Prion Misfolding: β-Sheet Destabilization

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ABSTRACT

Prion diseases are a group of fatal neurodegenerative disorders caused by the misfolding of the prion protein (PrP). The misfolded form propagates by converting native PrP into its pathogenic conformation, yet the precise molecular mechanisms underlying this process remain poorly understood. A key step in misfolding involves structural destabilization, particularly in the β-sheet regions of PrP. Molecular dynamics (MD) simulations provide atomic-level insights into protein misfolding, but conventional approaches are hindered by the large disparity between biologically relevant folding timescales and computational limitations. To overcome this barrier, we employ advanced sampling techniques to investigate the unzipping of the β-sheet in properly folded PrP, a potential early-stage event in misfolding. Our simulations capture conformational transitions that reveal key intermediates and energy barriers associated with β-sheet destabilization. By characterizing these molecular events, we provide novel insight into the earliest structural changes that may lead to prion propagation. Understanding these misfolding pathways is essential for elucidating prion disease mechanisms and could inform future therapeutic strategies aimed at stabilizing the native PrP conformation, thereby preventing disease onset.

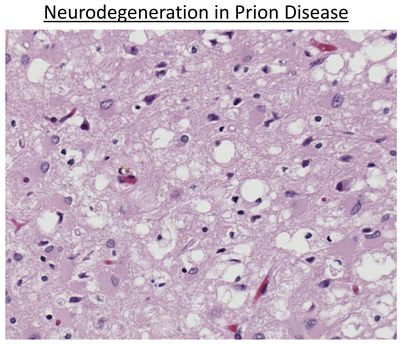
INTRODUCTION

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are fatal neurodegenerative disorders that affect both humans and animals. These diseases arise from the misfolding of the prion protein (PrP), where its normal α-helix-rich structure converts into a β-sheet-dominant pathogenic form. This misfolded PrP aggregates, leading to neurotoxicity and disease progression.

At the molecular level, a key factor in prion misfolding is the destabilization of β-sheet regions, which may serve as an early trigger for structural conversion. Computational techniques, particularly molecular dynamics (MD) simulations, provide atomic-level insight into this process. However, conventional MD approaches are limited by long folding timescales and high computational costs.

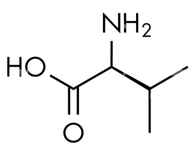
Prion Disease in Animals

Neurodegeneration in Prion Disease

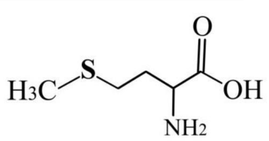


This study employs advanced sampling techniques to explore early-stage misfolding events, specifically focusing on β-sheet unzipping in native PrP. By identifying key intermediates and energy barriers, we aim to enhance our understanding of prion disease pathogenesis, which may inform therapeutic strategies targeting PrP stability.

Valine Chemical Structure



Methionine Chemical Structure



METHODS

We employed molecular dynamics (MD) simulations with umbrella sampling to investigate β-sheet destabilization in the prion protein (PrP), a potential early step in misfolding.

System Setup

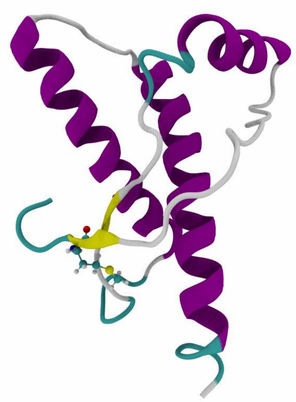
- The human PrP structure was obtained from the Protein Data Bank (PDB).
- The protein was solvated in explicit water using the TIP3P model and neutralized with counterions.
- CHARMM36m force field was used for accurate representation of PrP dynamics.
- The system underwent energy minimization followed by equilibration under constant temperature (310 K) and pressure (1 atm) (NPT ensemble).

Umbrella Sampling Protocol

To overcome the timescale limitations of conventional MD simulations, we applied umbrella sampling to enhance sampling of rare conformational transitions:

- A reaction coordinate was defined based on the distance between key backbone hydrogen bonds in the PrP β-sheet.
- Harmonic biasing potentials were applied at multiple windows along this coordinate.
- Each window was simulated for 100–200 ns, ensuring sufficient sampling of intermediate states.
- The weighted histogram analysis method (WHAM) was used to reconstruct the potential of mean force (PMF), providing a detailed energy landscape of β-sheet destabilization.

Figure 1: Prion Protein



Analysis & Structural Characterization

- Root mean square deviation (RMSD) was monitored to track structural deviations.
- Secondary structure evolution was analyzed to identify β-sheet disruption.
- Hydrogen bond occupancy was calculated to assess stability changes.
- Energy barriers for β-sheet unzipping were extracted from the PMF profile.
- Key intermediate conformations were identified to elucidate potential misfolding pathways.

By leveraging advanced sampling techniques, our study captures critical early-stage molecular events that may drive prion misfolding. These insights contribute to a deeper understanding of prion propagation mechanisms and may inform future therapeutic strategies.

RESULTS

The potential of mean force (PMF) profile, reconstructed using the Weighted Histogram Analysis Method (WHAM), reveals the free energy landscape of β-sheet destabilization. Figure 1 below shows the free energy as a function of the reaction coordinate, highlighting energy barriers associated with PrP unfolding.

The probability distribution of sampled conformations along the reaction coordinate further supports the presence of stable and intermediate states, which is shown in Figure 2.

Figure 2: Free Energy Curve

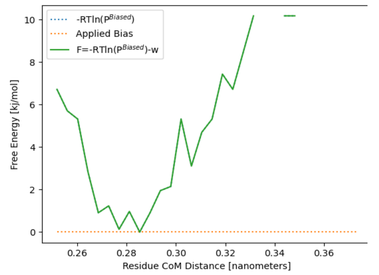
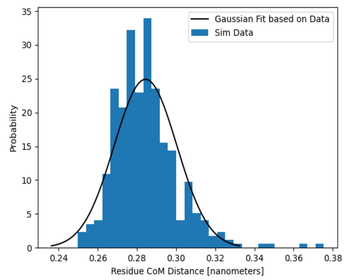


Figure 3: Probability Curve



Key Observations:

- A local minimum suggests a metastable intermediate.
- The highest energy barrier marks critical hydrogen bond disruption.
- The process appears cooperative, requiring significant energy.

Key Observations:

- The most probable state aligns with the native β-sheet.
- Intermediate peaks suggest transient unfolding states.
- A broader distribution at high values indicates flexibility.

CONCLUSION AND FUTURE WORK

Our study provides key insights into early prion misfolding by identifying intermediates and energy barriers associated with β-sheet destabilization. Using molecular dynamics simulations with umbrella sampling, we characterized structural transitions that may trigger pathological misfolding, offering a foundation for therapeutic strategies. Future work will explore the relationship between primary structure and stability, integrate AlphaFold to predict misfolded states, and incorporate intrinsically disordered protein (IDP) regions to better understand their role in misfolding, further refining our understanding of prion disease mechanisms and potential interventions.

ACKNOWLEDGEMENTS

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