LECTURE-29

Surface Plasmon Resonance

Handout

PREAMBLE

Surface plasmon resonance (SPR) is very promising and widely used label-free technique for studying molecular interactions. This label-free technique measures alterations in refractive index of dielectric layer adjacent to the metal film owing to the adsorption or desorption of molecules on surface in real-time mode. SPR is suitable for label-free analysis of several biomolecular interactions in a fast and high-throughput manner (Ray et al., 2010). Since, SPR-based biosensors are capable of detecting extremely minute amounts of target analytes with high selectivity; those are also very promising for discovery of disease biomarkers (Reddy et al., 2012). An introduction of SPR, its working principle, versatile applications and advantages and disadvantages will be discussed in this lecture.

OUTLINE OF LECTURE

I. Basic working principle of Surface Plasmon Resonance

II. Different applications of SPR-based biosensors

(a) Study of biomolecular interactions

(b) Detection of cancer biomarkers

(c) Detection of biomarkers for other human diseases

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(d) Screening of inhibitors of tumor targets

III. Advantages and disadvantages

IV. Conclusions

I. BASIC WORKING PRINCIPLE OF SURFACE PLASMON RESONANCE

SPR happens when energy from monochromatic incident light beam hits the metal-dielectric interface at a particular SPR angle (Englebienne et al., 2003), gets transformed into electromagnetic energy resulting into production of evanescent waves (Fig 1).

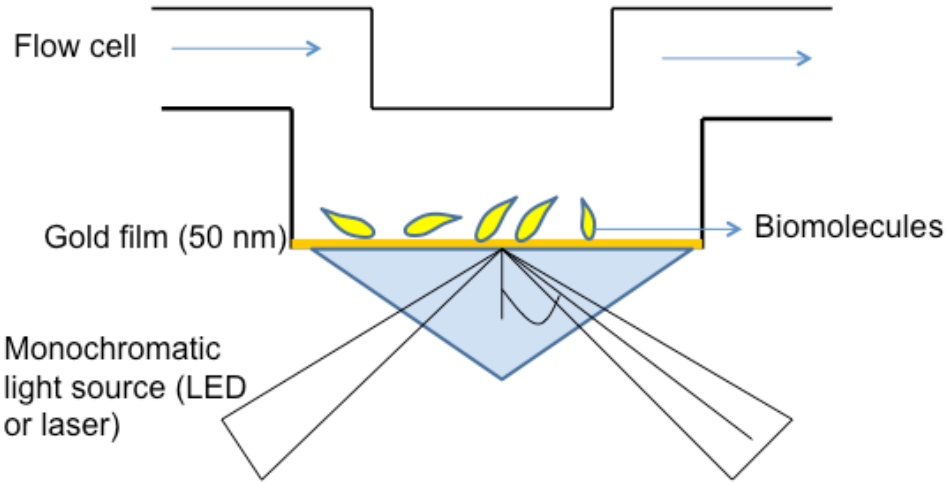

Fig 1. The basic principle of SPR: Measurement of alterations in refractive index of medium directly in contact with sensor surface.

Gold surface is generally used in SPR for immobilization of test proteins. The unlabelled query molecules are introduced in solution form and alterations in angle of reflection of light due to the binding of the probes to the immobilized protein provides real-time information regarding biomolecular interactions.

The angle at which the minimum intensity of the reflected light is achieved is called ‘‘SPR angle’’. SPR angle is directly related to the quantity of biomolecules bound

to the sensor surface (Fig 2). Different factors such as nature of the metal layer, angle of SPR, refractive index at the metal-dielectric interface, wavelength of the incident light, etc regulate the magnitude of surface plasmon resonance.

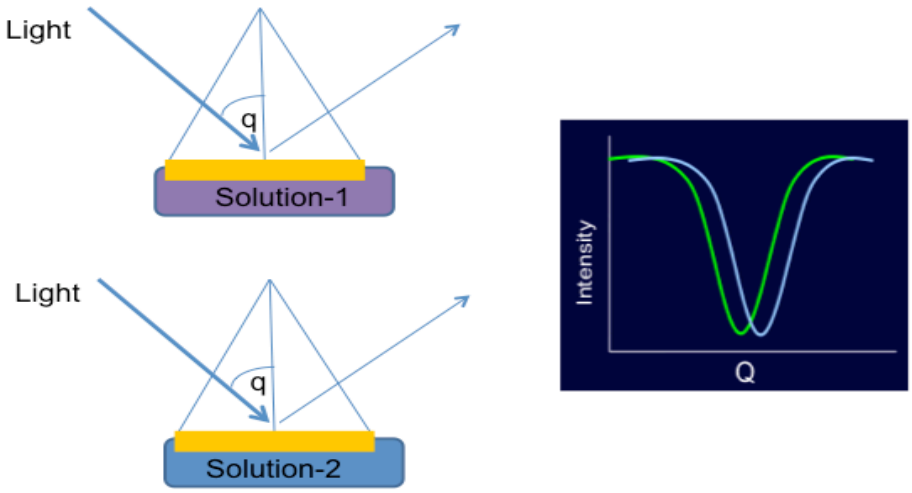

Fig 2. SPR angle; effect of refractive index near the surface on SPR angle

Illustration: Surface Plasmon Resonance*SPR is a highly sensitive spectroscopic tool that is increasingly being used for label-free detection studies. Test proteins such as antibodies are immobilized onto the gold-coated glass array surface. Incident light striking the surface is constantly reflected at a particular angle in this state. Unlabelled free antigens or other query proteins enter via the flow cell and move towards the immobilized antibodies or other test proteins. There is no change in reflected light upon entering into the system. Binding of antigen to antibody immediately brings about a change in the angle of reflection of light due to changes in the refractive index of the medium.*

In SPR measurement the sensorgram indicates the changes in reflection intensity with respect to incident angle before and after binding of the target molecule (Fig 3).

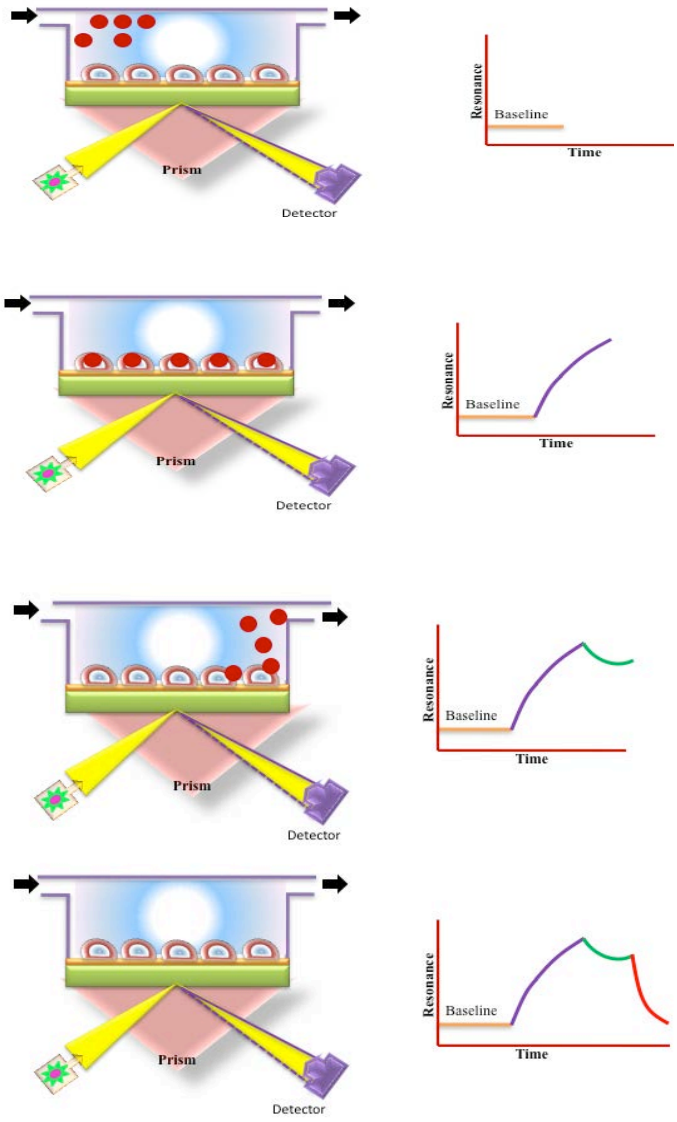

Fig 3. Different steps involved in biomolecular interaction analysis using SPR sensor. Overall SPR-based biosensors contain the incident light source, sensor gold surface, and the detector to capture the reflected light. The interaction between the molecules is measured by plotting the sensorgram (as shown in panels from top to bottom). The ligand molecules are immobilized on an activated gold sensor chip; while the query

molecule in buffer is flowed through the flow cell. Interaction along with binding kinetics is monitored in a real-time manner.

Illustration: Surface Plasmon Resonance Sensorgram

In SPR the changes can be continuously monitored to characterize biomolecular interactions in real-time. The SPR angle i.e. the angle at which minimum intensity of reflected light is obtained is indicative of the amount of biomolecule binding to the surface. The graph represents change in reflection intensity before and after antigen binding.

II. DIFFERENT APPLICATIONS OF SPR

In last ten years, several research groups have used SPR and related label-free techniques for real-time analysis of protein-protein and other biomolecular interactions, measurement of low abundance serum biomarkers, and screening of inhibitors of tumor targets and potential drug molecules (Table 1) (Ray et al., 2010; Reddy et al., 2012). SPR has also been applied extensively for many biomedical, food and environmental applications (Shankaran et al., 2007).

Ultra-sensitive detection is required for measurement of very low-abundance biomarkers. In a recent study Choi et al., have utilized SPR-based biosensor for detection of prostate-specific antigen (PSA) (Choi et al., 2008). In this study the authors have employed gold (Au) nanoparticle–antibody complex for signal enhancement of SPR and thereby effectively increased the sensitivity of the detection approach. SPR-based immunosensor have been designed, where a gold surface coated with PSA monoclonal antibodies (mAbs) and gold nanoparticle–conjugated antibody complex was used to capture PSA antigen (Fig 4). With this SPR-based biosensor the authors were able to detect PSA with a detection limit of 300 fM.

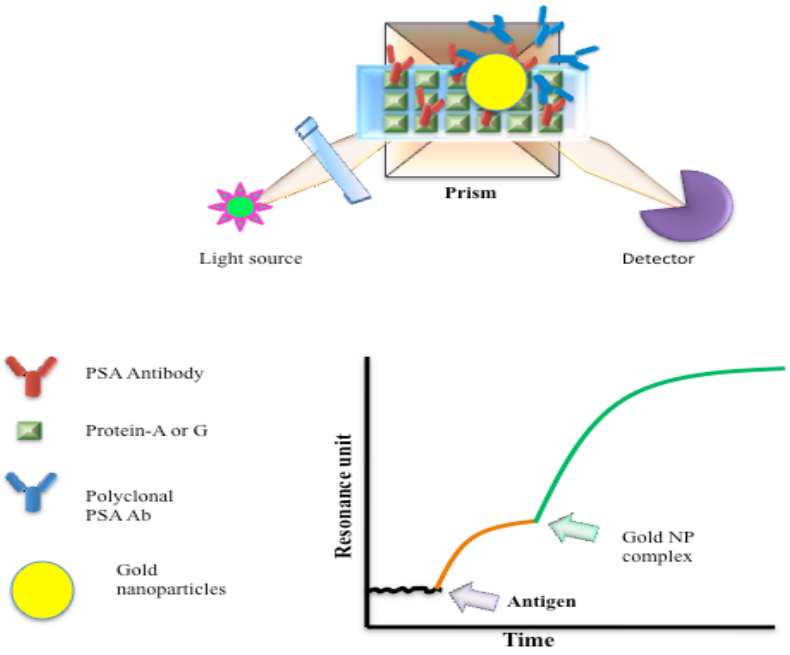

Fig 4. Sandwich immunoassay SPR platform for detection of cancer biomarker; prostate specific antigen (PSA) (Choi et al., 2008). PSA monoclonal antibodies were coated on a gold sensor chip functionalized with recombinant protein G via thiol groups. The sensorgram indicates the binding of the target antigen with immobilized antibodies. However, the change in SPR angle was not enough to monitor such interactions in complex clinical samples. A sandwich immunoassay approach using gold nanoparticle conjugated with PSA polyclonal antibody complex amplified the signal. Use of gold

nanoparticle-antibody complex as signal amplifier significantly improved the sensitivity level.

Table 1. Different applications of SPR-based biosensors (selected examples)

Application	Studies (References)
(a) Study of biomolecular interactions	1. Antigen-antibody interactions (Hiep et al., 2007) 2. Protein-glycan interactions (Yuk et al., 2006) 3. DNA-protein interaction (Zhu et al., 2000) 4. Studying association or dissociation kinetics (Wassaf et al., 2006)
(b) Detection of cancer biomarkers	1. Pancreatic cancer: CD166 (Vaisocherov et al., 2009) 2. Hepatocellular tumors: α-fetoprotein (Teramura et al., 2007) 3. Ovarian cancer: cancer antigen 125 (Suwansa-ard et al., 2009) 4. Colon cancer: CEA (Ladd et al., 2009)
(c) Detection of biomarkers for other human diseases	1. Type 2 diabetes: retinol binding protein 4 (Lee et al., 2008) 2. Heart diseases: B-type natriuretic peptide (Kurita et al., 2006); myoglobin and cardiac troponin I (Masson et al., 2007)
(d) Screening of inhibitors of tumor targets	1. uPAR-uPA interaction (Khanna et al., 2011) 2. Aurora B (Lang et al., 2010) 3. Pololike kinase 1, Akt and C-Src (Li et al., 2009; Ma et al., 2011)

III. ADVANTAGES AND DISADVANTAGES

SPR has its own advantages and limitations like other technologies.

Advantages

- Label-free detection eliminates the necessity of any secondary reactants and lengthy labeling process.
- Real-time measurements of biomolecular interaction.
- Multiplex analysis is possible; therefore, compatible with high-throughput assays.
- Sensitive to conformational changes; direct measurements and study of binding kinetics are possible.
- Both quantitative and qualitative measurements are possible.

Disadvantages:

- Requires sophisticated instrumentation.
- Restricted to choice of metal (gold/silver surfaces).
- Completely dependent on mass changes.
- Temperature sensitive; change in temperature affects refractive index.
- Non-specific interactions affect the signal.
- Works efficiently only with homogeneous surface.
- Sensitivity and specificity often become major concerns while handling very complex biological samples.

IV. CONCLUSIONS

It is evident that SPR-based label-free sensors are very promising for detection of disease biomarkers and HT real-time screening of biomolecules. However, SPR and related technologies have limited usage for large-scale applications in industries and clinics due to multiple technical limitations discussed above. Current advancements in the field of SPR have introduced quite a few new materials and methods for the improvement of sensitivity of the instruments (Halpern et al., 2011; Reddy et al., 2012). Application of different polarization methods for the incident light (such as p-polarised, s-polarised, TM waves, TE waves) can effectively enhance the coupling of incident light to plasmons. In addition, there are constant efforts to make reproducible and well-established surface chemistry for the generation of a selective sensing interface, which can reduce the binding of non-specific moieties that can alter the SPR signal. Multi-dimensional applications of SPR-based techniques have been achieved through successful coupling with different technological approaches, including SPR-MS, electrochemical SPR and surface plasmon fluorescence spectroscopy, which effectively circumvented some of the basic limitations associated with SPR. These efforts can expand the applications of SPR-based biosensors in clinical diagnosis.

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