Supporting Information

Water-soluble Clickable Nucleic Acid (CNA) Polymer Synthesis by Functionalizing the Pendant Hydroxyl

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Experimental Section

Materials and Instruments: All chemical reagents were purchased from Sigma Aldrich or Fisher and used without further purification. Unless otherwise noted, all reactions are run under atmospheric conditions. DNA (thiol-A10) was bought from Integrated DNA Technologies, Inc. The 10 nm gold nanoparticles were bought from TED Pella. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Advance-III 400 spectrometers. Mass spectra and analytical data were obtained via the PE SCIEX/ABE API QSTAR Pulsar Hybrid LC/MS/MS. Polymer molecular weights were estimated using a TOSOH ECO SEC HLC-8320GPC equipped with two polystyrene columns and UV and RI detectors. For these polymers, the UV detector was set at 260 nm and DMSO was used as the eluent at 50 ^{o}C. The output data of Mn were calibrated by short CNA oligomer [1]or PMMA standards. Photocuring was performed with the EXFO Acticure 4000 spot curing system with the 365 nm UV filter. Transmission electron microscopy (TEM) images were obtained from FEI Tecnai T12 Spirit TEM.

Synthesis of thymine-functionalized monomer containing a pendent TBS protected hydroxyl, a Trt protected thiol and a bromine propylamine functional group (compound 5).

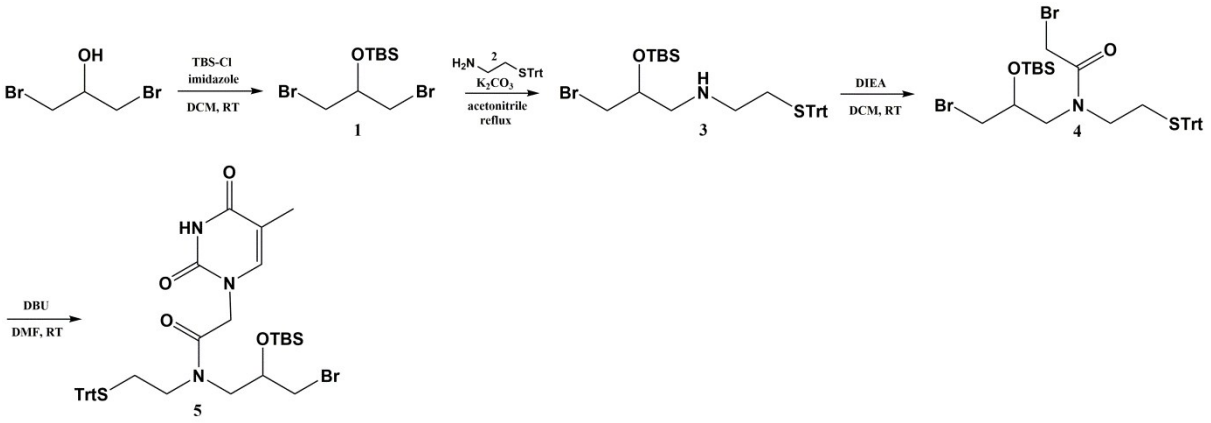

Figure S1. The synthesis route for compound 5 from 1,3-dibromo-2-propanol.

Compound 1. To a solution of 1,3-dibromo-2-propanol (1 g, 4.59 mmol) in DCM at 0 ^{o}C were added sequentially imidazole (280 μL, 5.05 mmol) and tert-butyldimethylchlorosilane (0.76 g, 5.05 mmol). The resulting mixture was allowed to warm to room termperature and was stirred for overnight at this temperature, at which point TLC analysis revealed that the starting material had been completely consumed. The reaction solution was washed by water three times, then purified by flash column with hexane. The product (7.26 g, 95% yield) was obtained as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.03 (tt, J = 5.6, 4.8 Hz, 1H), 3.56 – 3.47 (m, 4H), 0.93 (s, 9H), 0.15 (s, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 77.36, 77.04, 76.72, 36.93, 35.50, 25.68, 18.07, 11.16, -4.62.

ESI: 352.0013 [M+Na]

Compound 2 was synthesized by according to procedure found in literature[1].

Compound 3: compound 1 (0.3 g, 0.91 mmol) and compound 2 (0.1 g, 0.31 mmol) were dissolved in acetonitrile, potassium carbonate (0.05 g, 0.36 mmol) and catalytic amount TBAF and NaI were added into the above solution. This mixture was stirred overnight at 80 ^{o}C. After the starting material was completely consumed, the solution was evaporated and added DCM to extract three times. The product (94 mg, 60% yield) was purified by column chromatograph using hexane/ethyl acetate (3:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.39 (m, 6H), 7.35 – 7.26 (m, 6H), 7.26 – 7.18 (m, 3H), 4.38 (p, J = 6.2 Hz, 1H), 3.58 (td, J = 5.9, 2.1 Hz, 2H), 2.63 (ddd, J = 6.5, 5.6, 2.1 Hz, 2H), 2.44 (dd, J = 7.6, 6.5 Hz, 2H), 2.18 (dd, J = 7.5, 6.6 Hz, 2H), 0.89 (s, 9H), 0.04 (s, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 144.87, 129.64, 127.84, 126.59, 66.54, 64.51, 62.24, 58.44, 30.21, 25.80, 18.02, -4.98.

ESI: 570.2821 [M+H]

Compound 4: compound 3 (1.9 g, 3.75 mmol) was dissolved in DCM, DIPEA (2.6 mL, 15 mmol) and bromoacetyl bromide (1 mL, 11.25 mmol) were added consecutively into the above solution. This mixture was stirred overnight at room temperature. After the reaction completed, the reaction solution was washed 3 times with saturated $NaHCO_3$. The product (2.32 g, 90% yield) was purified by column chromatography with hexane/ethyl acetate (5:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.38 (m, 6H), 7.39 – 7.17 (m, 9H), 4.17 (ddd, *J* = 7.2, 4.7, 3.4 Hz, 0.5H), 4.11 (d, *J* = 10.6 Hz, 0.5H), 3.75 (d, *J* = 10.6 Hz, 0.5H), 3.67 (dddd, *J* = 9.6, 5.2, 4.2, 2.8 Hz, 0.5H), 3.58 – 3.40 (m, 2H), 3.42 – 3.20 (m, 3.5H), 3.08 (ddd, *J* = 20.7, 14.4, 4.9 Hz, 1H), 2.62 – 2.39 (m, 2.5H), 0.98 – 0.82 (m, 9H), 0.19 – -0.11 (m, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.48, 167.18, 144.67, 144.37, 129.70, 129.55, 128.13, 127.99, 127.07, 126.75, 68.40, 68.36, 67.57, 67.01, 53.10, 51.31, 49.82, 46.03, 36.82, 34.85, 30.27, 28.94, 27.19, 26.32, 25.82, 25.68, 17.96, 17.84, -4.46, -4.64, -4.71, -4.95.

ESI: 696.3968 [M+Li]

Compound 5: thymine (0.22 g, 1.75 mmol) was dissolved in 15 mL DMF. Compound 4 (0.8 g, 1.16 mmol) and DBU (175 μL, 1.16 mmol) were added into the above solutionsimultaneously. This mixture was stirred at room temperature for 3 h. After the reaction completed, DMF was evaporated and the product (0.68 g, 80% yield) was purified by column chromatograph with hexane/ethyl acetate (1:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (d, J = 3.7 Hz, 1H), 7.53 – 7.13 (m, 15H), 6.92 (q, J = 1.1 Hz, 0.4H), 6.83 (q, J = 1.2 Hz, 0.6H), 4.84 (d, J = 16.3 Hz, 0.5H), 4.32 – 4.01 (m, 2H), 3.75 (dt, J = 9.2, 3.6 Hz, 0.5H), 3.55 – 3.16 (m, 4.5H), 3.10 (dd, J = 15.2, 3.0 Hz, 0.5H), 2.91 (dd, J = 13.6, 7.8 Hz, 0.5H), 2.74 – 2.39 (m, 2.5H), 1.91 (dd, J = 7.3, 1.2 Hz, 3H), 0.90 (d, J = 1.5 Hz, 9H), 0.15 – -0.06 (m, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.83, 166.65, 164.09, 164.07, 150.89, 150.74, 144.63, 144.33, 141.04, 140.83, 129.68, 129.63, 129.59, 128.16, 127.99, 127.09, 126.79, 110.54, 110.40, 68.60, 68.51, 67.64, 67.07, 60.43, 51.65, 51.60, 48.62, 48.01, 47.83, 46.42, 36.11, 34.90, 30.13, 29.17, 25.76, 25.74, 21.10, 17.95, 17.87, 14.23, 12.46, 12.44, -4.46, -4.66, -4.85,

-4.94.

ESI: 742.1445 [M+Li]

Synthesis of Trt protected thiol/allylamine –terminated thymine-thymine dimer monomer containing a TBS protected hydroxy group (compound 7).

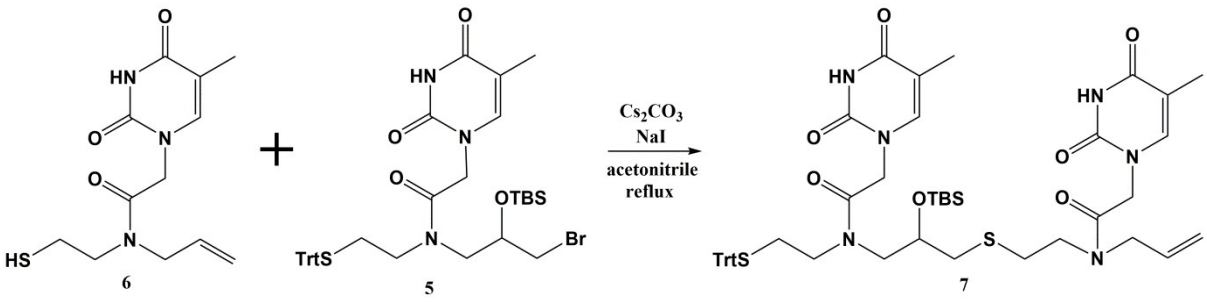

Figure S2. The synthesis route for Compound 7 by the thiol-bromine click reaction.

Compound 6 was synthesized by according to procedure found in literature[1].

Compound 7, trt protected dimer thiol-ene monomer: compound 6 (0.1 g, 0.35 mmol) and compound 5 (0.26 g, 0.35 mmol) were mixed together and dissolved in acetonitrile (10 mL), Cs_2CO_3 (0.17 g, 0.52 mmol) and a catalyic amount of NaI and TBAF were added into the above solution. This mixture was stirred overnight at 80 ^oC. After the reaction completed, the solvent was evaporated and using water to wash it three times. The crude product (0.19 g, 60% yield) was purified by column chromatograph with methanol/DCM (3%) and confirmed by ESI (961.3777 [M+Na]).

Synthesis of thiol functionalized allyl amine thymine-thymine dimer monomer (TTM).

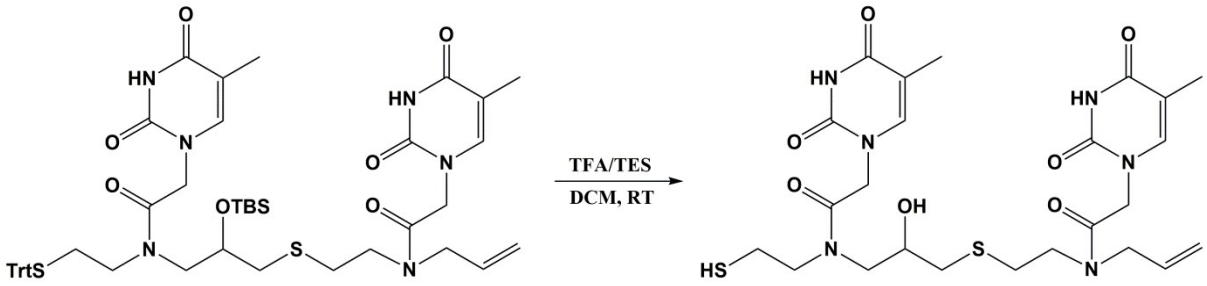

Figure S3. TTM was obtained by deprotecting thiol and hydroxy group.

TTM： Compound 7 (0.3 g, 0.36 mmol) was dissolved in 5 mL DCM, TFA (2 mL) and TES (1 mL) were added into the above solution. This mixture was stirred for 15 min at room temperature. After the starting material was consumed completely, the solvent was removed and the crude material was dissolved in DCM and washed by saturated $NaHCO_3$ solution three times. The product was finally purified using column chromatography and confirmed

by ESI (583.2103 [M+H]).

General procedure for polymerization.

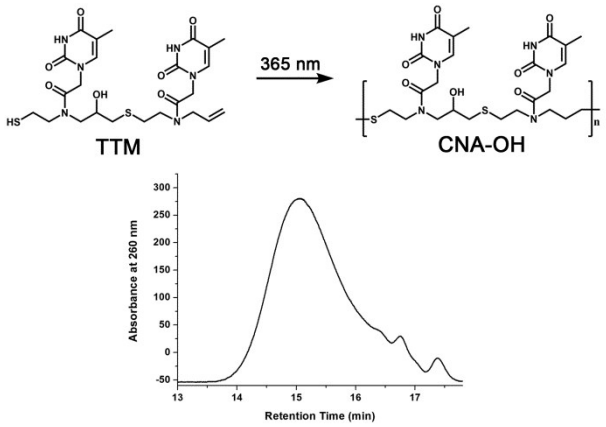

Figure S4. TTM was polymerized to form CNA-OH upon 365 nm light irradiation. SEC spectrum of CNA-OH which was precipitated in acetone (30% wt DMPA, 5% M DMPA).

Deprotected monomer TTM and 2,2-dimethoxy-2-phenylacetophenone (DMPA) 5% mol were dissolved in DMSO (30% wt). The solution was irradiated by UV light (365 nm). The final polymer CNA-OH was precipitated in acetone.

The polymerization process was monitored by SEC by removing and analyzing small samples (10 mL) of polymerization solution at various time intervals.

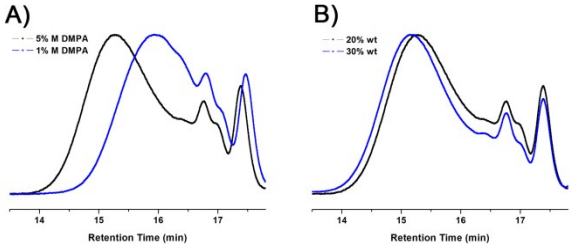

Figure S5. SEC traces of crude TTM thiol-ene polymerization system by changing different molar ratio of DMPA when the molecular weight concentration is 20% wt (A), different molecular weight concentration when the molar ratio of DMPA is 5% M (B).

The conjugate of CNA/ 8-arm PEG-Norb

TTM (35 mg), 8-arm PEG-Norb (31 mg, 10 KD) and 2,2-dimethoxy-2-phenylacetophenone (DMPA) 2% M were dissolved in DMSO (20% wt). This mixture was irradiated by UV light (365 nm) for 15 min. this sample was directly analyzed by SEC without any purification.

General procedure for synthesis of CNA-sulfonate

CNA-OH, glycine-Fmoc (1.1 equivalent comparing to TTM), EDC (1.2 equivalent

comparing to TTM) and DMAP (1.5 equivalent comparing to TTM) were dissolved in DMF and stirred for overnight at room temperature. The formed CNA-glycine-Fmoc was precipitated with ether and washed with DCM for three times to remove the left small starting materials. 20% piperidine/DMF was used to deprotect the Fmoc group. Then CNA-NH_2 and 1,3-propanesultone (0.9 equivalent relative to TTM) were dissolved in DMF and stirred for overnight at 130 ^oC. After reaction completed, di-H_2O was added into the above DMF solution and used dialysis bag (Mw=2k) to remove the solvent and other low molecular weight compounds. CNA-sulfonate polymer was obtained as a white solid after lyophilization.

Synthesis of CNA-sulfonate modified silica (CNA-SiO_2)

The CNA-SiO_2 was prepared according to previous reports[2]. Briefly, 0.5 mg silica (500 nm) were first reacted with 3-aminopropyltriethoxysilane (APTES) in ethanol (5% APTES) for 5 h to obtain amine-modified SiO_2 (NH_2-SiO_2). The particles were then reacted with 1.24 mg succinimidyl 4-(N-maleidomethyl) cyclohexane-1-carboxylate (SMCC) in DMSO for 5 h. This mixture was centrifuged and washed, resuspended in 10 μL di-H_2O. 14 μL of polyT CNA-sulfonate solution (1 mg in 200 μL di-H_2O) was mixed with 5 μL TCEP. This TCEP treated CNA was added into the SMCC modified SiO_2, this mixture was stirred for overnight. The final CNA-SiO_2 were purified and dispersed in di-H_2O for further use.

DNA-Au. The DNA-Au was prepared using previously published procedures.[2] The citrate on the surface of Au nanoparticles (10 nm, Au NPs) were first replaced by bis-p-sulfonatophenyl phenylphosphine dehydrate dipotassium salt (BSPP). 4.5 mg BSPP was added into 7 mL AuNPs solution and this mixture was stirred overnight. Then 3 M NaCl solution was added slowly into the above solution. The formed BSPP-Au were purified by 30K Nanosep centrifuge filter for three times. For DNA-A10 attachment to the 10 nm AuNPs, freshly TCEP treated A10-thiol and BSPP-Au (thiol-A10:BSPP-Au=200:1) were first mixed in 100 mM NaCl. Next, at half hour increments, and added another 50 mM NaCl solution every half an hour until the final concentration of NaCl is 500 mM. After centrifugation and washing with water three times, the pure A10-Au was obtained. It was dispersed in di-H_2O and kept at 4 ^oC for further use.

CNA-SiO_2 conjugated with A10-AuNPs: The prepared CNA-SiO_2 and A10-AuNPs are

mixed together (1 μL CNA-SiO_2 and 5 μL A10-AuNPs (100 nM)), and NaCl solution was added to reach a final concentration of 50 mM. Then this mixture was then heated to 40 ^oC for 10 min, then cool down to room temperature and kept for 3-5 h. The CNA-SiO_2/A10-AuNPs conjugate was stored at 4 ^oC for further analysis.

Reference:

1. W. Xi, S. Pattanayak, C. Wang, B. Fairbanks, T. Gong, J. Wagner, C. J. Kloxin and C. N. Bowman, *Angew. Chem. Int. Ed.*, 2015, **54**, 14462-14467.
2. L. He, C. Mao, S. Cho, K. Ma, W. Xi, C. N. Bowman, W. Park and J. N. Cha, *Nanoscale*, 2015, **7**, 17254-17260.