Procedure

Overview of kinetic analysis using Biacore™ systems

Kinetic analysis determines the rate of complex formation and dissociation. Reliable determination of kinetic constants requires careful experimental work, in both preparation of samples and analysis of the interaction.

Single-Cycle Kinetics (SCK)™

Single-Cycle Kinetics (SCK)™ needs less assay development and can be used when it is difficult to find suitable regeneration conditions. In addition, the method increases the chance for slow interactions to approach saturation and reduces the risk for inconsistent surface performance. Single-Cycle Kinetics (SCK)™ however is more sensitive to drift since the long cycle duration makes more stringent demands on stability. A typical sensorgram from a Single-Cycle Kinetics (SCK)™ analyse can be seen in Figure 1.

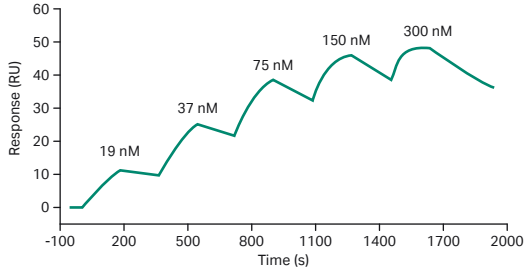

Fig 1. Sensorgram from a typical Single-Cycle Kinetics (SCK)™ analyse with five sample injections from a range of different concentrations (19–300 nm).

Multi-Cycle Kinetics (MCK)™

Multi-Cycle Kinetics (MCK)™ is the classical standard approach. The method is less sensitive to drift but cannot be used when it is difficult to find suitable regeneration conditions. Moreover, slow interactions do not approach saturation with Mult-Cycle Kinetics (MCK)™ and the method is depending on consistent surface performance between cycles. A typical overlay plot of sensorgrams from a Multi-Cycle Kinetics (MCK)™ analyse can be seen in Figure 2.

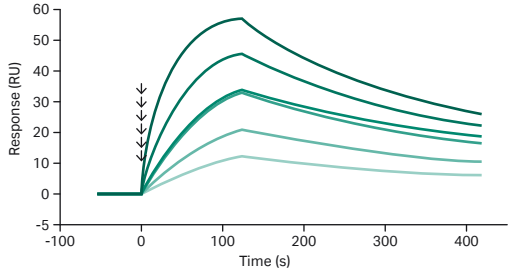

Fig 1. Overlay plot of sensorgrams from a typical Multi-Cycle Kinetics (MCK)™ analyse with five sample injections from a range of different concentrations. Arrows indicate sample injections.

Experimental design

1. Attach an amount of ligand that gives a R_{max} value in the range of 10–30 RU or less.

$$R_L = (MW_L/MW_A) \times (R_{max}/s)$$

R_L (RU) = Response level of attached ligand
R_{max} (RU) = Maximum binding capacity
MW_A (Da) = Molecular weight of analyte
MW_L (Da) = Molecular weight of ligand
s = number of binding sites per ligand

2. Include a reference surface for subtraction of bulk effects. In systems with serial flow cells, place the reference upstream of the active surface.
   - Using a blank surface as reference is often sufficient. If using a capturing approach attach the capturing molecule on the reference surface in the same way as for the active surface.
3. During assay development:
   - Check activity, specificity and non-specific binding.
4. Check for mass transfer limitations and linked reactions, either during assay development or the kinetic experiment itself.
5. Use high flow rates (30 µL/min or higher).
6. Inject a number of analyte concentrations [5–9 for Single-Cycle Kinetics (SCK)™ and 5–8 for Multi-Cycle Kinetics (MCK)™] over both active and reference surface.
   - Use a broad range of concentrations so that the lowest gives a measurable response, while ideally the highest approaches steady-state. Alternatively, the injection time can be prolonged to allow the highest concentration time to approach steady-state.
7. Include at least one zero-concentration cycle using blank samples (running buffer) for all sample injections. In Single-Cycle Kinetics (SCK)™ all injections are made in the same analysis cycles. Include a blank cycle with zero-concentration samples that contains the same number of injections as in the sample cycle. Inject the zero-concentration samples over both active and reference surfaces for double referencing.

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Important considerations

- Avoid use of multivalent molecules as analyte. Avidity effects resulting from binding of one analyte to several ligands may complicate the evaluation.
- Preferably use pure reagents. If the reagents are not pure, bear possible complications in mind (e.g. non-specific binding, heterogeneity).
- Include control samples if possible to monitor the activity of the surface when comparing multiple analysis run over the same surface.

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