Characterization and modulation of the hierarchical self-assembly of nanostructured DNA tiles into supramolecular polymers†‡

Marco Brucale,[a,c] Giampaolo Zuccheri,*[a,c,d] Luca Rossi,[b,e] Armando Bazzani,[b,e] Gastone Castellani[b,e] and Bruno Samorì[a,c,e]

Received 24th April 2006, Accepted 5th June 2006
First published as an Advance Article on the web 19th June 2006
DOI: 10.1039/b605744f

We present a set of DNA supramolecular architectures based on the polymerization of discrete DNA tiles having the shape of parallelograms and designed to have a one-dimensional inter-tile connectivity. Tiles bind to each other with two connections, which have different thermal stabilities. We discuss how this difference in stability implies that the same monomeric tile can yield supramolecular polymers of different shapes just by changing the polymerization conditions. We show how this system reacts to external stimuli by interconverting between some of its possible states. Concurrently, we show how performing the polymerization on a surface can influence its outcome.

Introduction

It is becoming increasingly evident that a convergence of the bottom-up and top-down construction strategies will prove of fundamental importance for the production of functional nanotechnological devices.[1–3]

In the last two decades, nucleic acids received an increasing amount of attention as an ideal tool for molecular fabrication tasks due to their extremely rich self-assembly capabilities. The reversible coupling of DNA molecules is governed by a highly programmable informational code, the rules of which are well-known. The resulting DNA helixes are extensively characterized objects with respect to their geometrical and mechanical properties.

An ample library of novel DNA motifs[4] has been described in the literature, which can be combined to create geometrically defined nanoscale architectures by design.[5] Since these architectures can be quite easily decorated with different objects such as metal nanoparticles, proteins, carbon nanotubes, or organic dyes,[6–15] structural DNA nanotechnology is asserting itself as a very promising way towards bottom-up construction of nanoscale structures with functional capabilities. Regardless of the existence of many structural motifs and of many proofs that they can self-assemble with high efficiency, the mechanistic knowledge of the involved processes is still fairly limited.

DNA nanoarchitectures can be designed to be monomeric or polymeric in nature. The former approach focuses on obtaining the desired shape directly in one hierarchical step from discrete oligonucleotides. Remarkable implementations of this approach appeared only recently in the literature.[16,17] Most published DNA nanoarchitectures are however designed as polymers created by the assembly of monomeric repetitive units, often called 'tiles,' capable of binding to each other in a programmed way towards higher and higher hierarchical levels of structural organization.[5] The overall shape of the architecture is critically dependent on the topological connectivity of the tiles. This means that tiles that are structurally very similar can assemble into radically different architectures thanks to the choice among possible connectivities. For example a line (1D connectivity) or a plane (2D connectivity) can be obtained with virtually the same tile.[10,18,19] Structures obtained by the 2D assembly of tiles (planes and tubes) comprise the majority of those reported in literature,[4,5] while 1D assemblies are comparatively less abundant.[5,18–21]

At each level of hierarchy, all the aforementioned DNA architectures are bound by reversible interactions, thus they can also be viewed as supramolecular polymers.[22,23] It has been shown that the polymerization of DNA-based monomers yields well-behaved, reversible polymers, the structure of which depends on a variety of factors including the strength and specificity of the association, the rigidity of the monomers, their concentration, and the environment in which the polymerization occurs.[24] This implies that DNA supramolecular polymers can be classified as 'constitutionally dynamic materials,'[23,25] as their organization is never permanently defined but it can change in response to the environment. Rarely, the attention has been focused on the sequential order of formation of cohesive-end connections with different stabilities and on the effect that this has on the final result of the polymerization

Herein, we present a family of DNA supramolecular architectures based on the polymerization of discrete DNA tiles having the shape of parallelograms[20] and designed to have a one-dimensional inter-tile connectivity. We show how one type of monomer can

[a]*Department of Biochemistry "G. Moruzzi" University of Bologna, Via Irnerio, 48, Bologna, Italy 40126. E-mail: Giampaolo.zuccheri@unibo.it.; Fax: +39 0512094387; Tel: +39 0512094388*
[b]*Department of Physics and National Institute for Nuclear Physics, University of Bologna, Italy*
[c]*National Center on nanoStructures and bioSystems at Surfaces (S3) of INFM-CNR, (Modena), Italy*
[d]*National Consortium of Materials Science and Technology (INSTM), Italy*
[e]*Interdepartmental Center "C. Galvani" for the Integrated Studies on Bioinformatics, Biophysics and Biocomplexity at the University of Bologna, Italy*

† This paper was published as part of a themed issue on DNA-Based Nano-Architectures and Nano-Machines.

‡ Electronic Supplementary Information (ESI) available: Cooking recipe for taralli with fennel seeds. See 10.1039/b605744f

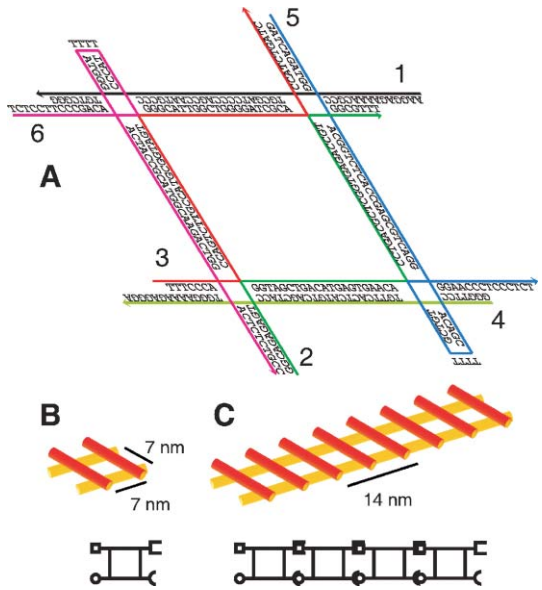
yield classes of supramolecular polymers of different shapes and size just by changing the polymerization conditions. We also show how changing the base composition by just one base pair (among 142) in the monomer leads to dramatically different behavior. Moreover, we show how this system adapts in response to external stimuli by interconverting between some of its possible states.

The described system could prove an interesting model for extending the knowledge of self-assembly processes.

Results

Design of a structurally controlled supramolecular polymer based on nanometric DNA parallelogram tiles

A mechanically rigid and structurally controlled supramolecular polymer is obtained thanks to the assembly of flat and rigid parallelogram-shaped tiles (see Scheme 1) as first suggested by Seeman and coworkers.[20] Each tile self-assembles into a single possible structure from the designed interaction of six different oligonucleotides. The assembly occurs on cooling the solution from a state where no interaction is stable to one where individual tiles form (below the melting temperature, T_m, of the extended interactions amongst oligonucleotides). A slow cooling rate ensures that the assembly takes place at equilibrium and only the most stable adduct forms.



Scheme 1 (A) Oligonucleotide sequences and structure of a DNA parallelogram tile. The tile depicted here has two pairs of 7-nt-long sticky ends with different sequences and base composition and thus a different thermal stability. In this study, we also employed another parallelogram tile, identical to the previous one except for the sequence of one pair of sticky ends, which is in this case designed to have the same base composition of the other pair of sticky ends, but a different sequence. (B) 3D model of one parallelogram tile, where helices are represented as cylinders. Below, its schematic representation used in the next schemes. (C) 3D model of a one-dimensional array of parallelograms linked to each other by means of both pairs of sticky ends (and schematic representation below).

As showed in Scheme 1, each tile is made of 4 four-way junctions flanking two turns of double-stranded DNA (dsDNA). On two opposite sides, connections are predisposed through 7-nucleotide (nt) long single-stranded DNA oligonucleotides, commonly named 'sticky ends,' protruding from 7-base pair (bp) long dsDNA segments. The T_m of these dsDNA segments is considerably lower than that characterizing the extended interactions that hold the tile together. The marked difference between these two regions of thermal stability makes the assembly hierarchical and enables the separation (in time or in space) of the two assembly events. In our experiments, this feature allowed us to assemble the tiles in optimal conditions, and then study the polymerization in different conditions.

As shown in Scheme 1C, the assembly resulting from polymerization of one tile around its two couples of aligned "sticky" ends is a 1D rail-like structure that should be rigid due to the mechanical coupling among all the possible deformations. In-plane deformations seem to be particularly difficult. Compared to previous implementations of this same DNA motif,[20] we implemented a smaller tile, thus a smaller separation between the junctions (two turns compared to four) and longer, more stable sticky ends in order for the assembly to result more rigid and stably connected.

The resulting assembled 1D rail-like system has two rows of 4-way junctions separated by 7 nm along the longitudinal axis of the chain, and each couple of junctions is spaced 7 nm on the width of the chain. Each repeating unit thus occupies 14 nm of length along the chain (see Scheme 1). The joining of two tiles around only one of the two possible connections should guarantee that the minimum energy structure is still the same flat rail as for the doubly-connected system, with the significant difference that the structural flexibility would be so much higher that many different conformations would be available to the incompletely connected polymers.

In the final, completely connected polymer, each tile is linked to another by up to 35 hydrogen bonds, plus the stacking energy of the newly-formed base pairs. The bonds are reversible, implying that the degree of polymerization can be changed by thermal treatment or modifications of the concentration of macromonomers.

Assembling parallelograms with two sticky ends having the same sequence

Our first attempt towards the making of rigid and linear rail-like polyparallelogram structures was made by joining two consecutive parallelograms by means of two 7-bp long dsDNA segments with the same base sequence. The two sticky ends on both sides of the tiles have the same sequence but different orientation (one has a 5′-overhang, while the other a 3′-overhang).

We performed a thermal assembly of a 0.1 μM solution of all the constituent oligonucleotides from 90 °C (where all interactions are unstable) to 15 °C (where polymers should be stable) at a cooling rate of 0.01 °C s^{-1} (the entire process takes slightly over 2 hours). The success and efficiency of the assembly was verified *via* electrophoretic analysis.

The resulting DNA nanostructures were observed with the atomic force microscope (AFM) at room temperature after spreading at 20 °C. The AFM analysis gives information on the structure and size of polymeric chains. As can be seen in the AFM micrographs (a sample is shown in Fig. 1), the assembly proceeds with high conversion in the conditions employed, as very few dot-like monomeric objects are observed (some dot-like single parallelogram objects have been characterized by us before).[5]

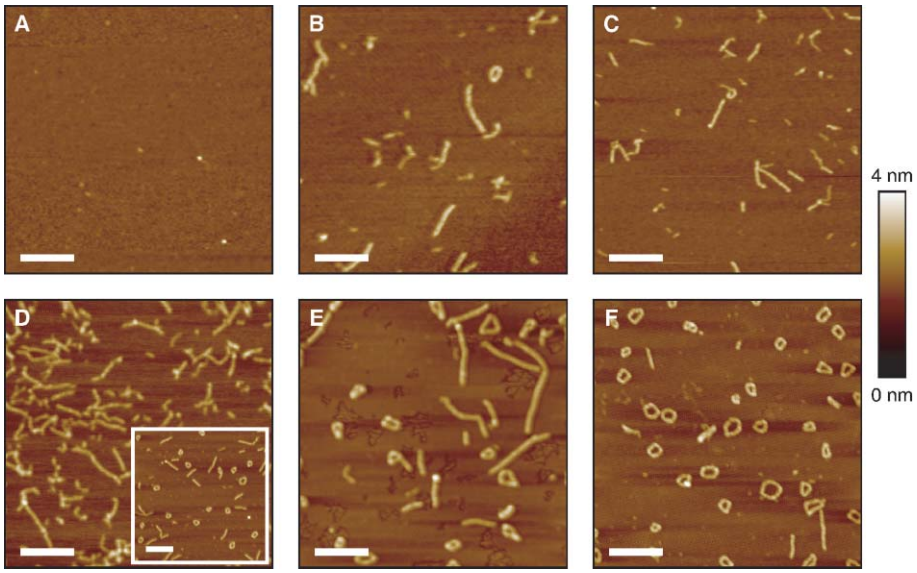


Fig. 1 AFM images representative of the result of the parallelogram polymerization in different conditions. (A–C) Annealing from 50 °C to 20 °C of the tile in which both pairs of sticky ends have the same thermal stability, at the same rate (0.01 °C s^{-1}) but different monomer concentrations: from left to right 1 nM, 10 nM, and 100 nM. (D–F) Annealing from 50 °C to 20 °C of the tile in which the two pairs of sticky ends have a different thermal stability at the same concentration (100 nM) but different cooling rates: from left to right 3.00 °C s^{-1}, 0.01 °C s^{-1}, approx. 0.0003 °C s^{-1}. The inset of panel D shows the result of a brief (5 min) heating of the same sample at 37 °C. All the scale bars of the images measure 200 nm.

The images show straight or slightly curved rod-like shapes, rods with short branchings and a very limited number of small ring-like cyclical objects (cyclical structures have an average contour length of 87 nm and represent as little as 2% of the imaged objects). The average dimension of the rod-like objects is 106 nm (66% of the sample) thus, considering a repeating unit of 14 nm, 7.6 units. Branched or otherwise "imperfect" rail objects represent about 32% of the population, while they probably represent the majority of the mass content of the sample, since they have a bigger size than rods and circular shapes.

All imaged objects show chains with a constant width compatible with the width of a parallelogram, demonstrating that, as designed, the connection among the tiles is one-dimensional.

Assembling parallelograms with same sticky ends at different oligonucleotide concentration

The above-described assembly-by-polymerization was performed also at reduced concentrations. Taking advantage of the hierarchically separated assembly steps, the concentration of preformed but disjointed parallelogram tiles was adjusted at 50 °C and then the temperature was lowered to 20 °C. Concentrations of 1, 10 and 100 nM were attempted. The results (reported in Fig. 1A–C) demonstrate that at 1 nM concentration, tiles are so dilute that they do not assemble at all in the conditions of the experiments, while at 10 and 100 nM, the assembly produces virtually the same results (AFM imaging is performed immediately after dilution to the same concentration) with comparable resulting topology (branching) and chain size.

Slow assembly of a DNA parallelogram with two different sticky ends

In order to improve and further characterize the assembly, we designed and implemented a polyparallelogram system based on a tile with two different pairs of sticky ends. The sticky ends have the same length while the base content is slightly different: one sticky end forms one G–C pair more than the other. This implies an approximately 2 °C melting temperature difference between them (see Scheme 1A). The size and sequence of the rest of the parallelogram tiles is exactly the same as that of the previously reported experiments.

The obtained single parallelograms have been assembled with the same procedure as described above (100 nM oligonucleotide concentration, 0.01 °C s^{-1} cooling rate from 90 °C to 20 °C). As evidenced by electrophoretic analysis, the assembly efficiency is comparable with the former case. The AFM study of the assembled products exhibits only two topologies of objects: rods and ring-like shapes of varying size (see Fig. 1E). By careful examination of high-resolution micrographs, it appears that the ring-like cyclical objects are not smoothly circular, but instead have curved and straight sections of varying number and extension. For this reasons, we will from now refer to them as "taralli," as they resemble the shape of these regional Italian bread-like snacks (see supplementary information for details‡). By digitization of the AFM images,[26] we could characterize the average size of the assemblies and the ratio between rods and taralli. Data have been obtained from 3441 objects, 31% of which have a rod-like shape and a median length of 96 nm *i.e.* 6.9 repeating units, while the other 69% are taralli, with a median length of 108 nm, *i.e.* 7.7 repeating units.

Very slow assembly of a DNA parallelogram with two different sticky ends

The same experiment in the same conditions was repeated with an ultra-slow cooling rate. The high temperature mix was taken from 90 °C to room temperature over a three-day time (0.0003 °C s^{-1},

approximately) by sealing a 2 l beaker of boiling water containing a floating tube with the mix in a thick styrofoam box and leaving it untouched at room temperature for 3 days.

The AFM imaging of this specimen revealed an overwhelming prevalence of taralli and very few rods (see Fig. 1F for a sample AFM image). After digitization of the molecule profiles, only 70 rod-like object (11% of the sample) out of 642 counted objects. Rod-like objects have a median length of 82 nm (*i.e.* 5.9 repeating units), while taralli shapes, 89% of the sample, have a median length of 116 nm (*i.e.* 8.3 repeating units) thus slightly longer than with the faster cooling rate described above.

Very fast assembly of a DNA parallelogram with two different sticky ends

The constituted DNA parallelograms with different sticky ends were assembled at high rate through a cooling rate of 3 °C s^{-1}.

Curiously, a distribution of shapes and topologies very similar to the result of assembly of DNA parallelograms with two sticky ends with the same stability and sequence is obtained. Branched and imperfect linear structures are obtained (Fig. 1D). Very few cyclical taralli structures are obtained. An accurate quantitative description of this type of system is arduous: from the visual inspection of a few images, it appears that 47% of the objects are rod-like, 3% only are taralli, while 50% of the objects are branched structures (comprising evidently imperfect rods).

In a qualitative type of experiment, we treated the same specimen by keeping it at 37 °C for a few minutes. As evident from the inset of Fig. 1D, the assemblies reorganize and the system then resembles the results of a slow assembly, with more perfectly assembled rods and a growing proportion of taralli, while branched structures tend to disappear. From the visual inspection of a few AFM images, 51% of the images objects are rod-like and 37% are taralli and only 12% are left as branched shapes.

Preliminary results on the assembly of parallelograms on a surface

A preliminary attempt at assembling the polymers directly on the surface of mica was performed by layering a solution of parallelograms at a concentration sub-critical for polymerization (1 nM, see above) at a temperature lower than the melting temperature of both sticky ends.

Over different intervals, the growth of polymers was quenched and characterized by AFM. The resulting structures are exclusively straight rods (no taralli or branched structures) of a size proportional, on average, to the allowed growth time. At any time, also individual monomeric objects are visible on the surface, together with longer polymeric objects (see Fig. 2).

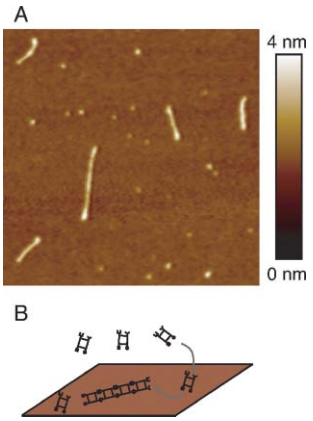


Fig. 2 (A) AFM image of the result of parallelogram polymerization on mica. A 1 nM solution of the tile with two different sticky ends in TAE–Mg^{2+} buffer was layered on a freshly cleaved mica surface at 20 °C and incubated for 10 minutes. AFM imaging of the same solution not exposed to the mica surface evidenced the complete absence of polymerization. Micrograph side is 1 μm. (B) Proposed scheme for the polymerization on the surface, showing monomers continuously adsorbing on the mica surface and binding to each other by lateral diffusion.

Discussion

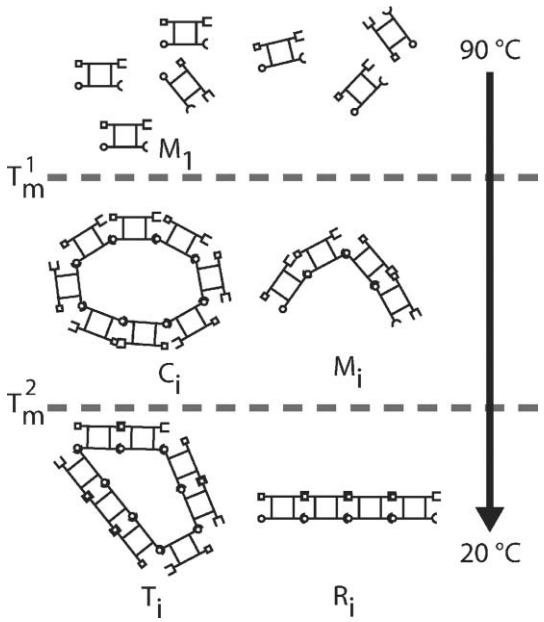
The DNA rhombus or parallelogram motif has been described first by Seeman and coworkers and used for the creation of 1D and 2D arrays.[20] It appears that this is not one of the most efficient and treatable DNA structural motifs, and, especially, 1D arrays have seldom been obtained with high efficiency. In the last few years, this motif has been used less and less, in favour of several others that can yield the assembly of micrometer-long 1D (and 2D) objects.[18,27] Even with its inherent limitations, this tile, more than many others, allows a high degree of structural control, as with very little changes in the structure and base sequence it permits the assembly in 1D, 2D or branched topologies (and also in cyclical ones, as shown here). The concept of hierarchical assembly is also directly applicable.[5] With respect to the published constructs based on the DNA parallelogram, we implemented significantly smaller tiles (with 2 double helical turns separating the 4 junctions, instead of 4, more commonly used by Seeman and coworkers) that are connected through longer sticky ends (7 nt instead of 4) in an attempt to generate more stable and more mechanically rigid polymeric structures.

From our reported data, it is evident that the assembly of DNA parallelogram macromonomers into supramolecular polymers is an efficient process, and while the size of the assembled objects does not compare to what was recently achieved with helix bundles[18] or various types of 2D systems, the conversion from single parallelograms to polymers seems complete under the investigated conditions. It is also apparent that the assembled structures are characterized by a high degree of structural rigidity, due to the designed mechanical coupling between all the possible deformations of the chain. Even though the polymeric chain is highly nicked (as it is made of many separated oligonucleotides), it appears to have a persistence length in the order of at least several hundred nanometers, probably at least one order of magnitude higher than dsDNA. For even the longest imaged 1D polymers, it appears that the orientational correlation along the chains is not lost, and so the persistence length is higher than the chain contour length.

As data obtained in various conditions show, the self assembly mechanism is rather complex, possibly involving thermodynamic and kinetic effects, and it is possible to direct its results to the formation of linear or circular or branched structures only by the subtle change of such tested variables as the cooling rate, the oligonucleotide concentration, and the insertion of point mutations in the sequence that forms the inter-tile connections.

On the structural origin of rods and taralli

The design of the monomers implies that the maximization of the interactions among them affords a linear, rigid structure where each parallelogram is linked to the successive by means of both of the sticky ends on one side. The occurrence of circular polymeric structures with straight sections (taralli) plausibly derives from the cyclization of a sufficiently long and flexible linear chain possibly formed by linking each parallelogram monomer only through one of the two sticky ends on each side (see Scheme 2). This must happen before the second, unutilized couple of sticky ends binds, and so rigidifies the chain, impairing its circularization. Once a flexible chain of singly-connected tiles is circularized, the joining of a subset of all the free sticky ends is still possible, and it leads to straightened sections of rail-like structure within circular objects. The rigid rail-like domains are separated by more flexible and incompletely connected domains that are necessary for the chain to change direction in the circular shape.



Scheme 2 Schematic representation of the polymerization of the parallelogram monomer with sticky ends having different melting temperatures, $T_m^1 > T_m^2$. During the annealing, the system spends a finite time during which only one of the connections can be stably joined. The only possible structures in this condition are flexible, singly-joined chains, which can remain linear (M_i) but will ultimately give rise to circular structures only (C_i). Upon approaching T_m^2 the second pair of sticky ends can join, transforming flexible linear chains M_i and flexible rings C_i into, respectively, rigid rods R_i and polygonal objects T_i. Since the amount of time elapsed at a temperature between T_m^1 and T_m^2 depends on the cooling rate, the occurrence of the different shapes in the final population of polymers observed at a temperature lower than T_m^2 is influenced strongly by the temperature program used to anneal the sample.

For the above to be a relevant phenomenon, there must be a finite time during the polymerization in which only one connection between each couple of tiles is set (and so the chain is flexible, see Scheme 2): this is certainly true when the two sticky ends on each side of a tile are different in sequence and so in thermal stability. In this case, the two melting temperatures of the two dsDNA sections formed are different: $T_m^1 > T_m^2$ so, during the cooling, a finite time is spent when one of the two sticky ends can be stably joined, while the other cannot. For the same couple of sticky ends, the slower the cooling rate, the longer this interval and thus the more chains have a chance to cyclize. At a temperature between T_m^2 and T_m^1, circularization is a favorable process for flexible chains, as it maximizes the number of base-pairs.

When the temperature nears the lower melting temperature T_m^2, the second type of connection becomes stable, leading to straight sections in the taralli and to rod-like linear chains, derived from the completion of the connections in the uncircularized fraction of the polymeric chains.

The qualitative results of a molecular-dynamics type of numerical simulation show the coexistence of linear chains and circular chains of different lengths when the simulation is run at a temperature where only one type of connection is stable (see Fig. 3A). When such a molecular conformation is quenched to a temperature lower than both melting temperatures, then all chains tend to form extended portions of rail-like structures, leading to straight and rigid objects and to polygonal circular ones (see Fig. 3B), as observed in our experiments.

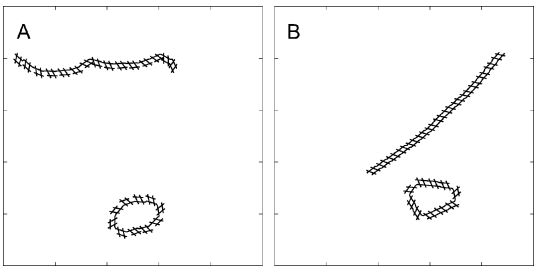


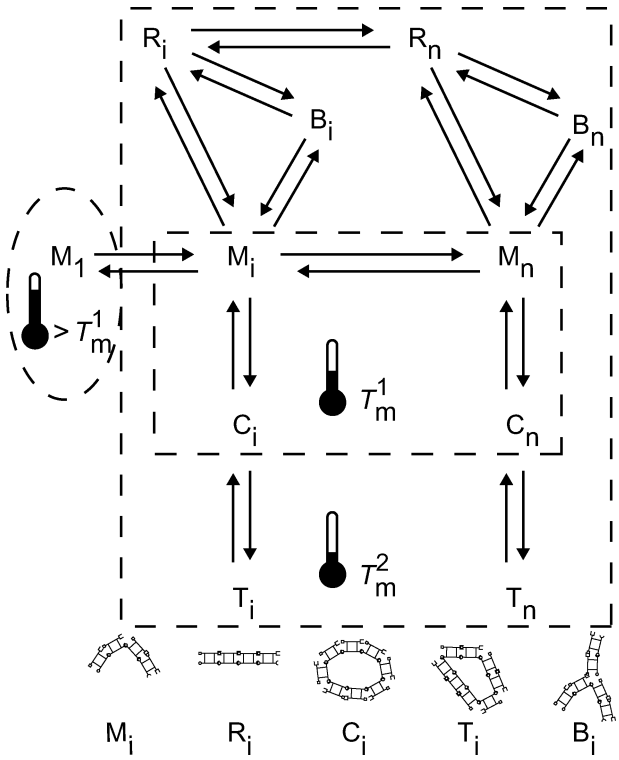
Fig. 3 Example snapshots of a molecular dynamics simulation of the parallelogram with sticky ends having different thermal stability. The side of the boxes is 350 nm. (A) Snapshot of a system composed by 25 parallelograms equilibrated at a temperature between T_m^1 and T_m^2. (B) Snapshot of the same system after equilibration at a temperature lower than both T_m^1 and T_m^2.

The bifurcation between taralli and rods and factors influencing it

As also derived on simpler polymeric systems,[28] when the assembly is done through thermodynamic equilibrium states, only cyclical shapes should be obtained. Our experiments prove this assertion, as the slower the cooling rate, the higher the fraction of taralli with respect to the other accessible shapes (see Fig. 1D–F).

A layout of the relevant concurrent reactions is proposed in Scheme 3. At a temperature higher than T_m^1, only the single parallelogram tile (M_1) can be found in solution, as no binding is thermodynamically accessible. At a temperature approaching T_m^1, the chain growth $M_1 \rightarrow M_i \rightarrow M_n$ occurs together with the equilibrium cyclization $M_i \rightarrow C_i$, where C_i represents a flexible cyclical chain, the result of the cyclization of a linear flexible chain M_i made of i monomers. Successively, at a temperature around T_m^2, each M_i can also rigidify to rod-like structures, R_i, while the C_i forms will turn into taralli shapes, described above (T_i). The thermodynamic constants of each equilibrium depend only of the temperature. Chain growth can only take place from linear chains (M_i or R_i).

If the assembly is undertaken through quasi-equilibrium states, then chain growth will proceed to the highest molecular weights



Scheme 3 Schematic outline of the polymerization reactions operating in solution. Dashed boxes include the sub-sets of reactions that are at the equilibrium at either T^1_m or T^2_m.

(M_n), and all linear chains will be converted to cyclical forms, as the temperature decreases, since the equilibria will be shifted in favor of the C_n shapes.

If, on the other hand, the assembly proceeds very fast, under kinetic control, irreversible ring closure can occur while chain size is still limited. As also the second type of connection becomes stable while the molecular weight of the chains is still low, the $M_i \rightarrow R_i$ transformation prevents the formation of larger cyclical structures (C_n) and yields a larger fraction of rods, even longer ones.

Finally, if the assembly is done at intermediate cooling rates, the result depends on both kinetics and thermodynamics. In these conditions, a more varied distribution of types of polymers is found, and the size of rods and taralli is intermediate between the two extreme cases described above. Examples of these three cases are observed experimentally (compare Fig. 1D–F and see the Results section for average sizes).

The emergence of branched structures

As of the reaction layout (Scheme 3), branched structures (B_i) can also arise. In order to obtain a branched structure in this system, it must occur that both connections are stable while in the presence of a high concentration of species that can bind to them. This can happen for parallelograms with two different sticky ends only if they are assembled under kinetic control. Under these conditions, mainly branched and straight linear forms will emerge, with a very small fraction of cyclical ones. This is experimentally witnessed in conditions of fast cooling (Fig. 1D). Branched structured can, in principle, derive also from cyclical forms, even though such structures are seldom observed experimentally.

How the supramolecular system adapts to environmental conditions

All the described polymeric forms can be reset to the monomer state simply by heating the system to above both the T_m of the connections but below the temperature leading to disassembly of the parallelograms into oligonucleotides (in our case, 50 °C was used). Moreover, as described in the Results section, the thermal equilibration of kinetically-assembled polyparallelograms at a temperature intermediate between the two T_m, can lead to a distribution of forms similar to that obtained after an exclusively thermodynamically-controlled assembly. This system proves to be adaptive to the environment and its state can be interconverted repeatedly.

Assembling polyparallelograms with sticky ends of the same thermal stability

All the above mentioned considerations withstanding, the entire reaction layout of Scheme 3 is accessible simultaneously for parallelograms with two sticky ends with the same melting temperature. Around T_m and below, the system will try to maximize all the possible interactions, leading to mainly branched and rigid linear structures. Only very small cyclical shapes are possible, as longer ones require a somewhat long singly connected chain to be present for a finite time. Due to this behavior, in this system, there is a negligible difference among the assemblies obtained at slow or fast cooling rate. We confirmed this behavior on a qualitative basis (data not shown).

It is our understanding that in principle a reduction of the monomer concentration would allow the complete formation of the double connections among tiles and thus lead to a reduced branching. We thus assembled the parallelograms at a 10-fold reduced concentration, evidencing no significant improvement in the structural regularity. A further 10-fold reduction yielded no assembly in the time scale of an experiment. In our hands, this strategy towards a better structural control still lacks an experimental proof.

When assembly takes place in a system with reduced dimensionality

As the dimensionality of the environment of a system is reduced from 3 to 2 dimensions, the degrees of freedom of motion of its components are reduced, while their effective concentration is increased.[29] If the encounter of two objects takes place through 2D diffusion from adsorbed states, numerous chain conformations are forbidden. As the parallelogram monomers and the perfectly assembled rail-like 1D chains are essentially flat objects, it is conceivable that they will be adsorbed in a condition propitious to their proper assembly.

As preliminary evidence, we layered a diluted solution of individual parallelograms on the surface of freshly cleaved mica kept at a temperature lower than the T_m of both sticky ends of the parallelograms. The concentration of monomers is such that no solution assembly is witnessed in the time scale of our longest experiments. The experimental evidence is that the growth of 1D rigid unbranched chains takes place with a good efficiency (see Fig. 2A), and chain length is proportional to the incubation time. No cyclical taralli are seen in this case, while monomers are still found on the surface at any stage of growth, as these continuously adsorb on the surface during chain growth (see Fig. 2B for a

possible route to surface growth). Further experiments will be necessary to better characterize the growth on the surface.

How to drive the system towards a higher level of structural control

On the basis of the discussed experimental evidence and the hypothesized reaction system, we can propose guidelines towards a better control of the assembly of these supramolecular chains. A system where T_m^1 and T_m^2 define two well-separated stability domains appears far more controllable than one where only one T_m regulates the assembly of both connections. In this case, the relative abundance of rods and taralli can be determined by a fast or slow cooling rate.

This system also proves to be adaptive, as expected from supramolecular polymers:[23] selective depolymerization of branched forms can be achieved, for instance by annealing at a temperature intermediate between T_m^1 and T_m^2. By heating at around T_m^1, the system is effectively reset and ready for a novel polymerization.

The control of the dimensionality of the assembly medium proves as an additional tool towards a finer level of structural control, as the growth of certain types of structures can be completely inhibited. This tool has never been used before in the context of DNA structural nanotechnology.

Our polymer could provide a DNA implementation towards the type of constitutionally dynamic materials proposed by Jean-Marie Lehn.[25]

Experimental

Sequence design

All the sequences of the oligonucleotides used in this work were designed by applying the sequence symmetry minimization feature of the program NANEV 1.1[30] run with a SSM search size of 4 and 1000 generations.

Oligonucleotides were purchased from MWG (Ebersberg, Germany), HPLC purified and lyophilized by the supplier, and suspended in MilliQ H_2O (Millipore Simplicity) prior to use. The concentration of each strand was estimated by UV spectroscopy by measuring OD_{260}.

Nanostructure assembly

DNA parallelograms were assembled in solution by mixing stoichiometric quantities of each component strand in TAE–Mg^{2+} buffer (20 mM Tris, 2 mM EDTA, 12.5 mM $MgCl_2$, pH 8.4). The final concentration of each strand was 0.1 μM. The mixtures were then heated to 90 °C for 5 minutes and cooled to 20 °C following one of the protocols described below. The slowest, 'quasi-equilibrium' annealing was performed by placing the mixtures in a 2 l water bath at 90 °C in a styrofoam box and left to cool to room temperature (around 20 °C) over approximately 72 hours, resulting in a cooling rate of ≈0.0003 °C s^{-1}. Other annealings were performed in a PCR thermocycler (PCR Sprint, Thermo Electron Corp., Waltham, MA, USA) by cooling the mixtures at a rate of 0.01 °C s^{-1} from 90 °C to 50 °C to ensure correct parallelogram formation. Then the cooling to 20 °C was completed at a rate of either 3.0 °C s^{-1} (fastest, 'kinetic' annealing) or 0.01 °C s^{-1} (intermediate case). When needed, the concentration of the monomer was changed only after performing the annealing from 90 °C to 50 °C at the usual strand concentration of 0.1 μM to ensure complete monomer formation.

Assemblies on the surface are performed after complete formation of the monomer at 0.1 μM in TAE–Mg^{2+}. The monomer solution is then diluted to 1 nM in the same buffer and equilibrated at 20 °C for a few hours. Polymerization is performed by depositing 10 μl of this solution on a piece of freshly cleaved mica and leaving it at 20 °C for at least 10 minutes.

AFM imaging

Atomic force microscopy imaging was performed in tapping mode with PointProbe noncontact silicon probes (NanoSensors, Wetzlar-Blankenfeld, Germany) on a NanoScope IIIa SFM system equipped with a Multimode head and a type E piezoelectric scanner (Veeco, Santa Barbara, CA, USA). Raw SFM images have been processed only for background removal (flattening) by using the microscope manufacturer's image-processing software. DNA molecule profiles have been measured from the SFM images with the software package ALEX.[26] Annealing mixtures were deposited on freshly cleaved mica (Ruby Red Mica Sheets, Electron Microscopy Sciences, Fort Washington, USA) and left to adsorb for 2 min at room temperature (≈20 °C). The mica surface was then rinsed with ≈500 μl of MilliQ H_2O (Millipore Simplicity) at the same temperature and dried with dry nitrogen.

Molecular dynamics simulations of parallelogram polymerization

The basic elements used to perform the molecular dynamics simulations are mass points which interact by means of a shielded Coulomb potential and are confined within a box. Four of these "particles" are rigidly linked together to form the DNA parallelogram units. Additional particles represent the heat reservoir used to set the temperature of the system. These are subjected to a type of dynamics described by the Langevin equation. Each of the four ends of a parallelogram unit can bind to the proper end of other units, with a probability that depends on the temperature. When two units bind together, restoring forces tend to optimize their conformation. It is possible to change the temperature during the simulation in order to investigate the emergence of different forms when the system is equilibrated above or below the melting temperature of one or both the sticky ends. Due to the simplifications introduced in the physical description of the system, it is possible to conveniently run simulations even on a personal computer.

Conclusions

In this experimental effort, we showed that a good degree of control can be achieved in the assembly of a 1D supramolecular polymer made of rigid DNA tiles. Very rigid rod-like structures or circular ones can be obtained from one tile only thanks to the comprehension of the inner workings of the self-assembly of this system.

As the tile design for the assembly of this 1D polymer enables further controlled branching or decoration with functional moieties, we believe that our results could prove useful towards the

realization of functional nanoscale materials, such as prototype nanoelectronic circuitry or nanoscale molecular factories.

Acknowledgements

We thank Dr Flaviana Mosca for her help in some of the preliminary experiments. We wish to acknowledge financial support from EUROCORES-SONS program BIONICS *via* funds from the Italian National Research Council (CNR), FISR D.M. 16/10/20-1999, EU FP6-STREP program NMP4-CT-2004-013775 NU-CAN, and Progetto Pluriennale 2004 Dipartimento di Biochimica, Università di Bologna.

References

1 *USA Pat.*, 6 652 808, 2003.
2 L. Mazzola, *Nat. Biotechnol.*, 2003, **21**, 1137–1143.
3 G. M. Whitesides and B. Grzybowski, *Science*, 2002, **295**, 2418–2421.
4 N. C. Seeman and P. S. Lukeman, *Rep. Prog. Phys.*, 2005, **68**, 237–270.
5 M. Brucale, G. Zuccheri and B. Samori, *Trends Biotechnol.*, 2006, **24**, 235–243.
6 C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607–609.
7 C. M. Niemeyer, *Curr. Opin. Chem. Biol.*, 2000, **4**, 609–618.
8 S. J. Park, A. A. Lazarides, C. A. Mirkin and R. L. Letsinger, *Angew. Chem., Int. Ed.*, 2001, **40**, 2909–2912.
9 K. A. Williams, P. T. Veenhuizen, B. G. de la Torre, R. Eritja and C. Dekker, *Nature*, 2002, **420**, 761.
10 H. Yan, S. H. Park, G. Finkelstein, J. H. Reif and T. H. LaBean, *Science*, 2003, **301**, 1882–1884.
11 E. Katz and I. Willner, *Angew. Chem., Int. Ed.*, 2004, **43**, 6042–6108.
12 C. M. Niemeyer, *Biochem. Soc. Trans.*, 2004, **32**, 51–53.
13 R. Singh, D. Pantarotto, D. McCarthy, O. Chaloin, J. Hoebeke, C. D. Partidos, J. P. Briand, M. Prato, A. Bianco and K. Kostarelos, *J. Am. Chem. Soc.*, 2005, **127**, 4388–4396.
14 G. Zuccheri, M. Brucale and B. Samorì, *Small*, 2005, **1**, 590–592.
15 B. Samorì and G. Zuccheri, *Angew. Chem., Int. Ed.*, 2005, **44**, 1166–1181.
16 P. W. Rothemund, *Nature*, 2006, **440**, 297–302.
17 W. M. Shih, J. D. Quispe and G. F. Joyce, *Nature*, 2004, **427**, 618–621.
18 F. Mathieu, S. Liao, J. Kopatsch, T. Wang, C. Mao and N. C. Seeman, *Nano Lett.*, 2005, **5**, 661–665.
19 S. H. Park, R. Barish, H. Li, J. H. Reif, G. Finkelstein, H. Yan and T. H. LaBean, *Nano Lett.*, 2005, **5**, 693–696.
20 C. Mao, W. Sun and N. C. Seeman, *J. Am. Chem. Soc.*, 1999, **121**, 5437–5544.
21 R. Sha, F. Liu, D. P. Millar and N. C. Seeman, *Chem. Biol.*, 2000, **7**, 743–751.
22 A. W. Bosman, R. P. Sijbesma and E. W. Meijer, *Mater. Today*, 2004, **7**, 34.
23 J. M. Lehn, *Prog. Polym. Sci.*, 2005, **30**, 814.
24 E. A. Fogleman, W. C. Yount, J. Xu and S. L. Craig, *Angew. Chem., Int. Ed.*, 2002, **41**, 4026–4028.
25 J. M. Lehn, *Science*, 2002, **295**, 2400–2403.
26 C. Rivetti and S. Codeluppi, *Ultramicroscopy*, 2001, **87**, 55–66.
27 Y. He, Y. Tian, Y. Chen, Z. Deng, A. E. Ribbe and C. Mao, *Angew. Chem., Int. Ed.*, 2005, **44**, 6694–6696.
28 G. Ercolani, L. Mandolini, P. Mencarelli and S. Roelens, *J. Am. Chem. Soc.*, 1993, **115**, 3901–3908.
29 G. Adam and M. Delbrück, in *Structural Chemistry and Molecular Biology*, ed. A. Rich and N. Davidson, W. H. Freeman, San Francisco, 1968, pp. 198–215.
30 R. P. Goodman, *BioTechniques*, 2005, **38**, 548–550.

