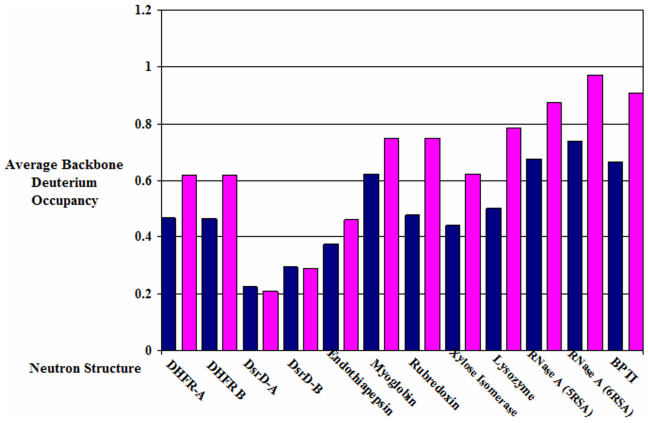
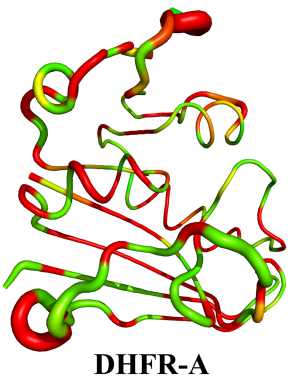
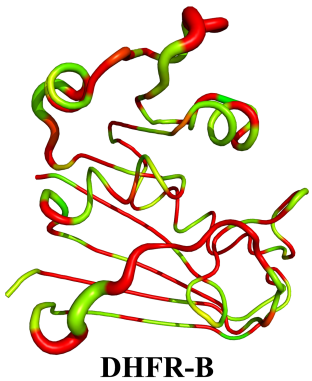
Supplementary Material



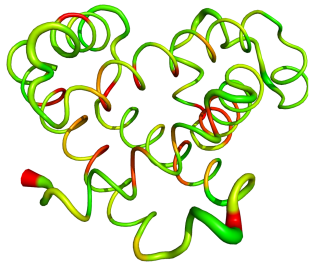
Supplementary Figure 1: Comparison of the average refined backbone amide deuterium occupancy for structured and unstructured regions in neutron protein structures. Averages for α-helical and β-strand (blue) regions have been combined. Shown in pink are the average deuterium occupancy values for amides in unstructured or randomly ordered regions (these regions are defined as "unassigned" by the secondary structure prediction program DSSP (Kabsch & Sander, 1983)). Paired with the calculated protection factors, it is clear that amides within regions with secondary structure have a suppressed propensity to undergo HDX. The global average backbone deuterium occupancies for α-helical, β-strand and unstructured regions are 0.57, 0.42 and 0.65, respectively. (The combined average occupancy for α-helical and β-strand regions is 0.5).



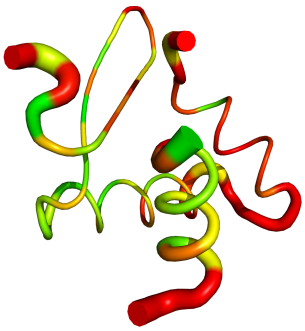
DHFR-A



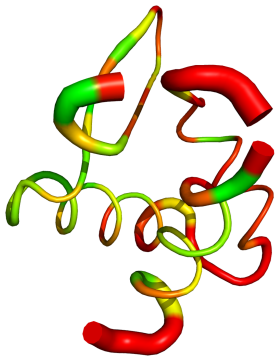
DHFR-B



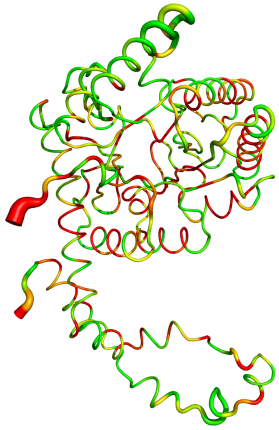
Myoglobin



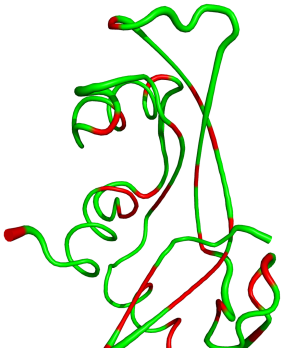
DsrD-A



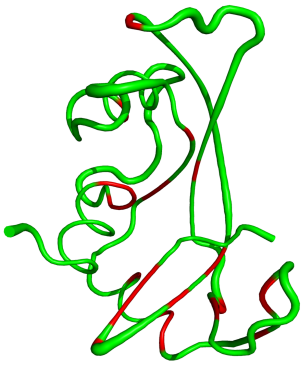
DsrD-B



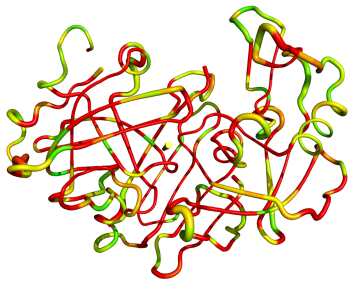
Xylose Isomerase



RNase A (5RSA)

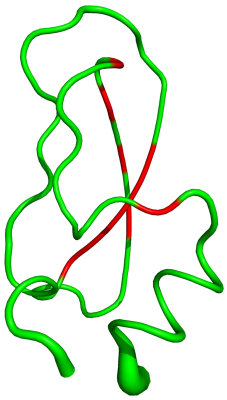


RNase A (6RSA)

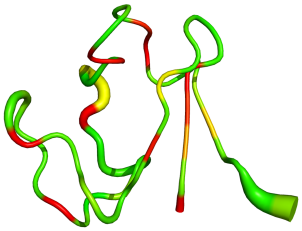


Endothiapepsin

Lysozyme

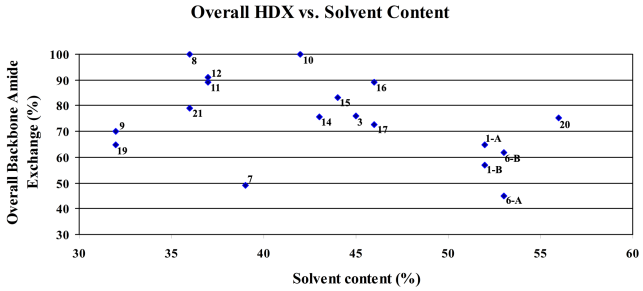


BPTI

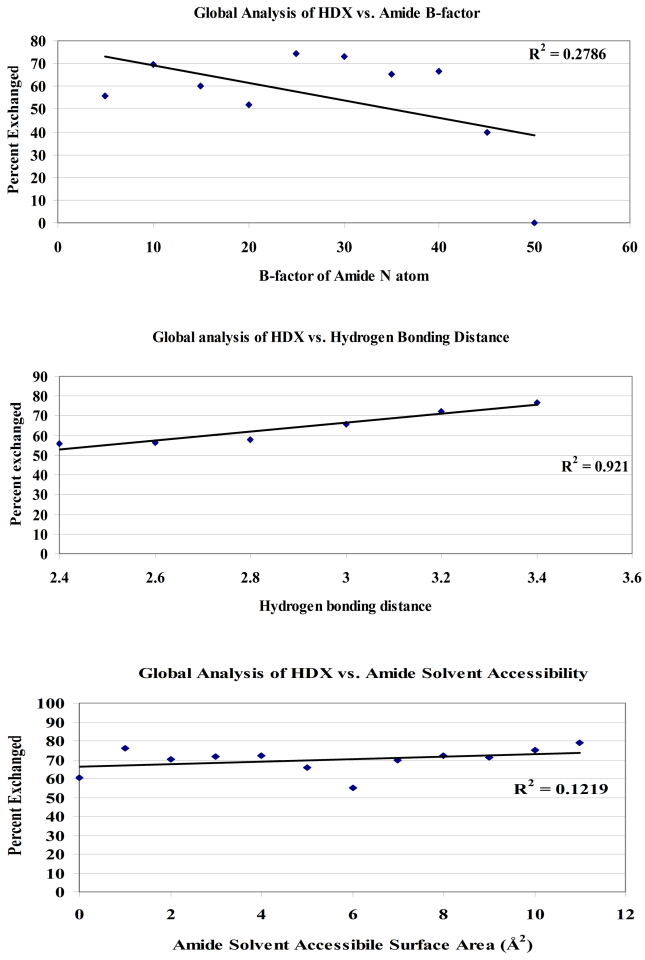


Rubredoxin

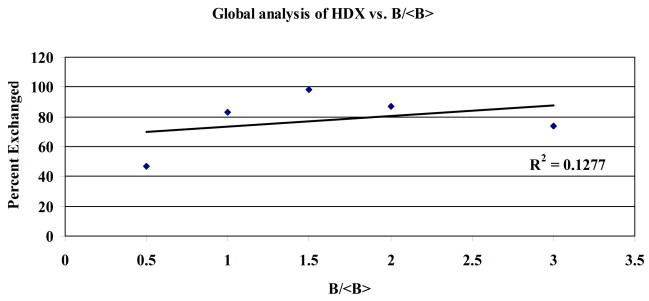
Supplementary Figure 2: Secondary structure and atom depth correlate strongly to the degree of HDX. Cartoon representations of the neutron structures considered in this analysis are colored on a gradient of red to green according to the refined deuterium occupancy of their backbone amides: red indicates fully hydrogenated (little to no exchange for D) and green indicates deuterated (partial to full exchange for D). The tube width corresponds to the amide nitrogen *B*-factor. Graphically, it is evident that β-strand structure provides strong protection from exchange and that depth is generally protective.



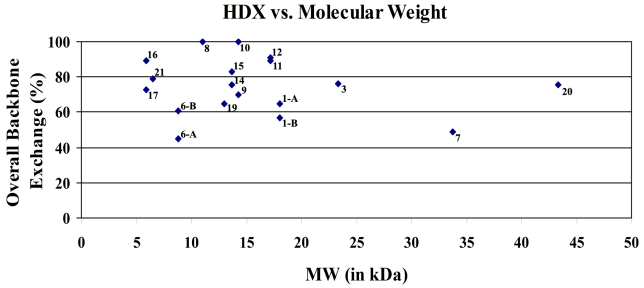
Supplementary Figure 3: The extent of backbone exchange of a protein is not affected by the solvent content of the crystal. Solvent content was calculated using the method of Matthews (Matthews, 1968).



Supplementary Figure 4: Global correlation fits of HDX against (top) amide *B*-factor, (middle) amide hydrogen bonding distance and (bottom) amide solvent accessibility. Analysis was performed as described for Figure 7 of the main manuscript.



Supplementary Figure 5: A global correlation plot of HDX and normalized *B*-factors. Individual amide *B*-factors were divided by the arithmetic mean of the amide *B*-factors for normalization. The magnitude of exchanged amides at each ratio bin (X-axis) were summed and a percent exchanged was calculated (Y-axis).



Supplementary Figure 6: The extent of backbone exchange of a protein is not affected by the overall size of the protein. Proteins were analyzed as monomers, so the MW shown is the size in kDa for one monomer of the given protein.