Protein interactions probed by mass spectrometric techniques: hydrogen deuterium exchange, limited proteolysis and crosslinking

Protein-protein interactions are vital for living systems to function properly. Although x-ray crystallography and other biochemical techniques are frequently used to study these interactions, there are limitations. Mass spectrometry techniques provide a unique ability to study dynamic interactions between proteins that may not be possible using NMR or crystallography techniques. Our group uses solution phase hydrogen/deuterium exchange, crosslinking, and limited proteolysis to understand how proteins interact. Because these questions are of such vital interest to biochemist, we have the opportunity for many collaborations, both at the University of Arizona and with other universities. Several collaborations are described below.

Wales, T. E., Engen, J. R., Mass Spectrometry Reviews, 2006, 25, 158– 170

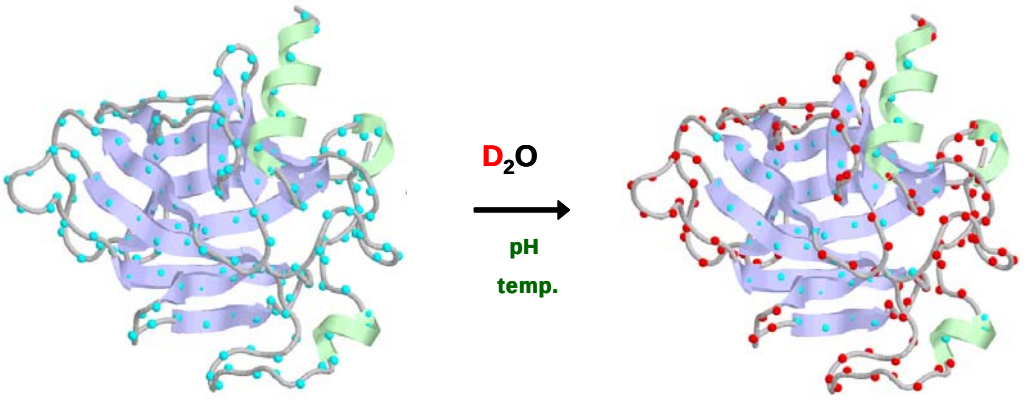
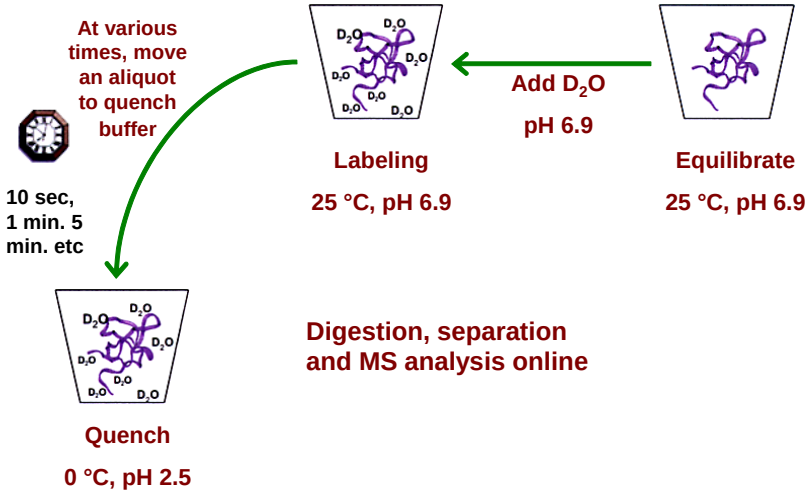
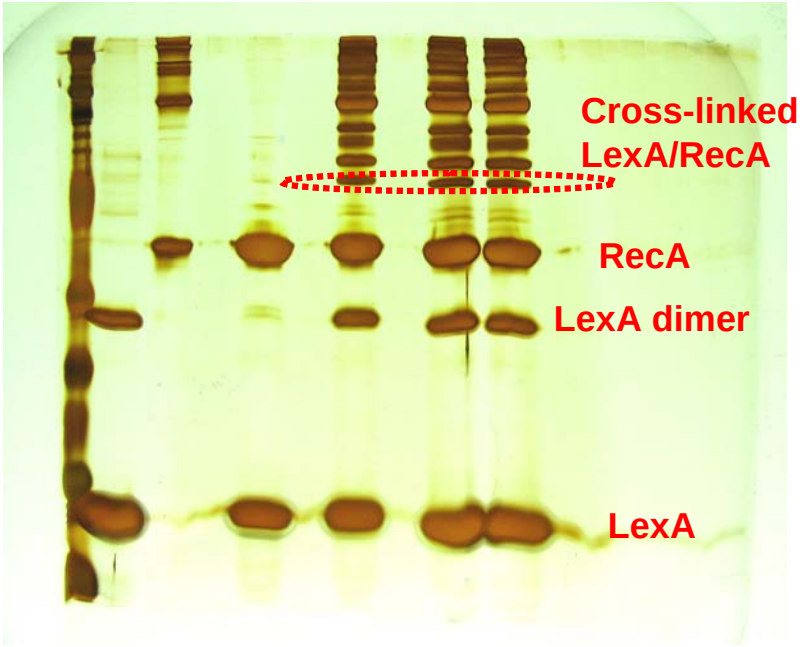


Figure courtesy of John R. Engen, University of New Mexico



LexA / RecA

A project in collaboration with the Little group at the U of A http://www.biochem.arizona.edu/little/Littlelab.htm focuses on a protein dimer, LexA/RecA, that is involved in the SOS response of E.Coli to conditions that damage DNA or inhibit DNA replication. This system models the SOS response in humans and can be used to shed light on how the human body responds to DNA damage. When the SOS response is triggered, an activated form of RecA can bind with LexA. This binding stimulates self-cleavage of LexA, which deactivates the repression of the SOS genes. Despite extensive mutagenesis studies, the binding interface of LexA/RecA interaction is still not clear. Currently, the interaction between LexA and RecA is being investigated by solution phase H/D exchange, cross-linking, and limited proteolysis in order to better understand the binding interface.



P53 tumor suppressor protein

In this collaborative project with Dr.Matthew Gage at Northern Arizona University's http://jan.ucc.nau.edu/~mjg95/ we are investigating the structure and dynamics of the p53 tumor suppressor DNA-binding core region by hydrogen/deuterium exchange and limited proteolysis. If damaged DNA replicates within the cell, the resulting mutations can cause cancer and other disease states within the body. The p53 tumor suppressor protein is involved in preventing cells with damaged DNA from replicating. A better understanding of how it functions within the body may provide pathways for future cancer therapies. H/D exchange allows us to measure the degree of "flexibility" by measuring the rate of exchange along the protein backbone. Using an online

digestion system in which the protein is digested on a pepsin column immediately before separation through a reverse phase LC column and elution to a mass spectrometer, we are able to minimize the back-exchange (loss of deuterium) and get dynamic information at the peptide level.