Hydrogen Deuterium Exchange Mass Spectrometry

Protein footprinting is a major research theme of the WU Research Resource in Mass Spectrometry. One approach that is under development and being implemented in applications is hydrogen/deuterium exchange (HDX). The goals are to map protein-ligand and protein-protein interactions, to follow changes in conformation as a function of pH, for example, and to measure protein-ligand affinities. There are several fine reviews of this method (*1-4*).

HDX takes advantage of the three types of hydrogens in proteins: those in carbon-hydrogen bonds, those in side-chain groups, and those in amide functional groups (also called backbone hydrogens). The exchange rates of hydrogens in carbon-hydrogen bonds are too slow to observe, and those of side-chain hydrogens (e.g., OH, COOH) are so fast that they back-exchange rapidly when the reaction is quenched in H_2O-based solution, and the exchange is not registered. Only the backbone hydrogens are useful for reporting protein structure and dynamics because their exchange rates are measureable and reflect hydrogen bonding and solvent accessibility.

When a target protein solution is diluted in D_2O, the protein mass will increase, owing to the exchange between backbone hydrogens and the deuterium in the solution. In a typical HDX experiment, the relative deuterium uptake rates are measured and compared for the protein under different conditions, most commonly with or without ligands. When a solution containing a protein-ligand complex is diluted in D_2O, the extent of HDX decreases for regions involved in ligand binding. This occurs because hydrogen bonding increases and/or solvent accessibility decreases in the bonding regions. By comparing changes in extent of exchange (i.e., ΔD) between ligand free and ligand bound states of the protein, ligand binding regions can be identified.

In a method termed global HDX, the extent of exchange can be determined by measuring the mass of the protein (and thus the deuterium uptake) in the presence and absence of ligand. This determines the overall extent of binding, if any, under the experimental conditions employed. Binding can be probed in a variety of experiments by altering salt concentrations, changing pH, adding other molecules (e.g., DNA), or mutating a protein.

To provide a more detailed picture of binding interactions, the protein can be rapidly digested with pepsin and analyzed using standard proteomics methods (Figure 1). This peptide level HDX is a very powerful technique since it can be applied to most biological interactions including those that involve multiple large proteins. It is important to get broad coverage of the protein, but advances in proteomics and the focused nature of the studies of these interactions (i.e., it is not a shotgun proteomics experiment – the binding partners and other biomolecules are carefully controlled) make this a straightforward goal. This works also demonstrates that large proteins can be studied at the resource by HDX. The peptide level HDX experiment is also a valuable probe for studying varying conditions, such as pH dependent folding and the effects of ionic strength. A recent example of this application is our study with Cole and Kendall at the University of Connecticut to determine the interface of a large oligomeric protein (~100 kDa), Sec A (*5*).

The HDX experiment has been developed to provide other details beyond binding site information. Kinetic information can be discovered by performing a titration experiment, originally called PLIMSTEX and this too has been expanded to cover large proteins (dPLIMSTEX), self-association (SIMSTEX), and other systems. HDX can also be used to determine the binding order for ligands to understand more about protein dynamics. It has also been developed to study protein aggregation, an important phenomenon in many diseases (e.g Alzheimer's Disease). HDX is now being used to inform homology modeling studies where the data can be used to adjust models or choose between differing results.

Compared to nuclear magnetic resonance (NMR), MS coupling with HDX has the advantage of less sample consumption, and higher upper mass limit on protein, as proteins can be digested by protease after deuteriums have been incorporated. In addition, HDX-MS can work with proteins under physiological relevant condition, to some extent. Proteins with impurities can also be studied directly by HDX-MS as well, as long as the impurities do not interfere with protein ligand interactions.

The highlighted links provide detailed information on these methods.

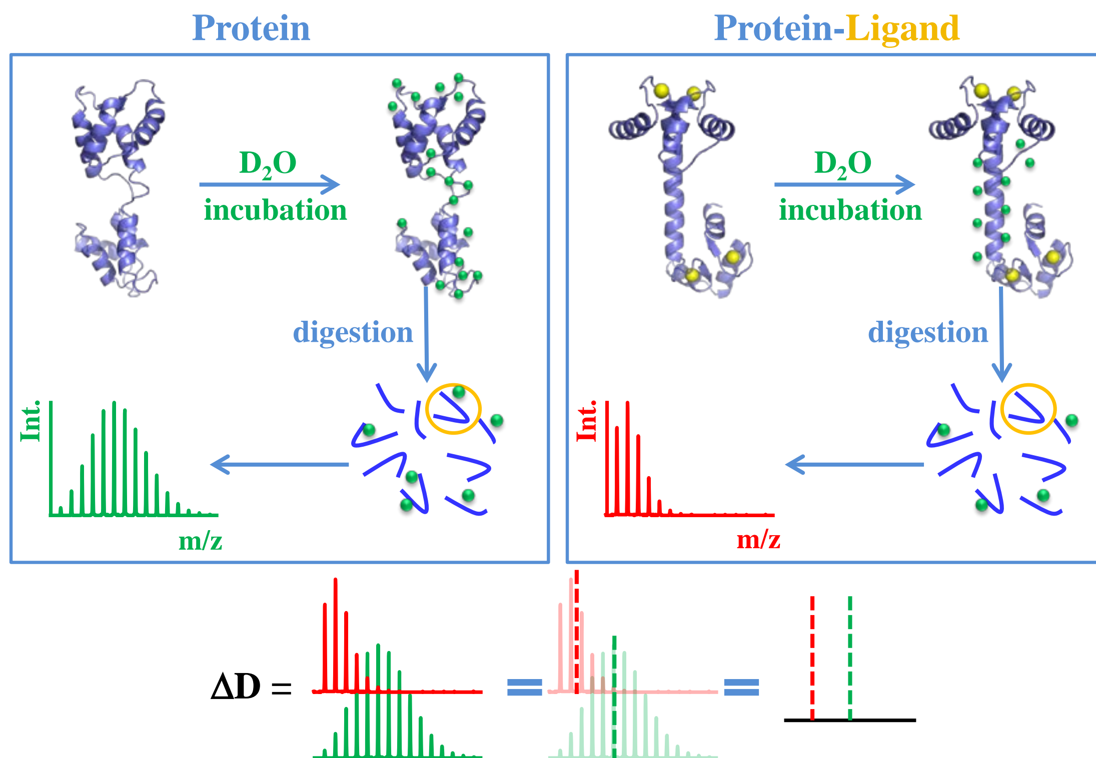

Figure 1. HDX-MS study of protein ligand interaction. The upper left panel shows the protein without ligand exposed to deuterium (green spheres), digested, and measured by MS. The upper right panel shows the same experiment, but in the presence of ligand (yellow spheres, for example calcium ions binding EF-hands). The lower panel shows the shift in mass due to the protection induced by the conformational change upon ligand binding. The circled peptide was not accessible for exchange as indicated by the reduction in the mass of this peptide.

References

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