# Detailed Methods Description – Optogenetic Kill Switch

The ordinary differential equations are constructed using mass action kinetics and quasi steady state approximations (Michaelis Menten). Here we provide the more detailed system representations of all four systems: pDusk, pDawn, pDusk + const. mazF, pDawn + const. mazE. The data and Matlab scripts were also made available online for future iGEM Teams to built other in silico optogenetic tools: https://github.com/marioisbeck/iGEM_Wageningen_UR_2016.

To evaluate how well a certain parameter set describes the response of the Ohlendorf *et al.* (2012) system, we used the sum of squared residuals to score each parameter set as described in Raue *et al.* (2009).

$$\chi^2(\theta) = \sum_{k=1}^{m} \sum_{l=1}^{d} \left( \frac{y_{kl}^d - y_k(\theta, t_l)}{\sigma_{kl}^d} \right)^2$$

where $y_{kl}^d$ represents $d$ data points for each observable $k$ at time points $t_l$. $\sigma_{kl}^d$ are the corresponding measurement errors and $y_k(\theta, t_l)$ the $k^{th}$ observable as predicted by parameters $\theta$ for time point $t_l$.
To construct a corresponding score from the same parameter set for pDusk and pDawn we used the weighted means approach accordingly:

$$\frac{\zeta_u + \zeta_a}{2}$$

where $\zeta_u$ represents the score of a parameter set for pDusk and $\zeta_a$ the corresponding score of the same parameter set for pDawn.

## Light On - Optogenetic Tool

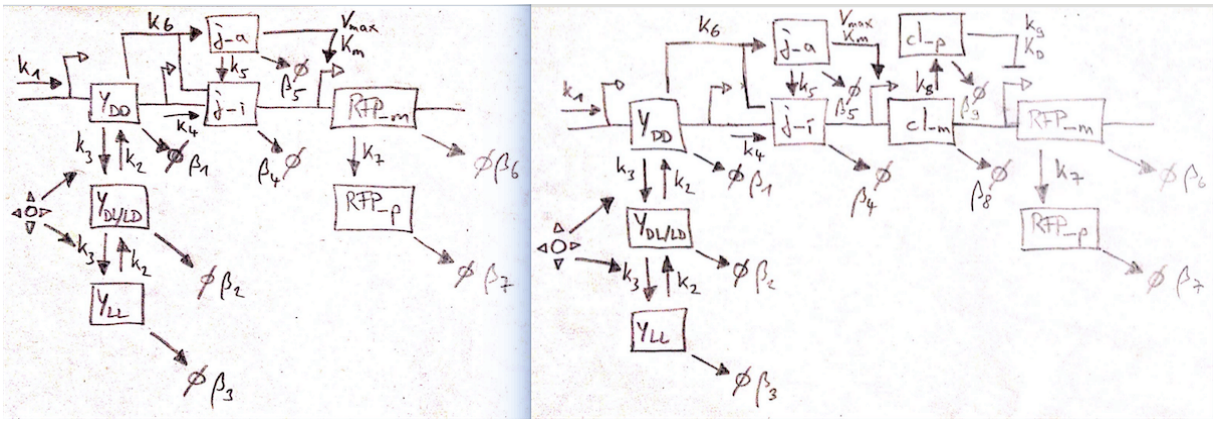


**Figure 1**: Detailed pDusk/ pDawn system design including all parameters. Where $y_{DD}$: Yf1 homodimer in dark-dark state, $y_{DL/LD}$: lumped Yf1 homodimer in both dark-light (DL) and light-dark (LD) state, $y_{LL}$: Yf1 homodimer in light-light state, $j_i$: inactive form of FixJ (mRNA stage of FixJ is lumped), $j_a$: active form of FixJ, $cI_m$: lambda phage inhibitor mRNA, $cI_p$: lambda phage inhibitor protein, $RFP_m$: mRNA form of Red Fluorescent Protein (RFP), and $RFP_p$: protein form of RFP.

In Figure 1 we show the pDusk (left) and pDawn (right) system with all parameters included. The outcome of the parameter estimation procedure and their meaning can be found in Table 1. All equations describing the systems are given in the equations 1 – 7 (pDusk) and 8 – 11 (pDawn).

1. $$\frac{dy_{DD}}{dt} = k_1 + 2 \cdot k_2 \cdot y_{DL,LD} - 2 \cdot (N \cdot k_3) \cdot y_{DD} - \beta_1 \cdot y_{DD}$$

2. $$\frac{dy_{DL,LD}}{dt} = 2 \cdot (N \cdot k_3) \cdot y_{DD} + 2 \cdot k_2 \cdot y_{LL} - 2 \cdot k_2 \cdot y_{DL,LD} - 2 \cdot (N \cdot k_3) \cdot y_{DL,LD} - \beta_2 \cdot y_{DL,LD}$$

3. $$\frac{dy_{LL}}{dt} = 2 \cdot (N \cdot k_3) \cdot y_{DL,LD} - 2 \cdot k_2 \cdot y_{LL} - \beta_3 \cdot y_{LL}$$

4. $$\frac{dj_i}{dt} = k_4 + k_5 \cdot j_a - \beta_4 \cdot j_i$$

5. $$\frac{dj_a}{dt} = k_6 \cdot y_{DD} \cdot j_i - \beta_5 \cdot j_a$$

6. $$\frac{dRFP_m}{dt} = \frac{V_{\max} \cdot j_a}{K_m + j_a} - \beta_6 \cdot RFP_m$$

7. $$\frac{dRFP_p}{dt} = k_7 \cdot RFP_m - \beta_7 \cdot RFP_p$$

8. $$\frac{dcI_m}{dt} = \frac{V_{\max} \cdot j_a}{K_m + j_a} - \beta_8 \cdot cI_m$$

9. $$\frac{dcI_p}{dt} = k_8 \cdot cI_m - \beta_9 \cdot cI_p$$

10. $$\frac{dRFP_m}{dt} = k_9 \cdot \frac{1}{1 + \left(\frac{cI_p}{K_d}\right)^2} - \beta_6 \cdot RFP_m$$

11. $$\frac{dRFP_p}{dt} = k_7 \cdot RFP_m - \beta_7 \cdot RFP_p$$

**Table 1**: Parameter explanation and values of outcome of parameter estimation pDusk/ pDawn.

| Parameter | Value | Description |
|---|---|---|
| $k_1$ | $2.6921 \frac{\mu \text{ mol}}{h}$ | production rate of $y_{DD}$ |
| $k_2$ | $0.0008 \frac{1}{h}$ | relaxation rate of $y_{DL,LD}$ and $y_{LL}$. We assumed a search space for τ of 5900 ± 25 s based on data from Möglich *et al*. (2009). This was mathematically transformed to $k_2 = \frac{\log(2)}{\tau} \cdot 3600 \frac{s}{h}$. |
| $k_3$ | $0.4219 \frac{m^2}{\mu\text{mol}}$ | conversion cross-section σ of light intensity activated production rate of $y_{DL,LD}$ and $y_{LL}$. The search space for this parameter was defined as 1,000 ± 250 in Klose *et al*. (2015) and Rausenberger *et al*. (2010). |
| $\beta_1$ | $0.3049 \frac{1}{h}$ | degradation rate of $y_{DD}$ |
| $\beta_2$ | $0.8406 \frac{1}{h}$ | degradation rate of $y_{DL,LD}$ |
| $\beta_3$ | $0.1477 \frac{1}{h}$ | degradation rate of $y_{LL}$ |
| $k_4$ | $0.2040 \frac{\mu\text{mol}}{h}$ | production rate of $j_i$ |
| $k_5$ | $2.1623 \frac{1}{h}$ | de-phosphorylation rate of $j_a$ |
| $\beta_4$ | $0.5205 \frac{1}{h}$ | degradation rate of $j_i$ |
| $k_6$ | $2.0838 \frac{1}{\text{h} \times \mu\text{mol}}$ | production rate of $j_a$ depending on the concentration of $y_{DD}$ and $j_i$ |
| $\beta_5$ | $0.6615 \frac{1}{h}$ | degradation rate of $j_a$ |
| $V_{max}$ | $2.9063 \frac{\mu\text{mol}}{h}$ | $V_{max}$ of production rate of $RFP_m$ based on $j_a$ |
| $K_M$ | $0.7130 \mu \text{ mol}$ | $K_M$ of production rate of $RFP_m$ based on $j_a$ |
| $\beta_6$ | $2.0224 \frac{1}{h}$ | degradation rate of $RFP_m$ |
| $k_7$ | $0.0460 \frac{1}{h}$ | translation rate from $RFP_m$ to $RFP_p$ |
| $\beta_7$ | $0.2903 \frac{1}{h}$ | degradation rate of $RFP_p$ |
| $\beta_8$ | $1.1579 \frac{1}{h}$ | degradation rate of lambda phage inhibitor $RNA_m$ |
| $k_8$ | $3.8073 \frac{1}{h}$ | production rate of $cI_p$ depending on $cI_m$ |
| $\beta_9$ | $0.6563 \frac{1}{h}$ | degradation rate of $cI_p$ |
| $k_9$ | $0.9920 \frac{\mu\text{mol}}{h}$ | maximal production rate of $RFP_m$ |
| $K_D$ | $0.1384 \mu\text{mol}$ | dissociation constant of $cI_p$ at $RFP_m$ promoter. The Hill coefficient was chosen to be 2 as the $cI_p$ regulated promoter BBa_R0051 has 2 binding sites for $cI_p$. |

## Light On, BeeT Off – Optogenetic Kill Switch

In Figure 2 we show the pDusk + const. mazF (left) and pDawn + const. mazE (right) system with all parameters included. The outcome of the parameter estimation procedure and their meaning can be found in Table 2. All equations describing the systems are given in the equations 12 – 16 (pDusk + const. mazF) and 17 – 23 (pDawn + const. mazE).

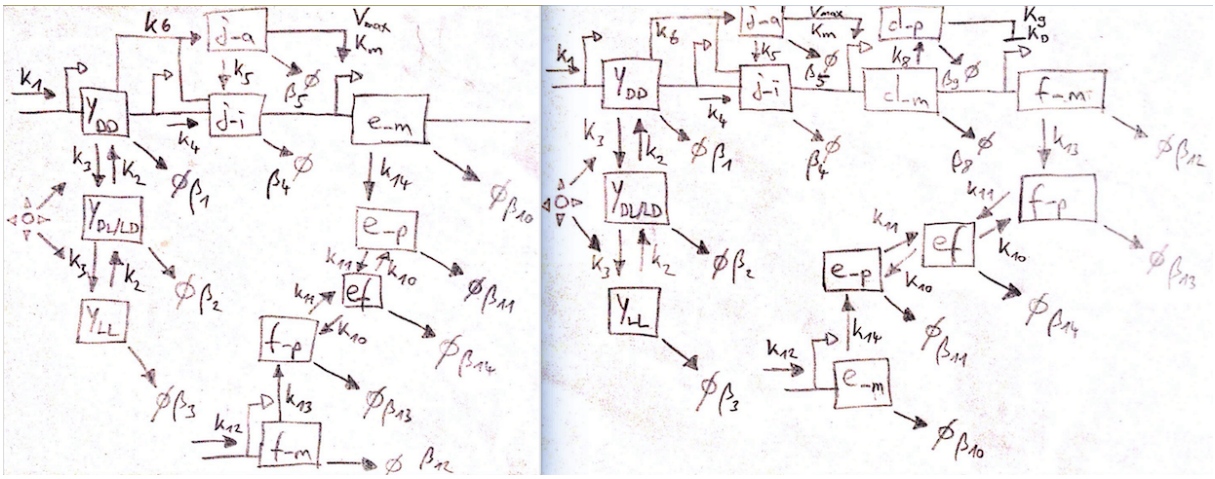

**Figure 2**: Detailed system design of pDusk + constitutive mazF and pDawn + constitutive mazE including all parameters.

12. $$\frac{de_m}{dt} = \left(\frac{V_{\max} \cdot j_a}{K_M + j_a}\right) - \beta_{10} \cdot e_m$$

13. $$\frac{de_p}{dt} = k_{14} \cdot e_m + k_{10} \cdot (2 \cdot ef) - k_{11} \cdot \left(2 \cdot (e_p)^2\right) \cdot (f_p)^4 - \beta_{11} \cdot e_p$$

14. $$\frac{df_m}{dt} = k_{12} - \beta_{12} \cdot f_m$$

15. $$\frac{df_p}{dt} = k_{13} \cdot f_m + k_{10} \cdot (4 \cdot ef) - k_{11} \cdot (e_p)^2 \cdot \left(4 \cdot (f_p)^4\right) - \beta_{13} \cdot f_p$$

16. $$\frac{def}{dt} = k_{11} \cdot (e_p)^2 \cdot (f_p)^4 - k_{10} \cdot ef - \beta_{14} \cdot ef$$

17. $$\frac{dcI_m}{dt} = \left(\frac{V_{\max} \cdot j_a}{K_M + j_a}\right) - \beta_8 \cdot cI_m$$

18. $$\frac{dcI_p}{dt} = k_8 \cdot cI_m - \beta_9 \cdot cI_p$$

19. $$\frac{df_m}{dt} = k_9 \cdot \left(\frac{1}{1 + \left(c \frac{I_p}{K_d}\right)^2}\right) - \beta_{12} \cdot f_m$$

20. $$\frac{df_p}{dt} = k_{13} \cdot f_m + k_{10} \cdot (4 \cdot ef) - k_{11} \cdot (e_p)^2 \cdot \left(4 \cdot (f_p)^4\right) - \beta_{13} \cdot f_p$$

21. $$\frac{de_m}{dt} = k_{12} - \beta_{10} \cdot e_m$$

22. $$\frac{de_p}{dt} = k_{14} \cdot e_m + k_{10} \cdot (2 \cdot ef) - k_{11} \cdot \left(2 \cdot (e_p)^2\right) \cdot (f_p)^4 - \beta_{11} \cdot e_p$$

23. $$\frac{def}{dt} = k_{11} \cdot (2 \cdot e_p) \cdot (4 \cdot f_p) - k_{10} \cdot ef - \beta_{14} \cdot ef$$

**Table 2**: Parameter explanation and values of outcome of parameter estimation mazEF.

| **Parameter** | **Set 1** | **Set 2** | **Description** |
|---|---|---|---|
| $\beta_{10}$ | $196.0000\frac{1}{h}$ | $873.0000\frac{1}{h}$ | degradation rate of $e_m$ |
| $k_{14}$ | $1.4312\frac{1}{h}$ | $0.7103\frac{1}{h}$ | production rate from $e_m$ to $e_p$ |
| $\beta_{11}$ | $0.3028\frac{1}{h}$ | $0.0244\frac{1}{h}$ | degradation rate of $e_p$ |
| $k_{10}$ | $2.2664\frac{1}{h}$ | $0.2564\frac{1}{h}$ | dissociation rate of complex ef (lumped/ simplified) |
| $k_{11}$ | $0.7838\frac{1}{\mu mol^5 \cdot h}$ | $0.1827\frac{1}{\mu mol^5 \cdot h}$ | rate of ef-complex formation (lumped/ simplified - this is why the unit has a $^5$) |
| $k_{12}$ | $0.0188\frac{\mu mol}{h}$ | $0.0847\frac{\mu mol}{h}$ | production rate of $f_m$ based on constitutive promoter |
| $\beta_{12}$ | $1.6545\frac{1}{h}$ | $1.1927\frac{1}{h}$ | degradation rate of $f_m$ |
| $k_{13}$ | $0.0383\frac{1}{h}$ | $0.0656\frac{1}{h}$ | production rate from $f_m$ to $f_p$ |
| $\beta_{13}$ | $0.7383\frac{1}{h}$ | $1.9110\frac{1}{h}$ | degradation rate of $e_m$ |
| $\beta_{14}$ | $1.8231\frac{1}{h}$ | $0.5894\frac{1}{h}$ | degradation of complex ef |