Supporting Information for

Journal: Chemical Communications

Manuscript ID:

Title: "Facile and rapid manipulation of DNA surface density on gold nanoparticles using mononucleotide-mediated conjugation."

Author(s): Wenting Zhao,[a] and I-Ming Hsing*[a,b]

Address: Bioengineering Graduate Program[a] and Department of Chemical and Biomolecular Engineering[b], the Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong SAR

*** Corresponding author:** I-Ming Hsing, E-mail: kehsing@ust.hk, Telephone: (852) 2358-7131, Fax: (852) 3106-4857.

Supporting Information

I. Experimental Details.

Materials. 13 nm Au-nps were prepared by reduction of tetrachloroaurate as reported previously [1]; while hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \bullet 3H_2O$), sodium citrate, adenosine triphosphate (ATP) and poly(L-lysine) were purchased from Sigma. *O*-(2-Carboxyethyl)-*O'*-(2-mercaptoethyl)heptaethylene glycol (cited later as thiol-oligo(ethylene glycol)) was from Aldrich. Oligonucleotides were synthesized by Integrated DNA Technologies. Other salts were provided by USB Corporation. The water used in all experiments was purified with NANOpure Diamond TOC Analytical Ultrapure Water System (Barnstead, U.S.A.) and autoclaved by ES-215/ES-315 Autoclaves (Tomy, Japan) prior to use.

103bp thiolated double-stranded DNA molecules (Thiol-103bp) are generated by the polymeric chain reaction (PCR) of bacteriophage M13 vector with one thiolated primer (thiolated reverse primer is 5'-thiol-C6-CAG GAA ACA GCT ATG AC- 3', and forward primer is 5'-GTA AAA CGA CGG CCA G-3'). The PCR product is further purified by PCRquick-spin™ PCR Product Purification Kit and the resulting concentration of purified 103bp-dsDNA is determined by measuring the absorbance at 260 nm.

Conjugation of DNA/Au-nps. The procedure for mononucleotide-mediated conjugation of DNA/Au-nps was as reported previously.[2] In brief, 100 μL citrate-stabilized Au-nps were first incubated with ATP in a molar ratio (ATP/Au-nps) of 1000 for 15 min. The mixture was then brought to 10 mM sodium phosphate buffer (pH 8.0) and a specific concentration of NaCl as described in the study. After brief vortexing, thiol-DNA (thiol-T30, 5'-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT-C3-thiol-3', 500 μM; or thiol-A30, 5'-AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA-C3-thiol-3', 500 μM) was introduced in a molar ratio (thiol-DNA to Au-nps) of 500, and the stopping reagent (thiol-T5, 5'-TTT TT -C3-thiol-3'; or thiol-oligo(ethylene glycol) (OEG) was added into the mixture in a molar ratio (OEG to Au-nps) of 1000 and incubated for 15 min unless specified otherwise. It should be

noted that the ratio of thiol-DNA to Au-nps can be reduced accordingly for the low-density conjugation. Afterwards, the particles were washed three times in 10 mM sodium phosphate buffer (pH 8.0) through centrifugation (13,200 rpm, 20 min) to remove excess reagents. Finally, the resulting conjugates were resuspended in 100 μL sodium phosphate buffer (10 mM, pH 8.0). For BSPP coating approaches, the procedure is the same as previously reported. [3]

Nano-assembly of DNA/Au-nps conjugates. Conjugates with complementary sequences (thiol-T30, 5'-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT-C3-thiol-3'; and thiol-A30, 5'-AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA-C3-thiol-3') were prepared using ATP-mediated approach, where two NaCl concentration groups (0 mM and 50 mM) were compared with OEG introduced at a series of time point (0 min, 5 min, 10 min, and 20 min). Hybridization was performed by mixing equal moles of two conjugates overnight in 10 mM sodium phosphate buffer with 0.1 M NaCl (pH 8.0). The resulting nano-assembly mixtures were probed by gel electrophoresis where dimer and trimer bands were imaged with TEM.

Gel electrophoresis of DNA/Au-nps conjugates. The gel electrophoresis was performed in 3% agarose gel with 1X TBE as running buffer. Conjugate with shorter DNA was run for 60 min in 5 V/cm electric field, while conjugate with 103bp DNA and nano-assembly mixtures were run for 120 min.

Fluorescence-based quantification of the DNA loading density on Au-nps. Centrifugation pellets of 300 μl TET-T30-thiol/Au13 conjugates were incubated overnight with 50 μl mercaptoethanol (ME) (12 mM in 0.3 M PBS). The solution containing displaced TET-T30-thiol was separated from the Au-nps by centrifugation. The fluorescence signal of each sample was measured using Applied Biosystems 7300 Real-Time PCR System. Standard curves were prepared to convert the fluorescence reading to molar concentration.

Transmission Electron Microscopy (TEM). TEM were performed in a JEM 2010 transmission electron microscope (JEOL) operated at 200 kV. Images were taken by GATAN MSC 794 CCD

Camera and analyzed with *GATAN Digital Microscopy* software. The carbon-nonporous film supported by copper grid (SPI® Supplies Inc., 400 mesh) was pre-treated by 0.01 % poly (L-lysine), and then was inserted into the gel where the front edge of the desired bands was sharply cut with a surgical knife. By continuing to run the gel for another 10 min, Au-nps assemblies were transferred to the grid for inspection.[4]

II. Gel Electrophoresis Comparison of BSPP Coating Approach and ATP-Mediated Approach.

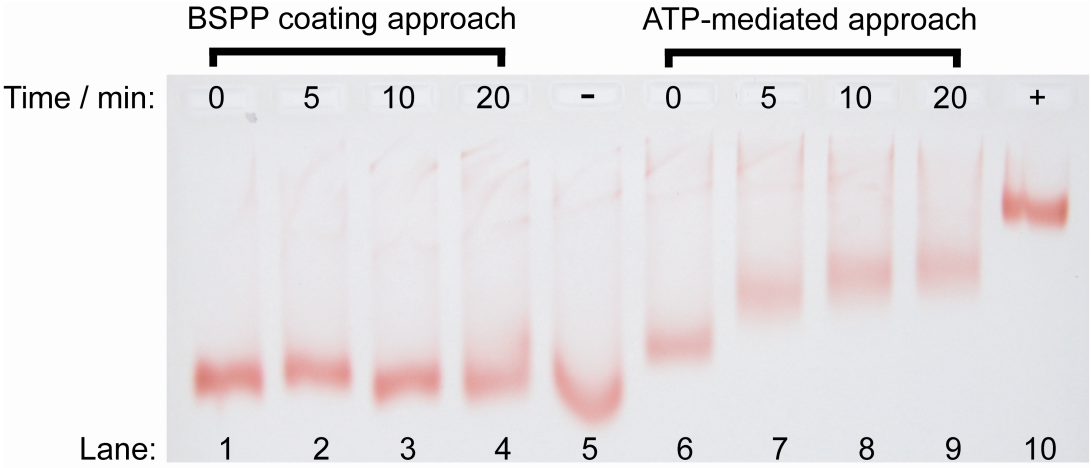

Figure S1. Gel electrophoresis comparison of thiol-T30/Au-nps conjugated through BSPP coating approach (Lane 1 to 4) and ATP-mediated approach (Lane 6 to 9) for different time (0 min, 5 min, 10 min, 20 min as shown from left to right in each approach group). The NaCl concentration in ATP-mediated approach is 50 mM. BSPP coated Au-nps without mixing with thiol-DNAs were served as negative control (marked "-"); while thiol-T30/Au-nps conjugated by ATP-mediated approach in 100 mM for 20 min were used as positive control (marked "+").

III. Gel Electrophoresis Validation of OEG Replacing Effect.

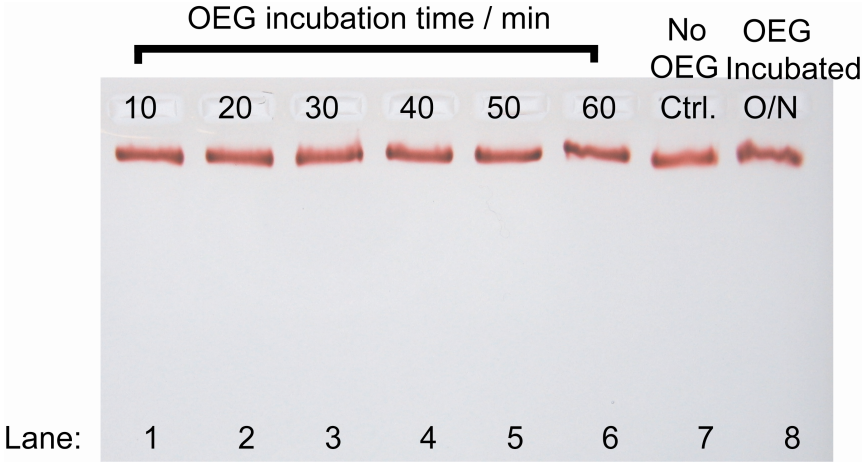

Figure S2. Gel electrophoresis of thiol-T30/Au-nps conjugates incubated with OEG for a series of times. Lane 1 to 6 represent for the incubation for 10 to 60 min, while Lane 8 is overnight incubation. Conjugates of thiol-T30 and Au-nps without any OEG incubated serve as control in Lane 7. No significant replacing effect of thiol-DNA by OEG was indicated as the mobility of conjugates didn't increase along with OEG incubation.

IV. References.

1. J. Turkevich, P. S. Stevenson and J. Hillier, *Discussions Faraday Soc.*, 1951, **11**, 55-75; G. Frens, *Nature Phys. Sci.*, 1973, **241**, 20-22.
2. W. T. Zhao, L. Lin and I. M. Hsing, *Bioconjugate Chem.*, 2009, **20**, 1218-1222.
3. C. J. Loweth, W. B. Caldwell, X. G. Peng, A. P. Alivisatos and P. G. Schultz, *Angew. Chem., Int. Ed.*, 1999, **38**, 1808-1812; S. A. Claridge, S. L. Goh, J. M. J. Frechet, S. C. Williams, C. M. Micheel and A. P. Alivisatos, *Chem. Mater.*, 2005, **17**, 1628-1635.
4. M. Hanauer, S. Pierrat, I. Zins, A. Lotz and C. Sonnichsen, *Nano Letter*, 2007, **7**, 2881-2885.