APPLICATION NOTE

Advanced HDX-MS Approaches for Target Binding Analysis in Biologics Development

BioAnalytix
A Protagen Protein Services Company

Introduction

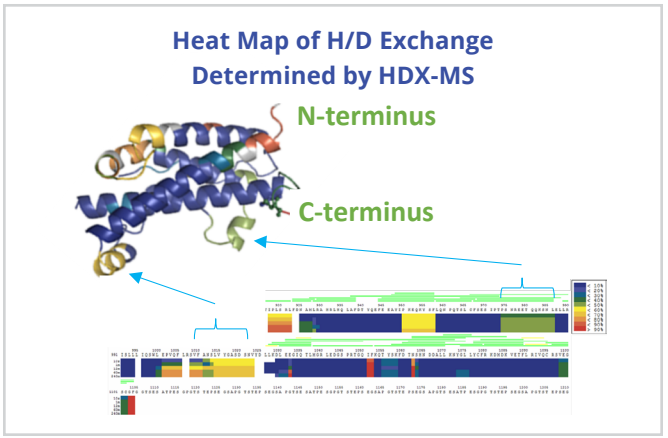
Target engagement is a critical factor in drug efficacy. Understanding and controlling how drug candidates interact with their expected targets can be enhanced using combined profiling approaches, specifically high-resolution analyses that provide details about the interaction. Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) and Native Mass Spectrometry (Native MS) are powerful, information-rich approaches that are increasingly used for binding site profiling and analysis of stoichiometry.

With interrogation and more accurate profiling of these properties, there is opportunity to de-risk and advance early development of biologic drugs by assessing target engagement and kinetics, particularly at different dose levels, and correspondingly, to leverage the understanding obtained to more reliably select and optimize candidates with greater probability of success. When combined with other well-established analytical techniques, there is further opportunity to utilize such detailed binding site profiling to better inform decisions and increase controls in CMC program development, to enhance clinical development success rates overall.

Highlights

We apply advanced MS approaches for enhanced development of complex biologics:

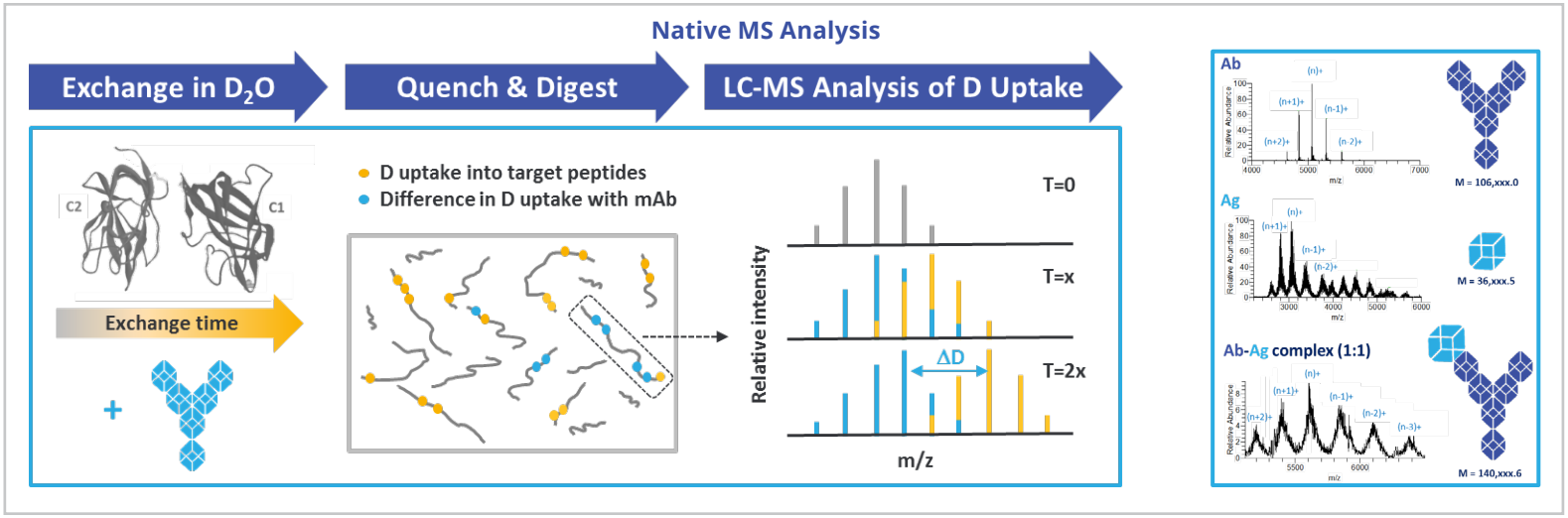
- ✓ Epitope Mapping
- ✓ Paratope Mapping
- ✓ Identification of Binding Regions and Assessment of Binding Mode
- ✓ Distinguish Regions Where Binding Induces Conformational Changes
- ✓ Complete Coverage of Highly Glycosylated Proteins
- ✓ Determine Stoichiometry



Applications of HDX and Native Mass Spectrometry in Biologic Development

HDX-MS analysis is used to map binding site interactions, including epitopes on antigens, paratopes on antibodies, protein-protein/ligand interfaces and self-association, as well as to identify conformational changes induced by binding. The H/D exchange data reflect three-dimensional structure and conformational dynamics of a biologic in solution and report on relative solvent accessibility of regions within the structured protein. The HDX-MS approach to epitope mapping is advantageous over other methods because the vast majority of epitopes (estimated at ~90%) are thought to be conformational epitopes comprised of noncontiguous sequence elements, which are not effectively represented in lower resolution analyses.

Studies can be conducted to identify changes to the biologic under different conditions, such as comparison of an antigen (alone and when bound by antibody) or an enzyme with and without an inhibitor to better understand the interaction and associated implications of binding. Native-MS is a gentle technique used to examine proteins in their folded states and can be applied to determine the size of macromolecules, complexes and non-covalent aggregates or associated proteins. When partnered with HDX-MS, more complete understanding of binding interactions may be obtained to inform factors that influence efficacy, including mechanism of action, dosing, potential synergies or competition in combined therapeutic approaches.



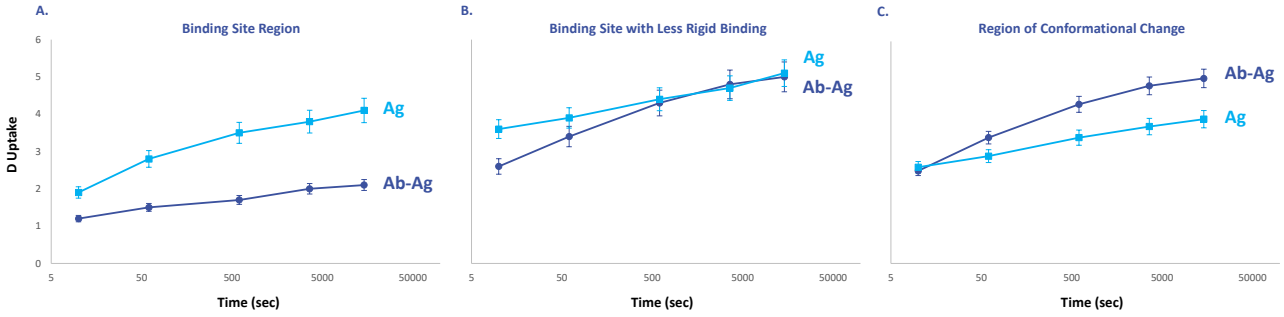
Use of advanced HDX and Native MS approaches can support improved identification of potentially successful biologic drugs. In early in development, these methods can enable de-risking of CMC and clinical designs. Specifically, applications of HDX-MS and Native-MS analyses may be used to obtain detailed assessments of target engagement by biologic drug candidates and provide improved understanding of binding site mapping and kinetic attributes that may influence efficacy; thus, supporting development decisions for complex biologics.

BioAnalytix – Your Strategic Development Partner

Each analytic program is customized to suit our individual client's particular molecules and the phase-appropriate regulatory demands and in keeping with excellent science and best practices of the industry. We successfully build integrated strategies through collaboration with our clients to ensure a productive and positive experience. Our key established areas of work using HDX-MS and Native MS include:

- Epitope Mapping for Binding Site Identification
- Determination of Target Binding Stoichiometry
- Paratope Mapping for Identification of Binding Site and Conformational Changes
- Assessment of Higher-Order Structure (HOS)
- Lot Comparability and Biosimilarity
- Process Development Changes

H/D Exchange Plots Indicating Types of Binding



ADVANCE. DE-RISK. ACCELERATE.

Schedule a call today with our PhD experts to discuss how we can help you advance and accelerate your biologic development programs.

Contact Us

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