A simple and sensitive colorimetric pH meter based on DNA conformational switch and gold nanoparticle aggregation†

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A simple and rapid colorimetric pH meter has been developed based on the conformational switch of i-motif DNA and non-crosslinking AuNP aggregation, the average accuracy of the nano-meter was found to be ±0.04 pH unit across the physiological operating range.

Over the past two decades, nucleic acids have become a powerful tool in nanostructure fabrication and nanodevice design due to their conformational polymorphism, programmable sequence-specific recognition and robust physicochemical nature.[1] Recently, a large number of novel nucleic acid structures which can act as enzymes (DNA/RNAzymes) and as receptors (aptamers) have been created through modern *in-vitro* directed selection approaches and have shown numerous advantages in terms of their applicability.[2] Nevertheless, few DNA or RNA based pH sensors have been reported to date.[3] pH-Sensor design has been a focus of many research endeavors for biomedical and bioprogress applications. An important step in this direction has been the recent development of nanoparticle-based optical pH sensors, which offer many advantages over conventional approaches including high sensitivity and ease of miniaturization. Emissive water solubilized CdSe/ZnS nanocrystals and environmentally-sensitive dye molecules were tethered to sense pH change by modulating a fluorescence resonance energy transfer (FRET) process.[4] A core–shell silica nanoparticle which comprises a shell of covalently bound sensor-dye molecules surrounding a core of sequestered, covalently-bound reference-dye molecule was used to detect pH by fluorescence.[5] By using Au nanoshell with a pH-sensitive molecular adsorbate, an all-optical nanosensor capable of measuring pH in its local vicinity over the range of 5.80–7.60 pH units was reported recently through the pH-dependent surface-enhanced Raman scattering spectra of the adsorbate molecule.[6] These approaches, although valuable, are offset by complicated sample preparation processes, requiring expensive sophisticated instrumentation and/or analyzing complicated spectrum, and in certain case, poor pH resolution. Therefore, a new strategy is needed to overcome these problems for the future development of pH sensors.

Herein we present our approach for achieving a very simple and promising colorimetric pH meter based on the conformational switch of i-motif DNA and the phenomenon of salt-induced unmodified gold nanoparticle (AuNP) aggregation. AuNPs have been successfully employed as a colorimetric probe for the detection of nucleic acids, enzymes, proteins, metal ions, and other small molecules based on their size-dependent surface plasmon resonance absorption.[2f,7] Notably, these assays rely on the modification of AuNPs, and the AuNP aggregation is induced by inter-particle crosslinking which is a relatively slow process. Recently, Li and Rothberg reported a different approach to develop colorimetric method for rapid detection of DNA by using unmodified AuNPs.[8] The mechanism behind their strategy lies in that unmodified AuNPs can differentiate single-stranded and double-stranded DNA, and that only ssDNA effectively binds to AuNPs, and stabilizes them against salt-induced aggregation. Most recently, this concept was extended to enzyme sensing and aptamer-based recognition.[9] I-Motif DNA is a four-stranded DNA structure with stretches of cytosine bases. At low pH, the C residues are partially protonated and the DNA folds into the closed i-motif structure; when the pH is increased to basic, the C^+ residues are deprotonated and it unfolds to a single-stranded form.[10] Since the i-motif undergoes a precise structural change driven by a pH change, we then sought to take advantage of the observed non-crosslinking AuNP aggregation phenomenon to develop a simple colorimetric assay for pH detection. To our knowledge, our method is the first example of the use of unmodified AuNPs and the conformational switch of DNA to fabricate a sensitive colorimetric pH sensor with high sensitivity and accuracy.

To demonstrate the utility of this approach, a 21-mer (CCCTAACCCTAACCCTAACCC) i-motif DNA which carries a piece of the human telomeric sequence was employed as a model system in this work. A DNA oligo with a random sequence (CTTCCTCTTCTCTCTCCTCTC) was used as the control. AuNPs were then treated with i-motif DNA at different pH. I-Motif DNA undergoes a structural switch from a close-packed quadruplex (pH 5.0) to a fully extended, open state ssDNA (pH 8.0). Upon the addition of salt, the former solution would show a color change from red to blue, while the latter retains its original red color. This process is illustrated in Fig. 1A and the isosbestic point means the coexistence of both aggregated and isolated Au NPs along with the change of pH value. On the other hand, introducing a random DNA sequence of the same length would not induce the formation of i-motif quadruplex DNA at low pH, so no aggregation and no color change is expected.

The assay begins with the mixing of 3.7 nM AuNPs with 110 nM i-motif DNA in 7.3 mM phosphate buffer at proper pH value for 3 min at room temperature. It did not show any color changes, which indicated that pH value itself did not cause the aggregation of AuNPs in the pH range investigated

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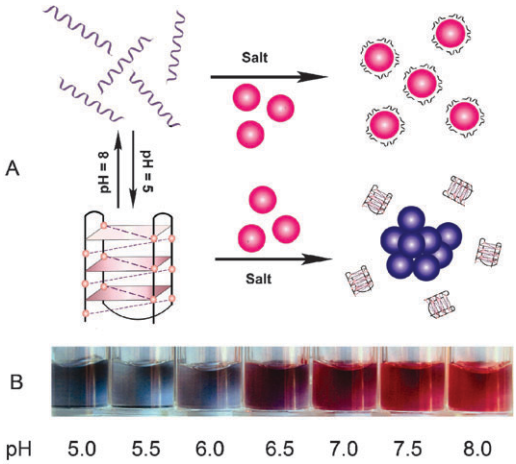

Fig. 1 (A) Schematic illustration of the colorimetric pH sensing based on conformational switch of i-motif DNA and non-crosslinking AuNP aggregation. (B) Visual color changes of the DNA/AuNPs system at different pH.

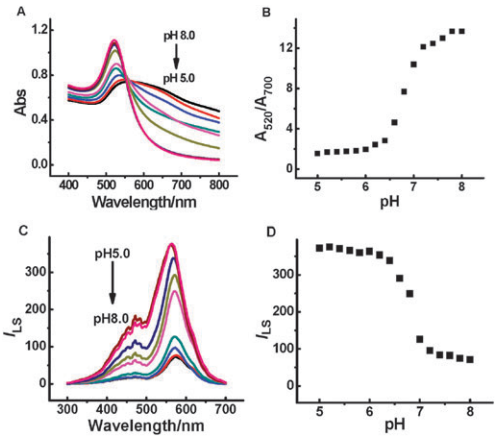

Fig. 2 (A) and (C): Representative UV-Vis absorption and light scattering spectra of the DNA/AuNPs mixed solution at different pH. (B) and (D): Plot of the absorption ratio (A_{520}/A_{700}) and the intensity of light scattering at 569 nm *versus* different pH values.

(data not shown). The solution was then challenged with appropriate amount of salts (NaCl, final concentration of 150 mM). Interestingly, the solution almost instantaneously changed its color upon pH-change. At pH 5.0, a red to blue color change was observed within seconds, while at pH 8.0, the solution retained its original red color. Moreover, we observed that the color change was sensitive to pH change. AuNPs gradually turned blue along with the decrease of pH value, implying the increased aggregation state of AuNPs (Fig. 1B). The aggregation of the AuNPs is attributed to the formation of a rigid quadruplex in acidic conditions. The stiff i-motif structure is similar to duplex DNA and cannot wrap around and stabilize the AuNPs, leading to their aggregation in the high ionic-strength medium. In contrast, the color change could not be observed for the control DNA sequence under the same conditions (Fig. S1 in the ESI†). This clearly indicated that the unmodified AuNPs can effectively differentiate the random coil and the rigid quadruplex DNA, thus serving as a very simple and promising colorimetric probe for pH sensing. This novel strategy has the advantage that i-motif/AuNPs need not to be labeled, thus one could simply identify a pH change with the naked eye. Moreover, compared with the AuNP aggregations induced by inter-particle crosslinking, which sometimes take a few hours to observe the color change, the AuNP test assay in the present study was completed in 1 min.

In addition to visual analysis, the sensitivity of the assay was further quantified by UV-Vis spectroscopy. The peak located at 520 nm is characteristic for the fully extended, open state ssDNA system (red color) at higher pH (8.0), while the 520 nm peak shifted to 530 nm and a new, broad absorption (540–750 nm) appeared for the folded i-motif system (blue color) at lower pH (Fig. 2A). In particular, the shifted absorbance to a longer wavelength with decreasing pH value of the solution correlated well with the color changes seen in Fig. 1B. When pH is decreased, the red-shifted band corresponding to the aggregation of AuNPs is intensified, and this is accompanied by a decrease in the plasmon absorbance of the individual AuNPs. Upon aggregation, the absorbance at 520 nm decreased, while the absorbance at 700 nm increased. We chose to use the absorbance ratio at these two wavelengths to quantify the color of the system. As can be seen in Fig. 2B, the assembly state of AuNPs was greatly dependent on pH value and a sigmoid-shaped calibration curve could be obtained. Significantly, the absorption ratio showed a sharp transition from pH 6.0 to 7.5, indicating that i-motif DNA underwent a significant conformational change in this pH range, which is agreed well with previous report that the formation of the i-motif folded structure showed a sharp transition around pH 6.5.[10a] The slightly higher mid-point in our system (pH 6.7–6.8) might due to the effect of the interaction between AuNPs and i-motif DNA. The most important feature to note in Fig. 2B is that the assay is very sensitive with a narrow range in the steeper part of the curve, which is expected to have an advantage in terms of detection in a biological relevant pH range.

An AuNP-based light scattering technique has been recently demonstrated to be a simple and sensitive tool for screening DNA binders in homogeneous solution.[7e] To further evaluate the performance and consistency of our assay, we used the light scattering method to investigate the pH sensing. As shown in Fig. 2C, resonance Rayleigh scattering (RRS) intensities for i-motif DNA were highly dependent on pH values. Weak RRS can be observed at 569 nm for the ssDNA/AuNPs system at pH 8.0. When the pH was decreased, the RRS peak gradually enhanced and eventually showed a distinct and strong RRS peak at pH 5.0. In contrast, the RRS intensities were little changed for the random DNA sequence in different pH, confirming the absence of AuNP aggregation (data not shown). The intensity of light scattering of i-motif/AuNPs mixed solution at different pH was shown in Fig. 2D. Remarkably, the sigmoid-shape curve also showed a sharp transition over the range of 6.0 to 7.5 pH unit, with a mid-point around pH 6.6–6.7. Therefore, the RRS results are fully consistent with the absorption results, and strongly support our other findings and the idea that our method can be an additional simple but sensitive approach for pH sensing in a biological environment.

An ideal pH sensor should have high sensitivity and accuracy to change in pH. A simple pH-dependent spectral output of the sensor (displayed in Fig. 2) may be effective to monitor

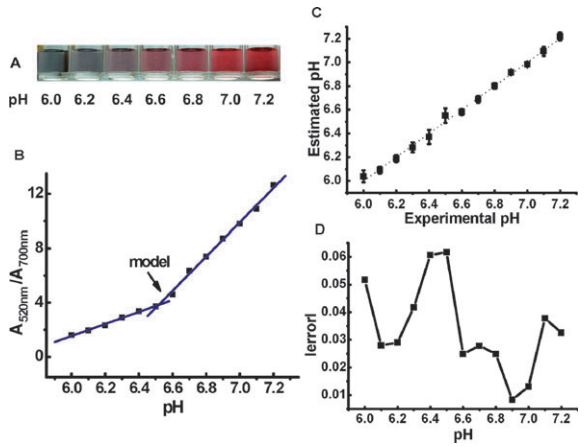

Fig. 3 (A) Photograph of the DNA/AuNPs system from pH 6.0 to 7.2 with increments of 0.2 pH units. (B) Plot depicting the relationship between absorbance ratio and pH value from pH 6.0 to 7.2. A locally linear approximation to the curve is built by finding the best collection of straight line segments to fit a collection of the data samples. (C) Model performance for the nanofabricated pH sensor between estimated and experimental pH values. Cross-validation was used to evaluate the quality of the model. (D) Magnitude of the average error of the cross validation estimation procedure shown in (C). The average error across the pH range is 0.04 pH.

large changes between pH 5.00 and 8.00, but in order to access the accuracy of the system quantitatively, it is essential to employ well-characterized and fully-validated pH measurements to yield reliable results. So, a finer data set (either the absorption ratio or RRS intensity) was collected in increment of 0.10 pH unit over the narrow pH range (6.00–7.20). This pH range was chosen because of its biological relevance and the dramatic optical responses of the system by subtle pH changes (Fig. 3A). To parameterize the relationship between absorbance ratio and pH value, a locally linear approximation was built by finding the best collection of straight line segments to fit the collection of data samples,[11] and a two-segment piecewise linear curve was found to fit our data with high accuracy and minimal complexity (shown in Fig. 3B and 3C). Then, cross validation method was used to judge the quality of our model and determine the average error magnitude across the pH range. As showed in Fig. 3D, the average error magnitude over the pH range of the sensor is 0.04. The pH accuracy has been markedly improved compared to the previously reported Au nanoshell-based optical nanoscale pH sensors with accuracy ranging from 0.10 to 1.0 pH unit.[6] Although we only addressed here the precious pH sensing over the range of 6.00 to 7.20, it is possible to apply to other pH range by just simply changing the stability of the i-motif structure through sequence modification. And furthermore, it's probably possible to improve the sensitivity by choosing proper AuNPs materials and detection system.

In summary, we have demonstrated a pH sensing assay that involving the principle of non-crosslinking AuNP aggregation and the use of DNA structural conformational transition that occurs when the pH changes. This novel assay is simple in design and fast in operation, avoiding either AuNPs modification or oligonucleotide labeling, and taking less than 3 min to complete the test. Due to the unique optical properties of AuNPs, the assay is easy to implement for visual detection. More importantly, operationally facile colorimetric and resonance RRS approaches have been developed with superior sensitivity for pH monitoring, which providing much higher accuracy and sensitivity in pH detection than the previously reported approaches. We expect that this strategy may offer a new approach for developing low cost, sensitive and rapid pH sensor that is likely to be highly useful in a wide range of applications within biology, biomedicine, process control and nanotechnology. Furthermore, since i-motif DNA used here was found in the human telomeric sequence and the color change is sequence specific to it, this colorimetric change can also be used as a potential tool to explore the possible biological significance of the i-motif.

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