

Based on the Microbial Technology's Three-dimensional Structure of the DNA Self-assembly

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Microbial technology has become the focus of cutting-edge scientific research and hotspots. Using this technology, molecular beacons and DNA self-assembly as DNA computing model can be in a depth study. Around these two technologies to discuss, on the one hand, a summary of recent molecular letter standard technology and DNA self-assembly technology development; on the other hand, the molecular beacon technology advantages into DNA self-assembly model and proposed a tetrahedron of DNA, and use the structure to solve the practical problems.

Key words: Microbial technology; Molecular beacons;
DNA self-assembly model; DNA tetrahedron.

In 1996, Tyagi and Krammer firstly establish a molecular beacon technology¹, after that this technology widely used in medicine, biology, molecular biology and many other areas. Molecular beacon technology has high specificity, high sensitivity, simple operation, especially during the real-time quantitative detection, clinical diagnosis, genetic testing has strong advantages. At present, the research is of great significance for the molecular beacons: In 2003, Zhi-xiang Yin solved the satisfiability problem using molecular beacon structure of DNA computing model². In 2008, Wen-bin Liu and Zhi-xiang Yin et al studied computing model based on molecular beacons logic gates³. After that many people used the molecular beacons into DNA computing⁴⁻⁵.

The principle of molecular beacons technology

The molecular beacon is fluorescently labeled oligonucleotides, generally it has 25-35 nucleotides. In terms of structure, it is composed of three parts: a ring-shaped area, stem area, the fluorophore and quencher groups. The ring-shaped area is generally 15-30 nucleotides, and it can specifically bind to the target molecule. In the stem area, there are 5-8 base pairs, reversible dissociation may occur in the molecular beacon and the target molecule binding process. The fluorophore is connected to the end of 5', and the quencher moiety generally attached to the end of 3'. In a free state, the molecular beacon was hairpin structure, when the fluorophore and quencher are in close proximity, the energy of the fluorescence resonance transfer to the group, The group issued fluorescence quenching group absorbed and distributed in the form of heat, the fluorescence was almost completely quenched, and when the molecular beacons in conjunction with the target molecules, the distance of the fluorophore and the quencher is larger, the fluorescence is restored (Fig. 1).

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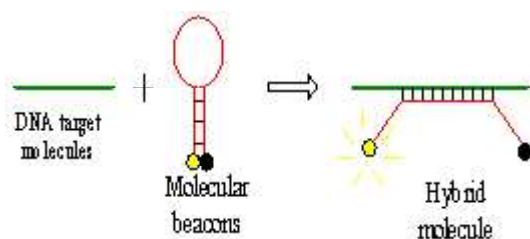


Fig. 1. The principle of molecular beacons technology

Based on current research, how to find a better method to maximize mining the molecular beacons expression and the ability to process information, and how to find the relationship between the length of the molecular beacon ring identification zone and fluorescent colors mild or with the length of the stem, in order to find the information capacity is greater, the sensitivity is higher, the structure is more complex, more generic model of the molecular beacon is the focus of the next research.

DNA self-assembly

With the development of the DNA computing, the DNA self-assembly technique has also been developing. The reason is that it is highly likely to become the next generation of Turing machine. Seeman first proposed the use of DNA molecules to start the self-assembly tile structure⁶, many people began to join the research. Mao et al gave a way of the the cumulative XOR operation⁷; Seeman used the DNA self-assembly to solve the Boolean logic⁸; Rothmund and others to achieve one- dimensional cellular automata⁹, then he encoded DNA to construct a variety of nanostructures¹⁰; Brun proposed two - dimensional self-assembly method for solving satisfiability problem, non-deterministic algorithm, the problem of the subset¹¹⁻¹³. These studies mainly the structure of one-dimensional and two- dimensional self-assembly model are still in its infancy¹⁴⁻¹⁵.

In fact, there is no difference between the self-assembly of the two-dimensional and the three-dimensional structure in the concept. The principle of the two-dimensional structure can also be applied to the three-dimensional structure. Seeman, for example, the synthesis of the cube and to the corner octahedral nanostructures¹⁶; Turberfiel and his friends constructed the DNA tetrahedron and dodecahedron¹⁷. Despite these examples of research are still relatively time-

consuming, inefficient, but they proved that the DNA can be used to construct a three-dimensional structure as a scaffold.

3D three-dimensional structure of DNA self-assembly

Molecular beacon technology is simple and convenient, real-time monitoring and high sensitivity. Along with the continuous development, many researchers are also considering this technology to be combined with other related models. For example, it can be replaced with chain technologies, and then the DNA logic gate can be constructed¹⁸; it combines with the DNA allosteric, the controllable fluorescent nano-devices can be constructed by DNA molecules of different forms, by detecting the fluorescence intensity, the changes in the DNA molecule can be known¹⁹; it can be combined with some digestion technology to detect, and sometimes it is more sensitive than PCR²⁰. The combination of these technologies enables to play an important role with the advantages of both, they will be able to achieve exciting results.

From a structural point of view of self-assembly, the three-dimensional structure compared to the structure of one-dimensional linear and two-dimensional planar structure in the development of nanotechnology, drug delivery, DNA computing has a huge advantage. In 2008, Weizmann combined DNA self-assembly and fluorescence techniques, used of DNA molecules complementary base pairing, hybrid and ligase, and then got the ring-chain structure²¹. In 2009, Andersen et al applied DNA fluorescence to detect a switch of nano-devices²². These attempts of the three- dimensional structure for the development of DNA self- assembly of the three- dimensional structure are of great significance.

On the basis of these studies, we tried to explore a more stable, higher yield of DNA self-assembled three-dimensional structure. In 2005, Goodman proposed on a nano DNA structure. Since the three-dimensional structure of the DNA tetrahedron is a natural "building blocks"²³, and they can quickly synthesize a single stereoisomer, high yield, transmission stability, and so on. A DNA tetrahedron (Figure 2) was designed. Firstly, three short DNA chain and a molecular beacon can be assembled, six pairs of complementary domain of hybrid into six sides (different colors to distinguish

in Figure 2). Each chain consists of three areas and runs for a week. In the skeleton of the DNA, there is a gap at the end of the 5' and 3'. Given the appropriate conditions, molecular beacons will find its target molecules; eventually a hairpin structure is formed (shown in Figure 2(b) and (c)).

In this structure, the five edges are 20 bases complementary to a paired double helix structure, and its length is 6.8 nm, the sixth section length is 3.4 nm, which contains 10 bases and forms a ring with the formation of a hairpin loop of the 4 nucleotides and 12 nucleotides after the molecular beacon binding domain.

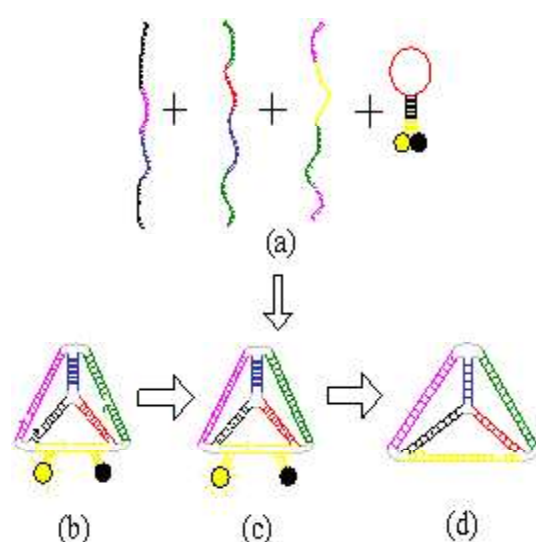


Fig. 2. DNA self-assembled three-dimensional structures

The process of assembling the tetrahedron is placed in the salt buffer, heated to 95°C, and then cooled to room temperature, pay special attention to where the annealing temperature to ensure that the hybridization does occur in the molecular beacon and DNA chains. The process of forming a hairpin structure of other three chains are phosphorylated acidified, and then the T4 DNA ligase is added, after processing, the self-assembly reaction occurs. At first, molecular beacons react with the other three chains, and then forming a hairpin structure. This hairpin structure can be reconstructed. Containing 5 base-paired stem of the hairpin structure of the chain and molecular beacons will be the formation of a gap in the closed state when the hairpin is formed. And then forming a hairpin loop, the energy of this

process is released by 12 nucleic acid hybridization. Figure 2(d) shows the two notches 30 of two consecutive double-stranded complementary base pairing. The nucleic acid molecular beacon as the release of energy is no hybridization complexes formed with the tetrahedron. Figure 2 (b), (c) and (d) shows the hairpin model of the tetrahedral opened and closed. Of course, you can also use polyacrylamide gel electrophoresis (PAGE) to observe and examine its reconfigurability, so that the hairpin loop is identified to form correctly.

The application of the three-dimensional structure

This three-dimensional structure not only has the picture structure information, but also it can be applied to nano-technology, pharmaceutical carrier and DNA computing, etc. With the change of the structure, it can be developed a lot of new application. In the DNA computing, the research of three-dimensional structure application is relatively small. But we can see from its unique advantage, it is suitable for solving NP-hard problems. Because of its complicated three-dimensional structure can shorten the time and space complexity of the self-assembly, enzyme usage, thus it can improve the success rate of experiments and precision. Apply it to logical operations²⁴⁻²⁶, and integrating it into DNA chips, the use of its efficient parallel computing ability, promote the development of DNA computer. In Boolean logic operation, for example, the simple introduces the application of the three-dimensional structure.

Boolean logic operation gets its name from the British mathematician George Boole, are widely used in electronics, computer software and hardware. A cloth in a computer's arithmetic logic defines some Boolean logic functions, or operators. Each function can be based on one or more input, use logic algorithm to get the output value (true value is 1, false value 0). So logic operation is of great importance. Discussed at the molecular dimensions using the three-dimensional structure to solve the problem of simple Boolean logical expression, for general, do not break conjunctive normal form:

$$W = (X_1 \vee X_2 \vee \overline{X_3}) \wedge (X_1 \vee X_3 \vee \overline{X_4}) \wedge (X_2 \vee \overline{X_3} \vee X_4) \wedge (\overline{X_1} \vee \overline{X_2} \vee \overline{X_4})$$

$\overline{X_i}$ is non- X_i , each argument values for $\{0, 1\}$.

" \vee " is the logical OR operation, therefore, $X_i \vee X_j = 0$ if and only if $X_i = X_j = 0$; " \wedge " is the logical

AND computation: $X_i \wedge X_j = 1$ if and only if $X_i = X_j = 1$.

if $X_i = X_j = 1$. Any group assignment is given, the value of W of the Boolean logic operation can be solved. Concrete ideas (Figure 3) as follows: At first, the value of each clause is calculated. Then the DNA self-assembled and bottom-up features are used in calculating the value of the second sentence respectively. Finally the whole logic operation the value of W is calculated. Application of the three-dimensional structure, we first construct the first layer of DNA strands clause operations. Because the 3D structure itself has the characteristics of calculating the multiple variables at the same time, so, in this case, the results will be got in three steps. Keep the strands of DNA in the saline buffer, heated to 95°C , then cool to room temperature, at this time, the molecular beacon will be added, and phosphoric acid acidification. Concentration and temperature needs to be concerned in the process, to ensure the formation of hairpin structure. Next, the solution is added with T4 DNA ligase. Then we can be detected in different three regions of fluorescence, and an area with no a hairpin structure. Again such processing, again for testing, there is not a hairpin structure. Above all, you can get the final value of W is 0. Application of this structure, it can simplify the structure of two-dimensional in operation for many times, on the other hand, because of reconstruct and repeated, it has a certain practical value. But in actual operation, the control of temperature, solution concentration and the enzyme usage and how to prevent the mismatch problem are needed to the focus of further research.

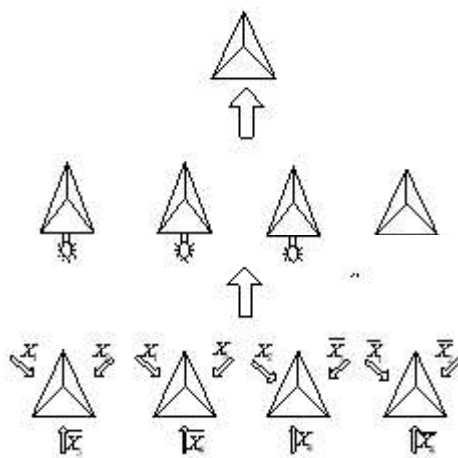


Fig. 3. The process of DNA self-assembly and bottom-up

CONCLUSIONS

In this paper, based on existing molecular beacon and DNA self-assembly model research, mainly studies the three-dimensional structure of a DNA self-assembly-DNA tetrahedron with molecular beacon. It can complete self assembly, and the experimental operation is simple. In addition, this model as well as related fields such as computer science, cryptography, nano intelligence provides a new train of thought and combining site. With in-depth study of 3D model of DNA self-assembly, DNA computing in practical application of the future will play a big role.

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