Nanotechnology **18** (2007) 125305 (4pp) doi:10.1088/0957-4484/18/12/125305

Scalable, low-cost, hierarchical assembly of programmable DNA nanostructures

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Received 30 November 2006, in final form 25 January 2007
Published 23 February 2007
Online at stacks.iop.org/Nano/18/125305

Abstract
We demonstrate a method for the assembly of fully programmable, large molecular weight DNA complexes. The method leverages sticky-end re-use in a hierarchical fashion to reduce the cost of fabrication by building larger complexes from smaller precursors. We have explored the use of controlled non-specific and specific binding between sticky-ends and demonstrate their use in hierarchical assembly. We conclude that it is feasible to scale this method beyond our demonstration of a fully programmable 8960 kD molecular weight 8 × 8 DNA grid for potential application to complex nanoscale system fabrication.

S Supplementary data are available from stacks.iop.org/Nano/18/125305

(Some figures in this article are in colour only in the electronic version)

The application of nanoscale phenomena in photonic and electronic devices is widely considered to be an important development for the future of computer systems. The material limitations of silicon and photolithography that have begun to curtail the historically steady advance of solid-state device performance are making this an increasingly important topic to industry and researchers [1]. However, few methods exist that can organize nanoscale and molecular components with the control and degree of asymmetry required to yield usefully complex circuit topologies in a scalable and low-cost manner[3].

DNA and RNA have gained popularity as a material system for creating complex, aperiodic nanostructures due to the ease with which these materials can be synthesized and controlled [2–7]. The pioneering development of the DNA crossover enables the rationale design and synthesis of structurally rigid molecular complexes from DNA [8–13]. Such methods rely on the programmability of oligonucleotide interactions and leverage the control that complementary nucleotide sequences exert over the thermodynamics of the assembly process. Recent advances in this field have produced many examples of periodic planar DNA lattice [12, 14–18]. However, to form aperiodic 2D structures these methods require the number of unique DNA sequences (and therefore cost) to scale with the area of the structure or the development of algorithmic self-assembly [19, 20]. To overcome such limitations we have demonstrated a low-cost hierarchical method to fabricate large molecular weight, aperiodic structures by DNA self-assembly.

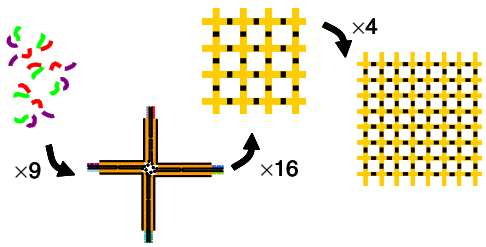

Figure 1. The hierarchical approach to build motifs from individual strands and then into 4 × 4 or 8 × 8 grids.

Briefly, we build on our prior work with aperiodic DNA self-assembled nanostructures [4, 5, 21] to create a set of four uniquely edged 4 × 4 grid structures (see figure 1). Since each motif is assembled from five common and four unique oligonucleotides in an individual vessel, each grid can be independently modified and can create arbitrary patterns as shown in figure 2. The grids are selectively functionalized with streptavidin by using a biotin-functionalized core-oligo during the annealing of the component motifs and introducing free streptavidin afterwards.

[3] We consider an approach to be scalable if it can assemble aperiodic structures beyond the size limitations imposed by the finite sequence space of the sticky-ends used during the assembly.

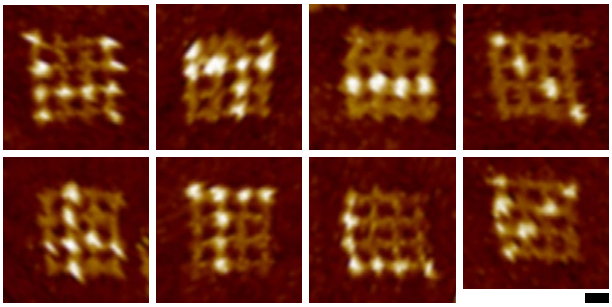

Figure 2. AFM images of streptavidin patterned 4 × 4 DNA grids. Scale bar is 20 nm.

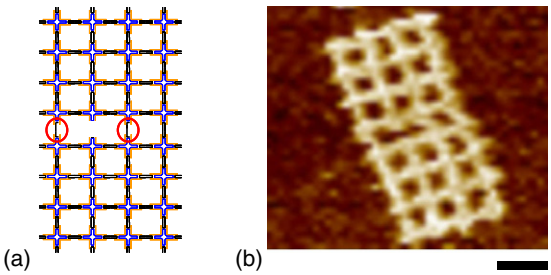

Figure 3. (a) Schematic and (b) AFM image of a two-grid (4 × 8) assembly. Only one linkage at the interface between the two distinct 4 × 4 grids is specific (rightmost) and the remaining two are generic (circled). Scale bar is 40 nm.

The cost of our technique scales with the number of unique oligos in the final nanostructure (e.g. 69 oligos in this case). The origami method [7, 22] suffers from this scaling law as well but in principle could use the methods outlined here to reduce the cost of larger multi-shape structures. Our methods go beyond the origami work to organize programmable structures from multiple long-strand motifs and can be applied to the dense plasmid-based motifs described in [7].

Regardless of the method employed to assemble the basic motif we must bypass the requirement of a unique set of sticky-ends required to assemble a structure unambiguously and reuse strands to decouple the cost of a structure from the linear dependence on its area. Thus, such a method is scalable in terms of the size of the nanostructure that can be fabricated from a finite DNA sequence space. In the limit, a single oligonucleotide sequence might be used to form large supramolecular structures [23]. However, to retain maximal programmability we use multi-strand designs with a variable degree of re-use. Strand re-use is exploited to some extent in both methods [5, 7, 22] but has not previously been demonstrated as a viable alternative for assembling large aperiodic structures.

We have explored two methods to achieve scalable DNA self-assembly. Each method builds larger structures from smaller motifs in a hierarchical manner. Figure 1 illustrates the hierarchical approach we have investigated.

1. Generic linkers

A series of 'generic' sticky-ends along the periphery of a DNA grid aid in binding a grid to an adjacent grid. The generic linkers[4] are designed to bind with only one helix (typical arms have two helices) to introduce a relatively unstable interaction. This is to prevent the generic linkers from dominating the specific interactions we will introduce later to programmably organize two distinct grids. The generic linkers are replicated along the grid-edge to liberate the few otherwise specific sequences and thereby enable stable binding between two grids. It is then possible to apply an incremental graph-colouring method, with a constant number of specific binding sites, to sequentially add motifs to the growing structure [24].

The specific binding of the two distinct grids is achieved by using non-generic sticky-ends at selective locations, in this example at the lower right and upper right corners of the two 4 × 4 grids, respectively (this will become the middle-right edge in the 32-motif structure). Figure 3 shows the relationship between generic and specific arms. The gap along the edge between the two 4 × 4 grids is introduced to disambiguate the identity of each grid in the assembly.

This demonstrates that multiple weak interactions between oligos along the edge of a grid (generic linkers) can be controlled (or dominated) by a single strong specific interaction. Moreover, this is evidence that the free energies among distal nucleotide interactions constructively add between bound motifs. We infer that the gap introduced for identification purposes may disrupt cumulative non-specific binding induced by the generic oligos. Further, the stability (at room temperature) of such assemblies is compromised because of the weakness of the generic linkages and gap. This may contribute to a reduced apparent yield of the 32-motif structure. However, this demonstration illustrates that the use of generic linkers along the edge of a DNA nanostructure can be dominated by a single specific interaction and enables the development of scalable sequential assembly methods.

2. Fully specific linkers

Our second method uses fully specific sticky-ends along the periphery of each sub-grid. The re-use of sticky-end sequences from within each grid reduces the number of unique oligos required to assemble larger arrays. This does not induce ambiguous assembly because the sticky-ends are re-used after the constituent pieces of the grids have already formed. For example, we can re-use sticky-ends from the four tetramers in each grid after the grid has assembled. We conclude that this is only possible because strand exchange between the re-used sticky-ends and the intra-grid sticky-ends does not occur.

We have tested the sticky-end re-use by assembling a 2 × 2 array of grids. Figure 4 illustrates a typical AFM scan of the assembled 64-motif product on cleaved mica. The molecular weight of these structures is 8960 kD and therefore one of the largest synthetic nanostructures ever synthesized. The method used to pattern the 4 × 4 grids from figure 2 is also applicable here.

The use of AFM to determine the 'yield' of assembly is not an accurate method since the mica surface used as a substrate (with the imaging buffer) will preferentially bind large, flat, charged DNA structures. Further, the simple motifs

[4] The left linker in figure 3 is 5′-TAGATGATAGAGTGGTACATCT-3′ and the right is 5′-ATCTAACGGATGAGTAGTGGGCTCAGTCGGAT-3′.

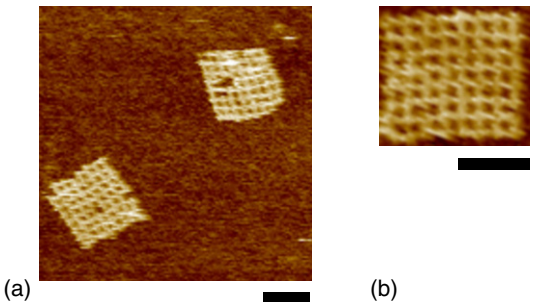

Figure 4. (a) An AFM scan of the 2 × 2 array of grids. (b) The assembled array demonstrates stability even under repeated AFM scans. Scale bars are 100 nm.

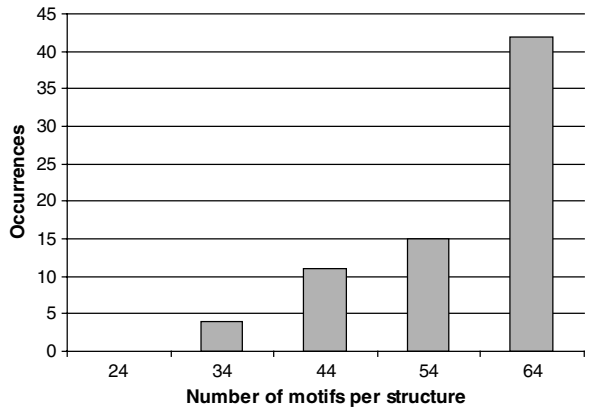

Figure 5. Histogram of identifiable structures as observed by AFM ($N = 73$).

used here, unlike those in [7], are not easily imaged alone by AFM but must be assembled into larger structures to be reliably observed.

Thus, to determine the relative merit of our technique we must evaluate the defect rate of assembled structures since the 'raw' motif yield is unknown. Figures 5 and 6 are histograms of the AFM-observed, surface-bound assembly products. Structures were classified as either *identifiable* (i.e. as a 64-motif grid, $N = 73$) or *unidentifiable* (i.e. fragments, $N = 43$)[5].

The binning used in figures 5 and 6 is 10- and 5-motifs wide, respectively. For example, incomplete structures with 55 motifs will be binned with fully intact structures with 64 motifs in figure 5. Structures were disregarded if any of the following was observed: (i) clipping by the scan window, (ii) piling into agglomerations, or (iii) manipulation away from the surface during a line scan.

The presence of non-ideal, defective structures is not surprising. As we note in [5] the availability of purification methods is a key advantage of DNA self-assembly in the face of defects. The use of solid-support or affinity binding purification may be able to remove defective structures. The *yield scalability* of our technique will depend on the efficiency with which defective material can be removed. However, the *size scalability*, of our technique depends only on sequence and motif re-use. Recent work in error-resilient nanostructure design may be applicable within this framework [25–29].

[5] N is the number of structures in each category.

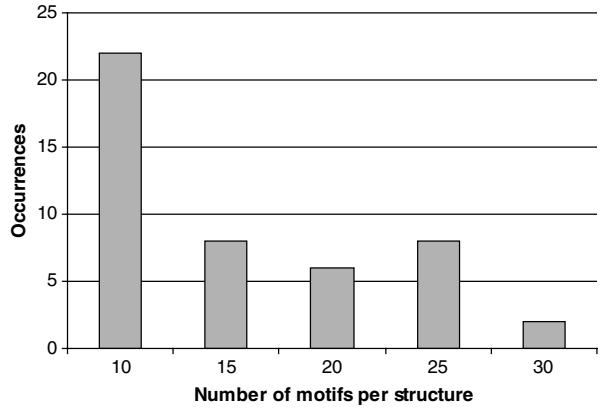

Figure 6. Histogram of unidentifiable structures as observed by AFM ($N = 43$).

3. Experimental procedures

The simple DNA motifs we use have nine ssDNA sequences: one core (150-nt), four shells (∼40-nt), and four arms (∼35-nt). The core and shell sequences were generated with the program SEQUIN [30]. The sticky-end sequences that coordinate the binding of motifs into larger assemblies were generated using our DNA design automation software [6] to minimize the chance of undesired hybridization. Synthetic oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA) and purified by polyacrylamide gel electrophoresis. The list of nucleotide sequences is provided in the supplementary materials available at stacks.iop.org/Nano/18/125305.

The motifs were formed by mixing a stoichiometric quantity of each strand in buffer, 1 × TAE/Mg^{2+} (tris acetate, 40 mM, pH 8.0), EDTA, 2 mM and magnesium acetate, 12.5 mM. The final motif concentration was 1 μM. The equimolar strand mixture was annealed by heating to 95 °C, slowly cooling to 4 °C over 24 h and then incubating at 4 °C for 12 h.

The 4 × 4, 16-motif grid was formed by mixing a stoichiometric quantity of each motif and annealing at a constant 23 °C for 4 h. The sample was then incubated at 4 °C for 12 h. The same annealing procedure was used to create the 64-motif 2 × 2 array by mixing equimolar quantities of the four constituent 16-motif 4 × 4 grids.

Biotinylated core strands and streptavidin (SA; invitrogen) interaction were used to demonstrate full addressability. SA was added to the aqueous solution of the assembled DNA nanostructures. The mix was incubated for 1 h at 27 °C and then 12 h at 4 °C. The concentration of SA:core ratio was 1.2:1 (20% excess SA).

AFM was performed under 1 × TAE/Mg^{2+} buffer in tapping mode. Each sample to be imaged was deposited (3 μl) onto freshly cleaved mica and left for 2 min, then 25 μl of 1 × TAE/Mg^{2+} buffer was added to the mica and another 25 μl was placed on the AFM tip. AFM images were obtained on a digital instruments nanoscope IIIa instrument with a multimode fluid-cell head with NP-S oxide-sharpened silicon nitride tips (Vecco).

4. Conclusion

We have demonstrated the assembly of a 2 × 2 array of DNA grids using sticky-end re-use to decouple the cost of the structure from its area. Our statistical analysis of the assembly method by AFM reveals that it is common to find missing motifs from large structures. This underscores the importance of defect-tolerant designs that can mitigate such defects at a higher level when using this method. This approach is applicable to larger structures and is limited by the ability of such structures to diffuse through solution and assemble with the same degree of selectivity as demonstrated by smaller structures. Thus, the yield of such assemblies will challenge the synthesis of larger structures and demand refinement in the process. The diminishing apparent yield of the method is fundamental to all self-assembly methods that organize molecular scale components from motifs (DNA origami included) and may require purification methods to achieve larger structures. Clearly, there is some size scale at which non-specific interactions begin to dominate the process but promising results in periodic DNA crystal formation suggest that this scale is near-macroscopic [16].

Our method is a low cost synthetic mechanism because of motif and strand re-use but can also leverage complex motifs assembled by other means, such as with the DNA origami method. Our method can achieve the fabrication of larger programmable structures than have been previously demonstrated by tile- or motif-based methods. The ability of DNA self-assembly to leverage existing and on-going investment in biotechnology and pharmaceuticals makes a strong case for the eventual application of DNA nanostructures to the fabrication of future computer systems.

Acknowledgments

We would like to acknowledge support from the NSF (CCR-03-26157), AFRL (FA8750-05-2-0018), Microsoft Research, Agilent Technologies, Intel and IBM.

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