Assembly of Two-Dimensional DNA Crystals Carrying N^4-[2-(*tert*-Butyldisulfanyl)ethyl]cytosine Residues

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DNA Lattices carrying two cytosine residues bearing the N^4-[(*tert*-butyldisulfanyl)ethyl] group at the apex of hairpin topological markers are prepared for first time. We show that these residues are important for the deposition of DNA lattices on gold surfaces.

Introduction. – In recent years, the use of the self-assembly properties of DNA to generate addressable nanostructures in one, two, and three dimensions has become increasingly popular [1–3]. A remarkable development in this field was the use of stable DNA *Holliday* junctions with addressable sticky ends to form two-dimensional (2D) DNA crystals [4]. The principles of construction described by *Seeman* have since been used and adapted to generate systems with fine control of shape and function [1–8]. For example, large DNA lattices have been transformed into highly regular 2D DNA networks on surfaces that provide templates for the deposition of gold nanoparticles in a regular square network by using biotin–streptavidin recognition system [7][8]. Fullerene arrays have been assembled on DNA templates [9], as well as gold nanoparticle arrays [10][11] and proteins [12–14]. Recently, 2D arrays of quantum dots with controlled periodicity have been prepared using DNA-tile arrays [15].

Before new applications for DNA nanostructured surfaces can be developed, there are several issues to be addressed. First, it is important to form DNA nanostructures on surfaces on which electrical contacts such as gold, silicon oxide, and other semiconductor oxides can lie. Most research on programmed DNA lattices involves mica and buffers that contain Mg^{2+} ions. Mica is an excellent substrate for the formation of 2D DNA lattices. This is because mica has a flat atomic structure, and, by using Mg^{2+}, it facilitates the deposition of negatively charged DNA on the negatively charged layers of mica. Unfortunately, electrical contacts cannot be used with mica.

Another important issue is the site-specific functionalization of the DNA lattices with molecules such as peptides, proteins, and nanomaterials. This involves the preparation of DNA conjugates so that a particular molecule or nanomaterial can be deposited upon a particular site of the DNA nanostructure without modifying the self-assembling properties of the DNA. For example, biotinylated DNA lattices were used

to obtain DNA-templated arrays of streptavidin and gold nanoparticles [7][8] as well as quantum dots [15]. However, the development of other types of conjugates that will not require streptavidin is becoming increasingly attractive and would broaden the scope of further applications. In this direction, gold nanoparticles [10][11] and the *c-myc* [14] peptide have been introduced into 2D DNA lattices by using 5′-functionalized oligonucleotides. This strategy needs to break in two parts one of the oligonucleotides involved in the formation of the topological hairpin markers with a subsequent second annealing step to ensure the incorporation of the small oligonucleotide to the DNA lattice.

We became interested in the preparation of sulfanylated 2D DNA arrays, because the special reactivity of the sulfanyl group will allow the functionalization of 2D DNA arrays. Sulfanyl groups have a strong affinity for gold surfaces, and they can also be used to introduce peptides and proteins as well as large number of molecules that have been functionalized with maleimido groups or bromo- and iodo-acetyl groups. Sulfanyl groups can be introduced relatively easily at the 5′-end or 3′-end of oligonucleotides by using commercially available chemicals. But these ends are usually needed for the assembly of the DNA nanostructure, and we do not want to increase the number of oligonucleotides as described [11][12][14]. For these reasons, we planned to insert reactive sulfanyl groups at the nucleobase. We used a well-characterized 2D DNA lattice [4] to study the formation of the DNA lattices on gold, a surface that allows electrical contacts. In this article, we show that DNA lattices carrying a single sulfanyl derivative in each topological hairpin marker can be prepared and deposited on mica substrates. But most importantly, we also demonstrated that these sulfanylated 2D DNA arrays are readily deposited on gold surfaces, while unmodified 2D DNA arrays cannot.

Results and Discussion. – *Oligonucleotide Design and Synthesis.* We focused on the A-B* system described by *Winfree et al.* [4] which comprises two DNA tiles, A and B*, formed by the assembly of five oligodeoxynucleotides (A: 21 – 48 bases; B*: 22 – 70 bases). This system produces a very compact structure in which the DNA occupies any available space. Tile B* has two loops protruding out of the plane of the DNA lattice in opposite directions. These two loops were used as topographic labels to indicate the position of tile B* on the DNA lattice during the process of visualization by atomic force microscopy (AFM). We modified tile B* by introducing one N^4-[2-(*tert*-butyldisulfanyl)ethyl]cytosine residue to replace one thymidine residue at the unpaired loop positions of the oligodeoxynucleotides (*Fig. 1*).

This involved preparing the two longer oligodeoxynucleotides of tile B* (69 and 70 bases), which carry one single N^4-[2-(*tert*-butyldisulfanyl)ethyl]cytosine residue in the middle of the sequence. To successfully obtain the modified tile B*, the appropriate N^4-[2-(*tert*-butyldisulfanyl)ethyl]cytosine phosphoramidite was prepared as described in [16]. The desired sulfanyl-modified oligodeoxynucleotides were purified by denaturing polyacrylamide gel electrophoresis (PAGE). The resulting modified oligonucleotides were hybridized with equimolar amounts of the other three oligodeoxynucleotides of tile B*. A single band was observed by native PAGE, which indicated the presence of the modified N^4-[2-(*tert*-butyldisulfanyl)ethyl]cytosine residue did not affect the formation of tile B*.

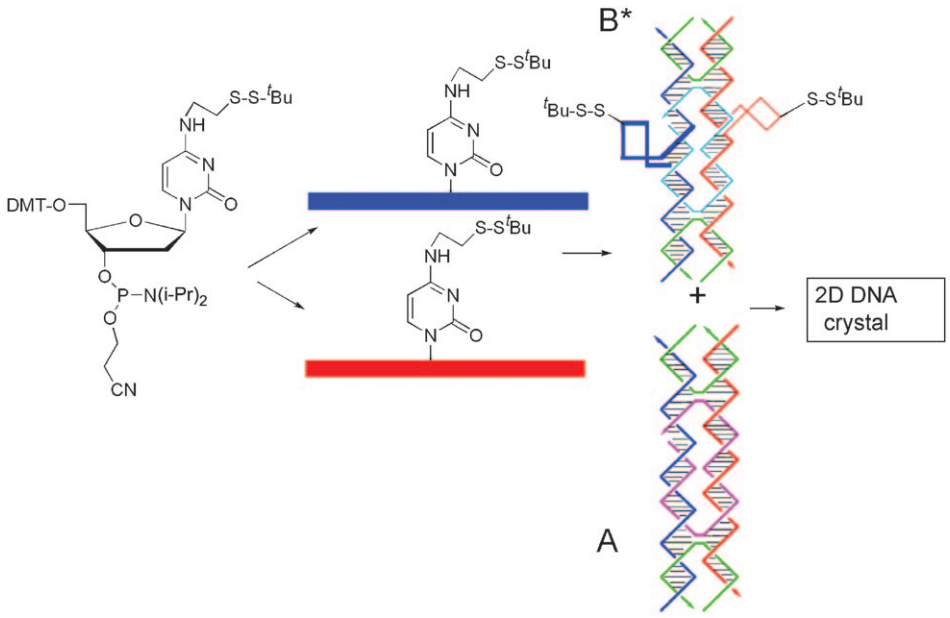

Fig. 1. *Steps used in the formation of the disulfanyl-modified A-B* DNA array.* First, the phosphoramidite of N^4-[2-(*tert*-butyldisulfanyl)ethyl]-2′-deoxycytidine was prepared [16]. Two oligonucleotides carrying the N^4-[2-(*tert*-butyldisulfanyl)ethyl]-2′-deoxycytidine residues at the apex of the topological loops of tile B* described by *Winfree et al.* [4] were synthesized. Annealing of these oligonucleotides with eight unmodified oligonucleotides described in [4] yielded the desired DNA lattice that was then deposited on gold and mica substrates.

Surface Preparation. Freshly cleaved mica was used for AFM imaging. In the case of gold, several gold surfaces were examined by AFM. Surfaces based on gold [1.1.1] were considered not suitable due to the formation of small terraces with step heights of more than 2 nm that will make difficult the visualization of the DNA topological markers of A-B* of 2–3-nm height. We selected gold evaporated on mica (template-stripped gold), the preparation of which is described in [17]. Template-stripped gold has a large flat area with a roughness of *ca.* 0.5 nm.

AFM Imaging of DNA Lattices. Atomic force microscopy (AFM) was used to visualize the modified 2D DNA lattices deposited on surfaces. The ten oligodeoxynucleotides (two sulfanyl-modified and eight unmodified) were hybridized, and the resulting solution was deposited on mica and gold substrates. The modified DNA lattice carrying two N^4-[2-(*tert*-butyldisulfanyl)ethyl]cytidine residues (one in each loop) was readily formed on gold surfaces (*Fig. 2*). As it can be seen from the AFM images in *Fig. 2*, the vertical columns of the lattice appear as stripes, which is the position of the loops of tile B*. The spacing of the decorated columns was 26 ± 1 nm, which is smaller than the expected value of 32 nm. This may be due to the roughness of the surface. The modified DNA lattice was also observed on mica (*Fig. 3*). The spacing of the decorated columns on mica was 32 ± 1 nm, which was the same value as on the unmodified DNA lattices [4]. In contrast, the unmodified lattice carrying all ten unmodified oligodeoxy-

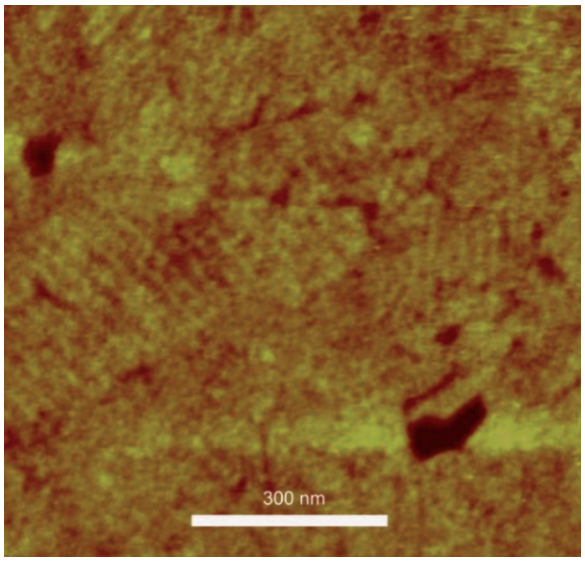

Fig. 2. *Topological AFM image of the disulfanyl-modified A-B* DNA lattice assembled on template-stripped gold*. Brighter (taller) diagonal ridges correspond to rows of the topological marker (hairpins present on tile B*) and fainter bands to DNA tile A.

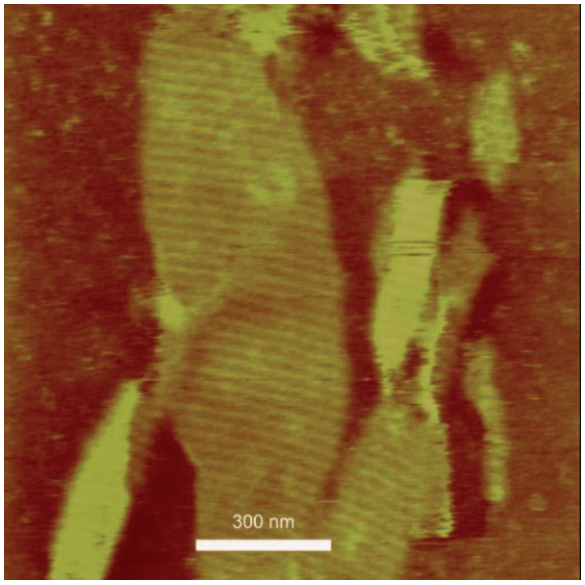

Fig. 3. *Topological AFM image of the disulfanyl-modified A-B* DNA lattice assembled on mica*. Brighter (taller) diagonal ridges correspond to rows of the topological marker (hairpins present on tile B*) and fainter bands to DNA tile A.

nucleotides was formed only on mica. There was no evidence of the formation of any unmodified lattice on gold. This observation indicates that the introduction of one single protected sulfanyl group in each loop of the B* DNA tiles induces a dramatic change in the affinity of DNA to the surfaces. The affinity of sulfanyl groups to gold surfaces facilitates the attachment of the sulfanyl-modified DNA crystals to gold.

Conclusions. – DNA Lattices were prepared using both B* loops functionalized with one protected sulfanyl residue. In this way, the DNA lattice can bind to the gold surface at both sites. An interesting possibility for future research would be the preparation of asymmetric DNA lattices carrying a protected sulfanyl group at one site (to bind to a gold surface) and a molecule of interest at the other site. Previous research has demonstrated that it is indeed feasible to obtain DNA conjugates by removing the *tert*-butylsulfanyl group of the sulfanyl moiety and reacting the resulting free sulfanyl with maleimido groups [16].

In the literature, the use of self-assembling properties of synthetic oligonucleotides for the construction of periodic arrays on the nanoscale as a potentially interesting technology has been described [2–8]. There is a wide range of literature describing a large variety of periodic arrays and their specific patterns [1–14]. The results of this study demonstrate that DNA lattices carrying a single protected sulfanyl group at specific internal DNA sites can be assembled in aqueous buffers and then deposited onto gold surfaces obtaining sulfanyl-modified DNA arrays that may provide starting points to template the deposition of molecules or nanomaterials with nanometric precision. This result opens new possibilities for the preparation of DNA-templated periodic lattices on electrically relevant surfaces as well as for the preparation of DNA-templated arrays of molecules and nanomaterials of interest.

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Experimental Part

Oligodeoxynucleotide Synthesis. Oligodeoxynucleotide sequences are shown in the *Table*. The syntheses were performed on the *Applied Biosystems Model 3400* DNA synthesizer using a scale of 0.2 μmol and standard 2-cyanoethyl phosphoramidites as monomers. The N^4-[2-(*tert*-butyldisulfanyl)ethyl]-2′-deoxycytidine phosphoramidite was prepared as described in [16]. After the assembly of sequences, ammonia deprotection was performed overnight at 55°. Oligodeoxynucleotides were purified by polyacrylamide gel electrophoresis (PAGE; see below).

Oligodeoxynucleotide Purification. Oligodeoxynucleotides were purified using denaturing gel electrophoresis. The gels contained 20% acrylamide (acrylamide/bisacrylamide 19:1) and 8.3M urea, and were run at 55° on a *Hoefer SE 600* electrophoresis unit. The running buffer comprised 89 mM *Tris* base, 89 mM boric acid, and 2 mM EDTA at pH 8.0. The sample buffer contained 10 mM NaOH, 1 mM EDTA, and a trace amount of *Xylene Cyanol FF* tracking dye. Gels were stained with ethidium bromide (EB), and the target band was excised and eluted in a soln. containing 500 mM $AcONH_4$, 10 mM $(AcO)_2Mg$, and 1 mM EDTA. The eluates were extracted with BuOH, which removes EB, followed by EtOH precipitation.

Table. *Sequences of Oligonucleotides Prepared*

Entry	Name	Sequence (5′–3′)[a])
1	A1	GATGGCGACATCCTGCCGCTATGATTACACAGCCTGAGCATTGACAC
2	A2	GTAGCGCCGTTAGTGGATGTC
3	A3	TGTAGTATCGTGGCTGTGTAATCATAGCGGCACCAACTGGCA
4	A4	GACTGCGTGTCAATGCTCACCGATGCAACCAG
5	A5	CTGACGCTGGTTGCATCGGACGATACTACATGCCAGTTGGACTAACGG
6	B1	CGCTACCGTGCATCATGGACTAACCAGTGCTCGCTGATTTTTCAGC-GAGTTACCGCATCGGACAGCAGC
7	B2	CGTCAGGCTGCTGTGCTCGTGC
8	B3	AGTACAACGCCACCGATGCGGTCACTGGTTAGTGGATTGCGT
9	B4	GCCATCCGTCGATACGGCACCATGATGCACG
10	B5	GCAGTCGCACGACCTGGCGTCTGTTGGCTTTTGCCAACAGTTTGTAC-TACGCAATCCTGCCGTATCGACG
11	B1-thiol	CGCTACCGTGCATCATGGACTAACCAGTGCTCGCTGATT**X**TTCAGC-GAGTTACCGCATCGGACAGCAGC
12	B5-thiol	GCAGTCGCACGACCTGGCGTCTGTTGGCTT**X**TGCCAACAGTTTGTAC-TACGCAATCCTGCCGTATCGACG

[a]) **X** = 2-(*tert*-Butyldisulfanyl)ethyl-dC

Formation of H-Bonded Complexes and DNA Lattices. Complexes were formed by mixing a stoichiometric quantity of each strand, which was estimated by measuring the optical density at 260 nm. All ten strands (A1 – A5 and B1 – B5) were mixed in 10 mM HEPES (pH 7.8), 12 mM $MgCl_2$, and 2 mM EDTA. The final concentration of DNA was 0.2 – 0.4 μM. The final volume was 50 μl. Mixtures were annealed from 90° to r.t. for 40 h in a 2 l water bath insulated in a styrofoam box. Prior to the array formation, the correct assembly of tile A and modified tile B* was checked by nature PAGE.

AFM Imaging. A sample of between 5 – 7 μl was spotted on freshly cleaved mica (*Ted Pella, Inc.*) and gold, resp. The arrays were imaged in tapping mode in a buffer. The sample was deposited for 1 – 3 min, and an additional 35 μl of fresh buffer was added to both the mica and gold, and to the liquid cell. All AFM imaging was performed on a *NanoScope III* (*Digital Instruments*) using commercial cantilevers with Si_3N_4 tips.

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