Supplementary Information

Growing functional artificial cytoskeletons in the viscoelastic confinement of DNA synthetic cells

Weixiang Chen[1,2], Siyu Song[1], Avik Samanta[3], Soumya Sethi[1], Christoph Drees[1], Michael Kappl[4], Hans-Jürgen Butt[4], Andreas Walther[1,2]*

[1]Life-Like Materials and Systems, Department of Chemistry, University of Mainz, Mainz, Germany.

[2]Max Planck Institute for Polymer Research, Mainz, Germany.

[3]Department of Chemistry, Indian Institute of Technology Kharagpur, Kharagpur, West Bengal 721302, India.

[4]Department of Physics at Interfaces, Max Planck Institute for Polymer Research, Mainz, Germany.

*Corresponding author. Email: andreas.walther@uni-mainz.de

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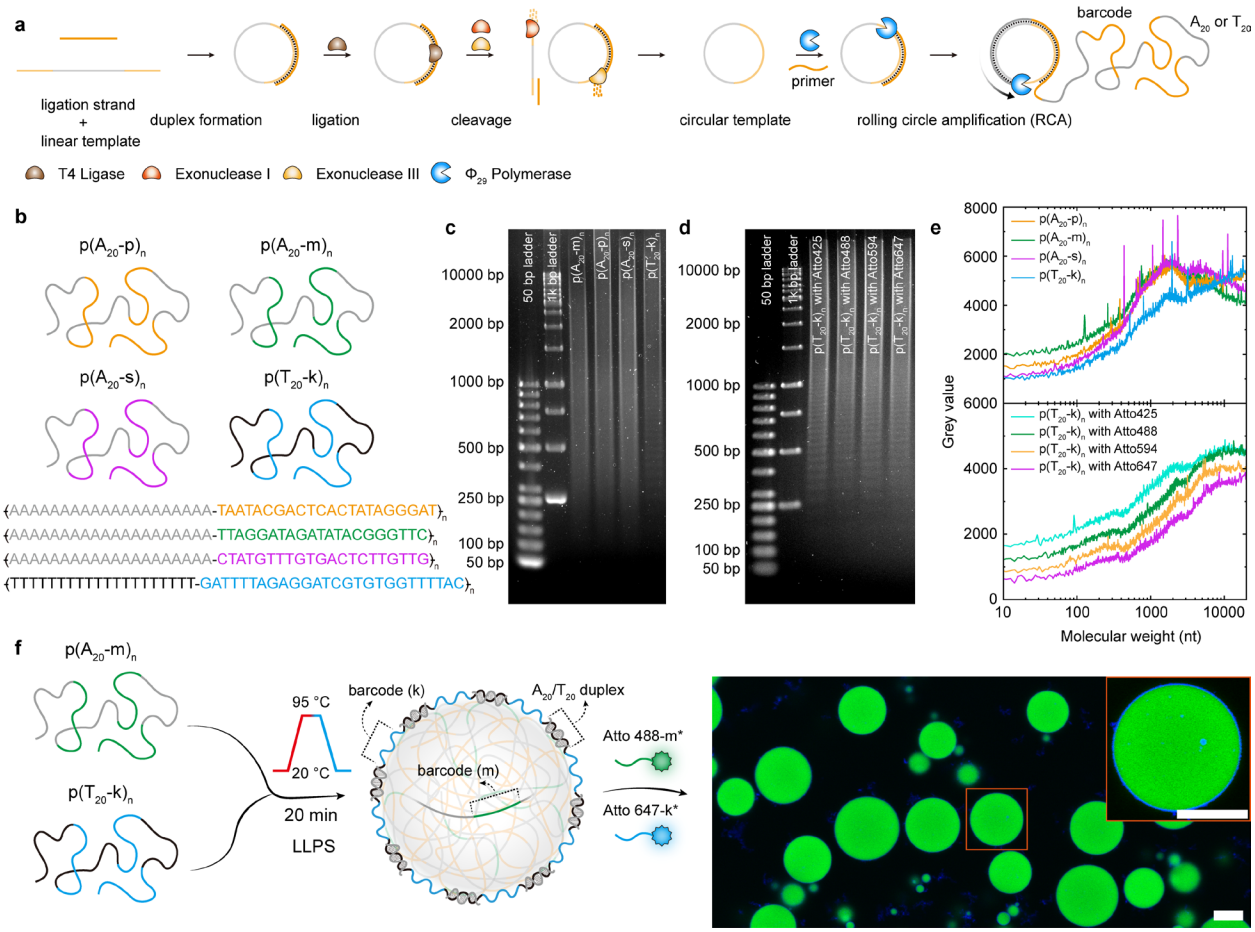
Supplementary Note 1. Estimation of total barcode concentration in SCs

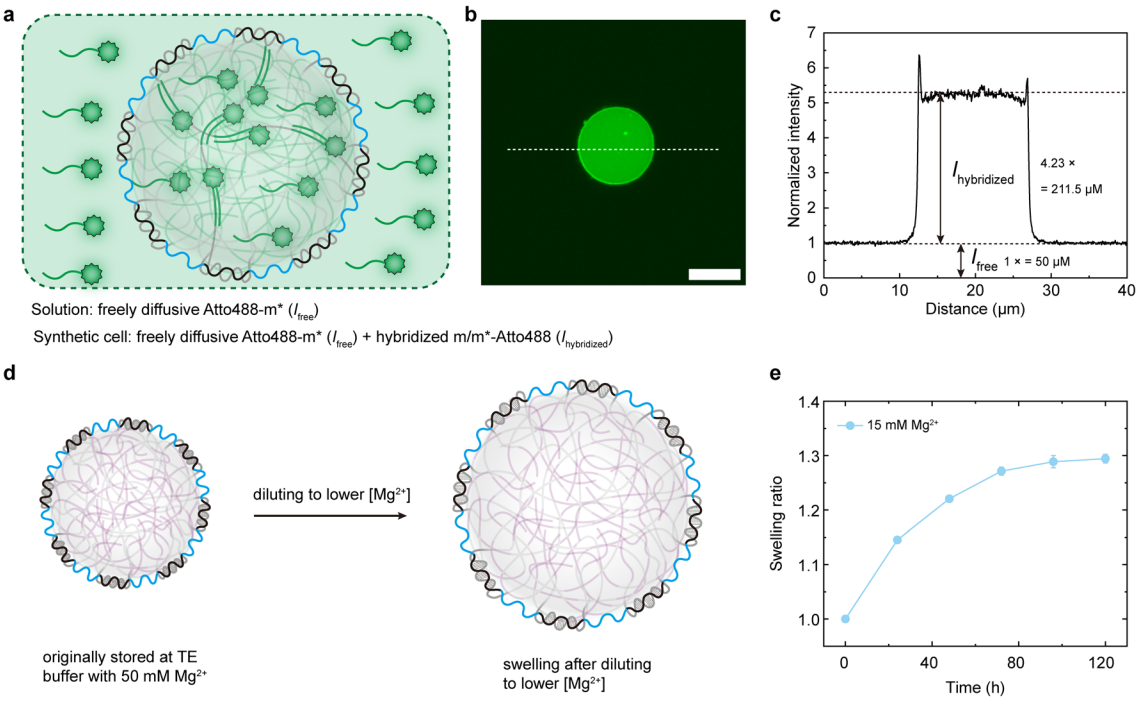
In Supplementary Fig. 2, we determined the local concentration of total barcode inside SCs to be 850 μM in TE buffer containing 50 mM Mg^{2+}. Given a roughly 2.2 times volumetric swelling after lowering the salinity from 50 mM Mg^{2+} to 15 mM Mg^{2+} (Supplementary Fig. 2e), the total barcode concentration inside the SCs is about 400 μM, which is relevant to the salinity used throughout this study and corresponds to 100% of barcode inside SCs.

The molecular weight of the repeating unit of $p(A_{20}\text{-}x)_n$ (x = p, m, or s) is in the range of 12600 – 13000 g/mol depending on the barcode. Therefore, 400 μM barcode in the SC core corresponds to a local concentration of 5.04 – 5.20 g/L.

For SCs made by mixture of adenine-rich DNA polymers having different barcodes, the concentration of a specific barcode inside the SCs scales with the fraction of this particular barcode used in the mixture. For instance, 10% of m barcode corresponds to 40 μM, 1% of m barcode corresponds to 4 μM, and 0.1% of m barcode corresponds to 400 nM, etc.

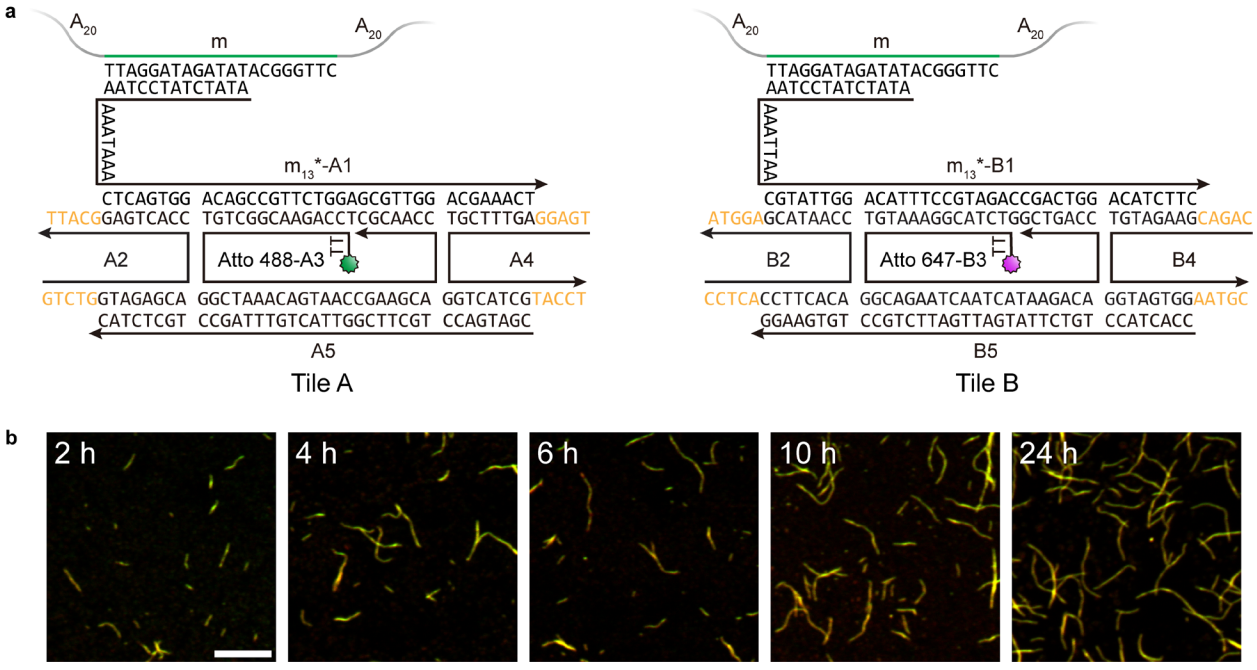


Supplementary Fig. 1: Synthesis of ssDNA multiblock copolymers by rolling circle amplification (RCA) and formation of synthetic cells by liquid-liquid phase separation. **a**, Schematic representation for the synthesis of DNA polymers including preparation of circular template and RCA. **b**, All DNA polymers synthesized and used in this work and their sequences. **c**, Representative gel electrophoresis of the generally used adenine-rich and thymine-rich DNA polymers, which are thermally heated at 95 °C for 30 min and used to make SCs. **d**, Gel electrophoresis of $p(T_{20}\text{-}k)_n$ containing different in-chain fluorophores, which are thermally heated at 95 °C for 30 min and used to make SCs. **f**, Scheme and representative CLSM image for the formation of SCs by LLPS of DNA polymers, resulting in core-shell SCs with addressable barcodes in the core and at the shell, which are labeled by Atto488-m* (green channel) and Atto647-n* (blue channel). The inset shows a zoomed-in SC corresponding to the orange box with visible shell. Scale bars are all 10 μm.



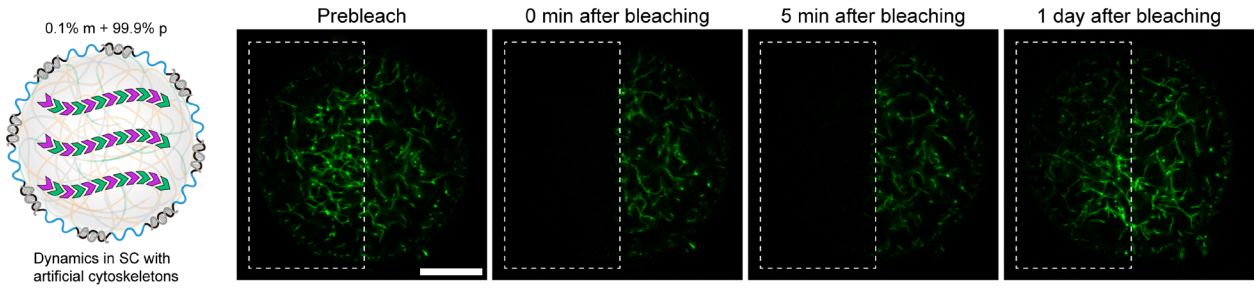
Supplementary Fig. 2: Determination of total barcode concentration in DNA SC.

a, Schematic representation of the titration experiment for determining the total barcode concentration in DNA SC. DNA SCs with 100% m barcode were mixed with high concentrations of Atto488-m* (50 μM) with a total concentration of m barcodes in the solution at 0.4 μM. Within the DNA SCs, all m barcodes are hybridized by Atto488-m*, and the SC is in a swollen state. The fluorescence intensity in the solution is only due to freely diffusing Atto488-m*, yielding I_{free}. In the SC, there are both hybridized Atto488-m* and freely diffusing Atto488-m*, giving $I_{hybridized}$ + I_{free}. The intensity ratio (I_R) inside and outside the SC is then ($I_{hybridized}$ + I_{free})/$I_{hybridized}$. **b**, Representative CLSM image of a SC in solution containing 50 μM Atto488-m*. **c**, Corresponding cross-sectional line profile along the dashed line in (b). The intensity is normalized to the solution intensity, *i.e.*, divided by I_{free}. Thus, normalized intensity = 1 means the intensity contributed from freely diffusive Atto488-m*, while the additional contributions above 1 within a DNA SC stem from the hybridized Atto488-m*, $I_{hybridized}$. As the solution contains 50 μM Atto488-m*, given the intensity ratio in and outside SC, ($I_{hybridized}$ - I_{free})/I_{free} = 4.23, the SC therefore contains 50 μM × 4.23 = 211.5 μM, reflecting the concentration of m barcode within the SCs after hybridization and swelling. Based on a roughly 4-fold swelling ratio in volume, the barcode concentration can be calculated to be ca. 850 μM in the non-hybridized pristine SCs. **d**, Schematic representation of the swelling of the SC induced by diluting the salinity (Mg^{2+}) in the solution. **e**, Swelling kinetics of the SCs after diluting to [Mg^{2+}] = 15 mM. The SCs are in equilibrium swollen state without further swelling after ca. 120 h. n = 5 for (e). Error bars represent standard deviation. The scale bar is 10 μm.



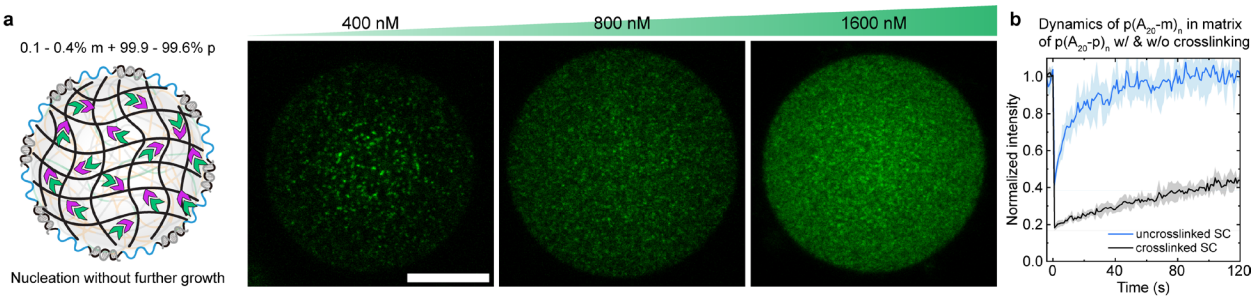
Supplementary Fig. 3: Complementary tile A and tile B form DNA nanotubes (DNTs) with low degree of branching in plain solution.

a, Design of the mutually complementary DNA tiles, tile A and tile B. Both contain a dangling strand partially complementary to a part of the m barcode of $p(A_{20}\text{-}m)_n$. Tile A contains Atto488 and tile B contains Atto647 as fluorescent labels. **b**, Time series CLSM images showing the formation of DNTs in solution over time. The scale bar is 5 µm.



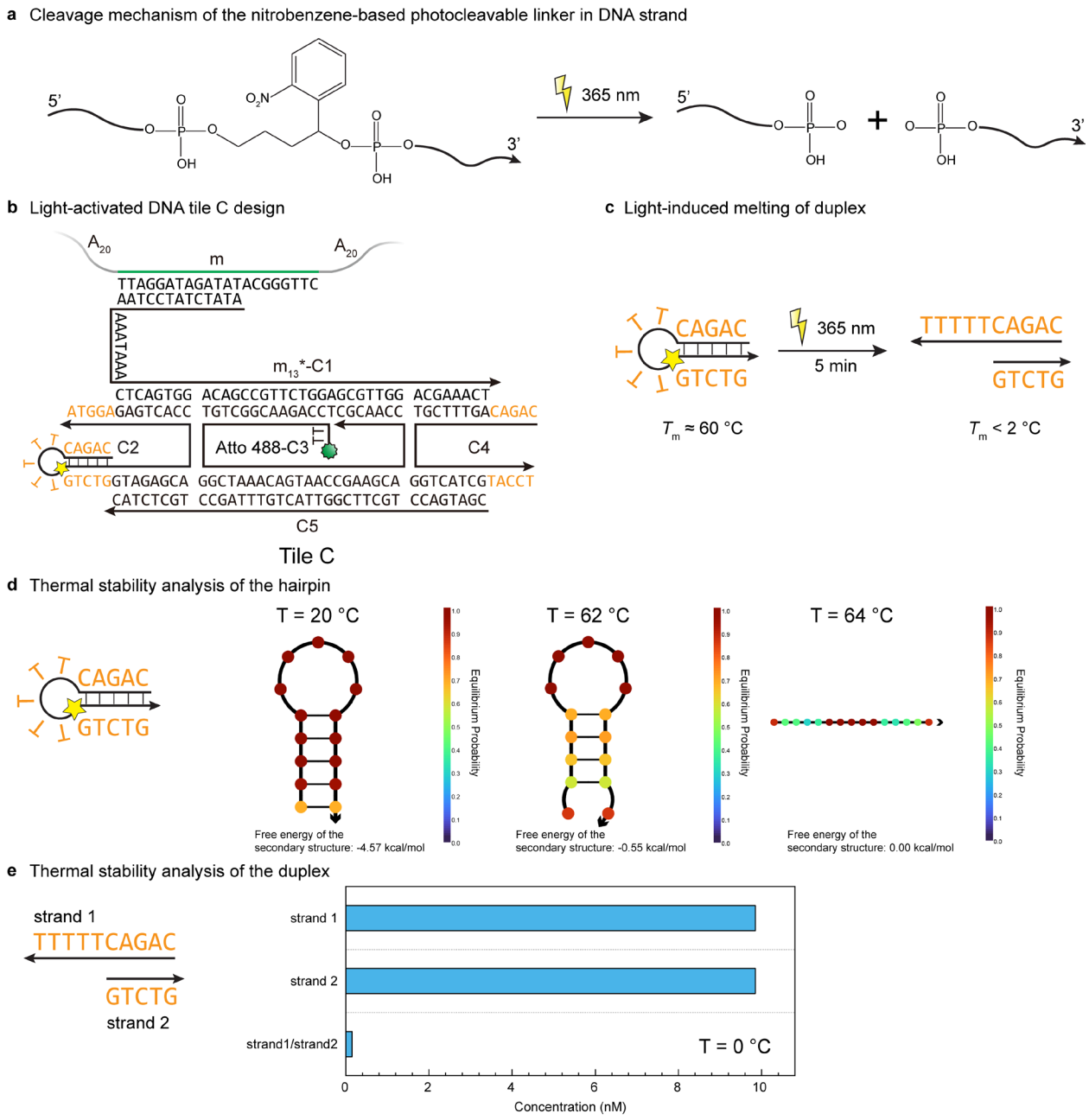
Supplementary Fig. 4: Dynamics of DNT inside SC by fluorescence recovery after photobleaching (FRAP) measurements.

FRAP on the formed artificial cytoskeletons inside a SC (white dashed box) reveals rather low dynamics, which shows no recovery in the first 5 min after bleaching and complete recovery after 1 day.

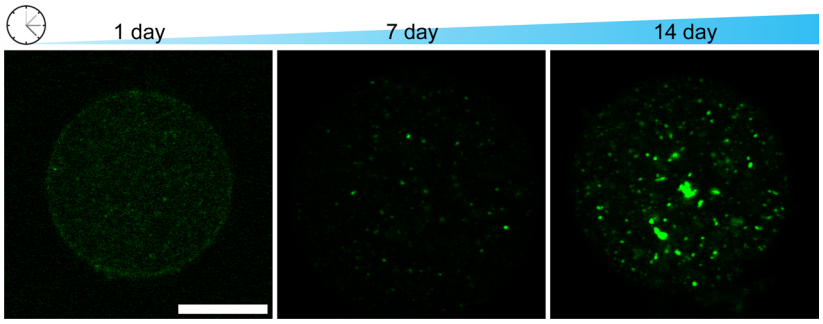


Supplementary Fig. 5: Crosslinking prevents cytoskeleton assembly.

a, Scheme and CLSM images depicting arrested growth of DNTs in fully crosslinked $p(A_{20}\text{-}p)_n$/p*-A_5-p* SCs (21 days) for different [m]. **b**, FRAP on the $p(A_{20}\text{-}m)_n$ (0.1%) chains labeled by Atto488-m* in the SCs with crosslinked and uncrosslinked $p(A_{20}\text{-}p)_n$ matrix (99.9%). The blue and black curves correspond to SCs in crosslinked and uncrosslinked states before the growth of artificial cytoskeletons. The shaded regions represent standard deviation. For all CLSM images, the DNTs have two co-localized fluorescent labels (Atto488, Atto647). Only Atto488 (green) is shown for highest resolution. $n = 4$ for (b). Error regions represent standard deviation. The scale bar is 10 μm.

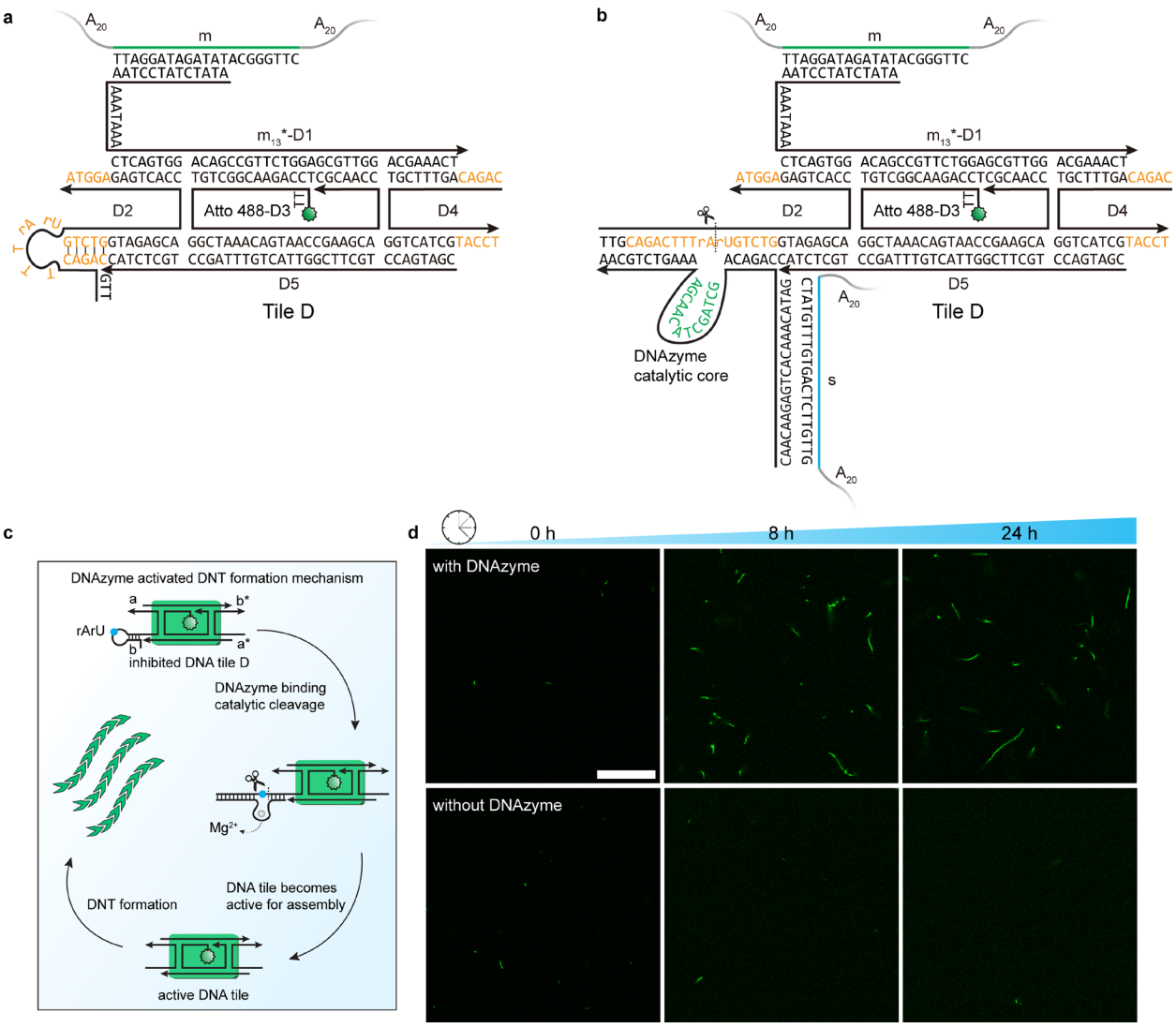


Supplementary Fig. 6: Light-activated DNT assembly using photocaged DNA tile C. **a**, Cleavage mechanism of the photocleavable linker (nitrobenzene) used in this work. **b**, Design of the light-activated DNA tile, containing Atto488 and a dangling strand complementary to a part of the m barcode of $p(A_{20}\text{-}m)_n$. **c**, Schematic representation for the cleavage-induced melting of the duplex utilizing the significant difference in their melting temperatures, T_m. **d**, NUPACK analysis of thermal stability of the hairpin. **e**, NUPACK analysis of thermal stability of the duplex after the hairpin is cleaved. NUPACK condition for both analysis: 10 nM of strand, 50 mM Na^+, 15 mM Mg^{2+}.

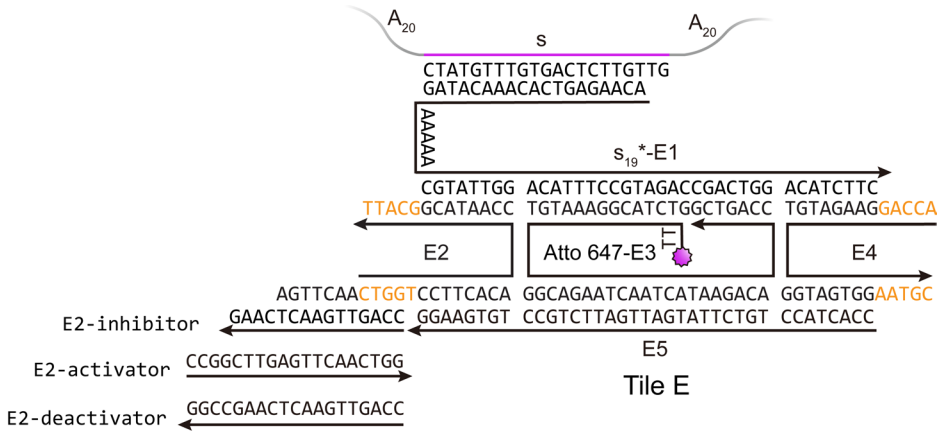


Supplementary Fig. 7: Control for light-activated artificial cytoskeleton inside SC shows absence of assembly.

Time-series CLSM images showing the absence of artificial cytoskeleton formation by tile C in a SC without UV illumination. Small aggregates appeared inside SC after 14 days, likely caused by ambient light pollution over long experimental time, which may have activated a small fraction of the initially inhibited tile C. The scale bar is 10 μm.

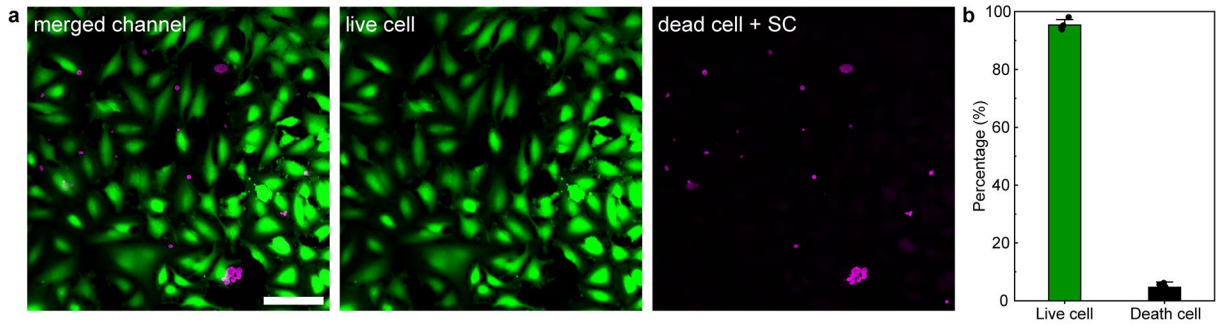


Supplementary Fig. 8: DNAzyme-catalyzed DNT assembly using DNA tile D in plain solution. **a**, Design of the DNAzyme-catalyzed DNA tile D containing a dangling strand complementary to a part of the m barcode of $p(A_{20}\text{-}m)_n$. **b**, Design of DNAzyme and how the DNAzyme can bind to the tile D on its sticky end for catalytic cleavage of the RNA substrate (rArU unit). **c**, Schematic representation for the cleavage and assembly of tile D, activated by DNAzyme. **d**, Time-series CLSM images showing the formation of DNT in solution with DNAzyme over time. The control experiment without DNAzyme shows the absence of DNT formed. The scale bar is 20 μm.



Supplementary Fig. 9: DSD-activated DNT assembly using DNA tile E.

Design of the DSD-activated DNA tile E containing a dangling strand complementary to a part of the s barcode of $p(A_{20}\text{-}s)_n$. This tile has a E2-inhibitor strand, which binds to one of the sticky ends and avoid autonomous formation of artificial cytoskeletons. The assembly of tiles can be triggered by adding E2-activator strand, which displaces the E2-inhibitor to set free the sticky end. The formed artificial cytoskeletons can be further deconstructed by adding E2-deactivator. E2-deactivator will displace the E2-activator so that the E2-inhibitor is available again in solution and will bind to the sticky ends to deconstruct the formed structures.



Supplementary Fig. 10: Cell viability after co-incubation with SC.

a, Representative CLSM images in different channels for cell viability assay of co-incubated cells and SCs. The live cells are shown in green channel, and the dead cells and SCs are shown in magenta channel. The dead cells were labeled by SYTOX™ Deep Red Nucleic Acid Stain, which also labeled the SC on the shell. **b**, Live and dead cell percentage for over 400 cell counts. $n = 4$ for (b). Co-incubation of cells and SCs was performed in a 96 well plate at 25 °C with 5 % CO_2 for 3 h. The co-incubation condition used here is the same as the experiments for SC-mammalian cell contact. Error bars represent standard deviation. The scale bar is 100 μm.

	SC composition	Starting materials	[m] or [s] in SC	[m] or [s] in solution (100× diluted)[1]	[DNA tile] in solution	Stoichiometry ([DNA tile] / [m] in solution)
Fig. 1	10% m 90% p	0.05 g/L $p(A_{20}\text{-}m)_n$ 0.45 g/L $p(A_{20}\text{-}p)_n$	40000 nM [m]	40 nM [m]	10 nM tile A 10 nM tile B	0.5
	5% m 95% p	0.025 g/L $p(A_{20}\text{-}m)_n$ 0.475 g/L $p(A_{20}\text{-}p)_n$	20000 nM [m]	20 nM [m]	10 nM tile A 10 nM tile B	1
	1% m 99% p	0.005 g/L $p(A_{20}\text{-}m)_n$ 0.495 g/L $p(A_{20}\text{-}p)_n$	4000 nM [m]	4 nM [m]	10 nM tile A 10 nM tile B	5
	0.5% m 99.5% p	0.0025 g/L $p(A_{20}\text{-}m)_n$ 0.4975 g/L $p(A_{20}\text{-}p)_n$	2000 nM [m]	2 nM [m]	10 nM tile A 10 nM tile B	10
	0.4% m 99.6% p	0.002 g/L $p(A_{20}\text{-}m)_n$ 0.498 g/L $p(A_{20}\text{-}p)_n$	1600 nM [m]	1.6 nM [m]	10 nM tile A 10 nM tile B	12.5
	0.2% m 99.8% p	0.001 g/L $p(A_{20}\text{-}m)_n$ 0.499 g/L $p(A_{20}\text{-}p)_n$	800 nM [m]	0.8 nM [m]	10 nM tile A 10 nM tile B	25
	0.1% m 99.9% p	0.0005 g/L $p(A_{20}\text{-}m)_n$ 0.4995 g/L $p(A_{20}\text{-}p)_n$	400 nM [m]	0.4 nM [m]	10 nM tile A 10 nM tile B	50
	0.05% m 99.95% p	0.00025 g/L $p(A_{20}\text{-}m)_n$ 0.49975 g/L $p(A_{20}\text{-}p)_n$	200 nM [m]	0.2 nM [m]	10 nM tile A 10 nM tile B	100
	0.025% m 99.975% p	0.000125 g/L $p(A_{20}\text{-}m)_n$ 0.499875 g/L $p(A_{20}\text{-}p)_n$	100 nM [m]	0.1 nM [m]	10 nM tile A 10 nM tile B	200
Fig. 2	0.1% m 99.9% p	0.0005 g/L $p(A_{20}\text{-}m)_n$ 0.4995 g/L $p(A_{20}\text{-}p)_n$	400 nM [m]	0.4 nM [m]	10 nM tile C	25
	0.1% m 10% s 89.9% p	0.0005 g/L $p(A_{20}\text{-}m)_n$ 0.05 g/L $p(A_{20}\text{-}s)_n$ 0.4495 g/L $p(A_{20}\text{-}p)_n$	400 nM [m] 40000 nM [s]	0.4 nM [m] 40 nM [s]	10 nM tile D	25
Fig. 3	0.1% m 0.1% s 99.9% p	0.0005 g/L $p(A_{20}\text{-}m)_n$ 0.0005 g/L $p(A_{20}\text{-}s)_n$ 0.4995 g/L $p(A_{20}\text{-}p)_n$	400 nM [m] 400 nM [s]	0.4 nM [m] 0.4 nM [s]	10 nM tile C 10 nM tile E	25
Fig. 4[2]	100% p	0.5 g/L $p(A_{20}\text{-}p)_n$	0 nM	0 nM	10 nM tile C 10 nM tile E	12.5
	0.1% m 99.9% p	0.0005 g/L $p(A_{20}\text{-}m)_n$ 0.4995 g/L $p(A_{20}\text{-}p)_n$	400 nM [m]	0.4 nM [m]		
	0.1% s 99.9% p	0.0005 g/L $p(A_{20}\text{-}s)_n$ 0.4995 g/L $p(A_{20}\text{-}p)_n$	400 nM [s]	0.4 nM [s]		
	0.1% m 0.1% s 99.9% p	0.0005 g/L $p(A_{20}\text{-}m)_n$ 0.0005 g/L $p(A_{20}\text{-}s)_n$ 0.4995 g/L $p(A_{20}\text{-}p)_n$	400 nM [m] 4000 nM [s]	0.4 nM [m] 0.4 nM [s]		
Fig. 5	0.15% m 99.85% p	0.00075 g/L $p(A_{20}\text{-}m)_n$ 0.49925 g/L $p(A_{20}\text{-}p)_n$	600 nM [m]	0.6 nM [m]	10 nM tile A 10 nM tile B	33.33

[1] SCs were always used in 100× dilution with respect to the starting materials.

[2] For experiment shown in Fig. 4 in main text, 100× diluted SCs of each type are mixed together, resulting in 0.8 nM m barcode and 0.8 nM s barcode in the solution, with 10 nM tile C and 10 nM tile D added to grow two distinct artificial cytoskeletons.

Supplementary Table 1. Details about composition of SCs with varied internal barcode concentrations, barcode concentration in solution, DNA tile concentration in solution, and stoichiometry between DNA tile and barcode in individual experiments.

	Name	Sequence (5' → 3')	Purification	Modification	Supplier
Template and RCA	Tp(A_{20}-p)	/Phosphate/ATA GTG AGT CGT ATT ATT TTT TTT TTT TTT TTT TTT ATC CCT	HPLC	5'-Phosphorylation	Biomers
	Tp(A_{20}-m)	/Phosphate/ATC TAT CCT AAT TTT TTT TTT TTT TTT TTT TGA ACC CGT AT	HPLC	5'-Phosphorylation	Biomers
	Tp(A_{20}-s)	/Phosphate/CAC AAA CAT AGT TTT TTT TTT TTT TTT TTT TCA ACA AGA GT	HPLC	5'-Phosphorylation	Biomers
	Tp(T_{20}-k)	/Phosphate/ATC CTC TAA AAT CAA AAA AAA AAA AAA AAA AAA GTA AAA CCA CAC G	HPLC	5'-Phosphorylation	Biomers
	ligation-p	TAA TAC GAC TCA CTA TAG GGA T	HPLC	None	Biomers
	ligation-m	TTA GGA TAG ATA TAC GGG TTC	HPLC	None	Biomers
	ligation-s	CTA TGT TTG TGA CTC TTG TTG	HPLC	None	Biomers
	ligation-k	TTT TAG AGG ATC GTG TGG TTT T	HPLC	None	Biomers
	primer-p	TAA TAC GAC TCA CTA TAG GG*A*T	Desalting	Phosphorothioated twice (*)	IDT
	primer-m	TTA GGA TAG ATA TAC GGG T*T*C	Desalting	Phosphorothioated twice (*)	IDT
	primer-s	CTA TGT TTG TGA CTC TTG T*T*G	Desalting	Phosphorothioated twice (*)	IDT
	primer-k	TTT TAG AGG ATC GTG TGG TT*T*T	Desalting	Phosphorothioated twice (*)	IDT
Label, Crosslinker, and RGD	Atto488-m*	/ATTO488/TGA ACC CGT ATA TCT ATC CTA A	HPLC	5'-Atto488	Biomers
	Atto647-s*	/ATTO647N/CAA CAA GAG TCA CAA ACA TAG	HPLC	5'-Atto647N	Biomers
	Atto565-p*	/ATTO565/ATC CCT ATA GTG AGT CGT ATT A	HPLC	5'-Atto565	Biomers
	Atto647-k*	/ATTO647N/AAA ACC ACA CGA TCC TCT AAA A	HPLC	5'-Atto647N	Biomers
	Atto425-k*-r	/ATTO425/AAA ACC ACA CGA TCC TCT AAA ACC AAT CCA ATC ACA GCA CGG AGG CAC GAC ACA AAA AAA AA	HPLC	5'-Atto425	Biomers
	p*-A5-p*	ATC CCT ATA GTG AGT CGT ATT A AAAAA ATC CCT ATA GTG AGT CGT ATT A	HPLC	None	Biomers
	DBCO-r*	/DBCO/TTT TTT TTT GTG TCG TGC CTC CGT GCT GTG	HPLC	5'-DBCO	Biomers

Supplementary Table 2. Oligomers for SCs and labels, with their names, sequences, purification methods, modifications, and suppliers.

	Name	Sequence (5' → 3')	Purification	Modification	Supplier
Tile A	m_{13}*-A1	ATA TCT ATC CTA AAA ATA AAC TCA GTG GAC AGC CGT TCT GGA GCG TTG GAC GAA ACT	HPLC	None	Biomers
	A2	GTC TGG TAG AGC ACC ACT GAG GCA TT	HPLC	None	Biomers
	A3	/ATTO488/TTC CAG AAC GGC TGT GGC TAA ACA GTA ACC GAA GCA CCA ACG CT	HPLC	5'-Atto488	Biomers
	A4	TGA GGA GTT TCG TGG TCA TCG TAC CT	HPLC	None	Biomers
	A5	CGA TGA CCT GCT TCG GTT ACT GTT TAG CCT GCT CTA C	HPLC	None	Biomers
Tile B	m_{13}*-B1	ATA TCT ATC CTA AAA ATT AAC GTA TTG GAC ATT TCC GTA GAC CGA CTG GAC ATC TTC	HPLC	None	Biomers
	B2	CCT CAC CTT CAC ACC AAT ACG AGG TA	HPLC	None	Biomers
	B3	/ATTO647N/TTT CTA CGG AAA TGT GGC AGA ATC AAT CAT AAG ACA CCA GTC GG	HPLC	5'-Atto647N	Biomers
	B4	CAG ACG AAG ATG TGG TAG TGG AAT GC	HPLC	None	Biomers
	B5	CCA CTA CCT GTC TTA TGA TTG ATT CTG CCT GTG AAG G	HPLC	None	Biomers
Tile C (Light control)	m_{13}*-C1 (same as m_{13}*-A1)	ATA TCT ATC CTA AAA ATA AAC TCA GTG GAC AGC CGT TCT GGA GCG TTG GAC GAA ACT	HPLC	None	Biomers
	C2-PC Linker	CAG ACT TTT T/PC Linker BMN/G TCT GGT AGA GCA CCA CTG AGA GGT A	HPLC	Photocleavable Linker	Biomers
	C3 (same as A3)	/ATTO488/TTC CAG AAC GGC TGT GGC TAA ACA GTA ACC GAA GCA CCA ACG CT	HPLC	5'-Atto488	Biomers
	C4	CAG ACA GTT TCG TGG TCA TCG TAC CT	HPLC	None	Biomers
	C5 (same as A5)	CGA TGA CCT GCT TCG GTT ACT GTT TAG CCT GCT CTA C	HPLC	None	Biomers
Tile D (DNAzyme control)	m_{13}*-D1 (same as m_{13}*-A1)	ATA TCT ATC CTA AAA ATA AAC TCA GTG GAC AGC CGT TCT GGA GCG TTG GAC GAA ACT	HPLC	None	Biomers
	D2-substrate	TTG CAG ACT TT**rA rU**GT CTG GTA GAG CAC CAC TGA GAG GTA	HPLC	rA, rU	Biomers
	D3 (same as A3)	/ATTO488/TTC CAG AAC GGC TGT GGC TAA ACA GTA ACC GAA GCA CCA ACG CT	HPLC	5'-Atto488	Biomers
	D4 (same as C4)	CAG ACA GTT TCG TGG TCA TCG TAC CT	HPLC	None	Biomers
	D5 (same as A5)	CGA TGA CCT GCT TCG GTT ACT GTT TAG CCT GCT CTA C	HPLC	None	Biomers
	s*-DNAzyme	CAA CAA GAG TCA CAA ACA TAG CAG ACA GGC TAG CTA CAA CGA AAA GTC TGC AA	HPLC	None	Biomers
Tile E (DSD)	s_{19}*-E1	ACA AGA GTC ACA AAC ATA GAA AAA CGT ATT GGA CAT TTC CGT AGA CCG ACT GGA CAT CTT C	HPLC	None	Biomers
	E2	AGT TCA ACT GGT CCT TCA CAC CAA TAC GGC ATT	HPLC	None	Biomers
	E3 (same as B3)	/ATTO647N/TTT CTA CGG AAA TGT GGC AGA ATC AAT CAT AAG ACA CCA GTC GG	HPLC	5'-Atto647N	Biomers

	E4	ACC AGG AAG ATG TGG TAG TGG AAT GC	HPLC	None	Biomers
	E5 (same as B5)	CCA CTA CCT GTC TTA TGA TTG ATT CTG CCT GTG AAG G	HPLC	None	Biomers
	E2-inhibitor	CCA GTT GAA CTC AAG	HPLC	None	Biomers
	E2-activator	CCG GCT TGA GTT CAA CTG G	HPLC	None	Biomers
	E2-deactivator	CCA GTT GAA CTC AAG CCG G	HPLC	None	Biomers

Supplementary Table 3. Oligomers for all DNA tiles, with their names, sequences, purification methods, modifications, and suppliers.