

Performance study – Prostate-specific antigen (PSA) / Anti-PSA kinetics

The b-screen device is the only label-free reader compatible to real high-throughput. It utilises SCORE technology to measure binding curves from biomolecular interactions on glass type microarrays with up to 22500 spots simultaneously. The aim of this study was the adaption of a well-known kinetic assay towards the b-screen. For this purpose, standard protocols “stated in Kinetic analysis of a high-affinity antibody/antigen interaction performed by multiple Biacore users” by Katsamba *et al.* were followed as close as possible. Few parameters had to be adapted, e.g. because of the different flow cell geometry and the need for an offline immobilization procedure. The obtained data was evaluated using commonly known algorithm and commercially available data evaluation Software.

Benchmarking SCORE against SPR

SCORE technology enables the label-free screening of biomolecular interactions on glass type microarrays. This study shows how binding and kinetic data obtained with the b-screen system compare to the gold standard SPR.

A well characterised high-affinity antibody/antigen system – anti-PSA/PSA – was used as a benchmark for the Biametrics b-screen device.

Spot-to-spot reproducibility was measured and kinetic rate constants and affinity were benchmarked against SPR data.

Performance

Affinities, on-rates and off-rates of the anti-PSA/PSA interaction measured with SCORE technology compare well to SPR data (Table 1). Although the off-rate is varying slightly between the results obtained with b-screen and the reference, the overall error and therefore uncertainty is significantly lower for SCORE than from the reference.

Overall there is a good correlation between the results using the Biametrics' b-screen device compared to the reference.

- High reproducibility
- Results well comparable to SPR
- High-throughput compatible

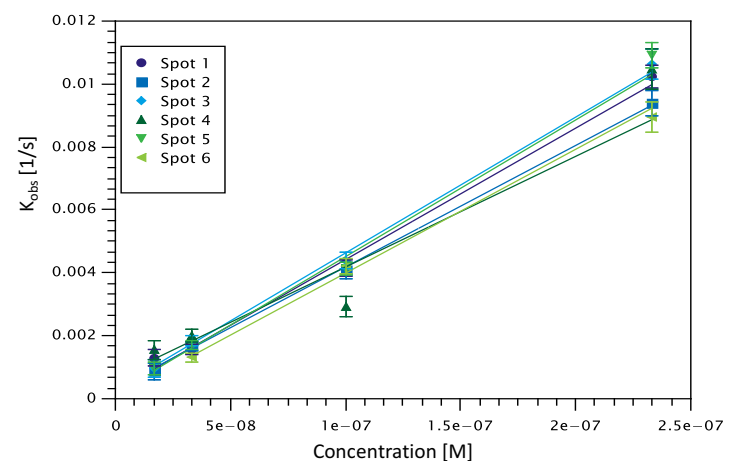


Figure 1: A good linearity validates the correct experimental setup and that a 1:1 binding model describes the kinetic behaviour. Reproducibility between the different slopes is very high, which correspond to the association rate constant.

Table 1: Kinetic and thermodynamic data obtained using the b-screen reader compared to published data measured with SPR technology by Katsamba *et al.*

	Biametrics SCORE Technology	Reference SPR (Biacore)
on-rate	$(4.03 \pm 0.32) \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$	$(4.1 \pm 0.6) \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$
off-rate	$(7.94 \pm 0.00) \times 10^{-5} \text{ s}^{-1}$	$(4.5 \pm 0.6) \times 10^{-5} \text{ s}^{-1}$
affinity	$(1.97 \pm 0.16) \text{ nM}$	$(1.1 \pm 0.2) \text{ nM}$

Binding curves

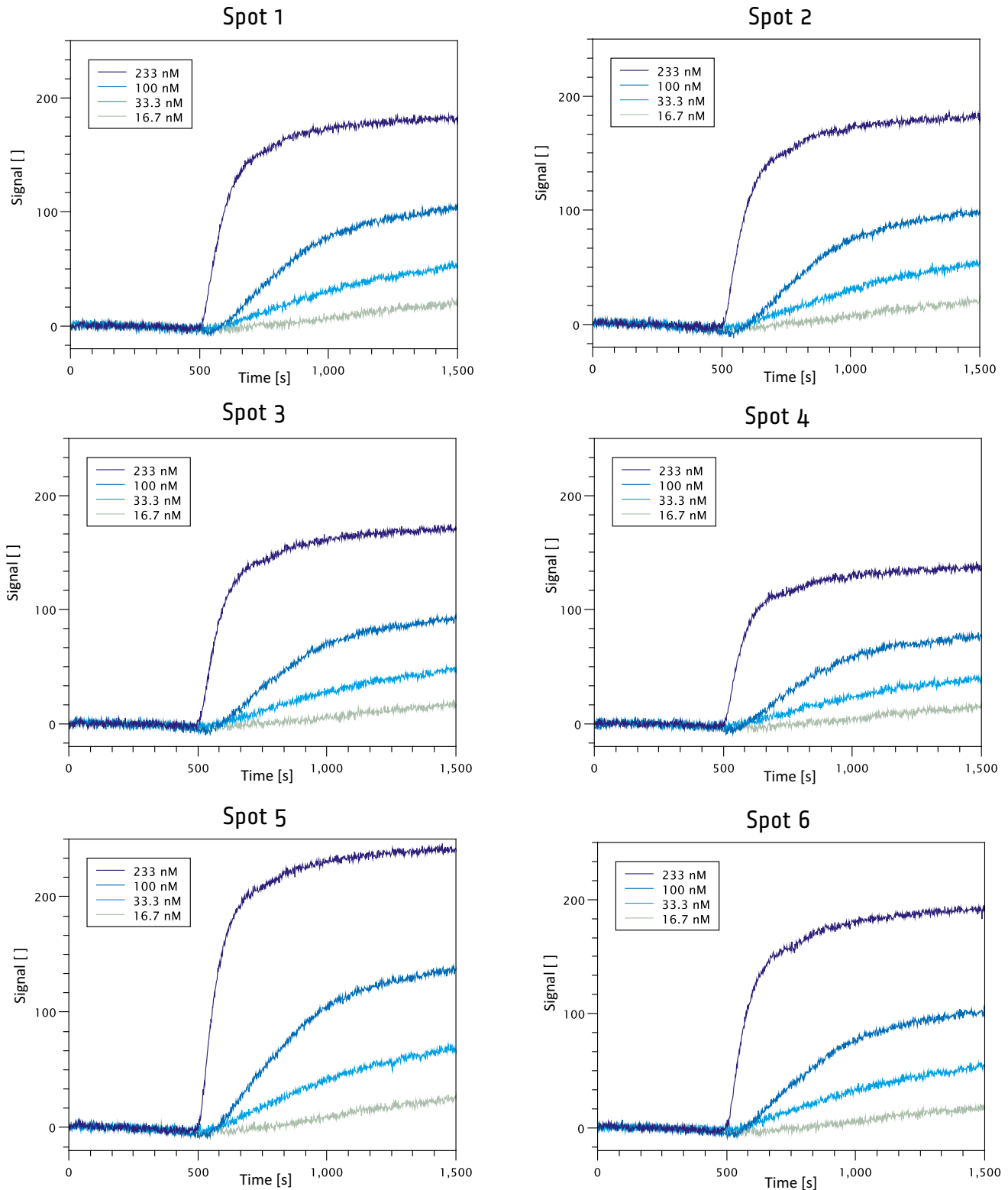


Figure 2: Spot-to-spot reproducibility. All spots show concentration-dependent response. The difference in maximum signal intensity is probably due to slightly different immobilization levels of anti-PSA, but does not influence the validity of the kinetics.

Experimental details

Immobilization

Spotting was performed at a humidity of 55% at 8°C. Anti-PSA was diluted with 10 mM Sodium-Acetate Solution pH 5 containing 0.01 % CHAPS to a final concentration of 1 mg/mL. BSA was diluted with PBS pH 7.4 containing 0,01% CHAPS to a final concentration of 1 mg/mL. Both solutions were spotted as hexaplicates. The spots were allowed to dry in after the spotting process and rehydrated in a humid chamber (saturated) at 8 °C over night.

Preconditioning of surfaces

Slides were quickly rinsed with deionised water and placed wet into the b-screen. To get rid of not properly immobilized anti-PSA, 1 mL of regeneration solution was injected as sample with the following parameters. Flow speed: 5 µL/s

Baseline:	500 s
Association:	200 s
Dissociation:	60 s
Regeneration:	-
Endline:	150 s

Kinetic measurements

The flow speed of all measurements was set to 0.85 µL/s. The fluidic parameters for determination of the association rate constant were:

Baseline:	500 s
Association:	1200 s
Dissociation:	60 s
Regeneration:	200 s
Endline:	150 s

PSA samples with the final concentration of 16.7, 33.3, 100, or 233 nM were injected in ascending order. The dissociation rate constant was determined by a single injection of 233 nM PSA followed by a dissociation phase of 6000 s.

Data evaluation

The anti-PSA spots were referenced towards the BSA spots. No filtering, smoothing, drift correction or x-alignment was applied to the data. The obtained sensorgrams were fitted using Origin. The fitting interval for the determination of the observable rate constant was adjusted to each concentration individually: 16.7, 33.3 nM (951-1550 s), 100 nM (850-1200 s), and 233 nM (600-850 s) applying a pseudo first kinetics function. These values were then plotted vs. their respective concentration and a linear fit was applied. The dissociation was fitted globally assuming first order kinetics with possible rebinding with an interval from 2000-5000 s.

Devices

All interaction analysis were performed on a b-screen by Biametrics. Generation of microarrays was performed on a BioOdyssey Calligrapher (Bio-Rad) using a 310 µm diameter ceramic solid pin. Printed arrays were stored at 8°C in a reffridgerator. Ultrapure water was collected from a Purelab-Classic (ELGA-Labwater).

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Biochemicals and chemicals

PSA and anti-PSA (clone M612166) was purchased from Fitzgerald and stored at -20°C until use. Glycine, CHAPS, hydrochloric acid, potassium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, Tween, and sodium-acetate were purchased from Sigma-Aldrich.

Consumables

Pipette tips were purchased from Eppendorf. 3D-NHS chips (BMV18307) from Biametrics were used for all experiments.

Buffers and solutions

Running buffer:

PBS pH 7.4 with 200 µg/mL BSA was used as running buffer.

Spotting buffer:

10 mM Sodium Acetate pH 5 containing 0.01% CHAPS.

Regeneration:

10 mM Glycine pH 3.

Literature

Katsamba *et al.*, "Kinetic analysis of a high-affinity antibody/antigen interaction performed by multiple Biacore users.", *Anal Biochem.* 2006, 15;352(2):208-21.