



# Nanotechnology and Its Applications in Molecular Detection

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## Abstract

Nanotechnology deals with the manipulation of single atoms and molecules to build devices of nano- or microscale to use for various purposes in several fields. Through its unique properties, it has unraveled an array of new opportunities in molecular diagnostics to explore. Using several particles and structures on the nanoscale exhibits different properties when compared to their bulk counterpart and such properties are appropriate to use in molecular detection. Lab-on-a-chip is an advanced technology that encodes nanodevices on a disposable chip which is very significant in biological detections. Gold on the nanoscale has appropriate properties that can be used for several biological processes. Similarly, magnetic nanoparticles react when a magnetic field is applied, which would have great applications in bio-separation. Other such technologies include quantum dot technology, nanobarcodes, and nanoparticle probes. Structures like nanowires are used in various ways to detect and separate the target analyte. Nanopore technology makes use of a pore (in nanoscale) in appropriate (Nanotechnology in molecular detection) substrates to detect anomalies in the target nucleic acid molecules. Cantilever arrays, nanosensors, and DNA nanomachines are also very useful technologies in biological detection. This field of study has a lot more to explore in the future.

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## **6.1 Nanotechnology and Molecular Detection: Importance of Nanotechnology in Molecular Detection**

Nanotechnology is helping to improve many industries and technology sectors. The applications of nanotechnology include the detection of molecular disease markers, implant technology, molecular imaging, tissue engineering, and devices for drug, protein, and gene, and radionuclide delivery. By extending the limits of molecular diagnostics, nanotechnology has the potential to improve the performance of biological tests. Nanotechnology is being widely used in early disease detection, in vivo diagnosis, predictive medicine, and genomic technologies. Nanotechnology-on-a-chips are becoming more prevalent in diagnostic tests. They allow for faster, more sensitive, and flexible results. Magnetic nanoparticles can be used to label various molecules and organisms. They can also be used to detect a genetic sequence in a sample. For the investigation of nucleic acids, nanopore technology can be utilized to change over the strings of electrochemical nucleotides into electronic marks (Jain 2003). In this chapter, we have divided the major applications of nanotechnology in molecular detection into three main sections, nanotechnology on a chip, nanoparticle technologies, and other main inventions/technologies (Fig. 6.1).

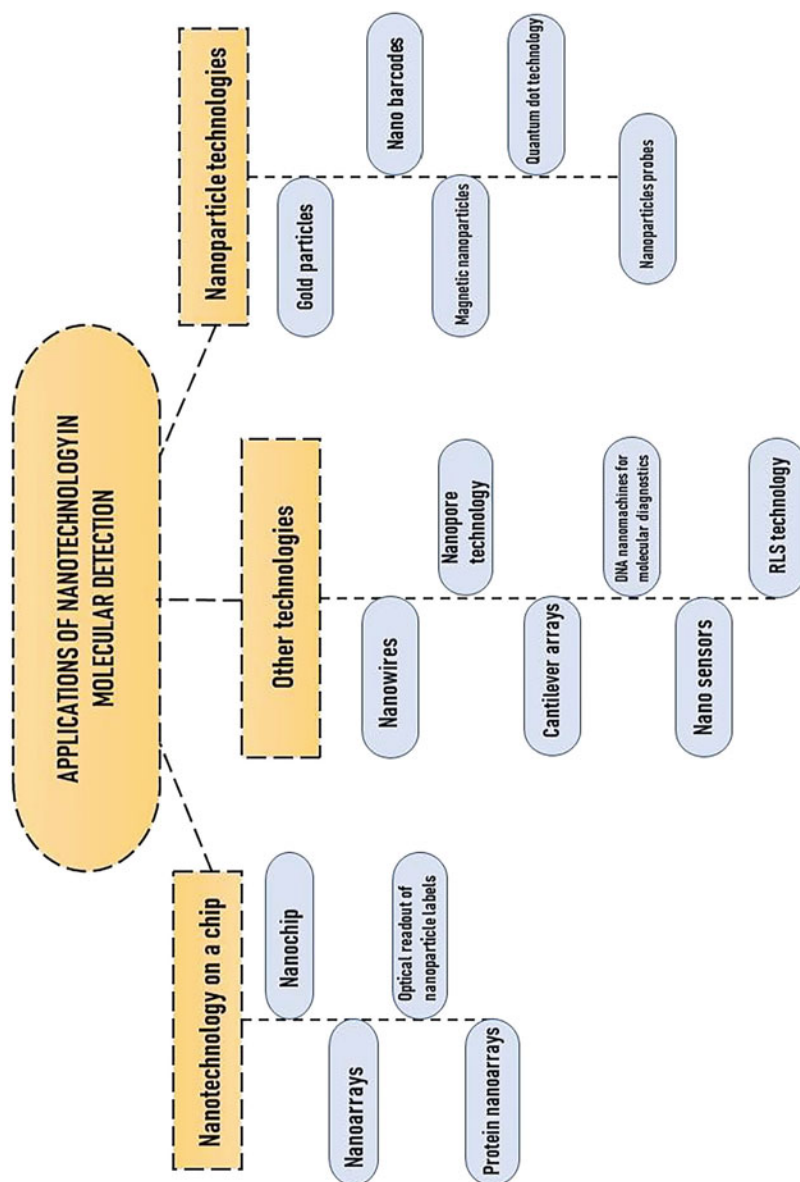
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## **6.2 Applications**

### **6.2.1 Nanotechnology on a Chip**

#### **6.2.1.1 Microfluidic Chips for Nanolitre Volumes: Nanochip**

A microfluid chip is a device that consists of multiple microchannels that are connected together to form a single cohesive unit. The microchannels are etched into materials such as glass, silicone, or polymer. These channels should be sealed tightly. The sample or liquid is injected into the chip via a syringe pump or a peristaltic pump. Microfluidic systems are used in various diagnostic procedures, nucleic acid detection, immunoassays, etc. Due to the development of processes like a deposition, electrodeposition, etching, bonding, injection molding, embossing, etc., the use of different materials (polymers, glass, silicon, etc.) for microfluidic chips has been possible. Microfluidic chips are widely used in many fields. The ability to integrate diverse medical tests on a single chip has proved to be useful in the biomedical field. These chips are also used in protein crystallization due to their



**Fig. 6.1** Classification of applications of nanotechnology in molecular detection

ability to generate on a single chip a large number of crystallization conditions. As microchannels have the same characteristics as that of biological cells, it allows easy manipulation of single cells and rapid drug changes and thus have found their application in cell biology research.

The **NanoCHIP®** is a small, lab-on-a-chip silicon product that helps in the identification and analysis of DNA/RNA. NanoCHIP® (Nanogen, San Diego, CA) has integrated test sites for 100 hydrogel-coated electrodes containing streptavidin. Programming is performed on a biologically evaluated target-binding probe that moves to a specific electrode and remains bound to streptavidin when a positive charge is applied. The electric field also concentrates the target molecule on the electrode, accelerates hybridization, and removes the non-specific binding material. Fluorescence is used for final detection (Martel et al. 2005).

### **6.2.1.2 Optical Readout of Nanoparticle Labels**

The study of molecular interactions with the help of optical readout of nanoparticles has emerged recently and it is essentially a parallel detection of DNA hybridization with the help of microarrays. The molecular interactions and binding events of DNA probes incubated with nanoparticle-labelled target DNA probes having a complementary sequence assembled on a glass chip are monitored optically using light. By counting on optical transmission and reflection, the process of detection is simplified to a great extent by stabilizing the samples. Using high light intensities for readout results in a much shorter detection time than using fluorescent dyes. Reanalysing the samples after months or years is possible due to the stabilization of the samples (Reichert et al. 2000).

### **6.2.1.3 Nanoarrays**

Existing DNA microchips/microarrays use cumbersome detection tools, making sample amplification and labelling very difficult, increasing analysis costs, and slowing down the time to obtain results. By showing higher sensitivity and simpler methodology, nanoarrays have taken over microarrays. Sensitivity, specificity, speed, portability, throughput, and cost are advantages of using nanoarrays accorded due to their small size which accounts for a large surface-to-volume ratio. Nanoarrays have specific target-binding properties and are structurally sturdy and reduce the need for skilled manpower. A nanoarray is read using a Nanoreader, an atomic force microscope, which is needed because of the ultra-miniaturization of nanoarrays. The probe used in the microscope scans the surface, records the surface interactions and detects topography and chemical interactions. These nanoarrays are used in the field of diagnostics, proteomics and drug discovery by allowing large-scale screening of drug and molecular interactions. There are two main strategies for generating nanoarrays. First, one can change the properties of an area, such with the help of light to create different chemical functionalities. Second, chemical components can be locally deposited directly onto the surface, for example, by mechanical means such as Nanometre “pencil”—a strategy similar to “new generation lithography” (Nicolau et al. 2005). Carbon Nanotube Technology (CNT) is an ideal nanoelectrode array platform. Carbon Nanotube Technologies (CNTs) are

structurally equivalent to a two-dimensional sheet of graphene encased in a column-shaped carbon tube. It consists of a single-walled carbon nanotube or a multiwalled carbon nanotube and has high aspect ratios (the length of the microarray being many times greater than its width). CNTs being critical components of the nanodevices are incorporated either directly or indirectly during fabrication routes (Chen and LI 2007).

#### 6.2.1.4 Protein Nanoarrays

A group of nanoscale protein domains on solid surfaces are protein nanoarrays and these are useful in ultra-miniaturized bioanalysis. The conception and program of protein arrays were presented in antibody arrays. The chip comprises a support surface such as glass, a nitrocellulose membrane, beads, and microtiter plates to which a chain of capture proteins are bound. A probe molecule labelled with a fluorescent dye is usually added to a matrix. The reaction occurring between the probe and the immobilized protein effuses a fluorescent signal which is scrutinized by a laser scanner. An aqueous environment is vitally important at all steps of chip manufacturing and functioning in order to inhibit protein denaturation. The sample buffer contains a high percentage of glycerol that helps to decrease the freezing point. The humidity of the production medium is also adjusted.

There are essentially three major types of protein microarrays—Analytical microarrays, [Reverse phase protein microarray \(RPPA\)](#), and functional microarrays. In Analytical microarrays, antibodies, aptamers, or affibody banks are placed on the surface of the holder. They are used to capture molecules as each molecule specifically binds to a specific protein. The array is probed with complex protein solutions such as cell lysates. Binding reactions obtained using an assortment of detection systems can be analysed to obtain information on the expression levels of specific proteins in a sample. Reverse (RPPA) arrays contain tissue lysates. Isolated cells from various tissues of concern are lysed and the lysate is set down on a microarray and probed with an antibody to the protein of interest. Chemiluminescence, Fluorescence, or colorimetry is used for detection. Control peptides are printed on slides to allow the quantification of proteins in sample lysates. In Functional microarrays, the target protein arrays are developed by immobilizing purified proteins. It determines the various interactions of proteins with DNA, RNA, phospholipids, other small molecules, and with other proteins too.

Researchers have fostered another kind of protein array for contemplating connections among proteins and different atoms on a nanoscale. The method used for the creation of the protein array is termed dip-pen lithography which uses an instrument to modify the gold film surface of the arrays. This method has proven to be highly sensitive and straightforward in the detection of DNA (Lee et al. 2002). A study done by [Minsu Lee et al.](#), using the dip-pen nanolithography (DPN) method, studied the single molecular nanopatterning and molecular interaction of proteins that were immobilized on the prolinker surface of gold-coated silicon wafer (Lee et al. 2002). Protein nanoarrays have been highly beneficial in proteomic and genetic screening, providing important leads for therapeutic agents in the pharmaceutical industry. Deviating from the mainstream applications of protein nanoarrays, a

protein-based light-harvesting system was established using protein nanoarrays that inspected the process of photosynthesis using enzyme-triggered protein nanosheets that resembled thylakoids (Zhao et al. 2017). The main advantage of using a protein nanoarray is that it has high throughput and can parallelly track a large number of proteins.

## 6.2.2 Nanoparticle Technology

### 6.2.2.1 Gold Particles

#### Introduction

Gold nanoparticles (AuNPs), also called colloidal gold, possess attributes like shape and size, large surface-to-volume ratio, multiple surface functionality, and low toxicity that make them widely compatible in several applications of bio-nanotechnology. AuNPs' unique surface chemistry and functionalization properties provide a versatile platform for nano-biological assemblies with several biological compounds like antibodies, proteins, and oligonucleotides, which makes AuNPs a promising candidate for molecular diagnostics. In diagnostics, AuNPs bind with the analytes which alters several physicochemical properties of the AuNPs like their resonance, conductance, and redox behaviour which can be studied to detect any particular analyte (Yeh et al. 2011).

#### Synthesis of AuNPs

Generally, gold nanoparticles are suspended in a solvent, most often water, which gives it the name colloidal gold. A broad range of approaches has been developed in the past decades to control the size, shape, and functionality of AuNPs. Turkevich et al. developed an appropriate artificial method for synthesizing AuNPs, where citric acid was treated with hydrogen tetrachlorocuprate in boiling water. In this technique, citric acid has two important roles; it acts as a stabilizing agent and as a reducing agent and this redox reaction gives rise to AuNPs. This method was refined by altering the citrate-to-gold ratio to control particle size (Turkevich et al. 1951). This protocol was only feasible when the required product was a dilute solution of moderately stable AuNPs with a diameter of 10–20 nm. AuNPs synthesis with citrate stabilizing mechanism had a drawback—these AuNPs can undergo an irreversible aggregation with thiolate ligands during the functionalization process. Several strategies such as using a surfactant prior to the modification and using thiotic acid as an intermediate via two-step functionalization solved the problem of aggregation (Aslan and Perez-Luna 2002). Even after nullifying such drawbacks, this protocol was not effective when the product needs to be synthesized in large amounts because of the high dilution rates.

In 1994, Brust and Schriffin formulated an effective protocol to synthesize AuNPs by producing alkanethiol-stabilized AuNPs through biphasic reduction with sodium borohydride (NaBH<sub>4</sub>) as the reducing agent and tetraoctylammonium bromide (TOAB) as the transfer reagent. Low dispersive AuNPs of 1.5–5 nm were synthesized by this methodology and the size could be altered by altering gold-to-

thiol ratios. These alkanethiol-protected AuNPs were more stable when compared to citrate-stabilized AuNPs and this stability was due to the van der Waals attractions between the ligands and the synergic effect of the strong thiol-gold interactions (Yeh et al. 2011).

### Properties of AuNPs

AuNPs synthesized for nano-biological purposes are generally spherical in shape. Spherical AuNPs possess several attributes: sufficient surface-to-volume ratio and improved biocompatibility and shape-related optoelectronic properties. Spherical AuNPs have the ability to quench fluorescence. They also exhibit the ability to emit a range of colours which is dependent on the size of the particle. The colours emitted range from brown, orange, and red to purple as the size of the particle increases from 1 to 100 nm and they have an absorption peak of 500–550 nm. This is called the “surface plasmon band” which indicates that the absorption band arises from the collective oscillation of conducting electrons due to the resonant excitation by the incident photons. This band is significant for nanoparticles because the band is absent in both small nanoparticles ( $d < 2$  nm) and bulk materials. Other physical properties the except size like shape, solvent, surface ligand, temperature, and proximity of other particles contribute to the colour-emitting phenomenon.

### Applications of AuNPs

Unique functionalization properties of gold nanoparticles form a versatile platform to form nano-biological assemblies with several biological compounds. These assemblies would alter the physicochemical properties of the nanoparticle, and these changes are detected to identify the analyte. This phenomenon makes AuNPs more effective in the field of molecular diagnostics. AuNPs also have applications in therapeutic drug delivery aided by the property of conjugation and applications in diagnostic imaging aided by its ability to quench fluorophores (Yeh et al. 2011).

#### 6.2.2.2 Nanoparticle Probes

Nanoparticle probes are nanoparticles (generally gold) to which DNA is attached through a proprietary modification procedure. These probes help to signal the presence of the target DNA sequence. This technology makes use of the advantageous properties of the nanoparticle to which the DNA is attached. AuNPs are used in the technology because of their versatile functionalization property and their ability to emit different wavelengths of light when their sizes are varied. Technically this technology is an extension of AuNP (Jain 2003). Based on this extended application of AuNPs, several products have been developed for molecular detection, which includes:

- A method called nanosphere spot assay is used for colorimetric detection of amplified DNA sequences. The identification and differentiation of single nucleotide proteins (SNPs) are achieved through the specificity of nanoparticle probes.

- Gold nanoparticle probe assay is used for DNA target analysis. This technique eliminates the time-consuming process of amplification and hence proves to be cost-effective.

Nanoparticle probes are very advantageous in several other fields like detection of SNPs, infectious disease diagnostics and antibiotic-resistant bacterial infection diagnostics.

### 6.2.2.3 Nanobarcodes

Nanobarcodes are the miniature version of barcodes in terms of their applications. This technology was introduced by researchers at Nanoplex Technologies (CA, USA) who have produced submicrometric metallic barcodes using sequential electrochemical deposition of metal to produce stripping patterns. These adjacent stripes have differential reflectivity and this enables the identification of the unique stripping pattern by the light of fluorescent microscopy. The standout of this technology is that this procedure of readout mechanism does not interfere with the use of fluorescence for the detection of analytes bound to particles by affinity capture. With the technology being appropriate, Nanobarcodes are used for population diagnostics and in point-of-care handheld devices. This technology has a wide range of applications that are to be explored. However, SurroMed Inc. has attempted to use this technology to develop a phenotyping platform with access to a large clinical population. This attempt from the company shows us the potential of nanobarcodes and how they would enable the development of personalized medicine through bio-marker-based drug development (Jain 2003).

### 6.2.2.4 Magnetic Nanoparticles: Ferrofluid

Magnetic nanoparticles have a core made up of magnetic substances which are generally iron (Fe), cobalt (Co), manganese (Mn), or nickel (Ni). The core is surrounded by biocompatible polymer chains and this polymeric layer is coated with affinity molecules that have the ability to capture the biological targets (analytes) from blood or other fluid samples. The size of magnetic nanoparticles is generally around 25–100 nm and they behave in liquids as a solution, not as suspension. Magnetic nanoparticles have unique properties that support their use in molecular diagnostics, such properties include large surface-to-volume ratio, high saturation magnetization, and importantly they can be manipulated by magnetic fields (Khizar et al. 2020). Magnetic nanoparticles are synthesized in appropriately sized cores which can be altered along with diverse surface coatings; these coatings provide the functionalization to the nanoparticle. The organic or inorganic coating makes the nanoparticle biocompatible to target the biomolecule (analyte). The interesting property of magnetic nanoparticles is that they exhibit super magnetism when the size is reduced. Such magnetically labelled biomolecules can be isolated from their carrier fluid on application of a magnetic field. These properties make the magnetic nanoparticles appropriate for molecular detection. The nanoparticles which have captured the analyte from the bloodstream or any tissue system can be isolated by applying magnetic fields and can be detected in several ways. Magnetic



nanoparticle technology is used in several detection procedures like NMR and MRI in medical fields. A wide array of applications of magnetic nanoparticles in bio-nanotechnology is available in the fields of sensing, detection, cell sorting, gene/protein analysis, and microfluidic mixing; the unique properties of magnetic nanoparticles make them the right candidate for all such applications (Jain 2003).

#### 6.2.2.5 Quantum Dot Technology

Quantum dots are generally nanocrystals made up of cadmium selenide coated with zinc sulphide and range from 2000 to 10,000 atoms wide in size. They are often referred to as tiny man-made semiconductor particles and their size normally does not exceed 10 nm. The extremely small size alters their optical and electronic properties which makes them different from the same material found in bulk. The frequency of the light emitted when irradiated with low-energy light is proportional to the size of the quantum dots. Quantum dots were observed to be unstable during their initial discovery but embedding the dots in the pores of latex beads solved the problem and made them more stable and ready for use. The majority of such nanoparticles can emit lights of specific wavelengths when excited with electricity or light; typically smaller dots emit shorter wavelengths generating colours like violet, blue or green. Bulk semiconductor materials exhibit the property of fluorescence but the quantum dots are scattered away from each other to create continuous conduction and valence bands. The fluorescence produced by the quantum dots can be readily controlled by changing their size during their synthesis, which makes their detection possible in any system. The emission wavelengths of quantum dots range from ultraviolet to infrared. Quantum dots also have high quantum yield, high photo stability, and high molar extinction coefficients. Recent studies also suggest that the fluorescent yield of quantum dots can be improved by building a “shell” of a larger band of semiconductor material around them. With their semiconductor-like properties, they have a wide application in photovoltaic devices and light-emitting devices. Their properties are also manipulated to use in bio-detection or bioimaging. In the generic process of bioimaging various types of organic dyes are used. However, these dyes suffer from low quantum yields and photostability. Quantum dots for their ability to have high quantum yields have been considered over the traditional dyes in many aspects. Quantum dots are found to be 100 times more stable and 20 times brighter than traditionally used fluorescent dyes. For bioimaging applications, the probes made of quantum dots in this case have to remain well dispersed and stable in the aqueous medium with extreme pH and ionic strength. In recent times, numerous techniques have been developed to make the quantum dots water-dispersible. Quantum dots are employed for *in vitro* and *in vivo* imaging, which in turn proves to be very important to diagnose many diseases and helps to understand embryogenesis and lymphocyte immunology (Jain 2003).

## 6.2.3 Other Nanoparticles

### 6.2.3.1 Nanowires

A nanowire is another type of nanostructure, with a diameter measuring in nanometres. Nanowires have similar structures to nanotubes, but they differ from nanotubes as they have different aspect ratios. The constraint that the nanowires follow is that their ratio of length to width is always larger than 1000. At such dimensions (like nanometre) quantum mechanical effects tend to be altered and are significant; for this reason, nanowires are also called quantum wires. Nanowires are manufactured by a variety of processes such as alternating current electrodeposition, chemical vapour deposition (CVD), and thermal evaporation (Mahbub and Hoque 2020). They also have high sensitivity and comparatively lower response times compared to other sensor systems. The properties of such structures when manufactured with non-toxic materials can serve a great purpose in bio-nanotechnology. Compared to their bulk counterpart, nanowires have significant electron transport properties and their charge carrier motions are improved (Rabbani et al. 2020). Nanowires are more efficient than nanotubes in several ways. One being the modifications in their design are controllable during the synthesis process to alter and manage operational parameters; another being many materials that are used to produce nanowires are compatible to open the cops for functionalization. For their property of small size and capability, they are appropriate for bio-detection of pathogens and biological chemical species. Wang and Joseph developed a biosensor to determine the existence of toxicants within the living cell where the sensor had optical fibers with nanowires covered with antibodies (Wang 2005). Cullum et al. synthesized nanowires over gold electrodes and coated ZnO for the detection of hydrazine by amperometric responses (Cullum et al. 2000).

### 6.2.3.2 Cantilever Arrays

Cantilevers are rectangular beams that are about 1  $\mu\text{m}$  thick. These micromechanical cantilevers are functionalized with receptor molecules that adsorb or recognize molecules on their surface, which causes surface stress and bends the cantilevers. And a laser beam detects such nano bending of cantilevers. Their major advantage of such cantilever systems is that they can function in various environments like liquid, air, or vacuum. Such technology is utilized in molecular detection where silicon cantilevers are used to detect the analyte. In biological applications, the surface of each cantilever is coated with DNA which binds to the target sequence. These DNA-coated cantilevers are exposed to the sample and the surface stress bends the beam by some nanometres and this indicates the presence of the target in the sample. When these cantilevers are assembled in a microarray assembly, they have the ability to detect multiple unlabelled biomolecules simultaneously within minutes (Mckendry et al. 2002). The ligand-receptor interactions occurring on the microfabricated silicon beams lead to nano chemical bending and this is detected optically *in situ*. Differential measurements along with control cantilevers on an array of eight cantilevers can specifically detect DNA targets in 75-fold excess of non-matching DNA background. These are also used to investigate the

thermodynamic characteristic of the biomolecular interactions happening on the array. Because of such properties exhibited by the cantilever array system, they allow multiple binding assays in parallel and they have the ability to detect femtomoles of DNA at a background of a complex solution. One significant application of microcantilever is in the detection of prostate-specific antigen (PSA). The cantilevers are coated with PSA antibodies and are exposed to the sample. The bending due to surface stress is detected by the laser and this detection indicates the presence of PSA. The advantage of this detection technique includes the ability to detect PSA in clinically relevant concentrations in the background of other proteins and this technique is more cost-effective as it eliminates the requirement of labelling. This technique demonstrated lesser false positives which are caused due to irrelevant binding of foreign molecules to the PSA antibodies (lang et al. 2005). The development of such successful detection techniques using cantilever arrays attracts a lot of research to exploit the capacity of this technology. Rockville, Inc., MD, USA, is striving to develop a cantilever technology that would allow multiple tests to be performed. on a single disposable chip (Jain 2003).

### 6.2.3.3 DNA Nanomachines for Molecular Detection

One of the most advanced protocols for manipulating DNA has led to performing computational operations. A radio antenna is fixed to a specific DNA molecule and when the relevant radio frequency is captured by the antenna, the DNA molecule seems to be boosted with energy and as a result, the DNA molecule responds. The functional antenna attached to the DNA molecule is made up of a combination of metals and its size is around 100 atoms wide and 1 nm long. A radio signal sent to such a system of DNA attached to an antenna has been shown to cause dehybridization of the DNA strand (Hamad-Schifferli et al. 2002). The major advantage of this technique is that the switching process is reversible in certain conditions and it does not affect the neighbouring molecules without the antenna. This revolutionary demonstration opened a wide range of possibilities to explore. This technique should show promising results for proteins, peptides, or other biomolecules. In June 2001, this technology was licensed to EngeneOS, Inc. by the Institute of Technology (MIT, Cambridge) (Lee et al. 2002). Though the capacity of this technique is not exhausted yet, its applications in molecular detection are identified and they include biomolecular detectors and the direct electronic readout of biomolecular interactions.

### 6.2.3.4 Nanopore Technology

An advanced sequencing technique involving a nanopore is a traceable technique that enables real-time analysis of long DNA fragments without amplification. The basic principle of such a biosensing protocol involves the construction of a pore structure that is a few nanometres wide through a planar membrane that divides two chambers of saline solution. This setup is made in such a way that the only path between the two chambers is through the nanopore. When a relevant voltage is passed, ions from both the chambers move through the nanopore which thus creates a stable ionic circuit with an open nanopore. Generally, the transmembrane current is

of the order picoamperes (pA) to nanoampere (nA). In such a system, if we introduce a negatively charged DNA fragment to the chamber connected to the cathode, due to electrophoresis the DNA molecule tends to translocation from one chamber to another through the nanopore. While the translocation of DNA the transmembrane movement of the ions is decreased as the electrophoresis mobility of DNA is less than that of the ions. Due to this phenomenon, the transmembrane conductivity will be decreased and it produces a blockage signal which is recorded as the transmembrane current. This blockage signal helps us to detect the DNA molecule. In theory, each nucleotide has the ability to generate specific current blockage signals which could be detected and this detection could theoretically permit DNA or RNA sequencing. Agilent Laboratories is collaborating with Harvard University to develop such a technology (Jain 2003). The major advantage of nanopore sequencing is that it eliminates the need to amplify a DNA strand and instead can give out the desirable product even with a single DNA molecule. The thickness of Si<sub>3</sub>N<sub>4</sub> or SiO<sub>2</sub> nanopore substrates is generally several tens of nanometres. But when a DNA strand travels through the nanopore, more than a single nucleotide is present in the nanopore which prevents the detection of each nucleotide and thus prevents sequencing. Graphene serves as an ideal substrate for single nucleotide resolution as it is a 2D nanomaterial with a thickness of a single atom. The current through a graphene field-effect transistor (FET) can be measured with the transmembrane ionic current. Radenovic et al. observed a change in carrier concentration of graphene nanoribbon due to the translocating DNA which thus led to spikes in drain current and transmembrane current of the graphene transistor. This technology can be exploited in biosensing and molecular detection. Nanopore technology has the capacity to distinguish and count a variety of specific biomolecules in a complex mixture. A biosensor has been designed that forms a DNA nanopore by covalently attaching one single DNA nucleotide to the lumen of  $\alpha$ -haemolysin ( $\alpha$ -HL) (Howorka et al. 2001). The binding of single-stranded DNA (ssDNA) to the tethered DNA strand causes a change in ionic signals. With this technique, we are able to discriminate between individual DNA strands up to 30 nucleotides in length (Shi and Fang 2018). This technology can also be applied to protein analysis. Nanopore technology's advantage of speed and simplicity has the potential to facilitate the development of molecular detection through nanotechnology.

### 6.2.3.5 Nanosensors

Nanosensors are high-sensitivity nanoscale biosensors that can convert physical quantities to detectable signals. Sensors play a main role in observing the unique changes of biomolecules that encode significant data that aids in interpreting basic biological processes. With an increasing number of studies in which intelligent nanosensors based on molecular markers are effectively applied in the pharmaceutical field, this superior technology has become a promising approach for pharmaceutical use. There are mechanical nanosensors and chemical nanosensors, both of which have different detection mechanisms. Currently, top-down lithography, bottom-up assembly, and molecular self-assembly are some of the ways used to make biosensors. Owing to their size-dependent physicochemical properties,

nanoscale materials have materialized as potential contenders for biosensor applications by providing unique information about real-time changes in crucial physiological parameters. Nanotubes, nanoprobes, nanoparticles, nanosensors having nanowires, cantilever involving nanosystems, and nano-electromechanical systems (NEMS) are a few different types of nanosensors (Shawon et al. 2020). Some of the advantages of nanobiosensors that make them useful and efficient in their detection of biomolecules include small size, high adsorption surface area and mobility of the particles, replicability of the particles, and greater surface-to-volume ratio, etc. The sensor can be electronically controlled in response to the binding of molecules. Prototype sensors have confirmed their ability to detect nucleic acids, proteins, and ions (Jain 2012). Fluorescence-based nanosensors, optode-based nanosensors, carbon-nanotube-based fluorescent nanosensors, and quantum dot-based fluorescent nanosensors are a few developments in the area of bio/nanosensors (Rong et al. 2019).

### 6.2.3.6 Resonance Light Scattering (RLS) Technology

Resonant light scattering (RLS) is elasticized scattering and occurs when the energy of the incident beam is immediate to the absorption band. First, we implemented RLS technology for studying biopolymers using traditional fluorescence spectroscopy. RLS has become a very interesting technique used to monitor molecular clusters and characterize chromophores. In recent years, RLS technology has been used to identify a variety of pharmaceutical and biological macromolecules such as nucleic acids, proteins, metal ions, and bacteria (Jiang et al. 2007). Incident wavelengths are particularly described in the absorption shell and amplified signals are being observed. Numerous RLS-derived approaches have been developed. The RLS technology has been used to study the aggregation of chromophores due to its simplicity and versatility. The observed RLS effect is an increase in the scattering intensity at the absorption wavelength of the aggregate molecule. If there is a strong electronic bond between the chromatophores, the effect can be improved many folds. RLS can benefit from particle size strategies established for current light scattering. DLS (Dynamic Light Scattering), a physics approach for determining the size distribution profile of tiny particles suspended in fluid or polymers in solution, measures the frequency spectrum of scattered light (Pasternack and Collings 1995).

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## 6.3 Conclusion

With the advent of recent developments in the field of biotechnology, numerous fields of research have opened up new avenues for investigation. Nanotechnology is one such field and its applications in molecular detection can pave the way to identify, diagnose and treat diseases. In the near future, more advanced applications of nanotechnology could emerge facilitating a multitude of discoveries. The promise of non-invasive, increased sensitivity and specificity, small sample size and reduced

time highlights the vital role of this technology in nanodiagnostics and the field of scientific research.<sup>1</sup>

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