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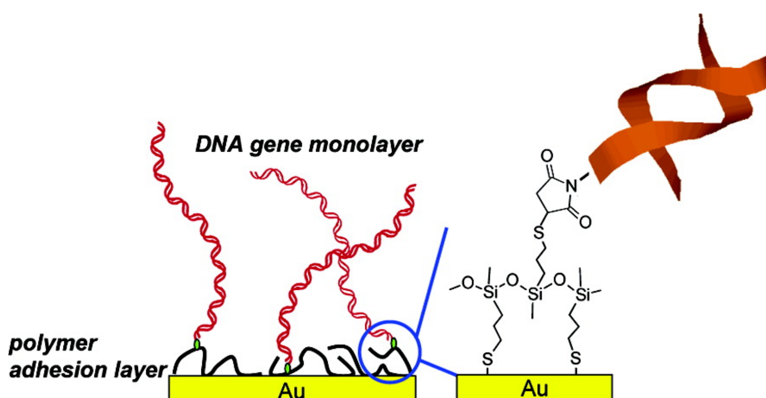

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Polymer-Anchored DNA Gene Monolayers

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The tremendous growth in biosensing and microarray technologies based on surface-tethered nucleic acids has heightened urgency to fundamentally understand how nucleic acids behave at surfaces. From oligonucleotide film studies it is known that hybridization is suppressed relative to bulk solution.[1−3] Theoretical descriptions are also emerging to complement experimental observations.[4−6] However, less scrutiny has been applied to monolayers of DNA chains that are truly macromolecular; we will refer to this case as "polymeric" DNA and consider it to imply chains of 100 monomers or longer. In this report we address preparation and preliminary characterization of model monolayers of polymeric DNA. These films may be useful in complementary DNA (cDNA) microarray technologies,[7] DNA vaccines,[8] and a spectrum of solid-phase molecular biology techniques.[9−12] They are also versatile experimental models for investigating surface-confined charged macromolecules, whose physicochemical behavior is often cast in terms of asymptotic laws reached at large chain lengths.[13]

Prevalent approaches for immobilizing long DNA fragments to surfaces are based on baking and UV cross-linking.[14] These methods leave surface-bound molecules in poorly understood conformations that likely involve multiple attachment points per chain. Site-specific, covalent attachment of polymeric DNA via one terminus has been reported on glass,[9] agarose,[12] and gold,[15] though stability limitations can constrain applications if regeneration (e.g., at elevated temperatures, as in PCR) of single-stranded molecules is required or if long-term trends need to be characterized. We therefore developed a methodology for gold supports combining stringent attachment via chain terminus with excellent stability, sufficient to withstand thermal denaturation at 90 °C without loss of surface-linked strands.

Our approach generalizes a recently reported strategy based on "anchor films" of the polymer poly(mercaptopropyl)methyl siloxane (PMPMS).[16] PMPMS forms a highly multidentate thiolate attachment to gold[16,17] and assembles in a nanometer-thin film that also provides a thiol-rich surface for further modification. Polyfunctional thiolate grafting is known to greatly enhance layer stability.[18] PMPMS films were derivatized with 1943 base pair (bp) double-stranded DNA (dsDNA) chains. These molecules, prepared by PCR from plasmid precursors (pT7LUC, Promega), contained the gene for firefly luciferase (LUC; 1650 bp) under the control of a T7 promoter. Disulfide-modified primers were used to introduce a disulfide terminus to the genes which was subsequently reduced with dithiothreitol to liberate a terminal thiol, followed by reaction with bis-maleimidotetraethylene glycol (BMPEO4, Pierce Biotechnology) to yield maleimide-terminated gene constructs, "LUC-MAL." A 100-fold excess of BMPEO4 to LUC amplicons was used in a 2 h reaction in pH 7.0 0.015 M citrate buffer, 1 M NaCl (SSC1M buffer). LUC-MAL was immobilized on 2 to 3 nm thick PMPMS films from $\sim 1 \times 10^{-8}$ M solutions in SSC1M (Figure 1).

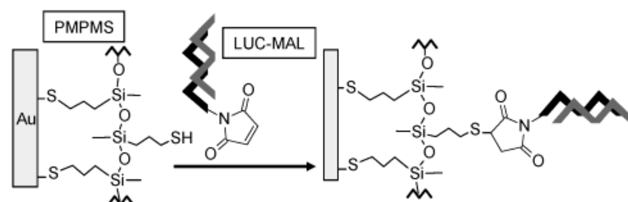

Figure 1. Attachment of LUC-MAL to a PMPMS anchor film.

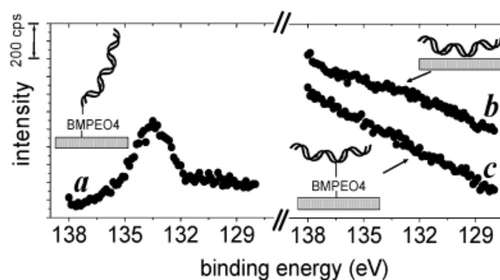

Figure 2. Raw P 2p traces from PMPMS films reacted with LUC amplicons. The inset schematics illustrate the tested mechanism of attachment (see text). All data are for 36 h immobilization from $\sim 1 \times 10^{-8}$ M solutions of the DNA in 0.015 M sodium citrate, 1.0 M NaCl, pH 7.0.

Control "LUC" chains, without a reactive terminus, were prepared from unmodified primers of the same sequence.

Compared to immobilization of oligonucleotides, attachment of polymeric DNA exacerbates prospects of side reactions because thousands of potentially competing reactive sites exist along the chain. For example, although aromatic amines on nucleic bases are not known to be highly reactive, there are ∼10 000 of these moieties in LUC-MAL. This excess, relative to a single endgroup, raises concern that a cross-linker like BMPEO4 may also react at internal positions leading to loss of control over final attachment geometry. To test for such side reactions we carried out expression of the LUC gene as a diagnostic screen (TnT Wheat Germ Extract, Promega), reasoning that modifications within the gene should interfere with RNA polymerase during transcription and block synthesis of the firefly luciferase enzyme. Quantification of the level of expressed enzyme was accomplished by addition of beetle luciferin, which is digested by luciferase with concomitant emission of light. Luminescence from bulk solutions of LUC chains after the standard BMPEO4 treatment was within ±15% of that from untreated genes. The comparable gene activity suggests that little if any modification took place at internal DNA positions.

X-ray photoelectron spectroscopy (XPS) was used to examine whether LUC-MAL attached to PMPMS site-specifically through one end. An end-tethered geometry is expected to be least interfering with hybridization or enzymatic addressing of immobilized molecules. Figure 2, trace *a*, shows P 2p emissions after attachment of LUC-MAL. The integrated intensity of this trace translates to a surface coverage of 4.1×10^{10} chains/cm^2. This coverage, calculated from absolute intensity using an independently measured instrument

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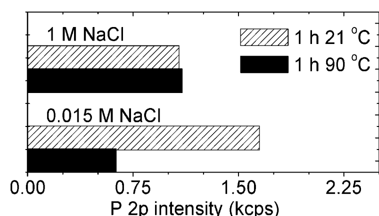

Figure 3. Integrated P 2p intensity from LUC-MAL monolayers following immersion in hot buffer (1.5 mM sodium citrate, pH 7) at the indicated ionic strength.

response factor, actually represents a lower limit because the calculation assumed zero attenuation of P 2p intensity. As described in the Supporting Information, we estimate that true coverage could be higher by up to 30%. Sample *b* is a control for physical adsorption; these strands were not end-modified or treated with BMPEO4. Trace *c* is a control for immobilization through internal sites; these strands did not carry a terminal disulfide but otherwise underwent the standard BMPEO4 treatment. The absence of P 2p emission in samples *b* and *c* indicates that, within sensitivity of the XPS ($\sim 5 \times 10^9$ chains/cm^2), attachment of LUC-MAL is site-specific.

The ability to denature bound chains without loss of surface-linked strands is essential to applications in nucleic acid diagnostics and solid-phase PCR protocols.[9,10,19] Figure 3 shows changes in P 2p intensity after immersion of LUC-MAL monolayers for 1 h at 90 °C in citrate buffers at two ionic strengths, 1.0 and 0.015 M NaCl. The high ionic strength data reveal excellent stability, with no loss of DNA evident, in agreement with earlier data on oligonucleotide films.[16] In contrast, direct chemisorption of DNA to gold through a single thiol fails under similarly aggressive conditions.[16] At the lower ionic strength, an ~60% decrease of P 2p signal is observed. This decrease is attributed to melting of the double helix. The lower added salt is insufficient to screen strand−strand electrostatic repulsions, causing loss of the strand not covalently linked to the solid support. The observed decrease does not exactly equal the expected value of 50%. The cause for this is not clear but is suspected to arise from different arrangements of immobilized single-stranded DNA (ssDNA) and dsDNA that alter attenuation of P 2p emission within these films.

The melting temperature T_M of polymeric dsDNA can be estimated from[20]

$$T_M = 81.5\ °C + 16.6 \log M + 0.41(\%GC) - 500/n$$

M is monovalent salt concentration (mol/L), % GC is percentage of GC base pairs, and n is duplex length. For LUC-MAL, n = 1943 bp and %GC = 45%, leading to T_M = 100 °C in 1 M NaCl and 69 °C in 0.015 M NaCl. The calculated T_M values agree with the data reported in Figure 3, namely stability under 1 M salt but dissociation of the strands when ionic strength decreases to 0.015 M. Melting of the dsDNA is also additional evidence that it is not internally cross-linked by BMPEO4, since cross-linking would have suppressed strand separation.

The behavior of interfacial polymeric systems is in large part governed by chain−chain interactions, which are intensified due to "crowding" of the chains at the surface. A quantitative measure of crowding is obtained by defining an "overlap density" $\sigma°$,[21,22]

$$\sigma° = 1/(\pi R_g^{\ 2}) \qquad R_g^{\ 2} = pL/3$$

R_g is the polymer's radius of gyration, p, its persistence length, and L, the contour length. For surface densities above $\sigma°$, chains are expected to come into physical contact. For LUC chains, L = 1943 bp × 0.34 nm/bp = 660 nm and, under moderate to high ionic strengths, $p \approx 50$ nm.[23] Therefore, $\sigma° \approx 3 \times 10^9$ chains/cm^2. The highest surface densities realized in our studies were for 60 h attachment. At these long times, hydrolysis of the maleimide function limited further increase in immobilized DNA. The 60 h XPS-derived coverage was 6.1×10^{10} chains/cm^2, about 20 times the overlap threshold. Therefore, the chains will interact, and their behavior will be collective rather than representative of isolated molecules. Importantly, the overall rigidity of LUC dsDNA is fairly high as it only contains ~13 persistence lengths. In future studies, chain rigidity and thus conformational statistics can be adjusted by controlling the ratio L/p. Systematic variations of this parameter will be central to examining crossover behaviors between rodlike and flexible polyelectrolytes tethered at surfaces.

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Supporting Information Available: Complete description of experimental procedures, data analysis, and calculations (5pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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