Great stretches for your antibody workout

DNA origami unravel the role of target spacing in antibody binding

Multivalency is ubiquitous in biology. Proteins in living organisms hold together through multiple weak interactions, creating dynamic, yet stable complexes. Antibodies are a good example. Each antibody carries two binding sites for antigen and their simultaneous engagement is critical for strong binding, clearing of pathogens and efficacy in therapy. However, the exact benefits of bivalent binding, called avidity, have been difficult to predict across different antibodies. This is because avidity depends both on the antibody structure and on the spacing and orientation of the antigens on the target, such a viral particle or a diseased cell[1,2]. In this issue of Nature Nanotechnology[3], Shaw et al. use DNA origami to develop an elegant system to precisely control antigen spacing and thus establish its effects on antibody avidity. The results open new avenues to engineer antibodies to engage better with multivalent targets.

The two antigen binding sites on antibodies are located on two antigen binding fragment (F_{ab}) arms swinging on flexible hinges, which are anchored to the central fragment crystallizable (F_c) portion (Figure 1A). Hinges of different antibody classes, such as IgM, IgD, IgG, IgA or IgE, differ in structure and length, resulting in different ranges of F_{ab} arm movement. This allows the immune system to selectively produce antibodies that specialize in recognizing pathogens with certain surface features. Additionally, some antibody classes form dimers (IgA), or pentamers and hexameters (IgM). The high valency and avidity of these antibodies are important early in the immune response, before selection generates antibodies with optimal complementarity to the antigen. Once the antibodies bind, specific arrangements of their F_c portions dictate how they recruit effector systems that kill and clear the pathogen. Formation of IgG hexameters on the surface of the pathogen, for example, is required to activate the C1q component of the complement[4], while opposite-facing molecules in IgA dimers enchain growing bacteria[5].

Despite the general knowledge of antibody structure, and the mechanism by which they engage antigens, avidity has been difficult to generalize. Experimentally, it is difficult to measure association and dissociation rates of one F_{ab} arm, while the other is bound. Interestingly, observations of single antibodies binding to arrayed antigens using high-speed atomic force microscopy revealed that the antibody is not statically bound to one location, but walks around[6]. This can be explained by dynamic instability of the bivalent state caused by stretching or twisting of the antibody. The mechanical stress releases one of the F_{ab} arms and upon subsequent re-binding to a different antigen, the antibody takes a step. Understanding of this phenomenon is essential to comprehend how ordering of antigens on targets influences antibody avidity and activation of effector mechanisms.

To unequivocally determine the effects of antigen spacing on antibody binding, Shaw et al. used DNA origami techniques to produce nanoscopic rods and bricks, which they decorated with small antigens, termed haptens, at precise locations (Figure 1B). The origami could be directly deposited on surface-plasmon resonance chips, allowing antibody binding to be measured without the complication of cross-linked, higher-order complexes. As expected, when the haptens were far apart, antibody binding was monovalent. For haptens spaced 3 to 17 nm apart, antibodies bound bivalently, but the avidity varied with spacing, gradually improving up to an optimum at 16 nm, and sharply dropping thereafter. This is the

first time that nanometer changes in antigen spacing were shown to dramatically affect antibody binding. The findings are consistent with the antibody hinge both constraining sampling of the antigenic surface and acting as an entropic spring, providing a destabilizing tension on the bivalent state.

The results also extend our understanding of antigen binding by different antibody classes and subclasses. Of IgG antibodies, the IgG_3 subclass bound best to sub-optimally spaced antigens, and this depended on the length of its hinge. Monomeric IgM, interestingly, could reach antigens furthest apart, up to 29 nm. The significance of this finding may be important to consider in the context of IgM expressed on the surface of B cells, where the structure of its hinge has been linked to B cell activation and tolerance[7].

The authors go one step further and develop a framework to model multivalent binding using Markov chains. After calibration for a single antigen-antibody bond, the model predicted ensemble binding to an arbitrary antigenic pattern. This modelling approach may thus assist studies of dynamics in a broad range of multivalent systems.

Is the hinge the only structure that dictates antibody spatial tolerance? Since the bivalent state is under mechanical stress, its stability is predicted to depend on the resistance of the antigen binding sites to mechanical forces[8]. This is consistent with the authors' observation that binding to a low-affinity antigen had much worse spatial tolerance than binding to a high-affinity antigen that also has better mechanical resistance. Avidity thus integrates information not just from antigen spacing and the structure of the hinge, but also from the biomechanics of the antigen-binding site. A better characterization of the mechanics of antibody-antigen bonds, for example using DNA origami nanosprings[9], will be helpful in the future.

The work of Shaw et al. advances us towards a possibility to design antibodies and antibody-like molecules that better target pathogens or cancer cells. It may be beneficial to engineer flex into antibodies to robustly bind antigens irrespectively of spacing, or the opposite, make antibodies selective for specific antigen arrangements. After all, these molecules do seem to enjoy just the right amount of stretch.

Figure Legend:

Figure 1. The effect of antigen spacing on bivalent antibody binding to pathogens is often unknown (A). By modelling the system using DNA origami, target sites can be precisely positioned and the effect of their spacing quantified (B).

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Figure 1