Supporting Information for

Fuel-Driven Transient DNA Strand Displacement Circuitry with Self-Resetting Function

Jie Deng,[a-c] and Andreas Walther[a-d]*#*

[a.] A^{3}BMS Lab – Active, Adaptive and Autonomous Bioinspired Materials, Institute for Macromolecular Chemistry, University of Freiburg, Stefan-Meier-Straße 31, 79104 Freiburg, Germany

[b.] Freiburg Materials Research Center (FMF), University of Freiburg, Stefan-Meier-Str. 21, 79104 Freiburg, Germany

[c.] Freiburg Center for Interactive Materials and Bioinspired Technologies (FIT), University of Freiburg, Georges-Köhler-Allee 105, 79110 Freiburg, Germany

[d.] Cluster of Excellence livMatS @ FIT – Freiburg Center for Interactive Materials and Bioinspired Technologies, University of Freiburg, Georges-Köhler-Allee 105, D-79110 Freiburg, Germany

[#] Present address: A^{3}BMS Lab, Department of Chemistry, University of Mainz, Duesbergweg 10-14, 55128 Mainz, Germany.

E-mail: dengj.jie@gmail.com, andreas.walther@uni-mainz.de.

Materials and methods

Instrumentation: ThermoMixer (Eppendorf), INTAS *CHEMOSTAR touch* fluorescence imager (INTAS Science Imaging), gel electrophoresis chambers (biostep), Enduro power supply 300 V (Labnet International, Inc.), Image J, ScanDropR UV-VIS spectrometer (Analytik Jena), Confocal laser scanning microscopy (Leica TCS SP8, Mannheim), and Spark® Multimode Microplate Reader (Tecan).

Reagents: T4 DNA Ligase (HC, 20 Weiss units/µL (WU), recombinant *E. coli* strain) was supplied by Promega and BsaI-HF®v2 (20 units/µL (U), *NEB #R3733*) was ordered from New England Biolabs (*NEB*). ATP solution (10 mM in 1 mM Tris-HCl pH 7.5) was purchased from Invitrogen. Agarose low EEO was supplied by PanReac AppliChem. DNA gel loading dye (6×), and streptavidin coated magnetic microparticles (Dynabeads™ MyOne™ Streptavidin C1) were supplied by ThermoFisher Scientific. Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, biology grade) was supplied by CALBIOCHEM. Sodium chloride (NaCl, 99%), hexadecane (99%), tris (hydroxymethyl)aminomethane hydrochloride pH 8.0 (Tris-HCl), trizma base, and black 384-well microplate (Corning, CLS3540) were ordered from Sigma-Aldrich. Acetic acid glacial (ACS, Reag. Ph. Eur. Analytical reagents) was supplied by VWR Chemicals. All oligonucleotides were supplied by Integrated DNA Technologies Inc. (IDT).

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.

BsaI-HF®v2 storage buffer (NEB): 10 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/mL BSA, 50% glycerol.

NEB CutSmart® Buffer: 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 100 µg/mLBSA.

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

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

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

CSF buffer: *CutSmart® Buffer* supplemented with 0.05% Pluronic® F-127.

Milli-Q water was used throughout of this study.

Supplementary Table 1: Oligonucleotide sequences used, with their name and color codes for their first appearance in individual Figure.

	Name	Oligonucleotide sequence	#nt	Purification
Figure 2	Complex1	5'-CATGAGAATTCCATTCACGGTCTCT-3'	25	HPLC
		5'-/5Phos/GATTAGAGACCGTGAATGGAATTCTCATG-3'	29	HPLC
	Input1	5'-/5Phos/AATCTAACTGTAA-3'	13	HPLC
	Reporter1	5'-TGTAATATCGTGCCC-3'	15	HPLC
		5'-/5Cy5/GCACGATATTACAGTTA-3'	17	HPLC
Figure 3	Reporter2	5'-TGTAATATCGTGCCC/3Cy5/-3'	15	HPLC
		5'-/5IAbRQ/GCACGATATTACAGTTA-3'	17	HPLC
	DoS	5'-/5BiosG/TTTTTTGGGCACGAT-3'	15	HPLC

Figure 4	Input2	5’-/5Phos/AATCTTTATGTAGA-3’	14	HPLC
	Activator	5’-TGTAGATGACATGTAATATCG-3’	21	HPLC
		5’-ATTACATGTCATCTACATAAA-3’	21	HPLC
	Reporter3	5’-TGTAATATCGTGCCC/3Cy5/-3’	15	HPLC
		/5IAbRQ/GCACGATATTACATGTCA-3’	18	HPLC
Figure 5	Input3	5’-/5Phos/AATCTAACTGTAAT-3’	14	HPLC
	Reporter4	5’-/5Phos/TGTAATGTAATCTA/3Cy5/-3’	14	HPLC
		5’-TAGATTACATTACAGTTA-3’	18	HPLC
	Complex5	5’-CGTCAAGCTTTCTATTCGCATCAGCATACTATTCAGGTCTCT-3’	42	HPLC
		5’-/5Phos/TACAAGAGACCTGAATAGTATGCTGATGCGAATAGAAAGCTTGACG-3’	46	HPLC
	Reporter5	5’-/5Phos/AATCTAACTGTAAT-3’	14	HPLC
		5’-/5IAbRQ/ATTACAGTTAGATTACAT-3’	18	HPLC

Experimental protocol

Hybridization of the double-stranded DNA (dsDNA) tiles. All DNA strands were used as received, to which certain amounts of annealing buffer (10 mM Tris-HCl (pH 8.0), 50 mM NaCl) were added to make stock solutions with a concentration of ca. 1 mM calculated by the amounts of DNA given by IDT 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. The annealed stock solutions were stored at -20 °C for further use.

Transient DNA strand displacement and its quantification by agarose gel electrophoresis

The transient DNA strand displacement (DSD) experiments were first performed by using different concentration of BsaI (0.4, 0.8, or 1.2 U) in 1× *NEB* CutSmart buffer at 37 °C containing 10 μM Complex1, 10 μM Input1, 10 μM Reporter1, 0.8 WU T4 DNA ligase, and 80 μM ATP. The experiments were carried out in a total volume of ca. 40 μL. At different time intervals (0 min, 10 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, and 24 h), 2 μL aliquots of the reaction solution were collected and quenched by 3 μL of quenching buffer (200 mM EDTA, 10 mM Tris-HCl (pH 8.0), 50 mM NaCl).

To investigate ATP-dependent lifetime for the transient DSD, 120 μM ATP was further used to fuel a system containing 10 μM Complex1, 10 μM Input1, 10 μM Reporter1, 0.8 WU T4 DNA ligase, and 1.2 U BsaI. At different time intervals (0 min, 10 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, and 24 h), 2 μL aliquots of the reaction solution were collected and quenched by 3 μL of quenching buffer.

Afterwards, all the quenched samples were mixed with 1 μL of 6× DNA gel loading dye (10 mM Tris-HCl (pH 7.6) 0.03 % bromophenol blue, 0.03 % xylene cyanol FF, 60 % glycerol 60 mM EDTA.) and then analyzed by 4 wt.% agarose gel electrophoresis (AGE) at 75 V for 3 h run time at room temperature in TAE buffer (40 mM Tris, 20 mM acetic ccid, 1 mM EDTA). The results were recorded by an INTAS *CHEMOSTAR touch* fluorescence imager by only exciting Cy5 in the DNA species. The ratio of DSD was further quantified by ImageJ software. Briefly, rectangular selections were made for every gel band and the integral grayscale values for each gel band were

then measured and calculated. For the two gel bands in each lane of the gel image, the lower one corresponds to the emission of Cy5 in Reporter1 (non-strand-displaced state) while the upper one corresponds to the emission of Cy5 in Complex2 (strand-displaced state). Hence, the fraction of DSD at each time point was calculated as the ratio between the integral grayscale value for Complex2 and the summed grayscale value of Reporter1 and Complex2.

Adaptive dynamic steady state for the ATP-fueled DSD monitored by FRET

Tuning the concentration of T4 DNA ligase. The experiments were first performed by using different concentration of T4 DNA ligase (0.2, 0.4 or 0.8 WU) in 1× *NEB* CutSmart buffer at 37 °C containing 1 μM Complex1, 1 μM Input1, 1 μM Reporter2, 0.2 U BsaI, and 6 μM ATP. For the control experiment, 0.2 WU T4 DNA ligase was used and no ATP was added. The experiments were carried out in a volume of ca. 25 μL in a black 384-well plate, and the results were recorded after every 2 min by a plate reader with an excitation at 630 nm and an emission at 665 nm (10 nm for both bandwidths). Note: a layer of hexadecane (8 μL) was added on top of the reaction solution to prevent evaporation at 37 °C.

Tuning the concentration of BsaI and Complex1. The experiments were further carried with fixed concentration of T4 DNA ligase (0.8 WU) but varied concentration for BsaI (0.2 or 0.4 U) and Complex1 (1 or 2 μM) in 1× *NEB* CutSmart buffer at 37 °C containing 1 μM Input1, 1 μM Reporter2, and 6 μM ATP. The control experiments were performed with 1 μM Complex1, 0.8 WU T4 DNA ligase, 0.2 U BsaI, and 0 μM ATP. The results were recorded by a plate reader (see above).

Transient DSD with ATP-dependent lifetime

The experiments were performed in 1× *NEB* CutSmart buffer at 37 °C containing 2 μM Complex1, 1 μM Input1, 1 μM Reporter2, 0.8 WU T4 DNA ligase, 0.2 U BsaI, and varied concentration of ATP (2, 4, and 6 μM). The results were recorded by a plate reader (see above).

Immobilization of the docking strand on streptavidin-coated magnetic colloids

10 μL of streptavidin-coated magnetic particles (Dynabeads™ MyOne™ Streptavidin C1, ThermoFisher Scientific, 10 mg/mL) were washed with 10 μL CSF buffer (*CutSmart® Buffer* supplemented with 0.05% Pluronic® F-127) for 3 times. Afterwards, the particles were re-suspended in 20 μL CSF buffer with 0.05 mM docking strand (DoS) and the reaction was carried out at room temperature for 1 h. The particles were then washed by 10 μL CSF buffer 5 times, re-suspended in 10 μL CSF buffer, and stored in the refrigerator for further use.

Transient colloid functionalization using fuel-driven DSD

The experiments were performed in 1× *NEB* CutSmart buffer at 37 °C containing 2 μM Complex1, 1 μM Input1, 1 μM Reporter2, 0.8 WU T4 DNA ligase, 0.2 U BsaI, ca. 66.8 μg/mL DoS-modified colloids (ca. 4.68-6.68×10^7 beads/mL) as well as 2 μM ATP. The experiments were in a total volume of ca. 30 μL. At different time intervals (0 min, 10 min, 1 h , 2 h, 4 h, 6 h, and 8 h), 3 μL aliquots of the suspension were extracted. The particles were collected by a magnet and the supernatant was discarded. The collected microparticles were washed with 10 μL CSF buffer at 37 °C for two times and then re-suspended in 10 μL CSF buffer. The CSF buffer for washing was incubated at 37 °C in the thermoshaker. For each washing cycle, 10 μL CSF buffer was added to the microparticles, vortexed for 5 seconds, incubated in the thermoshaker for 2 min, and then the supernatant was

removed via a magnet. Afterwards, the microparticles were characterized by a confocal laser scanning microscopy, always using the same microscopy settings for quantification.

Transient DSD circuitry

Typical experiments were performed in 1× *NEB* CutSmart buffer at 37 °C containing 1 μM Complex1, 1 μM Input2, 1 μM Activator, 1 μM Reporter3, 0.1 WU T4 DNA ligase, 0.4 U BsaI, and 6 μM ATP. Additionally, the concentration for Complex1, Input2, and Activator was further increased to 1.5 μM and 2 μM to further study the adaptive dynamic steady state of the DSD circuitry. The results were recorded by a plate reader (see above). Then, the lifetime of the transient circuitry was further programmed by ATP concentration. The experiments were performed 1× *NEB* CutSmart buffer at 37 °C containing 2 μM Complex1, 2 μM Input2, 2 μM Activator, 1 μM Reporter3, 0.2 WU T4 DNA ligase, 0.4 U BsaI, and varied concentration of ATP (4, 6, and 8 μM). The results were recorded by a plate reader (see above).

Transient feedback systems for ATP-fueled automaton

Typical experiments were performed in 1× *NEB* CutSmart buffer at 37 °C containing 1 μM Complex1, 1 μM Reporter4, 1 μM Complex5, 1 μM Reporter5, 0.8 WU T4 DNA ligase, 0.2 U BsaI, 6 μM ATP and varied concentration of Input3 (0.4, 0.2, and 0.1 μM). Additionally, similar experiments were also performed by using 0.4 U BsaI to investigate the adaptive steady state for the ATP-fueled automaton. The control experiment without Complex5 was carried out the same as above by using 0.1 μM Input3 (Figure S2). The results were recorded by a plate reader (see above).

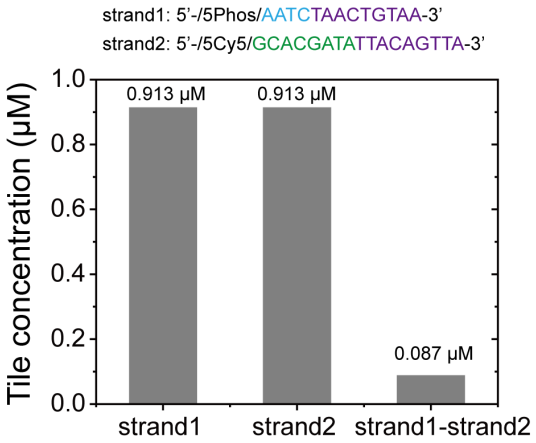

Figure S1. Nupack simulation for melting of Intermediate1. Equilibrium concentration for the ssDNA and dsDNA of the dsDNA cleaved from Complex2 (Intermediate1) in Figure 2 (main manuscript). Condition: 1 μM each ssDNA, 50 mM Na^+, and 10 mM Mg^{2+} at 37 °C. This confirms the self-dissociation of Intermediate1 into two ssDNA after BsaI restriction due to its low $T_{m.}$

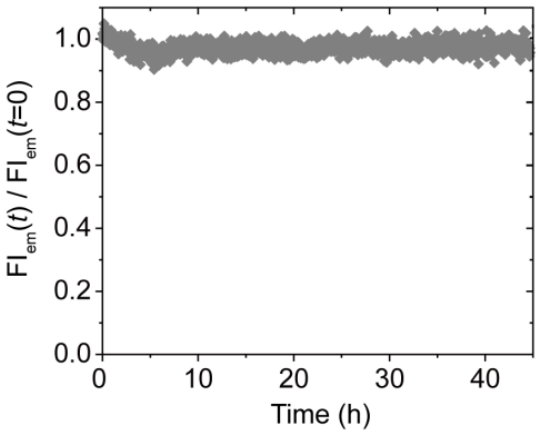

Figure S2. Feedback DSD systems without Complex5. Time-dependent FI for monitoring the FRET signal from ATP-driven automaton using 0.1 μM Input3, 6 μM ATP, 0.8 WU T4 DNA ligase, 1 μM each DNA species but without Complex5.

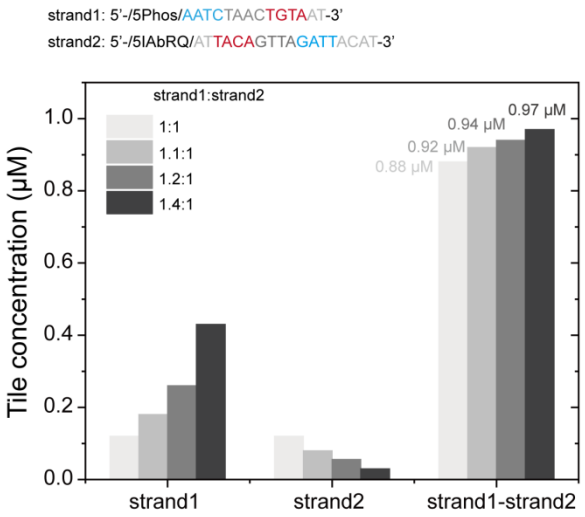

Figure S3. Nupack simulation for pairing of Reporter5. Equilibrium concentration of each ssDNA and dsDNA with different ratio of strand1 and strand2 were simulated. Condition: 1, 1.1, 1.2, or 1.4 μM strand1, 1 μM strand2, 50 mM Na^{+}, and 10 mM Mg^{2+} at 37 °C. The simulation indicates 12 % leakage of Reporter5 when those two ssDNA are at the same stoichiometry, while increasing the ratio of strand1 significantly decreases the leakage (Only 3 % leakage exists when the ratio is 1.4:1).