Interactions between proteins, DNA and RNA

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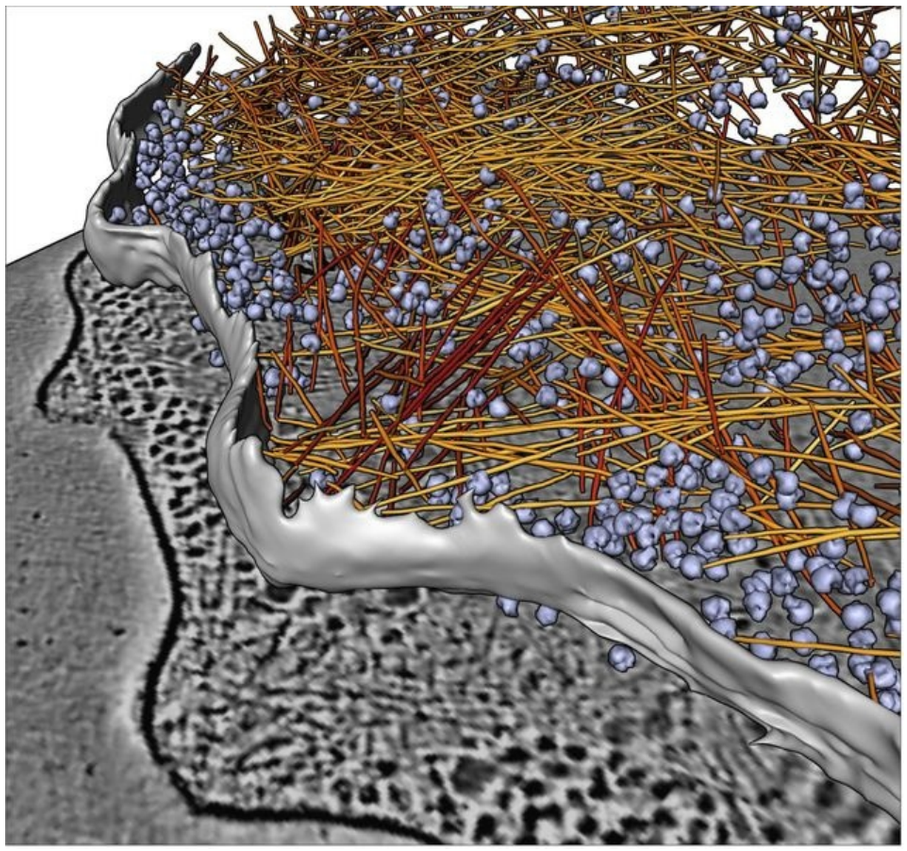
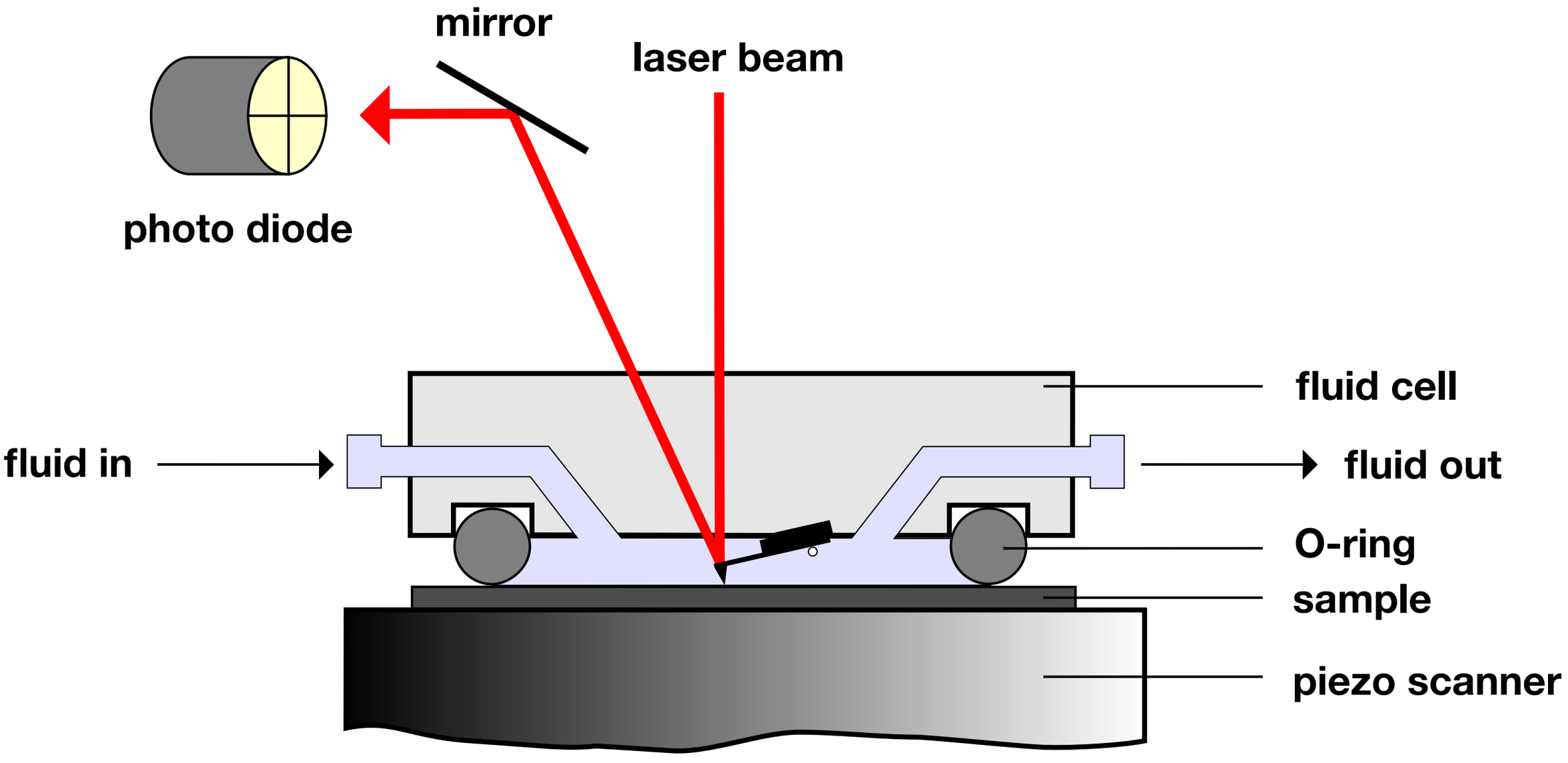
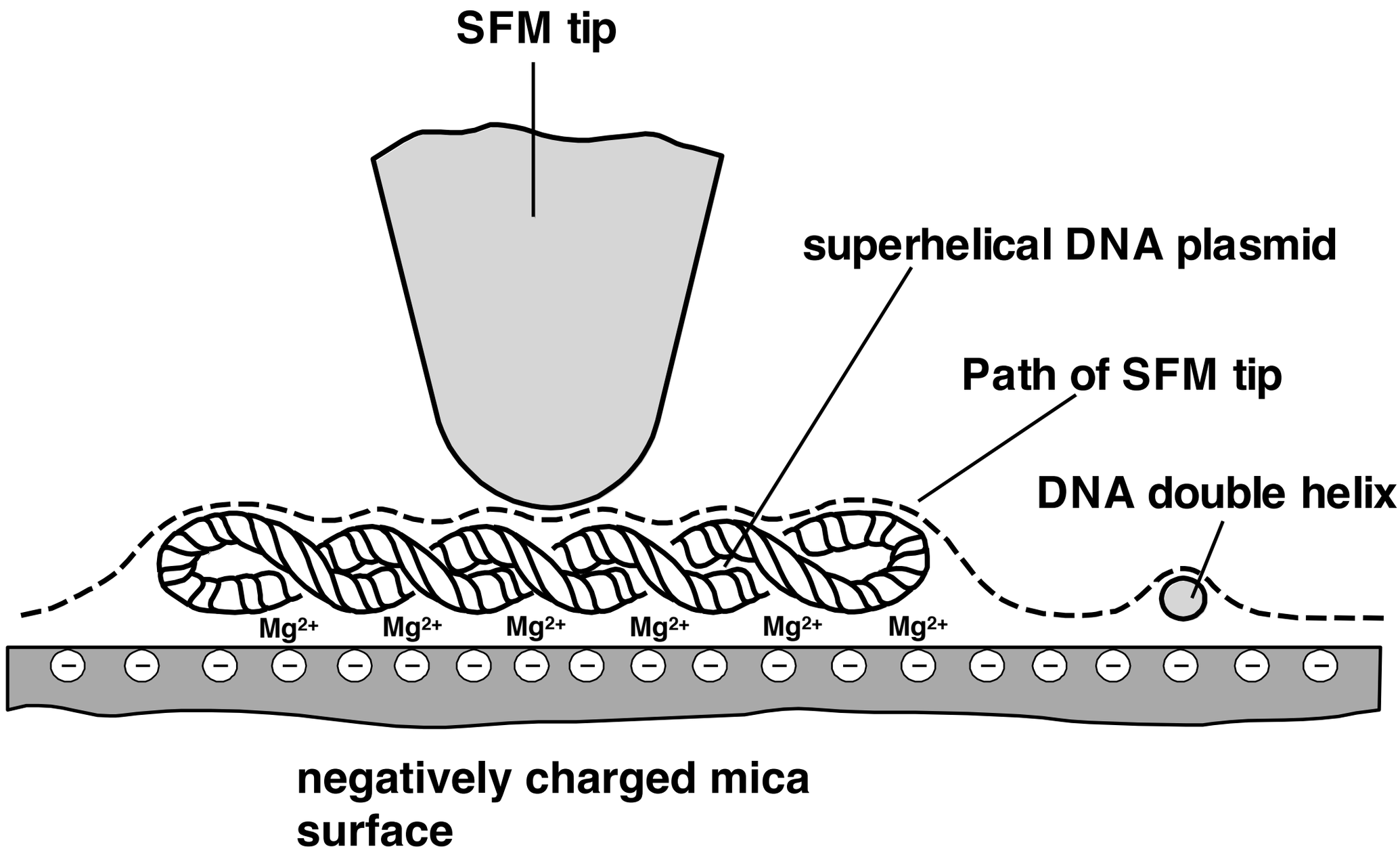
The energy, length and **time** coordinate system to find your way in the cell



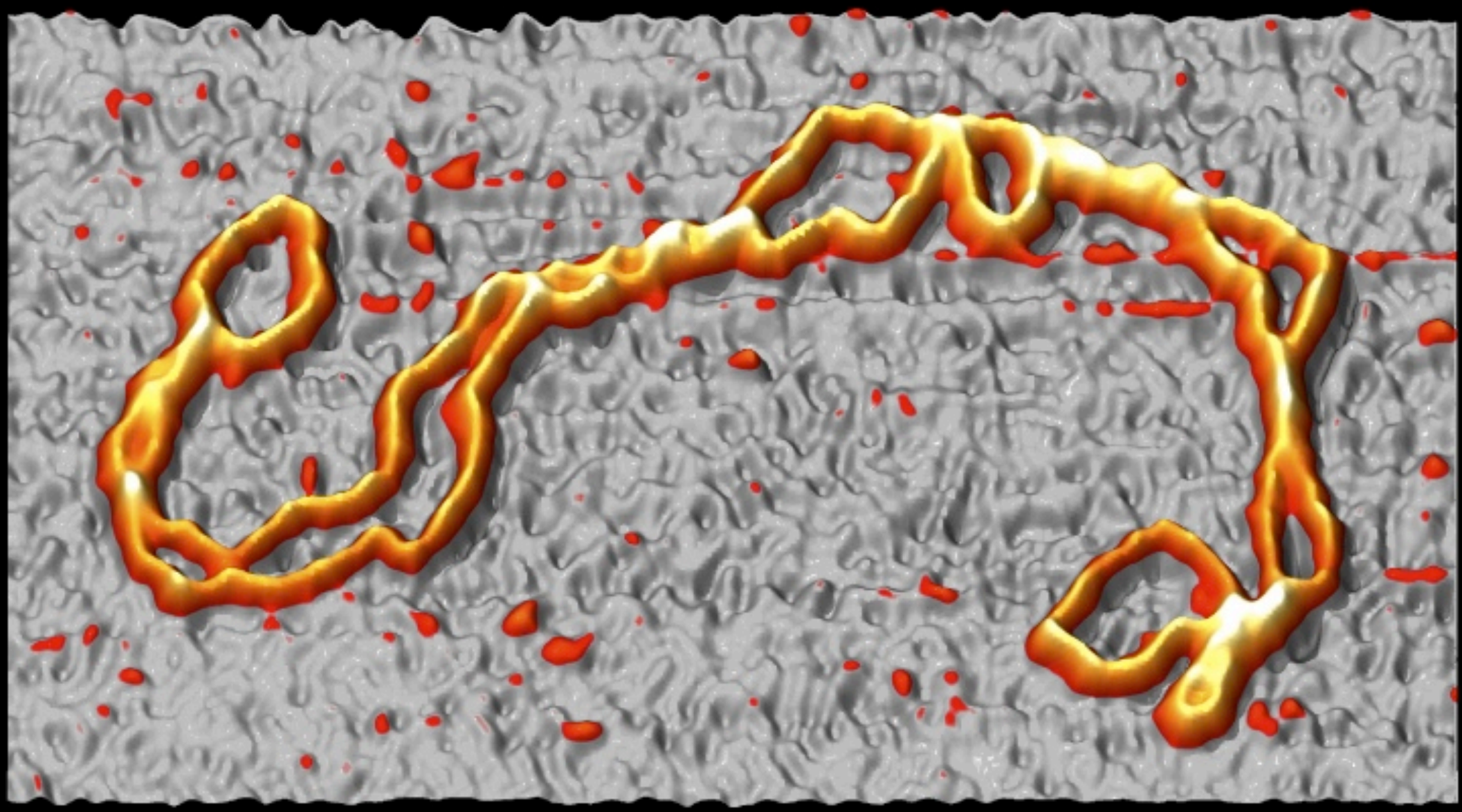
Scanning/atomic force microscope (SFM/AFM)



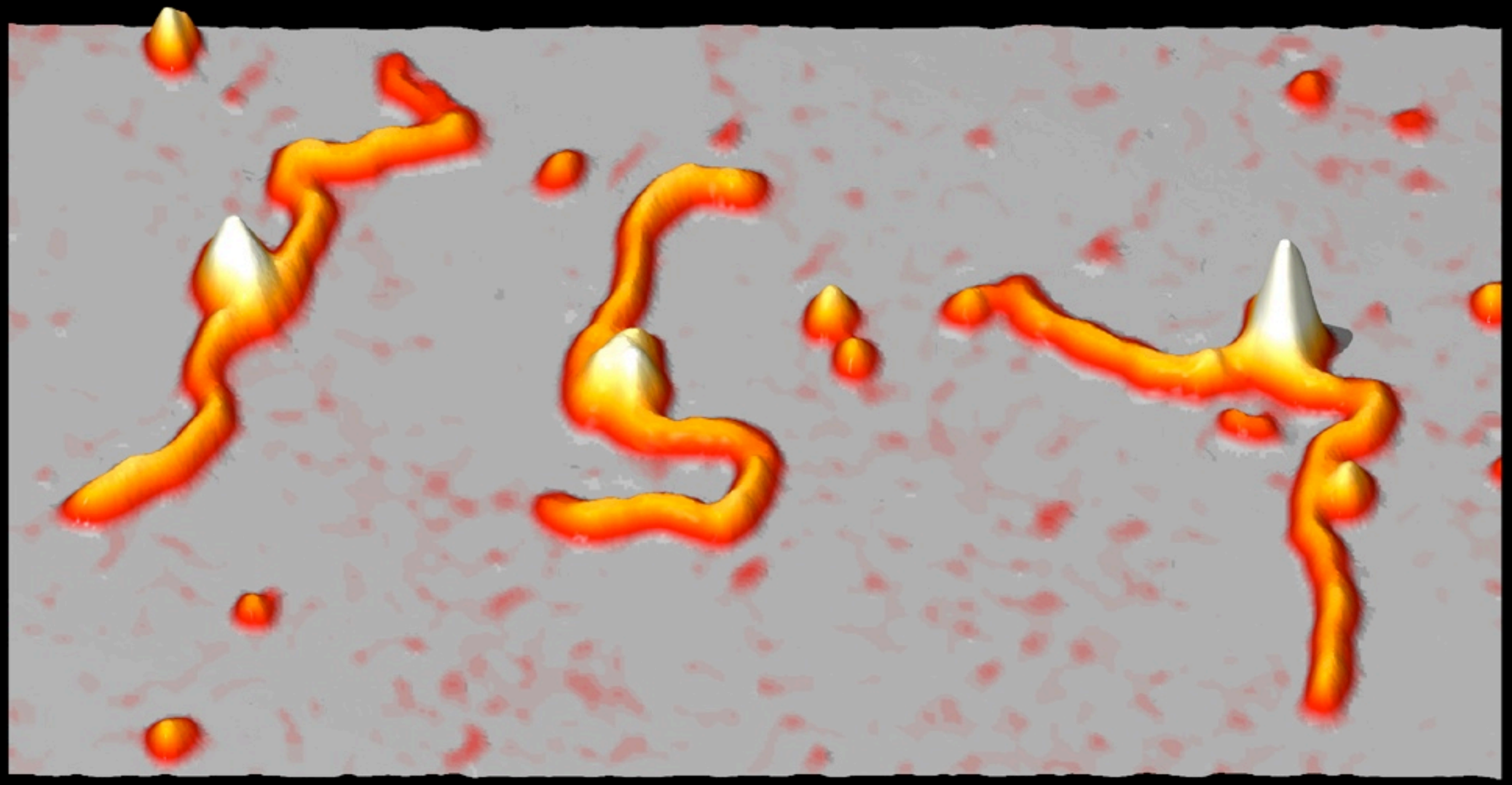
Movement of the SFM tip along the sample



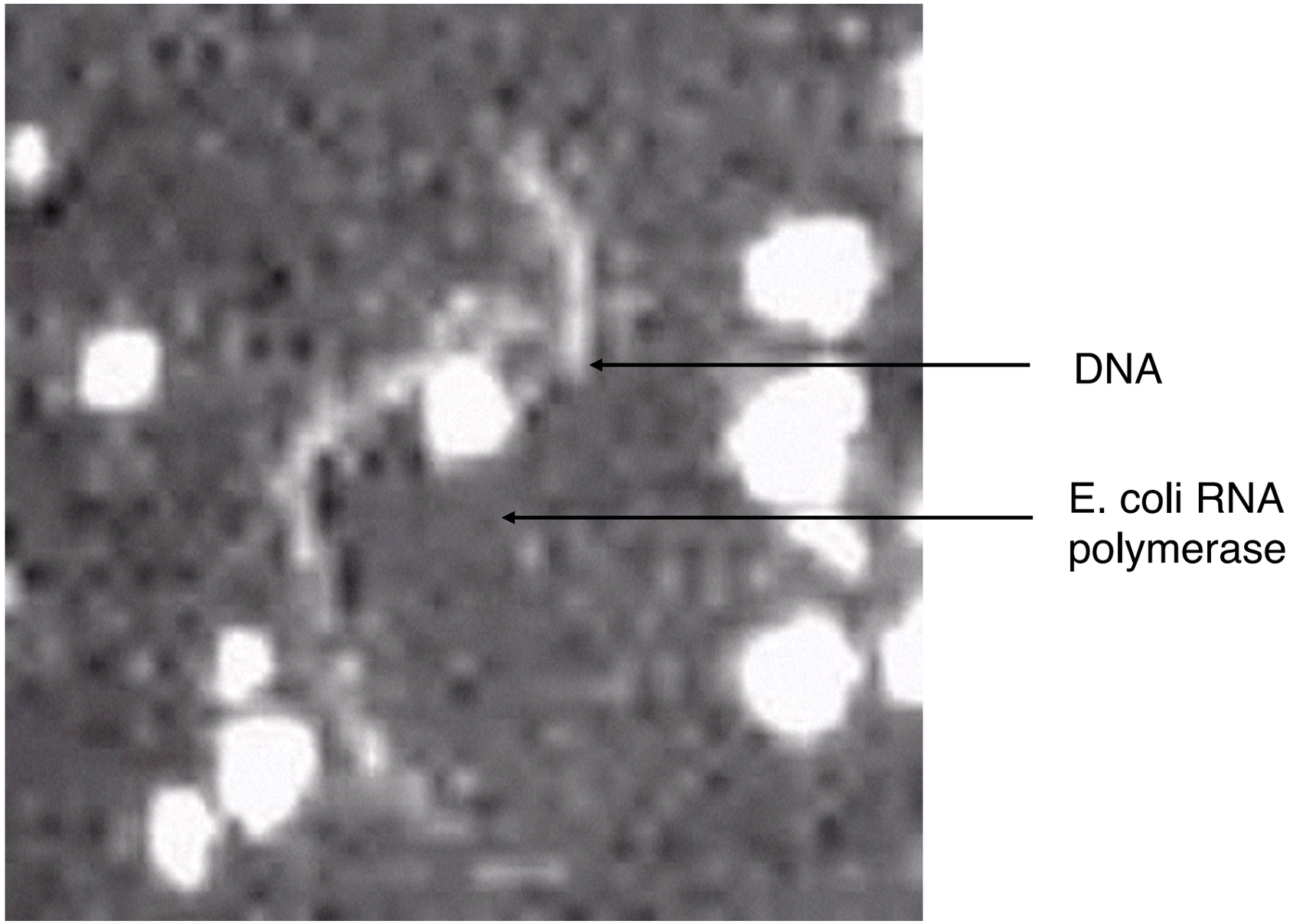
SFM image of a 6.8 kb superhelical plasmid



E. coli RNA polymerase at the promoter of a 1036 bp DNA

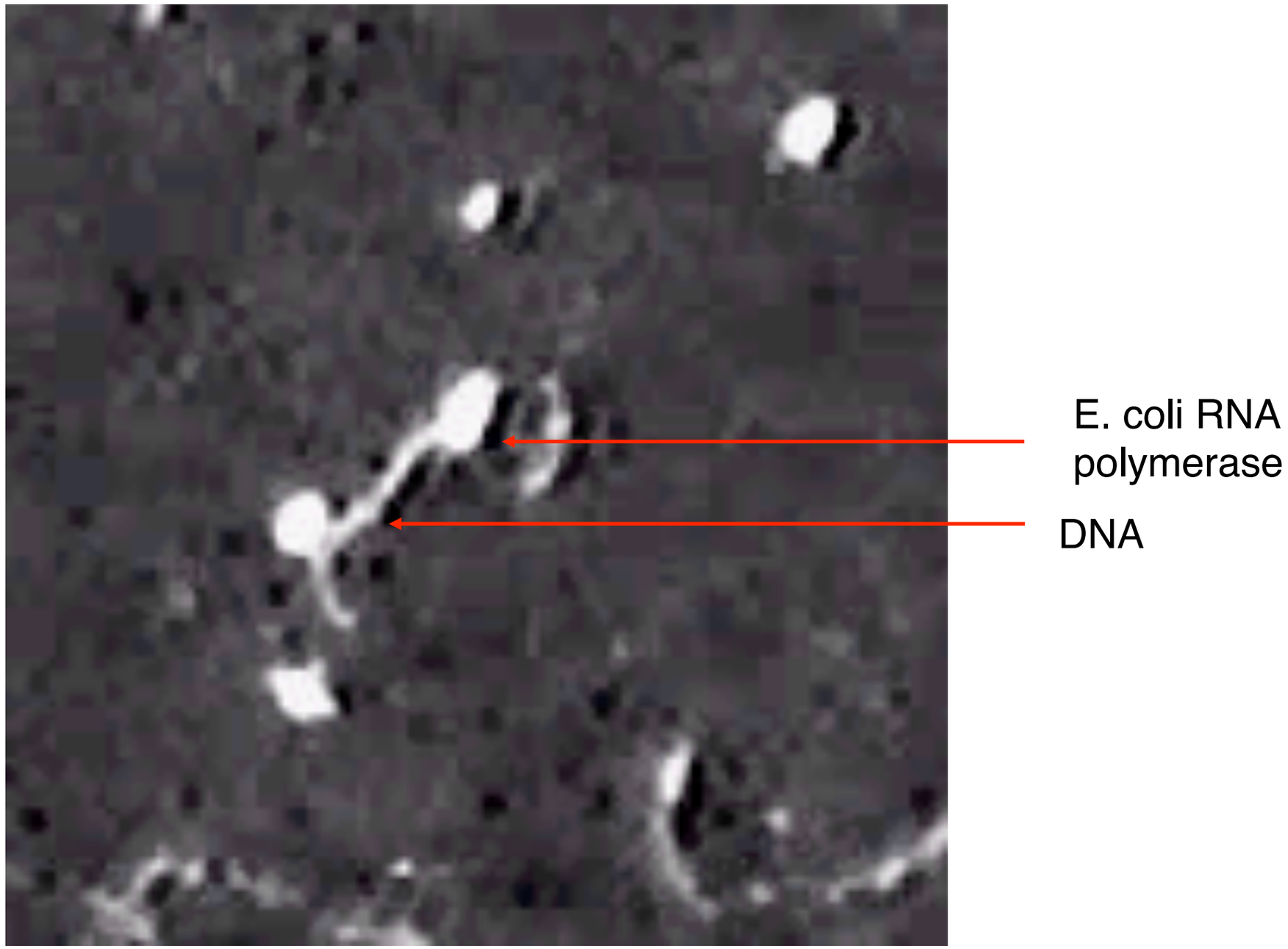


RNA polymerase finds its promoter by “sliding” along the DNA as visualized by SFM



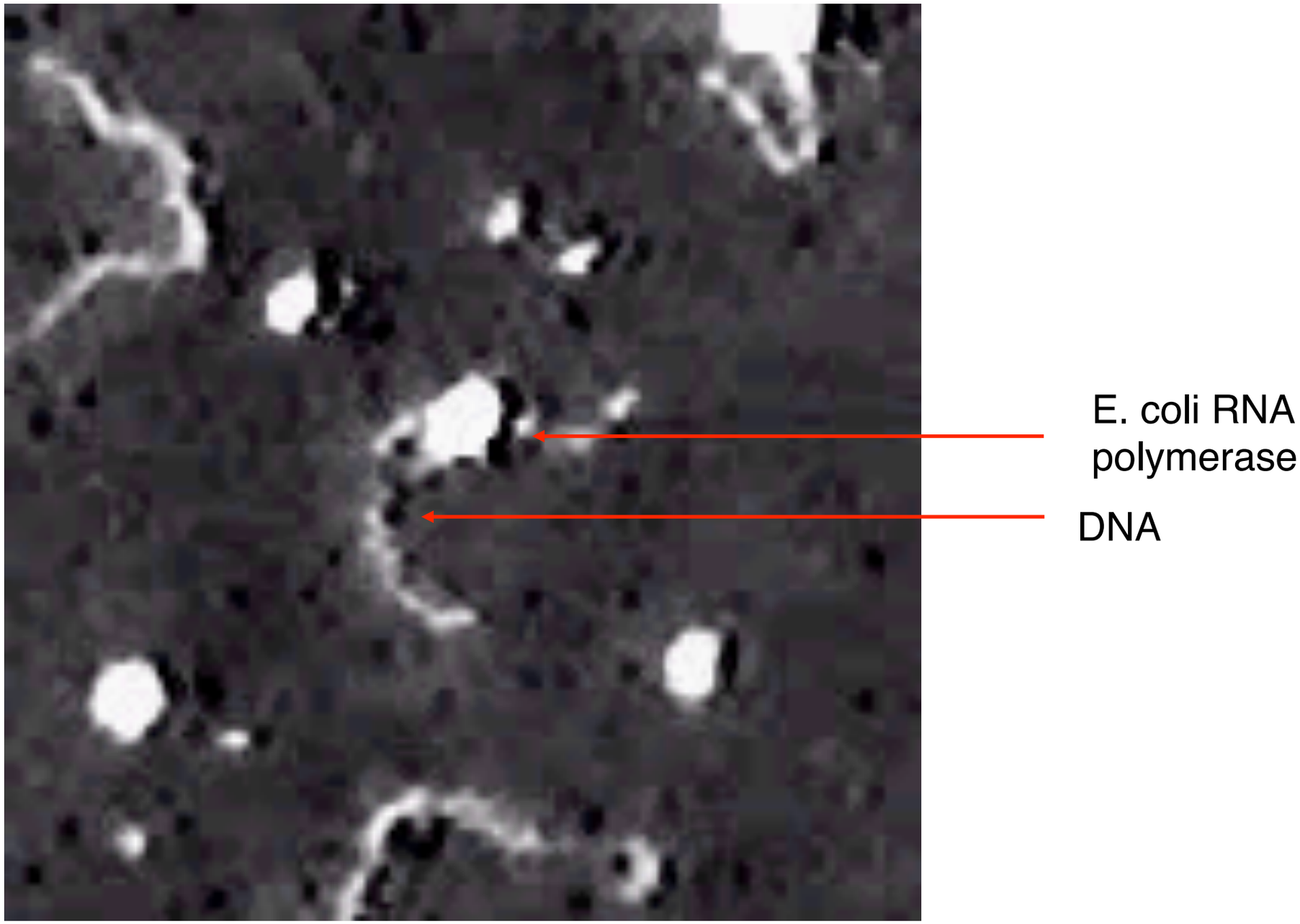
Guthold, M. et al. (1999). Direct observation of one-dimensional diffusion and transcription by escherichia coli RNA polymerase. Biophys J 77, 2284-2294.

Synthesis of RNA by E.coli RNA polymerase immobilized on the surface, Example 1



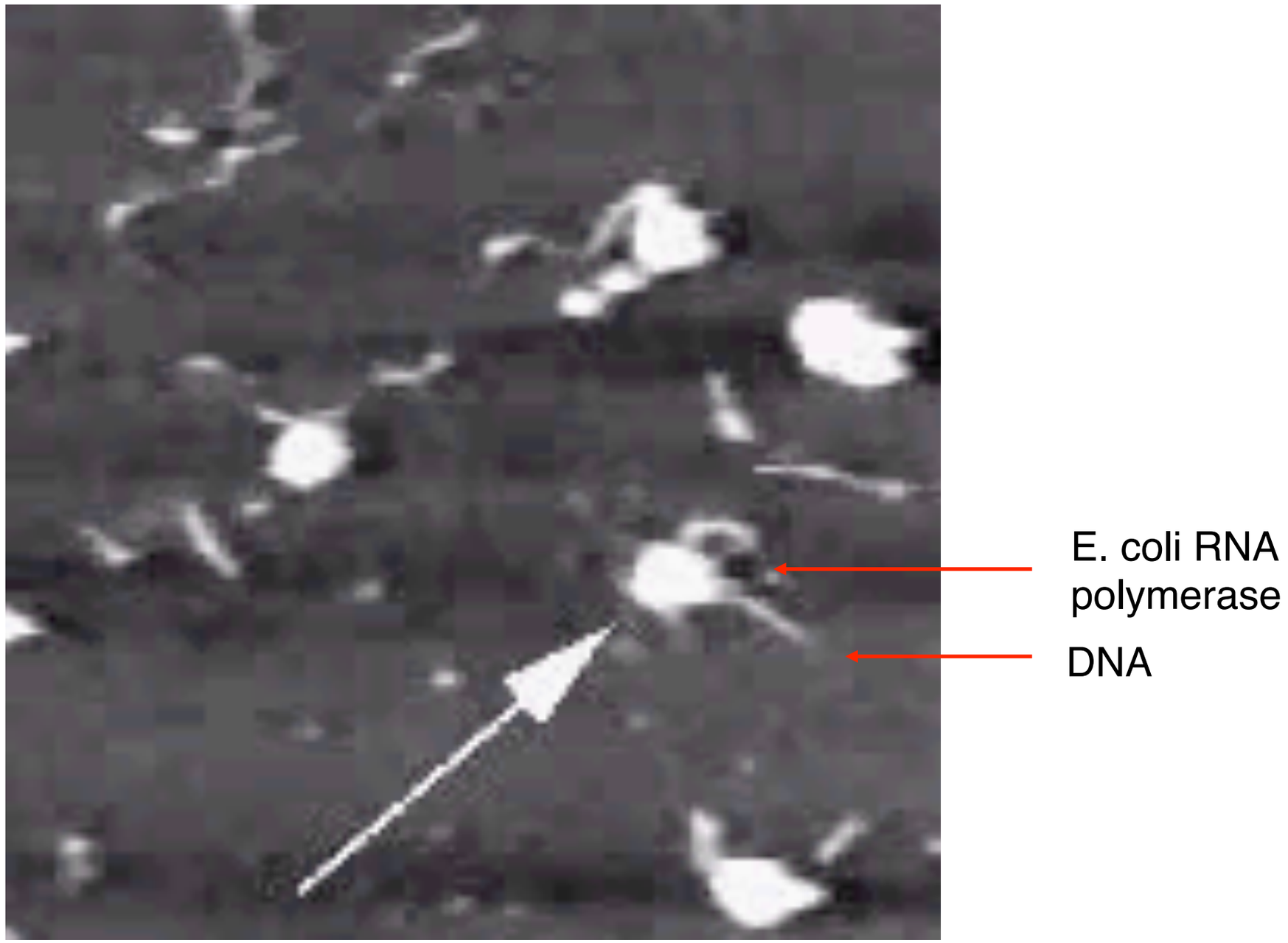
Kasas, Guthold, Bustamante, C.

Synthesis of RNA by E.coli RNA polymerase immobilized on the surface, Example 2



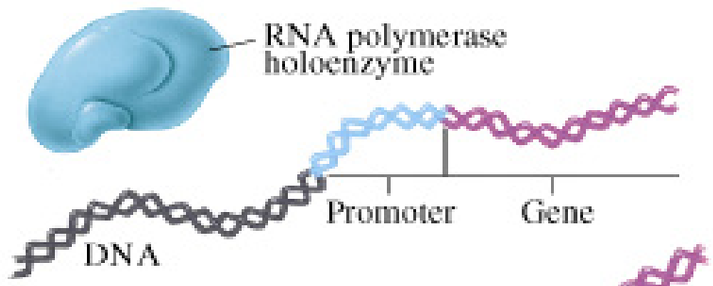
Kasas, Guthold, Bustamante, C.

Synthesis of RNA by E.coli RNA polymerase immobilized on the surface, Example 3



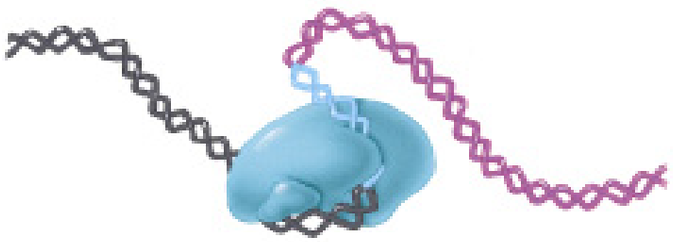
Kasas, Guthold, Bustamante, C.

Different intermediates in the transcription initiation process

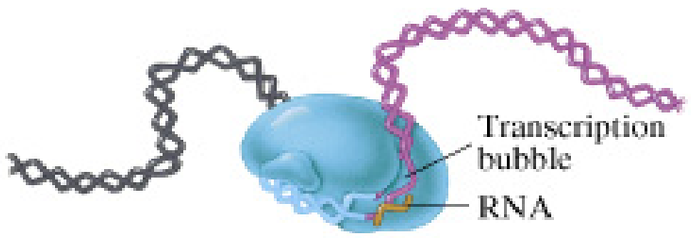


(a) RNA polymerase holoenzyme binds nonspecifically to DNA.

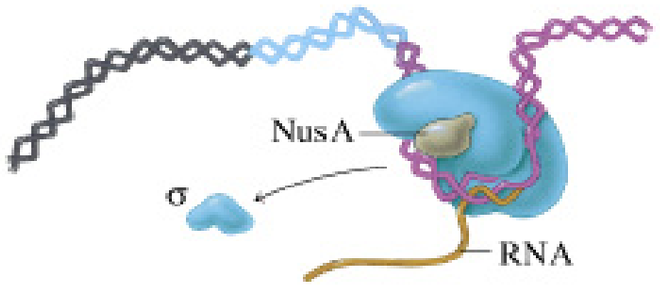
(b) The holoenzyme conducts a one-dimensional search for a promoter.



(c) When a promoter is found, the holoenzyme and the promoter form a closed complex.



(d) A conformational change from the closed complex to an open complex produces a transcription bubble at the initiation site. A short stretch of RNA is then synthesized.

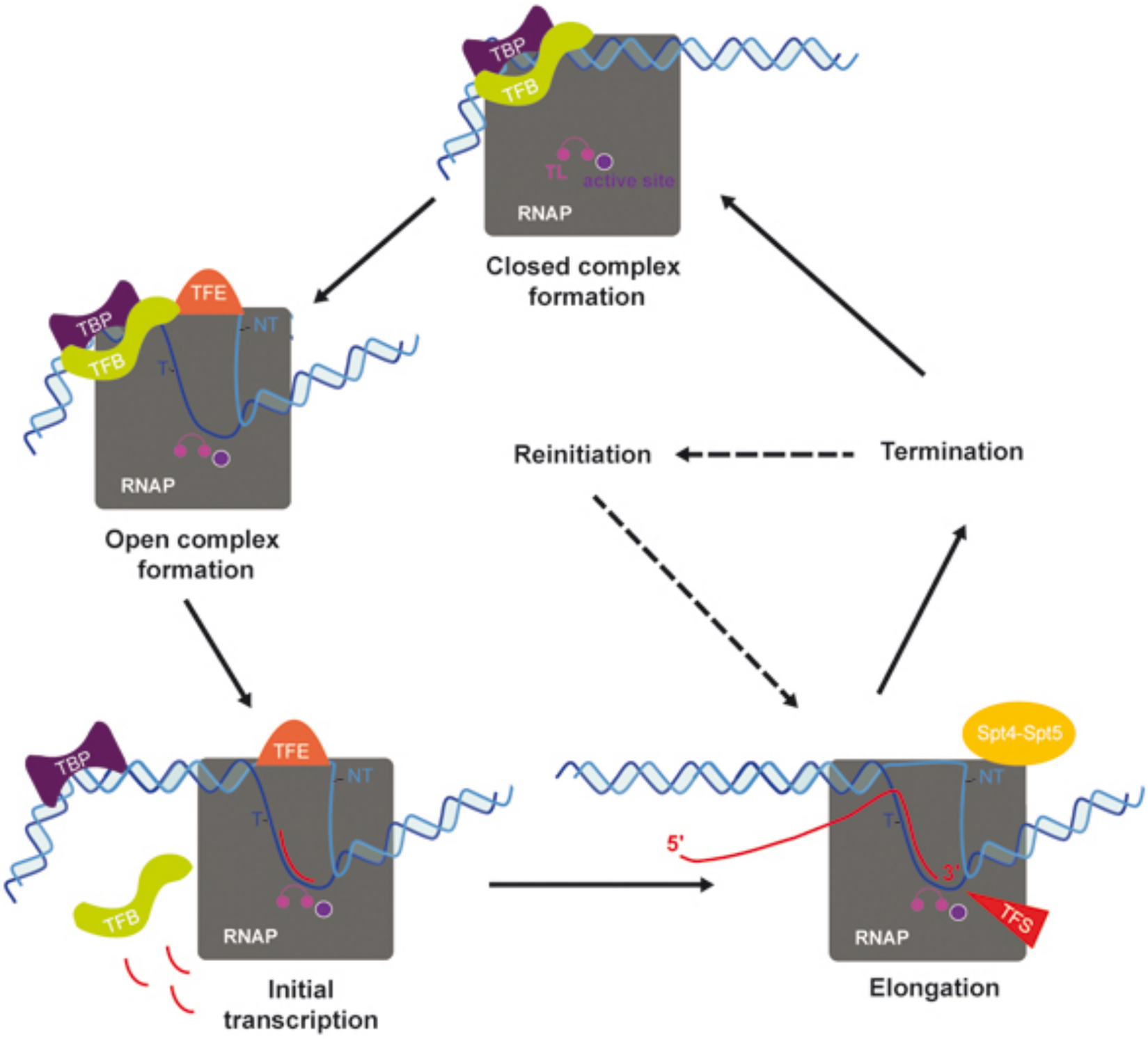


(e) The σ subunit dissociates from the core enzyme, and RNA polymerase clears the promoter. Accessory proteins, including NusA, bind to the polymerase.

Reaction mechanism of transcription by yeast RNA polymerase II

http://www.cramer.genzentrum.lmu.de/assets/Lab-Cramer/Lab-Cramer-Publications/txnmovie.mov

Intermediate steps during transcription that could be rate limiting for gene expression

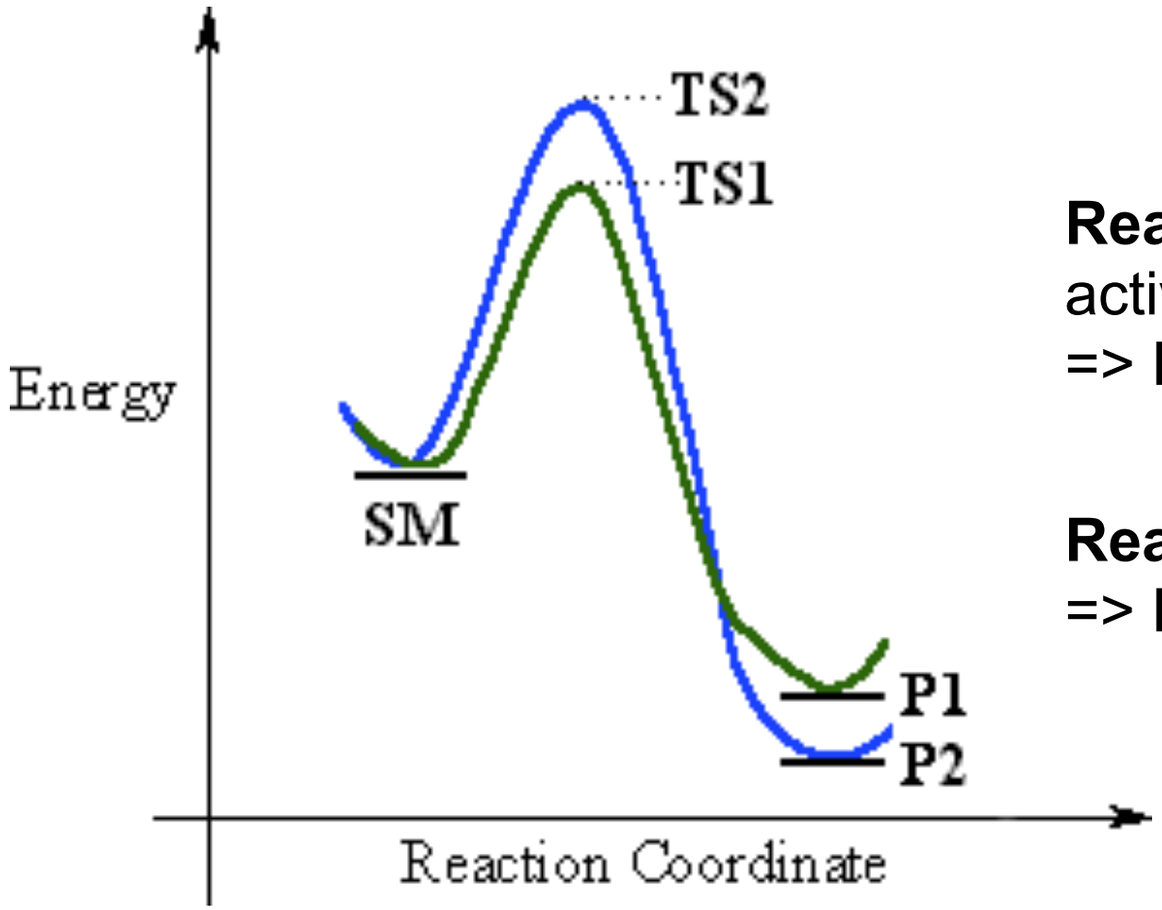


Scheme: Michael Thomm

Why is it important to study kinetics?

a) Thermodynamic versus kinetic control

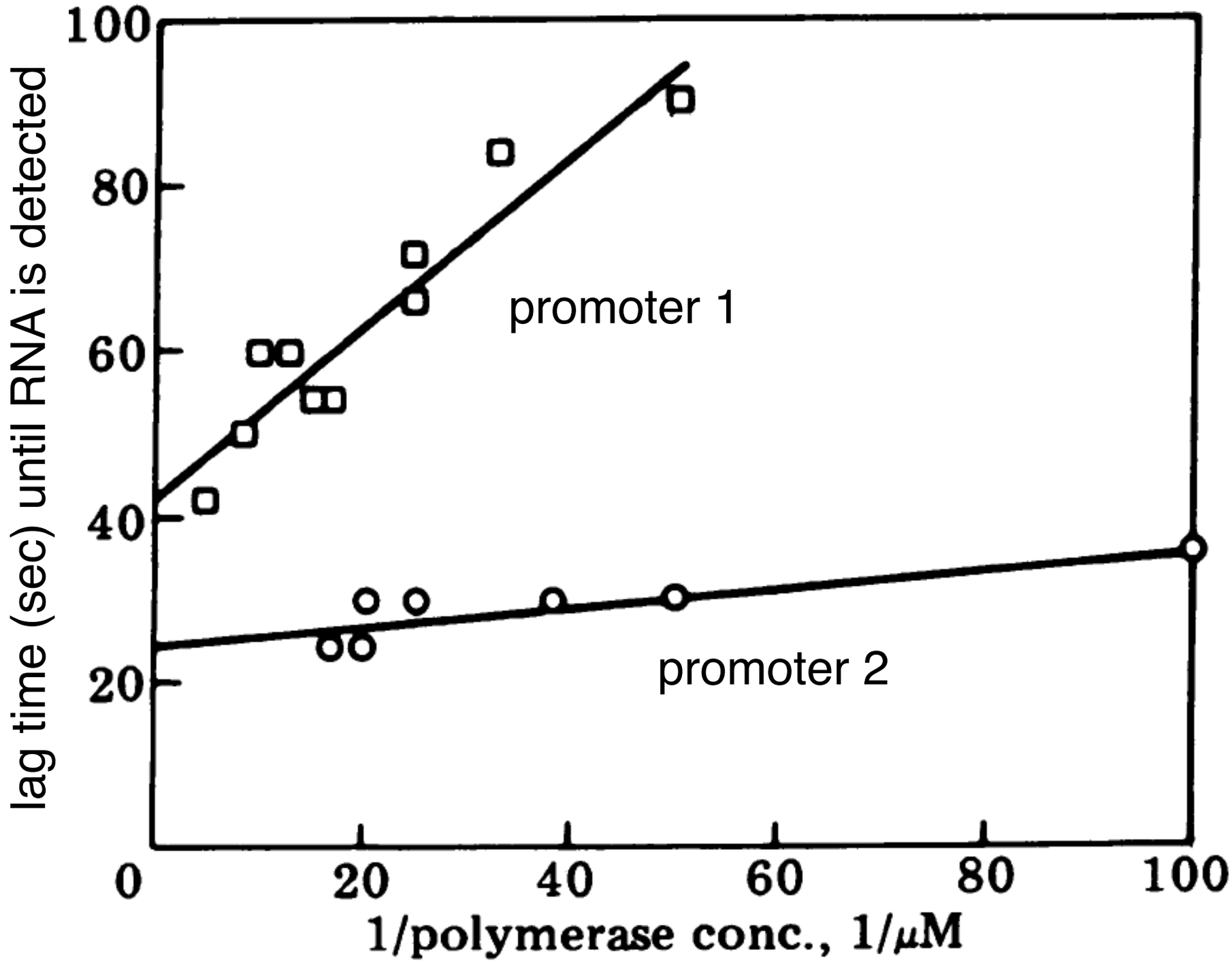
b) Understanding the reaction mechanism



Reaction 1 (**green**) is the *faster reaction* since the activation energy is lower.
=> **P1** is the **kinetic product**.

Reaction 2 (**blue**) generates a more stable product
=> **P2** is the **thermodynamic product**.

Kinetic analysis of two different promoters (McClure)



Calculating reaction kinetics

A very, very simple reaction

$$A \xrightarrow{k_{on}} B$$

k_{on} in s^{-1} is the reaction rate constant

$\frac{1}{k} = \tau$ decay time of the reaction

$$-\frac{d[A]}{dt} = k\,[A]$$

rate equation for decrease of A over time

$$-\frac{d[A]}{[A]} = k\,dt$$

separate variables

$$-\int \frac{1}{[A]}\,d[A] = k\int dt$$

integrate

$$-\ln[A] = kt + \text{constant}$$

succesful integration!

but what about the constant?

A simple reaction, 2nd try

$$-\int_{[A]_0}^{[A]_t} \frac{1}{[A]}\, d[A] = k\int_0^t dt$$

boundary condition: at t = 0

the initial concentration of A is $[A]_0$

we already know the indefinite integral

and we calculate it with our boundaries

$$-\ln[A] = kt + \text{constant}$$

$$-\left(\ln[A]_t - \ln[A]_0\right) = kt - k0 \quad \text{and} \quad -\ln[A]_t = kt - \ln[A]_0$$

$$\ln\left(\frac{[A]_t}{[A]_0}\right) = -kt \quad \text{and} \quad [A]_t = [A]_0\, e^{-kt}$$

hurray!

Irreversible bimolecular reaction

$$A + B \xrightarrow{k_{on}} AB$$

k_{on} in $M^{-1}\ s^{-1}$ or $\frac{\text{liter}}{\text{mol s}}$

$$\frac{d[AB]}{dt} = k_{on} \cdot [A] \cdot [B]$$

separating three variables is not good...

$$x = \left([A]_0 - [A]_t\right) = \left([B]_0 - [B]_t\right)$$

but we can do a trick...

$$\frac{1}{\left([A]_0 - [B]_0\right)} \ln\left(\frac{[B]_0[A]_t}{[A]_0[B]_t}\right) = kt$$

to get something useful

Reversible bimolecular reaction

$$\mathrm{AB} \underset{k_{\mathrm{on}}}{\overset{k_{\mathrm{off}}}{\rightleftarrows}} \mathrm{A+B}$$

k_{off} in s^{-1} is the reaction rate constant for dissociation

k_{on} in $M^{-1}\ s^{-1}$ is the reaction rate constant for binding

$$\frac{k_{\mathrm{off}}}{k_{\mathrm{on}}} = K_{\mathrm{d}}$$

relation to the equilibrium dissociation constant

$$\frac{1}{k_{\mathrm{off}}} = \tau$$

decay time of the complex

$$\frac{d[\mathrm{AB}]}{dt} = \mathrm{k_{on}} \cdot [\mathrm{A}] \cdot [\mathrm{B}] - \mathrm{k_{off}} \cdot [\mathrm{AB}]$$

rate equation for complex formation,
can be solved but it is already difficult

The “simple” Michaelis-Menten reaction

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} \mathrm{ES} \underset{k_{-2}}{\overset{k_{+2}}{\rightleftarrows}} \mathrm{E} + \mathrm{P}$$

$e_0 - x \qquad s_0 - x - p \qquad x \qquad p$

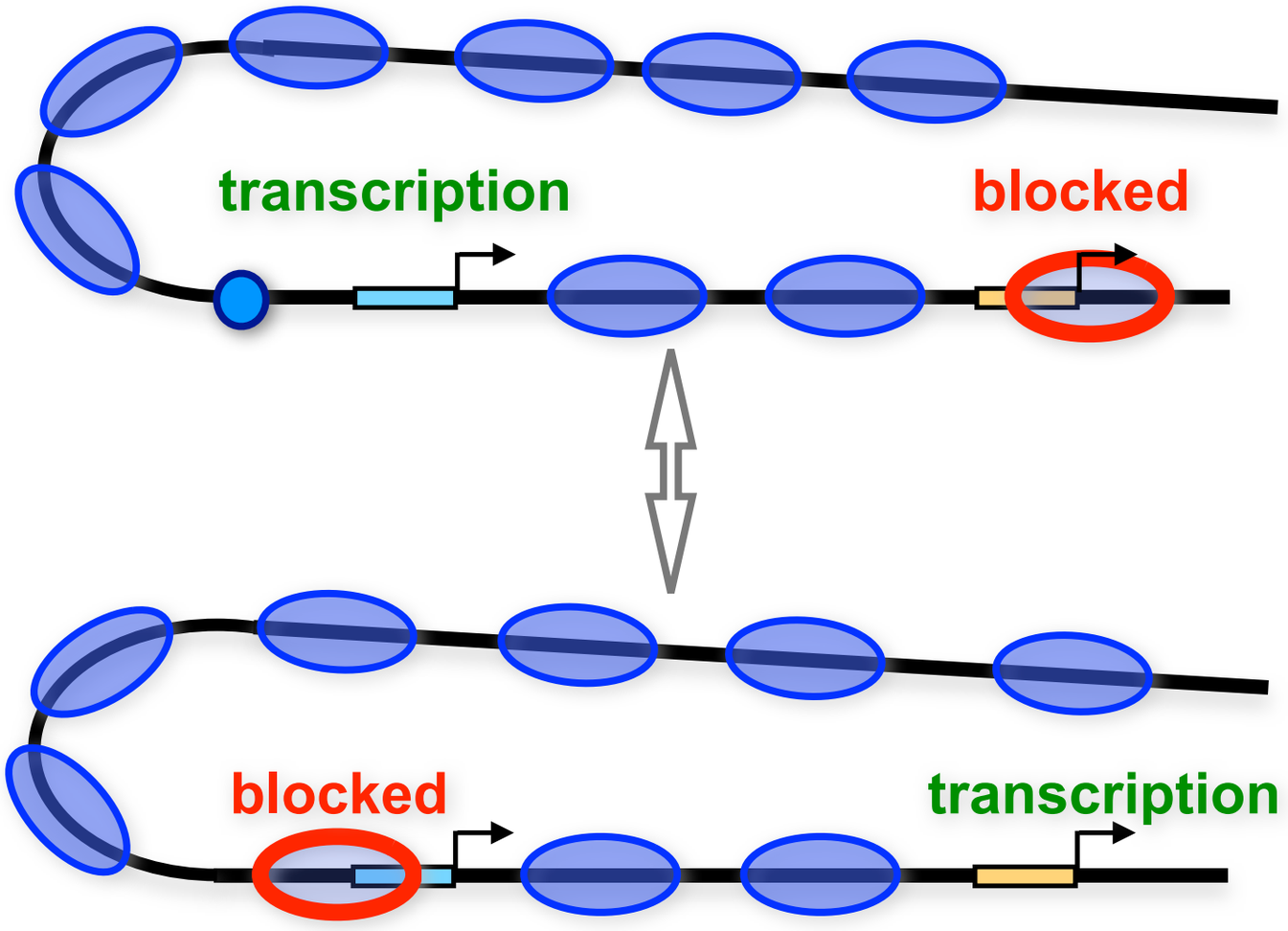
$$\frac{dx}{dt} = k_{+1} \cdot (e_0 - x) \cdot (s_0 - x - p) - k_{-1} \cdot x - k_{+2} \cdot x + k_{-2} \cdot (e_0 - x) \cdot p$$

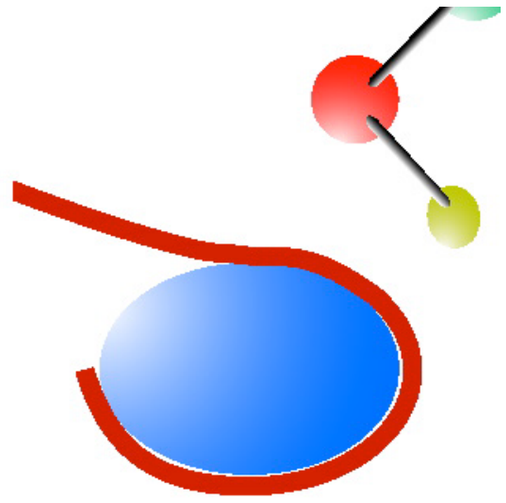
$$\frac{dp}{dt} = k_{+2} \cdot x - k_{-2} \cdot (e_0 - x) \cdot p$$

The second equation can be used to express x and dx/dt in dependence of p and dp but the resulting equation has no solution in p and t

⇒ simplifications like s0 >> e0 or dx/dt = constant

But can you calculate how ATP consuming chromatin remodeling complexes translocate nucleosomes?

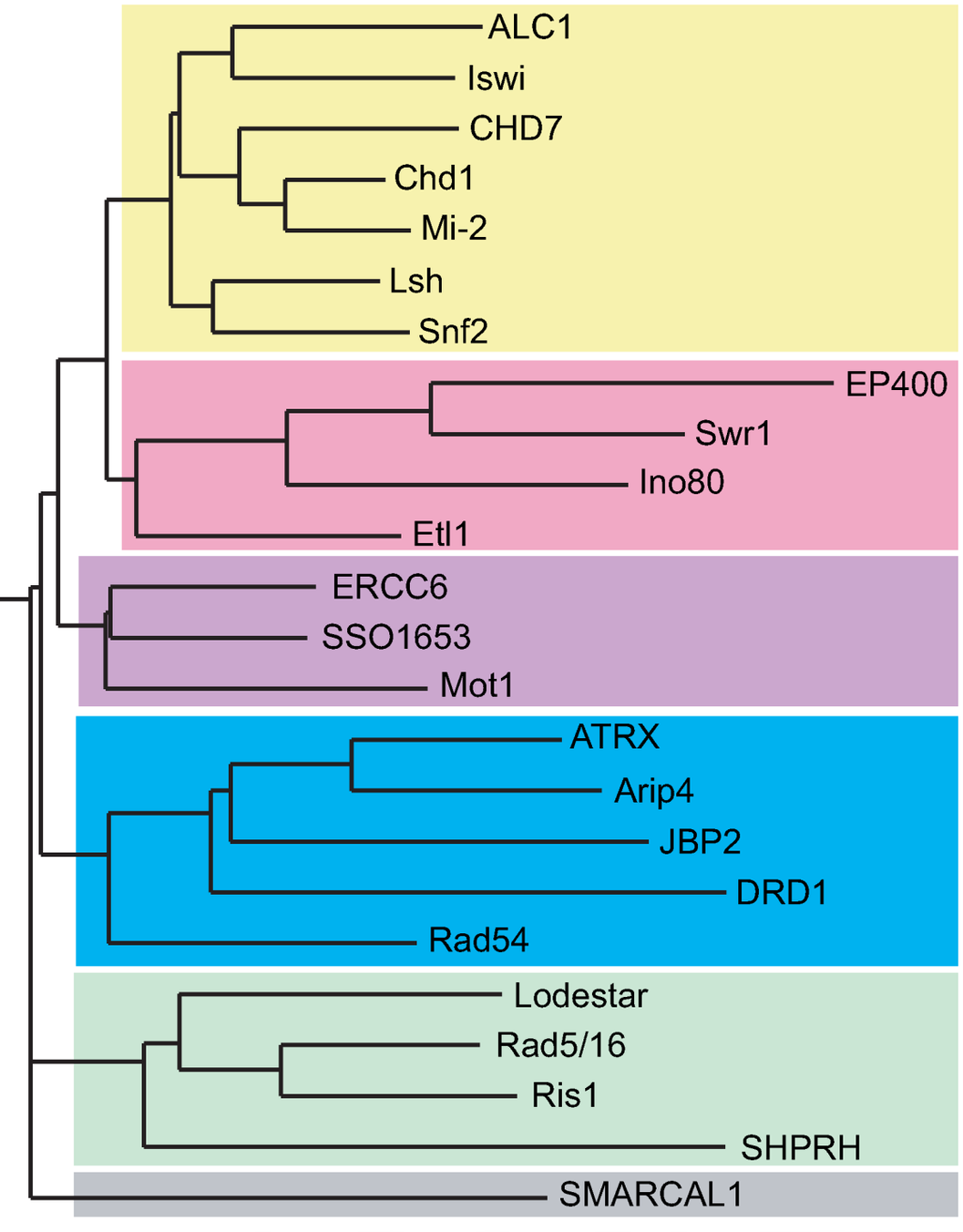




- ATP hydrolysis *in vitro*: 2-5 s
- nucleosome translocation (3-5 bp) *in vitro*: ~20 s

Chromatin remodeling complexes are diverse and abundant

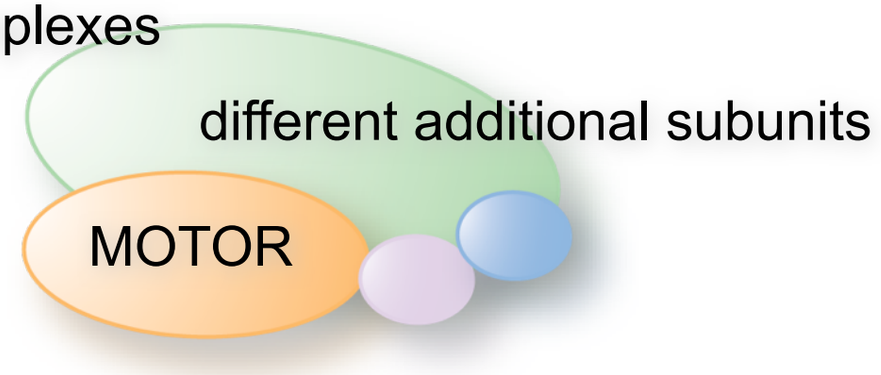
ATPase subfamilies with many members



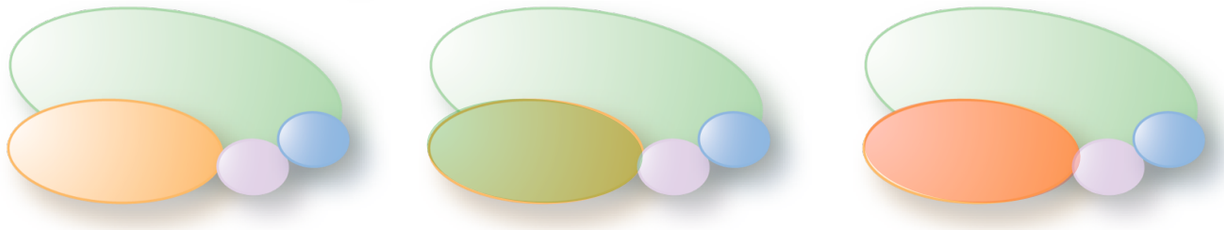
adapted from Owen-Hughes, NAR 2006

Diversity of chromatin remodeling complexes

Different complexes



Motor exchange within the same ATPase subfamily



Splice variants of complex subunits

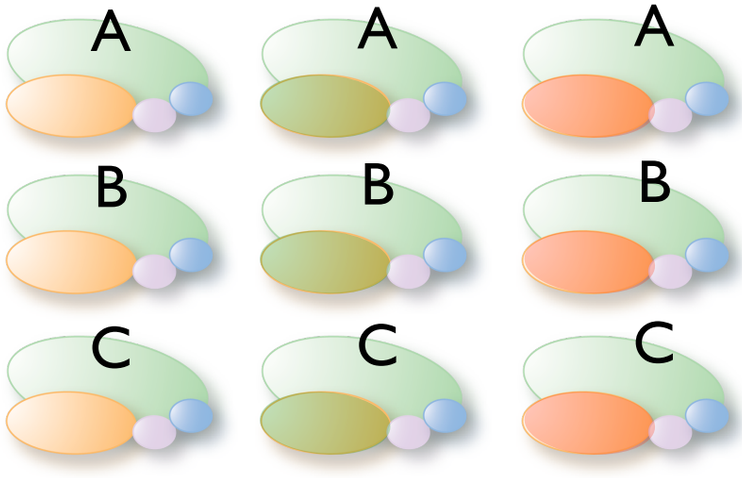
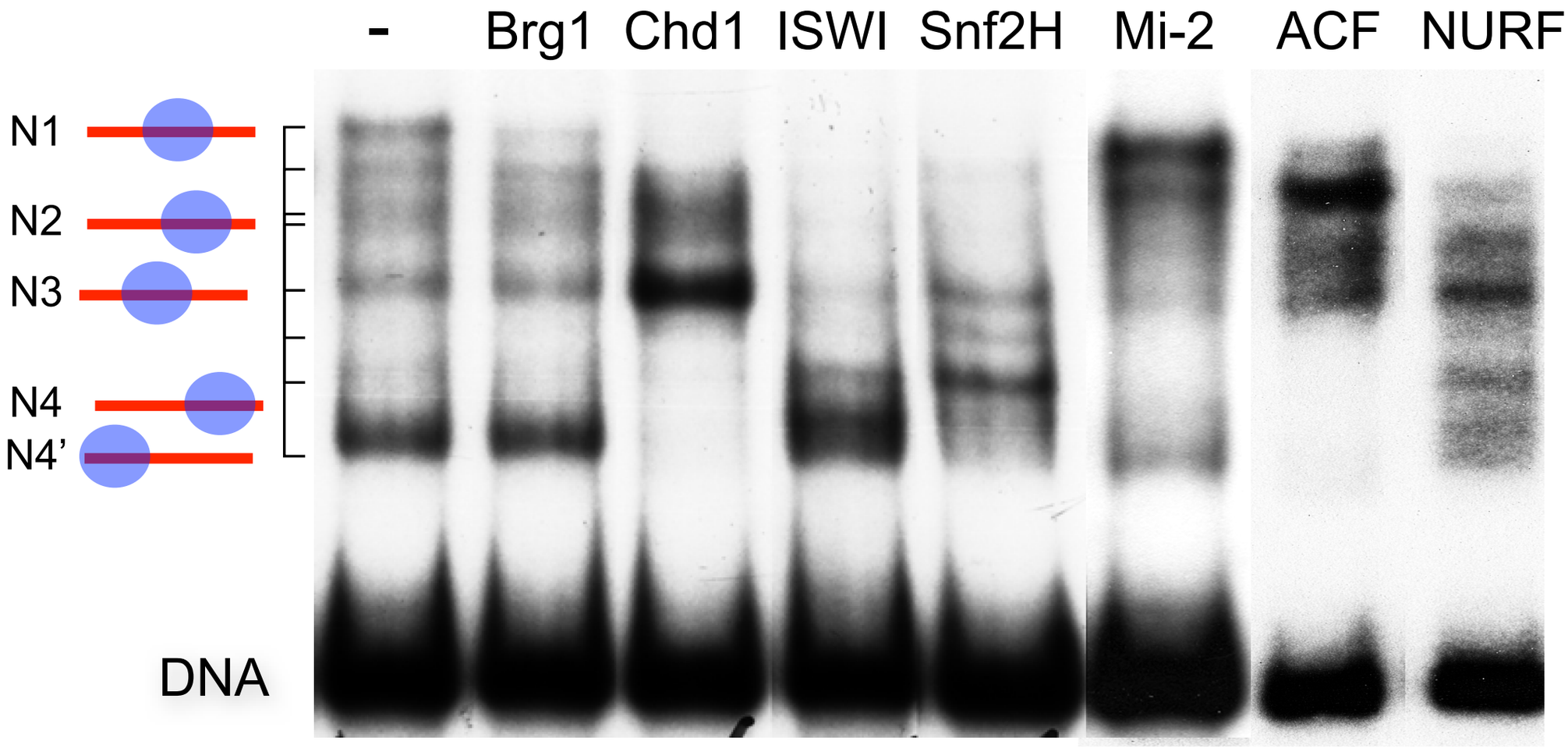


Figure by Gernot Längst

Different chromatin remodeler position nucleosomes to different sites on the same substrate (hsp70 promoter)



Rippe, Schrader, Riede, Strohner, Lehmann & Längst (2007). *PNAS* **104**, 15635-15640

Nucleosome translocation as a Michaelis-Menten reaction

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} \mathrm{ES} \xrightarrow{k_{+2}(\text{or } k_{\mathrm{cat}})} \mathrm{E} + \mathrm{P}$$

E: enzyme = remodeler
S: substrate = nucleosome at initial position
P: product = translocated nucleosome
P could sever as the substrate for a new translocation cycle

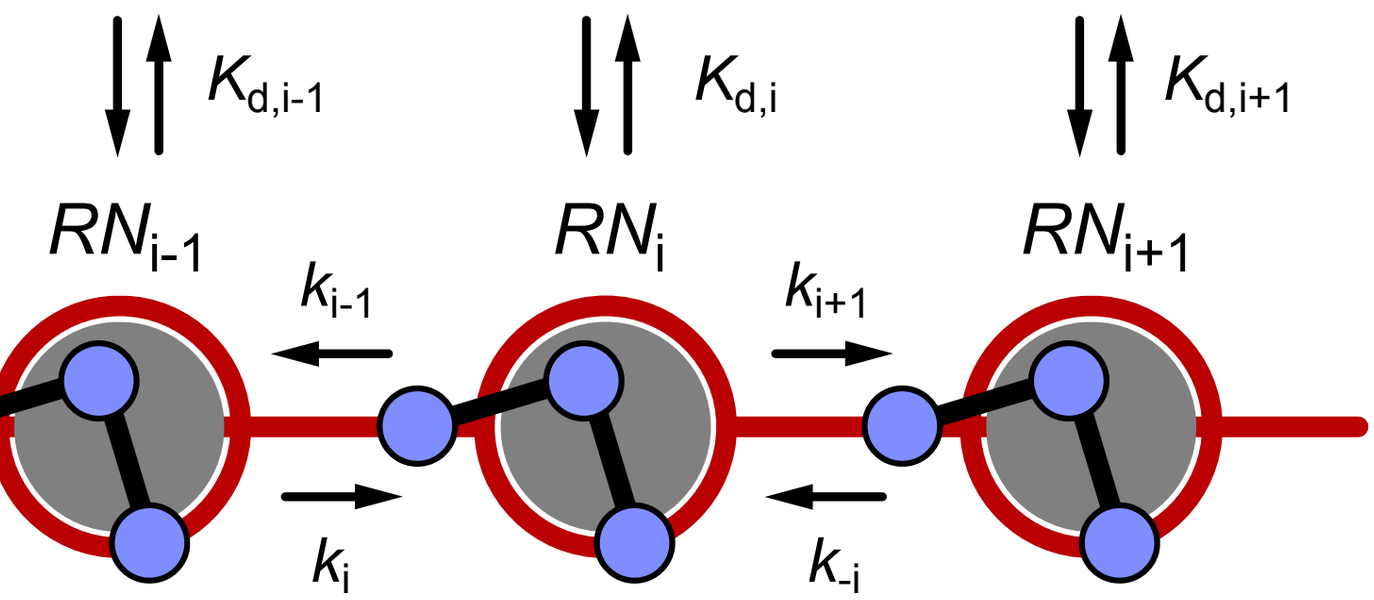
$$K_{\mathrm{M}} = \frac{k_{-1}+k_{+2}}{k_1} = \frac{\text{dissociation rates of ES}}{\text{formation rate of ES}} \left(\text{in } \frac{\text{mol}}{\text{liter}} \right)$$

concentration of substrate at which half the active sites of the enzyme are filled

$$\text{"reaction efficiency"} = \frac{k_{\mathrm{cat}}}{K_{\mathrm{M}}} = \frac{\text{catalysis rate}}{\text{binding site saturation}}$$

high k_{cat} = good catalysis rate
low K_{M} = good binding of substrate to enzyme

Copasi (www.copasi.org) to the rescue: Numerical simulations of binding kinetics (Question 2 and 3)



Standard conditions

$k_{on,i-1} = k_{on,i} = k_{on,i+1} = 10^8\ M^{-1}{\cdot}s^{-1}$

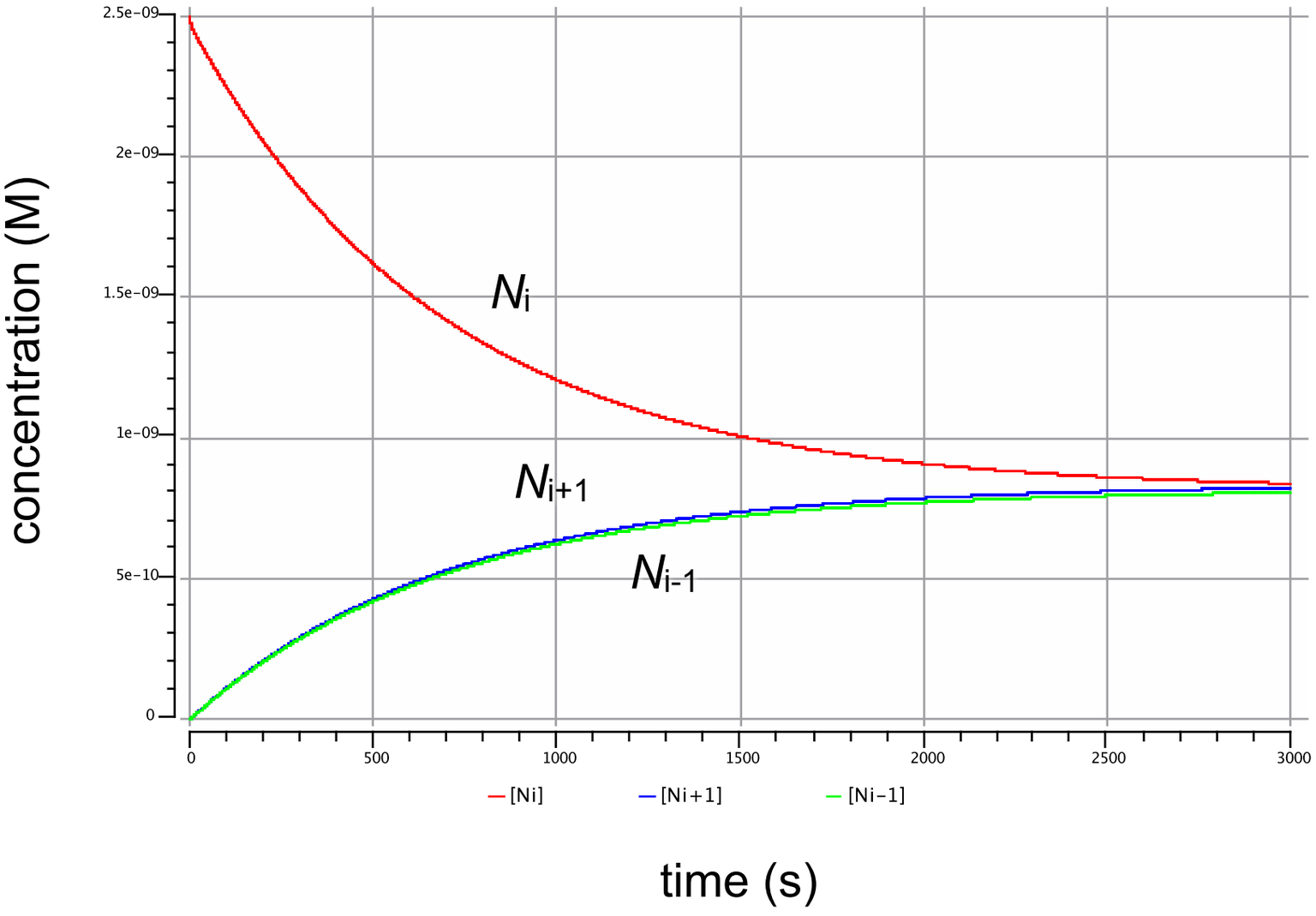
$k_{off,i-1} = k_{off,i} = k_{off,i+1} = 0.1\ s^{-1}$

$K_d = k_{off}/k_{on} = 10^{-9}\ M$

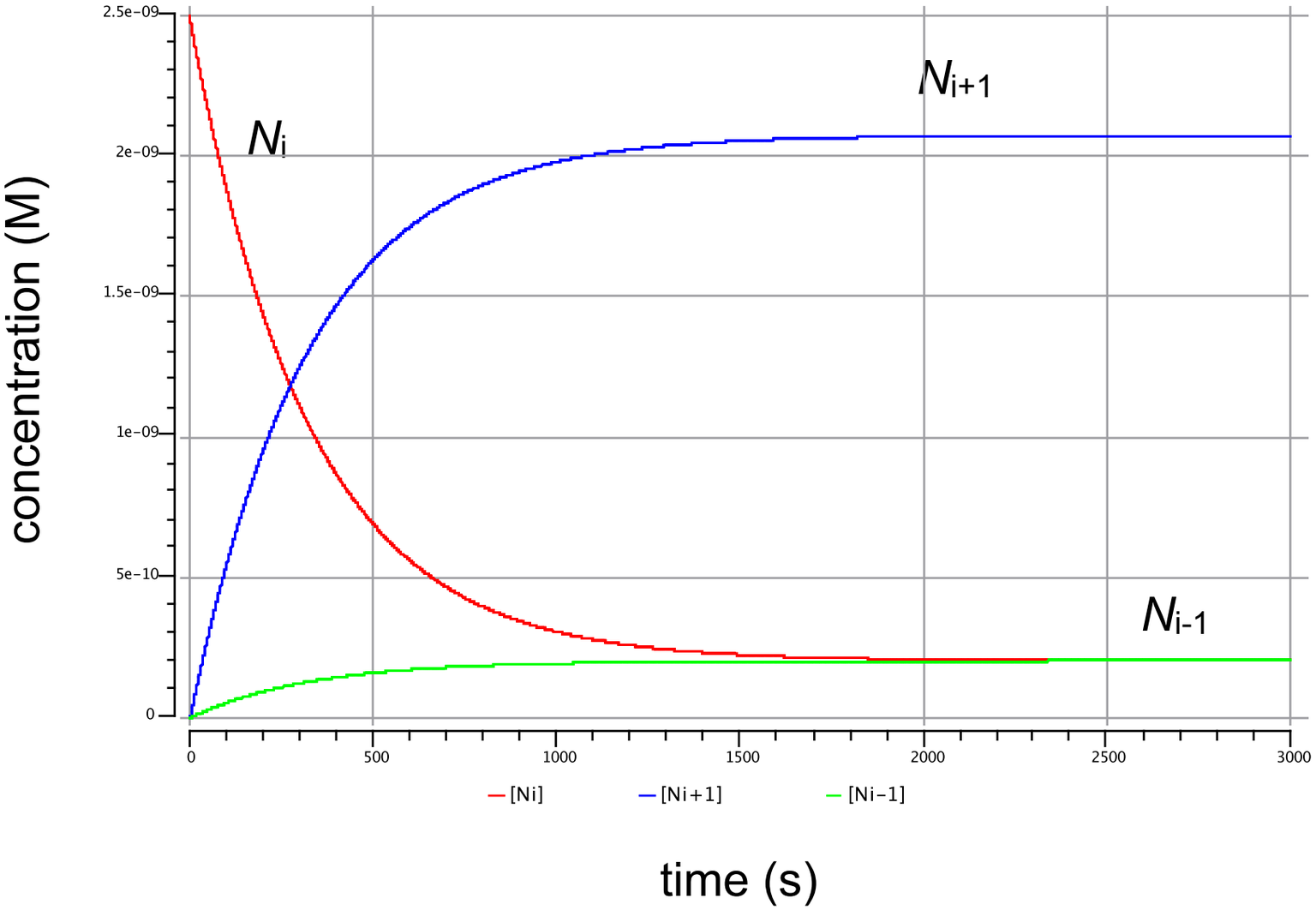
$k_{i-1} = k_{i+1} = k_{-i} = k_i = 1\ s^{-1}$

$N_i = 2.5{\cdot}10^{-9}\ M \quad R = 5{\cdot}10^{-11}\ M$

Standard conditions: k_{off} = 0.1 s^{-1}



10x reduced binding affinity at N_{i+1}: $k_{off,i+1}$ = 1 s^{-1}



this works perfect and is beautifully simple

Karsten's good and bad substrate model for nucleosome translocation

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} \mathrm{ES} \xrightarrow{k_{+2}(\text{or } k_{\mathrm{cat}})} \mathrm{E} + \mathrm{P}$$

$$\text{"reaction efficiency"} = \frac{k_{\mathrm{cat}}}{K_{\mathrm{M}}} = \frac{\text{catalysis rate}}{\text{binding site saturation}}$$

good nucleosome substrates:

- high remodeler binding affinity (= low K_M)
- high translocation rate away from this position (= high k_{cat})

=> high k_{cat}/K_M

bad nucleosome substrates:

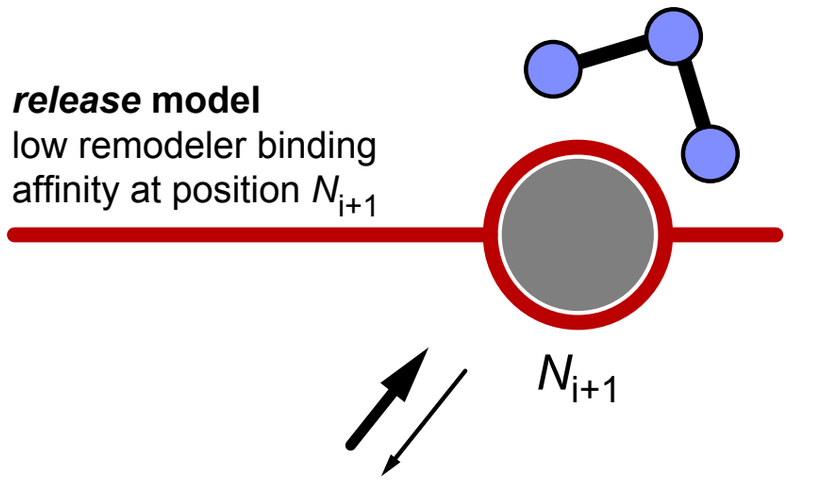
- low substrate binding affinity (= high K_M)
- low translocation rate away from this position (= low k_{cat})

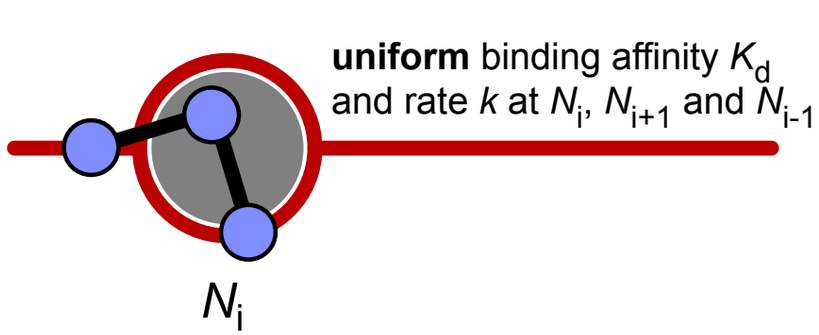
=> low k_{cat}/K_M

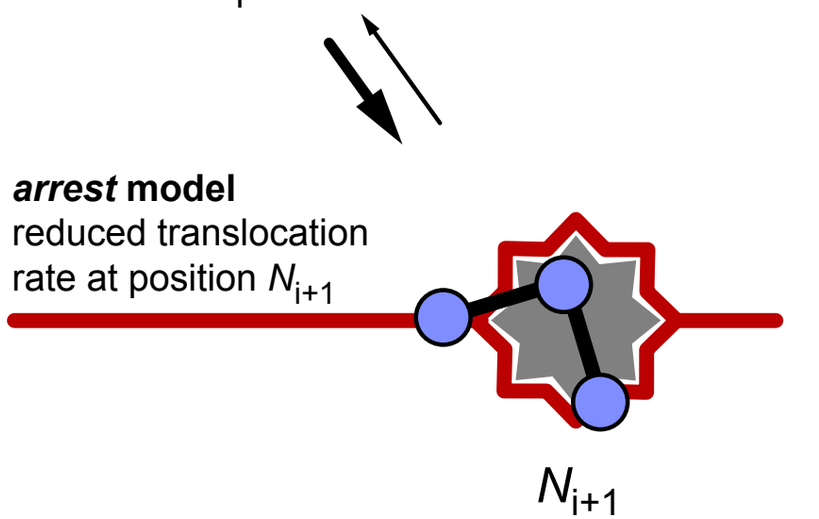
Hypothesis:

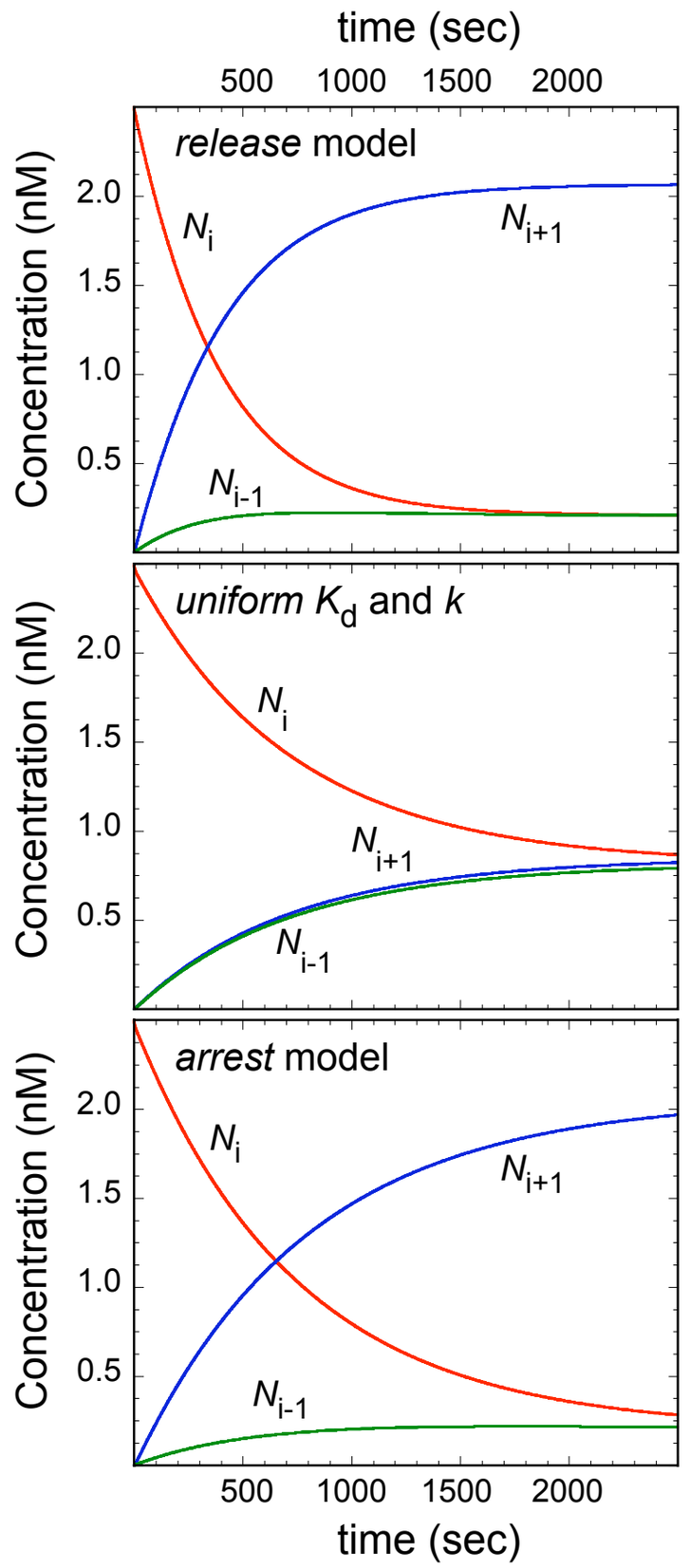
The remodeler move good substrate nucleosomes (high k_{cat}/K_M) to positions where they are bad substrates (low k_{cat}/K_M)

Two mechanisms to









Finding a nucleosme substrate: 3D search versus 1D sliding along the DNA