Regulatory networks

1 Mechanisms of regulation

2 Introduction to lactose regulation in *E.coli*

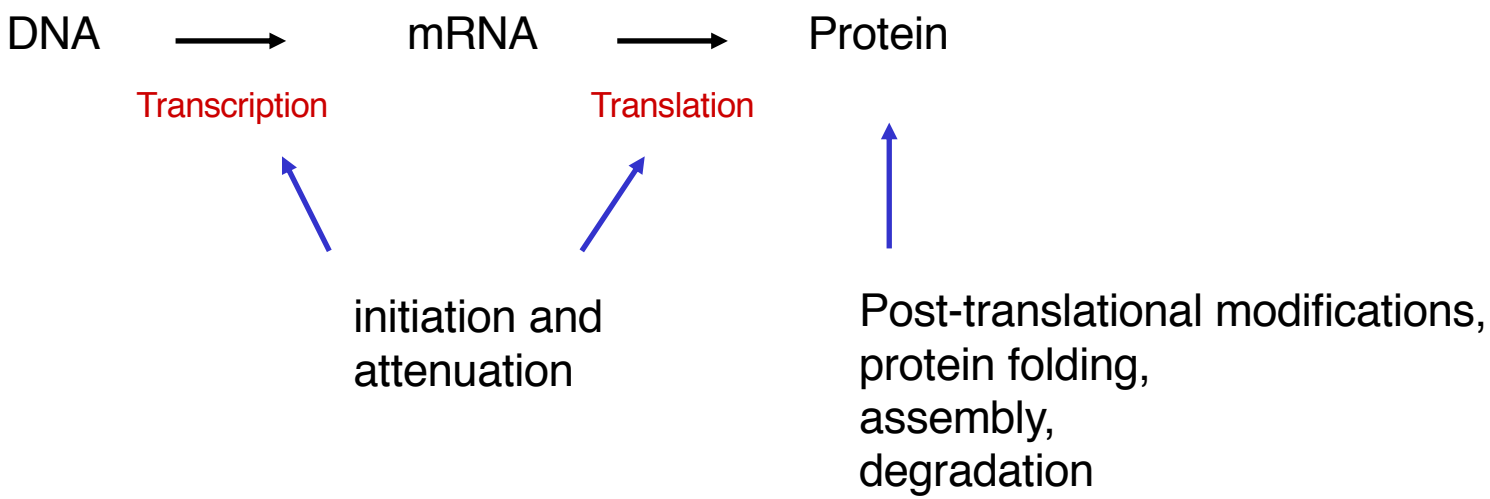
3 Modelling regulatory networks

4 Race to operator sequence

Part 1

Mechanisms of regulation

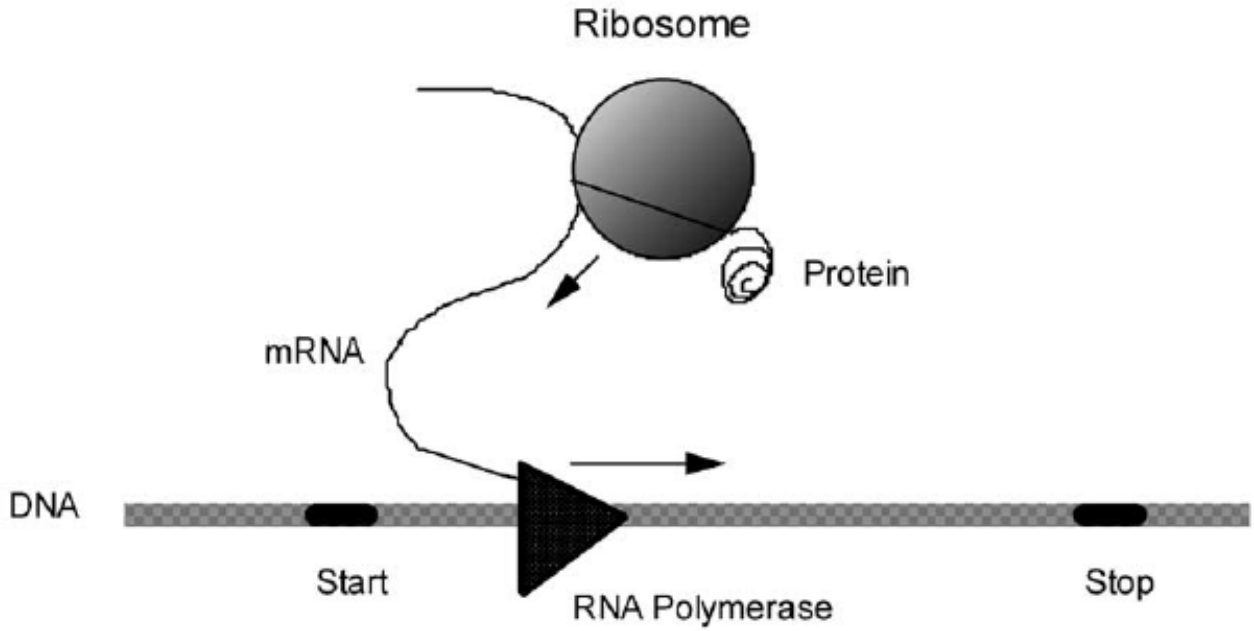
Different possibilies to adapt the metabolic activities of a cell to its environment



Procaryotes: regulation at the level of transcription

Eucaryotes: several different mechanisms

Transcription and translation of genes in bacteria



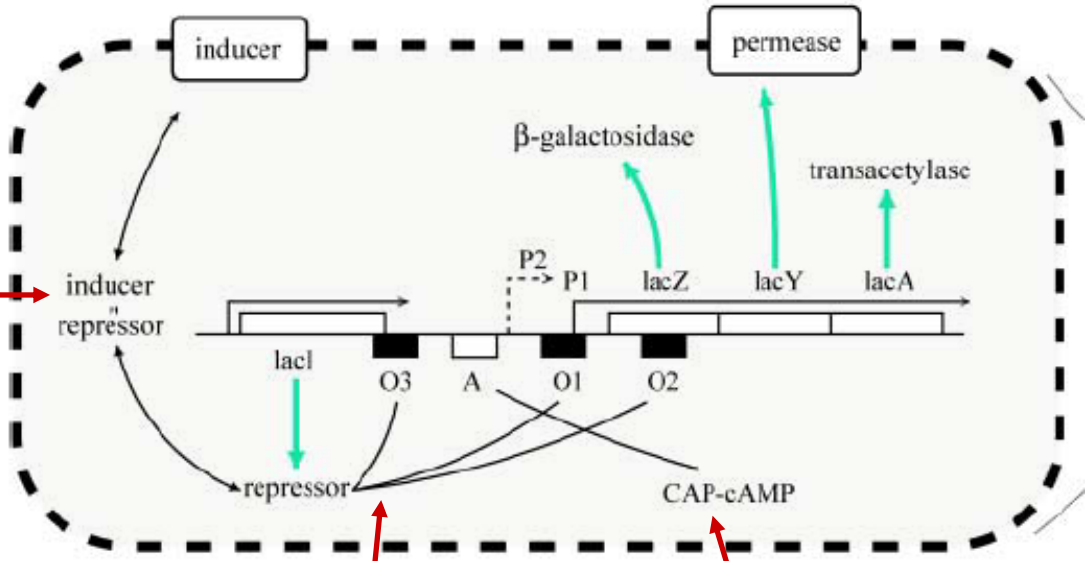
E. coli: procaryot, uni-cellular organism, one chromosome of 4.5 Mb, ~2000 genes, under standard conditions about 100 genes are actively transcribed

Part 2

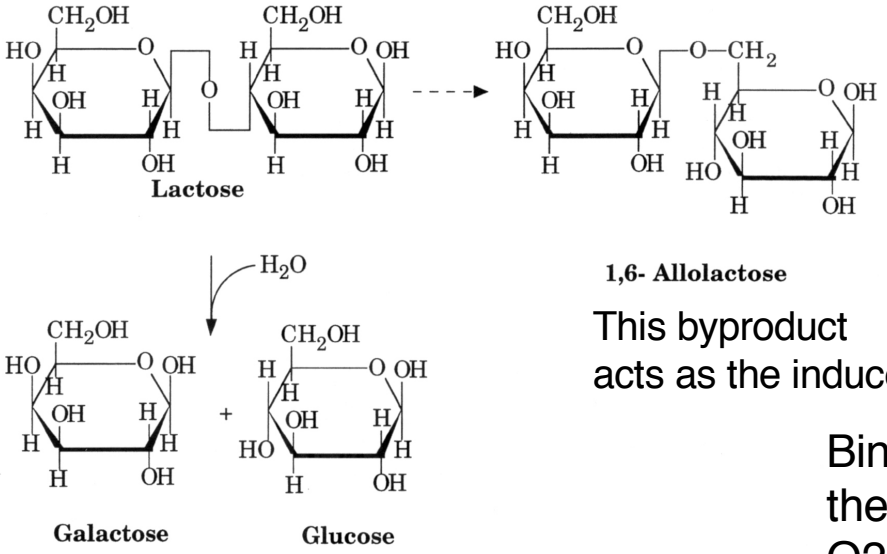
Introduction to lactose regulation in *E.coli*

The lactose operon

Binding of the inducer inactivates the repressor



β-galactosidase catalyses the hydrolosis of lactose to galactose and glucose:



This byproduct acts as the inducer.

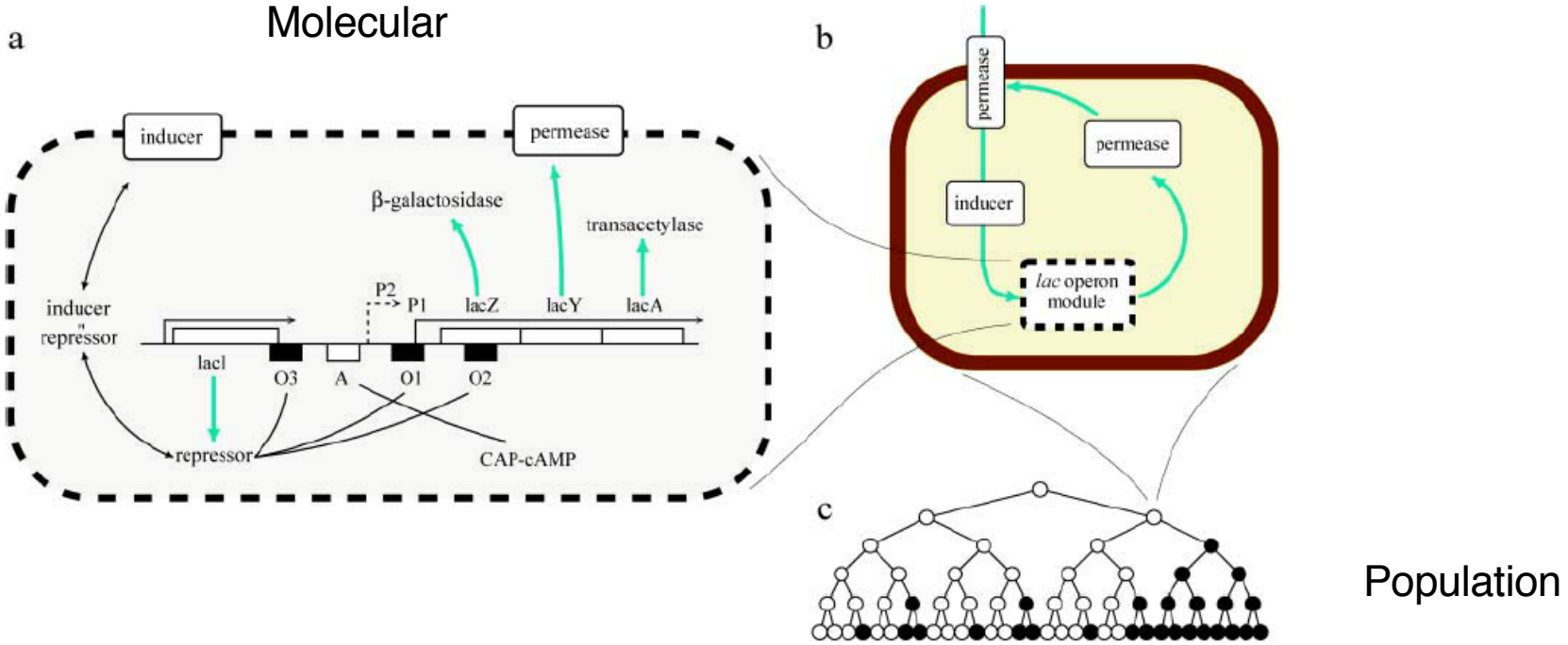
This complex must bind to the activator site A for efficient transcription.

Binding of the repressor to an operator site inhibits the transcription. O1 is the major operator, O2 et O3 are auxiliaire operators. O3 regulates itself.

Three levels of organisation

Cellular

Molecular



Population

Reaction of β-galactosidase and X-Gal

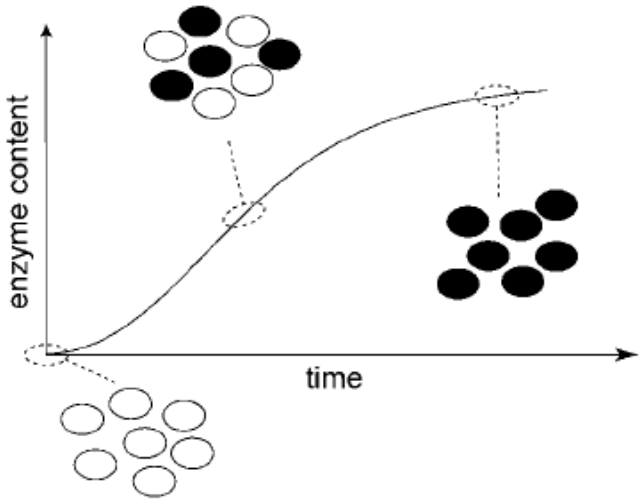
Color changes from transparent to indigo blue

X-Gal: $C_{14}H_{15}BrClNO_6$

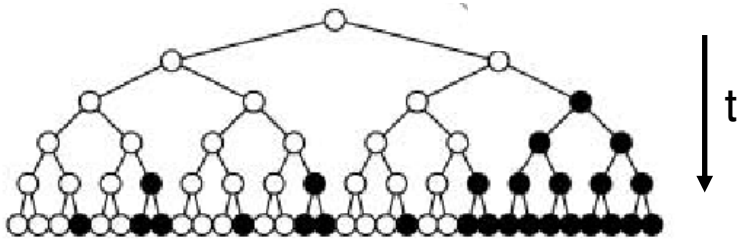
Parallel induction of β-galactosidases and permeases

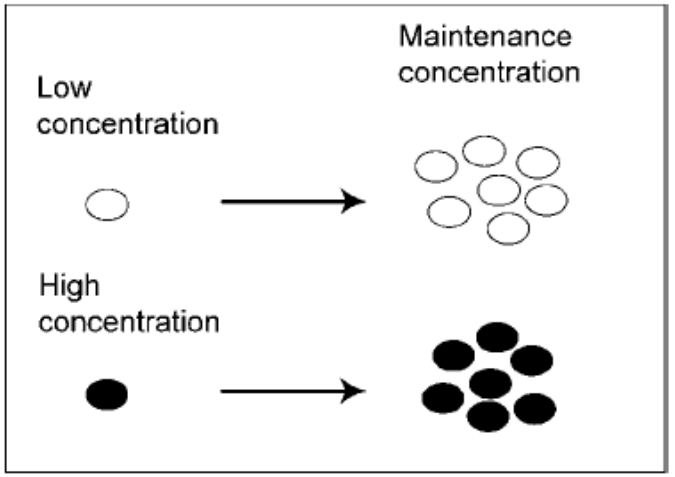
2 possible states for each bacterium

Maintenance concentration



Multiplication of a bacterium



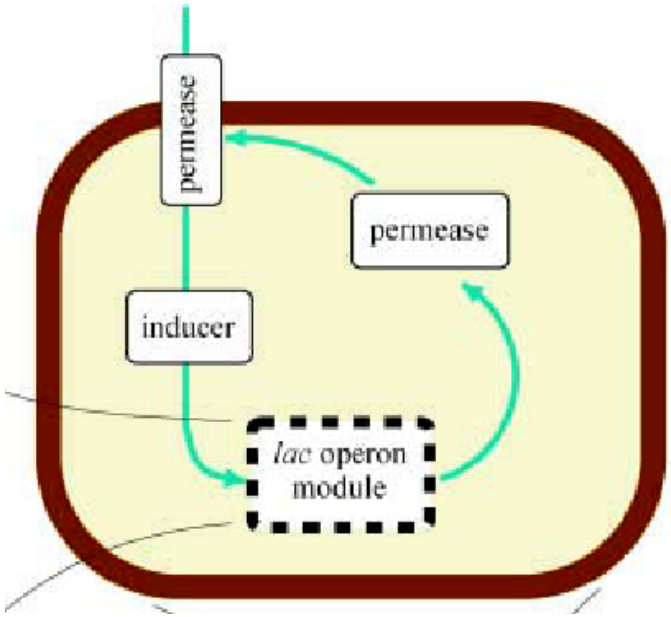


Part 3

Modelling regulatory networks

Simulating the dynamics of a regulatory network

5 concentrations: inactive permeases Y
active permeases Y_f
internal inducers I
external inducers I_{ex}
β-galactosidases Z -> color



synthesis

$$\frac{dY}{dt} = f_1(I) - a_1 Y,$$

Incorporation and activation

$$\frac{dY_f}{dt} = b_1 Y - a_2 Y_f,$$

Active export

Passive export

$$\frac{dI}{dt} = [f_2(I_{ex}) - f_3(I)]\, Y_f + b_2 I_{ex} - a_3 I,$$

$$\frac{dZ}{dt} = g\, f_1(I) - a_3 Z.$$

Active import

Passive import

synthesis

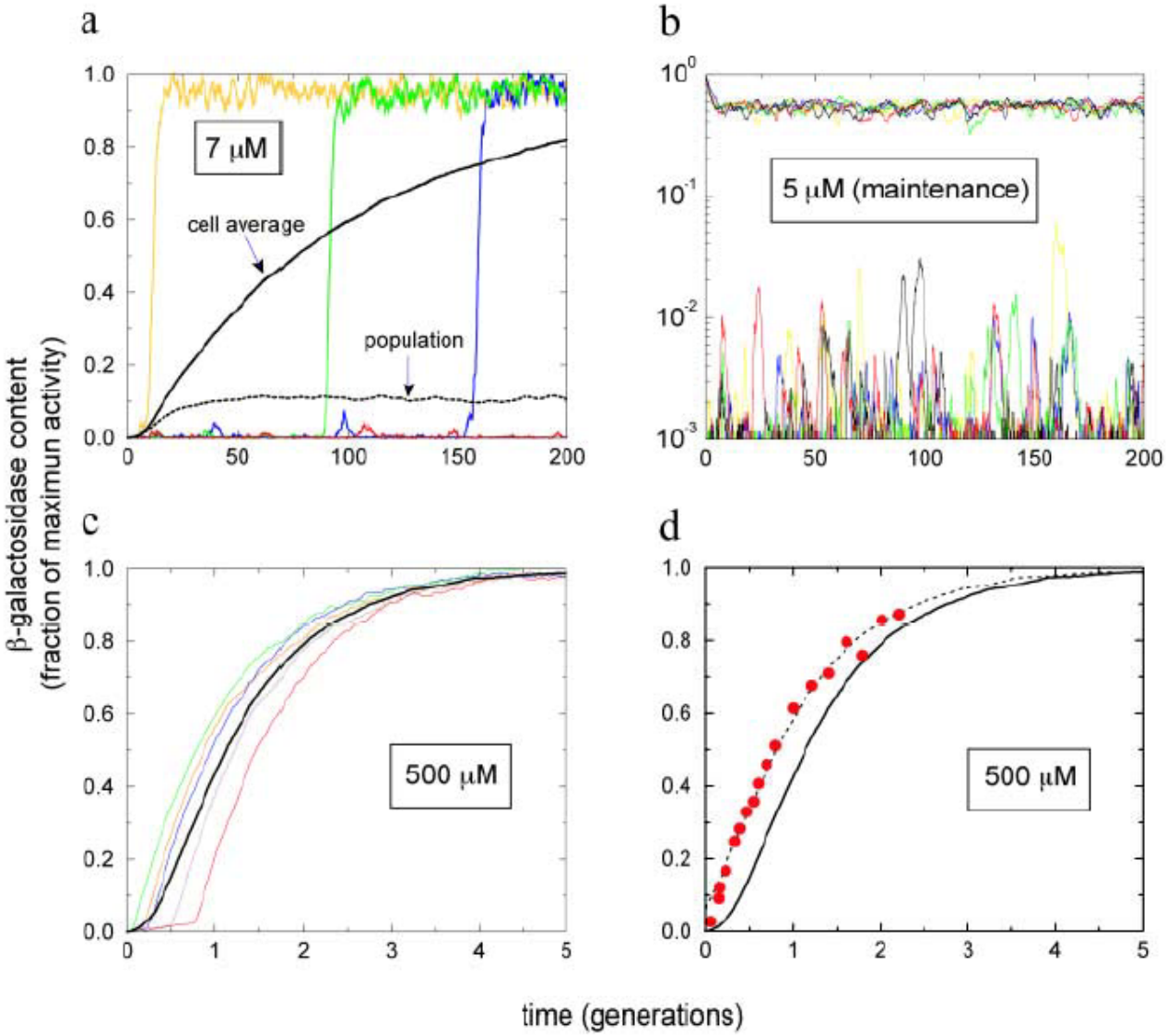
Vilar, Guet, Leibler, J. Cell Biol. 161, 471 (2003)

Resultats

Eqs 1-4

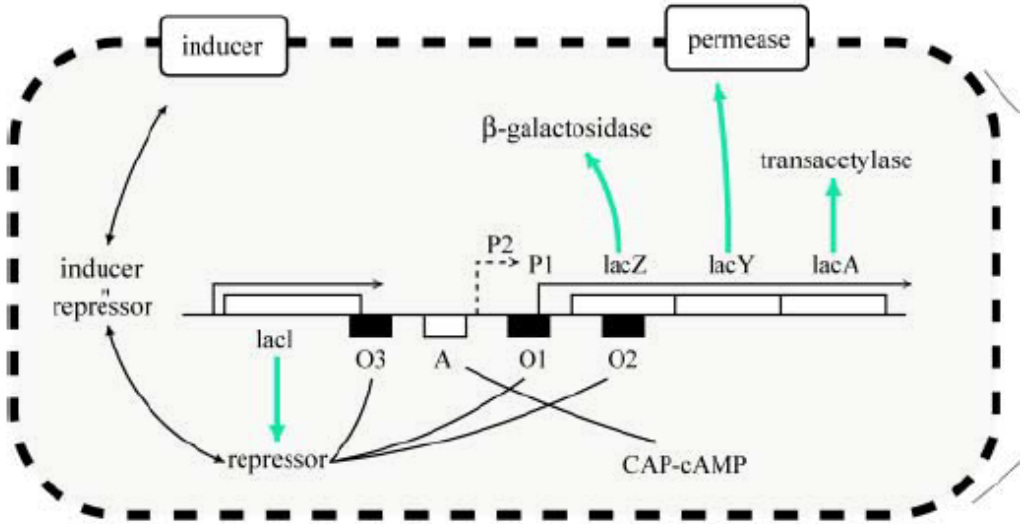
Numerical solution assuming stochastical events

Concentrations -> discret numbers



Requirements for good functioning of a gene regulatory network

- Specificity
- Reversibility
- Reactivity



Part 4

Race to operator sequence

Thermodynamics I
Specific interaction between repressor and DNA

$$R + ADN \underset{k_d}{\overset{k_a}{\rightleftharpoons}} R|ADN \qquad K_{eq}=k_d/k_a=10^{-10}\ M$$

Order of magnitude

Volume of *E.Coli* cell: ~ 1 μm^3

A repressor and an operator in the bacterium: $c_R=c_{ADN} \approx 10^{-9}$ M

Probability of operator occupation:
[RIDNA]/ [DNA] = [R] / K_{eq} = $10^{-9}/10^{-10}$ = 10 -> P= 90%

$\Delta G = \ln 10^{-10} \approx$ - 23 kT

Mesurement of K_{eq} by electrophoresis

Putting a DNA repressor mix in a gel followed by rapid migration. The equilibrium state is conserved, the mesh of the gel inhibits dissociation of the DNA-repressor complex.

Thermodynamics II
Non-specific interaction between repressor and DNA

$K_{eq} = 10^{-4}$ M

Six orders of magnitude weaker than the specific interaction

$\Delta G = \Delta H - T\Delta S = \ln 10^{-4} = -9.2$ kT

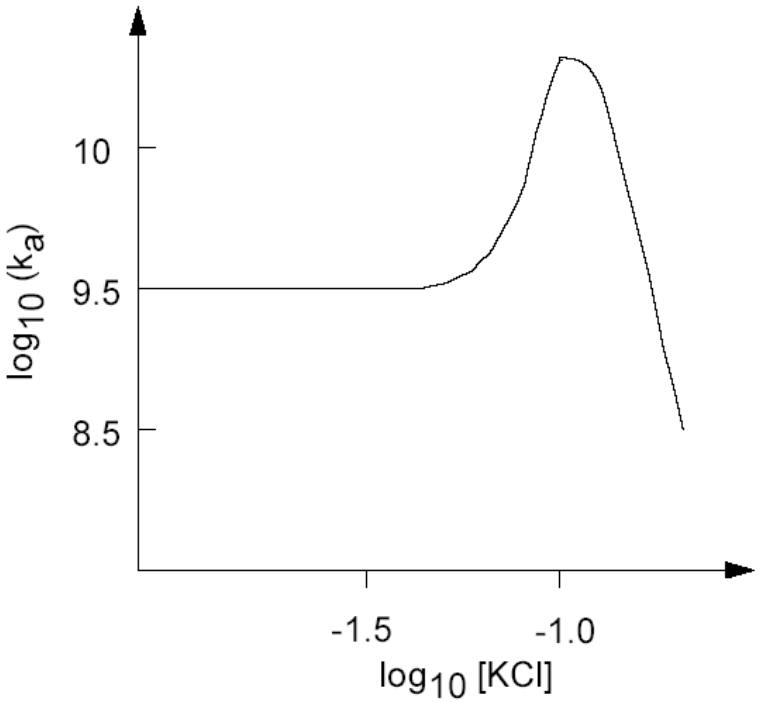
This interaction is of electrostatic origin and exhibits dominantly an entropic character
Protein binding chases away counter-ions.

WHAT IS THE BIOLOGICAL UTILITY OF THIS NON SPECIFIC INTERACTION?

Kinetics

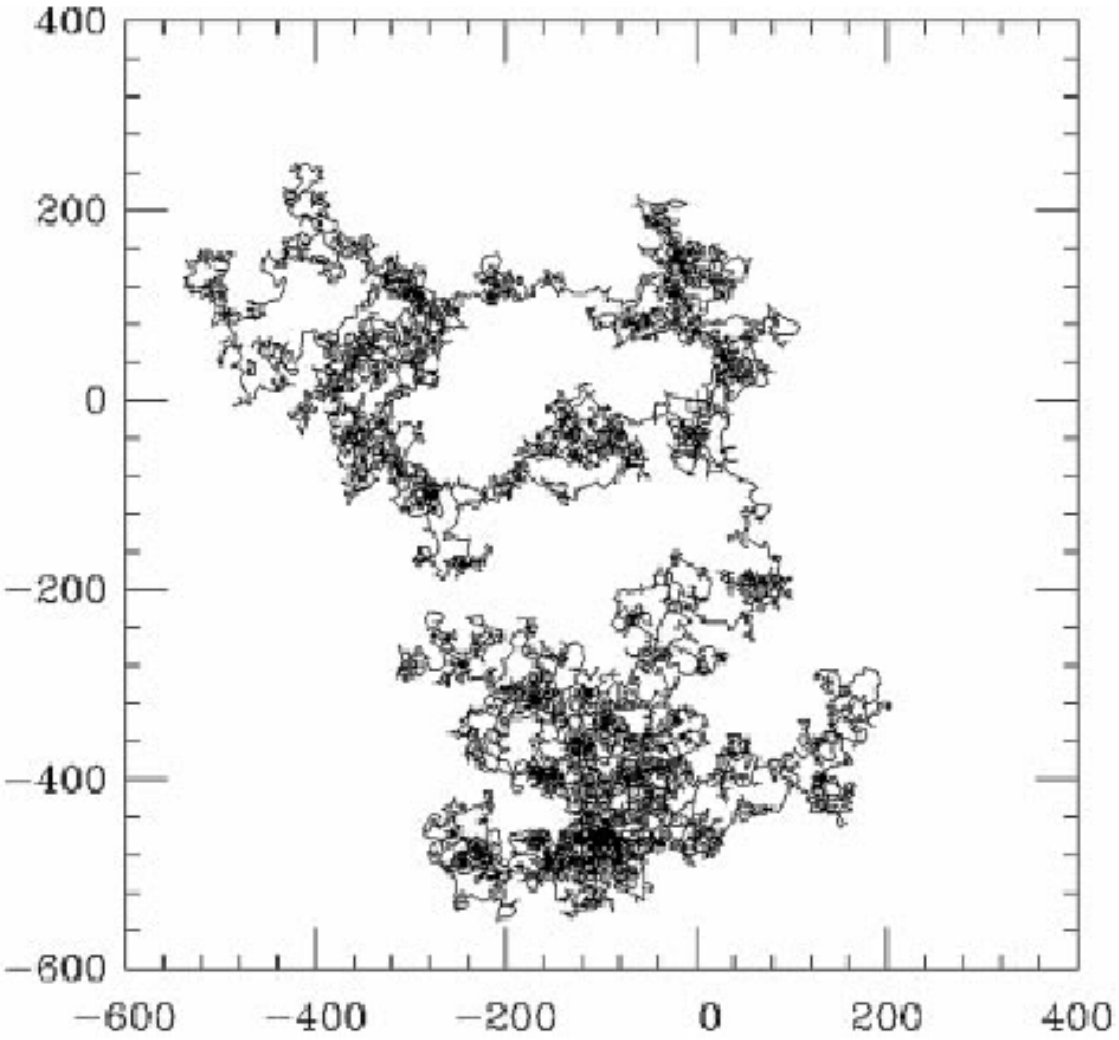
Measuring k_a of the specific interaction:
Mix repressor and operator DNA-^{32}P at t=0.
Withdraw samples at different time points t.
Adsorbing the complexes by the repressor to a nitrocellulose filter.
Wash the filter and measure its radioactivity with a phospho-imager.

$k_a=10^{10}\ M^{-1}s^{-1}$



How does the repressor rapidly finds the operator sequence ?
(typical search time: a few seconds in *E. coli*)

Searching the operator by 3D diffusion



A 3D random walk of 10^6 steps.

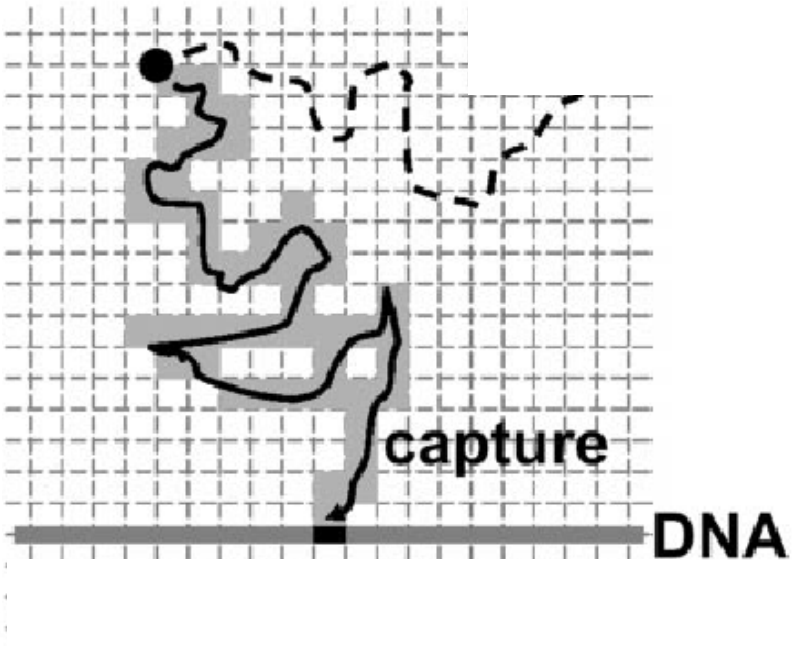
Step size=1.

Projection to the plane.

Average size ~1000 pas.

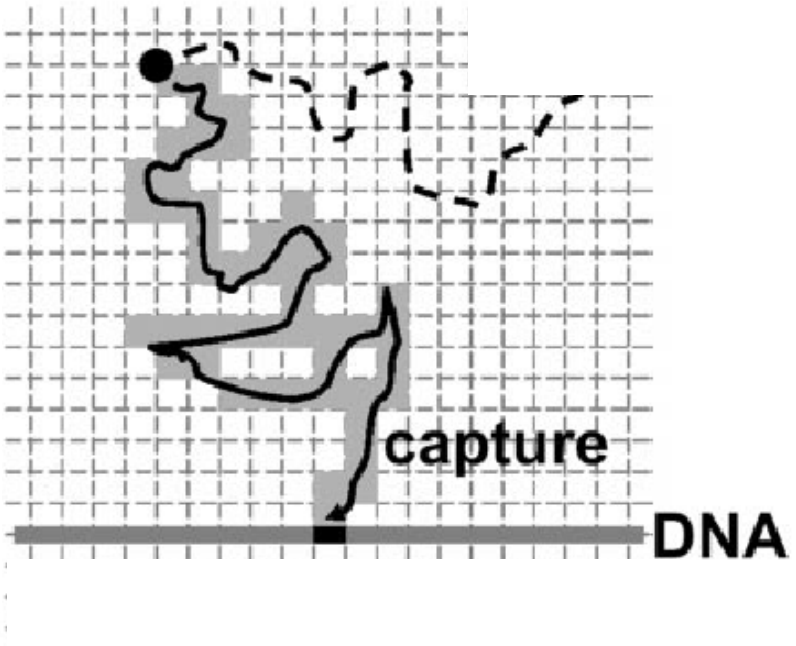
Many holes.

Debye-Smoluchowsky limit



Operator site fixed at the centre of a sphere, 3D diffusion equation, concentration is constant far from the centre, viscosity of water, repressor as a sphere of diameter b=5 nm, without non-specific interaction between repressor and DNA.

Debye-Smoluchowsky limit



Operator site fixed at the centre of a sphere, 3D diffusion equation, concentration is constant far from the centre, viscosity of water, repressor as a sphere of diameter b=5 nm, without non-specific interaction between repressor and DNA.

$kT/(6\pi\eta b) \cong 40\ \mu m^2/s$

$k_a = 4\ \pi\ D\ b = 2/3\ kT/\eta = 10^9\ M^{-1}s^{-1}$

Represents an upper limit → the process of 3D diffusion is too slow

Non-specific interaction between repressor and DNA

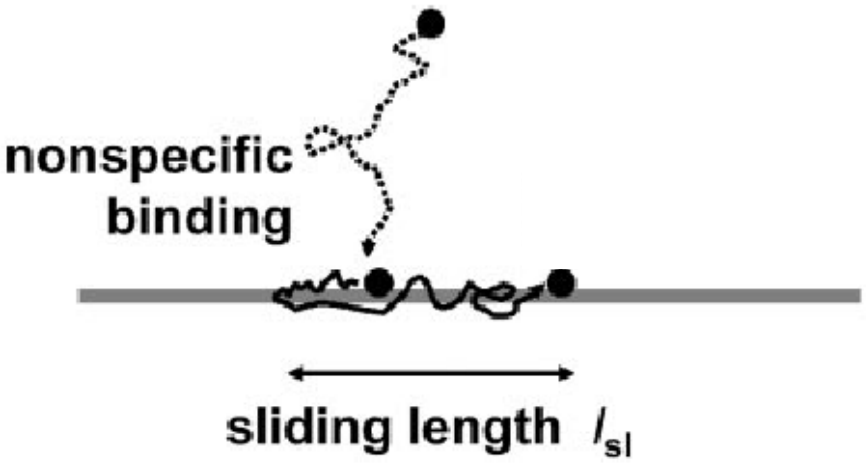
$K_{eq} = 10^{-4}$ M

Six orders of magnitude weaker than the specific interaction

Nevertheless important since the number of sites is huge:
$N_{bp} = 4.5\ 10^{6}$ for the *E.Coli* DNA.

→ the probability to find a repressor on the DNA amounts to 99%

Research by 1D diffusion



The 1D diffusion constant is ~100 times smaller than the 3D diffusion constant.

Major problem: $l_{sl} \propto t^{1/2}$

→The search time t increases rapidly with DNA size: $t \propto l_{sl}^2$

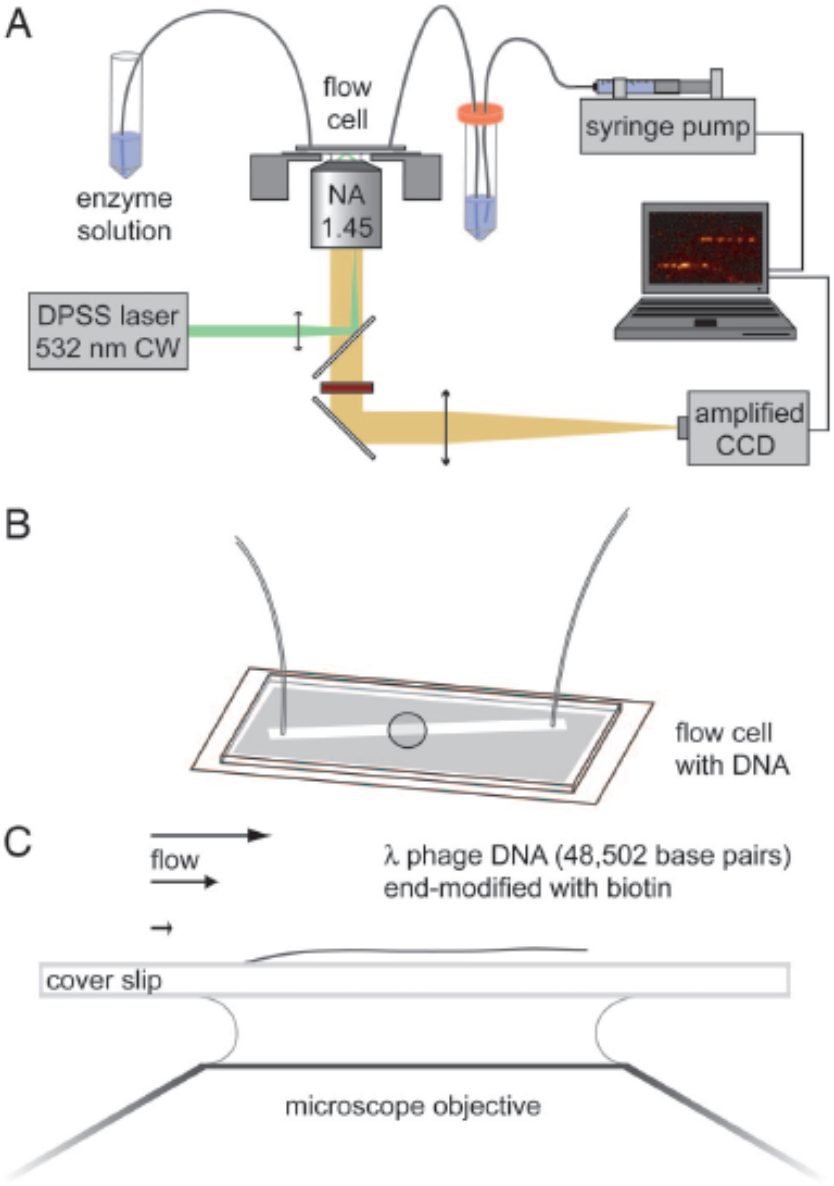
For l_{sl}=10 μm (33kb) and D_{1D}=0.5 $\mu m^2/s$ → $t = l_{sl}^2 / D_{1D} \cong 200$ s

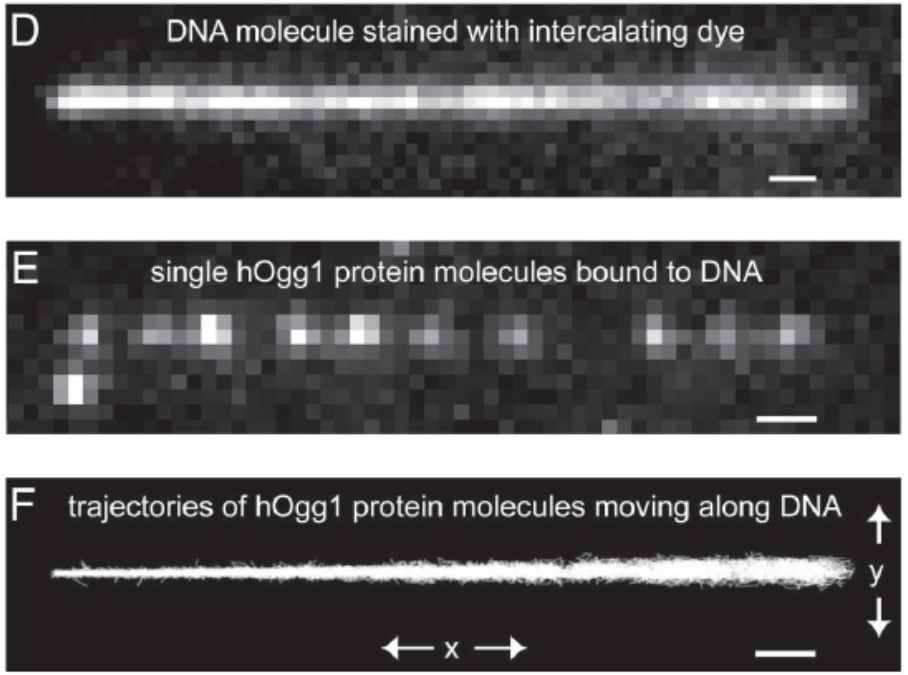
Predication for *E.coli* : 100 evenly distributed repressors take ~200 s to inhibit transcription

Typical value

Too slow

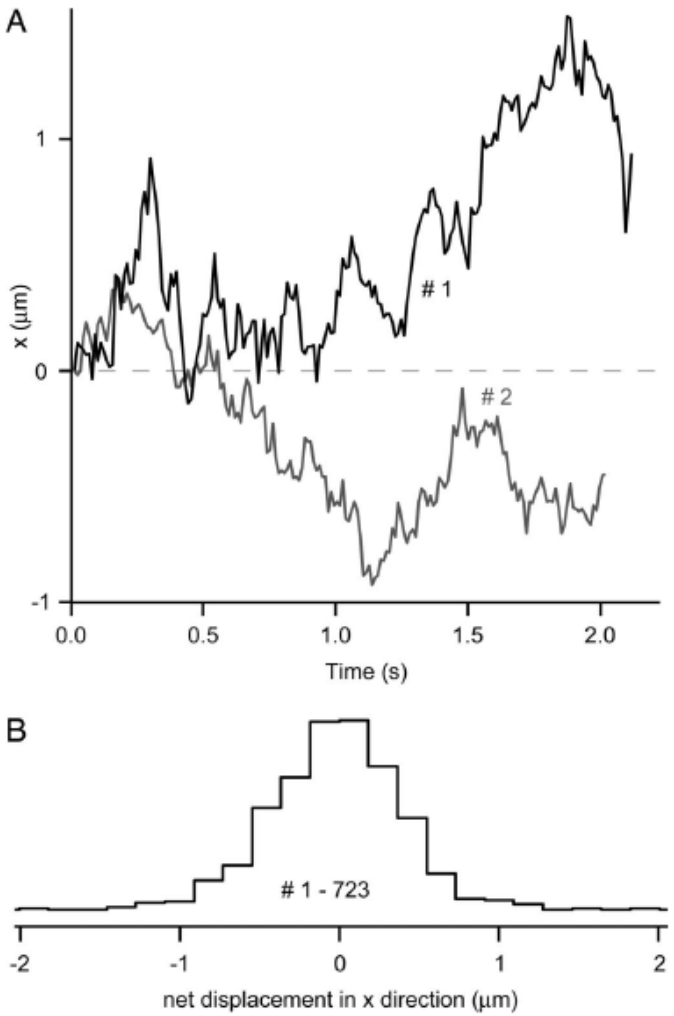
Diffusion of proteins on DNA: single molecule fluorescence



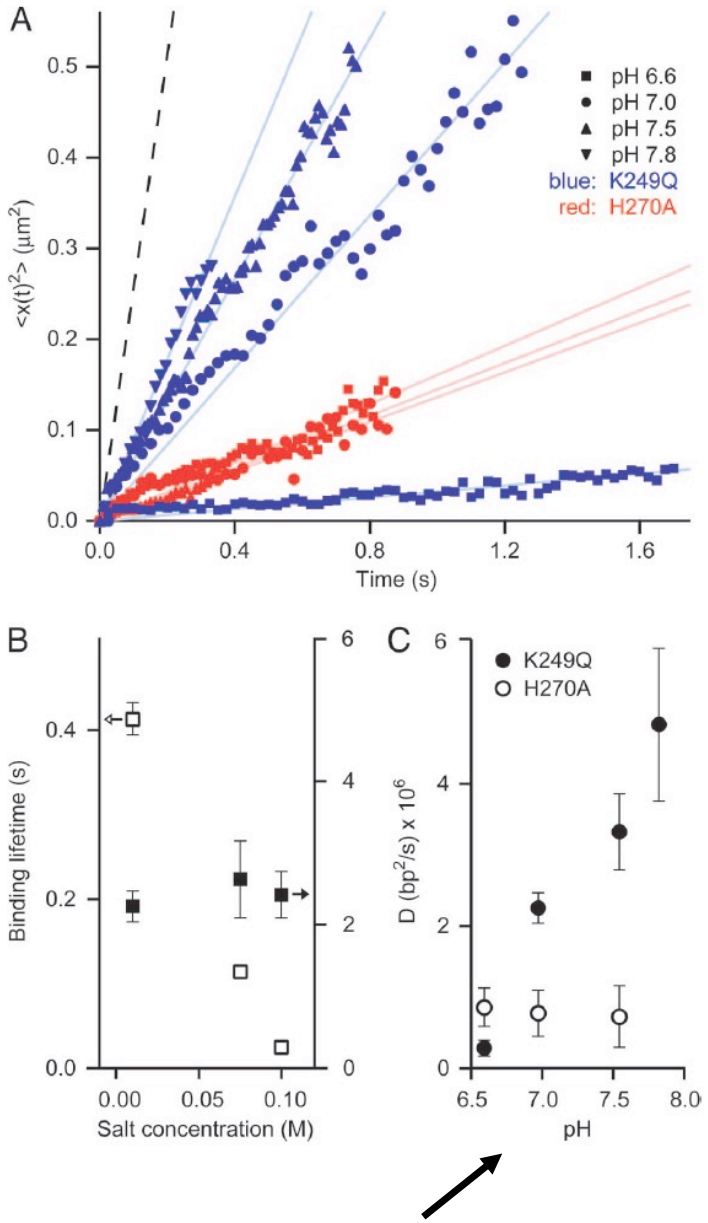


Blainey et al, Proc. Natl. Acad. Sci. 103, 5752 (2006)

Trajectories of two hOgg1 proteins



Mean-square displacements

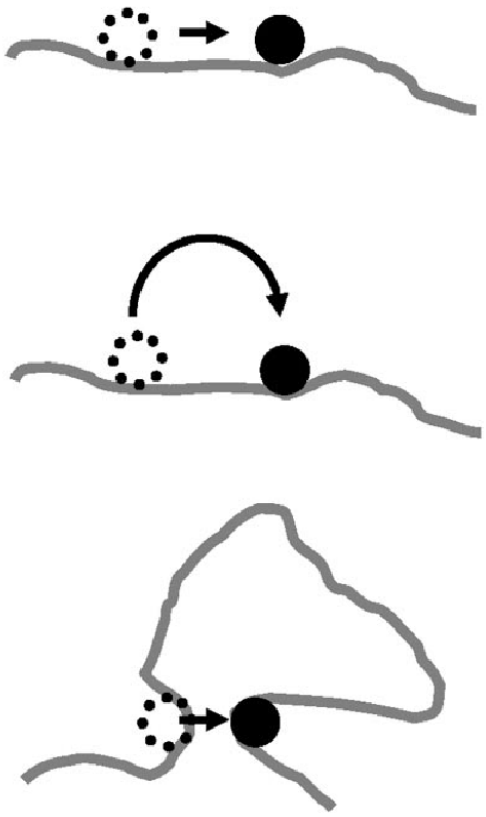


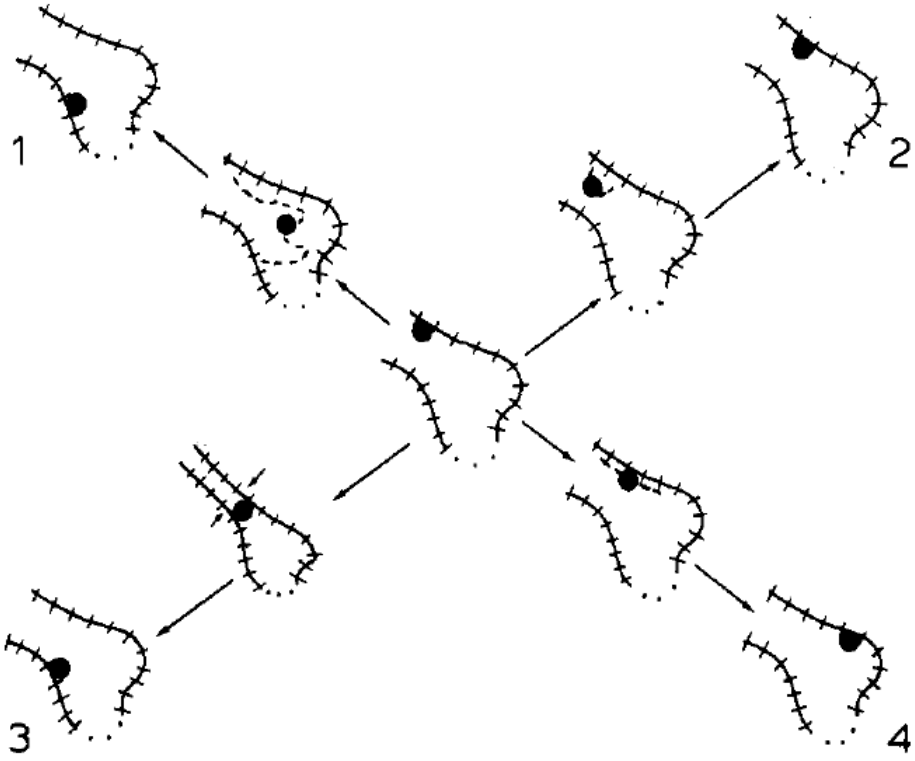
Binding lifetime depends on salt, while D is constant.

A conserved histidine at position 270 causes the pH dependence.

Research by combined 3D and 1D diffusion

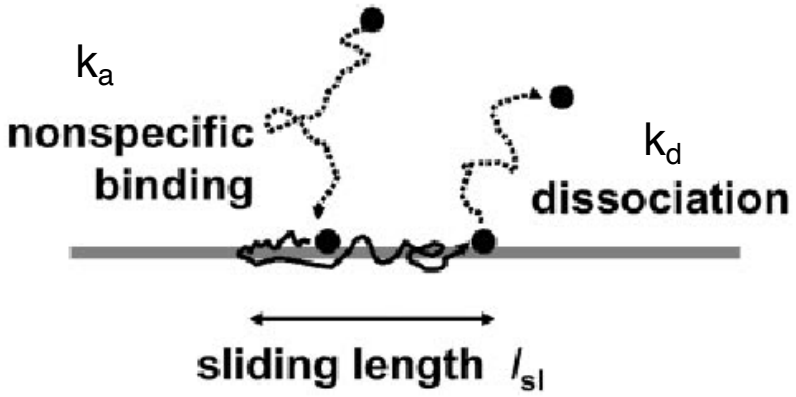
Berg, Winter et van Hippel (BWH), Biochemistry 20, 6929 (1981)





BWH theory

Mixed 1D and 3D diffusion



A 1D random walk:

Typical duration: $k_{d,ns}^{-1} \cong 100$ ms (measured value)

Explored DNA length: $l_{sl} = (D_{1D} / k_{d,ns})^{1/2} = 200$ nm $= 4\ l_p$ (D_{1D}=0.5 $\mu m^2/s$)

After a duration $T >> k_{d,ns}^{-1}$:

A number of $k_{d,ns}$ T random walks occurred

Explored DNA length: $L(T) \cong T\ (D_{1D}\ k_{d,ns})^{1/2}$ linear in time!

Search time

$T(L) = L / (D_{1D}\ k_{d,ns})^{1/2}$, for L=10 μm (33kb) we obtain $T \cong 5$ s

(cp. pure 1D diffusion: $T \cong 200$ s)

This strategy is used by many proteins to find a specific binding site on DNA or RNA

“research engine” for the genomics database in the cell

SUMMARY

The Lac operon of the *E.coli* bacterium as a simple illustration of the complexity of regulation networks in the biological cell

Transcriptional regulation

Simulation of regulation networks

Coupling diffusion and inter-molecular interactions
Mechanism of Berg, Winter and van Hippel describing the search for a specific sequence in DNA and RNA.