33-767, SS 2019, HW 1 (Feb.-1, 2019)
due: Feb.-08, 2019 (at start of class)

(1) "Bleach-chase" determination of protein degradation   (10 pts)

Pulse-chase experiments to determine rates of continuously replenished processes are discussed in the textbook by *Phillips et al.* on p. 94/95. Following *Phillips*, problem 3.6, here we look at such experiments more quantitatively.

Protein production in a cell is offset by active degradation and by dilution in cell division. Eden and coworkers ([Science 331, 2011, 764-768](#)) discriminate these mechanisms by an equivalent approach, dubbed "*bleach-chase*", in which they instantaneously *deplete* the fluorescent label in a population of YFP-fused proteins. Another labeled protein is coexpressed for which the protein label is insensitive to the spectrum of the bleach pulse. Because the fused proteins are both replenished by protein production, presumed to occur at the same rate, observing the *difference* in the temporal development of two differently labeled protein populations, of which one was bleached and one was not, reveals the rate of protein degradation.

Let the rate of protein degradation be α and that of production be β. This leads to the kinetic equations for the protein populations:

$$dN_f/dt = \beta - \alpha N_f \qquad (1)$$

$$dN_u/dt = \beta - \alpha N_u \qquad (2)$$

$$dN_p/dt = -\alpha N_p \qquad (3)$$

for the population not affected by the bleach pulse (fluorescent, f), bleach-sensitive but unbleached (u), and bleached (p).

(a) Discuss the models of protein production and degradation embodied in Eqns. (1)–(3).

(b) Because the proteins *per se* are not destroyed by the bleach pulse, the temporal development of the populations p and u jointly is assumed to equal the time development of the population f:

$$dN_f/dt = dN_u/dt + dN_p/dt \qquad (4)$$

The fluorescence intensity of f and u can be monitored in different spectral channels by automated video microscopy of a population of cells (see paper by Eden *et al.*). Thereby, N_f and N_u can be separately determined:

$$d\left(N_f - N_u\right)/dt = -\alpha\left(N_f - N_u\right) \qquad (5)$$

Show how the observation of the temporal development, $\Delta N(t) = \left(N_f(t) - N_u(t)\right)$, and the fluorescence intensity difference post-bleach, $\Delta N(0)$, is used to determine α.

(c) How is dilution of proteins in cell division discriminated against protein degradation in the experiment?

(2) The Stirling approximation   (20 pts)

To derive the Stirling approximation, $\ln n! \approx n\ln n - n$, begin with an integral representation of $n!$

(a) Show that $n! = \int_0^\infty x^n e^{-x}\, dx$ by representing the integrand as $x^n d\left(e^{-x}\right)$ and finding the recursive identity

$$\int_0^\infty x^n e^{-x}\, dx = n\int_0^\infty x^{n-1} e^{-x}\, dx \tag{1}$$

through integration by parts.

(b) Sketch the function $g(x) = x^n e^{-x}$ on $[0,\infty]$ and show that its maximum occurs at the same position, x_0, as the maximum of $\ln(g(x))$. Expand $\ln(g(x))$ about x_0 up to the second order in δ.

(c) The exponential of your expansion provides an approximation to the integrand in the left hand integral in Eq. (1) that is valid near x_0. Integrate the approximation (in which limits?) and show that

$$n! \approx n^n e^{-n} \int_{-\infty}^\infty e^{-\delta^2/2n}\, d\delta \tag{2}$$

(d) Evaluate the integral in Eq. (2) and show that $n! = n^n e^{-n} \cdot \sqrt{2\pi n}$. When you take the logarithm, explain under which conditions one may drop the $(2\pi n)^{-1/2}$ term.