pH-driven conformational switch of "i-motif" DNA for the reversible assembly of gold nanoparticles†

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Herein we demonstrate that gold nanoparticles conjugated to "i-motif" DNA behave like a pH dependent switch that undergoes reversible aggregations which can be easily visualized by the naked eye.

Colloidal gold nanoparticles (Au NPs) have useful optical and optoelectronic properties that can be easily tailored by modifying the size, shape and local environment of the nanoparticles.[1] When in proximity, Au NPs exhibit plasmon coupling that red-shifts the resonance wavelength depending on the inter-particle separation.[2,3] The aggregation of Au NPs with controlled inter-particle distances has been achieved using molecular recognition properties of DNA, where DNA oligos are chemically linked to the surface of Au NPs by the well known gold–thiol chemistry.[4,5] Plasmon coupling of DNA functionalized Au NPs has successfully been exploited in making a molecular ruler,[6] to detect DNA,[7–10] proteins,[11] triplex DNA binders,[12] metal ions,[13,14] and high-throughput screening of DNA-binding molecules.[15] Au NP aggregation can also be triggered by environmental changes, for example the pH of the solution.[16,17] A recent report has demonstrated the use of gold nanoparticles as a colorimetric sensor to detect protein conformational changes.[18] Herein, we demonstrate a reversible colorimetric switch using DNA–Au NP conjugates to respond to changes of the solution pH by a color change that can be observed with naked eye. This switch is based on reversible aggregation of DNA–Au NP conjugates that can be triggered by an environmental change.

Our system comprises two different sets of DNA–Au NP conjugates, NP1 and NP2 (Fig. 1A). The NP1 consists of Au NPs conjugated with a cytosine-rich DNA strand (30-mer), containing 4 stretches of CCC known as the "i motif", and the NP2 consists of Au NPs conjugated with a DNA strand (27-mer) complementary to the "i motif" but with 3 C–T mismatches. These mismatches are necessary to prevent the otherwise G-rich strand on NP2 to fold into G-quartet. These sequences have proved to be useful in making other DNA based nanodevices.[19–23] Both the sequences on NP1 and NP2 contain a stretch of poly (T_9) residues at the 3′-end to improve hybridization of the sequences on the Au particle surfaces.[24] In acidic pH $\sim$ 5, the C residues are partially protonated. The "i motif" on NP1 forms a DNA quadruplex by systematic intercalation of the semi-protonated $C{\cdot}C^+$ bases, while the other strand on NP2 adopts a random-coil conformation (Fig. 1A). When the pH of the solution is increased to $\sim$8, the C^+ residues are all deprotonated, and the "i motif" unfolds. Hybridization between the strands on NP1 and NP2 takes place, leading to aggregation of the Au NPs. This conversion between states of intra-molecular quadruplex and inter-molecular duplex of DNA can be achieved by alternate addition of H^+ and OH^-.

In a typical experiment, 15 nm Au NPs conjugated with the DNA strands were prepared separately at pH $\sim$ 8, as described previously[3] (see details in ESI†) and they were finally dispersed in PBS buffer (10 mM, 0.3 M NaCl, pH 8.0). The salt concentration is optimized here to avoid random aggregation of Au NPs and to improve the hybridization of the DNA to its complementary strand.

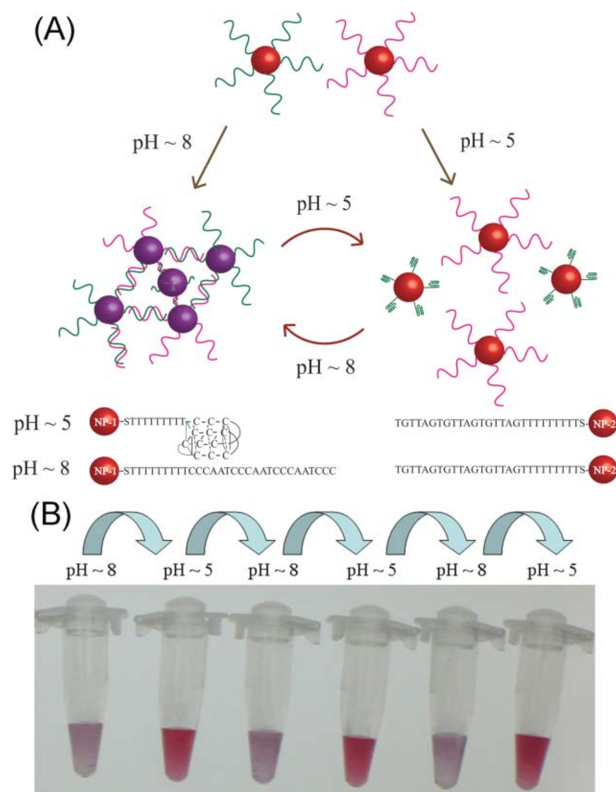

Fig. 1 (A) A Schematic illustration of the working cycle and color change of gold nanoparticle-DNA assembly at different pH of the solution and DNA sequences used herein, where NP1 indicates the gold nanoparticle modified with "i-motif" DNA and NP2 is nanoparticle modified with DNA complementary to "i-motif". The sequences of the DNA on each Au NPs are written out at the bottom of the scheme. (B) A picture showing the reversibility and color change of the DNA–gold conjugates at different pH. The pictures are taken $\sim$30 min after each pH change.

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The two samples both appeared red, separately, with the UV-vis absorption maximum at ~525 nm. When they were mixed, the DNA sequences on the two NPs hybridized to form a DNA duplex and brought the particles in proximity. The apparent color of the solution became purple (Fig. 1B) within 25 min, due to the gradual assembly of Au NPs into 3-dimensional macroscopic structures, which have a UV-vis absorbance maximum at ~563 nm (Fig. 2A).

When the solution pH was changed to ~5 by adding aliquots of 1 M HCl, the "i motif" on NP1 formed a quadruplex structure due to base intercalation. As a consequence, separation of the strands occurred, which led to deaggregation of the NPs, so that the apparent color of the solution turned red. By addition of aliquots of 1 M NaOH to increase the solution pH to ~8, the "i-motif" quadruplex reopened. As a result, the strands on the two NPs rehybridized and the color of the solution turned purple again. Fig. 1B shows the reversibility of the color change of the sensor in alternating additions of aliquots of H^+ and OH^-. UV-Vis absorbance was measured after each pH change, which showed a transition between ~525 nm and ~563 nm (Fig. 2A).

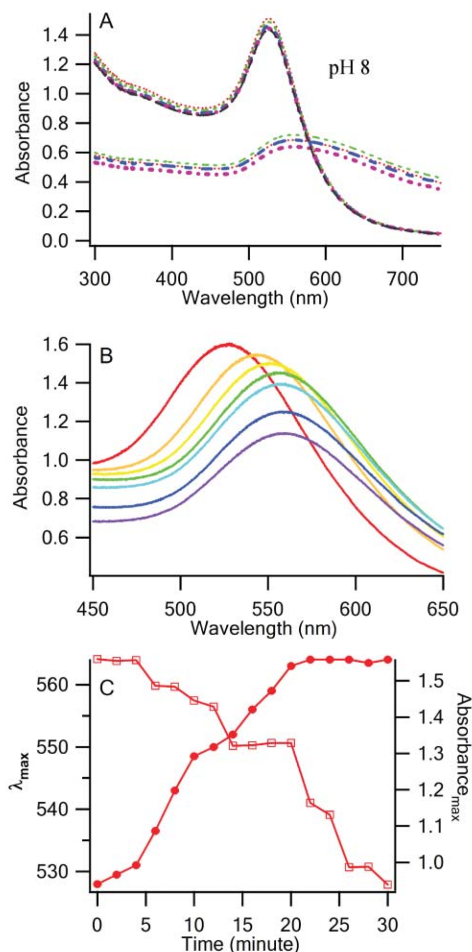

Fig. 2 (A) The UV-vis absorbance spectra of DNA–gold NP conjugates at different pH of the solution, ~525 nm is at pH ~ 5 and ~563 nm is at pH ~ 8. Here four cycles of pH and color changes are shown. Cycle 1, red traces; cycle 2, green traces; cycle 3, blue traces; cycle 4, purple traces. Each cycle takes ~30 min to finish. (B) After the pH of the solution is changed from ~5 to ~8, the UV-vis spectra shows the gradual aggregation of Au NPs with red shifting of the resonance peak and dampening of the absorbance. From red to purple, the spectra are taken in 5 min intervals. (C) The kinetics of the DNA–Au NP aggregation as the pH changes from ~5 to ~8. Solid circles indicate the absorbance peaks shifting to longer wavelength (left axis), and the empty squares show the maximum absorbance decreasing with time (right axis).

A native polyacrylamide gel (12%) was run at room temperature. A single band of the 1 : 1 mixture showed complete hybridization between these two sequences (see data in ESI†). This confirms the color change is indeed due to the hybridization between the "i motif" and its complementary sequence, even with three mismatches.

The color change of the Au NPs from red to purple upon an instant pH increase is a gradual process. The UV-vis absorbance spectrum of the sample was taken at regular time intervals (every 2 or 5 min), immediately after the NP1 and NP2 mixture had a pH change from ~5 to ~8. Fig. 2B and 2C follow the transitions with time. The gradual shift of the maximum wavelength of the absorbance and the decrease in the amplitude of the absorbance correspond to the color change. The shift in the maximum absorbance stops at ~20 min. The final decrease in the absorbance after 20 min is due to eventual precipitation of aggregates with time.

To further investigate the sequence specificity of the "i motif" with regards to pH change, we did control experiments with a pair of randomly sequenced complementary strands consisting of the same number of mismatches. DNA–Au NP conjugates were prepared using these two strands, and when they were mixed at pH ~ 8 in PBS buffer, the color of the solution changed to purple due to the DNA strands' hybridization. But when the pH of the solution was changed to acidic (~5), no purple to red color change was observed. This indicates the sequence specificity of the "i motif" sequences in serving the detection purpose (see ESI†).

In conclusion, we have demonstrated the use of DNA–Au NP conjugates as a colorimetric switch to show a color change upon a pH change of the solution. This colorimetric sensor is based on pH-dependent DNA conformational change on DNA–Au NP conjugates. At acidic pH ~ 5, the color observed was red, while in pH ~ 8, it appeared purple. Using this system, the detection of pH changes in solution is easy because the color change is obvious to the naked eye and does not need any sophisticated instrumentation to visualize. This color sensor is sensitive in the range of pH 5 to 8 and can be used reversibly and repeatedly. This may find applications in sensing pH changes in cells, plants or animals and environment. Since the color change is sequence specific to "i motif" sequences, which are found in the human telomeres and centromeres,[25] this colorimetric switch can also be used as a potential tool to explore the possible biological significance of the "i motif".

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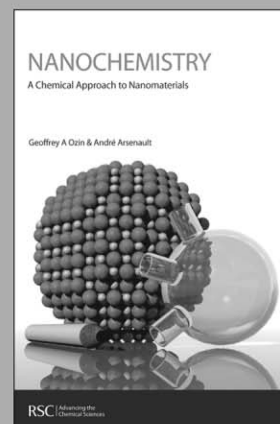

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