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Supporting Information

Molecular logic gate operations using one-dimensional DNA nanotechnology

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Abstract

Here, we have listed “materials and methods” and the results of additional experiments as supplementary information for the manuscript.

Table of Content

1. Materials and Methods

1.1. Materials: All DNA sequences were purchased from Integrated DNA Technologies (IDT), USA with the following sequence information,

(1) **Cp_{OR}**: 5'-/5ThioMC6-D/GTTCTGTCTGTCACTCCACT-3'

(2) **Cp_{AND}**: 5'-GTCTGTCTTG/3ThioMC3-D/-3'

(3) **I_{Hg}'**: 5'-AGTCTAGGATTCGGCGTGGGTTAACTGTCTGTTC-3'

(4) **I_{Ag}'**: 5'-AGTCTAGGATTCGGCGTGGGTTAAACTCCACTCA-3'

(5) **I_{Hg}"**: 5'-CTTGTCTGTCTCACTCCACT-3'

(6) **I_{Ag}"**: 5'-AGTCTAGGATTCGGCGTGGGTTAAACTCCACTCA-3'

(7) **H1**: 5'-TTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGGATTCGGCGTG-3'

(8) **H2**: 5'-AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACTACTTTG-3'

Ethidium bromide (EtBr) and certified genetic quality tested DNA grade agarose were purchased from Bio-Rad (Hercules, CA, USA). A 100-base pair (bp) DNA ladder and Gel Loading Dye, Purple (6X) (Catalog#B7025S) were purchased from New England BioLabs (NEB), Inc (Ipswich, MA, USA) for gel electrophoresis studies. Mercury (II) perchlorate trihydrate ($Hg(ClO_4)_2 \cdot 3H_2O$) was purchased from Alfa Aesar (Ward Hill, MA 01835, USA) and silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich (St. Louis, MO 63103, USA). The remaining inorganic metal ions (nitrate salts) were purchased from Acros Organics or Thermo Fischer Scientific (NJ, USA). All other reagents were purchased from Sigma-Aldrich, St. Louis, MO 63103, USA and were used as received without further purification. Ultrapure water was obtained from the RNA Institute, The State University of New York at Albany, NY, USA water purification system (18.2 MΩ cm) and used in all studies. All DNA concentrations were determined using a NanoDrop ND-1000 Spectrophotometer.

1.2. Methods:

1.2.1. Gel Electrophoresis. All DNA stock solutions for gel electrophoresis were freshly prepared using nuclease-free water. 100 μL of 2 μM of H1 and H2 hairpins in a fresh 50 mM sodium phosphate buffer (50 mM Na_2HPO_4/0.5 M $NaNO_3$, pH 7.5) were heated separately to 95 °C in 2.0 mL clear sterilized microtubes for 3 mins using a CORNING® LSE™ Digital Dry Bath Heater. Subsequently, the hairpin solutions were snap-cooled on ice and then stored at room

temperature (RT) for 2 hrs before use. In order to demonstrate that the initiators are necessary for HCR, two separate gel electrophoresis studies were performed in four combinations for both the **OR,** and **AND** gates. For the **OR** gate, the following order was used: a) H1+H2, b) H1+H2+ $\mathbf{I_{Ag}}$', c) H1+H2+$\mathbf{I_{Hg}}$' d) H1+H2+$\mathbf{I_{Hg}}$'+$\mathbf{I_{Ag}}$'. For the **AND** gate, the same order was followed by using **AND** gate initiators: (a) H1+H2, b) H1+H2+ $\mathbf{I_{Ag}}$", c) H1+H2+$\mathbf{I_{Hg}}$" d) H1+H2+$\mathbf{I_{Hg}}$"+$\mathbf{I_{Ag}}$"). The H1 and H2 were mixed in a 1:1 ratio for a final concentration of 1 μM and were incubated with 0.3 μM of the corresponding initiator DNA strands (for **OR** gate: $\mathbf{I_{Hg}}$' and $\mathbf{I_{Ag}}$', and for **AND** gate: $\mathbf{I_{Hg}}$" and $\mathbf{I_{Ag}}$") at 4 °C for at least 4 hrs. The mixture containing only H1&H2 (1:1) was performed as a control experiment. Briefly, the gel electrophoresis study was performed by mixing 15 μL of each hairpin mixture product and 10 μL of Purple Gel Loading Dye (6X) in a PCR strip tube at RT and then loading the solution into the gel pocket.

Preparation of 1% agarose gel: 1% agarose gel was prepared by heating 1.0 g of agarose in 100 mL of freshly prepared 1x sodium borate (SB) buffer, pH 8.5 (pH was adjusted with Boric Acid), for 85 secs using a microwave. 10 μL of 10 mg/mL EtBr was added to the gel solution in the gel box as the DNA staining dye before polymerization. 600 mL of 1x SB buffer was used as the running buffer and an additional 10 μL of 10 mg/mL of EtBr was added to both the anode and cathode sides before running the gel. The electrophoresis was performed for 70 mins at 4 °C and 110 V settings. The gel images were visualized using a Bio-Rad ChemiDoc™ MP Imaging System with Image Lab ™ 5.2.1 software.

1.2.2. Nanoparticle preparation, functionalization and characterization. Gold nanoparticle (AuNP, 13 nm) synthesis was performed using the standard citrate reduction method. Briefly, 50 mL of 1 mM $HAuCl_4$ was prepared using ultrapure, nuclease-free DI water and boiled in a 125 mL Erlenmeyer flask under vigorous magnetic stirring on a hotplate. 7 mL of 38.8 mM sodium citrate was added rapidly to the mixture, which was left stirring on hotplate until the color of the colloidal suspension turned to wine-red. The final AuNP solution (~13 nM) was cooled to RT and stored in a sterilized glass bottle (previously washed with aqua regia and 12 M NaOH) at 4 °C until use. The AuNPs were functionalized with thiol modified capture DNA probes (**AuNP-Cp$_{AND}$** or **AuNP-Cp$_{OR}$**) as previously reported.[1-5] The resulting **AuNP-Cp$_{Hg\text{-}OR\text{-}Ag}$** and **AuNP-Cp$_{Hg\text{-}AND\text{-}Ag}$** were washed and purified by benchtop centrifugation. The supernatants were discarded and the nanoprobe pellets were re-suspended in an equal volume of

ultrapure DI water; a second centrifugation was thereafter performed and the pellets were re-suspended in the reaction buffer. The bare and thiol-modified nanoparticles were characterized using a Cary 60 UV-Vis Spectrophotometer (Agilent Technologies Inc., USA).

To perform the HCR experiments, the **AuNP-Cp$_{Hg\text{-}OR\text{-}Ag}$** and **AuNP-Cp$_{Hg\text{-}AND\text{-}Ag}$** were re-suspended in sodium phosphate buffer (50 mM Na_2HPO_4/0.2 M $NaNO_3$, pH 7.5 as the **AND** gate reaction buffer, and 50 mM Na_2HPO_4/0.4 M $NaNO_3$, pH 7.5 as the **OR** gate reaction buffer) before use. The size of the nanoparticles was recorded by Dynamic Light Scattering (DLS) using DynaPro Titan (Wyatt technology Corporation, USA).

1.2.3. Operation of OR Logic Gate. The **OR** logic gate was operated using **AuNP-Cp$_{Hg\text{-}OR\text{-}Ag}$** which was functionalized with thiol-modified **Cp$_{OR}$** DNA.[3] For the **OR** logic gate operation, 1.0 μM of initiator (**I$_{Hg}$'+I$_{Ag}$'**) strands and 1 nM of Hg^{2+} and/or Ag^{+} metal ions were incubated with 200 μL of **AuNP-Cp$_{Hg\text{-}OR\text{-}Ag}$** at 4 °C for ~90 mins. This nanoprobe assembly was centrifuged at 12,100 rpm until a clear supernatant was observed (~10 mins). The supernatant was discarded and the nanoparticle pellet was re-suspended in the **OR** gate reaction buffer. 5.50 μL of a 200 μM **H1&H2** stock solution was added to the nanoparticle assembly for a final concentration of 5.5 μM. The samples were incubated at 4 °C for at least 4 hrs. Later, the samples were centrifuged at 12,100 rpm for 9 mins, the supernatants were discarded and the nanoparticle pellet was re-suspended in the **OR** gate reaction buffer. Finally, 1.0 μL of 1 M of $Mg(NO_3)_2$ solution was added to 20 μL of the nanoparticle assembly for a final concentration of ~50 mM Mg^{2+} in a PCR tube; soon after, each sample was diluted to equivalent amounts of buffer (final volume of 40 μL) in a PCR well-plate. The color-transition was observed over time. UV-Vis spectra were recorded in a 384 PCR well-plate (VWR) using a BioTek Synergy microplate reader. Hg^{2+} and Ag^{+} were employed as Boolean logic inputs, where "0" represents the absence of input, and "1" defines the presence of input. In this logic gate operation, four possible combinations of inputs were triggered: (i) 1 nM Hg^{2+} + 1 nM Ag^{+} (1,1); (ii) 1 nM Hg^{2+} (1,0); (iii) 1 nM Ag^{+} (0,1); (iv) neither one of the two metal ions (0,0). The kinetics measurements were performed using the Cary 60 UV-Vis spectrophotometer with 50 μL of individual nanoprobe HCR assembly diluted to 80 μL using the reaction buffer.

1.2.4. Operation of AND Logic Gate. The AND logic gate was operated using **AuNP-Cp$_{Hg\text{-}AND\text{-}Ag}$** which was functionalized with thiol-modified **Cp$_{AND}$** DNA. First, 1.0 μM of initiator

($\mathbf{I_{Hg}}$"+$\mathbf{I_{Ag}}$") strands and 1 nM of Hg^{2+} and/or Ag^{+} metal ions were incubated with 200 μL of **AuNP-Cp$_{\mathbf{Hg\text{-}AND\text{-}Ag}}$** at RT for ~60 mins. This nanoprobe assembly was centrifuged at 14,200 rpm until a clear supernatant was observed (~10 mins). The supernatant was discarded and the nanoparticle pellet was re-suspended in the **AND** gate reaction buffer. 5.50 μL of a 200 μM **H1&H2** stock solution was added to the nanoparticle assembly for a final concentration of 5.5 μM. The samples were incubated at 4 °C for at least 4 hrs. Later, the samples were centrifuged at 14,200 rpm for 9 mins, the supernatants were discarded and the nanoparticle pellet was re-suspended in the **AND** gate reaction buffer. Finally, 0.70 μL of 1 M of $Mg(NO_3)_2$ solution was added to 20 μL of the nanoparticle assembly in a PCR tube for a final concentration of ~35 mM Mg^{2+}; soon after, each sample was diluted to equivalent amounts of buffer (final volume of 40 μL) in a PCR well-plate. The color-transition was recorded over time after the addition of $Mg(NO_3)_2$. For a quantitative measurement, the UV-Vis spectra were recorded in a 384 PCR well-plate (VWR) using a BioTek Synergy microplate reader. The kinetics measurements were performed using the Cary 60 UV-Vis spectrophotometer with 50 μL of individual nanoprobe HCR assembly diluted to 80 μL using the reaction buffer.

1.2.5. Absence Test. The necessity of the HCR for generation of output signal was confirmed by performing an absence test, as follows: the gate operations were performed without (a) the initiators of **OR** or **AND** gates, (b) H1+H2 and (c) Hg^{2+} + Ag^{+}. In the absence of any set of these parameters, HCR was not expected to occur on the nanoparticle surface resulting in a color transition. These data also demonstrated that even though both Hg^{2+} + Ag^{+} inputs are present, the gates were shut down by excluding initiators or hairpins.

1.2.6. Selectivity Studies. The selectivity of both the **OR** and **AND** logic gate systems was evaluated for both metal ions (Hg^{2+} and Ag^{+}) by monitoring the response of the assay to a series of divalent metal ions (nitrate salts): Cd^{2+}, Mn^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+}, each at a 1 nM concentration in the final nanoparticle assembly, and a blank (no metal ion). All metal ions were freshly prepared in reaction buffer and stored at 4 °C. For the **OR** logic gate, the selectivity test was performed in the presence of both initiators, H1&H2 and either with 1 nM of Hg^{2+}, Ag^{+} or each of the respective metal ions.

Separately, to demonstrate the **AND** logic gate specificity towards coexistence of Hg^{2+} and Ag^{+}, each one of these two metal ions was separately tested with the same series of divalent

metal ions. The specificity studies were performed by adding 200 μL of **AuNP-Cp$_{Hg\text{-}OR\text{-}Ag}$** or **AuNP-Cp$_{Hg\text{-}AND\text{-}Ag}$**, 1.0 μM corresponding initiator pair and 5.5 μM **H1&H2** to each of the above solutions. The color-transitions were recorded ~30 mins after Mg^{2+} treatment.

1.2.7. Statistical Analysis. Data were expressed as mean ± SD. Experiments were performed in triplicate.

2. References.

1. J. Liu and Y. Lu, *Nat Protoc*, 2006, **1**, 246-252.

2. M. Rana, M. Balcioglu, M. Kovach, M. S. Hizir, N. M. Robertson, I. Khan and M. V. Yigit, *Chem. Commun.*, 2016, **52**, 3524-3527.

3. M. Rana, M. Balcioglu, N. M. Robertson, M. S. Hizir, S. Yumak and M. V. Yigit, *Chem. Sci.*, 2017, **8**, 1200-1208.

4. M. Rana, M. Balcioglu and M. V. Yigit, *BioNanoScience*, 2014, **4**, 195-200.

5. M. Balcioglu, M. Rana, M. S. Hizir, N. M. Robertson, K. Haque and M. V. Yigit, *Adv. Healthc. Mater.*, 2017, **6**.

3. Supporting Figures

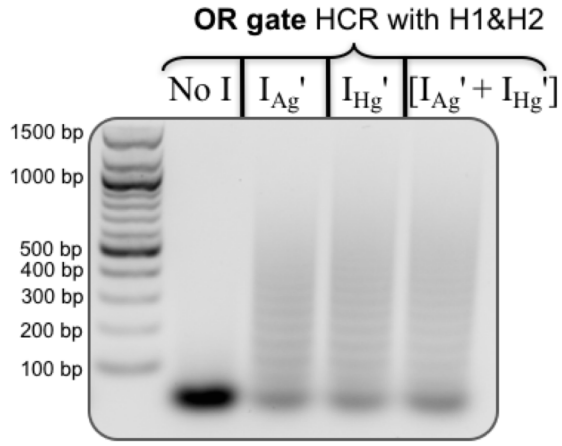

Fig. S1 Gel electrophoresis data showing the polymerization of the H1&H2 hairpin pair with OR gate initiators.

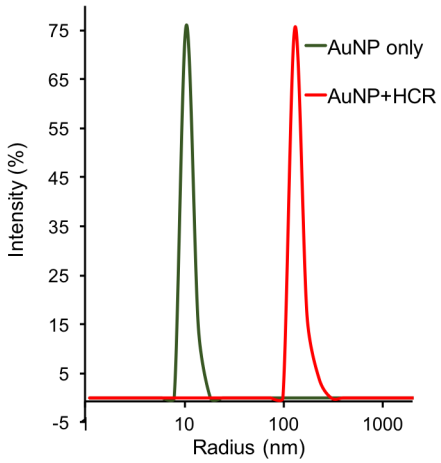

Fig. S2 The increase in hydrodynamic radius of the AuNPs before and after assembly of HCR-product on the surface.

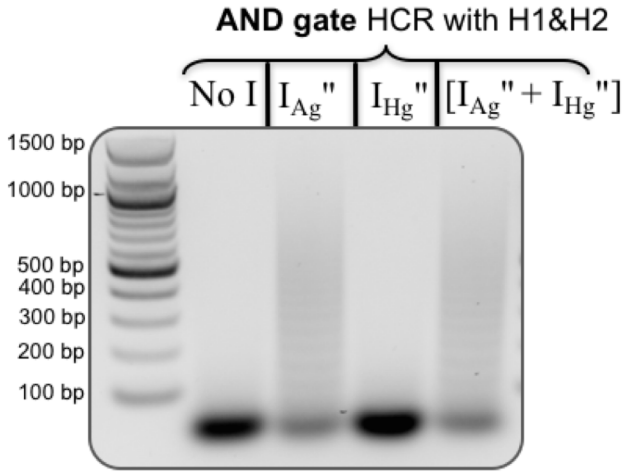

Fig. S3 Gel electrophoresis data showing the polymerization of the H1&H2 hairpin pair with AND gate initiators. While the I_{Ag}" is required for initiating HCR the I_{Hg}" is needed only for bridging the HCR product to AuNP-$Cp_{Hg\text{-}AND\text{-}Ag}$ and is not capable of triggering HCR

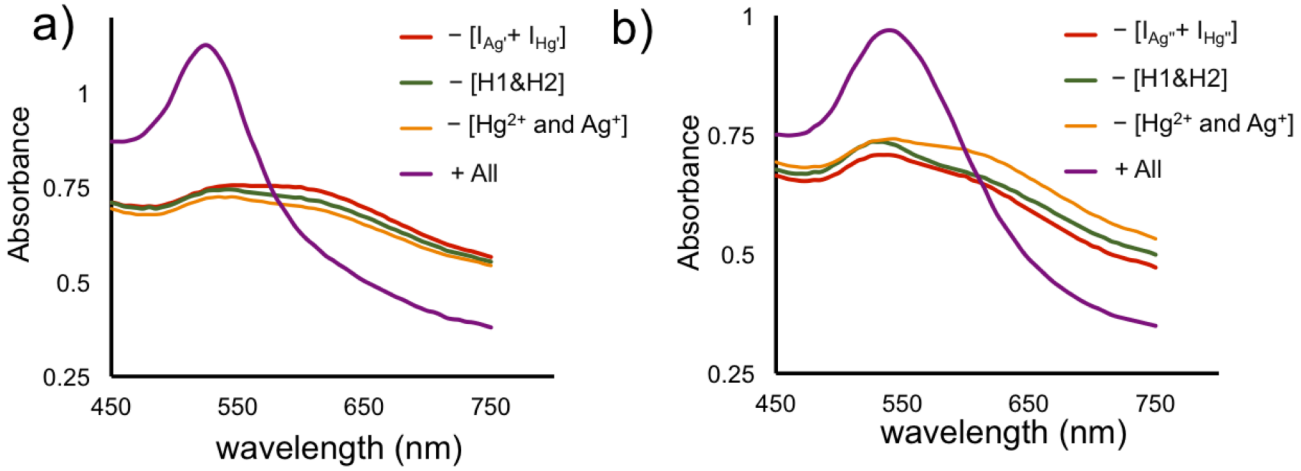

Fig. S4 Absence test suggesting the requirement of HCR for output signal. The HCR is required for generating the output signal for both a) **OR,** and b) **AND** logic gates. In the absence of initiators or H1&H2 hairpins; the output signal is not received even though both Hg^{2+} and Ag^{+} inputs were present.