Biophysical Chemistry

Justin Benesch
Jonathan Doye
Mark Wallace

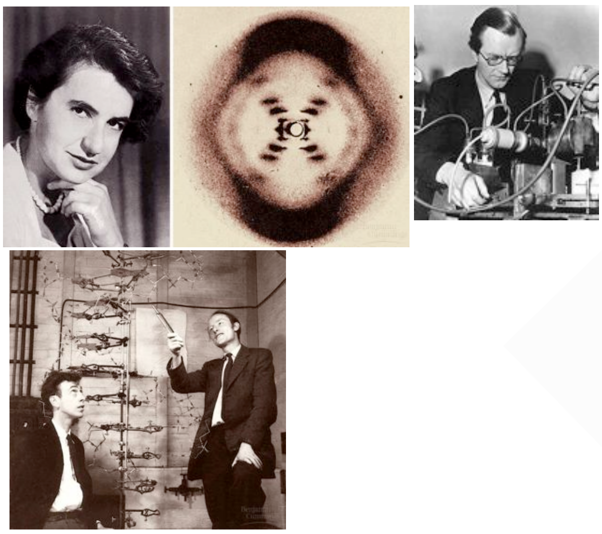
Introduction

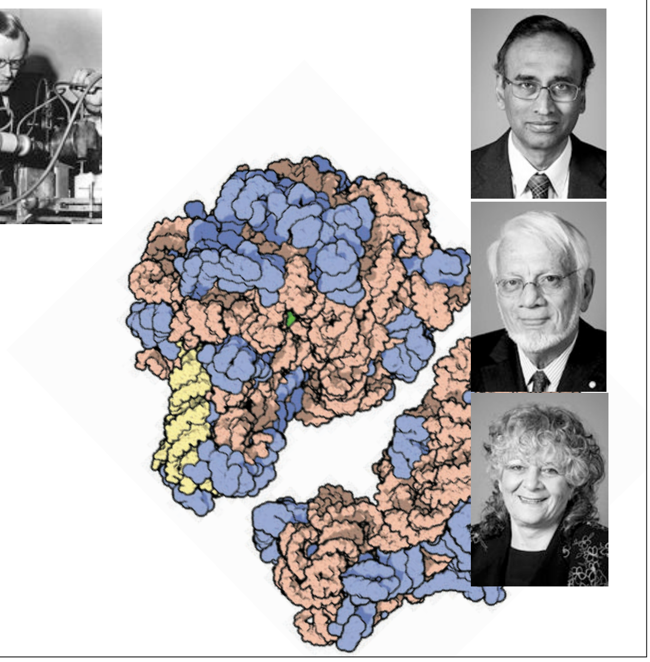
- What is Biophysical Chemistry?
  - Biophysical chemists seek to understand biological phenomena at the molecular level.

Biophysical Chemistry?

1962

2009





This course

- How physical science methods can be applied to give real insights into biological problems.
  - Not comprehensive!
  - Highlighted by examples.
  - Method-based exam questions.
  - Quantitative basics.

Syllabus

- Single molecule techniques.
- Biological applications of polymer theory.
- Biomolecule simulation.
- Biomolecular mass spectrometry.
- Gas-phase structural biology.

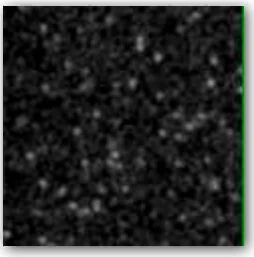

Mark Wallace


Jonathan Doye

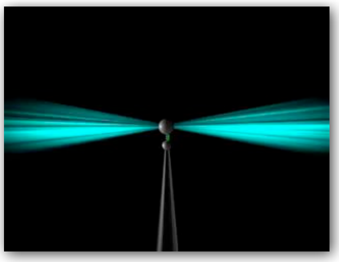

Justin Benesch

Single molecule techniques

- Applications of single-molecule techniques to biology.
- Single molecule Fluorescence Methods.
- Molecular Rulers.

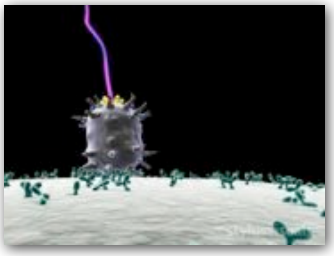


- Atomic Force Spectroscopy.
- Optical Tweezers.
- Single-channel electrical recording.

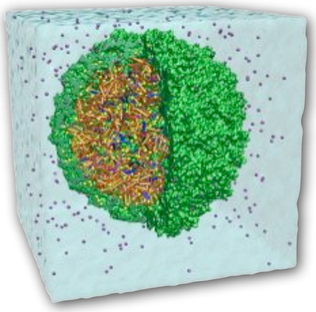


Biopolymer theory & simulation

- The freely-jointed chain and polymer elasticity.
- Applications to DNA: genome size & packaging.
- Beyond the freely-jointed chain: the worm-like chain model.

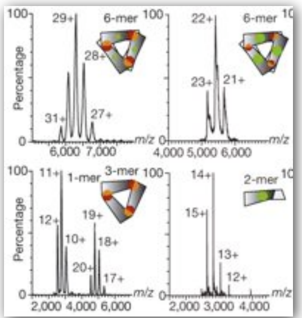


- Molecular Dynamics and Monte Carlo simulations.
- Protein folding.

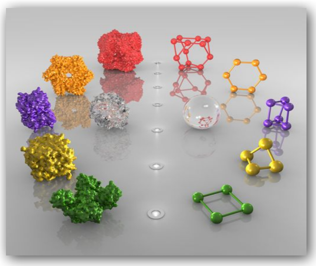


Gas-phase structural biology

- Mechanisms of ion formation.
- Methods for ion separation, and activation.
- Mass measurement of biomolecules, both intact molecules and and fragments.
- Cross-linking. H/D exchange. Oxidative footprinting.



- Mass spectrometry of protein assemblies.
- Ion mobility spectrometry.
- Collision cross sections.
- Electron microscopy.



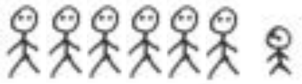
Resources

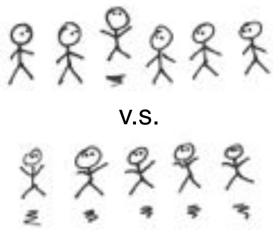
- Notes, Slides, & Problems
  - course.chem.ox.ac.uk.
- Books
  - **Biophysical Techniques** Iain Campbell, OUP.
  - **Principles of Fluorescence Spectroscopy** Lakowicz, Springer.
  - **Physical Biology of the Cell** Phillips, Garland.
  - Mass Spectrometry: Principles and Applications De Hoffman and Stroobant, Wiley.
  - Transport Properties of Ions in Gases Mason and McDaniel, Wiley
  - Single molecule Biology, Knight, Springer.

Single molecules

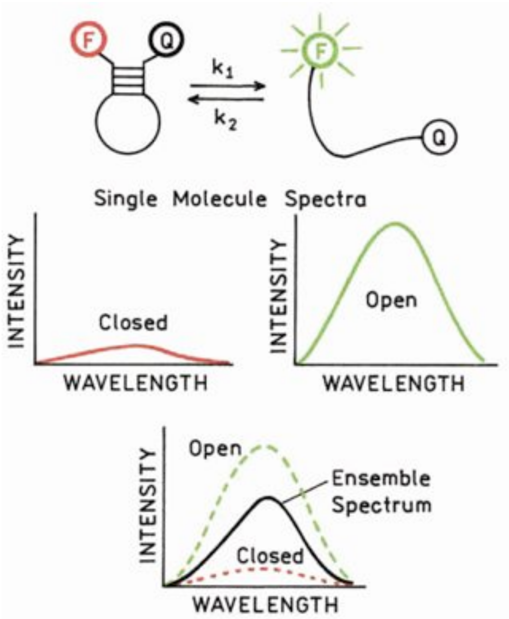
Why single molecules

- No Ensemble Averaging
  - Measure the distribution of a property.
  - Useful if population is heterogeneous.
- Kinetics at Equilibrium.
  - Measure the time trajectory.
  - Useful if dynamics is difficult to synchronise.
- Detect Rare Events.

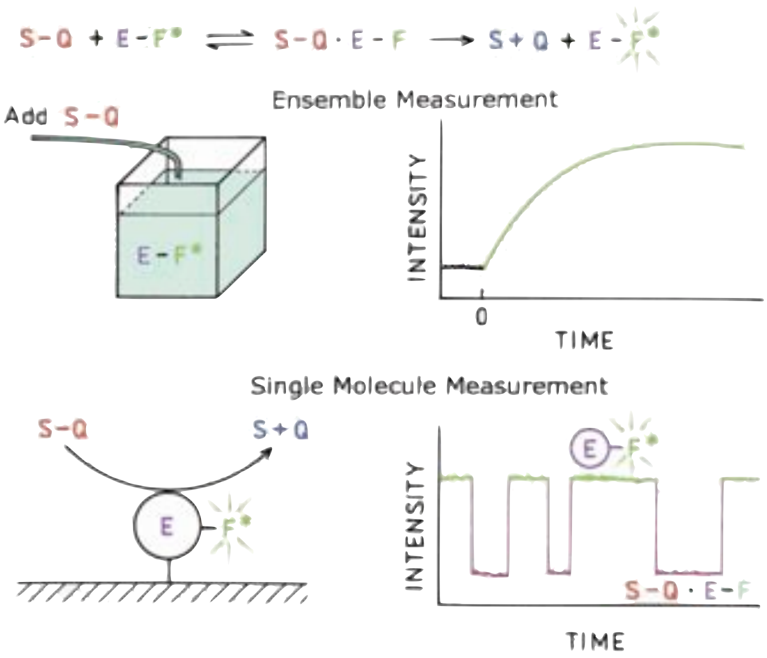




Ensemble averaging



Kinetics



Many types of measurement

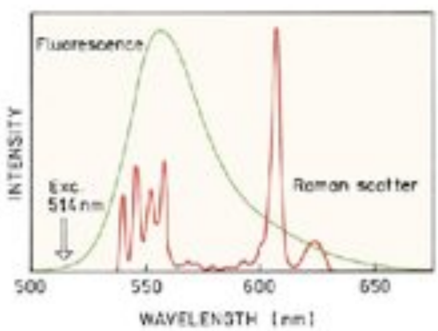
- Force
  - Atomic Force Microscopy
  - Optical Tweezers
- Light
  - Fluorescence
  - Absorption
- Charge
  - Scanning Tunnelling Electron Microscopy
  - Single-channel electrophysiology

Many types of measurement

- Force
  - **Atomic Force Microscopy**
  - **Optical Tweezers**
- Light
  - **Fluorescence**
  - Absorption
- Charge
  - Scanning Tunnelling Electron Microscopy
  - **Single-channel electrophysiology**

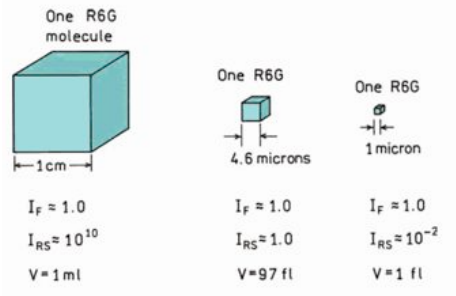
Single Molecule Fluorescence

- Fluorescence provides amplification
  - Easier to detect 10,000's of photons (per second per molecule) than detecting the molecule itself.
  - We can exploit Stoke's shift to separate excitation light from emission.
- It's still just one molecule vs. a lot of background!
  - Impurities
  - Scattered excitation light
  - Detector noise
  - Raman scattering



Detection from small volumes

- Consider the relative cross sections for absorption v.s. Raman scattering.
- To make this easy, let's assume fluorescence quantum yield = 1 (Intensity then proportional to cross-section and the number of molecules).
- Raman cross section for water is ~10^{-28} cm^2
- Absorption cross section of a typical fluorescent dye ~10^{-16} cm^2
- Calculate relative signal intensities as our volume of water changes



Total Internal Reflection Fluorescence

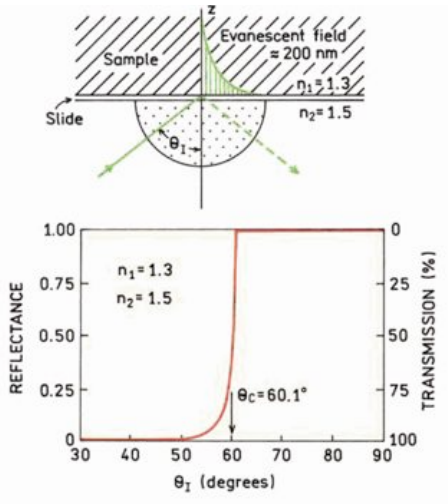


Figure 23.5. Top: Optical geometry for total internal reflection (TIR). **Bottom:** Calculated reflectance and transmittance for $n_2 = 1.5$ and $n_1 = 1.3$.

Critical Angle

$$\theta_c = \sin^{-1}\left(\frac{n_1}{n_2}\right)$$

Evanescent Intensity

$$I(z) = I(0)\exp\left(-\frac{z}{d}\right)$$

Penetration Depth

$$d = \frac{\lambda_0}{4\pi}\left(n_2^2 \sin\theta_2 - n_1^2\right)^{-1/2}$$

Total Internal Reflection Fluorescence
Annual Review of Biophysics and Bioengineering Vol. 13: 247-268 (1984)

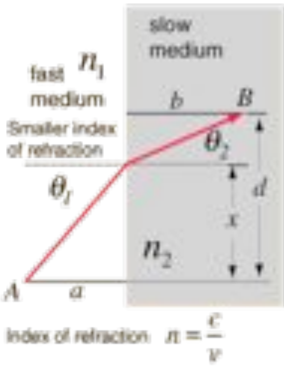
Snell's law

$$t = \frac{\sqrt{a^2 + x^2}}{v} + \frac{\sqrt{b^2 + (d - x)^2}}{v'}$$

$$\frac{dt}{dx} = \frac{x}{v\sqrt{a^2 + x^2}} - \frac{(d - x)}{v'\sqrt{b^2 + (d - x)^2}}$$

$$0 = \frac{\sin\theta_1}{v} - \frac{\sin\theta_2}{v'}$$

$$\boxed{\text{Snell's Law} \quad \frac{n_1}{n_2} = \frac{\sin\theta_2}{\sin\theta_1}}$$



Total Internal Reflection Fluorescence

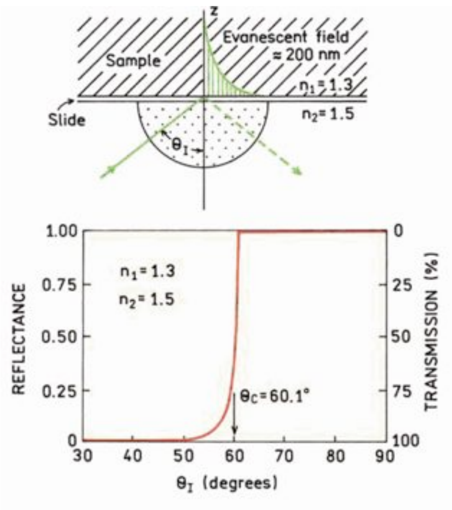


Figure 23.5. **Top**: Optical geometry for total internal reflection (TIR). **Bottom**: Calculated reflectance and transmittance for n_2 = 1.5 and n_1 = 1.3.

Critical Angle

$$\theta_c = \sin^{-1}\left(\frac{n_1}{n_2}\right)$$

Evanescent Intensity

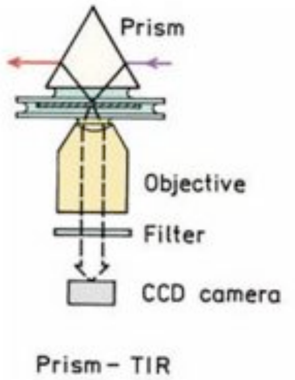
$$I(z) = I(0)\exp\left(-\frac{z}{d}\right)$$

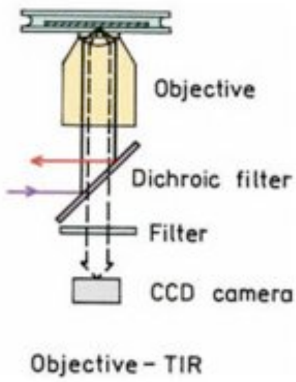
Penetration Depth

$$d = \frac{\lambda_0}{4\pi}\left(n_2^2 \sin\theta_2 - n_1^2\right)^{-1/2}$$

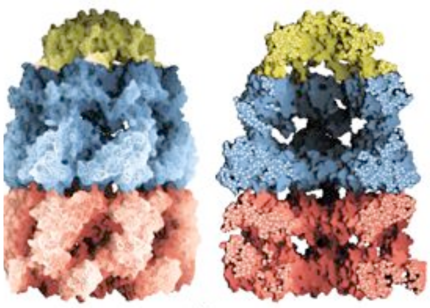
Total Internal Reflection Fluorescence
Annual Review of Biophysics and Bioengineering Vol. 13: 247-268 (1984)

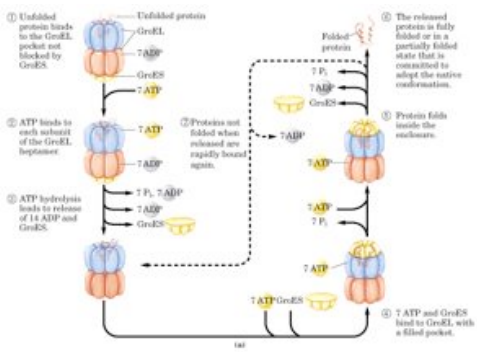
Total Internal Reflection Fluorescence





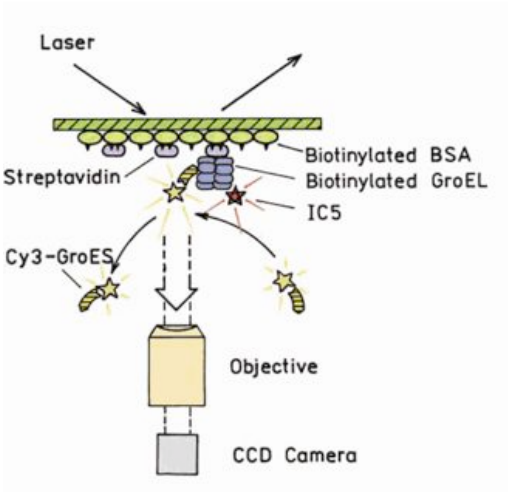
Example I: The GroEL/GroES chaperone

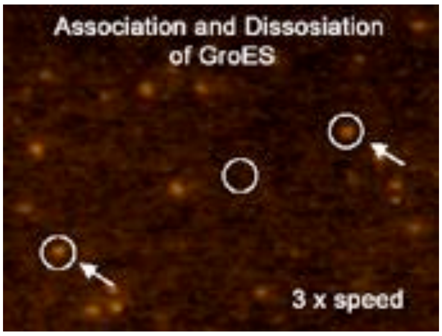


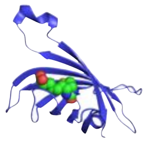


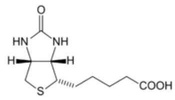
(Nelson & Cox, *Lehninger Principles of Biochemistry, 3rd ed*. Don't learn this!)

Experimental Setup



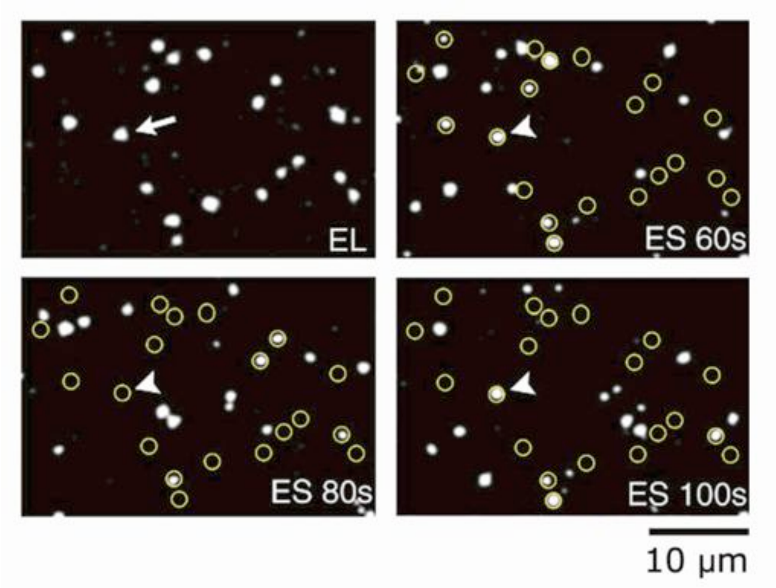




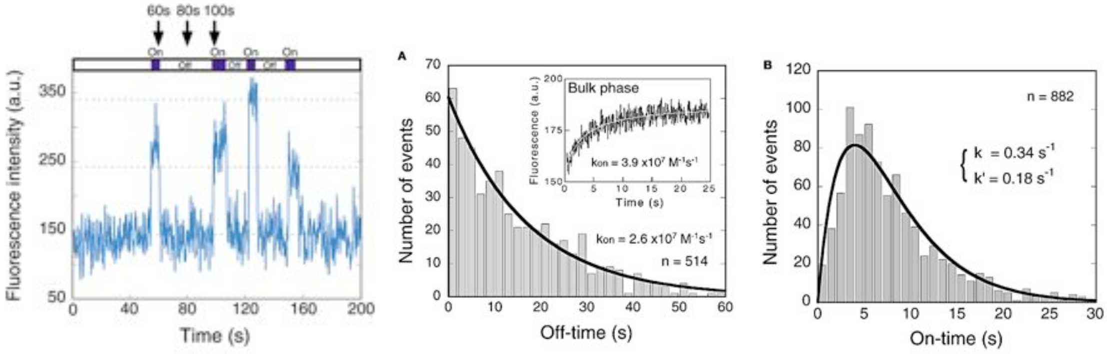


Single-molecule observation of protein–protein interactions in the chaperonin system
Hideki Taguchi, Taro Ueno, Hisashi Tadakuma, Masasuke Yoshida & Takashi Funatsu
Nature Biotechnology **19**, 861 - 865 (2001)
doi:10.1038/nbt0901-861

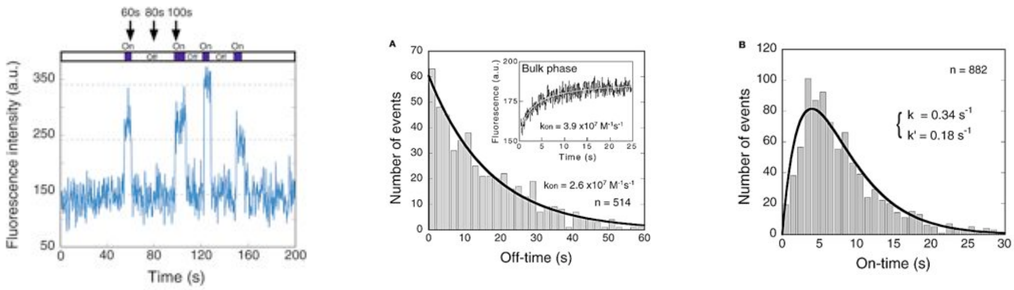
Raw Data



Analysis



Single-molecule kinetics

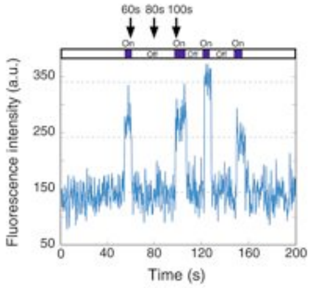


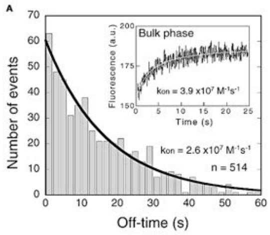
$$\text{EL} + \text{ES} \xrightleftharpoons{k_{\text{on}}} [\text{EL.ES}] \xrightarrow{k} \text{EL} + \text{ES}$$

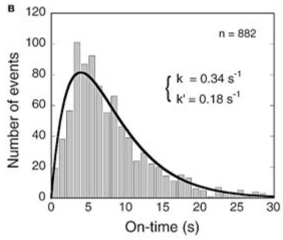
$$\frac{d[\text{EL.ES}]}{dt} = k_{\text{on}}[\text{EL}][\text{ES}]$$

$$-\frac{d[\text{ES}]}{dt} = k''[\text{ES}] \qquad \frac{N}{N_0} = e^{-k''t}$$

Single-molecule kinetics



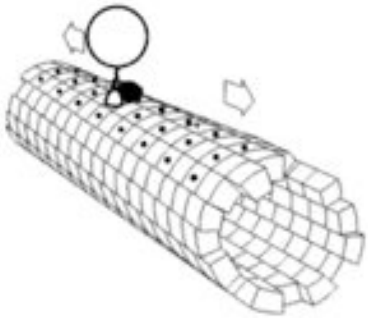


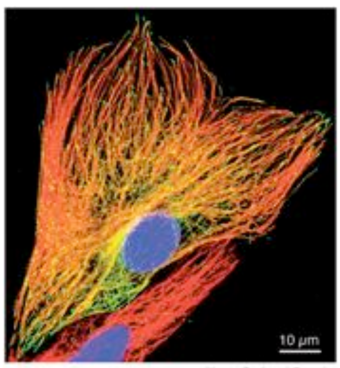


$$\mathrm{EL + ES} \xrightarrow{k_{\mathrm{on}}} [\mathrm{EL.ES}] \xrightarrow{k'} [\mathrm{EL.ES}]^* \xrightarrow{k} \mathrm{EL + ES}$$

$$\frac{N}{N_0} = \frac{kk'}{k'-k}\{e^{-kt} - e^{-k't}\}$$

Example II: Kinesin stepping



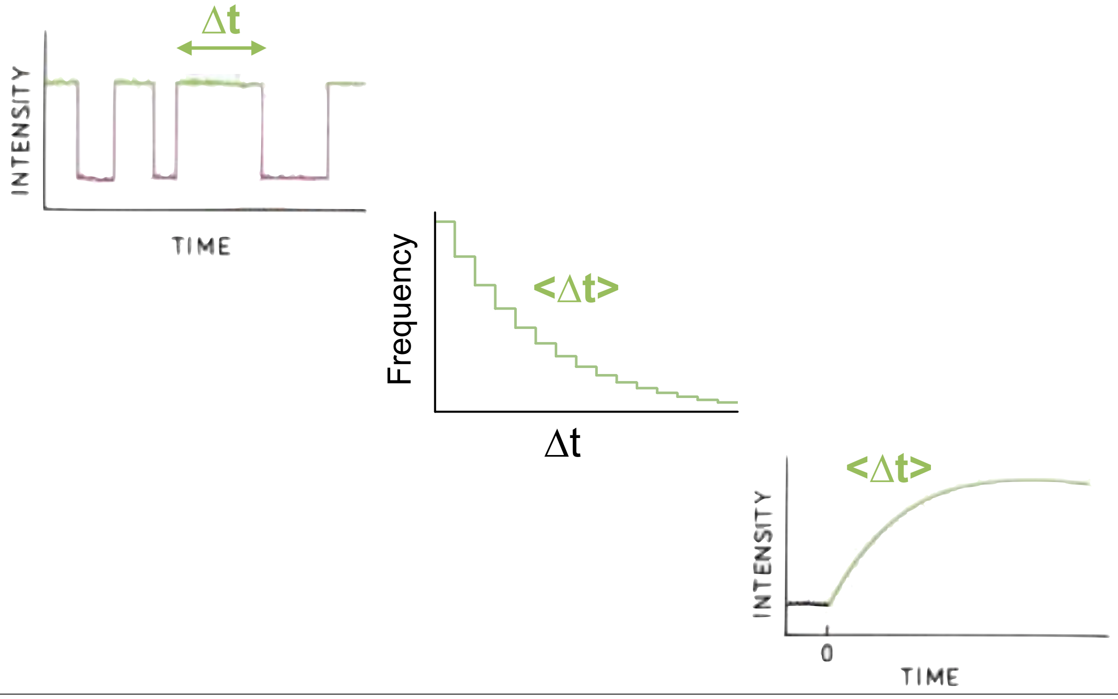


Nature Reviews | Genetics

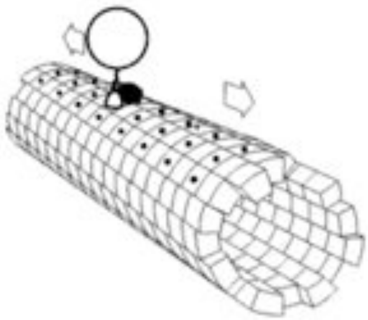
Biophysical Chemistry

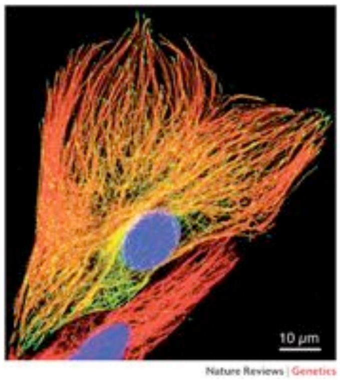
Justin Benesch
Jonathan Doye
Mark Wallace

Linking single-molecule & ensemble

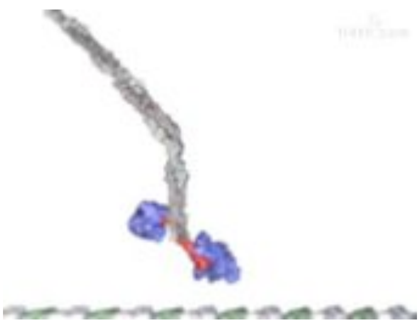


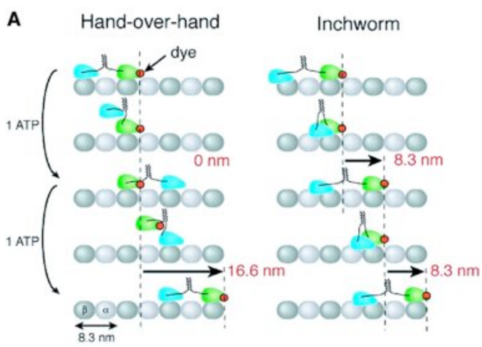
Example II: Kinesin stepping



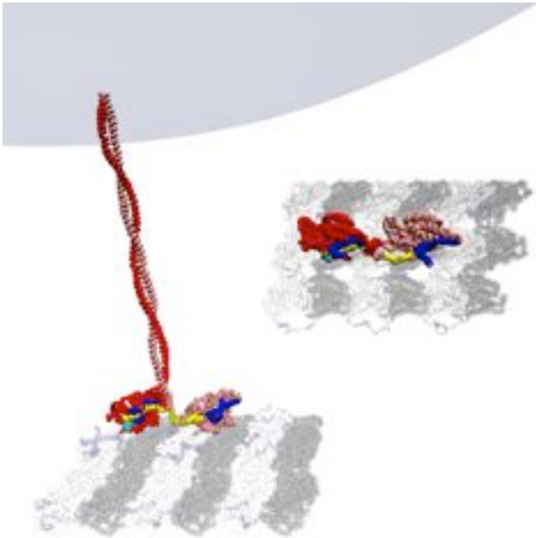


Example II: Kinesin stepping



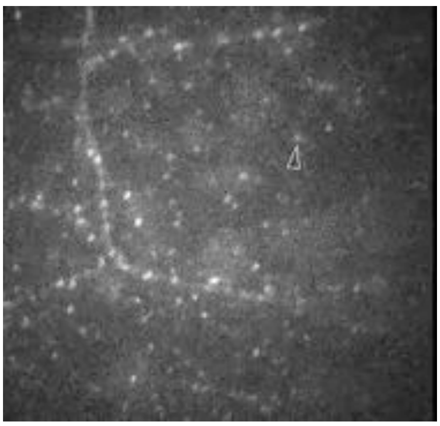


Hydrodynamic modelling



Zhang Thirumalai U.Maryland

Real data

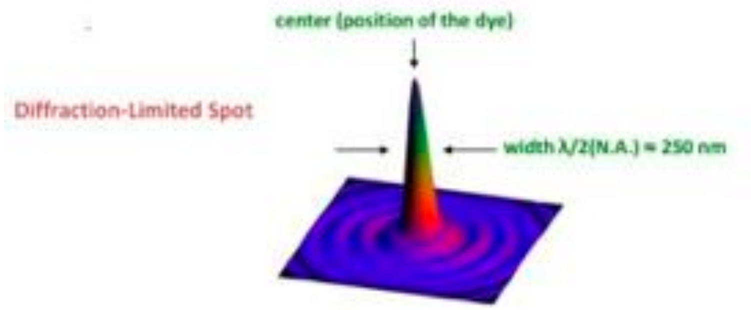


http://people.umass.edu/rossj

Photochemistry Revision

Imaging the nanoworld: localization

Image of a single dye molecule



Can determine the centre of mass of the molecules image with very high precision - down to 1 nm.
Therefore, can determine the molecules position with 1 nm accuracy, despite that it's image is 250 nm large!

Photochemistry Revision

Precision determined by

$$(\sigma_{x,y}^{2})_{m} = \frac{s^2 + a^2/12}{N_m} + \frac{4\sqrt{\pi}s^3 b_m^{2}}{a N_m^{2}}$$

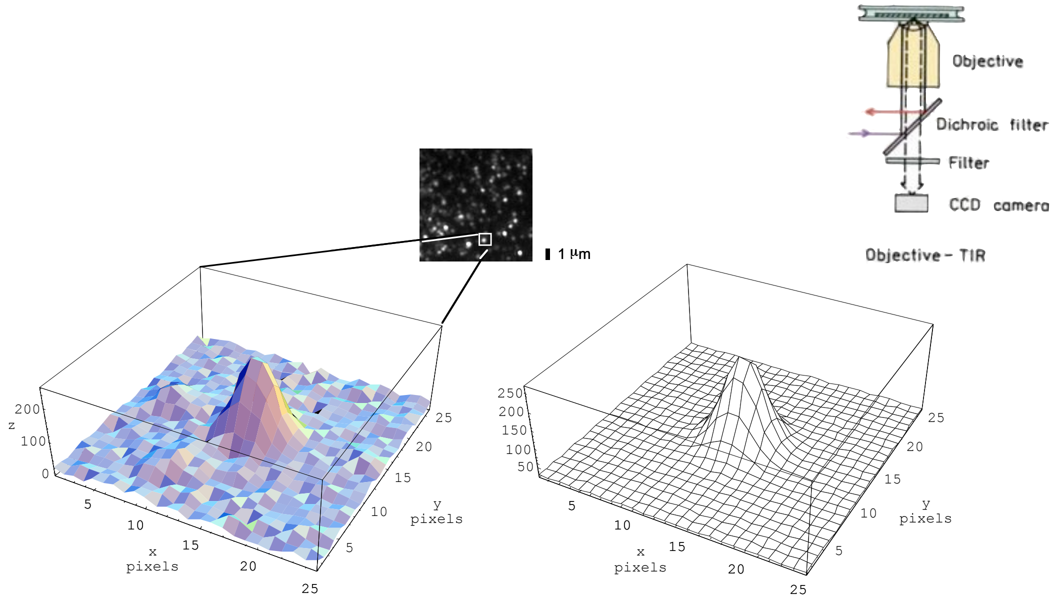
R. E. Thompson, D. R. Larson, W. W. Webb, Biophys. J. 82, 2775 (2002)

s:	standard deviation of the PSF
a:	pixel size in the image
N_m :	number of photons
b_m:	number of background photons

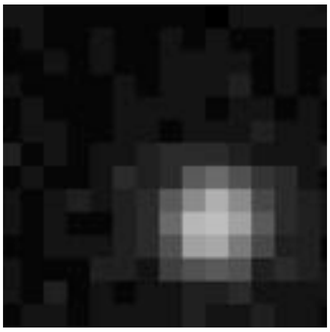
If background noise is negligible compared to the molecular signal, the error in the fitted position is:

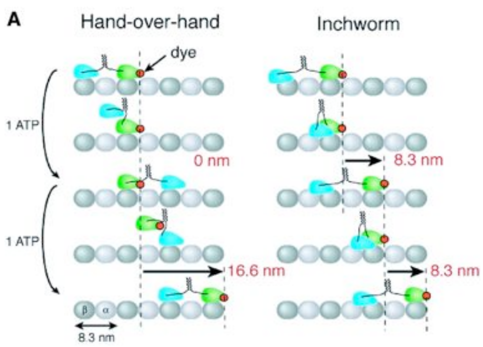
$$\boxed{\sigma_{x,y} \approx s/(N^{1/2})}$$

Single molecule fuorescence localisation

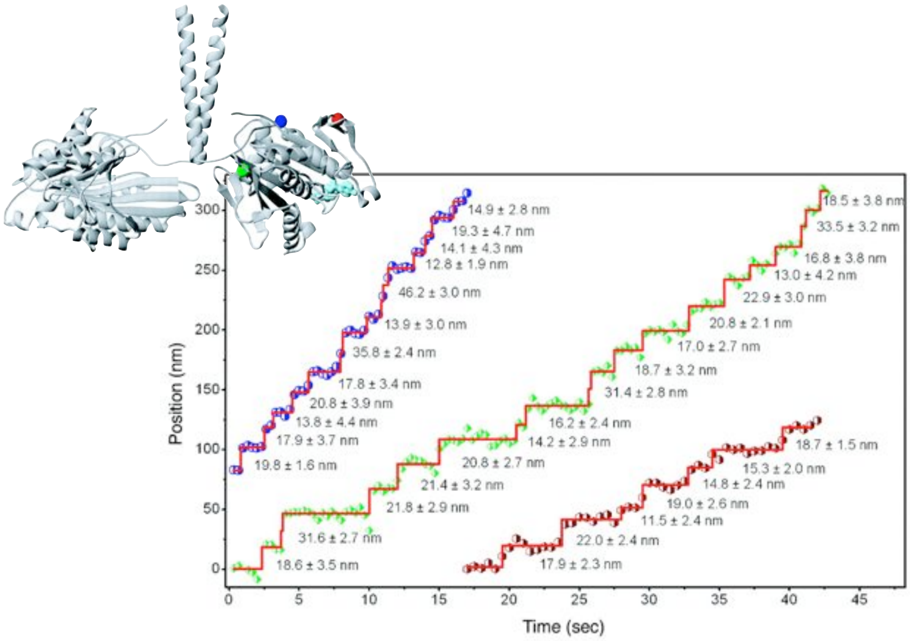


Nanometre Localisation of Kinesin Stepping



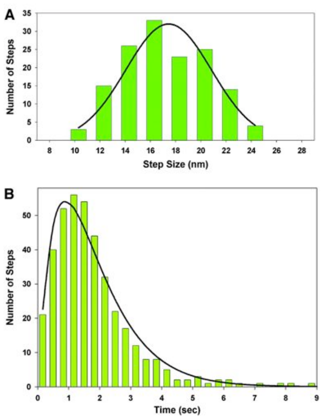


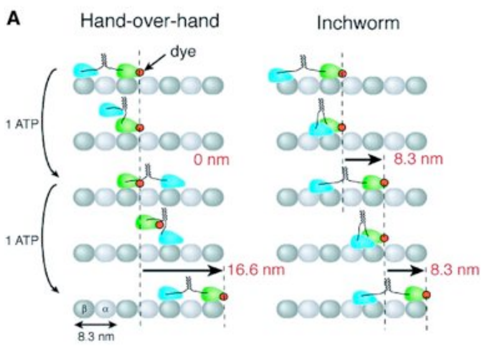
Tracking Kinesin Stepping



Yildiz et al. Kinesin walks hand-over-hand. 2004. *Science* 303, 676

Tracking Kinesin Stepping

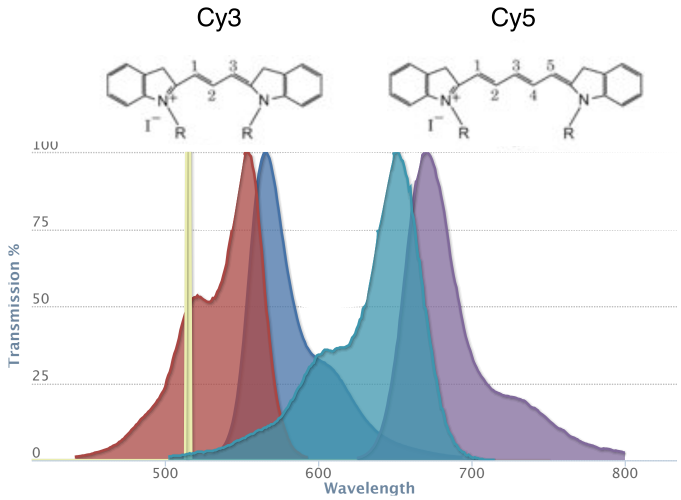




- Single step size
- Propose a kinetic scheme?
- Which model do you think is most appropriate?

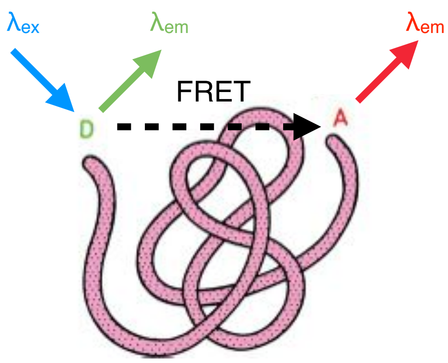
Yildiz et al. Kinesin walks hand-over-hand. 2004. *Science* 303, 676

FRET summary



- Non-radiative energy transfer between chromophores.
- Dipole-dipole interaction.
- r^{-6} distance dependence.

Using FRET as a molecular ruler



Energy Transfer Efficiency, E:

$$E = \frac{k_{ET}}{k_f + k_{ET} + \sum_i k_i}$$

in terms of dye separation:

$$E = \frac{R_0^6}{R_0^6 + r^6}$$

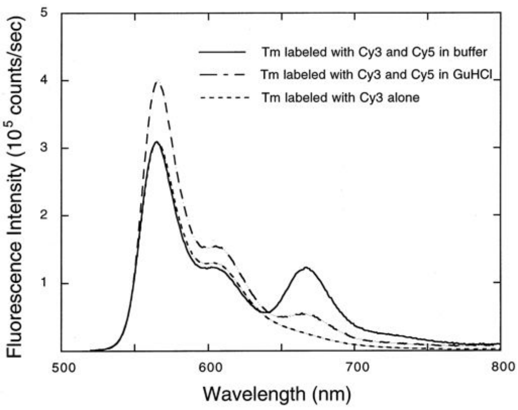
and experimentally:

$$E = \frac{I_A - \beta I_D}{I_A + \gamma I_D}$$

β fraction of donor signal in acceptor channel

γ fraction of acceptor signal in donor channel

Using FRET as a molecular ruler



Energy Transfer Efficiency, E:

$$E = \frac{k_{ET}}{k_f + k_{ET} + \sum_i k_i}$$

in terms of dye separation:

$$E = \frac{R_0^6}{R_0^6 + r^6}$$

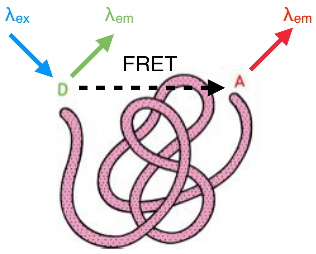
and experimentally:

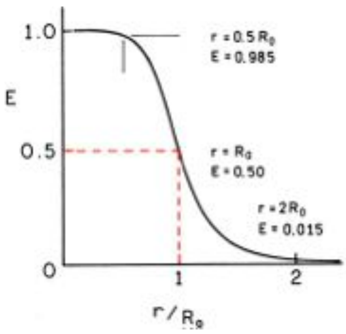
$$E = \frac{I_A - \beta I_D}{I_A + \gamma I_D}$$

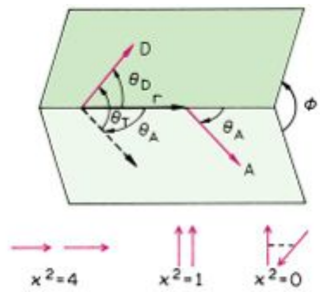
β fraction of donor signal in acceptor channel

γ fraction of acceptor signal in donor channel

Using FRET as a molecular ruler







$\kappa^2 = (\cos\theta_T - 3\cos\theta_D \cos\theta_A)^2$

relative orientation factor

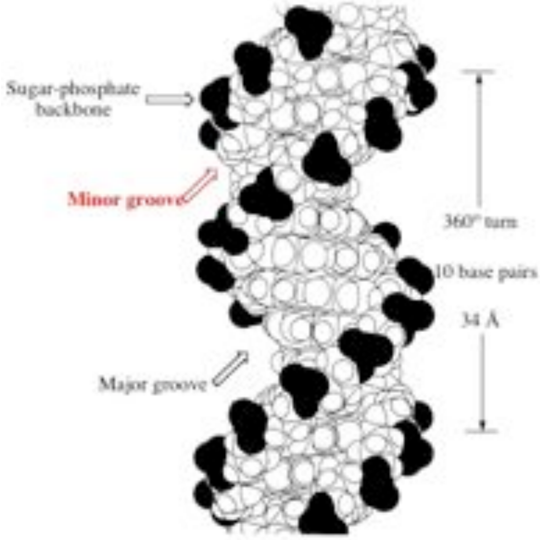
donor emission spectrum

$$E = \frac{R_0^6}{R_0^6 + r^6} \qquad R_0{}^6 = \frac{9\, Q_0\, (\ln 10) \kappa^2\, J}{128\, \pi^5\, n^4\, N_A} \qquad J = \int f_D(\lambda)\, \epsilon_A(\lambda)\, \lambda^4\, d\lambda$$

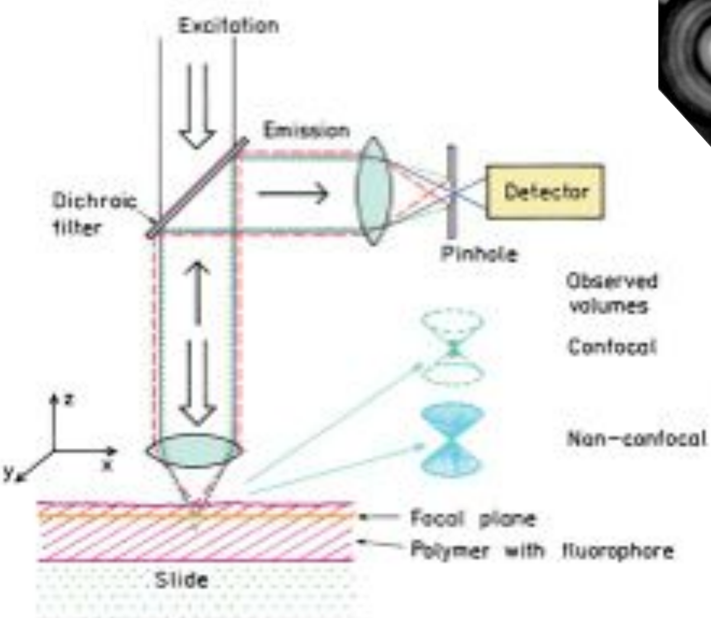
'overlap' integral

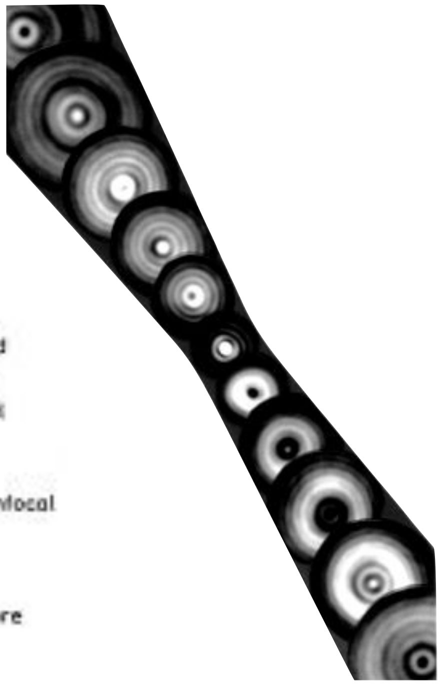
acceptor absorption spectrum

DNA: Molecular Ruler Example

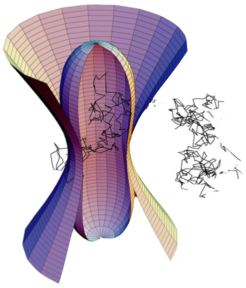


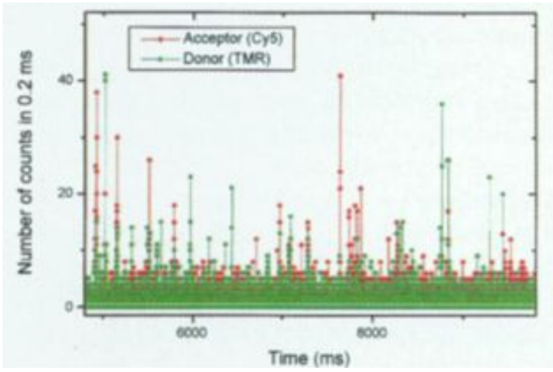
Confocal Microscopy

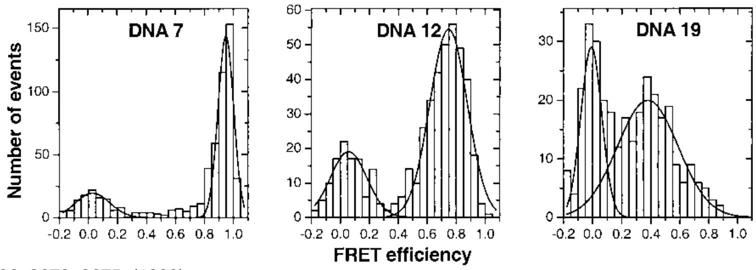




DNA: Molecular Ruler Example



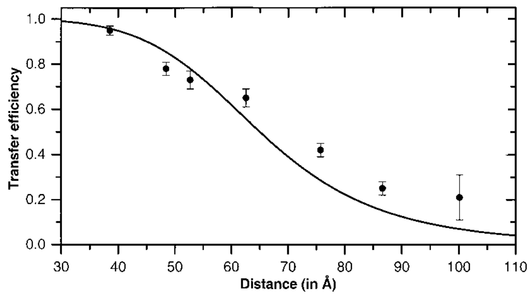




Deniz et al. PNAS 96, 3670–3675, (1999)

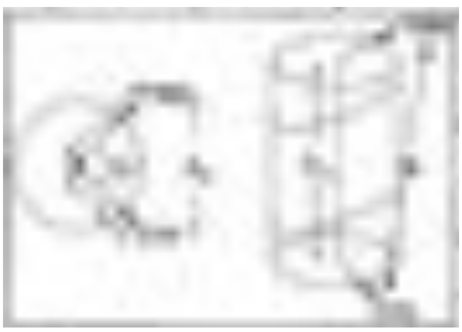
Why zero peak?

DNA: Molecular Ruler Example



Let's construct a (very) simple helical model:

- Assume helix runs around a cylinder.
- Typical diameter 20 angstroms.
- rise per base pair is 3.4 angstroms.
- 10 bp per turn.
- Calculate separation (r) as vector sum of distances parallel and perpendicular to cylinder axis.

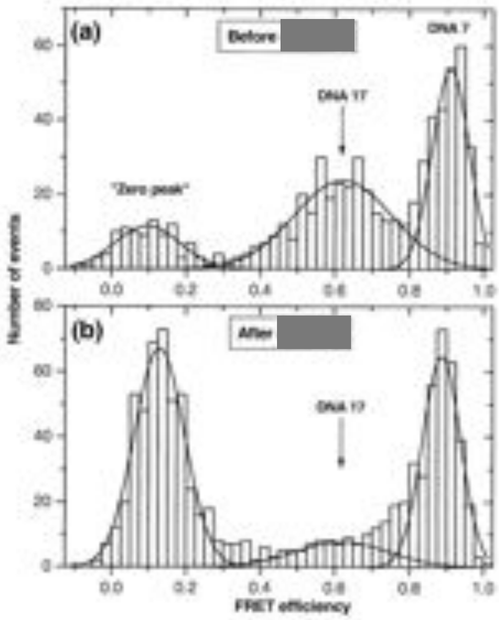


$$R_a = 3.4N + L$$

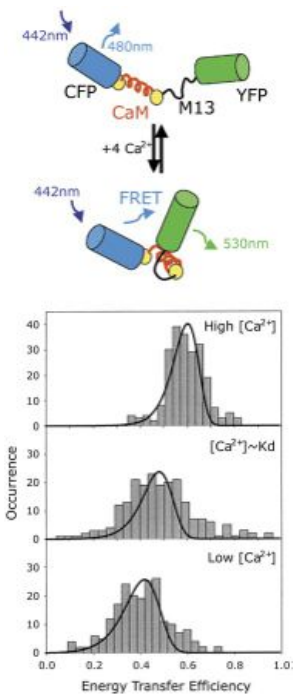
$$R_b = a^2 + d^2 + 2ad\cos\theta$$

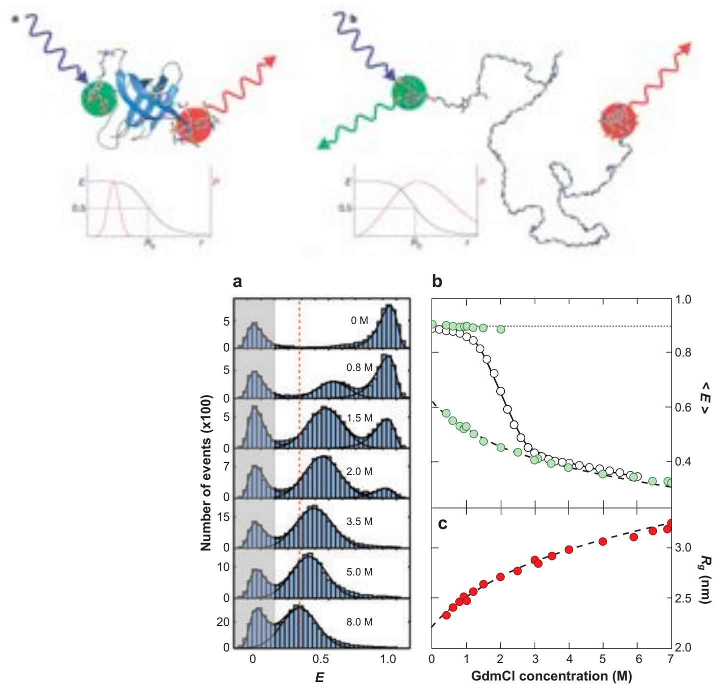
$$\theta = 36N + \phi$$

Can you interpret this?

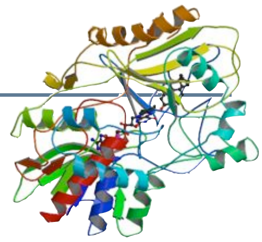


Conformational changes FRET



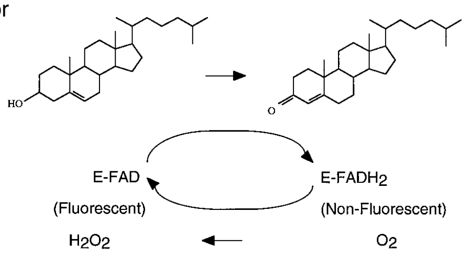


Single molecule enzyme turnover



Cholesterol Oxidase

Fluorescent Flavin Cofactor



‘Ping pong’ mechanism

$$E\text{-FAD} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\text{-FAD}\cdot\mathrm{S} \overset{k_2}{\longrightarrow} E\text{-FADH}_2 + \mathrm{P}$$

on　　　on　　　off

$$E\text{-FADH}_2 + \mathrm{O}_2 \underset{k_{-1}'}{\overset{k_1'}{\rightleftharpoons}} E\text{-FADH}_2\cdot\mathrm{O}_2 \overset{k_2'}{\longrightarrow} E\text{-FAD} + \mathrm{H}_2\mathrm{O}_2$$

off　　　off　　　on

Single-molecule Enzymology Xie & Lu 1999 JBC, 274,15967-15970.

Scanning confocal single-molecule

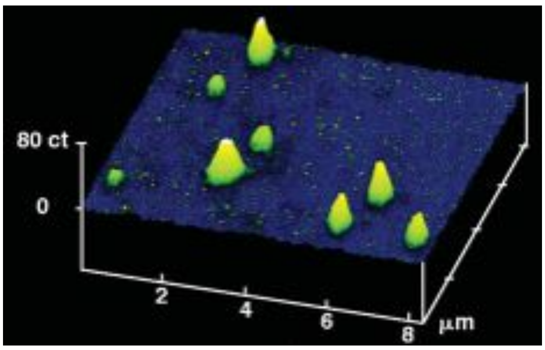
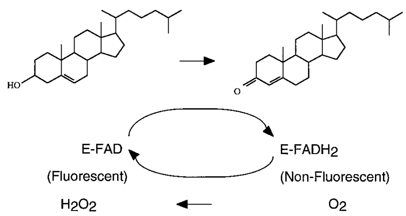
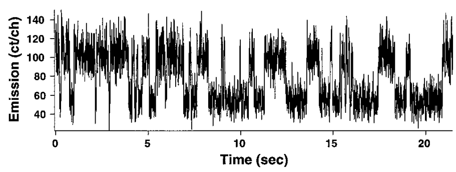
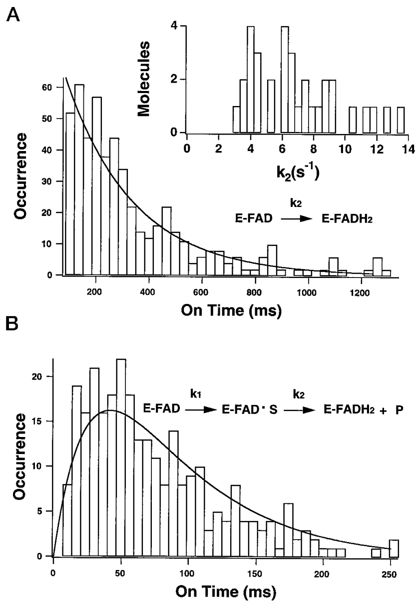


Figure 23.43. Single-molecule images of cholesterol oxidase as seen from the emission of FAD. Reprinted from [79].





Single-molecule histograms



Simplify kinetic scheme with cholesterol derivative that make k_2 rate limiting.

$$E\text{-FAD} \cdot \mathrm{S} \xrightarrow{k_2} E\text{-FADH}_2 + \mathrm{P}$$

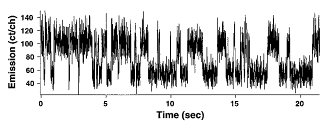
Even in this case we see a **distribution** of enzyme turnover rates.

With cholesterol it's more complex and we need original scheme.

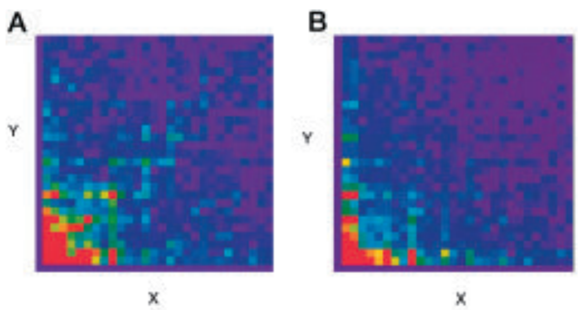
$$\begin{array}{ccccc} E\text{-FAD} + \mathrm{S} & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & E\text{-FAD} \cdot \mathrm{S} & \xrightarrow{k_2} & E\text{-FADH}_2 + \mathrm{P} \\ \text{on} & & \text{on} & & \text{off} \end{array}$$

Enzyme memory effects

...going beyond Michaelis-Menten. Static and dynamic heterogeneity.



2 turnover separation   10 turnover separation



$$E \underset{k_{E'}}{\overset{k_E}{\rightleftharpoons}} E'$$

$$\begin{array}{ccccc} E\text{-FAD} + \mathrm{S} & \xrightarrow{k_1} & E\text{-FAD} \cdot \mathrm{S} & \xrightarrow{k_{21}} & E\text{-FADH}_2 + \mathrm{P} \\ \updownarrow & & \updownarrow & & \updownarrow \\ E'\text{-FAD} + \mathrm{S} & \xrightarrow{k_1} & E'\text{-FAD} \cdot \mathrm{S} & \xrightarrow{k_{22}} & E'\text{-FADH}_2 + \mathrm{P} \end{array}$$

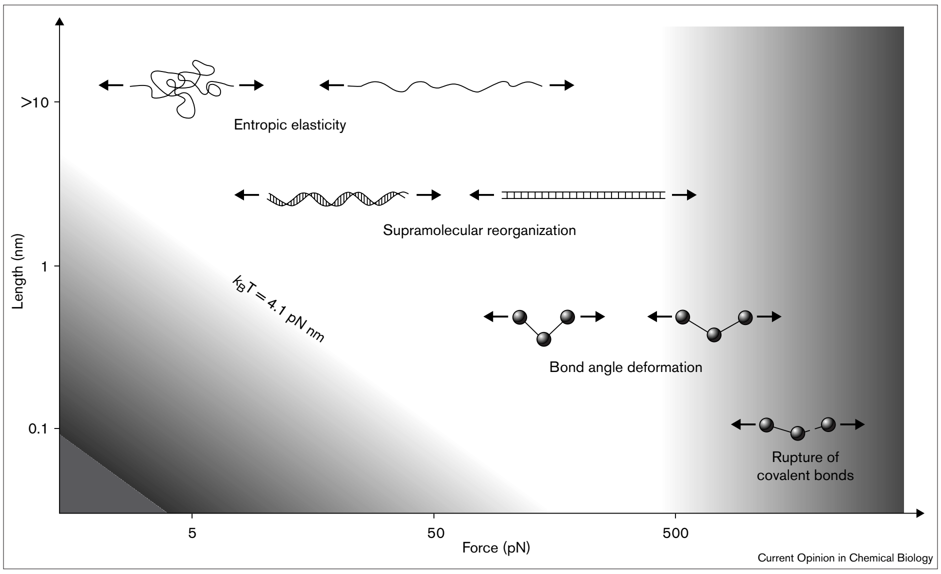
Slowly interconverting conformational states of the enzyme?

Biophysical Chemistry

Justin Benesch
Jonathan Doye
Mark Wallace

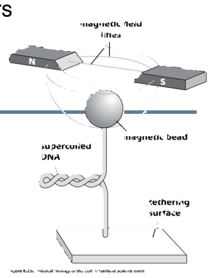
Experimentally accessible forces

Figure 1

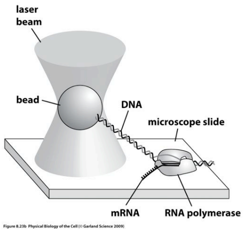


Typical forces and length scales (both scales are logarithmic) in single-molecule force spectroscopy. The experimental accessibility of mechanical information is limited to the light areas of the plot. The shaded area in the lower left corner indicates the region of limited thermal stability of molecular structures (length multiplied by force = thermal energy, k_BT = 4.1 pN nm at room temperature). The upper limit to the accessible experimental force range is determined by the rupture of covalent bonds at several nanonewton.

Magnetic Tweezers



Force Spectroscopy

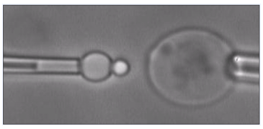


Optical Tweezers

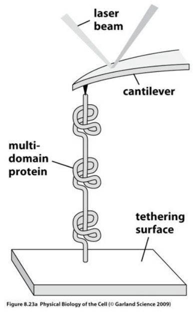
Comparison of techniques used for the mechanical characterization of bio-molecules*.

Method	Force range (pN)	Dynamical range	Typical applications
Magnetic beads	0.01–100	≥1 s	Stretching and twisting DNA
Optical tweezers	0.1–150	≥10 ms	Actin, DNA, molecular motors, proteins
Microneedles	>0.1	≥100 ms	Actin, stretching, unzipping and twisting DNA
BFP	0.5–1000	≥1 ms	Membrane anchors, receptor–ligand pairs
AFM	>1	≥10 μs	DNA, proteins, receptor–ligand pairs

*Typical values for accessible force range and experimental time window (dynamical range) are given.



Biomembrane Force Probe

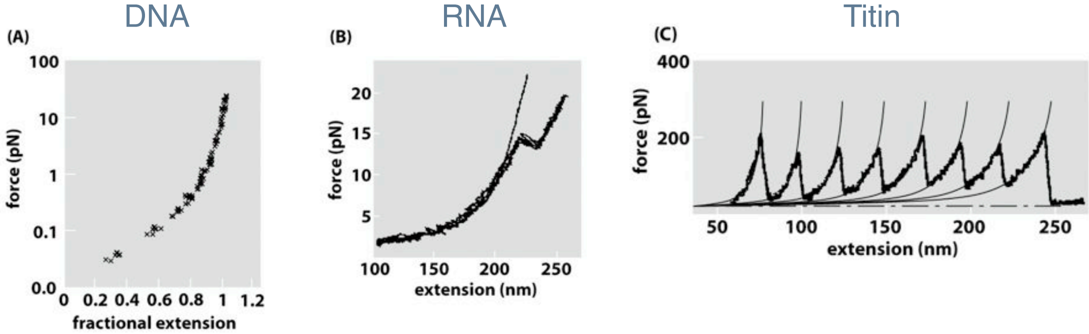


Atomic Force Microscopy

Force-extension of biopolymers

- Measuring the forces on a biomolecule as it is stretched can reveal information about it's structure and folding.

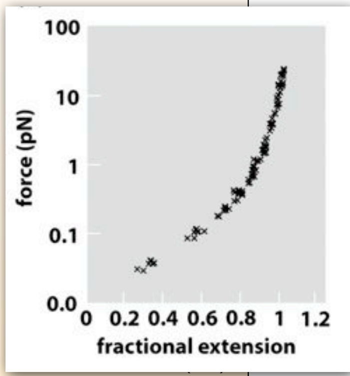
DNA RNA Titin



Recall biopolymer lecture:

This can then be used to obtain a expression for the force-extension curve:

$$\begin{aligned}\langle z \rangle &= -\frac{\partial A}{\partial F} = kT\frac{\partial \log Z}{\partial F} = NkT\frac{\partial}{\partial F}\left(\log\left(\sinh\left(\frac{Fl}{kT}\right)\right) - \log F\right) \quad (9)\\ &= Nl\left(\coth\left(\frac{Fl}{kT}\right) - \frac{kT}{Fl}\right)\end{aligned}$$



At low force ($F \ll kT/l$) this simplifies to Hooke's Law:

$$F = \frac{3kT}{Nl^2}z$$

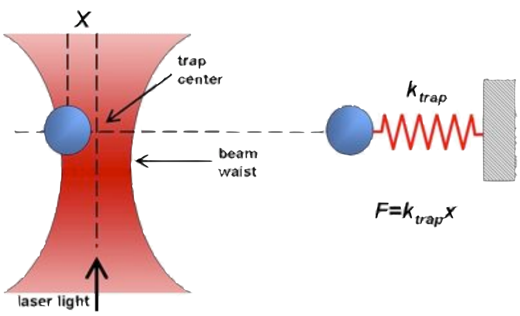
and at high force ($F \gg kT/l$) to

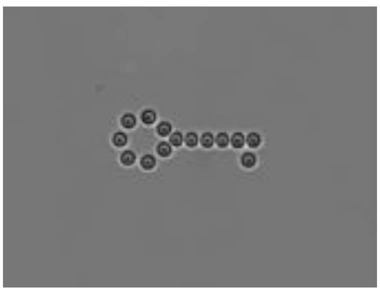
$$F = \frac{kT}{l}\frac{1}{1 - z/L}$$

The force diverges as the polymer approaches its contour length.

Introduction to optical tweezers

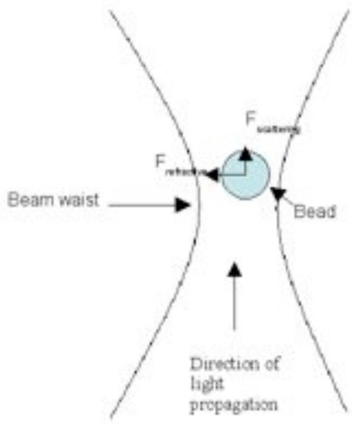
- Use radiation pressure to trap micron-sized objects in a focused laser.
- For a gaussian beam, force is proportional to displacement.
- Applies 0.01-100 pN forces.





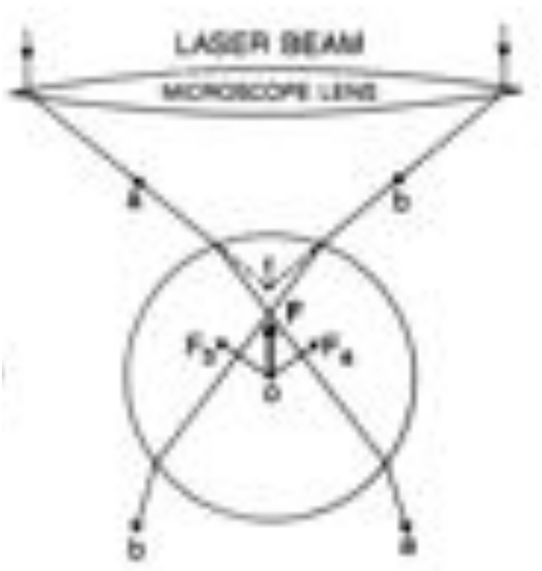
Optical Tweezers Theory

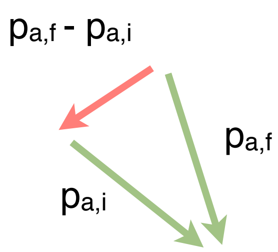
- Consider the momentum associated with light acting on a particle.
  - **Scattering** gives rise to force component in the direction of incident light.
  - **Refraction** gives rise to force component in the direction of intensity gradient.
  - Condition for stability of an object in the field is that the ratio of the gradient force to the scattering force be greater than unity.
  - For trapping the particle must have a higher index of refraction than that of the surrounding medium.



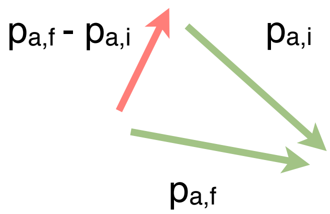
Axial gradient forces

Consider conservation of momentum following the momentum change due to light being refracted through the bead....

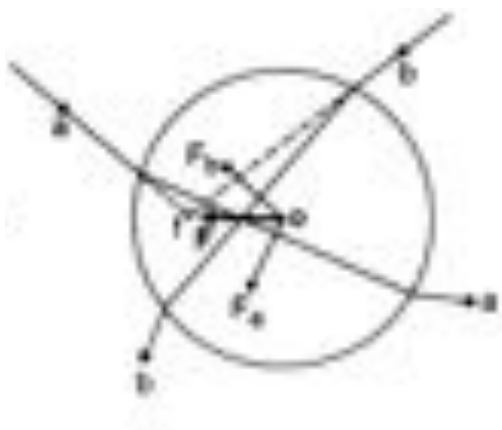


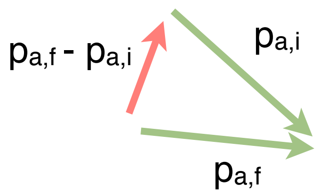


Axial gradient forces



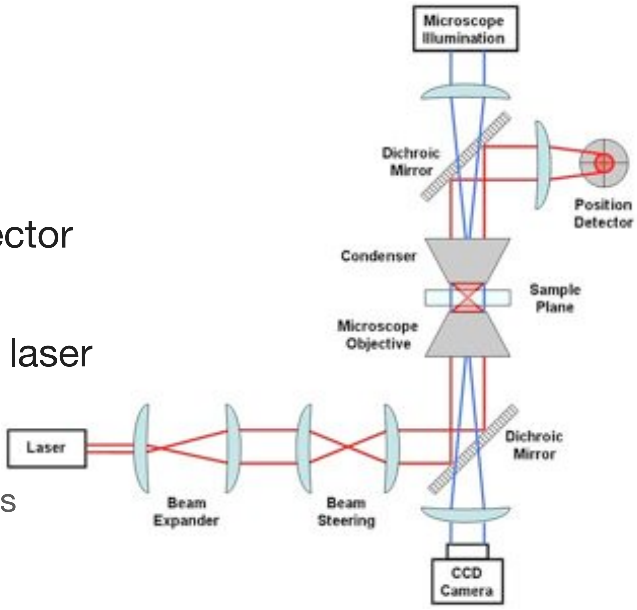
Lateral gradient forces





Instrument design

- Infrared
  - Sample transparency
  - High power
- Position sensitive detector
  - Fast feedback
- Can control sample or laser position
  - scanning mirrors
  - acousto-optic deflectors
  - piezo-electric stage



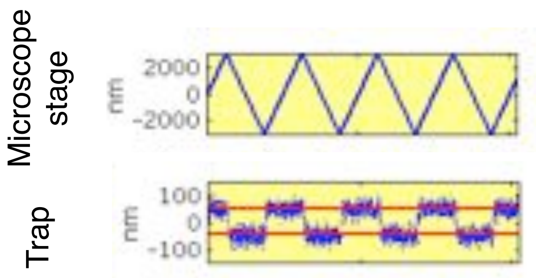
How about calibration?

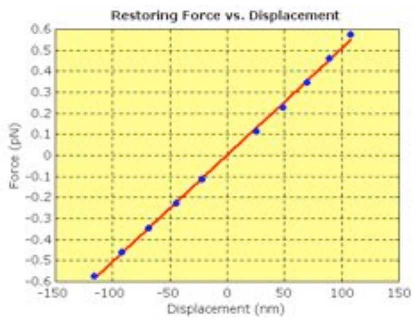
- Calibrate by examining thermal fluctuations (use equipartition).

$$k = k_B T / \langle x^2 \rangle$$

- Calibrate by measuring displacement when particle subject to viscous drag.

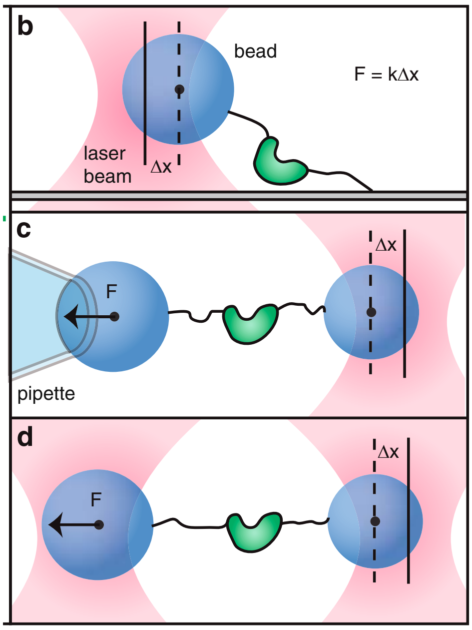
$$F_{\text{drag}} = \beta v = \alpha x \qquad \beta_{\text{sphere}} = 6\pi\eta a$$



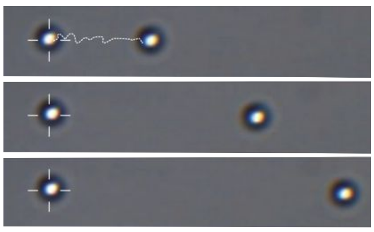


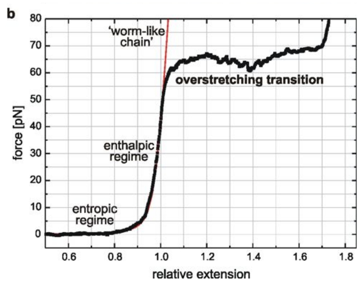
http://www2.bioch.ox.ac.uk/~oubsu/ebjknight

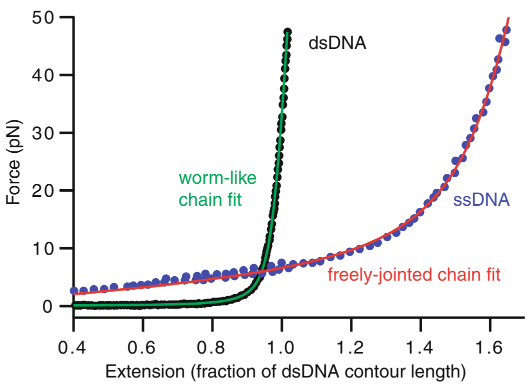
Trap Geometries



Example 1: DNA pulling







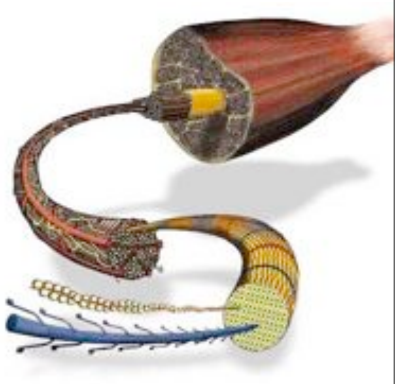
dsDNA:
- Approximate with WLC
- Persistence length ~ 50 nm

ssDNA:
- Approximate with FJC
- Persistence length ~ 2 nm

$$p_l = \frac{l_K}{2}$$

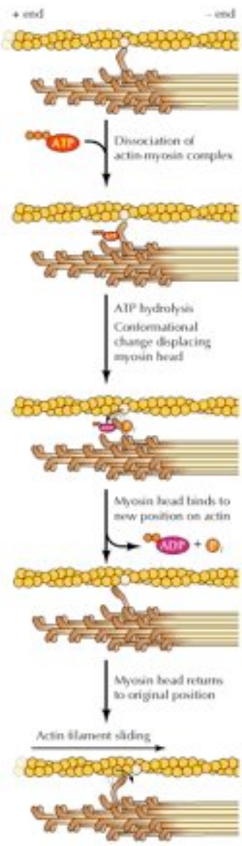
Example 2: Myosin & Actin

- Myosin motor protein that moves along an actin filament.
- ATP dependent motor.
- Actin myosin (II) interaction governs muscle contraction.
- Other myosins also involved in cell motility, cell division, hearing adaptation, organelle transport

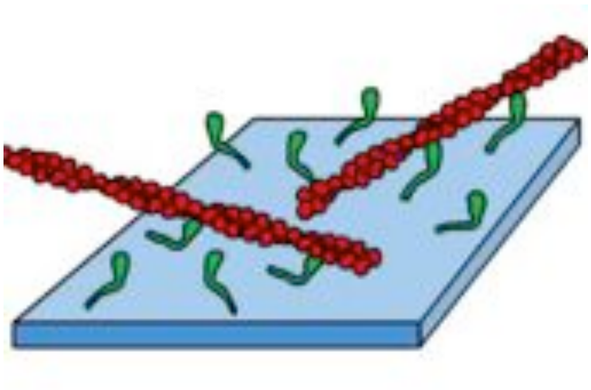


Myosin & Actin

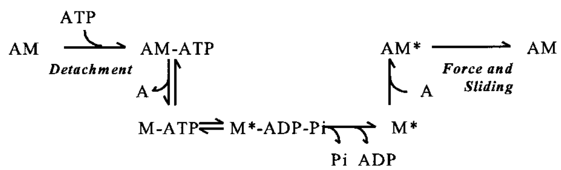
- Myosin motor protein that moves along an actin filament.
- ATP dependent motor.
- Actin myosin (II) interaction governs muscle contraction.
- Other myosins also involved in cell motility, cell division, hearing adaptation, organelle transport

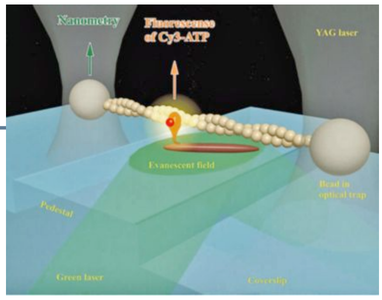


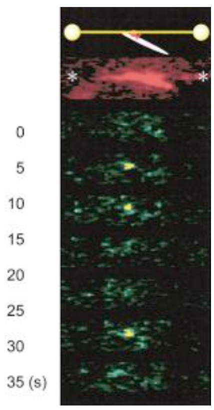
Motility assay

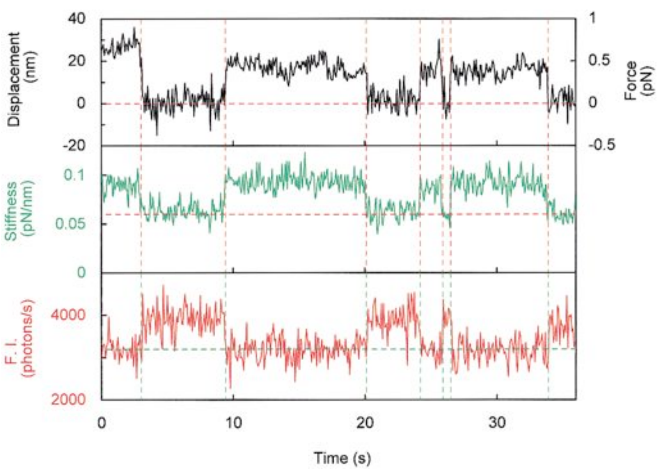


Optical tweezing actomyosin



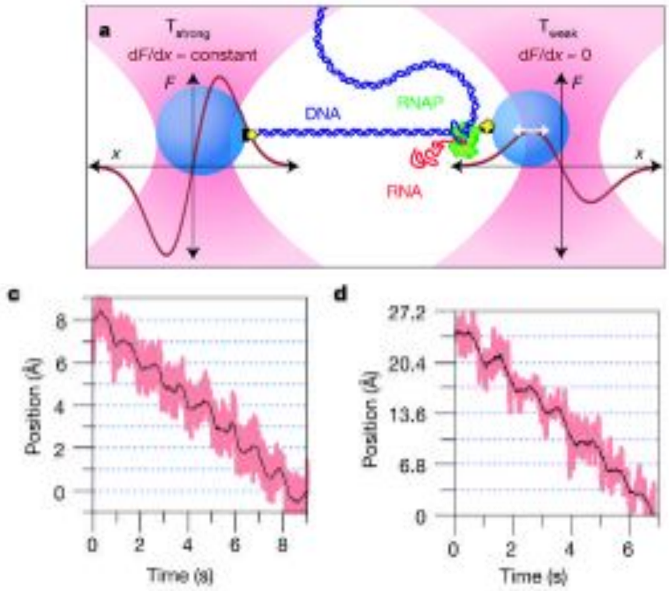


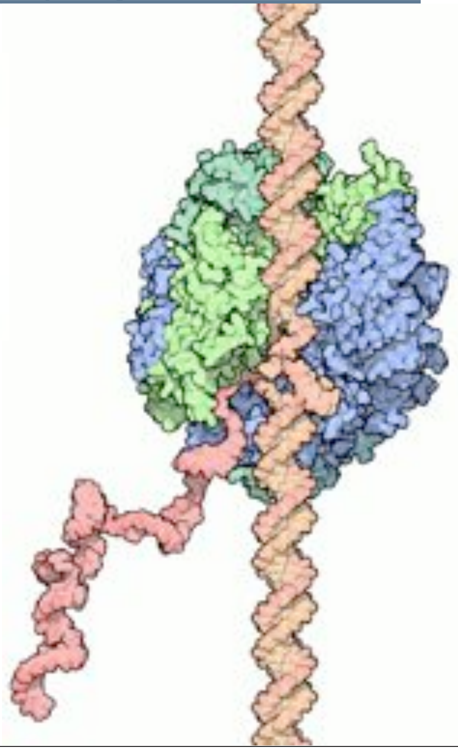




Ishijima et al. Cell, 92, 161-171, 1998

Example 3: 7 years later, RNA polymerase



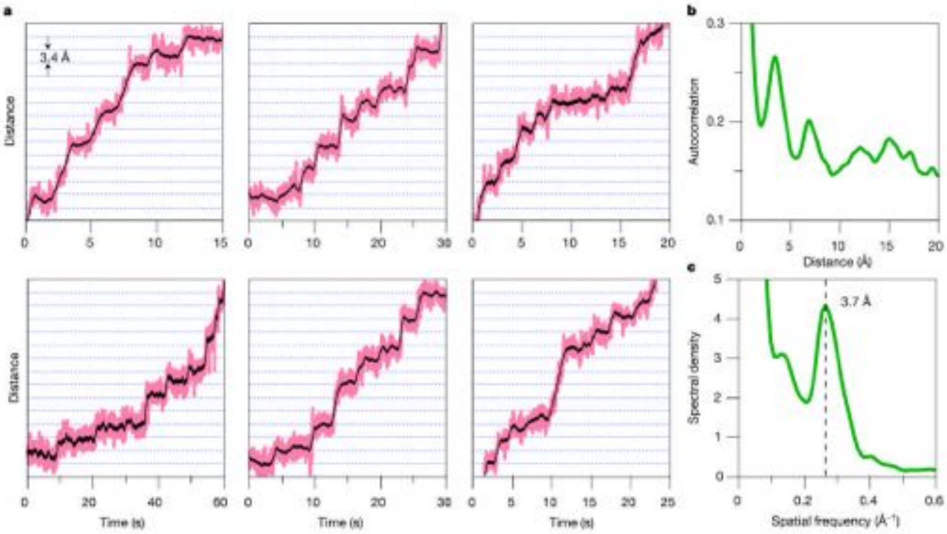


Abbondanzieri et al. Nature 2005 438 460–465.

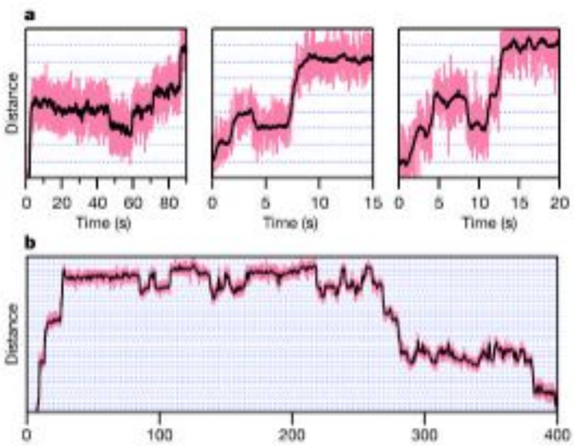
Molecular motors, polymerase

To isolate the detection and trapping beams from the effects of random air currents, which introduce density fluctuations that perturb the positional stability of laser beams, we enclosed all optical elements external to the microscope in a sealed box filled with helium gas at atmospheric pressure. Because the refractive index of helium is closer to unity than that of air (n_{He} = 1.000036 compared with n_{air} = 1.000293), density fluctuations introduce smaller deflections. Using helium, we realized a tenfold reduction in the noise spectral density at 0.1 Hz for a stiffly trapped, 700-nm diameter bead (k = 1.9 pN nm^{-1}), and the integrated system noise power remained below ~1 Å over the bandwidth of interest (Fig. 1b). To illustrate the

RNAP moves in discrete steps



RNAP backstepping and backtracking



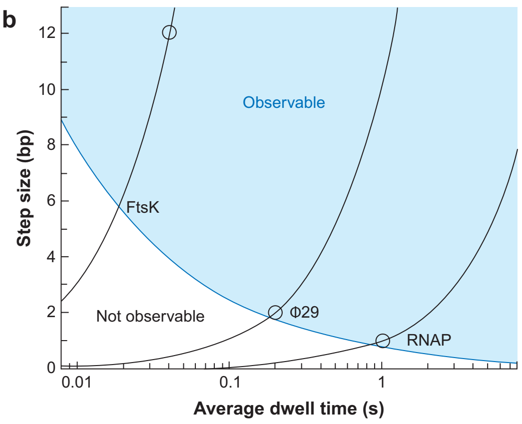
Under assisting loads, RNAP sometimes backsteps.

Under hindering loads DNAP sometimes backtracks.

Signal vs Brownian noise

$$SNR \leq \frac{\kappa_{tether}\Delta\ell}{\sqrt{4k_B T B \gamma}}$$

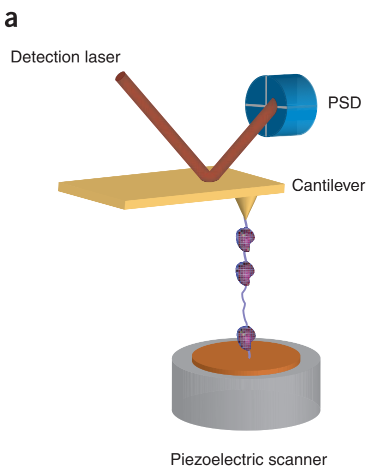
κ_{tether} = stiffness of tether
Δl = extension
B= bandwidth of measurement
γ= $6\pi\eta a$ =drag coefficient

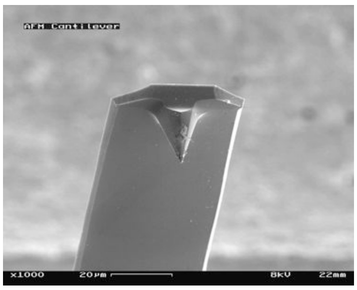


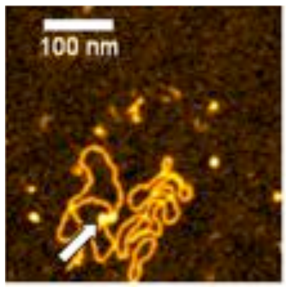
Resolution does not depend on Trap Stiffness

www.annualreviews.org • Recent Advances in Optical Tweezers

Atomic Force Microscopy & Spectroscopy



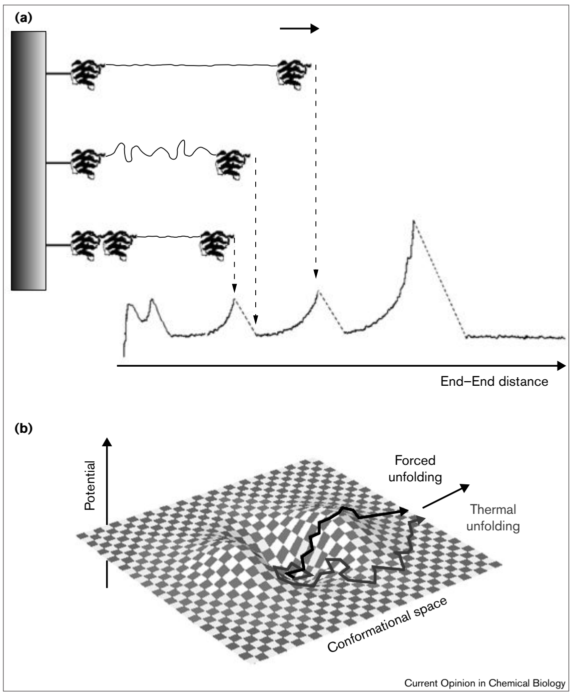




Leng et al. PNAS 2011 108 19973

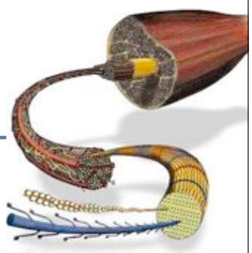
Protein unfolding

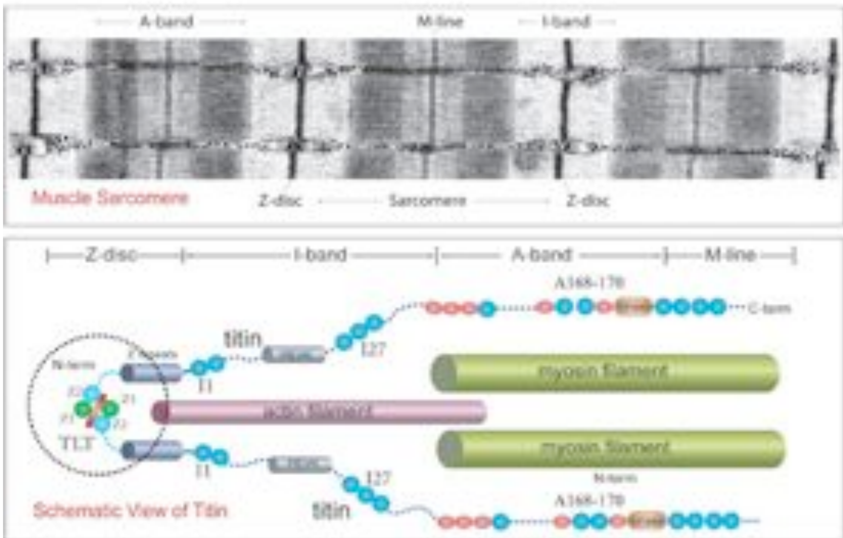
- Saw-tooth pattern from individual domain unfolding.
- Distance between peaks reflects contour length.
- An applied force enables unfolding over an energy barrier.



Current Op. Chem. Biol.

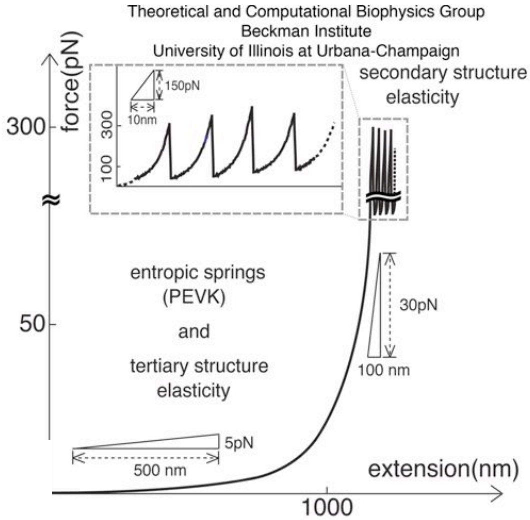
An example, Titin





Theoretical and Computational Biophysics Group, Beckman Institute, University of Illinois at Urbana-Champaign

Mechanical Unfolding of Titin

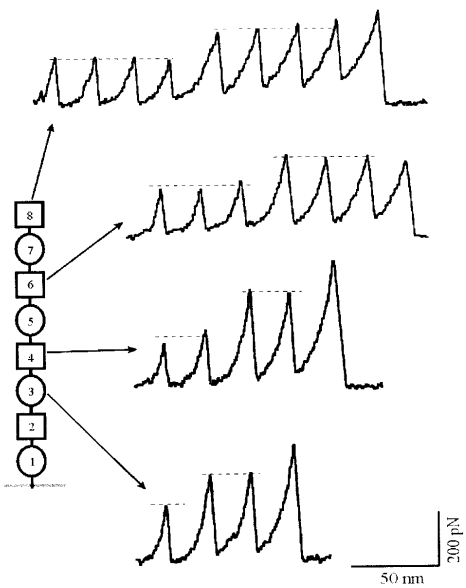


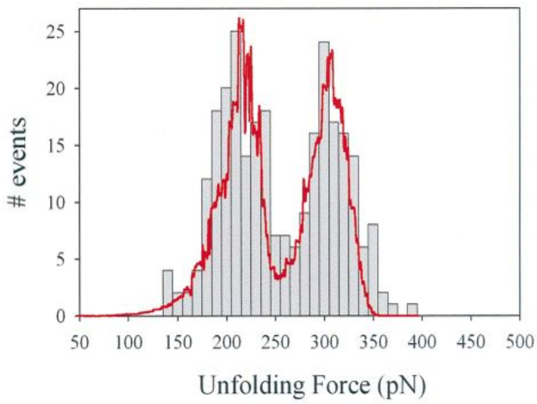
Tertiary protein structure gives rise to entropic spring response.

Secondary protein structure gives rise to protein unfolding peaks.

Engineering a model protein

(I27-I28)n poly protein as a model 'titin'





Li et al. PNAS 2000 97 6527

Biophysical Chemistry

Justin Benesch
Jonathan Doye
Mark Wallace

The cell membrane, a reminder...

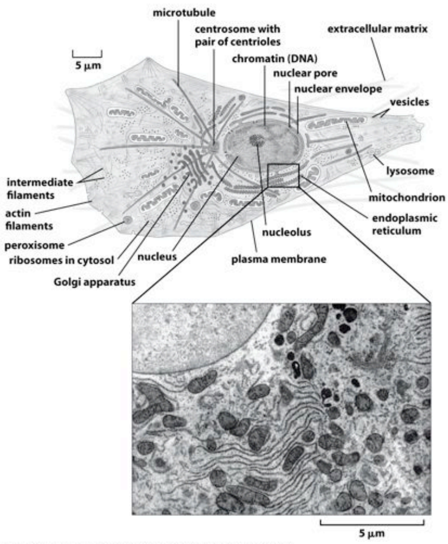


Figure 2.13 Physical Biology of the Cell (© Garland Science 2009)

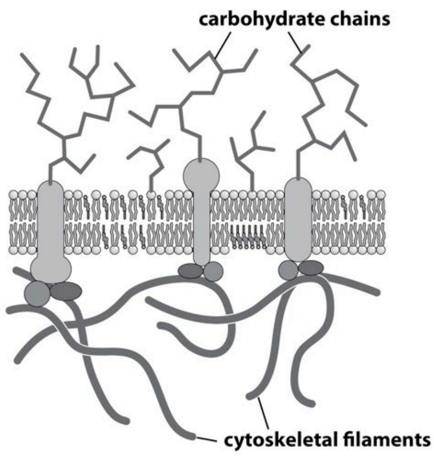


Figure 11.4c Physical Biology of the Cell (© Garland Science 2009)

Constituents of the cell membrane

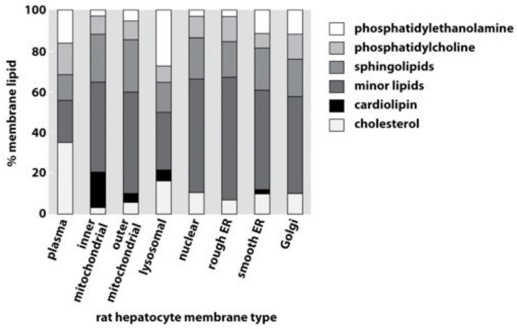
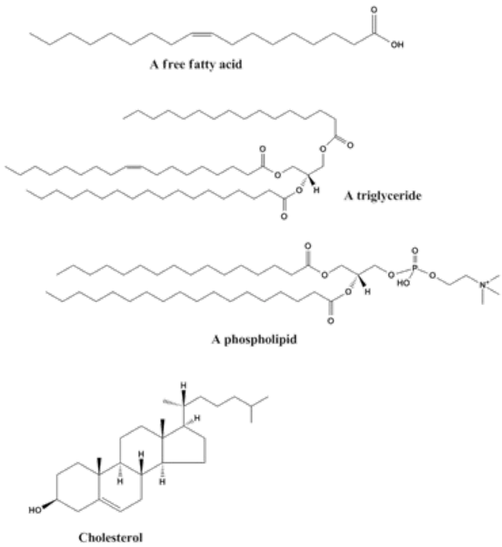
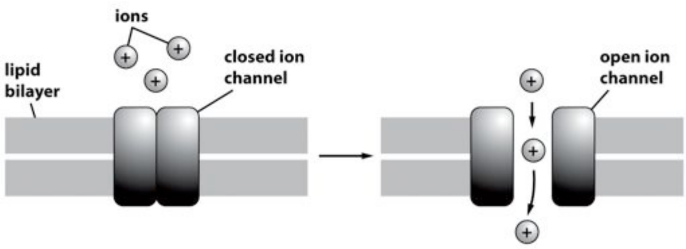
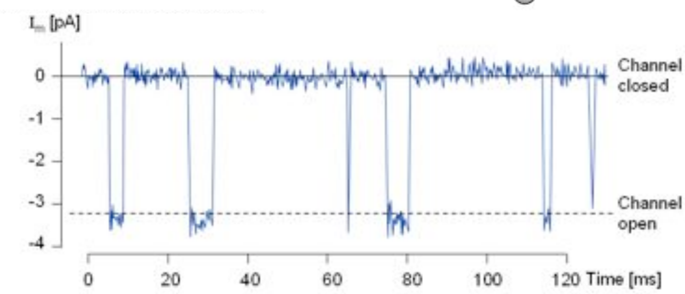


Figure 11.6a Physical Biology of the Cell (© Garland Science 2009)



Ion channels

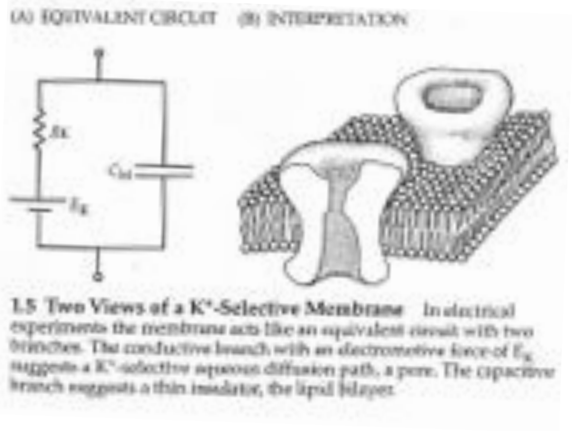




- Integral membrane protein forming a conductive channel.
- ‘Open’ and ‘closed’ states.
- Usually selective for particular ions.

An electrical model of the cell membrane?

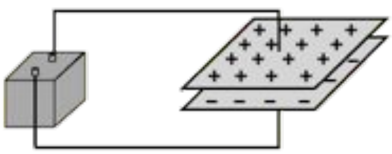
- Lipid bilayer
  - Capacitor
- Ion channel
  - Resistor
- Ionic gradients
  - Applied Potential



1.5 Two Views of a K+-Selective Membrane In electrical experiments the membrane acts like an equivalent circuit with two branches. The conductive branch with an electromotive force of E_K suggests a K^+-selective aqueous diffusion path, a pore. The capacitive branch suggests a thin insulator, the lipid bilayer.

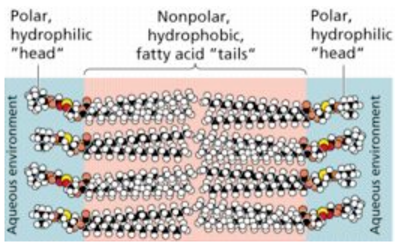
- Conventions
  - Membrane potential is measured as V_{in}-V_{out}
  - For cations an outward flow of ions is a positive current.
  - Outside of the cell is usually drawn at the top of any diagram.

The lipid bilayer as a capacitor



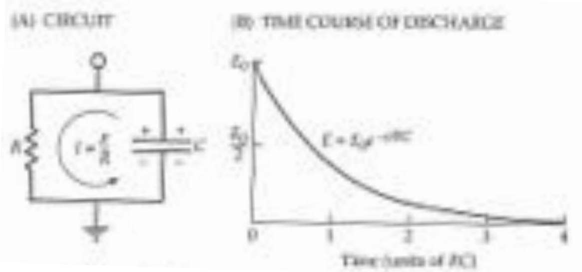
Capacitor

A battery will transport charge from one plate to the other until the voltage produced by the charge buildup is equal to the battery voltage.



$$C = \frac{Q}{V}$$

$$I = \frac{dQ}{dt} = C\frac{dV}{dt}$$



Membrane specific capacitance

$$C = \epsilon \frac{A}{d}$$

We can relate capacitance to dielectic permittivity, area, and separation.

$$C_m = \frac{\epsilon}{d}$$

But for a lipid bilayer often more useful to define a specific capacitance (per unit area).
Large, typically 1 uF cm^{-2}

Note that in the case of the cell membrane a small amount of charge separation is able to generate a large potential difference.

e.g. to obtain a membrane potential of 100 mV
$Q_m = C_m \times V_m = 1\ \mu F\ cm^{-2} \times 0.1\ V = 0.1\ \mu C\ cm^{-2}$

How many monovalent ions is this? (divide by charge on an electron..)
$6.25 \times 10^{11}\ cm^{-2} = 6250\ um^{-2}$
Not many when you consider the size of a cell!

e.g. see http://www.brainavm.uhnres.utoronto.ca/staff/Tymianski/THE_NERVE_IMPULSE.htm

Hille model of pore conductance

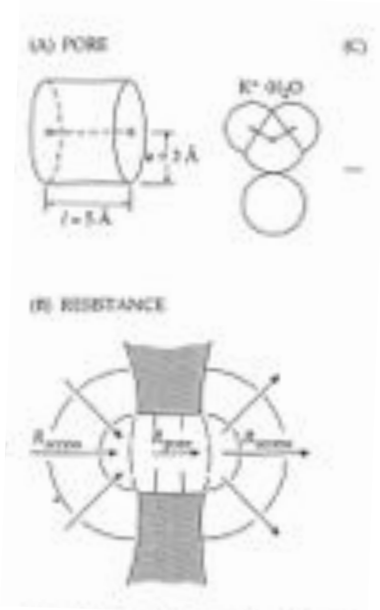
Recall that resistance depends on resistivity, ρ, length and area.

$$R = \frac{\rho l}{A}$$

Two contributions to resistance in an ion channel, the pore itself, and the access resistance.

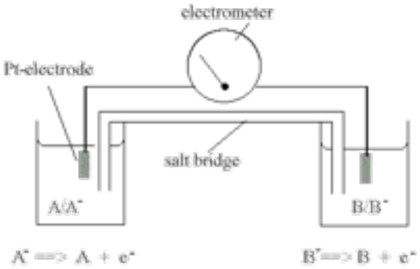
Treat the pore as a cylinder, and the access as the integral from infinity to the pore radius over two hemispherical caps.

$$R_{total} = R_{pore} + R_{access} = \frac{\rho}{\pi a^2} + \frac{\rho}{4\pi a}$$



For a = 0.3 nm, l = 0.5 nm, ρ = 80 Ω cm^{-1} this gives a R_{pore} of ≈ 1.4 GΩ

Nernst equation



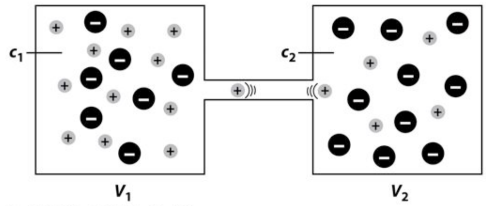


Figure 17.2 Physical Biology of the Cell (© Garland Science 2009)

$$E_{cell} = E^{\ominus}_{cell} - \frac{RT}{\nu F} \ln Q$$

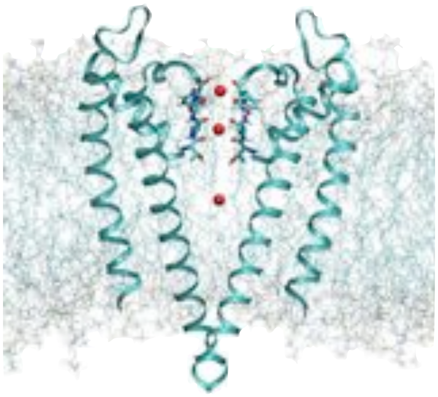
$$E_2 - E_1 = \frac{RT}{\nu F} \ln \frac{c_1}{c_2}$$

Ion species	Intracellular concentration (mM)	Extracellular concentration (mM)	Nernst potential (mV)
K^+	155	4	−98
Na^+	12	145	67
Ca^{2+}	10^{-4}	1.5	130
Cl^-	4	120	−90

Concentration imbalance leads to a resting potential in the cell.

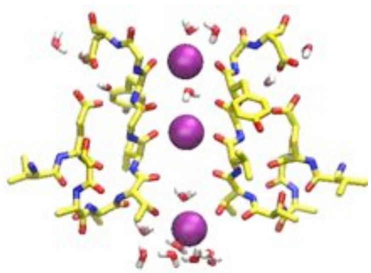
life.illinois.edu

Ion channels are selective



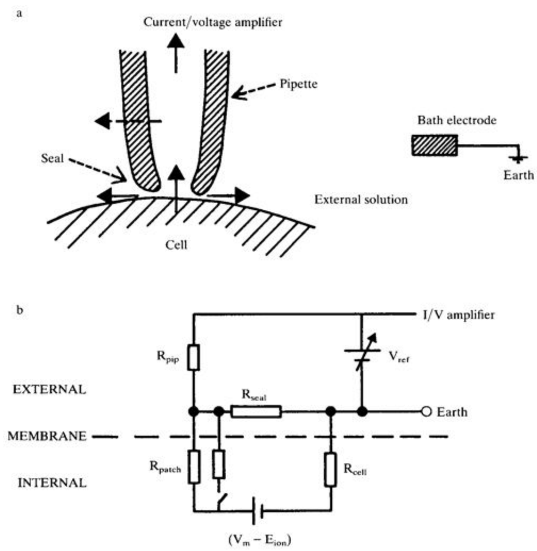
e.g. for Potassium channels such as KcsA, the hydration shell is removed from the ion when it enters the selectivity filter

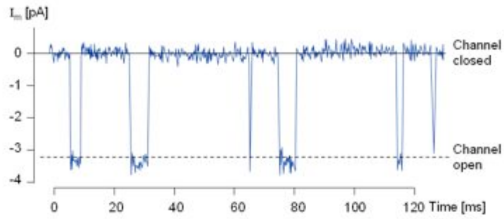
TVGYG electro-negative carbonyl oxygen atoms aligned toward the centre of the filter pore



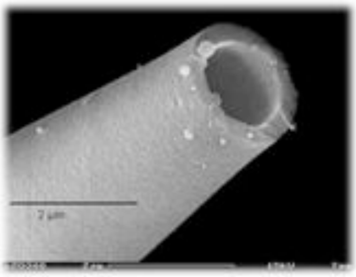
http://domene.chem.ox.ac.uk

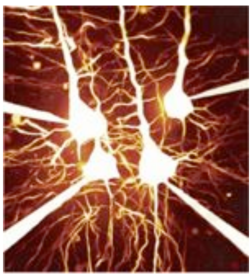
The patch clamp

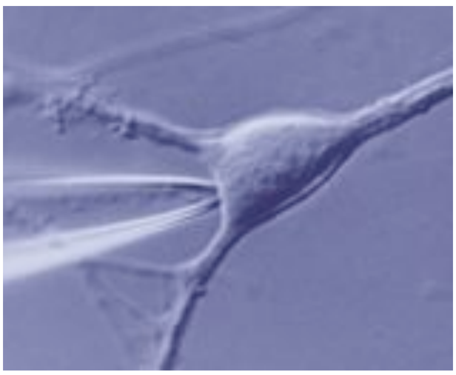


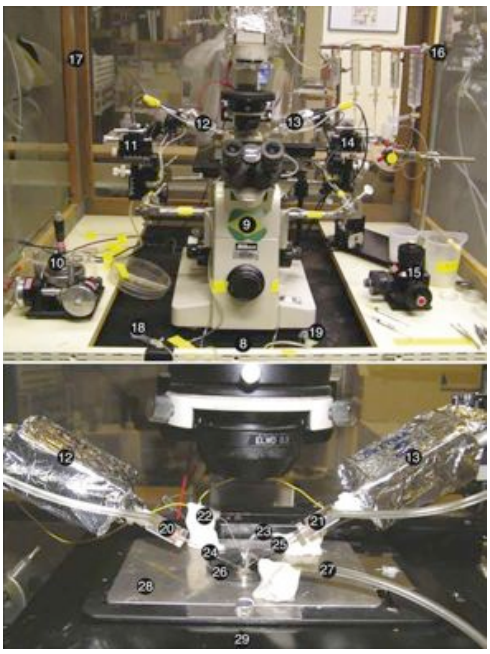


Patch clamping, reality.









Voltage-gated channels

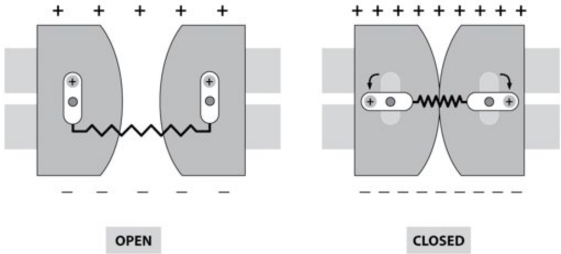
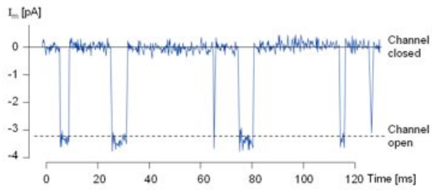


Figure 17.6 Physical Biology of the Cell (© Garland Science 2009)



Voltage-gated channels

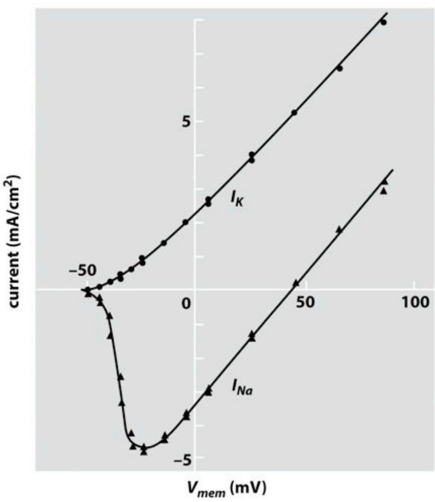


Figure 17.12 Physical Biology of the Cell (© Garland Science 2009)

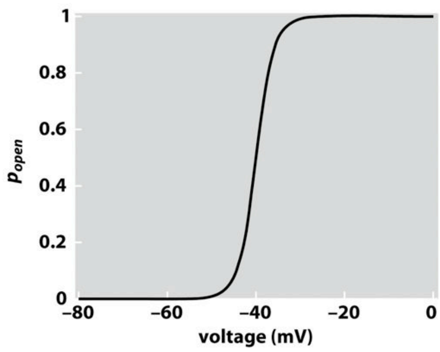


Figure 17.7 Physical Biology of the Cell (© Garland Science 2009)

a little stat mech...

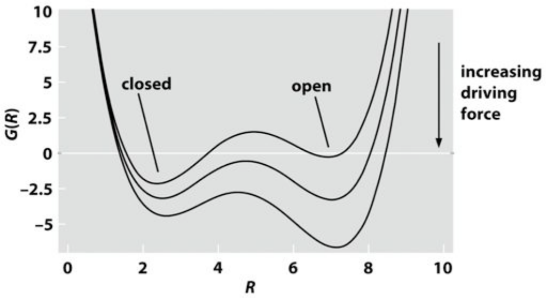


Figure 7.6 Physical Biology of the Cell (© Garland Science 2009)

ϵ_{cl}

ϵ_{op}

$$q = \sum_i e^{-\beta E}$$

$$q = e^{-\beta\epsilon_{cl}} + e^{-\beta\epsilon_{op}}$$

$$p_{op} = \frac{e^{-\beta\epsilon_{op}}}{e^{-\beta\epsilon_{cl}} + e^{-\beta\epsilon_{op}}}$$

$$p_{op} = \frac{e^{-\beta\Delta\epsilon}}{1 + e^{-\beta\Delta\epsilon}}$$

Open probability varies with applied potential

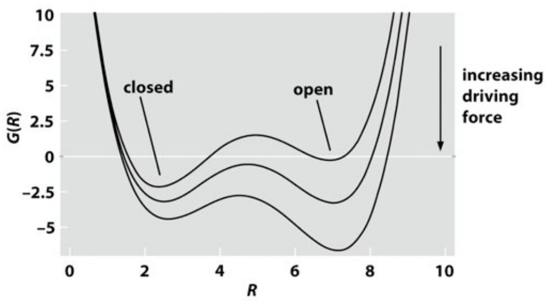


Figure 7.6 Physical Biology of the Cell (© Garland Science 2009)

Separate energy difference into conformational difference, and that due to different charge motions.

$$\Delta\epsilon = \Delta\epsilon_{conf} - \mu\frac{V_{mem}}{d}$$

dipole moment

electric field across membrane

Let’s define a midpoint value of the potential at which p_0=0.5

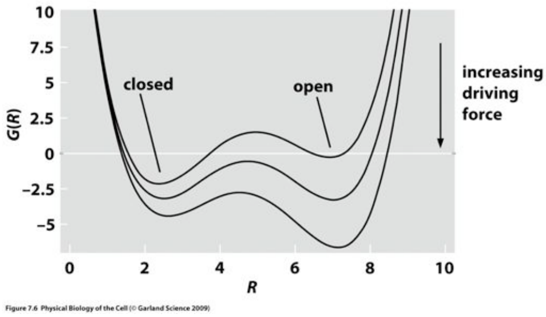
$$V^* = \frac{d\Delta\epsilon_{conf}}{\mu}$$

$$p_{op} = \frac{e^{-\beta\Delta\epsilon}}{1 + e^{-\beta\Delta\epsilon}}$$

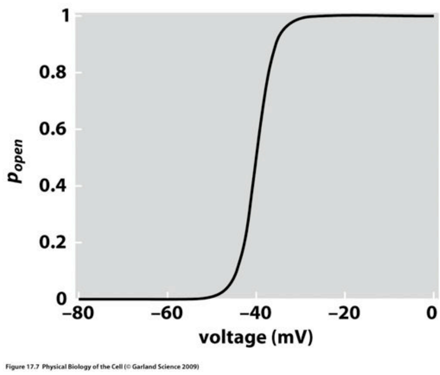
$$q = \mu/d$$

$$p_{op} = \frac{1}{1 + e^{\beta q(V^* - V_{mem})}}$$

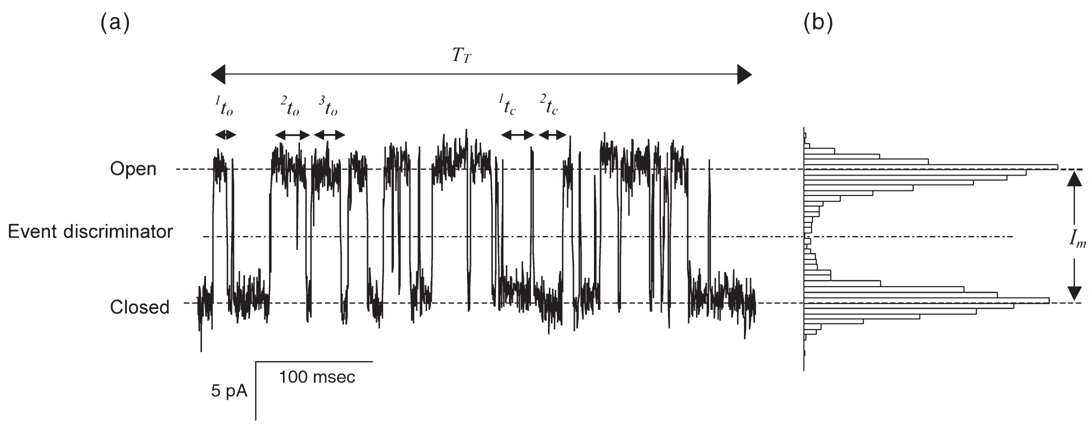
Open probability varies with applied potential



$$p_{op} = \frac{1}{1 + e^{\beta q (V^* - V_{mem})}}$$

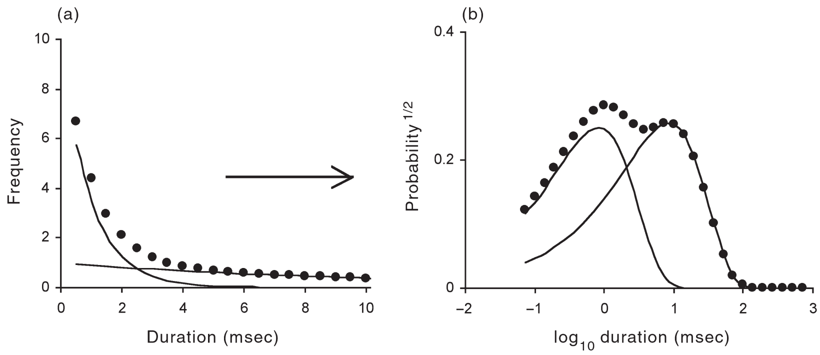


Single-channel analysis



$$P_o = \frac{{}^1t_o + {}^2t_o + {}^3t_o + {}^4t_{o\ldots\ldots} + {}^nt_o}{T_T}$$

Single channel analysis



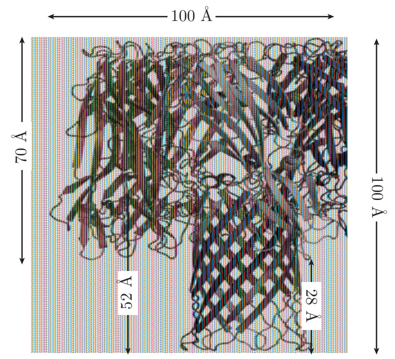
A distribution of dwell-times, here comprised of two exponential components with distinctly different decay constants.

The probability distribution is calculated by normalizing the frequency distribution according to the area under the curve. Note the logarithmic binning.

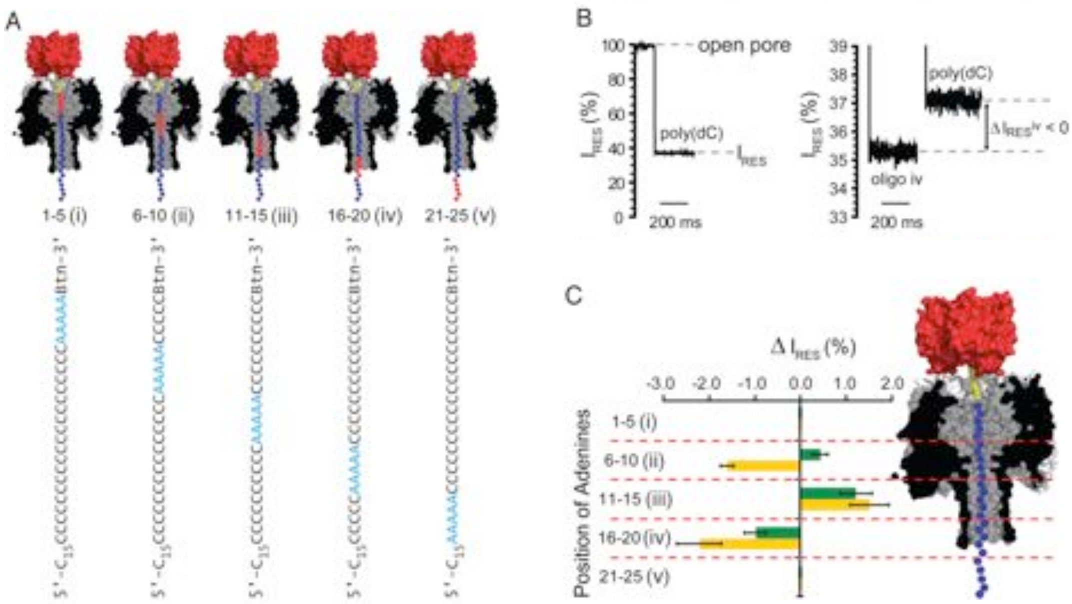
The locations of the peaks on the time scale approximately correspond to the time constants of each exponential.

DOI: 10.1046/j.1440-1681.2001.03503.x

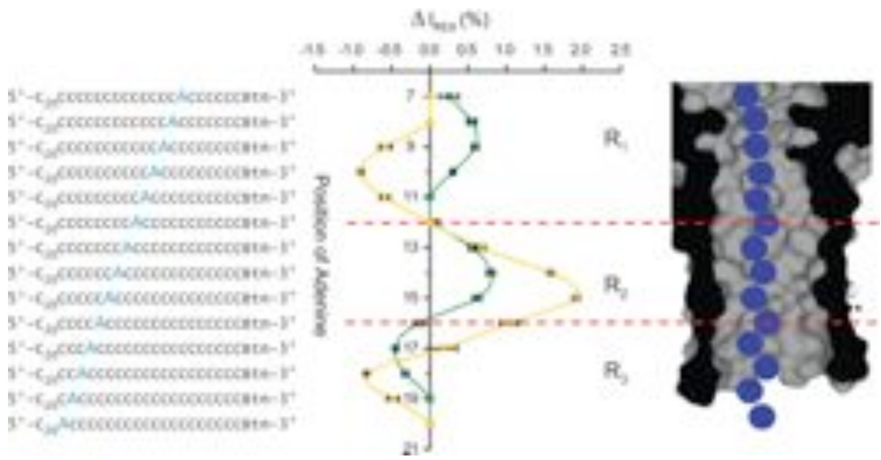
An example, the α-hemolysin nanopore



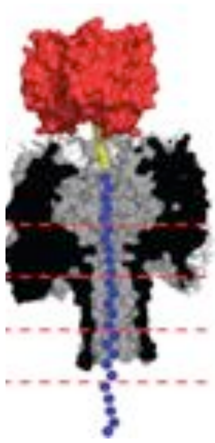
An example, the α-hemolysin nanopore

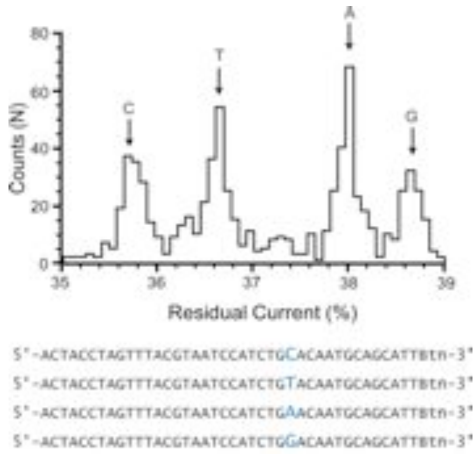


An example, the α-hemolysin nanopore



An example, the α-hemolysin nanopore





Finally, some self-promotion... SMF & SCR