BIOREPS 2016 Project C
Hamsters, Cows, and Infectious Dimers: the Secret Life of Prions

1 Background

Prionic diseases are a subset of fatal neurological diseases caused by the spontaneous misfolding of endogenous proteins (PrP^C) into a degenerative state known as prions (PrP^{Sc}). The mechanism behind the misfolding of the proteins is yet unknown. However, regardless of the precise trigger causing the initial reaction $PrP^C \rightarrow PrP^{Sc}$, wherein a healthy host protein is misfolded into a prion, it is believed that the presences of the prion encourages the misfolding of the surrounding proteins. This is because the prion possibly acts as a template to more easily convert the endogenous proteins [1].

Therefore, the system becomes autocatalytic, resulting in the reaction $PrP^C + PrP^{Sc} \xrightarrow{K} 2PrP^{Sc}$, wherein prions and proteins moving through the system meet and cause one another to refold at a rate K. In addition, because the misfolded state places the protein's hydrophobic material on its exterior, it is attracted to other prions, causing it to aggregate and over time form plaque-like structures known as fibrils on the neurological tissue [2]. As the following problems will elaborate, the incubation time of the disease, or the time from initial infection with PrP^{Sc} to the time at which a specified concentration PrP^{Sc} is achieved, directly correlates to the initial concentration of healthy PrP^C proteins , which act as "fuel" for the reaction. As the reaction progresses, the concentration of the pathogenic PrP^{Sc} increases, causing the reaction to occur more readily, theoretically until all PrP^C proteins are removed from the system or realistically until host death occurs [3].

Prion diseases are perhaps most commonly recognized in forms such as bovine spongiform encephalopathy (BSE; the so-called "mad cow disease") and through fears of contaminated food, but they are recognized as contributing to a variety of highly transmittable, fatal diseases in both humans and animals. Since PrP^{Sc} is a particularly stable form of the protein, it is known to be resistant to a variety of treatments, including high temperatures. Because of these transmittable and resistant characteristics, research into this prion reaction problem holds significant application in a wide variety of fields, ranging from the theoretical to the medical.

Most approaches to the problem agree that describing the $PrP^C \rightarrow PrP^{Sc}$ reaction is necessary to understanding the system; however, the details of this reaction require a wide array of parameters and complicated mechanisms, including possible chaperone systems [4]. So, though more complex models of the system have been explored, the 2003 paper by Ferriera, da Silva, and Cressoni proposes a simplified model which expresses the system's necessary characteristics in a compact, solvable analytical expression [3]. Unlike other models, which propose complex analytical models of fibril formation and breakage [5], the Ferriera *et al.* approach concentrates on simulating realistic incubation times for the disease by creating a simple mean-field model. In particular, it narrows the field of parameters to those which they deemed to be strictly necessary to achieving analytical results which correspond with and describe previous experimental data. In the following problems, you will use Ferriera *et al.*'s simplified approach, recreating and comparing with their calculations and simulations, to find an analytical solution for the prion reaction problem.

References

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2 Questions

2.1 Simple Analytic Model for Prion Concentration

To solve analytically for the prion concentration as a function of time we must clarify our notation and make a number of simplifying assumptions. Let A represent a host protein (PrP^C), and B represent a prion (PrP^{Sc}). Let *a* = [A], and *b* = [B], denoting the volume concentrations. Then the autocatalytic conversion reaction whereby a B converts an A into a copy of itself is

$$A + B \xrightarrow{K} 2B \tag{1}$$

where K is the reaction rate. Further we assume that the total concentration

$$a + b = \rho \tag{2}$$

is held constant, and that the volume concentrations are uniform. We assume that metabolic decomposition of A is compensated for by the host's generating new A, that B does not decay, and that each A is not replaced when it is converted into B. Finally, we assume the reaction is unidirectional in the direction of B. No B is ever converted back into an A.

a) Use these two expressions to derive the kinetic evolution of the prion concentration, $\frac{db(t)}{dt}$. Express the kinetic evolution without any dependence on the concentration of A.

b) Now that you have an expression for the kinetic evolution of the prion concentration, solve it to find $b(t)$ subject to the initial conditions $a(0) = a_0$, and $b(0) = b_0$. Use the expression for $b(t)$ to express T as a function of $b(T)$, a_0, and b_0.

c) Assume there is some threshold of concentration at which the prion has "incubated", call it b_I. At what time does incubation occur? Are there any simplifying approximations we can make for this expression? Consider that $b_0 \ll a_0$.

2.2 Relationship between Incubation Time and Initial Dose

d) By assuming that the initial concentration of host protein is much greater than the pathogenic ones (i.e. $a_0 \gg b_0$), we could have a simple expression for the incubation time [3]:

$$T_I \approx \frac{1}{Ka_0} \ln \left[\frac{b_I}{b_0} \left(\frac{1}{1 + \frac{b_I}{a_0}} \right) \right]. \tag{3}$$

From this equation, we can find out the Incubation time have a log dependence on the initial concentration of the pathogenic prion (PrP^{Sc}). Similarly, by assuming that when **B** reaches certain concentration (i.e. $b(T_D) = b_D$) will lead to the death of the host, we can obtain the Death time T_D. Give the analytical expression of $T_D - T_I$. Does this time difference depend on the b_0? Why?

We can also obtain the incubation time by using the well-known "Michaelis−Menten" kinetics, a model describing the rate of enzymatic reactions by relating reaction rate to the concentration of a substrate (a) as

$$\frac{db}{dt} = v_{max} \frac{a}{K_M + a} = K_T b \frac{a}{K_M + a}, \tag{4}$$

where K_T is the turnover number, the maximum number of substrate molecules (A) converted to product per enzyme molecule (B) per second (i.e. the max reaction rate $v_{max} = K_T b$), and K_M is the Michaelis constant. Direct integration of this equation leads to the expression for T. Show that this new expression of T reduces to the result of part (b) while we set $K_m \gg a_0 \gg b_0$ and $K \approx \frac{K_T}{K_M}$.

e) Once we have the model, it is natural to check if the model is consistent with the experiments. In 1984, Stanley B. Prusiner (who received the Nobel Prize in Physiology or Medicine in 1997 for prion research) quantitatively observed the dependence of the incubation time / Death time on the initial concentration of the pathogenic prion (b_0) from the inoculation of a form of scrapie in hamsters [6]. In his experiments, the initial concentration $b_0 = 10^x \beta_{min}$ ($x =$ dose), where β_{min} represents the smallest experimental concentration he injected to the hamster and x is allowed to vary from 0 to 10. The experimental data is approximately

Dose	1	2	3	4	5	6	7	8	9	10
Incubation (days)	129	112	102	94	87	80	73	65	58	51
Death (days)	149	132	117	110	103	96	88	81	74	67

Plot time T_I and T_D versus the dose. Does the time difference $T_D - T_I$ depend on the dose? Is this consistent with the results of part (d)?

Plug $b_I = I\beta_{min}$, $b_D = D\beta_{min}$, and $b_0 = 10^x \beta_{min}$, Eq (3) yields

$$T_I = C - \frac{\ln 10}{Ka_0} x, \tag{5}$$

where C is a phenomenological constant. To substitute b_I with b_D, we found that the T_D share the same Ansatz with T_I. Find an analytical form for C of both T_D and T_I (in terms of I or D, b_I or b_D, a_0). Ignoring $x \leq 2$ region, what are the rough values of C and Ka_0 for T_D and T_I which make this model fits the experimental data. Compare the fitting and the experimental data in a plot.

2.3 Simulation for Incubation Time Distribution

f) Based on Equation 3, the incubation time also varies with the initial concentration of the PrP^C proteins. Write a program (in your favorite language) to simulate the incubation time and observe the effect of varying the initial concentration of PrP^C proteins. Allow the particles to diffuse randomly to their nearest neighbor sites and react if a PrP^C protein approaches a PrP^{Sc} prion at a distance $d \leq \sqrt{massPrP^{Sc}}$ Use a 200 x 200 square lattice as the simulation matrix. Use the following assumptions:

- N_{B0}, the initial number of PrP^{Sc} misfolded proteins, equals 6.
- The diffusion takes place over the entire matrix, followed by the reactions. The time unit is then increased by 1.
- In each diffusion step, the proteins will diffuse to their nearest neighbor sites with equal probability. The proteins can only diffuse to immediately adjacent positions, not diagonal positions. If a site is filled, the protein will not diffuse. If the protein is at the edge of the lattice and its diffusion site is off the lattice, it will not diffuse.
- When a PrP^C approaches a PrP^{Sc} at a distance $d \leq \sqrt{massPrP^{Sc}}$, the PrP^C disappears and the PrP^{Sc} has its mass increased by 1.
- The reaction stops when one of the masses reaches m = 40. This is the computer time characterizing the incubation time.

Run at least 25 iterations. This may take awhile. Normalize the incubation times by dividing by the mean incubation time. Using this data, produce a log normal plot. Repeat this procedure for the following N_{A0} concentrations (percent of lattice sites filled with N_{A0}): 2%, 0.8%, and 0.6%. The graphs should be bell-shaped curves, with the smaller N_{A0} values having the smaller peaks.

g) Since we have now determined how the incubation time is influenced by the initial number of PrP^{Sc} seeds, it would be beneficial to develop an analytical form for the incubation time distribution. Using approximate observed incubation times from BSE-infected cattle born in 1987 in the United Kingdom, we can develop a distribution for that data from the following equation:

$$G(t_I) = \frac{1}{\sigma\sqrt{2\pi}} t_I^{-1} exp[-\frac{1}{2}(\frac{\ln t_1 + (1/2)\sigma^2}{\sigma})^2], \tag{6}$$

where σ is the unknown standard deviation of the log of the reaction rate K and t_I is $T_I/\bar{T}_I$, when $\bar{T}_I$ is the mean time for the cattle become infected with BSE. Determine the value for σ given the United Kingdom cattle data below and generate a graph of the function. How does this graph compare to the graphs developed in part f?

t_I	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8
G(t_I)	0	0	0.375	1.6	1.5	0.8	0.375	0.2	0.5

$Hint$: Consider using non-linear fit modeling