A Density Functional Approach to Iron-Binding in Prions

Danielle Marie McDermott
UC Davis, REU Project

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The prion protein has been implicated in numerous neurodegenerative diseases. The function and behavior of this protein, in both its normal and infective states, is subject to much debate within the scientific community. Experimental, *in vitro*, results indicate a high-binding affinity of Cu^{2+} to a normal prion, PrP^{C}. Previous *ab initio* work examined the binding of the octarepeat segment of PrP^{C} to various metal ions, and witnessed a trend of binding energies that agreed with both *in vitro* results and the theoretical Irving-William series. Fe^{2+} was predicted to fall between $E_B(Ni^{2+})$ and $E_B(Mn^{2+})$ in this work. Utilizing density functional theory (DFT) via the SIESTA code, the binding energy of the iron-prion system was calculated. The result broke the trend, falling between $E_B(Cu^{2+})$ and $E_B(Ni^{2+})$. Further work consequently will involve a repeat of the SIESTA calculations with more accurate pseudopotentials (implements of the code). It is yet to be determined whether the observed high-binding affinity of iron is repeatable, and could have interesting implications in the involvement of ferritin in the co-transport of PrP^{Sc} across epithelial cells that line the stomach.

I. Introduction

A great deal of scientific research has focused on prions, or *proteinacious-infectious agents*, over the last several decades. The "protein-only" hypothesis describes the onset of certain neurodegenerative diseases such as scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and Creutzfeldt-Jakob Disease (CJD) in humans in which infectious proteins are folded differently then their normally functioning counterparts. These 'misfolded' proteins interact with and convert other proteins to an infectious state. The observation of PrP (prion-related protein) in the normal form of PrP^{C} and infectious form of PrP^{Sc} brought the notion of prion diseases to serious investigation. In recent years this hypothesis of misfolded protein infection has been used to describe other neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases. All of these diseases show evidence of aggregations of the infectious prion in plaques in the brains of deceased patients.

Prion proteins are anchored to neurons by their C-terminal. About half of the protein is unstructured in extracellular material, and this unstructured region contains a sequence of repeating amino acids PHGGGWGQ called the octarepeat region. This region is believed to be important in normal functioning of PrP^{C} because it is found in all mammalian prions.

Metal binding to prions has become increasingly interesting due to experimental examinations of levels of metal concentrations in

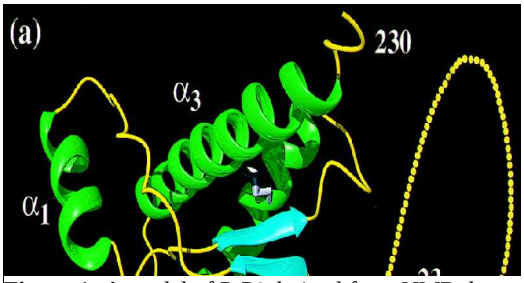

Figure 1: A model of PrP^{C} derived from NMR data. The octarepeat region is on the right hand side of the figure, falling between 23 & 121 in the unstructured region of the prion.

diseased brains. An *in vitro* (test tube) study looked specifically at the binding of trace metals to the prion, and observed the octarepeat region to be a metal binding site.

II. Density Functional Theory and the SIESTA Code

Density Functional Theory (DFT) is a method that casts the multi-electron Schrodinger equation in terms of single electron wavefunctions through the use of Slater Determinants. It depends on finite eigenfunctions to compose the basis set and casts everything in terms of matrices in order to solve for eigenvalues. Each Slater determinant represents a different electronic configuration, or filling of shells. Once the initial configuration is chosen, the total energy is chosen in terms of a Hamiltonian. The variational principle is used to determine the ground state of the system: where the total energy is changed with respect to electron density, and the minimum value is chosen. Essentially the electrons are treated in an average way, in a local approximation, and reduced to a collection of single-electron wavefunctions.

SIESTA Computational Details

(Ref: www.uam.es/departamentos/ciencias/fismateriac/siesta/)

ab initio code SIESTA by *Ordejón, Artacho, Soler et al:*

1. Pseudopotential
   - Troullier-Martins (norm-conserving)
2. Basis set
   - double-ζ with polarization orbitals (DZP) for metals (Cu, Ni, Zn, Mn) and double-ζ for other elements H, O, C, N
3. Exchange-Correlation Energy Functional
   - Generalized gradients approximation (GGA) by Perdew, Burke and Ernzerhof
4. Used Parameters:
   - Force-tolerance 0.02 eV/Ang in CG (Coordinate optimization by conjugate gradients)
   - Finite electronic temperature $k_B T = 0.01eV$
   - Mesh cutoff > 120 Ry

Figure 2: SIESTA computational details

The SIESTA code is available online, and uses DFT to calculate the coordinates and total energy of a system (and many other useful things not involved with this study). The initial inputs to the code are a geometry given by x-ray data of copper ions binding to the octarepeat region of a prion. The details of the inputs to SIESTA are detailed in the figure.

Because these calculations are largely concerned with the binding that occurs, a pseudopotential is a useful and valid approximation. The idea is to use a function that is less computationally expensive to approximate the wavefunction and potential of an atom. Outside of a chosen core radius, the function matches the original function. Choosing a small core radius is more accurate, and thus more expensive, than a larger core radius.

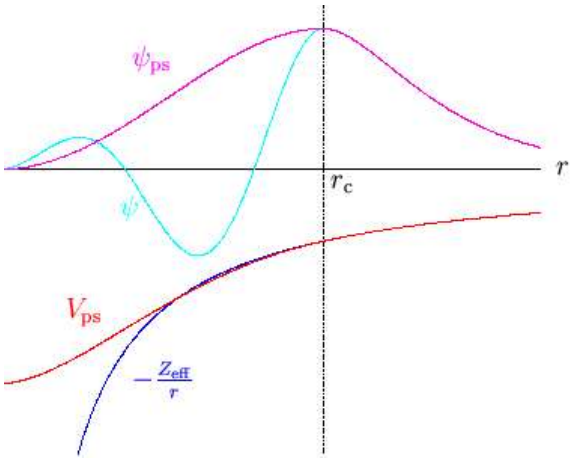

Figure 3: A pseudopotential example

III. Experimental Procedures

The PrP^C protein is expressed in a wide variety of vertebrates, and its structure and function are still under a great deal of scrutiny. How the protein "misfolds" into PrP^{Sc} remains a serious area of contention among the scientific community. Previously *ab initio* (first principle) calculations were performed to examine the binding energy of trace metals to known binding sites of the normal prion protein. The calculation was performed utilizing the SIESTA code employing density functional theory (DFT). The initial inputs to the code came from x-ray data studying the binding of Cu^{2+} to PrP^C, and were altered for systems involving Mn^{2+}, Zn^{2+}, and Ni^{2+}. To test different metals, the number of protons and spin was altered in the input of the code. The resulting trend agreed with *in vitro* outcome and seemed to agree with the predictions of Jahn-Teller theory and the Irving-William series. These methods were applied to the Fe^{2+}-prion system with the expectation that the binding energy would follow the trend predicted in the Irving-William series.

The intent was to put the information into SIESTA in order to calculate the binding energy of the system. The original input to the SIESTA code came from x-ray data of Cu^{2+} binding to the octarepeat region. SIESTA essentially disturbs the system, and calculates energy and the energy gradient. It adjusts to find the lowest energy of the system and runs through self-consistency loops to ensure the density put into the code matches with the density that comes out. To get out the binding energy, the total system was run through SIESTA: the section of the prion (HGGGW), 6 water molecules, and the metal ion into SIESTA. This same procedure was applied to a system involving merely the water molecules and metal ion. The third system involved merely $HGGGW^{2-}$. To calculate the binding energy, the difference was taken between the total system energy, and the system energies of the smaller systems.

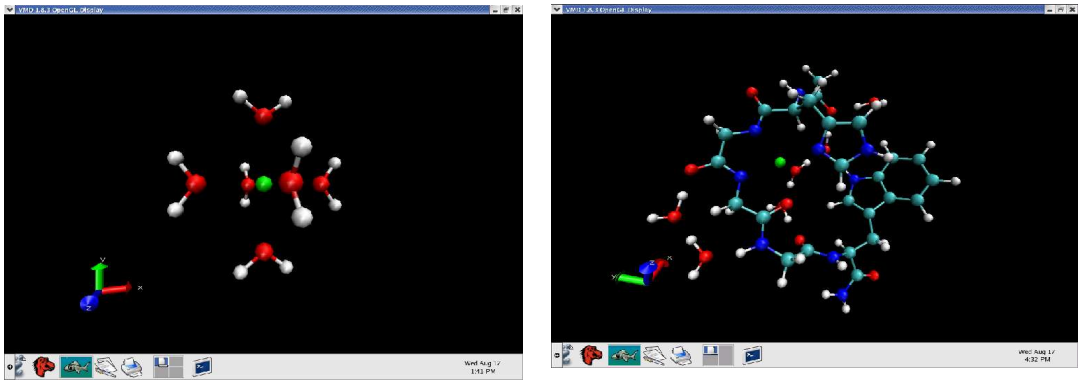

Figure 4: (a) Six water molecules and Fe^{2+} in the groundstate calculated by SIESTA. (b) The total system comprised of the prion backbone $HGGGW^{2-}$, 6 H_2O, and Fe^{2+} also in the groundstate.

The binding energy of the Iron-Prion system was predicted to correspond to the Irving-Williams series and fall between the binding energies of Ni^{2+} and Mn^{2+}. Previous calculations had been performed for Cu^{2+}, Ni^{2+}, Mn^{2+}, and Zn^{2+}. All of these metals had followed a trend predicted by the Irving-Williams series: $E_B(Cu^{2+}) >> E_B(Ni^{2+}) > E_B(Zn^{2+}) >> E_B(Mn^{2+})$, which agreed with the experimental data of the study which yielded the x-ray coordinates to input in SIESTA.

IV. Conclusion

The resulting binding energy of the SIESTA code fell between the binding energies of Cu^{2+} and Ni^{2+}. It is not necessarily satisfying that the trend was violated, but it may have interesting implications. Iron was added to this research initially because of an *in vitro* study revealing the involvement of ferritin (an iron storage protein) in binding to the scrapie prion and transporting across the epithelial walls of the stomach. A major research question centered looks to understand how the infected prions make their way to the brain. A high binding affinity of iron may be an important component in that study. Before a conclusive result is determined, the tests will be run again utilizing greater accuracy in the SIESTA code. The new runs will involve more accurate pseudopotentials that were developed and tested after the iron calculation was completed, a higher meshcutoff, and ungodly hours of calculations.

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The SIESTA Manual