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Synthesis of DNA block copolymers with extended nucleic acid segments by enzymatic ligation: cut and paste large hybrid architectures
Synthesis of DNA block copolymers with extended nucleic acid segments by enzymatic ligation: cut and paste large hybrid architectures†

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Ultra-high molecular weight DNA/polymer hybrid materials were prepared employing molecular biology techniques. Nucleic acid restriction and ligation enzymes were used to generate linear DNA di- and triblock copolymers that contain up to thousands of base pairs in the DNA segments.

Starting in the late twentieth century, DNA has doubtlessly been one of the most extensively studied biopolymers, not only for its critical biological role but for its unique and intriguing structural properties. For instance, it has famously been utilized for the self-assembly of complex and beautiful nanostructures.1,2 The same self-recognition properties have also been widely investigated in nucleic acid hybrid materials as a powerful tool to alter material structure and other properties on demand. In a medical context, an RNA aptamer covalently bonded with poly(ethylene glycol) (PEG), known as pegaptanib (MACugen), is already available in clinics for the treatment of age-related macular degeneration of the retina.3

Indeed, the pairing of oligodeoxynucleotides (ODNs) with synthetic polymers has far-reaching potential applications, from drug delivery and diagnostics to nanotechnology.4

One innovative method to overcome this barrier is solid phase coupling, in which the hydrophobic units are attached to a polymer block, whether PEG (Mn = 20 kDa), poly(ethylene glycol) (PEG) or poly(propylene oxide) (PPO) blocks.5,6 More recently, a method from molecular biology has been applied to the generation of DNA–polymer hybrids in order to attain longer ODN blocks than those possible through chemical synthesis (~200 bases). Namely, the polymerase chain reaction (PCR), when performed using plasmid DNA and either di- or triblock DBCs as the primer, enabled the production of well-defined multiblock copolymers with double-stranded (ds) DNA blocks of ~1578 base pairs (bp) and a total number average molecular weight (Mn) of more than 1000 kDa, including the polymer.7 The introduction of a thermostresponsive polymer, poly(N-isopropylacrylamide) (PNIPAM), into the primer in this technique allowed the rapid and facile isolation of the specific, desired amplicons from the complex reaction mixture.7

Here we present a novel synthetic strategy for the preparation of ultra-high molecular weight di- and triblock DBCs with even longer DNA blocks using another molecular biology technique, enzymatic restriction and ligation. Just as one builds paper models by cutting and pasting paper strips, we have prepared large polymer–DNA architectures by digestion and ligation of DNA using the corresponding enzymes as tools (Fig. 1). The family of hydrophilic and hydrophobic polymers used in this study proves the feasibility of this method to extend DBCs by different lengths of dsDNA, from 800 to 2300 bp, yielding highly defined block architectures with molecular weights in the range of three million dalton.

The five single-stranded (ss) di- and triblock DBCs used as starting materials in this study were prepared by solution coupling or solid phase synthesis, as previously reported by our group (Fig. 1A).1,8 The diblock architectures (ODN-b-polymer) consisted of a 22mer ODN segment (see Table S1, ESI† for the sequence) where the 5′-end is covalently bonded to a polymer block, whether PEG (Mn = 5 kDa or 20 kDa, ssDB1 or ssDB2), PNIPAM (Mn = ~6 kDa, ssDB3) or PPO (Mn = 6.8 kDa, ssDB4). In the triblock architecture, a PEG (Mn = 4 kDa) block was conjugated at both termini to identical ssODNs (ODN-b-PEG-b-ODN, ssTB).

To prepare the longer DNA segments for the extension of the ss copolymers above, a circular plasmid DNA (pBR322, 4361 bp) was digested by a DNA restriction enzyme (Alw26I) into three dsDNA segments, named short (S, 772 bp), middle (M, 1311 bp) and long (L, 2279 bp). The different fragments were isolated by agarose gel electrophoresis (Fig. 1B). Each gel band of the restriction products was subsequently purified using a gel extraction kit.

Hybridization of DNA block copolymers (ssDB1-4) with complementary sequences designed to introduce short overhangs and ligation with the restricted dsDNA segments were
Fig. 1 Schematic of DBCs, the large dsDNA segments and the enzymatic extension strategy for di- and triblock architectures. (A) Four single-stranded DNA diblock (ssDB-4) and triblock (ssTB) copolymers with synthetic polymer segments. (B) Digestion of pBR322 by a DNA restriction enzyme, Alw26I (also known as BsmAI), results in three dsDNA segments (S, M and L, depicted as red, green and blue bands, respectively) of the lengths shown (unit: bp). The restricted four-nucleotide sticky ends are complementary to the S’-overhang of corresponding phosphorylated cDNAs (cDNA-S: CGGT-, cDNA-M: GGGA- and cDNA-L: CTCA-). (C) In the presence of T4 DNA ligase, the mixture of a chosen ssDB (or ssTB), the appropriate cDNA, and dsDNA segments (S/M/L) results in extended dsDB or dsTB architectures. Asterisks (*) denote the S’-ends of the DNAs.

Carried out in one pot through simple mixing and incubation. Each complementary DNA (cDNA-S, cDNA-M and cDNA-L, respectively) can be hybridized with any of the DBCs used here because the 22mer DNA blocks are the same in each. In addition to a segment complementary to the DBC 22mer sequence, each cDNA contains an additional four-nucleotide overhang at the phosphorylated S’-end for recognition of the sticky end of the desired dsDNA segment. These three DNA components, i.e. ssDB, restriction products for extension (S, M or L) and the corresponding cDNA, were mixed in the presence of T4 DNA ligase, adenosine triphosphate (ATP) and hybridization buffer and incubated at 16 °C for 48 h (see ESI for experimental details). This ligation resulted in the controlled extension of the DNA blocks (S/M/L-dsDB4, Fig. 1C).

After gel extraction of the ligation products, 15 different products and controls were characterized by agarose gel electrophoresis under ethidium bromide (EtBr) staining (Fig. 2). First, it should be noted that all bands were discrete through the entire gel, demonstrating that the materials thus produced have high purity. The success of the ligation is clear from the trend in electrophoretic mobility, which, as anticipated, was dominated by the length of the extended DNA segments. A secondary determining factor in the mobility was the nature of the polymer attached. The widest variation due to the hydrophobicity and molecular weight of the appended polymer was observed for the materials extended with the shortest segments (group S in Fig. 2A), all of which exhibited the fastest mobilities. Interestingly, the bands of the three hydrophilic polymers (S-dsDB1 to 3, lanes S1–S3 in Fig. 2A) didn’t differ significantly although the Mn of the PEG segment in S-dsDB2 is four times higher than that of S-dsDB1. Moreover, the PNIPAM conjugate (S-dsDB3) had the lowest electrophoretic mobility of the hydrophilic polymer architectures in this group in spite of the low Mn of PNIPAM used (6 kDa). Indeed, the markedly lower mobility of the PPO-containing material (lane S4, S-dsDB4) leads to the conclusion that polymer block hydrophobicity determines the movement of the whole construct under electrophoresis conditions. Similar trends with few discrepancies were observed for M- (lane M0 to M4) and L-extended (lane L0 to L4) materials. However, this phenomenon merits further investigation with ionic polymers, as the nonionic polymers used in this work do not directly alter mobility under electrophoresis conditions.

The triblock architecture dsDNA-b-PEG-b-dsDNA (dsTB) was prepared in an analogous ligation procedure and was also characterized by gel electrophoresis (Fig. 2B). Not surprisingly, the electrophoretic mobility of extended dsTB is significantly lower than that of pristine dsDNAs due to the incorporation of two extended DNA segments. Once more, the gel bands were discrete, attesting to the purity of the products.

We have further characterized one of the triblock architectures (S-dsTB) using AFM. Fig. 2C shows a representative image of the triblock on a mica surface in fluid. Long structures with consistent height (~1 nm) were observed, and the measured average contour length (~550 nm) agrees well with the calculated length (541 nm). The ligated products found with the AFM tended to have kinks in the middle (marked in Fig. S3, ESI†), implying the presence of a flexible PEG block bridging the two long arms of the construct.9

Employing the method presented here, we achieved large triblock architectures with molecular weight of up to...
concentrations of employed to demonstrate successful scission (Fig. 3).

For both the di- and triblock architecture, digestions were
extensions (Fig. 2B and Fig. S5, ESI†). A distinct result from this triblock experiment is that a new band (the second band from the top of lanes S4 in Fig. 2B and M4 in Fig. S5, ESI†) of partially digested fragment appears on the gel as well. These bands can be interpreted as singly-cut materials in which the other restriction site across the polymer was not affected. A plausible explanation, taking into consideration a recently revealed DNA polymerase mechanism,10 is that this singly-cut triblock architecture is due to PEG-induced discontinuation of molecular recognition between the enzyme and DNA as the endonuclease slides along the dsDNA 'rails' in search of restriction sites. However, gel electrophoresis results alone are insufficient to shed light on this phenomenon. This observation may nonetheless pave the way to further studies of the mechanisms of DNA–enzyme interactions through the combination of the variation of DBC properties, such as polymer structure and DNA sequence, with another physical technique like single molecule spectroscopy.

In conclusion, we have demonstrated a new strategy to precisely prepare and alter extremely large DNA/polymer hybrid structures. The enzymatic manipulation of complex, non-natural DNA architectures constructed exclusively from DNA is already known,11,12 however, polymer–DNA chimeras have never been generated by enzymatic digestion and ligation. Moreover, it was successfully demonstrated that hydrophilic, hydrophobic and even thermoresponsive polymers are well compatible with the respective enzymes. Our findings represent a significant step forward for the facile preparation of high precision polymers for the application in life- and materials science.

Notes and references