CHARACTERISING MULTIVALENT COMPLEX FORMATION USING CONTROLLED COMPLEX LIFETIME IN FIDA 1

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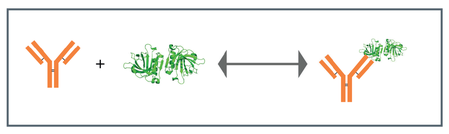
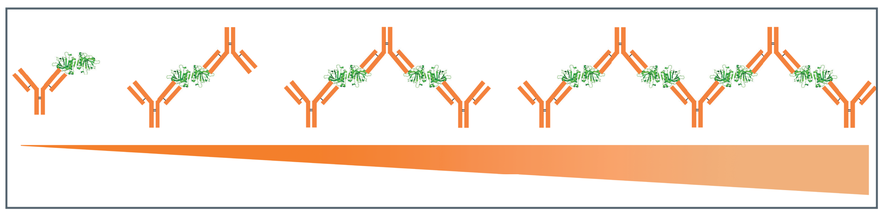
Introduction

Multivalent interactions often lead to the formation of random, disorganised networks that make it extremely challenging for current biophysical methods to characterise the primary interaction event. Such interactions are commonly seen in immune response related systems, cellular regulations and antibody interactions with dimeric target leading to multiple binding events. Formation of agglutination prone hetero-oligomeric networks make it challenging to decouple the 'true' binding event from the agglutination process and can skew the biophysical characterisation as well as affinity determination of the system.

Here, we demonstrate how Fida 1 can be used to probe the initial interaction and the impact of agglutination between bovine β-lactoglobulin (BLG) & polyclonal anti-BLG rabbit IgG by using Flow induced dispersion analysis (FIDA) with capillary mixing method. Controlled mixing of the binding partners in the Fida 1 capillary enables complex formation during sample measurement. The adjustable mobilisation pressure allows controlled complex lifetime prior detection, allowing for quantification of interaction, aggregation, soluble agglutination, complex size and affinity (K_D) determination.



Primary interaction



Aggregation propensity

Method

The experiments were performed on a Fida 1 instrument employing 480 nm LED-fluorescence detection. FIDA is a capillary-based technology which measures biomolecular size of fluorescently labelled proteins / complexes, by converting dispersion profiles to a size readout of hydrodynamic radius (R_h).

In this Fida 1 assay, alc480-labelled BLG was used as fluorescent "indicator" at 26 nM concentration in PBS buffer. The anti-BLG rabbit polyclonal IgG was used as "analyte" and was titrated from 0 - 6.3 µM.

The same set of samples were measured at 3 different mobilisation pressures, i.e., 100, 400 and 600 mbar which corresponds to reaction times of 1.2, 1.8 and 7.7 minutes. All measurements were run on a pre-coated capillary (L: 1 m, ID: 75 µm, L_{eff}: 84 cm).

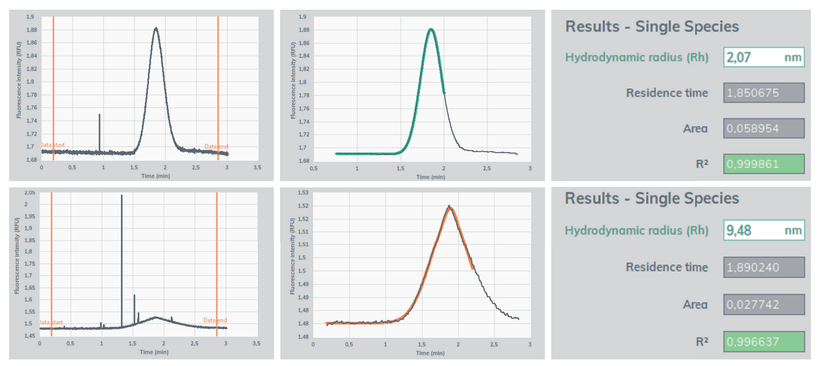
In-capillary mixing time	1.2 min	1.8 min	7.7 min
K_D (nM)	13	36	51
Indicator size	2.32 nm	2.03 nm	2.1 nm
Complex size	11.2 nm	10.2 nm	9.69 nm

Results

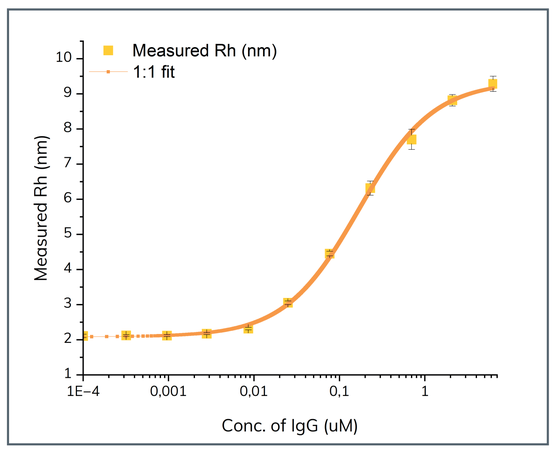
Binding assay between anti-BLG IgG and alc480-BLG was performed as a capillary mixing (Capmix) experiment such that indicator-analyte mixing times can be controlled. The current assays were performed for 3 different mixing times of 1.2, 1.8 and 7.7 minutes. Alc480-BLG was first measured in presence and absence of 6.3 µM anti-BLG IgG. Complex formation was confirmed as a clear increase in R_h from 2.09 ± 0.03 nm to 9.23 ± 0.23 nm. The measured complex size, however, is much larger than expected size of the 336 kDa quaternary complex i.e., ~6.6 nm (assuming a spherical shape).

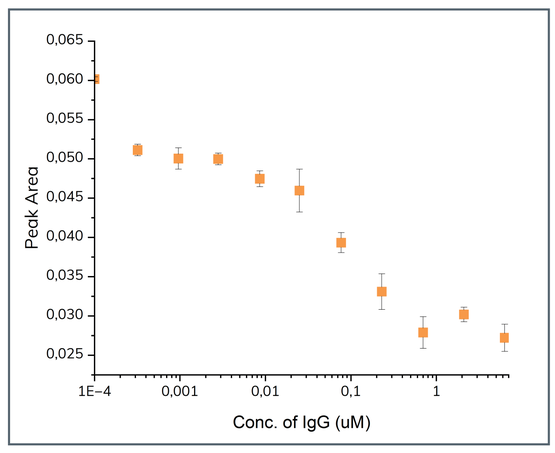
Interaction outcomes suggests:

- formation of higher order oligomers
- oligomers appear to be unstable (seen as aggregation spikes)

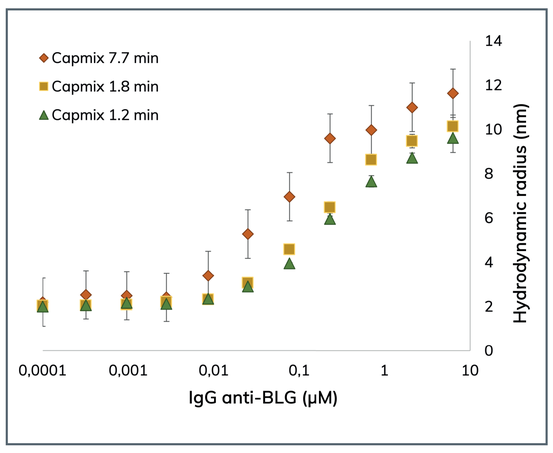


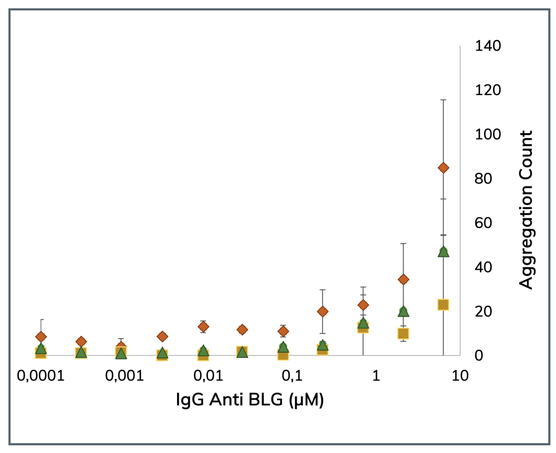
Full titration of anti-BLG was performed from 0 – 6.3 µM as Capmix and a mixing time of 1.8 min (at 400 mbar mobilisation pressure). The obtained binding curve was analysed as 1:1 binding (sigmoidal curve on left). Further, analysis of fluorescent peak area from same measurements indicated loss of soluble complexes as a function of IgG concentration (area curve on the right).





The complex lifetime of same samples was measured at different mixing times of 1.2, 1.8 and 7.7 minutes. At mixing time of 7.7 min, the observed complex sizes were much bigger, indicating more advanced aggregation. This also impacted the measured KD. Furthermore, the number of aggregates were observed to increase with higher IgG concentration.





Conclusions

Fida 1 provides in-solution characterisation of large complexes formed by multivalency of the antigen/antibody. The controlled capillary mixing times allows for probing the influence of aggregation on **complex size and affinity**, and offers an easy way of analysing complex interactions with minimal sample consumption.