APPLICATION NOTE

Assessing C1q interaction using Gator BLI

ABSTRACT

Therapeutic monoclonal antibodies (mAbs) exert their effects through a variety of mechanisms, including antigen neutralization, receptor modulation, and Fc-mediated immune effector functions. Complement-dependent cytotoxicity (CDC) is an Fc-mediated effector mechanism initiated through the classical complement pathway, in which C1q serves as the recognition molecule, binding to appropriately arranged Fc regions of antibodies on the target cell surface. This initiation triggers a proteolytic cascade that leads to membrane attack complex (MAC) formation and target cell lysis. Typical approaches for IgG-C1q interaction measurements often rely on the presence of a κ-light chain for antibody immobilization, and this application note presents this methodology as well as alternatives for when this light chain is absent. Here, we provide a protocol for biolayer interferometry (BLI)-based comparison of IgG-C1q binding using the Gator® platform.

INTRODUCTION

C1q: An early indicator of classical complement pathway engagement

Complement-dependent cytotoxicity (CDC) is the outcome of the classical complement pathway, not a pathway itself. The pathway begins when C1q, the recognition component of C1, binds Fc regions arranged on a target surface, and antibody density and spatial organization govern that recruitment [1-3]. Multivalent binding activates C1r and C1s, starting the cascade that ends in membrane attack complex (MAC) formation and cell lysis. C1q binding is the first checkpoint: necessary for activation, not sufficient on its own, and therefore a sensitive comparability attribute for biosimilars.

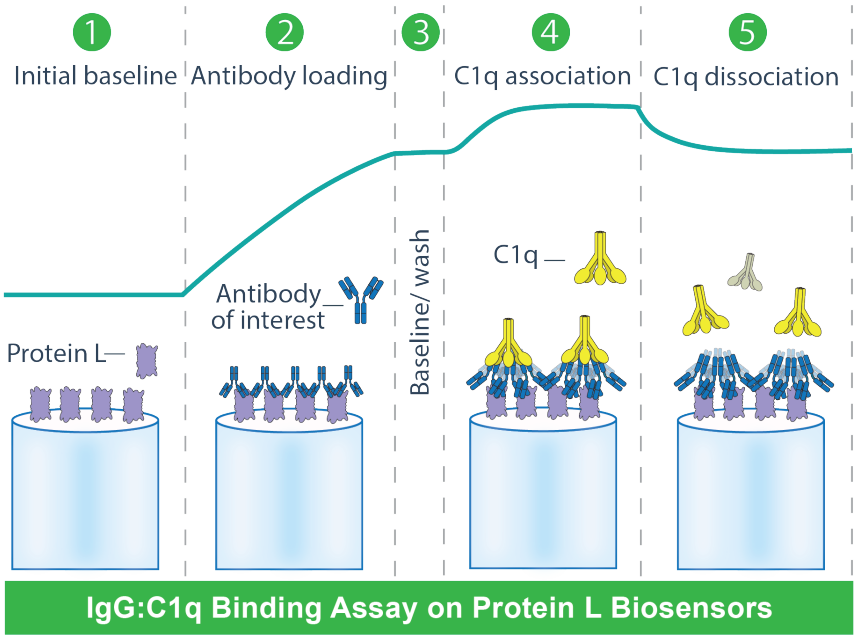

Figure 1: C1q binding assay on Protein L biosensors. Captured antibody is exposed to a C1q dilution series, then buffer. One C1q engages several adjacent Fc regions, so the response reflects antibody density as well as affinity. Traces are schematic; this note measures the recognition step only.

Biolayer Interferometry (BLI) for C1q binding assessment

For any κ-light-chain-containing antibody, Protein L is often the best capture strategy for two reasons. First, C1q is a 410 kDa hexamer and gives a substantial response per binding event, but one C1q molecule engages several adjacent Fc regions at once. Capacity is therefore set by footprint rather than by the amount of IgG immobilized, and the high capture density of a ProL biosensor gives a correspondingly larger C1q response. Second, ProL captures via the light chain, leaving the Fc exposed for the conformational arrangements that enable C1q binding. Published BLI approaches load IgG onto a Protein L sensor to ~4 nm shift before C1q addition [4, 5]. Our internal testing found that a 2-4 nm shift on a high-density ProL biosensor gives adequate antibody density to observe C1q binding under the conditions tested. The approach also requires no labeling of C1q or antibody, and ProL is a reusable capture surface.

However, because Protein L only binds to κ-light chains (and among those, only the VκI, VκIII and VκIV subtypes, and not the VκII subtype), this approach is not broadly applicable. This presents a challenge for CDMOs that need a way to characterize C1q binding activity on a variety of candidates without requiring a particular κ light chain. Thus, a more consistent approach that is suitable for both κ- and λ-light chain-containing IgGs would be 1) to biotinylate and immobilize the IgGs onto SA probes, or 2) direct amine-coupling to the AR probes. In this application note, due to the similarity in protocols, we will give a protocol for validating C1q interaction using ProL and SA biosensors together within the main text, as well as Amine Reactive biosensors (Appendix 1). Gator BLI biosensors do not show noticeable non-specific binding of C1q to the AR biosensor after quenching, reducing data processing time and improving overall accuracy of the resulting data.

MATERIALS AND METHODS

Reagents and equipment

Item	Origin	Specification / Part Number
Gator® Pivot	Gator Bio	PN 110055
BLI 384-Tilt Plate	Gator Bio	PN 130161
ProL Probes	Gator Bio	PN 160007
Streptavidin (SA) Probes	Gator Bio	PN 160002
PBST Buffer	User supplied	-
Q Buffer	Gator Bio	PN 120010
Regen buffer (No salt, pH 2.0)	Gator Bio	PN 120008
Antibody	User supplied	-
C1q	Abcam	AB282858
EZ-Link NHS-PEG4-Biotin	Thermo Fisher Scientific	#A39259

Biosensor selection

For the reasons given in the introduction, this protocol will primarily focus on a Protein L biosensor approach, and will give adaptations for an SA biosensor approach as well as for antibodies that lack a κ-light chain. An amine-reactive biosensor can also be used to attach the antibody directly to the biosensor (See Appendix 1 for protocol).

While SA and AR approaches do not require a specific light chain type, they do not have the Fc orientation advantage inherent in a light-chain capture approach. However, the resulting C1q interaction K_D for Adalimumab using SA capture showed only small deviations relative to the established ProL method (Table 1), suggesting that these differences in Fc availability are minor provided that a sufficient amount of antibody is immobilized on the sensor surface.

Added step for SA biosensors: biotinylation protocol

Antibody samples were prepared in a BSA-free PBS buffer at pH 7.4. EZ-Link NHS-PEG4-Biotin (ThermoFisher #A39259) was added at a ratio of 3:1 molar coupling ratio of biotin-to-antibody and incubated on ice for 30 minutes. The reaction was terminated by running samples through washed desalting columns to remove free biotin.

At this ratio, biotinylation did not prevent C1q binding. We recommend biotinylating using a biotin molecule that contains a linker (e.g., PEG4 or LC) to distance the antibody from streptavidin for stable capture and flexibility to enable C1q access.

BLI C1q affinity assay workflow overview

The C1q assay is structured in a five-step process, beginning with antibody immobilization followed by C1q association. Previous investigations have shown that C1q activity can diminish when immobilized onto the biosensor as ligand through biotinylation or amine coupling [5]. Our internal investigations with immobilizing C1q via either amine coupling or biotinylation observed a similar loss of C1q activity, which informed this assay workflow.

The protocol below is compatible with ProL and SA biosensors; AR requires additional activation/quench steps due to covalent coupling chemistry – see Appendix 1 for protocol. Figure 2 shows the full assay workflow for Adalimumab on SA and ProL biosensors.

All steps were performed on a Gator® Pivot instrument at 30 °C, 1000 rpm shaker speed.

Step 1 – Biosensor equilibration and baseline.

- Pre-wet biosensors in PBST for 600 seconds.
- Instrument transfers biosensors to PBST buffer well.
- Baseline time: 60 seconds.

Step 2 – Antibody immobilization.

- Instrument transfers biosensors into antibody wells.
- Antibody concentration: typically 100 µg/mL for faster assay times.
- Association time: 600 seconds (ProL) or 1200 seconds (SA) – higher antibody concentrations may reduce the time required to achieve the desired loading response.

Step 3 – Second baseline.

- Instrument transfers IgG-loaded biosensors to buffer blank well.
- Second Baseline time: 30 seconds.

Note: if second baseline loses more than 20% of its signal after loading, ProL engagement may be poor. Consider switching to a streptavidin-based protocol if this is the case.

Step 4 – C1q association.

- Instrument transfers IgG-loaded biosensors to a series of wells containing C1q at 20, 10, 5, 2.5, 1.25, and 0.625 µg/mL (48.8, 24.4, 12.2, 6.10, 3.05, and 1.52 nM).
- Association time: 60 seconds.

Step 5 – C1q dissociation.

- Instrument transfers biosensors to well containing only PBST to observe C1q dissociation.
- Dissociation time: 60 seconds.

Biosensor regeneration (ProL workflow only).

- Instrument transfers biosensors between Regen Buffer (no salt, pH 2.0) and PBST buffer 3-5 times in 5-second pulses.
- ProL biosensors can typically be reused for up to 20 cycles depending on regeneration efficiency and assay conditions.

Note: Optimize regeneration conditions for unpurified supernatants or for antibody formats other than intact IgG.

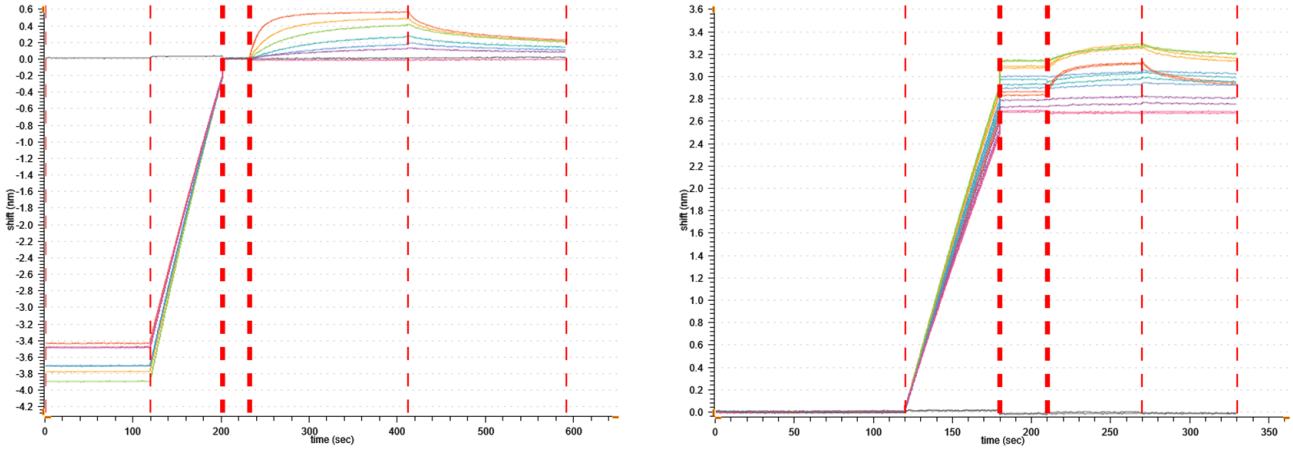

Figure 2: Full assay workflow result for Adalimumab C1q assay using Streptavidin (left) or Protein L (right) immobilization strategies. The SA graph is aligned by beginning of association, while the ProL graph is aligned to first baseline (see Figure 3 for the aligned association and dissociation steps). Channel 8 (grey trace) is an unloaded sensor that interacts with 10 µg/mL C1q, which shows no **non-specific binding** of C1q at highest concentration to the biosensor.

Calculating interaction affinity

Assays were assessed using the following parameters in the GatorOne analysis software.

1. Align to association, average 0.
    - Inter-step correction: On.
    - Averaging range set to 0 for both association and dissociation.
2. Single reference subtraction using loaded biosensor interacting with buffer blank.
3. Fitted using a 1:1 Langmuir fitting model.

RESULTS AND DISCUSSION

Comparison of ProL vs SA based methods for C1q interaction study

Figure 3 compares C1q binding to Adalimumab immobilized using ProL and SA biosensors. The same biotinylated antibody preparation was used for both methods, allowing comparison of the capture strategies without differences in antibody labeling between samples. Both methods produced concentration-dependent C1q binding responses, although differences in response magnitude and apparent binding kinetics were observed. Similarly, activity using an Amine-Reactive (AR) biosensor will also yield similar results because the attachment/orientation configuration is highly similar to the streptavidin approach, though without the 3D polymer matrix present upon the surface of SA biosensors (Appendix 1). Despite biosensor differences, no non-specific binding was observed in any of the biosensors tested (Figure 2, Figure 5).

Instead, the differences in these kinetics measurements can be attributed to the sensors used. The Streptavidin biosensors do not preferentially capture via the light chain, meaning that Fc orientation may not always be favorable. High antibody density is easy to achieve on an AR sensor or ProL biosensors, which have high capture site density. Gator SA biosensors are optimized for conventional kinetics and space streptavidin apart on a neutrally charged 3D polymer matrix. The lower capture density leaves fewer adjacent Fc regions within reach of a single C1q molecule, limiting both the response at saturation and the stability of the complex once formed (Table 1).

For Adalimumab, ProL and SA capture give values within 2x of one another (Table 1), suggesting that the method of interaction is largely irrelevant if broad comparison of C1q binding activity is the sole purpose of the experiment. When comparing against a reference antibody, both antibodies should be immobilized in the same manner for C1q binding studies to limit this minor deviation.

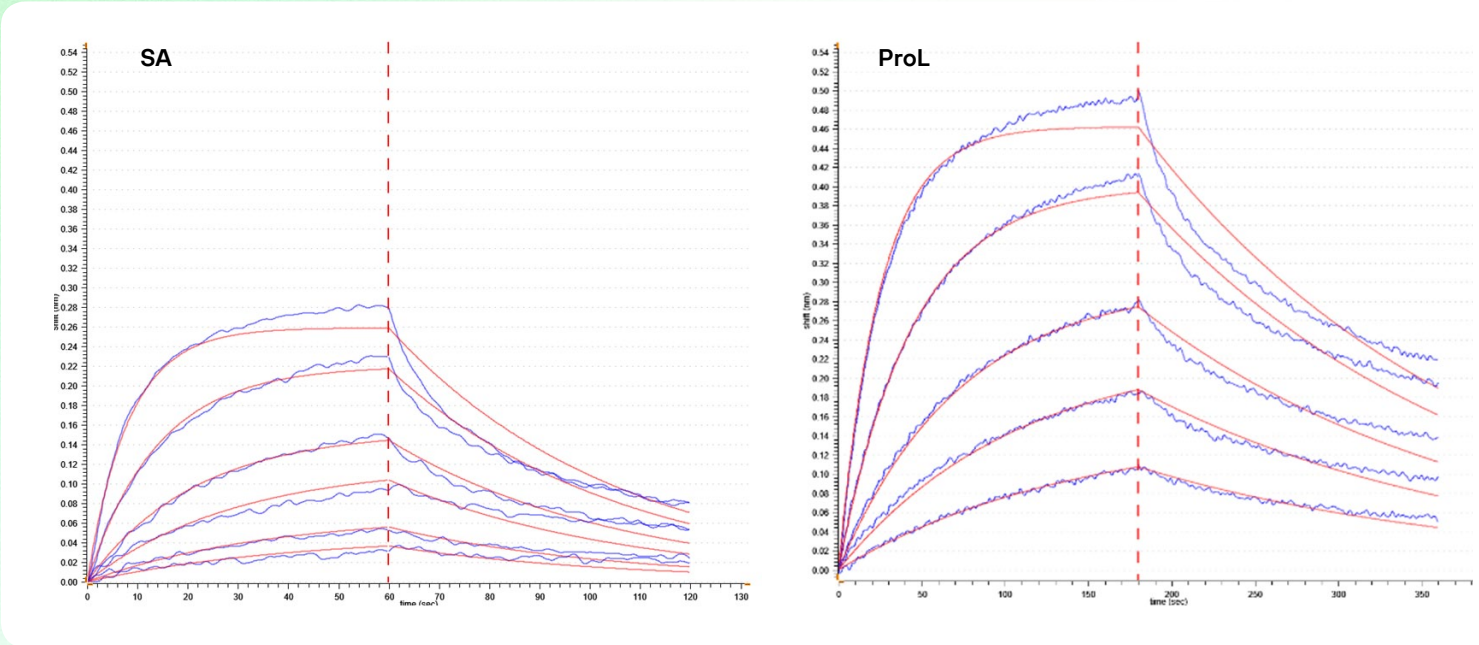

Figure 3: Protein L (right) and SA (left) capture method comparison using biotinylated Adalimumab for both samples. K_Ds are given in Table 1. The two panels are plotted on different time scales; signal amplitudes may be compared directly, but durations may not.

Affinity ranking for antibody isotypes aligns with literature

In addition to comparing the ProL vs SA methods, Figure 4 below shows the C1q association and dissociation steps for IgG1, IgG2, IgG3, and IgG4 to show the suitability of this method to various antibody isotypes to determine whether Gator BLI results align with literature for different isotypes.

Gator BLI results using the ProL approach for C1q binding kinetics align with established literature for ranking of different isotypes' affinity for C1q. Affinities are ranked with IgG3 being the strongest interaction, followed by IgG1 then IgG2 [6]. The IgG2-C1q interaction was minimal because its affinity is weak relative to the C1q concentration range tested, and the IgG4-C1q interaction is so weak that it wasn't observed at all at a 20 µg/mL top concentration, even on the high-capacity SA XT biosensor. Soluble, antigen-free IgM also showed no C1q binding, as expected, because its C1q binding sites are not exposed in its planar conformation until it engages antigen. Fitting results are shown in table 1 below. Note: the IgG1, IgG2, and IgG3 isotype assays were run to maximum possible load on ProL in order to enable direct comparison with sufficient signal for IgG2 visualization. The K_D value obtained for IgG1 at this high load is consistent with the measurement taken at a 3.45 nm load for Adalimumab.

C1q binding is hexavalent and avidity-driven, so a 1:1 model does not describe the underlying mechanism. The constants reported here are apparent values tied to a defined antibody surface density, and the purpose of this assay is to show that a test antibody matches its reference under identical capture and loading conditions rather than to report intrinsic kinetics. Table 1 below compares different biosensors and IgG subtypes and their interactions with C1q using a similar format. As mentioned above, the deviation between Adalimumab datasets between ProL and SA biosensors aligns with previous experience with C1q assays with other collaborators (a difference of about 2x, with SA biosensors appearing to have a weaker apparent affinity, a larger K_D).

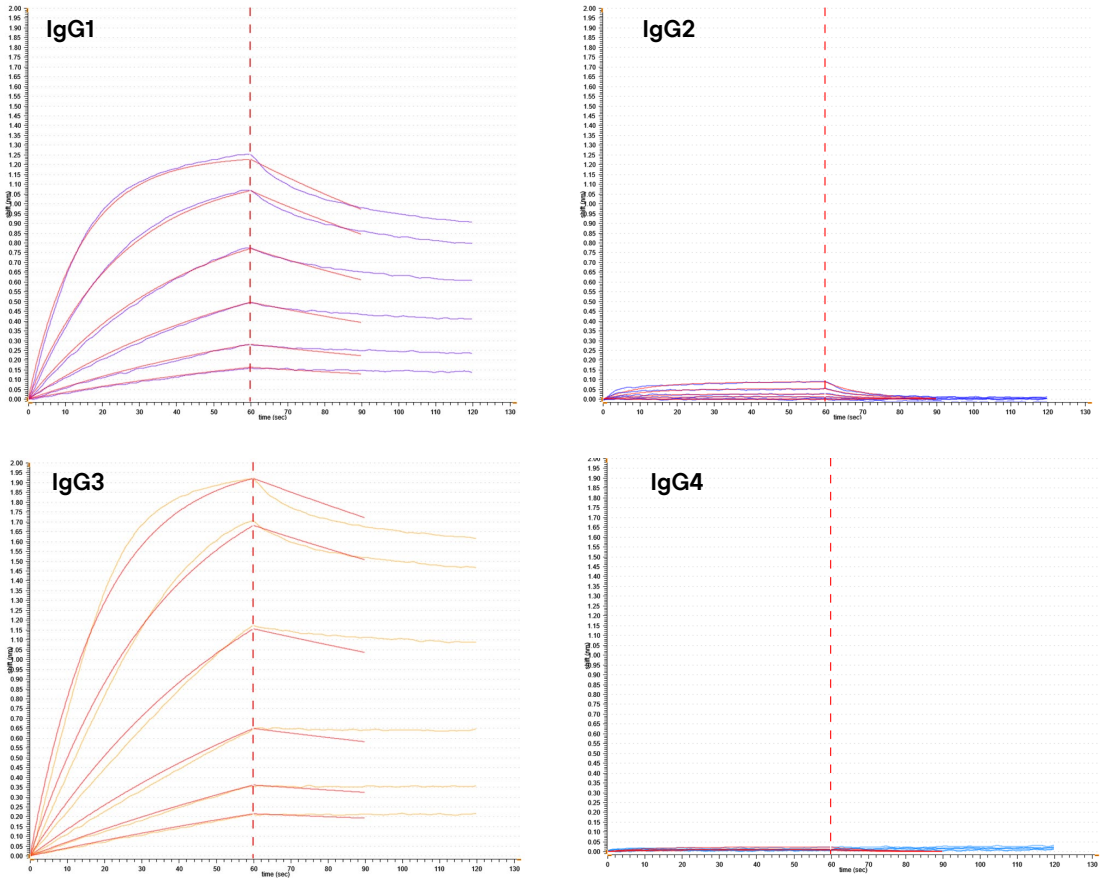

Figure 4: Sensorgrams for IgG1, IgG2, IgG3, and IgG4 using this method on ProL sensors. Loading responses, R_{max} and kinetics fitting values are contained in Table 1.

Antibody name	Isotype	Biosensor used	Avg load nm shift	C1q binding R_{max} (nm)	k_{off} (s-1)	k_{on} (M-1s-1)	K_D (M)
Adalimumab	IgG1	ProL	3.45	0.52	$7.08*10^{-3}$	$1.25*10^{6}$	$5.67*10^{-9}$
Adalimumab	IgG1	SA	2.68	0.32	$1.79*10^{-2}$	$1.99*10^{6}$	$9.01*10^{-9}$
Matuzumab	IgG1	ProL	2.91	0.43	$1.77*10^{-2}$	$1.25*10^{6}$	$1.42*10^{-8}$
Anti-C5	IgG1	ProL	3.05	0.52	$1.99*10^{-2}$	$1.46*10^{6}$	$1.36*10^{-8}$
IgG1 isotype	IgG1	ProL	8.33	1.30	$7.83*10^{-3}$	$1.30*10^{6}$	$6.04*10^{-9}$
IgG2 isotype	IgG2	ProL	6.74	0.09	ND	ND	ND
IgG3 isotype	IgG3	ProL	7.26	2.00	$2.47*10^{-3}$	$1.06*10^{6}$	$2.33*10^{-9}$
IgG4 isotype	IgG4	SA XT	3.91	0.00	ND	ND	ND
IgM	IgM	SA XT	5.62	-0.02	ND	ND	ND

Table 1: Affinity results for numerous IgGs of various isotypes as well as IgM tested using Protein L (ProL), Streptavidin (SA), or Streptavidin XT (SA XT). Differences in measured affinity are explained in the Results and Discussion section. Though some IgG2:C1q binding was observed (Figure 4), assay concentrations used were too low to calculate a K_D for IgG2 (ND). IgG4 and IgM binding was not observed (ND). Note: IgG1, 2, and 3 isotype experiments were performed at high load to enable detection of IgG2-C1q binding at the same concentrations of C1q while still being directly comparable. The IgG1 isotype and Adalimumab K_D values are consistent with one another despite this difference in load.

CONCLUSION

Herein we demonstrate a method to characterize C1q binding to IgG antibodies using the Gator® BLI platform using the traditional Protein L approach as well as alternative approaches featuring biotinylation or amine-reactive chemistries. IgG-C1q interaction has previously been shown to have high reproducibility [5], and while small deviations were observed between ProL and SA methods, these deviations (within 2x) are to be expected given the change in capture orientation. The binding data generated using this protocol are consistent with previously published results, showing strong agreement with reported IgG–C1q interaction measurements from similar, avidity-driven assay formats [4, 5].

This application note uses SA biosensors, ProL biosensors, and AR biosensors (Appendix 1) to show how C1q-antibody interactions can produce different kinetics values as a result of the differences in biosensor chemistry and composition. Regardless of the biosensor used, no non-specific binding of C1q to the biosensor was noted for any of the selected biosensors at appropriate assay concentrations. This positions Gator BLI as a well-suited label-free method for CDMO customers that need to perform C1q validation experiments without introducing the uncertainties inherent in double-reference subtraction. Get in touch with us to discuss your C1q characterization workflow.

REFERENCES

[1] C. A. Diebolder, F. J. Beurskens, R. N. de Jong, R. I. Koning, K. Strumane, M. A. Lindorfer, M. Voorhorst, D. Ugurlar, S. Rosati, A. J. R. Heck, J. G. J. van de Winkel, I. A. Wilson, A. J. Koster et al., "Complement is Activated by IgG Hexamers Assembled at the Cell Surface," Science, vol. 343, pp. 1260-1263, 2014. doi: 10.1126/science.1248943

[2] J. Strasser, R. N. de Jong, F. J. Beurskens, G. Wang, A. J. R. Heck, J. Schuurman, P. W. H. I. Parren, P. Hinterdorfer and J. Preiner, "Unraveling the Macromolecular Pathways of IgG Oligomerization and Complement Activation on Antigenic Surfaces," Nano Letters, vol. 19, no. 7, pp. 4787-4796, 2019. doi: 10.1021/acs.nanolett.9b02220

[3] S. J. Cleary, Y. Seo, J. J. Tian, N. Kwaan, D. P. Bulkley, A. E. Bentlage, G. Vidarsson, E. Boilard, R. Spirig, J. C. Zimring and M. R. Looney, "IgG hexamers initiate complement-dependent acute lung injury," The Journal of Clinical Investigation, vol. 134, no. 11, e178351, 2024. doi: 10.1172/JCI178351

[4] W. Zhou, S. Lin, R. Chen, J. Liu and Y. Li, "Characterization of antibody-C1q interactions by Biolayer Interferometry," Analytical Biochemistry, vol. 549, pp. 143-148, 2018. doi: 10.1016/j.ab.2018.03.022

[5] D. Jiao, Y. Hu, L. Liao, H. Zhang, L. Wang, Y. Xiao and J. Tian, "Qualification and robustness study of a Biolayer interferometry-based C1q binding assay for therapeutic antibodies," Journal of Pharmaceutical and Biomedical Analysis, vol. 268, 117229, 2026. doi: 10.1016/j.jpba.2025.117229

[6] G. Vidarsson, G. Dekkers and T. Rispens, "IgG Subclasses and Allotypes: From Structure to Effector Functions," Frontiers in Immunology, vol. 5, no. 520, 2014. doi: 10.3389/fimmu.2014.00520

APPENDIX 1 – PROTOCOL AND RESULTS FROM GATOR AMINE REACTIVE BIOSENSORS

Methods: Amine labeling and IgG-C1q interaction kinetics

All steps carried out at 1000 rpm, 30 °C.

Step 1 – Biosensor equilibration and baseline

- Pre-wet Amine Reactive (AR) biosensors in PBST for 600 seconds.
- Instrument transfers biosensors to PBST buffer well.
- Baseline time: 60 seconds.

Step 2 – Activation

- Instrument transfers biosensors into EDC/NHS mixture
- Mixture composition:
  - 400 mM EDC.
  - 200 mM sNHS.

Step 3 – Antibody loading

- Instrument transfers biosensors to well containing purified antibody.
- Antibody concentration: 100 µg/mL.
- pH: 6.0 (pH scouting carried out beforehand with the specific antibody used).
- Time: 300 seconds.

Step 4 – Quench

- Instrument transfers biosensors to ethanolamine wells to quench remaining active amine groups on sensor surface.
- Composition: 1 M ethanolamine in ddH2O.
- Time: 300 seconds.

Step 5 – Equilibration baseline

- Instrument transfers IgG-loaded biosensors to Q buffer well.
- Equilibration baseline time: 300 seconds.

Step 6 – Assay baseline

- Instrument transfers IgG-loaded biosensors to Q buffer well.
- Assay baseline time: 120 seconds.

Step 7 – C1q association

- Instrument transfers IgG-loaded biosensors to a series of wells containing C1q at 20, 10, 5, 2.5, 1.25, and 0.625 µg/mL (48.8, 24.4, 12.2, 6.10, 3.05, and 1.52 nM).
- Association time: 60 seconds.

Step 8 – C1q dissociation

- Instrument transfers biosensors to well containing only PBST to observe C1q dissociation.
- Dissociation time: 60 seconds.

Reagent	Supplier / brand	Specification / Part Number
EDC	Thermo Fisher Scientific / Pierce	22980
sNHS / sulfo-NHS	Thermo Fisher Scientific / Pierce	24510
Ethanolamine	MilliporeSigma / Sigma-Aldrich	E9508-100ML
PBS	Thermo Fisher Scientific / Gibco	10010023
Desalting columns	Thermo Fisher Scientific / Zeba	89882

Results and discussion:

Amine Reactive biosensors generated a similar result for IgG-C1q interactions, with a faster initial association and dissociation (Figure 5). This is consistent with capture orientation. Amine coupling attaches the antibody at random surface lysines, including those in the Fc region, so a portion of the immobilized IgG presents its Fc in a way that limits C1q access, as with the SA approach. ProL captures via the light chain and leaves the Fc exposed, making it more likely that C1q engages several adjacent IgGs at once. This produces a smoother dissociation curve and a slower off-rate (Figure 3).

As mentioned in the conclusion section above, for users striving to show C1q binding data as part of an antibody QC/potency measurement, ProL, SA, or AR methods may be used to compare the antibodies of interest, and results will be comparable provided that the same biosensor is used in each experiment.

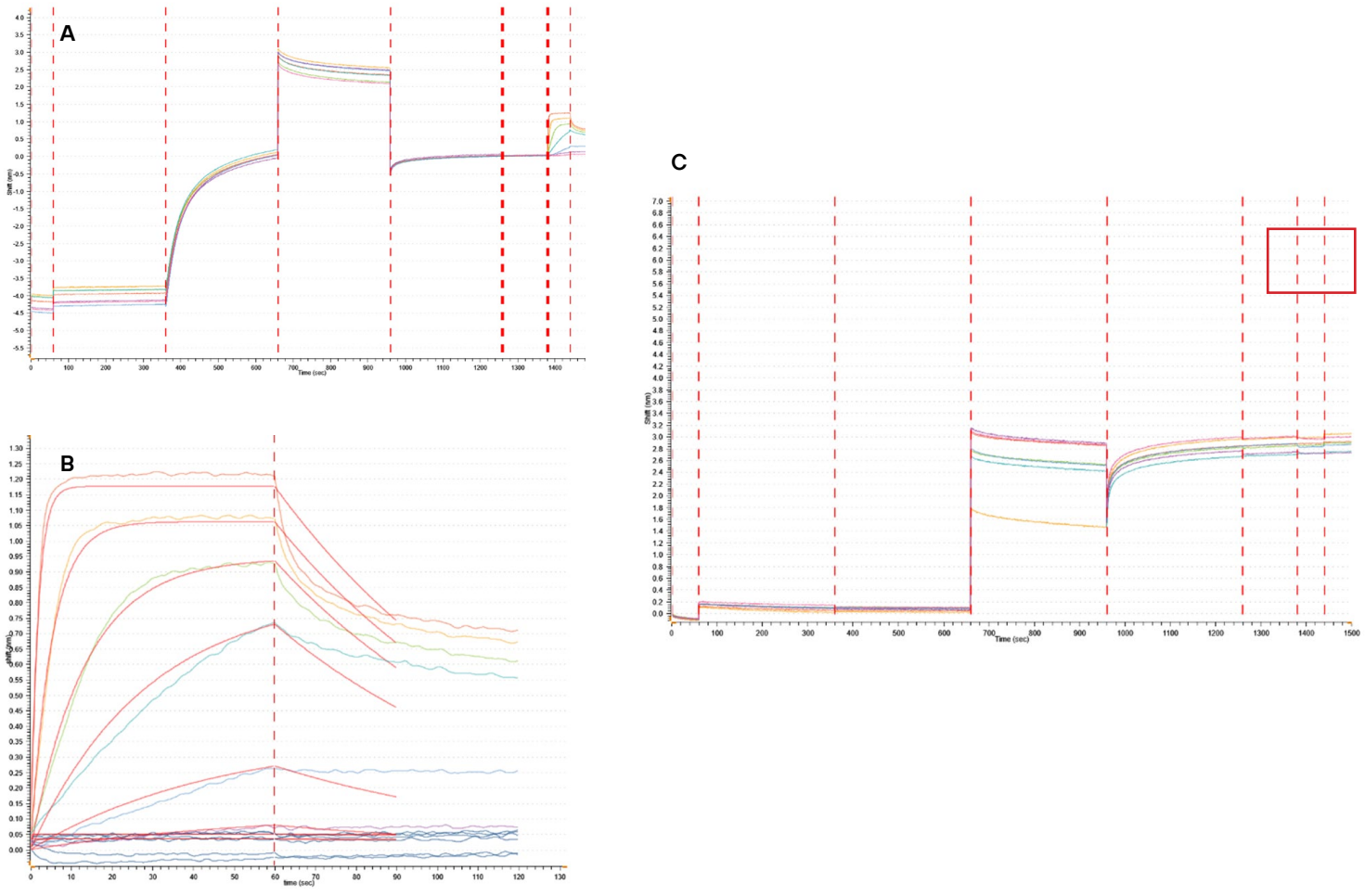

Figure 5. A: Full sensorgram of Amine Reactive sensor for IgG3 as ligand. **B**: processed data from A showing C1q binding to immobilized IgG3 and with reference traces from Figure 5C in dark blue below. **C**: Amine reactive sensor with no antibody ligand loaded shows no C1q binding activity (step 7, red box).