Single Molecule Techniques &
Single Molecule Biological Physics

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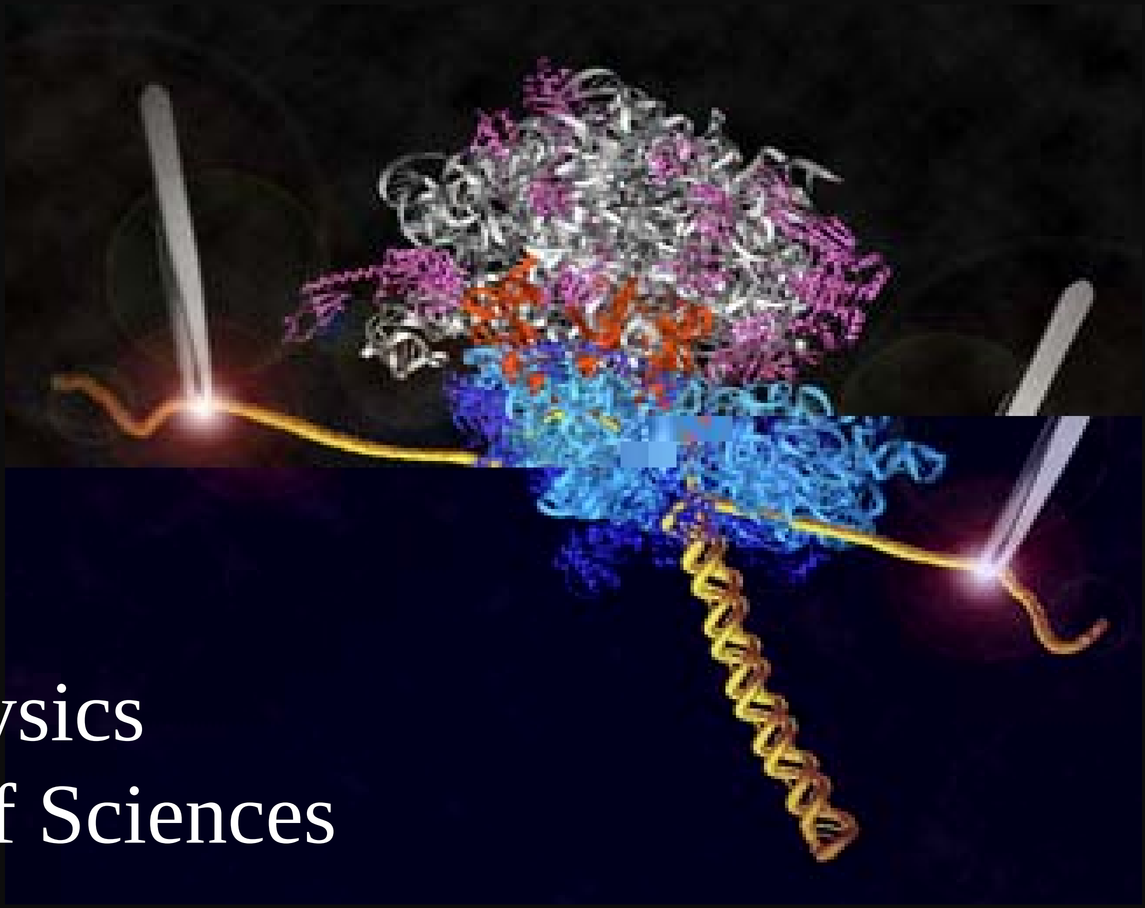

Institute of Physics
Chinese Academy of Sciences

Why bio? And how?

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(Cambridge, and Bentley House, London)
Agents for Canada and India: Macmillan

WHAT IS LIFE?

The Physical Aspect of the Living Cell

BY

ERWIN SCHRÖDINGER
SENIOR PROFESSOR AT THE DUBLIN INSTITUTE FOR ADVANCED STUDIES

Based on Lectures delivered under the auspices of the Institute at Trinity College, Dublin, in February 1943

CAMBRIDGE
AT THE UNIVERSITY PRESS
1948

$$ih\frac{\partial\psi}{\partial t} = E\psi$$

...

The Cell Is a Collection of Protein Machines.

B. Alberts, Cell <u>92</u>, 291-294 (1998)

...

It has become customary to explain molecular mechanisms through simple cartoons.

BUT fully understanding the mechanism will require returning to where the studies of DNA first began — in the realms of chemistry and physics.

B. Alberts, Nature 421, 431-435 (2003)

Bruce Alberts, biologist, Editor-in-Chief of Science

Watson & Crick

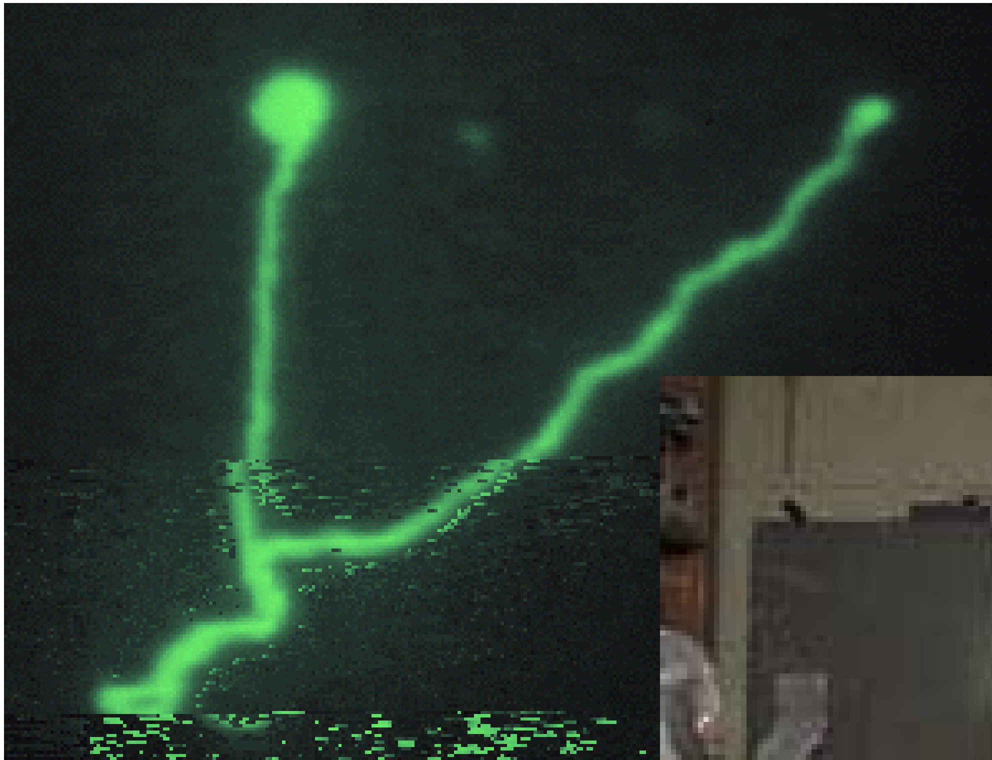
Feynman's suggestions….

"It is very easy to answer many of these fundamental **biological** questions; you just look at the thing!"

from R. P. Feynman's talk

There's plenty of room at the bottom. Dec. 29th, 1959

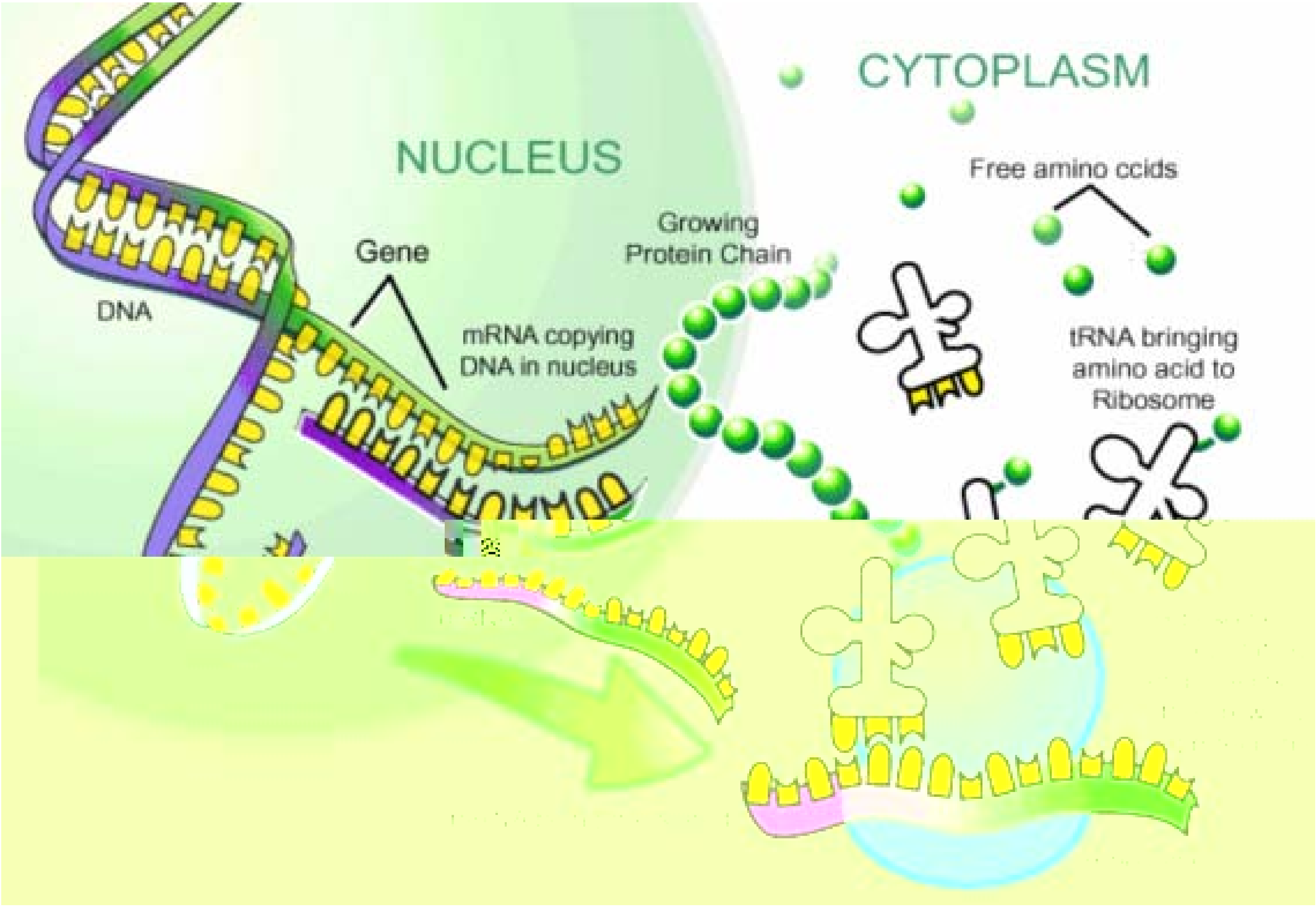
Steven Chu's Action



Other solutions:
Watching the action of a molecule!

Why single molecule?

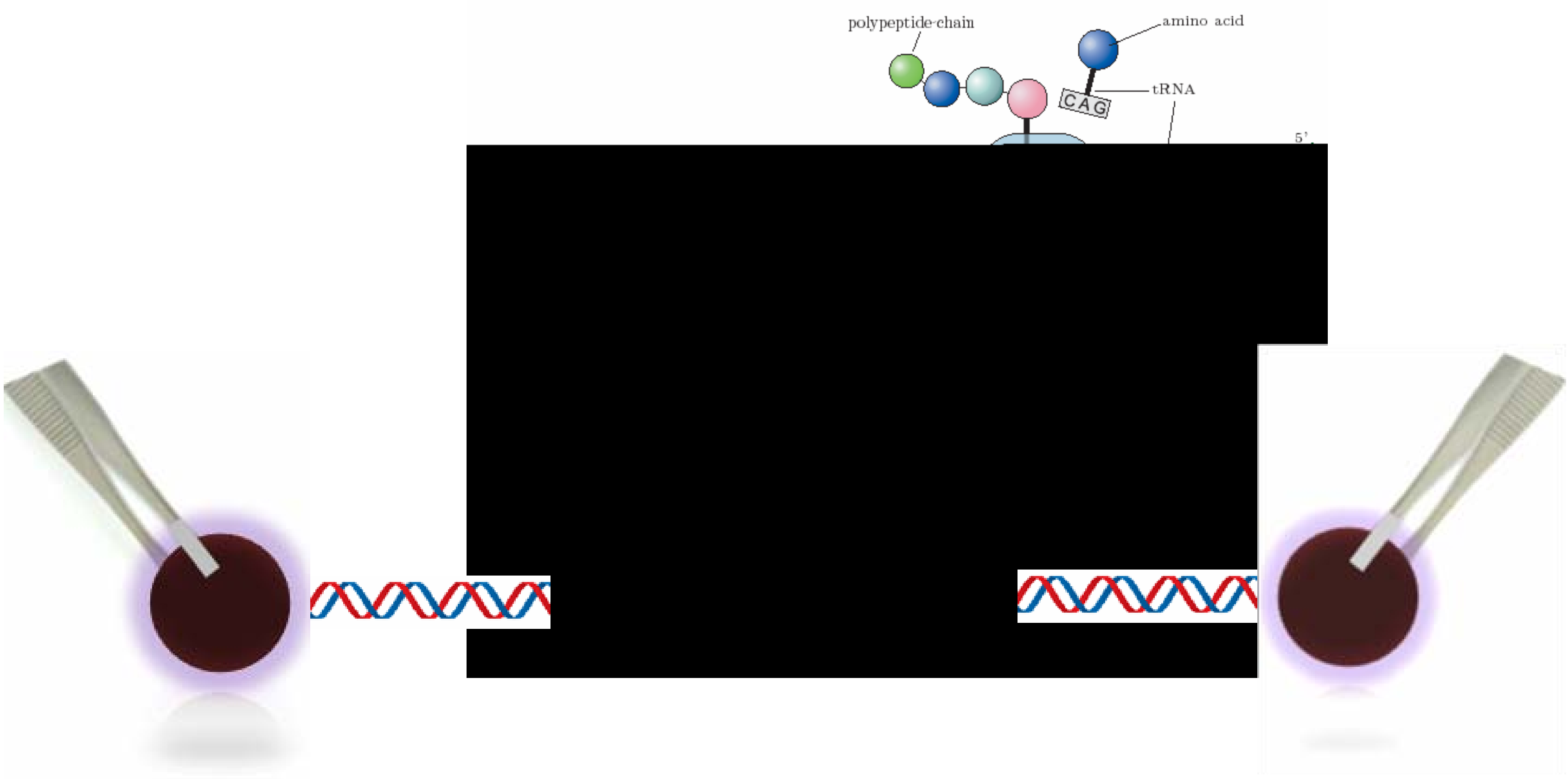
People are used to thinking about biological problems in a single molecular way.



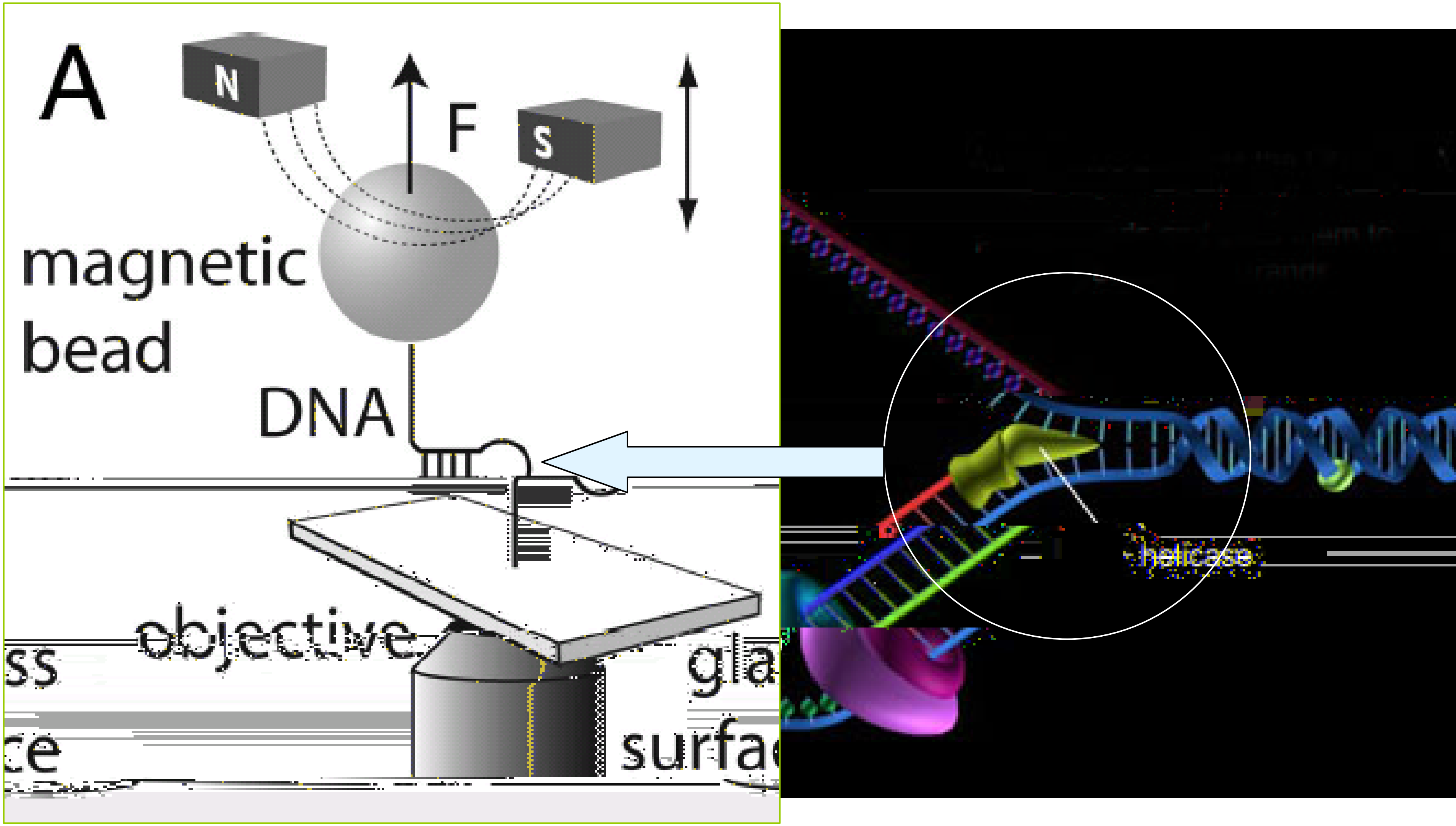
The common language for physicists and biologists.

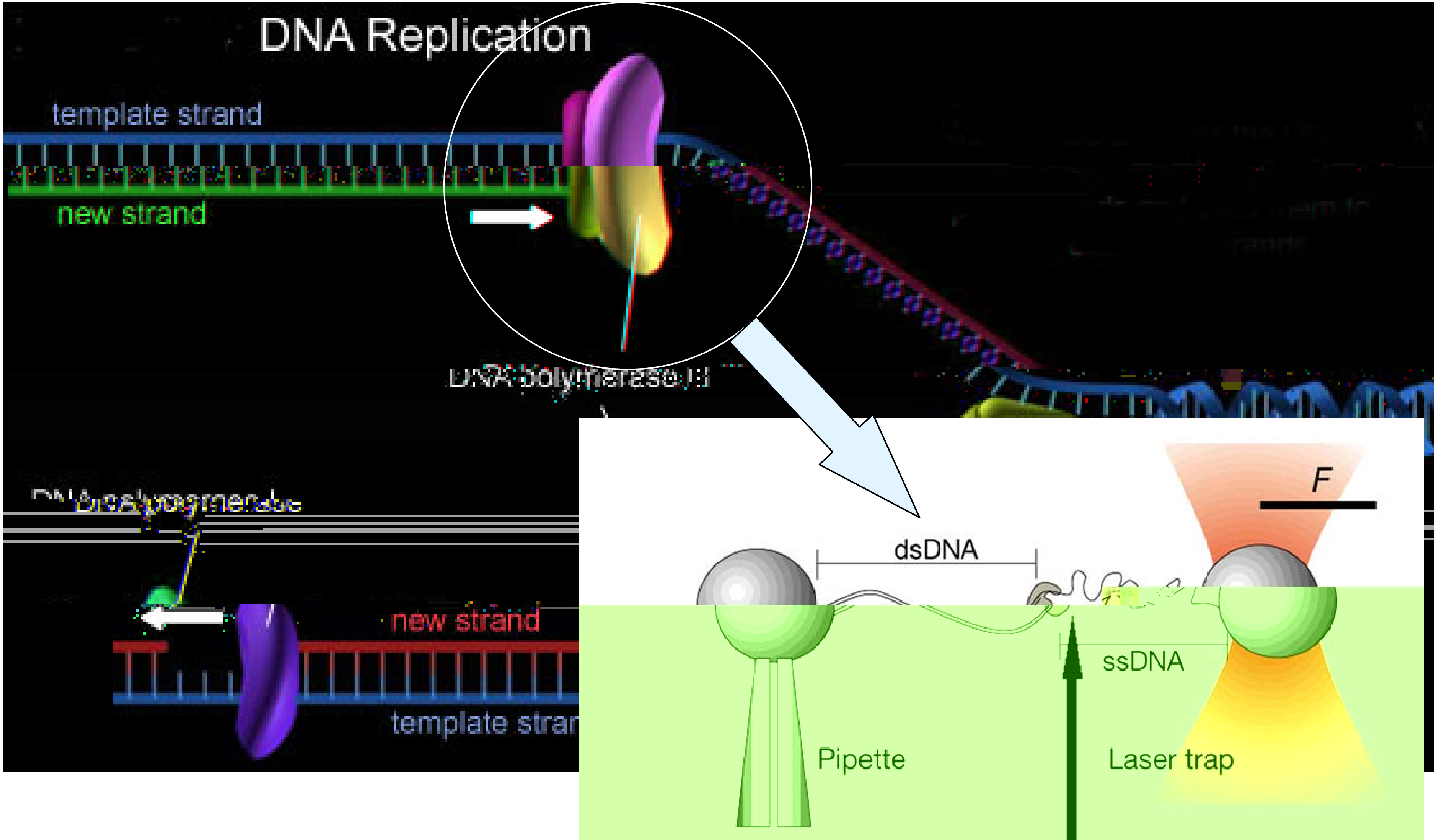
The single molecule roadmap towards quantitative life science.

A simplified view of the basic life processes



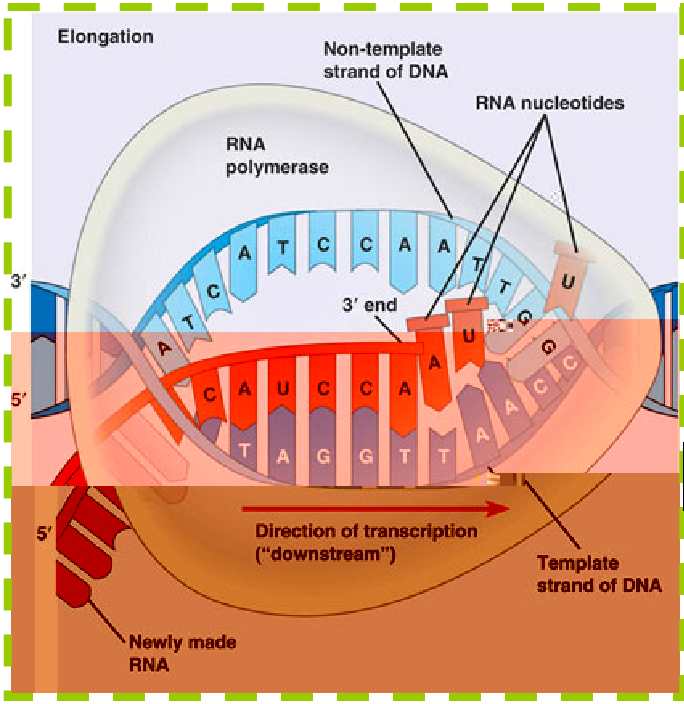
The 2 strands of a DNA must be separated in order for the genes to be duplicated.

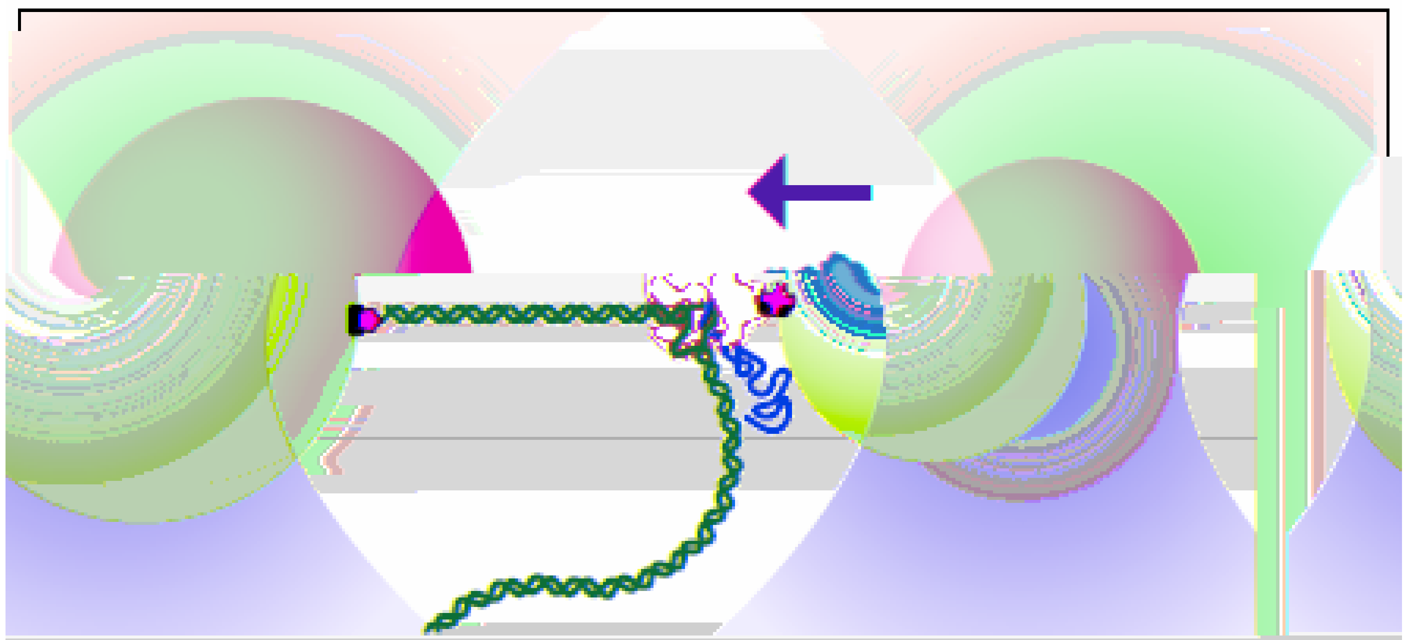


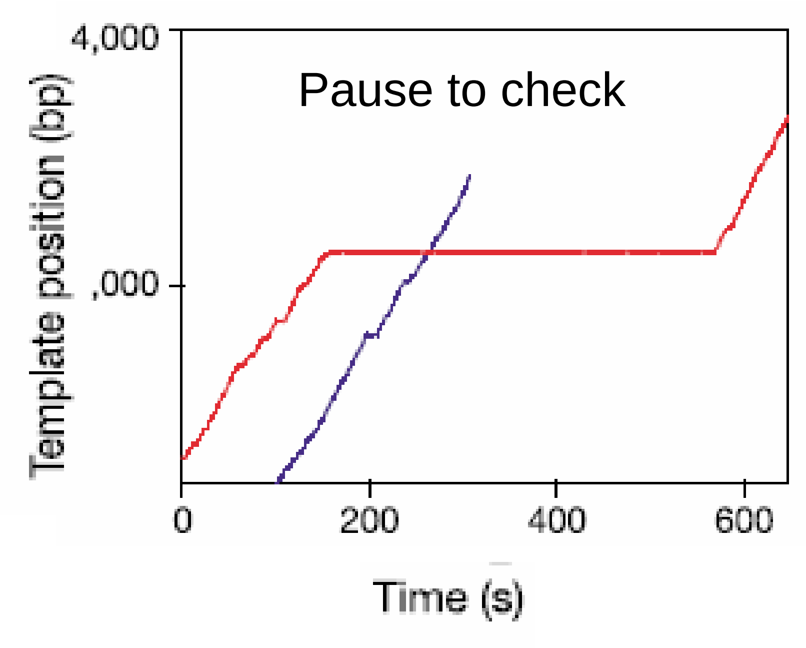


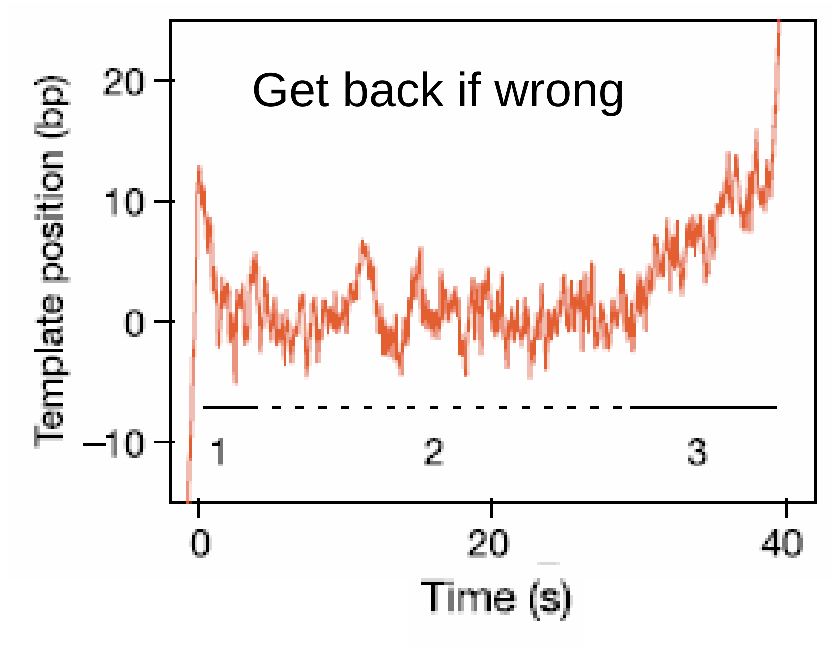
After the separation, there comes the synthesis of new ssDNA, using a ssDNA as template…

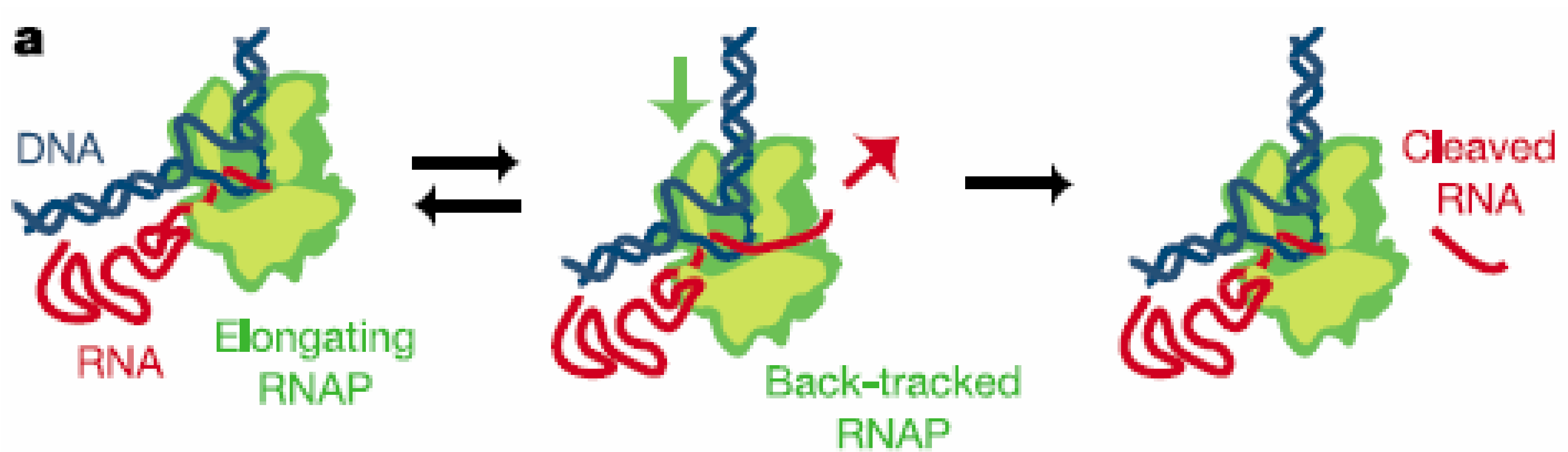
....Then comes the production of mRNA





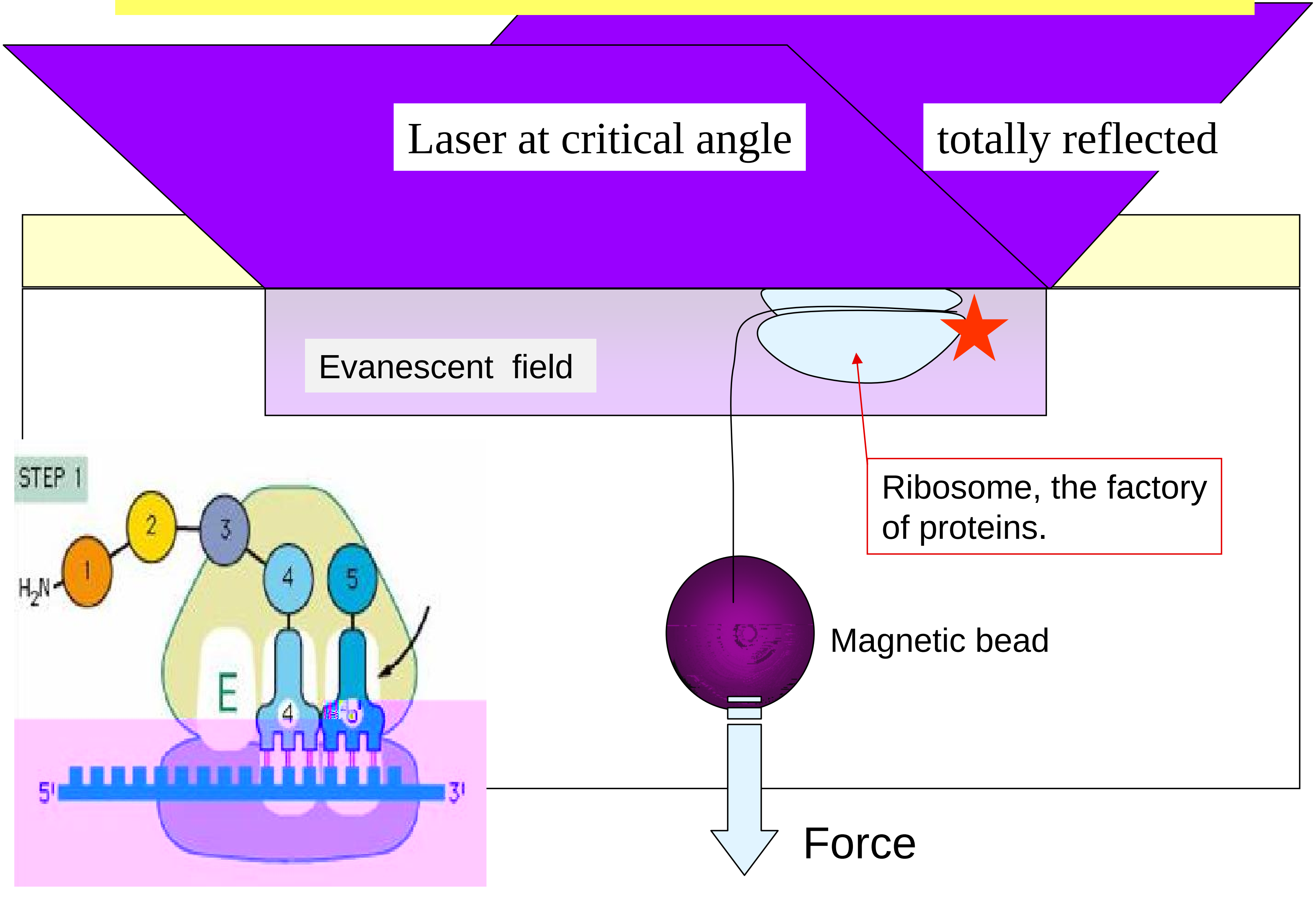


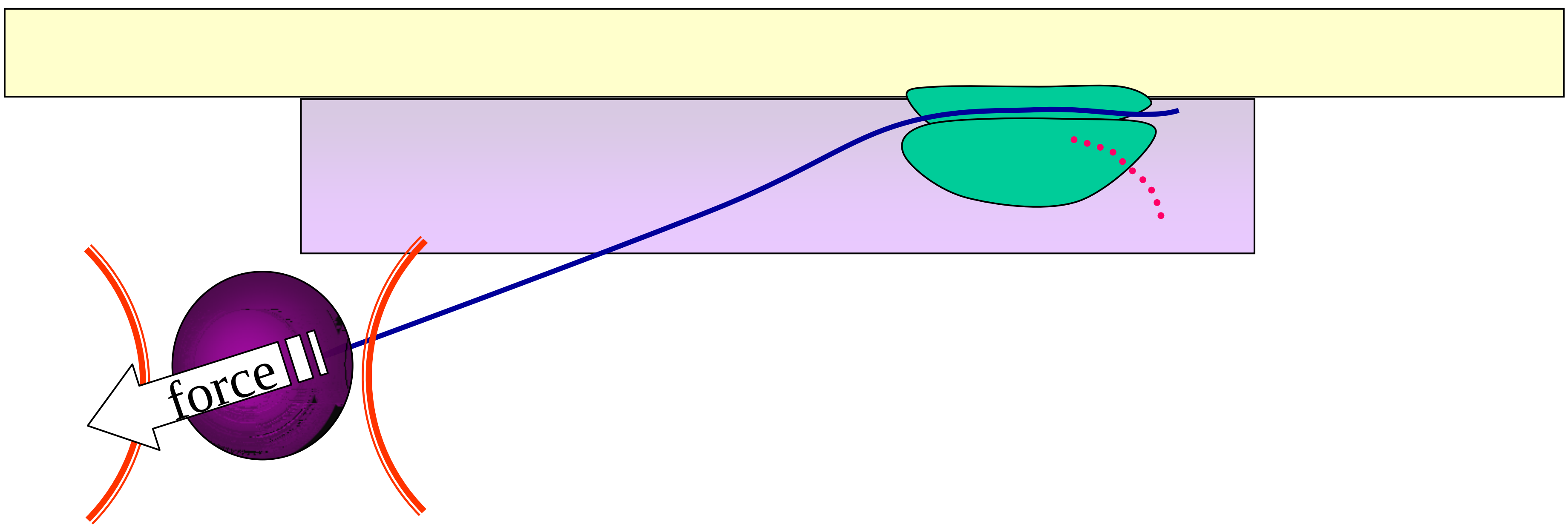


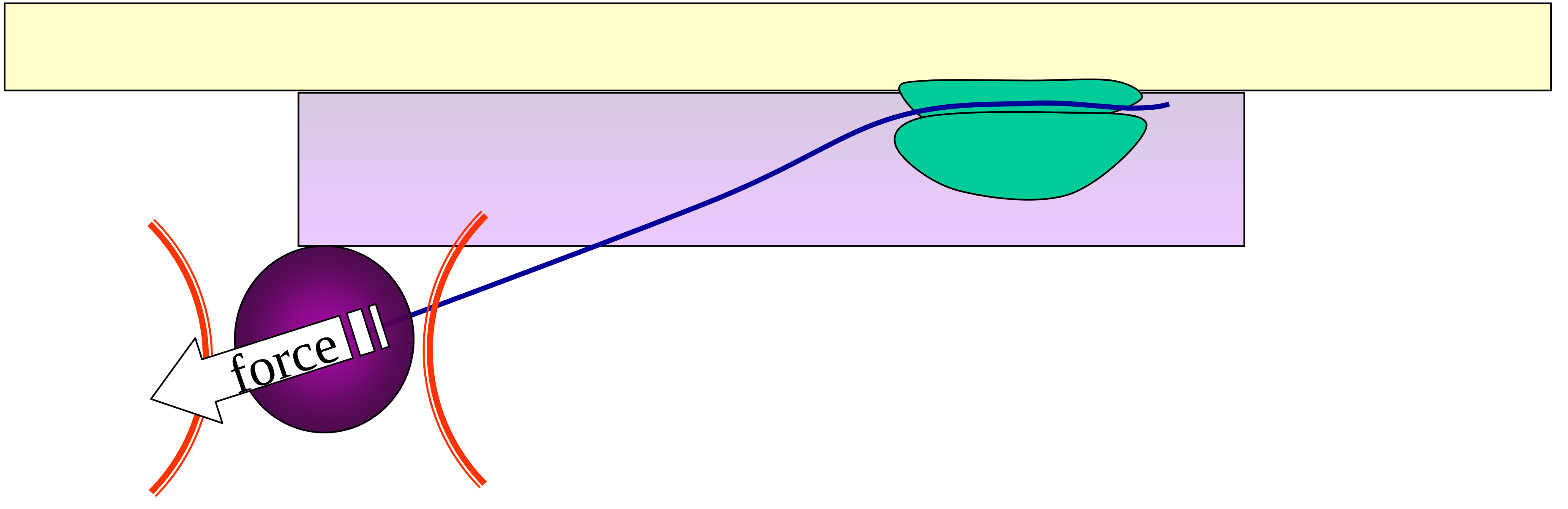


Shaevitz JW, nature 426 684 (2003)

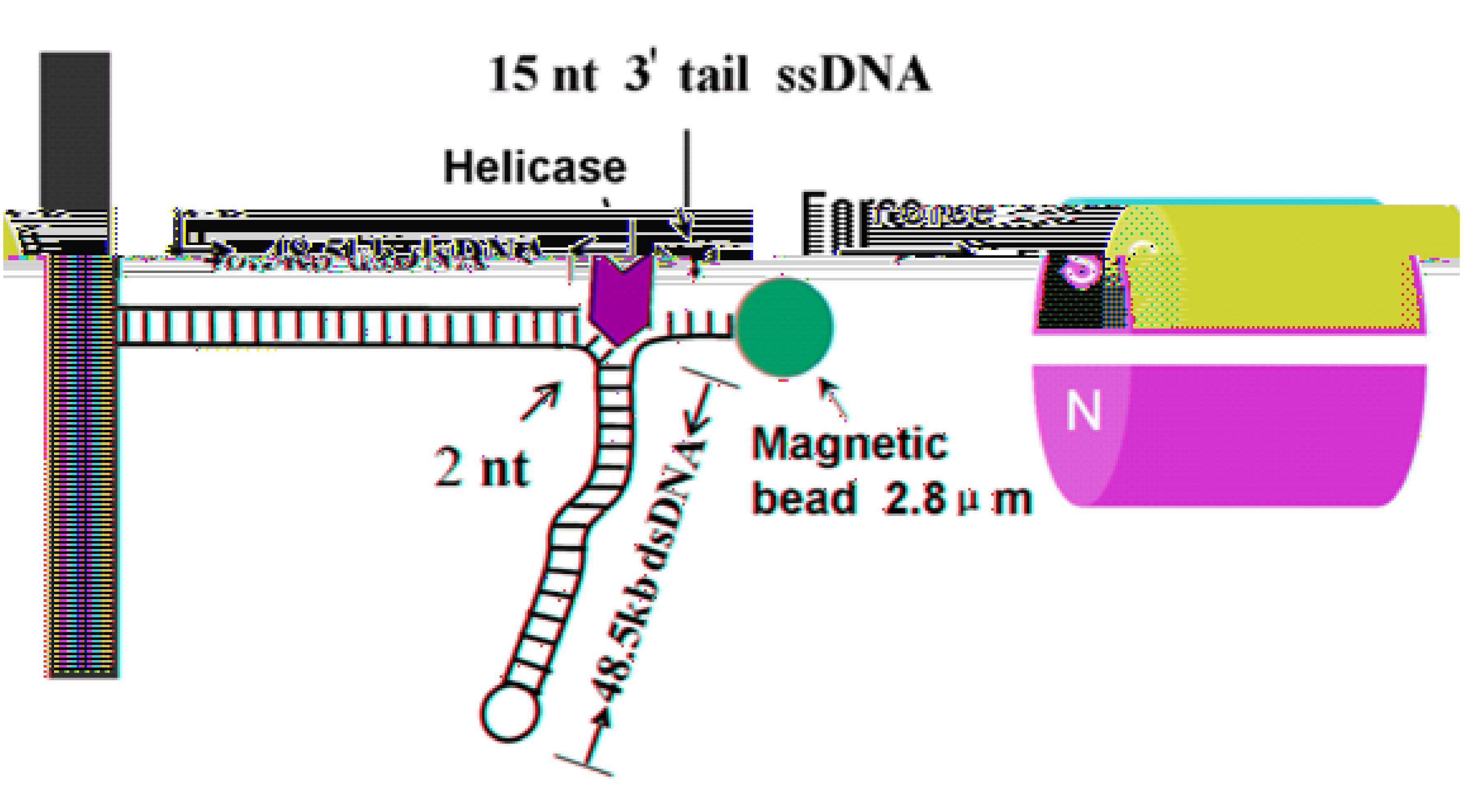
…And almost lastly, the synthesis of proteins

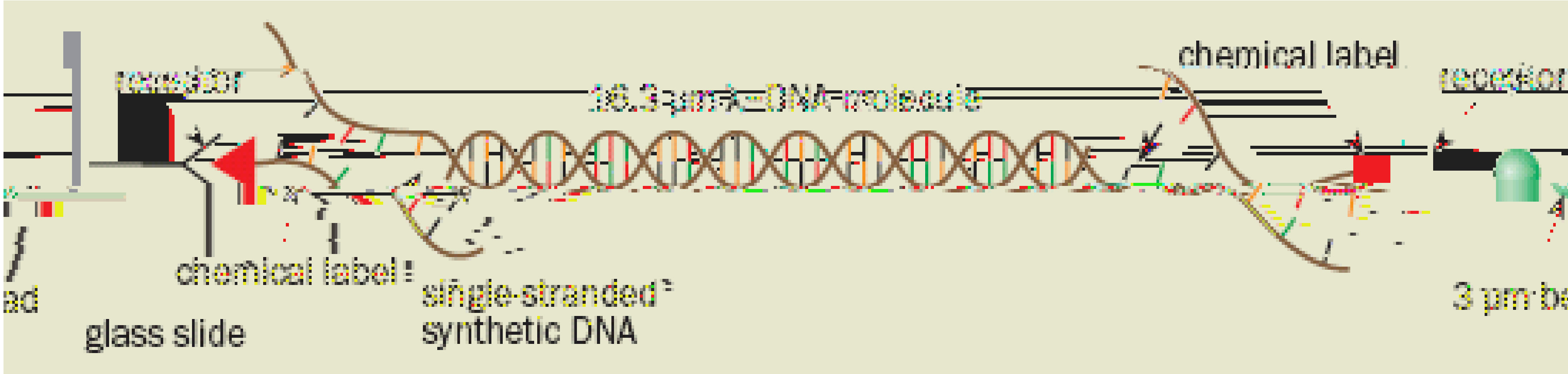



force

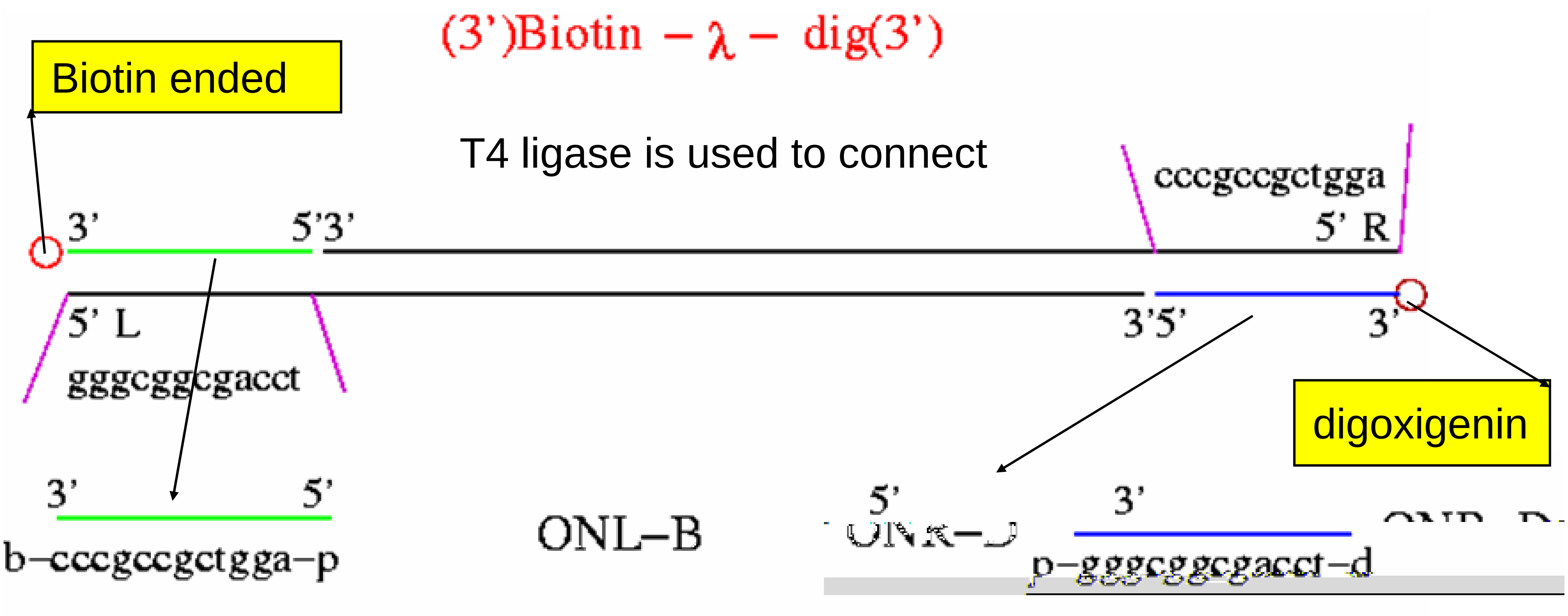

force

Some biochemistry is needed to connect a DNA to a surface and hold it via a handle.





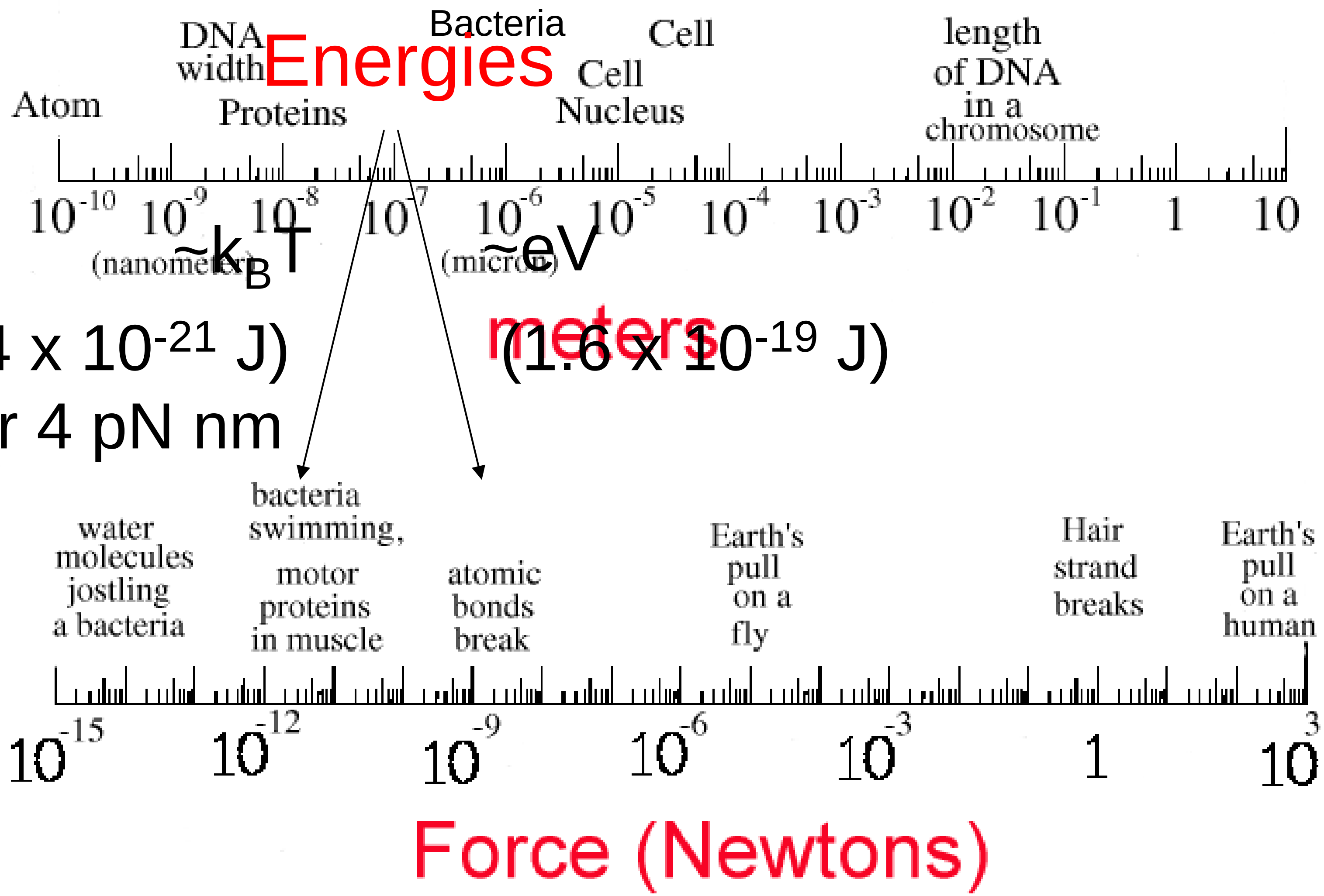
chemical label
receptor
chemical label
single-stranded
synthetic DNA
glass slide



(3')Biotin – λ – dig(3')
Biotin ended
T4 ligase is used to connect
cccgccgctgga
3'
5'3'
5' R
5' L
3'5'
3'
gggcggcgacct
digoxigenin
3'
5'
b–cccgccgctgga–p
ONL–B
5'
3'
p–gggcggcgacct–d

Instrumentation

Length, energy- and force-scales



Energies

~$k_B T$

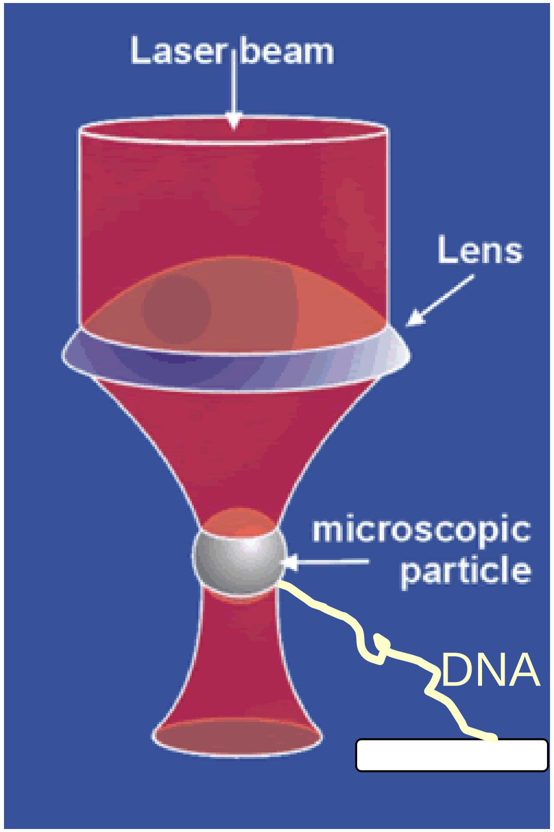
(4×10^{-21} J)

or 4 pN nm

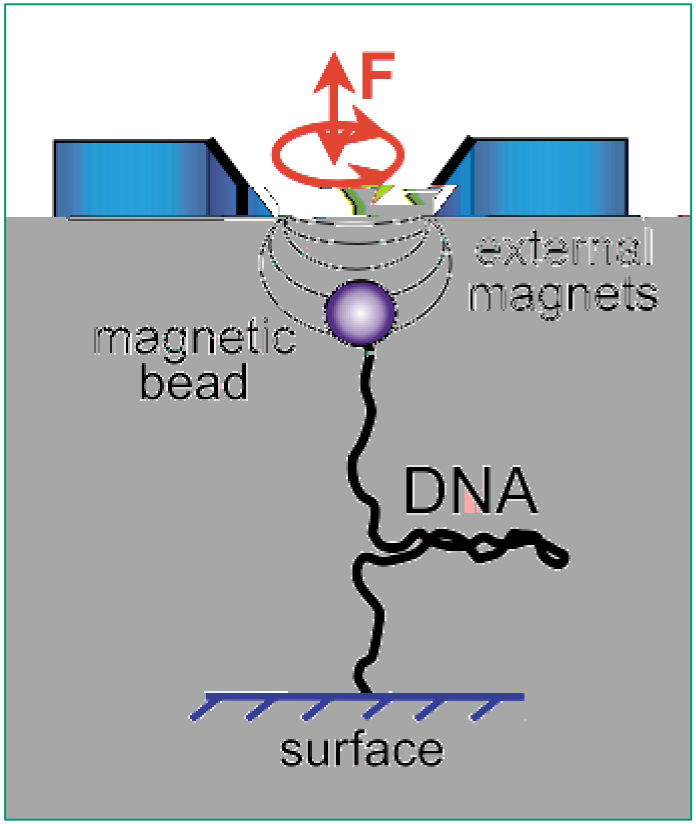
~eV

(1.6×10^{-19} J)

Optical tweezers

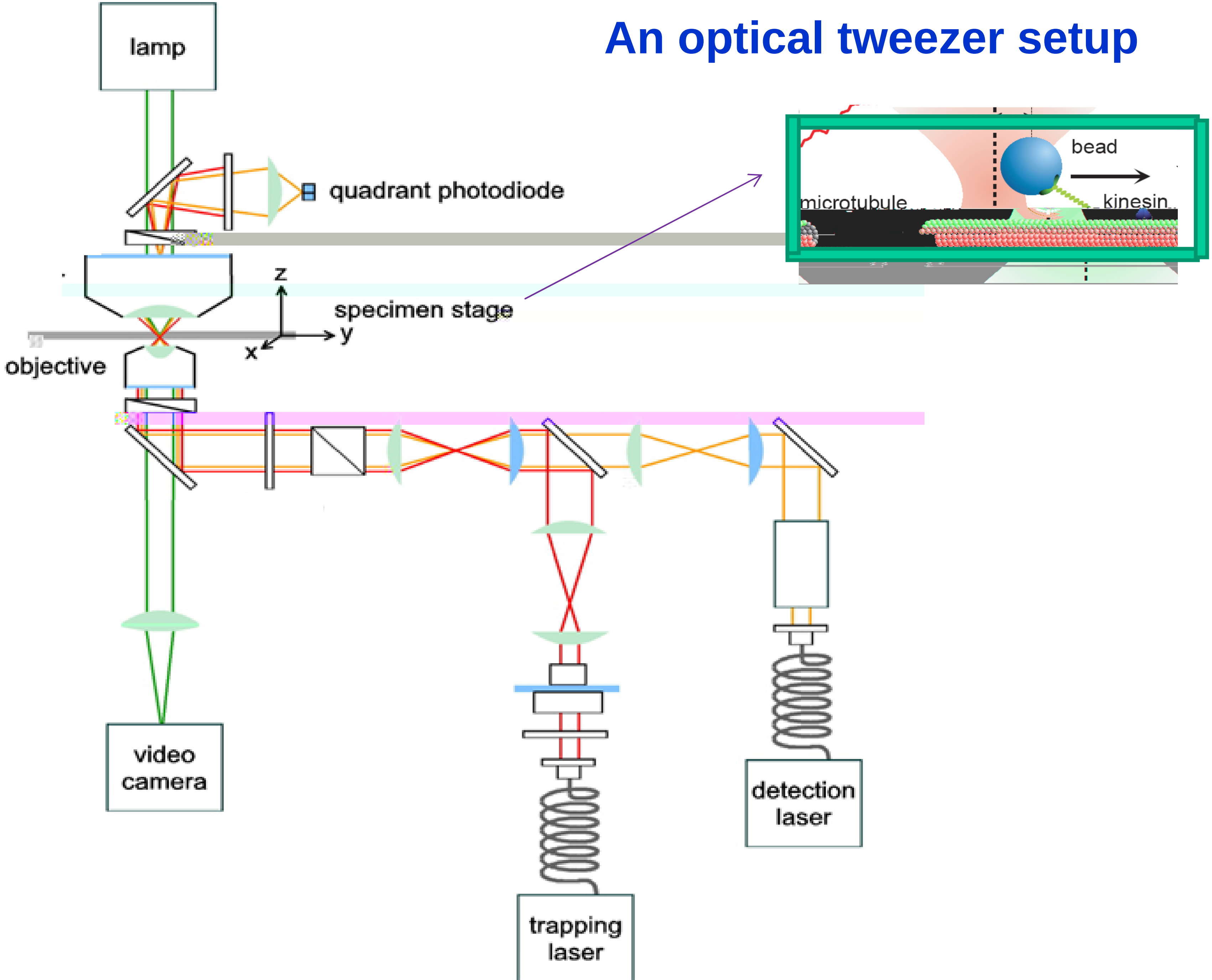


Magnetic tweezers



Manipulating the microscopic world

An optical tweezer setup



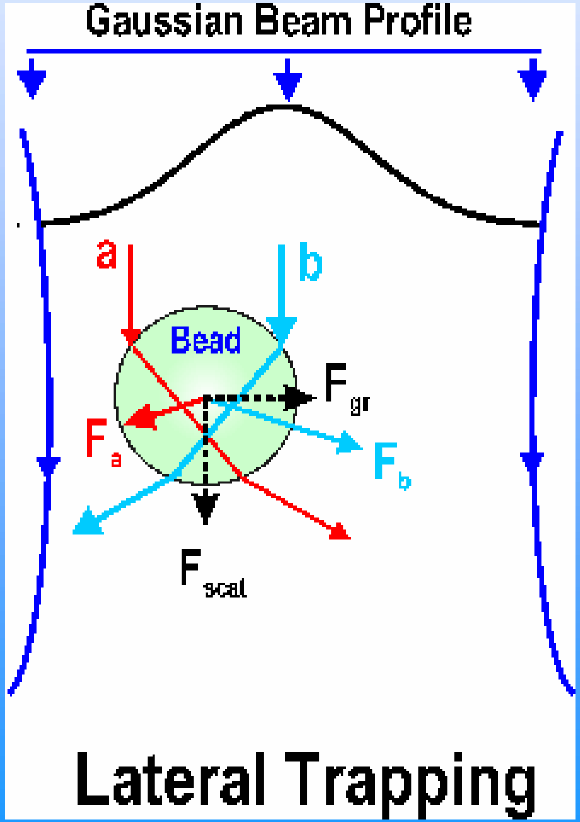
Working principle of optical tweezers

- One photon carries momentum $p = h/ \lambda$
- photon refraction ⟹ momentum change

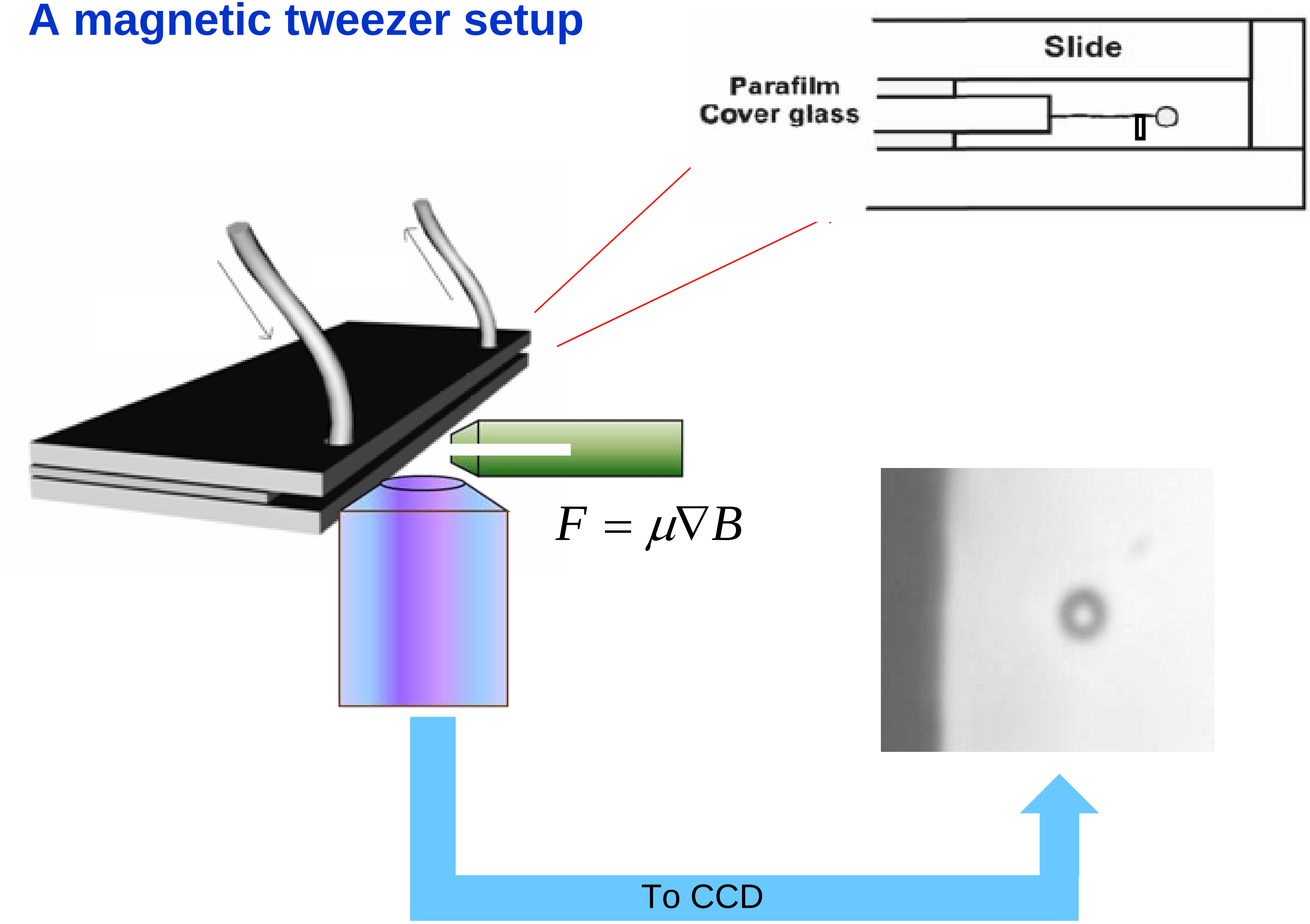
- Transparent particle of large refractive index ⟹ lens
- Gaussian beam: intense center
- momentum conservation

Lateral trapping: refraction of Gaussian beam ⟹ gradient force (F_{gr}) and a scattering force (F_{scat}).

- The lateral gradient force pulls particle to beam center

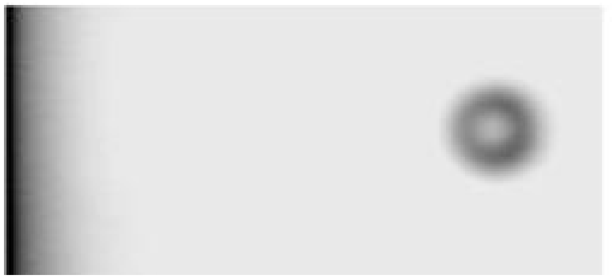


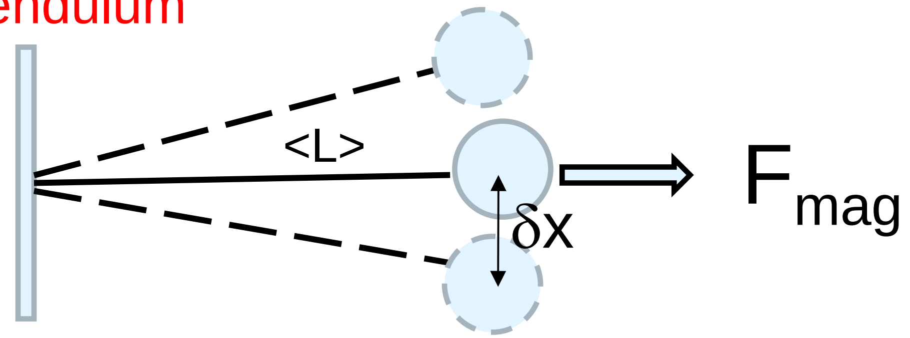
A magnetic tweezer setup

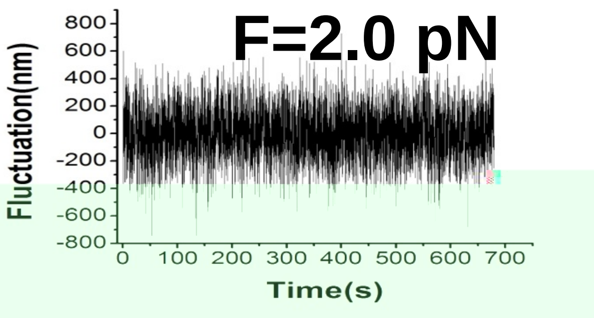


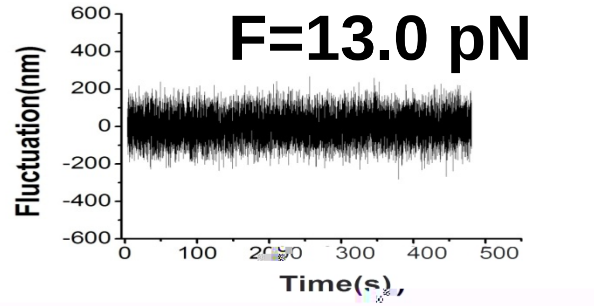
Force measurement

Overdamped pendulum









$$F_{mag} = \frac{k_B T \langle L \rangle}{\langle (\delta x)^2 \rangle}$$

A short story of a helicase

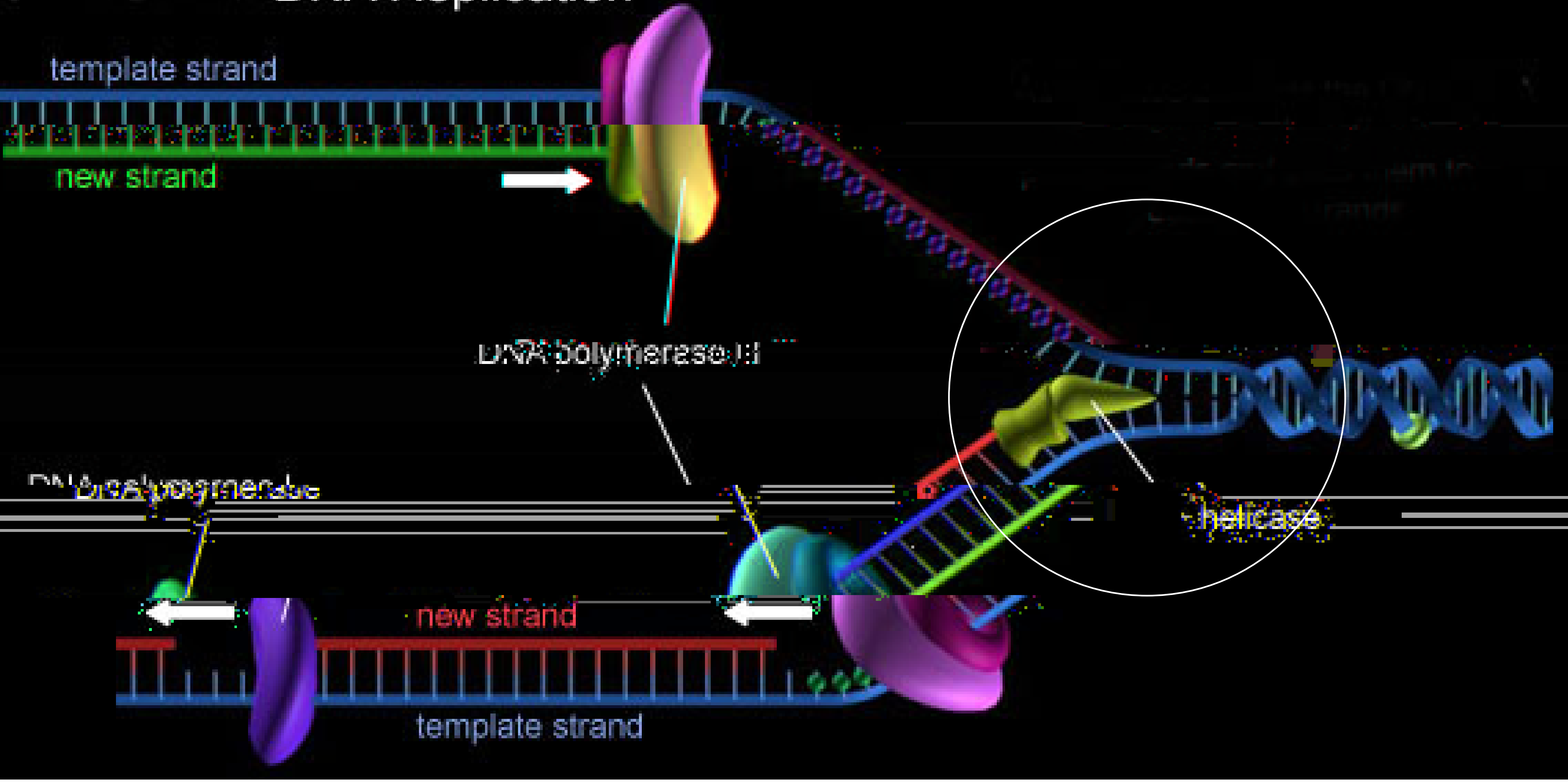
The EMBO Journal (2008) 00, 1–9 | 0261-4189/08
www.embojournal.org

THE EMBO JOURNAL

Impediment of *E. coli* UvrD by DNA-destabilizing force reveals a strained-inchworm mechanism of DNA unwinding

Fu WB, Wang XL, Zhang XH, Ran SY, Yan J, Li M, DNA condensation dynamics, **J Am Chem Soc**, 128,15040(2006).

DNA Replication

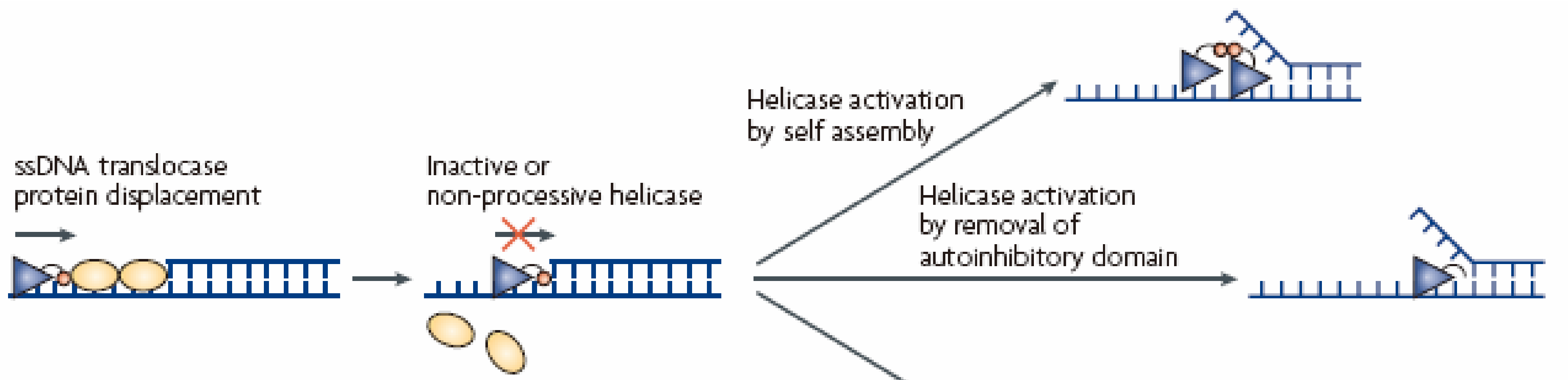


NATURE REVIEWS | MOLECULAR CELL BIOLOGY VOLUME 9 | MAY 2008 | 391

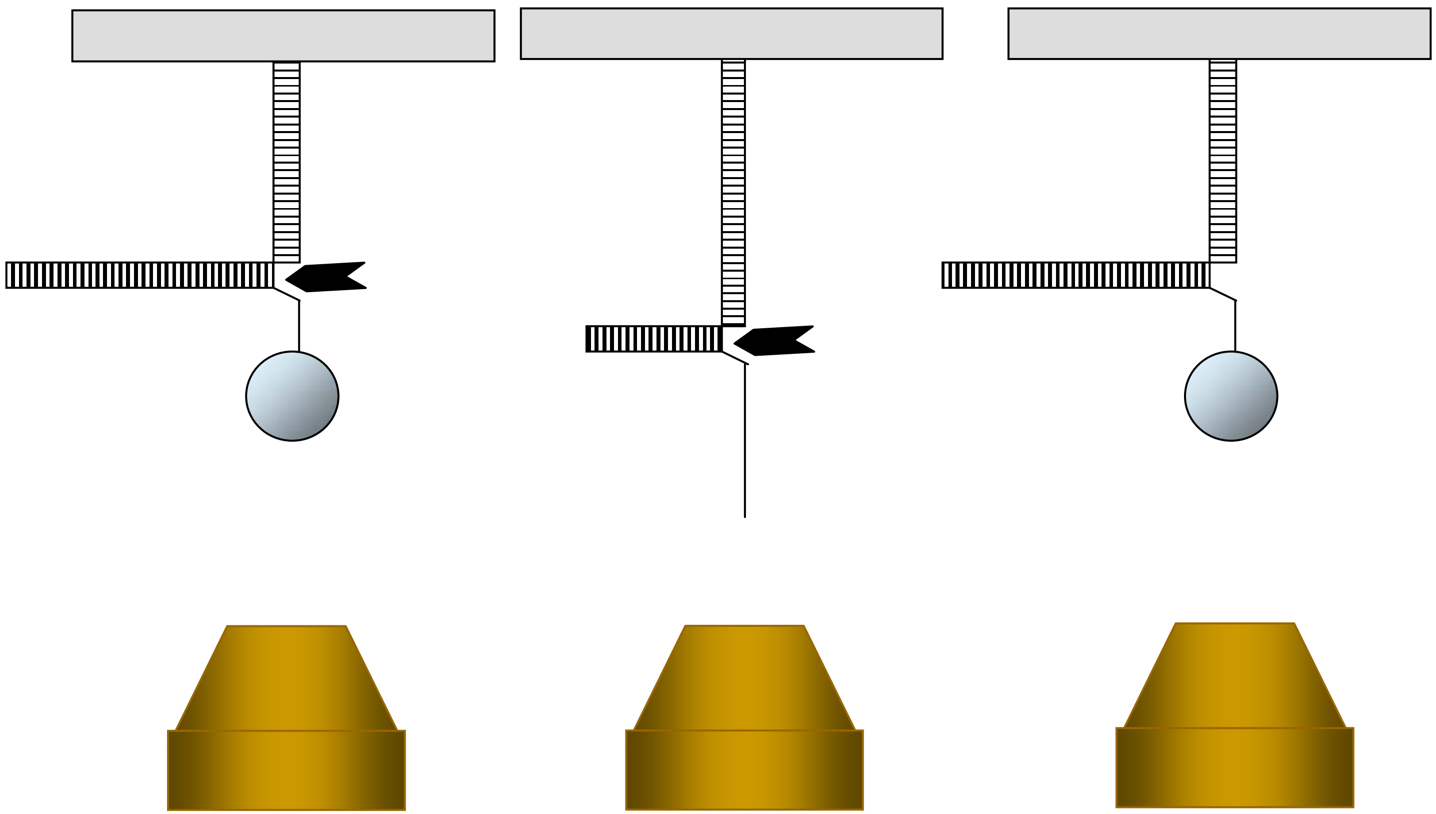
Nature Reviews

Non-hexameric DNA helicases and
slocases: mechanisms tra
regulation an

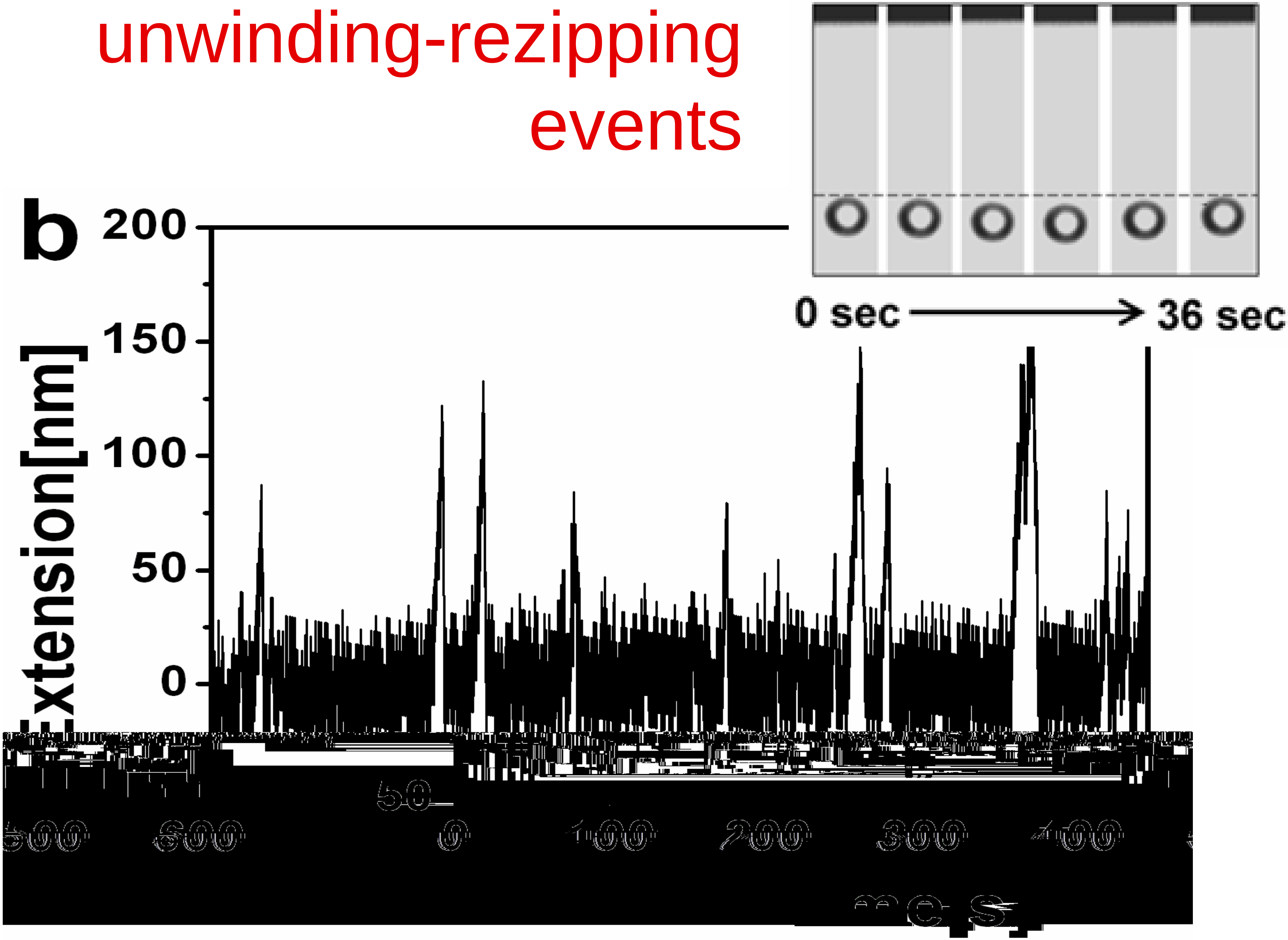
M. Lohman, Eric J. Tomko and Colin G. Wu
Timothy I



Binding



unwinding-rezipping events



1)

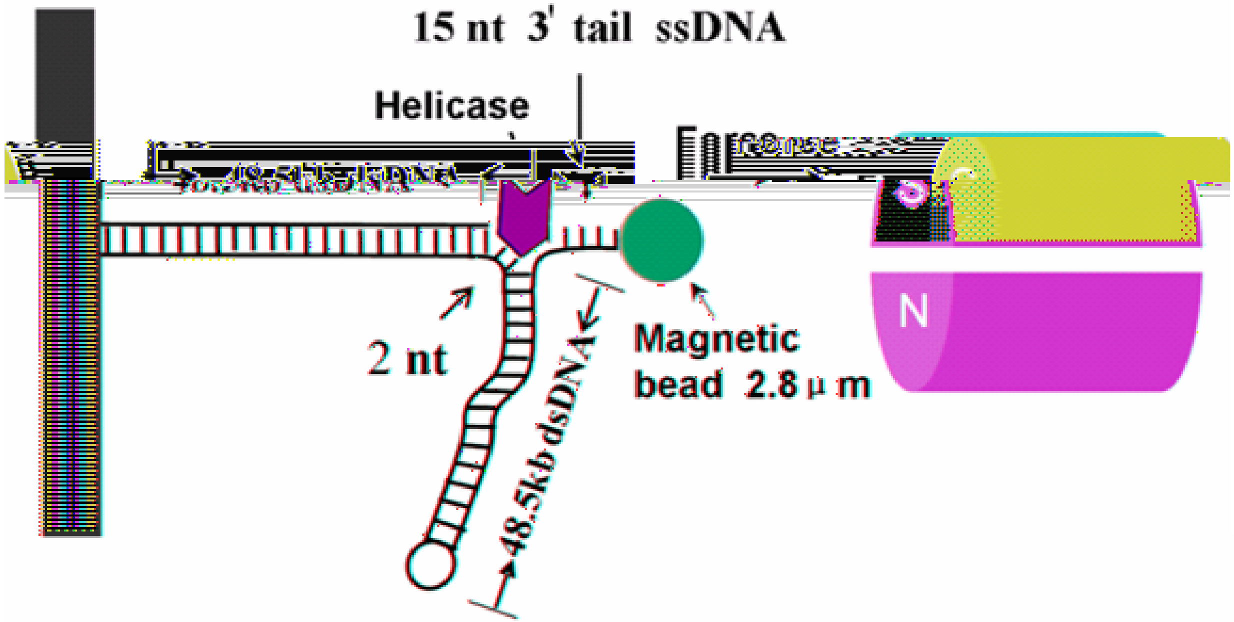
Dimer is the functional form of UvrD,

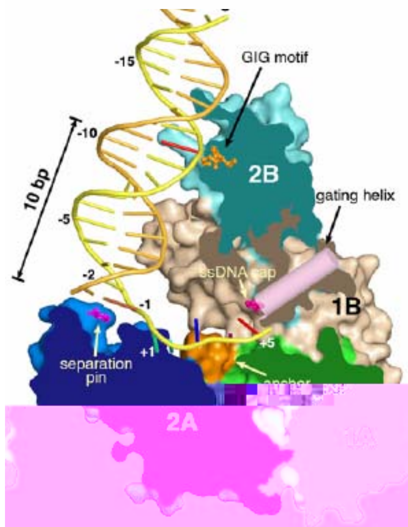
although UvrDs exists in solution as monomers.

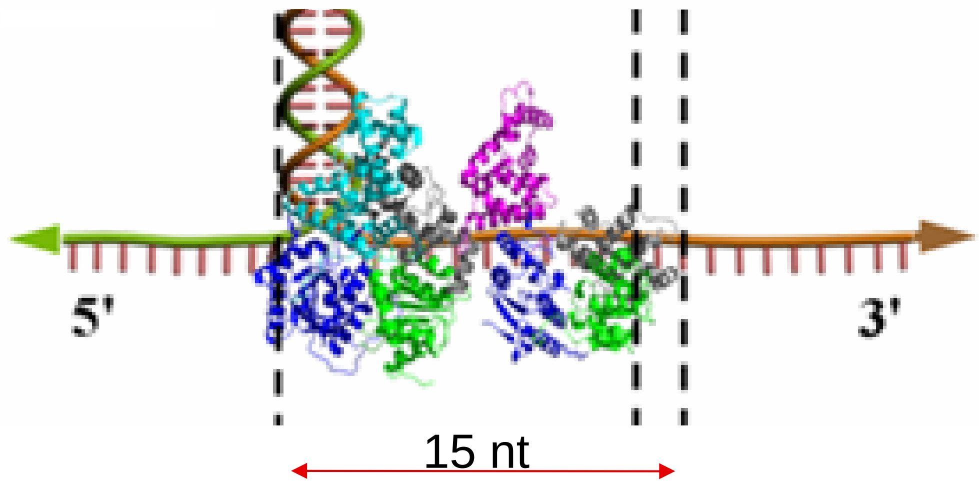
[UvrD]=5 nM and 10 nM

[ATP]=1 mM

A loading tail longer than 15 nt is required!

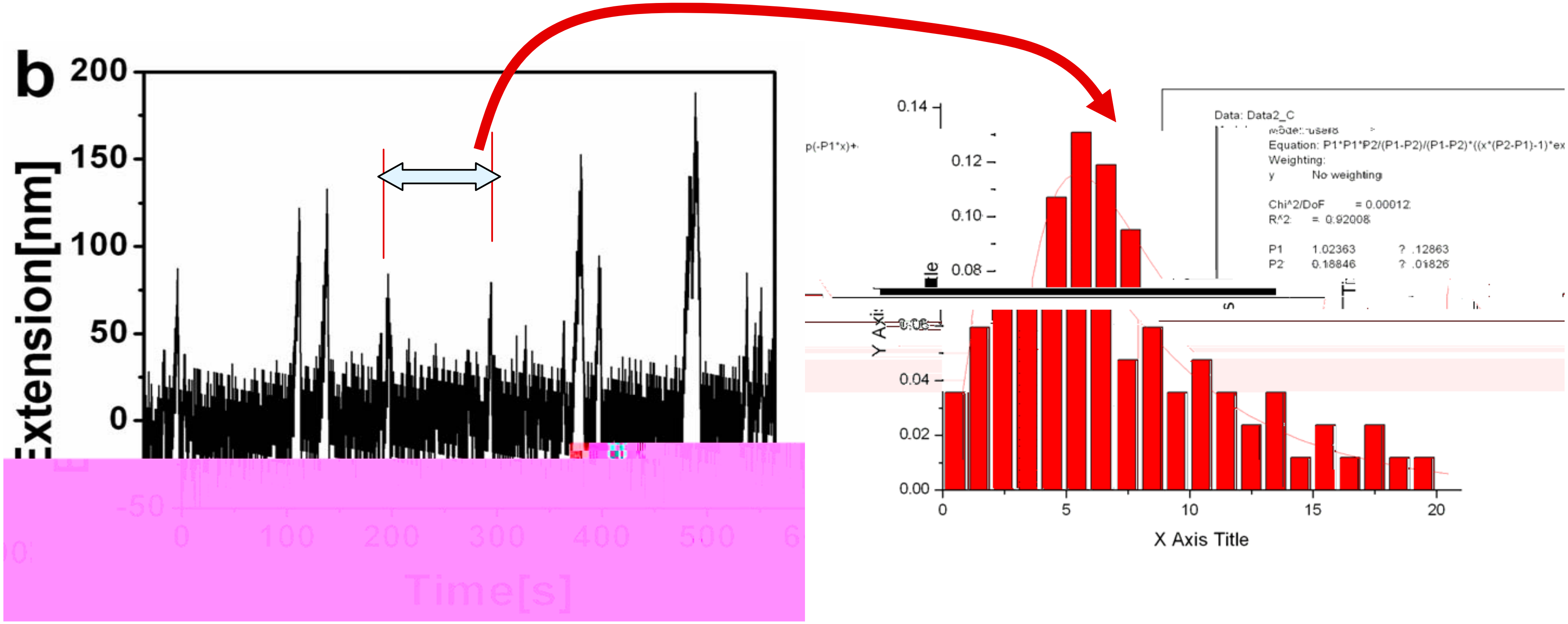






2)
There are two binding events before dimerization occurs at the DNA junction

Binding kinetics



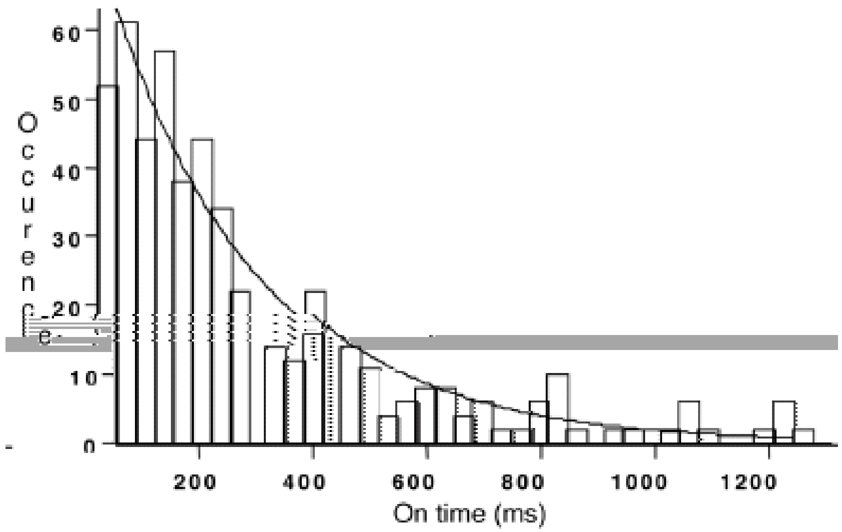
Single-Molecule Enzymatic Dynamics

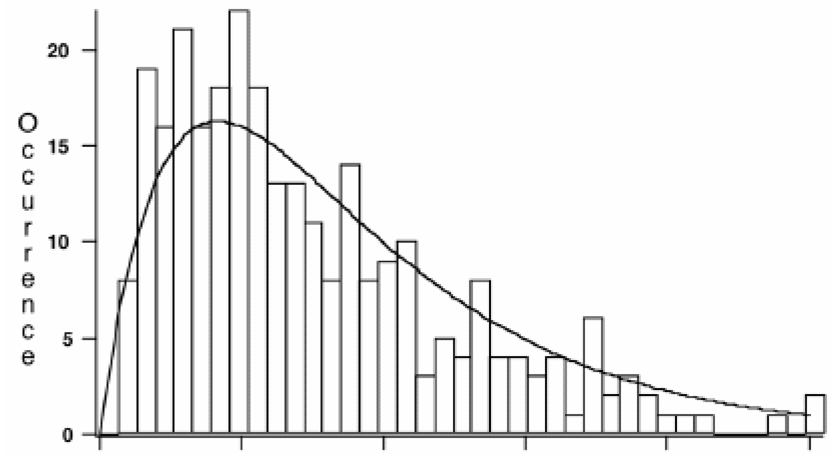
SCIENCE VOL 282 4 DECEMBER 1998

REPORTS 1877

H. Peter Lu, Luying Xun, X. Sunney Xie*

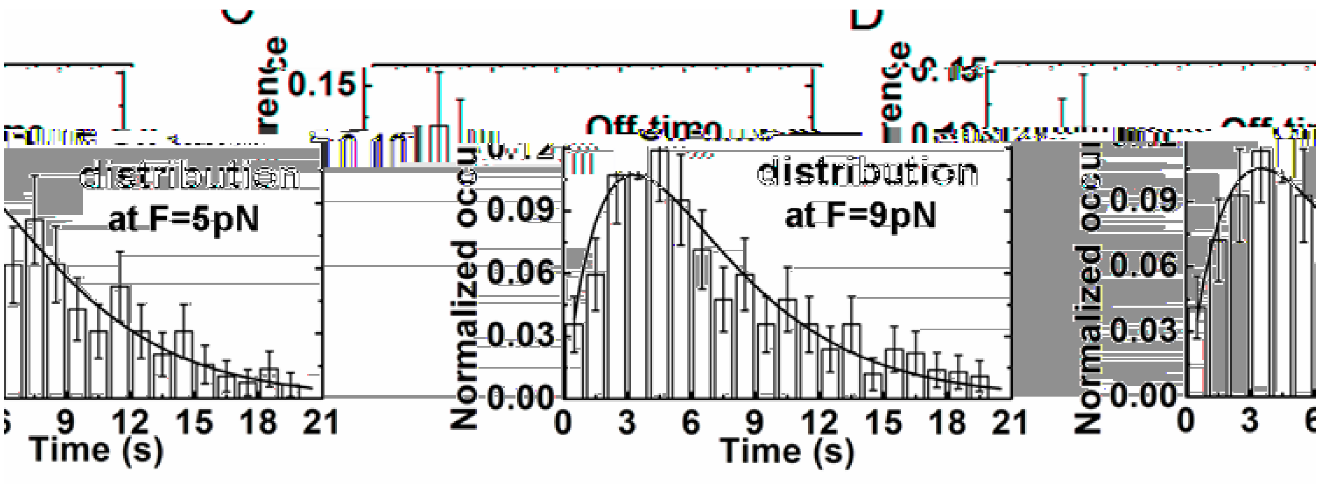
$$E + DNA \xrightarrow{k_1} E \cdot DNA,$$





$$f(t) = \frac{k_1 k_2}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right)$$

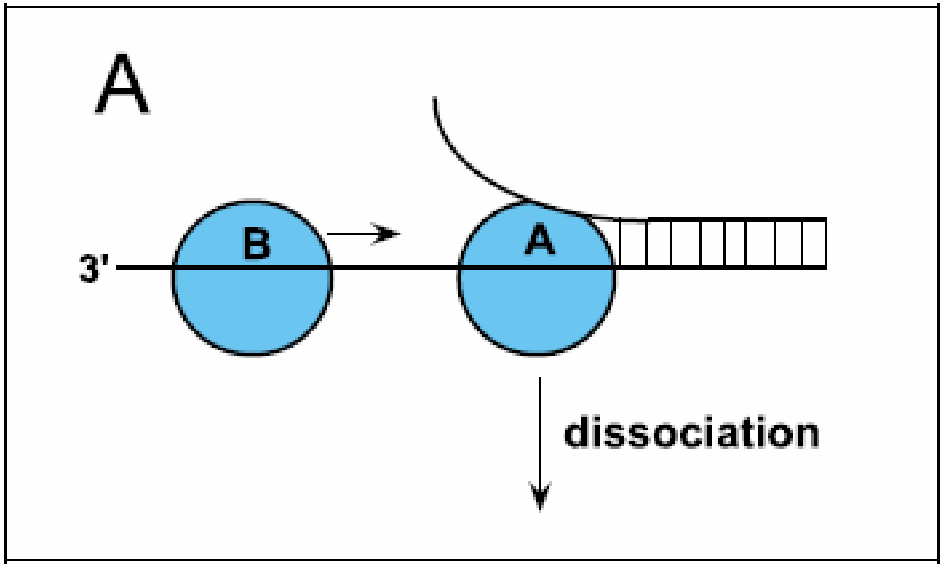
$$f(t) = \frac{k_1 k_2}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right)$$



K_1=0.23 ± 0.05 /s; K_2=0.38 ± 0.08 /s @ [UvrD]=5 nM

K_1=0.05 /s; K_2=0.07 /s @ [UvrD]=1 nM

$1/K_1$=20 Sec; $1/K_2$=14 Sec

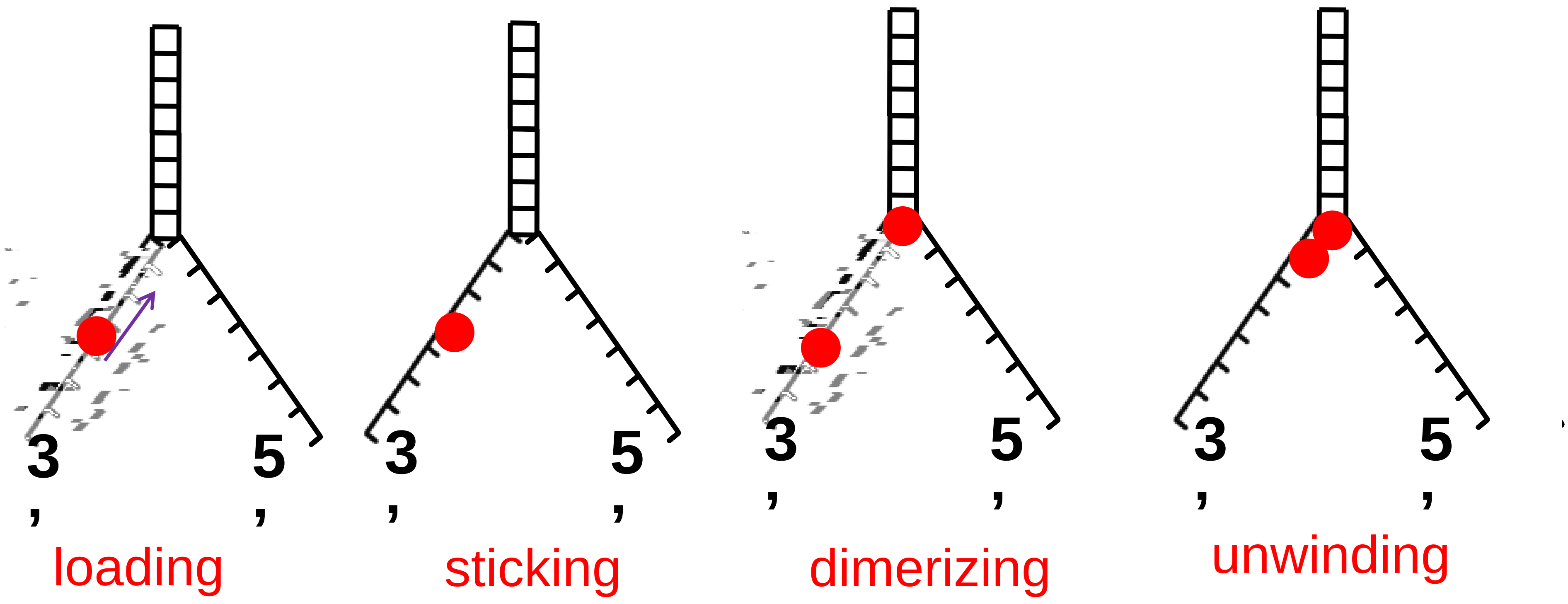


K_{-1}=0.12 /s @ [UvrD]=1 nM

$1/K_{-1}$=8.3 Sec

Maluf NK, Ali JA, Lohman TM (2003a) Kinetic mechanism for formation of the active, dimeric UvrD helicase-DNA complex. *J Biol Chem* **278:** 31930–31940

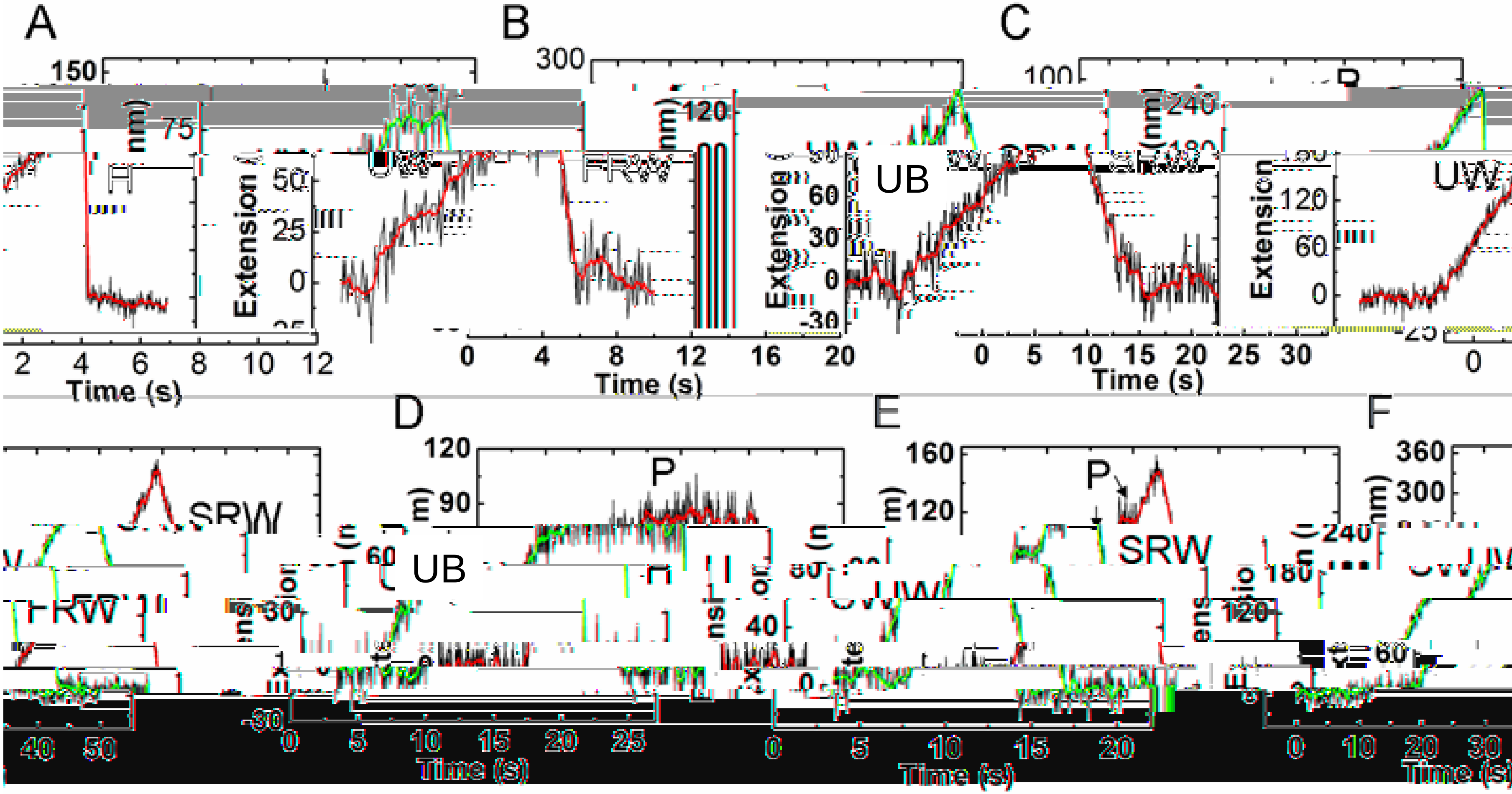
Model of
the assembly of a dimer at the DNA junction



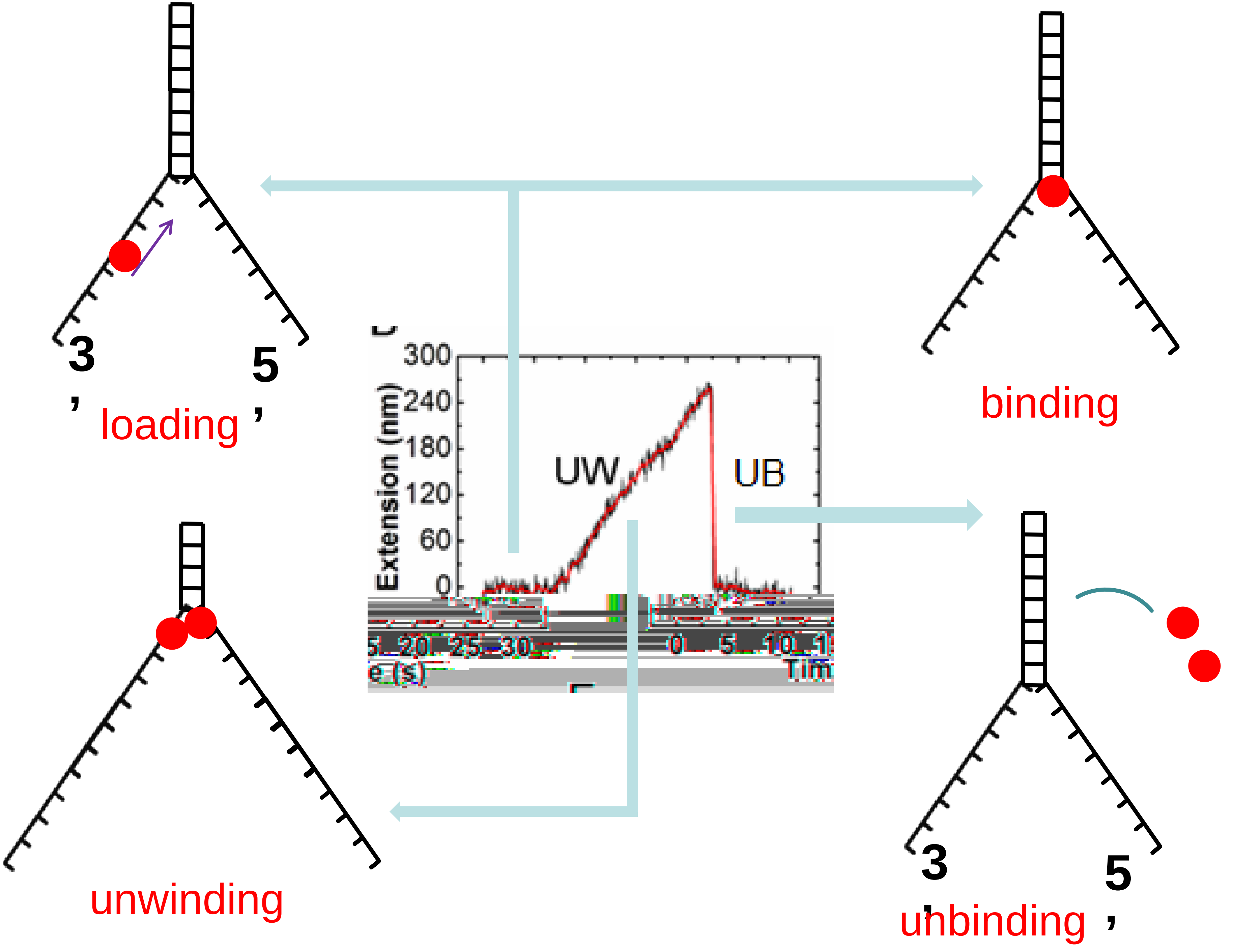
3)
Dimerization process is dynamical.

Details of the unwinding events

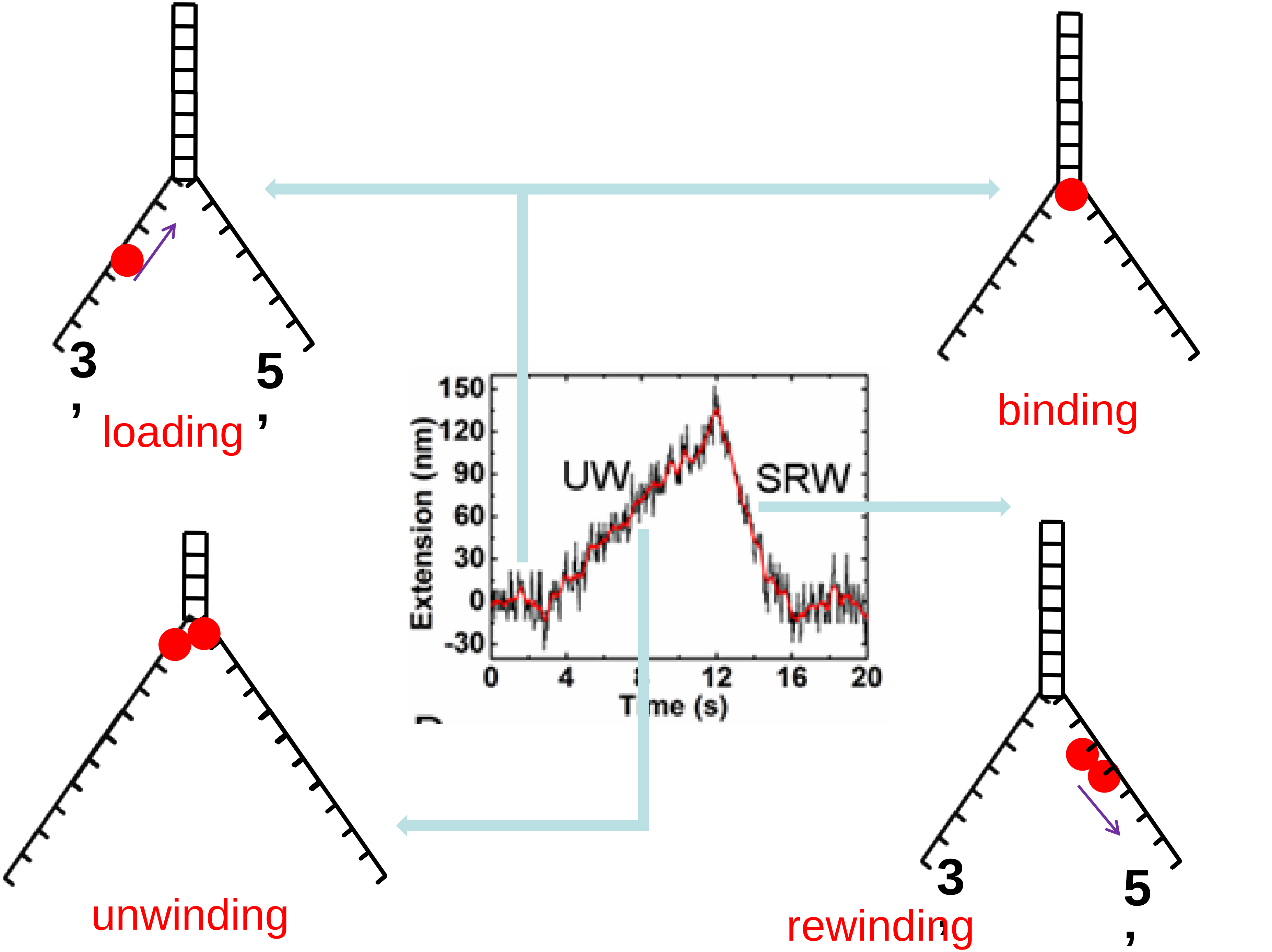
Details of the unwinding events



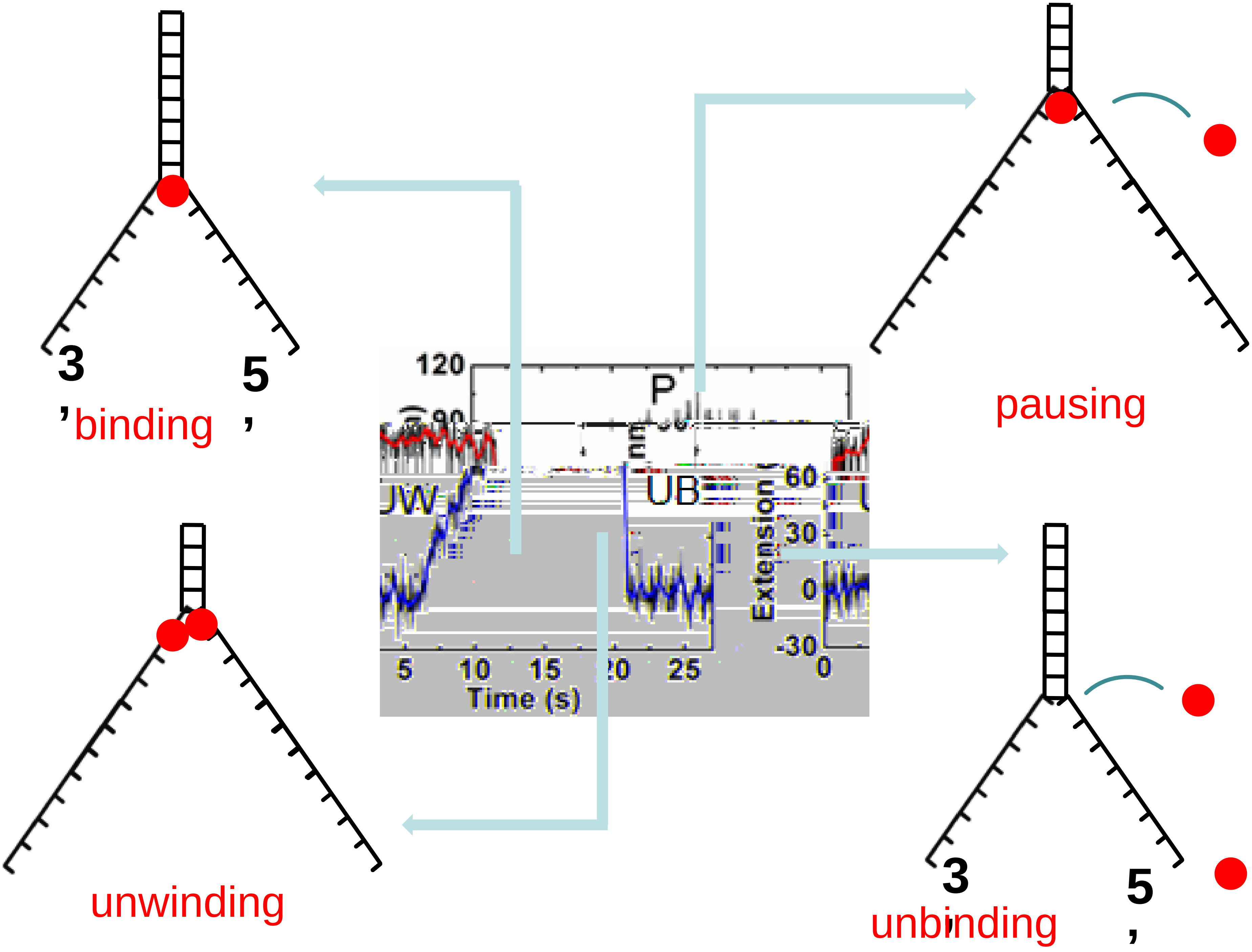
UW=unwinding; SRW=slow rewinding;
FRW=fast rewinding; P=pausing; UB=unbinding

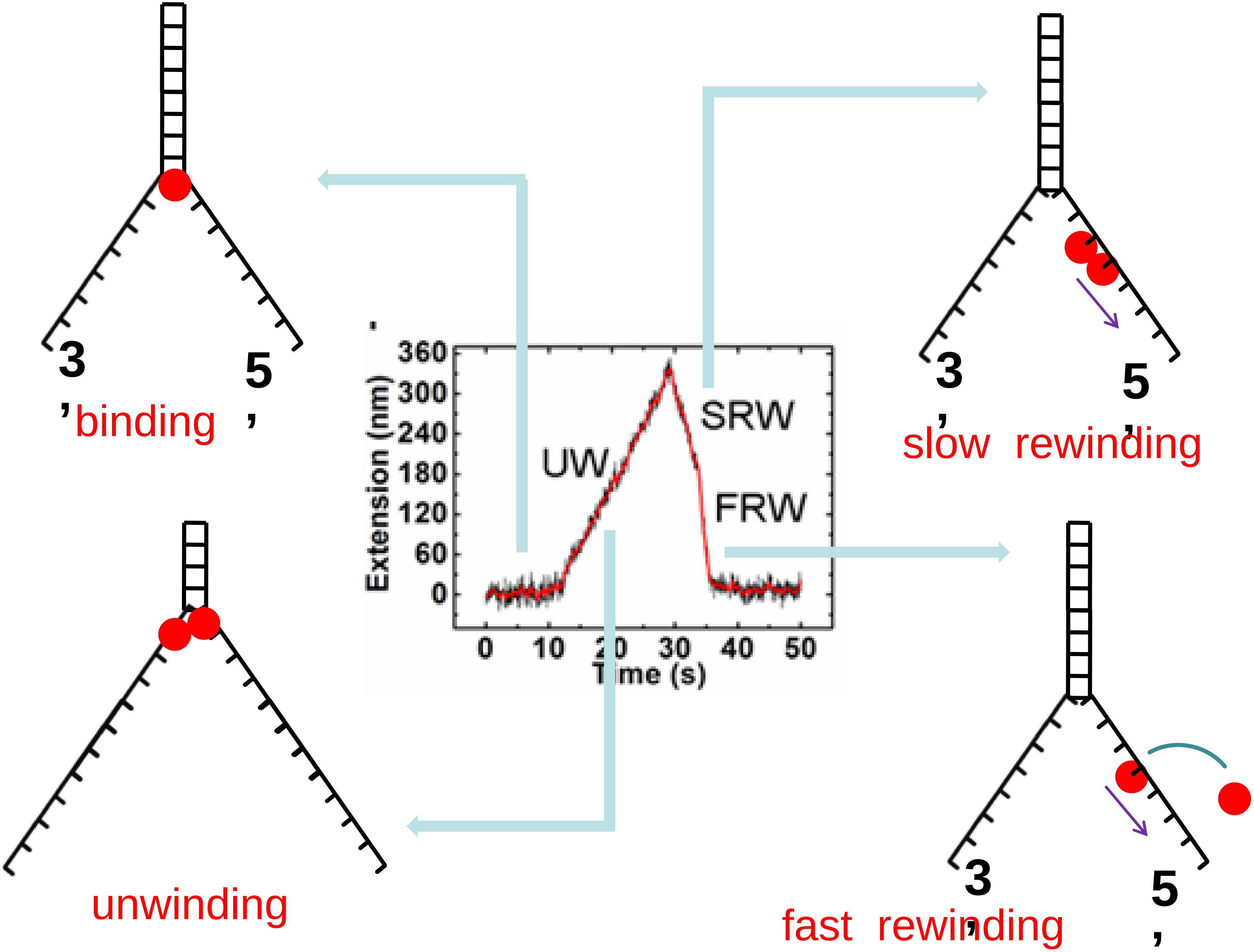


3'
5'
loading
binding
Extension (nm)
300
240
180
120
60
0
UW
UB
unwinding
3'
5'
unbinding



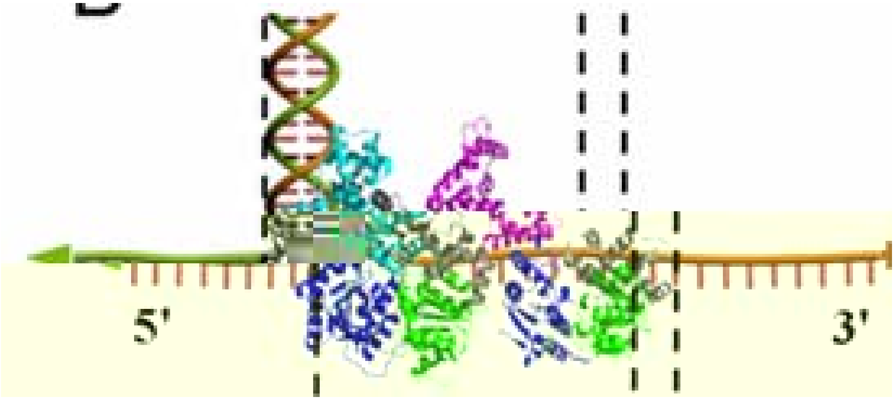
3’
5’
loading
binding
UW
SRW
Extension (nm)
150
120
90
60
30
0
-30
0
4
8
12
16
20
Time (s)
unwinding
rewinding
3’
5’

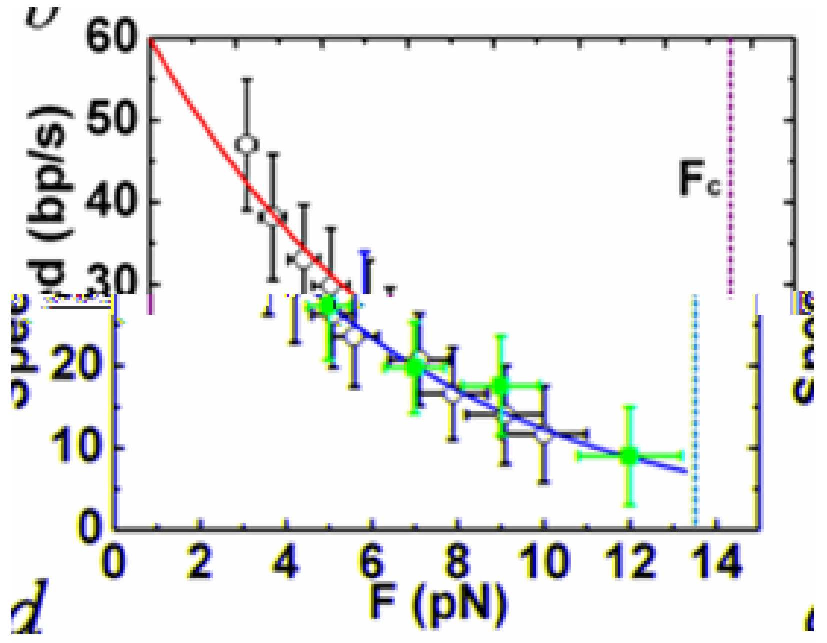

3'
binding
5'
pausing
120
P
UB
Extension
60
30
0
-30
5
10
15
20
25
Time (s)
unwinding
3'
unbinding
5'



3'
5'
binding
Extension (nm)
360
300
240
180
120
60
0
0
10
20
30
40
50
Time (s)
UW
SRW
FRW
3'
5'
slow rewinding
unwinding
3'
5'
fast rewinding

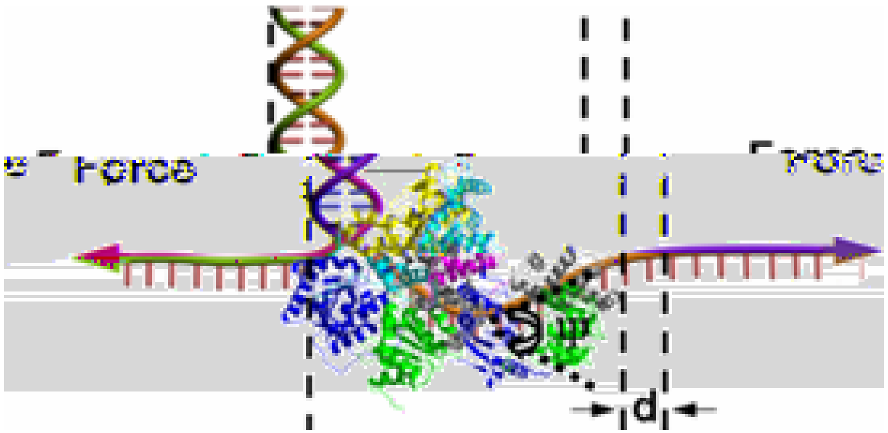
4)
Dimer undergoes a configurational change to become active.

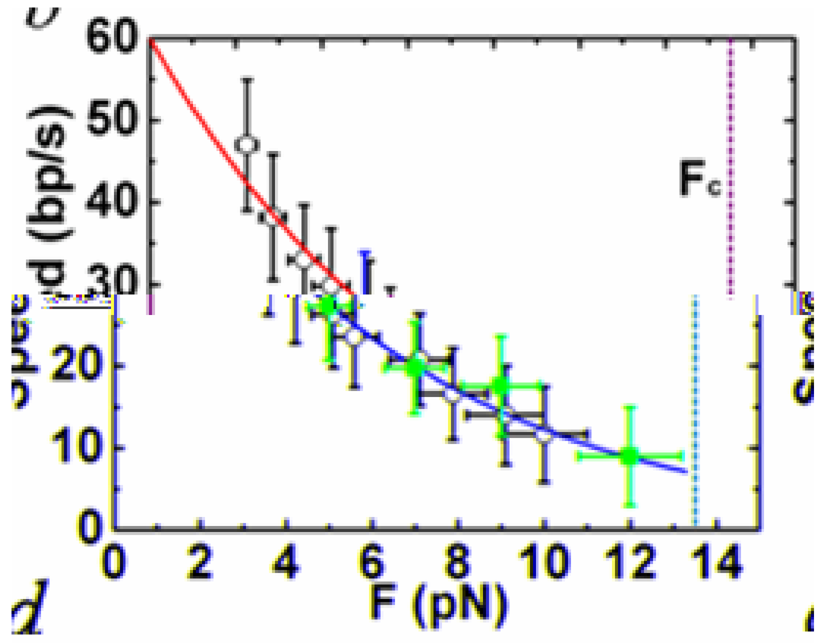




Configurational change of the dimer bends the ssDNA tail.

Force performs negative work!

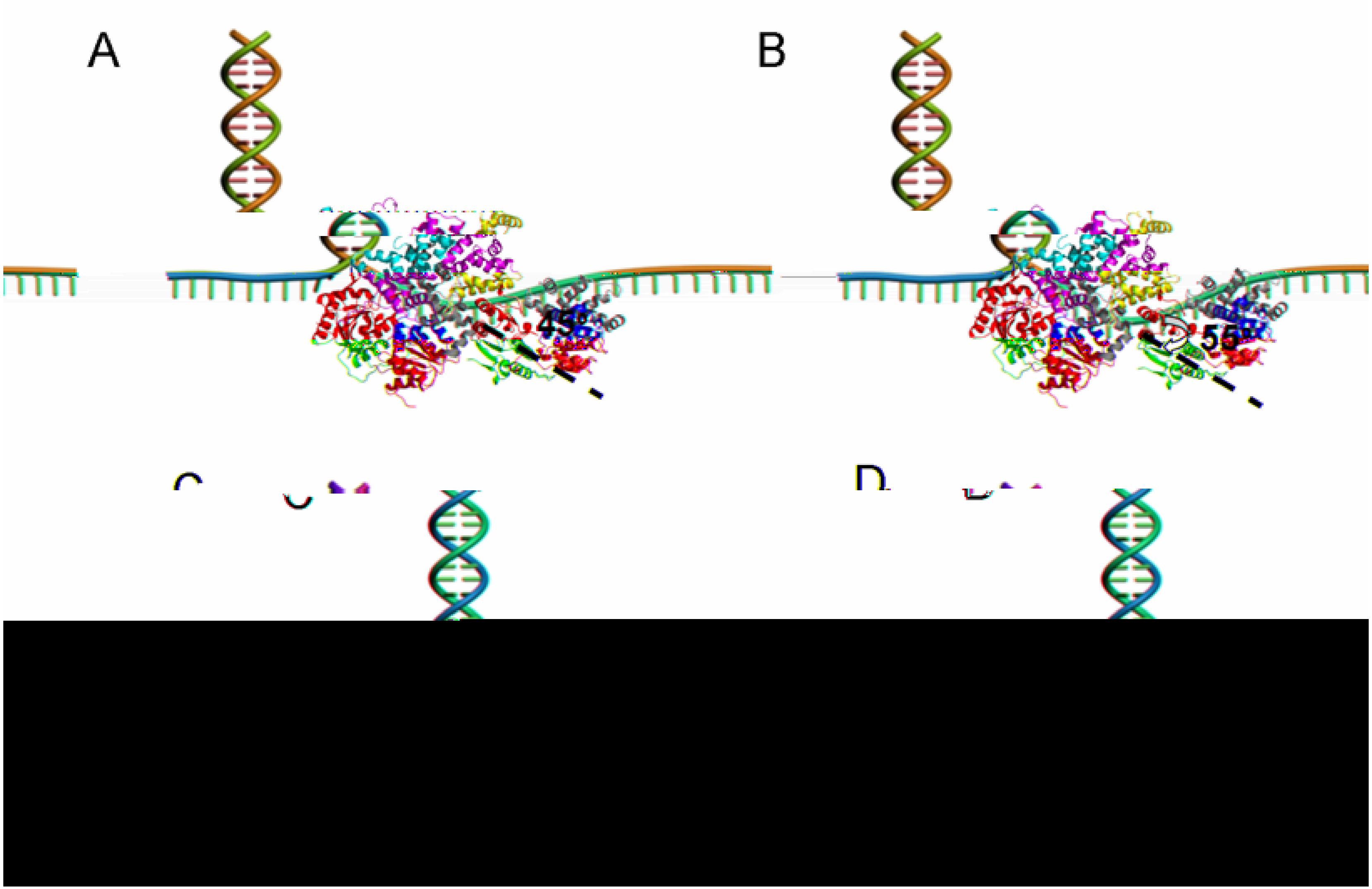




Configurational change of the dimer bends the ssDNA tail.

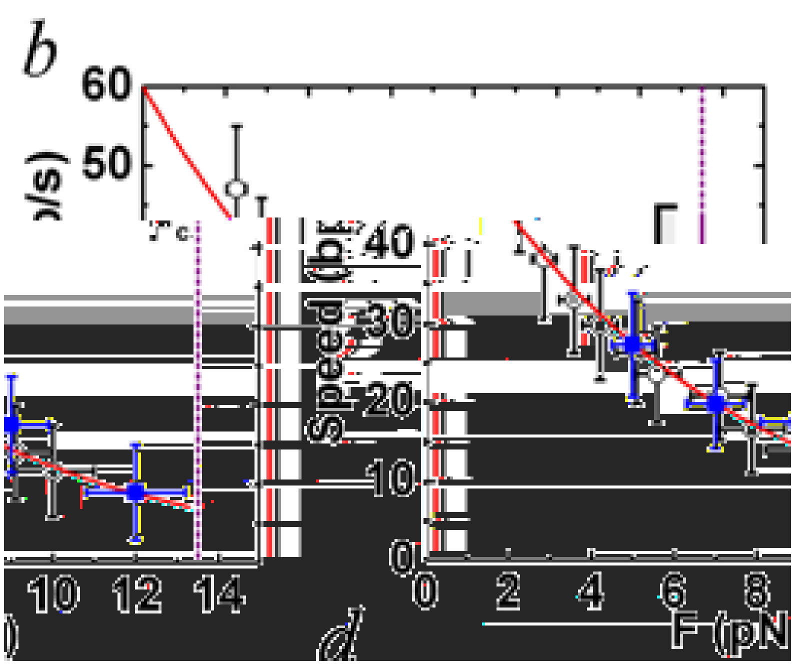
Force performs negative work!

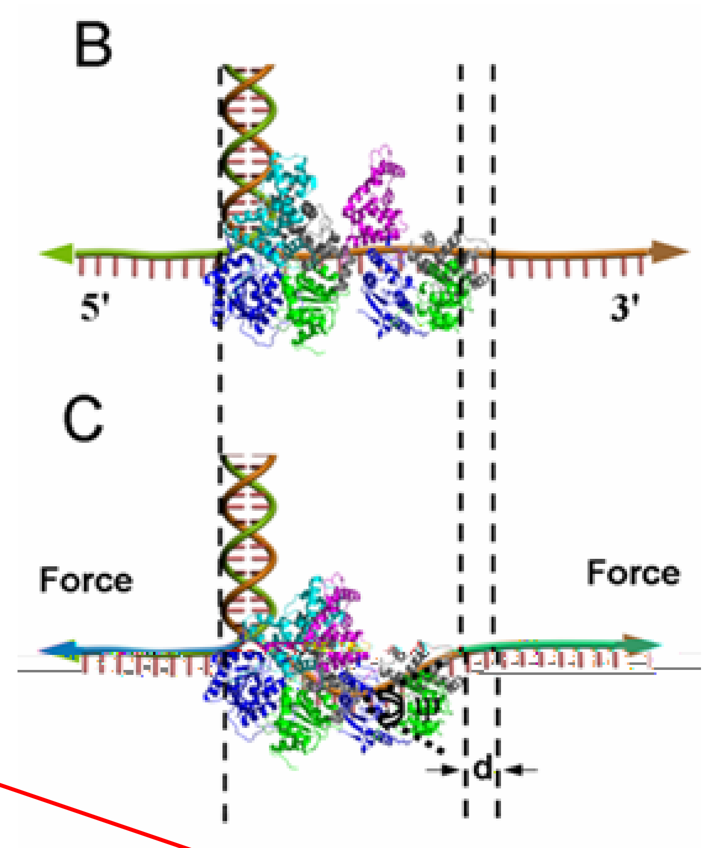
Docking of two UvrDs supports the mechanism.



Structures were from the PDB

Configurational change bends the ssDNA tail by ~50deg.





$v=v_0 \exp(-F*d/k_BT)$

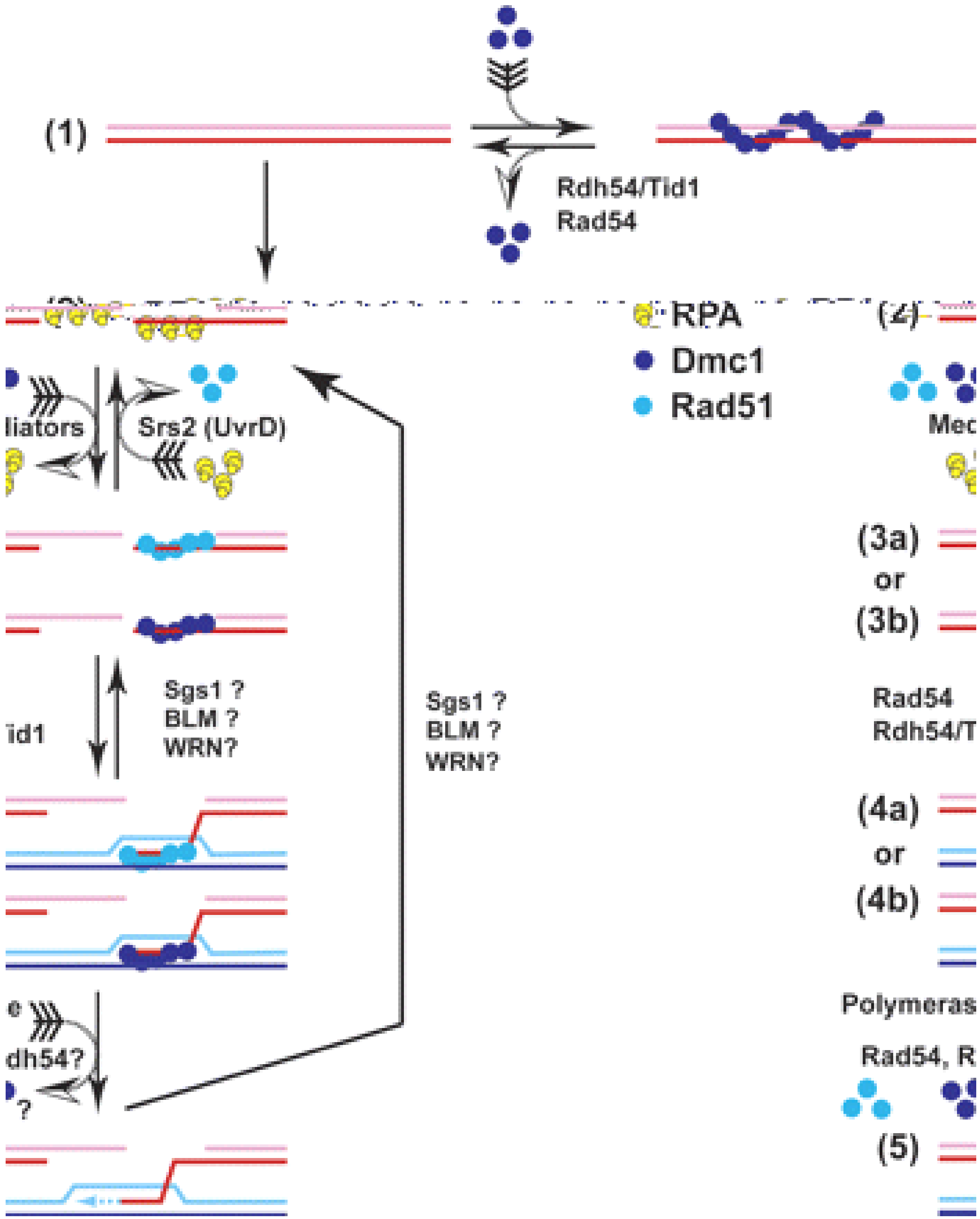
v_0=68 bp/s; JMB(2003)

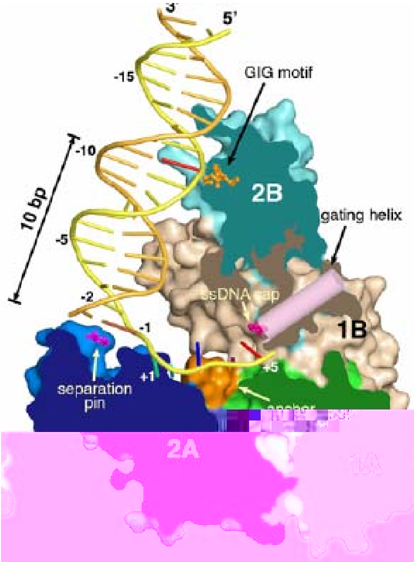
d=0.7 nm

d~0.7 nm

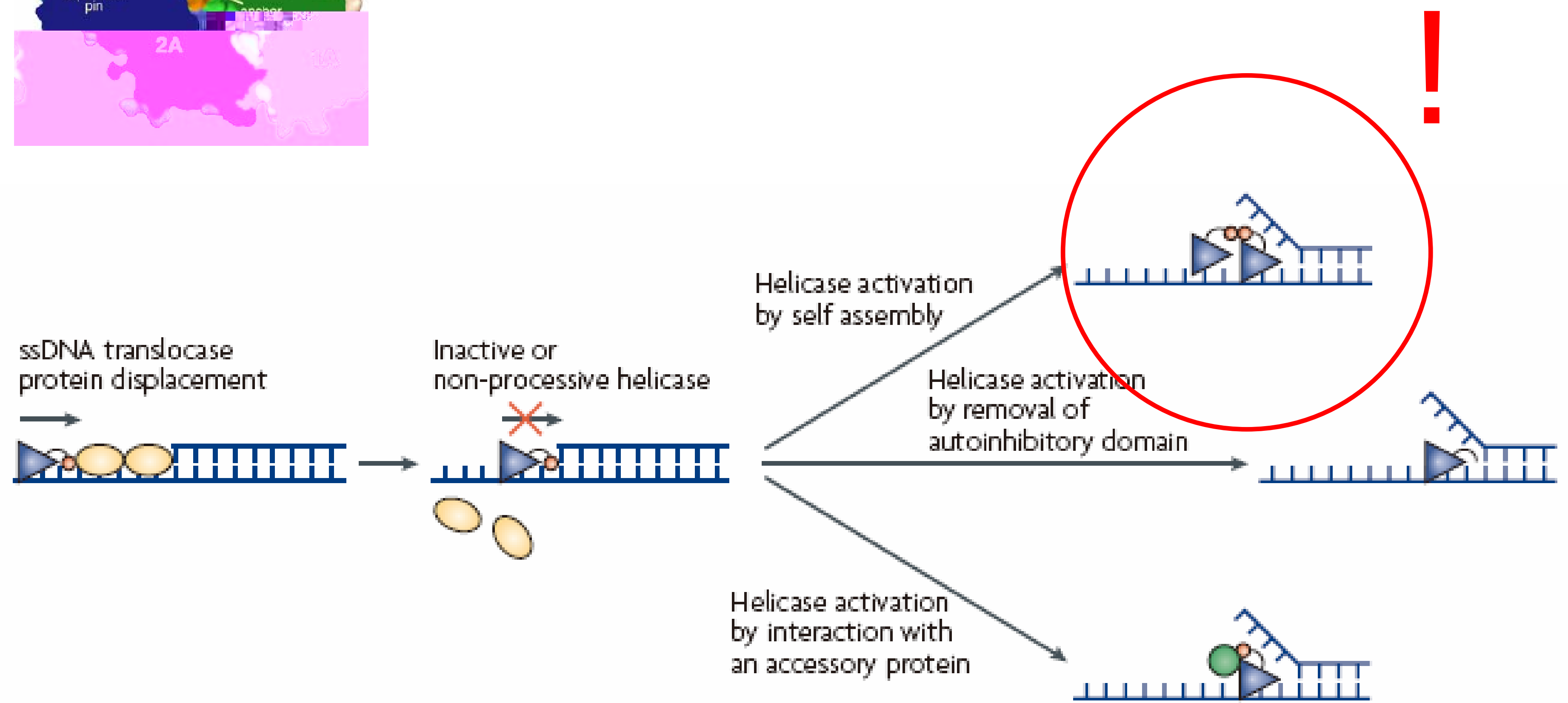
Biological significance

A road cleaner!

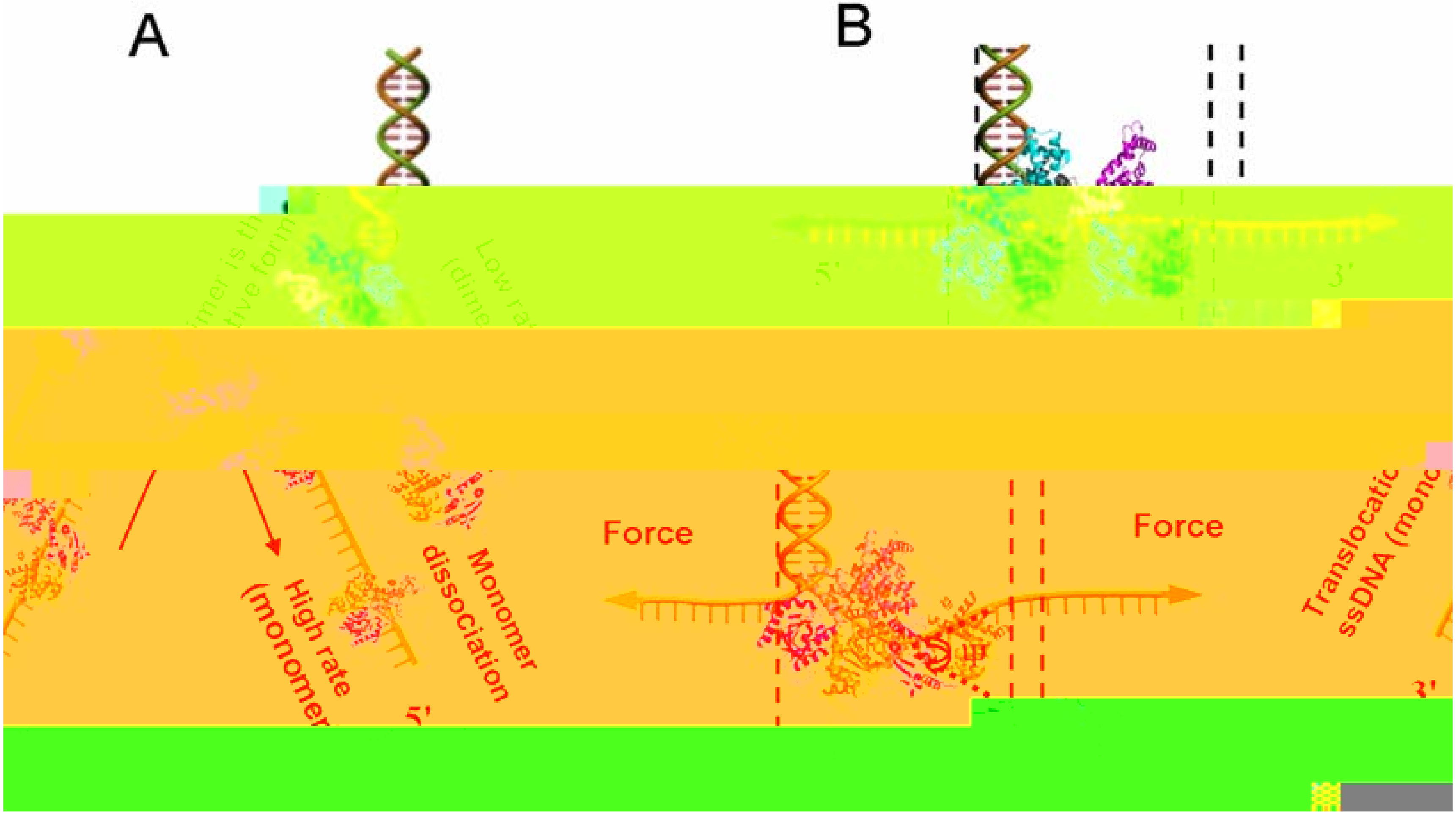




Autoinhibitory 2B domain must be released to activate the helicase.



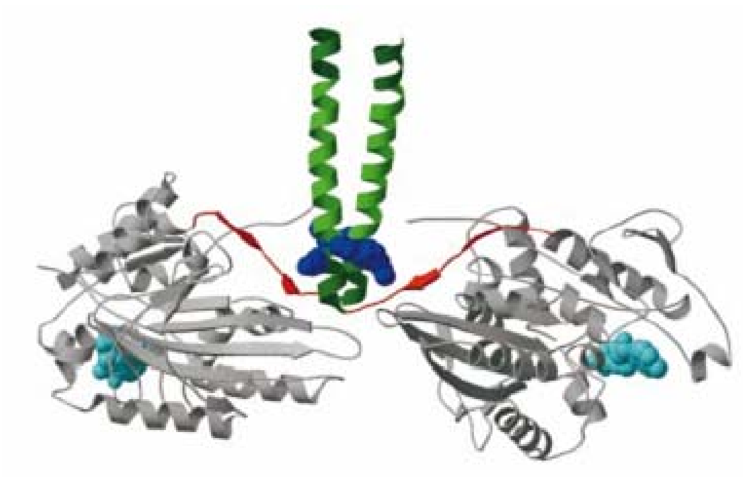
Proposed molecular mechanism of UvrD



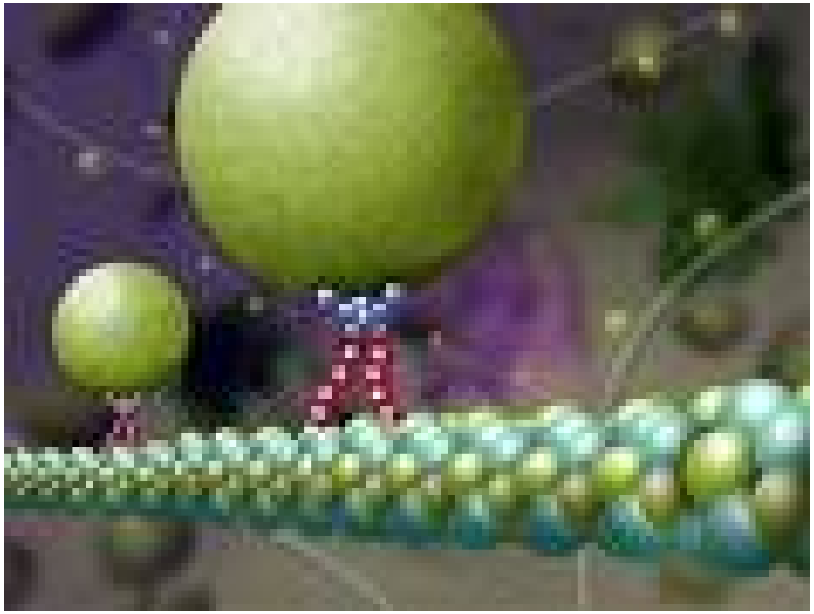
Sun *et al*. EMBO Journal 2008, 27, 3279

Thermodynamics of Kinesin

Kinesin is a motor protein moving along microtubule
toward the plus end

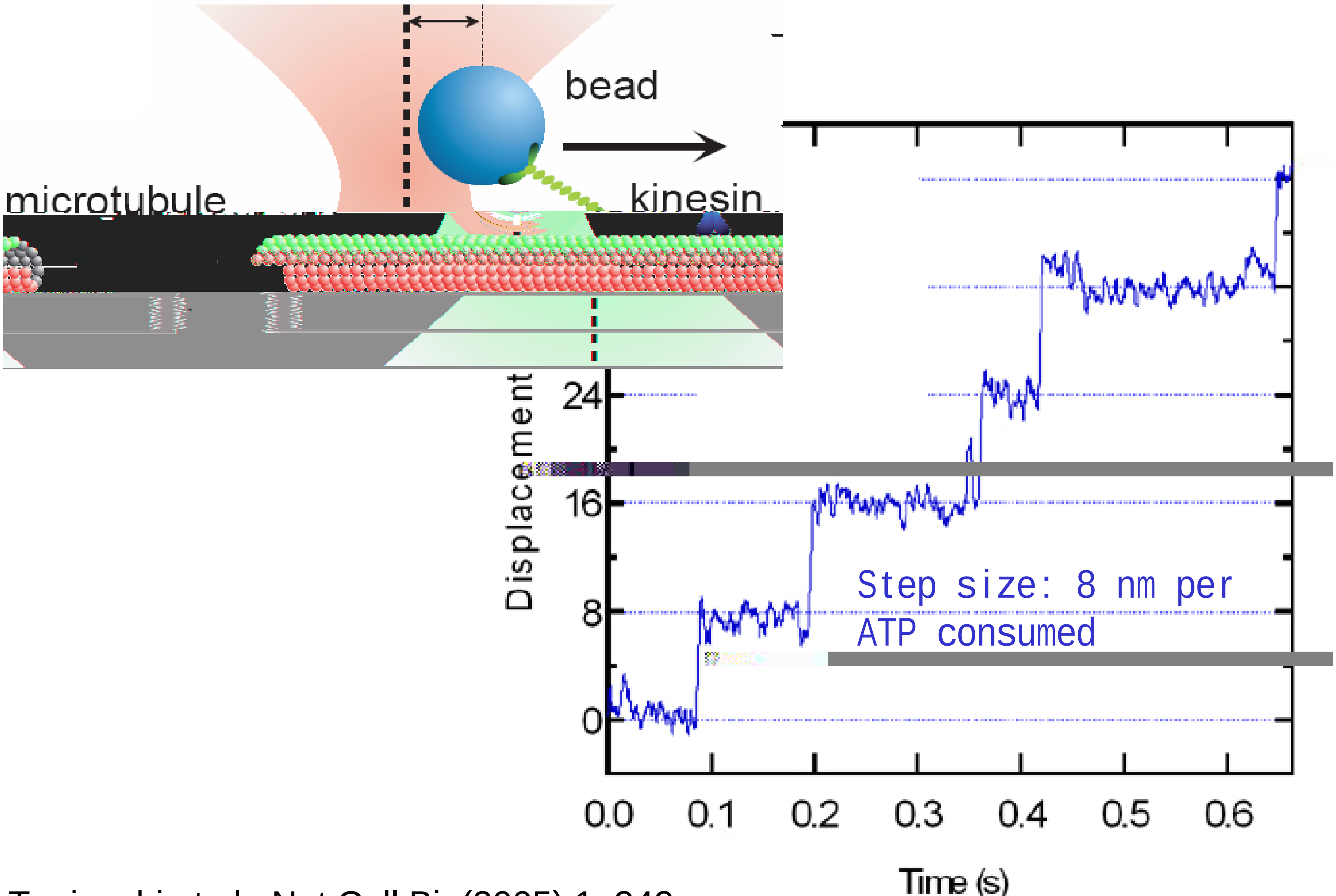


kinesin



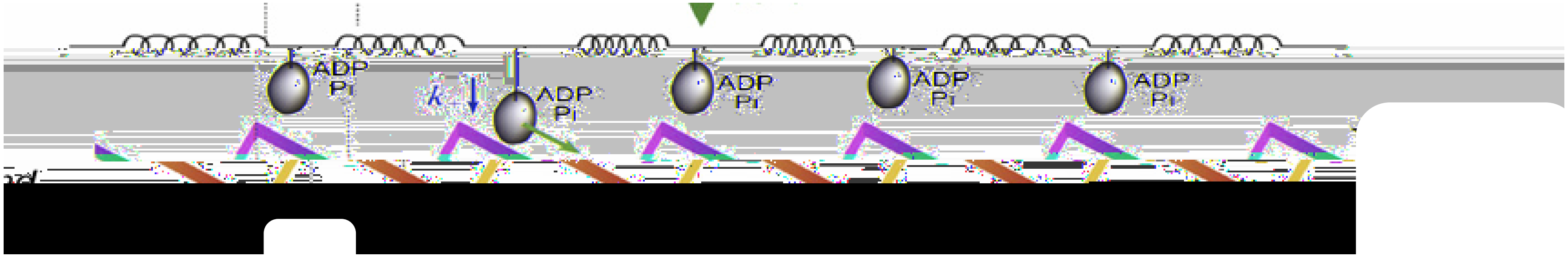
Walking on a microtubule

Stepwise movement of kinesin

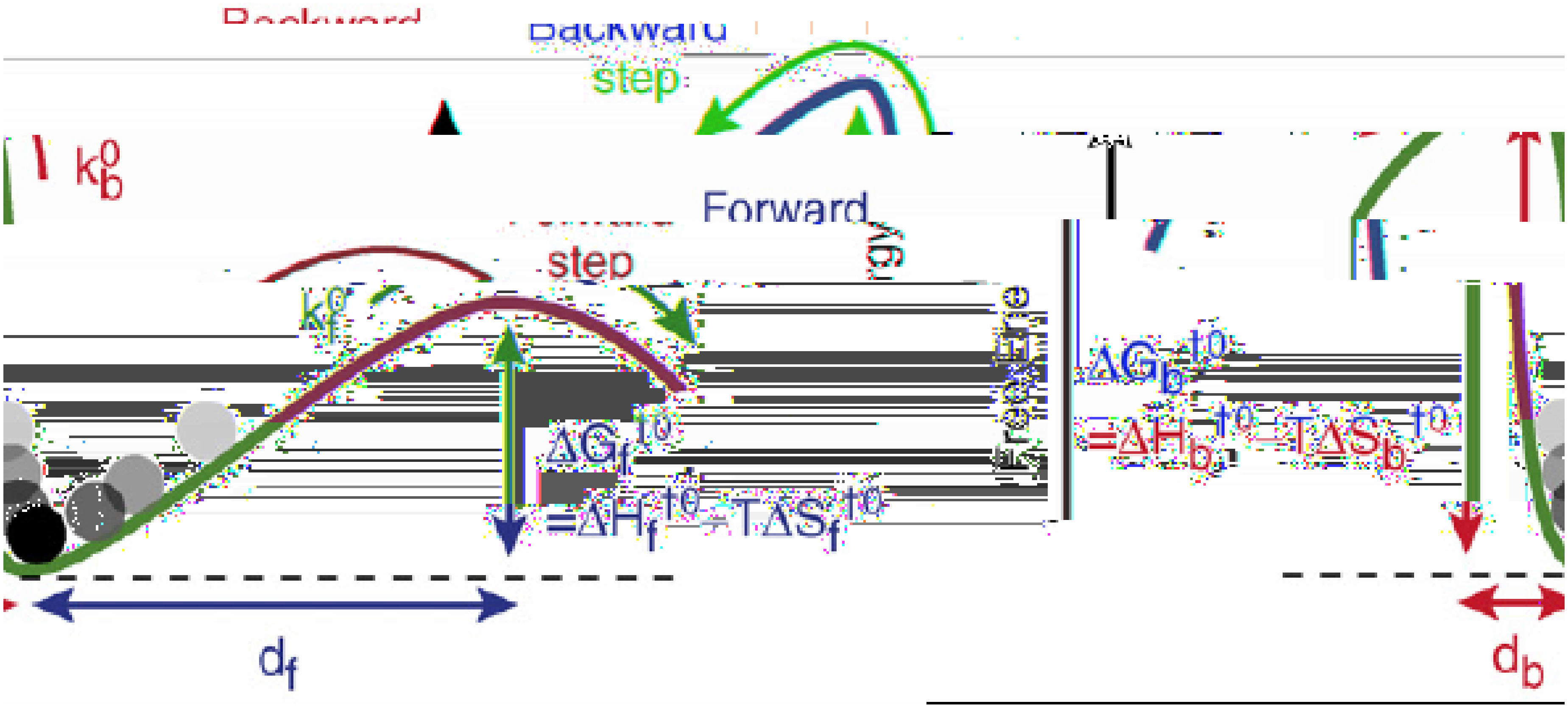


Taniguchi et al., Nat Cell Bio(2005) 1, 342

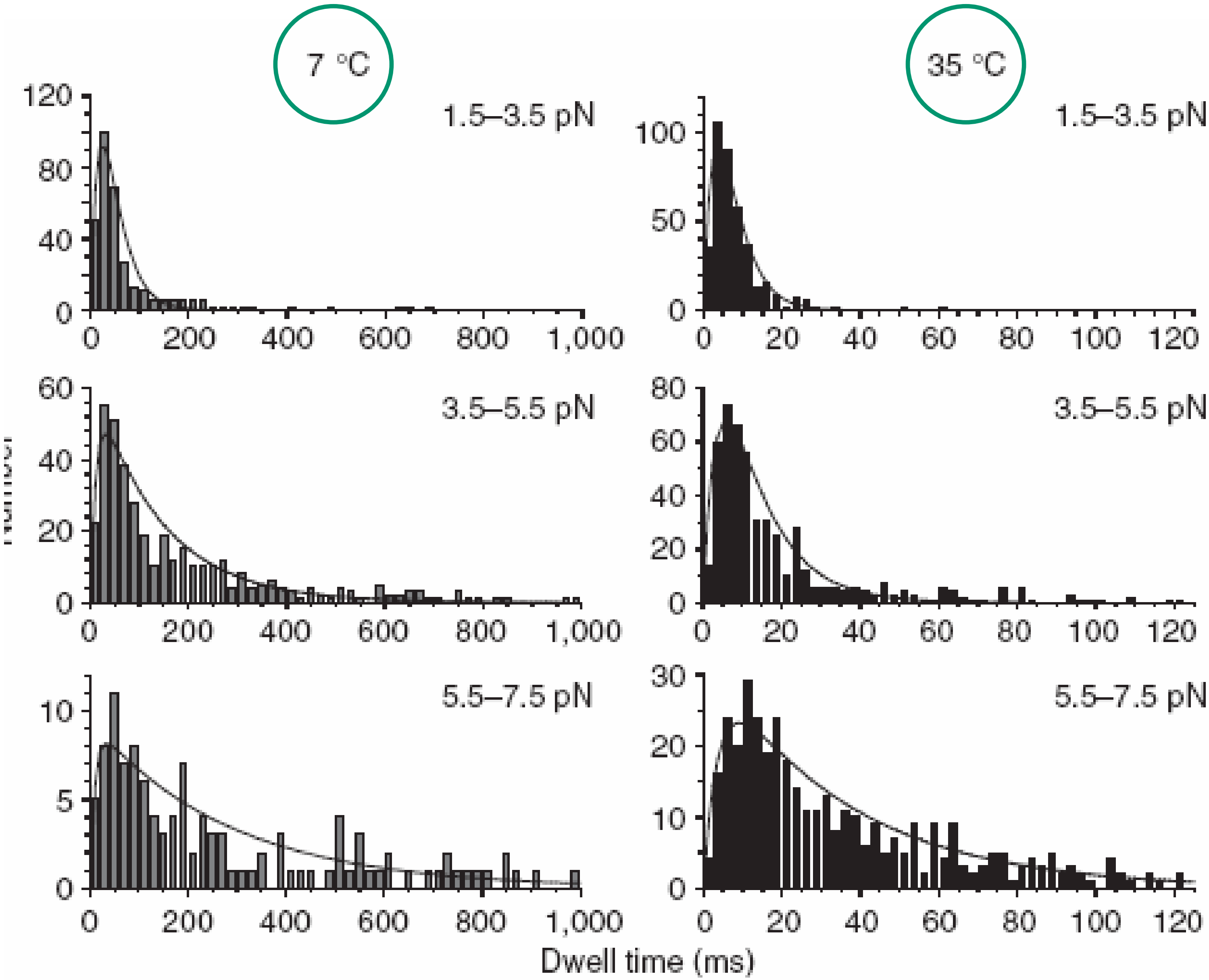
rachet



Can we measure the free energy landscape?
What drives the motor? enthalpy or entropy?



Dwell time distributions…, not exponential *!*



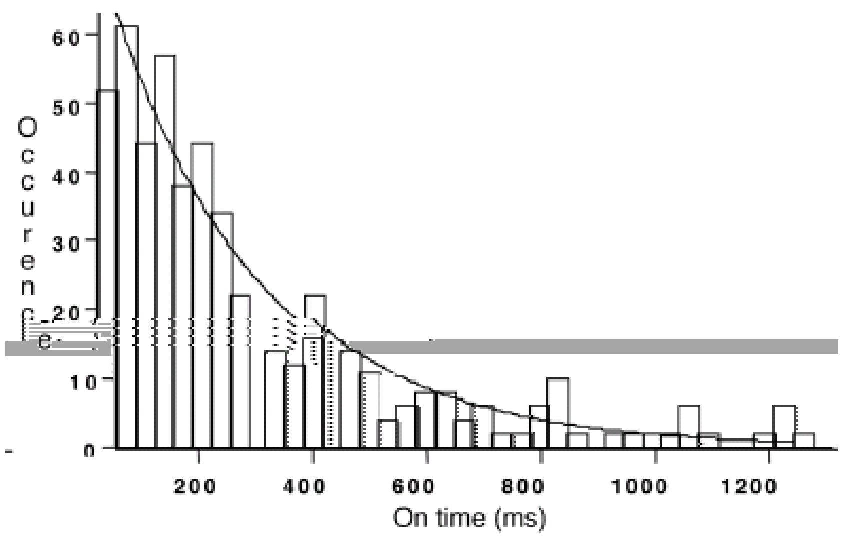
Single-Molecule Enzymatic Dynamics

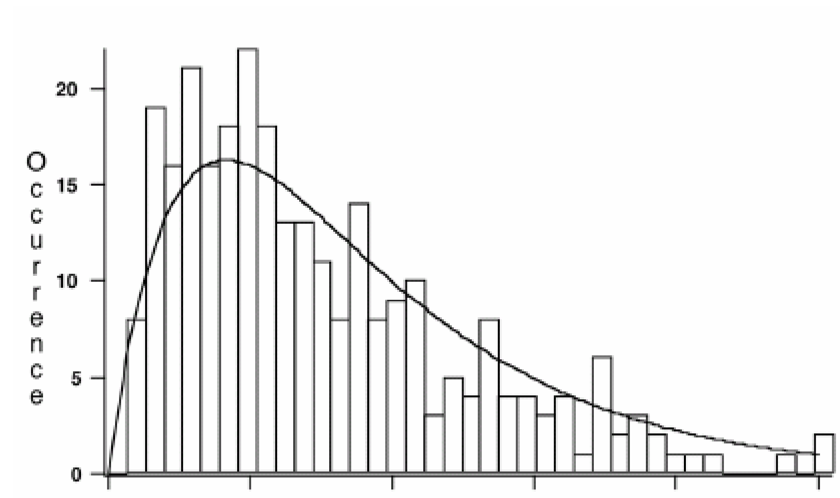
H. Peter Lu, Luying Xun, X. Sunney Xie*

SCIENCE VOL 282 4 DECEMBER 1998

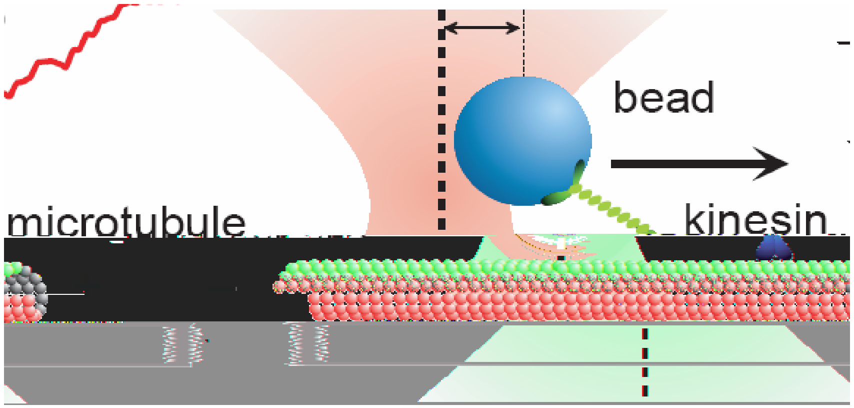
REPORTS 1877

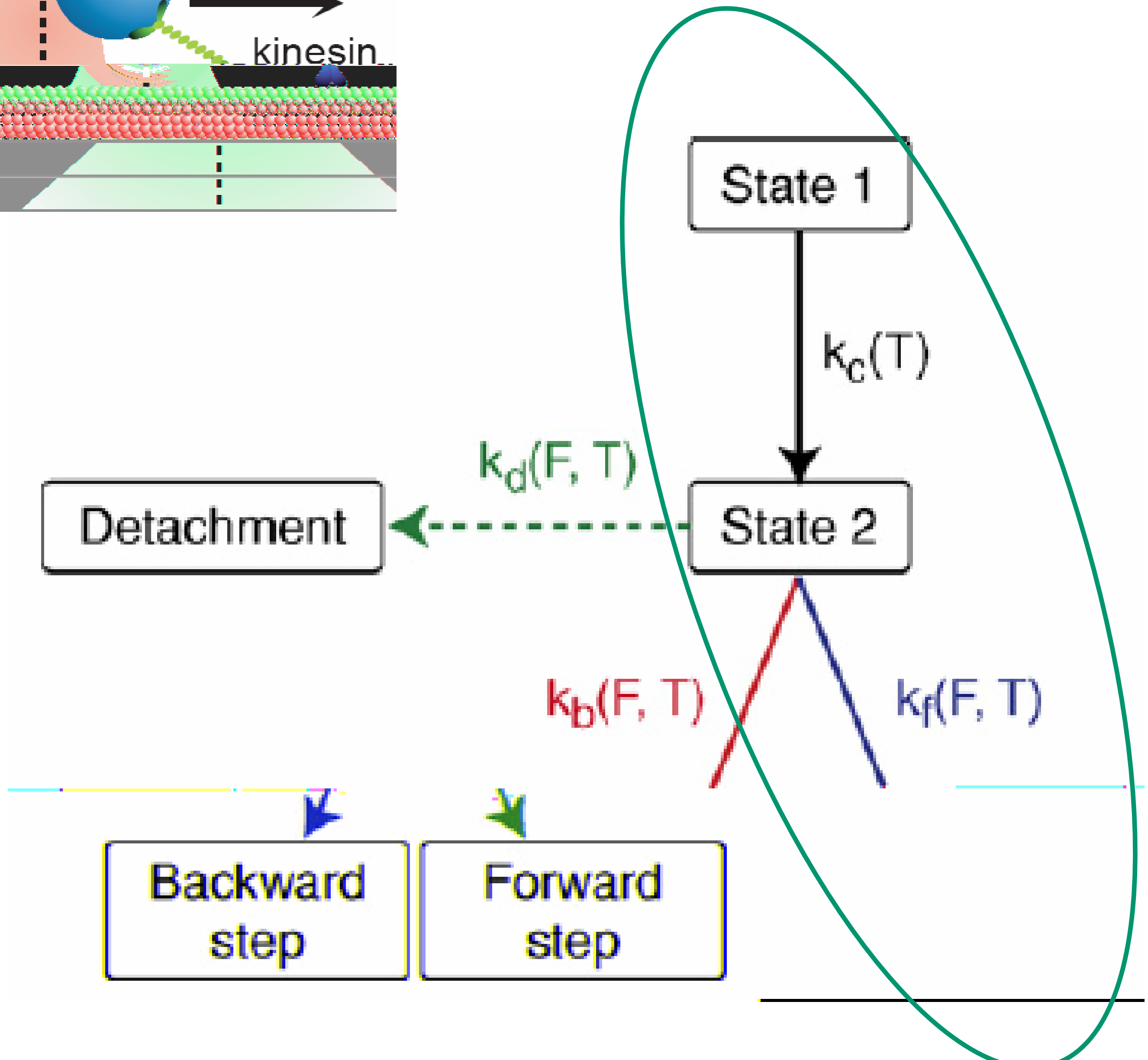
$$state1 \xrightarrow{k_1} state2$$



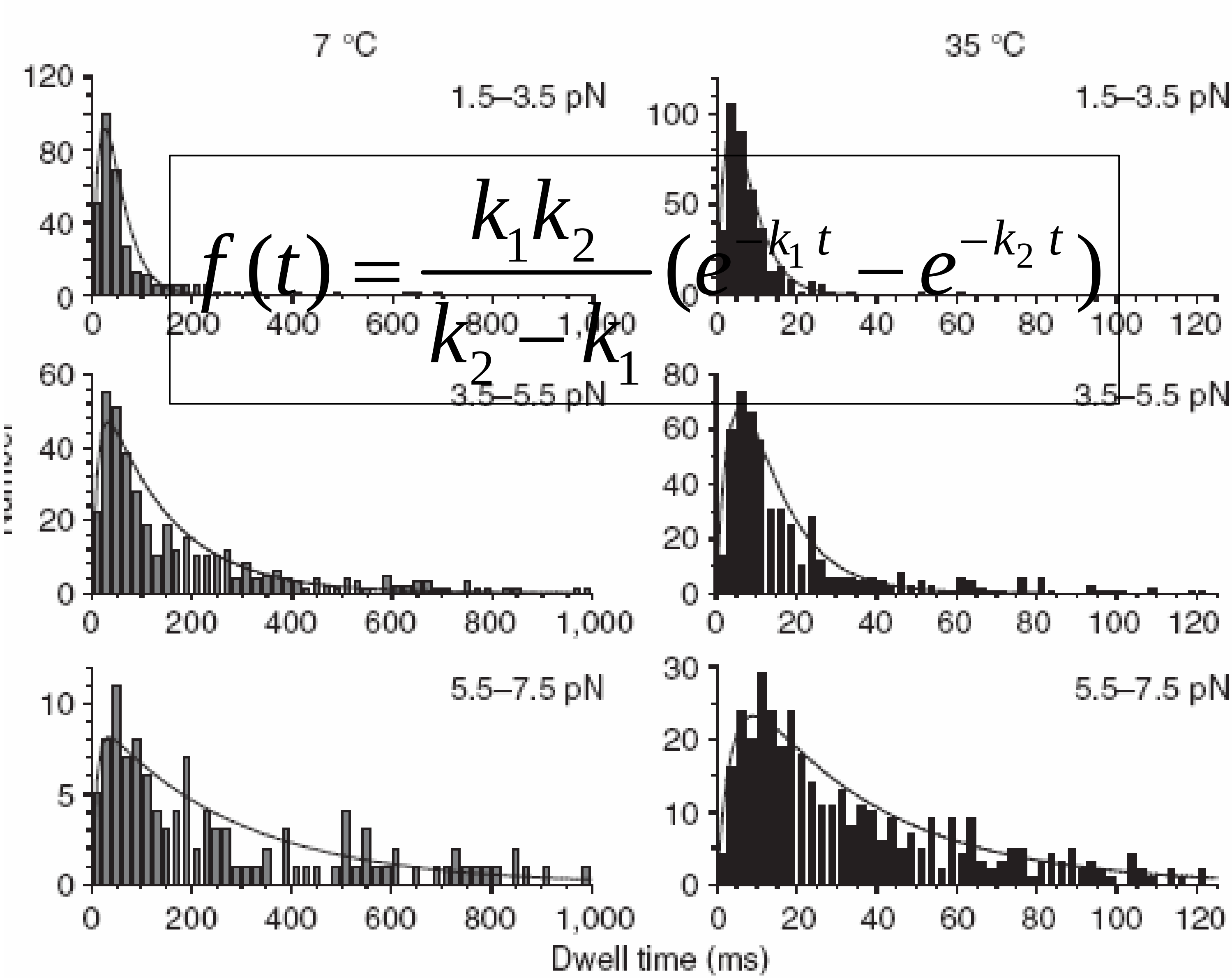


$$f(t) = \frac{k_1 k_2}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right)$$


bead
microtubule
kinesin

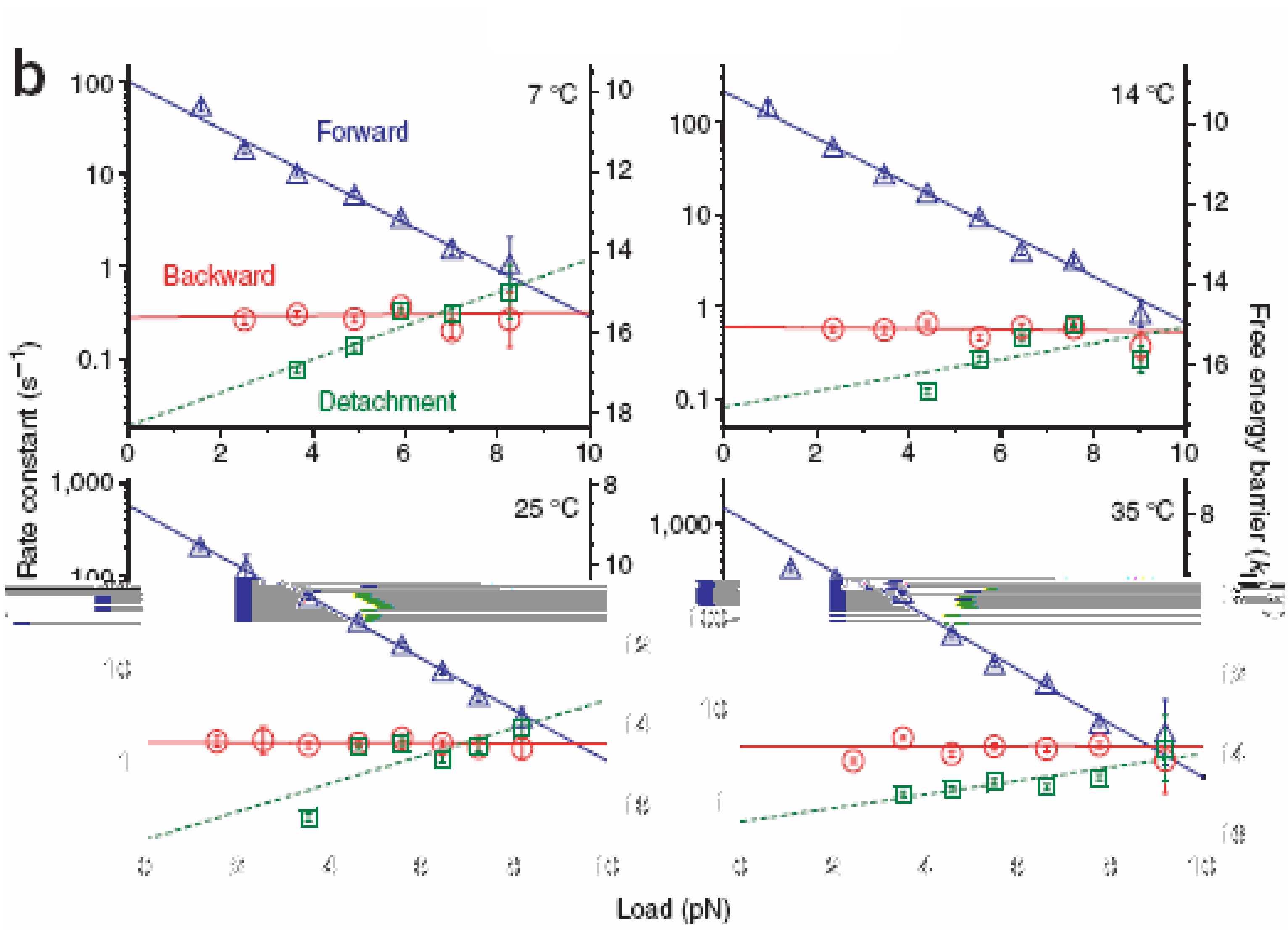

State 1
$k_c(T)$
$k_d(F, T)$
Detachment
State 2
$k_b(F, T)$
$k_f(F, T)$
Backward step
Forward step

Dwell time distributions ➔ rate constants



$$f(t) = \frac{k_1 k_2}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right)$$

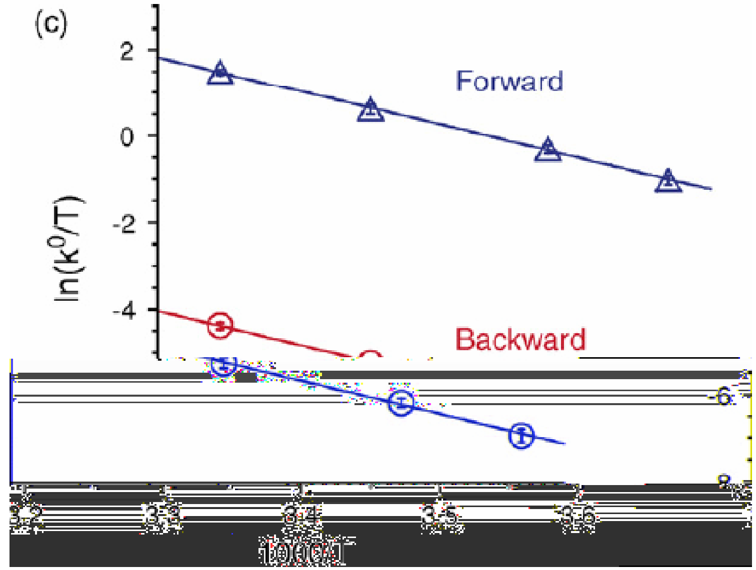
$$k(F,T) = A\cdot T e^{\left(-\frac{\Delta G(T)+Fd}{k_B T}\right)} \Rightarrow k(0,T) = A\cdot T e^{\left(-\frac{\Delta G(T)}{k_B T}\right)}$$



$$k(0,T) = A \cdot T e^{\left(-\frac{\Delta G(T)}{k_B T}\right)}$$

$$\ln\left(\frac{k_f(0,T)}{T}\right) = \ln(A) + \frac{\Delta S_f}{k_B} - \frac{\Delta H_f}{k_B T}$$

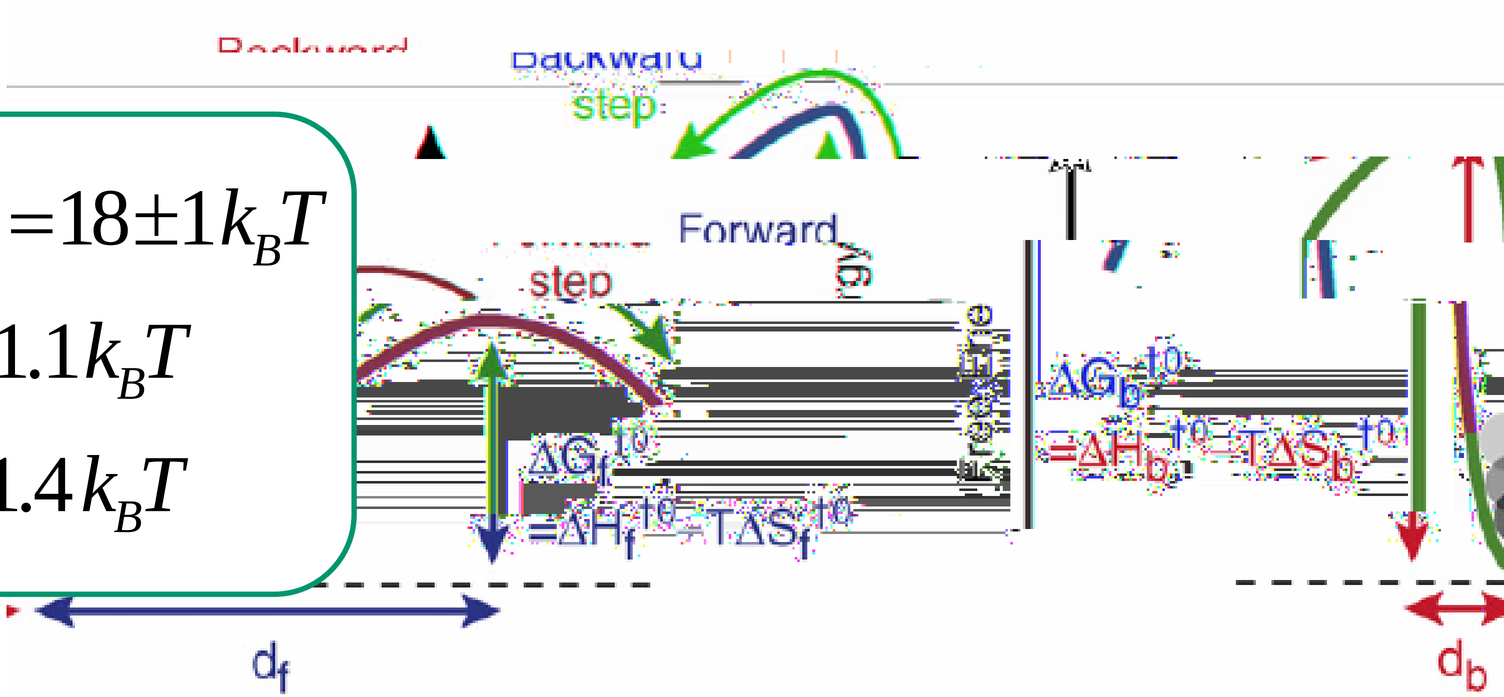
$$\ln\left(\frac{k_b(0,T)}{T}\right) = \ln(A) + \frac{\Delta S_b}{k_B} - \frac{\Delta H_b}{k_B T}$$



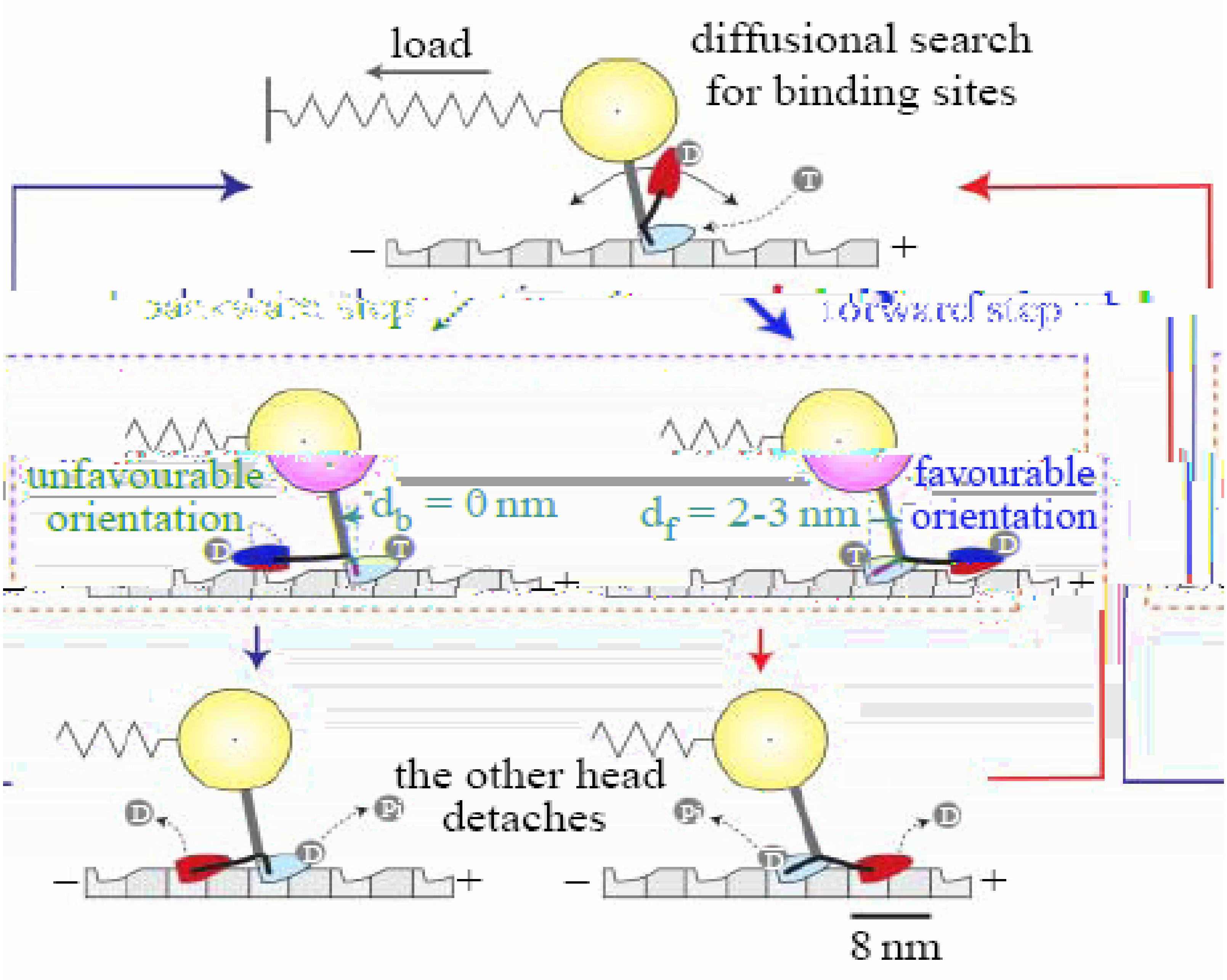
$$\Delta H_f = \Delta H_b = 18 \pm 1 k_B T$$

$$\Delta S_f = 9.9 \pm 1.1 k_B T$$

$$\Delta S_b = 3.9 \pm 1.4 k_B T$$



Mechanism of stepping



Summary

- Single molecule biological physics is an efficient pathway towards quantitative life science.
- It opens the door for physicists to enter the biological realm quickly.
- Its language can be understood by both the physicists and the biologists.
- It is a nice choice for people who love bio-x.

Acknowledgement

http://softmatter.iphy.ac.cn

Thank you!

In collaboration with Dr. XG Xi of the Institut Curie, France

UvrD Helicase Unwinds DNA One Base Pair at a Time by a Two-Part Power Stroke

Jae Young Lee[1] and Wei Yan

Cell *127*, 1349–1360, December 29, 2006 ©2006 Elsevier Inc. 1349