A direct and non-destructive approach to determine the folding structure of the I-motif DNA secondary structure by NMR

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Supporting Information

METHODS

Sample preparation. The DNA oligonucleotides were synthesized as described previously [1-3]. 6 % ^{15}N-cytosine or ^{15}N-thymine phosphoramidites were used for site-specific C- or T-labeled DNA synthesis. The uniformly ^{15}N-labeled cytosine and thymine phosphoramidites were purchased from Cambridge Isotope Laboratories. The NMR samples contained ~ 0.5mM DNA oligonucleotides in 95% H_2O and 5% D_2O. Each DNA sample was adjusted to pH 5.5 by using NaOH or HCl. No buffer was used in sample preparation. The final salt concentration was about 5 mM.

NMR experiments. NMR experiments were performed on a Bruker AVANCE 600 MHz spectrometer using a 5mm TXI gradient probe head. All spectra were acquired at 7°C, unless otherwise specified. Identifications of cytosine imino protons in site-specific labeled oligonucleotides were performed by one-dimensional ^{15}N-filtered experiments [1-4]. The GE-JRSE HMQC were used to measure ^{15}N-filtered spectra of Cytosine imino protons [5, 6]. To avoid signal loss due to saturation transfer, the jump-return spin-echo and gradient pulses were used to suppress the water peak. The jump-return delay time was tuned to have the maximum intensity at the frequencies of the peaks of interest around 15 ppm. The gradient pulses were applied for 1 ms with a strength of 6 G/cm. The 2JNH transfer time was adjusted to take into account of the relaxation loss. The carrier frequencies were set at water peak in the ^{1}H dimension and at 210 ppm in the ^{15}N dimension. The repetition delay was 1.5 s.

The imino proton in a C^+–C base pair has one-bond coupling to the N3 atoms of the two base-paired cytosines and can be readily detected by 1D ^{15}N-filtered HMQC experiments, using the site-specifically ^{15}N labeled DNA sample at each cytosine. The detection of an imino peak of a site-specifically C-labeled DNA by 1D ^{15}N-filtered HMQC experiments between 15-16 ppm, a region characteristics of C^+–C base pairs in I-motif structures, will indicate that this cytosine is involved in

the C^+-C base pairing. The cytosine imino proton which is not involved in the C^+-C base pairing will not be detected in this region. As the two cytosines of a C^+–C base pair share one imino H3 hydrogen, the imino proton involved in the hydrogen bonding distributes equally between the two base-paired cytosines. Through the $^1H - ^{15}N$ one bond coupling, the two C-labeled DNA samples at each of the two base-paired cytosines will give rise to a ^1H resonance peak in the 1D HMQC experiments at the same location.

References:

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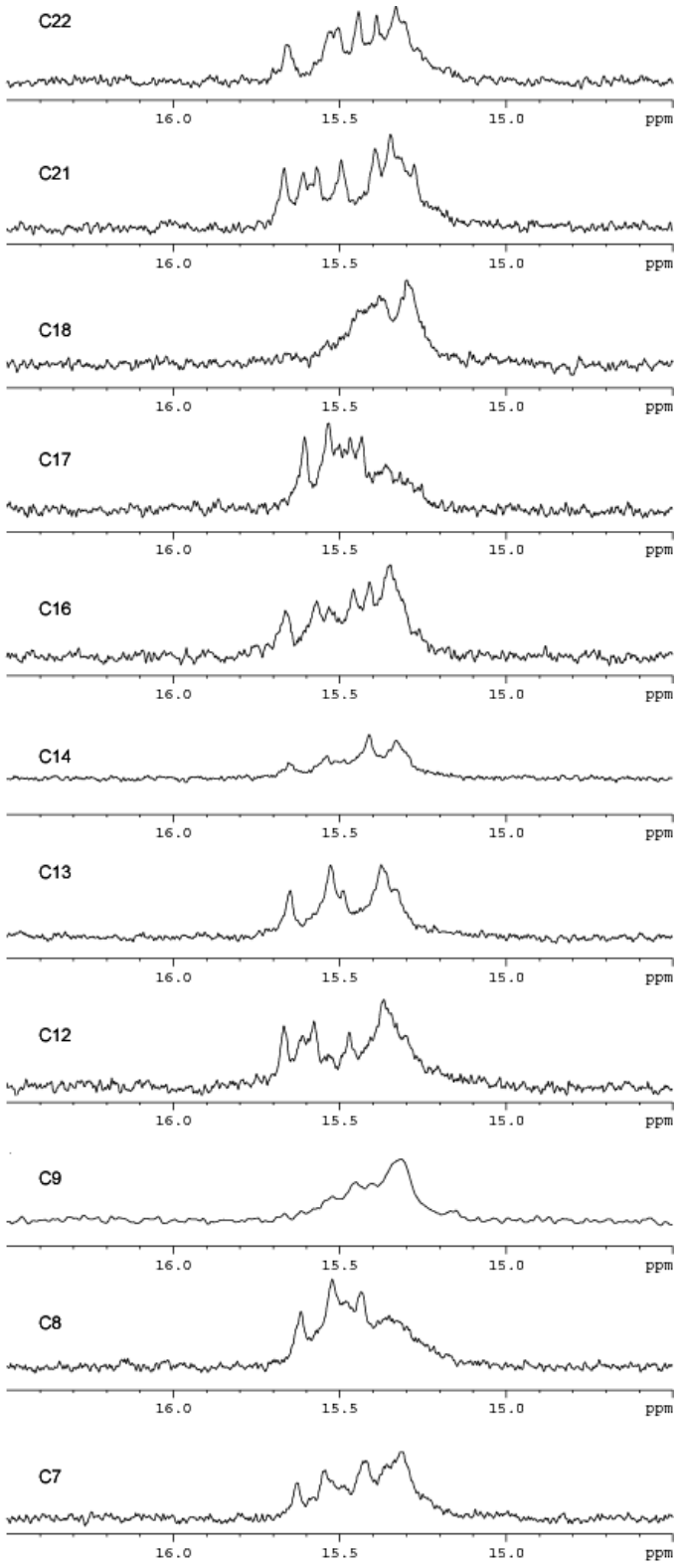
Figure S1. The cytosine-imino proton region of the 1D ^{1}H NMR of site-specific 5mC-substituted Py23 DNA samples at 7 °C.


C22
16.0
15.5
15.0
ppm
C21
16.0
15.5
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C18
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C17
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C9
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15.5
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C8
16.0
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15.0
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C7
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Figure S1.