Electronic Supplementary Material (ESI) for ChemComm.
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Electronic Supplementary Information

for

Reversible regulation of metallo-base-pair interactions for DNA dehybridization by ultrasound

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1. Materials

All chemical reagents were of analytical grade and were used without further purification if not stated otherwise. 3-morpholinopropanesulfonic acid (MOPs, ≥ 99.5%, Sigma-Aldrich), $AgNO_3$ (≥ 99.0%, Sigma-Aldrich), $NaNO_3$ (≥ 99.0%, Sigma-Aldrich), Agarose (Yuanye Biotech), GeneRuler Ultra Low Range DNA Ladder (Thermo-Fisher) and YeaRed Nucleic Acid Gel Stain (Yeasen Biotech) were used as received. Milli-Q water was used throughout the experiments.

All the Ag^+-base-paired DNA oligonucleotide (ODN) sequences (Table S1) were synthesized, HPLC-purified and MALDI-tested by Biomers Co. Ltd. (Germany). The C bases in the sequence are the binding site for Ag^+. For further quenching study, each

ODN was labeled with a fluorescent moiety (fluorescein, F) or a quencher (dabcyl, D) at the 3'- and 5'-ends, respectively.[1]

Table S1. Ag^+ base-paired ODNs used in this work.

ODN name	Sequences
1.0T-ODN-F	5'CACACAACACAACT(**F**)3'
2.0T-ODN-Q	3'CTCTCTTCTCTTCA(**Q**)5'
3.10T-ODN-F	5'TTTTTTTTTTCACACAACACAACT(**F**) 3'
4.10T-ODN-Q	3'CTCTCTTCTCTTCA(**Q**)TTTTTTTTTT5'
5.30T-ODN-F	5'TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCACA CAACACAACT(**F**)3'
6.30T-ODN-Q	3'CTCTCTTCTCTTCA(**Q**)TTTTTTTTTTTTTTT TTTTTTTTTTTTTT5'
7.50T-ODN-F	5'TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTCACACAACACAACT(**F**)3'
8.50T-ODN-Q	3'CTCTCTTCTCTTCA(**Q**)TTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT5'
9.50T-ODN-L	5'TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTCACACAACACAACT3'
10.50T-ODN-R	3'CTCTCTTCTCTTCATTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT5'
ODN: oligodeoxyribonucleotide F: fluorescein Q: dabcyl	

2. Methods

Fluorescence assays

Fluorescence spectra of fluorescein DNA structures were measured with a spectrophotometer (SpectraMax M3, Molecular Devices). Briefly, Ag^+ of different concentrations was incubated with Ag^+ base-paired ODNs (fluorescein- and quencher-labelled, 1:1 in molar ratio, c = 10 nM) in MOPs buffer (pH = 7.0) containing 50 mM of $NaNO_3$ for 5 min at 25 °C. Then, the fluorescence measurement of each mixture was carried out under an excitation wavelength λ = 490 nm at 25 °C.

For studying the mechanochemical response of the DNA sequences, the fluorescence emission intensity was measured at λ = 520 nm immediately after ultrasonication, under an excitation wavelength λ = 490 nm at 25 °C. For the temperature-dependent experiments, the fluorescence intensity of each mixture was measured every 30 s during the temperature increase (25 °C to 60 °C) and decrease (60 °C to 25 °C).

Calculation of normalized fluorescence intensity

Normalized fluorescence intensities of each test were determined by dividing the fluorescence intensity of the sample by the fluorescence intensity of the free fluorophore-labeled oligodeoxyribonucleotide without any treatment, multiplying the result by 100.

Sonication experiments

Ultrasonication experiments on Ag^+ base-paired ODNs (c = 10 nM) were performed in a 1 mL ultrasonication vessel (Test tube heavy-walled, 2775/2, Assistant) with a Qsonica Q125 sonicator (USA) equipped with a 3 mm diameter microtip probe (A12628PRB20). Sonication was performed using pulsed ultrasound (1.0 s on, 1.0 s off at 50% Amplitude) at f = 20 kHz. The vessel was placed in an ice bath to maintain a temperature inside the vessel of 6-9 °C throughout sonication.

Agarose gel electrophoresis

4% agarose gel was prepared and stained with YeaRed Nucleic Acid Gel Stain, then used to determine the hybridization and US-responsive dehybridization of Ag^+-base-paired ODNs with 50T tail. As indicated in Figure S1, each sample was mixtured with 5 μL loading dye, and then loaded into each well, respectively. Ultra-low range DNA ladder was loaded in lane 1 as gel marker. Electrophoresis was carried out at a voltage of 120 V for 40 min in TAE (1×) running buffer. Finally, the results were pictured and analyzed.

3. Supplementary figures

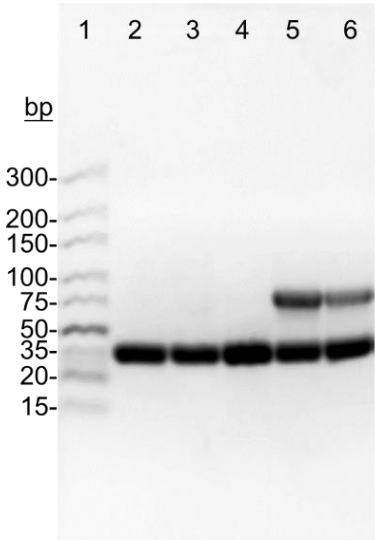

Figure S1. Agarose gel (4%) electrophoresis of Ag^+-base-paired ODNs with 50T tail. Lane 1: ultra-low range DNA marker; lane 2: 50T-ODN-L; lane 3: 50T-ODN-R; lane 4: 50T-ODN-L + 50T-ODN-R; lane 5: 50T-ODN-L + 50T-ODN-R + Ag^+; lane 6: 50T-ODN-L + 50T-ODN-R + Ag^+ with US treatment. The lateral annotation of ultra-low range DNA marker indicates the number of base pairs.

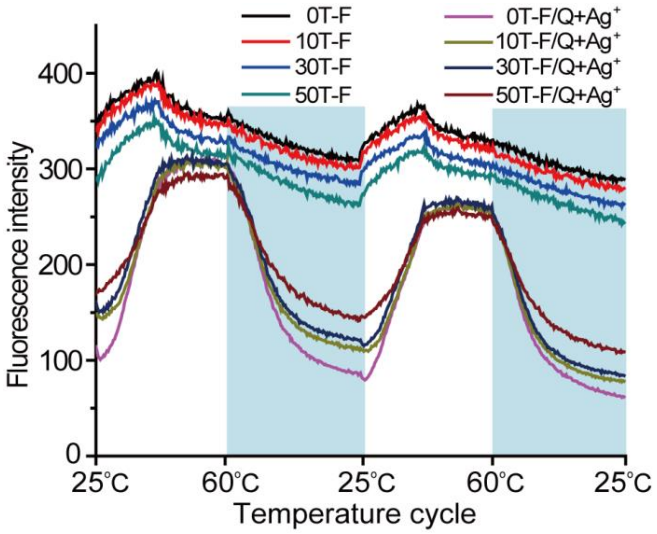

Figure S2. Real time temperature-dependent fluorescence intensity recording of fluorescein-labelled ODNs and Ag^+ base-paired ODNs with varying length of DNA tails. The temperature ramp rate was 0.5 °C·min^{-1}.

4. References

1 Y. Tanaka, J. Kondo, V. Sychrovský, J. Šebera, T. Dairaku, H. Saneyoshi, H. Urata, H. Torigoe and A. Ono, *Chem. Commun.*, 2015, **51**, 17343–17360.