Lecture #2
The Cell as a Machine

How Biomachines Work at Low Reynolds Number (diffusion and transport)

Background reading:
Berg (Random Walks in Biology, 1993),
Boal (Mechanics of the Cell, 2001) Appendix C (Elementary Statistical Mechanics)
Howard (Mechanics of Motor Proteins and the Cytoskeleton, 2001)

Summary: For all protein functions there is no role of momentum and diffusive motions of proteins are biased to produce work.

An important feature of cellular functions that is different from similar functions in the macroscopic world is the low Reynolds number at the cellular length scale. Low Reynolds number refers to the relative scales of the object, its inertial energy and the pressure fluctuations in the medium (water in this case). A practical consequence of life at low Reynolds number is that inertial movements are nonexistent. Instead, Brownian motions perturb many movements and provide the stochastic fluctuations that provide the basis for nanoscale functions. Fluctuations in protein conformation are harnessed by cells for productive activities. As we will discuss today even the hydrolysis of the high energy compound, ATP, to produce muscle contraction relies upon the stabilization of thermally induced conformations. ATP hydrolysis in one step causes the cycle to be directional and force producing.

Reynold's number $R = vL\rho/\eta$ (1)

See Berg (Random Walks in Biology, 1993, pp. 75-77). For a fish of density approximately that of water (ρ = 1 gm/cc), length of 10 cm (L), moving at a velocity of 100 cm/sec (v) in water (η = 0.01 g/cm sec), we calculate R to be about 10^5. In contrast, for a bacterium of the same density, length of 1 micron (L = 10^{-4} cm), moving at a velocity of 10^{-3} cm/sec through water, we calculate R to be 10^{-5}. Another way of thinking of this is in terms of the relative magnitude of the energy in the moving particle and the magnitude of thermal fluctuations.

Thermal Motions Drive Enzyme Functions

A major implication of the absence of momentum on a cellular scale is that the cell functions without momentum. Many of our macro-world machines rely on momentum and therefore, cannot be scaled down for use in cells. Further, all protein

functions rely upon thermal motions of the protein for mechanical force development. We will talk about this in the context of the movement of myosin on actin.

Viscous Drag on Particles

An important equation for understanding diffusion in general is the Einstein-Smoluchowski relation, which relates the friction coefficient of a particle moving through a medium to the diffusion coefficient of the particle in that medium. A case in point is a magnetic bead of 2 microns in diameter in a magnetic field (force on the particle is F_x). The drift velocity of the particle (v_d) is related to the force by a constant called the frictional drag coefficient (ϕ_d):

$$v_d \ \phi_d = F_x \qquad (2)$$

Because the drag is the same for diffusion as for externally applied forces, the diffusion coefficient can be derived from (2) as

$$D = kT/\phi_d \qquad (3)$$

The generality of this relationship makes it possible to move directly from knowledge of the frictional drag coefficient to the diffusion coefficient.

As spherical particles move through water they encounter viscous drag that limits diffusive steps. The Stokes' formula describes the relationship between viscous drag and spherical particle size.

$$f = 6\pi\eta r\, v \qquad (4)$$

Note that this can be related to equation (2) and $\phi_d = 6\pi\eta r$. Thus, the diffusion coefficient for a sphere is given by:

$$D_{sphere} = kT/6\pi\eta r \qquad (5)$$

For a sphere of one micron diameter in water at room temperature $\phi_d = 9.5 \times 10^{-6}$ g/sec and $D_{sphere} = 4.4 \times 10^{-9}$ cm^2/sec.

Diffusion of Small Particles

Brownian motion describes the basic motions of subcellular particles and all but the largest of cells. Brownian movements result from the local fluctuations in pressure on the particles. For a one micron particle, those fluctuations can produce movements of 1-3 microns in a second and they have been nicely described in Berg's book. To get an idea about the important aspects of diffusion, we will analyze in detail the process of diffusion of drunks away from the door of the bar.

One-dimensional diffusion (the objects in this case are drunks)

Assumptions: 1. Steps of r length occur at regular intervals (τ)

2. The direction of each step is equally likely to be + or – independent of previous steps.

3. Each object moves independent of other particles.

If 128 objects start at X = 0 and move r length, then 64 will be at +r and 64 at –r.
After 2t, there will be 32 at –2r, 64 at 0 and 32 at +2r.
After 3t, there will be 16 at –3r, 48 at –r, 48 at r and 16 at +3r
After 4t, there will be 8 at –4r, 32 at –2r, 48 at 0, 32 at +2r, and 8 at +4r
After 5t, there will be 4 at –5r, 20 at –3r, 24 at –r, 24 at +r, 20 at +3r, and 4 at +5r

Notice that with increasing time the width of the distribution increases (with the square root of time) and the height decreases (also with the square root of time). The distribution can be described as a Gaussian (we will come back to this point).

IMPORTANT FEATURES OF DIFFUSION

1. No net movement occurs (the average position is always zero)
2. Distribution is symmetrical (a corollary of 1)

Root-mean-square displacement $<\Delta X^2(n)>^{1/2}$

It is useful to describe the distribution of diffusing particles over time by the average of the square of the displacement, since it is not dependent upon sign.

Mathematically, the diffusion of particles in one dimension is given by

$$2D_1 t = <\Delta X^2> \qquad (6)$$

two dimensions is given by

$$4 D_2 t = <\Delta X^2+\Delta Y^2> \qquad (7)$$

and three dimensions is given by

$$6 D_3 t = <\Delta X^2+\Delta Y^2+\Delta Z^2> \qquad (8)$$

where D is the diffusion coefficient, t is the elapsed time, and $<\Delta X^2>$, $<\Delta X^2+\Delta Y^2>$, or $<\Delta X^2+\Delta Y^2+\Delta Z^2>$ is the average displacement squared. An important aspect of diffusion that is evident from this equation is that the average distance moved scales with the square of time.

Gaussian Distribution of Diffusing Particles

If all of the particles at time equals zero are at the origin, then the distribution of the particles after many elemental steps assumes a Gaussian.

$$P(x)dx = (1/(4\pi Dt)^{1/2})\, e^{-x^2/4Dt}\, dx \qquad (9)$$

For a normal curve, the fraction of the area which is within one standard deviation ($s = (2Dt)^{1/2}$) is approximately 68% of the total area. The probability that it has

wandered as far as two standard deviations is 4.5% and for three is 0.26%. These numbers provide a useful way to estimate changes in the concentration of proteins upon diffusion.

Practical Implications of the Diffusion Equation.

If we take the typical dimensions of a cell (volume of 4000 μm^3 or a cylinder 2 μm high with a diameter of 50 μm), then the distance for diffusion from one point to another in the cell will be on the order of 20 μm. With a typical protein diffusion coefficient of 10^{-7} cm^2/sec, the time for diffusion would be on the order of 40 seconds. Thus, for rapidly diffusing components, diffusive transport can be used to bring proteins from sites of synthesis to function.

In the case of highly asymmetric cells such as neurons, the distance of the tip of a process from the cell body is on the order of a meter in man and many meters in larger animals. For our typical protein to diffuse over a meter, would require 10^{11} seconds (3600s/hr, 86,400s/day, and 31,536,000s/yr) or about 3,000 years. Thus, we can safely say that diffusion cannot supply material to the ends of axons.

At a molecular level diffusion processes are extremely important. Motor proteins can not work by diffusive mechanisms alone but the efficiency of molecular motors can be high through mechanisms that involve the biasing of diffusive movements, i.e. a ratchet that is ATP dependent can bias movement of a motor.

Non-ideal Diffusive Processes

The recent advent of computer-based imaging and single photon detection methods to biological systems enables us to measure the detailed random walk of individual particles and even individual molecules (using single molecule fluorescence methods). The area of Single Particle Tracking has therefore arisen to and it provides a detailed view of diffusive movements of cellular components. Often the movements are not ideal in that the plots of the mean-squared displacement versus time (Equation 2) are non-linear. Two major types of non-ideal behaviors are observed in cells and can provide evidence of important aspects of cytoplasmic organization. Flow of the medium (cytoplasm or membrane) that the particle is diffusing in will give rise to an average velocity of movement.

$$< \Delta X^2> \; = 2D_1 t \; + (vt)^2 \qquad (10)$$

where v is the velocity of the flow. In these situations, one can easily document a flow that would otherwise be very difficult to determine.

Diffusion within a Corral

The other common feature of diffusion in cytoplasm or membranes is that particles are restricted to diffusing in certain regions and cannot enter other spaces because of barriers (membrane or cytoskeletal). There is no single equation to describe such diffusion but an approximation of the radius of the corral (r_c) comes from the equation

$$< \Delta X^2> = (r_c{}^2)[1 - A_1 \exp (-4A_2 Dt/(r_c{}^2))] \qquad (11)$$

where A and A are constants determined by the corral geometry (Saxton and Jacobson, 1997).

Problems

In general, I will give a biological system with appropriate references and then ask a question about the system. You can get more information on the cell biology by looking at the PubMed web site (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed) and then find the paper with the authors last names (separated by commas). Once you find the article open the abstract and you will find "books" in the upper right area near the title. Open the books link and you will find that many of the words are highlighted in blue. Double clicking on those words will open the Molecular Biology of the Cell descriptions of those terms and related materials.

Problem #1

In a recent article by Dayel et al (1999) they measure the diffusion of a green fluorescent protein by fluorescence recovery after photobleaching in the lumen of the endoplasmic reticulum (ER) and in cytoplasm and find a 3 fold higher diffusion coefficient in cytoplasm (1.5×10^{-7} cm^2/sec) than in the ER. My lab has measured diffusion of small (0.2 micron diameter) beads along microtubules (Wang and Sheetz, 2000) using single particle tracking and have found a one-dimensional diffusion constant of 2×10^{-10} cm^2/sec. Two proteins (both 5 nm in diameter) are synthesized in cytoplasm at a rate of 1000 molecules per minute in a thin region of cytoplasm (many lamellipodia are only 0.2 microns thick) that is essentially two dimensional. One protein, S, is soluble and the other, M, is rapidly bound to the microtubule. After 100 seconds of diffusion (assume that all molecules are at one point and start diffusing at t = 0), what is the average displacement of protein S and of protein M? Remembering that the distribution is Gaussian, what are the average concentrations of the two proteins within the region from the origin to the average displacement point? Now calculate the average displacement and concentration, if 1000 molecules of soluble protein, S, are expressed with a signal sequence so that they are translocated into the ER lumen which is a tube 0.1 microns in diameter and hundreds of microns in length.

Answer: After 100 sec., $\Delta X = \pm 7.6 \times 10^{-3}$ cm for S and $\pm 2.0 \times 10^{-4}$ cm for M. The volume for S is a cylinder 37 microns in radius and 0.2 microns thick ($2\pi r^2 l = 7.2\ 10^{-9}$ cm^3) whereas for M the volume is a cylinder of the microtubule plus the protein ($2\pi r^2 l =$ $3.8\ 10^{-15}$ cm^3).

Problem #2

A neuron (70 microns in diameter) is sprouting an axonal process and there are new proteins synthesized that are needed at the tip of the growing process. If a protein is synthesized (1000 molecules) at the cell end of an axon that is one hundred microns long (one micron in diameter) and the protein has a diffusion coefficient of 2×10^{-7} cm^2/sec, the average displacement of the protein molecules will equal the length of the axon after how many seconds? What will be the average protein concentration in the axon after that time? Now repeat the calculations for axons that are 1 mm and 1 m long (remember that

you may have some axons a meter in length). If there is a flow of cytoplasm into the axon as it is growing (the axon is elongating at 1 micron per minute typically), what will be the root-mean square average displacement of the 1000 molecules of protein that are placed at the end of a 1mm axon after 100 minutes?

References

Dayel, M. J., E. F. Hom, and A. S. Verkman. (1999) Diffusion of green fluorescent protein in the aqueous-phase lumen of endoplasmic reticulum. *Biophys J.* 76:2843-2851. http://www.biophysj.org/cgi/content/full/76/5/2843

Saxton, M. J., and K. Jacobson. 1997. Single-particle tracking: applications to membrane dynamics. *Annu Rev Biophys Biomol Struct.* 26:373-399.

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Books:
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