Polymer-Anchored DNA Gene Monolayers
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Supporting Information.

Materials. Poly(mercaptopropyl)methylsiloxane (PMPMS, degree of polymerization ~ 40) was purchased from Gelest Inc., dithiothreitol (DTT) from Fisher Scientific, and the crosslinker bis-maleimidotetraethylene glycol (BMPEO4) and Ellman's reagent (5,5'-dithio-bis-(2-nitrobenzoic acid)) were from Pierce Biotechnology. Oligonucleotides were from Qiagen Operon and included purification by HPLC. Three oligonucleotide sequences were used: a disulfide-terminated sequence 5’ HO(CH2)6-S-S-(CH2)6-CAA TAC GCA AAC CGC CTC TCC 3’ (P1-S-SR), and unmodified sequences 5’ CAA TAC GCA AAC CGC CTC TCC 3’ (P1) and 5’ TCG GTG ATG TCG GCG ATA TAG G 3’ (P2). TnT® wheat germ extract system for coupled transcription/translation was from Promega. Buffers were prepared using 18.4 MΩ cm water from a Millipore Biocell purification system.

Linear, 1943 bp long double-stranded (dsDNA) molecules were prepared by PCR amplification from plasmid precursors (pT7LUC, Promega). These chains consisted of a 64 bp spacer sequence in front of a T7 promoter, the promoter sequence, the firefly luciferase gene of 1650 bp, a poly(A) termination region, and a 63 bp tail. Chains without a terminal modification, referred to as "LUC" chains, were amplified using primers P1 and P2. Disulfide-terminated chains, "LUC-S-SR", were prepared using P1-S-SR and P2 primers. The disulfide was introduced to the promoter end of the gene. The PCR cocktail consisted of 1 X PCR Master Mix (Promega), 1 μM concentrations of each primer, 0.5 ng of pT7LUC DNA template, and nuclease-free water. PCR settings (Eppendorf Mastercycler) included an initial ramp to 95 ºC for 3 min, 30 amplification cycles of [94 ºC, 45 sec; 57.3 ºC, 45 sec; 72 ºC, 2min], and a final extension cycle at 72 ºC for 7 min. Samples were then held at 4 ºC until collected. LUC chains were purified on QIAquick PCR Purification spin columns (Qiagen) following manufacturer instructions. Their monodispersity was confirmed via agarose gel electrophoresis (E-C Minicell) using 0.5 μg/ml ethidium bromide stain and visualization under UV light. LUC-S-SR PCR product was processed as described below. DNA concentrations were determined from A260 absorption measurements on a Cary 50 spectrophotometer.

dsDNA constructs with a terminal maleimide moiety, "LUC-MAL", were prepared from LUC-S-SR precursors. A 500-fold excess of DTT in 100 μL of SSC1M (SSC1M: 0.015 M sodium citrate, 1 M NaCl, pH 7.0) to total disulfide was added to 700 μL of crude PCR product, and allowed to react for 1 h to reduce disulfide to thiol groups, producing "LUC-SH". The LUC-SH mixture was loaded on QIAquick spin columns, twice washed according to manufacturer instructions, and eluted with a 1 × 10⁻⁴ M sodium citrate pH 7.5 elution buffer. The elution buffer differed from that recommended in order to avoid amine groups in the eluent, which could potentially react with crosslinker imides in subsequent steps. Typical recovery was 400 μL containing ~ 3 × 10⁻⁸ M LUC-SH. The eluent was adjusted to 1M NaCl. BMPEO4 at a concentration of 2.8 × 10⁻³ M in SSC1M was added to a 100-fold excess over LUC-SH and, after a 2 h reaction, LUC-MAL was purified by passage through NAP-5 columns (Amersham Biosciences) with SSC1M as elution buffer. 750 μL of recovered LUC-MAL, at a typical concentration of 1 × 10⁻⁸ M in SSC1M, was immediately used to derivatize PMPMS-coated supports. Figure 1 illustrates the above sequence of chemical steps.
Figure 1. (a) Maleimide-terminated chains, LUC-MAL, were prepared from disulfide-terminated LUC-S-SR precursors in two steps: disulfide reduction with dithiothreitol (DTT) followed by addition to maleimide olefinic bond on BMPEO4. (b) Attachment of LUC-MAL to gold supports involved chemisorption of a PMPMS layer in a first step, followed by reaction of LUC-MAL with remnant PMPMS thiols to form thioether linkages.

Sample Preparation. Glass slides were cleaned by immersion in hot "piranha" (70/30 mixture of conc. H₂SO₄ and 30 % aqueous solution of H₂O₂) for a minimum of 20 minutes, rinsed thoroughly with deionized water, and dried with a nitrogen stream. WARNING: piranha solution is extremely oxidizing and should never be stored in tightly capped containers on account of gas evolution. Cleaned slides were coated with a 20 nm Cr adhesion sublayer and a 300 nm Au toplayer by thermal evaporation. Just prior to use, gold surfaces were cleaned for 20 minutes in a UV-ozone cleaner system (Jelight Company, Model 342) followed by 30 min immersion in ethanol to reduce any gold oxide that may have formed. The surfaces were washed with toluene and transferred, without drying, into 1 mM (monomer residues/volume) PMPMS solutions in toluene. Typical immersion times were 1 h. Following adsorption of PMPMS, slides were rinsed with toluene, dried under a nitrogen stream, and used immediately for DNA immobilization.

For DNA attachment a silicone gasket with cut-out "reaction" wells was sandwiched between a PMPMS-modified and a plain slide to create a set of circular chambers 0.54 cm² in area. DNA solutions in SSC1M were introduced by needle and syringe through the gasket into the sealed wells. After a designated time, the wells were drained and rinsed with SSC1M buffer four times while still sealed, disassembled, and subjected to a final rinse with deionized water. The surfaces were then dried and characterized by XPS (see below).

The time available for immobilization of LUC-MAL is limited by hydrolysis of the maleimide function. The hydrolysis rate was determined by using mercaptoethanol to titrate solutions of 1.7 × 10⁻⁴ M BMPEO4 in 0.01 M pH 7.2 buffer, after various times of storage. Decrease in mercaptoethanol concentration due to consumption by unhydrolyzed BMPEO4 maleimides was quantified spectrophotometrically using Ellman's reagent following provider instructions. Ellman's reagent undergoes an exchange reaction with free mercaptoethanol to produce the strongly absorbing species 2-nitro-5-thiobenzoic acid, which is detected at 412 nm and correlated with mercaptoethanol concentration through a calibration curve. About 50 % of
maleimide groups hydrolyzed within 24 h, after 72 h only 20% of maleimides remained active, and after 7 days no active maleimides were detected.

**XPS Characterization and Analysis.** XPS measurements were performed on a Physical Electronics PHI 5500 instrument equipped with an Al X-ray monochromatic source (Al Kα line, 1486.6 eV) and a spherical capacitor energy analyzer. Elemental scans were carried out for gold (Au 4f), carbon (C 1s), silicon (Si 2p), oxygen (O 1s), sulfur (S 2p), phosphorus (P 2p), and nitrogen (N 1s) at a 45° takeoff angle. Typical integration times were 3 min for Au, 6 min for C and O, 15 min for Si, S, and N, and 60 min for P. Elemental detection limits were approximately 0.1% of total photoelectron intensity. XPS traces were baseline corrected with the program XPSPeak, with baselines modeled as a combination of Shirley and linear functions.

XPS data were analyzed to derive DNA surface coverage using equation (1),

\[ I_m = \frac{R(\theta) m \ r \ \sigma_m \ X_m}{\sin \theta} \quad (1) \]

\( I_m \) is the integrated signal intensity from a monolayer of atoms, \( R(\theta) m \) is instrument response function at takeoff angle \( \theta \) for spectral line \( m \), \( \sigma_m \) is surface density of emitting atoms (atoms/true area), \( r \) is a roughness-correction factor (ratio of true to geometric area; \( r \geq 1 \)), and \( X_m \) is the differential photoionization cross-section. The product \( r \sigma_m \) is the surface density of atoms as seen by the instrument's analyzer, and is written as such in equation (1) to emphasize that the reported DNA coverage values are on a per geometric area basis. The takeoff (grazing) angle \( \theta \) is defined between path of detected photoelectrons and the sample surface. The intensity \( I_m \) refers to baseline-corrected peak area. Details of derivation of expression (1) are available in the literature. As further discussed below, we note that equation (1) assumes that the emitted intensity \( I_m \) is not attenuated by the presence of an overlayer.

LUC-MAL coverages (per geometric area) were estimated from absolute P 2p intensities by calculating the phosphorus atom coverage,

\[ r \sigma_P = \frac{I_{P2p} \ \sin \theta}{R(\theta)_{P2p} \ X_{P2p}} \quad (1b) \]

and dividing \( r \sigma_P \) by 3886, the number of P atoms per LUC chain. \( X_{P2p} \) = 1290 barns was taken from Scofield's tables. The instrumental response function \( R_{P2p} \) (at 133 eV) was interpolated from \( R_{Si2p} \) (at 100 eV) and \( R_{Si2s} \) (at 151 eV). Values of \( R \) at the two silicon lines were measured from fused silica reference slides cleaned in situ with a beam of Ar ions until C 1s emission was negligible, and using equation (2) to calculate \( R^3 \)

\[ R(\theta)_{Si} = \frac{I_{Si}}{\rho_{Si} \ X_{Si} \ \Lambda_{Si}^S} \quad (2) \]

The number density of silicon atoms in fused silica \( \rho_{Si} = 2.2 \times 10^{22} \) atoms/cm\(^3\), the attenuation lengths of photoelectrons in the silica support \( \Lambda_{Si2s}^S = 3.4 \) nm and \( \Lambda_{Si2p}^S = 3.5 \) nm, and the cross-sections \( X_{Si2s} = 1030 \) barns and \( X_{Si2p} = 884 \) barns were taken from Scofield. \( R \) changed by less than 3% over the range of interpolation.

Equation 1b assumed that P 2p emission from DNA films was not attenuated. This assumption was partially motivated by the expectation that the DNA chains are the topmost layer of the sample. Nevertheless, experimental P 2p emissions are expected to be attenuated as they
need to pass through the DNA layer itself. The expected error resulting from the assumption of no attenuation can be approximately calculated as follows. For a full DNA monolayer in which the chains lie side-by-side as aligned horizontal cylinders that completely cover the surface, the film thickness would be \( t \approx 2 \text{ nm} \) (diameter of dsDNA), and the density of phosphorus atoms \( \rho_P = \frac{\#P}{h A} \approx 1.5 \text{ nm}^{-3} \). Here, the number of P atoms (\#P) is 2 per volume \( hA \), where \( h \) is the length of one base pair (0.34 nm) along the cylinder axis and \( A \approx (\text{DNA diameter})^2 = 4 \text{ nm}^2 \) is the cross-sectional area of the film attributed to one chain. For such a full monolayer, the corresponding surface density of P atoms is \( \sigma_P = \frac{2}{(0.34 \times 2)} = 2.9 \text{ nm}^{-2} \). In estimating the error due to attenuation one also needs the attenuation length of P 2p photoelectrons in the DNA layer, \( \Lambda_{P2p}^O \), which is taken as 3.9 nm from experiments of Inagaki \textit{et al} \(^6\) as tabulated in Tanuma \textit{et al} \(^7\). With these values, the P 2p intensity from a full DNA monolayer, including effects of attenuation, can be calculated from

\[
I_{P2p} = R(\theta)_{P2p} \rho_P X_{P2p} A_{P2p}^O (1 - \exp[-t/\Lambda_{P2p}^O \sin\theta])
\]  

Comparison of values calculated from equations (3) and (1) for \( \theta = 45^\circ \) predicts that, for a full monolayer of dsDNA with \( \rho_P = 1.5 \text{ nm}^{-3} \), the intensity would be attenuated by 28% compared to intensity from the equivalent coverage of P atoms (\( \sigma_P = 2.9 \text{ nm}^{-2} \)) but without attenuation. In other words, neglecting corrections for signal decrease due to attenuation would result in an underestimate of DNA coverage by \( \sim 30\% \). We note that our experimental samples, allowing for a 30% increase in coverage, approach those of a complete monolayer of horizontal cylinders considered above. Thus multiplication of calculated values from equation (1) by 1.3 provides values close to those that would have been estimated from equation (3).

For reporting coverage values we prefer the physical clarity of the key assumption (no attenuation) involved in equation (1). This assumption translates to a lower limit on coverage, since some attenuation is always expected. In contrast, equation (3) assumes homogeneous, planar, uniformly thick DNA films. The impact of these assumptions on calculated coverage is more ambiguous; for example, the DNA chains might pile over one another, and there may be effects due to roughness and voids that are difficult to assess without more detailed information on sample structure.

**Luciferase Expression Measurements.** The ability of RNA polymerase to produce active luciferase enzyme by transcribing LUC dsDNA was used as a diagnostic for potential side reactions with BMPEO4. 4 \( \mu \)L of solution containing LUC DNA at a concentration of 31 \( \mu \)g/ml \((2.6 \times 10^{-8} \text{ M})\) in 1 X SSC buffer (0.015 M sodium citrate, 0.15 M NaCl, pH 7.0) was added to 96 \( \mu \)L of TnT® wheat germ extract mixture (Promega) prepared according to manufacturer instructions. After 2 h of incubation at 30 °C to allow synthesis of the luciferase enzyme, 5 \( \mu \)L of extract were withdrawn and added to 50 \( \mu \)L of assay solution (Promega) containing luciferin. Intensity of the resultant chemiluminescence was measured on a Lumat LB9501 luminometer using a 12 sec integration time.

LUC chains used in the luminescence assays were prepared using primers P1 and P2. The disulfide end-group was deliberately left off to avoid conjugation with species present in the transcription/translation solution. The PCR dsDNA product was purified on QIAquick spin columns (Qiagen) using 0.1 mM pH 7.5 sodium citrate buffer for elution. The eluent was adjusted to 1 M NaCl strength by addition of 20 X SSC. One aliquot (untreated LUC) was set
aside, while to a second aliquot (treated LUC) was added 1 mg/ml (2.8 × 10^{-3} M) BMPEO4 in SSC1M to produce a 100-fold excess over LUC chains. This aliquot was kept 2 h under the BMPEO4 solution before purification by a second pass through QIAquick spin columns. Both aliquots were adjusted to a final concentration of 31 µg/ml in 1 M NaCl citrate buffer before performing the luciferase assay.

References for Supporting Information