Supporting Information for:

Rapid Detection of MicroRNA by a Silver Nanocluster DNA Probe.

Seong Wook Yang[†,*], *Tom Vosch*[‡,*]

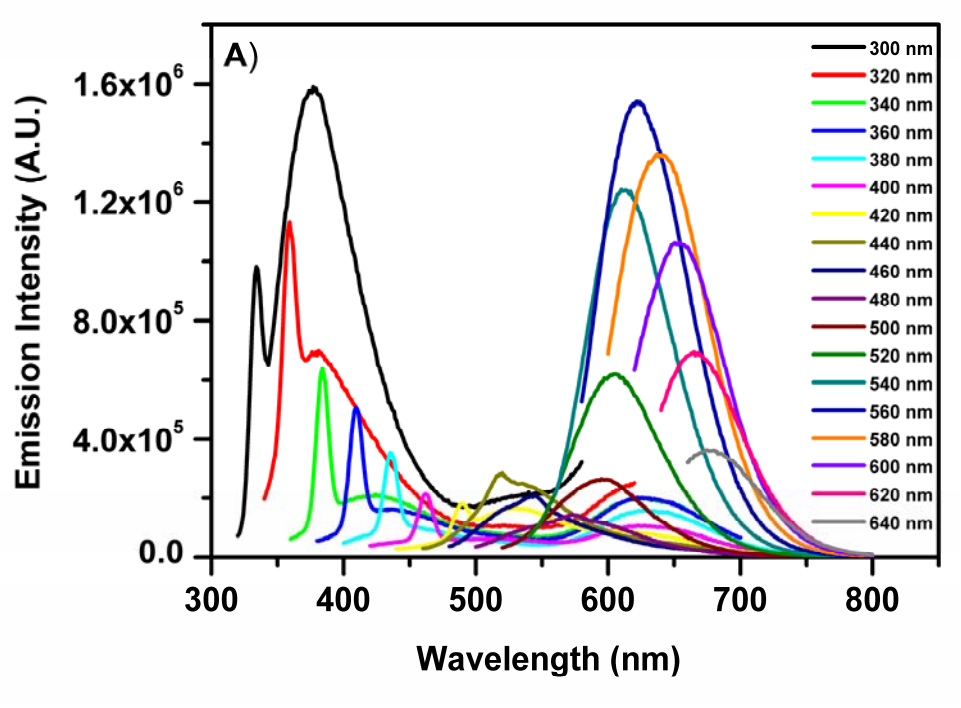
†Department of Plant Biology and Biotechnology, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Copenhagen, Denmark.

‡Nano-Science Center / Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark

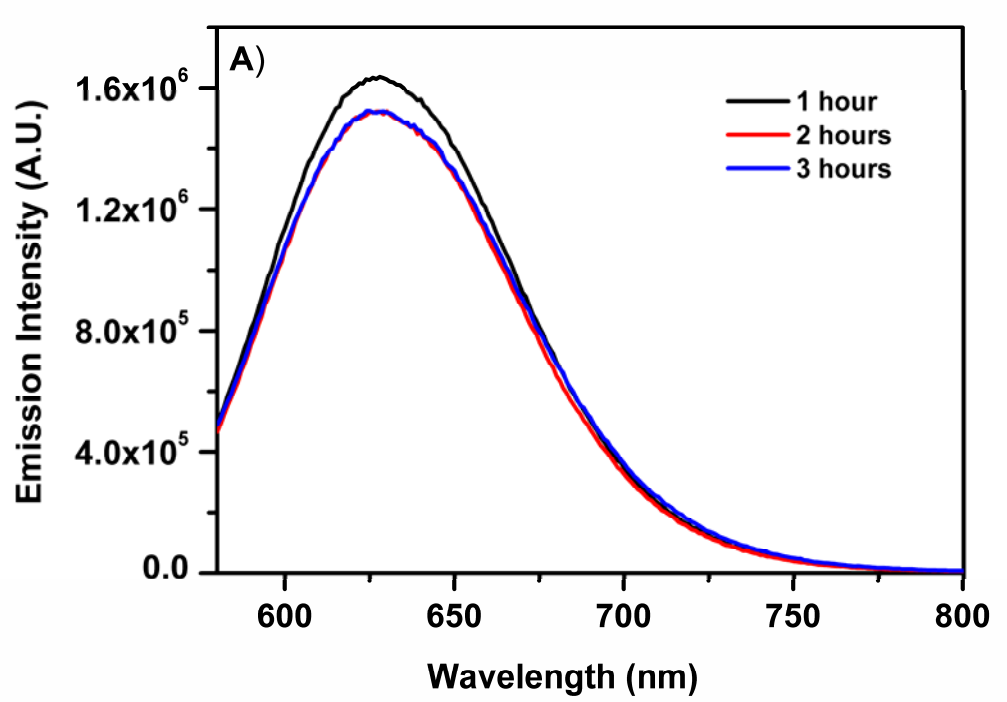
E-mail: swyang@life.ku.dk and tom@chem.ku.dk

AgNC synthesis procedure.

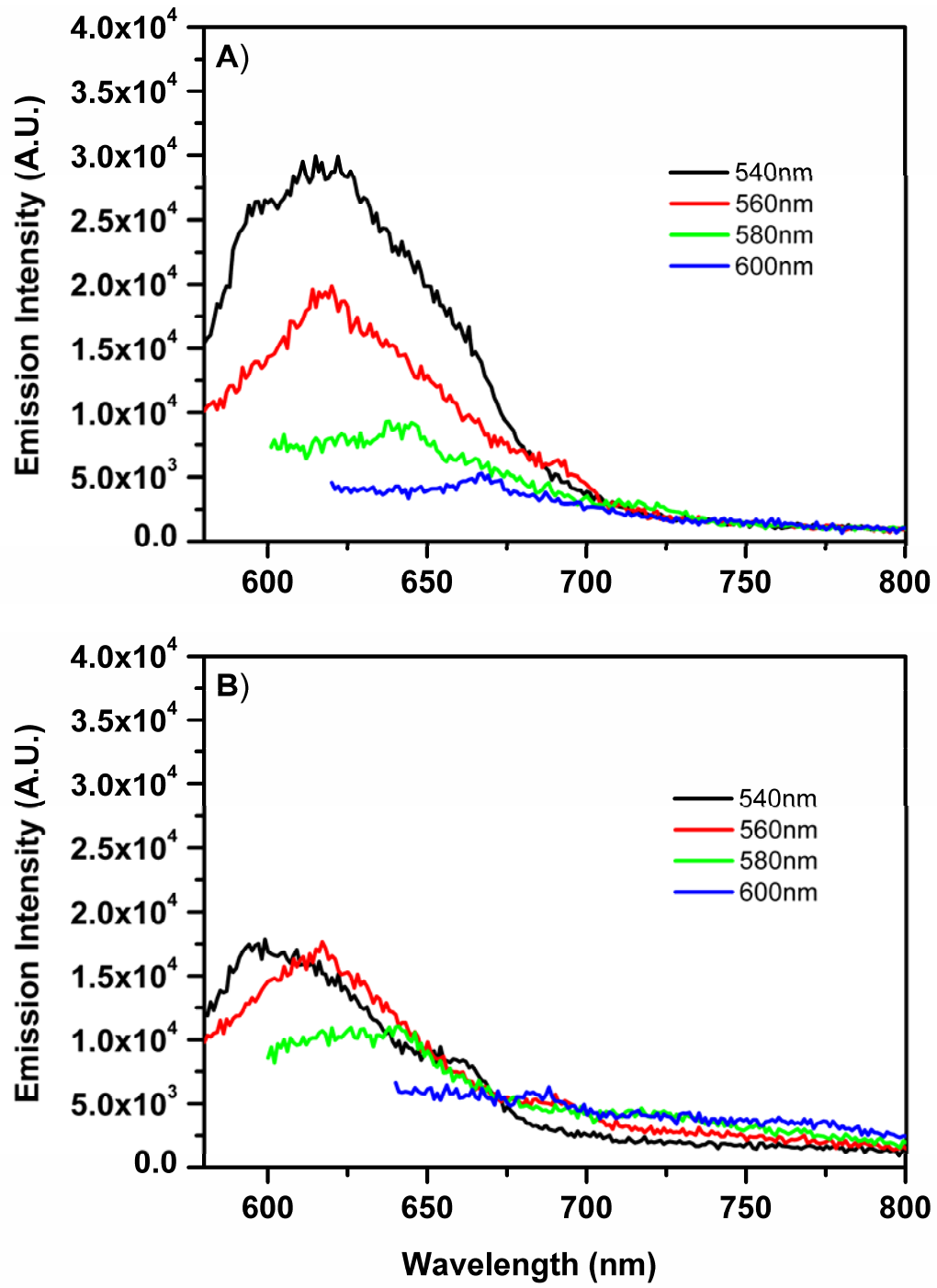
The DNA and miRNA sequences used in the publication are described in Figure 1A and Figure 3A. To make fluorescent AgNCs, we placed the DNA-12nt-RED-160 (15 μM) probe at 25°C for 10 min followed by an addition of $AgNO_3$ (250 μM) and $NaBH_4$ (250 μM), (1:17:17) to a final volume (50 μl). For the miRNA detection assay, we added various concentrations of miRNA160 (0.2 μM to 15μM) or a fixed amount of RNA-miR163, RNA-miR166, RNA-miR172 and RNA-RY-1(5 μM) to the DNA-12nt-RED-160 (15μM) and incubated for 15 min at 25°C. Then, $AgNO_3$ (250 μM) and $NaBH_4$ (250 μM) were added to the RNA/DNA mixtures to a final volume (50 μl). All the DNA/AgNCs were incubated for 1h at 25°C and diluted with 450 μl of distilled water before measurement by a fluorimeter (Horiba Jobin Yvon, Fluoromax-4) in a 1 mm quartz cuvette. Purification of small RNAs of WT and *hyl1-2* plants and northern blot analysis were performed as described in Yang et al.[1] For the plant experiments, 20 μg of total endogenous RNA (Total endogenous RNA was redissolved in RNAse free distilled water, instead of a 50% formamide solution, in the final step of purification) was incubated with the DNA-12nt-RED-160 probe (15μM) for 15min at 25°C and followed by the same AgNC creation procedure as described above. Possible AgNC creation/emission and immanent fluorescence of endogenous plant RNAs were tested with 20 μg of total RNAs of WT and *hyl1-2* as shown in SI Figure 5. For AgNC formation in DNA-12nt-RED (150μM and 15μM in 50 μl), we added $AgNO_3$ and $NaBH_4$ in a 1:6:6 to a final volume (50 μl). For AgNC formation in DNA-160 (15μM in 50 μl), we added $AgNO_3$ and $NaBH_4$ in a 1:17:17 ratio to a final volume (50 μl). Before measuring the fluorescence, the volume was increased from 50 μl to 500 μl for both DNA-12nt-RED and DNA-160. See SI Table 1 for emission intensities of the different synthesized compounds. The different ratios reflect the different DNA length and were chosen so that overall a 1 Ag^+ ion for 2 nucleotides ratio is obtained.



SI Figure 1: A) Emission spectra of 1.5μM DNA-12nt-RED-160 probe, 1 hour after preparation, as a function of excitation wavelength (from 300 to 640 nm, in 20 nm step size).



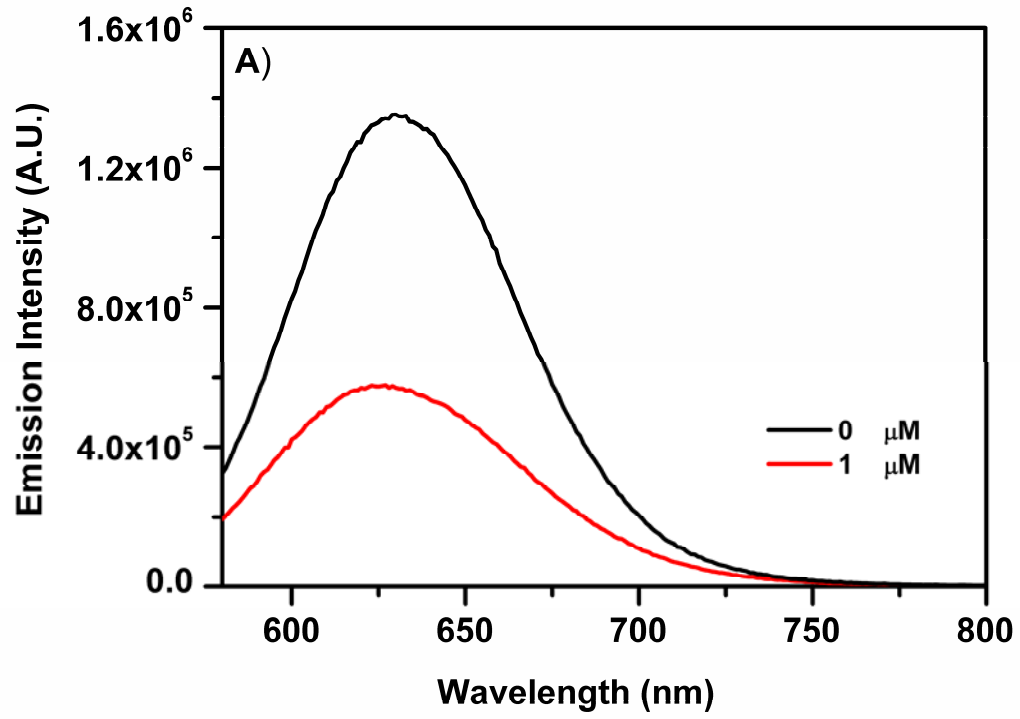
SI Figure 2: A) Time evolution of the fluorescence intensity of 1.5µM DNA-12nt-RED-160 probe after 1, 2 and 3 hours.



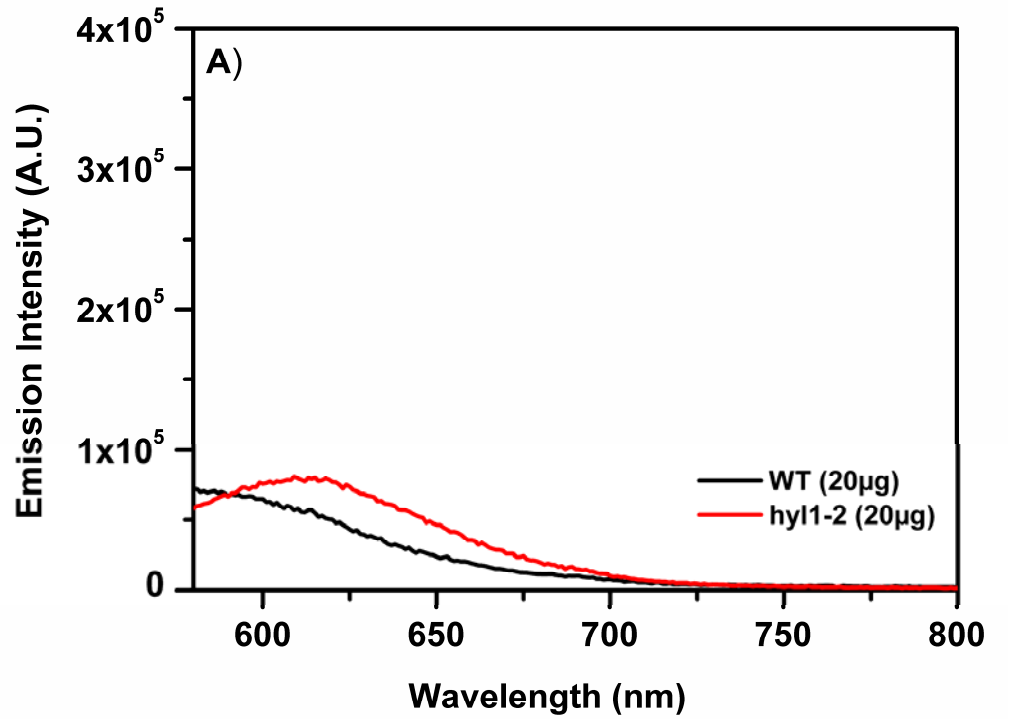
SI Figure 3: Emission spectra, 1 hour after preparation, as a function of excitation wavelength (from 540 to 600 nm, in 20 nm step size) for A) 1.5µM DNA-12nt-RED and B) 1.5µM DNA-160.

Sequence	Conc.	Ratio	Int. at 620 nm	Time
DNA-12nt-RED-160 probe	1.5 µM	1:17:17	1.60E+06	1h.
DNA-160	1.5 µM	1:17:17	1.70E+04	1h.
DNA-160	1.5 µM	1:17:17	2.40E+04	3h.
DNA-160	1.5 µM	1:17:17	4.60E+04	6h.
DNA-12nt-RED	1.5 µM	1:6:6	2.00E+04	1h.
DNA-12nt-RED	1.5 µM	1:6:6	3.50E+04	3h.
DNA-12nt-RED	1.5 µM	1:6:6	4.80E+04	6h.
DNA-12nt-RED	15 µM	1:6:6	1.61E+05	1h.
DNA-12nt-RED	15 µM	1:6:6	3.55E+05	3h.
DNA-12nt-RED	15 µM	1:6:6	4.70E+05	6h.

SI Table1: All samples were excited at 560 nm. The ratio is given as DNA conc.:$AgNO_3$ conc.:$NaBH_4$ conc.



SI Figure 4: A) Emission spectra of 1.5µM DNA-12nt-RED-160 probe, 1 hour after addition of $NaBH_4$ and $AgNO_3$ (black curve). After this spectrum was recorded, RNA-miR160 target (final concentration 1µM) was added to the DNA-12nt-RED-160 probe solution. A new emission spectrum was recorded 20 minutes later (red curve). The RNA-miR160 target reduced the original fluorescence intensity of the DNA-12nt-RED-160 probe a factor of 2.3. This is substantially less then when mixing DNA-12nt-RED-160 probe and RNA-miR160 target together before addition of $NaBH_4$ and $AgNO_3$ (see figure 2 in main text).



SI Figure 5: A) Emission spectra, excited at 560 nm wavelength, of AgNC emission formed when mixing and reducing 20 µg of endogenous RNA from a WT (black curve) and *hyl1-2* mutant plant with $AgNO_3$ and $NaBH_4$ (same amounts were used as in the experiments depicted in Figure 4, but in this case no DNA-12nt-RED-160 probe was present). Emission spectra were recorded 1 hour after the reduction step (addition of $NaBH_4$).

(1) Yang, S. W.; Chen, H. Y.; Yang, J.; Machida, S.; Chua, N. H.; Yuan, Y. A. *Structure* **2010**, *18*, 594-605.