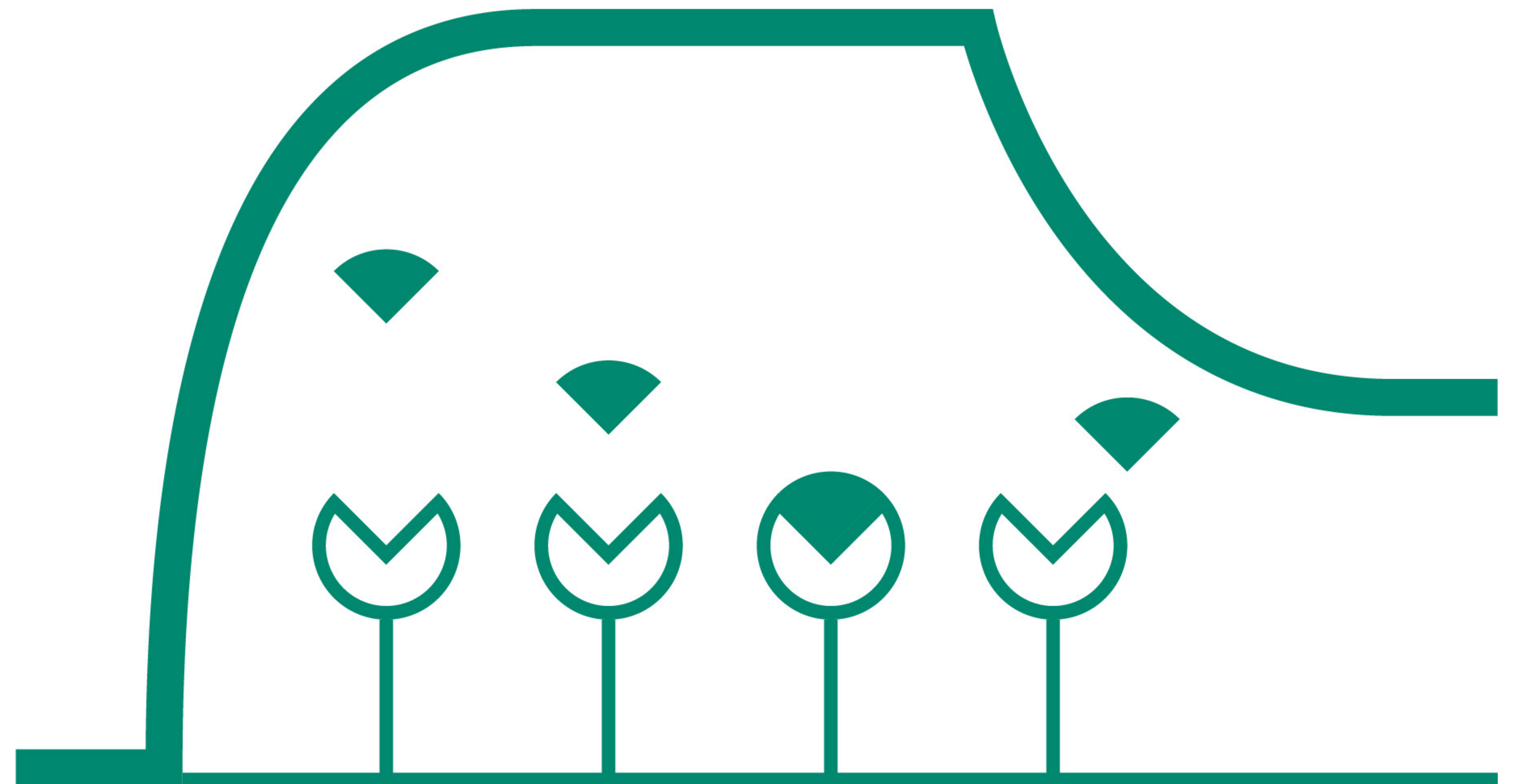
Biacore application guides

Solvent correction: principles and practice



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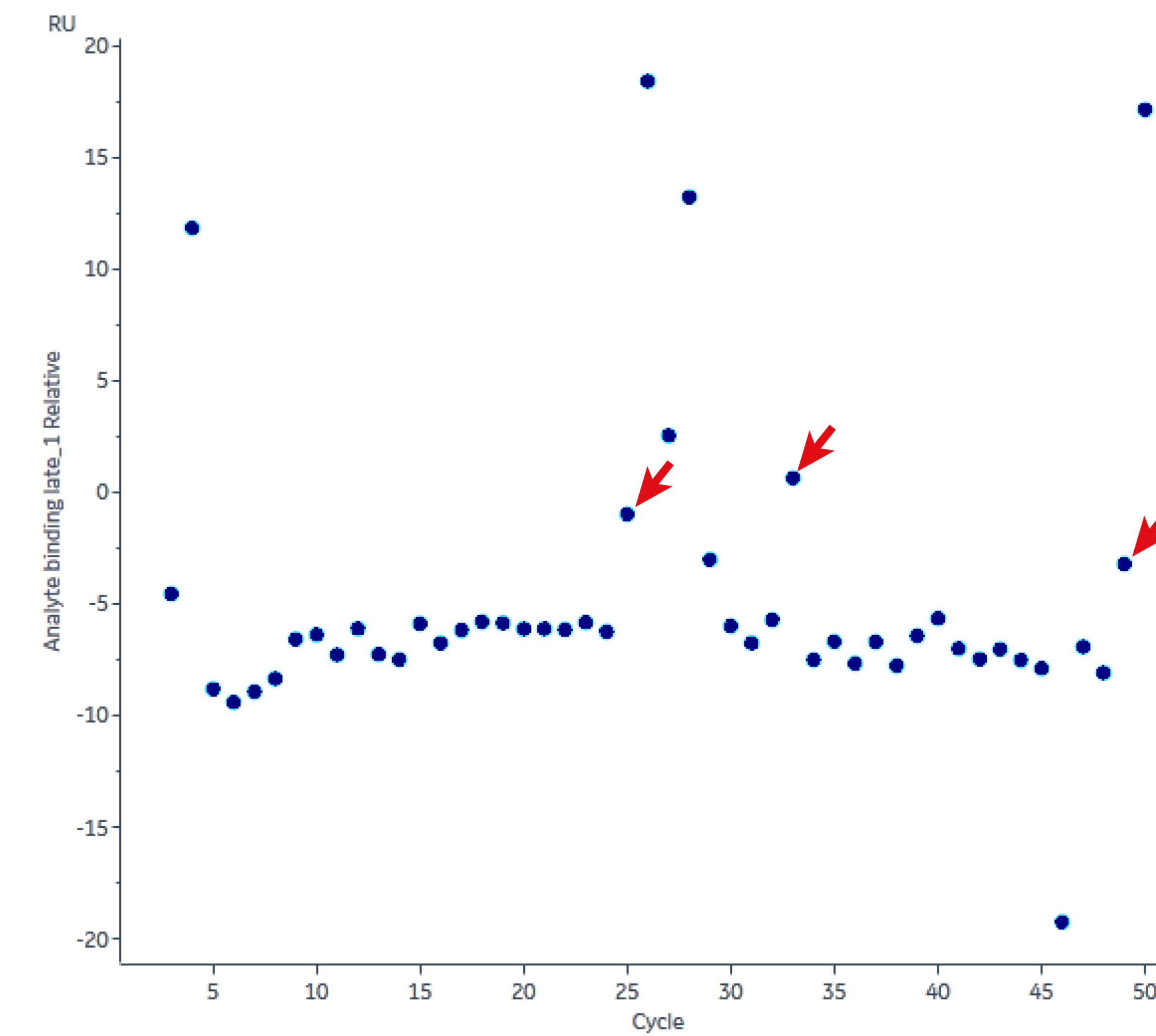
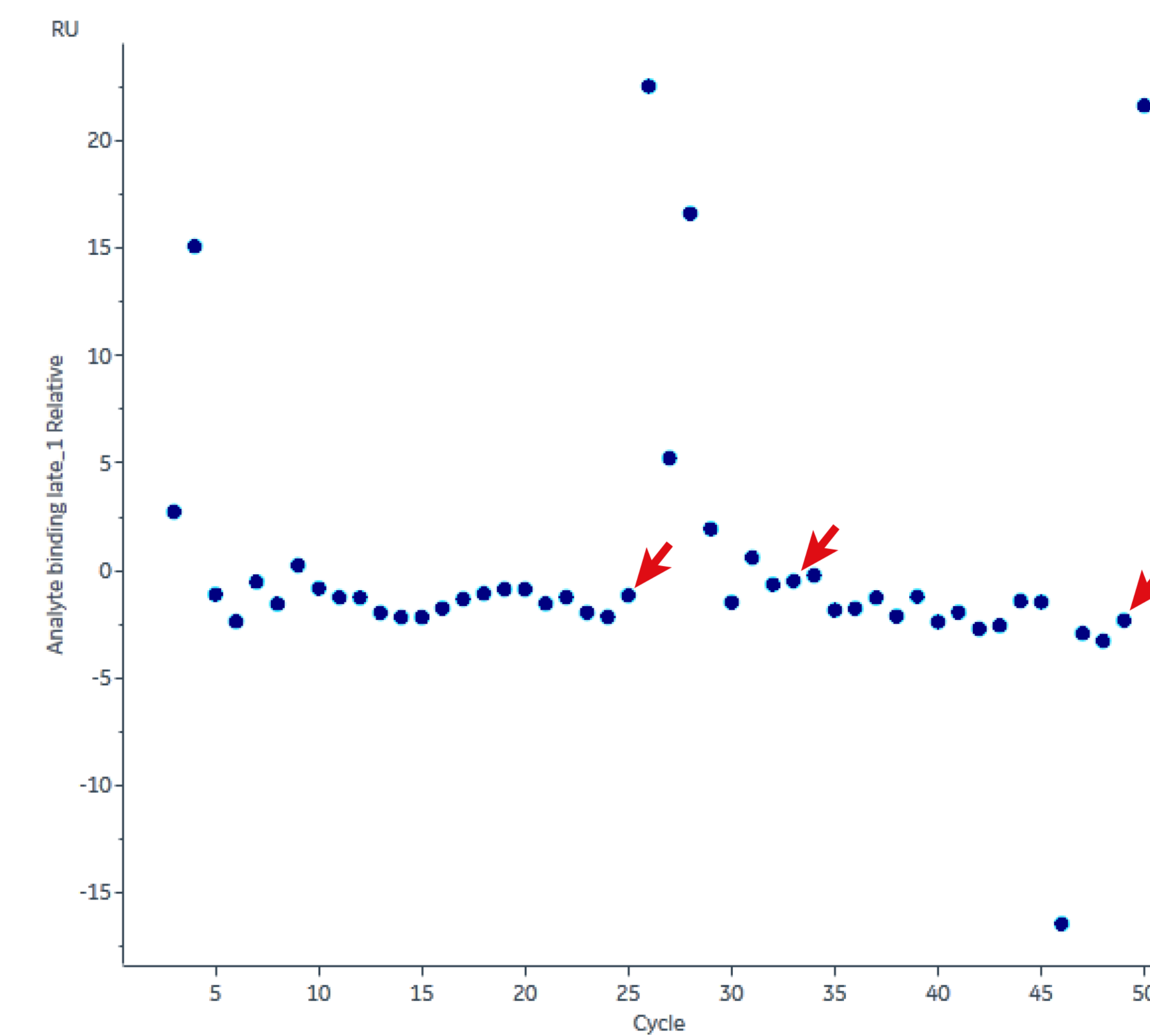
Solvent correction is a technique for correcting responses for variations in the bulk refractive index of the samples. In practice, solvent correction is used when the analytes are small molecules or fragments that require organic solvents, usually dimethyl sulfoxide (DMSO), to maintain solubility. Responses from such analytes are intrinsically low and the refractive index contribution from the solvent is high, so that variations in bulk contribution can introduce significant artefacts. Solvent correction is not relevant to work with macromolecular analytes or aqueous buffers with no high refractive index additives.

The illustration to the right shows the effect that solvent correction can have on the binding response. The example is taken from a fragment screening run on Biacore™ 8K. Arrows indicate some of the samples where correction has a significant effect.

Solvent correction is supported by all Biacore systems that are suitable for work with small molecules.



Before solvent correction.



After solvent correction.

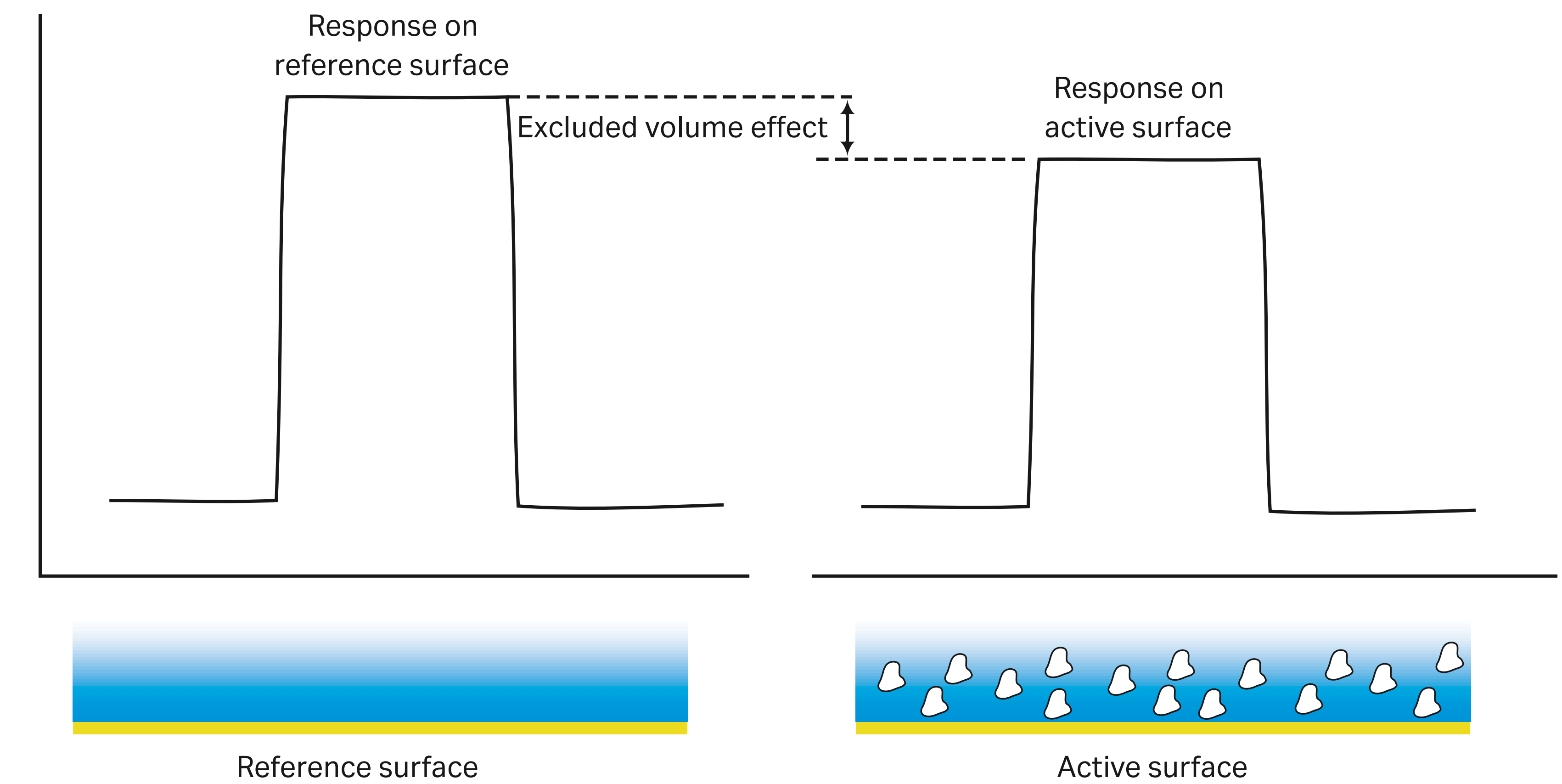
Why is solvent correction necessary?

Background

The need for solvent correction arises when the amount of ligand on the active surface is high compared with the reference, and the bulk refractive index contribution of the solvent is high compared with the expected analyte response. Bulk solution is excluded from the volume occupied by ligand on the active surface, so that bulk contributions on the active and reference surface will be slightly different, introducing a small error in the reference-subtracted response. This is illustrated schematically to the right.

As long as the refractive index of the samples is constant, the error in the reference-subtracted response is also constant and may be ignored for practical purposes. However, if the refractive index of the samples varies, the magnitude of the error will also vary.

Addition of 1% DMSO to buffer gives a bulk response of about 1200 RU, so that small variations in the DMSO content lead to significant variations in the bulk response, in relation to the expected response from low molecular weight samples (which may be as little as 5 to 10 RU). Such variations are difficult to avoid in the preparation of samples such as fragments and drug candidates for screening applications.



Principle of solvent correction

Solvent correction is determined by injecting a series of solutions containing a range of solvent concentrations (above and below the nominal solvent concentration in the running buffer and samples) over the active and reference surfaces. The injections are performed sequentially in a single cycle (illustrated below with 4 solvent concentrations).

A plot of the relative reference-subtracted response on the active surface against the unsubtracted response on the reference surface calibrates the error in reference subtraction against the bulk contribution. This calibration is then used to correct the measured sample responses.

In the schematic illustration to the right, samples that gave a response of 500 RU on the reference surface would be corrected by about -9 RU on the reference-subtracted response.

It is recommended that solvent correction cycles are run at the beginning and end of a run and at regular intervals during the run. In this way, any given sample cycle will lie between two solvent correction cycles. In newer Biacore systems, the correction factor for a given sample cycle is determined by interpolation between the curves from preceding and following correction cycles, to compensate for any drift in the solvent correction factors during the course of the run. Older Biacore systems do not use interpolated values.

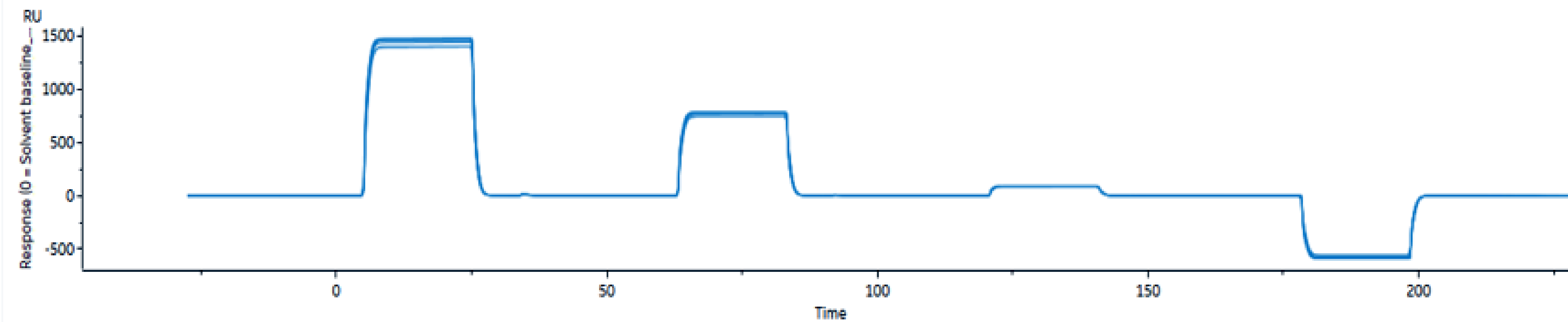
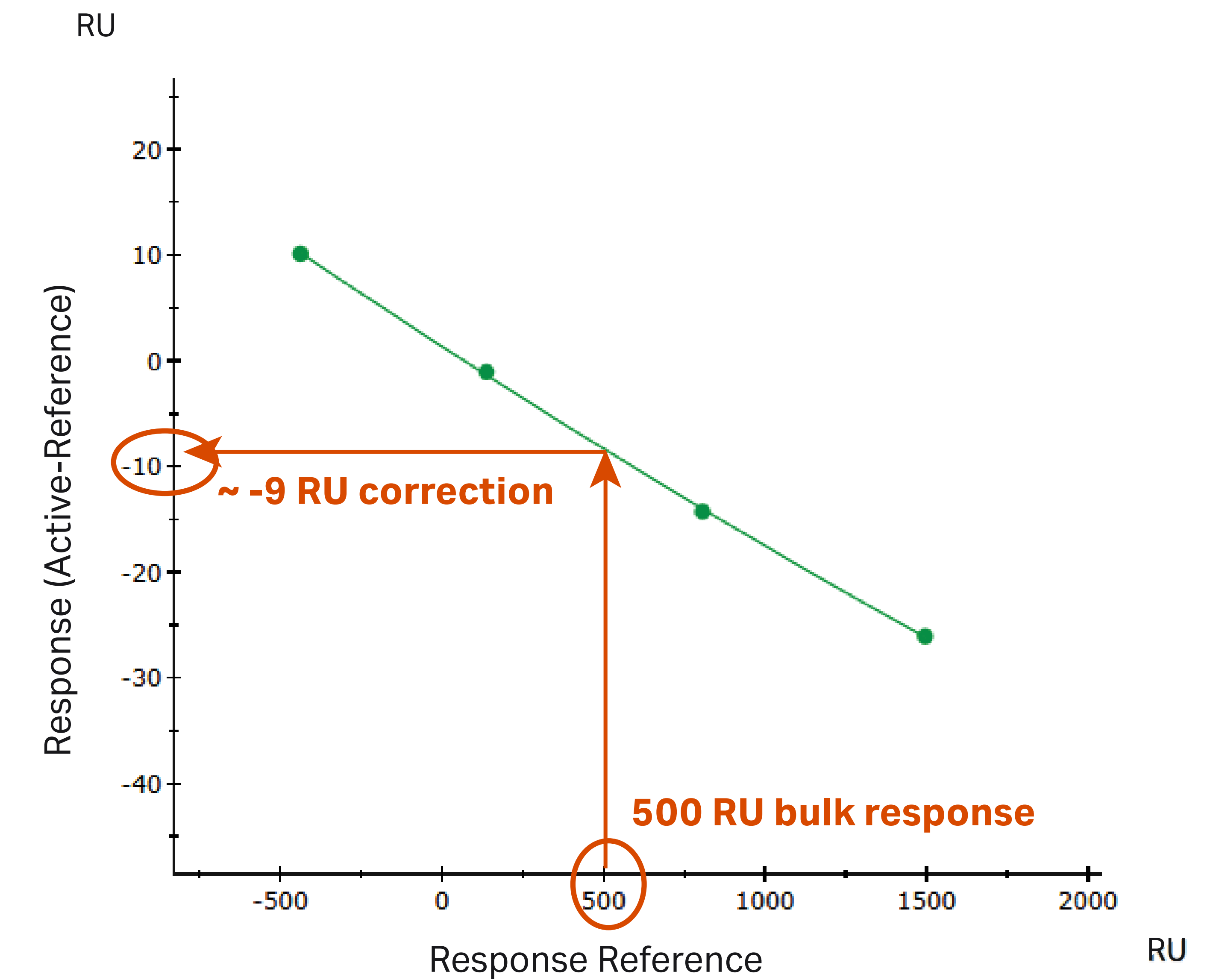


Illustration with 4 solvent concentrations.



Schematic illustration.

Solvent correction in practice

Preparing samples and solvent correction solutions

Because solvent correction compensates for very small variations in DMSO concentration between samples, it is important that solvent correction solutions and analyte samples are prepared using identical dilution protocols. It is not sufficient to simply dilute the solutions to the same nominal DMSO concentration. Protocols for preparing solvent correction solutions are described in separate Laboratory Guidelines.

Note: DMSO is hygroscopic and will absorb water if left exposed to the air. Keep all stock solutions and samples covered as much as possible.

As a general rule, solvent correction samples should cover a range of bulk responses from about -500 to +1500 RU. Solvent correction outside this range may not be reliable.

Solvent correction has become more predictable as Biacore instruments have developed, and the recommended number of solutions differs between different Biacore systems. Laboratory protocols for preparing solvent correction solutions and samples for different systems are available separately from Cytiva.

Including solvent correction in a run

Solvent correction cycles should be run at the start and end of an experiment and repeated at regular intervals during the run. Solvent correction solutions can be pooled provided that the microplate or vial is sealed with a septum, which reseals adequately after penetration by the needle.

Do not pool the solutions in positions covered with microplate foil. The foil does not reseal after needle penetration, and evaporation through the opening will introduce variations in the exact DMSO concentration.

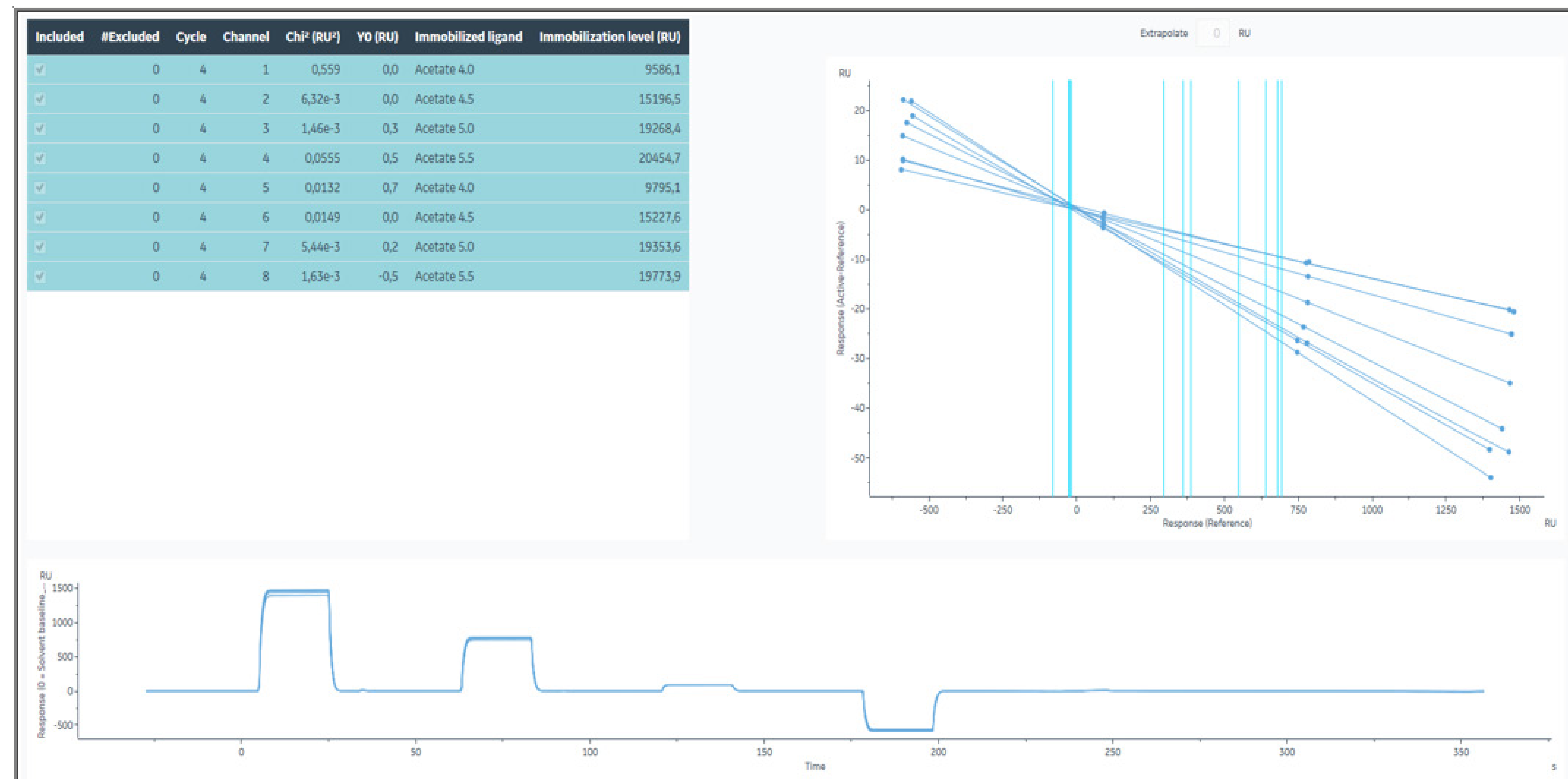
Applying solvent correction

Solvent correction is applied as the first step in evaluation. Once applied, the solvent correction settings cannot be changed. Corrected and uncorrected data are however both available for evaluation in the same session.

Solvent correction presentation

The presentation interface for solvent correction data differs between different Biacore systems, but there are three main components in all systems. The illustration shows an example from Biacore 8K.

Component	Description
Table	Lists solvent correction cycles and essential fitting parameters for the solvent correction curve derived from each cycle. Cycles can be selected and deselected in the table.
Curves	Displays an overlay plot of the solvent correction curves, with the range of sample report points marked. Individual points can be included or excluded.
Sensorgrams	Displays the sensorgrams from the solvent correction cycles. This component may not be displayed by default. Use this display to examine the quality of the sensorgrams.



Included	#Excluded	Cycle	Channel	Chi² (RU²)	Y0 (RU)	Immobilized ligand	Immobilization level (RU)
✓	0	4	1	0,559	0,0	Acetate 4.0	9586,1
✓	0	4	2	6,32e-3	0,0	Acetate 4.5	15196,5
✓	0	4	3	1,46e-3	0,3	Acetate 5.0	19268,4
✓	0	4	4	0,0555	0,5	Acetate 5.5	20454,7
✓	0	4	5	0,0132	0,7	Acetate 4.0	9795,1
✓	0	4	6	0,0149	0,0	Acetate 4.5	15227,6
✓	0	4	7	5,44e-3	0,2	Acetate 5.0	19353,6
✓	0	4	8	1,63e-3	-0,5	Acetate 5.5	19773,9

Solvent correction workflow

Follow the steps below to apply solvent correction. See *Assessing solvent correction quality,* on page 7 for how to assess the solvent correction data.

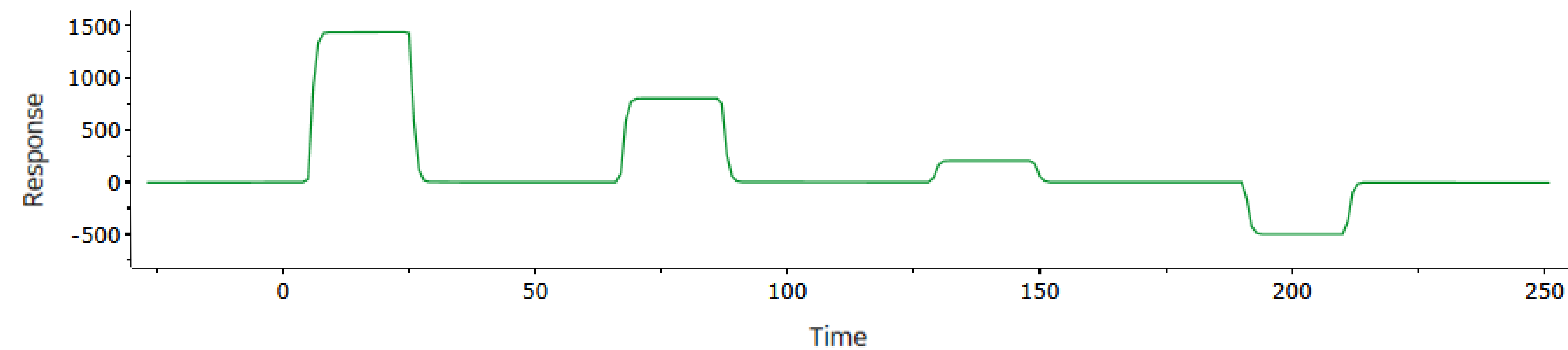
Note: Each solvent correction curve must have at least 3 included points.

Step	Action
1	Select the ***Solvent correction*** function if it is not automatically selected when the results are opened.
2	Examine the solvent correction curves and sensorgrams for quality.
3	Exclude cycles with disturbed sensorgrams or that give bad curves.
4	Exclude individual outlying points.
Note: Some Biacore systems may remove outliers automatically.	
5	Apply or cancel the solvent correction according to your assessment.

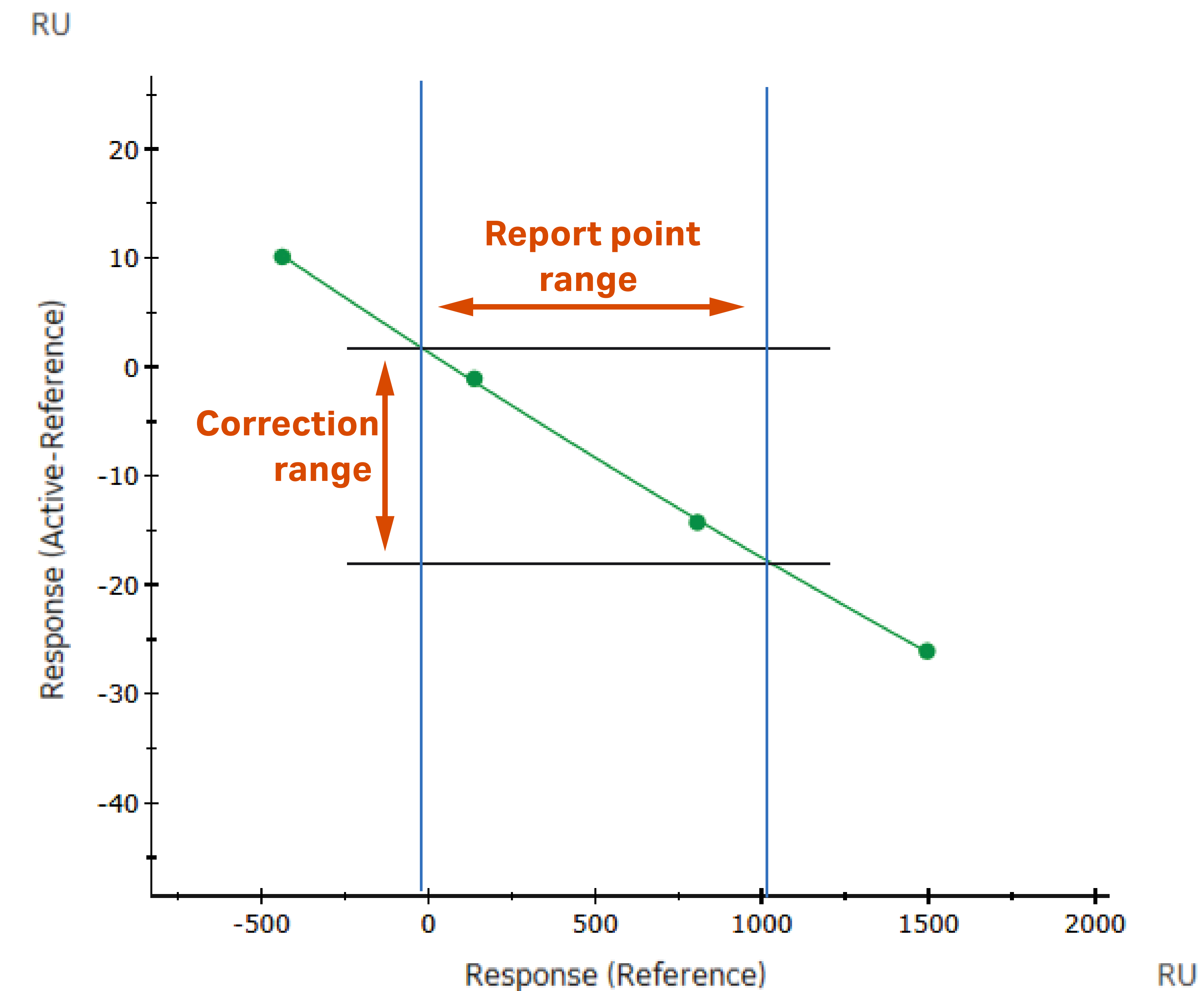
Assessing solvent correction quality

Judge whether to use solvent corrected or uncorrected data in evaluation items according to the guidelines below.

Property	Recommendation
Solvent correction sensorgram quality	Check the appearance of the solvent correction sensorgrams on the active and reference surfaces. Each injection of solvent correction solution should give a "square-wave" response, positive or negative, with rapid transition to and from the baseline and essentially constant response during the injection (as illustrated below). If there are any disturbed injections, exclude the corresponding points from the solvent correction curves.
Solvent correction curve range	The solvent correction curve should normally cover a response range of approximately -500 to +1500 RU on the x-axis (reference flow cell).



Property	Recommendation
Correction range	The y-axis range of the curves between the report point range lines gives an indication of the range of solvent correction for report points, as indicated schematically to the right. Compare this with the range of measured response values to judge the effect of solvent correction on the data. For example, if the response values are of the order of 20 to 30 RU and the solvent correction range is 1 to 2 RU, solvent correction can probably be ignored.
Curve quality	The solvent correction curves for Biacore 8K systems and later should be a reasonably close fit to the experimental points. As a rule of thumb, chi-square values should be below 2 RU. Exclude any isolated outlying points from the curves. Older systems may show greater deviation between the experimental and fitted curves and greater variation in the shape of the curves. **Note:** Solvent correction curves with only 3 data points will fit the experimental data exactly and will have a chi-square value of 0. Beware of using solvent corrected data if the correction curve does not fit the experimental points satisfactorily. Scatter in the correction points can distort the corrected responses.
Data point range	Sensorgrams with points that lie outside the solvent correction range will not be corrected. Such data points are omitted from sensorgram displays of corrected data. Report points that are not corrected are either excluded from plots of corrected data or identified by a curve marker. Solvent correction curves can be extrapolated if necessary to include points outside the default range. Extrapolation is based on the fitted solvent correction curve and should be used with caution. Do not use extrapolation unless the curve fits the experimental correction points closely.



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