and shows that approximately 13% of the HOMO density is located on the outer pyrrole ring, see ref. [12b].
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[25] To minimize adsorption phenomena, a high scan rate of $0.5\,V\,s^{-1}$ was used.

Gold/DNA Nanostructures

Towards Multistep Nanostructure Synthesis: Programmed Enzymatic Self-Assembly of DNA/Gold Systems**

*Antonios G. Kanaras, Zhenxin Wang, Andrew D. Bates, Richard Cosstick, and Mathias Brust**

The programmed self-assembly of nanostructures from well-defined units is an important aim in nanoscience.[1,2] The use of gold nanoparticles stabilized by thiol-modified DNA is a promising approach towards this goal.[3–8] The specificity of DNA base-pairing provides a precise means of programming interactions between particles by hybridization with specifically designed linker strands. To introduce an additional level of control, we have developed a general method by which the reactivity of initially latent DNA linking sites can be switched on deliberately. We have adapted well-developed methods of molecular biology to produce a nanoscale analogue of protecting groups. We show that linking sites can be protected by hybridization with complementary strands and deprotected by cleaving these double strands at predetermined sites with restriction enzymes. This results in cohesive ends of single-stranded DNA, which can bind by hybridization to complementary sequences present in the system. In a second enzymatic step the DNA phosphodiester backbones at the hybridization sites are covalently joined using a DNA ligase. This approach represents a generic protocol that will enable multistep nanostructure syntheses.

In addition to DNA, further biomolecular interactions have been exploited for programmed assembly. These include other specific recognition motifs such as antibody–antigen and biotin–avidin binding.[8–11] A number of nonbiomolecular systems with various degrees of complexity have also been reported.[12–16] These approaches generally have in common that the reactivity of the binding sites is determined by the initial design of the system. A reaction, once started, proceeds until all reactive sites have been consumed by binding to complementary motifs. This essentially limits programmed nanostructure assembly to single-step reactions. Conversely, modern preparative chemistry is characterized by complex multistep syntheses, which can routinely be carried out by selectively addressing certain reactive sites while others are left temporarily unreactive. This is achieved by the use of protecting groups, which are essential to practically all modern chemical syntheses. Here we introduce a comparable concept to programmed nanostructure assembly, which uses the restriction sites in double-stranded DNA as protected linking motifs and restriction endonucleases as selective deprotecting agents. The complete reaction sequence carried out to demonstrate this principle is illustrated schematically in Figure 1.

The starting point for this work was the preparation of an aqueous solution of 15 nm gold nanoparticles, which were coated and stabilized by a ligand shell of thiol-modified

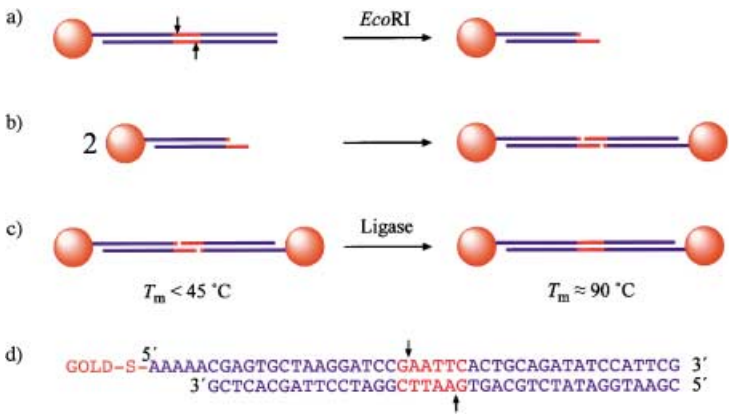

Figure 1. Schematic description of the method. a) Gold nanoparticles derivatized with double-stranded DNA are treated with restriction enzyme *Eco*RI, which cleaves the DNA to yield cohesive ends. The red color represents the recognition site of the enzyme, and the arrows indicate the sites of cleavage on each strand. In reality, each 15 nm particle has around 100 DNA ligands; b) Two cohesive ends hybridize, which leads to a weak association of particles; c) The DNA backbones are covalently joined at the hybridized site by DNA ligase to yield a stable 40-base-pair double-stranded link between particles; d) The sequences of the oligonucleotides are illustrated, together with the *Eco*RI site and positions of backbone cleavage, as in (a).

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single-stranded DNA of 45 bases. This was achieved by essentially following the methods described by Mirkin and co-workers.[3,5,17,18] A noteworthy modification is that the derivatization of the particles with DNA was carried out while concentrating the sample in a vacuum centrifuge. This led to extraordinarily stable particles, which were unaffected by the various conditions of the subsequent enzymatic reactions. The particle-bound DNA was then converted to a double-stranded form by hybridization with a complementary single-stranded DNA. The DNA sequence was chosen so that the double strands formed on the particles would contain recognition sites for a number of restriction endonucleases. The system did not now contain any single-stranded DNA capable of hybridization with complementary linker strands. In order to create reactive sites by deprotection, the restriction enzyme *Eco*RI was used to cleave the double strands on the particles at specific recognition sites to leave a cohesive end capable of hybridization. After inactivation of the enzyme the DNA remaining on the particle surface was isolated and analyzed by gel electrophoresis. As shown in Figure 2, almost all the DNA present in the system was cleaved by the enzyme. Importantly, preliminary experiments with other restriction enzymes such as *Eco*RV, *Pst*I, *Nla*IV, *Bam*HI, and *Apo*I gave comparable results when the respective recognition sites were present.

After this first enzymatic step, hybridization between the newly created cohesive ends through the relatively labile overlap of only four complementary base pairs resulted in the formation of weakly associated aggregates of particles. This process has been investigated by transmission electron microscopy (TEM) as shown in Figure 3a and b. The formation of small aggregates is clearly seen in comparison with randomly distributed individual particles prior to enzyme treatment. In order to stabilize the system at this stage the DNA backbones at the hybridized cohesive ends were covalently joined in a second enzymatic step using T4 DNA ligase. Inspection with TEM now showed the presence of significantly larger aggregates, which are formed as a consequence of much stronger interparticle binding by the newly formed 40-base-pair double-stranded linker (Figure 3c).

The simplest and most powerful analytical tool to monitor this enzymatic two-step reaction is the "Northwestern" spot test, developed by Mirkin and co-workers.[19] This is an extremely sensitive method to discriminate between aggregated and nonaggregated gold nanoparticles in aqueous solution, based on a perceptible color change from red to blue upon aggregation. The test consists of the evaporation of a droplet of an aqueous solution of particles on a reverse-phase thin-layer chromatography plate. A blue spot indicates aggregation, while a red spot indicates the presence of freely dispersed particles. Figure 4 shows the results of the spot tests and the visible absorption spectra of the corresponding solutions at various temperatures for the free particles in solution (a), the loosely aggregated particles after the enzymatic deprotection step (b), and the strongly bound aggregates after enzymatic ligation (c). The results are consistent with the TEM study but contain important additional information regarding the stability of the aggregates, both before and after the ligation step. The free particles complexed with double-stranded DNA always give a red spot test, even at room temperature, which confirms that no aggregation of particles takes place. The absorption spectrum is typical for freely dispersed particles of this size range showing a sharp plasmon absorption band at 524 nm. After treatment with *Eco*RI, a blue spot is obtained at 25 °C indicating particle aggregation, but the aggregates dissociate (red spot) at slightly elevated temperatures (45 °C) as a result of the melting (dehybridization) of the weak four-base-pair cohesive ends. The melting temperature (T_m) of a single cohesive end will be close to room temperature, but the loose aggregates of particles will be maintained by multiple transient links that will only dissociate completely at a higher temperature. In accordance with the spot tests, the visible spectra show a slightly broadened plasmon band which is red-shifted to 534 nm at 25 °C, and the typically sharp band at

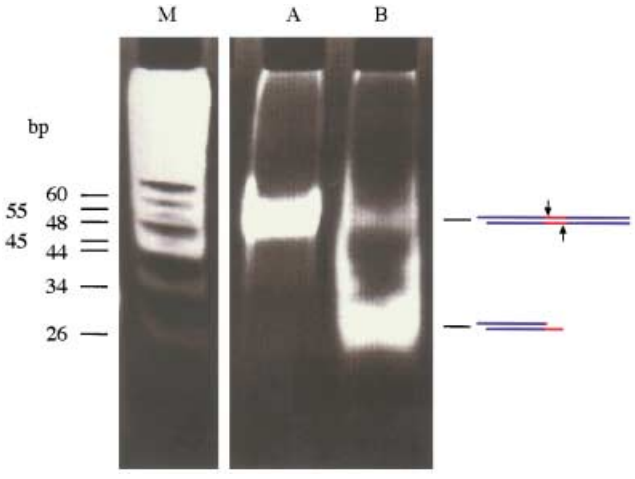

Figure 2. DNA cleavage analyzed by polyacrylamide gel electrophoresis. Particle-bound DNA was isolated before (A) and after (B) treatment with *Eco*RI and subjected to electrophoresis on a 20% polyacrylamide gel, alongside marker DNAs of known sizes (M). The numbers indicate DNA sizes in base pairs and the pictograms indicate the DNA species present, as in Figure 1.

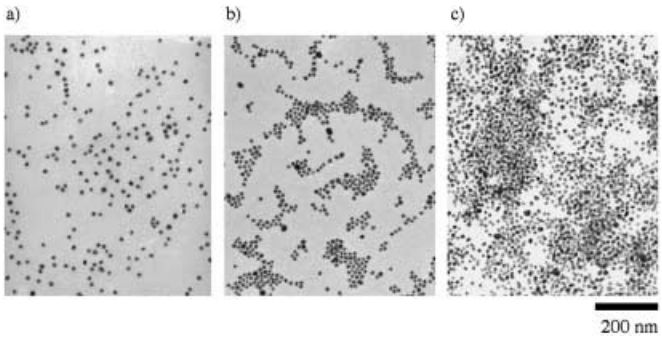

Figure 3. TEM analysis of DNA–nanoparticle complexes. Micrographs represent DNA–nanoparticle complexes analyzed before (a) and after (b) treatment with *Eco*RI, and after subsequent treatment with DNA ligase (c).

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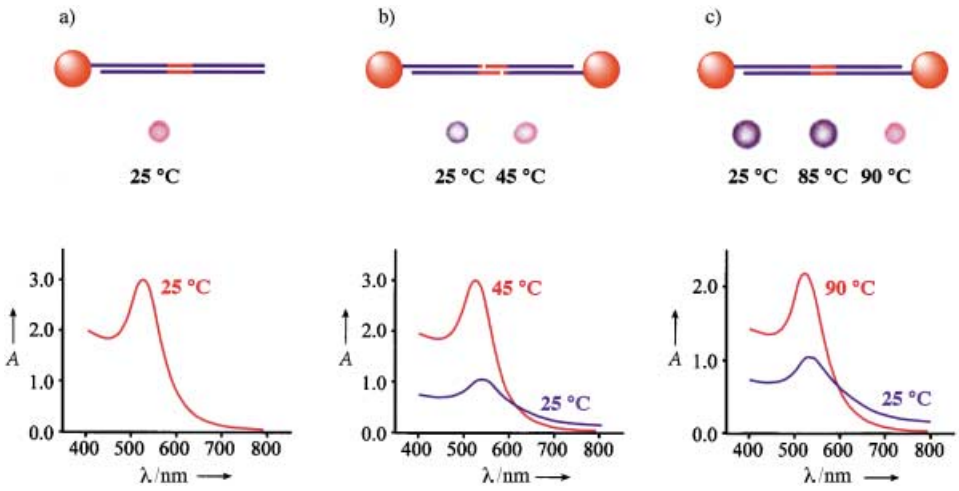

Figure 4. Spot test and absorbance spectra of DNA–nanoparticle complexes. "Northwestern" spot tests (see text) and visible absorption spectra are illustrated for DNA–nanoparticle complexes, analyzed at the temperatures shown, both before (a) and after (b) treatment with *Eco*RI, and after subsequent treatment with DNA ligase (c).

524 nm at 45 °C. A dramatic difference in the melting behavior is observed after the ligation step, which has converted the labile four-base-pair overlap into a continuous 40-base-pair double strand. The spot test remained blue up to 85 °C and changed to red sharply at 90 °C, at which temperature, the 40-base-pair double-stranded linker dehybridized. The same behavior is reflected in the absorption spectra of the solutions. This shows unequivocally that the second enzymatic step has indeed taken place and modified the properties of the system significantly. This was further confirmed by the gel electrophoresis of DNA released from the particles (not shown).

What has been demonstrated here is a new method of nanostructure manipulation. This is illustrated by a series of reactions in which randomly dispersed DNA-coated gold particles are converted first to weakly associated structures, and subsequently to an aggregated system through simple and predictable manipulation with DNA-processing enzymes. In particular, we have introduced the powerful concept of protecting and deprotecting specific reaction sites, which is new to nanoscience. The simplest possible system has deliberately been chosen for these "proof of principle" experiments. The preparation of geometrically more challenging structures by our new approach may now become possible using, for example, tailored nanoparticles with precisely one, two, or three DNA strands attached, as shown by Alivisatos and co-workers.[20] We have shown that enzymes can be employed as tools to provide crucial additional control (selective cleavage and ligation) over nanostructure assembly. This opens vast possibilities for the future use of enzymes in this area. Restriction endonucleases are very important research tools in molecular biology, and their well-established ability to cleave DNA at specific recognition sites is beginning to be applied to nanostructures.[21–25] To date, over 3000 restriction enzymes have been discovered and hundreds are commercially available.[21] This will allow for the preparation of nanostructure building blocks with a large number of protected binding sites, each of which is addressable by a different enzyme, so that the multistep syntheses of nanostructures can now be envisaged. It is to be expected that many other sophisticated biomolecular research tools will also become invaluable to nanoscience in the near future.

Experimental Section

Preparation of DNA-complexed gold nanoparticles: Near-monodisperse gold nanoparticles of 15 nm diameter were prepared in aqueous solution by the classical citrate reduction route following standard procedures.[26,27] Prior to use, the hydrosols were filtered with a 0.45 μm pore-size Millex-HA microfilter (Millipore).[5] DNA 45-mers with a 5′-thiol modification ($OPO_3(CH_2)_6$–S–S–$(CH_2)_6OH$)) were either purchased (Sigma Genosys Ltd.) or prepared following standard procedures.[4,5] In a typical preparation the gold nanoparticles were derivatized with thiolated single-stranded oligonucleotides by incubating a gold sol (500 μL, 6 nM) overnight with disulfide-protected oligonucleotides (25 μL, 40 μM) in aqueous solution. Then the solution was diluted to a final volume of 1 mL containing NaCl (0.1 M) and sodium phosphate buffer (5 mM, pH 7.0) solutions. After further incubation for 2 h the volume was slowly reduced to 150 μL by vacuum centrifugation over 3–4 h at 40 °C. This is an important modification of previously published procedures and ensures a gradual and simultaneous increase in ionic strength and oligonucleotide concentration, which leads to unusually stable DNA-complexed particles. The loading of particles with DNA achieved by this method has been estimated by UV/Vis spectroscopy to be in the range of 100 ± 30 DNA molecules per particle, somewhat more than previously reported.[18] Nonspecific aggregation (precipitation) may occur due to the presence of excess oligonucleotides. This is fully reversible by diluting to 500 μL and heating briefly to 50 °C. Unbound oligonucleotides were then removed by repeated centrifugation and suspension of the pellet (Sigma 1-13 Centrifuge, 13 000 rpm, ×2). The DNA-complexed particles were stored in a solution (500 μL) containing NaCl (0.5 M) and sodium phosphate (10 mM, pH 7.0).

Hybridization of particle-bound single-stranded DNA: The single-stranded DNA oligonucleotides present on the gold particles were hybridized with non-thiolated complementary DNA (64 μL of a 15.5 μM solution) by incubating for 2 h in a hybridization buffer (500 μL, NaCl (0.5 M), sodium phosphate (10 mM, pH 7.0)). Excess free oligonucleotides were then removed by centrifugation and

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suspension in the hybridization buffer (13000 rpm, ×3). Particles were stored in 500 μL of the hybridization buffer.

Enzymatic digestion of particle-bound DNA: The first enzymatic reaction step was carried out by incubating the particles overnight at 37°C in a 100 μL solution containing NaCl (50 mM), Tris-HCl (100 mM, pH 7.5), and $MgCl_2$ (10 mM) containing 200 units of *Eco*RI enzyme (New England Biolabs Ltd.).

Enzymatic ligation of particle-bound DNA: The enzymatic ligation step was carried out by incubating the particles overnight at room temperature in a buffer solution comprised of Tris-HCl (50 mM, pH 7.5), $MgCl_2$ (10 mM), and ATP (1 mM) containing 1600 units of T4 DNA ligase (New England Biolabs Ltd.).

Gel electrophoresis: After the first enzymatic reaction the enzyme was deactivated by addition of EDTA (20 mM final concentration). The particles were isolated by repeated centrifugation and redispersion into an aqueous EDTA solution (20 mM) and then incubated overnight in 20 μL of an aqueous solution of dithiothreitol (0.5 M) and EDTA (20 mM). Gel electrophoresis was carried out using a 20% polyacrylamide (19:1 acrylamide:bisacrylamide) gel in TBE (Tris-borate (90 mM, pH 8.0) and EDTA (1 mM)) and the bands were visualized by staining with ethidium bromide.

Transmission electron microscopy: Specimens for inspection by TEM were prepared by the slow evaporation of one drop of an aqueous solution of the particles onto a carbon-coated copper mesh grid. All samples were examined in a JEOL 2000 EX TEM operating at 200 kV.

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Oxidation of Alcohols in Water

Catalytic Oxidation of Alcohols in Water under Atmospheric Oxygen by Use of an Amphiphilic Resin-Dispersion of a Nanopalladium Catalyst

Yasuhiro Uozumi and Ryu Nakao*

Oxidation of alcohols to form carbonyl compounds is one of the most fundamental and important yet immature processes in organic chemistry. Thus, although a variety of methods and reagents for the oxidation have been developed, until recently the traditional oxidation reactions have been performed with stoichiometric amounts of heavy metal reagents (e.g. Cr, Mn)[1] or moisture-sensitive expensive oxidants (e.g., *N,N′*-dicyclohexylcarbodiimide (DCC), oxalyl chloride),[2] and often in environmentally undesirable media like chlorinated solvents which render them impractical. There is good reason to believe that alcohol oxidation might be feasible by aerobic oxidation in water in a reaction promoted by a heterogeneous catalyst under atmospheric pressure conditions. This would represent a much cheaper, safer, and more environmentally benign oxidation protocol. Recently, much work has appeared on the catalytic oxidation of allylic or benzylic alcohols with molecular oxygen,[3,4] and several palladium catalyst systems have been developed for the oxidation.[5,6]

We have previously reported that amphiphilic polystyrene-poly(ethylene glycol) (PS-PEG) resin-supported palladium–phosphane complexes catalyzed various palladium-mediated reactions smoothly in water.[7] With the resin-supported catalyst in aqueous media, organic substrates must

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