Novel multifunctional glycan probes for glycan recognition studies on microarrays and cells

Jin YU [1], Alexiane DECOUT [2], Antonio DI MAIO [1], Jodie ABRAHAMS [1], Ten FEIZI [1], Yan LIU [1]

[1] Glycosciences Laboratory, Department of Metabolism, Imperial College London, U.K. [2] Reproductive and Developmental Biology, Department of Metabolism, Digestion and Reproduction, Imperial College London, U.K.

jin.yu@ic.ac.uk

Glycans mediate key biological processes through specific glycan-protein interactions. Glycan microarrays have revolutionized the elucidation of glycan ligands in diverse recognition systems. Two major slide-based array systems are covalent arrays with amino-terminating glycans immobilized on amine-reactive glass[1,2] and noncovalent neoglycolipid (NGL)-based arrays with lipid-linked glycans immobilized on nitrocellulose-coated glass[3,4]. There are few reports on cross-platform comparisons, and the data are largely with covalent arrays and proteins that give robust binding signals[5,6]. Here we present the design of a novel tri-functional Fmoc-amino-azido (FAA) linker which enables efficient derivatization of free reducing glycans and their conversion into amino-terminating or lipid-tagged probes for constructing covalent arrays and NGL-based noncovalent arrays, respectively. This provides a unique opportunity for a close comparison of the two array platforms with a variety of glycan recognition systems. Whilst similar results are obtained with most plant lectins and antibodies investigated, a striking difference is observed with several viral adhesins and Siglecs which gave binding to low affinity sialyl glycan ligands only in the NGL platform. Our findings highlight that the mode of display and clustering of glycans can dramatically affect microarray readout with certain recognition systems, an important consideration for the glycan recognition knowledgebase. The FAA glycan probes can moreover be rendered fluorescent for detecting glycan binding by proteins on cell surfaces.

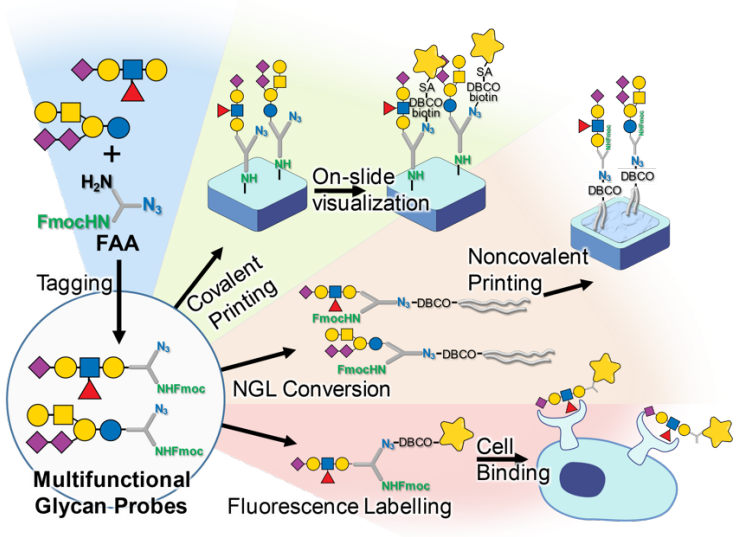

Figure. Schematic representation of the design and applications of FAA linked glycan probes.

Supported by UK Medical Research Council grant (MR/R010757/1) and Wellcome Trust Biomedical Resource grant (218304/Z/19/Z).

Bibliographic references:

1. O. Blixt, et al., (2004) PNAS, (101), 17033-17038.
2. C. Gao, et al., (2019) Front Chem (7), 833.
3. S. Fukui et al, (2002) Nat. Biotechnol(20), 1011-1017
4. A.S. Palma, et al. (2014) Curr. Opin. Chem. Biol. (18), 87-94.
5. L. Wang, et al. (2014) Glycobiology (24), 507-517.
6. L. Klamer, et al. (2022) Glycobiology (32), 679-690.