tx} / d_m \quad (28)$$