Structural Mass Spectrometry for a deeper characterization of mAb-derived therapeutics

Oscar Hernandez Alba

Institut Pluridisciplinaire Hubert Curien, Université de Strasbourg, France

Email : ahernandez@unistra.fr

Monoclonal antibodies (mAbs) are the fastest growing class of therapeutics with a wide range of clinical applications among which, cancer treatment, cardiovascular disorders, and immune diseases. Currently there are more than 170 approved mAbs world-wide, and more than 200 mAb biotherapeutics entering clinical studies per year since 2021. Due to their success, mAbs have not only reshaped the treatment of several diseases but also encouraged the design of highly engineered mAb-derived constructs to improve their therapeutic properties. Thereby, efforts in protein engineering have been focused on the conception of next generation of biotherapeutics to widen their therapeutic applications. Among them, antibody-drug conjugates (ADCs), bispecific antibodies (bsAbs), antibody-oligonucleotide conjugates (AOCs), and Fc-fusion proteins are considered the most promising mAb-based therapeutic formats. However, these proteins are still considered a real analytical challenge, mainly due to their size (around 150 kDa proteins), their flexible and dynamic conformation, and their intrinsic heterogeneity resulting from several tens of post-translational modifications (including glycosylation) that can be contained in their sequence. Although the comprehensive characterization of mAbs-related compounds requires the use of different techniques, native mass spectrometry (nMS), and more generally, structural mass spectrometry approaches, are particularly well-suited to afford structural insights at different levels, enabling a deeper characterization of therapeutic proteins. In this presentation, several techniques encompassed under the structural mass spectrometry term will be introduced, highlighting the advantages, and disadvantages associated to them, and how they can be used to elucidate therapeutic protein proteoforms, to relatively quantify different charge-, and size-variants, and unravel the effect of bio-conjugation process on the conformation and stability of the therapeutic protein.