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A portable surface plasmon resonance sensor system for real-time monitoring of small to large analytes

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Abstract Many environmental applications exist for biosensors capable of providing real-time analyses. One pressing current need is monitoring for agents of chemical- and bio-terrorism. These applications require systems that can rapidly detect small organics including nerve agents, toxic proteins, viruses, spores and whole microbes. A second area of application is monitoring for environmental pollutants. Processing of grab samples through chemical laboratories requires significant time delays in the analyses, preventing the rapid mapping and cleanup of chemical spills. The current state of development of miniaturized, integrated surface plasmon resonance (SPR) sensor elements has allowed for the development of inexpensive, portable biosensor systems capable of the simultaneous analysis of multiple analytes. Most of the detection protocols make use of antibodies immobilized on the sensor surface. The Spreeta 2000 SPR biosensor elements manufactured by Texas Instruments provide three channels for each sensor element in the system. A temperature-controlled two-element system that monitors for six analytes is currently in use, and development of an eight element sensor system capable of monitoring up to 24 different analytes will be completed in the near future. Protein toxins can be directly detected and quantified in the low picomolar range. Elimination of false positives and

increased sensitivity is provided by secondary antibodies with specificity for different target epitopes, and by sensor element redundancy. Inclusion of more than a single amplification step can push the sensitivity of toxic protein detection to femtomolar levels. The same types of direct detection and amplification protocols are used to monitor for viruses and whole bacteria or spores. Special protocols are required for the detection of small molecules. Either a competition type assay where the presence of analyte inhibits the binding of antibodies to surface-immobilized analyte, or a displacement assay, where antibodies bound to analyte on the sensor surface are displaced by free analyte, can be used. The small molecule detection assays vary in sensitivity from the low micromolar range to the high picomolar.

Keywords Surface plasmon resonance · Environmental monitoring · Portable SPR biosensor · Real-time toxin detection

Introduction

Surface plasmon resonance (SPR) biosensors are based on the fundamental Kretschmann [11] design where the intensity of transverse magnetic (TM) polarized light reflected off a thin layer of gold (~50 nm) on the surface of a prism shows a dependence on the angle of incidence or wavelength of the incident light (Fig. 1a). A plot of the intensity of reflection against the angle of reflection produces an SPR curve or profile (Fig. 1b). A minimum in the SPR curve is observed when the frequency and momentum of the incident light are matched by that of the surface plasmons (free electrons on the metal surface capable of undergoing a resonant oscillation with the illuminating light at angles above the critical angle), at which point the energy is absorbed by the surface plasmons and is not reflected back. The angle or wavelength at which the minimum of reflection occurs is dependent on the refractive index of the medium in contact with the outside of the gold layer.

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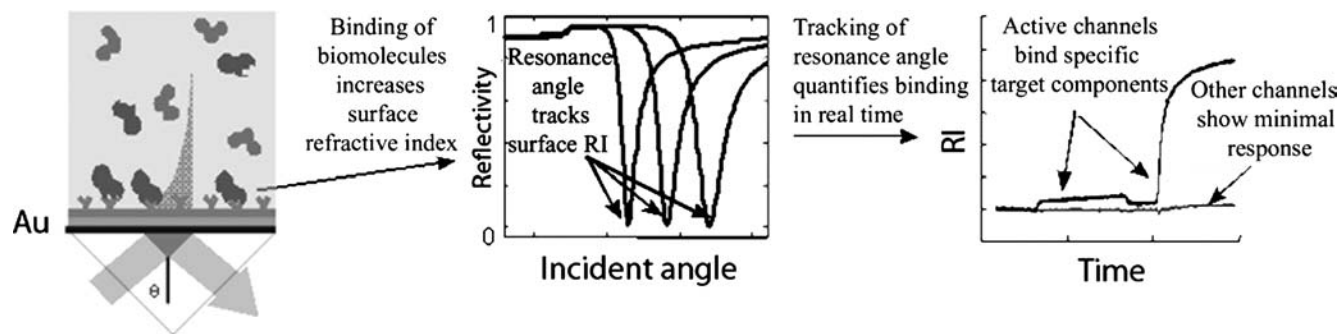


Fig. 1 **a** Representation of a surface plasmon resonance (SPR) sensor surface with attached antibodies that are binding specific analytes. **b** Minima of SPR curves moving to the right as analyte is bound. **c** Data are converted into plots of refractive index (RI) as a function of time by the system software

Attachment of specific recognition elements on the gold surface (usually antibodies), and passivation of the gold surface to non-specific binding, provides a condition for monitoring for the presence of specific targets in real-time (Fig. 1a). Since the refractive index (RI) of protein ($n = 1.45$) is greater than that of usual aqueous buffers (n about 1.334), when an analyte of RI greater than that of water/buffer and of sufficient size is bound at the surface, the change in RI is sufficient to result in a change in the position of the minimum of the SPR curve. The instrumentation software converts the change in SPR minima into RI as a function of time (Fig. 1c), thus allowing the binding event to be analyzed in real time [6].

The first commercial instruments available were both large and expensive and thus not suitable for applications that require portability [2]. Recent advances in miniaturization of SPR technology has made possible the development of portable systems [16, 17] that are adaptable to many different uses, including the following as examples: (1) continuous monitoring of water systems; (2) continuous monitoring of air supply systems, when coupled with a sample collection device that transfers analytes into the aqueous phase; (3) rapid identification of possible agents of chemical or biological warfare; (4) general laboratory analyses of intermolecular interactions, e.g., protein/protein interactions, protein/ligand interactions, and protein/nucleic acid interactions; (5) environmental monitoring; (6) drug discovery; (7) in-office diagnostics; (8) in-emergency-vehicle analyses, e.g., rapid detection of plasma levels of cardiac enzyme levels; and (9) automated protein purification protocols.

This brief review will focus on the development of portable SPR sensor systems and approaches used to detect environmental pollutants and agents of chemical and biological warfare/terrorism that vary in size from small organics to whole microbes or spores.

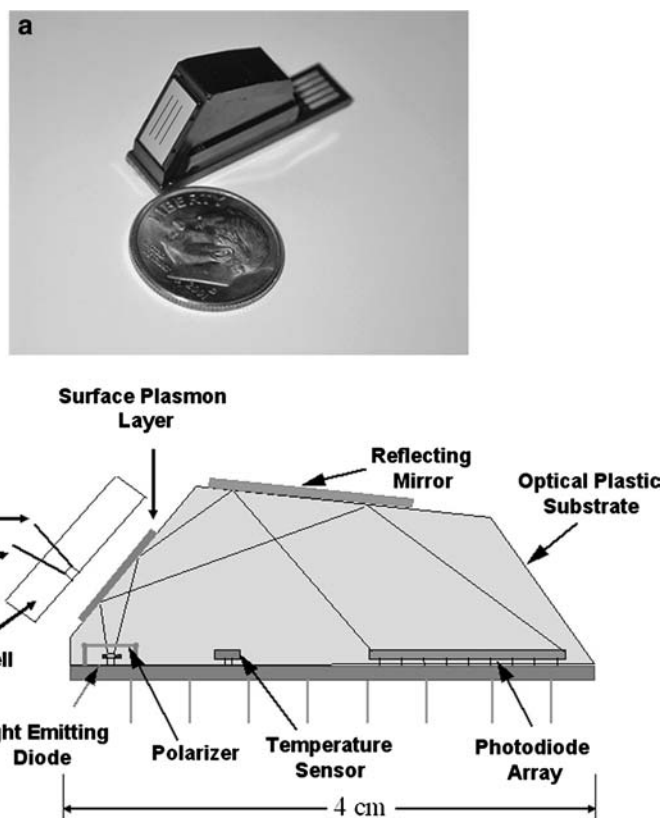
The Spreeta sensor elements

The portable SPR sensor system used for these applications is built around the Spreeta sensor elements manufactured by Texas Instruments (Dallas, TX) [5, 13, 14, 21]. Figure 2 shows a three-channel Spreeta sensor element, and a diagram of the sensor element. The miniature Spreeta SPR sensing chip is a fully integrated SPR sensor element containing an LED light source, a gold SPR surface, a reflecting mirror that directs the reflected light to a photodiode array and a temperature sensor. The output signal from the sensor element is processed through a digital signal processing chip, then fed to a computer, which generates and displays both the SPR curves and a plot of RI versus real-time. Each Spreeta sensor element has three separate channels that are individually derivatized with a specific antibody using a cast polydimethylsilane (PDMS) fluidics system. Several sensor elements can be derivatized with antibodies in parallel. Following the conjugation of antibodies to the sensor surface, the sensor elements are fitted into a cast PDMS flow cell where the input flow covers all three channels of one sensor element before flowing to the next element. It is also possible to make use of flow cells that keep the channels separated.

The sensor system

The sensor elements and fluidics are encased in a temperature-controlled, insulated cell. A thermoelectric temperature controller maintains the temperature to within approximately $\pm 0.01^\circ\text{C}$. The SPR sensor element module is enclosed in a case that includes the temperature control circuitry, a miniature peristaltic pump, a vacuum pump and the digital signal processors.

Fig. 2 a Photograph of a three-channel miniature Spreeta sensor element. A US dime (18 mm) is placed by the sensor element for scale. **b** Diagram of the components of the Spreeta sensor element



Preparing the sensor surface

Gold surfaces are inert and therefore present some challenges for attachment of recognition elements. One commonly used approach is to take advantage of the high affinity of thiols for gold [1]. This mode of attachment has been used for different applications [10]. Yet another approach became available when a peptide domain was engineered to have a very high affinity for elemental gold [4]. A protocol was developed for attaching specificity elements to the SPR sensor surfaces using the gold-binding peptide as a foundation layer [21]. This peptide domain was used for most of the applications described below [16, 17]. The gold-binding domain was fused to alkaline phosphatase in order to easily follow the binding of the gold-binding domain to gold surfaces [4]. In the applications described below, the fusion protein was first attached to the gold surface and then released from the alkaline phosphatase domain by gentle trypsin treatment (Fig. 3a). This step is carried out twice and results in a peptide foundation layer with free carboxyl and amino groups that can be used for coupling of antibodies, receptors, other recognition elements or antigens using standard coupling reactions (Fig. 3b). This peptide foundation layer also serves to reduce non-specific binding of non-target analytes to the gold surface [16, 17, 21].

Detection of small organics

The specific detection of small organic molecules depends on the acquisition of a recognition element specific for the target analyte. The latter is usually an antibody; however, it can be any molecule or structure capable of high-affinity, specific binding of the analyte. Antibodies have been used for the examples described below.

Two different approaches were adapted for the SPR detection/quantitation of small molecular weight analytes: a displacement assay (2-formats) and a competition assay.

For the displacement assay, two different implementations have been used. In the first, the sensor surface is derivatized with anti-target antibodies. The antibodies on the sensor surface are then “loaded” with a large reporter group (e.g., protein) to which target molecules have been covalently attached. Free analyte then displaces the bound target-conjugated reporter group at a rate that is proportional to the concentration of analyte (Fig. 3c). The second implementation involves attachment of the target to the sensor surface either as a target-protein-conjugate, or via direct linkage to the GBP foundation layer. Antibodies are then bound to the immobilized target molecules. Free target molecules displace the bound antibodies at rates proportional to the concentration of analyte (Fig. 3d). Antibodies with moderate affinities for analyte work best for this ap-

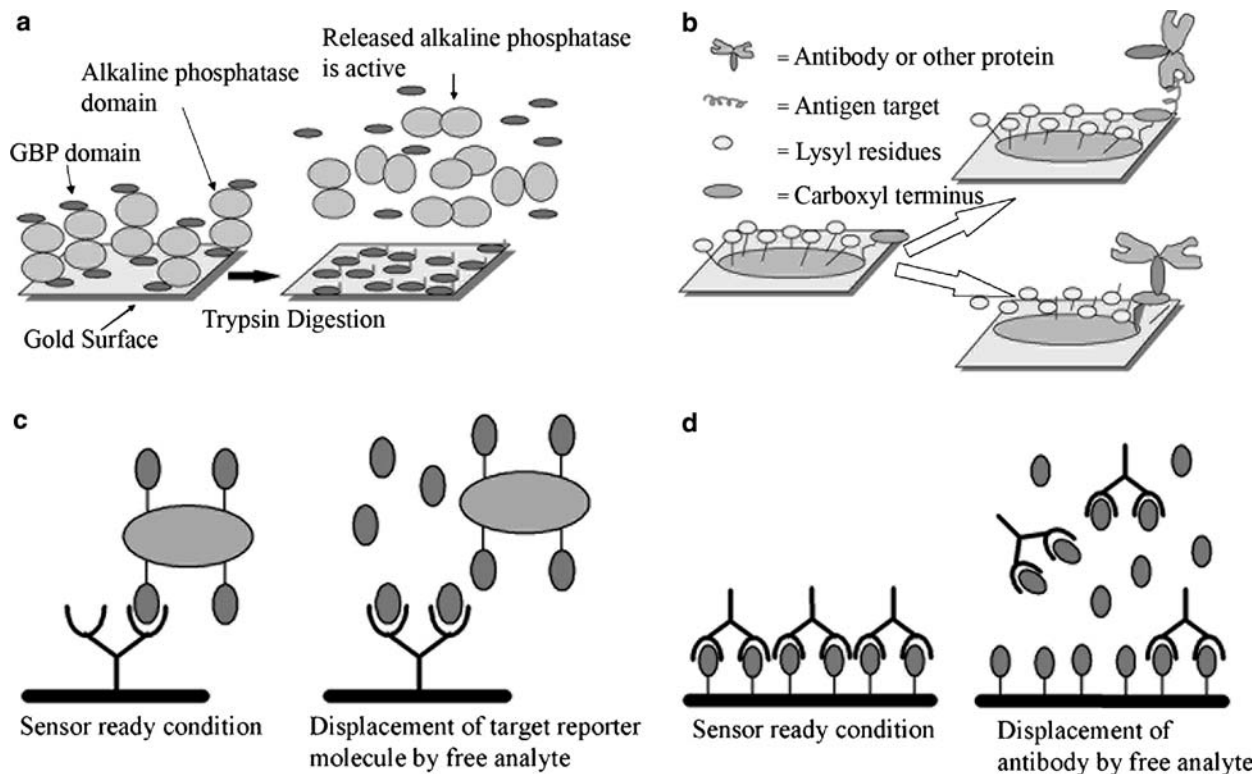


Fig. 3a–d Deposition of the gold-binding peptide layer. **a** A gold-binding peptide (*GBP*) domain attached to alkaline phosphatase is bound to the gold sensor surface followed by removal of the alkaline phosphatase domain by gentle proteolysis. **b** Attachment of targets (antigens) or antibodies to the GBP foundation layer. **c** Displacement assay where antibodies are immobilized on the

sensor surface, a large reporter molecule haptenized with target is bound, then displaced by analyte at a rate proportional to analyte concentration. **d** Displacement assay where analyte is immobilized on the sensor surface, antibodies are bound to the immobilized target molecules, then displaced by analyte at a rate proportional to analyte concentration

proach. It is possible to design fluidics where there is little consumption of antibodies using this method. This approach has been used for the detection of dinitro-

phenol (DNP)-lysine and domoic acid—the amnesic shell fish poison—in the low micromolar range of concentrations (data not shown). Elkind et al. [7] reported detection of DNP-glycine using the displacement approach.

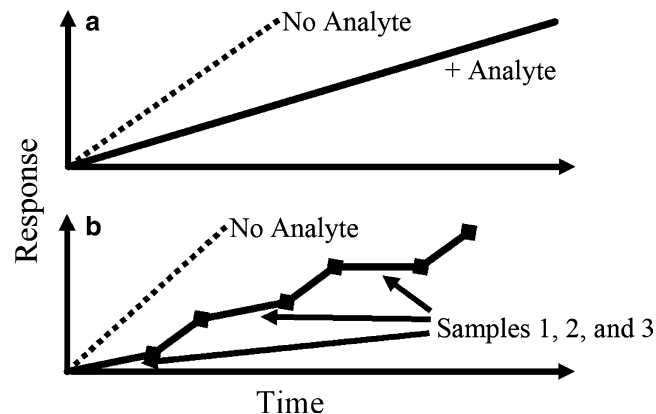


Fig. 4 a Competition assay where target is immobilized on the sensor surface and antibodies at a fixed concentration bind to the sensor surface in the absence (*dotted line*) or presence (*solid line*) of a given concentration of analyte. **b** Assay where different levels of analyte (1, 2, and 3) inhibit antibody binding during the same experimental run or binding isotherm

For the competition assay, an initial rate of antibody binding to immobilized target is established, then analyte is introduced directly into the antibody feed stream or preincubated with antibodies prior to flow through the SPR detection system. This approach (outlined in Fig. 4) has the advantage of a high level of sensitivity, especially if used with very high affinity antibodies. It has a disadvantage of consuming some antibody reagent. The range of sensitivity can be set by varying the concentration of the antibody feed stream. To achieve the highest level of sensitivity requires setting the antibody concentration in the feed stream to a level that provides a low but detectable rate of antibody binding to the target immobilized on the sensor surface. This protocol has been used for the detection of cortisol, domoic acid, and analyte conjugated to proteins used to generate the specific antibodies (data not shown). Cortisol levels as low as 750 pmol have been detected to date using this approach (data not shown). Strong et al. reported detection of trinitrotoluene (TNT) in soil samples to the ppm level [20].

Detection of protein toxins

Protein toxins are of sufficient size to detect directly by SPR. Figure 4 illustrates the binding of analyte to an antibody on the sensor surface. This binding event generates a binding curve with an initial rate of binding that is proportional to the concentration of analyte. For *Staphylococcus* enterotoxin B binding to the portable Spreeta-based system, the current lower limit of detection is approximately 100 pM [16]. Using this standard protocol, the A chain of ricin and botulinum toxoid A (denatured botulinum toxin A) were easily detected (data not shown). This same general protocol was used to test the suitability of the Spreeta based system for deployment in unattended air vehicles (UAVs) using the non-toxic proteins ovalbumin and horseradish peroxidase [18].

Detection of low concentration analytes and verification of analyte detection

It is very important to avoid false positives when monitoring for toxic agents. The same approach that is used to detect low levels of analytes can be used to both amplify detection signals and verify that a specific analyte was detected to a high level of certainty (Fig. 5).

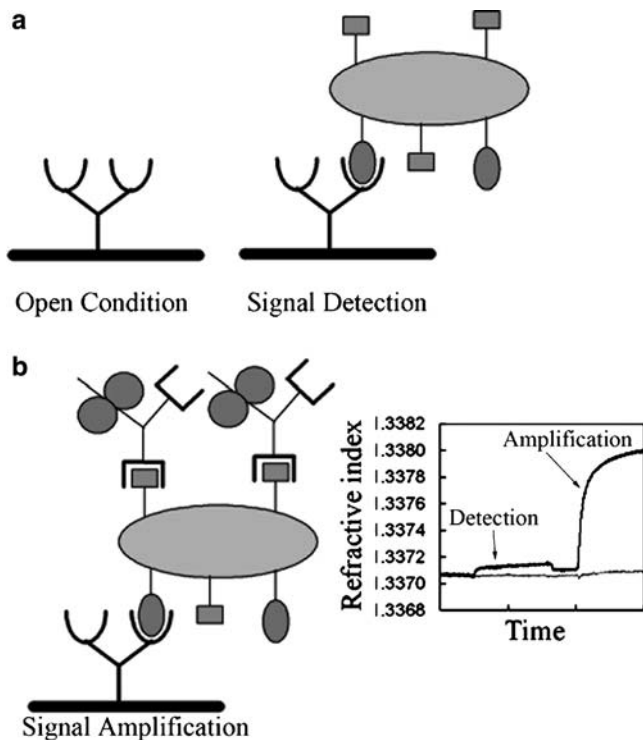


Fig. 5 a Direct detection of analyte. b Subsequent verification and amplification of signal with secondary antibodies specific for a different target epitope. Labeling of the secondary antibodies with colloidal gold can provide even higher ratios of amplification. This detection mode works well for larger analytes such as toxic proteins, viruses, spores and whole microbes

Once a putative detect signal has been observed, a second antibody with specificity for a different target epitope than was used to capture the analyte is flowed through the sensor system. Specific binding of this second antibody both verifies that the target analyte was bound and at the same time amplifies the original signal. It is also possible to design protocols with multiple steps of amplification when the concentration of analyte is very low. For example, if a mouse monoclonal antibody is used on the sensor surface as the target capture antibody, rabbit polyclonal antibodies to the same target can be used to provide a significant amplification to the first signal. If this signal is still small, goat anti-rabbit antibodies can provide a second step of amplification. A third stage of amplification can be achieved with donkey anti-goat antibodies if necessary and so on. Having a dedicated reference channel with an antibody with a non-target specificity is very important to detect any non-specific binding that might occur. Using this approach, as noted above, we have been able to detect *Staphylococcus* enterotoxin B at a level of 100 pM [16].

Figure 5 also illustrates the use of antibodies labeled with colloidal gold, which provide yet further amplification. He et al. [9] have reported amplification factors as high as 1,000-fold using gold nanoparticles. We have seen 20-fold amplification in preliminary experiments using this protocol.

Detection of viruses, microbes and spores

Related protocols have been used to detect larger target analytes such as viruses [3], microbes [8] and spores (data not shown). Again, to avoid false positives, or to speciate the bound target, secondary antibodies with specificity for different target epitopes are used. For example, a general antibody for *Salmonella* strains could be used as the capture antibody on the sensor surface and, when a signal is detected, antibodies specific for different strains can be used to identify the specific serological strain of organisms bound on the sensor surface. The speciation step will also provide amplification of the original signal.

Conclusions

Portable SPR-based detectors are unique in offering real-time detection of analytes that range in size from small organics to analytes as large as whole cells. These biosensors provide high sensitivity and high selectivity. The analyte capture element on the sensor surface can be virtually any molecule or structure that provides specific binding of the target analyte [15]. Antibodies are the usual choice of specificity element; however, aptamers [19], receptors, antigens or specific ligands may also be used. The inexpensive, portable, SPR-based sensors are also useful for general labora-

tory research. For example, they can be used to characterize the binding of protein transcription factors to regulatory regions of DNA [12]. Most intermolecular interactions that would generate a change in RI on the gold surface of the SPR sensor element can be followed with this technology [15].

The availability of these inexpensive, small, portable systems will find many different applications in monitoring for environmental pollutants, including analyzing for agents used in chemical and biological terrorism activities. The fundamental core system is adaptable for continuous monitoring of water supplies and air supplies when coupled with a sample collector device that transfers analytes into the aqueous phase. The small size of the system also makes it an excellent candidate for UAV applications [18] and employment in stand-alone ground sensor units.

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