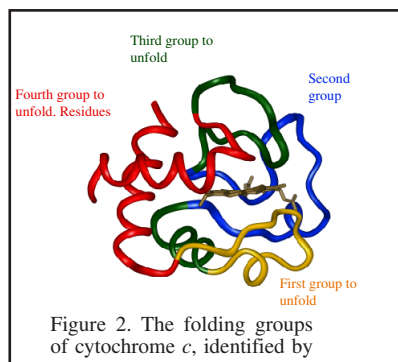


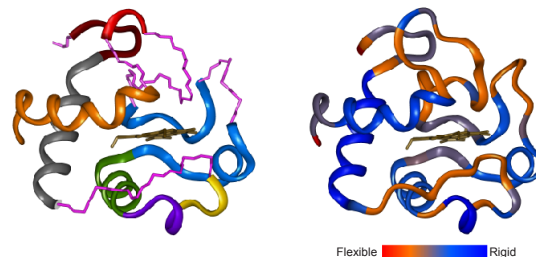
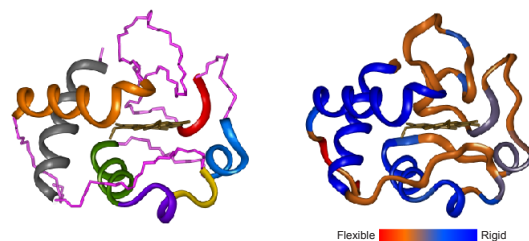
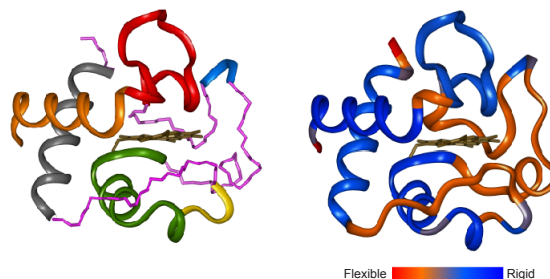
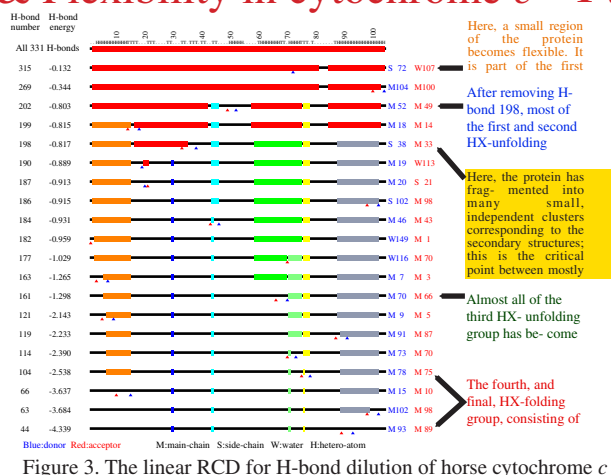
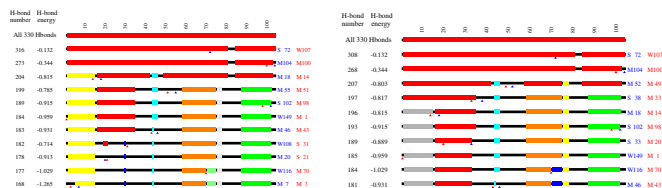
Results: Hydrogen Exchange, Folding & Flexibility in cytochrome *c* IV

Englander, *et al.*, have identified four distinct folding groups in cytochrome *c* based on HX studies. If we assume the unfolding pathway of horse cytochrome *c* is the inverse of the HX folding pathway, then we would expect the groups to unfold in the order shown in Figure 2. In Figure 3, As you follow figure 6 from top to bottom, the order in which regions of the protein become flexible (the colored bars disappear)



Results: Random Dilution of the H-bond Network V

To ensure that the method is not highly sensitive to the H-bond energy function, we mimic thermal fluctuations in the breakage of H-bonds by randomly removing one of the 10 weakest H-bonds, instead of always the weakest. The results (shown below) are quite similar to the strictly energy-based dilution (Figure 3), showing the robustness of the method.



Results: Flexibility in Cytochromes VI

The results of *FIRST* flexibility analysis for cytochrome *c* from horse (PDB code: 1hrc), yeast (PDB code: 1ycc) and bacteria (PDB code: 1co6) are shown in figures 4A-C. The figures depict the proteins at the point when they initially fragment into several small clusters.

In the lower left panel of each figure, we see that the location and size of the rigid and flexible regions that comprise these three cytochromes *c* are quite similar. Especially in the region of the N- and C-terminal and "60s" (colored green) helices. These regions are known to be quite stable based on hydrogen-exchange data.

In the lower right hand panel of each figure, a ribbon diagram for each protein has been colored according to a flexibility index. This quantitative measure is calculated by *FIRST* as the number of remaining rotational degrees of freedom in the cluster divided by the number of bonds. Interestingly, the right edge of the protein, where the protein most likely transfers its electrons, shows a considerable range in flexibility among the three proteins.

Conclusions VII

- Flexibility analysis of proteins as H-bonds are removed can yield insight into the folding pathway.
- Random dilution of the H-bond network yields similar results as the standard incremental dilution, showing insensitivity to the H-bond energy. Also, all random dilutions exhibit the same critical fragmentation from a mostly rigid protein into primarily secondary structures. This fragmentation is consistent with a two-state folding mechanism.
- Flexibility analysis of cytochrome *c* from different species shows conservation of rigid and flexible regions, and corresponds to sections of the protein predicted to be stable or unstable, respectively.

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