Nanobiotechnology

Reversible Switching of DNA–Gold Nanoparticle Aggregation**

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The utilization of the tremendous recognition properties and functionality of proteins and nucleic acids as building blocks in the "bottom-up" self-assembly of nanometer-scaled functional devices has led, in the past few years, to a new research discipline, descriptively termed as nanobiotechnology.[1] Applications include, for instance, the organization of metal and semiconductor nanoclusters,[2] numerous bioanalytical techniques,[3] as well as biomolecular electronics[4] and nanomechanical devices. In the development of the latter, an increasing number of reports is currently being devoted to the construction of nanomechanical devices from DNA.[5] Examples include the construction of devices from DNA molecules whose functionality is based on conformational changes induced by the binding of intercalators,[6] Co^{3+}-ion-dependent B- to Z-DNA transformation,[7] Mg^{2+}-ion-dependent DNA supercoiling,[8] pH-dependent formation of intramolecular cytosin quartet structures,[9] or by intermolecular hybridization with so-called fueling oligonucleotides.[10–14] In this concept, a given DNA conformation is changed upon hybridization with an effector oligomer, which, in turn, can be removed from the complex by hybridization with a second oligomer. Although various examples have proven the suitability of this approach for switching dye-tagged DNA molecules, to the best of our knowledge, its application to the reversible formation of materials has not been realized yet.

Herein we report the reversible aggregation of DNA-modified gold nanoparticles by taking advantage of two complementary fueling oligonucleotides, F_a and F_d (Figure 1). The base sequence of F_a is comprised of three stretches, a′, b′ (which are complementary to ≈23-nm Au nanoparticle-bound 12-mer oligomers a and b, respectively) as well as stretch c′ (which promotes hybridization of F_a and F_d). The transformation cycle starts with state I (Figure 1) in which the DNA nanoparticles are dispersed and reveal a characteristic plasmon absorption maximum at 526 nm (Figure 2). Upon addition of 32 equivalents of oligomer F_a, the particles

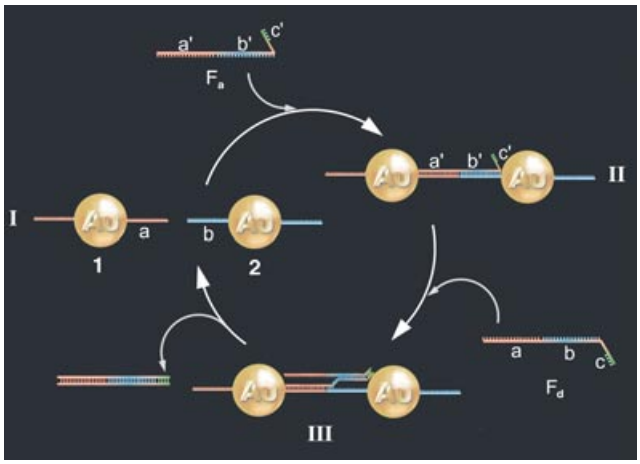

Figure 1. Schematic drawing of the reversible aggregation of DNA-modified gold nanoparticles utilizing fueling oligonucleotides F_a and F_d. F_a contains three stretches (a′, b′, and c′, which are complementary to the gold-nanoparticle-bound 12-mer oligomers, a and b). Stretch c′ forms a dangling end in the aggregated particles (state II), which promotes the hybridization of F_a and F_d (intermediate state III).

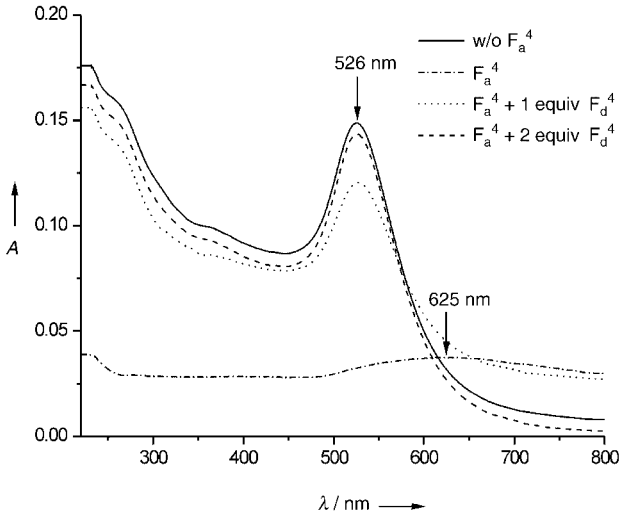

Figure 2. UV/Vis spectra of nanoparticle aggregation and redispersion. The absorbance maxima at 526 nm and 625 nm indicate the formation of dispersed and aggregated particles, respectively. The fueling oligomers F_a^4 and F_d^4 employed contain a stretch c′ and c that are 4 bases in length. Notably, 2 equivalents of oligomer F_d^4 (with respect to F_a^4) are required to restore the original extinction of the dispersed particles.

aggregate (state II, Figure 1), and, consequently, the plasmon absorption band is damped and shifted towards longer wavelengths (≈625 nm; Figure 2). In the next step, oligomer F_d is added; F_d is fully complementary to and hybridizes with F_a, starting at the dangling-end stretch c′ of the duplex DNA that interconnects the nanoparticles. This process, schematically shown as the intermediate state III in Figure 1, leads to the formation of a waste duplex and the redispersion of the nanoparticles. Hence, an increase in absorbance at 526 nm is observed (Figure 2).

Experimental variations revealed that 2 equivalents of oligomer F_d (with respect to F_a) were needed to retain the

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extinction of the original nanoparticle solution (Figure 2). It is likely that the excess F_d is necessary owing to a limited accessibility of linking strands F_a within the aggregates and, hence, decreased hybridization kinetics. Additional studies on the influence of the length and base composition of sequence stretch c within the fueling oligomer F_a and F_d showed that four base pairs are sufficient to promote complete removal of the DNA strands that interconnect the particles (see Supporting Information).

To demonstrate the reversible switching of the nanoparticle aggregation, we carried out seven consecutive cycles of aggregation and redispersion by adding fuelling oligomers F_a and F_d, respectively. After each addition, UV/Vis spectra of the samples were recorded (see Supporting Information) and the extinction at 526 nm and 700 nm, which are indicative for dispersed and aggregated particles, respectively, was plotted against the number of steps (Figure 3). The results clearly

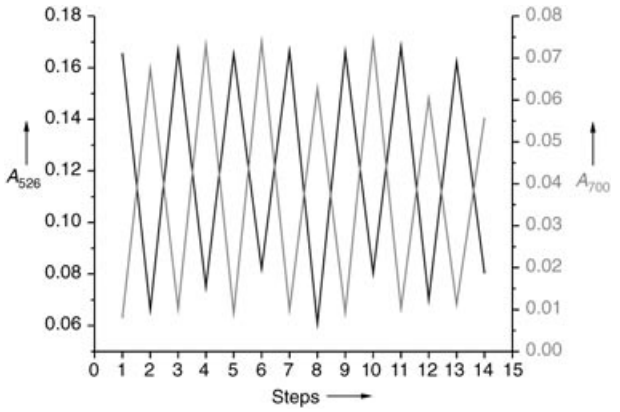

Figure 3. Reversible switching of the nanoparticle aggregation. Plot of the extinction measured at 526 nm (black) and 700 nm (gray) versus the number of steps of the addition of either oligomer F_a^4 (even numbers) or F_d^4 (odd numbers). Notably, a high extinction at 526 nm and 700 nm indicates the presence of dispersed and aggregated particles, respectively.

demonstrate the feasibility of using fueling oligomers for the reversible switching of nanoparticle aggregation. Nonetheless, careful analysis of the absorbance maxima observed for the aggregated particles indicated a decrease in the red shift of the plasmon absorption band from 619 nm in cycle 1 to 556 nm in cycle 7 (Supporting Information). As the magnitude of the shift is indicative of the size of the particle aggregates formed,[15,16] this observation suggests that the aggregation efficiency is diminished owing to increasing concentrations of free F_a oligomers.

In conclusion, we have shown that the reversible switching of DNA–gold nanoparticle aggregation can be carried out by the employment of complementary fueling oligonucleotides that contain a short dangling-end sequence for the initiation of strand removal from the aggregates. Further optimization of this concept by variation of temperature, salt concentration, and pH value, by using oligofunctional DNA nanoparticles[17] and, in particular, by careful design of the oligomer sequences[18,19] should pave the way towards nanostructured materials with programmable functionalities for a broad range of applications in nanobiotechnology.

Experimental Section

The preparation of DNA–gold nanoparticle conjugates was carried out as described earlier.[17] The size of the citrate-stabilized gold nanoparticles used was 22.9 ± 5.7 nm. Thiol-modified DNA oligomers (sequence a: 5′-SH-GGT GAA GAG ATC-3′; sequence b: 5′-AAG ACC ATC CTG-SH-3′) were purchased from Thermo Electron, Germany.

To aggregate the DNA-modified particles, generally 32.5 equivalents[20] of oligonucleotide F_a^4 (5′-TAC GCA GGA TGG TCT TGA TCT CTT CAC C-3′) was added to a mixture of DNA–gold nanoparticle conjugates **1** and **2** (each 1.54 nM) in TETBS buffer (0.3 M NaCl, 20 mM Tris-HCl, pH 7.35 containing 5 mM EDTA and 0.05 % Tween 20). The solution was incubated for 24 h at room temperature. Subsequently, the aggregates were redispersed by adding oligonucleotide F_d^4 (5′-GGT GAA GAG ATC AAG ACC ATC CTG CGT A-3′; 2.2 equiv with respect to F_a^4), and the solution was incubated for 24 h at 37 °C.

For the repeated switching of particle aggregation, seven cycles of aggregation/redispersion were performed as described above with the following amounts of fuelling oligomers: Cycle 1: 32.5 equiv (1.5 pmol) F_a^4 and 73.7 equiv (3.4 pmol) F_d^4; cycle 2: 84.5 equiv (3.9 pmol) F_a^4 and 192.8 equiv (8.9 pmol) F_d^4; cycle 3: 212.3 equiv (9.8 pmol) F_a^4 and 467.9 equiv (21.6 pmol) F_d^4; cycle 4: 511.2 equiv (23.6 pmol) F_a^4 and 1109.0 equiv (51.2 pmol) F_d^4; cycle 5: 1193.5 equiv (55.1 pmol) F_a^4 and 2558.1 equiv (118.1 pmol) F_d^4; cycle 6: 2729.2 equiv (126.0 pmol) F_a^4 and 5796.3 equiv (267.6 pmol) F_d^4; cycle 7: 6138.5 equiv (283.4 pmol) F_a^4. To prevent changes in absorbance due to dilution of the samples, 14 different aliquots of gold nanoparticles were prepared and F_a^4, F_d^4 oligonucleotide solutions were added until a distinct step was reached. In the remaining steps, only buffer was added to these particular samples. After the final addition of either oligomer or buffer solution to all 14 aliquots of the nanoparticle samples, the UV/vis spectra were recorded on a Varian-Cary 100 UV/Vis spectrophotometer.

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