On the statistical mechanics of prion diseases

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We present simulation results for a simple two-dimensional, lattice based, protein-level statistical mechanical model for prion diseases (e.g., Mad Cow disease) with concommitant prion protein misfolding and aggregation. Our simulations lead us to the hypothesis that the observed broad incubation time distribution in epidemiological data reflect fluctuation dominated growth seeded by a few nanometer scale aggregates, while much narrower incubation time distributions for inoculated lab animals arise from statistical self averaging. We model ‘species barriers’ to prion infection and assess a related treatment protocol.

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Transmissible neurodegenerative prion diseases, such as Mad-Cow Disease (BSE) and Creutzfeldt-Jakob Disease (CJD) in humans, increasingly represent a serious public health threat. Prusiner and collaborators have shown that the infectious agent (prion) in these diseases consists of a quantity of a misfolded form (PrPSc) of the ~200 amino acid PrPC protein which is expressed ubiquitously in mammalian neurons. The PrPC proteins normally reside on neuron surfaces, and the more hydrophobic PrPSc forms tend to aggregate: large (micron scale) PrPSc amyloid plaques are a common post-mortem feature of brain tissues, and fibrils are observed in in vitro experiments. Nucleic acid free propagation demands that PrPSc autocatalyze their own formation by helping to convert more PrPC into PrPSc. Given that mammalian PrPC differ by only 5-10% in amino acid composition and that variant CJD correlated with BSE has been observed in England, the efficacy of “species barriers” in limiting transmission is of considerable interest.

Several facts suggest that prion disease may be driven less by complex biology (as in Alzheimers’ disease) than by physico-chemical processes, including: (1) The “universal character” of sporadic CJD, observed globally without evident spatio-temporal clustering at an annual background death rate of 0.5-1.5/million and mean onset age of about 63 years. (2) Highly reproducible incubation time vs. infectious dose distributions observed in laboratory animal studies, with a power law relation between mean incubation time and dose concentration D. (3) The close temporal proximity of disease onset and death. (4) PrPSc may be grown in vitro without biological processing and is toxic to neurons, though not yet demonstrably infectious. The following question is thus raised: is the incubation time of prion disease dominated by a fundamental physico-chemical nucleation and growth of PrPSc protein aggregates?

We present simulation results for a simple two-dimensional, lattice based statistical mechanical model for prion disease based at the protein level. It is all but hopeless to develop an atomic level simulation capable of spanning the 21 temporal orders of magnitude between picosecond scale intra-protein motion and the potentially decades long incubation times of large prion aggregates. Our simulation is a bridge between the short distance and time scales covered in individual protein models and the long time, macroscopic realm of chemical kinetics. We use our simulations to identify the protectorate of principles necessary to describe the aforementioned universal features of prion disease. Specifically, we find: (1) Concomitant PrPSc autocatalysis and stable aggregation can explain the long disease incubation times, and the difference between sporadic (unseeded) and infectious (seeded) onset. This principle is consistent with data for yeast prions, is closely related to the earlier nucleation theory of Lansbury, and avoids the need to fine tune model parameters with separated autocatalysis and aggregation. (2) The distributions of disease incubation times (well defined onset edge, broad fluctuation driven spectrum for dilute prion doses, narrow short time shifted distribution for more concentrated prion doses) strongly suggest a central role for growth from a minimally nanometer scale seed (with order 10 PrPSc proteins). With these assumptions we obtain a one parameter fit to the BSE incubation time data, an order of magnitude correct estimate of the mean incubation time for BSE and infectious CJD, and we argue that self-averaging explains a narrowing of incubation time distributions observed in the laboratory. Finally, by considering both sticking and interconversion rate differences for prions of different species, we provide a theoretical underpinning to data for the species barrier efficacy and to proposed treatments based upon coating of incipient prion plaques.

Our assumption of two-dimensionality is consistent with the observation that PrPC resides on neuron surfaces and autocatalysis and aggregation must certainly initiate there. The lattice structure should be irrelevant at long times. By assuming a triangular lattice we minimize the anisotropy (which appears first in rank six tensors for hexagonal lattices). This simulation yields the crucial qualitative features necessary to specify the “prion protectorate.” Our resulting prion aggregates are essentially amorphous, rather than quasi-one-dimensional filaments as found in some in vitro experiments. We shall discuss an anisotropic simulation yielding fibrils elsewhere.
Our model is based upon the energy landscape schematized in Fig. 1. Individual lattice cells take on three values: unoccupied (implicitly filled with water), occupied by a PrP protein, or occupied by a PrPSc protein. At the beginning of each simulation, we choose a monomer PrP configuration distributed randomly, with a number (0-4) of PrPSc seeds located randomly as well.

In the spirit of cellular and molecular automata, we eschew an explicit Hamiltonian based molecular dynamics in favor of the following updating rules, specified in time units of one full lattice sweep (d is the nearest neighbor coordination number):

1. Identify each occupied cell as PrP or PrPSc by checking its number of nearest neighbors.
2. For each PrP, move one step in a random direction if that adjoining site is unoccupied.
3. Identify aggregates (size N_p) by PrPSc presence; move them per Rule (2) with probability 1/\sqrt{N_p}.
4. For d = 0, 1, the protein remains PrPSc.
5. For d = 2, the protein is PrP or PrPSc with equal probability.
6. For d \geq 3 the protein is PrPSc.

Rules (1-3) ensure diffusive motion of PrP or PrPSc monomers and PrPSc aggregates. Rules (4-6) specify protein interactions and autocatalysis, and reflect the relative hydrophobicity of PrPSc. To assure the biologically plausible condition of constant average PrP concentration, at the start of a new sweep we randomly place a new PrPSc for each lost in the previous update.

This procedure is repeated for up to millions of full sweeps through the lattice. Note that a sweep defines the minimum time scale for conversion of PrP to PrPSc, which is presumably of order a second or less in real time. We work with maximum lattice sizes of N = 4 \times 10^4. Practically, we are able to run at areal monomer concentrations c \geq 0.1\%, which are likely to be about three orders of magnitude larger than in the brain.

We run our seeded simulations repeatedly following the largest nano-aggregates until they reach a size of up to 0.4\% of the lattice. Each seed consists of 10 PrPSc monomers. For a given aggregate size, we have studied the properties of the distribution of lifetimes to reach that size. For a final aggregate size of A=0.2\%N (80 monomers) we display the corresponding distributions P_D in Fig. 2(a) for several values of c and D = 1. P_D displays greater fluctuations for small c, and increasing c shifts P_D to short times. Fluctuations dominate the growth from the small seed aggregate. For larger A we find that the onset time grows sublinearly in A, while the distribution width grows weakly with A. To within fluctuations, the P_D curves for different c collapse when scaled by the mean time t_m. (The curves broaden somewhat for D = 2, 3, 4.) This implies the scaling law

\[ P_D(t, c) \approx \frac{1}{t_m(c)} F_D\left(\frac{t}{t_m(c)}\right) \]  \quad (1)

In the range of concentrations studied, we can fit t_m(c) well by either: (a) the polynomial form t_m(c) \sim Ac^{-1} + Bc^{-2} (the first term reflecting monomer aggregation, the second representing dimer aggregation), or (b) a power law form, t_m(c) \sim c^{-\alpha(D)}, with \alpha(D) = 1.66(D = 1), 1.49(D = 2). For finite \alpha and c \rightarrow 0 the exponent \alpha(D) should tend to two, reflecting dominant dimer aggregation. Fit (b) is consistent with a nontrivial scaling limit as \alpha \rightarrow \infty, c \rightarrow 0. Both fits to t_m(c) give order of magnitude equivalent extrapolations to lower c relevant to biological contexts. Assuming t_m(c) \sim D^{-\beta} at fixed c, we estimate \beta \leq 1 with large numerical uncertainty. Taken together, these data lead us to a conjecture of asymptotic compression of P_D: for c \rightarrow 0, an increasingly large range of \alpha will have essentially the same scaled shape, because the time t_2 it takes to go from \alpha to say, 2\alpha will be much smaller than t_m for going from 10 \rightarrow \alpha.

We believe that other processes, such as protease attack or competition between PrPSc-PrPSc and PrPSc-neuron binding will limit prion aggregation on the neural surface and cause fissioning and spreading of nano-aggregates to other neurons. Such fission is necessary for exponential growth of infectious agents, as noted previously. At the order-of-magnitude level t_2 sets the doubling time for disease spread, assuming that intracellular diffusion rates exceed intra-cellular ones. Given t_2 << t_m (at biological c values) both in our fission hypothesis and experiment, slow growth fluctuations for small seed aggregates can dominate the overall incubation time for disease. For example, in hamster experiments the first observable levels of PrPSc in brain tissues occur at about 90 days, with symptom onset at 120 days, and a doubling time of 2 days. Given the conjectured asymptotic compression, the incubation time distribution will be relatively insensitive to a wide range of nano-aggregate sizes for fission parents or progeny.

We stress several points of agreement with observation and laboratory data. First, we always find death (runaway growth of a killer aggregate) in the presence of infection, in agreement with experiment and the aggregate nucleation hypothesis for prion disease. Second, our rough scaling of t_m with D agrees qualitatively with the extraordinarily reproducible dose vs. incubation time data of Prusiner and collaborators for prion infection in laboratory mice, for which \tau_1 \sim D^{-0.62}. Third, our lifetimes are in order of magnitude agreement with observed BSE incubation times and new variant CJD death ages, which we can prove by exploiting our concentration scaling. For a single seed, at our lowest concentration of 0.1\% our distribution peaks at about 10^4 sweeps. Assuming a literature estimate of concentration of in vivo prions in solution of 100 nanomole/liter and a protein diameter...
ter of order one nanometer suggests a dimensionless areal
concentration of about $10^{-3}\%$. With the above
concentration scaling this gives an incubation time of about $10^8$
sweeps. If we take the 5 year mean incubation time for
BSE this implies our basic misfolding time (one sweep)
is about one second, which is reasonable at the order of
magnitude level. Finally, by simply scaling time for
$A = 80$ by the mean time $t_m$, we achieve an exceptionally
good fit (A) of the inferred incubation time distribution
for BSE in the United Kingdom (for cattle born in 1987)
to our scaling curve as shown in Fig. 2(b). This
supports our hypothesis that runaway autocatalytic ag-
gregation seeded by a few prion nano-aggregates triggers
the disease. This is very plausible since (i) infection is
believed to have come from rendering plant offal derived
from many animals after considerable processing,leading
to substantial dilution, and (ii) prion transmission by
oral consumption is estimated to be less efficient than
innoculation by a factor of $10^6$.

Sporadic CJD develops in the absence of any infec-
tion or genetic predisposition to prion disease. We have
studied the lifetime distributions in this case and find
that they are very different in shape than the seeded
runs, being much broader and dimer fluctuation domi-
nated (peaks scale as $c^{-2}$). For our estimated biological
concentration of $c = 10^{-3}\%$, this could give a mean onset
time as large as $10^4$ years. Assuming a constant height
tail from the minimum single seed onset to the mean hu-
man lifespan taken with the 1 part in $10^6$ odds of dying
from sporadic CJD gives an estimate of a total death
probability of a part in $10^9$ up to this mean on-set time.
This is consistent with our simulations, but not resol-
vable currently.

In contrast to the breadth of our single seed lifetime
distributions, in the event of dosing by a greater number
of seeds, self-averaging will narrow the distribution, col-
lapsing it towards the onset (shorter times). Assuming
growth proceeds independently for different seeds (which
are quite reasonable for the low concentrations in living or-
organisms) distributions would narrow by $\sqrt{D}$. Several in
vivo experiments show distribution time narrowing with
increased dosage, but are not amenable to quantitative
analysis at this time.

We turn now to the species barrier. To simulate inter-
species transmission we replace the integer-valued coor-
dination, the key parameter which determined the stabil-
ity of different protein conformations in the earlier stud-
ies, by a new variable $x = N + N' \times P$ for PrP$^{C}$ and
$x' = N' + N \times P'$, for PrP$^{C',Sc}$, where $N$ is the number of
PrP$^{C,Sc}$ neighbors and $N'$ the number of PrP$^{C,Sc}$ neigh-
bors. The parameters $P$ and $P'$ (chosen less than unity)
provide a measure of the reduced effectiveness of inter-
species conversion. To deal with the continuous variable
$x$, rather than the integer coordination used earlier, we
represent the PrP$^{C}$ to PrP$^{Sc}$ conversion probability $f(x)$ by

$$f(x) = \frac{1}{e^{\beta(x-2)} + 1} \tag{2}$$

with a similar definition for PrP$^{C'}$ to PrP$^{Sc'}$ conversion.

A separate parameter describes physisorption to an ag-
gregate of alien prions. We allow a PrP$^{C,Sc'}$ cluster of mass $M$ ($M = 1$ being a monomer) to desorb from an
alien prion aggregate with probability $q^R/\sqrt{M}$, where
$q$ is the physisorption parameter, and $R$ is the number of
units in the cluster adjacent to the alien aggregate. We
define $q'$ for PrP$^{C,Sc}$ similarly. Thus, we describe
species barriers in terms of the two parameters affecting
conversion rates ($P, P'$) and physisorption ($q, q'$). Mak-
ing $P \neq P'$ and $q \neq q'$ allows for an asymmetry in infect-
tivity, and we can model one of the most striking aspects
of the species barrier, namely its asymmetry (e.g. mice
infect hamsters well, hamsters infect mice poorly).

We have carried out several simulations relevant to the
species barrier. First, we have placed alien prion `walls'
on a boundary, representing a single large aggregate from
another species. With suitable choice of parameters,
this leads to the asymmetric species barrier between two
species. Namely, disease onset in one species (with large
aggregates) and only sub-critical PrP$^{Sc}$ concentration
near the wall in the other. Recent experimental data
has also shown the build up of such sub-critical PrP$^{Sc}$
conversion in the asymmetric interspecies transmission.

Second, suppose the asymmetry is such that $P << P'$.
In this case, PrP$^{Sc'}$ is more favorably formed from PrP$^{C'}$
in the presence of a seed aggregate of PrP$^{Sc}$ than PrP$^{Sc}$
is formed from PrP$^{C}$ in the presence of a PrP$^{Sc'}$ seed.
Thus, by injecting a large enough initial dose of alien
PrP$^{C'}$ proteins into the organism, it should be possible
for PrP$^{C'}$ to compete favorably with PrP$^{C}$ for aggrega-
tion, and thus extend the incubation time. Such proto-
cols have been tested experimentally: (i) Experiments
with the coating dye molecule Congo Red [18] reveal
a surprising non-monotonic dose vs. lifetime relation:
small Congo Red concentrations yield a reduced time for
incubation, while larger Congo red concentrations signifi-
cantly extend the incubation time. (ii) In vitro exper-
iments show that the above coating scenario with alien
prions works [13] when the initial PrP$^{C'}$ concentration
$c' > c$.

We find both of these experimental features in our sim-
ulation as shown in Fig. 3. For $c'/c < 2$, the incuba-
tion time is slightly shortened, while for $c'/c > 2$ the
incubation time is increased. We find that the lifetime
shortening arises because once a few PrP$^{C'}$ aggregate,
they partially block the motion of adjacent PrP$^{C}$ pro-
teins, enhancing the misfolding probability of the latter.
However, further PrP$^{C}$ misfolding is significantly reduced
once PrP$^{Sc}$ coats the seed.

A crucial test of our theory would be to monitor, in
vitro, the lifetime distributions of normal prion proteins
seeded by small aggregates as a function of both $c$ and $D$ to look for the scaling behavior. If it is not possible to easily achieve $D = 1, 2$, presumably the distribution of Fig. 2(b) could be estimated by extrapolation from successively diluted PrP$^{Sc}$ doses. This would also test the extent to which the self-averaging applies. If our single seed hypothesis is correct, it is important to assess how passage occurs upon infection.

Theoretically, it is important to extend our model by including internal degrees of freedom for the proteins which can provide an orientational dependence to the aggregation, allowing for linear fibril formation as opposed to amorphous aggregates. This connects naturally with simulations on individual proteins, and also allows for the study of different protectorates for collective protein phenomena.

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FIG. 3. Mean incubation time $t_m$ vs. relative concentration of alien prions $c'/c$ (test of coating treatment; see text for discussion).