Antibody analyses with Biacore™ system - convenient, sensitive, versatile

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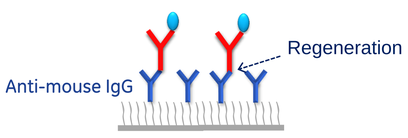
Conclusion

Easy and time-efficient sample preparation, unrivaled sensitivity, comprehensive information in a variety of applications make SPR technology one of a kind platform for the antibody characterization.

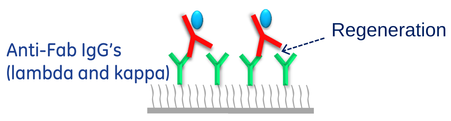
Convenience

Label-free interaction analysis on a Biacore system offers an array of possibilities for an in-depth antibody characterization. A range of capture kits are available, ranging from antibody to different types of tag capture kits. Regular sensor surfaces may also be used for convenient immobilization of a capture molecule of choice.

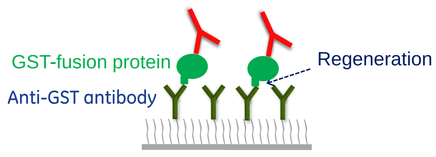
Mouse Antibody Capture Kit



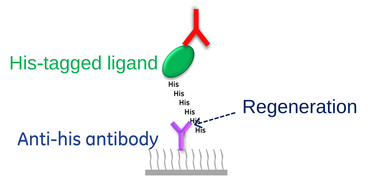
Human Fab Capture Kit



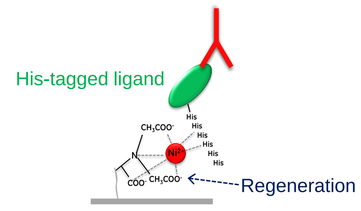
GST Capture Kit



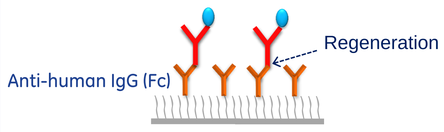
His Capture Kit



Sensor Chip NTA



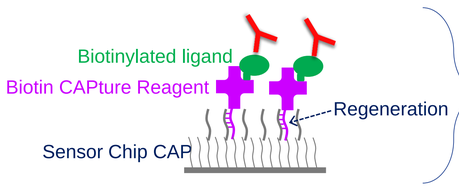
Human Antibody Capture Kit

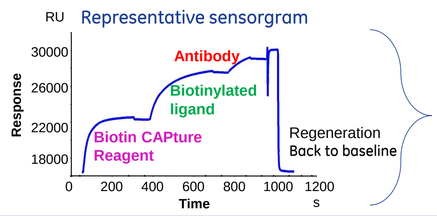


Benefits

- Orientated immobilization of ligand from complex solution
- Captured ligand is easily changed
- All subclasses captured in antibody capture kits
- High quality reagents and optimized protocols that save time and effort
- For ligands that are difficult to immobilize or regenerate

Biotin CAPture Kit

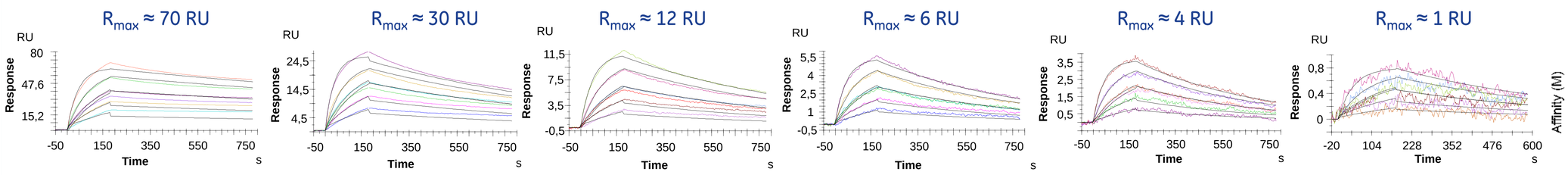


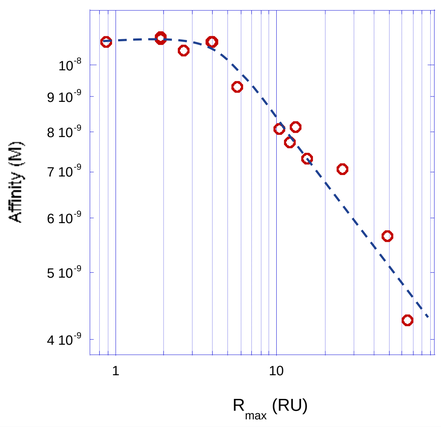


- Biotin CAPture reagent is bound (captured) to the surface by oligo hybridization
- Biotinylated ligand is immobilized via biotin capture
- Analyte (here: antibody) interaction with ligand is studied
- The surface is regenerated and rebuilt in next cycle

Sensitivity

In many cases, a better mimic of the biological situation would be to inject antibody and allow it to react with immobilized antigen. However, the bivalent nature of antibodies gives rise to avidity effects and kinetic analysis becomes challenging. Biacore system offer sensitivity that made it possible to eliminate avidity effects by immobilizing very low amount of antigen on the surface and perform a confident interaction analysis at extremely low response levels. As shown below, at the maximal observed response (R_{max}) below 6 RU, the avidity effects start to disappear and the interaction is described by 1:1 binding model.

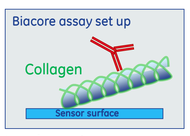




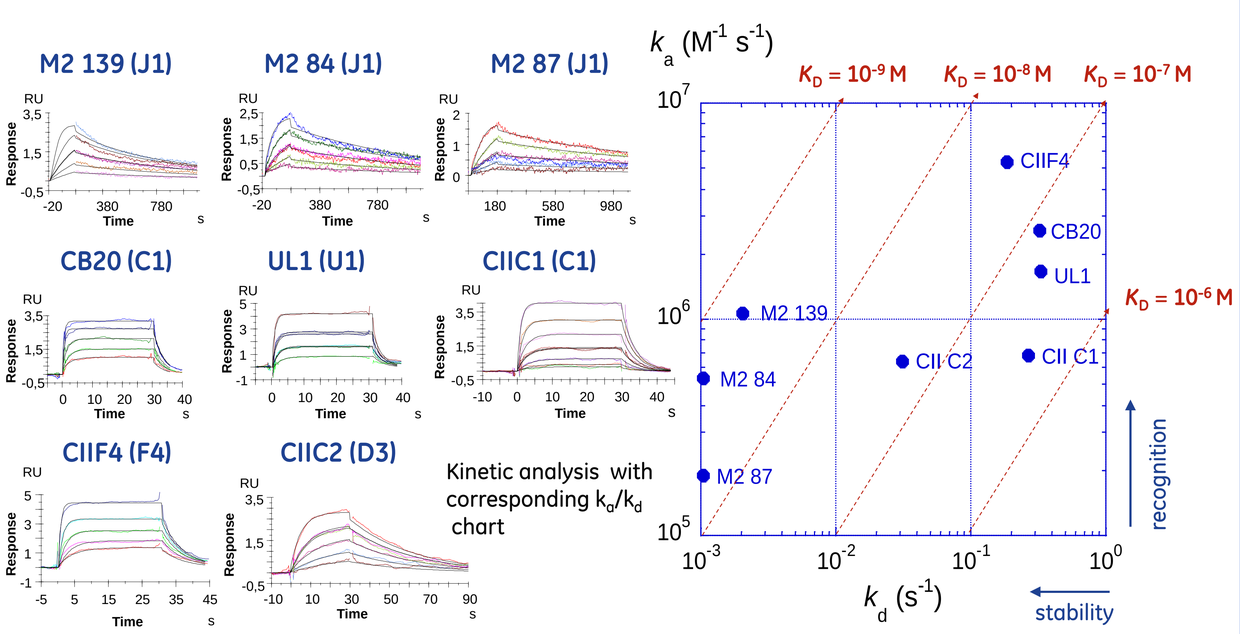
Decreasing response gives more reliable kinetic and affinity data

Versatility

Antibody characterization in the investigation of a disease mechanism



The autoimmune inflammatory disease Rheumatoid arthritis (RA) is characterized by development of autoantibodies to collagen type II. The disease can be mimicked in a collagen induced arthritis animal model, in which the animal develops similar symptoms and autoimmune antibodies. To study the importance of specific collagen epitopes, we analyzed the interactions of antibodies, purified from mouse serum, with various epitopes on collagen: C1, U1, J1, D3 and F4.
In the Biacore assay, collagen was immobilized on the sensor surface, because fibrillar collagen has a tendency to aggregate, and if used in solution (a reversed assay set up) the concentration would be underestimated, which would lead to an underestimation of the association rate constant and thereby also affinity.



Kinetic analysis with corresponding k_a/k_d chart

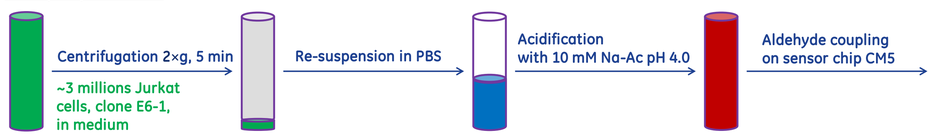
M2 antibodies against J1 epitope are secondary strongly mutated antibodies. They are highly pathogenic, they induce strong clinical athritis, and they also display the highest affinity against J1 epitope.
CB20, UL1 and CIIC1 induces clinical and subclinical artritis and are less pathogenic than M-antibodies. All three antibodies show considerably lower affinity against collagen than M antibodies.
The pathogenicity of CIIF4 and CIIC2 antibodies is being investigated. According to these results, the higher affinity indicates the higher pathogenicity. Antibodies with longer residence times are likely to be stronger inducers of downstream destructive processes as for example immuno complex formation and complement activation.

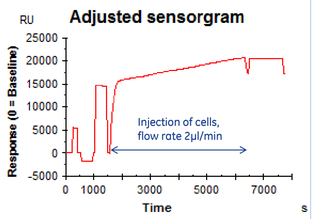
Acknowledgements

Prof. Rikard Holmdahl and Dr. Christoph Kessel, Medical Inflammation Research, The Karolinska Institute in Stockholm and Dr. Andrew Sanderson, GlaxoSmithKline in Cambridge, are acknowledged for providing reagents and for valuable discussions on rheumatoid arthritis and cell assay, respectively.

Antibodies binding to surface-attached cells

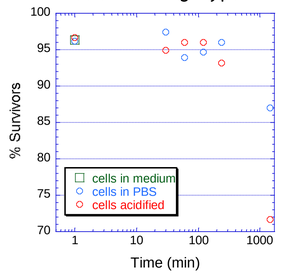
Cell preparation





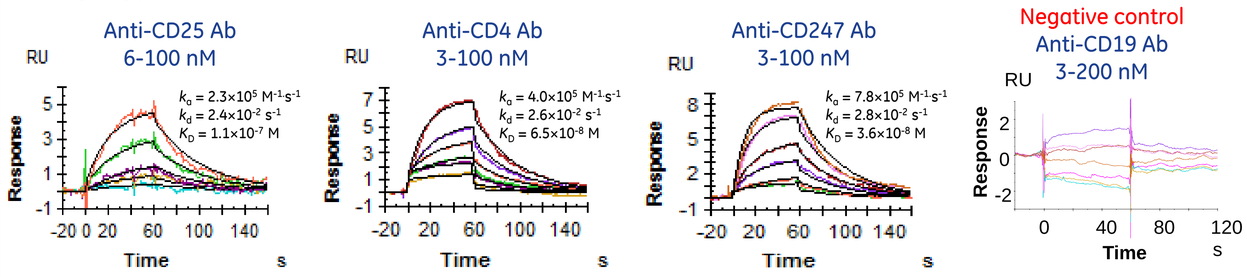
Cell survival test

Measuring the number of surviving cells over time in solution using Trypan blue stain

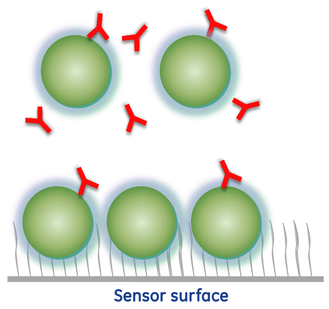


Studies of antibody interactions with cell surface proteins are hampered by the difficulties of creating stable and relevant conditions for proteins when isolated from the cell membrane. This could be circumvented by analyzing antibody binding to whole cell.
Here, we present our initial results from the development of a cell assay including (1) the cell preparation protocol, (2) the results from the examination of the cell survival ability when exposed to various steps of preparation procedure, (3) the binding of three antibodies, anti-CD25, anti-CD4 and anti-CD247, to the glycoproteins on cell surface and (4) competition experiment aiming to confirm the specificity of observed interaction.

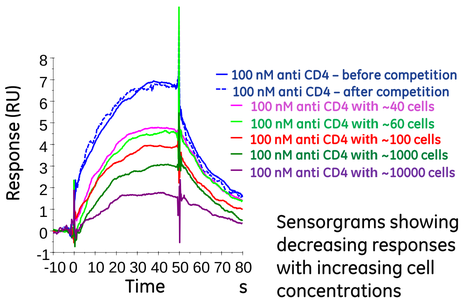
Binding to glycoproteins on cell surface



Confirming binding specificity by cell competition assay



In the competition assay, the antibody is mixed with increasing number of cells and thus prepared samples are sequentially injected over the cell surface. If the cells in the solution and on the surface compete for the binding to the same antibody, the response decrease, proportional to the number of cells, is observed.



Sensorgrams showing decreasing responses with increasing cell concentrations

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