Lecture #8

1. 1. If we have a transcription factor that is already bound to DNA near a transcription initiation site, then an enhancer can bind to it and activate transcription. From in vitro measurements of binding, the enhancer binds to the transcription factor with a binding constant of 10^6 M^{-1} in the presence of an enhancer DNA piece only 20 base pairs long. The enhancer binds to the appropriate DNA sequence with an affinity of 10^4 M^{-1}. Because binding to the DNA is a prerequisite for the enhancer binding to the transcription factor and chromosomal DNA has mobility, the entropy term for the enhancer-transcription factor binding is not the full 29 kJ/mole (assume it is 12 kJ/mole). Estimate the binding constant of chromosomal DNA-bound enhancer for the transcription factor.

For in-vitro binding of enhancer (E) to TF (which is already bound to DNA) $K=10^6$ 1/M, so $\Delta G_{binding}=-RT\ln K= -34KJ/mole=\Delta H_{TF\text{-}E\ bond}+\Delta H_{DNA\text{-}E\text{-}bond}-\Delta S$

In case of chromosomal DNA we need to correct this change in entropy by $\Delta S_{20bp\ DNA} -\Delta S_{\ chromosomal\ DNA}=29-12= 17KJ/mole$, so

$\Delta G_{binding\ chromosomal\ DNA}= -51KJ/mole$, and $K=\sim 8x10^8$ 1/M

2. What is the off-rate of the enhancer-transcription factor binding (assume that the on rate is decreased by 10 fold from a diffusion controlled on rate, because enhancer binds in complex with DNA and DNA diffuses quite slowly)? When the enhancer-transcription factor bond is broken, what is the rate of the dissociation of the enhancer from the DNA (assume here that the on rate is diffusion controlled)?

Kinetic constant of dissociation k_{-1} can be calculated from binding constant (K-thermodynamic constant of association reaction) and kinetic constant of association ("association rate", k_2). k_2 is controlled by diffusion in our case and equal $\sim 2*10^6$ 1/(M*s) decreased by factor of 10 (so $k_2=2*10^5$).

$k_{-1}=k_2/K$

For E-TF binding $K=8x10^8$ 1/M, $k_{-1}=\sim 2.5x10^{-4}$ 1/s

For E-DNA binding (TF-E bond is broken) $K=10^4$ 1/M, $k_{-1}=20$ 1/s

Lecture #9

1. If the bilayer has an elastic modulus of 10 mN/m for dA/A = 0.04 and we assume that half of the bilayer has half of that elastic modulus, then the addition of an amphiphilic compound that expands one half of the bilayer by 0.5% will cause an expansive tension in the other half of the bilayer of ___?

Elastic modulus E=10mN/m, and half of bilayer has half of this elastic modulus $E_{1/2}$ = 5 mN/m. dA/A=.5%=0.005, so tension $T=E_{1/2}*dA/A$ = 25 uN/m

2. In the video, we observed that a tube of membrane could go to several beads on a string. If we consider only bending stiffness of the membrane (2×10^{-19} Nm), then which is the lower energy configuration, a tube of constant diameter (0.2 microns) or a combination of a sphere and a tube of smaller diameter (overall length is 10 microns, i.e. 6.28 microns squared of membrane). (a partial proof is fine using the formula (14) above to calculate the energy needed to form a couple different spheres and tubes from a flat bilayer).

In this problem we will consider only changing in bending energy of the membrane (membrane is practically nonexpendable in normal conditions): $dE = (B/2)(1/R_t)^2 dA$

We will compare two different shapes with constant curvature (cylinder in case of thin tube and sphere with peace of tube of negligible membrane area), so we can integral form of this equation $E = (B/2)(1/R_t)^2 A$
The net area of membrane A remains constant.

Cylinder: $A=2\pi rl=6.26\mu m^2$, r=0.1 μm, l = 10 μm
Sphere (radius R) and small peace of tube (cylinder l_1-length, r_1-radius): $A=4\pi R^2 + 2\pi r_1 l_1$
The second term can be considered negligible for this problem (in principle we are interesting in complete beading – l_1 small), so $R^2=0.5\ \mu m^2$
$E_{sphere}/E_{cylinder}= [(B/2)(1/R)^2 4\pi R^2] / [(B/2)(1/r)^2 2\pi rl] = 2r/l = 0.02$ (sphere 50-times more energetically favorable than thin tube)

3. A membrane protein has a diffusion coefficient of 10^{-10} cm^2/sec and yet it travels 1.5 microns in 25 sec toward the leading edge. What is the probability that the protein would move in this way from a simple consideration of one-dimensional diffusion (remember the Gaussian (Normal) distribution curve)?

Gaussian distribution in case of diffusion has physical meaning of density of the probability to be at certain interval in space (one dimensional space for this problem).
$\langle \Delta X^2 \rangle = 2Dt$, and for given D and t $\sigma = \langle \Delta X \rangle = 7*10^{-5}$ cm = 0.7 μm
Given actual displacement is 1.5 μm ≈ 2 σ. But we know from Gaussian distribution table, that probability to have value more 2 σ is less then 4%.

Lecture #10

1. A membrane channel has a large cytoplasmic domain that covers a circular area 4 nm in diameter (pore is in the center) at the membrane surface and neutralizes the negative lipid charges beneath it. If the potential at the uncovered membrane surface is –50 millivolts, and the solution contains 0.1 M of monovalent salt. What is the pH at the mouth of the pore?

To find the pH at the cytoplasmic surface, we can assume $[H^+] = 4x10^{-8}M$ (normal pH in cytoplasm is ~7.4).
Concentration of counterion at the surface with membrane potential -50mV can be calculated by using Boltzmann distribution:

$c_s = c_c\, e^{+ze^{0.05/kT}} = 4x10^{-8} * \exp[+8 \times 10^{-21} / (1.38*10^{-23} * 300°)] = c_c\, e^{1.9} = \sim 3x10^{-7}$ M,

so $pH = -\lg c_s = \sim 6.5$
At the edge of protein the potential drops from -50mV by factor e every Debye length (~1nm for 0.1mM salt), so potential at the edge is equal $50/e^2 = 7$mV, and

$c_s = c_c\, e^{+ze^{0.007/kT}} = 5.2x10^{-8}$M, pH = ~7.3

2. We want to understand the importance of the cytoplasmic surface potential to the transmembrane potential. Transmembrane potentials are typically –100 millivolts (negative inside the cell). If the cytoplasmic surface has a charge of –50 millivolts, what is potential gradient across the 5 nm of the bilayer? Dielectric breakdown of biological membrane occurs at about 1 volt across the membrane. What is the fraction of the potential gradient across the membrane at dielectric breakdown that is contributed by the cytoplasmic surface potential given above?

Cytoplasmic surface is responsible for 50mV of potential difference across membrane (100mV), so there is another 50mV of potential difference across bilayer itself. Anf gradient is 50mV/5nm
Cytoplasmic surface contributes 5% of 1V dielectric breakdown.

Lecture #11

1. We talked about cell depolarization as the change from being permeable to potassium to being permeable to sodium. If the only ions that move during the depolarization of a cell are sodium ions, then how many ions will move in a cell with a membrane area of 4000 microns squared and a volume of 4000 microns cubed (assume outside K+ is 3 mM and inside Na+ is 4 mM).

Nernst equation gives us resting membrane potentials for Na^+ and K^+
$\Psi = 2.3(RT/zF)\log(c_1/c_2)$ (See text of lecture for itra- and extracellular ion concentrations).
V_{Na}=+87mV V_K=-93mV, and voltage jump will be V~180mV
Assuming membrane capacitance per surface unit $dC/dA=1\mu F/\mu m^2$ charge will be $Q=V*A*dC/dA = 7.2x10^{-12}$Columns. Dividing this value on elementary charge and cell volume give us concentration of moved charges: ~43200000 charges moved = $7.2x10^{-17}$ moles, which corresponds to ~18uM change in sodium concentration inside.

2. If we assume that 100 mM of anionic charges are immobilized on DNA in the nucleus, then what will be the potential developed by a Donnan equilibrium from the inside to the outside of the nucleus with a cytoplasmic concentration of 100 mM NaCl? Remember that a diffusion potential can be generated by either positive or negative charges.

The resting potential will be defined by concentration gradient sodium and chloride. Finally the electric potentials of sodium and chloride should be equal each other in equilibrium (at least for preserving stability of macro-electroneutrality of the internal content of cell). So by writing two Nernst equations for sodium and chloride and making them equal each other we will get Donnan ratio:
$C_{Na\ inside}/C_{Na\ outside} = C_{Cl\ outside}/C_{Cl\ inside}$, Also we know that $C_{Na\ outside}=C_{Cl\ outside}$=100mM
Another equation – electroneutrality inside the cell: $C_{Na\ inside}=C_{Cl\ inside} + A$ (A-concentration of fixed anions inside the cell)

Solving these equations gives us quadratic equation:
$X^2+100X-10^4=0$, where is $X= C_{Na\ inside}$
X=~62mM
By using Nernst equation we can find potential difference: V~12mV

Lecture #12

1. The transport of actin mRNA to the periphery has an unknown purpose. However, some people have suggested that actin is being synthesized to increase the concentration at the site of synthesis. If we assume that each actin mRNA (1050 bases in the coding region) is fully active as noted above (i.e. there is 1 active ribosome per every 60 bases of mRNA and the polymerization rate is 20/s), then we can calculate the concentration of actin in the periphery after 12 hours of synthesis from one message (1/2 the cell cycle). If the actin is polymerizing into filaments as it is synthesized in the periphery (in a space of 20 μm^2 by 0.4 μm thick), then the effective concentration will be? If the new actin is in the globular form that has a diffusion coefficient of 10^{-9} cm^2/s, then how long will it take the new proteins to diffuse to the other side of the cell, about 40 μm away? Will the concentration at the mRNA be significant higher at the end of the 12 hours or can we assume that all of the globular actin is uniformly distributed in cytoplasm?

Rate of polymerization 20 a.a./s per ribosome translates into 60bp/s (3bp per a.a.). So it takes ~1050/60 = 17.5s for one ribosome to finish mRNA translation. But every 60bp (or every second) new ribosome can start and practically it means that we have rate of synthesis 1 actin molecule per second from one message RNA. After 12 hours we will have N = 12*60*60 = 43200 molecules of actin synthesized. By dividing on the volume of cell we can find actin concentration: $N/[N_a*20um^2*0.4um]$=~3.6mM
The time for 2-dimensional diffusion for 40um: $t=\sim l^2/4D$=4000s=~1 hour
t<<12 hours, so probably it means that globular actin will be uniformly distributed in the cell.

2. If we have a multi-subunit protein such as myosin, which has 4 light chains and 2 heavy chains, then how would you coordinate the assembly of a functional molecular complex?

N/A

Lecture #13

1. In the theoretical filaments that are linear assemblies of subunits where the binding constant for the dimer is the same as the binding constant for the n + 1 mer, the filament length is limited because of what? For an extra credit of 10 points, describe how you would compute the average filament length from the dissociation constant and the concentration of the protein (all forms).

The length of filament will be limited mostly by concentration of available monomer.

Lecture #14

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).

2. A classic experiment in the microtub ule 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?