Supplementary Information

Discrimination between 5-hydroxymethylcytosine and 5-methylcytosine by a chemically designed peptide

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Experimental Section

Synthesis of hydroxymethylated DNA. The 5-hydroxymethyl-2′-deoxycytidine phosphoramidite was synthesized according to the facile synthetic protocol (*Org. Biomol. Chem.*, 2011, **9**, 4176.). A hydroxymethylated DNA was synthesized by the conventional phosphoramidite method by using an Applied Biosystems 392 DNA/RNA synthesizer. Synthesized DNA was purified by reverse phase HPLC on a 5-ODS-H column (10 × 150 mm, elution with a solvent mixture of 0.1 M triethylammonium acetate (pH = 7.0), linear gradient over 20 min from 5% to 20% acetonitrile at a flow rate 3.0 mL/min).

Peptide Synthesis and Characterization. The peptide **1** was synthesized on an automatic peptide synthesizer (Model 433A, Applied Biosystems) using the Fmoc solid-phase method on an amide resin (Rink Amide MBHA resin, Novabiochem). After synthesis, the peptides were cleaved from the resin and deprotected by treatment with trifluoroacetic acid/triisopropylsilane/1,2-ethanedithiol/water (94/1/2.5/2.5 v/v) and purified by HPLC on Chemcobond 5-ODS-H (10 × 150 mm, Chemco Scientific). The obtained peptide was characterized by MALDI-TOF mass spectrometry. $[M + H]^+$ calcd, 7335.5; found, 7335.3.

Gel Mobility Shift Assays. The DNA strands used in the assay were as follows: G-strand, ^{32}P-5′-TTT ATA TTA AAT ATT ATG GGG [C/^{m}C/^{hm}C]GG GGC CAA TAT ATT A-3′; C-strand, 5′-TAA TAT ATT GGC CC[C/^{m}C/^{hm}C] GCC CCA TAA TAT TTA ATA TAA A-3′ (underlines, the sequences to be recognized by the peptide). The ^{32}P-labeled G-strand was annealed with the unlabeled complementary C-strand. The reaction mixture containing the hybridized DNA (50 pM, 500 cpm) and the zinc finger peptide (0–10 μM) was incubated in 20 mM Tris-HCl (pH 8.0), 100 mM sodium chloride, 100 μM zinc chloride, 1 mM tris(2-carboxyethyl)phosphine (TCEP), 0.05% Nonidet P-40, 5% glycerol, 40 ng/μL bovine serum albumin, and 100 ng/μL poly(dI-dC) for 30 min at 4 °C. The reaction mixture was analyzed by polyacrylamide gel electrophoresis in Tris-borate buffer (pH 8.3) at 4 °C. The bands were visualized by autoradiography and quantified using Image Gauge version 4.01 software (Fujifilm). The dissociation constant (K_d) of the peptide for the target DNA was evaluated by curve-fitting the band intensities to the equation: $F = [P]/([P] + K_d)$, where F and [P] represent the fraction of the peptide-bound DNA and the total peptide concentration, respectively.

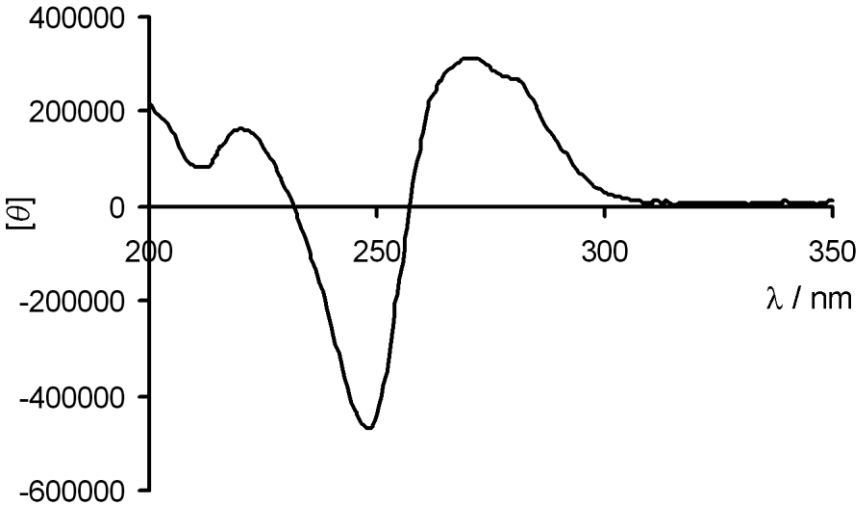

Fig. S1 CD spectrum of 5′-TTT ATA TTA AAT ATT ATG GGG ^{m}CGG GGC CAA TAT ATT A-3′/5′-TAA TAT ATT GGC CC^{m}C GCC CCA TAA TAT TTA ATA TAA A-3′. The measurement of the duplex (10 μM) was carried out in 20 mM Tris-HCl buffer (pH 8.0) containing 100 mM sodium chloride at 4 °C.

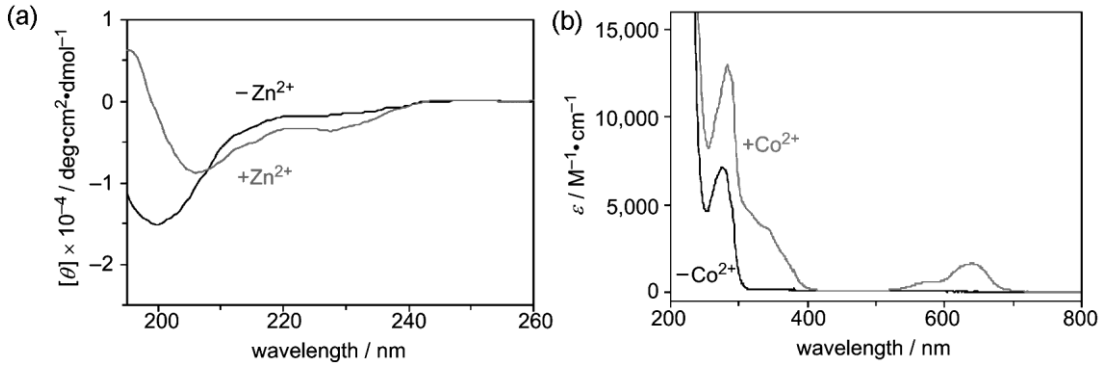

Fig. S2 Structure of peptide **1**. (a) CD spectra of the peptides in the absence (apo forms, black) or presence (zinc finger forms, gray) of 3 equiv zinc chloride. The peptide concentrations were 18 μM for **1**. Measurements were carried out in 10 mM Tris-HCl buffer (pH 7.5) containing 50 mM sodium chloride and 0.1 mM TCEP at 4 °C under nitrogen. (b) Absorption spectra of **1** (72 μM) in the absence (apo forms, black) or presence (cobalt complex forms, gray) of 300 μM cobalt chloride in 10 mM Tris-HCl buffer (pH = 7.5) containing 50 mM sodium chloride at 20 °C under nitrogen.

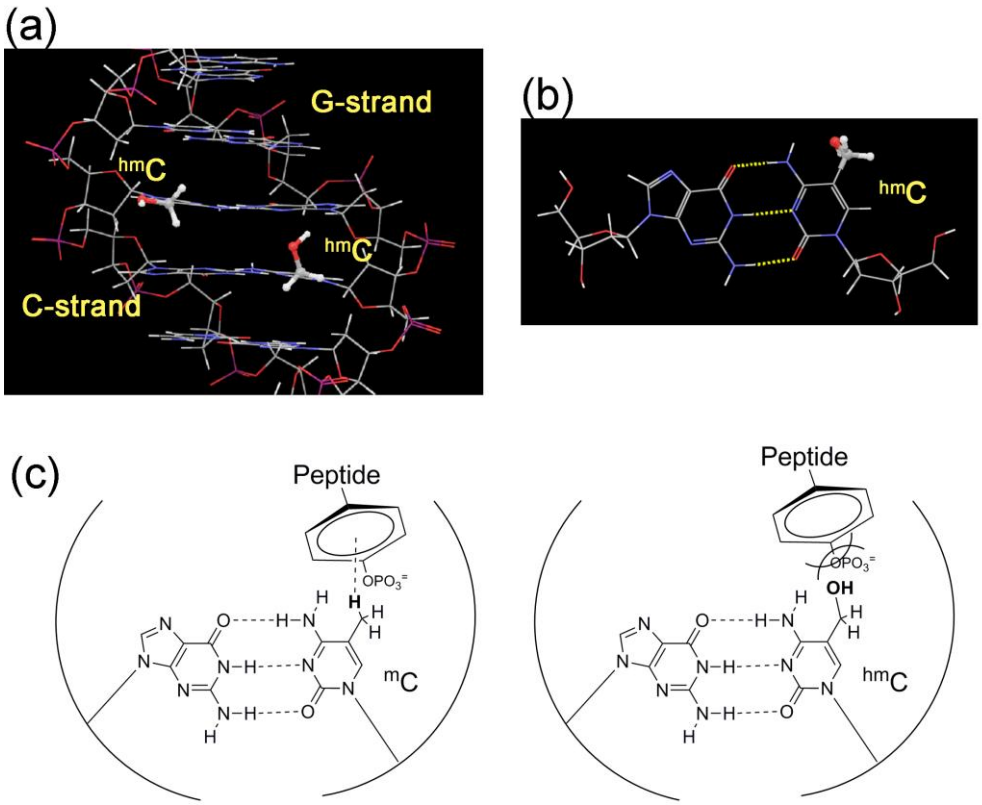

Fig. S3 A plausible structure of an ^{hm}C-containing DNA duplex. (a) Side view of G^{hm}CGG/CG^{hm}CC DNA and (b) top view of the ^{hm}C/G base pair in (a). The structures were minimized with OPLS2005 in MacroModel 9.8. (c) Interaction with peptide. The hydroxy group of ^{hm}C was extruded toward the outside of the DNA major groove. The location of the hydroxy group would sterically hinder the interaction between peptide pY and DNA.