Electronic Supplementary Material (ESI) for Chemical Communications.


# Supplementary Information

## Reprogrammable Multiplexed Detection of Circulating OncomiRs Using Hybridization Chain Reaction

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**Abstract**

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

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## 1. Materials and Methods

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

(1) Thiol-modified 36mer capture probe,
5'-TACTGGAAGATGTCTGATAAGCTATTCTACAGGGTA/3ThioMC3-D/-3'

(2) miR-10b, 5'-UACCCUGUAGAACCGAAUUUGUG-3'

(3) miR-21, 5'-UAGCUUAUCAGACUGAUGUUGA-3'

(4) miR-141, 5'-CAUCUUCCAGUACAGUGUUGGA-3'

(5) I-10b, 5'-AGTCTAGGATTCGGCGTGGGTTAACACAAATTCGG-3'

(6) I-21, 5'-AGTCTAGGATTCGGCGTGGGTTAATCAACATCAG-3'

(7) I-141, 5'-AGTCTAGGATTCGGCGTGGGTTAATCCAACACTG-3'

(8) H1, 5'-TTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGGATTCGGCGTG-3'

(9) H2, 5'-AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACTACTTTG-3'

Ultrapure RNase-free water was used in all studies. Ethidium bromide (EthBr), laemmli loading dye, and certified genetic quality tested DNA grade agarose were purchased from Bio-Rad (Hercules, CA, USA). 100 bp DNA ladder was purchased from New England BioLabs, Inc (Ipswich, MA, USA) for gel electrophoresis studies. RNase Away was purchased from Sigma-Aldrich and used to avoid any RNase contamination. All other reagents were purchased from Sigma-Aldrich, St. Louis, MO 63103, USA.

**1.2. Methods:**

**1.2.1. Gel Electrophoresis.**

Stock solutions of oligonucleotides were prepared using nuclease-free water. 100 µL of 2 µM H1 and H2 solutions in 50 mM sodium phosphate buffer (50 mM $Na_2HPO_4$/0.5 M NaCl, pH 6.8) were heated separately to 95 °C in 2.0 mL clear microtubes for 3 mins. The solutions were snap-cooled on ice for 3 mins and incubated at room temperature (RT) for 2 hrs before use. The H1 and H2 were mixed to a final concentration of 1 µM and incubated with 0, 100 nM, 300 nM, 500 nM, 700 nM or 1000 nM of initiator strands (I-10b, I-21 or I-141) at RT overnight. 15 µL of each product and 10 µL

of 6x loading dye were mixed in a PCR tube and loaded in the gel. A 1% agarose gel was used in gel electrophoresis studies and prepared by heating 1 g of agarose in 100 mL of freshly prepared 1x sodium borate buffer, pH 8.5, for 45 secs using a microwave. 10 µL of 10 µg/mL EthBr was added to the gel solution before polymerization. 1x sodium borate buffer was used as the running buffer and an additional 10 µL of 10 µg/mL of EthBr was added to the buffer before running the gel. The electrophoresis was performed for 60 mins at 150 V and gels were visualized using a Bio-Rad ChemiDoc™ XRS Imaging System with Quantity One 4.6.1 software.

**1.2.2. Nanoparticle synthesis and functionalization.**

Gold nanoparticles (AuNPs) were prepared using the standard citrate reduction method.[1] Briefly, 2 mL of 50 mM $HAuCl_4$ was added into 98 mL of boiling DI water in an Erlenmeyer flask. 10 mL of 38.8 mM sodium citrate was added and the mixture was stirred until the color turned wine-red. The solution was cooled to room temperature and stored at 4 °C. The AuNPs were functionalized with thiol-modified capture DNA probes (*AuNP-36mer*) according to the methods described in the literature.[1] The resulting *AuNP-36mer* was purified by centrifugation and re-suspended in an equal volume of RNase-free water. The nanoparticles were characterized using a Cary 60 UV-Vis Spectrophotometer (Agilent Technologies Inc., USA). For HCR experiments, the AuNP-36mer was re-suspended in sodium phosphate buffer (50 mM $Na_2HPO_4$/0.3 M NaCl, pH 6.8) before use. Dynamic light scattering (DLS) measurements were recorded to determine the size of the nanoparticles using a DynaPro Titan (Wyatt technology Corporation, USA).

**1.2.3. HCR-induced DNA polymerization for oncomiR detection.**

All RNA and DNA concentrations were determined using a NanoDrop ND-1000 Spectrophotometer. 50 mM sodium phosphate buffer ($Na_2HPO_4$/0.3 M NaCl, pH 6.8) was used for all DNA polymerization studies with nanoparticles. The 50 nM target oncomiR (miR-10b, miR-21 or miR-141), 2.2 µM initiator (I-10b, I-21 or I-141), and 5.5 µM H1/H2 concentrations were used in each study. Initially 50 nM of an oncomiR target was incubated with 100 µL of *AuNP-36mer* at RT for 20 mins and then at 4 °C for 10 mins. Initiator DNA strand (2.2 µM) was added to the resulting *AuNP-36mer-oncomiR* assembly, followed by incubation at 4 °C for 10 mins and at RT for 30 mins. The sample *AuNP-36mer-oncomiR-Initiator* assembly was centrifuged at 12,100 rpm until a clear supernatant is observed (~5 mins), and the nanoparticle pellet was re-suspended in the phosphate buffer. 3.5 µL of a 157 µM H1/H2 stock solution was added to the nanoparticle assembly for the final concentration of 5.5 µM. The samples were incubated at RT overnight. Finally, 1.4 µL of a 1 M $MgCl_2$

solution was added to 20 μL of the nanoparticle assembly to a final concentration of 70 mM in phosphate buffer. The change in color of the nanoparticle suspension was recorded by a digital camera 0 and 60 mins after the addition of $MgCl_2$. For characterization using UV-Vis spectroscopy the samples were diluted to 40 μL with phosphate buffer immediately before and after the $MgCl_2$ addition and, UV-Vis spectra of samples were recorded in 96 well plate.

Studies were also performed using various amounts (0, 10, 20, 40, 60, 100, 200, 500, 1000 and 2000 fmol) of target miRNAs in 20 μL of solution and, color changes were recorded four hours after incubation with 50 mM of $MgCl_2$. Control experiments were performed in the absence of target oncomiRs, correct initiators, and/or H1/H2 hairpins.

**1.2.4. Detection of target oncomiRs in endogenous total RNA.**

The MCF-7 and 4T1 cell lines were cultured in DMEM (Invitrogen) supplemented with 10% fetal bovine serum. The cells were propagated in media supplemented with L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Life Tech Corp., Grand Island, NY) at 37 °C in a 5% $CO_2$ incubator for two days. The cells were removed from the plate using dissociation buffer and used as liquid biopsy mimic of circulating breast tumor cells. The cells were lysed with the addition of QIAzol after being washed with ice cold PBS twice and total endogenous RNA was isolated according to the supplier's (QIAGEN) instructions.[2] In order to demonstrate the detection of target oncomiRs in MCF-7 cell line, 50 nM of exogenous miR-10b, miR-21 or miR-141 was added separately to 0.45 ng/μL of endogenous RNA resulting in 0.9 ng/μL of total RNA. Detection was carried out with this RNA concentration. As a negative control, 0.9 ng/μL of endogenous RNA without any oncomiR enrichment step was used. 100 μL of *AuNP-36mer* (~13 nM) was incubated with RNA samples and experiments were performed similar to the procedure described above. The color changes were recorded 0 and 60 mins after the addition of 70 mM $MgCl_2$.

In order to detect an endogenous oncomiR (miR-10b) isolated directly from the breast cancer cells, 50 ng/μL of total RNA extracted from 4T1 (experimental) and MCF-7 (control) cells was incubated with 100 μL of AuNP-36mer (~13 nM), separately. The resulting AuNP assemblies were purified by centrifugation. Later, the AuNP assemblies were incubated with 2.2 μM initiator (I-10b) and 5.5 μM of H1/H2 at 4 °C for 3 hours for each cell line as described above. The OD value at 520 nm and the pictures of the nanoparticle suspensions were recorded. All experiments were performed in triplicate (n=3) using 384 well plates.

### 1.2.5. Stability of HCR-induced DNA polymerization on AuNPs.

For monitoring the stability of the DNA assembly on AuNPs, HCR-induced DNA polymerization was achieved on AuNPs using 100 μL of *AuNP-36mer* (~13 nM), 50 nM of miR-10b, 5.5 μM of H1/H2 and 2.2 μM of I-10b. The stability of the HCR assembly on AuNPs was monitored by the addition of 70 mM of $Mg^{2+}$ to the resulting *AuNP-36mer-oncomiR-HCR* assembly three hours, one day, two days, one week or two weeks after the formation of HCR on gold nanoparticles. The control experiments were performed in the absence of target oncomiR. All measurements were performed in a clear 384 well PCR plate.

### 1.2.6. Atomic force microscopy (AFM).

The hybridization chain reaction was initiated using 1 μM of H1/H2 and 0.7 μM of I-10b in 50 mM sodium phosphate buffer (50 mM $Na_2HPO_4$/0.5 M NaCl, pH 6.8). For the control study, only 1 μM of H1/H2 mixture was used in 50 mM sodium phosphate buffer (50 mM $Na_2HPO_4$/0.5 M NaCl, pH 6.8). The samples were diluted 7 times in 10 mM $NiSO_4$ and 5 μL of the HCR product was deposited on a freshly cleaved mica surface and incubated for 5 mins. In addition, 100 μL of 10 mM $NiSO_4$ buffer was added onto the mica surface to perform imaging using the liquid peak force mode. Measurements were performed using BioScope Catalyst-Bruker with Bruker's Sharp Nitride Lever (SNL-10-C) probes. Topographic images were obtained with 3 μm scan sizes, 512 × 512 $pixels^2$ at a scan rate of 0.5 Hz.

## 2. Supporting Figures

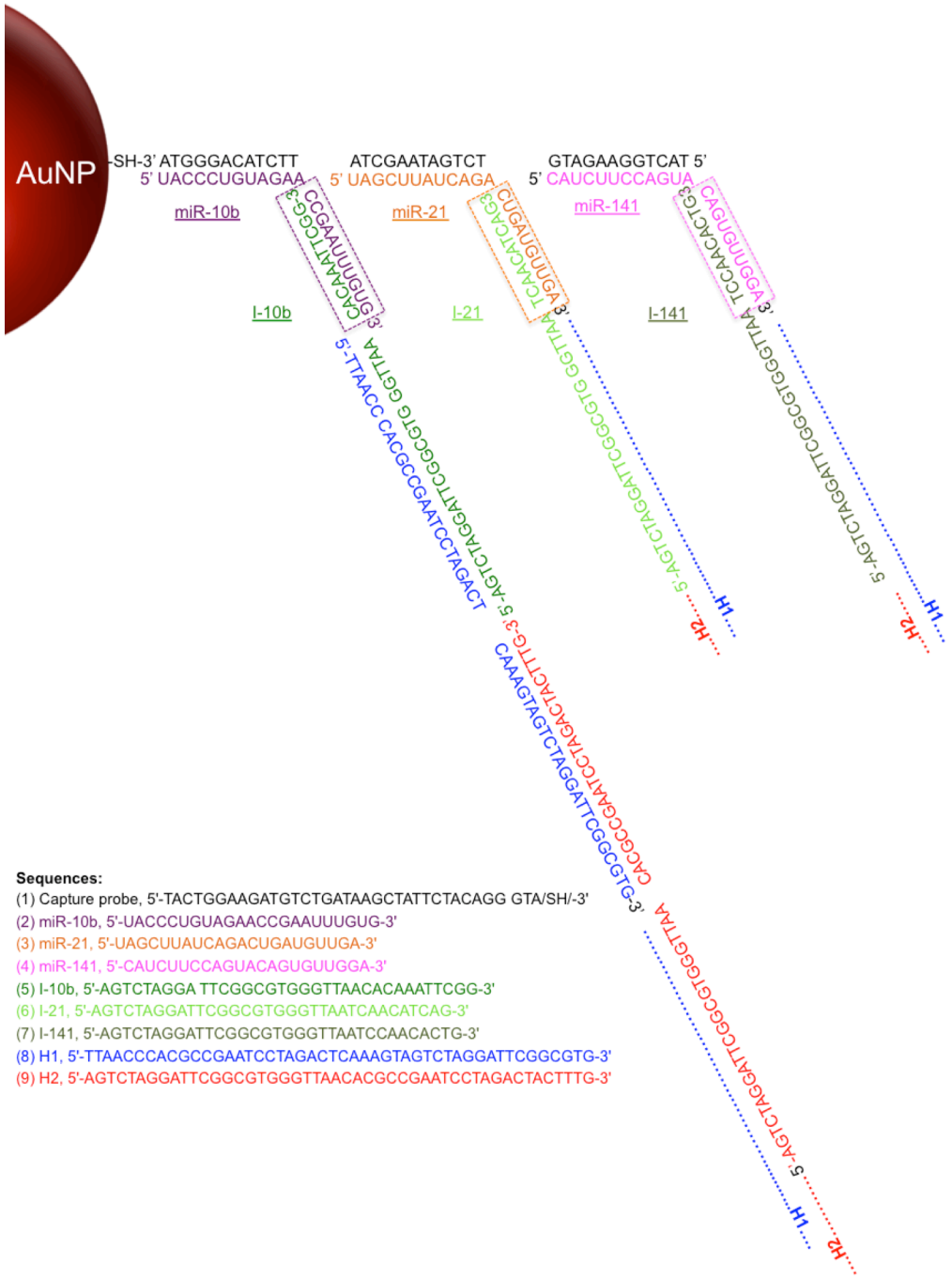


**Figure S1.** Illustration and design of miR-10b, miR-21 or miR-141 initiated DNA polymerization on the gold nanoparticle surface using initiator probes (I-10b, I-21 or I-141) and H1/H2 hairpins.

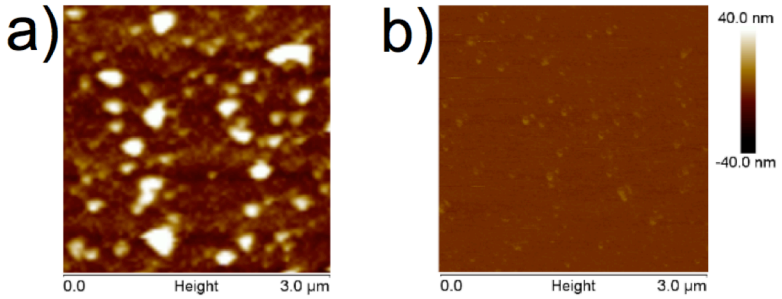


**Figure S2.** AFM images of DNA assemblies composed of H1 and H2 hairpins a) with and b) without an initiator strand (I-10b). The HCR occurs in the presence of the I-10b observed as clusters of DNA polymers.

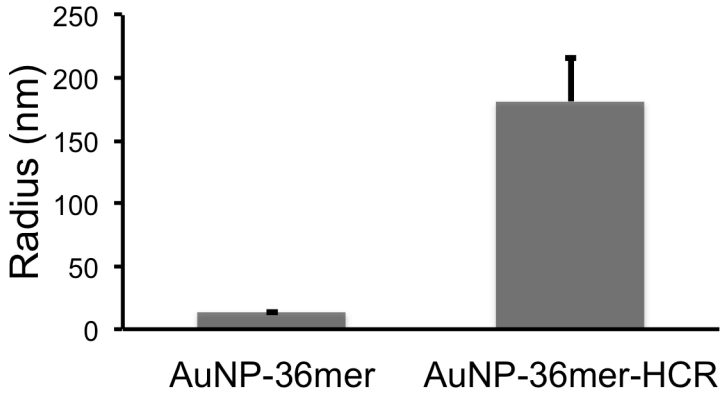


**Figure S3.** Average hydrodynamic size of the *AuNP-36mer* with and without HCR-induced DNA polymerization using dynamic light scattering.

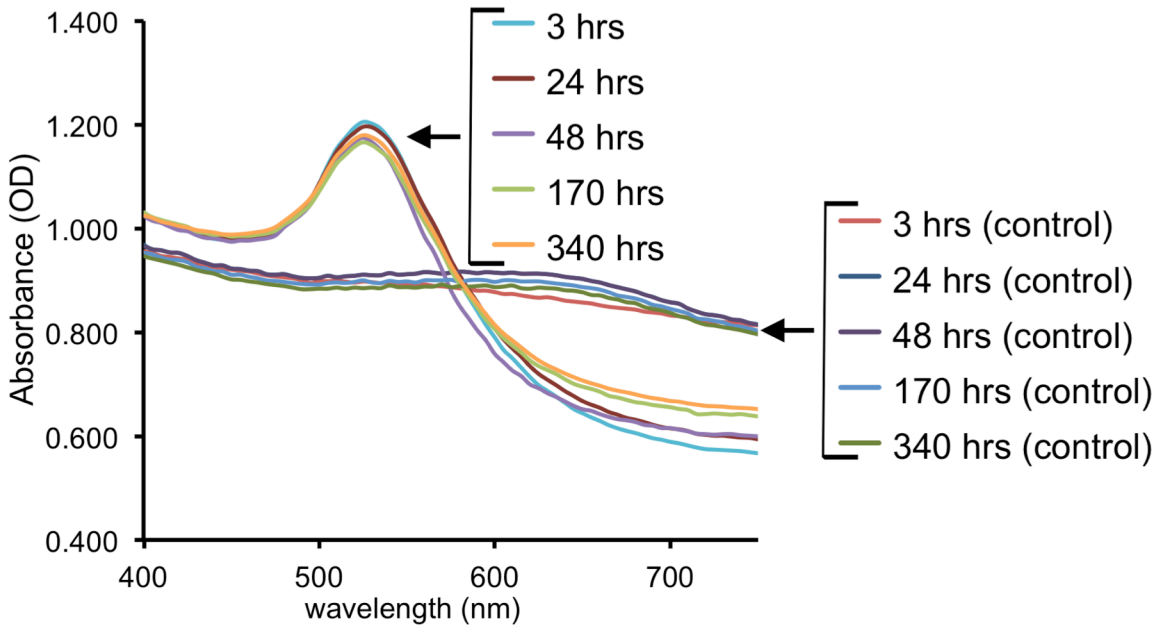


**Figure S4.** Stability of the HCR on the AuNP surface over time. Shift in the 520 nm plasmonic band of AuNPs to higher wavelengths is not observed with $Mg^{2+}$ treatment three hours, one day, two days, one week or two weeks after the formation of the HCR product on AuNPs. This suggests that the HCR-induced DNA polymers remain stable on the AuNP surface after formation of *AuNP-36mer-oncomiR-HCR* assembly. AuNPs (control) without HCR formation are not stable against $Mg^{2+}$ treatment as expected.

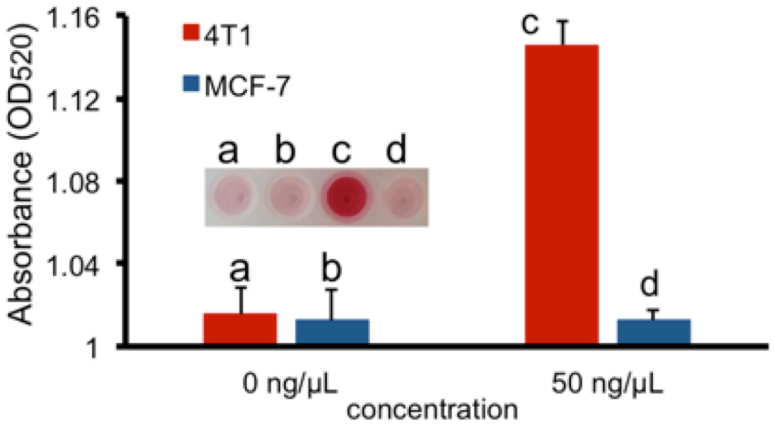


**Figure S5. Detection of miR-10b in a total RNA pool from cancer cells.** Visual and spectroscopic detection of miR-10b in 50 ng/uL of total RNA extracted from 4T1 (experimental) and MCF-7 (control) cells.

## 3. References


1. J. Liu and Y. Lu, *Nat. Protoc.*, 2006, **1**, 246-252.
2. N. M. Robertson, M. Salih Hizir, M. Balcioglu, R. Wang, M. S. Yavuz, H. Yumak, B. Ozturk, J. Sheng and M. V. Yigit, *Langmuir*, 2015, **31**, 9943-9952.