

N-GLYCANS: HOW TO ANALYSE AND HOW TO MODIFY?

Roland Contreras

*Fundamental and Applied Molecular Biology, Ghent University and Flanders
Interuniversity Institute for Biotechnology,
Technologiepark 927, B-9052 Ghent, Belgium
Roland.Contreras@dmb.rugent.be*

Protein linked N-glycans have a wide range of functions and changes in their structure has been linked several times to human disease and several other biological phenomena. Especially remarkable is their complexity and their heterogeneity, two aspects that are poorly understood. To develop approaches to answers these complexity and heterogeneity questions, we have developed systems to produce homogeneous, well defined engineered protein N-glycans. During this process, we needed and developed the powerful DSA-FACE technology for analysis of N-glycans and applied it for several biological questions.

Lower eukaryotes are very interesting organisms for glycan engineering as they only synthesise N-glycans of the high-mannose type. Thus, they are excellent hosts for this engineering work as they don't have competing complex glycosyl transferases coded for in their genome. Redirection of the fungal pathway, even to a simple hybrid or complex mammalian type structure requires several genetic interventions such as gene knock-outs and heterologous expression of mammalian glycosyl transferases. Furthermore, additional in vitro enzymatic manipulations may be required.

Our DSA-FACE technology (Callewaert et al., 2001) is very sensitive, high resolution and high-throughput. It was applied for diagnostic purpose in CDG children, for diagnosis of liver disorders and in auto-immune diseases. CDG types I and II have been detected in the total serum N-glycome. For liver failure, high sensitivity and specificity markers for both detection of cirrhosis (79 and 86 respectively; both are 100% for decompensated cirrhosis) and follow-up of fibrosis were characterized. Furthermore, studies with human serum, Hsp70 KO mice and caloric restricted mice and rats showed that the serum N-glycome can be used as an aging biomarker. Many examples are available where we used our DSA-FACE technology to obtain information on protein N-glycans s.a recombinant proteins, TrkA receptor, engineered HEK294 line (Reeves et al, 2002), etc.

-Callewaert et al.,(2003) Increased fucosylation and reduced branching of serum glycoprotein N-glycans in all known subtypes of congenital disorder of glycosylation I. *Glycobiology* **13**, 367-375.

-Callewaert, N., Van Vlierberghe, H., Van Hecke, A., Laroy, W, Delanghe, J and Roland Contreras. (2004) Noninvasive diagnosis of liver cirrhosis using DNA-sequencer-based total serum protein glycomics. *Nature Medicine*, 10, 429-434.

-Reeves, P.J., Callewaert, N., Contreras, R., Khorana, H.G. (2002) Structure and Function in Rhodopsin : High-level expression or rhodopsin with restricted and homogeneous N-glycosylation by a tetracycline-inducible N-acetylglucosaminyl transferase I-negative HEK293S stable mammalian cell line. *Proc Natl Acad Sci U S A* **99:21** 13419-24

-Vervecken, W., Kaigorodov, V., Callewaert, N., Geysens, S., De Vusser, K. and Roland Contreras (2004). *In vivo* synthesis of mammalian like hybrid type N-glycans in *Pichia pastoris*. *Environmental and Applied Microbiology*, 70(5):2639-46.