ProMoSCOPE™: Revealing the Sweet Truth Between Glycosylation and Immunity

Each cell of the human body is covered in a layer of simple and complex carbohydrates called glycans (Figure 1A), the majority of which are bound to protein or lipids through a process called glycosylation. The vast structural diversity, evolution and abundance of these cell surface proteins are dependent upon cell type and states, therefore are considered as 'cellular signatures' reflecting the characteristics of different cells (1,2).

Glycosylation is known to be involved in different aspects of the immune system, such as T cell biology, and is vital for the activation and function of T cell receptors (TCRs). TCRs are proteins on the surface of T cells that recognize and bind to foreign substances, such as pathogens or toxins, and play a key role in activating T cells. Glycosylation can influence the activation and function of TCRs by altering their conformation and stability, as well as, modulating their interactions with other proteins (3).

T cell metabolism is known to be regulated by glycans. T cells undergoing clonal expansion or proliferation requires a change in metabolism to withstand the increased bioenergetic demand for the synthesis of nucleotides, amino acids, and lipids through aerobic glycolysis and glutaminolysis (4). T cell activation also upregulates the hexosamine pathway, a component of glucose metabolism, to increase a nucleotide sugar donor substrate UDP-GlcNAc. This pathway is required for N-glycosylation, O-GlcNAcylation and the production of glycosyaminoglycans, a requirement for functional T cells (5).

Glycosylation is also one factor that can affect the immunogenicity of a protein which is affected by several factors, including its structure and the presence of antigenic determinants. When sugars are added to a protein, this can alter the shape and charge of the protein, potentially affecting its recognition by the immune system as foreign. This can affect the ability of the immune system to produce antibodies and memory cells in response to vaccines, impacting its effectiveness. Changes in the glycosylation of T cell surface proteins can also affect the production of cytokines, signalling molecules that help to coordinate the immune response (6). The immune system must be able to distinguish between self and non-self in order to function properly. Failure of this process can lead to the development of autoimmune disorders. This can result in a range of symptoms, depending on the tissues being attacked. Changes in the glycosylation pattern of self-antigens can alter their antigenic determinants which can lead to autoimmunity, as observed in murine models (7,8).

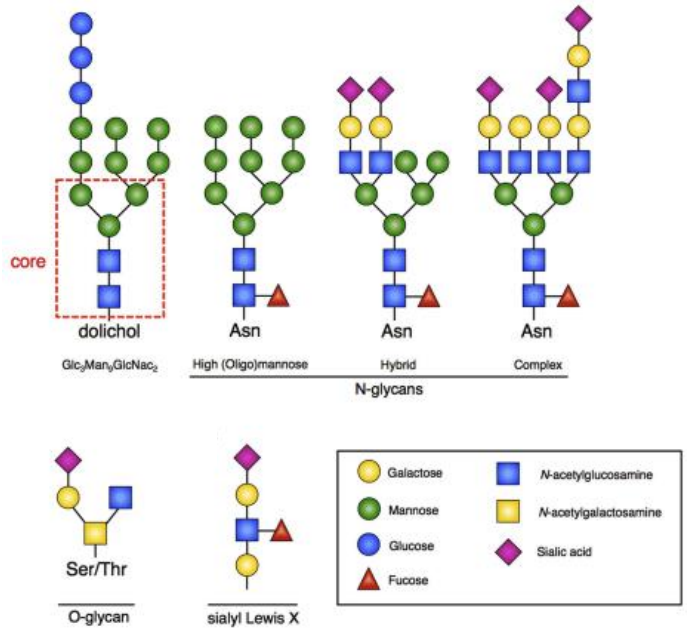

Figure 1. Schematic representation displaying the complex structure of different glycans (Figure is taken from (9)).

Given the abundant presence of glycans and the importance of normal glycosylation for functional immunity, multiple immunodeficiency disorders can potentially arise from defective glycosylation. Developing treatments for such disorders requires understanding the structure of these glycans and how they affect cellular functions. Additionally, quantifying glycosylation levels with gene expression profiles at a single cell resolution would be great asset towards treatment development. This has recently been made possible with **Singleron's** latest technology, **ProMoSCOPE™,** which allows the analysis of cell surface glycosylation levels together with the whole transcriptome at a single cell level. This novel method utilises specifically designed ProMoSCOPE Tags to chemoenzymatically label cells which contain a chemical component capable of recognising and binding to N-acetyllactosamines (LacNAc), a common disaccharide of cell surface glycans (Figure 2). Used in combination with **Singleron's SCOPE-chip®,** this allows a high throughput approach linking glycosylation abundance with gene expression.

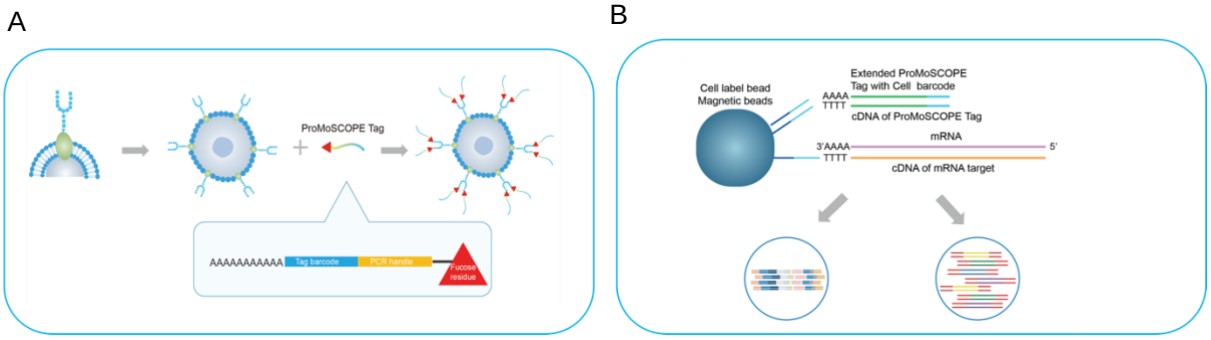

Figure 2. Schematic representation showing the principle of ProMoSCOPE™ technology.
Cell are chemoenzymatically labelled with ProMoSCOPE Tag that contains barcode and poly A sequences. ProMoSCOPE Tag specifically recognizes N-acetyllactosamine (LacNAc) at the cell surface (**A**). Upon cell lysis, barcoding beads capture both ProMoSCOPE Tags and mRNA by poly A tails and two libraries are prepared that can be sequenced together **(B)**.

To determine if the labeling process effects the transcriptome of cells involved in the immune system, a comparison was performed between ProMoSCOPE™ labelled cells taken from the thymus of mice and untreated controls. The thymus is a vital gland for T cell development, where the diverse generation of TCR repertoires to recognize numerous antigens are developed. The chemoenzymatic labelled group displayed similar cell composition and gene expression profiles as the untreated group (Figure 3A-C), confirming the minimal influence of labelling on the transcriptional status cells extracted from the thymus. This latest innovative technology combining accurate quantification of glycosylation levels together with gene expression profiles of single cells adds an invaluable addition in addressing the important role of glycosylation in immunity.

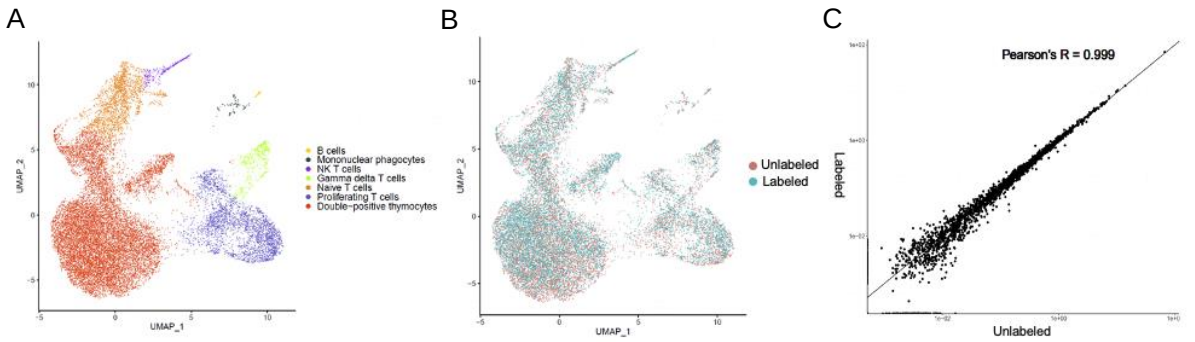

Figure 3. Enzymatic ProMoSCOPE™ labelling has a minimal effect on the transcriptome in mouse bone marrow.
Mouse bone marrow was extracted, single cell suspensions were loaded onto the SCOPE-chip® and processed by ProMoSCOPE™ Single Cell Glycosylation Detection Kit. Major cell types are clustered and showed in a UMAP plot. **(A).** UMAP plot of labelled (blue) and unlabelled (red) samples shows little difference in the cell type composition. **(C)** Scatter plot displaying the correlation between labelled and unlabelled samples to demonstrate no obvious effect of labelling on the transcriptome.

Immune cells sense environmental signals through cell surface-associate glycoproteins, glycolipids, as well as other glycan binding proteins and molecules. Cells of the adaptative and innate immune system express receptors capable of recognising surface glycans of different micro-organisms, where this recognition of glycosylated microbial patterns has greatly benefitted vaccine development (10). Quantifying cell surface glycosylation, with Singleron's ProMoSCOPE™ kit, at single cell level has the potential to aid in vaccine development, as well as understanding the mechanisms of host-pathogen interactions. Development of lymphoid progenitors into T cells occurs in the thymus. Thymus mice tissue processed using ProMoSCOPE™, has eight different cell types including various T cell subtypes (Figure 4A), where proliferating and gamma delta (GD) T cells displayed higher levels of glycosylation (Figure 4B).

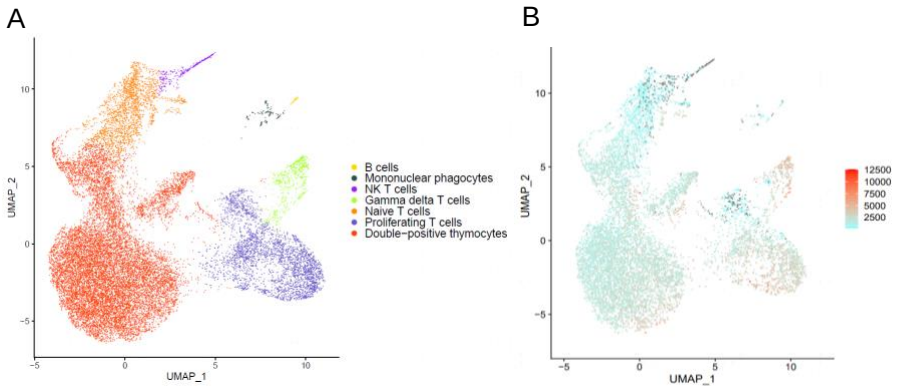

Figure 4. ProMoSCOPE™ reveals heterogeneity of glycosylation abundance in the thymus.
(A) Identification of 8 different cell types detected in thymus tissue. (B) UMAP displaying the tag UMI counts to indicate the levels of glycosylation for each cell type.

Similar to other products from Singleron's single cell multi-omics product portfolio, the ProMoSCOPE™ workflow can be performed either manually without specific equipment, or automated on the the Singleron Matrix® single cell processing system, giving users high flexibility to choose based on their applications and throughput.

To learn more about the **ProMoSCOPE™** technology and other single cell multi-omic approaches Singleron offers, visit our website at **www.singleron.bio** or email us at **info@singleronbio.com** and get in touch with one of our single cell specialists to discuss how we can further your research.

Product	Tissue
	2 RXNs / 16 RXNs
ProMoSCOPE™ Single Cell Glycosylation Detection Kit Tissue	1251011 /1251012
ProMoSCOPE™ Single Cell Glycosylation Detection Kit Tissue for Matrix	1251021/1251022

References

1) Varki, A. Biological roles of glycans. *Glycobiology.* 2017. doi.org/10.1093/glycob/cww086.
2) Fujitani, N. et al. Total Cellular Glycomics Allows Characterizing Cells and Streamlining the Discovery Process for Cellular Biomarkers. *Proc. Natl. Acad. Sci. U.S.A.* 2013, doi:10.1073/pnas.1214233110
3) Elien De Bousser, Human T cell glycosylation and implications on immune therapy for cancer, Human Vaccines & Immunotherapeutics, 2020, 16:10, 2374-2388
4) Almeida L, et al. Metabolic pathways in T cell activation and lineage differentiation. *Semin Immunol*, 2016, doi: 10.1016/j.smim.2016.10.009
5) Wellen KE, Thompson CB. A two-way street: reciprocal regulation of metabolism and signalling. *Nat Rev Mol Cell Biol*, 2012, doi: 10.1038/nrm3305
6) Germain, R. N., & Stefanova, I. T cell antigen recognition and activation. *Annual review of immunology*, 2010
7) Purcell AW, van Driel IR, Gleeson PA. Impact of glycans on T-cell tolerance to glycosylated self-antigens. *Immunol Cell Biol.* 2008, doi: 10.1038/icb.2008.48. Epub 2008 Jul 15. PMID: 18626489.
8) Lee SU, et al. N-glycan processing deficiency promotes spontaneous inflammatory demyelination and neurodegeneration. *J Biol Chem,* 2007, doi: 10.1074/jbc.M704839200
9) Lyons JJ, Milner JD and Rosenzweig SD. Glycans instructing immunity: the emerging role of altered glycosylation in clinical immunology. *Front. Pediatr*. 2015, doi: 10.3389/fped.2015.00054
10) Jasminka Krištić et al. Glycans are a novel biomarker of chronological and biological ages. *J Gerontol A Biol Sci Med Sci*. 2014. doi: 10.1093/gerona/glt190.