

Supplementary Information

Cationic polymers for DNA origami coating – examining the binding efficiency and tuning the enzymatic reaction rates

Jenny K. Kiviaho^{†a}, Veikko Linko^{†a}, Ari Ora^a, Tony Tiainen^b, Erika Järvihaavisto^a, Joonas Mikkilä^a, Heikki Tenhu^b, Nonappa^c and Mauri A. Kostiainen^{*a}

^aBiohybrid Materials Group, Department of Biotechnology and Chemical Technology, Aalto University, P.O. Box 16100, 00076 Aalto, Finland. *E-mail: mauri.kostiainen@aalto.fi †Equal contribution
^bLaboratory of Polymer Chemistry, Department of Chemistry, University of Helsinki, P.O. Box 55, 00014 Helsinki, Finland
^cMolecular Materials, Department of Applied Physics, Aalto University, P.O. Box 15100, 00076 Aalto, Finland

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1. Materials for polymer synthesis

Poly(ethylene glycol) (PEG, 4000 g/mol) (Fluka) and poly(ethylene glycol) monomethyl ether (mPEG, 5000 g/mol) (Fluka) were dried in a vacuum oven before the macroinitiator syntheses. Triethylamine (TEA) (Sigma-Aldrich, ≥ 99 %), α -bromoisobutyryl bromide (BiBB) (Aldrich, 98 %), ethyl α -bromoisobutyrate (EBiB) (Aldrich 98 %), copper(I)bromide (Aldrich, 99.999 % trace metal basis), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) (Aldrich, 97%), magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$) (Sigma-Aldrich ≥ 99 %), agarose (type I, low EEO), Tris-acetate-EDTA (TAE) buffer (10x, USB ultrapure MB grade), sodium hydroxide (NaOH) (Sigma-Aldrich, ≥ 98 % reagent grade), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), (Sigma, ≥ 99.5 %), magnesium sulfate (MgSO_4) (Sigma-Aldrich, ≥ 99.5 %), sodium hydrogen carbonate (NaHCO_3) (Riedel-de Haën), ethidium bromide (EthBr) (Sigma), deuterated chloroform (CDCl_3) (Euriso-top) and dichloromethane (DCM) (VWR, AnalaR NORMAPUR) were used as received. Tetrahydrofuran (THF) (VWR, AnalaR NORMAPUR) was dried over molecular sieves and 2-(Dimethylamino) ethyl methacrylate (DMAEMA) (Aldrich, 98 %) was passed through aluminum column prior polymer synthesis.

The M13mp18 single-stranded DNA (New England Biolabs or Tilbit Nanosystems) and the oligonucleotides (IDT, standard desalting) for DNA origamis were used as received.

2. Characterization of the polymers

MALDI-ToF. Sinapic acid (SA, Sigma-Aldrich), matrix solution was prepared by mixing ~20 mg of SA in 1 ml of solvent (0.1 % TFA, 50 % acetonitrile). Then 1 μ l of matrix-polymer (2:1 v/v) was spotted on a MALDI plate and allowed to dry before analysis.

PEG-macroinitiators. 1 μ l SA and 0.5 μ l sample. Spotted 1 μ l onto a steel plate.

PDAMEMA-PEG copolymer.

Trans-2-[3-(4-t-butyl-phenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) in THF. NaTFA 10 mg/ml. 1:1:10 = sample:NaTFA:matrix, spotted 0.5 μ l onto a steel plate.

PDMAEMA homopolymer. 2,5-dihydroxybenzoic acid (DHB) (20 mg/ml). (0.1 % TFA, 50 % acetonitrile). 1 μ l of matrix-polymer (2:1 v/v) was spotted on a MALDI plate and allowed to dry before analysis.

3. Syntheses

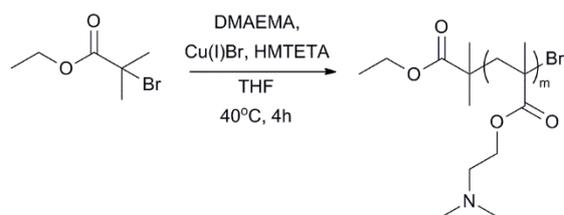
Synthesis of poly(dimethylaminoethyl methacrylate) homopolymer. The reagents were measured in three separate flasks in following order: the first flask was charged with copper bromide (0.045 g, 0.3 mmol), the second flask was filled with HMTETA (0.173 ml, 0.6 mmol) and DMAEMA (2.14 ml, 12.7 mmol), and the third flask was filled with EBiB (0.046 ml, 0.3 mmol) and dry THF (2.64 ml). All the reagents were degassed in their respective flasks and then the content of the second (HMTETA, DMAEMA) and third (EBiB, THF) flasks were transferred to the first flask (CuBr). The polymerization was allowed to proceed under nitrogen atmosphere for four hours at 40 °C after which the reaction was stopped by exposing the mixture to the air. The reaction mixture was diluted with THF and passed through aluminum column to remove the copper. The mixture was then precipitated into cold hexane and the product was collected and dried *in vacuo*.

^1H NMR (400MHz, 298K, CDCl_3): δ (ppm) 0.9, 1.05 (br, PDMAEMA backbone CH_2 and CH_3), 2.28 (s, $\text{N}(\text{CH}_3)_2$), 2.56 (br, $\text{CH}_2\text{N}(\text{CH}_3)_2$), 4.06 (br, OCOCH_2).

IR ν (cm^{-1}): 2940, 2860 ($>\text{CH}_2$ and $-\text{CH}_3$, C-H stretching), 2820, 2770 ($>\text{NCH}_2-$, C-H stretching), 1720 (C=O stretching), 1450 ($>\text{CH}_2$ and $-\text{CH}_3$, C-H bending), 1390 ($>\text{C}(\text{CH}_3)_2$, C-H bending), 1270, 1240 ($-\text{CO}_2-$, C-O and $-\text{CO}_2$ stretching), 1150 (R_3N , C-N absorption).

$M_n(\text{GPC})=4600$ g/mol, PDI=1.3

MALDI-ToF, $m/z_{\text{theor}}=5697.4$, $m/z_{\text{obs}}=5706.9$



Scheme S1. The synthesis of PDMAEMA.

Synthesis of difunctional poly(ethylene glycol) macroinitiator, Br-PEG-Br. A round-bottom flask, equipped with calcium chloride tube, was charged with poly(ethylene glycol) (5 g, 2.5 mmol), TEA (1.045 ml, 7.5 mmol) and dry THF (100 ml). BiBB (0.46 ml, 3.75 mmol) was carefully added through septum to the reaction mixture. The reaction was allowed to proceed for 24 hours at ambient temperature. The mixture was filtered to remove salts and the solvent was removed *in vacuo*. The crude product was dissolved into dichloromethane and the mixture was washed with saturated NaHCO₃ solution and dried over MgSO₄. The product was precipitated into cold diethyl ether and dried in a vacuum oven for 7 hours at 40 °C.

¹H NMR (400MHz, 298K, CDCl₃): δ (ppm) 1.95 (s, ω-terminal CH₃), 3.65 (s, backbone CH₂), 4.33 (t, CH₂OCO).

IR ν (cm⁻¹): 2880 (>CH₂, C-H stretching), 1730 (C=O stretching), 1470 (>CH₂, C-H bending), 1100 (C-O stretching).

M_n(GPC)=10 900 g/mol, PDI=1.03

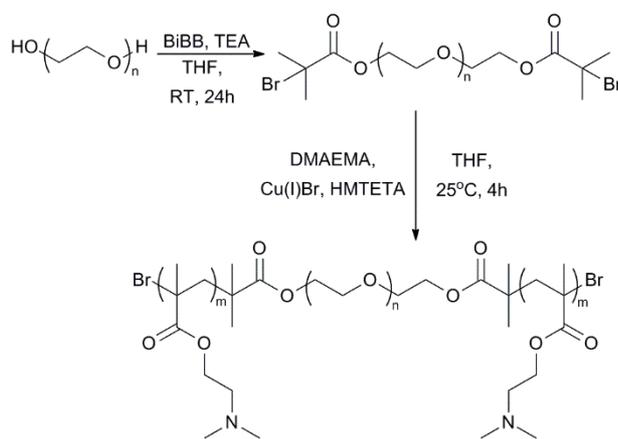
MALDI-ToF, m/z_{theor}=4523.906, m/z_{obs}=4523.099

Synthesis of PDMAEMA-PEG-PDMAEMA triblock copolymer. The reagents were measured in three separate flasks in following order: the first flask was charged with copper bromide (0.015 g, 0.11 mmol), the second flask was filled with HMTETA (0.06 ml, 0.22 mmol) and DMAEMA (2.14 ml, 12.7 mmol), and the third flask was filled with difunctional macroinitiator Br-PEG-Br (0.46 ml, 0.11 mmol) and dry THF (2.14 ml). All the reagents were degassed in their respective flasks and then the content of the second (HMTETA, DMAEMA) and third (Br-PEG-Br, THF) flasks were transferred to the first flask (CuBr). The polymerization was allowed to proceed under nitrogen atmosphere for four hours at 25 °C after which the reaction was stopped by freezing the solution with liquid nitrogen and exposing it to the air. The crude product was dissolved in water and the pH was adjusted first to pH = 4 and then rose to pH = 12 after which the polymer was precipitated by heating the solution to 65 °C.

¹H NMR (400MHz, 298K, CDCl₃): δ (ppm) 0.90, 1.06, 1.26, 1.83, 1.91 (br, PDMAEMA backbone CH₂ and CH₃), 2.28 (s, N(CH₃)₂), 2.56 (br, CH₂N(CH₃)₂), 6.65 (s, PEG backbone CH₂), 4.05 (br, OCOCH₂)

IR ν (cm⁻¹): 2920, 2850, 2820, 2770 (>CH₂ and >NCH₂-, C-H stretching), 1720 (C=O stretching), 1450 (>CH₂, C-H bending), 1270, 1240 (C-N stretching), 1150 (C-O stretching).

M_n(GPC)=18 800 g/mol, PDI=1.3



Scheme S2. The synthesis of difunctional macroinitiator and the following triblock copolymer synthesis.

Synthesis of monofunctional poly(ethylene glycol) macroinitiator, mPEG-Br. A round-bottom flask, equipped with calcium chloride tube, was charged with poly(ethylene glycol) monomethyl ether (7.5 g, 1.5 mmol), TEA (1.25 ml, 9.0 mmol) and dry THF (100 ml). BiBB (0.56 ml, 4.5 mmol) was carefully added through septum to the reaction mixture. The reaction was allowed to proceed for 17 hours at ambient temperature. The mixture was filtered to remove salts and the solvent was removed *in vacuo*. The crude product was dissolved into dichloromethane and the mixture was washed with saturated NaHCO₃ solution and dried over MgSO₄. The product was precipitated into cold hexane and dried in a vacuum oven for 7 hours at 40 °C.

¹H NMR (400MHz, 298K, CDCl₃): δ (ppm) 1.95 (s, ω-terminal CH₃), 3.39 (α-terminal CH₃), 3.65 (s, backbone CH₂), 2.40 (t, CH₂OCO).

IR ν (cm⁻¹): 2880 (>CH₂ and CH₃, C-H stretching), 2860 (-OCH₃, C-H stretching), 2740, 2690 (-CHO, C-H stretching), 1730 (C=O stretching), 1460 (>CH₂ and -CH₃, C-H bending), 1340 (-COCH₃, C-H bending), 1280, 1240 (-CO₂-, C-O and -CO₂ stretching), 1100, 1060 (C-O stretching).

M_n(GPC)=12 200 g/mol, PDI=1.04

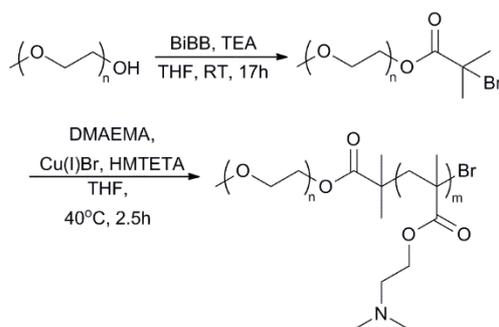
MALDI-ToF, m/z_{theor}=5231.86, m/z_{obs}=5229.9

Synthesis of PDMAEMA-PEG diblock copolymer. The reagents were measured in three separate flasks in following order: the first flask was charged with copper bromide (0.014 g, 0.098 mmol), the second flask was filled with HMTETA (0.054 ml, 0.198 mmol) and DMAEMA (1.00 ml, 5.93 mmol), and the third flask was filled with monofunctional macroinitiator mPEG-Br (0.514 ml, 0.099 mmol) and dry THF (1.5 ml). All the reagents were degassed in their respective flasks and then the content of the second (HMTETA, DMAEMA) and third (mPEG-Br, THF) flasks were transferred to the first flask (CuBr). The polymerization was allowed to proceed under nitrogen atmosphere for 2.5 hours at 40 °C after which the reaction was stopped by exposing the mixture to the air. The reaction mixture was diluted with THF and passed through aluminum column to remove the copper. The mixture was then precipitated into cold hexane and the product was collected and dried *in vacuo*.

$^1\text{H NMR}$ (400MHz, 298K, CDCl_3): δ (ppm) 0.90, 1.06, 1.27, 1.90 (br, PDMAEMA backbone CH_2 and CH_3), 2.28 (s, $\text{N}(\text{CH}_3)_2$), 2.56 (br, $\text{CH}_2\text{N}(\text{CH}_3)_2$), 3.38 (s, PEG α -terminal CH_3), 3.65 (s, PEG backbone CH_2), 4.06 (br, OCOCH_2).

IR ν (cm^{-1}): 2950, 2890 ($>\text{CH}_2$ and $-\text{CH}_3$ C-H stretching), 2820, 2770 ($>\text{NCH}_2-$, C-H stretching), 1720 (C=O stretching), 1460 ($>\text{CH}_2$ and $-\text{CH}_3$, C-H bending), 1340 (C-H bending), 1280, 1240 ($-\text{CO}_2-$, C-O and $-\text{CO}_2$ stretching), 1150 (R_3N , C-N absorption), 1110, 1060 (C-O stretching).

$M_n(\text{GPC})=15\ 800$ g/mol, PDI=1.13



Scheme S3. The synthesis of macroinitiator and the following diblock copolymer synthesis.

4. DNA origami

DNA origami folding for (60-Helix Bundle & Hexagonal Tube). DNA origami structures were folded in 100 μl quantities using 20 nM M13mp18 scaffold strand and a set of staple strands (strands listed in Refs. [S1 and S2]) at 10x excess (200 nM). The folding took place in a buffer solution containing 1x TAE (40 mM tris(hydroxymethyl)aminomethane) (Tris), 1 mM ethylenediaminetetra acetic acid (EDTA) and acetic acid for adjusting the pH to 8.3), 20 mM MgCl_2 . The thermal folding ramp (G-Storm G1 Thermal Cycler) was the following:

- From 65°C to 59°C : 1.0°C decrease in 15 minutes.
- From 59°C to 40°C : 0.25°C decrease in 45 minutes.
- Store at 12°C .

Agarose gel electrophoresis for verifying the quality of the folding. Agarose (0.8 g) was mixed with 100 of 1x TAE buffer containing 11 mM MgCl_2 , and the gel was stained with ethidium bromide (80 μl of 0.625 mg/ml stock solution). The samples were prepared by mixing DNA origami solution (10-20 μl) with 6x Blue Loading Dye (2-4 μl). 10 μl of each sample was loaded into a gel well and M13mp18 scaffold strand was used as a reference. 1x TAE including 11 mM MgCl_2 was used as a running buffer. The gel was run at a constant voltage of 90V for 45 minutes.

DNA origami purification. The excess amount of staple strands was removed from the DNA origami solution by the spin-filtering procedure (0.5 ml filter columns, with molecular weight cutoff = 100 kDa, Millipore Amicon Ultra YM-100). The filtration procedure was following:

- 50 μ l of DNA solution was diluted to 500 μ l in a filter column placed in a 2ml tube with HEPES/NaOH buffer (6.5 mM HEPES, pH 6.8), and the diluted solution was centrifuged with 14 000 rcf for 3 minutes.
- The flow-through was discarded and 450 μ l of fresh buffer was added into the filter.
- The above mentioned steps were repeated twice and at the last filtration round the centrifugation time was adjusted to 5 minutes.
- The sample was collected from the filter by turning the filter upside down in a fresh 2ml tube and centrifuging for 2 minutes at 1000 rcf.

After each centrifugation round, the volume of the DNA origami solution was reduced to 15-20 μ l. The same recipe was again used to remove excess LUC-enzymes from the LUC-origami solution.

Transmission electron microscopy (TEM) for characterizing plain DNA origami nano-objects.

The micrographs were taken with Tecnai 12 Bio-Twin microscope. The samples were prepared on carbon only copper grids (Electron Microscopy Sciences) by pipetting a 3 μ l drop of the sample solution onto the grid. The droplet was left on the grid for 1 minutes which after the excess solution was blotted away using filter paper. Samples were negatively stained by first applying 3 μ l of staining solution (0.5 % uranyl acetate in Milli-Q water) and then removing the excess stain with a filter paper. Additional 3 μ l of uranyl acetate was added onto the grid and after 20 seconds the excess stain was blotted with filter paper. The samples were dried at room temperature for minimum of 10 minutes before imaging. TEM image of the 60HB structures is shown in Fig. S1. Hexagonal tubes, their quality and the TEM images are reported in Ref. [S2].

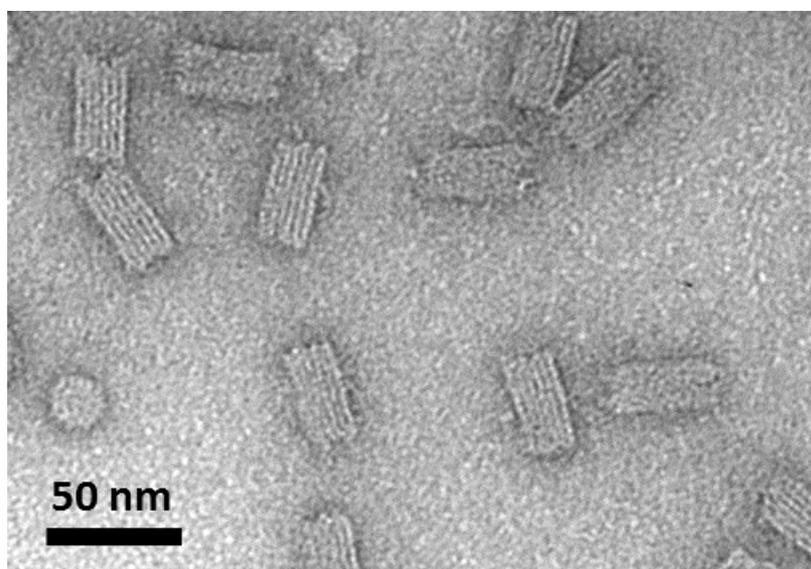


Figure S1. A TEM micrograph of 60HB DNA origami nanostructures after purification by spin filtering. Negatively stained sample on a copper only grid.

5. DNA origami-polymer complexes

Preparation of 60HB-polymer complexes. DNA origami solution was first diluted with HEPES/NaOH buffer (6.5 mM HEPES, pH 6.8) after which polymer solution was added so that the final volume of the sample was 30 μ l and the DNA origami concentration in sample solution 1 nM. Different amounts of polymer solutions and concentrations were used to obtain the desired $n_{\text{polymer}}/n_{\text{origami}}$ ratios.

Cell viability with the polymers and polymer-origami complexes. A549 cells were cultured on 24-well plates at 37 $^{\circ}$ C in 5 % CO₂. Growth medium was replaced with the sample solution consisting of 180 μ l of fresh media and 20 μ l of aqueous polymer solution (polymers alone or in complex with the DNA origami). The cells were incubated for 1 or 9 h, after which time the MTT assay was carried out. The sample media from each well was replaced with 200 μ l of fresh media and 20 μ l MTT (5 mg/ml) solution. The cells were incubated for 4 h and the MTT solution was replaced by 150 μ l of DMSO and mixed to dissolve the formazan crystals. Absorbance was measured at 540 nm with a BioTek Cytation 3 and the viability percentage was calculated by comparison to blank cells (100% survival).

Preparation of LUC-origami-polymer complexes. Spin-filter-purified hexagonal tube origami solution was incubated with Streptavidin-LUC enzymes (excess: 20 enzymes per each DNA origami) at least 6 hours, after which the excess and unbound LUC was removed by spin-filtering (as described above, see also Fig. S2). LUC origamis were mixed with HEPES/NaOH buffer (6.5 mM HEPES, pH 6.8) and different amounts of polymers (dissolved in the same buffer) in such a way that the final concentration of the origamis in each sample was 35 nM. The origamis were incubated 2 hours. The enzymes in the reference sample (free LUC) were diluted to correspond the behavior of LUC-origami samples without added polymers (similar decay kinetics and parameters). The reference sample was then treated exactly same way as the LUC-origami samples.

In order to ensure that above-mentioned filtering procedures work appropriately for the LUC-enzymes, luminescence decay curves for free unfiltered and filtered enzymes were measured. The concentration of the filtered enzyme was brought back to its theoretical initial value after the spin-filtering procedure. The graph below (Fig. S2) shows that 4x filtering removes free LUC-enzymes efficiently.

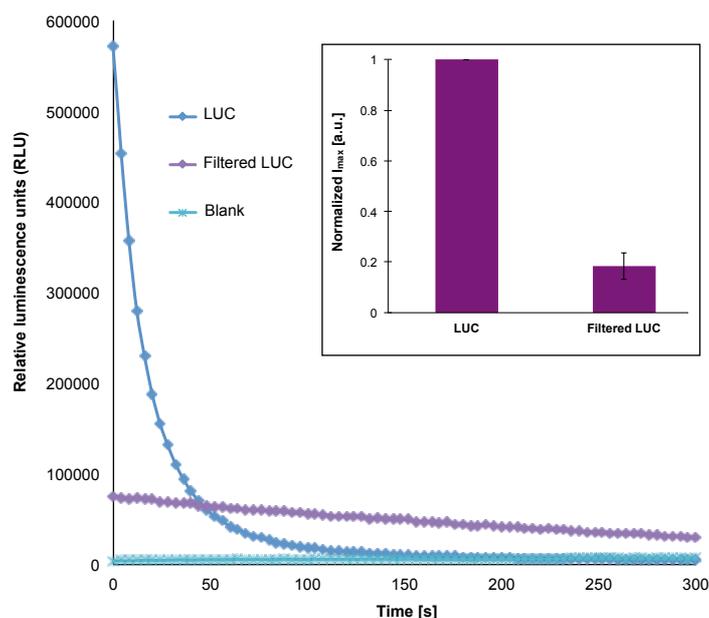


Figure S2. Luminescence decay and the maximum intensities for the plain free Streptavidin-Lucia (LUC) and the spin-filtered Streptavidin-Lucia (Filtered LUC).

Parameters from the decay assays of LUC-origami-polymer complexes

The luminescence decay of LUC-origami-polymer complexes was analyzed using the equation (1) (see the main article). All the fitting parameters are listed in the Table S1 below.

Table S1. Fitted normalized time constant T and the stretching exponent β for the luminescence decay assays that obey stretched exponential behavior. T and β have been calculated for the enzymes (Streptavidin-Lucia) loaded into / attached to the tubular DNA origami (LUC-origami) and the similar amount of enzymes that are free in the solution (free LUC).

Polymer (amount / DNA origami)	LUC-origami	Free LUC
No polymer (0x)	$T = 1 \pm 0.1$ (normalized), $\beta = 0.81 \pm 0.02$	$T = 1 \pm 0.1$ (normalized), $\beta = 0.85 \pm 0.07$
HP (10x)	$T = 1.0 \pm 0.1, \beta = 0.84 \pm 0.03$	$T = 1.0 \pm 0.1, \beta = 0.85 \pm 0.05$
HP (100x)	$T = 1.1 \pm 0.1, \beta = 0.83 \pm 0.03$	$T = 1.0 \pm 0.1, \beta = 0.85 \pm 0.05$
HP (1000x)	$T = 2.1 \pm 0.6, \beta = 0.87 \pm 0.03$	$T = 1.0 \pm 0.1, \beta = 0.85 \pm 0.05$
AB (10x)	$T = 1.6 \pm 0.2, \beta = 0.85 \pm 0.03$	$T = 1.0 \pm 0.1, \beta = 0.85 \pm 0.05$
AB (100x)	$T = 1.6 \pm 0.3, \beta = 0.76 \pm 0.07$	$T = 1.0 \pm 0.1, \beta = 0.85 \pm 0.05$
AB (1000x)	$T = 2.0 \pm 0.2, \beta = 0.80 \pm 0.07$	$T = 1.0 \pm 0.1, \beta = 0.85 \pm 0.05$
ABA (10x)	$T = 1.6 \pm 0.2, \beta = 0.73 \pm 0.04$	$T = 1.0 \pm 0.1, \beta = 0.85 \pm 0.05$
ABA (100x)	$T = 1.8 \pm 0.2, \beta = 0.79 \pm 0.03$	$T = 1.0 \pm 0.1, \beta = 0.85 \pm 0.05$
ABA (1000x)	$T = 1.7 \pm 0.1, \beta = 0.81 \pm 0.07$	$T = 1.0 \pm 0.1, \beta = 0.85 \pm 0.05$

Agarose gel electrophoresis for studying complexation. Agarose (0.8 g) was mixed with 100 of 1x TAE buffer containing 11 mM $MgCl_2$, and the gel was stained with ethidium bromide (80 μ l of 0.625 mg/ml stock solution). The samples were prepared by mixing DNA origami solution (25 μ l) with 6x Blue Loading Dye (3 μ l). 25 μ l of each sample was loaded into a gel well and native 60-helix bundle was used as a reference. 1x TAE including 11 mM $MgCl_2$ was used as a running buffer. The gel was run at a constant voltage of 75 V for 25-45 minutes.

Transmission electron microscopy (TEM) for polymer-origami complexes. The samples were prepared on carbon only copper grids (Electron Microscopy Sciences) as described above. The TEM images were taken with JEM 3200FSC field emission microscope (JEOL) operated at 300 kV in bright field mode with Omega-type Zero-loss energy filter. The micrographs were acquired with Gatan Digital Micrograph software while the specimen temperature was maintained at -187 °C.

Ethidium bromide displacement assay to study the binding of the polymer on the origami surface (see also Supplementary Section 6). First, the fluorescence of 0.9 μ M ethidium bromide

(EthBr) solution (HEPES 6.5 mM + NaOH, pH 6.8) at $\lambda=610\text{nm}$ was measured ($\lambda_{\text{ex}}=546\text{nm}$, $\lambda_{\text{em}}=580\text{-}650\text{nm}$, emission and excitation slit 20 nm, emission PMT (V) = 600). After that, 25 μl of 1 nM 60HB origami solution (HEPES 6.5 mM + NaOH, pH 6.8) was added to the EthBr-solution and the fluorescence of this mixture was measured. This was followed by small additions of polymer and the recording of the changes the in fluorescence of the solution. The relative fluorescence of the solutions was calculated as reported by Mikkilä *et al.* [S3]:

$$F_{\text{rel}}(\%) = \left(\frac{F_{\text{observed}} - F_{\text{EthBr}}}{F_{\text{origami+EthBr}} - F_{\text{EthBr}}} \right) \times 100\%$$

6. Ethidium bromide displacement assay

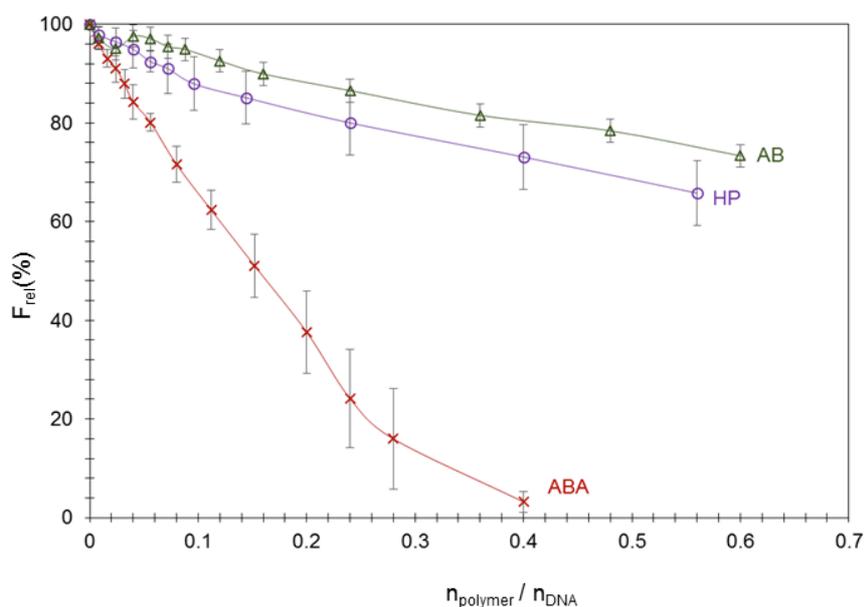


Figure S3. Ethidium displacement assay shows how the fluorescence of the EthBr decreases upon polymer addition. The polymers either quench the fluorescence or squeeze out the EthBr from the DNA double helix thus causing the decrease in fluorescence.

The EthBr fluorescence decreases upon polymer addition due to the quenching or the removal of EthBr from the DNA structure (Fig. S3). As the agarose gel experiment, also the EthBr assay indicates that the ABA-type copolymer binds most efficiently on the origami surface. With the displacement assay, the difference between ABA and AB/HP is more pronounced, but the trend is clearly the same and binding is observed. It could be argued that the ABA-structure of the PDMAEMA-PEG-PDMAEMA block copolymer enables the full utilization of the whole polymer chain. The flanking cationic blocks are able to anchor the middle PEG-block tightly to the origami surface, thus providing more extensive polymer coating than lower molecular weight PDMAEMA homopolymer and AB-type PDMAEMA-PEG block copolymer.

Supplementary information references:

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[S2] V. Linko, M. Eerikäinen and M. A. Kostiaainen, *Chem. Commun.*, 2015, **51**, 5351-5354.

[S3] J. Mikkilä, A.-P. Eskelinen, E. H. Niemelä, V. Linko, M. J. Frilander, P. Törmä and M. A. Kostiaainen, *Nano Lett.*, 2014, **14**, 2196-2200.