Review Article www.ijcps.com

International Journal of Chemical and Pharmaceutical Sciences
2013, Sep., Vol. 4 (3)

ISSN: 0976-9390
IJCPS

DNA Nanorobots: The complete healthcare package

Parva Jani*, Gunjan Patel, Priyanka Sharma, Rinkal Patel, Hitesh Jain and Yunus Pasha.

Sigma institute of pharmacy, Baroda, Gujarat, India.

***Corresponding Author:** E-Mail: prvjani@gmail.com.

ABSTRACT

Nanotechnology is an emerging technology with enormous potential in information and communication technology, biology and biotechnology, medicine and medical technology. Novel nano- and bio-materials, and nanodevices are fabricated and controlled by nanotechnology tools and techniques, which investigate and tune the properties, responses and functions of living and non-living matter, at sizes below 100 nm. The exploitation of DNA for the production of nanoscale machines or robots is possible which are known as DNA nanorobots. These nanorobots find their applications in medicine as smart drug delivery systems. They can be also used as biosensors.

Keywords: DNA, Nano robots, Origami, Apoptosis.

1. INTRODUCTION

1.1. DNA: The molecule of life

Deoxyribonucleic acid is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses as shown in figure 1. Along with RNA and proteins, DNA is one of the three major macromolecules essential for all known forms of life. Genetic information is encoded as a sequence of nucleotides (guanine, adenine, thymine, and cytosine) recorded using the letters G, A, T, and C. Most DNA molecules are double-stranded helices, consisting of two long polymers of simple units called nucleotides, molecules with backbones made of alternating sugars (deoxyribose) and phosphate groups (related to phosphoric acid), with the nucleobases (G, A, T, C) attached to the sugars.

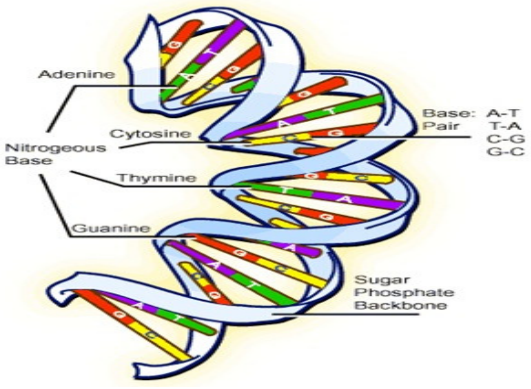

Figure - 1: structure of DNA.

DNA is well-suited for biological information storage, since the DNA backbone is resistant to cleavage and the double-stranded structure provides the molecule with a built-in duplicate of the encoded information.

These two strands run in opposite directions to each other and are therefore anti-parallel, one backbone being 3′ (three prime) and the other 5′ (five prime). This refers to the direction the 3rd and 5th carbon on the sugar molecule is facing. Attached to each sugar is one of four types of molecules called nucleobases (informally, *bases*). It is the sequence of these four nucleobases along the backbone that encodes information. This information is read using the genetic code, which specifies the sequence of the amino acids within proteins.

Within cells, DNA is organized into long structures called chromosomes. During cell division these chromosomes are duplicated in the process of DNA replication, providing each cell its own complete set of chromosomes. Eukaryotic organisms (animals, plants, fungi, and protists) store most of their DNA inside the cell nucleus and some of their DNA in organelles, like mitochondria or chloroplasts. In contrast, prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm. Within the chromosomes, chromatin proteins such as histones compact and organize DNA. These compact structures guide the interactions

between DNA and other proteins, helping control which parts of the DNA are transcribed.[1]

DNA is an excellent material for the construction of nanomachines because it is very biocompatible, it can be easily adjusted and programmed, and it has the ability to self-assemble in to a multitude of 2D and 3D structures. This rational self-assembly relies on the attractive forces between the Watson-Crick base-pairs that are characteristic of DNA. An adenine (A) on one strand will instinctively pair with a thymine (T) on the other strand; likewise, guanine (G) will pair with cytosine (C).

2. DNA-NANOROBOT

- It is a type of nano robotwhich is used to deliver the drug to the targeted cell only, not to the adjacent cell so no side effects damage to the healthy cells occurs.
- The aim of DNA nanorobotics is the design and fabrication of dynamic DNA nanostructures that perform specific tasks via a series of state changes.
- State changes can be done from the hybridization/denaturing of a single base to hybridization/denaturing of entire strands.
- These state changes can be effected autonomously, in which case the system switches state without external intervention while in other cases precise amounts of specific species, such as DNA strands or enzymes, are introduced to enforce state changes.
- It should be noted that different copies of the nanostructures might be in different states at the same time and we are generally interested in the overall average behavior system. The two halves of the hexagon are latched together to form the vessel where the payload (i.e. the drugs) will be placed.[2]

2.1. Components of DNA nanorobot

The components of DNA nanorobots[3] are shown in table 1.

2.1.1. Aptamers

- Aptamers are artificial nucleic acid strands that only bond to specific molecules as shown in figure 2. (includes proteins, drugs, amino acids, etc.).
- Aptamers is use to latch the two halves together on one end, & a DNA hinge on the other end. By using aptamers as latches, it have essentially created a lock for the vessel which will only unlock, and release the payload, when it comes into contact with the "keys"; in their case the "keys" were specific antigens.
- The aptamer locks can have even better identification abilities when used in combination having several different aptamer locks on the latch.
- Aptamers help in identification of antigens located on the cancerous cells.

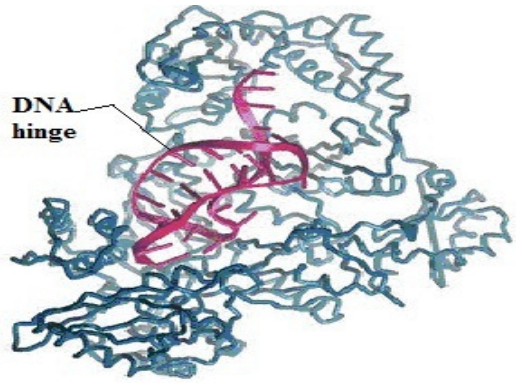

Figure - 2: Aptamer

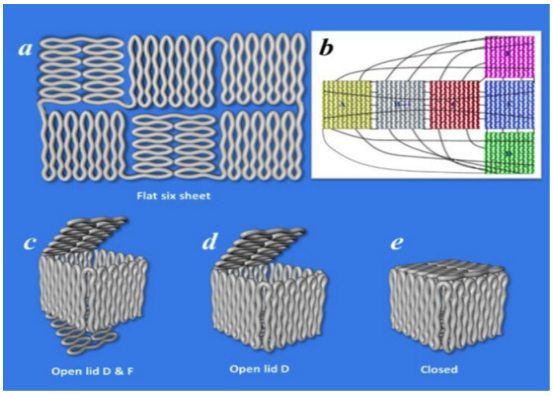

Figure - 3: 3D DNA box origami. (a) Six flat square-shaped origami domains which by their connection (black lines in (b)) will form a 3D DNA box origami; (c–e) DNA box origami in different states.

1.3.2. DNA Origami

The term origami refers to the Japanese folk art of folding paper into a special shape. The method is called DNA origami since one long strand of DNA is folded to produce the desired structure by the help of smaller staple strands. The origami folding process is the method is based on folding of the large ssDNA (usually the 7.3 kilobase genome of the M13 bacteriophage) with an excess of smaller complementary strands (typically 32 bases). These small strands are called "staple" strands and are complementary to at least two distinct segments of the long ssDNA. Long ssDNA and an excess of staple strands are then heat-annealed in a specific buffer with high

concentration of magnesium to form the origami as shown in figure 3.[4 - 6]

2.2. Preparation Of Dna Structures (DNA Origami)

- DNA origami begins by designing how to fold the long strand of DNA to form the desired shape. The shorter staples strands are placed to hold together the desired shape. The long strand folds as the staples search for the right genetic coding to fill all of its bonding sites with the bases of the long strand.
- Finally, the genetic sequencing of the long strand DNA and the folding sequence designed in the first step are put into computer software. It is used to determined the genetic sequences of the hundreds of staple strands. The genetic sequence of the staple strands is determined so that the entire structure can really only form and fold in the desired way by taking advantage of the complimentary pairs (A-G and C-T).
- DNA nano robot is madeup of DNA which contains payload. Is has capability to discriminate between healthy & disease cell & selectively deliver medicinal payload. Nano scale DNA cage release fab-antibody in presence of target cell.
- DNA nanotechnology that allowed the construction of sophisticated multidimensional structures and of devices capable of robot-like functions such as molecular sensing, logical computation and activation. The nanostructures are made by using 'DNA origami', an approach for creating two- or three-dimensional nanoscale shapes by folding a long single-stranded DNA molecule along a predetermined path using oligo nucleotide 'staples'6.
- Nanostructures made by DNA origami can be designed to present specific surface features at which other particles or molecules can be precisely positioned. This capability has been used to generate simple two-dimensional structures that direct the motion of robots constructed from DNA ezymes.
- Three-dimensional DNA box with a lid that can be opened by a DNA 'key' showed that DNA-origami structures are capable of acting as dynamic containers.

2.3. How does a DNA nanorobot reach the target

A major challenge in nanotechnology is to precisely transport a nanoscale object from one location on a nanostructure to another location following a programmable path. DNA has been explored as an excellent building material for the construction of both large scale nanostructures and individual nano mechanical devices[7].

The successful constructions of two dimensional DNA lattices and one dimensional DNA arrays made from DX molecules [8],TX molecules [9], rhombus molecules [10],and 4x4molecules [11] provide the structural base for realization of the above goal. However, the existing DNA nano mechanical devices only exhibit localized nonextensible motions such as open/close [12 –14], extension/contraction[15-17], and reversible rotation motion [18,19].

Table - 1: components of DNA nanorobot.

Component	Macro Robots	DNA Nano Robots
Structural Elements - Links	Metal, Plastic Polymer	DNA Nanotubes
Joints	Metal, Plastic Polymer material	*DNA* hinge *Molecular* bonds, Synthetic joints
Actuators	Electric motors, Pneumatic motors, Hydraulic motors, Smart material-based actuators	ATPase protein flagella motors, DNA actuators, Viral protein motors etc.
Transmission Elements	Springs (Metal,Polyvinyl) Bearings Gears	β Sheets
Sensors	Light sensors, force sensors, position sensors, temperature sensors	Rhodopsin Heat Shock Factor

Furthermore, these motions are not autonomously executed but rather mediated by external environmental changes such as the addition and removal of DNA fuel strands or the change of ionic strength of the solution. Autonomous unidirectional DNA devices executing linear translational motions are hence desirable.DNA fuels are designed which are autonomous and free running DNA machines[20].

There are two designs of autonomous DNA walking devices. Each device consists of a track and a walker. The track of each device contains a periodic linear array of anchorage sites. A walker sequentially steps over the anchorages in an autonomous unidirectional fashion. Eachwalking device makes use of alternating actions of restriction enzymes and ligase to achieve unidirectional translational motion. The action of ligase consumes ATP as energy source[21]. Each walking device makes use of alternating actions of restriction enzymes and ligase to achieve unidirectional translational motion. The action of ligase consumes ATP as energy source as shown in figure 4.[22]

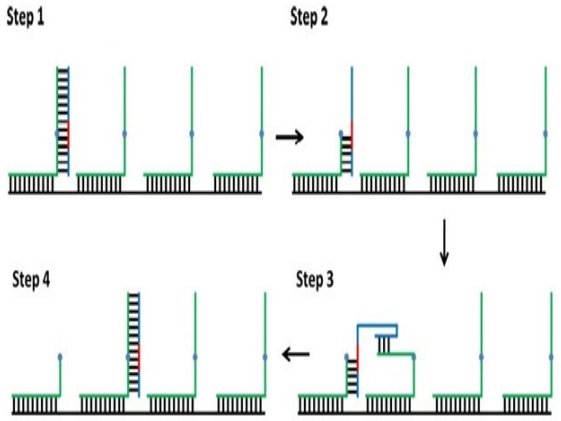

Figure - 4: Enzymatic DNA walking device

Firstly, they demonstrate unidirectional motion rather that random bidirectional motion. Secondly, the moving part (walker) in each walking device is a physical entity with a flexible body size rather than a symbolic entity, and thus the walker can serve not only as an information carrier butalso as a nanoparticle carrier.

Two basic operational events driving the unidirectional motion of the devices are ligations and cleavages.Two neighboring danglers with complementarysticky ends can associate with each other via the hybridization of their sticky ends. Subsequent to this hybridization, the nicks at either end of the hybridized section can be sealed by a ligase and the two duplex fragments are joined into one in a process referred to as ligation.

In *cleavage*, an approximately reverse process to ligation,a duplex DNA fragment is cut into two separateduplex parts (with each usually possessing a complementary sticky end) by enzymes known as restrictionendonucleases. Following cleavage, the two duplex DNA fragments (each with a sticky end) can go apartin a process known as melting. When the context is clear, the whole process of cleavage and subsequentmelting is referred to as cleavage.

Cleavage by an endonuclease usually requires that the substrate DNA fragment contains recognition site (specific DNA sequences) corresponding to the endonuclease and that the cleavage happens at specific cleavage*site* along the DNA fragment.

In contrast to cleavage, ligation does not require specific recognition sites, but it requires complementary sticky ends from the two parts to be joined together. Cleavage uses no energy input from external environment while ligation consumes one molecule of ATP as energy source.

DNA nanorobot can walk along a track of a single strand DNA. Three of legs enzymatic DNA which bind to and cut the DNA stands attached to origami. When a leg become free, after cutting , it explores the origami surface to find another DNA strand to bind to. By repeating this binding and cutting process a robot can walk.

Molecular nanorobot pick up a reactant along a way and react it at a specific location, molecular nanorobot can move along a cell membrane surface and interact with different receptor for disease treatment.

Such a assembly lines are inspired by the way the ribosomes in the cell make peptides by moving along m-RNA to produce a corresponding chain of amino acid. The DNA track will effectively do the job of m-RNA[23,24].

2.4. Applications of DNA nanorobot in treatment of cancer

DNA nanorobot is used as a targeted DDS this can improve medical treatments , such as the case of cancer and chemotherapy. Although there are many chemotherapy drugs, many of them are design to indiscriminately kill fast dividing cells . Of course, fast dividing cells include cancer cells, but they also includes stomach lining, hair follicles, blood cells, etc. Because chemotherapy drugs attack all of these fast dividing cells, they usually lead to various side effect like nausea &vomitting, hair loss, low blood cell counts, etc .So this is the main drawback of chemotherapy drugs.

2.4.1. Mechanism of destruction of cancerous cells

- By stimulation of death receptors causing apoptosis.
- Destruction of cancer cells by targeted delivery of anti-cancer drugs.
- By direct damage to the cancerous cells[25].

2.4.2. Targeted drug delivery system

The robot is made from DNA, and is in the shape of a hexagonal barrel that could carry a variety of payloads. It is held together by two "locks" comprised of DNA aptamers, which are short strands that can bind to antigen targets. The locks open when they encounter antigens on the surface of certain cells, and the robot delivers its cargo. These nanobots could be used for targeted drug delivery as shown in figure 5[26].

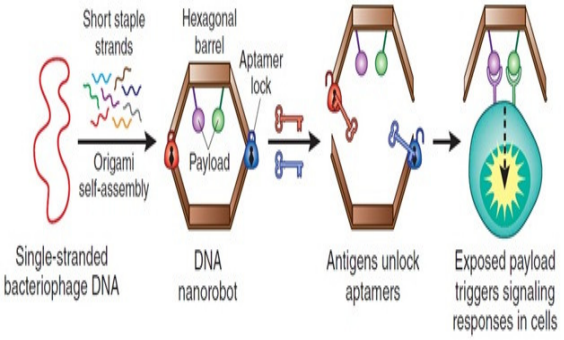

Figure - 5: DNAnanorobot used as targeted drug delivery system

2.4.3.DNA Nanorobots as Biosensors

The technology of nanosensing is also under development. For sensing certain analytes, genetically engineered versions of pore-forming proteins like Staphylococcus aureus alpha-hemolysin are AQ5 also being studied.Efforts to detect biological warfare agents like cholera toxins by utilizing their ability to bind to a bilayer membrane in the presence of gangliosides are another example .Light sensors could be made using certain photoreceptive polypeptides containing azobenzene or spyropyran units as they respond to light or dark environmental conditions by undergoing conformational change, for example, transition from random coil to a a-helix. An optical DNA biosensor platform has been reported using etched optical fiber bundles filled with oligonucleotide functionalized microsphere probes. Finally, work is in progress to develop sensors for brain implantation, which would foretell the development of a stroke and be useful for perioperative online monitoring during coronary by-pass surgery [27,28].

3. CONCLUSION

DNA nanorobots have a great potential for use as diagnostic and therapeutic agents as they can be programmed as per the requirements. The DNA nanorobot technology is in the initial stage. With the development of more advanced and complex DNA nanorobots they will be used for clinical purposes in the future.

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