A Fresh Look at DNA Nanotechnology

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Synthetic DNA structures for nanotechnological applications have experienced substantial success during the past decades benefiting from Seeman and his coworkers' pioneering work. In the last few years, some new branches have been emerging in this field. This review will summarize some recent progress in the authors' group.

1 Two-Dimensional DNA Triangle Arrays Designed with a Tensegrity Strategy

Forming crystalline DNA lattices in one, two and even three dimensions has long been a hot topic of DNA nanotechnology. These artificially designed lattices are the basis for a variety of applications. The first success with a 2D DNA lattice was achieved in [22] with building blocks of double-crossover (DX) DNA molecules. Following that, rhombus motifs, triple-crossover molecules, and a cross motif were also constructed from branched four-arm Holliday junctions [19]. Here we present a tensegrity strategy for the construction of well-structured DNA triangle molecules [14]. A DNA triangle consists of three vertices (DNA four-arm junctions) and three sides (DNA duplexes). Although individual four-arm junctions are flexible, the rigidity of the three duplex edges restricts the freedom of the component four-arm junctions and only triangles can form. The shape of such a triangle is fully defined by the lengths of the three edges. By rational use of sticky-end cohesion, we have successfully assembled triangle arrays in one and two dimensions. Fig. 1 shows the design of some such triangle arrays and some atomic force microscope (AFM) images of such triangle arrays.

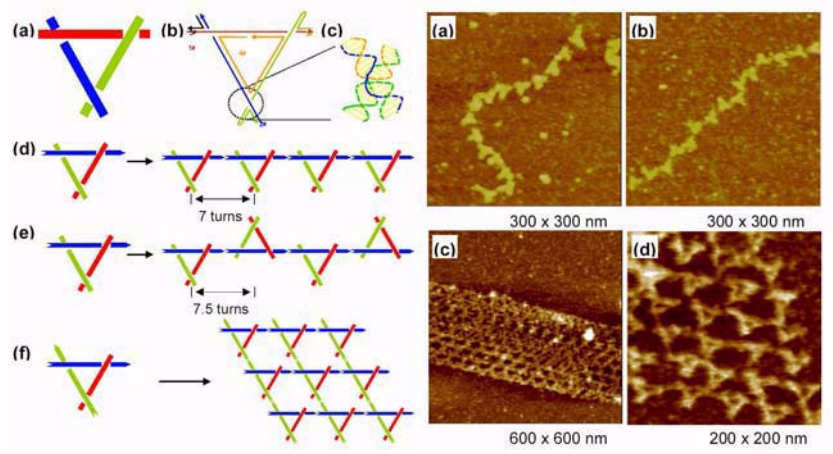

Fig. 1. DNA triangle arrays. *Left panel*: Schematic representation of one- and two-dimensional arrays. (**a**) A DNA triangle contains three DNA duplexes, shown as rods of different colors. (**b**) Strand structure of a DNA triangle. Each thin line represents a single DNA strand. An arrow indicates the 3′ end of a DNA strand. (**c**) The detailed structure of a triangle vortex. (**d**, **e**) 1D and (**f**) 2D self-assembly of DNA triangles. *Right panel*: AFM images of one-dimensional (**a**, **b**) and two-dimensional (**c**, **d**) DNA triangle arrays. (Reproduced from [14] with permission).

2 DNA Molecular Motors

Molecular motors are a very attractive topic in many scientific fields, because they are expected to be mechanical parts of future nanorobots. Complementarily to other molecular systems, DNA motors afford rational design, easy construction, and, most importantly, good control of the motions that they generate. Earlier models of DNA motors include a nanomechanical device based on B–Z transition upon a change of the ionic strength of a solution [17], and molecular tweezers with their opening and closing controlled by sequential addition of DNA strands [23]. Inspired by those early successes, the authors' group has worked intensively in this field. Comparison between cellular protein motors and macroscale man-made machines leads us to ask four questions, as listed below. Answering these questions is fundamental for the further development of molecular motors.

2.1 Can DNA Motors Perform Complicated Motions? — Modeling Gear Motion at Molecular Scale

Gears have many useful functions such as changing the direction and speed of movement, and are important parts in real machines. It is reasonable to expect that gears might play similar roles in small motor systems such nano motors and molecular motors. This notion has motivated us to model gears

with circular DNA molecules, which can roll controllably against each other by use of a strand displacement strategy (Fig. 2) [21]. A DNA gear has a central, circular single strand of DNA, which base-pairs with three linear DNA strands, leaving three unpaired tails as cogs for the gears. When a linker strand L1 is added to the sample, the two gears are bridged together and become ready for rolling. Upon addition of another linker strand L2, two linkages are built between the two gears and the gears roll mutually through 60°. By use of a strand displacement method, a removal strand R1 is then added, which forms a duplex with strand L1 and strips off L1 from the gear pair. This step creates a further 60° rolling between these two gears. If the above process is repeated, a continuous rotation of the two gears is realized.

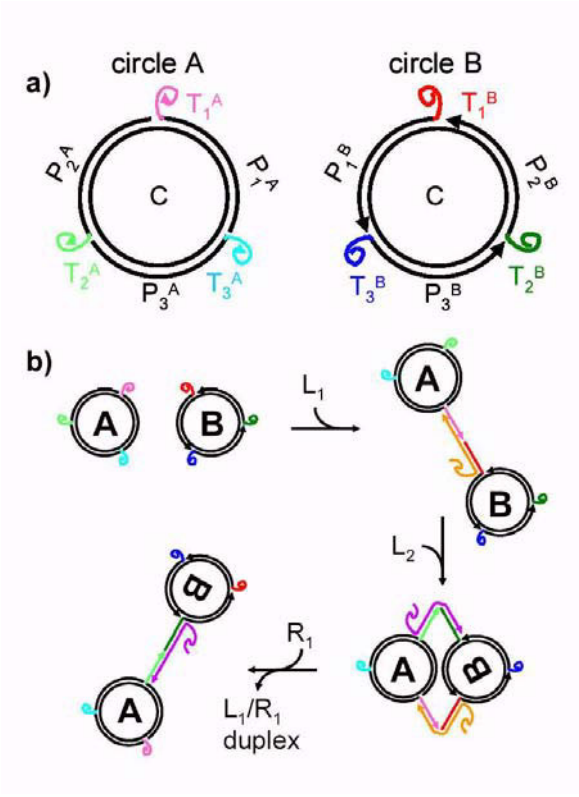

Fig. 2. Design and rolling mechanism of a pair of molecular gears. (**a**) Structures of the individual gears. C and P indicate DNA strands, and T indicates teeth. (**b**) Operation of the gears. L and R represent linker and removal strands, respectively. L_1 and R_1 are complementary to each other. Both circles remain intact during the rolling process. The only changed strands are the linker (L) and removal (R) strands. Note that no twisting motion is generated in the central strands during the rolling process. (Reproduced from [21] with permission).

2.2 Can DNA Motors Work Autonomously?

A DNA Machine Contains a DNAzyme Domain

Working autonomously is an essential feature for cellular protein motors and man-made macroscale machines. It is also desirable for nanomachines to be autonomous. This section describes our initial efforts to address this challenge. The key of our strategy is the introduction of a DNA enzyme domain, which extracts chemical energy and powers a DNA machine. We have tested this notion first with a construct that performs a simple opening and closing motion. This DNA machine undergoes continuous, autonomous motion in the presence of a fuel strand. The motion is controlled by the addition of a brake strand [7, 5]. Fig. 3 illustrates this process. This motor has a triangular shape. It contains a V-shaped dual arm spaced by a single DNA strand at the top. The single strand has a special sequence, corresponding to the core part of a DNA enzyme (E) and its flanking recognition arms on both sides. When this strand is base-paired with its substrate (S), which is a DNA strand with two RNA bases in the middle, the V-shaped arms will be opened owing to the increase in the rigidity of the single-stranded linkage after forming a duplex with its substrate. The DNAzyme then cleaves its substrate, and the cleaved products are short and dissociate from the DNAzyme, which virtually closes the two arms of the motor. If there is substrate in the solution, the above opening and closing process will continue until all substrate molecules (fuel) have been consumed. This process can be regulated by addition of a brake strand (B). The brake molecule is a DNA analog of the substrate, but has base pairs extending into the catalytic core of the enzyme. The brake strand can form a slightly longer duplex with the DNAzyme than the substrate does. The DNA machine will preferentially bind brake strands and further incorporation of fuel strands is then disabled. Therefore, the motor will be frozen in its open state. Note that the brake strand has an unpaired tail, which is designed for removal of the brake. Upon addition of a removal strand (R), the removal strand completely base-pairs with the entire brake strand. As a result, the brake will be removed from the motor system, and the motion of the machine will be resumed.

A DNAzyme-Containing DNA Walker

Recent work has shown that relatively complex motions can be realized with DNA nanocontructions, including walkers and gears. In the following, we show further that we can introduce a DNAzyme into a DNA walker. Such a DNAzyme-containing walker can move autonomously in either direction along a linear track in a controllable fashion [20]. This design takes advantage of the RNA-cleaving function of a DNAzyme. Details of this walker and its mechanism of movement are presented in Fig. 4. The enzyme strand (the red parts are base-recognizing arms, and the orange part is the catalytic core)

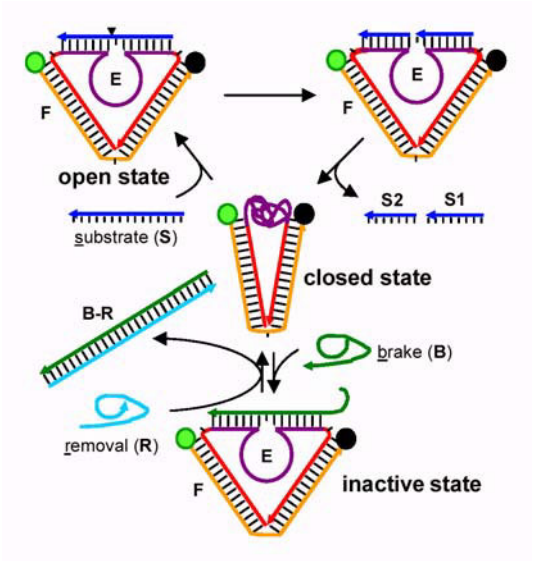

Fig. 3. Schematic of an autonomous DNA nanomotor based on a DNA enzyme. The DNA motor consists of two single strands, E and F. The strand E contains a 10–23 DNA enzyme domain, which is colored purple. The strand F has a fluorophore at the 5′ end (labeled as a solid green circle) and a quencher at the 3′ end (labeled by a solid black circle). (Reproduced from [5] with permission).

base-pairs with one of its substrates on the linear track. The blue dots on the green substrates depict the cleavage points, where the RNA bases are located. After the enzyme cleaves the substrate, the shorter product will be released into the solution owing to its relatively weaker bonding. This gives an opportunity for the exposed part of the enzyme strand to seek another substrate within its vicinity. Gradually, through branch migration, the whole enzyme strand will shift to the next neighboring substrate on the track, and the above process will be repeated until the DNA enzyme moves to the other end of the track. This process can be purposely chosen to start from either end of the track and will continue to the opposite end, but the walker cannot move backwards once it has been determined which end of the track it will start from, because the enzyme will destroy all the substrates that it has passed.

2.3 Can DNA Motors Work with Inexpensive Fuel Molecules? — pH-Switched DNA Motor Based on a Duplex–Triplex Transition

In the DNA machines described so far, DNA and RNA are used as fuel, but they quite expensive. It would be desirable to use inexpensive common chemicals as fuel. This motivation has guided us to design a pH-triggered DNA machine. Under certain conditions, a DNA triplex rather than a duplex

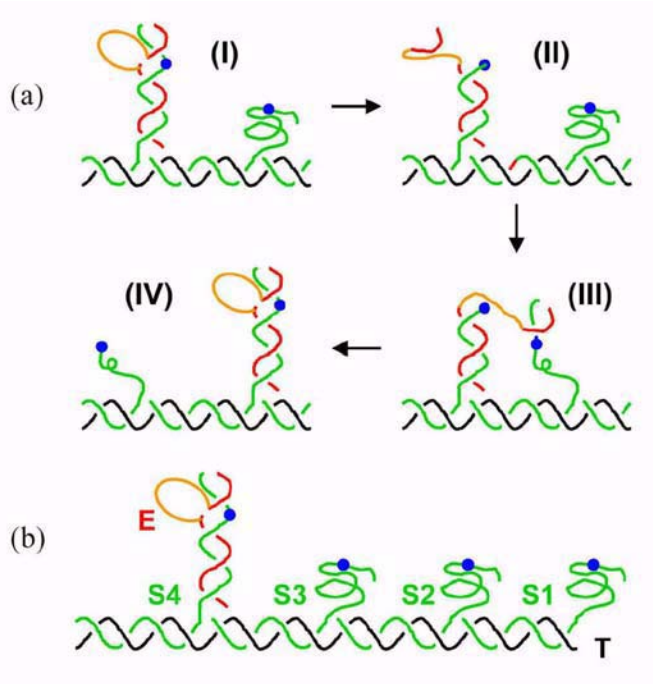

Fig. 4. Schematic of a walking DNAzyme and its track. (**a**) Principle of walking. (**b**) A construction where the walking DNAzyme is at one end of its track. Black lines, template (T); green lines, substrate (S); red–gold lines: a 10–23 DNAzyme; gold lines, the catalytic core. Blue dots indicate the bonds to be cleaved by the DNAzyme. (Reproduced from [20] with permission).

is a more stable conformation for certain DNA molecule assemblies. On the basis of this phenomenon, a DNA motor with a structure as shown in Fig. 5 can be generated [4]. This figure shows a DNA assembly that contains a long strand L (red) and two short strands (black) in an open and closed state. Strand S forms a duplex with one segment of strand L. At pH 8.0, this duplex is the dominant conformation. When the pH is switched to 5.0, one originally dangling single-strand segment within the strand L base-pairs back with the duplex part formed between S and L to give a triplex structure. The formation of this triplex contracts the whole assembly into a closed state. In the closed state, a prelabeled fluorescent dye (green) and a quencher (black) are brought together, resulting in efficient fluorescence quenching. Therefore, by measuring the fluorescent emission of the sample, the real-time operation of this motor can be easily monitored.

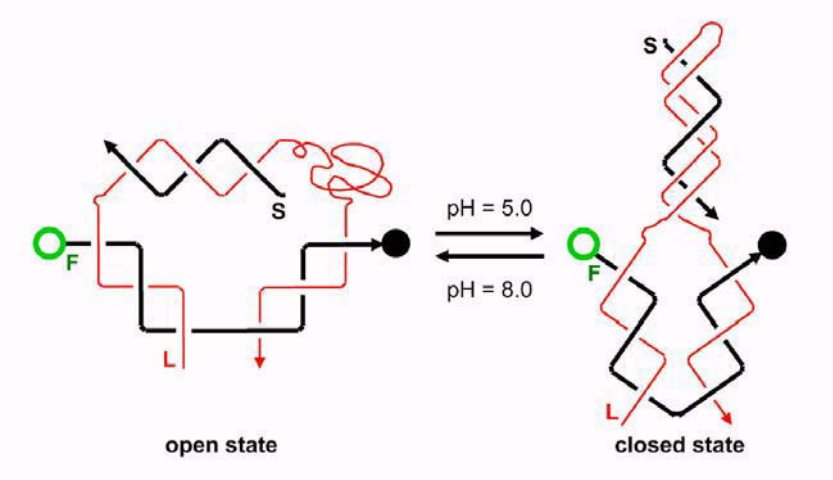

Fig. 5. A DNA nanomotor based on a DNA duplex–triplex transition. The DNA machine consists of three strands: a strand with a fluorescent label (strand F), a long strand (strand L), and a short strand (strand S). The open and solid circles represent a fluorophore and a quencher, respectively. Note the formation and dissociation of a DNA triplex involving the S and L strands upon change of the pH of the solution. (Reproduced from [4] with permission.)

2.4 Can DNA Motors Perform any Useful Work? — Programming Chemical Reactions by a Two-State DNA Switch

Various DNA machines have been demonstrated, but there are very few reports of their applications. Very fundamentally, we would like to ask: are they useful? This section describes one of the few reported examples of an attempt to address this question. DNA-templated organic reactions has been pursued over the years [13]; the following example will demonstrate that a DNA motor can be employed to control the path of a chemical reaction [6]. As shown in Fig. 6a, the DNA structure used consists of three strands: a long strand c (red), which can be divided into three domains, c1, c2, and c3. There are another two shorter strands, N1 (green) and N2 (blue) that base-pair with domains c1 and c2, respectively. These three strands are modified with either amine or carboxylic groups, as indicated in Fig. 3. A pH change between 5.0 and 8.0 determines whether a triplex structure will be formed or dissociated between domain c3 and the duplex formed between N2 and domain c2. At pH 8.0 or 5.0 (Fig. 6b), the carboxylic group on strand c will be brought closer to the amine group on strand N1 or on strand N2 respectively, and thus allow a corresponding amide bond to be formed at the designated position upon addition of a condensing reagent.

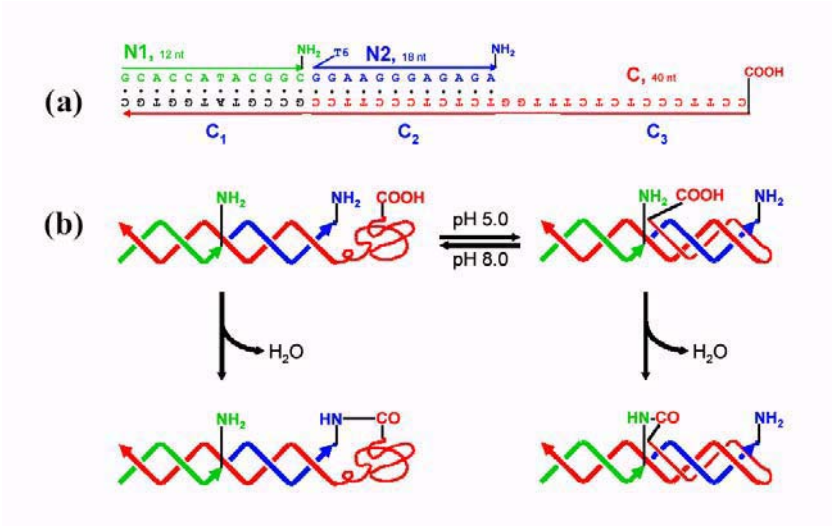

Fig. 6. Schematic illustration of the switching of a chemical reaction based on a DNA duplex–triplex transition. (**a**) DNA sequences, and the positions of the amino groups and carboxylate group of interest. Note that there is a string of unpaired T6 at the 5′ end of strand N2. Addition of the extra six bases to strand N2 causes strands N1 and N2 to have different molecular weights and electrophoretic mobilities, which allows identification of strands N1 and N2 by polyacrylamide gel electrophoresis (PAGE). (**b**) Switching of a chemical reaction by switching the location of the carboxylate group. This behavior is triggered by a change of the pH value of the solution. Note the formation and dissociation of a DNA triplex. (Reproduced from [6] with permission).

3 DNA Encoded One-Dimensional Array of Nanogold

A very challenging aspect of nanotechnology is the development of an efficient and potentially universal way to organize nanosized building blocks into designed architectures. Among the various possible materials, DNA is a superior molecule for this purpose for the following reasons: (1) DNA can be made to form well-defined nanostructures by rational design; (2) DNA can be chemically modified and operated on by enzymes; (3) DNA itself is an environmentally benign biochemical reagent. By choosing gold nanoparticles as model materials for the assembly, we have demonstrated the successful preparation of 1D gold nanoparticle arrays with lengths up to 4 ⎵m [10]. It has been previously shown that gold nanoparticles can be assembled into small, discrete structures [1, 16, 12, 24] through hybridizing mono-DNA-modified gold particles with a DNA template. However, creating a gold nanoparticle array containing hundreds of nanoparticles does not seem to be an easy matter because of difficulties with the availability of long, single-stranded DNA templates. Fortunately, a rolling-circle DNA polymerization technique [11, 15] developed

ten years ago can help. With the help of this rolling-circle polymerization, we can obtain a single-stranded DNA template with a tandemly repetitive sequence defined by the circular DNA template (Fig. 7). Also, gold nanoparticles can be modified with a thiolated single-stranded oligonucleotide, and mono-DNA-modified particles can be isolated simply by agarose gel electrohporesis using a protocol developed by Alivisatos et al. [1, 16, 12, 24]. After combining the mono-DNA-modified gold particles with the rolling-circle-synthesized long DNA template, 1D gold nanoparticle linear arrays with lengths up to several micrometers can be obtained.

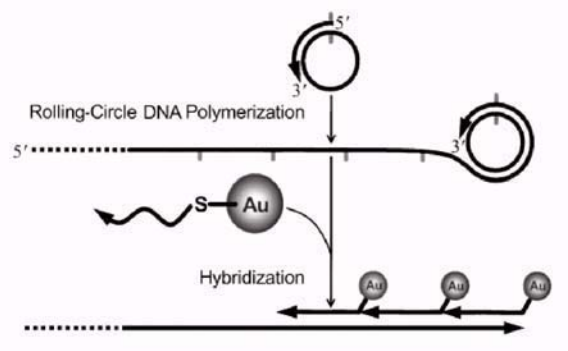

Fig. 7. Synthesis of an extended gold nanoparticle array by combining DNA-encoded self-assembly and rolling-circle polymerization of DNA. (Reproduced from [10] with permission).

4 DNA as Templates for Nanofabrication

4.1 Oriented Metallic Nanowire Networks Templated by DNA

Besides the use of self-assembly to form various structures, DNA molecules can also be used as scaffolds for nanofabrication. The first example that we have demonstrated is related to DNA metallization (Fig. 8). It is known that lambda-phage DNA, a linear DNA with a natural length of 16μm, can be aligned on a surface [2]. It is also known that DNA strands can be metallized through electroless metal reduction in solution or on a surface [3, 18]. This provides a fundamental possibility of fabricating 1D or 2D oriented metal wire networks. The method described here integrates a molecular combing technique and DNA metallization [8]. In the first step, DNA is aligned on a mica surface by a fluid flow in the presence of magnesium ions, which enhance the binding between the DNA and the mica surface and thus minimize DNA detachment during the metallization process. Note that both the DNA and the

mica surface are negatively charged. After alignment, the DNA sample is then used for metal deposition. Palladium was chosen as the metal for this purpose. To avoid the formation of nanowires with abundant branches, removal of the palladium solution from the surface before adding the reduction bath solution is helpful. The incubation time for the reduction process must be controlled within a range of several minutes to as short as tens of seconds, otherwise DNA will begin to detach from the surface, and the originally created DNA network structures will be partially destroyed.

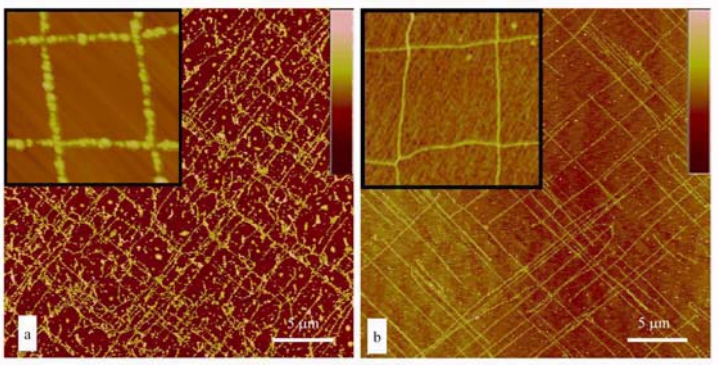

Fig. 8. AFM images of 2D aligned Pd nanowires (**a**) and the corresponding precursor DNA molecules (**b**). The insets in (**a**) and (**b**) give closer views of a 2D square of metal nanowires and of DNA molecules, respectively. Height scale: (**a**) 30 nm and (**b**) 3.0 nm. (Reproduced from [8] with permission).

4.2 Molecular Lithography with DNA Nanostructures

Another example of DNA-templated nanofabrication is the molding of DNA patterns with a metal film, resulting in a negative replica of the DNA structure (Fig. 9) [9]. The first step in realizing the replication of DNA nanostructures is to deposit DNA samples onto a mica surface. Since mica has an atomically flat surface, DNA structures on the mica surface show significant topographic patterns even though they are only about 1 nm high. Immediately after the sample has been deposited and the surface has been dried, a layer of gold metal is thermally evaporated onto the mica surface until a continuous film with a thickness of about 20 nm is formed. The weak bonding between the gold film and the mica surface offers the possibility to easily peel off the gold film to release the replica. By this strategy, DNA structures, both one-dimensional and two-dimensional can be successfully transferred to a metal substrate. Since good control over the DNA structure could be achieved by rational design, it is not a dream that in the future we might use the replicated

patterns for various purposes, from ultrafine display panels and integrated sensors to nanoelectronic applications.

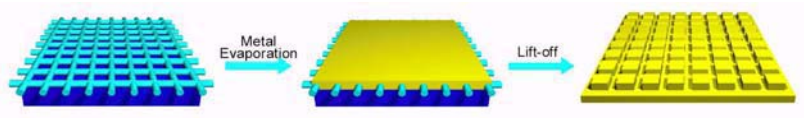

Fig. 9. A molecular lithography technique that transfers DNA nanostructures into metallic structures. (Reproduced from [9] with permission).

5 Final Remarks

This review has summarized our recent research results regarding DNA nanotechnology. DNA nanotechnology has clearly been developed into various research fields. Despite some difficulties that lie ahead, DNA, as a unique material, is paving the way towards molecular motors, directed material self-assembly, and nanofabrication, which may, potentially, solve the challenges in nanotechnology. We are expecting to see more and more breakthroughs in the coming years that will bring new content to this field, towards more practical applications of DNA nanostructures.

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