High-affinity capturing of antibodies on the switchSENSE® biochip using CaptureSelect™ affinity ligands

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For biomolecular interaction analysis non-covalent ligand immobilization strategies are commonly used as they require no chemical modification of the ligands and as the sample consumption is usually small. Furthermore, capture molecules that possess a specific affinity towards a certain ligand molecule can be used to immobilize a specific ligand from a crude mixture.

Yet, non-covalent immobilization can be problematic, if the complex of capture molecule and ligand is not sufficiently stable. Especially the rate of dissociation is crucial as the ligand might dissociate faster from the capture molecule than the analyte dissociates from the ligand.
In this application note, we introduce CaptureSelect™ capture molecules, which specifically immobilize antibodies from three species (rabbit, mouse, human) at very high affinities.

Background

Kinetic characterization of biomolecular interactions using biosensor systems relies on the stable immobilization of ligand molecules on the biosensor surface. Notably, non-covalent immobilization methods, such capture approaches using surface immobilized antibodies, can be error-prone, if the dissociation of the captured ligand from the antibody occurs at a fast rate. In such a case, the dissociation from capture antibody can affect or even completely mask the dissociation trace of the secondary interaction of interest.

Here, we describe the use of CaptureSelect™ ligands to capture different antibody types with very high affinity, allowing an easy kinetic characterization of antibody-antigen interactions.

CaptureSelect™ ligands are camelid-derived single domain antibody fragments. These relatively small fragments (approx. 13kDa) are very stable and can capture antibodies at high specificity and high affinity, via either their Fab or their Fc domains. They are used both as monomers or as covalently linked bi-functional ligands with increased affinity and target specificity.

In this application note, we present three different CaptureSelect™ ligands, targeting human, mouse and rabbit antibodies (CaptureSelect™ anti-IgG-Fc human, CaptureSelect™ anti-LC-kappa murine and CaptureSelect™ anti-IgG-Fc rabbit). We demonstrate the stability of the antibody captures on the switchSENSE® biochip and illustrate their use with an example kinetic measurement of an antigen-antibody interaction (hCA1-Antibody interaction).

Methods

Measurements were performed in PE140 buffer (pH 7.4, order no. BU-PE-140-10) with a dual-color DRX² instrument using the red fluorescence channel in static measurement mode. The obtained data was analyzed with the kinetics module of the **switch**ANALYSIS software. For all measurements, a multi-purpose **switch**SENSE® chip (order no. MPC2-96-2-G1R1) was used. The sensor surface was functionalized with the indicated conjugated CaptureSelect™ ligand and their affinities to the respective antibody type was determined using three antibodies as analytes: Humira® (human), anti-biotin IgG (mouse) and IgG (rabbit).

Association measurements were performed at 3 different antibody concentrations between 0 and 12.5 nM (depending on the antibody) while dissociations were only observed for the highest antibody concentrations by pumping buffer over the sensor surface. For both association and dissociation measurements flow rates of at least 200 µl/min were used. Before each association the sensor was regenerated by exposure to a high pH regeneration solution (order no. SOL-REG-12-1), which denatures the double stranded DNA and leaves only the covalently attached single-stranded nanolevers on the surface while the conjugate is washed away.

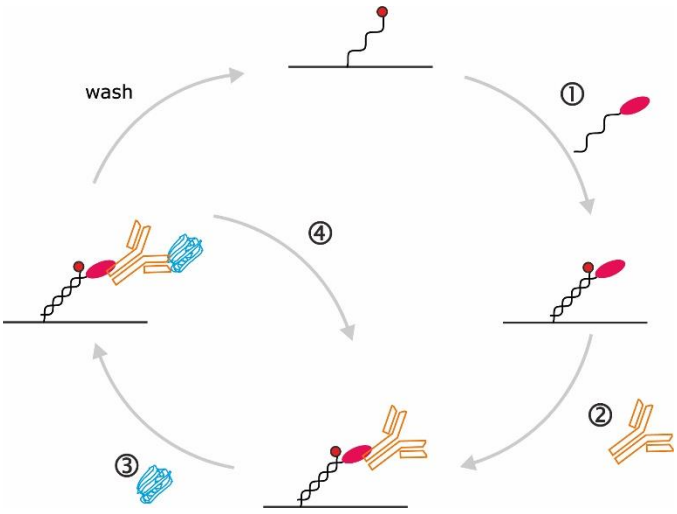

Figure 1 | Measurement workflow. ① Sensor functionalization with CaptureSelect™: Covalently attached single-stranded DNAs on the measurement electrode are functionalized by hybridizing complementary DNA - CaptureSelect™ conjugates to the surface. ② Capture of the antibody. ③ Associations and ④ dissociations of the antigen.

Measurements of a secondary interaction were carried out using human carbonic anhydrase (hCA1), which was associated to a specific antibody. Figure 1 shows the experimental workflow for the analysis of the hCA – anti-hCA interaction. After hybridization of the CaptureSelect™ conjugate (CaptureSelect™ anti-LC-kappa murine, ①), the antibody anti-hCA was captured at a concentration of 10 nM (②). Subsequently, hCA associations (③) and dissociations (④) were recorded at 5 different concentrations ranging from 0 to 40 nM.

Results

To characterize the affinities of the three CaptureSelect™ ligands, we measured the binding kinetics of each capture ligand towards a target antibody. Figure 2 shows the corresponding traces for: (a) Humira® on CaptureSelect™ anti-IgG-Fc human; (b) anti-biotin IgG on CaptureSelect™ anti LC-kappa murine; and (c) IgG on CaptureSelect™ anti-IgG-Fc rabbit.

All tested CaptureSelect™ capture ligands showed fast association rate constants (k_{on}) ranging from 1.5 x 10^6 $M^{-1}s^{-1}$ to 6.3 x 10^7 $M^{-1}s^{-1}$ combined with small values for the dissociation rate constant (k_{off}) ranging between 1 x 10^{-5} s^{-1} and 5 x 10^{-4} s^{-1}. This reflects high affinities, even in the sub-picomolar regime (Table 1) proving strong capture of the antibodies on the biosensor surface. Notably, the slow dissociation of the captured antibodies from the CaptureSelect™ ligands makes this immobilization approach suitable to measure antigen-antibody interactions with moderate to slow off-rates.

Table 1 | Kinetic parameters determined for the interaction of CaptureSelect™ ligands to their specific targets.

Interaction	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (pM)
Humira® / anti-Fc human	$1.5 \pm 0.06 \times 10^{6}$	$4.2 \pm 0.07 \times 10^{-4}$	280 ± 12
anti-biotin IgG / anti-LC-kappa murine	$6.3 \pm 0.31 \times 10^{7}$	$1.9 \pm 0.05 \times 10^{-5}$	0.30 ± 0.02
IgG / anti-Fc rabbit	$2.0 \pm 0.45 \times 10^{7}$	$2.2 \pm 0.02 \times 10^{-5}$	1.1 ± 0.25

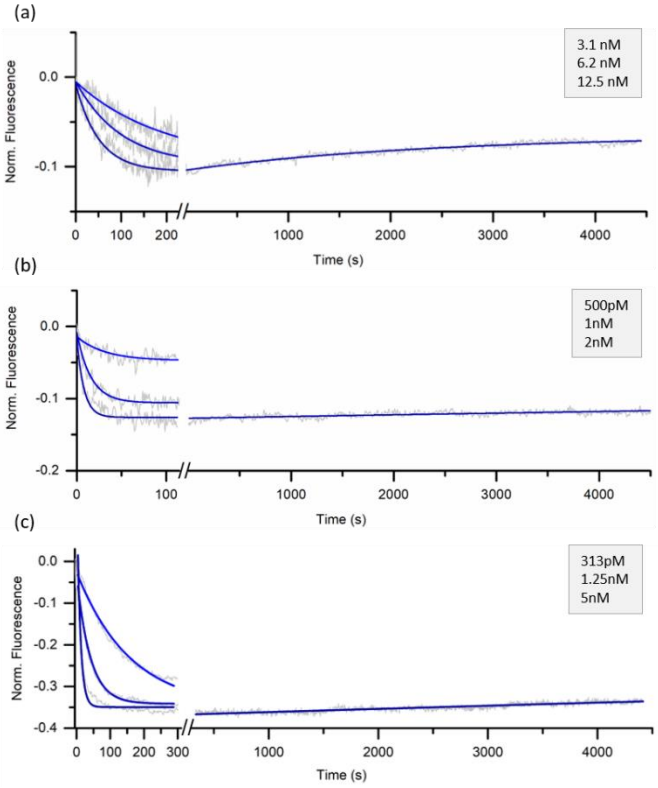

Figure 2 | Fluorescence intensity signals during the capture of three different antibodies to their corresponding CaptureSelect™ ligands: (a) capture of Humira® on anti-Fc human; (b) capture of anti-biotin IgG on anti-LC-kappa murine; and (c) capture of IgG on anti-Fc rabbit. In all cases the slow dissociation reflects the stability of the complex on the surface. Blue lines are global exponential fits using a 1:1 binding model.

As an example of such a characterization, Figure 3 shows the kinetic measurement of hCA towards anti-hCA captured by the CaptureSelect™ anti-LC-kappa murine on the biochip surface (refer to Figure 1 for the corresponding workflow). In this case, five complete kinetic cycles (association and dissociation for five concentrations) could be performed on the same surface, without regeneration and new antibody capture.

The kinetic traces could be well evaluated using a mono-phasic exponential global fit model. The resulting kinetic values are identical to those obtained for covalently conjugated anti-hCA antibody (data not shown). Furthermore, the sensor signal during the buffer blank injection shows no significant changes during the observed time scales, indicating that no significant dissociation of the captured antibody occurred.

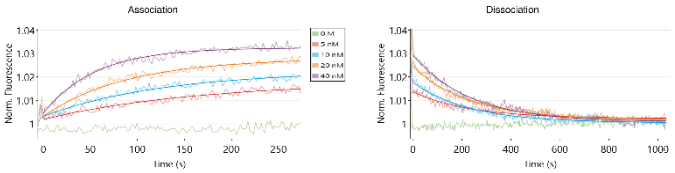

Figure 3 | Fluorescence intensity signals during association and dissociation of hCA to anti-hCA captured by CaptureSelect™ anti-LC-kappa murine. Global fitting of the measured curves yields $k_{on} = 3.44 \pm 0.12 \times 10^{5}$ $M^{-1}s^{-1}$, $k_{off} = 4.32 \pm 0.05 \times 10^{-3}$ s^{-1} and $K_D = 12.6 \pm 0.5$ nM

Conclusion

Covalently immobilized CaptureSelect™ capture ligands are well suited for the noncovalent immobilization of antibodies from human, mouse and rabbit. In all tested cases the dissociation of the captured antibodies was very slow with small signal changes during more than one hour of dissociation, which allows for the use of this immobilization approach to study secondary interactions with fast and moderate dissociation rates.

Advantages compared to other immobilization approaches

- Antibody ligands can be used in native state as no chemical modification is required.

- Reduced ligand consumption compared to covalent immobilization.
- Faster than covalent immobilization.

Capture Select™ is a product of ThermoFisher.

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