Supporting Information

Highly Sensitive DNA Hybridization Detection with Single Nanoparticle Flashlight Darkfield Microscopy.

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Chemicals and Materials. Chloroauric acid, sodium phosphate, trisodium citrate were purchased from Sinophrarm Chemical Reagent Corpration (ShangHai, China). dATP, thiol-DNA probes, fully matched DNA targets and single base pair mismatched DNA targets were purchased from TaKaRa corporation (DaLian, China). The DNA sequences are: probe1, 5' TAA CAA TAA TCC CTC-C_3-SH 3'; probe2, 5' HS-C_6- ATC CTT ATC AAT ATT 3'; fully matched target DNA:, 5' GAG GGA TTA TTG TTA AAT ATT GAT AAG GAT 3'; one base pair mismatched target DNA in the middle, 5' GAG GAA TTA TTG TTA AAT ATT GAT AAG GAT 3'; one base pair mismatched target DNA at the end, 5' GAG GGA TTA TTG TTA AAT ATT GAT AAG GAC 3'.

Flashlight darkfield microscopy (FLDM). For FLDM, a Nikon 80i microscope with a NA 1.20~1.43 darkfield condenser and a NA 0.75 40X objective was used. The original 100W Halogen Tungsten lamp for transmitted light illumination was replaced with a 1000W professional studio flashlight (PN1000, JinBei Co., Shanghai, China), which features a flash duration time of 1/2000 s and a recharging time of less than 2 s. The detector was a Photometrics CoolSnap HQ2 CCD camera, whose chip has a pixel size of 6.45 μm and a dimension of 1392 by 1040 pixels. When operated in normal interline mode with the exposure time over 90 ms, the sensing and data transfer module of the camera can work in parallel, allowing continuous no gap exposure. Thus, even though there is no synchronization between the flashlight and CCD camera, continuous flashes can be captured with no lost. In our FLDM experiments, 10 or 20 continuous snaps were usually acquired from one AuNP solution sample. The images were then processed using ImageJ software and the counts and standard deviation were obtained using the particle analysis module.

Synthesis of gold nanoparticles (AuNPs). 40 nm citrate-stabilized AuNPs were synthesized using a layer-by-layer seed growth method. Firstly, AuNP seed 1 was prepared by boiling 100.0 mL 2.5×10^{-4} M $HAuCl_4$ solution for 10 min, followed by adding 3.0 mL 3.919×10^{-2} M trisodium citrate. The solution was boiled for another 30 min as the color changed from pale yellow to navy blue and finally wine red. The seed 1 solution was then cooled to room temperature. Secondly, AuNP seed 2 was prepared by mixing 1 mL fresh prepared AuNPs seed 1 solution and 1 mL 3.919×10^{-2} M trisodium citrate together with 9 mL water and boiling for 5 min, followed by adding 200 μL 2.346×10^{-2} M $HAuCl_4$ solution and boiling for another 30 min. The seed 2

solution was then cooled to room temperature. Finially, 40 nm AuNPs were synthesized by mixing 5 mL AuNP seed 2 solution and 1 mL 3.919×10^{-2} M trisodium citrate solution together with 19 mL water and boiling for 5 min, followed by adding 200 μL 2.346×10^{-2} M $HAuCl_4$ solution and boiling for another 30 min. The obtained 40 nm AuNPs solution was then cooled to room temperature and stored in 4℃ for the further use.

One pot AuNPs probe DNA functionalization and target DNA hybridization. The as-prepared citrate-stabilized AuNPs were incubated with dATP at a dATP/AuNP molar ratio of 1000 for 15 min at room temperature. After that, the mixture was brought to pH 8.0 with 10 mM sodium phosphate buffer. With brief shaking, thiol-DNA probes was introduced at a molar ratio of thiol-DNA1/thiol-DNA2/AuNP equal to 50:50:1, followed by mixing at 50□and 300 rpm for 2 hours using an Eppendorf thermomixe. This particular procedure was chosen because it provides a much faster method (~ 2 hr) to prepare DNA functionalized AuNP probes than the traditional Mirkin method (~ 48 hr). At length, DNA targets of various concentrations were added into this solution without any further treatment. The hybridization reaction was performed at 25□and 300 rpm for 4 hours.

2D random walk simulation. To simulate the behaviors of single AuNPs diffuse in and out of an ultrasmall detection volume in 2D, 120 particles were generated arbitrarily within a large circle of 60 μm in diameter and were allowed to diffuse freely in 2D with a diffusion coefficient of 13.6 $\mu m^2/s$. The diffusion coefficient was calculated from the Stokes-Einstein equation for a 36 nm AuNPs. There was a small circle of 1 μm in diameter at the center of the large circle to serve as the detection area. As a result, there was in average 0.033 particles within the detection area at any time. The trajectory of each AuNP was then recorded and the times it passed through the detection area during certain amount of accumulation time was counted. Figure S1 shows the results from one set of simulation in which the step size was 1 ms and the accumulation time varies from 1000 ms to 10000 ms. It can be seen that while the total counts versus accumulation time increases gradually, the majority of counts in each simulation comes from just a few particles diffuse in and out of the detection area repeatedly.

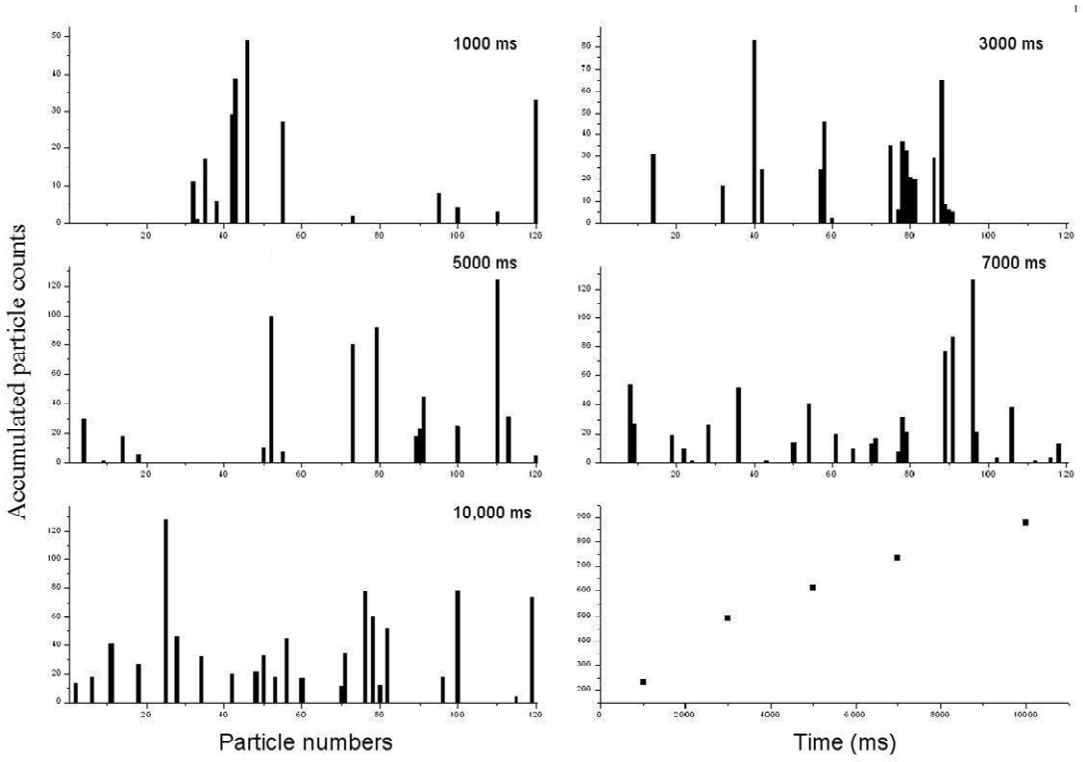

Figure S1. 2D random walk simulation.

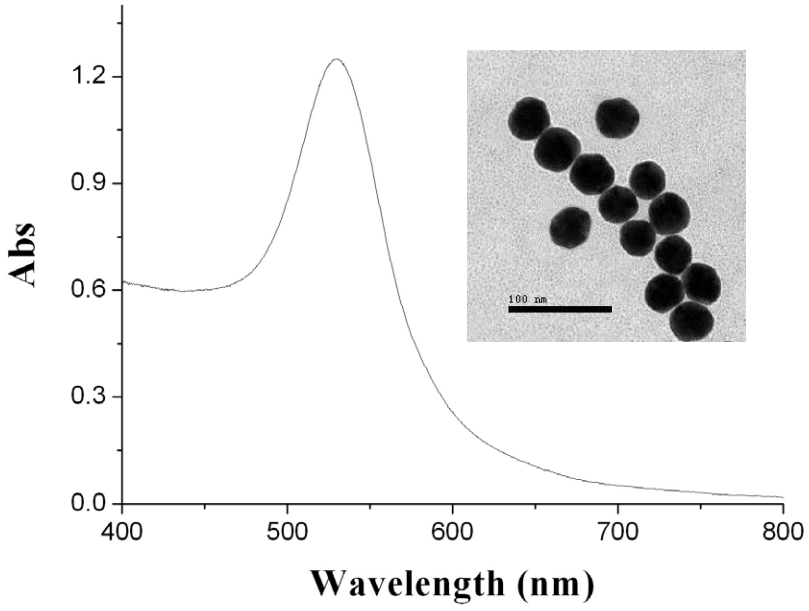

Figure S2. Absorption spectrum of as-prepared 40 nm gold nanoparticles. Inset image: TEM image of gold nanopartilces.