Supporting Information for:

Synthesis and Direct Observation of Thermo-Responsive DNA Copolymers

Songsong Li[†], and Charles M. Schroeder*[†,‡]

†Department of Materials Science and Engineering, ‡Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois, 61801

Supporting Text

Experimental

Materials and Reagents

N-Isopropylarylamide (NIPAM, 97 %, Sigma Aldrich) was purified by recrystallization from a mixture of toluene and n-hexane (1:3, toluene:n-hexane, v:v). 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid 3-azido-1-propanol ester, bacteriophage λ-DNA (New England Biolabs, Ipswich, MA), PCR Extender System (5 PRIME, Gaithersburg, MD), Taq DNA Polymerase with Thermopol Buffer (New England Biolabs, Ipswich, MA), 5-DBCO-dUTP (Jena Bioscience GmbH, Germany), Cy5-dUTP-PCR (Jena Bioscience GmbH, Germany), deoxynucleotide (dNTP) solution (New England Biolabs, Ipswich, MA), custom oligonucleotide primers (IDT DNA, Coralville, IA), Vivacon 2 with 100,000 MWCO Hydrosart Membrane (Vivaproducts, Littleton, MA), SYBR Gold Nucleic Acid Gel Stain (Invitrogen, Grand Island, NY), SYTOX Green Nucleic Acid Stain (Molecular Probes, Grand Island, NY) and all other reagents were used as received. 2,2-Azoisobutyronitrile (AIBN) was recrystallized from 95% ethanol.

Synthesis of PNIPAM-azide

For a 30 kDa PNIPAM polymer, NIPAM (1.13 g, 10 mmol), CTA (14.9 mg, 33 μmol), AIBN (0.54 mg, 33 μmol), and 1,4-dioxane (1.5 mL) were charged into a reaction tube with a magnetic stirring bar. The tube was degassed by three freeze-pump-thaw cycles and then sealed under vacuum. After reacting 3.5 h at 70 °C, the reaction tube was quenched into liquid nitrogen and diluted with 1,4-dioxane. Then the mixture was precipitated into an excess of diethyl ether to yield a white powder. By repeating the precipitation for three times and drying in a vacuum oven overnight, PNIPAM-azide was obtained as a white powder.[1] 13 kDa PNIPAM polymer was obtained with similar

method but different reagents ratio (NIPAM (1.13 g, 10 mmol), CTA (30 mg, 66 μmol), AIBN (1.08 mg, 66 μmol), and 1,4-dioxane (1.5 mL)). PNIPAM-azide absolute molecular weights (number average molecular weight, M_n) were determined on a gel permeation chromatography (GPC) system using dimethylformamide (DMF) containing 0.1 M LiBr as the mobile phase equipped with an additional miniDAWN TREOS 3-angle laser light scattering detector (MALLS, Wyatt Technology, CA). Infrared spectra were recorded on a PerkinElmer 100 serial FTIR spectrophotometer (PerkinElmer). Absorbance measurement of PNIPAM-azide was carried out by UV-Vis spectrophotometer (Hewlett–Packard 8453) with water bath to control the temperature.

Synthesis of DNA backbone & strain-promoted alkyne-azide cycloaddition

Polymerase chain reaction (PCR) was used to generate monodisperse DNA with functional groups, as previously reported[2] (Table S1, Table S2). Purified DNA products and PNIPAM-azide were mixed in solution (10 mM Tris/Tris-HCl, pH 8.0, 1 mM EDTA, and 1 M NaCl) based on a 25-fold molar excess of branches relative to backbone. Then after reacting at 30 °C for 48 hours, the products were washed with buffer (10 mM Tris/Tris-HCl, pH 8.0, 1 mM EDTA, and 300 mM NaCl) and purified with Vivicon column three times. The resulting products are shown in Figure S1.

Microfluidic device fabrication

PEGylated coverslips and flow cell were prepared as previously described[3]. Briefly, PEGylated glass coverslips (24×50 mm) were incubated with NeutrAvidin (0.125 mg/mL in 10 mM phosphate buffer, pH 8.0). Next, they were rinsed with water and attached to a drilled quartz microscope slide with epoxy and double-sided tape to form a flow channel (approximately 50 mm×4 mm×0.5 mm). Flow cell surfaces were initially incubated with 1 mg/mL bovine serum albumin (BSA) solution for 10 min and DNA sample (10-40 pM) in incubation buffer (10 mM Tris/Tris-HCl, pH 8.0, 1 mM EDTA, and 5 mM NaCl) for 40 min. The channel was rinsed with 1 mg/mL bovine serum albumin solution before imaging. Before constructing the flow cell, coverslips were functionalized with PEG/PEG-biotin for DNA attachment and reduction non-specific protein absorption. Polyethylene tubing (PE60) was epoxied into holes (0.048'' OD) drilled into quartz slides for buffer exchange and DNA stretching.

Single molecule fluorescence microscopy

The imaging buffer was mixed with 10 mM Tris/Tris-HCl (pH 8.0), 1 mM EDTA, 5 mM NaCl, 5 mg/mL glucose, 150 mM β-mercaptoethanol, 0.3 mg/mL glucose oxidase, and 0.3 mg/mL catalase to minimize photobleaching. DNA molecules (10-40 pM) were labeled with SYTOX Green (200 nM). DNA-copolymer samples were imaged with a 488 nm laser (50 mW SpectraPhysics Excelsior Laser), detected by a 1.45 NA, 100x oil immersion objective and collected by an Andor iXon EMCCD camera. Single molecule experiments are operated using the flow cell described above. A small amount of trace DNA was added to the imaging buffer to check the fluid drift in the system; videos are discarded when we observed the drift. Surface-tethered DNA copolymers are stretched under strong flow, and videos are acquired during and following the

cessation of flow. By fitting the relaxation data to a single exponential decay, the longest relaxation time of each molecule was obtained, as described in the main text. Average and standard deviation of relaxation times for each sample were then calculated.

Structural characterization: scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used for characterization of polymer morphologies. In these experiments, DNA and polymer solutions were rapidly dried on silica wafer under 45 °C to maximally retain sample morphology in solution. Then images were taken using a Hitachi S4800 high-resolution scanning electron microscope (SEM).

Dynamic light scattering (DLS)

A Malvern zetasizer Nano ZS instrument was used for DLS measurements. For these experiments, 10 kbp DNA-PNIPAM (13 kDa PNIPAM branches) copolymer was diluted in buffer (10 mM Tris/Tris-HCl, pH 8.0, 1 mM EDTA, and 300 mM NaCl) and maintained at 25 ^{o}C and 40 ^{o}C for measurements.

Table S1: DNA oligonucleotide sequences for PCR

Primer name	Target length	Sequence (5' to 3')	Modification
bb-F-biotin	n/a	5' biotin / CTGATGAGTTCGTGTCCGTACAACTGGCGTAATC	5'-biotin
bb-F	n/a	CTGATGAGTTCGTGTCCGTACAACTGGCGTAATC	None
10R	10052 bp	ATACGCTGTATTCAGCAACACCGTCAGGAACACG	None
30R	30052 bp	GAAAGTTATCGCTAGTCAGTGGCCTGAAGAGACG	None

Table S2: PCR parameters

Reaction composition	PCR step	10 kbp	30 kbp
50 uL per reaction	Initial denaturation	93 °C / 3 min	93 °C / 3 min
1X Tuning buffer with Mg^{2+}	Denaturation	93 °C / 15 sec	93 °C / 15 sec
0.4 uL PCR Extender Polymerase Mix	Anneal	62 °C / 30 sec	62 °C / 30 sec
400 nM BackboneF primer	Extension	68 °C / 8 min	68 °C / 20 min
400 nM 10R, or 30R primer	# cycles constant	10	10
20 ng λ-DNA	# cycles ramping	8	8
500 uM dATP, dCTP, dGTP, dTTP*	Increase per cycle	+20 sec	+20 sec

*dTTP was substituted with DBCO-dUTP according to branch loading from 1% to 10%.

Table S3: Average end-to-end distance of surface-tethered 10 kbp DNA-PNIPAM copolymers stretched in shear flow

Primer name	Branch loading (% DBCO substitution)	Branch molecular weight (kDa)	Temperature (°C)	Average extension (μm)	Standard deviation of extension (μm)
DNA	0	0	25	3.3	0.3
DNA	0	0	40	3.3	0.2
DNA	0	0	25 (back)	3.4	0.3
DNA-PNIPAM	1	13	25	3.0	0.6
DNA-PNIPAM	1	13	40	2.1	0.7
DNA-PNIPAM	1	13	25 (back)	3.1	0.6
DNA-PNIPAM	10	13	25	2.1	0.6
DNA-PNIPAM	10	13	40	1.2	1.2
DNA-PNIPAM	1	30	25	3.1	0.3
DNA-PNIPAM	1	30	40	1.9	0.5

Supporting Figures

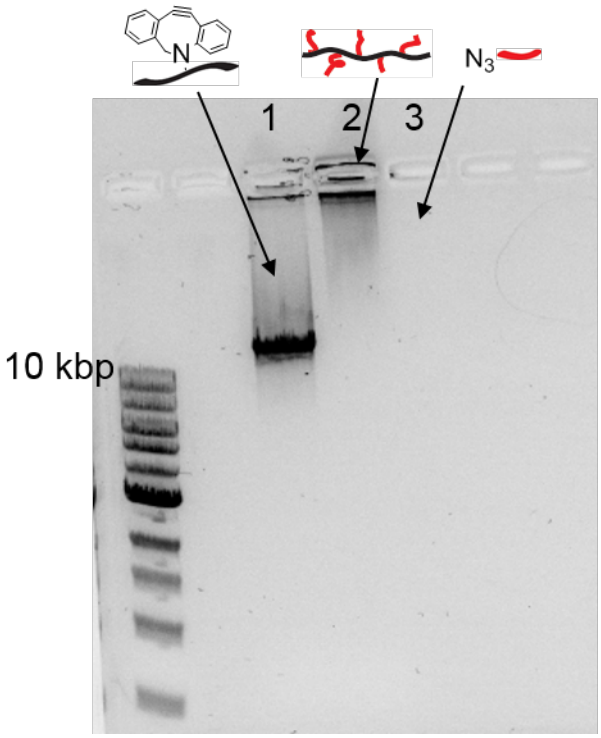

Figure S1: Agarose gel electrophoresis of DNA-PNIPAM formed by Cu-free click reaction. Lane 1: 30 kbp DBCO-DNA with 1 % DBCO substitution, Lane 2: 30 kbp DNA-PNIPAM with 1 % DBCO substitution reacted with 13 kDa PNIPAM-azide branch, Lane 3: 13 kDa PNIPAM-azide.

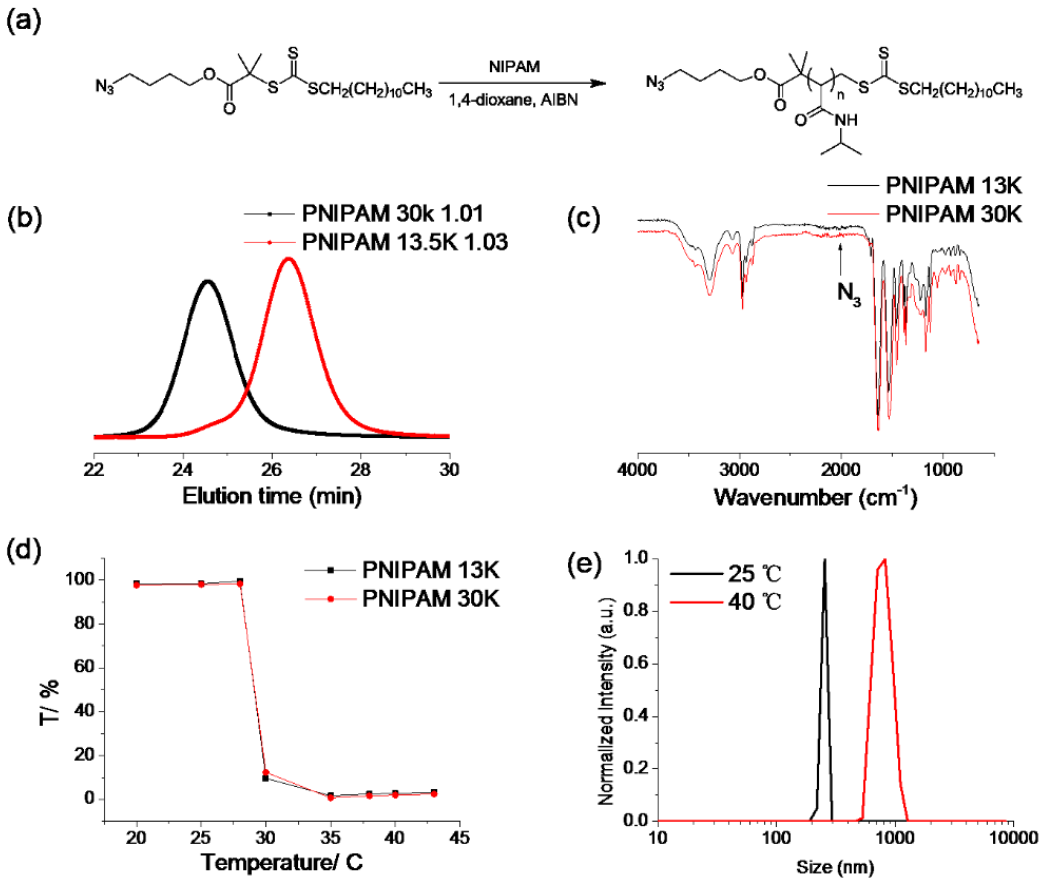

Figure S2: Synthesis and characterization of PNIPAM-azide branches and DNA-PNIPAM thermo-responsive copolymers. (a) Scheme for synthesizing PNIPAM-azide using RAFT, (b) gel permeation chromatography (GPC) spectra for PNIPAM-azide for 13 kDa and 30 kDa molecular weights (M_n) (solvent dimethylformamide, DMF), (c) infrared spectroscopy absorption spectra for PNIPAM-azide (13 kDa and 30 kDa), and (d) temperature dependence of optical transmittance obtained for 1.0 g/L aqueous solution of PNIPAM-azide in buffer (10 mM Tris/Tris-HCl, pH 8.0, 1 mM EDTA, and 300 mM NaCl). An abrupt decrease in transmittance at 30 ^{o}C is indicative of the lower critical solution temperature (LCST) for PNIPAM. (e) Dynamic light scattering (DLS) measurements for 10 kbp DNA-PNIPAM copolymers (13 kDa PNIPAM branches) at 25 ^{o}C and 40 ^{o}C in buffer (10 mM Tris/Tris-HCl, pH 8.0, 1 mM EDTA, and 300 mM NaCl).

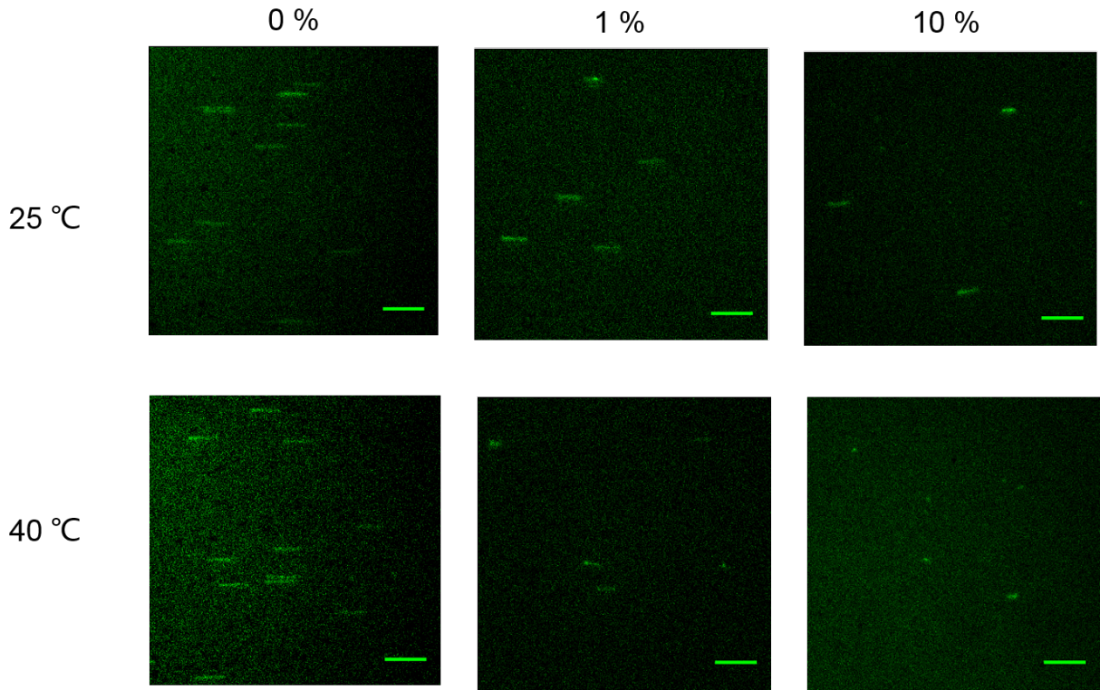

Figure S3: Single molecule imaging of surface-tethered DNA-PNIPAM copolymers and DNA polymers stretched under shear flow to study the branch loading effect to the polymer length. Stained with SYTOX Green. Backbone contour length is ≈4.3 μm for stained 10 kbp DNA. Scale bar: 5 μm

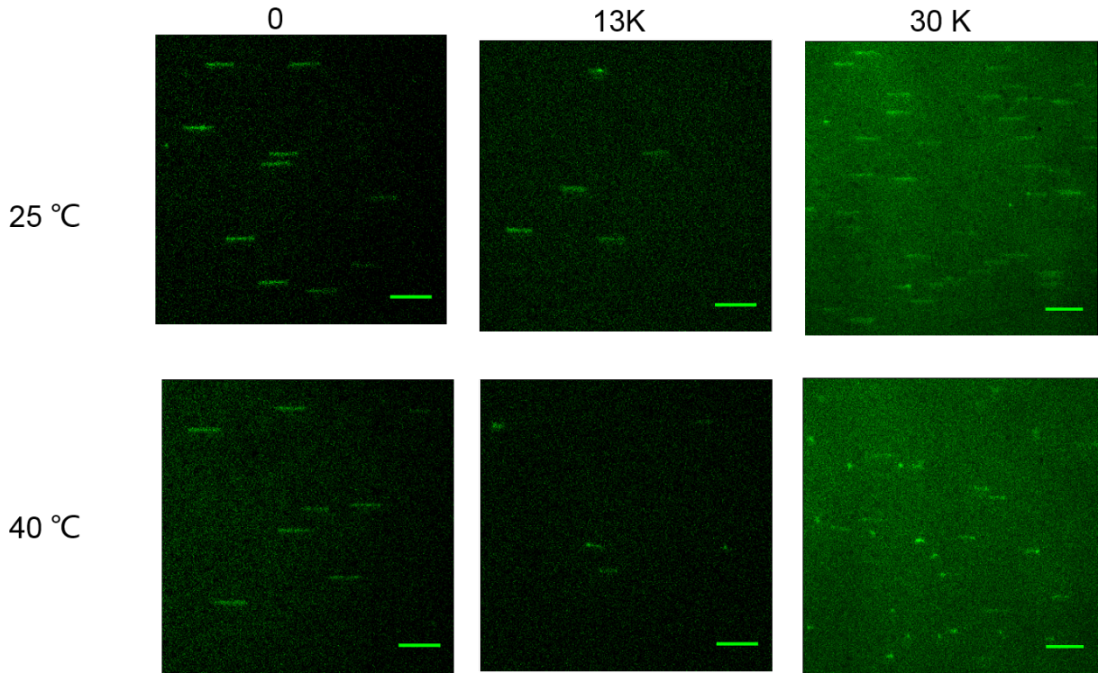

Figure S4: Single molecule images of surface-tethered DNA-PNIPAM copolymers and DNA polymers stretched under shear flow to study the branch size effect to the polymer length. Stained with SYTOX Green. Backbone contour length is ≈4.3 μm for stained 10 kbp DNA. Scale bar: 5 μm

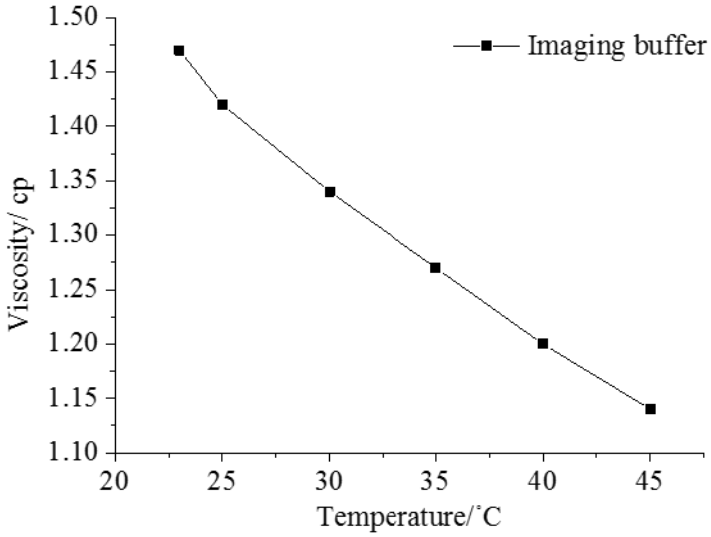

Figure S5: Shear viscosity of imaging buffer as a function of temperature.

References

(1) Li, C.; Zhang, Y.; Hu, J.; Cheng, J.; Liu, S. *Angewandte Chemie* **2010**, *49*, 5120.

(2) Mai, D. J.; Marciel, A. B.; Sing, C. E.; Schroeder, C. M. *ACS Macro Letters* **2015**, *4*, 446.

(3) Selvin, P. R.; Ha, T. *Single-molecule techniques*; Cold Spring Harbor Laboratory Press, 2008.