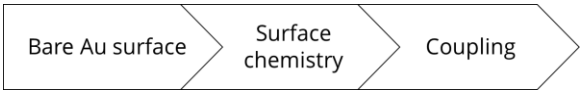
SPR sensor surface functionalization for biomolecular interaction investigation with P4SPR

Study of binding with SPR

The P4SPR is based on the surface plasmon resonance (SPR) phenomenon which allows the study of label-free interactions between a surface capture molecule and a target molecule in real time. This requires that a capture molecule is immobilized on the surface of the SPR sensor chip with the target molecule flown through over the surface (Figure 1). The capture and target molecules can range from small to large biomolecules, including nucleic acids (e.g. DNA) and peptides (e.g. proteins). Binding of a capture molecule with its target molecule can generate a specific SPR response, which translates into refractive index changes near the sensor surface. For more information on P4SPR basic functions, refer to Technical note – Key concepts of SPR and SPR with Affinité Instruments.

Figures 1 – Key elements of an SPR biosensor.

Overview of functionalizing an SPR sensor



Figures 2 – Steps to functionalize the SPR sensor with a biomolecular element of recognition.

There is a variety of strategies to functionalize an SPR sensor depending on what the capture molecule (element of recognition). While antibodies and peptides are the most prevalent capture molecules in SPR, biosensors with other biomolecules such as DNA/RNA, lipids and carbohydrates are also employed. Here, we focus on protein as capture elements first. Most strategies to functionalize sensors with proteins can be broken down into three steps to build a specific biosensor: bare Au surface, surface chemistry, and coupling (Figure 2). The first step is to have a metallic sensor, Au in this case, as pure as possible. Then, a 2D layer such as a self-assembled monolayer (SAM) or a 3D layer like dextran or PEG is coated on the sensor surface typically using the strong Au-S bond. And finally, the capture molecule (e.g., an antibody) is coupled to the surface chemistry either covalently (e.g., EDC/NHS coupling) or via strong binding (e.g., Ni-NTA and streptavidin/biotin).

Why surface functionalization is important

When considering SPR, selecting the right surface chemistry is key to properly immobilize the capture molecule, retain a high activity, and get SPR data specific to the studied biomolecular interaction.

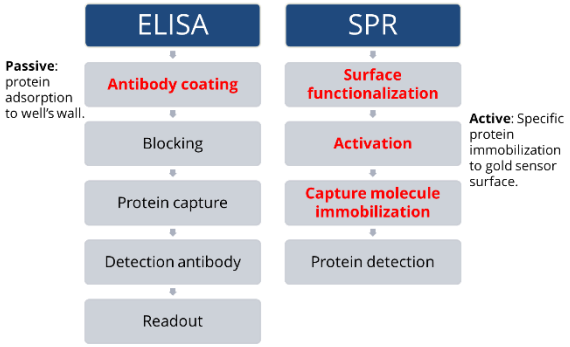


Figure 3 – Difference in functionalization steps in ELISA vs SPR.

Functionalization of SPR sensors can be compared to ELISA (Figure 3). ELISA and SPR involve a layer-by-layer approach that includes anchoring a capture molecule on the sensor or well wall and capturing a target molecule. In ELISA, the capture molecule is an antibody, and the antibody coating step is a passive process as antibodies adsorb to the well's wall. However, in SPR, immobilizing a capture molecule (antibody, peptide, DNA, enzyme, etc.) is an active process requiring a careful selection of linker, activation reagents and immobilization conditions (e.g., buffer, time, pH). The increased complexity in the functionalization of SPR sensors allow for more strategy options to play with this process and optimize the interactions with the target molecule.

Role of surface chemistry in SPR

Surface functionalization is essentially the process of chemically coating the surface with self-assembled monolayers (SAM) or linker molecules to further facilitate the immobilization of the capture molecule. A reliable surface chemistry is crucial in ensuring the specificity of the interaction of the capture and target molecule at the surface. Diverse chemistries for functionalizing the sensor surface such as SAM, hydrogel, and PEG, have been previously explored for SPR analysis.

The subsequent coupling or immobilization of the capture molecule can occur through covalent or non-covalent interactions of the capture molecule with the SAM or linker molecules. The binding of the target molecule with the capture molecule is then evaluated. The functionalized sensor chip plays a critical role when developing or optimizing a SPR assay, as selection of the appropriate surface chemistry, as well as immobilization conditions, will contribute to the quality of the data. This is due to the fact that the quantity and activity of the capture molecule on the surface will affect the binding response as well as sensitivity of the method. Therefore, sensor chips of consistently good quality and low cost would be favorable.

The objective of this technical note is to provide background knowledge on SPR sensor chip design as well as discuss the importance of the sensor chip surface functionalization and coupling approaches for SPR analysis in various applications.

P4SPR sensor chip design

The sensor chip used with P4SPR systems is made with a glass prism coated with a thin layer of gold. By inserting the sensor chip into the P4SPR cavity, it creates the optimal condition to generate the SPR signal as shown in Figure 4. The sensor surface can then be modified with different surface chemistries available for the immobilization of a variety of capture molecules.

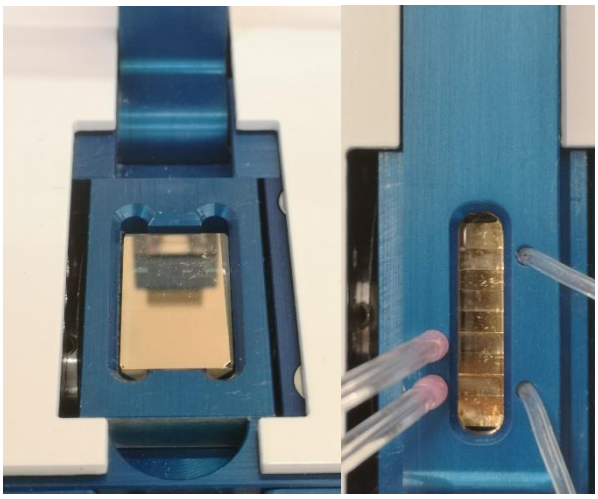


Figure 4 –P4SPR Au sensor in open (left) and closed (right) device.

There are 3 essential features of the sensor chips:

1. **Glass chip:** Visually the glass chip is a miniature dove prism with the longer edges available for optical path and the shorter edges are convenient for easy handling of the sensor chip. The uncoated glass chip is available for user who wishes to prepare their own metallic surfaces for plasmonic material studies, nano- or micro patterned surfaces, other metallic composition as well as surface material studies.
2. **Metallic thin film:** The thin layer of metallic thin film is generated by a consistent process of metal deposition evaporation, which ensures the reproducibility of the sensor chip from batch to batch. A certificate of analysis for the thickness of the thin layer is provided by the manufacturer to ensure that the fabricated chip is of the highest quality. There are other possibilities of metallic thin film with other compositions, thickness or patterns.
3. **Au sensors + surface functionalization:** The specificity is determined by the chemical properties of the linker molecules on the surface. Affinité Instruments technology ensures a consistent surface coating with minimal non-specific interations caused by disorientation of the capture molecules as well as pinhole defects often seen with nanoparticle-based surfaces. More details on

each type of Au sensors are given below, as well as a few examples of experimental needs that can be addressed through a versatile selection of Affinité Instruments' sensor chips (Table 1).

Table 1 – Recommendations for application of P4SPR sensors.

Au Sensors	Recommended for
Au sensors + metal ion-NTA	chelation of recombinant proteins
Au sensors + Affinicoat	covalent coupling and analysis in complex matrix (serum, cell lysate)
Au sensors + 16-MHA	covalent coupling and hydrophilic (proteins, DNA/RNA) and hydrophobic capture molecules (lipids and membrane-associated molecules)
Au sensors + streptavidin	non-covalent high affinity binding of biotinylated capture molecules (DNA, oligomers, aptamers, proteins, carbohydrates and lipid)
Au sensors	thiolated capture molecules (DNA/RNA, protein) and novel SAM material, biofouling molecules, molecular imprinted polymers

Surface functions of various Au sensors

**Please note that all the listed surface chemistry below are offered by Affinité*

Au sensors + Metal ion-NTA

This type of sensor has linker molecules coupled with nitrilotriacetic acid (NTA) chelating to a metal ion (Co2+ or Ni2+), providing means to attach polyhistidine-tagged (His-tag) proteins as for capturing target molecule. This is commonly used for applications involving recombinant proteins. The surface can be re-generated with EDTA, which removes the metal ion in order to release the chelated protein. This approach is simple and convenient for His-tag proteins that are sufficiently stable in comparison to covalent coupling which requires multiple steps before the capture molecule can be immobilized on the sensor surface.

Au sensors + Affinicoat ™

Affinité Instruments' proprietary peptide-modified Afficoat ™ sensor surface greatly reduces non-specific interaction and maintains the activity or function of the immobilized biomolecule. Given the complex nature of biological samples, non-specific interaction is of particular concern. Afficoat ™ is a zwitterionic peptide with high hydrophilicity, which was previously demonstrated to favor resistance to non-specific adsorption. The α-helical conformation found in Afficoat ™ is proven to drive unwanted biofouling away from the surface. By rendering non-specific interaction to a minimum, Afficoat ™ provides increased sensitivity and low background noise for the analysis.

Au-sensors + 16-MHA

The 16-MHA coated Au-sensor presents a hydrophobic and uncharged surface consisting of long-chain alkanethiol molecules attached directly to the gold film. This provide a more hydrophobic surface which may be useful in reduced non-specific interaction of highly charged biomolecules. This sensor chip is also designed for work with lipids and membrane-associated molecules.

Au-sensors + Streptavidin

Streptavidin-coated Au-sensor carries a surface with linker molecules to which streptavidin has been covalently attached. Streptavidin is a tetrameric protein with a high affinity for biotin (dissociation equilibrium constant $K_D \approx 10^{-15}$ M), so that the surface is prepared for high affinity non-covalent binding of biotinylated target molecules. The interaction of biotin with streptavidin is so strong that the biotinylated capture molecule cannot normally be removed to regenerate the streptavidin surface. This provides an alternative for capture molecules that are difficult to immobilize covalently.

Au sensors

Bare gold surfaces can be functionalized with self-assembled monolayers and biomolecules using electrostatic forces or gold and thiol-containing proteins, DNA and RNA formation. This also allows

flexibility for the users to investigate their own surface chemistry.

Common coupling approaches

The coupling approaches should be chosen to allow sufficient immobilization and preserved activity of the capture molecule on the surface. The capture molecule is immobilized on the functionalized sensor chip during the experiment via different coupling procedures which depend on the characteristics of the capture molecules. Table 2 presents different type of capture molecules and suggested coupling methods. Development of the coupling procedure may require investigation into several approaches such as direct covalent coupling, His-tag chelation and streptavidin-biotin binding (Figure 5). The main objective is to have a considerable density of the capture molecules in its active form for further binding interactions with the target molecule.

The first coupling procedure, and most common, is the direct covalent coupling of the capture molecules to the linker molecules. The capture molecule is directly coupled using EDC/NHS chemistry to form a covalent amide bond with the thiolated linker molecule. The covalent bond is formed during the SPR run which requires a few steps to prepare the linker molecule to couple covalently when the capture molecules have flown through. Secondly, the thiolated linker molecule forms a covalent bond with nitrilotracetic acid binding to metal ions. The Histidine-tagged protein is then chelated to the metal ions. In this case, no additional steps are required. The coupling occurs as soon as the His-tag protein solution is flown over the sensor surface. Lastly, the thiolated linker molecules is chemically linked to streptavidin. Similarly to His-tag protein chelation, biotinylated capture molecules bind to streptavidin as soon as the solution is flown through the surface.

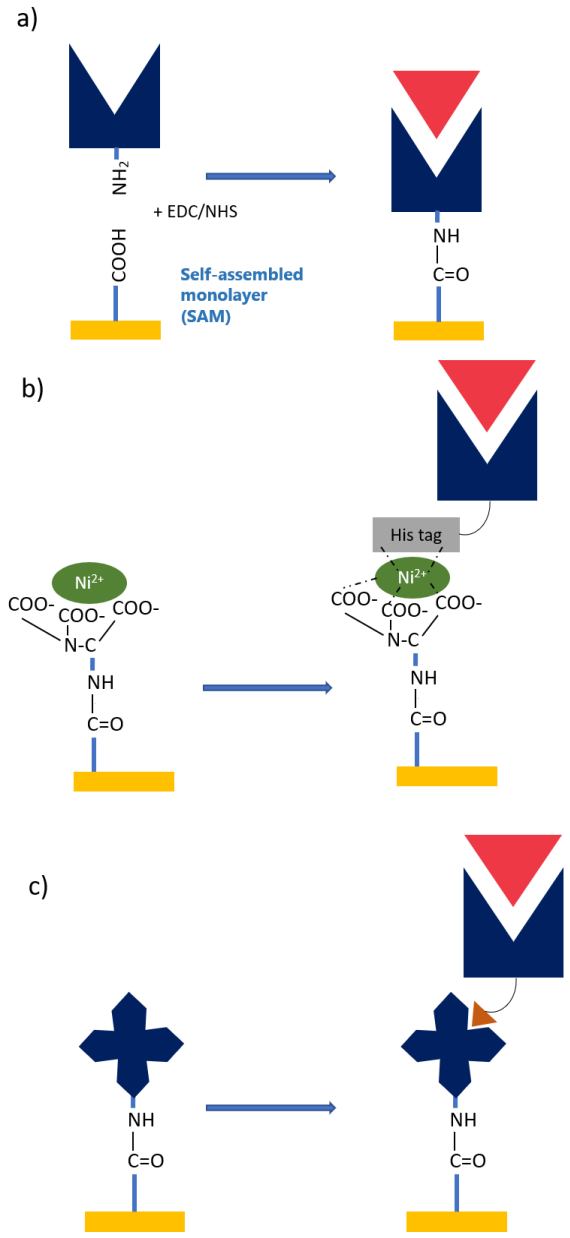


Figure 5 –Coupling methods a) covalent coupling b) His-tag chelation c) streptavidin-biotin binding .

Examples of interaction studies:

The different choices of surface coating have been used in SPR analysis for the study of biomolecular interactions. Table 3 provides examples of interactions that were previously investigated using the SPR technology. Affinité Instruments offers reliable and high quality sensors to better characterize these

interactions. In addition to applications to study biomolecular interactions, the rich sensor variety also allows flexibility in accommodating uses in other application areas such as assay development, clinical analysis, and environmental as well as material science.

Conclusion

In this technical note, an introduction to surface functionalization for SPR analysis as well as different types of P4PSR sensors were presented. The variations in functionalized P4SPR sensors, namely in surface chemistry, will dictate the analytical performance and can be engineered for optimization for an application of interest. Coupling approaches have also been described to accommodate the immobilization of diverse capture molecules.

About Affinité Instruments

Established in 2015 as a spin-off of the Université de Montréal, Affinité instruments' foundation is built on deep knowledge accrued throughout more than a decade of research in SPR. The commercialization of promising innovations is spearheaded by a leadership team experienced in business, science and engineering.

Table 2 – Suggested coupling methods for various capture molecules

	Coupling approches		
Capture molecules/biomolecules	direct coupling	His-tag chelation	streptavidin -biotin binding
Antibody	✓	✓	✓
Antigen	✓	✓	✓
antibody fragment	✓	✓	✓
other proteins	✓	✓	✓
Peptides	✓		
small molecule	✓		✓
DNA/RNA	✓		✓
Oligonucleotides	✓		✓
Aptamers	✓		✓
Carbohydrates	✓		✓
Nanoparticles	✓		

Table 3 – Summary of biomolecular interactions

	Target Molecules										
Capture molecules	antibody	antigen	antibody fragments	other proteins	peptides	small molecule	DNA/RNA	oligonucleotides	aptamers	carbohydrates	cells
antibody		✓		✓	✓	✓					
antigen	✓		✓	✓							
antibody fragments		✓	✓	✓	✓	✓					
other proteins	✓	✓	✓	✓	✓	✓	✓				✓
peptides	✓	✓	✓	✓							
small molecules	✓	✓	✓	✓					✓	✓	
DNA/RNA				✓			✓				
oligonucleotides								✓	✓		
aptamers	✓	✓	✓	✓	✓	✓					
carbohydrates				✓							
cells				✓							
nanoparticles				✓	✓	✓	✓	✓	✓		