Communication

Modular Access to Structurally Switchable 3D Discrete DNA Assemblies

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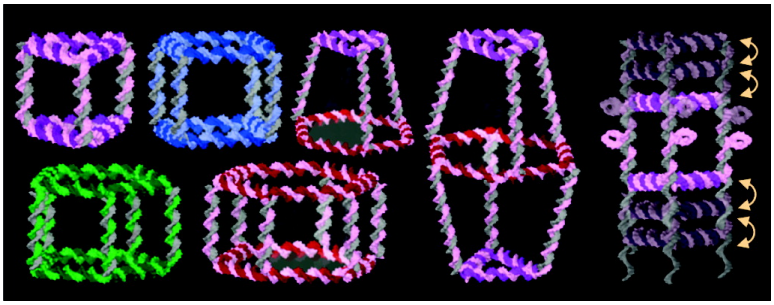

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Modular Access to Structurally Switchable 3D Discrete DNA Assemblies

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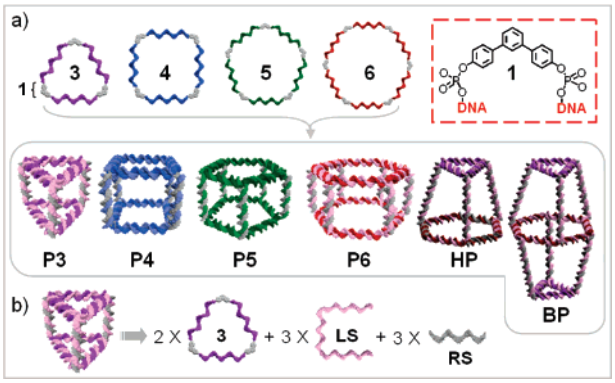
DNA has recently emerged as a promising construction material for the creation of discrete and periodic two-dimensional assemblies.[1] An immediate challenge, however, is the use of this molecule to generate three-dimensional structures. DNA polyhedra present tremendous potential for drug encapsulation and release, regulation of the folding and activity of encapsulated proteins,[2] selective encaging of nanomaterials, and assembly of 3D networks for catalysis and biomolecule crystallization. Only five reports have appeared on this subject over the past fifteen years, each presenting the synthesis of a single assembly,[3] illustrating the need for a systematic and straightforward method to access these materials. Moreover, none of these have been on structurally dynamic or stimuli responsive systems. In this contribution, we present a simple method that uses a small number of building blocks to quantitatively access a large number of 3D DNA assemblies. These include a triangular prism, a cube, pentameric and hexameric prisms, as well as more complex structures such as a heteroprism and biprism (Scheme 1). We also demonstrate the addressability of these assemblies by constructing a dynamic triangular prism capable of structural oscillation between three predefined lengths. In our approach, the target 3D object is modularly assembled in a minimum number of steps using a set of single-stranded and cyclic DNA building blocks that contain rigid organic molecules as their vertices (i.e., triangle **3**, square **4**, pentagon **5**, and hexagon **6**, Scheme 1). In principle, any discrete assembly that can be retrosynthetically broken down into our set of building blocks can also be generated, and thus this approach represents a highly straightforward and economical way to access libraries of discrete and structurally dynamic 3D DNA assemblies.

Initial work focused on the construction of our toolbox of cyclic single-stranded DNA building blocks triangle **3**, square **4**, pentagon **5** and hexagon **6**.[9] This involves the synthesis of a single continuous strand of DNA containing the appropriate number of vertex **1** molecules[4,5] (i.e., three for **3**, four for **4**, etc.), and its subsequent DNA-templated chemical ligation. The single-stranded and cyclic nature of **3**, **4**, **5**, and **6** was confirmed using ExoVII enzymatic digestion assays.[9]

With building blocks **3**, **4**, **5**, and **6** in hand, we proceeded to examine their self-assembly potential to generate 3D discrete DNA objects. Initially, triangular **P3**, cubic **P4**, pentameric **P5**, and hexameric **P6** prisms were constructed.[9] For example, prism **P3** is assembled using two units of triangle **3**, three linking strands (**LS**) and three rigidifying strands (**RS**) (Scheme 1b). Polyacrylamide gel electrophoresis (PAGE) analysis reveals the clean generation of all intermediates leading to the single stranded analogue of prism **P3** (**P3ss**) and the subsequent quantitative synthesis of fully double-stranded **P3** (Figure 1a). The connectivity of prism **P3** was confirmed using enzymatic digestion assays.[9] Mung bean nuclease (MBN) is selective for single-stranded DNA, while ExoVII only digests open uncyclized single-stranded DNA. As expected, prism **P3** was unaffected by either MBN or ExoVII, **P3ss** was digested by only MBN, while **P3-3** (structure in Figure 1a) was fully

Scheme 1 [a]



[a] For clarity, the assemblies are represented with longer DNA arms.[6]

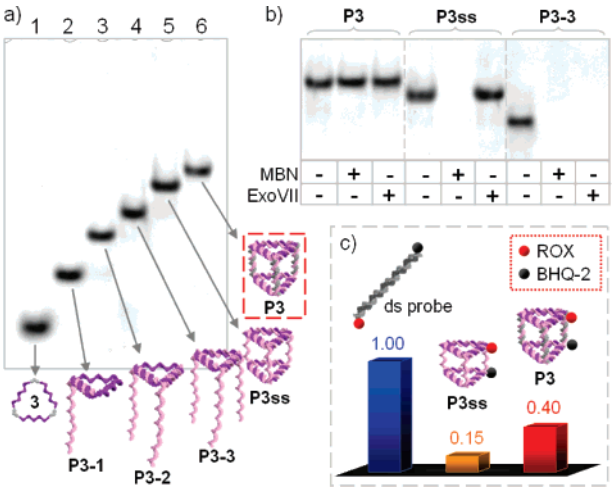


Figure 1. Assembly (a) PAGE analysis of triangle **3** (lane 1), all the possible intermediates leading to assembly of **P3** (lanes 2−5), and **P3** (lane 6). (b) **P3** is unaffected by either MBN or ExoVII, **P3ss** is only degraded by MBN, while **P3**−**3** is digested by both ezymes. (± indicate enzyme presence/absence). (c) FRET analysis of the formation of **P3** from **P3ss**.

degraded by both enzymes (Figure 1b). The assembly of prism **P3** was further confirmed using fluorescence resonance energy transfer (FRET) studies.[9] One of the linking strands was end-labeled with the fluorophore ROX and the quencher BHQ-2 (Figure 1c). When compared to fully unquenched double-stranded probe, a large drop in fluorescence is observed upon generation of **P3ss** (FRET calculated distance 4.4 nm), which increases following assembly of double stranded prism **P3** (5.2 nm, Figure 1c). Cube **P4**, pentameric **P5**, and hexameric **P6** prisms were also quantitatively accessed from square **4**, pentagon **5**, and hexagon **6**, respectively (Figure 2a). The connectivity in these assemblies was confirmed enzymatically.[9] Interestingly, an earlier synthesis of a DNA cube required multiple steps, with a yield of less than 1%.[3d]

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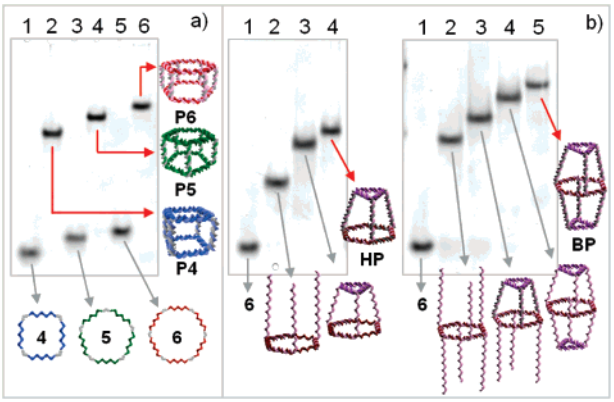

Figure 2. Diversity (a) square **4**, pentagon **5,** and hexagon **6** (lanes 1, 3, 5) quantitatively generate cube **P4**, pentagonal **P5**, and hexagonal **P6** prisms (lanes 2, 4, 6). (b-left) hexagon **6** (lane 1), assembly intermediates leading to construction of **HP** (lanes 2, 3), quantitative assembly of **HP** (lane 4). (b-right) **6** (lane 1), intermediates leading to **BP** (lanes 2−4), **BP** (lane 5).

In principle, any target 3D object that can be broken down into a combination of our building blocks **3**−**6**, can also be readily assembled. Heteroprism **HP** contains triangle and hexagon faces, and its synthesis was thus conducted using triangle **3**, hexamer **6**, and three linking strands (Figure 2b). Similarly, biprism **BP** contains triangular apexes and a hexameric core, and as such was constructed from two units of **3**, one unit of **6**, and six linking strands. The clean self-assembly of **HP** and **BP** was studied sequentially using PAGE (Figure 2b) and confirmed enzymatically.[9] The two-step construction of biprism **BP**, that is, assembly of two triangular apexes to the hexameric core, was also monitored using FRET studies.[9] Our approach of using a small number of presynthesized single-stranded cyclic units as the sides or planes of discrete DNA objects, provides for a facile method for generating large numbers of relatively complex 3D discrete assemblies.[7]

DNA building blocks that are cyclic can maintain their structural integrity in their single-stranded form, and thus, their use provides for an opportunity to generate structurally dynamic addressable assemblies. To demonstrate this, we constructed a triangular prism **dynP** that contains single-stranded regions separating both triangular faces (Figure 3). This synthetic intermediate was then used to generate three well-defined triangular prisms of different lengths **dynP10**, **dynP14**, and **dynP20**. Assembly of each prism was achieved using strands **7**−**9**, capable of introducing internal loops within **dynP** of different lengths, while real-time oscillation between each structure is conducted using eraser strands **10**−**12** (Figure 3a). One of the linking strands was end-labeled with ROX/BHQ-2 so that in addition to PAGE, the resulting assemblies could also be probed using FRET analysis (Figure 3b). Starting with **dynP**, addition of **7** generates triangular prism **dynP10** with 10 base internal loops and a calculated FRET length of 5.2 nm. The **dynP10** is converted into prism **dynP14** (6.9 nm) following addition of **10** to regenerate **dynP**, and then **8** which incorporates six base internal loops within **dynP14**. This assembly is similarly converted into triangular prism **dyn20** (8.9 nm) using **9** and **11**. Strands **12** regenerate the initial **dynP** and complete this structural cycle (Figure 3).[8] This is the first example of a dynamic 3D discrete DNA object that is controllably oscillated between three predetermined dimensions. Many applications can be anticipated for these switchable capsules including molecule-triggered drug delivery and dynamic 3D DNA crystals.

We have thus presented a new method, which allows for the facile and quantitative construction of a library of discrete three-dimensional DNA structures from a small set of building blocks.

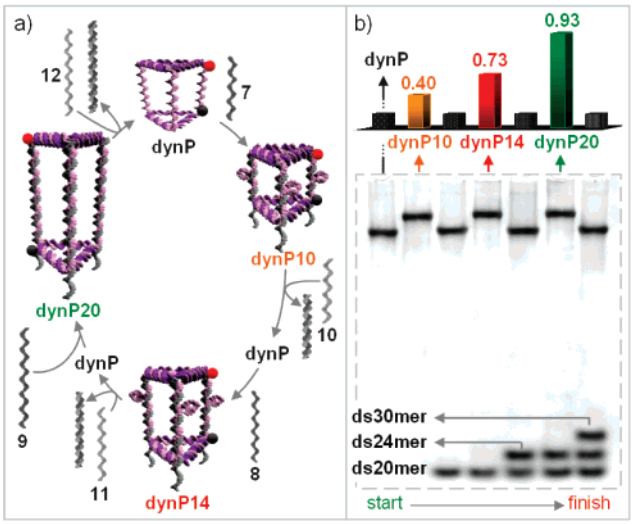

Figure 3. Switching (a) strands **7**, **8**, and **9** are used to generate **dynP10**, **dynP14**, and **dynP20** from **dynP**, with respective lengths of 10, 14, and 20 bases. Real-time structural oscillation between these structures is achieved by regenerating the intermediate **dynP** using strands **10**, **11**, and **12**. (b) This structural cycle is confirmed using PAGE and FRET analysis.

Moreover, reversible structural switching of these 3D-DNA objects in response to external agents was demonstrated. The approach involves the use of building blocks that are cyclic and single-stranded, as the faces or sides of the target 3D-DNA object. In principle, any structure that can be retrosynthetically broken down to such DNA cycles can be readily obtained. As such, this represents a highly economical method to access a large number of dynamic three-dimensional DNA objects and will significantly expand the applications of 3D-DNA construction.

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Supporting Information Available: **2-6**, **P3-6**, **HP**, **BP** synthesis; enzymatic and FRET studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) Rigid organic vertices provide the necessary balance of rigidity for structural definition and enough flexibility to achieve quantitative yields.

(6) With the exception of the **RS** within **HP** and **BP** that are 20 bases long, the length of each side within all of these assemblies is 10 bases.

(7) Encapsulating inner volumes of **P3**, **P4**, **P5**, **P6**, **HP**, and **BP** are calculated to approximately be 8, 39, 80, 102, 110, and 220 nm^3, respectively

(8) All 3D assemblies are thermally stable below 27 °C. **dynP14** and **dynP10** are also stable below 27 °C, but their sides contain hairpin loops (stable < 10 °C, 7 °C, resp). This stability can be increased to values that are well above room temperature following incorporation of longer arms.

(9) See Supporting Information for details.

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