In vivo DNA nanostructure synthesis for disease screening

**Abstract**

DNA has emerged as a promising material for the creation of novel functional nanostructures. Here we present DNA nanostructures capable of simultaneous detection of multiple microRNA (miRNA) targets which are identified as promising disease biomarkers. Logic gates can be easily incorporated into our designs to test various combinations of miRNA targets. G-quadruplexes form when the specified target hybridizes with the probe, generating fluorescence in the presence of substrate. We endeavor to demonstrate intracellular synthesis, self-assembly and functioning of our nanostructures inside E. coli. Our constructs open up new possibilities in future research on DNA nanotechnologies as diagnostic tools, and promote the applications of miRNA testing in clinical conditions.

**Design rationale**

**miRNA**

miRNAs are important biomarkers in our body. For example, miR-34b have elevated serum levels before onset of symptoms for Huntington’s disease, miR-15b-5p, miR-338-5p and miR-764 are also significantly over-expressed in plasma of patients with hepatocellular cancer.

**Tetrahedron**

Tetrahedrons have various advantages over other nanostructures. The following lists some of them.

- Reduces non-specific binding
- Smaller, simpler than DNA origami
- Efficiently endocytosed
- Reduced non-specific binding
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- Reduced non-specific binding
- Reduced non-specific binding

**Our design**

We generated sequences of 5 oligonucleotides (O1 to O5 in the diagram above) that can be self-assembled into a tetrahedron using Tiamat, a software for DNA sequence generation. Base pairing between the oligonucleotides are shown in the above diagram.

**Results and discussion**

We assembled the tetrahedron by mixing equal molar of oligonucleotides 1-5 (O1 to O5).

The DNA/RNA input strand, which is of 30nt and is based on the miRNA biomarker of Huntington disease miR34b) was put in equal molar with the tetrahedron for strand displacement and the formation of Gq. The product from the displacement yields a double stranded 50-nt input dimer of 30bp. After considerable number of runs with DNA, RNA was used as the input, otherwise constant.

To test the sensitivity of our probe, an limitation of detection analysis was conducted by varying the concentration of DNA & RNA input from 0nM to 100nM. LOD was calculated from the standard error of the regression line y=0.0009x+0.1298; R²=0.9739. LOD=17.2nM.

**Strand displacement**

Two strands with partly complementary sequences hybridize to each other at an unhybridized region called toehold, displacing one or more prehybridized strands.

**Split G-quadruplex**

G-quadruplex (Gq) are tertiary structures formed by sequences rich in guanine. When Gq forms a complex with hemin, it exhibits peroxidase activity and functions like a DNAzyme. Its catalytic activity is utilized in many DNA nanostructures where a colour change is produced by target-induced conformational change.

The ABTS assay is based on this system.

**The big picture**

Putting strand displacement, tetrahedron and Gq principles together yields the final product. A target miRNA binds to the toehold of the tetrahedron, displacing the prehybridized strand, forming Gq with peroxidase activity.

**Future Work**

We will be using transmission electron microscopy to visualize the tetrahedron for a complete verification of formation of the correct tetrahedral shape.

Optimization is another aspect that is hindering the efficiency of the production of a specific and sensitive device. A software could be made based on previous findings to aid in optimization.

We are also planning to incorporate the concept of logic gates into the design to increase specificity and to expand the design’s scope of diagnosing.

Of course, the final step we hope to achieve is to get the functional 3D nanostructure synthesised in-vivo and apply to the diagnostic world.