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Large Sequence-Defined Supramolecules Obtained by the DNA-Guided Assembly of Biohybrid Poly(phosphodiester)s

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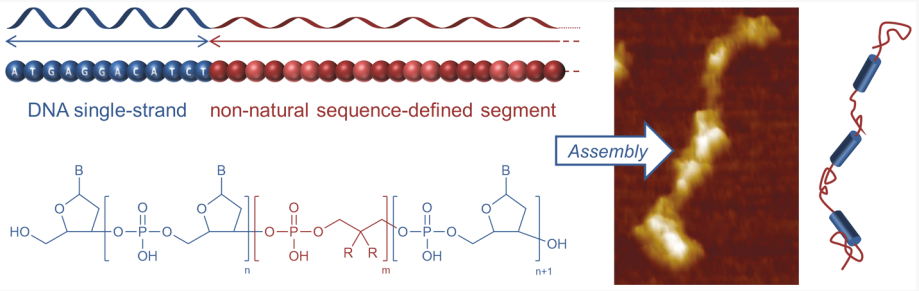

ABSTRACT: The DNA-guided assembly of biohybrid sequence-defined poly(phosphodiester)s was investigated. These polymers contain long non-natural segments covalently connected to single-stranded DNA sequences. These biohybrid structures were synthesized by automated phosphoramidite chemistry using both nucleoside and abiological phosphoramidite monomers. Using complementary DNA strands, the precursors were then assembled in aqueous buffer into linear or star-like superstructures. For instance, linear supramolecules containing 442 (352 non-natural monomers connected by three double-stranded DNA bridges of 15 base pairs) and 990 monomers (720 non-natural monomers connected by nine double-stranded DNA bridges of 15 base pairs) were prepared. A four-arm star structure containing 488 monomers (352 non-natural monomers connected by a four-arm junction of 68 base pairs) was also achieved. The formed supramolecules were characterized by electrophoresis, UV spectroscopy, and atomic force microscopy. All these techniques evidenced the formation of the expected supramolecules, although some defects were also evidenced. These results open up interesting avenues for the design of two-dimensional (2D) and three-dimensional (3D) constructs based on informational poly(phosphodiester)s.

INTRODUCTION

Since the early days of polymer science that started 100 years ago,[1,2] polymer chemists have predominantly synthesized polydisperse macromolecules, in which polymer chains have nonuniform molecular structures. Synthetic polymers are obtained via two main mechanisms, namely, step-growth and chain-growth polymerizations.[3] These approaches are straightforward but obey statistical laws leading to polydispersity. Even advanced chain-growth methods such as living anionic polymerizations and controlled radical polymerizations lead to polydisperse samples.[4,5] To achieve the chemical synthesis of uniform macromolecules, more precise, but demanding, multistep growth protocols have to be employed.[3] For example, solid-phase iterative chemistry,[6] dendrimer synthesis,[7] self-interrupted polymerization,[8] and iterative exponential growth[9] are all methods that allow the synthesis of uniform polymers. However, such multistep strategies are inherently limited by the yields of their repeated chemical steps. Thus, they are usually restricted to small-scale synthesis and to the preparation of oligomers, although a few examples of larger macromolecules have been reported.[10−14] Overall, it is challenging to prepare a perfectly uniform linear polymer chain containing more than 100 monomer units by chemical synthesis. In comparison, nature can achieve ultralong macromolecules with a perfectly uniform molecular structure. The human chromosome 1 is, for example, composed of two hybridized DNA chains, each of which contains a perfectly defined sequence of 248956422 monomers (i.e., more than 248 million).[15] It is currently impossible to synthesize (and even to conceive) a synthetic macromolecule with similar features by man-made polymer chemistry.[16,17] Here, we report a simple method to prepare large sequence-defined macromolecules. This approach combines a multistep growth phosphoramidite approach[18] with single-strand DNA hybrid-

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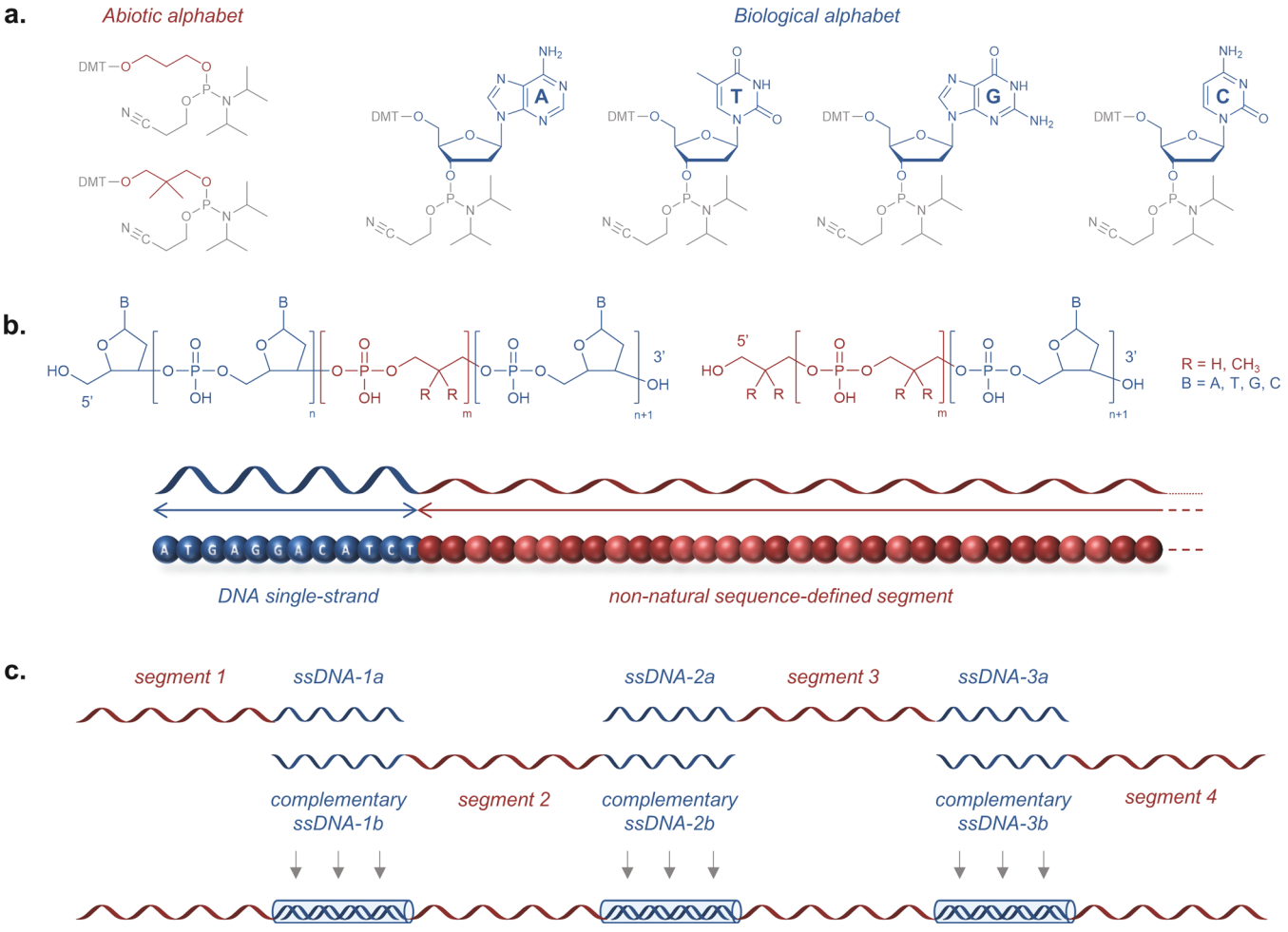

Figure 1. (a) Molecular structure of the phosphoramidite monomers used in this work. (b) Molecular structure of the different types of biohybrid poly(phosphodiester)s used for DNA-guided self-assembly. (c) General concept investigated herein for the synthesis of large sequence-defined supramolecules.

ization.[19] Using this strategy, supramolecules with different chain lengths, topologies, and sequences were prepared.

RESULTS AND DISCUSSION

Design and Synthesis of Biohybrid Polymers. Figure 1 shows the concept explored in this work. Since multistep growth approaches are limited in length by reaction yields, one possible solution to attain longer uniform macromolecules is to ligate preformed uniform precursors into a larger construct. Both covalent and noncovalent strategies may be considered for the ligation of preformed macromolecules. For instance, covalent approaches, such a native chemical ligation, have been already successfully developed for the total synthesis of large man-made proteins.[20] However, the covalent ligation of macromolecular reagents is usually affected by a kinetic exclusion effect that strongly limits the reactivity of high-molecular-weight precursors.[21] An interesting alternative to stepwise covalent ligation would be the development of a supramolecular strategy. Over the last two decades, the sequence-specific hybridization of single-stranded DNA (ssDNA) segments has been evidenced to be a versatile strategy for material self-assembly.[19] This concept can be used not only to create large two-dimensional (2D) and three-dimensional (3D) DNA constructs[22−24] but also to assemble nonbiological matter such as nanoparticles, nanotubes, and synthetic polymers.[25] In the latter case, ssDNA-hybridization has been mostly used to ligate nonuniform segments into supramolecular block copolymers.[26−28] In particular, remarkable progress in that area was achieved by Herrmann and co-workers for the synthesis of DNA (multi)block copolymers using selective hybridization[29] but also other tools such as enzymatic ligation[30] and the polymerase chain reaction.[31,32] However, to date, the construction of DNA assemblies containing molecularly uniform synthetic elements has only been investigated by Sleiman and co-workers.[33,34] Overall, DNA self-assembly has not been fully exploited for the synthesis of non-natural polymer architectures, in particular for the preparation of sequence-defined constructs.

Automated phosphoramidite chemistry is a tool of choice for preparing uniform polymers containing synthetic segments attached to DNA sequences. Indeed, both biological and nonbiological monomers can be used in this approach.[18,35,36] Thus, using a DNA synthesizer, it is possible to synthesize biohybrid macromolecules containing a long non-natural segment connected to one or two ssDNA extremities (Figure 1). Figure 1a shows the biological and nonbiological monomer alphabets used in this work to construct these polymers. The ssDNA segments were synthesized using standard commercial nucleoside phosphoramidites A, T, G, and C. To prepare the abiotic sequence-defined poly(phosphodiester) (PPDE) seg-

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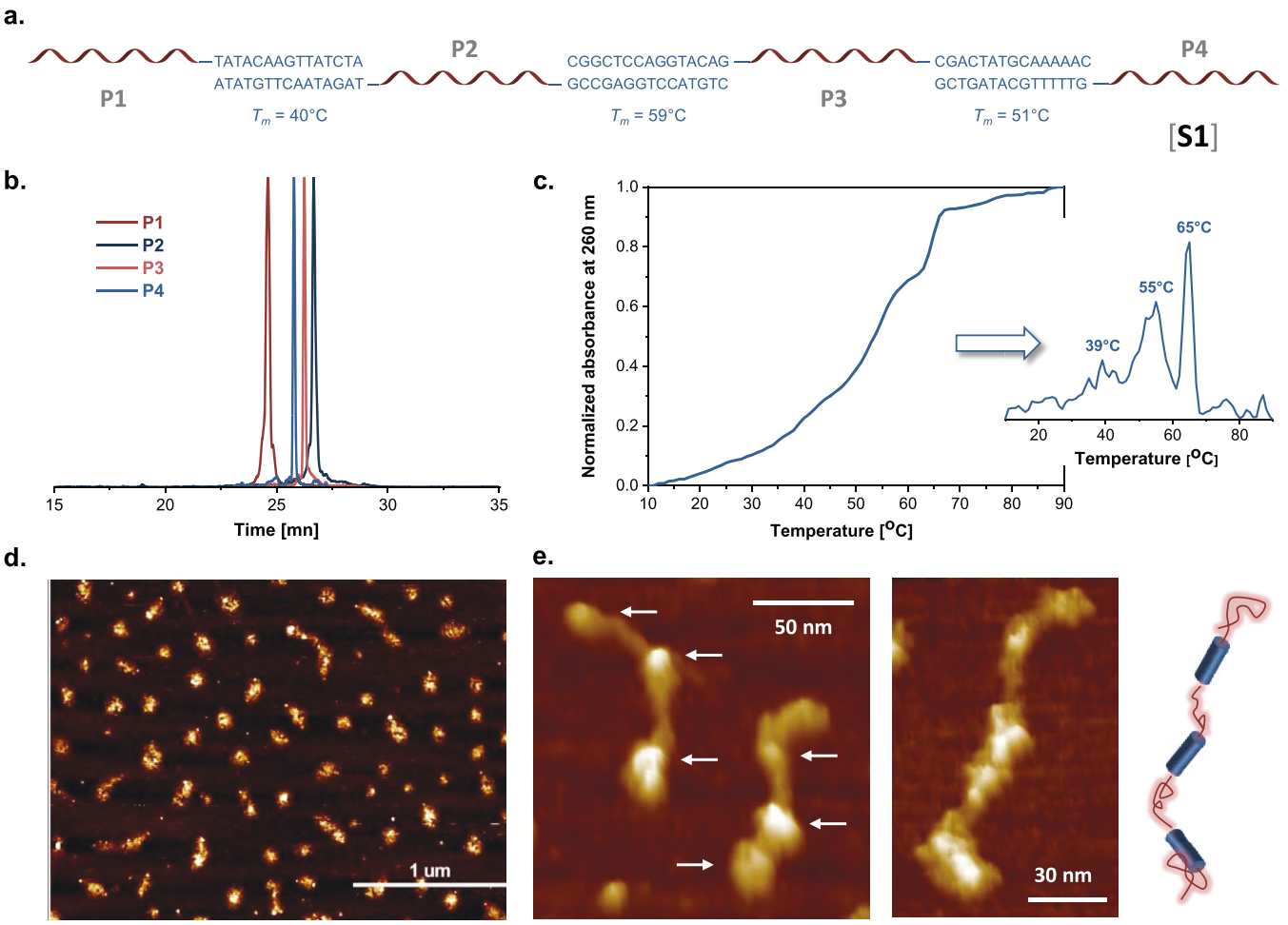

Figure 2. Linear supramolecule **S1** obtained by DNA-guided assembly of polymers **P1**−**P4**. (a) Schematized representation of the hybridized superstructure. The displayed melting temperatures T_m are theoretical values calculated with the software OligoCalc. (b) Ion-exchange HPLC traces recorded for the individual polymers **P1**−**P4**. (c) UV melting curves recorded for the assembled structure **S1**. The inset shows the first derivative of the melting curve. (d) Height AFM micrographs of **S1** recorded on a dried substrate. (e) Height AFM micrographs of **S1** recorded in aqueous solution.

ments,[37] two phosphoramidite monomers containing either propyl or dimethyl-propyl motifs were employed. These building blocks have been used in previous works for the synthesis of digital sequences[12,38] and are therefore convenient models for this first proof-of-concept. Abiotic digital poly(phosphodiester)s can be decoded using different sequencing techniques[39] such as tandem mass spectrometry[40] and nanopore sequencing.[41] Figure 1b shows the general molecular structure of the biohybrid polymers synthesized herein. Two different types of macromolecules were prepared: triblocks 5′-DNA-*b*-PPDE-*b*-DNA-3′ and diblocks 5′-PPDE-*b*-DNA-3′. In these structures, the directionality of the ssDNA sequences was adjusted to allow selective hybridization.[37] In the DNA-guided assembly process schematized in Figure 1c, the triblocks are inner segments that can be connected on both sides, whereas the diblocks are terminal outer segments. For linear constructs, the chain lengths of the non-natural PPDE and ssDNA segments were set at 88 and 15 residues, respectively. The motivation behind this choice is that although the biohybrid macromolecules contain mainly non-natural information, the oligonucleotide sequences shall remain of sufficient size to achieve sequence-specific hybridization. For nonlinear constructs, 34-mer DNA segments were utilized to achieve multistranded four-arm junctions.[42,43] The defined monomer sequences of the non-natural PPDE segments were shaped following previously reported encoding rules.[40,44] The ssDNA sequences were selected from the literature based on two main criteria: (i) sequence-selective duplex formation of complementary strands and (ii) formation of duplexes with noticeably different melting temperatures T_m to characterize the DNA-guided assembly by UV spectroscopy.[43,45,46] Table S1 shows the monomer sequence of the polymers **P1**−**P18** that were studied in this work. All these polymers were synthesized by automated phosphoramidite chemistry, purified using a reverse-phase column, and characterized by high-performance liquid chromatography (HPLC) and size exclusion chromatography (SEC) (Table S2).[12] In all cases, HPLC evidenced the formation of monodisperse biohybrid macromolecules. Due to the high chain length and high fraction of nucleotides of these polymers, it was not possible to detect most of them by mass spectrometry. However, when detected by electrospray mass spectrometry, the spectrum revealed uniform single species (Figure S1), as evidenced by HPLC results. These biohybrid macromolecules were then used for programmed DNA assembly, as described below.

Preparation of Linear Supramolecules. DNA assembly was first explored for the preparation of linear supramolecules. Polymers **P1**−**P4** were conceived for constructing a sequence-

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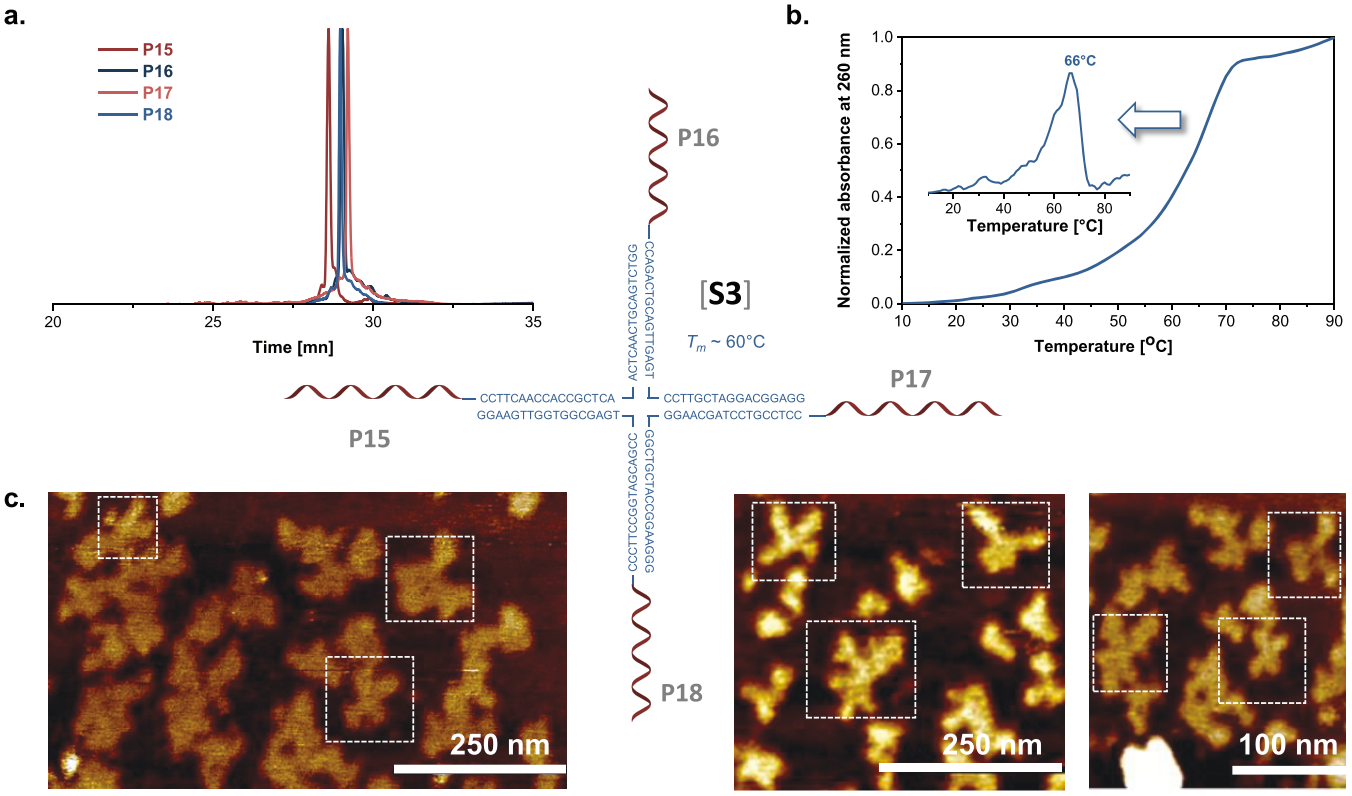

Figure 3. Star-shaped supramolecule **S3** obtained by DNA-guided assembly of polymers **P15**−**P18**. (a) Ion-exchange HPLC traces recorded for the individual polymers **P15**−**P18**. (b) UV melting curve recorded for the assembled structure **S3**. The inset shows the first derivative of the melting curve. (c) Height AFM micrographs of **S3**.

defined supramolecule **S1** containing 442 monomers, i.e., four blocks of 88 non-natural monomers connected by three double-stranded DNA sections of 15 base pairs (bp) each (Figure 2a). Assembly was performed by mixing four individual solutions (5 μ molar concentration) of the starting polymers in phosphate-buffered saline (PBS), followed by heating at 80 °C and slow cooling. The resulting superstructure **S1** was characterized by UV spectroscopy, electrophoresis, and atomic force microscopy (AFM). Figure 2c shows the UV melting curve recorded for **S1** and its first derivative. As mentioned in the previous section, the DNA sequences were selected to obtain distinguishable T_m. In the case of **S1**, the three 15 bp double-stranded DNA sections were selected from the literature[45] and, in the studied experimental conditions (PBS, pH ~ 7.4, 100 mM NaCl), have theoretical T_m of 40, 51, and 59 °C, according to the online prediction software OligoCalc. The first derivative of the experimental UV melting curve evidenced three distinct melting temperatures at 39, 55, and 65 °C, which are consistent with the reported values.[45] It shall be noted that software-predicted T_m values are only indicative and do not take into account the influence of polymer conjugation on T_m. The DNA-guided self-assembly was also confirmed by electrophoresis, which evidenced the formation of a superstructure of a much higher molecular weight than the individual precursors **P1**−**P4** (Figure S2). **S1** was also studied by AFM, which is a tool of choice for the imaging of discrete supramolecules.[47,48] The assembly solutions were diluted in deionized water and deposited on a mica substrate. After washing off salt crystals that prevent imaging,[49] individual superstructures were observed (Figure 2d). Some of these structures have a length of about 250 nm, which is consistent with the calculated **S1** contour length of an extended supramolecule. Yet, others appear folded and globular. The observed conformations might be due to the flexibility of the non-natural PPDE segments, which have a smaller persistence length than double-stranded DNA. In addition, the observation of smaller granular shapes confirms the presence of defects, which can be partially formed superstructures. **S1** was further studied by AFM in solution (Figure 2e).[50] This study indicated that the elongated structures contain long flexible domains (1 nm thick) and three shorter and thicker regions (2.4 nm thick), which is in good accordance with the thickness of double-stranded DNA and the expected morphology of **S1**.

The concept was then explored for the fabrication of larger linear constructs based on precursors **P5**−**P14** (Table S1 and Figure S3). The expected superstructure **S2** shall contain 990 monomers, i.e., ten blocks of 72 non-natural monomers connected by nine double-stranded DNA sections of 15 bp each (Scheme S1). Since it is unlikely to observe nine distinct T_m in melting curves, three groups of three double strands, which exhibit theoretical T_m around 40, 55, and 65 °C, were used.[46] Figures S4 and S5 show the UV spectroscopy and AFM characterization of the resulting superstructure **S2**, which was prepared in the same experimental conditions as **S1**. The first derivative of the UV melting curves clearly evidences three events with T_m of 36, 56, and 70 °C. Yet, AFM images (]Figure S5) show large clusters coexisting with smaller defects and therefore a pronounced dispersity. Probably due to the flexibility of the PPDE chains, the formed superstructures do not appear as elongated objects but fold into aggregated clusters.

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Preparation of Nonlinear Supramolecules. The concept described in this communication is not restricted to linear constructs. DNA hybridization also potentially enables the preparation of a wide variety of nonlinear architectures.[19,25] As a proof-of-concept, the construction of a four-arm star superstructure based on a Holliday junction was examined. The four-arm junction utilized herein is based on four ssDNA 34-mer (Figure 3) and is a non-migrating model of the biological four-way junction with well-documented conformations, dynamics, and denaturation.[43,51,52] The expected superstructure **S3** shall contain 488 monomers (352 non-natural monomers connected by a four-arm junction of 68 base pairs). In the present experimental conditions, the formed junction has a theoretical T_m of about 60 °C.[40] As shown in Figure 3b, the PBS assembly of polymers **P15−P18** leads to a superstructure **S3** with an experimental T_m of 66 °C. Electrophoresis (Figure S6) also evidenced the formation of an assembly with a different migration rate than the original precursors, even though the band of S3 is relatively faint. AFM studies also confirmed the star-like morphology and evidenced the presence of four-arm stars with an arm length of about 50 nm (Figure 3c), which roughly correspond to the expected extended length. These superstructures have a tendency to aggregate and only a few supramolecules could be imaged individually.

CONCLUSIONS

In summary, these results indicate that DNA hybridization allows the assembly of macromolecular building blocks with precisely controlled chain length and monomer sequences. Due to the versatility of automated phosphoramidite chemistry, it is relatively easy to synthesize sequence-defined biohybrid DNA/PPDE block copolymers and to assemble them into precise linear or nonlinear constructs. Yet, the quality of the assembly seems to depend on the starting number of macromolecular precursors. Although well-controlled assembly was observed with four biohybrid components, a higher amount of defects was detected when 10 components were used. Still, the method is valid and opens up interesting perspectives for the spatial organization of abiotic informational sequences, including digitally encoded polymers that show promises in data storage applications.[53] This strategy is indeed not restricted to the two nonbiological monomers used herein and can be applied to a broad variety of other phosphoramidite monomers.[35,54] Furthermore, the simple linear and star-like topologies examined in this work are only selected examples among a vast number of possibilities.[25] For example, precisely organized 2D or 3D information networks can be foreseen with this approach.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.macromol.0c02581.

Full experimental part. Tables S1−S2, Scheme S1, and Figures S1−S6 (PDF)

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Notes

The authors declare no competing financial interest.

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