Polyvalent DNA Nanoparticle Conjugates Stabilize Nucleic Acids (Supporting Information)

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Experiment Design and Methods

Materials. All bases and reagents used to prepare oligonucleotides were purchased from Glen Research. Deoxyribonuclease I (DNase I) was purchased from New England Biolabs Inc. (cat. # M0303L). Turbo DNase was purchased from Applied Biosystems (cat. # AM2239).

Instrumentation. Fluorescence measurements were recorded on a Photon Technology International FluoDia T70 fluorescence plate reader. Zeta potential measurements were performed on a Malvern Instruments Zetasizer Nano.

Oligonucleotide synthesis. Oligonucleotides were synthesized on an Expedite 8909 Nucleotide Synthesis System (ABI) using standard solid-phase phosphoramidite methodology. All oligonucleotides were purified by reverse-phase high performance liquid chromatography (HPLC). The oligonucleotides sequences used in this study are:

Thiol-modified:	5'-CCCAGCCTTCCAGCTCCTTG-(**A)$_{10}$-propylthiol**-3'
Molecular:	5'-CCCAGCCTTCCAGCTCCTTG-**dabcyl**-3'
Complement:	5'-CAAGGAGCTGGAAGGCTGGG-**fluorescein**-3'

DNA-gold nanoparticle conjugates. Thiol-modified oligonucleotides (final concentration = 3.5 μM) were added to a 10 nM solution of 13 ± 1 nm gold NPs.[1] To form PEG-diluted conjugates, (1-mecaptoudec-11-yl)tri(ethylene glycol); final concentration = 60, 30, 15 or 7.5 μM) was added immediately after oligonucleotides.

After 12 hours, sodium dodecylsulphate (SDS), phosphate buffer (pH = 7.4), and sodium chloride were added to achieve final concentrations of 0.1 % SDS, 10 mM phosphate, and 0.1 M sodium chloride. Six additional aliquots of sodium chloride were added over an eight-hour period to achieve a final sodium chloride concentration of 0.3 M, and the mixture was shaken overnight. The particles were purified from unreacted materials by three successive rounds of centrifugation (16,000 rcf, 20 min), supernatant removal, and resuspension in phosphate buffered saline (PBS) (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4, Hyclone).

DNA loading determination. The concentrations of purified DNA-Au NPs were measured with a UV-vis spectrophotometer (λ = 524, ε = 2.7 x 10^8 $L \cdot mol^{-1} \cdot cm^{-1}$). The gold particles were treated with KCN solution (0.1 M) to oxidatively dissolve the particles and liberate the surface-coordinated oligonucleotides. Oligonucleotide concentration was determined using a commercially available single-stranded oligonucleotide quantification kit (Oligreen; Invitrogen) following the manufacturers' recommendations. Oligonucleotide loading was calculated by dividing the concentration of oligonucleotides by the concentration of nanoparticles.

Nuclease assay protocol. Fluorophore-labeled complementary sequences and DNA-functionalized nanoparticles were hybridized at 1,000 nM and 100 nM in PBST, respectively. For the dabcyl-labeled molecular system, both sequences were 1,000 nM. The solutions were heated to 70 ^{0}C and allowed to cool slowly to room temperature (~12 hours). A 5 μL aliquot of these samples was diluted in 85 μL of assay buffer (10 mM tris

(pH = 7.5), 2.5 mM $MgCl_2$, and 0.5 mM $CaCl_2$) and placed in a 96-well fluorescence microplate at 37°C. After allowing the sample to equilibrate (10 minutes), 10 μL of DNase I in assay buffer (2 units/L) was added. To prevent evaporation, the reaction was covered with 40 μL of mineral oil. The fluorescence of the sample (excitation = 490 nm, emission = 530 nm) was measured every 74 seconds for 12 hours. All samples were measured in triplicate. To determine the beginning and ending points for the reaction, fluorescence was measured from aliquots of reaction buffer without enzyme and highly concentrated DNase I (114.5 units/L, Invitrogen cat. # 18047-019, data taken after 300 min when the reaction is complete) (Figure S2). For the Lineweaver-Burk analysis, degradation reactions were conducted at DNA duplex concentrations of 500, 100, 50, 25, 20, 15, and 10 nM (from 5000 nM stock solutions). The initial reaction velocity was determined from the slope of the progress curve from the first 10 data points (740 seconds) after nuclease addition.

Zeta potential measurements. Purified, functionalized Au NPs were diluted in Nanopure™ water at a concentration of 1 nM. Measurements were performed at 25°C. Each sample was measured in triplicate.

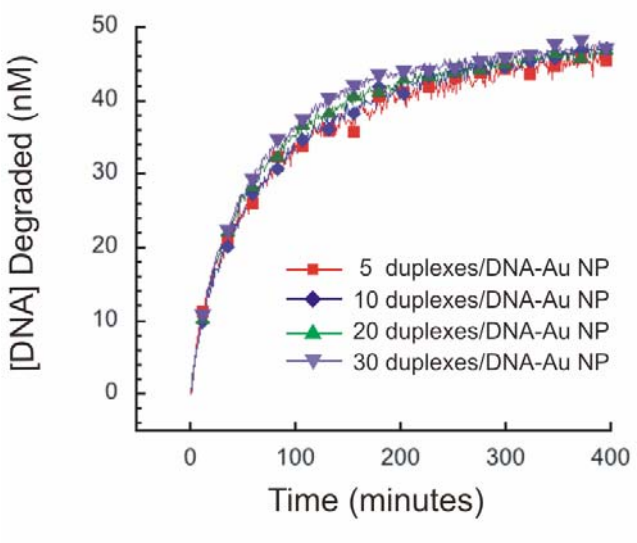

Figure S1. Comparison of the degradation rates of DNA-Au NP hybridized with 5,10, 20, and 30 duplexes per nanoparticle. The total fluorophore labeled duplex concentration is made similar by adjusting the concentration of nanoparticles.

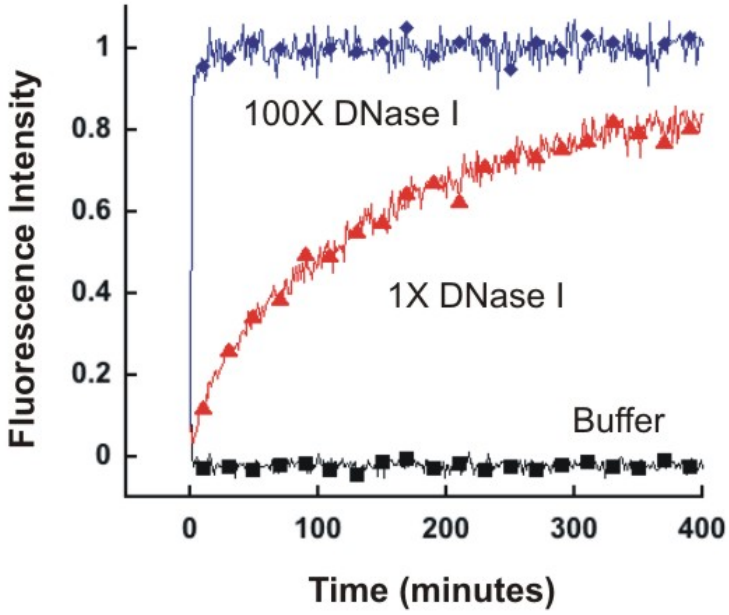

Figure S2. Fluorescence-based progress curve of nanoparticle conjugates (a representative nanoparticle functionalized with DNA is shown). A buffer curve (bottom) and a 100X DNase I curve (top) were used to establish the baseline and maximum fluorescence for each sample.

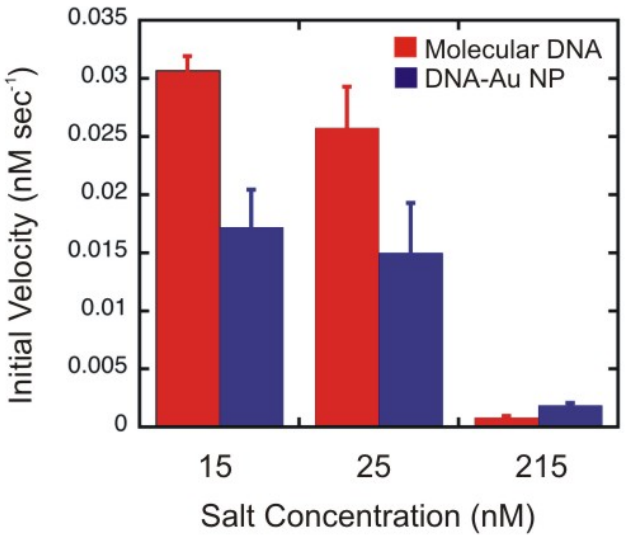

Figure S3. Initial degradation reaction velocity as a function of solution salt concentration for molecular DNA and DNA-Au NPs.

References

(1) Frens, G. *Nature: Phys. Sci.* **1973**, *241*, 20-22.