Quantifying molecular glycan-lectin binding parameters for efficient cross-presentation

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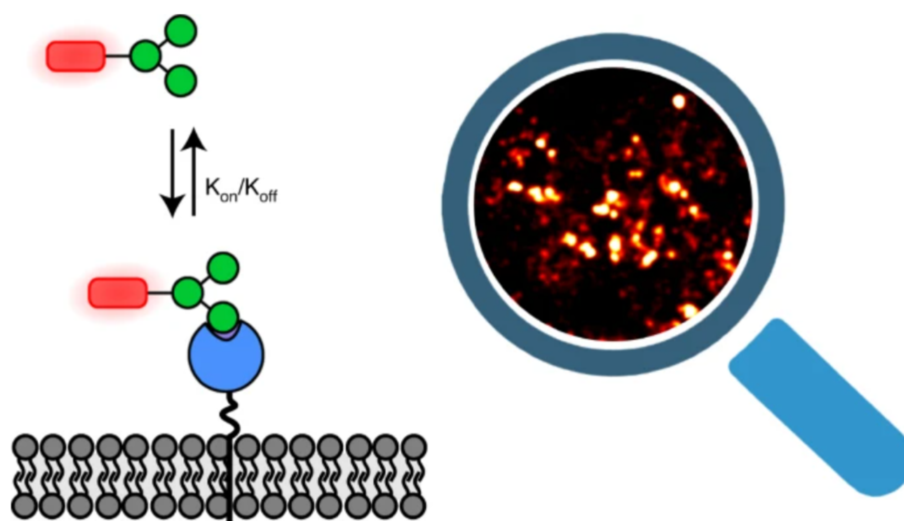
Obtaining glycan binding kinetics on living cells is hampered by their intrinsic weak lectin affinity and is therefore limited to *in vitro* techniques. To address this, we have developed the super-resolution microscopy technique Glyco-PAINT [1]. This novel methodology allows for the direct readout of single-molecule on and off-rates of labelled glycans on living cells and hereby provides the possibility for SAR studies on difficult-to-target lectins.

In this talk, I will showcase the Glyco-PAINT technique, including recent developments towards its use in studying the interaction of immune cells with glycans. In these studies, we investigate binding of high mannose and Lewis-type glycans to endocytic lectins such as the MR and DC-SIGN. Using a library of molecularly defined carbohydrate clusters we were able to obtain live-cell receptor density maps and correlate single-molecule binding events with glycan uptake. In a similar approach, these optimized glycan ligands were conjugated to synthetic long peptide (SLP) vaccines and evaluated for binding to primary murine immune cells. Here, a correlation of single-molecule binding parameters with the individual T cell cross-priming capabilities of the SLPs could be established obtaining crucial SAR insights. In conclusion, we present a novel method for measurement of live-cell binding of glycans that can be broadly applied to gain a further understanding of the efficacy of (synthetic) glyco-conjugates in many immunological synapses.



Graphical abstract of the GlycoPAINT technique

Bibliographic references:

[1] Riera, R. et al. Single-molecule imaging of glycan–lectin interactions on cells with Glyco-PAINT. Nat. Chem. Biol. 17, 1281–1288 (2021)