Selection of rabbit B-cell generated recombinant antibodies using a highly sensitive SPR instrument

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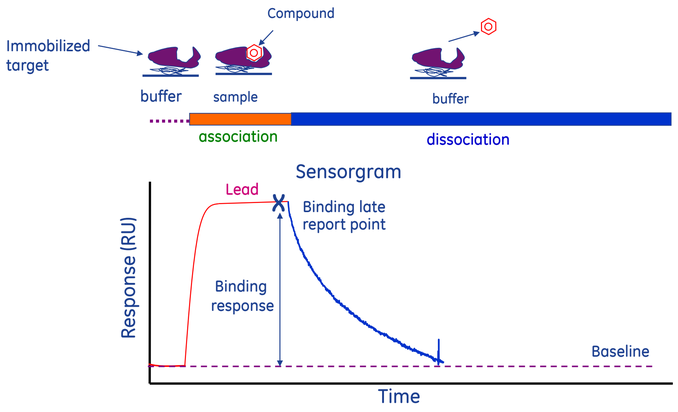
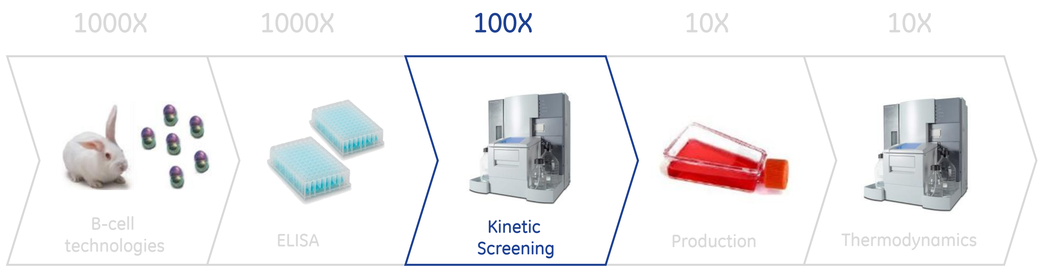
Antibodies produced using Rabbit B-cells are often obtained in low concentrations. When performing kinetic screening of these antibodies towards small peptides or small molecules, the sensitivity of the analysis systems becomes very important. Another major hurdle has been how to efficiently evaluate the data and select the best candidates for further evaluation. In this study, the highly sensitive Biacore™ T200 equipped with a new software was used to efficiently select the top candidates for further characterization.

What does Biacore™ measure



The sensorgram provides real time information about the entire interaction. Kinetics can be calculated from the curvature during injection (orange) and dissociation (blue).
Antibodies are normally characterized by very strong binding with very slow dissociation.

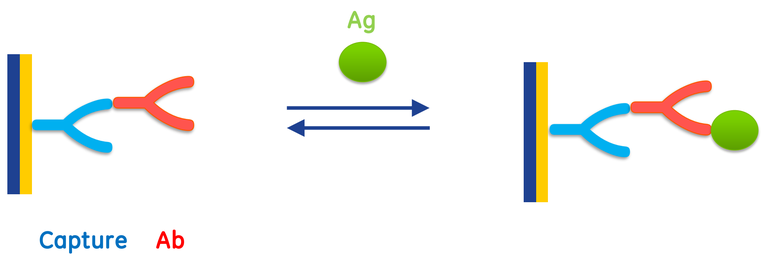
Selection process



- Rabbits were immunized with a 13 kDa chemokine
- 64 Rab-mAb:s were selected and produced for kinetic screening
- Kinetic screening using three different recombinant chemokine derivatives was performed
- Rab-mAb:s with desired binding kinetics to all three chemokines were selected

Kinetic screening assay set-up

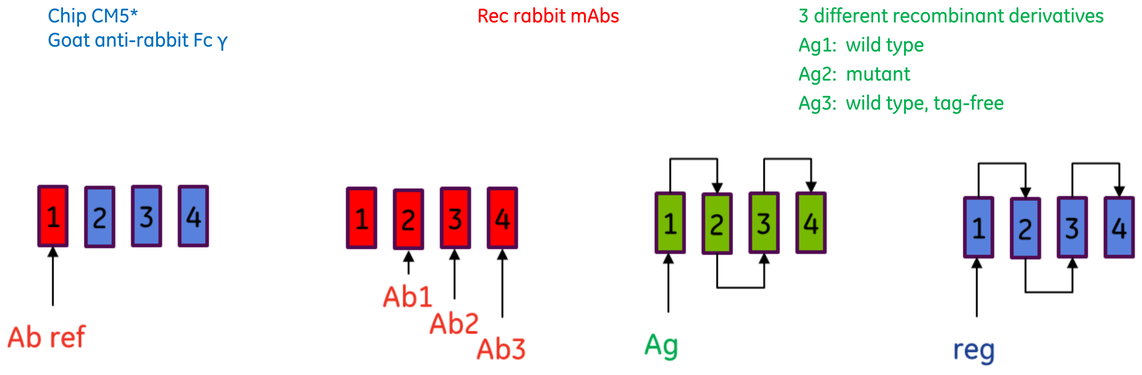
The kinetic screen was performed using an efficient capture approach optimizing the throughput of the system. By this approach up to 130 Rab-mAbs can be screened and evaluated in 24h.



Capture
Chip CM5*
Goat anti-rabbit Fc γ

Ab
Rec rabbit mAbs

Ag
3 different recombinant derivatives
Ag1: wild type
Ag2: mutant
Ag3: wild type, tag-free

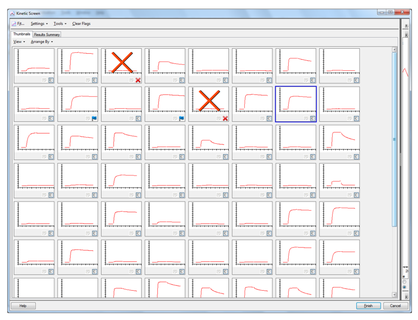


- A reference Ab was captured in flow cell 1
- Three Rabbit-mAbs were captured in every cycle, one in each of three flowcells
- All four flow cells were addressed with antigen
- The whole surface was regenerated and ready for another cycle

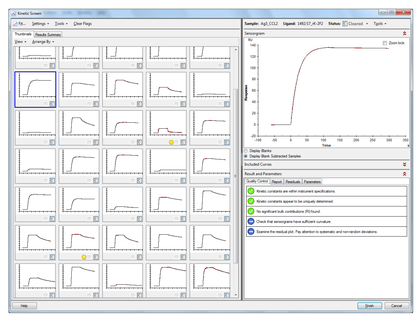
Kinetic Screening data evaluation

Assess data quality - Overview the series

Enhanced evaluation software allow several runs to be co-evaluated if required. Up to 200 concentration series can be simultaneously evaluated. Rapid qualification and pre-fit selection of only relevant data in the thumbnail view sped up the evaluation by minimizing the fitting time. In addition, automated QC tools provided fast fit-quality assessments, enabling confident interpretation of results.

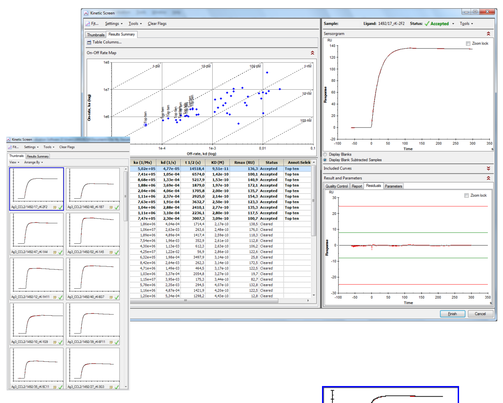


Pre-fit overview allows rapid pre-qualification of the data set.



Post-fit overview with automated fit assessment allows for confident result interpretation.

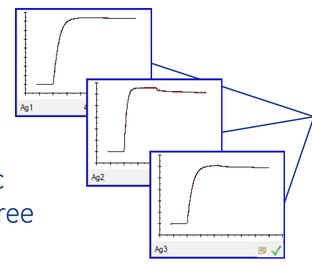
Result summary and antibody selection



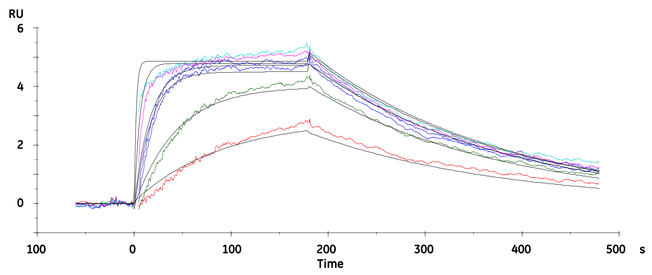
The results are visualized in an on-off rate map and displayed in a customizable result table. Using these tools the top ten antibodies towards each antigen could be quickly identified.

The on-off rate map gives a full overview of the kinetic parameters and affinities of the analyzed antibodies.

Five antibodies were identified that exhibited promising binding kinetic properties towards all three antigens.



Rab-mAb No.	k_a x 10^5	k_d x 10^5	K_D x 10^{-11}
48	7.3 – 14	6.1 – 20	8.4 – 14
47	8.6 – 17	13 – 23	11 – 25
40	11 – 25	24 – 26	10 – 21
39	10 – 22	29 – 34	14 – 32
35	11 – 23	31 – 35	15 – 28



The high sensitivity of the system allows kinetic information to be retrieved from antibodies expressed in low concentrations minimizing the number of false negatives.

Using Biacore T200 it was possible to retrieve kinetic information from this 1.2kDa glycosylated peptide with only 400RU of rabbit-mAb captured on the surface. The analysis was performed at 37°C.

Conclusions

Kinetic screening using Biacore™ T200 has proven very valuable in the selection of rabbit B-cell generated antibodies for diagnostic purposes. The high sensitivity and the new evaluation tools have improved the ability to select promising antibodies based on their kinetic characteristics also for antibodies expressed at low levels and antibodies interacting with small molecules.

- Five Rab-mAb:s were rapidly identified for further characterization (e.g. binding thermodynamics)
- Valuable kinetic data was obtained also at very low expression levels
- The new Biacore T200 software fully supported the kinetic screen evaluation by means of overview, functionality and flexibility

imagination at work