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# Conductive composites for oligonucleotide detection☆

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ABSTRACT

A new method for oligonucleotide detection is presented based on oligonucleotide cross-linked polymer composites. Conductive carbon nanoparticles are incorporated into a DNA-functionalised polymer, containing partially complementary oligonucleotide cross-linkers, which is polymerised *in situ* upon interdigitated electrodes. In the presence of an aqueous solution of a specific analyte oligonucleotide sequence, the cross-linkers are cleaved, leading to increased swelling. As the polymer swells the relative density of the conductive particles decreases, leading to an easily measurable decrease in electrical conductivity. We demonstrate that such are capable of discriminating between analyte and control solutions, with single-base specificity, in under 3 min. The lower detection limit of these composites is of the order of 10 nM. The swelling characteristics of these composites is confirmed by optical imaging and the effects of varying temperature upon such composites are also reported.

## 1. Introduction

Conductive composites, wherein conductive particles are mixed into a non-conductive polymer matrix, have long been applied to chemical sensing in fields such as electronic noses and tongues [1–8]. In such applications, the conductive component is added to the non-conductive polymer in sufficient quantities to form conductive pathways throughout the composite, referred to as a percolating state [8]. The presence of certain molecules, often volatile organic compounds (VOCs), cause the polymers to swell, resulting in the breaking of conductive pathways and a decrease of the electrical conductivity. Generally such chemical sensors are ambiguous in their response, but arrays of these, with each element prepared with different polymers, coupled with pattern recognition techniques can provide specific discrimination. To date there have been few successful applications of such swelling, percolating composites to biosensing [9]. This may be due to the lack of specificity of an adsorptive swelling mechanism coupled to the often low concentration of biological molecules of interest.

We hypothesized that designing the system to inherently amplify the swelling from a biomolecule could permit percolating composite biosensing. Stokke et al. have developed DNA-functionalised polymers that demonstrate selective swelling in the presence of specific oligonucleotide sequences [10–12]. Such polymers contain both conventional chemical cross-linkers and oligonucleotide cross-linkers, comprised of two, partially complementary, oligonucleotide sequences (Fig. 1). In the presence of analyte oligonucleotides (to which the 'sensor' strand is perfectly complementary) the 'blocker' strand will be displaced, causing the cross-link to break. This selective cleavage of cross-links leads to changes in the swelling behaviour of the polymer, resulting in increased swelling.

This paper outlines preliminary efforts to develop conductive composites based on such DNA-functionalised polymers, in the hope of providing a simple, rapid and low-cost means of transduction for multiplexed oligonucleotide sensing applications ranging from point-of-care diagnostics [13–15] to forensic science [16,17]. To date, only optical transduction methods have been applied to these polymers [10–12], which, whilst effective, presents potential cost, portability and multiplexing challenges.

We report the development of an Oligonucleotide Cross-linked Polymer Composite (OCPC) that demonstrates highly selective swelling in the presence of analyte oligonucleotides and whose resistance decreases with swelling. Analyte solutions can be clearly differentiated from control solutions, with single-base specificity, in under 3 min *via* differences in the rate of transition between percolating and non-percolating states.

## 2. Materials & methods

### 2.1. Materials

Acrylamide (AAM), methylene-bisacrylamide (MBA), hydroxycyclohexyl phenyl ketone (HPK), ethylene glycol, trimethoxysilyl propyl methacrylate (TPM), hydrochloric acid (HCl), phosphate buffer

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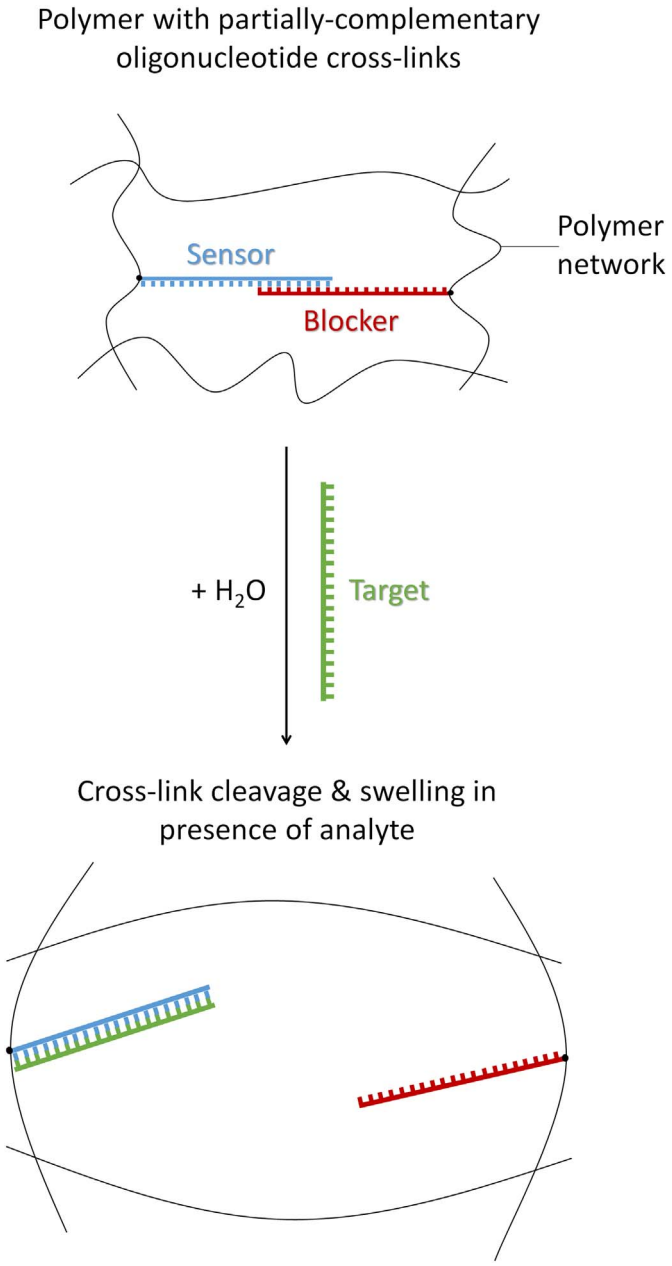


**Fig. 1.** The sensing mechanism of oligonucleotide-functionalised polymers. (Adapted from Tierney and Stokke [10]).

(1 M, pH 7.4) and carbon nanopowder (< 50 nm) were purchased from Sigma-Aldrich and used as received. Aqueous stock solutions of AAM and MBA were prepared (150 mM NaCl, 1 mM phosphate buffer) as was a stock solution of the HPK photo-initiator in ethylene glycol.

The oligonucleotide sequences were purchased from Integrated DNA Technologies. They include sensor (S) and blocker (B) sequences (Fig. 1), a fully complementary analyte sequence ($A_0$), a five-mismatch sequence ($A_5$), a single-mismatch sequence ($A_1$) and a random control sequence (R). The S and B sequences were supplied functionalised with an Acrydite modifier and have a 12-base complementary region at their respective 3′ ends (Fig. 2). The S and B sequences were mixed together in equal concentrations, before being extracted from solution using a standard ethanol precipitation. All other oligonucleotide sequences were suspended at various concentrations (ranging from 1 nM to 10 μM) in aqueous buffer solutions (150 mM NaCl, 1 mM phosphate buffer).

### 2.2. Composite synthesis

The AAM, MBA and HPK stocks were mixed together in appropriate volumes to form a 10 wt% AAM gelator solution (0.6 mol% w.r.t. monomer MBA, 0.125 mol% w.r.t. monomer HPK). Carbon nanopowder was added to this solution at concentrations ranging between 0 and 20 mg/mL and dispersed *via* manual agitation. This mixture was added to the dried S/B oligonucleotides and the oligonucleotides allowed to fully dissolve, producing an oligonucleotide concentration of 0.4 mol% w.r.t. monomer. Full details of the composite synthesis can be found in the Supplementary information.

After mixing, droplets of this pre-composite solution were pipetted onto substrates and polymerised by exposure to UV radiation for 60 s using an arc lamp (Dymax Bluewave 75, 280–450 nm, > 19 W/cm$^2$) to form an OCPC.

### 2.3. Electrode fabrication

The electrode devices consisted of platinum Interdigitated Electrodes (IDEs) fabricated on a silicon/silicon dioxide substrate. The IDE arrays covered an area of 1.5 × 1.5 mm and contained 30 digits, each 10 μm wide and separated by 40 μm. They were fabricated using standard photolithography procedures. The substrates were silanised using a procedure adapted from Tierney et al. [18], so as to improve the adhesion of the composites. The devices were immersed in a 0.01 M HCl solution for approximately 10 min before immersing them in TPM in acetone (0.02 M) for 1 h. Further details on the electrode fabrication and silanisation can be found in the Supplementary information.

### 2.4. Optical measurements

Optical volume measurements enabled the simple determination of OCPC swelling responses before testing their conductivity changes as a result of that swelling. OCPC droplets were prepared as described in Section 2.2 and polymerised *in situ* on the electrode devices described in Section 2.3. These samples were partially dried out under refrigeration (3 ± 1)°C before being immersed in various oligonucleotide solutions ($A_0$, $A_1$, $A_5$ and R) at concentrations ranging between 1 nM and 10 μM. The samples were either imaged as they swell in these solutions (as shown in Fig. 3) or swollen to equilibrium at temperatures ranging from 3 to 37 °C, removed from solution, patted dry and imaged in air (Fig. 3). The carbon concentrations were either 0 or 10 mg/mL and for these experiments carbon was added solely as a contrast agent. Further details on the optical measurements can be found in the Supplementary information.

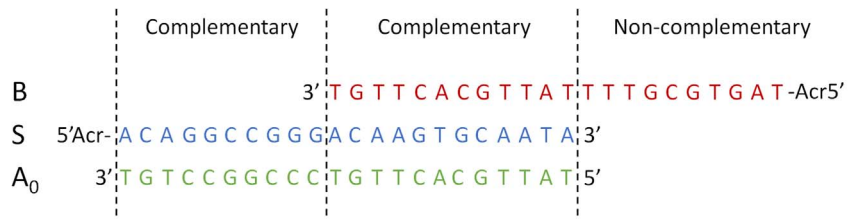


**Fig. 2.** The sequences and respective complementarity of the S, B and $A_0$ oligonucleotide sequences. 'Acr' denotes the acrydite modifier. The vertical dashed lines delineate the respective regions of complementarity.