Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Silver(I) Ions Modulate the Stability of DNA Duplexes Containing Cytosine, Methylcytosine and Hydroxymethylcytosine at Different Salt Concentrations

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Table S1: Synthesized DNA duplexes containing a C-C, 5mC-C or 5hmC-C base pair mismatch and their melting temperatures (*T*m, °C) with different KNO_3 concentrations.

5'- AATAAAATA**X**TATAAA-3' (X=C, 5mC or 5hmC)				
3'- TTATTTTATCATATTT-5'				
	*T*m(°C), -Ag(I)	*T*m(°C), +Ag(I)	Δ*T*m(°C)	*T*m(°C, simulation in NaCl)[a], -Ag(I)
C-C (100mM)	20.0±0.0	35.0±0.0	15.0±0.0[b]	18.6
5mC-C (100mM)	21.7±0.4	34.3±0.3	12.6±0.5	
5hmC-C (100mM)	18.0±0.5	33.2±0.5	15.2±0.7	
C-C (1M)	28.5±0.6	43.5±0.6	15.0±0.8	31.1
5mC-C (1M)	29.1±0.8	42.4±1.0	13.3±1.3	
5hmC-C (1M)	27.6±0.3	39.9±1.8	12.3±1.8	
C-C (10mM)	N.O.[c]			0.6

[a]: The *T*m values were calculated by the program (http://biophysics.idtdna.com) with different NaCl concentrations;

[b]: Standard deviations for these changes were calculated by error propagation equation;

[c]: "N.O.", no observation. This is because the 16-bp DNA duplex contains no C-G pairs and has a mismatch, and the calculated *T*m was 0.6°C, which suggests rare duplex formation at 10mM salt.

Experimental measurements were repeated at least three times for each sample. Data were presented as average ± standard deviation.

Materials and Methods

DNA oligonucleotides (Table S1) were synthesized and electrophoresis-purified by Integrated DNA Technologies Inc, CA. Before testing, the mixtures of ssDNAs were heated to 90 °C for 5 minutes, then gradually cooled down to room temperature and stored at 4 °C. Samples contained 1 μM duplex, 100 mM KNO_3 and 10 mM Mops, pH=7.1[1]. KNO_3 was used because it can be applied to other experimental methods, like nanopore single-channel recording[2]. Using the nanopore sensor[3], we are currently working on the detection of silver(I) using DNA duplex containing C-C mismatch. KCl was used as the buffer in the majority of previous nanopore experiments, but is cannot be used for silver(I) detection. In this situation, we are using KNO_3 as the buffer.

Melting Temperature Determination for DNA Duplexes.

The melting temperatures of duplexes containing cytosine-cytosine, methylcytosine-cytosine, or hydroxymethylcytosine-cytosine mismatches were determined by monitoring the increase in absorbance at 260nm as a function of temperature. The temperature was increased from 4°C to 50°C (for samples without silver(I)), or from 10°C to 60°C (for samples with silver(I)), at a rate of 0.5°C/min[1]. 2 μM silver(I) were used in the experiment, because previous studies found that the melting temperature reached a plateau when the silver(I) concentration was 1.5 fold higher than the DNA[1]. The *T*m was measured by the Cary 100 Bio UV-Visible spectrophotometer. The melting temperature was calculated from the collected data using the Cary WinUV Thermal software.

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