Speedy, On-the-spot DNA Sensors with Fluorescent Polymers

Anupama Rao Gulur Srinivas[1], David Barker[1], Jadranka Travas-Sejdic[1,2]

[1]Polymer Electronics Research Centre (PERC), School of Chemical Sciences, University of Auckland,
[2]MacDiarmid Institute for Advanced Materials and Nanotechnology, New Zealand

The need for providing highly accurate, sensitive, economical and pain-free sensors (e.g. pregnancy tests, glucose testing kits) has driven the growth of the biosensor market. Modern day sensors have moved away from their predecessors who used bulky semi-conducting materials as transistors, towards miniaturization of biosensors. Biosensors for medical applications remain a hugely untapped market, which we aim to target with the first of its kind binary DNA sensor using fluorescent polymers. Proven to be highly sensitive for the target DNA, the system exhibits a number of desirable characteristics such as ease of handling (through magnetic deployment), facile, low-cost, sensitive and selective for DNA sensing. Potential applications of this sensing device include the detection of a huge range of biological entities: genetic diseases, bacteria (like E. coli) and other DNA-containing microorganisms.

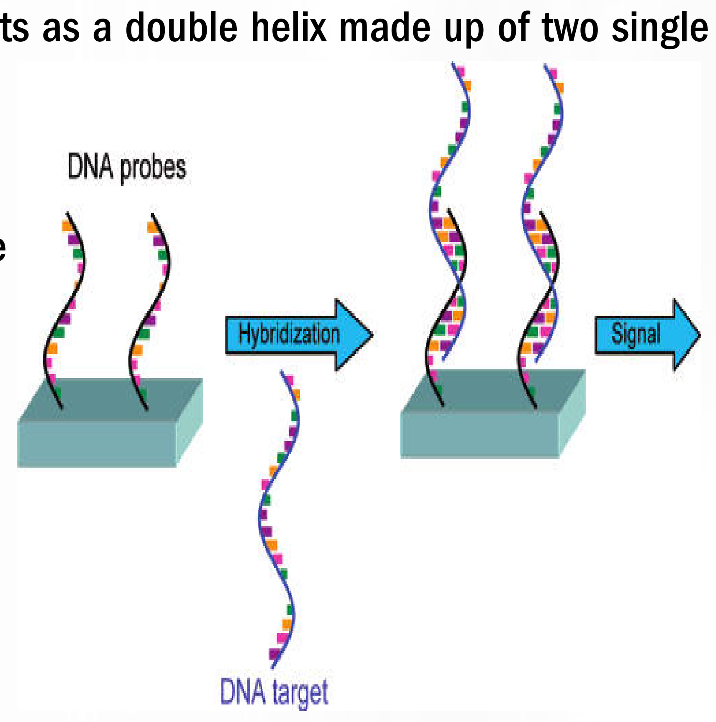
DNA Sensors

What is DNA?

Deoxyribonucleic acid (DNA) is the most important biomolecule as it contains genetic information critical for the functioning of all living organisms. DNA exists as a double helix made up of two single DNA strands consisting of base pairs.

How do DNA sensors work?

DNA sensors use the ability of single stranded DNA molecule to 'seek out'/hybridize with its complementary strand forming a double helix.
This recognition event is then converted into a measurable signal and can be used to detect target DNA (when all the bases match up perfectly), non-complementary DNA (when no bases match up) and mutated DNA (when most of the bases match up).



What are the applications of DNA sensors ?

DNA sensors have uses in medical diagnostics, forensics, monitoring of food contaminants and security/biodefense as any DNA sequence can be detected.

Fluorescent polymers

What are fluorescent polymers?

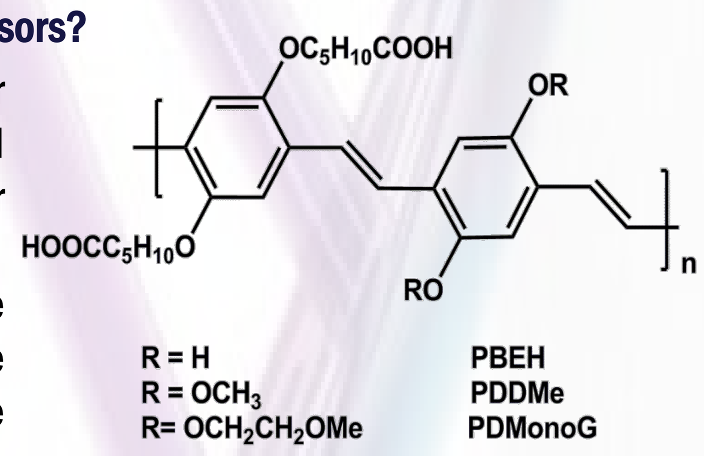
Fluorescent polymers are long-chain molecules consisting of many small molecules (repeat units) linked together to make solid materials which emit light (fluorescence).

Which fluorescent polymers were used in this study?

We have selected a fluorescent polymer called poly(p-phenylene vinylene)(PPV) as it is highly fluorescent and is easily produced. Fluorescence and chemical functionality of PPV were designed to be used as a dual support in the sensing design.

What are the advantages of using fluorescent polymers in DNA sensors?

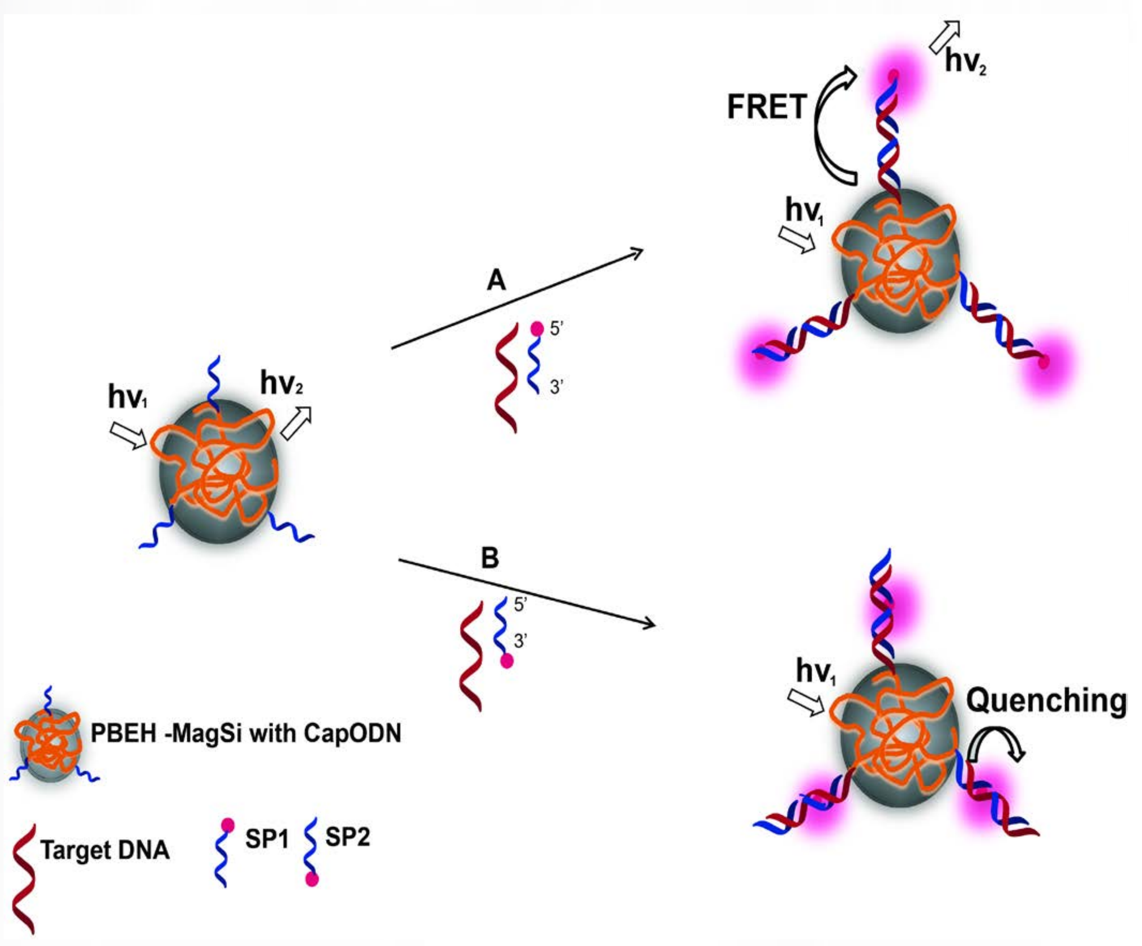
The fluorescence of polymers is sensitive to minor changes in their environment. The polymers exhibit a collective fluorescence signal compared to single molecules and thus can detect smaller amounts of DNA.
These polymers can be used as passive supports (DNA can be attached to the polymer) or active supports (presence of the target DNA is indicated by the change in fluorescence of the polymer) in the sensor.



DNA Sensing Results

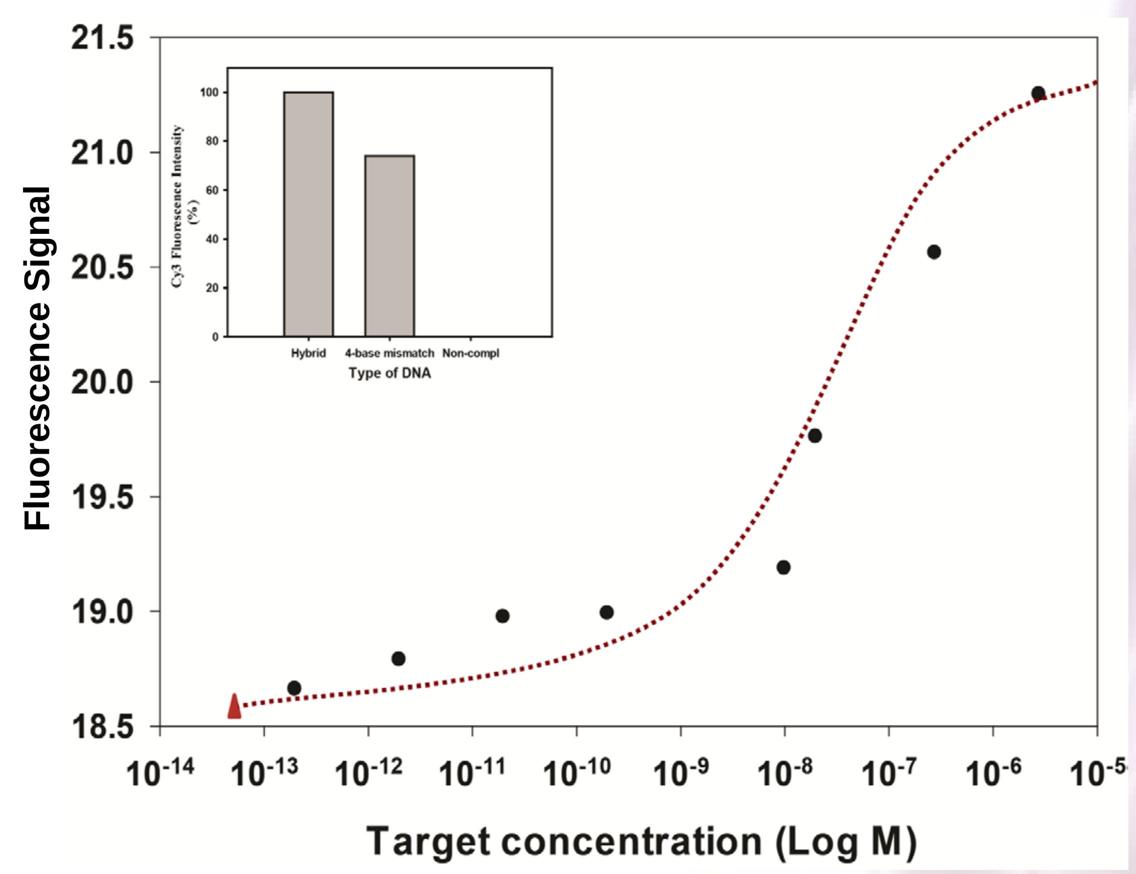
We have developed a first of its kind binary DNA sensor - based on a bio-compatible fluorescent polymer bound to a magnetic bead having a DNA probe attached to it. This strand of DNA forms half of the recognition sequence, the other a covalently bound dye-DNA strand free in solution. These two DNA sequences initiate an optical signal when they hybridize with the complementary target DNA. The optical signal can be either "switch-on" (the energy from the fluorescence of the polymer is transferred to the dye causing the dye's fluorescence to be switched-on) or "switch-off"(the energy from the fluorescence of the polymer is transferred to the dye but the dye does not fluoresce, thus switching it off).

Binary DNA sensor design



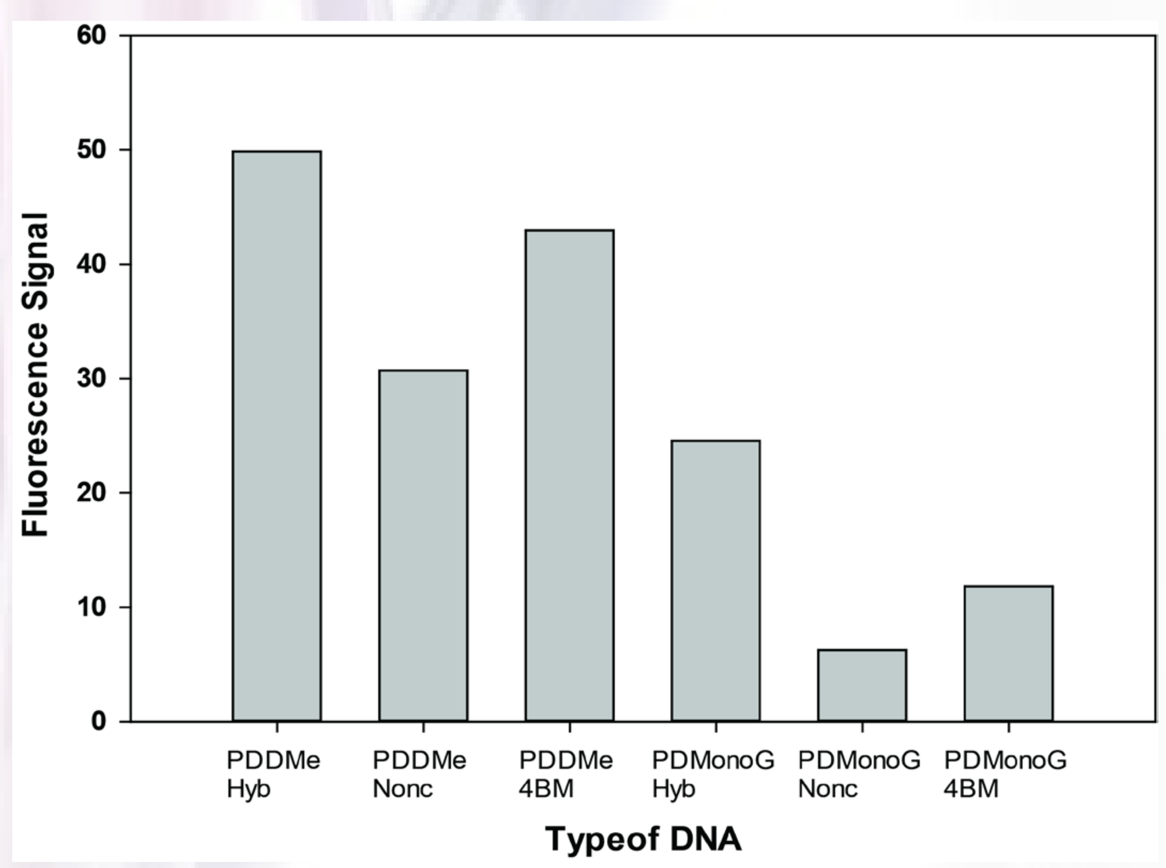
Design of our novel binary DNA sensor with either "switch-on"(A) or "switch-off") (B) optical readout.[1]

"Switch-on" sensor



The optical signal obtained with various concentrations of target DNA and with different types of DNA (Inset): Fully complementary target, Mutated (MM) and non-complementary DNA. PBEH was used for this study.[1]

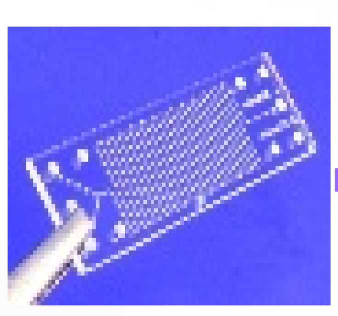
"Switch-off" sensor

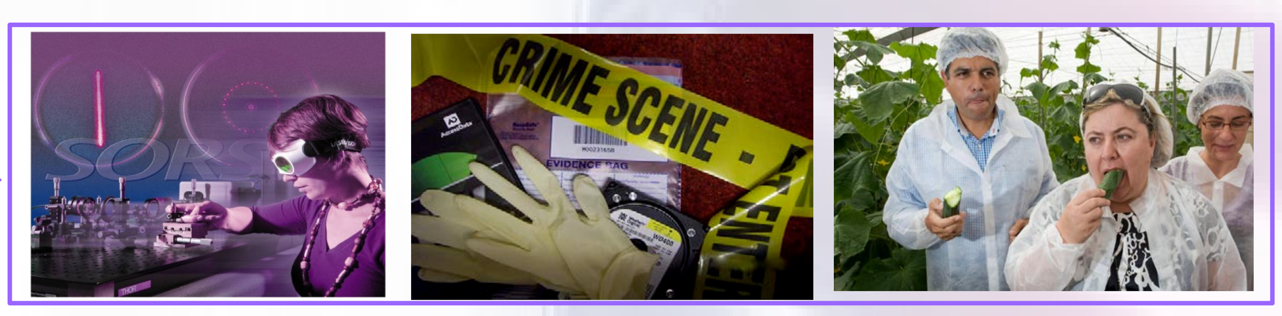


The optical signal obtained with different types of DNA: Fully complementary target (Hyb), Mutated (4BM) and non-complementary DNA (Nonc). PDDMe and PDMonoG were used for this study.

Conclusions: What do these results mean?

- The binary DNA sensor has demonstrated excellent ability to distinguish target DNA (when all the bases match up perfectly) when compared to non-complementary DNA (when no bases match up) and mutated DNA (when not all but most of the bases match up) strands.
- The "switch-on" sensor has proven high sensitivity as it was able to detect target DNA in low concentrations (240 fmol), typical of working concentrations encountered in the real world.
- The DNA sequences used in this sensor can also be tailored to allow for detection of any given sequence (e.g. E.Coli, Breast cancer gene, Anthrax), and mutations there of with applications in medical diagnostics, monitoring of food contaminants, forensics and biodefense.
- The fluorescence properties of the synthesized fluorescent polymers; PBEH, PDDMe and PDMonoG can also be integrated towards applications in light-emitting diodes (LEDs), light-emitting cells (LECs), solar cells and flexible electronics.





References
1. Gulur Srinivas, A.R.; Peng, H; Barker, D; Travas-Sejdic, J., *Biosensors & Bioelectronics* 2012, *35*, 498-502

Acknowledgements
This work was supported by the NERF Subcontract grant, Industrial Research Limited (IRL)

The MacDiarmid Institute for Advanced Materials and Nanotechnology

INDUSTRIAL RESEARCH LIMITED Te Tauihu Pūtaiao