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Chemokines and SARS-CoV-2 Nucleocapsid Protein

A Model of Biomolecular Interaction for Protein Domain Mapping Utilizing Octet® Bio-Layer Interferometry

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Abstract

Chemokines (chemotactic cytokines; CHK) are a class of cytokines that control the migration of immune cells to sites of infection and inflammation and have a role in anti-viral response. Binding of chemokines to glycosaminoglycans (GAGs) such as heparan sulfate on endothelial cells and in the extracellular matrix in tissues is required to create a concentration gradient to drive the migration of immune cells. Targeting chemokine-GAG and chemokine-receptor interactions is a promising approach to inhibit chemokine activity and inflammation. Newer strategies in cell therapy to employ the chemokine expression system and engineered chemokine receptors to aid homing of CAR-T (chimeric antigen receptor-T cell) cells to enhance the effectiveness of CAR-Ts in the treatment of solid tumor further highlights the importance to understand chemokine-GAG and chemokine-receptor binding interactions. CHKs generally present two major protein sites to exert their properties, the GAG-binding site and the receptor-binding site. Characterization of protein domains participating in biomolecular interactions between antigens (example, viral proteins) and key components of the innate immune system is instrumental for antigen cartography, drug discovery and vaccine development. Identifying and mapping these domains of interaction within a given protein can be challenging, laborious and time consuming, requiring the use of techniques such as alanine scanning, generation of escape mutants, and deep mutational scanning. The Octet® Bio-Layer Interferometry (BLI) platforms' fluidic-free assay format enables the rapid, label-free and high-throughput analysis of the interacting antigen domains and epitope diversity. This application note discusses and comprehensively characterizes the interaction between the SARS-CoV-2 Nucleocapsid (N) protein and human CHKs as a model for protein domain mapping of biomolecular interactions on the Octet® BLI platform. It also provides guidance and considerations for assay development and optimization.

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Introduction

Chemokines (CHKs) are secreted from cells and immobilized on the cell surface by interaction with GAGs through the GAG-binding domain of CHKs. This is required for retention on the endothelium and interaction with the CHK receptors, G protein-coupled receptors present on leukocytes' surface, through the receptor-binding domain of CHKs. Due to the essential role of CHK in the antiviral response, viruses have developed counteractive proteins (viral chemokine binding proteins, vCBP) that are able to interfere with the host CHK network, modulating its activity and thus altering leukocyte migration. The vCBPs can modulate the CHK activity by interaction with the CHK GAG- or receptor-binding domain, or both. The importance of vCBPs as viral countermeasure against the host immune response is emphasized by the large number of inhibitory vCBPs **[1, 2]**. For example, myxoma virus M-T7, vaccinia virus A41, ectromelia virus E163, murine gammaherpesvirus-68 M3, and animal alphaherpesviruses gG, are all traditional examples of secreted vCBPs binding CHKs through the CHK-GAG-binding site. These vCBPs block or impair the GAG-CHK or CHK-receptor interactions which are translated into a disruption of the chemotactic gradient, hence the inhibition of leukocyte infiltration.

The SARS-CoV-2 N-protein which binds viral RNA and plays pivotal roles in packaging and transcribing viral RNA also plays the role of a vCBP. The N-protein is divided into three major structural domains: an N-terminal domain (NTD), a central linker, and a C-terminal domain (CTD) (Figure 1). Both the NTD and CTD are RNA-binding sites. Two N-protein monomers have been reported to form a stable dimer through their CTD **[3]**. The N-protein was believed to be restricted to the intracellular compartment during infection. However, it has recently been reported that the N-protein is abundantly expressed on the surface of both virus infected and neighboring uninfected mammalian cells, where it enables activation of Fc receptor-bearing immune cells with anti-N-protein antibodies (Abs), and inhibits leukocyte chemotaxis by binding 11 human CHKs with high affinity, inhibiting their chemoattractant properties **[4]**. The N-protein has also been presented as the first secreted human coronavirus vCBP able to bind CHKs through the GAG-binding site of CHK, shown by binding studies performed on the Octet® BLI platform.

However, it has not been characterized whether the N-protein binds human CHKs preferentially through a specific or multiple protein domains, i.e., mapping which N-protein domains participate in CHK binding.

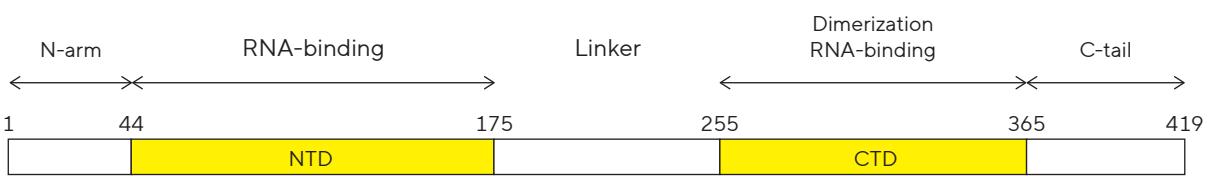
In this application note we present the workflow, assay design strategy, data, and proposed model of N-protein – CHK by domain mapping of the interaction using a simple, direct, qualitative assays performed on the Octet® BLI platform.

Materials and Methods

Materials

- Octet® BLI system (RH16 instrument) and Octet® BLI Discovery and Analysis Studio Software
- Octet® Streptavidin (SA) Biosensors (Sartorius), Cat. Nos. 18-5019 (tray); 18-5020 (pack); 18-5021 (case)
- 96-well, black, flat bottom microplate, Greiner Bio-One, Cat. No. 655209
- Assay buffer: The biomolecular interaction assays were performed with in-house made buffer containing DPBS (Gibco Cat. No. 14190-144), 1% BSA, and 0.05% Tween 20
- HEK293-FT cells (ATCC Cat. No. CRL-11268)
- DMEM with GlutaMAX (Thermo Fisher Scientific, Cat. No. 10566016)
- SARS-CoV-2 N-protein 2xStrep mammalian expression vector (Addgene Cat. No. 141391)
- PeproTech® human CHKs: CCL26 (Cat. No. 300-48), CCL28 (Cat. No. 300-57), CXCL10 (Cat. No. 300-12)
- Lysis buffer: The crude lysates from transiently transfected cells were obtained with in-house made lysis buffer containing 50 mM tris-HCl (pH 7.4), 150 mM NaCl, 5 mM KCl, 5 mM MgCl2, 1% NP-40, and 1x protease inhibitors (Roche, Cat. No. 4693159001)
- GeneTex® anti-SARS-CoV-2 N Antibodies: Ab 1 (Cat. No. gtx635686), Ab 2 (Cat. No. gtx635688), Ab 3 (Cat. No. gtx635685), Ab 4 (Cat. No. gtx635679), Ab 5 (Cat. No. gtx635687), Ab 6 (Cat. No. gtx635808), Ab 7 (Cat. No. gtx635689)
- MilliporeSigma® GAGs: chondroitin sulfate A (Cat. No. C9819), chondroitin sulfate B (Cat. No. C3788)

Figure 1: *Domain Architecture of the SARS-CoV-2 N-Protein*



Note. NTD: N-Terminal Domain; CTD: C-Terminal Domain

Methods

- HEK293-FT cells were grown in DMEM with GlutaMAX supplemented with 8% (v/v) not heat-inactivated fetal bovine serum (FBS) (HyClone, Cat. No. SH30071.03)
- Batches of SARS-CoV-2 N-protein 2xStrep tag in crude lysates were obtained from 30 × 10^6 HEK293-FT cells transfected with 30 µg of Addgene plasmid # 141391 with TransIT-LT1 Transfection Reagent (Mirus Bio). After 24 hours, transfected cells were selected with puromycin (10 µg/mL; InvivoGen, Cat. No. ant-pr-1). After 48 hours, transfected cells were trypsinized, washed with DPBS, and lysed for 30 min at 4 °C in 1 mL of lysis buffer, followed by centrifugation at 1000*g* at 4 °C. Clarified supernatants (crude lysates) were collected, aliquoted, stored at –20 °C, and characterized by immunoblotting using IRDye® 680RD Streptavidin (LI-COR Cat. No. 926-68079)
- CHKs were reconstituted in DPBS 0.1% BSA at 10 µM, aliquoted and stored at –80 °C
- GAGs were reconstituted in deionized H_2O at 1 mg/mL, aliquoted and stored at 4 °C
- All BLI assays were performed at a shake speed of 1000 rpm at 30 °C.

The Antibody (Ab)-Blocking Assay Workflow (Table1, Figure 2)

a. Biosensor hydration: SA biosensors were dipped in assay buffer for at least 10 minutes before use.
b. Initial baseline (biosensor equilibration): Hydrated SA biosensors were dipped in assay buffer for 60 seconds.
c. Ligand loading: 30 µL of crude lysate containing SARS-CoV-2 N 2xStrep protein was combined with 170 µL of assay buffer and was loaded onto SA biosensors for 300 seconds to achieve a load response of 5 nm. The loading step time can be modified (shortened or extended) to achieve a load response of 5 nm.
d. Baseline/wash step after ligand loading (to monitor stability of ligand capture): 90 seconds in assay buffer.
e. Antibody loading: SA biosensors were dipped for 180 seconds in 200 µL of assay buffer containing 0.02 mg/mL of anti-N Ab.
f. Baseline equilibration after Ab loading: 90 seconds in assay buffer.
g. CHK association and dissociation: association of each CHK sample in assay buffer at indicated concentration was carried out for 300 seconds, followed by dissociation for 180 second in assay buffer.

Table 1: *Antibody Blocking Assay Workflow Steps*

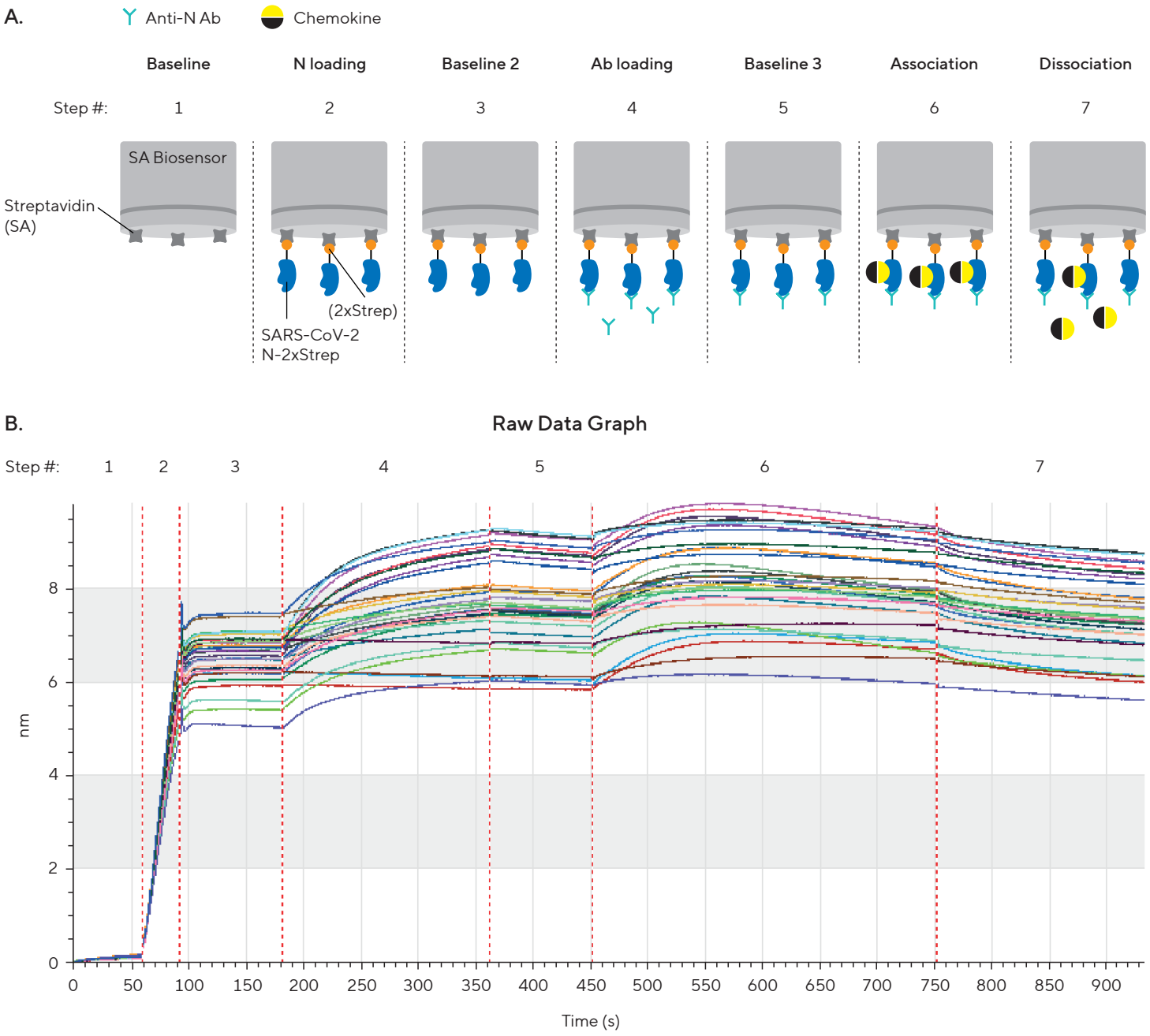
Step No.	Step Name	Step Time (s)	Step Type Name	Step definition
1	Baseline	60	Baseline	Sensor check
2	Ligand loading	300 (load response of 5 nm)	Loading	SARS-CoV-2 N 2xStrep immobilization
3	Baseline 2	90	Baseline	Baseline
4	Antibody loading	180	Association	Ab loading
5	Baseline 3	90	Baseline	Baseline
6	CHK association	300	Association	Association with chemokines
7	CHK dissociation	180	Dissociation	Dissociation

Note.

i. The binding response of each CHK to N-protein, in the absence Abs (control), was considered 100% of binding response.

ii. The volume of crude lysate (30 µL) containing SARS-CoV-2 N 2xStrep protein is arbitrary and was determined based on protein yields for each batch.

Figure 2: *Antibody Blocking Assay Workflow*



Note. Antibody blocking assay workflow: Anti-N-protein Abs bound to immobilized N-protein into SA biosensors reduce N-protein - CHK binding activity.
A. Illustration of the assay workflow highlighting the assay events at each step.
B. Octet® BLI sensorgrams of binding assays showing all phases (raw data) of the interaction between the chemokine and immobilized N-protein in the presence of anti-N antibodies.

The GAG-Competition Assay Workflow (Table 2, Figure 3)

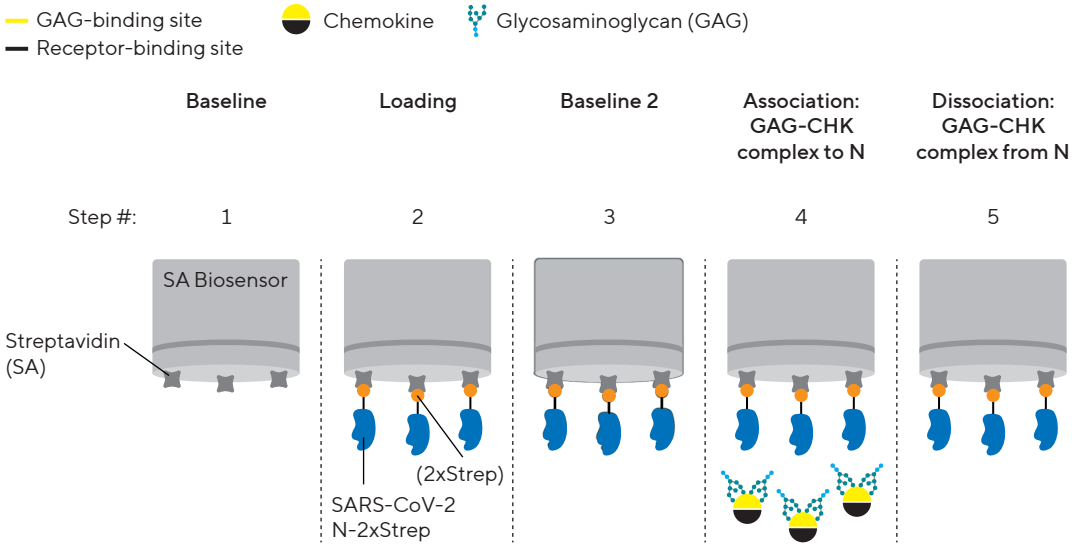
a. Biosensor hydration: SA biosensors were dipped in assay buffer for at least 10 minutes before use.
b. Chemokine sample preparation: Selected chemokines (100 nM) were incubated in assay buffer alone or with indicated concentrations of soluble GAGs for 10 min at room temperature.
c. Initial baseline (biosensor equilibration): Hydrated SA biosensors were dipped in assay buffer for 60 seconds.
d. Ligand loading: 30 µL of crude lysate containing SARS-CoV-2 N 2xStrep protein was combined with 170 µL of running buffer and was loaded into SA biosensors for 300 seconds to achieve a load response of about 5 nm.
e. Baseline equilibration after ligand loading: 90 seconds in assay buffer.
f. CHK association and dissociation: association of each CHK in assay buffer at indicated concentration was carried out for 300 seconds, followed by dissociation for 180 seconds.

Table 2: *GAG-Competition Assay Workflow Steps*

Step No.	Step Name	Step Time (s)	Step Type Name	Step definition
1	Baseline	60	Baseline	Sensor check / Sensor equilibration
2	Ligand loading	300 (load response of 5 nm)	Loading	SARS-CoV-2 N 2xStrep immobilization
3	Baseline 2	90	Baseline	Baseline
4	CHK association	300	Association	Association with chemokines-GAG complex to N-protein
5	CHK dissociation	180	Dissociation	Dissociation of chemokines-GAG complex from N-protein

Note.
i. The binding response of each CHK to N-protein, in the absence GAGs, was considered 100% of binding response.
ii. The volume of crude lysate (30 µL) containing SARS-CoV-2 N 2xStrep protein is arbitrary and was determined based on protein yields for each batch.

Figure 3: *The GAG-competition assay workflow*



Note. The GAG-competition assay workflow. Schematic of the assay workflow highlighting the assay events at each step, illustrating all sequential steps listed in Table 2.

Assay Optimization Tips

Optimization of ligand (e.g., SARS-CoV-2 N-protein 2xStrep) density on the biosensors surface is critical to promote a 1:1 binding event in order to avoid artefactual avidity effects. The fact that a reduction in ligand density on the biosensor surface that does not help to recover a 1:1 binding event between ligand and analyte, may be indicative of a bivalent (non-1:1) analyte interaction (**See Application Note: Optimizing Kinetics Assays to Avoid Avidity Effects**). Most CHKs evaluated did not show a 1:1 binding interaction behavior by decreasing N-protein density on the biosensors surface **[4]**.

- For optimal binding activity, CHKs should be freshly reconstituted in DPBS with 0.1% BSA and used immediately. *PeproTech® indicates that the average shelf life after CHK reconstitution is 1 week, if kept at 4 °C.
- Assay buffer should be pre-warmed to 30 °C before use.
- Batches of recombinant protein (ligand) in crude lysates should be stored at –20 °C. Use of Protease inhibitors are critical to prevent protein degradation during freezing/thawing cycles. Each crude lysate should be tested to determine the ligand's concentration before performing BLI biomolecular interaction assays. The concentration of ligand thus measured would help determine the ideal ligand concentration to use during the loading step. Ligand loading onto the biosensors should be steady and gradual, rather than have a fast and steep loading response slope.
- Minimum volume needed for samples is 200 µL/well for flat bottom whole area 96 well black plates.
- For BLI biomolecular interaction assays, old batches of CHKs should not be used, since these might aggregate showing abnormal | aberrant curves (e.g., increase in binding response immediately follow by a decrease during the association step). Additionally, CHK concentration should not be higher than 200 nM for the same reason.

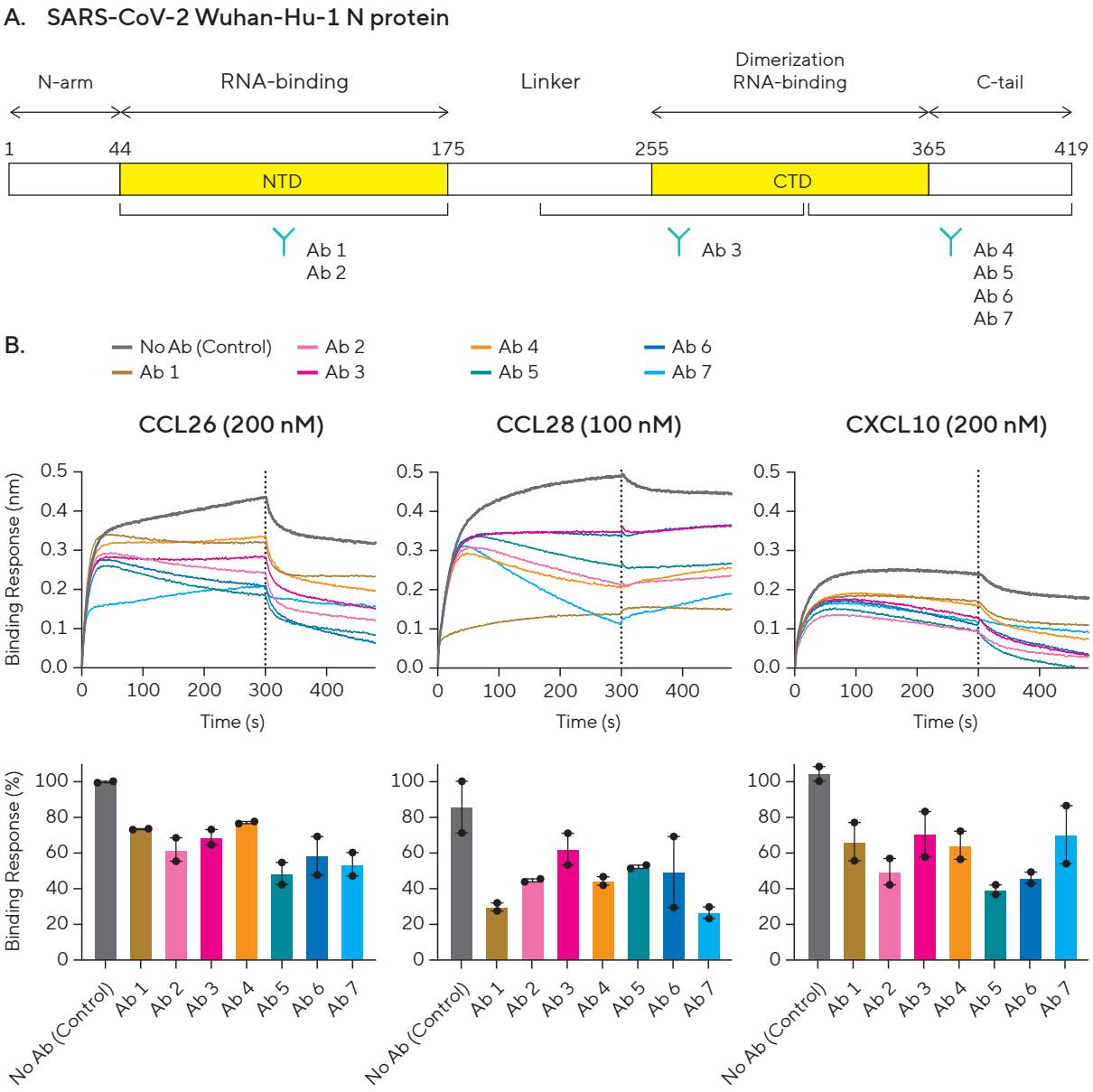
Results and Discussion

Antibody-Blocking Assays: SARS-CoV-2 N-protein uses both the NTD and CTD RNA-binding domains to bind CHKs

A previous study reported that, among 64 human cytokines examined on Octet® BLI, the N-protein binds CCL5, CCL11, CCL21, CCL26, CCL28, CXCL4, CXCL9, CXCL10, CXCL11, CXCL12β, and CXCL14 with micromolar to nanomolar affinities **[4]**. For this application note, 3 CHKs (CCL26, CCL28, and CXCL10) were selected to investigate whether the N-protein binds human CHKs preferentially through a specific or multiple protein domains, i.e., mapping which protein domains participate in CHK binding. Interaction of multiple protein domains might imply that each N-protein molecule could bind more than one CHK at the same time.

Antibody-blocking assays on the Octet® BLI platform were performed by immobilizing N-protein on the surface of Octet® SA Biosensors, followed by dipping these biosensors in samples containing specific monoclonal antibodies against the N-protein's NTD or CTD (Figure 4A), and finally, incubating the immobilized N-protein-antibody complex with each of the 3 selected CHKs. The binding response obtained from each CHK binding to immobilized N-protein in the absence of antibodies (control condition) was considered 100% of binding. A variable decrease in the ability of N-protein to bind CHKs, ranging from 75% to 25%, which was antibody-specific was observed (Figure 4B). Immobilized N-protein on the surface of biosensors was incubated with each anti-N-protein antibody up to saturation, meaning that all available binding sites on the N-protein NTD or CTD were blocked and inaccessible for potential CHK binding. These preliminary results suggested that both the N-protein NTD and CTD have the ability to bind and retain CHKs. Based on this data (Figure 4B), we selected two anti-N-protein antibodies (Ab 2 and Ab 6), which decreased binding of the N-protein to CHKs by about 50% each.

Figure 4: *Anti-N-Protein Antibody Binding Sites on SARS-CoV2 N-Protein*

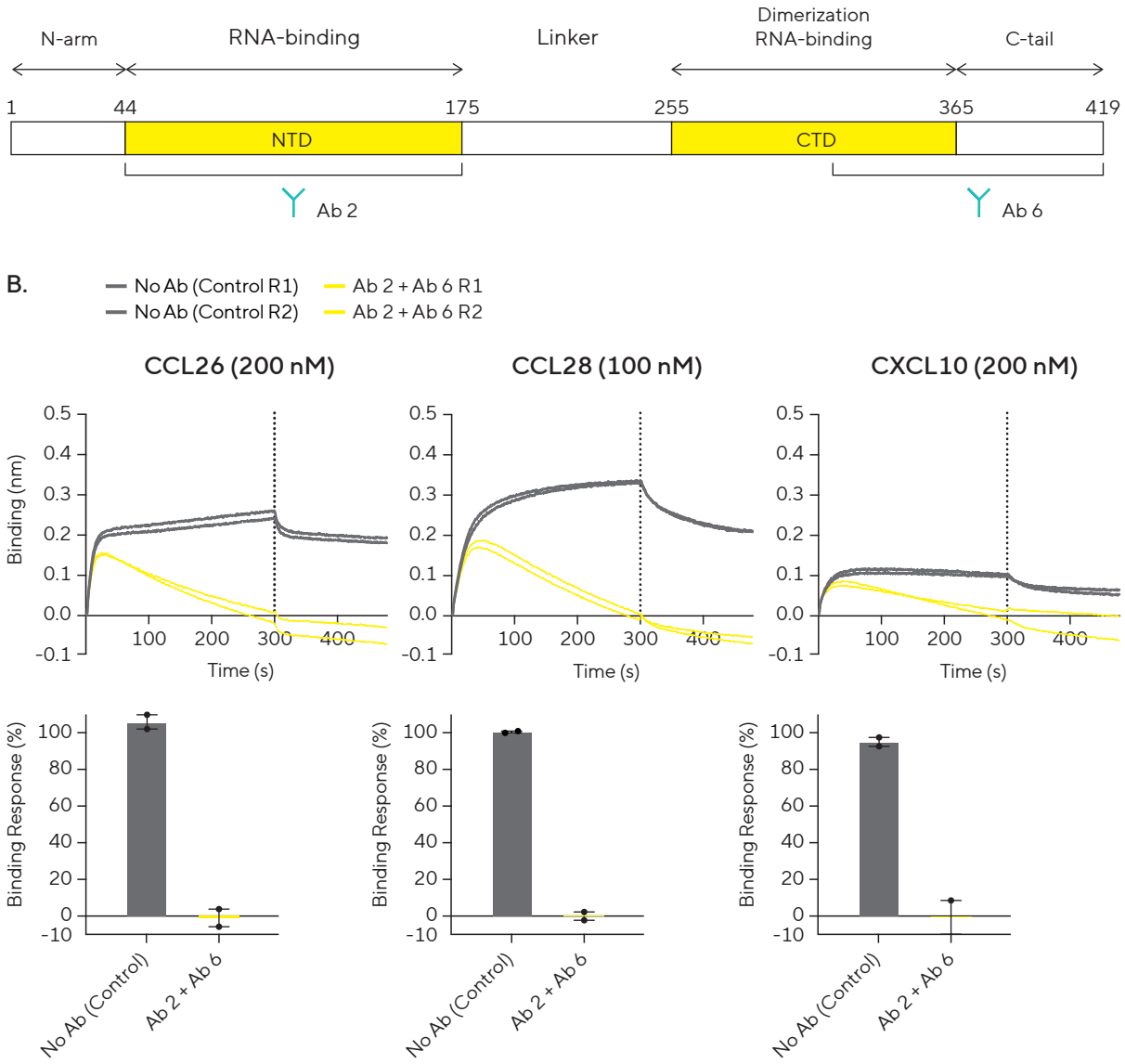


Note. Anti-N antibodies bound to N-protein immobilized onto SA biosensors reduce N-protein-CHK binding activity.
A. Schematic of the N-protein domain architecture and binding sites of each anti-N-protein Ab used in this study.
B. BLI sensorgrams of binding assays showing association and dissociation phases of the interaction between the N-protein and 3 positively bound CHKs (upper graphs). Prior to the association phase with CHKs, immobilized N-protein was incubated with one of each anti-N Ab up to binding saturation. The dotted line indicates the end of the association phase. The binding response of each CHK to N-protein, in the absence of prior incubation with an anti-N-protein Ab, was considered 100% binding (lower graphs). Data represent the mean +/- SEM (n = 2). One representative assay out of three independent assays performed in duplicate is shown.

To confirm the contribution of the N-protein NTD and CTD to CHK binding, we performed antibody-blocking assays utiizing Octet® BLI by incubating biosensors with immobilized N-protein with Ab 2 (anti-N-NTD) and Ab 6 (anti-N-CTD) simultaneously up to saturation, and then, incubating the immobilized N-protein- antibody complex with each of the 3 selected CHKs to assess the decrease in binding response.

We found that by blocking the N-protein NTD and CTD simultaneously, the ability of the N-protein to bind and retain CHKs was not partially, but completely abrogated (Figure 5A-B). Together, these findings indicate that the N-protein binds CHKs through both its RNA-binding domains, NTD and CTD, where both contribute equally to the N- protein CHK-binding activity (Figure 5C).

Figure 5: *Blocking of Chemokine Binding by Anti-N-Protein Antibodies*



Note. Both the NTD and CTD RNA-binding domains of the N-protein contribute to CHK binding.
A. Schematic of binding sites for Anti-N Ab 2 and Ab 6, blocking NTD and CTD domains respectively.
B. BLI sensorgrams of binding assays showing association and dissociation phases of the interaction between the N-protein and CHKs (upper graphs), Prior to the association phase with CHKs, immobilized N-protein was simultaneously incubated with both anti-N Abs up to binding saturation. The dotted line indicates the end of the association phase. The binding response of each CHK to N, in the absence of prior incubation with anti-N Abs, was considered 100% of binding response (lower graphs). Data represent the mean +/- SEM (n = 2). One representative assay out of two independent assays performed in duplicate is shown. (C) Proposed model of biomolecular interaction between N-protein and CHKs, where both the NTD and CTD RNA-binding domain of N act as CHK-binding sites.

GAG-Competition Assays: The SARS-CoV-2 N-protein binds CHKs through the GAG-binding site of CHKs

To exert their function, CHKs need to interact with both surface GAGs and their receptors. To investigate whether the GAG-binding or the receptor-binding site of CHKs is involved in the interaction with N-protein, we performed GAG-competition assays with 3 CHKs binding to N-protein on Octet® BLI (Figure 6A). CHKs (CCL26, CCL28, and CXCL10) were pre-incubated with soluble GAGs (chondroitin sulfate A, and B). CHKs bind GAGs through its GAG-binding site, which blocks and makes this CHK binding site inaccessible for additional interaction with the N-protein. It was previously reported that N-protein does not bind these particular GAGs, i.e. chondroitin sulfate A, and B and therefore any binding observed would be from the specific interaction of the chemokine to N-protein **[4]**. In this experimental scenario, the N-protein might bind CHKs through the receptor-binding site of CHKs, where the GAG-binding site of CHKs (blocked by soluble pre-incubated GAGs) would not affect the N-protein - CHK interaction (Figure 6A, Scenario 1). On the other hand, if the N-protein bound CHKs through the GAG-binding site of CHKs, the N-protein - CHK interaction would be impaired, since this binding site would be unavailable for interaction due to the presence of pre-bound soluble GAGs (Figure 6A, Scenario 2). By blocking the GAG-binding site of these CHKs with increasing concentrations of soluble GAGs, the ability of N-protein to bind and retain these CHKs was abrogated in a concentration-dependently manner (Figure 6B). The binding response obtained from each CHK binding to immobilized N-protein in the absence of GAGs was considered 100% of binding. These results indicate that N-protein binds CHKs through the GAG-binding site of CHKs, in accordance with Figure 6A, Scenario 2.

The SARS-CoV-2 N-protein was shown as the first secreted vCBP from human coronaviruses **[4]**. Our results show that N-protein is able to bind CHKs with both of its RNA-binding domains, through the GAG-binding site of CHKs. N-protein binding to CHKs has on average a higher affinity (nanomolar range) than for ssRNA (micromolar range) **[3-5]**.

Previous analysis of Octet® BLI kinetic data between N-protein and each of the 11 CHK bound by N-protein showed heterogeneous binding profiles, deviating from a classical 1:1 interaction **[4]**. The best performing binding model to calculate the affinity constants was the heterogeneous ligand model (2:1), which assumes CHK binding at two independent sites on the N-protein (**See Application Note: Biomolecular Binding Kinetics Assays on the Octet® BLI Platform**) Therefore, it seemed likely that a N-protein molecule could be binding two CHKs at the same time, one CHK through each RNA-binding domain.

Conclusion

Elucidating biomolecular interactions, the binding mechanism and the structural components facilitating the interaction are key aspects in understanding receptor-ligand interactions and its effect on cellular signaling pathways, many of which play a crucial role in the progression of diseases and infections such as cancers, chronic inflammation, auto-immune disease, and viral infections. The Octet® BLI platform allows one to map the protein domains involved in signaling and enable the identification of potential targets for therapeutic development. In this application note, we use the biomolecular interaction between human chemokines (CHK) and SARS-CoV-2 N-protein as a model for protein domain mapping by using the Octet® BLI platform, to show that each RNA-binding domain of the SARS-CoV2 N-protein is a CHK-binding site. We provide evidence of the ability of N-protein to independently bind CHKs through the N-terminal and C-terminal domains. Further, competition assays with GAGs were performed which indicated that N-protein bound CHKs through the GAG-binding site of CHKs, showing the capabilities of the Octet® BLI platform to perform competition assays of biomolecular interaction, and by doing so, mapping of protein domains/sites of interaction between interacting partners.

The application note discusses the setup of the assay formats used in the study such as competition assays, antibody blocking assays and provides guidance on optimizing key factors such as ligand loading on the Octet® biosensors and samples preparation and handling.

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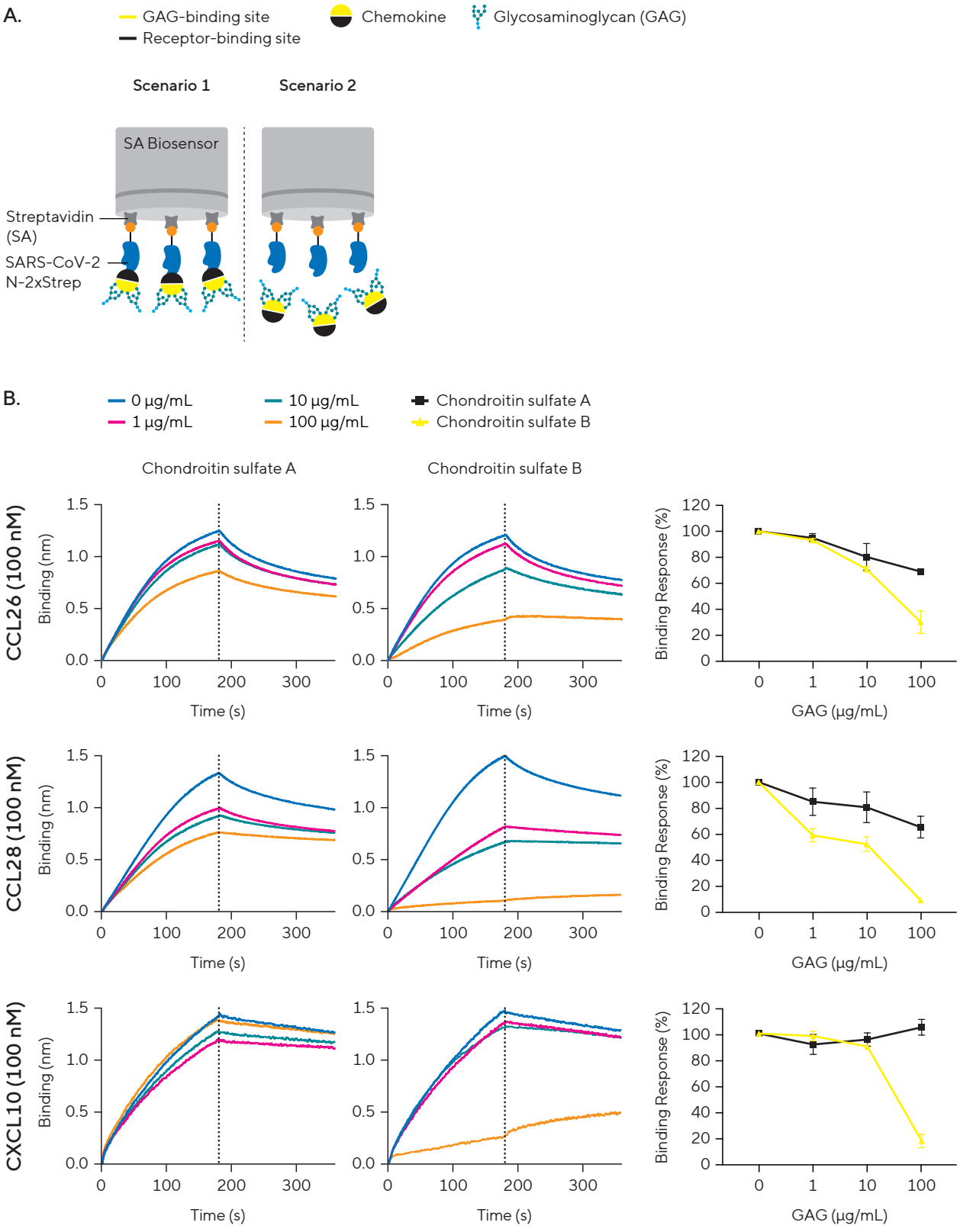
Figure 6: *Mapping GAG Binding Site on SARS-CoV2 N-Protein*



Note. The GAG-binding domain of CHKs mediate the interaction with the N-protein.
A. Proposed scenarios of CHK-N-protein biomolecular interaction, where CHKs may bind to N-protein through their receptor-binding site (scenario 1), or through their GAG-binding site (scenario 2). Prior incubation of CHKs with soluble GAGs blocks the GAG-binding site of CHKs, i.e., the CHK availably for interaction through its GAG-binding site.
B. BLI sensorgrams of binding assays showing association and dissociation phases of the interaction between the N-protein and CHKs, in the presence of GAGs (left and middle graphs). Prior to the association phase, CHKs were incubated with increasing concentrations of each soluble GAG. The dotted line indicates the end of the association phase. The binding response of each CHK to N, in the absence GAGs, was considered 100% of binding response (right graphs). Data represent the mean +/- SEM of 2-3 independent experiments. The N-protein does not bind chondroitin sulfate A/B, as previously described [4].

References

1. Gonzalez-Motos, V., K.A. Kropp, and A. Viejo-Borbolla, Chemokine binding proteins: An immunomodulatory strategy going viral. Cytokine Growth Factor Rev, 2016. 30: p. 71-80.

2. Hernaez, B. and A. Alcamí, Virus-encoded cytokine and chemokine decoy receptors. Curr Opin Immunol, 2020. 66: p. 50-56.

3. Zinzula, L., et al., High-resolution structure and biophysical characterization of the nucleocapsid phosphoprotein dimerization domain from the Covid-19 severe acute respiratory syndrome coronavirus 2. Biochem Biophys Res Commun, 2021. 538: p. 54-62.

4. López-Muñoz, A.D., et al., Cell surface SARS-CoV-2 nucleocapsid protein modulates innate and adaptive immunity. Science Advances, 2022. 8(31): p. eabp9770.

5. Dinesh, D.C., et al., Structural basis of RNA recognition by the SARS-CoV-2 nucleocapsid phosphoprotein. PLoS Pathog, 2020. 16(12): p. e1009100.

6. Crijns H, Vanheule V and Proost P. Targeting Chemokine–Glycosaminoglycan Interactions to Inhibit Inflammation. Front. Immunol., 2020, Vol. 11:483. **https://doi.org/10.3389/fimmu.2020.00483**

7. Foeng J, Comerford I, McColl SR. Harnessing the chemokine system to home CAR-T cells into solid tumors. Cell Rep Med. 2022 Feb 28;3(3):100543. **https://doi.org/10.1016/j.xcrm.2022.100543**

8. **https://www.sartorius.com/en/pr/octet/optimizing-kinetics-assays-to-avoid-avidity-application-note**

9. **https://www.sartorius.com/en/pr/octet/biomolecular-binding-kinetics-assays-on-the-octet-platform**

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