Why sensitivity matters: reliable hit characterization for challenging targets using high-sensitivity SPR systems

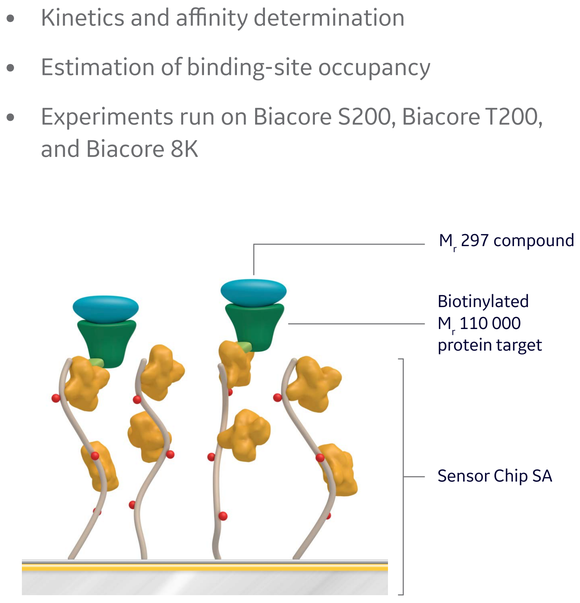
Anna Moberg[1], Maria Lindgren[1], Cynthia Shuman[2], and Paul Belcher[1]

[1]GE Healthcare Bio-Sciences AB, Uppsala, Sweden, [2]GE Healthcare Life Sciences, Marlborough, MA, USA

Introduction

The drug discovery community is moving towards analysis of more challenging targets such as GPCRs and other membrane proteins. These targets are often low in abundance or only partially active, resulting in low signals in binding experiments. To detect these signals, high sensitivity surface plasmon resonance (SPR) equipment is crucial. Working with high sensitivity SPR instruments enables screening and analysis at low surface densities, reducing coupling-related artifacts, and simplifying data interpretation. SPR also allows screening at low concentrations, minimizing solubility issues and effects from secondary interactions.

Here we present a study comparing the sensitivity of Biacore™ S200, Biacore T200, and Biacore 8K SPR systems. Using a previously well-characterized binding interaction, limitations in determination of reliable affinity and kinetics at low surface densities were investigated. Using these results, we also describe how the sensitivity of the equipment reflects on hit detection with regards to binding-site occupancy.



Experimental setup

Model system obtained from Novartis Institutes for BioMedical research (NIBR), Cambridge, USA.

- Varying immobilization levels of target protein, pushed to the lowest limit where affinity and kinetics for the interaction can be determined
- 19.5 nM to 5 µM of the compound in two-fold dilution series in HBS-N
- Kinetics and affinity determination
- Estimation of binding-site occupancy
- Experiments run on Biacore S200, Biacore T200, and Biacore 8K

Comparing Biacore instruments at low response levels

Equal data generated down to 1 RU response level.

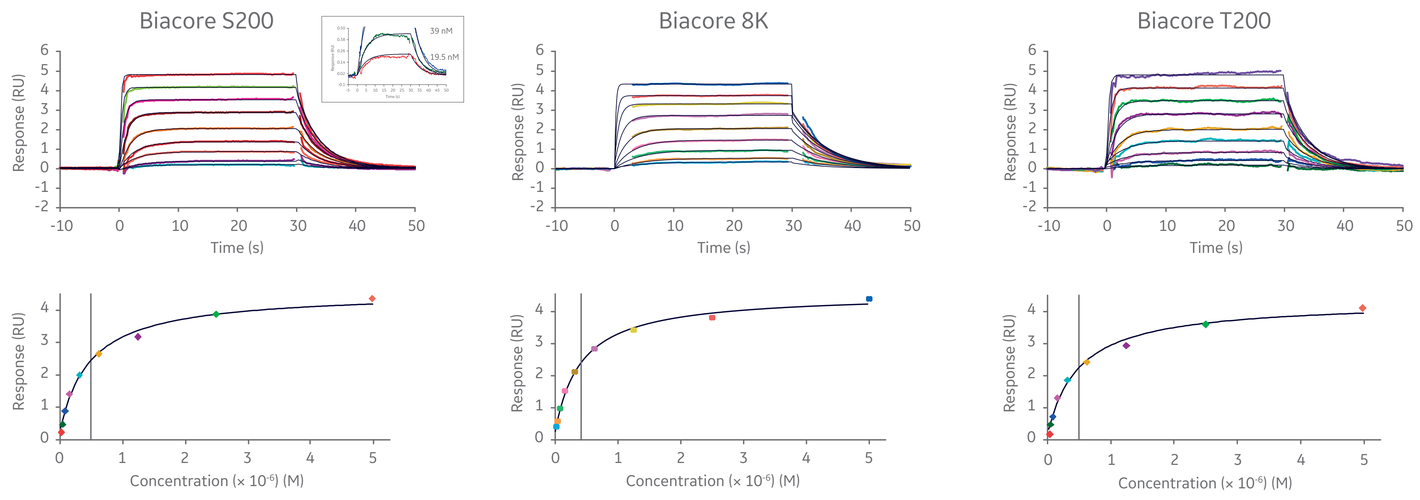


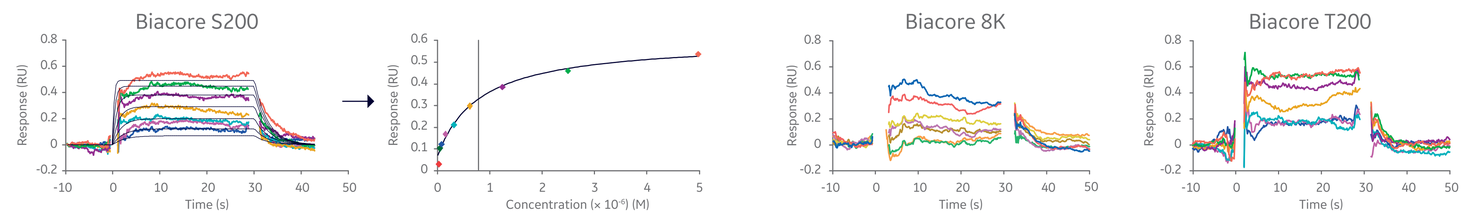
Table 1. Kinetics and affinity at R_{max} 4 RU

Instrument	Kinetics Chi² (RU²)	k_a (1/Ms)	k_d (1/s)	K_D (M)	R_{max} (RU)	Steady-state affinity (M)
Biacore S200	0.002	5.7×10^5	0.24	4.2×10^{-7}	4.1	4.7×10^{-7}
Biacore T200	0.008	5.9×10^5	0.34	3.3×10^{-7}	3.3	5.0×10^{-7}
Biacore 8K	0.007	9.5×10^5	0.17	1.8×10^{-7}	3.0	4.1×10^{-7}

Table 2. Kinetics and affinity at R_{max} < 1 RU

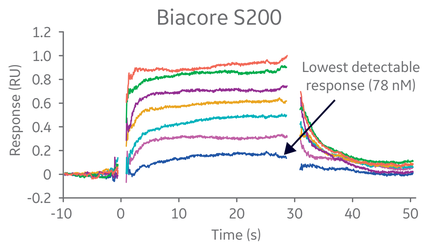
Instrument	Kinetics Chi² (RU²)	k_a (1/Ms)	k_d (1/s)	K_D (M)	R_{max} (RU)	Steady-state affinity (M)
Biacore S200	0.001	7.6×10^5	0.28	3.7×10^{-7}	0.9	3.7×10^{-7}
Biacore T200	0.005	7.7×10^5	0.26	3.3×10^{-7}	0.9	5.2×10^{-7}
Biacore 8K	0.004	6.3×10^5	0.33	5.3×10^{-7}	0.8	5.4×10^{-7}

At responses < 0.5 RU, Biacore S200 still delivers reliable kinetics and steady-state affinity data.
The resolution between curves for Biacore 8K and Biacore T200 is insufficient at these low responses.



Sensitivity expressed as required binding-site occupancy

- Binding-site occupancy is defined as quantity of ligand-bound binding sites divided by the total quantity of ligand binding sites
- Dependent on molecular weight of interactants and immobilization level
- Biacore S200 allows for lower binding-site occupancy than Biacore 8K and Biacore T200 for reliable hit detection using the same assay setup



Lowest detectable analyte conc (M)	7.8×10^{-8}
Lowest detectable response (RU)	0.1
R_{max} observed (RU)	1
MW of compound for R_{max} (M_r)	297.3
MW of immobilized/captured ligand (M_r)	110 000
Immobilization/capture level (RU)	646

Instrument	Binding-site occupancy at R_{max} 1 RU
Biacore S200	10%
Biacore 8K	26%
Biacore T200	30%

Conclusions

- The determined affinity for the interaction correlated well with previous assays for all instruments down to R_{max} 1 RU
- Only Biacore S200 delivered reliable kinetics and affinity data at extremely low R_{max} values (< 0.5 RU)
- The high sensitivity of Biacore S200 enables analysis of challenging targets with low activity and low abundance

Acknowledgement

This comparison study was performed in collaboration with Dr. Kirk Wright, Senior Investigator I at Novartis Institutes for BioMedical Research (NIBR), Cambridge, USA, who also provided the model system and confirmed correlation of data with previous experments.

KA3197230318PO