DNA origami reveals the spatial tolerance of antibodies.

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Rigidly organized protein patterns are typically either foreign or intracellular in most mammals and the immune system has thus evolved an efficient response to such structures. Notably, both the HPV and the HBV vaccines employ a particle display of dense arrangements of proteins to elicit a strong immune response. However, the molecular understanding for why antigen positioning appears important in early immune response remains largely unanswered. By precisely controlling the spatial display of antigens, coupled to a dynamic model that is able to recreate the data, we are now able to rigorously dissect what is bivalent binding, what is monovalent binding, what are the rates for mono- to bivalent, and in particular; how does the distance between antigens affect the affinity of different isotypes and classes of Abs.
We introduce a method where molecularly precise nanoscale patterns of antigens are displayed using DNA origami and immobilized in a surface plasmon resonance (SPR) setup. Using human antibodies where all carry identical binding domains, we find that all subclasses and isotypes studied here, bind bivalently according to a unique separation distance dependent curve spanning 3-17 nm with a distinct preference for antigens separated by approximately 16 nm, and that considerable differences in this *spatial tolerance* exist between IgM and IgG and between low and high affinity antibodies.

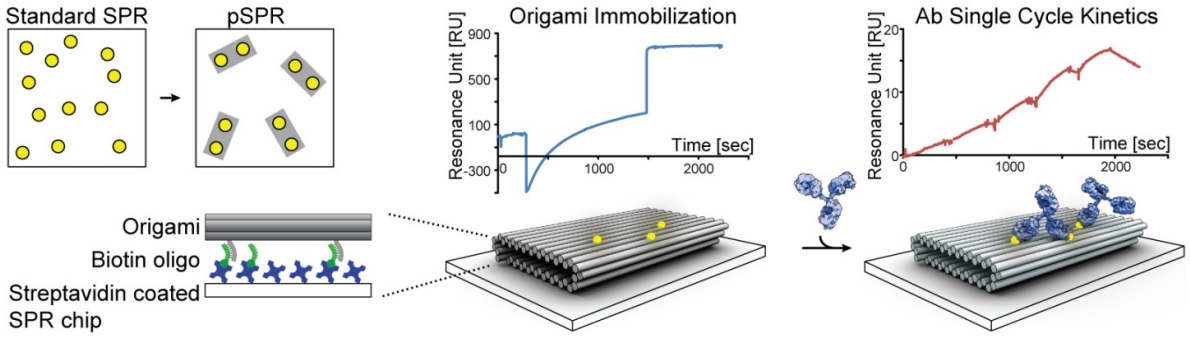

Figure 1: **The Patterned Surface Plasmon Resonance method.** In contrast to conventional SPR, which randomly arranges its ligands on the surface, the PSPR method utilizes DNA origami to pre-pattern the molecule of interest prior to immobilization. The antigen nanopatterns were fabricated using different combinations of antigen-decorated staple oligonucleotides. The antigen nanopatterns were immobilized onto a streptavidin-biotinlylated oligonucleotide surface via oligo hybridization to sequences protruding from the bottom of the origami, followed by an injection of increasing antibody concentrations, and finally a dissociation phase and the ensemble kinetic data for each pattern or distance can be obtained by fitting the binding curves.

References

[1] Binding to nanopatterned antigens is dominated by the spatial tolerance of antibodies, Shaw A, Hoffecker IT, Smyrlaki I, Rosa J, Grevys A, Bratlie D, Sandlie I, Michaelsen TE, Andersen JT and Högberg B, *Nature Nanotechnology*, **14**, p. 184–190 (2019)