APPLICATION NOTE

Label-Free Characterization of the Therapeutic Antibody Tocilizumab with Digital SPR™

Overview

Therapeutic monoclonal antibodies are one of the fastest growing classes of drugs, and interleukins and their signal pathways are a popular target for treatments due to the vast number of problems their improper regulation present. Surface plasmon resonance (SPR) is a powerful tool that is well-suited for analyzing the binding interactions associated with signaling proteins, their receptors and related effectors. In this application note, we demonstrate the ability of Nicoya's Digital SPR to advance the development of antibody therapeutics, with a focus on interleukin and interleukin-related therapies.

Introduction

Interleukins are a specialized group of cytokines essential for coordinating immune responses, including cell proliferation, differentiation, and inflammation.[1] Among these, Interleukin-6 (IL-6) acts as a critical "alarm system" for the body, expressed in response to environmental stressors such as infection or tissue damage.[1,2]

Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is a pleiotropic cytokine with wide-ranging effects on immune regulation, hematopoiesis, inflammation, and oncogenesis.[3,4] It is secreted by a variety of cell types, including T cells, B cells, monocytes, and fibroblasts, often in response to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs).[4,5] Under physiological conditions, IL-6 acts as an essential mediator of the acute-phase response, coordinating the body's defense against infection and tissue injury.[5]

However, the dysregulation of IL-6 production is a hallmark of chronic inflammatory pathologies. Elevated serum levels of IL-6 are correlated with disease activity in rheumatoid arthritis, where it drives synovial inflammation and joint destruction, as well as in the "cytokine storm" associated with CAR-T cell therapy and severe viral infections.[5]

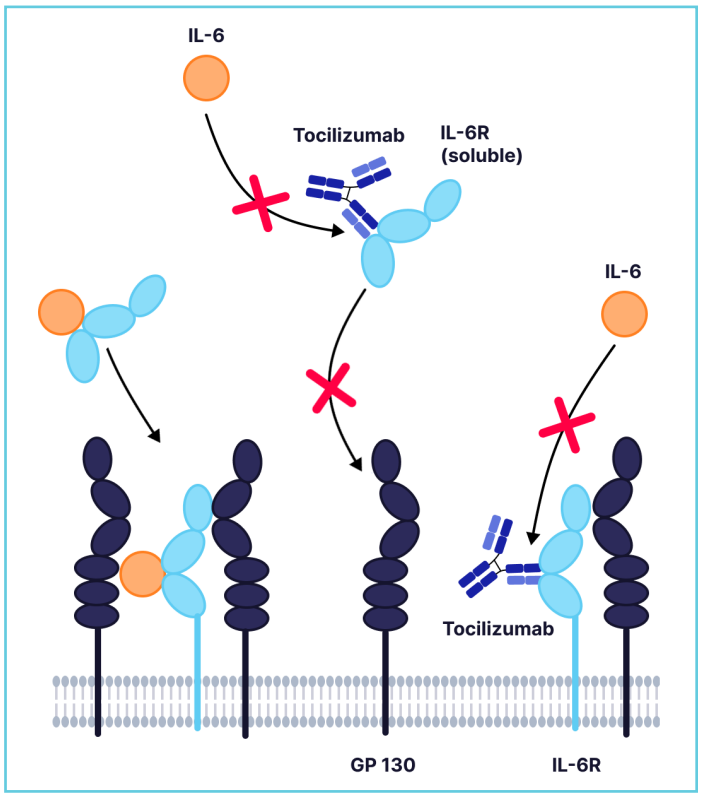

Figure 1: *Inhibition of IL-6 Signaling by Tocilizumab. IL-6 binding to IL-6R or the soluble IL-6R (sIL-6R) causes the dimerization of gp130 and triggers a downstream signal cascade. Tocilizumab binds to the IL-6R (or sIL-6R), preventing the binding of IL-6, which mediates IL-6 trans-signaling and reduces the chance of a cytokine storm.*

Tocilizumab: Mechanism of Action (MoA)

When IL-6 binds to the IL-6 receptor (IL-6R), it recruits glycoprotein 130 (gp130), triggering its signal cascade.[6] Tocilizumab is a recombinant humanized IgG1κ antibody derived from a mouse anti-human IL-6R antibody (clone PM-1), grafted onto a human framework to minimize immunogenicity.[7] Tocilizumab works by binding to the IL-6R with high affinity, effectively outcompeting IL-6, blocking the recruitment of gp130 and the signalling pathways that drive chronic inflammation (Figure 1).[7-9] This mechanism of action is distinct from antibodies that target the cytokine itself such as Siltuximab.[8,9]

Kinetic analysis with Digital SPR

As the demand for monoclonal antibody drugs grows, scientists require advanced tools to characterize these complex interactions. Traditional methods like ELISA and Western Blot can be time-consuming and require external labels, while Surface Plasmon Resonance (SPR) offers real-time, label-free data on binding kinetics. This application note explores the use of Nicoya's Digital SPR, a high-throughput SPR platform powered by digital microfluidic (DMF) technology. Digital SPR automates assays and reduces sample consumption by up to 200X compared to traditional SPR and BLI, providing a highly efficient solution for kinetic analysis of IL-6 and Tocilizumab and accelerating the development of life-saving therapeutics.

Materials

- Alto 16-Channel with Nicosystem Pro (DSPR16-PRO)
- 16-Channel Carboxyl Cartridge (DSPR-CBX-CMD-16)
- 16-Channel Streptavidin Cartridge (DSPR-STV-CMD-16)
- Running Buffer: PBS-T (DSPR-PBST) + 5 mM EDTA
- Carboxyl Surfacing Kit: cleaning, normalization, activation (DSPR-CBX-SURF)
- Regeneration Solution: 10 mM Glycine-HCl, pH 1.5 (DSPR-GLYHCl-1.5)
- Capture Surface: Nicoya human/rabbit VHH kit (DSPR-VHH-HR-KIT)
- Ligand: IL-6 (Sino Biological, Cat: 10395-HNAE)
- Ligand: Tocilizumab (Selleckchem, Cat: A2012)
- Analyte: IL-6R (Sino Biological, Cat: 10398-H08H)

Methods

Experiment design

The experimental setup was completed remotely on the Digital SPR Nicosystem™ User Portal, followed by run initiation on the instrument:

1. From a laptop, the experiment was designed and saved in the Nicosystem.
2. On the instrument, the designed method was selected to launch the Digital SPR on-screen setup guide.
3. A Digital SPR 16-Channel Cartridge was placed in the instrument, and samples were loaded into the cartridge following the experiment setup guide.
4. The experiment was initiated on the Digital SPR by selecting "Run Method".

Assay Protocols

Nicoya's eBook: Mastering kinetic binding assays walks through the assay development steps for determining the optimal experimental conditions for analyzing kinetics on Digital SPR platforms. This includes guidance on ligand loading density, analyte concentration, buffer conditions, and regeneration for obtaining accurate and reliable kinetics and affinities.

Direct kinetics (IL-6)

1. Carboxyl sensors were normalized with normalization solutions.
2. Carboxyl sensors were primed with 10 mM HCl for 60 s.
3. Carboxyl sensors were activated with 200mM EDC/NHS for 600 s.
4. 10 µg/mL IL-6 diluted in 10 mM Sodium Acetate, pH 5.5, was immobilized onto even sensors for 600 s.
5. All sensors were blocked with 1 M ethanolamine for 300 s to quench any remaining active carboxyl groups.
6. Functionalized carboxyl sensors were conditioned with 10 mM Gly-HCl, pH 1.5 for 60 s.
7. Digital SPR automatically executed three-fold dilutions from stock concentrations loaded. 450 nM IL-6R was loaded to produce concentrations of 150 nM, 50 nM, 16.7 nM, 5.56 nM and 1.85 nM.
8. The five concentrations of IL-6R were exposed to each sensor from low to high for 180 s, followed by a single dissociation in the running buffer for 600 s, and a single 60 s regeneration step with 10 mM glycine-HCl, pH 1.5. This constitutes a full single-cycle kinetics (SCK) round.

Capture Kinetics (Tocilizumab)

1. Streptavidin sensors were normalized with normalization solutions.
2. 5 µg/mL biotinylated Fc-specific VHH diluted in PBST was immobilized onto all sensors for 300 s.
3. 10 µg/mL Tocilizumab diluted in 10 mM Sodium Acetate, pH 5.5, was captured on to even sensors for 600 s.
4. All sensors were conditioned with 10 mM Gly-HCl, pH 1.5.
5. To perform a buffer blank, even sensors were exposed to 5 µg/mL Tocilizumab or biosimilar for 300 s. All sensors were then exposed to running buffer for 900s and regenerated with 10 mM Gly HCl pH 1.5 for 60s.

6. Digital SPR automatically executed three-fold dilutions from stock concentrations loaded. 300 nM IL-6R was loaded to produce concentrations of 100 nM, 33.3 nM, 11.1 nM, 3.70 nM, and 1.23 nM.
7. Even sensors were exposed to either 5 µg/mL Tocilizumab or biosimilar. All sensors were then exposed to the lowest IL-6R concentration for 180 s, allowed to dissociate in the running buffer for 900 s, and regenerated with 10 mM Gly HCl, pH 1.5 for 60 s. This step was repeated for the remaining four IL-6R concentrations, which then constituted a full multi-cycle kinetics (MCK) round with a buffer blank.

Results & Discussion

In this study, Digital SPR was used to measure the binding kinetics of IL-6 and Tocilizumab to IL-6R using direct amine coupling. The immobilization of ligands on the carboxyl surface was optimized to obtain a low ligand density while allowing for detection of all analyte concentrations sampled. Figures 2a & 2b show examples of raw association and dissociation of analytes and regeneration of the sensor surface for two of the assay setups tested. Full regeneration was achieved for both assays using Gly-HCl pH 1.5.

All kinetic values were calculated using a 1:1 Langmuir fit since the binary IL-6 and IL-6R complex has been shown to be a 1:1 interaction in previous studies.[10] The kinetics parameters measured for both interactions are summarized in Table 1 and representative sensorgrams are shown in Figures 2c & 2d. The K_D values determined were 27.2 nM for IL-6 and 6.25 nM for Tocilizumab, agreeing closely with previous SPR studies that report K_D values of 32.4 nM for IL-6 and 9.9 nM for Tocilizumab.[11,12]

Since IL-6 is the natural binding partner for IL6-R and Tocilizumab is an antibody designed to block IL-6 binding, it is expected that Tocilizumab will bind with a higher affinity than IL- 6. The data collected here indicate that the affinity of Tocilizumab is >4-fold higher than IL-6. Furthermore, the association and dissociation rate constants k_a and k_d show that the difference in affinity is due to a ~3.5-fold faster association rate of IL-6 and a >16-fold slower dissociation rate of Tocilizumab. The slower dissociation rate will allow Tocilizumab to stay bound for longer and block IL-6 binding of IL-6R.

Conclusion

This study demonstrates that Nicoya's Digital SPR platform enables rapid, label-free characterization of ligand-receptor and antibody–receptor interactions, providing high-resolution kinetic data with minimal sample consumption. Using Digital SPR, the binding of IL-6 and Tocilizumab to IL-6R was measured, revealing that Tocilizumab binds with >4-fold higher affinity than IL-6, and stays bound much longer due to its slower dissociation rate. These results confirm Tocilizumab's effectiveness in blocking IL-6 receptor binding and illustrate its mechanism of action in blocking IL-6-mediated signaling. Overall, Digital SPR proves to be a powerful tool for evaluating therapeutic antibodies, offering precise insights into binding kinetics that can guide drug design and optimization in cytokine-targeted therapies.

Table 2: *Kinetic parameters measured for IL6 and Tocilizumab binding to IL-6R*

Ligand	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
IL-6	$1.82 \times 10^5 \pm 1.41 \times 10^4$	$4.91 \times 10^{-3} \pm 2.79 \times 10^{-4}$	27.2 ± 2.8
Tocilizumab	$5.21 \times 10^4 \pm 1.48 \times 10^4$	$3.03 \times 10^{-4} \pm 1.05 \times 10^{-4}$	6.25 ± 2.70

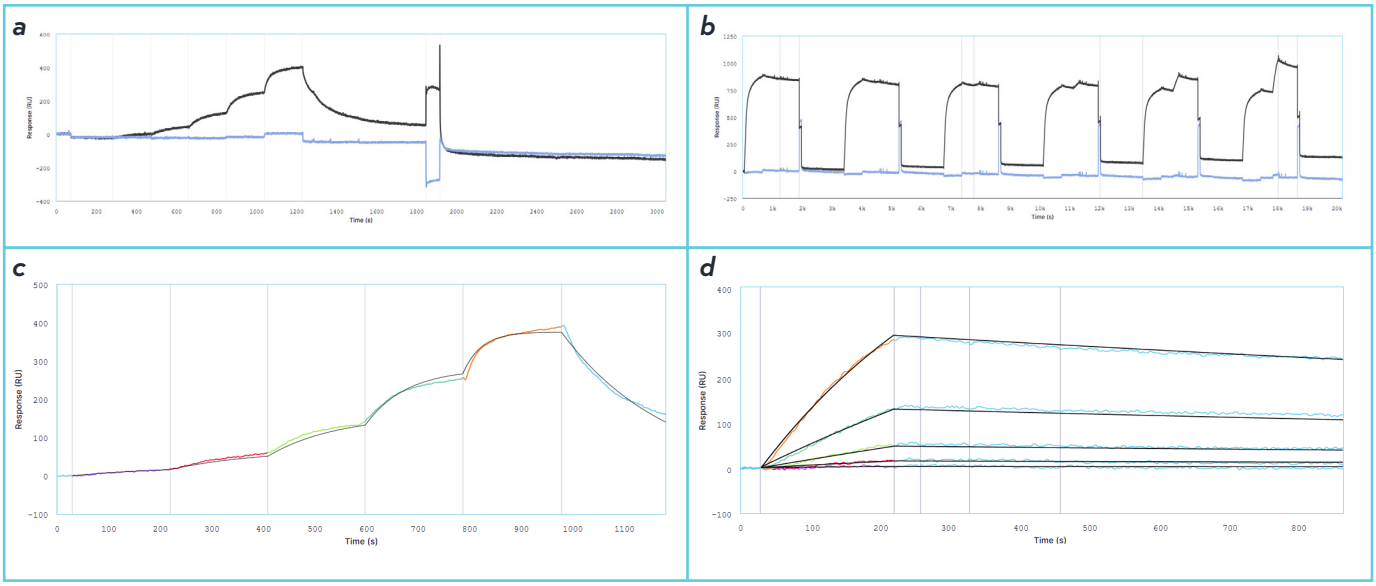

Figure 2: *Representative raw sensorgrams showing analyte binding and regeneration for immobilized (a) IL-6 and (b) Tocilizumab. IL-6R concentrations ranged from 1.85 nM to 150 nM for IL-6 binding as described in the figure legend. The analyte concentrations of the five Tocilizumab curves from low to high were 1.23 nM, 3.7 nM, 11.1 nM, 33 nM and 100 nM. Active and reference channels are shown in black and blue respectively, with 10 mM Glycine-HCl, pH 1.5 used to achieve full regeneration of the sensor surface. Panels (c) and (d) show the corresponding reference-subtracted data for IL-6 and Tocilizumab, respectively. The binding curves are fitted with a 1:1 Langmuir model (black curve), calculated using the Nicosystem Pro Software.*

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