Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

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

Masayuki Endo* and Tetsuro Majima*

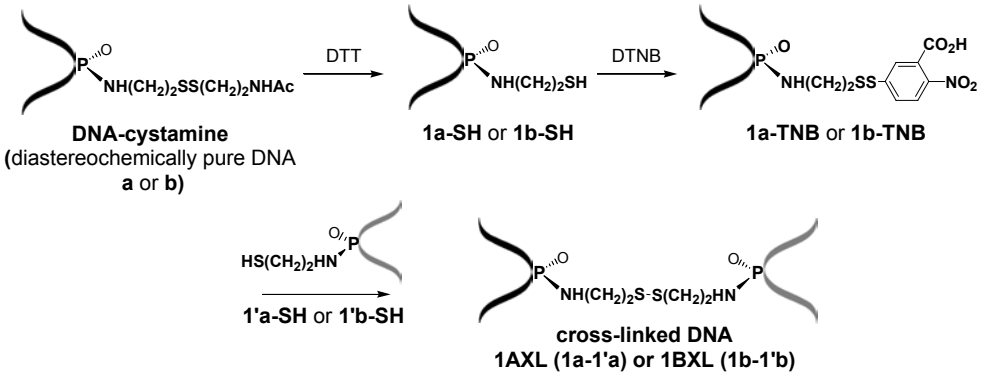
The Institute of Scientific and Industrial Research, Osaka University

8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan

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 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 a 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 (Fig. S1A).



Scheme S1 Synthesis of cross-linked oligonucleotides (XL-DNA). Cystamine-tethered oligonucleotides (DNA-cystamine) employed for the synthesis are diastereochemically 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 ^{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, Nacalai Cosmosil C18 reversed-phase column (7.5 x 150 mm), 2.0 mL/min,

260 nm]. 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).

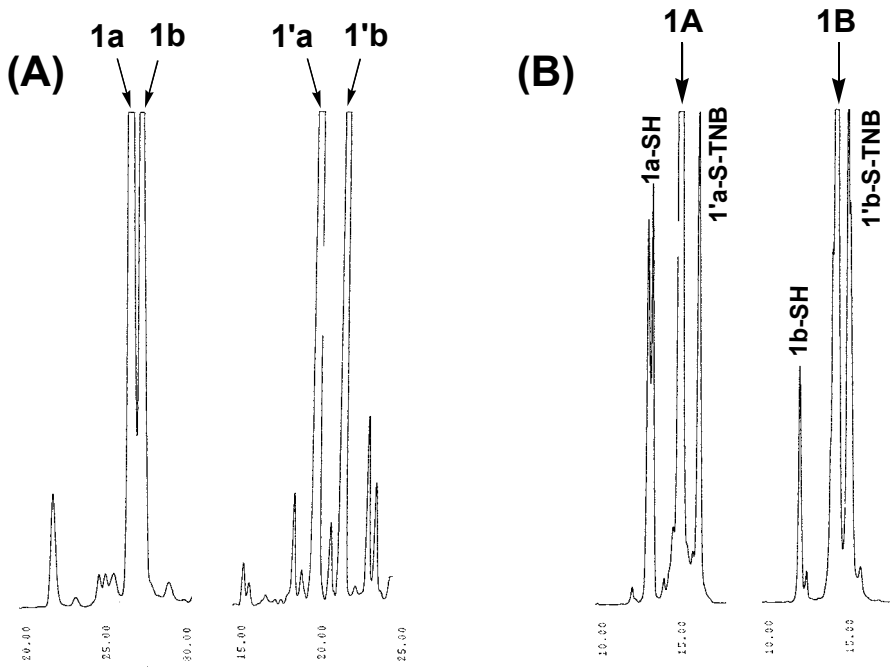


Fig. S1 HPLC profiles of the reaction mixture of diastereomers (A) [2-11% (left) and 2-12% (right) CH_3CN (30 min)], and the reaction mixtures of **1A** and **1B** (B) [2-15% CH_3CN (20 min)].

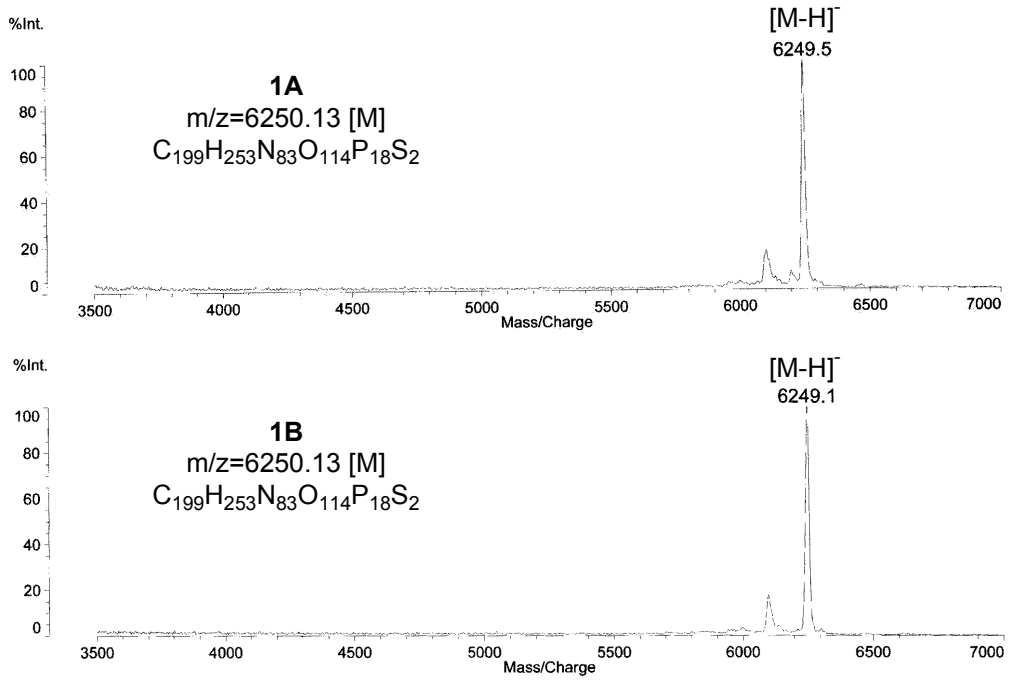


Fig. S2 MALDI-TOF mass spectra of XL-DNAs **1A** and **1B**.

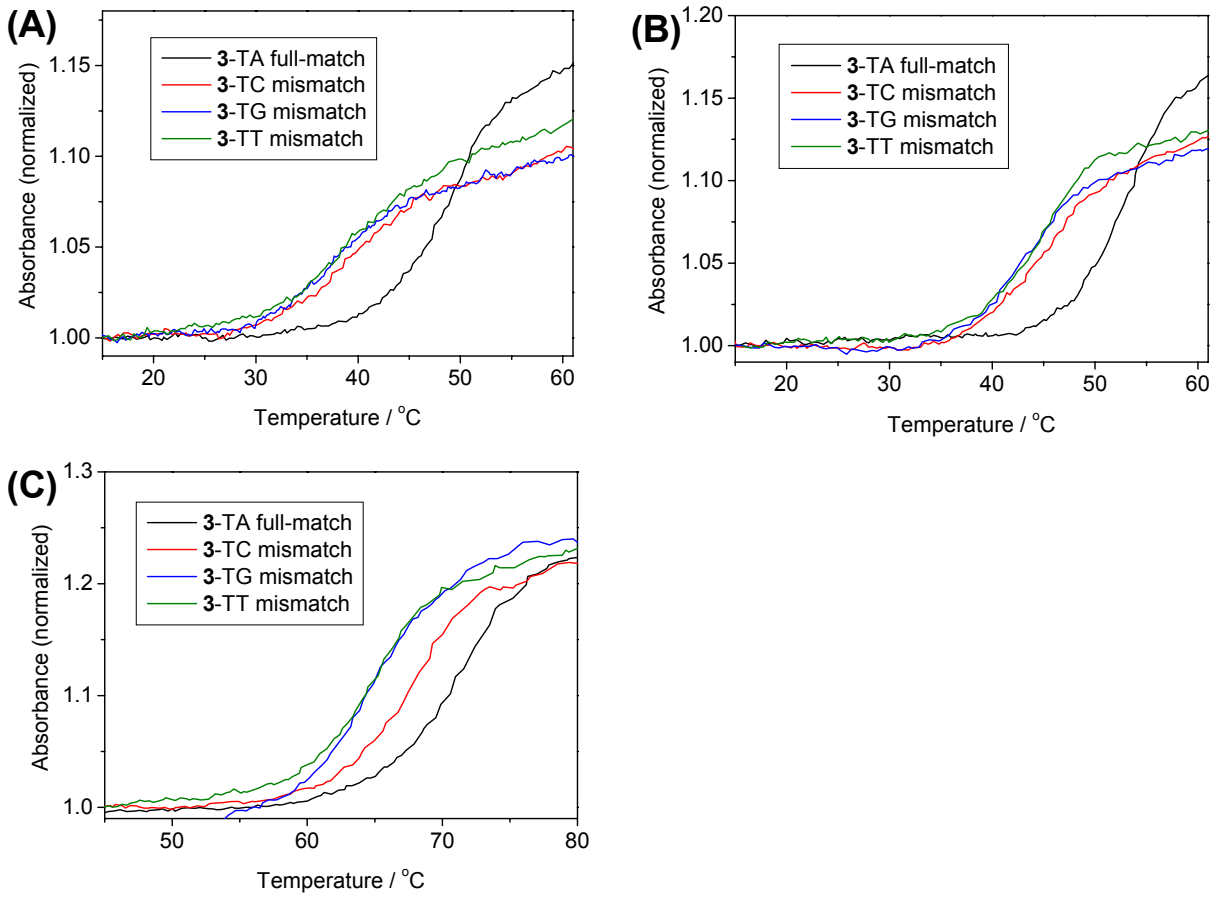


Fig. S3 Melting temperature profiles of the XL-DNA with full-matched and mismatched DNA strands. (A) **1A** with four counterpart DNA strands. (B) **1B** with four counterpart DNA strands. (C) 20mer native DNA **2** with four counterpart DNA strands.

References

1. M. Endo, T. Majima, *J. Am. Chem. Soc*. 2003, **125**, 13654.

2. M. Endo, T. Majima, *Chem. Commun*. 2004, 1308.