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Supporting Information

Multiple Light Control Mechanisms in ATP-Fueled Non-equilibrium DNA Systems

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Contents

1. Materials and methods

Instrumentation: ThermoMixer (Eppendorf), UVsolo *touch* Gel electrophoresis documentation system (Analytik Jena), INTAS *CHEMOSTAR touch* fluorescence imager (INTAS Science Imaging), gel electrophoresis chambers (biostep), power source 250 V (VWR), Image J, ScanDropR UV-VIS spectrometer (Analytik Jena). Mightex LED (λ = 365 nm, and λ = 420 nm, 270 mW output for both light sources). Spark® Multimode Microplate Reader (Tecan).

Reagents: All chemicals were purchased from Sigma-Aldrich unless otherwise stated. ATP determination kit and 3-O-(1-(4,5-dimethoxy-2- nitrophenyl)ethyl) adenosine-5'-triphosphate (DMNPE-ATP) were purchased from ThermoFisher Scientific. T4 DNA ligase (HC, 20 units/µL) and BamHI (HC, 80 units/µL) were supplied by Promega. ATP solution (10 mM in 1 mM Tris-HCl pH 7.5) was purchased from Invitrogen. Agarose low EEO was supplied by PanReac AppliChem. Gene Ruler 1k bp and 50 bp DNA ladders (ready to use) were ordered from Thermo Scientific. Fluorescent log scale 1k bp and 50 bp DNA ladders were ordered from Jena Bioscience. Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, biology grade) was supplied by CALBIOCHEM. Sodium chloride (NaCl, 99%) was ordered from ABCR. Tris (hydroxymethyl)aminomethane hydrochloride pH 8.0 (Tris-HCl) and trizma base were ordered from Sigma-Aldrich. Acetic acid glacial (ACS, Reag. Ph. Eur. Analytical reagents) was supplied by VWR Chemicals. RotiR-GelStain (1,1'3,3',5,5'6,6'-Octamethyl-2,2'-spiro(2,3-dihydro-1H-Benzimidazol) was supplied by Carl Roth. Sterile Milli-Q water with conductivity less than 0.055 mS/cm was used throughout all DNA experiments.

Conditions: All the experiments for DNA ligation and transient polymerization were performed at 25 °C with 300 rpm shaking. The scale for all the DNA experiments was ranging from 20 to 100 µL. Photouncaging was carried out at 25 °C without shaking.

Buffer compositions

T4 DNA Ligase Storage Buffer (Promega): 10 mM Tris-HCl (pH 7.4 at 25 °C), 50 mM KCl, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, 50 % glycerol.

BamHI Storage Buffer (Promega): 10 mM Tris-HCl (pH 7.4), 300 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.5 mg/mL BSA, 50% glycerol.

Restriction Enzyme 10x Buffer E (Promega): 60 mM Tris-HCl (pH 7.5), 1 M NaCl, 60 mM $MgCl_2$, 10 mM DTT.

Annealing Buffer: 10 mM Tris-HCl (pH 8.0), 50 mM NaCl.

TAE Buffer: 40 mM Tris, 20 mM acetic acid, 1 mM EDTA.

Quenching Buffer: 200 mM EDTA, 10 mM Tris-HCl (pH 8.0), 50 mM NaCl.

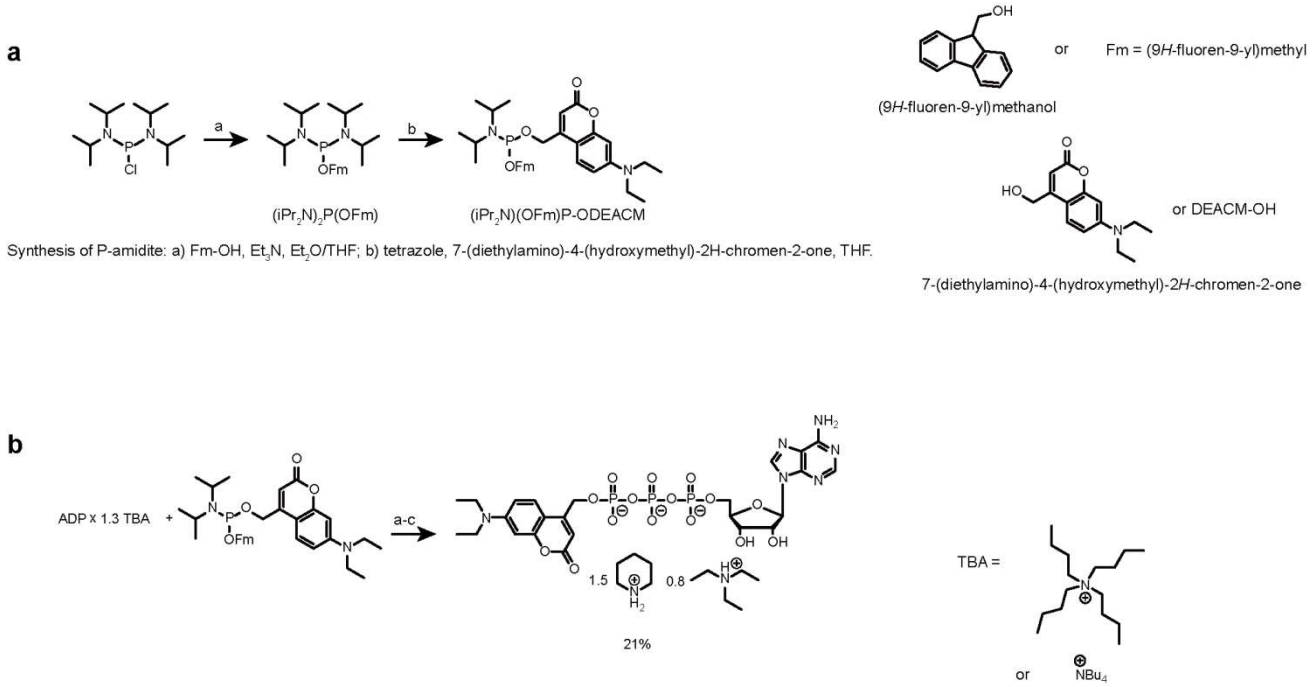
Oligonucleotides: All oligonucleotides were supplied by Integrated DNA Technologies Inc. with HPLC purification.

Supplementary Table 1: Oligonucleotide sequences used, with their name in individual Figure, the sequence codes used for ordering at IDT. Sequences marked in red indicate the sticky ends for individual tiles.

	ID	Oligonucleotide sequence	#nt	Purification
M1	D_1	5'-/5Phos/GATCCCTTACGGTGAATGGAATTCTCATGCGAATAGAG-3'	38	HPLC
	D_2	5'-/5Phos/GATCCTCTATTCGCATGAGAATTCCATTCACCGTAAGG-3'	38	HPLC
M2	D_3	5'-/5SpPC/ATCCTCTATTCGCATGAGAATTCCATTCACCGTAAGGG-3'	38	HPLC
	D_4	5'-/5SpPC/GATCCCTTACGGTGAATGGAATTCTCATGCGAATAGAG-3'	38	HPLC
M3	D_1	5'-/5Phos/GATCCCTTACGGTGAATGGAATTCTCATGCGAATAGAG-3'	38	HPLC
	D_3	5'-/5SpPC/ATCCTCTATTCGCATGAGAATTCCATTCACCGTAAGGG-3'	38	HPLC
M4	D_5	5'-CCTTACGGTGAATGGAATTCTCATGCGAATAGAG-3'	34	HPLC
	D_2	5'-/5Phos/GATCCTCTATTCGCATGAGAATTCCATTCACCGTAAGG-3'	38	HPLC
M5	D_6	5'-GTAGGTACAGGTCGTCATAACTGGTCTCG-3'	29	HPLC
	D_7	5'-CGAGACCAGTTATGTAGCTATGATACTCTAGTCAG-3'	35	HPLC
	D_8	5'-/5Phos/GATCCTGACTAGAGTATCACGACCTGTACCTACTTT-ATTO488-3'	36	HPLC
M6	D_6	5'-GTAGGTACAGGTCGTCATAACTGGTCTCG-3'	29	HPLC
	D_9	5'-/5Phos/GATCCGAGACCAGTTATGTAGCTATGATACTCTAGTCAG-3'	39	HPLC
	D_8	5'-/5Phos/GATCCTGACTAGAGTATCACGACCTGTACCTACTTT-ATTO488-3'	36	HPLC
M7	D_3	5'-/5SpPC/ATCCTCTATTCGCATGAGAATTCCATTCACCGTAAGGG-3'	38	HPLC
	D_{10}	/5Phos/AGGATCCCTTACGGTGAATGGAA/iCy5/TTCTCATGCGAATAG	38	HPLC

2. Experimental protocols

Synthesis of P3-[7-(diethylamino)coumarin-4-yl]methyl adenosine 5'-triphosphate (DEACM-ATP). The synthesis of (iPr$_2$N)$_2$P(OFm) and (iPr$_2$N)(FmO)P-ODEACM were the same as our previous report (Scheme S1a).[1] Commercially available adenosine 5'-diphosphate (ADP) sodium salt was first converted into its tetrabutylamonium salt (TBA) by ion exchange on *Dowex® 50WX8* (H^+ form) followed by neutralization with TBA-OH and subsequent lyophilization. The accurate amount of TBA counterions was determined by ^{1}H-NMR to be 1.3. Afterwards, ADP × 1.3 TBA salt (74.0 mg, 100 µmol, 1.0 eq.) was coevaporated with dry MeCN (2 × 1.0 mL) and dissolved in dry DMF (1.0 mL). (iPr$_2$N)(FmO)P-ODEACM (103 mg, 180 µmol, 1.8 eq.) and 5-(ethylthio)-1*H*-tetrazole (0.77 M in DMF, 260 µL, 200 µmol, 2.0 eq.) were added. After stirring for 30 min the mixture was cooled to 0 °C and 3-chloroperbenzoic acid (77 %, 33.0 mg, 150 µmol, 1.5 eq.) was added slowly. After stirring for another 10 minutes the solution was transferred to a centrifugation tube and Et$_2$O (40 mL) was added to precipitate the intermediate. The suspension was then centrifuged and the precipitate was removed and dissolved in DMSO (2.0 mL). Piperidine (0.1 mL) was added and the mixture was stirred for 15 minutes. Then Et$_2$O (40 mL) was added, and the suspension was centrifuged and the obtained precipitate was washed with Et$_2$O (5.0 mL). The crude product was purified by preparative HPLC (water/MeCN gradient) to obtain the desired DEACM-caged ATP (1.5 eq. of $C_5H_{12}N^+$, 0.8 eq. of $C_6H_{16}N^+$, 19.7 mg, 20.8 µmol, 21 %) as yellow fluffy solid (Scheme S1b).



Scheme S1. Synthesis of DEACM-ATP.

Photolysis of caged ATPs. DMNPE-ATP and DEACM-ATP were first dissolved in Tris-HCl buffer (10 mM, pH 7.2) to make stock solutions with a concentration of 10 mM and stored at -25 °C. Afterwards, the stock solutions were diluted to desired concentrations in Milli-Q water for kinetic studies. The diluted solutions were aliquoted, 10 µL for each, and then photolyzed for predetermined time at 365 nm and 420 nm (Mightex LED, 270 mW) for DMNPE-ATP and DEACM-ATP, respectively. The photolyzed samples were then diluted 100 fold with water to fit

to the measurement range of the ATP determination kit. The ATP determination kit was prepared according to the protocol by the supplier. Afterwards, 5 µL of the diluted sample was mixed with 95 µL of ATP detection kit, and the bioluminescence was measured by a plate reader. ATP concentrations were then calculated from a calibration curve.

DNA tiles annealing. All DNA strands were HPLC purified and were used as received, to which certain amounts of annealing buffer were added to make stock solutions (1 mM as specified by the shipped specification) and the real concentrations were calculated via UV-VIS spectroscopy. The double-stranded (ds) DNA tiles in this study were annealed from two complementary single-stranded (ss) DNA with the same stoichiometry at room temperature overnight, and the T-shaped tiles were annealed from 3 ssDNA. The annealed stock solutions with a concentration ranging from 0.3 to 0.5 mM were stored at -25 °C for further use.

Ligation by ATP released from caged ATPs. The ligation of the dsDNA tiles (M1) were performed at 25 °C in 1× *buffer E* containing 0.05 mM dsDNA tiles, 0.1 mg/mL BSA acetylated bovine serum (BSA), 0.46 WU/µL T4 DNA ligase, and 0.6 mM caged ATP (DEACM-ATP or DMNPE-ATP). Afterwards, the prepared solutions were separately photouncaged either at 365 nm (for DMNPE-ATP) or at 420 nm and 27 mW (for DEACM-ATP) for 30 s, 1 min, 2 min, and 4 min. After being ligated for 1 h and 2 h, 6 µL aliquots of the reaction solutions were quenched by 8 µL quenching buffer, and analyzed by agarose gel electrophoresis (AGE) (2 wt. %, 105 V for 70 min run time). The results were recorded by a gel imager (Analytik Jena, Germany).

Uncaging DMNPE-ATP at 420 nm for DNA ligation. The experiments were performed at 25 °C in 1× *buffer E* containing 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/µL T4 DNA ligase, and 0.6 mM DMNPE-ATP. Afterwards, the prepared solution was photouncaged at 420 nm and 27 mW for 4 min. At 10 min and 1 h, 6 µL aliquots of the reaction solution were collected and quenched with 8 µL quenching buffer. Afterwards, the samples were analyzed by AGE (see above).

Light-activated transient DNA polymerization. The experiments were performed at 25 °C in 1× *buffer E* containing 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/µL T4 DNA ligase, 10 units/µL BamHI, and 0.6 mM caged ATP (DEACM-ATP or DMNPE-ATP). Then, the prepared mixtures were separately illuminated by LED light (420 nm and 365 nm for DEACM-ATP and DMNPE-ATP, respectively) at 27 mW for 30 s, 1 min, 2 min, and 4 min. At different time intervals, 6 µL aliquots of the reaction solution for each sample were collected and quenched by 8 µL quenching buffer. Afterwards, the prepared samples were analyzed by AGE (see above). To further investigate the light-power controlled speed for chain growth, the above prepared reaction solution supplemented with DEACM-ATP was uncaged at 420 nm and 13.5 mW. After being uncaged for 30s, 1 min, 2 min, and 4 min, samples were collected and quenched at different time intervals, and then analyzed by AGE (see above).

Investigation of side effects on the enzymes by LED illumination. 0.05 mM M1, 0.1 mg/mL BSA, and 0.46 WU/µL T4 DNA ligase were dissolved in 1× *buffer E*. The obtained solutions were separately illuminated at 365 nm and 420 nm (27 mW) for 4 min. afterwards, 0.6 mM DMNPE-ATP was added to each tube and then

uncaged at 365 nm and 27 mW for 1 min. Samples at different time intervals (1 h, 2 h) were collected and quenched by quenching buffer followed by analysis with AGE (see above).

To test the side effects on both enzymes by LED illumination, 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, and 10 units/μL BamHI were dissolved in 1× *buffer E*. Then, the prepared systems were illuminated by LED light (365 nm and 420 nm, each for 4 min) at 27 mW. Afterwards, 0.6 mM DMNPE-ATP was added and uncaged for 1 min at 365 nm and 27 mW. At different time intervals, aliquots were collected and quenched by quenching buffer followed by analysis with AGE (see above).

Multiple uncaging and refueling by DEACM-ATP. 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, and 1 mM DEACM-ATP were dissolved in 1× *buffer E*. The prepared system was first fueled by uncaging at 420 nm and 27 mW for 30 s. At different time intervals, aliquots were collected and quenched. After the DNA polymers were degraded (2 days), the system was again uncaged for another 2 min at 420 nm and 27 mW. Aliquots for the second round were collected at different time intervals, quenched with quenching buffer, and finally all the samples were analyzed with AGE (see above).

Multiple activation by combination of DEACM-ATP and fresh ATP. 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, and 0.3 mM DEACM-ATP were dissolved in 1× *buffer E*. Then, the prepared system was first illuminated at 420 nm and 27 mW for 4 min. At different time intervals, aliquots of the reacted solution were collected and quenched by quenching buffer. After all the DNA polymers were degraded (4 days), another 0.54 mM fresh ATP solution was added to the system, and samples were quenched at different time intervals. Afterwards, the prepared samples were analyzed by AGE (see above).

Repeated activation of the system by DMNPE-ATP. 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, and 1 mM DMNPE-ATP were dissolved in 1× *buffer E*. For the repeated uncaging at 365 nm and 27 mW was first used for both rounds. The combinations of uncaging time include 30 s + 4 min, and 1 min + 4 min. Then the light power was decreased to 13.5 mW and the system was uncaged at 365 nm for 30 s and 2 min for uncaging round 1 and round 2, respectively. For another experiment, 13.5 mW was used for the 1st round uncaging of 30 s but 27 mW was used for the 2nd round uncaging of 2 min. Aliquots at different time intervals for all the experiments were collected, quenched, and analyzed by AGE (see above).

Multiple activation by combination of DMNPE-ATP and fresh ATP. 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, and 1 mM DMNPE-ATP were dissolved in 1× *buffer E*. Then, the prepared system was first illuminated at 365 nm and 13.5 mW for 1 min. At different time intervals, aliquots of the reacted solution were collected and quenched by quenching buffer. After all the DNA polymers were degraded (4 days), another 0.6 mM fresh ATP solution was added to the system, and samples were quenched at different time intervals. Afterwards, the prepared samples were analyzed by AGE (see above).

Wavelength-selective sequential activation. 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, and 0.3 mM DEACM-ATP, and 0.3 mM DMNPE-ATP were dissolved in 1× *buffer E*. Then, the prepared system was first illuminated at 420 nm and 27 mW for 4 min. At different time intervals, aliquots of

the reacted solution were collected, and quenched using the quenching buffer. After 4 days, the system was uncaged at 365 nm and 27 mW for another 4 min, and samples at different time intervals were collected and quenched. Afterwards, all the samples were analyzed by AGE (see above).

Kinetic study of the photouncaging of the photocaged DNA tiles

The stock solution of M2 was diluted to 1× *buffer E* with a final concentration of 0.05 mM. Afterwards, the diluted solutions were aliquoted, 10 μL for each, and then photouncaged for predetermined time at 365 nm and 135 mW. Afterwards, all the uncaged samples were analyzed via HPLC.

HPLC analysis was performed using a Dionex HPLC UltiMate 3000 Liquid Chromatography system with integrated single wavelength UV detection unit. Oligomers were separated on a DNAPac™ RP HPLC column (Thermo Fisher Scientific). The DNA tiles were analyzed using a gradient of two on-line degassed solvents (A, B). The injection volume was 100 μL with a dsDNA concentration of 0.005 mM.

Solvent A: triethylamine/acetic acid pH 7.8 at 100 mM (TEAA) with 5 % acetonitrile (MeCN)

Solvent B: MeCN

The used gradient ran from 100 % A and 0 % B to 70 % A and 30 % for 30 minutes. The flowrate was 0.2 mL/min at 40 °C and the DNA tiles were detected at 260 nm continuous flow absorption.

Ligation of the single-side and double-side caged DNA tiles

The ligation of single-side caged DNA tiles was performed at 25 °C in 1× *Buffer E* with 0.05 mM M3, 0.46 WU/μL T4 DNA ligase, 0.1 mg/mL BSA, and 1 mM ATP. 6 μL aliquots of the reaction solution before ligation and after 1 h ligation were collected and quenched with 8 μL of quenching buffer. The aliquots were then analyzed by 2 wt.% AGE at 105 V with 70 min run time. The results were recorded by a gel imager (Analytik Jena, Germany). The ligation of the double-side caged DNA tiles was performed at the same conditions as above, except M2 was used. After all the components were added to Milli-Q water and reacted for 1 h, an aliquot of the reaction solution was collected and quenched as above. Then, the photocaged dsDNA tiles were uncaged by illuminating at 365 nm and 135 mW for 15 min to activate the monomers and initiate the polymerization (Mightex LED, 270 mW). The ligated sample was collected again after 1 h of ligation. The aliquots were analyzed by AGE (see above).

Light-activated transient DNA polymerization with programmable lifetimes

The experiments were performed at 25 °C in 1× *Promega Buffer E* with 0.05 mM M2, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, 0.1 mg/mL BSA, and 0.1, 0.3, 0.6, or 0.9 mM ATP. The photocages were removed by irradiation with LED light at 365 nm and 135 mW for 15 min. At different time intervals, 6 μL aliquots of the reaction solution were collected and quenched by 8 μL of quenching buffer. Afterwards, all the quenched samples were analyzed by AGE (see above).

Light-modulation of ATP-driven multiple transient dynamic steady-states for DNA assembly

The experiments were performed at 25 °C in 1× *Promega Buffer E* containing 0.03 mM M2, 0.01 mM M1, 0.01 mM M4, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, and 0.3 or 0.6 mM ATP. At different time intervals, 6 μL aliquots of the reaction solution were collected and quenched by 8 μL of quenching buffer.

After 5 h, the photocaged DNA building blocks in the system were further uncaged by illuminating at 365 nm and 135 mW for 15 min (Mightex LED, 270 mW) to activate M2. Then, 6 μL aliquots of the reaction solution for the second round were collected again and quenched by 8 μL of quenching buffer. Afterwards, all the collected samples were analyzed by AGE (see above).

Self-sorting in ATP-driven multicomponent self-assembling systems

The experiments were performed at 25 °C in 1× *Promega Buffer E* containing 0.02 mM M7, 0.01 mM M5, 0.005 mM M6, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 15 units/μL BamHI, and 0.6 mM ATP. At different time intervals, 6 μL aliquots of the reaction solution were collected and quenched by 8 μL of quenching buffer. Afterwards, all the collected samples were analyzed by 2 wt.% AGE at 80 V for 2.5 h run time. The results were recorded by a fluorescence imager (INTAS Science Imaging, Germany).

Light-modulation of multicomponent non-equilibrium self-sorting systems

The experiments were performed at 25 °C in 1× *Promega Buffer E* containing 0.02 mM M7, 0.01 mM M5, 0.005 mM M6, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 15 units/μL BamHI, and 0.6 mM ATP. At different time intervals, 6 μL aliquots of the reaction solution were collected and quenched by 8 μL of quenching buffer. After 5 h, the system was illuminated at 365 nm and 135 mW for 15 min (Mightex LED, 270 mW). Then, 6 μL aliquots of the reaction solution at different time intervals were collected again and quenched by 8 μL of quenching buffer. Afterwards, all the collected samples were analyzed by AGE (see above).

Wavelength-orthogonal control of multiple dynamic steady states

The experiments were performed at 25 °C in 1× *Promega Buffer E* containing 0.02 mM M7, 0.01 mM M5, 0.005 mM M6, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 15 units/μL BamHI, and 0.6 mM DEACM-ATP. The system was first activated by illuminating at 420 nm and 27 mW for 4 min (Mightex LED, 270 mW). At different time intervals, 6 μL aliquots of the reaction solution were collected and quenched by 8 μL of quenching buffer. After 5 h, the system was illuminated at 365 nm and 135 mW for 15 min (Mightex LED, 270 mW). Then, 6 μL aliquots of the reaction solution at different time intervals were collected again and quenched by 8 μL of quenching buffer. Afterwards, all the collected samples were analyzed by AGE (see above).

3. Supplementary Note 1. Characterization of DEACM-ATP

^{1}H-NMR (400 MHz, D_2O) δ = 8.16 (s, 1H), 7.95 (s, 1H), 7.02 (d, *J* = 9.0 Hz, 1H), 6.39 (d, *J* = 9.0, 2.1 Hz, 1H), 6.18 (d, *J* = 2.4 Hz, 1H), 6.08 (s, 1H), 5.73 (d, *J* = 4.5 Hz, 1H), 4.98 (q, *J* = 15.4, 15.0 Hz, 2H), 4.38 – 4.14 (m, 5H), 3.26 (q, *J* = 7.2 Hz, 4H), 1.07 (t, *J* = 7.0 Hz, 6H). (Signals from the counterions are not shown.) ^{31}P-NMR (162 MHz, D_2O) δ = −11.20 (br s, 2P), −22.62 (br s, 1P). HRMS (ESI) calcd for $C_{24}H_{32}N_6O_{15}P_3$ $[M+H]^+$: 737.1133, found: 737.1133.

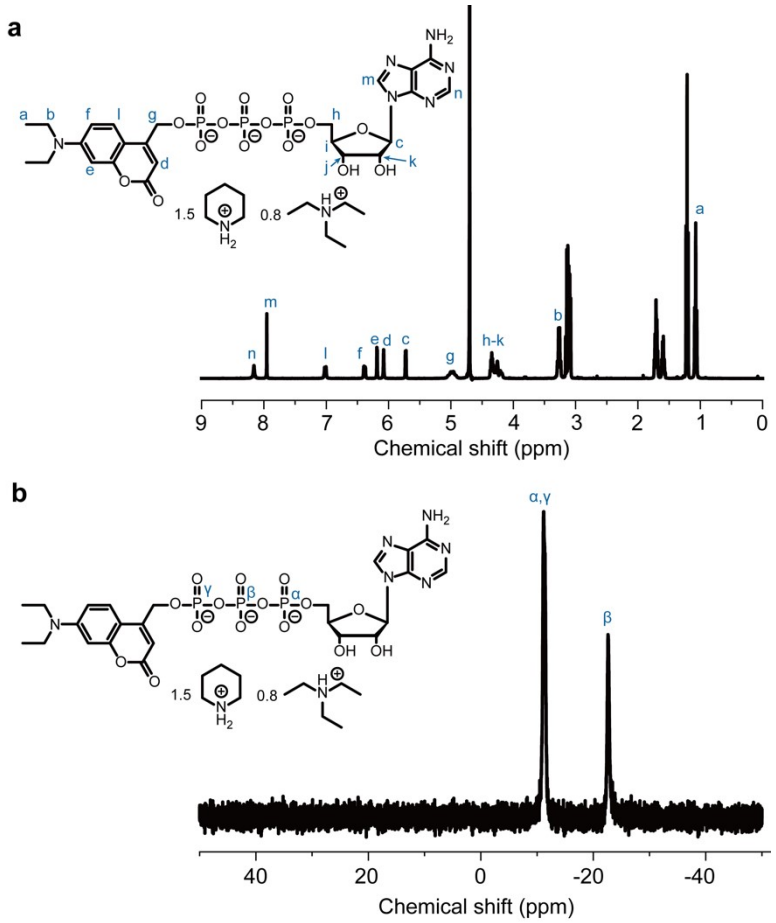


Figure S1. Characterizations of the DEACM-ATP. (**a**) ^{1}H NMR spectrum of DEACM-ATP in D_2O. (**b**) ^{31}P spectrum of DEACM-ATP in D_2O.

4. Supplementary Note 2. Photolysis of DMNPE-ATP and DEACM-ATP for DNA ligations

The difference in absorption between DMNPE-ATP and DEACM-ATP provides the rational for orthogonal and stepwise uncaging of these caged ATPs (Figure S2a). DMNPE-ATP shows almost no absorption at 420 nm; thus one can choose to specifically uncage DEACM-ATP with the presence of DMNPE-ATP by 420 nm LED. Subsequently, the second energy source, DMNPE-ATP, can be further uncaged at 365 nm.

We systematically investigated both ATP photocages for their photolysis at different starting concentrations by varied light powers (27 and 13.5 mW), and by subsequent detection of the released ATP via a bioluminescence assay.[2] For DMNPE-ATP, quantitative release of ATP can be achieved in a few minutes at a low concentration of 0.2 mM by illuminating at 365 nm (27 mW). For higher concentrations, such as 1.0 mM DMNPE-ATP, however, quantitative release of ATP requires extended illumination (Figure S2b).[3] For instance, only about 78 % of the caged ATP is released after 4 min of irradiation. The ATP release can further be controlled by varying the light power. A drastically lower release speed is observed when reducing the light power to 13.5 mW (Figure S2c). Similar dependencies are obtained for the uncaging of DEACM-ATP at 420 nm via a LED light (Figure S2d,e).

Then, both caged ATPs were applied to DNA polymerization initiated by photouncaging. Critically, the uncaging of DMNPE-ATP at 420 nm (27 mW) cannot induce efficient DNA polymerization, which is attributed to poor ATP releasing due to a wavelength mistake (Figure S3a). Thus, the uncaging of DEACM-ATP can proceed at 420 nm without efficiently activating the DMNPE-ATP, which requires shorter wavelength for photo-activation. For both caged ATPs, upon irradiations with right colors of light, the ligations proceed quickly to very high chain length above 1k base pairs (bp), rather independent of the photolysis times (varied from 30 s to 4 min) (Figure S3b,c). This can be rationalized by the fact that the DNA tile concentration is 50 µM, and the full ligation only requires 100 µM of ATP, while the supplied total caged ATP concentration is 600 µM, which is more than enough for the ligation.

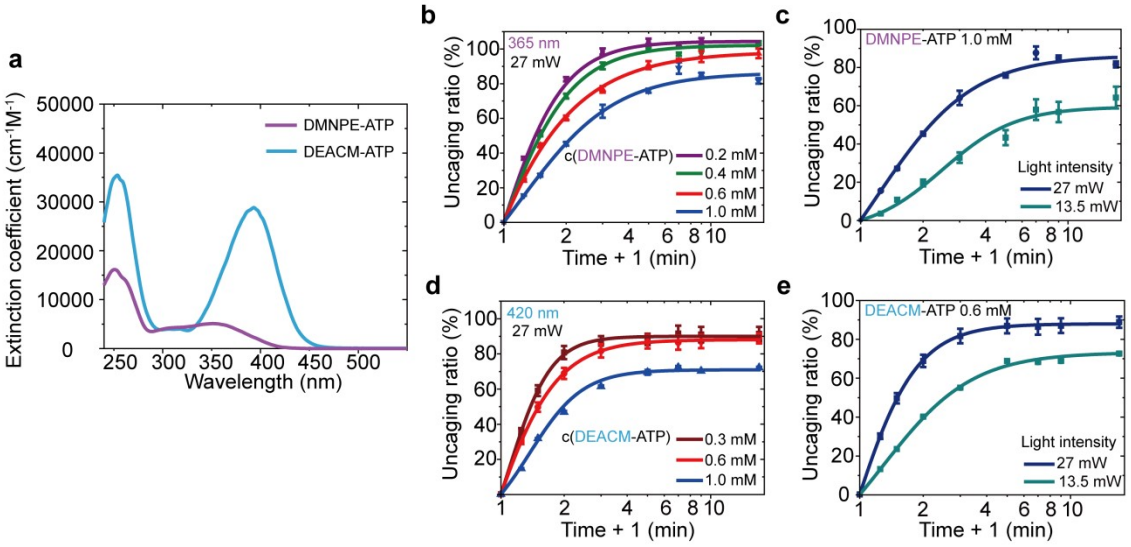


Figure S2. Photolysis investigations of caged ATPs. (**a**) Absorption spectra of DEACM-ATP and DMNEP-ATP. Measurements were taken in Milli-Q water with a concentration of 0.05 mM. Absorption intensities are represented as extinction coefficient as function of wavelength (full lines). (**b**) Kinetic study of DMNPE-ATP photolysis at 365 nm and 27 mW. (**c**) Uncaging of DMNPE-ATP (1.0 mM) at 365 nm and 27 or 13.5 mW. (**d**) Uncaging of DEACM-ATP at 420 nm and 27 mW. (**e**) Uncaging of DEACM-ATP (0.6 mM) at 420 nm and 27 or

13.5 mW.

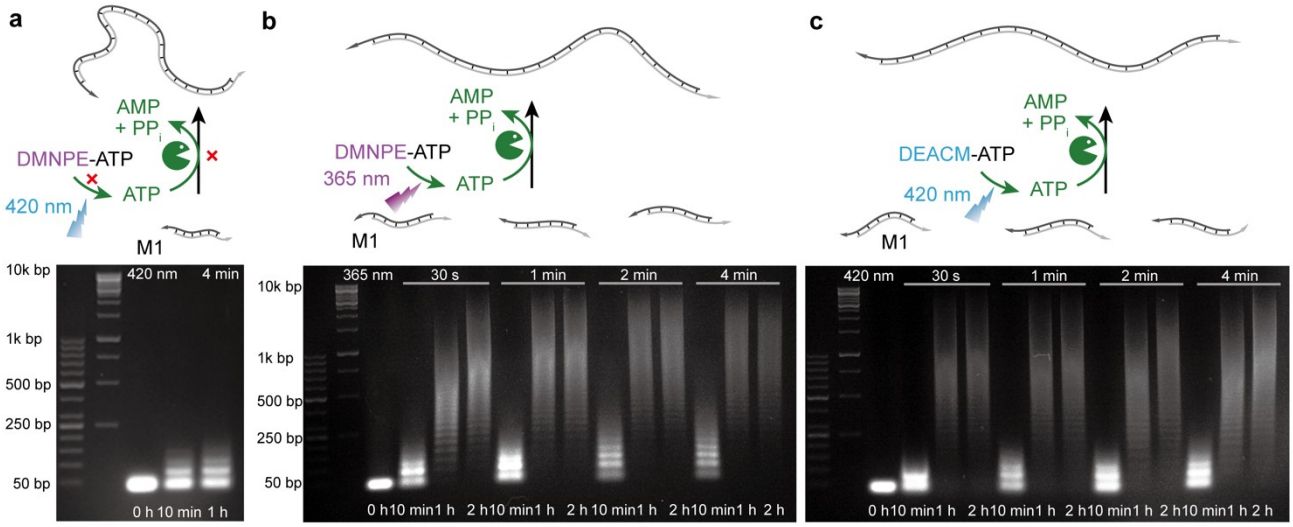


Figure S3. Light-activated DNA polymerizations via ligation of the M1 tile using T4 Ligase (no BamHI added). (**a**) Wavelength mismatch ligation by ATP attempted to be uncaged from DMNPE-ATP at 420 nm for 4 min at 27 mW; analyzed by AGE (2 wt.% agarose gel, 105 V, 70 min). (**b**) Ligations by ATP released from DMNPE-ATP via photolysis at 365 nm and 27 mW for different time, analyzed as above. (**c**) Ligations by ATP released from DEACM-ATP via photolysis at 420 nm and 27 mW for different time, analyzed as above. Conditions: (**a**) 25 °C, 1× *buffer E*, 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, and 0.6 mM DMNPE-ATP; uncaged at 420 nm and 27 mW for 4 min. (**b**) Same as (**a**) but uncaged at 365 nm and 27 mW for 30 s, 1 min, 2 min, and 4 min, respectively. (**c**) 25 °C, 1× *buffer E*, 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, and 0.6 mM DEACM-ATP; uncaged at 420 nm and 27 mW for 30 s, 1 min, 2 min, and 4 min, respectively.

5. Supplementary Note 3. Light-activated transient DySS DNA polymerization

The photolysis of DEACM-ATP at 420 nm and 27 mW leads to release of free ATP, which drives the system into a non-equilibrium DySS with the formation of dynamic DNA polymers (Figure S4a). Grayscale profiles extracted from AGE at 2 min photolysis display the monomer band at ca. 40 bp before photolysis (Figure S4b). After photolysis, the chains rapidly grow and reach a DySS plateau of ca. 2000 bp after 1h. After three days, the DySS DNA polymers are degraded and the system returns to its original state. In the beginning, the ligation is favored by copious release of ATP via photolysis and the high concentration of substrates (M1), while a low quantity of the restriction sites limits the speed for restriction. Upon reaction progress the mutual feedback of both processes in the ERN leads to a balance in the DySS and to a constant frequency of ligation and cutting. Once ATP is increasingly consumed, the ligation process seizes and the kinetic balance shifts towards the degradation.

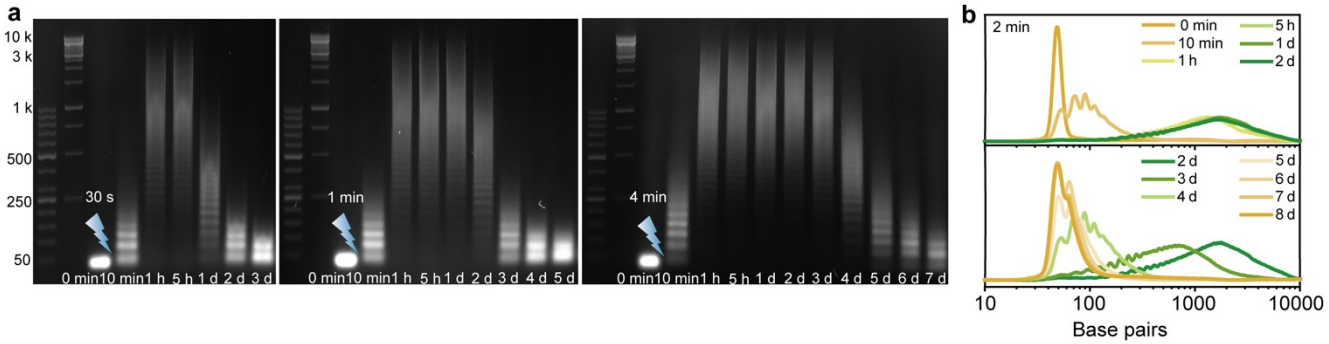


Figure S4. Transient DNA polymerizations fueled by photolysis of DEACM-ATP. (**a**) AGE analysis of the DNA chain growth with time by varied uncaging time (2 wt.% agarose gel, 105 V, 70 min). (**b**) DNA chain length distribution with time by 2 min irradiation. Conditions: 25 °C, 1× *Promega Buffer E*, 0.05 mM M1, 0.6 mM DEACM-ATP, 0.46 WU/µL T4 DNA ligase, 10 units/µL BamHI, and 0.1 mg/mL BSA; uncaged at 420 nm and 27 mW for 30 s, 1 min, and 4 min.

6. Supplementary Note 4. Light-activated transient DySS DNA polymerization using low light power for photolysis

Here we show the transient DNA polymerization fueled by ATP as released by photolysis of DEACM-ATP at 420 nm and 13.5 mW. Compared to photolysis at 27 mW, the photolysis at 13.5 mW yields a much slower speed for ATP release (Figure 2d in main manuscript). As a result, by photolysis at 420 nm and 13.5 mW for 30 s, 1 min, and 2 min, no efficient DNA polymerization is observed. Significant DySS polymers occur only when the photolysis time is increased to 4 min.

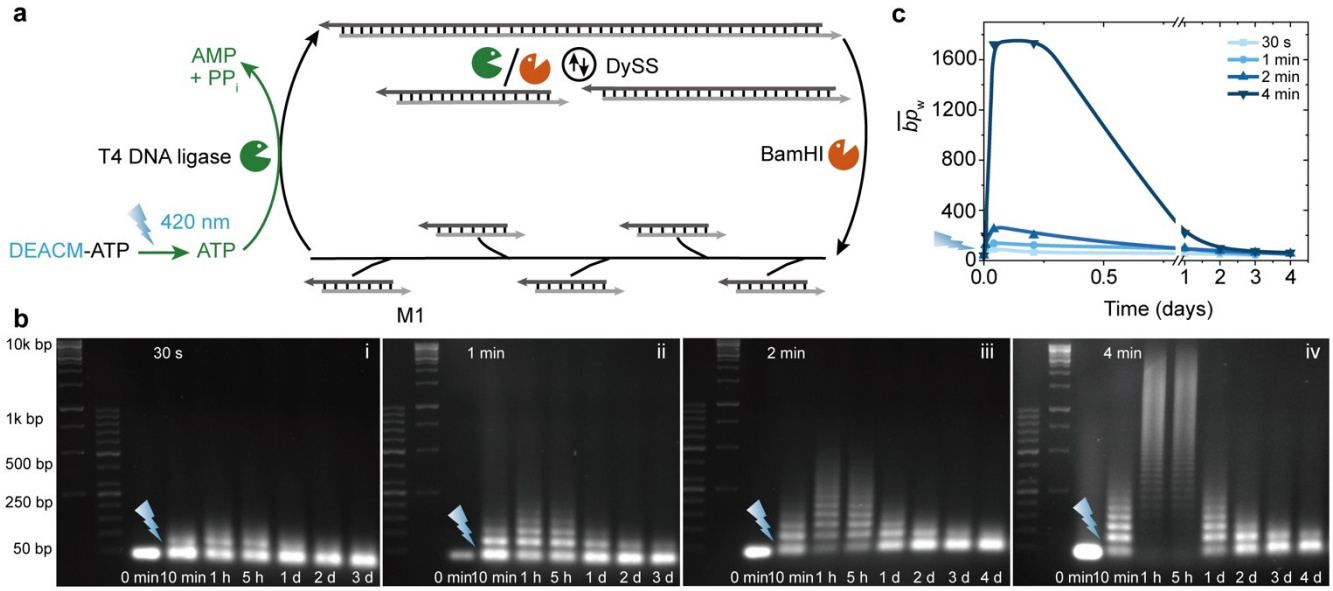


Figure S5. Light-activated transient DySS DNA polymerization via photolysis of DEACM-ATP at 420 nm and 13.5 mW. (**a**) Schematic illustration of light-activated transient DNA polymerization fueled by uncaging DEACM-ATP. (**b**) AGE analysis of the DNA distributions with time by varied uncaging time (2 wt.% agarose gel, 105 V, 70 min). (**c**) The $\overline{bp}_w$ development with time by varing uncaging time from 30 s to 4 min. Conditions: 25 °C, 1× *buffer E*, 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, and 0.6 mM DEACM-ATP; uncaged at 420 nm and 13.5 mW for 30 s, 1 min, 2 min, and 4 min.

7. Supplementary Note 5. Light-activated transient DySS DNA polymerization by photolysis of DMNPE-ATP

The kinetic study of the uncaging of DMNPE-ATP in Figure S2b shows fast ATP release upon irradiation at 365 nm. Thus, we thought the ATP released from DMNPE-ATP can also efficiently fuel the transient DNA polymerization. The experiments were performed at 25 °C in 1× buffer E containing 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/µL T4 DNA ligase, 10 units/µL BamHI, and 0.6 mM DMNPE-ATP by uncaging at 365 nm and 27 mW for 30 s, 1 min, 2 min, and 4 min (Figure S6). Overall, by different photolysis time at 365 nm and 27 mW, transient DySS DNA polymerizations can be achieved. However, by photolysis for 30 s (ca. 0.14 mM ATP released from DMNPE-ATP), dsDNA tiles cannot polymerize to long DNA polymers, which is due to inefficient ligation at a low ATP concentration. Further increasing the uncaging time to 2 min and 4 min promotes the DNA DySS polymers up to ca. 10k bp and induces delayed degradations of the DNA polymers. However, all these DNA polymers enter less defined DySS like DEACM-ATP fueled systems (Figure S4), which can be attributed to less efficient ligation reactions here. Thus, we assume that the by-product during photouncaging of DMNPE-ATP can inhibit the activity of T4 DNA ligase. As a result, the degradations of the DNA polymers are also significantly delayed compared to that for the systems by DEACM-ATP and complete degradations of the DNA polymers need more time.

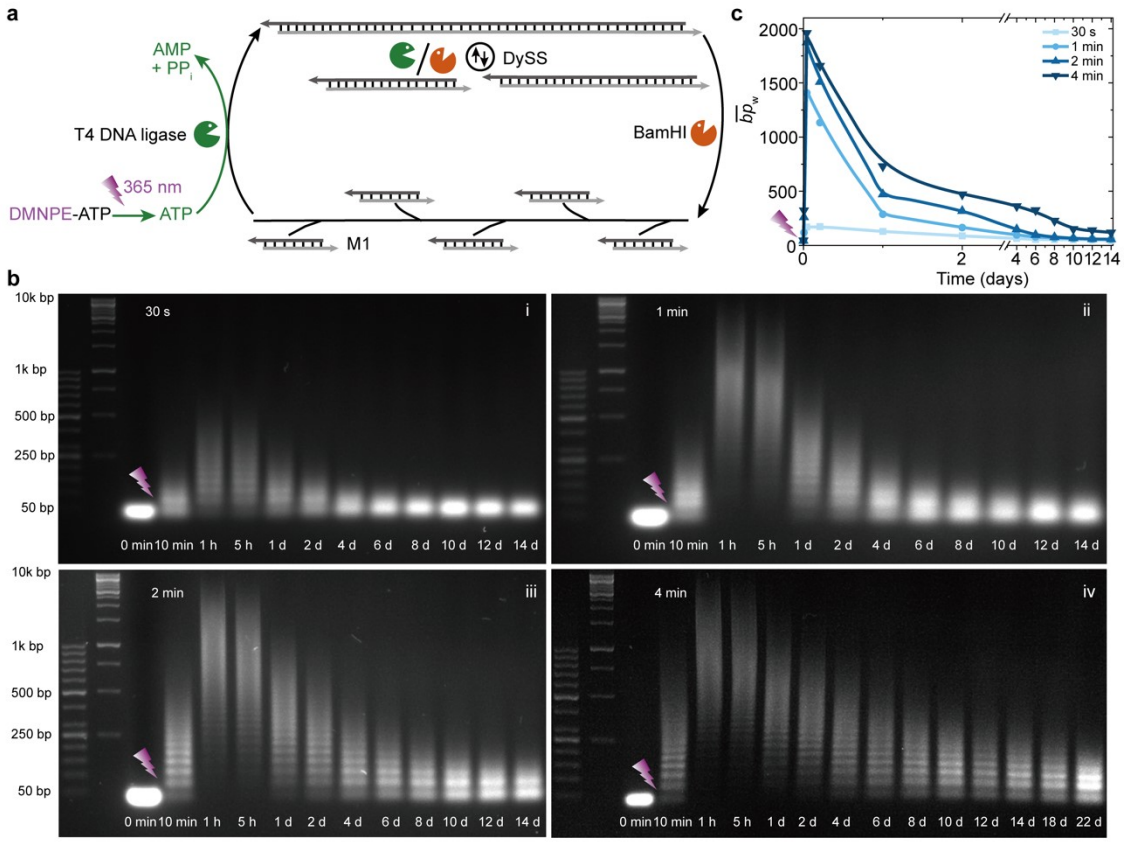


Figure S6. Light-activated transient DySS DNA polymerization via photolysis of DMNPE-ATP. (**a**) Schematic illustration of light-activated transient DNA polymerization fueled by uncaging DMNPE-ATP. (**b**) AGE analysis of the DNA polymer distributions with time by varied uncaging time (2 wt.% agarose gel, 105 V, 70 min). (**c**) The $\overline{bp}_w$ development with time by varying uncaging time from 30 s to 4 min. Conditions: 25 °C, 1× *buffer E*, 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/µL T4 DNA ligase, 10 units/µL BamHI, and 0.6 mM DMNPE-ATP; uncaged at 365 nm and 27 mW for 30 s, 1 min, 2 min, and 4 min.

8. Supplementary Note 6. Effects of light illumination on T4 DNA ligase and BamHI

Here we discuss the effects of UV and Vis light illumination on both enzymes. DMNPE-ATP was applied for the ligation. Two batches of solutions containing 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase were prepared in 1× *buffer E*. After being illuminated at 365 and 420 nm and 27 mW for 4 min, 0.6 mM DMNPE-ATP was added to the solutions, followed by uncaging at 365 nm and 27 mW for 30 s. AGE in Figure S7a indicates efficient DNA polymerizations upon photolysis, and the DNA polymers show comparable length, bp_w, to the DNA polymers from non-preilluminated sample by 30 s uncaging in Figure S3b. Thus, we conclude that the UV and Vis light illuminations do not have significant side effects on the ligase. Additionally, the transient DNA polymerization by pre-illumination of both enzymes show the same results as without any pre-illumination (Figure S7b, Figure S6b-ii). Hence, we conclude that the UV and Vis light illuminations do not show any significant side effects on both enzymes.

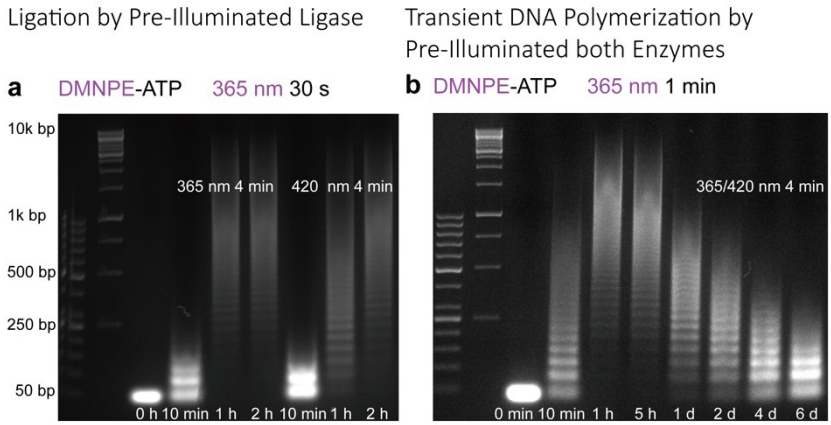


Figure S7. Effects of UV and Vis illumination on T4 DNA ligase and BamHI. (**a**) Ligation fueled by 30 s uncaging at 365 nm and 27 mW after 4 min of pre-illumination at 365 or 420 nm (AGE, 2 wt.% agarose gel, 105 V, 70 min). (**b**) Transient DNA polymerization fueled by 1 min uncaging at 365 nm and 27 mW after 4 min pre-illumination at 365 and 4 min pre-illumination at 420 nm. Conditions: (**a**) 25 °C, 1× *buffer E*, 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, and 0.6 mM DMNPE-ATP. (**b**) 25 °C, 1× *buffer E*, 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, and 0.6 mM DMNPE-ATP.

9. Supplementary Note 7. Addition of ATP for the second round activation of the transient DySS DNA polymerization

The transient DySS DNA polymerization was first started by uncaging DEACM-ATP in the system. After the DNA polymers were degraded, a batch of fresh ATP solution was added to the system to activate the second round of the transient DNA polymerization. The efficient DNA polymerization and transient behavior in the second round indicates that the enzymes remain active and the refueling of the system by second uncaging of DEACM-ATP in Figure 2e,f (main manuscript) is indeed due the second release of ATP from photolysis.

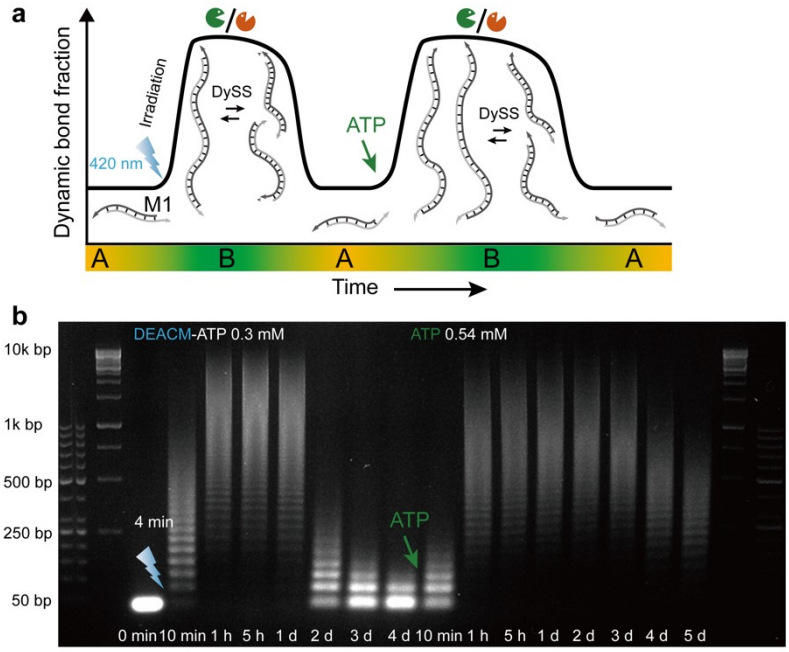


Figure S8. Multiple activations of transient DNA polymerization by DEACM-ATP and free ATP solution. (**a**) Schematic illustration of multiple activations of transient DNA polymerization by DEACM-ATP and free ATP. (**b**) AGE analysis of the DNA polymerization with time of the multiple activations of transient DNA polymerization (2 wt.% agarose gel, 105 V, 70 min). Conditions: 25 °C, 1× *buffer E*, 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, and 0.3 mM DEACM-ATP, uncaged at 420 nm and 27 mW for 4 min, followed by the addition of 0.54 mM ATP after the degradation of the first round.

10. Supplementary Note 8. Repeated fueling of transient DySS DNA polymerization by multiple uncaging of DMNPE-ATP

We tried to switch the system on demand to repeatedly control the transient DNA polymerization via multiple uncaging of DMNPE-ATP. 1 mM DMNPE-ATP was stored in the system, and the system was uncaged at 365 nm and 27 mW for 30 s. According to Figure S2c, this should lead to an uncaging of only 15 %. As expected, the DNA polymerization was not very efficient due to the low concentration of released ATP (Figure S9c). However, by uncaging for 1 min (ca. 27 % uncaging) for the first round long DNA polymers can be obtained (Figure S9d). Afterwards, both systems were uncaged for the second round at 365 nm and 27 mW for another 4 min. However, the second round uncaging failed to fuel the DNA polymerization. Then, the light power for the multiple uncaging was decreased to 13.5 mW (Figure S9e). AGE analysis shows weak transient behaviors for both rounds, but 2 min uncaging for the second round uncaging still cannot induce efficient ligation. Then, the light power for the second round uncaging was increased to 27mW (Figure S9f), leading a bit stronger transient behavior of the DNA polymerization for the second round activation, but still with low efficacy. A previous report showed multiple uncaging of DMNPE-ATP by UV irradiations,[4] which indicated that the UV irradiation itself should not damage the remaining photocaged ATP. However, it is obvious that the byproduct, a nitroso compound, shows strong inhibition on the enzymes, especially T4 DNA ligase. Thus, the systems show less defined steady states (Figure S6). We also show that less byproduct production in the first round leads to less inhibition for the second round activation (Figure S9e-f vs c-d). As a result, the only reason for the failure of multiple activations of DNA polymerization should be attributed to the accumulated inhibition of T4 DNA ligase by the byproduct from photolysis. In order to further directly prove this, we performed another control experiment, for which we utilized UV light to uncage DMNPE-ATP for the first round activation, and then fresh ATP was added to fuel the second round polymerization. The result in Figure S9g shows the failure of ligation for the second round, which confirms the inhibition of T4 DNA ligase as the ligase would be otherwise still active without the photolysis of DMNPE-ATP. Overall, the T4 DNA ligase shows strong inhibition after long time incubation with the byproduct from photolysis.

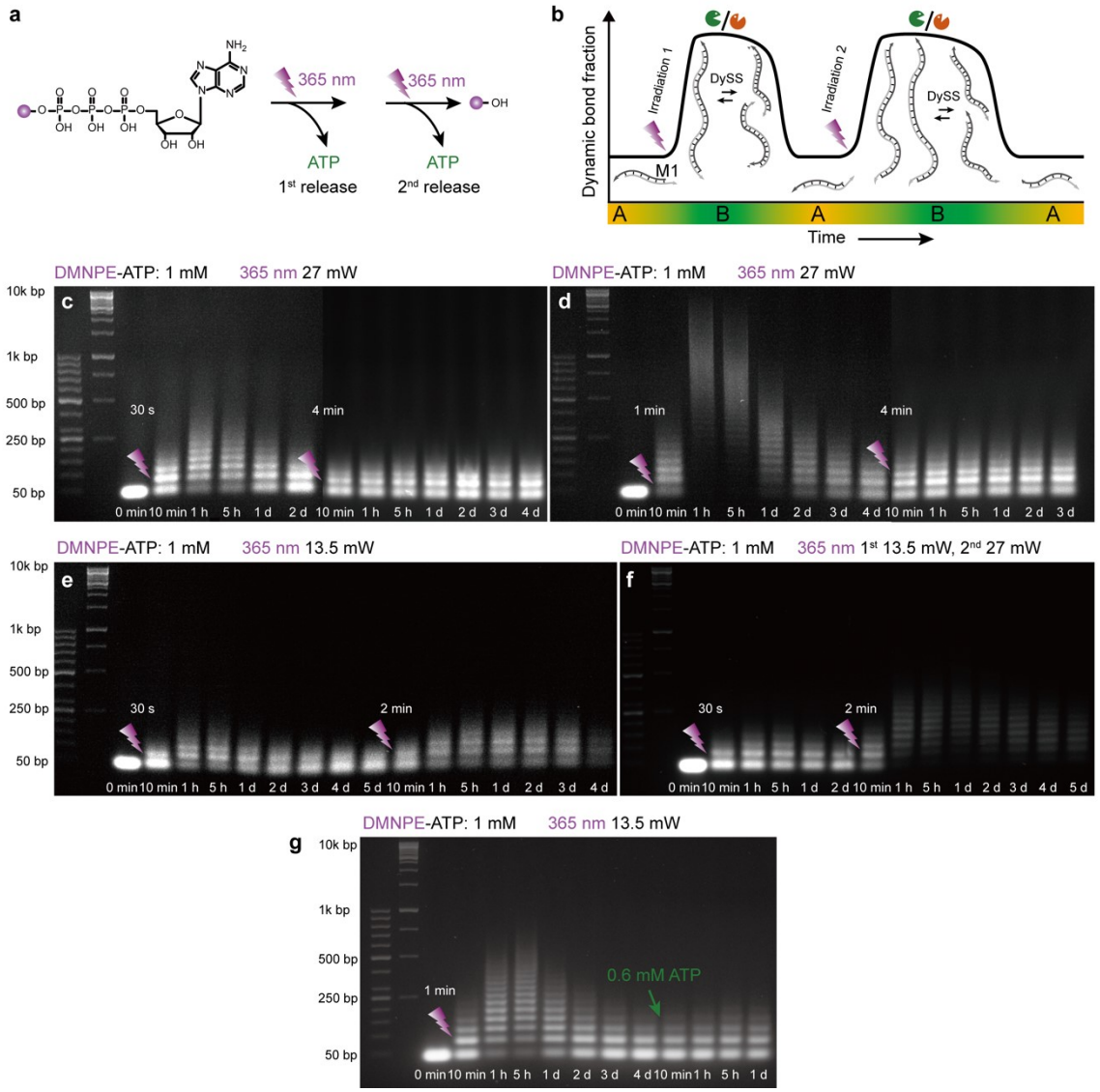


Figure S9. Multiple uncaging of DMNPE-ATP for repeated activation of the transient DNA polymerization. (**a**) Schematic illustration of multiple uncaging of DMNPE-ATP. (**b**) Schematic illustration of multiple activation of transient DNA polymerization by multiple uncaging of DMNPE-ATP. (**c**) AGE analysis of the DNA polymerization with time by multiple uncagings of DMNPE-ATP at 365 nm and 27 mW for 30 s and 4 min for the first and second rounds, respectively. (**d**) AGE analysis of DNA polymerization with time by multiple uncaging of DMNPE-ATP at 365 nm and 27 mW for 1 min and 4 min for the first and second rounds, respectively. (**e**) AGE analysis of DNA polymerization with time by multiple uncaging of DMNPE-ATP at 365 nm and 13.5 mW for 30 s and 2 min for the first and second rounds, respectively. (**f**) AGE analysis of DNA polymerization with time by multiple uncaging of DMNPE-ATP at 365 nm 13.5 mW for 30 s for the first round activation and 27 mW for 2 min for the second round activation. (**g**) AGE analysis of DNA polymerization with time by uncaging of DMNPE-ATP at 365 nm and 13.5 mW for 1 min for the first round activation and adding 0.6 mM fresh ATP for the second round activation. Conditions: 25 °C, 1× buffer E, 0.05 mM M1, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, and 1.0 mM DMNPE-ATP.

11. Supplementary Note 9. Photouncaging of the caged DNA tiles

The photocleavage of the PCGs from double-side photocaged dsDNA tile (M2) was investigated by HPLC. The caged DNA tiles with a concentration of 0.05 mM were illuminated with an LED at 365 nm (135 mW) for different time. Figure S10 shows fast photocleavage of the photocages from the caged DNA tiles. After 2 min UV irradiation, 96.2 % of the photocaged tiles are uncaged. Longer UV irradiation time leads to higher ratio of photouncaging, and which reaches 98.2 % after 15 min UV irradiation. It is worth noting that fast releasing of the dsDNA tiles is needed for efficient ligation and polymerization. Thus, 15 min illumination at 365 nm and 135 mW was used to uncage the caged DNA tiles throughout the study.

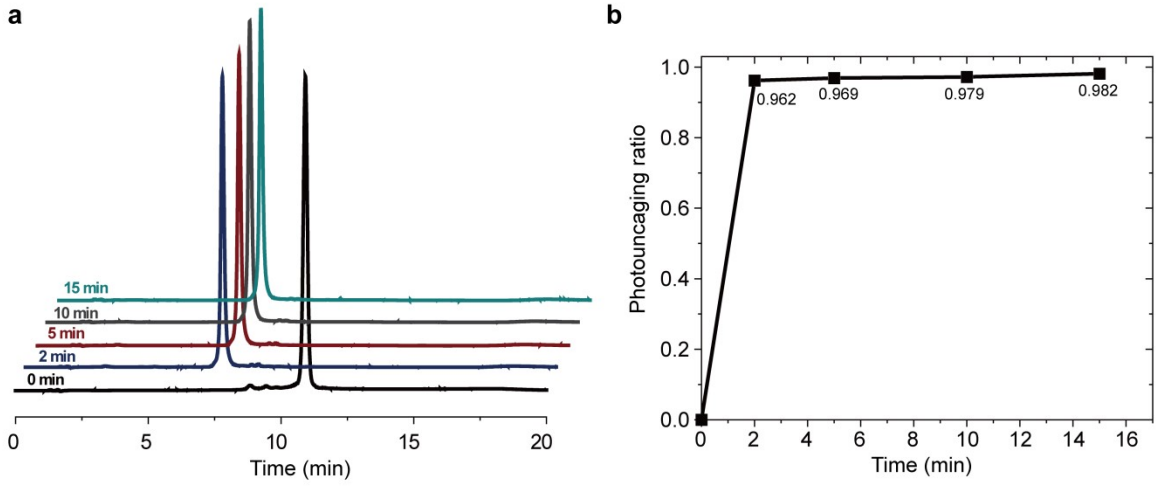


Figure S10. Investigation of the photouncaging of double-side caged dsDNA tiles. (**a**) HPLC track of the photouncaging at 365 nm and 135 mW. (**b**) Quantified photouncaging ratio for the photocaged tiles after different time irradiation at 365 nm. Conditions: Photouncagings were performed in 10 µL solution aliquot for each spectrum with a concentration of 0.05 mM.

12. Supplementary Note 10. ATP-fueled polymerization of photocaged DNA tiles

We investigated the effect of the PCG to block the ligation of the sticky-end overhangs in dsDNA building blocks. The PCGs are situated as a photocleavable end group on the phosphate at 5' end of the DNA. As long as this PCG is attached, the T4 ligase is unable to join the phosphate at 5' end with an adjacently situated 3' end hydroxyl group into a covalent phosphodiester bond. Since previous studies indicated that one internal caging group (attached to the base) is enough to prevent the dimer formation from DNA tiles with self-complementary overhangs of 6 or 8 nt,[5] we assumed that a PCG on one side of the dsDNA building blocks could be enough to prevent the hybridization and the ligation between the caged 3 nt sticky end and its free counterpart. Hence, in the beginning only one side of the dsDNA tiles was caged (M3). The ligation of this single-side caged dsDNA tiles was afterwards conducted at 25 °C in *Promega Buffer E* with 0.05 mM M3, 0.46 WU/µL T4 DNA ligase, 0.1 mg/mL BSA, and 1 mM ATP. It turns out that one photocage for each tile is not enough to prevent the ligation of the DNA tiles (Figure S11a,b). AGE in Figure S11b shows efficient ligation of M3. After one hour ligation, the DNA chains grow up to 10k bp, which means that the 3 nt sticky end with a PCG on its terminal end is still able to hybridize with its complementary counterpart and the remaining phosphate and hydroxyl groups can be efficiently joined by T4 DNA ligase. The result is an incompletely ligated dsDNA part with a remaining PCG sitting at the 5' end in the recognition area for the restriction enzymes.

This result led us to cage both sides of the DNA tiles (M2). Since there is no free phosphate group left, the ligation of the caged DNA tiles is indeed fully inhibited (Figure S11c,d, lane 1). The ligation can only happen upon the removal of the photocages by UV illumination (Figure S11c,d, lane 2).

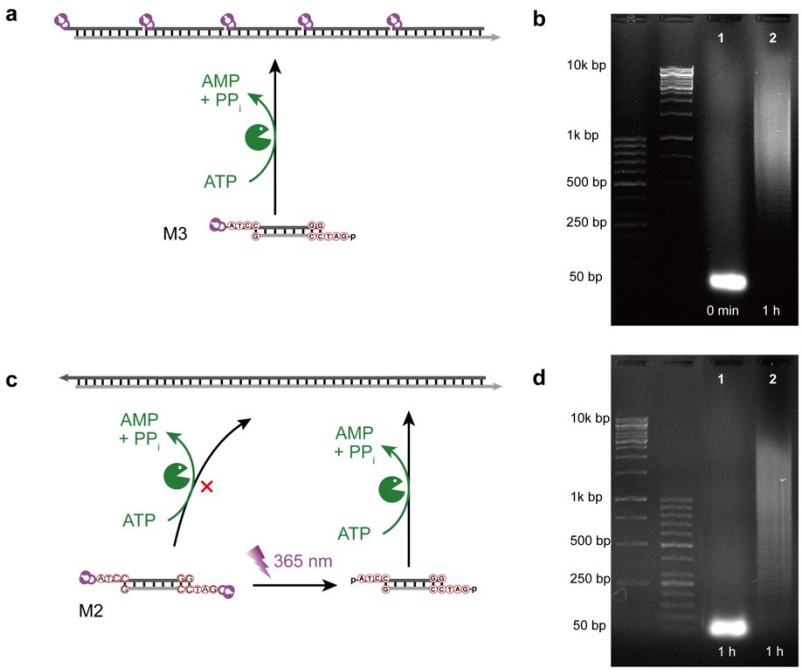


Figure S11. Ligation of the photocaged DNA building blocks: Single-side caged tiles vs. double-side caged tiles. (**a**) Schematic illustration of ATP-fueled polymerization of single-side photocaged DNA tile. (**b**) AGE analysis for single-side photo-caged DNA tiles (lane 1) before and (lane 2) after ligation. (**c**) Schematic illustration of light-activated, ATP-fueled polymerization of double-side photocaged DNA tile. (**d**) AGE analysis for 1 h ligation of double-side photo-caged DNA tiles (lane 1) before and (lane 2) after photouncaging at 365 nm at 135 mW. All AGEs were performed with 2 wt.% agarose gel under 105 V for 70 min run time. Conditions: (**a**) 25 °C, 1× *Promega Buffer E*, 0.05 mM M3, 0.46 WU/µL T4 DNA ligase, 0.1 mg/mL BSA, and 1 mM ATP. (**c**) The same conditions as (**a**) except that M2 was used, and the uncaging was performed at 365 nm and 135 mW.

13. Light-activated transient polymerization of photocaged DNA tiles

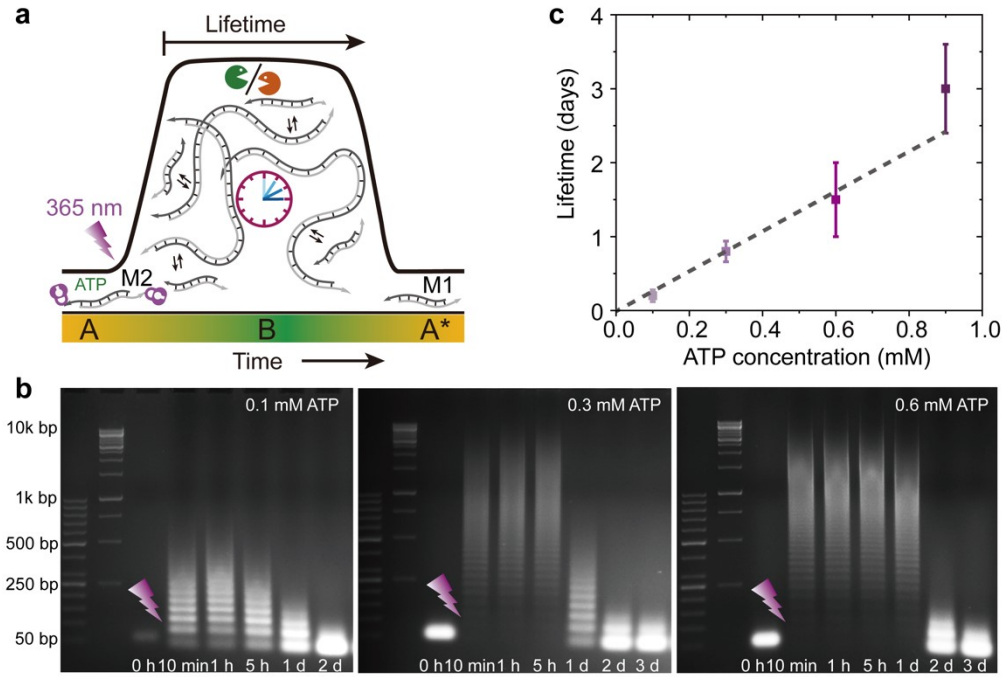


Figure S12. Light-activated transient polymerization of double-sided photocaged DNA tiles. (**a**) Schematic illustration of light-activation in ATP-fueled transient DNA polymerization. (**b**) AGE analysis of the light-activated DNA chain growth with time fueled by 0.1, 0.3, 0.6 and 0.9 mM ATP (2 wt.% agarose gel, 105 V, 70 min). (**c**) Lifetime of the DNA assemblies with varied ATP concentrations. Conditions: 25 °C, 1× *Promega Buffer E*, 0.05 mM M2, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, 0.1 mg/mL BSA, and varied concentrations of ATP.

14. Light-modulated multiple transient DySSs of DNA assemblies

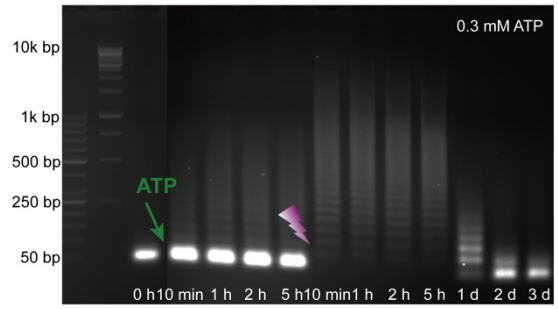


Figure S13. Light-modulated multiple transient DySSs of DNA assemblies. AGE analysis for light-modulated two transient DySSs of DNA assemblies fueled with 0.3 mM ATP (2 wt.% agarose gel, 105 V, 70 min). Condition: 25 °C, 1× *Promega* Buffer E, 0.01 mM M1, 0.01 mM M4, 0.03 mM M2, 0.1 mg/mL BSA, 0.46 WU/μL T4 DNA ligase, 10 units/μL BamHI, and 0.3 mM ATP.

Reference

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