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Mass Spectrometry & Purification Techniques

Perspective

Established Methods for Analyzing Protein Complex Structures

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DESCRIPTION

Understanding protein function and the processes of action in a biological system requires the characterization of protein structures and protein complexes. All biological activities are governed by these proteins, the networks and complexes they build, the many interactions that take place during the creation of complexes, and the folding they do. X-ray crystallography, cryo-Electron Microscopy (cryo-EM), and nuclear magnetic resonance are examples of established methods for analyzing protein complex structures, folding, and protein-protein interactions. These methods, however, are restricted to looking at structures in their static forms and may not be able to analyze proteins in their natural settings.

The size of the biomolecule under investigation further restricts the use of these conventional methods, and many proteins are simply ineligible for these kinds of study. Necular Magnetic Resonance (NMR) is an example of a technology that can only be used with proteins that have fewer than 200 amino acids, are soluble for analysis, and can be labelled with isotopes. It is sometimes quite difficult to make the well-diffracting crystals required for investigation in X-ray crystallography. For a long time, protein complexes, protein architectures, and protein-protein interactions were studied using Mass Spectroscopy (MS) methods; nevertheless, these studies were only conducted in highly specialized and instrument-savvy research labs. Additionally, due to their complexity, the need for specialized sample preparation, sophisticated MS characteristics, and data analysis that is appropriate for the application. The Hydrogen/Deuterium exchange-Mass Spectrometry (HDX-MS), a powerful tool for studying protein structures, dynamics, folding, complexes and interactions. The advantages of HDX-MS, the information it provides and the complementary role it plays with traditional techniques, as well as the latest tools and workflows that have been developed to simplify HDX-MS.

There are several MS technologies available for determining the structures of proteins or protein complexes. Regarding protein and protein complex structures, each method offers complementing information. One such MS technique for structural biology is HDX-MS, which makes use of the labile nature of protons found on amides of protein backbones. Proteins exchange these protons with hydrogen groups found in the buffer when they are dissolved in solution. Protons from the protein are swapped for deuterium in a deuterated buffer.

Protons on the functional groups of amino acid side chains interchange too quickly for measurement, and those on the carbons exchange too slowly, therefore only the protons on the backbone amides are detected.

Data about solvent accessibility, which may 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 may be determined via MS. HDX-MS may be utilized to gather data on the structure, protein-protein or protein-ligand interaction sites, allosteric effects, inherent disorder, and conformational changes brought on by post translational modifications for individual proteins as well as protein complexes. Unlike conventional structural techniques, HDX-MS has the extra benefit of not being constrained by the size of proteins or protein complexes. It also benefits from excellent sensitivity and the ability to recognize coexisting protein conformations.

CONCLUSION

Information on protein structure and conformation may be obtained via HDX-MS. Additionally, HDX-MS can reveal protein-protein or protein-ligand interaction sites as well as conformational changes brought on by PTMs for protein complexes. Additionally, it is a supplement to established methods like cryo-EM, X-ray crystallography, NMR, and MS structural biology methods like crosslinking mass spectrometry.

For instance, HDX-MS experiments can aid in directing choices on the complimentary methods to employ in order to gather more data. Recent advancements in hardware have made it possible for scientists to study structures that were previously inaccessible. It is now possible for the HDX-MS workflow to leave specialized research facilities and eventually join the mainstream due to the improvements in software and the entire workflow.

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