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Site-Specific Assembly of DNA and Appended Cargo on Arrayed Carbon Nanotubes

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Arrayed carbon nanotubes (CNTs) represent an ideal scaffold for the generation of ordered structures featuring biomolecular components. These structures, exhibiting high electrical conductivity,[1,2] also constitute a useful base material for nanoscale biosensors.[3−10] While CNTs have been interfaced with proteins and DNA,[3−19] most of the hybrid structures reported to date used unordered CNTs that were functionalized by nonspecific and random adsorption of biomolecular components.[3−8,11−13,15−19] Here, we describe a rational strategy that permits discrete regions of arrayed CNTs to be functionalized simultaneously and specifically with DNA oligonucleotides. We have exploited the different chemical properties of two regions on single CNTs and orthogonal chemical coupling strategies to derivatize CNTs within highly ordered arrays with multiple DNA sequences. Through duplex hybridization, we then targeted different DNA sequences with appended metal nanoparticles to distinct sites on the CNT architecture with spatial control. The materials generated from these studies represent the first CNTs with bipartite functionalization. Moreover, the approach described provides a high level of precision in parallel and directed assembly of DNA sequences and appended cargo.

Highly ordered CNT arrays[20,21] are generated through the growth of multiwalled nanotubes (MWNTs) within an aluminum oxide nanopore template. The template defines the geometric features of the CNT array, and in the studies described here, the CNTs are 50 nm in diameter, have walls of 3 nm thickness, and exhibit an exposed length of 40 nm, a total length of 20 μm, and a center-to-center spacing of 110 nm between adjacent tubes.[21] The closed sidewall and the open end of a CNT exhibit inherently different physical and chemical properties.[1,2] Nitric acid etching introduces carboxylic groups at defect sites (primarily at the tips), while the graphitic sidewalls are hydrophobic and chemically inert.[1,2,22] We therefore envisioned that the bifunctional chemical structure of CNTs would facilitate the selective attachment of multiple DNA sequences using two distinct DNA−CNT linking strategies. In one strategy, by accessing the free carboxyl groups of CNTs, single-stranded, amine-terminated DNA oligonucleotides are attached to the CNT array using amide-coupling chemistry in aqueous/organic solvent mixtures. A second and orthogonal modification strategy involves the attachment of oligonucleotides to the sidewalls of the CNTs through hydrophobic interactions. For this scenario, we introduced pyrene (a functional group that has been shown to promote adsorption to CNTs)[17,23] onto the 5′ end of a DNA oligonucleotide. To visualize the immobilized strands, complementary sequences were thiolated[24] and attached to gold nanoparticles.[25] This visualization strategy permits the DNA−CNT conjugates to be analyzed by scanning electron microscopy (SEM). Additionally, the delivery of gold nanoparticles to CNTs using the self-assembly properties of DNA represents an advance toward building higher-order nanostructures with rational control.

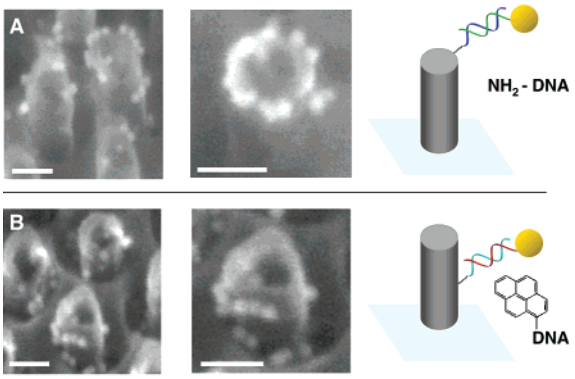

Figure 1. Two strategies for selective attachment of DNA to an aligned CNT array. Images shown correspond to CNT arrays following single-stranded DNA attachment and hybridization of nanoparticle-labeled complements. **(**A**)** Amide coupling of single-stranded DNA followed by hybridization of a complementary strand attached to a 10 nm Au particle. **(**B**)** Adsorption of pyrene-modified single-stranded DNA followed by hybridization of a complementary strand attached to a 10 nm Au particle. All scale bars represent 50 nm. See Supporting Information for details concerning the preparation of the samples shown.

The two DNA−CNT linking strategies were tested individually to evaluate the selectivity achieved with each approach. As shown in Figure 1A, the tips of arrayed CNTs were successfully modified with amine-functionalized DNA oligonucleotides. The SEM images depict the CNTs after DNA attachment via amide coupling and following the hybridization of the complementary 15-mer attached to a gold nanoparticle label. Importantly, the nanoparticles are localized at the tips of the CNTs, where the majority of the carboxylic acids are expected to be found.[22,26] When large areas of the array are examined, tip-to-sidewall particle ratios approach 4:1. The adsorption of the gold nanoparticles is specific and DNA-dependent, as control experiments utilizing unlabeled nanoparticles, nanoparticles labeled with a noncomplementary sequence, or unmodified CNTs did not produce Au−DNA−CNT hybrid structures.

Likewise, the sidewalls of arrayed CNTs were successfully targeted using DNA functionalized with a hydrophobic appendage. Figure 1B shows SEM images of CNT samples prepared with the pyrene-based attachment strategy. Here, the ratio of tip-to-sidewall attachment is reversed from the amide-coupling experiments, with sidewall-to-tip particle ratios approaching 3:1.[26] The immobilization of DNA through the interaction of the hydrophobic pyrene group with the graphite-based sidewalls of the CNTs was also a highly specific and DNA-dependent process, as CNT samples that were

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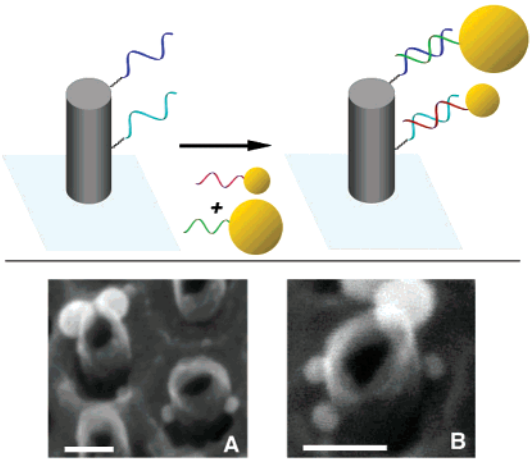

Figure 2. Site-specific delivery of DNA oligonucleotides and appended Au nanoparticles. SEM images shown in (A) and (B) correspond to nanoparticles introduced through parallel hybridization following the serial attachment of two DNA single strands. The 30 nm particles represent sites where amide coupling of strand X occurred, and the 15 nm particles represent sites where strand Y was adsorbed via pyrene functionality. All scale bars represent 50 nm. See Supporting Information for sample preparation protocols.

hybridized with noncomplementary DNA or subjected to hybridization with a complementary sequence without prior pyrene−DNA attachment did not retain Au nanoparticles.

To demonstrate that the two orthogonal attachment strategies could be used simultaneously, we functionalized a CNT array with two different DNA oligonucleotides. This dual functionalization then allowed us to use differential hybridization to deliver two gold nanoparticles with distinct dimensions to discrete regions of an individual CNT (Figure 2). Single-stranded DNA attachment was performed in series, while the hybridization of complementary nanoparticle-labeled strands was performed in parallel. An amine-functionalized oligonucleotide (strand X) was attached to the CNTs with the amide-coupling strategy as described above. However, in this experiment, before nanoparticle-labeled strands were introduced for hybridization, a pyrene-functionalized oligonucleotide (strand Y) was attached through hydrophobic adsorption to the CNT sidewalls. The thiol-modified complement to strand X was attached to a 30 nm Au particle, and the complement to strand Y was attached to a 15 nm Au particle. After simultaneous hybridization of the array with both Au-labeled complements, SEM imaging revealed the successful and specific delivery of the two sequences as shown in Figure 2. The 30 nm particles, which serve as the label for the amide-attached oligonucleotides, are located primarily at the tips, and the 15 nm particles, the label consistent with hydrophobic attachment, are primarily on the sidewalls. For the experimental demonstration to be more convincing, we intentionally placed the larger and heavier gold nanoparticle label on the tip. The results clearly show that the specificity of DNA duplex formation permits each Au−DNA conjugate to be selectively directed toward a target site on the CNT.

These experiments illustrate that individual CNTs can be functionalized with spatial selectivity and can be used to differentiate between two DNA sequences. In addition, they represent an augur for using DNA to controllably produce assemblies of hybrid nanostructures. Delivering different payloads to specific areas of functionalized nanotubes may facilitate the production of new nanomaterials. The growth of nanostructures can be templated by nanoparticles, and this work demonstrates control over their placement on CNTs with enhanced spatial specificity.

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Supporting Information Available: Experimental protocols, SEM images of unfunctionalized CNT arrays, and structures of DNA oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) While the amide-coupling- and pyrene-adsorption-based attachment strategies were selective for the tips and sidewalls of the tubes, respectively, some cross-reactivity was observed. Defect sites displaying carboxylates along the sidewalls of CNTs are known to exist (Jiang, K.; Schadler, L. S.; Siegel, R. W.; Zhang, X.; Zhang, H.; Terrones, M. *J. Mater. Chem.* **2004**, *14*, 37−39), which explains the small amount of DNA attachment in this region of the CNT when amide coupling was used. The low levels of attachment of pyrene DNA to the tips of CNTs likely reflects incomplete oxidation or a low level of affinity of the modified oligonuclotides for the oxidized sites. It is noteworthy that when the dual functionalization procedure was carried out, almost no cross-reactivity was observed, indicating that the specificity of each coupling strategy predominated under competitive conditions.

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