Supporting Information

Utilization of a Multiple Cloning Site as a Versatile Platform for DNA Triblock Copolymer Synthesis

*Jeehae Shin and Sheng Li**

Department of Chemical and Biomolecular Engineering
Korea Advanced Institute of Science and Technology (KAIST)
Daejeon, 34141, South Korea

E-mail: shengli@kaist.ac.kr

1. Characterization of DNA sequences and DNA diblock copolymers

Table S1. DNA sequences used in the synthesis of DNA block copolymers. All DNA sequences are shown in 5′ to 3′ direction. Bold letters correspond to the sequences participate in the generation of sticky ends when hybridized with complementary DNA strands.

amine-5'Bam(21mer)3'	**/C6amine/**TCA GCA GTC TGA GTG CAA CC**G**
amine-5′cDNA(21mer)3'	**/C6amine/**CGG TTG CAC TCA GAC TGC TGA
amine-5'Eco(34mer)3'	**/C6amine/**TCA GCA GTC TGA GTG CAA CCC CGT TGG TGC TCT **G**
amine-5'EcoXho(35mer)3'	/**C6amine**/ AGT CTG AGT GCA ACT GCA GGA **GAA TTC** CCC GTT C**C**
5'Eco(30mer)3'-amine	**AAT TC**A TTG AGA TTG GGA GTG GCA CTT CCA **/amine/**
5′XhoBam(21mer)3′-amine	**TCG AG**T GCA GGA **GGA TCC** GGT /**amine**/
5′Xho(21mer)3'-amine	**TCG AG**A TTG AGA TTG GGA GTG /**amine**/
5'Bam(21mer)3'-amine	**GAT CC**G GTG CAC TGG CGG TTG /**amine**/
5'Eco(72mer)3'	TCA GCA GTC TGA GTG CAA CCC CGT TGG TGC TCT GCA GGA GCT GGT GGT GCA GAA AGG CTG GCG GTT GCC GG**G**
5′BamXhoEco(65mer)3'	**GAT CC**A GTC TGA GTG CAA CCC CGT **CTC GAG** TCT GCA GGA **GAA TTC** GGT GCA CTG GCG GTT GCC GG

Table S2. DNA diblock copolymers prepared via solution phase coupling reaction or AuNP conjugation method.

DNA Diblock Copolymer	**DNA Sequence**	**Synthetic Polymer**
PEG(1k)-5′cDNA(21mer)3′	amine-5′cDNA(21mer)3′	PEG(1k)
PEG(1k)-5′Eco(34mer)3′	amine-5′Eco(34mer)3′	PEG(1k)
biotin-PEG(1k)-5′Eco(34mer)3′	amine-5′Eco(34mer)3′	biotin-PEG(1k)
5′XhoBam(21mer)3′-PEG(1k)	5′XhoBam(21mer)3′-amine	PEG(1k)
5′Eco(30mer)3′-PEG(1k)	5′Eco(30mer)3′-amine	PEG(1k)
5′Xho(21mer)3′-PEG(1k)	5′Xho(21mer)3′-amine	PEG(1k)
5′Eco(30mer)3′-P4VP(6.3k)	5′Eco(30mer)3′-amine	P4VP(6.3k)
5′Bam(21mer)3′-PMMA(6k)	5′Bam(21mer)3′-amine	PMMA(6k)
AuNP-PEG(10k)-5′Bam(21mer)3′	amine-5′Bam(21mer)3′	PEG(10k)
AuNP-PEG(10k)-5′EcoXho(35mer)3′	amine-5′EcoXho(35mer)3′	PEG(10k)
5′Eco(30mer)3′-PEG(10k)-AuNP	5′Eco(30mer)3′-amine	PEG(10k)

2. Synthesis and purification of DNA-PEG diblock copolymers

DNA-PEG(1k) diblock copolymers were synthesized by solution phase coupling reaction between amine-functionalized DNA and NHS-functionalized PEG. Following the coupling reaction, the product was ran through HPLC for purification (Figure 1a-b). The HPLC purified diblocks were then confirmed by gel eletrophoresis (Figure 1c).

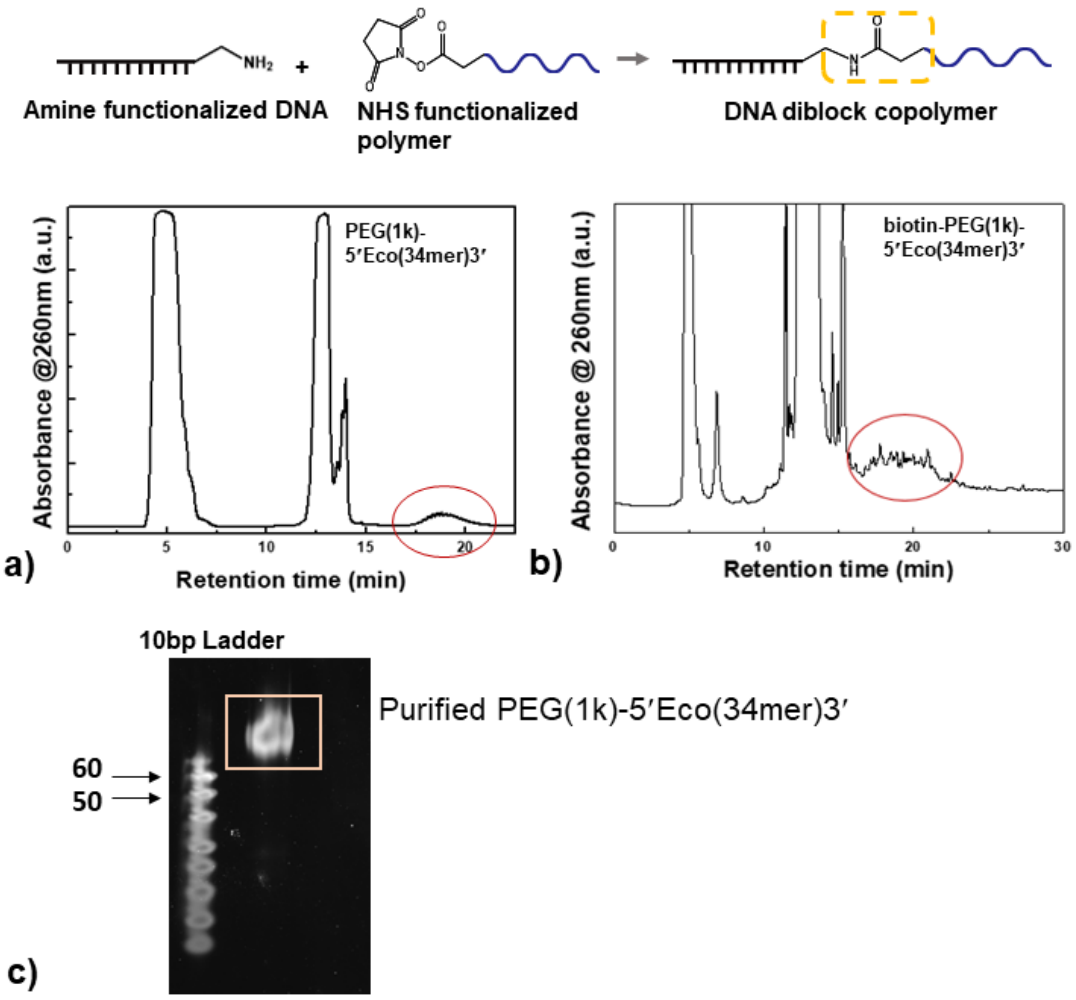

Figure S1. HPLC elution profiles for (a) PEG(1k)-ss5′Eco(34mer)3′ and (b) biotin-PEG(1k)-ss5′Eco(34mer)3′. Materials are eluted in the order of free DNA, amine-functionalized DNA, and DNA-PEG diblock copolymers. Red circled fractions are collected, dialyzed, and freeze dried, then used as diblock copolymers for further experimentation. (c) HPLC purified PEG(1k)-ss5′Eco(34mer)3′ is confirmed by 15% polyacrylamide gel containing 8 M urea.

3. Synthesis and purification of DNA-P4VP and DNA-PMMA diblock copolymers

Amine-functionalized DNA was coupled to NHS-activated hydrophobic polymer in mixed water/DMF solvent. The recovered DNA diblock copolymer crude mixture was purified using 15% polyacrylamide gel containing 8 M urea (Figure S2). Grey boxed regions were cut and extracted by 0.3 M NaCl and isopropanol. Product purification by gel extraction was repeated until all free DNA fragments were eliminated.

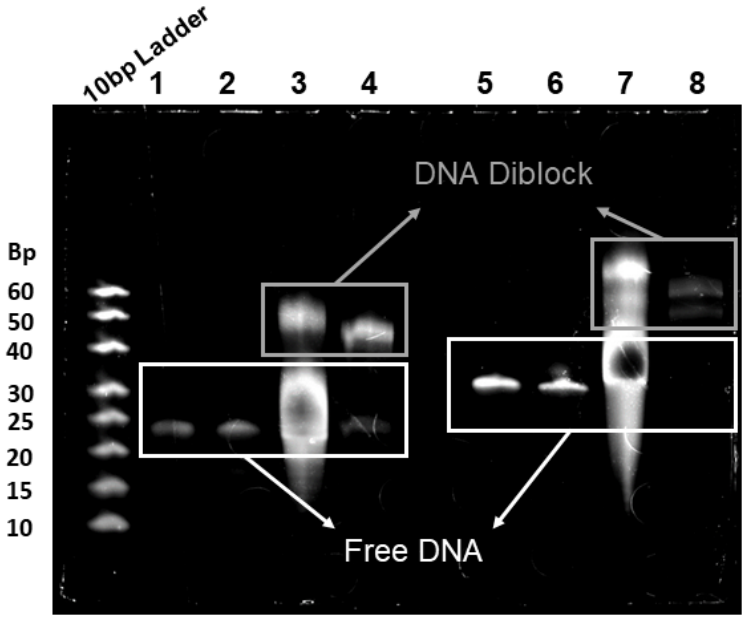

Figure S2. 15% polyacrylamide gel analysis of 5′Bam(21mer)3′-amine (lane 1); 5′Bam(21mer)3′-amine and PMMA(6k) mixture (lane 2); crude reaction mixture of 5′Bam(21mer)3′-PMMA(6k) (lane 3); purified 5′Bam(21mer)3′-PMMA(6k) (lane 4); 5′Eco(30mer)3′-amine (lane 5); 5′Eco(30mer)3′-amine and P4VP(6.3k) mixture (lane 6); crude reaction mixture of 5′Eco(30mer)3′-P4VP(6.3k) (lane 7); purified 5′Eco(30mer)3′-P4VP(6.3k) (lane 8). Grey boxes indicate the positions of the DNA diblock copolymers, and white boxes indicate the positions of free DNAs.

4. Synthesis and characterization of AuNP-conjugated DNA-PEG(10k) diblock copolymers

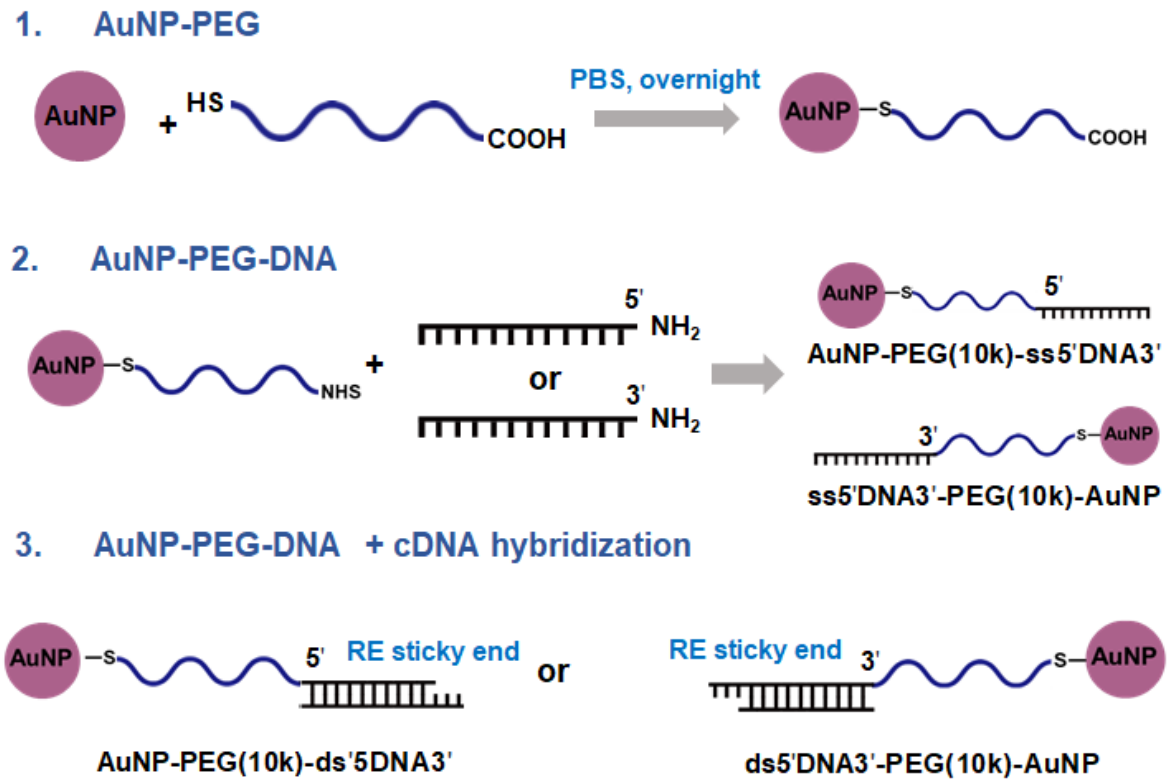

Figure S3. Schematic for preparation of AuNP-conjugated DNA-PEG(10k) diblock copolymers

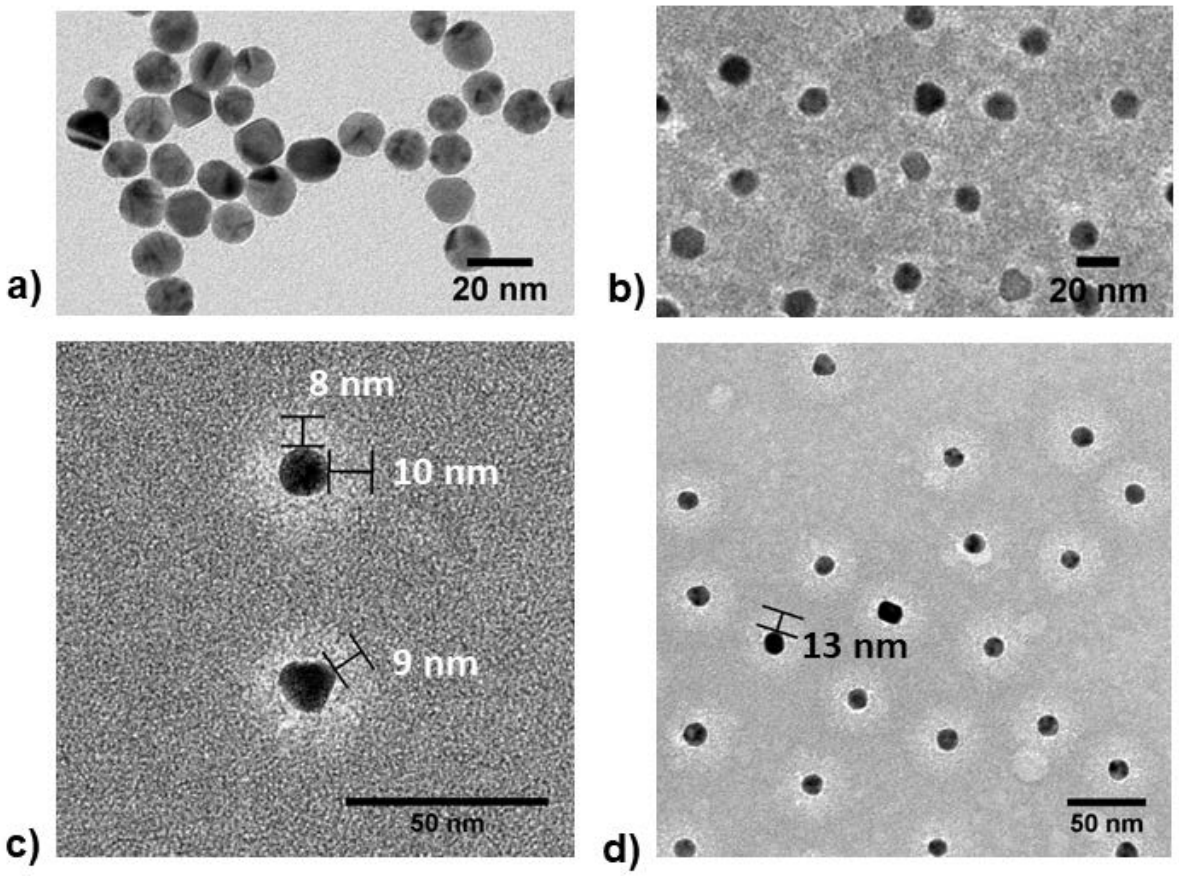

Figure S4. TEM images of (a) citrate stabilized 15nm AuNPs, (b) PEG polymer conjugated AuNPs, (c) AuNP-PEG(10k)-ss5′Bam(21mer)3′, and (d) ss5′Eco(30mer)3′-PEG(10k)-AuNP. All materials are stained by 2% uranyl acetate.

5. Gel electrophoresis of extended DNA diblocks prepared with and without T4 ligase

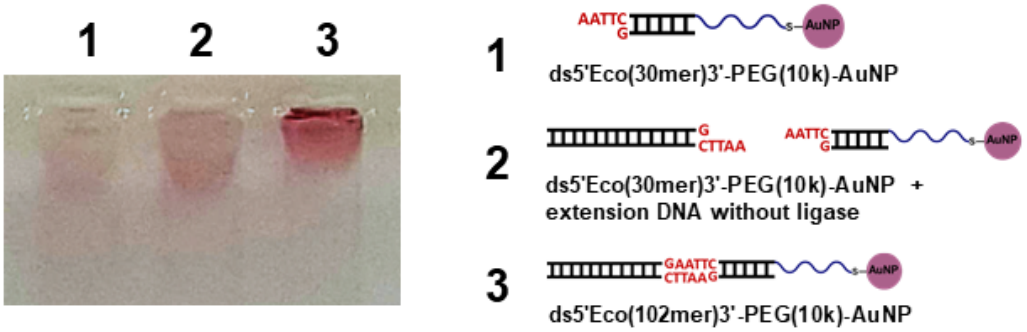

Figure S5. 0.8% agarose gel analysis of differently prepared DNA-PEG diblock copolymers. Lane 1 contains the non-ligated starting material ds5′Eco(30mer)3′-PEG(10k)-AuNP. Lane 2 and 3 contain the reaction product of ds5′Eco(30mer)3′-PEG(10k)-AuNP and ds5′Eco(72mer)3′ extension DNA, conducted without and with T4 ligase, respectively.

6. Urea treatment to determine the extent of self-ligated byproducts formation

The crude reaction products following ligation of AuNP-PEG(10k)-ds5′Bam(21mer)3′ and ds5′BamXhoEco(65mer)3′ were examined before and after 8 M urea treatment (Figure S6). Furthermore, the DNA fragments were visualized using Safe-Red™ stain. Note when Safe-Red™ DNA staining was used, bands corresponding to AuNP-conjugated block copolymers were extinguished due to AuNP quenching of the DNA stain.

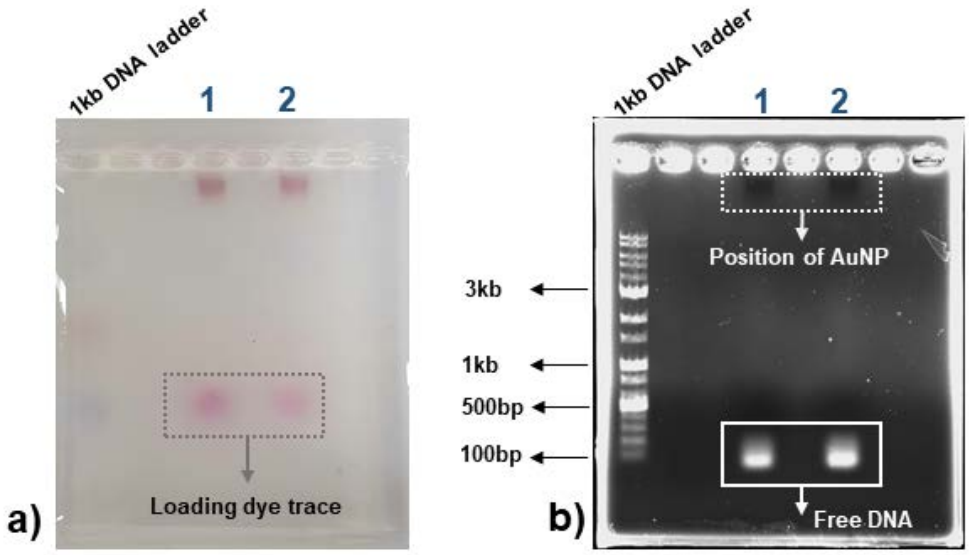

Fig. S6 (a) 0.8% agarose gel analysis of crude AuNP-PEG(10k)-ds5′BamXhoEco(86mer)3′ ligation products before (lane 1) and after (lane 2) 8 M urea treatment. (b) Same 0.8% agarose gel with DNA staining, then visualized by Chemi Doc. 1kb ladder is shown on the left.

7. Gel electrophoresis of MCS containing parent DNA diblock copolymer and digested diblocks

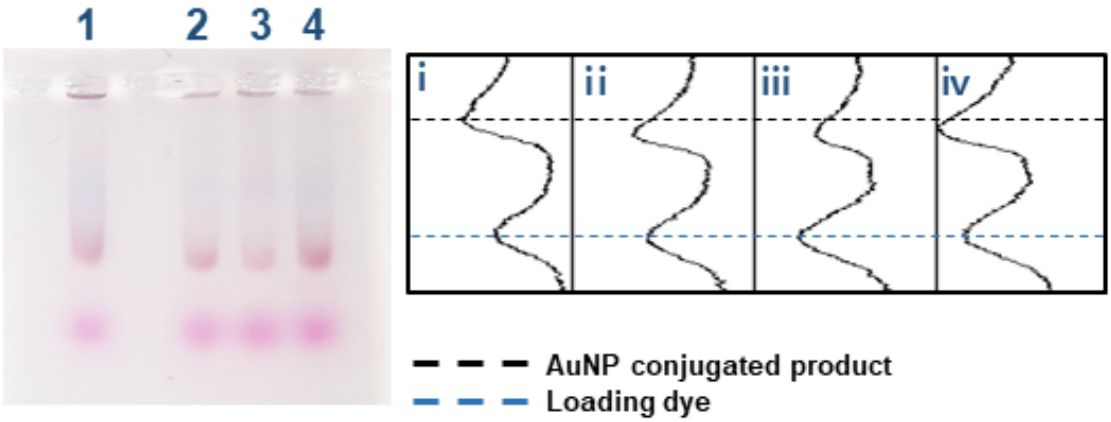

Fig. S7 0.8% agarose gel analysis of AuNP-PEG(10k)-ds5′BamXhoEco(86mer)3′ parent DNA diblock (lane 1); BamH1-cut AuNP-PEG(10k)-ds5′Bam(21mer)3′ (lane 2); Xho1-cut AuNP-PEG(10k)-ds5′BamXho(46mer)3′ (lane 3); and EcoR1-cut AuNP-PEG(10k)-ds5′BamXhoEco(61mer)3′ (lane 4). Gel band migration distances measured using image J software are shown next to the gel image. Black dotted line and blue dotte line represent AuNP-conjugated materials and the corresponding loading dye traces, respectively.