Application Note

July 2021

Rapid measurement of Antibody-Antigen Kinetics using The Bolt Analysis Platform

Brice Darroch, Camilla Trevor, Christoph von Bieberstein, Lukas Vasadi, Peter Ball, Ruizhi Wang

HexagonFab, Cambridge, United Kingdom

SUMMARY

In Drug Discovery and Process Monitoring, there is an unmet need for low-cost, rapid, and accurate methods for analysing interactions between molecules. Label-free assays are the most widely used method which offers several benefits to the user, including real-time data read-out, quantitative data, and high sensitivity. However, current label-free, optical-based systems like surface plasmon resonance (SPR) and bio-layer interferometry (BLI), suffer from loss of sensitivity with decreasing molecular size, high cost, and complex operating procedures. The key aim of this application note is to showcase the HexagonFab Bolt as a new user-friendly tool for Biomolecular Interaction Analysis (BIA), usually performed with SPR or BLI instruments. Here we used Bolt and Biacore T200 to assess the affinity of a commercial anti-Avidin antibody by measuring the association (k_a) and dissociation (k_d) constants. The K_D of 48 nM determined with Bolt agreed with the value obtained by the Biacore - the industry-standard SPR instrument.

Acknowledgements: We thank Katherine Stott (Biochemistry Department, University of Cambridge) for performing the SPR measurements reported in this application note.

THE HEXAGONFAB BOLT

Rapid measurement of association/dissociation rates

To address the limitations of optical-based, label-free analysis platforms, HexagonFab has developed a graphene-based sensor system, named Bolt. The sensor measures changes in the electrical properties of a charge-sensitive graphene membrane which are induced by the presence of molecules at the sensor-analyte interface (e.g., proteins, small molecules, or DNA/RNA). Those changes are translated into response units and the magnitude of the response depends on the number of charges at the sensor interface, thus the higher the concentration, the higher the response change. The system offers rapid readout and a single kinetics measurement was completed in as little as 40 minutes.

In this application example, we used Bolt to measure the binding interaction between a target (Avidin) immobilised on the sensor surface and its complementary antibody.

MATERIALS & METHODS

Sensor fabrication and Avidin immobilisation

Graphene sensors were fabricated according to the method developed by Wang *et al*. (Wang, 2018; Wang, 2019) and functionalised with a carboxyl-terminated linker (proprietary). Avidin (1 mg/mL; Sigma-Aldrich) was immobilised on Bolt amine-reactive sensor and Biacore chip (CM5, Cytiva) using EDC/NHS-chemistry (Fischer, 2010)(Sigma reagents).

Binding conditions

Binding experiments were carried out in a binding buffer (PBS, pH 7.4, Sigma). The anti-Avidin antibody (monoclonal antibody, ThermoFisher) was measured on Bolt at 3 concentrations (75 nM, 150 nM, 250 nM; 21 °C) and 4 concentrations on Biacore T200 (4 nM, 20 nM, 100 nM, 500 nM; 30 µL/min, 25 °C). The bovine serum albumin (BSA, Sigma-Aldrich) used for the specificity experiment was dissolved in binding buffer to 150 nM.

Data Acquisition

The affinity and kinetic parameters were determined by HexagonFab Bolt (in-house) and Biacore T200 (external facility) instruments. Data was acquired in 3 steps: baseline (binding buffer, 15 min), association (analyte in binding buffer, 6 min), dissociation (binding buffer, 15 min). Binding signals were analysed with the Biacore Evaluation or in-house developed software (Bolt data). Curves were fitted with the standard 1:1 Langmuir binding model.

RESULTS & DISCUSSION

Target-specific binding signal

To demonstrate the sensor's ability to discriminate between a specific and non-specific binding event, the sensor surface was coated with Avidin and subsequently incubated either with BSA or anti-Avidin antibody. The sensor showed a low response for binding of BSA to the surface (Figure 1A), which proved the signal selectivity for specific binding events.

Antibody affinity determined with HexagonFab Bolt and Biacore T200

The analysis was performed by running an anti-Avidin antibody as the analyte. The generated signals (Figure 1B) were identical to a typical association/dissociation curve recorded by an SPR/BLI instrument. The sensor was able to distinguish the signal response for each concentration. All of the K_D values were within the same order of magnitude, as shown in Table 1. Moreover, the determined mean association (k_a), dissociation (k_d), and binding constants (K_D) were comparable to the results obtained by a Biacore T200 instrument (Table 1).

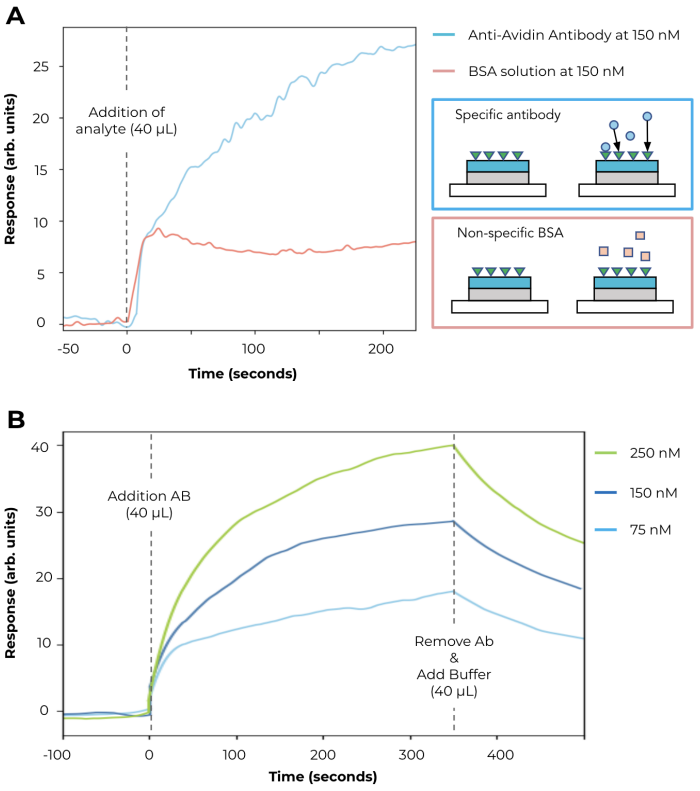

Figure 1. Binding kinetics measured on HexagonBolt. **(A)** Sensor shows high signal specificity for antibody against Avidin-coated sensor. **(B)** Affinity determination of anti-Avidin antibody. The measurement was done for 3 antibody concentrations in the binding buffer.

Table 1. k_a and k_d values measured with HexagonFab Bolt and the mean values for data measured on Biacore T200 across the concentration range stated in Materials and Methods.

Instrument	IgG (nM)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
HexagonFab Bolt	75	1.3×10^5	9.0×10^{-3}	70
	150	1.7×10^5	2.8×10^{-3}	25
	250	5.4×10^4	2.4×10^{-3}	43
	Mean:	9.8×10^4	4.7×10^{-3}	48
Biacore T200		9.5×10^3	2.0×10^{-4}	21

CONCLUSION

The affinity for the described Avidin/anti-Avidin interaction was measured to be 48 nM, which is similar to the values determined by an established instrument, in this case the Biacore T200. Due to simplicity of operation, low cost, and rapid signal acquisition, the HexagonFab Bolt offers an attractive alternative to standard SPR/BLI techniques for analysing antibody-antigen interactions. With the use of an innovative mass-independent signal detection strategy, Bolt will become a useful tool for analysing interactions between a wide range of biomolecules, including small molecules, DNA, and more.

REFERENCES

Fischer MJ., Methods Mol Biol. 2010; 627:55-73.

Wang R. *et al,* patent WO2020099890A1, 2018.

Wang R. *et al.*, ACS Nano. 2019 Feb 26; 13(2):2114-2126.

HexagonFab

HEXAGONFAB LIMITED

UNITED KINGDOM
Unit 1, Cambridge House
Oakington Road
Cambridge, CB3 0QH

SWITZERLAND
Impact Hub Zurich
Sihlquai 131
8005 Zurich