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Ultrasensitive label-free detection and quantitation of DNA hybridization via terahertz spectrometry

Anis Rahman[1*], Bruce Stanley[2], Aunik K. Rahman[1]

[1]*Applied Research and Photonics Inc. (ARP), 470 Friendship Road, Suite 10, Harrisburg, PA 17111*
[2]*Penn State College of Medicine, 500 University Drive, Hershey, PA 17033*
**e-mail: a.rahman@arphotonics.net*

ABSTRACT

Time-domain terahertz (THz) spectrometry has been used to analyze DNA hybridization state and its quantitation in a label-free manner. Time-resolved THz signal (or temporal signal) converted to frequency domain constitutes a signature of a given molecular “event” (e.g., a vibrational or a conformational state). The temporal signal provides a means of probing a molecular event in an appropriate time window. This is a unique ability of this technique because different molecular events exhibit different time response based on their physical and chemical nature. Conformational difference of a given molecule results in different signature with an appropriate time response that can be accurately probed by a terahertz temporal signal. In this work we discriminate between single stranded and double stranded 25-mer oligonucleotides via spectral signature. For each species, different peaks were identified; however, the peaks are distinctly different allowing an easy comparison. Additionally, temporal transmission spectra of the DNA specimens were collected at normal temperature and atmosphere. The peak value extracted from the temporal spectra exhibit a power law behavior over a region spanning from 13.6 femto-molar to 0.136 nano-molar. The results clearly demonstrate the ability of the spectrometer to detect a minute amount of biomolecules in a label-free fashion. This capability can be used as a diagnostic tool, as well as for studying molecular reactions such as mutation.

Keywords: Terahertz Spectrometry, Label-free detection, DNA hybridization state, DNA Quantitation, terahertz temporal signal, Fourier analysis, Dose dependence, Single- and double-stranded DNA.

1. INTRODUCTION

Terahertz (THz) spectrometry has the potential to analyze DNA-DNA and other biomolecular interactions in a label-free fashion. THz measurement is conducted in time domain where the temporal signal is acquired over femto-second to a few tens of pico-second range. An optical delay-line is used to generate time delay on sub pico-second scale while the sample response is detected with a technique known as terahertz sampling. Therefore, an important advantage of this technique is that the delay time can be adjusted from tens of femto-seconds to tens of pico-seconds. This gives a useful means of probing a molecular “event” (e.g., a vibrational state or a conformational state) in an appropriate time window. This is an important ability that is not available from any other technique. This is also a powerful ability because different molecular event requires appropriate time window, based on its physical and chemical nature. For example, a molecular vibration occurs over a longer time scale compared to a bond vibration. Similarly, compositional or conformational difference of a given molecule results in different signature with appropriate time response that can be accurately probed by a terahertz temporal signal. The temporal signal (also called a pulse or transient), when converted to frequency domain via Fourier transform, constitutes the signature of a given event. This signature is unique and represents a means of identifying a molecular event, thus forms the basis of detection of molecular interactions such as a single nucleotide polymorph or molecular chirality.

Detecting hybridization of unknown DNA molecules is important for many biomedical applications because, by detecting hybridization of unknown target DNA molecules to known single-stranded oligo- or polynucleotide probe molecules, DNA sequences are readily identified [1]. The dominant approach to detect hybridization is based on fluorescent labeling of the target denatured DNA. Although fluorescent labeling has given rise to extremely efficient diagnostic systems, especially since the emergence of gene chips [2–3], a strong interest in developing alternative

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label-free detection schemes exists: labeling not only constitutes an additional costly and time-consuming preparatory step, it can introduce modifications in DNA strand conformation that lower the precision of gene detection [4]. Additionally, label degradation, labeling yield fluctuations, fluorescence efficiency site dependence and fluorophore quenching inhibit the quantificability of genetic diagnostics [5–6], which would be very important for various analyses, especially in cancer research. A large interest in label-free genetic diagnostic systems therefore exists.

Theoretical calculations predict a number of resonances in the THz frequency range associated with inter-backbone excitations of DNA molecules, such as propeller-twist, hydrogen-bond breathing, and base-roll and base-shift vibrational modes [7–9]. The presence of these modes indicates a unique potential of THz technologies for the label-free detection of the DNA binding state. Numerous investigations by Raman, Fourier-transform and time-domain THz techniques on hybridized DNA molecules have been performed in the past [10–12]; however, few experiments have addressed binding state specific analysis. Recently, it has been pointed out that the dependence of the binding state of DNA to its dielectric properties at THz frequencies can be used as a potential method for label-free gene detection [13].

In this report label-free detection of DNA hybridization state has been investigated. Biologically available small quantity DNA detection and quantitation has also been conducted via THz spectrometry. In the following we first briefly describe a terahertz source derived from electro-optic dendrimer. Subsequently we describe use of a pulsed terahertz spectrometer to investigate the DNA hybridization and quantitation with relevant results.

2. ELECTRO-OPTIC DENDRIMER BASED TERAHERTZ SOURCE

The electro-optic (EO) dendrimer was produced at ARP by doping commercially available PAMAM dendrimer with a suitable chromophore and then corona-poling at high electric field. The electro-optic coefficient (EOC) was determined to be 130 pm/V [14]. Such high EOC dendrimer is suitable for terahertz generation via electro-optic route, specifically (i) by electro-optic rectification (EOR) and (ii) by difference-frequency generation (DFG). Fig. 1 shows the tunable terahertz power generated via EOR [15]. To determine the terahertz waveform and the generated THz range of the present dendrimer emitter, a standard experimental arrangement of electro-optic sampling was used as shown in Fig. 2. The femto second laser beam from a mode locked Ti:Sapphire laser (FemtoLasers, Vienna, Austria) was split in to two arms with a 60:40 beam splitter. The 60-arm (the pump beam) was modulated by a chopper at low frequencies and then passed through a delay stage before focusing on the terahertz emitter. The 40-arm (the probe beam) was passed through an ITO coated lens and focused on the sampling sensor. The ITO is transparent to the 800 nm laser but reflects the major portion of the terahertz beam originating from the emitter.

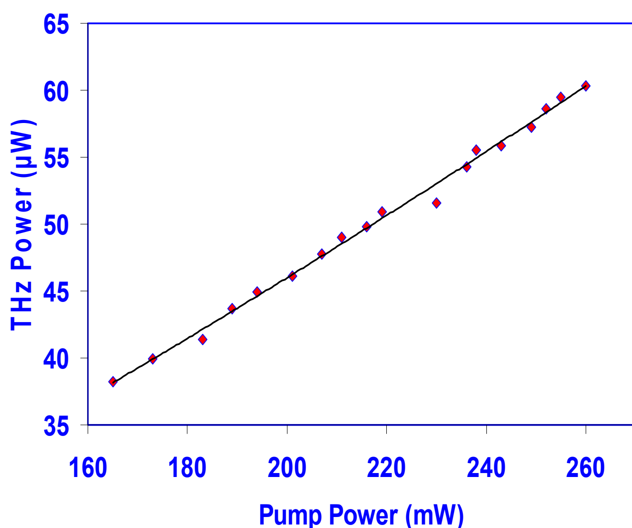

Fig. 1. Generated terahertz power vs. Pump power from a femto-second laser.

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Both the terahertz beam and the probe beam finally overlap on the sampling sensor (see Fig. 2). A pyroelectric detector (Spectrum Detectors, Lake Oswego, OR) was used to sense the sampling sensor's response. The detector entrance was covered by a 3.3 mm thick polyethylene filter that stops any IR from entering the detector. The chopper's frequency is detected by a lock-in amplifier that also reads in the pyroelectric detector's responses at the reference frequency. The lock-in amplifier is controlled by a computer to read the transient (or temporal) pulse; a typical pulse is shown in Fig. 3(a) and the corresponding Fourier transform magnitude spectrum is shown in Fig. 3(b). The dendrimer emitter of the present investigation produced a broadband terahertz radiation spanning up to 7 THz with the major peak of the magnitude spectrum around 2.5 THz.

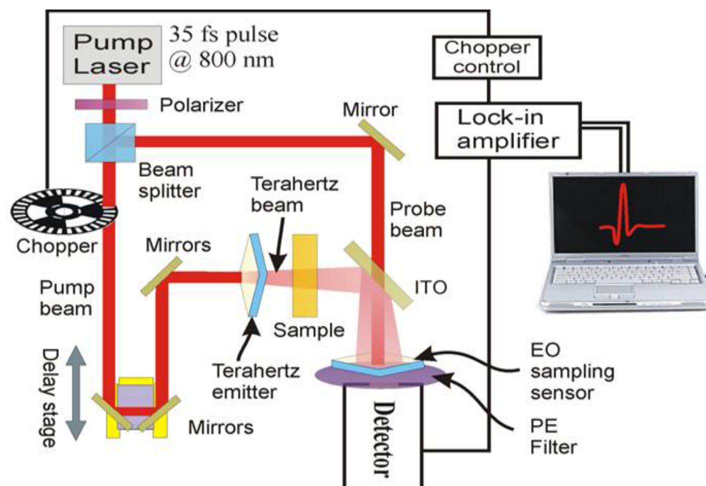

Fig. 2. Terahertz time-domain spectrometer setup. Terahertz detection is accomplished via electro-optic sampling technique.

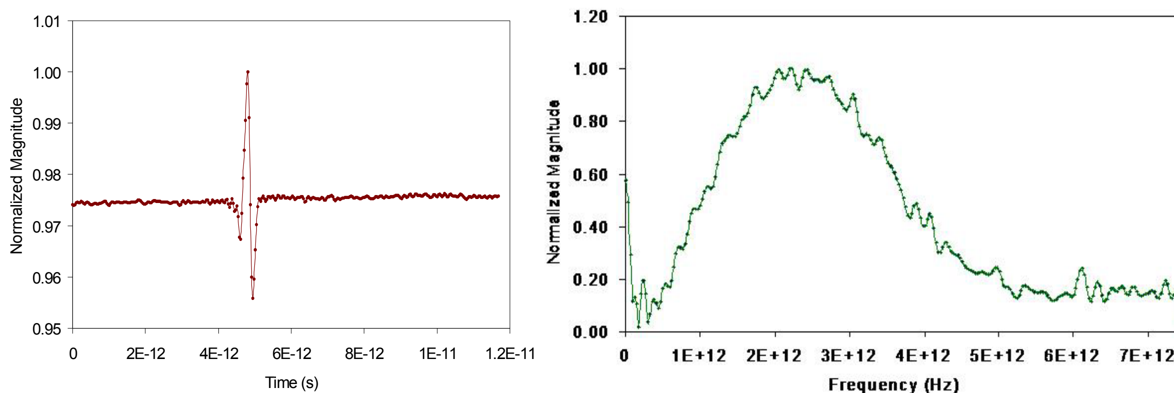

Fig. 3. (a) Typical temporal pulse envelope detected by a lock-in amplifier (without specimen). Sub-pico-second pulse is generated that is suitable for probing molecular events. (b) Broadband magnitude spectrum obtained by Fourier transform of the temporal pulse (a) showing a range of >7THz.

3. DNA HYBRIDIZATION DETECTION

Two complementary 25-mer oligonucleotides were synthesized at the Penn State College of Medicine Macromolecular Synthesis Lab, having the sequences: TCT TCG CAC TAT CCC AAG ATC TGA G (MW 7576.9 g) and CTC AGA TCT TGG GAT AGT GCG AAG A (MW 7746.1 g). Aqueous solution of these samples was applied onto microscope slides to make ~1 cm spots. Slides were prepared for both individual strands and hybridized duplex DNAs at different concentrations. The concentration of both single stranded DNA (ssDNA) and double stranded DNA (dsDNA) were varied over a range from nano-molar down to femto-molar.

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The setup of Fig. 2 was used to measure the terahertz spectra of 25-mer oligonucleotide samples. Terahertz time-domain signals were collected by an automated setup. Here the glass slides containing the DNA samples were placed in the terahertz beam path for a direct transmission under ambient conditions. The T-rays were transmitted through the specimen and then focused on a dendrimer sampling sensor that was probed by a femto-second pulsed beam. Both the pump and the probing beams were obtained from a single femto-second pulsed laser; therefore, both are coherent beams. Fig. 4 exhibits representative normalized temporal spectra of a blank glass slide. Fig. 5 shows the normalized temporal spectra of ssDNA and dsDNA samples obtained by subtracting the blank glass slide background. Fig. 6 shows the magnitude spectra that were generated by fast Fourier analysis of the temporal time-domain spectra shown in Fig. 5. Three different peaks can be identified for both ssDNA and dsDNA (Fig. 6); however, the peaks are significantly shifted compared to each other, thus allowing an easy comparison.

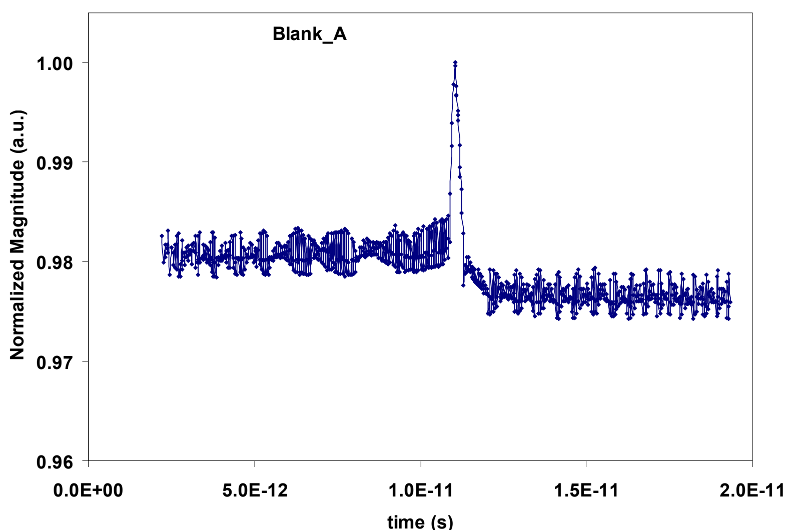

Fig. 4. Terahertz temporal spectrum of a blank glass slide.

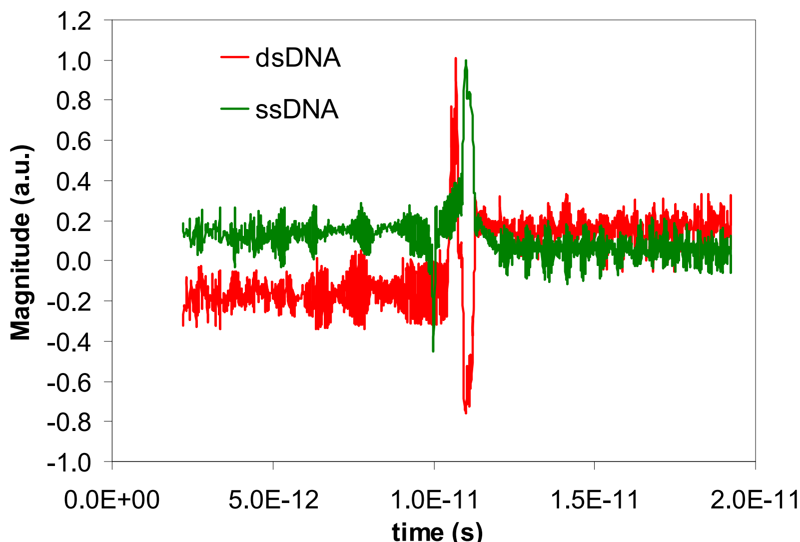

Fig. 5. Temporal transmission spectra of ssDNA and dsDNA samples.

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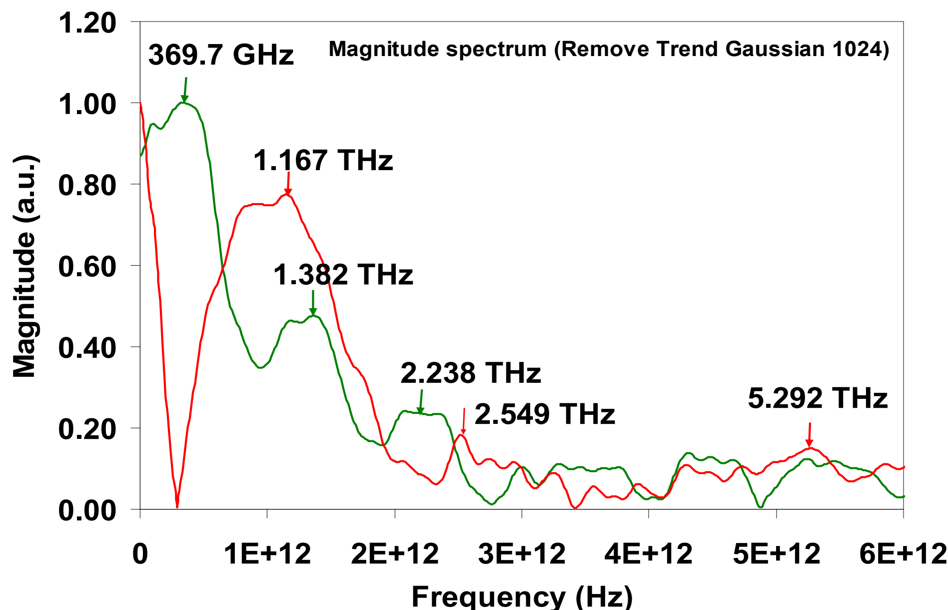

Fig. 6. Magnitude spectra of the ssDNA (green) and dsDNA (red) computed from the pulses of Fig. 5. Three different peaks can be identified for both ssDNA and dsDNA; however, the peaks are significantly shifted compared to each other, thus allowing distinct comparison.

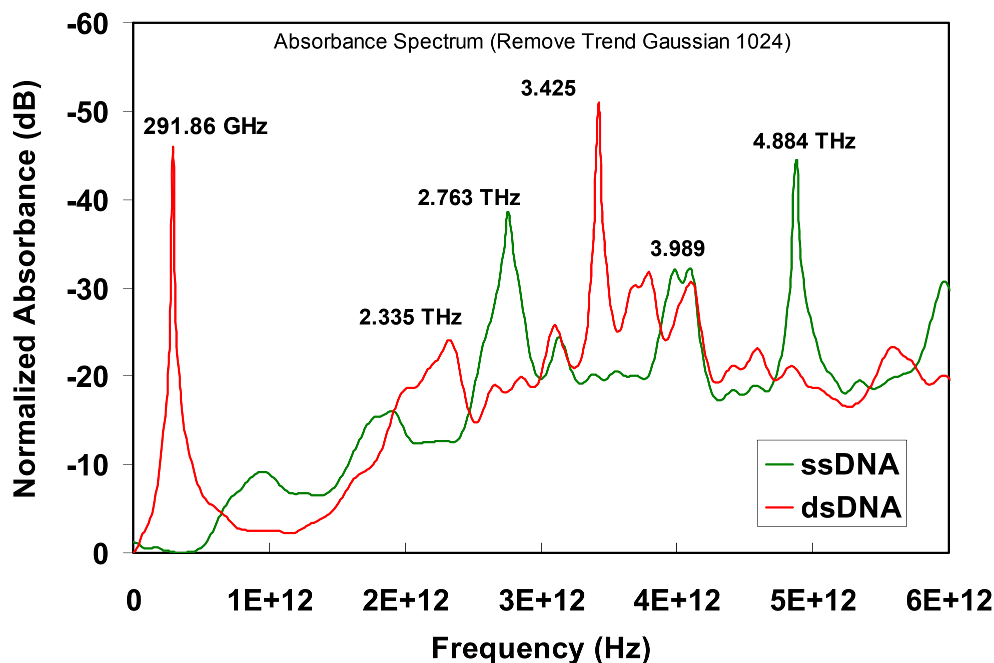

Fig. 7. Absorbance spectra of the ssDNA (green) and dsDNA (red). Sharp absorbance peaks are present that are shifted with respect to each other. These peaks are likely arising from unique vibrational and/or conformational states of the present oligonucleotides and the spectrum forms a unique signature of this particular sequence.

Fig. 7 shows normalized absorbance spectra of the DNA samples under study that were also obtained by fast Fourier analysis of the terahertz temporal pulses of Fig. 5. Here strong absorption peaks observed that are clearly

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distinguishable for the ssDNA and dsDNA. The origin of these peaks needs to be further investigated for conclusive inference. However, it is speculated that the different peaks are indicative of different hydrogen bonds between the oligonucleotide constituents. The stronger absorption peak at low frequency (~291 GHz) of the dsDNA samples has most like originated from the moisture content of the sample under ambient conditions. However, further investigation is necessary to validate the foregoing hypothsesis.

4. DNA DOSE DEPENDENCE AND QUANTITATION

Temporal transmission spectra of the dsDNA specimens on glass slides were collected at normal temperature and atmosphere. Fig. 8(a) shows the measured spectra that are normalized w.r.t. the highest concentration (lowest transmission). The peak transmission extracted from the temporal spectra of the dsDNA samples exhibit a power law behavior over a region spanning from 13.6 femto-moles to 0.136 nano-moles (see Fig. 8(b)). The results clearly demonstrate the ability to discern a minute amount of biomolecules in a label-free fashion. From measurements of known concentration, calibration curves can be established for different molecular species. The importance of this ability is in the fact that it will be able to identify a disease causing pathogen that may bind to the DNA causing unwanted changes (e.g., mutation). This capability can be used as a diagnostic tool, as well as for studying molecular reactions such as mutation. Further, Fourier transform of these temporal pulses will reveal the frequency domain signature of a given species (not shown here). Therefore, a combined knowledge of the temporal pulse and the frequency domain spectrum will establish a unique capability of detecting and quantifying molecular species.

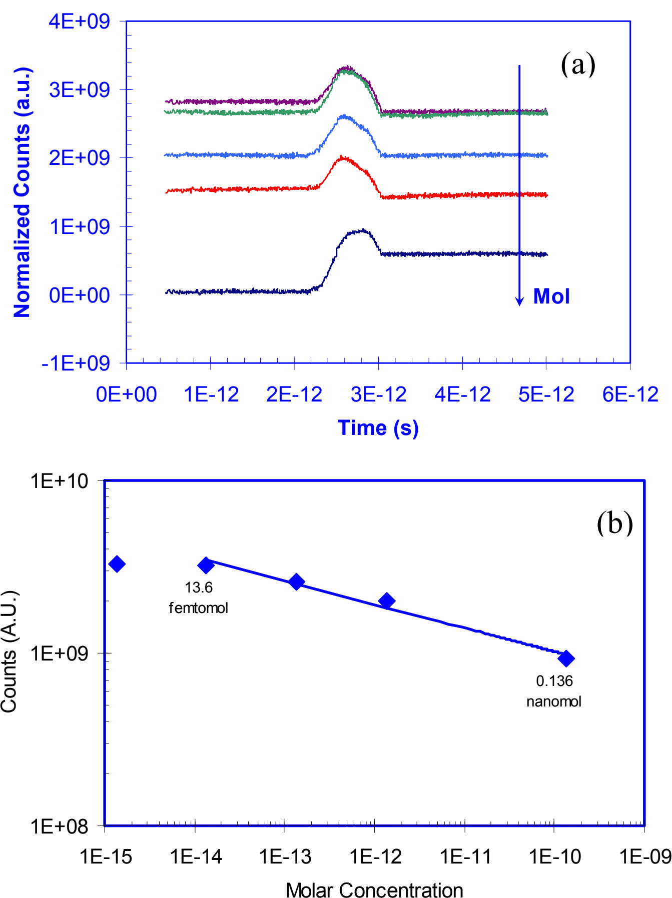

Fig. 8. (a) Temporal pulse of double stranded DNA at different concentrations (obtained from a DFG based setup [14]. Here the measured spectra are normalized w.r.t. the highest concentration (lowest transmission). (b) Concentration dependence of transmission peak of double stranded DNA samples. The data follow the Magnitude-Concentration inverse relationship within experimental error. Similar curve can be used to determine the concentration of an unknown from its transmission peak.

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5. SUMMARY

In this paper identification of DNA hybridization states and quantitation of very small amount of DNA have been demonstrated via terahertz time-domain spectrometry. It was found that ssDNA and dsDNA exhibit distinguishably different transmission (absorption) behavior over a frequency range of ~0 to 7 THz. It was also found that DNA concentration as small as ~14 femto-molar can be detected. This capability can be used as a diagnostic tool or for studying molecular interactions such as mutation and chirality.

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