Supplementary Information for

Hybrid enzymatic *graft-through* and *graft-from* polymerization of DNA bottlebrush polymers

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Table S1. Materials with vendors and catalog information

Material	Vendor	Catalog #
DNA oligos with standard desalting (for initiator screening) or 5'-azide and HPLC purification (for *graft-onto* SPAAC)	IDT DNA	n/a (custom)
Lambda DNA	New England Biolabs	N3011L
Taq DNA Polymerase with ThermoPol® Buffer	New England Biolabs	M0267L
Deoxynucleotide (dNTP) Solution Set	New England Biolabs	N0446S
Terminal Transferase	New England Biolabs	M0315S
Nuclease-free water	Fisher Scientific	FERR0582
5-dibenzylcyclooctyl-PEG_4-uridine-5'-triphosphate, triethylammonium salt (5-DBCO-PEG_4-dUTP)	Jena Bioscience GmbH	CLK-048-L
5-dibenzylcyclooctyl-PEG_4-deoxycytidine-5'-triphosphate, triethylammonium salt (5-DBCO-PEG_4-dCTP)	Jena Bioscience GmbH	CLK-060-L
Agarose	Fisher Scientific	BP160-500
Tris base	Fisher Scientific	BP152-1
Acetic acid, glacial	Fisher Scientific	A38-500
Ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA)	Fisher Scientific	BP120-1
40% acrylamide and bis-acrylamide, 29:1	Bio-Rad	1610146
Ammonium persulfate (APS)	Bio-Rad	1610700
N,N,N',N'–tetramethylethylenediamine (TEMED)	Bio-Rad	1610801
Boric acid	Thermo Scientific	A16624.0B
SYBR Gold Nucleic Acid Gel Stain (10,000X Concentrate in DMSO)	Invitrogen	S11494
QIAquick PCR Purification Kit for PCR Cleanup	Qiagen	28104
Vivacon® 2 Centrifugal Concentrator	Sartorius	VN02H42
Grade V4 muscovite mica discs, 12.7 mm (Electron Microscopy Sciences)	Fisher Scientific	50-949-195
Scotch Double Sided Tape, 0.5 in x 400 in	3M	7100188678
SCANASYST AIR-HR AFM tips	Bruker	SCANASYST -AIR-HR

Table S2. PCR parameters for 2.2 kbp *graft-through* products with 5-DBCO-PEG$_4$-dNTP.

Reaction composition	**PCR step**	**2.2 kbp target**
50 µL per reaction		
1X ThermoPol® reaction buffer	Initial denaturation	95 °C / 30 sec
0.25 µL *Taq* DNA Polymerase	*Denaturation	95 °C / 15 sec
200 nM UnivF* primer	*Anneal	60 °C / 30 sec
200 nM 2.2kR** primer	*Extension	68 °C / 4 min
20 ng λ-DNA	Final Extension	68 °C / 5 min
200 µM dATP 200 µM dGTP (200−X) µM dCTP† (200−X) µM dTTP X µM 5-DBCO-PEG$_4$-dCTP X µM 5-DBCO-PEG$_4$-dUTP	*cycle steps 30x	

*UnivF Sequence: 5’-CTG ATG AGT TCG TGT CCG TAC AAC TGG CGT AAT C-3’

**2.2kRSequence: 5’-CTG ACC CAG CCA CGG AAC ACA TCG-3’

†For 1:1 dNTP substitution ratio, X = 100, for 1:2 dNTP substitution ratio, X = 50.

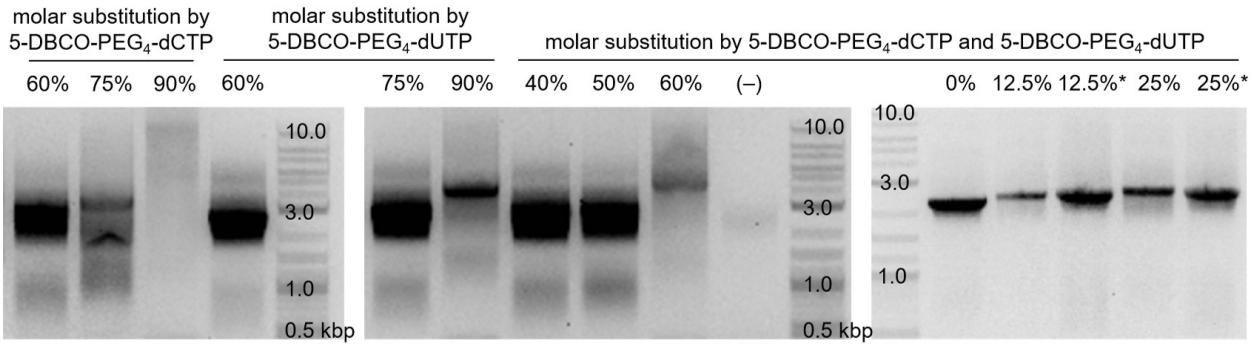

Figure S1. Tolerance of *graft-through* PCR to DBCO-modified dNTPs. 2.2 kbp targets from λ DNA are successfully amplified with up to 75% molar substitution of dCTP with 5-DBCO-PEG$_4$-dCTP, 90% molar substitution of dTTP with 5-DBCO-PEG$_4$-dUTP, or 50% molar substitution of both dCTP and dTTP with 5-DBCO-PEG$_4$-modified analogues. The lane marked with (–) is a negative control (no λ template added to the PCR mixture). Lanes marked with asterisks (12.5%* and 25%*) indicate samples purified using QIAquick PCR cleanup kits.

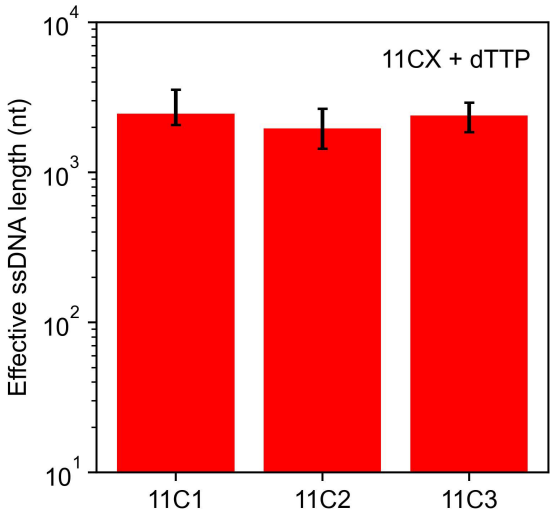

Figure S2. GC content has negligible impact on TdT-catalyzed polymerization. Initiators 11C1, 11C2, and 11C3 (45.4, 63.6, and 54.5 %GC content, respectively) yield similar dTTP addition after 30 min reactions.

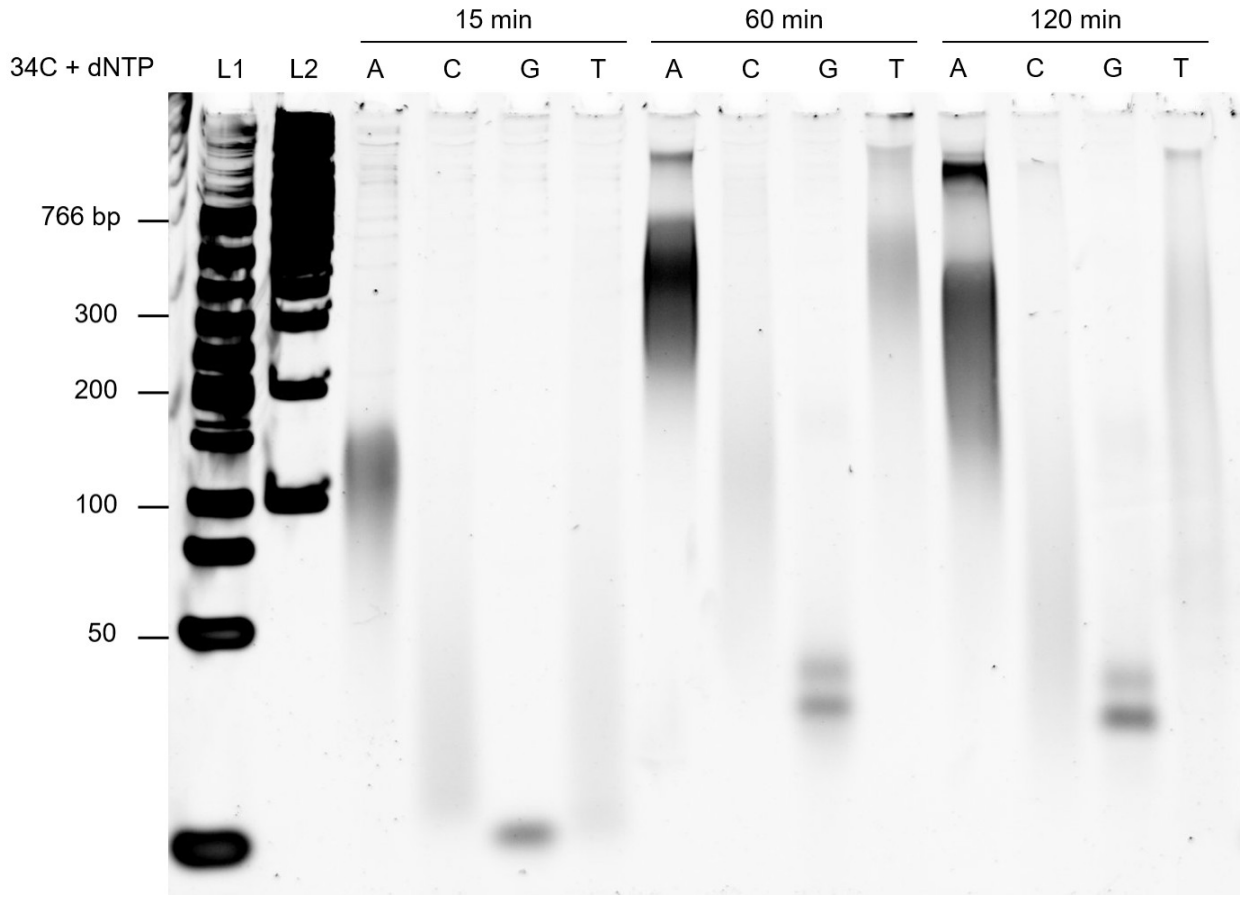

Figure S3. Polyacrylamide gel electrophoresis after TdT-catalyzed polymerization of initiator 34C with varying dNTPs and reaction times. Lane L1 contains a low molecular weight DNA ladder, and lane L2 contains a 100 bp DNA ladder.

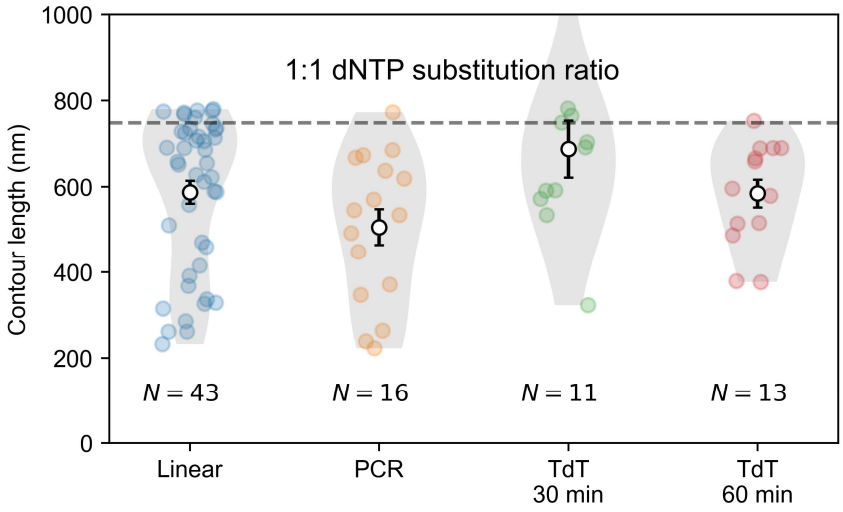

Figure S4. Contour length distributions for 1:1 dNTP substitution ratio.