

Supplementary Information

**DNA/RNA chimera indicates the flexibility of backbone influences
encapsulating fluorescent AgNCs emitters**

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Method and Materials

Materials and Reagents

DNA, RNA and DNA/RNA chimera probes were obtained from commercial supplier: IDT (Integrated DNA Technologies, BVBA, Interleuvenlaan 12A, 3001 Leuven, Belgium). The synthesis of emissive AgNCs was carried out using AgNO₃ (99.9999%) and NaBH₄ (99.99%) from Sigma Aldrich. Tris-Acetate buffer (pH 7, 0.5 M) was prepared with TRIZMA® acetate salt (≥99.0%, from Sigma Aldrich) in pure Milli-Q water (18.2MΩ.cm). NaCl (5mM) solution was prepared with NaCl salt (≥98.0%, from Sigma Aldrich) in pure Milli-Q water.

Synthesis of nucleic acid/AgNCs

Fluorescent nucleic acid/AgNCs were prepared by incubating nucleic acids (15μM) with 10mM Tris Acetate buffer and 0.5mM NaCl for all the probes with Let-7a target sensing sequence. Fluorescent nucleic acid/AgNCs were prepared by incubating nucleic acids (15μM) with 20mM Tris Acetate buffer for all the probes with 160 target sensing sequence. NaCl addition does not affect fluorescence emission from 160 target sensing probes but enhanced emission was observed for nucleic acid probes with Let-7a target sensing sequences. Nucleic acids incubated with buffer were denatured by incubating at 95°C/10 mins. The solutions were then incubated at 25°C/20 mins to allow them to form structure. The renaturation step was followed by an addition of AgNO₃ (250 μM) and NaBH₄ (250 μM), (1:17:17) to a final volume (50 μl). All the nucleic acid/AgNCs were incubated for 1h at 25°C and diluted with 450 μl of distilled water just before measurement on a fluorimeter (Horiba Jobin Yvon, Fluoromax-4) in a 10 mm disposable cuvette. We here designated the concentrations of nucleic acids and buffer in the 50 μl reaction mixture.

Circular dichroism (CD) spectroscopy

All CD spectra were recorded twice on a Jasco J-815 spectropolarimeter, in the data interval 330 nm - 190 nm with a 1 nm step and a scan rate of 50 nm/min, a 4 sec integration time and a 2 nm

band width. A sample column of 180 μ L was used in a 1 mm Hellma Quartz cell. Spectra were collected at 25 deg C. Conditions and buffer for Let-7a: 3.75 μ M nucleic acid, in 2.5 mM tris acetate buffer at pH 6.5 with 0.125 mM NaCl. For R160 the nucleic acid concentration was 15 μ M in 5 mM Tris-acetate at pH 6.5. The R160 data was divided by x4 for allow for direct comparison with Let-7a data (e.g. concentration normalization). For all spectra equivalent reference spectra were recorded and subsequently subtracted in the Jasco Software Spectra Analysis, where a Savitzky–Golay smoothing was also applied.

Capillary electrophoresis

Capillary electrophoresis (CE) is a useful tool in the analysis of DNA/AgNC probes. Experiments were performed with a Hewlett Packard 3D Capillary Electrophoresis apparatus with an internal diode array Uv-vis spectrophotometer as detector. A silica capillary (64.5 cm x 50 μ m i.d.) with 56.0 cm to the detection window was used. The capillary was in every run flushed with 0.1 M NaOH for two minutes, followed by wash with buffer for another two minutes. Samples were applied for 10 s at a pressure of 50 mbar and the run was started with positive to negative potential. All experiments were performed at 20 kV and 15 $^{\circ}$ C. Buffer was in all runs 10 mM Tris-Acetate, 0.5 mM sodium chloride, pH = 6.5 in the RED-let7a system and 20 mM Tris-Acetate pH 6.5 in the RED-160 system. The buffers in the inlet and outlet reservoirs were changed after each run to avoid pH changes. All CE experiments were performed at least twice.

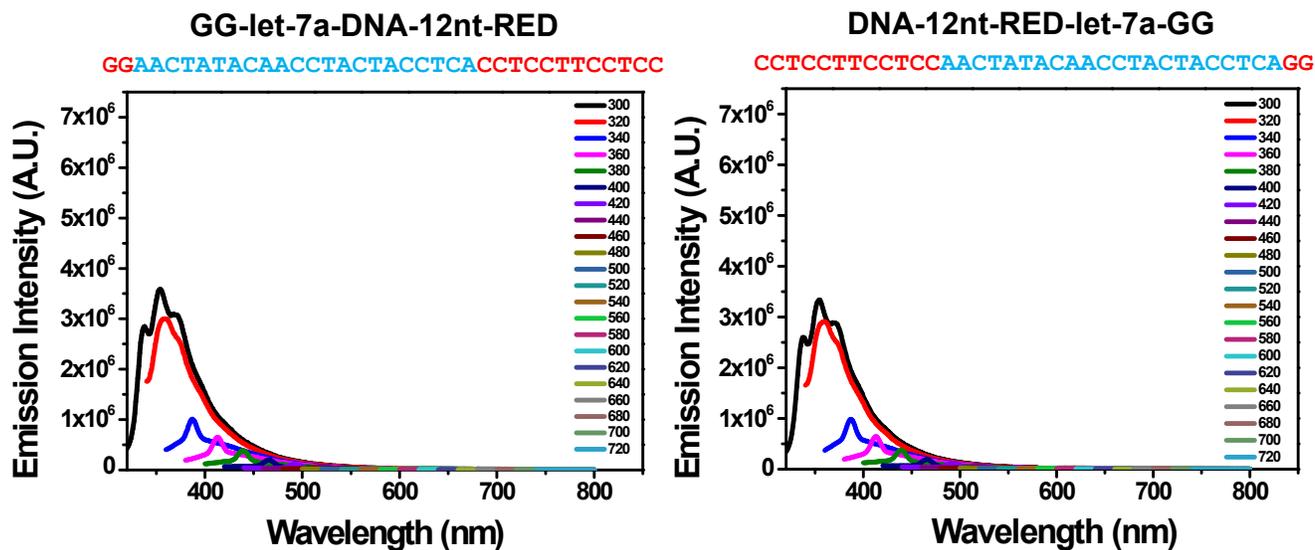


Figure S1. Emission spectra of AgNCs modified GG-let-7a-DNA-12nt-RED (1.5 μ M) and DNA-12nt-RED-let-7a-GG (1.5 μ M) probes. The probes were reconstituted to provide a possible secondary structure to let-7a sensors. Extra two guanine bases added at the opposite end of DNA-12nt-RED scaffold to induce self-dimer or hair-pin structures. The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C.

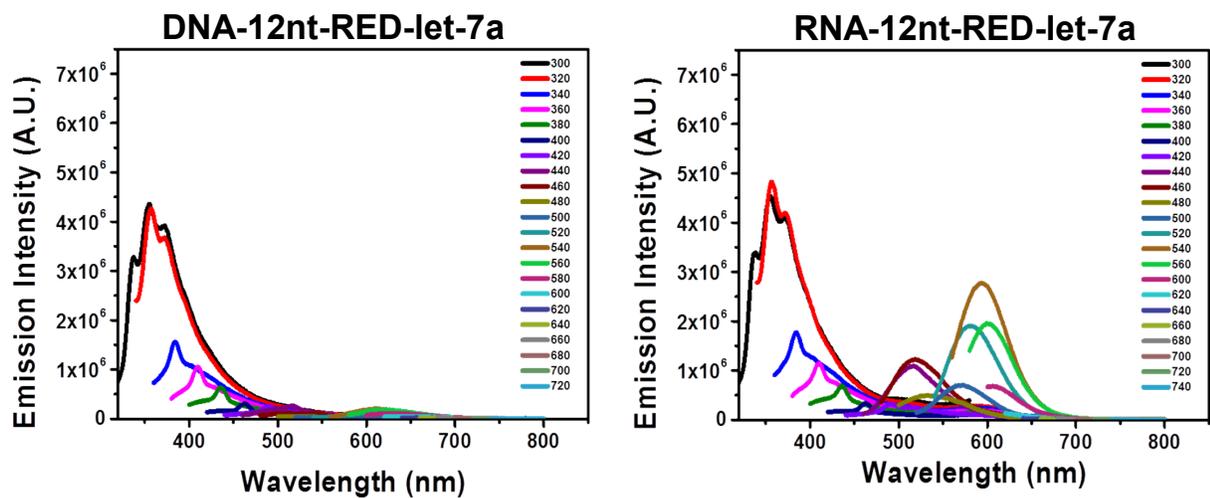


Figure S2. Emission spectra of AgNCs modified RNA-12nt-RED-let-7a (1.5 μM) and DNA-12nt-RED-let-7a (1.5 μM) probes. The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C.

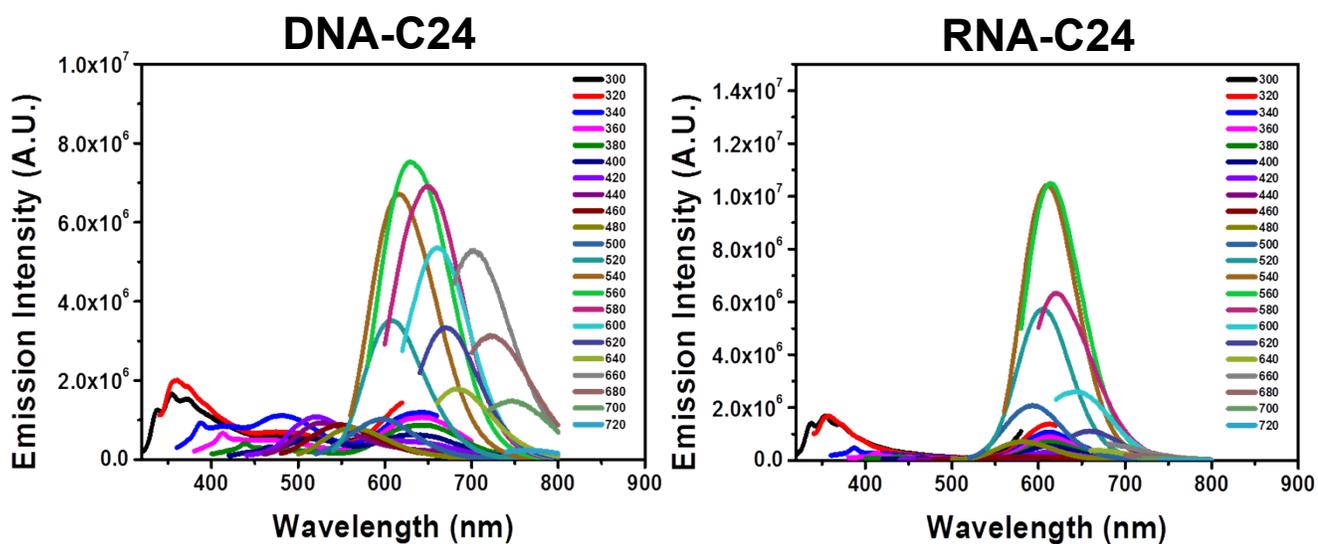


Figure S3. Emission spectra of AgNCs modified DNA-C24 (15 μM) and RNA-C24 (15 μM) probes. The probes were constituted with to poly $[\text{dC}_n]_{n=24}$ and poly $[\text{rC}_n]_{n=24}$, respectively. The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C.

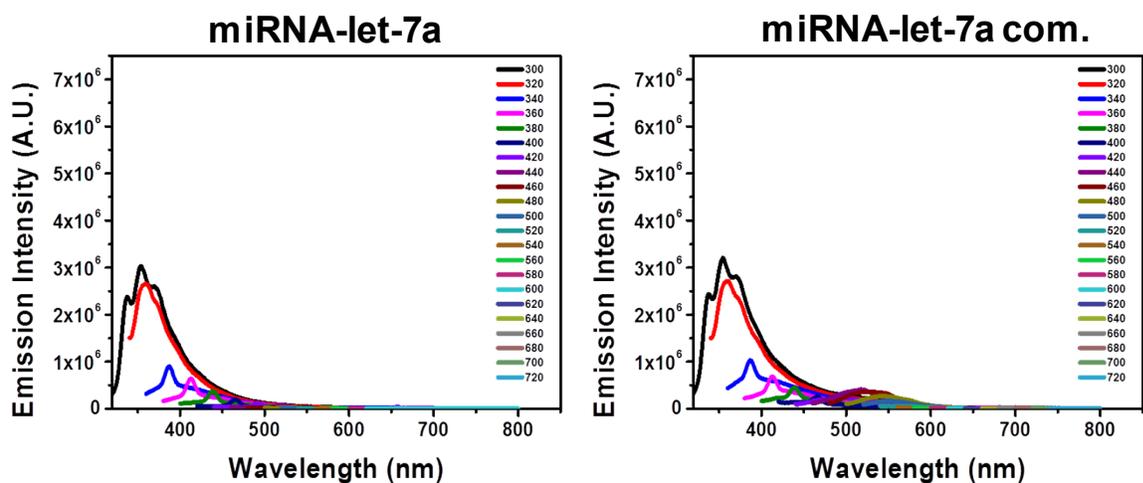


Figure S4. Emission spectra of AgNCs modified miRNA-let-7a (1.5 μM) and miRNA-let-7a complementary sequences (1.5 μM). The former is miRNA-let-7a sequences (UGAGGUAGUAGGUUGUAUAGUU) and the latter is complementary RNA sequences against miRNA-let-7a (AACUAUACAACCUACUACCUCA), respectively. The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C. 40 miRNAs were tested as templates for emissive AgNCs but none of them was fluorescence. We show two examples of them.

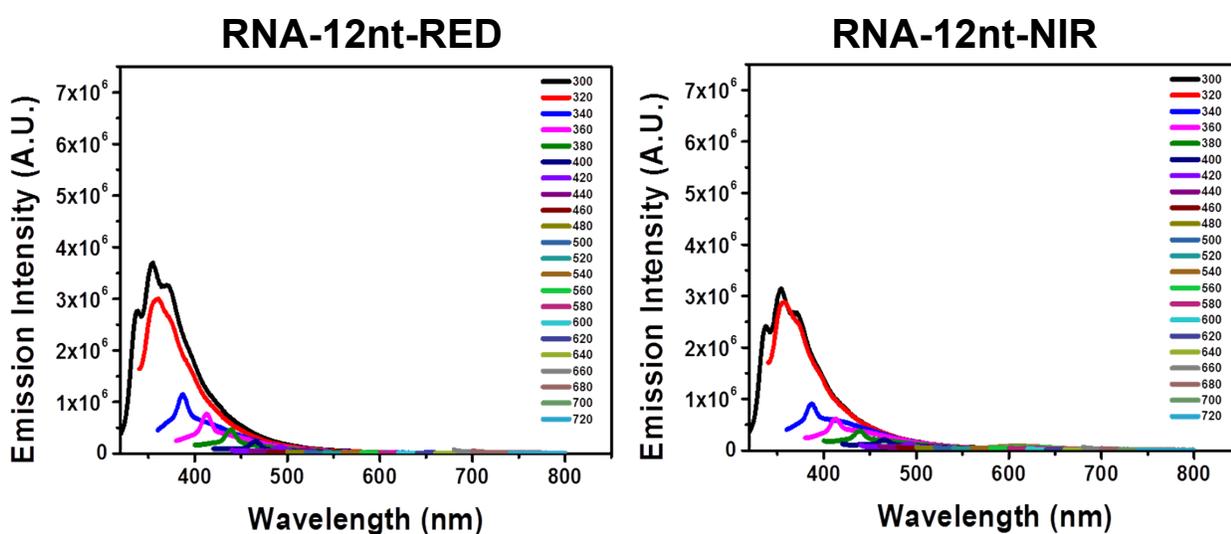


Figure S5. Emission spectra of AgNCs modified RNA-12nt-RED (1.5 μM) and RNA-12nt-NIR (1.5 μM) sequences. The former is the RNA conversion of DNA-12nt-RED scaffold (CCUCCUCCUCC) and the latter is the RNA conversion of DNA-12nt-Near Infra-Red scaffold (CCCUAACUCCCC), respectively⁷. The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C.

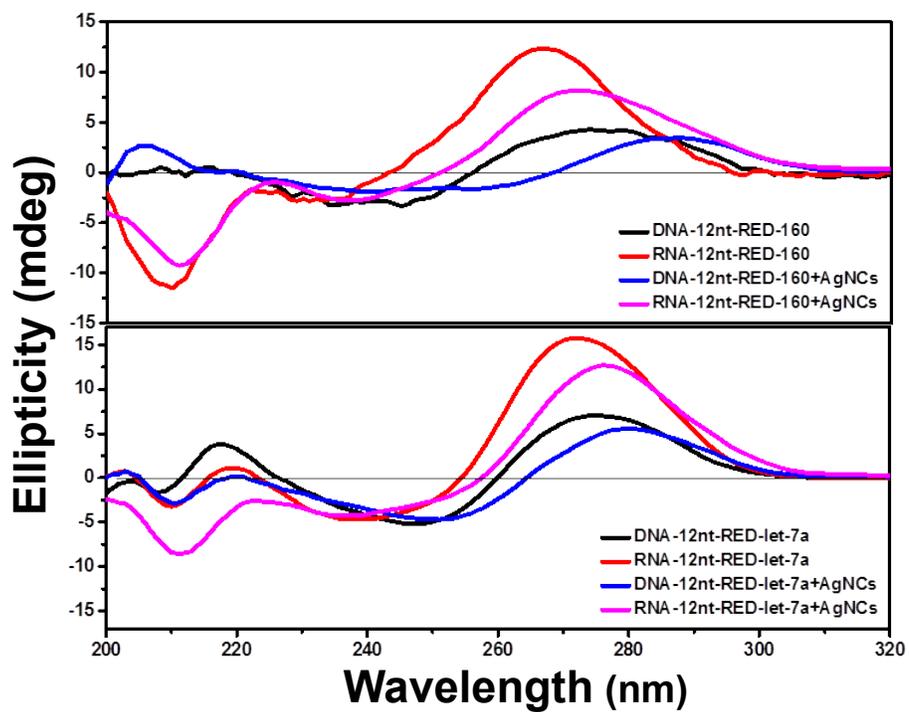


Figure S6. UV-Circular dichroism spectra for nucleic acid/AgNCs probes. Top panel: DNA-12nt-RED-160 and RNA-12nt-RED-160 with and without AgNCs. Bottom panel: DNA -12nt-RED-let7a and RNA-12nt-RED-let7a with and without AgNCs.

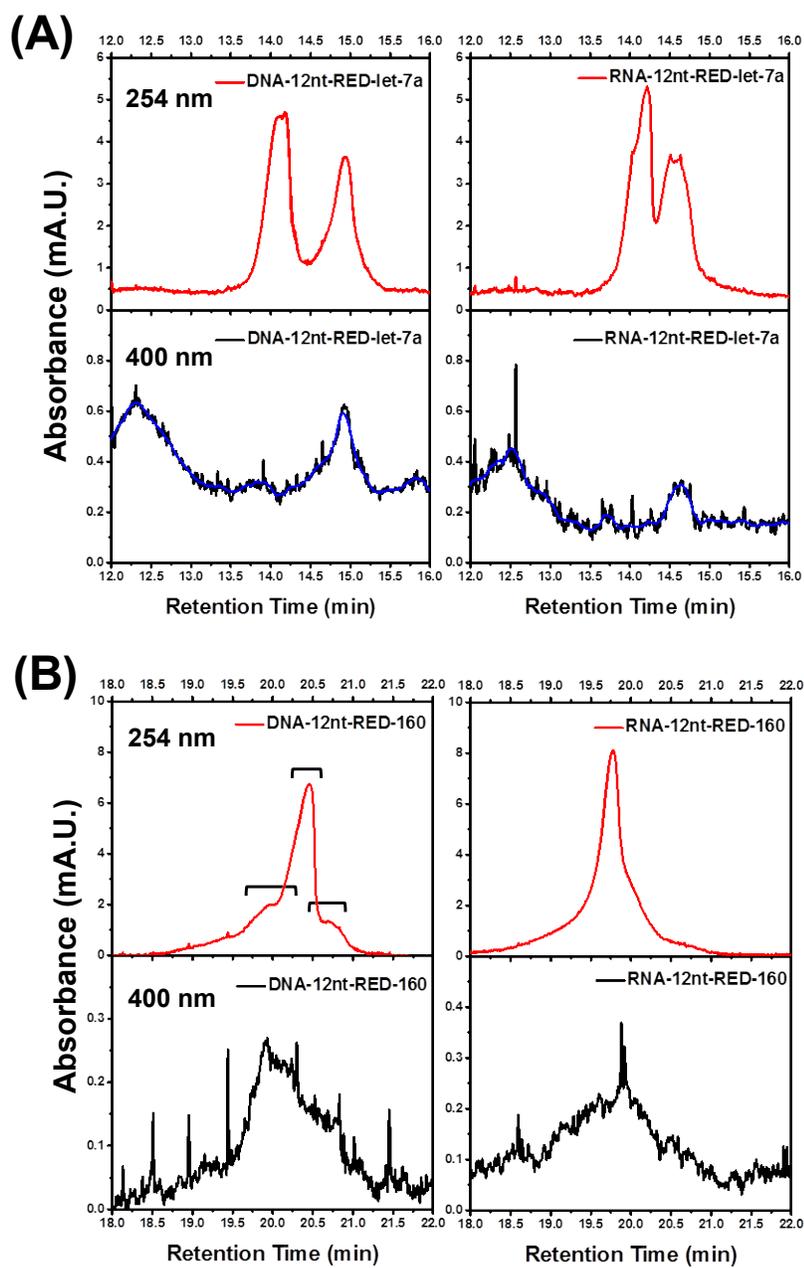


Figure S7. (A) Electropherograms by capillary electrophoresis. Retention peaks of DNA-12nt-RED-let7a and RNA-12nt-RED-let7a modified with AgNCs. (B). Retention peaks of DNA-12nt-

RED-160 and RNA-12nt-RED-160 modified with AgNCs. Absorbance measured at 254nm wavelength (shown in red) and 400nm wavelength (shown in black).

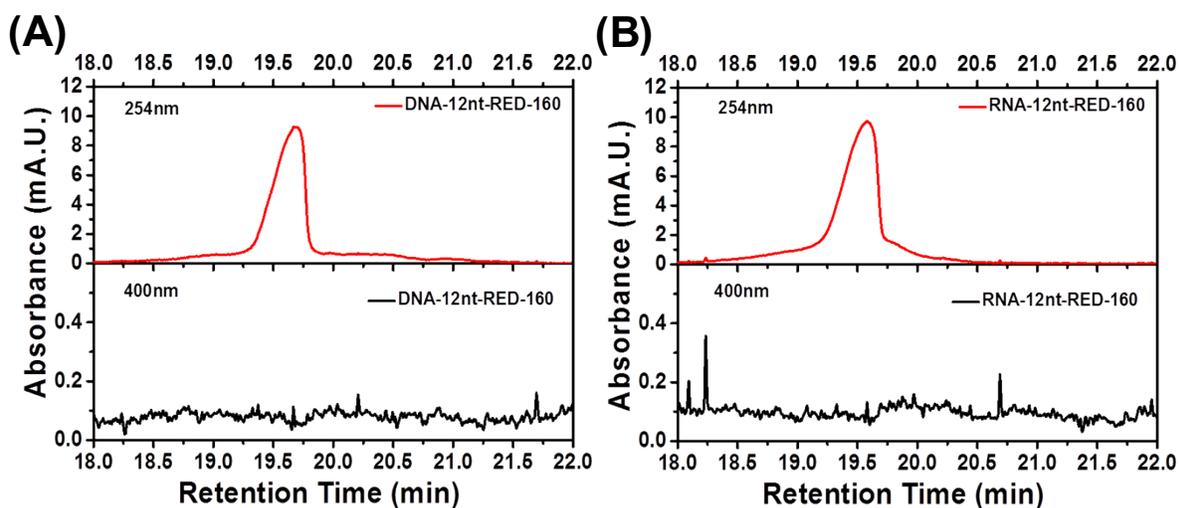


Figure S8. Electropherograms by capillary electrophoresis. (A) Retention peaks of DNA-12nt-RED-160 and (B) RNA-12nt-RED-160 without AgNCs (unmodified). Absorbance measured at 254nm wavelength (shown in red) and 400nm wavelength (shown in black).

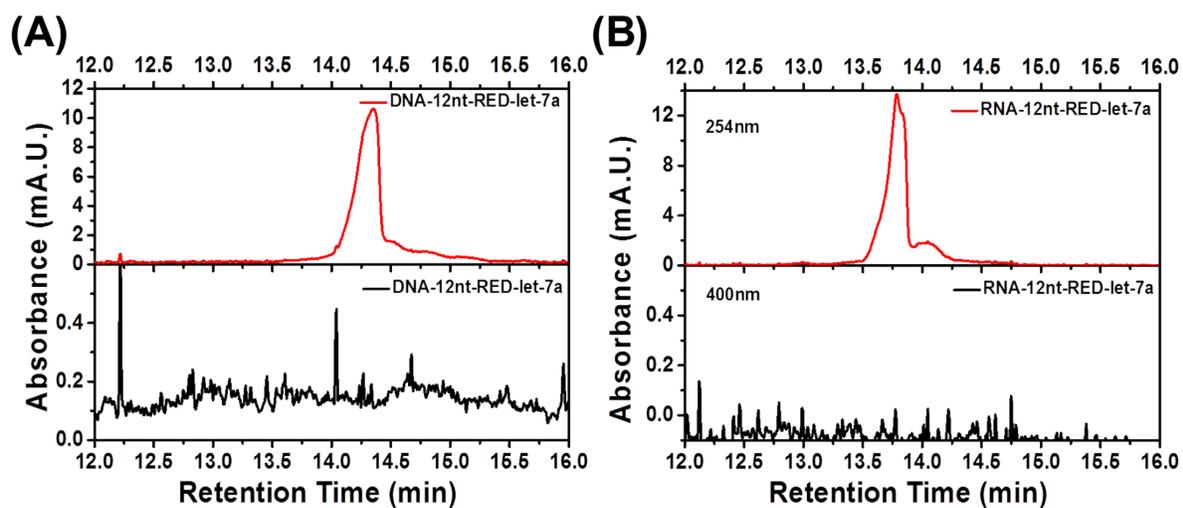


Figure S9. Full electropherograms by capillary electrophoresis. (A) Retention peaks of DNA-12nt-RED-let-7a and (B) RNA-12nt-RED-let-7a without AgNCs (unmodified). Absorbance measured at 254nm wavelength (shown in red) and 400nm wavelength (shown in black).

DNA-12nt-RED-let-7a	d _d C _d d _d T _d d _d C _d d _d T _d d _d T _d d _d C _d d _d T _d d _d C _d d _d A _d d _d A _d d _d C _d d _d T _d d _d A _d d _d T _d d _d A _d d _d C _d d _d A _d d _d A _d d _d C _d d _d T _d d _d A _d d _d C _d d _d T _d d _d A _d d _d C _d d _d T _d d _d C _d d _d A
DNA-22nt-R12-let-7a	d _d C _d d _d T _d d _d C _d d _d T _d d _d T _d d _d C _d d _d T _d d _d C _d d _d T _d d _d C _d d _d A _d d _d A _d d _d C _d d _d T _d d _d A _d d _d T _d d _d A _d d _d C _d d _d A _d d _d A _d d _d C _d d _d R _d d _d C _d d _d U _d d _d A _d d _d C _d d _d U _d d _d A _d d _d C _d d _d U _d d _d A _d d _d C _d d _d U _d d _d C _d d _d A
DNA-17nt-R17-let-7a	d _d C _d d _d T _d d _d C _d d _d T _d d _d T _d d _d C _d d _d T _d d _d C _d d _d T _d d _d C _d d _d A _d d _d A _d d _d C _d d _d T _d d _d A _d d _d U _d d _d A _d d _d C _d d _d A _d d _d A _d d _d C _d d _d U _d d _d A _d d _d C _d d _d U _d d _d A _d d _d C _d d _d U _d d _d C _d d _d U _d d _d C _d d _d A
DNA-12nt-R22-let-7a	d _d C _d d _d U _d d _d C _d d _d U _d d _d U _d d _d C _d d _d U _d d _d C _d d _d A _d d _d A _d d _d C _d d _d U _d d _d A _d d _d U _d d _d A _d d _d C _d d _d A _d d _d A _d d _d C _d d _d U _d d _d A _d d _d C _d d _d U _d d _d A _d d _d C _d d _d U _d d _d C _d d _d U _d d _d C _d d _d A
RNA-12nt-RED-let-7a	r _r C _r r _r U _r r _r C _r r _r U _r r _r U _r r _r C _r r _r U _r r _r C _r r _r C _r r _r A _r r _r A _r r _r C _r r _r U _r r _r A _r r _r U _r r _r A _r r _r C _r r _r A _r r _r A _r r _r C _r r _r U _r r _r A _r r _r C _r r _r U _r r _r A _r r _r C _r r _r U _r r _r C _r r _r A

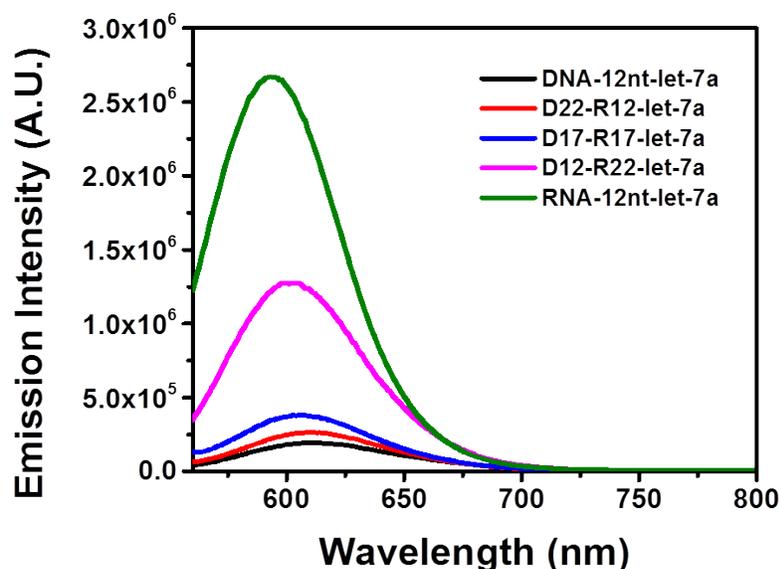


Figure S10. Sequence of DNA-RNA chimera-let-7a probes for the flexibility test. Each DNA-RNA chimera sequence is given in two colors: red shows cytosine-rich scaffold (DNA-12nt-RED) while blue shows target miRNA sensing sequence (miR-Let7a). Prefix d-and r-refers to deoxyribose and ribose sugar bases of DNA and RNA, respectively. Graph; Emission spectra of

the three AgNCs modified DNA/RNA chimera let-7a-RED probes together with the two AgNCs modified controls (RNA-12nt-RED-let-7a and DNA-12nt-RED-let-7a).

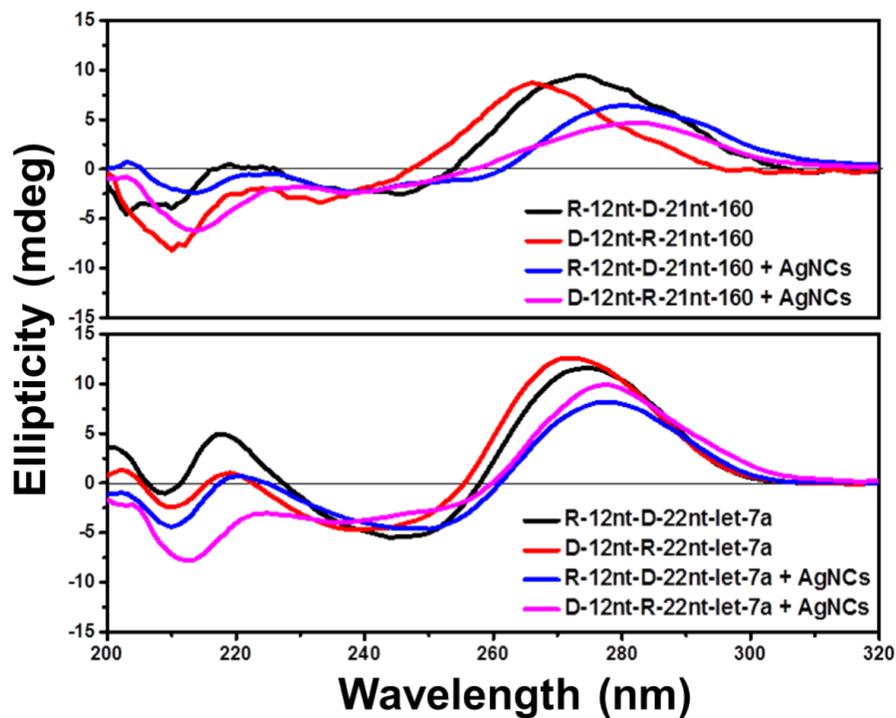
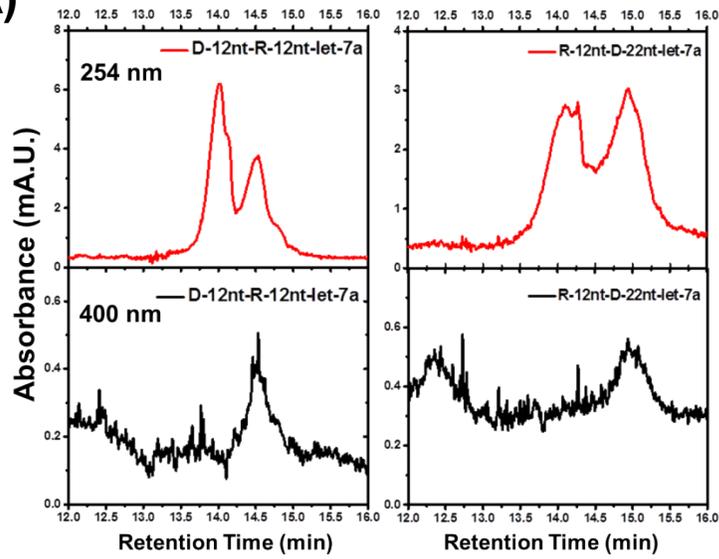


Figure S11. UV-Circular dichroism spectra for DNA-RNA chimera/AgNCs probes. Top panel: D-12nt-R-21nt-160 and R-12nt-D-21nt-160 with and without AgNCs. Bottom panel: D-12nt-R-21nt-let7a and R-12nt-D-21nt-let7a with and without AgNCs.

(A)



(B)

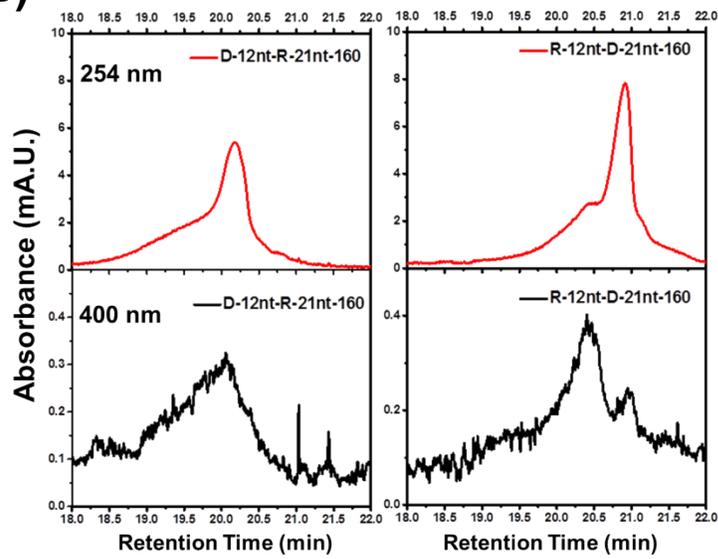


Figure S12. Electropherograms by capillary electrophoresis. (C) Retention peaks of D-12nt-R-21nt-let7a and R-12nt-D-21nt-let7a modified with AgNC. (D) Retention peaks of D-12nt-R-21nt-160 and R-12nt-D-21nt-160 modified with AgNC. Absorbance measured at 254nm wavelength (shown in red) and 400nm wavelength (shown in black).

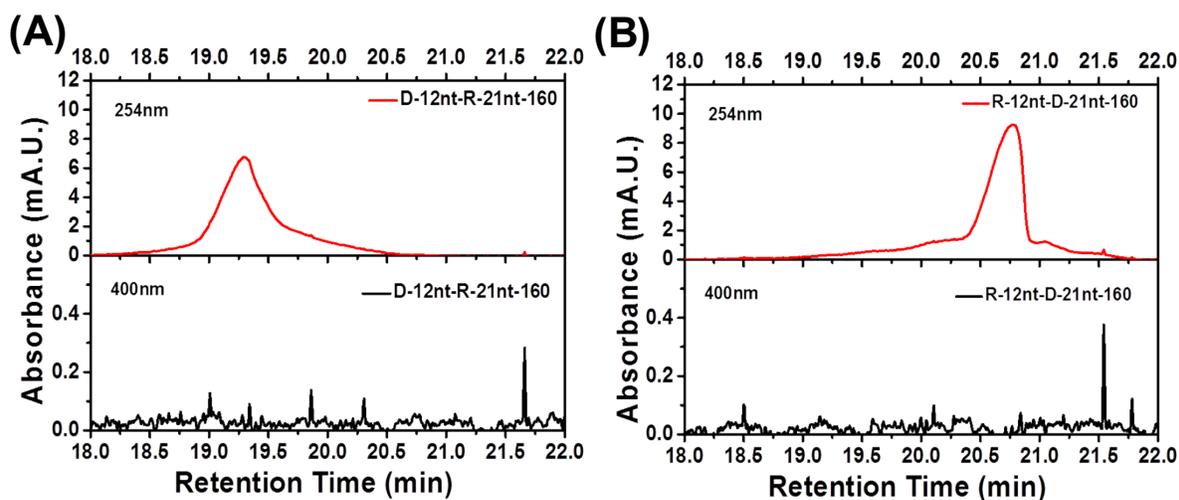


Figure S13. Electropherograms by capillary electrophoresis. (A) Retention peaks of D-12nt-R-21nt-160 and (B) R-12nt-D-21nt-160 without AgNCs (unmodified). Absorbance measured at 254nm wavelength (shown in red) and 400nm wavelength (shown in black).

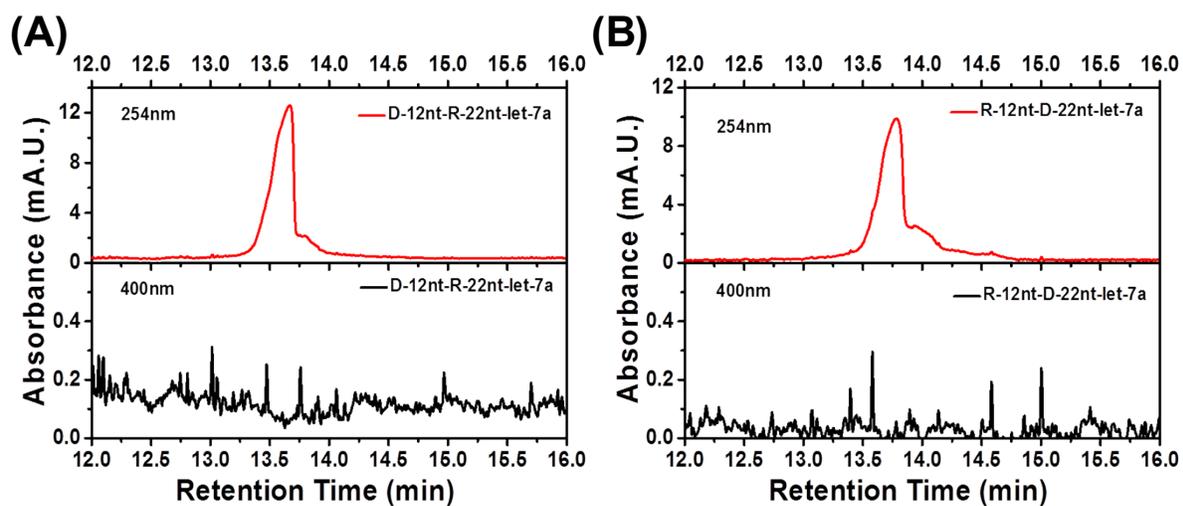


Figure S14. Electropherograms by capillary electrophoresis. (A) Retention peaks of D-12nt-R-22nt-let7a and (B) R-12nt-D-22nt-let7a without AgNCs (unmodified). Absorbance measured at 254nm wavelength (shown in red) and 400nm wavelength (shown in black).

RNA-12nt-RED-let-7a C→G

C_r**C**_r**U**_r**C**_r**C**_r**U**_r**U**_r**C**_r**C**_r**U**_r**C**_r**C**_r**A**_r**A**_r**G**_r**U**_r**A**_r**U**_r**A**_r**G**_r**A**_r**A**_r**G**_r**G**_r**U**_r**A**_r**G**_r**U**_r**A**_r**G**_r**G**_r**U**_r**G**_r**A**

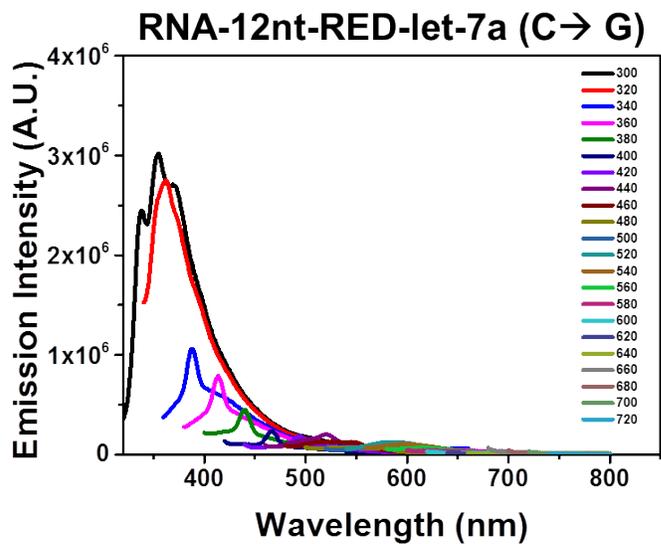


Figure S16. Emission spectra of AgNCs modified RNA-12nt-RED-let-7a (C→G) (1.5 μ M). The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C. To induce possible stable structures (A-form configured structures) by C≡G base pairs, 8 cytosines in the complementary sequences against miRNA let-7a were substituted with 8 guanines.

RNA-12nt-RED-160 G->C

C C U C C U U C C U C C U C C C A U A C A C C C A C C C A C C C A

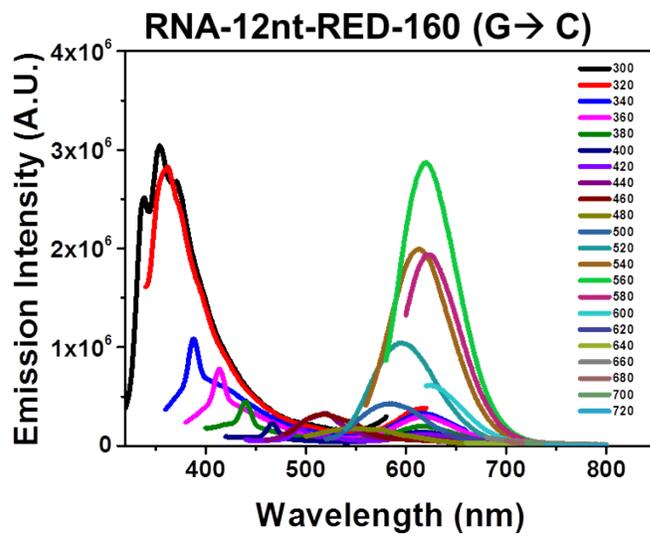


Figure S17. Emission spectra of AgNCs modified RNA-12nt-RED-160 (G→C) (1.5 μM). The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C. To block stable hair-pin or dimer structures through C≡G base pairs, 8 guanines in the complementary sequences against miRNA miR160 were substituted with 8 cytosines.

DNA-12nt-RED-let-7a-Uracil C_dC_dT_dC_dC_dT_dT_dC_dC_dT_dC_dC_dA_dA_dC_dU_dA_dU_dA_dC_dA_dA_dC_dC_dU_dA_dC_dU_dA_dC_dC_dU_dC_dA_d

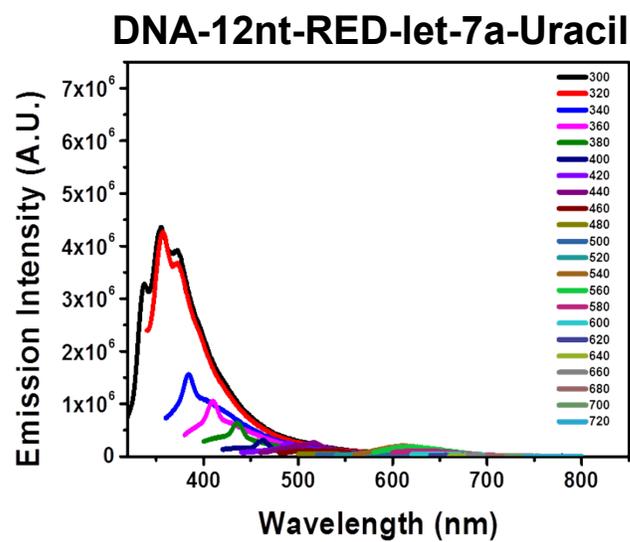


Figure S18. Emission spectra of AgNCs modified DNA-12nt-RED-let-7a-Uracil (1.5 μ M). The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C.

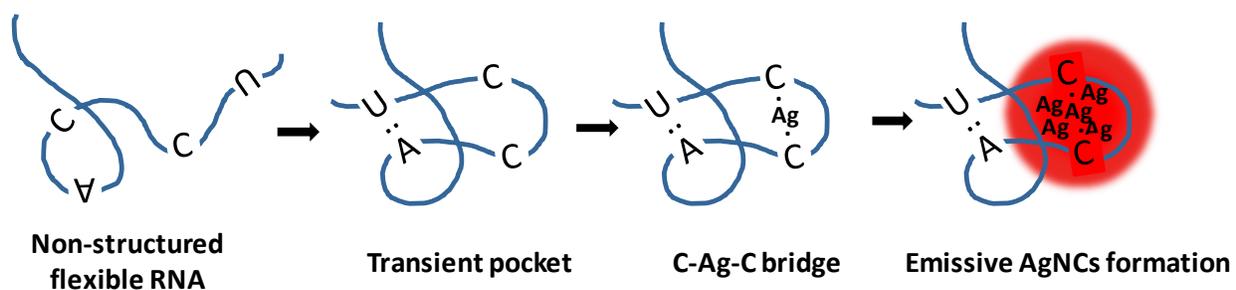


Figure S19. A schematic cartoon shows the suggested hypothesis of emissive AgNCs formation in a flexible RNA strand. Non self-complementary RNA is competent to form a transient pocket if it contains random A and U bases. The flexibility of RNA backbone appears to be important to induce the transient structure. The transient structure may increase the probability of cytosine to cytosine encountering and subsequent C-Ag⁺-C formation. Although only one base pairs of A=U and C≡G were exemplified in the cartoon, we speculate that serial cytosine bases may be required to generate strong fluorescence.