How Hydrogen Bond Redundancy Affects Protein Flexibility

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Abstract # 387

Introduction

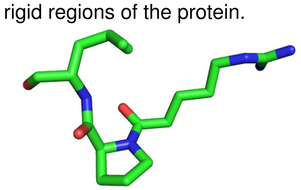
Main Question: Stability in proteins is the resistance to denaturation, or unfolding. A protein that is highly stable has a high tolerance to bonds breaking before unfolding; an unstable protein has less tolerance. In this study, we focus on the question, how many hydrogen bonds can be lost without destabilizing the protein structure?

Approach: We assume a protein that is highly stable is highly resistant to changes in flexibility. We use rigidity theory to analyze the flexibility of the system with different sets of constraints imposed by hydrogen bonds. We vary the set of hydrogen bonds modeled and measure the fraction of hydrogen bonds which are *redundant;* a hydrogen bond is considered *redundant*, if when it is removed as a constraint, the protein does not become more flexible.

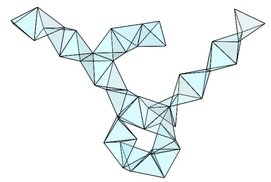
Results: There is a phase transition in the hydrogen bonds in a protein from highly redundant to highly non-redundant that can be observed given any reduction in the subset of hydrogen bonds.

Modeling a Protein as a Body-Bar-Hinge and Associated Graph

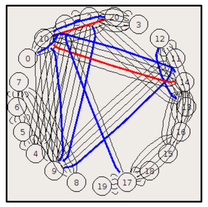
We utilize a pebble game algorithm to determine the rigidity of different regions of a protein [1,3]. In order to use the pebble game algorithm, we first model the protein as a body-bar-hinge structure, and then convert the body-bar-hinge structure to an associated graph, on which the pebble game is performed. After the pebble game algorithm completes, we analyze the graph to determine regions of the graph that are rigid to each other, and from this information we determine rigid regions of the protein.



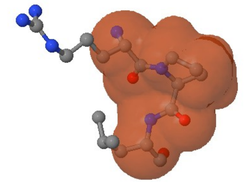
Protein Representation (original protein)



Body Bar Hinge Representation



Associated Graph (on which the pebble game is run)



Body Bar Hinge Post Pebble Game (the largest rigid cluster is shown)

Experimental Setup & System Description

Hypothesis and Contributions

We want to validate the assumption that protein stability correlates with hydrogen bond redundancy. To do so, we focus on two new novel concepts: (1) we analyze random subsets of hydrogen bonds and (2) we introduce the concept of hydrogen bond redundancy.

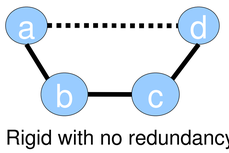
The Redundancy Ratio

H = Full set of Hydrogen Bonds
H'= a subset of Hydrogen Bonds involved as constraints
R = a subset of H', which is the set of Hydrogen Bonds that are redundant

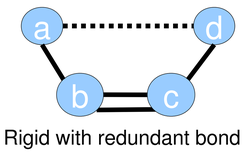
Redundancy Ratio = |R| / |H'|

Hydrogen bonds and redundancy

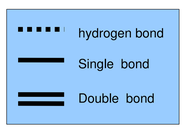
A strong hydrogen bond, like a covalent bond, maintains the distance and bond-bending angles between two atoms, only allowing torsional motion and effectively acting as a hinge.



Rigid with no redundancy



Rigid with redundant bond



Molecular modeling variables

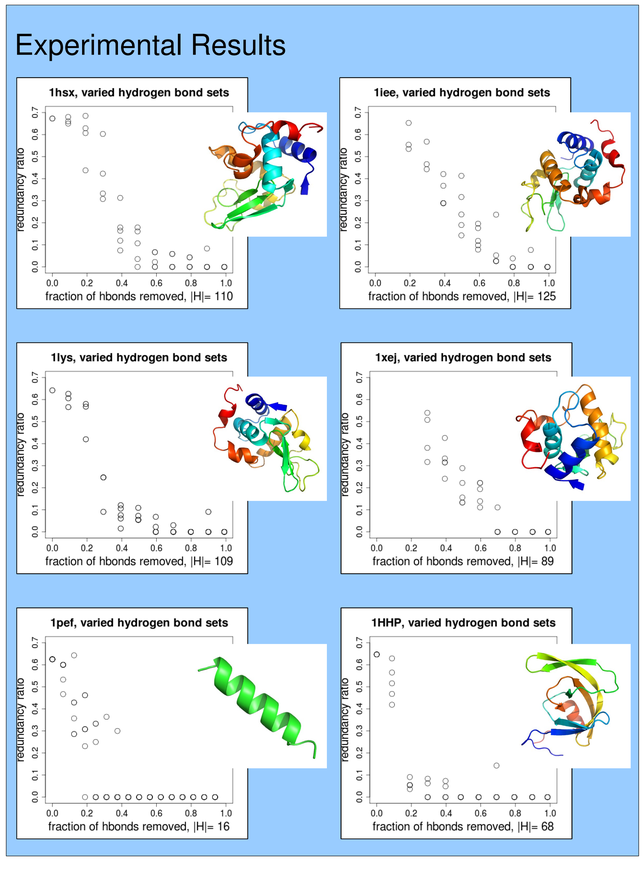
	Fixed or variable?	How modeled
Covalent bonds	fixed	Modeled as rotatable hinges or non-rotatable (peptide bonds).
Disulfide bonds	Placement is based on geometry	Modeled as hinges.
Hydrogen bonds	Placement based on energy cutoff	Randomize which modeled as hinges and which excluded.
Hydrophobic interactions	Placement based on cutoff distance	Modeled as removing 2 degrees of freedom.

Experimental Setup

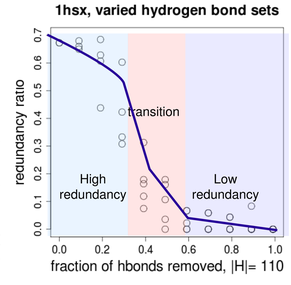
What we control: Placement of covalent bonds, disulfide bonds, and hydrophobic interactions.
What we vary: The subset of hydrogen bonds included as constraints by uniform sampling.
What we measure: The fraction of redundant bonds for each subset of hydrogen bonds.

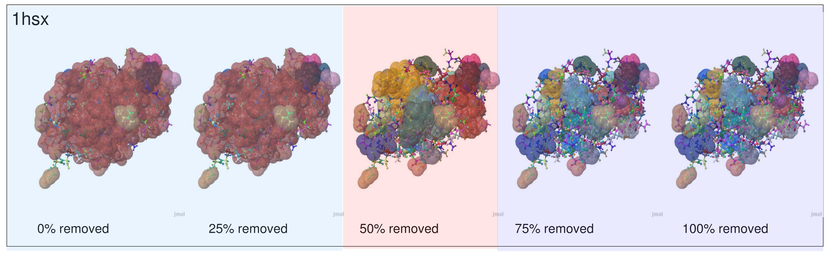
Results and Discussion

Experimental Results



We focused on several proteins, ranging in size from 18 to 130 residues, having approximately 150-1000 atoms, excluding hydrogens Due to programmatic constraints we excluded proteins that were not a single contiguous polypeptide chain. **Left:** We show data for six of the analyzed proteins. **Right:** For protein 1HSX, a phase transformation occurs as the number of hydrogen bonds included is decreased, from highly redundant to highly non-redundant. **Below:** For 1HSX, the phase transition between highly redundant to highly non-redundant bonds may correlate with the transition from highly stable to highly unstable proteins.





Discussion: This work aims to further understand the protein (un)folding pathway. Related studies focus on the loss of rigidity, monitoring the rate of increase in degrees of freedom as hydrogen bonds are removed [2]. This study focuses on the link between protein stability and rigidity, and monitors the rate that redundancy decreases as hydrogen bonds are removed. Generally, in terms of rigidity, in going from 0% to 25% h-bond removal, the distribution of rigid bodies does not change significantly, which suggests that that 25% of the h-bonds are dispensable without altering the overall fold of the protein. Notice also that the transition region for different conformations of the lysozymes occurs at different levels of hydrogen bond removal. This is also interesting in light of the fact that while some surface mutations have no effect on structure, others are critical.

Future work: Characterize the phase transition from stable to unstable for different protein families.

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References

[1] D. J. Jacobs and M. F. Thorpe Generic Rigidity Percolation: The Pebble Game, Physics Review Letters, 75, 4051-4054, 1995.
[2] A. J. Rader, B. M. Hespenheide, Leslie A. Kuhn, and Michael F. Thorpe, Protein Unfolding: Rigidity Lost, in: PNAS 99, pages 3540-3545, 2002.
[3] Audrey Lee and Ileana Streinu, Pebble Game Algorithms and Sparse Graphs EuroComb 2005 , Sept. 2005.

Public Rigidity Web Server

The public web server for performing rigidity experiments on Protein Structures will be available in September, 2009. Visit http://rigdyn.linkage.cs.umass.edu