High throughput detection of antibody self-interaction by bio-layer interferometry

Tingwan Sun, Felicia Reid, Yuqi Liu, Yuan Cao, Patricia Estep, Claire Nauman, and Yingda Xu*

Protein Analytics; Adimab, LLC; Lebanon, NH USA

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Self-interaction of a therapeutic monoclonal antibody may cause aggregation, high viscosity, or low solubility, which limits its likelihood of being successfully developed as a drug (i.e., the developability of the drug). Ideally, antibodies with self-interaction tendencies could be rapidly eliminated early in the discovery stage, with minimum material consumption, to minimize downstream risks. This screening process is usually performed by low to medium throughput assays such as self-interaction chromatography (SIC) or cross-interaction chromatography (CIC). SIC measures the retention time of an antibody as it flows across a column conjugated with the same antibody of interest (or “self”). A longer retention time results from stronger self-interaction of the molecule of interest, and this in turn is usually correlated with lower solubility. Material consumption and throughput, however, greatly limit the general applicability of this assay to a range of tens to hundreds of candidate molecules. Similar to SIC, CIC measures the retention time of an antibody as it flows across a column conjugated with polyclonal human serum antibodies. Later elution of an antibody in CIC indicates exposure of surfaces prone to formation of non-specific interactions, and this usually serves as an indicator of undesirable solution properties, including lower solubility. CIC is a more attractive option in that a single column can be used to screen multiple antibodies, which minimizes sample consumption and individual column preparation and improves throughput. Some antibodies do show strong binding to the unconjugated, quenched SIC or CIC columns, which limits analyses of them by either assay. Recently, non-chromatographic methods such as self-interaction nanoparticle spectroscopy (SINS), and affinity capture (AC)-SINS using gold nanoparticles, and surface plasmon resonance (SPR) based assays, have been reported to predict or confirm antibody self-interaction. Concurrently, bio-layer interferometry has emerged as a technology for the detection of biomolecular interactions using label-free biosensors. Here, we describe a high throughput method to detect antibody clone self-interaction by bio-layer interferometry (CSI-BLI) with low material consumption.

Nine antibodies, including seven human IgG1 antibodies against multiple targets that were discovered in-house and two control antibodies with known solubility in phosphate buffered saline (PBS) were used in this study. The control antibodies, CNTO607 (negative solubility control, ~13 mg/ml as reported by Wu et al.) and adalimumab (positive solubility control), were made recombinantly as human IgG1 from published V region sequences and expressed in HEK293 cells. The in-house discovered antibodies were pre-selected based on SIC and CIC assays to represent different levels of self-reactivity. The SIC column was prepared by coupling > 1 mg of each antibody to a 1 ml HiTrap column (GE Healthcare # 17-0716-01), followed by ethanolamine quenching. Approximately 5 ug of that antibody was tested at a flow rate of 0.1 ml/min using PBS as a mobile phase on an Agilent 1100 series HPLC system. During the SIC assay, Mab4 and 7 showed similar retention times to adalimumab while Mab1, 2, 6 and CNTO607 showed delayed retention time (Fig. 1A). Mab3 and 5 showed significant column binding, whereas retention time on the blank column was greater than that on the SIC column. The same set of antibodies was also tested by CIC. The CIC column was prepared by coupling ~30 mg of human serum polyclonal antibodies (Sigma #I4506) to a 1 ml HiTrap column, followed by quenching with ethanolamine. Other experimental conditions were identical to those of SIC. Good correlation was observed between these two assays (Fig. 1A). Presumably the polyclonal human serum antibodies conjugated to the CIC column provide a sufficiently diverse sampling population to include the type(s) of interaction responsible for the original antibody self-interaction. These correlations were observed between the SIC and CIC assays for the seven in-house antibodies. The two control antibodies were included to confirm results. Strong self-interaction responses in the CSI-BLI assay also show delayed retention times in SIC and CIC. This method allows hundreds of candidates to be screened in a matter of hours with minimal material consumption.
antibodies were later characterized by DLS at ~50 mg/mL in PBS (except CNTO607). Detectable large particles were observed for Mab1, 2, 3, and 6, but not for Mab4, 5, 7 and adalimumab (data not shown).

Figure 1. (A) Comparison of retention time for 9 antibodies on CIC vs. SIC. (B) Self-binding response difference between adalimumab and CNTO607 on AHC, AHQ, and AR2G sensors. (C) Self-binding response difference between CNTO607 and adalimumab under varied AHQ sensor loading densities (0.3–1.0 nm) and solution antibody concentrations (0.1–1.3 μM). (D) Self-binding response for 9 antibodies under optimized conditions. Measurements were made in quadruplicate. (E) Comparison of BLI self-binding response vs. SIC retention time for 9 antibodies.
To further improve throughput and reduce material consumption, CSI-BLI using the Octet® RED384 was developed to assess antibody self-interaction. In this assay, the antibody of interest was loaded to a BLI biosensor to measure any significant self-binding response. High solubility antibodies such as adalimumab generally have minimal self-interaction and generate a low self-binding response in this assay. On the other hand, antibodies with poor solubility, such as CTNT607, have stronger self-interaction, and we postulate that avidity effects further increase the binding response because this weak self-interaction is usually not observable when monovalent Fab is used in solution. To test which biosensor type generates the greatest response difference between adalimumab and CTNT607, anti-human Fc capture (AHC), anti-human IgG quantitation (AHQ), and amine reactive second-generation (AR2G) biosensors were evaluated. Control antibodies were loaded onto AHC or AHQ sensors, followed by blocking with an in-house produced human IgG1 Fc. The antibodies were also covalently coupled to AR2G biosensors followed by quenching. The self-binding test was performed with 1 uM antibody in 100 ul PBSF (0.1% BSA in PBS) solution on the Octet® RED384. The AHQ biosensor generated the greatest response difference (Fig. 1B), and thus was chosen for further signal optimization in biosensor loading density and solution antibody concentration. This observation aligned with our previous experience during kinetic screening of antigen binding to antibody-loaded sensors. Even though AHC sensors allowed higher IgG loading capacity than AHQ sensors, the antigen binding responses were usually lower. AHC and AHQ sensors are constructed in a very similar fashion, both using goat anti-human Fc polyclonal antibodies for human IgG capturing, but with different linker types and lengths. A shorter linker is used and there is no cross-linking of polyclonal goat anti-human Fc antibodies to AHQ sensors. In the case of AHC sensors, a longer linker and cross-linking treatment provide higher loading capacity and a more stable baseline for kinetic screening. Crowding effects, however, may lower the active surface binding concentration of IgGs on the sensor and decrease antigen binding response.

Control antibodies were loaded on an AHQ biosensor to 0.3, 0.5, 0.8, or 1.0 nm (by controlling loading time), then tested for self-binding in a 0.1, 0.2, 0.5, 1.0, or 1.3 uM antibody solution after blocking the biosensor with human IgG1 Fc. As shown in Figure 1C, a greater response difference was obtained at higher biosensor loading densities. More importantly, the antibody concentration in solution had a larger effect on the CSI response difference between the positive and negative control antibodies. A loading density of ~0.8 nm and 1 uM antibody solution concentration were chosen as the final screening conditions.

Under these optimized conditions, CSI-BLI was used to test self-interaction in quadruplicate for the set of nine human monoclonal antibodies discussed above and the results were compared with the SIC and CIC data. As shown in Figure 1D, Mab4 and 7 have responses similar to that of adalimumab, while Mab1, 2, 6 and CTNT607 have responses significantly greater than that of adalimumab. These four antibodies also show delayed retention times on SIC and CIC (Fig. 1A), indicating that CSI-BLI has a positive correlation with these column-based chromatographic assays (Fig. 1E). When performing DLS for Mab1, 2, and 6 at 50 mg/mL in PBS, large particles were also observed, confirming their aggregation propensity (data not shown). Moreover, CSI-BLI also works well with antibodies that are incompatible with SIC or CIC, such as Mab3 and 5, where severe blank column binding is observed. In CSI-BLI, Mab5 showed a low self-binding response and Mab3 showed a higher self-binding response (Fig. 1D), which correlates well with the DLS data (data not shown).

In this study, all antibodies were formulated in PBS buffer at pH 7.4 prior to SIC, CIC or CSI-BLI assays. Buffer composition is a critical factor that influences antibody self-interaction, and consequently developability attributes, including solubility. If an antibody shows minimal self-interaction in a relatively harsh formulation buffer such as PBS, it usually suggests good developability properties. Adalimumab was chosen as a positive control for developability, and antibodies with significantly higher self-binding responses (greater than 0.1 nm) in CSI-BLI than adalimumab are considered potentially problematic downstream. The choice of response cutoff value is dependent upon selection output. A less stringent response cutoff may be used when fewer biologically relevant clones are available.

In CSI-BLI, the Fc region of the antibody is directionally captured on the biosensor so that the Fab region is accessible for binding by the Fc or Fab portion of an antibody in solution via hydrogen bond pairing, ionic interaction, or hydrophobic interaction. Rather than relying on cumulative effects on retention time in the chromatographic methods to detect these weak interactions, sensitive BLI technology allows for direct monitoring of antibody self-binding. CSI-BLI has unparalleled throughput compared with CIC or SIC in that a plate of 96 antibodies can be tested for self-binding within 2 h, rather than a few days. This high throughput is contributed partly by the design of the Octet® RED384, where 16 channels are available for data collection simultaneously. Furthermore, only 15 ug of each antibody is necessary for this assay, making CSI-BLI even more attractive for early-stage discovery screening, where a large number of candidates are produced at low quantity.

Disclosure of Potential Conflicts of Interest
No conflicts of interest were disclosed.

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