Preparation of branched structures with long DNA duplex arms†‡

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Branched structures with long DNA duplex arms have been constructed through biotin–streptavidin binding and characterized by gel electrophoresis and atomic force microscopy (AFM) imaging.

This paper reports the construction of branched DNA–streptavidin (DNA–STV) structures through biotin–streptavidin interaction. All branches are synthesized by polymerase chain reaction (PCR), ~700 nm long and one of the primers is biotinylated. The branched complexes with different numbers of branches have been separated by electrophoresis and characterized by atomic force microscopy (AFM). The resulting branched DNA–STV complexes could be potentially metallized into branched metallic nanowires and used in multi-terminal nanoelectronic devices.

DNA is an excellent building block for nanomaterials.[1] A variety of self-assembled nanostructures[2,3] and simple nanomechanical devices[4] have been developed from DNA. In addition, DNA can organize nanoparticles[5] and biomacromolecules,[6] and template nanofabrications.[7] For instance, linear DNA molecules can be metallized into conductive nanowires.[7b,c,g] Sequence-specific metallization[7c,g] allowed the construction of a field effect transistor. However, more complex DNA templates are desired in order to develop nanoelectronics. Woolley and coworkers recently reported using three-armed DNA nanostructures as templates to fabricate metal junctions from chemically synthesized DNA single strands.[7f] The arms in these junctions were around 20 nm in length (~60 bp). It would be quite challenging to connect such nano-junctions into electronic circuits.

In order to obtain long-armed DNA junction structures, three methods have been explored: (1) using protein RecA to generate junctions between two double-stranded DNA molecules with homologous regions;[7c,g] (2) to elongate small DNA junctions by ligating short arms with long dsDNA fragments;[8a,b] and (3) using multiple long single DNA strands to directly form large branched structures.[8c,d] However, the production yields were low or required long experimental processes. Herein, we have constructed DNA–STV complexes with long dsDNA branches through biotin–STV interaction.

Our strategy consists of two steps (Fig. 1): (i) We use PCR to prepare long double-stranded DNA (dsDNA) in which one primer is 5′-biotinylated; (ii) the obtained biotinylated long dsDNA molecules are then complexed with STV, forming DNA–STV complexes with different numbers of branches, which can be separated by agarose gel electrophoresis. Niemeyer and his coworkers have used a similar strategy on short DNA molecules to create DNA–STV complexes.[9] Biotin–STV binding is one of the strongest, noncovalent interactions. Because of its superior strength and specificity, this interaction has become one of the most widely used affinity pairs in biotechnology. STV is a tetrameric protein with one biotin-binding site per subunit. Theoretically, it can bind up to four biotinylated DNA molecules to form four-branched DNA–STV complexes. In reality, DNA–STV complexes with different numbers (1–4) of DNA branches would be expected.

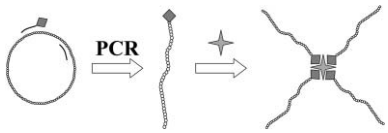

Fig. 1 Formation of branched DNA junctions. Rhombuses and stars represent biotins and streptavidins (STVs), respectively.

PCR is a commonly used technique in molecular biology for the preparation of DNA samples. Compared to chemical synthesis, PCR is more suitable for synthesizing long DNA fragments, which can contain thousands of base pairs. In this work, two 20-base-long primers (one with a biotin at the 5′ end) and a DNA template of pUC19 plasmid were used to prepare 2000 base pairs-long (2 kb) biotinylated dsDNA. Electrophoretic analysis showed that the PCR products were mainly dsDNA with a length of about 2 kb, as we expected (see Fig. 1S in the ESI‡).

After purification by agarose gel electrophoresis, the 2 kb biotinylated dsDNA was mixed with STV at a molar ratio of 5 : 1 and incubated for 15 h. Excess dsDNA was used in order to promote each STV to bind more biotinylated dsDNA. The final DNA–STV complexes were isolated by gel electrophoresis (Fig. 2). It was clear that DNA–STV complexes with 2, 3, or 4 branches were well separated and each appeared as a sharp band.

The purified DNA–STV complexes were analyzed by atomic force microscopy (AFM) in air after being deposited on mica surfaces. Fig. 3 shows the images of the conjugates with two, three, and four dsDNA branches. Each branch is about 680 nm, which is consistent with the calculated length of the 2 kb-long-dsDNA branch. The branches are 0.4 nm high and the junction point is much higher (around 2 nm, consistent with the height of streptavidin, see Fig. 2S in the ESI‡), which confirmed that the conjugates were formed in the way we designed. Note that the heights of dsDNA and STV are lower than those when they are under their native conditions. However, it is well documented that biomacromolecules decrease their height substantially when dehydration occurs.[2]

In conclusion, we have prepared branched DNA structures through biotin–STV interaction. The method reported here should be easily adapted to prepare DNA junctions with any arbitrarily designed length by PCR. We also speculate that the specifically

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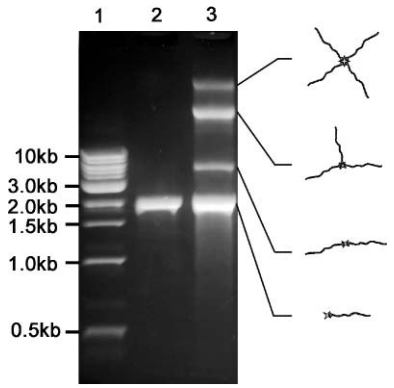

Fig. 2 Eletrophoretic isolation of DNA–streptavidin complexes with different numbers of dsDNA branches. Lane 1: 1 kb DNA ladder (size markers); lane 2: biotinylated dsDNA (2000 base pairs, 2 kb) from PCR; lane 3: a mixture of dsDNA–streptavidin complexes. The structures of dsDNA–streptavidin complexes are shown on the right. Note that because the dsDNA is much larger than streptavidin in physical size, agarose gel is unable to differentiate the dsDNA from the streptavidin conjugated with one dsDNA branch under the current conditions.

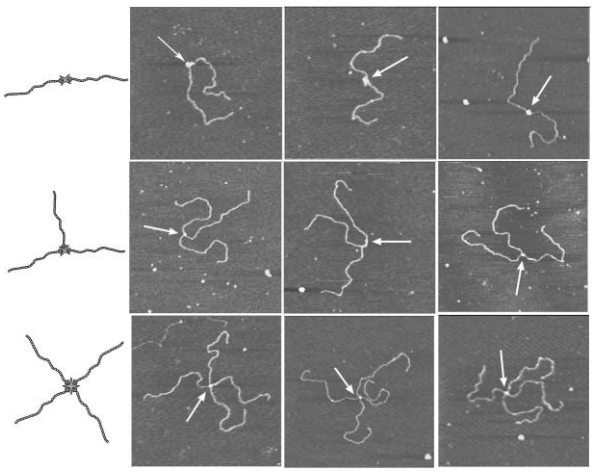

Fig. 3 Representative atomic force microscopy (AFM) images of the DNA–STV complexes with 2, 3, and 4 dsDNA branches. All images have the same scanning size (1 × 1 μm) and height scale (3 nm). The left side shows the models of these complexes. STV molecules appear as bright spots and are indicated by arrows.

localized STV could provide us with a potential opportunity for further modification of this junction structure.[7f] Furthermore, functional groups could be introduced into dsDNA during PCR. These would increase the complexity and versatility of the designated nanostructures. We realize that the DNA duplexes are quite flexible. This problem has to be solved for many potential applications.

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