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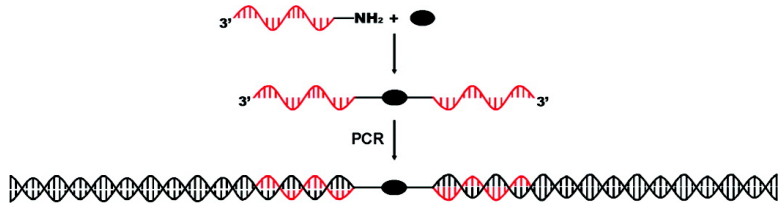

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Synthesis of DNA−Organic Molecule−DNA Triblock Oligomers Using the Amide Coupling Reaction and Their Enzymatic Amplification

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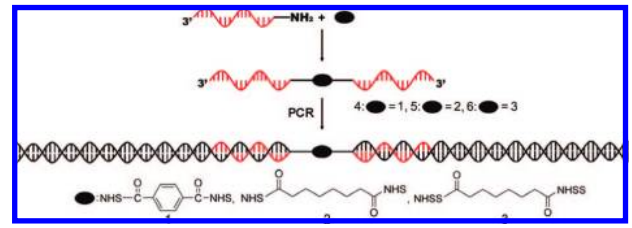
Molecular electronics, which involves molecular building blocks as active electronic elements, has been the subject of intense research for many years because of the fundamental interests in molecular charge transport[1] and potential applications, such as (bio)nanosensors[2] and molecular memory devices.[1b,3] Molecular electronics requires a method for making reliable electrical contacts to single-molecules.[4] To date, several approaches have been reported: scanning-probe microscopy,[5] mechanical break junctions,[6] nano patterning,[1] and direct deposition of electrode on a self-assembled molecular monolayer.[7] However, most methods are laborious and difficult for large-scale application and more importantly cannot control the number of molecules in the junction.[4,8] Recently, DNA has been used as a template for metallic nanostructures, such as Ag, Au, Pd, and Cu nanowires.[9] Moreover, oligodeoxynucleotides (ODNs) have been tethered to organic molecules to screen libraries of synthetic molecules,[10] for DNA detection,[11] and nanostructure constructions.[12] Collectively, these techniques should provide an efficient route toward large-scale molecular electronics.

We envision using a DNA−organic molecule−DNA (DOD) triblock architecture, where the DNAs are subsequently "metallized" to enable contacts to a single molecule. Long double strand DNAs (dsDNAs) (>a few-hundred nanometers) are needed to tether individual molecules to lithographically patterned microscopic electrodes. However, the synthesis of long triblock DOD is challenging and has not been reported. As a key first step, we demonstrate the synthesis of such triblock adducts and their subsequent elongation to construct molecular electronic suitable DOD architectures using polymerase chain reaction (PCR) (Scheme 1). The PCR approach is also the first example in the extension of ODNs on both sides of a single molecule and a simple method to prepare DODs with various organic molecules.

To construct the triblock adducts, compared with solid-phase reactions[13] and DNA-templated organic reactions,[10] traditional coupling reactions provide high purity triblock molecules when purified by gel electrophoresis[14] and allow the preparation of 3′ terminated ODNs needed for the subsequent PCR. An amide-coupling reaction has advantages to link an organic molecule with two ODNs,[15] due to the easy accessibility of 5′ (or 3′) aminated ODNs and the high nucleophilicity of primary amines toward carbonyl groups (pK_aH of $-NH_2 = 35$).[16] Furthermore, we varied the substrate molecule, pH level, and solvent to optimize the reactivity. Our amide-coupling reaction generally involved 5′ aminated 24-mer ODNs (12 nmol) in 6 μL of MOPS (pH 8.0, 50 mM) and an organic molecule (3.6 nmol) in 20 μL of DMSO for 3 days at room temperature. The products of the reaction were isolated[17] and then successfully confirmed by electron spray ionization mass spectrometry (ESI-MS).

Scheme 1. Schematic Representation of Building Triblock Architectures, DNA−Organic Molecule−DNA[a]



[a] PCR = polymerase chain reaction; NHS = *N*-hydroxyl succinimide; NHSS = *N*-hydroxyl sulfosuccinimide.

In our first attempt, 1,4-disuccinimidyl terephthalate (DST) was utilized and gave product **4** in 6.8% yield with an observed mass value at 15416.0 Da (calculated value: 15416.1 Da) (Scheme 1). With less steric hindrance than DST, disuccinimidyl suberate (DSS) increased the reactivity under the same reaction condition discussed above; as a result, it afforded product **5** in 8% yield with an observed mass value at 15424.3 Da (calculated value: 15424.2 Da). However, the yield of product **5** at pH 7.4 was half-that at pH 8.0 because of the reduced nucleophilicity of the primary amine at lower pH. Taking the solubility effect into consideration, we introduced bis (sulfosuccinimidyl) suberate (BS^3) because of its good solubility in both water (up to 10 mM)[18] and DMSO. When compared to water-insoluble DSS, it noticeably increased the reactivity to afford product **6** in 15.3% yield with an observed mass value at 15422.3 Da (calculated value: 15424.2 Da). This result indicates that the solubility of organic molecules in an appropriate solvent mixture has a direct correlation to the reactivity of the amide-coupling reaction. On the other hand, in an aqueous solution system (20 μL of water and 6 μL of pH 8.0 MOPS), the yield of product **6** was decreased to 5.8% yield, even though NHS-ester and NHSS-ester have almost identical reactivity to primary amines.[18] The lower yield is due to faster hydrolysis of NHSS-ester in aqueous solution versus the DMSO-water mixture;[18] we monitored this difference in hydrolysis rate by a deconvolution of the mass spectrum (Supporting Information).

The PCR technique is not only a fast way to amplify a specific DNA fragment, but can also be used for the DNA-templated polymerization of ODNs (i.e., primers) using a DNA polymerase as a catalyst, a counter-primer, and a DNA-template. The latter approach has been employed to elongate the length of ODNs forming up to 35 kbp dsDNA.[19]

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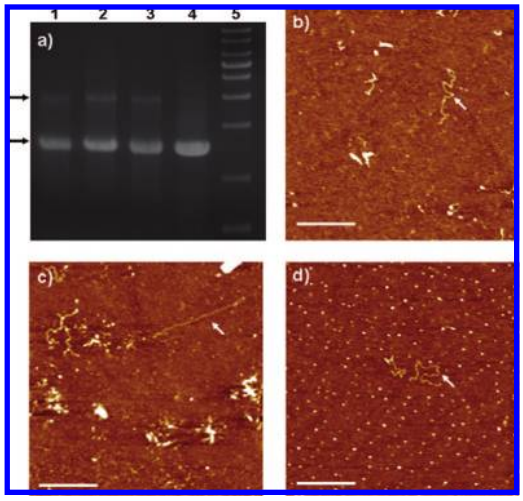

Figure 1. Characterizations of PCR results by 1% agarose gel electrophoresis and AFM. In the gel image (a): (lane 1) 1.5 kbp dsDNA-DST-1.5 kbp dsDNA; (lane 2) 1.5 kbp dsDNA-DSS-1.5 kbp dsDNA, (lane 3) 1.5 kbp dsDNA-BS^3-1.5kbp dsDNA; (lane 4) control experiment with a pair of 5′ amine-terminated primers; (lane 5) 1 kbp DNA ladder. The arrows indicate 3 kbp band (top) and 1.5 kbp band (bottom). The AFM height images show (b) 1.5 kbp dsDNA-DST-1.5 kbp dsDNA, (c) 1.5 kbp dsDNA-DSS-1.5 kbp dsDNA, and (d) 1.5 kbp dsDNA-BS^3-1.5kbp dsDNA. The scale bars are 500 nm, and the heights are 2.5 nm in all images. See Supporting Information for detailed procedures.

For PCR, both forward and reverse primer sequences were obtained from λ-phage DNA and 5′ amine-modified: 5′ NH_2−C6-TCCGATAGTGCGGGTGTTGAATGA (forward primer, 24-mer) and 5′ NH_2−C6-TGCATGTGGAAAGTCCTACGGTCA (reverse primer, 24-mer). In addition, the forward primer was used to synthesize the triblock molecules (i.e., triblock primers) (Scheme 1, **4**−**6**). In our experiments, a reaction mixture containing 5′ aminated forward and reverse primers amplified approximately a 1.5 kbp dsDNA fragment from λ DNA. In contrast, the triblock primers and the 5′ aminated reverse primer amplified about 3 kbp DOD architectures from λ DNA. The PCR results were analyzed by 1% agarose gel electrophoresis; 1.5 kbp dsDNA adducts and triblock architectures appeared at 1.5 kbp and 3 kbp band on the gel, respectively (lanes 1−3, Figure 1a).[20] As a control experiment, 5′ aminated forward primer was used instead of the triblock primer, but a 3 kbp band was not observed on the gel (lane 4, Figure 1a). The PCR products were further confirmed by atomic force microscopy (AFM): in AFM height images, the average length of the triblock architectures was estimated to be ∼940 nm (calculated length: 990 nm) (Figure 1b−d).

In summary, we have successfully developed a simple and versatile approach using PCR to synthesize triblock architectures comprising "long dsDNA-organic molecule-long dsDNA." The amide-coupling reactions allowed us to incorporate molecules with 3′-ODN end substitutions and can be easily extended to introduce other organic molecules to build various ODN nanostructures, for example three-armed ODN nanostructures.[21,22] Moreover, the PCR method is a powerful tool to rapidly build micrometer-scaled DNA structures from the ODN precursors. By incorporating organic molecules with interesting electronic properties, this strategy could constitute a versatile biomolecule-based platform for single-molecule electronics.

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Supporting Information Available: Complete ref 1c, detailed experimental procedures, the spectra and deconvolution of ESI-MS, and AFM images. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Tao, N. J. *Nat. Nanotechnol.* **2006**, *1*, 173. (b) Reed, M. A.; Lee, T. *Molecular Nanoelectronics*; American Scientific Publishers: Stevenson Ranch, CA, 2003. (c) Guo, X.; et al. *Science* **2006**, *311*, 356. (d) Qin, L.; Park, S.; Huang, L.; Mirkin, C. A. *Science* **2005**, *309*, 113. (e) Liu, S.; Tok, J. B.-H.; Bao, Z. *Nano Lett.* **2005**, *5*, 1071.

(2) (a) Patolsky, F.; Zheng, G.; Lieber, C. M. *Anal. Chem.* **2006**, *78*, 4260. (b) Joachim, C.; Gimzewski, J. K.; Aviram, A. *Nature* **2000**, *408*, 541.

(3) Lu, W.; Lieber, C. M. *Nat. Mater.* **2007**, *6*, 841.

(4) Hipps, K. W. *Science* **2001**, *294*, 536.

(5) (a) Cui, X. D.; Primak, A.; Zarate, X.; Tomfohr, J.; Sankey, O. F.; Moore, A. L.; Moore, T. A.; Gust, D.; Harris, G.; Lindsay, S. M. *Science* **2001**, *294*, 571. (b) Reddy, P.; Jang, S.-Y.; Segalman, R. A.; Majumdar, A. *Science* **2007**, *315*, 1568. (c) Moore, A. M.; Dameron, A. A.; Mantooth, B. A.; Smith, R. K.; Fuchs, D. J.; Ciszek, J. W.; Maya, F.; Yao, Y.; Tour, J. M.; Weiss, P. S *J. Am. Chem. Soc.* **2006**, *128*, 1959. (d) Quinn, J. R.; Foss, F. W., Jr.; Venkataraman, L.; Breslow, R. *J. Am. Chem. Soc.* **2007**, *129*, 12376.

(6) (a) Reed, M. A.; Zhou, C.; Muller, C. J.; Burgin, T. P.; Tour, J. M. *Science* **1997**, *278*, 252. (b) Smit, R. H. M.; Noat, Y.; Untiedt, C.; Lang, N. D.; van Hemert, M. C.; van Ruitenbeek, J. M. *Nature* **2002**, *419*, 906.

(7) (a) Zhitenev, N. B.; Meng, H.; Bao, Z. *Phys. Rev. Lett.* **2002**, *88*, 226801. (b) Nitzan, A.; Ratner, M. A. *Science* **2003**, *300*, 1384. (c) Akkerman, H. B.; Blom, P. W. M.; de Leeuw, D. M.; de Boer, B. *Nature* **2006**, *441*, 69.

(8) Martin, C. R.; Baker, L. A. *Science* **2005**, *309*, 67.

(9) (a) Keren, K.; Krueger, M.; Gilad, R.; Ben-Yosep, G.; Sivan, U.; Braun, E. *Science* **2002**, *297*, 72. (b) Becerril, H. A.; Stoltenberg, R. M.; Wheeler, D. R.; Davis, R. C.; Harb, J. N.; Woolley, A. T. *J. Am. Chem. Soc.* **2005**, *127*, 2828. (c) Lund, J.; Dong, J. C.; Deng, Z. F.; Mao, C. D.; Parviz, B. A. *Nanotechnology* **2006**, *17*, 2752.

(10) Li, X. Y.; Liu, D. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 4848 and references therein.

(11) Ergen, E.; Weber, M.; Jacob, J.; Herrmann, A.; Mullen, K. *Chem.*−*Eur. J.* **2006**, *12*, 3707.

(12) (a) Alemadrouglu, F. E.; Herrmann, A. *Org. Biomol. Chem.* **2007**, *5*, 1311. (b) Nielsen, M.; Dauksaite, V.; Kjems, J.; Gothelf, K. V. *Bioconjugate Chem.* **2005**, *16*, 981. (c) Waybright, S. M.; Singleton, C. P.; Wachter, K.; Murphy, C. J.; Bunz, U. H. F. *J. Am. Chem. Soc.* **2001**, *123*, 1828.

(13) Miller, G. P.; Kool, E. T. *J. Org. Chem.* **2004**, *69*, 2404.

(14) We observed a side product from CPG-supported 24-mer ODN itself during the purification of the 24-mer ODN using 15% TBE-urea gel electrophoresis after the treatment of NH_4OH. The position of the side product overlapped with desired products on the gel.

(15) Abdalla, M. A.; Bayer, J.; Radler, J. O.; Mullen, K. *Angew. Chem., Int. Ed.* **2004**, *43*, 3967.

(16) Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*; Oxford University Press Inc.: New York, 2001; pp. 286.

(17) The products were isolated by 15% TBE-urea gel electrophoresis, and then the yields were measured by the NanoDrop spectrophotometer (ND-1000, Thermo Scientific) after the purification using NAP-25 columns.

(18) Instructions for DSS and BS^3: http://www.piercenet.com/files/0418as 4.pdf (accessed Aug 27, 2008).

(19) Barnes, W. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2216.

(20) The 3 kbp DODs were observed as weak bands, but undesired 1.5 kbp dsDNA−organic molecule−ODN adducts were dominantly monitored. We presume that the priming ability of the remaining ODN was reduced by the increased steric hinderance from the elongated ODN. When we introduced 1.5 kbp dsDNA (synthesized in the previous PCR) in place of λ DNA-template, we observed the intensified 3 kbp bands.

(21) Kuroda, T.; Sakurai, Y.; Suzuki, Y.; Nakamura, A. O.; Kuwahara, M.; Ozaki, H.; Sawai, H. *Chem. Asian J.* **2006**, *1*, 575.

(22) DOD triblock architectures can also potentially be obtained using two different functional groups on a single molecule, such as−NHS and−SH to allow the attachment of different type of ODNs.

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