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Unravelling Functional Changes in Antibody Proteoforms Using Affinity CE-MS

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Summary

Antibodies recruit immune responses via interaction with different Fcγ receptors (FcRs). These interactions are strongly influenced by structural features including glycosylation. Unfortunately, common approaches, such as SPR provide an overall affinity response for all different glycoforms and assessment of their individual binding require tedious production or enrichment of specific forms. In particular, assessment of the influence of Fab glycosylation in binding represents a huge challenge due to the complexity of generating homogeneous forms.

In our lab, we have exploited the capabilities of mobility-shift affinity CE-MS to study the binding of antibodies and FcRs in a proteoform-resolved fashion. To this end, the FcR receptors were added to the background electrolyte whereas the mixture of antibody glycoforms were injected in the CE. We will show that the approach is able to determine the relative binding of different glycoforms based on the shifts on their mobility. For FcγRIIIa the obtained affinity profiles were benchmarked towards affinity LC using the same constructs providing similar results. Due to low amounts of receptor required, the developed affinity CE-MS platform was ideal for testing a large variety of FcRs, namely FcγRIIa, FcγRIIb, FcRn and FcγRIIIa and including different allotypic variants. As anticipated, Fc glycosylation was key for the binding. Hemiglycosylated antibodies showed strong decrease in the binding towards the FcγRs while non-Fc glycosylated forms showed near no binding. Fc-glycoforms behaved differently between receptors with clear differences for afucosylated and high mannose variants. Interestingly, different receptor allotypes also revealed some glycan-sensitive differences. Furthermore, we explored the potential of the approach to investigate the influence of Fab glycosylation in FcR binding. Our results showed an altered binding for the Fab-glycosylated variants. As Fab glycans are far from the binding site, the change in binding properties most likely correlates to a change in protein conformation in the interaction surface.