Electronic Supplementary Material (ESI) for ChemComm.
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Facile Synthesis of DNA-polymer Amphiphiles and their Self-assembly

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Materials and methods

Phorsphoramidites and supplies for DNA synthesis were purchased from Glen Research Co. All other materials were purchased from Sigma-Aldrich Co., VWR International LLC., or Fisher Scientific Inc., and were used without further purification unless otherwise indicated. DLS (Dynamic Light Scattering) data were acquired from a MALVERN Zetasizer Nano-ZSP. MALDI-ToF MS measurements were carried out on a Bruker Microflex LT mass spectrometer (Bruker Daltonics Inc., MA, USA). ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer (Varian Inc., CA, USA). Chemical shifts (δ) were reported in ppm. Gel permeation chromatography (GPC) measurements were performed on a TOSOH EcoSEC HLC-8320GPC system equipped with an RI and an UV-Vis detector at 40 ºC and with a flow rate of 0.5 mL/min. *N*, *N*-Dimethylformamide (DMF) with 0.2 M LiBr was used as the eluent. The GPC was calibrated based on polystyrene standards (706 kDa, 96.4 kDa, 5970 Da, 500 Da). Gel electrophoresis was performed using 0.5% agarose gel in 0.5x Tris/Borate/EDTA (TBE) buffer with a running voltage of 100 V. Gel images were acquired on an Alpha Innotech Fluorochem Q imager. TEM images were obtained on a JEOL JEM 1010 electron microscope utilizing an accelerating voltage of 80 kV.

Oligonucleotide synthesis

Oligonucleotides were synthesized on a Model 391 DNA synthesizer (Applied Biosystems, Inc., Foster City, CA) using standard solid-phase phosphoramidite methodology. The 5'-alkyne group is installed using a hexynyl phosphoramidite (Glen Research, Co.) as the final base in the DNA synthesis.

Table S1. Oligonucleotide sequences used in this study.

$DNA_{6\text{-mer}}$	5'-alkyne-AAAACC-3'
$DNA_{19\text{-mer}}$	5'-alkyne-AAAAAATCCTTATCAATAC-3'
$DNA_{26\text{-mer}}$	5'-alkyne-AAAAAATCCTTATCAATACCCCCCCC-3'
$DNA_{19\text{-mer-C}}$	5'-alkyne-GTATTGATAAGGATTTTTT-3'

General method for ATRP synthesis of polymer precursors for NAPAs

In a 25 mL Schlenk flask, a stir bar and CuBr (1 equiv.) were placed. Next, the flask was thoroughly purged by vacuum and flushed with nitrogen before toluene (4 mL) (styrene), *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (1.1 equiv.), ethyl 2-bromopropionate (1 equiv.) and monomer (pre-filtered through a alumina column, 30-400 equiv.) were sequentially added to the flask *via* syringe. Then the reagents were degassed by 3x freeze-pump-thaw cycles. The reaction mixture was stirred for 50 min-7 h at room temperature (*t*-butyl acrylate) or 100 °C (styrene). Polymerization was stopped by opening the flask and exposing the catalyst to air. Polymers were purified by precipitation and dried *in vacuo*.

Table S2. Molecular weight and polydispersity as determinted by DMF GPC.

Polymer	M_n (g/mol)	M_w (g/mol)	PDI
3.9 kDa PS	3900	4400	1.13
5.5 kDa PS	5500	6400	1.16
8.5 kDa PS	8500	10000	1.17
14 kDa PS	14500	16400	1.13
2.2 kDa	2200	2600	1.18

General method for the synthesis of azide-terminated polymers

In a 20 mL flask, ATRP polymer (0.5 mmol) and sodium azide (5.0 mmol) were dissolved in DMF (5.0 mL). The reaction mixture was allowed to stir over night at room temperature. The polymer was then purified by precipitation and washing with water, and was dried *in vacuo* in quantitative yield.[1]

General method for the synthesis of NAPAs from ATRP polymers

Polymer (2.5 µmol) and DNA-CPG (17 mg, 0.5 µmol) were placed in a glass vial. CuI/*N*,*N*-diisopropylethylamine/acetic acid[2] (0.9 µmol/0.9 µmol/0.9 µmol) were dissolved in 600 µL DCM and added *via* syringe. The mixture was shaken gently for 8 h at room temperature on an Eppendorf Thermomixer C. The resulting CPG beads were washed with dichloromethane (20 mL) to remove unreacted polystyrene (PS). Thereafter,

the CPG beads were treated with aqueous ammonium hydroxide (28-30% NH_3 basis) at 55 ºC for 17 h. Afterwards, the ammonia was removed by passing a steam of N_2 over the conjugates solution until the smell of ammonia was disappeared. The resulting solution was loaded into an agarose gel (1 %) and was electrophoresed in 0.5x TBE buffer under 100 V for 30 min. Desired bands were cut and recovered from the gel by using a GenElute™ spin column. Conjugates solution was desalted by dialysis against Nanopure™ water (MWCO 6-8 kDa, 24 h). A stream of N_2 was applied on the solution to concentrate dialyzed sample. Conjugate solutions were stored in 4 ºC fridge.

Micellization methods

Kinetic micelles were prepared during the ammonium hydroxide cleavage process (*vide infra*). After exaction from agarose gel and dialysis, the micelles were diluted to 4 OD/mL prior to characterization by DLS and TEM. Thermodynamically generated micelles were achieved by gradual addition of water (1.8 mL, 0.01 mL/min) to a DMF solution of DNA-*b*-PS (35 OD/mL, 0.2 mL) *via* a syringe pump (Cole-Parmer infusion/withdraw pump, EW-74900-40). Thereafter, DMF was removed by dialysis against Nanopure™ water (MWCO 6-8 kDa, 24 h). A small amount of large, insoluble aggregates were present for some samples, and they were removed by centrifugation (5 krpm, 10 min) prior to TEM and DLS analyses. More than 67% of sample is retained as determined by UV-Vis spectroscopy monitoring absorption at 280 nm.

TEM characterization

TEM samples were prepared by following procedure. Onto a carbon-coated copper grid (treated with glow-discharge prior to use), samples were placed as a drop of dispersion in aqueous solution. Excess solution was carefully blotted off using filter paper, followed by addition of a drop of the staining solution (2% uranyl acetate). After the removal of excess staining solution, copper grids were air dried before TEM analysis.

DNA micelle hybridization and melting

A pair of complementary NAPA kinetic micelles (precursor NAPA: $DNA_{19\text{-mer}}$-*b*-$PS_{3.9kDa}$ and $DNA_{19\text{-mer-C}}$-*b*-$PS_{3.9kDa}$) were lyophilized separately, and were re-dissolved and mixed in 0.5 mL of 300 mM NaCl solution in a centrifuge tube to give a final concentration of 2 OD/mL. The solution was incubated in a hot water bath at 45 ºC for *ca.* 12 h. After the incubation, the sample was mounted on a Dynamic Light Scattering instrument (MALVERN Zetasizer Nano-ZSP), and the particle number-average hydrodynamic diameter and scattered light intensity were measured as a function of temperature, from 22 to 63 ºC, and the scan rate was set to 0.5 ºC/min.

Additional Figures

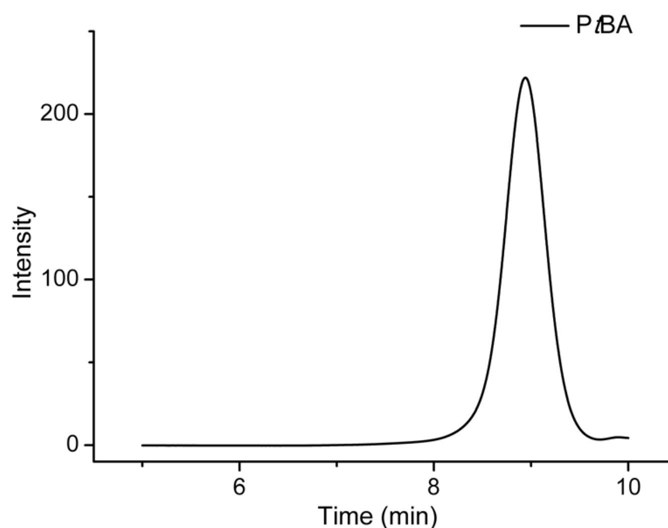

Figure S1. GPC chromatogram of the P*t*BA used for coupling reactions with DNA (eluent: tetrahydrofuran, M_n: 2.2 kDa, PDI: 1.18, a five-point polystyrene standard system was used).

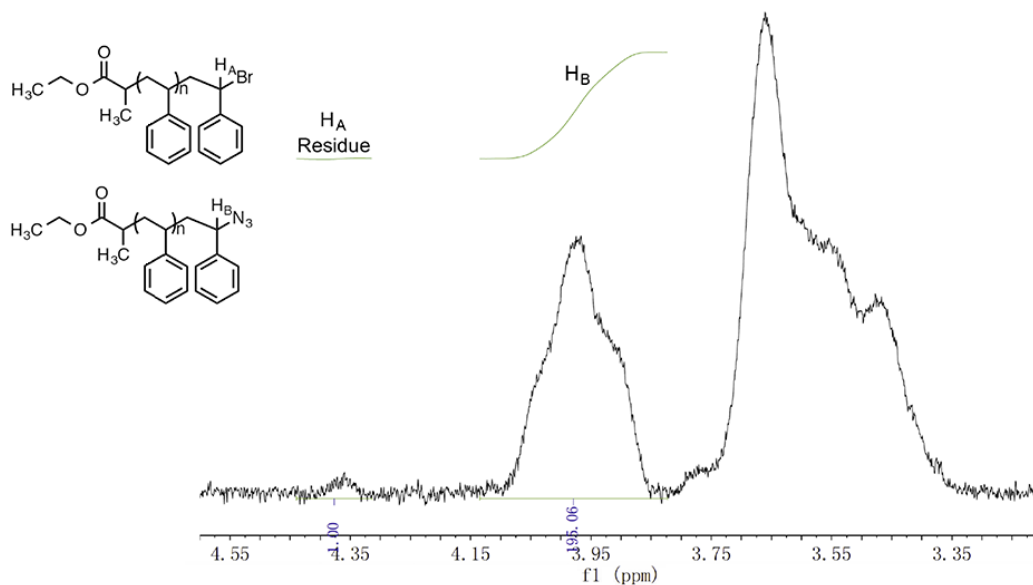

Figure S2. ^{1}H NMR spectrum of azide-terminated PS (14 kDa) in chloroform-d expanded in the region of the chain-end protons, showing the complete conversion of the chain-end bromides to azides.

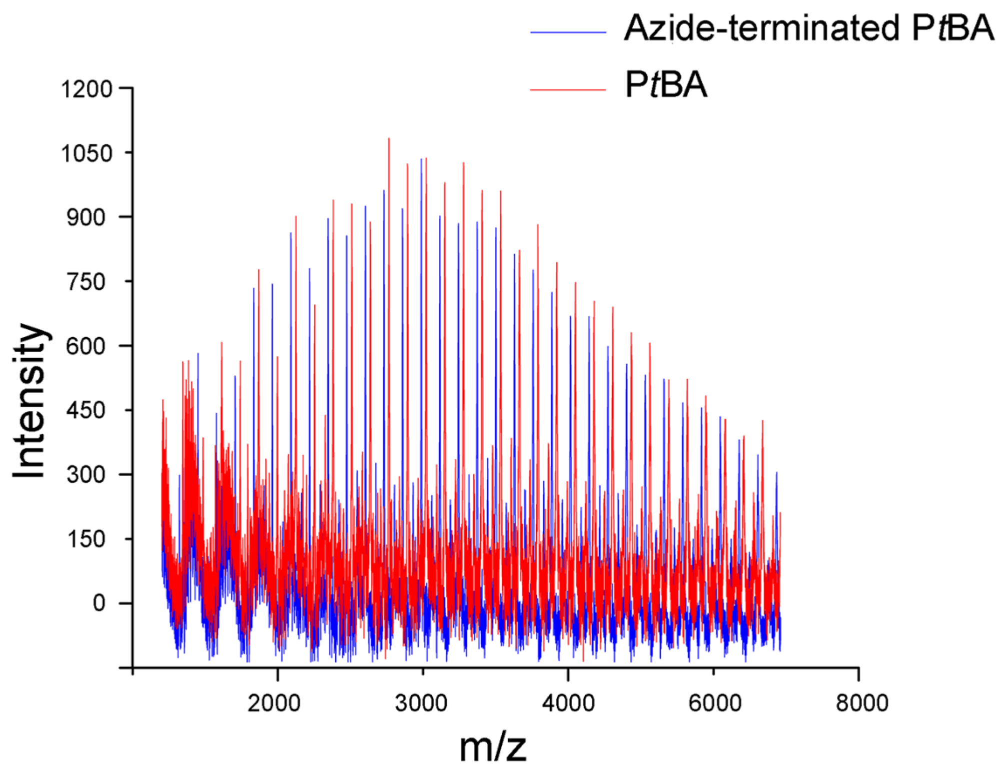

Figure S3. MALDI-ToF MS of 2.2 kDa P*t*BA before the azide substitution reaction (red) and after (blue).

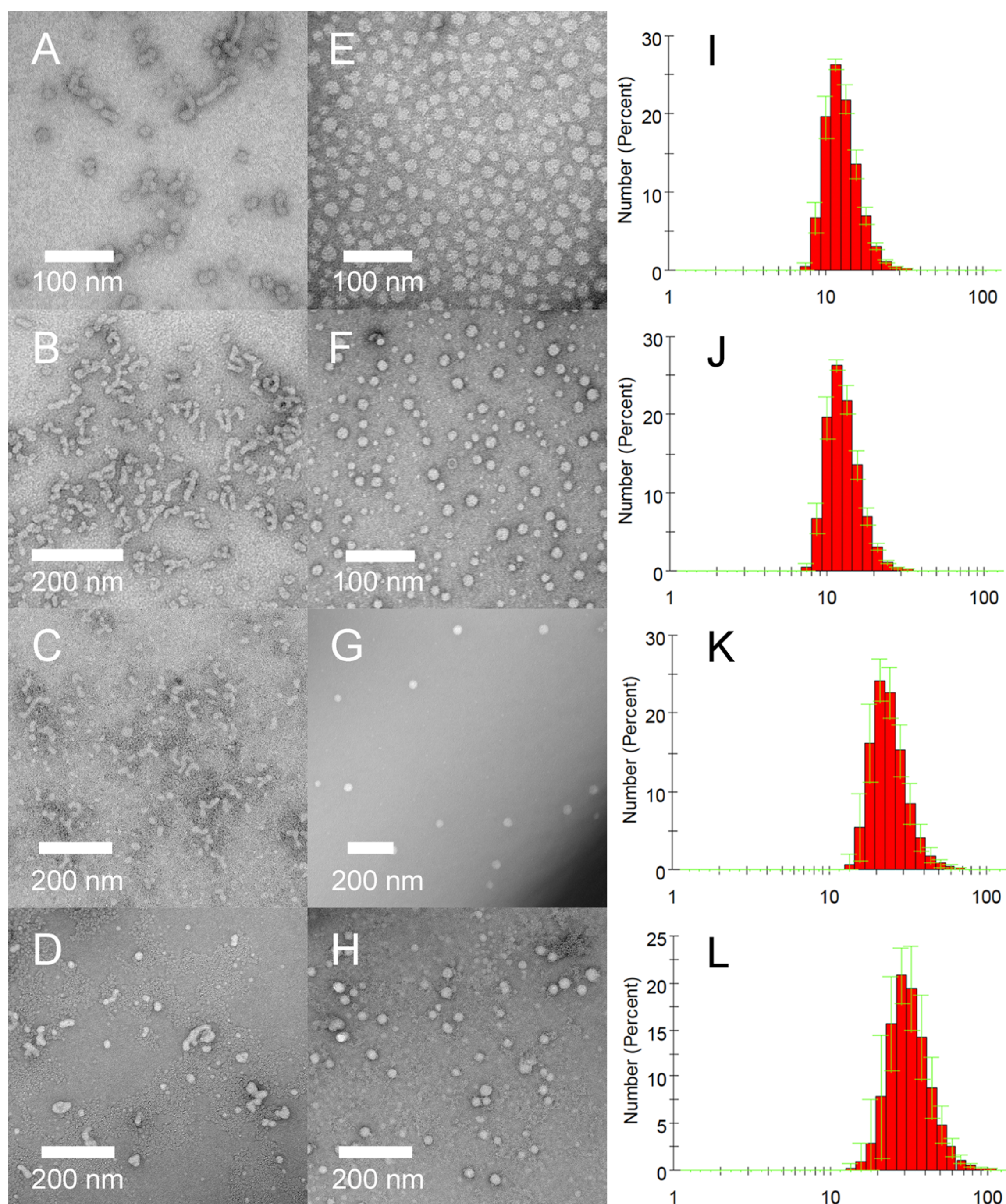

Figure S4. TEM images of micelles formed from two different methods. Each row refers to a different NAPA. From top to bottom: $DNA_{6\text{-mer}}$-*b*-$PS_{5.5kDa}$, $DNA_{6\text{-mer}}$-*b*-$PS_{8.5kDa}$, $DNA_{19\text{-mer}}$-*b*-$PS_{8.5kDa}$, and $DNA_{6\text{-mer}}$-*b*-PS_{14kDa}. A-D: kinetic micelles generated during DNA cleavage. E-H: thermodynamic micelles formed by gradual addition of water to a DMF solution of NAPAs. I-L: DLS number-average hydrodynamic diameters of the thermodynamic micelles.

Reference

1 (a) V. Coessens, T. Pintauer, and K. Matyjaszewski, *Prog. Polym, Sci.,* 2001, **26**, 337. (b) J.-F. Lutz, H. G. Börner and K. Weichenhan, *Macromol. Rapid Commun.*, 2005, **26**, 514.

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