On diagonal

$i = 1$

$j = 1$

$I_{i,j} = 1$

$$\alpha_{p,\,p_{GFP}} = \alpha^{0} \cdot \frac{K_r^{\,n}}{K_r^{\,n} + \frac{[C_{d}1]^{n/2}}{[C_{d}1]^{n/2}}}$$

$$= \alpha^{0} \cdot \frac{K_r^{\,n}}{K_r^{\,2} + 1}$$

Not the same as concentration

& No I_{ij}

$$= \alpha^{0} \cdot \frac{K_r^{\,n}}{K_r^{\,n} + [C_d 1]^{n} \cdot I_{i,j} \cdot \frac{[C_{d1}]^{n/2}}{[C_{dj}]^{n/2}}}$$

$$= \alpha^{0} \cdot \frac{K_r^{\,n}}{K_r^{\,n} + [C_d 1]^{n} \cdot 1 \cdot 1}$$

Off diagonal

$i = 1$

$j = 2$

$I_{i,j} = 0$ → there should be no modulation

$I_{j,i} = 0$

$$\alpha_{0} \cdot \alpha_{i,i} \cdot \alpha_{ij} \cdot \alpha_{jj} \cdot \alpha_{ji}$$

↓

$$\frac{K_r^{\,n}}{K_r^{\,n} + [C_{di}]^{n} \cdot I_{i,j} \cdot \frac{[C_{di}]^{n/2}}{[C_{dj}]^{n/2}}}$$

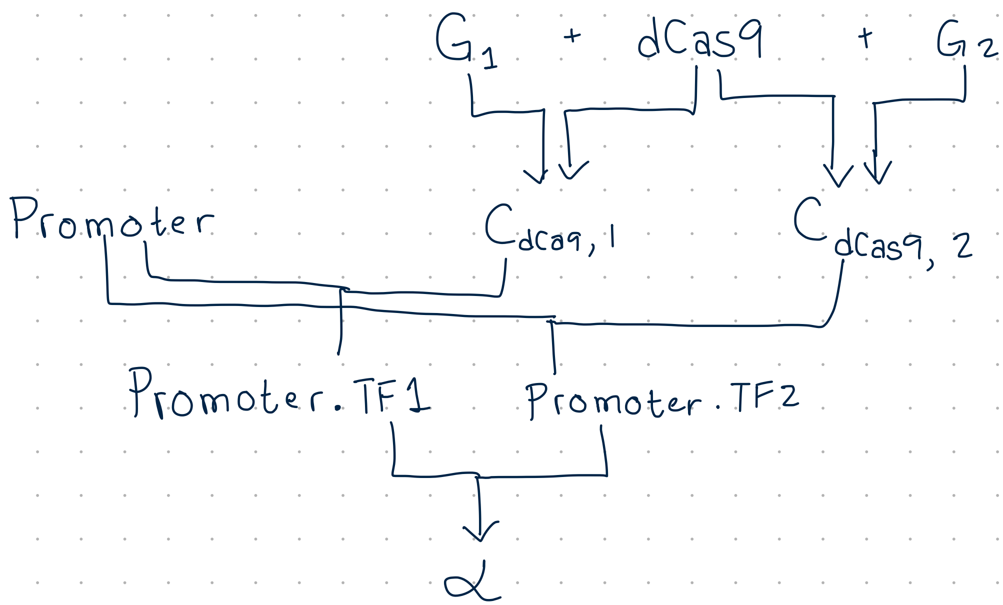
high i & high j

$$\frac{\varepsilon}{\varepsilon + [C_{di}]^{n} \cdot 0 \cdot 1}$$

Re-deriving the Hill equation with multiplexing

$$dCas9 + G_i \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} C_{dCas9,\,i}$$

$$C_{dCas9,\,i} + \text{Promoter} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{Promoter.TF} \xrightarrow{\alpha_p} GFP \xrightarrow{\delta_p} \emptyset$$



$$\begin{array}{cc|ccc} & & & j & \\ & & 1 & 2 & 3 \\ \hline & 1 & 1 & 0 & 0.5 \\ i & 2 & 0 & 1 & -0.5 \\ & 3 & 0.5 & -0.5 & 1 \end{array}$$

Doesn't necessarily have to be symmetrical

On diagonal

$i = 1$

$j = 1$

$I_{ij} = 1$

$$u_1 = 1 \cdot [P_{d1}]$$

$$g = \frac{1}{2} \cdot \frac{[P_{d1}]}{\sqrt{[P_{d1}]+1}} + 1$$

only good for activation

Off diagonal

No interaction

(1 & 2)

$$\begin{array}{ccc} & j=1 & & j=2 \\ u_1 = & \left(1 \cdot [P_{d1}]\right) & + & \left(0 + [P_{d2}]\right) \\ u_2 = & \left(0 \cdot [P_{d1}]\right) & + & \left(1 + [P_{d2}]\right) \end{array}$$

$$g = \frac{1}{2} \cdot \left(\frac{[P_{d1}]}{\sqrt{[P_{d1}]+1}} + 1\right) \cdot \left(\frac{[P_{d2}]}{\sqrt{[P_{d2}]+1}} + 1\right)$$

Synergy

(1 & 3)

$$\begin{array}{ccc} & j=1 & & j=3 \\ u_1 = & \left(1 \cdot [P_{d1}]\right) & + & \left(0.5 \cdot [P_{d3}]\right) \\ u_3 = & \left(0.5\ [P_{d1}]\right) & + & \left(1\ [P_{d3}]\right) \end{array}$$

$$g = \frac{1}{2} \cdot \left(\frac{[P_{d1}] \cdot 0.5\,[P_{d3}]}{\sqrt{[P_{d1}] \cdot 0.5\,[P_{d3}] + 1}} + 1\right) \cdot \left(\frac{[P_{d3}] \cdot 0.5\,[P_{d1}]}{\sqrt{[P_{d3}] \cdot 0.5\,[P_{d1}] + 1}} + 1\right)$$

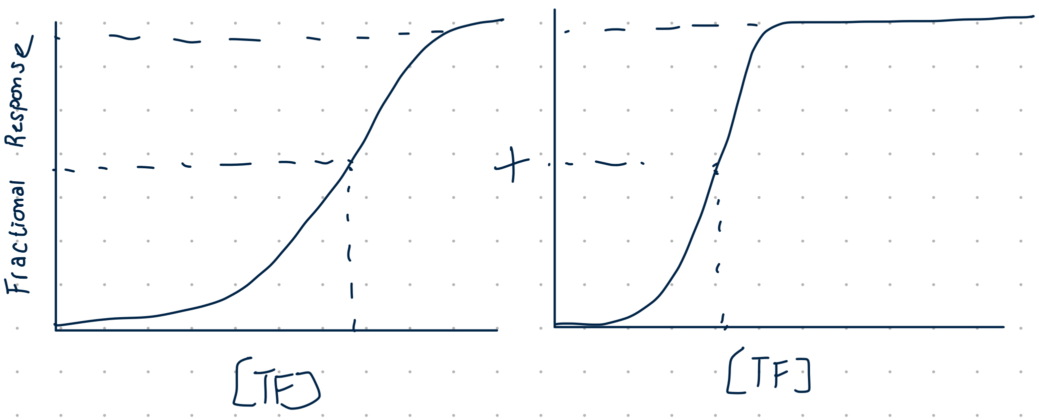
Interference

(2 & 3)

$$\begin{array}{ccc} & j=2 & & j=3 \\ u_2 = & \left(1 \cdot [P_{d2}]\right) & + & \left(-0.5 \cdot [P_{d3}]\right) \\ u_3 = & \left(-0.5\ [P_{d2}]\right) & + & \left(1 \cdot [P_{d3}]\right) \end{array}$$

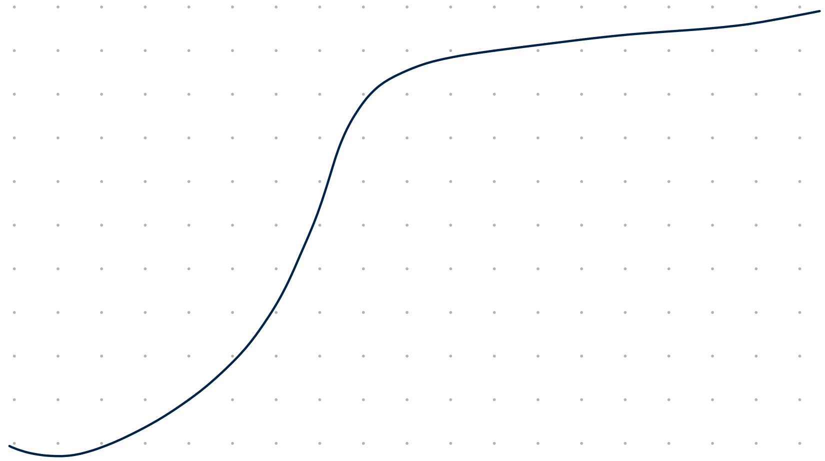
$$g = \frac{1}{2} \cdot \left(\frac{[P_{d1}] \cdot -0.5\,[P_{d3}]}{\sqrt{[P_{d1}] \cdot -0.5\,[P_{d3}] + 1}} + 1\right) \cdot \left(\frac{[P_{d3}] \cdot -0.5\,[P_{d1}]}{\sqrt{[P_{d3}] \cdot -0.5\,[P_{d1}] + 1}} + 1\right)$$

How to model multiplexed CRISRa



Is RNAp bound to Promoter	Is TF1 bound to its binding site
Yes	Yes
	No
No	Yes
	No

n_R = # of RNAP molecules available for binding = [RNAP]?
n_2 = " TF1 "



$$\alpha_{GFP} = \alpha^0 \cdot \prod_{i=1}^{n} g_i$$

$$g_i = \qquad + \frac{(K_r \cdot I_{ij})^n}{(K_r \cdot I_{ij})^n + \frac{[C_{d1}]^{n/2}}{[C_{d2}]^{n/2}}}$$

$$\sum_{j=1}^{n} 1 \cdot$$

1	0	0.5
0	1	-0.5
0.5	-0.5	1

$$K_r$$

$$g_{GFP} = g_1 \cdot g_2$$

$$= \frac{1}{2}\left(\frac{u_1}{\sqrt{u_1^2+1}} + 1\right) \cdot \frac{1}{2}\left(\frac{u_2}{\sqrt{u_2^2+1}} + 1\right) \cdot$$

$$u_1 = 1 \cdot [Pd1] + -0.5 \cdot [Pd3]$$

$$u_2 = 1 \cdot [Pd2] + -0.5\,[Pd1]$$