GE Healthcare

Biacore™ 8K – meeting the toughest challenges in screening and characterization

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Introduction

The demands on the pharmaceutical industry are increasing by the day. Targets are increasingly complex and there is an escalating pressure to produce better hits at a faster rate.

Here, we introduce Biacore 8K, a surface plasmon resonance (SPR) system that combines high sensitivity and high throughput to meet the needs for increased efficiency. This poster describes a variety of applications run by customers on prototype systems during the development of Biacore 8K.

Biacore 8K

Biacore 8K efficiently delivers binding data with the quality you expect while meeting tomorrow's challenges in small molecule and biotherapeutic screening and characterization.

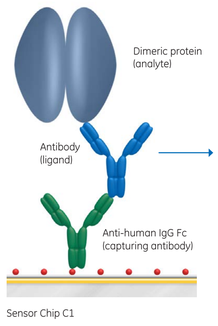
This eight needle, high-sensitivity SPR system rapidly provides kinetics and affinity data, shortening time to results by up to eight times compared to single-needle systems. The blend of system flexibility and throughput reduces the experimental cycle time, even for complex targets and new drug formats such as bispecific antibodies.

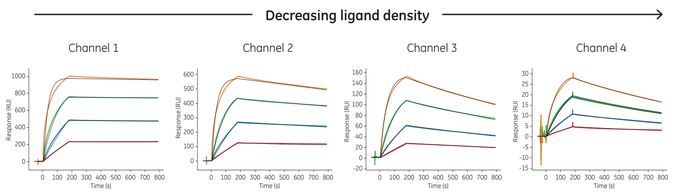
Application examples

Low surface ligand density minimizes avidity effects for bivalent analytes

Working with analytes with more than one binding site for the ligand imposes challenges as there are avidity effects to consider. One way of handling avidity is to decrease ligand density on the surface to the point where it is no longer possible for bivalent binding to occur.

Sensor Chip C1 and Human Antibody Capture Kit were used to capture decreasing amounts of ligand in order to establish conditions where no avidity effects could be observed, that is, capture level < 10 RU. Concentration series of the bivalent analyte were then injected over the surface in single-cycle kinetics mode.





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}
Chi²*	214	56.1	2.2	0.12

*Lower Chi² indicates improved data quality.

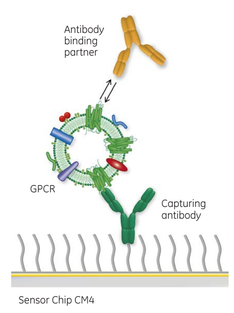
- Off-rate increases with lower ligand densities due to decreasing avidity.
- Avidity effect minimized for lowest capture level (< 10 RU).

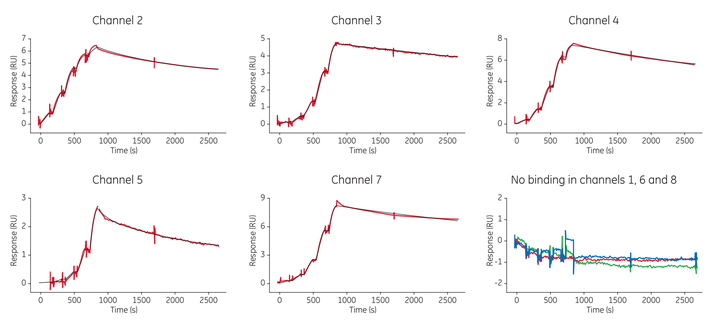
Data courtesy: Schräml, Biehl, von Proff, Roche Diagnostics GmbH, Centralised and Point of Care Solutions, Pentzberg, Germany.

Reliable kinetic characterization of antibodies binding to GPCRs captured from crude membrane preparations

The purpose of the experiment was to capture a GPCR target from a crude membrane preparation from CHO cells and characterize the binding of an antibody against it.

Ligands were amine coupled on Sensor Chip CM4 (one ligand per channel). A crude membrane preparation was captured on the surface followed by concentration series of antibodies binding to the target.





- Eight membrane protein/antibody interactions characterized in < 2 h.
- Reliable kinetic analysis of GPCRs directly from crude membrane preparations.
- The high sensitivity of Biacore 8K enabled resolution of kinetics at low response levels.

Fragment screening against a GPCR target in 2.5 h

A solubilized GPCR was covalently coupled to Sensor Chip NTA in all channels (8000 RU). 320 fragments were screened at 50 µM for their binding to the target. Evaluation was performed in the plot tool using the automated binding behavior markers to rapidly select binders with the highest reponses and square-shaped sensorgrams typical for well-behaving fragments. Cutoff was set to include 10% of fragments without binding behavior markers.

- 320 fragments were analyzed for their binding to the GPCR in 2.5 h, eight times faster than the corresponding run on a single-needle system.
- 32 fragments were selected for further analysis.

Reliable parallel differentiation between high affinity binders and resolution into on- and off-rates

The purpose of the experiment was to characterize and rank eight high affinity protein-protein interactions. 55 to 70 RU of target protein were coupled to Sensor Chip SA (all channels). Concentration series of the binders were injected over the surface in single-cycle mode. Data was fitted to the 1:1 binding model.

Ligand	Analyte	k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (M)	Off-rate ranking order
Ligand A	Analyte 6	4.1×10^6	3.6×10^{-6}	8.7×10^{-11}	1
Ligand B	Analyte 6	2.7×10^6	6.4×10^{-6}	2.3×10^{-10}	2
Ligand A	Analyte 1	2.4×10^5	1.2×10^{-5}	4.8×10^{-11}	3
Ligand B	Analyte 1	1.2×10^5	1.6×10^{-5}	1.3×10^{-10}	4
Ligand C	Analyte 2	2.5×10^5	2.7×10^{-5}	1.1×10^{-10}	5
Ligand C	Analyte 3	3.3×10^5	5.7×10^{-5}	1.8×10^{-10}	6
Ligand A	Analyte 4	6.3×10^5	6.8×10^{-5}	1.1×10^{-10}	7
Ligand C	Analyte 5	4.2×10^5	8.8×10^{-5}	2.1×10^{-10}	8

- The parallel configuration of the instrument allowed for simultaneous analysis of eight high-affinity interactions in 4.5 h.
- The low baseline drift of Biacore 8K enabled off-rate determination down to 4×10^{-6} s^{-1}.
- Single-cycle kinetics omitted the need for optimization of regeneration conditions.

Acknowledgements

We acknowledge all customers that kindly participated in prototype evaluations of Biacore 8K, thereby providing us with invaluable feedback on hardware, software, and user experience as well as data from numerous interesting applications that demonstrates the versatility of Biacore 8K.

Thank you all!

Conclusion

- Biacore 8K offers a great combination of high quality data, high throughput, and high sensitivity.
- Biacore 8K significantly shortens time to results by combining the parallel configuration with efficient assay setup and rapid data handling.
- Biacore 8K facilitates work with all types and sizes of targets and samples, in buffer as well as in crude sample matrices.
- The parallel configuration of the instrument facilitates quick scouting and assay development.

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