Thermodynamic properties of branched DNA complexes with full-matched and mismatched DNA strands

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**Materials.** 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 carried out on a JASCO LC-2000Plus series HPLC system. Melting profiles were obtained on a JASCO V-530 UV/vis spectrophotometer equipped with a JASCO ETC-505T temperature controller.

**Synthesis of cross-linked oligonucleotides containing a diastereochromically 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 a reversed-phase HPLC [linear gradient with 2-12 % acetonitrile/water (30 min) containing 50 mM ammonium formate, Nacalai Cosmil 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 (Fig. S1A).

**Scheme S1** Synthesis of cross-linked oligonucleotides (XL-DNA). Cystamine-tethered oligonucleotides (DNA-cystamine) employed for the synthesis are diastereochromically pure, and these diastereomers were used separately for the preparation of the cross-linked oligonucleotides.

Reduction of a disulfide linkage of cystamine-tethered oligonucleotides (20 nmol) was carried out in a solution containing 10 mM dithiothreitol (DTT) and 50 mM Tris-HCl buffer (pH 8) at 50 °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, Nacalai Cosmil C18 reversed-phase column (7.5 x 150 mm), 2.0 mL/min,
A SH-DNA (20 nmol) was treated with 5 µmol of 5,5'-bis(thio-2-nitrobenzoic acid) (DTNB) in a 10 % DMF/water solution at 50 °C for 2 h, and a TNB-attached-DNA was purified by HPLC. The TNB-attached DNA 1 and a SH-DNA 1' of the different sequence were then reacted in a 1:1 mole ratio at 37 °C overnight. The disulfide XL-DNA was purified by HPLC (Fig. S1B). The production of XL-DNAs 1A and 1B was confirmed by MALDI-TOF mass spectroscopy (Fig. S2).

**Fig. S1** HPLC profiles of the reaction mixture of diastereomers (A) [2-11% (left) and 2-12% (right) CH₃CN (30 min)], and the reaction mixtures of 1A and 1B (B) [2-15% CH₃CN (20 min)].

**Fig. S2** MALDI-TOF mass spectra of XL-DNAs 1A and 1B.
Fig. S3 Melting temperature profiles of the XL-DNA with full-matched and mismatched DNA strands. (A) 1A with four counterpart DNA strands. (B) 1B with four counterpart DNA strands. (C) 20mer native DNA 2 with four counterpart DNA strands.

References