2D binding properties as function on the applied force and the interaction time of single domain antibodies binding tumor markers

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Therapeutic antibodies are now a common treatment of major diseases, especially in cancer. While capable to bind soluble antigens, antibodies do often bind their target at the interface between an immune cell and a target cell or a pathogen surface. While affinity is usually measured with one reactant in solution at least (*i.e.*, three dimensional environment or 3D), these measurements do not take into account the physical aspects of cell-cell interface (*i.e.*, 2D) that include force and relative motion of molecules constrained at surfaces, which can modify the interaction time available before binding. Our purpose is to look for links between 2D affinity or kinetics and cellular response. To quantify 2D binding properties, we perform kinetic measurements of five single domain antibodies (sdAbs) against the tumor marker HER-2 using a Laminar Flow Chamber (LFC). In the LFC, HER-2 is coated to a microbead surface and interacts with single sdAbs bound to the chamber bottom surface in the presence of flow. This allows us to measure at single molecular level the association kinetics (as the number of bond formed during a given interaction time between molecules) and the dissociation kinetics (as bonds lifetimes) under forces. Forces vary in the piconewton range and interactions times in the millisecond range.

Our results suggest to classify measured sdAbs in two groups. In one group, binding decreases non-linearly when the interaction time decreases and bond lifetimes are not affected by force. In a second group, binding decreases linearly with interaction time and bond lifetimes are modified by force.

Our anti-HER-2 sdAbs can be fused with an anti-CD16 sdAb, forming bi-specific antibodies able to recruit NK cells toward HER-2 positive breast cancer cells. We selected one sdAbs of each group and produced the corresponding bi-specific antibodies. We will perform NK-tumor cell cytotoxicity experiments with these, and try to correlate the tumor cell killing with 2D binding properties. This study provides a physical characterization of antigen-antibody interactions and could be useful for the selection of antibodies in therapeutics.