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Mass Spectrometry & Purification Techniques

Opinion Article

# Basic Principle of Hydrogen/Deuterium Exchange Mass Spectrometry

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## DESCRIPTION

Deuterium/Hydrogen exchange a rapidly developing method for examining the structural characteristics and dynamic properties of proteins is mass spectrometry (HDX-MS). It could stand alone or work in conjunction with structural biology techniques such as Cryo-Electron Microscopy (EM). Both small protein complexes and big protein aggregates can be studied using HDX-MS. Due to recent methodological developments and expanding equipment availability, HDX-MS is increasingly being used on a regular basis for various applications.

An important method for examining protein structure and conformational dynamics has emerged: H/D-Exchange-MS. H/D-Exchange-MS is a potent mapping technique for:

- Protein folding
- Protein-ligand interactions
- Protein-protein interactions and
- Conformational changes

Companies have started to use HDX-MS data to characterise the higher order structure of protein therapies for regulatory agency filing. In order to expose information on the tertiary structure of proteins, H/D-Exchange studies rely on the chemical reaction of swapping covalently bound hydrogens with deuterium atoms. The many labile hydrogens (O-H, N-H, and S-H) in peptides interact with those in the surrounding water molecules. Proteins are diluted into an excess of deuterium oxide to observe the exchange of amide hydrogens with the solvent ($D_2O$). The proteins are then exchanged, sorted, ionised, and analyzed.

### Principle

Since several good earlier papers have already reported on the fundamentals of HDX-MS, we will only quickly touch on them here. Following the dilution of the protein into $D_2O$, which is typically buffered to a neutral or basic pH, deuterons (2H+ or D +; cations of deuterium) exchange with protons (1H+; cations of protium) in a time-dependent manner. The pH adjustment ensures faster exchange rates compared to those observed under acidic conditions. The isotope impact during pH measurement should also be considered while creating the $D_2O$ buffer. This may be done with a typical glass electrode by simply increasing the reading (at 25°C) by 0.4 pH units. A correlation between the different exchange rates and the conformation/topology of the protein side chains may also be inferred from the experimentally recorded rates of the HDX phenomenon. The exact size of the HDX impact is dependent on a number of variables, including pH, ionic strength, the make-up of the side chains, the proximity of the neighboring residues, and interactions with other molecules. Most of the amide hydrogens eventually undertake this exchange process as a result of the conformational shifts in the protein because protein structures are typically quite dynamic entities; however, there will always be certain sites that are going to exchange very slowly (only after years). Since backbone amide hydrogen exchange rates in HDX-MS may be described on a timeline of seconds to days and can be further accelerated by structural obstruction, an acidic pH, and low temperatures.

A valuable method for studying protein structure is the HDX-MS protocol, which takes advantage of the labile nature of protons found on protein backbone amides. Proteins swap these protons with hydrogen groups found in a deuterated buffer when they are dissolved in a solution, and the protons from the protein exchange protons with deuterium. Only the protons found on the amides' backbones are counted. Data about solvent accessibility, which can be used to infer details about protein structure and conformation, is provided by the rate of hydrogen to deuterium exchange. The rate of deuterium uptake can be measured using mass spectrometry.

Structure, protein-protein or protein-ligand interaction sites, allosteric effects, intrinsic disorder, and conformational changes brought on by posttranslational modifications can all be learned via HDX-MS analysis (PTMs). The advantage of HDX-MS is that it is highly sensitive and can identify coexisting protein conformations. It is also not constrained by the size of proteins or protein complexes.

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