From ELISA to Biacore SPR

Principle ELISA

In an enzyme-linked immunosorbent assay (ELISA) a target antigen (analyte) must be either immobilized to a solid surface or bound to an immobilized capture antibody. Immobilization is mediated by hydrophobic interaction or covalent bonds. The target antigen is then complexed with an antibody that is linked to an enzyme (directly or via secondary antibody). The conjugated enzyme activity is determined via incubation with a substrate converted to a product, which can be colorimetrically measured. Depending on the set-up, the assay is a direct, indirect, sandwich, or competitive ELISA (Figure 1).

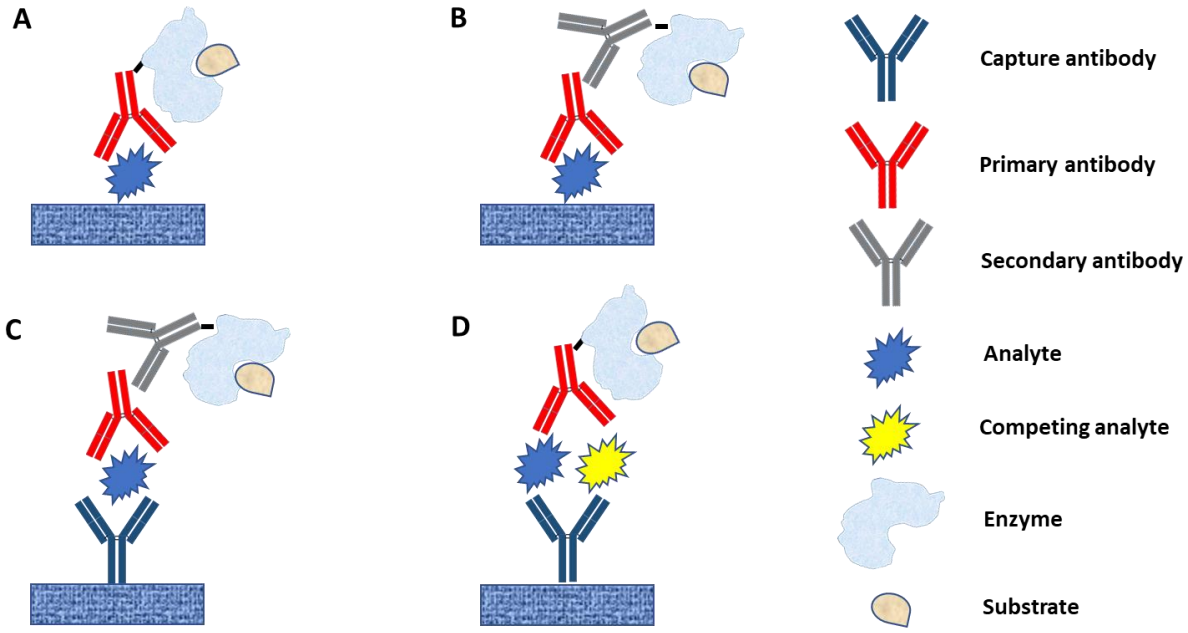

Figure 1. Schematic illustration of ELISA assay formats: (A) direct; (B) indirect; (C) sandwich; (D) competitive.

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Principle Biacore SPR

Biacore surface plasmon resonance (SPR) monitors the interaction between molecules in real time. One interaction partner (ligand) is covalently immobilized or captured onto the surface of a sensor chip, while the second interaction partner (analyte) is injected over the bound ligand. Then binding is monitored. SPR measures the adsorption of material onto planar metal (typically gold or silver) or metal nanoparticles surfaces.

SPR is the resonant oscillation of conduction electrons at the interface between negative and positive permittivity material, which is stimulated by incident light. When the surface plasmon wave interacts with a local particle or irregularity, such as a rough surface, part of the energy can be re-emitted as light, which can be measured.

Depending on the set-up, the Biacore assay is direct, direct with enhancement molecule (antibody), indirect solution competition, or indirect surface competition (Figure 2).

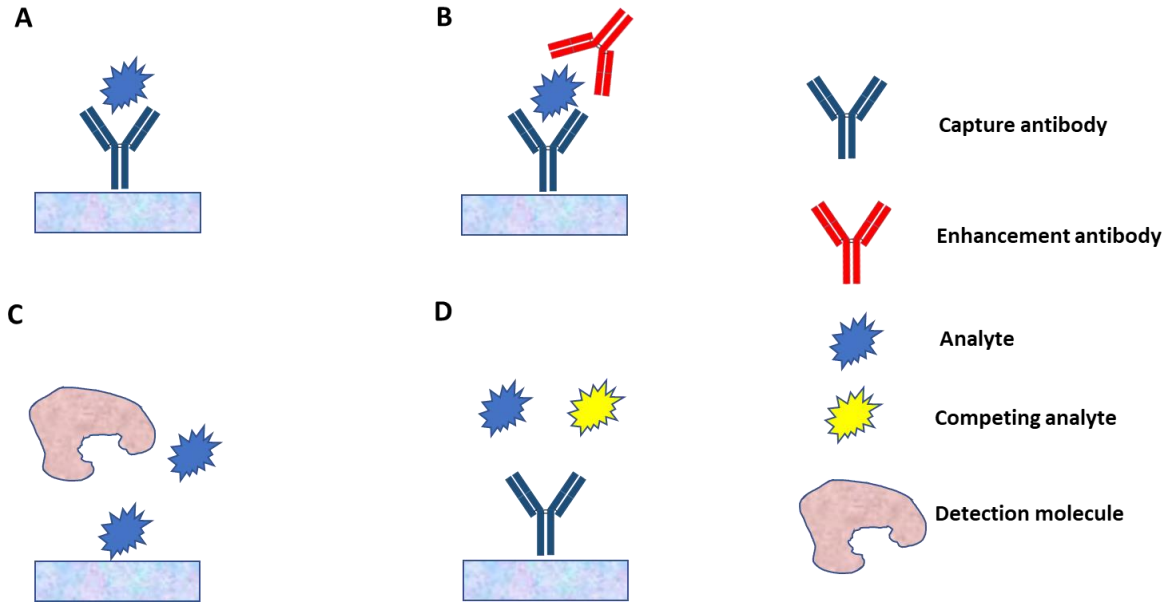

Figure 2. Schematic illustration of Biacore assay formats: (A) direct; (B) direct with enhancement molecule (antibody); (C) indirect solution competition; (D) indirect surface competition.

Similarities between ELISA and Biacore SPR

Though having a completely different instrumental set-up, ELISA and Biacore SPR assays are based on the same analytical principles. Both are carried out on a solid support in which one interaction partner is immobilized and the analyte free in solution. Conversion from ELISA into Biacore SPR may thus only require the transfer of the antibodies and the buffer conditions. Sometimes issues with sensitivity may arise.

Conversion from ELISA to Biacore SPR Assays

Biacore SPR assays increase operational efficiency by enhancing automation and eliminating time-consuming washing steps. The ability to run in sequence several methods and to link the surface preparation to the assay step increases the degree of automation. As can be seen in Figure 3, SPR-based assays can be carried out with about half of the number of steps and time as compared to ELISA (96-well plate).

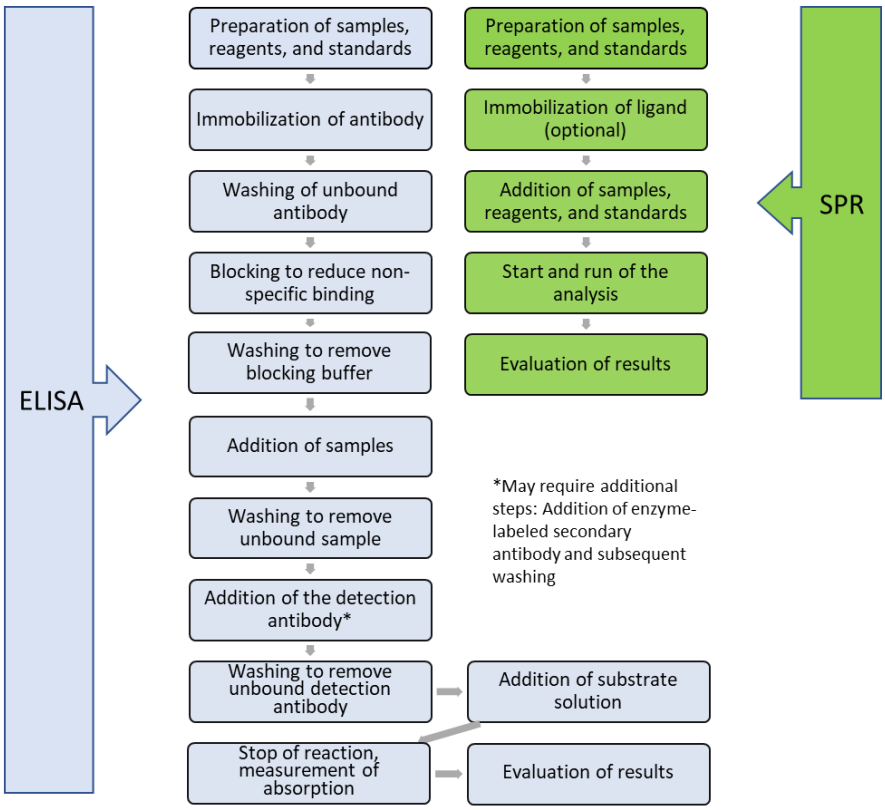

Figure 3. Analysis of 40 samples (in duplicate) take with a typical Biacore SPR assay about half of the number of steps and time as compared to ELISA.

Key Points for Developing a SPR Assay

Selection of Assay Format

Depending on the format, the signal reported in the assay is either directly or inversely proportional to the amount of active analyte bound to the ligand. The conversion of an ELISA to a Biacore SPR assay may involve re-qualification and validation of the conditions and configurations.

Assay sensitivity, range, precision, and throughput are major aspects for selecting a format (Figure 2). Direct binding eliminates the need of a secondary reagent step (A). Direct binding combined with an enhancement molecule such as a polyclonal antibody, enhances sensitivity and specificity (B). If immobilization or regeneration of the ligand is not satisfactory, an indirect solution competition (C) or surface competition (D) assay may also be carried out.

Determination of the Optimal pH for Immobilization of the Ligand

The positively charged ligand is electrostatically coupled to the negatively charged surface of the sensor chip. The optimal pH, which is the highest pH that allows ligand pre-concentration, has to be determined.

Selection of Sensor Surface and Immobilization Procedure

Covalent immobilization of the ligand results into a stable sensor surface that can be utilized repeatedly, provided that the surface can be regenerated between cycles. Amine coupling chemistry through lysine groups is most widely used. Coupling through thiol groups and for ligands containing glycosyl residues, carbohydrazine-based aldehyde coupling are alternative approaches.

Analyte Preparation

Precise sample preparation is required for accurate measurement, particularly when the analyte is dissolved in organic solvent. The purity of the analyte is of utmost importance. Furthermore, even a subtle difference of organic solvent concentration between the sample and running buffer may lead to inaccuracy.

Regeneration

Regeneration is the process of removing bound analyte from the sensor surface after analysis. For analytes with slow dissociation (sensorgram does not go back to the background level at the end of the dissociation step), a regeneration step must be carried out. The optimal regeneration condition should not change the activity of the immobilized ligand, i.e. the responses obtained from binding assays before and after regeneration should be the same.

For low-affinity interactions, regeneration of the surface is not necessary as the analyte often dissociates by itself within seconds to a few minutes. For interactions with higher affinity the number of possible regenerations depends on the nature of the attached ligand. Antibodies can often be regenerated over hundreds of cycles.

Minimizing Nonspecific Binding

Non-analyte components that bind to the surface or to the detecting molecule can also lead to response signals. When the sample can be diluted to a high enough extent, nonspecific binding is often negligible. However, if the analyte concentration is low, high dilutions may not be possible and nonspecific binding can be reduced for example by an extra wash injection step. Optimizing buffer conditions, for example by increasing the salt content is another method to reduce nonspecific binding.

Advantages of Biacore SPR in Comparison to ELISA Assays

- Highly automated setup decreasing inter-and intraassay variability
- No biotinylated antibodies, streptavidin-HRP reagent and other developing reagents (no labeling) nor wash buffer preparation and repetitive washing steps needed
- Lower sample consumption
- Reduced consumables expenses: after regeneration reuse of components possible for additional assays
- High reproducibility and robustness minimizing the need for assay reruns
- Improved quantitation and/or affinity analysis of low affinity/high KD analytes
- Broader selection of assay reagents (low affinity reagents)
- Suitable for more complex assays, such as bispecific antibodies
- Not prone to edge effects or the hook effect as in ELISA assays

Conclusion

ELISA and Biacore SPR assays are based on the same analytical principles and conversion from ELISA into Biacore SPR requires most often only the transfer of the antibodies and the buffer conditions. Biacore SPR assays have higher automation leading not only to a reduction of hands-on-time and reduced operating costs but also to a number of analytical enhancements such as for example higher reproducibility and robustness.