# DNA multiblock copolymers†

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**Single stranded (ss) DNA block copolymers were applied to synthesize DNA multiblock architectures by hybridization; these polymeric bioorganic hybrids were characterized by gel electrophoresis and MALDI-TOF mass spectrometry.**

DNA block copolymer structures, morphologies and applications have generated considerable scientific interest over the past decade. These hybrids consist of DNA as the biological component covalently linked to organic polymer segments either in linear or graft architectures. Applications of the linear topologies range from gene delivery,[1–5] DNA detection,[6,7] and biomaterial purification,[8,9] to nanoscopic scaffolds for template directed organic reactions.[10] In extension to linear diblock structures only two A–B–A type DNA triblock architectures have been reported.[6,11] Their central organic units consisting of fluorene and ethylene oxide moieties are limited with respect to molecular weight. Furthermore, no complex DNA multiblock copolymers have been reported so far. Herein, we describe a novel concept for the fabrication of DNA multiblock architectures by hybridization. Thereby Watson–Crick base pairing is employed for the formation of triblock and pentablock structures (Fig. 1).

For the generation of DNA triblock copolymers, two ss-DNA diblock polymers were synthesized. The sequences of the two oligodeoxynucleotides (ODNs) (22 mer) were selected to be complementary to each other. The DNA–*b*-poly(ethylene glycol) (PEG) diblock copolymers were synthesized by reacting carboxyl chain-end functionalized PEGs ($M_n$ = 5000 and 20000 g $mol^{-1}$, polydispersity index (PDI) < 1.1) with 5′ amino-modified ODNs to yield the corresponding conjugates DNA–*b*-PEG(5 K) and DNA–*b*-PEG(20 K). The resulting conjugates were purified by denaturing polyacrylamide gel electrophoresis (PAGE) and characterized by MALDI-TOF mass spectrometry and HPLC (see ESI† for experimental details). The DNA–*b*-PEG block copolymers bearing complementary sequences were hybridized in TAE buffer (20 mM tris(hydroxymethyl)aminomethane–HCl, pH 8.0; 10 mM acetic acid; 0.5 mM EDTA) in the presence of 100 mM NaCl and 20 mM $MgCl_2$. Equimolar quantities of these block copolymers were mixed, heated to 95 °C and then slowly cooled to room temperature over the course of three days by using a thermocycler. The resulting triblock architectures were characterized by 5% denaturing PAGE (Fig. 2(A)). In order to assess the electrophoretic mobility, these hybrids were compared with DNA–*b*-PEG containing ss or double stranded (ds) nucleic acid segments. Lanes 1 and 2 contain the ss-DNA–*b*-PEGs where the organic polymer segment exhibits a molecular weight of 5000 and 20000 g $mol^{-1}$, respectively. Lanes 3 and 4 consist of the corresponding ds-DNA–*b*-PEGs that were generated by hybridization of the ss-DNA–*b*-PEGs from lanes 1 and 2 with the complementary ODN. Lanes 5–7 represent the triblock structures of PEG(5 K)–ds-DNA(22 bp)–PEG(5 K), PEG(5 K)–ds-DNA-(22 bp)–PEG(20 K) and PEG(20 K)–ds-DNA(22 bp)–PEG(20 K). With increasing molecular weight of the synthetic polymer segments, reduced electrophoretic mobilities were detected.

In order to realize more complex multiblock architectures containing ds-DNA, a novel building block was prepared by a straightforward synthetic route. This triblock architecture is composed of a central PEG domain ($M_n$ = 1000 g $mol^{-1}$, PDI < 1.1) onto which two identical ss-ODNs were covalently attached at their 5′ ends. These ODNs encode the complementary sequence of the ss-DNA–*b*-PEG. For the generation of the triblock copolymer, a bis-phosphoramidite functionalized PEG was synthesized and attached to the 5′ terminus of the nucleic acid fragment employing solid phase synthesis similar as reported previously.[10] This ss DNA triblock architecture was analyzed and purified by denaturing PAGE and the molecular weight was confirmed by MALDI-TOF mass spectrometry (see ESI† for experimental details). This building block was used to construct ds DNA pentablock copolymers with varying molecular weights of the terminal synthetic polymer units. These multiblock architectures were synthesized by hybridizing two equivalents of the ss-DNA–*b*-PEG with one equivalent of the ss-DNA triblock copolymer applying the same conditions as described above. The multiblock bioorganic hybrids were analyzed by denaturing PAGE (Fig. 2(B)). Lanes 1 and 2 correspond to the triblock architectures DNA–PEG–DNA exhibiting either ss or ds nucleic acid segments, respectively. Lanes 3 and 4 represent the A–B–A–B–A type pentablock structures with terminal PEG segments of 5000 and 20000 g $mol^{-1}$, respectively. Again, an increase in the molecular weight of the DNA block copolymers resulted in lower gel shifts. As an additional structural proof, MALDI-TOF mass spectrometry was used to confirm the formation of the ds-DNA pentablock structures (Fig. 3).

Multiblock copolymers are very attractive materials due to their rich varieties of morphologies in bulk and in selective solvents. However, the synthesis of well-defined multiblock architectures, usually prepared by living polymerization techniques, is difficult and laborious. These complex structures can be realized by sequential addition of monomers, the use of difunctional linking agents or difunctional initiators and by combinations thereof.[12]

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† Electronic supplementary information (ESI) available: I: General considerations. II: Synthesis of DNA–PEG diblock copolymers. III: Synthesis of phosphoramidite functionalized polymers. IV: Synthesis of ss-DNA–PEG–ss-DNA triblock copolymer. V: Preparation of multiblock architectures by hybridization. VI: Scanning force microscopy (SFM) measurements of DNA triblock copolymer micelles. See DOI: 10.1039/b615276g

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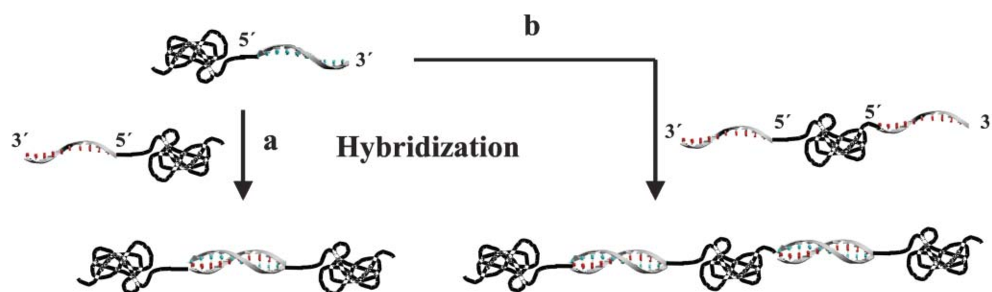


**Fig. 1** Schematic representation of building up DNA multiblock copolymers by hybridization. Fabrication of (a) triblock- and (b) pentablock architectures.

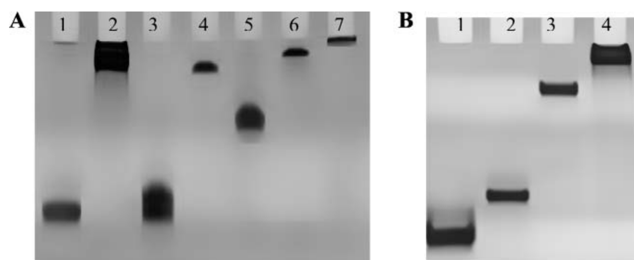


**Fig. 2** Polyacrylamide gels of the di-, tri-, and pentablock copolymers. (A) Lanes 1–4 contain diblock copolymers of ss-DNA–*b*-PEG(5 K), ss-DNA–*b*-PEG(20 K), ds-DNA–*b*-PEG(5 K) and ds-DNA–*b*-PEG(20 K), respectively. Lanes 5–7 contain the triblock architectures of PEG(5 K)–DNA–PEG(5 K), PEG(5 K)–DNA–PEG(20 K) and PEG(5 K)–DNA–PEG(20 K), respectively. (B) Lanes 1 and 2 contain the triblock copolymers of ss-DNA–PEG–ss-DNA and ds-DNA–PEG–ds-DNA, respectively. Lanes 3 and 4 contain the pentablock architectures of PEG(5 K)–DNA–PEG–DNA–PEG(5 K) and PEG(20 K)–DNA–PEG–DNA–PEG(20 K), respectively.

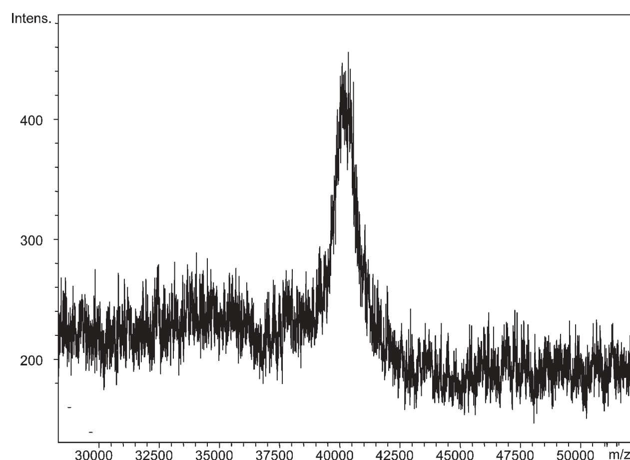


**Fig. 3** The MALDI-TOF mass spectrum of the pentablock copolymer, PEG(5 K)–DNA–PEG–DNA–PEG(5 K) (found: 40 500 g $mol^{-1}$, calc. 41 000 g $mol^{-1}$).

Nevertheless, control over the molecular weight and low polydispersity are hard to achieve.[13] Moreover, the products are sometimes contaminated with homopolymers and further purification is crucial to obtain pure materials.[12] In contrast, the assembly of DNA multiblock copolymers by molecular recognition has some striking advantages. First, contamination with homopolymers is avoided when pure ss building blocks are employed. Second, dry and inert conditions for multiblock assembly are not required. Third, highly well defined structures are obtained due to the monodispersity of the nucleic acid segments. To elucidate the resulting morphologies in a selective solvent, preliminary experiments with a ds DNA triblock copolymer, were carried out. Scanning force microscopy analysis revealed formation of inverse spherical micelles in dichloromethane (ESI†). A detailed study of the influence of the molecular parameters such as ss and ds nucleic acid segments or block length ratios on structural properties in solution as well as the investigation of bulk morphologies are the subject of forthcoming publications.

In conclusion, Watson–Crick base pairing was employed to construct multiblock copolymer architectures in a highly modular manner. In the future, this approach will be also used to connect synthetically incompatible organic polymer segments.

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