Fidabio

RAPID SIZING AND CHARACTERISATION OF BISPECIFIC ANTIBODIES

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Introduction

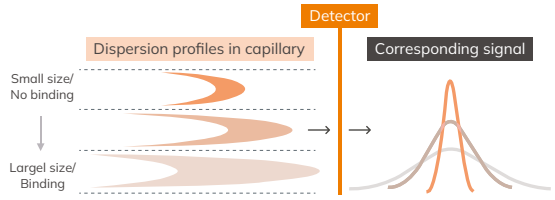
We present how the Fida 1 characterises the formation of ternary complex between bispecific antibodies and target antigens. Using µL of sample, we carried out full titrations and measured the affinity of the antigens, the size of the binary and ternary complex, as well as the cooperativity. The cooperativity is a parameter of utmost importance as values much smaller than 1 would disqualify any bispecific antibody for use as a therapeutic. Sixty individual bispecific antibody samples were analysed in a fully automated way within 12 hours, using

40 nL of sample per sizing

Methods

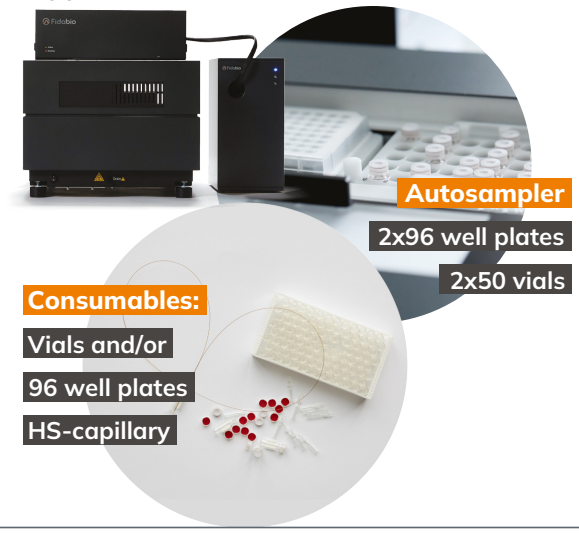
Two antigens (Ag1, Ag2) are tested for ternary complex formation with 12 different bispecific constructs. Further, full titration is done with increasing concentration of a selected antibody and constant concentration of Ag1 and labelled Ag2.
In the Fida 1 assay, either one of two antigens serves as fluorescent "indicator" while the bispecific constructs serve as the "analyte". Experiments were performed on a Fida 1 instrument employing 480 nm LED detection. FIDA is a capillary-based technology which measures biomolecular size of protein/complexes, by converting dispersion profiles to a size (hydrodynamic radius, Rh) readout. The sensitive measurement of size changes reveal binding events.

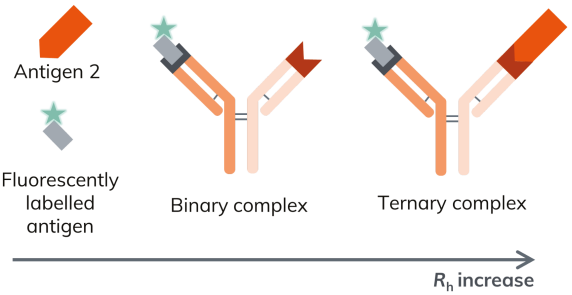
FIDA METHOD



$$D = \frac{a^2}{24\sigma^2} t_R \qquad R_h = \frac{k_b \cdot T}{6 \cdot \pi \cdot \eta \cdot D}$$

Fida 1





Results

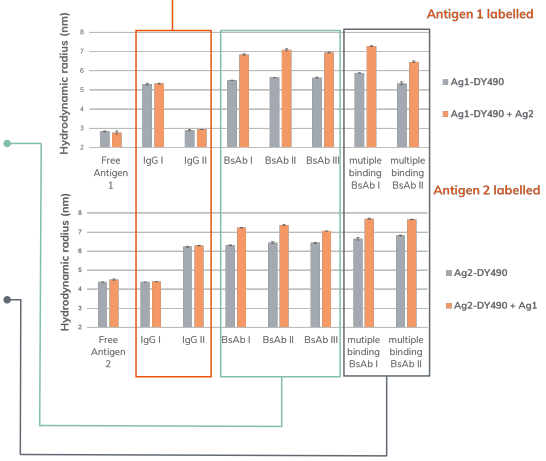
TERNARY COMPLEX FORMATION CONFIRMATION

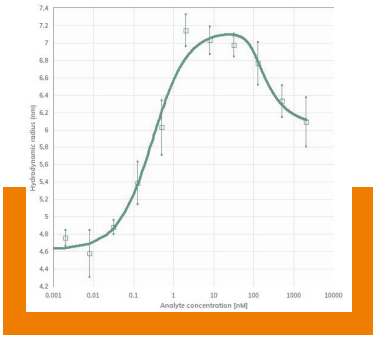
To confirm that the overall architecture of the bispecific antibody has not inhibited the ability to form the ternary complex, all constructs were screened with labelled Antigen 1 and subsequently with labelled Antigen 2.

The positive IgG controls cause a change in hydrodynamic radius upon binding to the labelled target antigen. Based on the size changes, we infer that IgG I binds to Antigen 1, while IgG II binds to Antigen 2.

When it comes to bispecific antibodies BsAb I, BsAb II, and BsAb III, we see additive increase in size when both Ag are present, indicating binding to both antigens.

Finally, the bispecific antibodies that has multiple fragments specific for the same antigen doesn't cause a further size increase, indicating that extra binding sites for each antigen are not beneficial.





FULL TITRATION FOR CHARACTERISATION OF TERNARY COMPLEX FORMATION

A single bispecific antibody (BsAb I) was chosen for full binding characterisation. The size of the free labelled Ag2 is 4.61 ± 0.09 nm, K_D of Ag1 is 7.8 nM, K_D of Ag2 is 0.3 nM and the size of the binary complex is 6.06 nm corresponding well with the previously measured value. The cooperativity is 1.6 indicating no detrimental effects from the binary architecture.

Conclusions

The present study shows how FIDA enables rapid screening for both binary and ternary complex formation, determining the size of bispecific antibodies using 40 nL of sample per sizing. This offers a clear advantage over conventional methods in terms of information gained in a single experiment and sample usage. FIDA can be further applied to fully characterise all binding parameters of ternary complexes using only µL of bispecific antibody and nL of target antigen saving precious and costly material.