The possible reason responsible for the difference between two C_{60}-based derivatives is very complicated. One explanation is that the difference results from different joint-groups. $C_{60}(OH)_x(O)_y$ might have a high affinity for bone. It is reported that empty fullerols and other polyhydroxylated compounds have a high affinity for cortical bone[5]. Another explanation for the accumulation of ^{99m}Tc-$C_{60}(OH)_x(O)_y$ in liver and spleen is that ^{99m}Tc-$C_{60}(OH)_x(O)_y$ is recognized by reticuliendothelial cells. When the compound entered tissues accompying the flow of the blood, ^{99m}Tc-$C_{60}(OH)_x(O)_y$ is licked up or enwrapped by reticuliendothelial cells, and thus retained in those tissues. Analo-reticuliendothelial tissues exist in bone red marrow, so ^{99m}Tc-$C_{60}(OH)_x(O)_y$ could accumulate in bone. From the results of experiment of mice and rabbits, we can see that radioactivity accumulated in red marrow-contained backbone, extremity honeycomb, breastbone and coronal bone, and that radioactivity did not accumulate in long bones and ribs.

4 Conclusion

$C_{60}(OH)_x(O)_y$ could be absorbed by biological bodies, and was eventually excreted through urine and enteron. The blood clearance of the compound was slow.

Since no more studies about biodistribution of fullerene derivatives in live organisms were reported, the further study is needed to find out to what extent C_{60} itself determines the biodistribution of derivatives.

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Assemble four-arm DNA junctions into nanoweb

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Abstract **DNA is of structural polymorphism, which is useful in nanoarchitecture; especially, four-arm DNA junctions can be used to assemble nanowebs. The static four-arm DNA junctions were designed and synthesized. One-arm DNA and two-arm DNA came out simultaneously with the four-arm DNA junction's formation. A new method, termed the two-step method, was proposed and the productivity of four-arm DNA junctions was increased. A nanoweb was assembled successfully, but it showed irregularity itself. It was not the same as we expected. We consider that it is a result from the flexibility of four-arm DNA junction.**

Keywords: nucleic acid, self-assembly, nanotechnology, atomic force microscopy.

DNA is well known as the polymeric molecule that contains the genetic information for life. Moreover, it is important and cannot be substituted in nanoarchitecture with unique advantages. (i) DNA is a kind of nanowire itself naturally which can be used as template in nanofabrication[1-3]. For example, the diameter of canonical B-double helical nucleic acid is 2 nm. (ii) DNA is of structural polymorphism, triplex and quadruplex DNA have been discovered subsequently[4]. Some special structures can be synthesized such as four-arm DNA junctions[5,6]. A large number of potentially useful rigid nanoparts made of polymerized DNA have been built, including molecular scale rods, rings, cubes[14], tetrahedrons, hollow tubes, branched joints, etc.[7,8]. (iii) DNA is available for arbitrary programmed sequences due to convenient solid support synthesis. (iv) DNA can also be easily chemically modified, so the application of DNA is increased for more potential nanotechnological purposes. (v) Particularly, the interactions between DNA and enzymes as well as lipids have been studied in detail and widely. DNA can be recognized and manipulated by a large battery of enzymes, including ligase, restriction endonucleases, kinases and exonucleases. This advantage can be used in biocomputation and nanofabrication[9-11].

Recently, great progress has been made in using DNA to build nanostructures. Mirkin used DNA as a

linker to direct the formation of macroscopic architecture from Au nanoparticles[2]. Braun made a 100 nm silver wire with DNA as a template. Particularly, many shapes of pure DNA have been synthesized by Seeman, such as 2-dimensional crystals[12], actuators[13] and cubes[14].

In this work, 2-dimensional nanowebs were synthesized from four-arm DNA junctions. If oligonucleotides with particular sequences are designed, the static four-arm DNA junction can form with the branch joint immobile. The static four-arm DNA junction is of a planar cruciform structure in condition of no multivalent cations in buffer, so it is an ideal nanopart. We used the four-arm DNA junction as the core and the double helical DNA with sticky ends as the linker to synthesize 2-dimensional nanolattices.

1 Materials and methods

(i) Materials. Tris buffer, polyacrylamide gel, T4 DNA ligase, ethanol, superpure water.

(ii) Design of DNA's sequences. The Holliday DNA junction is a molecular paradigm of homologous recombination[14]. It is also a kind of four-arm DNA junction, but its branch joint is mobile. In the Holliday DNA junctions, the sequences' homologies play two contradictory roles. On the one hand, only when the sequences are homologous can this four-arm DNA junction form. On the other hand, branch migration is a direct consequence of homology or two-fold sequence symmetry. A four-arm DNA junction would disassemble when the branch joint migrates to the end of one double helical DNA strand. So, this mobile DNA junction is not proper for nanofabrication. But if we change the sequence symmetry, we can get an analogue of Holliday DNA junction, a new kind of four-arm DNA junction, termed static DNA junction, and the joint could be fixed precisely. A four-arm junction's stabilization depends on temperature, the length of arm, and properties of the buffer, while joint's fixation profits from the sequences' asymmetry.

If used as a nanopart, a four-arm DNA junction must be small and stable. So the construction of static four-arm DNA junction should accord with the following principles: (i) CG base pair is stable than AT. So it is essential to increase the CG content as highly as possible. (ii) The repeated units should be few, so that the mismatch could be avoided. (iii) The other DNA structures should be kept away. For example, a sequence with three or more consecutive G could form quadruplex DNA, which should be avoided. (iv) Symmetry entails branch migration. The sequence around the junction site should be of minimum two-fold symmetry.

The sequences were designed as follows (5′-phosphorylation, 3′-hydroxylation):

5′-GGCTCGCAATCCTGAGCACG-3′ (1)
5′-GGCTCGTGCTCACCGAATGC-3′ (2)
5′-GGCTGCATTCGGACTATGGC-3′ (3)
5′-GGCTGCCATAGTGGATTGCG-3′ (4)
5′-AGCCAACCCGTTCTCGGAGCACTGCAGAAC-3′ (5)
5′-AGCCGTTCTGCAGTGCTCCGAGAACGGGTT-3′ (6)

The structure of the double helical DNA linker with sticky ends and the static four-arm DNA junction with sticky ends with the designed sequences are shown in fig. 1.

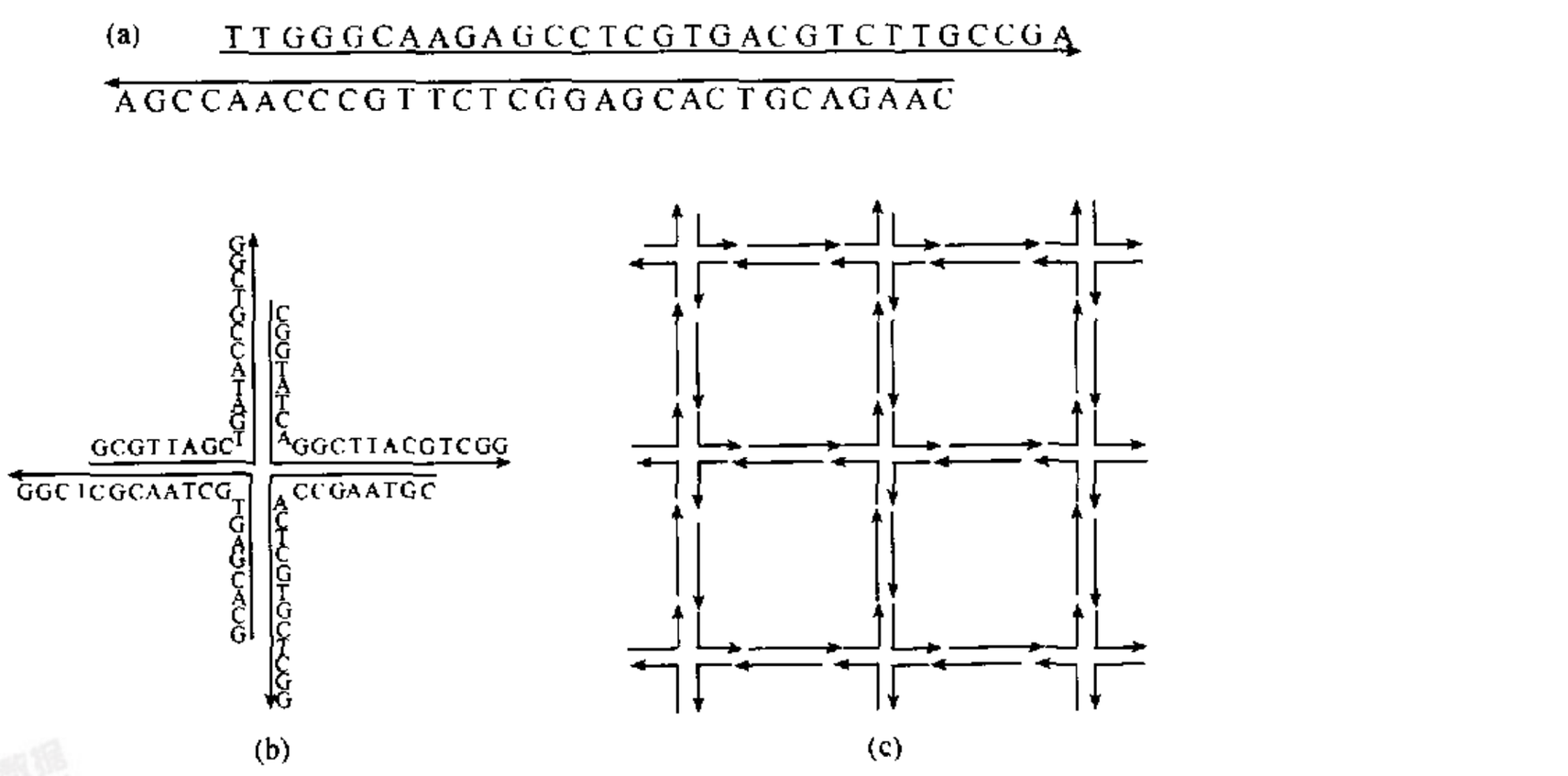

Fig. 1. Sketch maps showing how to synthesize the 2-dimensional DNA web. (a) Double helical DNA linker with sticky ends. (b) Static four-arm DNA junction with sticky ends. c) Sketch of a 2-dimensional DNA nanoweb, side length is 50 bp, 17 nm.

(iii) Synthesis of the four-arm DNA junctions and double helical DNA linkers. The four-arm DNA junctions were synthesized from the former four strands (1—4) and the linkers from the latter two strands (5, 6). Samples were purified from polyacrylamide gel. The bands of four-arm DNA junctions and double helical DNA linkers were cut out and eluted in the eluant buffer for 5—6 h at room temperature on the shaking table. The products were centrifugated to eliminate the gel, and precipitated by adding iced ethanol, then washed twice by 70% ethanol.

(iv) Synthesis of the nanoweb. The four-arm DNA junctions and double helical linkers with sticky ends were mixed at ratio of 1 : 2 (mol : mol), and annealed at 15℃ (without multivalent cations such as Mg^{2+}). Then T4 DNA ligases were added to ligate the sticky ends. 12 h later, the reaction was stopped by adding EDTA to final concentration of 20 mmol/L. The products were precipitated by adding iced ethanol and stored in Tris buffer at 0 — 4℃.

(v) Imaging with AFM. The nanoweb samples were diluted to 1 ng/uL. A 1—5 uL aliquot of this solution was deposited on a freshly cleaved mica surface and dried for 2 min, then washed twice with superpure water and dried for use. Samples were imaged with the NANOSCOPE III type (Digital Instruments) in tapping mode. The scanning frequency was about 2 Hz. The free amplitude was 1.5 V and the resonant frequency was about 320 kHz.

2 Results and analysis

(i) Synthesis of the double helical DNA linkers and the four-arm DNA junctions. It was simple to synthesize double helical DNA linkers. The two strands (5, 6) were mixed at stoichiometric ratio (1 : 1, mol : mol), and heated to 90℃, cooled slowly to 55℃ and kept constant for 2 h, then cooled to room temperature. The four-arm DNA junctions formed with byproducts (one-arm and two-arm DNA junctions) from the four strands (1—4). One-arm DNA junctions were the stable product from any two of strands (1—4), and two-arm DNA junctions were from any three of strands (1—4). Although the activation energy was optimum, the formation of four-arm DNA junction involved four molecules, so the yield was not high. A new method, termed the two-step method, was developed to synthesize four-arm DNA junctions. At the first step, any two of the four strands (1—4) were mixed at stoichiometric ratio (1 : 1, mol : mol), and heated to 90℃, cooled slowly to 55℃ and kept constant for 2 h, then cooled to room temperature. So did the other two strands. Two kinds of one-arm DNA junction were got at high yield. Then the two intermediates were mixed at 1 : 1 (mol : mol), incubated at proper temperature for 2 h, and then cooled slowly to room temperature. The DNA linkers and junctions were purified from polyacrylamide gel.

Fig. 2 shows the 12% polyacrylamide gel electrophoresis, from which we can state that one-arm, two-arm and four-arm DNA junctions were stable in proper buffer. A large number of one-arm DNA junctions formed when only two strands were involved in reaction (lane 3). While three strands were involved, one-arm and two-arm DNA junctions formed simultaneously, and it is worthy of notice that the formation of two-arm DNA junctions was preponderant (lanes 2, 8). If all the four strands (1—4) mixed at the same time and the three junctions including one-arm, two-arm, four-arm ones formed after incubation (lanes 1, 9). But the one-arm and two-arm DNA junctions decreased when we used the two-step method to synthesize four-arm DNA junctions (lanes 4—7). It was found that the incubation temperature (from 35℃ to 55℃) at the second step did not affect the formation and yield of four-arm DNA junctions. In conclusion, when four strands (1—4) were mixed simultaneously, the two-arm DNA junctions would form as a stable byproduct. If DNA were measured accurately, at the first step of the two-step method, only trace of single strand DNA existed, so at the second step, two-arm DNA junctions formed less for shortage of raw materials and four-arm DNA junctions should be synthesized at very high yield. In this work, DNA's concentration was measured by UV spectra and it was inaccurate for the bases' bad-distribution. So the excrescent single strand DNA would retain when only two strands went in reaction and the two-arm junctions were obvious (lanes 4—7).

Fig. 2. 12% polyacrylamide gel electrophoresis at 60 V. After 6 h, the gel was dyed in 0.5 ug/mL EB for 10 min. All the reactants were mixed at stoichiometric ratio (1 : 1, mol : mol). Lane 1. The four strands (1—4) were mixed simultaneously, heated to 90℃, cooled slowly, incubated at 55℃ and kept constant for 2 h, then cooled slowly to room temperature. Lane 2. Reactants were three strands (1—3), under the same conditions as of lane 1. Lane 3. Reactants were strands (1, 2) with the same conditions as of land 1. Lane 4. Two-step method was adopted. One-arm DNA junctions were synthesized from strands (1, 2). So did strands (3, 4), under the same conditions as of lane 1. Then the two one-arm DNA junctions were mixed at 1 : 1 (mol : mol), incubated at 55℃ which was kept constant for 2 h, then cooled slowly to room temperature. Lane 5. The same as lane 4, but the temperature was changed to 45℃. Lane 6. The same as lane 4, but the temperature was changed to 40℃. Lane 7. The same as lane 4, but the temperature was changed to 35℃. Lane 8. The same as lane 2, but reactants were strands (2—4). Lane 9. The same as lane 1.

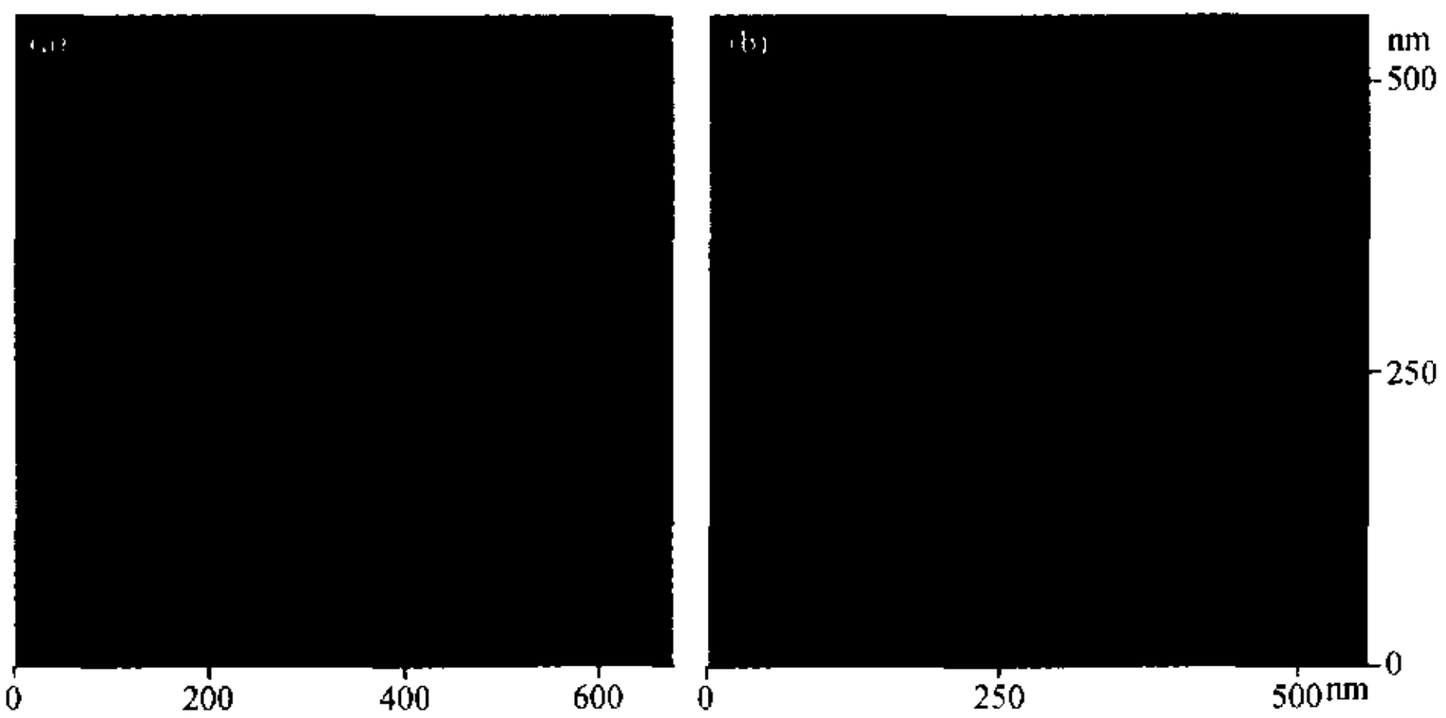

Fig. 3. AFM images. The nanoweb was irregular. The lattice corresponding to what we designed as fig. 1(c) can be found in a small area (in the white frame in fig. 3). The distance between neighboring holes' centers was about 18 nm, which is near 17 nm (the side length of designed lattice = 0.34 nm/bp × 50 bp).

(ii) Imaging with AFM. Fig. 3 is the AFM image of the nanowebs assembled from the four-arm DNA junctions and the double helical DNA linkers. It can be stated that the web was irregular; some holes are big and some small. The lattice corresponding to what we designed as fig. 1(c) can be found in a small area (in the white frame in fig. 3). The distance between neighboring holes' center was about 18 nm, which is near 17 nm (the side length of designed lattice = 0.34 nm/bp × 50 bp). Large regular structure was not found. Although without bivalent and multivalent cations, the four-arm DNA junction is of a planar cruciform structure and the angles between neighboring arms is 90° when the arms were not too long (<3 turns)[14−16]. When the nanowebs formed, the four-arm DNA junctions were annealed and ligated with the DNA linkers. The arm's length increased and the rigidity decreased, so the arm bend and cannot form regular 2-dimensional lattices.

3 Discussion

The four-arm DNA junctions were thermodynamically stable as a nanopart, but their structure is flexible. It was reported recently that one enzyme, named RuvA[17], could specially bind with a four-arm DNA junction as tetramer or octamer[18] even if with bivalent or multivalent cations. The assembly of RuvA and four-arm DNA junction was a planar cruciform structure but more rigid than four-arm DNA junctions alone. A series of enzymes have been found recently, which can specially recognize and manipulate four-arm DNA junctions[19,20]. This knowledge made a solid basis for the use of four-arm DNA junctions in nanofabrication and biocomputation.

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