Supporting Information

MicroRNA Conjugated Gold Nanoparticles and Cell Transfection

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Experimental Details:

Chemicals. Hydrogen tetrachloroaurate (III) hydrate (HAuCl₄) (Strem), sodium citrate (99%) (Sigma-Aldrich), diethyl pyrocarbonate (DEPC) (Sigma-Aldrich), oligoethylene glycol thiol (OEG) (ProChimia), sodium chloride (Sigma-Aldrich) and phosphate buffer (0.05M, pH=7) (Fisher Scientific) were used as received. Water was purified with a Millipore Milli-Q water system to achieve a final resistance of 18.2 MΩ.

MiRNA oligonucleotides were obtained from Integrated DNA Technologies. The sequences included a sense strand (miR-130b, i.e., 5′-/Phos/CAG UGC AAU GAU GAA AGG GCA UAC/iSp18//iSp18//3ThioMC3-D/-3′), anti-sense strand (miR-130b*, 5′-ACU CUU UCC CUG UUG CAC UAC GCA U-3′), Cy3 luciferase (5′-/5cy3/ACU CUU UCC CUG UUG CAC UAC GCA U-3′), and Cy5 luciferase (5′-/5cy5/ACU CUU UCC CUG UUG CAC UAC GCA U-3′). MiRNAs were reconstituted using phosphate buffer, to final concentrations between 200 and 515 μM and used as received. No further purification was performed.

A multiple myeloma cell line (MM.1S) was used for testing cell transfection. The cells were grown in suspension in RPMI-1640 supplemented with 10% fetal bovine serum (GIBCO/Invitrogen).

Methods. The synthesis of citrate-capped gold nanoparticles of 13-nm diameter (AuNPs) followed the reported procedure described previously¹. Briefly, freshly prepared sodium citrate (38.8 m, 5 mL) was added to a boiling solution containing 1 mM HAuCl₄ (45 mL). The solution

was heated for an additional 30 min. The resulting nanoparticle solution displayed a ruby-red color.

The surface modification of the citrate-capped AuNPs with miRNAs followed a procedure similar to that reported for modifying AuNPs with siRNA². Briefly, sense and antisense miRNA were combined and heated at 60 °C to form double stranded RNA. Then the RNA duplexes were added to a solution of AuNPs that were previously treated overnight with 0.1% diethyl pyrocarbonate (DEPC) to deactivate RNase and subsequently autoclaved to remove the DEPC and to sterilize the solution. The amount of miRNA added to the solution was sufficient for 10 to 25 miRNA molecules per nanoparticle. Then oligoethyleneglycol thiol (OEG) (30 μ mol/mL final concentration) was added to further stabilize the surface of the particle, by keeping the RNA from folding and electrostatically binding to the surface of the nanoparticle. Following synthesis, the particles were purified using centrifugation, and the supernatant was removed. The nanoparticles were re-suspended in phosphate buffered saline (PBS) to achieve a final particle concentration of 25 nM, with a miRNA concentration of 0.25-0.62 μ M.

Gel electrophoresis using a 1% agarose gel was performed to confirm the nanoparticle modification was complete. Four samples were run: unmodified, citrate capped nanoparticles; citrate capped nanoparticles with bis(p-sulfonatophenyl)phenylphosphine potassium dihydrate (BP); the miRNA-modified nanoparticles; and miRNA-modified particles after reaction with the BP. A voltage of 110 V was applied for approximately 1 hour.

Passive nanoparticle uptake was performed using the following protocol: Myeloma cells were suspended in RPMI medium at a concentration of ~0.5 million cells/mL. 2-mL of a cell suspension was placed in each well of a 6-well plate. The samples of nanoparticles are added drop-wise to the wells to achieve the desired final concentration (0 to 2 nM). The plate was gently tilted to homogenize the well contents. Cells were incubated and nanoparticle uptake was analyzed under a fluorescent microscope at 48 hours. Since the miRNAs were tagged with fluorescent Cy3 or Cy5, the delivery of the miRNA to target sites was monitored.

Analysis of expression of target genes was performed using a functional luciferase assay³. One nanomole of GR3'UTR Luciferase Reporter was cotransfected with Renilla Luciferase using Amaxa Nucleofector 1 and Nucleofector Kit V. The Cy3 and Cy5 labeled miRNA-NPs were introduced to the MM.1S cells 24 hours after reporter transfection, no additional transfection agent was used. A miR-130b mimic was used for a positive control experiment. Final solution concentrations of 0.25, 0.5, 1 and 2 nM of miRNA-AuNPs were used, and 0, 0.2, 0.6, 2 nM final miRNA concentration for the mimic experiment. Cells were harvested 48 hours after transfection for analysis, and lysed using buffer from Promega Dual Luciferase Reporter Kit following the manufacturer's protocol. The ratio between firefly luciferase and renilla was recorded.

Instrumentation. Ultra-violet Visible (UV-Vis) spectra were acquired with a Hewlett-Packard 8453 spectrophotometer. The spectra were collected over a range of 480 to 1100 nm.

Transmission Electron Microscopy (TEM) was performed on a Hitachi H-7000 electron microscope (100 kV). TEM samples were collected for assembly solutions of miRNAs and nanoparticles. The samples were prepared by casting onto a carbon coated copper grid sample holder followed by evaporation at room temperature.

SERS spectra were measured by an Advantage 200A Raman spectrometer (DeltaNu). The instrument collects data over 200-3400 cm^{-1} . The laser power was 5 mW, and the wavelength of the laser was 632.8 nm. The spectral resolution of the system was about 10 cm^{-1} . Signal integration times of 30 or 60 seconds were used. Samples of dried nanoparticle solutions on a gold surface were analyzed.

Gel electrophoresis data were obtained using a Pharmacia voltammeter. A typical experiment used 1% agarose gel at 110 V, and phosphate buffer (pH 10) as the running buffer with a run time of up to 2 hours.

Confocal microscopy was done using a Zeiss confocal UV LSM Meta microscope. As a cellular uptake control for the imaging, a Cy5 labeled NP containing no RNA was used (not included).

A Turner Designs 20/20 model luminometer was used to measure the luciferase activity for the functional luciferase assay.

Estimate of the theoretical surface coverage of miRNAs on the AuNPs: For a typical 10-mL solution of 16 nM AuNPs, the total nanoparticle surface area is $4\pi(6.5 \times 10^{-7} \text{ cm})^2 \times (0.01 \text{ L}) \times (1.62 \times 10^{-8} \text{ M}) \times (6.02 \times 10^{23}) = 5.2 \times 10^2 \text{ cm}^2$. Assuming a diameter of 2.6 nm for miRNA duplexes, each duplex covers $\pi(1.3 \times 10^{-7} \text{ cm})^2 = 5.3 \times 10^{-14} \text{ cm}^2$. Assuming hexagonal close packing, the packing density would be $1/((\sin 60^\circ) \times (1.3 \times 10^{-7} \text{ cm})^2) = 7 \times 10^{13}/\text{cm}^2$. Therefore, a full monolayer coverage of miRNA would require $5.2 \times 10^2 \text{ cm}^2 \times 7 \times 10^{13}/\text{cm}^2 = 4 \times 10^{16}$ miRNAs, which corresponds to a final miRNA concentration of $\sim 6 \text{ }\mu\text{M}$ in the 10-mL solution, or ~ 350 miRNA per nanoparticle.

Additional schemes:

Scheme S1. An illustration of the interaction between miR-130b and an antisense strand for the double-stranded miRNA.

Scheme S2. The structures for the fluorescent dyes, Cy3 and Cy5, which were used for labeling miRNAs (A), the oligo ethylene glycol thiol (B), and the bis(p-sulfonatophenyl)phenylphosphine ion (BP).

Additional data:

Figure S1. UV-Vis spectra of AuNPs before (black) and after (red) conjugation of miRNAs.

Figure S2. SERS spectra. (I) in 400 - 1800 cm^{-1} region for miRNA-AuNPs (A) and citrate-capped AuNPs (B) (60 second integration), and (II) in 400 - 1100 cm^{-1} (a) and 1000 - 1800 cm^{-1} regions (b) (30 second integration). (Samples were deposited on a gold thin film substrate.)

Figure S3. Microscopic images of the MM.1S cells prior to cell transfection (A) and after cell transfection with the Cy5-miRNAs-AuNPs (B).

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S.I. References:

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