

Editorial

Advanced Analytical Strategies for Recombinant Therapeutic Proteins

Therapeutic proteins produced using recombinant DNA technologies are generally complex, heterogeneous, and subject to a variety of enzymatic or chemical modifications during expression, purification, and long-term storage. Hence the analytical strategies for characterization, quantitation, purity assay and evaluation of the biological activity of recombinant proteins still represent a big challenge and a matter for debate.

The aim of the proposed special issue is to point out the main applications as well as future potentialities of the most advanced analytical techniques in the different aspects of the quality assessment of therapeutic proteins and in particular for conformation analysis, aggregates and impurities detection and quantitation, intact protein characterization, post-translational modifications (PTMs) identification and biological activity assessment. The special issue contains six in depth reviews and two original papers.

Protein conformation is a key aspect to be assessed, because a specific conformation is essential for the biological function of the protein. The paper by Bertucci *et al.* points out the growing role of circular dichroism (CD) as a valuable and reliable technique to obtain this information, representing a useful tool for the study of pharmaceutical peptides themselves, in new formulations, after new processes of derivatization, production and storage. The paper also contains examples on the use of CD spectroscopy in the structural characterization of free and formulated recombinant proteins, looking at the prediction of the secondary structure, propensity to conformational changes, stability, and tendency to aggregation. Characterization of protein conformation by high resolution mass spectrometry (direct ESI-MS and hydrogen/deuterium exchange) is reviewed by Bobst & Kaltashov. The paper provides an overview of the MS techniques and current trends for the characterization of the higher order structure and dynamics of biopharmaceutical products.

Recombinant proteins often fail to reach their native conformation and in such cases they form different kinds of aggregates which are unsuitable for the intended applications. This has pressed, on one hand, the exploration of different approaches to favour protein folding, and on the other hand, the development of analytical strategies aimed at detecting soluble and insoluble aggregates. In the review by Garcia-Fruitos *et al.* the biological aspects of protein folding and unfolding are addressed together with an overview of the most advanced analytical techniques suitable for the fast evaluation of conformational quality and aggregation of recombinant drugs, even if showing apparent solubility.

Determining the purity of recombinant proteins presents considerable analytical challenges to the biopharmaceutical industry and the results are highly dependent on the specific method used. Even after sophisticated steps, significant levels of protein impurities such as host cell proteins may still remain present in the final purified biopharmaceutical. Regulatory agencies require demonstration that impurities are not only minimized but also analyzed by the most sensitive available methods. The review by Righetti *et al.* describes the basics and application of a novel approach for the capture and amplification of low-level contaminants in purified preparations of recombinant DNA products based on combinatorial solid-phase hexapeptide ligand library.

Recombinant therapeutic proteins undergo different PTMs and chemical reactions during expression, purification, and long-term storage. A full detailed characterization of the protein is a very challenging analytical task and to date, mass spectrometry (MS) has attained a central role because of the wealth of structural and molecular information that can be obtained from small amounts of sample. The paper by Carini *et al.* reviews the most advanced mass spectrometric strategies for the molecular weight determination of intact recombinant therapeutic proteins which furnishes valuable information not only for protein structure characterization but also to assess purity and heterogeneity. Moreover, mass spectrometry is the method of choice for sequencing peptides and proteins and is the preferred choice for characterizing PTMs. The paper by Zhou *et al.* reviews Electron transfer dissociation (ETD) as an emerging dissociation method to characterize PTMs, which in most cases overcomes the limitations of the most commonly used collision induced dissociation (CID). The review outlines the most recent applications of ETD for characterizing peptides and PTMs, highlighting advantages and complementarities over CID.

The original paper by Xie *et al.* describes the application of the most modern MS techniques for simultaneous identification of hemagglutinin (HA) proteins and process-related impurities in a trivalent influenza candidate vaccine. In particular, the combination of reversed-phase LC-MS and targeted glycopeptides analysis by hydrophilic interaction LC (HILIC)-multiple reaction monitoring (MRM) was found a suitable approach to (1) identifying protein impurities, (2) verifying the sequence of the HA proteins, (3) profiling N-linked glycosylation patterns of the HA molecules, and (4) identifying potential protein-related degradants

Assessment/evaluation of the biological activity or potency is a key component in the quality control of biopharmaceuticals. The original paper by Zumpe *et al.* reports the development of an innovative method based on a rapid intracellular phosphorylation assay to assess interleukin-7 potency which is characterized by different advantages over the classical proliferation assay. The study also serves as an example for the typical steps during development and validation of a potency assay for quality control testing.

In summary the special issue provides an overview of the most advanced analytical techniques with a view to ensuring the quality of recombinant therapeutic proteins.

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