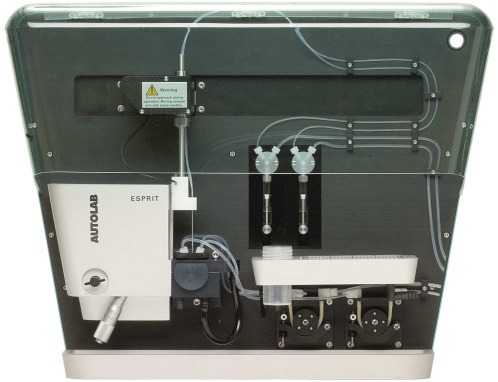

Study of the interaction between Bovine Serum Albumin and Rabbit anti-Cow Albumin antibody

The Autolab ESPRIT can monitor binding events in real time without labeling and the system can therefore be used to determine both affinity and rate constants of interactions between any molecules.
This application note will show data of the binding interaction between immobilized protein BSA and antibody Rabbit Anti-Cow Albumin IgG1 in solution. Binding curves were obtained at several concentrations of IgG1 (1-274 nM) to determine the association and dissociation rate constants, and hence the overall affinity constant of the interaction.

Experimental Conditions

The bare gold disk is prepared with a self-assembled monolayer (SAM), 1mM - 11-mercaptoundecanoic acid (MUA, Aldrich) in Ethanol. The coupling buffer solution, 10 mM NaAc pH4.5 in H_2O (Fluka), is used for measuring the baseline. The pH is set with acetic acid. Immobilization of 2.5µg Bovine Serum Albumin (BSA, Fluka) (dissolved in coupling buffer) is performed after the activation of the MUA with a mixture of 0.1M EDC in H2O and 0.1M NHS in H2O (Fluka). Remaining reactive esters on the surface were deactivated with 1M-ethanolamine pH8.0 (Fluka). Tightly associated, but non-covalently bound protein was removed with 100mM HCl. To prepare the immobilized disk for the kinetic experiment, the disk is washed with PBS (Fluka) with 0.05% Tween-20 added.

The PBS with 0.05% Tween-20 is also used to establish the baseline for the interaction analysis as well as for diluting the antibody Rabbit anti-Cow Albumin.

Results

Immobilization of BSA in channel 1 and 2.
Cleaning the bare gold disk is followed by a 4 hours incubation of the MUA. The monolayer is stabilized by washing with coupling buffer and HCl, followed by activation with a mixture of EDC/NHS for 5 minutes, which is then washed away with coupling buffer, and at last a 15 minutes immobilization of 2.5 µg BSA protein (see Figure 1). After washing, no decay of angle was visible. So, all the BSA molecules were coupled.

Interaction of anti - Cow Albumin antibody
Stabilizing the prepared surface is done first before starting the baseline with PBS–Tween for 5 minutes. After the baseline, the first concentration of anti-Cow Albumin was added. The interaction was monitored for 1 hour, followed by dissociation in PBS-Tween. The interaction complex was regenerated with 100mM HCl for 5 minutes. After restoring the PBS-Tween baseline, the interaction cycle was repeated for the other antibody concentrations (see Figure 2).
The reference measurements were performed with the Rabbit anti-human Alpha-2-macroglobuline antibody. The concentration of the reference antibody was equivalent to the anti-BSA antibody of every individual experiment.

Evaluation of the data

The interaction system;

$$BSA + \alpha BSA \underset{K_{dissociation}}{\overset{K_{association}}{\rightleftarrows}} Complex$$

$$[A] + [B] \Leftrightarrow [AB]$$

The rate formation of the complex;

$$\frac{d[AB]}{dt} = ka[A][B] - kd[AB]$$

The integrated rate equation for the association phase is

$$R_t = \frac{K_a \cdot [C] \cdot R_{max}}{K_a \cdot [C] + K_d} (1 - e^{-(k_a[C]+K_d)\cdot t}) + R_0,$$

In which R0 is the signal at time 0 and [C] is the concentration of the analyte, Rabbit anti- Cow BSA. The equation can be adapted to

$$R_t = E\,(1 - e^{-K_s t}) + R_0$$

In which E is the signal at equilibrium of the association (t=:) corrected for R0 and *Ks* is K_a[C] + K_d.
The association rate constant (K_a) is obtained from the slope of a plot of *Ks* vs the concentration of Rabbit anti- Cow BSA. Theoretically, the dissociation rate constant (K_d) can be obtained from the intercept of this plot; however, K_d values obtained in this way have to be treated with care (1).

For the interaction of protein BSA and Rabbit Anti-Cow Albumin IgG1, Ka was 9.36 x 10^3 $M^{-1}s^{-1}$and the intercept value, kd, was 7.85 x 10^{-3} s^{-1}.

Discussion

The association rate constant *K*a and the dissociation rate constant *K*d are then obtained from linearization of data from the association phase. The value of *K*d can also be obtained from the dissociation phase (1).
It has become apparent that there are more ways to evaluate the data as for example the mass transport limitations or conformational change after binding has not been taking into account in this simplified evaluation.

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3. Nieba, L., Krebber, A., and Plückthun, A. (1996) *Anal. Biochem.* **234,** 155–165.
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Figure 1. Immobilization of 2.5 ug BSA on MUA.

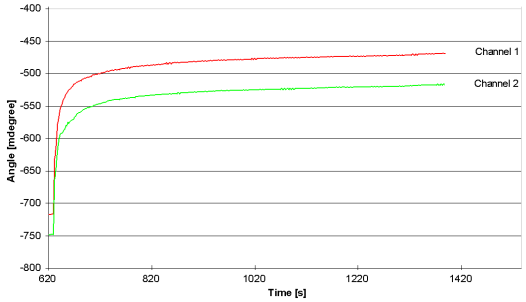

Figure 2. Interaction of anti-Cow Albumin.

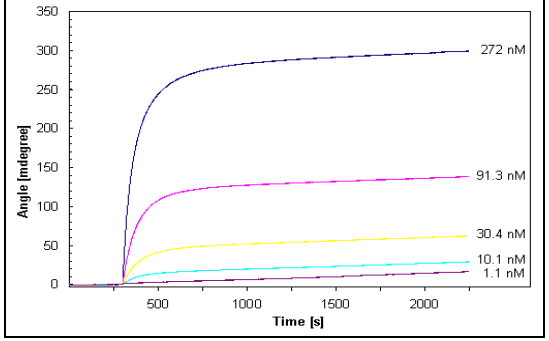

Figure 3. Determination of Equilibrium angle.

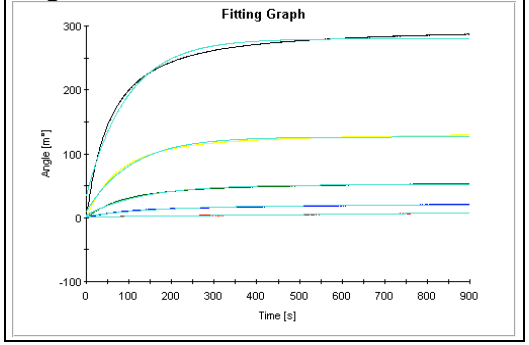

Figure 4. Fitting of the concentration vs equilibrium angle.