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Measurements of SARS-CoV-2 antibody dissociation rate constant by chaotrope-free biolayer interferometry in serum of COVID-19 convalescent patients

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1. Introduction

The coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is still rampant in much of the world with the appearance of new variants. In the race to develop analytical assays that characterize the production of SARS-CoV-2 antibodies, kinetics measurements of antigen-antibody binding interactions are critical to understanding the functional efficiency of SARS-CoV-2 antibodies. Previously reported chaotrope-based avidity assays that rely on artificial disruption of binding do not reflect the natural binding kinetics. This study developed a chaotrope- and label-free biolayer interferometry (BLI) assay for the real-time monitoring of receptor binding domain (RBD) binding kinetics with SARS-CoV-2 spike protein in convalescent COVID-19 patients. An improved conjugation biosensor probe coated with streptavidin-polysaccharide (SA-PS) led to a six-fold increase of signal intensities and two-fold reduction of non-specific binding (NSB) compared to streptavidin only probe. Furthermore, by utilizing a separate reference probe and biotin-human serum albumin (B-HSA) blocking process to subtracted NSB signal in serum, this BLI biosensor can measure a wide range of the dissociation rate constant ($k_{off}$), which can be measured without knowledge of the specific antibody concentrations. The clinical utility of this improved BLI kinetics assay was demonstrated by analyzing the $k_{off}$ values in sera of 24 pediatric ($\leq 18$ years old) and 63 adult ($>18$ years old) COVID-19 convalescent patients. Lower $k_{off}$ values for SARS-CoV-2 serum antibodies binding to RBD were measured in samples from children. This rapid, easy to operate and chaotrope-free BLI assay is suitable for clinical use and can be readily adapted to characterize SARS-CoV-2 antibodies developed by COVID-19 patients and vaccines.

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2 antibodies following infection and vaccination, there has been an increasing interest in assessing the quality and affinity maturation of the antibody responses (Clark et al., 2021; Hurlburt et al., 2020). The strength of antibody binding could influence the protective efficiency against SARS-CoV-2, not least by determining neutralization potency, a major correlate of protection after vaccination (Benner et al., 2020; Klasse, 2014; Luo et al., 2020a). The ratio of the dissociation rate constant ($k_{off}$) [1/s] over the association rate constant [1/M s] is equal to the equilibrium dissociation constant $K_D$ [M], the reciprocal measurement of affinity. Thus, $k_{off}$ is one determinant of affinity: the lower $k_{off}$ all other things being equal, the higher the affinity (Klasse, 2016). One advantage of $k_{off}$ is that because of its dimension (reciprocal time), it can in principle be determined as without knowledge of the concentration of specific antibodies in a polyclonal serum. The $k_{off}$ measurement potentially could be applied as routine clinical test to assess antibody dissociation kinetics, related to avidity, after SARS-CoV-2 infection or vaccination.

Unfortunately, the concept of avidity has been used inconsistently in the literature. One strict definition is the degree to which antibody binding is strengthened through multivalency. If the intrinsic affinity is the affinity of monovalent binding of one paratope to one epitope, and the functional affinity describes the binding of, e.g., a bivalent IgG, influenced by the extent to which the antigen spacing allows bivalent binding, then the avidity can be quantified as (intrinsic $K_{D}$/functional $K_{D}$). How much greater than 1 that ratio is depends, incidentally, on how much the bivalency reduces the $k_{off}$ of the binding and thereby increases the functional affinity. Paradoxically, the term avidity has come to be used to describe the binding property that increases with affinity maturation. This phenomenon in reality describes the intrinsic affinity. Indeed, the use of low coating density, which disfavors bivalent binding, was a rational means of approximating measurement of intrinsic affinity, although it was labeled avidity (Wei et al., 2010). Against this background it is clear that $k_{off}$ is a binding parameter that influences intrinsic affinity – which tracks affinity maturation – as well as functional affinity and avidity in the original sense. If $k_{off}$ is measured for polyclonal antibodies in serum, it would constitute an important means of characterizing the development of antibody responses during infection and post vaccination.

Previously, other methods have been employed in what became known as “avidity assays.” Most antibody avidity methodologies on different platforms employ a denaturing agent (such as urea) to artificially disrupt antibody-antigen binding (Bauer, 2021; Huang et al., 2020; Liu et al., 2020; Luo et al., 2020a). However, the accuracy of chaotrope-avidity assays relies on the assumption that only interaction, not the structural integrity of the paratope or the epitope is affected by the chaotrope. Variations in chaotrope resistance are subject to complex influences to measure binding strength or avidity, which may cause conflicting results (Alexander et al., 2013; Demnission et al., 2018; Klasse, 2016). It is technically challenging but of pressing need to develop rapid, accurate and reliable kinetics measurements to evaluate avidity in a non-denaturing condition in order to investigate the functional efficiency and efficacy of SARS-CoV-2 antibodies.

Biolayer interferometry (BLI) is a label-free and fluids-free technology for direct measurement of biomolecular interactions (Conception et al., 2009; Sun et al., 2008). When antibody concentrations are known, e.g., for monoclonal antibodies, BLI measures the antibody-antigen association rate constant ($k_{on}$ [1/M s]), dissociation rate constant ($k_{off}$/[s]), and equilibrium dissociation constant ($K_D$ [M]) in real-time without the need of time-consuming steps of conjugating fluorophores, enzymes or other signaling elements to the protein. However, the BLI technology is not commonly used for clinical sample measurements, primarily because of the challenge of non-specific binding (NSB) interactions from the complex composition of the serum (Auer et al., 2015). NSB generates a signal that cannot be easily distinguished from the specific binding signal, leading to the inaccurate measurements. Since NSB interactions are resistant to chaotrope agents, chaotrope-avidity assays could not be properly used for complex clinical specimens. In that case, minimizing the NSB interference without adding chaotropic agents is challenging for the clinical application of BLI kinetics measurement.

This study describes the development of a novel BLI assay that could directly monitor changes in the dissociation kinetics between SARS-CoV-2 antibody in human sera and the receptor binding domain (RBD) of the viral spike protein. This new BLI assay does not apply any chaotropic agent and is designed to minimize NSB in serum matrix by the use of the streptavidin-poly saccharide (SA-PS) coated probe and a reference probe. This novel design allows for a direct, real-time quantification of $k_{off}$ for monitoring the kinetics of antibody dissociation. Using this new BLI assay, we compared the SARS-CoV-2 antibody dissociation rate constant between pediatric and adult convalescent patients to better understand the age-related immune responses against SARS-CoV-2 virus.

2. Materials and methods

2.1. Reagent and materials

Streptavidin (SA) probe (Gator Bio, CA, USA 160002), 96-well black plates (Greiner Bio-One, Germany 655,209), 96-well Max plates (Gator Bio 130,018), SARS-CoV-2 receptor binding domain protein (RBD, Fc Tag) (SinoBiological, China 40,592-V02H), Albumin Human (Sigma Aldrich, MO, USA A9731), EZ-Link NHS-LC-LC-Biotin (Thermo Fisher Scientific, MA, USA 21343), 10K MWCO Dialysis Cassette (Thermo Fisher Scientific 87,730). PD-10 column (GE Healthcare, IL, USA 17-0851-01). Q Buffer consists of 0.2% BSA, 0.02% Tween-20, 0.05% Sodium azide in PBS pH 7.4, Gator Bio 120,010. Rabbit SARS-CoV-2 Spike antibody (Sino biological 40,591-T62).

2.2. Streptavidin-poly saccharide (SA-PS) conjugate

For SA-PS conjugation, a synthetic polysaccharide with a molecular weight of approximately 400 kDa with 5% of the sugar residues modified to contain amino groups was reacted with succinimidyl 6-[3-[2-pyridyl dithio]propionamido] hexanoate (SPDP; Invitrogen, CA, USA) in phosphate buffered saline (PBS; pH 7.4) for 1 h, then purified with a PD-10 column (GE Healthcare, IL, USA). The thiol on the PS-SPDP were de-protected by adding dithiothreitol (Sigma Aldrich, MO, USA), incubating for 1 h at room temperature, followed by purification of the polysaccharide-SH on a PD-10 column. Succinimidyl 4-[N-maleimidomethyl]cyclohexan-1-carboxylate (SMCC; Thermo Fisher Scientific) was incubated with the streptavidin (Agilent SA10-100) at a 10 to 1 M ratio for 1 h at room temperature in PBS, followed by purification on a PD-10 column. The PS-SH and Cy5-Streptavidin-SMCC were mixed at a 1 to 1 wt ratio and allowed to react overnight at room temperature. N-ethylmaleimide (Sigma Aldrich) was then added to react with residual thiols and after 30 min the conjugate was purified with a Sepharose CL-4B (GE Healthcare) column to remove unconjugated streptavidin.

2.3. Streptavidin and streptavidin-poly saccharide probes preparation for BLI

Aminopropylsilane probes (Gator Bio, CA, USA 160005) were equilibrated in sodium phosphate buffer (pH 3.8), then incubated for 2min with 1 mg/ml bis (sulfosuccinimidyldimethyl)cyclohexan-1-carboxylate (SMCC; Thermo Fisher Scientific A39266) in sodium phosphate buffer. Probes were incubated in SA (Agilent SA10-100) or SA-PS conjugate for 5min, then exposed to sodium phosphate with 15% sucrose and dried for 30 min at 40 °C.

2.4. Biotin labeling of RBD and human serum albumin (HSA)

To generate biotin-RBD (B-RBD): 1 mg RBD was reconstituted to 1 mg/ml in PBS pH 7.4, and then dialyzed at 4 °C overnight against PBS.
pH 7.4 using a 3 ml 10 K MWCO Dialysis to create dialyzed RBD. EZ-Link NHS-LC-LC-Biotin was dissolved in Dimethylformamide (DMF) to 10 mg/ml just before use. 10 μl of 10 mg/ml NHS-LC-LC-Biotin was added to 1 mg of dialyzed RBD (15:1 M ratio), incubated for 1 h at room temperature, and purified by a PD-10 column. The concentration was measured using UV–Vis adsorption measurement at 280 nm (extinction coefficient of RBD is 1.3 (mg/ml)⁻¹ cm⁻¹).

To generate biotin-HSA: 3 mg HSA was dissolved in PBS pH 7.4 to 2 mg/ml and filtered through a 0.2 μm PES syringe filter (Corning, 431,229). EZ-Link NHS-LC-LC-Biotin was dissolved in DMF to 10 mg/ml just before use. 40 μl of 10 mg/ml NHS-LC-LC-Biotin was added to 1.5 ml of 2 mg/ml HSA (15:1 M ratio), incubated for 1 h at room temperature, and purified using a PD-10 column. The concentration was measured using UV–Vis adsorption measurement at 280 nm (extinction coefficient of HSA is 0.53 (mg/ml)⁻¹ cm⁻¹).

### 2.5. Biolayer interferometry assay

The BLI assay was performed using the Gator™ Label-Free Bioanalysis instrument (Gator Bio, Palo Alto, CA, USA) and software version 1.January 6, 1203. Two SA-PS probes (one sample probe and one reference probe) were used for each sample measurement. To set up the assay, the probes were prewetted in Q buffer for 300 s. The measurement with sample probe started with 60 s baseline measurement in Q buffer, then 60 s probe loading with 1 μg/ml B-RBD, 60 s probe wash in Q buffer, 240 s probe saturation with 40 μg/ml B-HSA, 60 s probe wash in Q buffer, 300 s probe incubation in sample well (association step), followed by 1200 s probe incubation in Q buffer (dissociation step). Reference probe measurements were performed identical to the probe measurement but no biotin-RBD loading was applied (reference probe is incubated in Q buffer instead) (Fig. 1). To measure serum samples, serums were 1:9 diluted in Q buffer. Residual values chosen were all under ±10% of the maximum signal of the fitted curve, and R² and X² values were above 0.90 and below 3 (Sultana and Lee, 2015). The imprecision was determined by running a low specific binding signal sera sample three times per day on three different days. The imprecision of specific binding signal and \( k_{off} \) measured by coefficient of variation (CV) was 8.85% and 16.32%, respectively. The method detection limit (MDL) was reported by measuring specific signal of multiple negative samples mean + 2*SD (Standard deviation), 20 replicates of three different pooled negative samples in three days. The MDL of specific binding signal was 0.0069 nm, the MDL of \( k_{off} \) was 9.97E-6.

### 2.6. Clinical serum specimens

This study was performed at NewYork-Presbyterian Hospital/Weill Cornell Medical Center with approval by the Institutional Review Board. Analysis was performed on de-identified remnant serum samples collected between April and July 2020 from 63 adult (age: 25–85, median: 51 years old) and 24 pediatric (aged: 0–15, median: 11 years old) patients confirmed positive with SARS-CoV-2 total antibody and IgG assays (Yang et al., 2021a,b). Pooled-negative serum contained a mixture from 10 serum samples confirmed negative by the SARS-CoV-2 total antibody assay.

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Fig. 1. Schematic illustration and polyclonal antibody validation of the SARS-CoV-2 antibody kinetics assay. A) BLI kinetics measurement in SARS-CoV-2 antibody positive serum samples. The SA-PS probe is loaded with Biotin-RBD and then saturated with Biotin-HSA as blocker. The probe is subsequently incubated into the sample well and then dissociation buffer to capture RBD specific antibodies and measure the dissociation rate (\( k_{off} \)) respectively. another probe with unloaded RBD is used as reference probe for NSB measurement. B) Data processing steps to calculate the specific binding signal and the \( k_{off} \) values.
2.7. Statistical analysis

Correlations between two numerical variables were assessed by the Spearman correlation coefficient. Summary statistics are presented as mean with SD or median with interquartile range (IQR) for continuous variables and frequency with proportion for categorical variables. Paired or unpaired 2-tailed $P < 0.05$ was considered statistically significant in comparison of two age groups. Analyses were performed using Prism version 9.0.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Assay development

The principle of the BLI assay was illustrated in Fig. 1. The sample probe measured the total binding signal which the sum of specific antibody binding to RBD on the probe and any NSB interaction. The reference probe measured the NSB interactions only (Fig. 1A). To generate the specific binding signal, the reference probe signal was aligned with and then subtracted from the sample probe signal (Fig. 1B). The maximum specific binding signal was measured at the end of the association step, which represented the total amount of antibody bound to the probe at the beginning of dissociation $([AB]_0)$. In the dissociation step, binding signal at each point of measurement $[AB]$ was compared to $[AB]_0$. The 1st order reaction kinetic equation was fit to the dissociation curve and the dissociation constant ($k_{\text{off}}$) was calculated according to $\frac{[AB]}{[AB]_0} = e^{-k_{\text{off}}t}$. The measured $k_{\text{off}}$ value was the average dissociation rates of polyclonal SARS-CoV-2 antibodies dissociating from the RBD on the BLI probe.

3.2. Assay optimization and validation using polyclonal antibody

To demonstrate the advantage of SA-PS probe, signal from the SA-PS probe and streptavidin probe were compared in pooled SARS-CoV-2 negative serum samples (Fig. 2A). The SA-PS probe showed more than two-fold lower NSB signal than that of the SA probe (0.13 nm ± 0.002 and 0.33 nm ± 0.011, respectively) indicating that the SA-PS probe have the capability to improve specificity by reducing NSB. The performance of the two probes was subsequently tested in spiked purified rabbit polyclonal IgG antibody (PAb) samples (Fig. 2B). After subtracting NSB, the SA-PS probe generated reproducible, specific binding signal (0.18 nm ± 0.01) with well fitted dissociation curves. In contrast, SA probe did not generate specific binding signal (0.03 nm ± 0.025) thus $k_{\text{off}}$ could not be calculated.

To determine the measurable range, three concentrations (300 μl/ml, 150 μl/ml, 75 μl/ml) of PAb were spiked in pooled negative serum, respectively, within the range of antibody levels in patient sera (Supplementary Fig. 1A). The pooled negative serum without the spike was
used to measure NSB. The specific binding signals were calculated by subtracting NSB signal from the total binding signal of each sample. The specific binding signals showed a linear relationship with concentrations ($R^2 = 0.997$). In contrast, the $k_{off}$ values in the spiked sera were independent of the concentrations of the polyclonal antibodies using proper negative references and then subtraction of NSB from the measurements (Supplementary Fig. 1B).

### 3.3. Assay validation in patient serum samples

The assay parameters, including RBD loading and HSA blocking, were optimized for a proper detection in serum samples (Supplementary Figs. 2 and 3). Extending RBD loading achieved better fitting $R^2$ (Supplementary Fig. 2D). Addition of HSA coating helped further in reducing but not completely eliminating NSB (Supplementary Fig. 3E). To achieve the best combination of assay time and assay performance, 60s RBD loading and 240s HSA blocking were applied for all sample tests. The detection of SARS-CoV-2 RBD and antibody binding kinetics was assessed in 3 SARS-CoV-2 antibody positive (Fig. 3A) and 3 negative (Fig. 3B) patient serum samples. The response signals and $k_{off}$ could be detected in both positive and negative samples using the reference probes without specific RBD binding due to NSB (Fig. 3A and B). The SARS-CoV-2 specific binding signal was obtained by subtracting the NSB from the total binding signal (Fig. 3C). The $k_{off}$ calculated from the specific binding signal of 3 positive samples was 1.10E-3, 7.98E-4, 3.82E-4 respectively. In contrast, the $k_{off}$ of negative samples could not be calculated.

When analyzing the SARS-CoV-2 polyclonal antibody-antigen
binding kinetics, $k_{on}$ and $K_D$ cannot be measured when the absolute concentration of polyclonal antibodies is unknown in serum samples. However, $k_{off}$ can be obtained without the measurement of antibody concentration. To prove that the $k_{off}$ is concentration independent, five representative serum samples were serially diluted using the SARS-CoV-2 negative pooled human serum as diluent (2- and 4-fold dilutions). The specific binding signals decreased after dilutions whereas the $k_{off}$ values did not show significant change (Fig. 4A and B). Furthermore, fifteen additional samples with different SARS-CoV-2 antibody levels were tested undiluted and with 2-fold dilution to show that the specific binding signals dropped significantly with dilution ($p < 0.0001$) (Fig. 4C), whereas the $k_{off}$ measurements showed no significant difference between original and diluted samples ($P = 0.783$) (Fig. 4D). These findings demonstrated that in the case of SARS-CoV-2 polyclonal antibody-antigen interaction, the $k_{off}$ constant was independent of concentration and could be measured without dilution in patient serum samples using our BLI method.

Fig. 4. The $k_{off}$ measurement is SARS-CoV-2 antibody concentration independent. A) Specific binding signal measurement in original, 2-fold and 4-fold diluted samples. B) specific binding signal and $k_{off}$ measurement of the original and diluted samples. C) Comparing specific binding signal of patient samples between original and 2-fold diluted samples (paired, two-tail $t$-test). D) Comparing $k_{off}$ values between original and 2-fold diluted patient samples (paired, two-tail $t$-test).
3.4. Kinetics measurement in SARS-CoV-2 antibody positive serum of convalescent children and adults

To further evaluate the clinical utility of the novel BLI SARS-CoV-2 antibody dissociation kinetics assay, we tested 24 children and 63 adult convalescent patients who had positive SARS-CoV-2 antibody results. The $k_{off}$ values of SARS-CoV-2 antibodies were in the range of 1E-4 to 2E-3 $s^{-1}$, suggesting a wide variation in SARS-CoV-2 $k_{off}$ among different patient samples (Fig. 5A). No correlation was observed between $k_{off}$ and specific binding signal in adults ($r = -0.1894, P = 0.1372$) or pediatric ($r = -0.4584, P = 0.0243$) sample sets (Fig. 5A). There was no significant difference ($P = 0.1466$) in the specific binding signal among different age groups (Fig. 5B, Supplementary Fig. 4). Notably, antibodies from children exhibited significantly lower $k_{off}$ (median: 3.12E-4; IQR: [2.55E-4 to 4.07E-4]) compared to adult (median: 6.36E-4; IQR: [3.80E-4 to 9.53E-4]; $P < 0.0001$) (Fig. 5C).

4. Discussion

This study describes a novel BLI technique that directly measures the SARS-CoV-2 antibody dissociation kinetics from the RBD of virus spike protein in clinical serum samples. The BLI platform has advantages in kinetics analysis, and is widely used in the research field, but has not been routinely applied to analyze complex clinical specimens. We introduced a new BLI conjugate probe that reduces BLI NSB interactions in serum, and demonstrated the clinical utility of this chaotrope-free and label-free kinetics assay to evaluate SARS-CoV-2 antibody dissociation rate constant in COVID-19 convalescent patients.

So far, various SARS-CoV-2 antibody kinetics and avidity assays in human sera have been developed on different platforms, such as lateral-flow (Valdivia et al., 2021), enzyme-linked immunosorbent assay (ELISA) (Klein et al., 2020; Pichler et al., 2021), cyclic enhanced fluorescence assay (CEFA) (Racine-Brzostek et al., 2021; Yang et al., 2021b), and nanoplasmonic assay (Liu et al., 2020). However, none of them is both chaotrope-free and label-free. Surface Plasmon Resonance (SPR) is a label free platform but is more challenging in serum kinetics measurements because of the NSB issue. Specifically, the SPR microchannel flow cells are easily clogged, which limits the clinical application in serum or other crude samples. Orlov et al. developed a label-free microarray biosensor based on the technique of spectral-correlation interferometry (SCI) to measure the concentration and native kinetic of the autoantibodies in human serum (Orlov et al., 2020). Compared with complex functionalization of biochips and a multiplex setup of the SCI technique, the BLI assay is more flexible to use in clinical settings. Moreover, BLI is a fluids-free assay in which the sensor tips merely dip into sample containing microwells and does not need to maintain or optimize the fluids. Using BLI assays, Dzimianski et al. developed a BLI immunosorbent assay to measure the SARS-CoV-2 Spike RBD antibody levels in human plasma, but because of their dip-read format, NSB issue causes ambiguous results in some negative samples, a problem that was not resolved (Dzimianski et al., 2020). Luo et al. reported a two-step measurement of SARS-CoV-2 relative avidity index (Luo et al., 2020b). However, this assay used the chaotropic agent (urea). Dennison et al. performed a chaotrope-free BLI avidity measurement of recombinant monoclonal antibodies induced by malaria vaccine, but this assay was developed for purified monoclonal antibodies in PBS buffer and highly
diluted pooled sera (Dennison et al., 2018). In contrast, the present method monitors unperturbed binding kinetics in natural conditions of the crude human sera without using any chaotropic agents or labels.

To get through the “bottleneck” of label-free BLI biosensing which is high NSB in the complex human serum matrix, our BLI assay introduced the SA-FS coated probe to reduce the NSB signal. The polysaccharide is highly branched and when immobilized on the probe, forms a network reducing exposure of the surface to sample NSB. Since the polysaccharide has minimal interaction with the sensor surface, prior conjugation with streptavidin enables immobilization to protein. We have previously reported a CEFA assay for SARS-CoV-2 antibody characterization (Yang et al., 2021a). Importantly, there is a distinction between how the high molecular weight polymer is used in the CEFA assay previously reported and the current assay. In the CEFA, the high molecular weight polysaccharide carries multiple Cy5 label streptavidin to amplify the immuno-specific signal compared to monomeric Cy5-streptavidin. The signal amplification is achieved by embedding multiple copies of Cy5-SA in an inert polysaccharide matrix. Furthermore, in the present BLI assay, by utilizing a separate reference probe and B-HSA blocking process to subtracted NSB signal in serum, our BLI biosensor can measure a wide range of specific signal and k₉ off whereas the CEFA assay measures only the relative dissociation rate.

Utilizing the BLI assay, we demonstrated that SARS-CoV-2 antibody binding has slower dissociation kinetics in the convalesce serum samples of children than those of adults, which may partly account for the different clinical outcome between children and adults. Most children with SARS-CoV-2 infection are either asymptomatic or exhibit mild symptoms, and have a lower risk of developing severe respiratory syndrome. The stronger antibody binding (lower k₉ off) in children could play a role in modulating the different clinical manifestation of SARS-CoV-2 infection in children compared to adults. This finding is congruent with our previous study (Yang et al., 2021a) using a fluorescent immunoassay (Pylon 3D analyzer). In the present study, we detected lower k₉ off value in pediatric samples with significant difference (P < 0.0001). Our results demonstrated heterogeneous, age-dependent dissociation kinetics in convalescent COVID-19 patients. Overall, kinetics measurement of SARS-CoV-2 antibody interaction could be of great value to better understand the functional efficiency of antibodies in different populations. Our study was limited by a relatively small sample size and its retrospective nature. Future research, both in larger prospective studies and of long-term clinical outcomes is warranted. This study paves the way for future research of immune response after SARS-CoV-2 infection or vaccination in different age groups.

5. Conclusion

Understanding the kinetics of SARS-CoV-2 specific antibody binding to the virus S antigen is a key element in characterizing the human immune response against the SARS-CoV-2 infection. This novel BLI antibody kinetics assay can also support vaccine development and efficacy characterization of SARS-CoV-2 antibody in different patient populations. This assay is simple to operate and can be easily extended to other clinical applications requiring real-time measurement of antibody-antigen interactions.

CRediT authorship contribution statement

Ying Hao and He S. Yang for performing the BLI experiment, data analysis and writing the manuscript; Sabrina E. Racine-Brzostek for conceptualization and manuscript editing; Molsen Karbuschi for the BLI assay development and writing the manuscript; Pu Li for editing the manuscript; Robert Zuk methodology, for method development; Yawei J. Yang for reviewing the manuscript; P.J. Klasse analysis of binding kinetics and manuscript editing; Yuanyuan Shi for conceptualization and supervision of the project; Zhen Zhao for conceptualization, investigation, supervision of the project, writing and editing the manuscript.

Authors’ disclosures or potential conflicts of interest

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Further reading

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