Label-free probing of the binding state of DNA by time-domain terahertz sensing

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We present a promising approach for the label-free characterization of genetic material. Time-resolved terahertz (THz) transmission analysis of polynucleotides demonstrate a strong dependence of the complex refractive index on the binding state (hybridized/denatured) of deoxyribonucleic acid (DNA) molecules. By monitoring THz transients, one can thus infer the binding state of oligo- and polynucleotides, and hence identify polynucleotides by detecting the binding of unknown polynucleotide DNA sequences to known probe molecules. A broadband experimental demonstration in a free-space configuration, as well as a discussion of the potential application for next generation gene chips is presented. © 2000 American Institute of Physics.

One of the prominent challenges of biotechnology is the development of simple and flexible systems for the rapid analysis of genetic material. Widespread applications in medicine and biology with a weighty economic impact are envisaged, like, e.g., the detection of changes in the DNA-encoded chain in patient’s blood for the early diagnosis of diseases or gene therapy, the detection of specific signatures of viruses and bacteria, or the analysis of messenger ribonucleic acids (mRNA) for the analysis of metabolic processes. Traditional laboratory diagnosis of genes and mRNA require great expertise, expensive equipment, and can be extremely time consuming. Biochip approaches integrating large arrays of complementary deoxyribonucleic acid (cDNA) or synthesized oligonucleotides as probe molecules have greatly enhanced analytic capabilities by scaling up the number of genes which can be analyzed simultaneously. However, the basic processes necessary for detecting the presence of a gene is still complicated and requires the fluorescent labeling of one strand of DNA to detect its match (hybridization) with a sense cDNA strand or sense oligonucleotide. Labeling with fluorescent chromophores is not extremely time consuming. Biochip approaches integrating large arrays of complementary deoxyribonucleic acid (cDNA) or synthesized oligonucleotides as probe molecules have greatly enhanced analytic capabilities by scaling up the number of genes which can be analyzed simultaneously. However, the basic processes necessary for detecting the presence of a gene is still complicated and requires the fluorescent labeling of one strand of DNA to detect its match (hybridization) with a sense cDNA strand or sense oligonucleotide. Labeling with fluorescent chromophores is not only an unwanted additional preparatory step which complicates genetic analysis, but eventually introduces modifications in the DNA strand conformation, which lower the precision of gene detection. Labeling also deteriorates the quantifiability in comparative studies, as the fluorescence efficiency of labels is strongly site dependent and the additionally required processing steps induce labeling yield fluctuations. Calculations have predicted a multitude of resonances in the terahertz (THz) frequency range associated with phonon (base twisting, helix, and librational) or plasmon modes for DNA molecules. Although previous calculations are not precisely accurate as the underlying strengths of van der Waals, electron exchange, and Coulomb interactions are not precisely known, the presence of these interheli-cal excitations indicates the potential of THz measurements for the label-free detection of the binding state of DNA molecules. Various studies by Raman or Fourier-transform infrared techniques have been performed. However, some disagreement in the observed transitions indicates the difficulties of these techniques in reliably analyzing DNA, as indicated by recent time-domain analysis of hybridized DNA for different humidity conditions. In this presentation we demonstrate the potential applicability of transient THz transmission analysis to identify directly, without any labeling, the binding state of DNA strands. Measurements of the time-resolved THz transmission of polynucleotides demonstrate a dependence of the complex refractive index on the hybridization state (hybridized=double stranded/denatured =single stranded) of the DNA molecules. By monitoring THz transients one can thus infer the binding state of oligo- and polynucleotides, and hence detect the presence of genes by monitoring their binding to a known single-stranded probe polynucleotide.

In order to ensure identical experimental parameters when comparing hybridized and denatured DNA samples exactly the same material for both types of samples is used. This is important in order to exclude any influence of preparatory artifacts or variations due to contaminations. In our experiments we use the vector pcDNA3 (Invitrogen, Carlsbad, CA) as a DNA probe with a size of 5.4 kb. The DNA was amplified in E.coli and purified using a Nucleobond PC500 system to obtain pure, supercoiled DNA. DNA concentration was adjusted to 17 μg/μl in bidestilled H₂O. Denaturation of a part of the solution was performed by rising the temperature 5 min to 95 °C and immediately stopping on ice, in order to avoid renaturation. 2 μl of either native or denatured DNA was applied onto specially prepared sapphire substrates and dried in a desiccated atmosphere over 48 h to reach a steady state. This is important, as the THz refractive index of DNA was demonstrated to depend drastically on humidity. Thin films with a diameter around 2 mm and an
FIG. 1. THz transients (segment around time delay zero) of the transmission through hybridized DNA on sapphire, through the sapphire reference, and the difference $\Delta t(\tau)$ of both (top). The transmission spectrum through hybridized DNA illustrates the bandwidth of the system (lower plot).

approximate thickness of 30 $\mu$m were obtained. Transmission spectra were taken at 300 K in a N$_2$ atmosphere by standard time-domain THz spectroscopy$^{14,15}$ using either a solid-state or a Ar$^+$-ion laser pumped 100 fs Ti:Al$_2$O$_3$ laser source and photoconductive detectors.

Figure 1 presents typical THz transmission transients taken through a hybridized DNA on sapphire sample. The analysis of the absorption and refraction by the DNA films is performed by taking alternating measurements (20 times) of the transmission under normal incidence through DNA on the sapphire substrate ($t$), and through the substrate alone as a reference ($t_0$), in order to avoid experimental fluctuations. The difference $\Delta t(\tau)$ between sample and reference transients is calculated. The Fourier transformation of this time-domain difference determines the complex transmission difference $\Delta t_{\text{complex}}$, which reflects the transmission change (amplitude and phase change) induced by the absorption and refraction of the DNA film. The amplitude of this transmission change is plotted in Fig. 2 for various DNA samples, normalized to the transmission through their respective references to account for the spectral characteristic of the measurement system [only the region of the spectra with a signal to noise $\geq 50$ dB is taken into account in order to minimize errors]. A much higher change $|\Delta t_{\text{complex}}|/t_0|$ of the THz transmission induced by hybridized DNA samples in comparison to denatured samples is observed. This clear distinction between denatured and hybridized samples was found in all samples tested, which included diverse samples sets made from both the original and newly prepared DNA solutions and even measurements with different laser systems. One can clearly see from the transmission spectra in Fig. 2, that while denatured DNA has a small influence on the THz signal, hybridized DNA modifies the transmission of the THz pulse much stronger. This can plausibly be due to the predicted phonon or plasmon modes for DNA in the THz frequency range, which are absent or strongly modified in denatured material, or by a change in the eventually present hydration chain along the polynucleotide molecules. Nevertheless, regardless of the physical reason for the detected change, the observation of a transmission change indicates that THz sensing could provide a method for the label-free detection of the binding state of DNA.

In order to better understand the nature of the transmission change, we calculate the complex refractive index of the DNA films by numerical solution of Fresnel equations (including Fabry–Perot interferences) with a similar approach as in Ref. 16. One observes a clear difference as a function of the binding state in both the real and absorptive part of the refractive index. Figure 3 depicts the real part of the refractive index of such calculations, which demonstrates a broadband lowering of the refractive index of the native DNA material by $\Delta n \approx 0.1$ due to denaturing. The absorption coefficient of denatured DNA is correspondingly also lower. The only uncertainty entering this calculation of the complex refractive index is the absolute thickness of the not perfectly homogeneous DNA films. While this could slightly modify the absolute values for the refractive indices, calculations with different thickness indicate that this uncertainty shifts the refractive index of both denatured and hybridized samples parallel and has thus a small influence on $\Delta n$. Furthermore, this broadband change of the refractive index by $\Delta n \approx 0.1$ is reproduced in a wide variety of analyzed samples, including longer DNA segments of 15 kb. It is clear...
that the probed THz resonances are not expected to be sharp, due to the distribution of different base pairs along the DNA chain. Further analysis, e.g., in homo-based polynucleotides, are however required to fully understand the nature of the observed complex refractive index change.

The presented experiments represent a first observation of binding-state-dependent properties of polynucleotides in the THz frequency range. This observation does not only point out a probing concept for the fundamental analysis of biomolecules which can easily enable dynamic conformation analysis, it potentially also represents the basis for the development of future THz-based gene probing techniques. Although the sensitivity of our approach cannot yet compete with the superb sensitivity of fluorescence-labeled approaches, it is nevertheless straightforward to develop alternative detection geometries which increase the THz sensing drastically—details of such an approach surpassing a $10^3$ sensitivity increase will be presented later on. Label-free THz based polynucleotide probing will additionally become increasingly attractive as THz technologies mature and become more efficient, compact and easily used.

In conclusion, the presented time-domain analysis demonstrates the capability of THz techniques for detecting the binding state of genetic material directly, without requiring markers, potentially giving rise to label-free methods for genetic analysis which could be used in future biochip technologies.

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