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DNA-Programmed Modular Assembly of Cyclic and Linear Nanoarrays for the Synthesis of Two-Dimensional Conducting Polymers

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Supporting Information

ABSTRACT: Nanometer-scale arrays of conducting polymers were prepared on scaffolds of self-assembling DNA modules. A series of DNA oligomers was prepared, each containing six 2,5-bis(2-thienyl)pyrrole (SNS) monomer units linked covalently to N4 atoms of alternating cytosines placed between leading and trailing 12-nucleobase recognition sequences. These DNA modules were encoded so the recognition sequences would uniquely associate through Watson−Crick assembly to form closed-cycle or linear arrays of aligned SNS monomers. The melting behavior and electrophoretic migration of these assemblies showed cooperative formation of multicomponent arrays containing two to five DNA modules (i.e., 12−30 SNS monomers). The treatment of these arrays with horseradish peroxidase and H_2O_2 resulted in oxidative polymerization of the SNS monomers with concomitant ligation of the DNA modules. The resulting cyclic and linear arrays exhibited chemical and optical properties typical of conducting thiophene-like polymers, with a red-end absorption beyond 1250 nm. AFM images of the cyclic array containing 18 SNS units revealed highly regular 10 nm diameter objects.

There is extensive interest in the development of nanostructures composed of self-assembling molecular components that adopt predictable shapes and have unique chemical or material properties. Because of its easy manipulation, sequence programmability, and well-known self-organizing abilities, DNA is often employed as a template or scaffold upon which these structures are built.[1−8] We have reported a fusion of the templating and scaffolding roles of DNA in the directed assembly of nanowires. In this approach, monomer moieties of conducting polymers (e.g., aniline) are covalently linked to nucleobases and thus preorganized in the major groove of duplex DNA. This prearrangement of monomers facilitates their subsequent oxidative polymerization by horseradish peroxidase (HRP) and H_2O_2 to form molecular wires with controlled lengths and structures having tailored optical and electronic properties.[9−13] In this paper, we report the use of an encoded modular DNA assembly strategy[14] enabling the programmed polymerization of 2,5-bis(2-thienyl)-pyrrole (SNS) monomers into a variety of linear and closed-cycle arrays of conducting polymers.

We prepared a series of DNA oligomers by means of the previously described postsynthetic modification method.[12,15] These oligomers had six SNS monomer units linked covalently to the N4 atoms of six cytosine nucleobases through trimethylene groups. These modified bases (designated "X" in Figure 1) were separated by thymines at alternating positions to

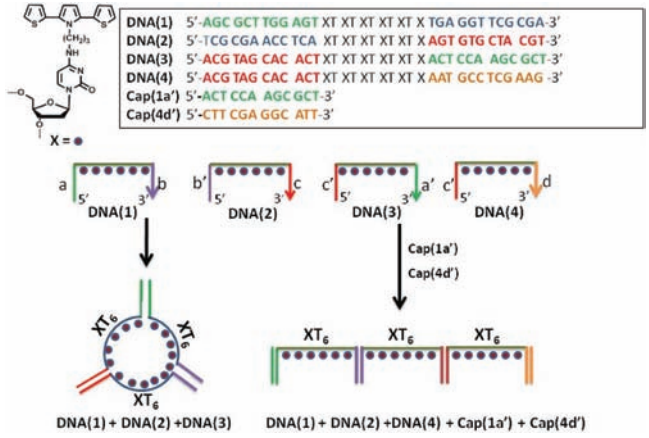

Figure 1. Structures of the DNA oligomers used in this work and schematic representations of the programmed assembly of these modules into cyclic and linear arrays containing ordered, DNA-linked SNS monomers.

form $(XT)_6$ units and placed in the middle of the oligomers between flanking "recognition sequences". Previous experiments showed that the alternating XT arrangement appropriately spaces the SNS monomers for efficient head-to-tail coupling.[12] The 12-nucleotide sequences on the 5′ and 3′ sides of the XT-containing segments were designed to recognize and hybridize uniquely with oligonucleotides containing complementary sequences by Watson−Crick self-assembly. For example, as shown in Figure 1, the last 12 bases on the 3′ side of DNA(1) (designated *b*) were complementary to the first 12 bases on the 5′-side of DNA(2) (designated *b*′). Similarly, the sequences *a*/*a*′ and *c*/*c*′ were also complementary. Thus, when the three oligomers DNA(1), DNA(2), and DNA(3) were combined in buffer solution, they were expected to self-assemble into a closed cycle array with the adjacent SNS-modified segments held in place by three double stranded DNA

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"arms". In contrast, the self-assembly of oligomers DNA(1), DNA(2), and DNA(4) was expected to form a linear array wherein the recognition sequences *a* and *d* would be "capped" by the complementary 12-mer oligomers Cap(1*a*′) and Cap(4*d*′). Clearly, encoded DNA modules may be combined to form a variety of cyclic and linear arrays. We describe below the formation of arrays composed of three, four, and five modules and their reaction with HRP/H_2O_2 to form conducting polymer nanostructures.

The hybridization of oligomers DNA(1), DNA(2), and DNA(3) was examined by measurement of melting temperatures (T_m) and gel electrophoresis. Shown in Figure 2 is the

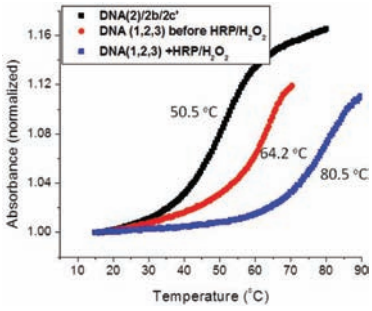

Figure 2. Thermal melting behavior of the partial duplex of DNA(2) with Cap(2*b*′) and Cap(2*c*) and of the cyclic assembly DNA(1,2,3) before and after reaction with HRP/H_2O_2.

melting behavior of DNA(2) with the recognition sequences at its 5′ and 3′ sides capped with their complementary 12-mers Cap(2*b*) and Cap(2*c*′).[16] A melting transition with T_m = 50.5 °C was observed, corresponding to denaturation of the capped duplex regions. As expected, the melting behaviors of capped DNA(1) and DNA(3) were similar to that of DNA(2). In contrast, the cyclic assembly DNA(1,2,3) showed a single transition at 64.2 °C. This striking increase in T_m (13.7 °C) is indicative of the additional cooperative stabilization expected to result from the formation of the proposed cyclic structure. In support of this interpretation, the linear assembly DNA(1,2,4) with Cap(1*a*′) and Cap(4*d*′) did not show a significant increase in T_m in comparison with capped DNA(2).[17]

Polyacrylamide gel electrophoresis (PAGE) is able to separate DNA assemblies according to their size and total charge. Figure 3a shows the results of nondenaturing (duplex regions are maintained as duplexes) PAGE analysis of the cyclic and linear DNA arrays. As the components of these structures are assembled, they move more slowly on the gel because of increasing size. Lane 3 shows the essentially complete formation of the expected cyclic assembly DNA(1,2,3), and lane 4 shows the linear assembly DNA(1,2,4) as a band at approximately the same position as that of cyclic assembly. These two structures can readily be distinguished by their different responses to the inclusion of capping sequences. Figure 3b shows that the closed cycle DNA(1,2,3) is unaffected by addition of Cap(1*a*′) and Cap(3*a*) but that the linear assembly DNA(1,2,4) is significantly retarded by inclusion of Cap(1*a*′) and Cap(4*d*′). Evidently, addition of the complementary caps does not disrupt the cyclic array, but the caps do hybridize with and increase the size of the linear array.

PAGE analysis also confirmed the finding from the T_m measurements that the linear array was less thermally stable than the cyclic array. Weak bands corresponding to the modules constituting the linear assembly DNA(1,2,4) were

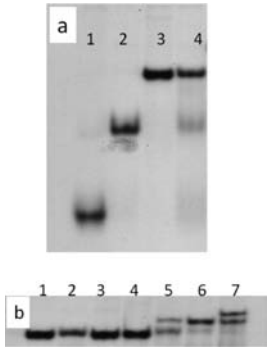

Figure 3. Autoradiography of the nondenaturing PAGE analysis of cyclic and linear DNA assemblies. (a) Lane 1, DNA(1) only; lane 2, DNA(1,2); lane 3, DNA(1,2,3) cyclic assembly; lane 4, DNA(1,2,4) linear assembly. (b) Lane 1, DNA(1,2,3); lane 2, DNA(1,2,4); lane 3, DNA(1,2,3)/Cap(1*a*′); lane 4, DNA(1,2,3)/Cap(3*a*); lane 5, DNA-(1,2,4)/Cap(1*a*′); lane 6, DNA(1,2,4)/Cap(4*d*′); lane 7, DNA(1,2,4)/Cap(1*a*′)/Cap(4*d*′).

observed in its gel; analogous bands were not seen in the analysis of the cyclic array DNA(1,2,3). This modular approach was easily expanded to enable the assembly of larger cyclic arrays. Figure 4 presents the PAGE analysis showing the

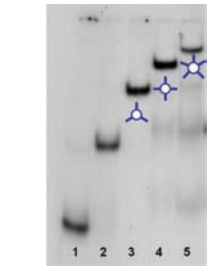

Figure 4. Autoradiography of nondenaturing PAGE analysis of cyclic DNA assemblies. Lane 1, DNA(1) only; lane 2, DNA(1,2); lane 3, DNA(1,2,3) trimer; lane 4, DNA(1,2,4,5) tetramer; lane 5, DNA-(1,2,4,6,7) pentamer.

formation of cyclic structures composed of three, four, and five DNA modules. These structures were efficiently formed in high yield from their components and contained 18, 24, and 30 ordered SNS monomers, respectively.

Treatment of the DNA assemblies with HRP/H_2O_2 resulted in covalent bond formation between the SNS monomers that ligated the component DNA modules and formed arrays of conducting polymers (Figure 5). Assembly DNA(1,2,3) was treated with HRP/H_2O_2 in 10 mM sodium citrate buffer (pH

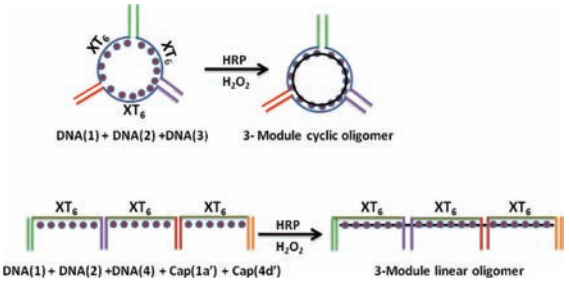

Figure 5. Schematic illustration of the ligation of DNA modules to form cyclic and linear polymers from the programmed self-assembly of DNA with linked SNS monomers.

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4.5) containing 200 mM NaCl and a catalytic amount of ABTS.[18,19] The formation of polymer from the arrayed SNS monomers was analyzed first by its effect on the T_m of the assembly. Bond formation between the SNS monomers results in ligation of the modules constitute the assembly, as evidenced by a dramatic increase in its stability. The product of the reaction of the DNA(1,2,3) assembly with HRP/H_2O_2 showed a single melting transition at 80.5 °C, an increase of 16.3 °C in T_m, indicating ligation of all three modules (see Figure 2). In contrast, the melting curve observed after reaction of a solution of DNA(2) with Cap(2*b*) and Cap(2*c*′) with HRP/H_2O_2 showed no change in the T_m.[20] The T_m profile of assembly DNA(1,2) with Cap(1*a*′) and Cap(2*c*′) exhibited two transitions.[17] The first appeared at ∼45 °C, corresponding to denaturation of the capping oligomers. The second appeared at ∼80 °C, which is close to the T_m of the linked cyclic assembly DNA(1,2,3) and corresponds to melting of ligated modules that had been cross-linked by bond formation between SNS monomers. Similarly, two melting transitions were observed for all of the other capped ligated linear assemblies.

The UV−vis absorption spectra of these assemblies also changed characteristically upon treatment with HRP/H_2O_2. Before the reaction, DNA(1,2,3) showed absorptions typical of the UV bands of DNA and the 320 nm band characteristic of the SNS monomer (Figure 6). After reaction with HRP/H_2O_2, the absorption spectrum shifted, revealing a maximum at 580 nm and a band in the near-IR region with a maximum at 1070 nm. These spectral features are typical of the oxidized, conducting form of poly(SNS). Reduction of the oxidized polymeric assembly with hydrazine shifted its absorbance to ∼405 nm, which is characteristic of the fully reduced *leuco* form of the polymer.[21] The absorption maxima of the oxidized poly(SNS) assemblies shifted as expected with the conjugation length of the polymer.[22] Oxidation of the capped single module DNA(1) gave bands at 560 and 1030 nm, and the reaction of the capped, two-module linear array DNA(1,2) gave bands at 570 and 1050 nm. The systematic 20 nm red shift observed as the number of monomer units in the assembly was increased from 6 to 12 to 18 indicates that HRP/H_2O_2 oxidation resulted in the expected length increase of the polymers.[21]

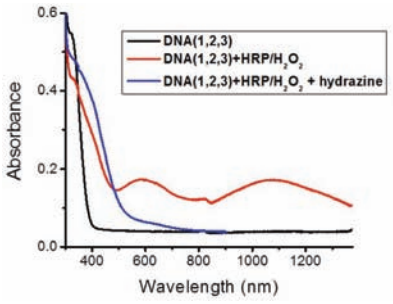

Figure 6. UV−vis−NIR absorption spectrum of the DNA(1,2,3) cyclic assembly. Black curve: DNA(1,2,3) before reaction with HRP/H_2O_2, showing the absorption of SNS monomers. Red curve: DNA(1,2,3) after reaction with HRP/H_2O_2, showing the absorption of the oxidized conducting polymer (the "blip" at ∼810 nm is an instrumental artifact due to a filter change). Blue curve: absorption spectrum of the reduced *leuco* form of the conducting polymer obtained by adding hydrazine after the reaction with HRP/H_2O_2.

HPLC analysis of the DNA(1,2,3) assembly before treatment with HRP/H_2O_2 revealed two partially resolved peaks with retention times of ∼16 and ∼18 min whose UV−vis spectra corresponded to the modules of DNA linked to unreacted SNS monomers.[23] Reaction of this assembly with HRP/H_2O_2 and subsequent reduction resulted in the essentially complete disappearance of the peaks assigned to the individual modules and the appearance of a broad new peak eluting at ∼10 min whose UV−vis spectrum displayed the characteristic features of the *leuco* form of poly(SNS). These findings suggest that bond formation between SNS monomers on separate but adjacent modules in the DNA(1,2,3) assembly ligates the three modules into an intact cyclic array, a result consistent with the optical absorption data and supported by PAGE analysis.

The covalent ligation of the DNA modules resulting from the HRP/H_2O_2-catalyzed polymerization reaction was studied by denaturing PAGE. The DNA(1) module was labeled at the 5′ terminus with ^{32}P for visualization by autoradiography. Under the conditions of this experiment, normal duplex DNA separates into single strands. Lane 1 of Figure 7 shows the position of DNA(1), and lane 2 shows that HRP/H_2O_2 treatment of a solution of DNA(1) hybridized with Cap(1*a*′) and Cap(1*b*′) resulted in no measurable change in its position, notwithstanding the oligomerization of the SNS monomers as evidenced by characteristic changes to its absorption spectrum.[24] Lane 3 shows results from the reaction of a solution containing DNA(1,2), Cap(1*a*′), and Cap(2*c*′) with HRP/H_2O_2. Despite the denaturing conditions of the experiment, this assembly migrated considerably slower than DNA(1), indicating that DNA(1) and DNA(2) had become irreversibly ligated by covalent bond formation between SNS monomers. Similarly, lanes 4, 6, and 8 show the results of the HRP/H_2O_2 reaction on cyclic arrays containing three, four, and five modules, and lanes 5, 7, and 9 correspond to capped three-, four-, and five-module linear arrays. The cyclic arrays showed nearly complete ligation; only trace amounts of individual modules were visible in the gel. The decreased stability of the unreacted linear arrays was evidenced by the presence of shorter ligated segments in the gel. For example, reaction of the five-component linear array shown in lane 9 resulted in a mixture containing 1−5 ligated modules.

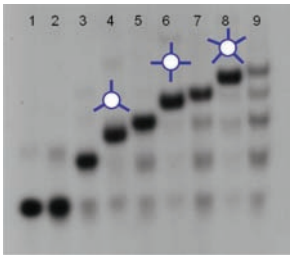

Figure 7. Autoradiography of denaturing PAGE analysis of the ligation of cyclic and linear DNA assemblies through the formation of cyclic and linear conducting polymers formed by reaction with HRP/H_2O_2. Lane 1, DNA(1) only; lane 2, DNA(1)/Cap(1*a*′)/Cap(1*b*′) + HRP/H_2O_2; lane 3, DNA(1,2)/Cap(1*a*′)/Cap(2*c*′) + HRP/H_2O_2; lanes 4, 6, and 8, DNA(1,2,3), DNA(1,2,4,5), and DNA(1,2,4,6,7) (corresponding to cyclic trimer, tetramer, and pentamer, respectively) + HRP/H_2O_2; lanes 5, 7, and 9, DNA(1,2,4), DNA(1,2,4,6), and DNA-(1,2,4,6,8) (corresponding to linear trimer, tetramer, and pentamer, respectively) + HRP/H_2O_2.

AFM images corroborating the structure of the polymerized cyclic assembly formed by treatment of DNA(1,2,3) with HRP/H_2O_2 are shown in Figure 8. The expected diameter of

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these structures, including the three duplex DNA arms, was ~10 nm, and the expected height was 1.0–2.0 nm. The structures observed by AFM were highly uniform with an

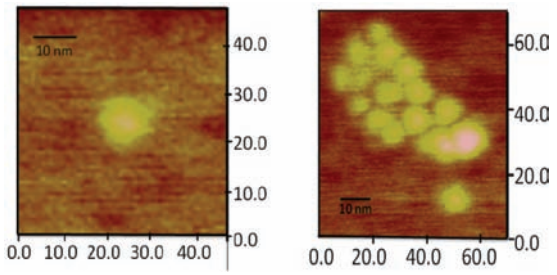

Figure 8. AFM images of the cyclic assembly DNA(1,2,3) after reaction with HRP/H_2O_2.

average diameter of 12.7 ± 1.9 nm and an average height of 0.8 ± 0.2 nm.

The modular assembly process described here[25] enables the simple synthesis of nanometer-scale objects composed of a DNA scaffold linked to and supporting a linear or cyclic conducting polymer. The contrasting properties of linear and cyclic arrays of conducting polymers have been of interest for a long time.[26] In particular, it has been suggested that "giant" conjugated molecular rings of conducting polymers may sustain persistent periodic currents in strong magnetic fields.[27] Bäuerle and co-workers have recently reported a general approach to the synthesis of giant cyclic oligothiophenes containing up to 35 covalently linked thiophene rings.[28] In this report, we have described an approach that enables the precise preparation of a cyclic conducting polymer array of 90 aromatic rings. The modular nature of this approach allows for the ready control of the synthetic process and permits the formation of arrays of defined dimensions. Furthermore, the immense power of DNA self-assembly may enable the construction of ordered periodic superarrays consisting of cyclic or linear conducting polymer nanostructures arranged in regular one- and two-dimensional patterns.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental section and supporting figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The structures of the DNA oligomers used in this work that are not shown in Figure 1 are described in the Supporting Information (SI) along with the characterization of these compounds (see Figure S-1 and Tables S-1 and S-2).
(16) T_m was monitored at 260 nm in 10 mM sodium citrate buffer (pH 4.5) and 200 mM NaCl.
(17) See Figure S-3.
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(22) See Figure S-4.
(23) See Figure S-5.
(24) The reaction of uncapped DNA modules with HRP/H_2O_2 resulted in uncontrolled ligation giving complex mixtures of oligomers.
(25) Also see: O'Sullivan, M. C.; Sprafke, J. K.; Kondratuk, D. V.; Rinfray, C.; Claridge, T. D. W.; Saywell, A.; Blunt, M. O.; O'Shea, J. N.; Beton, P. H.; Malfois, M.; Anderson, H. A. *Nature* **2011**, *469*, 72.
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