

## N-Glycosylation in the Green Microalgae *Chlamydomonas reinhardtii*

**Authors :** Pierre-Louis Lucas, Corinne Loutelier-Bourhis, Narimane Mati-Baouche, Philippe Chan Tchi-Song, Patrice Lerouge, Elodie Mathieu-Rivet, Muriel Bardor

**Abstract :** N-glycosylation is a post-translational modification taking place in the Endoplasmic Reticulum and the Golgi apparatus where defined glycan features are added on protein in a very specific sequence Asn-X-Thr/Ser/Cys where X can be any amino acid except proline. Because it is well-established that those N-glycans play a critical role in protein biological activity, protein half-life and that a different N-glycan structure may induce an immune response, they are very important in Biopharmaceuticals which are mainly glycoproteins bearing N-glycans. From now, most of the biopharmaceuticals are produced by mammalian cells like Chinese Hamster Ovary cells (CHO) for their N-glycosylation similar to the human, but due to the high production costs, several other species are investigated as the possible alternative system. In this purpose, the green microalgae *Chlamydomonas reinhardtii* was investigated as the potential production system for Biopharmaceuticals. This choice was influenced by the facts that *C. reinhardtii* is a well-study microalgae which is growing fast with a lot of molecular biology tools available. This organism is also producing N-glycan on its endogenous proteins. However, the analysis of the N-glycan structure of this microalgae has revealed some differences as compared to the human. Rather than in Human where the glycans are processed by key enzymes called N-acetylglucosaminyltransferase I and II (GnTI and GnTII) adding GlcNAc residue to form a GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>2</sub> core N-glycan, *C. reinhardtii* lacks those two enzymes and possess a GnTI independent glycosylation pathway. Moreover, some enzymes like xylosyltransferases and methyltransferases not present in human are supposed to act on the glycans of *C. reinhardtii*. Furthermore, the recent structural study by mass spectrometry shows that the N-glycosylation precursor supposed to be conserved in almost all eukaryotic cells results in a linear Man<sub>5</sub>GlcNAc<sub>2</sub> rather than a branched one in *C. reinhardtii*. In this work, we will discuss the new released MS information upon *C. reinhardtii* N-glycan structure and their impact on our attempt to modify the glycan in a Human manner. Two strategies will be discussed. The first one consisted in the study of Xylosyltransferase insertional mutants from the CLIP library in order to remove xyloses from the N-glycans. The second will go further in the humanization by transforming the microalgae with the exogenous gene from *Toxoplasma gondii* having an activity similar to GnTI and GnTII with the aim to synthesize GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>2</sub> in *C. reinhardtii*.

**Keywords :** *Chlamydomonas reinhardtii*, N-glycosylation, glycosyltransferase, mass spectrometry, humanization

**Conference Title :** ICC 2018 : International Conference on Carbohydrate

**Conference Location :** Paris, France

**Conference Dates :** October 29-30, 2018