B. Basic Models of Transcriptional Control

1. tsx init by RNAp alone

\[
\text{RNAp} + \text{promoter} \rightleftharpoons_{K_p} \text{RNAp} \cdot \text{promoter} \rightarrow \alpha \text{RNAp} + \text{promoter} + mRNA
\]

• mRNA level:

\[
\frac{\text{d} \left[ m \right]}{\text{d}t} = \alpha \cdot \mathcal{P} - \beta \cdot \left[ m \right]
\]

probability of promoter occupation by RNAp

mRNA degradation

• steady-state mRNA level (measurable):

\[
\left[ m^* \right] = \frac{\alpha \cdot \mathcal{P}}{\beta}
\]

• from protein-DNA interaction, expect \( \mathcal{P} = \frac{1}{1 + \frac{K_p}{[P]_{av}}} \)

where \([P]_{av} = \text{avail RNAp conc} \approx 0.5 \sim 1 \mu M \)

\( K_p = N \cdot K_p / K_{ns} = 10^4 \sim 10^7 \text{nM} \)

\( \Rightarrow \) for RNAp by itself, \( \mathcal{P} \approx \frac{[P]_{av}}{K_p} \ll 1 \)

\( \Rightarrow \) TF can modulate \( \mathcal{P} \) or \( \alpha \)

2. Activation by recruitment

How does gene expression depend on [A]?

Strategy: [Shea & Ackers, 1985]

-- assume \( \left[ m^* \right] = \alpha \cdot \mathcal{P}([A],[P]) / \beta \)

-- \( \mathcal{P} \) computed according to thermodynamics (assumes thermal equilibrium)

Recall for operator site alone:

\[
p_A = \frac{[A]_{tot}}{[A]_{tot} + \tilde{K}_A}
\]

[will drop tilde and subscript “tot” from here on]

Total probability of RNAp binding to promoter in the presence of A:

\[
\mathcal{P}([A],[P]) = \frac{W(0,1) + W(1,1)}{W(0,0) + W(0,1) + W(1,0) + W(1,1)}
\]

where \( W(\sigma_A, \sigma_p) = \text{weight of} \)

\[
\begin{align*}
\text{operator } A \text{ is occupied } (\sigma_A = 1) \text{ or unoccupied } (\sigma_A = 0) \\
\text{promoter is occupied } (\sigma_p = 1) \text{ or unoccupied } (\sigma_p = 0)
\end{align*}
\]
Dependence of the total probability of RNAp-promoter binding on A:

\[ \mathcal{P}([A],[P]) = \frac{W(0,1)+W(1,1)}{W(0,0)+W(0,1)+W(1,0)+W(1,1)} \]

Form of \( W(\sigma_A, \sigma_P) \): let \( W(0,0)=1 \) (since only ratio of weights matter)

\[
W(0,1) = [P] / K_p, \quad W(1,0) = [A] / K_A
\]

\[
W(1,1) = \omega \cdot \frac{([A] / K_A) \cdot ([P] / K_p)}{[A] + [P] / K_p}
\]

check: \( P \) by itself, i.e., \([A]=0\), \( p_P = \frac{W(0,1)}{W(0,0)+W(0,1)} = \frac{[P] / K_p}{1+[P] / K_p} \)

\( P \) given \( A \), i.e., \([A]=\infty\), \( p_{P|A} = \frac{W(1,1)}{W(1,0)+W(1,1)} = \frac{\omega \cdot [P] / K_p}{1+\omega \cdot [P] / K_p} \)

\( \Rightarrow \) promoter strength effectively increased (\( K_p \Rightarrow K_p / \omega \))

Compact notation: \( W(\sigma_A, \sigma_P) = ([A] / K_A)^{\sigma_A} \cdot ([P] / K_p)^{\sigma_P} \cdot \omega^{\sigma_A, \sigma_P} \)

then \( \mathcal{P}([A],[P]) = \sum_{\sigma_A} W(\sigma_A, \sigma_P) = 1 / \sum_{\sigma_A, \sigma_P} W(\sigma_A, \sigma_P) \)

\[ \mathcal{P}([A],[P]) = \frac{[P] / K_p + \omega \cdot ([A] / K_A) \cdot ([P] / K_p)}{1+[A] / K_A + [P] / K_p + \omega \cdot ([A] / K_A) \cdot ([P] / K_p)} \]

- function of \([A]\) and \([P]\), parameterized by \( K_p, K_A, \omega \)
- typical parameter range:
  - promoters weak: \( [P] / K_p \ll 1 \)
  - TF concentration: \([A]=1 \sim 1000 \text{ nM} \)
  - operators tunable: \( K_A = 1 \sim 1000 \text{ nM} \)
  - cooperativity weak: \( \omega = 10 \sim 100 \) (typically \( \sim 20 \))

\( \Rightarrow \) want promoter activity as function of \([A]\)

- expected behavior
  - low state: for \([A]=0\), \( \mathcal{P} = \frac{[P] / K_p}{1+[P] / K_p} = [P] / K_p \ll 1 \),
    \[ \Rightarrow \mathcal{P} = \mathcal{P}_b \] as long as \( \omega \cdot [A] / K_A \ll 1 \)
\[
\mathcal{P}(\{A\}, \{P\}) = \frac{[P]/K_p + \omega \cdot ([A]/K_A) \cdot ([P]/K_p)}{1 + [A]/K_A + [P]/K_p + \omega \cdot ([A]/K_A) \cdot ([P]/K_p)}
\]

- function of \([A]\) and \([P]\), parameterized by \(K_p, K_P, \omega\)
- typical parameter range:
  - promoters weak: \([P]/K_p \ll 1\)
  - TF concentration: \([A] = 1 \sim 1000 \text{ nM}\)
  - operators tunable: \(K_A = 1 \sim 1000 \text{ nM}\)
  - cooperativity weak: \(\omega = 10 \sim 100\) (typically \(\sim 20\))

\(\Rightarrow\) want promoter activity as function of \([A]\)

- expected behavior
  - low state: for \([A] = 0\), \(\mathcal{P} = \frac{[P]/K_p}{1 + [P]/K_p} = [P]/K_p \ll 1\)
    \(\Rightarrow\) \(\mathcal{P} = \mathcal{P}_l\) as long as \(\omega \cdot [A]/K_A \ll 1\)
  - high state: for \([A] \gg K_A\), can consider \(A\) always bound to \(O_A\)
    \(\Rightarrow\) \(\mathcal{P}_h = \frac{\omega \cdot [P]/K_p}{1 + \omega \cdot [P]/K_p} \leq 1\)
  - maximal fold-change (“capacity”): \(\frac{\mathcal{P}_h}{\mathcal{P}_l} = \omega \cdot \frac{1 + [P]/K_p}{1 + \omega \cdot [P]/K_p} \leq \omega\)

\(\Rightarrow\) for maximal control, want weak promoter such that \(\omega \cdot [P]/K_p \ll 1\)

\[
\ln \left( m^* \right) = \alpha \cdot \mathcal{P} / \beta = m_0 \frac{1 + \omega \cdot [A]/K_A}{1 + [A]/K_A}, \quad m_0 = \frac{\alpha \cdot [P]}{\beta K_p}
\]

log-log slope ("sensitivity")

max fold change ("capacity")
3. Repression by promoter occlusion

\[
W(\sigma_R = 1, \sigma_P = 0) = [R] / K_R,
\]
\[
W(\sigma_R = 0, \sigma_P = 1) = [P] / K_P,
\]
\[
W(\sigma_R = 1, \sigma_P = 1) = 0
\]

[promoter and O\(_R\) cannot be simultaneously occupied]

\[
\mathcal{P} = \frac{W(0,1) + W(1,1)}{W(0,0) + W(0,1) + W(1,0) + W(1,1)} = \frac{[P] / K_P}{1 + [P] / K_P + [R] / K_R} \approx \frac{1}{1 + [R] / K_R}
\]

-- large [R] can provide arbitrarily strong repression according to model

-- “promoter leakage” provides the lower limit on [m*]

-- high TF conc often generate toxic side effects

4. Activation by catalysis (rather than recruitment)

\[
\text{RNAP} + \text{promoter} \xrightarrow{K_p} \text{RNAP} \cdot \text{promoter} \xrightarrow{\alpha} \text{RNAP} + \text{promoter} + \text{mRNA}
\]

• mRNA level:

\[
\frac{d}{dt}[m] = \alpha \cdot \mathcal{P} - \beta \cdot [m]
\]

• steady-state mRNA level (measurable):

\[
[m^*] = \frac{\alpha \cdot \mathcal{P}}{\beta}
\]

for \(\sigma^{54}\) promoters, the rate of promoter opening catalyzed by activator
4. Activation by catalysis (rather than recruitment)

\[
\text{RNAP + promoter } \xrightarrow{K_p} \text{RNAP} \cdot \text{promoter} \xrightarrow{\alpha} \text{RNAP + promoter + mRNA}
\]

“p”

“m”

• mRNA level: \( \frac{d}{dt} [m] = \alpha \cdot P - \beta \cdot [m] \)

\( \text{tsx init rate} \) \hspace{1cm} \( \text{mRNA degradation} \)

probability of promoter occupation by RNAP

• steady-state mRNA level (measurable): \( [m^*] = \frac{\alpha \cdot P}{\beta} \)

for \( \sigma^{54} \) promoters, the rate of promoter opening catalyzed by activator

model:

\[
\alpha \Rightarrow \alpha_{\sigma_A}
\]

\[
\alpha \cdot P \Rightarrow \sum_{\sigma_A, \sigma_P} \alpha_{\sigma_A} \cdot W(\sigma_A, \sigma_P) = 1 / \sum_{\sigma_A, \sigma_P} W(\sigma_A, \sigma_P)
\]

\[
[m^*] \approx m_0 \frac{1 + \frac{\omega \cdot [A]}{K_A}}{1 + [A] / K_A}, \hspace{0.5cm} m_0 = \frac{\alpha_0 \cdot [P]}{\beta \cdot K_A}
\]

\( \Rightarrow \) same form as recruitment, but capacity increased by \( \frac{\alpha_1}{\alpha_0} \)

\( \Rightarrow \) large fold change, but dedicated components

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• “Advantages of the \( \sigma^{54} \) system:
  – very low basal rate for small \( \alpha_0 \)
    (activators need to consume ATP to catalyze open complex)
  – large capacity w/o need for large \( \omega \)
    (recall also that very large \( \omega \) can reduce capacity)
  – can activate from a long distance away (via DNA looping -- later)

• but in most bacteria species, there is at most one \( \sigma^{54} \) factor
  (compared to many families of \( \sigma^{70} \) factors)

• possible disadvantages?
  long distance activation can create unintentional cross talk unless different promoters are kept far apart (require long chromosomes) or separated by “insulating elements” (not available for prokaryotes)

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5. Induction of TF

\[ X + I \xrightarrow{k_+} XI \]

\[ \text{dissociation constant} \quad K_i = \frac{[X][I]}{[XI]} = \frac{k_-}{k_+} \]

\[ [XI] = [X]_{tot} \frac{[I]}{[I]+K_i} = [X]_{tot} \frac{[I]_{tot}}{[I]_{tot} + K_i} \]

usually \([I]_{tot} \gg [X]_{tot}\), so \([I] = [I]_{tot}\)

will drop the subscript "tot" from here on

"activated TF" \(X^*\) = form of TF able to bind specifically to DNA
or able to activate RNAp

if \(X^* = XI\), then \([X^*] = [X]_{tot} \frac{[I]}{[I]+K_i}\)

if \(X^* = X\), then \([X^*] = [X]_{tot} \frac{K_i}{[I]+K_i}\)

---

often TF are dimers \((X_2)\)

\[ K_1 = \frac{[X_2][I]}{[X_2I]} \]

\[ [X_2]_{tot} = [X_2] \left( 1 + 2 \frac{[I]}{K_1} + \frac{[I]^2}{K_1K_2} \right) \]

- non-cooperative \((K_1 = K_2)\): \([X_2]_{tot} = [X_2]_{tot} \left( 1 + \frac{[I]}{K_1} \right)^2\)

- strongly cooperative \((K_2 \ll K_1)\): \([X_2]_{tot} = [X_2]_{tot} \left( 1 + \frac{[I]^2}{K_1K_2} \right)\)

(e.g., binding of 2nd molecule much easier after 1st is bound)

\(\Rightarrow\) active TF could be \(X_2\), \(X_2I\), or \(X_2I_2\)

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Hill function
C. Cooperativity in Transcriptional Control

\[ [m^*] = \frac{\alpha \cdot P}{\beta} = m_0 \cdot \frac{1 + \omega \cdot [A]/K_A}{1 + [A]/K_A}, \quad m_0 = \frac{\alpha \cdot [P]}{\beta \cdot K_P} \]

\[ \log \log \text{slope} \leq 1 \]

("sensitivity")

\[ \text{max fold change} \]

("capacity")

\[ K_A \text{ tunable; } \omega \text{ constrained; } \text{slope}?? \]

\[ \Rightarrow \text{need sensitivity} > 1 \text{ for nontrivial circuits (later)} \]

1. Dimerization: \( X^e = X_2 \)

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

\[ = \sqrt{\kappa \cdot [X_2]} + 2 \cdot [X_2] \]

for \( [X]_{tot} \ll \kappa \), \( [X_2] = [X]_{tot}^2 / \kappa \)

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

\[ \sqrt{\kappa K_A} \]

\[ \kappa \]

\[ \text{slope} \leq 2 \]

\[ \ln([X]_{tot}) \]

\[ \text{slope} = 1/2 \]

\[ \text{requires } K_A \ll \kappa \]

\[ \text{(strong site, weak dimer)} \]

\[ \text{most bacterial TFs: } \kappa = 1 \sim 10 \text{ nM} \]

\[ [X]_{tot} \sim [X_2] \]

\[ \Rightarrow \text{bacteria do not seem to use this source of cooperativity} \]

\[ \Rightarrow \text{possible cost: need } [X]_{tot} \gg [X_2] \]

i.e., lots of (useless) monomers

\[ \kappa \]

\[ \text{slope} = 1 \]

\[ \ln([X]_{tot}) \]
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 $q_x = [X]/K_X$

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

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

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

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

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

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2. Synergistic activation

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

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

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

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

- 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 $\lambda$ ($A = CI$)

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

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

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

$$\mathcal{P}(\sigma, \omega, q_\sigma) \approx \frac{W_{on}}{W_{off}} = q_p \frac{1 + q_{A1} + \omega_{2p}q_{A2} + \omega_{12}\omega_{2p}q_{A1}q_{A2}}{1 + q_{A1} + q_{A2} + \omega_{12}q_{A1}q_{A2}}$$

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

parameter dependence? (universal problem for q-bio)

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

$$\mathcal{P}(\sigma, \omega, q_\sigma) = q_p \cdot \frac{1 + \frac{\omega_{2p}[A]}{K_{A2}}}{1 + [A]/K_{A2}}$$

$$\mathcal{P}(\sigma, \omega, q_\sigma) \approx \frac{W_{on}}{W_{off}} = q_p \frac{1 + q_{A1} + \omega_{2p}q_{A2} + \omega_{12}\omega_{2p}q_{A1}q_{A2}}{1 + q_{A1} + q_{A2} + \omega_{12}q_{A1}q_{A2}}$$

$$= q_p \cdot \frac{1 + \frac{K_{A2}}{K_{A1}} \frac{[A]}{K_{A2}} + \frac{\omega_{12}\omega_{2p}}{K_{A1}K_{A2}}}{\frac{[A]}{K_{A2}} + \frac{\omega_{12}[A]^2}{K_{A1}K_{A2}}}$$
3. Cooperative activation

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

parameter dependence? (universal problem for q-bio)

- $K_{A1} = \infty$ (i.e., remove $O_{A1}$ site)
- $K_{A1} = 0$ (i.e., fix $A$ to $O_{A1}$ site)
- $K_{A1} = \infty$ (i.e., remove $O_{A1}$ site)

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

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

3. Cooperative activation

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

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

- $\omega_{12} \approx 100$, $\omega_{2p} \approx 10$
- $K_{A2} / K_{A1} \approx 25$
- close to the optimal range
- sensitivity $\approx 0.93$ 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??
4. Cooperative repression

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

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

\[
W_{\text{off}} \begin{cases} 
W(0,0,0) = 1 \\
W(1,0,0) = q_{r_2} \\
W(0,1,0) = q_{r_1} \\
W(1,1,0) = \omega_{12} \cdot q_{r_1} \cdot q_{r_2}
\end{cases}
\]

\[
W_{\text{on}} \begin{cases} 
W(0,0,1) = q_p \\
W(1,0,1) = 0 \\
W(0,1,1) = 0 \\
W(1,1,1) = 0
\end{cases}
\]

\[
\mathcal{P}(\{R\}) = W_{\text{on}} / W_{\text{off}} = \frac{q_p}{\left(1 + q_{r_1} + q_{r_2} + \omega_{12} q_{r_1} q_{r_2}\right)}
\]

\[
= \frac{q_p}{\left[1 + \left(K_{r_1}^{-1} + K_{r_2}^{-1}\right) \cdot [R] + \omega_{12} [R]^2 / (K_{r_1} K_{r_2})\right]}
\]

\[
= \frac{q_p}{\left[1 + \omega_{12} [R]^2 / (K_{r_1} K_{r_2})\right]}
\]

if \(\omega_{12} \gg \left(\sqrt{K_{r_2} / K_{r_1}} + \sqrt{K_{r_1} / K_{r_2}}\right)^2 = K_{\text{larger}} / K_{\text{smaller}}\)

for phage $\lambda$, $O_{A1}=O_{R2}$ and $O_{A2}=O_{R1}$ $\Rightarrow \omega_{12} \approx 100$; $K_{r_1} / K_{r_2} \approx 25$

---

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

\[
\mathcal{P}(\{R\}) = \frac{q_p}{\left(1 + [R] / K_{r_1}\right) \cdot \left(1 + [R] / K_{r_2}\right)}
\]

\[
= \frac{q_p}{[R]^2 / (K_{r_1} K_{r_2})} \quad \text{for} \quad [R] \gg K_{r_1} + K_{r_2}
\]

$\Rightarrow$ cooperative repression does not require interaction
c.f. “collaborative competition” (Jon Widom)
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 σ54-promoters (e.g., glnALG operon)

Consider regulation of the lac promoter (P_{lac})

LacI coding seq
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

\[ W_{on} = q_p, \quad W_{off} = 1 + 2[R] / K_1 \equiv 1 + 2q_1 \]
\[ \Rightarrow P([R]) = \frac{W_{on}}{W_{off}} = \frac{q_p}{1 + 2q_1} \]

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

\[ W_{off} = \sum \frac{C_L[R]}{K_1 K_3} \]
\[ W_{on} = q_p + 2q_1 q_3 \]
• include O1 and O3 (dissoc constants $K_1$ and $K_3$)

\[
\mathcal{P}(\{R\}) = q_p \frac{1 + 2q_3}{(1 + 2q_1)(1 + 2q_3)} + \frac{C_L}{K_1K_3}
\]

What is $C_L$?

-- suppose O1 and O3 are not linked statistical weight

\[
\frac{[O3 : R : O1]}{[O3 : O1]} = \frac{[O3][R : O1]}{[O3][O1]} = \frac{[R]}{K_1K_3} \frac{[O3][O1]}{[O3 : O1]}
\]

$C_L \approx 1/V_{cell} \approx 1 \text{nM}$

$\Rightarrow C_L$ 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 $R$.

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

\[
\mathcal{L}_{13} = 92 \text{ bp} \approx 30 \text{ nm}
\]

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

\[
C_{L_{13}} \approx \frac{1}{\left(\frac{4\pi}{3} \mathcal{L}_{13}^3\right)} \approx 10^4 \text{ nM}
\]

(for $\mathcal{L}_{12} = 400 \text{ bp} \approx 130 \text{ nm}$, $C_{L_{12}} \approx 10^2 \text{ nM}$)

(for $\mathcal{L} = 1000 \text{ bp}$, $C_L \approx 6 \text{ nM}$, negligible)

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

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

displacement of RW given by

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

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

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

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

for small $\mathcal{L}$s, need to consider the details of DNA bending
• How should DNA looping be used?

\[
\mathcal{P}(\frac{[R]}{K_1}) \approx q_p \frac{1 + 2q_3}{(1 + 2q_1)(1 + 2q_3) + 2C_L \frac{[R]}{K_1 K_3}}
\]

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

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

for \( q_3 \ll 1 \) \([R] \ll K_1\), \( r.f. \approx 1 + \left( \frac{C_L}{K_1} \right) \frac{2[R]}{K_1} \)

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: \([R] \approx 10 \text{nM}, K_1 \approx 0.5 \text{nM}, K_3 \approx 250 \text{nM} \)  
ref: Oehler et al., 1990, 1992  
Vila & Leibler, 2003

r.f. with loop \( \approx 400 \)  
r.f. w/o loop \( \approx 20 \)

\( C_L/K_3 \approx 20 \quad C_L \approx 5000 \text{nM} \)

direct determination: \( C_L \approx 3000 \text{nM} \)

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: \( 1 + (2[R]/K_1) \)