The head of a human flea
Review and Prep for Final

Same as Mid-term
Final: Monday, Dec 14, 1:30-4:30pm
Office Hrs: Friday 10 am-12 pm
        Sunday 3 – 5pm
In Biophysics office (179 LLP?)
   (good?)
What you’ve learned

New Stuff (post-mid-term)
Lec 16, 17: Optical Traps (processive, but non-processive too; pN force, nm distances)
Lec 18-20: Fluorescence (super-accuracy, measuring dynamics super-resolution, images w nanometer res.)
Lec 21-22: Diffusion (short distances, fast, e.g. nerve communication, breathing:
  Long distance—can’t rely on—molecular motors )
Lec 23-25: Ion Channels (Nerves relies on “batteries” which are membranes with salt imbalance) Calculate Nernst potential)
Lec 26: Cool High Resolution Techniques: E.M., cryo-E.M., X-rays)

Old Stuff (pre-midterm but subject to testing)
F_{1}F_{o} ATPase: turning the chemical-electrical energy of proton flow into ATP

Really old stuff but have to know
Free energy = energy plus entropy. They both count.
ATP: typical unit size: 25k_B T = 100 pN-nm
Thermal energy matters a lot!

Everything (which goes like $x^2$ or $v^2$ in PE or KE) has $\frac{1}{2} kT$ of energy.

If a barrier has on this order, you can jump over it and you will be a mixture of two states.

Boltzman distribution $= Z^{-1} \exp \left(-\frac{\Delta E}{k_B T}\right)$

$$K_{eq} = \frac{k_f}{k_b}$$
Entropy also matters (if lots of states can go into due to thermal motion)
Probability of going into each state increases as # of states increases

Add up the # of states, and take logarithm: \( \ln \sigma = S = \text{Entropy} \)
Free energy

$$\Delta G = \text{free energy} = \Delta E - T\Delta S$$
(Technically $\Delta G = \Delta H - T\Delta S$: $\Delta H = \text{enthalpy}$ but doesn’t make a difference when dealing with a solution)

Just substitute in $\Delta G$ for $\Delta E$ and equations are fine.
Diffusion

Kinetic thermal energy: $\frac{1}{2} \, m v^2 = \frac{1}{2} \, k_B T$ (in one D; 3/2 in 3D).
Things move randomly.
Simple derivation $x^2 = 2^n D t$ (where $n = \# \, \text{dimensions}; \, t = \text{time}$).
Where $D = kT/f$ is the diffusion constant
$f = \text{friction force} = 6\pi \eta r$. ($\eta = \text{viscosity}, \, r = \text{radius}$)

[Note: when trying to remember formulas, take limit $\rightarrow 0$ or $\rightarrow \infty$.]
Diffusion

Efficient at short distances, not-so at long distance

Distances across nerve synapses is short (30-50 nm) and neurotransmitters are small (like an amino acid). Diffusion is fast enough for nerve transmission. In bacteria, typically ≈1 um. Fast enough. In eukaryotes, typically ≈10-100 um, too slow.
The cell uses packets of energy of $\approx 25kT$

**ATP:**

Small enough amounts that you can use it efficiently.

- Molecular motors (kinesin, $F_1F_0$ ATPase: like $>50\%-100\%$. Car motor- $< 20\%$.

- Mitochondria came from an ancient bacteria that was engulfed (has it’s own DNA).
Paul Boyer (UCLA) had predicted that some subunits in the ATP synthase rotated during catalysis to produce ATP from ADP+ Pi. John Walker (MRC, Britain) crystallized the ATP. They won Nobel Prize in 1997.
At minimum, how many charges need to be used up to generate 1 ATP?

ATP = 20 – 25 \( k_B T \) of energy.

If 100% efficient, need 3 \( (x 7 \ k_B T) \) charges to cross membrane. Amazingly:

ATP Synthase = \( F_1 F_0 \) ATPase operates at \( \sim 100\% \) efficiency!

Takes 3 protons and converts that energy into 1 ATP (from ADP+ P\(_i\)) !!

Does it by “turning a wheel”, 3 x 120\(^\circ\).

ATP Synthase: A rotary engine in the cell that drives you!
Molecular Motors

Instead of relying on diffusion, where $x^2 \propto (D)(\text{time})$, and therefore $x \propto [Dt]^{1/2}$, you have $x \propto (\text{velocity})(\text{time})$.

Translating motors (myosin, kinesin, dynein)

Rotating motors ($F_1F_0$ ATPase)

Combination (a little: DNA or RNA polymerase, helicases)
How to measure?

Lots of ways.
Magnetic Tweezers  Bead fluctuating
Optical Traps     Limit your bandwidth (Fourier Transform)
Fluorescence     Inherent photon noise, Poisson – √N
Patch-clamping    Inherent open/closing of channels

You have to worry about getting reasonable signal/noise.
Noise – motion due to diffusion, photon noise
Neat video: kinesin, dynein, myosin walking in a “city”

https://www.youtube.com/watch?v=tMKIPDBRJ1E
Dielectric objects are attracted to the center of the beam, slightly above the beam waist. This depends on the difference of index of refraction between the bead and the solvent (water). Can measure pN forces and (sub-) nm steps! Vary $k_{\text{trap}}$ with laser intensity such that $k_{\text{trap}} \approx k_{\text{bio}}$ ($k_{\text{bio}} \approx 0.1\text{pN/nm}$).

Optical Traps (Tweezers)
Basepair Resolution—Yann Chemla @ UIUC

1bp = 3.4Å

UIUC - 02/11/08

UIUC - 02/11/08

3.4 kb DNA
F ~ 20 pN
f = 100Hz, 10Hz

unpublished
You can get beautiful pictures
Super-Accuracy: Photon Statistics

If you’re collecting many photons, you can reduce the uncertainty of how well you know the average. You can know the center of a mountain much better than the width.

Standard deviation (w)

vs.

Standard Error off the Mean (center)

$SEM = \frac{w}{\sqrt{N}} = \frac{250}{100}$ nm = few nm
Super-Accuracy: good for telling about Dynamics

Kinesin: Hand-over-hand or Inchworm?

16 nm

Kinesin walking on microtubule
Don’t over generalize!
Biology is filled with amazing “tricks”, adaptations

Just when you think that everything “walks”, you find that things slide.
About HCV NS3 helicase

1. **Hepatitis C Virus** (HCV) is a deadly virus affecting 170 million people in the world, but *no cure or vaccine*.

2. **Non-Structural protein 3** (NS3) is essential for viral replication.

3. NS3 is composed of serine protease and a helicase domain

4. NS3 unwinds both RNA and DNA duplexes with 3’ overhang

5. In vivo, NS3 may assist polymerase by *resolving RNA secondary structures or displacing other proteins*. 
FRET: measuring conformational changes of (single) biomolecules

FRET useful for 20-80Å

Distance dependent interactions between green and red light bulbs can be used to deduce the shape of the scissors during the function.
See (from Myong Science, 2007):

http://www.sciencemag.org/content/suppl/2007/07/26/317.5837.513.DC1/1144130s1.mov

Cat video!
Resolution: Photon: the diffraction limit

There is an “Inherent” uncertainty

– width = $\lambda/2\text{N.A.}$ or 250 nm

This is the best at which you can tell where a photon is going to land. It doesn’t matter how many photons you collect.
You can get super-resolution to a few 10’s nm as well

Turn a fluorophore on and off.
Super-Resolution Microscopy
Inherently a single-molecule technique

Huang, Annu. Rev. Biochem, 2009

STORM
STochastic Optical Reconstruction Microscopy

PALM
PhotoActivation Localization Microscopy (Photoactivatable GFP)
Bates, 2007 Science
Diffraction Limit beat by STED

If you’re clever with optical configuration, you can make width smaller: STED.
You get down to 50 nm or-so.
Nerves & Action Potentials

Rush of $\text{Na}^+$ in, followed by $\text{K}^+$ out.

At resting (negative) potential, channels closed.
At less negative potential (0mV), channels open.

At one end of neuron, some chemical released
$\rightarrow$ causes some charges ($\text{Ca}^{2+}$) injected/depolarize membrane.

Low $\text{K}^+$, High $\text{Na}^+$

High $\text{K}^+$, Low $\text{Na}^+$

$-0.1 \text{ V (at rest)}$

~10 million ions/sec go through single channel
S4 has lots of amino acid charge
Feels effect of external voltage

S5, S6: Notice
Selectivity Filter (GYG)

C=O binds to K⁺, displaces OH₂

For K channels: Energy for K⁺ dehydration is close to zero, but very high for Na⁺ (or any other ion). Same for Na⁺ channels (see calculation).
Three-dimensional map of the \textit{T. thermophilus} ATP synthase

See you at the Final
Dec 14, 1:30-4:30pm, Here (158 LLP)

Don’t forget to fill out course evaluation