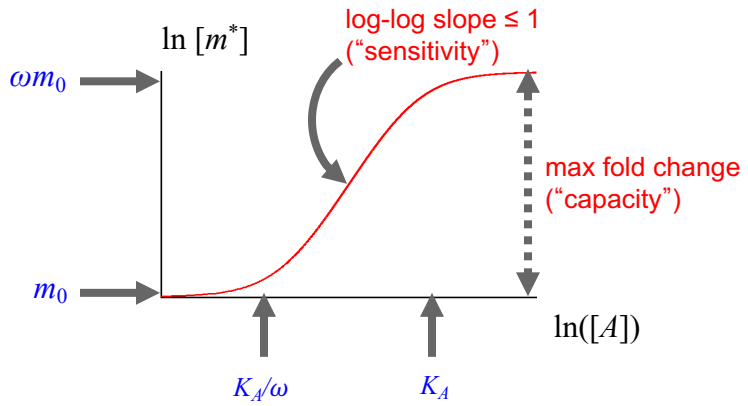
## C. Cooperativity in Transcriptional Control

$$[m^*] = \alpha \cdot \mathcal{P} / \beta \approx m_0 \cdot \frac{1 + \omega \cdot [A]/K_A}{1 + [A]/K_A}, \qquad m_0 \equiv \frac{\alpha\,[P]}{\beta\,K_P}$$




➔ $K_A$ tunable; $\omega$ constrained; slope??
➔ need sensitivity > 1 for nontrivial circuits (later)

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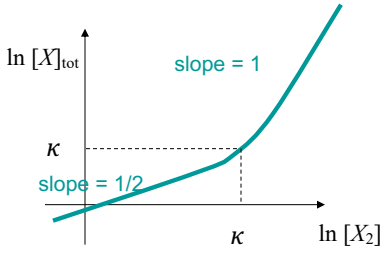
### 1. Dimerization: $X^* = X_2$

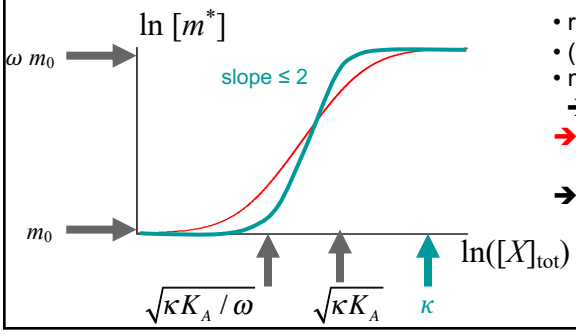
$$X + X \rightleftharpoons X_2$$
$$\kappa = [X]^2 / [X_2]$$

$$[X]_{tot} = [X] + 2 \cdot [X_2]$$
$$= \sqrt{\kappa [X_2]} + 2 \cdot [X_2]$$

for $[X]_{tot} \ll \kappa$, $[X_2] \approx [X]_{tot}^2 / \kappa$

and $[m^*] \propto \frac{1 + \omega \cdot [X_2]/K_A}{1 + [X_2]/K_A} \approx \frac{1 + \omega \cdot [X]_{tot}^2/(\kappa K_A)}{1 + [X]_{tot}^2/(\kappa K_A)}$




- requires $K_A \ll \kappa$
- (strong site, weak dimer)
- most bacterial TFs: $\kappa$ = 1 ~ 10 nM
  ➔ $[X]_{tot} \sim [X_2]$,

➔bacteria do not seem to use this source of cooperativity

➔ possible cost: need $[X]_{tot} \gg [X_2]$
i.e., lots of (useless) monomers

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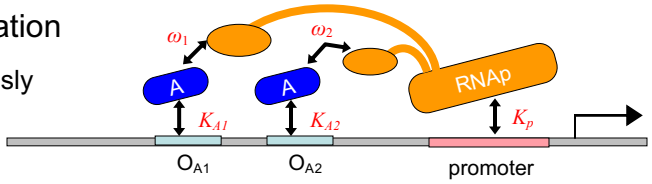
## 2. Synergistic activation

RNAp can simultaneously contact two TFs (e.g., Crp at positions -61.5 and -91.5)




statistical weight $W$ for each configuration $\{\sigma_1, \sigma_2, \sigma_p\}$, with $q_X = [X]/K_X$

$$W_{off}\begin{cases} W(0,0,0)=1 \\ W(1,0,0)=q_{A1} \\ W(0,1,0)=q_{A2} \\ W(1,1,0)=q_{A1}\cdot q_{A2} \end{cases} \qquad W_{on}\begin{cases} W(0,0,1)=q_p \\ W(1,0,1)=\omega_1\cdot q_{A1}\cdot q_p \\ W(0,1,1)=\omega_2\cdot q_{A2}\cdot q_p \\ W(1,1,1)=\omega_3\cdot q_{A1}\cdot q_{A2}\cdot q_p \end{cases}$$

3-body interaction: $\omega_3 = \omega_1\,\omega_2$ (independent); $\omega_3 > \omega_1\,\omega_2$ (pre-bending by Crp)

tsx level: $[m^*] = m_0 \cdot \mathcal{P}([A])$

$$\mathcal{P}([A]) = W_{on}/(W_{on}+W_{off}) \approx W_{on}/W_{off} \quad \text{since } \mathcal{P} \ll 1$$

$$= q_p \cdot \frac{(1+\omega_1 q_{A1})\cdot(1+\omega_2 q_{A2}) + (\omega_3-\omega_1\omega_2)\cdot q_{A1}q_{A2}}{(1+q_{A1})\cdot(1+q_{A2})}$$

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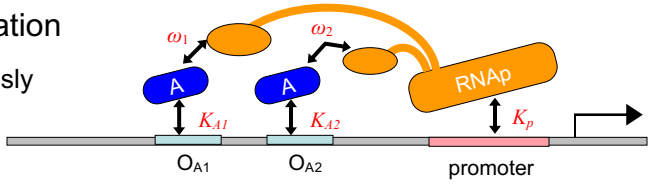



$$\mathcal{P}([A]) = q_p \cdot \frac{(1+\omega_1 q_{A1})\cdot(1+\omega_2 q_{A2}) + (\omega_3-\omega_1\omega_2)\cdot q_{A1}q_{A2}}{(1+q_{A1})\cdot(1+q_{A2})}$$

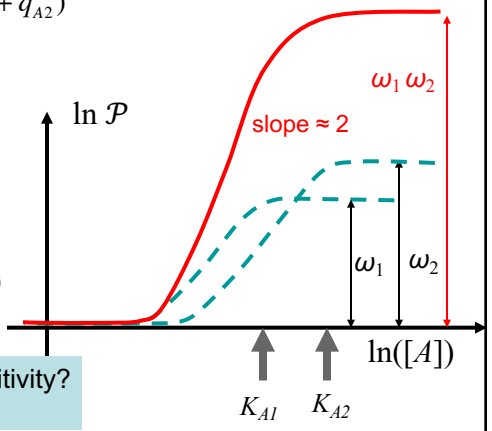
where $q_X = [X]/K_X$

- for $\omega_3 \approx \omega_1\,\omega_2$ (no interaction)

$$\mathcal{P}([A]) \approx q_p \cdot \frac{1+\omega_1 q_{A1}}{1+q_{A1}} \cdot \frac{1+\omega_2 q_{A2}}{1+q_{A2}}$$

capacity of response = $\omega_1\,\omega_2$
sensitivity = 2
➔ effective Hill form with Hill coeff 2

- for $\omega_3 > \omega_1\,\omega_2$ (positive cooperativity)
capacity of response = $\omega_3$




a great way to boost capacity & sensitivity?
but not widely seen in *E. coli*

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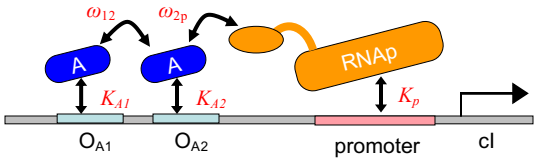
## 3. Cooperative activation

widely seen in bacteria;
e.g., $P_{RM}$ promoter of phage λ
(A = CI)




statistical weight $W$ for each configuration $\{\sigma_1, \sigma_2, \sigma_p\}$, with $q_X = [X]/K_X$

$$W_{off}\begin{cases} W(0,0,0)=1 \\ W(1,0,0)=q_{A1} \\ W(0,1,0)=q_{A2} \\ W(1,1,0)=\omega_{12}\cdot q_{A1}\cdot q_{A2} \end{cases} \qquad W_{on}\begin{cases} W(0,0,1)=q_p \\ W(1,0,1)=q_{A1}\cdot q_p \\ W(0,1,1)=\omega_{2p}\cdot q_{A2}\cdot q_p \\ W(1,1,1)=\omega_{12}\cdot\omega_{2p}\cdot q_{A1}\cdot q_{A2}\cdot q_p \end{cases}$$

(3-body interaction insignificant!)

$$\mathcal{P}([A]) \approx W_{on}/W_{off} = q_p \frac{1+q_{A1}+\omega_{2p}q_{A2}+\omega_{12}\omega_{2p}q_{A1}q_{A2}}{1+q_{A1}+q_{A2}+\omega_{12}q_{A1}q_{A2}}$$

$$= q_p \cdot \frac{1+\left(\omega_{2p}+\frac{K_{A2}}{K_{A1}}\right)\cdot\frac{[A]}{K_{A2}}+\omega_{12}\omega_{2p}\frac{[A]^2}{K_{A1}K_{A2}}}{1+\left(1+\frac{K_{A2}}{K_{A1}}\right)\cdot\frac{[A]}{K_{A2}}+\omega_{12}\frac{[A]^2}{K_{A1}K_{A2}}}$$

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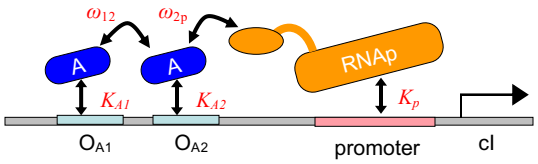


parameter dependence? (universal problem for q-bio)

– $K_{A1} = \infty$ (i.e., remove $O_{A1}$ site)

$$\mathcal{P}([A]) \approx q_p \cdot \frac{1+\omega_{2p}[A]/K_{A2}}{1+[A]/K_{A2}}$$

$$\mathcal{P}([A]) \approx W_{on}/W_{off} = q_p \frac{1+q_{A1}+\omega_{2p}q_{A2}+\omega_{12}\omega_{2p}q_{A1}q_{A2}}{1+q_{A1}+q_{A2}+\omega_{12}q_{A1}q_{A2}}$$

$$= q_p \cdot \frac{1+\left(\omega_{2p}+\frac{K_{A2}}{K_{A1}}\right)\cdot\frac{[A]}{K_{A2}}+\omega_{12}\omega_{2p}\frac{[A]^2}{K_{A1}K_{A2}}}{1+\left(1+\frac{K_{A2}}{K_{A1}}\right)\cdot\frac{[A]}{K_{A2}}+\omega_{12}\frac{[A]^2}{K_{A1}K_{A2}}}$$

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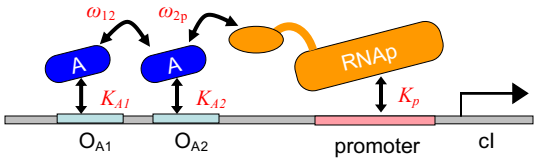


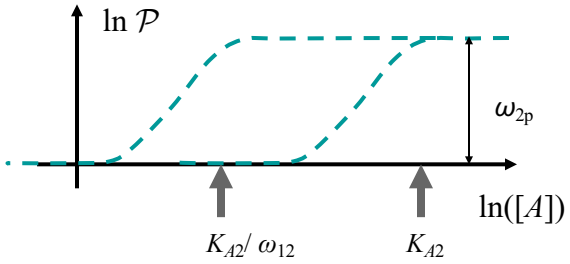

parameter dependence? (universal problem for q-bio)

– $K_{A1} = \infty$ (i.e., remove $O_{A1}$ site)

$$\mathcal{P}([A]) \approx q_p \cdot \frac{1+\omega_{2p}[A]/K_{A2}}{1+[A]/K_{A2}}$$

– $K_{A1} = 0$ (i.e., fix A to $O_{A1}$ site)
single site with $K_{A2}$ ➔ $K_{A2}/\omega_{12}$

$$\mathcal{P}([A]) \approx q_p \cdot \frac{1+\omega_{12}\omega_{2p}[A]/K_{A2}}{1+\omega_{12}[A]/K_{A2}}$$




– intermediate $K_{A1}$: capacity fixed ($\omega_{2p}$); can at most have a steeper slope

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## 3. Cooperative activation

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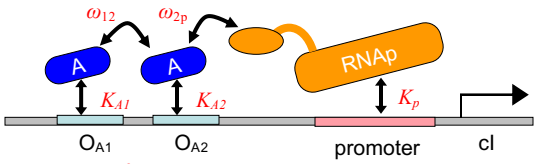
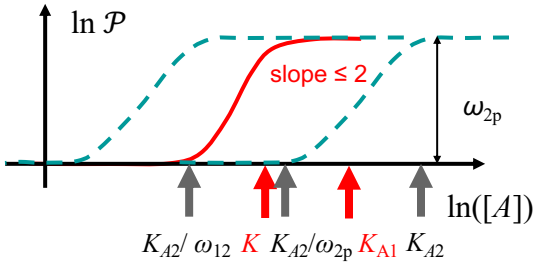
(A = CI)




can show that $\mathcal{P}([A]) \approx q_p \cdot \dfrac{1+\omega_{2p}([A]/K)^2}{1+([A]/K)^2}$ where $K \approx \sqrt{K_{A1}K_{A2}/\omega_{12}}$

if $\omega_{12} \gg \omega_{2p} \gg 1$ and $K_{A2} \gtrsim K_{A1} \gtrsim K_{A2}/\omega_{2p}$




parameters for $P_{RM}$ promoter:
$\omega_{12} \approx 100$, $\omega_{2p} \approx 10$,
$K_{A2}/K_{A1} \approx 25$
– close to the optimal range
– sensitivity ≈ 0.93 limited by $\omega_{2p}$
(single-site sensitivity: 0.54)

– need to increase both $\omega_{12}$ and $\omega_{2p}$ for more sensitivity
– much larger $\omega_{12}$ may be a problem for TF-DNA dynamics
– is a slightly larger sensitivity really significant physiologically??

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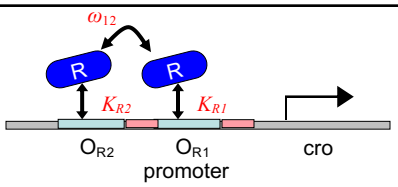
## 4. Cooperative repression

e.g., $P_R$ promoter of phage λ
(R = CI)

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$$\mathcal{P}([R]) \approx W_{on}/W_{off} = q_p/\left(1+q_{R1}+q_{R2}+\omega_{12}q_{R1}q_{R2}\right)$$
$$= q_p/\left[1+\left(K_{R1}^{-1}+K_{R2}^{-1}\right)\cdot[R]+\omega_{12}[R]^2/\left(K_{R1}K_{R2}\right)\right]$$
$$\approx q_p/\left[1+\omega_{12}[R]^2/\left(K_{R1}K_{R2}\right)\right]$$
$$\text{if } \omega_{12} \gg \left(\sqrt{K_{R2}/K_{R1}}+\sqrt{K_{R1}/K_{R2}}\right)^2 \approx K_{\text{larger}}/K_{\text{smaller}}$$

for phage λ, $O_{A1}$=$O_{R2}$ and $O_{A2}$=$O_{R1}$ ➔ $\omega_{12} \approx 100;\ K_{R1}/K_{R2} \approx 25$

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## 4. Cooperative repression

e.g., $P_R$ promoter of phage λ
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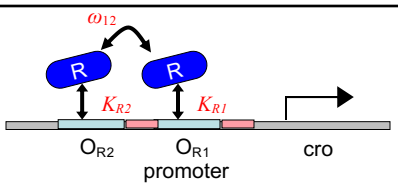


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Note that even if $\omega_{12}=1$ (i.e,. no interaction)

$$\mathcal{P}([R]) \approx \frac{q_p}{\left(1+[R]/K_{R1}\right)\cdot\left(1+[R]/K_{R2}\right)}$$
$$\approx \frac{q_p}{[R]^2/\left(K_{R1}K_{R2}\right)} \quad \text{for} \quad [R] \gg K_{R1}+K_{R2}$$

➔ cooperative repression does not require interaction
c.f. "collaborative competition" (Jon Widom)

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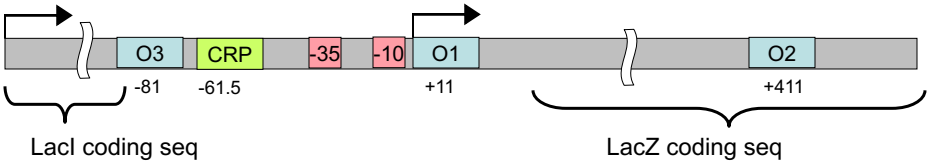
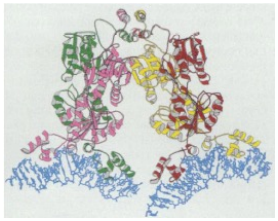
## 5. Transcriptional control via DNA looping

- discovered in the study of araBAD regulation (Schleif, 1984)
- also involved in the repression of *lac, deo, mel, gal, …* operons
- activation of $\sigma^{54}$-promoters (e.g., *glnALG* operon)

### Consider regulation of the *lac* promoter ($P_{lac}$)




Lac repressor = dimer of dimers



– each dimer unit can bind specifically to operator
– the two dimeric units are (approximately) uncoupled
i.e., can bind DNA independently of the other unit
– enables DNA looping

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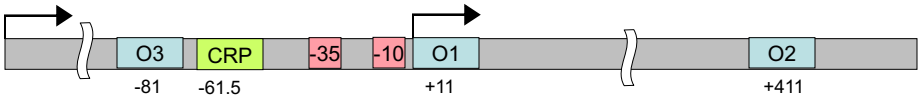
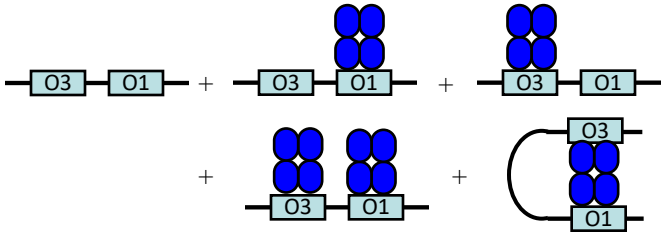



- effect of O1 alone (tetramer conc = $[R]$; dissoc const = $K_1$)

$$W_{on} = q_p, \quad W_{off} = 1 + 2[R]/K_1 \equiv 1 + 2q_1$$

$$\Rightarrow \quad \mathcal{P}([R]) \approx \frac{W_{on}}{W_{off}} = \frac{q_p}{1 + 2q_1}$$

- include O1 and O3 (dissoc constants $K_1$ and $K_3$)

$W_{\text{off}} =$ 


$$= 1 + 2q_1 + 2q_3 + 4q_1q_3 + 2C_L \frac{[R]}{K_1K_3}$$ [note: $C_L$ has dimension of conc]

$$W_{on} = q_p + 2q_pq_3$$

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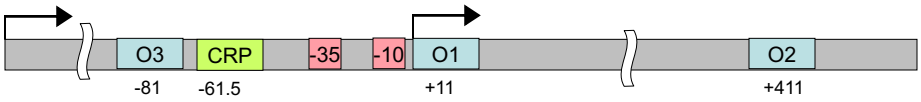
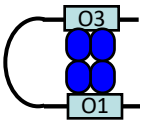



• include O1 and O3 (dissoc constants $K_1$ and $K_3$)

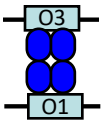
$$\mathcal{P}([R]) \approx q_p \frac{1+2q_3}{(1+2q_1)\cdot(1+2q_3)+2C_L\dfrac{[R]}{K_1K_3}}$$




What is $C_L$?

-- suppose O1 and O3 are not linked

statistical weight




$$= \frac{[\mathrm{O3}:R:\mathrm{O1}]}{[\mathrm{O3}-\mathrm{O1}]} = \frac{[\mathrm{O3}][R:\mathrm{O1}]}{K_3[\mathrm{O3}-\mathrm{O1}]} = \frac{[R]}{K_3K_1}\cdot\frac{[\mathrm{O3}][\mathrm{O1}]}{[\mathrm{O3}-\mathrm{O1}]}$$

conc of O1 O3 in the same config but without R

$C_L \sim 1/V_{\mathrm{cell}} \sim 1$ nM

➔ $C_L$ gives probab. that two operators are in the required config by chance; or the effective conc seen at one site given the other site is occupied by $R$

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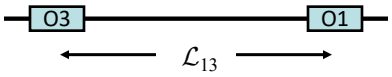
-- next consider two operators linked by the DNA backbone:




$\mathcal{L}_{13} = 92$ bp $\approx 30$ nm

crude approximation 1: "tether" two operators with flexible linker of length $\mathcal{L}$

$$C_{L13} \sim 1\Big/\left(\tfrac{4\pi}{3}\mathcal{L}_{13}^3\right) \approx 10^4 \text{ nM} \qquad \begin{pmatrix}\text{for } \mathcal{L}_{12} = 400 \text{ bp} \approx 130 \text{ nm}, C_{L12} \sim 10^2 \text{ nM} \\ \text{for } \mathcal{L} = 1000 \text{ bp}, C_L \sim 6 \text{ nM, negligible}\end{pmatrix}$$

crude approximation 2: linker = flexible polymer of persistence length $L_p$

for $\mathcal{L} \gg L_p$, $\quad (L_p = 50 \text{ nm} \approx 150 \text{ bp})$

displacement of RW given by

$$P(r) \approx \left(2\pi\overline{r^2}\right)^{-3/2} \exp\left[-\left(r^2/2\overline{r^2}\right)\right], \quad \text{where } \overline{r^2} \sim L_p^2\cdot\left(\mathcal{L}/L_p\right) = L_p\cdot\mathcal{L}$$

$$\Rightarrow C_L = P(r=0) \approx 1\Big/\left(2\pi L_p\mathcal{L}\right)^{-3/2} \quad \text{(increases more slowly with } \mathcal{L})$$

for $\mathcal{L}_{12} = 400$ bp $\approx$ 130 nm, $C_{L12} \approx 120$ nM

$\mathcal{L} =$ 1000 bp, $C_L \approx 30$ nM

for small $\mathcal{L}$s, need to consider the details of DNA bending

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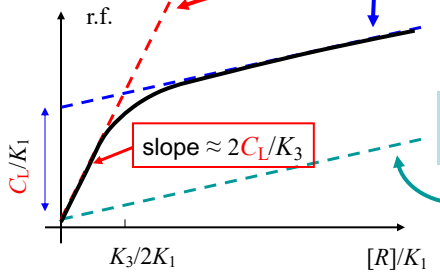
• How should DNA looping be used?

$$\mathcal{P}([R]) \approx q_p \frac{1+2q_3}{(1+2q_1)\cdot(1+2q_3)+2C_L\dfrac{[R]}{K_1K_3}}$$

$$\text{repression factor (r.f.)} \equiv \frac{\mathcal{P}(0)}{\mathcal{P}([R])} = 1+2q_1+\frac{C_L}{K_1}\frac{2q_3}{1+2q_3}$$

for $q_3 \gg 1$ $([R] \gg K_3)$, $\text{r.f.} \approx 1+\dfrac{2[R]+C_L}{K_1}$

for $q_3 \ll 1$ $([R] \ll K_3)$, $\text{r.f.} \approx 1+\left(1+\dfrac{C_L}{K_3}\right)\cdot\dfrac{2[R]}{K_1}$




for large fold-repression, want
$C_L \gg K_3 \gg [R] \gg K_1$

no DNA looping:
$1 + (2[R]/K_1)$

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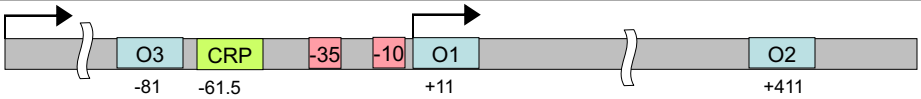


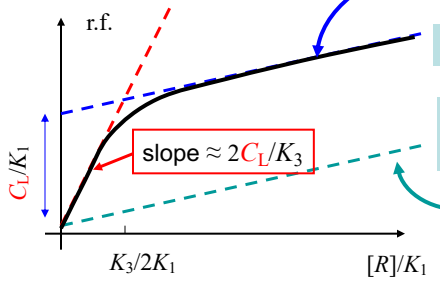

expt: $[R] \approx 10$ nM, $K_1 \approx 0.5$ nM, $K_3 \approx 250$ nM

ref: Oehler et al, 1990, 1992
Vila & Leibler, 2003

r.f. with loop ≈ 400
r.f. w/o loop ≈ 20
} $C_L/K_3 \approx 20$ ➔ $C_L \approx 5000$ nM

direct determination: $C_L \approx 3000$ nM
further enhancement (~5x) due to Crp-mediated DNA bending


weak O3 needed to prevent "squelching"




for large fold-repression, want
$C_L \gg K_3 \gg [R] \gg K_1$

no DNA looping:
$1 + (2[R]/K_1)$

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