O-glycan Variability of Glycoproteins Expressed by *Pichia pastoris*

and its Effects on Mannose Receptor Binding Properties

**Magnus Sjöblom¹, Anki Gustafsson², Lena Strindeli², Tomas Johansson²,
Linda Björnström³, Ulrika Rova¹, Jan Holgersson⁴**

¹Luleå University of Technology, Biochemical and Chemical Process Engineering, Luleå, magnus.sjoblom@ltu.se; ²Recopharma AB and ³AbSorber AB, S-141 57 Huddinge; and ⁴Karolinska Institute, Clinical Immunology & Transfusion Medicine, S-141 86 Stockholm, Sweden

Abstract: Mannosylated model antigens have been shown to have an increased capability to stimulate antigen specific T-cell proliferation compared to their non-mannosylated counterparts [1, 2]. Mannosylation is thought to target antigens to mannose receptors (MR) which subsequently mediate their up-take and MHC presentation [3, 4]. In this perspective, yeast and fungi are interesting production platforms for recombinant mannosylated vaccines due to their natural ability to mannosylate proteins in combination with their attractive cultivation characteristics. However, structural and functional characterizations of the glycans involved are poor, specifically for O-glycans. P-selectin glycoprotein ligand 1/mouse Immunoglobulin G2_b (PSGL-1/mIgG2_b) is a chimeric homodimeric mucin like protein with 106 theoretical sites for O-glycans and six N-glycans [5]. In the context of mannose receptor targeted vaccines, glycoproteins with this type of glycosylation expressed by *P. pastoris* have not been studied before. The purpose of this project was to investigate how changes in bioprocess parameters (i.e. pH, temperature) affect O-glycan biosynthesis of PSGL-1/mIgG2_b expressed in *P. pastoris* and its significance for MR binding. Preliminary studies indicate that the cultivation pH influence O-glycan structure. Biacore analysis revealed specific binding between PSGL-1/mIgG2_b and immobilized MR. To demonstrate how O-glycans synthesized under different conditions influence the affinity of PSGL-1/mIgG2_b for the MR, additional Biacore studies where N-linked oligosaccharides are removed by PNGase F treatment, will be presented. Structures of relevant glycans, determined by MS, will also be presented.

References:

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