DNA-based nanotechnology with no system poisoning

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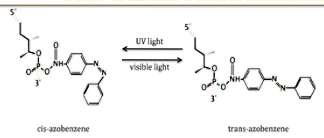
Introduction

DNA is a versatile molecule, which presents very interesting characteristics: it has a high specificity of recognition of the complementary strand and a well-defined structure; it is easily modified by a number of enzymes and cheap to synthesize. All these characteristics have allowed it to become a widely used material for the construction of nanostructures and nanodevices the last thirty years.
The problem of the first generated structures is the amount of waste DNA produced, which quickly poison the system and allowed only few actions before it becomes unusable. Moreover, it is necessary to add new fuel every operational cycle, frequently in stoichiometric amounts for the correct functionality of the system. For that reason, new solutions are being investigated. Some of them involve the use of different wavelengths to switch the device and the use of pH modifications.

Light

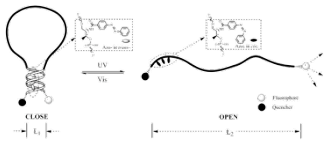
The Basis: Azobenzene

Azobenzene is a chromophore with two phenil rings connected by an azo bound (-N=N-). It is found in *cis* form under UV radiation (300-400 nm) and in *trans* under visible light radiation (>400 nm). This photoreaction is clean and reversible.
Trans azobenzene fits into the DNA helix and is very stable. When it passes to *cis* form, DNA destabilizes and the strands are dissociated[1].

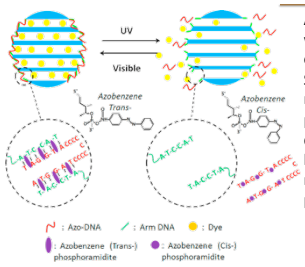


DNA Nanomotor

DNA nanomotors are devices that alternate between two states when there is a certain change of conditions. The present nanomotor uses the alternation of two wavelengths for producing this movement. It consists of a DNA strand with three azobenzene moieties incorporated at the 3' end. In order to follow the movement of the device, researchers incorporated a Fluorescein at 5' end and a Dabcyl to 3'. Under visible light irradiation, the structure forms a hairpin and a quenching of fluorescence takes place. When UV is applied, the DNA strand opens and the fluorescence increases[2]. The addition of silver nanowires may increase the rate of azobenzene isomerization, achieving an open-close conversion rate of 85%[3].



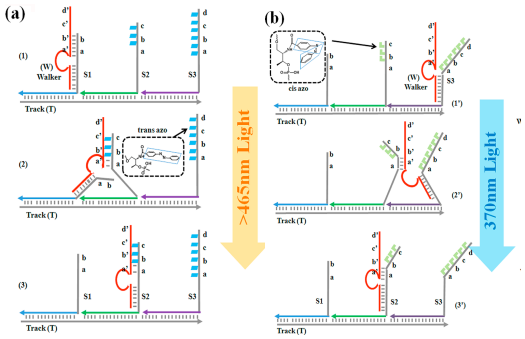
Drug Delivery



A mesoporous silica sphere has been functionalized with azobenzene-modified DNA strands in order to obtain a photon-manipulated releasing system. Silica sphere is filled with rhodamine 6G, as a model molecule. DNA strands are covering the porous preventing the dye from diffusion. The DNA is dissociated by UV light irradiation and the drug can be released. This releasing is slow: 91% of the dye is released after 1500 min. Under visible light, the porous get closed[4].

DNA Walker

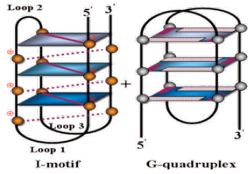
Walkers are pieces of DNA that move over artificial tracks. In this case, the energy source is the light. A walker (W) consisting of two legs, the searching and the holding legs, starts its way in the first anchorage point (S1). When illuminated by visible light, azobenzene adopts a *trans* conformation. The searching leg of W has more complementarity with S2 than with S1, and it moves completely to S2. The holding leg prevents W going off the track.
A similar process takes place with UV irradiation. Azobenzene adopts *cis* form, so W has more complementarity with the previous anchorage site.
By varying the wavelength it is possible to choose the directionality of the walker[5].



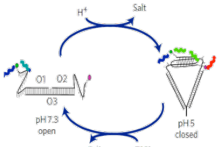
pH variation

The Basis: i-motif

Some cytosine-rich DNA strands can adopt an i-motif structure under acidic conditions. When pH is reduced, the i-motif adopt a random coil structure. I-motif can be both inter and intramolecular. The complementary strand is rich in Gs, and it forms the so-called G-quadruplex form[6].



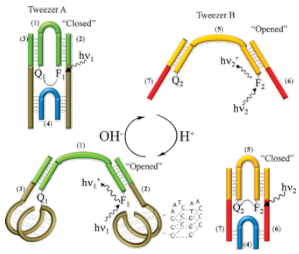
DNA Nanomachine



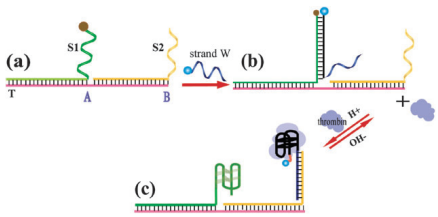
DNA nanomachines can be used as pH sensors inside living cells. The nanomachine consists of three DNA strands. O1 and O2 strands have a cytosine-rich overhang, which form an i-motif at acidic pH. To observe this phenomenon, O1 and O2 are labeled with Alexa-488 and Alexa-647, respectively. Because of the formation of the i-motif, these dyes show fluorescence resonance energy transfer (FRET) at pH 5, but not at pH 7, when the strands are random coiled. O3 is for maintaining the structure.
This nanomachine has been tried inside a living cell. Researchers bound the nanomachine to transferrin, and incorporated it inside the cell. Then, they could observe the changes of pH in the endosomes where the nanomachine was[7].

DNA Tweezer

This tweezer can alternate an open-closed state by the changes in pH. A first tweezer, tweezer A, is found in the closed form under basic conditions. If pH is reduced, domains 2 and 3 acquire their i-motif structure and strand 4 is dissociated.
Tweezer B does the opposite. It is found opened when pH is basic, and closed with strand 4 at an acidic pH. Hybridization of domain 4 with domains 6 and 7 is energetically less favorable than with domains 3 and 4, and for this reason the amount of opened tweezers A at basic pH is low[8].



DNA Walker



Ionic strength changes can also be used for building a walker. In this case, the track has two anchorage sites. S1 consists in a cytosine-rich region, which forms an i-motif structure under acidic pH conditions. Part of strand W corresponds to a thrombin-binding aptamer. W is bound to S1, but when pH is reduced, S1 will change its structure, forcing W to hybridize with S2 and bind thrombin. In acidic conditions, although thrombin is also present, the interaction between S1 and W is more favored thermodynamically.
In order to follow the reaction progress, S1 and W were bound to rhodamine and a black hole quencher, respectively. Under alkaline conditions, FRET is observed, while upon acid addition, the fluorescence increases[9].

Conclusions

DNA-based nanotechnology is still a developing area, where only proofs-of-concept have been made. Nonetheless, DNA has emerged as a material of high interest, with which many applications may be possible, for instance drug delivery or even computational procedures.
Use of renewable sources such as, light and ionic strength, is pivotal in terms of ecology and economy. These sources have other advantages, as there is no need to interfere directly with the system, and no/few toxicity for the biological system upon their use.
In conclusion, application of these or similar approaches can still give innovative and surprising results.

References

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