



Monitoring of interactions between aptamers and human IgE by Surface Plasmon Resonance imaging

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Aptamers are *in vitro*-generated, short, single-stranded nucleic acids that selectively bind to target compounds such as small molecules or proteins^{1,2}. Their main advantage over monoclonal antibodies is their resistance to denaturation and degradation. Being very robust and easy to synthesize, they are becoming widely used for the development of aptamer-based biosensors in the diagnostics field³.

This application note shows that the HORIBA Scientific SPRi platform is suitable for the analysis of aptamer-based molecular interactions. For this purpose, the interaction between human IgE protein, an antibody involved in allergic reactions, and a human IgE specific aptamer⁴ is presented as proof-of-concept.

Key words:

Aptamer - Aptamer, protein interactions - Human IgE - Kinetics analysis - Affinity - Surface Plasmon Resonance imaging

Material and Method

DNA aptamers

The IgE specific DNA aptamer sequence (5'-GGG GCA CGT TTA TCC GTC CCT CCT AGT GGC GTG CCC C-3') and the negative control DNA (5'-GGG GCA CGT TTA TTT TTT TTT TGT GGC GTG CCC C-3') were ordered with TTT TTT T-(CH₂)₆-SH spacer at the 3' end from Sigma-Aldrich.

The (1-mercaptopundec-11-yl)tetra(ethylene glycol) (MUTEG) was purchased from Sigma-Aldrich.

Immobilization of the human IgE (hIgE) aptamer on the SPRi-Biochip™

The thiol modified IgE aptamer and reference DNA was immobilized on the planar gold SPRi-Biochip™ sensor through thiol-Au chemisorption. The aptamer solutions were prepared at 10 μM in 20 mM PBS pH 7.4. Four replicate spots were printed simultaneously for each aptamer using the SPRi-CFM (Figure 1) during a 15-minute back-and-forth cycling. The SPRi-CFM uses flow deposition to immobilize 48 molecules in a single run (up to 144 spots per chip). Immobilizing with flow generates increased spot homogeneity and higher immobilization levels. After the immobilization procedure, the SPRi-Biochip™ surface was blocked with a 0.1 mM MUTEG solution prepared in 10 mM PBS pH 7.4. While MUTEG has good resistance against non-specific adsorption, it is sterically short enough not to hinder the specific binding event.

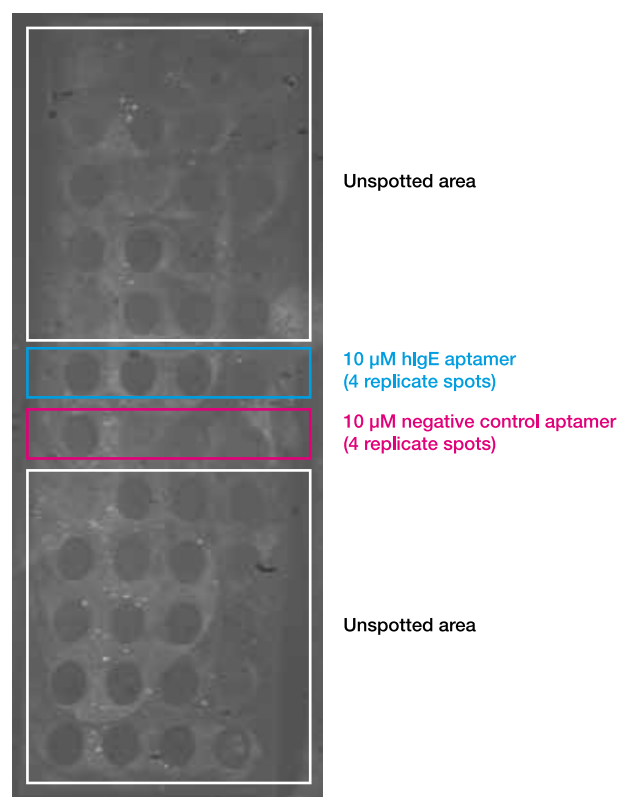


Figure 1: Spotting map and image of the SPRi-Biochip™ surface after spotting

SPRi experiment

The spotted biochip was inserted into the SPRi-PlexII™ instrument from HORIBA Scientific. The system is equipped with a 200 µL sample loop, a continuous flow pump and an in-line degasser. The flow rate was set to 50 µL/min, and the working temperature was fixed at 25°C. Different concentrations of hlgE prepared in a two-fold dilution in the running buffer (10 mM PBS + 1 mM MgCl₂) were injected in order to determine the affinity between hlgE and its aptamer (see Table 1). The SPRi-Biochip™ surface was regenerated using a 12 mM NaOH with 1.2 % EtOH solution after each hlgE injection.

	c0	c1	c2	c3	c4	c5
hlgE concentration (ng/mL)	0	63	125	250	500	1000

Table 1: Concentrations of hlgE passed over the SPRi-Biochip™ surface

Results and Discussion

Specific aptamer-hlgE interaction

Figure 2 shows a SPRi difference image obtained after the injection of 1000 ng/mL hlgE (concentration c5). Only hlgE aptamer spots become white, which confirms that the aptamer-hlgE interaction is highly specific. The SPRi difference image provides a quick confirmation of the presence of binding and its specificity.

The SPRi signals obtained on the hlgE aptamer spots after the subtraction of the negative control signal were compared for the 5 different concentrations of hlgE (see Table 1). Figure 3 shows the calibration curve obtained; the plot was fitted with a linear model ($R^2 = 0.9842$).

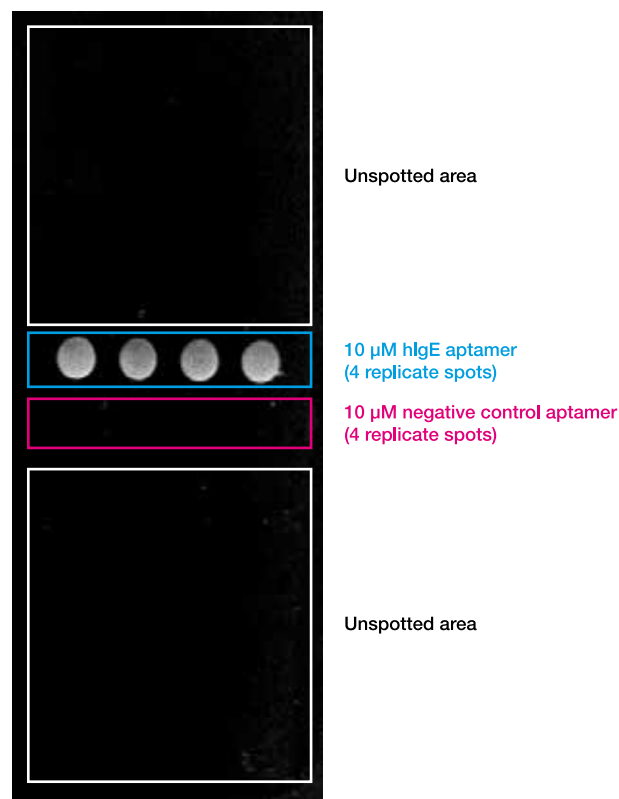


Figure 2: SPRi difference image obtained after the injection of 1000 ng/mL hlgE

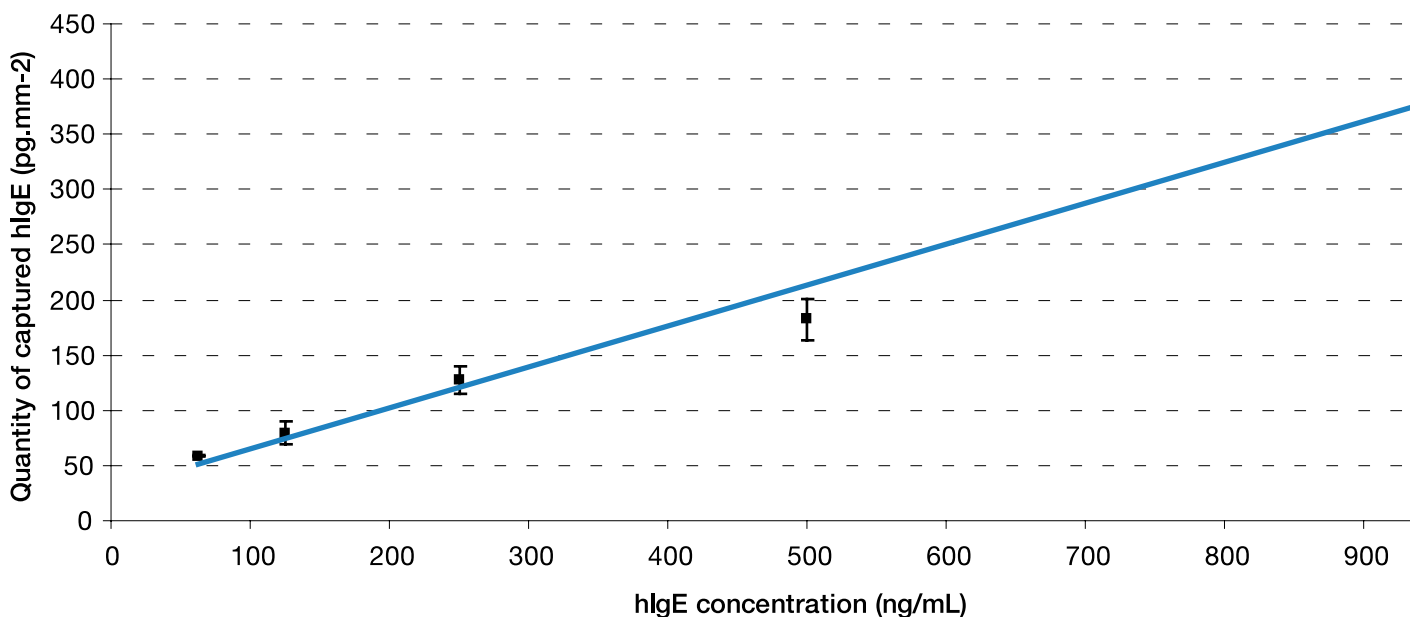


Figure 3: Calibration curve obtained for the aptamer – hlgE interaction

Kinetics analysis of the aptamer-hlgE interaction

The SPRi data was then processed using ScrubberGen software in order to determine the kinetic parameters (association k_a and dissociation k_d rates) and the affinity (K_D) of the aptamer-hlgE interaction. The data was fitted using a 1:1 interaction model (Figure 4). The affinity between the aptamer and hlgE was about 730 pM.

References

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Conclusion

This application note demonstrates that the SPR imaging platform from HORIBA Scientific is well adapted to the characterization of aptamer-protein interactions. The SPRi difference image gives meaningful information regarding the presence of binding and its specificity. The real-time monitoring of the interaction gives access to the kinetics (association and dissociation rates) and the affinity of the interaction. The multiplexing capabilities of the platform will bring very promising applications for the development of aptamer-based biochips in biomedical diagnostics.

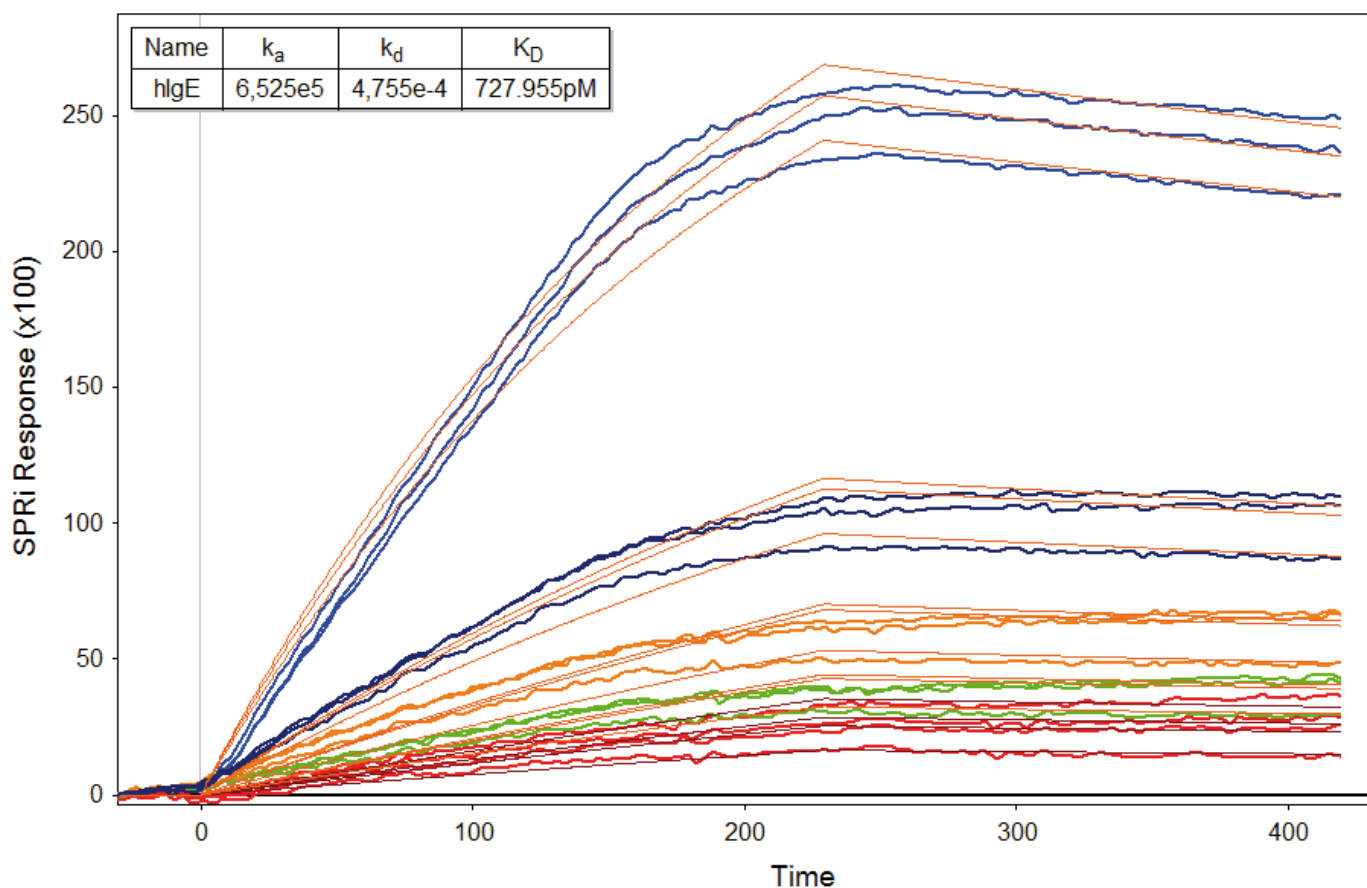


Figure 4: Kinetics analysis of the aptamer – hlgE interaction using ScrubberGen



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