



# Surfaces for tuning of oligonucleotide biosensing selectivity based on surface-initiated atom transfer radical polymerization on glass and silicon substrates

April K.Y. Wong, Ulrich J. Krull\*

Chemical Sensors Group, University of Toronto Mississauga, 3359 Mississauga Rd. N., Mississauga, ON, Canada L5L 1C6

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## ABSTRACT

Covalently immobilized mixed films of oligonucleotide and oligomer components on glass and silicon surfaces are reported. This work has investigated how such films can improve selectivity for the detection of multiple base-pair mismatches. The intention was to introduce a “matrix isolation” effect on oligonucleotide probe molecules by surrounding the probes with oligomers, thereby reducing oligonucleotide-to-oligonucleotide and/or oligonucleotide-to-surface interactions. Thiol-functionalized oligonucleotide was coupled onto (3-aminopropyl)trimethoxysilane (APTMS) via a heterobifunctional linker, succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (sulfo-SMCC). Using a variety of monomers such as 2-hydroxyethyl methacrylate (HEMA), oligomers were grown by surface-initiated atom transfer radical polymerization (ATRP) from a bromoisobutyryl NHS ester initiator which was immobilized onto APTMS sites that coated glass and oxidized silicon substrates.

Various surface modification steps on silicon substrates were characterized by ellipsometry, wettability, atomic force microscopy, X-ray photoelectron spectroscopy, and time-of-flight secondary ion mass spectrometry. Polymerized HEMA (PHEMA) in mixture with oligonucleotide probes was evaluated for fluorescence transduction of hybridization. The presence of PHEMA was found to provide a sharper melt curve for hybrids containing both fully complementary and three base-pair mismatched targets, and this surface derivatization also minimized non-selective adsorption. The maximum increase in slope was improvement by a factor of 3-fold. An increase of up to 30% in difference of melting temperatures between fully complementary and 3 base-pair mismatched targets was achieved using PHEMA. The results suggest that the presence of oligomers dispersed among DNA hybrids can improve selectivity through what is believed to be a reduction of dispersity of interactions of probes with targets, and probes within their local environment at a surface.

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## 1. Introduction

The screening of nucleic acids remains an area of great interest as the medical and environmental communities demand diagnostic technologies that are sensitive, selective, reusable, and rapid to determine the presence of targets such as disease-causing genes, or the presence of pathogenic bacteria in food/soil samples [1–3]. As a biological recognition agent, oligonucleotide probes offer chemical stability, high affinity, versatility in terms of sequence choice and length, opportunity for chemical modification, reusability, and facile synthesis [1]. Such selective chemistry can be coupled to an electrochemical, piezoelectric, or optical transduction system to achieve biosensing capability [1,2]. The interest herein is the further development of surfaces for immobilized oligonucleotide

probes which can enhance selectivity for the detection of multiple nucleotide polymorphisms.

The thermal melting temperature ( $T_m$ ), the temperature at which half of any available DNA duplexes have denatured, is characteristic of the stability of hybridization [4]. It is known that for oligonucleotides in solution, every extra A-T base-pair provides about 2 °C increase in  $T_m$  while every G-C base-pair contributes to a 4 °C increase [4]. Therefore, a window of 4–8 °C typically exists for determination of 2–3 mismatches when operating in bulk solution, and even less when considering single base polymorphisms (SNPs). SNP studies have been explored by groups led by, for example, Corn and Mirkin [5,6]. The ability to discriminate between closely related oligonucleotide sequences is also influenced by the steepness of the melt curve.

Hybridization that occurs on surfaces rather than in solution tends to significantly broaden melt curves as there are a wide diversity of physical conformations that can exist at an interface, meaning that there is a distribution of hybridization energetics at a

\* Corresponding author. Tel.: +1 905 828 5437; fax: +1 905 828 5425.

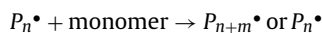
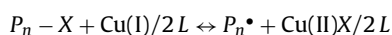
E-mail address: [Ulrich.krull@utoronto.ca](mailto:Ulrich.krull@utoronto.ca) (U.J. Krull).

surface [7]. The local environment of the probe oligonucleotides is a key factor that influences selectivity and efficiency [8,9]. In fact, the very process of hybridization on a surface when it occurs to a substantial extent can dynamically change the surface charge density and the corresponding melt curve [8,9]. Orientation, steric conformation, and mobility of immobilized oligonucleotide probes can be affected by the surface charge density due to probe-to-probe and probe-to-surface interactions.

The present work explores a covalently immobilized mixed oligomer and oligonucleotide film in order to ameliorate nearest-neighbour interactions of probes and to block interactions with the substrate. The concept is one of “matrix isolation”, such that oligomers, immobilized through one end, on average surround oligonucleotide probes so that probes are isolated from one another and from the surface.

An earlier attempt by our team to develop a mixed oligonucleotide and oligomer film was based on a photochemical approach, where partial activation of a linker which had been protected by a photolabile group was done using UV irradiation [7]. Ethylene glycol phosphate was immobilized on hexaethylene glycol linkers, and then the remaining photolabile group was deprotected for immobilization of oligonucleotide probes. Melt curves from the resulting mixed films tentatively suggested that the presence of an oligomer did exert a useful effect on the  $T_m$  of the DNA hybrids, while still maintaining expected trends for decreasing ionic strength and  $T_m$  differences between fully complementary hybrids and those containing a single base-pair mismatch (bpm). However, the synthetic approach was not very practical during sensor fabrication as the photolabile protecting group had to be synthesized and purified in a darkroom to prevent photodegradation. Sensitivity to UV light leads to the potential for photodegradation [10].

In this new study, we are exploring a different synthetic approach to achieve a matrix isolation effect. In general, end-functionalized polymers can be directly grafted to a surface via a specific chemical linkage. However, this “grafting to” method cannot yield a high surface density of polymers [11], which is undesirable if one wants to build a highly controlled environment for oligonucleotide probes. A more effective way to graft polymer brushes of high density onto surfaces is called a “grafting from” method, in which immobilized initiators are used to grow the polymer chains from the substrate one monomer at a time. One such “grafting from” method can be achieved using atom transfer radical polymerization (ATRP). This is a form of controlled “living” polymerization that is capable of polymerizing various acrylate- and methacrylate-based monomers to achieve a narrow polydispersity index ( $M_w/M_n \ll 1.5$ ) with controlled molecular weight [12]. Controlled/“living” polymerization is achieved by having a fast dynamic equilibrium between a higher concentration of dormant species ( $P_n - X$ ) than of active species ( $P_n^*$ ) usually at ppm concentrations [13], and is described by the following reaction:



where Cu(I) abstracts the halogen atom (X) from the initiator or dormant polymer chain ( $P_n - X$ ) and causes a homolytic cleavage of the halide-carbon bond, leaving a radical at the terminal end of the polymer chain. Cu(I) becomes oxidized to Cu(II) upon complexing with the halide and ligands (L), the latter being used to adjust the strength of the catalyst [14]. Wherever a radical alkyl or polymer chain is available, an acrylate or methacrylate-based monomer can be added at the end of this chain, thus propagating the radical to the end of this new monomer. The increase in Cu(II) will deactivate the polymerization process and revert the active species ( $P_n^*$ ) back to its dormant state ( $P_n - X$ ) [13]. This reversible reaction is key to

the controlled polymerization in ATRP. Surface-initiated ATRP has been used in numerous applications such as the modification of quantum dots, nanoparticles and microbeads, biosensing, protein adsorption studies, and improvement of biocompatibility by means of surface coatings [15].

Here we report a strategy for creating mixed films using surface-initiated ATRP of 2-hydroxyethyl methacrylate (HEMA) and other monomers on 3-aminopropyltrimethoxysilane (APTMS)-modified glass and oxidized silicon substrates. Surface characterization methods were used to determine the success of each reaction step. The resulting films provided an advantageous biosensing surface for detection of hybridization of oligonucleotides by fluorescence. The melt curves collected from mixed films showed sharper transitions than those observed for immobilized films of oligonucleotides alone. Greater melting temperature differences for mismatches were noted, and the mixed films ameliorated the problem of non-selective adsorption.

## 2. Experimental

### 2.1. Materials

Redistilled benzaldehyde (BZ, >99.5%), 4-nitrobenzaldehyde (98%), (3-aminopropyl)trimethoxysilane (APTMS, 97%), redistilled N,N'-diisopropylethylamine (99.5%), 2-bromoisobutyl bromide (98%), N-hydroxysuccinimide (NHS), triethylamine (99%), 2-hydroxyethyl methacrylate (HEMA, 97%), ethylene glycol methyl ether methacrylate (EGMEM, 99%), ethylene glycol methacrylate phosphate (EGMP), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), styrenesulfonic acid (SSA), Cu(I) chloride, Cu(II) bromide, 2,2'-dipyridyl, sodium bicarbonate, sodium sulfate, sodium dodecyl sulfate, sodium carbonate, sodium chloride, sodium orthophosphate were from Sigma-Aldrich (Oakville, ON). Tris hydrochloride was from EM Science (Gibbstown, NJ, USA) (Succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate) (sulfo-SMCC) was from Fisher Canada (Nepean, ON). Ammonium hydroxide (30%) and hydrogen peroxide (30%) were from EM Science (Gibbstown, NJ, USA). An inhibitor present in the HEMA was monomethyl ether hydroquinone MEHQ. HEMA was purified by using MEHQ inhibitor remover (Sigma-Aldrich, Mississauga, ON), which was packed in a column. All salts were dissolved in Millipore purified water (Milli-Q water, 18 MΩ cm). All solvents including methanol (MeOH), dichloromethane (DCM), deuterated chloroform ( $\text{CDCl}_3$ ), diethyl ether, dimethylformamide (DMF), dioxane, toluene, and silica gel for chromatography were from Sigma-Aldrich (Oakville, ON). Argon gas was from BOC Canada Limited (Oakville, Canada).

Substrates included silicon wafers from International Wafer Service (Limerick, PA, USA), and glass microscope slides (3 mm × 1 mm × 1 mm) from Fisher Scientific (Pittsburgh, PA, USA).

Table 1 lists the oligonucleotide sequences from Integrated DNA Technologies (Coralville, IA). A small aliquot of the disulfide form of each thiol-modified oligonucleotide were first reduced by TCEP and purified through a Sephadex G-25 DNA grade column from GE Healthcare (Baie d'Urfé, Québec, Canada).

### 2.2. Instrumentation

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) experiments were done at Surface Interface Ontario, University of Toronto. The instrument used was an Ion-ToF ToF-SIMS IV (Muenster, Germany), operated in static mode. The ion source was a  $^{69}\text{Ga}$ -liquid metal ion gun (LMIG) with a potential of 25 keV and a maximum current of 2.5 pA. A spot area of 500 μm × 500 μm was sampled. Both negative and positive ion modes were investigated

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