# Label-Enhanced SPR: A primer on technology & applications

*Surface Plasmon Resonance (SPR) is the physical principle utilized in most optical biosensors. SPR is widely used in biomolecular analysis and is particularly valuable for monitoring binding, affinity, kinetics, and concentration in real time. However, conventional SPR technology still suffers from a number of technical limitations, mainly related to limited sensitivity and limited specificity.*

*The label-enhanced SPR technology of Episentum breaks the limitations of conventional SPR technology, resulting in significantly improved sensitivity and specificity. Here we briefly describe the functional principles behind label-enhanced SPR and how its strongly improved performance translates into benefits in different applications.*

## Introduction

Conventional SPR [1-3] involves determining the refractive index close to the sensor surface by measuring the angular movement of the SPR dip. The binding of molecules to an immobilised compound on the surface causes changes in the refractive index that are monitored in the time domain as a sensorgram. Such refractive index monitoring is hampered by two limitations:

- The differences between the refractive indices of substances are small, resulting in limited analytical sensitivity. This problem is especially pronounced when working with small molecules, low concentrations, weak binders, and low levels of immobilized compounds.
- Any substance binding to the surface causes a change in the refractive index, as do a number of disturbances, e.g. minute changes in the composition of the running buffer. This results in poor analytical specificity and strong influences from non-specific binding and bulk disturbances.

## Label-enhanced SPR technology

Label-enhanced SPR breaks the performance barriers of conventional SPR by introducing two new elements [4,5]:

- Specially developed Episentec™ dye labels that have an extremely high refractive index and a very high absorbance at the wavelength used for SPR measurements.
- EpiGrammer™ software to evaluate the full shape of the SPR dip curve, thereby extracting information about the absorbance as well as the refractive index.

The outstanding optical properties of our Episentec dye labels yield a very strong binding signal, up to 100 times stronger than that obtained using conventional SPR. The measured signal is further refined in EpiGrammer software, where the contribution of refractive index changes is removed. The result is a very strong and pure absorbance signal generated solely by the binding of labelled substances. This signal is essentially free from errors caused by bulk disturbances and non-specific binding of non-labelled substances.

## No special instrument hardware needed

It is important to note that label-enhanced SPR is based solely on the use of specialized dye labels and software, while all measurements are performed on standard, commercial SPR instruments, e.g. Biacore™ instruments. No changes to the standard SPR instrument

hardware are required. Furthermore, the method allows conventional, universal SPR detection to be performed simultaneously and in parallel with label-enhanced detection, effectively converting the instrument into a multimodal reader. This gives the user full flexibility in terms of running label-free and labelled assay formats.

### Epigram or sensorgram?

EpiGrammer software refines the data to produce an enhanced sensorgram or 'epigram'. The epigram resembles a conventional sensorgram, but while a conventional sensorgram displays refractive index changes, the epigram displays a strong, specific measure of absorbance changes close to the sensor surface.

Whereas the unit of conventional sensorgrams is RU (Resonance Units), the unit of enhanced sensorgrams is eRU (enhanced Resonance Units). Since RU and eRU reflect different parameters they cannot be quantitatively compared, but the eRU scale is generally 10-100 times stronger than the RU scale in a corresponding experiment.

### Enhanced sensitivity

The enhanced sensitivity is advantageous when working with low sample concentrations and with small molecules, and enables the monitoring of weaker molecular interactions than is possible with conventional SPR. In addition, lower levels of molecules can be immobilized, which reduces crowding and undesirable cooperative binding effects, and also minimises problems with mass transport. The enhanced sensitivity is also beneficial when proteins with low activity are immobilized on the surface.

### Enhanced specificity

The enhanced specificity reduces the problem of non-specific adsorption of sample compounds, which is highly advantageous when working with crude biochemical samples. Refractive index variations between crude samples are also cancelled out. Furthermore, the extra information obtained when running enhanced dye-labelled SPR and conventional SPR in parallel turns SPR into a significantly more information-rich technique.

### Label-free or label-enhanced? Have the best of both worlds!

There are many established applications where label-free SPR performs satisfactorily, with no need to turn to label-enhanced SPR. However, there is an even greater range of hitherto unapproachable applications where label-enhancement makes SPR analysis a feasible alternative: small molecules, low concentrations, weak interactions, very fast or very slow kinetics, low immobilization levels, low-activity surfaces, etc.

In addition, no information is lost when running label-enhanced SPR: conventional SPR is always run simultaneously, and conventional sensorgrams are recorded in parallel. The information content increases, and any non-labelled reaction step, such as the immobilization step, can be monitored in standard SPR mode.

### … "we cannot use labels that alter the binding properties of our analyte"…

Yes, it's true that labels may alter the binding properties of the labelled molecule. However, the Episentec dye labels are small molecules that come in hydrophobic as well as hydrophilic variants, so the chances are good that you will find a label that suits your particular application. Care has to be taken to validate the use of labels in any specific application, but we have found that there are many applications where the influence of the label on the binding affinity and kinetics is negligible.

## Even better: run a label-free format using label-enhanced SPR

If possible, we usually recommend the use of a competitive assay format [6], where a non-labelled analyte and a labelled analogue compete for the same binding site on the surface. In this format, the labelled analogue only functions as a tool compound or reporter compound. Monitoring tool compound binding gives indirect information about analyte binding, and the interaction between the analyte and the binder on the surface remains totally label-free!

Using the well-established principles of competitive analyses [7-9], the affinity, kinetics, and concentration of the non-labelled analyte can be deduced with high accuracy. And with the added advantage of outstanding sensitivity!

## Know your signal – absolute ranking made simple

SPR is usually described as a mass-sensitive technique. This causes problems, for example, when a number of differently sized molecules are screened for binding. For comparison purposes, the binding signal of every single molecular species has to be calibrated according to its molecular mass [10].

Actually, things are even worse: different molecules have different refractive indices, so the mass-sensitive approach is not very accurate. For example, saturated molecules and substances containing fluorine atoms have a low refractive index, while aromatic molecules and substances containing bromine and iodine atoms have a high refractive index.

In contrast, the absorbance of a specific Episentec dye label is constant and well defined. Running a screening series in competition with a dye-labelled binder yields a constant signal per dye-labelled molecule bound – or not bound because of competition. The epigrams give a quick visual, but still accurate, ranking of affinities irrespective of the size and refractive index of the compounds screened.

## Higher throughput and lower chip consumption

Since label-enhanced SPR is immune to variations in bulk liquid composition, there is no need to run any reference channel or any complicated double-referencing procedure to compensate for, for example, variations in DMSO or glycerol between samples. This is because absorbance, not refractive index, is monitored.

This translates to a simplified experimental procedure and also a more efficient use of sensor chips, lower chip consumption, and higher sample throughput.

## Monitor two binders simultaneously

The EpiGrammer software usually produces a pure, enhanced absorbance sensorgram (epigram) emanating solely from the dye-labelled compound. But EpiGrammer can also be run in the opposite direction: to produce a pure refractive-index sensorgram without contributions from the dye-labelled compound. In this way, the binding of one dye-labelled and one non-labelled compound can be studied simultaneously. This feature is unique to label-enhanced SPR.

Such monitoring of two binders simultaneously may be of great value, for example, when studying allosteric effects or the cooperative binding of two small molecules to two sites on a protein.

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## Summary

The label-enhanced SPR technology of Episentum offers the following benefits:

- Enhanced sensitivity: signal-to-noise ratio increased 100-fold.
- Enhanced specificity: measurement is fully specific with respect to the dye label.
- Compatibility with standard commercial SPR instruments.
- Label-enhanced and label-free SPR simultaneously and in parallel.
- Label-free analysis with outstanding sensitivity in competitive format.
- Constant signal per bound molecule.
- No bulk referencing needed.
- Possibility to monitor two binders simultaneously.



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