Review Lecture 2
Michaelis-Menten kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES]$$

$$\frac{d[E]}{dt} = -k_1[E][S] + (k_{-1} + k_2)[ES]$$

$$\frac{d[ES]}{dt} = k_1[E][S] - (k_{-1} + k_2)[ES]$$

$$\frac{dP}{dt} = k_2[ES] \equiv v$$

$$E_o = [E] + [ES]$$

$$\frac{d[S]}{dt} = -k_1 E_o [S] + (k_1[S] + k_{-1})[ES]$$

$$\frac{d[ES]}{dt} = k_1 E_o [S] - (k_1[S] + k_{-1} + k_2)[ES]$$

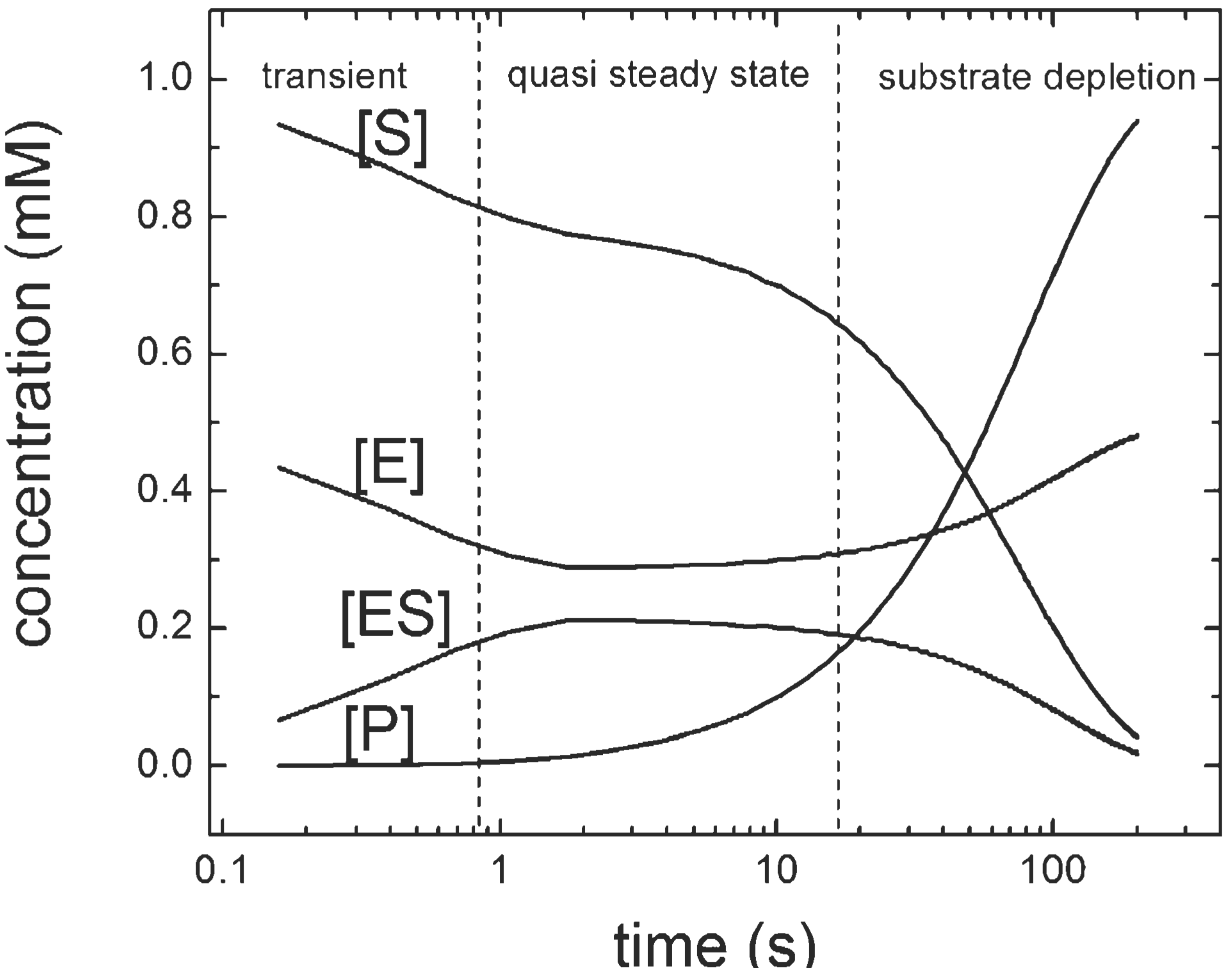
Initial conditions:

$$[S]_{t=0} = S_o$$

$$[E]_{t=0} = E_o$$

$$[ES]_{t=0} = 0$$

$$[P]_{t=0} = 0$$



transient
quasi steady state
substrate depletion
[S]
[E]
[ES]
[P]
1.0
0.8
0.6
0.4
0.2
0.0
0.1
1
10
100
concentration (mM)
time (s)

$$v_0 = \frac{v_{max} S_o}{K_m + S_o}$$

Good approximation if $S_o >> E_o$

in this case S_0 ~ [S] at the start of quasi-steady state

Review Lecture 2
Equilibrium binding and cooperativity

$$S + P_{j-1} \leftrightarrow P_j$$

Adair's Equation:

$$r = \frac{K_1[S] + 2K_1K_2[S]^2 + 3K_1K_2K_3[S]^3 + \ldots + nK_1K_2\ldots K_n[S]^n}{1 + K_1[S] + K_1K_2[S]^2 + \ldots + K_1K_2\ldots K_n[S]^n}$$

$$K_j = \frac{[P_j]}{[P_{j-1}][S]}$$

macroscopic association constant
for transitions between state j-1 and j

Note #1 Detailed balance

$$P_o \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} P_1 \underset{k_{-2}}{\overset{k_{+2}}{\rightleftarrows}} P_2 \quad \cdots \quad P_{n-1} \underset{k_{-n}}{\overset{k_{+n}}{\rightleftarrows}} P_n$$

$$0 = \frac{d[P_o]}{dt} = -k_{+1}[P_o][S] + k_{-1}[P_1]$$

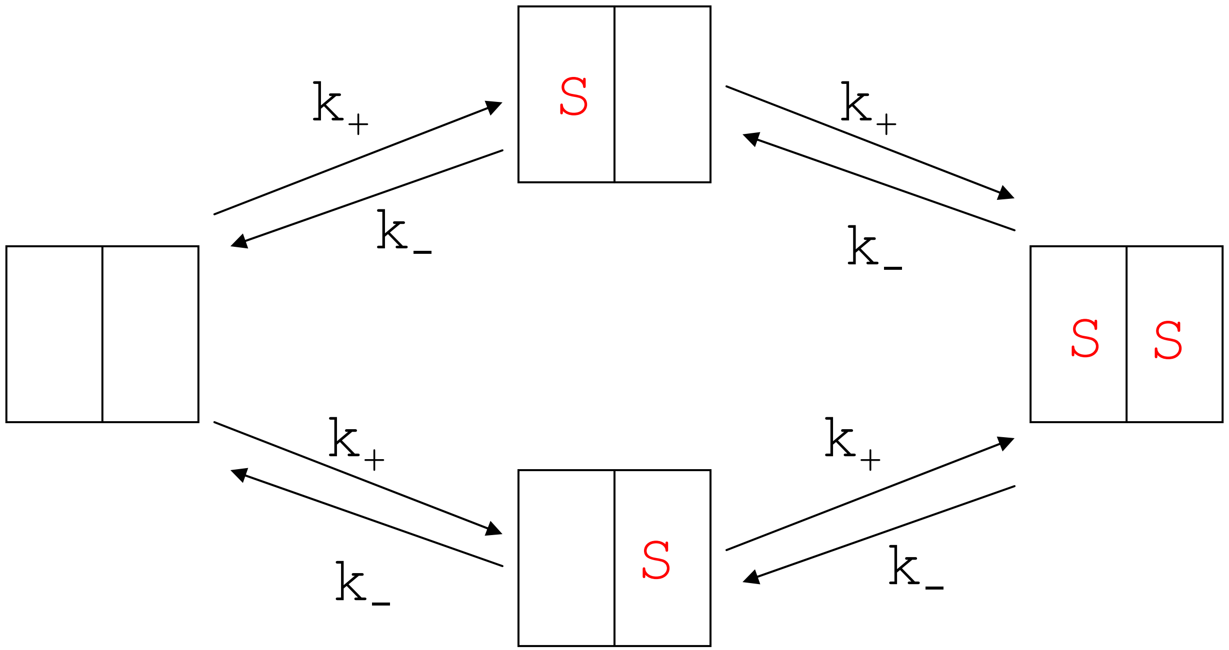
$$0 = \frac{d[P_1]}{dt} = -k_{+2}[P_1][S] + k_{-2}[P_2] + k_{+1}[P_o][S] - k_{-1}[P_1] =$$

$$= -k_{+2}[P_1][S] + k_{-2}[P_2]$$

etc.

$$\longrightarrow K_j \equiv \frac{k_{+j}}{k_{-j}} = \frac{[P_j]}{[P_{j-1}][S]}$$

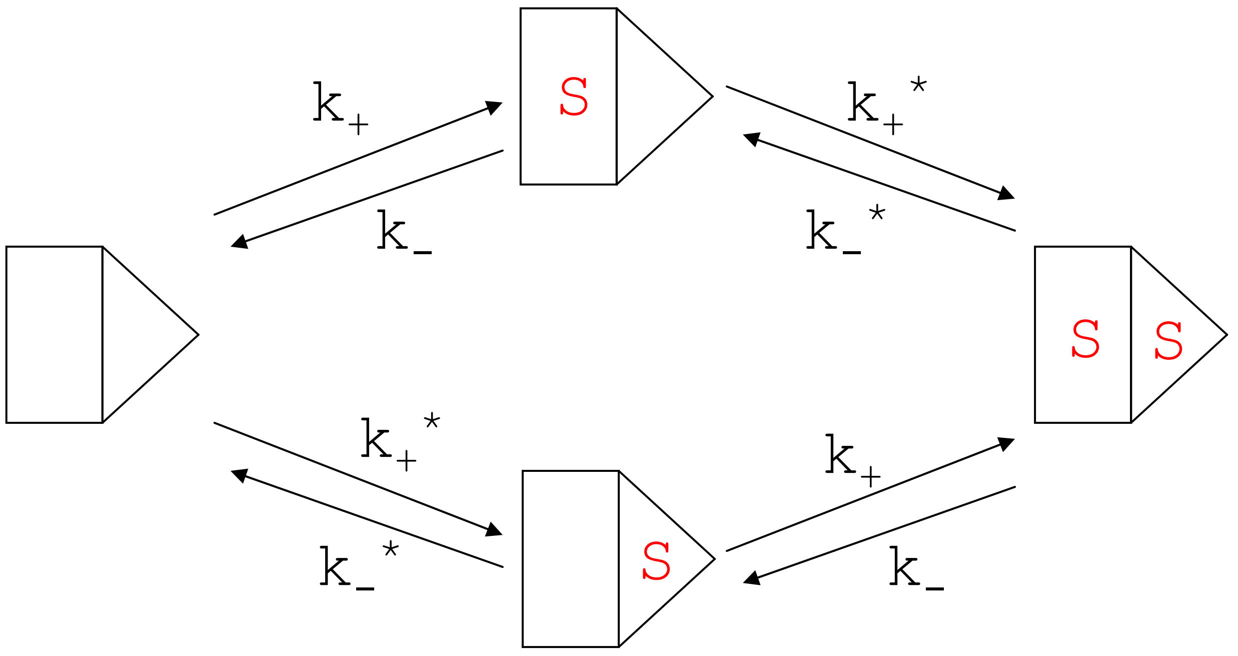
I Identical and independent binding sites



$K = k_+/k_-$ $K_1 = 2K$ $K_2 = K/2$

use Adair: $$r = \frac{2K[S] + 2K^2[S]^2}{1 + 2K[S] + K^2[S]^2} = \frac{2K[S]}{1 + K[S]}$$

II Non-identical and independent binding sites

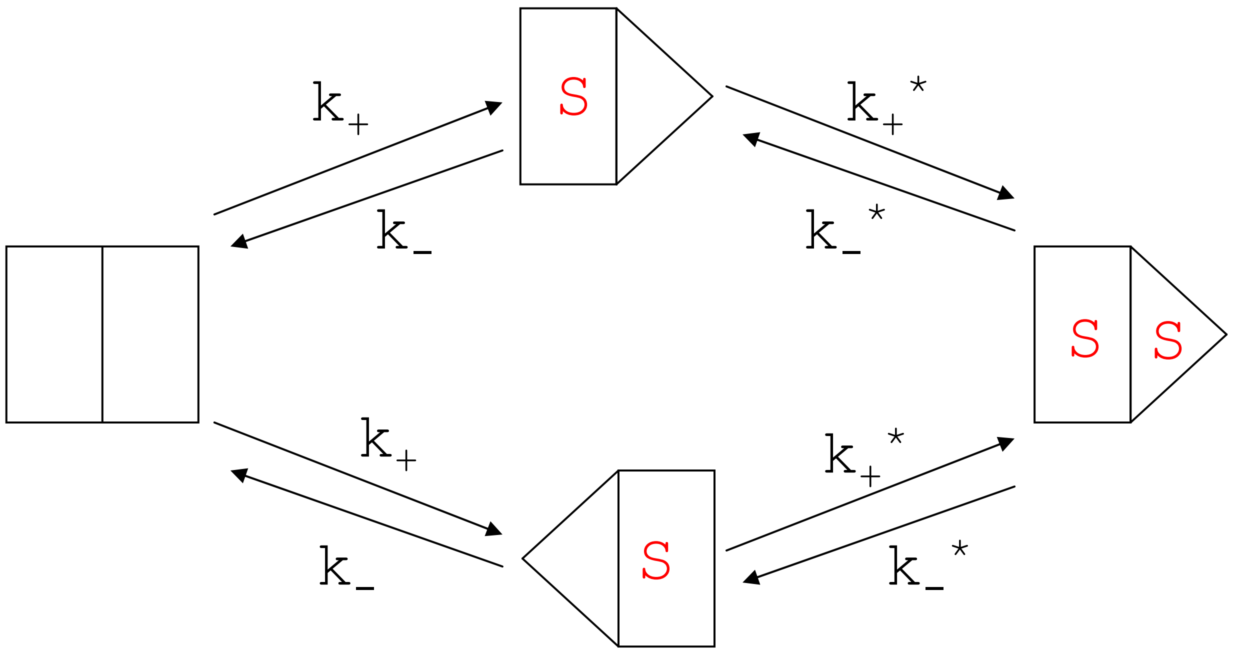


$K = k_+ / k_-$

$K^* = k_+^* / k_-^*$

Independent binding: $r = \dfrac{K[S]}{1+K[S]} + \dfrac{K^*[S]}{1+K^*[S]}$

III Identical and interacting binding sites



$K = k_+ / k_-$ $\quad K_1 = 2K$ $\quad K_2 = K^* / 2$

$K^* = k_+^* / k_-^*$

use Adair:

$$r = \frac{2K[S] + 2KK^*[S]^2}{1 + 2K[S] + KK^*[S]^2}$$

Cooperativity

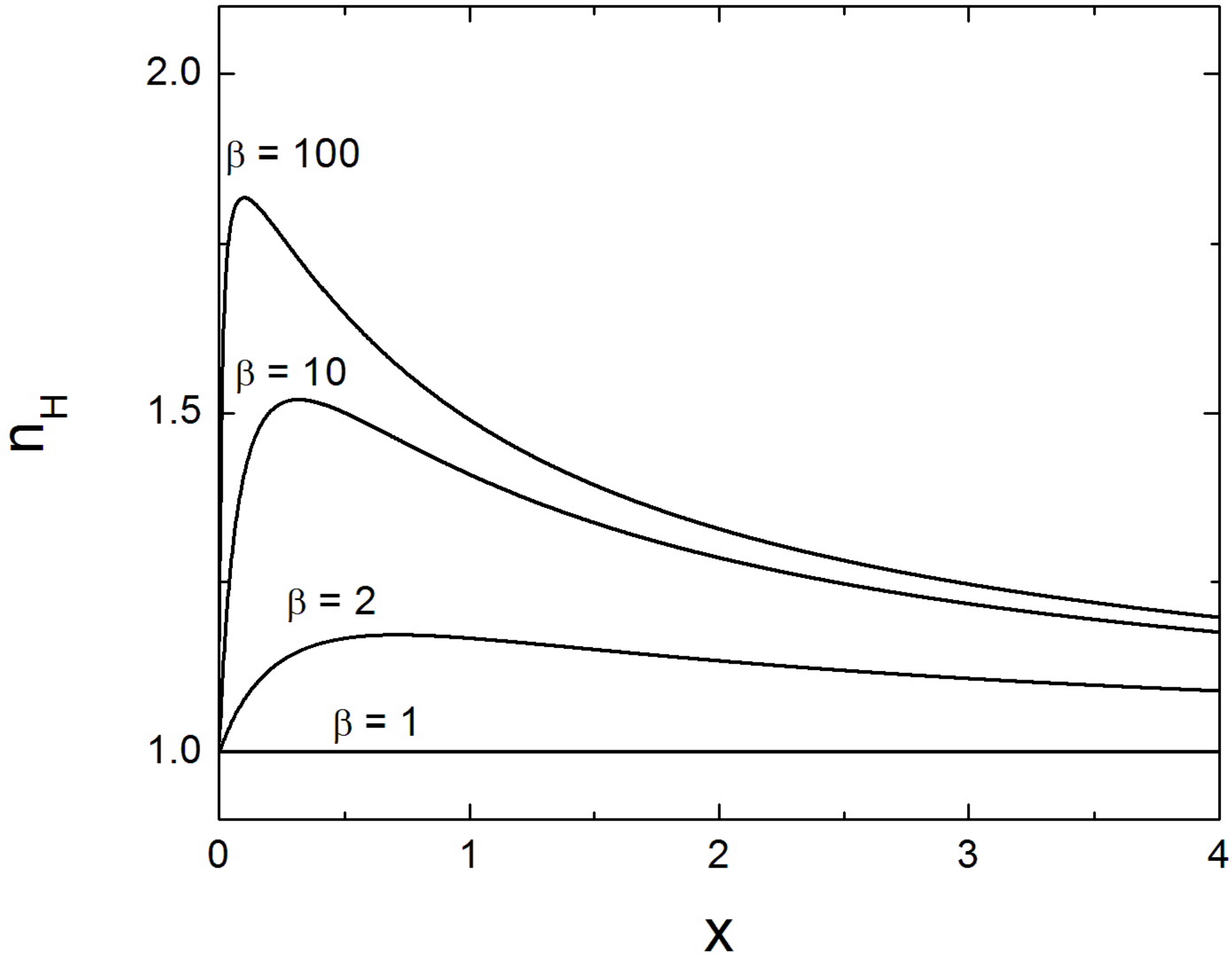
$$r = \frac{2K[S] + 2KK^{*}[S]^2}{1 + 2K[S] + KK^{*}[S]^2}$$

$$Y = \frac{x(1+\beta x)}{1 + 2x + \beta x^2}$$

$\beta = K^*/K$
$x = K[S]$

$\beta > 1$: positive cooperativity
$\beta > 2$: sigmoidal curve
$\beta < 1$: negative cooperativity
(always: $d^2Y/dx^2 < 0$)

Hill number for ‘real’ dimer



Introduction phage biology

Phage genome:
48512 base pairs ~ 12 kB
'phage.jpg' ~ 10 kB

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See Ptashne, Mark. *A genetic switch: phage lambda*.
3rd ed. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2004.

The lysis-lysogeny decision:

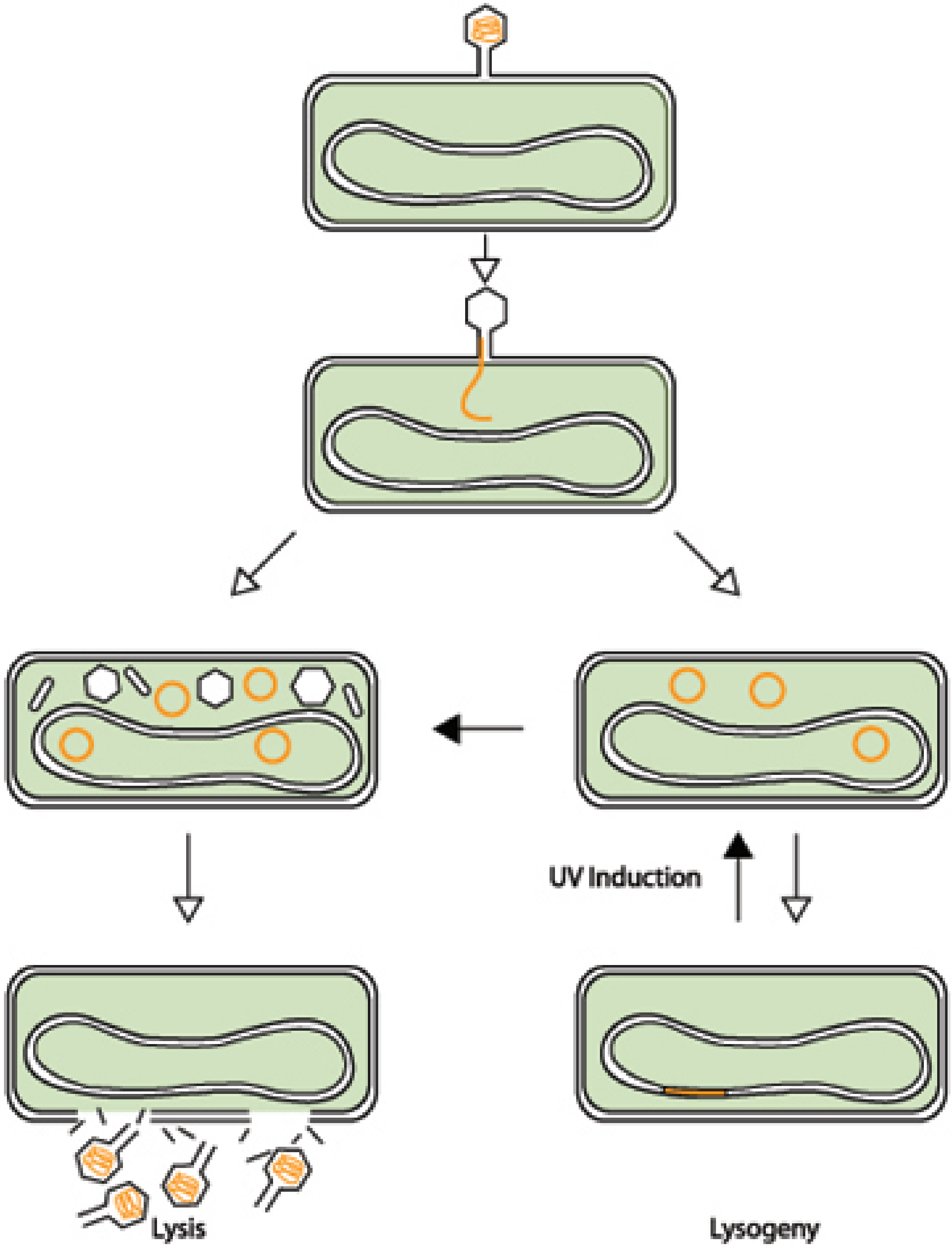

Image by MIT OCW.

As the phage genome is injected phage genes are transcribed and translated by using the host's machinery.

Which set of phage proteins are expressed determines the fate of the phage: lysis or lysogeny

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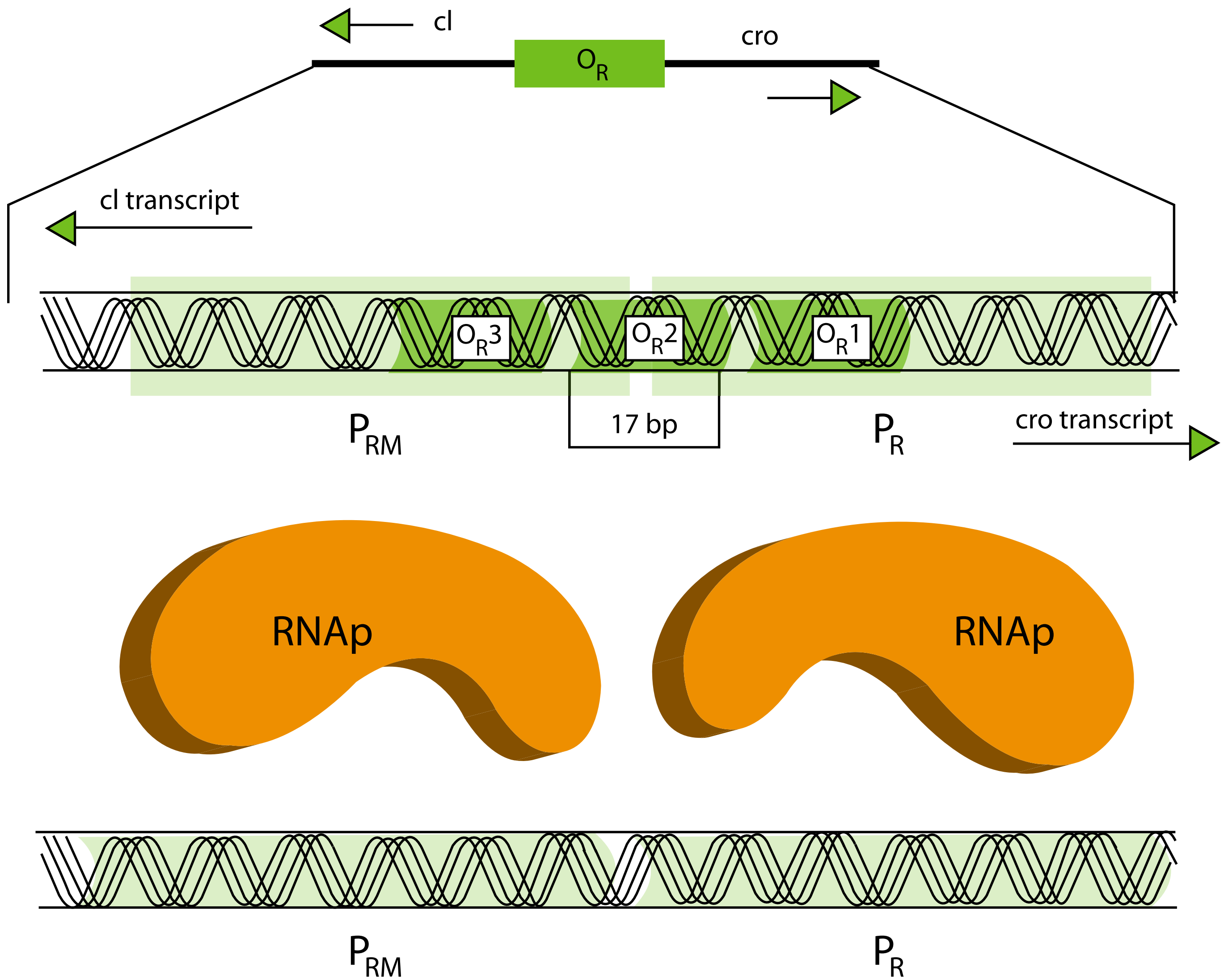
A lysogen is immune to invasion of another phage. Repressor dimers turn off genes in the injected phage chromosome. High concentration of repressor keeps cell in lysogenic state.

The lysis-lysogeny decision is a genetic switch



only 'space' for one RNA polymerase (mutual exclusion)

Image by MIT OCW. After Ptashne, Mark. *A genetic switch : phage lambda*. 3rd ed. Cold Spring Harbor, N.Y. : Cold Spring Harbor Laboratory Press, 2004.

Single repressor dimer bound - three cases:

I Negative control, dimer binding to OR2 <u>inhibits</u> RNAp binding to right P_R promoter.

Positive control, dimer binding to OR2 <u>enhances</u> RNAp binding to left P_{RM} promoter.

II Negative control, dimer binding to OR1 <u>inhibits</u> RNAp binding to right P_R promoter.

Negative control, dimer binding to OR1 <u>inhibits</u> RNAp binding to left P_{RM} promoter (too distant).

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See Ptashne, Mark. *A genetic switch: phage lambda*.
3rd ed. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2004.

III Negative control, dimer binding to OR3 <u>inhibits</u> RNAp binding to left P_{RM} promoter.

Positive control, dimer binding to OR3 <u>allows</u> RNAp binding to right P_R promoter.

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See Ptashne, Mark. *A genetic switch: phage lambda*.
3rd ed. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2004.

Repressor-DNA binding is highly cooperative

intrinsic association constants:

$K_{OR1} \sim 10\ K_{OR2} \sim 10\ K_{OR3}$

However $K_{OR2}^* >> K_{OR2}$ (positive cooperativity)

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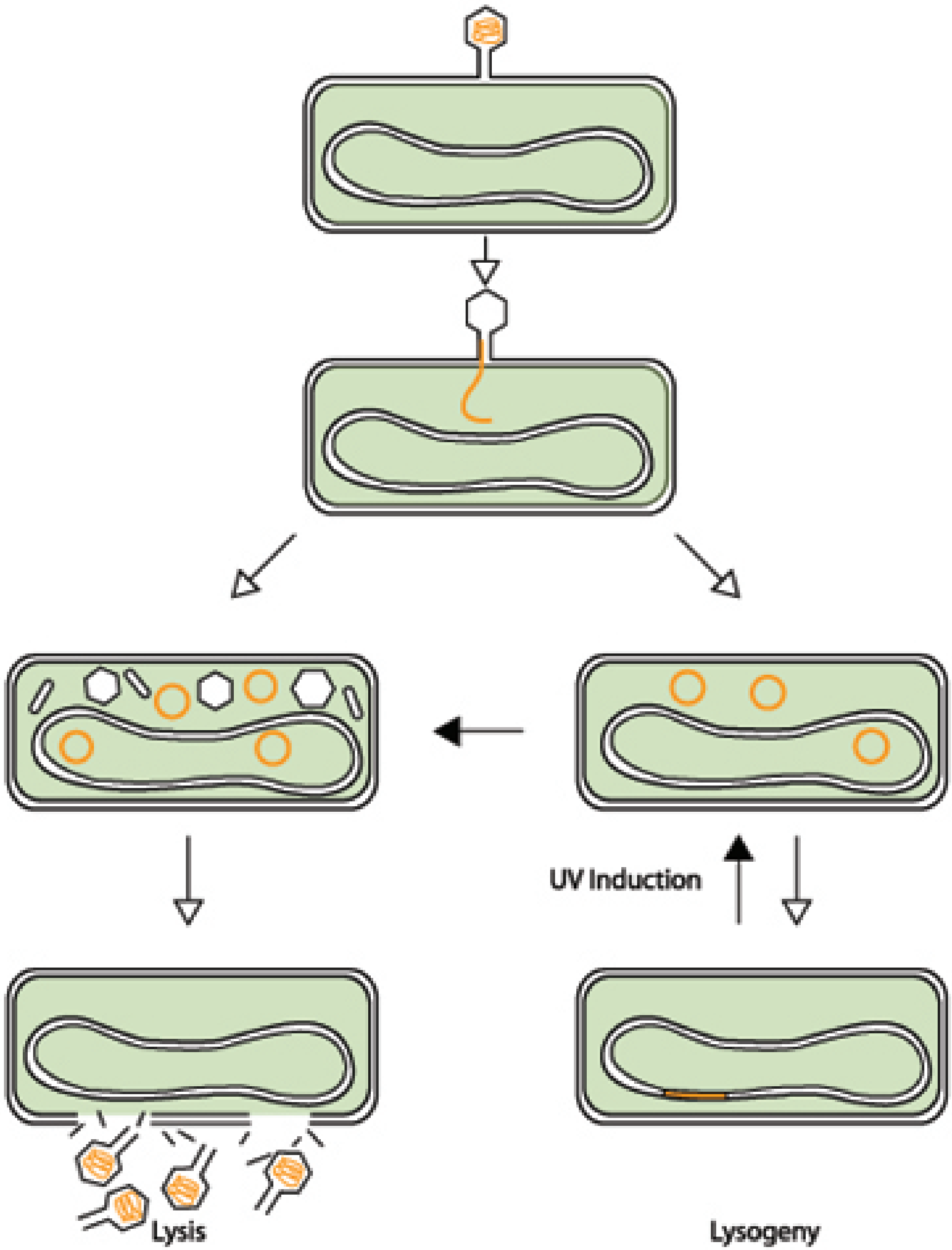
See Ptashne, Mark. *A genetic switch: phage lambda*.
3rd ed. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2004.

Flipping the switch by UV:



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See Ptashne, Mark. *A genetic switch: phage lambda*.
3rd ed. Cold Spring Harbor, N.Y.:
Cold Spring Harbor Laboratory Press, 2004.

In lysogenic state, [repressor] is maintained at constant level by negative feedback

Repressor-DNA binding is highly cooperative

intrinsic association constants:

$K_{OR1} \sim 10\ K_{OR2} \sim 10\ K_{OR3}$

However $K_{OR2}^* >> K_{OR2}$ (positive cooperativity)

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See Ptashne, Mark. *A genetic switch: phage lambda*.
3rd ed. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2004.

Cro dimers bind non-cooperatively to OR sites

$K_{OR3} \sim 10\ K_{OR2} \sim 10\ K_{OR1}$

Note for repressor:

$K_{OR1} \sim 10\ K_{OR2} \sim 10\ K_{OR3}$

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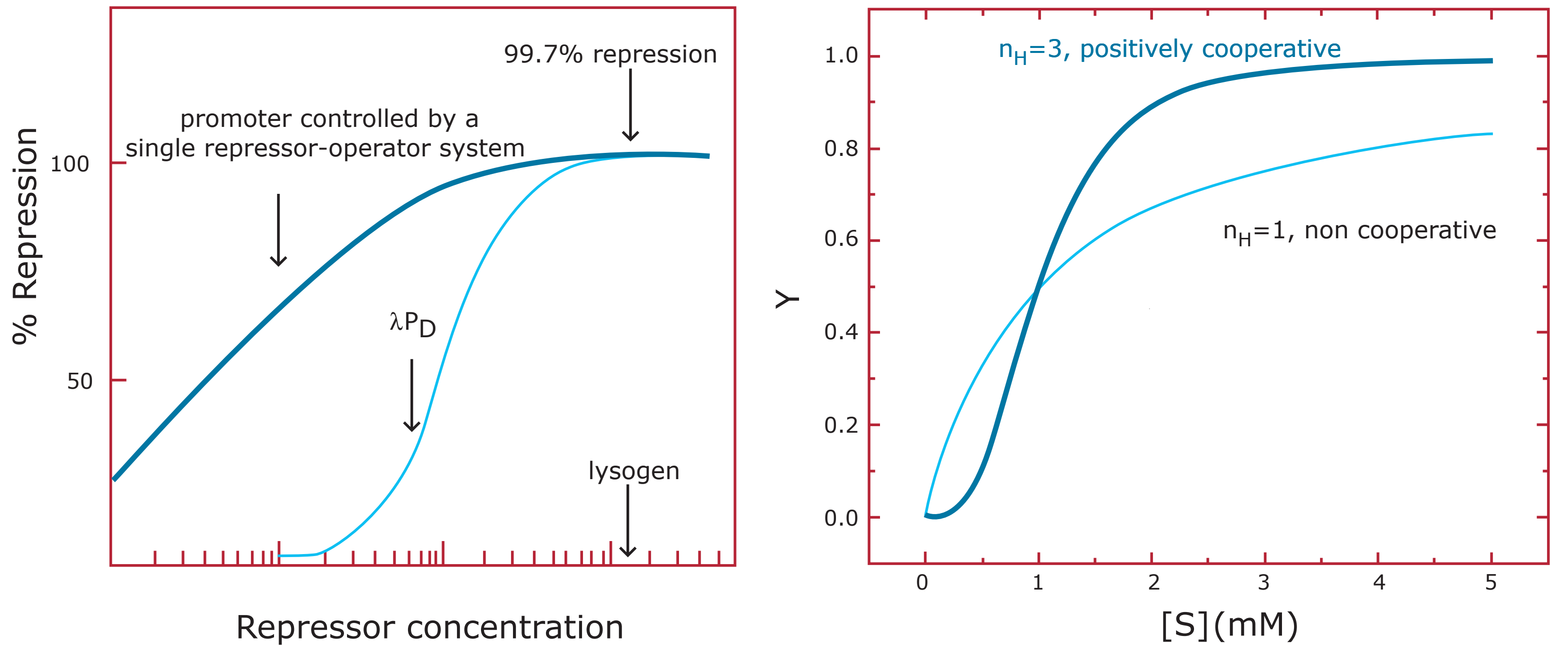
3rd ed. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 2004.

Cooperative effects make sharp switch
('well defined' decision)



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Note: several layers of cooperativity: dimerization, cooperative repressor binding

How to create a mathematical model that captures the essence of the switch ?

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