N-glycan xylosylation in the diatom *Phaeodactylum tricornutum*

Julia Van Bockstaele Fuentes[1], Carole Plasson[1], Olivier Perruchon[1], Corinne Loutelier-Bourhis[2], Carlos Afonso[2], Patrice Lerouge[1], Narimane Mati-Baouche[1], Muriel Bardor[1,3]

[1] *Université de Rouen Normandie, GlycoMEV UR 4358, SFR Normandie Végétal FED 4277, Innovation Chimie Carnot, GDR CNRS Chemobiologie F-76000 Rouen, France.*

[2] *Université de Rouen Normandie, INSA Rouen Normandie, Univ Caen Normandie, ENSICAEN, CNRS, Institut CARMeN UMR 6064, F-76000 Rouen, France.*

[3] *Alga Biologics, 25 rue Tesnières, 76821 Mont-Saint-Aignan Cedex, France.*

patrice.lerouge@univ-rouen.fr

In 2024, the biopharmaceutical market value reached above $469 billion. This dynamic market includes monoclonal antibodies (mAbs) either originators or biosimilars, hormones, nucleic acid and engineered cell-based products. Currently, most of the mAbs are produced in mammalian cells, such as Chinese Hamster Ovary (CHO) cells. However, production in CHO cells is expensive and present potential risk of viral contamination. This explains the growing interest for the development of cost-effective, safer and more sustainable expression systems, such as microalgae. Since they are photosynthetic eukaryotic cells, their culture in photobioreactors is inexpensive. In addition, microalgae perform efficient folding and *N*-glycosylation of proteins. Moreover, microalgae are classified as Generally Recognized as Safe strains and, therefore, microalgae, such as the diatom *Phaeodactylum tricornutum,* have emerged as alternative cell factories for the production of biologics. So far, analyses of glycans *N*-linked to *P. tricornutum* proteins revealed mainly high mannose type *N*-glycans both on endogenous glycoproteins[1] and mAbs[2]. However, bioinformatic approaches allowed the identification of a putative sequence coding for a xylosyltransferase (PtXylT) in the *P. tricornutum* genome[3]. *N*-glycan xylosylation of plant-derived therapeutic proteins has been demonstrated to induce immune responses[4]. Therefore, the present research project aims to investigate the xylosylation processing of *Phaeodactylum tricornutum N*-glycans, that is currently poorly understood. In contrast, plant core β(1,2)-xylosyltransferases (XylT) have been well-studied. In this context, we performed mass spectrometry analyses to identify xylosylated protein *N*-glycans in *P. tricornutum* Pt3 ecotype. Moreover, functional complementation of a tobacco mutant impaired in XylT activity with the gene coding for PtXylT confirmed that this glycosyltransferase is a core β(1,2)-xylosyltransferase.

This work is funded by the European Union under Grant Agreement 101119499 through the Marie Sklodowska-Curie Actions Glyco-N project. The authors gratefully acknowledge Nicole Raven and the Fraunhofer Institute for Molecular Biology and Applied Ecology IME (Aachen, Germany) for providing seeds of *Nicotiana benthamania* XylT/FucT double mutant.

(1) Dumontier et al. (2021) *Carbohydrate Polymers* 259, 117660. (2) Vanier et al. (2015*) PlosONE* 5, e0139282. (3) van Bockstaele-Fuentes et al. (2025) *Frontiers in Plant Science* Vol 16. (4) Bardor et al. (2003) *Glycobiology* 13, 427-434.