Binding Mechanisms of Metal Ions in Prion Proteins

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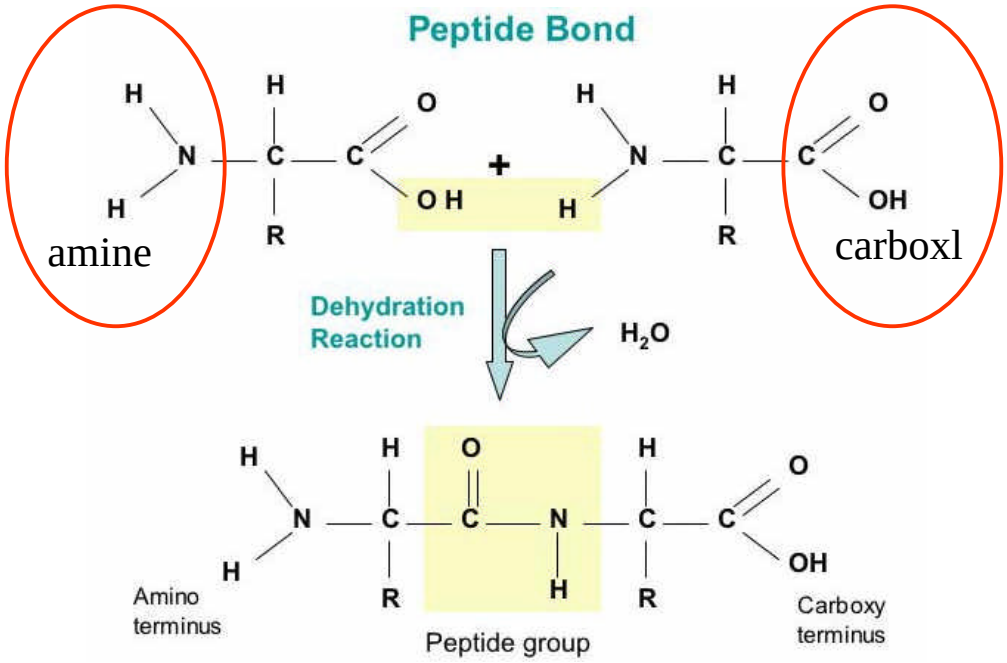
Amino acids: building blocks of proteins



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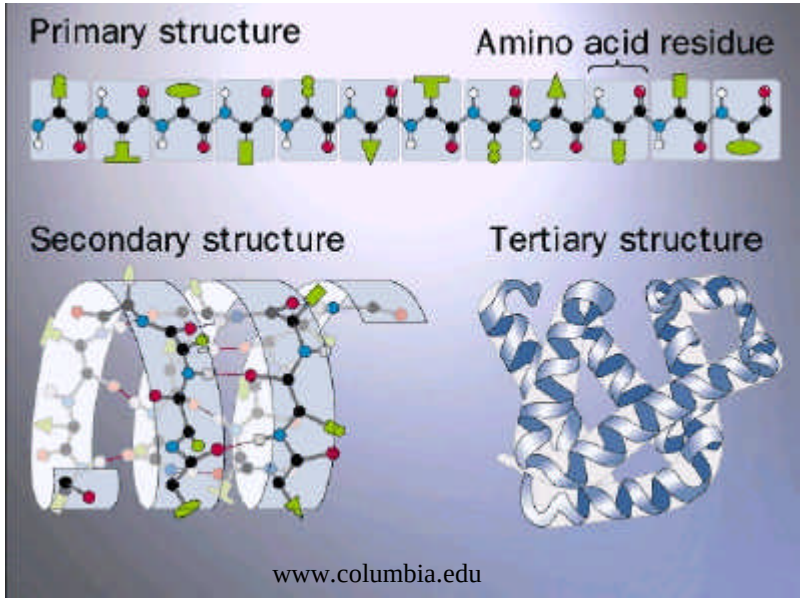
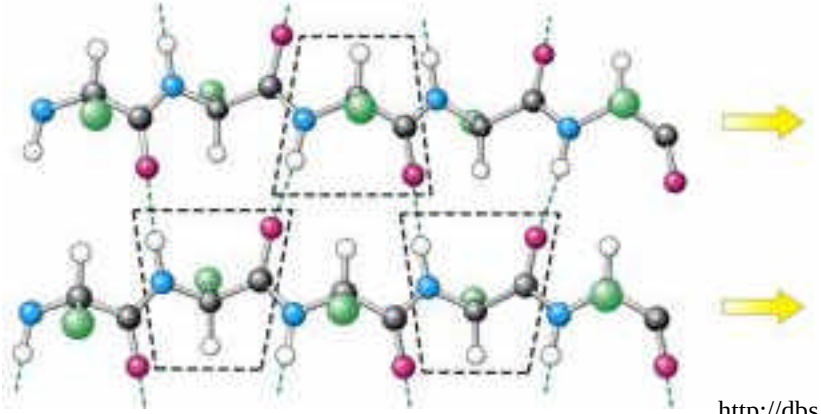
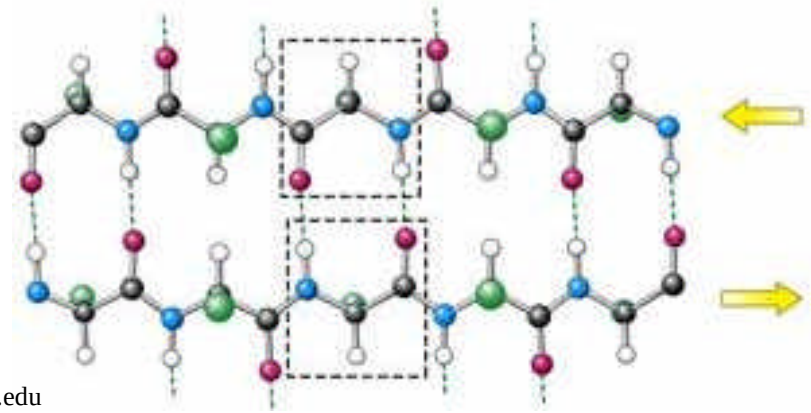
α-helix



Parallel β-sheet

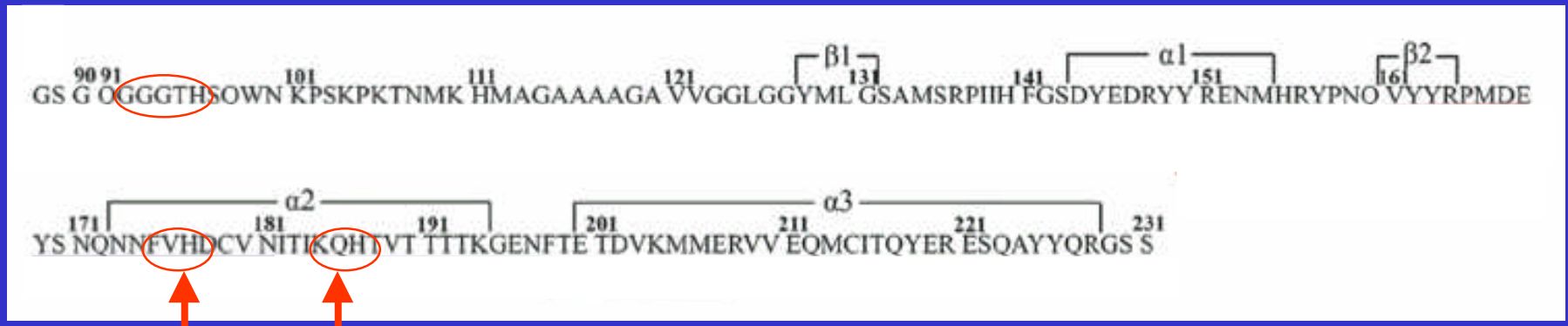


Anti-parallel β-sheet



http://dbs.umt.edu

PrP^C 88-231 primary structure



Currently exploring Cu^{2+} binding by histidine and neighbor amino acids in C-terminal region of prion fibrils.

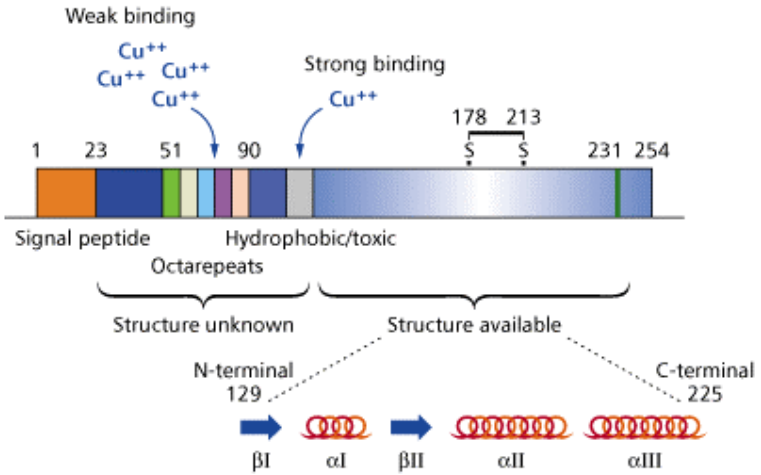


Fig. from chembytes e-zine website http://www.chemsoc.org/chembytes/ezine/2002/jones_apr02.htm

Why?

- Breakdown of metal homeostasis as key factor in many neurodegenerative diseases.
- Debate about whether binding of metals plays a neuroprotective or neurodegenerative role in disease.

Cu^{2+} binding could inhibit conformational change associated with diseased form of PrP (PrP^{Sc})

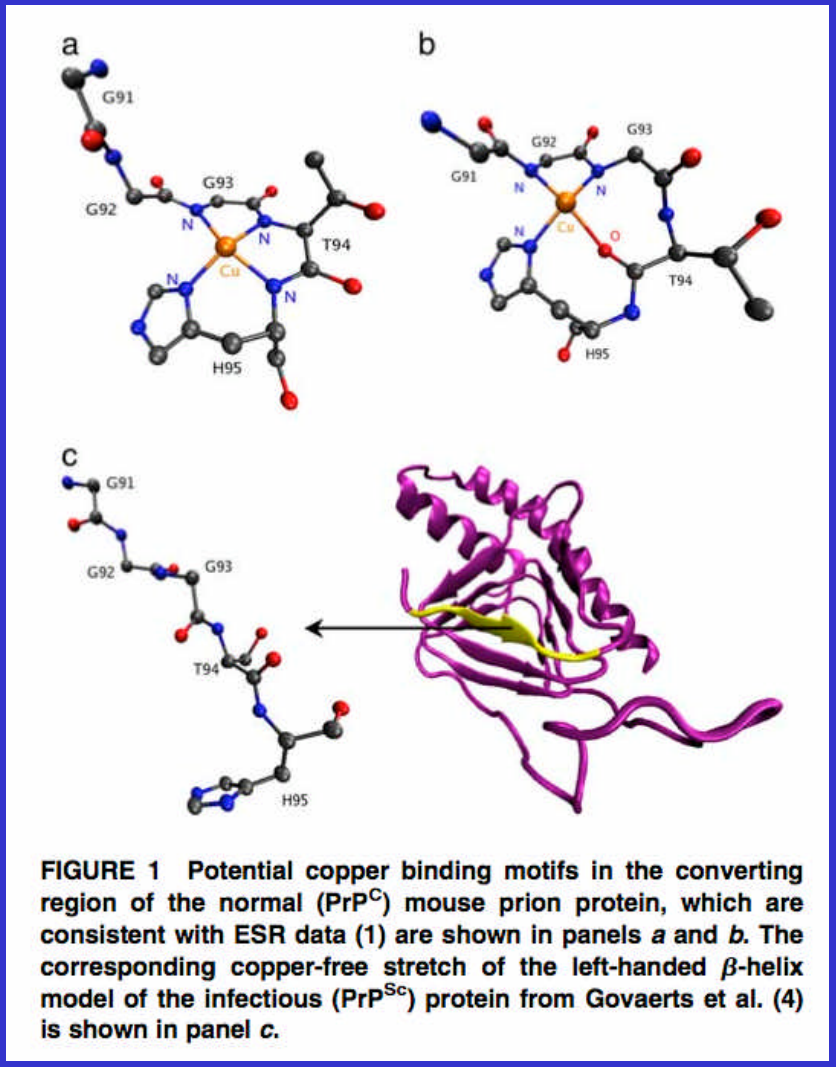
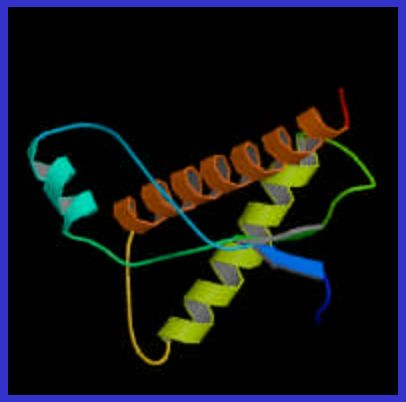


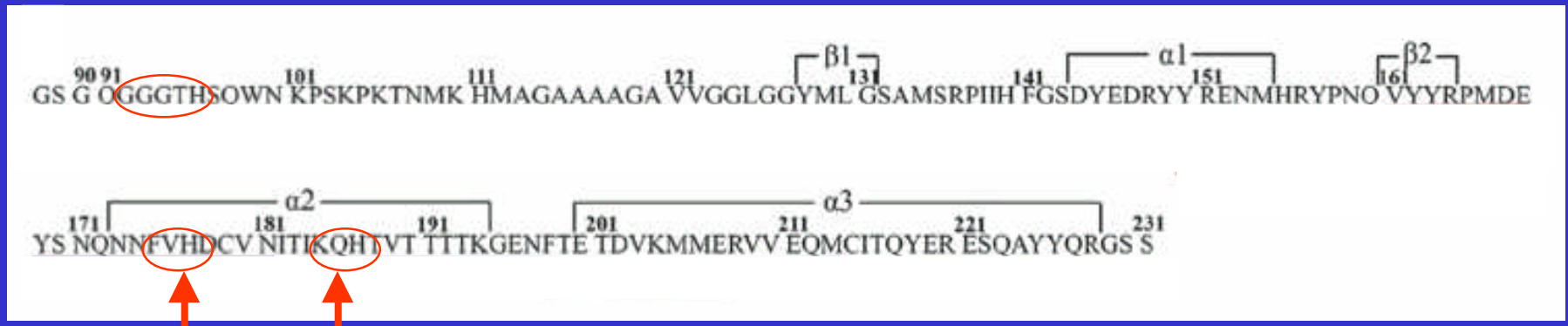
FIGURE 1 Potential copper binding motifs in the converting region of the normal (PrP^{C}) mouse prion protein, which are consistent with ESR data (1) are shown in panels *a* and *b*. The corresponding copper-free stretch of the left-handed β-helix model of the infectious (PrP^{Sc}) protein from Govaerts et al. (4) is shown in panel *c*.



Difference between PrP^{C} and PrP^{Sc} is *conformational*

Calculations by D. Cox, J. Pan and R. Singh predict structural change when Cu^{2+} binds to core region (sequence 92-96 GGGTH) of PrP^{C}. Bending is ***not*** compatible with the straight β-strand backbone structure associated with PrP^{Sc}.

PrP^C 88-231 primary structure



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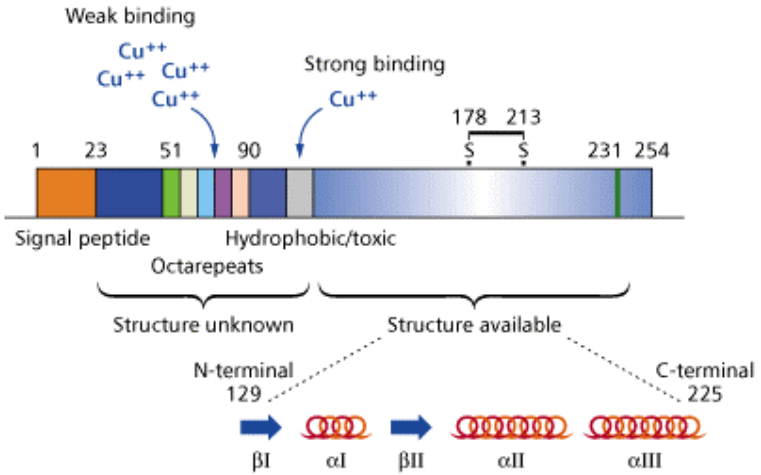

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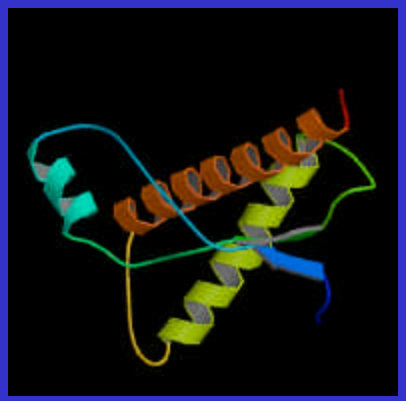
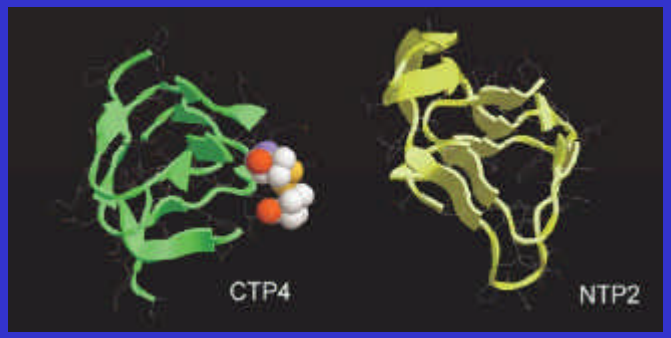
Cu^{2+} *will NOT* bind to α-helical structure of PrP.

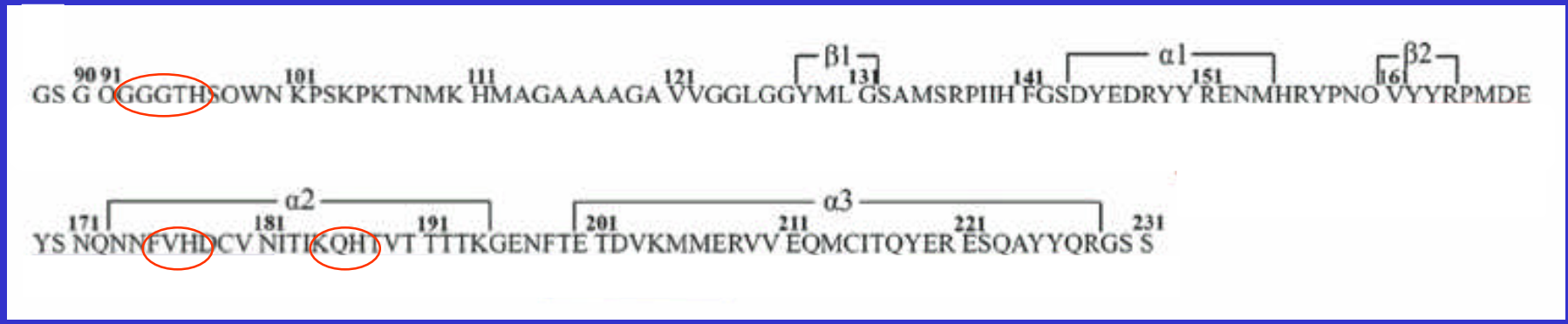
C-terminal & N-terminal left handed β–Helix (LHβH) models for prion fibril.



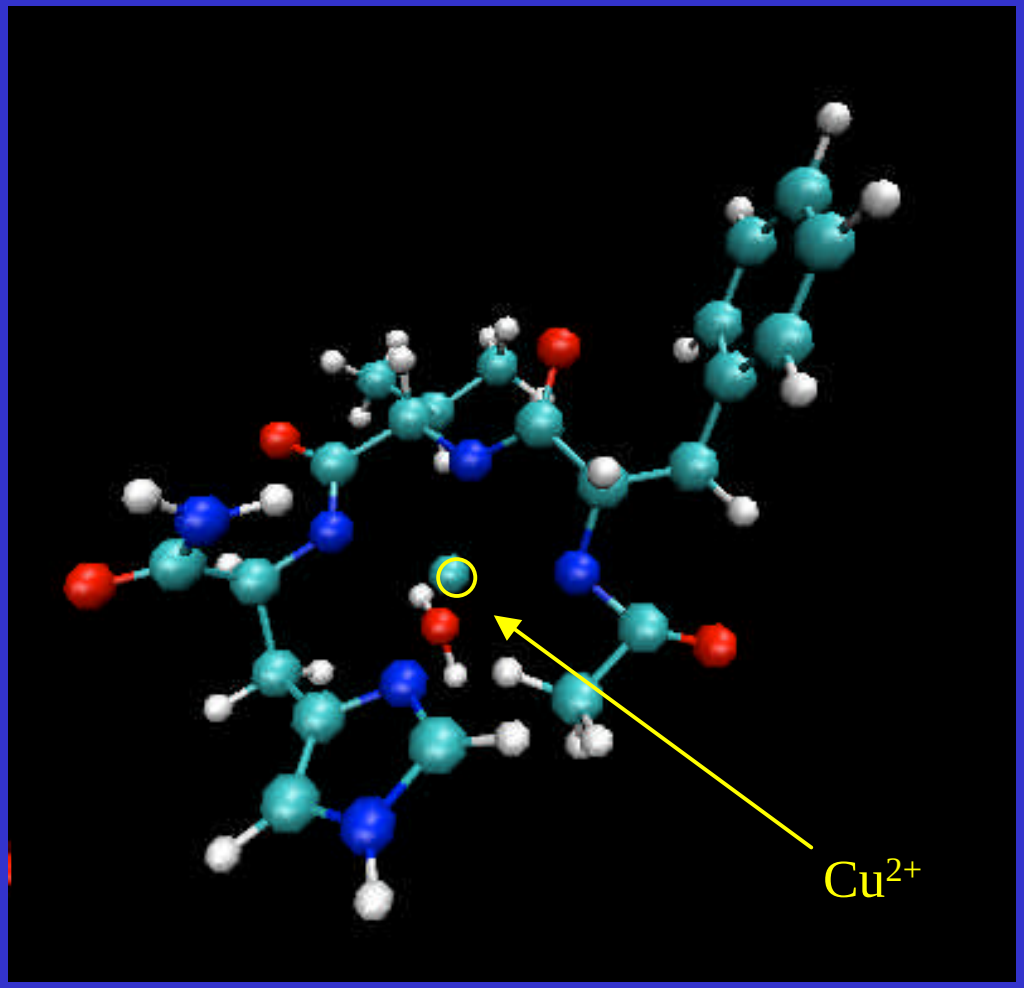


Conversion of PrP to amyloid fibrils involves disruption of α-helices *enabling Cu^{2+} binding at this stage* or refolding to β-structure.

Sequence 175-177 FVH in human PrP^C



Candidate structure for sequence 175-177 FVH: Cu^{2+} coordination by N atoms of HFV backbone, H side chain and O atoms of H_2O.



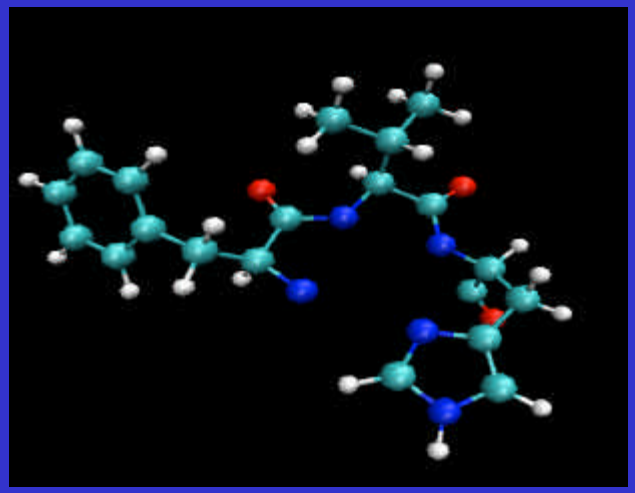
Candidate structures created using visualization software (e.g. VMD, PyMol, Swiss-PDB).

Local structure (geometry) minimized using quantum mechanical -Density Functional Theory calculations (SIESTA).

Goal: estimate energetics of Cu-PrP binding

($E_{binding} = E_{complex} - E_{fragment}$)

Peptide (FVH) fragment



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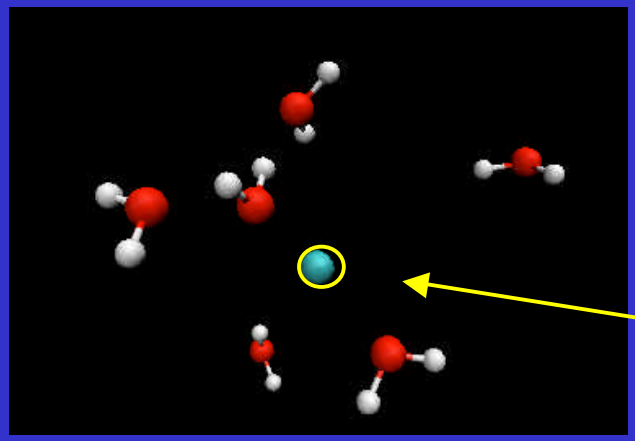
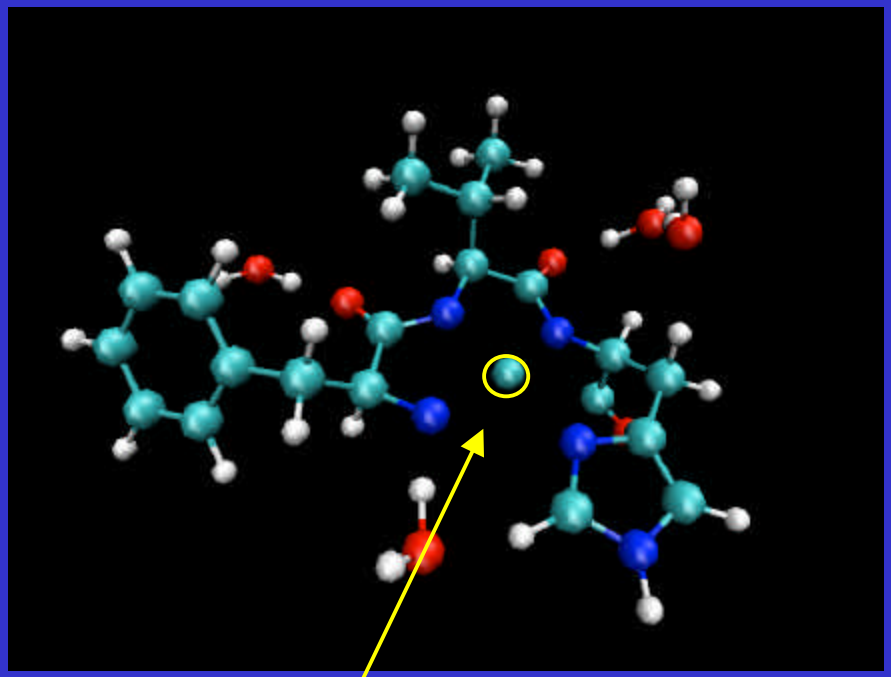


Cu^{2+}- H_2O fragment

Complex



Cu^{2+}

Which calculations will predict binding?

Studied PrP^C sequence 92-96 GGGTH, known experimentally to be a strong Cu^{2+} binding site:

- Molecular Dynamics (MD) calculations alone (using implicit solvent) DO NOT predict Cu^{2+} binding .
- Quantum Mechanical (QM) calculations (in vacuum) predict unphysically large binding energies.

Same outcome for the sequence 175-177 FVH.

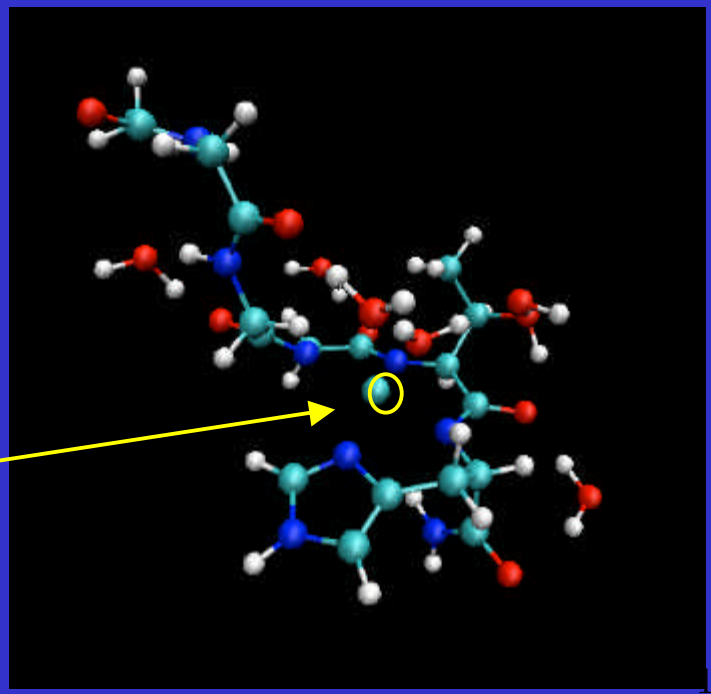
Embedded QM calculations in MD simulations using the Generalized Born (GB) approximation as implicit solvent.

Energies from QM calculations:

- Electrostatic energy (Coulomb interactions) between each atom of the system, which include exchange and correlation interactions between electrons.
- Total kinetic energy.

Energies from MD simulations:

- Van der Waals interactions.
- Solvation energy.



Cu^{2+}

Cu^{2+}- GGGTH - H_2O complex

Results obtained by embedding QM calculations in MD simulations

PrP sequence 92-96 GGGTH:

Binding energies of about 2.4 eV.

PrP sequence 175-177 FVH:

Binding energies of about 3.0 eV.

Prion protein sequence 175-177 FVH predicted to bind Cu^{2+} at least as strongly as the 92-96 GGGTH region.

More results

Explored the binding affinity of other transition metal ions to HGGGW of the octarepeat region of PrP:

Cu^{2+}: 1.8 eV

Ni^{2+}: 1.6 eV

Zn^{2+}: 1.3 eV

Mn^{2+}: Non-binding.

Follows trend observed experimentally, however…

Some unresolved issues:

- Lack of good force-field parameters for most transition metals.
- Na^{+}, Cl^{-} ions included in MD simulation may generate environment that differs from physiological salt concentrations in the brain.

Future work

- Explore other possible metal ion binding sites of the prion protein.
- Study Cu^{2+} binding to multiple His residues, as opposed to isolated binding motifs, to different regions of the PrP.
- Investigate both isolated and cooperative transition metal ion binding to proteins associated with other neurodegenerative diseases, e.g., amyloid-β (Alzheimer's Disease), α-synuclein (Parkinson's Disease).

Supplementary Slides

References

- D. L. Cox, J Pan and R. R. P. Singh, *Biophys. Lett.* ***91*** *L11* - L13 (2006).
- C. S. Burns et. al., *Biochemistry* **42**, 6794 - 6803 (2003)
- C. Govaerts, C. H. Wille, S. B. Prusiner and F. E. Cohen, *Proc. Natl. Acad. Sci. USA* **101**:8342 - 8347 (2004)

Summary of Calculation Method

1. Construction of candidate structure for the complex and each fragment.
2. QM calculations for the complex and each fragment. Obtain minimized local structure, electrostatic potential and QM energy contribution.
3. Create parameters for the Cu^{2+} bound residues and metal ion. Need atom-centered point charges for the complex and peptide fragment (derived by fitting electrostatic potential).
4. MD simulations for the complex and each fragment. Obtain non-bonded interactions and energies of solvation.
5. Calculate the total energy of the complex and each fragment by adding QM and MD contributions.
6. Estimate binding energy:

$$E_{binding} = E_{complex} - E_{fragments}$$

Construction of candidate structures:

Visualization software (VMD, PyMol, Swiss-PDB)

Quantum mechanical calculations:

Kohn-Sham self-consistent density functional method in the local density or generalized gradient approximation (SIESTA implementation).

- Geometry relaxation, total and partial energies, atomic forces, electron densities.

Molecular dynamics calculations:

Package of molecular simulation programs (AMBER)

- Molecular mechanics, normal mode analysis, molecular dynamics and free energy calculations to elucidate the structures and energies of molecules.

Amino Acids Cheat Sheet