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Supplemental Information

Translational Entropy and DNA Duplex Stability

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Materials and Methods

DNA duplex preparation

Custom-synthesized DNA oligonucleotides were purified by anion exchange FPLC on a Mono-Q column using a linear 0.1–1 M gradient of NaCl in 10 mM Tris–HCl, 1mM EDTA, 20% (v/v) acetonitrile (pH 7.4). To prepare duplex, equimolar amounts of complementary strands were mixed, placed in boiling water and slowly cooled. The molar DNA concentration was determined from the absorbance at 260 nm. For calorimetric studies, solutions of single-stranded oligonucleotides were dialyzed for 30 hr with three changes of buffer using a 500–1000 molecular mass cut-off membrane (Spectra/Por Biotech). Solutions of duplexes for calorimetric studies were dialyzed under the same conditions using 3500–5000 molecular mass cut-off membranes. The buffer used in all experiments was 5 mM Na phosphate (pH 7.4) with added 150 mM NaCl.

Differential scanning calorimetry (DSC)

Scanning calorimetric experiments were carried out on a capillary DSC instrument built at Johns Hopkins University, a prototype of the Nano DSC of TA Instruments. Details of the instrument's performance and data acquisition are given elsewhere (3). The heating and cooling rate was 1 K/min at a constant over-pressure of 2 atm., required to prevent appearance of bubbles upon heating and to expand the heating range of aqueous solutions up to 110°C. DNA duplexes and the separated strands were studied over concentrations from 0.5 to 3.5 mg/ml, the majority of the definitive measurements being made at 283 μM duplex. The partial specific volume of duplexes was taken as 0.54 cm^3/g. Calorimetrically determined heat capacity profiles of the DNA duplexes were analyzed using the CpCalc program, which was developed by Dr. George Privalov at Johns Hopkins University and is now provided with the Nano DSC from TA Instruments.

Spectroscopy

UV absorption was measured using a Perkin Elmer Lambda 25 UV/VIS spectrophotometer equipped with the PTP-6 Peltier System.