Current Opinion in Solid State and Materials Science 15 (2011) 208–224

Contents lists available at ScienceDirect

Current Opinion in Solid State and Materials Science

journal homepage: www.elsevier.com/locate/cossms

Surface plasmon resonance-based biosensors: From the development of different SPR structures to novel surface functionalization strategies

Edy Wijaya [a], Cédric Lenaerts [b], Sophie Maricot [a], Juriy Hastanin [b], Serge Habraken [c], Jean-Pierre Vilcot [a,*], Rabah Boukherroub [a,d], Sabine Szunerits [a,d,*]

[a] Institut d'Electronique, de Microélectronique, et de Nanotechnologie (IEMN), UMR 8520, Université Lille Nord de France, Avenue Poincaré, BP 60069, 59652 Villeneuve d'Ascq Cedex, France
[b] Centre Spatial de Liège, Université de Liège, Avenue du Pré Aily, 4031 Angleur, Belgium
[c] Université de Liège, Hololab, Allée du 6 Août, 17, 4000 Liège, Belgium
[d] Institut de Recherche Interdisciplinaire (IRI), USR-3078, Université Lille Nord de France, Parc de la Haute Borne, 50 Avenue de Halley, BP 70478, 59658 Villeneuve d'Ascq, France

ARTICLE INFO

Article history:
Received 21 December 2010
Accepted 14 May 2011
Available online 7 June 2011

Keywords:
Surface plasmon resonance
SPR
Biosensors
Surface functionalization

ABSTRACT

Surface plasmon resonance (SPR)-based biosensors are very powerful tools for the study of biomolecular interactions, chemical detection and immunoassays. This paper reviews the performance of various SPR structures and detection schemes focusing on propagating surface plasmons generated in planar structures. Some aspects of their surface functionalization, the key element which imparts biofunctionality to these structures and hence transforming them into biosensors, will also be discussed accordingly. The ultimate performance of SPR-based biosensors will thus be determined by both their inherent optical performance and suitable surface functionalization.

1. Introduction

The first documented observation of surface plasmons dates back as early as 1902 when R.W. Wood reported unexplained narrow dark bands in the diffracted spectrum of metallic gratings illuminated with polychromatic light [1]. This anomalous phenomenon, referred to as Wood's anomaly, was then explained in terms of surface plasmon resonance (SPR) in 1968 [2]. In the same year, optical excitation of surface plasmons by attenuated total reflection was introduced by Otto [3] and Kretschmann and Raether [4]. The application of surface plasmon resonance for gas detection and biosensing was later demonstrated in 1983 [5]. Since then, the great potential of SPR sensor technology for the detection of chemical and biological substances has been receiving growing interest from the scientific community. Today, SPR-based biosensors are increasingly employed, not only in gas sensing [6], but also in many other important applications in food safety, biology, and medical diagnostics. To cite just some examples: detection of low levels of *Escherichia coli* in fresh spinach by SPR spectroscopy [7], serotyping of *Salmonella* by SPR [8], and sensing of living cells [9].

We begin this review with a brief discussion of some physical fundamentals of surface plasmons and their optical generation scheme. The concept of SPR-based biosensors and their performance characteristics are then highlighted. Finally, advances in both optical performance of SPR sensors and their surface functionalization are discussed.

2. Physics of surface plasmons

Surface plasmons, also often known in the literature as surface plasmon polaritons (SPPs) or surface plasma waves (SPWs), are electromagnetic excitations in the form of charge density oscillations propagating at the interface between a dielectric and a metal, evanescently confined in the perpendicular direction (Fig. 1).

It can be shown that surface plasmons are longitudinal waves with magnetic vector perpendicular to the plane of incidence (i.e. transverse-magnetic (TM) or p-polarized) whose dispersion relation is given by:

$$k_{SP} = \frac{\omega}{c}\left[\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}\right]^{1/2} \quad (1)$$

where k_{SP} is the propagation constant of the surface plasmons, ω is the angular frequency, c is the speed of light in vacuum, and ε_m and ε_d are the dielectric constants of the metal and dielectric,

* Corresponding authors. Address: Institut de Recherche Interdisciplinaire (IRI), USR-3078, Université Lille Nord de France, Parc de la Haute Borne, 50 Avenue de Halley, BP 70478, 59658 Villeneuve d'Ascq, France. Tel.: +33 3 62 53 17 25; fax: +33 3 62 53 17 01 (S. Szunerits), tel.: +33 3 20 19 79 65; fax: +33 3 20 19 79 66 (J.-P. Vilcot).
E-mail addresses: jean-pierre.vilcot@iemn.univ-lille1.fr (J.-P. Vilcot), sabine.szunerits@iri.univ-lille1.fr (S. Szunerits).

1359-0286/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cossms.2011.05.001

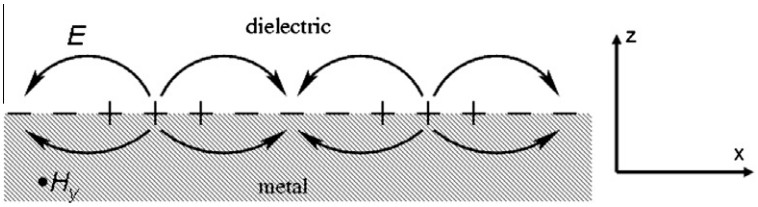

Fig. 1. Schematic illustration of charge density oscillations at a metal-dielectric interface propagating in the *x* direction.

respectively. This dispersion relation is sketched in Fig. 2 where k_x is the component along the *x*-axis of the incident light wave-vector.

Since ε_m is a complex entity, k_{SP} is also a complex entity. Its real part is related to the effective refractive index of the surface plasmons while the imaginary part is associated with the attenuation of the surface plasmons in the direction of propagation.

3. Optical excitation of surface plasmons

In optical sensors based on surface plasmon resonance (SPR), the surface plasmons are excited by light waves. Surface plasmons can be optically excited if the phase-matching condition is fulfilled, i.e., the projection along the *x*-axis of the incident light wave-vector matches the propagation constant of the surface plasmons k_{SP}. Methods of optical excitation of surface plasmons include prism, grating, and waveguide coupling.

3.1. Prism coupling

From the dispersion relation and as shown in Fig. 2, the wave vector of surface plasmons at a metal-dielectric interface is by nature larger than that of the light wave in the dielectric. This means that the surface plasmons cannot be excited directly by a light wave incident on the interface. To allow optical excitation of surface plasmons, the wave vector of the incident light must be increased. This can be accomplished by passing the light wave through an optically denser medium in the attenuated total reflection (ATR) method (Fig. 3).

A light wave passing through a high refractive index prism and totally reflected, for a specific angle, at the prism base generates an evanescent wave penetrating the thin metal film. If the thickness of the metal film is properly chosen, the evanescent wave can then tunnel through the metal film to excite surface plasmons at the other metal-dielectric interface. The resonance condition can be expressed by:

$$k_x = \frac{2\pi}{\lambda_0} n_p \sin\theta = \mathrm{Re}\{k_{SP}\} \tag{2}$$

where k_x is the component along the *x*-axis of the incident light wave-vector, λ_0 is the wavelength in vacuum, n_p is the prism refractive index, θ is the incident angle, and Re{} corresponds to the real part.

The optical excitation of surface plasmons is accompanied by the transfer of the light wave energy into the surface plasmons and its subsequent dissipation in the metal film. This process results in a drop in the intensity of the reflected light.

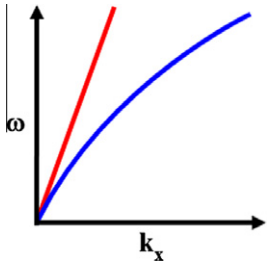

Fig. 2. Schematic illustration of surface plasmons dispersion relation (blue line). The corresponding light line in the dielectric is also shown (red line).

3.2. Grating coupling

The in-plane wave vector of the incident light wave impinging on a dielectric-metal interface can also be increased to match that of the associated surface plasmons by a diffraction grating (Fig. 4). The diffraction grating scatters the incident light wave and consequently modifies its k_x by an integer multiple of the grating wave number *G* depending on the diffraction order. The coupling condition when the *m*th diffraction is coupled to the surface plasmons can then be expressed by:

$$k_d = k_x + mG = \frac{2\pi}{\lambda_0} n_d \sin\theta + m\frac{2\pi}{\Lambda} = \mathrm{Re}\{k_{SP}\} \tag{3}$$

where k_d is the propagation constant of the diffracted light, *G* is the wavenumber of the grating, $m = 0, \pm1, \pm2, \ldots$ is the diffraction order, Λ is the grating period, and n_d is the dielectric refractive index. The detection in this case can be made for example by measuring the reflected intensity from a monochromatic light wave as a function of the angle of incidence (angular modulation).

3.3. Waveguide coupling

Surface plasmons can also be excited by guided modes of a dielectric waveguide (Fig. 5). When a guided mode propagating along the dielectric waveguide enters the region covered by a thin metal film, its evanescent field can penetrate through the metal film. Resonance can then take place if the wavelength-dependent propagation constant of the guided mode matches that of the surface plasmons at the outer metal-dielectric boundary. As this phase-matching condition is only satisfied for a narrow wavelength range, a dip in the transmitted spectrum can be observed.

4. SPR-based optical sensors

The promising potential of SPR sensors lies in the very high sensitivity of surface plasmons excited at a metal-dielectric interface to a change in the refractive index of the dielectric. A change in the refractive index of the sensed medium (the dielectric) results in a change of the propagation constant of the surface plasmons. This change subsequently alters the resonance condition between the surface plasmons and the interacting optical wave. Based on the measured characteristics of the optical wave interacting with the surface plasmons, SPR sensors can be categorized as sensors with angular, wavelength, intensity, or phase modulation.

In SPR sensors with angular modulation, a monochromatic light wave with a variable angle of incidence is used to excite surface plasmons. The excitation of surface plasmons is characterized by a dip in the angular spectrum of the reflected light at the angle

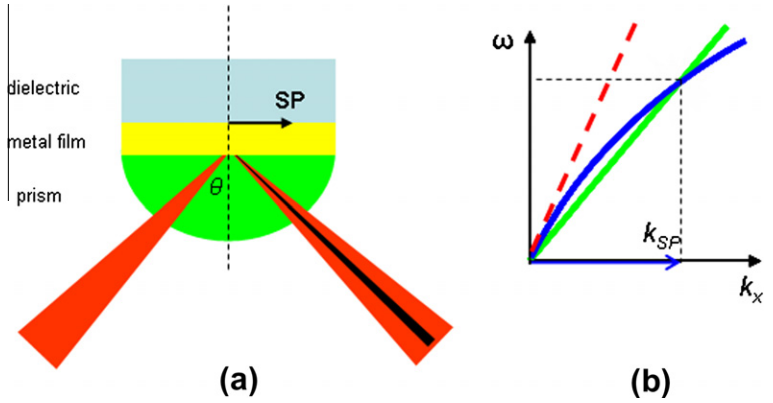

Fig. 3. (a) Excitation of surface plasmons by the attenuated total reflection (ATR) method. (b) The light line in the denser medium (green line) is tilted to the right relative to that in the dielectric (red dashed line) resulting in an increase of the incident light's wave vector. For a given wavelength, resonance takes place at a specific angle of incidence when this light line intersects the dispersion curve of the surface plasmons (blue line).

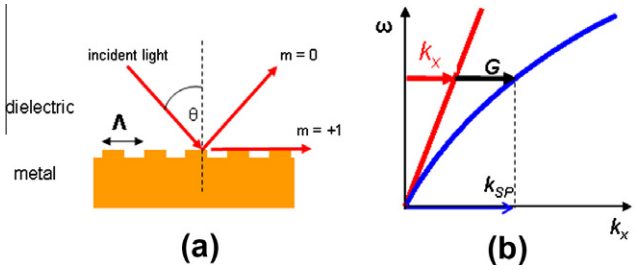

Fig. 4. (a) Excitation of surface plasmons by the diffraction grating method. (b) Dispersion diagram illustrating the phase-matching condition. The original x-component of the wave vector of the incident photons k_x is increased by G through $m = +1$ diffraction order to match that of the surface plasmons.

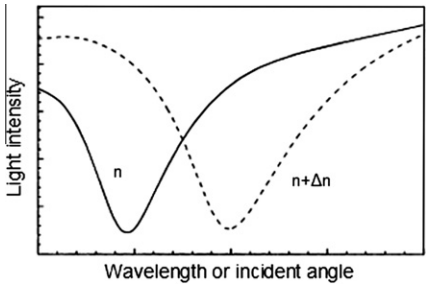

Fig. 6. Illustration of angular and wavelength modulation. The wavelength or angle of resonance shifts as the refractive index of the sensed dielectric changes from n (solid line) to $n + \Delta n$ (dashed line).

of resonance. The shift of the angle of resonance is monitored as the refractive index change of the sensed medium (Fig. 6); typically, this is the working scheme of configurations using prism coupling. In SPR sensors using wavelength modulation, a polychromatic optical wave is employed and the resonance wavelength corresponding to the surface plasmons excitation is monitored; this is, for example, the case for waveguide coupling configurations. Finally, intensity and phase shift of the interacting optical wave at fixed wavelength and angle of incidence is monitored in SPR sensors with intensity and phase modulation, respectively. Of these different modulation schemes, the angular and wavelength modulations are the most commonly employed in SPR sensors owing to their high resolution and relatively simple instrumentation. But intensity modulation can be also used, mainly in systems devoted to multiple analyses, under the SPR-i (surface plasmon resonance imaging) acronym.

5. SPR-based biosensors

A SPR-based biosensor is made up of a SPR sensor and suitable surface functionalization acting as the biorecognition element (Fig. 7). When a biomolecular interaction (e.g. specific binding of analytes) takes place, the refractive index near the surface is altered. This modification of refractive index can then be detected by the SPR sensor.

As a SPR sensor and appropriate surface functionalization form the building blocks of a SPR biosensor, it is clear that the overall

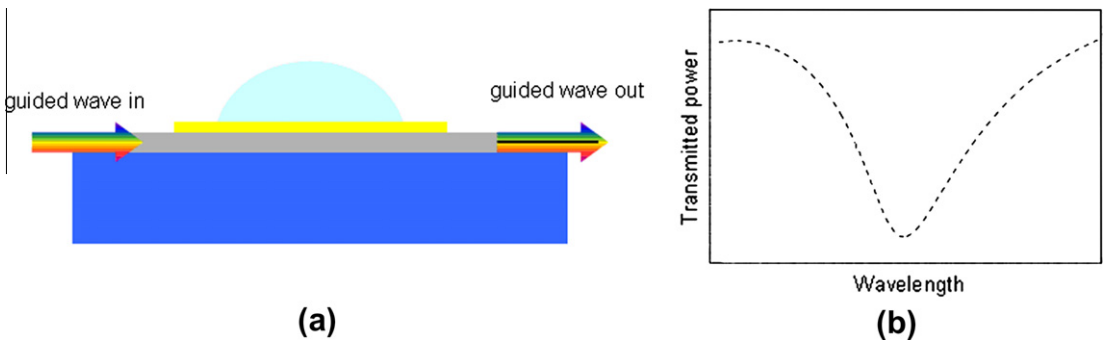

Fig. 5. (a) Excitation of surface plasmons by waveguide coupling. Dark blue layer indicates substrate, gray layer indicates waveguide, yellow layer indicates metal film, light blue layer indicates dielectric. (b) Schematic illustration of the transmitted spectrum showing a resonance dip.

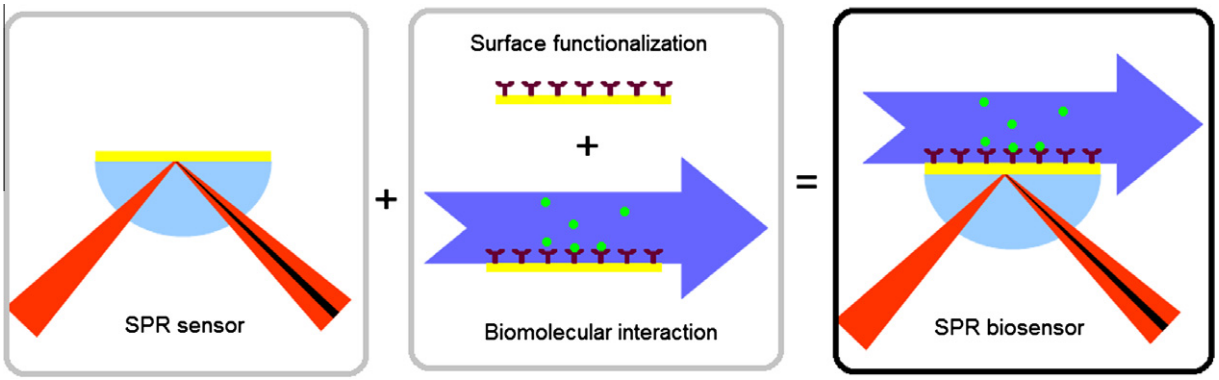

Fig. 7. A SPR sensor equipped with suitable surface functionalization as biorecognition element is transformed into a SPR biosensor. Biological analytes, represented as green dots, are shown to interact with the biorecognition elements, represented as brown Y. The large blue arrow indicates the flow of the solution to be analyzed; practically this flow is generated by a microfluidic system.

performance of a SPR biosensor depends on both the intrinsic optical performance of the SPR sensor and the characteristics of the surface functionalization. In what follows, both of these factors are separately discussed.

6. Optical performance of SPR sensors

6.1. Performance characteristics

The optical performance of SPR sensors is most frequently assessed by their sensitivity and resolution. Sensor sensitivity S is defined as the ratio of the change of the sensor output dY (e.g. angle or wavelength of resonance) to the change of the refractive index of the sensed medium dn (*RIU for Refractive Index Unit*).

$$S = \frac{dY}{dn} \tag{4}$$

To take into account the width of the resonance, sensor sensitivity is also often evaluated in terms of a figure of merit (*FOM*):

$$FOM = \frac{S}{\mathrm{FWHM}} \tag{5}$$

where FWHM is the full width at half maximum of the resonance.

Sensor resolution refers to the smallest change in refractive index of the sensed medium that produces a detectable change in the sensor output, which is determined by the noise in the sensor instrumentation. The limit of detection of SPR sensors is therefore dependent on the sensor resolution.

Changes in the calculated sensitivity between the physical and biosensor approaches are related to the biorecognition process, shown in Fig. 7. Usually, the physical approach allows one to calculate the sensitivity of a SPR sensor taking into account the global change of the refractive index of the sensed medium (Eq. (4)). Taking a closer look on Fig. 7, it can be seen that the biorecognition process only concerns a very thin layer; typically the interactions are localized in a layer whose thickness is only about 10 nm from the sensor surface. That is to say that the sensitivity of a biosensor shall be calculated taking into account a change of refractive index in this interaction region, the overall refractive index of the surrounding medium being often unchanged. Since the thickness of this interaction layer is much smaller than the common penetration depth of the plasmonic wave, the sensitivity value of a biofunctionalized sensor is likely to be different from the one that can be determined using a pure physical approach.

6.2. Advances in SPR sensor performance

Prism-based angular modulation with a conventional SPR structure consisting of a high refractive index glass prism and a thin metallic layer (typically around 50 nm of gold) on which the desired biorecognition elements can be immobilized is the most commonly used configuration in SPR sensing technology. Despite its apparent simplicity, this is actually the configuration adopted in the best commercial SPR sensor adopted by which boasts a refractive index resolution as low as 1×10^{-7} RIU (refractive index unit) [10]. In order to attain even higher sensitivity and lower limit of detection, novel SPR structures and approaches have been intensively investigated worldwide.

One of the most promising novel structures in SPR sensors is an optical multilayer structure supporting long range surface plasmons (LRSPs). Long range surface plasmons represent a special surface plasmon mode, which can be generated when a thinner metal film, typically 20–25 nm, is sandwiched between two dielectric media of similar refractive indices. This special mode can be optically excited in a modified Kretschmann geometry where a dielectric film whose refractive index is similar to that of the sensed medium is stacked between the high refractive index prism and the metal layer.

Optical field enhancement due to LRSPs has been shown to be an order of magnitude larger than that of conventional surface plasmons [11]. Compared to conventional surface plasmons, LRSPs also have lower propagation loss and longer field penetration. The lower propagation loss of LRSPs leads to a narrower resonance and the longer field penetration of LRSPs is particularly favorable for the detection of large-size analytes such as bacteria, viruses, and spores. Therefore, SPR sensors employing LRSPs are expected to offer better performance compared to sensors with conventional surface plasmons.

The exploitation of long range surface plasmons for high resolution SPR sensors was first reported in 1990 [12]. LRSP-based SPR sensors using different combinations of multilayer structures leading to a refractive index resolution of 2.4×10^{-7} RIU have been subsequently investigated [13]. Further improvement of LRSP-based SPR sensors resulted in a refractive index resolution as small as 5×10^{-8} RIU and sensitivity as high as 59,000 nm/RIU [14,15].

A novel approach to spectroscopy of surface plasmons based on the coupling of polychromatic light waves to surface plasmons and the simultaneous dispersion of the optical wave by a special diffraction grating referred to as SPR coupler and disperser (SPRCD) has been proposed [16]. In this method, a portion of the incident polychromatic light wave is coupled to surface plasmons via the second-order diffraction while the light wave simultaneously

dispersed via the first-order diffraction is projected onto a position-sensitive detector. The need for a spectrometer in this system is therefore effectively eliminated making this design attractive for simple and low-cost SPR sensors. Subsequent experimental demonstration of this approach shows a refractive index sensitivity of around 620 nm/RIU and resolution as low as 3×10^{-7} RIU [17].

In addition to higher sensitivity and lower limit of detection, compactness is also a desirable feature in SPR sensor technology. Therefore, different designs of miniaturized or integrated SPR sensors have been actively explored in recent years.

Optical fiber-based SPR sensors offer a possibility for sensor miniaturization and are extremely attractive for in vivo applications. Miniaturization of SPR sensors based on optical fibers with a refractive index resolution of 4×10^{-5} RIU has been proposed [18]. In these sensors, the cladding layer of the optical fiber is locally removed and subsequently covered by a thin gold film to enable the excitation of surface plasmons. A theoretical analysis shows that by using a Bragg grating, the refractive index resolution of optical fiber SPR sensors could be lowered to 2×10^{-6} RIU [19]. Tapering of the fiber core has also been theoretically shown to increase the sensitivity of optical fiber SPR sensors by a factor of 15 and this improved sensitivity could be further enhanced by a factor 4 by introducing a Teflon layer between the core and the metal layer to excite long-range surface plasmons [20].

Integrated optical waveguide SPR sensors are particularly promising candidates for the development of miniaturized multichannel sensing devices on a single chip. A SPR sensor based on an integrated optical waveguide with a sensitivity of 2100 nm/RIU and a resolution of 1.2×10^{-6} RIU has been reported [21]. The waveguide was fabricated by a $K^+ \leftrightarrow Na^+$ ion-exchange method on BK7 glass substrate. The original operating range of the sensor around a refractive index of 1.44 was then optimized for an aqueous environment (refractive index around 1.33) relevant to biosensing by using a tantalum pentoxide overlayer. A SPR sensor based on a germanium-doped silicon dioxide waveguide on silicon substrate with slightly higher sensitivity (2500 nm/RIU) has also been demonstrated [22]. The waveguide was fabricated by plasma-enhanced chemical vapor deposition (PECVD) method, which allows precise control of the refractive index difference between the core and the cladding layer.

In view of developing high-throughput fabrication with lower cost, a single-mode SPR waveguide sensor fabricated by polymer imprinting technique has been proposed [23]. This sensor is based on intensity modulation with a refractive index resolution of approximately 3.8×10^{-4} RIU. An optical waveguide SPR sensor with dual light-emitting diodes and a photodiode based on the detection of differential intensity shift at two different wavelengths has also been reported [24]. The refractive index resolution of this sensor is estimated to be around 2.3×10^{-5} RIU. The relatively poor resolution of these sensors is rather characteristic of sensors based on intensity modulation whose typical resolution is on the order of 10^{-5} RIU [25].

Lastly, combining the conventional Kretschmann geometry with metamaterials can potentially improve the performance of SPR sensors as well. The use of an array of gold nanorods to replace the continuous gold film in the Kretschmann geometry resulting in enhanced SPR performance with refractive index sensitivity as high as 32,000 nm/RIU has been experimentally demonstrated [26,27].

7. Commonly used and newly developed surface functionalization strategies

One of the first steps in any SPR-based protocol concerns the way the receptor molecules are anchored onto the SPR surface. The technique of SPR becomes indeed interesting when the metallic film, supporting the propagation of charge density waves (the plasmons), is chemically modified. The bioreceptors are either physisorbed or chemically attached onto the sensor surface. Covalent attachment is often preferred, as it provides strong and stable binding of the receptor to the SPR surface. This allows consequently easy regeneration of the sensor interface using conditions, which can remove the analyte from the surface, but not the attached ligand itself. The covalent immobilization strategies include chemical reactions such as amine, aldehyde or thiol coupling on previously formed functional self-assembled monolayers. The most widely used approach for the introduction of functional groups onto thin gold SPR films is based on thiolated organic compounds, which spontaneously form self-assembled monolayers (SAMs) on gold surfaces. The use of polymers is an alternative with associated advantages and disadvantages. The selection of one over the other depends on the application sought after. In general, polymeric layers such as carboxylated dextran (CM-dextran) thin films allow molecules to move freely on the surface, thus reducing some problems with binding interference. In addition, they provide more attachment points than a monolayer and act as a buffer to reduce nonspecific binding to the surface. However, it is more difficult for molecules to diffuse through such a hydrogel than through SAMs, which can potentially alter the measured kinetics. While monolayer films do not show such diffusion problems, they are prone to be less sensitive if not properly formed. However, this problem was addressed in the literature showing that, in fact, there is no difference in binding kinetics between monolayers and a dextan hydrogel [28]. Thus, whether the coating is a monolayer or a thin film, the selection of the immobilization procedure is mainly application driven. Generally, flat surfaces with SAMs are beneficial compared to polymeric layers both when the analytes of interest are larger than molecules, such as cells and viruses, and for kinetic parameter determination, when a low amount of non-specific binding is required and a low level of immobilization is recommended [29]. The achievement of negligible non-specific binding to the SPR sensor interface is an important factor contributing to the success of the sensor applications. Non-specific binding contributions lead to errors in concentration determination and in calculation of kinetic constants. Reduction of non-specific binding can be achieved by creating more hydrophilic interfaces or by including compounds such as polyethylene glycol derivatives in the immobilization steps or incorporated into the ligand to be attached onto the SPR surface [30].

Next to the selected immobilization strategy to bind the receptors to the SPR chip, the choice of the metal film is critical for sensitive SPR sensing. Gold is the substrate of choice for SPR measurements for mainly two reasons: it is relatively stable in aqueous environments required for monitoring biomolecular interactions and can be easily functionalized through the formation of alkanethiols SAMs. However, gold is not the best candidate for achieving high-sensitivity SPR sensing. Theoretical modeling of SPR in conducting metal oxide thin films has been performed by Franzen et al. who suggested that ITO could be a better suited substrate [31]. However, this would require excitation and detection in the infrared range. In the conventional visible range, silver substrates appear to be the most appealing, because plasmon coupling exhibits a sharper angular resonance as compared to that on gold, yielding an increased sensitivity [32]. Silver substrates can be functionalized similarly to gold ones, using thiol or disulfide molecules which adsorb from solution and self-assemble into densely packed monolayers or by depositing polymers. However, silver suffers from a poor chemical stability, which hampers its wide use for SPR sensing. One strategy to circumvent this limitation is to use bimetallic silver/gold layers [33,34]. In this case, the usual thiol-on-gold chemistry can be performed for coupling probes to the

sensor surface. Alternatively, lamellar structures, in which a thin layer of a dielectric is deposited onto the surface plasmon active metal thin film, were developed [35–47]. These overlayers allow an efficient protection of the underlying silver film and at the same time open the scope for new surface functionalization schemes, which can be employed for anchoring ligands to the SPR sensor chip. Currently, the investigated dielectric layers are either oxide based [35,37–45], where the attachment of ligands to the surface is mainly achieved through silanization of the surface hydroxyl groups. The other approach consists on the deposition of carbon [36] and amorphous silicon–carbon alloys [46,47]. These thin carbon-based films allow covalent immobilization of biomolecules of interest using well-developed and robust chemistry based on the attachment of alkene-containing molecules to the substrate through carbon–carbon or Si–C bonds. The different concepts will be presented in the following with additional highlights of newly developed surface functionalization routes, without being exhaustive.

7.1. Thiolated functional groups

While new strategies for the immobilization of bioreceptors have been developed in the last ten years, the most commonly used strategy for the introduction of surface functional groups onto SPR chips is still based on the attachment of sulfur-containing ligands. Gold–sulfur (Au–S) bonds are readily formed on gold enabling in an easy and fast manner the direct attachment of receptors to the gold surface by the formation of self-assembled monolayers. Several reviews are devoted to this topic and the reader is referred to them [48,49]. The Au–S chemistry has thus made possible the routine analysis of aqueous binding events to immobilize molecules at nearly neutral pH and moderate temperature.

Two strategies are employed for making bioreceptor SAMs on gold. The first is based on the separate synthesis of the bioreceptor derivative with a pendant alkanethiol group and subsequent formation of the SAM [50–52]. This is often used in the case of DNA, where a thiol (SH) group can be easily incorporated onto the 5′ end. The drawback of this strategy arises from the higher synthetic effort, especially for more complex bioreceptors. Furthermore, as the complexity of tethered bioreceptors increases, there is no guarantee that the molecules will pack to form a structurally well-defined monolayer. To control the surface density, the use of mixed monolayers by diluting with short chain thiols (e.g. mercaptohexanol) is thus often preferred [50].

The second strategy is based on the direct chemical transformation of the functionalized monolayer [53–57]. In most cases, 11-mercaptoundecanoic acid (MUA) is used and the gold film was modified by immersing the gold surface into a 1 mM ethanolic solution for at least 18 h followed by thorough rinsing with ethanol and water (Fig. 8) [55]. The presence of surface linked carboxylic acid groups enables the covalent immobilization of aminated or thiolated bioreceptors using a variety of different protocols. It was, for example, shown that thiolated DNA (HS-DNA) can be linked *via* electrostatically bound poly(L-lysine) onto the deprotonated acid groups, followed by exposing to a solution of a bifunctional linker (e.g. sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SSMCC)), which contains a N-hydroxysulfosuccinimide (NHS) ester and a maleimide functionality. The NHS ester end of the linker reacts with some of the free lysine residues on the electrostatically adsorbed poly-L-lysine, resulting in surface containing reactive maleimide groups. The maleimide functional groups react further with HS-DNA as schematically outlined in Fig. 8, with an absolute surface coverage of immobilized DNA $\Gamma = 3 \times 10^{12}$ molecules cm^{-2} [58]. The density of DNA probes is in the right order using this approach. It is to be noted that DNA hybridization can be monitored by SPR only for a surface coverage of immobilized DNA probe $\Gamma \approx 10^{11}$ molecules cm^{-2} or higher [58]. The presence of poly-L-lysine modified surface minimizes non-specific adsorption of DNA onto the gold surface. A different approach is based on the direct anchoring of amine-terminated DNA (NH_2–DNA) by the carbodiimide-catalyzed amide bond formation reaction with the surface linked carboxyl groups using EDC and NHS (Fig. 8).

Another possibility is the use of amine-terminated thiols such as 11-amino-1-undecanethiol (MUMA) (Fig. 9). The covalent linking of amine-terminated DNA can be achieved by the mentioned NHS/EDC reaction on electrostatically adsorbed poly-L-glutamic acid onto the formed 11-amino-1-undecanethiol monolayer, through amide bond formation [56]. Such an interface showed very high surface hybridization efficiency and very little non-specific adsorption. It was successfully used in complex and multistep biosensing assays.

Besides these rather common approaches, more complex thiolated structures can be immobilized on the metal surface of SPR chips. For example, thiolated triethylene–glycol units carrying a hydroxyl terminal function can be directly self-assembled onto gold using the same concept as outlined before (Fig. 10). Such SAMs are useful in preventing non-specific protein binding. The hydroxyl group of the SAM can be, for example, further reacted with amine-terminated nitrilotriacetic acid (NTA), to which His_6-tagged carboxyl-tails of the L-type Ca^{2+} channel α_1-subunit can be bound [59].

These are indeed merely some examples of bioreceptors immobilization using thiol chemistry. One of the limitations of this approach resides on the kind of functional thiolated molecules, which can be synthesized and the follow up reaction to bind the receptor. Recently, SAM templates incorporating alkyne terminal groups were used for covalent linking of bioreceptors carrying an azide-functional group using "click" chemistry (Fig. 11) [60]. "Click" chemistry is a generic term describing a range of chemical transformations characterized by high efficiency, selectivity and tolerance to a variety of solvents and functional groups. The concept was introduced by Sharpless [61] and is usually based on the copper(I) catalyzed triazole formation through the classic Huisgen 1,3-dipolar cycloaddition between azides and alkynes. The appealing characteristics of the azide-alkyne click reactions have led to its rapid utilization in a range of applications including organic, medicinal, polymer and materials chemistry.

Finally, cystein mediated immobilization strategies have to be mentioned as they offer in particular promising results for the immobilization of antibodies. Simple physical adsorption of antibodies was shown to suffer from random orientation and antibody denaturation, yielding poor reproducibility. More stable immobilization is obtained by covalent attachment, however, this causes disordered antibody orientation, which can result in the loss of biological activity. Orientated antibody immobilization, where antigen binding domains are well exposed to the assay solution, is one of the key issues in the development of immunosensors. Cystein-mediated protein immobilization has been widely used in this respect. Cystein residues can be genetically introduced into a specific site of the target protein [62]. Such modified proteins form properly oriented protein layers through the thiol group of the cystein function. This approach was for example successfully used for the direct immobilization of protein G variants onto gold SPR chip [62].

Currently the primary new research focus using thiolated SAMs in connection with SPR is either orientated towards a decrease in the number of surface reaction steps involved in bioreceptors linking or the formation of stimuli-responsive interfaces. In this respect, an oligo(ethylene glycol) molecule with twelve repeating ethylene glycol units modified to include a pyridyldisulfide end

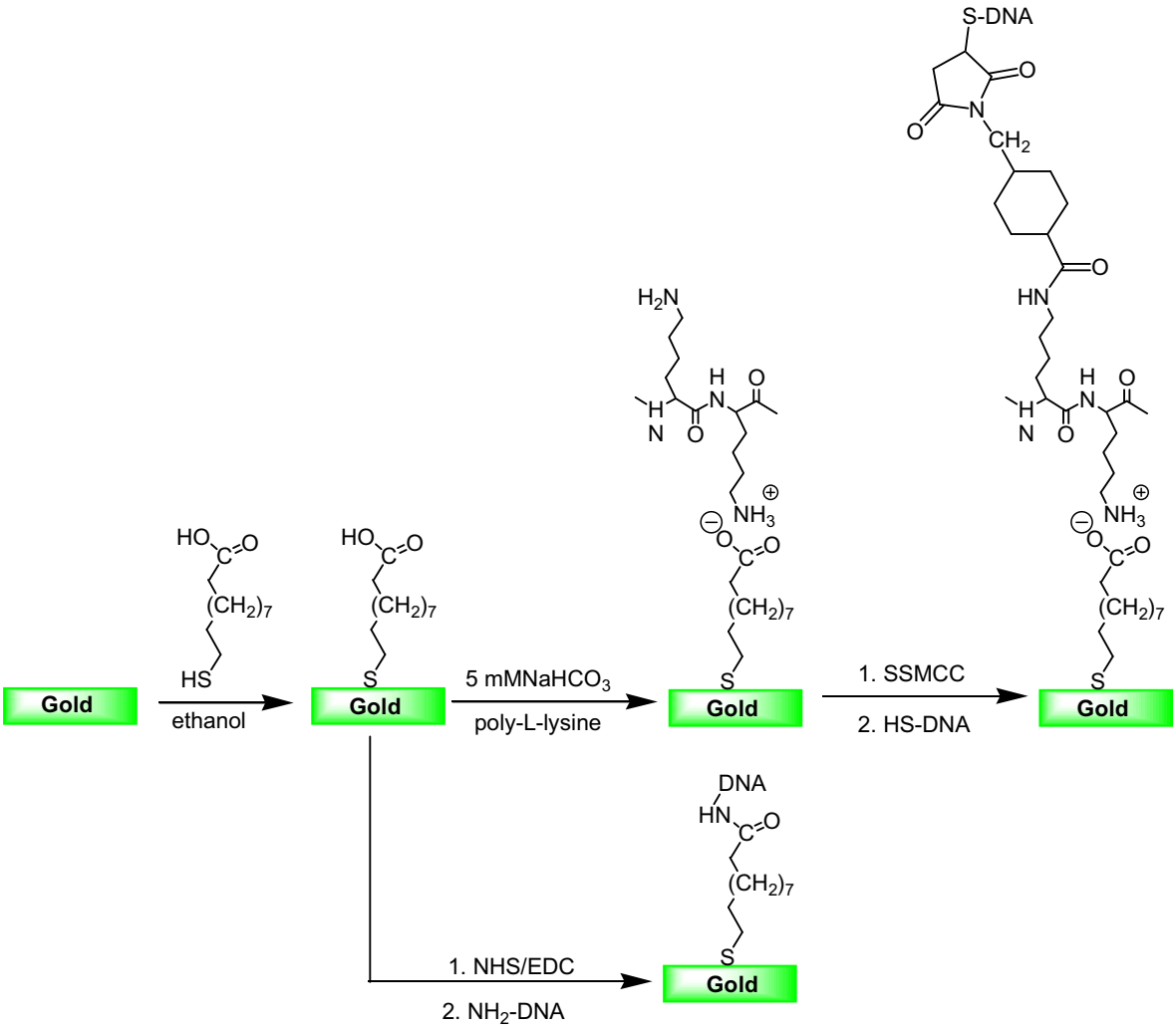

Fig. 8. Formation of 11-mercaptoundecanoic acid (MUA) on gold followed by linking of thiolated DNA or amine-terminated DNA molecules.

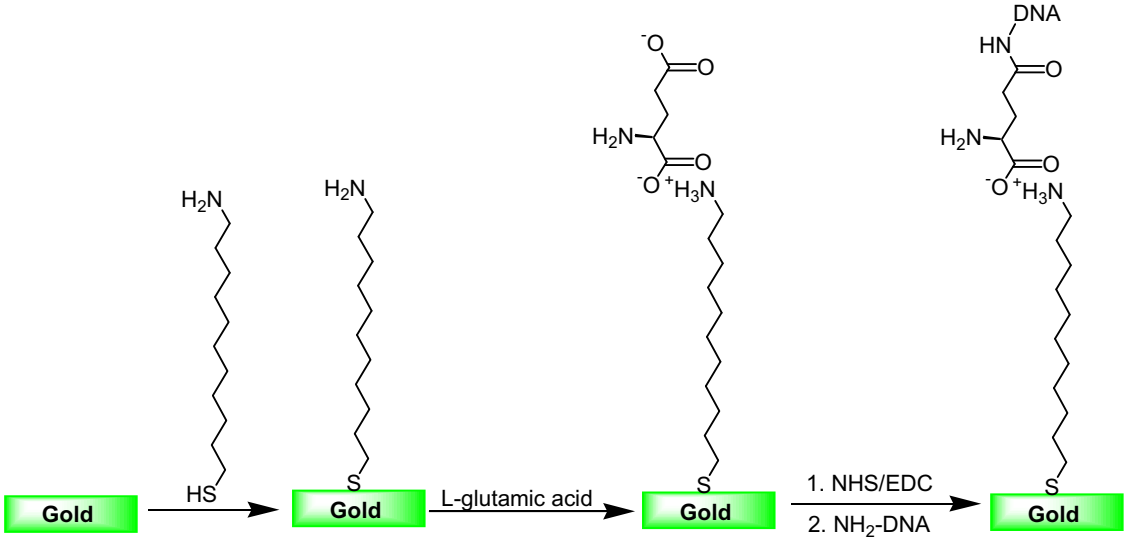

Fig. 9. Schematic illustration of gold functionalization with 11-amino-1-undecanethiol and subsequent linking of amine-functional DNA molecules.

group generated an interface highly responsive to temperature changes in the vicinity of the physiological temperature, 37 °C [30]. Such a SPR interface exhibits switchable physical properties, which are highly interesting for biomedical applications. It has been shown that such an interface allows controlling the affinity binding of streptavidin to biotin-tethered surface [30].

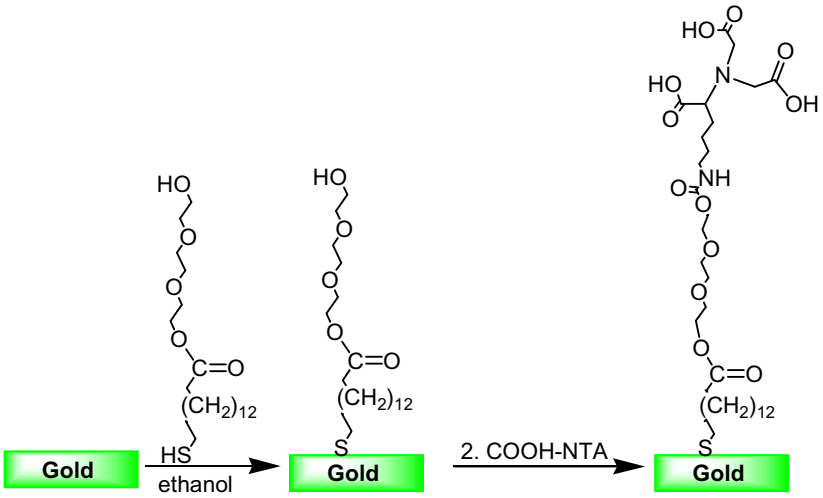

Fig. 10. Immobilization of thiolated triethylene–glycol units onto gold substrate followed by linking of nitrilotriacetic acid (NTA).

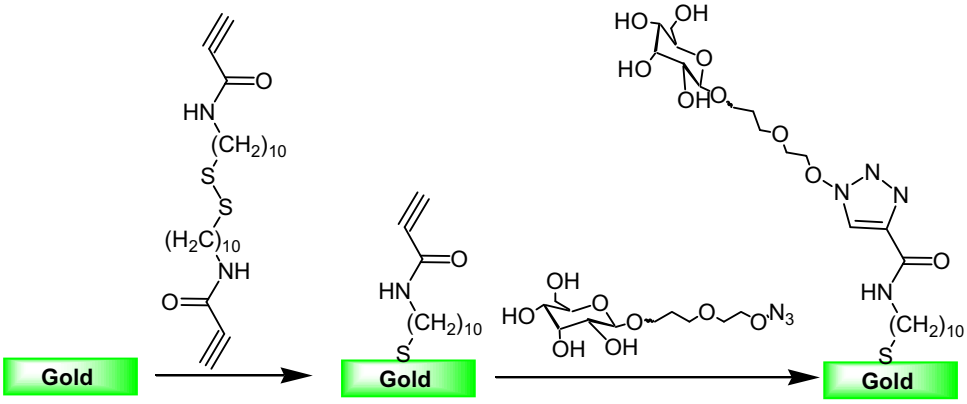

Fig. 11. "Clicking" carbohydrates carrying azide function onto alkyne-terminated gold SPR interfaces.

However, independent from the intensive use of thiolated compounds for the immobilization of bioreceptors, the susceptibility of the gold–sulfur bond to oxidation and photodecomposition is a real challange for thiol chemistry. Other functionalization schemes based on hydrogels and conducting polymers have been investigated in parallel over the years and will be discussed in more details in the following section.

7.2. Carboxymethylated dextran layers: the Biacore chip

Next to the formation of Au–S bonds, one of the most common strategies to immobilize bioreceptors is to use activated carboxymethylated dextran (CM-dextran). Dextrans are hydrophilic, non-charged neutral polymeric carbohydrates, soluble in water in any proportion forming highly hydrated hydrogels. Due to these properties, dextrans display very low non-specific interactions with bioreceptors. Owing to the high concentration of hydroxyl groups in the dextran matrix, chemical modification is possible without significantly affecting its hydrophilicity. The essentially non-branched polymer chains are highly flexible and ligands immobilized in dextran matrices are thus well accessible. Introduced by Lofas and Johnsoon [63], it is one of the commercially available SPR chips commercialized by Biacore. The matrix is constructed by self-assembly of 1,ω-hydroxyalkythiol (16-mercaptohexadecane-1-ol) onto gold, followed by covalently linking of the dextran polymer by activation of the hydroxyl groups with epichlorohydrin under basic condition to yield epoxides, which by further reaction with bromoacetic acid results in the formation of surface linked carboxymethylated dextran films (Fig. 12) [64]. The thickness of the polymer matrix is about 100 nm and the carboxylic acid groups can be further used to covalently link amine-terminated or thiol-terminated bioreceptors as discussed before hand for acid terminated SAMs [65].

The references which use this type of substrate are countless, as this kind of interface, known as CM5 chip, is commercialized by Biacore. More recently, carbohydrate epitopes were also immobilized on CM5 SPR interface using Staudinger ligation chemistry [66]. This was accomplished by first introducing azide functionalities to the CM5 interface, followed by reaction with phosphane-modified carbohydrate ligands (Fig. 13). The advantage of this approach is that the chemistry employed is extremely mild and can be easily adapted to any biosystem.

7.3. Polymer films

7.3.1. Conducting polymers

One important aspect when constructing biosensors is the suppression of non-specific adsorption of the bioreceptor on the sensing surface. In most cases, the degree of non-specific adsorption determines the sensitivity and specificity of a biosensor. Some effects such as hydrophobicity, surface charge and pH have been identified for being essential to decrease non-specific adsorption.

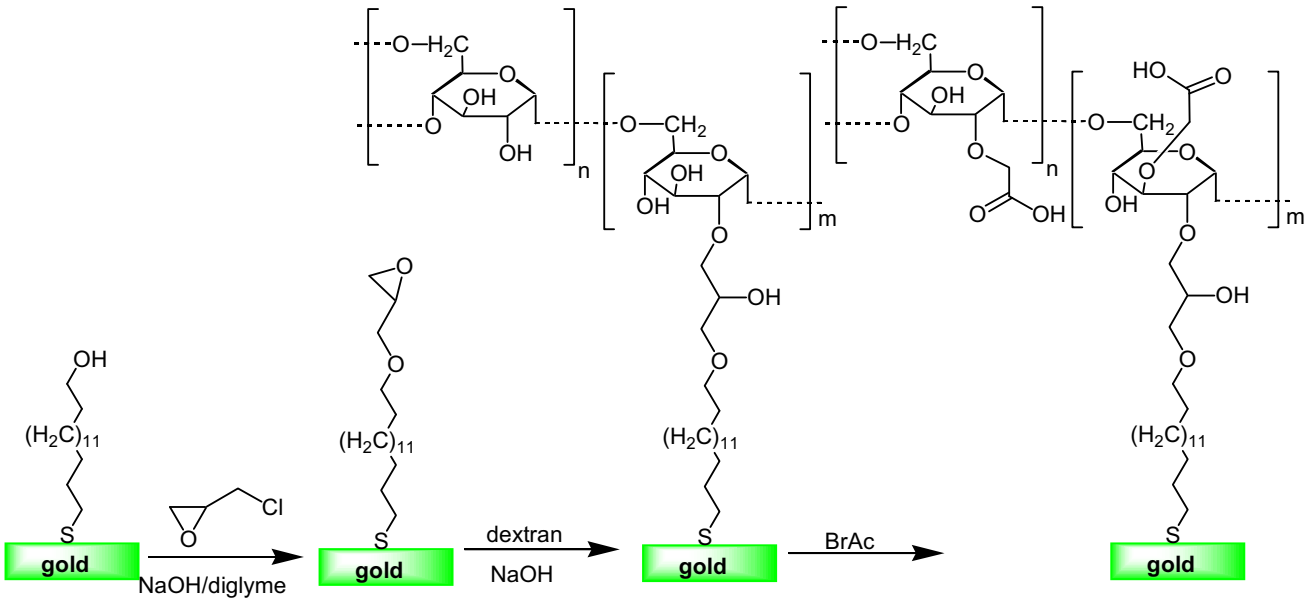

Fig. 12. Formation of surface-linked carboymethylated dextran polymer films on gold.

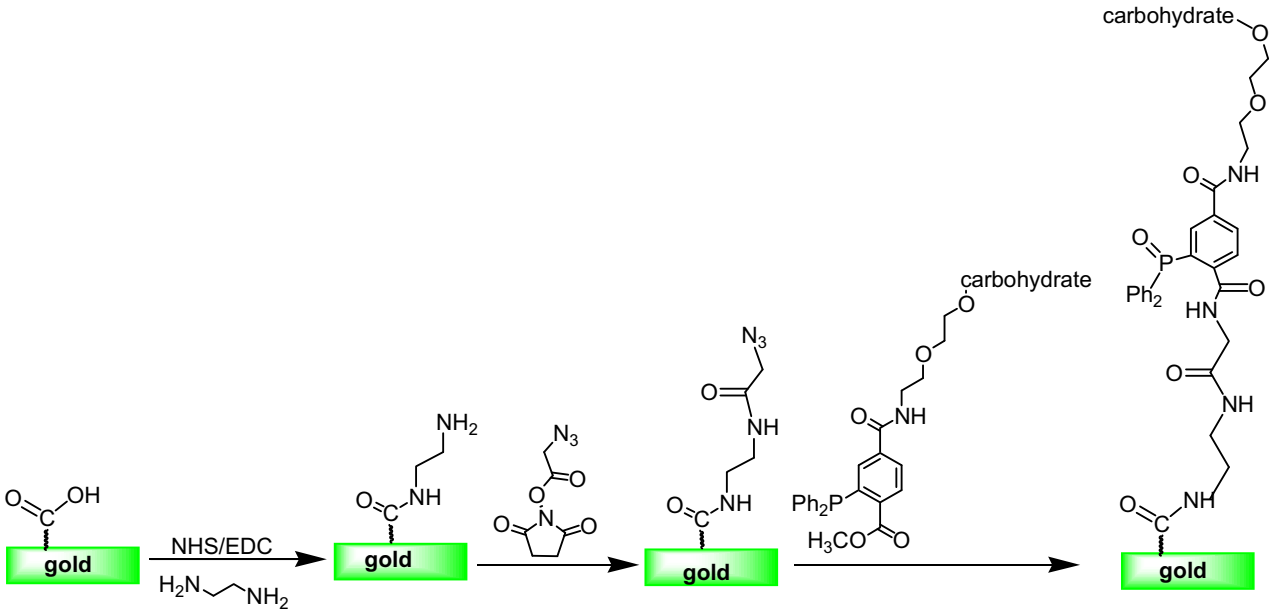

Fig. 13. Conjugation of carbohydrate epitopes to a CM5 interface using Staudinger ligation chemistry.

A convenient way to modify the surface of conducting materials such as the gold interface of the SPR chip is through electrodeposition of thin oligomeric or polymeric films from solution monomers [67–70]. The intensive use and interest in such interfaces for anchoring bioreceptors onto biosensor interfaces is driven mainly by the following factors: (i) the polymer films are uniform, (ii) their thickness can be readily controlled and (iii) the surface modification is limited to the surface of the electrode. In addition, electrochemistry can be easily integrated with SPR measurements [68,71,72]. Both methods are compatible in the sense that they both rely on a conductive substrate: for electrochemistry the gold film of the SPR interface is the working electrode, without disturbing its use for the generation of surface plasmons.

Polypyrrole is probably the most intensively investigated and widely used conducting polymer for biosensing, mainly owing to its stability, conductivity and biocompatibility [67]. The entrapment of a bioreceptor within the polymer constitutes a simple one-step method during the electrochemical polymerization, but suffers greatly from the poor accessibility of the target molecules due to the polymer hydrophobicity. The use of functional polypyrrole films is thus a more promising approach [67,69]. Functional monomers such as pyrrole propylic acid are commercially available by Sigma–Aldrich, where the abundant carboxyl groups provide a versatile platform for direct covalent binding of bioreceptors. However, beside pyrrole, which can be easily electropolymerized from aqueous solutions, the relative low conductivity of poly(pyrrole propylic acid) makes the film growth more difficult. Copolymerization with pyrrole monomers overcomes this disadvantage (Fig. 14) [69,73].

This approach is not limited to acid functions. It was, for example, reported that the generation of a SPR sensor specific to lectins such as PNA and Maackia amurensis based on the electrochemical

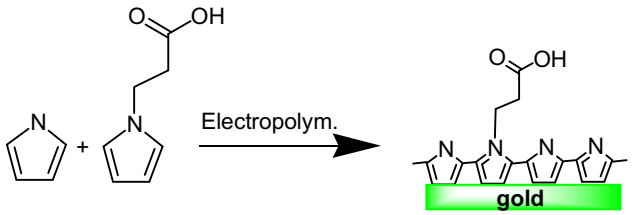

Fig. 14. Modification of SPR interfaces with a polypyrrole/poly(pyrrole propylic acid) copolymer.

polymerization of oligosaccharide derivatives functionalized with pyrrole groups shows specific binding characteristics with detection limits in the low nM range [68]. The polypyrrole based surface functionalization scheme in combination with electrospotting process can also be successfully applied for the fabrication of DNA arrays with SPR imaging read out [74], and has become a routine technique for the fabrication of SPR chips.

7.3.2. Plasma polymerized surface modification

Plasma polymerization techniques have been largely investigated over the last decade to deposit highly reactive, functional thin films with potential biomedical applications. One of the advantages of plasma polymerization techniques is that the density of a particular functional group and the degree of cross-linking within the polymer network can be largely tailored by careful control over the energy input during the deposition. The types of films that have been found to be particularly suitable for biosensing applications are those containing reactive groups such as carbonyl, amines, carboxylic acids and anhydrides. For example, amine-terminated or amine bearing bioreceptors such as bovine serum albumin (BSA) can be irreversibly linked to plasma-polymerized films carrying maleic anhydride functions (Fig. 15A) [75]. The introduction of esters has been more complicated due to the difficulties encountered to retain the active functional groups during the plasma deposition. However, it has been shown recently that thin films from pentafluorophenyl methacrylate can be deposited *via* plasma polymerization, where the highly reactive ester groups allow the subsequent coupling with aminated bioreceptors (Fig. 15B) [76].

7.3.3. Recent developments: intelligent polymeric materials for SPR

Recently, there has been great interest in the development and integration of intelligent materials, which are sensitive to physical, chemical and/or electrical stimuli into biosensing devices. Poly(N-isopropylacrylamide) (PNIPAAm) is one of most popular polymers used in this respect. It is a heat-sensitive polymer that changes its hydrophilic/hydrophobic state rapidly and reversibly, according to the temperature of the surrounding atmosphere. Under the lowest critical solution temperature (LCST), about 32 °C, the polymer is hydrophilic and extended, while it becomes hydrophobic in a form of a shrunken helix above the LCST. Anchoring NHS-functionalized PNIPAAm onto amine-terminated SAM modified gold interfaces allowed for example controlling the amount of anti-biotin antibody binding by the structural transformation of the polymer film by the temperature variation and monitoring the process by SPR [77].

An innovative approach for achieving a low level of non-specific interactions consists on the synthesis of a thiol-terminated reactive polymer, polythiol, suitable for protein covalent immobilization (Fig. 16) [29,78]. O-Phthaldialdehyde reacts with thiols to offer thioacetals, which in a further reaction with primary amines results in the formation of an isoindole complex. The interest in this approach is that the thioacetal groups do not need any specific activation to react with proteins, thus making it attractive for sensing. In addition, the polymer contains thiol groups that promote self-assembly on gold.

The same research group proposed an interesting alternative for the local structuring of SPR interfaces [79]. It is based on the *in situ* formation of a functional polymer film using the energy of the evanescent wave generated on the surface of a SPR device [79]. The method is based on the polymerization of a mixture of N,N-methylene-bis-acrylamide (MAA) and methacrylic acid using a photoinitiator couple consisting of methylene blue (sensitizing dye) and sodium p-toluenesulfinate as reducing agent at the focal point of the SPR. No polymerization occurred in solution or any other sites on the SPR surface. Varying the monomer concentration and

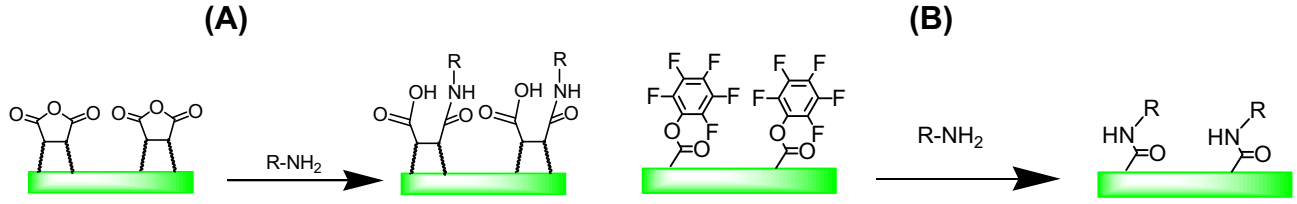

Fig. 15. Reaction of plasma polymerized thin films carrying (A) maleic anhydride or (B) pentafluorophenyl active ester functions.

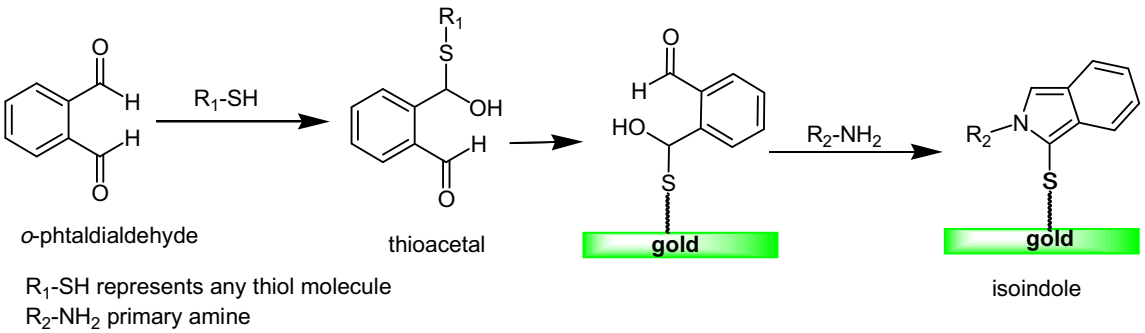

Fig. 16. The reaction between o-phthalialdehyde and thiol to yield thioacetal and isoindole derivatives.

exposure time allowed a precise control over the polymer thickness (20–200 nm), while standard coupling chemistry using EDC/NHS was used for the immobilization of Protein G [79].

Very recently, polysilsesquioxanes have been deposited as thin films onto SPR chips by spin coating and post-curing [80]. Polysilsesquioxanes are an emerging class of hybrid (organic–inorganic) nanostructured polymers containing nanosized cages. Chemical pre-functionalization of the polysilsesquioxane with pentafluorophenyl acrylate units allows the immobilization of amine-terminated bioreceptors by simple dipping into the solution of the analyte without the need of activation process (Fig. 17).

7.4. Lamellar SPR structures based on dielectric overcoatings

7.4.1. Glass and oxide-like overcoatings on gold

While SAMs are widely used due to the ease of incorporating carboxylate, amine or hydroxyl groups, the drawbacks of such films include limited chemical and electrochemical stability. Moreover, a poor orientation and potential problems of protein adsorption and fouling is often encountered if no synthetic effort in the synthesis of more sophisticated thiols is made. In addition, while the surface chemistry developed on gold has been of great value [48,49], the limitations of working on gold are becoming more noticeable with increasingly complex fabrication requirements for biometric systems and arrays. Alternative routes and improvements were thus sought after. Silicon dioxide-based materials such as glass (silicate) are standard materials for biosensing being inexpensive and benefiting from a rich variety of well-developed attachment schemes based on silane-coupling chemistry. If the knowledge on glass could be applied to SPR, many existing protocols and commercial products could be transferred without significant effort. In fact a number of strategies developed on glass have already been adapted to work on gold. However, adapting often proves to be a complex process, requiring time-consuming synthetic work and extensive manipulation of the existing protocols to be effective on the new surface. An ideal way to simplify the process is to coat the gold SPR substrate with thin silicate materials (Fig. 18A). Since the SPR signal decays exponentially within about 200 nm from the surface, the coating must have a thickness on the order of some nanometers to retain high detection sensitivity. The theoretically calculated influence of the thickness of a silica overlayer with a refractive index of $n_{SiOx} = 1.48$ is presented in Fig. 18B. Increasingly thick SiO_x layers results in a shift of the surface plasmon minimum to higher angles and is accompanied with a decrease of absorbed light at the resonance minimum [40]. A SiO_x overlayer of 5–10 nm in thickness shows the most favorable parameters in terms of SPR signal, homogeneity, pin hole free and chemical stability required for sensitive SPR sensing.

For many years, the use of such lamellar structured SPR chips for biosensing applications was hampered as the gold/SiO_2 interface was in most cases not stable upon immersion into water

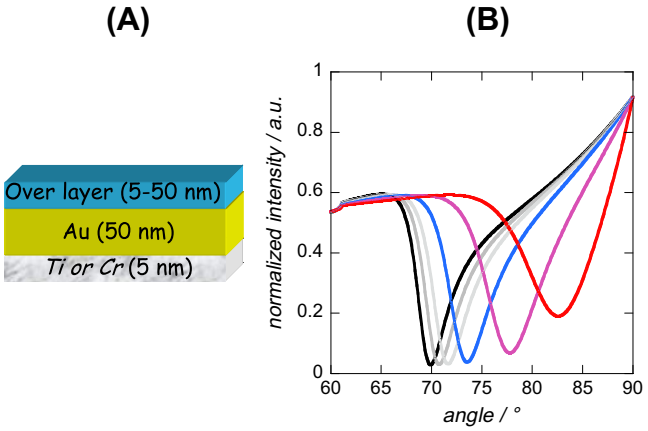

Fig. 18. (A) Schematic illustration of lamellar structure of dielectric-on-gold substrate, (B) Theoretical influence of silica thickness ($n_{SiOx} = 1.48$) on the SPR signal. The SPR chip consists of 5 nm titanium adhesion layer ($n = 2.36 + i3.47$) and 50 nm gold layer ($n = 0.197 + i3.67$) using a prism with $n = 1.52$: 0 nm (black), 5 nm (gray), 10 nm (bright gray), 20 nm (blue), 40 nm (magenta), 60 nm (red) thick silica layer.

and PBS buffer. The thin silica layer peels off the surface within a few minutes [35]. Different strategies for the formation of stable gold/SiO_2 substrates were reported recently. Knoll and co-workers used a sol–gel technique for the deposition of 3-(mercaptopropyl)trimethoxysilane layer onto gold followed by subsequent hydrolysis of the terminal trimethoxysilyl groups to generate surface silanol groups necessary for the condensation reaction of spin-coated tetramethoxysilane. The silica films were 3–100 nm in thickness and further functionalization with amine and biotin modified silanes introduced chemical functionalities onto the SPR interface.

A different bottom up approach was proposed by Cheng and co-workers, who showed that nanoscale silicate layers can be built up using layer-by-layer deposition of poly(allylamine hydrochloride) and sodium silicate, followed by calcination at high temperature to form stable films of 2–15 nm in thickness [81]. The resulting silicate films were used as platforms to construct supported bilayer membranes. The group of Kasemo demonstrated recently, that stable, smooth and very hydrophilic gold/SiO_2 interfaces can be prepared by spin coating poly(hydroxymethylsiloxane) onto the SPR chip, and post-treatment via plasma oxidation [82,83]. Some among us prepared stable silica films directly deposited on gold using plasma-enhanced chemical vapor deposition (PECVD) without the need of an additional titanium adhesion layer between the gold and the silica film. [40,41,44,45,84,85] The technique is based on the decomposition of a mixture of silane gas (SiH_4) and nitrous oxide (N_2O) near the substrate surface, enhanced by the use of a vapor containing electrically charged particles or plasma at 300 °C. The thickness and the refractive index of the silica layer

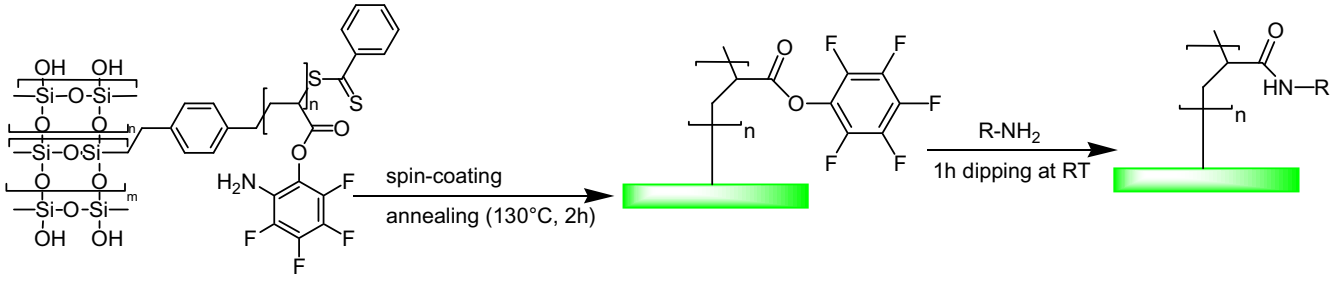

Fig. 17. Spin coating of polysilsesquioxane hydrid polymers onto gold and subsequent reaction with amine-terminated bioreceptors.

are controlled by the reaction time and stoichiometry of the film. Silica coatings as thin as 7 nm exhibited very good stability in both organic and aqueous solutions as well as in a piranha solution at 80 °C. This harsh treatment did also not induce any thickness or SPR response changes and produced considerable amounts of Si–OH, which allows chemical modification with functional silane molecules (Fig. 19). Amine-terminated oligonucleotides can be, for example, grafted on such an interface using a standard procedure developed for glass, including the following sequences: (i) reaction of the silicon oxide layer with 3-aminopropyltrimethoxysilane (APTES) to produce amine terminal groups on the surface, (ii) transformation of the amine to aldehyde termination by chemical coupling with a bifunctional linker such as glutaraldehyde and immobilization of ODNs bearing a terminal amine group [39].

One limitation of the silica coating concerns its insulating character, limiting its use for electrochemistry-SPR (E-SPR). A lamellar structure with 7 nm thick SiO_x showed charge transfer, which was rather sluggish [39,41].

Antimony-doped tin oxide (SnO_2:Sb) and indium tin oxide (ITO) thin films are a possible alternative to SiO_x layers for E-SPR, displaying a resistivity in the order of 10^{-4}–10^{-2} Ω cm [37,42,43]. On the other hand, while for a 10 nm SiO_x overlayer the SPR signal is not significantly changed, in the case of SnO_2:Sb the signal is largely deteriorated (Fig. 20A) [37,38]. This is linked to the higher imaginary refractive index of Sb-doped SnO_2 ($n = i0.249$), which confers the Au–SnO_2:Sb composite interfaces a yellow color and an additional light absorption. This problem is less severe in the case of a 10 nm ITO overlayer. While only a shift to larger angles is observed, the ITO films display several interesting advantages such as optical transparency, electrical conductivity, and excellent adhesion properties to metals. However, for the development of such novel lamellar SPR interfaces one important characteristic is the final refractive index sensitivity, *S*, of the interface (Eq. (4)). Fig. 20B depicts the experimentally determined refractive index sensitivity in the form of a bar diagram for a classical gold SPR interface and for different experimentally constructed lamellar surfaces where the film thickness was between 5 and 8.5 nm. The sensitivity of gold/SiO_x (7 nm) and gold/ITO (8.5 nm) are about the same and lower than the pure gold interface. The decrease is most drastically encountered on a gold/SnO_2:Sb (2%) (5.5 nm) interface.

Bioreceptors can be integrated on ITO and SnO_2:Sb (2%) interfaces in the same way as for silica-based lamellar structures. Fig. 21 shows how the silanization reaction can be used to incorporate surface carboxyl groups. There are indeed several advantages linked to the presence of carboxylic groups on the interface to which amine-terminated alkyl-chain silanes can be readily attached. In addition using longer functional alkyl chain silanes, such as undecenyltrichlorosilane (UETS), limits the formation of polycondensated side products of the hydrolyzed silane reagent as well as vertical polymerization [86]. This side reaction is unavoidable using aminopropyltrimethoxysilane. Also some bifunctional linkers, especially glutaraldehyde, besides being toxic, are prone to form polymers. The resulting imine bond is also chemically unstable and requires an additional reduction step using $NaBH_4$ solution. This is not the case if using EDC/NHS chemistry of acid terminated interfaces.

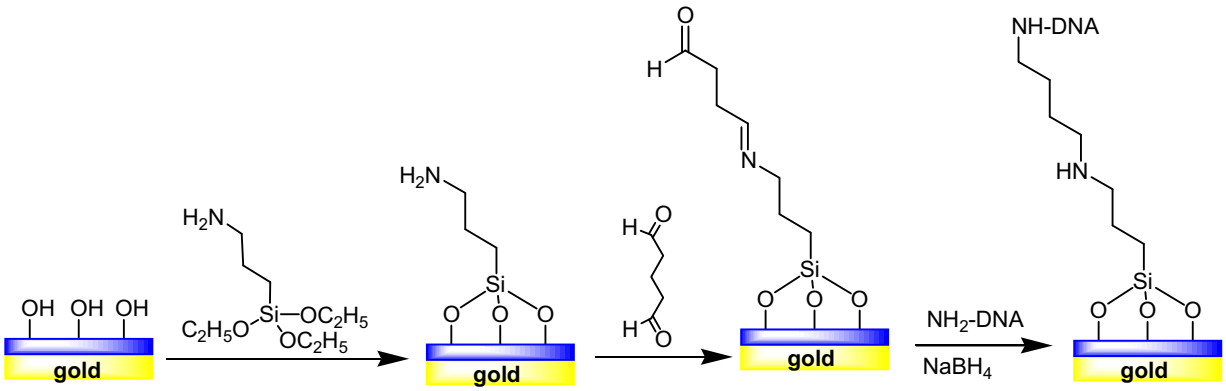

Fig. 19. Schematic outline of the covalent linking of amine-terminated bioreceptors to silica coated SPR interfaces.

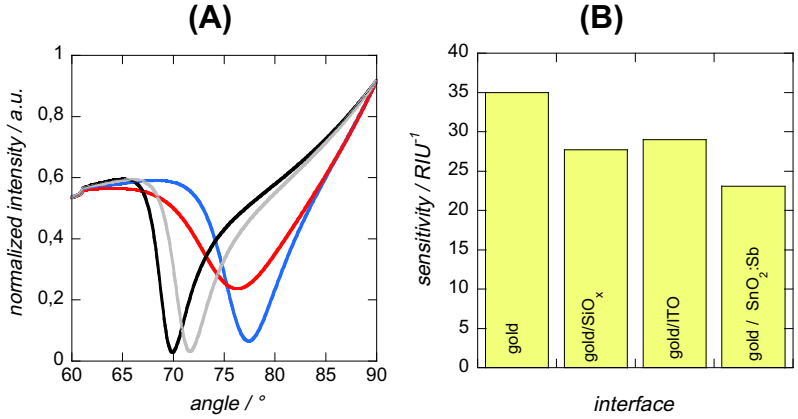

Fig. 20. (A) Influence of the refractive index of 10 nm thick dielectric overcoatings on the SPR signal using a prism with $n = 1.52$: naked gold (black), +SiO_x ($n = 1.48$) (gray),+SnO_2:Sb (2%) ($n = 1.91 + i0.249$) (red), ITO ($n = 2.0 + i0.001$) (blue); (B) Experimentally determined refractive index sensitivities S for a classical gold SPR interface before and after coating with different dielectric overlayers of SiO_x ($d = 7$ nm), ITO ($d = 8.5$ nm), SnO_2:Sb (2%) ($d = 5.5$ nm).

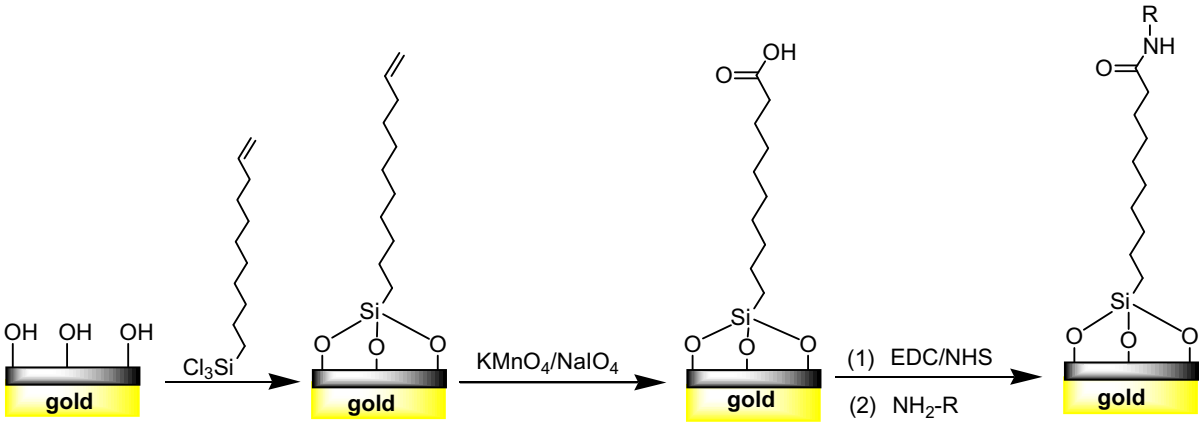

Fig. 21. Schematic illustration of the carboxylation of oxide-based overlayers such as SiO_x, ITO and SnO_2:Sb (2%).

7.4.2. Carbon-based overcoatings on gold

While stable and reusable devices are formed using silanization, one of the drawbacks of silane chemistry is that it is not entirely well controlled. In addition, the Si–O–Si bonds are prone to hydrolysis when exposed to harsh environments, such as highly saline conditions. Recent studies have demonstrated that carbon-based surfaces could overcome this hurdle. They can be also readily modified with bioreceptors using well-developed and robust chemistry [87]. One widely used approach for the modification of diamond thin films is based on the attachment of alkene-containing molecules to the substrate through UV light mediated carbon–carbon bond formation. It remained unknown for a while whether these functionalization strategies could be applied to other forms of carbon elaborated at low temperature. Indeed, diamond and most other forms of carbon such as glassy carbon, carbon nanofibres and pyrolyzed films involve high temperature, often in the range of 800–1000 °C, for growth deposition, limiting the ability to effectively integrate carbon with other materials. However, carbon also

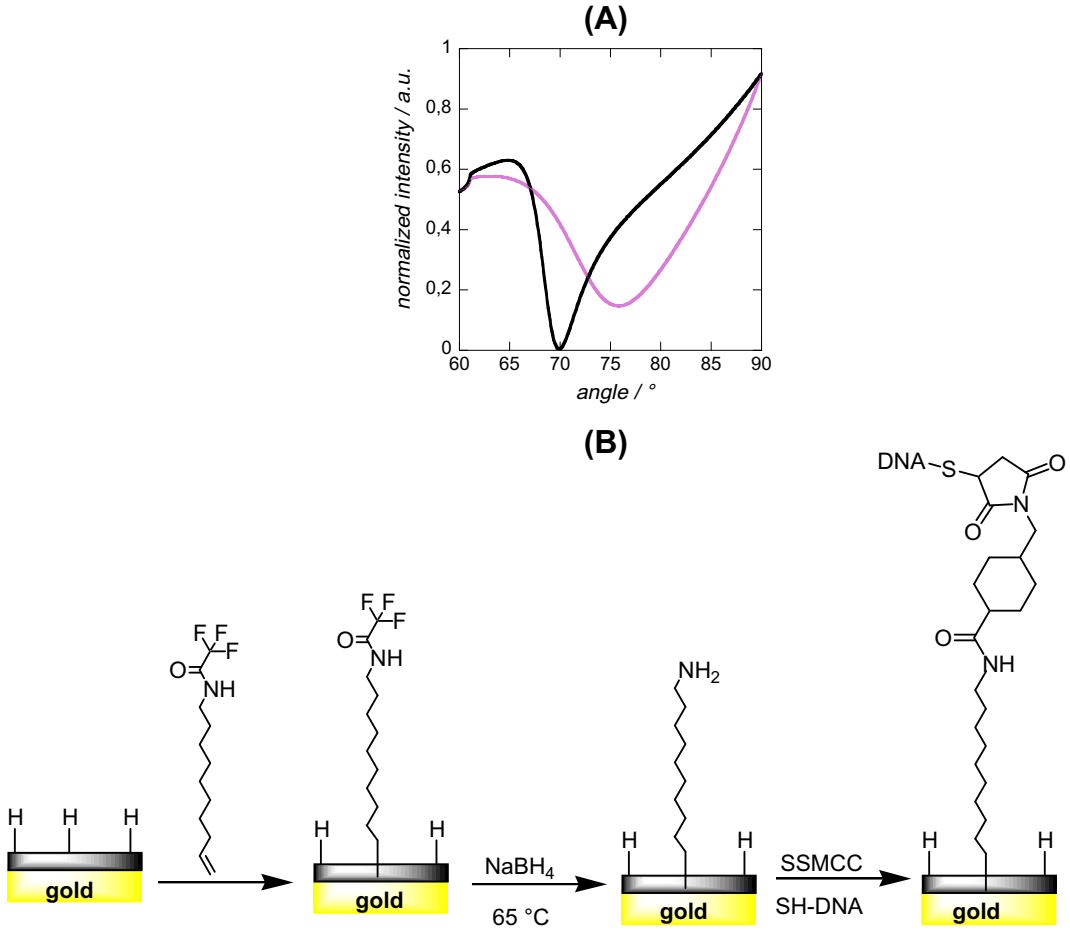

Fig. 22. (A) Theoretically calculated change of the SPR signal on gold (42.5 nm) with an amorphous carbon overcoating of 7.5 nm in thickness using Windspall (prism $n = 1.52$, amorphous carbon $n = 2.19 + i0.63$), (B) functionalization of amorphous carbon to produce TFAAD-modified surface, deprotection to produce amine-terminated surface and reaction with SSMCC linker and SH-DNA to covalently immobilize DNA on the carbon surface.

forms a range of less-crystalline materials, including diamond-like carbon (DLC) and other forms of amorphous carbon (*a*-C:H) films. Amorphous carbon is particularly an interesting material as it can be deposited at room temperature [88,89]. It can also be hydrogen-terminated using an inductively coupled hydrogen plasma, allowing the use of the same surface functionalization schemes developed for diamond. There are currently only few reports on the integration of amorphous carbon with gold-based SPR chips. Lockett et al. recently showed that thin layers of amorphous carbon (0–20 nm) can be deposited onto a surface plasmon active gold film using a sputtering technique [36]. A decrease of the photon-plasmon coupling efficiency and a broadening of the angle scanning SPR curves are ascribed to the complex dielectric function of amorphous carbon ($n = 2.19 + i0.63$). Theoretical determination of the refractive index sensitivity gave $S = 15$, which is significantly lower than that of the previously described interfaces (Fig. 22A). The loss in sensitivity caused by the amorphous carbon film could be slightly reduced by altering the thickness of the surface plasmon active gold film; a $S = 18$ was obtained in this case. The interface was further investigated for the *in situ* synthesis of oligonucleotide arrays utilizing photochemically protected oligonucleotide building blocks (Fig. 22B). This is in fact not possible with traditional gold SPR substrates, as extended exposure to ultraviolet light and oxidizing chemical conditions leads to the degradation of the gold thin film [36].

In a related approach, different *a*-C:H layers (nitrogenated and fluorinated) were deposited onto a gold SPR interface by radio-frequency sputtering from a graphite target in the presence of argon and an additional gas plasma of nitrogen or tetrafluoromethane [89]. Here, the resistance to protein adsorption was studied.

We and others have found that the sensitivity can remain the same as on gold by choosing amorphous silicon-carbon alloys (abbreviated as a-$Si_{1-x}C_x$:H) with the right chemical composition (Fig. 23A) [47]. Amorphous silicon–carbon alloys can be deposited as thin films, and changing the carbon content of the film allows for fine tuning the material properties. Increasing the carbon content leads to the optical band gap enlargement and to a transparent material, and at the same time decreasing the refractive index, which is beneficial for the fabrication of lamellar SPR interfaces. Stable hydrogenated coatings of about 5 nm in thickness of a-$Si_{0.63}C_{0.37}$:H film were thus formed on gold using PECVD in the "low-power" regime. Surface hydrogenated a-$Si_{0.63}C_{0.37}$:H can be conveniently functionalized by stable organic layers through robust Si–C covalent bonds in a similar way as crystalline silicon. Fig. 23B shows one possibility to introduce acid terminal function onto such a dielectric layer. Immersing surface-hydrogenated a-$Si_{0.63}C_{0.37}$:H films into undecylenic acid followed by UV irradiation leads to the formation of an organic monolayer covalently attached to the surface through Si–C bonds. By making a quantitative infrared data analysis, a molecular density of linked carboxydecyl groups of $N_A \sim 2 \times 10^{13}$ mol cm^{-2} was found [47]. This value is lower than that on crystalline silicon, as expected for a material incorporating a significant amount of methyl groups. The acid function can be further converted to an activated ester group using EDC/NHS chemistry, to which amine-terminated bioreceptors can be easily anchored.

7.4.3. Overcoatings on silver

For sensing applications, it is mandatory that the width, position, and height of the resulting SPR signal to be highly sensitive to any variation in the refractive index of the dielectric medium in the vicinity. For a SPR set up in the Kretschmann–Raether ATR configuration, reflectance depends strongly on the experimental conditions (e.g., polarization, wavelength of the coupled radiation,

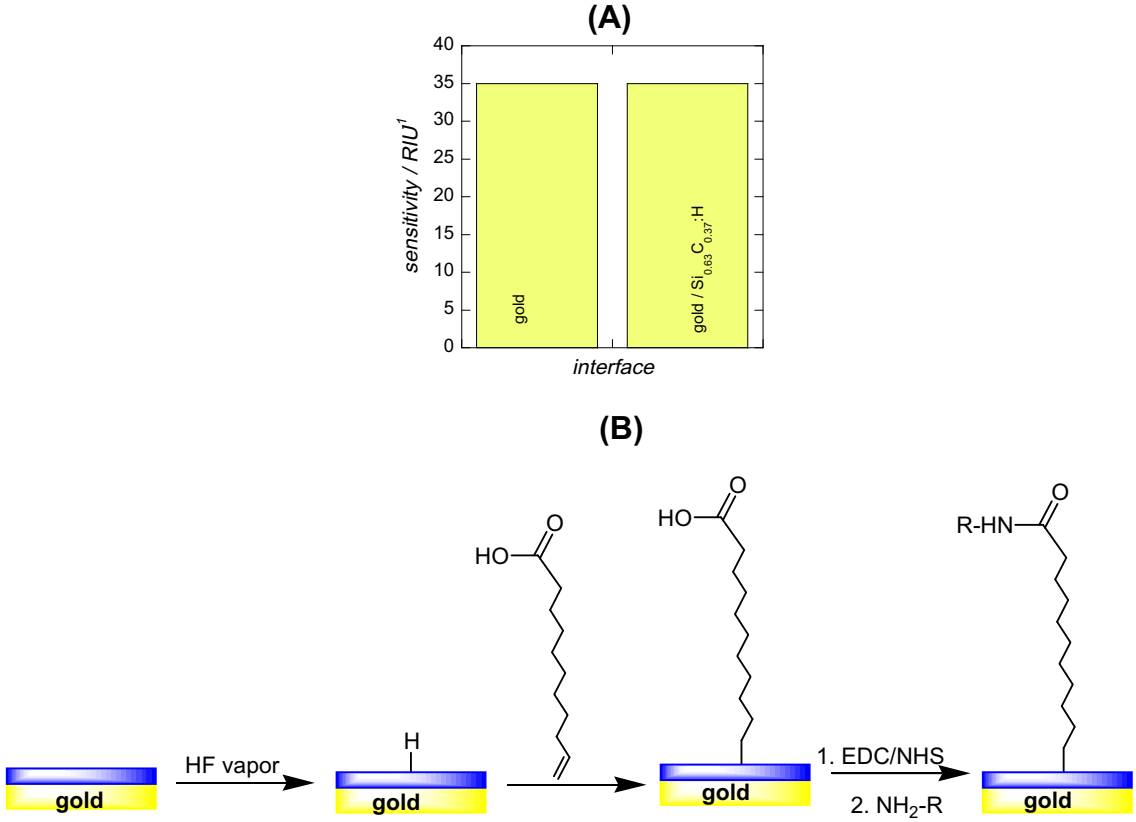

Fig. 23. (A) Experimentally determined refractive index sensitivities S for a classical gold SPR interface without and with 5 nm thick a-$Si_{0.63}C_{0.37}$ coating layer, (B) Surface hydrogenation of a-$Si_{1-x}C_x$:H thin film and subsequent functionalization with undecylenic acid.

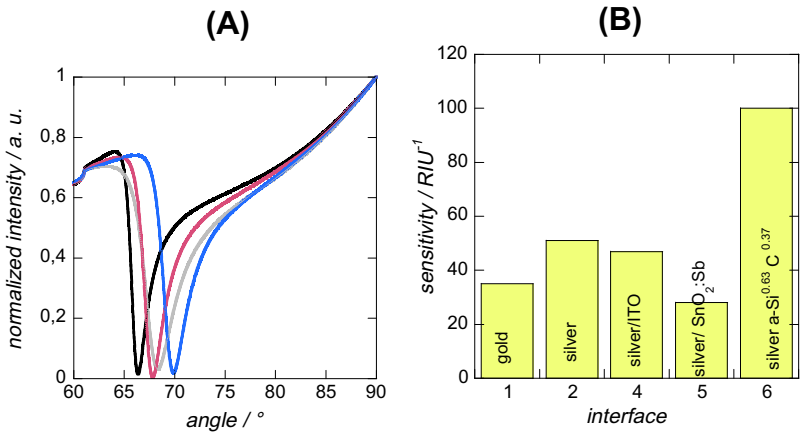

Fig. 24. (A) SPR spectra of different chips *d* = consisting of 5 nm titanium adhesion layer ($n = 2.36 + i3.47$) and 38 nm silver layer ($n = 0.14 + i4.581$) using a prism with $n = 1.52$ on glass before (black) and after deposition of dielectric overcoatings: SnO_2:Sb ($d = 5.5$ nm, gray), ITO ($d = 8.5$ nm, blue), a-$Si_{0.63}C_{0.37}$ ($d = 5$ nm, magenta), (B) Refractive index sensitivities for a classical gold and silver SPR interfaces before and after coating with ITO ($d = 8.5$ nm), SnO_2:Sb (2%) ($d = 5.5$ nm) and a-$Si_{0.63}C_{0.37}$ ($d = 5$ nm).

coupling prism) and material characteristics (e.g., refractive indexes n (n) $n' + in''$, with n' being the real part and n'' the imaginary part) of the metal films. For an optimized SPR signal, the choice of the thin metal film in contact with the sensing medium is thus one critical parameter, influencing considerably the shape of the plasmon curve. Gold is most commonly used as it possesses highly stable optical and chemical properties. Silver provides in contrast the sharpest SPR signal and is reported to have an enhanced sensitivity to thickness and refractive index variation in comparison to gold. In addition, the penetration length of a 50 nm thick gold film is about 164 nm with a light source of 630 nm, whereas a 50 nm thick silver film has an enlarged penetration length of 219 nm. Although the favorable optical properties of silver should favor its use over gold interfaces for SPR sensing, the main drawback of the silver-based SPR interfaces is its chemical instability. Silver rapidly oxidizes when exposed to air, and the process is accelerated in aqueous solutions, making it difficult to get reliable optical signals and to perform long time measurements needed to detect biological interactions. As a consequence, silver-based SPR interfaces can only be employed when coated with a thin and dense protecting layer, stabilizing the interface while keeping the favorable optical properties of silver. Only a few attempts have been reported until now to protect silver SPR surfaces and to take advantage of the optical properties of silver [33,34]. One approach to protect silver is based on the formation of bimetallic silver/gold layers (total thickness of 50 nm with the gold thickness comprised between 12 and 25 nm) taking advantage of the optical properties of both interfaces: the Ag underlayer sharpens the SPR signal, while the gold overlayer, being in contact with the solution, protects the silver due to its high chemical stability [33]. The bimetallic resonant films display a resonance angle translation on refractive index changes as gold SPR interfaces, but with a more narrow resonance dip, thus providing an enhanced signal/noise ratio.

A different approach consists on coating of silver substrates with previously described dielectric films. One requirement is that the deposition process has to be performed at ambient temperature to circumvent the oxidation of the silver thin film. Magnetron and radio frequency sputtering techniques are well suited for this purpose. Indeed, dense ITO as well as antimony-doped tin oxide hybrid thin films can be successfully formed on silver-based SPR chips, showing a long term chemical stability, together with better defined SPR signals as in the case of gold (Fig. 24A). The sharpest SPR signal was obtained on silver/a-$Si_{0.63}C_{0.37}$ lamellar interfaces, due to the favorable optical properties of the amorphous silicon–carbon layer. Compared to naked gold and coated gold interfaces, silver shows an increased sensitivity, which is however rather meaningless due to the instability of the interface. While coatings such as ITO and antimony-doped SnO_2 allow protecting the silver interface effectively, the refractive index sensitivity is lowered, being in the case of silver/ITO, still enhanced as compared to standard gold SPR interfaces (Fig. 24B). However, with a 5 nm thick a-$Si_{0.63}C_{0.37}$ overcoating the protection of the underlying silver thin film is ensured while at the same time the overall sensitivity of the interface was largely enhanced. Such an interface would thus represent an ideal candidate for sensitive SPR sensing, which is currently under investigation.

8. Conclusions

Novel SPR structures and approaches are being intensively investigated by many research groups to design sensors with higher sensitivity, better performance, and lower limit of detection compared to the commercially available SPR instruments. Structures making use of long-range surface plasmons (LRSPs) for which a refractive index resolution as small as 5×10^{-8} RIU have been demonstrated. SPR coupler and disperser (SPRCD) has been proposed as an attractive design for low-cost SPR sensors. In addition to higher sensitivity and lower limit of detection, compactness is also a desirable feature in SPR sensor technology. Portable SPR sensors are particularly useful for point-of-care applications. Therefore, different designs of miniaturized or integrated SPR sensors have also been actively explored in recent years. Optical fiber-based SPR sensors offer a possibility for sensor miniaturization and are extremely attractive for in vivo applications. Integrated optical waveguide SPR sensors are particularly promising candidates for the development of miniaturized multi-channel sensing devices on a single chip. SPR waveguide sensor fabricated by polymer imprinting technique has been proposed for low-cost, high-throughput fabrication. The use of plasmonic metamaterials can also potentially improve the performance of SPR sensors. Despite these developments, the utmost challenge to design compact and portable SPR biosensors with high performance, low limit of detection, low cost, and high throughput fabrication still stands out for the research community to address.

A variety of different functionalization strategies for gold-based SPR interfaces are currently available and the choice depends on the sensing applications. The fast development of different thin film deposition techniques opened the way for the preparation of

lamellar SPR structures. As some of the methods allow thin film deposition at ambient temperature, the coating of oxidizable metals such as silver was achieved. The coating protects the underlying silver interface and at the same time opens the way to employ different innovative surface functionalization strategies, overcoming the low stability of Au–S bond. In addition, through optimization of the film thickness and optical properties, a significant increase in refractive index sensitivity was achieved, opening the way to use such lamellar structures for real time biosensing. The glass-like SPR interfaces are currently used to link colloidal gold nanoparticles for enhancement of the SPR signal. Indeed, SPR is limited to high molecular weight analytes such as proteins or low molecular weight substances that undergo color changes upon chemical transformation that yield refractive index changes observable by SPR. This limitation can be overcome by the conjugation of labels, such as nanoparticles, that amplify the refractive index changes that occur. Such a strategy is about 100 fold more sensitive than the conventional ELISA method for antigen–antibody detection. The enhanced shift results from the coupling of the surface and particle plasmons. Such approaches have currently only been exploited on gold-based SPR interfaces. It is expected that on the newly fabricated silver/*a*-$Si_{0.63}C_{0.37}$ SPR substrate even more striking effects will be observed. One of the main challenges that face SPR sensing in the coming years is, however, probably to widen the scope of commercially available SPR interfaces, so that professionals other than specialist in surface chemistry, have access to the developed functionalization strategies widely employed in research laboratories.

Acknowledgements

The EU-ERDF via the Interreg IV programme (project "Plasmobio"), the Centre National de la Recherche Scientifique (CNRS), the Agence Nationale de la Recherche (ANR JCJC 2006) and the Nord-Pas-de Calais and Walloon regions are gratefully acknowledged for financial support.

References

[1] Wood R. On a remarkable case of uneven distribution of light in a diffraction grating spectrum. Phil Mag 1902;4:396–402.
[2] Ritchie R, Arakawa E, Cowan J, Hamm R. Surface-plasmon resonance effect in grating diffraction. Phys Rev Lett 1968;21:530–1532.
[3] Otto A. Excitation of surface plasma waves in silver by the method of frustrated total reflection. Z Physik 1968;216:398–410.
[4] Kretschmann E, Raether H. Radiative decay of non-radiative surface plasmons excited by light. Z Naturforsch 1968;23A:2135–6.
[5] Liedberg B, Nylander C, Lundstrom I. Surface plasmons resonance for gas detection and biosensing. Sens Actuat B 1983;4:229.
[6] Yang D, Lu HH, Chena B, Lin CW. Surface plasmon resonance of SnO_2/Au Bilayer films for gas sensing applications. Sens Actuat B 2010;145:832–8.
[7] Linman MJ, Sugerman K, Cheng Q. Detection of low levels of *Escherichia coli* in fresh spinach by surface plasmon resonance spectroscopy with a TMB-based enzymatic signal enhancement method. Sens Actuat B 2010;145:613–9.
[8] Mazumdara SD, Barlena B, Kämpferb P, Keusgen M. Surface plasmon resonance (SPR) as a rapid tool for serotyping of *Salmonella*. Biosens Bioelectron 2010;25:967–71.
[9] Chabot V, Cuerrier CM, Escher E, Aimez V, Grandbois M, Charette PG. Biosensing based on surface plasmon resonance and living cells. Biosens Bioelectron 2009;24:1667–73.
[10] Piliarik M, Homola J. Surface plasmon resonance (SPR) sensors: approaching their limits? Opt Express 2009;17:16505–17.
[11] Sarid D, Deck RT, Craig AE, Hickernell RK, Jameson RS, Fasano J. Optical field enhancement by long-range surface-plasma waves. J Appl Opt 1982;21:3993–5.
[12] Matsubara K, Kawata S, Minami S. Multilayer system for a high-precision surface plasmon resonance sensor. Opt Lett 1990;15:75–7.
[13] Nenninger G, Tobiska P, Homola J, Yee S. Long-range surface plasmons for high-resolution surface plasmon resonance sensors. Sens Actuat B 2001;74:145–51.
[14] Slavik R, Homola J. Ultrahigh resolution long range surface plasmon-based sensor. Sens Actuat B 2007;123:10–2.
[15] Vala M, Etheridge S, Roach J, Homola J. Long-range surface plasmons for sensitive detection of bacterial analytes. Sens Actuat B 2009;139:59–63.
[16] Telezhnikova O, Homola J. New approach to spectroscopy of surface plasmons. Opt Lett 2006;31:3339–41.
[17] Piliarik M, Vala M, Tichy I, Homola J. Compact and low-cost biosensor based on novel approach to spectroscopy of surface plasmons. Biosens Bioelectron 2009;24:3430–5.
[18] Slavik R, Homola J, Ctyroky J. Miniaturization of fiber optic surface plasmon resonance sensor. Sens Actuat B 1998;51:311–5.
[19] Spackova B, Homola J. Theoretical analysis of a fiber optic surface plasmon resonance sensor utilizing a Bragg grating. Opt Express 2009;17:23254–64.
[20] Jha R, Verma RK, Gupta BD. Surface plasmon resonance-based tapered fiber optic sensor: sensitivity enhancement by introducing a teflon layer between core and metal layer. Plasmonics 2008;3:151–6.
[21] Dostalek J, Ctyroky J, Homola J, Brynda E, Skalsky M, Nekvindova P, et al. Surface plasmon resonance biosensor based on integrated optical waveguide. Sens Actuat B 2001;76:8–12.
[22] Huang JG, Lee CL, Lin HM, Chuang TL, Wang WS, Juang RH, et al. A miniaturized germanium-doped silicon dioxide-based surface plasmon resonance waveguide sensor for immunoassay detection. Biosens Bioelectron 2006;22:519–525.
[23] Matsushita T, Nishikawa T, Yamashita H, Kishimoto J, Okuno Y. Development of new single-mode waveguide surface plasmon resonance sensor using a polymer imprint process for high-throughput fabrication and improved design flexibility. Sens Actuat B 2008;129:881–7.
[24] Suzuki A, Kondoh J, Matsui Y, Shiokawa S, Suzuki K. Development of novel optical waveguide surface plasmon resonance (SPR) sensor with dual light emitting diodes. Sens Actuat B 2005;106:383–7.
[25] Dostalek J, Vaisocherova H, Homola J. Multichannel surface plasmon resonance biosensor with wavelength division multiplexing. Sens Actuat B 2005;108:758–64.
[26] Kabashin AV, Evans P, Pastkovsky S, Hendren WA, Wurtz G, Atkinson R, et al. Plasmonic nanorod metamaterials for biosensing. Nature Mater 2009;8:867–871.
[27] Boltasseva A, Atwater HA. Low-loss plasmonic metamaterials. Science 2011;331:290–1.
[28] Lahiri J, Isaacs L, Tien J, Whitesides GM. A strategy for the generation of surfaces presenting ligands for studies of binding based on an active ester as a common reactive intermediate: a surface plasmon resonance study. Anal Chem 1999;71:777–90.
[29] Kyprianou D, Guerreiro AR, Nirschl M, Chianella I, Subrahmanyam S, Turner APF, et al. The application of polythiol molecules for protein immobilization on sensor surfaces. Biosens Bioelectron 2010;25:1049–55.
[30] Zareie HM, Boyer C, Bulmus V, Nateghi E, Davis TP. Temperature-responsive self-assembled monolayers of oligo(ethylene glycol): control of biomolecular recognition. ACS Nano 2008;2:757–65.
[31] Franzen S. Surface plasmon polaritons and screened plasma absorption in indium tin oxide compared to silver and gold. J Phys Chem C 2008;112:6027.
[32] Liu H, Wang B, Leong ESP, Yang P, Zong Y, Si G, et al. Enhanced surface plasmon resonance on a smooth silver film with a seed growth layer. ACS Nano 2010;4:3139.
[33] Zynio SA, Samoylov AV, Surovtseva ER, Mirsky VM, Shirshov YM, et al. Bimetallic layers increase sensitivity of affinity sensors based on surface plasmon resonance. Sensors 2002;2:62.
[34] Wang L, Sun Y, Wang J, Zhu X, Jia F, Cao Y, et al. Sensitivity enhancement of SPR biosensor with silver mirror reaction on the Ag/Au film. Talanta 2009;78:265.
[35] Kambhampati DKM, Robertson JW, Cai M, Pemberton JE, Knoll W. Novel silicon dioxide sol–gel films for potential sensor applications: a surface plasmon resonance study. Langmuir 2001;17:1169–75.
[36] Lockett MR, Weibel SC, Philips MF, Shortreed MR, Sun B, Corn RM, et al. Carbon-on-metal films for surface plasmon resonance detection of DNA arrays. J Am Chem Soc 2008;130:8611.
[37] Manesse M, Sanjines R, Stambouli V, Boukherroub R, Szunerits S. Preparation and characterization of antimony-doped SnO_2 thin films on gold and silver for electrochemical and surface plasmon resonance studies. Electrochem Commun 2008;10:1041.
[38] Manesse M, Sanjines R, Stambouli V, Boukherroub R, Szunerits S. Preparation and characterization of antimony-doped SnO_2 thin films deposited on silver substrates for surface plasmon resonance studies. Langmuir 2009;25:8036.
[39] Manesse M, Stambouli V, Boukherroub R, Szunerits S. Electrochemical impedance spectroscopy and surface plasmon resonance studies of DNA hybridization on gold/SiO_x interfaces. Analyst 2008;133:1097–103.
[40] Szunerits S, Boukherroub R. Preparation and characterization of thin films of SiO_x on gold substrates for surface plasmon resonance studies. Langmuir 2006;22:1660.
[41] Szunerits S, Boukherroub R. Electrochemical investigation of a novel interface for electrochemical surface plasmon resonance studies (E-SPR). Electrochem Commun 2006;8:439–44.
[42] Szunerits S, Castel X, Boukherroub R. Surface plasmon resonance investigation of silver and gold films coated with thin indium tin oxide (ITO) layers: influence on stability and sensitivity. J Phys Chem C 2008;112:15813–7.
[43] Szunerits S, Castel X, Boukherroub R. Preparation of electrochemical and surface plasmon resonance active interfaces: deposition of indium tin oxide on silver thin films. J Phys Chem C 2008;112:10883.
[44] Szunerits S, Coffinier Y, Janel S, Boukherroub R. Stability of gold/silica thin film interface. Electrochemical and surface plasmon resonance studies. Langmuir 2006;22:10716–22.

[45] Szunerits S, Nunes-Kirchner C, Wittstock G, Boukherroub R, Chantal G. Electrochemical investigation of the influence of thin SiO_x films deposited on gold on charge transfer characteristics. Electrochim Acta 2008;53: 7805–7914.
[46] Touahir L, Jenkins ATA, Boukherroub R, Gouget-Laemmel AC, Chazalviel J-N, Peretti J, et al. Surface plasmon-enhanced fluorescence spectroscopy on silver based SPR substrates. J Phys Chem C 2010;114:22582.
[47] Touahir L, Niedziółka-Jönsson J, Galopin E, Boukherroub R, Gouget-Laemmel AC, Solomon I, et al. Surface plasmon resonance on gold and silver films coated with thin layers of amorphous silicon–carbon alloys. Langmuir 2010;26:6058.
[48] Love JC, Estroff LA, Kriebel JK, Nuzzo RG, Whitesides GM. Thiolates on metals as a form of nanotechnology. Chem Rev 2005;105:1103.
[49] Ulman A. Formation and structure of self-assembled monolayers. Chem Rev 1996;96:1533.
[50] Peeters S, Stakenborg T, Reekmans G, Laureyn W, Lagae L, Van Aerschot A, et al. Impact of spacers on the hybridization efficiency of mixed self-assembled DNA/alkanethiol films. Biosens Bioelectron 2008;24:72.
[51] Malic L, Veres T, Tabrizian M. Biochip functionalization using electrowetting-on-dielectric digital microfluidics for surface plasmon resonance imaging detection of DNA hybridization. Biosens Bioelectron 2009;24:2218.
[52] Ananthanawat C, Vilaivan T, Mekboonsonglarp W, Hoven VP. Thiolated pyrrolidinyl peptide nucleic acids for the detection of DNA hybridization using surface plasmon resonance. Biosens Bioelectron 2009;24:3544.
[53] Wang J, Munir A, Li Z, Shou HS. Aptamer-Au NPs conjugates-enhanced SPR sensing for the ultrasensitive sandwich immunoassay. Biosens Bioelectron 2010;25:124.
[54] Frasconi M, Tel-Vered R, Riskin M, Willner I. Surface plasmon resonance analysis of antibodies using imprinted boronic acid-functionalized gold nanoparticle composites. Anal Chem 2010;82:2512–9.
[55] Liu Y, Dong Y, Jauw J, Linman MJ, Cheng Q. Highly sensitive detection of protein toxins by surface plasmon resonance with biotinylation-based inline atom transfer radical polymerization amplification. Anal Chem 2010;82:3679–85.
[56] Chen Y, Nguyen A, Niu L, Corn RM. Fabrication of DNA microarrays with poly(L-glutamic acid) monolayers on gold substrates for SPR imaging measurements. Langmuir 2009;25:5054–60.
[57] Hoa XD, Kirk AG, Tabrizian M. Enhanced SPR response from patterned immobilization of surface bioreceptors on nano-gratings. Biosens Bioelectron 2009;24:3043.
[58] Jordan CE, Frutos AG, Thiel AJ, Corn RM. Surface plasmon resonance imaging measurements of DNA hybridization adsorption and streptavidin/DNA multilayer formation at chemically modified gold surfaces. Anal Chem 1997;69:4939–47.
[59] Gamsjaeger R, Wimmer B, Kahr H, Tinazli A, Picuric S, Lata S, et al. Oriented binding of the His6-tagged carboxyl-tail of the L-type Ca^{2+} channel a-subunit to a new NTA-functionalized self assembled monolayer. Langmuir 2004;20:5885.
[60] Zhang Y, Luo S, Tang Y, Yu L, Hou K-Y, Cheng J-P, et al. Carbohydrate–protein interactions by "clicked" f carbohydrate self-assembled monolayers. Anal Chem 2006;78:2001–8.
[61] Kolb HC, Finn MG, Sharpless KB. Chemistry: diverse chemical. Function from a few good reactions. Angew Chem Int Ed 2001;40:2004–21.
[62] Lee J-M, Park HK, Jung YS, Kim JK, Jung SO, Chung BH. Direct immobilization of protein G variants with various numbers of cysteine residues on a gold surface. Anal Chem 2007;79:2680–7.
[63] Lofas S, Johnsoon BJ. A novel hydrogel matrix on gold surface plasmon resonance sensors. Chem Soc, Chem Commun 1990;21:1526.
[64] Lofas S, Johnsson B, Tegendal K, Ronnberg I. Dextran modified gold surfaces for surface plasmon resonance sensors: immunoreactivity of immobilized antibiotics and antibody-surface interaction studies. Colloids Surf B 1993;1:83–9.
[65] Jung S-H, Jung J-W, Suh I-B, Yuk J-S, Kim W-J, Choi E-Y, et al. Analysis of C-reactive protein on amide-linked N-hydroxysuccinimide-dextran arrays with a spectral surface plasmon resonance biosensor for serodiagnosis. Anal Chem 2007;79:5703–10.
[66] Loka RS, Cairo CW. Immobilization of carbohydrate epitopes for surface plasmon resonance using Staudinger ligation chemistry. Carbohydr Res, accepted for publication, doi:10.1016/j.carrs.2010.1009.1020.
[67] Le HQA, Sauriat-Dorizon H, Korri-Youssoufi H. Investigation of SPR and electrochemical detection of antigen with polypyrrole functionalized by biotinylated single-chain antibody: a review. Anal Chim Acta 2010;674:1.
[68] Gondran C, Dubois M-P, Fort S, Cosnier S, Szunerits S. Detection of carbohydrate-binding proteins by oligosaccharide-modified polypyrrole interfaces using electrochemical surface plasmon resonance. Analyst 2008;133:206.
[69] Dong H, Cao X, Li CM, Hu W. An in situ electrochemical surface plasmon resonance immunosensor with polypyrrole propylic acid film: comparisons between SPR and electrochemical responses from polymer formation to protein immunosensing. Biosens Bioelectron 2008;23:1055.
[70] Su X, Teh H, Aung KMM, Zong Y, Gao Z. Femtomol SPR detection of DNA–PNA hybridization with the assistance of DNA-guided polyaniline deposition. Biosens Bioelectron 2008;23:1715.
[71] Zhang N, Schweiss R, Zong Y, Knoll W. Electrochim Acta 2007;52:2869.
[72] Szunerits S, Knorr N, Calemczuk R, Livache T. A new approach to writing and simultaneous reading of micropatterns: combining surface plasmon resonance imaging (SPR-i) with scanning electrochemical microscopy (SECM). Langmuir 2004;20:9236–41.
[73] Hu W, Li M, Cui X, Zhou Q. In situ studies of protein adsorption on poly(pyrrole-co-pyrrole propylic acid) films by electrochemical surface plasmon resonance. Langmuir 2007;23:2761.
[74] Guedon P, Livache T, Martin F, Lesbre F, Roget A, Bidan G, et al. Characterisation and optimisation of a real time parallel, label free, polypyrrole based DNA sensor by SPRi. Anal Chem 2000;72:6003.
[75] Liu S, Vareiro MMLM, Fraser S, Jenkins ATA. Control of attachment of bovine serum albumin to pulse plasma-polymerized maleic anhydride by variation of pulse conditions. Langmuir 2005;21:8572.
[76] Duque L, Menges B, Borros S, Forch R. Immobilization of Biomolecules to plasma polymerized pentafluorophenyl methacrylate. Biomacromolecules 2010; xxx.
[77] Song SY, Choi HG, Hong JW, Kim BW, Sim SJ, Yoo HC. Selective antigen–antibody recognition on SPR sensor based on the heat-sensitive conformational change of poly(*N*-isopropylacrylamide). Colloid Surf A: Physicochem Eng Aspects 2008;313–314:504.
[78] Kyprianou D, Guerreiro AR, Chianella I, Piletska EV, Fowler SA, Karim K, et al. New reactive polymer for protein immobilization on sensor surfaces. Biosens Bioelectron 2009;24:1365.
[79] Chegel V, Whitcombe MJ, Turner NW, Piletsky SA. Deposition of functionalized polymer layers in surface plasmon resonance immunosensors by in-situ polymerization in the evanescent wave field. Biosens Bioelectron 2009;24:1270.
[80] Kessler D, Roth P, Theato P. Reactive surface coatings based on polysilsesquioxanes: controlled functionalization for specific protein immobization. Langmuir 2010;25:10068.
[81] Phillips KS, Han J, Martinez M, Wang Z, Carter D, Cheng Q. Nanoscale glassification of gold substrates for surface plasmon resonance analysis of protein toxins with supported lipid membranes. Anal Chem 2006;78:596.
[82] Satriano C, Edvardsson M, Ohlsson G, Wang G, Svedhem S, Kasemo B. Plasma oxidized polyhydroxymethylsiloxane – a new smooth surface for supported lipid bilayer formation. Langmuir 2010;26:5715–25.
[83] Satriano C, Marletta G, Kasemo B. Oxygen plasma-induced conversion of polysiloxane into hydrophilic and smooth SiO_x surfaces. Surf Interf Anal 2008;40:649.
[84] Zawica I, Wittstock G, Boukherroub R, Szunerits S. Polarization modulation infrared reflection absorption spectroscopy investigations of thin silica films deposited on gold. 2. Structural analysis of 1,2-dimyristoyl-sn-glycero-3-phosphocholine bilayer. Langmuir 2008;24:3922–9.
[85] Zawica I, Wittstock G, Boukherroub R, Szunerits S. PM IRRAS investigation of thin silica films deposited on gold. Part 1. Theory and proof of concept. Langmuir 2007;23:9303–9.
[86] Fadeev AY, McCarthy TJ. Self-assembly is not the only reaction possible between alkyltrichlorosilanes and surfaces: monomolecular and oligomeric covalently attached layers of dichloro- and trichloroalkylsilanes on silicon. Langmuir 2000;16:7268.
[87] Szunerits S, Boukherroub R. Review: chemical, photochemical and electrochemical surface modification of diamond. J Solid State Electrochem 2008;12:1205–18.
[88] Sun B, Colavita PE, Kim H, Lockett M, Marus MS, Smith LM, et al. Covalent photochemical functionalization of amorphous carbon thin films for integrated real-time biosensing. Langmuir 2006;22:9598.
[89] Akasaka H, Gawazawa N, Suzuki T, Nakano M, Ohshio S, Saitoh H. Evaluation of protein adsorption on hydrogenated amorphous carbon films by surface plasmon resonance phenomenon. Diamond Relat Mater 2010;19:1235.