BIAffinity Application Note

Rifs analysis of the interaction between antibody mAb1 and mAb1 – binding peptide pep1

1 Introduction

The BIAffinity used in these studies is based on the detection principle Rifs (Reflectometric Interference Spectroscopy). Rifs and the sensor chip technology combined within the BIAffinity offers a promising tool for unlabeled and time saving protein characterization.
In this application note the interaction of immobilized 150 kDa antibody mAb1 and mAb1 – binding peptide (pep1) with a molecular weight of 1700 Da is described. Thereby the coupling of an unrelated antibody (mAb2) to the reference channel via flow channel 2 is shown. Depending on this the prevention of unspecific binding effects and the adulteration of the reference signal can be observed.

2 Material and Methods

2.1 Immobilization of mAb1 and mAb2 antibody

Senor chip: carboxyl-functionalized surface (e.g. C_AMD, CMD, DC – PEG)
Running buffer: 10 mM HBS pH 7.4 without surfactant
Immobilization buffer: 10 mM acetate buffer pH 5.0
Activation: NHS / EDC (0.05 M / 0.05 M)
Ligand: mAb1 (Mw = 150 kDa); mAb2 (Mw = 150 kDa) each 30 µg/ml

A. Immobilization Protocol						
No.	**Step**	**Sample**	**Conditions**	**Flow rate [µl/min]**	**Flow cell**	**Port**
1	Baseline	HBS	10 mM; pH 7.4	5	1 - 2	Buffer
2	Activation	NHS/EDC in H_2O	0.05 M / 0.05 M, 60 µl	5	1 - 2	1
3	Ligand binding 1	mAb1 in acetate buffer pH 5	30 µg/ml	5	1	2
4	Deactivation 1	Ethanolamine – HCl	1 M; pH 8.5, 50 µl	5	1	3
5	Ligand binding 2	mAb2 in acetate buffer pH 5	30 µg/ml	5	1 – 2	4
6	Deactivation 2	Ethanolamine – HCl	1 M; pH 8.5, 50 µl	5	1 – 2	5
7	Baseline	HBS	10 mM; pH 7.4	5	1 - 2	Buffer
Running temperature:		25° C				

The carboxyl groups of the sensor surface are activated by using the carbodiimid chemistry. Thereby 60 µl of a 1:1 NHS/EDC mixture in deionized water is injected to the sensor chip. Immediately mAb1 in acetate buffer pH 5 is immobilized to flow channel 1, which is deactivated by an injection of ethanolamine – HCl subsequently.
The unrelated antibody mAb2 is then injected to both flow channels, but only immobilized to flow channel 2, because flow channel 1 is still deactivated. Another injection of ethanolamine – HCl is used to block reactive groups, which are still remaining on the sensor surface and especially on flow channel 2.
The flow rate is adjusted to 5 µl/min all the time.

2.2 Interaction of mAb1 – binding peptide (pep1) to immobilized mAb1 antibody

Senor chip: mAb1 immobilized senor chip
Running buffer: 10 mM HBS pH 7.4 without surfactant
Sample buffer: 10 mM HBS pH 7.4 without surfactant
Regeneration buffer: 10 mM glycine pH 3.0
Analyte: pep1 in 10 mM HBS pH 7.4 without surfactant

B. Measurement protocol								
Nr.	**Step**	**Sample**	**Conditions**	**Flow rate [µl/min]**	**Volume [µl]**	**Time [sec]**	**Port**	**AB**
1	Baseline	HBS	10 mM; pH 7.4	30	75	150		
2	Association	pep1	variable	30	120	-	1	x
3	Dissociation	HBS	10 mM; pH 7.4	30	450	900		
4	Regeneration	Glycine	10 mM; pH 3.0	30	30	-	2	x
5	Buffer	HBS	10 mM; pH 7.4	30	50	100		
Running temperature:		25° C						

The interaction between mAb1 and the mAb1 – binding peptide pep1 is determined in dependence on the analyte concentration (60 nM – 600 nM in HBS pH 7.4). Therefore 120 µl of the pep1 solution is used to observe the association. The mAb1 / pep1 complex is then dissociated within 900 sec. using running buffer HBS pH 7.4. After the dissociation time, the sensor surface is regenerated with a pulse of 10 mM Glycine pH 3.0 to condition the chip for the next measurement (not shown). Running buffer corresponds to dilution buffer of biological sample, whereby the method is carried out with a flow rate of 30 µl/min. Table B. shows a special method to measure the interactions between pep1 as analyte and mAb1 as ligand.

3 Results

3.1 Signal/ Noise plot

The Signal/ Noise plot (fig. 1) displays the baseline, which is detected under buffer conditions before starting the immobilization routine.

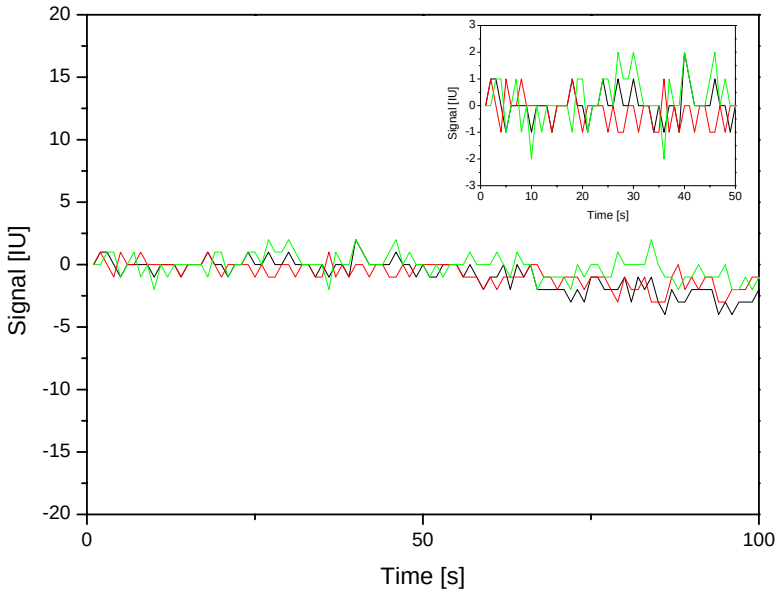

Fig. 1: Signal/Noise Sensorgram

Because of the low peak-to-peak noise and the stable baseline, high signals and low analyte concentrations can be detected, as shown in the following sectors.

3.2 Covalent linking of mAb1 antibody

The injection of the reactive NHS/EDC mixture to the sensor surface offers now active carboxylic groups, which are used for covalent immobilization (fig. 2) of mAb1 via the amino groups by forming amide bindings.

The reference surface for unspecific binding effects of the analyte pep1 is generated during the immobilization routine of the ligand. Therefore channel 1 is linked with mAb1 and the flow channel 2 (reference channel) is created by coupling of the unrelated antibody mAb2.

By rotation of the sensor chip to 90°, the reference sensor surface can be used during the performance of the analyte measurement as shown below (fig. 3).

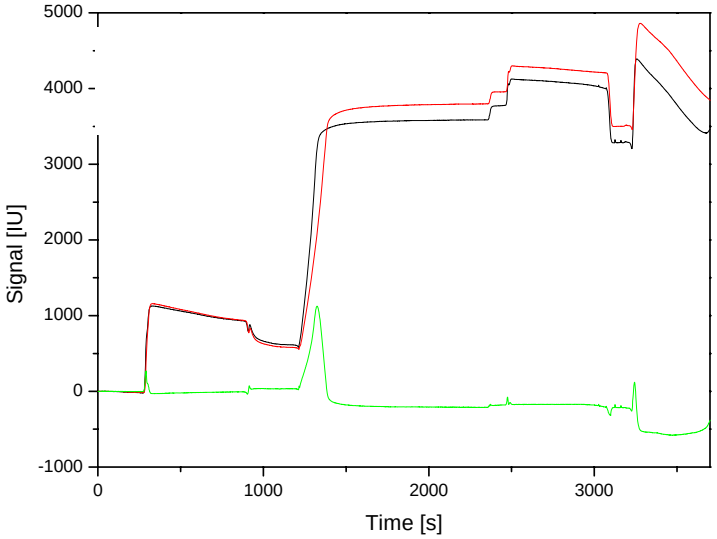

Fig. 2: Immobilization of mAb1 and mAb2 antibody to carboxylic functionalized sensor surface [(——) channel 1, (——) channel 2, (——) and difference C1 – C2

3.3 Measurement of mAb1 – binding peptide (pep1)

By using a second injection with an unrelated protein during the immobilization routine, the unspecific binding of the analyte can be repressed. Furthermore the adulteration of the signal can be reduced.

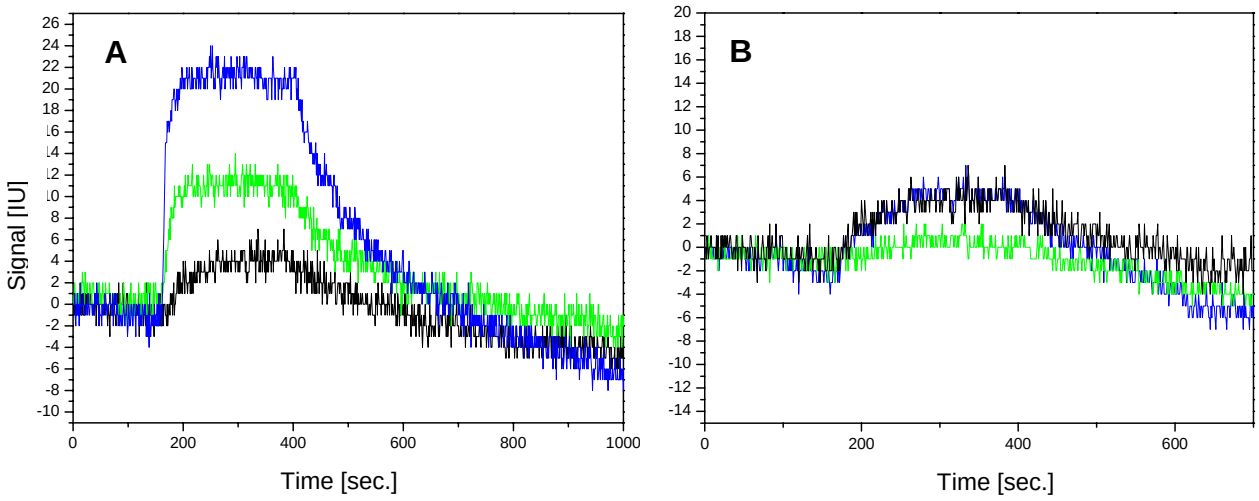

Fig. 3: Measurement of mAb1 – binding peptide (pep1)

A: Measurement of different concentrations pep1 (C1 – C2) [(——) 60 nM; (——) 240 nM; (——) 600 nM]

B: 60 nM pep1 [(——) specific interaction, (——) reference channel, (——) and difference C1 – C2 (referenced channel)]

Specific signals, which are used for analysis, are obtained by the substraction of the reference signal in channel two from the binding signal in channel 1. The so-called referenced channel is determined during the measurement by using control software of BIAffinity and shown within the sensorgram (fig. 3 A). The binding curves can be analyzed by global fitting of the association and dissociation phase to a 1:1 binding model (not shown).

4 Conclusion

The binding studies of mAb1 – binding peptide pep1 to immobilized antibody mAb1 could be observed well by using Rifs with the BIAffinity. Low unspecific binding effects and signal / noise conditions can be obtained within this measuring system.
Furthermore the second immobilization of the unrelated antibody mAb2 gives a closer comparison of specific and unspecific binding effects to the sensor surface. The adulteration of the referenced signal, which is used for analyses, can be reduced.

Publisher:
Analytik Jena AG
Konrad-Zuse-Straße 1
07745 Jena/ Germany

Phone +49 36 41 77-94 00
Fax +49 36 41 77-76 77 76

www.analytik-jena.com
biosolutions@analytik-jena.com