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Supporting Information

Enriching Adenosine by Thymine-Rich DNA Oligomers

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Materials and General Methods

Materials

Stains-all ($C_{30}H_{27}BrN_2S_2$) was purchased from Sigma Aldrich. Tris base, EDTA.Na_2.H_2O, magnesium acetate, acrylamide, bis-acylamide were purchased from Sangon Biotech Co., Ltd (Shanghai, China). *N,N,N′,N′*-Tetramethyl ethylenediamine (TEMED) and ammonium persulfate were purchased from Beijing Dingguo Changsheng Biotech Co., Ltd (Beijing, China). Acetic acid was purchased from Chengdu Kelong Chemical Co., Ltd (Chengdu, China). DNAs were purchased from Sangon Biotech Co. Ltd (Shanghai, China). Adenine were purchased from Shanghai Titan Scientific Co., Ltd (Shanghai, China). Adenosine were purchased from Shaoyuan Scientific Co., Ltd (Shanghai, China).

DNA sequences:

T_{10}: 5'-TTTTTTTTTT-3'
T_{20}: 5'-TTTTTTTTTTTTTTTTTTTT-3'
T_h: 5'-TTTTTTTTTTCGCGTTTTCGCGTTTTTTTTTT-3'
T_c: 5'-GCCGTTTTTTTTTTTTTTTTGCTTTTGCTTTTTTTTTTTTTTTTCGGC-3'

Gel electrophoresis

Native PAGE. Native PAGE containing 14% or 20% polyacrylamide (19:1 acrylamide/bisacrylamide) was run on HOEFER SE 600 electrophoresis system at 4 ℃ or 25 ℃ (220 V, constant voltage). The buffers for electrophoresis and in the DNA samples were same 1×TAE/Mg^{2+} buffer (40 mM Tris, 2 mM EDTA.Na_2.H_2O, 20 mM acetic acid, 12.5 mM magnesium acetate). In each well, 1 μg DNA sample was loaded. After electrophoresis, the gels were stained with Stains-All (Sigma, USA) and scanned with a Cannon LIDE 120 scanner.

Relative mobility calculation. The relative mobility was calculated as the mobility of the complex of DNAs and adenine/adenosine divided by the mobility of A_{20}.

Ultraviolet-visible spectra measurement

The thymine-rich DNAs and adenine/adenosine were dissolved in 1×TAE/Mg^{2+} buffer (20 μL, pH 8.0). The DNAs were heated to 95 ℃ for 5 min and cooled on ice for 10 min before being mixed with adenine/ adenosine. The solution was then cooled from 50 ℃ to 4 ℃ over 2 h (50 ℃ for 5 min, naturally cooled to 4 ℃ and kept at 4 ℃ for 2 h). Then the samples were diluted with 1×TAE/Mg^{2+} buffer (4 ℃) to 200 μL and mixed with vortex before measurement. 1×TAE/Mg^{2+} buffer was used as a blank. The ultraviolet spectra were measured at room temperature (230 to 350 nm range, 100 nm/min scan rate) with a Cary Series UV-Vis Spectrophotometer (Agilent, America).

Self-aggregation of adenine

Adenine in water was heated to 95°C to be dissolved at concentration of 40, 56 or 72 μM. Adenine was immediately mixed with or without 1.0 μM polyT in 1×TAE/Mg^{2+} in 96-well plates respectively, and then was cooled down to 25°C. The self-aggregation of adenine was followed as a function of time by measuring the turbidity of the solutions at 570 nm with a SYNERGY H1 microplate reader (BioTek, America). The optical density (OD) measurement was performed at room temperature. For all the experiments, at least three replicates.

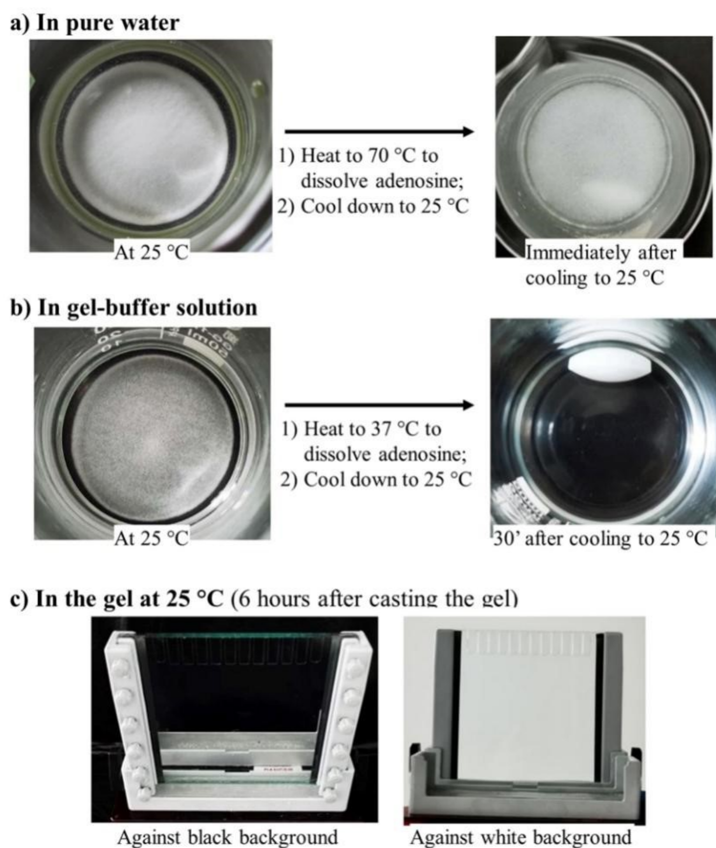


Figure S1. Investigation of the solubility of adenosine. In (a) pure water; (b) gel-buffer solution (polyacrylamide (19:1 acrylamide/bisacrylamide) and 1×TAE/Mg^{2+} buffer (40 mM Tris, 2 mM EDTA.Na_2.H_2O, 20 mM acetic acid, 12.5 mM magnesium acetate); (c) casted gel (the same composition at in solution except that the polyacrylamide was polymerized). *In pure water (Fig. S1a).* 60 mM adenosine is not soluble at 25 °C. When heated to 37 °C, adenosine dissolved; but as long as temperature decreased to 25 °C, adenosine precipitated out. *In gel-buffer solution (Fig. S1b)* that contains polyacrylamide (19:1 acrylamide/bisacrylamide) and 1×TAE/Mg^{2+} buffer (40 mM Tris, 2 mM EDTA.Na_2.H_2O, 20 mM acetic acid, 12.5 mM magnesium acetate). 60 mM adenosine is not soluble at 25 ℃. When heated to 37 °C, adenosine dissolved. When the solution cooled down to 25 ℃, the solution remains clear and no sign of solid precipitation for at least 30' (then, we stopped observation and went on to cast the gel. *In casted gel (Fig. S1c).* The gel-buffer solution was polymerized by adding ammonium persulfate and tetramethylethylenediamine. The casted gel is transparent and remains transparent for at least 6 hours at 25 ℃. No sign to suggest adenosine to precipitate. We have not observed it further.

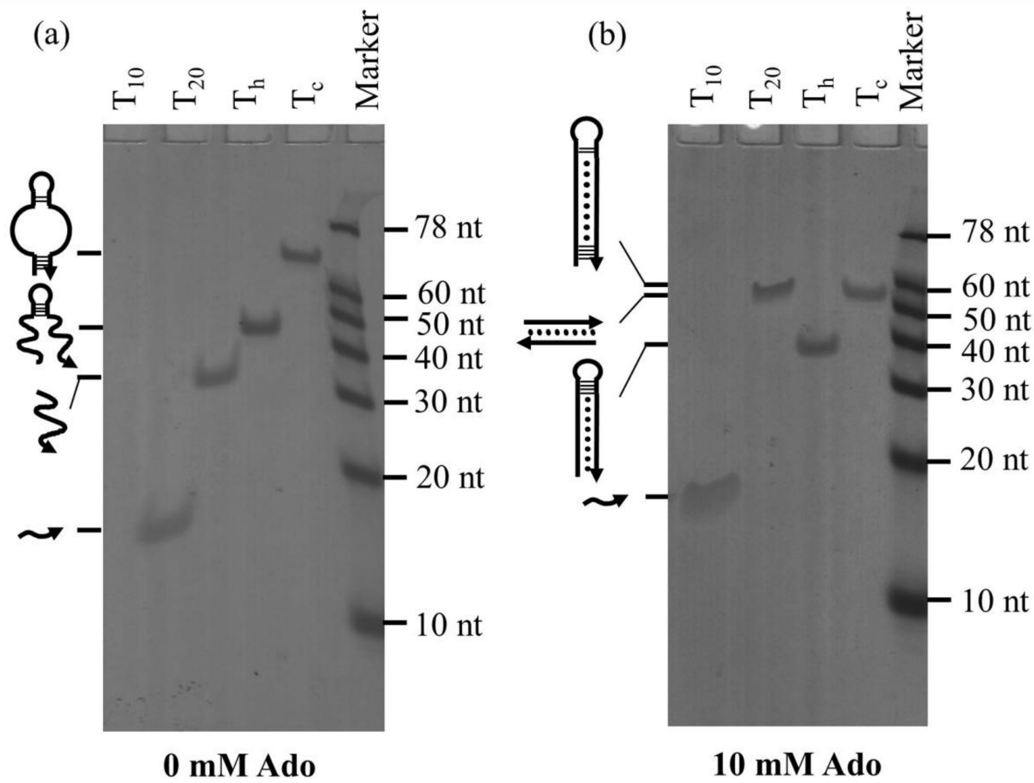


Figure S2. Analysis of the designed DNAs and Ado interaction by 20% native PAGE at 4 ℃. The gel contained 0 mM Ado (a) and 10 mM Ado (b).

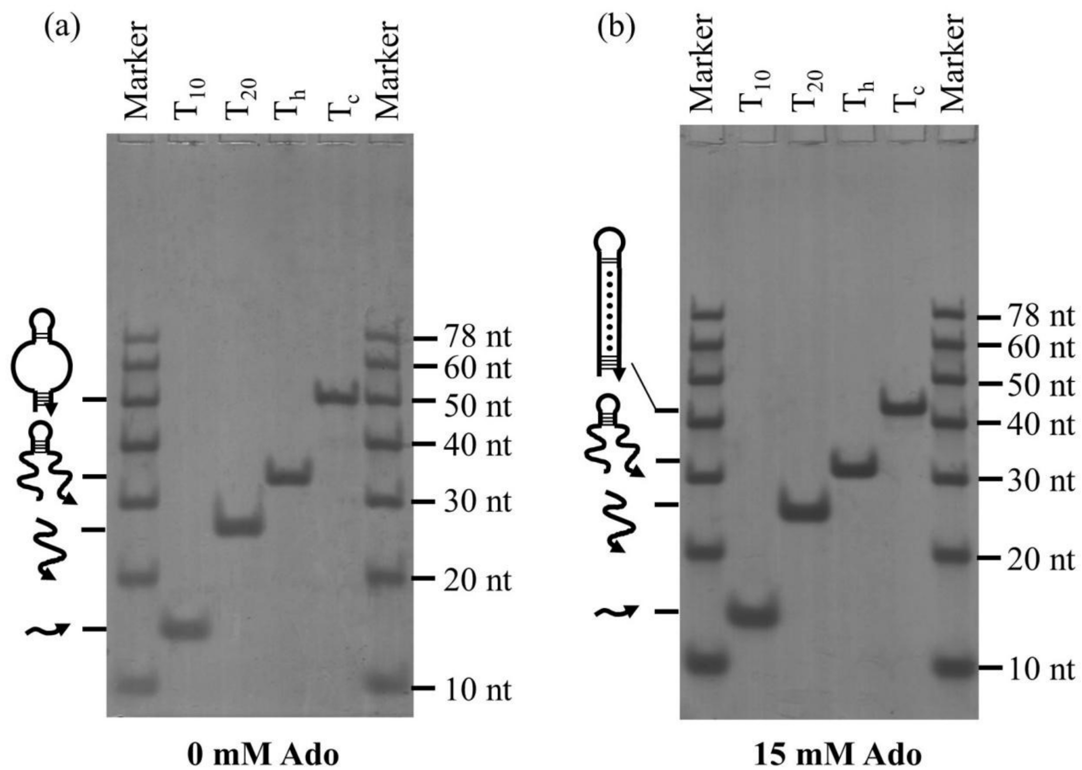


Figure S3. Analysis of of the designed DNAs and Ado interaction by 14% native PAGE at 25 ℃. 0 mM adenosine (a) and 15 mM adenosine (b) was added in the gel.

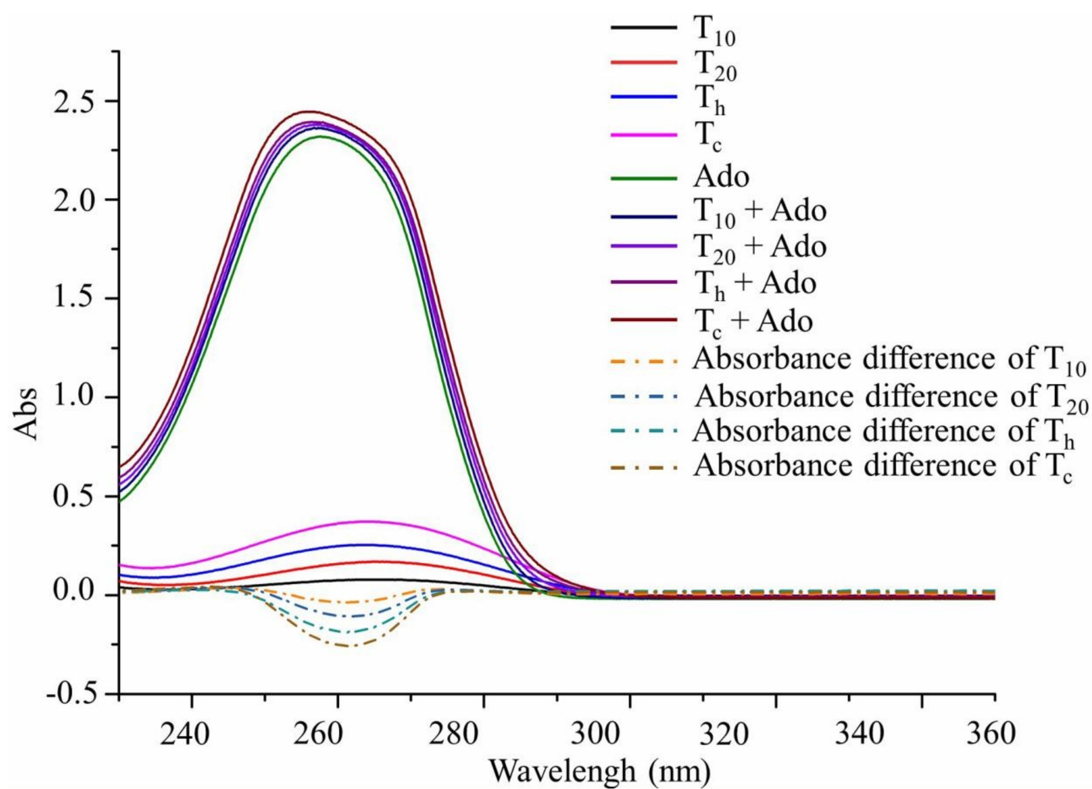


Figure S4. Ultraviolet absorbance of DNA-adenosine complexes at pH 5.0. The positive peaks are the UV absorbance of the complexes. The negative peaks represent the absorbance change (Abs change = $Abs_{(DNA\text{ - }Ado\ complex)}$ - Abs_{DNA} - Abs_{Ado}). DNA concentration: 1.0 μM; Ado concentration: 200 μM. The UV absorbance at pH 5.0 was nearly equal to that at pH 8.0.

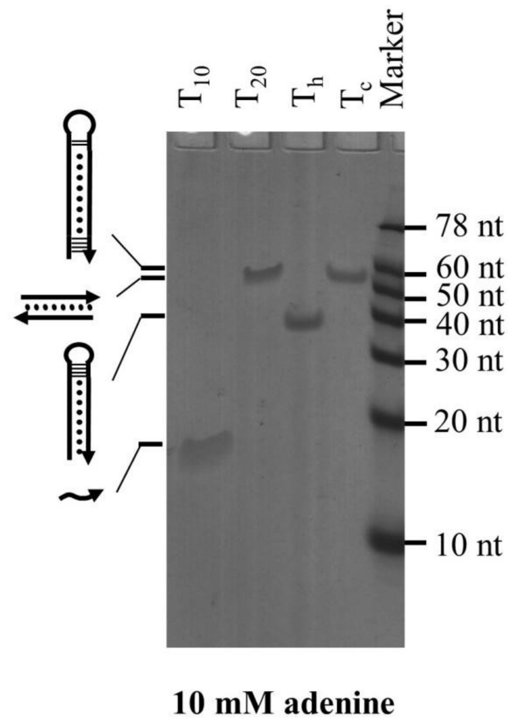
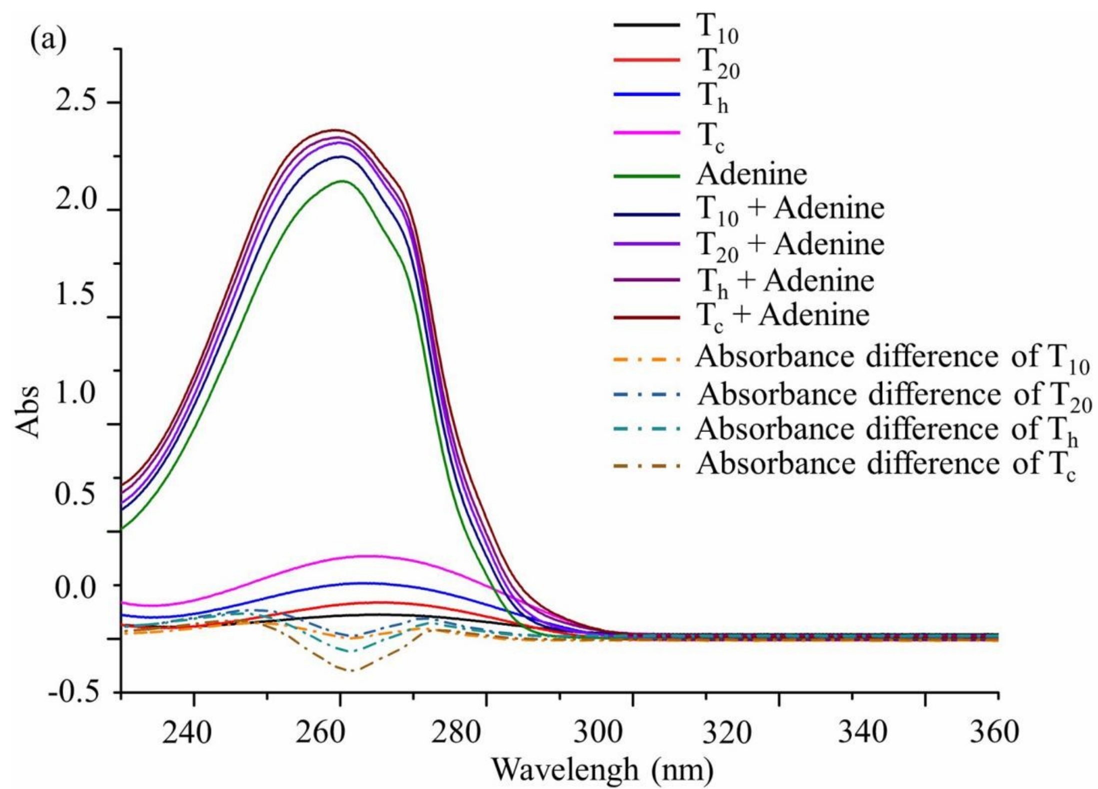


Figure S5. Analysis of the designed DNAs and adenine interaction by 20% native PAGE at 4 ℃. 10 mM adenine was added in the gel.



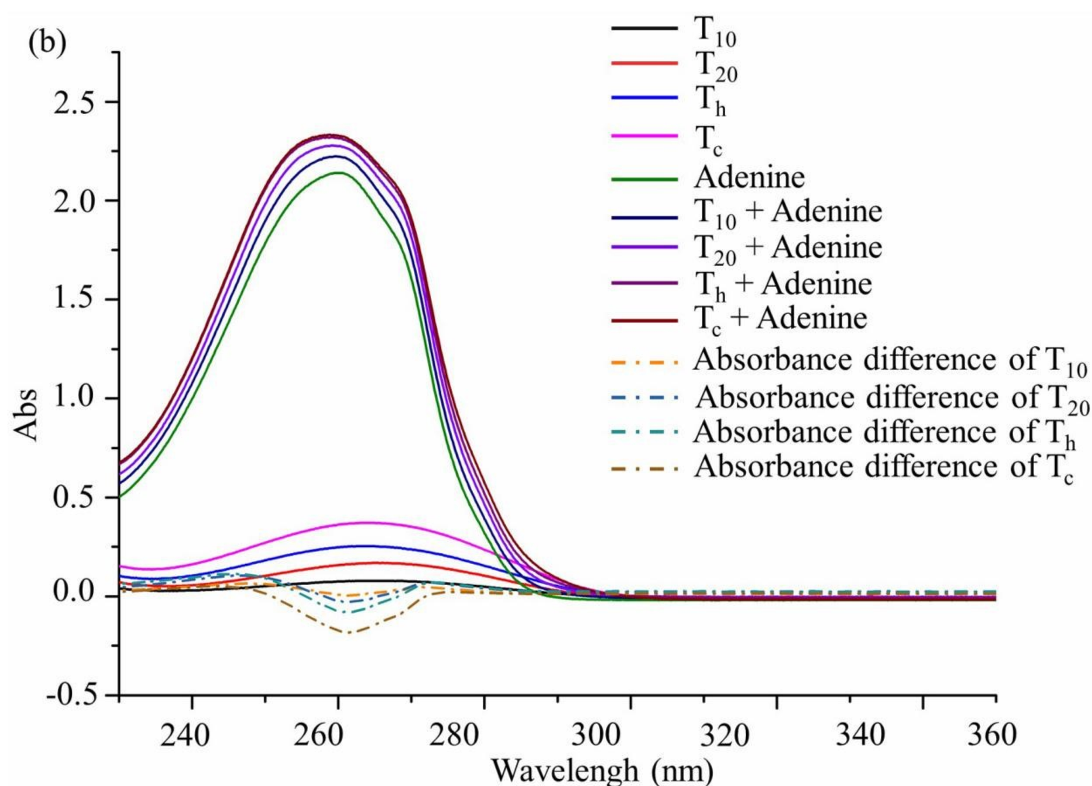


Figure S6. UV absorption spectra of the complexes of polyT and adenine, and the absorbance difference between complex and its monomers at pH 8.0 (a) and pH 5.0 (b). The positive peaks are the UV absorbance of the complexes, and the negative peaks are the absorbance change (Abs change = $Abs_{(DNA\text{ - }adenine\ complex)}$ - Abs_{DNA} - $Abs_{adenine}$). DNA concentration: 1.0 μM; adenine concentration: 200 μM.

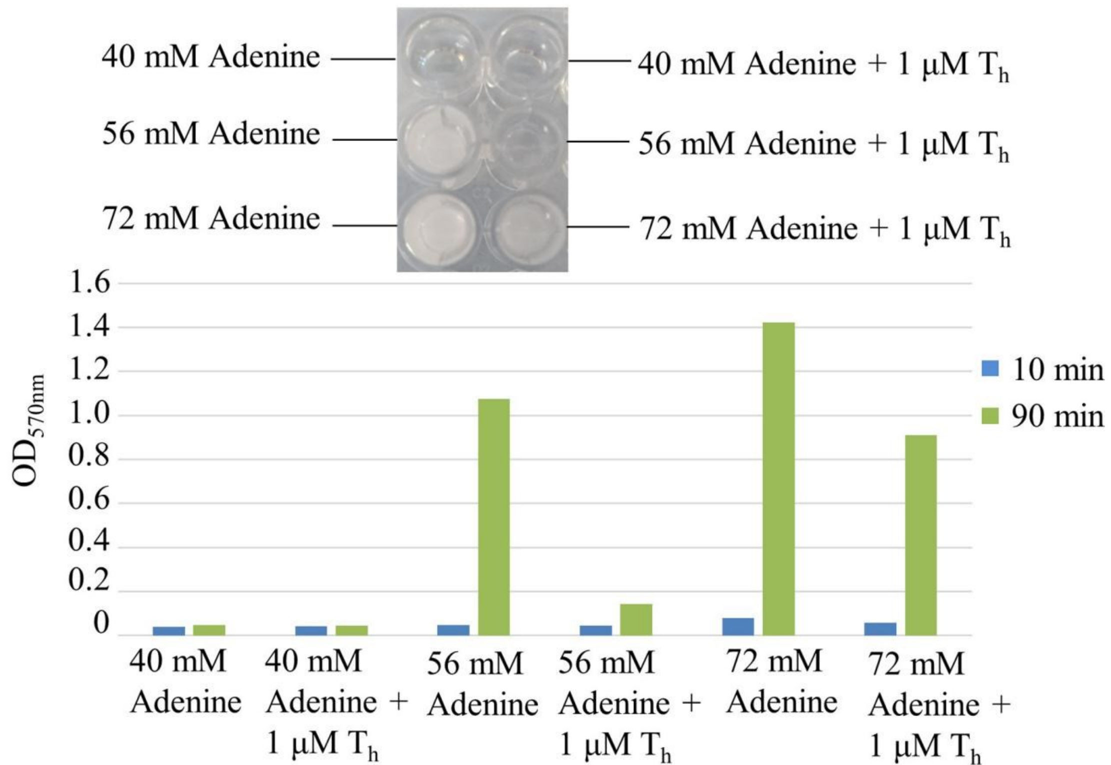


Figure S7. Solubility increase of adenine by thymine-rich DNAs. Adenine was dissolved in 1×TAE/Mg^{2+}, with or without T_h, and then allowed to stand at room temperature for 10 min or 90 min for turbidity measurement. The turbidity of the adenine solution containing T_h is significantly lower than that of adenine alone at 90 min. T_h could prevent the self-aggregation of adenine.