Application Note #5

iCLUEBIO

Kinetic Analysis of Fc Gamma Receptors and Nivolumab

Introduction

Fc gamma receptor & Nivolumab

Fc gamma receptors (FcγRs) recognize the Fc region of immunoglobulin G (IgG) antibodies. These receptors are classified into three main types—FcγRI, FcγRII, and FcγRIII—based on their binding affinity for the Fc region, with FcγRI exhibiting the highest affinity. Each receptor type comprises several subclasses with distinct properties.

Nivolumab is a human monoclonal antibody of the IgG4 isotype that targets the PD-1 receptor. It is widely used as an immune checkpoint inhibitor for cancer immunotherapy. Since its FDA approval in 2014, Nivolumab has been employed in the treatment of metastatic melanoma, non-small cell lung cancer, gastric cancer, and ovarian cancer.

This application note investigates the binding kinetics between the Fc region of Nivolumab and various FcγRs using surface plasmon resonance (SPR).

Materials and methods

Materials

- Instrument : iMSPR-ProX [CAT#INPX1000]
- Sensor chip : C-Dex100 [CAT#CDDH1100]
- Amine coupling kit [CAT#IMAM1000]
- Running buffer : PBS [CAT#RBPB1010-500]
- Ligand :FcγRI (CD64) / FcγRIIa (CD32a) / FcγRIIIa (CD16a) / FcRn
- Analyte : Nivolumab
- Regeneration solutions:
  Glycine-HCl (pH 3.0) [CAT#RGGH1030]
  1M Ethanol-amine (pH 8.5) [CAT#IMBK1000]
  PBS [CAT#RBPB1010-500]

Preparation

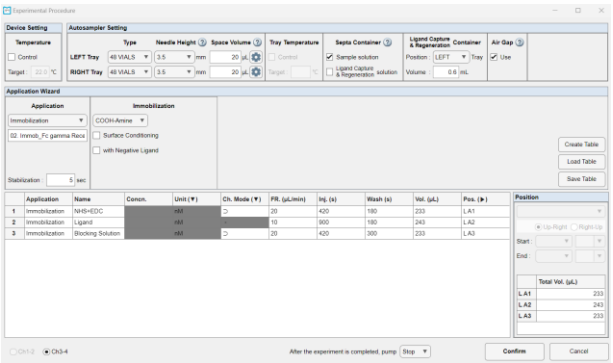
1. The C-Dex100 sensor chip was mounted onto the prism holder of the iMSPR-ProX system. After connecting 1× PBS as the running buffer, the system was primed.
2. The chip surface was then pre-conditioned by injecting 50 mM NaOH at a flow rate of 30 μl/min for 1 minute, repeated three times.

[Figure 1. Sensor chip loaded onto prism holder]

Immobilization: Amine coupling

1. In continuous flow mode, a mixture of 200 mM EDC and 100 mM NHS was injected at 20 μl/min for 7 minutes to activate carboxyl groups on both the ligand and reference channels.

2. In individual flow mode, FcγR proteins were diluted in 10 mM acetate buffer (pH 4.0) to a final concentration of 30–50 μg/mL and immobilized by injecting at 10 μl/min for 10–15 minutes.
3. Change back to continuous mode and residual COOH groups were blocked with 1M Ethanolamine-HCl at 20 μl/min for 7 minutes.



[Figure 2. Experimental procedure for ligand immobilization]

Kinetic analysis

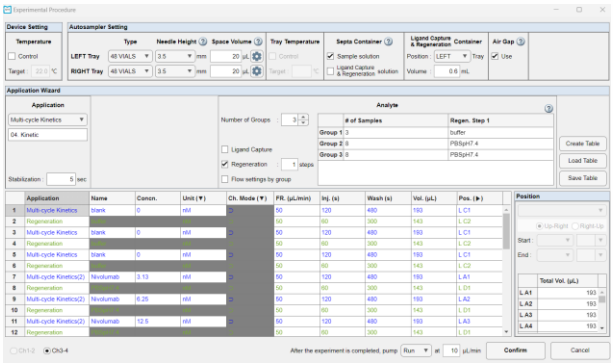
1. Nivolumab was serially diluted 2-fold in running buffer to prepare a concentration range as listed in Table 1.
2. Each diluted solution was injected over the surface at 50 μl/min for 2 minutes to observe the association phase.
3. After the injection is complete, wait for 8 minutes with the running buffer flowing to observe the dissociation phase.
4. The surface was regenerated under conditions specified in Table 2, ensuring complete removal of residual analyte.
3. After regeneration, the steps described in sections 2–4 were repeated using the next concentration of Nivolumab. [Figure 3]
4. Following completion of the experiment, kinetic analysis results were automatically evaluated. [Figure 4]

Ligand	Range of analyte conc.
FcγRI (CD64)	1.56 - 100 nM
FcγRIIa (CD32a)	7.8 - 500 nM
FcγRIIIa (CD16a)	15.6 - 1000 nM
FcRn	3.13 - 200 nM

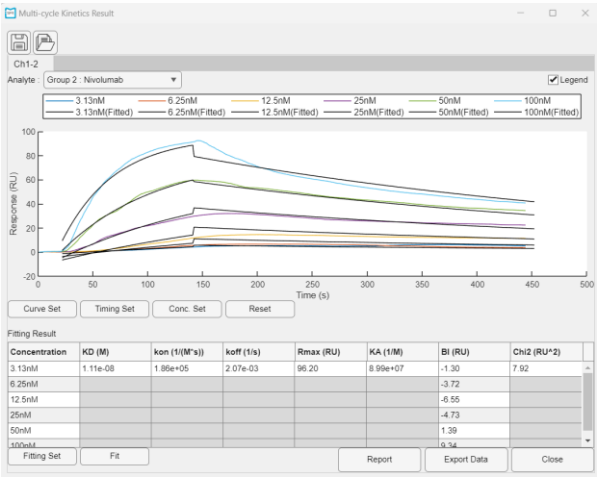
[Table 1. Concentration ranges of Nivolumab per ligand]

Ligand	Regeneration solution	Injection time/ Wash time
FcγRI (CD64)	Glycine-HCl (pH 3.0)	1 min / 10 min
FcγRIIa (CD32a)	1M Ethanol-amine (pH 8.5)	1 min / 3 min
FcγRIIIa (CD16a)	1M Ethanol-amine (pH 8.5)	1 min / 3 min
FcRn	1X PBS (pH 7.4)	1 min / 3 min

[Table 2. Regeneration conditions per ligand]

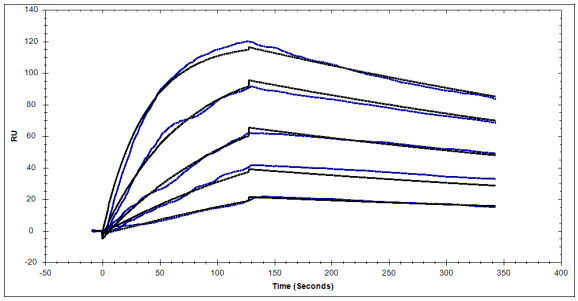


[Figure 3. Experimental procedure for kinetic analysis]



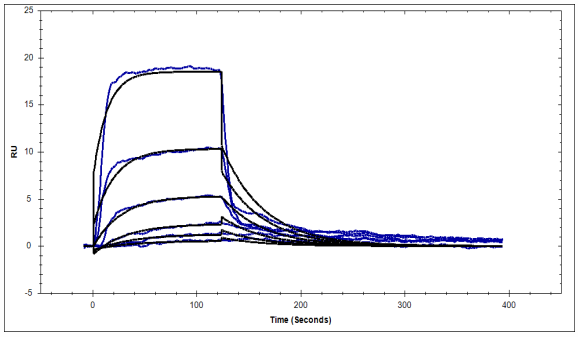
[Figure 4. Result of the kinetic evaluation]

Results



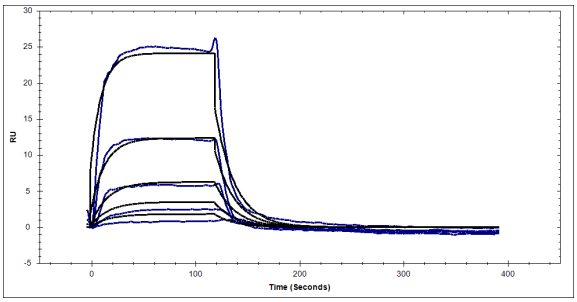
k_a (1/Ms)	k_d (1/s)	K_D (M)
1.02E+06	1.45E-03	1.43E-09

[Figure 5. Kinetic analysis of FcγRI and Nivolumab]



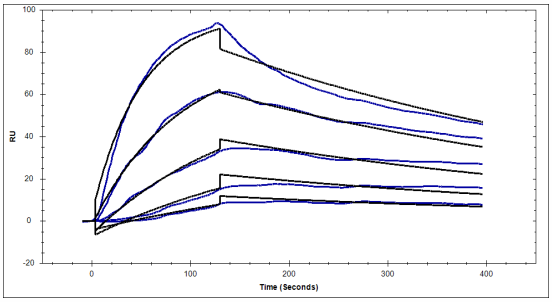
k_a (1/Ms)	k_d (1/s)	K_D (M)
9.84E+04	2.51E-02	2.56E-07

[Figure 6. Kinetic analysis of FcγRIIa and Nivolumab]



k_a (1/Ms)	k_d (1/s)	K_D (M)
4.23E+04	5.23E-02	1.24E-06

[Figure 7. Kinetic analysis of FcγRIIIa and Nivolumab]



k_a (1/Ms)	k_d (1/s)	K_D (M)
1.86E+05	2.07E-03	1.11E-08

[Figure 8. Kinetic analysis of FcRn and Nivolumab]

Conclusion

This study demonstrated that the binding affinity (K_D) of Nivolumab (IgG4 isotype) differs among Fc gamma receptor subtypes, with the strongest interaction observed for FcγRI, followed by FcγRIIa and FcγRIII. These differences in receptor-specific binding affinities were validated using the iMSPR-ProX.

Fc gamma receptors are actively being investigated as therapeutic targets for various immune-related diseases. SPR offers the advantage of real-time monitoring of molecular interactions without the need for fluorescent labeling or conjugation.

Reference

- Bournazos, Stylianos, Aaron Gupta, and Jeffrey V. Ravetch. "The role of IgG Fc receptors in antibody-dependent enhancement." *Nature Reviews Immunology* 20.10 (2020): 633-643.
- Bruhns, Pierre, et al. "Specificity and affinity of human Fcγ receptors and their polymorphic variants for human IgG subclasses." *Blood, The Journal of the American Society of Hematology* 113.16 (2009): 3716-3725.