Supporting Information for

Controlling the surface properties of nanostructures for studies of polymerases

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Materials

Reagents

Below, we list the reagents required for several distinct steps in the experiments.

- For PEGylation:
  - Solvents and buffers: acetone, ethanol, phosphate-buffered saline (PBS), bicarbonate (all from Sigma)
  - Alconox detergent (Sigma)
  - Vectabond (Brunschwig Chemie)
  - Polyethylene glycol (PEG)
  - The experiments described in this paper used the following PEGs:
  - M-PEG-SPA, MW 5000 Da (stored as 100 mg aliquots at -20 °C, Nektar Therapeutics)
  - Biotin-PEG-NHS, MW 3400 Da (stored as 10 mg aliquots at -20 °C, Nektar Therapeutics)
  - Similar PEGs can also be purchased from Iris Biotech:
  - MeO-PEG-COO-Su (MW 5000 Da)
  - Biotin-PEG-COO-Su (MW 3000 Da)

- For experiments involving DNA anchoring and interaction with polymerases:
  - Streptavidin (Sigma) and fluorescently labeled streptavidin (TMR-streptavidin, Invitrogen)
  - DNA oligonucleotides (Biolegio)
  - NEB2 buffer (10x): 100 mM Tris-HCl pH 7.9, 500 mM NaCl, 100 mM $MgCl_2$, 10 mM DTT
  - DNA polymerases: Klenow exonuclease minus (Promega, M2181)
    Sequenase 2.0 (USB, 70775Y/Z)
  - DNA polymerase buffer (10x): 500mM Tris-HCl (pH 7.2 at 25°C), 100mM $MgSO_4$, 1mM DTT.
  - Standard nucleotides (Invitrogen)
  - Fluorescently labeled nucleotides (tetramethylrhodamine-6-dCTP (Perkin-Elmer), phosphate-labeled Tetramethylrhodamine-dATP (Visigen)
  - Chemicals for PAGE gels: 10x Tris-Borate-EDTA (TBE) buffer (Promega), urea (Sigma), 40% acrylamide (Promega), bis-acrylamide (Promega), ammonium persulfate (Biorad), TEMED (Biorad), Blue/Orange loading dye (Promega), 10 bp DNA step ladder (Promega), agarose (Promega), SYBR gold nucleic acid stain (Invitrogen, 11494).

- For waveguide fabrication:
  - Positive e-beam resist ZEP 520A (ZEON Corp, Japan)
  - Ammonia Solution 28% (VLSI Selectipur, BASF)
  - Hydrogen Peroxide 31% (VLSI Selectipur, BASF)
  - O-xylene (JT Baker Chemical Co.)
  - N,N-dimethylacetamide (Fluka)
  - 2-propanol (JT Baker Chemical Co.)

Equipment

Below, we list the equipment required for several distinct steps in the experiments.

- For PEGylation:
  - Microscope slides (18 mm x 18 mm, Brauschweig)
  - Fused silica cover slides (Valleydesign, Cruz, CA, USA)
  - Water filtration system (Millipore). Throughout the paper, the term "water" always refers to pure water obtained with this system.
  - Sonicator (Branson 1510, Bransonic)
  - Oxygen Plasma cleaner (Structure Probe)
  - Syringe-driven filter units (0.22 μm, Millipore)
  - Nitrogen gun

- For surface characterization:
  - Atomic Force Microscope (Multimode AFM, Digital Instruments) and AFM tips (Olympus, OMCL-AC160TS-W2)
  - Setup for fluorescence microscopy, including an inverted microscope (Olympus) equipped with an Apochromat objective (Olympus, 60 X, NA 1.45), a transmitted light illuminator, a set of fluorescence filters for the observation of TMR (Chroma), a laser (532 nm, 50 mW, Crystalasers) used to create a wide-field illumination, a CCD camera (DV887, Andor) and a motorized stage (MicroStage-20E, Mad City Labs)

- For DNA preparation and detection on PAGE gels:
  - Thermal cycler
  - Temperature block
  - Adjustable Sequencing Kit, 20 cm x 42 cm (CBS Scientific, SG-400-20)
  - Low Fluorescence UN-Notched Glass Plate Set, 20x22cm (CBS Scientific, LFP20-020-UN)
  - Sequencing Spacer Set 1.5 mm x 22 cm (CBS Scientific, SGS-20-1520)
  - 1.5mm x 20 Well Comb for 20cm wide (CBS Scientific, SG20-1520)
  - Power supply (Consort E312)
  - Gel Imager (Syngene, BTS-20.M)

- For waveguide fabrication (clean room facilities):
  - Plasma system (TePla 100)
  - Evaporator (Temescal FC-2000)
  - Electron beam pattern generator (Leica EBPG5000+)
  - Etcher (Alcatel GIR300 RIE)

Methods

PEGylation of glass slides

Standard microscope glass slides (18 mm x 18 mm) were successively sonicated for 10 minutes in an Alconox detergent solution (1% in water) and for one hour in pure ethanol, then rinsed with water and dried with nitrogen. Next, the slides were introduced into an oxygen plasma cleaner (Structure Probe) for 10 minutes, at a pressure of about 0.1 mbar. Immediately following the plasma treatment, the slides were sonicated for 5 minutes in a freshly made aminosilane solution (1% Vectabond in acetone), rinsed with water and dried with nitrogen. PEG dilutions (in 100 mM Bicarbonate Buffer, pH 8.5, filtered through a 0.22 μm filter unit) were prepared immediately before use. Non-biotinylated PEG (M-PEG-SPA, MW 5000, Nektar Therapeutics) was diluted to 20% w/v, and supplemented with the appropriate amount of biotinylated PEG (Biotin-PEG-NHS, MW 3400, Nektar Therapeutics). Each glass slide was reacted with 50-100 μL of PEG solution for 3 hours. Finally, the slides were thoroughly rinsed with water, dried with nitrogen, and stored in sealed containers.

Waveguide fabrication

Cleaning. Fused silica cover slides were immersed in a cleaning solution containing 30 mL NH_4OH (27 %) and 150 mL water. The cleaning solution was heated to 75 °C, and supplemented with 30 mL of H_2O_2 (30 %). After 10 min, the slides were rinsed thoroughly with water, dried with nitrogen, and treated with an oxygen plasma.
Evaporation and coating with resist. A layer of aluminum (typically 100-120 nm thick) was evaporated on the slides. Evaporation was performed at a rate of 0.3 nm/s, at room temperature and with base pressure of about 1×10^{-6} mbar. The samples were then spin-coated with an electron beam resist (ZEP 520A) at a rotation speed of 5000 rpm for 1 min, and baked on a hot plate for 30 min at 175 °C. This procedure results in a resist layer with a thickness of about 300 nm.
Exposure and development. The samples were exposed with an electron beam pattern generator. A beam of approximately 3.3 nA at 100 kV acceleration (which corresponds to an estimated beam size of approximately 6 nm), a beam step size of 5 nm, and a dose of approximately 350 $\mu C/cm^2$ were typically used. The samples were developed in xylenes for 3 min, with mild agitation. They were rinsed with isopropanol immediately after development and dried with nitrogen.
Etching. The pattern was transferred into the aluminum using reactive ion etching. We typically etched for 4 minutes using 12.5 sccm N_2, 10 sccm BCl_3, and 5 sccm Cl_2. During etching, the pressure in the chamber was regulated to 16 μbar, the table temperature was maintained at 50 °C, and the RF power equaled 50 W. The samples were rinsed with distilled water immediately after etching and dried with nitrogen. Finally, the resist was removed by sonication for 1 min in dimethylacetamide, and the samples were rinsed with isopropanol and dried with nitrogen.

PEGylation of waveguides

PEGylation of waveguides was achieved with a protocol similar to the one used for the PEGylation of glass slides. In addition, brief sonications were applied each time the waveguides were covered with solutions, specifically to improve their wetting.

DNA templates

DNA templates suitable for Klenow and Sequenase polymerases (figure S2a) were prepared by hybridizing 20 pm of a 75-base, biotinylated oligonucleotide (5'biotinGCTACTGCTACTGCTACTGCTACTGCTACTGCTACTGCTACTGCTACTGACTGCTA CTAACACGGAGGTTCTA) and 20 pm of a 14-base, complementary oligonucleotide (5'TAGAACCTCCGTGT). The hybridization was performed in a total volume of 30 µl in NEB 2 buffer. Primers were hybridized by the application of a temperature cycle.

Bulk assay for polymerization

Reactions were performed in 50 µl solutions typically containing 0.2 µM hybridized DNA, 1 mM of regular dATP, dGTP and dTTP, 10 M TAMRA-dCTP and 0.1 U/µl Klenow or Sequenase polymerase, in DNA polymerase buffer (50mM Tris-HCl, pH 7.2, 10 mM $MgSO_4$, 0.1mM DTT for Klenow polymerase). The reaction was allowed to proceed for one hour at 37 °C, and stopped by heating the reaction mixture to 75 °C for 10 minutes.

A polyacrylamide gel was prepared by mixing 49.8 g urea (8.3 M), 50 mL 40% acrylamide, 1 g bis-acrylamide, 10 mL 10x TBE, 47.5 mg ammonium persulfate, and water to yield a total volume of 100 mL. This mix was dissolved by stirring and heating (30-40 °C). 90 µl of TEMED were added to the gel mix. The gel was poured, left polymerize for one hour at room temperature, and heated up to 60-65 °C during running.

The products of the polymerization reaction were run on the PAGE gel in 1x TBE. In our experimental conditions, in which a tension of about 600 V (delivered by two power supplies in series) was applied on the gel, the typical duration of the migration was 2.5-3 hours. Gel imaging was performed in two steps (figure S2). First, the unstained gel was imaged with a UV light source. This allows for the selective observation of the DNA products in which fluorescent nucleotides have been incorporated (figure S2b). Then, the gel was stained with Sybr Gold (staining was performed following the instructions given by the supplier), after which all DNA products become visible (figure S2c).

Polymerization on surface-anchored DNA molecules

On PEG-coated glass slides and waveguides, we incubated 100 µL of a streptavidin solution (0.1 mg/mL) for 10 minutes. The surfaces were rinsed with water and dried with nitrogen. Then, a 50 µM DNA solution in PBS was incubated on the surfaces. The surfaces were rinsed with water and dried with nitrogen. A supplementary passivation of the surfaces was performed by incubating a 1 mM solution of unlabeled dATP for 10 min. Next, the surfaces were rinsed with water and dried with nitrogen. Finally, the surfaces were covered with a 100 µL reaction

mix containing 0.4-40 U polymerase, unlabeled dATP, dTTP and dGTP (to a final concentration of 1 mM each), and base-labeled dCTP (to a final concentration of 0.1-1 µM), in polymerase buffer (see Materials, above). The reaction was allowed to proceed for 1-3 hours at 37 °C. In parallel, a negative test with no polymerase or with heat-inactivated polymerase was always run. Following the reaction, the slides were rinsed thoroughly with water and dried with nitrogen. Therefore, the fluorescence subsequently observed on the slides only comes from fluorescent molecules that were strongly bound to the surface.

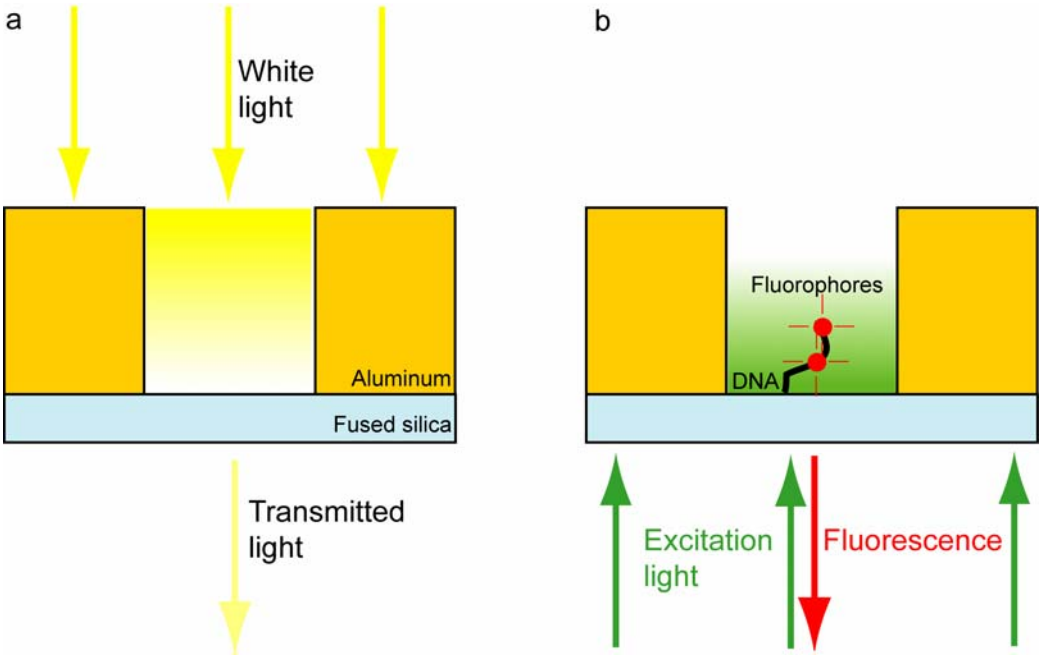

Figure S1 Optical measurements with waveguides
a) Monitoring the transmission of white light. Waveguides are illuminated from above by a beam of white light. The light transmitted can be used to make an image of the waveguide array under study (figure 6c and d), provided that large enough waveguides are present in the field of view. Indeed, light transmission by waveguides critically depends on their size.
b) Monitoring the fluorescence. The fluorophores present within the waveguides (for instance, fluorescently-labeled nucleotides incorporated onto surface-anchored DNA molecules, as in figure 6) are illuminated by a beam originating from a 532 nm laser. If the waveguides (referred to as “zero-mode waveguides” in this case) are small enough, the illuminated volume is essentially limited to the bottom part of the waveguides. The fluorescence emitted by fluorophores is collected through an objective, separated from the illumination light by spectral filtering and imaged onto a CCD camera (figure 6a and b).

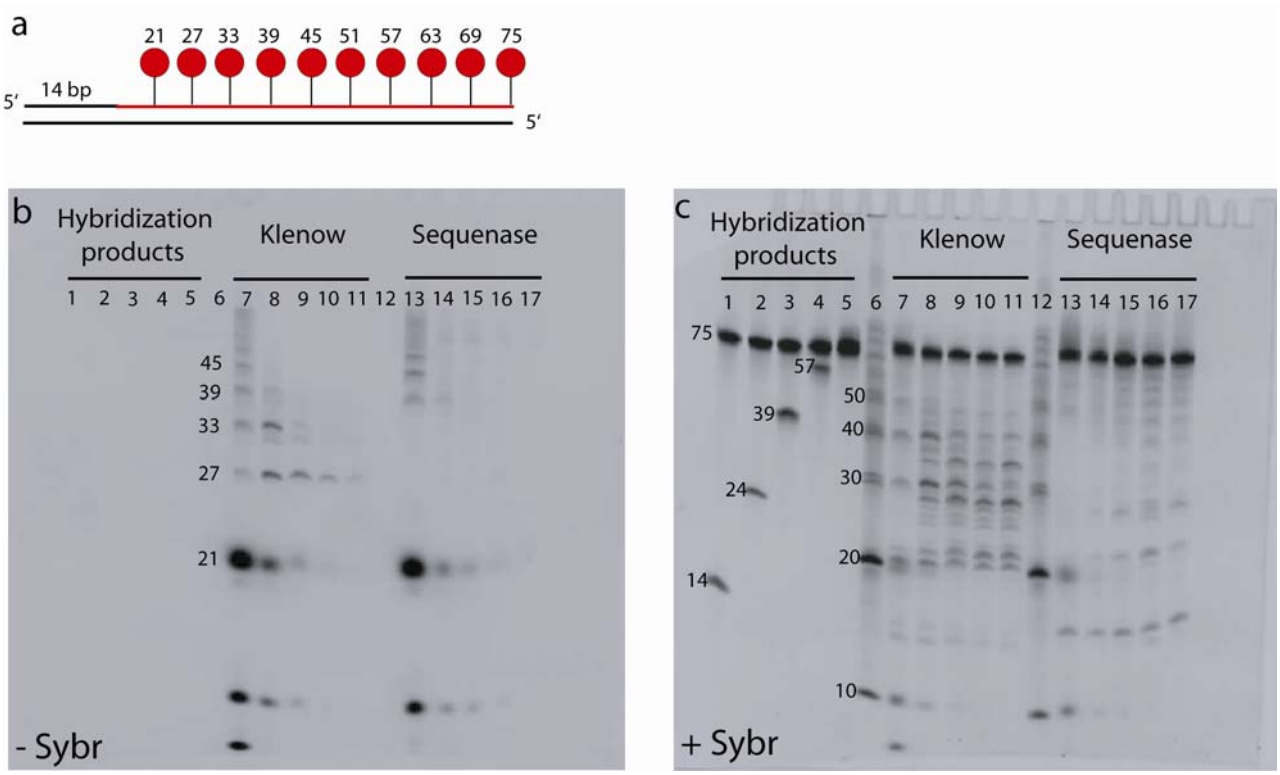

Figure S2 Polymerase: bulk fill-in reaction and detection on a denatured PAGE gel
(a) Experimental strategy. The DNA molecules used during the fill-in reaction are produced by the hybridization of a 75 nt oligonucleotide with a 14 nt complementary oligonucleotide (both shown in black) so as to form a suitable substrate for Klenow and Sequenase polymerases. The sequence to be polymerized is periodic, with one TAMRA-dCTP to be incorporated every 6 bases (the corresponding positions are shown in red). (b) and (c) show the migration of the products of the fill-in reaction on a 20% denatured PAGE gel. The images were obtained respectively before (b) and after (c) staining of the gel with Sybr Gold: therefore, only fluorescent nucleotides (free or incorporated) are observed in (b), whereas all DNA products become visible in (c). Lanes 1 to 5 correspond to the migration of the products of various hybridization reactions, in which the 75-base oligonucleotide had been annealed to 14 (1), 24 (2), 39 (3), 57 (4) and 75 (5) bases complementary oligonucleotides. Lanes 6 and 12 are 10 bp DNA ladders (invisible in the unstained gel shown in (b)). DNA in lanes 7 to 11 are the result of a fill-in reaction with Klenow polymerase and decreasing concentrations of TAMRA-dCTP: 10 μM (7), 3 μM (8), 1 μM (9), 0.3 μM (10) and 0.1 μM (11), while the products of lanes 13-17 are the result of a reaction carried out with Sequenase and the same decreasing concentrations of TAMRA-dCTP. Note that the brightness of the band at 75 bases in (c) is not meaningful in the context of the fill-in reaction, since this band includes the initial 75-base oligonucleotide of the DNA template.