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Application note

Characterization of COVID-19 antibodies by Bio-Layer Interferometry using Nano-CaptureLigands

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Content

1. Summary

This application note demonstrates that Nano-CaptureLigands™ are highly suitable capturing tools for the functional antibody characterization via Bio-Layer interferometry (BLI). In this case study, we determined the binding kinetics of human and mouse variants of the COVID-19 antibody CR3022 to the SARS-CoV2 Spike receptor-binding domain (RBD). Obtained data agree very well with published data.

2. Introduction

2.1. Bio-Layer interferometry

BLI is commonly used for studying biomolecular interactions. This label-free technology uses an optical method, which analyzes the interference pattern of white light. It detects changes in the number of molecules bound to the biosensor tip and/or the binding of ligands immobilized on the biosensor tip surface to analytes in solution. Applications include the analysis of protein-protein interactions, affinity, kinetic assays, epitope binning, and protein quantitation.

2.2. Nano-CaptureLigands as immobilization reagents

ChromoTek's Nano-CaptureLigands are capture reagents for the site-directed, gentle, and homogenous immobilization of antibodies in biosensor assays like BLI. Nano-CaptureLigands are biotinylated V_HHs, also known as Nanobodies. They capture non-biotinylated antibodies with high specificity for their immobilization to streptavidin/avidin coated surfaces.

Due to their advanced immobilization of antibodies, Nano-CaptureLigands provide a stable baseline with negligible antibody dissociation, can be frequently regenerated for multiple reuse and allow the selective immobilization of antibodies from crude liquids like hybridoma supernatant, serum, and plasma samples.

2.3. Anti-SARS-CoV/CoV2 Spike antibody clone CR3022

The SARS-CoV/CoV2 Spike human IgG1 antibody CR3022 was first reported by ter Meulen et al. in 2006. This human antibody was selected from a phage display scFv library derived from a convalescent SARS patient from Singapore. It binds to the receptor-binding domain (RBD) of the SARS-CoV Spike protein (also known as S protein or S glycoprotein) and neutralizes SARS-CoV. CR3022 was shown to be cross-reactive to the Spike RBD of SARS-CoV2 by Tian et al. 2020 and was thus one of the first known antibodies against SARS-CoV2.

SARS-CoV and SARS-CoV2 Spike RBD have a 73% sequence identity and screening of anti-SARS-CoV Spike antibodies using ELISA identified CR3022 as cross-reactive antibody. For binding kinetics determination by BLI, CR3022 was added in a scFv format to the immobilised SARS-CoV2 Spike RBD. A dissociation constant K_D of 6.3 nM with an on-rate of k_{on} 1.8 $\cdot 10^5$ M/s and an off-rate of k_{off} 1.2 $\cdot 10^{-3}$ s^{-1} was determined. No competition for binding to the human receptor ACE2 was found.

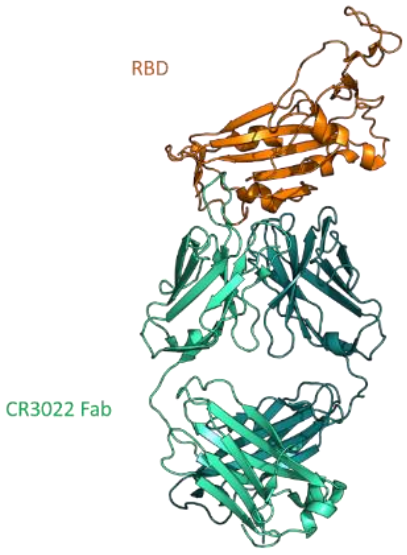

Figure 1: Crystal structure of CR3022 Fab fragment (green) bound to SARS-CoV2 Spike RBD (orange). PDB 6W41; Yuan et al. Science 2020.

The structural basis of the binding of CR3022 to the SARS-CoV2 Spike RBD was elucidated by Yuan et al. 2020 (Figure 1). The authors conclude that CR3022 binds to a conserved, cryptic epitope. Binding of CR3022 does not block the ACE2 interface but destabilizes the Spike prefusion-stabilized trimer instead. This finding is supported by very similar structural and functional data from Huo et al. 2020.

3. Materials and Methods

In this application note, we used two recombinantly produced formats of the CR3022 antibody, human IgG1 κ and mouse IgG2b κ. The non-biotinylated antibodies were immobilized on streptavidin biosensors using Nano-CaptureLigands. Binding kinetics and affinity of both antibodies to the SARS-CoV2 Spike receptor-binding domain were determined by BLI.

3.1. Anti-COVID-19 antibodies and Nano-CaptureLigands

Human IgG1 is the native format of the anti-SARS-CoV/CoV2 Spike antibody CR3022, whereas the chimeric mouse IgG2b κ antibody comprises the variable domains V_L and V_H of the original human IgG1 plus C_L & C_H1 domains and Fc fragment of mouse IgG2b. Both antibodies are monoclonal and were purified using Protein A (Figure 2).

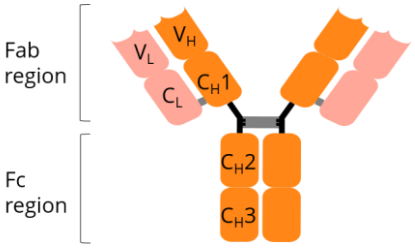

Figure 2: Antibody structure

Three Nano-CaptureLigands were used to capture the non-biotinylated CR3022 antibodies to streptavidin coated biosensors. For immobilization of mouse IgG2b, both a mouse IgG2b Fc-specific and a mouse IgG Fab-κ-LC-specific Nano-CaptureLigand were applied (Table 1).

Antibody	Nano-CaptureLigand
Anti-COVID-19 & SARS-CoV S glycoprotein [CR3022], Human IgG1, Kappa (Absolute Antibody Ab01680-10.0)	Nano-CaptureLigand™ human IgG/rabbit IgG, Fc-specific VHH, biotinylated (ChromoTek shurbGB-1)
Anti-COVID-19 & SARS-CoV S glycoprotein [CR3022], Mouse IgG2b, Kappa (Absolute Antibody Ab01680-3.0)	Nano-CaptureLigand™ mouse IgG2b, Fc-specific VHH, biotinylated (ChromoTek smsG2bB-1)
	Nano-CaptureLigand™ mouse IgG, Fab-kappa-LC-specific VHH, biotinylated

Table 1: Antibody variants of CR3022 and Nano-CaptureLigands used for antibody immobilization to streptavidin coated biosensors

3.2. Spike S protein

SARS-CoV2 Spike receptor-binding domain expressed in a human cell line was kindly provided by U. Rothbauer, NMI Tübingen, and used as analyte.

3.3. BLI

All samples were set up in a black 96-well plate (Greiner Microplate PP, flat-bottom, black, 655209) at room temperature, and 200 µL were used per well. FortéBio Streptavidin (SA) Biosensors (18-5019) were used for immobilization of Nano-CaptureLigands. Experiments were run on a FortéBio Octet® RED96e at +30°C, with a shaking speed of 1,000 rpm and a recording rate of 5 Hz.

BLI buffers:

- Kinetics Buffer: PBS, 0.01% (m/v) BSA, 0.002% (v/v) Tween-20
- Regeneration Buffer: 0.1 M glycine, pH 2

Protocol:

- Baseline 1: Incubation of the biosensors for 60 s in 1x Kinetics Buffer.
- Loading: Dilution of the Nano-CaptureLigand to a concentration of 1 µg/mL in 200 µL 1x Kinetics Buffer. Loading of the diluted Nano-CaptureLigand on the biosensors for 60 s.
- Quenching: Incubation of the biosensors for 60 s with Biocytin (10 µg/mL).
- Baseline 2: Incubation of the biosensors for 120 s in 1x Kinetics Buffer.
- Activation: Activation of the biosensors for 180 s with the antibody (20 nM).
- Baseline 3: Incubation of the biosensors for 120 s in 1x Kinetics Buffer.
- Association: Binding of different RBD concentrations in 1x Kinetics Buffer for 240 s.
- Dissociation: Incubation of the biosensors for 240 s in 1x Kinetics Buffer. Wells from step 6, Baseline 3 were used.

4. Results

4.1. COVID-19 antibody capture

The CR3022 antibodies were successfully captured to streptavidin coated sensors using Nano-CaptureLigands (Figure 3). Particularly the Fc-specific Nano-CaptureLigands for human IgG and mouse IgG2b captured the antibodies in a stable manner with negligible dissociation of the antibodies from the Nano-CaptureLigands (Figure 3 A & B).

The Fab specific Nano-CaptureLigand mouse Fab-kappa was also able to capture the mouse IgG2b κ antibody; the resulting baseline was less stable, however (Figure 3 C). Nonetheless, it was still possible to determine binding parameters of the CR3022 antibody to the Spike RBD.

4.1. COVID-19 antibody characterization

The kinetics data obtained from our BLI measurements correspond well with published data (Huo et al. 2020 and references therein, Tian et al. 2020, ter Meulen et al. 2006, and Yuan et al. 2020) indicating that the two CR3022 antibody variants were functionally immobilized to the streptavidin-coated biosensors using Nano-CaptureLigands (Table 2). The slight differences to literature data are likely caused by different experimental set-ups: In case of the antibody CR3022, it was used in formats as different as intact IgG, Fab fragment, and scFv in the published assays. Furthermore, the Spike RBD analyte used in these experiments was obtained from expression either in insect or in mammalian cells, which affects its glycosylation pattern and possibly its conformation (a human cell line was used for expression of the RBD in the present case study to ensure native glycosylation).

Almost the same on- and off-rates were measured for CR3022 human IgG1 and CR3022 mouse IgG2b immobilized with Nano-CaptureLigand human IgG/rabbit IgG, and Nano-CaptureLigand mouse IgG2b respectively, despite the different Fc domains of the antibodies. This reproducibility indicates the reliability of the generated data when using Fc-specific Nano-CaptureLigands for capturing to streptavidin. The immobilization of the CR3022 mouse IgG2b with the Fab-kappa-LC-specific Nano-CaptureLigand mouse IgG resulted in slightly deviating on- and off-rates, probably due to the less stable baseline; however, the measured data are still within the variation of the published data.

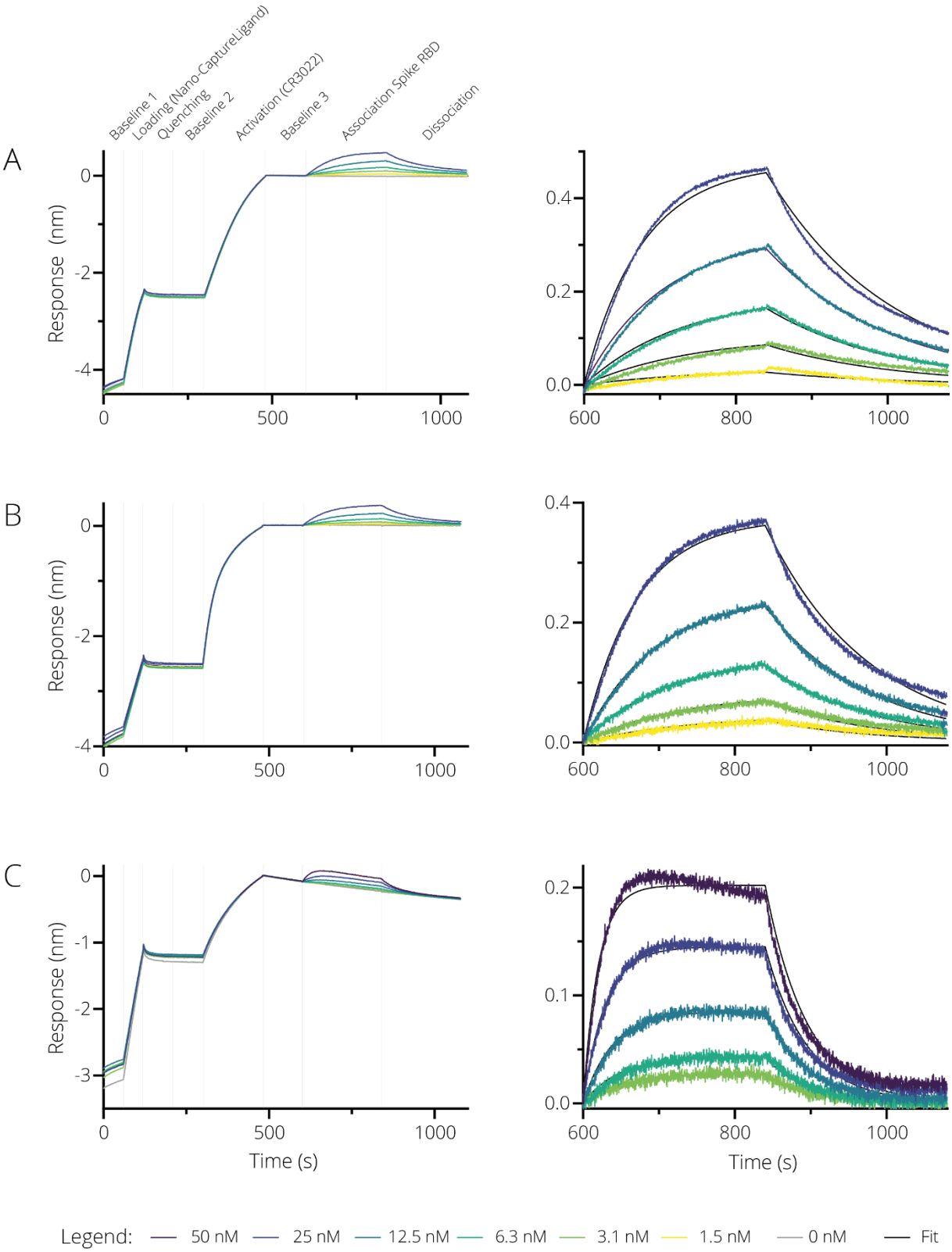

Figure 3: BLI kinetics of the binding of human and mouse antibody CR3022 to SARS-CoV2 Spike RBD. Human and mouse antibody CR3022 was captured on a streptavidin biosensor using biotinylated Nano-CaptureLigands and assayed with different concentrations of SARS-CoV2 Spike RBD. **A**: Human IgG1 antibody CR3022 was captured by Nano-CaptureLigand™ human IgG/rabbit IgG, Fc-specific VHH, biotinylated. **B**: A chimeric mouse IgG2b antibody CR3022 was captured by Nano-CaptureLigand™ mouse IgG2b, Fc-specific VHH, biotinylated. **C**: A chimeric mouse IgG2b κ antibody CR3022 was captured by Nano-CaptureLigand™ mouse IgG, Fab-kappa-LC-specific VHH, biotinylated. See legend (bottom) for colour coding of RBD concentrations.

Nano-CaptureLigand	Immobilized ligand	Free analyte	K_D (nM)	k_a (Ms^{-1})	k_d (s^{-1})	$\chi 2$	R^2
Nano-CaptureLigand™ human IgG/rabbit IgG, Fc-specific VHH, biotinylated	CR3022 hu IgG1	SARS-CoV2 Spike RBD	21	$2.9 \cdot 10^5$	$6.0 \cdot 10^{-3}$	1.17	0.994
Nano-CaptureLigand™ mouse IgG2b, Fc-specific VHH, biotinylated	CR3022 ms IgG2b		26	$2.8 \cdot 10^5$	$7.3 \cdot 10^{-3}$	0.45	0.996
Nano-CaptureLigand™ mouse IgG, Fab-kappa-LC-specific VHH, biotinylated	CR3022 ms IgG2b		29	$6.3 \cdot 10^5$	$1.8 \cdot 10^{-2}$	0.39	0.991

Table 2: Kinetic date from BLI measurements of CR3022 antibody variants. The 2 antibody variants were captured using 3 Nano-CaptureLigands; for immobilization of the mouse IgG2 chimeric antibody both Fc- and Fab-kappa-LC specific Nano-CaptureLigands were applied.

5. References

Huo J, Zhao Y, Ren J, et al. Neutralization of SARS-CoV-2 by destruction of the prefusion Spike. *BioRxives*. 2020.

Tian X, Li C, Huang A, et al. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. *Emerg Microbes Infect*. 2020;9(1):382-385.

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Yuan M, Wu NC, Zhu X, et al. A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. *Science*. 2020;368(6491):630-633.