Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

DNA duplex stabilization in crowded polyanion solutions

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1. Materials and Methods

Chemicals. The DNA samples were purchased from Integrated DNA Technologies (IDT, Coralville IA). The DNA sequences are shown in Figure S1. All the NaPAA polymers were purchased from Sigma-Aldrich and PEG from VWR. NaCl and HEPES were purchased from Mandel Scientific (Guelph, Ontario, Canada). Milli-Q water was used for all the experiments.

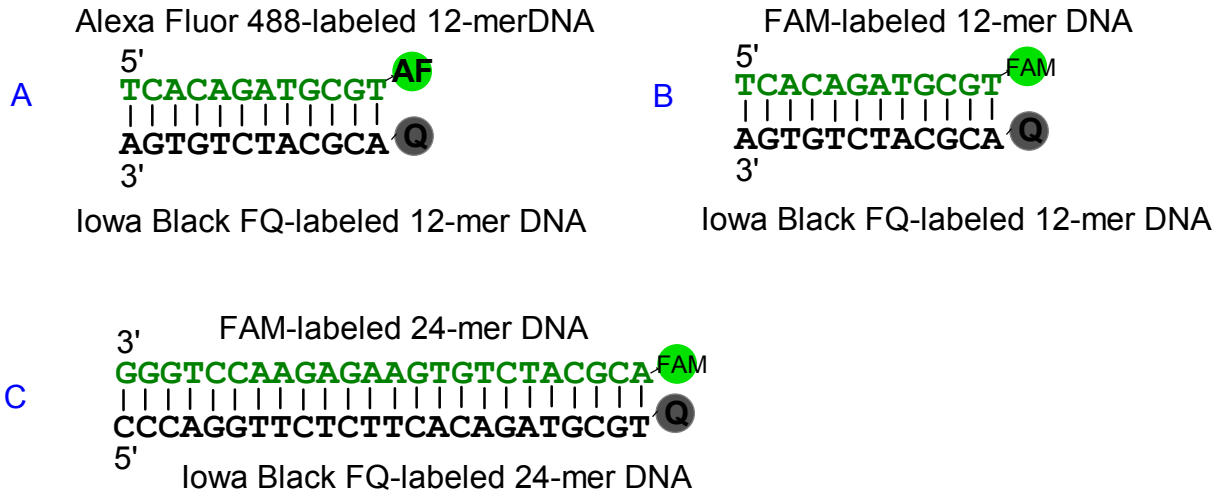

Figure S1.DNA sequences and modifications used in this work.

Melting curves. DNA samples were prepared by dissolving the fluorophore and quencher labeled DNA at final concentrations of 25 μM and 30 μM, respectively in 250 mM HEPES (pH 7.6). The sample was incubated at 4 °C for 30 min to allow DNA hybridization. Polymer solutions were prepared by diluting 45% NaPAA1200, 45% NaPAA8k, or 35% NaPAA15k with water. To 49 μL of the polymer solutions, 1 μL of the DNA solution was added and mixed so that the final DNA concentrations were 500 and 600 nM for the fluorophore and quencher-labeled DNA. The final HEPES

concentration was 5 mM. The samples were loaded into a 96-well PCR plate with each well containing 15 μL of the sample. The melting curves were analyzed using a real-time PCR thermocycler (CFX96, Bio-Rad) with 20 sec incubation at each temperature and 1 °C increment. Melting curves with different DNA concentrations or in the presence of PEG were collected using similar methods.

2. Original melting curves. To allow easy comparison of T_m, the melting curves in the paper have been normalized. Herein, the original curves are presented in Figure S2. At low salt or polymer concentration, the background fluorescence was high (e.g. the black traces) since the DNA hybridization reaction requires salt. Increase of NaCl or NaPPA resulted in the suppression of both background fluorescence as well as the final fluorescence. The final fluorescence suppression is likely due to fluorescence quenching. After reaching the maximal fluorescence indicating complete melting of DNA duplex, further increase of temperature caused fluorescence quenching for all the samples. This is because the quantum yield of fluorescence is lower at higher temperatures. These melting curves indicate that normalization did not change the conclusions about T_m values.

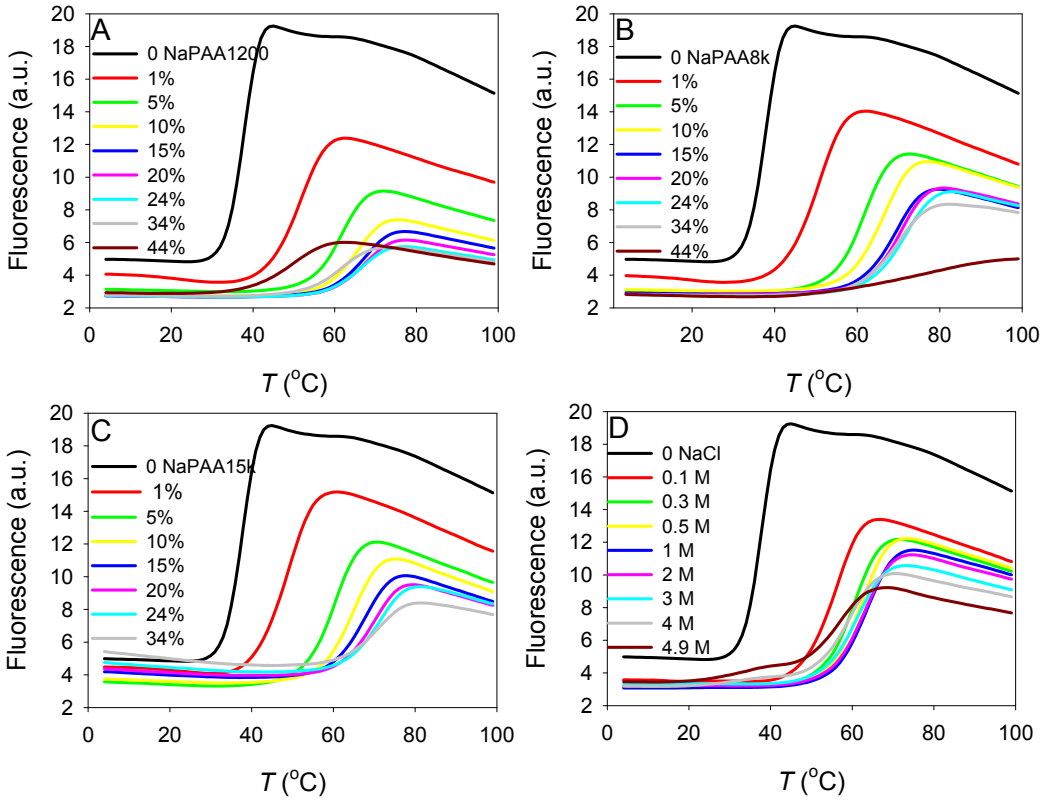

Figure S2. Original melting curves for the DNA in NaPAA and in NaCl.

2. Comparing FAM-labeled DNA with Alexa Fluor 488 labeled DNA. To test whether using fluorophore labeled DNA might introduce artifacts, we replaced the Alexa Fluor 488 with FAM. As shown in Figure S3, these DNAs showed almost identical T_m values under all tested NaPAA 8k concentrations. Considering the chemical difference between FAM and Alexa Fluor 488, this experiment strongly suggests that the effect of fluorophore is very small.

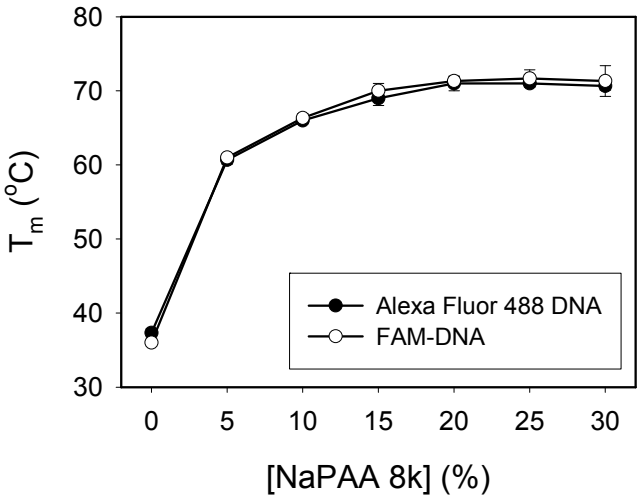

Figure S3. Melting temperature values of the DNAs shown in Figure S1A and S1B as a function of NaPAA 8k concentration.

4. Melting of a 24-mer DNA. To test the effect of DNA length, we further employed the 24-mer DNA shown in Figure S1C. As shown in Figure S4A, NaPAA 1200 also showed significant drop in Tm at high concentration and the highest T_m value that can be achieved in NaPAA 8k is ~86 °C, which is significantly higher than the highest T_m values (~~80 °C) observed with NaCl or PEG 4k with NaCl (Figure S4B).

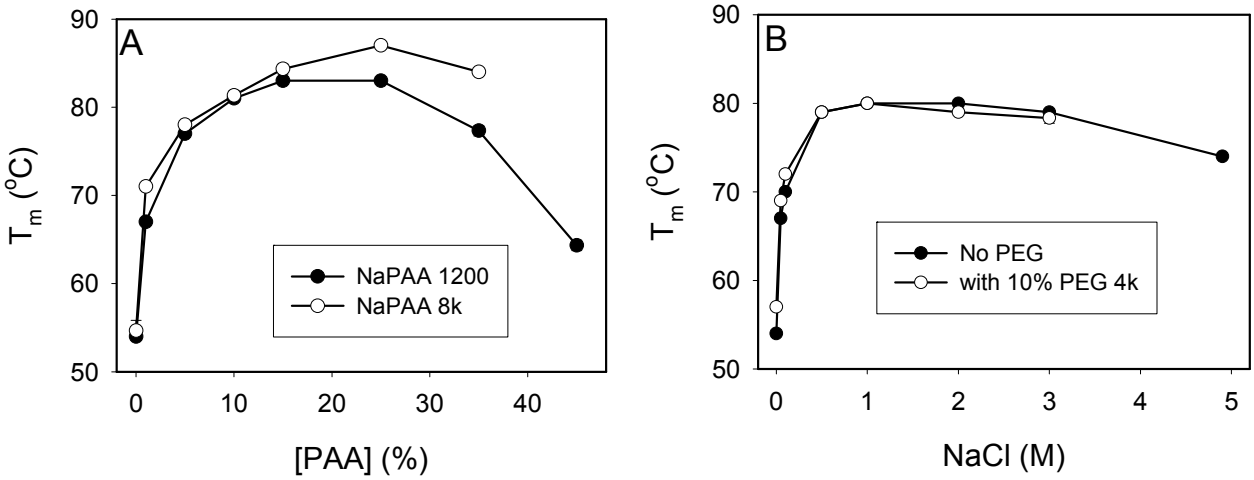

Figure S4. T_m of the 24-mer DNA in Figure S1C as a function of NaPAA concentration and MW (A) or as a function of NaCl concentration in the absence or presence of 10% PEG 4k.