Surface Plasmon Resonance Based Recent Advances in Understanding Plant Development and Related Processes

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Abstract

Since its introduction in the early 1990s, SPR has now become a powerful research tool for studying specificity, affinity and real time kinetics of a broad range of biomolecular interactions, including protein-DNA, protein-protein, protein-carbohydrate, protein-RNA and protein-lipid interactions. Surface plasmon resonance (SPR) has provided crucial information on the mechanisms of molecular interactions accompanying varied aspects of plant development. The structure-function relationship of various lectins depends on the quaternary arrangement of its monomers. Novel findings have been made in plant hormone research using SPR as a technique. Thus, new salicylic acid binding proteins (SABPs) have been identified in Arabidopsis. These include α-ketoglutarate dehydrogenase E2 subunit, glutathione S-transferases, the oligopeptidases TOP2 and TOP1, and members of GAPDH protein family. By immobilizing biotin-labelled DELLA peptides on the sensor chip and AtGID1a [*Arabidopsis* gibberellic acid (GA) receptor] as analyte, GA_4 has been observed to maximally enhance binding between DELLA and GID1. Molecularly imprinted monolayer (MIM)-decorated SPR detection method precisely differentiates between similar plant hormones, such as, IAA, [1]H-indole-3-butyric acid (IBA) and kinetin (KT), with detection limits around sub-picomolar range. Coronatine Insensitive-1 (COI1) has been shown to act as a jasmonic acid receptor using SPR. Ricin, a plant toxin, was detected at a concentration 2,500 times less than the minimum lethal dose (200 ng.ml^{-1}) using a SPR biosensor. Real time binding kinetic studies of viral proteins (VirE1 and VirE2) and ssDNA using SPR have shown that their binding is strongly influenced by substrate and it occurs at poly T sequences and not at polyA and dsDNA. An interaction between the replicase protein (p93) of cucumber necrosis tombusvirus (CNV) with the host protein, Hsp 70 (molecular chaperone), has revealed the potential role of Hsp90 in the assembly of viral replicase. SPR analysis from a small library of phytochemicals has shown ellagitannin geraniin as one of the most potent inhibitor of Hsp90 (a stabilizer of many oncoproteins). Future applications of SPR technique are likely to provide tremendous inputs into the molecular understanding of plant development and related processes.

Introduction

Applications of the currently available technologies and development of new technologies are crucial to understand the mechanisms of cell and tissue development, hormone interaction, functioning of signaling and metabolic cascades, mechanisms of cell wall formation and mechanisms of stress tolerance in plants. Although plants remain rooted at one place for their life, they can produce photoassimilates using constituents from soil and air; survive unfavorable conditions and herbivores, pests and pathogens attacks by synthesizing defense biochemicals. Like human beings, plants share numerous regulatory mechanisms and homologous genes, cell biological processes and biochemical pathways. Applications of technologies not only provide means to test hypothesis derived from less precise information, they also lead to generation of new hypotheses. Deciphering the molecular basis of inheritance through the discovery of the structure of DNA, transcription and translation remains a classical example of applications of technologies, like X-ray diffraction, in the current era of modern biology. A recent document entitled "A New Biology for the 21st Century" published by National Academy of Sciences (USA), highlights the major challenges in plant science research. Rapid advances in plant science research in the current era are putting forward new aspects for understanding plant growth and development, posing a challenge to decipher them through the applications of new technologies [1].

Significant new information has been obtained in plant biology in the recent past using high end technologies. This includes insights into hormone signaling and perception and its regulation by protein degradation, discovery of ion transporters of as receptors for specific ligands, characterization of flowering signal, discovery of small RNA, and molecular mechanism of guard cell movement. The pace with which DNA sequencing technology is improving, whole genomes can now be sequenced within hours and rapid progress is being made in decoding the genome sequence of thousands of *Arabidopsis thaliana* accessions [2,3]. Gene transfer into plant genomes another major challenge being faced by plant biologists. Although Arabidopsis transformation is quick and simple, extended tissue culture phases leading to somaclonal variations are required for another species. Future research in plant biology is expected to focus on and solve the issue of generation of plant mini-/ microchromosomes, synthesis of artificial chromosomes and development of effective transformation techniques for introduction of large synthetic DNAs into plant genomes. With greater availability of genome sequences, proteomics will be attainable and efficient in many plant species.

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In the recent past plant science research, has witnessed a surge in image analysis using super-resolution imaging techniques [4-6]. Understanding of plant growth through applications of new molecular and imaging technologies is one of the major challenges in the current era of "new biology". Imaging techniques are considered tools of highest interest in life science research because they offer contactless and non-invasive measurements. It is now possible to image entire roots or shoots at different stages of development, with precise cellular or subcellular spatial resolution. Techniques like stochastic optical resolution microscopy (STORM), three-dimensional structured illumination microscopy (TIRF), photoactivated localization microscopy (PALM) and stimulated emission depletion microscopy (STED), outperform classical diffraction limits to provide advanced imaging of molecules, cells, organs and even entire root or shoot system [7]. Techniques like optical coherence tomography (OCT) have been used in the recent past to examine seedling growth in *Arabidopsis thaliana* with a resolution facilitating discrimination of individual cells. Other imaging techniques, such as X-ray phase contrast imaging (X-ray PCT), have been used for microstructure analysis of voids in seeds and multiangle confocal microscopy has been employed for observing entire seedling growth [7]. High resolution images obtained from these technologies provide in depth information on various facets of plant development. Imaging for specific applications or plants, such as seed germination, can require long, time-lapsed acquisitions in dark [8,9]. It is, therefore, important to consider phototoxicity of probes by employing wavelengths, energy and duration of light being used in multiscale microscopic technologies.

An understanding of biomolecular binding interactions is fundamental to research in life sciences. Visualization of molecular interactions in plant cells is permitted using Raman spectroscopy, and field scanning optical microscopy [10]. A variety of light absorbing proteins in plants have been used to construct plant cell biological tools. Fluorescent tags based on various photoreceptors (eg. phytochrome and phototropin) have been designed. These light-based tools help us understand protein functions. Several instrument-dependent factors, however, limit the sought-after information from live-cell imaging. These include the capability to identify labeled structures above their background and instrument noise, the stability of the label, the spatial resolution of the microscope, etc. Multiphoton imaging has revolutionized *in vivo* imaging in various animal systems, and is being increasingly used in plant science research to reduce photobleaching, cytotoxicity and image generation through highly focused excitation and emission wavelengths [11,12].

During the last decade, various new analytical techniques have been developed to evaluate and characterize biomolecular interactions. Surface Plasmon Resonance (SPR) technology has revolutionized life science research by enabling real time detection and monitoring of biomolecular binding events without the use of labels, thus providing a better understanding of biochemical interactions. When used in an imaging mode, SPR can be very useful for quantifying and visualizing both fixed and live cells and their extracellular environment since it is label-free, can give good contrast at low levels of incident light, and can quantify very small amounts (up to 3 ng.cm^{-2}) of biological materials [13]. SPR is a phenomenon which occurs when a beam of light hits the metal surface at a certain angle. It offers several advantages over other conventional methods, such as ELISA, for analyzing biomolecular interactions. Present review provides an overview of the basic principles of surface plasmon resonance, followed by an in-depth analysis of SPR applications in understanding plant biological concepts and biomolecular interactions.

The Concept of Surface Plasma Resonance

When a beam of light travels from a high refractive medium to a low refractive index medium, the incident light gets partly reflected or refracted at the interface. However, at a critical angle of incidence, the incident light gets reflected completely into the high refractive index medium and no refraction is observed across the interface. This phenomenon is referred as Total Internal Reflection (TIR) [14]. When a thin layer of any suitable conducting material of a defined thickness is coated over the TIR interface, then light is not reflected fully. A fraction of the reflected incident light instead creates an electromagnetic field, called the *evanescent wave,* which extends about a quarter of wavelength beyond the reflecting surface and penetrates the low refractive index medium. The evanescent wave is so called because the amplitude of the wave decreases exponentially with increase in distance from the interface. Within the metal surface, the evanescent wave interacts with the delocalized free electrons, leading to induction of wave-like oscillation of free electrons (called 'surface plasmons'). This leads to reduction in the intensity of reflected light [15]. *Surface plasmons* or *surface plasmon polaritons* are thus, longitudinal electromagnetic surface waves which propagate within the conductor surface along the interface region. For a non-magnetic metal like gold, a highly polarized surface plasmon wave is generated, which further creates an enhanced evanescent wave, attributing propagating and electromagnetic nature to its surface. So, at an angle of incidence, energy of the photons gets transferred to the surface plasmons, leading to formation of *excited surface plasmon wave* at the interface [16]. This energy coupling between surface plasmon wave on the interface and the incident light produces a sharp drop in the reflected light intensity (called *surface plasmon resonance*). Whenever a plasmon is excited, a photon disappears, producing a sharp drop in reflected light intensity at that specific angle of incidence. The dip in reflected light is due to coupling of incident light into the surface plasmons, resulting in maximum absorption of the incident light by the gold film. SPR angle (also known as resonance angle) is the angle at which maximum loss in the intensity of reflected light occurs. SPR angle is strongly dependent on the refractive indices of metal film and analyte solution. It is in direct contact with the gold layer of the prism, and is measured with a charged couple device (CCD) chip. The angle at which minimum reflection occurs, is detected and converted to the resonance units [17].

A typical SPR biosensor has three major components: the sensor system, the optical system, and the detection system. The sensor system consists of the sensor chip made up of gold coating on glass surface to achieve TIR condition. The optical system is made up of high efficiency near-infrared lasers or light emitting diodes (LEDs), which serve as source of light during SPR measurements. A cylindrical lens is used to focus a range of incident light on prism. A change in refractive index occurring on the sensor surface (caused by interaction of molecules) is then detected. The detection system, which can be either charge-coupled device (CCD) or a position sensing detector (PSD), finally detects the changes in the reflected light intensity. For further analysis, the position of the resonance absorption peak is then recorded. The use of fixed linear arrays of charge-coupled detectors to monitor the response, enables measurements at a broad range of angles.

The sensitivity of SPR depends on the specificity of the biomolecules immobilized on the chip, their affinity constants, orientation, packing density and concentration. Another critical factor which affects SPR measurements is the thickness of metal layer coated over the prism surface. When a thick metal layer is used, bulk waves are not efficiently converted into surface plasmons. However, using a very thin metal

layer allows reconversion of plasmons to bulk waves. SPR analysis can be done under continuous-flow or non-flowing conditions. Generally, flow cells of small volume are used because they allow minimum usage of sample, control the thickness of diffusion layer, increase reproducibility in solution mixing, and decrease concentration variations in the cell [18].

Several methods are available for monitoring the coupling of light wave to the surface plasmons.

Measurement of angle of resonance

It is one of the most commonly used set-up and involves detection of changes in reflected light intensity with an alteration in the incident angle, thereby recording the angle of incidence (or the resonant angle). The angle of resonance can be measured very precisely and is used by the SPR system BIACORE (from GE Healthcare, USA).

Measurement of resonant or spectral wavelength

It involves measurement of resonant wavelength at a fixed angle of incidence and generating a reflectivity curve. This method is not so widely used.

Detection of phase shift of light

This method uses a reference beam and has maximum sensitivity but requires a more complex setup (a series of high frequency circuits) than other methods. Both the incident angle and wavelength are fixed and phase differences in the reflected and incident light are recorded.

Measurement of reflected light intensity

In this method changes in refractive index are measured while keeping both the angle of incidence and wavelength constant. This set-up makes it possible to simultaneously follow large numbers of SPR spots, thereby increasing sample throughput.

Sample Immobilization on Chip Surface

For SPR analysis, the ligand biomolecules need to be attached to the sensor chip surface. A range of robust and reproducible 'sensor-chips' are available commercially which can be used with SPR instruments. Chips with different densities are available to optimize sensitivity and non-specific interactions [19]. Several methods based on surface chemistry are available for immobilization of ligand molecules onto the chip for different applications.

Direct or covalent immobilization

It involves covalent attachment of ligand molecules to the sensor chip surface using various functional groups (amino, thiol, aldehyde or carboxyl) on the ligand. For example, free amine groups present in lysine residues or at the N-terminus of the protein allow coupling via amine linkage. There are several kinds of surfaces commercially available for covalent coupling of proteins. A carboxylated dextran matrix is most suitable for covalent immobilization because many ligands can be immobilized on it. Immobilization over a carboxylated surface must be performed in a buffer where the ligand is positively charged. The net charge of carboxy-methylated dextran is negatively charged, allowing concentration of ligand on the chip surface and, hence, its better immobilization. However, it is difficult to immobilize strong negatively charged proteins on a dextran surface. When using direct coupling approach, overcrowding, non-specific binding and aggregation should be avoided [20].

Indirect coupling using high affinity capture molecule

In this case, the ligand molecule is attached to a suitable molecule through non-covalent interactions. This intermediary molecule is further covalently immobilized on the sensor surface. Capture molecules, such as monoclonal antibodies, avidin-biotin or His tag protein- nitrilotriacetate (NTA) interactions, can be used. Streptavidin and NTA chips are basically used for conjugation of biotinylated protein and His-tagged protein, respectively. NTA surface has the inherent problem of leaching of the immobilized ligand, leading to dripping base line. Consequently, it is not preferred for kinetic analysis.

Hydrophobic adsorption

In the HPA sensor chip, long-chain alkane-thiol molecules are attached covalently onto the surface, and liposomes or micelles form a lipid monolayer on the surface. Additionally, a hybrid lipid bilayer format can also be used. This is generally used for investigations on transmembrane proteins eg. GPCRs (G-protein coupled receptors).

Chips can often be regenerated by washing off all analyte molecules and used again. Typical protein samples are around 1 $\mu g.\mu l^{-1}$, with volumes around 20 μl to 100 μl. PBS buffer is a good choice and TRIS should be avoided. Binding affinities in the range of 1 μM to 1 nm can be detected.

SPR as an Analytical Tool

For using SPR as a diagnostic instrument, it is necessary to observe a change in the refractive index thereby causing an alteration in angle corresponding to a sharp dip in reflected light intensity. The penetration of plasmon wave into the low refractive index medium results in a shift in the angle of reflected light intensity, which is recorded. The intensity of reflected light is measured as a time-dependent function of the angle of incidence. SPR is a natural phenomenon which occurs when monochromatic plane-polarized light hits an electrically conducting metal surface (generally gold) at the interface of two media having varied refractive indices [21].

In a typical SPR biosensing experiment, one of the interactants (usually a protein, termed as 'ligand') in the interacting pair is immobilized onto a sensor chip surface (usually a gold-coated glass slide). The other interacting partner (or the 'analyte') is injected in the form of an aqueous buffer solution continuously flowing through the flow cell over the sensor surface. As the analyte molecules interact with the immobilized ligand molecules, the mass change on the sensor surface leads to an enhancement in the refractive index at the metal layer interface between the sensor surface and solution flowing over it, which is measured as a change in the resonance wavelength or SPR angle. The refractive index changes are monitored in a time-dependent manner ('real time') by detecting changes in the reflected light intensity using an array of photodiodes or charge-coupled detectors (Figure 1). The result is displayed on the instrument as a graph plotted as change in response or resonance units (RUs) versus time ('sensorgram'). A linear relationship exists between the change in angle, caused by association or dissociation of molecules from the sensor surface, and the mass of bound material at the sensor chip surface (Figure 2). At the start of the experiment, the RU value corresponds to a starting critical angle 'a'. As the number of analyte molecules binding to the ligand molecules increases, an increasing response is recorded in the sensorgram [22]. The response, however, remains constant after attaining equilibrium. At steady state, the value of RU is equal to the changed final critical angle 'b'. It is important to first record a background response by injecting the analyte in the absence of any ligand molecule. Actual
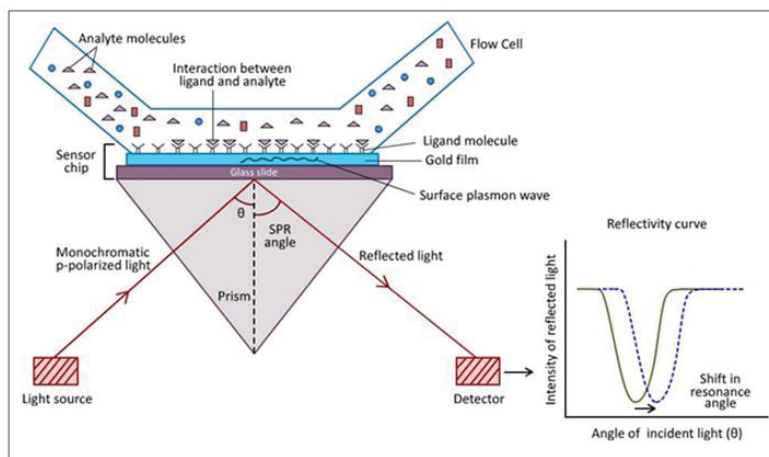
Figure 1: Diagram of the working principle of a SPR-based optical biosensor.
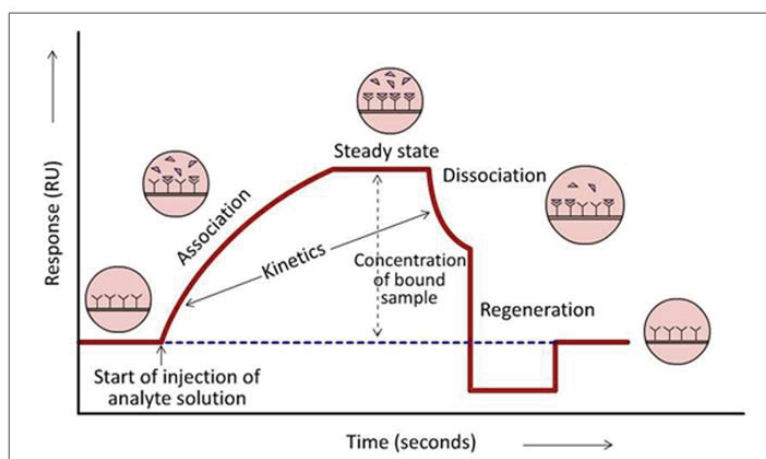

Figure 2: A SPR sensogram depicting different phases in a typical SPR experiment.

binding response is obtained by subtracting this background response form the observed values. One RU corresponds to an angle shift of 0.0001° upon binding of around 1 pg protein.mm^{-2}. After reaching equilibrium, the sample is replaced by buffer solution (or 'regenerating fluid'), which allows dissociation of the interacting partners, and the SPR response decreases. The surface can be regenerated back to the critical angle 'a' for restarting the experiment. Thus, a real time SPR experiment consists of an association phase, an equilibrium phase and a dissociation phase. SPR response depends on various factors, such as binding affinity, molecular weight of the analyte molecule and the number of receptor molecules on the chip. Maximum binding capacity of the receptor molecules on the sensor surface for analyte molecules is referred as its Rmax [23].

The rate and size of change of the SPR signal provides information about the apparent association and dissociation rate constants and the interaction stoichiometry. The ratio of association and dissociation constant gives the apparent equilibrium constant (affinity). There is a direct correlation between the immobilized mass on the chip surface and SPR signal. Signals can easily be acquired from very low quantities (less than a microgram) of the mass. It is also possible to study binding events of compounds in crude extracts because SPR signal depends only on binding of a specific compound on the immobilized template [24].

Limitations of SPR

It is difficult to analyse small molecules through SPR because changes in reflected light angle measured by SPR are directly proportional to the mass of the analyte. Thus, small molecules generate poor signal or weak sensogram. Several commercial SPR-based biosensors use carboxy-methylated dextran for immobilizing one of the partners of the interacting pair. Proteins with pI ranging from 4.0 to 8.0 can be easily immobilized on such a matrix. However, it is difficult to immobilize highly acidic proteins. Although different types of immobilization are technically feasible, immobilization of molecules that does not affect the biological functionality poses problems. The best approach to avoid issues related to alteration of functional property owing to immobilization, is to use monoclonal antibodies for directional or positional immobilization of interacting partners. This alternative however poses a technical limitation of development of target-specific monoclonal antibodies against either of the interacting partners. SPR-based biosensors require less sample volume and offer quick results. However, every assay requires rigorous standardization which, in turn, uses up a considerable amount of precious ligand/ analyte/ receptor. Nevertheless, the analyte needs to be of high purity to offer reliable data. Several proteins that have pI of more than 8.0, have non-specific interactions with dextran matrix due to charge phenomena.

Neutralization of non-specific signals requires incorporation of inhibitors or other competitive molecules. This complicates data analysis. SPR- based methods are very good for the analysis of 1:1, 1:2, rate limiting and heterogeneous ligand bindings. Viscous samples may clog flow cells, thereby permanently damaging the component. Drift in base line owing to gradual loss of immobilized molecules caused by improper immobilization, use of strong regeneration conditions or use of unstable molecules, can also occur. SPR-based commercial biosensors (Biacore and IAsys) are being increasingly used, but the high operation cost of the method and the system make it unaffordable to many researchers. Although commercial biosensors are easy to operate, but interpretation of binding kinetics is a difficult task [25]. Various experimental artifacts, such as mass transport, effects of matrix and non-specific binding further complicate the analysis [26,27]. The two most important limitations of SPR are 1. Use of metal surface as a prerequisite for SPR experiments for excitation of electrons. 2. Less sensitivity of low molecular weight molecules. Another potential limitation of the SPR technique is that the immobilized protein may be inactivated. Also, the interaction is studied close to a surface, thus the measured parameters may not reflect those in solution. Another problem with SPR is non-specific binding of the analytes to the chip matrix. Careful control experiments are needed to exclude binding to the surface in the absence of immobilized protein. Often blocking agents, such as BSA, gelatin, and casein are added to the buffers as are non-ionic detergents, such as Tween and Triton, to reduce non-specific binding. Ionic strength and pH can also have strong influence in non-specific binding and are often varied while optimizing experimental conditions.

SPR for Bio-molecular Interaction Analysis

SPR has now become the method of choice for investigating the dynamics of biological and chemical interactions. It offers several advantages for the verification and analysis of biomolecular interactions. The success of SPR application in analyzing biomolecular interactions can be attributed to the following factors:

• Requirement of very small amounts of sample (mg or sub-mg may suffice)

• Provision for pre-equilibrium kinetic data

• Availability of information on binding affinities and rates of association and dissociation

• Highly sensitive response

• Rapid and high throughput analysis

• Feasibility of identification of binding events with label-free molecules (without using any fluorescent or labelling interactants)

• Real-time kinetic measurements

• High spatial resolution

• Identification of specific vs. non-specific adsorption processes

• Anti-interference capability

• No pretreatment of samples required

Applications of SPR Technique in Plant Biology

Protein-carbohydrate interactions

Various biological processes, such as cell-cell recognition, signaling (receptor–ligand interactions) and catalysis, are dependent on protein-carbohydrate interactions. Despite their significance in the myriad of biological systems, these interactions have, so far, remained relatively unexplored. SPR facilitates an easy, real-time monitoring of kinetics of the protein-carbohydrate interaction [28]. Sugar-binding proteins (lectins) bind to cell walls or membranes. Lectin-carbohydrate interactions might influence the physiology of the cell wall and affect cell metabolism [29]. Lectins can also stimulate immune system and can be used as cell markers for diagnostic purposes, thus making them an interesting group of biomolecules for investigations. SPR technique has been exploited for qualitative and quantitative estimation of the interaction between an oligosccharide and various lectins [30]. A glycopeptide derived from asialofetuin (a glycoprotein containing three asparagine-linked triantennary complex carbohydrate chains with N-acetylgalactosamine residues at termini) was immobilized on the SPR chip, and binding specificity and affinity of six lectins [from *Sambucus sieboldiana*, *Maackia amurensis*, *Aleuria uuruntia*, *Ricinus communis* (RCA$_{120}$), *Datura stramonium* (DSA) and *Phaseolus vulgaris*] was monitored. The relative binding molar ratio of lectins to the glycopeptide has suggested the possible number of binding sites available for the respective interaction, thus providing information about the constituents of sugar chains. Two lectins with binding specificity for sialic acid epitopes Neu5Ac(α2-6)Gal and Neu5Ac(α2-3) Gal from *Sambucus nigra* and *Maackia amurensis*, respectively, were analyzed using SPR to characterize the specificity of lectins [31]. The presence of an endogenous lectin receptor in different soluble protein fractions (albumins, globulins and basic glutelines) obtained from the seeds of *Cratylia floribunda,* has also been examined using SPR. The sensor chip was covalently coated with CFL lectin (*Cratylia floribunda* lectin) through a carboxymethyl dextran layer and real-time kinetic analysis was carried out for the binding of protein fractions. The analysis revealed in determining that the CFL lectin interacts with soluble endogenous glycosylated receptor through its carbohydrate-binding site [32]. The glycan-binding specificities of structurally closely related mannose/glucose-specific lectins from the sub-tribe Diocleinae were also distinguished using SPR tool. It was, thus, established that despite very similar amino acid sequences and monomeric structures, the structure-function relationship of these lectins depends on the quaternary arrangement of monomers [33].

To overcome the hurdle of relative insensitivity of SPR to detect low-molecular mass analytes like carbohydrates, a new modification of labeling carbohydrates with an organoplatinum (II) complex of the type [PtCl-(NCN-R)] has been devised [34]. This labeling significantly enhances the signal without influencing the specificity of the biological interaction, though it does pose a limitation on the calculation of kinetic parameters using SPR. Novel and chemically synthesized carbohydrate-based ligands for developing potential ricin (bioterrorism agent) sensors has also been investigated [35]. Non-natural 6-substituted galactose derivatives have been assessed for the ligand specificity of plant lectin RCA$_{120}$ (*Ricinus communis* aglutinin 120) using SPR imaging (SPRi) of glycoarrays. The analysis has revealed that these novel galactosides are biologically and chemically more robust than natural glycans counterparts and hence can be used as novel ligands for fast and sensitive detection of ricin. Due to its additional advantages, like lower cost and flexibility, localized surface plasmon resonance (LSPR) has found increasing number of applications over SPR. For LSPR, the nanostructured metal surface of the transducer is immobilized with a receptor layer and local changes in the refractive index in response to binding of the biological analyte are monitored as variations in the optical response. PEG-thiol modified mannose was derivatized on Au island transducers and LSPR analysis was carried out to monitor, quantify, image and assess the

selectivity of mannose-Con A (lectin Concavalin A from *Canavalia ensiformis*) interactions [36]. Also for further sensitive detection of ConA, an array with double-layer dextran capped silver nanoparticles (Ag NP) was constructed. This modification improved the limitation of quantification of ConA from 3.42 (in single-layer Ag NP) to ~1.14 μg/ml [37]. An SPR-system wherein mannose and galactose carbohydrate arrays were immobilized on gold film to monitor the binding of lectins jacalin and concavalin-A, was developed [38]. Thus, SPR detection helps in analyzing the specificity, affinity and stoichiometry of varied lectin-based interactions and provides an insight into the fundamental mechanisms of molecular recognition.

Protein- chaperone interactions

Several techniques have been used to monitor the binding of molecular chaperones with unfolded proteins to facilitate proper folding, but very few investigations have been undertaken to examine the relative binding affinities of such protein-chaperone interactions. Hydrogen–deuterium exchange (H/D exchange) and NMR-based methods, fluorescence spectroscopy, back-scattering and surface plasmon resonance, are some of the techniques involved in analysing binding affinities of chaperones [39]. The binding affinity analysis using SPR sheds light on the underlying molecular mechanisms associated with varied cellular and biomolecular interactions involving chaperones. It also helps in understanding the structure-activity relationships and the analysis of the kinetic and thermodynamic parameters for the respective interactions. Furthermore, the driving force of these interactions can also be investigated using variable temperatures during the analysis [40]. SPR analysis has shown an interesting interaction between the replicase protein (p93) of cucumber necrosis tombusvirus (CNV) with the host protein (Hsp 70, a molecular chaperone), thus revealing the potential role of Hsp90 in the assembly of viral replicase [41]. Modulation of the activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs) can cause severe diseases, ranging from cancer to neurodegenerative disorders. Hence, numerous investigations have been done to discover HDAC inhibitors and enhancers. The effects of several polyisoprenylated benzophenone derivatives (PBDs, isolated from natural sources) on HAT activity was examined by SPR analysis. It led to the discovery of guttiferone A, guttiferone E, clusianone and nemorosome, which can be promising therapeutic agents [40]. The tetranortriterpenes from the root extracts of *Pseudricedrela kotschyi* and *Trichilia emetica* were analyzed for their binding affinity for the chaperone Hsp90. SPR data revealed the strong binding of two phragmalin limonoids, of which kotschyin D inhibited Hsp 90 activity and induced degradation of its client proteins [42]. SPR analysis from a small library of compounds (including plant polyphenols, like tanins, flavonoids and coumarins) showed ellagitannin geraniin as one of the most potent inhibitor of Hsp90. It was observed that geraniin, the main polyphenolic compound in *Geranium thunbergii*, shows a dose-dependent inhibitory effect on the ATPase activity of Hsp 90α [43]. The potential supporting function of Hsp70.1 or any other Hsp90 isoform during protein sorting was also investigated [44]. This was done by analysing their preferential binding with organelle-specific, tetratricopeptide repeat (TPR) domain-containing docking proteins (Toc64, OM64 and AtTPR7) localized on the outer envelope of chloroplasts, outer membrane of mitochondria and ER membrane, respectively. Through SPR analysis of binding affinities for docking protein-chaperone interactions, differential binding preferences were found. Thus, OM64 preferentially binds to Hsp70.1, and Toc64 and AtTPR7 bind to both Hsp70.1 and Hsp90 isoforms. AtTPR7 showed reduced binding with Hsp90.1, thereby, proving significance of SPR as a powerful tool to distinguish individual binding constants. Investigations were carried out on 2-Cys-Peroxidoxins (2-Cys-Prxs, thiol-based peroxidases) from the cyanobacterium- *Anabaena,* to understand their role in oxidative stress tolerance [45]. NADPH-dependent thioredoxin reductase (NTRC) was found to be the possible physiological reductant for the peroxidase activity of Alr4641 through SPR analysis. Combinatorial approach of spectroscopic ellipsometry and SPR, known as total internal reflection ellipsometry (TIRE), was used to analyze the binding behavior and affinities of plant plastidial chaperone receptor-OEP61 (outer envelope protein of 61 kDa) towards other chaperones. The golden surface of the chip was immobilized with OEP61 via an intermediate layer of polycations, and TIRE analysis was carried out. This investigation revealed binding capacity of OEP61 towards Hsp70 and its ability to differentially bind quite similar Hsp70 isoforms [46]. Various applications summarized above indicate that SPR data obtained from a wide myriad of research investigations involving molecular chaperones can be applied for drug designing, diagnostics, therapeutics and discovering novel biomolecular interactions.

Diagnosis of plant viruses

SPR provides a unique technology for investigations in areas like plant virology, characterization of novel disease biomarkers and development of drug-delivering nanoparticles [47]. Various features of SPR technology have helped in deciphering appropriate antibodies for research and diagnostic purposes, and can be extremely helpful in precise disease diagnostics and therapeutics of different plant viruses. Numerous techniques are available for detecting and quantification of virus-specific proteins and nucleic acids like, PCR or RT-PCR, REA, ELISA, which are effective but are also time-consuming and require specific sample preparation [47]. Only a few methods can detect intact viral particles, which indicate the beginning of infection process and can depict the intensity of infection by quantitative determination [48]. Enzyme-linked immunosorbent assay (ELISA), the most commonly employed technique for detecting intact viruses, poses the major constraint of providing no information on the kinetic parameters of the reaction [48]. In recent years, some non-destructive techniques like quartz microweighing [49-51], bioelectric recognition [52], atomic force microscopy [53] and interferometry [54], have been employed frequently for detection of viral interactions. Optoelectronic transducers, like surface plasmon resonance (SPR)-based biosensors, have been successfully used in the recent past for analyzing higher order biological entities like viruses and bacteria. However, relatively only a few SPR analyses pertaining to viruses of plant origin are available [55]. Apart from its benefits like rapidity and high sensitivity, SPR technique provides an edge over other techniques due to a real-time mode of detection of intermolecular interactions, their kinetic and thermodynamic evaluation and also evaluation of epitope interference [47,48,55].

Highly sensitive nature of SPR technology has been exemplified by showing the feasibility of detecting minute differences in the binding affinities of two recombinant Fabs (antigen-binding fragments), 57P and 174P (difference of 15 amino acids in their variable region) with peptide corresponding to the residues 134-146 of the coat protein of tobacco mosaic virus [56]. Diagnosis of intact tobacco mosaic virus (TMV) through detection and quantitative estimation of antigen and antibody (IgG) complexes using the SPR approach has been demonstrated [48]. An antiviral IgG-TMV complex was prepared during a pre-incubation step, and then specific binding between the complex and a protein A (IgG-specific) immobilized on a sensor substrate was detected using SPR. The applicability of SPR was also demonstrated to analyze the

serological variability of *Lettuce mosaic virus* (LMV) using monoclonal antibodies (mABs) [57]. It was found that LMV particles contain at least five independent recognition/binding regions, which coorelate with seven mABs reactivity groups used during the investigation. Mutation-prone nature of viruses poses limitations on the use of antibodies for SPR technique. For SPR technique, aptamers provide varied advantages over antibodies, like easy *in vitro* selection, chemical stability, and well controlled chemical modifications. SPR imaging has been used for specific detection of two close homologues of apple stem pitting virus (ASPV) coat proteins (PSA-H and MT32, with 81% identical amino acid sequence) using original DNA aptamers [58]. SPR analysis has been used to examine interactions of *Potato virus Y* (PVY) virus with antibodies, quantification of their interactions, detection of overlap of epitopes being targeted by monoclonal antibodies and evaluation of the inherent serological variability of the PVY. This approach can also help in detecting possible PVY interactions with other proteins from the host plant [55]. SPR-based biosensors can also be used to explore various aspects of viral binding to functionalized surfaces and to discover an anti-viral drug [47].

Detection of phytohormones and understanding their metabolic pathways and signaling

Phytohormones are small molecules which play important roles in regulating and controlling various stages of plant growth and development. Various traditional methods, such as immunological assays, genetic analysis using mutants and mass spectrometric detection, have been used in the past to provide an insight into the complex hormone signaling pathways in plants. These conventional biochemical and genetic approaches are, however, time-consuming and not very sensitive, thereby necessitating the development of high-throughput sensitive techniques. In recent years, SPR has become increasingly popular in the field of phytohormone study. SPR technology has been used understand the hormone metabolic pathways and their signal transduction.

Salicylic acid (SA): It is an important plant hormone which plays a crucial role in regulating various developmental processes in plants, such as disease resistance [59]. A key role of SA in systemic acquired resistance (SAR) has also been shown in plants [60,61]. Identification of several SA-binding proteins (SABPs) has provided an insight into the SA-mediated signaling. However, many aspects of the mechanism of action of SA are yet to be investigated. It is important to isolate additional SABPs to elucidate the mechanism of SA-mediated diverse effects in plants. Various genetic and biochemical approaches have been used to isolate and validate SA-binding proteins in Arabidopsis [62-65]. Using SA analogs in conjunction with surface plasmon resonance (SPR) based approach, several new SABP candidates have been identified in Arabidopsis, including the α-ketoglutarate dehydrogenase E2 subunit (KGDHE2), and glutathione *S*-transferases [66], the oligopeptidases TOP2 and TOP1 [67], and several members of GAPDH protein family [68]. This SPR-based approach utilizes a SA analog, 3-aminoethyl salicylic acid (3-AESA), which acts as a ligand and is immobilized onto the CM5 sensor chip via an amide bond. The solution containing the protein to be tested (analyte) is then allowed to flow over the sensor surface to detect SA-binding proteins. Thus, SPR has proved to be a more sensitive and effective tool for evaluation and validation of SABPs which cannot be detected by conventional methods due to their weak or transient interaction with SA [66,69].

Abscisic acid (ABA): It is a sesquiterpene plant hormone involved in various developmental and biochemical processes in plants [70]. Despite rapid progress in elucidating the molecular details of ABA signaling, knowledge about ABA receptors in plants is limited. Several indirect evidences have suggested the presence of multiple ABA receptors but none of these have been characterized [71,72]. SPR technology has been utilized to demonstrate functional and biochemical interactions of the monoclonal antibody JIM19 with plasma membrane and protoplasts of rice [73]. In protoplasts from barley aeurone layer, JIM19 has been shown to regulate ABA responses [74]. In the SPR experiment, JIM19 was covalently bound to the CM5 biosensor chip by amine coupling and a non-saturating solution of plasma membranes could flow over the chip to characterize the binding of PM epitopes to JIM19 [75]. JIM19 was shown to bind to glycoproteins in rice plasma membrane and this interaction between JIM19 and rice plasma membrane was shown to be antagonized by ABA but not by phaseic acid (structurally like ABA but biologically inactive) [73].

Gibberellins (GAs): It constitutes a group of tetracyclic diterpenoid phytohormones which control key aspects of plant development. SPR biosensors have been used to investigate GA perception and signaling mechanisms. The interaction of two DELLA proteins (SLR14–125 and RGL11–137 from rice and Arabidopsis, respectively) with the GA receptor (GID1), with or without bioactive gibberellins (GAs), has been demonstrated using SPR [76,77]. These investigations have provided evidence for GA-dependent and GA-independent interactions of GA receptor (GID) with DELLA proteins. N-terminal fragments of SLR1 (SLR14–125) and RGL1 (RGL11–137), fused with glutathione S-transferase (GST) and maltose-binding protein (MBP) tags, respectively, were produced and purified from *E. coli*. These were used as ligands immobilized using anti-MBP and anti-GST antibodies on the sensor surface while GID1 was used as analyte [77,78]. With these analyses, investigators were able to determine the open and closed state of GID for their binding affinity for DELLA proteins. However, SPR has not yet been employed for quantification of bioactive GAs. SPR was utilized to detect and quantify bioactive GAs in solution [79]. The specific binding property of GA and GA receptors enables detection of GAs using SPR. It is difficult to immobilize GA as ligand on the chip surface because GA might then lose its ability to bind to GA receptors. Since GAs are low-molecular-weight biomolecules (346 Da), it is difficult to directly use them as analyte molecules. Genetic and mutant analyses have shown that GA receptors (GID1) interact with DELLA proteins depending on GA concentration [80,81]. Thus, DELLA proteins can serve as potential ligand candidates for analysis of GA using SPR. DELLA proteins negatively regulate GA responses in plants [82,83]. To understand the interaction between GA receptor (GID1) and DELLA protein, biotin-labelled DELLA peptides were immobilized on the chip surface while AtGID1a with or without GAs was used as analyte molecule. GA_4 was shown to have highest bioactive ability to enhance binding between DELLA and GID1 (Figure 3) [79].

Auxins: These are important endogenous plant hormones involved in cell growth and division. Various methods are employed for detection and estimation of auxin, including HPLC, ELISA, GC-MS etc. An SPR-based detection method developed for identification of Indole-3-acetic acid (IAA) in plant tissues in real time manner used a combination of SPR sensing and a molecularly imprinted monolayer (MIM) [84]. This SPR detection method is precise and shows good recoveries and can also differentiate between similar molecules, such as IAA, ^{1}H-indole-3-butyric acid (IBA) and kinetin (KT), with a detection limit around sub-picomolar range [84]. SPR has also been used to show interactions between monoclonal antibodies (such as scFV12) and recombinant auxin binding protein in *Nicotiana tabacum* (NtABP1), by immobilizing biotinylated NtABP1 onto CM dextran by amino linkage and using purified scFV12 and Fab12 as analytes [85].
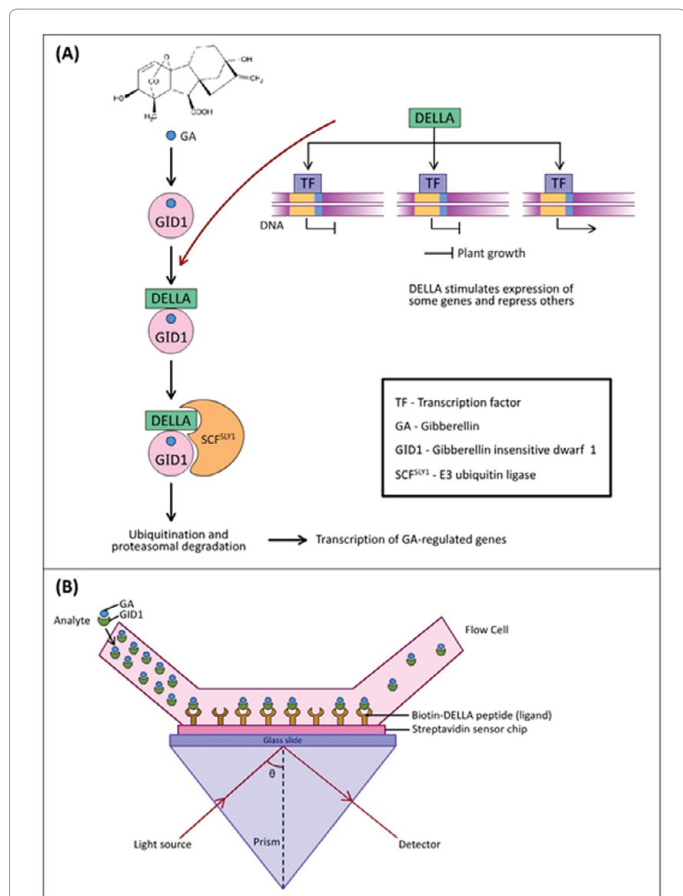
Figure 3: A-Simplified signal transduction pathway of GA-mediated responses. B-Principle of SPR-based functional analysis of bioactive GA detection [79].

Jasmonic acid (JA): It is an important plant defense hormone which mediates its effects through a multiprotein complex containing F-box protein Coronatine Insensitive-1 (COI1) [86]. This complex interacts with the jasmonate ZIM (JAZ1)-domain proteins and targets them for ubiquitin-mediated degradation by 26S proteasome [87]. Possible interaction analysis between COI1, JA and JAZ1 was investigated using SPR. Purified JAZ1-His protein was immobilized onto the sensor surface and a positive SPR signal was obtained only when COI1 pre-incubated with JA-Ile or coronatine was injected as analyte solution. However, no signal was observed when COI1 or JA-Ile was injected alone. This indicates that a significant interaction exists between COI1 bound to JA-Ile or COR and JAZ1 domain [88]. Thus, it was shown using SPR that COI1 acts as a JA receptor (Figure 4).

Protein-protein interactions

Protein-protein interactions are important for regulation of cellular processes, such as signal transduction events, metabolic pathways, cell cycle, cell death coordination and so on. SPR provides excellent information on protein-protein interactions, including their quantification, binding kinetics, interaction affinity and selectivity, and equilibrium studies. It also serves as an important technique for investigations on the effect of substitution of amino acids on the binding properties of proteins and to characterize interaction kinetics between proteins and antigen-antibody.

During replication, the replicase complex in plus-stranded RNA viruses is assembled because of protein-RNA and protein-protein

interactions and among the viral RNA, and host- and viral- proteins [89-91]. Various studies have been carried out to identify these protein-RNA and protein-protein interactions. The dynamics of interaction between replicase proteins in tomato bushy stunt virus (p33 and p92) has been analysed using SPR technique. It has already been shown that these replication proteins interact both *in vitro* and *in vivo* [92]. To analyse the interaction kinetics of these replicase proteins, recombinant fusion protein p33 was immobilized on sensor chip using amine coupling while the analyte solution containing the test protein (p33 or p92) was injected. The binding of C-terminal of p33 with S1 domain is stronger as compared to that with S2 domain and S1 is the major contributor of these interactions [92]. SPR has also been used to study the interaction between pectin methyesterase and its inhibitor in kiwi fruit. The effect of pH and ionic strength was studied by immobilizing the enzyme on sensor surface via amine coupling and it was found that binding of PME with its inhibitor is associated with inactivation of PME [93].

SPR has been used to understand the regulation of enzyme-enzyme interactions in Arabidopsis during cysteine biosynthesis in mitochondria. Serine acetyltransferase (SAT) and O-acetylserine (thiol)-lyase (OAS-TL) are the two important enzymes involved in cysteine biosynthesis [94]. It was first shown in Salmonella that one tetramer of SAT and two dimers of OAS-TL interact to form a heterooligomeric cysteine synthase complex [95,96]. Real time interaction kinetics of this multimeric enzyme complex was analysed using SPR with respect to the reaction intermediate O-acetylserine and quantitative paramaters, such as equilibrium rate and association and dissociation constants were determined [97].
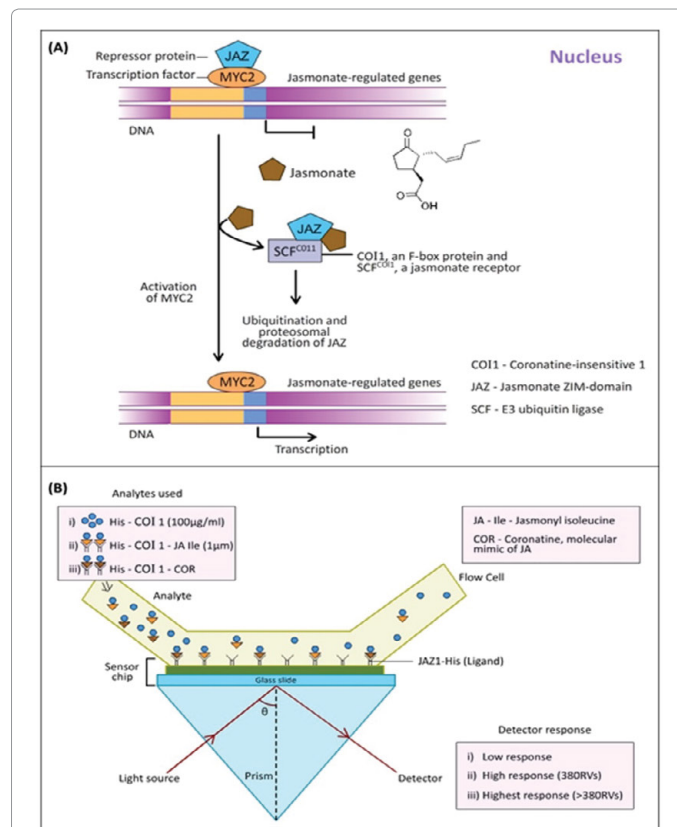

Figure 4: A-Simplified signal transduction pathway of JA-mediated responses. B-SPR analysis to determine possible interactions between COI1 (F-box protein), JA-Ile and JAZ1 [88].

An SPR strategy to characterize and localize interactions between photosystem II (PSII) and its putative, transiently bound interaction partners has also been developed [98]. The binding site of CyanoP, a cyanobacterial homolog of higher plant PsbP (an extrinsic PSII protein), was determined using SPR and it was found to be precisely localized at the same position as PsbO i.e., in the centre of PSII [98]. SPR has also been employed for characterizing biomolecular interactions between protein disulphide isomerase (an oxidoreductase enzyme belonging to the thioredoxin superfamily) from *Oldenlandia affinis* (OaPDI) and cyclotide precursor protein kalata (Oak1) [99]. Protein disulphide isomerase functions as an endoplasmic reticulum (ER) chaperone and is involved in oxidative folding of polypeptides in ER. Cyclotides are small disulphide rich peptides approximately 30 amino acids in length and have a cyclic backbone. They have a unique hydrophobic patch exposed on the surface and thereby require special conditions for their formation. The exact mechanism of biosynthesis and folding of cyclotides is not known. The role of PDI from *Oldenlandia affinis* was examined in oxidative folding of cyclotide kalata B1 using SPR [99].

Protein-nucleic acid interactions

Protein-DNA interactions are important for accessing the genetic information stored in DNA within the cell. Numerous conventional methods have been used to analyse the complex network of these interactions. SPR allows robust analysis of protein-DNA interactions wherein one biomolecule is attached on the sensor chip while the other interacting partner can flow over the chip surface. SPR was first used to characterize protein-DNA interactions to show lactose repressor and operator interaction [100].

This technique has been employed for analyzing the binding of transcription factors (proteins) to target sites on DNA. Earlier investigations were confined only to short DNA segments. However, it was later shown that SPR can be used to characterize DNA-protein interactions using entire gene promoters [101]. Interaction analysis between LEAFY (LFY, a transcription factor from Arabidopsis) and two promoters (APETELA1 and APETELA2) was done using SPR wherein biotinylated AP1 and AP2 were immobilized in separate channels on streptividin-coated SPR chips and different concentrations of LEAFY protein were injected in the analyte solution. This novel application of SPR to characterize interactions between proteins and long segments of DNA has both quantitative as well as qualitative benefits. SPR can also be used for quantitative characterization of interactions between the gene promoters and transcription factors.

SPR has been employed to analyse the binding between viral proteins and single-stranded DNA. VirE1 and VirE2 are bacterial proteins secreted into the host cell during infection by *Agrobacterium tumefaciens*. VirE2 binds to the single-stranded transfer DNA (T-DNA) during genetic transformation of host plants and protects it from cytoplasmic nucleases, thereby mediating its secure transport to the nucleus. VirE1 is a small, acidic chaperone which is responsible for maintaining VirE2 in its soluble form as a heterodimer by preventing its oligomerization. In order to understand the mechanism of infection of Agrobacterium, it is important to investigate the binding of VirE2 and T-DNA since these are the two most essential components in infection. Real time binding kinetic analysis of viral proteins (VirE1 and VirE2) and ssDNA using SPR has shown that binding is strongly influenced by substrate and it occurs at poly T sequences and not at polyA and dsDNA. Short segments of ssDNA of length comparable to protein-binding footprint were immobilized on sensor surface and it was found that both VirE2 and VirE1-VirE2 complex bind to this immobilized ssDNA. The VirE2-ssDNA interaction was shown to be electrostatic in nature since it dissociated at high salt concentrations [102].

Succinic semialdehyde dehydrogenase (SSADH) is an enzyme located in the mitochondria and is involved in γ-aminobutyric acid (GABA) shunt. Recombinant SSADH1 from Arabidopsis has been shown to function as a homotetramer and is inhibited by NADH and adenine nucleotides [103]. SPR has been employed for detection of enzyme-nucleotide interaction by immobilizing the enzyme (AtSSADH1) on the CM5 chip by amine coupling and further monitoring nucleotide binding. NAD^+ and ATP binding to AtSSADH1 were assessed by SPR and it was found that ATP and NAD^+ have different binding sites. GTP was shown to bind competitively to the ATP binding site [104]. MsRac1 and MsRac4 isolated from *Medicago sativa* belong to the class of GTP-binding Rho proteins in plants. All GTP-binding Rho proteins share common conserved regions. The GTP-binding property of MsRac1 and MsRac4 has been shown using SPR. The interaction between GTP and G-protein was confirmed and the inhibitory effects of EDTA, GTP, and Mg^{2+} on GTP binding were characterized [105].

SPR and secondary metabolites and xenobiotics

SPR is used extensively to monitor environmental contaminants, drugs and biomolecules [106]. A system comprising of the photosynthetic reaction center (RC) from the purple bacterium, *Rhodobacter sphaeroides*, and SPR was developed for the detection of atrazine, a triazine herbicide which inhibits photosynthetic electron transfer. The SPR sensor chip was immobilized with heavy-subunit-histidine-tagged RCs (HHisRCs) by nickel chelation. This immobilization has facilitated in the detection of atrazine and can thus be employed for identification of various photosynthetic inhibitors [107]. To overcome the handling of unstable nature of natural receptor molecules during SPR analysis, artificial receptors have been developed. Molecularly imprinted polymers (MIPs) which are extensively crosslinked polymers, consist of specific recognition sites for target analytes. MIPs offer varied benefits, like insolubility in water and most organic solvents, long-term stability and chemical inertness. Ametryn, a triazine herbicide, is regarded as ubiquitous environmental pollutant. Its detection in the soybean and rice was carried out by coupling MIPs with atom transfer radical polymerization (ATRP) [106]. A self-assembled terminated aminothiol monolayer on a gold chip was coupled with the initiator 2-bromo-2-methylpropionyl bromide, followed by grafting of MIP film by the surface-initiated way. SPR spectroscopy analysis demonstrated that the imprinted sensing film exhibited higher selectivity for ametryn than the non-imprinted film.

Organophosphorus pesticides (OPPs) are widely used for the control of pests but are fatal for humans and animals. SPR combined with MIP film is a promising method for detection of OPPs, like acephate. Good recoveries and precision were demonstrated using this method for the samples of cole and apple spiked with acephate solution [108]. On a gold surface coated with a monolayer of carboxyl terminated alkanethiol, a photoinitiator was bound covalently. Thereafter, an ultrathin MIP film was prepared on an SPR sensor chip using surface initiated radical polymerization and directly characterized by FT-SPR measurements. This demonstrated impressive selectivity for acephate. Similarly, profenofos, another OPP, was detected in tap water using a highly sensitive, selective and stable SPR sensor chip using molecularly imprinted thin film as the recognition element [109].

Recently, a biosensor with higher sensitivity, selectivity and stability has been designed using magnetic molecularly imprinted polymers to amplify SPR response. This system was designed by self-polymerization of dopamine on the Fe_3O_4 nanoparticles (NPs), leading to the detection of chorpyrifos (a pesticide) up to a limit of 0.76 nm using SPR. Thus,

this magnetic imprinted Fe_3O_4@polydopamine nanoparticles (Fe_3O_4@PDA NP) can serve as an attractive recognition element for detection of pesticide residuals and other environmental contaminants using SPR sensors [110].

SPR provides quick toxin detection and offers considerable advantages over other biophysical approaches. SPR assays have been devised to identify toxins with detection limits down to a femtomolar range. Ricin is a toxic protein present in the seeds of the castor bean plant (*Riccinus communis*). Ricin cleaves the glycosidic bond between adenine residue and sugar at a specific position on 28S ribosomal RNA, thereby inactivating the ribosome. Ricin was detected by SPR at a concentration about 2,500 times less than the minimum lethal dose of 200 ng.ml^{-1}. SPR sensor surface was prepared with an antibody fragment specific for the biological toxin ricin [111]. Similarly, two antibodies (7G12 and TFTA), with strong affinity to ricin variants, were identified to recognize both horticultural and commercial ricin variants' extracts from different *Ricinus communis* cultivars. In the SPR system, antibody 7G12 was used as the capture ligand and ricin was detected with a limit of detection of 0.5 ng.ml^{-1} [112]. Since ricin binds cell-surface oligosaccharides, synthetic analogues of β-lactosyl- and β-D-galactosyl ceramides were used as the ligands and SPR analysis was used for detection in a highly sensitive manner (10 pg.ml^{-1}, 5 min) [113]. A multi-analyte SPR instrument has been developed which demonstrated ricin A detection among five distinct analytes. In this system, samples flow over the surfaces of eight sensor chips, each with 3 sensing regions, thus a total of 24 areas of detection that can be concomitantly monitored by SPR [114]. Localized SPR and synthetic glycosyl cermaides (β-lactoside) attached to gold nanoparticles (20 nm) were used to detect ricin at 30 ng.ml^{-1} [115]. Bioconjugates of single domain antibodies (sdAb) derived from llama, thermostable recognition elements that can be manipulated using standard DNA methods, with robust quantum dots (QDs), were also applied in SPR immunoassays along with dihydrolipoic acid (DHLA) ligand for detection of ricin. The DHLA-QD-sdAb conjugates, when used as reporter elements in SPR sandwich assays, lead to ~10 fold signal enhancement over sdAb reporters for detection of ricin (Figure 5) [116].

A highly potent and fatal toxin from *Abrus precatorius*, abrin, which inactivates type II ribosome, was detected to the limit of 35 and 75 ng.ml^{-1} using E12 and RF12, two human monoclonal antibodies, capable of binding to native abrin. SPR studies have helped in determining the association and dissociation rate constants and cross-reactivity for both antibodies [117].

An effective way to assess the status of plant growth and control pests and diseases is by terpene vapors detection, as they act as biomarkers during plant growth. Detection of terpene vapors was carried out using a gas sensor based on the localized SPR (LSPR) of sputtered gold nanoparticles. On exposure to terpene vapors, a decline in transmittance and redshift in wavelength was detected. Further, gold NPs with the ring-structured thiolate-capping showed a high LSPR response to terpene vapors [118].

Thus, SPR biosensors provide a sensitive, selective and efficient method for environmental monitoring applications and assessing volatile organic compounds like terpenes.

Other applications

Surface plasmon resonance imaging has been used to visualise the dynamics of cell-substratum interactions and deposition of extracellular proteins using live cells [119,120]. High resolution SPR imaging using high numerical aperture objective lens has been demonstrated as a novel approach to visualise subcellular structures in proximity to cell surface such as nucleus, focal adhesions and cellular secretions [13]. Thus SPR has provided means to quantify interactions of cells with their extracellular matrix (ECM).

An SPR system to identify bacterium-bacteriophage interactions was developed [121]. Somatic coliphages are a type of bacteriophages which are known to be indicators of fecal contamination in water. These bacteriophages were detected in wastewater using host *E. coli* which was immobilized on sensor chip gold surface via avidin-biotin interaction. The binding of bacteriophage to the bacterium resulted in change in mass density over the chip surface thereby producing a positive SPR signal [121]. SPR biosensors have been also applied for detection of genetically modified organisms (GMOs). This detection method is based on the hybridization of an ssDNA probe attached on the sensor chip and its target oligonucleotides in the analyte solution. The immobilized probe sequences are specific for NOS terminator and 35S promoter sequences which are characteristic of GMOs [122].

Major findings leading to better understanding of plant development and related processes using SPR technique have been summarized in Table 1 presented below.

Perspectives, Discussion and Conclusion

Surface plasmon resonance technique offers tremendous scope for plant biology research. It can be used to visualize the dynamics of cell-substratum interactions and deposition of extracellular proteins using live cells. High resolution SPR imaging using high numerical aperture objective lens can be used as a novel approach to visualize
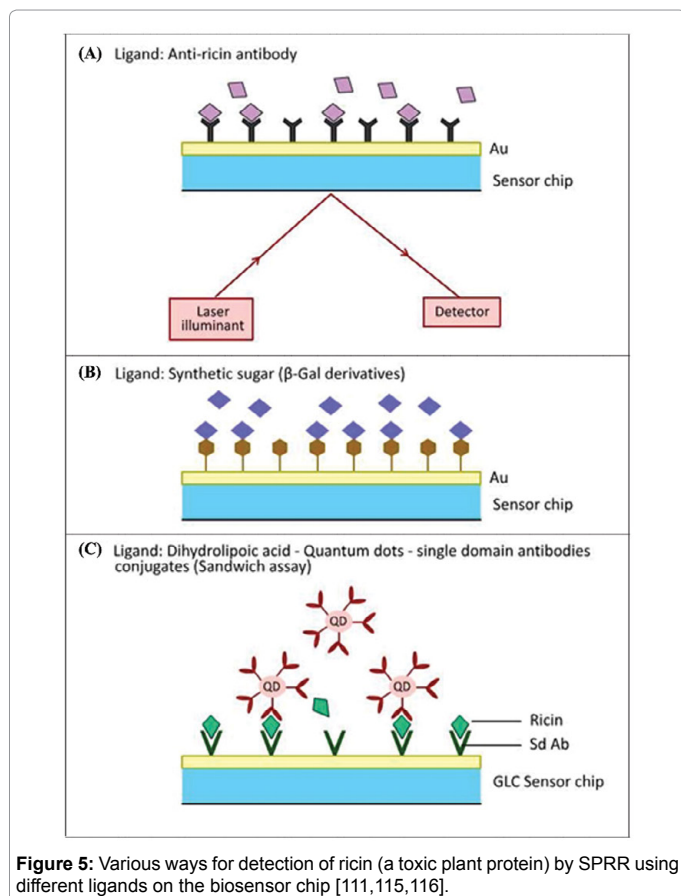

Figure 5: Various ways for detection of ricin (a toxic plant protein) by SPRR using different ligands on the biosensor chip [111,115,116].

Advancements	References
I. Lectin-carbohydrate interactions	
• Examining the relative binding molar ratios of six lectins from *Sambucus sieboldiana*, *Maackia amurensis*, *Aleuria uuruntia*, *Ricinus communis* (RCA$_{120}$), *Datura stramonium* (DSA) and *Phaseolus vulgaris*.	[30]
• Detection of lectin receptor in different soluble fractions (albumins, globulins and basic glutelins) from the seeds of *Cratylia floribunda*	[32]
• Developing potential ricin (bioterrorism agent) sensors using unnatural carbohydrates (6-substituted galactose derivatives)	[35]
• Improving the limitation of Concavalin A detection using dextran capped silver nanoparticles	[37]
II. Protein-chaperone interactions	
• Revealing the potential role of Hsp 90 (molecular chaperone) in the assembly of viral replicase protein (p93) of cucumber necrosis tombusvirus (CNV).	[40]
• Discovery of therapeutic agents (guttiferone A, guttiferone E, clusianone and nemorosome) which can modulate the ability of histone acetyltransferase (HATs), which cause severe diseases ranging from cancer to neuro-degenerative disorders.	[41]
• Discovery of kotschyin D (a tetranortriterpene from the root extracts of *Pseudricedrela kotschyi* and *Trichilia emetica)* as an inhibitor of Hsp 90 (heat shock protein 90).	[42]
• Discovery of ellagtannin geraniin (a polyphenol found in *Geranium thunbergii*) as a potent inhibitor of Hsp 90 tetratricopeptide repeat (TPR) domain-containing docking proteins (Toc64 in the outer envelope of chloroplasts, OM64 in the outer membrane of mitochondria and AtTPR7 in the ER membrane).	[43]
• Discovery of the reductant action of NADPH-dependent thioredoxin reductase (NTRC) on 2-Cys-peroxiredoxins from *Anabaena*.	[44]
• Analysis of the binding affinity of OEP61 (outer envelope protein of 61 kDa) towards Hsp 70.	[45]
	[46]
III. Diagnosis of plant viruses	
• Detection of minute differences in the binding affinities of two recombinant Fabs (antigen-binding fragments), 57P and 174P, with peptide corresponding to 134-146 residues of the coat protein of tobacco mosaic virus.	[56]
• Detection and quantitative estimation of binding TMV and antiviral IgG (protein-A) immobilized on a sensor.	
• Analysis of serological variability of *Lettuce mosaic virus* (LMV) using monoclonal antibodies.	[48]
• Detection of two closely homologous apple stem pitting virus coat proteins (PSA-H and MT32) with 81% identical amino acid sequence.	[57]
	[58]
• Evaluation of serological variability of *Potato virus Y* (PVY) and quantification of its interaction with antibodies.	[55]
IV. Phytohormones and associated metabolic pathways and signaling events	
• Identification of salicyclic acid binding proteins (SABPs).	[66]
• Functional and biochemical interactions of anti-ABA mAB (JIM19) with cytosolic proteins in rice and barley.	[73-75]
• Detection and quantification of bioactive GA in solution.	[79]
• Examining the interaction between GA receptor (GID1) and DELLA proteins using biotin-labelled peptide immobilized on the SPR sensor chip.	[79]
• Identification and quantification of ^{1}H-indole-3-acetic acid (IAA), ^{1}H-indole-3-butyric acid (IBA) and kinetin (KT) using molecularly imprinted monolayer (MIM) based SPR detection method.	[84]
• Analysis of interaction between recombinant auxin binding protein in *Nicotiana tabacum* (NtABP1) and monoclonal antibodies.	[84]
• Interaction of jasmonic acid with jasmonate domain protein (JAZ1) and F box protein (COI1).	[87,88]
V. Protein-protein interactions	
• Analysis of interaction between replicase proteins (p33 and p92) in tomato bushy stunt virus by immobilizing recombinant fusion protein on sensor chip using amine coupling as the analyte test protein (p33 or p92)	[92]
• Interaction between pectin methylesterase and its inhibitor in kiwi fruit	[93]
• Demonstration of formation of a hetero oligomeric cysteine synthase complex accompanying the biosynthesis of cysteine in mitochondria through an interaction of one tetramer of serine acetyltransferase (SAT) and two dimers of O-acetylserine (thiol) lyase (OAS-TL)	[94,95]
• Determination of the binding site of CyanoP, a cyanobacterial homolog of higher plant PsbP (an extrinsic PSII protein) in the centre of PSII	[98]
• Biomolecular interaction between protein disulphide isomerase from *Oldenlandia affinis* (OaPDI) and cyclotide precursor protein kalata (Oak1)	[99]
VI. Protein-nucleic acid interactions	
• Lactose repressor and operator interaction	100]
• Analysis of interaction between LEAFY (LFY, a transcription factor from Arabidopsis) and two promoters (APETELA 1 and 2) using biotinylated AP1 and 2, immobilized on streptividin-coated chips and different concentrations of LEAFY protein as analyte	[101]
• Analysis of binding between bacterial proteins (Vir E1 and Vir E2) secreted by *Agrobacterium tumefaciens* into the host cell and single stranded T-DNA during genetic transformation of host plants	102]
• Inhibition of succinic semialdehyde dehydrogenase	[103]
VII. Secondary metabolites and Xenobiotics	
• Detection of photosynthetic inhibitors, such as atrazine, a triazine inhibitor	[107]
• Ricin detection from different cultivars of *Ricinus communis* using antibody 7G12 as the capture ligand on SPR chip	[111-113]
• Detection of terpene, a biomarker of plant growth	[118]

Table 1: Recent advancement in plant biology using SPR technology.

subcellular structures in proximity to cell surface such as nucleus, focal adhesions and cellular secretions. An in depth understanding of signal transduction events, metabolic pathways, cell cycle and cell death coordination using SPR based protein-protein interaction, is likely to provide new information on protein-protein interactions, including their quantification, through binding kinetics, interaction affinity and selectivity, and equilibrium studies. It is difficult to immobilize GA as ligand on the sensor chip because it may cause GA to lose its binding ability to GA receptors. Also since GAs are low-molecular-weight biomolecules (346 Da), they are difficult to be directly detected as analyte on the SPR sensor chip with immobilized GA receptors. Thus, DELLA proteins can serve as potential ligand candidates for analysis of GA using SPR (Figure 4). Differentiating between structurally similar hormones, such as various indole-based hormones, is another challenge which holds promise for fruitful investigations using molecularly-imprinted monolayer (MIM)-decorated SPR detection technique. SPR data obtained from wide myriad of research investigations involving molecular chaperones can be applied for drug designing, diagnostics,

therapeutics and discovering novel biomolecular interactions. Novel and unnatural carbohydrate-based ligands for developing potential ricin (bioterrorism agent) sensors are biologically more robust than natural glycans counterparts for the sensitive and fast detection of ricin. SPR investigations in similar directions hold promise for detection of various plant toxins (Figure 5). Protein-DNA interactions are important for accessing the genetic information stored in the DNA within a cell. SPR can be used to identify binding of transcription factors (proteins) to target sites on DNA. SPR can be used for quantitative characterization of interactions between the transcription factors and gene promoters having multiple cis-acting sites. SPR can also be used to monitor environmental contaminants, drugs and biomolecules. A biosensor with magnetic imprinted Fe_3O_4@polydopamine nanoparticles (Fe_3O_4@PDA NP) can serve as an attractive recognition element for detection of pesticide residuals and other environmental contaminants using SPR sensors. SPR biosensors provide a sensitive, selective and efficient method for environmental monitoring applications and assessing volatile organic compounds like terpenes. Various features of SPR technology help in deciphering appropriate antibodies for research and diagnostic purposes, and can be extremely helpful in precise disease diagnostics and therapeutics of different plant viruses. SPR-based biosensors can also be used to explore various aspects of viral binding to functionalized surfaces and to discover an anti-viral drug.

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