Supporting Information

Temperature-Programmed Assembly of DNA:Au Nanoparticle Bioconjugates

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Experimental Methods.

Bioconjugate preparation. All DNA:Au nanosphere bioconjugates were prepared by derivatizing the surface of 12-nm diameter Au spheres with thiolated DNA oligonucleotides as previously described.[1-3] For particles with only one oligonucleotide sequence (ie. **A_9:Au**), 5 nanomoles of thiolated DNA was added to 950 μl of 12-nm Au colloid (17 nM). For the mixed-oligonucleotide particles (ie. **$C_{9/18}$:Au**), 2.5 nanomoles of each sequence were mixed before addition to the colloid solution. The solution was placed in a heat block at 37ºC for 30 minutes. Salt was added to bring the solution to 0.1 M NaCl, 10 mM phosphate pH 7. The conjugates were then allowed to "age" for at least 1.5 h before removing excess DNA by centrifugation. The conjugates were spun down twice using a Biofuge Pico centrifuge (Heraeus) at ~12,300 g for 35 minutes. After each centrifugation, the supernatant was removed, and the soft pellet containing the Au particles resuspended in 0.3 M NaCl, 10 mM phosphate pH 7.0 buffer.

Bioconjugate hybridization. All hybridizations were carried out in a high salt buffer (0.3 M NaCl, 10 mM phosphate buffer, pH 7.0). Equimolar amounts (1.3 picomoles each) of each purified conjugate were added to an eppendorf tube along with 1.5 nanomoles of each linking oligonucleotide (**A_9'C_9'** and **B_{18}'C_{18}'**). The mixture was left to hybridize at either 25ºC or at 65ºC overnight. For hybridization of the two-strand systems, in order to avoid cross hybridization, samples were briefly heated to 90ºC before allowing them to cool to 65ºC or 25ºC.

Thermal denaturation. All thermal denaturation curves were taken using 1ºC steps and holding at each temperature for five minutes. The 3-strand samples were heated from 25ºC to 80ºC in a microcuvette with a push-in cap. A thin layer of mineral oil was added to the top of the solution to help prevent evaporation of the solvent. Melts of the 2-strand samples were carried out in a larger cuvette with a screw-top cap. The larger volume and better sealing of these cuvettes were necessary to prevent evaporation, since these samples were heated to 90ºC.

Gel electrophoresis. Agarose gels for DNA:Au bioconjugate separations were prepared using 3% Agarose 1000 in 0.5X TBE buffer (44.5 mM Tris, 44.5mM boric acid, 1mM EDTA). Separations were carried out at a constant potential of 100 V for 2 h.

Fluorescence determination of DNA loss at 65ºC. Ten batches of **C_{18}f:Au** were prepared as described above. Two 400-µl aliquots were removed from each batch. One aliquot from each batch was heated to 65ºC, while the other was left at room temperature. After heating for 18 or 6 h, all samples were washed once by centrifugation to remove any DNA that had been lost to the solvent. The fluorescent DNA was then displaced from the particles using 12 mM mercaptoethanol,[1,4] and the DNA coverage was quantified using a SPEX Fluorolog 3 fluorimeter.

Kinetic Trace. Equimolar amounts (4.6 picomoles each) of **A_9:Au**, **B_{18}:Au**, and **$C_{9/18}$:Au** were added to a 4-ml cuvette, along with 0.8 nanomoles of each linking oligonucleotide (**A_9'C_9'** and **B_{18}'C_{18}'**). The sample was diluted to 4 ml with the hybridization buffer. This concentration was necessary for the initial absorbances to be within the limits of the detector. Spectra of the sample were taken once every ten minutes during hybridization over a 12-hour period.

Fluorescence determination of DNA exchange at 65ºC. Ten batches of **A_9:Au** were prepared as described above. Each batch was split into two samples that were 500 µl of a 6.64 x 10^{-9} M conjugate solution. The fluorescent, thiolated oligonucleotide **F_{10}** (1.66 x 10^{-10} moles) was added to both samples. One of these samples was heated to 65ºC, while the other was left at room temperature. After 6 h, all of the samples were washed by centrifugation to remove excess DNA. The fluorescent DNA was then displaced from the particles using 12 mM mercaptoethanol,[1,4] and the coverage of fluorescent DNA was quantified.

Identification of bioconjugates remaining in solution after 65ºC hybridization. Equimolar amounts (500 µl of 2.01x10^{-8} M) of **A_9:Au**, **B_{18}:Au**, and **$C_{9/18}$:Au** were added to an eppendorf tube along with 1.0 nanomoles of each linking oligonucleotide. The absorbance of the mixture (50 µl diluted to 200 µl) was measured, and the remaining mixture was the split into six identical samples (240 µl each), which were allowed to hybridize at 65ºC for 6 h. The absorbance of each of the six samples was measured, and a different sequence of free, non-thiolated DNA was then added to each sample. Buffer (15.34 µl) was added to Sample 1 as a control. Oligonucleotides **A_9'**, **B_{18}'**, **C_9'**, **C_{18}'**, and **N_9** (1.7 nanomoles, with a volume of 15.34 µl each) were added to Samples 2-6, respectively. Note that, in order to compete

with the linking oligonucleotide that should still be in solution after the 65ºC hybridization, the amount of DNA added in this step is an order of magnitude greater than the linker in each sample. The samples were photographed and then allowed to hybridize (with either the linking oligonucleotide or the complementary sequence) overnight. After this second hybridization step, the samples were photographed again, and the absorbance of each sample was measured.

For the case in which the aggregate is removed from the samples, the initial sample is prepared, and the absorbance was measured just as the sample above. This entire sample was allowed to hybridize at 65ºC for 6 h. After the absorbance of this sample was measured, six 100-µl were removed and placed into new tubes. Buffer (7.44 µl), complementary DNA and non-complementary DNA (0.77 nanomoles, with a volume of 7.44 µl each) were added to Samples 1-6 as above. The samples were allowed to hybridize overnight, and then the absorbance of each sample was measured.

(1) Pena, S. R. N.; Raina, S.; Goodrich, G. P.; Fedoroff, N. V.; Keating, C. D. *J. Am. Chem. Soc.* **2002**, *124*, 7314-7323.

(2) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078-1081.

(3) Mucic, R. C.; Storhoff, J. J.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 12674-12675.

(4) Demers, L. M.; Mirkin, C. A.; Mucic, R. C.; Reynolds, R. A., III; Letsinger, R. L.; Elghanian, R.; Viswanadham, G. *Anal. Chem.* **2000**, *72*, 5535-5541.

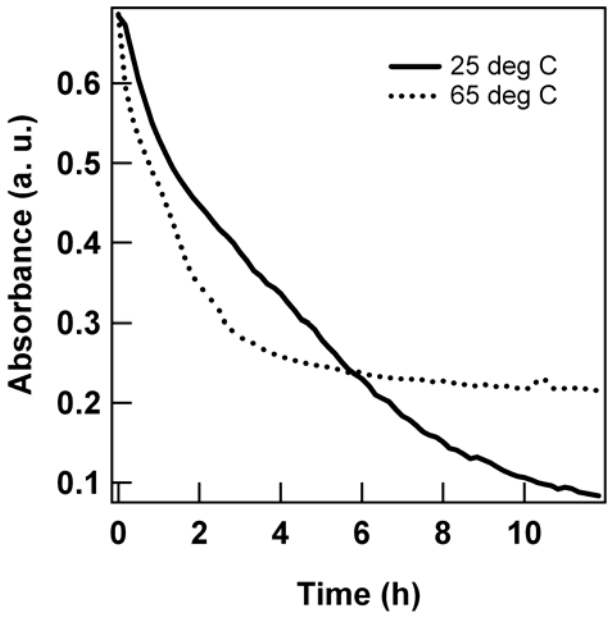

Figure S1. Kinetic trace of absorbance at 520 nm as the 3-strand TPA sample is allowed to hybridize over 12 h. Spectra were taken once every ten minutes.