Optical Transduction Mechanisms in DNA-based Nanobiosensors for Fluorescence Detection

The Fundamental Science of Nanotechnology Assignment 2

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The field of DNA nanotechnology has grown rapidly as investigators have increasingly harnessed the selectivity and self-assembly of DNA base pairing to form a wide range of applications. DNA-based nanobiosensors, for instance, are of major interest due to their significant breakthrough in the field of medical diagnostics, leading to the development of faster and more cost-effective detection methods than the tranditional method. With a focus on optical detection principle utilised by DNA-based nanobiosensors, the basic mechanisms and functionality of the fluorescence detection are outlined, alongside an illustrative example in the field of molecular beacons (MBs).

I Introduction:

Advances in nanotechnology have accelerated the process of integration of various scientific fields (e.g. biology, medicine and genetics), resulting in the prompt development of nanobiosensors [15]. With help of another rapidly developed of deoxyribonucleic acid (DNA) technology, nanobiosensors have been increasingly studied in medicine and healthcare, in addition to their applications in gene therapy at the molecular and cellular levels [10]. One of the most commonly investigated topics of research in this area is combining gene and biosensor technology, as a frontier science centred on designing novel types of optical DNA-based nanobiosensors [13]. Various optical detection principles also enrich the study of the functionality of the DNA-based nanobiosensors, including fluorescence, surface plasmon resonance (SPR), chemiluminescence, colourimetry, interferometry, and surface-enhanced Raman scattering (SERS) spectroscopy [11]. These methods of optical transduction provide an endless number of applications of nanobiosensors in vast array of subjects, especially in biomedical and diagnostic contexts. This report focuses on the optical transduction mechanism (especially the fluorescence detection method) employed in DNA-based nanobiosensors. Further providing an example implementation of molecular beacons (MBs), which has been investigated in detail in the context of fluorescent probes in optical nanobiosensors.

II Optical DNA-based nanobiosensors and Fluorescence Detection:

II.1 Optical DNA-based nanobiosensors:

DNA is an ideal material for nanofabrication of nanobiosensors because of its self-assembly and biocompatibility. Moreover, DNA hybridisation can be optically detected by measurement of changes in fluorescence, colourimetry, luminescence and interferometry, which provides a wide range of approaches to building optical DNA-based nanobiosensors [14]. The possibility to add genetic material into the intracellular fluid results in DNA-based nanobiosensor being more useful for the detection of biomolecules inside living cells [5]. In general, the detection method of the optical nanobiosensor is achieved via immobilised reagents, able to interact with the analytes to produce a complex with distinctive optical properties [21]. Another novel method of detection involves directly coding the fluorescence sequence-information into the genetic materials, allowing detection of a specific DNA sequence [8]. Because of the high sensitivity and flexibility of fluorescent coding, an even greater degree of control is possible over optical DNA-based

nanobiosensors detection of changes in optical responses. Hence DNA-based nanobiosensors are being increasingly applied in assay development, leading towards simple and rapid testing for genetic disease and infectious agents [5].

II.2 Principle of Fluorescence Detection:

The most common DNA-based nanobiosensors make use of fluorescence detection. Scientifically speaking, an electron emits a photon when it returns to its original ground state, which can provide a faint optical signal. Fluorescence detection is achieved by measuring the slight changes of transition energy when a valence electron is excited from its ground state to an excited state [15].

Nanobiosensors based on fluorescence detection techniques have been developed that rely upon target hybridization with labelled probes (e.g. MBs) [2]. A process of DNA hybridization in the fluorescent probes can be employed to detect a specific DNA sequence. Researchers use this technique to develop platforms allowing for the detection of mutations (the permanent alteration of the nucleotide sequence of the genome). In a general fluorescence detection process, an artificially manufactured probe is designed as a short strand of DNA, which contains a replacement base with fluorescent properties (e.g. anthracene) in place of one base in the sequence [8]. The strand is then added, as a probe, to the targeted DNA sequence, forming a double helix. If all the bases in the strand being analysed pair with those on the probe, a decreased fluorescence signal will be observed. If there is a mismatch, the fluorescence signal will increase. This change in intensity can help us identify locations of any mutations present in the targeted sequence. The fluorescence signal can be reduced or quenched in two possible ways: (1) through contact with the faces of the adjacent DNA base pairs or (2) through interaction with water molecules [10].

An example utilising fluorescence detection in optical nanobiosensor design is seen in Molecular beacons (MBs), which are single-stranded nucleic acid probes with specific detecting functions in optical DNA nanobiosensors [9]. MBs are widely used in fluorometric analysis of nucleic acids and single nucleotide polymorphism. They enable a high signal-to-noise ratio with hybridization, such that high specificity single base mismatches can be detected with ease [3]. Further information about MBs based nanobiosensors is presented in the next section.

III Molecular beacons as fluorescent probes in nanobiosensors:

In this section, an example of utilising the fluorescent detection principle in a novel designed nanobiosensors, which is a molecular beacons (MBs) coated optical-fibre nanotip for DNA detection [9] is discussed. The DNA detection mechanism using molecular beacons (MBs) as fluorescent probes is firstly introduced. The fabrication method of the MB-coated optical nanobiosensor is further provided, as a route to understanding the manner in which the product can be applied to various diverse fields. Finally, the use of MBs as probes in nano-chips is evaluated, and compared to previous non-nano methods of detecting the DNA sequence.

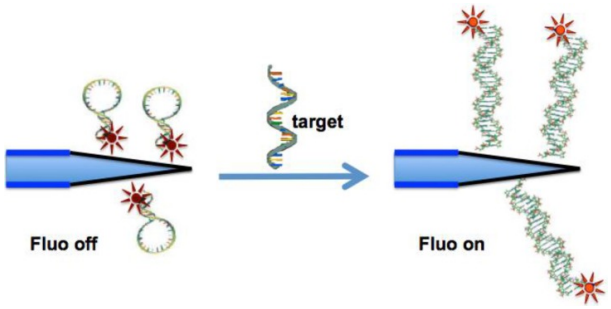

Figure 1: Optical fibre nanotips coated with molecular beacons (MBs) for DNA detection: scheme of the sensing mechanism [9]

An illustration of MB-coated fibre-optical nanotips for DNA detection is shown in figure 1. The solid support chosen as a substrate for the immobilisation of the MB was a tip of an optical fibre (diameter $\approx 500um$) tapered at nanoscale size (down to $\approx 30nm$ at the tip). This sharpened fibre-optical nanotip is designed as a nanobiosensor for mRNA/DNA detection inside the cytoplasm of living cells [17]. This is a novel non-radioactive

method for detecting specific sequences of nucleic acids. The fibre-optical nanotip was manufactured using a dynamic chemical etching method, which can be considered in detail in the fabrication stage, involving mechanical rotation and being dipped in a chemical etching solution (e.g. hydrofluoric acid) covered with a protection layer.

III.1 Molecular beacons (MBs)

Molecular beacons (MBs) are single-stranded nucleic acid probes, a polynucleotide molecule with specific detecting functions. An illustrative diagram of an MB is shown in the figure 2 [7], which is comprised of three functional domains including a stem, a loop, and a fluorophore/quencher pair ('F' and 'Q'). The signalling elements ('F' and 'Q') can produce on/off signals depending on the conformational state of MBs. Ideally, a hairpin structure of an MB comprises 4-7 nucleobase pairs (bps) in the stem region and 15-25 bps in the loop region [6]. The loop region is the detection element capable of inducing a conformational change in MBs based upon hybridization with its complementary target [20].

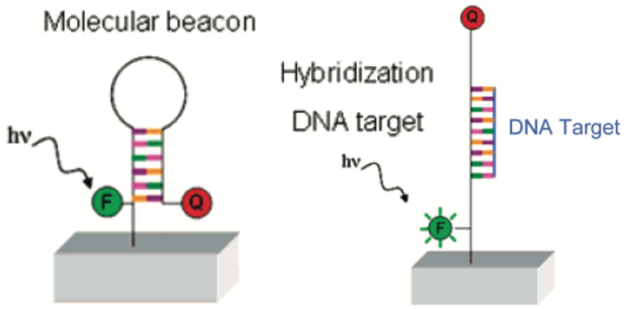

Figure 2: Schematic of molecular beacon (MB) and hybridization with targeted DNA. [1]

In an MBs fluorescence detection process, the MB undergoes a conformational reorganization upon hybridization of the target oligonucleotide with the loop region of MB[1]. The quencher ('Q') and fluorophore ('F') are spatially separated, causing the fluorescence signal to be restored [2]. The fluorophore is capable of being excited to a higher energy state. The fluorophore then rearranges from the high state back to the ground state, and the excess energy is released and emitted as light. Emission of light returns the fluorophore to its ground state such that the fluorophore can again absorb light energy. This technique could be adapted to detect changes in specific DNA sequences and single base mutations in a variety of genetic diseases [7].

III.2 MBs coated Fibre-optical nanotips

MB-coated fibre-optical nanotips are conceived as integrated nanobiosensors consisting of MBs as recognition probes, coupled with the interface of the optical fibre, designed to enable diagnostic tests at the level of individual living cells through a DNA detection system [9]. These nanobiosensors are fabricated with optical fibres pulled down to their tips (so-called nanotips) with distal ends in nanoscale dimensions, which can be coated with immobilised MB probes selective to target mRNA/DNA sequence. The resulting evanescent field at the tip of the fibre is used to excite target DNA bound to the detecting region of MBs upon introduction of laser light. A photometric detection system is used to measure the fluorescence signal originating from the MBs or from the polymerase chain reaction (PCR) [1]. The performance of the fibre nanotip as a nanobiosensor can be evaluated by monitoring the fluorescence signal as a function of time when exposed to different concentrations of the target [3].

III.3 Fabrication method of MBs coated fibre-optical nanotips:

III.3.1 Fibre-optical nanotips fabricated by a dynamic chemical etching

Fibre-optical nanotips can be produced traditionally by two main methods (1) the heating and pulling method or (2) the chemical etching method. The heating and pulling method is not recommended because of its high cost (e.g. expensive equipment) and complicated manipulations [19]. Instead, chemical etching is a more economical and convenient method for fabrication of nanotips. It offers a better control of the shape and roughness of a fibre-optical nanoprobe, allowing

the production of fibre tips with a higher optical throughput, due to shorter cones and larger cone angles. Here, a dynamic chemical etching method to produce optical-fibre nanotips is introduced, combining the mechanical movements together with chemical etching techniques [9]. This method uses different dynamic regimes of the mechanical movements during the chemical etching process to vary the cone angle, the shape and the roughness of the nanotip. In a detailed process, one of the tipped optical fibre ends is dipped into an aqueous hydrofluoric acid solution, with the other end being connected to a rotated motor. The precision translation stages are used to align the fibre on the rotation axis [3]. The aim of this is to control the rotation movements to change the behaviours of the hydrofluoric acid solution, further resulting in the shapes and surface characteristics of the obtained nanotips. Modifying the speed of extraction can alter the angle of the nanotip. Dynamic chemical etching methods can mass-produce fibre-optical nanotips with distal ends smaller than 30 nm, with high yield and cost effectivity (product with cone angle ranging from 15^o to 40^o , and roughness below 10 nm, as shown in Figure 3). This nanometric roughness serves as an optimal coating substrate for biosensing probes (e.g. MBs).

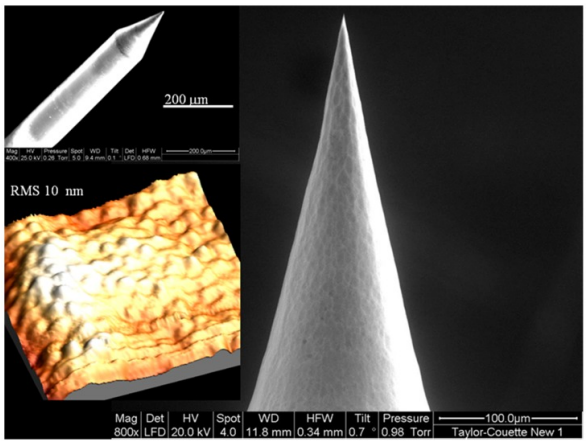

Figure 3: manufactured optical-fire nanotips [3]

III.3.2 MBs coating and immobilisation

Once the nanotips have been produced, the MB probes can be immobilised. Before the immobilisation, the MB is heated for 5 min in a water bath at about 70-80 ^oC (slightly higher than the melting temperature) and then left to slowly equilibrate down to room temperature in order to optimize the hairpin formation. The immobilisation onto the substrate of the nanotip was performed overnight at 4 ^oC [9]. Then the fibre-optical nanotips are covered with a protection silica layer, and the MB is attached via a covalent-binding procedure [3]. This immobilisation method is also widely used in a gene-chip design, which requires the MB probes to be stable and remain functional after attachment so that biomolecules can be immobilised with an appropriate orientation and configuration [18].

III.4 Evaluation: Comparison to the non-nano method for DNA detection

Even 20 years ago, genome sequencing technology employing nanobiosensors was simply non-existent [12]. Industrial-scale systems were required in order to sequence such technology, where today they can be sequenced in a tiny chip the size of a finger. In the postgenomic era, there is a continuing demand for highly sensitive and selective DNA probes, because DNA is stable, cost-effective and easily adaptable to optical fibres and microarray technology for device manufacture [17]. This has already prompted MB probes to be coated with optical nanotips and developed in nano-chips for gene-detection assays. In comparison to the transitional method of DNA detection, key disadvantages are that the nano-chip is expensive to buy, high cost and produced via a complex manufacture process. It is for these reasons that relatively few areas are using this technology [3]. However, after MBs were first introduced [19], this class of DNA probes have demonstrated many advantages, and have therefore undergone considerable development in nanobiosensors for DNA detection [16]. Their unique features such as ease of synthesis, molecular specificity, inherent signal transduction mechanisms and structural tolerance to various modifications, provide a tremendous promise for detection of disease and obtaining sequence-specific information in a faster, simpler and more cost-effective manner

compared to traditional hybridization techniques [22]. Some original methods for detecting DNA molecules (such as radioactive label and silver staining) have radioactive risk and toxicity. It is additionally impossible to achieve real-time monitoring with these methods [19]. Important applications of MBs in real-time polymerase chain reactions (PCR) have been found, which produce the fluorescent signal with high specificity [4]. MBs are designed to hybridize with the forward/reverse targeted DNA to monitor amplification of the detected sequence. This process generates increasing fluorescence from the MB probes to measure PCR progress in real time. MBs' unique structural and nano-sized properties provide a high degree of molecular sensitivity, with the ability to differentiate even a single nucleotide between two target DNA sequences [22].

IV Conclusion and Future development:

Nanobiosensors with optical transduction mechanisms demonstrate great potential for use as biosensing tools in DNA-based applications. Optical DNA-based nanobiosensors are being developed using novel fluorescent methods for DNA detection. Since the first appearance of molecular beacons (MBs), MB-coated nanobiosensors have been widely developed in many important bioanalytical fields, such as MB-coated fibre-optical nanotips and further utilisation of MBs as probes in gene-detection assays. The detailed fabrication method of MB-coated fibre-optical nanotips have been outlined, including the immobilisation methods, which can be employed in a range of different fields. MBs as fluorescent probes perform with higher sensitivity and flexibility relative to traditional chemical methods for DNA detection, especially for real-time monitoring and single nucleotide recognition. Future developments in the field are expected to include the development of improved diagnostics, capable of very high sensitivity through the employment of fluorescence detection in a real-time monitoring for specific disease diagnostics. In spite of the many unresolved issues and challenges foreseen in the coming future, multidisciplinary approaches have converged for the evolution of innovative concepts to utilise the properties of MBs for bioanalytical and biomedical applications.

Word count: 2562

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