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RNA-Mediated Metal-Metal Bond Formation in the Hexagonal Pd Nanoparticle Synthesis is not Influenced by 5'-Thiolation of RNA

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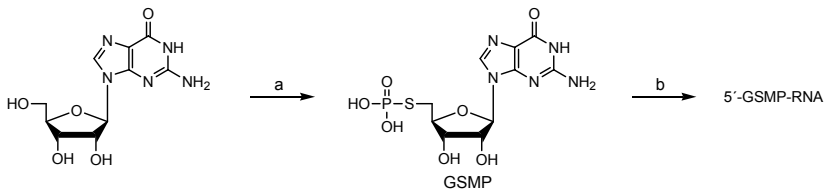
The ability to organize macromolecules, colloids, and nanocrystals at surfaces is of great interest in the fields of materials science, biology, and medicine. For example, quantum dots or light-harvesting dendrimers[1] into ordered arrays could be organized into high-density photonic solids[2] with tunable optical properties, and semiconductor nanorods and wires could be assembled to form the basis of ultra-high density logic and memory circuits.[3] An ability to introduce biomolecules (*i.e.* proteins, nucleic acids, *etc*) onto specific or ordered arrays would facilitate detection, as well as determination of their atomic structure.[4] Perhaps most challenging is the idea of combining biological molecules with inorganic nanostructures to form hierarchical multicomponent biological-inorganic hybrids, since this has the potential to lead to integration of functional biomolecular motifs into nanoelectronic devices, nanoelectronic components into *in vivo* medical devices for high sensitivity/selectivity applications. Despite its importance, practical methods for achieving controlled assembly of nanostructures are in their infancy.

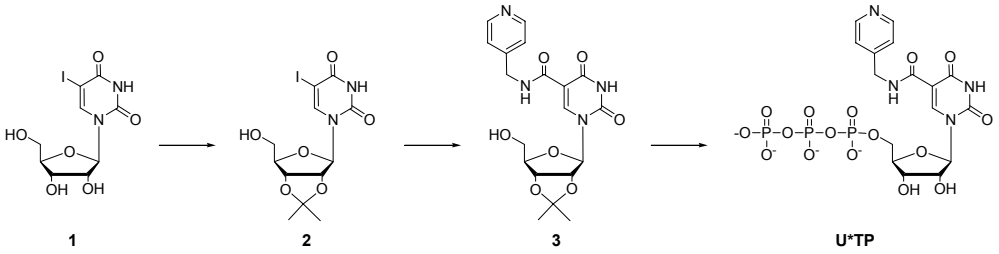
From this point of view, aptamers are interesting candidates as biomolecules which can be introduced to specific or ordered arrays. Aptamers are a special class of nucleic acids (RNAs or DNAs) selected from systematic evolution of ligands by exponential enrichment processes *in vitro* that specifically bind to a target molecule with high affinity.[5] Emerging as alternatives to antibodies, a wide range of DNA- and RNA-based aptamers have been found to specifically bind to target molecules,[5] such as proteins,[5,6] metal ions,[7] and small molecules,[8] and for target-specific delivery.[9] In addition, it was recently reported that RNA could even mediate the growth of hexagonal palladium nanoparticles as a catalyst, where 5-(4-pyridylmethyl)-uridine 5'-triphosphate (U*TP) was used to provide additional metal coordination sites beyond the heterocyclic nitrogens present in native RNA.[10] *In vitro* selection techniques were used to evolve an initial library of ~10^{14} unique RNA sequences through eight cycles of selection to yield a very active RNA sequence, referred to as Pdase 017, which could catalyze the formation of crystalline hexagonal palladium platelets.[10] Interestingly, the palladium particle growth was found to occur in aqueous solution at ambient temperature, without any endogenous reducing agent, and at low concentrations of metal precursor (70 μM), while the detailed atomic level structure, composition, and formation mechanism of these crystals are still being investigated.

Inspired by the work of Gugliotti *et al*.,[10(a)] we have investigated the effect of 5'-modification of the RNA molecule on the formation of crystalline hexagonal palladium platelets, because Pdase 017 is an attractive candidate for creating spatially well-defined patterns of materials on surfaces in the future study and because several 5'-modifications of RNA molecules have been shown to have broad applications in studying RNA structures and mapping RNA-ligand interactions. In light of organization of nucleic acids at surfaces, introduction of 5'-deoxy-5'-thioguanosine-5'-monophosphorothioate (GSMP) at the 5'-termini of the RNA molecules is useful because GSMP can be almost completely converted to a sulfhydryl group in the presence of alkaline phosphatase and the terminal sulfhydryl group can be reacted with the thiol-reactive functional groups, such as haloacetamides, maleimides, benzylic halides and bromomethyl ketones.[11] GSMP was prepared *via* a two-step synthetic method as recently reported (Scheme 1),[11] and used in the present study to investigate the effect of 5'-modification of the RNA molecule on the formation of crystalline hexagonal palladium platelets.

U*TP required for Pdase 017 mediator of nanoparticle synthesis was synthesized basically as reported[10(b)] (Scheme 2, also described in Experimental Section), and 5'-GSMP-functionalized Pdase 017 mediator was prepared according to the literature[10(b),11] (Experimental Section). Figure 1(a) shows that U*TP used in the present study is a good substrate for *in vitro* transcription. In addition, 5'-GSMP-functionalized Pdase 017^{-1} mediator having the reversed sequence compared to 5'-GSMP-



Scheme 1. Synthetic scheme for 5'-deoxy-5'-thioguanosine-5'-monophosphorothioate (GSMP) and 5'-GSMP-functionalized RNA. Reagents and conditions: (a) $P(Ph)_3$, I_2, imidazole, *N*-methyl-2-pyrrolidinone, followed by trisodium thiophosphate, water; (b) T7 RNA polymerase, GSMP:GTP:ATP:CTP:UTP



Scheme 2. Synthetic scheme for 5-(4-pyridylmethyl)-uridine 5'-triphosphate (U*TP)

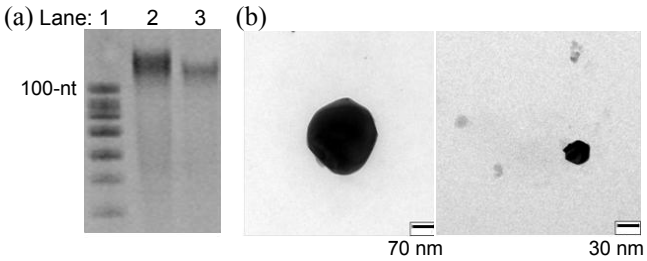


Figure 1. (a) Image of T7 RNA polymerase transcripts from the template to compare the *in vitro* transcription efficiency. Lane 1, ssDNA ladder (IDT, IA); lane 2,5'-GTP-RNA resulting from the transcript without GSMP; lane 3, mixtures of 5'-GTP-RNA and 5'-GSMP-RNA resulting from 20 eq. of GSMP. The image of the RNA products was obtained using Gel Doc 2000 Gel Documentation System (Bio-Rad, CA), following denaturing 7.5 M urea/8% polyacrylamide gel electrophoresis and EtBr staining. (b) Transmission electron microscopy (TEM) image of a hexagonal palladium (Pd) nanostructure formed in the presence of 5'-GSMP-terminated Pdase 017 (left) and 5'-GSMP-terminated Pdase 017^{-1} (right) in solution.

functionalized Pdase 017 mediator was also prepared in order to compare its efficiency of metal nanoparticle formation (Experimental Section). We found that ~95% of the 5'-end of the transcribed RNA molecule comprised of GSMP, which was confirmed by quantitation of the sulfhydryl group existent at 5'-end of the RNA molecule resulting from alkaline phosphatase treatment to convert 5'-GSMP-RNA to 5'-HS-RNA, as previously reported.[11] The obtained 5'-GSMP-functionalized Pdase 017 and 017^{-1} mediators were tested for their mediation of metal-metal bond formation in the hexagonal Pd nanoparticle synthesis. Figure 1(b) demonstrates that the 5'-GSMP-modified Pdase 017 and 017^{-1} are able to catalyze crystal growth and direct crystal shape. The metal complex precursor (66 μM $[Pd_2(DBA)_3]$) in an aqueous solution of 5% THF was incubated with the 5'-GSMP-modified RNA molecules (1 μM) for 2 h, followed by separation of the RNA-bound metal particles from free RNA and unincorporated metal precursor using size-exclusion membranes. The resulting RNA-bound metal particles were analyzed by transmission electron microscopy (TEM), revealing that the dominant Pd particle shape observed was thin hexagonal plates (~150 nm diameter for Pdase 017 and ~30 nm for Pdase 017^{-1}, respectively). We also found that 5'-HS-functionalized Pdase 017 and 017^{-1} mediators after alkaline phosphatase treatment could catalyze the Pd nanoparticle formation (data not shown). Taken together, the data show that the 5'-modified Pdase 017 and 017^{-1} mediators can mediate the metal-metal bond formation of hexagonal Pd nanoparticle synthesis as previously reported with Pdase 017.[10(a)]

To summarize, we have introduced GSMP to the 5'-end of the U*TP-containing RNA molecule, and shown that 5'-GSMP-modified RNA molecules can catalyze crystal growth and direct crystal shape. Since 5'-GSMP-modified Pdases 017 and 017^{-1} mediators can be easily converted to 5'-HS-functionalized ones, it is expected to orderly pattern 5'-modified RNA molecules on surfaces in order to mediate the formation of nanocrystals and to integrate nanoscale materials on surfaces. As more RNA sequences coding for different materials are discovered, it should be possible to introduce 5'-functionalized RNA catalysts in order to affect the orthogonal synthesis of nanoscale materials in ways that could lead to functional device architectures.

Experimental Section

Reagents were obtained from commercial suppliers and were used without further purification. Tris-(dibenzylideneacetone) dipalladium(0) ($[Pd_2(DBA)_3]$) was purchased from Aldrich, and GSMP was synthesized as reported.[11] Only commercially available, precleaned nuclease- and protease-free labware and chemicals were used. Diethyl pyrocarbonate (depc)-treated, autoclaved, double-distilled water was used to make all aqueous RNA and buffer solutions. RNA concentrations were measured by absorbance at 260 nm using Agilent 8453 UV-visible spectrophotometer.

U*TP was synthesized basically according to the reported protocols,[10(b)] but the alcohol protection step was modified to improve the synthetic efficiency, as follows:

Synthesis of 2. Under argon, 0.1 g of 5-iodouridine (0.27 mmol) was suspended in 4.67 mL of dry acetone, followed by addition of 0.015 mL of conc. H_2SO_4, and the reaction mixture was stirred at room temperature until complete conversion was obtained. After quenching with dry pyridine the solvents were evaporated in vacuo and the crude was purified *via* flash-column chromatography, using 9:1 CH_2Cl_2/MeOH, yielding 0.1 g of **2** (0.24 mmol, 88%) as a white solid.

Synthesis of 3. 3 was synthesized according to the literature.[10(b)] Briefly, in a heavy-walled glass bomb under argon, a solution of alcohol-protected 5-iodouridine in 3 mL of THF was prepared in the presence of 3 eq. of 4-(aminomethyl) pyridine, 5 eq. of triethylamine and 0.1 eq. of tetrakis-[triphenylphosphine]palladium. The bomb was evacuated and filled with 50 psi of carbon monoxide three times, then sealed and heated at 70 °C for 48 h. After the reaction bomb was cooled and vented carefully, the volatiles were removed in vacuo and the residue was purified on flash silica gel column with 19:1 CH_2Cl_2/MeOH, to give **3** in 90% yield.

Synthesis of U*TP. U*TP was prepared as reported.[10(b)] In brief, **3** (100 μmol) was dissolved in anhydrous pyridine (2 mL) and evaporated to dryness in vacuo. The residue was dried further over phosphorus pentoxide under vacuum for 1 h at room temperature. The reaction flask was filled with argon by introducing the gas into the desiccators and closed with a rubber septum. During all the following manipulations a small positive pressure of argon was maintained in the reaction vessel by connecting it with an argon-filled balloon. Anhydrous pyridine (100 μL) was injected through the septum followed by anhydrous dioxane (300 μL). A freshly prepared 1 M solution of 2-chloro-1,3,2-benzodioxaphosphorin-4-one in anhydrous dioxane (110 μL) was then injected into the well-stirred solution of the nucleoside. A white precipitate was formed when DMF was omitted from the solution. After 10 min a well-vortexed mixture of a 0.5 M solution of *bis*(tri-*n*-butylammonium) pyrophosphate in anhydrous DMF (300 μL) and tri-*n*-butylamine (100 μL) was quicjly injected, and the reaction mixture was stirred for 10 min. A solution of 1% iodine in pyridine/water (98:2, v/v) (2 mL) was then added. After 15 min excess iodine was destroyed by adding a few drops of a 5% aqueous solution of $NaHSO_3$, and the reaction solution evaporated to dryness. The residue was dissolved in water (25 mL) and glacial acetic acid (5 mL) to give a solution of pH 2.2. After 1 h, the solution was evaporated to dryness, the residue dissolved in water. U*TP was purified by DEAE Sephadex anion exchange column with a linear gradient of 800 mL each of 0.05 M and 1 M TEAB. The product was eluted between 0.50 and 0.55 M buffer, in 72% yield. The nucleoside precursors and U*TP were checked for purity by analytical HPLC (C18 column, 0 - 100% CH_3CN in 0.10 M triethylammonium) and ^{1}H NMR, and the triphosphate solution was quantitated on the basis of its UV absorbance at λ_{max}.

Preparation of RNA Mediators. GSMP-containing Pdase 017 and 017^{-1} mediators (87-mer: 5'-GSMP-CG GAC AAC ACU* CGG AGG ACA GCU* U*CC U*U*A U*GU* AAA AAA CAA CCA U*GU* AAC U*CC U*AU* CU*U* U*CC CGG CU*C GCA AAU* AAG AAC AGA GGG-3' and 5'-GSMP-GG AGA CAA GAA U*AA ACG CU*C GGC CCU* U*U*C U*AU* CCU* CAA U*GU* ACC AAC AAA AAA U*GU* AU*U* CCU* U*CG ACA GGA GGC U*CA CAA CAG GCG-3', respectively) were prepared by transcription of complimentary ssDNA templates[10] in an aqueous solution containing 4% (w/v) glycerol, 40 mM Tris-HCl (pH = 8.0), 12 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 1 mM spermidine, 0.01% Triton X-100, ~0.4 mM of adenosine 5'-triphosphate (ATP), cytosine 5'-triphosphate (CTP), guanosine 5'-triphosphate (GTP), and 5-(4-pyridylmethyl)-uridine-5'-triphosphate (U*TP), 250 nM T7 RNA polymerase (New England Biolabs), and 0.8 unit/μL RNase inhibitor (New England Biolabs). 5'-Deoxy-5'-thioguanosine-5'-monophosphorothioate (GSMP)-modified Pdase 017 and 017^{-1} RNAs were produced using this transcription protocol but incubated with 0.2 mM GTP and 3.8 mM GMPS.[11] The transcriptions were run at 37 °C for 20 h and stopped by the addition of 10 μL of 100 mM EDTA. After desalting the transcriptions on 10 K molecular weight cutoff filters (Microcon 10, 10-kD cutoff), Pdase 017 and 017^{-1} RNA mediators were purified using gel electrophoresis and stored at −80 °C prior to use.

Formation of Metal Nanoparticles. The metal complex precursor $[Pd_2(DBA)_3]$ was dissolved in freshly distilled THF to give a concentration of 1.3 mM. This THF solution was added to water (pH 7), giving an aqueous solution of 5% THF and 66 μM organometallic complexes. GSMP-modified RNA sequences (1 μM) were then incubated in the presence of the metal complex precursor. The incubation was performed in aqueous solution for 2 h at ambient temperature. Size-exclusion membranes (Microcon 100, 100-kD cutoff) were used to separate the RNA-bound metal particles from free RNA and unincorporated metal precursor. The reaction mixture was first concentrated onto the membranes by centrifugation and washed four times with buffer containing NaCl (1 mM), KCl (1 mM), $CaCl_2$ (1 mM), $MgCl_2$ (1 mM), and Na_3PO_4 (1 mM, pH 7.2) followed by washing twice with water (pH 7) to remove excess salts. The RNA-bound metal particles were recovered from the membrane by resuspension in water.

Electron Microscopy. TEM was performed at KIST using a Phillips CM30 transmission electron microscope operating at 50 - 300 kV accelerating voltage. To prepare samples for analysis, an aqueous solution of RNA-bound metal particles was drop-cast onto carbon-coated copper TEM grids (Pelco). Bright-field images were captured digitally with Digital Micrograph using a CCD camera.

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