

PH-driven Conformational Change of “i-motif” DNA for Reversible Assembly of Gold Nanoparticles

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Preparation of Au-DNA conjugates

Colloidal gold solution (15 nm) was purchased from Ted Pella Inc. and 3'-thiol modified DNA oligonucleotides were purchased from Integrated DNA Technologies (www.idtdna.com) and purified using 10% denaturing PAGE in 1X TBE buffer (89 mM Tris base, 89 mM Boric acid and 2 mM EDTA, pH 8.0) and their concentrations were estimated by OD at 260 nm. The two DNA-Au NP conjugates, NP1 and NP2, were prepared in separate tubes, by mixing the 3'-thiol modified DNA strands with the Au NPs in the ratio of 250:1 and stirred for 24 hrs. In each tube, PBS buffer (10 mM, 0.1 M NaCl, pH 8.0) was added to the mixture and stirred for another 24 hrs. Salt concentration was increased to 0.15 M and stirred for another 24 hrs. The mixture was centrifuged at 13,200 rpm for 30 minutes. The supernatant was removed and the red oily precipitate was washed with PBS buffer (10 mM, 0.15 M NaCl, pH 8.0) to remove excess of oligonucleotides, and finally the precipitate was redispersed in PBS buffer containing 0.3 M NaCl.

The two 3'-thiol modified strands used are:

5'-CCC TAA CCC TAA CCC TAA CCC TTT TTT TTT-SSH-3'

5'-TGT TAG TGT TAG TGT TAG TTT TTT TTT-SSH-3'

Where the red colored bases are the mismatches.

Hybridization confirmation The two 3'-thiol modified oligonucleotides were mixed in a 1:1 ratio at 1 μ M concentration in 1X TAE Mg²⁺ buffer (40 mM Tris base, pH 8.0, 20 mM EDTA and 12.5mM Mg(Ac₂)) and annealed by heating at 45 °C for 10 min, 37°C for 15 min, 22 °C for 20 min, and 4 °C for 2 hrs. A 12% Native PAGE gel was run to confirm the hybridization between two strands with 3 mismatches. A single band after annealing confirms the complete hybridization between the two strands.

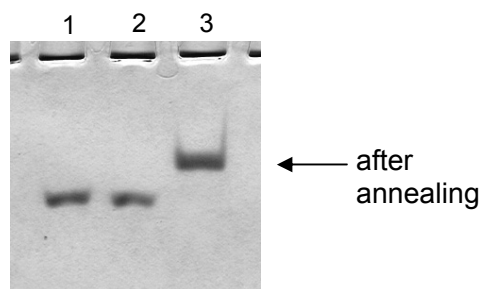


Figure S1. 12% non-denaturing gel showing the hybridization between the two DNA strands. Lane 1 and lane 2 are the two individual DNA strands and lane 3 is the 1:1 mixture after annealing.

Nanoparticle assembly and Reversibility of the sensor

The two Au-DNA conjugates, NP1 and NP2, were mixed in PBS buffer (10 mM, 0.3 M NaCl, pH 8) to a final concentration of 3 nM each. The color of the solution gradually changed from red to blue within 20 minutes. 100 μ L of the solution was separated out (pH 8 cycle 1). The pH of the solution was changed to \sim 5 by adding aliquots of 1 M HCl. Color of the solution was changed back to red in 1 minute due to quick access of H⁺ to DNA. Again, 100 μ L of the solution was separated out (pH 5 cycle 1), and the pH of the rest of the solution was changed to \sim 8 by adding 1M NaOH. Stock solutions of NP1 and NP2 were added to keep the final concentration of Au-DNA conjugates 3 nM each (to compensate for the dilution due to addition of the acid and base solutions). Several similar cycles were done to show the reversibility of the sensor with obvious color change. UV absorbances were taken after every step. A picture of the tubes after each pH change was taken using a digital camera to report the color change with the changes in pH.

Control Experiment

Two 3'-thiol modified DNA oligonucleotides with random sequences were used to confirm the specificity of “*i motif*”. DNA-Au conjugates were prepared as described and were mixed at pH \sim 8. The aggregates show a purple color. Upon addition of 1 M HCl, there was no apparent color change. This indicated the sequence specificity of the “*i motif*” for the conformational change with pH. UV-vis spectra were taken after mixing of DNA-Au conjugates and addition of HCl. The sequences used in the control experiment are:

Control-1: 5'-TGT GTC CAG TCG ATA GAT CCA TTT TTT TTT-SSH-3'

Control-2: 5'-TGA TCT TTC GAC AGG ACA TTT TTT TTT-SSH-3'

in which the red colored bases are the mismatches in the duplex.

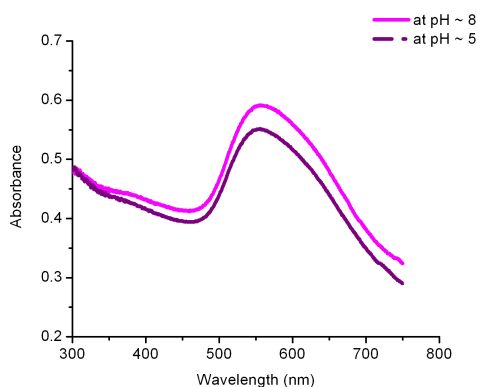


Figure S2. UV-vis spectra showing no spectral shift after mixing the control DNA-Au NP conjugates at pH \sim 8 and then change pH to \sim 5.