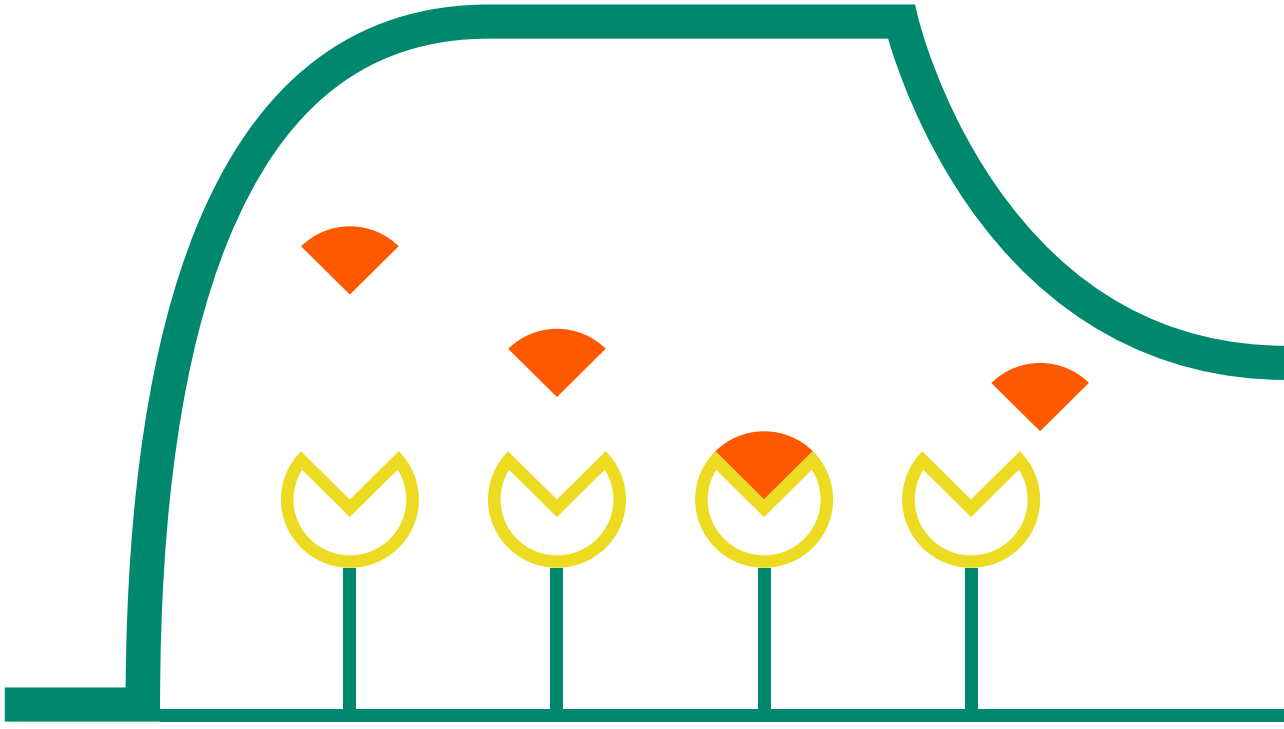
cytiva

Outstanding sensitivity for confident SPR interaction analysis



When you perform surface plasmon resonance (SPR)-based analyses with a high sensitivity instrument— you can also have greater data precision and better confidence in your results interpretation.

Higher sensitivity enables you to:

- work at the limit of kinetic, molecular weight and concentration ranges
- be more precise when measuring and studying interactions involving low molecular weight analytes
- better study unstable molecules like membrane proteins — optimization for high activity and high signal levels is generally not necessary
- use lowest level of attached ligand to more accurately measure on- and off-rates
- characterize binding of antibodies without the complicating effects of avidity

Expanding the measurable kinetic space

Interactions characterized by similar affinities can have very different on- and off-rates. On-rates may be is a measurement of recognition between interacting partners, or readiness with which interacting partners associate. Off-rates indicate the stability of the complex. By deconstructing affinity into on- and off-rates, you will get a much deeper understanding on how the dynamics of molecular interactions relate to protein function.

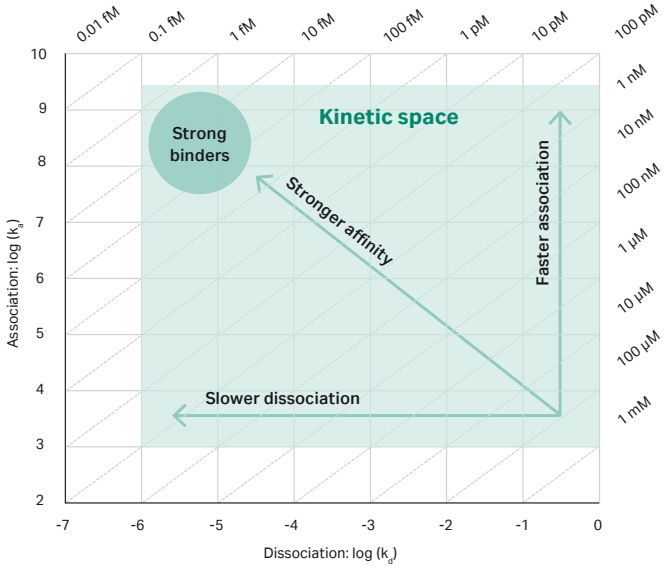


Fig 1. Kinetic measurements over the broadest range, from the fastest on-rates to the slowest off-rates. Interactions with apparently similar affinities can have very different kinetic profiles and resolution into component on- and off-rates can improve candidate selection. Even interactions at the extremes of kinetic behaviour, for example, with very slow off-rates and fast on-rates, can be detected and differentiated with confidence.

The wide range (Fig 1) of kinetic rate constants that can be determined using Biacore™ SPR systems allows resolution and ranking of strongly binding antibodies as well as the weakest binding fragment and the most rapidly associating compounds.

As on- and off-rates may be precisely measured even at the extremities of kinetic behavior, you aren't restricted to steady state analysis which requires high concentrations of interacting partner in solution.

The use of low concentrations enables analysis of low-solubility molecules as well as the avoidance of phenomena such as promiscuous binding.

Working with sensitive targets

The possibility to derive high quality data from low levels of attached interaction partners is advantageous in the analysis of sensitive proteins such as G protein-coupled receptors (GPCRs).

GPCRs are among the most important classes of drug targets, and the high sensitivity of Biacore SPR systems means that only a fraction of the attached target needs to remain active following immobilization (Fig 2). Rare targets may be used sparingly, allowing for reduced consumption without compromising data quality. Sensitive targets can be studied with greater confidence, reducing time-to-results in the drug discovery process.

Antibody binding to a GPCR target was studied using Biacore 8K. A capturing antibody was amine coupled to Sensor Chip CM4. A CHO cell membrane containing the GPCR target was then injected and captured on the surface. No prior purification of the cell membrane was performed. The binding of an anti-target antibody was then studied using Biacore Single Cycle Kinetics (SCK).

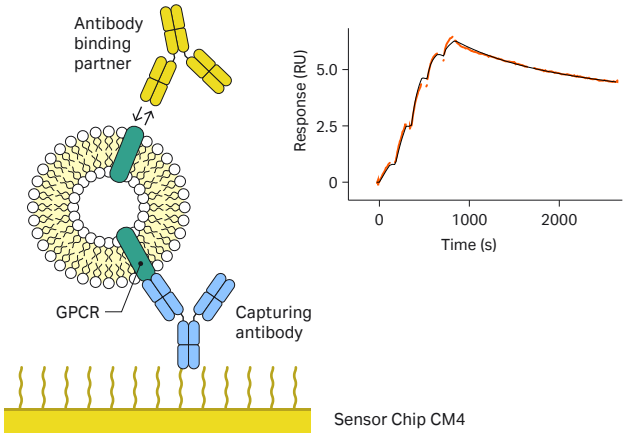


Fig 2. Antibody binding to a GPCR target captured on a Biacore SPR sensor chip directly from a crude CHO cell membrane prep without prior purification.

Precision through sensitivity: detect lowest concentrations of analyte with confidence

Confident measurement of very low protein concentrations is important in areas such as quality control during production of biotherapeutics or in the detection of trace quantities of unwanted proteins. Many intracellular proteins are expressed at extremely low concentrations and their quantitative analysis requires high precision even at the limits of sensitivity. A concentration series of an anti-TNFα antibody was injected over Sensor Chip Protein A docked in a Biacore 8K system to assess the detection limit of the interaction (Fig 3). Limit of detection was determined from 10 replicate blank injections. Antibody was injected for 1200 s at concentrations between 0.3 and 27 pM. The surface was regenerated according to instructions for Sensor Chip Protein A. Results showed that it was possible to confidently detect concentrations as low as 0.3 pM anti-TNFα antibody.

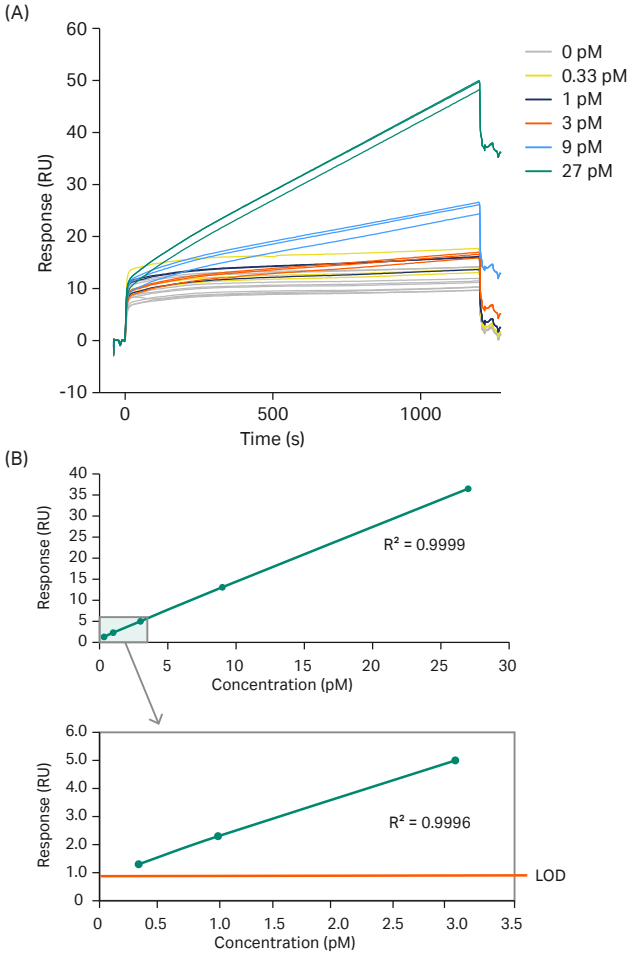


Fig 3. Assessment of limit of detection for an anti-TNFα antibody binding to Sensor Chip Protein A. The limit of detection for the anti-TNFα antibody was determined from the mean of 10 blanks + 3 SD (1 RU). Results show that with this antibody is possible to confidently detect concentrations below 1 pM.

Precision through sensitivity: detect the smallest molecules

The need for technologies to reliably detect and profile interactions involving very small compounds is increasing in areas such as small molecule and fragment-based drug discovery. The sensitivity of Biacore 8K allows the user to detect and precisely characterize any organic compound, regardless of its molecular weight. This means that an affinity analysis of the simplest analytes can be confidently performed (Fig 4).

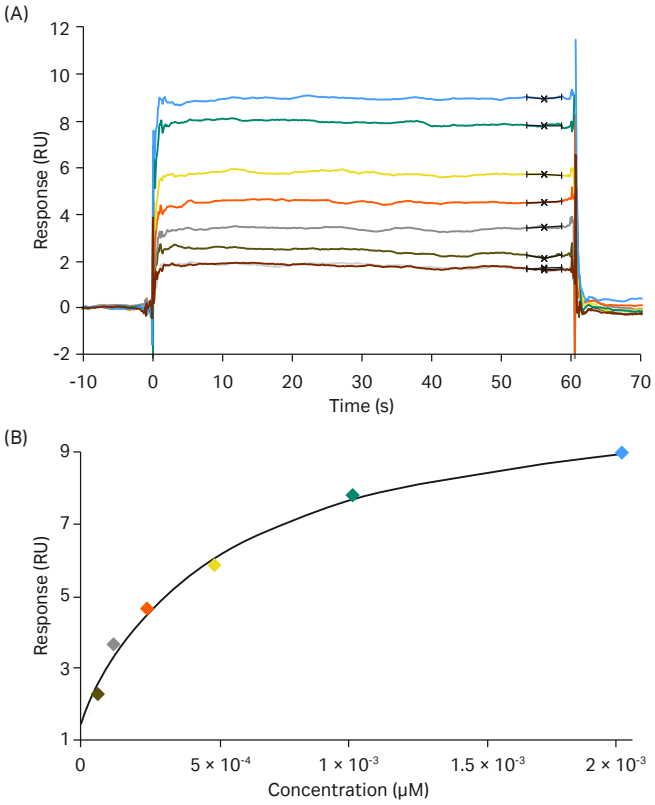


Fig 4. Binding of methanesulfonamide (M_r 95) to carbonic anhydrase immobilized at a level of 7000 RU. Affinity (K_D) was calculated to 0.49 mM.

Precision through sensitivity: working at the lowest ligand attachment levels

Working with low surface densities reduces coupling-related artifacts, mass transport limitation and simplifies data interpretation. However, low surface densities result in low binding signals and high sensitivity equipment is required to detect these signals. This is especially true for challenging targets such as GPCRs and other membrane proteins that are often low in abundance or only partially active. Biacore SPR systems are highly sensitive and enables screening and characterization at the lowest surface densities.

A study using Biacore 1S+ system explored the limitations in determining reliable kinetics and affinity at very low surface densities. Biacore 1 series systems feature high-quality instrument design, low short-time noise, and high signal stability, which allows sensorgrams to be distinctly separated down to the milli-resonance unit (mRU) range. The high sensitivity of Biacore 1S+ systems was utilized to assess the kinetics and affinity of a well-characterized interaction at R_{max} levels below 1 RU (Fig 5). The binding of a 201 Da analyte (CBSA) was characterized, and the data was fitted to a 1:1 binding model. The results demonstrate that it is possible to confidently resolve the kinetics and affinity of the interaction, even when the highest concentration yields a R_{max} response as low as 0.9 RU.

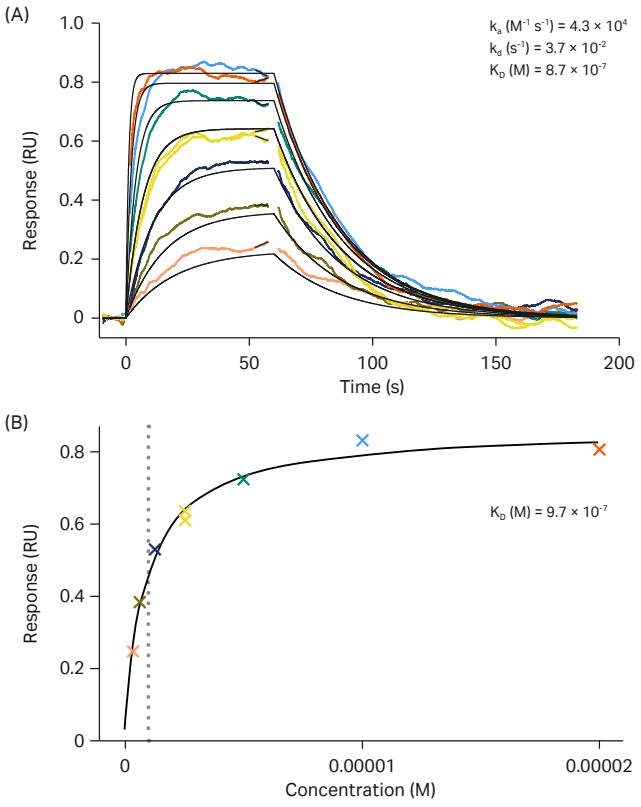


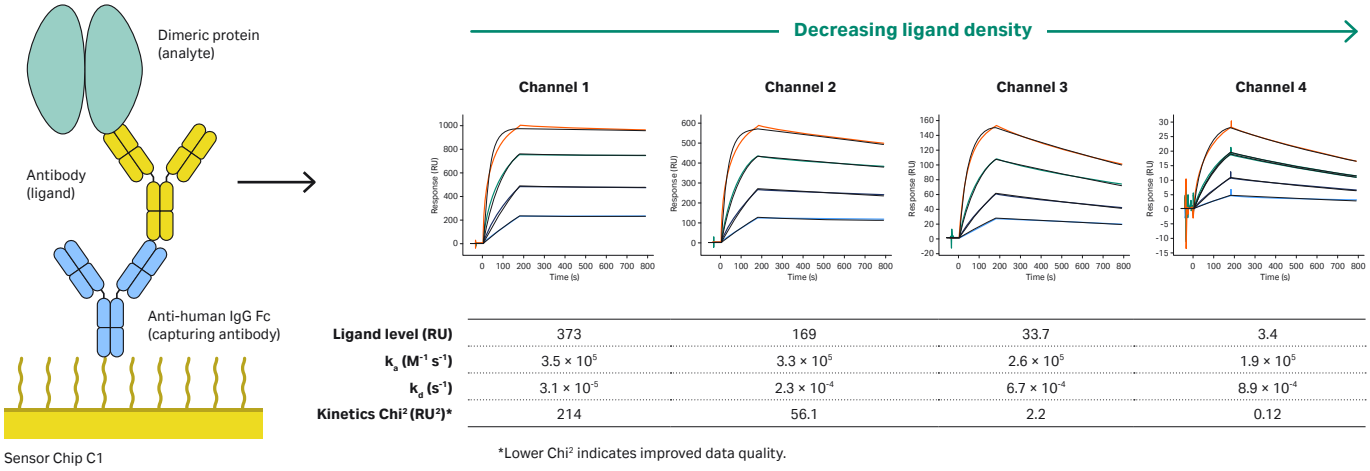
Fig 5. Biacore 1S+ system enables reliable assessment of kinetics (A) and affinity (B) of a 201 Da analyte (CBSA) binding to a 30 kDa protein (carbonic anhydrase isozyme II) immobilized at low level of 200 RU on Sensor Chip CM5.

Flexibility in assay design: avidity-free characterization with bivalent analytes

Full characterization of antigen-antibody interactions is important when assessing the suitability of antibodies as therapeutic, analytical, or diagnostic tools. When running Biacore assays involving antibodies, it is usually recommended to attach the antibody on the sensor surface to avoid avidity effects. Avidity causes the measured dissociation rate to appear slower than it really is, creating difficulties in ranking strong binders and complicating data interpretation.

In certain cases, it can be preferable to attach the antigen to save precious targets or because the antigen may be easier to attach or capture. There are also situations where both interactants are multivalent and you are forced to handle avidity effects. Avidity can be avoided by working at low ligand attachment levels. High sensitivity instrumentation allows one of the interactants to be attached on the sensor surface at a very low density — resulting in clean, avidity-free interaction studies.

Binding of a dimeric protein to an antibody was analyzed using Biacore 8K and Human Antibody Capture Kit. Anti-human antibody was covalently coupled to Sensor Chip C1 (Fig 6). Decreasing amounts of antibody was then captured to establish conditions where no avidity effects were observed, at capture levels below 10 RU. A concentration series of the dimeric protein was then injected over each surface. The effects of avidity due to bivalency progressively reduced with lower surface density, which is seen as an increased dissociation rate.



	Channel 1	Channel 2	Channel 3	Channel 4
Ligand level (RU)	373	169	33.7	3.4
k_a (M^{-1} s^{-1})	3.5×10^5	3.3×10^5	2.6×10^5	1.9×10^5
k_d (s^{-1})	3.1×10^{-5}	2.3×10^{-4}	6.7×10^{-4}	8.9×10^{-4}
Kinetics Chi² (RU²)*	214	56.1	2.2	0.12

*Lower Chi² indicates improved data quality.

Fig 6. Binding of a dimeric protein to a human antibody captured on an anti-human antibody coupled to Sensor Chip C1. Decreasing ligand levels reduce the effect of avidity which is seen as faster off-rate. Data courtesy: Schräml, Biehl, von Proff, Roche Diagnostics GmbH, Centralised and Point of Care Solutions, Penzberg, Germany.

Flexibility in assay design: reversible capture of biotinylated ligand using Biotin CAPture Kit

Biotinylation is the process of covalently attaching biotin (enzymatically or chemically) to a molecule for subsequent capture to streptavidin or NeutrAvidin™. The affinity of streptavidin and NeutraAvidin for biotin is extremely high, with an equilibrium dissociation constant of about 10^{-15} M. Capture is therefore irreversible and normally not possible to remove the ligand after coupling on Sensor Chip SA and Sensor Chip NA.

Sensor Chip CAP (Biotin CAPture Kit) is an alternative to Sensor Chip SA and Sensor Chip NA that enables reversible coupling of biotinylated molecules. This sensor chip is functionalized with an oligonucleotide. The complementary sequence conjugated with Streptavidin is injected over the surface, hybridizes and forms a regenerable streptavidin surface. Biotin CAPture Kit is suitable for scouting of capture levels of biotinylated ligands or when there is a need to test multiple biotinylated ligands on the same sensor surface. Reversible biotin coupling can also be beneficial for unstable ligands that lose activity during analysis.

Binding of small molecule inhibitors to biotinylated Thermolysin was characterized using Biacore T200 (Fig 7). Capture of the protein on Sensor Chip SA resulted in a rapid decline in protein activity during analysis and significant drift. Transfer of the assay to Sensor Chip CAP meant that fresh Thermolysin was captured in every cycle. This eliminated the problem with the declining activity and removed the assay drift.

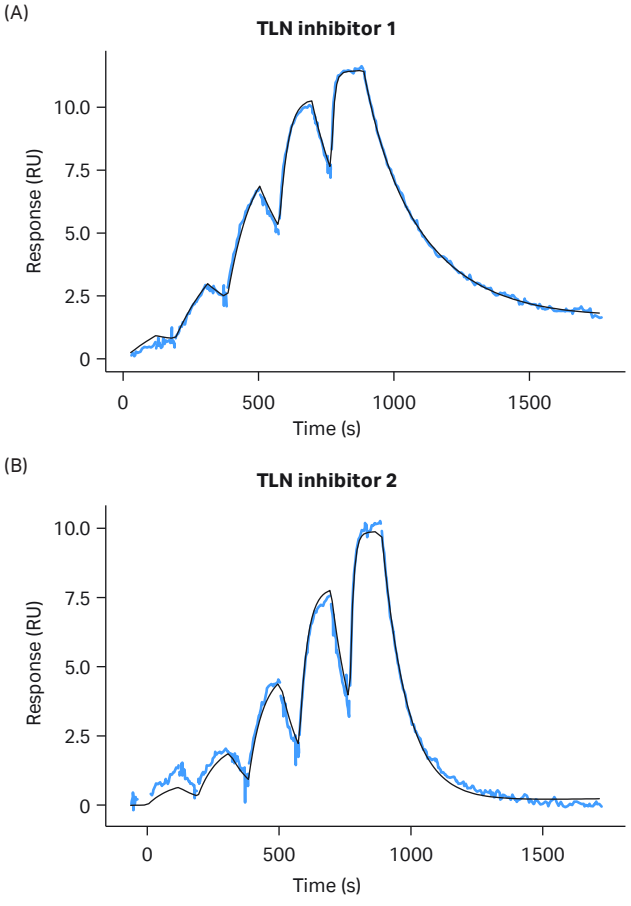


Fig 7. Coupling low levels of biotinylated Thermolysin on Sensor Chip CAP enabled rapid Biacore Single Cycle Kinetics (SCK) analysis of a series of small molecule Thermolysin inhibitors. Data courtesy: Klebe *et al.*, Philips Universität, Marburg, Germany.

Reliable characterization of antibody- fibrillar protein interactions

Human rheumatoid arthritis (RA) is a common autoimmune disorder that can be artificially induced in mice by immunization with type II collagen, leading to the appearance of potentially pathogenic autoantibodies.

Certain specific epitopes on type II collagen have been found to be strongly related to the pathogenicity of the induced antibodies. Biacore SPR system was used in an analysis of serum IgG to test whether the autoimmune responses of mice were directed to these epitopes.

Earlier studies have shown that SPR analyses studying the interactions of antibodies with collagen presents some practical difficulties (Table 1). Fibrillar proteins such as collagen tend to aggregate. If used in solution, their concentration can be underestimated, leading to a lower calculated rate of association. Attaching the collagen on the surface also leads to problems with avidity.

In this study, the sensitivity of Biacore SPR systems allow confident characterization of the collagen/antibody interaction, avoiding aggregation and avidity effects.

Collagen was attached at a non-detectable level and the very low R_{max} was verified using a reference antibody. Antibodies specific to several epitopes on type II collagen, isolated from mouse sera, were subsequently characterized (Table 1).

The appearance of the high affinity antibody, M2139, directed against the J1 epitope on type II collagen is linked to the most severe disease, whereas the lower affinity antibody F4 has been shown to protect against collagen-induced arthritis in mice (Fig 8).

The high sensitivity of the instrument and the very low surface density used in these experiments enabled precise characterization of the collagen/antibody interactions. As the target epitopes were not repetitive, binding was predicted to be monovalent. The ability to use extremely low-density surfaces also enables investigations into how avidity can affect binding of antibodies to large proteins with repetitive epitopes.

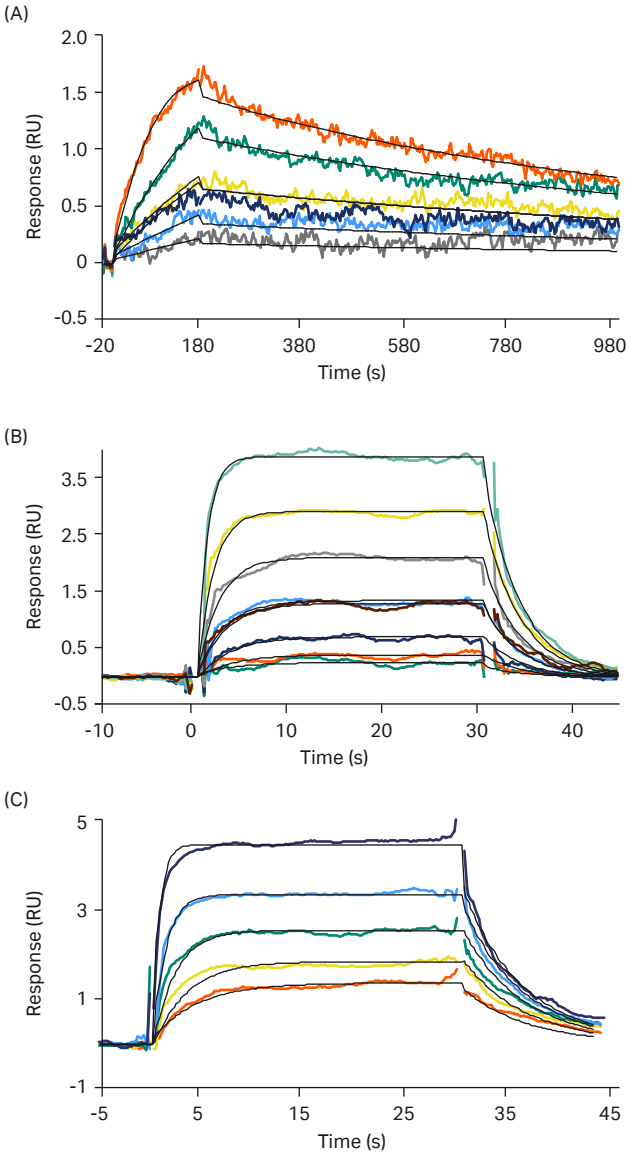


Fig 8. Different interaction profiles of high, medium, and low affinity antibodies. In many cases, the kinetic profiles are related to severity of disease induced by the antibodies generated after injection of mice with type II collagen.

Table 1. Collagen antibody characterization

Type II collagen epitope	Antibody designation	Affinity	Pathologic effect
J1	M2139	High	+++
	M287		+++
	M284		++
D3	CIIC2	Medium	Unknown
C1	CIIC1	Low	++
	CB20	Low	+
U1	UL1	Low	++
F4	F4	Low	-[1]

[1] Antibody F4 has been shown to protect against collagen-induced arthritis.

Discussion and conclusions

Biacore SPR systems are sensitive, label-free interaction analysis systems. They offer excellent opportunities to work confidently at the upper and lower limits of the kinetic range, and open up possibilities to analyze interactions complicated by phenomena such as avidity. The opportunity to attach interacting partners at a very low density is advantageous in applications such ranking high affinity antibodies.

When analyzing interactions involving very small molecules in drug discovery, there is no lower limit in terms of molecular weight. Previously borderline kinetic data can now be confidently accepted. The possibility to use a 40 Hz data collection enables kinetic analysis of very fast interactions. This reduces the risk of missing potentially interesting candidates as well as confirming early failures.

Reference

1. Karlsson R. *et al*. Binding of autoreactive mouse anti-type II collagen antibodies derived from the primary and the secondary immune response investigated with the biosensor technique. J Immunol Methods 188, 63–71 (1995).

Acknowledgements

- Collagen antibodies: Professor Rikard Holmdahl and Dr. Christoph Kessel, Department of Medical Biochemistry & Biophysics, Medical Inflammation Research, Karolinska Institute, Stockholm, Sweden.
- Reversible capture using Biotin CAPture Kit: Professor Klebe *et al*., Philips Universität, Marburg, Germany
- Working at the lowest ligand attachment leves: Dr. Kirk Wright, Senior Investigator I, Novartis, Cambridge, USA.
- Antibody characterization without avidity: Dr. Michael Schräml *et al*., Roche Diagnostics GmbH, Centralised and Point of Care Solutions, Penzberg, Germany.

cytiva

cytiva.com/biacore

For local office contact information, visit cytiva.com/contact

CY15166-03Jul25-WP

danaher