CIFRE Thesis proposal Sanofi R&D (Vitry-sur-Seine) and Structural Biology Institute (IBS Grenoble)

**Innovative NMR tools to assess and monitor robustness of bioproduction processes of monoclonal antibodies (mAb)**

This thesis will be held under a CIFRE convention funded by SANOFI R&D in collaboration with the Academic Biomolecular Spectroscopy group from the Structural Biology Institute (IBS) in Grenoble.

Monoclonal antibodies (mAbs) are biotherapeutic products that have achieved outstanding success in treating many life-threatening and chronic diseases. mAbs are commonly produced in Chinese Hamster Ovary (CHO) cells and the characterization of these biotherapeutics and especially of their Higher Order Structure (HOS) is very challenging. Preliminary studies conducted in Sanofi R&D and recent literature suggest that NMR could be a key method to better understand structure/functions relationships for this new medicines generation and this thesis subject comes within this scope.

The student will spent 80% of his time in the IBS laboratory in Grenoble and the rest of the time in the Sanofi R&D center of Vitry-sur-Seine close to Paris. This thesis will be conducted in a state-of-the-art academic NMR laboratory specialized in structural biology that is part of a robust international network providing access to all the necessary orthogonal skills and techniques. The team “NMR of large biomolecular assemblies” has a unique know-how in developing innovative isotopic labelling approaches. This group has also a strong expertise in cutting-edge NMR experiments that facilitate the characterization of high molecular weight assemblies by solution NMR spectroscopy. This NMR team is continuously devoting research efforts in order to tackle more and more challenging systems and overcome the current size and time limitation of NMR technology. Moreover, this laboratory is associated to a very innovative start-up, NMR Bio, which has a recognized expertise in the production of methyl-labelled samples for NMR studies and cell-free systems.

Sanofi R&D center of Vitry-Sur-Seine is gathering research in oncology as well as production/purification and all analytical activities necessary to the development of mAbs. The candidate will have an on-site initial training to unlabeled monoclonal antibody production and a general introduction to mAb characterization techniques and will then have regular meetings with a team of Sanofi researchers who will guide him during the whole PhD project duration.

The ultimate goal of this work is to develop innovative NMR tools that will allow better characterizing higher order structure (HOS) of complex proteins such as mAbs. The HOS of a therapeutic protein is a key parameter to be assessed when evaluating robustness of pharmaceutical bioproduction processes and its measurement represents a significant analytical challenge. Currently available biophysical methods are not sufficient to detect subtle conformational changes and it is often not possible to explain the structural origin of potency loss observed in bioassays after stress tests and thus to precisely understand biotherapeutic molecules degradation pathways. The purpose of this project is to further explore the capabilities of NMR to strengthen analytical toolbox for characterizing biotherapeutics such as mAbs and accelerate Sanofi biotherapeutic products development.

NMR is a very powerful and reproducible tool for providing information on the quality control of biological compounds, but is also a very reliable technique to provide a wealth of information on the structure and dynamics of biomolecules in solution at atomic resolution. As such, it could greatly contribute to our understanding of biological processes and could be a rational approach to resolving R&D processes issues at every step of the bio-drug lifecycle. NMR, in its standard labelling conditions, has long been limited to the study of small size proteins (<30 kDa). However these past 15 years huge progress in methyls specific labelling have been made enabling the study by NMR of high molecular weight assemblies up to 1 MDa. Natural abundance Methyl 2D-NMR approach has been demonstrated recently in literature to provide high resolution HOS data for the mAb therapeutic class (~150 kDa). Although this fingerprinting approach is a very powerful and effective tool for quality control, it does not grant access to in-depth structural information, highlighting the importance of specific labelling of NMR mAbs samples in CHO cells.

During the thesis, a first milestone to be reached will be the development of a new culture medium for the expression of methyl-labelled proteins in CHO cells. The IBS team recently succeeded to produce similar labelled-rich media for the culture of eukaryotic cells (Sf9 insect cells and human HEK cells). Giving these elements, we strongly believe the PhD student will be able to reformulate suitable media for the production of labelled antibodies in CHO Cells.

Once the sample prepared, a second milestone will be to perform the appropriate NMR experiments to assign the full-length antibody spectra. In order to facilitate this challenging task, a first approach will consist in a separate assignment of Fab and Fc Fragments. Usually, implemented assignment strategies rely on the production of methyl protonated protein in a fully perdeuterated background. In this case, the Fab and Fc NMR samples will be produced in CHO cells in a $H_2O$ buffer limiting the level of deuteration in produced proteins. The residual protonation may decrease the quality of NMR spectra and therefore affect the assignment process. If it is the case, a back-up solution will be used by producing methyl labelled proteins in vitro and the assignment will be transferred to the NMR spectra of the antibody produced in CHO cells. For resonances affected by post-translational modifications or located at the interface of domains, mutagenesis approaches will be used to complete the assignment.

A full mAb assignment will then give access to a wide range of information relative to mAb characterization. A third milestone will consist in the acquisition and interpretation of NMR binding and dynamics experiments on the labelled full-length mAb. This work will provide a more complete understanding of the conformational properties of local motions, binding with partners, epitope mapping and rationalization of the activity of the antibody from both a structural and dynamic perspective.

**Candidate profile:**

Benefit for candidate if granted:

- The candidate will benefit from the expertise of a state-of-the-art academic CEA laboratory in the field of NMR applied to very high molecular weight assemblies.
- Gain significant knowledge in the field of biotherapeutics development by the pharmaceutical industry
- Acquire a strong knowledge in the production of labelled antibodies in cell-free systems and mammalian cells.
- This thesis will be the opportunity to use LC-MS and other biophysical orthogonal techniques such as CD, FT-IR, SPR, nanoDSF, cryoEM and X-ray crystallography.

Required skills:

- MSc in biophysics or biochemistry
- Experience with NMR
- Experience in structural biology and / or biochemistry is an advantage
- Fluent in English
- Oral and written presentation
- Interest in translational pharmaceutical issues
- Team player and good communication skills

Expected qualification / experience

- Training in NMR and structural biology
- Lab experience in biophysics and biochemistry

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