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Computational Studies of the Structural Stability of Rabbit Prion Protein Compared to Human and Mouse Prion Proteins

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1. Introduction

Prion diseases are invariably fatal and highly infectious neurodegenerative diseases affecting humans and animals. The neurodegenerative diseases such as Creutzfeldt-Jakob disease, variant Creutzfeldt-Jakob diseases, Gerstmann-Sträussler-Scheinker syndrome, Fatal Familial Insomnia, Kuru in humans, scrapie in sheep, bovine spongiform encephalopathy (or 'mad-cow' disease) and chronic wasting disease in cattle belong to prion diseases. By now there have not been some effective therapeutic approaches to treat all these prion diseases. In 2008, canine mammals including dogs (canis familials) were the first time academically reported to be resistant to prion diseases Polymenidou et al. (2008). Rabbits are the mammalian species known to be resistant to infection from prion diseases from other species Vorberg et al. (2003). Horses were reported to be resistant to prion diseases too Perez et al. (2010). By now all the NMR structures of dog, rabbit and horse prion proteins had been released into protein data bank respectively in 2005, 2007 and 2010 Lysek et al. (2005); Perez et al. (2010); Wen et al. (2010a). Thus, at this moment it is very worth studying the NMR molecular structures of horse, dog and rabbit prion proteins to obtain insights into their immunity prion diseases.

The author found that dog and horse prion proteins have stable molecular dynamical structures whether under neutral or low pH environments, but rabbit prion protein has stable molecular dynamical structures only under neutral pH environment. Under low pH environment, the stable α -helical molecular structures of rabbit prion protein collapse into β -sheet structures. This article focuses the studies on rabbit prion protein (within its C-terminal NMR, X-ray and Homology molecular structured region RaPrP^C (120-230)), compared with human and mouse prion proteins (HuPrP^C (125-228) and MoPrP^C (124-226) respectively). The author finds that some salt bridges contribute to the structural stability of rabbit prion protein under neutral pH environment.

As we all know, the disease infectious prions PrP^{Sc} are rich in β -sheets (about 43% β -sheet) Griffith (1967) and the normal cellular prions PrP^C are predominant in α -helices (42% α -helix, 3% β -sheet) Pan et al. (1993). Prion diseases are believed caused by the conversion of

normal PrP^C to abnormally folded PrP^{Sc}, and prion diseases are so-called protein 'structural conformational' diseases. Thus, we may study the molecular structures of prion proteins to obtain some insights of prion diseases. Human prion diseases include the Creutzfeldt-Jakob disease, variant Creutzfeldt-Jakob diseases, Gerstmann-Sträussler-Scheinker syndrome, Fatal Familial Insomnia, and Kuru. The NMR solution structure of the human prion protein (1QLX.pdb) was released into the PDB bank (www.pdb.org) in 1999 and last modified in 2009 Zahn et al. (2000). Mice are popular experimental laboratory animals and the NMR structure of mouse prion protein (1AG2.pdb) was released into the PDB bank in 1997 and last modified in 2009 Riek et al. (1996). Rabbits, dogs and horses were reported to be resistant to prion diseases Perez et al. (2010); Polymenidou et al. (2008); Vorberg et al. (2003) and by the end of 2010 their NMR structures (2FJ3.pdb, 1XYK.pdb, and 2KU4.pdb respectively) were completed to release into the PDB bank Lysek et al. (2005); Perez et al. (2010); Wen et al. (2010a). The X-ray structure of rabbit prion protein (3O79.pdb) was released into PDB bank in 2010 too Khan et al. (2010). At this moment it is very worth studying these molecular structures of horse, dog, rabbit, human and mouse prion proteins to reveal some secrets of prion diseases. The author found that dog and horse prion proteins have stable molecular dynamical structures whether under neutral or low pH environments Zhang and Liu (2011); Zhang (2011a), but rabbit prion protein has stable molecular dynamical structures only under neutral pH environment Zhang (2010); Zhang and Liu (2011); Zhang (2011a;b). Under low pH environment, among all the prion proteins above-mentioned only for the rabbit prion protein, its stable α -helical molecular structures collapse into β -sheet structures Zhang (2010); Zhang and Liu (2011); Zhang (2011a;b). The conversion of disease PrP^{Sc} from normal PrP^C is just involving 'conformational change' from predominantly α -helical protein to one rich in β -sheet structure. This article specially focuses on the rabbit prion protein to obtain some insights into the immunity of rabbits to prion diseases.

For the rabbit prion protein, we have its NMR and X-ray structures (2FJ3.pdb and 3O79.pdb respectively). Early in 2004, Epa Zhang et al. (2006) made molecular modeling of a homology structure (denoted 6EPA.pdb) for rabbit prion protein RaPrP^C(120-229), which was constructed using the NMR structure of the human prion protein (1QLX.pdb) as the template. Besides all these structures of rabbit prion protein, the knowledge on their conformational evolution/dynamics is considered essential to understand rabbit prion and the molecular modeling (MM) and molecular dynamics (MD) approach takes advantage beyond the experimental limit. In Section 2, this article first briefly reviews the main MD results of the homology MM structure Zhang et al. (2006) at 500 K and of the wild-type NMR structure at 450 K Zhang (2011b), compared with the MD of the wild-type NMR structures of human (HuPrP^C (125-228), 1QLX.pdb) and mouse (MoPrP^C (124-226), 1AG2.pdb) prion proteins. Section 3 will present the wild-type NMR rabbit, human and mouse MD comparisons at 350 K. Because the X-ray structure 3O79.pdb was produced differently from the NMR structure 2FJ3.pdb and the Homology structure 6EPA.pdb, we will not do their MD comparisons; however, in Section 4, we will give detailed sequence and structure alignment analysis of all these three rabbit prion structures compared with human and mouse wild-type structures. Section 5 gives some concluding remarks on rabbit prion protein and prion diseases.

2. MD reviews on the 500 K homology rabbit prion protein and the 450 K NMR rabbit prion protein

2.1 The homology rabbit prion protein at 500 K Zhang et al. (2006)

Zhang et al. Zhang et al. (2006) studied the MD of RaPrP^C(120-229) homology structure (6EPA.pdb). The MD simulations used Amber 8 Case et al. (2004) PMEMD program, with explicit water at different temperatures and pH values. The simulation conditions are listed in Table 1 of Zhang et al. (2006). The RMSD (root mean square deviation) and radius of gyration results are shown in Figs 2-3 of Zhang et al. (2006), where we may see that rabbit prion protein has more stable structural dynamical behavior compared to the human and mouse prion proteins at 500 K under neutral pH environment. This is also shown in Fig. 4 of Zhang et al. (2006) of snapshots for human, mouse and rabbit prion proteins at 5ns, 10ns, 15ns, 20ns, 25ns, and 30ns respectively. Fig. 4 of Zhang et al. (2006) shows that the helices of HuPrP and MoPrP were unfolded but RaPrP still keeps the helical structures at 500 K under neutral pH environment. The performance of RaPrP also shows that 500 K is a not a very bad temperature chosen for theoretical research for RaPrP. Under low pH environment at 500 K, these helical structures of RaPrP were unfolded. One of the reasons of the rabbit prion protein unfolding is due to the remove of the salt bridges such as N177-R163 (Fig. 5 of Zhang et al. (2006)). We may see in Fig. 5 of Zhang et al. (2006) the salt bridge / hydrogen bond between Arginine 163 and Aspartic acid 177 is conserved through a large part of the simulations and contributes to the protein stability of rabbit prion protein structure. Simulations at low pH value, where this salt bridge is absent, show RMSD and radius of gyration values for the rabbit prion protein to be of the same magnitude as the human and mouse prion proteins.

2.2 The NMR rabbit prion protein at 450 K Zhang (2011b)

Zhang Zhang (2011b) did the MD studies on the NMR rabbit prion protein RaPrP^C(124-228) (2FJ3.pdb) at 450 K under both neutral and low pH environments for the simulations of 20 ns. Zhang Zhang (2011b) found that "the secondary structures under low pH environment at 450 K have great differences between rabbit prion protein and human and mouse prion proteins: the α -helices of rabbit prion protein were completely unfolded and began to turn into β -sheets but those of human and mouse prion proteins were not changed very much. These results indicate the C-terminal region of RaPrP^C has lower thermostability than that of HuPrP^C and MoPrP^C. Under the low pH environment, the salt bridges such as D177-R163, D201-R155 were removed (thus the free energies of the salt bridges changed the thermostability) so that the structure nearby the central helices 1U3 was changed for rabbit prion protein" Zhang (2011b). The author continued his MD simulations for another 10 ns. The secondary structures for the MD simulations of 30 ns (Fig. 1) shows the same conclusion as that of Zhang (2011b). We may say that the salt bridges such as D177-R163, N201-R155 contribute to the structural stability of wild-type rabbit prion protein (Fig. 1). At 450 K, whether in neutral or in low pH environments, the α -helical secondary structures of dog prion protein have not changed for the long 30 ns' simulations Zhang and Liu (2011); at 350 K, horse prion protein has the same molecular structural dynamics Zhang (2011a) during the 30 ns' long simulations.

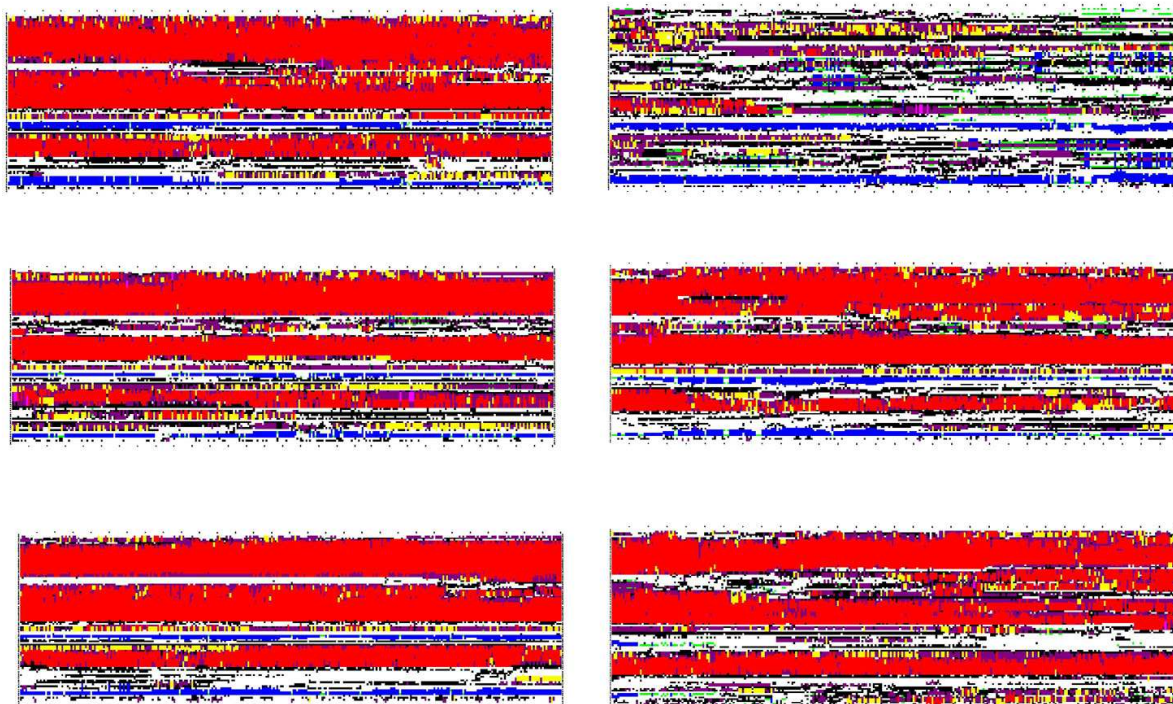


Fig. 1. Secondary structures of rabbit, human and mouse prion proteins (from up to down) at 450 K under neutral and low (from left to right) pH environments (red: α -helix, pink: π -helix, yellow: 3_{10} -helix, green: β -bridge, blue: β -sheet, purple: Turn, Black: Bend; x-axis: time (0-30 ns), y-axis: residue numbers).

3. 350 K

350 K might be a practical temperature for some experimental laboratory works. Zhang Zhang (2011c) did MD simulations for wild-type rabbit, dog and horse prion NMR structures at 350 K. The findings of 350 K are: “dog and horse prion proteins have stable molecular structures whether under neutral or low pH environments. Rabbit prion protein has been found having stable molecular structures under neutral pH environment, but without structural stability under low pH environment. Under low pH environment, the salt bridges such as D177-R163 were broken and caused the collapse of the stable α -helical molecular structures”. Here the MD simulations are done for wild-type human and mouse prion proteins in the use of the same Materials and Methods as in Zhang (2011c).

Seeing Fig. 2, we know that at 350 K human and mouse prion proteins have stable molecular structures whether under neutral or low pH environments.

Clearly the following salt bridges play an important role to the NMR structural stability of rabbit prion protein: (1) GLU210-ARG207-GLU206-LYS203 (99.78%, 88.85%, 82.74%, H3-H3), GLU210-HIS176 (74.31%, H3-H2), GLU206-HIS176 (57.10%, H3-H2), ARG207-HIS176 (0.52%, H3-H2), ASP177-ARG163 (19.54%, H2-S2); (2) ARG150-ASP146-ARG147-ASP143 (91.38%, 100%, 86.43%, H1-H1), HIS139-ARG150 (50.96%), HIS139-ASP146 (92.62%); (3) ASP201-ARG155 (10.07%, H3-H1), ASP201-ARG150 (2.61%, H3-H1), ASP201-ARG147

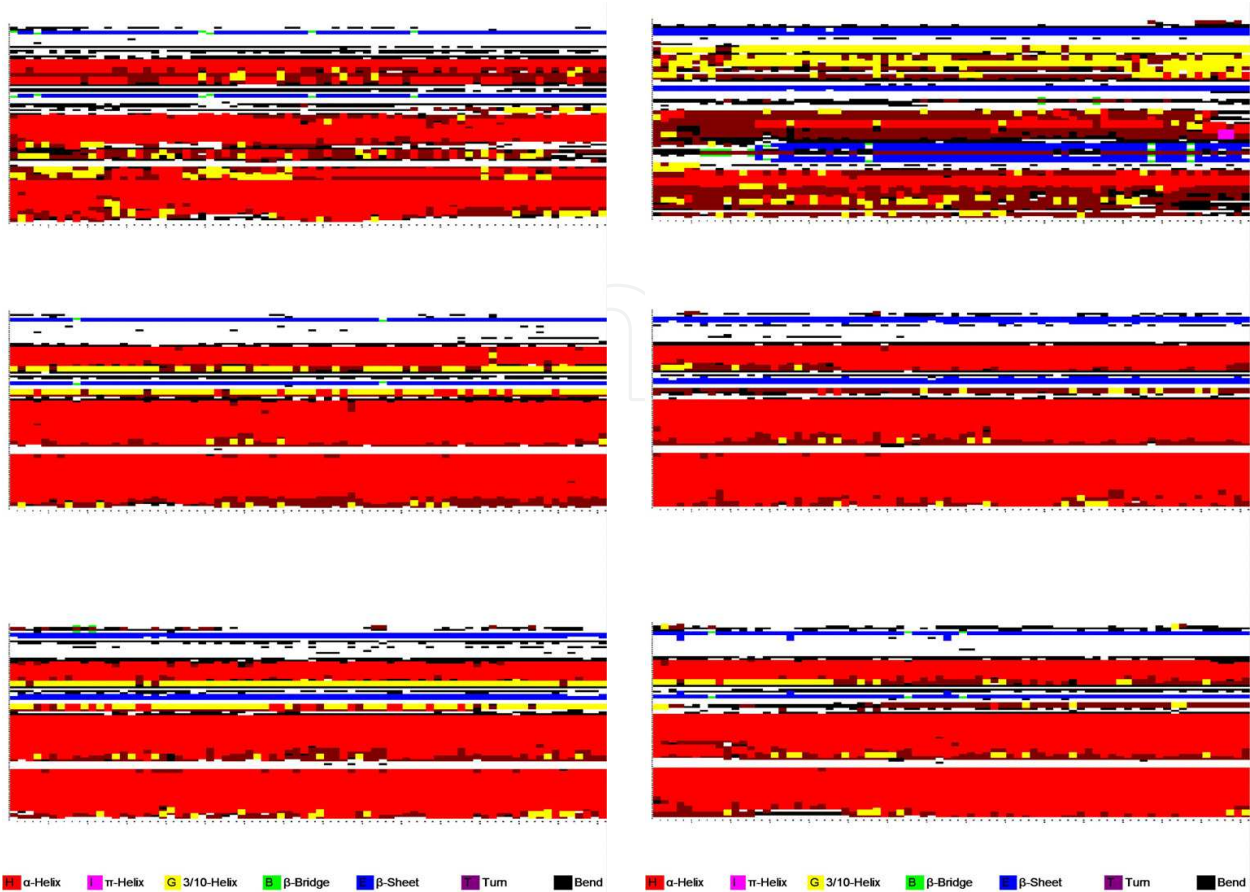


Fig. 2. Secondary structures of rabbit, human and mouse prion proteins (from up to down) at 350 K under neutral to low pH environments (from left to right) (X-axis: 0 ns - 30 ns (from left to right), Y-axis: residue numbers 124 - 228 / 125 - 228 / 124 - 226 (from up to down)).

(0.01%, H3-H1), ASP201-HIS186 (0.50%, H3-H2); and(4) ARG155-ASP201 (10.07%, H1-H3), TYR156-HIS186 (H1-H2, 71.69%), ARG155-GLU151 (20.70%, H1-H1), ARG155-GLU195 (0.06%), where H1, H2, H3 denote the α -helix 1, 2, 3 respectively, S1, S2 denote the β -strand 1 and 2 respectively, and '%' denotes the percentage during the whole simulation of 30 ns. Compared with human, mouse, dog and horse NMR prion proteins, rabbit NMR prion protein has some special salt bridges which contribute to its structural stability at 350 K during the simulation of 30 ns (Fig. 3) (human, mouse, dog and horse NMR prion proteins have not these salt bridges).

4. Alignment analyses

We make the sequence alignment of PrP from horse, dog, rabbit, human and mouse protein (Fig. 4).

In Fig. 4, "*" means that the residues in that column are identical in all sequences in the alignment, ":" means that conserved substitutions have been observed, "." means that semi-conserved substitutions are observed, the RED color takes place at small (small+ hydrophobic (incl.aromatic-Y)) residues, the BLUE color takes place at acidic residues, the MAGENTA color takes place at Basic-H residues, GREEN color takes place at

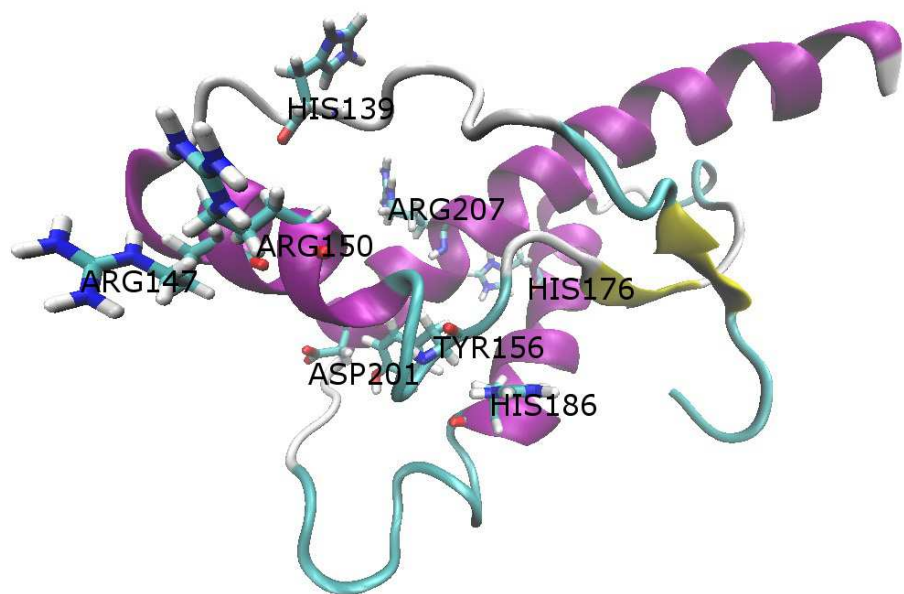


Fig. 3. Some special salt bridges ARG207-HIS176, TYR156-HIS186, HIS139-ARG150, ASP201-ARG147, ASP201-ARG150, ASP201-HIS186, ARG155-GLU151 of wild-type NMR rabbit prion protein at 350 K.

Hydroxyl+sulphydryl+amine+G residues, and Grey color takes place at unusual amino/imino acids etc.. For the structural domain, in Fig. 4 we can see some special residues listed in Table 1 for horse, dog, human and mouse prion proteins, which might contribute to characters of each structure respectively. Rabbits differ from horses, dogs, humans and mice at: S173 (N174

Horse	S167 (others are D), Y222 (others are S), Q226 (others are Y), V241 (others are I), F245 (others are S)
Dog	L129 (others are M), S165 (others are P), N170 (others are S), S173 (others are N), V244 (others are I)
Human	I138 (immunities are L), S143 (others are N), H155 (others are Y), M166 (others are V), I183 (immunities are V), E219 (others are Q), S230 (immunities are A)
Mouse	I183 (immunities are V), V215 (others are I), D217 (others are Q), S230 (immunities are A)

Table 1. Alignment analysis of special residues for HoPrP, DoPrP, HuPrP, and MoPrP.

for horse, T174 for dogs, N174 for humans and mice), Q219 (K220 for horses and humans, R220 for dogs and mice), A224 (F225 for horses, Y225 for dogs, humans and mice), L232 (I233 for dogs, V233 for horse, humans and mice), and G228 (others are S229). For rabbits, at positions 89 and 97 the residues are special from all others (G89 (others are N90), S97 (others are N98)). These special residues are illuminated in Fig. 5. Some recent researches are focusing on the loop between β 2 and α 2, i.e. PrP(164-171) Apostol et al. (2011); Fernandez-Funez et al. (2011); Khan et al. (2010); Perez et al. (2010); Wen et al. (2010a;b); we may see in Fig. 5 that the immune animals horses, dogs and rabbits have some residues in this loop different from humans and mice.

Lastly, we illuminate the figure (Fig. 6) of rabbit prion protein, including the homology, NMR and X-ray structures (6EPA.pdb, 2FJ3.pdb, and 3O79.pdb respectively). We superpose the homology structure onto the NMR structure and find the RMSD value is 3.2031669 angstroms.

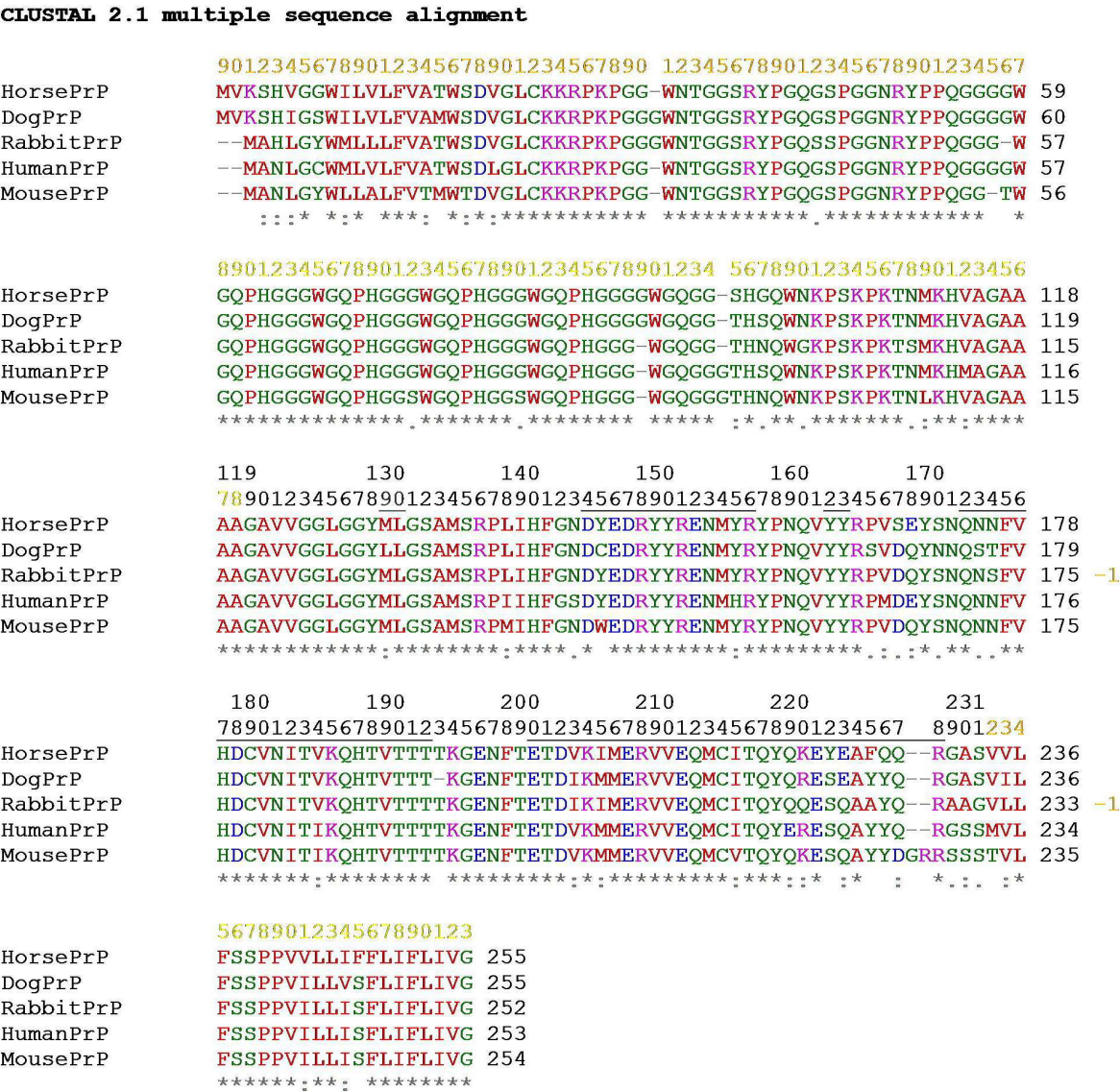


Fig. 4. Horse, dog, rabbit, human and mouse prion protein sequence alignment.

Similarly, we superpose the X-ray structure onto the NMR structure and we get their RMSD value is 2.7918559 angstroms. This implies the homology structure 6EPA.pdb made in 2004 by Epa Zhang et al. (2006) is as effective as the X-ray structure 3O79.pdb released recently on date 2010-11-24 (last modified on 2011-02-02).

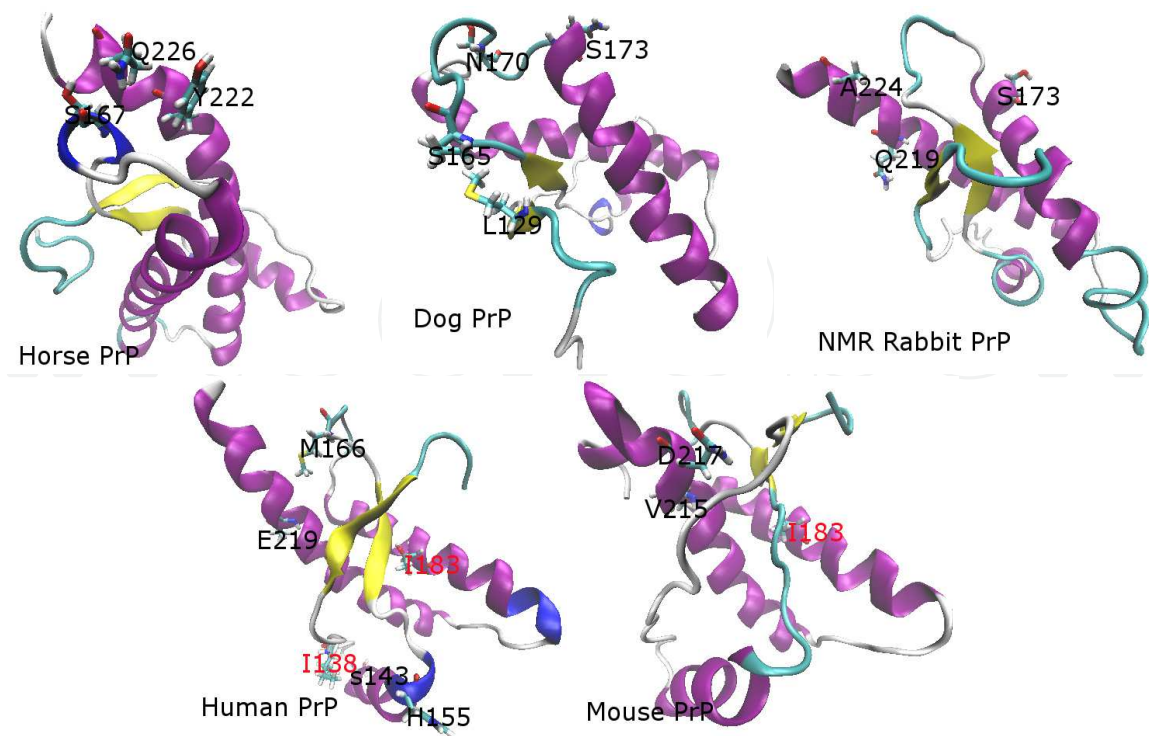


Fig. 5. Special residues owned only by HoPrP, DoPrP, RaPrP, HuPrP, and MoPrP respectively.

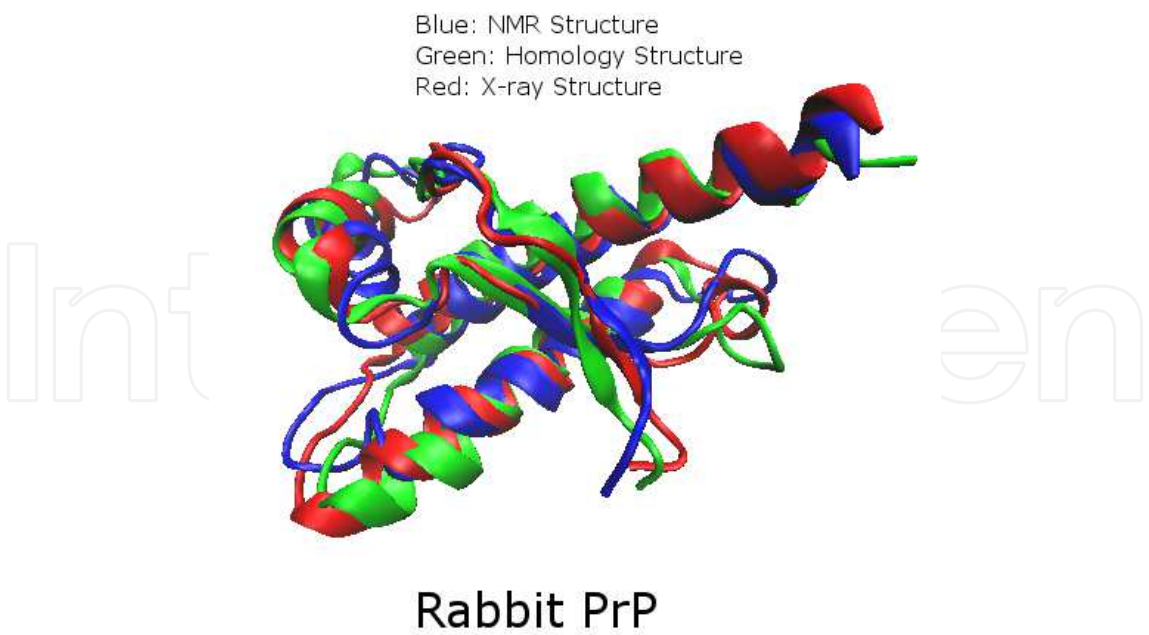


Fig. 6. Rabbit prion protein NMR, homology and X-ray structures (2FJ3.pdb, 6EPA.pdb, and 3O79.pdb).

5. Conclusion

To really reveal the secrets of prion diseases is very hard. Prion proteins have two regions: unstructured region and structured region. Rabbits, horses, and dogs were reported having immunity to prion diseases. Fortunately, by the end of 2010 all the NMR molecular structures of rabbit, horse, and dog prion proteins had been released into PDB bank already; for rabbit prion protein, its X-ray structure was also released into PDB bank in the end of 2010. Prion diseases are 'structural conformational' diseases. This paper timely presents a clue to reveal some secrets in the view of the dynamics of prion molecular structures. MD results of the author nearly in the passing 10 years show to us a common conclusion: under low pH environment at many levels of temperatures with different starting MD velocities, rabbit prion protein always unfolds its α -helical structures into β -sheet structures. Prion diseases are just caused by the conversion from predominant α -helices of PrP^C into rich β -sheets of PrP^{Sc}. Hence, we should furthermore study rabbits, horses and dogs, compared with humans and mice in order to reveal some secrets of prion diseases; for us, it is a long shot but certainly worth pursuing.

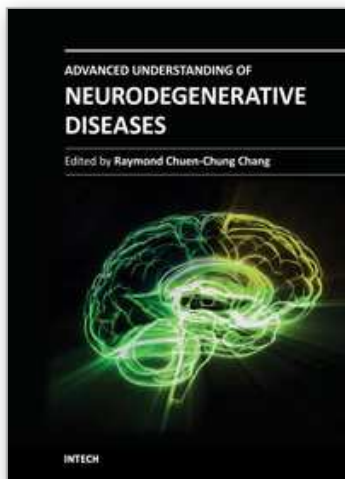
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Advanced Understanding of Neurodegenerative Diseases

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Advanced Understanding of Neurodegenerative Diseases focuses on different types of diseases, including Alzheimer's disease, frontotemporal dementia, different tauopathies, Parkinson's disease, prion disease, motor neuron diseases such as multiple sclerosis and spinal muscular atrophy. This book provides a clear explanation of different neurodegenerative diseases with new concepts of understand the etiology, pathological mechanisms, drug screening methodology and new therapeutic interventions. Other chapters discuss how hormones and health food supplements affect disease progression of neurodegenerative diseases. From a more technical point of view, some chapters deal with the aggregation of prion proteins in prion diseases. An additional chapter to discuss application of stem cells. This book is suitable for different readers: college students can use it as a textbook; researchers in academic institutions and pharmaceutical companies can take it as updated research information; health care professionals can take it as a reference book, even patients' families, relatives and friends can take it as a good basis to understand neurodegenerative diseases.

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