SPRI Horiba Scientific

Label-free molecular interaction analysis

providing information on kinetic processes (association and dissociation), binding affinity, analyte concentration and real-time molecule detection. A large variety of bio-interactions can be monitored, such as antibody/antigen, peptide/antibody, DNA/DNA, antibody/bacteria etc.

Optimization studies for biomolecular interaction analysis (immobilization concentration, pH…) are faster – Saving you time and consumables.

- SPRI Technology

SPRI Applications

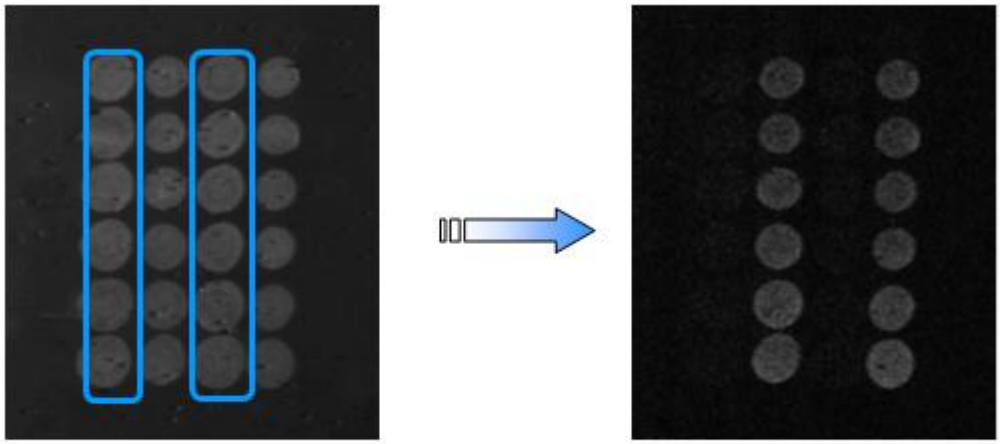
- **Real-time molecule detection**
- **Affinity – Kinetics analysis**
- **Concentration measurements**

Real-time molecule detection

Typical applications include:

- The detection of lymphocytes binding to an antibody chip
- Antibiotic detection for food analysis
- Detection of transgenic DNA involved in gene doping

The SPRi difference image gives a direct Yes/No answer of the binding. When injecting the sample, interacting spots appear as white on the SPRi difference image. The detection of the interaction is label-free, no need to modify your molecules.

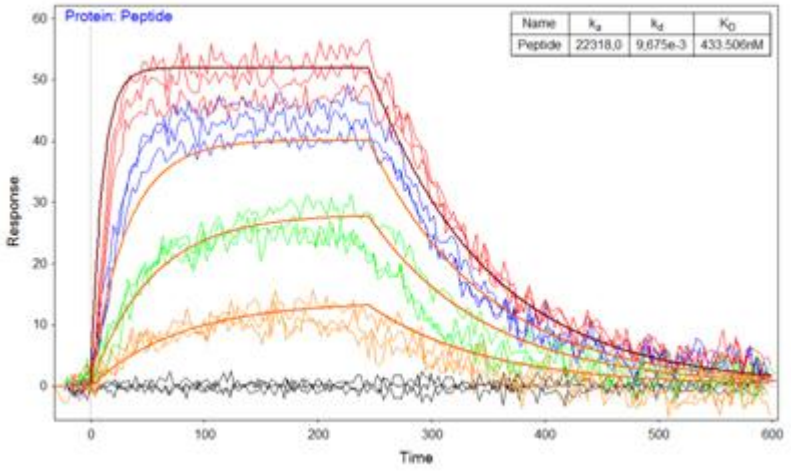


Detection of transgenic DNA involved in gene doping

The strategy exploits SPRi sensing to detect the transgenic event by targeting selected marker sequences, which are present on shuttle vector backbone used to carry out the transfection of human embryonic kidney (HEK) cell lines. Here, we identified DNA sequences belonging to the Cytomegalovirus promoter and the Enhanced Green Fluorescent Protein gene. System development is discussed in terms of probe efficiency and influence of secondary structures on biorecognition reaction on sensor; moreover, optimization of PCR samples pretreatment was carried out to allow hybridization on biosensor, together with an approach to increase SPRi signals by in situ mass enhancement. Real-time PCR was also employed as reference technique for marker sequences detection on human HEK cells.

Affinity – Kinetics analysis

The affinity (KD) of the interaction describes the strength of binding. However, similar affinities can result in different kinetics. The determination of the kinetic parameters from the sensorgrams (association ka and dissociation kd rates) better characterize the molecular interaction.



Affinity – Kinetics analysis

Unlike end-point measurements such as ELISA, SPR imaging allows the monitoring in real-time of the molecular interaction, providing information on the kinetics parameters. The high-throughput of SPR imaging allows the parallel analysis of multiple molecules immobilized on the biochip surface.

Typical applications include :

- Antibody screening/comparison
- Immunoassay development

Studying antibody-antigen binding using SPRI
(Immunoassay development)

For example : The detection and measurement of antibody binding by the type 1 diabetes autoantigen GAD65 represents an example of an antibody-antigen interaction where good structural, mechanistic and immunological data are available. Using SPRi we were able to characterize the kinetics of the interaction in greater detail than ELISA/RIA methods. Furthermore, our data indicate that SPRi is well suited to a multiplexed immunoassay using GAD65 proteins, and may be applicable to other biomarkers.

Concentration measurements

The active concentration of a biomolecule in a solution can be easily obtained using a specific binding partner. Because the measurement is based on the affinity interaction between the binding partners. the measured concentration is not influenced by sample heterogeneity and the measurement is fast (typical assay time: < 10 min).

Typical applications include:

- The detection of anti-bovine IgG in human fluids
- The detection of anti-rabbit IgG and ovalbumine

Experimental Conditions Optimization made EASY with SPRi

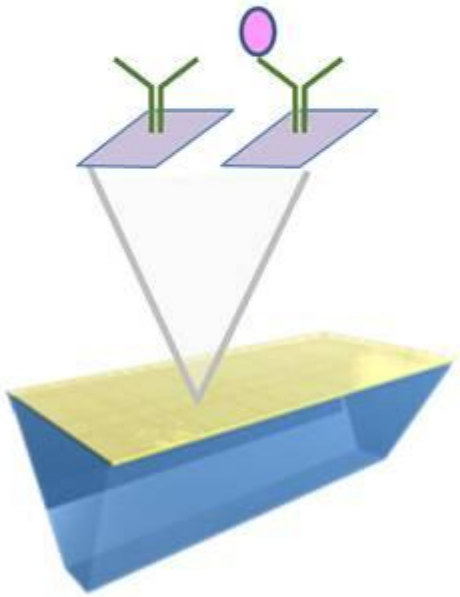
In addition to attaining the full kinetic profile of your interaction, the high-throughput (more than 100up to 768 samples per day) and multiplexing (monitor few 100 of 144 interactions simultaneously) capabilities of HORIBA Scientific SPRi systems allows you to optimize your experimental conditions in short period of time while reducing cost.

What are the applications of SPRi?

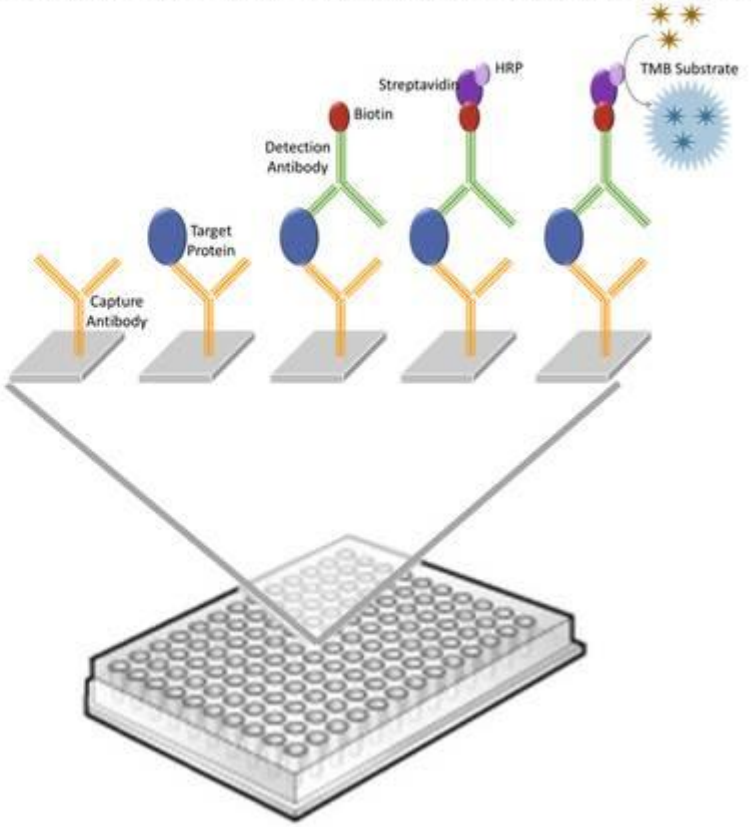
- Protein / protein interaction
- DNA/DNA interactions
- protein/carbohydrates
- Protein / DNA interactions
- RNA/RNA interactions
- Antibody (protein) / bacteria interactions
- Antibody / cell interactions
- Peptide / protein interactions
- Polymer / polymer interactions...

SPRi a Complimentary Technique to ELISA

SPRi



ELISA



SPRi is an alternative technique to traditional immunobased assay like ELISA

Kinetic Information:

	K_a (on rate)	K_d (off) rate	K_D (Equilibrium Dissociation Constant)
ELISA	NO	NO	YES
SPRi	YES	YES	YES

CONCENTRATION ANALYSIS – ELISA vs. SPR

ELISA

- Antigen immobilization (standards + unknowns)

Wash

- Blocking

Wash

- Incubation with primary antibody

Wash

- Incubation with secondary antibody

Wash

- Incubate with enzyme-specific substrate

Wash

- Quantify result with optical plate reader
- Generate calibration curves
- Evaluate unknowns

Disadvantages

- Multiple reagents
- End-point assay
- Plate-based denaturation
- Plate-based epitope inaccessibility
- Weak affinity antibodies are washedaway

CONCENTRATION ANALYSIS – ELISA vs. SPR

SPR

- Antibody immobilization
- Flow standard + unknownantigen using 4x1 mode
- BiOptix concentrationanalysis software processesthe data

Advantages:

- No washing
- No secondary reagents
- Real-time data
- Set up and walk away!

Throughput:

- In one overnight run, one can process
- g110 samples (or 55 in duplicate) and g8 replicates of a 7 point calibration curve

ANTIBODY SCREENING – ELISA vs. SPR

ELISA

- Antigen immobilization (standards + unknowns)

Wash

- Blocking

Wash

- Incubation with screening antibodies

Wash

- Incubation with secondary antibody

Wash

- Incubate with enzyme-specific substrate

Wash

- Quantify result with optical plate reader

Time Required:

- ~5 to 30 hours

Disadvantages

- Time consuming
- End-point assay
- Plate-based denaturation
- Plate-based epitope inaccessibility
- Weak affinity antibodies are washed away

ANTIBODY SCREENING – ELISA vs. SPR

SPR

- Antibody capture
- Flow antigen
- Regenerate surface
- Analyze with easy-to-use,intuitive software:
- Time Required:
- •30 mins per 2 mAbs

Advantages

- No washing - set up and walk away
- Real-time data
- No long wait steps
- ***Get kinetics and affinity as well!***

Throughput:

- Screen 96 samples in one day