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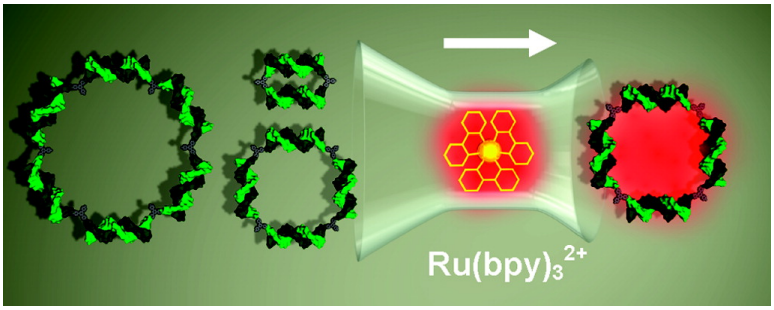

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Guest-Mediated Access to a Single DNA Nanostructure from a Library of Multiple Assemblies

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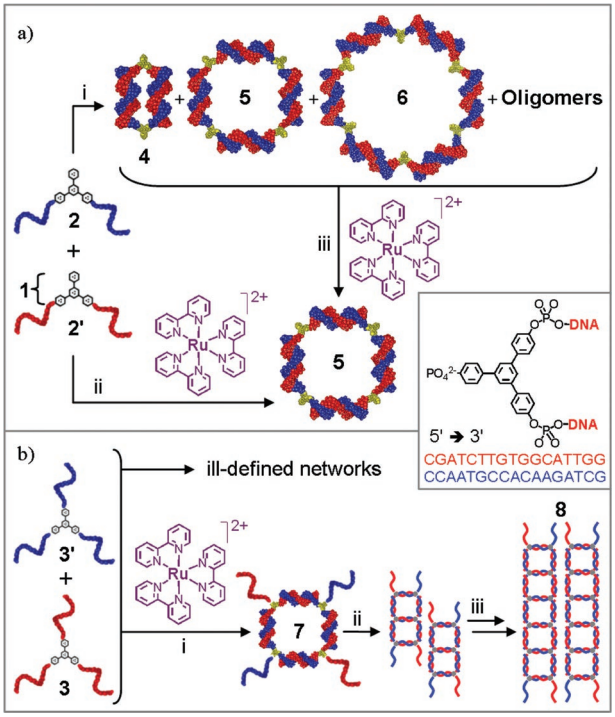
DNA has recently emerged as a promising construction material in nanoscience. Examples include the organization of 2D and 3D structures,[1] gold nanoparticle patterns,[2] memory circuits,[3] and stimuli-responsive nanomachines.[4] However, as the structural complexity of these systems increases, so will the number of DNA sequences that need to be incorporated. This inevitably results in overlapping, degenerate sequences which may assemble into undesirable products. A method to template access to a *single* product from sequence-degenerate DNA building blocks, which normally result in multiple assemblies, would aid in the design of more complex DNA systems.[5] The templated synthesis of a specific molecule from a dynamic library of possible structures has, in fact, been a recent subject of intense interest.[6] Although many elegant approaches have applied this concept to amplify the formation of small molecules, supramolecular assemblies, and macromolecules,[6] there are no examples in which this powerful method is used in the area of DNA nanotechnology. Here we report the guest-mediated self-assembly of a single DNA nanostructure, using two symmetrically branched building blocks **2** and **2′** that otherwise generate a library of products (Scheme 1a). We also show how this approach can be used to predictably construct well-defined 1D DNA fibers extending over tens of microns, using two trifunctional DNA building blocks that normally assemble into ill-defined oligomeric networks (Scheme 1b). Overall, this provides an attractive method for altering and refining product outcome in DNA self-assembly, by addition of a small DNA-binding molecule to generate a single nanostructure.

Dynamic libraries that adapt to external stimuli contain a number of equilibrating members under thermodynamic control.[6] The self-assembly of the complementary molecules **2** and **2′**, in which two identical DNA arms branch from the fully rigid organic vertex **1** (Scheme 1; inset), was found to generate a small dynamic library of DNA structures.[2a,b,7,8,13] In addition to oligomers, hybridization of **2** to **2′** (mixing at 90 °C and incubation at 0 °C for 15 min) results in three discrete assemblies assigned to dimer **4**, square **5**, and hexagon **6** (based on gel mobility as compared to a molecular weight marker; Scheme 1ai; Figure 1a). The cyclic nature of **4**, **5**, and **6** was ascertained using Mung Bean nuclease enzymatic assays.[13] We then examined the dynamic character of this library by first subjecting the entire mixture (i.e., **4**, **5**, **6**, and oligomers) to repeated heating and cooling cycles (20 to 90 °C). This results in the gradual decrease of oligomers, until their eventual disappearance following 66 cycles.[13] In addition, subjecting the individually isolated dimer **4**, square **5**, and hexagon **6** to 66 such heating/cooling cycles resulted in their re-equilibration into the mixture of **4**, **5**, and **6** but not oligomers.[13] These findings indicate that the oligomers are kinetic products, while the discrete assemblies **4**, **5**, and **6** are thermodynamically stable. Moreover, while the constituents of this library are stable at room temperature and equilibrium is slowed down ("frozen"), equilibration can be readily achieved by heating the mixture (55 °C, 16 h).[13] The assembly of **2** and **2′** thus creates a dynamic library of equilibrating structures that should in principle be environmentally responsive.

Scheme 1



Self-assembly of **2** with **2′** in the presence of excess $Ru(bpy)_3^{2+}$ as a small molecule template indeed results in dramatically different behavior. Although the hybridization of **2** with **2′** at room temperature exclusively forms oligomers, the same self-assembly in presence of $Ru(bpy)_3^{2+}$ generates square **5** quantitatively (Scheme 1aii; Figure 1b). In fact, when either of the preformed oligomers, dimer **4** or hexamer **6** are isolated (Figure 1c, lanes 1, 4, 7) and heated with excess $Ru(bpy)_3^{2+}$ at 55 °C for 16 h, quantitative re-equilibration into square **5** is observed (lanes 3, 6, 9; Scheme 1aiii). In contrast, heating these individual components (55 °C, 16 h) without the ruthenium template only converts each assembly into the initial library of products.[13] Interestingly, the ruthenium template is also found to accelerate the equilibration process itself. When added to isolated **4**, **6**, and oligomers at 25 °C for 16 h, re-equilibration into the library of **4**, **5**, **6**, and oligomers is observed (Figure 1c, lanes 2, 5, 8), while without the template, these members partially re-equilibrate into the oligomers only.[13] Heating any of these mixtures (55 °C, 16 h) in the presence of $Ru(bpy)_3^{2+}$ produces **5** quantitatively. We are currently further investigating the mechanism of this selection process.[9,13] Preliminary modeling shows that dimer **4** presents two DNA strands in close proximity and little to no space available for $Ru(bpy)_3^{2+}$ guests to reside in its central cavity. While square **5** easily accommodates a number of such

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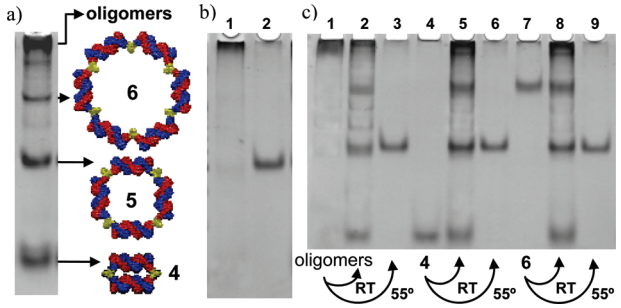

Figure 1. (a) **2** and **2′** generate cyclic dimer **4**, tetramer **5**, hexamer **6**, and oligomers. (b) Hybridization of **2** to **2′** at room temperature generates polymers (lane 1), while the same assembly in the presence of $Ru(bpy)_3^{2+}$ results in tetramer **5** quantitatively (lane 2). (c) Oligomers, **4** and **6** (lanes 1, 4, and 7), with $Ru(bpy)_3^{2+}$, re-equilibrate into all the cyclic structures and oligomers when incubated at 25 °C for 16 h (lanes 2, 5, and 8) and into **5** exclusively when incubated at 55 °C for 16 h (lanes 3, 6, and 9).

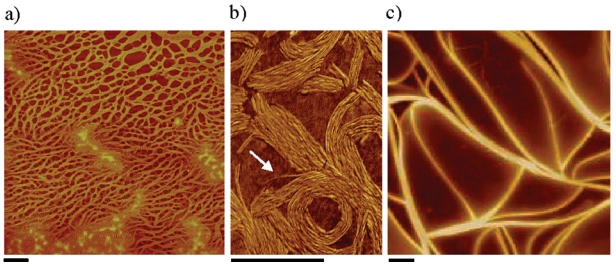

Figure 2. AFM analysis of the assemblies formed from **3** and **3′** reveals the generation of (a) ill-defined DNA networks in the absence of $Ru(bpy)_3^{2+}$, and (b) the predicted DNA ladders **8** (phase image)—which (c) further assemble into larger DNA fibers—in its presence. Bar is 1 μm.

complexes in its cavity, it is likely that hexagon **6** and higher-order structures present too much entropic cost to be favored over **5**.[13] In conclusion, $Ru(bpy)_3^{2+}$ not only provides access to a single member from the library generated with **2** and **2′** but can also re-equilibrate each already formed member into tetramer **5** quantitatively. A current challenge in the area of DNA nanotechnology, and self-assembly in general, is error correction.[10] The ability of this template to dynamically transform any number of assemblies into a single product provides a built-in correction mechanism that helps ensure the integrity of the construction process, even when errors are made.

3 and **3′** are the three-arm DNA analogues of **2** and **2′** and are thus expected to assemble into ill-defined oligomeric aggregates, similar to the assembly of **2** and **2′** into multiple structures. Analysis using electrophoresis confirms the formation of aggregate structures of little mobility, and atomic force microscopy (AFM) shows the generation of ill-defined DNA networks from **3** and **3′** (Figure 2a).[13] Since **2** and **2′** in the presence of $Ru(bpy)_3^{2+}$ generate a single structure, the use of $Ru(bpy)_3^{2+}$ with **3** and **3′** provides for the opportunity to predictably generate well-defined extended assemblies. **3** and **3′** with $Ru(bpy)_3^{2+}$ are expected to first exclusively assemble into cyclic tetramer **7**, similar to **5**, that contains four single-stranded complementary DNA arms capable of further hybridizing to one another (Scheme 1bi). In the presence of $Ru(bpy)_3^{2+}$, these structures are expected to further dimerize (Scheme 1bii) and continue to grow in 1D into extended periodic DNA ladders **8** (Scheme 1biii). Electrophoresis experiments confirm the role of $Ru(bpy)_3^{2+}$ in redirecting the self-assembly behavior of **3** and **3′** into a single molecule similar to square **5**.[13] AFM imaging shows the formation of the predicted DNA ladders **8** (Figure 2b) and reveals their subsequent assembly into 1D fibers extending over >50 μm (Figure 2c).[13] These 1D structures likely result from the assembly of thousands of squares (over 6000 would assemble to generate a 40 μm fiber).[11]

We have shown that the addition of a small molecule template can be used to quantitatively generate a single DNA nanostructure from a set of building blocks that otherwise results in a library of products. This guest template can also re-equilibrate any member of the self-assembled mixture into the selected nanostructure. This approach not only allows for the incorporation of symmetry to construct more complex systems but also presents the immediate advantage of autocorrecting errors that may form during the initial self-assembly process. We applied this concept to predictably generate well-defined DNA fibers that periodically extend over tens of micrometers, from two symmetrically tris-branched building blocks. The ability to access a single assembly from a library of possible combinations through the simple use of a small molecule introduces an additional level of control in DNA construction. Considering the wealth of DNA-binding molecules which can be employed to tune, modify, and correct the assembly of DNA structures, this approach promises to lead to significant advances in the field of DNA nanotechnology.[12]

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Supporting Information Available: Synthesis of **2**, **2′**, **3**, and **3′**, self-assembly, and AFM analysis of **3** and **3′**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) This is the first assembly study in which identical DNA arms branch from a fully rigid synthetic vertex. If dsDNA is assumed to be rigid, the 120° angle in **1** would result in DNA hexagons. However, **2** and **2′** also generate dimers and tetramers, thus although rigid vertices provide structural definition to an assembled system, they do not direct the actual assembly.

(9) Photophysical behavior of $Ru(bpy)_3^{2+}$ (steady-state and lifetime measurements) reveals no major change upon addition of pure **4**, **5**, **6**, building blocks **2** or **2′**, or regular single- or double-stranded DNA.

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(11) For detailed structural characterization of **8**, see Supporting Information.

(12) Preliminary studies show that bis-intercalator TOTO significantly increases the relative amount of dimer **4** from **2** and **2′**, as predicted from the parallel arrangement of two DNA duplexes in dimer **4**.

(13) See Supporting Information for details.

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