Instantaneous and Quantitative Functionalization of Gold Nanoparticles with Thiolated DNA Using a pH-Assisted and Surfactant-Free Route

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S *Supporting Information*

ABSTRACT: The attachment of thiolated DNA to gold nanoparticles (AuNPs) has enabled many landmark works in nanobiotechnology. This conjugate chemistry is typically performed using a salt-aging protocol where, in the presence of an excess amount of DNA, NaCl is gradually added to increase DNA loading over 1−2 days. To functionalize large AuNPs, surfactants need to be used, which may generate difficulties for downstream biological applications. We report herein a novel method using a pH 3.0 citrate buffer to complete the attachment process in a few minutes. More importantly, it allows for quantitative DNA adsorption, eliminating the need to quantify the number of adsorbed DNA and allowing the adsorption of multiple DNAs with different sequences at predetermined ratios. The method has been tested for various DNAs over a wide range of AuNP sizes. Our work suggests a synergistic effect between pH and salt in DNA attachment and reveals the fundamental kinetics of AuNP aggregation versus DNA adsorption, providing a novel means to modulate the interactions between DNA and AuNPs.

The field of nanobiotechnology has emerged since 1996 with the landmark work for attaching thiolated DNA to gold nanoparticles,[1,2] allowing the programmability, molecular recognition, and catalytic property of biopolymers to couple to the optical, thermal, electric and catalytic property of inorganic nanomaterials. Numerous applications have since been made possible, including the preparation of ordered nanoparticle structures,[3] biosensing,[4] separation,[5] and gene and drug delivery.[6] At the same time, many distance-dependent physical properties related to nanoparticles in terms of heat, electron, and energy transfer[7] as well as polyvalent binding[8] have been systematically studied using DNA as a rigid spacer, adding new insights into nanoscience.

To enable these applications, a crucial step is to attach thiolated DNA to gold nanoparticles (AuNPs) and to ensure that the conjugate is stable at least under physiologic buffer conditions for DNA hybridization.[9−11] The most commonly used 13 nm AuNPs are usually prepared using a citrate reduction method and are stabilized by the negative charges from the weakly adsorbed citrate ions. Even very low concentrations of salt (e.g., 50 mM NaCl) can induce irreversible aggregation of such AuNPs. DNA is highly negatively charged and thus repelled by AuNPs. This charge repulsion posed an initial technical difficulty, since if a high concentration of NaCl is added to screen the charge, AuNP aggregation occurs before a high density of DNA is attached. This problem has been elegantly solved by using a process known as "salt aging", where NaCl was stepwise added to the DNA/AuNP mixture.[9,10] It was later discovered that the density of attached DNA is proportional to the final NaCl concentration.[12−14] The attached DNA enhances AuNP stability so that even more NaCl can be added to further increase DNA loading. This iterative process (Figure 1A) takes
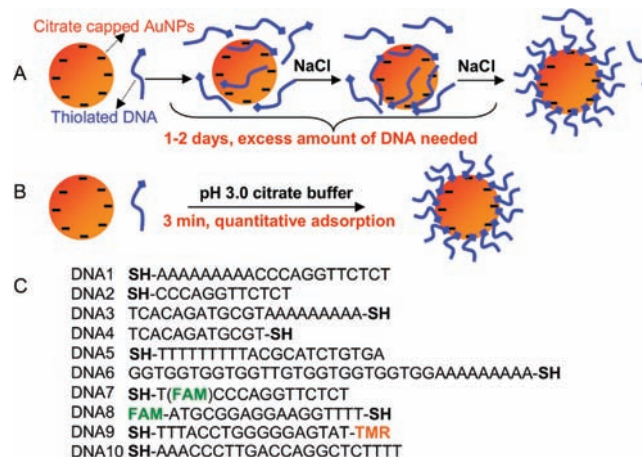

Figure 1. Schematics of attaching negatively charged thiolated DNA to negatively charged AuNPs using the salt aging (A) and the low pH assisted (B) methods. (C) Sequences (listed from 5′ to 3′) and modifications of the DNAs used in this work.

1−2 days to form a stable conjugate,[9,15,16] and the stability of this gold−thiol bond has also been systematically studied.[17,18]

This procedure works less well for larger AuNPs (e.g., 50 nm), and it requires even more steps of salt addition.[19,20] In 2006, it was reported that AuNPs up to 250 nm could be stably functionalized in the presence surfactants, such as sodium dodecylsulfate.[12] The stepwise addition of NaCl is still required though, taking a full day. This discovery has allowed for systematic size-dependent research to be carried out.[16,21] In 2009, a fluorinated surfactant was reported to achieve rapid

DNA loading in ∼2 h, where even 1 M NaCl could be added all at once.[22] Surfactants were first adsorbed so that AuNPs could withstand high NaCl concentration, which in turn facilitated rapid DNA adsorption and displacement of surfactants. The use of surfactants, especially fluorinated surfactants, is undesirable for many applications including drug delivery since surfactants might be toxic to cells.

In all the previous work, an excess amount of DNA was added, and nonconjugated DNA had to be removed. To quantify the number of DNA attached to each AuNP, fluorescently labeled DNAs are often used,[14] or DNA staining dyes have to be employed to prepare calibration curves. The situation is getting even more complicated when several different DNAs need to be attached at a certain ratio. Since different DNAs may have different adsorption rates, the added ratio is unlikely to be the final ratio on AuNP. Therefore, a method for fast, quantitative, and surfactant-free DNA loading is needed to further advance this field. Herein we communicate such a method to achieve all these goals (Figure 1B). The only required reagent is a low pH citrate buffer. Most previous work on tuning the AuNP/DNA interaction employs only salt,[19,23−25] while the effect of pH remains largely unexplored.[18,24,26] Our discovery reveals a new way to control DNA adsorption, which is quite different from using salt and is likely to find applications in many areas involving AuNPs and DNA.

To test the effect of pH, two tubes each containing 10 nM of citrate-capped 13 nm AuNPs were mixed with 3 μM DNA1 (see Figure 1C for DNA sequence) for 1 min before the samples were respectively adjusted to pH 7.6 and 3.0. After 3 min, 0.3 M NaCl was added. The sample in pH 7.6 immediately turned purple, indicating AuNP aggregation, but the one in pH 3.0 remained red (Figure 2A). Next, 200 μL of each AuNP was centrifuged to remove the supernatant, and the pallets were dispersed in a typical buffer for DNA hybridization (300 mM NaCl, 10 mM HEPES, pH 7.6). Again, the pH 7.6 sample turned blue right away, but the pH 3.0 one remained red even with 1 M NaCl (Figure 2B). Therefore, only the DNA
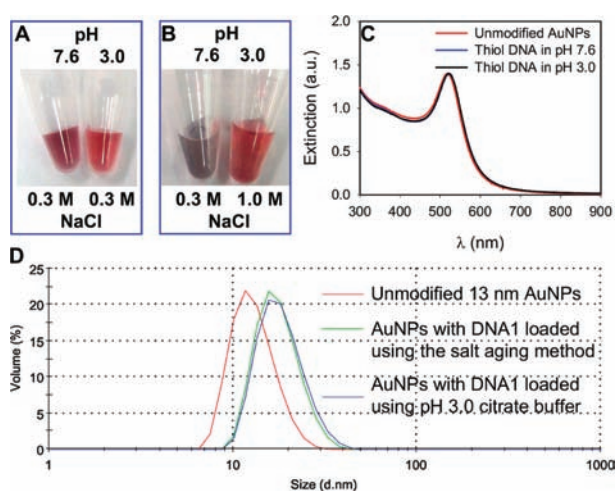
attachment step needs to be performed at low pH. After that, the pH can be adjusted to neutral. To confirm the quality of AuNPs after DNA attachment, the AuNPs were characterized using UV−vis spectroscopy (Figure 2C). The spectra were almost identical for the unmodified AuNPs, AuNPs attached with DNA1 using the conventional salt aging method and with the low pH method. Next the AuNPs were studied using dynamic light scattering (Figure 2D). The hydrodynamic size of unmodified AuNPs was 13.5 nm. Using the salt-aging method, the size changed to 18.6 nm due to the attached DNA. Using the pH 3.0 buffer, the size was 17.9 nm. Therefore, the quality of AuNPs was comparable for the two methods, and no AuNP aggregation was detected.

The above experiments indicate that stable conjugates can be achieved in a few minutes at pH 3. To gain quantitative understanding, the reaction kinetics was monitored in 10 mM citrate·HCl buffers of different pH. For this purpose, a thiol and FAM (6-carboxylfluorescein) dual labeled DNA was used (DNA8). DNA adsorption brings FAM close to AuNP to induce fluorescence quenching. Since FAM is pH sensitive, the kinetic measurement was performed by transferring a small volume of the DNA/AuNP mixture (DNA:AuNP = 75:1) into a large volume of pH 7.6 buffer at designated time points. As shown in Figure 3A, >80% DNA adsorption was achieved in
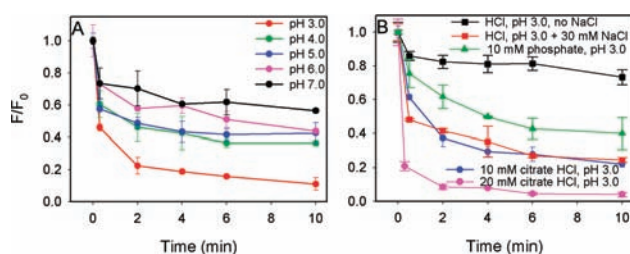

Figure 3. Kinetics of fluorescence decrease indicating DNA adsorption as a function of pH (A) and salt (B). In (A) all the buffers contained 10 mM citrate·HCl and therefore 30 mM Na$^+$.

the first 2 min at pH 3. DNA adsorption gradually decreased with increasing pH. At pH 7.0, only ∼40% DNA adsorbed. Therefore, DNA adsorption is a strong function of pH.

Since citrate is one of the components in the AuNP solution, using citrate buffer for pH adjustment avoids interference. For mechanistic understanding, we aim to test whether the fast adsorption is a pure pH effect or citrate itself plays a role. We next used HCl to adjust pH to 3.0. Interestingly, after the initial drop of ∼20% fluorescence, further DNA adsorption became much slower (Figure 3B, black squares), suggesting these initially adsorbed DNA hindered further adsorption. The 10 mM citrate·HCl buffer contained 30 mM Na$^+$ from trisodium citrate, but no additional Na$^+$ was present if pH was adjusted using HCl. For a fair comparison, 30 mM NaCl was added to the pH 3.0 HCl sample, and efficient adsorption was then obtained (red squares). Using 10 mM pH 3 phosphate (∼10 mM Na$^+$) produced intermediate adsorption (green triangles). Therefore, both low pH and salt were required for fast adsorption. If 20 mM of pH 3.0 citrate·HCl buffer was added (i.e., 60 mM Na$^+$), ∼100% adsorption was achieved in 2 min (pink dots), further confirming the role of salt concentration.

All the above experiments were performed by mixing DNA and AuNPs before adjusting pH. In the absence of DNA, we noticed that if HCl was used, AuNPs were stable even at pH 3.0. If citrate·HCl was used, however, AuNPs changed color to

Figure 2. (A) Photograph of AuNPs mixed with DNA1 for 3 min at pH 7.6 or 3.0 followed by adding 0.3 M NaCl. (B) The samples were prepared the same way as in (A), but the free DNAs were removed, and AuNPs were redispersed in buffers containing 0.3 M NaCl and pH 7.6 or 1.0 M NaCl and pH 7.6. Characterization of DNA1-functionalized AuNPs using UV−vis spectroscopy (C) and dynamic light scattering (D).

blue right away (Figure S1, Supporting Information). This again indicated the effect of salt. Since HCl is volatile, we employed citrate·HCl buffer for subsequent studies.

To test whether the AuNPs were homogeneously functionalized with such a short incubation time at low pH, DNA8 was mixed with AuNPs at various pH for 10 min, and then the samples were analyzed using gel electrophoresis (Figure 4A). The sample at pH 3.0 migrated as a single sharp band, indicating that each AuNP contained roughly the same number of DNA. The samples at pH 7 and 8, however, were smeared, suggesting a wide distribution of DNA density. The same gel was also imaged using UV light excitation to observe fluorescence from nonconjugated free DNAs (Figure 4B, white bands). The pH 3 sample had almost no free DNA, suggesting high adsorption efficiency. The higher pH samples showed more free DNA, consistent with the previous kinetic data. In this imaging mode, AuNPs appeared to be dark bands since they absorbed UV light.

In the above kinetic experiments, a DNA:AuNP ratio of 75:1 was used, and quantitative adsorption was achieved with 20 mM citrate·HCl. Quantitative adsorption is important for applications where a designated number of DNA need to be adsorbed or a mixture of different DNAs need to be loaded at a certain ratio. To further characterize DNA adsorption, the DNA:AuNP ratio was varied from 20 to 150. Close to quantitative adsorption was observed up to a ratio of 80 (Figure 4C). After that, more free DNAs were observed due to surface saturation. Such experiments cannot be carried out by adding salt at neutral pH in the absence of surfactants since the addition of 50−100 mM NaCl (e.g., typical for salt aging) induced AuNP aggregation even at a relatively high DNA:AuNP ratio (inset of Figure 4C and see Figure S2, Supporting Information for UV−vis spectra). This may explain why in the salt-aging method, DNA is always used in great excess, and a long incubation time is needed. We have previously studied DNA adsorption as a function of NaCl concentration at neutral pH, where <20 nonthiolated DNAs were adsorbed in the presence 90 mM NaCl.[24] Adsorbed DNA posed a strong repulsive barrier for incoming DNA, thus limiting the loading capacity. At pH 3, A and C bases are positively charged and even citrate is partially protonated, reducing charge repulsion with AuNPs and among DNA and allowing for fast adsorption.

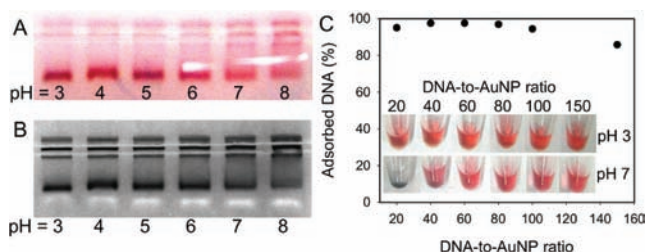Our results imply the fundamental reaction kinetics during AuNP functionalization. The negative charges on citrate capped AuNPs are essential for their colloidal stability, which also retard DNA adsorption. To achieve DNA attachment while still maintaining good dispersion of AuNPs, DNA adsorption needs to proceed before AuNP aggregation. In the salt aging method, the kinetics of DNA adsorption needs to be accelerated by a high DNA concentration. In other words, at neutral pH if DNA concentration is low, AuNPs aggregate before DNA is attached upon adding salt. At low pH, it is possible to achieve DNA binds to AuNPs before AuNP aggregate even when the DNA concentration is low (e.g., 20:1).

So far, we have established that low pH is essential for fast DNA adsorption. To test whether low pH has any effect on adsorption capacity, DNA8 and 13 nm AuNPs were incubated at a ratio of 200:1 in pH 3 and 7, respectively. The Na^+ concentrations were adjusted to 30, 100, and 300 mM, and the adsorbed DNAs were quantified (Figure 5A). At each salt
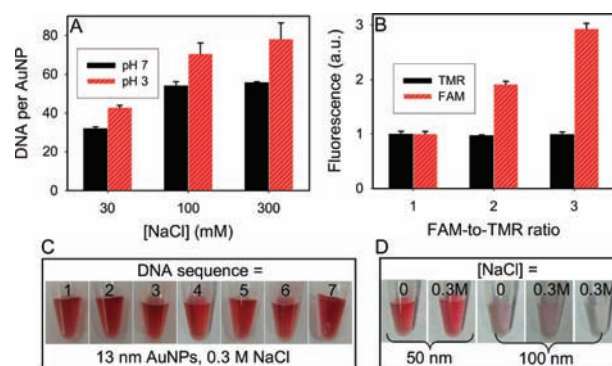

Figure 5. (A) DNA adsorption capacity as a function of pH and salt concentration. (B) Attachment of two DNAs at different ratios. (C) AuNPs attached with different DNA sequences at pH 3 with 300 mM NaCl. (D) Functionalization of 50 and 100 nm AuNPs with DNA10. The last tube did not contain any DNA.

concentration, the capacity of DNA at pH 3 was ~30% higher than that at pH 7. Higher NaCl concentrations resulted in increased capacity at both pH's, consistent with the literature.[12] A high DNA loading is one of the most important features of such DNA-functionalized AuNPs, giving multivalent binding and cooperative melting. However, if the DNA density is too high, it may inhibit DNA hybridization.[22]

To ensure that conjugates prepared at low pH were functional, we tested DNA-directed assembly of AuNPs. The AuNPs were modified by respectively mixing with DNA1 and DNA3 followed by adjusting pH to 3.0 for 3 min. For comparison, the normal salt aged samples were also prepared. In both cases, AuNPs changed color to purple in a few minutes after adding linker DNA (data not shown), indicating AuNP aggregation. Upon increasing temperature, sharp melting transitions were observed (Figure S3, Supporting Information), which is one of the hallmarks of such DNA-linked AuNPs.[10] Therefore, our pH method generates fully functional AuNPs.

Next we aim to test whether this method is general to other DNA sequences. A total of seven thiolated DNAs were used (see Figure 1C for DNA sequences) including ones with poly-A spacer, poly-T spacer, and various fluorophores. The length of DNA also varied from 12 to 35 nucleotides; all showed good protection just several minutes after adjusting pH to 3 (Figure 5C), confirming good generality to DNA sequence. Using DNA10, AuNPs of 50 and 100 nm were functionalized at pH 3 (Figure 5D). Both showed color similar to the control samples

Figure 4. Gel electrophoresis of AuNPs mixed with DNA8 in buffers of various pH. (A) Picture taken using a digital camera and AuNPs are red. (B) Picture taken using a gel documentation system with UV excitation. The free DNA appears as white bands; the AuNPs appear black. (C) Percentage of adsorbed DNA as a function of DNA:AuNP ratio at pH 3 in 20 mM citrate·HCl buffer. Inset: photographs of AuNPs in pH 3 citrate·HCl (no additional NaCl) and pH 7 (100 mM NaCl added a few minutes after mixing DNA with AuNPs).

where no NaCl was added. The 100 nm sample was purple even in the absence of salt. If NaCl was added in the absence of DNA, the tube turned clear immediately (the last tube in Figure 5D; see Figure S4, Supporting Information for UV–vis spectra). Therefore, fast adsorption of DNA at low pH is also generally applicable to various AuNP sizes.

For more advanced applications, it is often desirable to attach multiple different DNAs at designated ratios.[27] Current methods employ an excess amount of DNA, disallowing a rigorous control on the final ratio of adsorbed DNA.[27b] In this regard, quantitative DNA adsorption at low pH might be useful to achieve this goal. To test this, we mixed DNA8 and DNA9 at 1:1, 2:1, and 3:1 ratios, where the DNA9:AuNP ratio was maintained at 15:1. The fluorescence recovered after releasing the DNAs from AuNPs was measured, and all showed similar TMR fluorescence but the FAM intensity followed a 3:2:1 pattern (Figure 5B). Therefore, multiplexed functionalization with precise density control was achieved.

In summary, we reported a facile method for instantaneous attachment of thiolated DNA to AuNPs using a low pH buffer. The whole process takes just a few minutes, as compared to 1–2 days of using the salt-aging protocol. One important feature is to achieve quantitative DNA adsorption and maintain AuNP stability even at low DNA density, allowing the adsorption of designated numbers of DNA and the attachment of multiple DNAs at designated ratios. While the salt-aging method has driven the development of nanobiotechnology in the past 16 years, these new features brought by the low pH method will enable more applications. In addition to its technical importance, our work also has fundamental implications that can impact many other fields of research. For example, in colloidal and surface science, while electrostatic interactions can be controlled by either using high salt to reduce the Debye length or using low pH to reduce the surface charge density, this study reveals a possible synergistic effect between these two factors. So far, most of the work on DNA-functionalized AuNPs has been carried out at close to neutral pH. Our results indicated that pH provides a new way to tune the interaction between DNA and AuNPs.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Materials and methods, melting curves, UV–vis spectra, and AuNP stability assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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