Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Colorimetric Detection of MicroRNA miR-21 based on Nanoplasmonic Core-Satellite Assembly

Jiyun Park[a] and Jong-Souk Yeo [a*]

[a] School of Integrated Technology, Yonsei Institute of Convergence Technology, Yonsei University, Incheon, 406-840, Korea.

E-mail: jongsoukyeo@yonsei.ac.kr

Materials: H_2SO_4 (95-98%), H_2O_2 (30%), ethyl alcohol, 3-aminopropyltriethoxysilane (APTES, 99%), octadecyltrimethoxysilane (OTMS, 90%), NaOH were purchased from Sigma-Aldrich. Gold nanoparticles with mean diameter sizes of 30 and 50 nm were obtained from BBI International. Tris-HCl (hydroxymethylaminomethane) buffer (pH7.5) solution and dithiothreitol (DTT) were obtained from Thermo Scientific. Nap-10 column was purchased from GE Healthcare. RNase-free water was purchased by invitrogen. All oligonucleotides and miRNA were synthesized and purified with a HPLC method by Bioneer Co. Ltd. All reagents were used as received without further purification and treatment.

Immobilization and Functionalization of Core GNPs: Glass slide was cleaned in a Piranha solution (7:3, H_2SO_4:H_2O_2) to render glass surfaces highly hydrophilic and to hydroxylate the surface, followed by rinsing with DI water several times and drying the glass slide with nitrogen gas. To functionalize the surface of the cleaned glass slide with mixed self-assembled monolayer of APTES and OTMS, the glass substrate was treated in a 5mM of mixed solution of APTES and OTMS in anhydrous ethanol at room temperature for 6h. After incubation was complete, the substrate was rinsed and sonicated in ethanol to remove physically adsorbed APTES and OTMS molecules, followed by drying with a stream of nitrogen gas. We dropped 2 ml of 50 nm citrate-capped gold nanoparticles of 75 pM onto the substrate and washed with DI. We prepared 10 mM of Probe 1 solution in RNase-free water and 5 ml of the Probe 1 solution droplets were deposited onto core gold nanoparticles on the substrate for 1 h. After that, the glass slide was completely rinsed with RNase-free water and dried with nitrogen gas.

Conjugation of Probe 2 onto Satellite GNPs: We diluted Probe 2 with RNase-free water to yield a final concentration of 10 mM. For thiol modification at the end of the Probe 2, we activated the Probe 2 as previously described. To complete conjugation, we added 1 mM of the reduced Probe 2 solution to 100 pM of 30 nm gold nanoparticles and incubated the mixed solution for 12 h at room temperature. After incubation, to remove excess Probe 2, we had to spin down the solution at 12,000 rpm for 3 min

and redisperse the remaining satellite nanoparticles in 1 ml of Tris-HCl (0.01 M)/NaCl (0.05 M) buffer solution to yield a Probe 2 conjugated satellite AuNPs in a final concentration of 90 pM.

Hybridization of Target miR-21 onto Probe 2 Conjugated Satellite GNPs: A 10 mM of target miR-21 solution, stored at -20 degrees Celsius, was gradually diluted with RNase-free water for final concentrations in the range 1 pM – 10 mM. Each target miR-21 solution was then added into the same volume of Probe 2 conjugated satellite AuNPs solution and the mixed solution was incubated at room temperature for 1 h for exact hybridization. The solution with high concentration of target miR-21 had excessive residuals of miR-21 in solution.

Targeted Assembly of GNPs: The solution of target–satellite conjugates was applied over the Probe 1–core conjugates and incubated for 1h at room temperature. After incubation, the substrate was thoroughly washed with RNase-free water and dried with nitrogen gas.

Optical Characterizations: The nanoassemblies were observed using an inverted optical microscope (Ti-U, Nikon) with an oil-immersion dark-field condenser (NA = 1.23 - 1.43, Nikon) and a digital camera (DS-Ri1,Nikon). Spectra were then obtained using spectrophotometer (Monora 320i, DongWoo Optron Co., Ltd.) equipped with a CCD camera (DV420A-BV, Andor).

Selection of Negative Controls: Point mutations (small mutations) in mature microRNAs (miRNAs) are rare [1-4]. Even with point mutations, the effect on miRNA functions is not significant [5]. For a process of clinical detection, a target miR-21 is in competing situation not with a miR-21 having a point mutation but rather with other miRNAs (i.e., miR-16, miR-136, and miR-206) which have totally different sequences from the target miR-21. It means that distinguishing the target miRNAs from other miRNAs is more crucial in demonstrating our concept. Thus, we concluded that randomly generated oligonucleotide would be meaningful as negative controls to suggest the directions for future clinical studies.

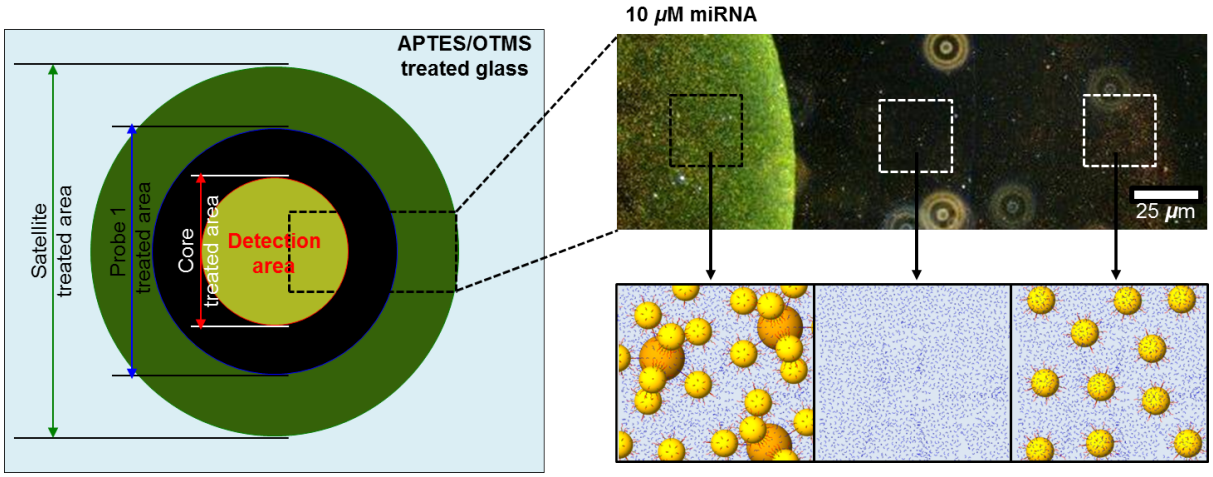

Figure S1 A concept of experimental configuration.

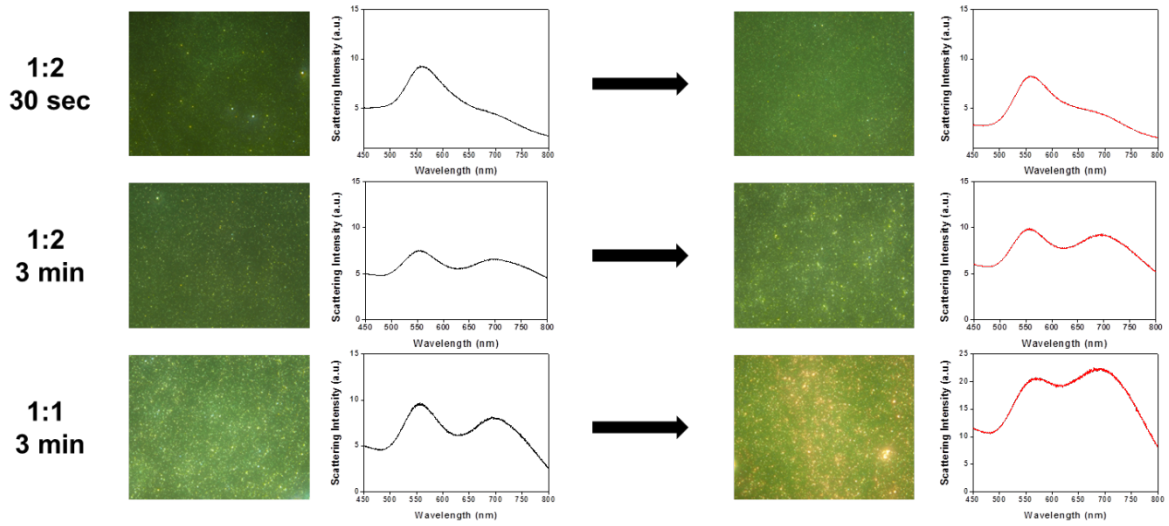

Figure S2 Effect of core nanoparticle density in detection area.

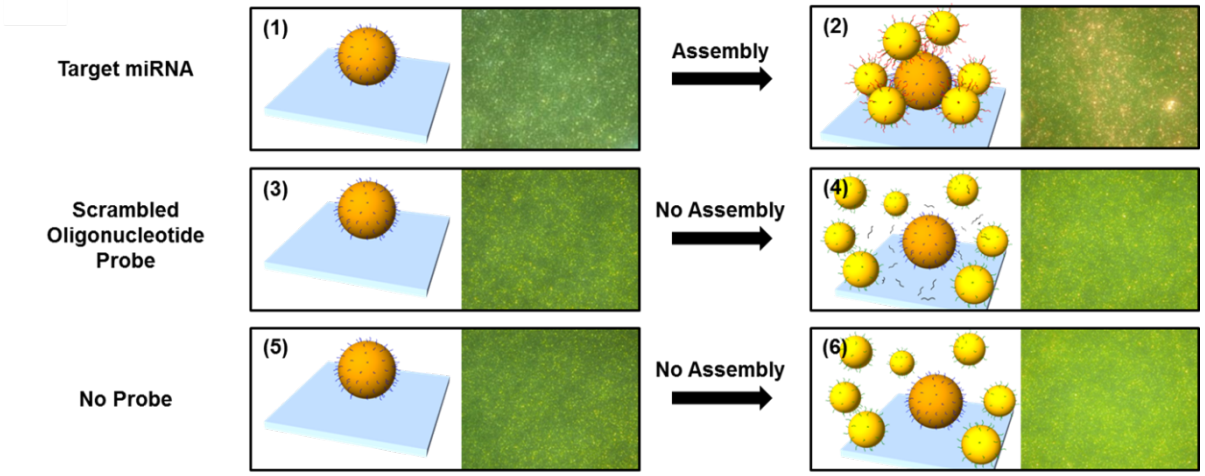

Figure S3 Selectivity for the target miR-21.

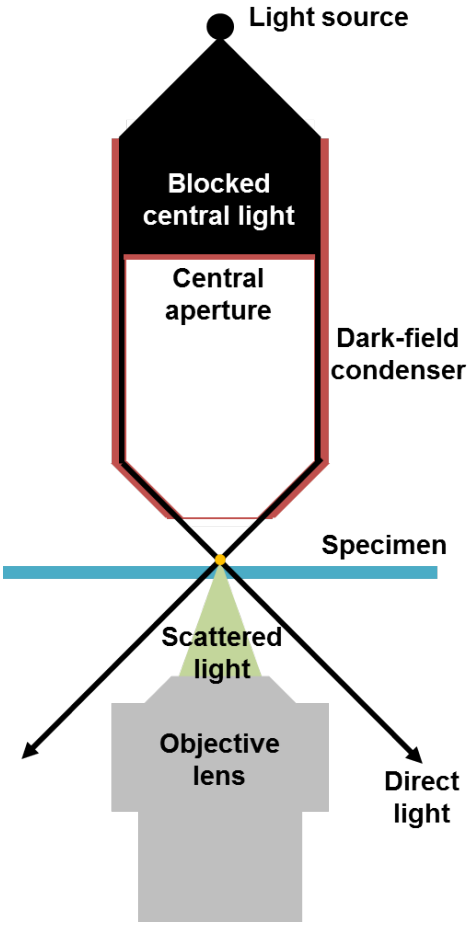

Figure S4 Principle of dark-field scattering experimental setup.

Supplementary References

1. S. Diederichs and D. A. Haber, *Cancer Res*, 2006, 66, 6097-6104.
2. S. A. Melo and M. Esteller, *Febs Lett*, 2011, 585, 2087-2099.
3. S. Q. Lv, Y. H. Kim, F. Giulio, T. Shalaby, S. Nobusawa, H. Yang, Z. Zhou, M. Grotzer and H. Ohgaki, *Brain Pathol*, 2012, 22, 230-239.
4. G. A. Calin and C. M. Croce, *Nat Rev Cancer*, 2006, 6, 857-866.
5. M. Q. Wu, N. Jolicoeur, Z. Li, L. H. Zhang, Y. Fortin, D. L'Abbe, Z. B. Yu and S. H. Shen, *Carcinogenesis*, 2008, 29, 1710-1716.