Two-dimensional Nanoparticle Arrays Show the Organizational Power of Robust DNA Motifs

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This supplementary information contains the experimental methods used in this work. It also contains three figures, S1, with the sequences used, S2, which illustrates the purification of nanoparticles with a single strand attached, and S3, which illustrates the purification of a 3D-DX triangle with a gold nanoparticle attached.

Experimental Section:

DNA sequences were designed using *SEQUIN*,[1] were synthesized by standard phorphoramidite techniques[2] and purified from denaturing polyacrylamide gels. 3D-DX triangle DNA molecules and its 2D assembly were constructed by annealing stoichiometric mixtures of the strands (estimated by OD_{260}) to a concentration of 0.5 μM in a buffer solution containing 10mM HEPES, 1mM EDTA, 3.5mM $MgCl_2$ and 100mM NaCl from 90 ℃ to room temperature.

Gold colloids with mean diameters of 5 and 10 nm were purchased (Ted Pella). Citrate-stabilized gold colloids were subsequently passivated with a monolayer of anionic phosphine molecules as described elsewhere. [3] The colloidal solution was concentrated up to the micromolar range after phosphine coating. ssDNA/Au conjugates were prepared by mixing gold nanoparticles with 5'-thiolated (-SH) ssDNA in a mole ratio of 3:1 and incubated for 2h in a buffer containing 10mM HEPES, 1mM EDTA and 50mM NaCl (HEPES buffer). The Au nanoparticles tethered with single DNA strands were purified by gel electrophoresis (3% agarose gel at 5V/cm, HEPES buffer 10mM HEPES, 1mM EDTA), and then recovered by cutting and extracting the appropriate band.[3] ~100uL red-color solution was collected and then diluted to a final volume of 500μL in a solution containing 100mM Na and HEPES buffer. After further incubation for 5h the volume was slowly reduced to 100μL by vacuum centrifugation at room temperature. This process produces a gradual increase in ionic strength, which leads to much more stable DNA/Au conjugates.

The 3D-DX Triangle DNA/Au conjugate was prepared by mixing the ssDNA-Au strands and other 21 component DNA strands in HEPES buffer for overnight annealing from 75 ℃ to room temperature. The final reaction volume was 50μL and the concentration of each oligonucleotide was 0.5μM, with the except for the Au/DNA

conjugates, which were present at 0.8μM. The 3D-DX triangle DNA/Au conjugates were purified and collected following the same procedure as above.

To form the array, the two conjugates were mixed in stoichiometric quantities, warmed to 45 ℃, and cooled slowly to room temperature in a 2L water bath in a Styrofoam box over 24h. The low initial temperature was used to ensure the stability of Au/DNA conjugate. Following this incubation, visualization of the particles was carried out by transmission electron microscope (TEM).

TEM imaging was performed using a Philips CM-10 instrument operated at 80kV. The particle sample was prepared on 400 mesh formvar-coated copper grids by dropping 5μL sample solution on grids and then wicking off excess solution using filter paper after 30s. All grids were dried in a desiccator at least overnight.

References for supplementary material.

1. Seeman, N. C. *J. Biomol. Str. & Dyns.* **1990** *8,* 573-581.
2. Caruthers, M. H. *Science* **1985** *230*, 281-285.
3. (a) Zanchet, D.; Micheel, C.; Parak, W. and Alivisatos, P. *Nano Lett.* **2001** *1*, 32-35. (b) Zanchet, D.; Micheel, C.; Parak, W. and Alivisatos, P. *J. Phys. Chem. B* **2002** *106*, 11758-11763.

Supplementary Figures

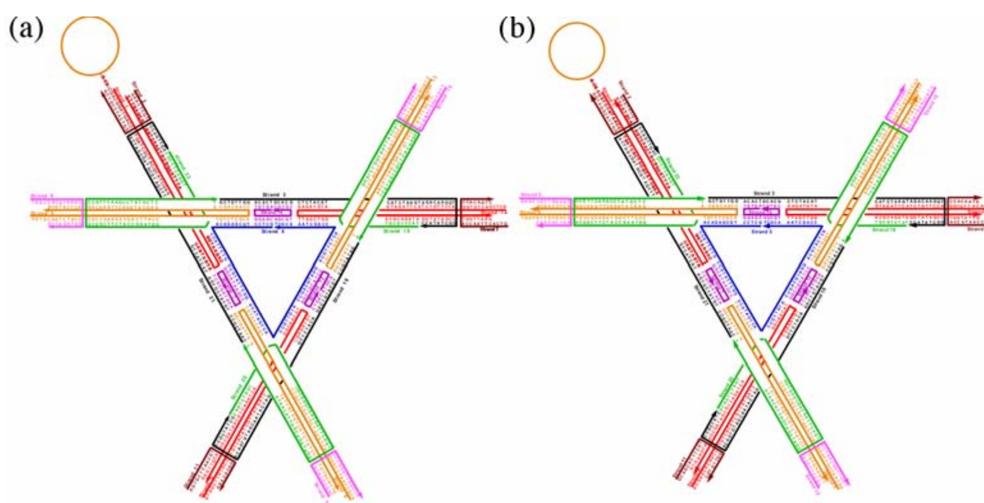

Figure S1. *The Sequences of the DNA Motifs Used in this Work.* The helices have been unwound and the strands are color-coded to match those in Figure 1. The thiol-attached gold nanoparticle is shown as a **labeled** gold-colored circle. 3' ends are indicated by arrowheads. The two molecules are illustrated.

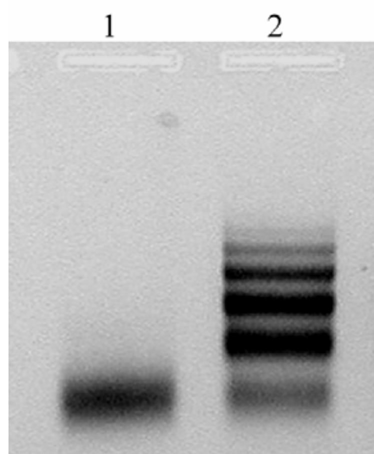

Figure S2. *Purification of Nanoparticles Attached to a Single DNA Strand.* This is a 3% agarose gel. Lane 1 contains 5 nm gold nanoparticles; Lane 2 contains a crude preparation of 5nm nanoparticles to which the thiolated DNA strand of the 3D-DX triangle has been attached.

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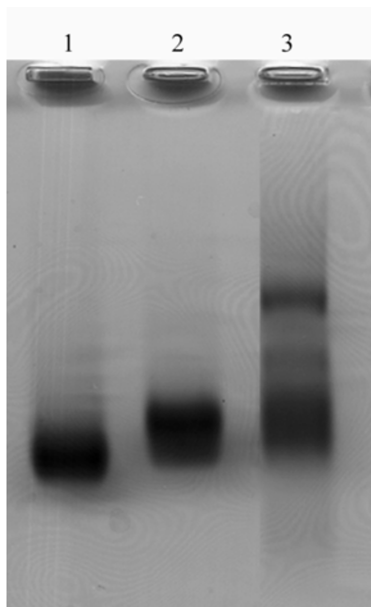

Figure S3. *Purification of the 3D-DX Triangle Containing a Single Gold Nanoparticle*. This is a 2.5% agarose gel. Lane 1 contains 5nm gold nanoparticles; Lane 2 contains 5nm nanoparticles derivatized with a single DNA strand; Lane 3 contains a crude preparation of 5nm nanoparticles to which the 3D-DX triangle has been attached. The dark band about 60% from the top contains the 3D-DX triangle attached to the gold nanoparticle.