Discovery and Development of Small Molecules by Information-rich Next Generation Multiplexing SPR

Cyrill Brunner[1], Sven Malik[2], Paul Ritter[2], Soumav Nath[2], Martin Kleinschmidt[3], 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; [3] Fraunhofer IZI, Weinbergweg 22, GER-06120 Halle (Saale)

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, selectivity and thermodynamic 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 small molecule discovery and development.

Affinity and Selectivity

We have tested a set of 8 compounds with a zinc-binding motif against two bacterial glutaminyl cyclases, P1 and P6. These enzymes carry a catalytic zinc ion and are a relevant disease factor in periodontitis [1,2]. Affinity has been determined in both multi-cycle and single-injection cycle kinetics format.

The two assay formats yielded similar results for the compound set against P1 (not shown) and P6 (Fig. 1B). We observed two subgroups of four compounds each in the interaction with P6. The tenfold difference in median affinity is reflected by a threefold improvement in on- and off-rate, respectively.

We also investigated the selectivity of the compound set. All compounds except for Compound 1 showed an increased affinity towards P6 compared to P1. Affinities can be up to a factor 20 different between the two proteins.

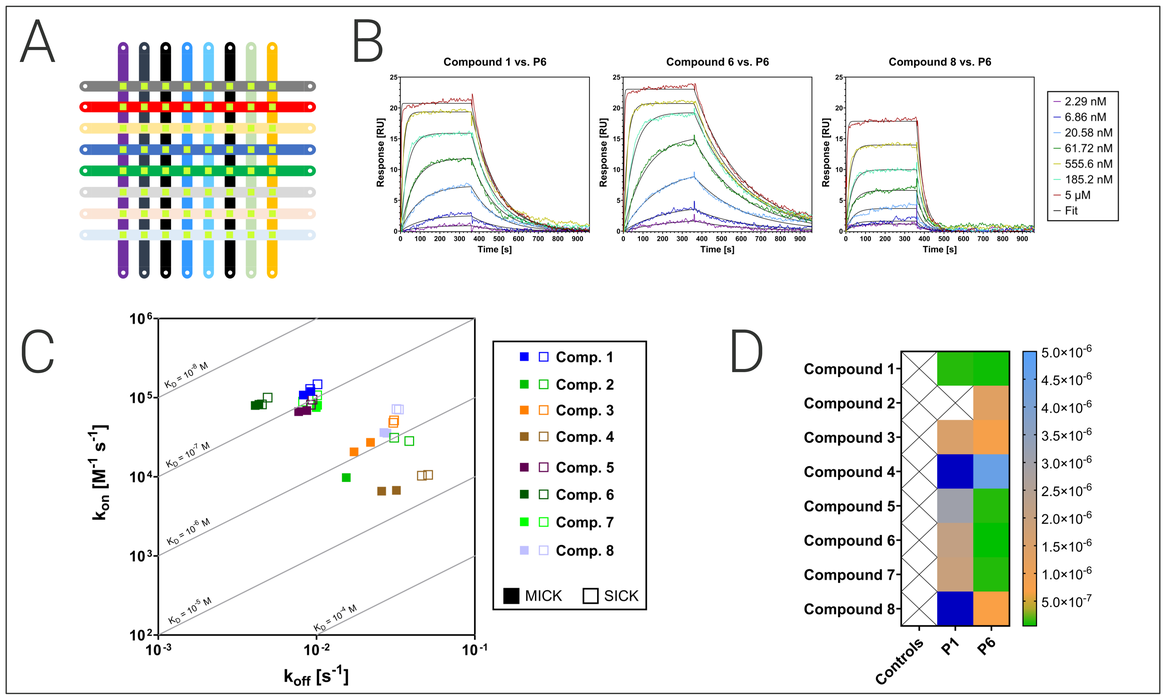

Fig. 1. A: Graphical representation of the assay set-up. P1 and P6 were immobilized on spot columns 7+8 and 4+5, non-binding control on columns 1+2 in vertical direction. Compounds 1-8 were then injected in horizontal direction. B: Representative sensorgrams. C: Kinetic map of compounds 1-8 vs. P6. D: Heat map of affinity (K_D in M) of the compound set measured for P1 and P6.

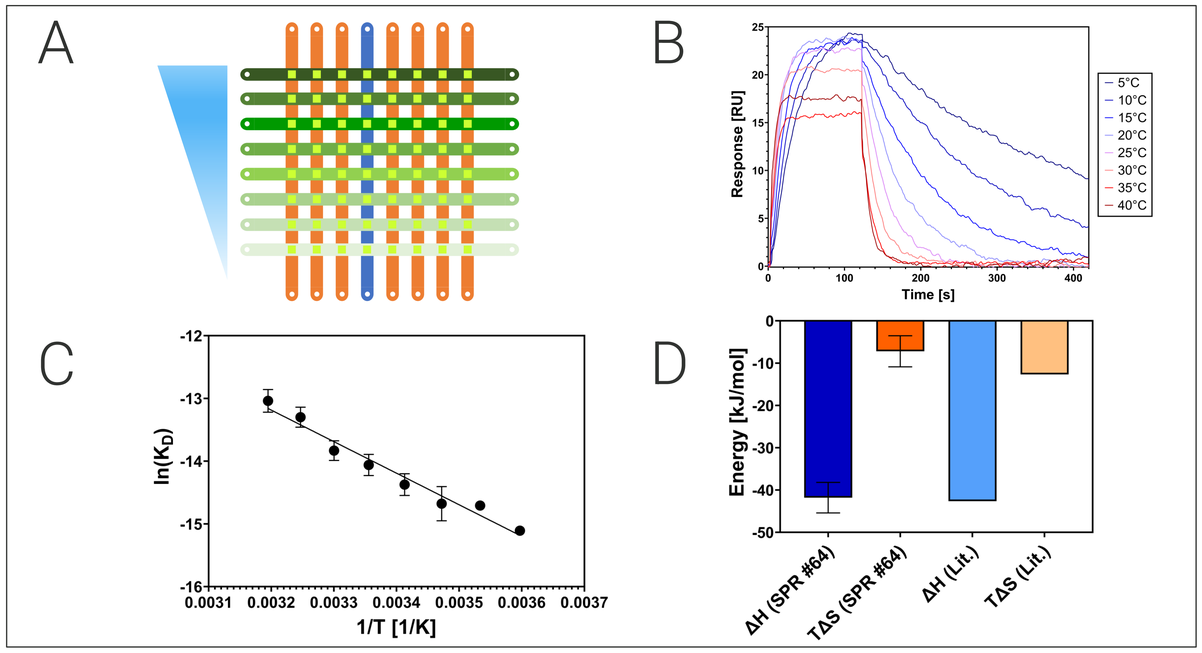

Fig. 2 A: Graphical representation of the assay set-up. Protein was immobilized on spot columns 1-3 and 5-8, the compound CBS was injected in single-injection cycle mode. B: Overlay of kinetic traces for CBS at 1.6 µM at variable temperatures. C: Van't Hoff plot of ln (K_D) vs 1/T for the determination of enthalpy and entropy. D: Bar plot showing determined and literature values for ΔH and TΔS .

Thermodynamics

The capability of the SPR #64 for the determination of thermodynamic constants was tested with the well-established interaction of bovine carbonic anhydrase II with 4-sulfamoylbenzoic acid (CBS). The protein was immobilized by amine coupling on all spot columns except for spot column 4 which served as reference. CBS was then injected at 8 temperatures ranging from 5-40 °C in the single-injection cycle kinetics mode (Fig. 2A). The affinity and the kinetic constants changed visibly with the assay temperature. Both association and dissociation rate constant were affected by the temperature increase (Fig. 2B).

Obtained affinities were then used to calculate from a van't Hoff plot the enthalpy and entropy constants for each spot columns (Fig. 2C). The values also correspond well with literature values reported by Day et al. (Prot Sci, 2002, 11::1017-25) as shown in Figure 2D.

This experiment further shows that the instrument delivers reproducible results across all spot columns and offers thus an attractive alternative to isothermal titration calorimetry. A full thermodynamic characterization was performed at a protein consumption of 20 µg protein within 9 hours of unattended run time. Furthermore, the assay automatically yield kinetic rate values at biologically relevant temperatures.

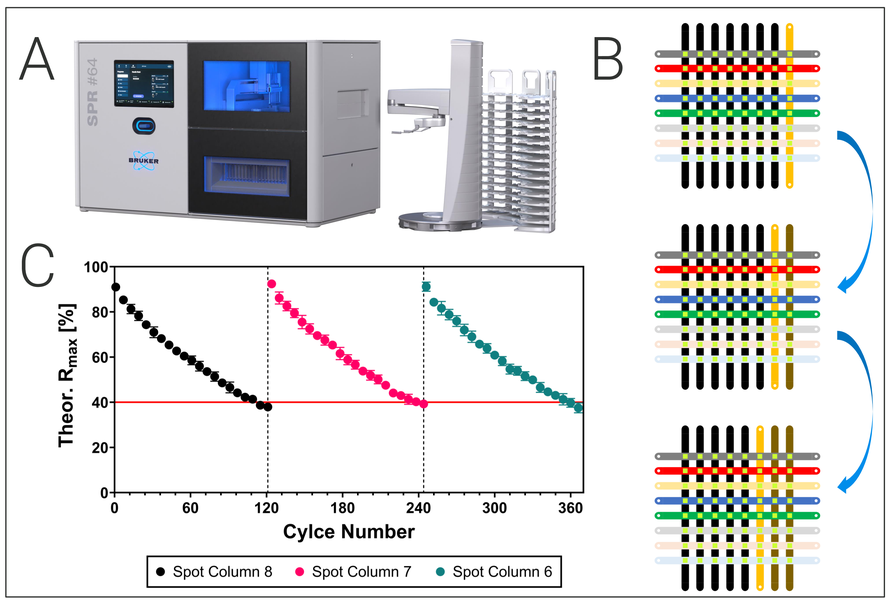

Fig. 3 A: The SPR #64 in combination with a plate robot (Thermo Spinnaker). B: Graphical representation of the extended screener principle. C: Graph showing the decrease in protein activity over the course of the assay. The red line represents the activity limit expressed in theoretical R_{max}, the dashed black line the switch to a new spot column.

Extended Screener

Loss in protein activity over the course of a screening is a major limitation for long, unattended and automated runs. The 8 spot columns of the SPR #64 extend screening options by its unique manifold design. We showcase here the concept of the "extended screener" at a screening example with a biotinylated carbonic anhydrase II immobilized. Acetazolamide as positive control was injected regularly after every 6th cycle resulting in two controls per plate. Cycles of 4 analyte injections with non-binding compounds, one buffer injection and one regeneration injection were used in between. This set-up was run on an SPR #64 combined with a Thermo Spinnaker robot. The screening started with protein being immobilized on spot column 8.

Prior tests showed that immobilized carbonic anhydrase II has a steady decrease in protein activity down to 40% after approximately 120 cycles. Consequently, we set up a method that automatically re-immobilizes biotinylated protein after 120 cycles on a yet unused spot column.

This concept allows to extend a screening run to up to eight immobilizations without exchanging the sensor or restarting a method. Thus, the SPR #64 can massively increase screening capacities for SPR for fragment-based screenings and small molecule screenings.

Acknowledgement

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[1] Taudte, N. et al., J Biol Chem, 296: 100263, 2011 [2] Ramsbeck, D. et al., Pharmaceuticals, 14, 2021

Summary

- The SPR #64 has a rotatable 8x8 microfluidics for high throughput and flexibility in drug development.
- Instrument provides detailed information on kinetics and selectivity of multiple small molecules vs. several proteins
- Thermodynamic constants can be determined accurately in short time
- The 8x8 array allows for extended screening runs.

Surface Plasmon Resonance