Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Supramolecular Assembly-Enabled Homochiral Polymerization of Short $(dA)_n$ Oligonucleotides

Sreejith Mangalath, Suneesh C. Karunakaran, Gary Newnam, Gary B. Schuster, and Nicholas V. Hud*

School of Chemistry and Biochemistry, Georgia Institute of Technology, 311 Ferst Drive, Atlanta, Georgia 30332-0400 (USA)

E-mail: hud@chemistry.gatech.edu

Materials and Methods

Oligonucleotides were purchased from IDT. Cyanuric acid, EDC, MES, HEPES and $MgCl_2$ were purchased from Sigma Aldrich. beta-L-Pac-dA-CE Phosphoramidite was purchased from Glen Research. L-oligonucleotide and all the oligos with mismatches were synthesized inhouse using a standard DNA/RNA synthesizer.

Construction of assembly: 500 μM of oligonucleotide (unless otherwise stated), Cyanuric acid (12.5 mM – 75 mM), 100 mM HEPES buffer (pH 6.8) and 10 mM $MgCl_2$ were mixed and made up to 10 μL by gentle heating to 80 °C for 5 min. The mixture is then allowed to cool to 4 °C for 15 min and used for analyses.

Polymerization Reactions: Samples for polymerization reactions were prepared by mixing oligonucleotide (500 μM, unless otherwise stated) and Cyanuric acid (12.5 mM – 75 mM), 100 mM HEPES buffer (pH 6.8) and 10 mM $MgCl_2$. After 15 min equilibration at 4 °C, EDC (250 mM, from an H_2O stock) was added, and samples were incubated for 24 h (unless otherwise stated) at 4 °C.

^{32}P Post-labeling assay: After reaction, a 1 μL aliquot of reaction mixture was removed and treated with T4 polynucleotide kinase and [γ^{32}P] ATP overnight, according to standard procedures. The ^{32}P labelled redaction products were analyzed using 20% denaturing PAGE analysis.

Ion exchange chromatography (HPLC) analysis of Reaction Products: Reactions contained 500 μM oligonucleotide, Cyanuric acid (12.5 mM – 75 mM), 10 mM $MgCl_2$, 100 mM HEPES buffer (pH 6.8), and 250 mM EDC. The reactions were incubated at 4 °C. At each time point, a 1 μL aliquot was removed and diluted in 99 μL 25 mM EDTA and 25 mM tris buffer. The aliquot was then immediately chromatographed (Thermofisher DNAPac 200 analytical column, ambient temperature). Gradient: Solvent A = 12.5 mM Tris buffer, pH 8. Solvent B = 12.5 mM Tris buffer + 1.5 M NaCl, pH 8. 0–15 min, 5% B. 15–25 min, 5–55% B. 25–30 min, 5% B.

CD Analysis: Circular dichroism (CD) spectra were collected using a JASCO 810 CD spectropolarimeter. Samples were contained within a strain free 0.1 mm quartz demountable cell (Starna).

AFM Analyis: AFM images were obtained with a Nanoscope IIIa (Digital Instruments) in tapping mode using Si tips (Vistaprobes, 48 Nm^{-1}) on freshly cleaved mica. The mica substrate was rinsed with water and dried under N_2. A 2 μL sample of the assembly solution was spread over the mica using N_2 flow and was dried with N_2 gas. The average length and height of supramolecular polymers from AFM images were calculated using the image analysis software Image J (NIH).

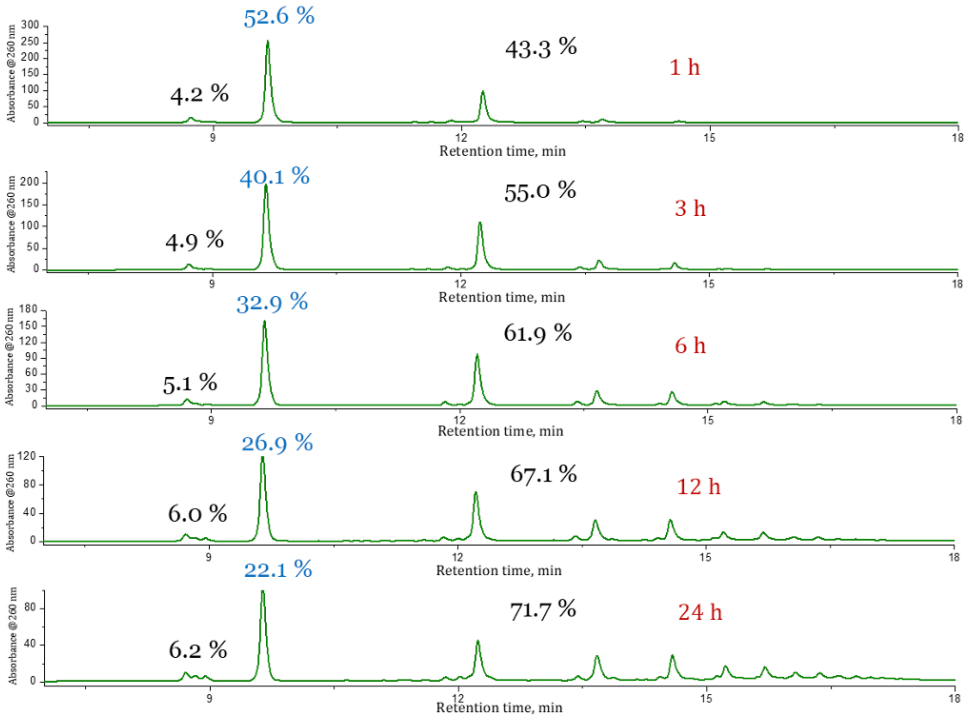

Figure S1. Ion exchange chromatograms of $(dA)_5$3'p- CA (500 μM, 25 mM CA, 100 mM HEPES, pH 6.8, 10 mM $MgCl_2$, 250 mM EDC, 4 °C) polymerization reactions at various time intervals (1, 3, 6, 12 and 24 h).

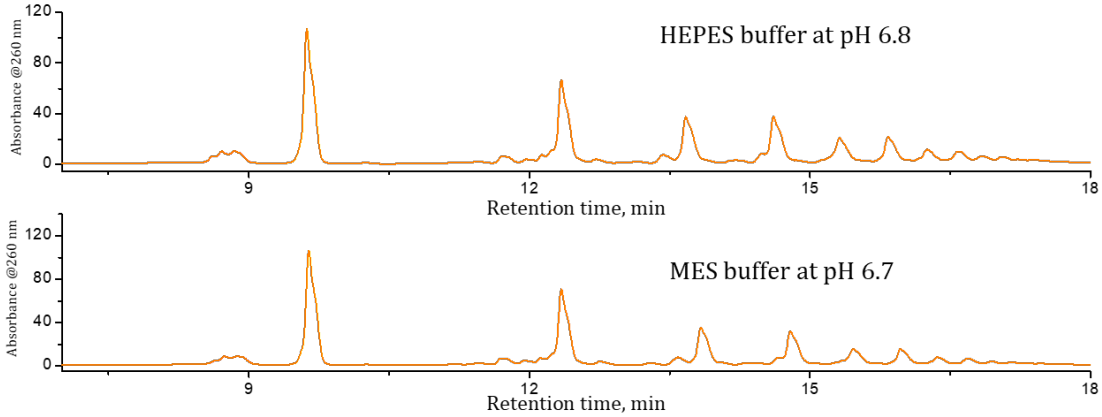

Figure S2. (a) Ion exchange chromatograms of $(dA)_5$ – CA assembly (500 μM$(dA)_5$3'p, 25 mM CA, 10 mM $MgCl_2$, 250 mM EDC, 4 °C) polymerization reactions in HEPES and MES buffer (100 mM, pH 6.7-6.8) after 24 h.

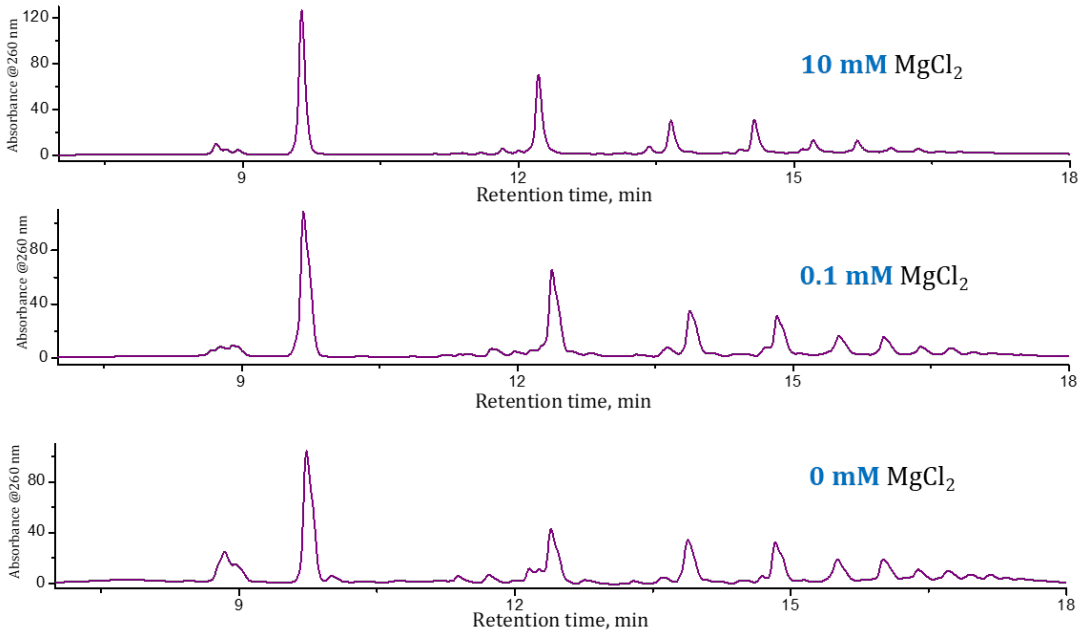

Figure S3. Ion exchange chromatograms of $(dA)_5$ – CA assembly (500 μM $(dA)_5$3'p, 25 mM CA, 100 mM HEPES, pH 6.8, 250 mM EDC, 4 °C) polymerization reactions at various $MgCl_2$ concentrations (10 mM, 0.1 mM and 0 mM) after 24 h.

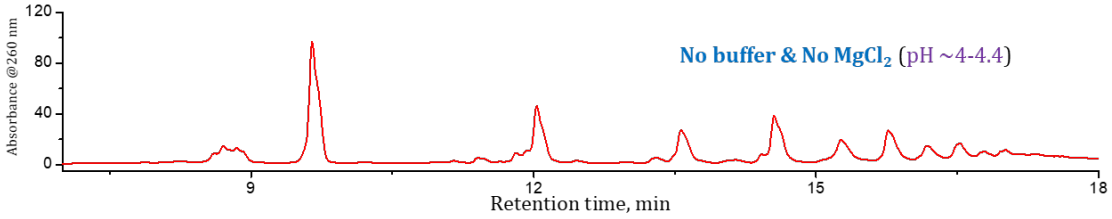

Figure S4. Ion exchange chromatograms of $(dA)_5$ – CA (500 μM $(dA)_5$3'p, 25 mM CA, 10 mM $MgCl_2$, 250 mM EDC, 4 °C) polymerization in aqueous solution (No buffer and $MgCl_2$) after 24 h.

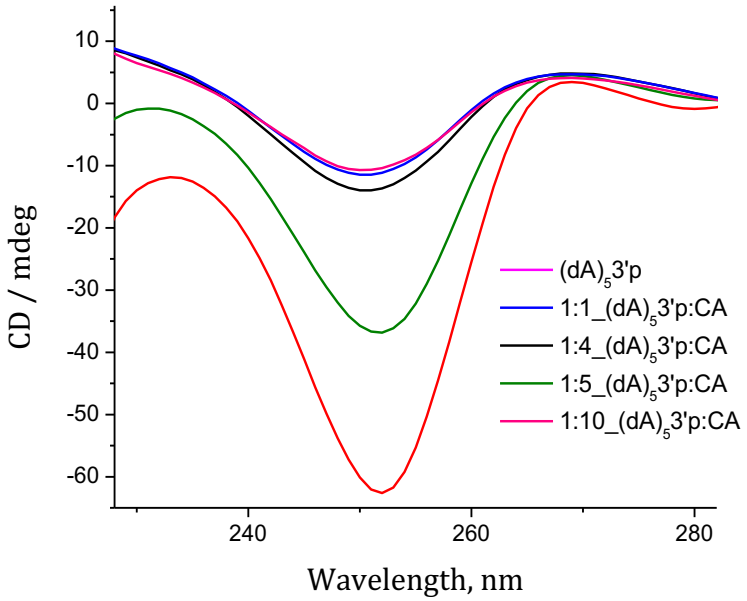

Figure S5. CD spectra of $(dA)_5$ – CA assemblies (500 μM $(dA)_5$3'p, 100 mM HEPES, pH 6.8, 10 mM $MgCl_2$) with varying CA concentrations 2.5 mM, 10 mM, 12.5 mM and 25 mM (dA:CA = 1:1, 1:4, 1:5 and 1:10) at 4 °C.

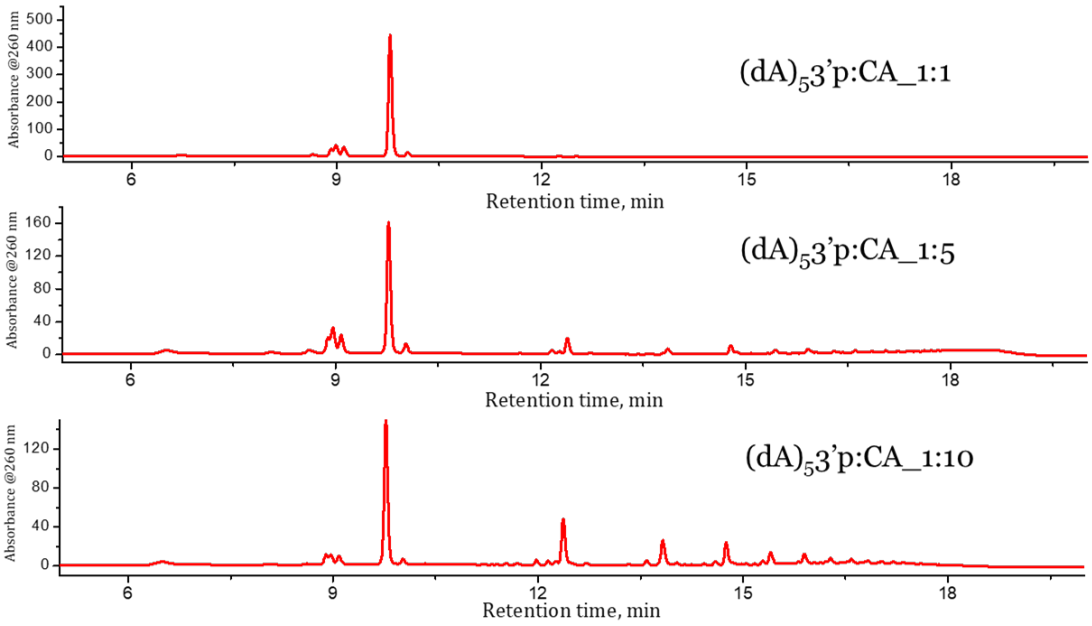

Figure S6. Ion exchange chromatograms of $(dA)_5$3'p – CA assembly polymerization reactions at various CA concentrations- 2.5 mM, 12.5 mM and 25 mM (dA:CA = 1:1, 1:5 and 1:10) after 24 h (500 μM $(dA)_5$3'p, 100 mM HEPES, pH 6.8, 250 mM EDC, 4 °C).

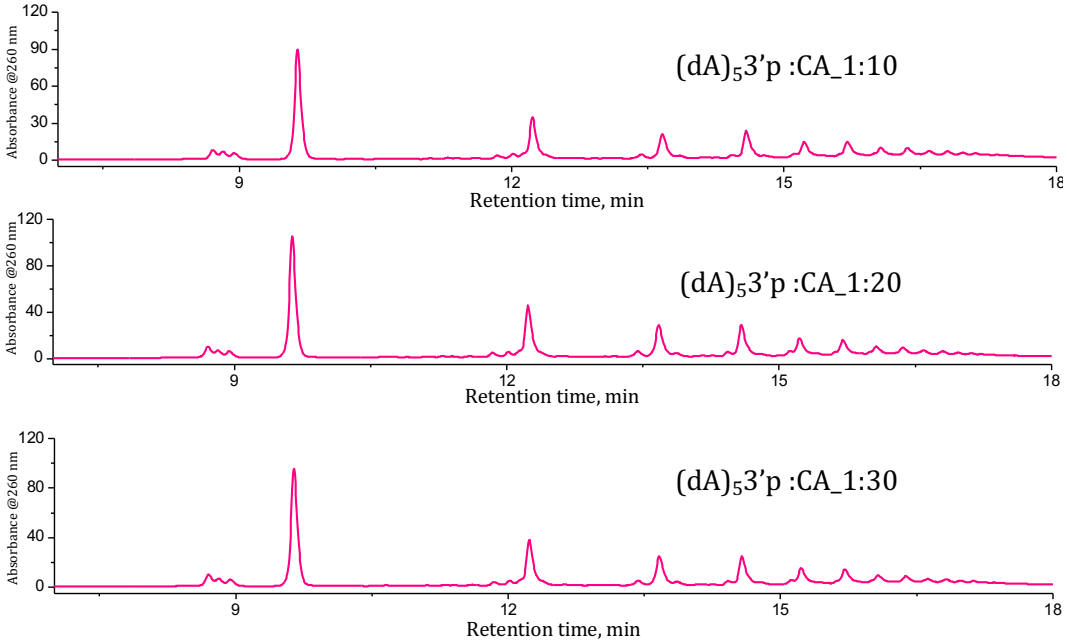

Figure S7. Ion exchange chromatograms of $(dA)_5$3'p – CA assembly polymerization reactions at various CA concentrations- 25 mM, 50 mM and 75 mM (dA:CA = 1:10, 1:20 and 1:30) after 24 h (500 μM $(dA)_5$3'p, 10 mM $MgCl_2$, 100 mM HEPES, pH 6.8, 250 mM EDC, 4 °C).

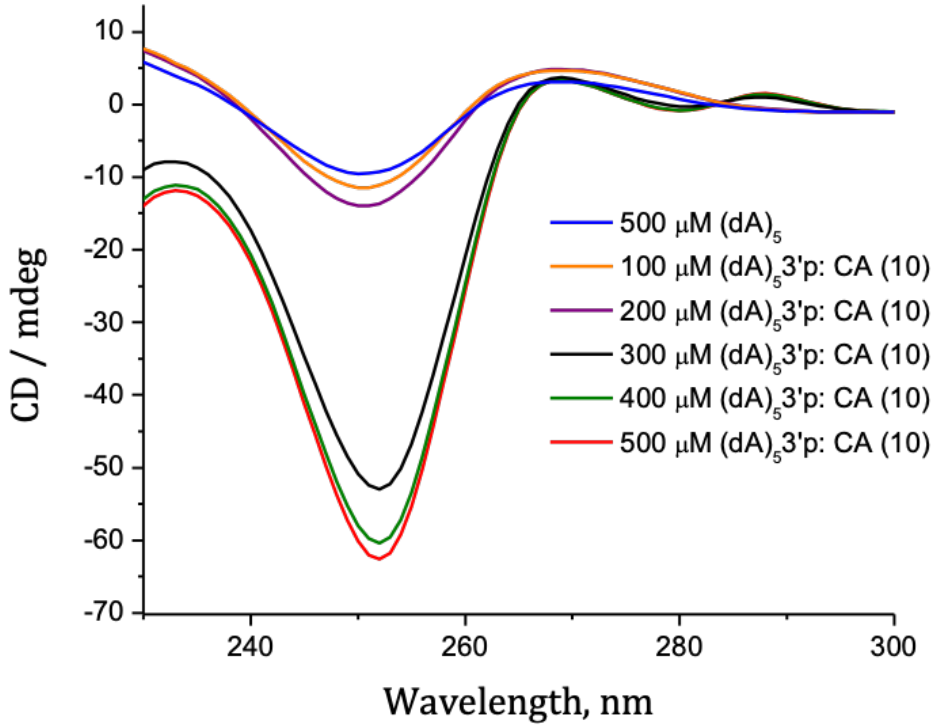

Figure S8. CD spectra of $(dA)_5$ – CA assemblies with varying $(dA)_5$3'p concentrations (100-500 μM $(dA)_5$3'p, 100 mM HEPES, pH 6.8, 10 mM $MgCl_2$) with 5 – 25 mM CA (dA:CA = 1:10) at 4 °C.

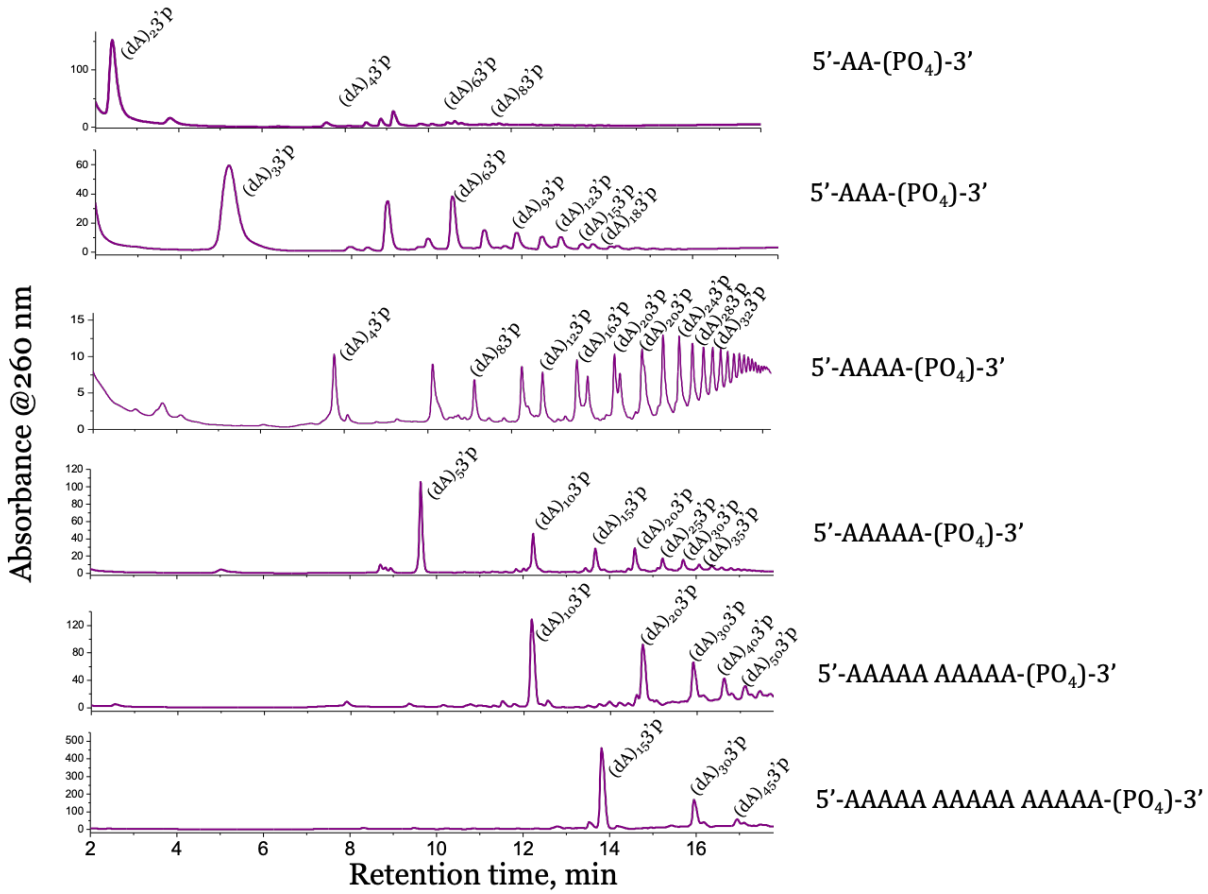

Figure S9. Ion exchange chromatograms of $(dA)_n$3'p-CA polymerization reactions with change in oligomer length ($(dA)_2$3'p -5 mM, $(dA)_3$3'p -1 mM, $(dA)_4$3'p -700 μM, $(dA)_5$3'p -500 μM, $(dA)_{10}$3'p -500 μM and $(dA)_{15}$3'p -500 μM) with CA concentrations of 25-100 mM (dA:CA = 1:10) after 24 h (100 mM HEPES, pH 6.8, 250 mM EDC, 4 °C). The concentrations of $(dA)_n$3'p, n>4 were fixed at 500 μM and higher concentrations of $(dA)_n$3'p, n<5 were chosen to ensure formation of stable assembly.

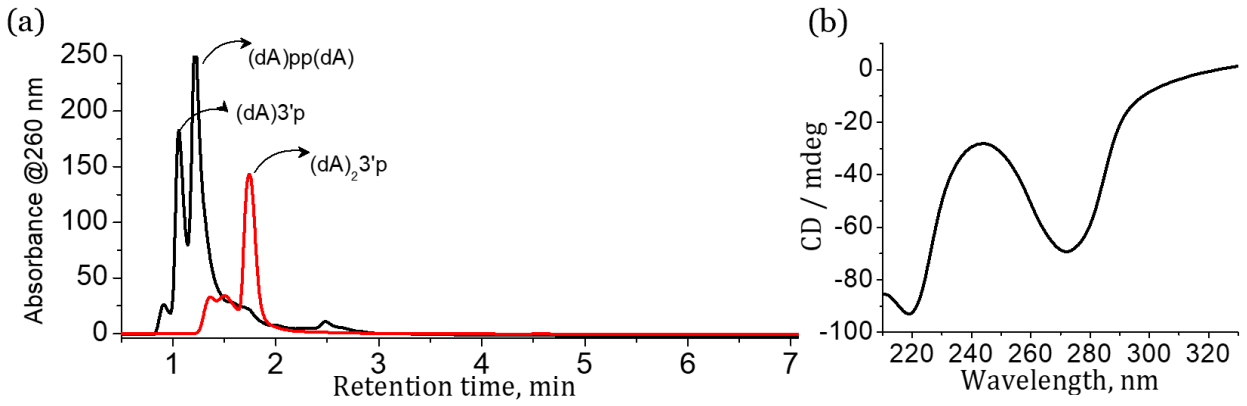

Figure S10. (a) Ion exchange chromatograms of (dA)3'p– CA assembly polymerization reaction (black trace) after 24 h (10 mM (dA)3'p, 100 mM CA, 100 mM HEPES, pH 6.8, 10 mM $MgCl_2$, 250 mM EDC, 4 °C) plotted with pre-synthesized $(dA)_2$3'p (red trace) for reference. (b) CD spectrum of (dA)3'p – CA assemblies (10 mM (dA)3'p, 100 mM CA, 100 mM HEPES, pH 6.8, 10 mM $MgCl_2$, 4 °C).

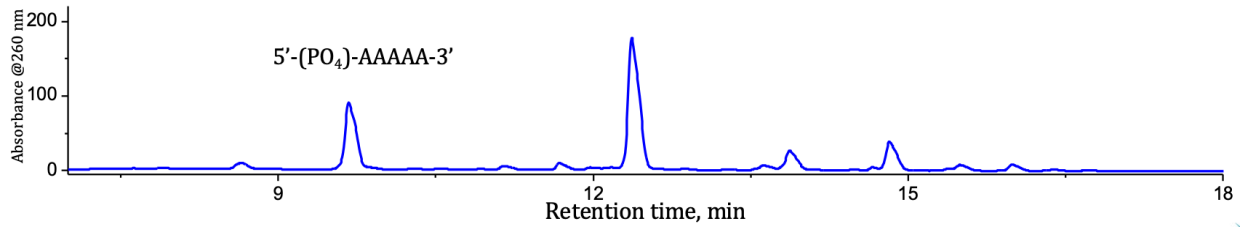

Figure S11. Ion exchange chromatograms of 5'p$(dA)_5$ – CA assembly polymerization reaction after 24 h (500 μM 5'p$(dA)_5$, 25 mM CA, 100 mM HEPES, pH 6.8, 10 mM $MgCl_2$, 250 mM EDC, 4 °C).

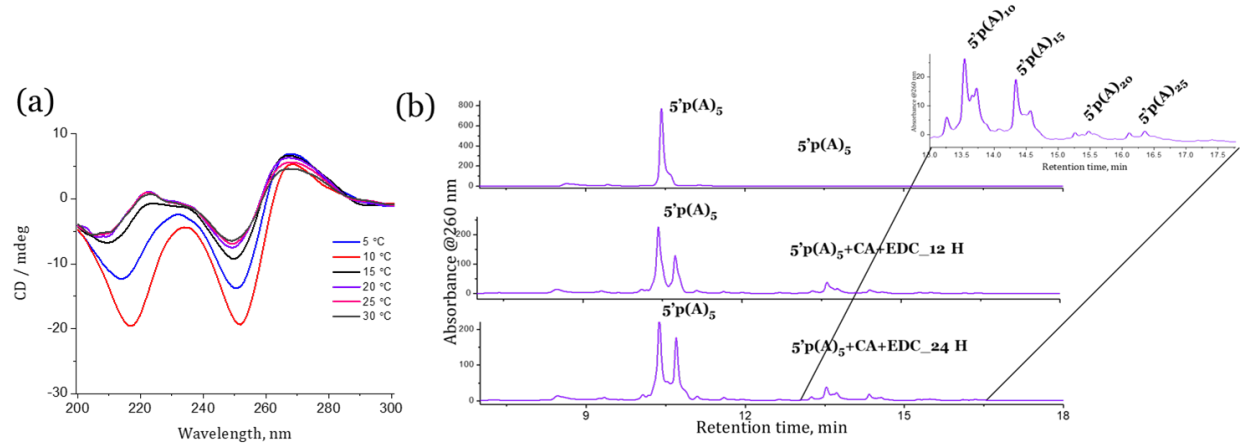

Figure S12. (a) Temperature dependent CD spectra of 5'p$(A)_5$-CA assembly (500 μM 5'p$(A)_5$, 100 mM HEPES, pH 6.8). (b) Ion exchange chromatograms of $(A)_5$5'p and 5'p$(A)_5$:CA polymerization reactions after 12 h and 24 h (500 μM 5'p$(A)_5$, 25 mM CA, 100 mM HEPES, pH 6.8, 250 mM EDC, 4 °C).

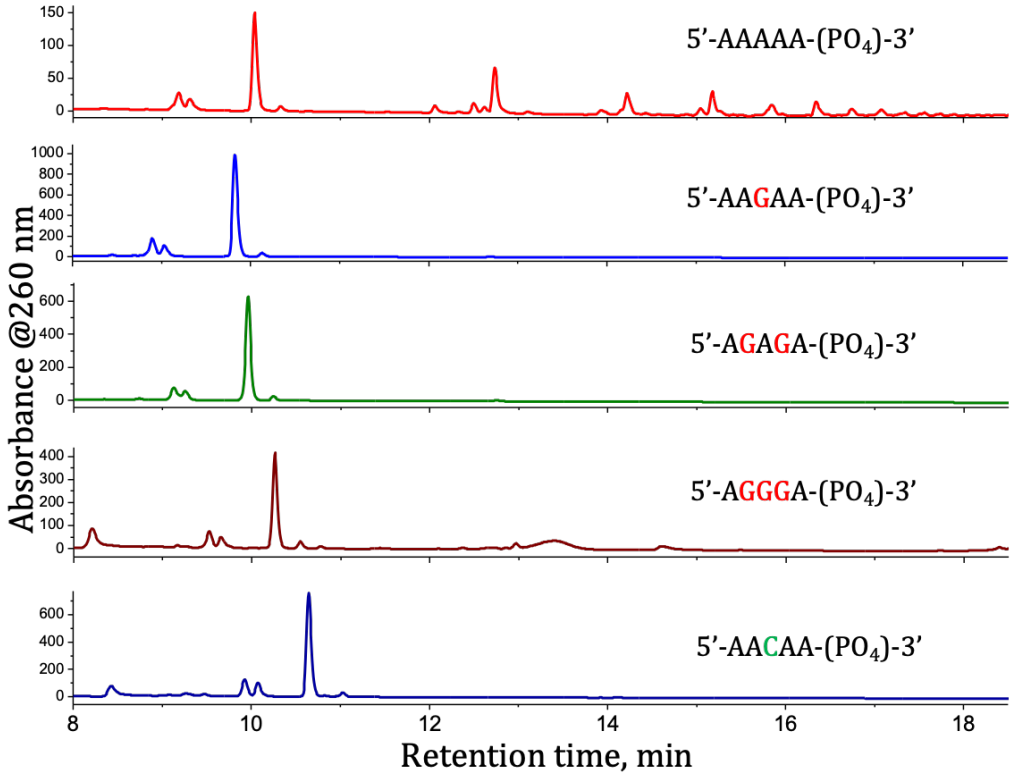

Figure S13. Ion exchange chromatograms of $(dA)_5$3'p and $(dA)_5$3'p with mismatches (G and C) polymerization reactions after 24 h (500 μM oligonucleotides, 25 mM CA, 100 mM HEPES, pH 6.8, 250 mM EDC, 4 °C).