B. Basic Models of Transcriptional Control

1. tsx init by RNAp <u>alone</u>

$$\underbrace{\text{RNAp} + \text{promoter}}_{\text{“}P\text{”}} \underset{}{\overset{K_p}{\rightleftharpoons}} \text{RNAp} \cdot \text{promoter} \xrightarrow{\alpha} \text{RNAp} + \text{promoter} + \underbrace{mRNA}_{\text{“}m\text{”}}$$

- mRNA level: $\frac{d}{dt}[m] = \alpha \cdot \mathcal{P} - \beta \cdot [m]$
  - α: probability of promoter occupation by RNAp
  - β: mRNA degradation
- steady-state mRNA level (measurable): $[m^*] = \alpha \cdot \mathcal{P} / \beta$
- from protein-DNA interaction, expect $\mathcal{P} = 1 \Big/ \left(1 + \widetilde{K}_p / [P]_{av}\right)$

  where $[P]_{av}$ = avail RNAp conc ≈ 0.5 ~ 1 μM

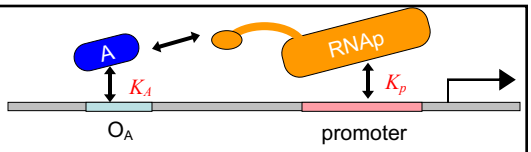
  $\widetilde{K}_p = N \cdot K_p / K_{ns} = 10^4 \sim 10^7$ nM

➔ for RNAp by itself, $\mathcal{P} \approx [P]_{av} / \widetilde{K}_p \ll 1$

➔ TF can modulate $\mathcal{P}$ or α

1

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: $p_A = [A]_{tot} \Big/ \left([A]_{tot} + \widetilde{K}_A\right)$

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

<u>Total</u> 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 $W(\sigma_A, \sigma_P)$ = weight of $\begin{cases} \text{operator } A \text{ is occupied } (\sigma_A = 1) \text{ or unoccupied } (\sigma_A = 0) \\ \text{promoter is occupied } (\sigma_P = 1) \text{ or unoccupied } (\sigma_P = 0) \end{cases}$

2

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)

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

} derived from stat mech

$= e^{-E_{\text{int}}/k_BT}$ ("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]=∞, $p_{P|A} = \frac{W(1,1)}{W(1,0)+W(1,1)} = \frac{\omega \cdot [P]/K_P}{1+\omega \cdot [P]/K_P}$

➔ promoter strength effectively increased ($K_p \rightarrow K_p/\omega$)

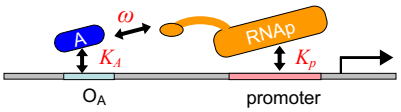
Compact notation: $W(\sigma_A,\sigma_P) = ([A]/K_A)^{\sigma_A} \cdot ([P]/K_P)^{\sigma_P} \cdot \omega^{\sigma_A\sigma_P}$

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

3

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

- function of $[A]$ and $[P]$, parameterized by K_A, K_P, ω
- 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 ~ 20)



➔want promoter activity as function of $[A]$

- expected behavior
  - low state: for $[A]=0$, $\mathcal{P} = \frac{[P]/K_P}{1+[P]/K_P} \approx [P]/K_P \ll 1$

"basal level" = $\mathcal{P}_{lo}$

$\Rightarrow \quad \mathcal{P} \approx \mathcal{P}_{lo}$ as long as $\omega \cdot [A]/K_A \ll 1$

4

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

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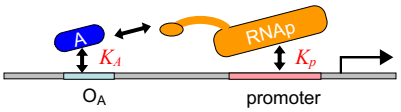

➔want promoter activity as function of $[A]$

"basal level" = $\mathcal{P}_{lo}$

- expected behavior
  - low state: for $[A]=0$, $\mathcal{P} = \frac{[P]/K_P}{1+[P]/K_P} \approx [P]/K_P \ll 1$

    $\Rightarrow \quad \mathcal{P} \approx \mathcal{P}_{lo}$ 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

    $\Rightarrow \quad \mathcal{P}_{hi} \approx \frac{\omega\cdot[P]/K_P}{1+\omega\cdot[P]/K_P} \le 1$
  - maximal fold-change ("capacity"): $\mathcal{P}_{hi}/\mathcal{P}_{lo} \approx \omega\cdot\frac{1+[P]/K_P}{1+\omega\cdot[P]/K_P} \le \omega$

➔ for maximal control, want weak promoter such that $\omega\cdot[P]/K_P \ll 1$

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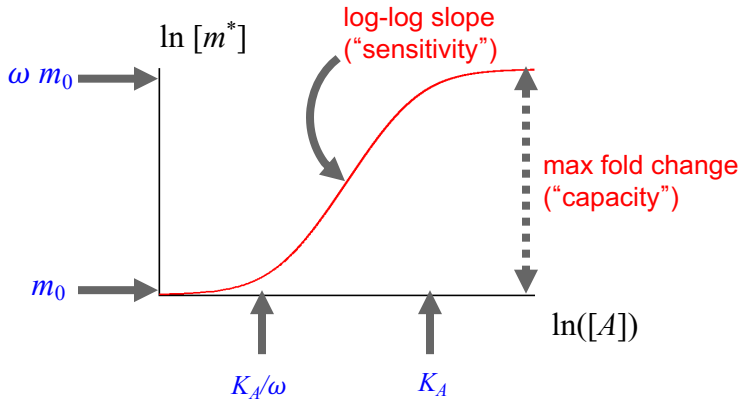
take $\omega[P]/K_P \ll 1$ from here on, then $\mathcal{P} \approx \frac{[P]}{K_P}\cdot\frac{1+\omega\cdot[A]/K_A}{1+[A]/K_A}$

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



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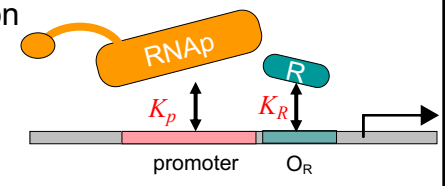
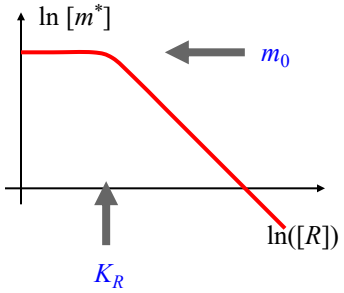
3. Repression by promoter occlusion

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



[promoter and O_R cannot be simultaneously occupied]

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



-- 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

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4. Activation by catalysis (rather than recruitment)

$$\underbrace{\text{RNAp} + \text{promoter}}_{\text{"}P\text{"}} \underset{}{\overset{K_P}{\rightleftharpoons}} \text{RNAp} \cdot \text{promoter} \xrightarrow{\alpha} \text{RNAp} + \text{promoter} + \underbrace{mRNA}_{\text{"}m\text{"}}$$

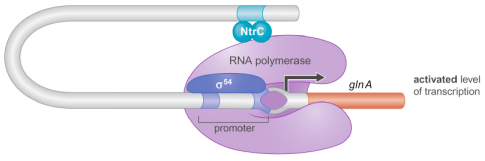
• mRNA level: $\frac{d}{dt}[m] = \alpha \cdot \mathcal{P} - \beta \cdot [m]$

tsx init rate ↑ ↑ mRNA degradation

probability of promoter occupation by RNAp

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

for σ54 promoters, the rate of promoter opening catalyzed by activator



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$$\underbrace{\text{RNAp} + \text{promoter}}_{\text{“}P\text{”}} \underset{}{\overset{K_P}{\rightleftharpoons}} \text{RNAp} \cdot \text{promoter} \xrightarrow{\alpha} \text{RNAp} + \text{promoter} + \underbrace{mRNA}_{\text{“}m\text{”}}$$

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

  tsx init rate ↑ ↑ probability of promoter occupation by RNAp; ↓ mRNA degradation

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

for σ54 promoters, the rate of promoter opening catalyzed by activator

model: $\alpha \Rightarrow \alpha_{\sigma_A}$

$$\alpha \cdot \mathcal{P} \Rightarrow \sum_{\sigma_A} \alpha_{\sigma_A} \cdot W(\sigma_A, \sigma_P = 1) \Big/ \sum_{\sigma_A, \sigma_P} W(\sigma_A, \sigma_P)$$

$$\Rightarrow \quad [m^*] \approx m_0 \cdot \frac{1 + \frac{\alpha_1}{\alpha_0} \cdot \omega \cdot [A]/K_A}{1 + [A]/K_A}, \qquad m_0 \equiv \frac{\alpha_0 \, [P]}{\beta \, K_P}$$

➔ same form as recruitment, but capacity increased by α_1/α_0
➔ large fold change, but dedicated components

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- “Advantages of the σ54 system:
  - very low basal rate for small α_0
    (activators need to consume ATP to catalyze open complex)
  - large capacity w/o need for large ω
    (recall also that very large ω can reduce capacity)
  - can activate from a long distance away (via DNA looping -- later)
- but in most bacteria species, there is at most one σ54 factor
  (compared to many families of σ70 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 $X + I \underset{k_-}{\overset{k_+}{\rightleftharpoons}} XI$

dissociation constant $K_I \equiv \frac{[X]\cdot[I]}{[XI]} = \frac{k_-}{k_+}$

$[X]_{tot} = [X] + [XI]$

$[XI] = [X]_{tot}\frac{[I]}{[I]+K_I} \approx [X]_{tot}\frac{[I]_{tot}}{[I]_{tot}+K_I}$

usually $[I]_{tot} \gg [X]_{tot}$, so $[I] \approx [I]_{tot}$

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 $[X^*] = [X]_{tot}\frac{K_I}{[I]+K_I}$

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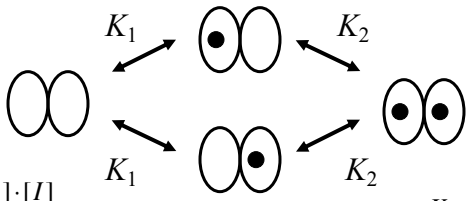
often TF are dimers (X_2)



$K_1 \equiv \frac{[X_2]\cdot[I]}{[X_2I]}$ $K_2 \equiv \frac{[X_2I]\cdot[I]}{[X_2I_2]}$

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

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

• strongly cooperative ($K_2 \ll K_1$): $[X_2] \approx [X_2]_{tot}\Big/\left(1+\frac{[I]^2}{K_1K_2}\right)$

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

Hill function

➔ active TF could be X_2, X_2I, or X_2I_2

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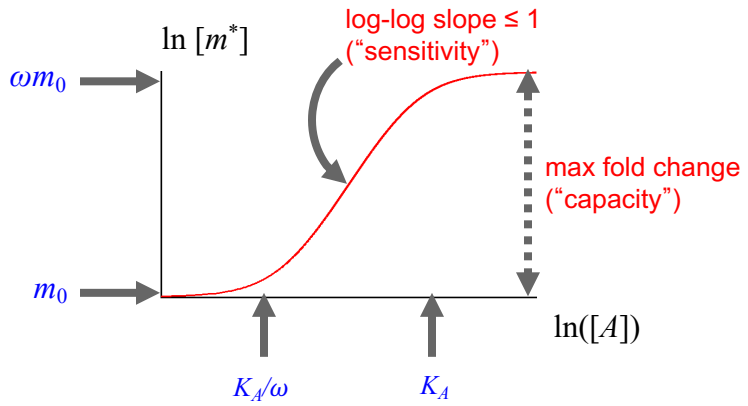
C. Cooperativity in Transcriptional Control

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



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

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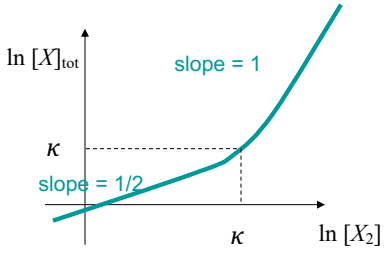
1. Dimerization: $X^* = X_2$

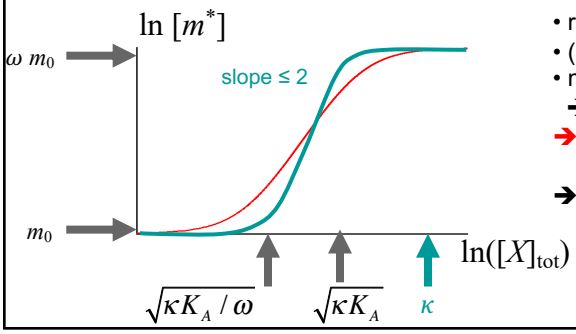
$$X + X \rightleftharpoons X_2$$
$$\kappa = [X]^2 / [X_2]$$

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

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: κ = 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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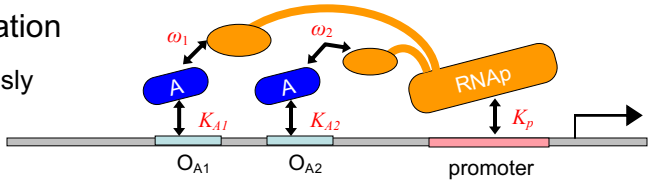
2. Synergistic activation

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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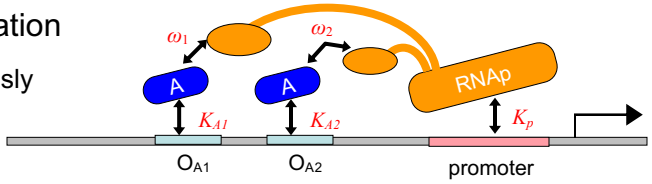


$$\mathcal{P}([A]) = q_p \cdot \frac{(1+\omega_1 q_{A1})\cdot(1+\omega_2 q_{A2}) + (\omega_3-\omega_1\omega_2)\cdot q_{A1}q_{A2}}{(1+q_{A1})\cdot(1+q_{A2})}$$

where $q_X = [X]/K_X$

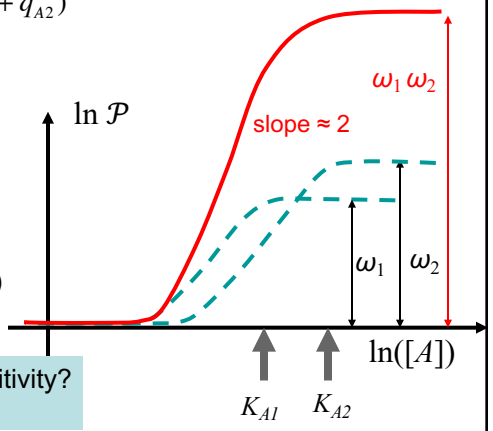
- for $\omega_3 \approx \omega_1\omega_2$ (no interaction)

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

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 = ω_3

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



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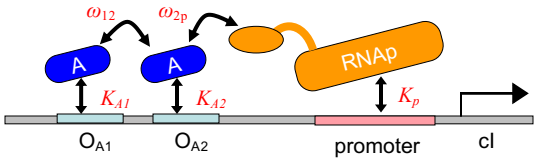
3. Cooperative activation

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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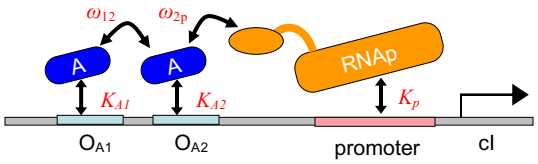
3. Cooperative activation

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



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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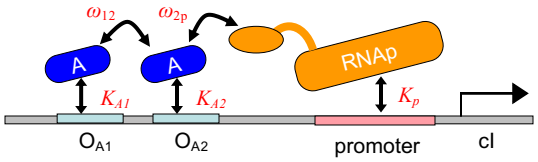


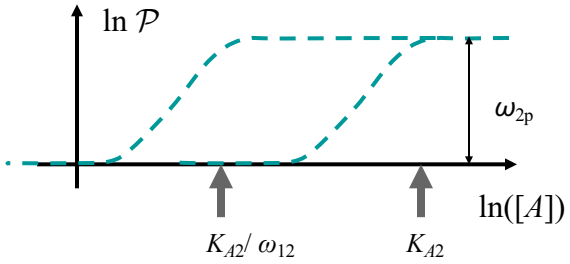
parameter dependence? (universal problem for q-bio)

– $K_{A1} = \infty$ (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}/ω_{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 (ω_{2p}); can at most have a steeper slope

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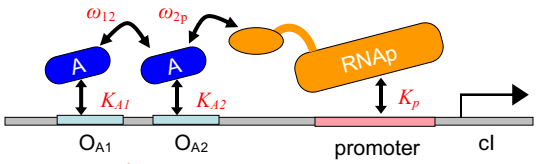
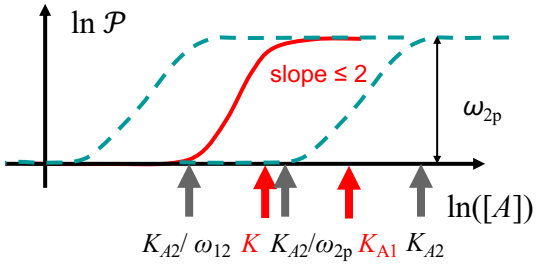
3. Cooperative activation

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



can show that $\mathcal{P}([A]) \approx q_p \cdot \frac{1+\omega_{2p}([A]/K)^2}{1+([A]/K)^2}$ 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 ω_{2p}
(single-site sensitivity: 0.54)

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

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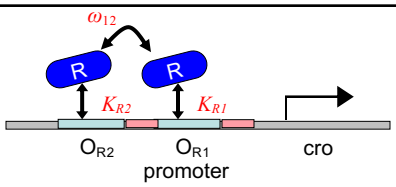
4. Cooperative repression

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



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

$$W_{off}\begin{cases} W(0,0,0)=1 \\ W(1,0,0)=q_{R2} \\ W(0,1,0)=q_{R1} \\ W(1,1,0)=\omega_{12}\cdot q_{R1}\cdot q_{R2} \end{cases} \qquad W_{on}\begin{cases} W(0,0,1)=q_p \\ W(1,0,1)=0 \\ W(0,1,1)=0 \\ W(1,1,1)=0 \end{cases}$$

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

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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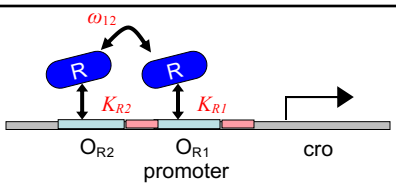
4. Cooperative repression

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



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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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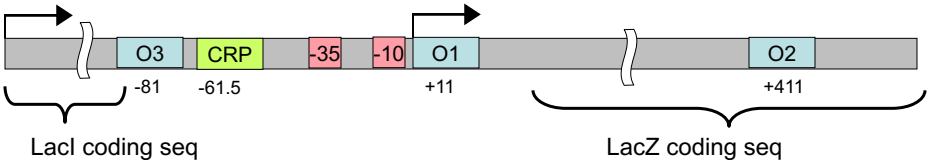
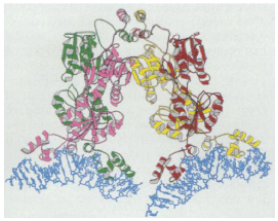
5. Transcriptional control via DNA looping

- discovered in the study of araBAD regulation (Schleif, 1984)
- also involved in the repression of *lac, deo, mel, gal, …* operons
- activation of 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

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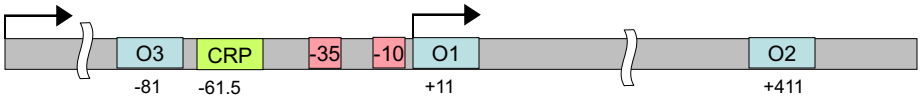
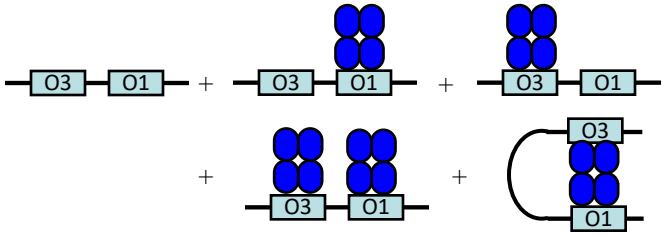


- effect of O1 alone (tetramer conc = $[R]$; dissoc const = K_1)

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

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

- 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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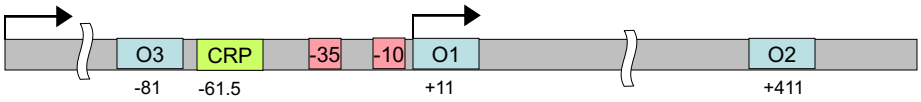
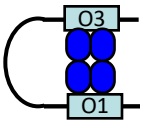


• include O1 and O3 (dissoc constants K_1 and K_3)

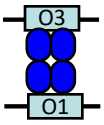
$$\mathcal{P}([R]) \approx q_p \frac{1+2q_3}{(1+2q_1)\cdot(1+2q_3)+2C_L\dfrac{[R]}{K_1K_3}}$$



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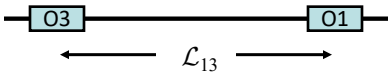
-- next consider two operators linked by the DNA backbone:



$\mathcal{L}_{13} = 92$ bp ≈ 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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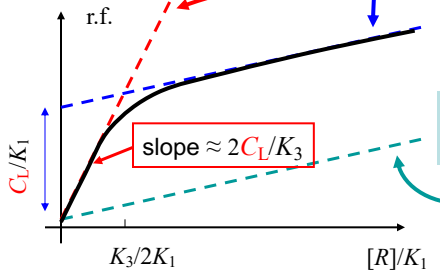
• How should DNA looping be used?

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

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

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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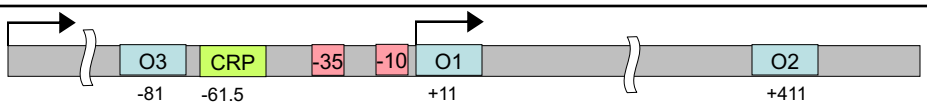
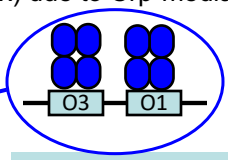


expt: $[R] \approx 10$ nM, $K_1 \approx 0.5$ nM, $K_3 \approx 250$ nM

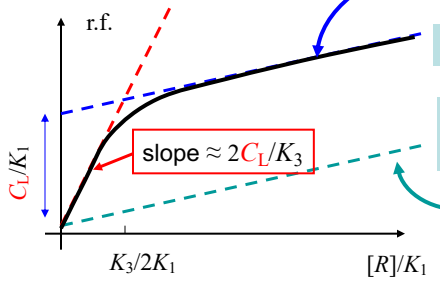
ref: Oehler et al, 1990, 1992
Vila & Leibler, 2003

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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