Supporting Information for

Specific and sensitive detection of nucleic acids and RNases using gold nanoparticle-RNA-fluorescent dye conjugates

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Experimental Details

Synthesis of gold nano particles

Au nanoparticles (AuNP) (~ 13 nm) were synthesized by citrate reduction[1] and phosphine capping.[2] Briefly, a 500 ml aqueous solution of 1 mM $HAuCl_4$ was prepared and brought to reflux under vigorous stirring to which 50 mL of 38.8 mM trisodium citrate was added. Heat removal after 15 minutes was followed by allowing the solution to cool to room temperature. BSP (bis (*p*-sulfonatophenyl) phenylphosphine dihydrate, dipotassium salt, Sterm Chemicals, 150 mg) was added over a period of 5 minutes followed by overnight stirring. Small amounts of a 2 M NaCl solution were added to precipitate the particles. After centrifuging, the solids were washed with 250 mM NaCl and brought up in 0.3 mM BSP in H_2O (~100 nM AuNP). Salt precipitation and NaCl solution wash was repeated, and then the solids were redispersed at 400 nM AuNP (using extinction coefficient at 522 nm of 2.43 x 10^8 M^{-1} cm^{-1}) in 10 mM sodium phosphate buffer (pH 7.0). The size and

monodispersity of the gold particles were confirmed by performing Transmission Electron Microscopy with JEM 1230 (JEOL).

Modification of gold nano particles with RNA

Thiols were activated by treatment with 10 mM TCEP (Pierce) in 10 mM sodium phosphate buffer (pH 7.0) for 30 minutes at room temperature. RNA molecules (4 μM, 200-fold molar excess) were mixed with 20 nM of GNPs in 1 mL of buffer for 16 hours at room temperature. To increase the density of RNA coverage on the gold surface, the NaCl concentration was gradually increased up to 0.1 M. After an additional 48 hours, the excess free RNA was removed by centrifugation at 14,000 rpm for 25 minutes in the same buffer, repeated four times. The RNA-modified GNPs were redispersed in 200 μL of the phosphate buffer containing 0.1 M NaCl at room temperature

Measuring fluorescence of free fluorescein

After detaching the immobilized RNA from the gold particles using a 24 hour incubation with 14 mM of 2-mercaptoethanol (Sigma Aldrich) at 37 ^{o}C, the fluorescence intensity of the free fluorescein was measured using an ND3300 instrument (NanoDrop Technologies Inc).

Fluoresence quenching efficiency was determined by measuring the fluorescence signal of both free and immoblized fluorescein and was calculated using equation (1),

$$Q = (1 - \frac{F_q}{F_o}) \times 100 \qquad (1)$$

where, Q is quenching efficiency, F_q and F_o are the fluorescence intensities with and without the GNP,

respectively. Fluorescence intensity of immobilized RNA on the gold nanopartilces and detached RNA from the gold nanoparticle were measured and used as F_o, and F_q, respectively.

Determination of number of immobilized RNA per nanoparticle

The number of immobilized RNA on single GNPs was measured by comparing the fluorescence intenisity of the detached RNA with the fluoresence of RNA which was not immobilized. Immobilized RNA on the gold nano particles were detached by incubating 3.2 nM of the RNA modified gold nanoparticles with 14 mM of 2-mercaptoethanol at 37^{o}C for 24 hours.

DNA/RNase H detection

The enzymatic digestion of RNA in the presence of different amounts of target DNA (0 to 10 μM) by RNase H was carried out in 10 μL of the modified RNase H buffer (50 mM Tris-HCl, 75 mM KCl, and 8 mM $MgCl_2$, pH 8.2) containing RNase H (0.043 unit/μL, Promega) with the RNA modified GNPs at ~1 nM for 1 hour at 37 ^{o}C (using a gold plasmon extinction coefficient of 2.4 x 10^8 M^{-1} cm^{-1})[3]. After incubation, the fluoresence of 2 μL of each sample was measured using a ND3300 instrument (NanoDrop Technologies, Inc. Wilmington, DE, USA). RNase H activity was determined with 1 μM of target DNA.

RNase A detection

RNase A (Sigma Aldrich) was added to 1 nM of RNA modified gold nano particles (10 μL total volume) in RNase A buffer (10 mM Tris HCl, , and 15 mM NaCl, pH 7.5).

Fluorescence intensity was measured after 1 hour incubation at 37 ^{o}C using a NanoDrop ND3300 instrument.

Time measurement

RNase A detection: The fluoresence intensity of 1 nM of the RNA modified gold nanoparticles in 70 μL of RNA buffer was measured for 2 minutes (Luminescence Spectrometer LS50B (PerkinElmer)) followed by addition of 1 μL of RNase A (10 ng/μL).

RNase H detection: The fluoresence intensity of 1 nM of the RNA mofidied gold nano particles in 70 μL of RNase H buffer containing the target DNA (14 nM) was measured, followed by the addition of 1 μL of RNase H (5 units/μL).

Calculated distances between immobilized fluoresceins

The approximate distance between immobilized fluoresceins (R_b) was calculated using equation (2) and a regular square array of RNA on the gold surface[3]

$$R_b = \sqrt{\frac{1}{C_s \times 4}} \quad (2)$$

where C_s is the surface density of the immobilized RNA (molecules/unit area). The number of immobilized RNA molecules per GNP was calculated to be 70 (see above section on number per particle), and thus the nearest distance between two immobilized RNA molecules is ~1.4 nm. Also, we note this number attached to this size nanoparticle (13 nm) is not fully close packed on the surface through a simple surface area comparison to reports from DNA thiolate monolayers being ~160 strands on a 15.7 nm (ref).[4] The predicted average distance between fluoresceins is largely dependent on the curvature of

the gold surface, as it is positioned off a terminal nucleotide. The maximum predicted length of the fully stretched 26 base single stranded RNA (~7.3 nm)[5] leads to the unit area changes from 531 nm^2 to 2391 nm^2 area. Thus, the average inter-fluorescein distance is predicted to be 2.9 nm or less.

Referemces

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