Lecture #14

Problems

1. If the K_d for the actin subunit-subunit interactions along a strand is 0.1 mM and the K_d for subunits at the ends of two-stranded filaments is 0.03 mM, then what is the K_d for a single inter-strand bond (assume that subunits that bind at the ends are bound by one intra- and one inter-strand bond).

k_d of subunit-subunit interaction gives us energy of intra-strand bond: $\Delta G_1 = -RT\ln 0.1$
k_d of subunit at the end of filament gives us total energy of one inter- and one intra-strand bonds: $\Delta G_{total} = -RT\ln 0.03$
Therefore the energy of inter-strand bond is: $\Delta G_2 = \Delta G_{total} - \Delta G_1$
And relative $k_d = \exp[-\Delta G_2/RT] = \exp[\ln 0.03 - \ln 0.01] = 0.03/0.1 = 0.3M$

2. A classic experiment in the microtubule field involved the dilution of the microtubule solution and the subsequent changes in microtubule number and length. The observation was that the dilution of an equilibrium solution of monomer and polymer resulted in the decrease in the number of microtubules and a slight increase in their length. How can you explain this result based upon the GTP hydrolysis after polymerization?

One explanation is that dilution decreases concentration of all components in system – the concentration of tubulin filaments and GTP. Hydrolysis of GTP to GDP leads to depolymerization, but decrease in GTP concentration slows down hydrolysis and depolymerization, so long filaments will be longer. There are also processes of polymerization and depolymerization – the short filaments will be completely depolymerized and lower concentration of tubuline monomers will lead to lower probability of seed formation (start of new short filaments)

Lecture #15

1. Consider a pie ce of spaghetti 1mm in diameter. Yung's modulus is ~10^8 J/m^3.
a. What is its persistence length at room temperature? Is the result consistent with your everyday observations?
b. Please calculate spring constant for 1 cm spaghetti piece. If you consider spaghetti as linear spring, how many molecules of ATP have to be hydrolyzed in order to displace the end of a spaghetti piece in 3mm?

a. Lp = EI/kT = ~10^{15}m – way too long for everyday experience – parts of spaghetti pieces usually loose mutual correlation at much shorter distances. It is because of the fact that formal equation for persistence length cannot be applied for macro-objects. Equations just shows the level of balance between object rigidity (EI) and the intensity of thermal motion (kT), but macro objects do not experience thermal motion.
b. K = $3EI/L_3$ = ~15N/m. Deformation of linear spring leads to change in energy: E = ~x^2K/2= 0.00007 J or N*m . Energy of one ATP molecule is 80pN*nm. So we need ~10^{15} molecules of ATP or ~10^{-9} moles

2. (extra credit) Suppose that the ratio of substrate to product in a mixture is ten times greater then the ratio at equilibrium. How much mechanical work could be obtained by converting one molecule of substrate to one molecule of product? Suppose that you have a total of N substrate plus product molecules and that the equilibrium ratio is 1. What is the total amount of mechanical work that could be done with mixture before it becomes completely spent?

Energy of transformation of one molecule is ~$kTln(P_e/S_e) – kTln(P_e/10S_e)$ = =kTln10=2.3kT

For full work of transformation to equilibrium ratio we have to integrate this equation over elementary steps of individual molecule transformation t~o N/2 molecules.

Lecture #17

1. A white cell with an initial diameter of 8 μm (R) in resting suspension culture is completely drawn into a pipette with an inner diameter of 2.5 μm (r).
(a) What is the change in the apparent surface area of the cell before and after aspiration?
(b) How can this technique be used to measure the amount of excess membrane area in the cell?

a. The easiest way to estimate change in membrane area with aspiration into pipette is to consider change in cell shape from sphere to cylinder with diameter equal to internal pipette diameter without changing the volume:
V_{cell} = 4/3πR3, $S_{initial}$= 4πR^2, V = ~πr^2h (h – length of the cylinder), so h = =~50μm, and S_{final} = 2*πr^2 + 2πrh, and change in area ~200μm^2
b. Systematic decrease of pipette diameters allows finding the minimal diameter before cell death – it will describe max excess membrane area.

2. Under micropipet aspiration, red cells do not .ow fully into the pipet like white cells.
Instead, red cells membranes exhibit a *shear elasticity* that allows it to resist aspirations where $L_p > R_p$. In this way, red cells, like most biological materials, exhibit characteristics of both solid and .uid materials. This deformation has been analyzed under the assumption of constant membrane area; Chien et al. (*Biophys J*, 24:463, 1978) linearized to the result to give: $P_p R_p \mu = 2.45$
L_p
R_p
, for $L_p > R_p$ (6)
where μ is the shear elastic modulus of the membrane.
(a) Use eq. 6 to determine the shear elastic modulus of a red cell from the .ctitious experimental data shown in .g. 8. Assume that the inner pipet diameter is 1.4 μm.
(b) Based on eq. 6, what is likely to be the largest source of error in measuring μ?

a. The slope of the curve gives the coefficient in equation: $P_p = k*L_p$, and k = = $2.45*\mu/R_p^2$
b. N/A

Lecture #18

1. We discussed processive motors in terms of the kinetic scheme for kinesin (Scheme 1 with MtK above). If we assume that the concentration of ATP is high, then ATP binding will not be the rate- limiting step (K_1). What is the rate- limiting step, if $K_2 = 1000\ s^{-1}$, $K_3 = 100\ s^{-1}$, and $K_4 = 400\ s^{-1}$? If the step length is 8 nm/ATP, then how fast can the motor move if it has one head (assume that between step 3 and 4, the kinesin head will come off the microtubule)? (same question for 2 heads like the normal molecule?)

Limiting step is 3rd – min kinetic constant
So the speed of overall process defined by $k_3 = 100$ events per second (ATP hydrolysis events). If one ATP hydrolysis event leads to moving of motor on 8 nm the overall speed will be 800nm/s
In simplest model addition of the second head will double speed = 1600 nm/s

2. (Extra Credit 10 Points) For muscle myosin, which is non-processive (see scheme 1 with AM above), the rate- limiting step is the hydrolysis of M.ATP to M.ADP.Pi (this is the forward rate constant of #3, which is typically 40 s^{-1}). M.ADP.Pi then binds to actin to produce a force by the swing of the crossbridge, Pi and then ADP are released. Myosin bound to actin then waits for another ATP to release it and start the cycle again. The series of steps from force production to release takes about 2 milliseconds without load in a maximally activated muscle. Assuming 0.002 sec is the time that the active heads are bound to the actin filament and that the forward swing of the crossbridge is 10 nm, then what is the maximal velocity of muscle contraction (you should also assume that without an external load, the myosins are pulling against themselves; i.e. half of the time is spent producing force to pull other myosins forward and half of the time is spent being pulled forward by other myosins).

Maximal velocity of muscle contraction is: ~10nm/2ms / 50% = ~10 μm/s – too slow, so model of 50/50 mutual pulling is probably wrong

3. (Extra Credit 10 Points) What happens to the time of force production in the muscle described in Problem 2, when a load is applied that slows contraction? Assuming that the velocity is slowed to 1/10 of the maximal velocity determined in Problem 2, what is the average time that a head is bound and producing force before it is released? For an additional 5 points, compute the fraction of the myosin heads (on average) that are bound to actin under these conditions.

Decrease in velocity 10 times will lead to 10 times increase in time of binding (to 20ms) in linear case.

Lecture #19

1. Polyribosomes represent an assembly line of protein synthesis on one mRNA, and clearly many copies are made in parallel. For this problem, we have induced synthesis of a protein by a stimulus. We want to determine how many proteins per cell are present at steady state if the half-time of the protein after synthesis is 30 hours and the rate of synthesis is 20 molecules per minute. (Assume that the degradation rate represents a first order decay process)

First order decay means exp law in concentration decrease. $C=C_0*\exp[-kt]$, and half time gives as kinetic constant of decay: $C=C_0/2$, so $k = \ln 2/\tau_{1/2}$
In steady state rate of synthesis is equal to the rate of decay:
dN/dt = 20 molecules/minute = $N*k$, so N = ~100 molecules

2. In the case of plasma membrane proteins, they need to be processed through the ER and Golgi before reaching the plasma membrane, which often takes 15-30 minutes. If a protein has a half- life of 10 minutes in the ER and 15 min in the Golgi, then what is the number of molecules of the protein in the ER and Golgi at steady state assuming a synthesis rate of 100 molecules per minute.

The same type of calculations as in the first problem:
$k_{1\ ER} = \ln 2/\tau_{1/2\ ER}$ = ~0.07 1/min
$k_{2\ goldgi} = \ln 2/\tau_{1/2\ goldgi}$ = ~0.05 1/min
$N_1 = 100 / k_{1\ ER}$ = ~1443 molecules
$N_2 = 100 / k_{2\ goldgi}$ = ~2164 molecules

Lecture #20

1. We will use a 14° temperature block to hold a GFP-tagged membrane glycoprotein in the ER until a significant amount is synthesized. When the temperature is raised to 37°, the protein will be released to transit to the Golgi and on to the plasma membrane. If further synthesis of the protein is blocked and we use the constants defined in the Hirschberg et al. 1998 study, then about how long will it take before 10% of the protein reaches the plasma membrane.

Every minute 2.8% of protein in ER goes to Golgi, and 3% of protein goes to PM form Golgi
In steady state it means that 0.028*0.03=0.0084 of protein goes from ER to PM per minute. In linear approximation of steady state it means that 10% of protein will go to PM in ~120minutes

2. If endocytosis is randomly sampling the surface and 4% of the surface is endocytosed every minute, then how long will it take to endocytose 80% of a membrane protein?

Practically here we have first kinetic order decay of the number of "non-sampled" proteins:
$N(t)=N_0*\exp[-k*t]$, 4% of area (=2% of membrane proteins) is sampled in 1 minute:
$0.96=\exp[-k*1\text{min}]$, so k=0.04 1/min
80% will be sampled in $t=-\ln(0.20)/k=$~40min

3. (Extra Credit, 10 pts) Many receptors are recycled after endocytosis but a fraction often moves on to the lysosome where it is degraded. If the endocytosis rate is the same as in problem 2 but 75% of the endocytosed protein is recycled, then what is the half-time for the degradation of the protein?

Rate of endocytosis is 4%=0.04 1/min, 75%=0.75 of receptors are recycled, and only p=0.04*(1-0.75)=0.01 part of protein pull is degraded, and P=1-p=0.99 are not degraded.
Exp low of degradation: $N=N_0*\exp[-t*k]$, $0.99=\exp(k*1\text{min})$, and $k=(-\ln[0.99])$ 1/min.
Half-life of protein $T=(\ln 2)/k=$~69min

Lecture #23

1. Cells are about 30 microns across and are on average about 2 microns thick. Assuming that the cell is rectangular, what is the resistance that the cell encounters from fluid drag when moving at the highest velocity observe for mammalian cells of 40 microns per minute. (assume that the medium has the viscosity of water at 25° C).

The Stokes' formula describes the relationship between viscous drag and spherical particle size.
$f = 6\pi hrv$

For all other geometries theory gives similar formula, which usually differs from Stock's law only by numerical factor (not 6π), so we can use just use Stock's formula as it is in order to estimate force roughly, but of course you can find in literature more precise solutions.

2. In the model of cell migration that we discussed in class, there were a number of critical parameters that could be controlled to effect the directed movement of neural crest cells to the proper location in the periphery. If we consider the important aspects of cell migration listed above, we can generate several different models for the process of cell migration. The one that I described in class involved the orientation of matrix fibers such that they mechanically directed the cells to the right location. In the description of the process (above) that was lifted from the review article, another model is outlined. Please describe the important differences between the two models and give one critical difference that you think could be tested experimentally.

N/A