Supporting Information

Nanoplasmonic molecular ruler for nuclease activity and DNA footprinting

Fanqing Frank Chen¹,§, Gang L. Liu²,*, Yadong Yin¹,³,⁵, Siri Kunchakarra¹, Bipasha Mukherjee¹, Daniele Gerion¹, Stephen D. Jett⁴, David G. Bear⁴, A. Paul Alivisatos¹,³, Luke P. Lee²

¹ Lawrence Berkeley National Laboratory, Berkeley, CA 94720
² Biomolecular Nanotechnology Center, Department of Bioengineering, University of California at Berkeley, Berkeley, CA 94720
³ Department of Chemistry, University of California, Berkeley, CA 94720
⁴ Department of Cell Biology and Physiology and the Cancer Research and Treatment Center, University of New Mexico Health Sciences Center, Albuquerque, NM 87131
⁵ Current address: Department of Chemistry, University of California at Riverside, Riverside, CA 92521

§ Correspondence should be addressed to:
Fanqing Frank Chen (f_chen@lbl.gov)
Life Sciences Division
Lawrence Berkeley National Laboratory
MS977R0225A, 1 Cyclotron Rd.
Berkeley, CA 94720

Materials and Methods

Synthesis of DNA oligonucleotides and preparation of thiolated, FITC labeled dsDNA.

Two complementing strands of DNA, each 54 nucleotides long, were synthesized (Operon, Alameda, CA). They are (1) the oligonucleotide SprAuFor with dual thiol modification at the 5’ end of the oligonucleotide (di-thiol-5’-AAAGATCCAAAGTTGAAATTCCTGACAGACGTCTCGGTACCCAAA-3’), and (2) the reverse complementing strand named SprAuRev (FITC-5’-TTTCCTAGGTTCAGACACGCTGCTATAGCAGCCATGGTTT-3’). For the preparation of double-stranded DNA, 5'-thiolated SprAuFor was mixed at 1:1 molar ratio with 5'-FITC-labeled SprAuRev, in a final volume of 100 μL. The mixture was first denatured by heating to 95 °C for 10 minutes, then the DNA strands were reannealed by cooling down slowly to room temperature. The quality of the DNA was checked by gel electrophoresis. Test digestion of DNA with restriction endonucleases HinDIII, KpnI, XhoI, and SalI were performed according to the manufacturer’s instructions (New England Biolabs, Beverly, MA) (Supplement Fig. S1). The cleavage sites for the various enzymes are shown (Figure 1b). Appropriate amounts of buffer and
water were mixed along with 1 μL of each of the enzyme in 100 μL final reaction volume. The reaction was incubated overnight at 37 °C. The digestion was then verified by gel electrophoresis on 5% NuSieve agarose gel in 1X TBE, with a 10 bp ladder as a molecular weight standard (Invitrogen, Carlsbad, CA) (Fig. S1).

**Solubilization of Au nanoparticles with phosphine surfactants**

In order to increase the stability of the Au nanoparticles for further manipulation, the surface capping of the 20nm Au colloids (Ted Pella, Inc., Redding, CA) has been modified with a phosphine moiety (Bis(p-sulfonatophenyl)phenylphosphine, STREM Chemicals, Newburyport, MA) to solubilize 20 nm gold nanocrystals (Ted Pella, Inc, Redding, CA) as described previously with slight modifications¹. 10 mL of 20 nm nanoparticle solution (2.32 nM) was concentrated by a factor of 200, by precipitating the nanoparticles using an ultracentrifuge (14,000 RPM for 20 min). The pellet was resuspended in 50 μL of phosphine buffer (0.3μM-4mM phosphine in DEPC-treated water). The mixture was left overnight on a rocking platform at room temperature to allow sufficient time for surfactant exchange. The final concentration of the phosphine-coated gold particles was ~0.46 μM. We have run the gold nanoparticles on a 1% agarose gel, and observed the successful surfactant exchange. It was observed that 1 μL of the concentrated phosphine-coated gold particle was sufficient for the visualization of the Au nanoparticles in the gel.

**Validation of endonuclease cleavage by fluorescence imaging**

In order to confirm that the plasmon resonance frequency shift of the Au-DNA nanoconjugate is due to the effect of the endonuclease, and that the nanoplasmonic shift can accurately reflect DNA cleavage-induced size change, we compared the dark-field images (Fig 3a, panels 2, and 3) and FITC fluorescence images (Fig. S3, panels 2, and 3) of the Au-DNA nanoconjugates with nuclease to those of the Au-DNA nanoconjugates without nuclease (Fig. 3a, panels 1 and Fig. S3, panel 1). After 1-hour (Fig. 3a, panels 2 and Fig. S3 panel 2) and 16-hour XhoI endonuclease reactions (Fig. 3a, panels 3 and Fig. S3, panel 3). The fluorescence in the Au-DNA nanoconjugates was strong before cleavage. Note the full length of the complete dsDNA (~20 nm) is much longer than the effective Forster transfer distance (<10 nm), and thus the FITC fluorophore was not quenched. The FITC fluorophore was detached from the Au nanoparticles and diffused into the buffer solution after the DNA cleavage, so that the overall fluorescence intensity at the image plane (where the Au nanoparticles are) decreased dramatically. The purpose of the FITC label removal experiment was solely for the validation of the enzymatic cleavage. The fluorescent label had no influence on the plasmon resonance measurement. The scattering spectra of the nanoconjugates were measured after the fluorescence was photobleached. In the first 3 minutes, there were a temporary red shift of the plasmon resonance wavelength, an increase of scattering intensity, and a flattening of the spectra. The brief oscillation is likely due to the initial loading of the enzyme molecules onto the dsDNA before incision.

**Estimate of diffusion rate**

The DNA cleavage we observed is not a diffusion-limited process. The diffusion rate of the endonuclease enzymes (number of enzyme molecules diffusing onto single
nanoconjugates per unit time) can be estimated as $\Delta N / \Delta t = 4 \pi D r C$, where $D = 5 \times 10^{-7} \text{cm}^2/\text{sec}$ is the diffusion constant of enzyme in water, $r = 28 \text{nm}$ is the radius of a single nanoconjugate, and $C = 3.5 \text{nM}$ is the molar concentration of enzymes. The diffusion rate is estimated to be 37 molecule/sec. Therefore the DNA digestion we observed is not a diffusion-limited process (otherwise the digestion of ~100 dsDNA on a single nanoconjugate would finish in a few seconds). On the other hand, the rate constant of the nuclease reaction on the nanoconjugate ($5.8 \times 10^{-3} \text{s}^{-1}$ for higher concentrations of XhoI) is comparable to that in free solutions. This implies that the Au nanoparticle has minimal impact on the enzyme activity and serves only as a highly sensitive sensor.

**Calculation of the refractive index of dsDNA in different lengths**

The plasmon resonance wavelength of the Au-DNA nanoconjugate can be calculated using the Mie scattering theory. The pertinent variables include the dielectric constant of Au and the effective thickness and refractive index of the biopolymer shell (phosphine+dsDNA). We used the dielectric constant of Au at various wavelengths provided by Johnson and Christy, though their results are considered more appropriate for bulk or polycrystalline Au. We estimated the thickness of the phosphine layer to be 2 nm. The length of the dsDNA is calculated as 0.34 nm/bp. The length of the dsDNA, before cleavage and after cleavage by KpnI, Sall, XhoI, and HinDIII, are respectively 54, 48, 36, 24, 12, and 0bp, and therefore the biopolymer shell thickness are 18.36, 16.32, 12.24, 8.16, 4.08, and 2 nm, respectively. Using the calculation program for coated particle by Bohren and Huffman, the dielectric constants of the biopolymer shell in different thickness are found by way of trial and error. The equivalent refractive index can be considered to be simply the square root of the dielectric constant. The dependence of the dielectric constant of dsDNA on its length has been studied and the quadratic Langevin model to describe the relationship has been established. We fitted the calculated refractive index according to the following empirical formula $\varepsilon (l) = n^2 (l) = a [\coth (b l^2)-1/b l^2] + c$, where $a$, $b$ and $c$ are three fitting variables. Our calculation results agree with the results provided by Mulvaney very well, as listed below.
Figure S1. Cleavage of DNA with endonucleases. Test digestions of the 54bp dsDNA with restriction endonucleases HindIII, KpnI, XhoI, and SalI were performed according to the manufacturer’s instructions (New England Biolab, Beverly, MA). The cleavage sites for the various enzymes are shown in Figure 1b. Appropriate amounts of buffer and water were mixed along with 1 µL of each of the enzyme in 100 µL final reaction volume. The reaction was incubated overnight at 37 °C. The digestion was then verified by gel electrophoresis here on 5% NuSieve agarose gel in 1X TBE, with a 10 bp ladder as a molecular weight standard (Invitrogen, Carlsbad, CA). Note that the DNA shown in the gel is not tethered onto Au nanoparticles.
Figure S2. Gel electrophoresis of nanoconjugates. Shown are: (1) Au-phosphine (Au-ph) nanoconjugates in different phosphine/Au ratios. The Au nanoparticles were stabilized through surface exchange with Bis(p-sulfonatophenyl)phenylphosphine (phosphine) to prevent aggregations. Left lane is a control of Au nanoparticle without phosphine surfactants. For purpose of simplifying the writing, the Au-ph is called Au in the main text, since all the Au nanoparticles used in the study have the phosphine surfactant coating.
Figure S3. Confirmation of endonuclease cleavage. Same reactions as in Figure 3a were carried out and imaged with fluorescence imaging. Shown are: fluorescence images of Au-DNA nanoconjugates (1) before, (2) 1 hr and (3) 16 hr after the cleavage reaction by enzyme XhoI. The fluorescence intensities at 4 different areas (red squares) in each case were measured and the statistics of the fluorescence intensities are shown in (4) with the average fluorescence intensity of the single Au nanoconjugates in the above three cases. Note the full length of the complete dsDNA (~20 nm) is much longer than the effective Forster transfer distance (<10 nm), and thus the FITC fluorophore at distal end of DNA was not quenched. The FITC fluorophore was detached from the Au nanoparticles and diffused into the buffer solution after the DNA cleavage, so that the overall fluorescence intensity at the image plane (where the Au nanoparticles are) decreased dramatically.
Figure S4

Figure S4. Simulated scattering spectra of the Au-DNA nanoconjugates after cleavage by different endonuclease enzymes. The wavelength-dependent dielectric constant of Au is interpolated from the tabulated data by Johnson and Christy\textsuperscript{3}. The simulation code is modified from the BHCOAT by Bohren and Huffman\textsuperscript{4}.
Table S1

Table S1. Back-calculated dielectric constant and refractive index of the biopolymer shell layer of the Au-DNA nanoconjugate based on the experimental results of SPR wavelength. For the first set of calculated $\lambda$ and $n$, the dielectric constant of Au is provided by Johnson and Christy\(^3\), and the approximation equation* by Mulvaney\(^5\) is used in calculations. For the second set of calculated $\lambda$ and $n$, the dielectric constant of Au is also provided by Johnson and Christy\(^3\), and the simulation code derived from BHCOAT\(^4\) is used. For the third set of calculated $\lambda$ and $n$, the dielectric constant is provided by Weaver et al\(^6\), and the approximation equation by Mulvaney\(^5\) is used in calculations.

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\lambda^2 = \lambda_p^2 (\epsilon_\infty + 2\epsilon_m),
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where $\lambda$ is the wavelength, $\lambda_p$ is the bulk plasma wavelength of Au, $\epsilon_\infty$ is the dielectric constant of Au at ultrahigh frequency, and $\epsilon_m$ is the dielectric constant of the shell layer.

References