Computational Analysis of Human Prion Protein's Allosteric Potential Reveals Potential Pharmacological Chaperone Binding Site for V189I and V203I PrP^{Sc}

Womack, Reagan M.
Dr. Joshua Ring, advisor
Department of Chemistry, Lenoir-Rhyne University

LENOIR-RHYNE UNIVERSITY

BACKGROUND

Prion Diseases

- formerly known as transmissible spongiform encephalopathies (TSEs)
- rare neurodegenerative diseases, classified by tissue deposition of misfolded isoform of cellular prion protein [1]
- most common human prion disease is Creutzfeldt-Jakob disease (CJD) [2]; other prion diseases include bovine spongiform encephalopathy (Mad Cow Disease) in cattle and scrapie in sheep

Prions: agents of prion diseases, occur when normal cellular prion protein (PrP^{C}) misfolds into abnormal isoform (PrP^{Sc}); current working theory of disease progression suggests prions attach to PrP^{C}, refold it into the abnormal isoform, and add to a growing amyloid fibril

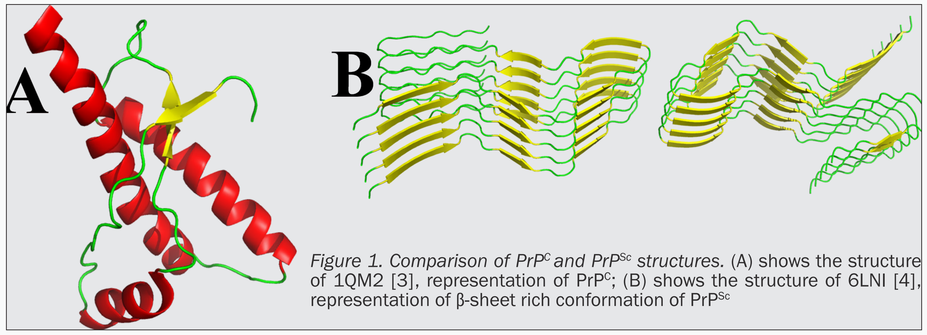


Figure 1. Comparison of PrP^{C} and PrP^{Sc} structures. (A) shows the structure of 1QM2 [3], representation of PrP^{C}; (B) shows the structure of 6LNI [4], representation of β-sheet rich conformation of PrP^{Sc}

Human Prion Protein

- structure consists of 3 α-helices and 2 β-sheets
- residues preceding non-conserved α2-α3 loop hypothesized to be region where misfold begins [5]
- folds under kinetic control (PrP^{C} is not the most stable conformation)
- can be divided into 2 distinct subdomains

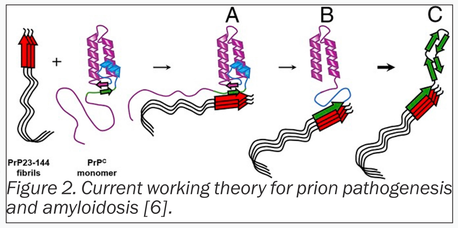


Figure 2. Current working theory for prion pathogenesis and amyloidosis [6].

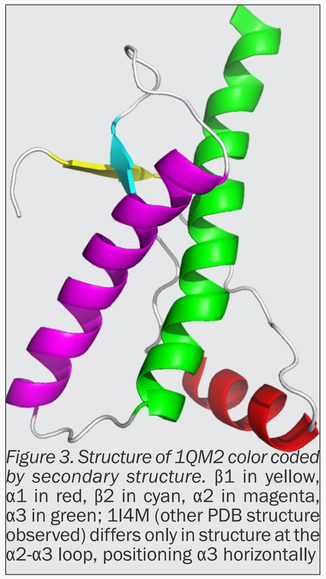


Figure 3. Structure of 1QM2 color coded by secondary structure. β1 in yellow, α1 in red, β2 in cyan, α2 in magenta, α3 in green; 1I4M (other PDB structure observed) differs only in structure at the α2-α3 loop, positioning α3 horizontally

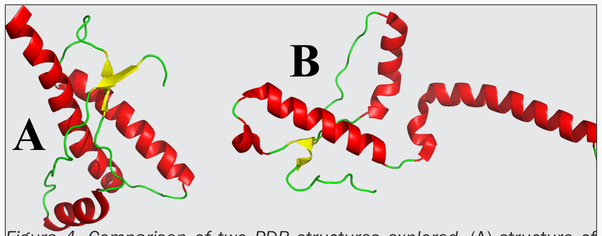


Figure 4. Comparison of two PDB structures explored. (A) structure of 1QM2, derived from solution NMR [3]; (B) structure of 1I4M, derived from x-ray diffraction [8]

Using Computational Models to Identify Potential Binding Sites for Pharmacological Chaperones

- models of allostery can be applied to non-allosteric proteins to identify residues that may be connected [7]
- many computational methods have been developed for exploring allosteric communication within a protein

RESULTS

V189I and V203I identified as mutations of interest (Eris Suite Webserver)

- infectious mutations aren't more destabilizing than non-infectious, also observed by Redler et. al [9]
- single factor ANOVA showed no significant difference in distributions, to be expected for a protein that folds under kinetic control
- no significant difference in ΔΔG distribution was found between two PDB structures used; proceeded to use 1QM2 for remainder of research
- V189I and V203I both had relatively positive ΔΔG values, indicating destabilization of PrP^{C}
- V189I and V203I have both been linked to sporadic Creutzfeld-Jakob Disease (sCJD)

Markov Transient Analysis reveals potential regulatory sites (ProteinLens Webserver)

- three potential regulatory sites were identified for each mutation residue set as the source site
- for residue 189, V180, G127, and Q172 identified as potential regulatory sites
- for residue 203, N197, G195, and D167 identified as potential regulatory sites
- residues identified showed strongest connectivity to source site, within 99% quantile of all residues on the protein

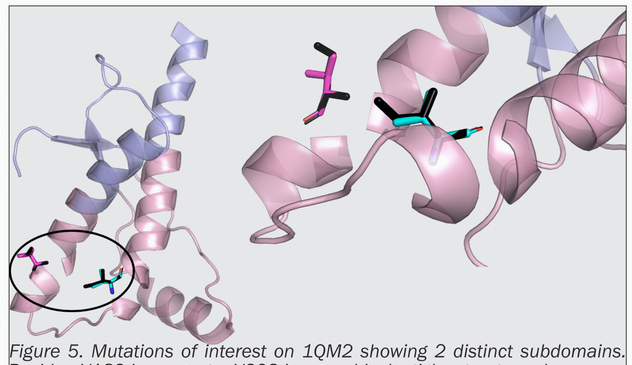


Figure 5. Mutations of interest on 1QM2 showing 2 distinct subdomains. Residue V189 in magenta, V203 in cyan; black sticks structure shows conformational change to isoleucine from mutation.

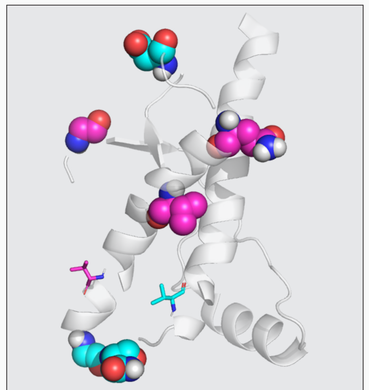
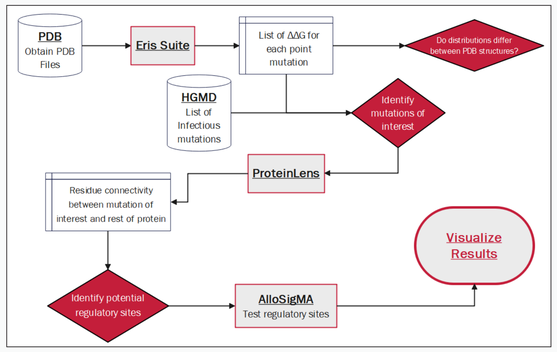


Figure 6. Potential regulatory sites for V189 (magenta) and V203 (cyan) identified through Markov transient analysis. Two residues identified for V203 are located on α2-α3 loop; other 4 residues are on opposite side of boundary plane from mutation sites



Proposed binding residues identified through simulated probe binding (AlloSigMA)

- small 3-residue probe was simulated binding to the proposed binding sites and the allosteric effect on mutation sites and hypothesized initiation site for misfolding [5] was recorded
- probing at residues 167, 195, and/or 197 showed constraining effect (Δg<0) on both mutation sites and α2-α3 loop and surrounding residues
- residues 167, 195, and 197 hypothesized to be potential binding sites for pharmacological chaperone molecules to stabilize the native conformation of PrP^{C}

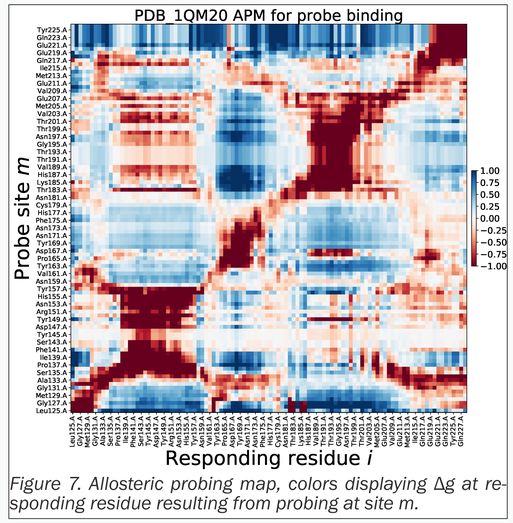


Figure 7. Allosteric probing map, colors displaying Δg at responding residue resulting from probing at site m.

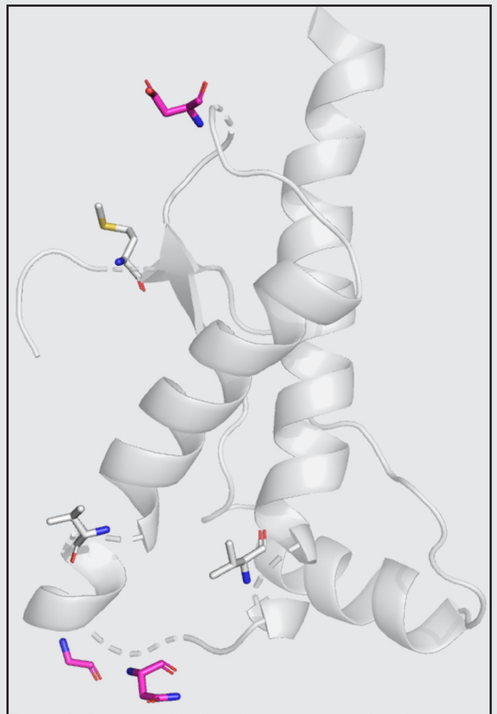


Figure 8. Proposed binding sites (magenta) for pharmacological chaperone molecules.

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REFERENCES

A full list of references can be found at linktr.ee/reaganwomack under "List of References."

GIT REPOSITORY

Scan the QR code below to access a Git repository with all data and script files used throughout this project.

More Information
Reagan M. Womack
reagan.womack@my.lr.edu
linktr.ee/reaganwomack