B. Basic Models of Transcriptional Control

1. tsx init by RNAP alone

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

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

- probability of promoter occupation by RNAP

\[ \text{mRNA degradation} \]

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

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

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

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

⇒ for RNAP by itself, \[ P \approx \frac{[P]_{av}}{K_p} \ll 1 \]

⇒ TF can modulate \( P \) or \( \alpha \)

2. Activation by recruitment

How does gene expression depend on [A]?

Strategy: [Shea & Ackers, 1985]

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

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

Recall for operator site alone: \[ p_A = [A]_{tot} / ([A]_{tot} + K_A) \]

[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 \( W(\sigma_A, \sigma_P) = \text{weight of} \)

- operator \( A \) is occupied (\( \sigma_A = 1 \)) or unoccupied (\( \sigma_A = 0 \))
- promoter is occupied (\( \sigma_P = 1 \)) or unoccupied (\( \sigma_P = 0 \))
Dependence of the total probability of RNAS-promoter binding on A:

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

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

\[
\begin{align*}
W(0,1) &= \frac{[P]}{K_p} \cdot \frac{[A]}{K_A} \cdot \frac{([P]/K_p)}{([A]/K_A)} \\
W(1,1) &= \omega \cdot \left( \frac{[A]}{K_A} \right) \cdot \frac{([P]/K_p)}{([A]/K_A)} \\
W(1,0) &= \frac{[P]}{K_p} \\
W(0,0) &= 1
\end{align*}
\]

\( \omega = e^{-\frac{E_m}{k_BT}} \) ("cooperativity factor")

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., \([A]=\infty\),

\[ P_{PA} = \frac{W(1,1)}{W(1,0) + W(1,1)} = \frac{\omega \cdot [P]/K_p}{1 + \omega \cdot [P]/K_p} \]

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

Compact notation:

\[
W(\sigma_A, \sigma_P) = \left( \frac{[A]}{K_A} \right)^{\sigma_A} \cdot \left( \frac{[P]}{K_p} \right)^{\sigma_P} \cdot \omega^{\sigma_A \sigma_P}
\]

then

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

• function of \([A]\) and \([P]\), parameterized by \(K_p, K_p, \omega\)

• typical parameter range:
  - promoters weak: \([P] / K_p \ll 1\)
  - TF concentration: \([A] = 1 \sim 1000\) nM
  - operators tunable: \(K_A = 1 \sim 1000\) nM
  - cooperativity weak: \(\omega = 10 \sim 100\) (typically \(\approx 20\))

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

• expected behavior
  - low state: for \([A] = 0\), \(P = \frac{[P] / K_p}{1 + [P] / K_p} \approx [P] / K_p \ll 1\)
  \(\Rightarrow P = P_{lo}\) as long as \(\omega \cdot [A] / K_A \ll 1\)
  - high state: for \([A] \gg K_A\), can consider \(A\) always bound to \(O_A\)
  \(\Rightarrow P_{hi} = \frac{\omega \cdot [P] / K_p}{1 + \omega \cdot [P] / K_p} \leq \omega\)

→ for maximal change ("capacity"):
  \(P_{hi} / P_{lo} = \omega \cdot \frac{1 + [P] / K_p}{1 + \omega \cdot [P] / K_p} \leq \omega\)

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

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

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]

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

-- 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)

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

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

\[ \text{tsx init rate} \quad \text{mRNA degradation} \quad \text{probability of promoter occupation by RNAP} \]

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

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

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

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

\( \text{tsx init rate} \quad \text{mRNA degradation} \quad \text{probability of promoter occupation by RNAP} \)

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

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

\[
\alpha \Rightarrow \alpha_{\sigma_5} \\
\alpha \cdot \mathcal{P} \Rightarrow \sum_{\sigma_5} \alpha_{\sigma_5} \cdot W(\sigma_5, \sigma_\alpha = 1) / \sum_{\sigma_5, \sigma_\alpha} W(\sigma_5, \sigma_\alpha) \\
\Rightarrow [m^+] \approx m_0 \frac{1 + \frac{\omega \cdot [A]}{K_A}}{1 + \frac{[A]}{K_A}}, \quad m_0 = \frac{\alpha_{\sigma_5} \cdot [P]}{\beta K_p}
\]

⇒ same form as recruitment, but capacity increased by \( \alpha_1 / \alpha_0 \)
⇒ 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

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

dissociation constant

\[ K_i = \frac{[XI][X]}{[XI]} = \frac{k_+}{k_-} \]

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

-usually \([I] \gg [X]_{tot}\), 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 (\(X_2\))

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

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

- non-cooperative (\(K_1 = K_2\)):

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

- strongly cooperative (\(K_2 \ll K_1\)):

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

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

\[ \Rightarrow \text{active TF could be } X_2, X_2I, \text{ or } X_2I_2 \]

Hill function
C. Cooperativity in Transcriptional Control

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

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

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

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

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

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

- requires \( K_A \ll \kappa \)
- (strong site, weak dimer)
- most bacterial TFs: \( \kappa = 1 \sim 10 \text{ nM} \)
- \( [X]_{tot} \sim [X_2] \)
- bacteria do not seem to use this source of cooperativity
- possible cost: need \( [X]_{tot} \gg [X_2] \)
  i.e., lots of (useless) monomers
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: $[m^*] = m_0 \cdot P([A])$

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

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

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

capacity of response = $\omega_1, \omega_2$

sensitivity = 2

effective Hill form with Hill coeff 2

$\Rightarrow$ 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
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 \( q_X = [X]/K_X \)

\[
W_{\text{eff}} = \begin{cases} 
W(0,0,0) = 1 \\
W(1,0,0) = q_{A_1} \\
W(0,1,0) = q_{A_2} \\
W(1,1,0) = \omega_{12} \cdot q_{A_1} \cdot q_{A_2} \\
\end{cases} \\
\frac{W_{\text{on}}}{W_{\text{off}}} = q_p \cdot \frac{1 + q_{A_1} + \omega_{2p} q_{A_2} + \omega_{12} \omega_{2p} q_{A_1} q_{A_2}}{1 + q_{A_1} + q_{A_2} + \omega_{12} q_{A_1} q_{A_2}}
\]

\[= q_p \cdot \frac{1 + \left( \frac{K_{A_2}}{K_{A_1}} \right) [A] + \frac{K_{A_2}}{K_{A_1}} \frac{[A]^2}{K_{A_1} K_{A_2}}}{1 + \left( \frac{K_{A_2}}{K_{A_1}} \right) [A] + \frac{K_{A_2}}{K_{A_1}} \frac{[A]^2}{K_{A_1} K_{A_2}}}\]
3. Cooperative activation

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

- parameter dependence? (universal problem for q-bio)
  - \( K_{A1} = \infty \) (i.e., remove \( O_{A1} \) site)

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

\[-K_{A1} = 0 \text{ (i.e., fix } A \text{ to } O_{A1} \text{ site)} \]

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

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

\[
\ln P \quad \omega_{2p} \quad \ln([A])
\]

\[
K_{A2}/\omega_{12} \quad K_{A2}
\]

\[
K_{A1}/\omega_{12}
\]

\[\text{parameters for } P_{\text{RM}} \text{ promoter:}
\]

- \( \omega_{12} \approx 100, \omega_{2p} \approx 10, \)
- \( K_{A2} / K_{A1} \approx 25 \)

- 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 $\lambda$

\[(R = Cl)\]

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

\[
W_{\text{off}} \begin{cases} 
W(0,0,0) &= 1 \\
W(1,0,0) &= q_{R2} \\
W(0,1,0) &= q_{R1} \\
W(1,1,0) &= \omega_{12} \cdot q_{R1} \cdot q_{R2}
\end{cases} \quad W_{\text{on}} \begin{cases} 
W(0,0,1) &= q_p \\
W(1,0,1) &= 0 \\
W(0,1,1) &= 0 \\
W(1,1,1) &= 0
\end{cases}
\]

\[
\mathcal{P}(R) = \frac{W_{\text{on}}}{W_{\text{off}}} = q_p / \left(1 + q_{R1} + q_{R2} + \omega_{12} q_{R1} q_{R2}\right)
\]

\[
= q_p / \left[1 + (K_{R1}^{-1} + K_{R2}^{-1}) \cdot [R] + \omega_{12} [R]^2 / (K_{R1} K_{R2})\right]
\]

\[
= q_p / \left[1 + \omega_{12} [R]^2 / (K_{R1} K_{R2})\right]
\]

if $\omega_{12} \gg \left(\sqrt{K_{R2} / K_{R1}} + \sqrt{K_{R1} / K_{R2}}\right)^2 = K_{\text{larger}} / K_{\text{smaller}}$

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

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4. Cooperative repression

e.g., $P_R$ promoter of phage $\lambda$

\[(R = Cl)\]

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

\[
W_{\text{off}} \begin{cases} 
W(0,0,0) &= 1 \\
W(1,0,0) &= q_{R2} \\
W(0,1,0) &= q_{R1} \\
W(1,1,0) &= \omega_{12} \cdot q_{R1} \cdot q_{R2}
\end{cases} \quad W_{\text{on}} \begin{cases} 
W(0,0,1) &= q_p \\
W(1,0,1) &= 0 \\
W(0,1,1) &= 0 \\
W(1,1,1) &= 0
\end{cases}
\]

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

\[
\mathcal{P}([R]) = \frac{q_p}{\left(1 + [R] / K_{R1}\right) \cdot \left(1 + [R] / K_{R2}\right)}
\]

\[
= \frac{q_p}{[R]^2 / (K_{R1} K_{R2})} \quad \text{for} \quad [R] \gg K_{R1} + K_{R2}
\]

\(\Rightarrow\) 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 σ^{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

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

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

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

\[ W_{off} = O3 + O1 + O3 + O1 + O3 + O1 \]

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

\[ W_{on} = q_p + 2q_1q_3 \]
• include O1 and O3 (dissoc constants $K_1$ and $K_3$)

$$P([R]) = q_p \frac{1 + 2q_3}{(1 + 2q_1, (1 + 2q_1) + \left(\begin{array}{c} C_L \cr K_3K_1 \end{array}\right)}$$

What is $C_L$?

-- suppose O1 and O3 are not linked

statistical weight

$$\text{conc of O1 O3 in the same config but without R}$$

$C_L$ 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 $\mathcal{L}$

$$C_{113} \approx \frac{1}{(4\pi/3 \mathcal{L}_{13}^3)} \approx 10^4 \text{ nM} \quad \text{for } \mathcal{L}_{12} = 400 \text{ bp} \approx 130 \text{ nm}, \, C_{112} \approx 10^2 \text{ nM}$$

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

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

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

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

for $\mathcal{L}_{12} = 400 \text{ bp} \approx 130 \text{ nm}, \, C_{112} = 120 \text{ nM}$

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

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

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

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

for \( q_3 \gg 1 \) \( ([R] \gg K_3) \),

\[
\text{r.f.} \approx 1 + \frac{2[R] + C_L}{K_1}
\]

for \( q_3 \ll 1 \) \( ([R] \ll K_3) \),

\[
\text{r.f.} \approx 1 + \left( 1 + \frac{C_L}{K_3} \right) \cdot \frac{2[R]}{K_1}
\]

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 \( \approx 20 \)

\[ C_L/K_3 \approx 20 \Rightarrow C_L \approx 5000 \text{ nM} \]

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

further enhancement (~5x) due to Crp-mediated DNA bending

weak O3 needed to prevent “squelching”