Temporal and reversible control of a DNAzyme by orthogonal photoswitching

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Methods/Materials

UV/Vis Spectrometry: UV/Vis absorbance spectrometry measurements were carried out at 1 μM of $C_{DM\text{-}Azo}$ or C_{AAP} in hybridization buffer in a Hellma Analytics High Precision 10 mm Quartz SUPRASIL® cuvette (Type No. 105-204-QS) and measured in a Jasco UV/Vis spectrophotometer V630 BIO equipped with a PAC 743 Auto Peltier 6-Cell Changer and a Julabo F250 recirculating cooler. Irradiations were performed with an in-house built LED machine at 50 °C, 700 mA, for 4 minutes with the exception of those at 350 nm lasting only 2 minutes at the LED's maximum current capacity of 30 mA. The baseline measurement for AAP samples used an unmodified C oligo (CTCACTCAGCAATTTCACCAGTACTACAGTTAAGGCAGC, Metabion GmbH) at 1 μM and hybridization buffer for DM-Azo samples, which were also used for auto-zeroing before measuring. Measurements were taken immediately after irradiation. The curves were zeroed and DM-Azo results were normalized to the DNA peak. A 2nd order smooth using 5 neighbors was performed with GraphPad Prism software.

Hybridization Studies: Hybridization was characterized via fluorescence without G quadruplex formation by replacing the G triplets with fluorophore and quencher pairs, namely Hex on the 3' end of A-ODN (A-Hex: ACAGGCGGCCTTAACTGTAGTT-Hex) and BHQ-2 on the 5' end of B-ODN (B-BHQ2: BHQ2-TCTGGTGAAATTGCTGCC), oligonucleotides ordered from Metabion GmbH. A-Hex and B-BHQ2 were combined at 0.6 μM with 0.4 μM C_{AAP}-ODN, and 0.2 μM $C_{DM\text{-}Azo}$-ODN in reaction buffer (25 mM HEPES, 20 mM KCl, 200 mM NaCl, 10% DMSO, pH 8). Separated controls were prepared with A-Hex, B-BHQ2, and either C_{AAP}- or $C_{DM\text{-}Azo}$-ODN in equimolar concentrations at 0.3 μM to avoid background fluorescence interference. The positive control used an unmodified C-ODN for maximum expected hybridization and the negative control used the reverse complement of the C-ODN (GCCTTAACTGTAGTACTGGTGAAATTGCT, Ella Biotech GmbH) for no hybridization. Irradiations at 365 nm, 590 nm, and 450 nm were performed with an in-house built LED machine at 50 °C, 700 mA, for 4 minutes and irradiations at 350 nm at 30 mA for 2 minutes. After irradiation, samples were incubated for 20 minutes at room temperature. 30 μL of each sample was added to a black flat bottom 384-well plate, excited at 535 nm, and emission at 556 nm measured using an EnSpire Multimode Plate Reader (PerkinElmer) at 25 °C. Results were normalized by subtracting the negative control's signal from each signal and dividing by the difference between the positive and negative controls' signals, effectively setting the positive control as 100% hybridization and the negative control as 0% hybridization.

Circular Dichroism: CD measurements were carried out on a Jasco 810 Spectropolarimeter with A-ODN, B-ODN, and $C_{DM\text{-}Azo}$- or C_{AAP}-ODN oligonucleotides at 1 μM in hybridization buffer without potassium (noK: 25 mM HEPES, 200 mM NaCl, 10% DMSO, pH 8). The samples were irradiated at 365 nm, 700 mA, 50 °C, for 4 minutes and measured (365 nm-noK). The DM-Azo samples were then irradiated at 450 nm and AAP samples at 590 nm, 700 mA, 50 °C, for 4 minutes and measured (450/590 nm-noK). NoK buffer was used for baseline correction. Potassium Chloride (4 μM) was added to each sample to 20 mM and each sample was measured again (450/590 nm-wK) using buffer with potassium for baseline correction. The samples were then irradiated at 365 nm, 700 mA, 50 °C, for 4 minutes and measured (365 nm-wK). Results were accumulated over 3 scans and normalized by zeroing the first 20 points and smoothed to the 2nd order, 5 neighbors, using GraphPad Prism software. The smoothed 365 nm-wK curves were subtracted from the smoothed 450/590 nm-wK curves.

Activity Assays: Experiments were performed at concentrations of 0.6 μM for both A-ODN and B-ODN oligo, 0.4 μM C_{AAP}, and 0.2 μM $C_{DM\text{-}Azo}$ in reaction buffer (25 mM HEPES, 20 mM KCl, 200 mM NaCl, 10% DMSO, pH 8). Strands A-ODN (ACAGGCGGCCTTAACTGTAGTT GGGTAGGGCGGG) and B-ODN (TGGGTCTGGTGAA ATTGCTGCC) were ordered from Metabion GmbH. The DM-Azo phosporamidite was synthesized in-house and provided to Ella Biotech GmbH for introduction into the DNA sequence for $C_{DM\text{-}Azo}$ (AGCAATTTCACCAGTA CxTAxCAxGTxTAxAGxGC, x=DM-Azo).

Strand C_{AAP} (AGCAATTTCACC AGTACToACAoGTToAAGoGC, o=AAP) was synthesized in-house using previously reported methods[14] and subsequently purified by reversed phase HPLC. Separated controls were prepared identically as the combined system, omitting either C_{AAP}- or $C_{DM\text{-}Azo}$-ODN. The experimental solution was aliquoted after every step; "dark adapted" (no irradiation), 365 nm (all *cis*), 590 nm (AAP *trans*), 450 nm (all *trans*), 350 nm (DM-Azo *trans*) over five cycles for the combined system and two cycles for the separated controls. Irradiations were performed with an in-house built LED machine at 50 °C, 700 mA, for 4 minutes with the exception of irradiations at 350 nm being at 30 mA for 2 minutes. After irradiation, hemin was added to 0.9 μM and the samples incubated at 25 °C for 90 minutes. Before measurement, 0.6 μL of 100 mM ABTS was added to 24.4 μL of each sample in a clear flat bottom 384-well plate and mixed. Measurements were performed on an EnSpire Multimode Plate Reader (PerkinElmer) at 25 °C measuring absorbance at 414 nm over time upon dispensing 5 μL of 12 mM H_2O_2 into each well. The experiment was independently repeated three times and the reaction rate was calculated by taking the slope during the linear phase of the reaction. Reaction rates were calculated by averaging the slopes during the linear phase of the reaction and normalized to the highest rate.

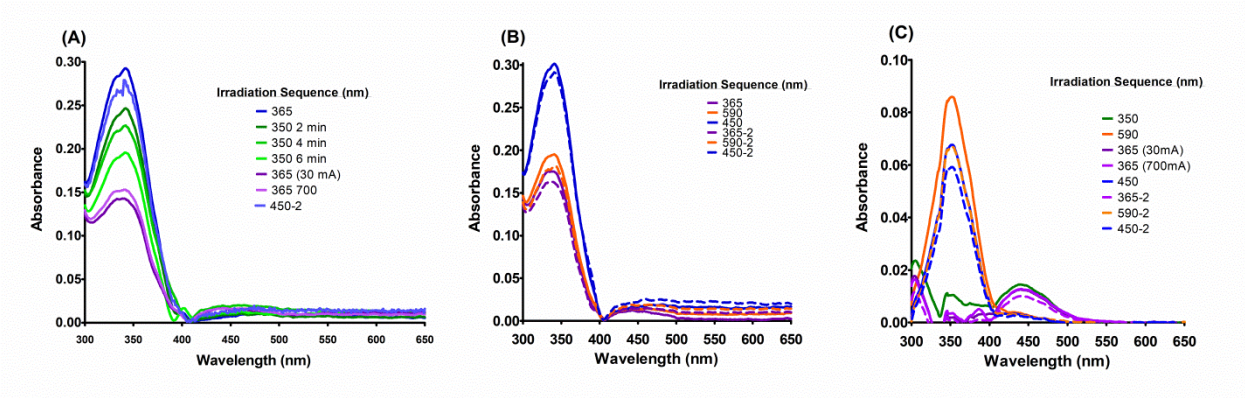


Figure S1. UV/Vis spectra for 1 µM (A, B) $C_{DM\text{-}Azo}$ and (B) C_{AAP} after irradiation at various wavelengths.

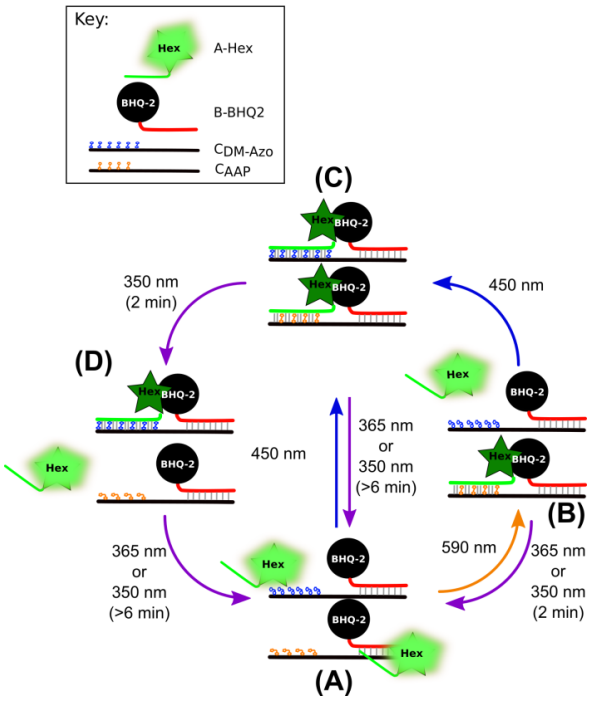


Figure S2. Schematic diagram for fluorescence hybridization assays. When AAP and DM-Azo are in *cis*, A-Hex cannot hybridize to $C_{DM\text{-}Azo}$- or C_{AAP}-ODN and fluoresces. When both photoswitches are in *trans*, A-Hex hybridizes and is quenched

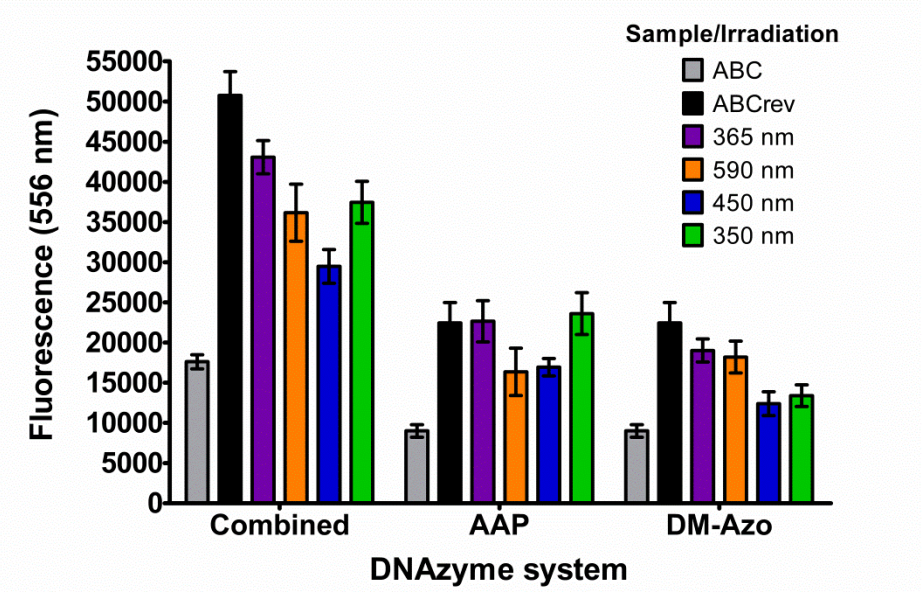


Figure S3. Non-normalized hybridization efficiency results. Hex fluorophore is on the A-ODN that hybridizes to the modified part of the C-ODN and BHQ-2 is on the B-ODN that hybridizes to the unmodified part of the C-ODN. When AAP or DM-Azo is in *cis*, A-Hex dehybridizes and fluoresces. When in *trans*, A-Hex hybridizes and is quenched. The positive control (ABC) uses an unmodified C-ODN oligo for maximum hybridization. The negative control (ABCrev) uses the reverse compliment of C-ODN for no hybridization. For combined samples, A-Hex, B-BHQ-2, C-ODN, and $C\text{-}ODN_{rev}$, are at 600 nM while $C_{DM\text{-}Azo}$ is at 200 nM and C_{AAP} is at 400 nM. All ODNs are at 300 nM in samples where AAP and DM-Azo are separate. Error bars: S.D. (n=3).

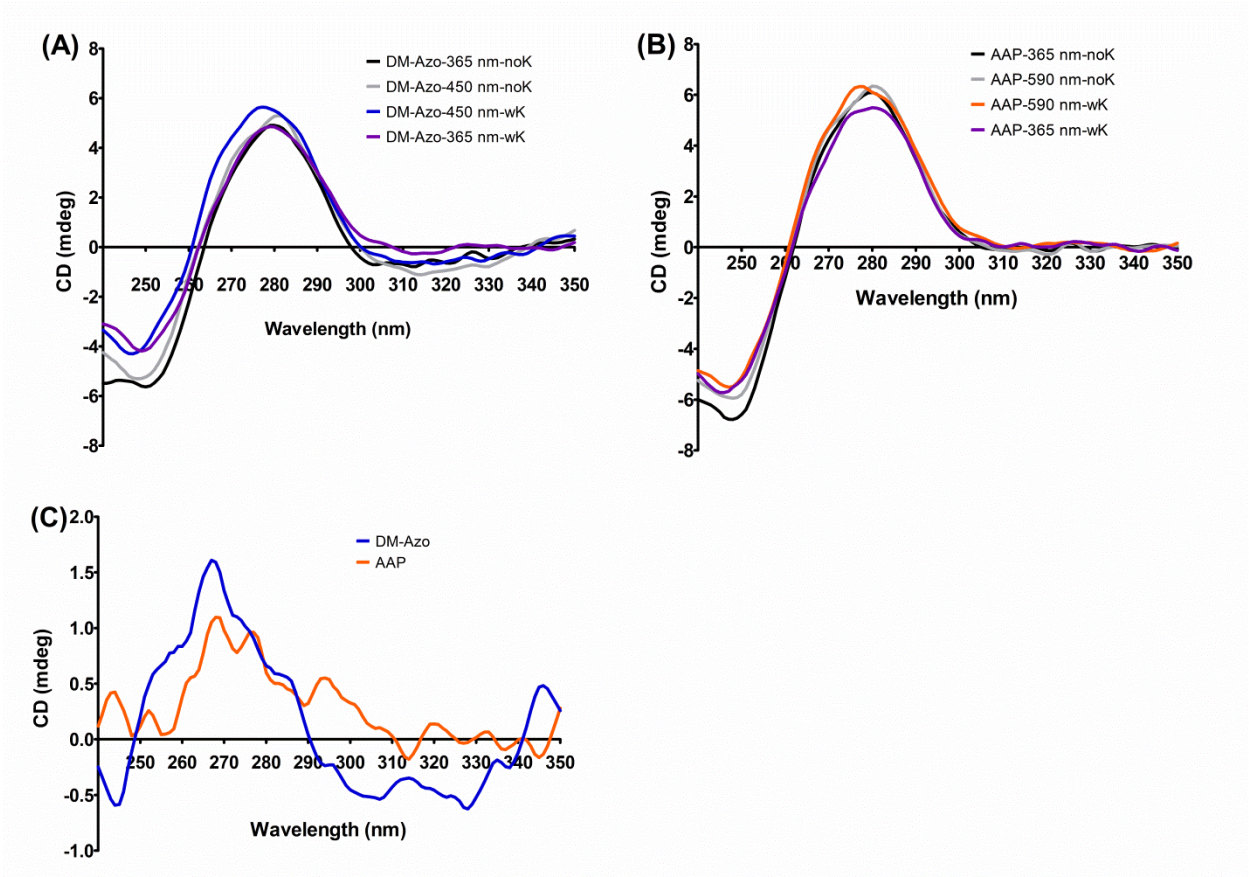


Figure S4. CD signals of (A) DM-Azo and (B) AAP DNAzymes. Samples were prepared at 1 µM in buffer without potassium and irradiated at 365 nm. The CD signal was measured, then DM-Azo samples were irradiated at 450 nm and AAP samples at 590 nm before measuring CD signals again. Potassium was added to each sample and the CD signal (photoswitches still in *trans*) was measured before irradiating at 365 nm and measuring again. Results were accumulated over 3 scans and smoothed to the 2nd order, 5 neighbors. (C) Smoothed *cis* signal subtracted from the smoothed *trans* signal for DM-Azo and AAP DNAzymes.

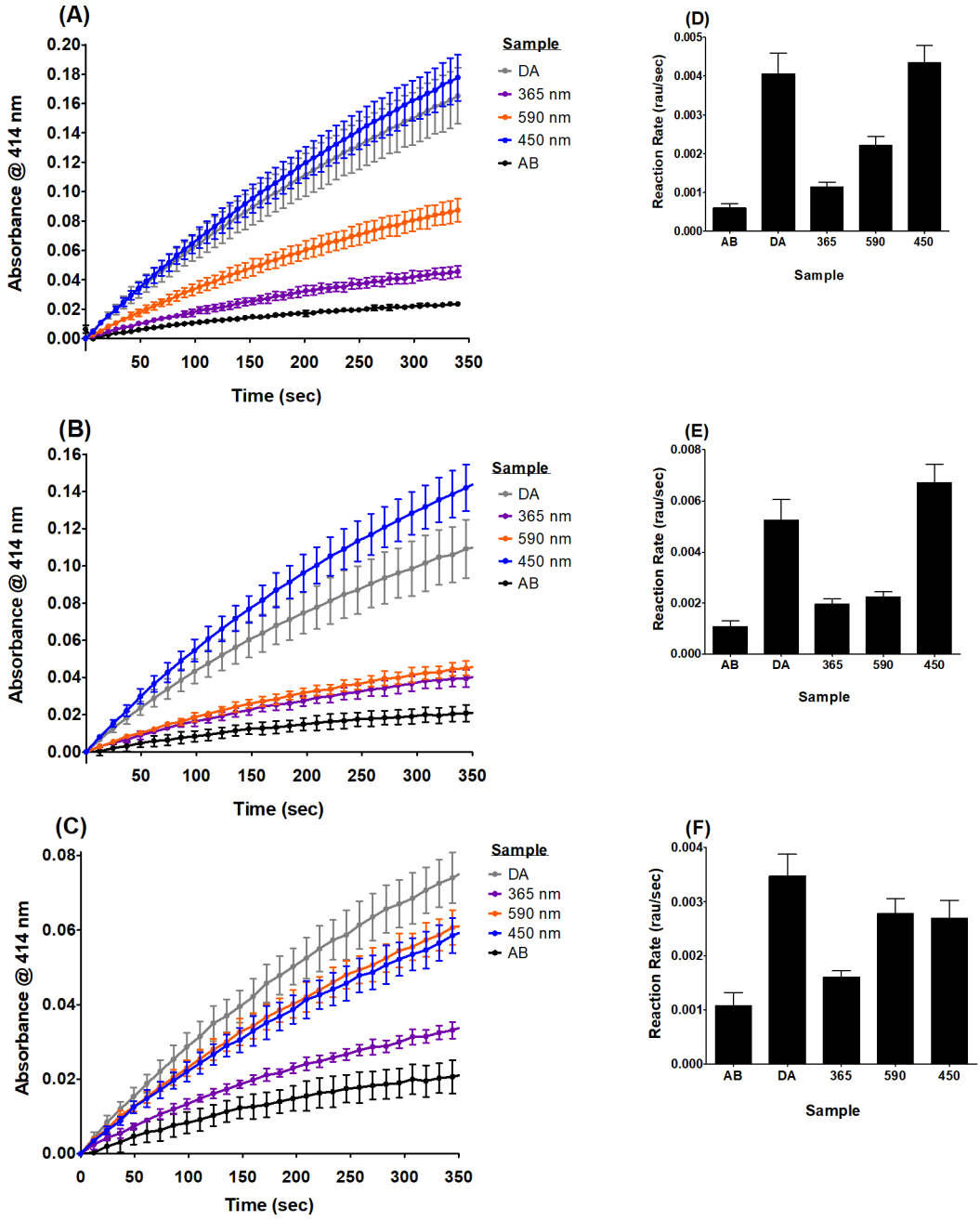
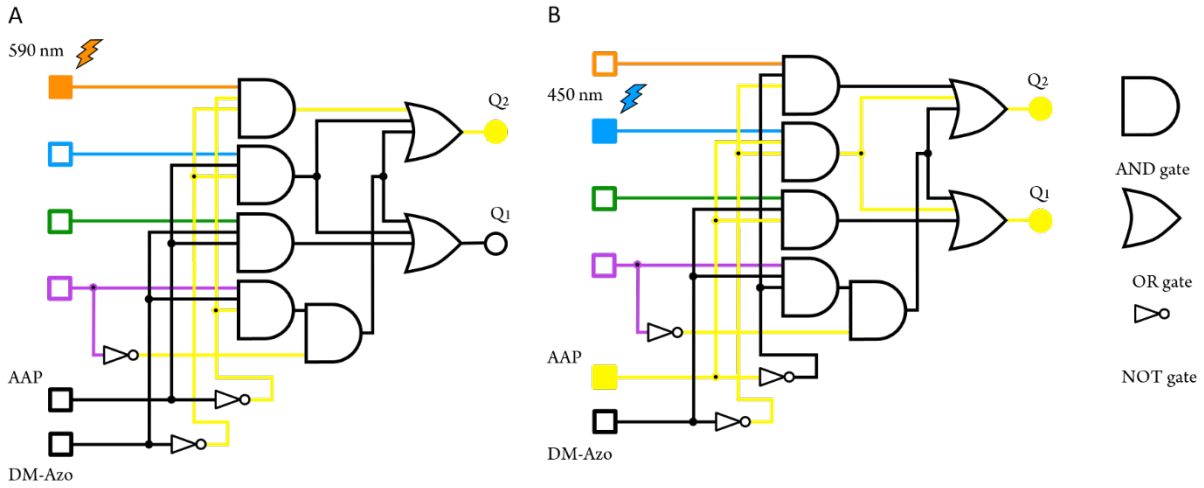
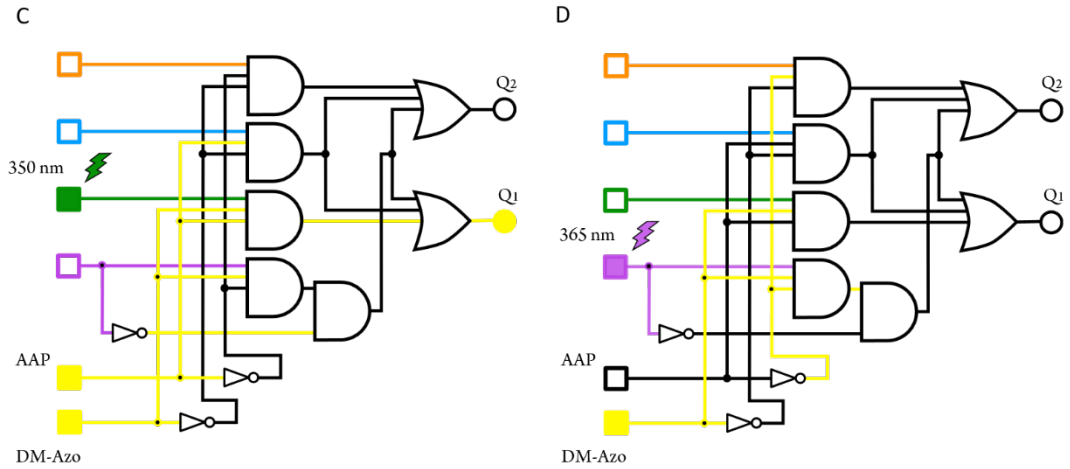


Figure S5. Activity assay results (curves, reaction rates) over one switching cycle for (A, D) AAP and DM-Azo combined, (B, E) DM-Azo only, and (C, F) AAP only DNAzymes. After 365 nm irradiation, the DNAzymes exhibit limited catalytic activity due to both AAP and DM-Azo being in *cis*, thus no hybridization and no catalytic G4. Upon 590 nm irradiation, AAP switches to *trans* and AAP DNAzymes are active. After 450 nm irradiation, DM-Azo switches to *trans*, both DNAzymes are active and the system exhibits maximum catalytic activity. A- and B-ODNs are at 600 nM while $C_{DM\text{-}Azo}$ is at 200 nM and C_{AAP} is at 400 nM. Error bars: S.D. (n=3).



Select Inputs (initial settings)		Data Inputs (Irradiation)				Output	
DM-Azo	AAP	590 nm	450 nm	350 nm	365 nm	Q_1	Q_2
0	0	1	x	X	x	0	1
0	1	x	1	X	x	1	1
1	1	x	x	1	x	1	0
1	0	x	x	x	1	0	0

Select Inputs (initial settings)		Data Inputs (Irradiation)				Output	
DM-Azo	AAP	590 nm	450 nm	350 nm	365 nm	Q_1	Q_2
0	0	1	x	X	x	0	1
0	1	x	1	X	x	1	1
1	1	x	x	1	x	1	0
1	0	x	x	x	1	0	0

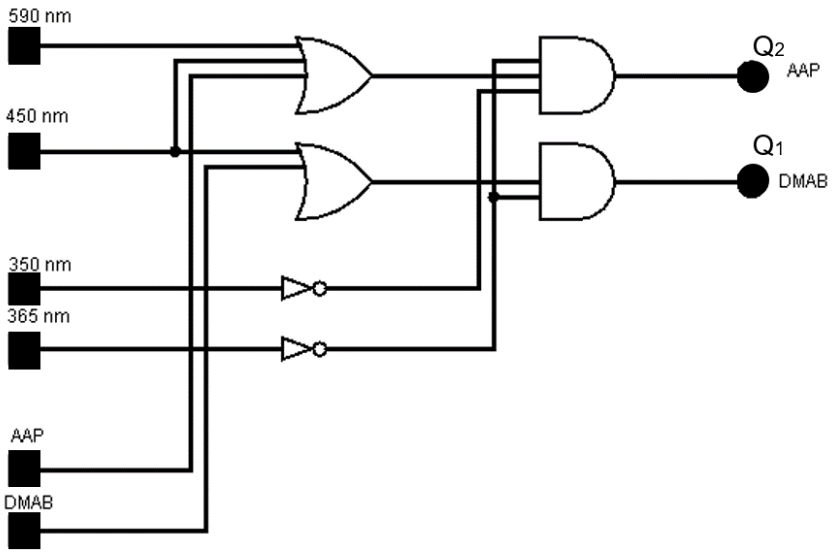


Select Inputs (initial settings)		Data Inputs (Irradiation)				Output	
DM-Azo	AAP	590 nm	450 nm	350 nm	365 nm	Q_1	Q_2
0	0	1	x	X	x	0	1
0	1	x	1	X	x	1	1
1	1	x	x	1	x	1	0
1	0	x	x	x	1	0	0

Select Inputs (initial settings)		Data Inputs (Irradiation)				Output	
DM-Azo	AAP	590 nm	450 nm	350 nm	365 nm	Q_1	Q_2
0	0	1	x	X	x	0	1
0	1	x	1	X	x	1	1
1	1	x	x	1	x	1	0
1	0	x	x	x	1	0	0

Figure S6. 4:2 multiplexer with different switching options. The corresponding input and output variables are highlighted in yellow in the truth table below the logic circuits. The output signal defines the new state of the select inputs considering the outputs Q1 and Q2 corresponding to the state DM-Azo and AAP respectively. A) Starting from the select inputs both set to "0" the 590 nm output is sent to the output Q_2 returning "1" and Q_1 returns "0." In B when the select inputs are set to DM-azo "0" and AAP to "1" the 450 nm input "1" is directed to the both outputs. Select inputs both set to "1" as in C directs the 350 nm input to the output Q_1 while the output Q_2 returns "0." D) Select input DM-Azo = "1" and AAP = "0" returns the input of 365 nm as "0" to both outputs.

A



B

Select Inputs (initial settings)		Data Inputs (Irradiation)				Output		Photostationary state of the output	
DMAzo	AAP	590 nm	450 nm	350 nm	365 nm	Q_1	Q_2	DMAzo	AAP
1	1	0	0	0	0	1	1	trans	trans
trans	trans	1	0	0	0	1	1	trans	trans
		0	1	0	0	1	1	trans	trans
		0	0	1	0	1	0	trans	cis
		0	0	0	1	0	0	cis	cis
0	1	0	0	0	0	0	1	cis	trans
cis	trans	1	0	0	0	0	1	cis	trans
		0	1	0	0	1	1	trans	trans
		0	0	1	0	0	0	cis	cis
		0	0	0	1	0	0	cis	cis
1	0	0	0	0	0	1	0	trans	cis
trans	cis	1	0	0	0	1	1	trans	trans
		0	1	0	0	1	1	trans	trans
		0	0	1	0	1	0	trans	cis
		0	0	0	1	0	0	cis	cis
0	0	0	0	0	0	0	0	cis	cis
cis	cis	1	0	0	0	0	1	cis	trans
		0	1	0	0	1	1	trans	trans
		0	0	1	0	0	0	cis	cis
		0	0	0	1	0	0	cis	cis

Figure S7. Theoretical application of the orthogonally and temporally controlled DNAzyme as a complex logic circuit. A) Representation of the logic circuit. B) Truth table associated to the logic circuit.