A systems approach to biology

SB200

Lecture 6
2 October 2008

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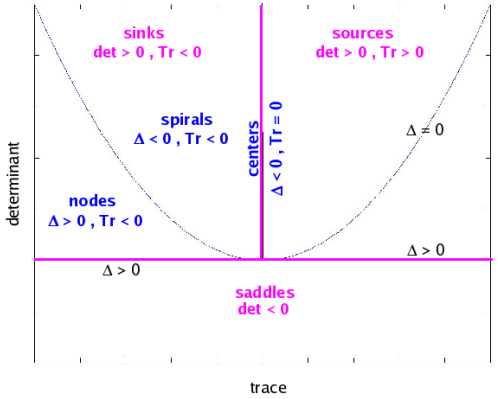
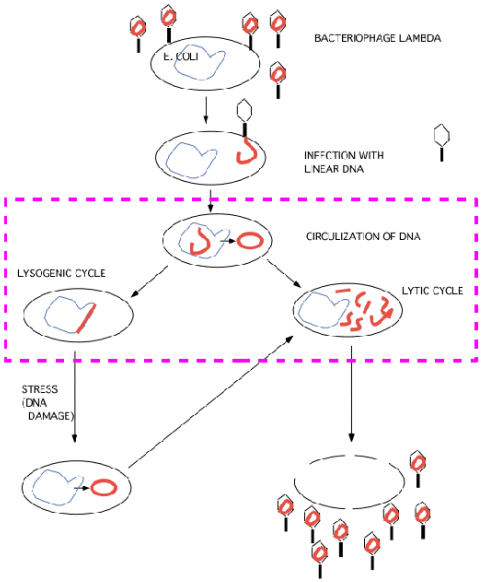
Recap of Lecture 5

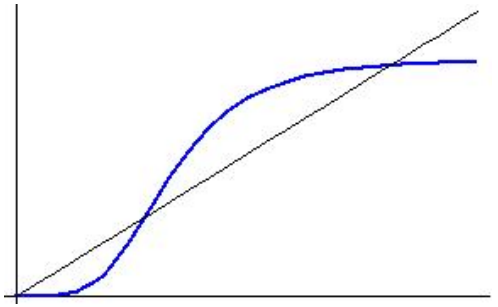
Det/Tr diagram



off state is unstable

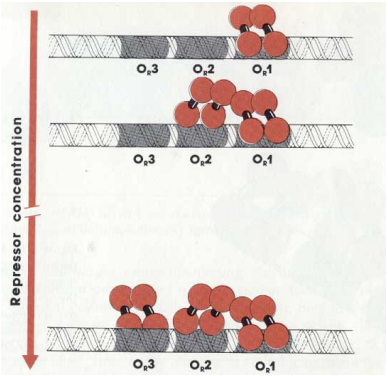


sigmoidal dose response gives a stable off state and sharper on-to-off switching

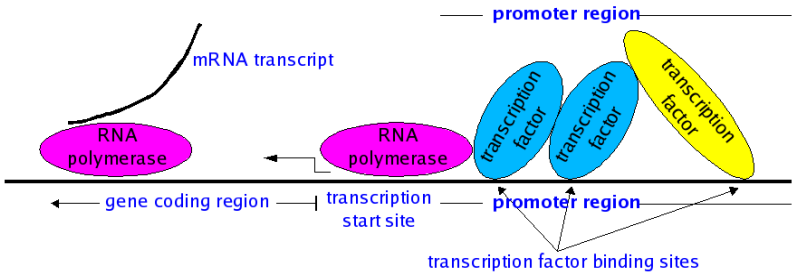


SIGMOIDAL
SWITCHING
THRESHOLDING
ULTRASENSITIVE
ALLOSTERY
COOPERATIVITY

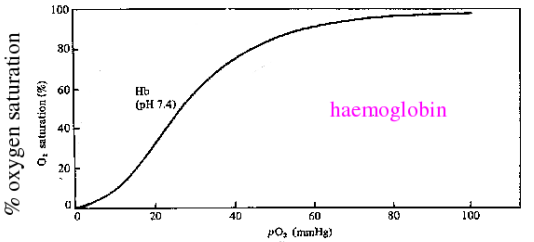
λ promoter



bacterial gene expression



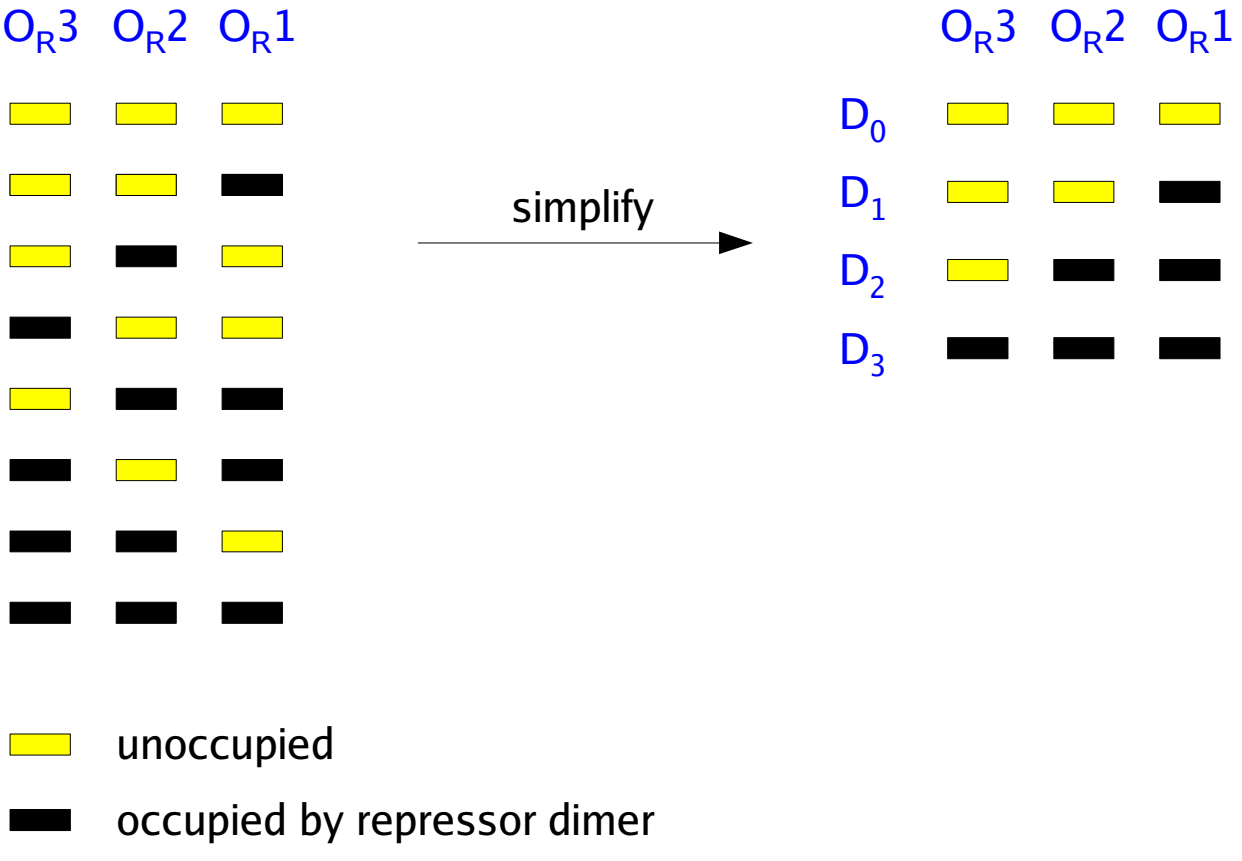
oxygen curve for haemoglobin



calculating the rate of repressor expression

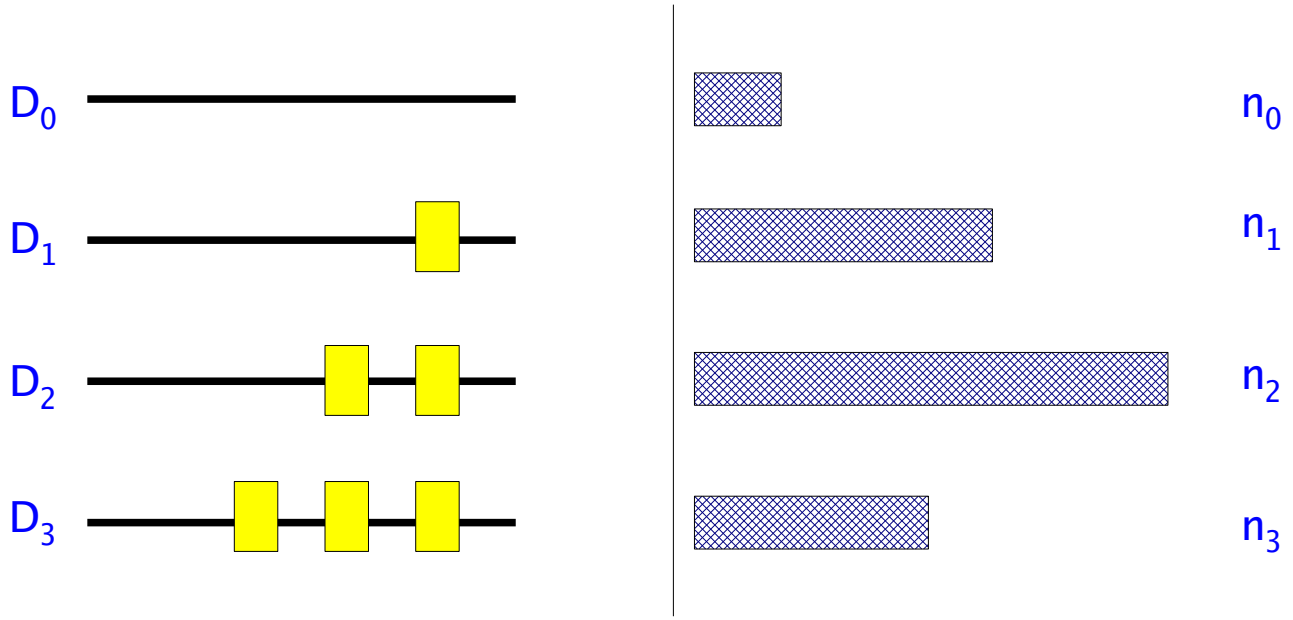
Shea-Ackers model
Ackers, Johnson & Shea, PNAS **79**:1129-33 1982

a general statistical mechanical model for transcription factor binding



probabilities as mean frequencies of occupation

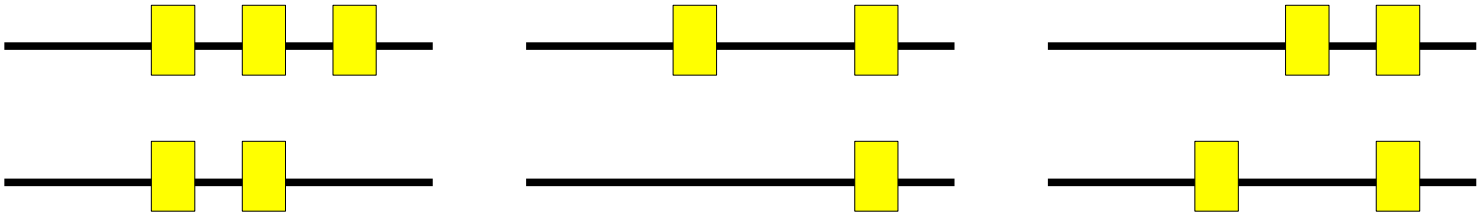
observe DNA over time and determine the occupation histogram



$$\text{probability}(D_i) = n_i / (n_0 + n_1 + n_2 + n_3)$$

probabilities as concentration ratios

observe many copies of DNA in equilibrium with repressor and determine concentrations of each state *(D_0, D_1, D_2, D_3)*



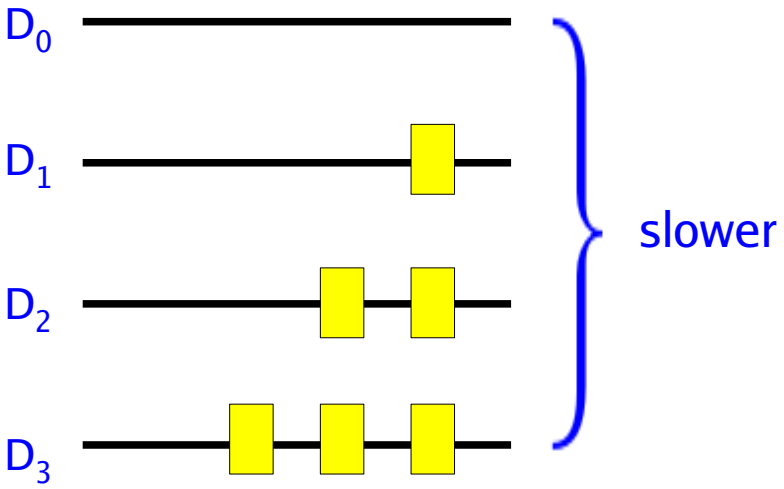
$$\text{probability}(D_i) = [D_i] / ([D_0] + [D_1] + [D_2] + [D_3])$$

the two ways of calculating probabilities give the same results ("ergodicity)"

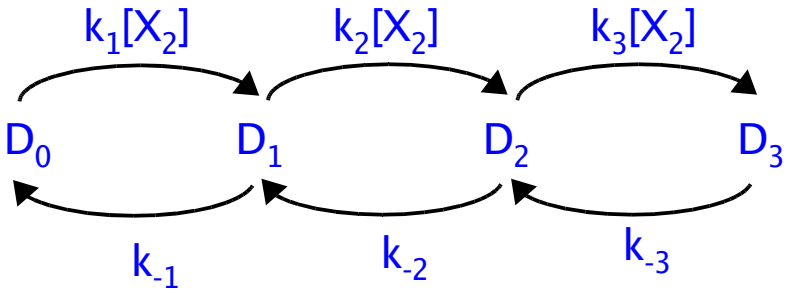
separation of time scales

$X + X \leftrightarrow X_2$ fast $[X_2] = K [X]^2$

equilibrium constant



assume repressor dimerisation is at equilibrium with respect to repressor-DNA binding



if a chain is at equilibrium, each individual loop is at equilibrium

$$[D_i] = (k_i[X_2] / k_{-i})\,[D_{i-1}]$$

$$K_i = k_i / k_{-i} \quad \text{equilibrium constant} \quad (M^{-1})$$

JG, "Multis ite protein phosphorylation makes a good threshold but can be a poor switch," PNAS **102**:14617-22 2005

$$[D_1] = K_1 [X_2][D_0] \quad [D_2] = K_1K_2[X_2]^2[D_0] \quad [D_3] = K_1K_2K_3[X_2]^3[D_0]$$

$$[D_0] + [D_1] + [D_2] + [D_3] = [D_T]$$

$$[X_2] = K[X]^2$$

if r_i = transcription rate in state D_i then the average transcription rate r is

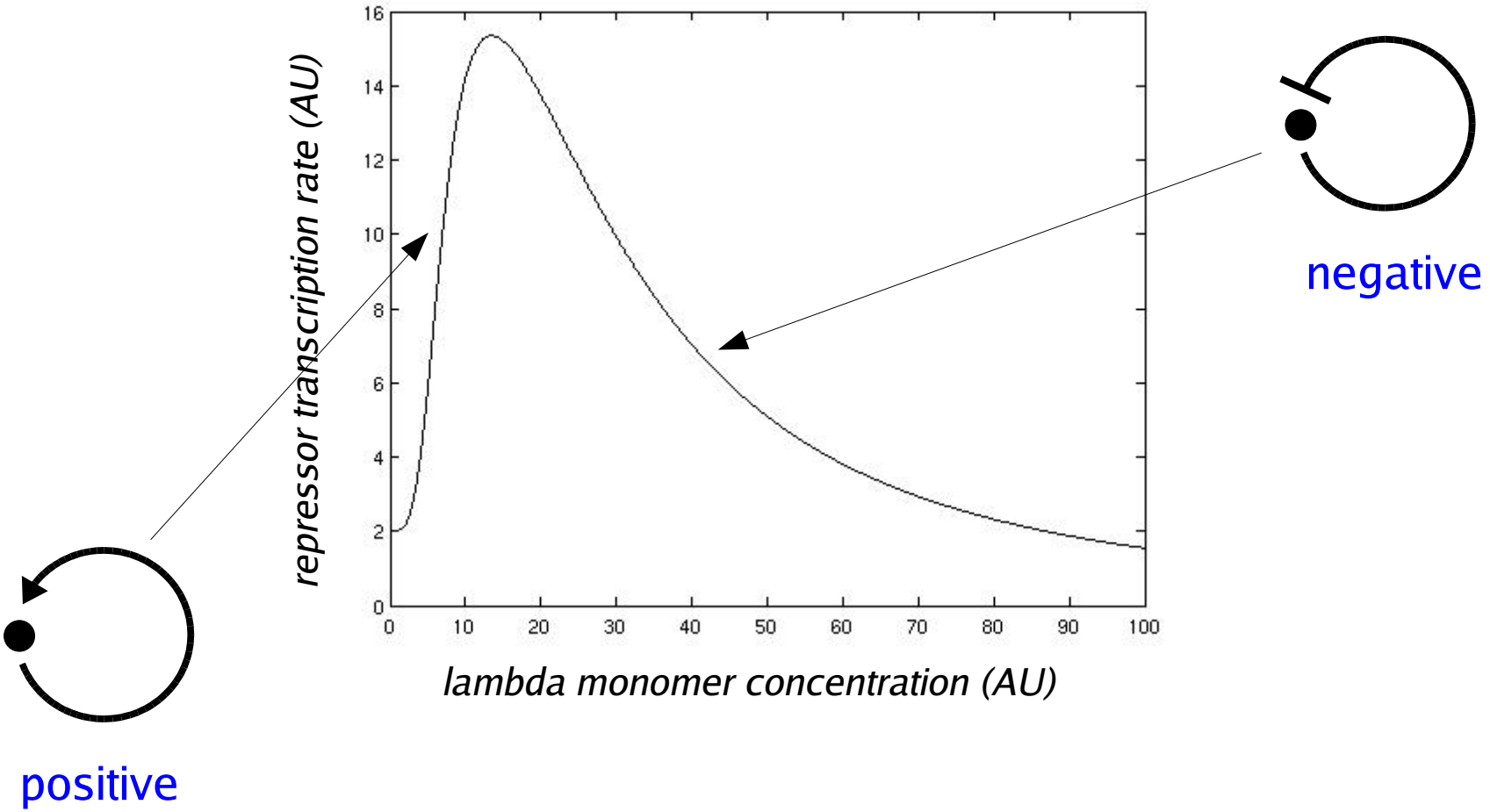
$$r = r_0\frac{[D_0]}{[D_T]} + r_1\frac{[D_1]}{[D_T]} + r_2\frac{[D_2]}{[D_T]} + r_3\frac{[D_3]}{[D_T]}$$

$$r = \frac{r_0 + r_1(K_1K)x^2 + r_2(K_1K_2K^2)x^4 + r_3(K_1K_2K_3K^3)x^6}{1 + (K_1K)x^2 + (K_1K_2K^2)x^4 + (K_1K_2K_3K^3)x^6}$$

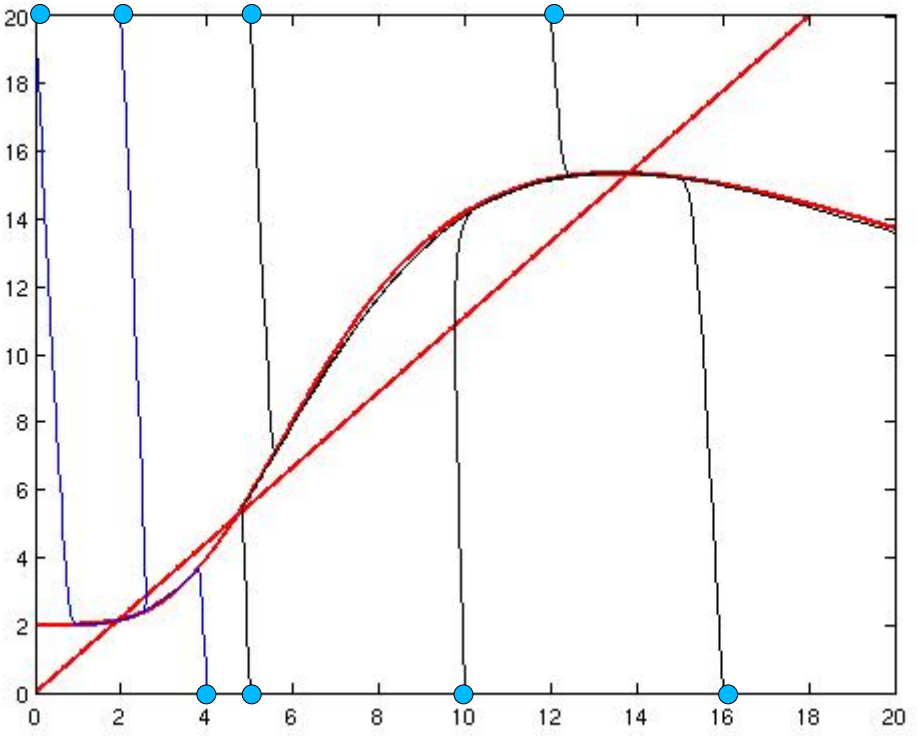
r_0	= basal transcription rate	K	= $5 * 10^7\ M^{-1}$
r_1	= r_0	K_1	= $3.3 * 10^8\ M^{-1}$
r_2	= $11 * r_0$	K_2	= $2 * K_1$
r_3	= 0	K_3	= $0.08 * K_1$

Ackers, Johnson & Shea, PNAS **79**:1129-33 1982

feedback is context dependent



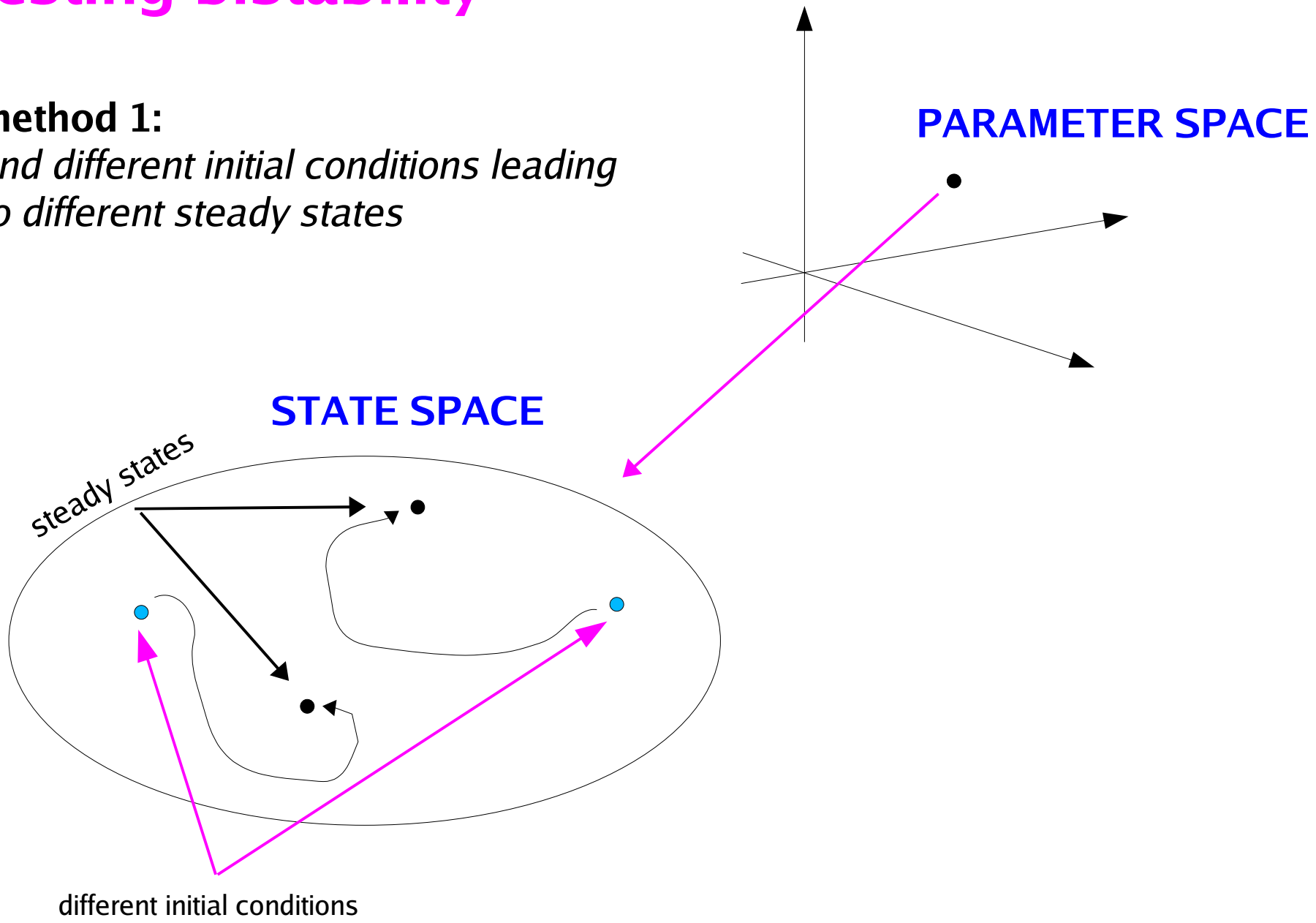
cooperative promoter design leads to bistability



λ	0.18	$(min)^{-1}$
a	0.02	$(min)^{-1}$
b	0.5	$(min)^{-1}$
r_0	1	$(nM)(min)^{-1}$

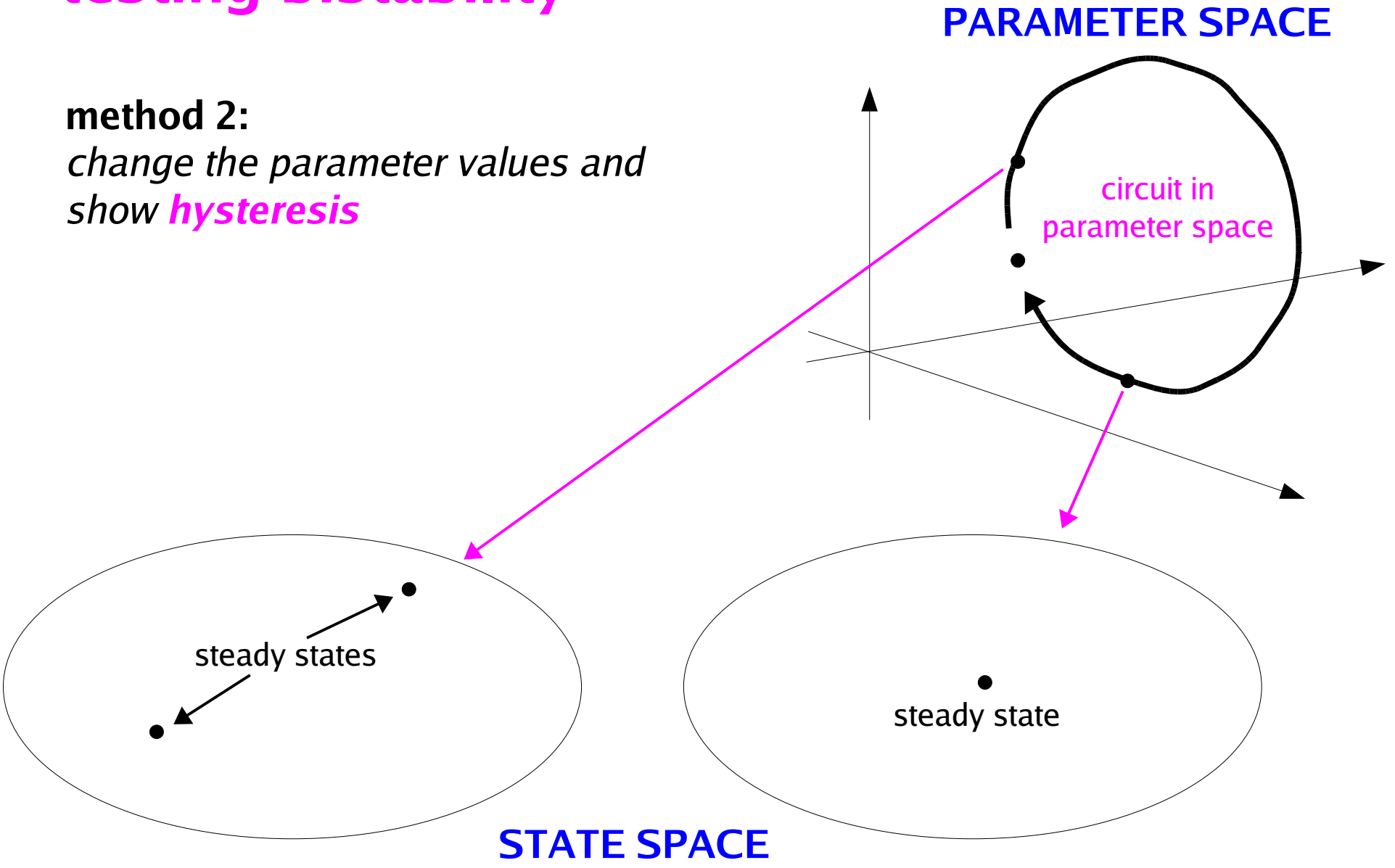
testing bistability

method 1:
find different initial conditions leading to different steady states

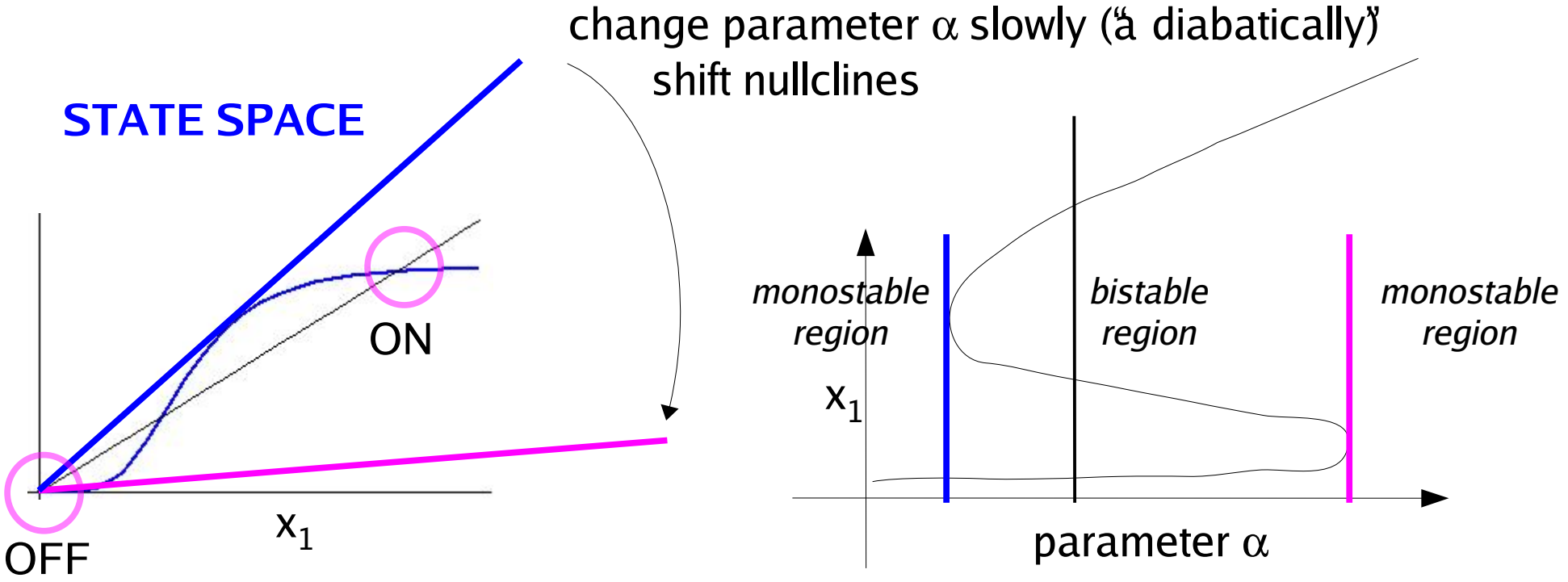


testing bistability

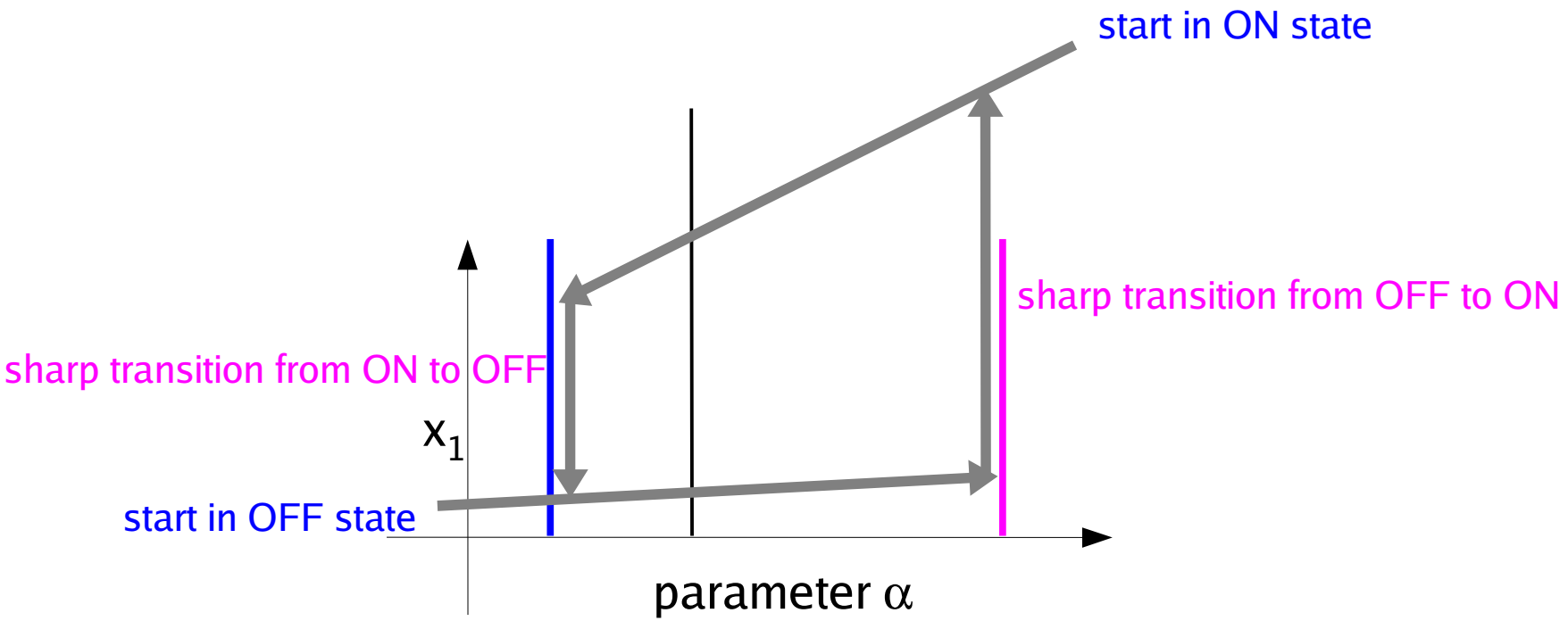
method 2:
change the parameter values and show ***hysteresis***



HYSTERESIS

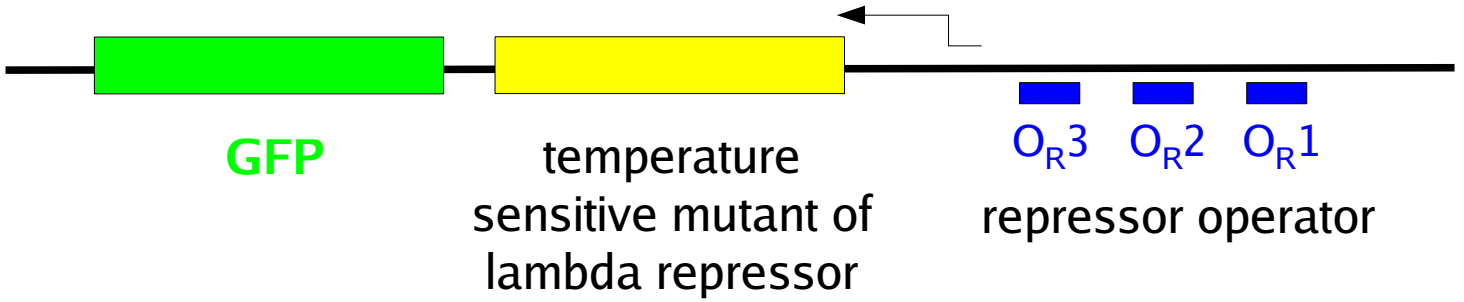

change parameter α slowly ("a diabatically")
shift nullclines
STATE SPACE
ON
OFF
x_1
monostable region
bistable region
monostable region
x_1
parameter α

A hysteretic cycle is evidence of creation/annihilation of stable **attractors**



experimental detection of bistability in phage lambda

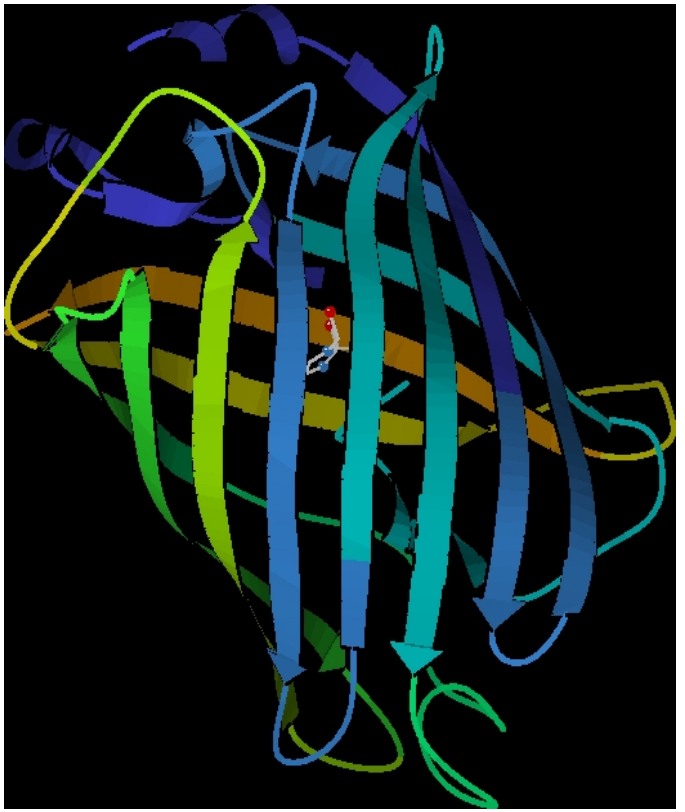
engineered phage lambda autoregulatory loop



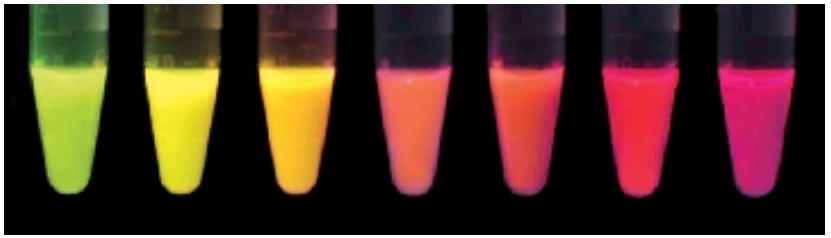
transfected into E coli

Farren Isaacs, Jeff Hasty, Charles Cantor and James Collins,
"Prediction and measurement of an autoregulatory genetic module"
PNAS **100**:7714-9 2003

Aequoria victoria



Green fluorescent protein
PDB 1QYQ

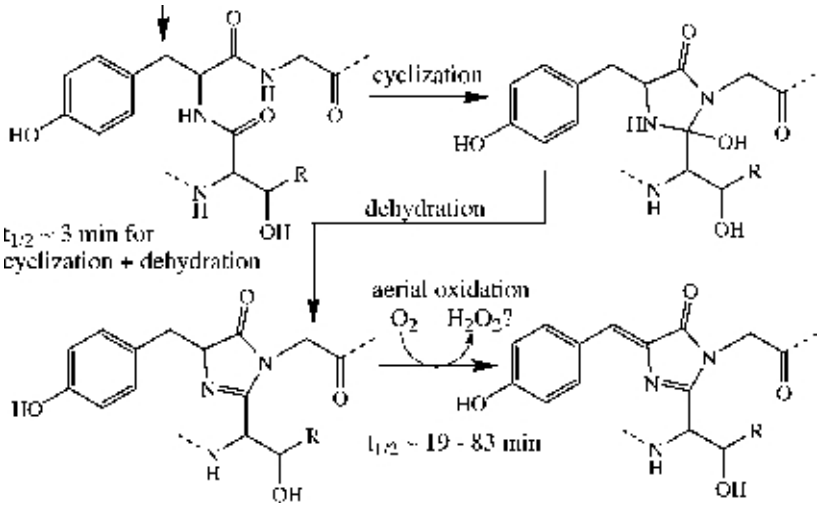


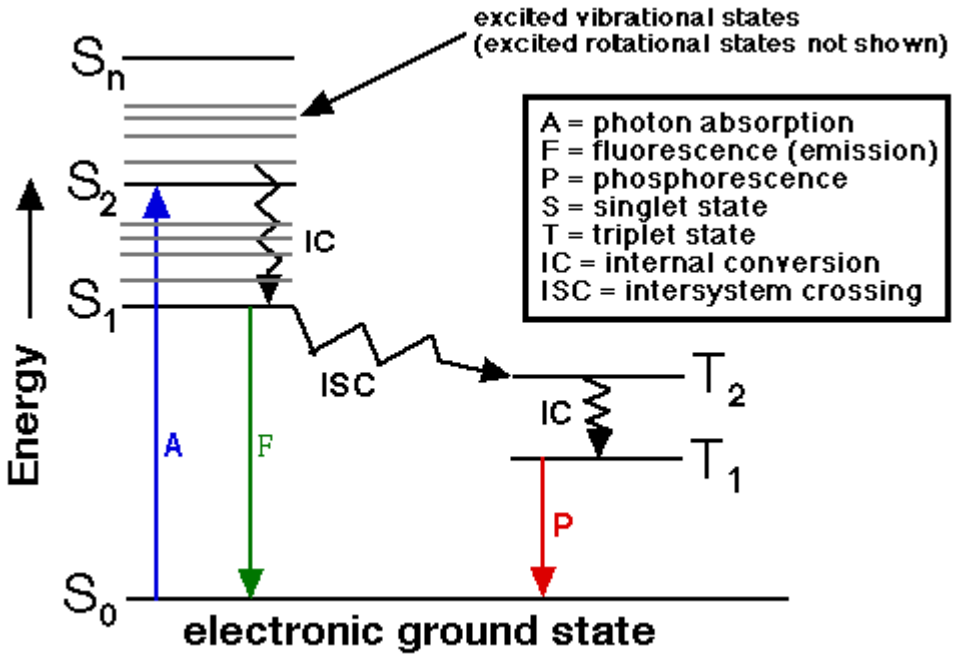
N. C. Shaner, R. E. Campbell, P.A. Steinbach, B.N.G. Giepmans, A.E. Palmer & R. Tsien *"Improved monomeric red, orange and yellow fluorescent proteins dervied from Discosoma sp. red fluorescent protein,"* Nature Biotech., **22**:1587-72 2004

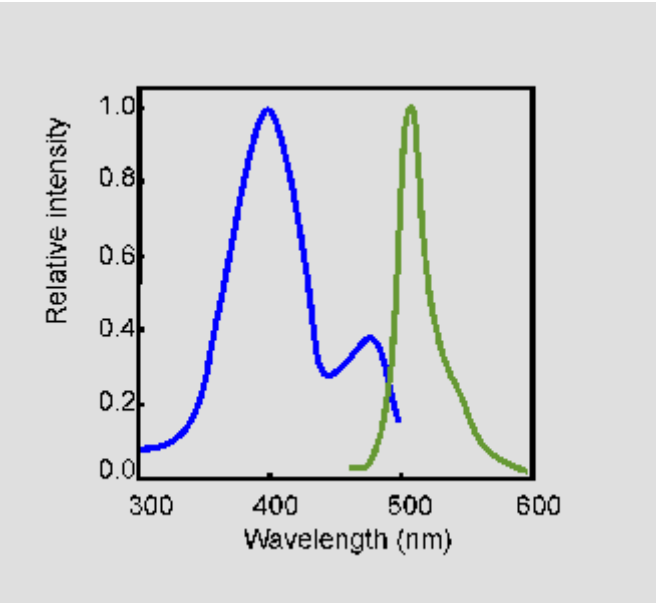
GFP matures post-translationally

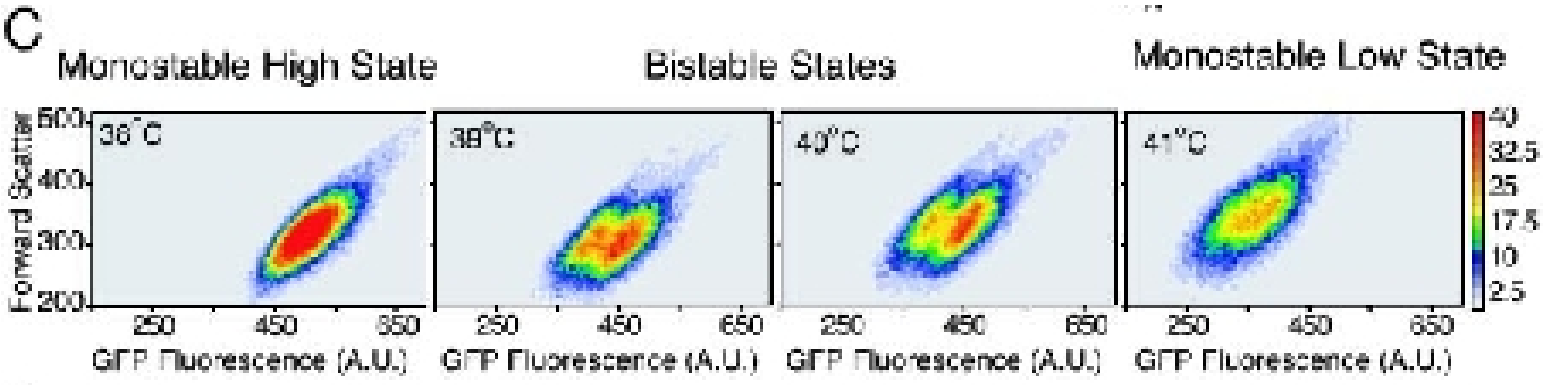
chromophore – SYG motif at residues 65-67

maturation – cyclisation, dehydration, oxidation

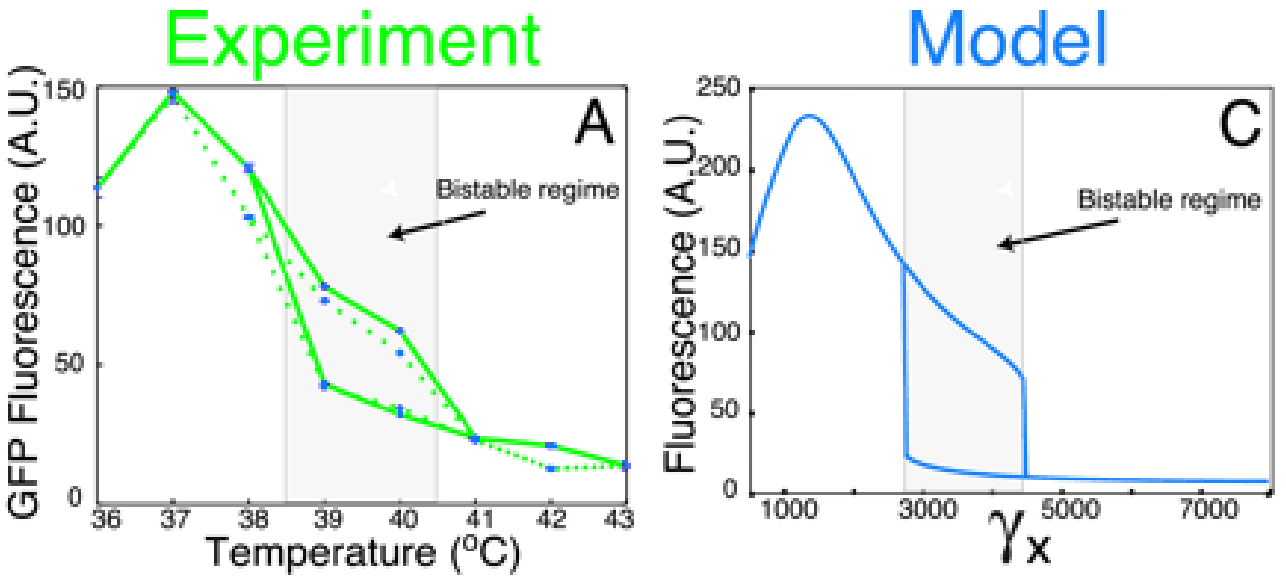








biphasic response to temperature shift

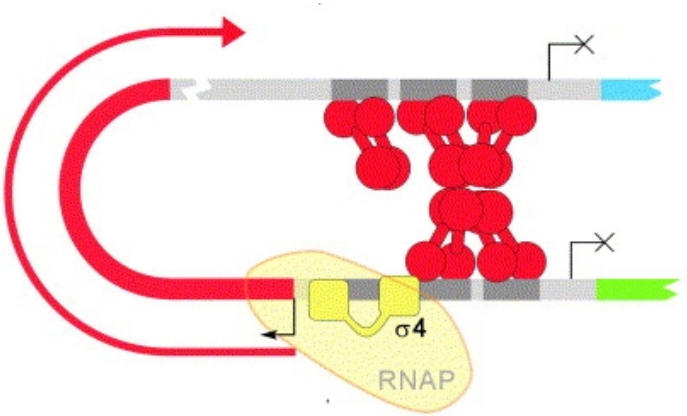


bistable response to temperature shift

phage lambda is still not fully understood !!

Measured frequency of spontaneous lysis
~ 10^{-8} per cell per generation
~ once per cell per 5000 years!
Not explained by current models

Aurell, Brown, Johanson & Sneppen
"Stability puzzles in phage λ"
Phys Rev E **65**:051914 2002



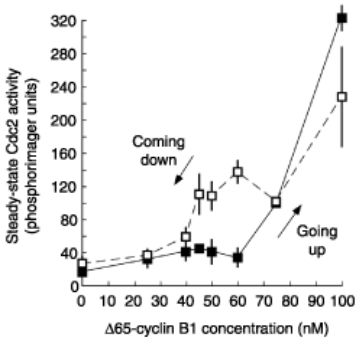
DNA looping creates more cooperativity

Dodd, Shearwin & Egan
"Revisted gene regulation in bacteriophage λ"
Cur Op Gen Dev **15**:145-52 2005

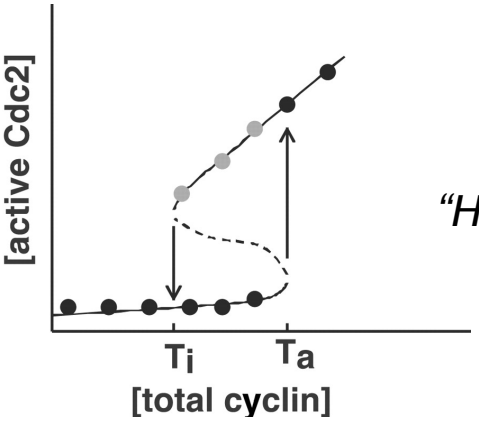
Redundancy?

Michalowski & Little
"Positive autoregulation of cI is a dispensable feature of phage λ gene regulatory circuitry"
J Bacteriology **187**:6430-42 2005

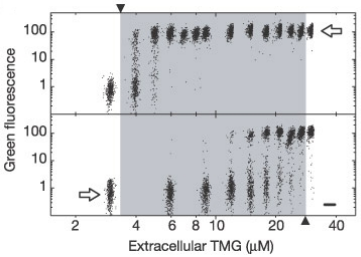
hysteresis has been widely used to test for bistability



Pomerening, Sontag & James Ferrell
"Building a cell cycle oscillator: hysteresis and bistability in the activation of CDC2"
Nature Cell Biology **5**:346-51 2005



Sha, Moore, Chen, Lassaletta, Yi, Tyson & Sible
"Hysteresis drives cell-cycle transitions in Xenopus laevis egg extracts"
PNAS **100**:975-80 2003

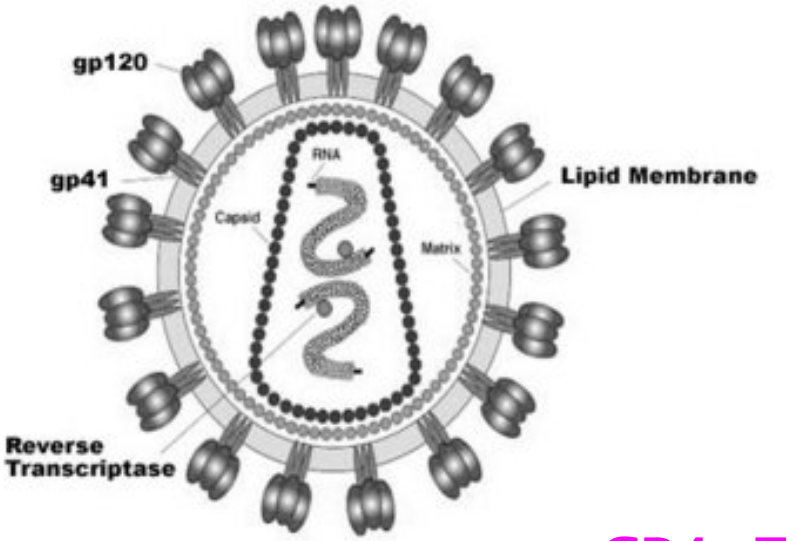


Ozbudak, Thatai, Lim, Shraiman & van Oudenaarden
"Multistability in the lactose utilization network of Escherichia coli"
Nature **427**:737-40 2004

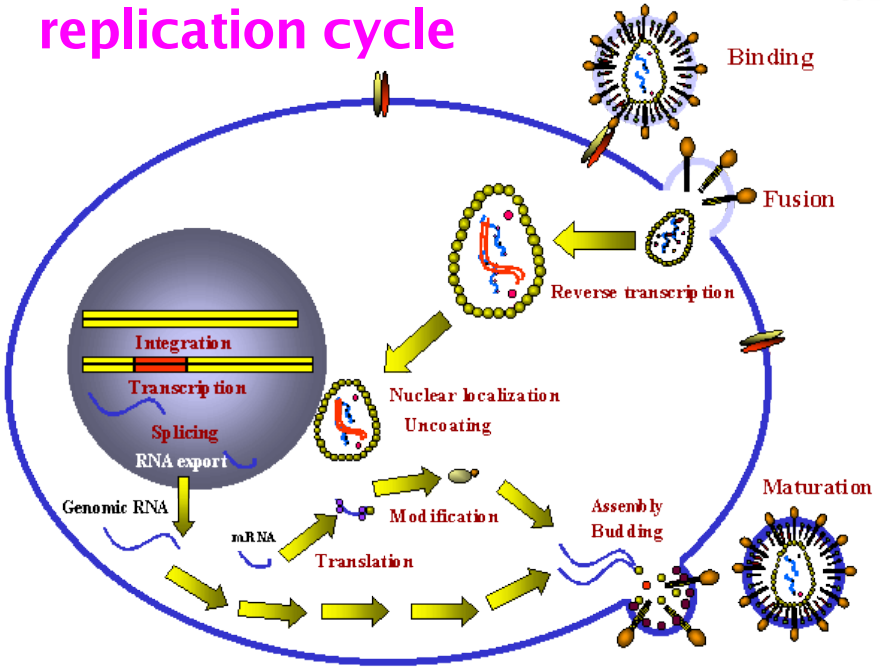
LATENCY SWITCH IN HIV-1

HIV-1

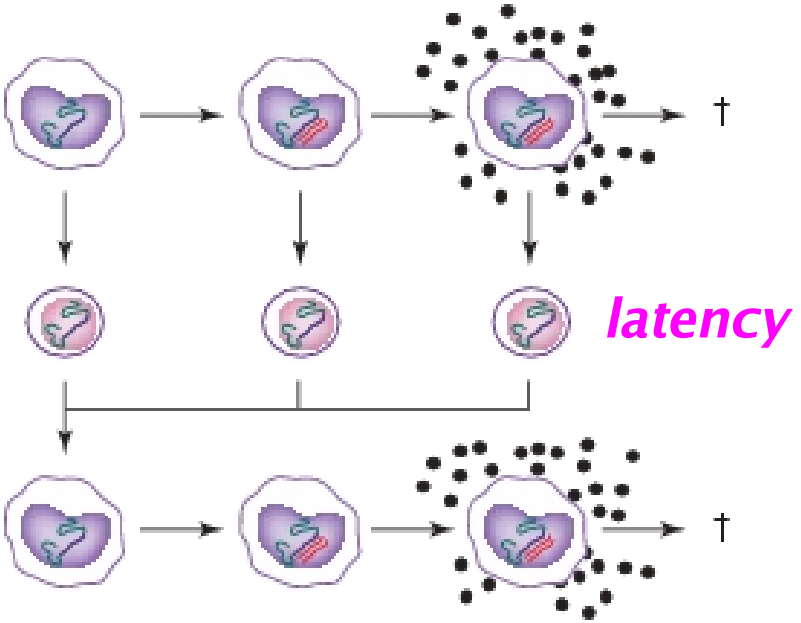
virion structure



replication cycle

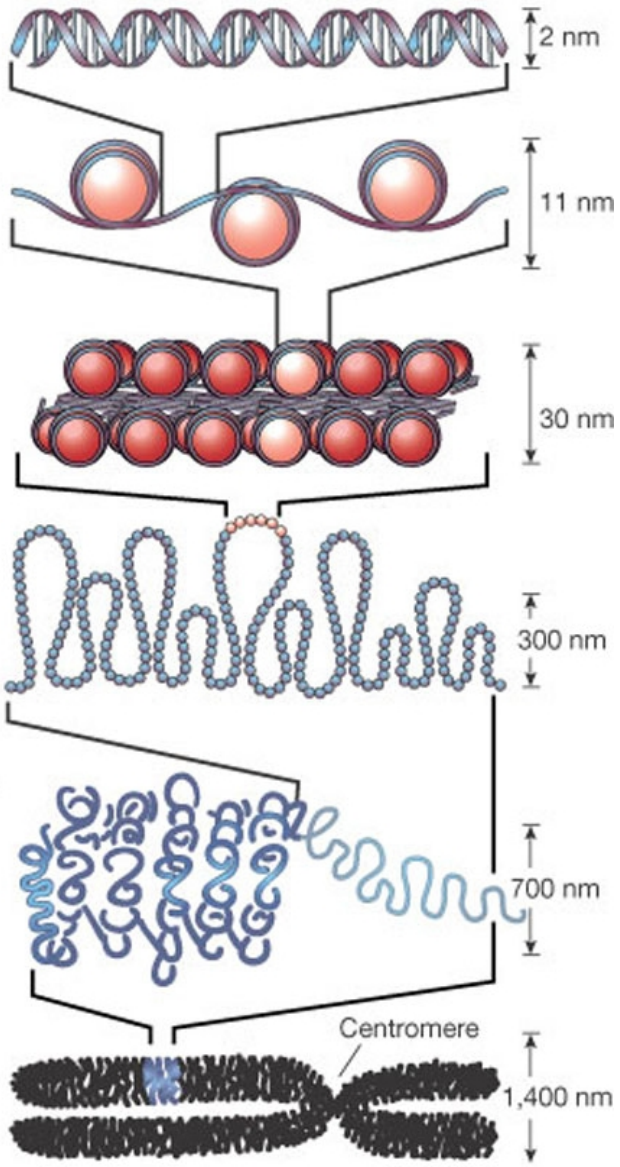


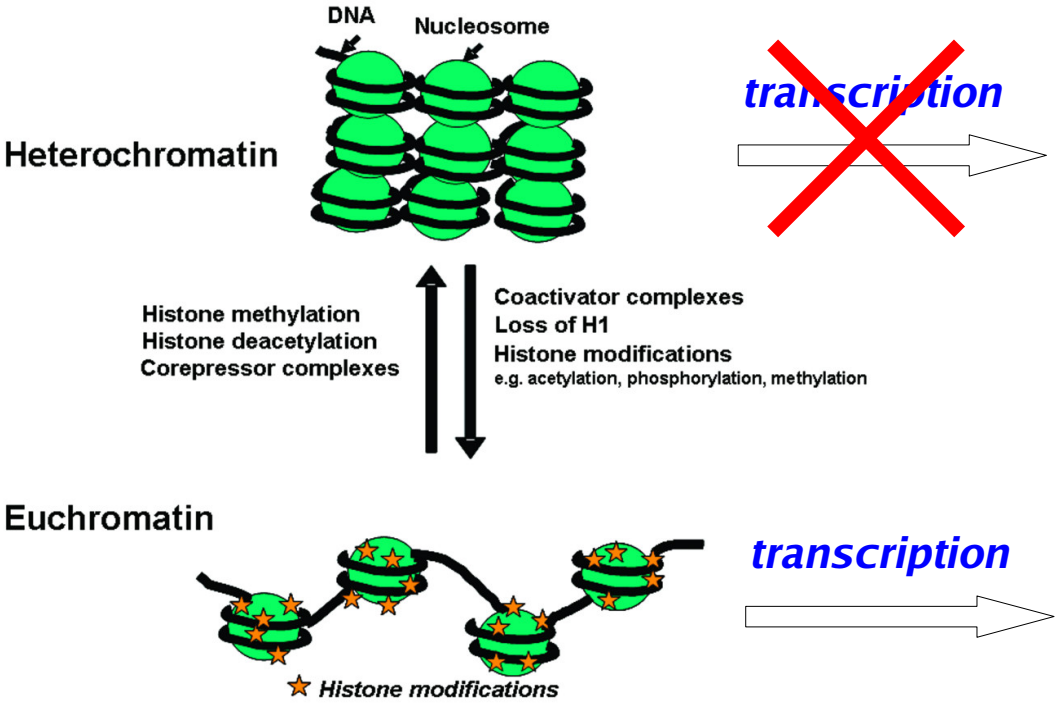
CD4+ T cell infection

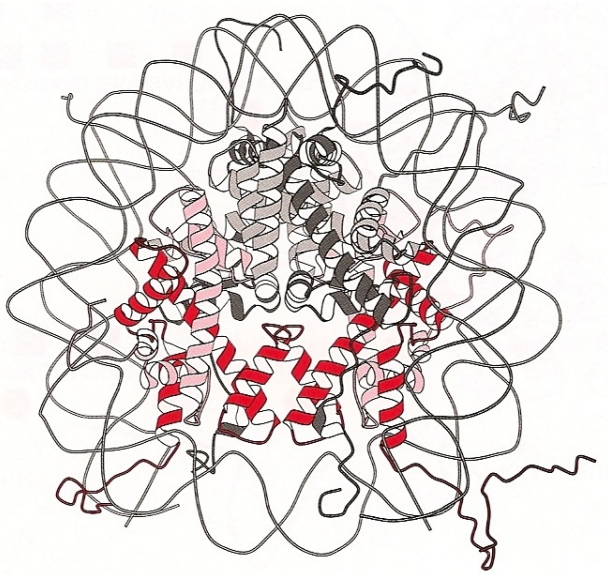


Lassen, Han, Zhou, Siliciano, Siliciano, *"The multifactorial nature of HIV-1 latency,"* Trends Mol. Med. **10**:525-31 2004

eukaryotic gene transcription is repressed by chromatin

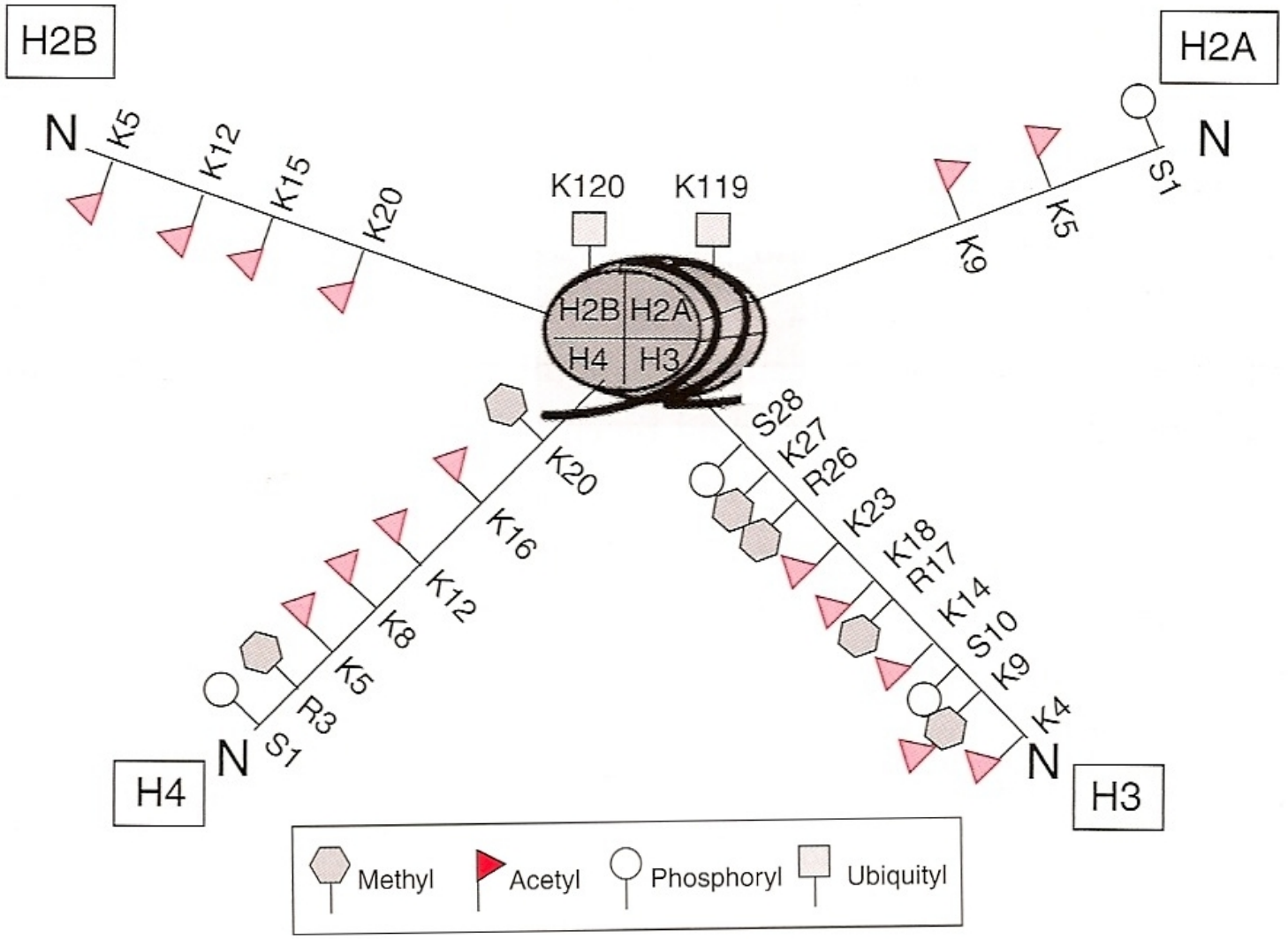






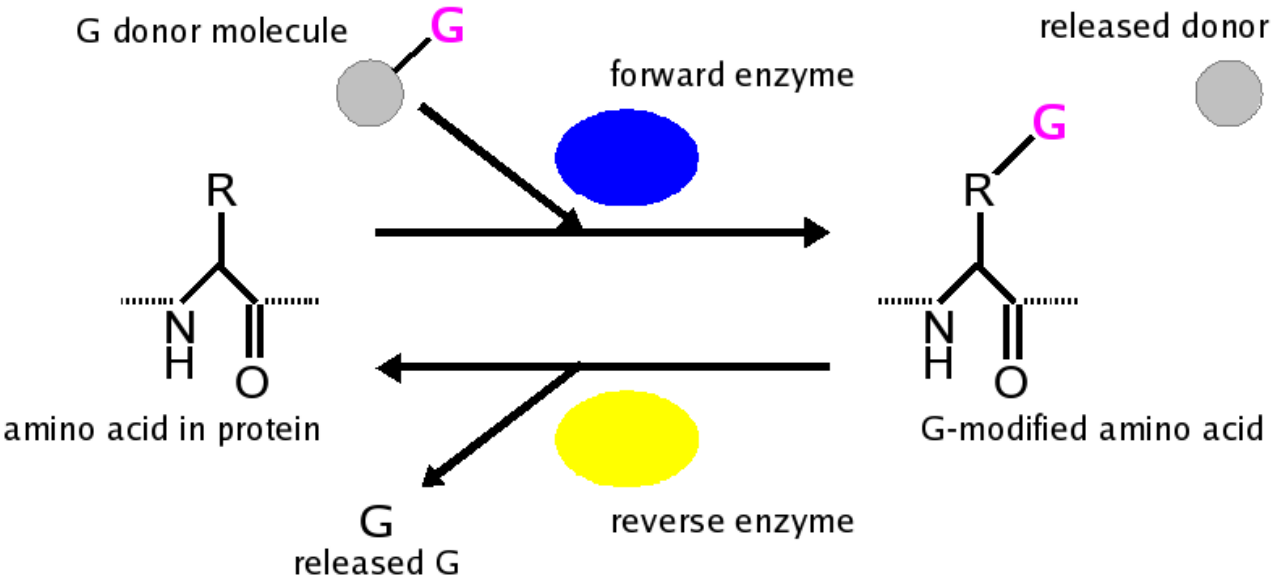
Felsenfeld & Groudine, *"Controlli ng the double helix"*, Nature **421**:448-53 2003

and regulated by a hypothetical "histone code"



Turner, *"Dec oding the nucleosome"*, Cell **75**:5-8 1993.
Strahl, Allis, *"The language of covalent histone modifications"*, Nature **403**:41-53 2000

most proteins are post-translationally modified

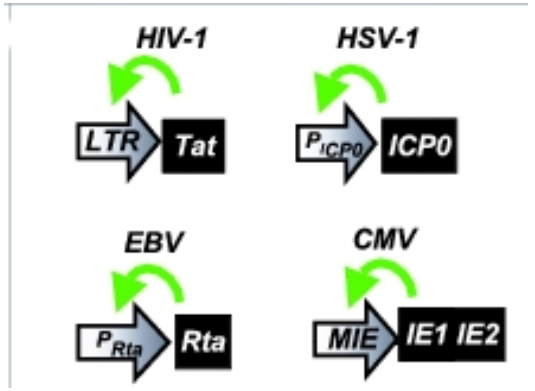


Modification	Modifier	Donor	Modified residue
phosphorylation	PO_3^{2-}	ATP	S, T, Y (H, D in bacteria)
sulfation	SO_3^-	PAPS	Y† (extracellular)
methylation	CH_3	SAM	E, K(1-3)†, R(1-2)†
acetylation	CH_3CO	AcCoA	K
ubiquitylation	Ubiquitin	-	K
ubiquitylation-like	SUMO, Nedd8, ...	-	K

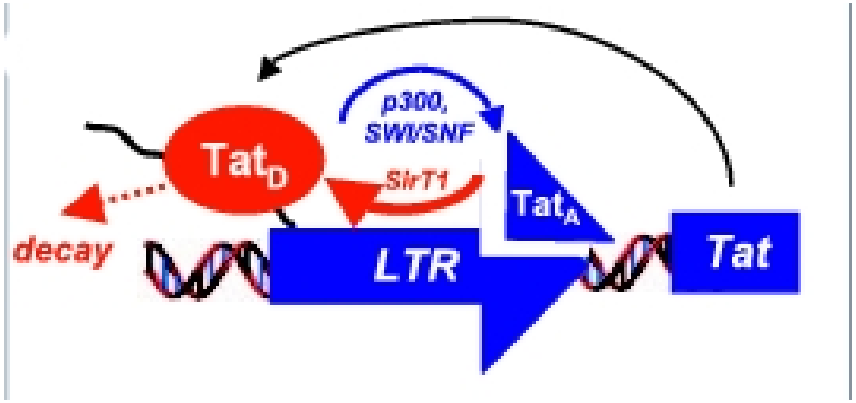
† = reverse enzymes not known

latent viruses use transcriptional activators to initiate lysis

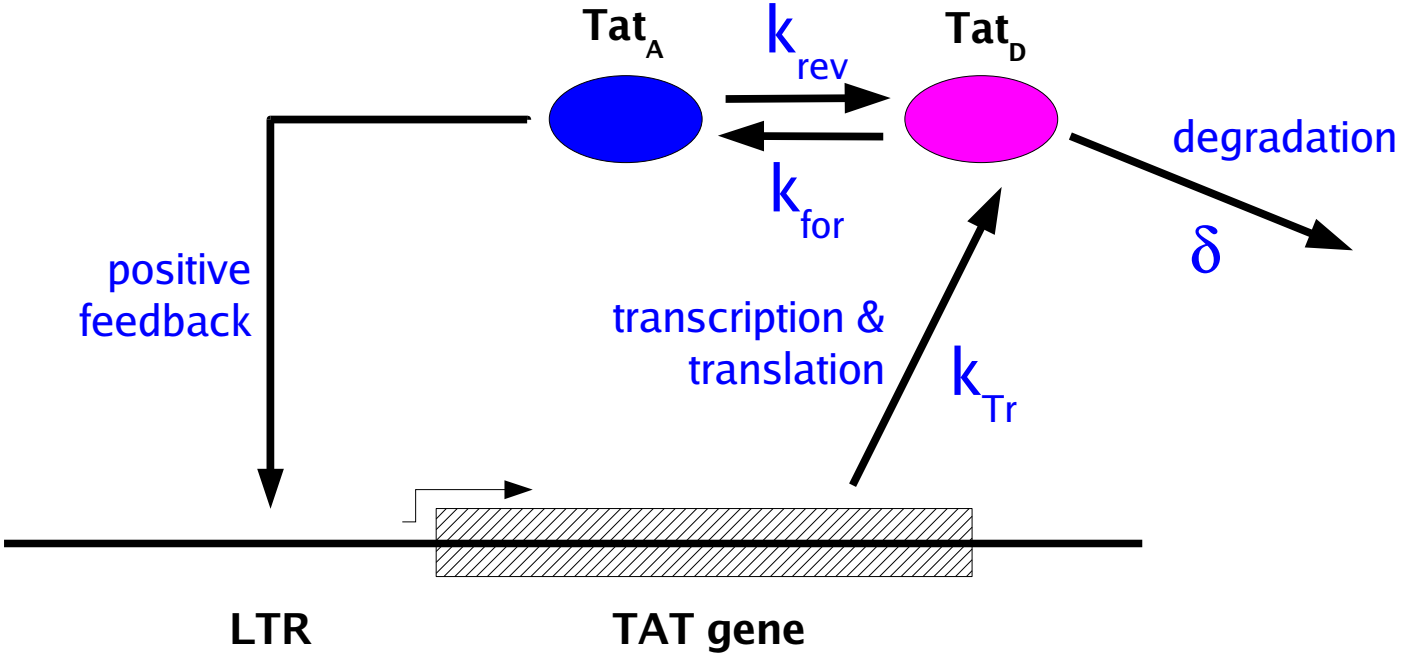
stable off states (latency) are not maintained by cooperativity



HIV-1 autoregulatory loop



Leor Weinberger and Thomas Shenk, *"A n HIV feedback resistor: auto-regulatory circuit deactivator and noise buffer,"* PLoS Biol. **5**:e91 2007



linear model

$$d/dt\,(Tat_D) = -k_{For} \cdot Tat_D + (k_{Rev} + k_{TR}) Tat_A - \delta \cdot Tat_D$$
$$d/dt\,(Tat_A) = k_{For} \cdot Tat_D - k_{Rev} \cdot Tat_A$$

$$\frac{d}{dt}\begin{pmatrix} \mathrm{Tat}_D \\ \mathrm{Tat}_A \end{pmatrix} = \begin{pmatrix} -k_{for} - \delta & k_{rev} + k_{Tr} \\ k_{for} & -k_{rev} \end{pmatrix} \begin{pmatrix} \mathrm{Tat}_D \\ \mathrm{Tat}_A \end{pmatrix}$$

$$\mathrm{Tr} = -(k_{for} + \delta + k_{rev}) < 0$$

$$\det = \delta k_{rev} - k_{for} k_{Tr}$$

condition for stability of the off state

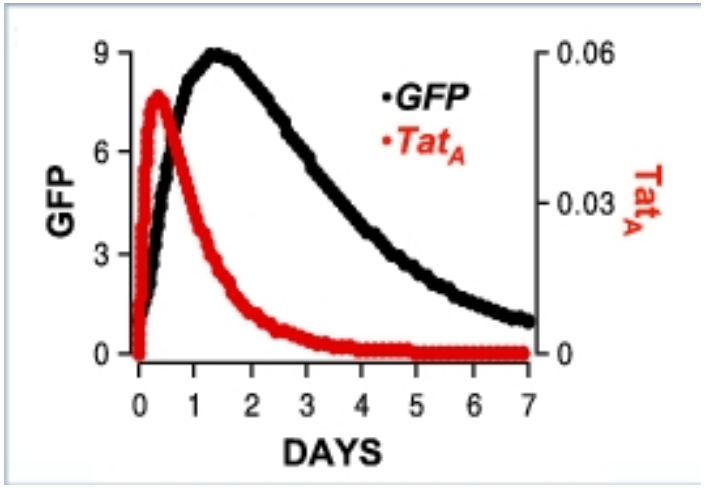
$$\boxed{\delta k_{rev} > k_{for} k_{Tr}}$$

engineered HIV-1 autoregulatory loop

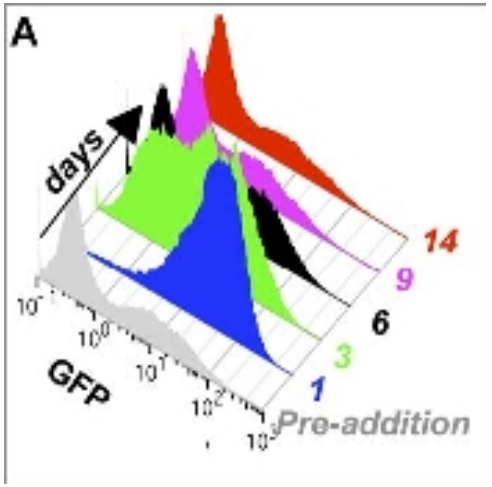
transfected into Jurkat cells (a T cell cancer line)

Weinberger, Burnett, Toettcher, Arkin, Schaffer, *"Stochastic gene expression in a lentiviral positive-feedback loop,"* Cell **122**:169-82 2005

addition of a pulse of Tat_D



simulation



data