Investigation of condition-dependent binding using Frame Inject

The traditional investigation of condition-dependent binding leads always to high reagent costs as well as a long experimental time. The new feature Frame Inject is a perfect solution to work cost- and time-effective at low sample consumption.

Introduction

Real-time, label-free (RT-LF) analysis utilizing SPR detection is a powerful tool for the biophysical characterization of small molecule drugs and drug candidates. The next generation RT-LF analysis platform, Sierra SPR-32, was evaluated for its assay flexibility and rapid kinetic characterization of binding interactions. Equipped with a 4 - Buffer separator, the Sierra SPR-32 system allows the investigation of up to four buffer conditions simultaneously. The Frame Inject requires just a fraction of each buffer condition, that replaces the system buffer while usage. The pre-association, as well as the dissociation occurs under the conditions, that should be investigated. Thus experimental time and reagent costs are kept low.

In the present study 4 buffer conditions were tested simultaneously with a small molecule inhibitor.

Keywords: Sierra SPR-32; Frame Inject; mechanistic studies; cofactor dependent binding; pH-dependent binding; cost-effectiv; low consumption

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Material and Methods

Instrumentation

All real-time, label-free (RT-LF) assays were completed using the Sierra SPR-32 system, from Bruker Daltonics SPR (Hamburg, Germany). The Sierra SPR-32 system contains 32 detection spots arranged in a 4x8 array. Eight samples are delivered simultaneously to the detection spots via a continuous flow microfluidic device. A high sensitivity surface plasmon resonance imaging detector, SPR+, is used to measure binding interactions in real time.

Surface Preparation

The target protein was attached to high-capacity-amine sensors (HCA, Part.-No.: 1862614, Bruker Daltonics SPR) using standard primary amine immobilization chemistry. Eight (8) detection spots, one per channel (Spot D), were immobilized with the target protein. To create a secondary control surface, detection Spot C in each flow cell was activated and blocked. See Figure 1 and 2 for the arrangement of active and control surface. Spot A and B in each flow cell was not used for the experiments. The final average immobilization response for the target protein on all detection spots is shown in Table 1.

Assay Conditions

Immobilization of the target protein was conducted using PBS buffer containing 0.05% Tween 20, pH 7.4. The cofactor binding assays were completed using PBS buffer containing 0.05% Tween 20, 3% DMSO, pH 7.4 and 1 mM of each Cofactor (A, B and C). In total four different buffer conditions were tested, see Table 2. Inhibitor 1 (200 Da) was injected across detection spots C and D (in series). All assays were conducted at 25°C.

Cofactor Binding Assay

Four buffer conditions were tested within a single Frame Inject, compare Figure 3 for the arrangement of buffer conditions.

During the Frame Inject a duplicate of inhibitor 1 was injected at 50 µM per buffer condition over the target. The monitored association was 120 sec at 30 µl/min and dissociation for 180 sec. A single DMSO calibration was conducted prior the Frame Inject.

Data analysis was completed using the Analyser R2 software (Bruker Daltonics SPR). All sensorgrams were double-reference subtracted and DMSO-corrected prior to data analysis. A Langmuir 1:1 binding model was used to fit the kinetic data.

Results

Surface Preparation

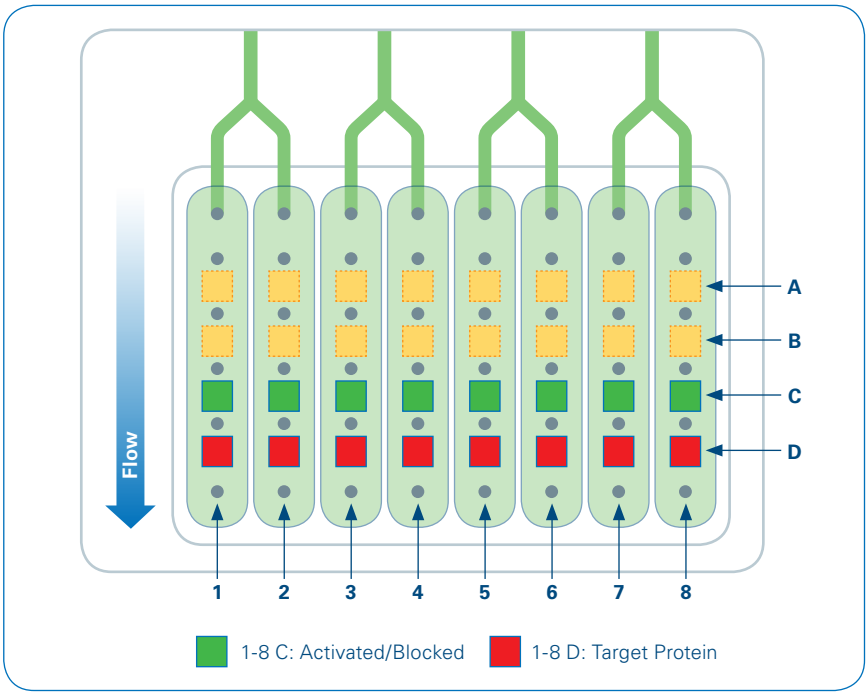

Figure 1: Flow Cell Schematic and Immobilization Pattern. One target protein was immobilized onto detection Spots D, within each flow cell. A control was created by activating and blocking Spot C in each flow cell

Table 1: Immobilization consistency across eight sensor spots

Protein (Spot)	Immobilization Level (RU)	% CV
Target Protein (1-8 D)	5317 ± 160	3.0

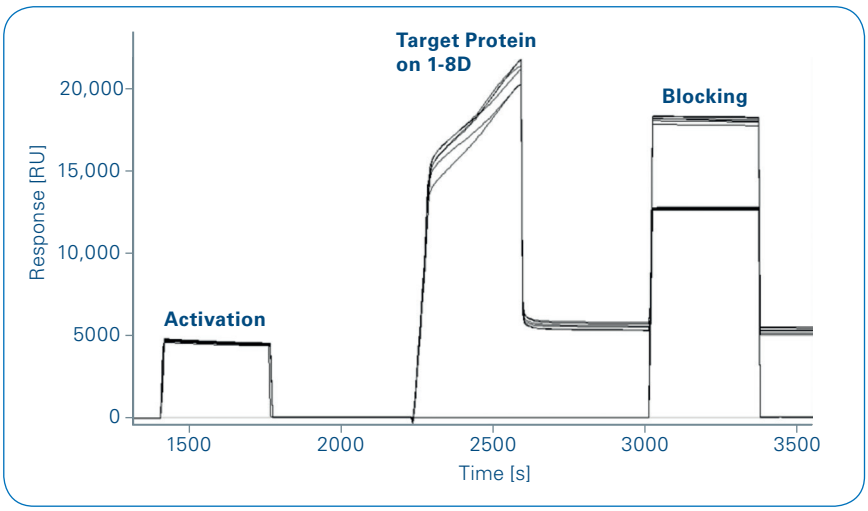

Figure 2: Immobilization of Control and Active Surfaces. Using a standard primary amine coupling protocol, the target protein was attached to 8 of 32 detection spots across 8 flow channels. One row of detection spots (Spot C) was activated and blocked for use as the control surface. The protein was injected across the desired Spot D (see schematic Figure 1a.). All detection spots were activated and blocked using a single injection. Preparation of 16 surfaces (8 controls + 8 active) was completed in ~ 1 hour. Average immobilization levels are reported in Table 1

Cofactor Binding Assay

Table 2: Buffer conditions

Buffer	Channel	Cofactor
1	1+2	PBS buffer containing 0.05% Tween 20, 3% DMSO, pH 7.4 and 1 mM Cofactor A
2	3+4	PBS buffer containing 0.05% Tween 20, 3% DMSO, pH 7.4 and 1 mM Cofactor B
3	5+6	PBS buffer containing 0.05% Tween 20, 3% DMSO, pH 7.4 and 1 mM Cofactor C
4	7+8	PBS buffer containing 0.05% Tween 20, 3% DMSO, pH 7.4 and no Cofactor

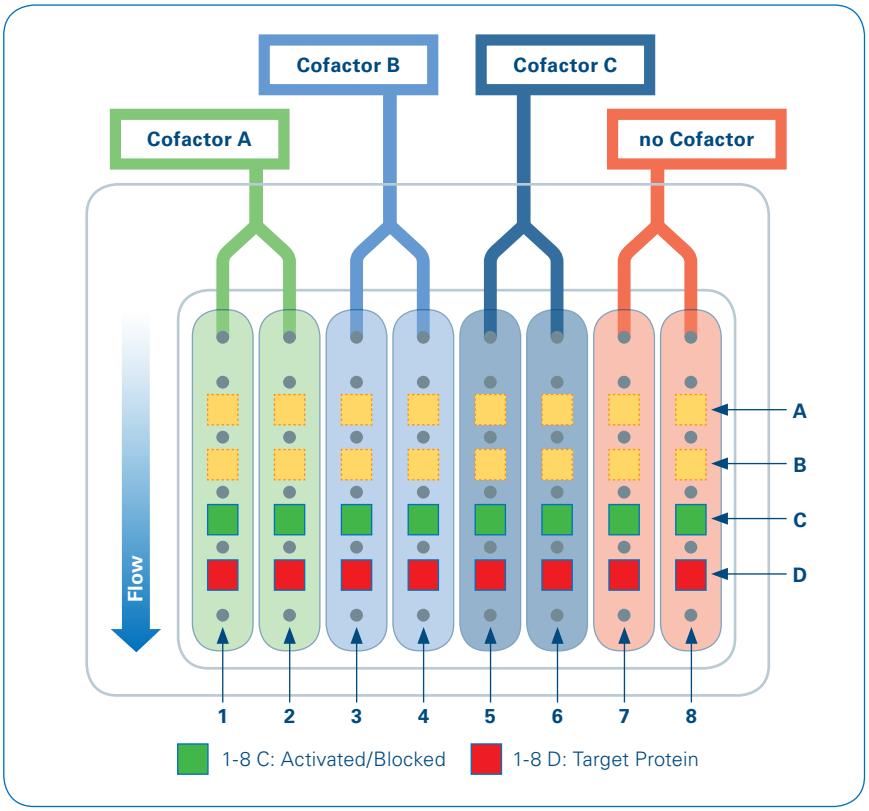

Figure 3: Arrangement of four buffer conditions during Frame Inject.
Cofactor A was used in Channel 1 and 2, Cofactor B was used in Channel 3 and 4, Cofactor C was used in Channel 5 and 6 and no Cofactor was used in Channel 7 and 8

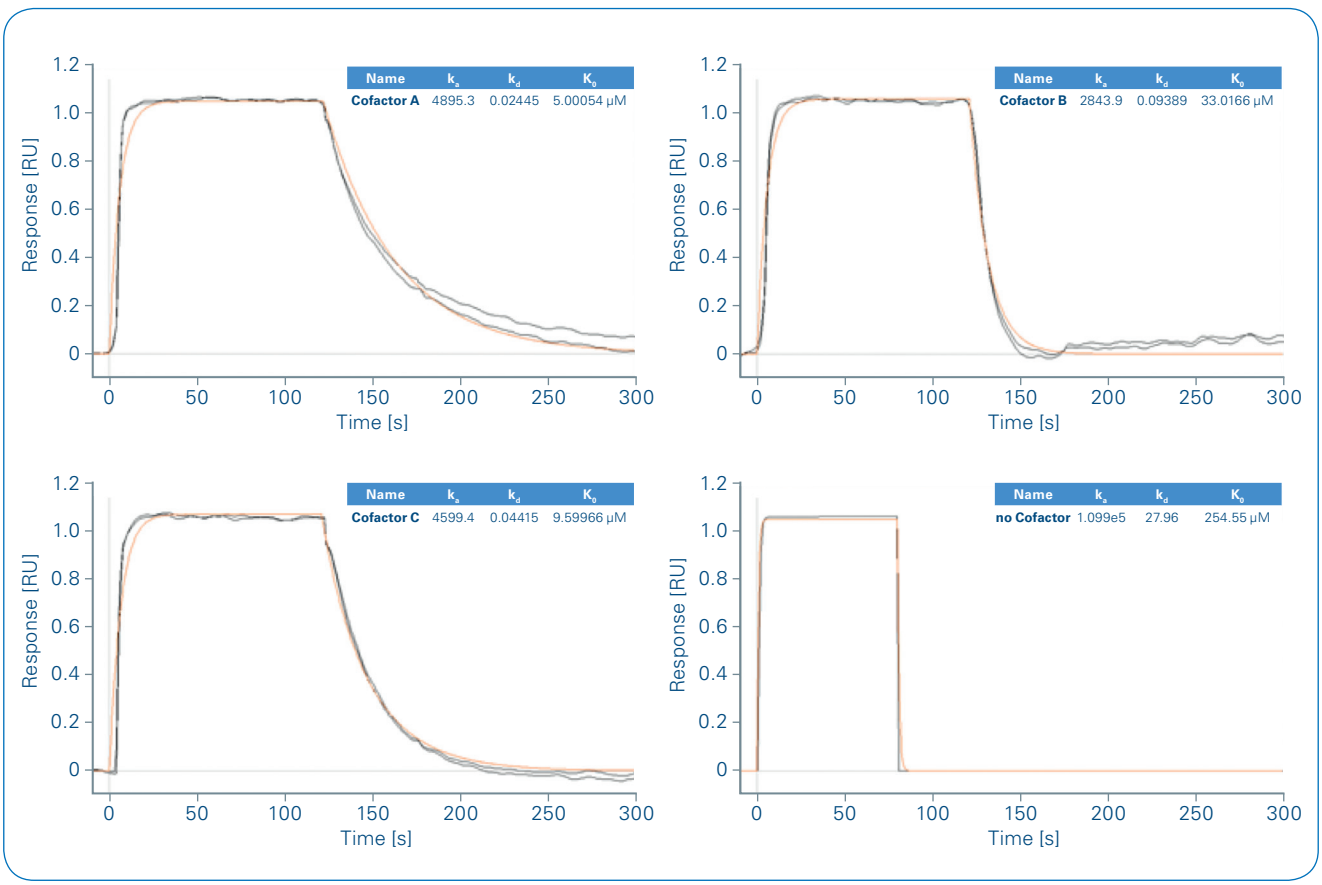

Figure 4: Inhibitor 1 diluted into four different buffers was injected for binding over the target protein. Using 120 seconds for association and 180 seconds for dissociation the entire Frame Inject was performed within 20 minutes. The binding signals were double subtracted, solvent corrected and normalized prior applying the Langmuir 1:1 fit

Table 3: Summary of kinetic parameters of Inhibitor 1, depending on buffer condition/cofactor

Cofactor	ka [M*s^{-1}]	kd [s^{-1}]	KD [µM]
Cofactor A	4895.3	0.02448	5.00
Cofactor B	2843.9	0.09389	33.02
Cofactor C	4599.4	0.04415	9.60
no Cofactor	1.099e5	27.98	254.51

Table 4: Comparison of reagent consumption and experimental time for the traditional investigation and Frame Inject. (Note: For the traditional investigation a sequential usage of the individual buffers was assumed)

	Buffer consumption per condition [mL]	Total Time [min.]
Frame Inject	~ 1.1	20
Traditional investigation	~ 70	100

Conclusions

- This study underlines the benefits of using the Frame Inject feature for the investigation of condition-dependent binding. Four buffer conditions have been tested within 20 minutes. The impact per cofactor on the inhibitor interaction was clearly demonstrated as well as the low reagent consumption and short experimental time.
- Frame Inject displays the outstanding flexibility and usability of the Sierra SPR-32 for daily challenges within the drug discovery process.
- The high consistency within the duplicate injection as well as the low %CV for the immobilization level across all eight channels shows the high performance of the Sierra SPR-32.

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