[PrP^X]

Research Proposal

Surya Sanjay[1] and Tanay Bhangale[1]
[1]Troy High School, Troy, MI 48098

Abstract

Prions are infectious proteins that cause diseases, such as Creutzfeldt-Jakob's and mad cow disease[1], through intracellular aggregation. They are noted for their ability to convert the wild-type, benign (PrP^C) prion into the infectious variant (PrP^{Sc}). Since their discovery in 1982[2], prions have had no viable treatment options[3], and only recently have the structures and replication method been fully deciphered[4]. The anti-prion has been hypothesized as a prion conversion inhibitor, but although this prolongs lifespan[5], this doesn't restore normal function, which is a pressing issue as prion function is not yet understood[6]. To prove the feasibility of a new treatment model, we propose a novel prion protein variant that is benign, as functional as PrP^C, and has the ability to catalyze the conversion of PrP^{Sc} into the novel variant. Using a model of the infectious murine prion[4] and many mutagenized variants of the benign murine prion as PrP^X, created with Rosetta's *ab initio* software[7], we plan to perform molecular dynamics (MD) simulations with GROMACS 2020[8] to determine the success of models based on the described criteria. A machine learning method will then be utilized, identifying protein parameters that contribute most to the success of chosen models in order to create a confidence interval containing possible treatment options. Although prion disease has comparatively low incidence[9], the success of this model could allow for similar proteins (termed "prion-like") like Aβ and tau that contribute to Parkinson's, Alzheimer's, and ALS to be targeted in a similar way in future studies[10].

Materials and Methods

Step 1: Generation of a Prion Conversion Model

Only recently has a strong, molecular model of prion replication been elucidated, and this will be redone as a control to compare to the PrP^X conversion model. Current knowledge suggests that the PrP^{Sc} protein is a 4-rung beta-solenoid (4βS), while the PrP^C is mostly composed of α-helix sections. The scrapie (infectious) protein binds to the N-terminus of the cellular (benign) protein and causes the latter's peptide sequence to refold into beta-solenoid rungs, effectively continuing the scrapie protein's structure[4]. Using this mechanism, prions form long, aggregate chains called amyloid deposits within the extracellular matrix (ECM), released after causing cell death[11]. We will use Gromacs 2020.1 and proven parameters to perform a molecular dynamics

(MD) simulation of this model of replication[8]. After modeling the hypothesized system, we plan to determine which exact amino acids the benign prion protein binds to on the infectious prion, and then create bonding parameters that would aid our search for possible PrP^X's.

Step 2: Creation of PrP^X Models

To create specific *de novo* protein structures based on our criteria, we will use RamaNet, a novel neural network-based protein design program. This program works by randomly generating a protein's Φ and Ψ angles to create a structure, while using a string of valines as a placeholder for the peptide sequence, which can be edited later[12]. The 30 models that we create will then be used in Gromacs to determine efficacy.

Step 3: Generation of a PrP^X Conversion Model

After generating the PrP^X models in RamaNet, we plan to recreate the environment used in the prion conversion model, except that we will replace the cellular protein with PrP^X. We will then test each of the models, collecting relevant data, such as total interaction energy and whether the beta-solenoid rungs refolded themselves into α-helices. Success will be determined mainly by evident refolding and partially by a more negative interaction energy.

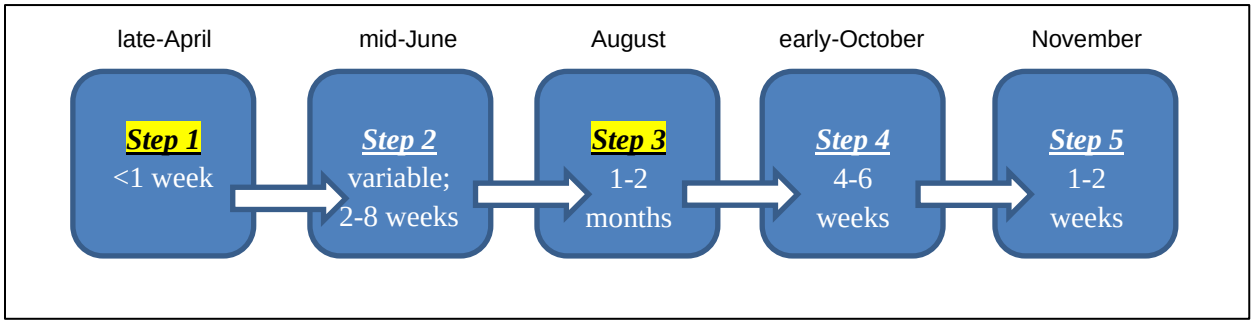
Step 4: Comparison of PrP^X Models to PrP^C

This step only requires modeling of superposition of PrP^X on PrP^C, making sure that the structures are similar. For this, we will use Rosetta to determine the root mean square deviation values of PrP^X models, and we will create a threshold value based on the variation in existent benign prion protein structures to designate successful structures[13]-[22].

Step 5: Application of Machine Learning to PrP^X Parameters

Once we have a set of PrP^X models validated to suit the target parameters, we will create a dataset containing relevant protein structure parameters, such as the percentage of α-helix within the structures, Φ and Ψ angles, peptide sequence (if varied), and stability. We will then create a machine learning model whose target classes will be termed "successful" and "unsuccessful." Therefore, even though we will have created successful models, we will have a confidence interval to suggest models that are more successful and have less risk when used for treatment.

Research Timeline (Flowchart)



Clinical Significance

Diseases that are directly caused by prions, although having low incidence, are of utmost importance to understand prions themselves: *Creutzfeldt-Jakob's disease (CJD)* is a fatal neurodegenerative disease associated with prions, that affects both humans and animals. There are two types of CJD, variant CJD, and spontaneous CJD. The variant CJD arises from the consumption of cows that contain the agent of bovine spongiform encephalopathy (mad cow disease), rarely occurring in humans. The most common type is the spontaneous CJD, which contains a quick disease course ranging from 6 to 12 months. This disease is not very common, as the annual incidence of CJD is 1 in 1,000,000 cases[23]. *Fatal familial insomnia (FFI)* is a commonly inherited prion disease caused by a mutation on codon 178 of the PRNP gene that leads to the proliferation of the PrP^{Sc} molecule in the thalamus of the brain[24]. *Gerstmann-Sträussler-Scheinker's disease (GSS)* is an autosomal dominant neurodegenerative disease associated with amyloid aggregations in the central nervous system. Since prions are classified as amyloid proteins, this disease stems from the mutation in the gene PRNP like the FFI disease[25].

Prion behavior is also similar to the pathology of other diseases, meaning that the results of this study could be applied to those diseases in a future study. *Huntington's disease* is a common neurodegenerative disease, about 1 in 10,000 people are affected. Proteins involved in this disease have similar behavior to prions, suggesting a link between these two mutations[26]. *Alzheimer's disease* is the most common neurodegenerative disease, affecting more than 44 million people worldwide. This disease is caused by aggregates of the amyloid-beta protein, and tau protein neurofibrillary tangles in the brain. The PrP^{Sc} molecule, which converts the PrP^{C} molecule into itself, can lead to the decrease in the regulation of the toxicity from the amyloid-beta accumulation leading to further mental decline[27]. *Parkinson's disease* is another common neurodegenerative disease stemming from the aggregation of Lewy bodies, with about 60,000 new cases in the US annually. The mechanism of the proliferation of these Lewy bodies is similar to that of the prion protein, opening different avenues for developing a new treatment for Parkinson's[28].

For these diseases, most attempted treatments and cures have not been effective, and therefore, the conceptualization of new mechanisms is important in the fight against these diseases.

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