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Supporting Information

Selective Nascent Polymer Catch-and-Release Enables Scalable Isolation of Multi-Kilobase Single-Stranded DNA

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Contents

Materials and Reagents

Solvents and reagents were purchased from commercial sources and used as received, unless otherwise specified. Molecular biology grade Acrylamide (AA, cat. #A9099), N N'-methylenebisacrylamide (BAA, cat. #M7279), sodium acrylate (A, cat. #408220), and ammonium persulfate (APS, cat. #A3678) were purchased from *Sigma Aldrich*. Ultrapure N,N,N´,N´-tetramethylethylenediamine (TEMED, cat. #15524010) was obtaind from *Thermo Scientific*. Methanol (MeOH, ACS reagent grade), ethanol (anhydrous), and isopropanol (laboratory grade) were obtained from *Fisher Scientific*. All reagents containing AA groups were stored at 4°C or -20°C, and protected from light. Desalted oligonucleotides were purchased from *Integrated DNA Technologies* and used without further purification. Taq DNA Polymerase, Q5, pBR322 vector, deoxynucleotides (dNTPs), and streptavidin-coated microbeads (#S1420S) were purchased from *New England Biolabs*. iProof High Fidelity PCR Kit was purchased from *Bio-Rad*. KOD Hot Start Polymerase Kit was purchased from *EMD Millipore*. The GeneJET PCR Purification kit was purchased from *Thermo Scientific*. 1 kB DNA ladders were purchased from *New England Biolabs* (cat. #N3232L) and Thermo Scientific (cal. #SM0312). Microfiltration was performed through Freeze 'N Squeeze™ DNA Gel Extraction Spin Columns from *Bio-Rad* or through 0.2 μm cellulose acetate syringe filters from *Corning*. Nitrogen gas (99.999%) from an in-house gas generator was used for experiments under inert conditions. Water was obtained from a *Milli-Q* system.

UV/Vis absorbance data was recorded on a Nanodrop 2000c Spectrophotometer (*Thermo Scientific*).

Agarose gel electrophoresis (AGE) was carried out in 0.5x TBE buffer containing 11 mM $MgCl_2$ as an eluent. Unless specificed otherwise, DNA samples were eluted at 90 V for 90 minutes in a 0.7% agarose gel, which had been pre-stained with SYBR Gold (*Thermo Scientific*). DNA origami structures were eluted at 60 V for 4 hours in a 2% agarose gel, which had been pre-stained with Ethidium bromide (*Bio Rad*). Densitometric quantification of DNA bands was performed in *ImageJ* (v. 2.0.0-rc-43/1.51h).

Transmission electron microscopy (TEM) was performed on a *JEOL JEM 1400* microscope operated at 80 kV. 3 μL of sample was applied to a Formvar/Carbon 400 mesh copper grid (*Electron Microscopy Sciences*). Samples were blotted after 3 minutes, stained for 30 seconds with a 2% uranyl formate solution containing 15 mM NaOH, thoroughly blotted and dried in air.

Polymerase chain reaction (PCR) and DNA origami folding reactions were performed on a PTC-225 Peltier Thermal Cycler (*MJ Research*). Primers for PCR were designed using *Primer3Plus* web-interface (v. 2.4.0)[1].

Gel permeation chromatography (GPC) was performed on a 1260 Infinity Bioinert Multi-Detector GPC/SEC System (*Agilent*) using TSKgel G5000PW_{xl} and G6000PW_{xl} SEC columns in tandem (*Tosoh*). Aqueous $NaNO_3$ (0.1 M) containing 0.05% NaN_3 was used as a mobile phase. Molecular weights were determined via triple detection, using refractive index, viscosity, and light scattering channels. Polyethylene oxide (PEO) was used as a standard (*Agilent*, M_w = 256,100 Da, PD =

1.05), and molecular weight data was validated using at least one different PEO standard (*Agilent*).

Procedures

General

All samples containing unreacted **AA** groups were protected from unnecessary exposure to light.

PCR

Short strands (<5 kbp)

PCR of short strands was performed with Taq DNA Polymerase in Standard Taq Reaction Buffer (*New England Biolabs*). Product concentrations were in the range of 50-150 ng/µL.

In a typical experiment, the following mixture was prepared in a 15 ml Falcon tube on ice:

Component	Volume [µL]
Nuclease-free water	8150
10X Standard Taq Reaction Buffer	1000
10 mM dNTPs	200
10 µM Forward Primer	200
10 µM Reverse Primer	200
Template (0.1 ng/µL)	200
Taq DNA Polymerase	50
Total volume	10000

The solution was gently mixed. Then 100µL aliquots were transferred into a 96-well PCR plate. The plate was tightly closed with an aluminum cover, transferred to a PCR instrument with the block preheated to 95°C, and subjected to 30-35 thermocycles:

Step	T [°C]	t [s]
Initial denaturation	95	30
Denaturation	95	20
Annealing	T_m – 5°C	40
Extension	68	60 s × length [kb]
Final extension	68	300
Hold	4	inf.

All wells were combined, aliquoted into 2 ml centrifuge tubes, and stored at -20°C, protected from light. The samples were used directly for SNAPCAR without prior purification. To reduce sample volume, the product can be concentrated up by using a centrifugal filter (e.g. Amicon Ultra-15, 100 kDa).

Long strands (>5 kbp)

PCR of long strands require long-range polymerases. We tested Q5, iProof, and KOD Hot Start DNA polymerase kits for amplification of a 7301 bp fragment from M13-derived p7308. We obtained highest product yields with KOD Hot Start (~100 ng/µL; Figure S2).

In a typical experiment, the following mixture was prepared in a 15 ml Falcon tube on ice:

Component	Volume [µL]
Nuclease-free water	1550.4
10X KOD Buffer	240
25 mM MgSO4	172.8
2 mM dNTPs	240
10 µM Forward Primer	72
10 µM Reverse Primer	72
Template (10 ng/µL)	4.8
KOD Hot Start DNA Polymerase	48
Total volume	2400

The solution was gently shaken. Then 100µL aliquots were transferred into 24 PCR tubes. The tubes were transferred to a PCR instrument with the block preheated to 95°C, and subjected to 30-35 thermocycles:

Step	T [°C]	t [s]
Initial denaturation	95	120
Denaturation	95	20
Annealing	T_m	20
Extension	70	25 s × length [kb]
Final extension	70	60
Hold	4	inf.

After amplification, the samples were purified with a PCR purification kit, eluted in a final volume of 300 µL TE buffer, and stored at -20°C, protected from light.

SNAPCAR

Route L

Materials

- Acrylamide-labeled **dsDNA** from PCR, unpurified, e.g. 100 ng/µL (concentration range: 10-1000 ng/µL)
  - Note 1: Avoid excessive light exposure of acrylamide-labeled DNA
  - Note 2: We produced the dsDNA by amplification with Taq Polymerase in Standard Taq Reaction Buffer from *New England Biolabs*. If another PCR buffer is being used that contains large amounts of thiol or detergent additives, it might be necessary to purify the PCR product prior to SNAPCAR.

- Acrylamide/Sodium acrylate mixture (**AA/A**): acrylamide (**AA**) mixed with sodium acrylate (**A**) at 99:1 mass ratio (prepare 40 wt% stock solution in the fume hood; store at 4°C protected from light)
- **TBE** buffer (5x): 500 mM Tris, 500 mM boric acid, 10 mM EDTA, pH 8.2 (approximate ionic strength: 310 mM)
- **TE** buffer (1x): 5 mM Tris, 1 mM EDTA, pH 8.0 (approximate ionic strength: 8.5 mM)
- NaCl, 5 M
- Washing solution (**WS**): 5 mM Tris, 1 mM EDTA, 30 mM NaCl, pH 8.0 + 1 vol. MeOH
- Tetramethylethylenediamine (**TEMED**, stored at 4°C protected from light)
- Ammonium persulfate (**APS**) powder (stored in small batches of ~50 mg in sealed containers, protected from moisture)
- Nitrogen gas (**N_2**)
- Thin syringe needle (e.g. 22G1.5) for N_2 purging
- Formamide denaturing buffer (**FDB**): 99% Formamide(aq.) containing 5 mM EDTA, pH 8.0
- Basic denaturing buffer (**BDB**): 0.2 M NaOH + 2 mM EDTA
- Sodium acetate buffer (3 M, pH 5.25)
  - Prepare by mixing 3 M NaOAc with 3 M HOAc
- Methanol (**MeOH**)
- Isopropanol (**iPrOH**)
- 75% Ethanol, -20°C
- H_2O
- 4 ml glass vial with septum cap
- 0.2 ml PCR tubes
- 1.5 ml centrifuge tubes
- 50 ml centrifuge tubes
  - Container size presumes a 2 ml reaction volume. Larger containers (e.g. 250 ml Nalgene centrifuge bottles) or smaller containers (e.g. 1.5 ml Eppendorf tubes) may be used when scaling up/down the reaction.

Parameters overview

Polymerization time [h]	≥3 h
APS concentration [wt%]	0.015
TEMED concentration [wt%]	0.015
Total AA concentration [wt%]	5.0
Total A concentration [wt%]	0.05
N_2-purging	Yes
Sample volume [µL]	2000
Buffer	TBE, pH 8.2

Procedure

1. Polymerization

A. Prepare SNAPCAR reaction **1** in a septum-sealed 4-ml glass vial and solutions **N** and **D** (native and denat. controls) in PCR tubes:

Sample ID	C_{DNA} final [ng/µL]	H2O [µL]	TBE, 5x [µL]	FDB [µL]	dsDNA, 100 ng/µL [µL]	A, 40% [µL]	TEMED, 10 wt% [µL]	APS, 10 wt% [µL]	Total volume [µL]
1	67.1	0	400		1341.5	252.5	3	3	2000
N	6.71	73.29	20		6.71				100
D	6.71			93.29	6.71				100

Important notes:

- If changing the production scale, ensure that the reaction container has the appropriate size: to achieve efficient N_2 purging, 1/4 to 2/3 of the container volume should be filled with the reaction solution.
- Mix first **H_2O**, **TBE**, **dsDNA**, and **A**, then bubble solution **1** for 10 min with N_2. In the meanwhile, prepare fresh 10 wt% stock solutions of **TEMED** and **APS**:
    - **TEMED**: 13 µL **TEMED** + 87 µL H_2O
    - **APS**: 50 mg **APS** + 0.5 ml with H_2O
- Add **TEMED** to sample **1**, swirl vial, then add **APS** while continuing N_2 bubbling

B. After adding all reagents, bubble solution with N_2 for another 5 minutes
    - The solution should become viscous during that time

C. Tightly seal valve and incubate for at least 3 h (shake gently during that time, if possible).
    - One may let the sample incubate overnight

D. After incubation, the solution should have turned highly viscous.
    - The viscous fluid can be handled by pipettors when cutting off the pipette tips, or by using a disposable syringe.

2. Precipitation under native conditions (**purification**)

A. Mix sample **1** + 18 mL (9 vol.) **TE** in 50-ml Eppendorf tube

B. Vortex or shake at 1500 rpm for 10 min or until fully dispersed (***TE_0***)
    - Note: Red IDs indicate that some of the solution (e.g. 50 µL) can be stored to enable later analysis.

C. Add 100 µL **NaCl** (5 M) and vortex for a few seconds
    - This brings up the ionic strength of the solution to a level that is required for the subsequent MeOH precipitation step

D. Rapidly add 15 mL (0.75 vol.) **MeOH** and vortex briefly
    - There should be no precipitate. If there's precipitate it should redisperse after some vortexing

E. Add another 5 ml (0.25 vol.) **MeOH**, vortex for 20 seconds
    - A white precipitate should form immediately

F. Let sample incubate for 2 min
G. Centrifuge at 100–150 g at r.t. for 5 min and decant supernatant (***S_1A***)
    - Avoid centrifugation faster than 150 g acceleration, since this will make it difficult to disperse the pellet
    - There should be a voluminous pellet
    - The supernatant is clear or slightly turbid
H. Wash the pellet by adding 20 ml **WS** and vortexing for 1 min, spin down at 100–150 g for 5 min, and discard supernatant (***S_1B***)
    - Make sure that there's as little supernatant left as possible

3. Precipitation under denaturing conditions (**ssDNA release**)

A. Add **H_2O** to the pellet until the total volume is ~16.4 ml, and shake until pellet is fully dispersed (this should not take more than 1 min). Then shake for another 5 minutes
B. Add 3.6 ml **BDB** and shake for 5–10 min
    - Important note: Do not incubate for more than 10 min!
C. Rapidly add 15 ml (0.75 vol.) **MeOH** to the solution, quickly shake and vortex briefly
    - There should be no precipitate after vortexing
D. Add another 5 ml (0.25 vol.) **MeOH** to the solution and vortex for 20 sec.
    - White precipitate should form immediately
E. Incubate solution at -20°C for 30–60 min
F. Spin down precipitate at 0–4°C and 20,000–24,000 g for 30–60 min
G. Transfer supernatant into a new Falcon tube. (***S_2***)
    - Be extra careful not to transfer any pellet fragments into the supernatant! If some visible material is being transferred to the new tube, centrifuge that tube again at 24,000 g, 4°C for 10 min
H. Optional: run preliminary agarose gel electrophoresis for analysis (See section 5A)

4. iPrOH precipitation

A. Add to the supernatant 4 mL (0.1 vol.) **sodium acetate** (3 M, pH 5.25) and vortex briefly
B. Split into two 50 ml Falcon tubes (2×22 ml)
C. Add 17.6 ml (0.8 vol.) **iPrOH** to each tube and vortex briefly
D. Incubate for 2 h on ice
E. Centrifuge at 20,000–24,000g for 45 min at 0–4°C
F. Decant supernatants
    - Depending on the initial DNA concentration, the pellets might be very small and barely visible.

G. Add 2 mL **EtOH** (75%, -20°C). Incubate pellet in EtOH for 10 min in the freezer. Do not vortex.
H. Centrifuge at 20,000–24,000g for 10 min at 0–4°C
I. Remove supernatant
J. Air-dry pellets, e.g. by shaking the open tubes for 30 min at 37°C
K. Add to each pellet 250 µL TE buffer and shake for 20 min at 37°C (***P_3***)
    - Note 1: For ≥ 1 kb long strands, heat the solution to 80°C for 2 min to fully disaggregate the ssDNA product.
    - Note 2: The ideal (100% yield equiv.) concentration of ssDNA product (in ng/µL) should be double the concentration of dsDNA in solution **1** (i.e., 200 ng/µL ssDNA product if the initial concentration of dsDNA in sample #1 is 100 ng/µL)
L. Analyze by Nanodrop and agarose gel electrophoresis
M. Store product at -20°C

5. Analysis

A. Preliminary agarose gel electrophoresis (optional)
    i. Gel conditions: 0.7% agarose, pre-stained with SYBR Gold, 90 V, 90 min, 12-well comb
    ii. Heat control sample **D** to 90°C for 1 min, then quickly cool to 4°C before proceeding
    iii. Mix 20 µL of each of samples **N**, **D**, **TE_0**, **S_1A**, **S_1B**, and **S_2** with 20 µL glycerol
        - Note: This is important to make all MeOH-containing samples sink into the wells
    iv. Add 4 µL loading dye to each
    v. Run different volumes on agarose gel: 5 µL of **N**, **D**, and **TE_0**; 10 µL of **S_1A** and **S_1B**; 20 µL of **S_2** (volume differences compensate for dilution steps in the procedure)
B. Nanodrop
    i. OD_{260}: Estimate ssDNA concentration
    ii. Abs_{260}/Abs_{280}: Indicates purity. Ideally ≥1.80
    iii. Abs_{260}/Abs_{230}: Indicates purity. Ideally ≥ 2.00. A low value might indicate contamination by residual copolymer
C. Agarose gel electrophoresis
    i. Gel conditions: 0.7% agarose, pre-stained with SYBR Gold, 90 V, 90 min, 12-well comb
    ii. Anneal sample **D** for 1 min to 90°C and quickly cool to 4°C before proceeding.
    iii. Run samples **N**, **D**, and **P_3**.
    iv. Assess product concentration and yield by densitometry

Route X

Materials

- Acrylamide-labeled **dsDNA** from PCR, unpurified, e.g. 100 ng/µL (concentration range: 10–1000 ng/µL)
  - Note 1: Always avoid excessive light exposure of acrylamide-labeled DNA
  - Note 2: Produce the dsDNA by amplification with Taq Polymerase in Standard Taq Reaction Buffer from *New England Biolabs*. If another PCR buffer is being used that contains large amounts of thiol or detergent additives, it might be necessary to purify the PCR product prior to SNAPCAR.
- Acrylamide/bis-acrylamide mixture (**9:1 AA**): acrylamide (**AA**) mixed with bis-acrylamide (**A**) at 9:1 mass ratio (prepare 40 wt% stock solution in the fume hood; store at 4°C protected from light)
- **TBE** buffer (5x): 500 mM Tris, 500 mM boric acid, 10 mM EDTA, pH 8.2 (approximate ionic strength: 310 mM)
- **TE** buffer (1x): 5 mM Tris, 1 mM EDTA, pH 9.0
- Tetramethylethylenediamine (**TEMED**, stored at 4°C protected from light)
- Ammonium persulfate (**APS**) powder (stored in small batches of ~10 mg in sealed containers, protected from moisture)
- 4 ml glass vials with Mininert® valve
- Nitrogen gas (**N_2**)
- Thin syringe needle for N_2 purging
- Cellulose acetate (**CA**) syringe filter, 0.22 µm pore size
- Sodium acetate buffer (3M, pH 5.25)
  - Prepare by mixing 3M NaOAc with 3M HOAc
- 100% Ethanol, -20°C
- 75% Ethanol, -20°C
- Ultracentrifuge tubes

Parameters overview

Polymerization time [h]	20 h
APS concentration [wt%]	0.002
TEMED concentration [wt%]	0.002
mono-/bisacrylamide ratio	9:1
Total acrylamide concentration [wt%]	1.25
N_2-purging	Yes, rigorous
Sample volume [µL]	2000
Buffer	TBE, pH 8.2

Procedure

1. Polymerization
   A. Degas 10 ml H_2O by bubbling with N_2 for 15 min
   B. Prepare 1wt% TEMED and 1wt% APS stock solutions in degassed water
   C. Prepare the following solution in a Mininert-valve equipped glass vial:
      - Mix first H_2O, TBE, DNA and AA/BAA mixture, then add TEMED and finally APS

H_2O [µL]	TBE, 5x [µL]	DNA, 100 ng/µL [µL]	9:1 AA [µL]	TEMED, 1wt% [µL]	APS, 1wt% [µL]	Total volume [µL]
529.9	400	1000	62.5	4	4	2000

D. Bubble solution with N_2 while reagents are being added, and subsequently bubble for another 30 minutes
E. Tightly seal septum valve and store vial in a desiccator for 20 h
F. After reaction time the material should have become a soft gel

2. Optional: Electroelution purification
   A. Add the gel into a well of a 1.2% agarose gel w/o stain, designed for electroelution (Figure S7)
   B. Elute for 30 min at 90V
   C. Transfer gel from the well back into a 15 ml test tube
   D. Add TE buffer (pH 9.0) to gel, until total volume is 3 ml
   E. Vortex for 1 min

3. Dehybridization and ssDNA retrieval
   A. Add tube into 80°C warm water bath and let equilibrate for 5 min
   B. Add boiling hot 1x TE buffer (pH 9) to 15 mL total volume
   C. Vortex for 20 seconds
   D. Transfer tube to ice to rapidly chill for 5 min
   E. Centrifuge at 150,000 g for 30 min at 4°C to spin down gel particles
   F. Filter supernatant over syringe filter into a 50 ml Falcon tube, to remove residual polyacrylamide
   G. Ethanol precipitation:
      - Add 1.25 ml NaOAc, pH 5.25, 3M to the filtrate
      - Fill up tube with -20°C ethanol and incubate in freezer for 2 h
      - Centrifuge in pre-cooled rotor at ≥ 5000 g for 30 min
      - Decant supernatant and add to pellet 1 ml of -20°C 70% ethanol
      - Centrifuge in pre-cooled rotor at ≥ 5000 g for 5 min
      - Air-dry pellets for 30 min at r.t.
   H. Add to each pellet 250 µL TE buffer and shake for 10 min at 37°C
   I. Analyze by Nanodrop and agarose gel electrophoresis
   J. Store in centrifuge tube at -20°C

SNAPCAR cost estimate

Costs for materials and reagents were estimated for three different scales, assuming a 1 kb long target strand:

1. **Small scale:** 10 pmol or 3 µg, starting from a 100 µL sample volume.
2. **Medium scale:** 1 nmol or 300 µg, starting from a 10 mL sample volume.
3. **Large scale:** 100 nmol or 30 mg, starting from a 1 L sample volume.

Costs were analyzed separately for four groups (Figure S17):

A. **PCR** (polymerase, dNTPs, primers, containers, pipette tips, etc.)
B. **SNAPCAR reagents** (Acrylamide, Acrylate, APS, TEMED, N_2, buffers, solvents, etc.)
C. **SNAPCAR materials** (containers, centrifuge tubes, pipette tips, etc.)
D. **Analysis** (Agarose, gel stain, buffers , containers, pipette tips, etc.)

Reference cost for *Thermo Scientific* Dynabeads® kilobaseBINDER™: 10 mg magnetic beads cost $430 and bind a maximum of 1 nmol dsDNA (manufacturer specifications). Assuming recovery of 80% ssDNA after denaturation, the cost is ~$540 per nmol. Svobodová et al. have previously estimated the cost of ssDNA generated from magnetic beads to be 600 € (~$700) per nmol[2].

Folding of DNA Origami

The single-layer DNA origami structure was folded following the original method by Rothemund[3]. Multi-layer DNA origami were folded following the honeycomb design by Douglas and coworkers[4]. All structures had been designed in CadNano software, developed by Douglas and coworkers[5].

Barrel

The barrel structure design has been previously reported (Figure S18).[6] It was folded from **ss3315**. The following samples were prepared:

	Barrel	**Scaffold**
Component	**Volume [µL]**	
50 mM Tris, 10 mM EDTA, pH 8.0	3.4	3.4
60 mM $MgCl_2$	4	4
ss3315 (46 nM)	6	6
Staple strands (500 nM each)	8	
Nuclease-free water	18.6	26.6
Total volume	40	40

Both samples were annealed: 80°C (10 min), 55°C→45°C (18h; -0.56°C/h), 45°C→25°C (1h; -20°C/h), 4°C (hold)

Rectangle

The rectangle is an adaptation from a previously reported structure (Figure S19)[3,7]. It was folded from **ss7301**. The following samples were prepared:

	Rectangle	**Scaffold**
Component	Volume [µL]	
50 mM Tris, 10 mM EDTA, pH 8.0	2.9	2.9
60 mM $MgCl_2$	4.0	4.0
ss7301 (35 nM)	11.4	11.4
Staple strands (8.1 µM each)	0.5	
Nuclease-free water	21.2	21.7
Total volume	40.0	40.0

Both samples were annealed: 95°C (1 min), 80°C (5 min), 65°C→45°C (18h; -1.11°C/h), 45°C→25°C (2h; -10°C/h), 4°C (hold)

6-Helix Bundle

The 6-helix bundle[8,9] was folded from **ss7301**. The strand diagram is depicted in Figure S20). The following samples were prepared:

	6-Helix Bundle	**Scaffold**
Component	Volume [µL]	
50 mM Tris, 10 mM EDTA, pH 8.0	2.9	2.9
60 mM $MgCl_2$	5.3	4.0
ss7301 (35 nM)	11.4	11.4
Staple strands (1 µM each)	4.0	
Nuclease-free water	16.4	21.7
Total volume	40.0	40.0

Both samples were annealed: 95°C (1 min), 80°C (5 min), 65°C→45°C (18h; -1.11°C/h), 45°C→25°C (2h; -10°C/h), 4°C (hold)

DNA capture by magnetic microparticles

To evaluate binding specificity and ssDNA isolation with microparticles, we followed a previously published procedure, which utilizes streptavidin-coated magnetic beads.[10] In short, the beads were washed three times in binding buffer (20 mM Tris-HCl, 1 mM EDTA, and 0.2 M NaCl, pH 8.0). The last supernatant was removed thoroughly, and a 1:1 mixture of unlabeled 7301-bp dsDNA (**ds7301**) and 3315-bp biotin-labeled dsDNA (**ds3315**) in binding buffer was added. The dispersion was vortexed shortly and incubated for 30 min while continuously inverting the tube. The supernatant was obtained for analysis of capture selectivity. The beads were washed 3x with binding buffer. After removing the last supernatant, 0.2M NaOH was added, and the suspension was continuously inverted for 6 minutes. Finally, the supernatant was isolated and neutralized with 3M sodium acetate (pH 7). A **ds3315** control solution, the **ds3315/ds7301** mixture, intermediate supernatants and final ssDNA product solution concentrations were analyzed by agarose gel electrophoresis. Results of binding selectivity and ssDNA product are shown in Figure S12.

Ionic strength

Ionic strength (I) of aqueous solutions was estimated by equation 1.

$$I = \frac{1}{2}\sum_{i=1}^{n} c_i z_i^2 \qquad (1)$$

, where c_i is the concentration of ion i, and z_i is the charge of that ion, in a solution containing n number of different ions.

Estimates took into consideration all mono-, di-, and trivalent ions, most typically: Na^+, K^+, $[Tris–H]^+$, Cl^-, OH^-, Mg^{2+}, $EDTA^{3-}$.

Figures

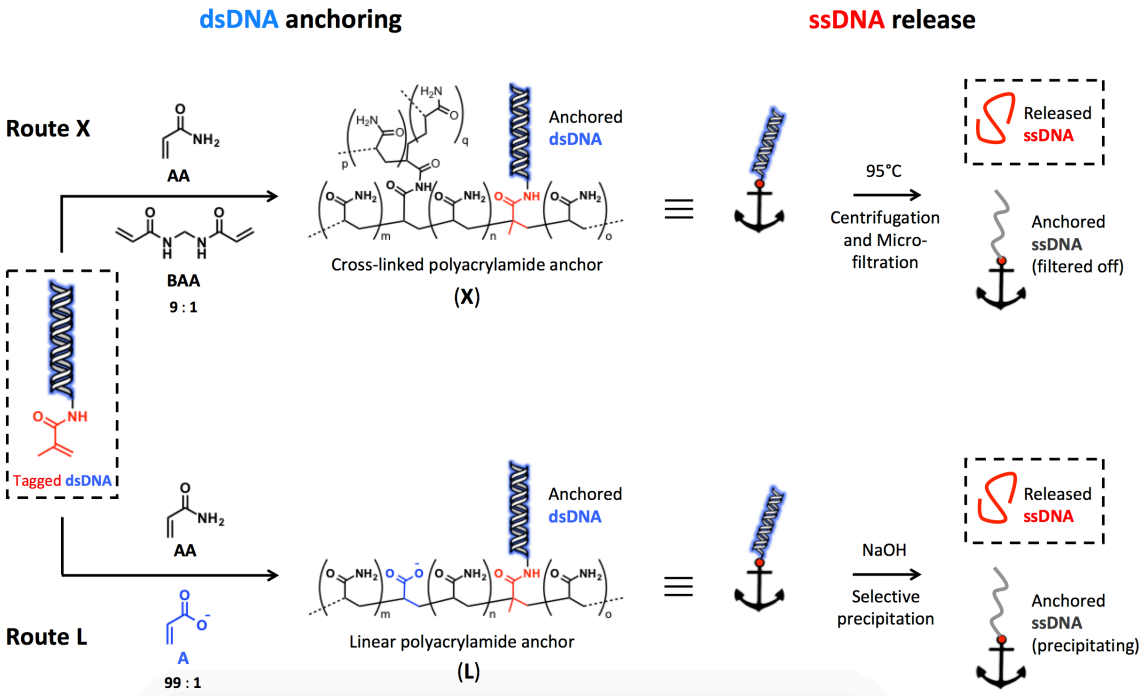

Figure S1. *SNAPCAR initial route using cross-linked copolymer (X), and improved route using linear copolymer (L).*

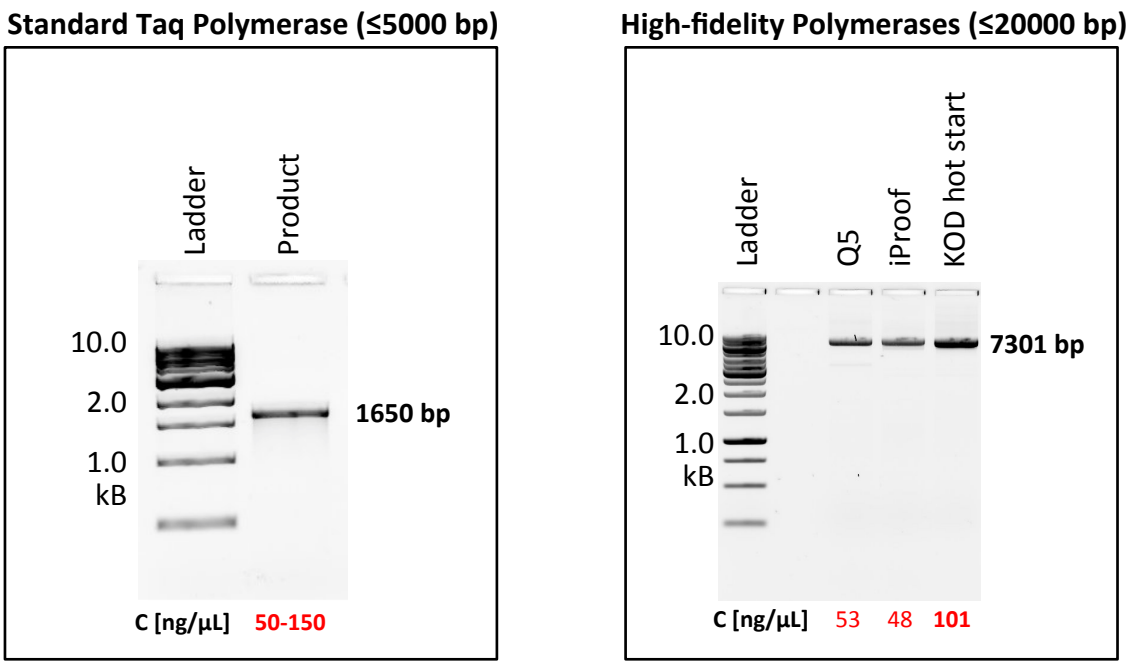

Figure S2. *PCR amplification for production of dsDNA starting material. Left: a typical ≤5 kbp product, commonly obtained at 50-150 ng/µL concentration (agarose gel stained with EtBr). Right: a 7.3 kbp product amplified with three different long-range polymerases, Q5, iProof, and KOD (agarose gel stained with EtBr).*

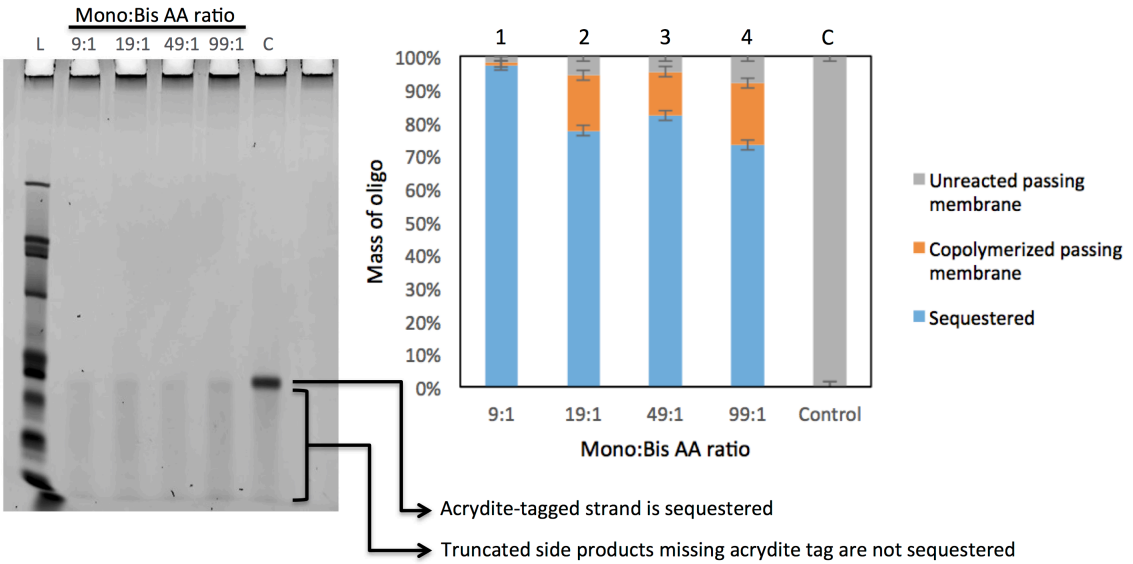

Figure S3. *Sequestration of an acrylamide-labeled oligonucleotide strand via copolymerization with mixtures of acylamide (AA) with bisacrylamide (BAA) in varying mass ratios. Each copolymer solution was filtered through a 0.45 µm cellulose acetate membrane.* ***Left****: polyacrylamide gel electrophoresis of the filtrates (C= unlabeled control strand).* ***Right****: Sequestration and filtration efficiencies. The acrylamide-labeled strand was sequestered with >97% yield. In contrast, unlabeled truncated byproducts of the oligonucleotide synthesis were not sequestered, demonstrating the selectivity of the process. Higher AA/BAA ratios resulted in similarly high conversion (>90%), but removal of the copolymer by microfiltration was incomplete.*

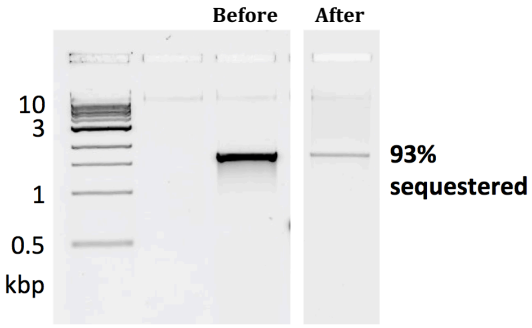

Figure S4. *Agarose gel of filtrates before and after capture of an* ***AA****-labeled 1650-nt dsDNA strand via Route X.*

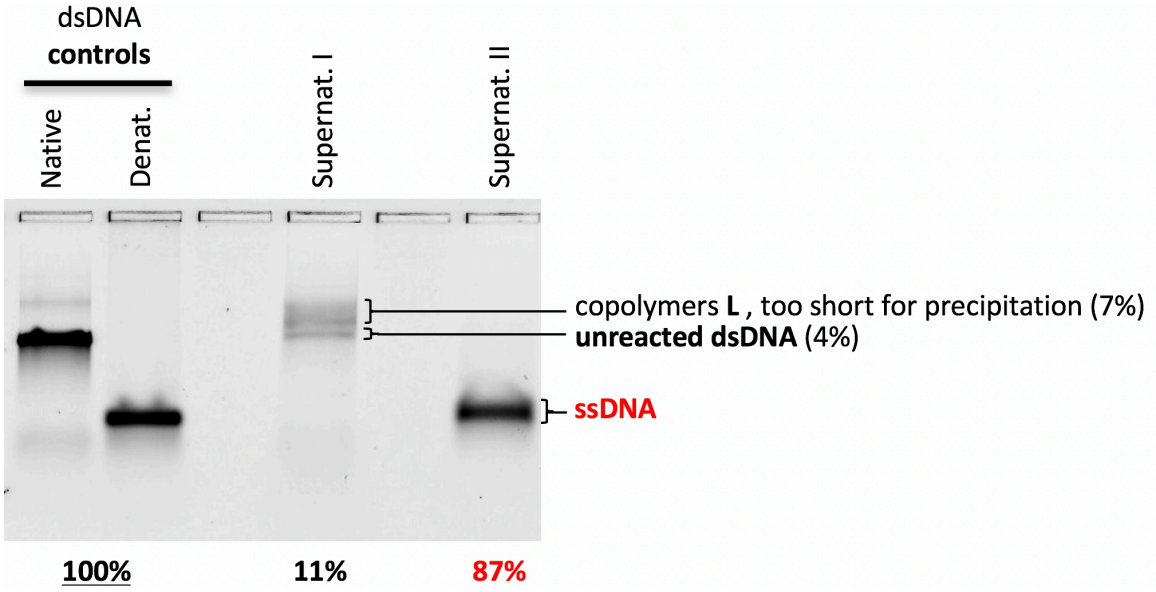

Figure S5. *Agarose gel of supernatants I and II, obtained from 3315-nt target dsDNA via Route L (enlargement from Figure 3d in the main text).*

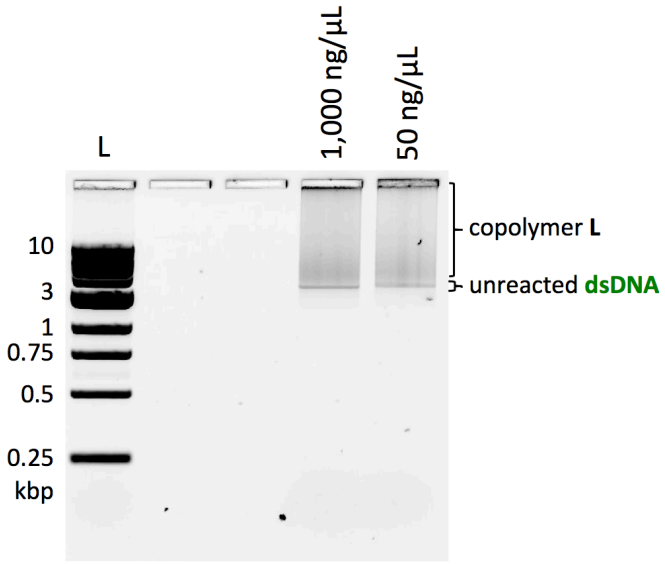

Figure S6. *Agarose gel of anchored 3315-nt dsDNA (crude product) after copolymerization via Route L at different target dsDNA concentrations. dsDNA capture efficiencies were 93% and 95% for 1,000 ng/µL and 50 ng/µL concentrated solutions, respectively.*

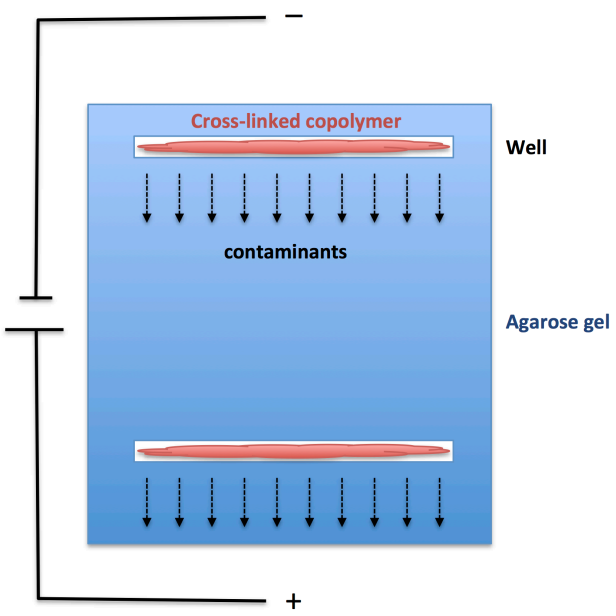

Figure S7. *Optional purification of the cross-linked copolymer (red; obtained in Route X), via electroelution within an agarose gel (blue). Total well loading capacity for a standard gel: 10 ml.*

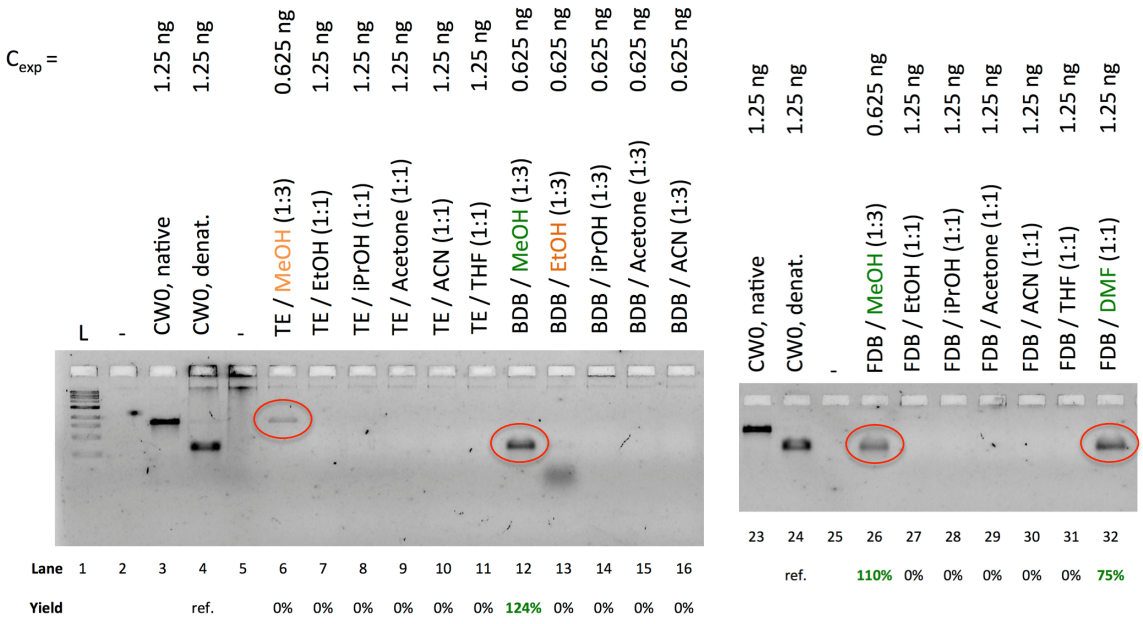

Figure S8. *Screening for an ideal "bad" solvent to precipitate polymer L without precipitating free DNA (agarose gels). Methanol precipitates the polymer under both native and denaturing conditions, while leaving dsDNA and ssDNA in solution. CW0: 1650nt DNA, ACN: Acetonitrile, THF: Tetrahydrofuran, DMF: Dimethyl formamide, BDB: Basic denaturing buffer (100 mM NaOH, 1 mM ETDA), FDB: Formamide denaturing buffer (99% formamide (aq), 5 mM EDTA, pH 8.0).*

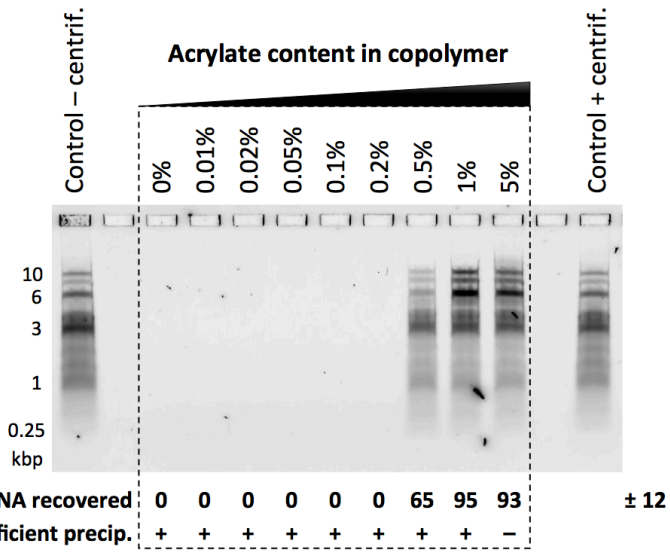

Figure S9. *Optimization of copolymer composition to suppress DNA co-precipitation: native AGE of supernatants after addition of 1 volume methanol to a 1-kB DNA ladder that was mixed with poly(**AA**-co-**A**) of different **AA**:**A** ratios.*

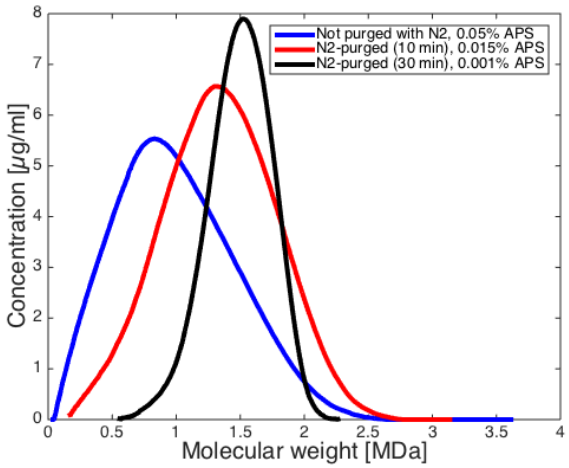

Figure S10. *Polymer size distributions of poly(**AA**-co-**A**) formed under different conditions, as determined by GPC. Blue line: copolymer formed without exclusion of oxygen, requiring high APS concentration. Red line: typical copolymer formed in an SNAPCAR experiment. Black line: copolymer formed under stringent exclusion of oxygen and with low APS concentration.*

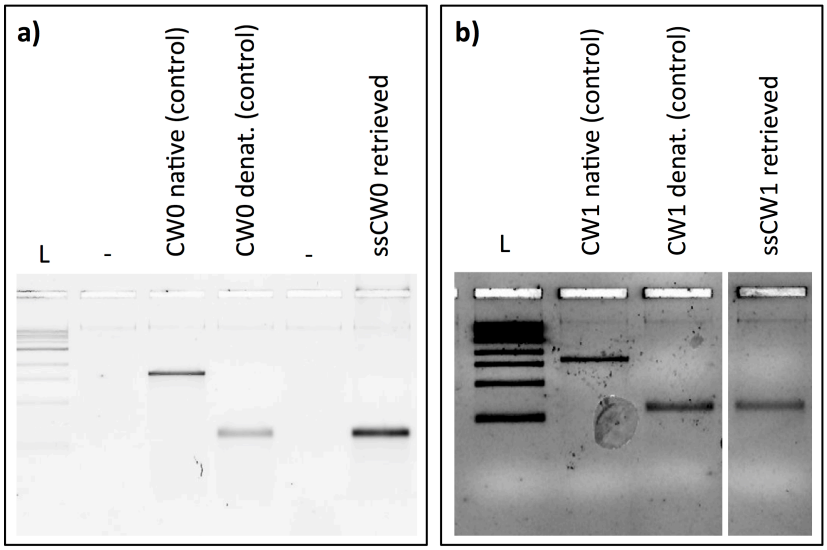

Figure S11. *Retrieval of two different 1650-nucleotide-long single-stranded DNA molecules. a) ss1650 obtained via Route X (yield: 72%). b) ss1650 obtained via Route L (yield: 71%). L = 1kB DNA ladder (NEB).*

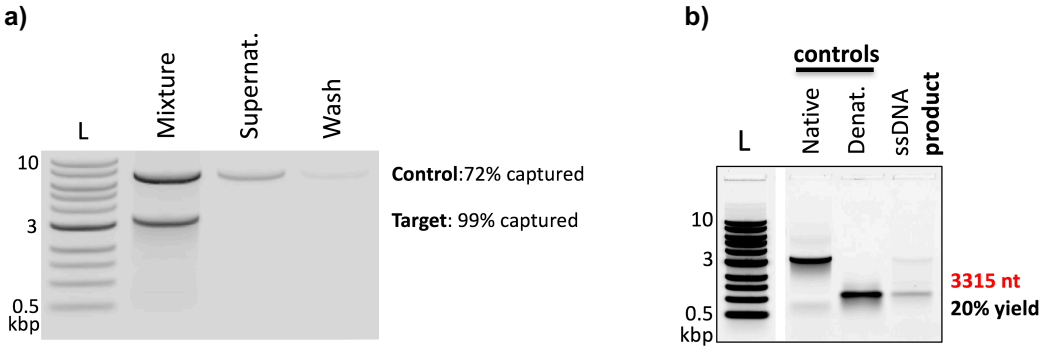

Figure S12. dsDNA binding selectivity (a) and ssDNA isolation (b) using streptavidin-coated magnetic microparticles. *a) Binding selectivity test for a mixture of unlabeled* ***ds7301*** *control and biotin-labeled* ***ds3315****. Target binding was highly efficient (%99%). However, significant nonspecific adsorption was observed for the unlabeled control strand. b) ssDNA isolation from bead-immobilized* ***ds3315****. The ssDNA product was obtained with 20% yield and 83% purity.*

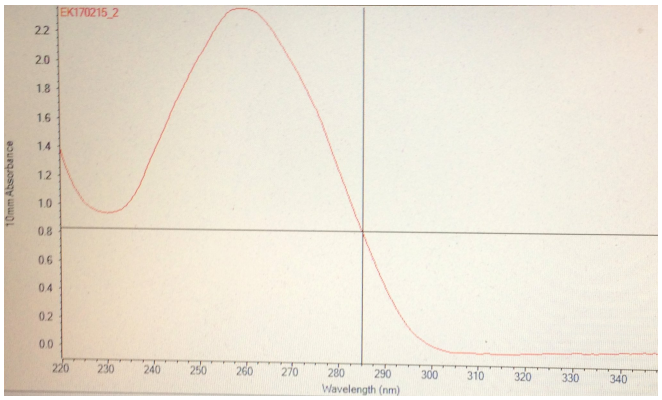

Figure S13. *UV/Vis spectrum of SNAPCAR ssDNA product* ***ss7301*** *(7.3 kB) in TE buffer. C = 77.9 ng/µL;* A_{260}/A_{230} *= 2.53;* A_{260}/A_{280} *= 1.90.*

Table S1. *UV absorbance values for several SNAPCAR ssDNA products in TE buffer (pH 8.0).*

Route	Strand	$A_{260/280}$	$A_{260/230}$	C [ng/µL]
X	**ss1650**	1.79 (fail)	0.35 (fail)	17.3
L	**ss1650**	1.89 (pass)	2.02 (pass)	62.2
L	**ss3315**	1.90 (pass)	2.13 (pass)	46.7
L	**ss7301**	1.90 (pass)	2.53 (pass)	77.9
	ideal:	*≥1.80*	*≥2.00*	

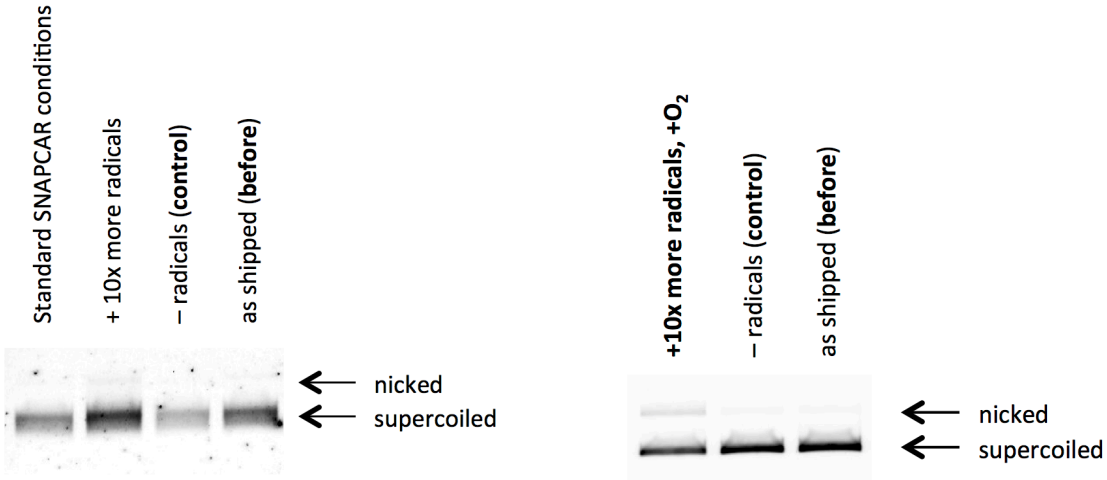

Figure S14. *Native AGE for the assessment of DNA backbone cleavage resulting from free radical polymerization. The major band of supercoiled pBR322 plasmid indicates intact DNA backbones. The distinct band of nicked plasmid is used to quantify backbone lesions.*

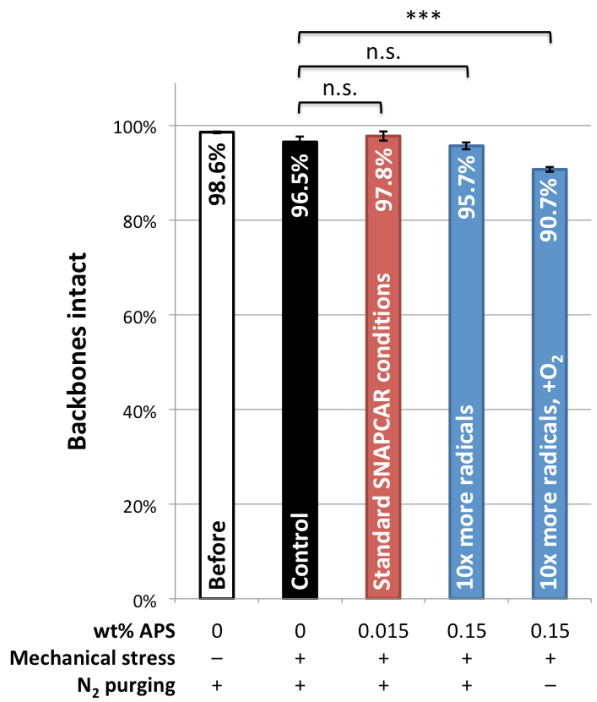

Figure S15. *Cleavage of DNA backbones during radical polymerization. White: pBR322 plasmid as received from supplier; Black: Control sample of pBR322 after the mechanical stress of sample handling (i.e. shaking, precipitation, centrifugation), but without radical initiator APS; Red: pBR322 after exposure to free radical polymerization under standard SNAPCAR conditions; Blue: pBR322 after free radical polymerization with 10-fold increased radical concentration in absence and presence of molecular oxygen. Error bars were determined from three to four quantifications. p-values were calculated using the one-tailed t-test. n.s.: p > 0.05; *** p = 0.00015.*

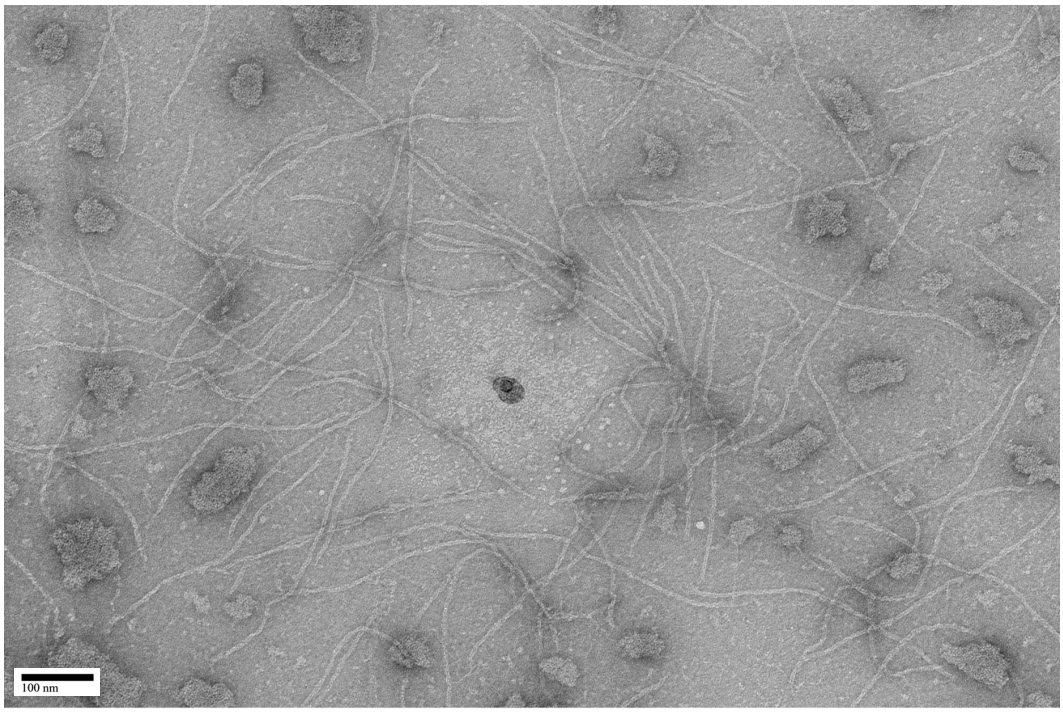

Figure S16. *TEM image of the crude **6HB** folding product.*

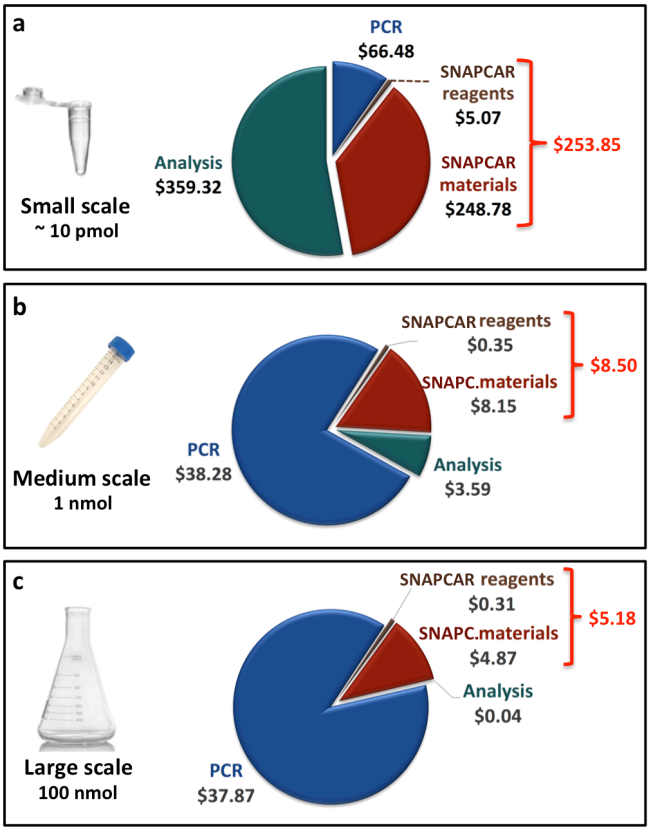

Figure S17. *Costs of materials and reagents for ssDNA production with SNAPCAR, including PCR for upstream synthesis of the dsDNA precursor. Costs per nanomole ssDNA product, assuming synthesis of 1kB ssDNA obtained at 50% overall yield. a) Small scale – 10 pmol, b) Medium scale – 1 nmol, c) Large scale – 100 nmol.*

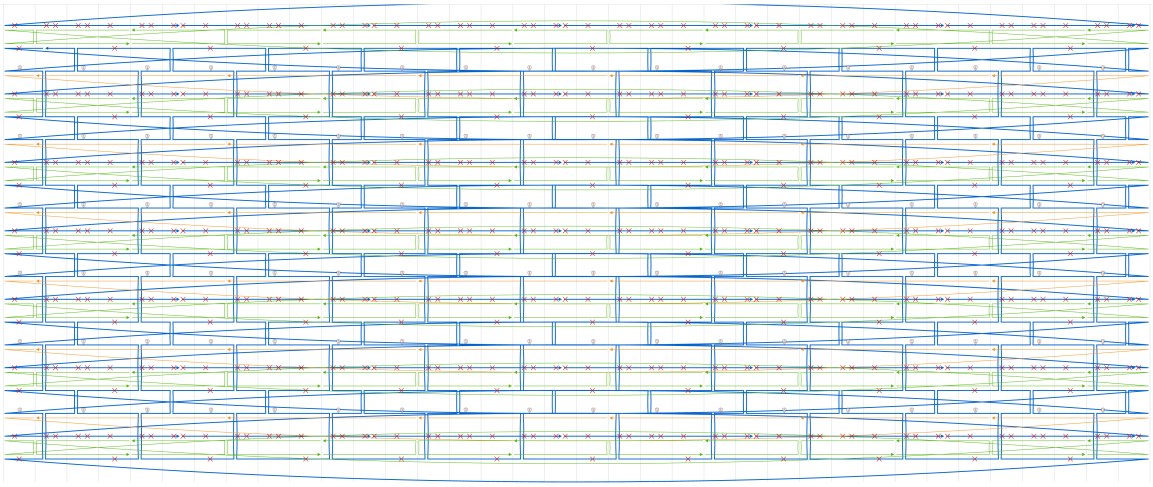

Figure S18. *DNA origami strand diagram of the Barrel.*

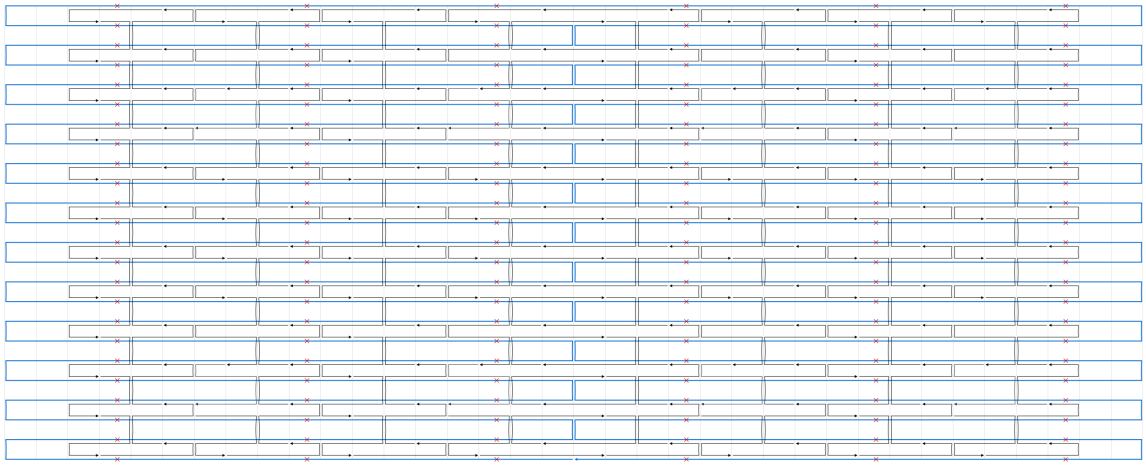

Figure S19. *DNA origami strand diagram of the Rectangle.*

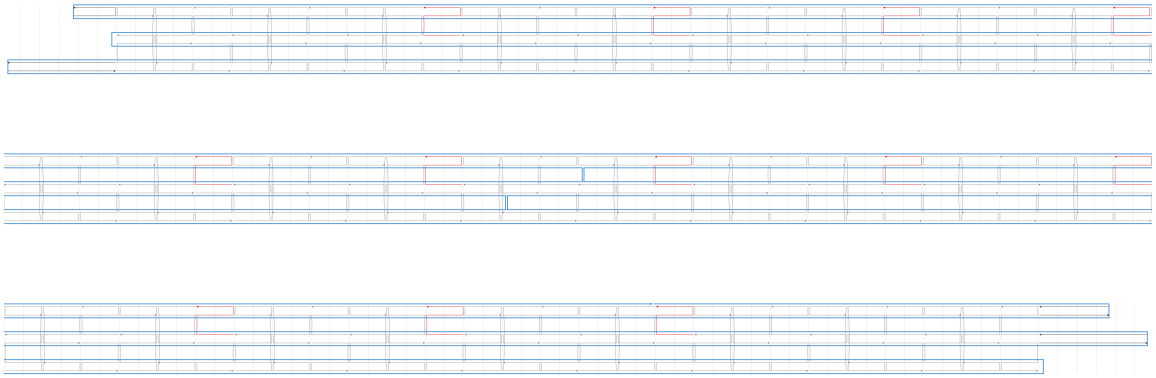

Figure S20. *DNA origami strand diagram of the 6-helix bundle.*

DNA Sequences

Primers

Table S2. *PCR Primers.*

ID	Sequence	Target	Product size
EK073	CCCTACCGAAACATCCGC	CW0	1650 bp
EK074	/5Acryd/GTCACAGCCTCAACAACAGC	CW0	1650 bp
EK086	AACAAGACAACCGCTCGTG	CW1	1650 bp
EK087	/5Acryd/CCTGAGTTCCTGTCGGAC	CW1	1650 bp
EK088	CGGGGATCCTTATACGGG	P7308	7301 bp
EK089	/5Acryd/AGCTCGAATTCGTAATCATGGTC	P7308	7301 bp
EK100	TCCGCTGAAACTGTTGAAAG	P7308	3315 bp
EK111	/5Acryd/GCAGAAGATAAAACAGAGGTGAG	P7308	3315 bp

Templates

CW0

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GTTGTTGAGGCTGTGAC

CW1

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p7308

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Staple strands

Barrel

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Rectangle

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6-Helix Bundle

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