From Ribbons to Networks: Hierarchical Organization of DNA-grafted Supramolecular Polymers

Yuliia Vyborna, Mykhailo Vybornyi and Robert Häner*

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern Switzerland

Supporting Information Placeholder

ABSTRACT: DNA-grafted supramolecular polymers (SPs) allow the programmed organization of DNA in a highly regular, one-dimensional array. Oligonucleotides are arranged along the edges of pyrene-based helical polymers. Addition of complementary oligonucleotides triggers the assembly of individual nanoribbons resulting in the formation of extended supramolecular networks. Network formation is enabled by cooperative coaxial stacking interactions of terminal GC base pairs. The process is accompanied by structural changes in the pyrene polymer core that can be followed spectroscopically. Network formation is reversible and disassembly into individual ribbons is realized either via thermal denaturation or by addition of a DNA separator strand.

The creation of functional nanoscale structures represents a major goal of today’s nanotechnology. DNA-based materials are of primary interest for the construction of functional platforms.1-4 Proper choice of the nucleotide sequence provides control over aromatic stacking and hydrogen bonding interactions,5-7 thus enabling the assembly of systems with a high degree of complexity.8-11 Approaches towards the preparation of functional DNA materials include the designed DNA self-assembly,12-15 the grafting of oligonucleotides onto metal nanoparticles (NPs)16 and other surfaces,17-19 as well as polymers.20-23 The latter class, DNA-grafted polymers, has been pioneered by Nguyen and Mirkin and gained increasing attention over the last years.24,25 We have recently introduced DNA-grafted supramolecular polymers (SPs).26 These self-assembled structures appear as one-dimensional (1D) ribbons, consisting of an oligopyrenotide core27 with arrays of single-stranded oligonucleotides appended onto its edges. The non-covalent nature of SPs brings the additional feature of reversibility of the polymerization process.26-34 Furthermore, it enables the formation of polymers with a high DNA grafting density.35,36 Herein we describe the hierarchical organization of DNA-grafted SPs. It is shown that individual ribbons assemble into extended networks through a highly cooperative mesh of DNA blunt end stacking interactions.

Chimeric oligomers Py-a, Py-b and Py-c (Scheme 1) are all composed of a heptapyrenotide part and an appended oligonucleotide. They were prepared via solid-phase synthesis, purified by RP-HPLC and characterized by MS (SI). The two complementary oligonucleotides 1a (separator strand) and 1b (connector strand) have the same nucleobase sequence as the respective corresponding chimeric oligomers Py-a and Py-b; 1c is complementary to the oligonucleotide part of Py-c.

Scheme 1. Left: Sequences of chimeric oligomers and oligonucleotides; top right: chemical structure of phosphodiesters-linked pyrene part; bottom right: illustrative representation of chimeric oligomers.

Figure 1. A) UV/vis of Py-a at 20°C (black) and 95°C (red). Temperature-dependent change of absorbance at 305 nm of Py-a (B), Py-a*1b (C) and Py-a + 1a (D). Arrows indicate cooling and heating. Cooling (black) and heating (red) were performed using a 0.1°C/min ramp. Conditions: 2 μM Py-a and 6 μM of 1b (C) or 1a (D); 10 mM phosphate buffer, pH=7.0, 250 mM sodium chloride. The insets in (B)-(D) show the first derivatives of the corresponding curves. The full width at half-maximum
The 305 nm band (Figures 1B-C; for monitoring at 260 nm see SI, Fig. bly/disassembly process is most conveniently followed by changes of annealing), suggests a high degree of cooperativity. Also this process is for a system containing reversible and shows hysteresis. In contrast, annealing/melting curves reflecting a conformational reorganization of the supramolecular pyrene below 30°C (Figure 1C). The change in the intensity of the J-band corresponds to the J-band of assembled pyrenes. 2 μM solution of Py-a in aqueous buffer (10 mM sodium phosphate, pH=7.0 and 250 mM sodium chloride) is cooled from 95° to 20°C using a gradient of 0.1°C/min. Stacking interactions between pyrenes drive the self-assembly of polymeric ribbons. The polymerization process leads to the development of two distinct absorption bands in the UV/vis spectrum at 335 (H-band; S0 →S1 transition) and 305 nm (J-band; S0 →S2 transition; see Figure 1A). The assembly/disassembly process is most conveniently followed by changes of the 305 nm band (Figures 1B-C; for monitoring at 260 nm see SI, Fig. S11). Figure 1B shows the assembly of ribbons from Py-a upon cooling. The polymerization occurs via a single transition that starts at approx. 85°C. The process is reversible, albeit some hysteresis is observed. Surprisingly, if the same procedure is performed in the presence of the complementary oligonucleotide 1b, a second transition appears below 30°C (Figure 1C). The change in the intensity of the J-band reflects a conformational reorganization of the supramolecular pyrene backbone. The sharpness of this transition, characterized by the full-width at half-maximum (fwhm) of 1.5°C (melting) and 2.5°C (annealing), suggests a high degree of cooperativity. Also this process is reversible and shows hysteresis. In contrast, annealing/melting curves for a system containing Py-a and the non-complementary oligonucleotide 1a exhibit only a single transition below 85°C, which coincides with the formation of Py-a nanoribbons (Figure 1D).

DNA-grafted SPs are typically performed by slow annealing. Thus, a 2 μM solution of Py-a in aqueous buffer (10 mM sodium phosphate, pH=7.0 and 250 mM sodium chloride) is cooled from 95° to 20°C using a gradient of 0.1°C/min. Stacking interactions between pyrenes drive the self-assembly of polymeric ribbons. The polymerization process leads to the development of two distinct absorption bands in the UV/vis spectrum at 335 (H-band; S0 →S1 transition) and 305 nm (J-band; S0 →S2 transition; see Figure 1A). The assembly/disassembly process is most conveniently followed by changes of the 305 nm band (Figures 1B-C; for monitoring at 260 nm see SI, Fig. S11). Figure 1B shows the assembly of ribbons from Py-a upon cooling. The polymerization occurs via a single transition that starts at approx. 85°C. The process is reversible, albeit some hysteresis is observed. Surprisingly, if the same procedure is performed in the presence of the complementary oligonucleotide 1b, a second transition appears below 30°C (Figure 1C). The change in the intensity of the J-band reflects a conformational reorganization of the supramolecular pyrene backbone. The sharpness of this transition, characterized by the full-width at half-maximum (fwhm) of 1.5°C (melting) and 2.5°C (annealing), suggests a high degree of cooperativity. Also this process is reversible and shows hysteresis. In contrast, annealing/melting curves for a system containing Py-a and the non-complementary oligonucleotide 1a exhibit only a single transition below 85°C, which coincides with the formation of Py-a nanoribbons (Figure 1D).

35°C leads to the disappearance of the signal (see SI, Figure S2), which clearly shows that it is linked to the transition taking place in this temperature range. The appearance of the exciton coupled signal represents a change in the relative orientation of the transition dipole moments. This suggests that the stacking arrangement of the pyrenes and, hence, their electronic interaction is altered during the observed process. In contrast, the samples prepared either from Py-a alone or Py-a + 1a are CD silent in the 300-320 nm region (Figure 2A, black and green curves). Thus, the low temperature transition only occurs when 1b (connector strand) is hybridized to the DNA part of Py-a.

Atomic force microscopy (AFM) was used to correlate the spectroscopic data with the morphological appearance of aggregates. Supramolecular polymers were deposited and visualized on amino-modified mica surface. Self-assembly of Py-a leads to the formation of ribbons that exhibit a length of several hundred nanometers and are randomly distributed on the surface (Figure 3A). The Py-a + 1a mixture leads to identical results (Figure 3B). In the complementary Py-a*1b system, however, polymers exist as high-density networks (Figure 3C), reminiscent of haystacks in a field, rather than as individual ribbons. The formation of networks is confirmed by transmission electron microscopy (TEM; Figure S10).

The two transitions appearing in the annealing curves of Py-a in the presence of complementary oligonucleotide 1b reflect the hierarchical structural organization of chimeric oligomers. Based on the combined spectroscopic and morphologic data, we propose a model for network formation as illustrated in Scheme 2. The first transition is due to the formation of nanoribbons via supramolecular polymerization of Py-a. The second cooperative transition occurs only in the presence of the complementary oligonucleotide 1b. DNA hybridization between 1b and the single-stranded oligonucleotides grafted onto the pyrene nanoribbons results in the formation of duplexes. These duplexes are arranged along the edges of the ribbons (see Scheme 2) and contain GC base pairs at their termini. Cooperative interactions between individual ribbons through coaxial stacking of these blunt-ended GC base pairs lead to network formation. We assume that each ribbon, through short patches of a few GC basepairs, is connected to neighboring ribbons; long-range collinear stacking of ribbons is unlikely. The importance of blunt end stacking interactions for the controlled assembly of DNA nanostructures and devices is well documented.56,65,68-60 Coaxial stacking of GC base pairs is significantly stronger than of AT base pairs.45,46 This is also observed in the present case. In oligomer 1c, the two 3’-terminal bases are switched in comparison to 1b (Scheme 1). The annealing curve of oligomer Py-c in the presence of 1c shows only a single transition occurring at 80±5°C (Figure S7). Thus, the blunt-ended AT base pairs, which are formed by hybridiza-
The disassembly process is also confirmed by the disappearance of the individual nanoribbons into extended networks, only takes place in the presence of a complementary oligonucleotide. Hybridization leads to the formation of duplexes along the helical nanoribbon core. Coaxial stacking interactions of blunt end GC base pairs trigger the formation of a network in a highly cooperative process. The networks can be disassembled by destroying the coaxial stacking interactions either by heating or through addition of a separator strand. Supramolecular polymeric networks of this type may be relevant for the development of DNA-based smart materials, such as stimuli-responsive carriers of active ingredients.

**REFERENCES**

(10) Ke, Y.; Ong, L. L.; Sun, W.; Song, J.; Dong, M.; Shih, W. M.; Yin, P. *Nat. Chem.* 2014, 6, 994-1002.

**ASSOCIATED CONTENT**

Supporting Information

Additional spectroscopic (UV-vis, CD) and microscopic (AFM and TEM) data. This material is available free of charge via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

* Corresponding Author
  * e-mail: robert.haener@dcb.unibe.ch.

**ACKNOWLEDGMENT**

This work was supported by the Swiss National Foundation (Grant 200020_149148).

**Figure 3.** AFM images and illustration of supramolecular assemblies formed from Py-a (A), Py-a + 1a (B) and Py-a*1b (C). Conditions as in Figure 2.

The supramolecular nature of the interaction of nanoribbons allows reversing the network formation under isothermal condition, as shown by competition experiments. Thus, addition of the separator strand (1a, 2-fold excess over 1b) to the Py-a*1b network at 20°C results in the disassembly of the aggregates (illustrated in Scheme 2). Formation of the duplex 1a*1b (Tm=47°C, SI Figure S5) leads to the removal of the connector strand 1b from the network. Complete disappearance of the network is accomplished within 2 hours (SI, Figure S4C). AFM imaging shows only individual ribbons after addition of 1a (Figure S8). The disassembly process is also confirmed by the disappearance of the CD signal centered around 305 nm (Figure 2B).

In conclusion, we have demonstrated that chimeric pyrene-DNA oligomers assemble into extended networks via hierarchical assembly pathways. The first step, self-assembly of oligomers into helical nanoribbons, is driven by aromatic stacking interactions among pyrene units. The supramolecular polymerization is independent from the nucleotide sequence of the DNA part. The second step, aggregation of individual nanoribbons into extended networks, only takes place in the presence of a complementary oligonucleotide. Hybridization leads to the formation of duplexes along the helical nanoribbon core. Coaxial stacking interactions of blunt end GC base pairs trigger the formation of a network in a highly cooperative process. The networks can be disassembled by destroying the coaxial stacking interactions either by heating or through addition of a separator strand. Supramolecular polymeric networks of this type may be relevant for the development of DNA-based smart materials, such as stimuli-responsive carriers of active ingredients.