

Multiplex miRNA detection using DNA-templated silver nanocluster probes

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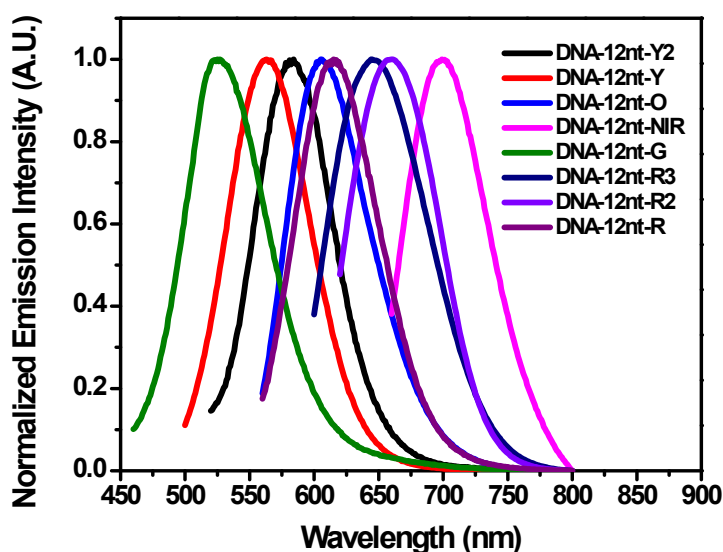
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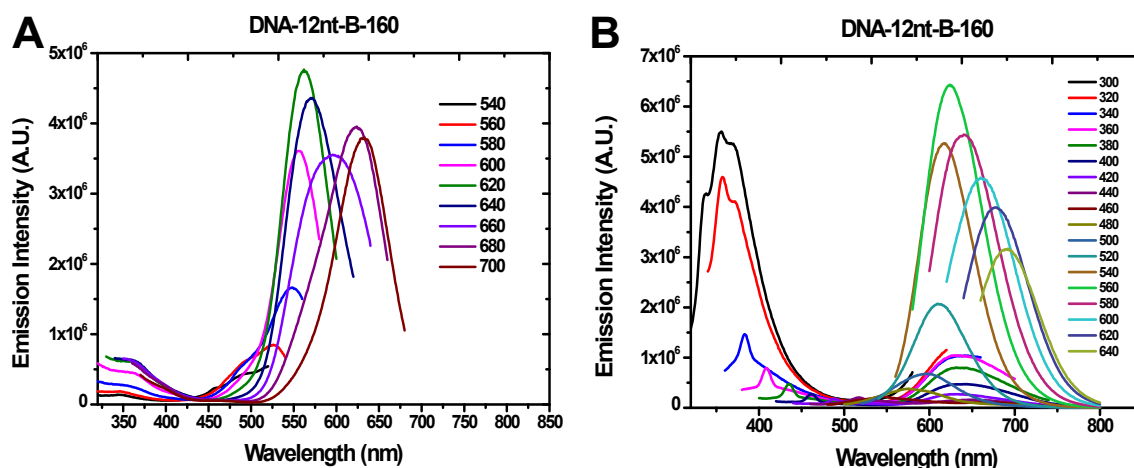
Scaffold	Sequence	Color	Reference	Em= λ max	Emission Intensity	Conc.
DNA-12nt-R	CCTCCTTCCTCC	Red	Richards et,al.	620nm	8.73E+05	15 μ M
DNA-12nt-B	CCCTTTAACCCC	Blue (ND)	Richards et,al.	ND	ND	ND
DNA-12nt-G	CCCTCTTAACCC	Green	Richards et,al.	520nm	1.04E+06	15 μ M
DNA-12nt-Y	CCCTTAATCCCC	Yellow	Richards et,al.	572nm	2.01E+06	15 μ M
DNA-12nt-Y2	CCCTTAATTCCTCC	Yellow2	This study	584nm	5.17E+05	15 μ M
DNA-12nt-NIR	CCCTAACTCCCC	NI-RED	Richards et,al.	700nm	6.30E+06	15 μ M
DNA-12nt-O	CCCTCACTCCCC	Orange	This Study	605nm	2.30E+06	15 μ M
DNA-12nt-R2	CCCATATTCCCC	Red2	Sandeep et, al.	657nm	2.33E+07	15 μ M
DNA-12nt-R3	CCCTATAACCCC	Red3	Sandeep et, al.	644nm	1.19E+07	15 μ M

R:RED, B:BLEU, G:GREEN, Y:YELLOW, Y2:YELLOW2, NIR:NEAR-INFRA RED,
O:Orange, R2:RED2, R3:RED3, ND:Not determined in this study

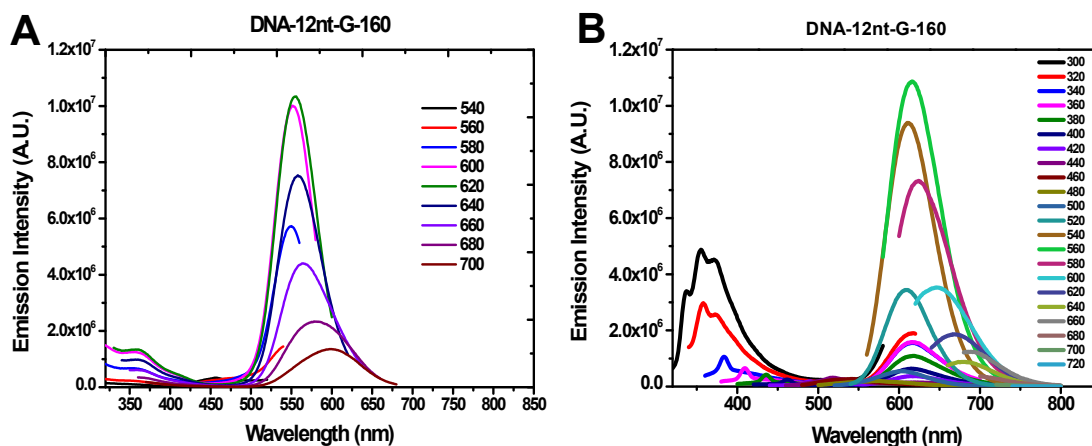
SI Table 1: Details of the DNA-12nt Scaffold sequences. Emission wavelengths and Emission intensity refers to the maximum emission measured after 24 hours of incubation at 4°C under the conditions mentioned in the reference. DNA concentration used for the experiments is 15 μ M. AgNO₃ and NaBH₄ were added in the ratio of (1:6:6).



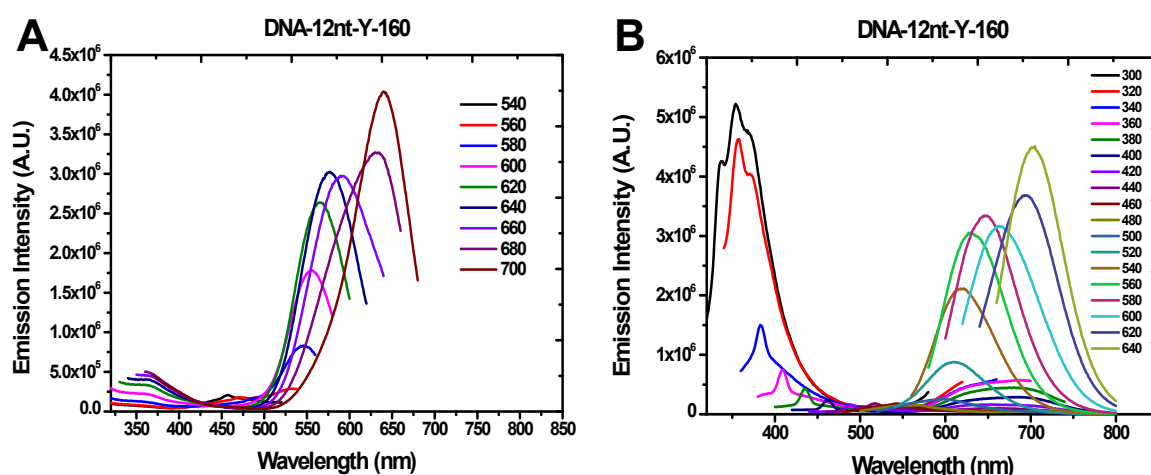
S1 Figure 1: Emission spectra of the eight DNA-12nt scaffolds. The maximum emission spectra of each DNA-12nt scaffold were recorded 24 hour at 4°C after mixing and reducing the DNA/AgNO₃ mixture with NaBH₄.



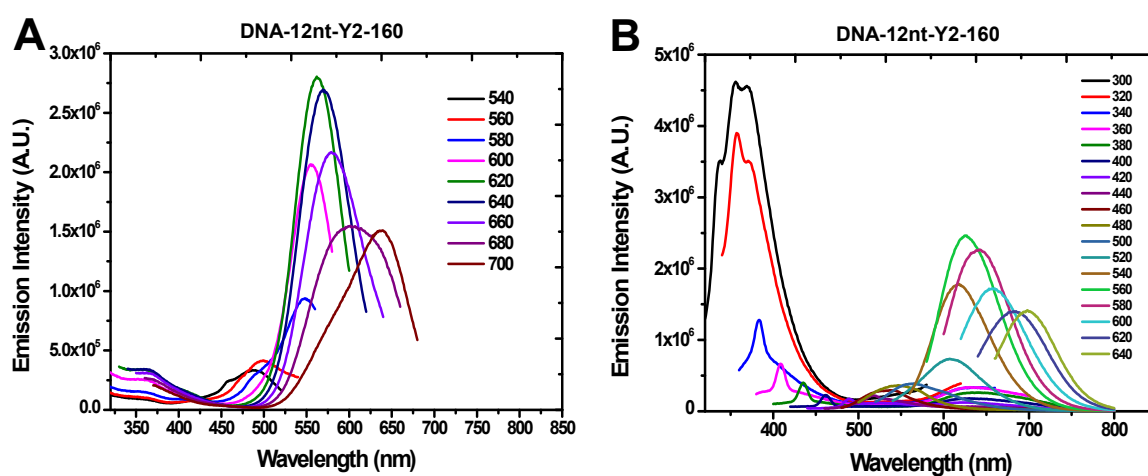
SI Figure 2: A) Excitation spectra of a 1.5 μM DNA-12nt-B-160 probe. The spectra were recorded by monitoring the emission from 540-700 nm in 20 nm steps. B) Emission spectra of a 1.5 μM DNA-12nt-B-160 probe. The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C.



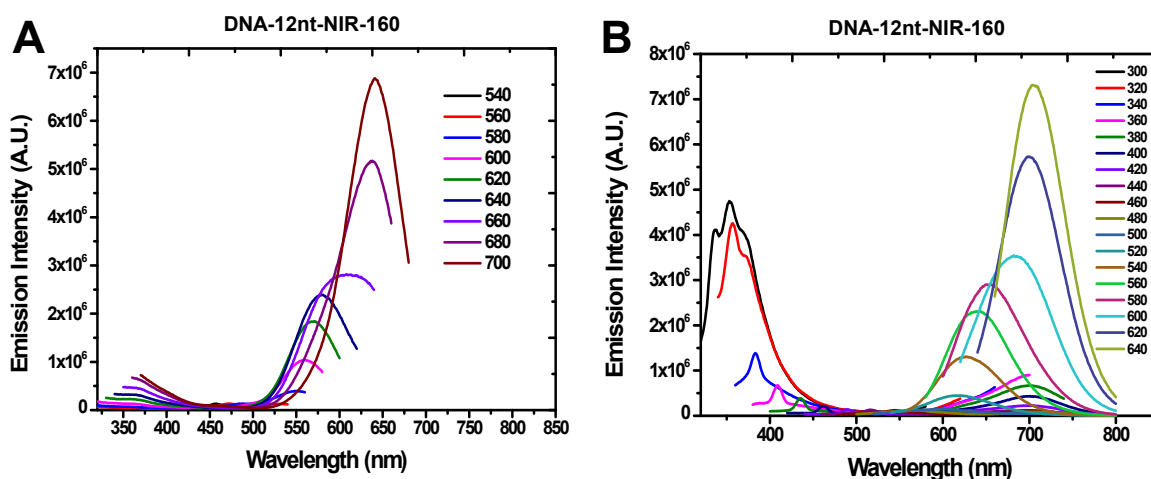
SI Figure 3: A) Excitation spectra of a 1.5 μM DNA-12nt-G-160 probe. The spectra were recorded by monitoring the emission from 540-700 nm in 20 nm steps. B) Emission spectra of a 1.5 μM DNA-12nt-G-160 probe. The spectra were recorded by exciting from 300-720 nm in 20 nm steps. All spectra were recorded at 25°C.



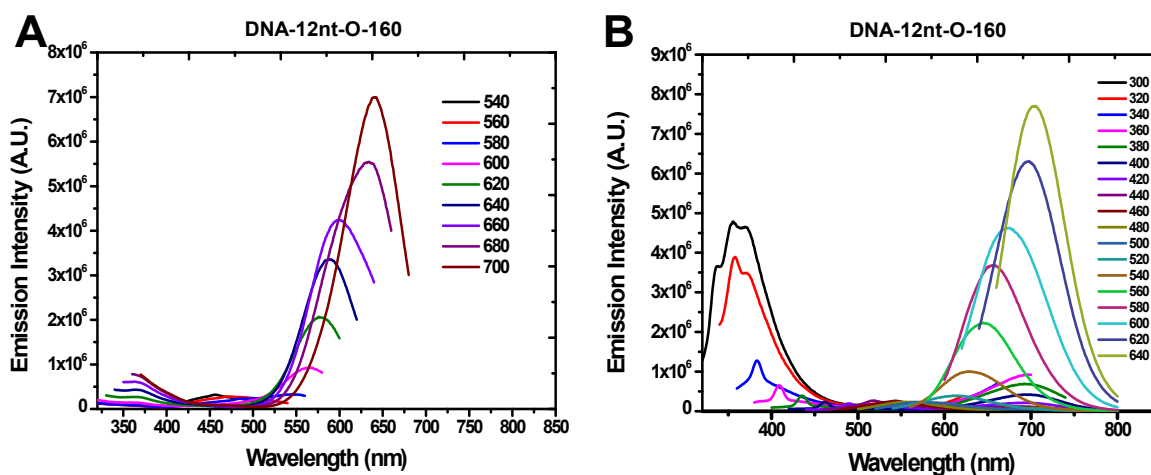
SI Figure 4: A) Excitation spectra of a 1.5 μM DNA-12nt-Y-160 probe. The spectra were recorded by monitoring the emission from 540-700 nm in 20 nm steps. B) Emission spectra of a 1.5 μM DNA-12nt-Y-160 probe. The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C.



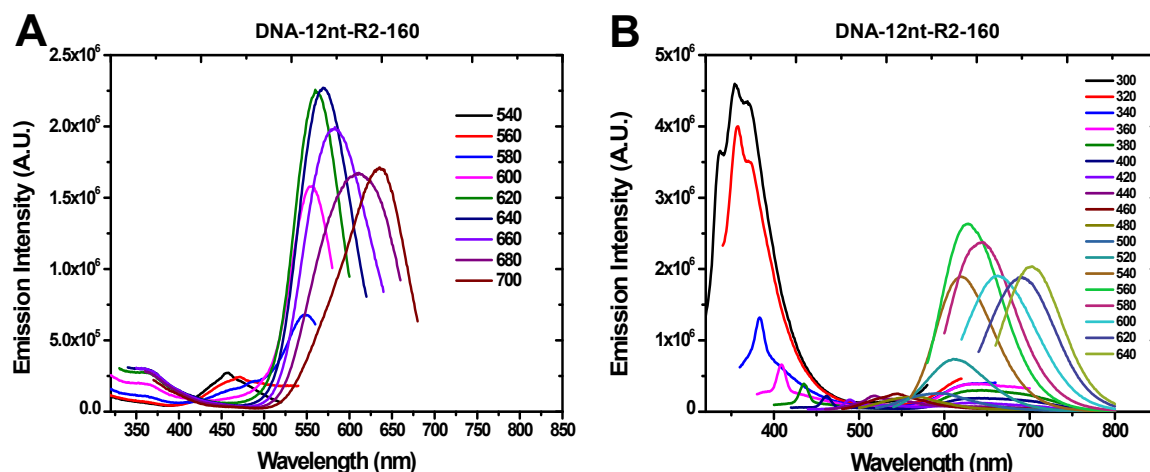
SI Figure 5: A) Excitation spectra of a 1.5 μM DNA-12nt-Y2-160 probe. The spectra were recorded by monitoring the emission from 540-700 nm in 20 nm steps. B) Emission spectra of a 1.5 μM DNA-12nt-Y2-160 probe. The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C.



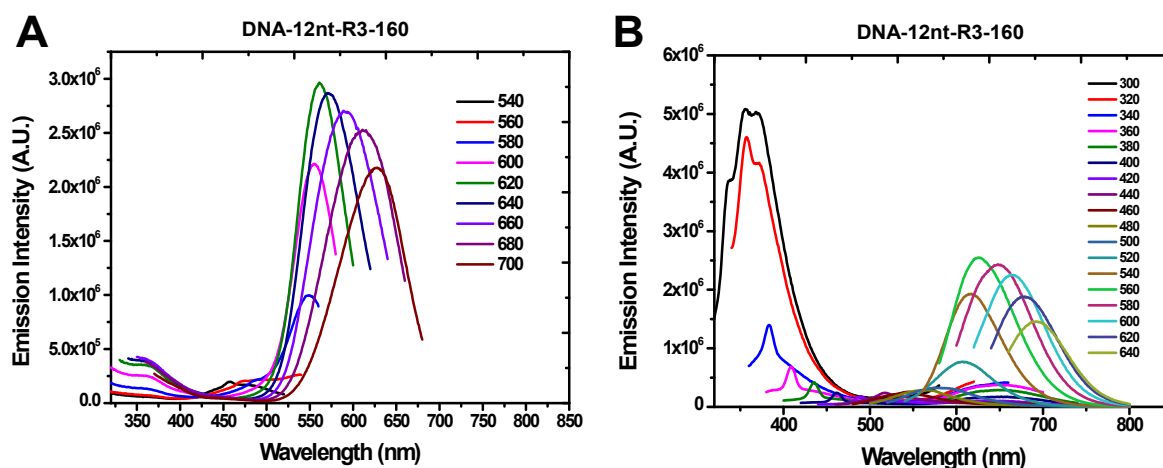
SI Figure 6: A) Excitation spectra of a 1.5 μM DNA-12nt-NIR-160 probe. The spectra were recorded by monitoring the emission from 540-700 nm in 20 nm steps. B) Emission spectra of a 1.5 μM DNA-12nt-NIR-160 probe. The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C.



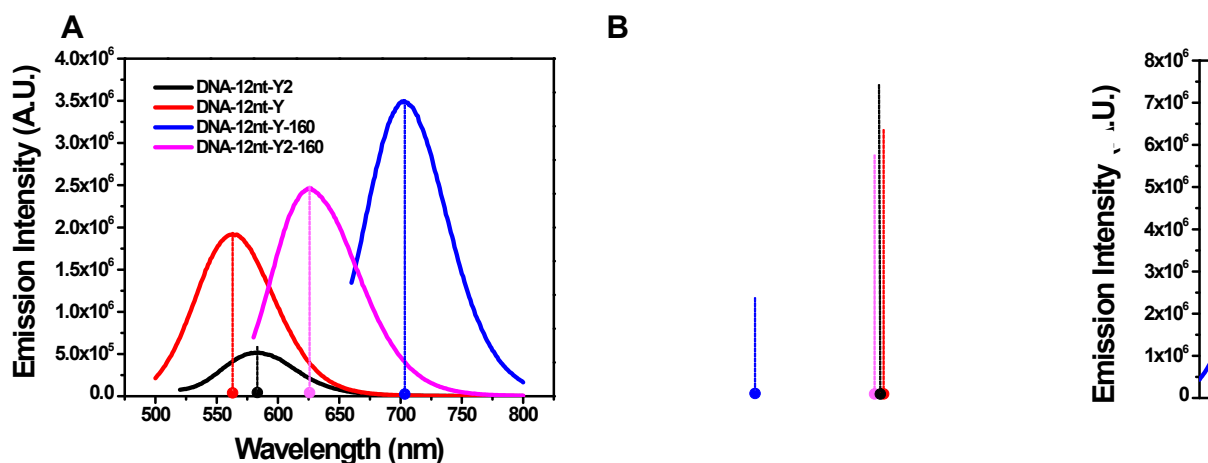
SI Figure 7: A) Excitation spectra of a 1.5 μM DNA-12nt-O-160 probe. The spectra were recorded by monitoring the emission from 540-700 nm in 20 nm steps. B) Emission spectra of a 1.5 μM DNA-12nt-O-160 probe. The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C.



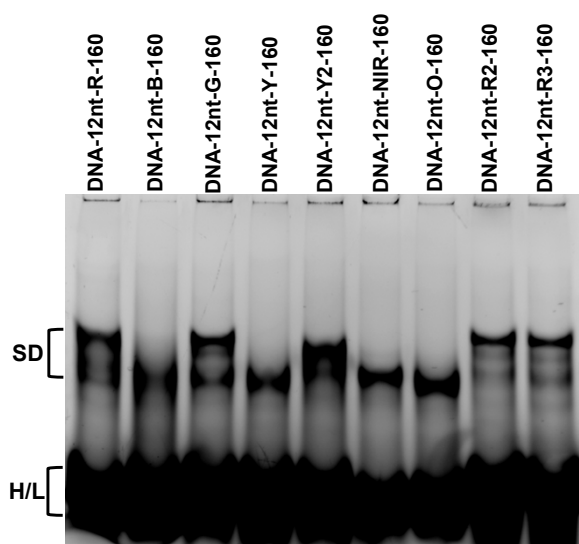
SI Figure 8: A) Excitation spectra of a 1.5 μM DNA-12nt-R2-160 probe. The spectra were recorded by monitoring the emission from 540-700 nm in 20 nm steps. B) Emission spectra of a 1.5 μM DNA-12nt-R2-160 probe. The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C.



SI Figure 9: A) Excitation spectra of a 1.5 μM DNA-12nt-R3-160 probe. The spectra were recorded by monitoring the emission from 540-700 nm in 20 nm steps. B) Emission spectra of a 1.5 μM DNA-12nt-R3-160 probe. The spectra were recorded by exciting from 300-640 nm in 20 nm steps. All spectra were recorded at 25°C.

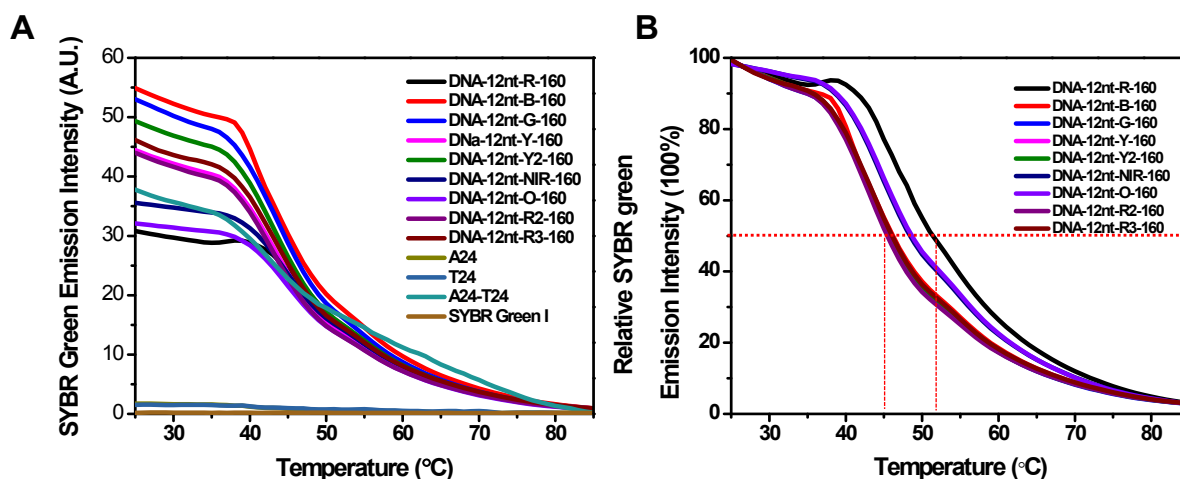


SI Figure 10: A) Emissions of a 1.5 μM DNA-12nt-Y-160, a 1.5 μM DNA-12nt-Y2-160, a 1.5 μM DNA-12nt-Y, and 1.5 μM DNA-12nt-Y2. B) Emissions of a 1.5 μM DNA-12nt-O-160, 1.5 μM DNA-12nt-NIR-160, 1.5 μM DNA-12nt-O, and 1.5 μM DNA-12nt-NIR. The highest emission of each DNA probe was recorded at 25°C. The emissions of DNA-12nt scaffolds were recorded after 24 hours incubation at 4°C.

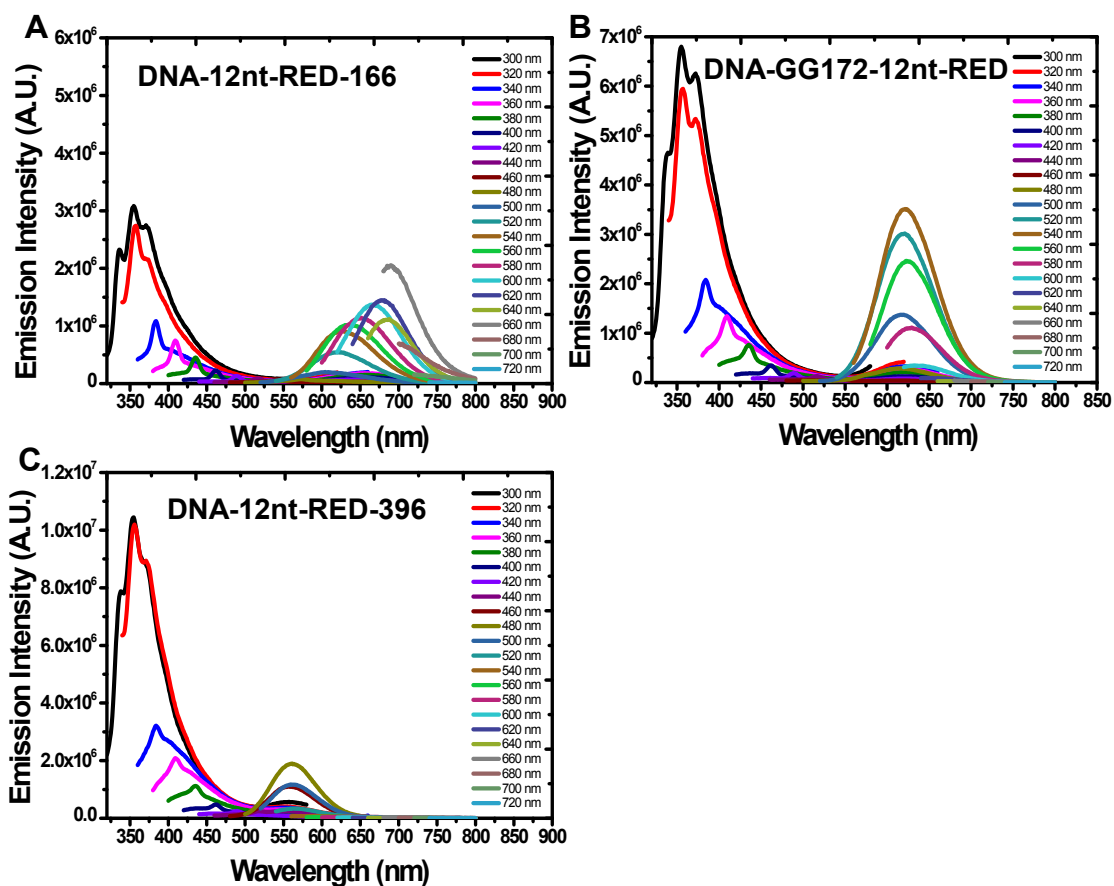


SI Figure 11: Native gel electrophoresis of the nine DNA/AgNCs probes. AgNO_3 and NaBH_4 were added to all the DNA samples before running the gel electrophoresis

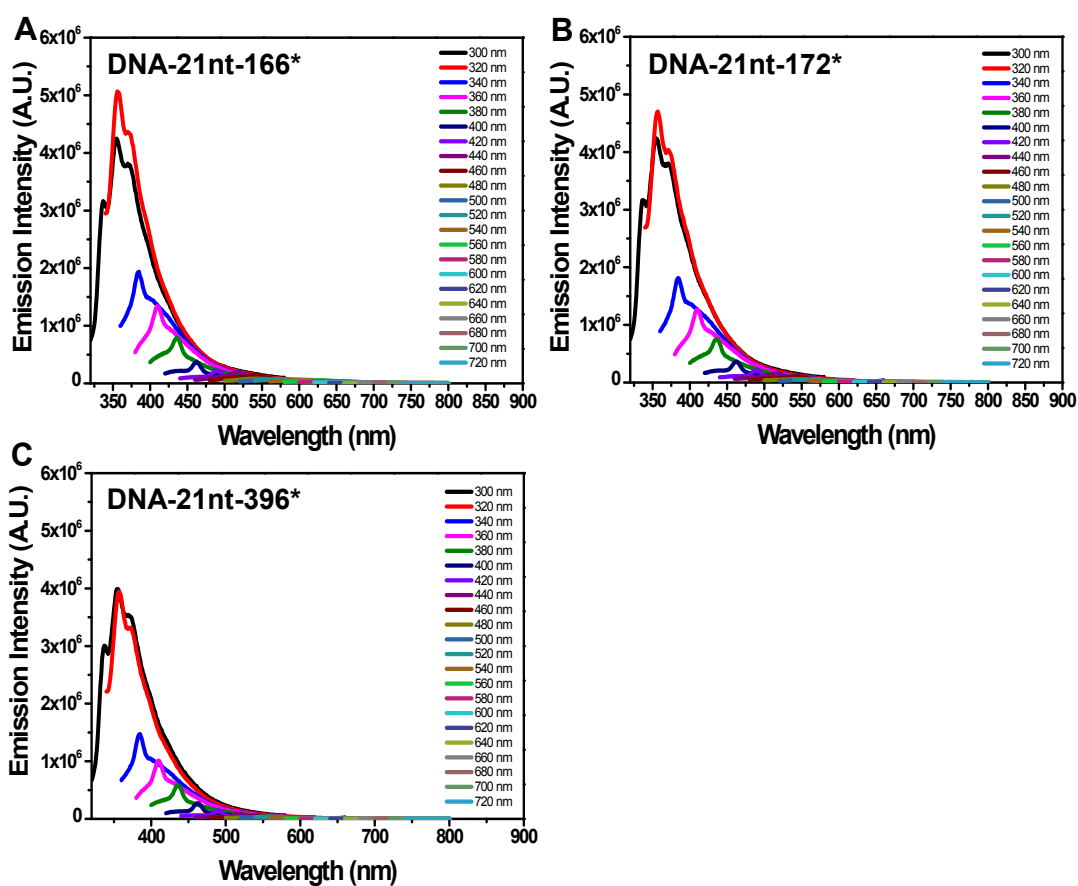
experiment. SD: mismatch self-dimer DNA or other secondary structures. H/L: hair-pin or single stranded DNA.



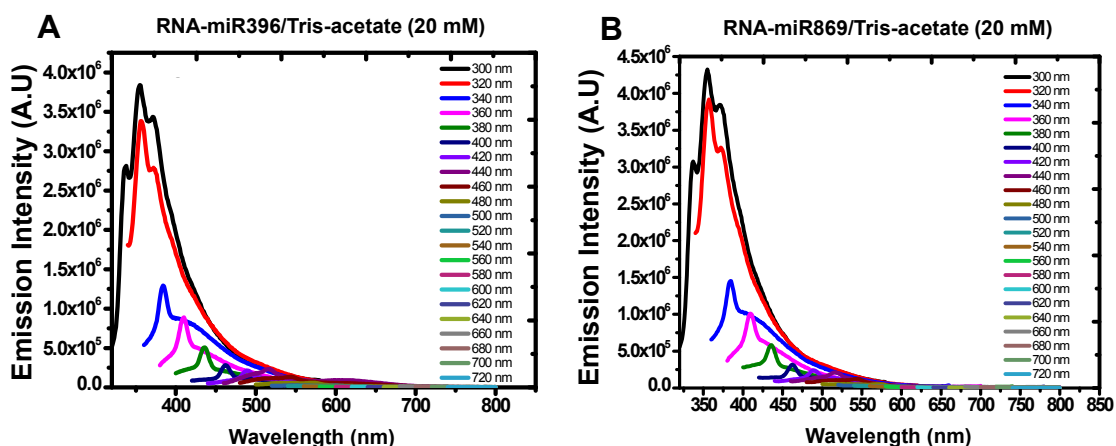
SI Figure 12: A) HRM analysis displays the presence of secondary structures in nine DNA/AgNCs probes. Native DNAs without AgNC's were used in the HMR experiments and the observed green emission (monitored at 510nm) is from the added SYBR green dye. Curves are the SYBR green emission intensity from 25°C to 85°C. Single stranded A24 and T24 were used as negative control. Double stranded A24-T24 was used as positive control. B) Normalized melting temperature curves of the nine DNA/AgNCs probes show their T_m values. Dashed red lines show the range of measured melting temperatures between 45°C and 53°C.



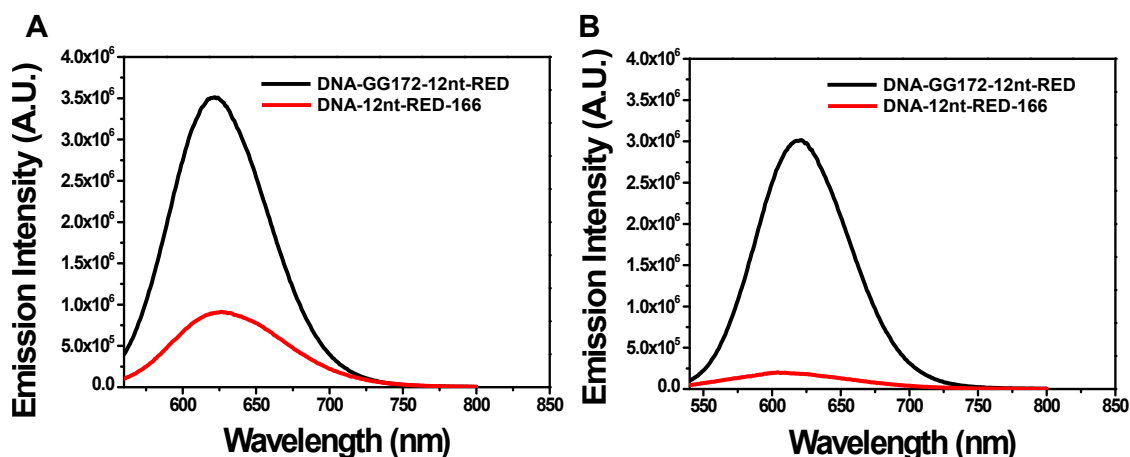
SI Figure 13: A) Emission spectra of 1.5 μM DNA-12nt-RED-166 probe in Tris-acetate buffer (2 mM), 1 hour after AgNCs creation, as a function of excitation wavelength (from 300 to 720 nm, in 20 nm step size). B) Emission spectra of 1.5 μM DNA-GG172-12nt-RED probe. C) Emission spectra of 1.5 μM DNA-12nt-RED-396 probe.



SI Figure 14: A) Emission spectra of 1.5 μM DNA-21nt-166* in Tris-acetate buffer (2 mM), 1 hour after AgNCs creation, as a function of excitation wavelength (from 300 to 720 nm, in 20 nm step size). B) Emission spectra of 1.5 μM DNA-21nt-172*. C) Emission spectra of 1.5 μM DNA-21nt-396*.



SI Figure 15: A) Emission spectra of 1.5 μ M RNA-miR396 in Tris-acetate buffer (2 mM), 1 hour after AgNCs creation, as a function of excitation wavelength (from 300 to 720 nm, in 20 nm step size). B) Emission spectra of 1.5 μ M RNA-miR869.



SI Figure 16: A) Overlapping Emission Spectrum of DNA-GG-172-12nt-Red and DNA-12nt-Red-166 when excited at 540nm and emission measured at 620nm. B) Excitation adjustment to avoid overlapping Emission Spectrum of DNA-GG-172-12nt-Red and DNA-12nt-Red-166 when excited at 510nm and emission measured at 620nm

REFERENCES

(1) Richards, C. I.; Choi, S.; Hsiang, J.-C.; Antoku, Y.; Vosch, T.; Bongiorno, A.; Tzeng, Y.-L.; Dickson, R. M. *J Am Chem Soc* **2008**, *130*, 5038.

(2) Patel, S. A.; Richards, C. I.; Hsiang, J. C.; Dickson, R. M. *J Am Chem Soc* **2008**, *130*, 11602.