Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

# Dissipative Organization of DNA Oligomers for Transient Catalytic Function

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**Materials and methods**

**Instrumentation:** ThermoMixer (Eppendorf), gel electrophoresis chambers (biostep), Enduro power supply 300 V (Labnet International, Inc.), INTAS *CHEMOSTAR touch* fluorescence imager (INTAS Science Imaging), ScanDropR UV-VIS spectrometer (Analytik Jena), 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. Gene ruler 50 bp DNA ladder (ready to use), and DNA gel loading dye (6×) were supplied by ThermoFisher Scientific. Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, biology grade) was supplied by CALBIOCHEM. Sodium chloride (NaCl, 99%), hexadecane (99%), and 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. RotiR-GelStain (1,1'3,3',5,5'6,6'-Octamethyl-2,2'-spiro(2,3-dihydro-1H-Benzimidazol) was supplied by Carl Roth. All oligonucleotides purified by high-performance liquid chromatography were supplied by Integrated DNA Technologies Inc. (IDT), and the sequences and modifications are shown below.

**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.

Milli-Q water was used throughout of this study.

**Supplementary Table 1:** Oligonucleotide sequences used, with their name in individual Figure, the sequence codes used for ordering at IDT.

| | Name | Oligonucleotide sequence (5'-3') | #nt |
|---|---|---|---|
| **Figures 2-4, S2** | Complex 1 | CATGAGAATTCCATTCACGGTCTCT | 25 |

| | | | |
|---|---|---|---|
| | | /5Phos/GATTAGAGACCGTGAATGGAATTCTCATG | 29 |
| **Figure 2** | Segment 1 | /5Phos/AATCCTTATC/3Cy5/ | 10 |
| | Segment 2 | /5IAbRQ/TTTTTATAAG | 10 |
| **Figures 3, S2** | Complex 3 | GAATAGAAAGCTTGACGAGGTCTCA | 25 |
| | | /5Phos/GCCATGAGACCTCGTCAAGCTTTCTATTC | 29 |
| **Figure 3** | Segment 3 | GATCTGATGTATGT | 14 |
| **Figures 3, S2** | Segment 4 | /5Phos/AATCACATACTGCATACTACGACT | 24 |
| | Segment 5 | /5Phos/TGGCAGTCGTTCAATACA | 18 |
| **Figure 3** | Segment 6 | /5IAbRQ/GATCTGATTTTTGTATGT | 18 |
| | Segment 7 | /5Phos/AATCACATACTTTTTGCATACTTTTTACGACT | 32 |
| | Segment 8 | /5Phos/TGGCAGTCGTTTTTTCAATACA | 22 |
| **Figures 3, S2** | Target 1 | TGTATTGATTTTTTTTAGTATGCATTTTTTTTATCAGATC/3Cy5/ | 40 |
| | Target 2 | TGTATTGATTTAGTATGCATTTATCAGATC/3Cy5/ | 30 |
| | Target 3 | GTATTGATTTGTATGCATTTTCAGATC/3Cy5/ | 27 |
| | Target 4 | TGTATTGATTTAGTTTGCATTTATCATATC/3Cy5/ | 30 |
| **Figure S2** | Segment 9 | /5IAbRQ/GATCTGATGTATGT | 14 |
| **Figure 4** | Segment 10 | TAGCTGAGCGATAATAA | 17 |
| | Segment 11 | /5Phos/AATCTTATTCACCCATGTTGAAGA | 24 |
| | Substrate | /5Cy5/TCTTCATrAGCAGCTA/3IAbRQSp/ | 15 |

## Experimental protocol

**DNA stock solutions.** All DNA strands were used as received. All single-stranded (ss) DNA oligos were dissolved in certain amounts of annealing buffer (10 mM Tris-HCl (pH 8.0), 50 mM NaCl) 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 a UV-VIS spectroscopy. Double-stranded (ds) DNA Complexes were annealed from two ssDNA strands with the same stoichiometry at room temperature overnight. All the stock solutions were stored at -20 °C for further use.

**ATP-fueled transient organization of DNA segments**

The experiments were performed in 1× *NEB* CutSmart buffer at 37 °C with a total volume of ca. 30 µL containing 10 µM Complex 1, 10 µM Segment 1, 10 µM Segment 2, 0.92 WU T4 DNA ligase, 1 U BsaI, and varied concentration of ATP (0.03, 0.06, and 0.12 mM). 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), 3 µL aliquots of the reaction solution were collected and quenched by 2 µL of quenching buffer (200 mM EDTA, 10 mM Tris-HCl (pH 8.0), 50 mM NaCl). All the collected samples were stored at -20 °C for further analysis.

Afterwards, 1 µL 6× DNA gel loading dye (10 mM Tris-HCl (pH 7.6), 0.03 % bromophenol blue, 0.03 % xylene cyanol FF, 60 % glycerol, and 60 mM EDTA) was added to all the quenched samples and then the mixtures were analyzed by 2 wt.% agarose gel electrophoresis (AGE) containing ca. 0.02 % (v/v) RotiR-GelStain at 105 V for 1.5

h run time at room temperature in TAE buffer. The results were recorded by an INTAS *CHEMOSTAR touch* fluorescence imager via UV excitation. The yield of Complex 2 was quantified by ImageJ software according to our previous report.[1]

**ATP-fueled transient Förster resonance energy transfer (FRET)**

The experiments were carried out in 1× *NEB* CutSmart buffer at 37 °C containing 10 μM Complex 1, 10 μM Segment 1, 10 μM Segment 2, 0.92 WU T4 DNA ligase, 1 U BsaI, and varied concentration of ATP (0, 0.03, 0.06, and 0.12 mM). The experiments were carried out in a total 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.

**ATP-fueled transient sequence-defined oligomers**

The experiments were performed in 1× *NEB* CutSmart buffer at 37 °C containing 10 μM Complex 1, 10 μM Complex 3, 10 μM Segment 3, 10 μM Segment 4, 10 μM Segment 5, 0.92 WU T4 DNA ligase, 1 U BsaI, and varied concentration of ATP (0.1, 0.2, and 0.4 mM). The experiments were carried out in a total volume of ca. 25 μL. At different time intervals (0 min, 10 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, and 48 h), 2 μL aliquots of the reaction solution were collected and quenched by 3 μL of quenching buffer. Afterwards, all the collected samples were analyzed by 2 wt.% AGE containing ca. 0.02 % (v/v) RotiR-GelStain at 80 V for 2.5 h run time at room temperature in TAE buffer. The results were recorded by an INTAS *CHEMOSTAR touch* fluorescence imager via UV excitation. Time-dependent yield of the sequence-defined oligomers was quantified by ImageJ software.

**Kinetically trapped ATP-fueled multivalent hybridization**

The experiments were conducted in 1× *NEB* CutSmart buffer at 37 °C using 10 μM Complex 1, 10 μM Complex 3, 10 μM Segment 9, 10 μM Segment 4, 10 μM Segment 5, 5 μM Target (1, 2, 3, or 4), 0.92 WU T4 DNA ligase, 1 U BsaI, and 0.1 mM ATP. The experiments were carried out in a total volume of ca. 25 μL in a black 384-well plate, and the results were recorded by a plate reader (see above) (see results in Figure S2).

Control experiments were performed the same as above by using Target 1 and 0 mM ATP.

**ATP-fueled transient multivalent hybridization**

The experiments were conducted in 1× *NEB* CutSmart buffer at 37 °C using 10 μM Complex 1, 10 μM Complex 3, 10 μM Segment 6, 10 μM Segment 7, 10 μM Segment 8, 5 μM Target (1, 2, 3, or 4), 0.92 WU T4 DNA ligase, 1 U BsaI, and 0.1 mM ATP. The experiments were carried out in a total volume of ca. 25 μL in a black 384-well plate, and the results were recorded by a plate reader (see above).

**ATP-fueled transient DNAzyme**

The experiments were carried out in 1× *NEB* CutSmart buffer at 25 °C containing 10 μM Complex 1, 10 μM Segment 10, 10 μM Segment 11, 0.46 WU T4 DNA ligase, 1 U BsaI, and 0.03 mM ATP. The experiments were carried out in a total volume of ca. 50 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), 3 μL aliquots of the reaction solution were collected and quenched by 2 μL of quenching buffer.

Afterwards, all the collected samples were analyzed by 2 wt.% AGE containing ca. 0.02 % (v/v) RotiR-GelStain at 105 V for 1.5 h run time at room temperature in TAE buffer. The results were recorded by an INTAS *CHEMOSTAR touch* fluorescence imager via UV excitation. The time-dependent yield of the transient DNAzyme was quantified by ImageJ software (see results in Figure S3).

**ATP-fueled dynamic transient DNAzyme for RNA cleavage**

The experiments were carried out in 1× *NEB* CutSmart buffer at 25 °C containing 10 μM Complex 1, 10 μM Segment 10, 10 μM Segment 11, 0.46 WU T4 DNA ligase, 1 U BsaI, 0.03 mM ATP, and varied concentration of RNA substrate (0.33, 0.67, 1.33, 2.0, and 4.0 μM). The experiments were carried out in a total volume of ca. 25 μL in a black 384-well plate, and the results were recorded by a plate reader (see above).

Control experiments were conducted the same as above by using 4.0 μM RNA substrate and 0 mM ATP (see results in Figure S5).

**ATP-powered static DNAzyme for RNA cleavage**

The experiments were carried out in 1× *NEB* CutSmart buffer at 25 °C containing 10 μM Complex 1, 10 μM Segment 10, 10 μM Segment 11, 0.46 WU T4 DNA ligase, 0.03 mM ATP, and varied concentration of RNA substrate (2.0 and 4.0 μM). The experiments were carried out in a total volume of ca. 25 μL in a black 384-well plate, and the results were recorded by a plate reader (see above).

**Multiple activation of DNAzyme for repeated catalytic functions**

The experiments were carried out in 1× *NEB* CutSmart buffer at 25 °C containing 10 μM Complex 1, 10 μM Segment 10, 10 μM Segment 11, 0.46 WU T4 DNA ligase, 1 U BsaI, 5 μM ATP, and 2.67 μM RNA substrate. The experiments were carried out in a total volume of ca. 25 μL in a black 384-well plate, and the results were recorded by a plate reader (see above). At ca. 90 min, 10 μM ATP was added to start the second cycle of DNAzyme activation. At ca. 200 min, another 10 μM ATP was added to start the third cycle of DNAzyme activation.

**Relative kinetics calculation**

The kinetics for RNA substrate cleavage via both dynamic and static DNAzymes were calculated from the linear regions of the fluorescence intensity plots in Figure 4c and d. The plots for the first 30 min were used for the calculations using the following equation: $P = k_{obs}t + b$, where P is the amount of product (overall fluorescence intensity for this case), $k_{obs}$ is the observed rate of product formation, and b is the initial background overlapping with the yielded product (fluorescence intensity at $t = 0$). By linear fitting of the plots in Figure 4c and d, we obtained $k_{obs}$ for all the RNA cleavage reactions. By normalizing those values to $k_{obs}$ for ATP-fueled transient DNAzyme using 0.33 μM RNA substrate, relative kinetics ($k_{relative}$) for all the reactions can be further calculated, which has been plotted and shown in Figure 4e.

**Supplementary Note 1. Kinetically trapped intermediate from BsaI restriction**

It is worth noting that the ATP-fueled transient sequence-defined oligomer in Figure 3a (main manuscript) fail to realize transient multivalent sensing with those barcodes embedded targets (Figure S2). The experimental

systems were set at 10 µM Complex 1, 10 µM Complex 3, 10 µM Segment 9, 10 µM Segment 4, 10 µM Segment 5, 5 µM Target (1, 2, 3, or 4), 0.92 WU T4 DNA ligase, 1 U BsaI, and 0.1 mM ATP. Time-dependent FI measurements of the systems using Targets 1-3 show quick FI decrease after adding ATP, indicating ATP-fueled multivalent hybridization between the sequence-defined oligomer and the targets, while the recovery of FI after depleting ATP is time-consuming. It is known from Figure 3b,c that the sequence-defined oligomer fueled by 0.1 mM ATP has a lifetime of ca. 5 h, and the presence of targets shows no effect on the lifetime of the ATP-driven enzymatic reaction network. However, in this investigation transient system is not achievable and no obvious FI recovery can be observed even after 22 h. Only a slight FI recovery is observed for the transient system using Target 1, which is due to the relatively weaker binding affinity with Target 1 due to its long junctions between barcodes (8 nucleotides (nt)). The system using Target 2 shows a quite flat plateau of the FI even after 22 h due to its even stronger binding affinity because of the 3 nt junctions between the barcodes, while the system using Target 3 shows a very slight recovery of FI after 22 h, which is due to a shortening of the barcodes length from 8 to 7 nt. The existing of two mutations in the barcodes in Target 4 disables its multivalent recognition by the sequence-defined oligomer. All the results indicate that after ATP is consumed, the intermediates from BsaI restriction are kinetically trapped, preventing their dissociation, and, thus, FI recovery. To prevent the kinetically trapped state of the intermediates after BsaI restriction, a spacer between each sticky end and the barcode in Segments 4, 5, and 9 is needed to weaken the binding after BsaI restriction (Figure 3d in main manuscript).

## Supplementary Figures

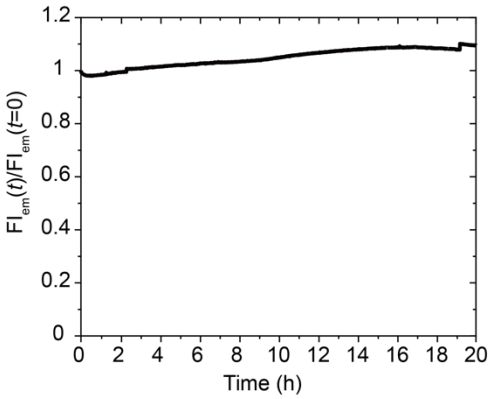


**Figure S1. Control for ATP-fueled transient FRET.** Time-dependent fluorescent intensity (FI) for monitoring the FRET signal from ATP-driven transient organization of functional Segments using 10 μM Complex 1, 10 μM Segment 1, 10 μM Segment 2, 0.92 WU T4 DNA ligase, 1 U BsaI, and 0 mM ATP.

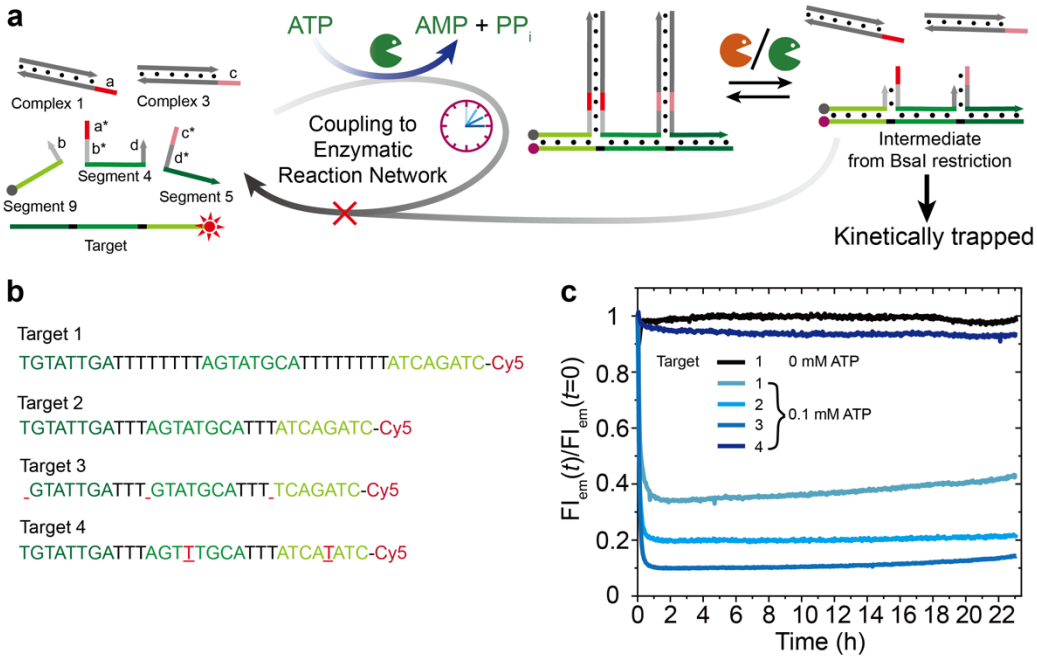


**Figure S2. ATP-fueled hybridization probe.** (**a**) Schematic representation of kinetically trapped ATP-driven hybridization probe. (**b**) Detailed sequences of the targets. (**c**) Time-dependent FI measurements of the ATP-driven multivalent hybridization systems using different targets. Conditions: 37 °C, 10 μM Complex 1, 10 μM Complex 3, 10 μM Segment 9, 10 μM Segment 4, 10 μM Segment 5, 5 μM Target (1, 2, 3, or 4), 0.92 WU T4 DNA ligase, 1 U BsaI, and 0.1 mM ATP. Control experiments were performed by using Target 1 and 0 mM ATP.

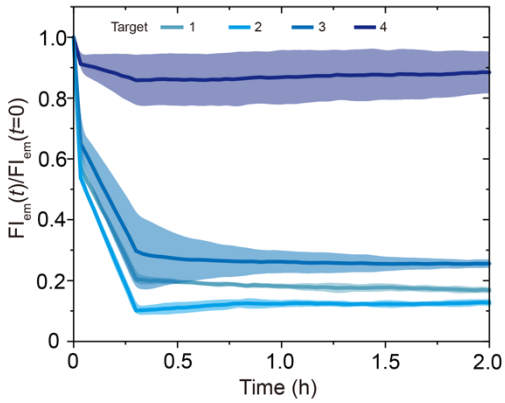


**Figure S3. Early stage of fluorescence quenching for Figure 3e in main manuscript.** Time-dependent FI measurements corresponding to transient sensing of different targets. The shaded areas correspond to the standard deviation of duplicate measurements. Conditions: 37 °C, 10 µM of each DNA species for the sequence-defined oligomer, 5 µM target, and 0.1 mM ATP.

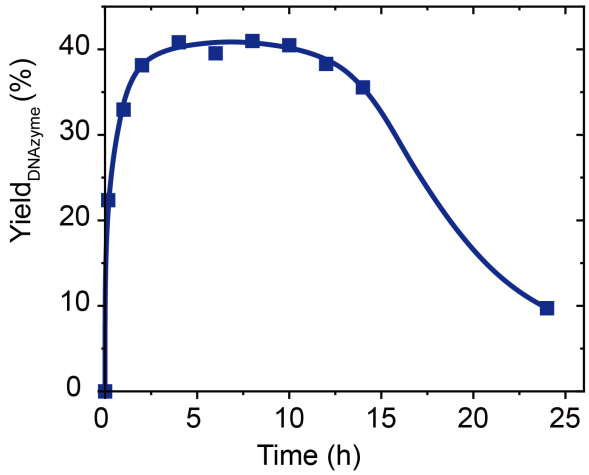


**Figure S4. ATP-fueled transient DNAzyme.** Time-dependent yield of DNAzyme in the ATP-fueled transient DNA ligation quantified from AGE data. Line is a guide to the eye. Conditions: 25 °C, 10 µM Complex 1, 10 µM Segment 10, 10 µM Segment 11, 0.46 WU T4 DNA ligase, 1 U BsaI, and 0.03 mM ATP.

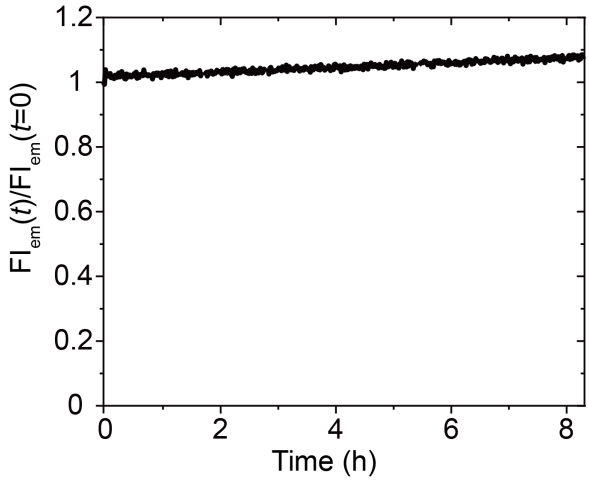


**Figure S5. Control for RNA cleavage by ATP-fueled transient DNAzyme.** Time-dependent FI measurements for monitoring RNA cleavage by DNAzyme using 10 µM Complex 1, 10 µM Segment 10, 10 µM Segment 11, 0.46 WU T4 DNA ligase, 1 U BsaI, 4.0 µM RNA substrate, and 0 mM ATP.

**Reference**

[1] J. Deng, A. Walther, *Nat. Commun.* **2020**, *11*, 3658.