Terahertz Biosensing Technology: Frontiers and Progress

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The accurate detection of minute amounts of chemical and biological substances has been a major goal in bioanalytical technology throughout the twentieth century. Over the years a wide variety of biosensing strategies have been developed to satisfy numerous needs. The general aim is to develop a scheme that is able to simplify sample preparation steps, provide high selectivity and sensitivity, respond in a continuous and reversible manner, and accomplish measurements without sample perturbation. Added to these conditions are other goals of convenience such as reusability, portability, low costs for mass production, miniaturization, and ease of use. These features are the most demanding parameters for biosensor designs. While there are several types of effective biosensors in the literature,[1, 2] it would be difficult, if not impossible, to find an individual device that can meet all the specified criteria.

In any biosensing strategy, a ligand should be allowed to interact specifically with its analyte to generate a signal that can be amplified and processed. Major detection schemes use antibodies, antigens, nucleic acids, cells, and membranes as molecular recognition species toward many analytes. For a receptor to recognize a specific target, its immobilization on a solid support must not interfere with molecular recognition. Although a variety of immobilization methods have been adapted from standard chromatographic support modifications, many sophisticated immobilization techniques have been developed recently.[3] Knowledge of the basic mechanisms by which the reactive groups couple to target functional groups provides the means to design a conjugation strategy.

Since the development of Clark’s oxygen-sensing electrode in the mid-twentieth century, numerous strategies for biosensor designs have emerged. New avenues with various approaches exploiting ongoing discoveries in biology, electronics, physics, and chemistry have significantly expanded the opportunities for biosensor applications. Miniaturization and implantable devices are still significant challenges facing biosensor technology, although progress is being made toward these goals. The application of miniaturization techniques to optical molecular sensors has resulted in their potential use for inaccessible, difficult, or dangerous locations, for example, in vivo sensors for arterial use.

In this Highlight, we will present a new detection modality based on terahertz time-domain spectroscopy (THz-TDS). We concentrate on a THz biodetection approach that expands over many disciplines and applications. The uniqueness, limitations, and potential capabilities of the THz biosensor will be reviewed in light of recent developments.[4, 5]

HIGHLIGHTS

Terahertz Biosensing Progress

Options for new detection methods caused the number of optical biosensors in the ultraviolet, visible, and mid-infrared regions of the electromagnetic spectrum to mushroom. This is essentially due to the availability of sources, detectors, and fiber optics, which have helped develop highly sensitive and portable biosensors for both civilian and military needs. Although the same fundamental design principals lie at the heart of the new developments, translation of those principals into the realm of newer technologies is a real challenge. Recently, THz-TDS has emerged as a successful method to probe the electrical properties of thin solid films in the spectral interval from 0.1 – 10 THz, between the infrared and microwave bands. It provides a new alternative to measure the refractive index of thin solid films without sample perturbation. The heart of THz system is a mode locked Ti:sapphire femtosecond laser, which generates 150 fs pulses at an 86 MHz repetition rate and 1.5 W average power. A beamsplitter separates the laser beam into excitation and reference pulses. The excitation pulse illuminates an unbiased GaAs semiconductor emitter wafer to generate a THz beam, which is collimated and focused onto an electro-optic sampling crystal, (110) ZnTe, with parabolic mirrors. A pellicle after the second parabolic mirror allows the reference beam to travel collinearly with the THz wave across the electro-optic crystal. A quarter wave plate (QWP), a Wollaston prism (P), and a pair of photodiodes are assembled for the balanced detection of the THz beam. When the sample is mounted in a galvanometer and dithered in and out of the THz beam, the technique is called terahertz differential time-domain spectroscopy (THz-DTDS), schemati-

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cally represented in Figure 1. The typical dimensions of a conventional THz system is about $1.2 \times 2.4 \text{ m}^2$, making on-site measurement virtually impossible. However, a newer compact version is now being refined. By integrating miniaturized optical technology with a fiber laser, we have been able to build a multitude of sensitive, reliable, and portable systems.

During the past decade, THz spectroscopy has witnessed increasing interest in the field of material science because of the difficulty in measuring the electrical properties of thin solid films in the far-infrared frequency range with conventional methods. Recently, several research groups attempt to expand THz technology to medical science to monitor the interactions between biological molecules. We performed a few experiments for label-free analyte – ligand binding and realized it may be used to detect numerous airborne chemical and biological agents before they become a biohazard. The system can detect chemical agents in vapor, gas, and solid phase. For chemical products, only a few steps are required for the sample to be tested, however, for biological materials it requires an antibody to specifically recognize the analyte. The THz system can be used as a rapid, instrument-based detection in which the need for a fluorescent dye will be eliminated. Although many biological molecules show an inherent fluorescence (associated with the amino acid derivatives of tryptophan or tyrosine, nucleic acids or other metabolites such as porphyrins) exploiting this inherent property for bioassay is not a simple procedure because the emitted signal is generally weak and often difficult to distinguish above a background signal. Other limitation of fluorescence indicators can also be fluorescence quenching by other solutes, or the insensitivity of fluorescence to certain binding events. Importantly, in current solid-phase immunoassay procedures, wash steps are labor intensive and time consuming when performed manually and would require complicated robotics in an automated format. In contrast, the portable THz spectrophotograph of the near future should be able to identify airborne chemical and biological materials in few seconds without need of a fluorescent tag.

Compared to the existing biosensors in the marketplace, the sensitivity of a standard THz system is still lacking, thus, improving the signal-to-noise ratio is one of the challenges facing the THz technology. In this regard, a new THz-DTDS setup has been developed, in which the difference between a reference and transmitted signal is plotted in real time. In this configuration, the sample is mounted in a galvanometer modulated at $10 \text{ Hz}$ over a peak-to-peak distance higher than $10 \text{ mm}$. The edge of the reference and sample moves back and forth laterally through the focus of the THz beam, and the difference signal is calculated. This setup has been shown to enhance the THz sensitivity from $10^6$ to $10^{10}$. In an effort to further increase the THz sensitivity, Michael Nagel and colleagues (at the the Rheinisch-Westfälische Technische Hochschule, Aachen) opted for a new flexible strategy based on a planar integrated wave-guide capable of detecting DNA – DNA interactions in the femtomole concentration range. An in-house developed channel waveguide was used to generate and detect THz signals with minimal distortion or loss; the interaction length between the THz rays and sample is greatly improved by using a thin film microstrip with an embedded band pass filter used as a resonator to guide the THz signal in plane with the sample. A particularly positive feature of the integration is its improved sensitivity, rapidity, accuracy, and small sample volume. With these advances, however, come drawbacks; for example, the smaller sample size makes testing more difficult. Current modern technology of robot-controlled pipettes could be the best way to overcome this problem.

The sensitivity of the integrated THz system is directly related to the surface chemistry of the microstrip, the loss in the resonator, and the homogeneity of the sample film. Thus, an adequate control of these parameters is of paramount importance for reliable and reproducible results. Interconnecting a large number of THz resonators to form a microarray, or a DNA-chip, for the simultaneous detection of thousands of genomes in a single experiment is not a straightforward task, therefore considerable research and development still needs to be done. Such efforts will open up new possibilities for miniaturization of conventional THz systems and provides a new starting point for THz-sensing biochip.

**Terahertz Biosensing Application**

Recently, our attempts to apply THz rays to biological and chemical materials have registered a noticeable success. In our laboratory, we have setup a long-term strategy for probing biomolecular interactions, including antigen – antibody binding, in the lower frequency part of the infrared spectrum using THz-DTDS, as described above. As a first step towards this objective, and in order to assess the sensitivity of THz system, we studied the binding between the eggwhite glycoprotein, avidin, and vitamin H, biotin.

Avidin’s most characteristic feature is its four identical binding sites for biotin, which provide the possibility of cross-linking between different biotin-containning molecules. The importance of the biotin – avidin system has been highlighted in numerous studies, and it has been widely used in a variety of biodetection methods, including affinity chromatography and binding assays. The procedure for covalent immobilization of avidin on glass slides and the subsequent biotin – avidin binding has been clearly described in detail in a previous paper. The surface of the glass slide has been derivatized using thiol-terminal silanes followed by a succinimide crosslinker to increase the density of bound avidin to glass cover slips. Only half of the avidin coated glass
slide was exposed to a solution containing biotin molecules, the other half of the slide was used as a reference. Switching THz rays rapidly between the sample and reference clearly gives a measurable signal, directly proportional to the difference signals between avidin and the avidin–biotin complexes. In order to control the effects of artifacts and avoid false positive results, we performed similar experiments as described above but in the absence of biotin. We found that a small measurable signal still exists, presumably, due to avidin surface inhomogeneity and/or instrument noise (Figure 2). The technique is able to detect films of submicron thickness with less than $0.1 \mu g/cm^2$ of biotin–avidin complex without sample perturbation, comparable to optical surface techniques such as ellipsometry, reflectometry, and standard integrated optics. Yet, we believe it is possible to reach higher sensitivity by controlling the glass surface chemistry and the biotin deposition procedure. Improvements are underway to monitor the interaction of a wide range of biomolecular interaction such as antigen–antibody, enzyme–coenzyme, and cell–cell specific affinity ligands using biochemically based methods for the amplification of the THz signal. By employing a secondary interaction with small silica beads conjugated with a ligand or receptor, which binds the surface-bound component, specific amplification of the optical signal can be obtained.

Using an integrated wave-guide, described above, to spot particular sequences of DNA, Nagel’s team has achieved sensitivity down to femtomolar levels. The authors used the frequency-dependent complex transmission coefficient $S_2(\omega)$ to show the agreement between their simulated and experimental results for an unloaded filter. Loading the filter with either denatured or hybridized DNA yields a frequency shift by less than 0.1 THz for denatured DNA and 0.2 THz for hybridized DNA. The transmitted signal in both cases is clearly dampened (Figure 3).

The most remarkable feature of their THz system is its enhanced sensitivity and the small amount of DNA material required to perform the experiment. As stated above, if these characteristics are adequately controlled, the newly developed THz system can be instrumental in an efficient detection protocol of genetic disorders, DNA hybridization and mutation, without using fluorescent tags. Previously, electrochemical detection of double-strand DNA, based on the amperometric or voltammetric transduction, has been reported. An acoustic-wave biosensor was also applied to sense the formation of complementary and non-complementary oligonucleotide complexes in solution. However, these non-optical approaches seem incapable of reaching the performance of the THz integrated biosensor reported by Nagel and colleagues. The ability of THz rays to detect single base mutation in a single copy of a gene will have tremendous impact on genomic technology, where the analytical challenges posed by small volume and high sensitivity are required. Integrated THz optical systems do not suffer from inherent problems related to electrical detection, such as solid contact, membrane adhesion, and pH sensitivity, as in miniaturized electrochemical and electrical biosensors.

Surface plasmon resonance (SPR) is another optical biosensor used extensively for investigating binding effects and determining the association/dissociation constants of a wide range of biomolecular interactions. Its ability to monitor the on–off binding of label-free antigen–antibody, DNA–RNA, and DNA hybridization in solution and in real time makes it one of the most successful biosensors in the marketplace. The capabilities of SPR seem unapproached with a THz biosensor, for reasons we will outline later. On the other hand, a new study at the Genome Technology Center in Palo Alto, California, showed that fluorescence based systems are able to detect small variations in DNA sequence irregularities with sensitivity 30 to 100 times greater than that achieved by the field-proven UV-absorption method, approaching or even exceeding the detection limits of THz rays. It is worth noting, however, that the sensitivity of fluorescence dye depends on several parameters directly related to properties of the chosen fluorophore, such as quantum yield and extinction coefficient. Therefore, the sensitivity limits in fluorescence-based detection methods should not be regarded as an absolute. Importantly, fluorescence spectroscopy is usually performed using fluorescent labels, which is impractical for in situ measurements. An obvious advantage of optical biosensor technology, however, is its ability to measure biomolecular interactions in a noninvasive manner.

**Perspectives and Limitations of THz Biosensors**

The technique of THz spectroscopy, which was conceived and delivered by physicists, has developed into a powerful biosensor for thin solid film measure-
ments. Although still in its infancy, the integrated THz biosensor has shown promise for detecting minute amounts of label-free DNA–DNA interactions at the femtomolar level. Continuing advancement and increasing affordability of laser sources, integrated optics, and surface chemistry will allow low-cost instruments with augmented multifaceted capabilities to be developed. Improving the instrumental signal-to-noise ratio is still a major challenge, and much work needs to be done to expand the THz technology to its limits. The major hurdle facing THz applications in biological science is its extreme sensitivity to water vapor, which is an enormous obstacle. The inability of the THz technique to monitor biomolecular interactions in solution is a serious limitation. Although the effect of water vapor can be significantly reduced using the THz-DTDS, several issues still need to be addressed to produce commercially viable devices able to compete with the success of SPR, as used in the highly successful BiACore instrument (Pharmacia). The binding kinetics of biologically relevant materials generally occurs at a time scale of a few milliseconds, higher than the THz time delay, and thereby it is practically impossible to determine the association and dissociation constants of ligand–analyte binding using THz systems, in spite of the fact that this is an advantage for following rapid chemical reactions, for example.

Although optical biosensors successfully monitor the interaction of biomolecules, it is still difficult to predict which optical method will be most advantageous for detecting a specific analyte for a particular application. Indeed, the most readily accepted consensus is toward an all-integrated molecular device capable of performing label-free recognition and measurement with few sample preparation steps. It is certain that future THz methods will breach the cross-discipline barriers and will find use in a number of nonconventional applications, including clinical, environmental, and military. There is no doubt that the curiosity-driven research in the molecular sciences will equally benefit from the future developments in this rather promising field.

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