Innovative isotopic labeling approaches enabling NMR investigation of monoclonal antibodies at atomic resolution

Arthur Giraud*[1], Jérôme Boisbouvier* , Oriane Frances , and Elodie Crublet

[1]Sanofi [Vitry-sur-Seine] – SANOFI Recherche – 13 Quai Jules Guesde, 94400, Vitry-sur-Seine, France

Résumé

Monoclonal antibodies (mAbs) are biotherapeutics products that have achieved outstanding success in treating manty life-threatening and chronic diseases. In pharmaceutical industries, mAbs are commonly produced in Chinese Hamster Ovary (CHO) cells. To ensure that each batch retains full therapeutical activities, it is particularly important to control the quality and the folding state of the proteins. mAbs can be characterized by 2D (1H,13C)-methyl NMR at natural abundance, to obtain a spectral fingerprint of these 150 kDa biologics. Although this fingerprint comparison approach is a very powerful and efficient tool for quality control of the mAb, it does not provide access to in-depth structural information, highlighting the need for NMR isotopic labeling and assignment strategies of mAbs to enable complete antibody characterization.

To solve this major bottleneck, we developed the production of isotopically labeled mAb sub-domains (e.g Fab and Fc domains) using a cell-free protein expression system. Contrary to the CHO expression system, D2O does not impede the *in vitro* production of proteins, allowing us to produce uniformly enriched Fab and Fc domains with 2H, 15N and 13C isotopes with a high isotope incorporation rate. Such optimized sample enables the acquisition of high quality 2D TROSY and 3D experiments required for the sequential assignment of backbone resonances. We also demonstrated that our cell-free expression approach allows the production of perdeuterated and methyl specific 13CH3-labeled proteins, a prerequisite to transfer the sequence specific assignment from backbone to methyl moieties. The assignment obtained with this cell-free approach will then be transferred to the full mAb produced un CHO cells. To achieve this last, we developed a new CHO culture medium allowing, for the first time, the expression of (1H, 13C)-methyl specific labeled mAbs in these eukaryotic cells.

In this presentation, I will describe our new isotopic labeling methods enabling the production of optimally labeled mAbs and Fab fragments. These new isotopic labeling approaches open the possibility to assign such large therapeutic proteins for future NMR investigations, giving NMR a key role in the characterization of biologics.

*Intervenant