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Optical sensors for hypermethylated DNA detection†

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Hypermethylated DNA is a potential biomarker for different kinds of cancer. It is found in bodily fluids and excreta which make it useful for noninvasive cancer diagnostics. The possibility of such a noninvasive test using optical bio-sensing methods has been investigated

1 Introduction

DNA methylation is the process by which a methyl (CH_3) group is covalently added to the cytosine base in DNA. DNA methylation is one of the ways that gene expression changes epigenetically rather than due to the order of base pairs.[1] Hypermethylated DNA (hmDNA) has been linked to tumor genesis[2] and has been used as a biomarker for lung[3], prostate[4], colorectal[5] and esophageal[6] cancers. It is found in bodily fluids and excreta which make it useful for noninvasive cancer diagnostics.[7] The possibility of such a noninvasive test using optical bio-sensing methods has been investigated in this work.

2 DNA Biosensors

The interaction between the complimentary bases of DNA is used to measure if, or how much, a specific DNA sequence is present in an analyte. A single stranded probe sequence, which can be DNA or RNA, is immobilised and forms a recognition layer. The analyte DNA is recognised, it bonds to the recognition layer, if it consists of the complimentary bases of the probe sequence (figure 1). The interaction between the recognition layer and the analyte

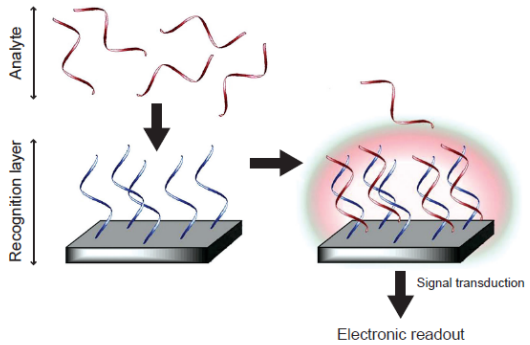

Fig. 1 General DNA sensor design, recognition layer[8]

needs to be transduced to a measurable and quantified signal. Besides optical signal transductio which is discussed in this paper, mechanical and electrochemical signal transduction can be used as well.[8]

2.1 Optical biosensors

Optical biosensors use light to convey information from the chemical to physical domain. They can be briefly classified as the following:

2.1.1 Traditional Fluorescence based

Introduction Fluorescence is a phenomenon where a material absorbs electromagnetic radiation such as light at a certain wavelength and then after a short period emits it at a higher wavelength, with lower energy.[9] This transition can be visualized by a Jablonski diagram (figure 2).[10]

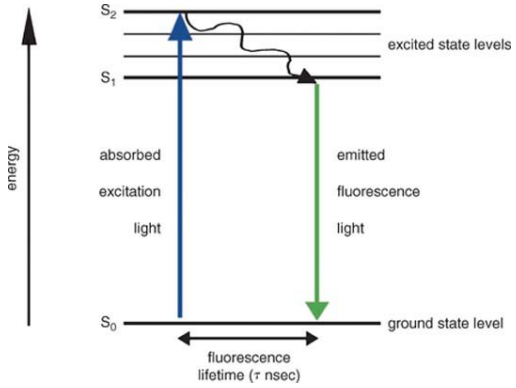

Fig. 2 Jablonski Diagram of a typical fluorescence process showing excitation, non radiative and radiative transitions[10]

Fluorescence DNA sensing makes use of a label attached to the target DNA sample with relatively simple chemistry.[11] Fluorescent labels can also be added during the methylation specific PCR process.[12] This technique, which is well established in biochemistry labs, has recently been applied to nanobiosensors using Iron-Gold nanoparticles with single stranded DNA (ssDNA) probe molecules and Dipyridamole fluorescent molecules to achieve magnetic separation and low limit of detetcion on $3.1 \cdot 10^{-16}$ M.[13] This sensor could also distinguish the level of methylation of ssDNA from fluorescence intensity (figure 3).

Another interesting application of fluorescence is Fluorescence/Förster Resonance Energy Transfer (FRET). This setup consists of two fluorophores: a donor and an acceptor. There exists an overlap in the absorption of the acceptor and emission of the donor molecule. When these molecules are close enough, energy

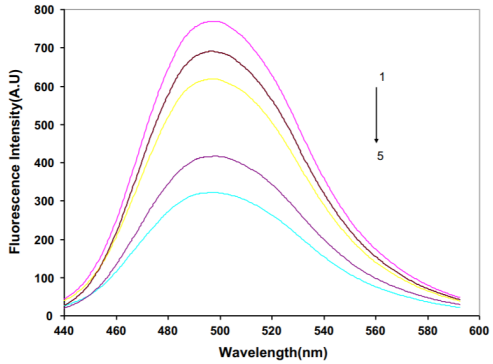

Fig. 3 As methylation increases from one to five base sequences, fluorescent signal intensity decreases[13]

may be transferred from donor to acceptor (figure 4). Thus when DNA molecule is captured by a probe molecule, the emission of the donor fluorophore reduces whereas, the emission of the acceptor fluorophore increases. FRET has a large dynamic range, and increases the signal to noise ratio of traditional fluorescent detection.[14] Zhang *et al.* described an FRET based analaysis of hmDNA found in blood, serum and stool to detect ovarian cancer.[15]. Here they used an enzyme, H*pa*II, to digest non methy-

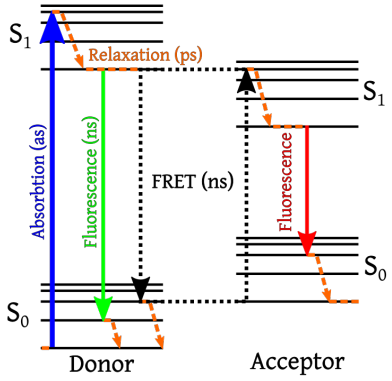

Fig. 4 Due to electrostatic coupling energy may be transferred non radiatively from the donor molecule to the acceptor molecule[16]

lated DNA, then regular PCR was used to amplify the remaining DNA. Thereafter cationic conjugated polymer (CCP) based fluorophore (donor) was used in conjunction with fluorescein (acceptor) labeled DNA to perform FRET analysis (figure 5 a-c). Similar studies of hmDNA quantification using FRET have also been done using quantum dots (QDs) as donors to avoid photobleaching, have higher stability and tuneable emission spectrum (figure 5d).[17] Unfortunately, due to limitations of the PCR digestion, this method is not entirely quantitative.

Another related variation of fluorescence based technology discussed in this paper is Fluorescence-lifetime imaging microscopy (FLIM). The lifetime of decay for fluorescence depends on the available decay channels. In case of FRET, the decay time is reduced from non FRET decay. This difference causes a blue shift which can be used as a detection mechanism for DNA hypermethylation.[18,19]

2.1.2 Terahertz sensing

Terahertz (THz) sensing is a label free spectroscopy technique that uses absorption at certain frequencies to characterize the analyte. Using terahertz time domain spectroscopy the characteristic frequency of methyl bond in CpG can be found. This resonance frequency is the frequency at which the bond absorbs energy the strongest. For methyl bond in CpG it is found to be around 1.7 Thz. THz detection has been used to characterize cancers from a variety of origins and cell lines.[20,21] Overall THz sensing in this provides a very high accuracy of the degree of DNA methylation when compared to the gold standard ELISA assay (figure 7).

2.1.3 Optofluidic Ring Resonators

Optofluidic Ring Resonators (OFRR) provide a label free, low cost, low limit of detection technique for biomolecule detection.[22] It is based on the principle of evanescent wave sensing wherein the refractive index depends upon the analytes on the interface of the waveguide.[23] In OFRR light is circulated in a circular waveguide. Photons with a frequency matching the resonance frequency couple into the waveguide. These resonances are known as whispering gallery modes (WGM). The spectral position of the WGMs depends on the refractive index of the media around it (figure 8).[24]

2.1.4 Surface Plasmon Resonance based

Surface Plasmon Resonance (SPR) can analyse the binding of molecules from the analyte to the probe sequences of the recognition layer via the metallic surface on which the probes are immobilised. If binding takes place, the refractive index of the metal changes (figure 9). How much binding takes place can thus be calculated from the change in refractive index.[25] There are multiple advantages of using the SPR method. It is real-time and label free. The receptor-target interactions can be analysed with a wide range of molecular weights and affinities. It is highly compatible with different environments like temperature and pH.[25] The systems that are used can be complex and expensive[8]. A disadvantage is that the SPR readout has a poor sensitivity.[25] Pan *et al.* and Li *et al.* have both proposed methods to overcome this disadvantage.

Pan *et al.* used SPR in combination of a double recognition mechanism. Target DNA was captured by the immobilised, methylation specific probe in the recognition layer. After that methyl-CpG binding domain (MBD) protein was added. The binding of the MBD to the methylated DNA that is captured before resulted in an increase of the refractive index. This increase resulted in a detectable optical signal. A solution of 5 pmol of methylated DNA was used to obtain this result. A lower limit of detection or sensitivity test are not reported. The time in which the DNA methylation detection can be done is compared with bisulfite treatment or methylation-sensitive restriction digestion, since time is a major disadvantage of those other methods. So this method has an advantage of rapid and automatic detection. In one hour the DNA methylation detection can be completed automatically using the method of Pan *et al.*.[26]

Li *et al.* developed an end-to-end nanorod assembly enhanced SPR system for detection of DNA methylation and adenine methy-

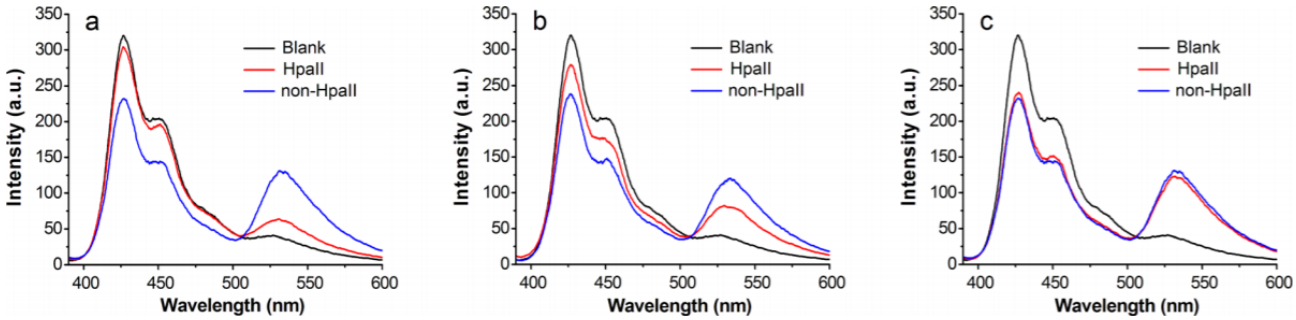

Fig. 5 In the red curve as the methylation of hmDNA increases, the emission intensity at 424 nm due to CCP decreases and at 530 nm due to fluorescein increases[15](d) Principle of QD-FRET illustrated[17]

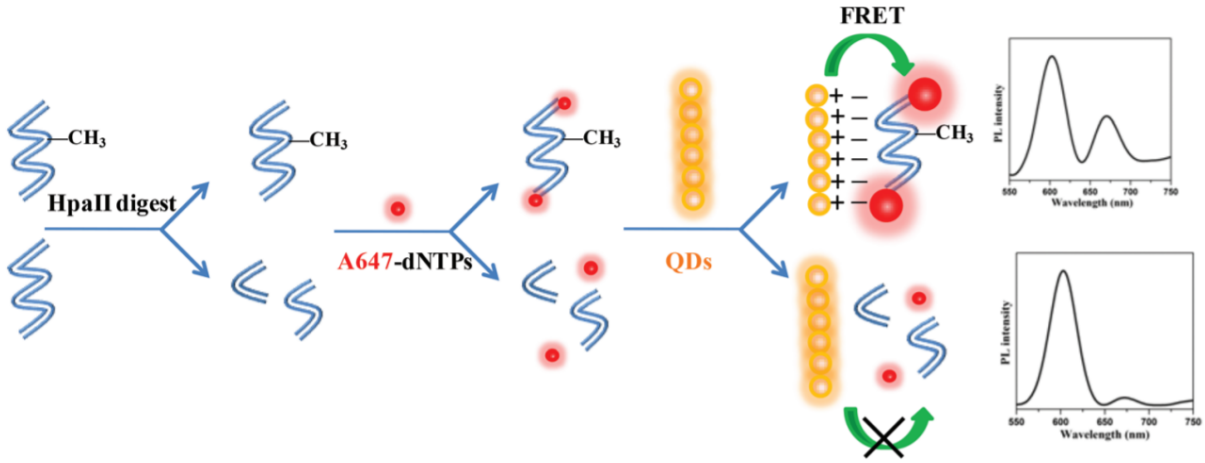

Fig. 6 Principle of QD-FRET illustrated[17]

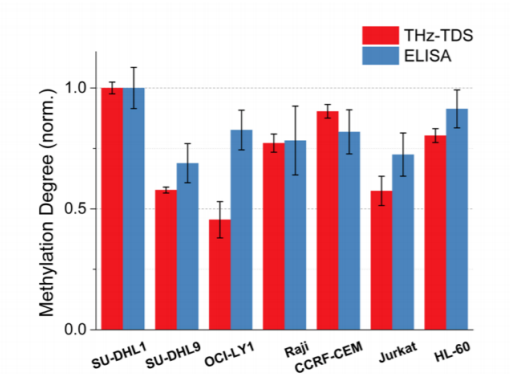

Fig. 7 Tetrahertz sensing vs ELISA Methylation quantification[20]

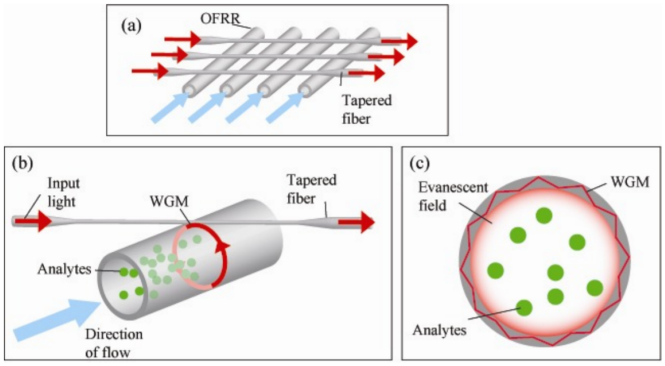

Fig. 8 (a) OFRR multiplexed array (b) Experimental setup (c) Principle of OFRR[22]

lation (Dam) methyltransferase (MTase). This resulted in a biosensor with a linear relationship between the concentration of Dam MTase and the SPR angle shift between 0.5 to 120 U/mL. A detection limit of 0.2 U/mL was estimated based on the slope and the standard deviation of the zero level. The specificity is evaluated by investigating the response of the system to other DNA MTases and this showed a high sensitivity for Dam MTase (figure 10).[27]

2.2 Colorimetric based

The methylated DNA itself does not show absorption in the visible region, so labels are used to make colorimetric evaluation possible. Commonly gold nanoparticles with probe DNA or with an aptamer are used. The gold nanoparticles change color when the target sequences are hybridized and the probe DNA or the aptamer is chosen or made so that it binds only to the target DNA.[25] The gold nanoparticle with the DNA probe hybridizes with the tar-

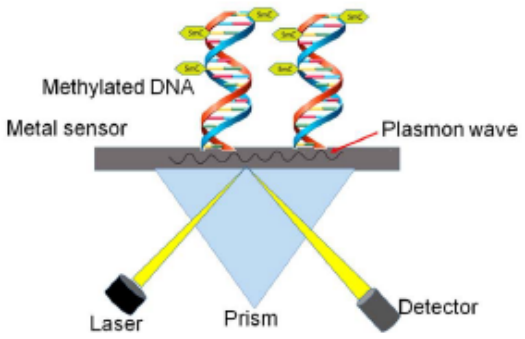

Fig. 9 SPR[25]

get DNA. Two probes are used that complement with both ends of the target DNA, which causes sandwich hybridization. Each gold nanoparticle has many DNA probes on it, so the sandwich hybridization will form a network aggregation. This aggregation creates a color change from red to blue which can be seen with the naked eye. A colorimeter can also be used to quantitively determine the absorption spectrum.[28]

Advantages of the colorimetric method are that it is simple, reliable, cost effective, portable and targets can be detected with the naked eye.[25]

Su *et al.* have used gold nanoparticle labelling and this had good results. They have reported that 0.01 fM methylated DNA could be determined. Besides that, they report the detection of 0.1% methylated DNA, target M, in the presence of a large excess of unmethylated DNA, target N (figure 11). The results were obtained after first employing bisulfite conversion, then using ligase chain reaction (LCR) to amplify the DNA samples and lastly monitoring the LCR products with gold nanoparticle-based colorimetric assay.[28]

Chen *et al.* have designed and constructed gold nanoparticle probes to use for the seni-quantative analysis of methylated tumor suppressor genes in cultured cells. The DNA was extracted from the cells and bisulfite treatment was employed. The resulting DNA was amplified by polymerase chain reaction (PCR) and added to the solution with the constructed gold nanoparticle probes after which the absorption spectra were determined. The ratio between the absorption at 620 nm and 520 nm was used as index to indicate the level of aggregation of the gold nanoparticle probes. This resulted in the data that is visualised and explained in figure 12 The lower limit of detection of the probe is 20%.[29]

3 Conclusion

Different strategies for the optical detection of methylated DNA were discussed.The specifications of the different methods can be found in table 1 The conclusion can be drawn that it is possible to use optical methods to detect methylated DNA. The extent of DNA methylation can also be detected via optical methods.[15,17] At least one of the discussed studies has shown that it can be used to distinguish cancer cells from cells without cancer[29]. Zhang *et al.* also report protocols that can achieve up to 85.7% sensitivity and 100% specificity. Colorimetric nanoparticle based methods as described by Su *et al.* and Fluorescence based methods described by Dadmehr *et al.* report detection limits in the femtomolar range. Additionally, Dadmehr *et al.* report a linear dynamic range of three orders of magnitude. Terahertz sensing by Cheon *et al.* provides a promising alternative for label free detection but suffers from complicated optics setups leading to high costs. The colorimetric method does need labels, but it is classified simple and cost effective[25]. Based on the discussion of the optical transduction principles only it is not possible to say which method is most promising, since the sensitivity and selectivity is also greatly affected by the way that the DNA sample is amplified before the

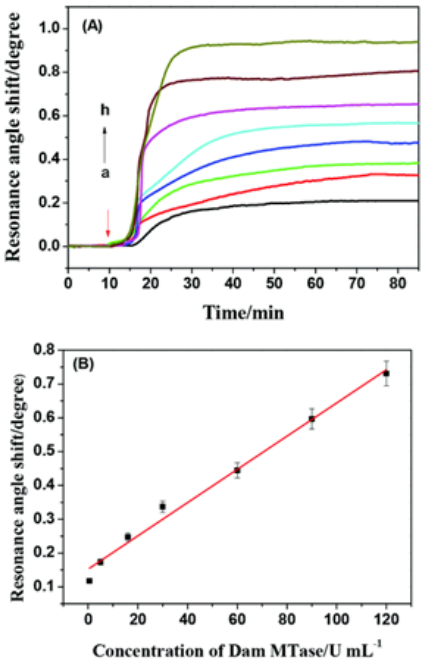

Fig. 10 (A) Real-time resonance angle responses of the biosensor for Dam MTase detection with ETE AuNR assembly probes. The concentration of Dam MTase from (a) to (h): 0, 0.5, 5.0, 15, 30, 60, 90, and 120 U/mL. (B) Calibration curve of the determination of Dam MTase with ETE AuNR assembly probes.[27]

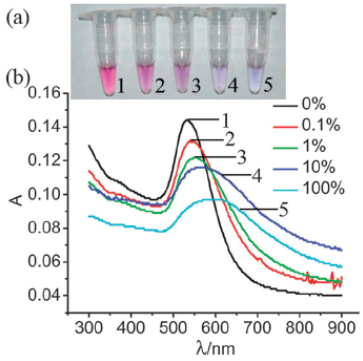

Fig. 11 (a) Visual detection of target M at different ratios in the mixtures of target M and target N with a total concentration of 10 fM. The ratio of target M in the mixtures was (1) 0%, (2) 0.1%, (3) 1.0%, (4) 10%, and (5) 100%, respectively. (b) The absorption spectra corresponding to the solutions shown in (a).[28]

Table 1 A comparison of the current hmDNA sensors in literature. The values with * added have determined the methylation semi-quantitively.

Dynamic range	Limit of detection	Transduction principle	Detector molecule	reference
$8.0{\cdot}10^{-13} - 3.2 \cdot 10^{-15} M$	$3.1{\cdot}10^{-16} M$	Fluorescence	Thiolated ssDNA on gold nanoparticles	Dadmehr *et al.*
-*	-*	FRET	fluorescein-labeled DNA	Zhang *et al.*
-*	-*	FLIM	DNAmethyltransferase	Choudhury *et al.*
-	-	Evanescent Field/OFRR	Label Free	Suter *et al.*
-	$5 \cdot 10^{-12} mol$	SPR	methyl-CpG binding domain	Pan *et al.*
0.5 to 120 U/mL	0.2 U/mL (estimated)	SPR	-	Li *et al.*
$0.01 \cdot 10^{-15} - 1 \cdot 10^{-15} M$	$0.01 \cdot 10^{-15} M$	Colorimetric	gold nanoparticles	Su *et al.*
20-100*%	20*%	Colorimetric	gold nanoparticles	Chen *et al.*

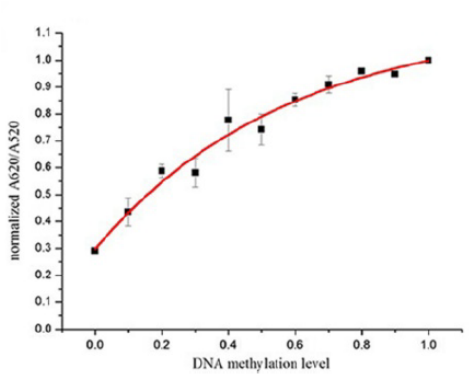

Fig. 12 Curve fitting results of A620 nm/A520 nm value to DNA methylation degree: Y = abX + c, a = -0.85, b = 0.18, c = 1.15, R2 = 0.98. Y is the A620nm/A520nm value and X is the Met-p16 concentration. [29]

detection is applied. Even if the different ways of amplifying the sample before the detection were discussed it would still not have been possible to declare one of the combinations the most promising, since it probably depends on the sort of bodily fluid or excreta that the DNA sample is taken of and which sort of cancer it concerns what the requirements of the sensor have to be. [7]

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