Large branched self-assembled DNA complexes

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Abstract. Many biological molecules have been demonstrated to self-assemble into complex structures and networks by using their very efficient and selective molecular recognition processes. The use of biological molecules as scaffolds for the construction of functional devices by self-assembling nanoscale complexes onto the scaffolds has recently attracted significant attention and many different applications in this field have emerged. In particular DNA, owing to its inherent sophisticated self-organization and molecular recognition properties, has served widely as a scaffold for various nanotechnological self-assembly applications, with metallic and semiconducting nanoparticles, proteins, macromolecular complexes, \textit{inter alia}, being assembled onto designed DNA scaffolds. Such scaffolds may typically contain multiple branch-points and comprise a number of DNA molecules self-assembled into the desired configuration. Previously, several studies have used synthetic methods to produce the constituent DNA of the scaffolds, but this typically constrains the size of the complexes. For applications that require larger self-assembling DNA complexes, several tens of nanometers or more, other techniques need to be employed. In this article, we discuss a generic technique to generate large branched DNA macromolecular complexes.

1. Introduction

Over the last two decades, researchers have synthesized and fabricated a large variety of nanoscale molecular complexes, most with fascinating and even novel physical properties. For example, molecular switches and transistors \cite{1,2}, functional devices from semiconducting and conducting carbon nanotubes \cite{3-5} and nanowires \cite{6,7}, single molecules that emulate conventional electronic circuit elements \cite{8-12}, \textit{inter alia}, have been reported. One of the driving forces for these enormous advances has been the development of readily usable scanning probe tools such as atomic force (AFM) and scanning tunneling microscopy (STM), which enabled the detailed and convenient investigation of the physical properties of nanoscale objects. However, despite the advances in fabrication and characterization of such functional nanoscale molecular objects, technologies to position and interconnect these objects at molecularly accurate addresses, and integrate them into already existing environments, do not exist.

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Almost six decades ago, Brattain, Bardeen and Shockley invented the first solid-state amplifier, the point-contact transistor [13]. Shortly after this invention, the first simple electronic devices, assembled manually to form a small number of individual transistors, were fabricated. But despite these early successes, it was not possible to fabricate complicated devices that required large numbers of transistors and other electronic building blocks, since powerful interconnection technologies were not available. It took more than ten years before integrated circuit technology was developed, which, arguably, was the key element that led to the revolutionary explosion in high-speed switching, logic, and memory circuitry that has had such an impact on the modern world. Nanotechnology has probably reached a similar crossroads; individual functional elements are available and well characterized, but integration technologies are still lacking.

Over the last decade, self-assembly concepts have received considerable attention as promising candidates for such integration technologies on the molecular scale. In order for molecular self-assembly concepts to be realized, scaffolds are required onto which the functional elements can be assembled. In this article, we will describe a method for fabricating large branched DNA complexes which are ideally suited to serve as scaffolds for the interconnection of nanoscale objects and their integration into appropriate environments.

Several groups have investigated the fabrication and use of DNA scaffolds in self-assembly applications. For example, Liu et al. fabricated ordered protein arrays on DNA scaffolds by using DNA aptamers to bind proteins onto specific locations on the scaffold [14]; metallic nanowires were constructed on DNA scaffolds by specific deposition of silver [15], gold [16,17], platinum [18], palladium [19], inter alia, onto the scaffold. In many of the published examples, the researchers used linear DNA molecules as scaffolds, i.e. DNA without branch-points. However, branch-points are essential for scaffolds to offer a high degree of flexibility.

In nature, DNA normally exists in linear form, but under certain circumstances, branching can occur. For example, Holliday junctions can form, where two linear DNA stands are joined together in a cross-like arrangement [20-22]. More than two decades ago, Seeman and co-workers [23] started to fabricate artificial DNA junctions by exploiting the inherent self-assembly properties of DNA using short, synthetic, single-stranded DNA oligonucleotides as building-blocks. Since then, several groups have fabricated three- [24,25] and four-arm [26] DNA junctions, and rigid DNA triangles [27]. Two-dimensional DNA lattices [28,29] and DNA cubes [30] were assembled from such small branched DNA junctions. The constituent DNA for all these complexes was derived through a synthetic route, which limits the lengths of the constituent molecules to around 30 nm and therefore also limits the size of the final construct. In many applications, for example where it is desired to integrate the scaffold into an existing device that has been fabricated using standard state-of-the-art nanofabrication tools such as electron-beam lithography, larger scaffolds can be advantageous.

Larger branched DNA junctions have previously been fabricated, for example by extending the arms of small DNA junctions through ligation of long fragments of DNA molecules onto the individual arms [31], by using large dendrimer-like structures [32], or by using proteins to promote the formation of DNA junctions [33]. Although these approaches may ultimately result in large branched DNA structures, they cannot be used in applications where large branched complexes comprising only DNA are required. In order to address this problem, we developed a method to fabricate branched DNA structures up to several hundreds of nanometers in size [34]. The method is based on an enzymatic approach to generate the constituent DNA molecules, and is inspired by the assembly methods generally used to generate small DNA junctions, where appropriate single-stranded DNA molecules are self-assembled into the desired geometry by exploiting the DNA’s inherent lock-and-key properties. The use of an enzymatic method to generate the single stranded DNA allows the fabrication of much larger structures than the ~30 nm that would be possible if synthetic constituent DNA was used.
2. Fabrication of branched DNA complexes

Figure 1 shows the schematic illustrations of the two different designs discussed here: a three-arm junction with a single branch point, and a four-arm junction with two branch-points. Each double-stranded arm is assembled from two different, reverse complement (RC) fragments of two different components. For example, arm A’s (blue) upper strand (labeled a-RC in figure 1(a)) is part of component β, and its lower stand (labeled a) is part of component α. The lower panels of figure 1 show the individual components (α, β, γ and α, β, γ, δ), and we note that in the case of the three-arm construct, each component consists of two different fragments, an upstream region (for example a in component α), and a downstream region (c-RC in component α). The downstream region is the reverse complement to a forward region of another component, such that in the case of the three-arm construct, α binds to β and γ; β binds to γ and α; and γ binds to α and β. The individual components are designed such that the desired multi-arm complex is the only conformation that can form upon self-assembly.

Figure 1. Design schematic of a simple three- (a) and four-way (b) DNA complex. The complexes are assembled from individual single-stranded components (α, β, γ and α, β, γ, δ, respectively). These single stranded individual components are designed such that each arm of the final complex possesses a single-stranded overhang of 20 bases, which can be used to integrate this DNA complex into existing environments (adapted from Ref. 34).

The procedure for generating the single stranded components using enzymatic processes to avoid the length-limitations imposed by the synthetic route is illustrated in figure 2. The individual fragments for each component (α, β, γ, and α, β, γ, δ for the three-arm and the four-arm construct, respectively) were generated by PCR using λ bacteriophage DNA as a template. The double-stranded fragments were then cut with the appropriate restriction enzymes to produce single-stranded overhangs, which were then used to ligate the individual fragments together (figure 2(b)). The ligated products were cloned into pGEM-T easy (Promega) and selected plasmids were sequenced to ensure that the templates for the generation of the constituent DNA were correct. The plasmids were used as templates to amplify the individual components by PCR with the forward primer modified with five phosphorothioates at its 5’ end (figure 2(d)). The resulting PCR products contain the five phosphorothioate modifications on the forward strand, which protect the modified strands from being digested by T7 exonuclease [35]. In order to generate the single stranded components, the partially protected double-stranded PCR products were digested with T7 gene 6 exonuclease (figure 2(e)) and
the phosphorothioate-modified ends were removed by using a short bridging oligonucleotide to make the single-stranded DNA locally double-stranded and subsequent cutting of the end with the appropriate restriction enzyme (figure 2(f)).

![Figure 2.](image)

**Figure 2.** Schematic illustration of the method used to generate the single-stranded component $\alpha$. (a) Each fragment (a and c-RC) of the component $\alpha$ is generated by PCR. (b) The two fragments (a and c-RC) are ligated together, and (c) cloned into a pGEM-T easy. (d) The plasmids were used as templates to generate the component $\alpha$ by PCR. The forward primers contain five phosphorothioate modifications at the 5' end. (e) The single stranded component is generated by digesting the unprotected strand of the double-stranded PCR product with T7 gene 6 exonuclease, and (f) the phosphorothioate-modified end is removed via restriction digestion (adapted from Ref. 34).

The final complexes were formed by mixing the individual single-stranded DNA components at equimolar quantities in 1X hybridization buffer consisting of 5 mM HEPES pH 7, 2 mM MgCl$_2$, and 0.5 mM EDTA [36], heating to 95°C for 10 minutes, and slowly cooling to room temperature. The products were assessed with an AFM and typical images are shown in figure 3. For the imaging, the DNA complexes were deposited onto freshly cleaved mica and imaged using tapping mode in air.

![Figure 3.](image)

**Figure 3.** AFM images of branched DNA structures. (a) A three-arm, single branch-point structure and (b) a four-arm, double branch-point structure assembled according to the design in figure 1 (adapted from Ref. 34).

3. **Conclusions**

In conclusion, we have developed a technique to generate large branched DNA complexes. The technique is based on enzymatic processes to generate the constituent DNA, rather than synthetic methods, thus allowing much larger complexes to be fabricated.
Acknowledgement
This work was in part funded by the EPSRC.

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