Functionalized Surfaces

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Selective Removal of DNA-Labeled Nanoparticles from Planar Substrates by DNA Displacement Reactions**

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With the great progress that has been made in the synthesis and assembly of nanomaterials during the past decades, there has been an ever-increasing interest in developing new methods to selectively manipulate nanomaterials that function as nanomachines[1] for promising applications in nanomechanics, nanoelectronics, nanobiotechnology, etc. To precisely control the nanomachines, both quantitative and kinetic studies of the manipulation processes are indispensable because they would provide not only determinative proof of the successful accomplishment of the manipulation, but also a deeper understanding of the process and a solid basis for further applications. One of the most promising ways to accomplish these aims is to fabricate nanomachines on planar substrates because, firstly, with the development of advanced lithographic techniques, predesigned micro or nanopatterns can be formed, which provides a fascinating way to integrate top-down and bottom-up techniques to fabricate nanomachines.[2] Secondly, with the development of modern surface techniques, different facile, reliable methods have been developed to characterize the nanostructures on planar substrates,[3] which would allow the convenient study of the surfaces quantitatively and in real time.[4]

Formed from four bases, A, T, C, and G, DNA molecules provide unlimited base sequences for selective molecular recognition and interaction. DNA has been widely applied to the assembly of nanomaterials into different nanostructures based on DNA hybridization reactions.[5] By combining DNA assembly and dissociation processes, exciting nanomachines may be developed.[6] Recently, much attention has been paid to the development of new methods for the disassembly of DNA-directed nanostructures. Compared with other disassembly methods such as use of temperature,[7] enzymes,[8] and aptamers,[9] the DNA displacement reaction (DDR)[10] shows some unique advantages. It provides a highly selective way to disassemble the nanostructures without the limitation of specific DNA sequences required by enzymes and aptamers. In addition, DNA sequences with similar melting temperatures could also be distinguished. Therefore, DDR may provide a general, facile, and highly selective method to manipulate DNA-assembled nanostructures.

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Pioneering progress has been made on the disassembly of the nanoparticle aggregations by DDR.[11] Niemeyer et al.[11a] proved that the disassembly of DNA–gold-nanoparticle aggregates by using DDR was feasible. Sleiman et al.[11b] applied DDR to control the geometry of the discrete gold nanoparticle assemblies. Milam et al.[11c] accomplished the DDR-based reversal of DNA-mediated adhesion of micron- to nanosized colloidal particles. However, these studies were based on homogeneous reactions in solution. It is of great interest to perform the disassembly (removal) of gold nanoparticles by heterogeneous DDR on a planar substrate. Moreover, the lack of quantitative and kinetic studies hinders further understanding and applications of these processes. One possible reason for this may be the difficulty of quantitatively characterizing the nanoparticle aggregates in solution. It is well known that a single layer of gold nanoparticles (GNPs) could form on planar substrates, which may provide an opportunity to carry out quantitative and kinetic studies of the DDR process.[4]

Herein, we report the successful demonstration and quantitative study of the selective removal of DNA-labeled GNPs from a planar substrate by DDR. The kinetics of the process were also monitored by the surface plasmon resonance (SPR) signal of the nanoparticles. The establishment of the method for quantitative and kinetic studies of the removal process, together with the demonstration of the ability to selectively manipulate DNA-labeled GNPs will serve as a basis for further applications of DDR in the fabrication of sophisticated nanomachines on planar substrates.

As shown in Figure 1 A, DDR refers to the displacement of a short DNA strand (S, complementary to a portion of a long DNA strand N) by another long DNA strand (K), which can form more complementary base pairs (bps) with the strand N.[10] Firstly, a single layer of GNP binds to the planar substrate by the hybridization reaction of DNA S (12-mer) with DNA N (20-mer). DNA N, which consists of two segments, a 12-mer segment to hybridize with DNA S and an 8-mer overhang segment at the side of the nanoparticle for subsequent DDR (through hybridization with DNA K). Poly(dA) spacer segments (the segment between the recognition sequence and the thiol functionality, of either 10 or 20 dA units, the dA value is shown as subscript) may also be introduced to DNA S or N. Upon addition of DNA K, DNA N hybridizes to DNA K and DNA S will be displaced. Therefore, DNA N labeled nanoparticles can be removed from the surface. According to our protocol, the reduction of the density of the GNPs on the planar surface should be a direct result of the DDR. Scanning electron microscopy (SEM) was used to characterize the changes of DNA-N_{20} labeled GNP density on a DNA-S_0 modified planar substrate.

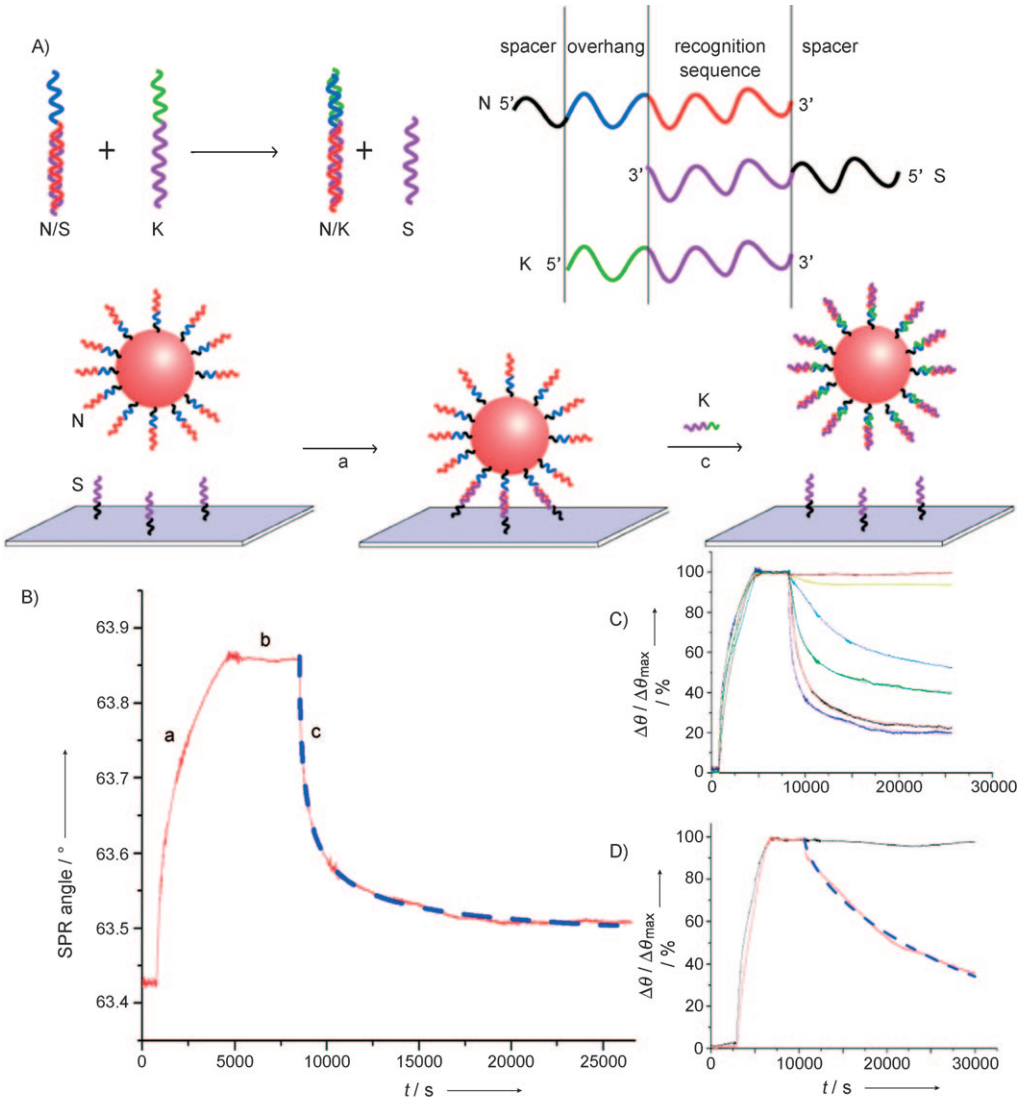

Figure 1. A) Schematic representation of the DNA displacement reaction and the principle of our work, B) The SPR curve of the process in which DNA-N_{20} labeled GNP bound to and then was removed from a DNA-S_0 modified planar gold substrate, C) dependence of removal efficiency on sequence: response to DNA K (orange, top line), and on DNA K concentration: 40 (yellow), 185 (cyan), 815 (green), 2950 (brown), and 5980 nM (blue, bottom line), and D) SPR sensograms of DNA-N_{20}' labeled GNP on DNA-S_0'-modified planar substrate treated with complementary DNA K′ (red) and noncomplementary DNA K (black). The dashed lines are curves calculated to fit the kinetics of the removal process.

The image shows a distinct edge between areas where displacement had and had not occurred (see Figure S2 in the Supporting Information, the dark and the light parts are where displacement had and had not occurred, respectively). Further studies revealed that the density of GNPs in the areas where displacement had and had not occurred were (22 ± 3) and (120 ± 11) particles μm^{-2}, respectively (statistical results from about 120 μm^2), which gave a removal efficiency of about 81.7%.

Surface plasmon resonance is a powerful tool for kinetic studies on planar substrates and has been used for the study of the kinetic processes of DNA hybridization.[12] GNP-enhanced SPR has also been applied to immunoassay[4c] and DNA detection.[4f] It was shown that the SPR angle shift $\Delta\theta$ had an approximately linear relationship with the density of GNPs.[4d,e] We used SPR to study the GNP removal process from the planar substrate, a typical SPR response curve of this process is shown in Figure 1 B. After a DNA-S_0 functionalized substrate was incubated with DNA-N_{20} labeled GNPs (1.2 nM) for 1 h, the SPR angle increased from $(63.427 \pm 0.003)°$ to $(63.859 \pm 0.001)°$ as a result of the hybridization process (segment a). Then the substrate was washed for 1 h with a buffer solution (segment b), DNA K (6 μM) was introduced into the flow cell (30 μL, diameter = 9 mm) and further incubated for 5 h. Remarkably, the SPR angle decreased to $(63.508 \pm 0.001)°$, which corresponded to the removal of GNP from the substrate (segment c) with a removal efficiency of approximately 81.3%. The kinetic curve proved the feasibility of applying DDR to remove DNA-labeled GNPs from planar substrates.

To further confirm that the decrease of the SPR angle was indeed induced by DDR, the effect of DNA-K concentrations and sequences on the removal efficiency was examined. As shown in Figure 1C, a higher DNA-K concentration induced a higher removal efficiency. When the DNA K concentration reached 6 μM, the removal efficiency reached the upper limit of about 80%. With a further increase of DNA-K concentration to 140 μM, the removal efficiency only reached 80.2%. The effect of the DNA-N_{20} labeled GNP surface density on the removal efficiency was also studied. The removal efficiency remained unchanged under the selected reaction conditions, which gave a nanoparticle surface density of up to 147 particles μm^{-2} (see Figure S3 in the Supporting Information). Further studies showed that the SPR angle shift caused by nonspecific absorption (DNA-N_{20} labeled GNP on noncomplementary DNA-S_0' modified surface) accounted for no more than 5% under the same reaction conditions. There were at least 15% DNA-N_{20} labeled GNPs hybridized on the planar surface that could not be removed, even at a high concentration of DNA K under the same reaction conditions. Possible reasons for this observation include various steric or electrostatic effects that prevent the complete breaking of all the duplex N–S pairs between GNPs and the substrate by DDR. On the other hand, the same concentration of DNA K′ (20-mer), which is not complementary to DNA N_{20}, showed no detectable decrease in SPR angle (Figure 1C, black line). Similar reactions were also performed with DNA-N_{20}' labeled GNPs, DNA-S_0' modified planar gold substrates, and DNA K′. As shown in Figure 1D, DNA-N_{20}' labeled GNP could only be removed by DNA K′ and no detectable decrease was observed with noncomplementary DNA K. The dependence of the removal efficiency on both the concentration and sequence of DNA K confirmed that the decrease of SPR angle was the result of DDR. Further studies revealed that, under our experimental conditions, the SPR angle shift $\Delta\theta$ (from 0 to 2.9°) had an approximately linear relationship with the surface coverage of GNPs (from 0 to 184 particles μm^{-2}; Figure S4 in the Supporting Information). This result serves as a solid basis for quantitative and kinetic studies of the removal process that are based on SPR in this range.

Based on the quantitative study, we have found that the spacer length of DNA N and S is a key factor in controlling the DDR efficiency (Figure 2A–C). It was observed that the removal efficiency of DNA N increased with the spacer length (for a detailed explanation, see the Supporting Information). In contrast to DNA N, the longer spacer on DNA S decreased the removal efficiency. To understand this result, we further tested the effect of a neutral spacer, PEG (poly(ethylene glycol), $(CH_2CH_2O)_6$), for DNA S, the length of which is similar to that of the 10 dA spacer.[13] As shown in Figure 2D, when neutral PEG is used as a spacer, the removal efficiency (79.5%) was similar to that of DNA S_0 (without a spacer). This result showed that the negative charge of the poly(dA) spacer (not the steric factor) was the main reason for the decrease of the removal efficiency. It has been reported that negatively charged spacers would increase the local salt concentration surrounding the duplexes and the frayed ends of the duplex portion will be stabilized.[7d] Based

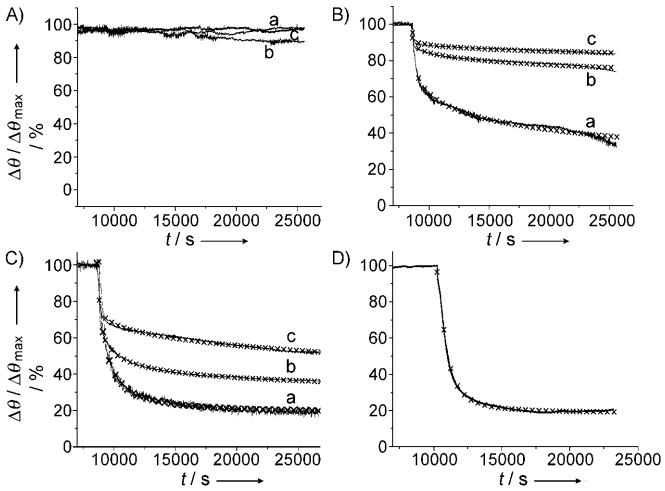

Figure 2. A) DNA-N_0 labeled GNP on the substrate modified by a) DNA S_0, b) DNA S_{10}, and c) DNA S_{20}. B) DNA-N_{10} labeled GNP on the substrate modified by a) DNA S_0, b) DNA S_{10}, and c) DNA S_{20}. C) DNA-N_{20} labeled GNP on the substrate modified by a) DNA S_0, b) DNA S_{10}, c) DNA S_{20}. D) DNA-N_{20} labeled GNP on the substrate modified by DNA S_{PEG}. The lines labeled × are the curves calculated to fit the kinetics of the removal process.

on these experimental results, DNA N_{20} with a 20dA spacer and DNA S_0 without a spacer were used in all other experiments in this work.

Novel methods for selective control of individual subunits without affecting others are highly desirable in nanomachine design and operation. One of the most attractive advantages of the DDR method reported here is its high selectivity. Figure 1C,D shows that DNA N_{20}/S_0 or N_{20}'/S_0' could only be opened by their corresponding complementary DNA strands. However, these two experiments were performed on two separate substrates functionalized only with DNA S_0 or S_0'. In nanomachines, different subunits labeled with different DNA strands may be very close and affect each other. Therefore, we tested the hybridization and displacement reactions further on a surface that was simultaneously modified with two different strands—DNA S_0 and S_0'. As shown in Figure 3B, DNA-N_{20} and -N_{20}' labeled GNPs could bind to the substrate sequentially (segments a and b). After the addition of DNA K′ (6 μM), the SPR angle decreased to a value only slightly larger than the angle induced by the first hybridization reaction of DNA-N_{20} labeled GNP (segment c). The removal efficiency of this first DDR reaction was 93.3% for DNA-N_{20}' labeled GNPs. Only after the addition of DNA K (6 μM), a further decrease was observed (segment d), which resulted from the removal of DNA-N_{20} labeled GNPs with an efficiency of 89.9%. These results clearly showed that the hybridization and displacement reactions could be applied to selectively control DNA-labeled GNPs on the basis of their DNA tags, which demonstrated the potential of the method for the fabrication and manipulation of complex nanomachines.

Kinetic curves were obtained from SPR measurements. An empirical equation that could fit all the observed removal kinetic curves has been derived (see the Supporting Information).

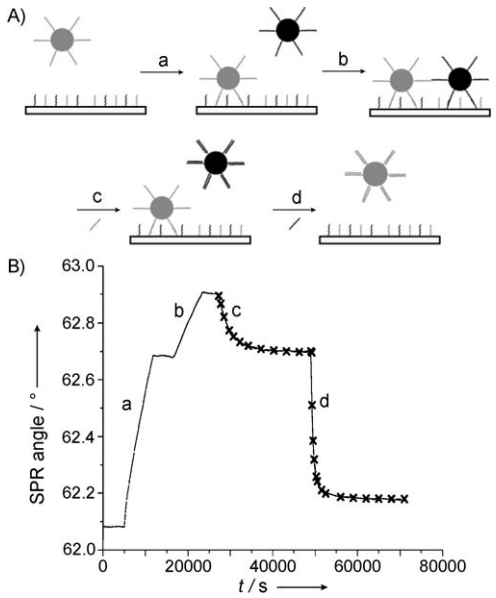

Figure 3. A) Illustration of subsequent binding/removing processes of DNA-N_{20} and -N_{20}' labeled GNP on a planar surface modified with the mixture of DNA S and S_0. B) SPR sensogram of the subsequent binding/removing processes illustrated in (A). The line labeled × is the curve calculated to fit the kinetics of the process.

In conclusion, the DDR-based selective removal of DNA-labeled GNPs from a planar substrate has been successfully demonstrated by quantitative and kinetic studies. The present work represents an initial but important step in the applications of DDR in the development of various novel nanodevices and nanomachines, such as large-scale parallel data-storage devices based on DNA-controlled nanoparticles. Finally, our results from a heterogeneous planar substrate may also provide a valuable reference for similar reactions in homogeneous solutions.

Experimental Section

All DNA oligomers were purchased from Integrated DNA Technologies, Inc. (see Table S1 in the Supporting Information for sequence listing). The gold nanoparticles were synthesized according to a previously reported procedure.[7d] The particle size was about (16.7 ± 1.9) nm (see Figure S1 in the Supporting Information) and the particle concentration of 2.8 nM was determined by using the inductively coupled plasma (ICP) technique according to a previously reported procedure.[12] The extinction coefficient of the nanoparticles was $4.5 \times 10^8\,M^{-1}\,cm^{-1}$ ($\lambda = 520$ nm). The DNA-N labeled GNPs were prepared by adding freshly cleaved thiolated oligonucleotides N with different spacer lengths to a solution of aqueous nanoparticles (2.8 nM) to give a final oligonucleotide concentration of 0.49 μM. After 16 h, the concentration of NaCl in the solution was adjusted to 0.1M by adding a phosphate buffer (2M NaCl, 10 mM phosphate, pH 7). After standing for an additional 40 h, excess reagents were removed by centrifugation for 10 min at 12000 rpm. After washing with a phosphate buffer (0.1M NaCl, 10 mM phosphate, pH 7) and successive centrifugation, the oily red precipitate was redispersed in the hybridization buffer (0.3M NaCl, 10 mM phosphate, pH 7) to the desired concentration.

Planar SPR substrates were prepared by sequentially depositing Cr (20 Å) and Au (470 Å) using an E-Beam Evaporator (Temescal BJD 1800 system) at the rate of 1 Å s^{-1} and 1.5–2.0 Å s^{-1}, respectively. DNA modification was performed by using similar procedures to those previously reported.[14] The substrates were prepared by immersing the nonmodified substrate into DNA S (0.73 μM) with different spacer lengths in a TE buffer (1M NaCl, 10 mM Tris–HCl, 1 mM EDTA, pH 8; Tris = tris(hydroxymethyl)aminomethane, EDTA = ethylenediaminetetraacetic acid) for 3 h. The samples were rinsed three times with TE buffer and once with nanopure water. Subsequently, the DNA-functionalized substrates were immersed in mercaptohexanol solution (1.0 mM) for 1 h. After rinsing three times with nanopure water, the substrates were dried with a stream of nitrogen. To prepare a doubly modified substrate, the planar gold substrate was immersed in a mixture of DNA S_0 (0.37 μM) and DNA S_0' (0.37 μM) for 3 h.

The surface plasmon resonance responses were measured on a SPR Kretschmann-type spectrometer Biosuplar-2 (Analytical-μSystem, Germany) with a light-emitting diode light source ($\lambda = 670$ nm, prism refraction index $n = 1.61$). A typical procedure for the measurement of sensograms is: Firstly, the flow cell was washed with hybridization buffer. After the baseline became stable, a solution of DNA-N labeled gold nanoparticles (1.2 nM) was introduced into the flow cell. The flow was then stopped and the sample was incubated for 1 h to measure the hybridization process. After that, the flow cell was washed with hybridization buffer for 1 h to wash away the DNA-N labeled GNPs. Subsequently, DNA K (6 μM) was introduced into the flow cell, the flow was stopped and the sample was incubated for about 5 h to monitor the displacement process. The standard deviations of the SPR angles were calculated from 20 continuous points on the SPR curve of the corresponding segments. For the measurement of the effect of spacer length on the displacement reaction, the concentration of DNA-N labeled GNPs and DNA K were fixed at 1.2 nM and 140 μM respectively. Selective removal of DNA-N_{20} and -N_{20}' labeled GNP from a doubly modified substrate were measured using a nanoSPR 321 instrument.

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