Supramolecular DNA nanotechnology

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The field of structural DNA nanotechnology utilizes DNA’s powerful base-pairing molecular recognition criteria to help solve a number of problems facing researchers in material science and nanotechnology. In it, DNA is stripped away from its preconceived biological role, and is treated as a synthetic polymer. A subarea of research that we have recently termed supramolecular DNA nanotechnology is emerging, and is proving to be a powerful complement to some of the already established rules of structural DNA nanotechnology. My work as a doctoral student helped establish this new area of research (Aldaye, Palmer & Sleiman, Science 2008, 321, 1795 – invited review), and can conceptually be divided into the following sections:

[1] Discrete nanoparticle assemblies. Gold nanoparticle assemblies have recently emerged as a promising class of materials, with novel optical, electronic, catalytic and sensing applications. Their assembly into well-defined discrete systems, however, remains challenging. We presented the first example of such a system, and organized six gold nanoparticles into a well-defined discrete hexagon (Aldaye & Sleiman, Angew. Chem. Int. Ed. 2006, 45, 2204 – this article was selected as a Hot Paper and received its own cover-page). The approach combines the rules of supramolecular chemistry with those of DNA nanotechnology, and involves labeling each nanoparticle with DNA molecules, modified with rigid organic vertices, to dictate their final position within a pre-programmed assembly (Fig 1). We then developed a second-generation platform that now provides economical access to libraries of discrete nanoparticle assemblies that are structurally and functionally addressable in real-time (Aldaye and

Fig 1. Gold nanoparticles tagged with DNA molecules containing a rigid organic vertex are sequentially assembled into a well-defined discrete hexamer.
Sleiman, *J. Am. Chem. Soc.* 2007, 129, 4130 – this contribution was highlighted by *Nature Nanotechnology: “DNA builds bridges”*). In this approach, we used single-stranded and cyclic DNA templates that are synthetically embedded with organic molecules (Fig. 2). Essentially, a single template can now be used to generate a large number of nanoparticle assemblies of different combination and geometry, and to also generate assemblies that can be structurally and functionally modulated in response to external stimuli. This contribution currently represents the highest degree of control over the construction and manipulation of discrete nanoparticle assemblies.

Fig 2. (a) Triangular-shaped DNA template 1 and square-shaped DNA template 2 generate triangular and square gold nanoparticle assemblies, while the linear analogous 5 and 6 result in open linear assemblies. (b) 1 can be used as a template to generate triangles of (i) 3 large (15-nm, red), (ii) 2 large / 1 small (5-nm, purple), (iii) 1 large / 2 small and (iv) 3 small particles. (c) 2 can be used as template to organize gold particles into (i) squares, (ii) trapezoids and (iii) rectangles. Inset: A loop is used to shorten the template’s arm. (d) A write/erase function is demonstrated using 1 by (i) assembling 3 gold nanoparticles (15-nm) into a triangular structure, (ii) removing a specific particle using an eraser strand, (iii) and re-writing with 5-nm gold nanoparticle. 50 nm bar.

[Reviewer 1, **complete review**] “A highly attractive and innovative piece of work that opens up a whole new array of possibilities in the nanoparticle world.”

[Reviewer 2] “This is really an impressive piece of work. The nanoparticle architectures built by DNA assembly are unparalleled in my opinion.”
Structurally tunable three-dimensional discrete DNA assemblies. Three-dimensional (3D) discrete DNA nanostructures can be used for the encapsulation and release of drugs, the regulation of protein folding and activity, and for the construction of 3D DNA networks. Despite all of their exciting potential, only five reports on the synthesis of this unique class of materials have been reported over the past 15 years. By using single-stranded and cyclic DNA templates of different geometry, as the faces or sides of the 3D objects to be generated, we developed a modular and straightforward approach to 3D DNA construction (Fig 3). Moreover, our three-dimensional assemblies are amenable to structural modulation between a number of pre-defined sizes, making them useful for molecule-triggered delivery and dynamic three-dimensional DNA crystallization (Aldaye & Sleiman, J. Am. Chem. Soc. 2007, 129, 10070 – this contribution was highlighted by Nature: “Gene boxes”, Nature Materials: “Unnatural life”, and ACS nano: “Inspiration for nature”). A patent entitled “Nucleotide modular assemblies and their use as delivery devices” was filed by Aldaye & Sleiman (50:50).

Fig 3. (a) Single-stranded and cyclic DNA templates are used in a face-centered approach to construct 3D DNA objects. (b) This involves weaving together discrete shapes via single-stranded DNA molecules. (c) Our 3D objects can also undergo structural modulation. For example, we show that strands 7, 8 and 9 are used to generate dynP10, dynP14 and dynP20 prisms, from dynP, with respective length of 10, 14 and 20 bases, while real-time oscillation is achieved by regenerating the intermediate dynP using strands 10, 11 and 12.
[3] Geometrically well-defined DNA nanotubes. DNA nanotubes can be used to template the growth of metallic nanowires, and to potentially act as stiff interconnects and drug nanocarriers. All current methods for their construction, however, result in symmetrical and cylindrical assemblies that are entirely double-stranded. We presented an approach to DNA nanotube synthesis that provides access to geometrically well-defined triangular- and square-shaped nanotubes, and generated, for the first time, DNA nanotubes that can exist in both a double-stranded “closed” form and in a partially single-stranded “open” form (Aldaye, Lo, McLaughlin & Sleiman, Nature Nanotech. 2009, submitted). Our approach involves weaving cyclic and single-stranded DNA building blocks (Fig 4), and provides a new parameter to fine-tune DNA nanotube construction, with potential applications, that include the synthesis of metallic nanowires of controlled shape and topology, and for the loading and release of cargo.

[4] Transition metal-mediated DNA self-assembly. As it becomes necessary to design larger and more complex DNA nanostructures, it inevitably becomes necessary to also incorporate degenerate DNA sequences that may assemble into undesirable side-products. This is a major bottleneck currently facing researchers in DNA nanotechnology. We applied the well-established supramolecular rules of host/guest

Fig 4. (a) Single-stranded DNA molecules are assembled using DNA into modules with addressable sticky-end overhangs that radiate above and below the plane. (b) These can then be polymerized longitudinally to generate 1D DNA nanotubes of well-defined triangular and square-shaped geometry. (c) We can also link these modules using single-stranded DNA modules to generate single-stranded DNA nanotubes that are “open” (bottom right) for the loading and release of cargo.
chemistry to DNA nanotechnology, and templated the selective synthesis of a “correct” DNA nanostructure from a dynamic library of many “incorrect” members using the small molecule Ru(bpy)$_3^{2+}$ (Aldaye & Sleiman, J. Am. Chem. Soc. 2007, 129, 13376). In a direct application, we used Ru(bpy)$_3^{2+}$ to template the construction of uniform one-dimensional DNA ladders from symmetrically branched DNA building blocks that otherwise assemble into ill-defined oligomeric networks (Fig 5). This is the first example in which a small molecule has been used to dictate the assembly outcome of DNA nanostructures, and presents a novel solution to a long standing problem, that is, “what do to do when sequences code for more than one assembly”.

[Reviewer’s comment] “This is a terrific work describing equilibrating DNA assemblies. Hanadi is to be congratulated for discovering how Ru(bpy)$_3^{2+}$ can effect this switch.”

[5] Molecule templated synthesis of higher-order DNA helices. Higher-order DNA assemblies with helices composed of more than two DNA strands provide an untapped wealth of potentially unique structural, physical, and chemical properties. Many areas of research will benefit from our ability to easily generate a large number of such motifs. To date, however, only two examples have emerged. Reprogramming DNA bases to produce a stable DNA strand can be synthetically difficult, and in some cases unattainable. We developed an approach to rationally reprogram the assembly outcome of unmodified DNA, and constructed a triple helix from poly-adenine strands using the tri-facial complementary self-assembling molecule cyanuric acid (Aldaye, Lo & Sleiman, in preparation). Considering the wealth of molecules that contain base-bonding faces, this approach allows for the true potential of this novel class of materials to now be systematically studied and exploited.
Collectively, this dissertation provides a set of simple solutions to some of the bigger challenges facing researchers in nanotechnology, and offers a snapshot of what is to be expected from the symbiosis that is supramolecular DNA nanotechnology. **Our work will impact the areas of synthesis, self-assembly, sensing, catalysis, delivery, storage, photonics, electronics, and scaffolding.**