Time to tweak tremendously tiny trisoligonucleotide-tetrahedrons

DNA is most commonly known as the carrier of genetic information in all living beings on earth. For a chemist DNA is nothing more than a large molecule that can be artificially recreated in the lab on a smaller scale using special equipment and techniques. It is an interesting material because DNA strands contain a unique informational pattern. This means that in a pool of hundreds of DNA strands one strand will interact primarily with only one other strand in the entire mix and form a double helix. Due to this specificity artificial DNA building blocks can be prepared with the ability to fold into unique nanometer sized motifs, like a smiley, a star, a cube or a tetrahedron. The latter two examples are of considerable interest in the field of DNA nanotechnology because they show a potential use as nanoscale containers. This containers can, for instance, transport medicine through the body and selectively release it at the desired location, which minimizes negative side effects.

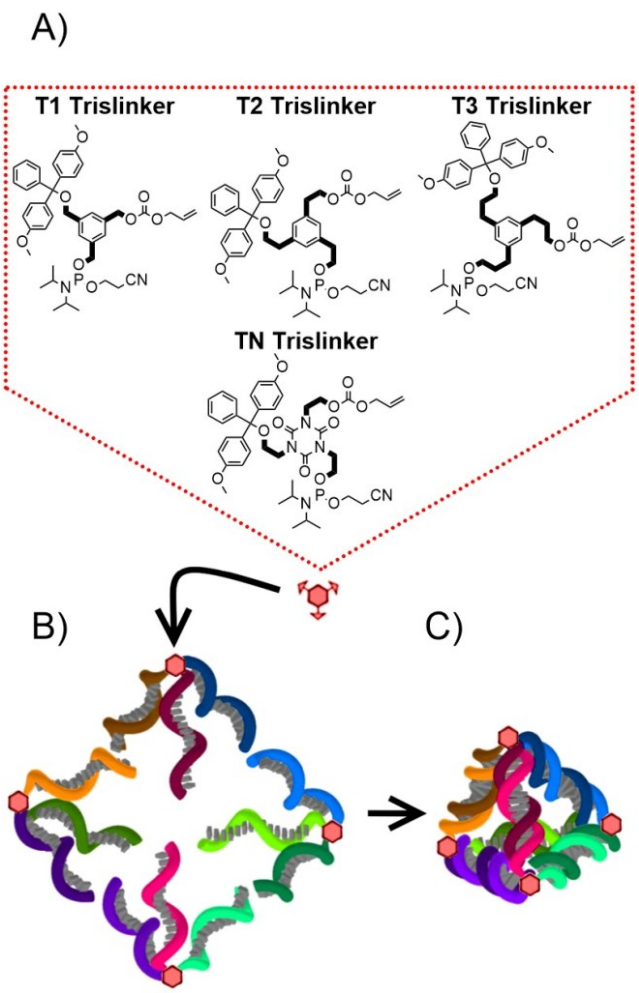

Fig. 1. Assembly of trisoligonucleotides (B) into tetrahedral scaffolds (C) and investigated linker

designs (A) of varying flexibility at the alkylene chains (A; bold lines).

One method to prepare DNA building blocks is based on so-called trisoligonucleotides. These building blocks contain three DNA arms attached onto a linker core. A set of four trisoligonucleotides can then assemble into a cage in the form of a tetrahedron (Figure 1; B and C). The linker needs to be flexible enough to allow folding of the trisoligonucleotides into the tetrahedral scaffold. If the linker is too rigid, the cage cannot close and is therefore not suitable as a potential cargo carrier. Flexibility is enabled by hydrocarbon chains located between the linker and the DNA arms (Fig 1, A: Bold segments).

The minimal required bendability of these chains necessary to allow stable folding into a closed tetrahedron was studied in this work, which shares an insight of the lower limits in nanoconstruction in general. Three chains with varying lengths were compared: Methylene (T1), ethylene (T2) and propylene (T3). So far studies on the flexibility of methylene- and propylene-chains exist, but not for ethylene. In a systematic study three comparable sets of four trisoligonucleotides were prepared with the only difference lying in the hydrocarbon chain length. Preparation involved synthesis of the trislinkers (Fig. 1, A), automated synthesis of the trisoligonucleotides, purification via preparative polyacrylamide gel electrophoresis and mass analysis via MALDI-TOF. All four building blocks of each set were then mixed together and assembled using a temperature protocol.

Assembly products were then digested with the enzyme mung bean nuclease and then studied with native agarose gel electrophoresis. This enzyme preferably digest DNA single-strands over double helices. A fully closed tetrahedron contains six double helices, whereas a partially open also contains single-strands. The assembly experiment with methylene-chains (T1) resulted as expected in degradation, whereas ethylene (T2) and propylene (T3) remained intact. In other words, linkers with at least ethylene-chains show sufficient flexibility, whereas methylene-chained linkers are too rigid to fold into tetrahedrons. However, trisoligonucleotides based on the T1 trislinker were still capable to form tetrahedral scaffolds in intermixing experiments with linkers of higher flexibility.

Because this study revealed ethylene chains as sufficiently flexible, an entirely new generation of trislinkers was established (TN) using the commercially available 1,3,5-tris(2-hydroxyethyl)-N,N’,N’’-isocyanurate (THEIC) being of similar geometry as the T2 trislinker core. The linker can be prepared faster and at a reduced cost compared to the previous linkers, which alleviates a bottle-neck in the preparation of trisoligonucleotides in bulk. Flexibility and stability of the TN trislinker in trisoligonucleotides was tested similarly to the T1-T3 sets and also led to stable tetrahedrons.

This study revealed a lower limit in DNA nanoconstruction and with THEIC a new generation of cheaper and versatile trislinkers is introduced ready for future use in trisoligonucleotide-based

nanostructures.

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