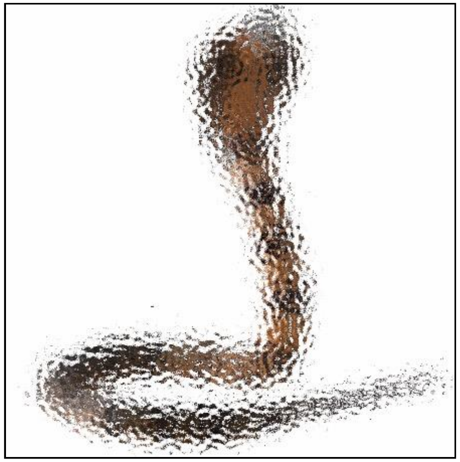
SINGLE MOLECULE TECHNIQUES

Roop Mallik
ASET Colloquium, TIFR, Feb 16, 2018

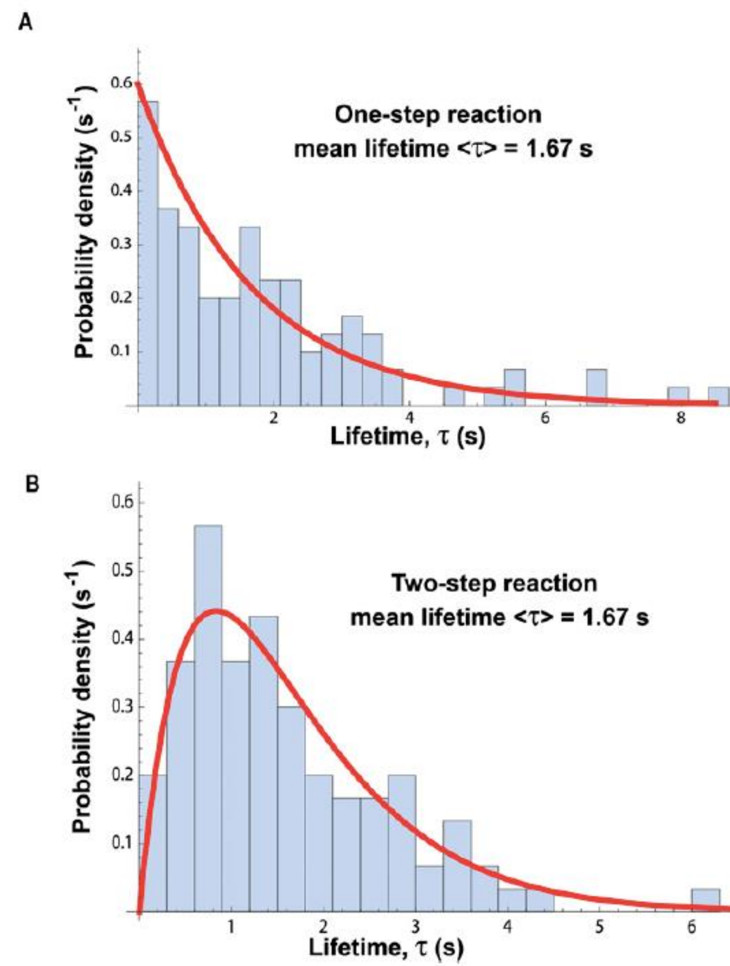
Outline

- ✓ Why Single Molecule
- ✓ Why NOT Single Molecule
- ✓ What Molecules ?
- ✓ Kinds of SM experiments
  - ➢ Imaging (Heisenberg ?) and Manipulation
- ✓ Examples
  - ➢ Ion channels
  - ➢ RNA Polymerase
  - ➢ Ribosome
  - ➢ Translational Motors

Problem of Synchronization and “blurring”

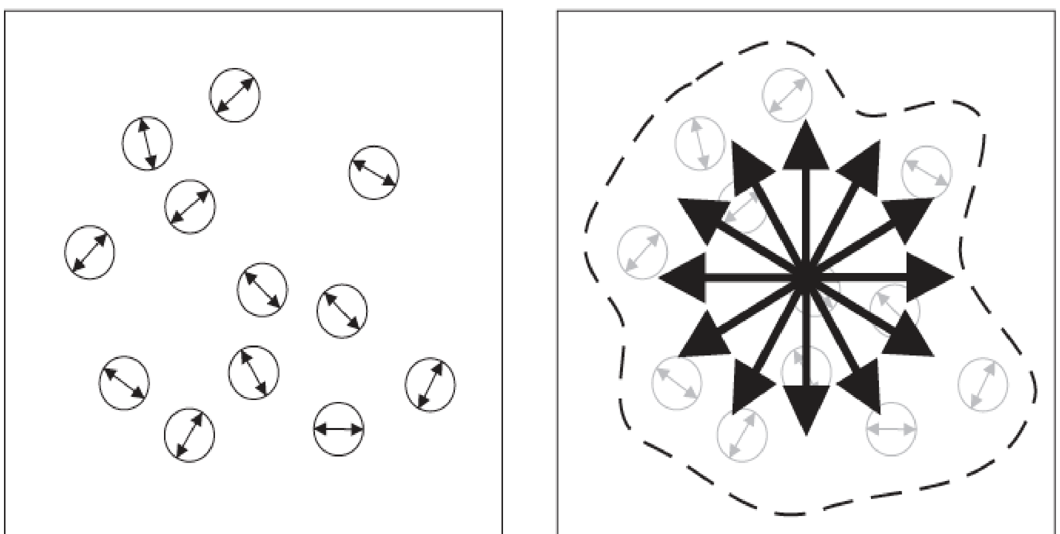


Histograms …
Individuality within the crowd



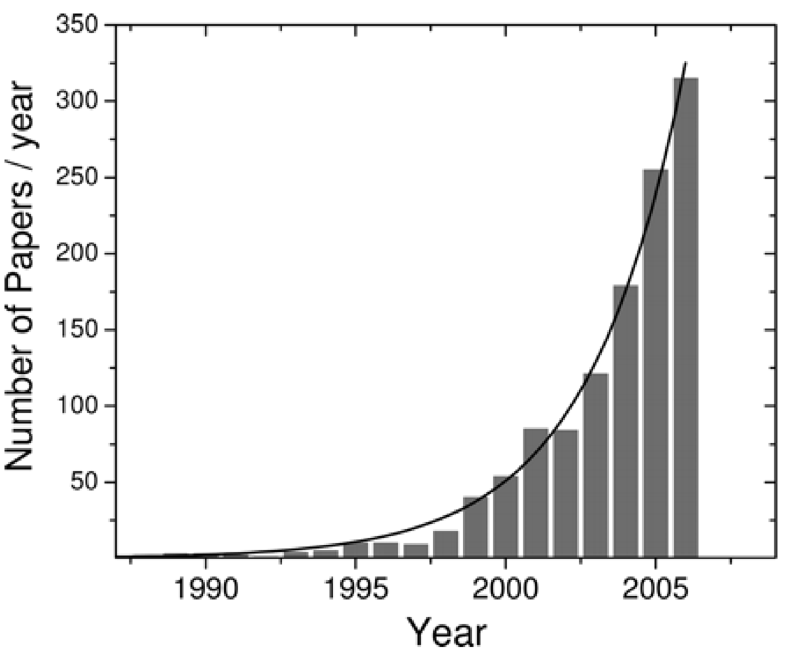
Tinoco et al, 2011

Single Molecule Biology, Knight



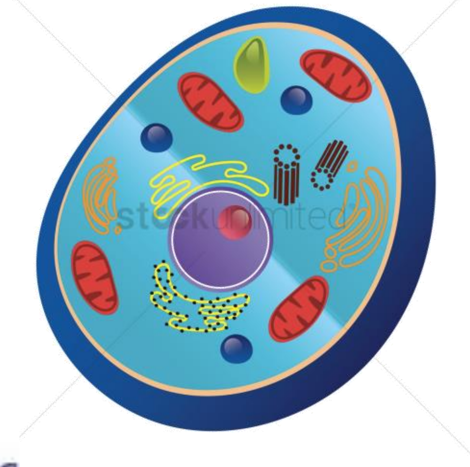
What can you do with SM

- Mechanical Properties of Bio Molecules
- Conformational changes
- Mechanochemical Coupling of enzymes (Vector properties eg Force)
- Folding pathways, Transition states along the way
- Enzyme stochasticity and Population heterogeneity



Why not *just* Single ?

10x10x10 (microns)



Inside Cells
$C_{PROTEINS} \approx 0.2$ gm/mL

$3 \times 10^6 \frac{\text{proteins}}{\mu m^3}$

organism	characteristic volume	number of proteins
E. coli	≈ 1 μm^3	≈ 3×10^6
budding yeast	≈ 30 μm^3	≈ 100×10^6
HeLa cell line	≈ 3,000 μm^3	≈ 10×10^9

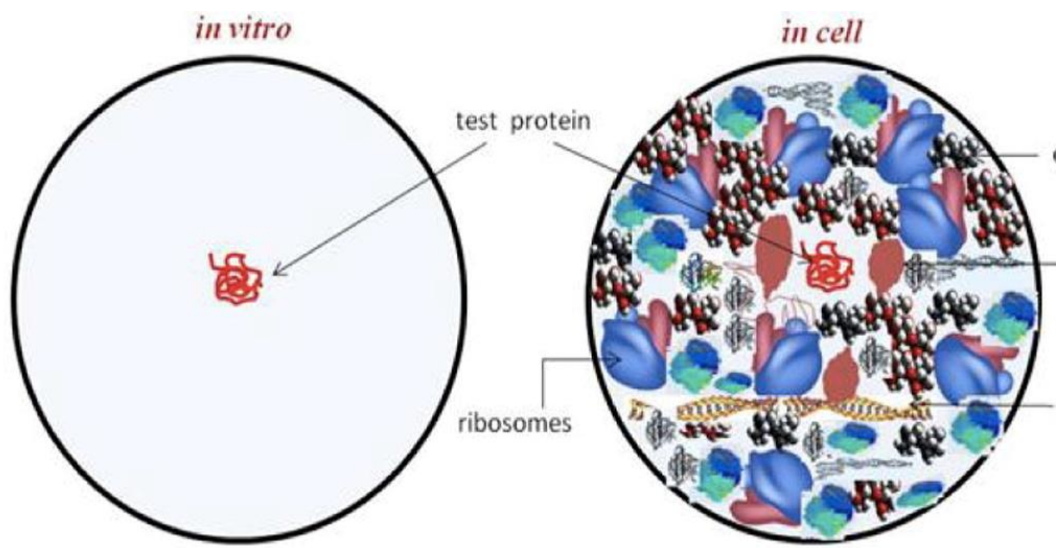
R. Milo, Bioessays 2013

Shahid, BBA 2017

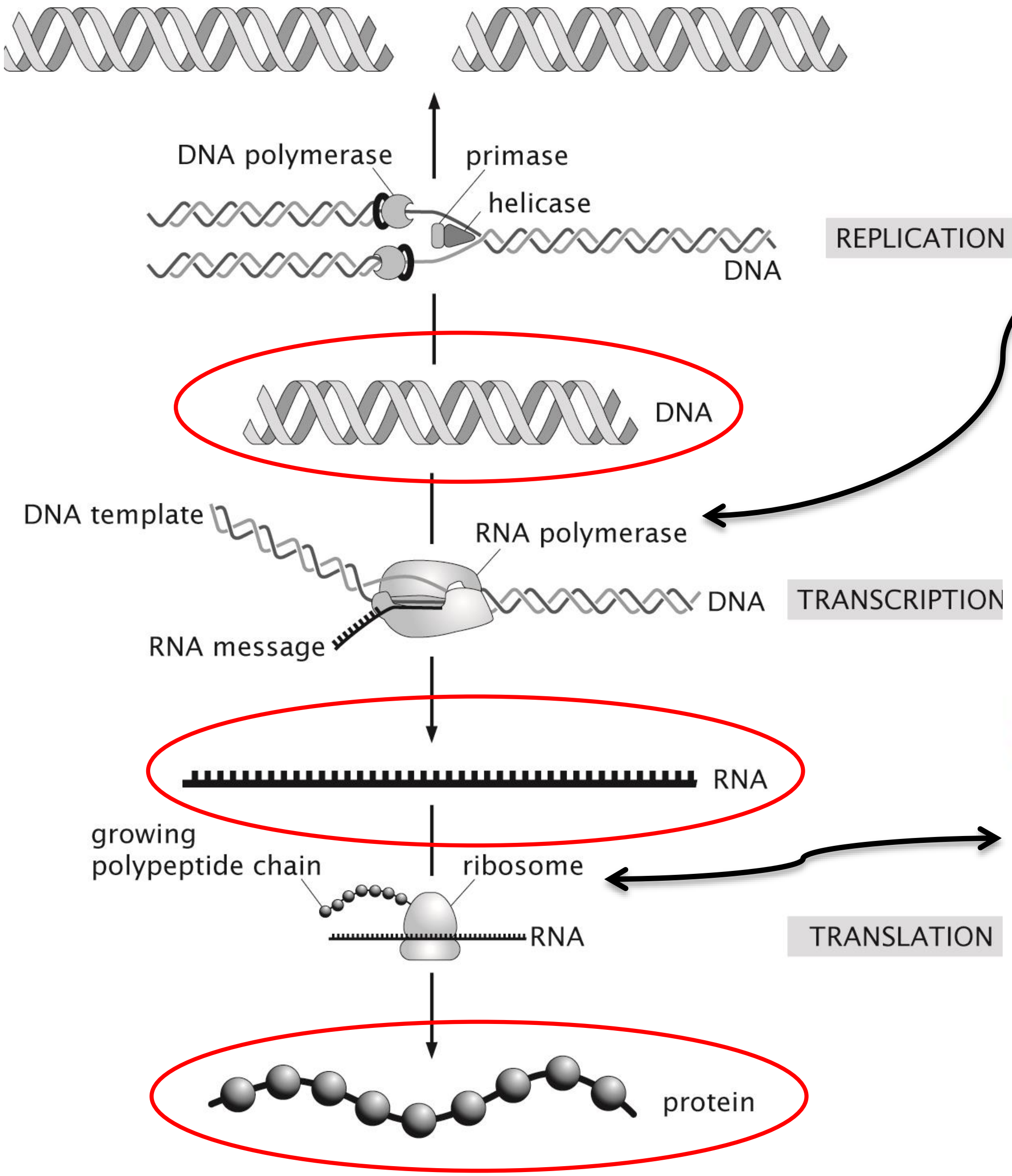
On average, a protein has 300nm^3
Available to itself

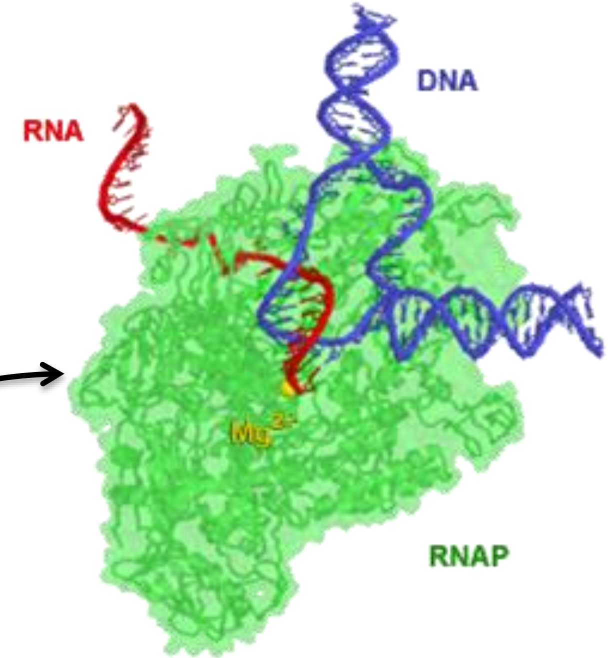
$\sqrt[3]{300} \sim 7$nm

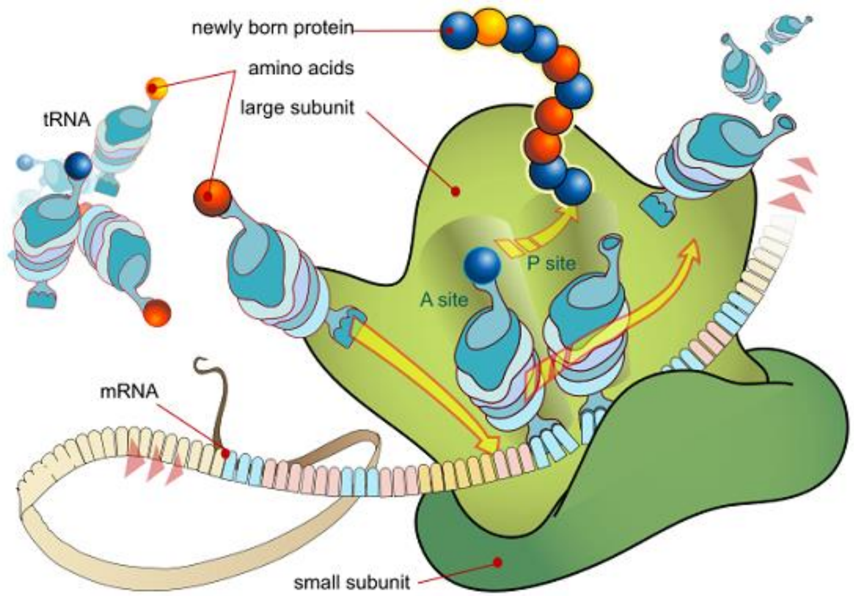
From EM images, proteins range from 10-100 nm in size



Central dogma and the Molecules







Google images

History

*MEASUREMENT OF ACTIVITY OF SINGLE MOLECULES OF β-D-GALACTOSIDASE**

BY BORIS ROTMAN†

DEPARTMENT OF GENETICS, STANFORD UNIVERSITY MEDICAL SCHOOL, PALO ALTO, CALIFORNIA

Communicated by Joshua Lederberg, October 16, 1961   PNAS

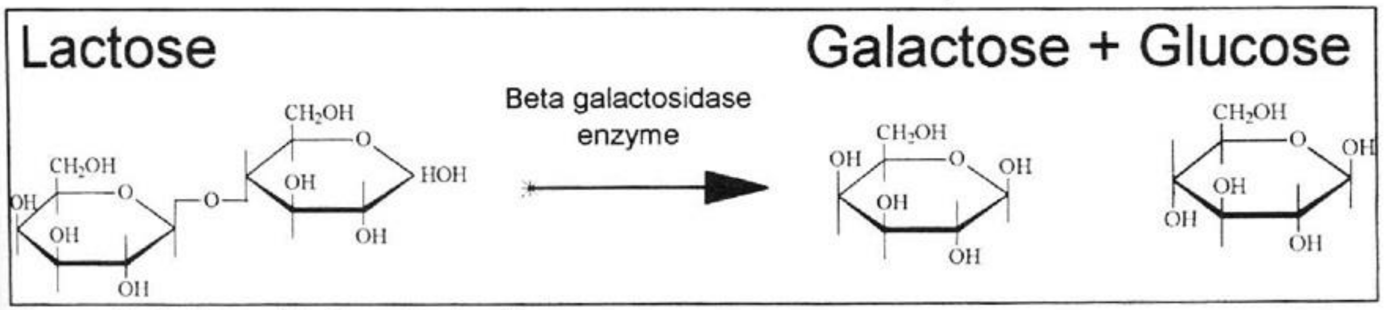
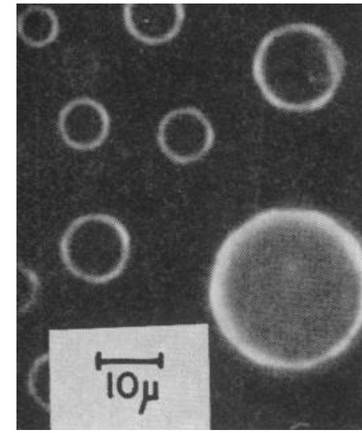


Figure 1: Action of beta-galactosidase



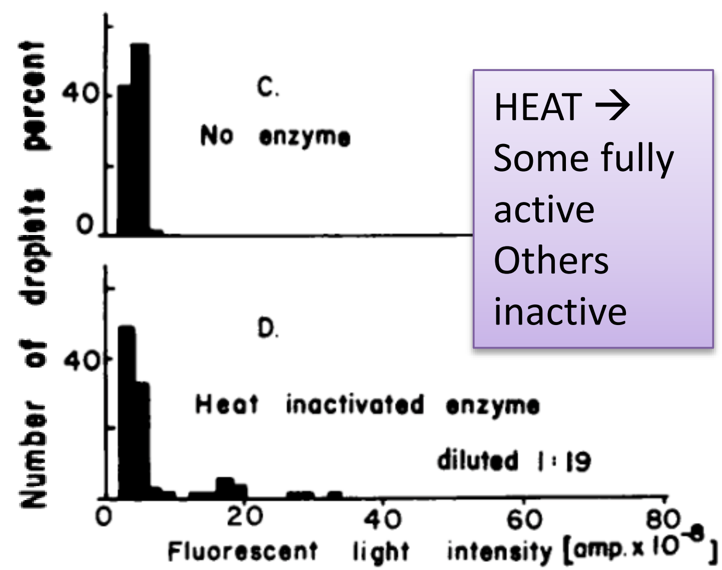
Enclose in oil droplets with fluorogenic substrate
Measure fluorescence

λ = Average number of Enzymes/Droplet

$$P(X=x)=\frac{e^{-\lambda}\lambda^{x}}{x!}$$

Then, prob of finding x enzymes in droplet

Fluorescence in droplets was Poisson distributed



HEAT → Some fully active
Others inactive

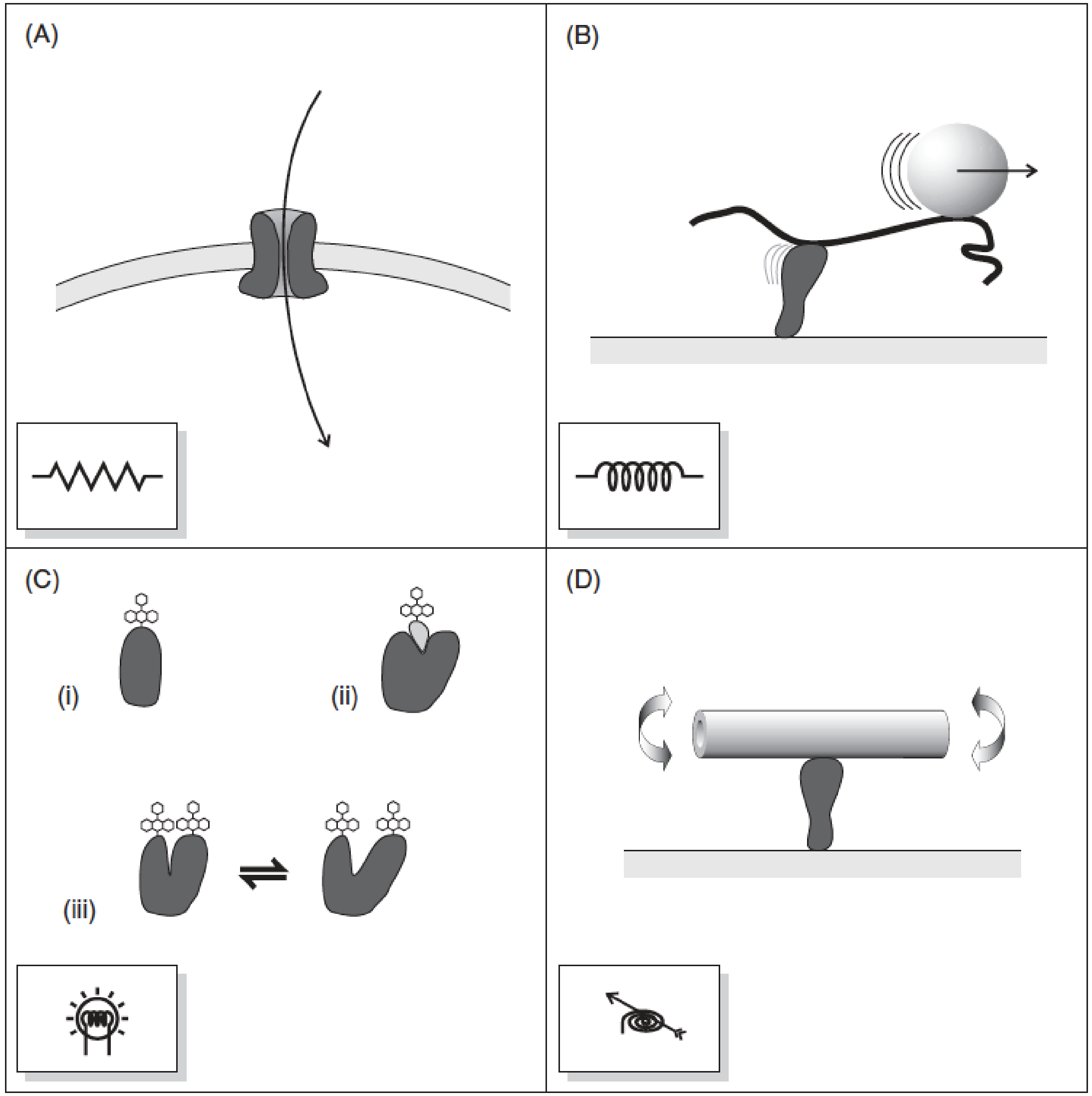
Types of SM studies

Ion Channels

Measure
+
Manipulate

AFM,
Optical Tweezer
Magnetic Tweezer

Measure
+
Manipulate



Single molecule
Fluorescence

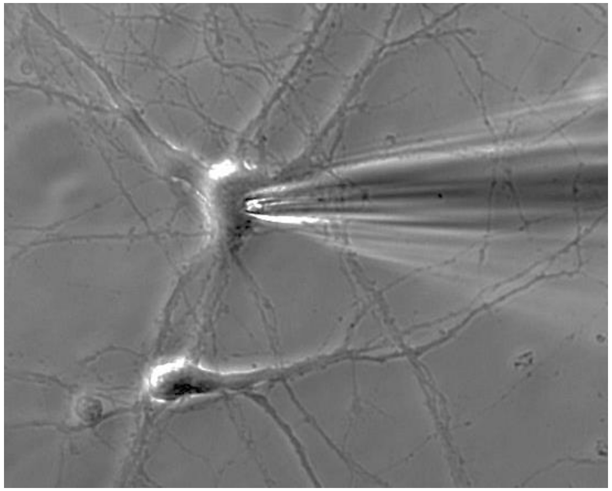
Watching
SPT, FCS, FRET

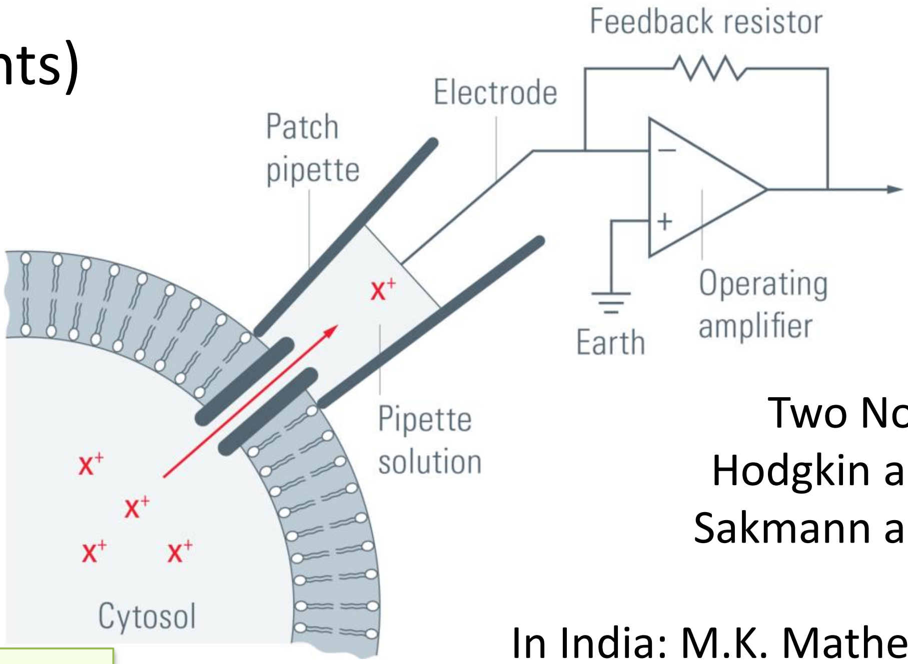
Large marker on
Small molecule

Watch Rotation
Diffusion etc.

Single Molecule Biology, Acad Press (Ed. Knight)

Ion channel (Measure currents)





Two Nobel Prizes
Hodgkin and Huxley
Sakmann and Neher

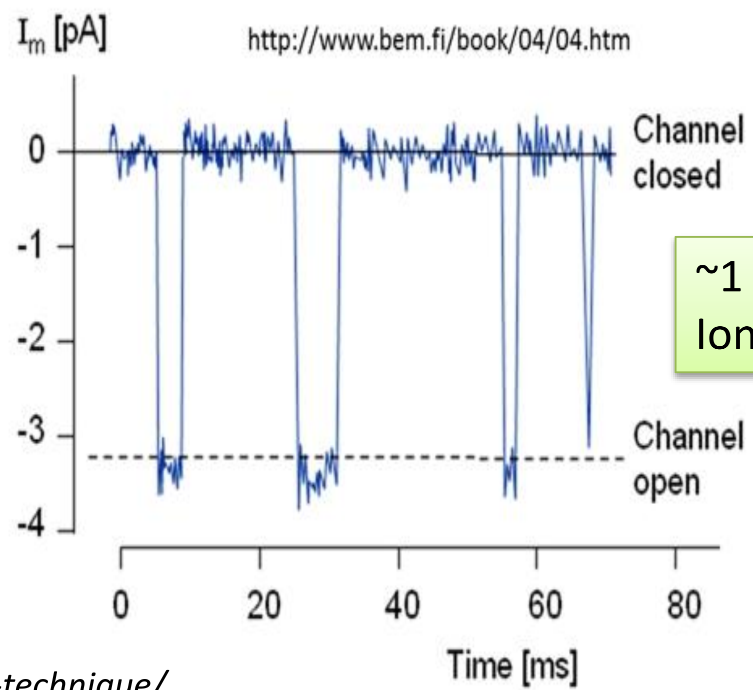
In India: M.K. Mathew (NCBS)

Snippet

Nav1.7 sodium channel target for new analgesics

Mutations → Complete inability to sense pain

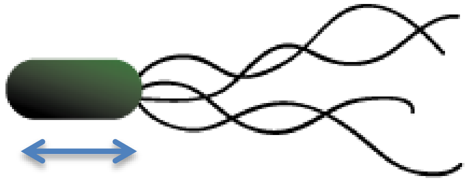
Patch clamp recording to screen for drugs that can block this channel and relieve pain

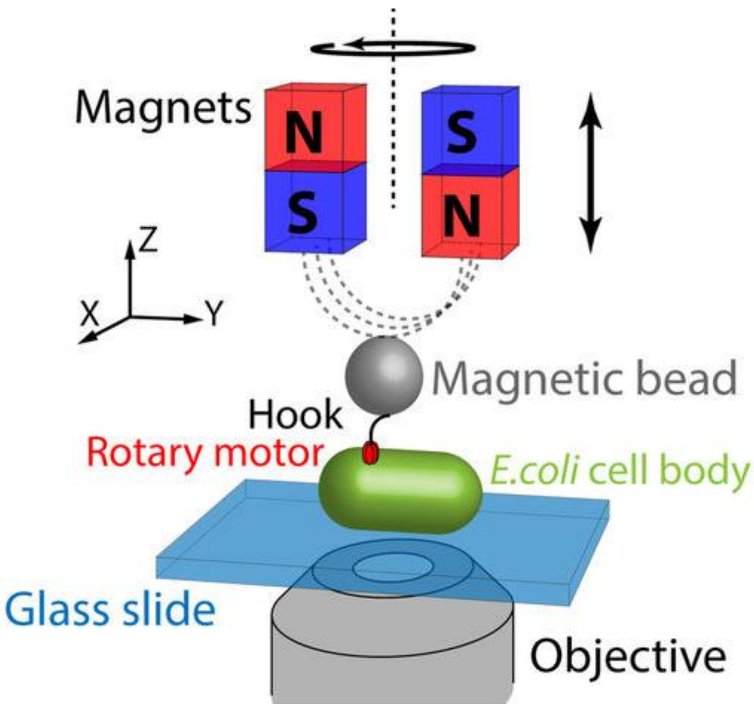


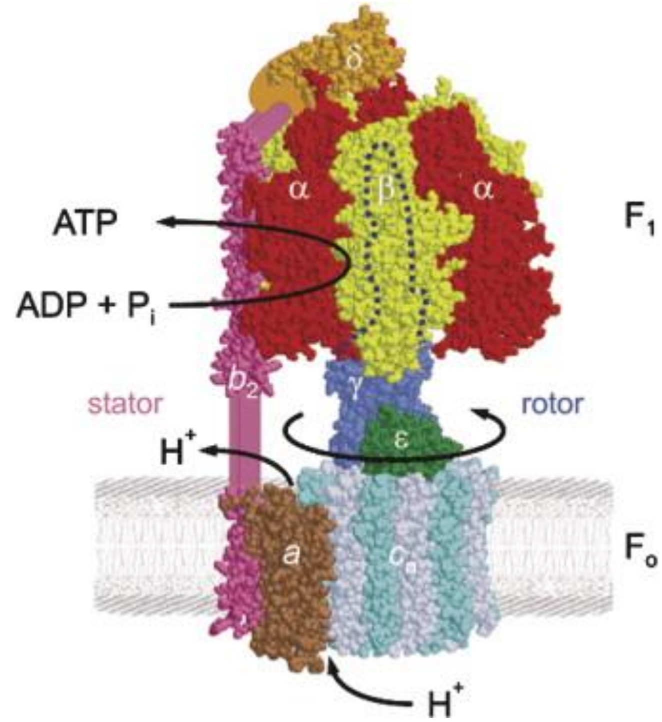
~1 million Ions/sec

www.leica-microsystems.com/science-lab/the-patch-clamp-technique/

Watching Rotation (Flagellar Motor)







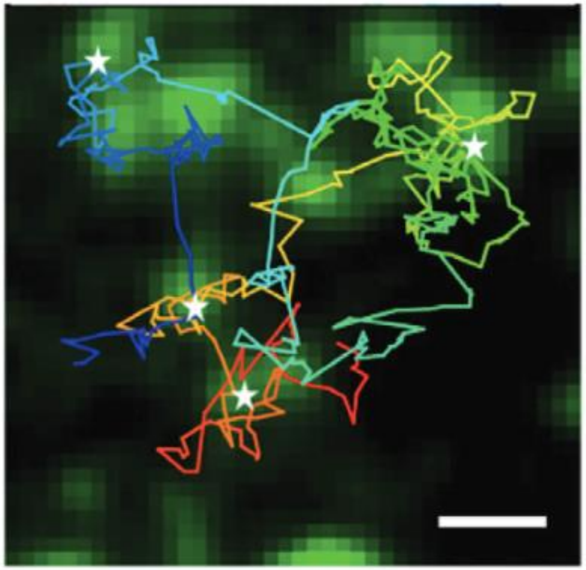
Work = *F R* θ = *T* θ

Torques are ~1000 pN-nm
Rotates at 18,000 rpm

Maarten M. van Oene,
SciRep 2017

SINGLE PARTICLE TRACKING

J. Phys. D: Appl. Phys. **49** (2016)



Interaction of voltage-gated potassium channels with clathrin-coated pits (CCPs) →

Cargoes and CCPs mature before internalization

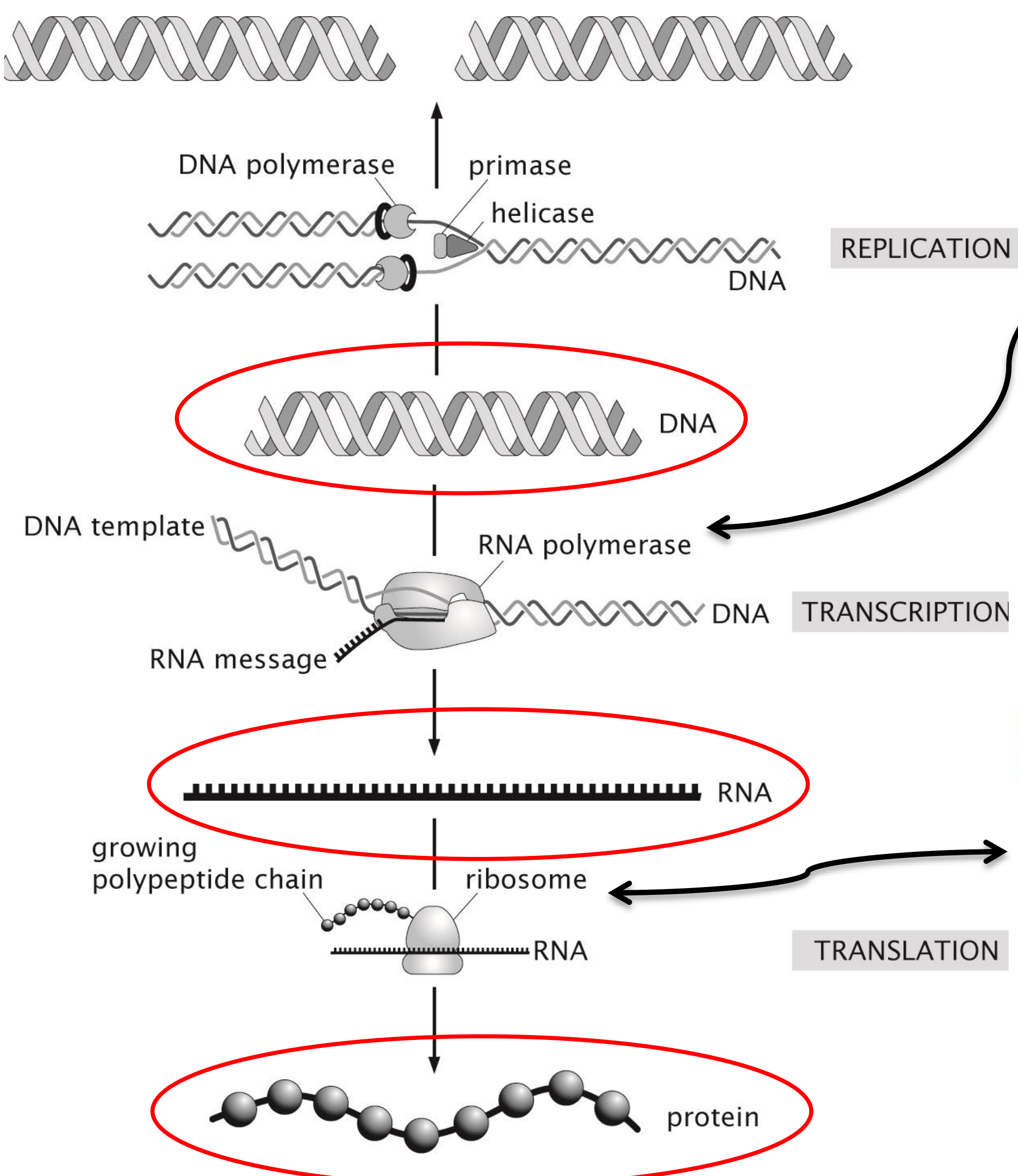
Does this localization even make sense ?

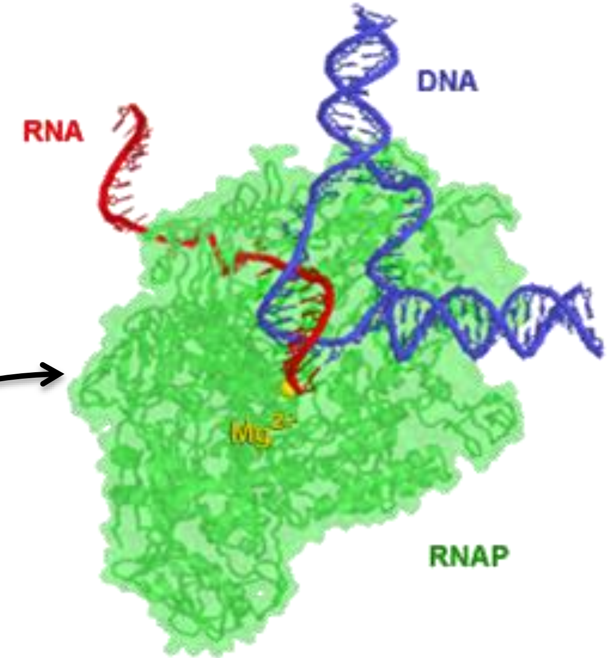
$$\Delta X \sim h / (m \, \Delta V)$$

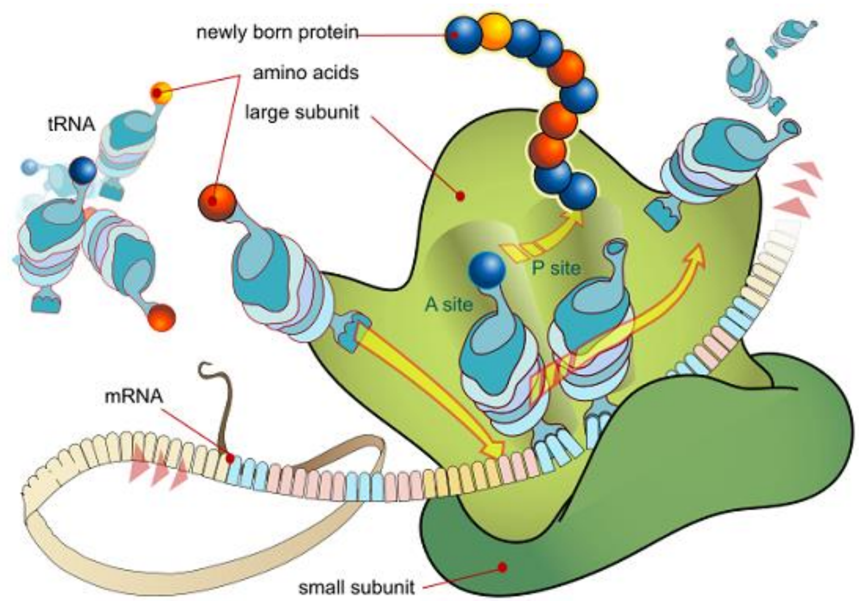
Use $\Delta V = 0.1 \times V$

Particle	**Mass (kg)**	**ΔV (m/sec)**	**ΔX (m)**
Electron	10^{-31}	10^{-6} (Using Drift Vel)	10^{3}
Protein (50 KDa)	10^{-15}	10^{-7} (Using Motor Vel)	10^{-12} (~0.001 nm)
Cricket ball	0.1	3 (Average of K. Yadav & B. Kumar	10^{-33}

Central dogma and the Molecules



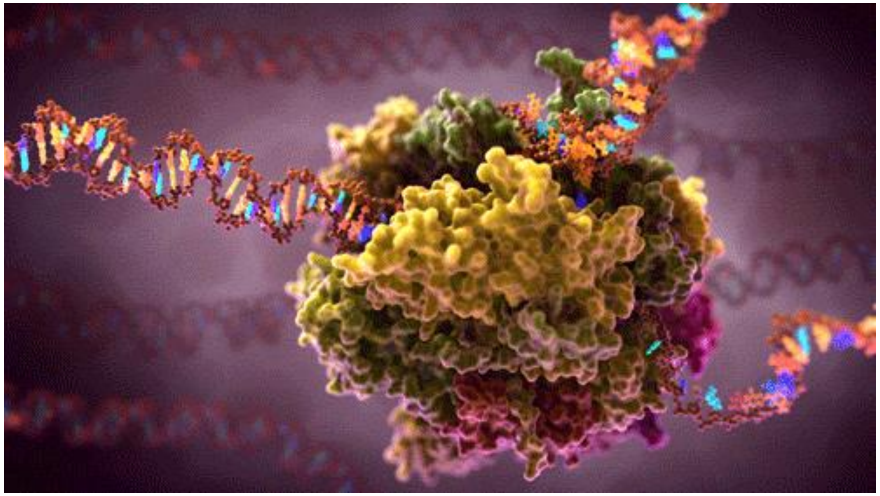


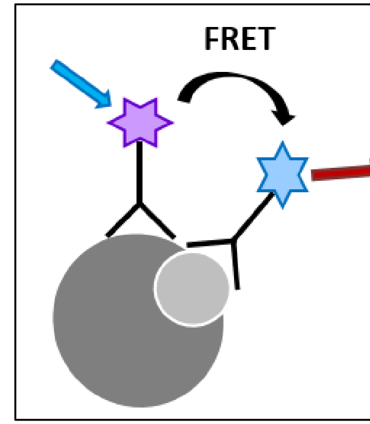


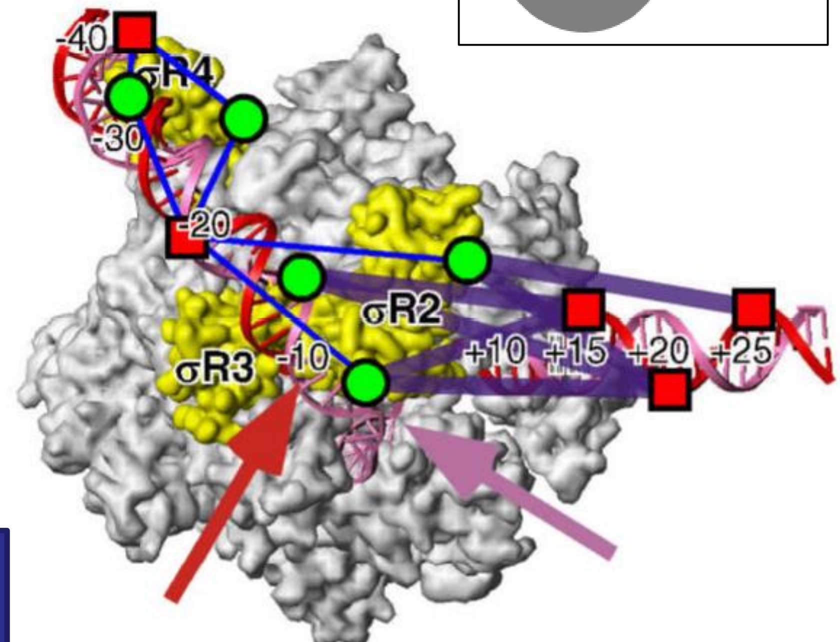
Google images

"FRET" ting over RNA Polymerase

https://giphy.com/





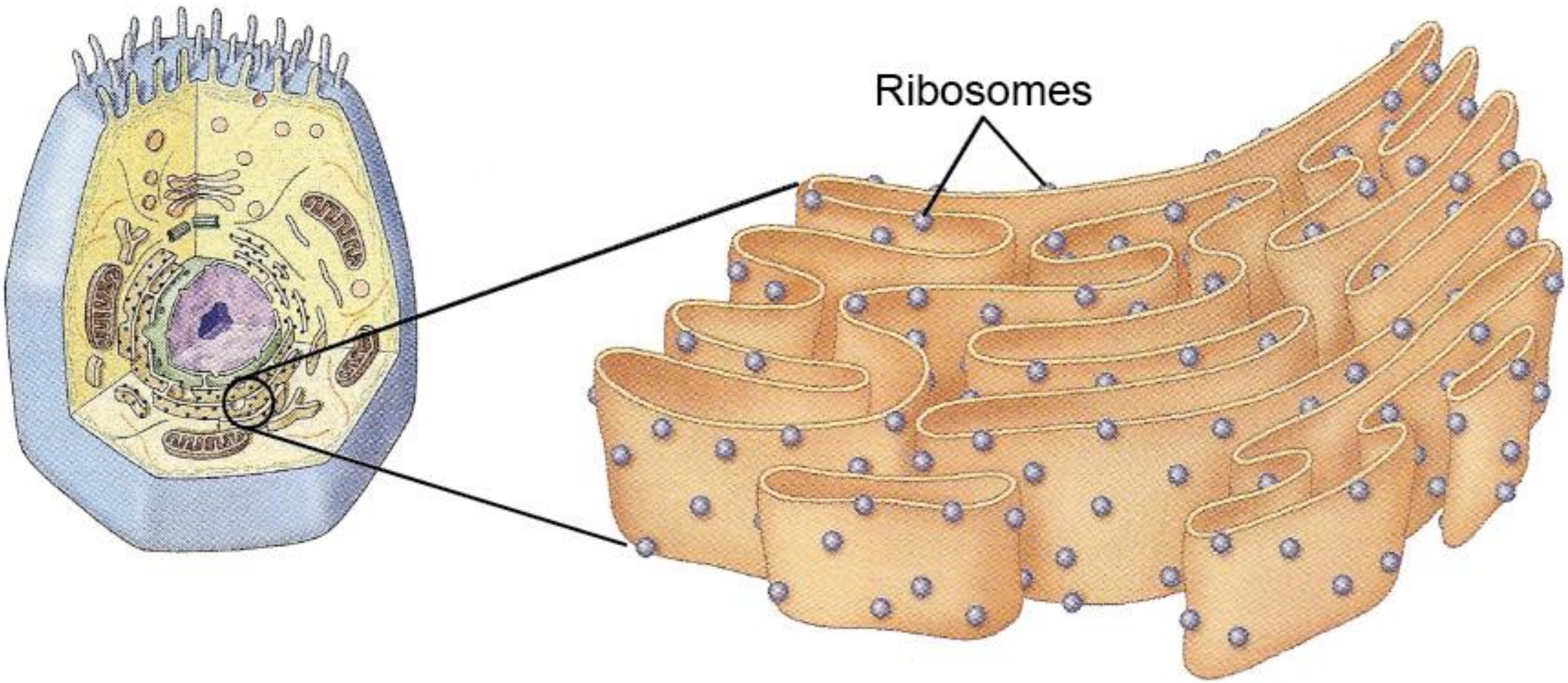


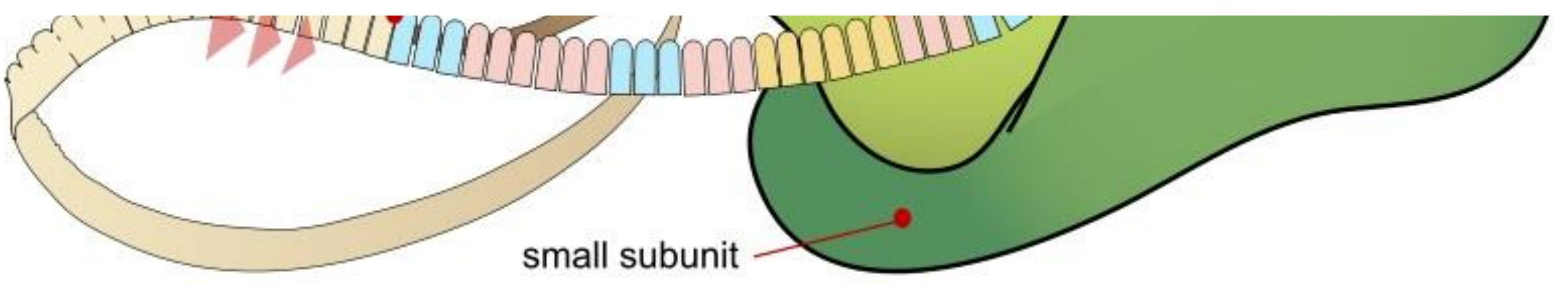
RNA Polymerase
Decked up (!!)

Kapanidis et al, Science 2006

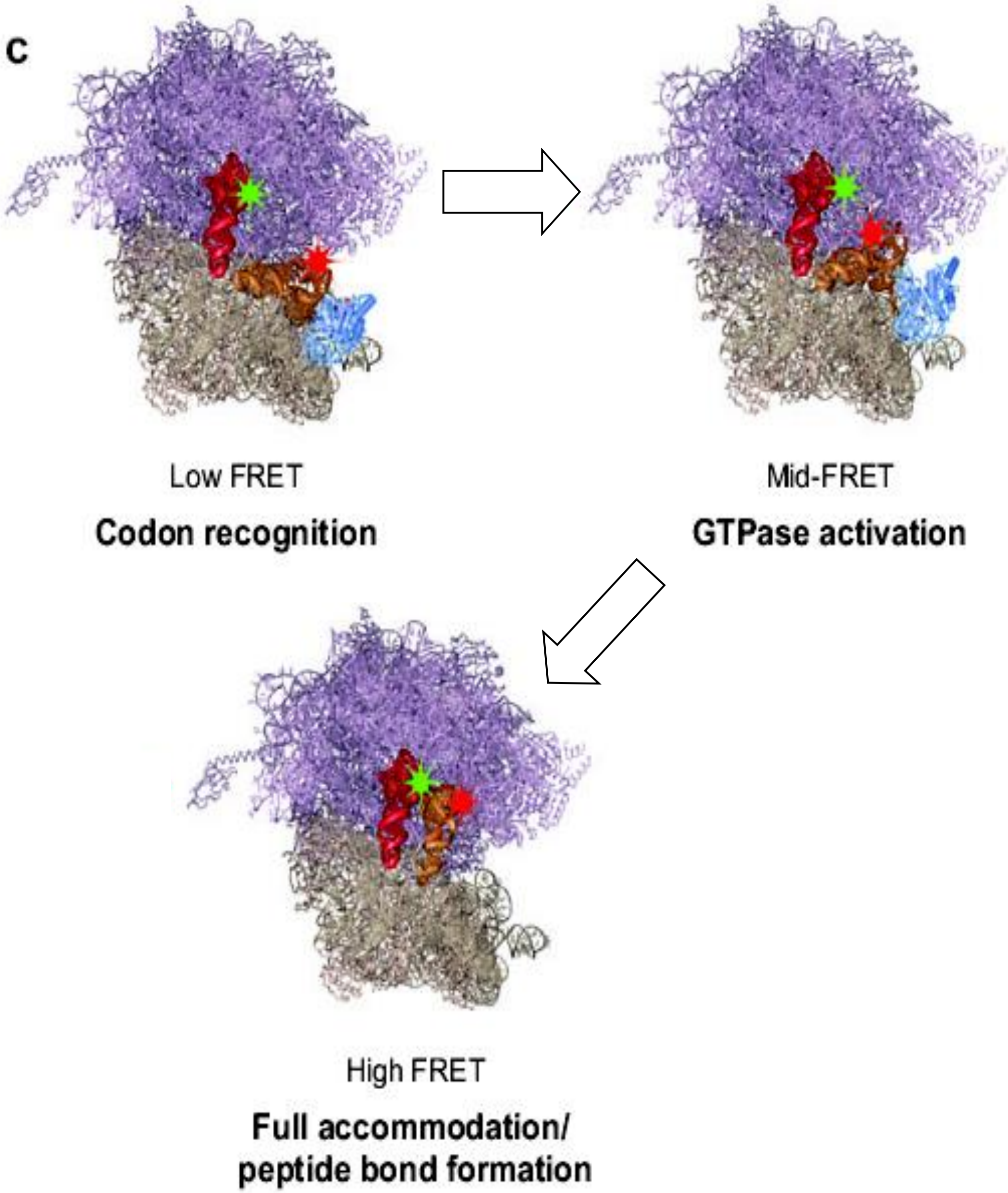
(A) Distance between the RNAP leading edge and a point on the downstream DNA
(B) Distance between the RNAP trailing edge and a point on the upstream DNA
(C) Expansion or contraction within RNAP itself
(D) Expansion or contraction between points on the upstream and downstream DNA

Ribosome

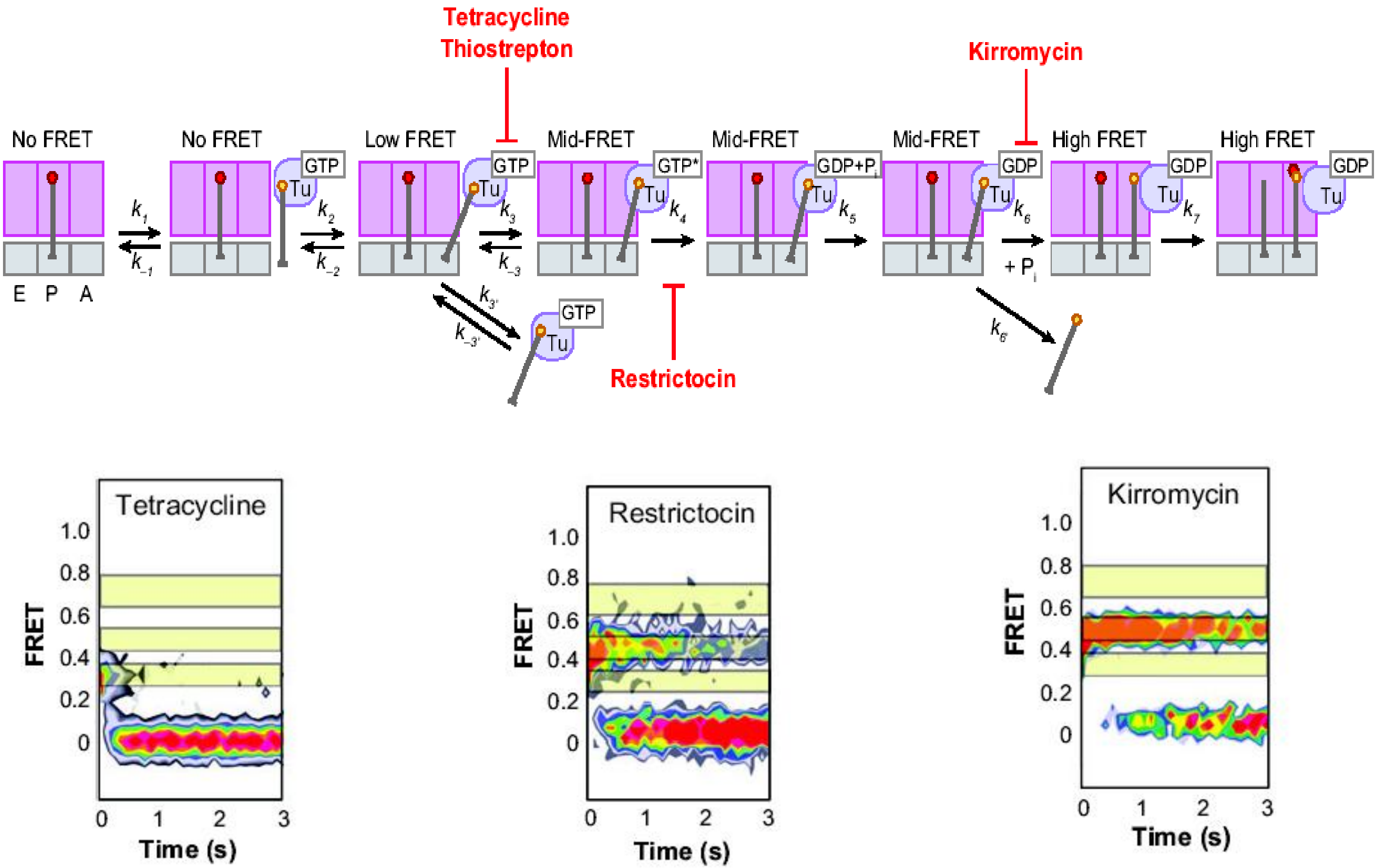




Understanding where Antibiotics work …



Blanchard et al
NSMB 2004



Tetracycline
Thiostrepton
Kirromycin
No FRET
No FRET
Low FRET
Mid-FRET
Mid-FRET
Mid-FRET
High FRET
High FRET
GTP
GTP
GTP*
GDP+Pi
GDP
GDP
GDP
Tu
E P A
k1
k−1
k2
k−2
k3
k−3
k4
k5
k6
k7
+ Pi
k3'
k−3'
GTP
Tu
k6'
Restrictocin
Tetracycline
Restrictocin
Kirromycin
FRET
Time (s)

MANIPULATION...

- Why apply force ?
- Stiffen the molecule to improve resolution
- Explore and manipulate the energy landscape of conformational changes/motion of a molecule
- The landscape for molecular motion is position. External force will perturb this landscape, and kinetics of motion
- Information about translocation steps in a mechanochemical cycle

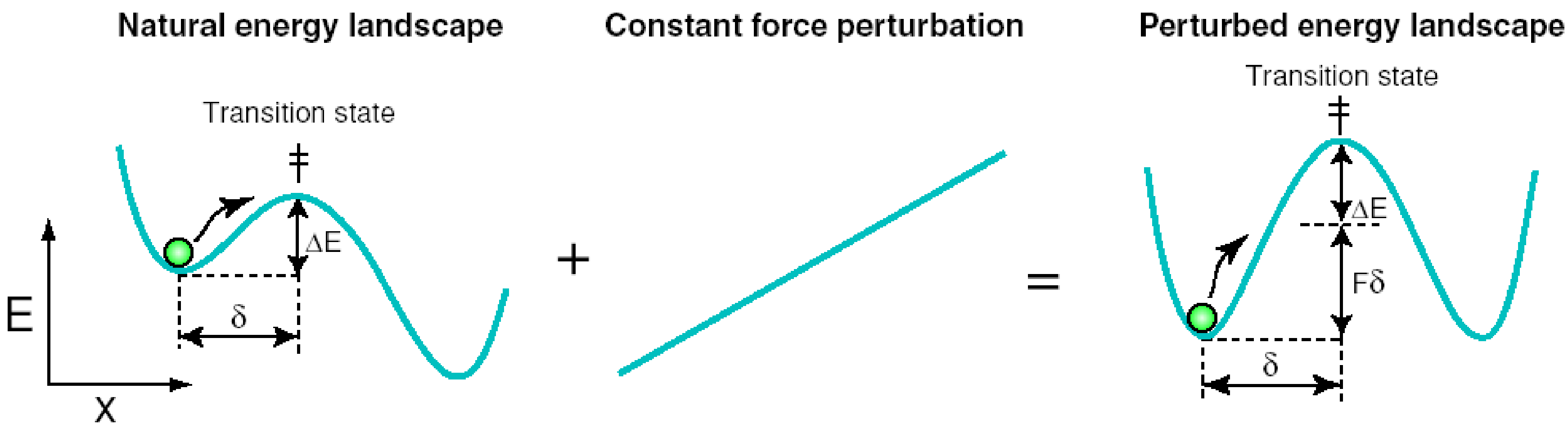
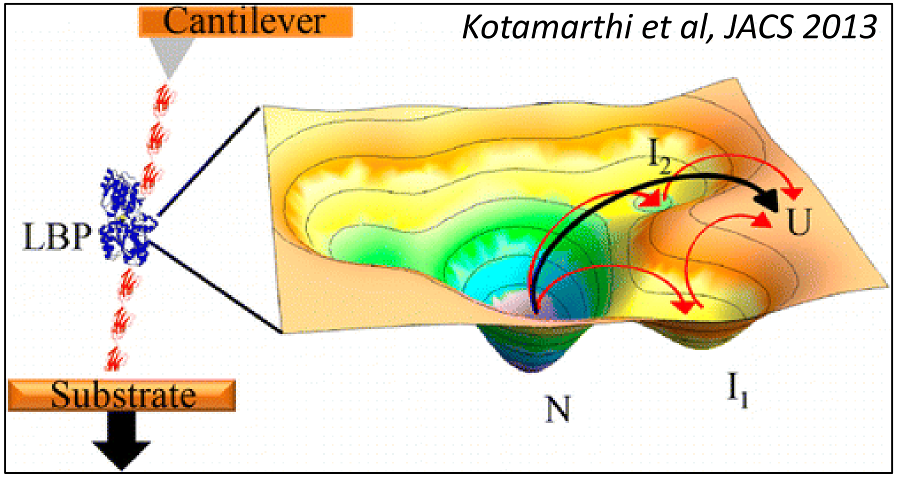


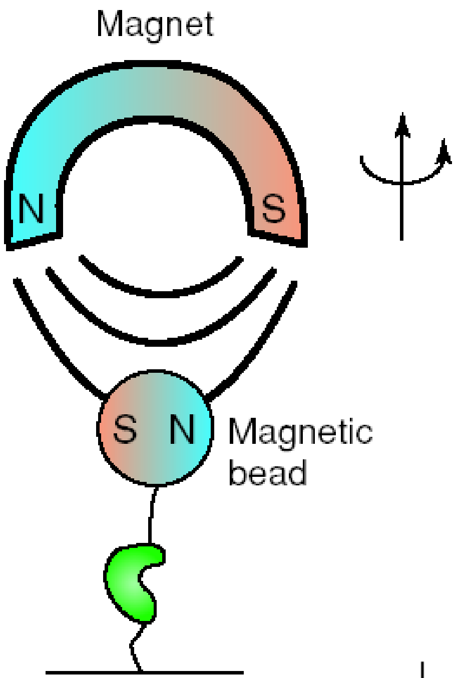
Figure 3

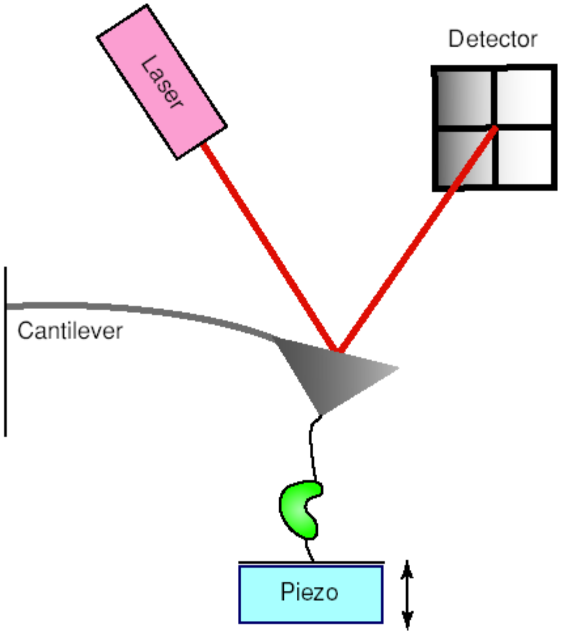
The effect of external force on the energy landscape. The natural energy landscape is altered by applying a constant force to produce a perturbed energy landscape. This perturbation changes the height of the energy barrier, ΔE, by an amount equal to $F\delta$.

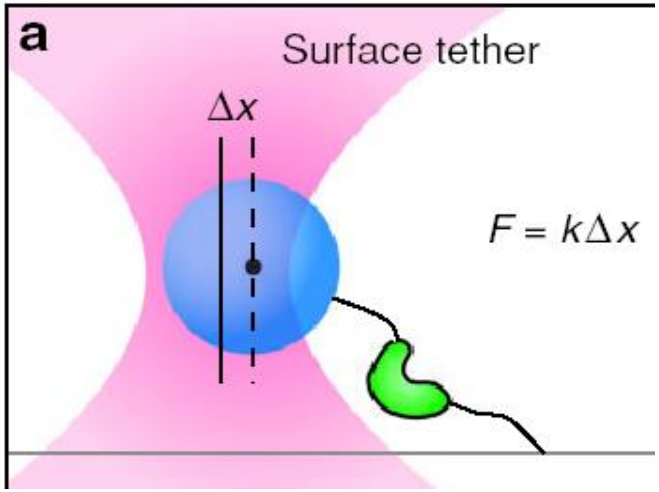
FORCE TRANSDUCERS

- Magnetic tweezers -- Constant force over molecular dimensions, Torque
- Atomic Force microscopes -- Larger force
- Optical tweezers -- Non contact

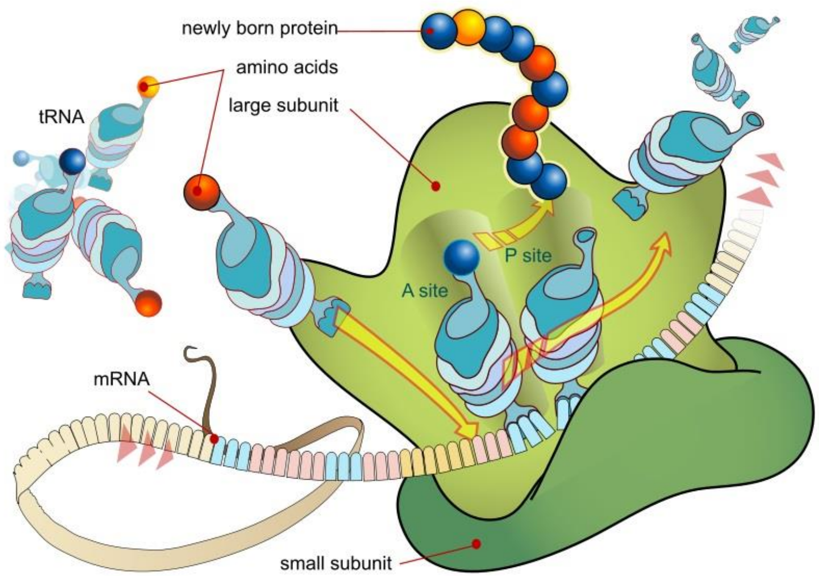


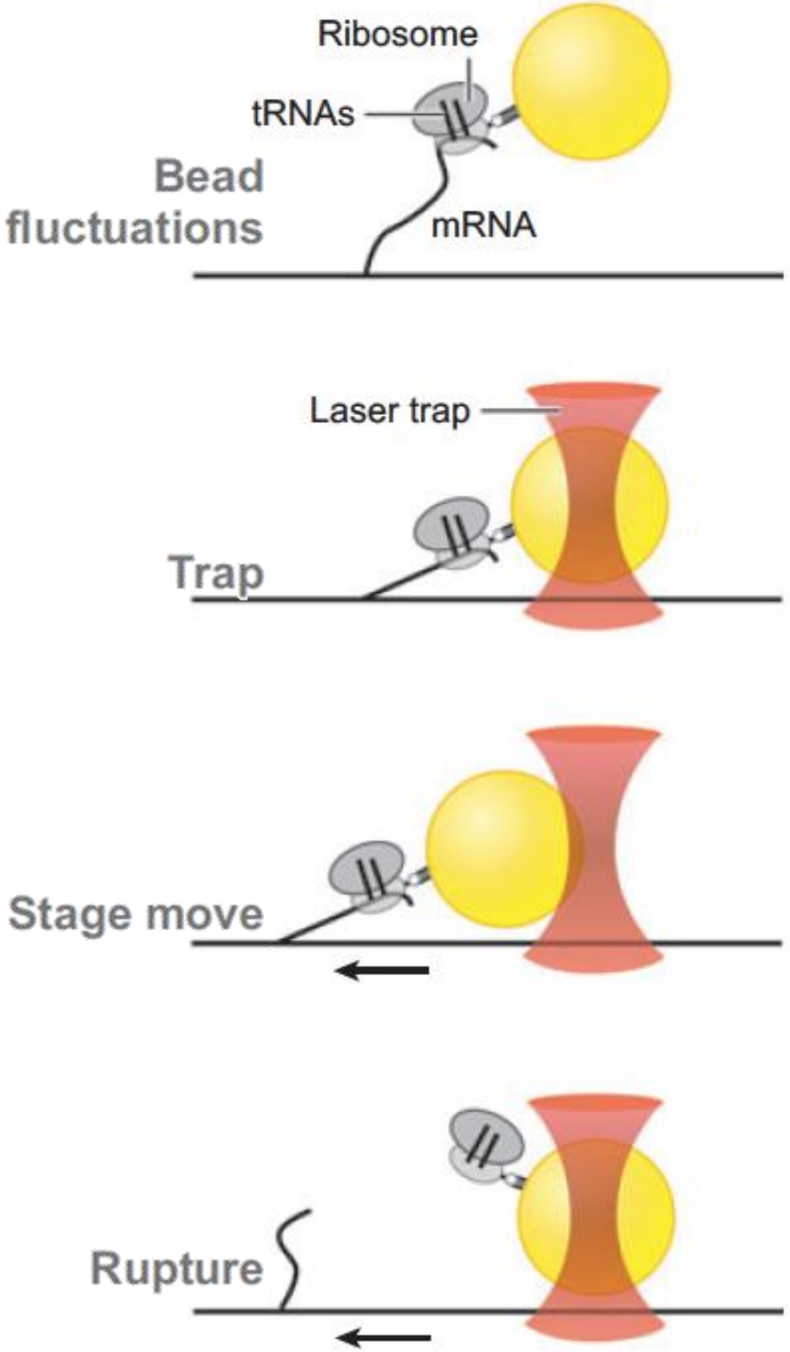


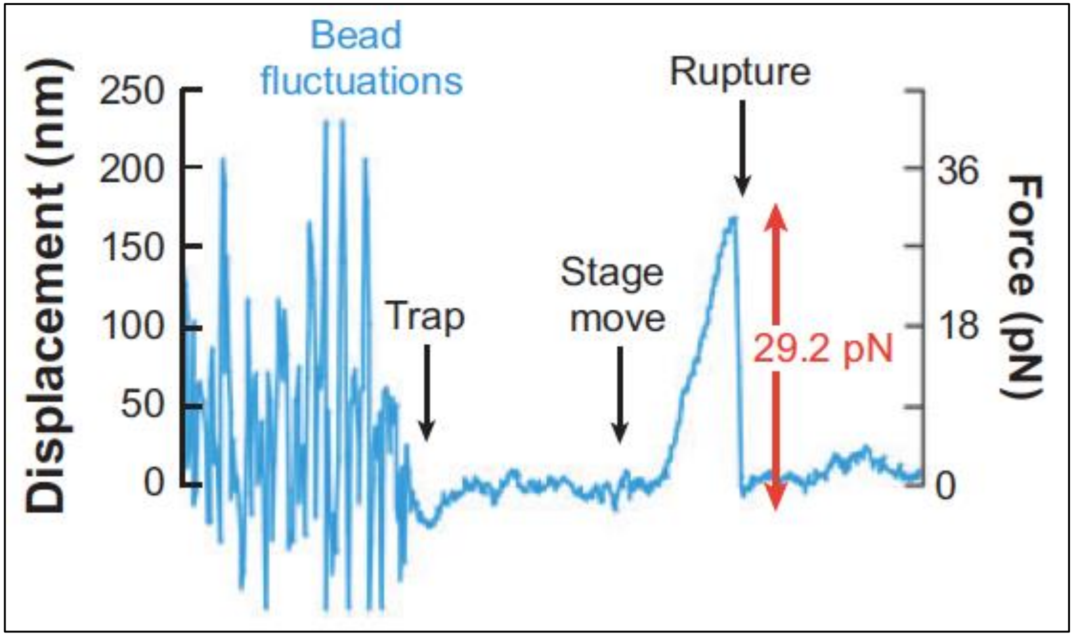




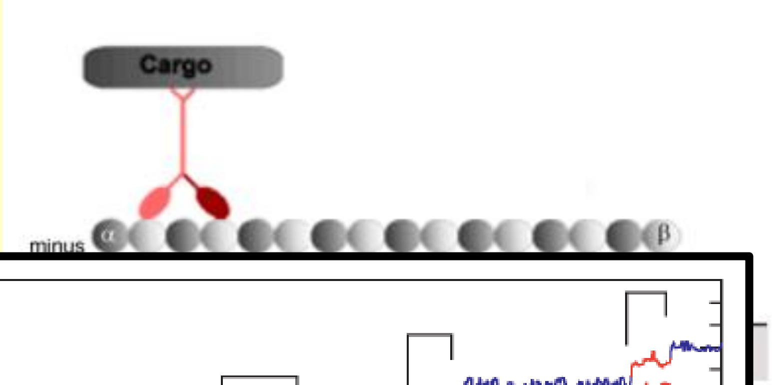
How strongly does mRNA bind the Ribosome ?

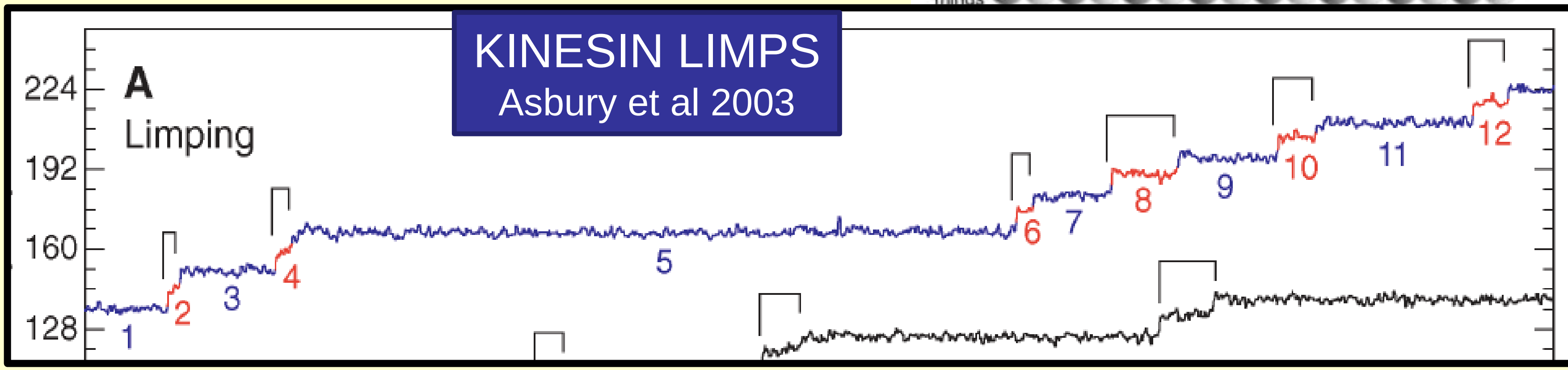




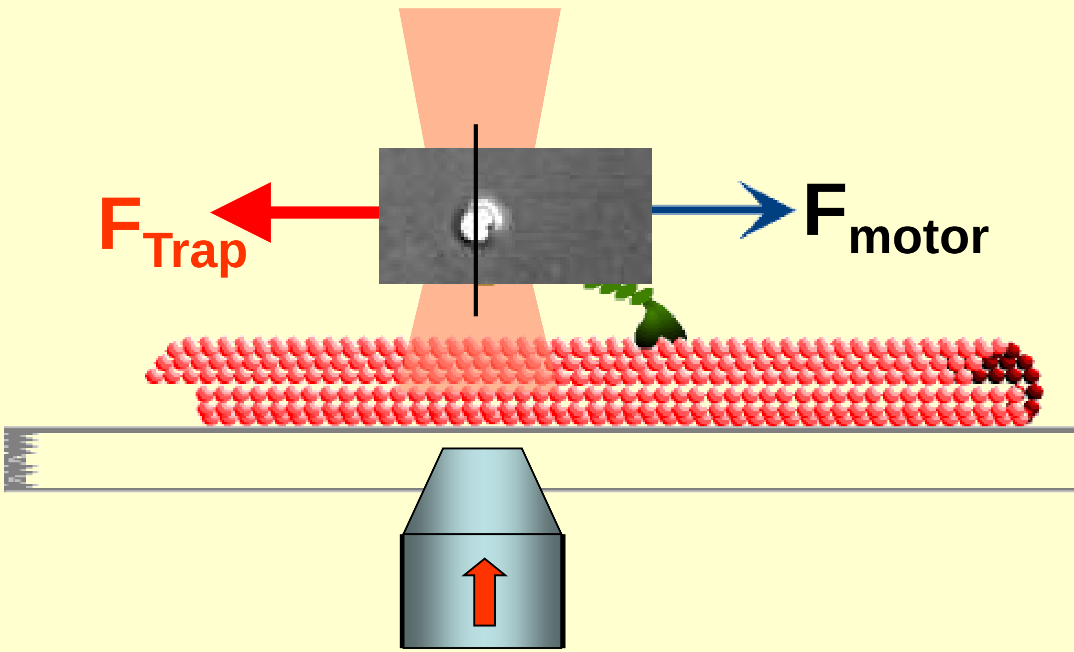


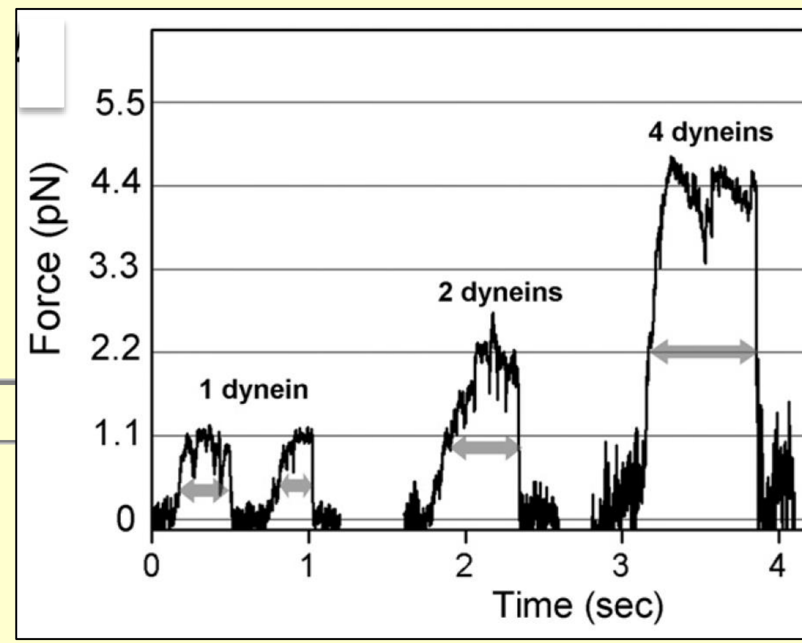
SM studies on Linear Motors



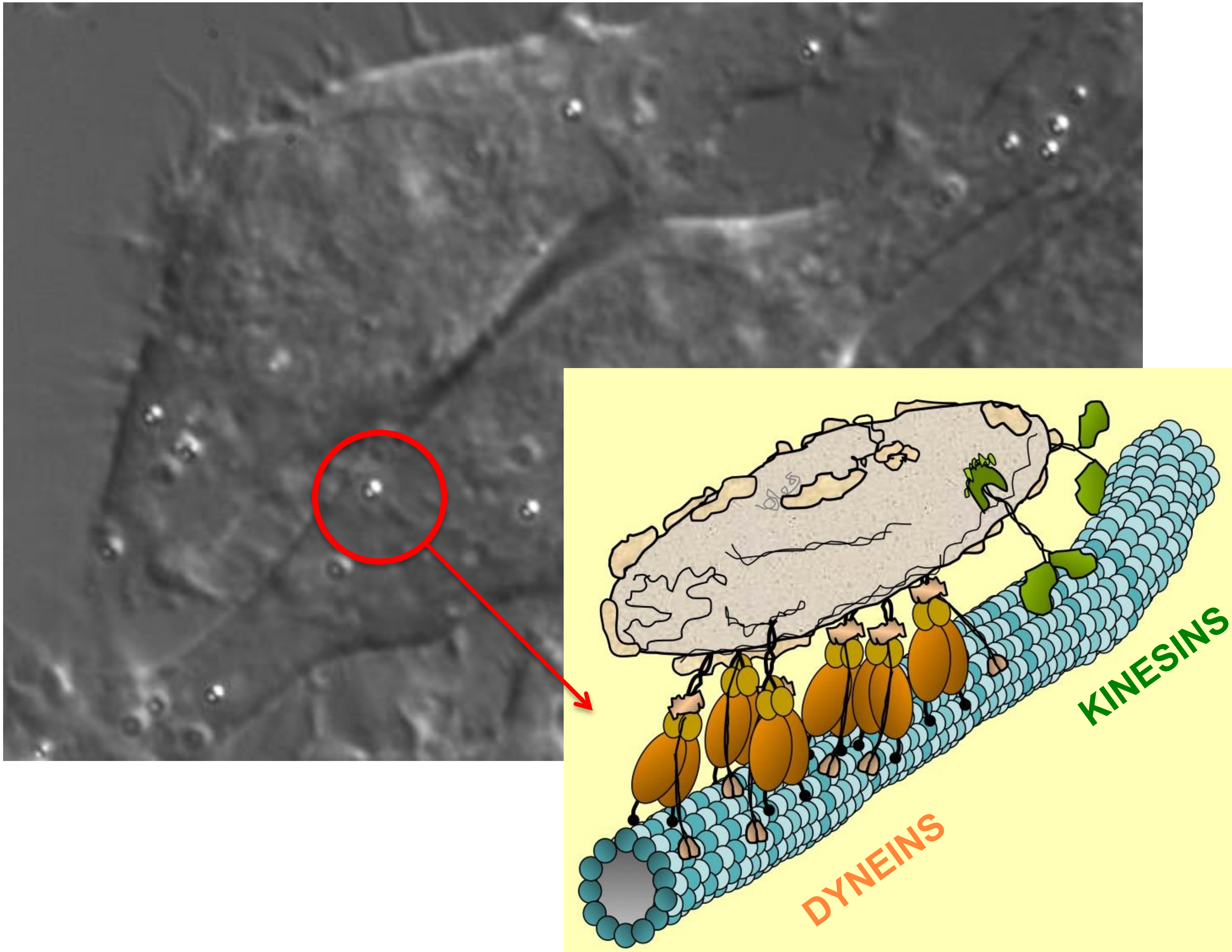


Soppina et al, 2009

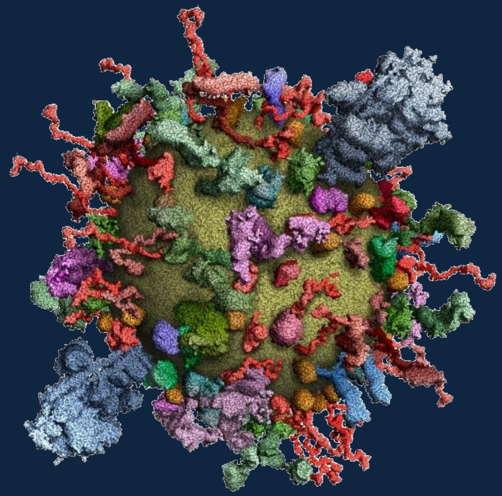




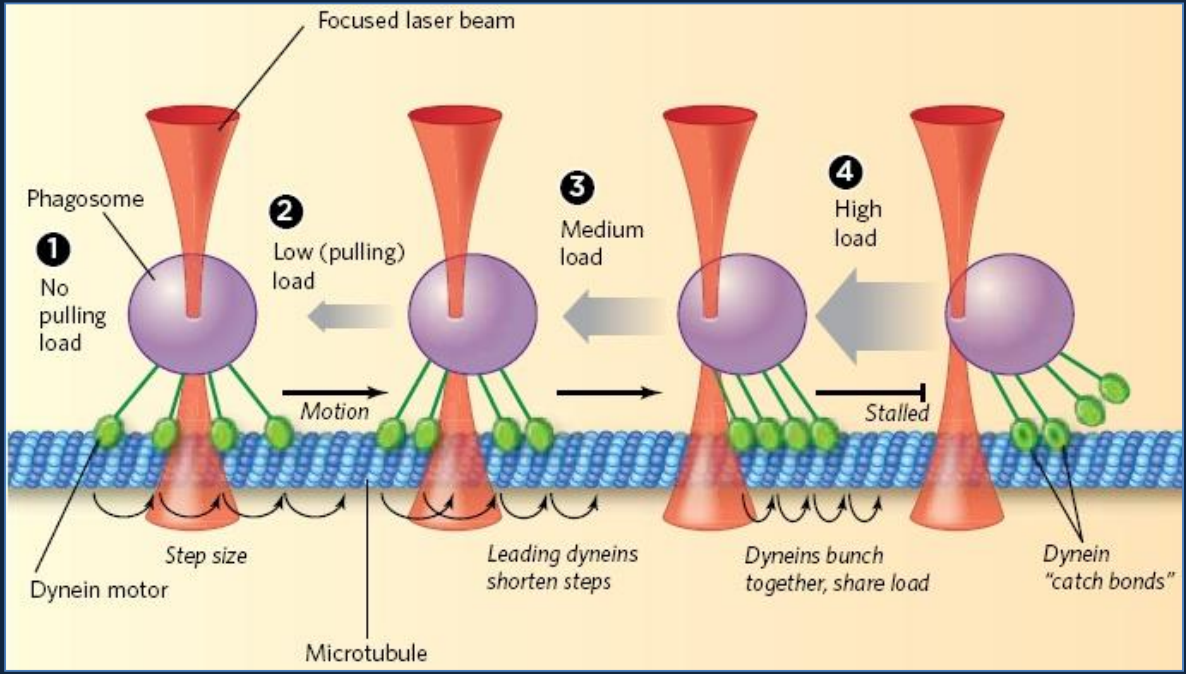
Ingested Bead = 1 micron diameter

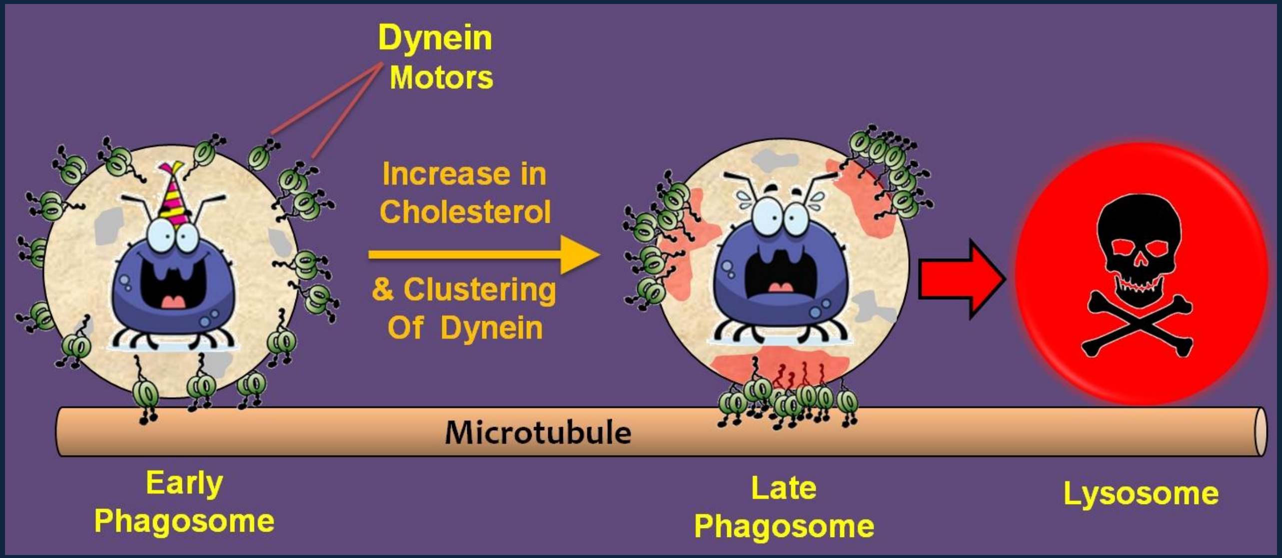


Motors and Lipids



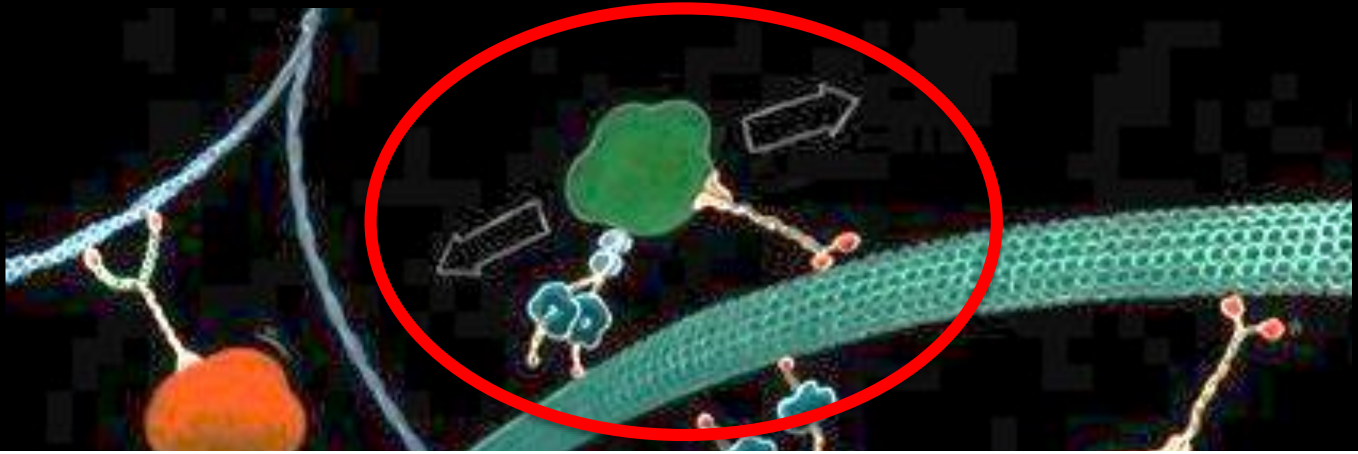
www.biotechnologie.de

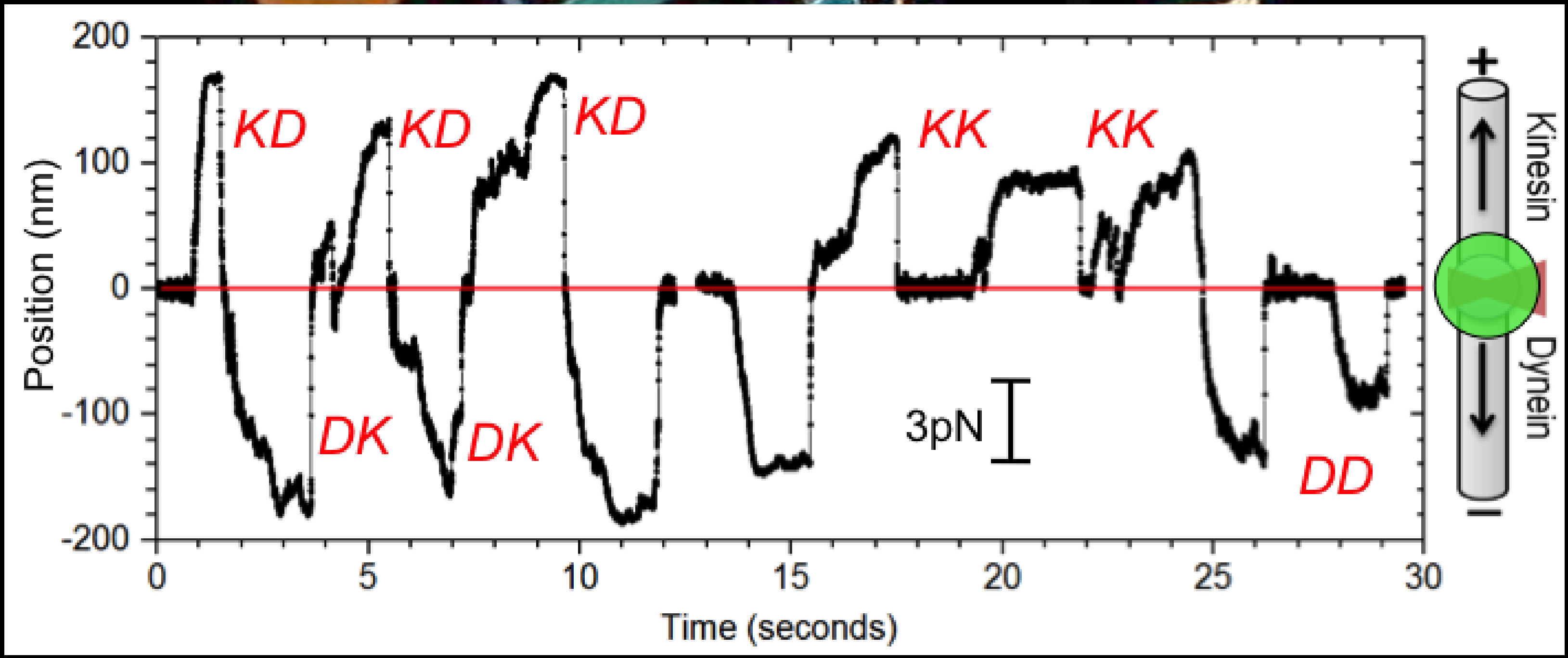




Mallik et al, Nature 2004
Soppina et al, PNAS 2009
Rai et al Cell, 2013
Rai et al Cell, 2016

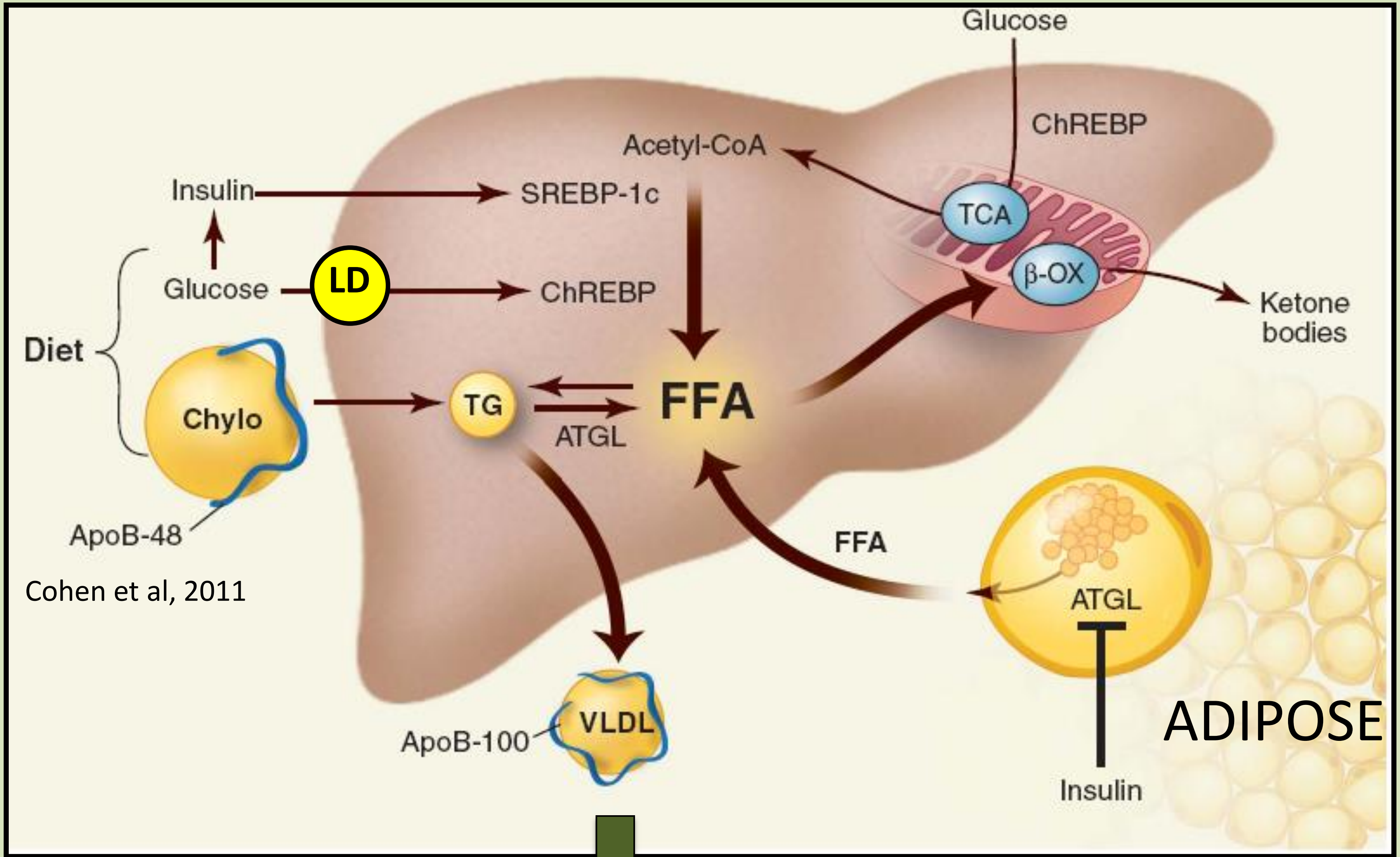
Tossing Coins on Cellular Vesicles





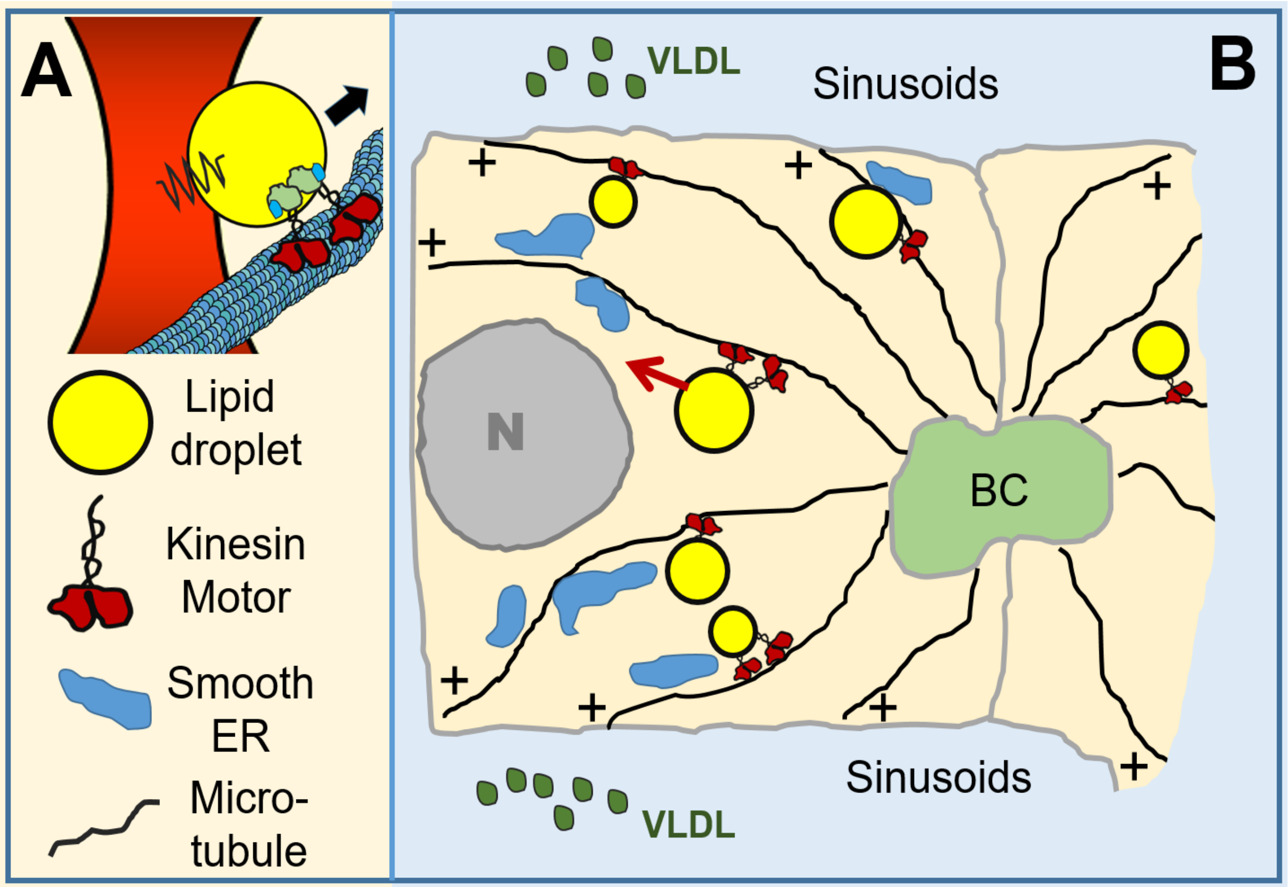
Paulomi Sanghavi et al, To appear in Current Biology

Who let the Fat out ?

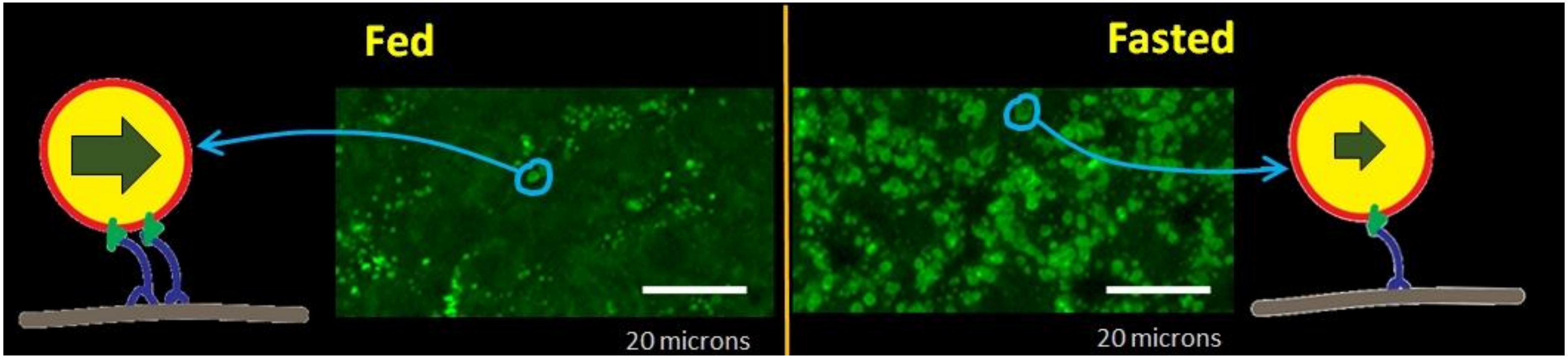


Cohen et al, 2011

10^{18} per day



Priyanka Rai et al
PNAS 2017



https://vagabond3.com