Unmodified gold nanoparticles as a colorimetric probe for potassium DNA aptamers†

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Unmodified gold nanoparticles effectively differentiate unfolded and folded DNA, thus providing a novel approach to colorimetrically probe aptamer-based recognition processes.

Modern *in-vitro* directed selection approaches have created a large number of novel nucleic acid structures such as aptamers or deoxyribozymes/ribozymes (DNAzymes/RNAzymes).[1] Aptamers are DNA or RNA structures possessing high binding affinity to various ligands,[2] while DNAzymes/RNAzymes are catalytic nucleic acids exhibiting activities just like protein enzymes.[3] These *in-vitro* selected nucleic acid structures have become increasingly important in developing biosensors for a variety of biologically, environmentally or security relevant targets including proteins (thrombin, growth factors and HIV-associated peptides),[4] small organic molecules (ATP and cocaine)[5] and metal ions (K^+, Hg^{2+} and Pb^{2+}).[6–8] Other novel exciting applications include aptamer-based protein chips aimed at high-throughput screening in proteomics,[9] and DNAzyme-based molecular-scale logic gates or molecular computation.[10,11]

We are particularly interested in developing molecular sensors by using aptamers, which offer several unprecedented advantages. First, any given target can in principle find its specific aptamer *via* SELEX (systematic evolution of ligands by exponential enrichment),[1,12] thus aptamer-based sensors can be conveniently generalized to a biosensor platform.[13] Second, aptamers usually possess high binding affinity,[9] which can often be directly translated into high detection sensitivity. Third, DNA (and certain modified RNA) are chemically stable, and can be readily immobilized on solid surfaces, which makes it possible to develop robust and reusable solid-state sensors.[14]

Aptamers usually undergo significant structural variations upon binding with specific ligands.[2] Differentiation of such structural change thus forms the basis of aptamer-based sensors. A typical aptamer-based sensor involves an oligonucleotide doubly labeled with a pair of electron-/energy-transfer donor and acceptor.[6,7] As a result, ligand-binding induced aptamer structural variation is directly coupled to the distance change between the donor and the acceptor, which can be optically or electrochemically interrogated with high sensitivity.[15] Very recently, Liu *et al.* reported the use of gold nanoparticles (AuNPs) as a colorimetric indicator for aptamer-based detection.[5,16] Their approach relies on the modification of AuNPs with thiolated DNA aptamers, and the concept of target-mediated aggregation of AuNPs, which was originally developed by Mirkin's and Alivisatos' group.[17,18] Other approaches for AuNPs-based DNA detection include conjugating biotinylated DNA on avidin-coated AuNPs.[19] We herein report that by exploiting interactions between AuNPs and DNA sequences, unmodified AuNPs can effectively differentiate unstructured and folded aptamer, thus serving as a very simple and promising colorimetric probe for aptamer-based sensing.

A DNA aptamer for potassium ions (K^+) which has a sequence of GGG TTA GGG TTA GGG TTA GGG was employed as the model system in this work. Two DNA oligo with random sequences (AGC AAC CTC AAA CAG ACA CCA TGG; TAG CTA TGG AAT TCC TCG TAG GCA) was used as the control. The K^+ aptamer is a G-rich ssDNA and is random-coil like in solution.[6] Upon binding to its target (K^+), the aptamer folds to a four-stranded tetraplex structure (G-quartet) *via* intramolecular hydrogen bonds between guanines.[8] It is worth noting that the K^+ aptamer carries a piece of the human telomere sequence, which is known to be physiologically important in protecting cells from recombination and degradation, and that G-quartet structures are indeed observed in the human telomere DNA.[20]

We demonstrate that color changes of unmodified AuNPs which are visible with the naked eye (Fig. 1) can be used to probe the formation of G-quartets. Solutions of unmodified AuNPs (3.5 nM) are red colored due to their specific and size-dependent surface plasmon resonance (SPR) absorption. Addition of salt screened electrostatic repulsion between negatively charged, unmodified AuNPs, and resulted in aggregation of AuNPs that

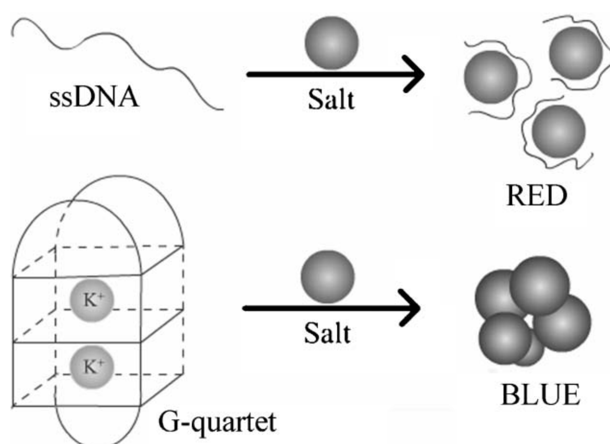

Fig. 1 Scheme of the K^+ aptamer and colorimetric detection of K^+-induced structural variation.

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led to red-to-purple color change.[17] We then treated AuNPs with the K^+ aptamer for 4 min, either in the presence or in the absence of K^+. Upon the addition of salt, the former solution showed a color change from red to purple, while the latter retained its original red color (Fig. 2). Apparently, the unstructured aptamer (ssDNA) stabilized AuNPs, which was because the adsorption of highly negatively charged ssDNA on AuNPs significantly increased their resistance to salt-induced aggregation.[21] However, this stabilization effect was insignificant in the presence of K^+, the facilitator for the formation of G-quartets. AuNPs treated with G-quartets changed color just like unmodified AuNPs, suggesting that the G-quartet structure did not significantly adsorb to AuNPs.

We propose that the differentiation ability of AuNPs comes from the complex interactions between DNA and AuNPs. This effect is in fact similar to recent findings by Rothberg and coworkers, who reported a colorimetric strategy for DNA detection by using unmodified AuNPs.[21] The mechanism behind their strategy lies in that unmodified AuNPs can differentiate single-stranded (ss-) and double-stranded (ds-) DNA; and that only ssDNA effectively binds to AuNPs, and stabilizes them in solutions of high ionic strength.[21] Interestingly, this differentiation ability of AuNPs towards ss- and ds-DNA also forms the basis of our recently developed "nanoparticle PCR", a novel AuNPs-enhanced PCR strategy with high selectivity.[22]

The interactions between DNA and AuNPs are rather complex. On the one hand, DNA bases possess high affinity to gold *via* coordination between Au and nitrogen atoms (favoring DNA adsorption); on the other hand, negatively charged surfaces of AuNPs electrostatically repel DNA phosphate backbones (disfavoring DNA adsorption).[22] Interestingly, formation of G-quartets not only increases surface charge density as compared to unstructured ssDNA,[8] but also prevents the exposure of DNA bases to AuNPs, thus disfavoring adsorption of G-quartets on AuNPs in both facets. In addition, unstructured ssDNA is soft and random-coil like, which is in sharp contrast to the rigid structure of G-quartets. As a result, ssDNA possess higher freedom to wrap on AuNPs than G-quartets, which may also contribute to the differentiation ability of AuNPs.[22]

Fig. 2 Colorimetric detection of K^+-induced structural variation of the K^+ aptamer. Four microliters of DNA solutions (8.3 μM), either in the presence or in the absence of K^+ (1.67 mM), were mixed with 200 μL of gold nanoparticles. The solutions were allowed to react for 4 min at room temperature and then 35 μL of buffered salt (10 mM phosphate, 0.5 M NaCl, pH 7.4) were added to each solution. From left to right: K^+ aptamer + K^+; control DNA + K^+; K^+ aptamer.

We observed that the color change of AuNPs was a sensitive function of K^+ concentration. AuNPs gradually turned to purple along with the increase of K^+ concentration, implying the increased aggregation state of AuNPs. UV-vis studies provided quantitative results, which clearly showed that adsorption at 522 nm gradually decreased while adsorption at 650 nm increased (Fig. 3). This blue shift in the SPR absorption suggested the formation of large-sized aggregates of AuNPs. Significantly, color change is visible at as low as ~1 mM of K^+, suggesting that AuNPs are sensitive probes for aptamer structures.

AuNPs are also selective probes for G-quartets. We found that the control DNA sequence, which could not form structures, did not show color change in the presence of K^+ (Fig. 2). We also examined the effect of a variety of monovalent cations (Li^+, Na^+, Rb^+, NH_4^+) and two physiologically relevant divalent cations (Ca^{2+} and Mg^{2+}) at 16.7 mM. We found that none of these ions could induce significant color change of AuNPs. This is markedly different from K^+, which showed visible purple color at a concentration more than an order of magnitude lower than these non-cognate ions (Supplementary Information).

Detection of G-quartets has traditionally been performed with spectral techniques such as UV-vis, circular dichroism (CD) and NMR.[23] More recently, Ueyama *et al.* reported a fluorescent sensor that could differentiate G-quartets from unstructured ssDNA by using doubly labeled aptamers.[6] Wang and coworkers developed an improved strategy by introducing fluorescent conjugated polymers and exploiting a variation of Forster resonance energy transfer (FRET) between polymers and the dye labeled on aptamers.[8] In contrast to these instrumentation-based detection techniques, we have shown that unmodified AuNPs can act as a colorimetric structural probe for K^+ aptamers. This novel strategy has the advantage that aptamers/AuNPs need not be labeled, thus one could identify aptamer structures simply with the naked eye.

While this work is in its preliminary stage, it could be improved by introducing several existing technologies, which might eventually lead to a cost-effective biosensor platform. Since the detection is homogeneous, it is easily adaptable for high-throughput assays in microwell-based plates and even automated analysis by using microfluidics. More importantly, this strategy is not limited to probing G-quartets and detection of K^+. In fact, it is a general phenomenon that aptamers predominantly exist in random coils in solution, while folding to a well ordered structure during ligand binding.[2] Therefore the principle described in this work can be conveniently generalized to other aptamer systems.

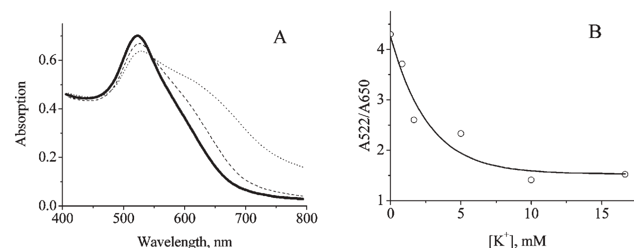

Fig. 3 A) Absorption spectra of gold nanoparticles/aptamer mixed solutions in the absence (solid line) and in the presence of K^+ with concentrations of 1.67 (dashed line) and 10 mM (dotted line). B) Plot of the absorption ratio (A522/A650) *vs.* K^+ concentration.

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Eventually, we expect that it will be possible to visually detect a large number of important analytes with unmodified AuNPs and aptamers generated from SELEX.

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