20.430/6.561/10.539/2.795
Fields, Forces, and Flows in Biological Systems
Fall 2015

Problem Set # 3 (Chemical Sub-System)

Issued: Friday 10/02/15
Due: 5pm Friday 10/09/15

Problem Sets should be turned in to the 20.430 FFF drop-off boxes, located to the right of the elevators on the 2nd floor of Building 16. Please turn Problem 1 into Box 1, and Problem 2 into Box 2.

Reading Assignment - Chapter 2: 2.1, 2.2; 2.3.1; 2.3.4; (skip 2.4), 2.5.1 (skip 2.5.2), and 2.6, FFF by Alan Grodzinsky

Problem 1: Extensions of the IGF-1 binding example from Lecture (Text examples 1.4.2 and 1.6.2. Figures correspond to slides that will be shown in lecture (week of October 5).)

Experiment: Assume that the tissue explant shown in Figure A has a homogeneous distribution of IGFBP-3 binding sites of density n = 50 nM. At the start of the experiment, you add ^{125}I-IGF-1 to the bathing solution at a concentration less than 0.1 nM (i.e., the red arrow on the x-axis of total bath IGF-1 concentration of Figure A) and then wait for sufficient time such that homogeneous binding equilibrium is obtained within the tissue.

Measurement shows that the ^{125}I-IGF-1 "Uptake Ratio", Ru ≈ 15. "Ru" is defined as the ratio of the concentration of total [bound + free] ^{125}I-IGF-1 inside the tissue (i.e., moles of solute per liter of intra-tissue water) to the concentration of ^{125}I-IGF-1 in the bath (the RED ratio on the upper right hand side of the "Ru" equation in Figure A). You then add eight successive aliquots of unlabeled "cold" (BLUE) IGF-1 until the final total bath concentration of IGF-1 is about 300 nM - you wait for equilibrium binding to be achieved after each addition. The solid line is a best-fit curve to the BLUE equation for Ru in terms of the two binding constants n and K_d, and the partition coefficient K_{part}. Note that the asymptotes for low and high bath concentration of IGF-1 involve n, K_d, and K_{part}, which makes the curve-fit more robust.

(a) From the definition of Ru given in terms of the RED ratio of radiolabeled concentrations, derive the BLUE expression for Ru given at the top right of Figure A. Your answer involves the concept and rationale for using radioisotope tracers to interpret the distributions of the related, "cold" non-labeled species. (Hint 1: note the two equations given on Figure B for K_{part} written for both labeled and unlabeled species; Hint 2: your derivation should involve only a few lines of simple algebra).

(b) Briefly explain why the concentration of ^{125}I-IGF-1 bound to sites in the tissue decreases as you add more unlabeled IGF-1 to the bath.

(c) Briefly describe the relation between the equation for Ru and the equation for the binding isotherm (FFF Eq, 1.49) for this reversible, 1storder, bimolecular reaction. Why is this experiment useful for assessing binding isotherms and binding constants?

(d) Eqns. 1.70 and 1.71 of the textbook (Figure C) define the effective diffusivity of IGF-1 including binding. Justify the incorporation of the "quasi-equilibrium" binding isotherm. Plug Eq. 1.67 into the continuity Eq. 1.65 and carry out the algebraic details to derive Eqns. 1.70 and 1.71.

Figure A

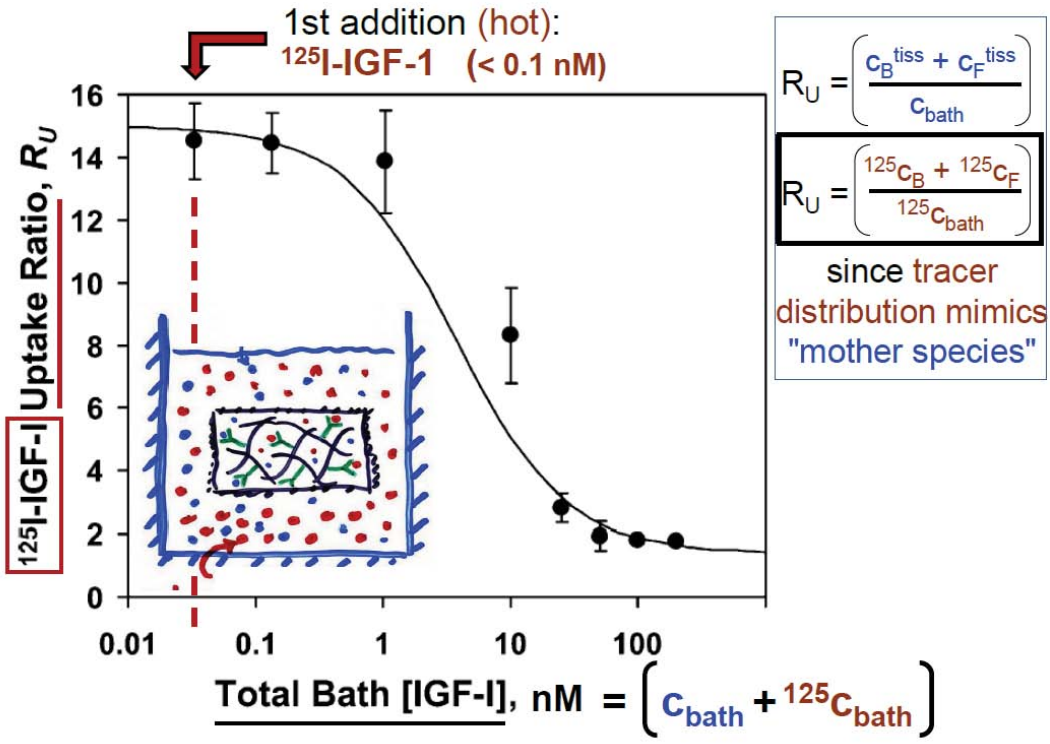

Figure B

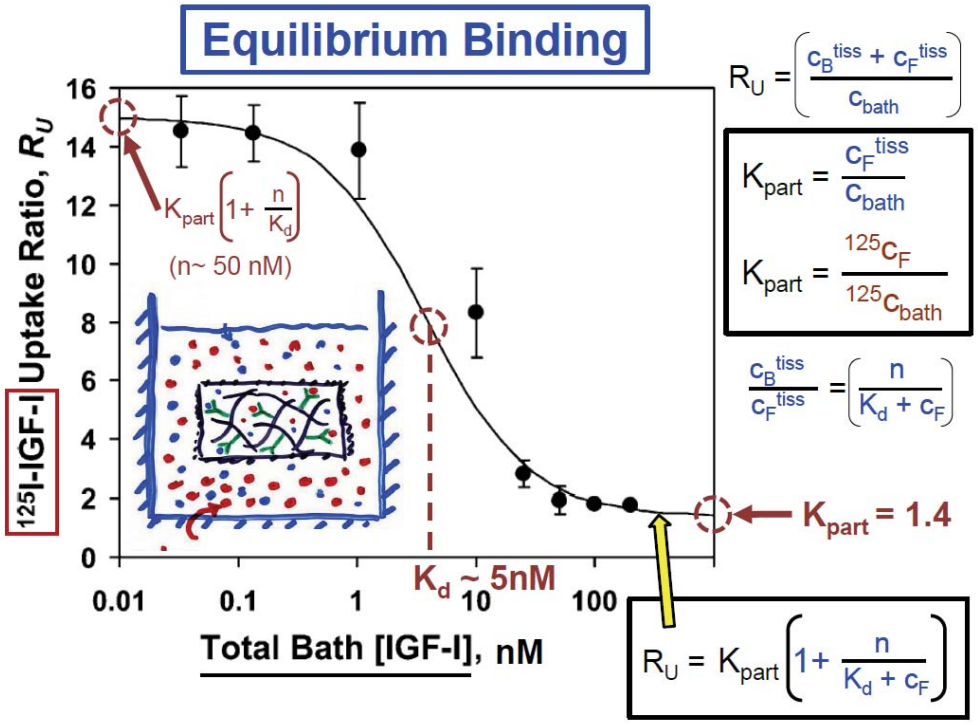

Figure C

The diffusion equation (1.65) then takes the form

$$\frac{\partial \bar{c}_F(x,t)}{\partial t} + \frac{\partial}{\partial t}\left(\frac{n\,\bar{c}_F(x,t)}{K_D + \bar{c}_F(x,t)}\right) = D_{\mathrm{IGF}}\frac{\partial^2 \bar{c}_F}{\partial x^2} \tag{1.68}$$

By using the chain rule for differentiation, (1.68) can then be written as

$$\frac{\partial \bar{c}_F}{\partial t}\left(1 + \frac{n\,K_d}{(K_d + \bar{c}_F)^2}\right) = D_{\mathrm{IGF}}\frac{\partial^2 \bar{c}_F}{\partial x^2} \tag{1.69}$$

The diffusion–reaction equation (1.69) is nonlinear in c_F; however, for small enough concentrations $c_F \ll K_d$, it can be written in the form

$$\frac{\partial \bar{c}_F}{\partial t} = D_{\mathrm{eff}}\frac{\partial^2 \bar{c}_F}{\partial x^2} \tag{1.70}$$

$$D_{\mathrm{eff}} = \frac{D_{\mathrm{IGF}}}{1 + n/K_d} \tag{1.71}$$

Problem 2: Revisiting morphogen gradients in early *Drosophila* embryogenesis

When *Drosophila* eggs are laid, they already contain mRNA for several maternal factors, including Bicoid (Bcd). The Bcd mRNA is localized at the anterior end of the embryo, serving as a source of Bcd protein, as discussed in lecture. It is essentially stable up until the end of what is called nuclear cycle 14, approximately 120 minutes after the egg has been laid, after which it gets actively degraded. In this problem, we want to estimate the number of mRNA molecules deposited in the embryo by its mother. Figure 1 provides some useful numbers for this problem (adapted from Physical Biology of the Cell, 2nd Edition).

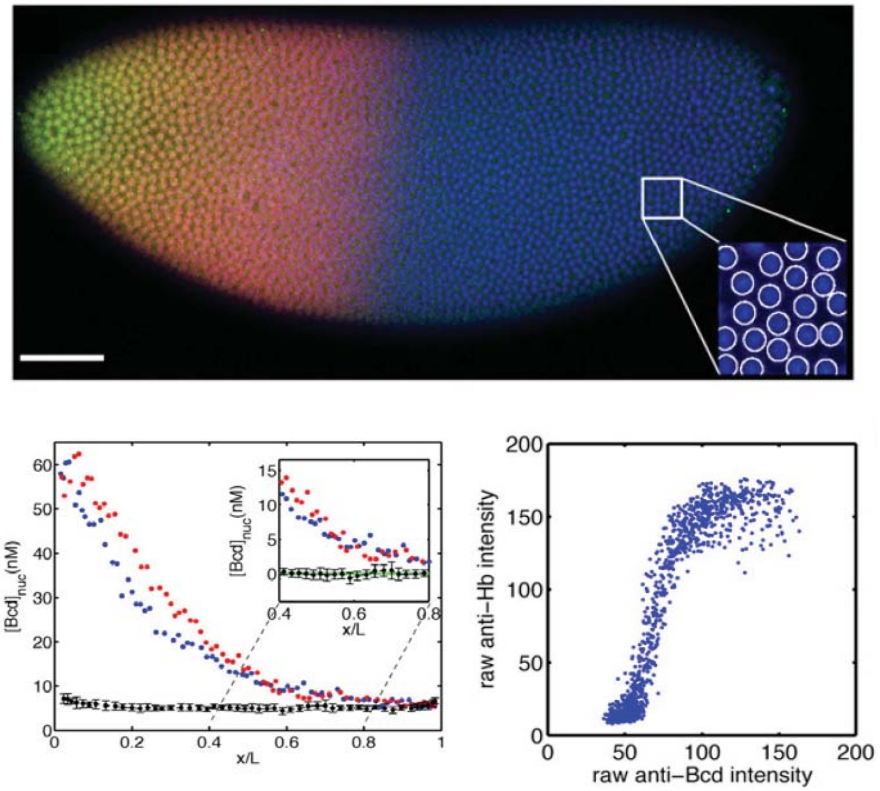

Figure 1 (Top) Fluorescence image showing distribution of Bicoid (Bcd, green) across nuclei (blue) together with the distribution of hunchback (Hb, red). (Bottom, left) Quantitative spatial distribution of Bcd protein in nuclei (blue and red denote ventral and dorsal cells, respectively, and black denotes control or background fluorescence in the absence of Bcd-GFP). (Bottom, right) Correlation between Hb and Bcd expression measured across the embryo using in situ immunofluorescent staining of 1299 nuclei in a single embryo. Figure reproduced from Gregor et al., *Cell* 130: 153, 2007.

a) To make this estimate, we appeal to measurements of the number of Bcd proteins at nuclear cycle 14. Assume that all Bcd is localized to the nuclei, which at cycle 14, have a radius of about 3.3 microns and have moved to the periphery of the embryo. The embryo can be modeled as a cylinder with length *L* = 500 microns and inner radius *a* = 100 microns. The nuclei form a sheet with thickness Δa = 6.6 microns (twice their radius) around the outside of this cylinder. How many Bcd molecules N_{Bcd} are in the embryo?

Hint 1: Recall from lecture that the Bcd concentration profile in 1-D is a decreasing exponential. In our 3-D cylinder, we can likewise treat the Bcd concentration as a function of *z* alone (state the assumptions that allow you to do this):

$$c(z, r, \theta) = c_0 e^{-x/\lambda}$$

where $\lambda = \sqrt{D/\mathrm{k}} = 200$ microns from class.

Hint 2: The volume element for an integral in cylindrical coordinates is ($rdrd\theta dz$).

b) We now need to estimate the number of mRNA that led to this number of proteins. The total amount of Bcd (N_{Bcd}) produced in the embryo can be described by the equation

$$\frac{dN_{Bcd}}{dt} = kM_{Bcd} - \beta N_{Bcd}$$

where M_{Bcd} is the number of Bcd mRNA molecules, *k* is the translation rate, and $\beta = 1/30$ min is the decay rate of Bcd. Assume that a single ribosome translates the Bcd mRNA in approximately 50 sec, and there are on average 13 ribosomes actively translating Bcd at any given time. How many Bcd mRNA molecules M_{Bcd} are in the embryo?

c) The average length of a gene in *Drosophila* is about 11 kilobases (kb), and the average elongation rate of an mRNA transcript is about 1.2 kb/min. How does the time to produce an average mRNA compare with the nuclear cycle times in the initial stages of embryo development? For example, nuclear cycles 9 through 11 last no more than 6 min, while nuclear cycle 12 lasts about 10 min, and cycle 13 lasts approximately of 12 min.

d) The data in the bottom right panel of Figure 1 show the expression of Hunchback (Hb) as a function of Bcd. What inferences can you make about the impact of Bcd level on Hb expression? Hint: See Eq. 4 in Gregor et al., Cell 130: 153, 2007 for more information.

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