Supporting Information for

Controlled Spacing of Cationic Gold Nanoparticles by Nano-Crown RNA

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Materials and Methods

Preparation of DNA templates for RNA in vitro transcription

DNA has been purchased from Integrated DNA Technologies, Inc (www.idtdna.com). DNA templates for in vitro transcription were generated by amplifying a synthetic DNA molecule (RNAn.tmp), coding for the antisense sequence of the desired RNA molecule, with a reverse primer (RNAn.rev) and a forward primer (RNAn.fwd) containing the T7 RNA polymerase promoter. The forward and reverse primers were designed to hybridize to the template sequence (RNAn.tmp) with *Tm* ~54 °C. Typical PCR reactions were carried out in a total volume of 200 µL and contained 2 mM $MgCl_2$, 50 mM KCl, 10mM Tris pH 8.9, 0.5% NP-40, 1 mg/mL gelatin, 0.5 mM of each dNTP, 2 nM of RNAn.mat, and 1 µM RNAn.fwd and RNAn.rev. The reactions were calibrated to produce 150 pmol of DNA template after 20 cycles (94 °C for 1 min 15 s; 53 °C for 1 min 15 s; 72 °C for 1 min 15 s). PCR products were purified using the QiaQuick PCR purification kit from QiaGen.

Preparation of RNA molecules and self-assembling RNA ladders

RNA molecules were prepared in vitro by run-off transcription of DNA templates using T7 RNA polymerase. 90 pmol of each DNA template was incubated for 4 h at 37°C with T7 RNA polymerase (4 U/µL) in a buffer containing 10 mM $MgCl_2$, 2 mM spermidine, 50 mM Tris pH 7.5, 2.5 mM of each NTP, 1 mM DTT, 0.01 U/µL inorganic pyrophosphatase and 0.8 U/µL RNasin. After the reaction, the DNA templates were degraded by incubating with FPLC pure RQ1 DNase (0.4 U/µL) for 30 min at 37 °C. The RNA products were purified using denaturing polyacrylamide gel electrophoresis (10% acrylamide, 8 M urea). After elution overnight at 4°C in (200 mM NaCl, 10 mM Tris pH 7.5, 0.5 mM EDTA), the RNA was ethanol precipitated, rinsed twice with 90% ethanol, dried and dissolved in water.

Optical Densities of all the RNAs were determined by the 260 nm absorbance measurements done on a 1/70 diluted sample in a 70 µL quartz cuvette. Once the concentration of all the RNAs was established, a 1:1:1:1 mixture of 200 nM monomers was mixed for supra-molecular assembly of tetramers. The mixtures were first heated at 90 ^{o}C for 1 min, immediately cooled on ice for 3 min, and then incubated at 30^{o}C for 2 min. RNA tetramers were assembled at 30^{o}C for 30 min in 89 mM Tris borate buffer (pH = 8.3), 0.2 mM of $Mg(OAc)_2$ and 50 mM KCl and then cooled on ice.

The tail connectors used to link the tectosquares are thermodynamically less stable by at least two orders of magnitude than the loop-loop interactions that promote the self-assembly of the four different RNA units into a tectosquare. Thus, by varying the magnesium concentration at constant room temperature, it is possible to monitor the hierarchical assembly of RNA. At room temperature, 0.2 mM magnesium will only

promote RNA assembly through loop-loop interactions, whereas 15mM magnesium will allow assembly through tail-tail as well as loop-loop connectors. Squares form through loop-loop interactions in 0.2 mM magnesium buffer at 30°C.[1] Ladders assemble through tail-tail interactions by cooling a solution of squares from 50°C to 4°C overnight on a freshly cleaved AFM mica in 89 mM Tris borate buffer 15 mM $Mg(OAc)_2$ and 50 mM KCl. 40 ul of 5 nM solution of two squares was deposited on the surface. To avoid condensation and allow a good control of the temperature mica discs and TEM grids were placed in a 50 mL Falcon tube and placed inside a Styrofoam box filled with water.

Gold nanoparticles preparation

Hexadecylamine-stabilized gold nanoparticles with a diameter of 3.5 ± 1 nm were first prepared using a two phase synthesis system.[2] Then a ligand exchange reaction was performed to coat the nanoparticles with thiocholine, possessing a positively charged ammonium group, which gives a positive charge to the ligand shell of the nanoparticle and making the overall size of the nanoparticle to be 5 ± 1 nm.

3.5 nm thiocholine Au nanoparticle preparation: The synthesis of alkylamine stabilized Au nanoparticles is based off a previous publication.[2] A 25mL aqueous solution of 112mg (0.284mmol) $HAuCl_4$:3H2O was combined with 0.749g (3.10mmol) hexadecylamine (HDA) in 50mL toluene and stirred rapidly. A reducing solution of 0.165g (4.36mmol) $NaBH_4$ in 25mL water was added and the mixture was stirred overnight. The organic phase was removed and reduced to ~15mL, followed by the addition of 350mL 95% ethanol. This mixture was stored overnight at -80°C to precipitate HDA-Au nanoparticles. Solids were isolated by centrifugation at 4°C and washing three times with 200-proof cold ethanol. Following the last centrifugation the

solids were lightly dried and dissolved in a minimum amount of $CHCl_3$ and centrifuged several times at 14000 rpm to remove insoluble material. The solvent was evaporated and the nanoparticles were redispersed in CH_2Cl_2. The resulting solution contained ~15mg/ml of HDA-Au product. The dark burgundy solution of nanoparticles in CH_2Cl_2 underwent a biphasic ligand exchange reaction (CH_2Cl_2/aqueous) by stirring with excess aqueous 30mM thiocholine 500mM NaCl solution, which was prepared by base hydrolysis of acetylthiocholine chloride (Sigma). Over a period of several hours the color transferred completely to the aqueous layer, similar to the ligand exchange of triphenylphosphine-stabilized 1.5 nm Au NP with an aqueous thiocholine solution.[3] The aqueous layer was removed, washed twice with CH_2Cl_2 and dialyzed against nanopure water over a two day period with three water exchanges. The nanoparticle solution was then diluted with filtered nanopure water and stored in the refrigerator. Characterization by TEM showed the average size of thiocholine Au (tcAu) to be 3.5 ± 1nm (Figure S3).

Preparation of linear double stranded DNA

A derivative of pHSHW5 plasmid was prepared. pHSHW5 was transformed and expressed in ER1727 bacteria cells. The plasmid was isolated using "QIAprep Spin Miniprep Kit" from Qiagen according to manufacturer specifications. Plasmid DNA was precipitated with ethanol and resuspended in 10 mM/1 mM Tris-EDTA buffer for concentration determination through absorbance at 260 nm. Plasmids were digested to completion with the restriction enzyme EcoRI, for which there is a single site in the plasmid. Digested DNA was purified using multiple phenol/chloroform and ethanol precipitations DNA, and resuspended in 50 mM phosphate buffer at pH 7.4. A stock solution containing (40 ng/uL) DNA was diluted to (1ng/uL) immediately before use by

dilution of 5 uL stock into 195 uL of 20 mM bis-tris propane buffer at pH 9.0. Silicon wafers with an oxide layer functionalized with hydrophobic 3-aminopropyldimethylethoxysilane were dipped for 10-15 seconds in this solution, and pulled through the liquid-air interface to stretch DNA through molecular combing. Samples were incubated with 3.5nm thiocholine Au NPs for 5 min, washed with 5 drops of nanopure water and dried under a nitrogen stream. Analysis by AFM took place under ambient conditions.

Decoration and visualization of RNA and DNA assemblies

5 uL of 40 nM solution of 3.5 nm thiocholine Au nanoparticles were deposited into the RNA solution on top of the AFM mica and TEM grids (Ted Pella). Gold nanoparticles were left to equilibrate and settle for five minutes. AFM mica and TEM grid were washed with 1 mL of 15 mM Mg 50 mM KCl 1X TB buffer (pH 8.3) and 1 mL of millipore water. Substrate was then wicked with a Kimwipe and dried with nitrogen.

AFM imaging was performed with Nanoscope IV and Dimension 5000 (Digital Instruments) using tapping mode in air. Silicon tips (MULTI 40 from NanoDevices) without reflective coating had a resonant frequency of 60 kHz and spring constant of 1 N/m. TEM imaging was performed with a FEI Tecnai G2 Sphera Microscope operated at 200 kV.

AuNPs bind isolated squares. As determined by UV-Vis spectroscopic method, we have observed a shift in one of the peaks of absorption from 520 nm to 530 nm after binding of RNA squares to the gold. However, as the association is very efficient, the concentration range of gold and RNA allowing accurate measurement is still too high for determining a Kd. We can only conclude that the Kd is below 60 nM.

Supporting Tables

Table 1. List of DNA oligonucleotide sequences used to generate PCR templates for transcription. Oligo.tmp is amplified with oligo.fwd and oligo.rev.

Forward primer for S211abcd and S212abcd : TTC TAA TAC GAC TCA CTA TAG GGA AAG CCT GGA TGA AG

S211a.tmp GCC TCG TAG TGT CCA CTT CTA CGA GAC TTG CCT GGA CGT GCC TCC TTC ATC CAG GCT TTC CC

S211a.rev CTC TGG TTT TGC CTC GTA GTG TCC ACT T

S211b.tmp GCC TCG TAG TGT GGA CTT CTA CGA GAC TTG CCT GGA CGT GCA GGC TTC ATC CAG GCT TTC CC

S211b.rev CGG TGA TTT TGC CTC GTA GTG TGG ACT T

S211c.tmp GCC TCG TAG TGC TCG CTT CTA CGA GAC TTG CCT GGA CGT GCC TGC TTC ATC CAG GCT TTC CC

S211c.rev GGA TGC TTT TGC CTC GTA GTG CTC GCT T

S211d.tmp GCC TCG TAG TGC GAG CTT CTA CGA GAC TTG CCT GGA CGT GGA GGC TTC ATC CAG GCT TTC CC

S211d.rev CCT GTC TTT TGC CTC GTA GTG CGA GCT T

S212a.tmp GCC TCG TAG TGT CCA CTT CTA CGA GAC TTG CCT GGA CGT GCC TCC TTC ATC CAG GCT TTC CC

S212a.rev TCA CCG TTT TGC CTC GTA GTG TCC ACT T

S212b.tmp GCC TCG TAG TGT GGA CTT CTA CGA GAC TTG CCT GGA CGT GCA GGC TTC ATC CAG GCT TTC CC

S212b.rev CCA GAG TTT TGC CTC GTA GTG TGG ACT T

S212c.tmp GCC TCG TAG TGC TCG CTT CTA CGA GAC TTG CCT GGA CGT GCC TGC TTC ATC CAG GCT TTC CC

S212c.rev GAC AGG TTT TGC CTC GTA GTG CTC GCT T

S212d.tmp GCC TCG TAG TGC GAG CTT CTA CGA GAC TTG CCT GGA CGT GGA GGC TTC ATC CAG GCT TTC CC

S212d.rev GCA TCC TTT TGC CTC GTA GTG CGA GCT T

Table 2. List of RNA molecules used in this study.

S211a 5’- GGG AAA GCC UGG AUG AAG GAG GCA CGU CCA GGC AAG UCU CGU AGA AGU GGA CAC UAC GAG GCA AAA CCA GAG

S211b 5’- GGG AAA GCC UGG AUG AAG CCU GCA CGU CCA GGC AAG UCU CGU AGA AGU CCA CAC UAC GAG GCA AAA UCA CCG

S211c 5’- GGG AAA GCC UGG AUG AAG CAG GCA CGU CCA GGC AAG UCU CGU AGA AGC GAG CAC UAC GAG GCA AAA GCA UCC

S211d 5’- GGG AAA GCC UGG AUG AAG CCU CCA CGU CCA GGC AAG UCU CGU AGA AGC UCG CAC UAC GAG GCA AAA GAC AGG

S212a 5’- GGG AAA GCC UGG AUG AAG GAG GCA CGU CCA GGC AAG UCU CGU AGA AGU GGA CAC UAC GAG GCA AAA CGG UGA

S212b 5’- GGG AAA GCC UGG AUG AAG CCU GCA CGU CCA GGC AAG UCU CGU AGA AGU CCA CAC UAC GAG GCA AAA CUC UGG

S212c 5’- GGG AAA GCC UGG AUG AAG CAG GCA CGU CCA GGC AAG UCU CGU AGA AGC GAG CAC UAC GAG GCA AAA CCU GUC

S212d 5’- GGG AAA GCC UGG AUG AAG CCU CCA CGU CCA GGC AAG UCU CGU AGA AGC UCG CAC UAC GAG GCA AAA GGA UGC

Supporting Figures

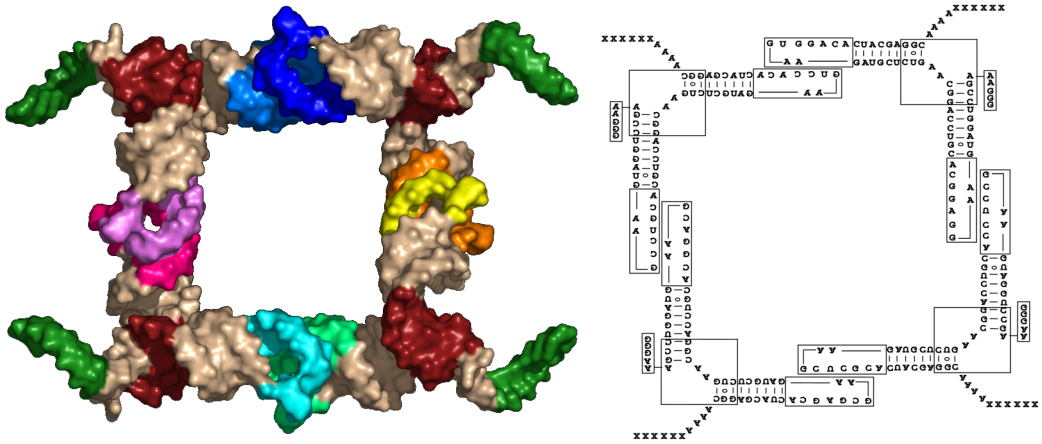

Figure S1. 3D model (left) and 2D diagram (right) of a tectosquare (TS) used to assemble ladders. 3' tail is colored in green. Kissing loop motifs[5] are colored in dark/light blue, purple/magenta, cyan/bluegreen, and yellow/orange.

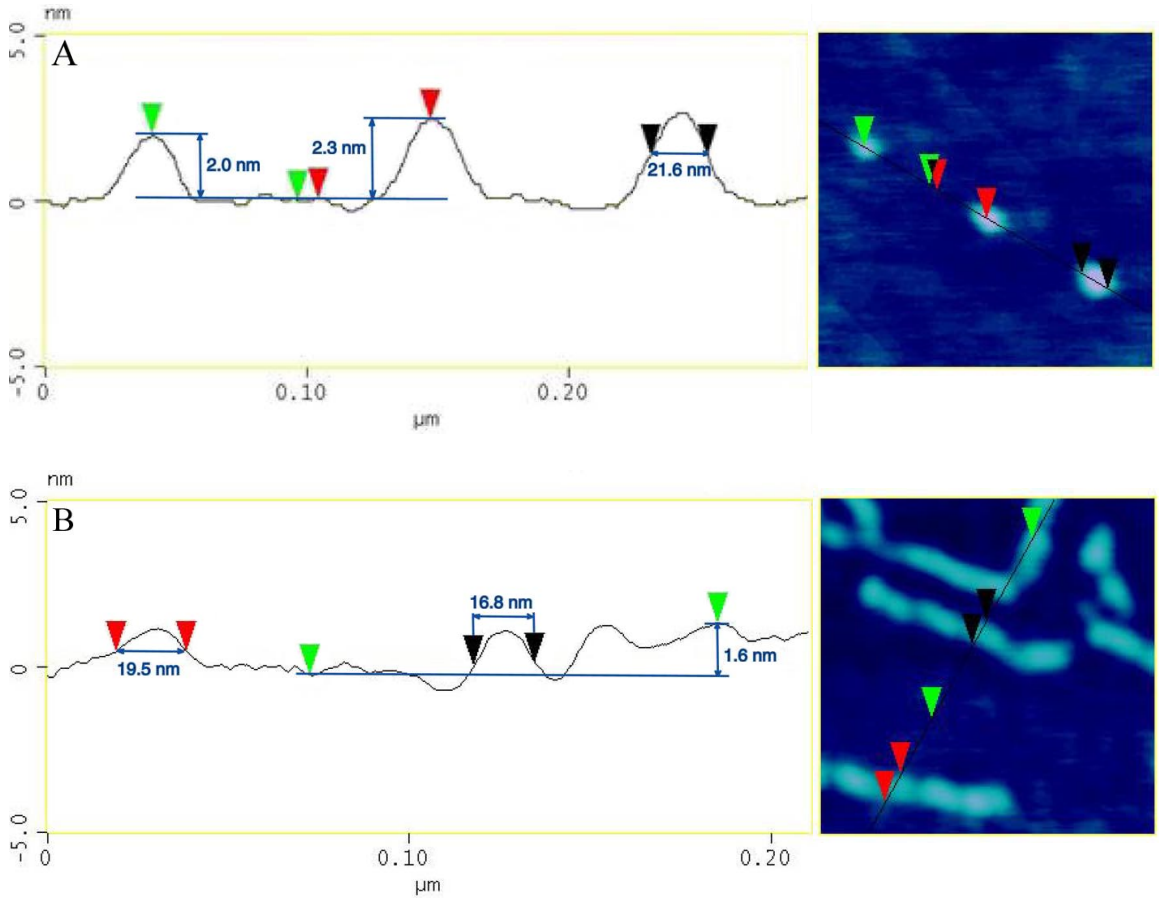

Figure S2. (A) AFM image of RNA tetramers. Zoom in and section analysis of the three RNA tetramers. (B) AFM image of RNA ladder assembled in solution. Zoom in and section analysis of the ladders.

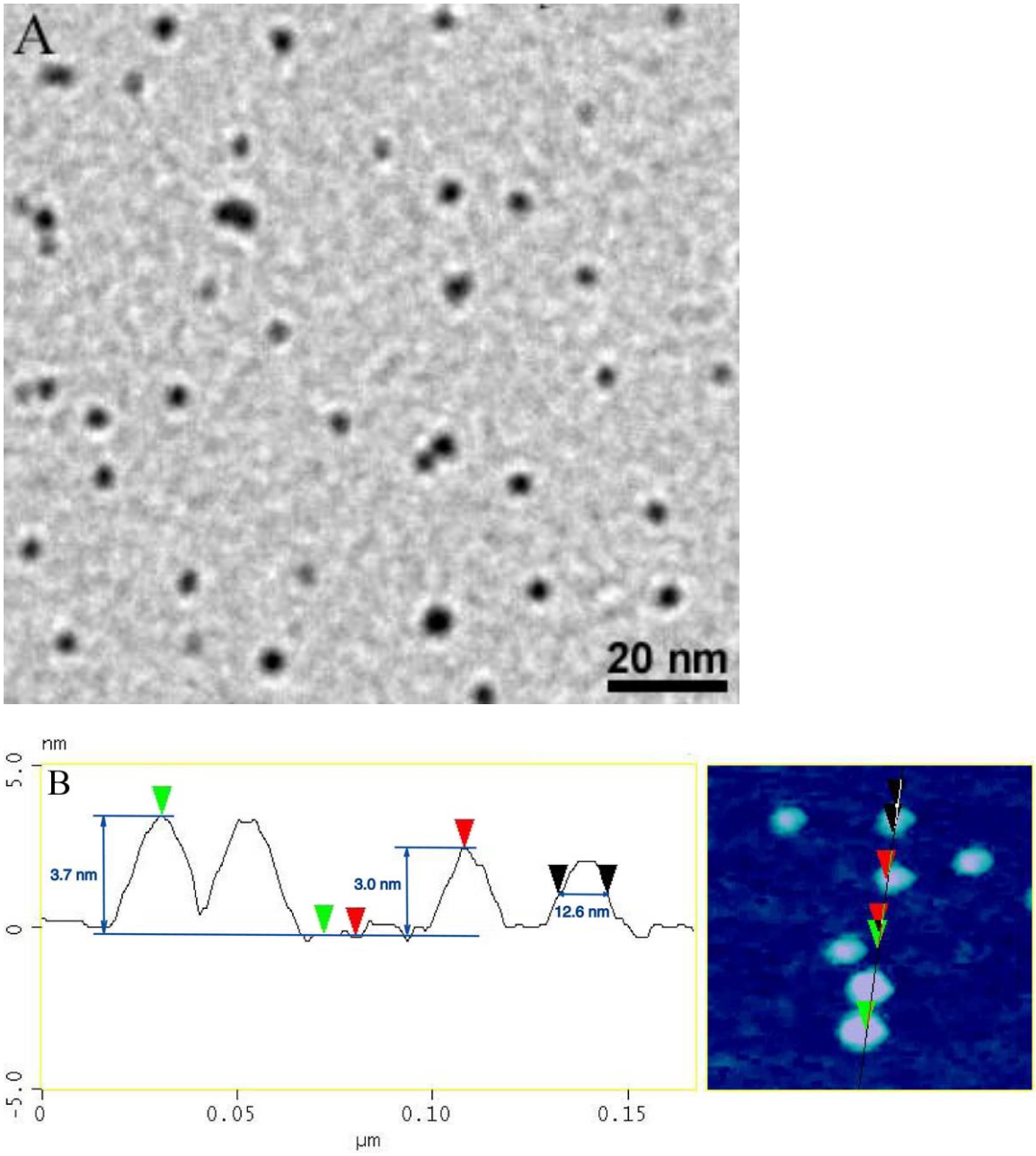

Figure S3. (A) TEM and (B) AFM of 3.5 nm thiocholine modified gold nanoparticles. Zoom in and section analysis of four gold nanoparticles.

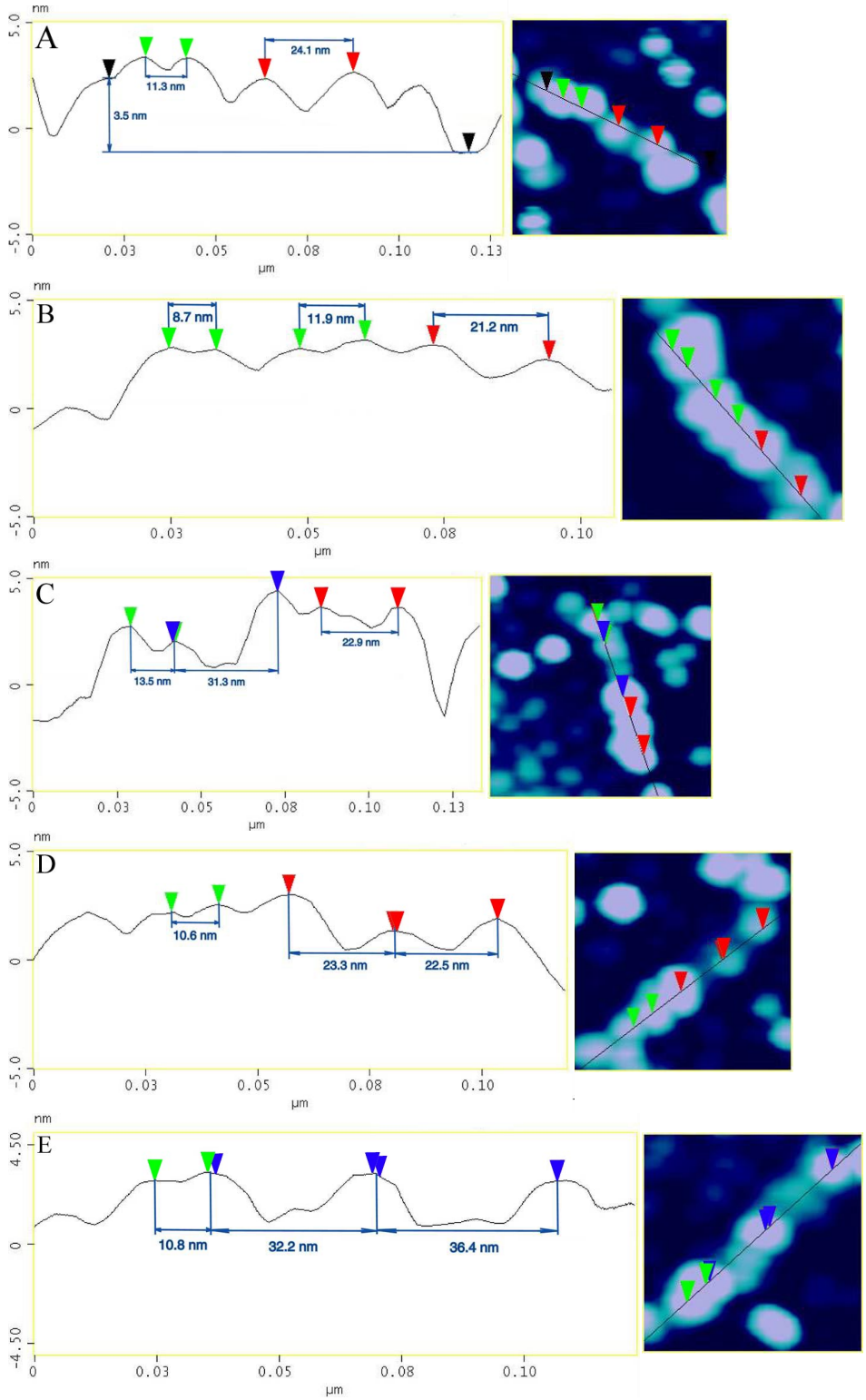

Figure S4. AFM image of RNA ladder decorated by gold nanoparticles. Section analysis of the decorated ladders along the black line. RNA height varies from 1.7 to 2 nm where no gold nanoparticles are present. The height of gold nanoparticles varies from 3 to 5 nm. Representative distances of 11.3, 22.6 and 33.9 nm are indicated in green, red and blue respectively.

References:

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5) Ennifar, E.; Walter, P.; Ehresmann, B.; Ehresmann, C.; Dumas, P. *Nature Struct. Biol.* **2001**, 8, 1064.