Electronic Supplementary Information

for

Freezing promoted hybridization of very short DNA oligonucleotides

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Materials and methods

Chemicals and Instrumentation. Sodium chloride and 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) were purchased from Mandel Scientific (Guelph, ON, Canada). Thiazole orange, thioflavin T, and SYBR Green I were from Sigma-Aldrich. All of the DNA samples were purchased from Integrated DNA Technologies (IDT, Coralville, IA). The sequences of the DNAs are shown in Table S1. Milli-Q water was used for all of the experiments to prepare buffers and solutions. The fluorescence was measured using a plate reader (SpectraMax M3) with 490 nm excitation and 534 nm emission. The photographs were taken by a digital camera in a dark room with 470 nm LED excitation (Safe Imager BlueLight Transilluminator, Invitrogen).

Staining DNA with dyes. All the DNA (see sequence in Table S1) were dissolved in 5 mM HEPES buffer (pH 7.6). To screen the DNA dye, 1 μM DNA (A5, T5, or A5+T5, 0.5 μM each) was incubated with 10 μM dye in 5 mM HEPES (pH 7.6). The samples were then frozen at -20 °C for 2 h. A LED excitation (470 nm) was used to excite the DNA-dye samples. To test the hybridization of poly-A and poly-T, DNAs in 5 mM HEPES (pH 7.6) were used with a total adenine concentration of 9 μM, and TO was 10 μM. To test the effect of salt concentration, various concentration of NaCl from 1 mM to 200 mM was added into the 5 mM HEPES. To test the effect of ice, various amounts of glycerol from 15 to 50 % (w/v) were added into the samples before freezing. The fluorescence of the samples was recorded using a digital camera with excitation at 470 nm in a dark room.

Fluorescence spectroscopy. To measure fluorescence in the solution state, 100 μL sample (DNA and TO mixed in 5 mM HEPES buffer) was added in a 96 well black half-area plate. The fluorescence spectroscopy was then measured by a plate reader by excitation at 490 nm. To measure fluorescence in the frozen state, the 96 well black plate was frozen in a -20 °C freezer for 2 h. Then the spectra were collected quickly (in about 10 sec) by excitation at 490 nm with the plate reader. To avoid freezing-induced heterogeneity, we measured the fluorescence intensity at 12 spots of each well in solution phase and in the frozen state, respectively. For the FRET experiment, excitation was at 440 nm.

Table S1. The sequences of the DNA used in this work.

DNA names	Sequences and modifications (from 5′ to 3′)	
A_5	AAA AA	Poly A
A_7	AAA AAA A	
A_{10}	AAA AAA AAA A	
A_{12}	AAA AAA AAA AAA	
A_{15}	AAA AAA AAA AAA AAA	
T_5	TTT TT	Poly T
T7	TTT TTT T	
T_{10}	TTT TTT TTT T	
T_2CT_2	TTC TT	Mismatch
TC_2T_2	TCC TT	
TC_3T	TCC CT	
T_3CT_3	TTT CTT T	
T_4CT_5	TTT TCT TTT T	
Sub-5	ACT AT	Random Sequence
C-Sub-5	ATA GT	
Sub-7	TCA CTA T	
C-Sub-7	ATA GTG A	
Sub-10	AGA TCA CTA T	
C-Sub-10	ATA GTG ACT C	
30 mer	Cy3-GTC ACG AGT CAC TAT AGG AAG ATG GCG AAA-FAM	
P1	CGCCATC	
P2	TCCTATA	
P3	TGACTCG	
M1	CGCAATC	
M2	TCCCATA	
M3	TGAATCG	

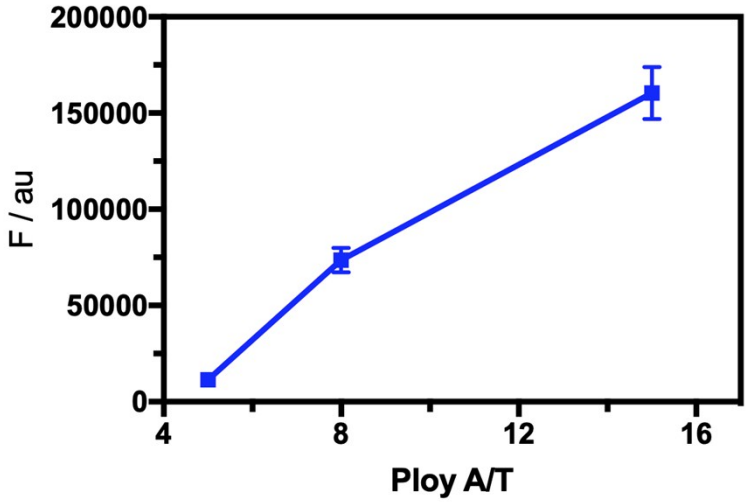

Fig. S1. SG staining with different lengths of poly-A/T DNA. All measurements performed in 10 mM HEPES (pH 7.6) buffer. DNA concentrations were adjusted so that the number of base pairs were the same in each case. For 5 AT base pairs, [A5]= 200 μM, [T5]= 400 μM. For 8 AT base pairs, [A8]= 125 nM, [T8]= 250 nM. For 15 AT base pairs, [A15]=67 nM, [T15]= 134 nM. The result shows that at least 8-mer DNA was required to see a strong fluorescence.

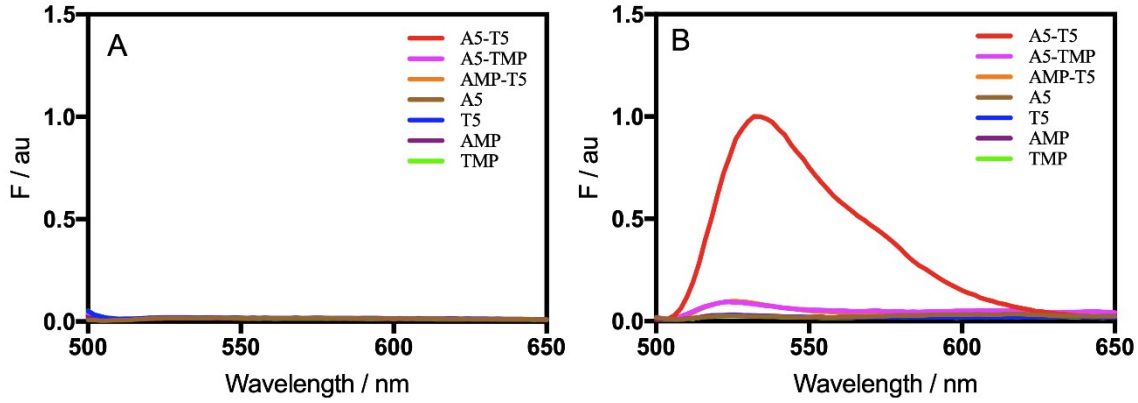

Fig. S2. Hybridization of A/T DNA followed by TO staining. Fluorescence of A/T DNA (A) at room temperature and (B) after freezing at -20°C. The total nucleotide concentration was fixed at 15 µM (e.g. 1.5 µM of A5 and T5 DNA) in 5 mM HEPES buffer (pH 7.6), and TO was 10 µM. Nucleoside monophosphate was used as 1-mer but we did not observe any fluorescence enhancement for these samples.

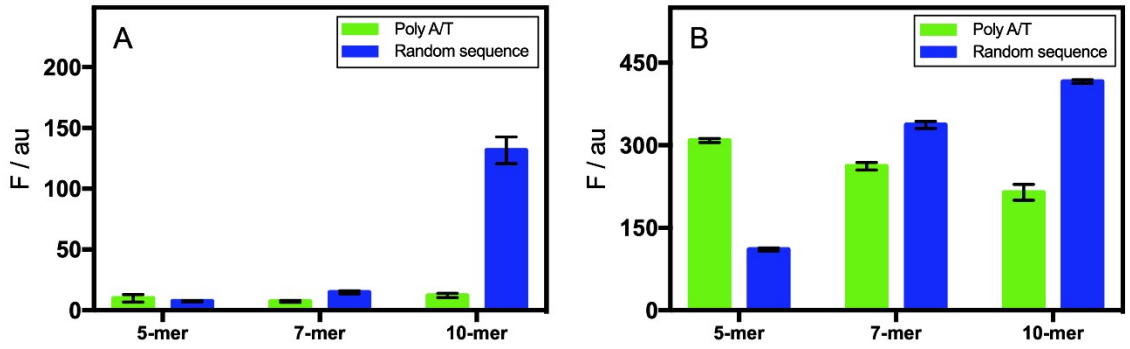

Fig. S3. Hybridization of short DNA studied by TO staining (A) at room temperature, and (B) after freezing at -20 °C. The total nucleotide concentration was fixed at 9 μM (e.g. 0.9 μM of A5 and T5 DNA, or 0.9 μM of Sub-5 and C-Sub-5 DNA), and TO was 10 μM. The fluorescence was detected in 5 mM HEPES (pH 7.6). At room temperature, we observed strong fluorescence only with the 10-mer random sequence DNA. Since poly A/T DNA duplexes have the lowest stability, and likely that the random sequence 10-mer can be stable at room temperature but the A10/T10 duplex was unstable at room temperature. Note no salt was added. Upon freezing, all the samples showed strong fluorescence, indicating that freezing works not only for the poly A/T DNA but also for other sequences.

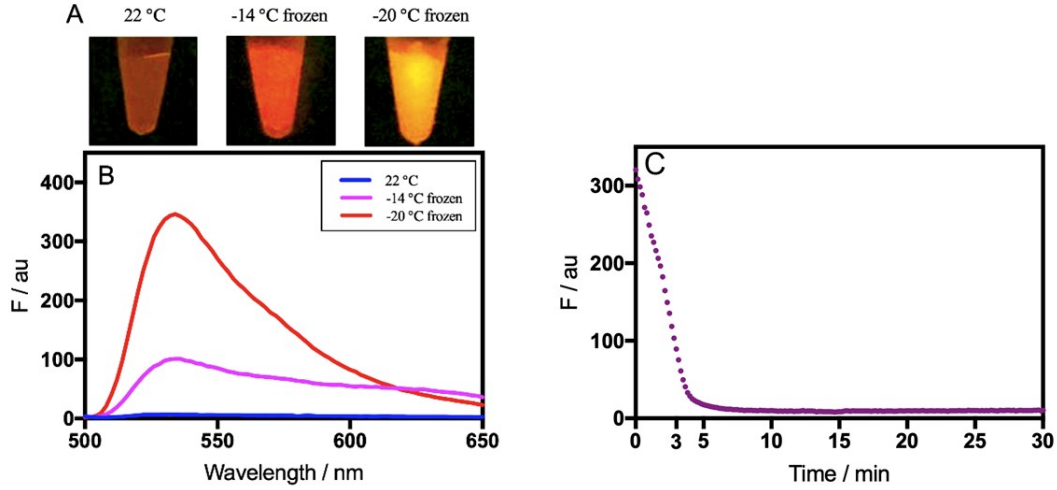

Fig. S4. Fluorescence (A) photographs and (B) spectra of A5-T5 DNA at different temperatures. The total nucleotide concentration was fixed at 15 μM (e.g. 1.5 μM of A5 and T5 DNA) in 5 mM HEPES buffer (pH 7.6), and TO was 10 μM. In this case, the emission of -14 °C was weaker than that at -20 °C, indicating that temperature was important for stable formation of such very short duplex. (C) Time-dependent fluorescence change of A5-T5 DNA at 534 nm with 0 mM NaCl after freezing and taken out of -20 °C to room temperature. The total nucleotide concentration was fixed at 9 μM (e.g. 0.9 μM of A5 and T5 DNA) in 5 mM HEPES buffer (pH 7.6), and TO was 10 μM. After 5 min, the fluorescence disappeared due to thawing of the sample. This kinetics also reflects the effect of temperature for DNA hybridization.

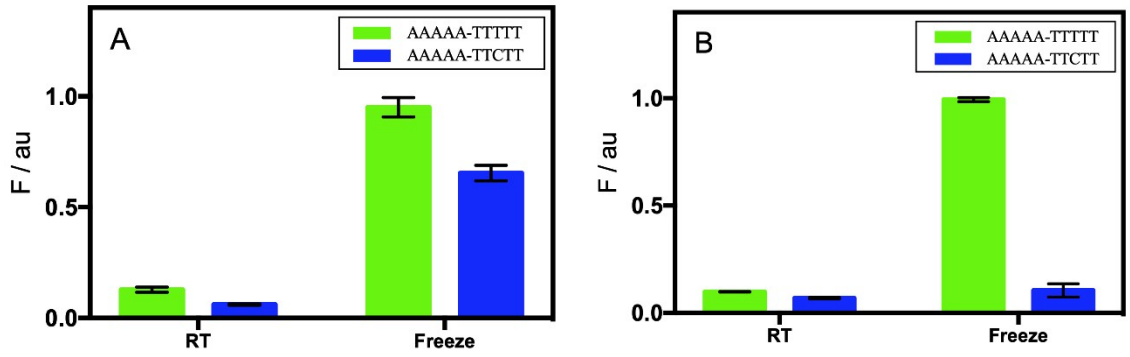

Fig. S5. Effect of mismatch in T5 DNA with (A) no NaCl and, (B) 50 mM NaCl. The nucleotide concentration was fixed at 5 µM (e.g. 0.5 µM of A5 and T5 DNA), and TO was 10 µM. After freezing, the fluorescence intensity of mismatched A5/T2CT2 decreased, but the differences were not significant with T5. With 50 mM NaCl (Figure S5B), only poly A5-T5 had fluorescence after freezing. The emission of the mismatched sample was as low as the room temperature samples. Therefore, specificity was improved by salt and our experiment in the main paper was performed with 50 mM NaCl.