# LE-SPR improves the performance of high-end SPR instruments

*Surface Plasmon Resonance (SPR) technology has been continuously developed and refined during the last 25 years. Owing to improvement of optical hardware as well as data evaluation software, modern SPR instruments can quantify signals well below 1 Resonance Unit (RU). However, with the introduction of the latest generation of SPR instruments in recent years, the achievable instrument improvements have most likely reached the end of the road. Even so, in this Application Note we show how Label-Enhanced SPR (LE-SPR), applied to a high-end instrument, contributes a further significant improvement of the performance.*

## Summary

In this Application Note we show how LE-SPR significantly enhances the performance of the high-end Biacore™ T200 instrument. The raw LE-SPR epigrams show considerably less disturbances than the raw conventional sensorgrams. The cleaner raw data reduces the need for complex data manipulation, thereby minimizing the risk of introducing uncontrolled data distortion. The final epigrams are superior in terms of short term noise as well as general disturbance level. Dissociation rate plots show the epigrams to be far more accurate than the conventional sensorgrams.

## Introduction

Today's top-notch SPR instruments are generally regarded to be the Biacore T200/S200 series. The short term baseline noise level of these instruments is specified to an impressively low 0.015-0.03 RU. However, it is often not the short term noise that limits the precision of a SPR experiment. Other disturbances, such as bulk refractive index effects, temperature and pressure effects, and general drift, e.g. due to spurious adsorption or desorption of molecules to/from the chip surface, may amount to much higher levels. Other significant disturbance factors are compression or expansion of the hydrogel matrix on the sensor chip, or mass redistribution due to conformation changes of immobilized proteins. In summary, these other disturbance factors may amount to several RU.

Further, the cited low noise levels require advanced processing of raw data [1]. Such data processing procedures may *per se* introduce distortion to the raw data. Since data processing usually occurs inside the instrument software, concealed from the user, it may be difficult to inspect and judge the level of disturbances present in the raw data and in what way the data processing procedures distort the data.

In this Application Note we show how LE-SPR markedly improves the performance of high-end SPR instruments. LE-SPR is entirely based on specialized reagents and software; hence it provides a simple and low-cost means of improving the performance of high-end, high-cost instruments.

For the demonstration we utilize binding of the small molecule furosemide to immobilized CAII enzyme on a Biacore T200 instrument. The furosemide is studied alone in conventional SPR mode, and in mixtures with a dye-labelled competitor in LE-SPR mode.

## Conventional SPR

Raw data

Figure 1a shows the raw data from the sample flow channel. The sensorgrams contain many peculiarities and the information content is clearly limited.

First step: Reference cell subtraction

Figure 1b shows the data after reference cell subtraction; this procedure is most often a necessity in conventional SPR. Otherwise, inevitable bulk refractive index (RI) differences between sample and running buffer cause very large disturbances to sensorgrams. However, reference cell subtraction is an error-prone procedure. Different amounts of adsorbed or immobilized material in the sample and reference flow

cells, respectively, cause different bulk RI contributions; this is called the excluded volume effect. This effect may to some extent be overcome by a tedious solvent correction procedure [1] – but also the outcome of this procedure is questionable on the sub-RU level.

Reference cell subtraction is also commonly used to compensate for non-specific binding of sample components. However, this method is strictly only applicable when the surface composition is exactly the same in the two cells – which by definition is not the case, since the sample analyte is supposed to bind to the sample cell surface but not to the reference cell surface. Of course, also background sample components will in general bind differently to the two surfaces. In Figure 1b, the remaining level of disturbances after reference cell subtraction amounts to about 2 RU.

Second step: Blank subtraction

After reference cell subtraction, the blank sample should of course only yield a horizontal straight line with superimposed baseline noise. However, from Figure 1b, this is obviously not the case. Diverse, unspecified factors contribute to a blank disturbance level (even excluding the sharp spikes at start and end of injection) of about 1 RU. To further reduce the level of disturbances, blank subtraction is usually performed – combined with reference cell subtraction this is termed double referencing [1].

The blank sensorgram may conveniently be subtracted from the sample sensorgrams – see Figure 1c - but this is another dangerous procedure. What are the unknown disturbance factors in the blank sensorgram? Are the exact same disturbances hidden in the sample sensorgrams, so that they can be eliminated by simple subtraction? Will simple subtraction of the blank yield "correct" sensorgrams? The short answer is: probably not.

In Figure 1c, the baselines are still not reproducible or close to zero, even after blank subtraction. The remaining disturbance is on the order of 2 RU.

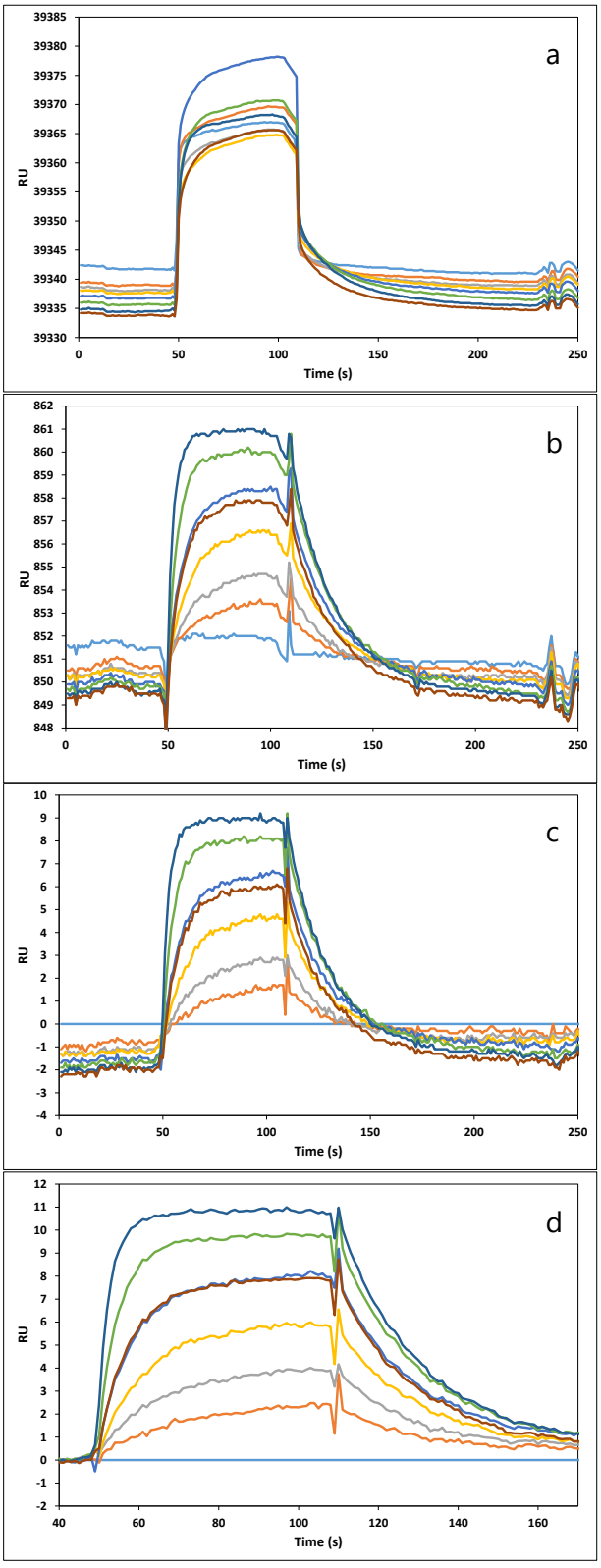


**Figure 1** Conventional sensorgrams. Blank is light blue curve. From top: a) Raw data. b) After reference cell subtraction. c) After blank subtraction. d) After baseline correction.

Third step: baseline correction

The final data manipulation step is usually a simple baseline correction: all sensorgrams are adjusted to the same (zero) value with respect to the initial buffer level as is shown in the close-up in Figure 1d.

However, the mere presence of baseline drift is a symptom of incongruity. What is the cause of the drift? Adsorption on the surface? Insufficient regeneration? Hydrogel matrix or protein conformation changes? Simple baseline correction – without knowing the cause of the drift – may introduce further errors.

The resulting sensorgrams in Figure 1d may superficially look adequate, but from this figure only it's impossible to judge the quality of the raw data and to what degree the data manipulation procedures have introduced errors. The short term noise is clearly visible, but may still be acceptable.

## Label-Enhanced SPR

In contrast, Figure 2 shows enhanced sensorgrams (epigrams) of the same analyte at the same concentration level scaled to the same visual level, but now run in competition with a dye-labelled analogue and evaluated with EpiGrammer™ software.

The epigrams are presented without reference cell subtraction, since the bulk contributions are small and reproducible. Thus, any data distortion contribution from reference cell subtraction is avoided.

The epigrams are also shown without blank subtraction. The blank only yields a straight zero line with negligible baseline noise. Thus, any data distortion due to blank subtraction is also avoided.

Also the inter-cycle baseline drift is essentially zero at this scale. Still, a minute baseline correction is applied to adjust all baselines to exactly the same level.

Even restricting the perspective to short term noise only, the epigrams show far superior performance compared to the conventional sensorgrams. Taking also the larger disturbances (on the order of 2 RU) in the raw sensorgrams into account, which are not present in the epigrams, the signal-to-disturbance ratio is enhanced about 100-fold in the epigrams.

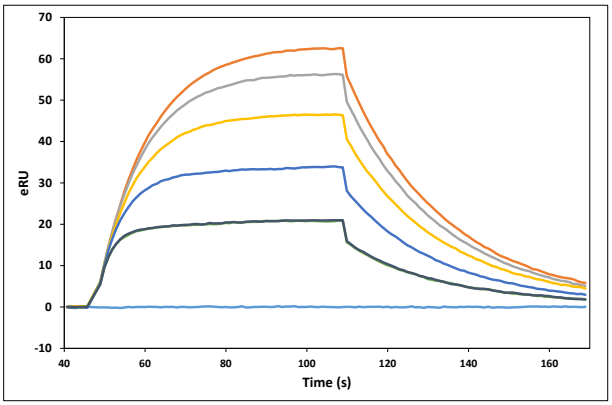


**Figure 2** Enhanced sensorgrams (epigrams). Raw data with slight baseline correction. Blank is light blue curve. Note that the duplicates (lowest curves at 20 eRU) completely overlap.

## Comparing the performance

The signal-to-noise ratio or signal-to-disturbance ratio of sensorgrams are important measures of performance, which can be directly translated to minimum detection level. However, these measures don't give any actual information as to the accuracy or 'correctness' of the data.

One objective way of evaluating the accuracy is examining the dissociation ratio. By focusing on the dissociation phase, any errors or complications attributable to sample injection and association are excluded, and the evaluation model is greatly simplified. For a number of sensorgrams of the same molecule, dissociating with the same rate constant $k_{diss}$ to a zero baseline level, the ratio between the curves should be constant over time.

The dissociation ratio for the conventional sensorgrams and the epigrams is shown in Figure 3. The uppermost curve is the highest concentration, set to a constant value of 1, and the other curves are ratioed against this curve.

For the conventional sensorgrams, there are severe deviations from the ideal case. The lowermost curve, 0.1 µM, increases from about 0.2 to over 0.4 – the deviation is more than 100%. The duplicate curves at 0.8 µM are diverting: one crosses the lower 0.4 µM curve, while the other crosses the higher 1.6 µM curve and even touches the highest 3.2 µM curve. The concentration error is about 4-fold.

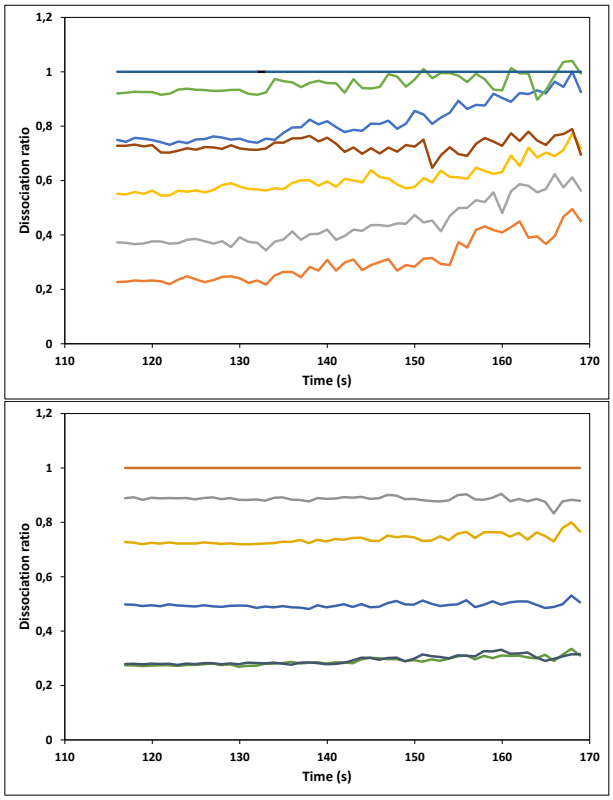


**Figure 3** Dissociation ratio of conventional SPR (top panel) and Label-Enhanced SPR (bottom panel).

For the epigrams, the case is quite different. The dissociation ratio graphs are essentially horizontal straight lines with some low noise at long times (= low signals). The drift is only a few percent, the duplicates overlap well, and there is no crossing of lines.

In summary, this verifies that the epigrams give a true representation of the dissociation process. It is reasonable to assume that the epigrams give a truer picture of the association phase as well.

## Material and Methods

All experiments were performed on a Biacore T200 instrument (GE Healthcare) with a carboxymethyldextran sensor chip. The enzyme carbonic anhydrase II (mw 30 000 Da) was immobilized at a low level of 1200 RU. Running buffer, blank and sample solvent was PBS-P+ with 2% DMSO. Furosemide (Sigma-Aldrich, mw 331 Da) was injected alone at concentrations 0, 0.1, 0.2, 0.4, 2x0.8, 1.6, and 3.2 µM, and at concentrations 0, 0.1, 0.3, 1, and 2x3 µM in mixtures with 5 µM aminoethylbenzylsulfonamide (Sigma-Aldrich) labelled with Episentec™ dye B23 (Episentum). Data was evaluated using T200 Evaluation Software (GE Healthcare) and EpiGrammer 3.0 (Episentum).

## References

1. R. Karlsson "Experimental design", Chap 2. in M.A. Cooper (ed.) "Label-Free Biosensors", Cambridge University Press, New York, 2009.

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## Summary

**LE-SPR significantly enhances the performance of the high-end Biacore™ T200 instrument in terms of short term noise as well as general disturbance level. The cleaner raw data eliminates the need for complex data manipulation procedures, thereby minimizing the risk of distortion of the raw data.**