Rapid evaluation of Human IgG and Anti-IgG binding affinity using P4SPR

Introduction

Immunoglobulin G (IgG) is an important contributor to the immune system. Its function has been extensively studied in fields such as medicine and engineering. Specific interactions between the IgG and anti-IgG monoclonal antibody have been studied with various techniques. The binding affinity determination of the antigen-antibody interactions are often vital in various research areas such as development of biotherapeutics.

P4SPR is a robust instrument capable of providing label-free assessment of a variety of different molecular/biomolecular interactions. In this study, we have employed our 4-channel P4SPR to characterize a high affinity antigen-antibody interaction, IgG and anti-IgG in real-time. High quality results were generated rapidly and easily with real-time data monitoring along with reference channel background correction.

Measurement Setup[1]

- Affinité Instruments P4SPR 4-channel sensor
- Au + AffiCoat sensor (Affinité Instruments proprietary peptide-coated sensor)
- Running buffer (PBS , 0.1% BSA, 0.005% Tween, pH 7.4)
- EDC:NHS solution (400mM : 100mM in H_2O)
- Acetate solution (10mM in H_2O, pH 4.5)
- Ethanolamine solution (1M in H_2O, pH 8.5)
- Glycine solution (10mM in H_2O, pH 2.2)
- Goat anti-human IgG (20 µg/mL in acetate solution)
- Human IgG in Running buffer:
  - Set A: 1, 10, 20, 40, 80, 100 pM
  - Set B: 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4,5 nM
- Control: BSA 0.1% in Running buffer

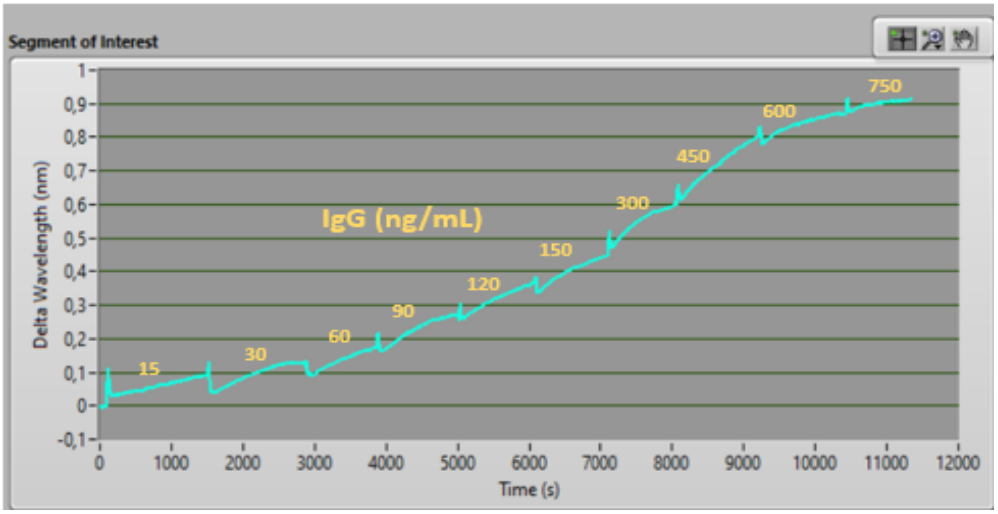

Figure 1. Real-time sensorgram of IgG of different concentrations (set B) binding with Anti-IgG immobilized on an AffiCoat surface using P4SPR. SPR response is the average signal of all 3 sample channels subtracted from the signal of the reference channel.

[1] See Appendix for more details

Procedure

1. Rinse the sensor chip with H_2O and let the signal stabilize for 2 minutes.

2. Inject 300 µL of EDC/NHS into both injector ports for 2 minutes.

3. Inject 300 µL of acetate solution into both injector ports for 10 seconds.

4. Inject 300 µL of Anti-IgG for immobilisation to take place for 20 minutes.

5. Rinse with 300-400 µL of acetate solution.

6. Block the surface with 500 µL of ethanolamine solution for 10 minutes.

7. Passivate the surface with the running buffer and allow signal to stabilize for 15-20 minutes.

8. Inject 300 µL of control in the reference injection port and 300 µL of IgG 1pM in the sample injection port for about 15 minutes.

9. Inject increasing concentrations of IgG (Set A) for 15 minutes each.

10. Regenerate with the glycine solution and passivate the surface with the running buffer.

11. Repeat steps 8 and 9 by injecting concentrations from Set B.

12. Save shifts from each of the concentration of IgG and plot against concentration of IgG for evaluating binding affinity.

Results and Discussion

In this experiment, Anti-IgG was immobilized on the surface and was exposed to increasing concentrations of IgG for the evaluation of binding affinity. In the reference channel, a control solution of BSA 0.1% was injected. The signal obtained from the reference channel was subtracted to eliminate background signal caused by non-specific interactions. The increasing concentration of IgG solutions were injected into the sample injection port. The individual SPR shifts from all three sample channels can be averaged, background corrected and isolated from the real-time raw data which could save tremendously on post-analysis processing time.

The Anti-IgG was immobilized on AffiCoat sensor chip which provides proven function to reduce non-specific interaction. Figure 1 shows the sensorgram of Anti-IgG binding to increasing concentrations of IgG. The regeneration of the surface was successful and excellent results were presented for Set B after surface regeneration. Note that even though the injections were made manually, consecutive injections were reproducible. This extends the capabilities of the system in providing stability and precision as well as robustness of the sensor surface.

Figure 2 shows the binding curve which demonstrates concentration dependence. Minimal binding was occurred in the reference channel. An algorithm fit of 1:1 binding was employed and are overlaid. The K_D is determined to be 3.07 nM for this interaction which are in concordance with other studies involving Anti-IgG and IgG.

The P4SPR advantage

Affinité instruments' P4SPR is a modular, label-free multichannel system with great application versatility. Its accessibility and ease of use allow for rapid high quality SPR results in antibodies characterization. Its multichannel feature increases result precision and additionally, with the AffiCoat coated sensor chips, non-specific interactions are greatly reduced. This would be especially interesting for studying interactions in crude samples.

The wider application of the P4SPR to more complex systems has considerable potential to study a variety of antigen-antibody interactions and can be extended to other biomolecular interactions. Examples of real-life clinical and research applications are development of serological testing and DNA-based vaccine.

About Affinité Instruments

Established in 2015 as a spin-off of the Université de Montréal, Affinité instruments' foundation is built deep knowledge accrued on over a decade of research in SPR. The commercialization of promising innovations is spearheaded by a diverse leadership experienced in business, science and engineering.

Appendix

- PBS: Phosphate Buffer Saline
- BSA: Bovine Serum Albumin
- EDC: N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
- NHS: N-hydroxysuccinimide