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What do we know about (your) antibodies?
Novel insights from novel techniques in mass spectrometry

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In our body we produce every day huge amounts of antibodies, of which many end up in circulation. It has been estimated that humans can make about 10^{15} distinct antibody clones, all exhibiting a slightly different sequence. This huge number has so far refrained many from charting whole serum antibody, or immunoglobulin (Ig), repertoires. We recently developed an LC-MS based antibody repertoire profiling method for studying immunoglobulins in a quantitative manner. We analyzed a variety of samples from both healthy as well as diseased donors and made some paradigm-shifting observations. Firstly, circulating Ig repertoires are much simpler than anticipated, dominated by a few hundred clones. Second, the clonal repertoires are entirely unique for each donor, both for IgG1 and IgA1 we found virtually no overlap between individuals. Conversely, longitudinal samples from the same (healthy) donor showed a far-reaching overlap even when samples were taken months apart. In the repertoires of severely ill patients, more plasticity was observed. Charting the Ig repertoires in diseased donors allowed the selection of Ig clones of interest, i.e., those emerging after the onset of disease. We demonstrate that these latter serum clones can be fully *de novo* sequenced by combining top-down and bottom-up analysis and iterative software algorithms to connect these layers of data. In this manner antigen-directed antibodies could be identified and developed into novel therapeutics.

All (sub)classes of immunoglobulins have unique structural features. In our work we also investigate the structures of IgA1 and IgM, and reveal that they are not always as described in text-books. I will present work through which we redefine the molecular composition of circulatory IgM. Using single-particle charge-detection mass spectrometry, mass photometry, proteomics, and immunochemical assays, we reveal that circulatory IgM is (re)defined by the universal presence of an additional protein component. We study the covalent attachment of this protein and evaluate its effect on the binding of IgM to several receptors. Lastly, our data reveal the distinctiveness of the circulatory and secretory IgM.