Supporting Information for

Attomole Detection of Mesophilic DNA Polymerase Products by Nanoparticle-Enhanced Surface Plasmon Resonance Imaging on Glassified Gold Surfaces

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This file contains:

Experimental section

Figures S1—S5

EXPERIMENTAL SECTION

All chemicals were obtained from commercial sources and used as received. All DNA oligonucleotides were synthesized by Integrated DNA Technologies. MilliQ-purified water (Millipore) was used throughout.

Klenow Fragment Reactions. Reactions containing 16 μM target, 5´—GTT CTG ATC AGT TCA CGC ATA GAC CAC TTG AGG CTA ACA C—3´, 200 μM dNTPs, and Klenow fragment of *Escherichia coli* DNA Polymerase I (New England Biolabs, 0.5 U/μl) were placed in 9 x 9 mm Frame-Seal slide chambers (Bio-Rad) that had been affixed to the glassified gold-spotted slides. The slides were heated to 37 °C for up to 20 min on a Hybaid OmniSlide (Hybaid Ltd.). The slide temperature was increased to 75 °C for 20 min to heat-kill the enzyme. The Frame-Seals were removed, the slides were rinsed with water and placed in 6 M urea warmed at 75 °C for 10 min to ensure the denaturation of the target from the probe DNA. The slides were rinsed with water and dried with N_2 gas.

Fluorescence Microscopy. The slides were prepared as described previously[1] with the following changes. The surface of the CodeLink slide was photopatterned with UV radiation through a chromium quartz mask to produce a surface with square areas of N-hydroxysuccinimide (NHS) active esters for binding to aminated DNA. Microarrays were fabricated with amine-modified single-stranded DNA probes: 5´—$NH_2(CH_2)_{12}(T)_{15}$GTG TTA GCC TCA AGT CCT CT—3´ or a T_{30} control sequence. The surface-attached single-stranded DNA molecules were reacted as described above. The slides were exposed to 100 nM detector (5´—Cy3-GTT CTG ATC AGT TCA CGC AT—3´) for 30 min under a glass coverslip at room temperature. To remove the

unannealed detector, the slides were soaked once in 4X SSC, 0.1% SDS to remove the coverslip and rinsed once in each of the following solutions: 4X SSC, 0.1% SDS; 4X SSC; 2X SSC, 0.1% SDS; 2X SSC and 0.2X SSC, 0.01% SDS (stock 20X SSC, US Biological: 3 M NaCl, 300 mM sodium citrate, pH 7.0). Fluorescence images were taken on an Olympus IX71 inverted microscope (Olympus America Inc.); line profiles were drawn using ImageJ (NIH).

Preparation of the slide for SPRI experiments. A gold-spotted SF10 glass surface was glassified using a modified version of the method developed by Phillips et al.[2] as follows. The gold-spotted slide was reacted with 1 mM ethanolic solution of 11-mercaptoundecylamine (MUAM; Dojindo) for 24 h. The slide was rinsed with ethanol to remove unbound thiol and dried with nitrogen. The gold-spotted slide was then exposed to an aqueous polyglutamic acid (pGlu) solution for 30 min (2 mg/mL, Sigma). After the slide was rinsed with water and dried with nitrogen, an ultrathin silicate film was built using a layer-by-layer assembly of polyelectrolyte as follows: the clean dry slide was immersed in 2 mg/mL polylysine solution for 5 min followed by rinsing with water and drying with nitrogen. The slide was then dipped into sodium silicate solution (40 g/L) for 5 min followed again by rinsing and drying. This process was repeated 3 times to build up layers of polylysine and sodium slilicate. The slide was calcinated in the furnace by heating it at 450 °C for 3 h. A (3-aminopropyl)trimethoxysilane (50 mM, Sigma) in ethanol/water (95%/5%) solution was prepared and was allowed to stand for 20 min before filtering with a 0.22-μm cutoff syringe filter. The chip was immersed in the ethanol/water solution for 45 min. The slide was then reacted with pGlu solution for 30 min followed by reaction with two amine-modified oligonucleotides, both in phosphate buffer solution (10 mM phosphate, 0.1 M NaCl, 5 mM $MgCl_2$, pH 7.2), 5′—$NH_2(CH_2)_{12}(T)_{15}$GTG TTA GCC TCA AGT CCT CT—3′ or a T_{30} control sequence.

SPR Imaging. Aqueous sodium citrate-stabilized Au nanoparticles (NPs) with a mean diameter of 13±1 nm were synthesized following the Turkevich method[3] with modifications as described in Sendroiu and Corn.[1] A 3´-thiol-modified single-stranded oligonucleotide detector with the sequence 5´ —$SH(T)_{15}$GTT CTG ATC AGT TCA CGC AT—3´ was attached to the nanoparticles. The assembly formed of AuNP and detector ssDNA is named detector—NP. Surface plasmon resonance imaging experiments were performed using an SPRimager (GWC Technologies) instrument using protocols that have been described in detail elsewhere.[4-7] Hybridization of 5.43 nM detector—NP was performed in the SPR flow cell for up to 15 min. SPR images were processed using Image J (NIH).

REFERENCES

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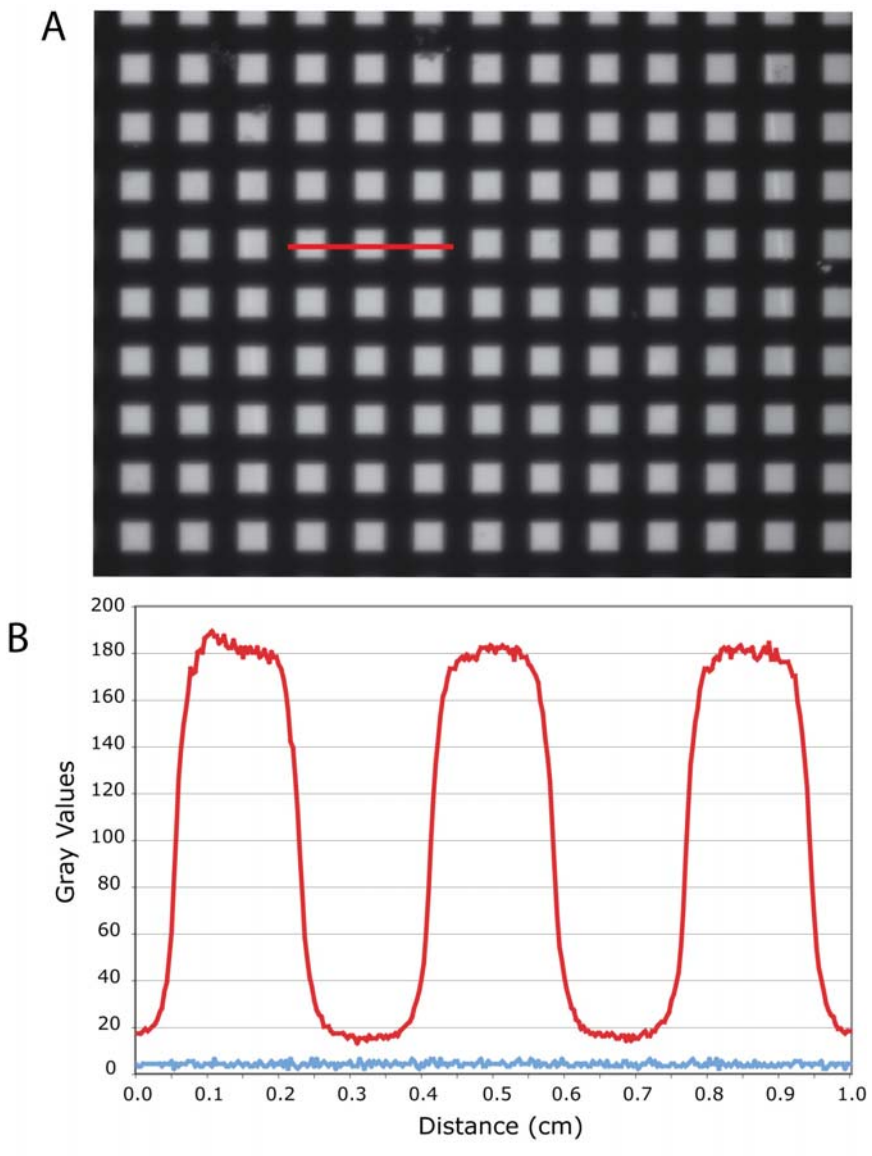

Figure S1. (A) The surface-bound Klenow fragment reaction product was detected by hybridization to a Cy3-labeled complementary DNA sequence. Fluorescence images were taken using an inverted fluorescence microscope with 550 nm excitation/570 nm emission wavelengths. (B) Line profile showing measurement of fluorescence from both the reaction (red) and control slide (blue).

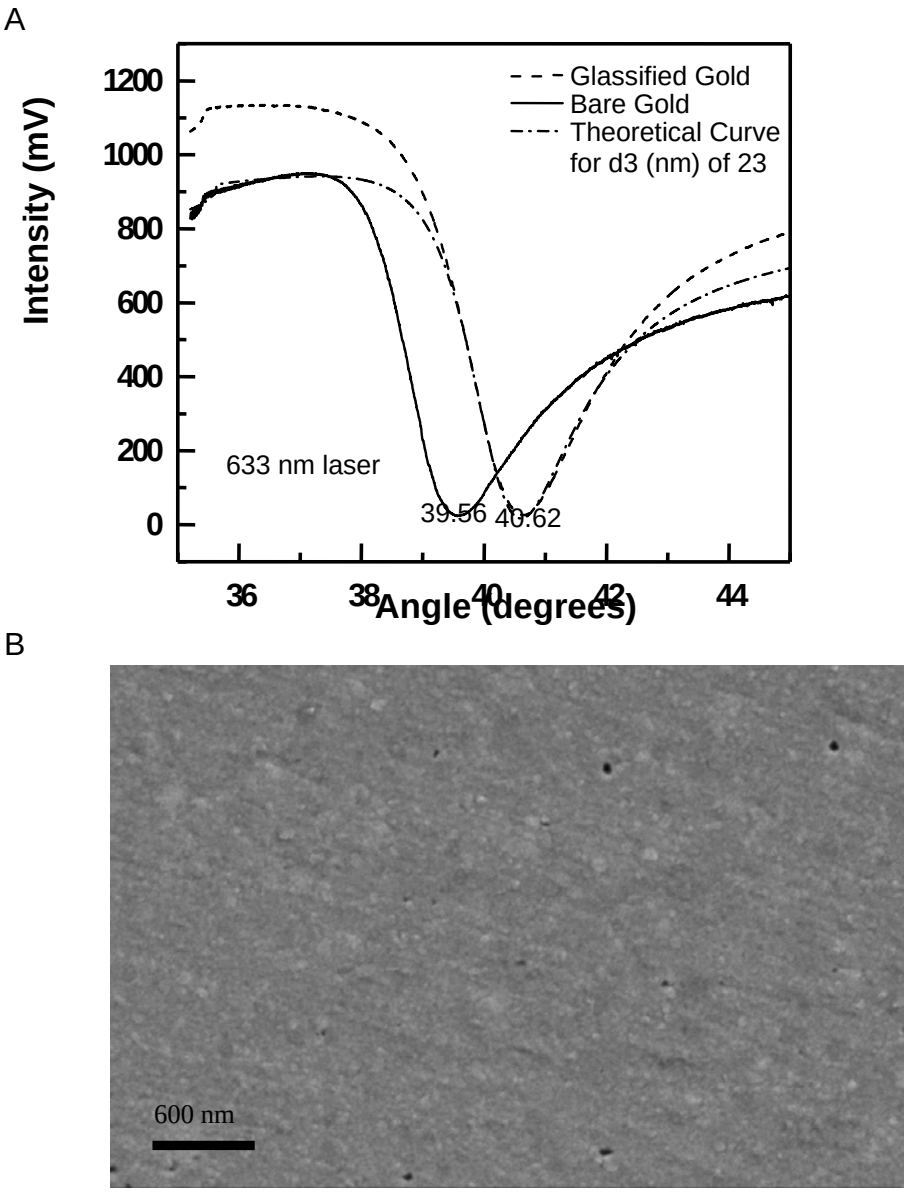

Figure S2. Characterization of the calcinated slides. (A) The thickness of the glass layer was measured by fitting the experimental SPR reflectivity curves with the data obtained using the four-phase Fresnel reflectivity calculation macro for IgorPro (http://corninfo.ps.uci.edu/calculations.html). A HeNe laser, 633 nm, and a diode laser, 814 nm, were used to obtain the experimental SPR curves. Results from both laser sources indicated a thickness of 23 nm. (B) The morphology of the calcinated surface was evaluated by SEM. The SEM image shows that the silicate surface appears smooth with no visible cracks.

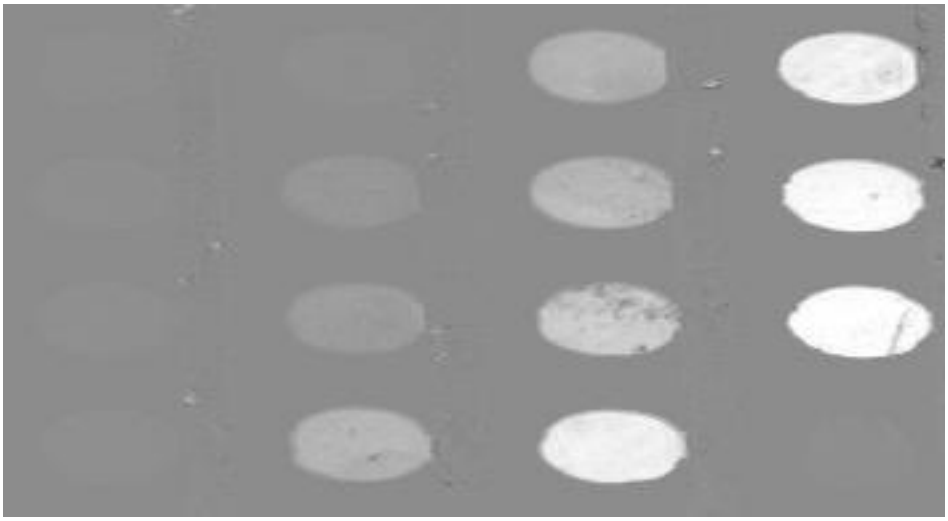

Figure S3. Unmodified SPR difference image corresponding to the false color image shown in Figure 2, left inset. Difference image was obtained by subtracting the initial background from the image taken after 15 min of detector–NP binding to the reaction product.

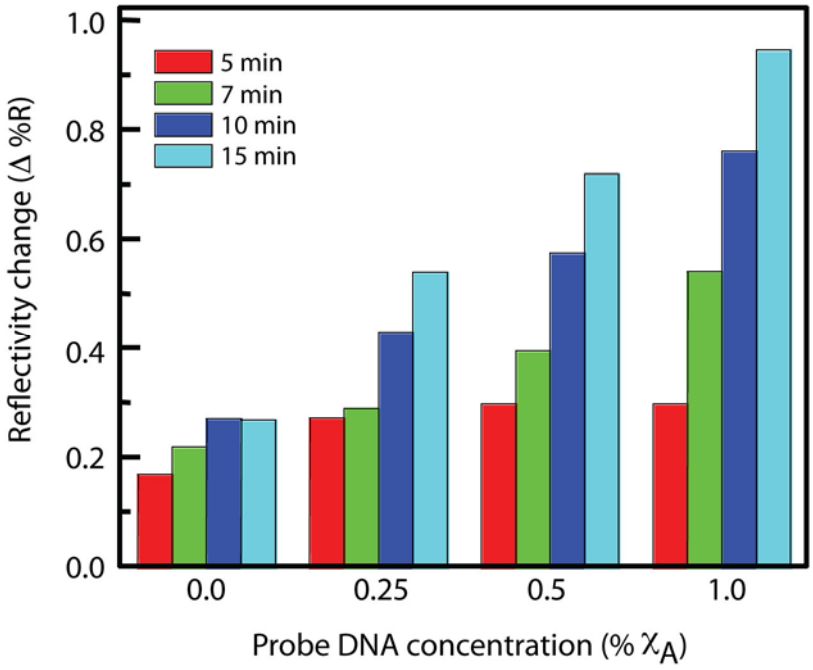

Figure S4. Percent reflectivity change (Δ%R) as a function of probe DNA concentration at 5, 6, 7 and 15 min of detector–NP binding. After 15 min of detector–NP binding to the reaction product in each spot, it is possible to distinguish binding of nanoparticles to the spot containing 0.25% DNA probe. In contrast to the other spots with probe DNA, the Δ%R of the 0% spot does not increase after 10 min.

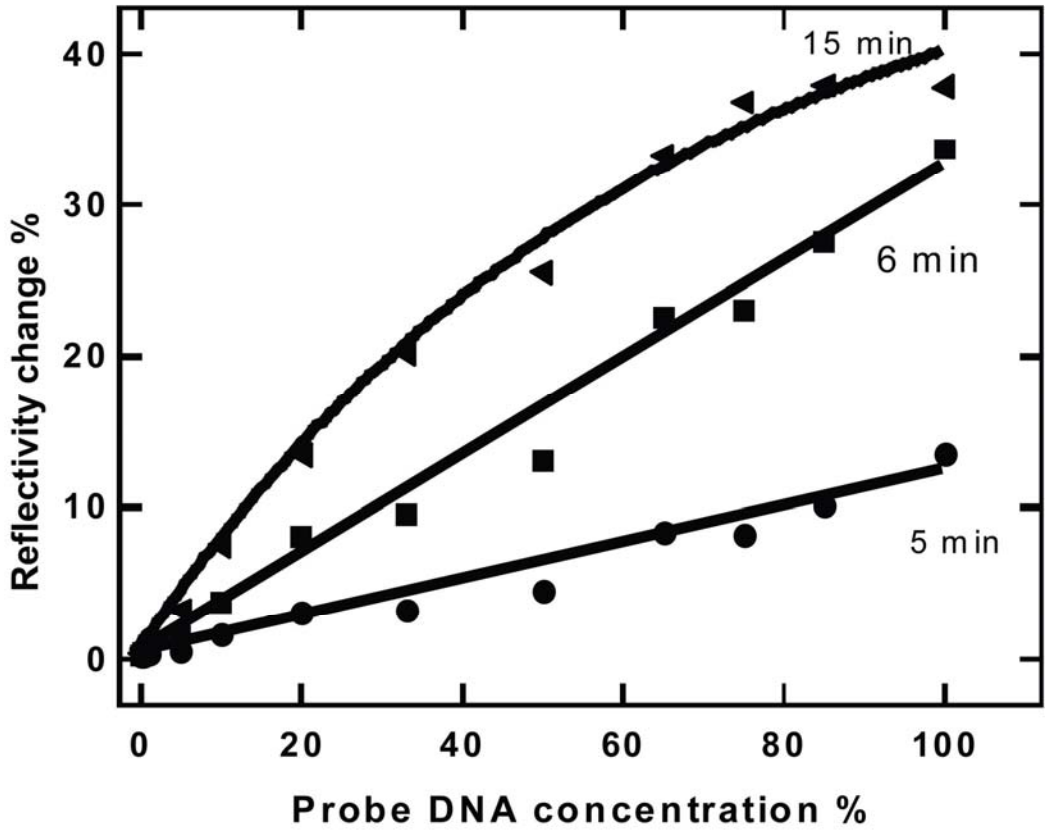

Figure S5. Adsorption of the detector–NP to the enzymatically extended probe DNA concentration as measured by percent reflectivity change (Δ%R) at three different times: 5, 6, and 15 min. When the detector–NPs are allowed to bind for 15 min, the higher percentages of probe DNA (65% and above) reach the maximum percent reflectivity change observed. The data for this adsorption reaction were fit to a first-order exponential curve. At 5 and 6 min, the lines are linear, indicating that the binding of nanoparticles has not yet reached saturation at the spots with the highest concentrations of DNA.