Supporting information

for

A Synthetic Light-Driven Substrate Channeling System for Precise Regulation of Enzyme Cascade Activity Based on DNA Origami

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1. Experimental details

Synthesis of aozbenzene-modified DNA. Azobenzene-modified oligonucleotides were synthesized on a 12-Column DNA Synthesizer (PolyGen GmbH) based on the synthesis protocol provided by the reagents' manufacturers.[1] Briefly, azobenzene groups were incorporated into DNA using the phosphoramidite monomer. After machine synthesis, all the DNA products were cleaved from the solid support by incubating with ammonia and methylamine (1:1, v/v) at 65 ℃ for 30 min in a water bath. In order to precipitate DNA, 40 μL 3.0 M NaCl and 1.2 mL ethanol were added and incubated at -20 ℃ for 30 min. After removing the supernatant, the precipitated DNA product was purified by reversed phase HPLC (Agilent 1100) using a C18 column with the elution gradient of 200 mM TEAA (pH 7.5) and acetonitrile (0-4 min: 0-0%; 4-30 min: 10-65%). After HPLC purification, the DNA product was detritylated with 200 μL 80% acetic acid for 20 minutes and desalted using NAP-5 (GE healthcare). The purified strand was dissolved in ultrapure water and quantified by UV absorption at 260 nm. The HPLC of azobenzene-modified DNA is shown in Fig S3.

Gel electrophoresis characterization. For agarose gel electrophoresis, 1.2% agarose was used for separation of different assemblies of DNA origami. The gel was run in iced 1×TAE/Mg buffer at 100 V for 1 hour in the dark. For polyacrylamide gel electrophoresis (PAGE), 12% native-PAGE was used for characterizing the duplex or single-strand oligonucleotides. The gel was run in 1×TBE buffer at 120 V for 1 hour at room temperature in the dark.

AFM imaging. 20 μL 4 nM sample was deposited on a freshly prepared mica surface. After 3 min, excess DNA strand or enzyme was removed with washing with 40 μL 1× TAE/Mg buffer for three times. AFM imaging was performed using BL-AC40TS (Olympus) tips in AC water mode on a cypher S instrument (Asylum Research)

Light irradiation conditions for photoisomerization of azobenzene. UV light (Spectroline Model SB-100P/FA UV lamp) at 365 nm was used in the experiment. The visible light was provided by a 500 W high-pressure mercury lamp (CHF-XM-500 W, Beijing, China) with 450 nm band pass filter (Giai Photonics co., Shenzhen, China). The distance between the samples and the light resource was fixed, and the optical density was measured by the optical densitometer (CEL-NP2000, Beijing Au-light Co., China) at 4 cm from the center of the lamp. The power density of the UV lamp was 50 mW/cm^2, while the power density of mercury lamp with 450 nm filter was 20 mW/cm^2. During irradiation, the sample was kept at room temperature.

2. Enzyme cascade reaction scheme

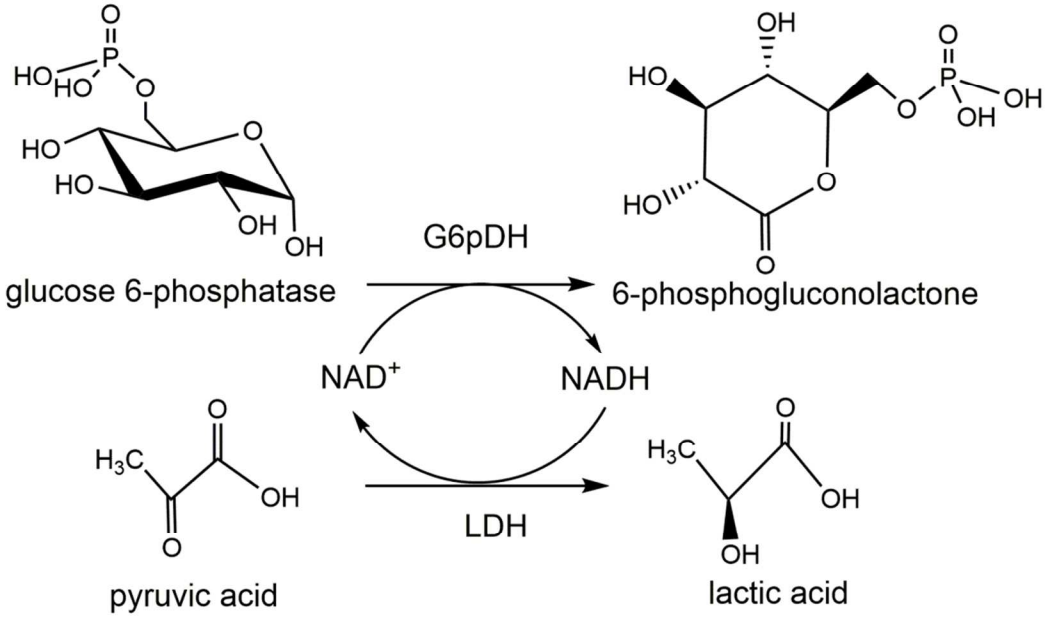

Figure S1. Scheme of G6pDH-LDH cascade reaction. First the glucose-6-phosphate is catalyzed by G6pDH with NAD^+ reduced to NADH. Subsequently, pyruvic acid is reduced by LDH catalysis to lactic acid, while NADH is oxidized to NAD^+ to continue cascade reaction.

3. Holliday junction assembly

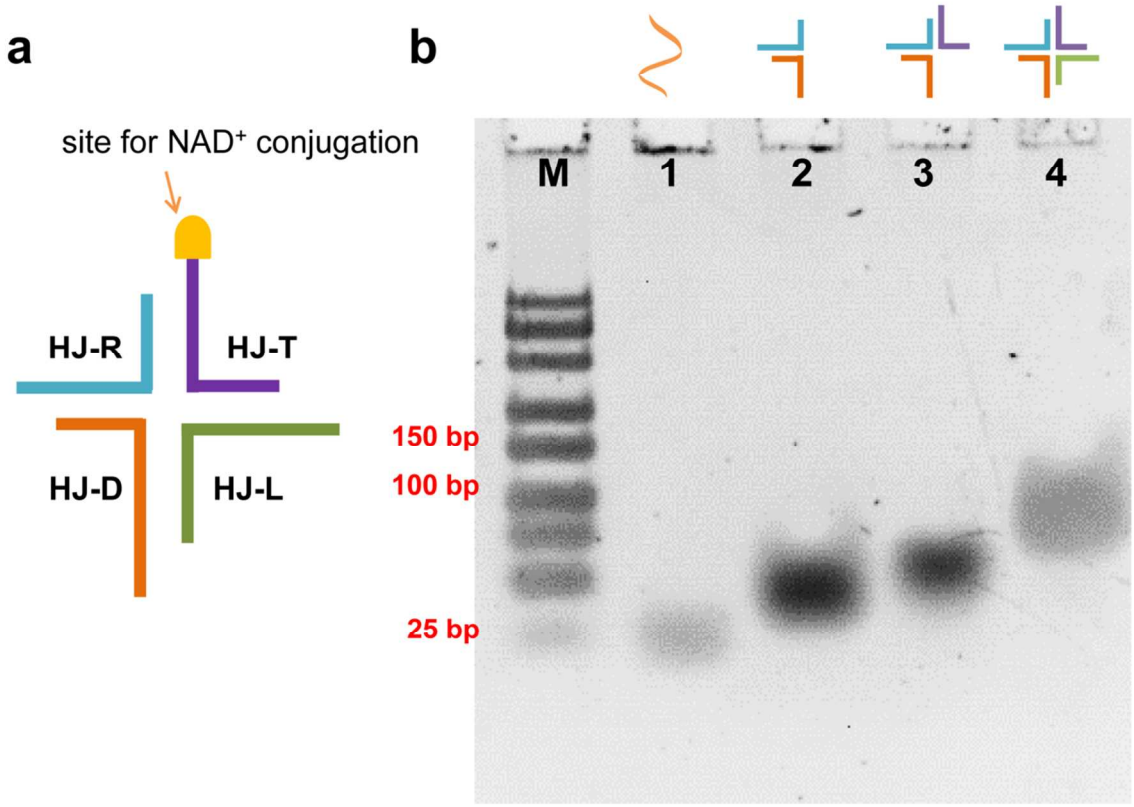

Figure S2. (a) Schematic of the HJ design. (b) 12% Native-PAGE for HJ assembly characterization. M: DNA maker; lane 1: HJ-D; lane 2: HJ-D+HJ-R; lane 3: HJ-D+HJ-R+HJ-T; lane 4: HJ-D+HJ-R+HJ-T+HJ-L. The sequences of the HJ are listed in Table S2.

4. HPLC characterization of azobenzene-modified DNA

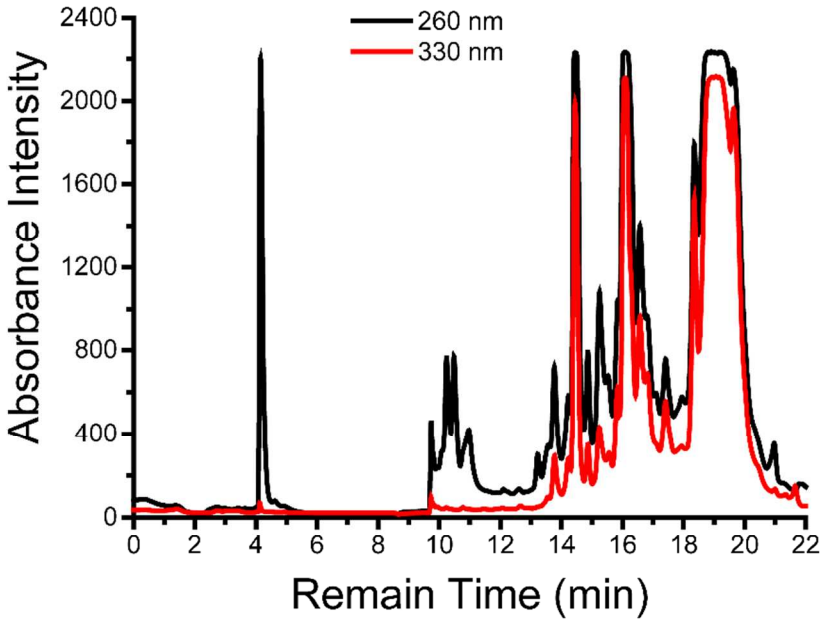

Figure S3. A typical HPLC for the purification of azobenzene-modified oligonucleotides (e.g. A3X). The signal of DNA and azobenzene were monitored by the absorbance at 260 nm (black line) and 330 nm (red line), respectively. Since azobenzene is a hydrophobic group, products with different number of azobenzene exhibited different remain time. More importantly, according to the principle of DNA synthesizer, only target oligonucleotide is labeled with a 5'-terminal, highly hydrophobic 4,4'-Dimethoxytriphenylmethyl group (DMT), which make the reman time of target oligonucleotide is longer than that of byproducts with less base number. Finally, the last elution peak belonging to target oligonucleotide was collected for further use.

5. Schematic of Ori-FAM

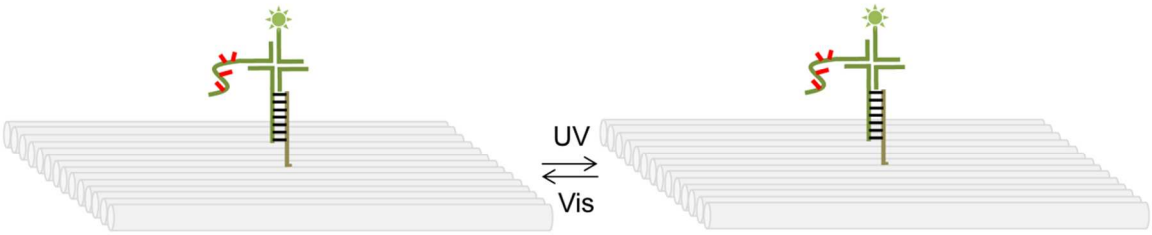

Figure S4. Schematic of Ori-FAM. The hybridization sequences between HJ-FAM and DNA origami were native DNA strands rather than azobenzene-modified DNA strands.

6. Enzymes purity verification

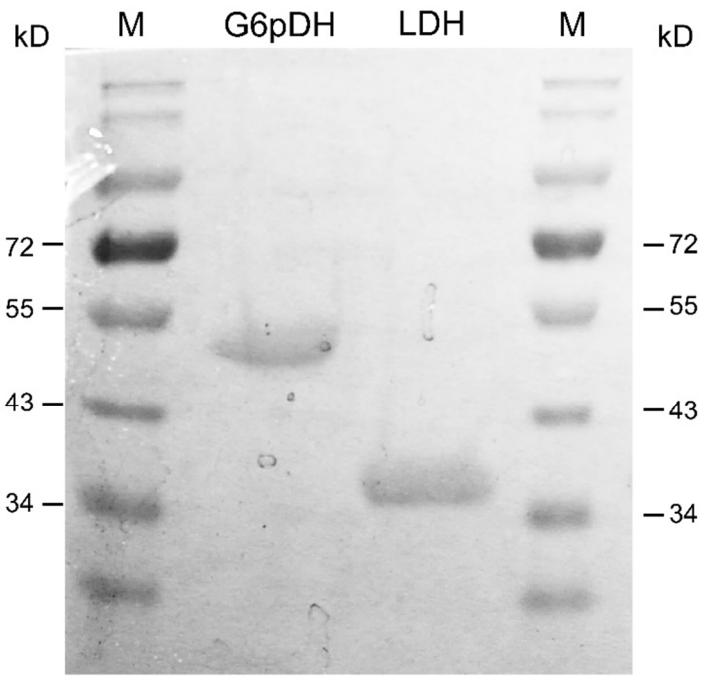

Figure S5. 10% SDS-PAGE for characterization of the purity of G6pDH and LDH. G6pDH is a dimer and its molecular mass is about 110 kD. While LDH is a tetramer with 140 kD of molecular mass. Both G6pDH and LDH show one single lane belonging to the subunit with expected molecular mass, suggesting the high purity of original proteins.

7. Enzymes coupled with DNA by SPDP crosslinker

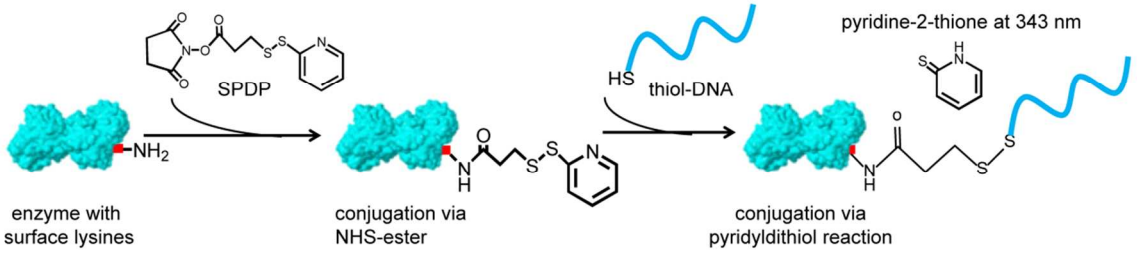

Figure S6. The schematic of the SPDP crosslinker through NHS-ester and pyridyldithiol reactive group to couple a lysine group of the enzyme and thiol-modified oligonucleotide. The coupling efficiency was evaluated by the absorbance of pyridine-2-thione at 343 nm.

a

DNA	$\varepsilon_{260}/\varepsilon_{280}$	ε_{260} ($*10^5$ M^{-1} cm^{-1})	ε_{280} ($*10^5$ M^{-1} cm^{-1})	enzyme	$\varepsilon_{260}/\varepsilon_{280}$	ε_{260}	ε_{280}	conjugate	A260/A280	A260	A280	DNA to protein ratio	enzyme conc.(uM)
WS3	1.56	1.285	0.8237	G6pDH	0.52	0.6240	1.200	GWS3	0.95	1.31	1.38	1.02	6.75
WS2	1.60	1.301	0.8131	LDH	0.57	1.115	2.203	LWS2	1.02	4.97	4.87	1.94	13.52

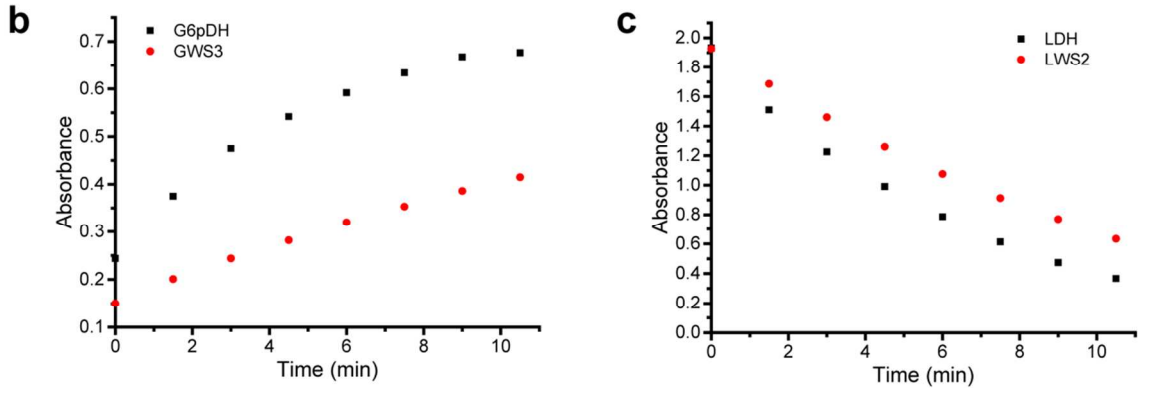

Figure S7. (a) Quantification of the ratio of DNA on each enzyme and the concentration of enzyme after SPDP coupling. The extinction coefficient of oligonucleotide was obtained using the OligoAnalyzer tool of Integrated DNA Technology (IDT), while the extinction coefficient of enzyme was based on references.[2,3] The concentration of DNA-enzyme was calculated based on below listed equations.[2] (b) The activity of G6pDH before and after conjugation with WS3 DNA (GWS3). The activity showed about 40% percent loss. Assay test conditions: 1 µL 200 nM enzyme was incubated with

1 μL 200 mM G6P, 1 μL 100 mM NAD^+ in 100 μL 100 mM HEPES buffer. Enzyme activity was measured by the increasing absorbance of NADH at 340nm. (c) The activity of LDH before and after conjugation with WS2 DNA (LWS2). The activity showed minor change after conjugation. Assay test conditions: 1 μL 200 nM enzyme was incubated with 1 μL 200 mM pyruvate, 1 μL 100 mM NADH in 100 μL 100 mM HEPES buffer. Enzyme activity was measured by the decreasing absorbance of NADH at 340nm.

The equations for calculation of the concentration of DNA-enzyme:

A_{260} (DNA_enzyme) = ε_{260}(enzyme) × Conc. (enzyme) + ε_{260} (DNA)×Conc.(DNA)

A_{280} (DNA_enzyme) = ε_{280}(enzyme) × Conc.(enzyme) + ε_{280} (DNA) ×Conc.(DNA)

Ratio $\left(\frac{DNA}{protein}\right) = \frac{Conc.(DNA)}{Conc.(protein)}$

8. Influence of different light irradiation on enzyme activity

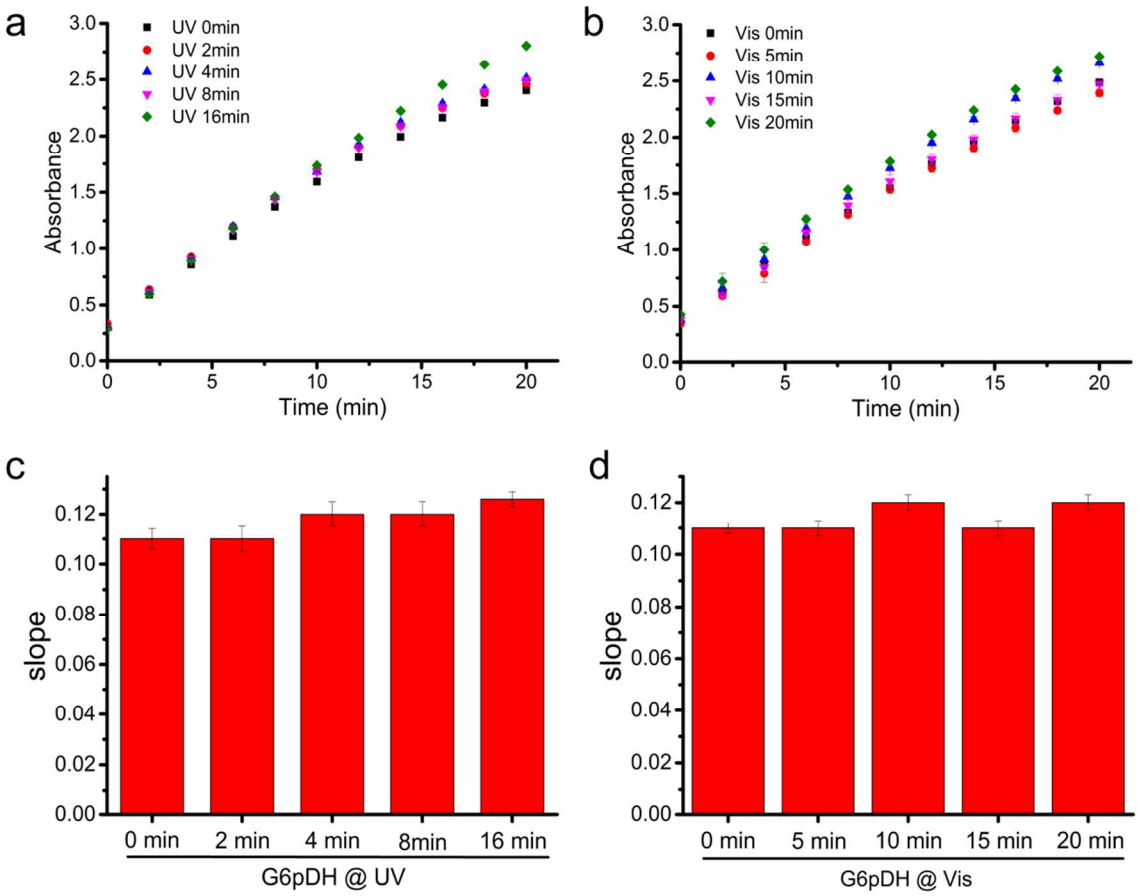

Figure S8. G6pDH activity after different time of visible or ultraviolet light irradiation. Raw absorbance spectra data under ultraviolet light irradiation (a) or visible light exposure (b). The catalytic rate of G6pDH activity under ultraviolet light irradiation (c) and under visible light exposure (d). The enzyme array conditions are the same as those in Figure S7b.The enzyme activity of G6pDH was estimated using the slope of the kinetic curve of (a) and (b).

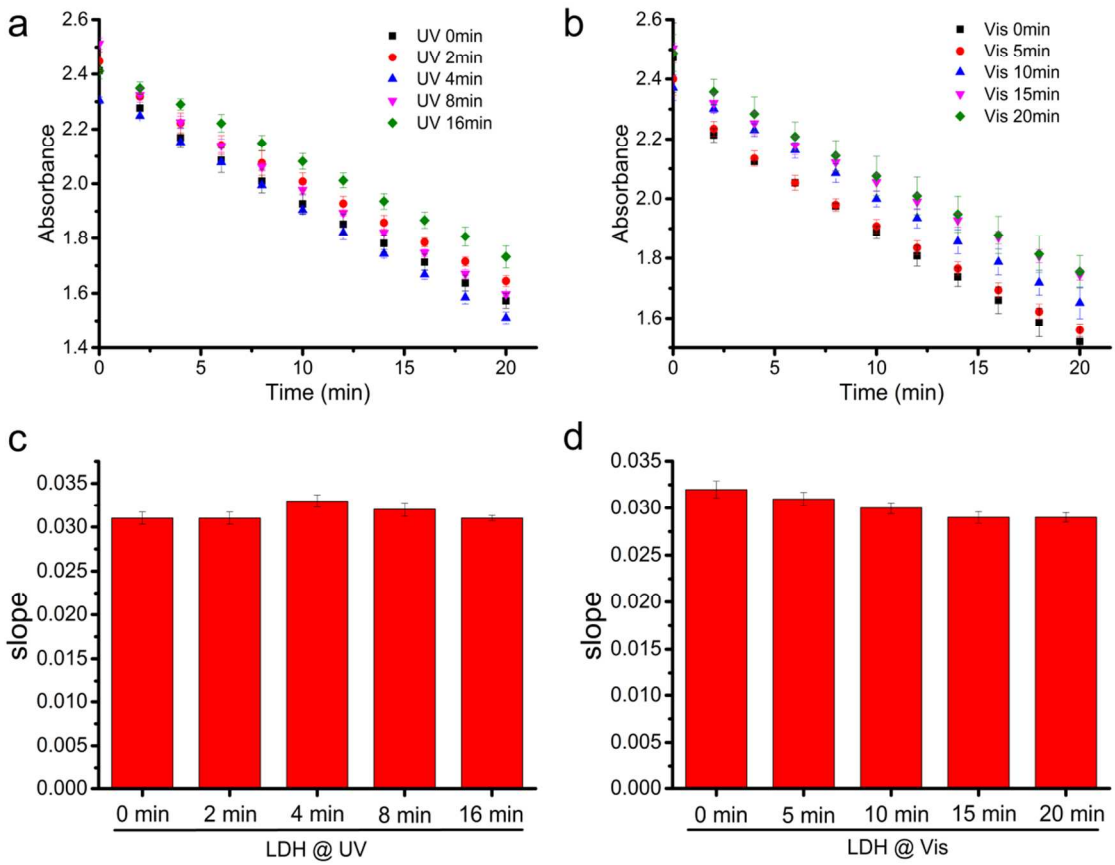

Figure S9. LDH activity after different time of visible or ultraviolet light irradiation. Raw absorbance spectra data under ultraviolet light irradiation (a) or visible light exposure (b). The catalytic rate of LDH activity under ultraviolet light irradiation (c) and under visible light exposure (d). The enzyme array conditions are the same as those in Figure S7c.The enzyme activity of LDH was estimated using the slope of the kinetic curve of (a) and (b).

9. Design details about enzyme cascade assembled on DNA origami

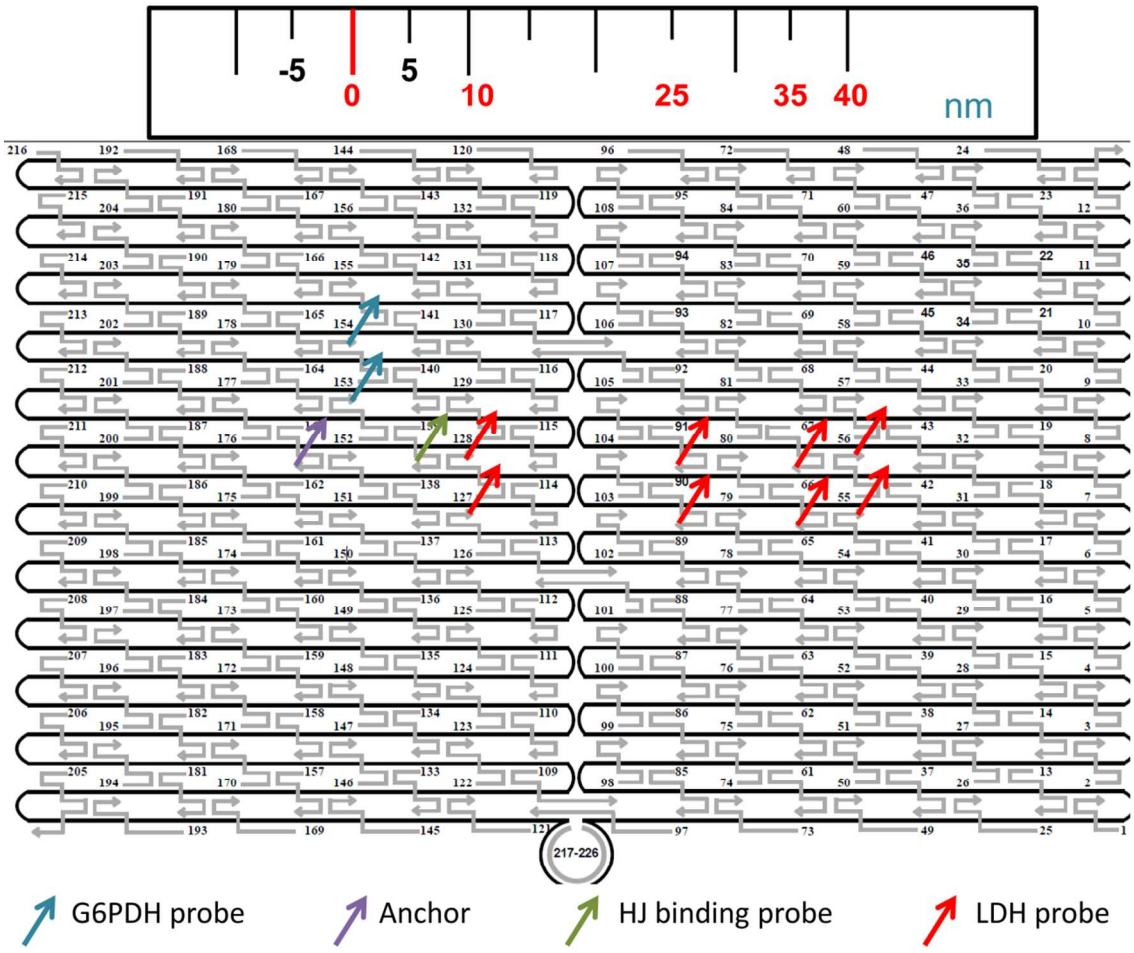

Figure S10. Schematic of the DNA origami tiles design for G6pDH-LDH enzyme cascade complex assembly with different horizontal distance (10nm, 25nm, 35nm, 40nm). The colored arrows represent the extended docking tiles from the surface of DNA origami which hybridizes with the DNA crosslinked enzyme and HJ-D arm. Here two docking tiles are designed for each enzyme to hybridize, ensuring a high capture yield.

10. Raw kinetic data

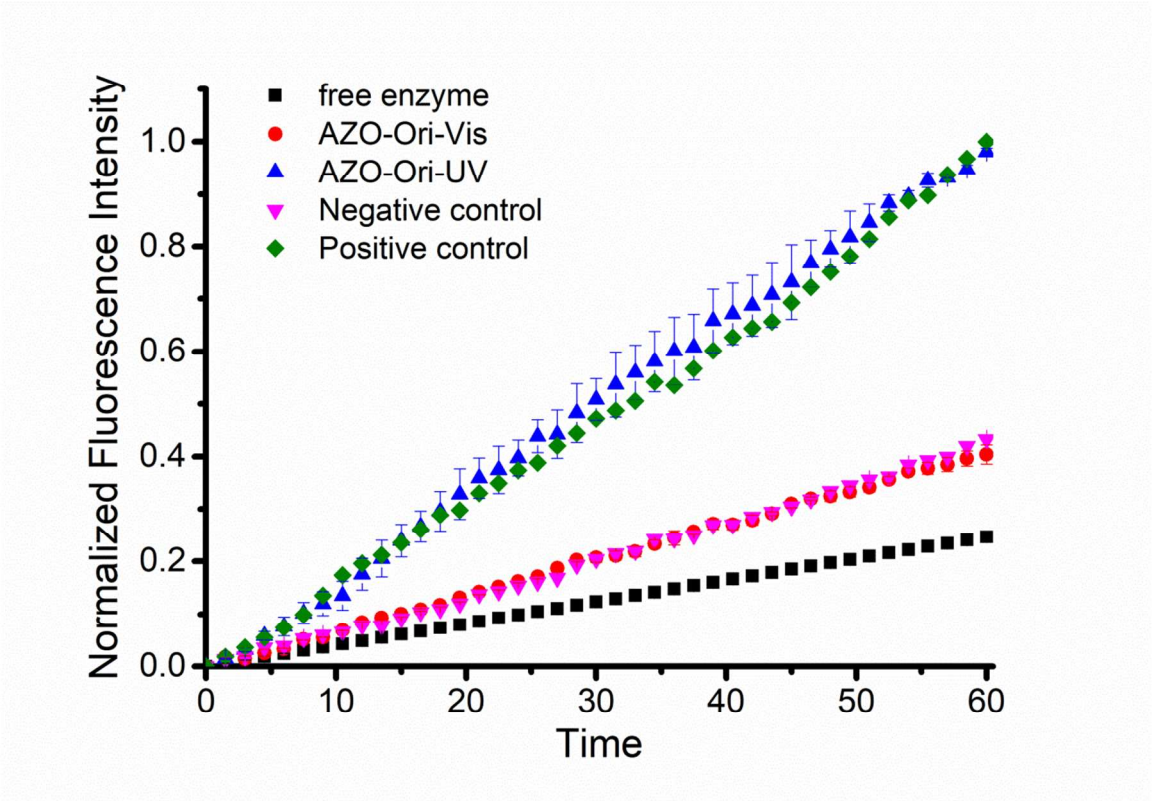

Figure S11. Raw fluorescence traces for comparison of four assemblies with different DNA bonding sites on DNA origami. To obtain the net activity from G6pDH-LDH origami assembly (as shown in maintext), the activity of the same amount of unassembled enzymes (without origami, named as free enzyme) was also measured and subtracted from the mixture's raw activity.

Summary: the slope of S11

slope/*10^{-2}	free enzyme	Azo-Ori-Vis	Azo-Ori-UV	negative control	positive control
	0.41	0.68	1.68	0.71	1.62

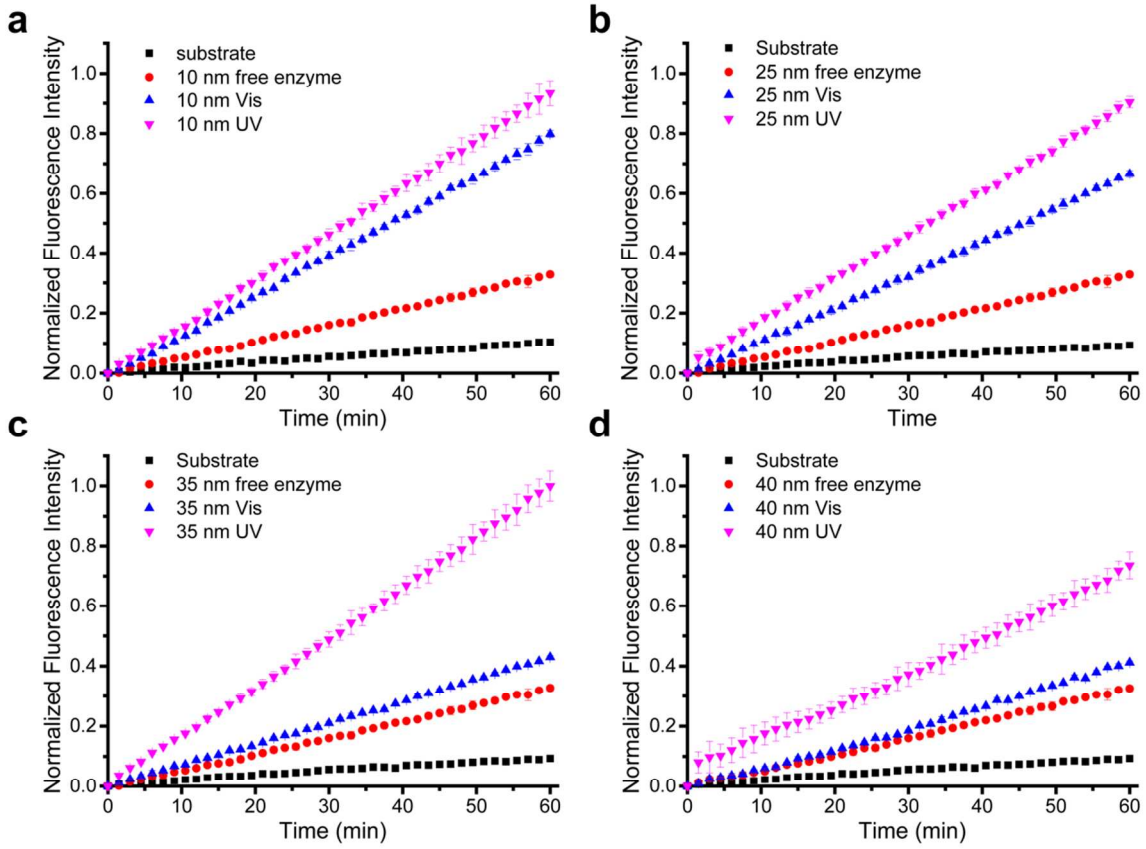

Figure S12. Raw kinetic data for G6pDH-LDH origami assembly activity with different distances between G6pDH and LDH. The distances are 10 nm (a), 25 nm (b), 35nm (c) and 40 nm (d). The reaction is monitored by the increasing fluorescence of resorufin (ex= 544 nm/em=590 nm). Substrate indicates the autocatalyzed reaction without the G6pDH-LDH origami assembly. Vis indicateds the G6pDH-LDH origami assembly activity under irradiation by visible light. UV indicates the G6pDH-LDH origami assembly activity under irradiation by ultraviolet light. To obtain the net activity from G6pDH-LDH origami assembly (as shown in maintext), the activity of the same amount of unassembled enzymes (without origami, named as free enzyme) was also measured and subtracted from the mixture's raw activity.

Summary: the slope of S12

slope/*10^{-2}	substrate	free enzyme	10 nm Vis	10 nm UV
	0.15	0.55	1.34	1.56
	substrate	free enzyme	25 nm Vis	25 nm UV
	0.15	0.55	1.11	1.46
	substrate	free enzyme	35 nm Vis	35 nm UV
	0.15	0.55	0.71	1.65
	substrate	free enzyme	35 nm Vis	35 nm UV
	0.15	0.55	0.7	1.12

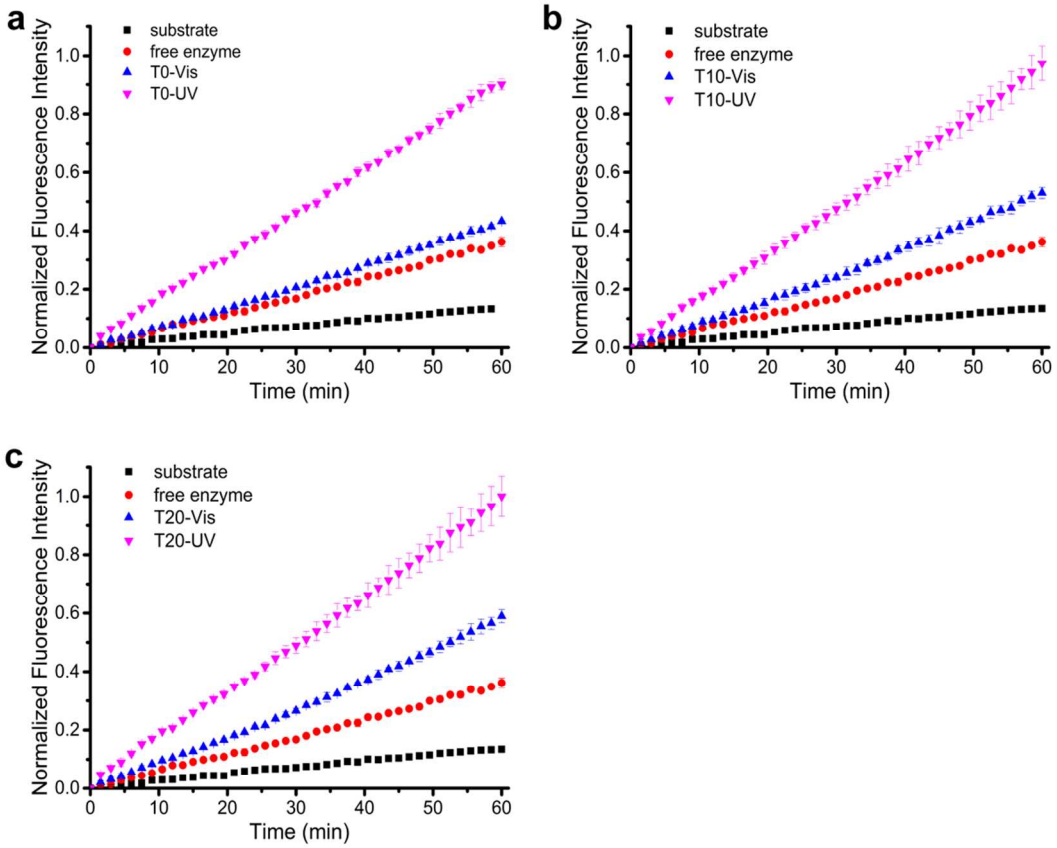

Figure S13. Raw kinetic data for G6pDH-LDH origami assembly activity with different polyT spacer between G6pDH- LDH and NAD^+. The spacer lengths were T0 (a), T10 (b), T20 (c). The reaction is monitored by the increasing fluorescence of resorufin (ex:544 nm/em:590 nm). Substrate indicates the autocatalyzed reaction without the G6pDH-LDH origami assembly. Vis indicates the G6pDH-LDH origami assembly activity under irradiation by visible light. UV indicates the G6pDH-LDH origami assembly activity under irradiation by ultraviolet light. To obtain the net activity from G6pDH-LDH origami assembly (as shown in maintext), the activity of the same amount of unassembled enzymes (without origami, named as free enzyme) was also measured and subtracted from the mixture's raw activity.

Summary: the slope of S13

slope/*10^{-2}	substrate	free enzyme	T0 Vis	T0 UV
	0.22	0.59	0.71	1.49
	substrate	free enzyme	T10 Vis	T10 UV
	0.22	0.59	0.88	1.59
	substrate	free enzyme	T20 Vis	T20 UV
	0.22	0.59	0.97	1.61

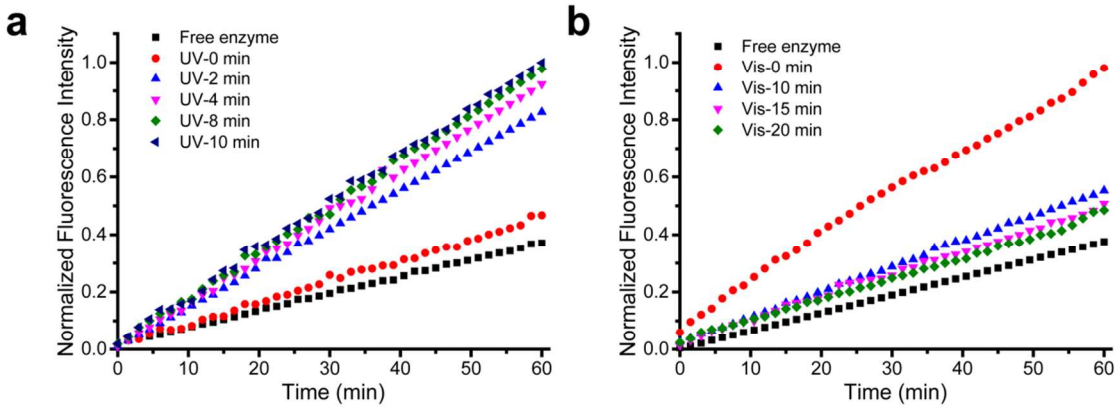

Figure S14. Raw kinetic data for enzyme cascade assembly activity for different irradiation time under UV light irradiation (a) or Vis light irradiation (b). To obtain the net activity from G6pDH-LDH origami assembly (as shown in maintext), the activity of the same amount of unassembled enzymes (without origami, named as free enzyme) was also measured and subtracted from the mixture's raw activity.

Summary: the slope of S14

slope/*10^{-2}	free enzyme	UV-0 min	UV-2 min	UV-4 min	UV-8 min	UV-10 min
	0.59	0.74	1.36	1.52	1.59	1.63
	free enzyme	Vis-0min	Vis-10min	Vis-15min	Vis-20min	
	0.62	1.47	0.88	0.78	0.73	

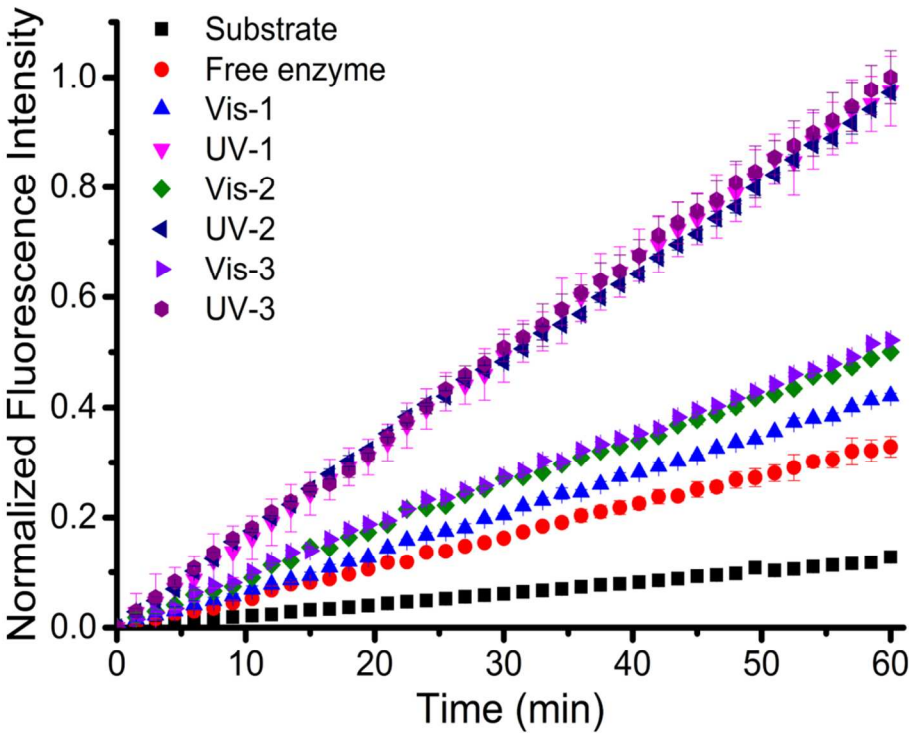

Figure S15. Raw activity traces for reversible regulation of the enzyme cascade on DNA origami. To obtain the net activity from G6pDH-LDH origami assembly (as shown in maintext), the activity of the same amount of unassembled enzymes (without origami, named as free enzyme) was also measured and subtracted from the mixture's raw activity.

Summary: the slope of S15

slope/*10^{-2}	substrate	free enzyme	Vis	UV	Vis	UV	Vis	UV
	0.21	0.56	0.71	1.65	0.82	1.59	0.85	1.67

11. DNA origami tiles design

Table S1. Azobenzene modified DNA sequences.

DNA name	DNA sequence	comment
A3X	CTCGT X TA X GT X TC A	X means azobenzene
B4X	ACTG X AA X CT X AA X CG	
HJ-R-A3X	CTCGTXTAXGTXTCATTTACAGCCCATAGCGGATT GAATTAAT	
163-B4X	TTTTGTTTAAGCCTTAAATCAAGAATCGAGAATTT ACTGXAAXCTXAAXCG	

Table S2. Holliday Junction sequences.

DNA name	DNA sequence	comment
HJ-L	AGGTTAGTGCTCATCGAATCCGTTCT	
HJ-R	TACAGCCCATAGCGGATTGAATTAAT	
HJ-D	CGACCGACCGACCGAGAACGGATTC GAGCTATGGGCTGTA	the binding site on DNA origami (indicated by red texts)
HJ-T	ATTAATTCAATCCTGAGCACTAACCT	
HJ-T-FAM	ATTAATTCAATCCTGAGCACTAACCT-FAM	
HJ-T-NH_2-T0	NH2-ATTAATTCAATCCTGAGCACTAA CCT	The amino group is used for further modification with NAD^+; with different length of poly T
HJ-T-NH_2-T10	NH2-TTTTTTTTTT ATTAATTCAATCCTGAGCACTAACCT	
HJ-T-NH_2-T20	NH2-TTTTTTTTTTTTTTTTTTTTATTAA TTCAATCCTGAGCACTAACCT	

Table S3. DNA origami probe sequences design.

DNA name	DNA sequence	comment
139-HJ probe	ATTATTTAACCCAGCTACAATTTTCAA GAACGTTTTTCGGTCGGTCGGTCG	binding with HJ
163-anchor	TTTTGTTTAAGCCTTAAATCAAGAATC GAGAATTTTT GCTTCGTTGTCATACTAGGAGTG	binding with 163
152-ws3	TATTTTGCTCCCAATCCAAATAAGTGA GTTAATTTTTGCACGCACGC	binding with G6pDH
153-ws3	GCCCAATACCGAGGAAACGCAATAGG TTTACCTTTTTGCACGCACGC	

10nm-127-ws2	CTAATTTATCTTTCCTTATCATTCATCC TGAATTTTTCCAGCCAGCC	binding with LDH at a distance of 10 nm
10nm-126-ws2	AATTACTACAAATTCTTACCAGTAATC CCATCTTTTTCCAGCCAGCC	
25nm-91-ws2	AAGAGGAACGAGCTTCAAAGCGAAGA TACATTTTTTTCCAGCCAGCC	binding with LDH at a distance of 25 nm
25nm-90-ws2	TCGCAAATGGGGCGCGAGCTGAAATA ATGTGTTTTTTCCAGCCAGCC	
35nm-67-ws2	TCAGAAGCCTCCAACAGGTCAGGATCT GCGAATTTTTCCAGCCAGCC	binding with LDH at a distance of 35 nm
35nm-66-ws2	CGAGTAGAACTAATAGTAGTAGCAAA CCCTCATTTTTCCAGCCAGCC	
40nm-55-ws2	CAATAAATACAGTTGATTCCCAATTTA GAGAGTTTTTCCAGCCAGCC	binding with LDH at a distance of 40 nm
40nm-54-ws2	GGTAGCTAGGATAAAAATTTTTAGTTA ACATCTTTTTCCAGCCAGCC	

Table S4. Tiles sequences for the unmodified DNA origami.

DNA name	sequence
13	TGGTTTTTAACGTCAAAGGGCGAAGAACCATC
14	CTTGCATGCATTAATGAATCGGCCCGCCAGGG
15	TAGATGGGGGGTAACGCCAGGGTTGTGCCAAG
16	CATGTCAAGATTCTCCGTGGGAACCGTTGGTG
17	CTGTAATATTGCCTGAGAGTCTGGAAAACTAG
18	TGCAACTAAGCAATAAAGCCTCAGTTATGACC
19	AAACAGTTGATGGCTTAGAGCTTATTTAAATA
20	ACGAACTAGCGTCCAATACTGCGGAATGCTTT
21	CTTTGAAAAGAACTGGCTCATTATTTAATAAA
22	ACGGCTACTTACTTAGCCGGAACGCTGACCAA
23	GAGAATAGCTTTTGCGGGATCGTCGGGTAGCA
24	ACGTTAGTAAATGAATTTTCTGTAAGCGGAGT
25	ACCCAAATCAAGTTTTTTGGGGTCAAAGAACG
26	TGGACTCCCTTTTCACCAGTGAGACCTGTCGT
27	GCCAGCTGCCTGCAGGTCGACTCTGCAAGGCG
28	ATTAAGTTCGCATCGTAACCGTGCGAGTAACA

29	ACCCGTCGTCATATGTACCCCGGTAAAGGCTA
30	TCAGGTCACTTTTGCGGGAGAAGCAGAATTAG
31	CAAAATTAAAGTACGGTGTCTGGAAGAGGTCA
32	TTTTTGCGCAGAAAACGAGAATGAATGTTTAG
33	ACTGGATAACGGAACAACATTATTACCTTATG
34	CGATTTTAGAGGACAGATGAACGGCGCGACCT
35	GCTCCATGAGAGGCTTTGAGGACTAGGGAGTT
36	AAAGGCCGAAAGGAACAACTAAAGCTTTCCAG
37	AGCTGATTACAAGAGTCCACTATTGAGGTGCC
38	CCCGGGTACTTTCCAGTCGGGAAACGGGCAAC
39	GTTTGAGGGAAAGGGGGATGTGCTAGAGGATC
40	AGAAAAGCAACATTAAATGTGAGCATCTGCCA
41	CAACGCAATTTTTGAGAGATCTACTGATAATC
42	TCCATATACATACAGGCAAGGCAACTTTATTT
43	CAAAAATCATTGCTCCTTTTGATAAGTTTCAT
44	AAAGATTCAGGGGGTAATAGTAAACCATAAAT
45	CCAGGCGCTTAATCATTGTGAATTACAGGTAG
46	TTTCATGAAAATTGTGTCGAAATCTGTACAGA
47	AATAATAAGGTCGCTGAGGCTTGCAAAGACTT
48	CGTAACGATCTAAAGTTTTGTCGTGAATTGCG
49	GTAAAGCACTAAATCGGAACCCTAGTTGTTCC
50	AGTTTGGAGCCCTTCACCGCCTGGTTGCGCTC
51	ACTGCCCGCCGAGCTCGAATTCGTTATTACGC
52	CAGCTGGCGGACGACGACAGTATCGTAGCCAG
53	CTTTCATCCCCAAAAACAGGAAGACCGGAGAG
54	GGTAGCTAGGATAAAAATTTTTAGTTAACATC
55	CAATAAATACAGTTGATTCCCAATTTAGAGAG
56	TACCTTTAAGGTCTTTACCCTGACAAAGAAGT
57	TTTGCCAGATCAGTTGAGATTTAGTGGTTTAA
58	TTTCAACTATAGGCTGGCTGACCTTGTATCAT

59	CGCCTGATGGAAGTTTCCATTAAACATAACCG
60	ATATATTCTTTTTTCACGTTGAAAATAGTTAG
61	GAGTTGCACGAGATAGGGTTGAGTAAGGGAGC
62	TCATAGCTACTCACATTAATTGCGCCCTGAGA
63	GAAGATCGGTGCGGGCCTCTTCGCAATCATGG
64	GCAAATATCGCGTCTGGCCTTCCTGGCCTCAG
65	TATATTTTAGCTGATAAATTAATGTTGTATAA
66	CGAGTAGAACTAATAGTAGTAGCAAACCCTCA
67	TCAGAAGCCTCCAACAGGTCAGGATCTGCGAA
68	CATTCAACGCGAGAGGCTTTTGCATATTATAG
69	AGTAATCTTAAATTGGGCTTGAGAGAATACCA
70	ATACGTAAAAGTACAACGGAGATTTCATCAAG
71	AAAAAAGGACAACCATCGCCCACGCGGGTAAA
72	TGTAGCATTCCACAGACAGCCCTCATCTCCAA
73	CCCCGATTTAGAGCTTGACGGGGAAATCAAAA
74	GAATAGCCGCAAGCGGTCCACGCTCCTAATGA
75	GTGAGCTAGTTTCCTGTGTGAAATTTGGGAAG
76	GGCGATCGCACTCCAGCCAGCTTTGCCATCAA
77	AAATAATTTTAAATTGTAAACGTTGATATTCA
78	ACCGTTCTAAATGCAATGCCTGAGAGGTGGCA
79	TCAATTCTTTTAGTTTGACCATTACCAGACCG
80	GAAGCAAAAAAGCGGATTGCATCAGATAAAAA
81	CCAAAATATAATGCAGATACATAAACACCAGA
82	ACGAGTAGTGACAAGAACCGGATATACCAAGC
83	GCGAAACATGCCACTACGAAGGCATGCGCCGA
84	CAATGACACTCCAAAAGGAGCCTTACAACGCC
85	CCAGCAGGGGCAAAATCCCTTATAAAGCCGGC
86	GCTCACAATGTAAAGCCTGGGGTGGGTTTGCC
87	GCTTCTGGTCAGGCTGCGCAACTGTGTTATCC
88	GTTAAAATTTTAACCAATAGGAACCCGGCACC

89	AGGTAAAGAAATCACCATCAATATAATATTTT
90	TCGCAAATGGGGCGCGAGCTGAAATAATGTGT
91	AAGAGGAACGAGCTTCAAAGCGAAGATACATT
92	GGAATTACTCGTTTACCAGACGACAAAAGATT
93	CCAAATCACTTGCCCTGACGAGAACGCCAAAA
94	AAACGAAATGACCCCCAGCGATTATTCATTAC
95	TCGGTTTAGCTTGATACCGATAGTCCAACCTA
96	TGAGTTTCGTCACCAGTACAAACTTAATTGTA
97	GAACGTGGCGAGAAAGGAAGGGAACAAACTAT
98	CCGAAATCCGAAAATCCTGTTTGAAGCCGGAA
99	GCATAAAGTTCCACACAACATACGAAGCGCCA
100	TTCGCCATTGCCGGAAACCAGGCATTAAATCA
101	GCTCATTTTCGCATTAAATTTTTGAGCTTAGA
102	AGACAGTCATTCAAAAGGGTGAGAAGCTATAT
103	TTTCATTTGGTCAATAACCTGTTTATATCGCG
104	TTTTAATTGCCCGAAAGACTTCAAAACACTAT
105	CATAACCCGAGGCATAGTAAGAGCTTTTTAAG
106	GAATAAGGACGTAACAAAGCTGCTCTAAAACA
107	CTCATCTTGAGGCAAAAGAATACAGTGAATTT
108	CTTAAACATCAGCTTGCTTTCGAGCGTAACAC
109	ACGAACCAAAACATCGCCATTAAATGGTGGTT
110	CGACAACTAAGTATTAGACTTTACAATACCGA
111	CTTTTACACAGATGAATATACAGTAAACAATT
112	TTAAGACGTTGAAAACATAGCGATAACAGTAC
113	GCGTTATAGAAAAAGCCTGTTTAGAAGGCCGG
114	ATCGGCTGCGAGCATGTAGAAACCTATCATAT
115	CCTAATTTACGCTAACGAGCGTCTAATCAATA
116	AAAAGTAATATCTTACCGAAGCCCTTCCAGAG
117	TTATTCATAGGGAAGGTAAATATTCATTCAGT
118	GAGCCGCCCCACCACCGGAACCGCGACGGAAA

119	AATGCCCCGTAACAGTGCCCGTATCTCCCTCA
120	CAAGCCCAATAGGAACCCATGTACAAACAGTT
121	CGGCCTTGCTGGTAATATCCAGAACGAACTGA
122	TAGCCCTACCAGCAGAAGATAAAAACATTTGA
123	GGATTTAGCGTATTAAATCCTTTGTTTTCAGG
124	TTTAACGTTCGGGAGAAACAATAATTTTCCCT
125	TAGAATCCCTGAGAAGAGTCAATAGGAATCAT
126	AATTACTACAAATTCTTACCAGTAATCCCATC
127	CTAATTTATCTTTCCTTATCATTCATCCTGAA
128	TCTTACCAGCCAGTTACAAAATAAATGAAATA
129	GCAATAGCGCAGATAGCCGAACAATTCAACCG
130	ATTGAGGGTAAAGGTGAATTATCAATCACCGG
131	AACCAGAGACCCTCAGAACCGCCAGGGGTCAG
132	TGCCTTGACTGCCTATTTCGGAACAGGGATAG
133	AGGCGGTCATTAGTCTTTAATGCGCAATATTA
134	TTATTAATGCCGTCAATAGATAATCAGAGGTG
135	CCTGATTGAAAGAAATTGCGTAGACCCGAACG
136	ATCAAAATCGTCGCTATTAATTAACGGATTCG
137	ACGCTCAAAATAAGAATAAACACCGTGAATTT
138	GGTATTAAGAACAAGAAAAATAATTAAAGCCA
139	ATTATTTAACCCAGCTACAATTTTCAAGAACG
140	GAAGGAAAATAAGAGCAAGAAACAACAGCCAT
141	GACTTGAGAGACAAAAGGGCGACAAGTTACCA
142	GCCACCACTCTTTTCATAATCAAACCGTCACC
143	CTGAAACAGGTAATAAGTTTTAACCCCTCAGA
144	CTCAGAGCCACCACCCTCATTTTCCTATTATT
145	CCGCCAGCCATTGCAACAGGAAAAATATTTTT
146	GAATGGCTAGTATTAACACCGCCTCAACTAAT
147	AGATTAGATTTAAAAGTTTGAGTACACGTAAA
148	ACAGAAATCTTTGAATACCAAGTTCCTTGCTT

149	CTGTAAATCATAGGTCTGAGAGACGATAAATA
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152	TATTTTGCTCCCAATCCAAATAAGTGAGTTAA
153	GCCCAATACCGAGGAAACGCAATAGGTTTACC
154	AGCGCCAACCATTTGGGAATTAGATTATTAGC
155	GTTTGCCACCTCAGAGCCGCCACCGATACAGG
156	AGTGTACTTGAAAGTATTAAGAGGCCGCCACC
157	GCCACGCTATACGTGGCACAGACAACGCTCAT
158	ATTTTGCGTCTTTAGGAGCACTAAGCAACAGT
159	GCGCAGAGATATCAAAATTATTTGACATTATC
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161	CATATTTAGAAATACCGACCGTGTTACCTTTT
162	CAAGCAAGACGCGCCTGTTTATCAAGAATCGC
163	TTTTGTTTAAGCCTTAAATCAAGAATCGAGAA
164	ATACCCAAGATAACCCACAAGAATAAACGATT
165	AATCACCAAATAGAAAATTCATATATAACGGA
166	CACCAGAGTTCGGTCATAGCCCCCGCCAGCAA
167	CCTCAAGAATACATGGCTTTTGATAGAACCAC
168	CCCTCAGAACCGCCACCCTCAGAACTGAGACT
169	GGAAATACCTACATTTTGACGCTCACCTGAAA
170	GCGTAAGAGAGAGCCAGCAGCAAAAAGGTTAT
171	CTAAAATAGAACAAAGAAACCACCAGGGTTAG
172	AACCTACCGCGAATTATTCATTTCCAGTACAT
173	AAATCAATGGCTTAGGTTGGGTTACTAAATTT
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176	AGGTTTTGAACGTCAAAAATGAAAGCGCTAAT
177	ATCAGAGAAAGAACTGGCATGATTTTATTTTG
178	TCACAATCGTAGCACCATTACCATCGTTTTCA

179	TCGGCATTCCGCCGCCAGCATTGACGTTCCAG
180	TAAGCGTCGAAGGATTAGGATTAGTACCGCCA
181	CTAAAGCAAGATAGAACCCTTCTGAATCGTCT
182	CGGAATTATTGAAAGGAATTGAGGTGAAAAAT
183	GAGCAAAAACTTCTGAATAATGGAAGAAGGAG
184	TATGTAAACCTTTTTTAATGGAAAAATTACCT
185	AGAGGCATAATTTCATCTTCTGACTATAACTA
186	TCATTACCCGACAATAAACAACATATTTAGGC
187	CTTTACAGTTAGCGAACCTCCCGACGTAGGAA
188	TTATTACGGTCAGAGGGTAATTGAATAGCAGC
189	CCGGAAACACACCACGGAATAAGTAAGACTCC
190	TGAGGCAGGCGTCAGACTGTAGCGTAGCAAGG
191	TGCTCAGTCAGTCTCTGAATTTACCAGGAGGT
192	TATCACCGTACTCAGGAGGTTTAGCGGGGTTT
193	GAAATGGATTATTTACATTGGCAGACATTCTG
194	GCCAACAGTCACCTTGCTGAACCTGTTGGCAA
195	ATCAACAGTCATCATATTCCTGATTGATTGTT
196	TGGATTATGAAGATGATGAAACAAAATTTCAT
197	TTGAATTATGCTGATGCAAATCCACAAATATA
198	TTTTAGTTTTTCGAGCCAGTAATAAATTCTGT
199	CCAGACGAGCGCCCAATAGCAAGCAAGAACGC
200	GAGGCGTTAGAGAATAACATAAAAGAACACCC
201	TGAACAAACAGTATGTTAGCAAACTAAAAGAA
202	ACGCAAAGGTCACCAATGAAACCAATCAAGTT
203	TGCCTTTAGTCAGACGATTGGCCTGCCAGAAT
204	GGAAAGCGACCAGGCGGATAAGTGAATAGGTG
217	AACATCACTTGCCTGAGTAGAAGAACT
218	TGTAGCAATACTTCTTTGATTAGTAAT
219	AGTCTGTCCATCACGCAAATTAACCGT
220	ATAATCAGTGAGGCCACCGAGTAAAAG

221	ACGCCAGAATCCTGAGAAGTGTTTTT
222	TTAAAGGGATTTTAGACAGGAACGGT
223	AGAGCGGGAGCTAAACAGGAGGCCGA
224	TATAACGTGCTTTCCTCGTTAGAATC
225	GTACTATGGTTGCTTTGACGAGCACG
226	GCGCTTAATGCGCCGCTACAGGGCGC

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