Throughput Meets Flexibility: The Next Generation Multiplexing SPR for Efficient Therapeutic Protein Discovery and Development

Cyrill Brunner[1], Sven Malik[2], Paul Ritter[2], Soumav Nath[2], Christopher Pelczar[2], and Meike Hamester[2]

[1] Bruker Switzerland AG, Industriestrasse 26, CH-8117 Faellanden

[2] Bruker Daltonics GmbH & Co. KG , Fahrenheitstraße 4, GER-28359 Bremen

Introduction

Surface Plasmon Resonance is an established and widely used biophysical technology in drug development. The real-time, label-free analysis of interactions offers insights into kinetics, thermodynamics and more. We developed a novel rotatable 8x8 microfluidics that allows to determine affinity, kinetics and conditional binding constants in a single assay at high throughput. Partnered with our established SPR+ detection system for state-of-the-art sensitivity, the SPR #64 provides multiple assay formats perfectly suited for therapeutic protein discovery and development.

High Affinity Kinetics

The development of therapeutic proteins often result in binders of picomolar affinities and off-rates close to 10^{-6} s^{-1}. This requires an instrument with a stable base-line and temperature control as minimal differences in temperature can already result in signal disturbances. Since the slow off-rates require long measurement times, an efficient assay set-up without any compromise on data quality is beneficial.

We have tested a single oligonucleotide against 8 slightly modified complementary oligonucleotides. Affinities of 100-300 pM and off-rates of 6 x 10^{-5} s^{-1} at 37 °C. The assay was performed in the single-injection cycle kinetics (SICK) assay format which allowed to determine eight affinities within a single injection. This reduced measurement time by about 80% compared to standard multi-cycle kinetics assay and offers an efficient, attractive alternative especially for affinity maturation tests of high affinity therapeutic proteins.

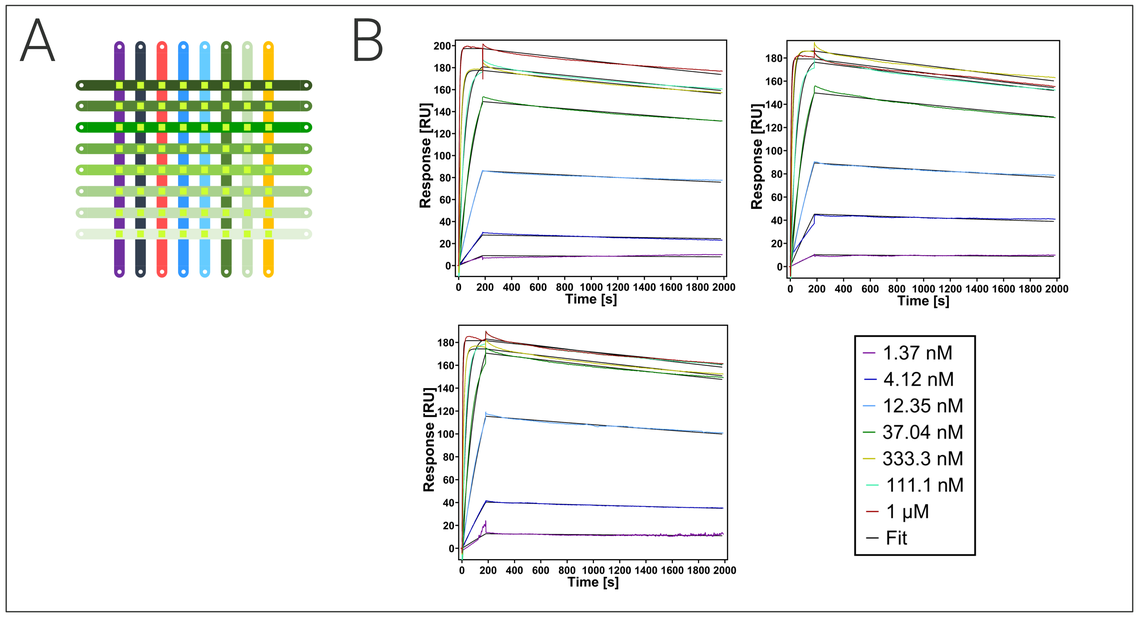

Fig. 1. A: Graphical representation of the assay set-up. Oligonucleotides were immobilized by biotin capture with one per spot column in vertical direction. The complementary oligonucleotides were injected in horizontal direction with a single injection cycle kinetics assay format with seven concentrations and one buffer control in horizontal direction. B: Representative sensorgrams.

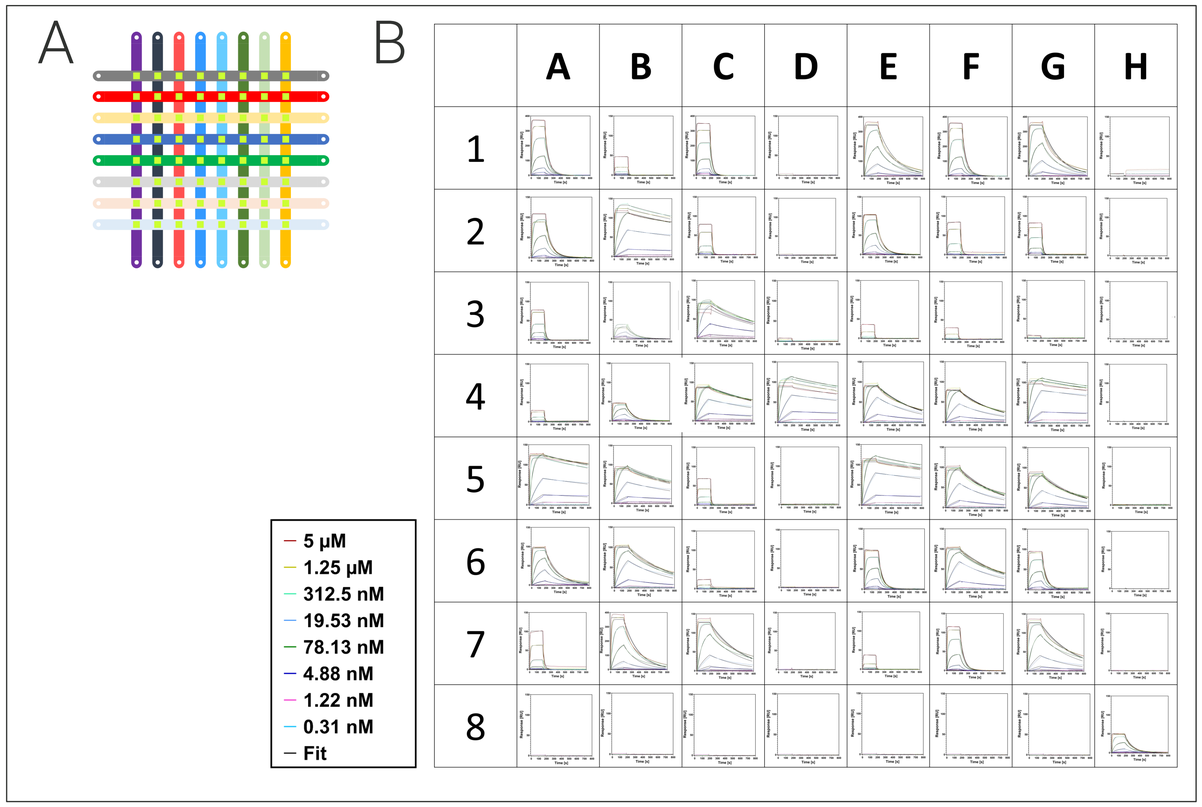

Fig. 2 A: Graphical representation of the assay set-up. Oligonucleotides A-H were immobilized by biotin capture on spot columns 1-8, the oligonucleotides 1-8 were injected in multi-cycle kinetics in horizontal direction. B: 8x8 matrix of all measured 64 interactions.

Multiplexing SPR-Assay

Lead discovery and confirmation assay require higher throughput and benefit from multiplexing assays to identify favorable hits. We have tested a set of 8x8 oligonucleotides in a multi-cycle kinetics assays against each other, a total of 64 interactions (Fig. 2B), in a single assay. Sensorgrams on the diagonal show the interaction between the complementary pairs. While most oligonucleotide pairs had only minimal mismatches resulting in slightly changed kinetics, oligonucleotides D and H were specifically designed to show the least degree of binding to the others. Hence, the oligonucleotide 8 was also a complete negative control and did not bind to the others, whilst oligonucleotide 4 maintained some affinity towards the other immobilized oligonucleotides. This mimics typical negative controls used in SPR-assay for therapeutic protein testing such as mutants or antigens from different species.

The assay showcases how the SPR #64 helps specifically in multiplexing assays were several different interaction partners need to be measured simultaneously.

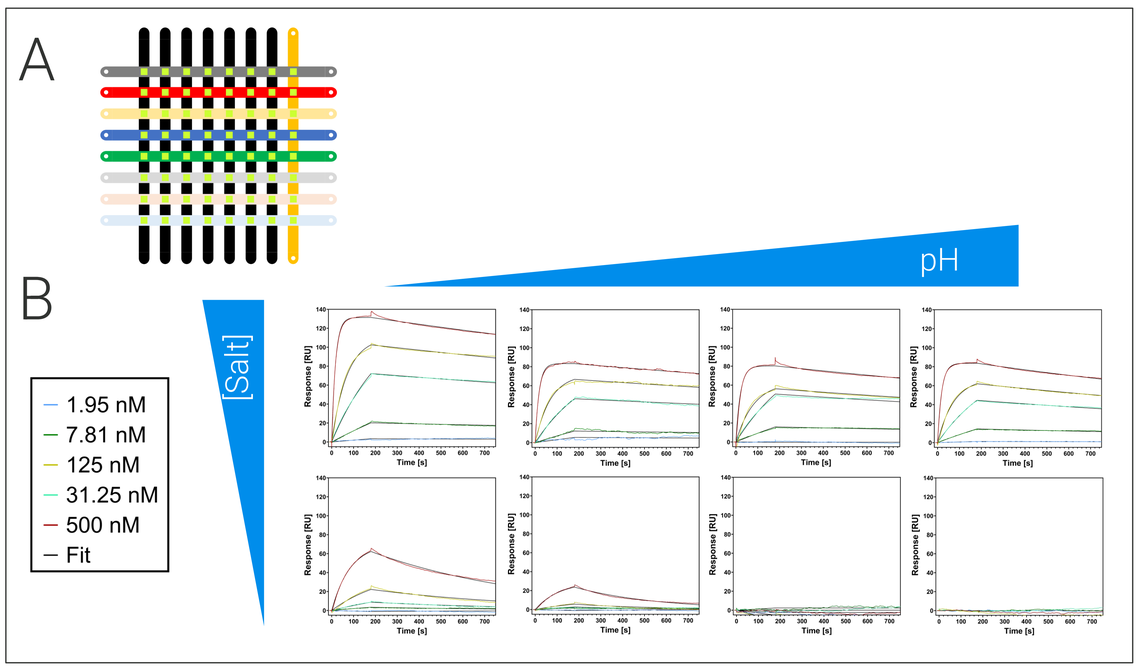

Fig. 3 A: B: Graphical representation of the assay set-up. B: Sensorgrams of the oligo-oligo interaction measured at four different pH and two salt concentrations.

Conditional Binding with Multi-Buffer Tool

Determination of kinetics of an interaction at variable buffer conditions can be crucial for certain disease areas such as cancer. Similarly, the testing of different buffer conditions might be necessary in an assay development. We have made use of the in-built multi-buffer tool to analyze the interaction of two oligonucleotides at four different pH (7.4, 8.0, 8.5 and 9.0) and two salt concentrations (0 and 150 mM). One binding partner was immobilized by biotin-capture, the complementary strand used as analyte. Analytes were injected in a multi-injection cycle kinetics assay format at seven concentrations.

Figure 3B shows clearly how a reduction in pH affects especially the maximal response whilst the overall kinetic is comparably unaffected by increases in the pH. A complete abundance of salt on the other side decreases the affinity clearly and abrogates the interaction completely at pH 8.5 and 9.0.

This example showcases how the flexibility and additional features like the multi-buffer tool of the SPR #64 can expand assay capabilities. Eventually, the SPR #64 helps in performing information-rich assays

Summary

- The SPR #64 has a rotatable 8x8 microfluidics for high throughput and flexibility in drug development.
- The determination of high affinity interactions is done with a single injection.
- Instrument provides detailed information on kinetics for multiplexing assays.
- The multi-buffer tool allows to test for the impact of different buffer conditions in an assay.

Surface Plasmon Resonance