

Kinetic and concentration analysis in all directions with Biacore™ 8K and Biacore™ 8K+

M. Lindgren, V. Fridh, A. Moberg, H. Roos, H. Bergling, M. Tidare, Å. Lundström, O. Karlsson, L. Nygren-Babol, and C. Holmgren
Cytiva, Björkgatan 30, 751 84 Uppsala, Sweden

Introduction

Reliable measurement of binding kinetics and protein concentration is fundamental in all areas of biological research and development, ranging from studies on mechanism of action to drug release testing. Biacore™ systems have a wide dynamic range which enables assessment of binding rate constants and active concentration for basically all biological interactions.

With the introduction of Biacore™ 8K and Biacore™ 8K+, new possibilities on how to perform kinetic and concentration analysis open up. These eight-needle high-sensitivity SPR systems not only shorten time to results by up to eight times compared to single-needle systems, they also allow for

kinetic and concentration analysis in parallel approach. The 2D Kinetics™ functionality offers detailed kinetics from only one sample cycle, thereby significantly reducing assay development time. For concentration measurements, the use of a parallel calibration curve provides shorter run times, which results in data with very good precision enhanced by inter-channel normalization.

This poster presents the high flexibility in assay set-up offered with Biacore™ 8K and Biacore™ 8K+ when determining binding rate constants, or in concentration analysis.

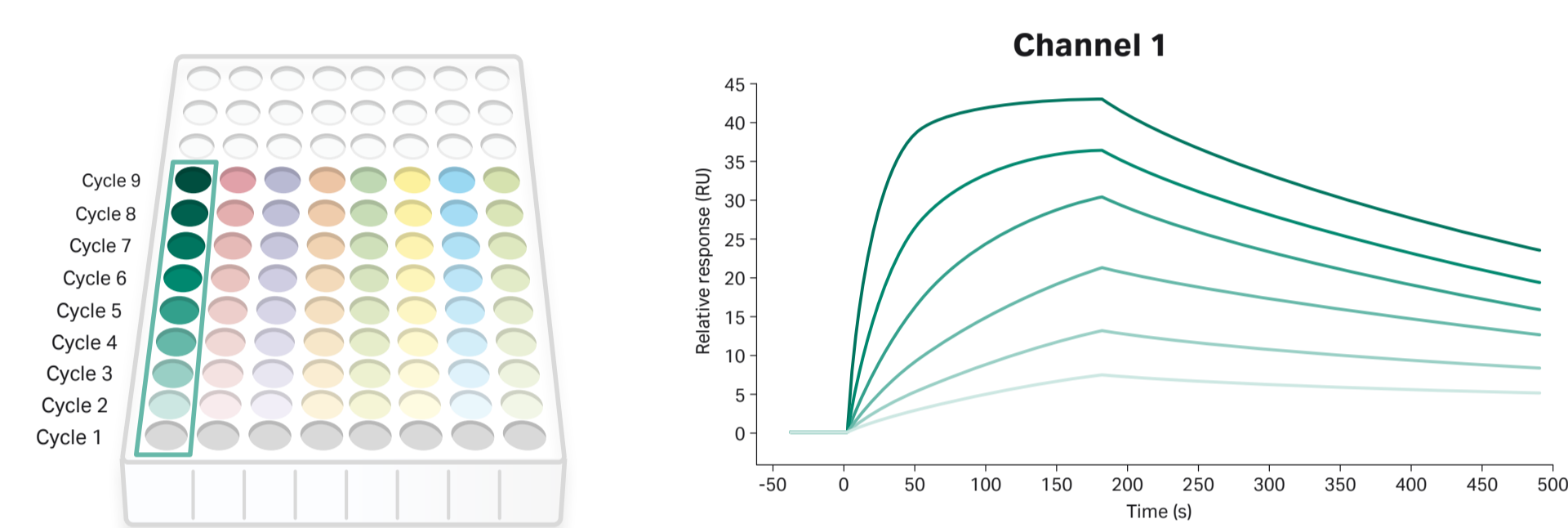


Kinetic analysis

Depending on the preferences and circumstances of each assay, kinetic analysis can be performed in four different ways.

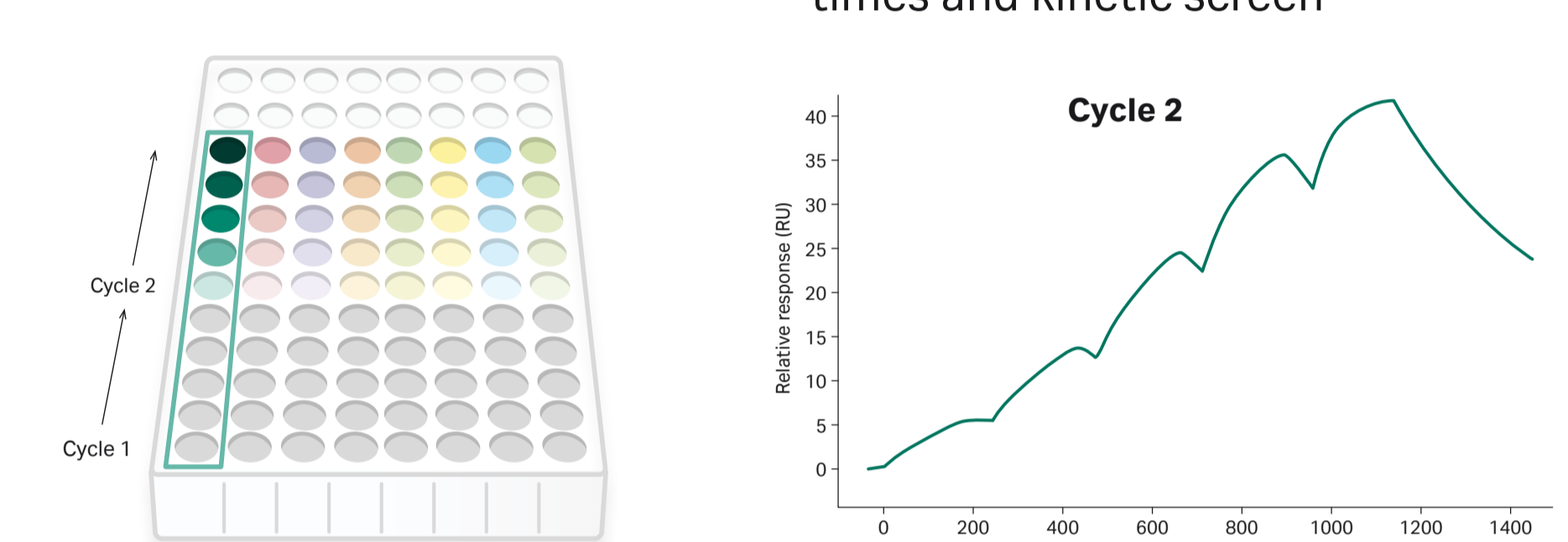
Multi-Cycle Kinetics (MCK)™

- Suitable when different ligands are to be immobilized
- Suitable also for many samples against one ligand



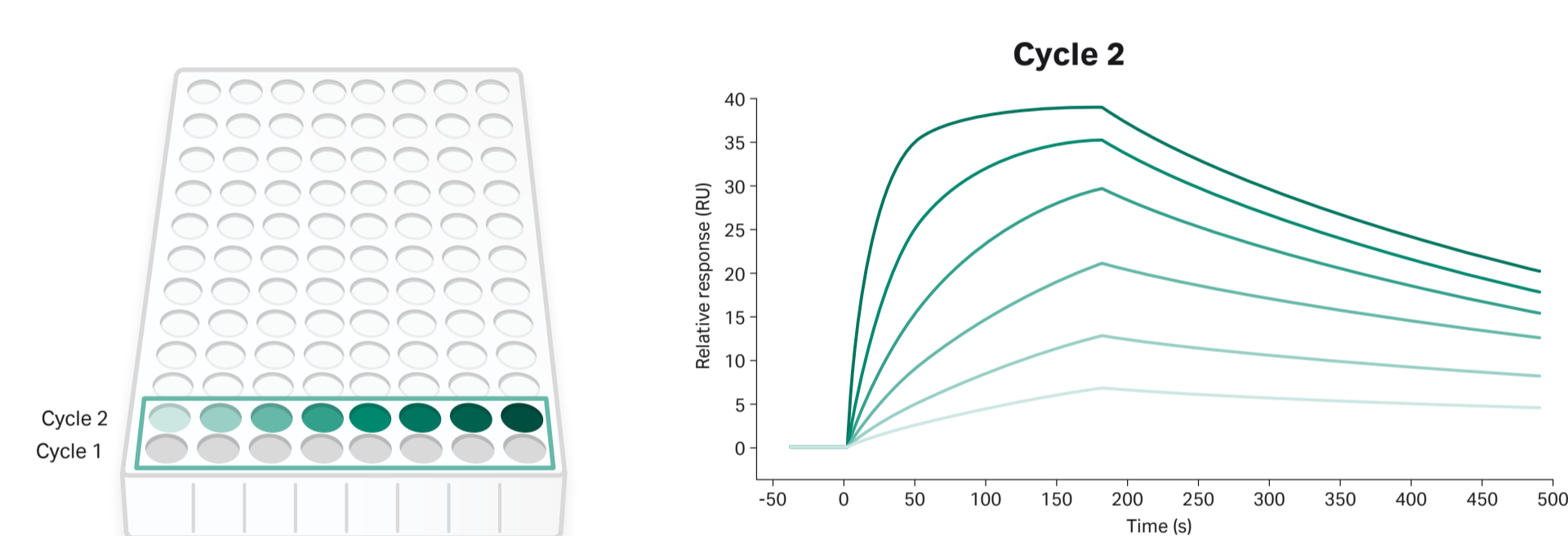
Single-Cycle Kinetics (SCK)™

- Fast run time
- No regeneration needed
- 2–5 concentrations per injection
- Beneficial for long dissociation times and kinetic screen



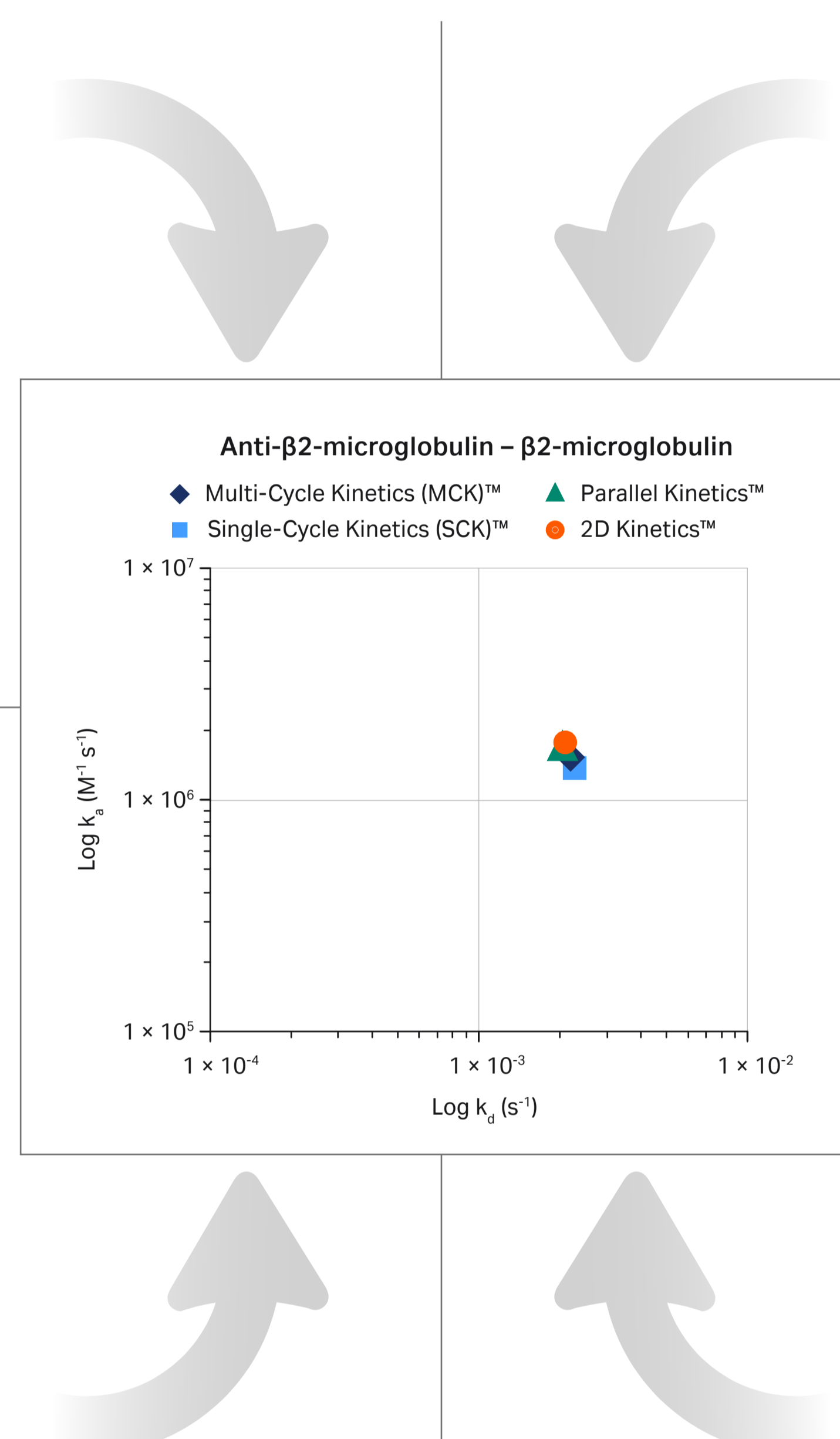
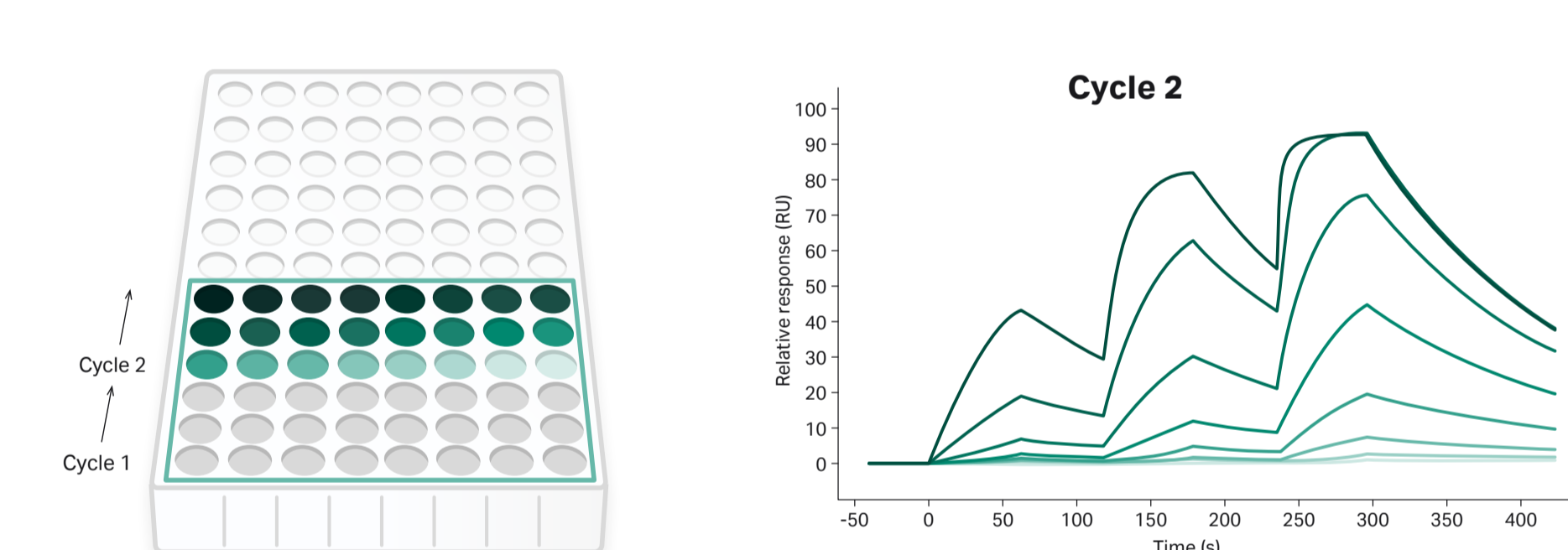
Parallel Kinetics™

- Short run time for few samples
- Kinetic analysis in only two cycles (one blank cycle)
- Beneficial for samples with long dissociation times
- Alternative set-up: Two samples, four concentrations



2D Kinetics™

- In depth analysis in only one sample cycle
- Sample diluted directly into plate
- Sample diluted in two dimensions to cover a wide range
- No preknowledge about affinity or regeneration needed



Concentration analysis

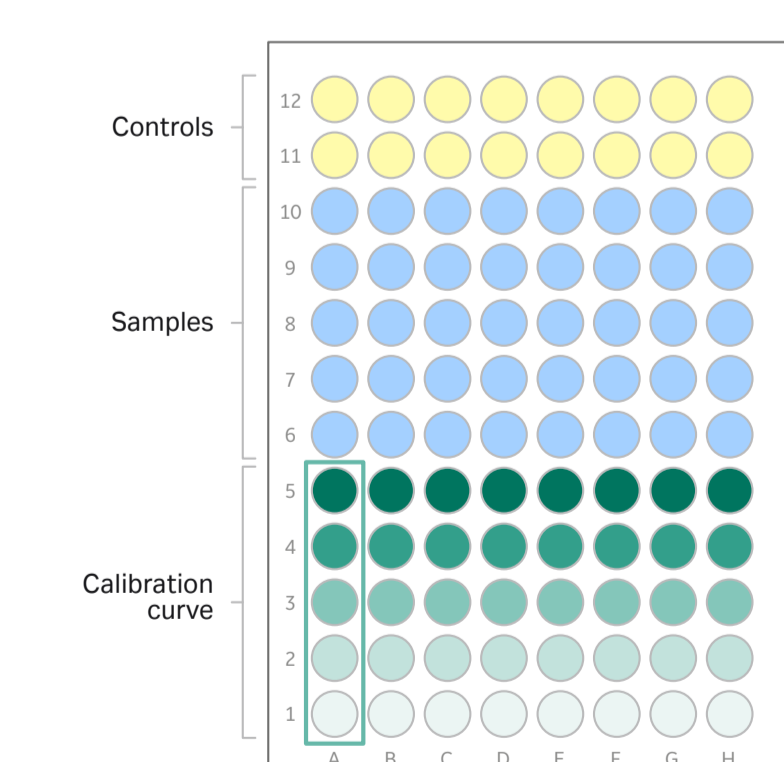
Concentration analysis can be performed using calibration curves for each channel separately (serial approach) or with a calibration curve common for all channels (parallel approach). The parallel approach involves a channel normalization step to compensate for small inter-channel variations.

The ligand used in the experiments was Protein A (Sensor Chip Protein A, Cytiva) and the analyte was human IgG.

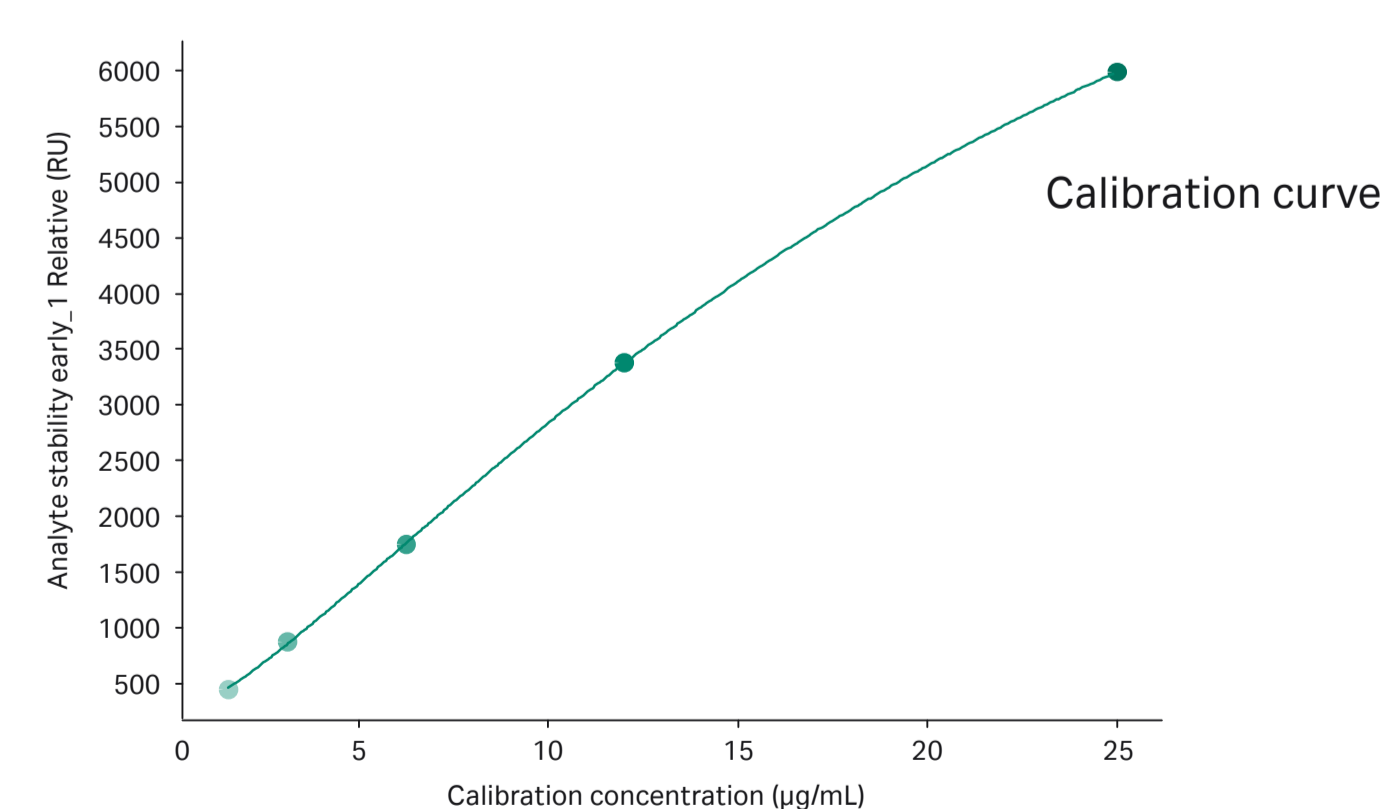
Serial concentration analysis

- Providing data with excellent precision
- Each channel holds its own calibration curve
- Beneficial when analyzing a large number of samples, several ligands and/or high precision is required

Experimental setup



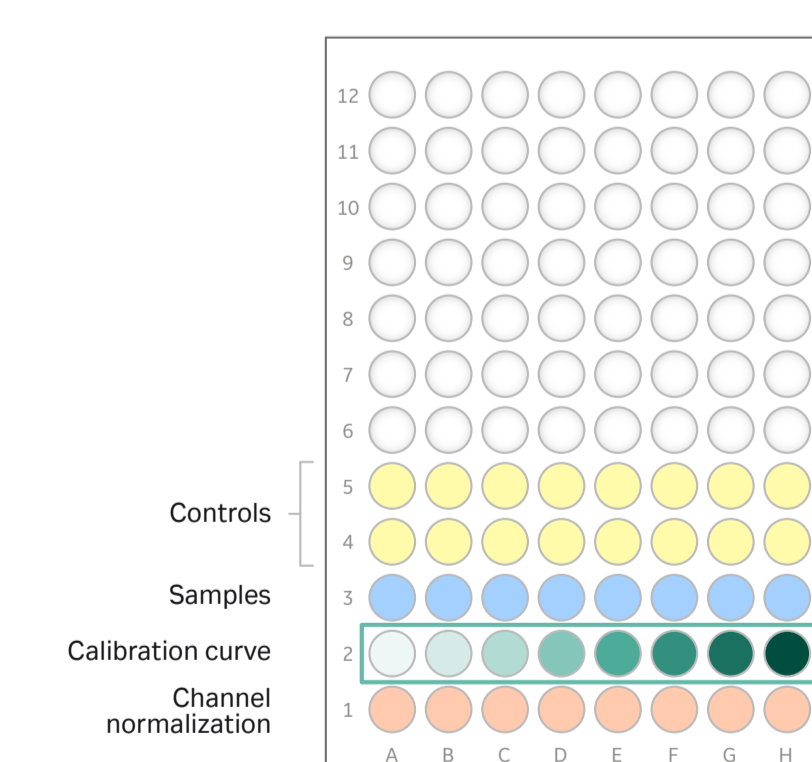
Results channel 1 (example)



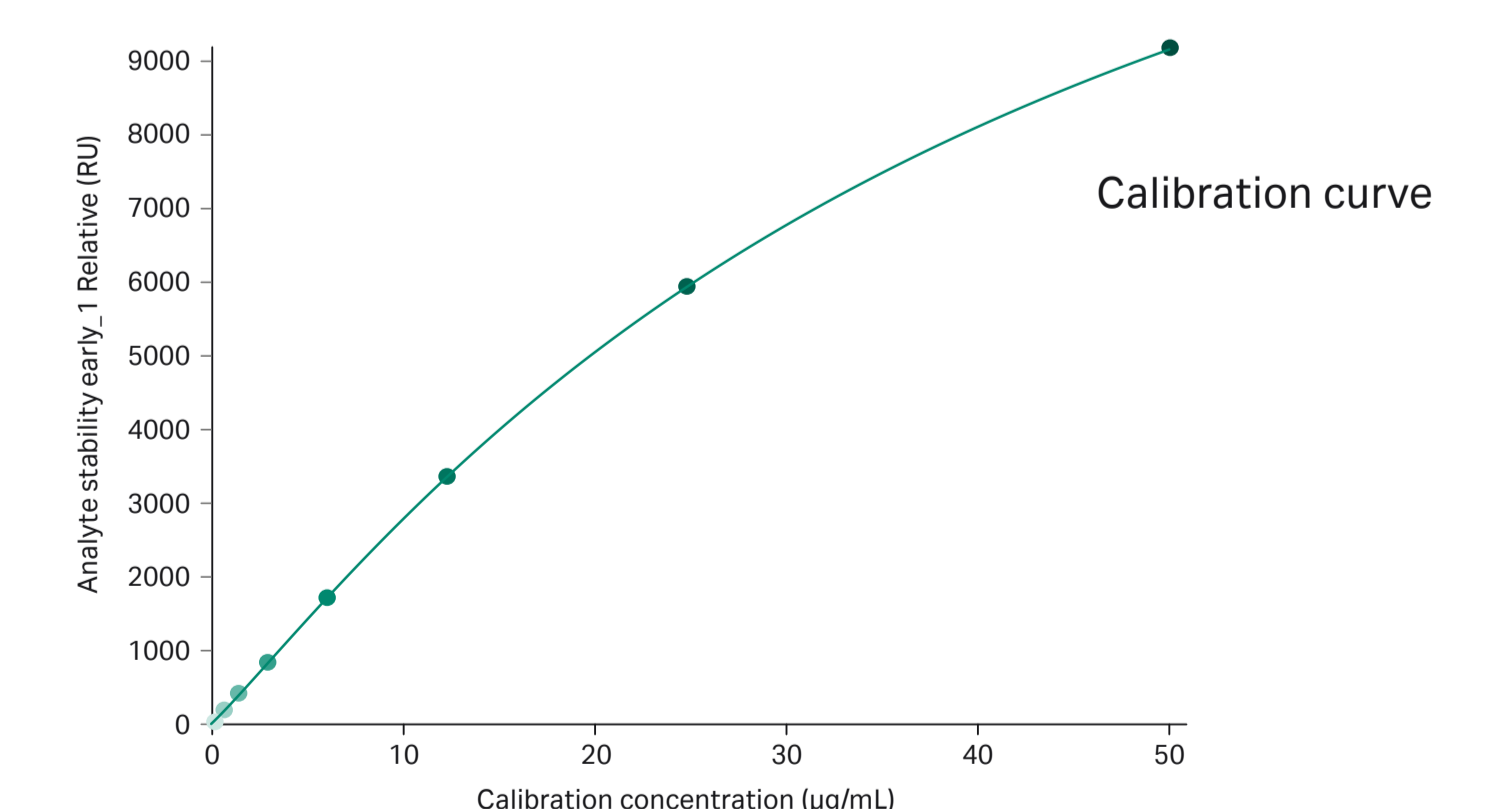
Parallel concentration analysis

- Providing data with very good precision
- Calibration curve across channels, only one cycle needed for calibration curve
- Shorter run time than serial concentration analysis
- Channel normalization accounts for differences in response from the used channels

Experimental setup



Results channel 1–8 (example)



Biacore™ 8K and Biacore™ 8K+ using Biacore™ Insight Evaluation Software offers

- Analysis of binding kinetics and protein concentration in both serial and parallel fashion
- 2D Kinetics™ for kinetic analysis in a single sample cycle without prior knowledge of the characteristics of the interaction, saving valuable assay development time
- Automated and reproducible real-time determination of active concentration providing results in less than half the time compared to ELISA
- Seamless handling and presentation of data from one or more Biacore™ systems with easy access to the evaluation tool by multiple users in the lab