Supplementary Material for Chemical Communications

Electronic Supplementary Information

Programmable DNA translation system using cross-linked DNA mediators

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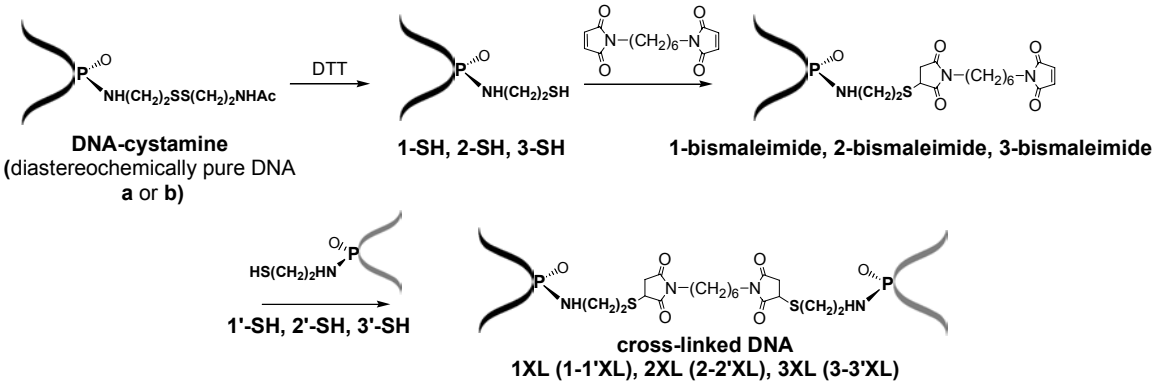
Materials. The reagents used in the experiment were purchased from the following companies; dithiothreitol (DTT), Nacalai Tesque (Kyoto, Japan); bismaleimidohexane, Tokyo Kasei (Tokyo, Japan); T4 polynucleotide kinase and T4 DNA ligase, Toyobo (Osaka, Japan), [γ-^{32}P] ATP, Amersham Biosciences (Piscataway, NJ). All the solvents in the experiment were distilled from calcium hydride. All the reagents for DNA synthesis were obtained from Glen Research (Sterling, VA). DNA synthesis was performed on a PerSeptive Biosystems Expedite 8900 DNA synthesizer. Purification of oligonucleotides was performed on a JASCO LC-2000Plus series HPLC system. Imaging and quantification of radioactive gels were carried out on a Fujix BAS 1000 imaging analyzer.

Oligonucleotide synthesis and purification.

Oligonucleotides (10 mer and 20 mer strands) used in the experiments were purified by a reversed-phase HPLC, and 30 mer and 40 mer strands were purified by a 10 % denaturing polyacrylamide gel electrophoresis (PAGE).

Synthesis of cross-linked oligonucleotides with a bismaleimide linker containing a diastereochemically pure phosphoramidate.

A disulfide tether was introduced via a phosphoramidate linkage in the center of a 10 mer single strand DNA according to the previously reported method.[1-2] Two adjacent diastereomer peaks were separated by reversed-phase HPLC [linear gradient with 2-12 % acetonitrile/water (30 min) containing 50 mM ammonium formate, Nacalai Cosmosil C18 reversed-phase column (7.5 x 150 mm), 2.0 mL/min, 260 nm]. Here the faster and slower eluted diastereomers on HPLC are denoted as **a**- and **b**-diastereomers, respectively.



Scheme S1. Synthetic scheme for cross-linked oligonucleotides. Cystamine-tethered DNAs (DNA-cystamine) employed for the synthesis are diastereochemically pure, and these diastereomers were used separately for preparation of the cross-linked DNAs.

Cleavage of a disulfide linkage of cystamine-attached oligonucleotides (10 nmol) was carried out in a solution containing 10 mM dithiothreitol (DTT) and 50 mM tris(hydroxymethyl)aminomethene hydrochloride (Tris-HCl) (pH 8) at 50 ^{o}C for 30 min. A thiol-tethered oligonucleotide (SH-DNA) was purified by HPLC [linear gradient with 2-15 % acetonitrile/water (20 min) containing 50 mM ammonium formate, Nakalai Cosmosil C18 reversed-phase column (7.5 x 150 mm), 2.0 mL/min, 260 nm]. A SH-DNA (5 nmol) was treated with 1 μmol of 1,6-bismaleimidohexane in a 10 % DMF/water solution at 50 ^{o}C for 2 h and a mono-capped bismaleimide-DNA conjugate was purified by HPLC. This DNA conjugate and a SH-DNA of the different sequence were then reacted in a 1:1 mole ratio at 37 ^{o}C overnight. After treatment with 10 mM DTT at 37 ^{o}C for 2 h to remove a trace of an undesired disulfide XL-DNA, the bismaleimide XL-DNA was purified by HPLC. All the reaction steps were quantitative. The cross-linking was confirmed by 20 % denaturing PAGE, and the gel was visualized by ethidium bromide staining (Figure S1C).

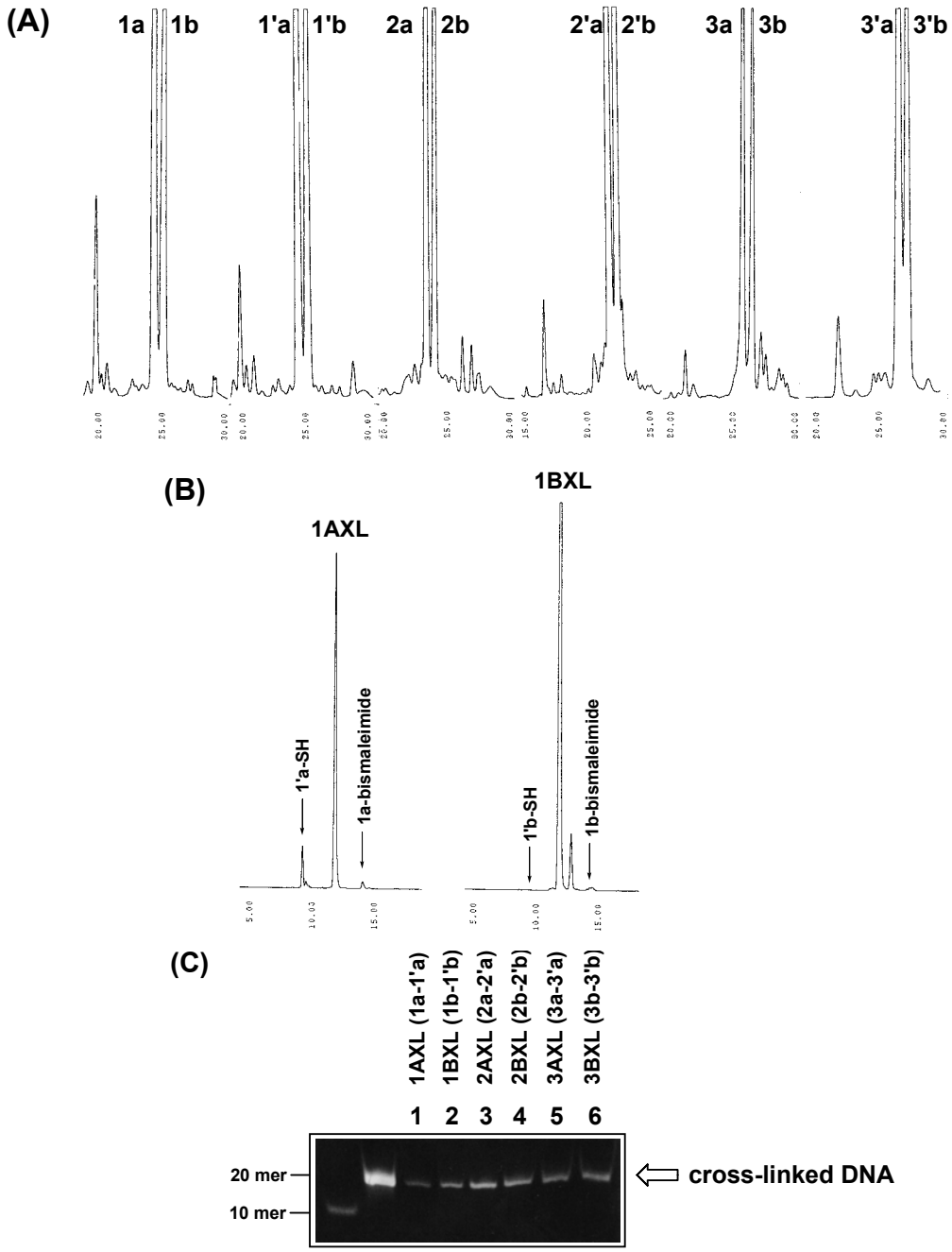


Fig. S1. HPLC profiles of the reaction mixture of diastereomers (A) [2-12 % CH_3CN (30 min)], and the reaction mixtures of **1AXL** and **1BXL** (B) [2-15 % CH_3CN (20 min)]. (C) 20 % denaturing polyacrylamide gel electrophoresis (PAGE) of the purified XL-DNAs.

Thiol-tethered strands (diastereochemically pure oligonucleotides)

1a and **1b**	GCGCTpACACC
1'a and **1'b**	CGGCTpACTCC
2a and **2b**	GTGCTpAGCGG
2'a and **2'b**	CCCGTpAGGAG
3a and **3b**	GAGGTpAGCCC

3'a and **3'b** GCCGTpAGTCG

Cross-linked oligonucleotides

1AXL and **1BXL**	**2AXL** and **2BXL**	**3AXL** and **3BXL**
GCGCTpACACC	GTGCTpAGCGG	GAGGTpAGCCC
CGGCTpACTCC	CCCGTpAGGAG	GCCGTpAGTCG

Template DNA strands

1t-2t-3t TTTTT-GGTGTAGCGC-CCGCTAGCAC-GGGCTACCTC-TTTTT

1t-3t-2t TTTTT-GGTGTAGCGC-GGGCTACCTC-CCGCTAGCAC-TTTTT

2t-1t-3t TTTTT-CCGCTAGCAC-GGTGTAGCGC-GGGCTACCTC-TTTTT

2t-3t-1t TTTTT-CCGCTAGCAC-GGGCTACCTC-GGTGTAGCGC-TTTTT

3t-1t-2t TTTTT-GGGCTACCTC-GGTGTAGCGC-CCGCTAGCAC-TTTTT

3t-2t-1t TTTTT-GGGCTACCTC-CCGCTAGCAC-GGTGTAGCGC-TTTTT

10 mer DNA fragments

1c GGAGTAGCCG

2c CTCCTACGGG

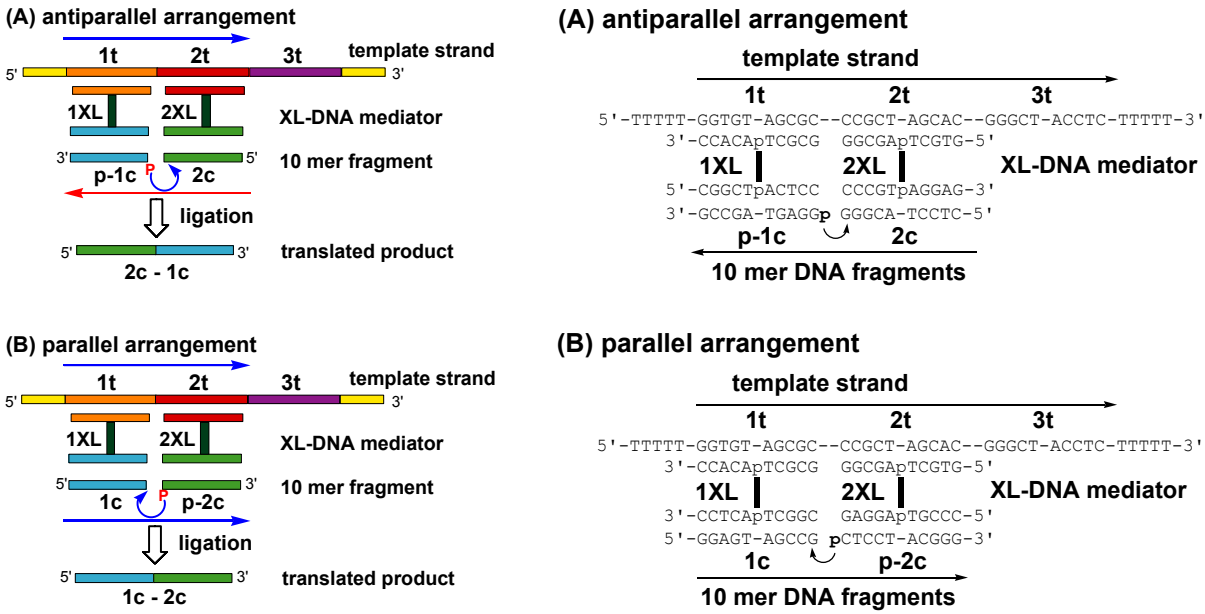
3c CGACTACGGC

Fig. S2. DNA sequences of thiol-tethered strands, cross-linked DNAs, template DNAs, and 10 mer DNA fragments. P denotes a phosphoramidate linkage where a thiol-tether is introduced.

DNA ligation for two crosslinked DNAs and two 10 mer DNA fragments .

Phosphorylation of 10 mer DNA strands was carried out at 37 °C for 2 h in a solution (60 μL) containing 10 mer DNA (1.0 nmol), 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT, 1 mM ATP, and T4 polynucleotide kinase (10 units). After the heat inactivation at 65 °C for 20 min, the phosphorylated product was purified by HPLC [linear gradient with 2-15 % acetonitrile/water (20 min)].

A sample solution (20 uL) containing 2 μM template DNA, 2μM cross-linked DNAs, 2μM 10 mer DNA strand, 2μM 10 mer phosphorylated DNA strand, 66 mM Tris-HCl buffer (pH 7.6), 6.6 mM $MgCl_2$, 0.1 M NaCl, 10 mM DTT, and 1 mM ATP was heated at 85 °C for 5 min, then gradually cooled down to 15 °C (1.0 °C/min) by a thermal cycler. T4 DNA ligase (8 units) was added to the mixtures, and the reaction was carried out at 16 °C for 16 h. After the reaction, the same volume of formamide was added, and the mixture was heated at 85 °C. The reaction mixture was analyzed and quantified by HPLC (Figure S3).



Scheme S2. The DNA translation system using two XL-DNAs. The DNA assemblies with template DNA (**1t-2t-3t**), two XL-DNAs **1AXL** and **2XL**, and two 10 mer DNA fragments. (A) For antiparallel arrangement, 10 mer strands **p-1c** and **2c** were employed for ligation. (B) For parallel arrangement, 10 mer strands **1c** and **p-2c** were employed.

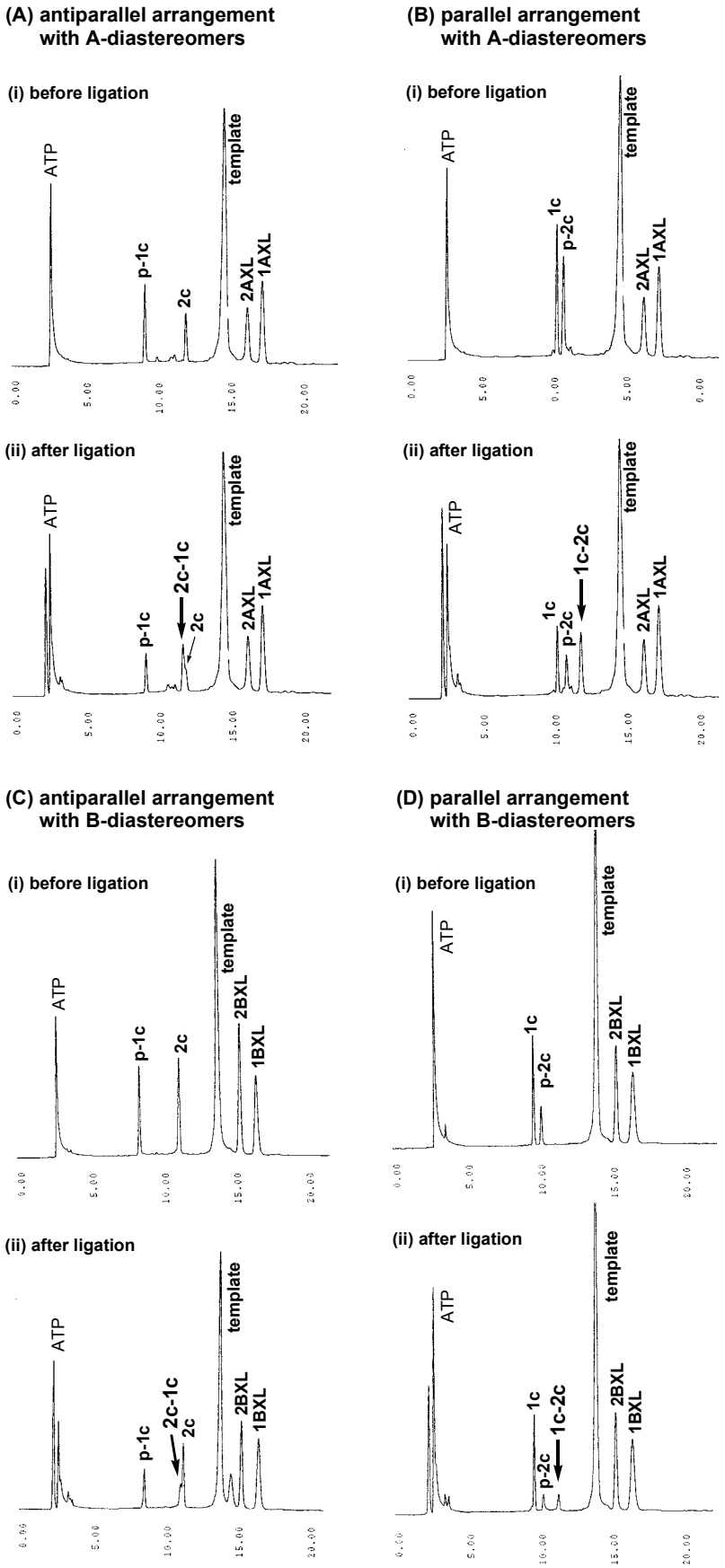
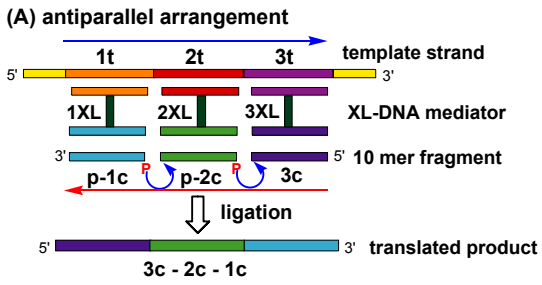


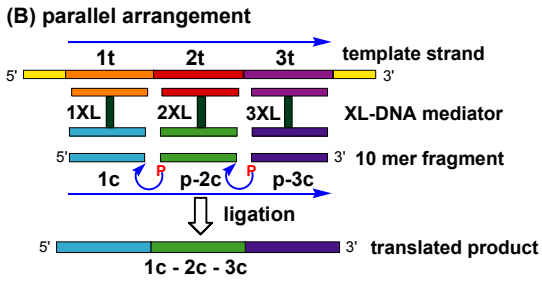
Fig. S3. HPLC profiles for analysis of the ligation reactions with template DNA, two crosslinked DNAs (**1XL** and **2XL**), and two 10 mer DNA fragments. (A) Antiparallel arrangement with **1AXL**, **2AXL**, **p-1c**, and **2c**. (B) Parallel arrangement with **1AXL**, **2AX**L, **1c**, and **p-2c**. (C) Antiparallel arrangement with **1BXL**, **2BXL**, **p-1c**, and **2c**. (D) Parallel arrangement with **1BX**L, **2BXL**, **1c**, and **p-2c**.

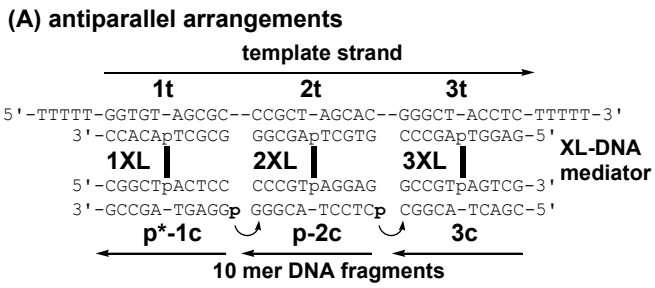
DNA ligation for three cross-linked DNAs and three 10 mer DNA fragments.

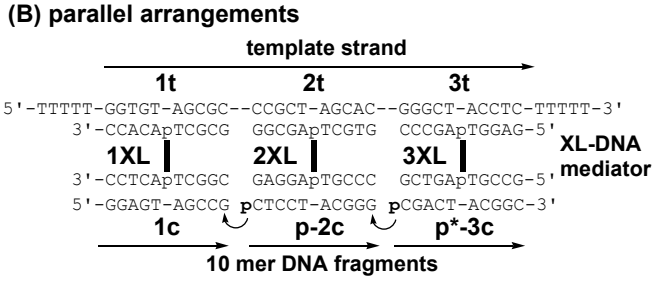
Radioactive labeling (^{32}P) of 10 mer DNA strands was carried out at 37 ^{o}C for 2 h in a solution (20 μL) containing 10 mer DNA (0.01 nmol), 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT, [γ-^{32}P]-ATP (20 μCi), and T4 polynucleotide kinase (10 units). After the heat inactivation at 65 ^{o}C for 20 min, the excess of [γ-^{32}P]-ATP was removed by a gel filtration column (BioRad, micro spin column P-6). The radioactivity of the ^{32}P-phosphorylated DNA strand was quantified by a scintillation counter, and the ^{32}P-DNA strand was diluted with 20 μM of the same sequence of the phosphorylated DNA strand (cold sample).

A sample solution (20 uL) containing 2 μM template DNA, 2μM cross-linked DNAs, 2μM 10 mer ^{32}P-phosphorylated DNA strand, 2μM 10 mer phosphorylated DNA strand, 2μM 10 mer DNA strand, 66 mM Tris-HCl buffer (pH 7.6), 6.6 mM $MgCl_2$, 0.1 M $MgCl_2$, 10 mM DTT, and 1 mM ATP was heated at 85 ^{o}C for 5 min, then gradually cooled down to 15 ^{o}C. T4 DNA ligase (8 units) was added to the mixtures, and the reaction was carried out at 16 ^{o}C for 16 h. After the reaction, the same volume of formamide was added and heated at 85 ^{o}C. The reaction mixture was analyzed by a 20 % denatured PAGE, and the gel was quantified by an imaging analyzer (Figure S5).









Scheme S3. The DNA translation system using three XL-DNAs. The DNA assemblies with template DNA (**1t-2t-3t**), three XL-DNAs **1AXL**, **2AXL**, and **3AXL**, and three 10 mer DNA fragments. (A) For antiparallel arrangement, 10 mer strands **p*-1c**, **p-2c** and **3c** were employed for ligation. (B) For parallel arrangement, 10 mer strands **1c**, **p-2c**, and **p*-3c** were employed. P* denotes the ^{32}P-labelled phosphate.

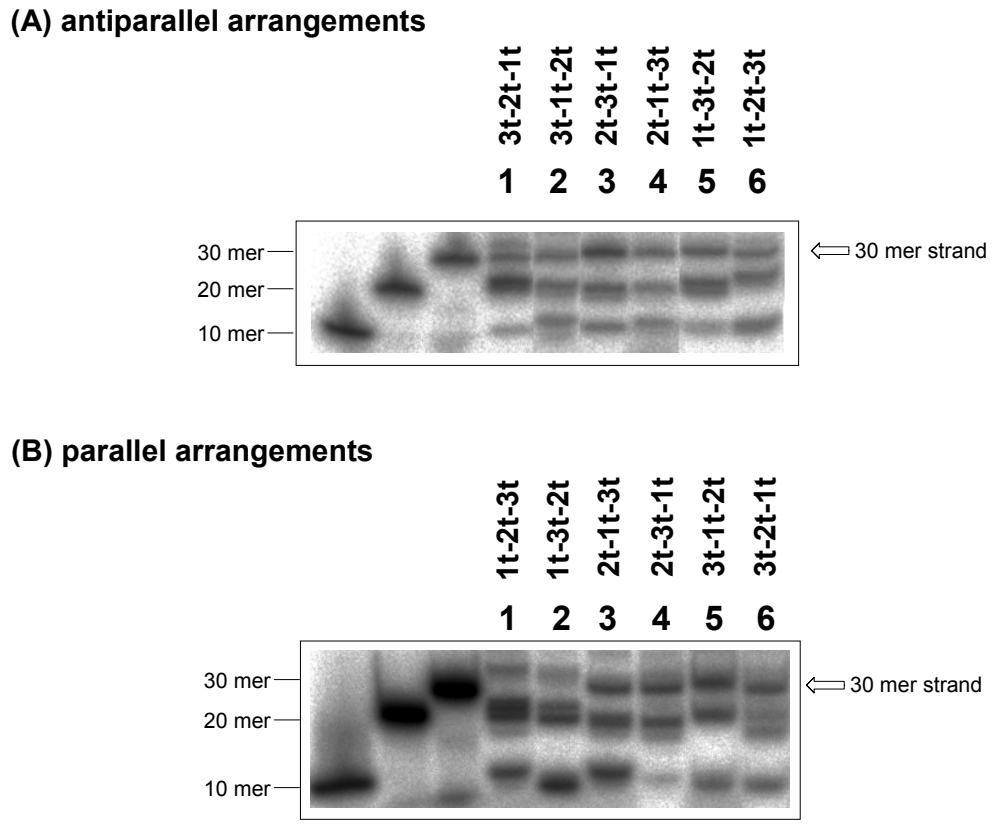


Fig. S4. PAGE analysis of the ligation reactions using six combination of template DNAs with three cross-linked DNAs (**1AXL**, **2AXL**, and **3AXL**), and three 10 mer DNA fragments. (A) Antiparallel arrangements. (B) Parallel arrangements.

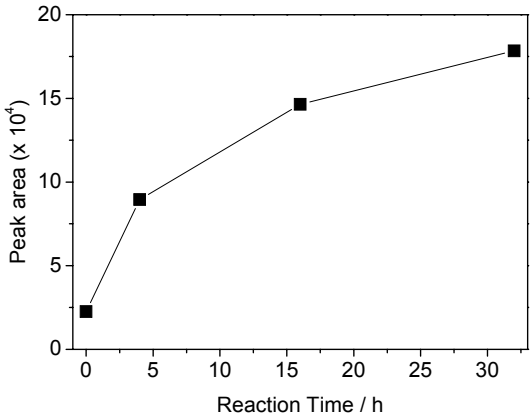


Fig. S5. Time dependence of ligation reaction with template DNA, **1AXL**, **2AXL**, **1c**, and **p-2c** (parallel arrangement). The reaction was monitored by HPLC and quantified by peak area of HPLC profile.

References

1. J. A. Fidanza, L. W. Mclaughlin, *J. Org. Chem*. **1992**, *57*, 2340.

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3. M. Endo, T. Majima, *Angew. Chem. Int. Ed*. **2003**, *42*, 5744.