Communication

G-Quartet-Induced Nanoparticle Assembly

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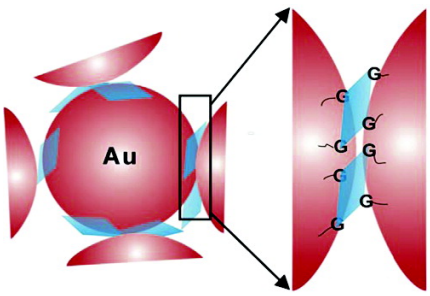

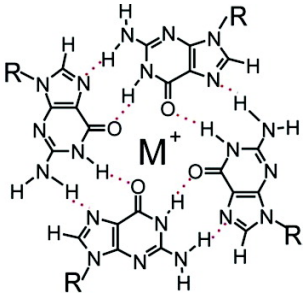

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G-Quartet-Induced Nanoparticle Assembly

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Nanoparticles heavily functionalized with oligonucleotides have been widely used as building blocks in a variety of rational bottom-up assembly schemes and as probes in ultra-sensitive assays.[1,2] These particle probes, when bound to complementary oligonucleotides, exhibit extraordinarily sharp melting transitions due to cooperative binding of the oligonucleotides between the particles.[3] This sharp transition translates into a major technological advantage in terms of high assay selectivity and, specifically, the ability to distinguish perfectly complementary targets from ones with single-base mismatches.[3a]

A fundamental unanswered question in this field pertains to the minimum number and types of bases or recognition elements required to effect assembly. In the case of deoxyguanosine (G), it is conceivable that a small number or a series of single bases could effect nanostructure assembly. This is possible for three reasons. First, G is a tighter binder to C than A is to T. Second, G can form quartet structures, which are more stable than duplex structures based upon comparable length sequences. Third, the nanoparticles, in principle, provide a substrate that can tether G-containing sequences and promote cooperative binding. Herein, we describe studies aimed at delineating the binding and melting properties of nanoparticles functionalized with G-rich sequences and compare them to free strands of the same sequence.

In a typical experiment, a thiolated guanosine phosphate derivative (3′-thiolpropyl deoxyguanosine phosphate, **1**) was immobilized on the surface of 13 nm Au nanoparticles by mixing an aqueous solution containing **1** with an aqueous solution of the nanoparticle (∼10 nM) at an 800:1 molar ratio for 1 day. The resulting Au nanoparticles are stable at room temperature in the absence of additional salt and remain dispersed with no spectroscopic evidence of degradation for over 1 month. However, upon increasing the solution salt concentration to 0.1 M NaCl (by adding aliquots of 2 M NaCl), the nanoparticles gradually assemble into macroscopic architectures (Figure 1A). This assembly process occurs with a concomitant color change from red to purple, a consequence of decreased interparticle distance and increased plasmon coupling.[1c] Eventually, the aggregates precipitate from solution, and the reaction medium is colorless. The assemblies are composed of discrete nanoparticles with no evidence of particle fusion (TEM) and are presumably held together via interactions between the base-terminated surface ligand. Consistent with this conclusion, these aggregates exhibit melting transitions when heated from room temperature to 80 °C (Figure 1B,C). The assembly process can also be reversed by lowering the ionic strength of the medium. This is accomplished by centrifugation of the nanoparticle assembly and redispersion in a medium of lower ionic strength (e.g., water, Figure 1D). In comparison, unmodified citrate-stabilized particles exhibit irreversible salt-induced particle aggregation, eventually resulting in the formation of bulk gold precipitates.

In an effort to understand this assembly process, we synthesized a series of G-rich sequences that could be used to modify gold

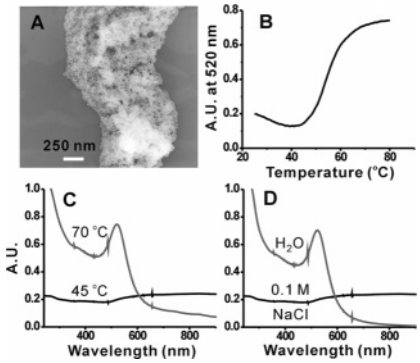
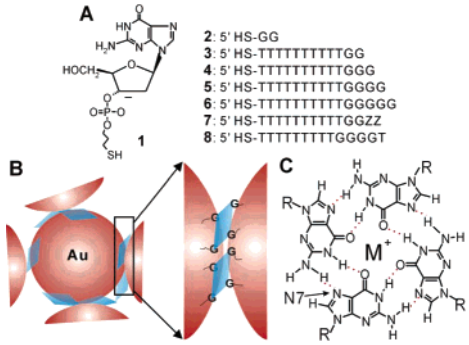


Figure 1. (A) A typical TEM image of formed microscopic Au nanoparticle assembly. (B) Melting transition of **1**-modified Au nanoparticle assembly. (C) UV−vis spectra of **1**-modified Au nanoparticle assembly before (45 °C) and after (70 °C) thermal dissociation. (D) UV−vis spectra of **1**-modified Au nanoparticle assembly before and after redispersion using water.

Scheme 1



nanoparticle surfaces, **2**−**8** (Scheme 1A). Having G groups near the point of attachment to the particle surface, as in **1** and **2**, is not ideal because interactions between the G-base and the surface can compete with interactions between two particles.[3b] The T-10 tether in **3**−**7** is commonly used in the design of probes to further stabilize the particle and push a recognition sequence away from the particle surface to enhance recognition and hybridization.[3] However, it destabilizes aggregates due to an increase in particle charge. This is evidenced by the observation that particles modified with a 2G sequence without a T-10 tether (**2**) assemble into an aggregate that cannot be thermally disassembled in 0.3 M NaCl PBS solution; however, particles with a T10G2 sequence **3** will not assemble under identical conditions. Note that by decreasing the ionic strength of the solution containing the aggregates assembled by the **2**-modified particles, complete disassembly can be effected.

If one examines the assembly properties of the particles modified with T10-G-rich sequences, one observes the following trends. The particles terminated with strands containing 3Gs, **4**, melt at 35.7 °C, while those terminated with 4 and 5Gs, **5** and **6**, melted at 53.0

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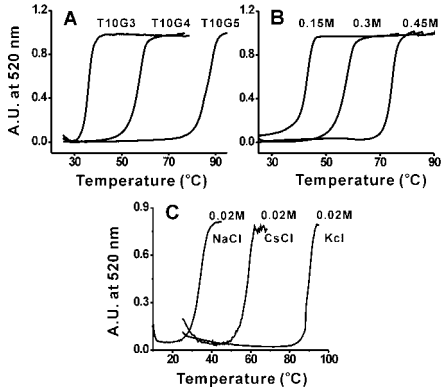

Figure 2. (A) Melting transitions of G-rich DNA (**4**−**6**) modified Au nanoparticle assemblies in 0.3 M NaCl, 0.01 M sodium phosphate buffer (pH = 7.0). (B) Melting transitions of **5**-modified Au nanoparticle assembly in solutions with different ionic strengths (0.15, 0.3, or 0.45 M NaCl PBS). (C) Melting transitions of **5**-modified Au nanoparticle assembly in 0.2 M LiCl, 0.01 M lithium phosphate buffers (pH = 7.24) with 0.02 M different cations (NaCl, KCl, or CsCl).

and 86.8 °C, respectively, in a 0.3 M NaCl PBS solution (Figure 2A). The solution ionic strength also significantly affects the G-directed assembly process. For example, assemblies formed from particles modified with the T10G4 sequence **5** melt at 43.0 °C in 0.15 M NaCl PBS and 74.3 °C in 0.45 M NaCl PBS solution (Figure 2B). All of these data are consistent with a chemically specific interaction between the G-terminated surface ligands on different particles overcoming the electrostatic repulsion between the particles and being responsible for the aggregate assembly process.

We propose that the chemical interaction between the G-modified particles is the formation of G-quartet structures (Scheme 1B,C). Two additional experiments that support this conclusion include the following: (1) a cation dependence on the assembly process, and (2) the observation that particles modified with sequences where the Gs have been replaced with an unnatural analogue, 7-deaza-dG (Z), do not result in particle assembly.[4] The N7 nitrogen atom of G in the unnatural base Z, which is a key structural element for the G-quartet formation, is replaced by a carbon atom. The assembly process is dramatically affected by the nature of the cation. It is accelerated, and the resulting aggregates are stabilized according to the following trend: $K^+ \gg Cs^+ > Na^+$, as one could explain if G-quartets were involved in the assembly process.[4] For example, after adding NaCl, KCl, or CsCl (to a final concentration of 0.02 M) to a solution of **5**-modified gold nanoparticles dispersed in 0.2 M LiCl buffer, particle assemblies gradually formed. For the three different metal ions, the solution with 0.02 M NaCl exhibited the slowest assembly rate (overnight), and the resulting structures melt at the lowest temperature (33 °C). The solution with 0.02 M KCl exhibited the fastest assembly process (within 20 min) and yielded structures with the highest melting temperature (90 °C) (Figure 2C). Seela and co-workers recently reported that Na^+ ion is more efficient than K^+ for effecting aggregate formation with gold nanoparticles modified with a G-rich DNA strand.[5a] However, in their case, very high concentrations of monovalent cations were used (e.g., 0.5 M NaCl), and therefore, the chemical interactions between the nanoparticles inside the aggregates may be nonspecific and irreversible. Substitution of two of the Gs in **5** with the unnatural base Z that does not support quartet formation dramatically destabilizes the assemblies. For example, the melting temperatures of aggregates formed from **5**-modified particles exhibit a melting temperature of 90 °C (0.02 M KCl, 0.2 M LiCl, 0.01 M Li phosphate buffer, pH = 7.24), while the analogue with the two unnatural bases **7** will not form aggregates, even at room temperature. Note that we could not use circular dichroism spectroscopy to characterize the nanoparticle aggregates because of their efficient light-scattering properties.

If one assumes the formation of G-quartets, the trend of increasing stability with increasing number of contiguous Gs is predictable. However, the sharpness of these melting transitions, the conditions (e.g., the formation kinetics, the number of contiguous Gs required, the salt conditions) under which they occur, and the magnitude of increase in T_m with the addition of each G are striking.[4] All these observations are suggesting a highly cooperative assembly process that involves the formation of multiple G-quartets between nanoparticles.[3a]

In summary, this work is significant for the following reasons. First, it provides experiments aimed at delineating the binding and melting properties of nanoparticles functionalized with G-rich sequences. Highly cooperative transitions have been identified and are proposed to be the consequence of multiple G-quartet links between the particles. Second, guanosine phosphate is an essential building block used in nanoparticle probe design. This work shows that one should avoid G-rich sequences in nanoparticle probe design, especially at the termini of the oligonucleotide strands on the particle surface. For example, particles modified with **5** will naturally self-aggregate at room temperature, while those modified with **8** will not under identical conditions (0.3 M NaCl PBS). The work also suggests that one should avoid the use of potassium salts in hybridization buffers for particle probes modified with G-rich sequences to avoid self-aggregation. Third, this work shows how one can use recognition schemes involving oligonucleotides that do not rely on Watson−Crick double helix formation to effect inorganic particle assembly.[5] Finally, the unique ion species-dependent assembly properties and related sharp melting profiles of the aggregates point toward the potential development of novel metal ion detection schemes.

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