In Vitro Selection of Shape-Changing DNA Nanostructures Capable of Binding-Induced Cargo Release

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SUPPORTING INFORMATION



Figure S1. Loading into the abasic site of SSA/cargo strand duplex structures efficiently quenches ATMND fluorescence. Duplex-loaded ATMND yields 7- and 9-fold lower fluorescence intensities with SSA-1 (red) and SSA-2 (blue), respectively, compared to free ATMND in solution (black).



Figure S2. Calibration curve for SSA-1 structure-switching derived from fluorescence intensities detected at increasing ATP concentrations yields an EC₅₀ value of $29.9 \pm 5.8 \mu$ M at 5 μ M of SSA-1/cargo strand (1:1.5 in ratio) concentration.



Figure S3. Comparison of ATP-induced cargo release by SSAs in solution. When challenged with 1 mM ATP, SSA-1 generates a strong fluorescent signal (170% increase relative to no ATP), indicating efficient cargo release as a result of binding-induced structure-switching. By comparison, SSA-2 and SSA-2 where the T-to-C mutation is reverted achieve only limited ATMND release in solution (15% and 9% increase, respectively). These results indicate that this mutation is not responsible for the difference in cargo release, which may instead be attributable to SSA-2's dependence on the presence of a solid support.



Figure S4. Comparison of SA-binding between the two SSAs. We incubated both SSAs at 100 nM concentration with SA-coated beads, and heat eluted bound DNAs after thorough washing. Eluted DNAs were quantified *via* RT-PCR, and we observed that SSA-2 binding to SA beads was ~20-fold greater than for SSA-1.



Figure S5. EC₅₀ of SSA-1 in response to ATP is dependent on cargo strand concentration. As the cargo strand:SSA-1 ratio increases, EC₅₀ also increases; For cargo strand:SSA-1 ratios of 1.5 (black), 7.5 (red) and 30 (blue) with a 30 nM concentration of SSA-1, we observed EC₅₀ values of $31.8 \pm 4.8 \mu$ M, $50.2 \pm 10.8 \mu$ M and $92.0 \pm 31.7 \mu$ M, respectively.



Figure S6. Threshold cycle (C_T) values of SSAs released from magnetic beads after binding to ATP. The template concentration and C_T were fitted with linear regression. We calculated a PCR efficiency of 86.3%.

Name	Sequence	Description			
ssDNA library	5'-AGCAGCACAGAGGTCAGATG-N ₁₇ -	DNA sequence with two			
	TTTTGTGGGTAGGGCGGGTTGGTTTT	randomized 17-base			
	-N ₁₇ -CCTATGCGTGCTACCGTGAA-3'	recognition domains			
FP	5'-AGCAGCACAGAGGTCAGATG-3'	Forward primer			
RP	5'-TTCACGGTAGCACGCATAGG-3'	Reverse primer			
RP-biotin	5'-biotin-	Biotinylated reverse			
	TTCACGGTAGCACGCATAGG -3'	primer for ssDNA			
		generation			
FP-	5'-CATCTGACCTCTGTGCTGCT-3'	Blocking strand of			
complementar		forward primer binding			
У		site.			
M12-T12-	5'-biotin-	Biotinylated cargo strand			
biotin	TTTTTTTTTTTTTAACCCGCCCTAC-3'	with T_{12} extension			
M12	5'-AACCCXCCTAC-3'	Cargo strand with an			
		abasic site			

Table S1. Sequences of DNA molecules used in these experiments

	R 1	R2	R3	R4	R5	R 6	R 7	R8	R9	R10
SA			1 µM	1 µM	1 µM	1 µM	1 µM	1 µM	1 µM	1 µM
BSA			1 µM	1 µM	1 µM	1 µM	1 µM	1 µM	1 µM	1 µM
GTP							1 mM	1 mM	1 mM	1 mM
ATP	1 mM	1 mM	1 mM	0.8 mM	0.4 mM	0.2 mM	0.1 mM	70 μΜ	20 μΜ	20 µM

Table S2. Selection conditions during ten rounds of SSA selection.

Table S3. 50 cloned sequences from the SSA library synthesized from machine-mixed nucleotides.

	1 10	20	30	40	50	60	70	80	90	100
1	AGCAGCA CAGAGGTCA	GATGATAA	ATCCAGGAGTAGAT	TTTGT	GGGTAGGGGGG	TTGGTT	ITCATGACAAA	GACGACCCCTA	TGCGTĠC	ACCGTGAA
2	AGCAGCACAGAGGTCA	GATGTAAC	A TAAGATGAG TGCT	TTTGT	GGGTAGGGGGG	TTGGTT	ITCGTAGAATT	AAAACCATCCTA	TGCGTGC	ACCGTGAA
3	AGCAGCACAGAGGTCA	GATGGAAA	A TAG TTCGAGGTT T	TTTGT	GGG TAGGG CGG C	TTGGTT	ITCATTAAAAG	TTAAACACCCTA	TGCGTGC	ACCG TGAA
4	AGCAGCACAGAGGTCA	GATGAACT	CTAGGATCGGCCTT	TTTGT	GG TAGGG CGG	TTGGTT	ITCGACGTATA	GCAGTGGCCTA	TGCGTGC	ACCGTGAA
5	AGCAGCA CAGAGGTCA	GATGCCCA	TCTGGTAGCCGGAT	TTTGT	GGG TAGGG CGGC	TTGGTT	IT CCCGCCACG	TAAGAAATCCTA	TGCGTGC	ACCGTGAA
6	AGCAGCACAGAGGTCA	GATGGGTG	AAGAGTTGTCACTT	TTTGT	GG TAGGG GGG	TTGGTT	TGCGTAAAAC	TGTTGTATCCTA	TGCGTGC	ACCGTGAA
7	AGCAGCACAGAGGTCA	GAAGG	GACGAATGAAACG	TTTGT	GGGTAGGGGGG	TTGGTT	PTGGTCAACGT	GGATGATCCTA	TGCGTGC	ACCGTGAA
8	AGCAGCACAGAGGTCA	SATGTTAT	TCATCTGAGAAAG	TTTGT	GGGTAGGGGGGG	TTGGTT	TCTGTACGAC	GGAGGCACCTA	TGCGTGC	ACCGTGAA
ğ	AGCAGCACAGAGGTCA	CACAA	TCCACCGGGAAG	TTTGT	GG TAGGG CGGC	TTGGTT	TCGTAAGAAT	TATTAATACCTA	TGCGTGC	ACCGTGAA
10	AGCAGCACAGAGGTCA	GATGACAT	AGGCGTGGATCTCT	TTGT	GG TAGGG GGG	TTGGTT	TCCAAACAAC	GGTGTAACCTA	TGCGTGC	ACCGTGAA
11	AGCAGCACAGAGGTCA	SATGATGT	AAGGAGGACTTGAT	TTTGT		TTGGTT	TTAATCATAC	ATTCTGTGCCTA	TGCGTGC	ACCGTGAA
12	AGCAGCACAGAGGTCA	SATGGGCG	AGGCTGG CGG AGAT	TTTGT		TTGGTT	TAAGAGACGA	TAATCAGACCTA	TGCGTGC	ACCGTGAA
13	ACCACACACACCTCA	CATCCATC	CCAACC CACCTAT	TTTCT		TTCCTT	TTACCGAGAGG	TCCTAAAACCTA	TCCCTCC	ACCC TCAA
14	ACCACACACACACC	CATCCOR		TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT		TTCCTT	TCCAACACAA	TCCCATTACCTA	TCCCTCC	ACCC TCAA
15	ACCACACACACACOTCAC	ATCTCAA	A A A C C C T A A C C C C	TTTT		TTCCTT	TACTCAAACAC	CAATCCCCCTA	TCCCTCC	ACCC TCAA
16	ACCACCACACACO ICAC	CATCICCA		TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT		TTCCTT	THETTACEATT	CATTCACCCTA	TCCCTCC	ACCCTCAA
17	AGCAGCACAGAGGICA		AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	TTTTTTTTTTT		TTOOTT	TTOTTCOTATCA	TTACACCOCTA	TCCCTCC	ACCC TCAA
10	AGCAGCACAGAGGICA	CCCCA	CCAALCALLCLCCCL			TTGGTT		TACAGCACCTA	TCCCTCC	ACCO TCAA
10	AGCAGCACAGAGGICA	JALGUGGA	JACCARAGAICGCI			TIGGII		TAGACACACCETA	TCCCTCC	ACCG IGAA
19	AGCAGCACAGAGGICA	JALGGUGA	ACCAGACACCACG	TTTTT		TIGGII	TAGCATCICI	IGAAACGGCCIA	TGCGIGC	ACCGIGAA
20	AGCAGCACAGAGGICA	JAI GGAIC	IGIGIGACAACGGI			TIGGII	THUGUGICAAI		TCCCTCC	ACCGIGAA
21	AGCAGCACAGAGGICA	JALGGAAA	IGAAICI IAIGI			TIGGII.		GAGACAICCIA	IGCGIGC	ACCG IGAA
22	AGCAGCACAGAGGICA	JALGAGIG					I GAAGGIACA	AGIGAAGGUUIA	IGUGIGU.	ACCG IGAA
23	AGCAGCACAGAGGICA	JALGALAG					I GGAGGAAGU	AIGGIAGUCUIA	IGCGIGU.	ACCG IGAA
24	AGCAGCACAGAGGICA	JALGGALG	ALLAGAGCALGA				AUGGGUAGG	AAA IGGGACCIA		ACCG IGAA
25	AGCAGCACAGAGG ICA	JAIGALLI	GGAATTGGGAAT					AGGUGAILCUIA	IGCGIGC.	ACCG IGAA
26	AGCAGCACAGAGGICA	JAIGUCAA	AGCG TAAG ACGG				I I CCAGAACGC	TAAAGAGACCTA	IGCGIGC	ACCG IGAA
27	AGCAGCACAGAGGICA	JA GAG G	AGIIIIGGAAG	111 _G 10		IIGGII	I I GA I GAGACI	ACAGUCIGULIA	IGCG IGC	ACCG IGAA
28	AGCAGCACAGAGG ICA	JAIGIIAI	AAAIGIACGIGAAI	111GI	JGG DAGGG GGG	IIGGII	IIGGAGGUIGA	IGTATTIGCCTA	IGUGIGU.	ACCG IGAA
29	AGCAGCACAGAGG ^{II} CA	JATGCCTT	TTACGTACCTACGT	IIIGI(GG TA GGG CGG	IIGGTI.	I IGGAAGG CAT	CATACTCCCCTA	IGCG IGC	ACCG IGAA
30	AGCAGCACAGAGG"CAG	GATGTCGC	CCCAAGATIGGITTI	TTIGIC	GGG TAGGG CGG C	TIGGTT	ITTAGAAIGCI	TCGGTGCACCTA	IGCGIGC	ACCG IGAA
31	AGCAGCACAGAGG <mark>T</mark> CA	JATGGTAC	AGMAGCGGCAGATT	IIIGI(GG HA GGG CGGC	I I GG I I	TTCTACTAGCT	CTG TCTAGCCTA	IGCGIGC.	ACCG IGAA
32	AGCAGCACAGAGG <mark>T</mark> CA	GATGGAGT	IGGICICGCGGACI	TTTGT	GGG MAGGG CGGC	III GG III		TTACCTGACCTA	IGCGIGC.	ACCGTGAA
33	AGCAGCACAGAGGICA	GATGGTTA	AAACGATGATGGGI	TTTGT	GGG TAGGG CGG C	TIGGTT	IIGGCAC AAG I	ICGGIG IGCCTA	IGCGIGC	LACCG TGAA
34	AGCAGCACAGAGGTCA	GATGGTAG	CGTTCAG TGA TTT I	TTTGT	GGG TA GGG CGG C	TTGGTT	ITAAG TCG CAG	TACGATTACCTA	TGCGTGC	ACCG TGAA
35	AGCAGCACAGAGGICA	GATGAACC	TCTTATAGACCAG	TTTGT	GGG TAGGG CGG C	TTGGTT	IT TCCG TG TAA	AGCCGAACCCTA	TGCGTGC	LACCG TGAA
36	AGCAGCACAGAGGTCA	GAIGGGIG	GTCATCATIGCACI	TTTGT	GGG MA GGG CGG C	TTGGTT	ITCTTGTGCAG	TAGCCAAGCCTA	TGCGTGC	ACCG TGAA
37	AGCAGCACAGAGGTCA	GATGGAGC	GACGG <mark>TT</mark> AAACAG <mark>T</mark>	TTTGT(GGG TAGGG CGG C	TTGGTT	ITGACACGCCG	CAACGGCTCCTA	TGCG TGC	ACCG TGAA
38	AGCAGCACAGAGGTCA	GATGTCAG	AGAACAAGCAAAC	TTTGT	GGGTAGGGCGGC	TTGGTT	ITACAGTTACGO	GCCTG TACCTA	TGCGTGC	ACCG TGAA
39	AGCAGCACAGAGG TCAG	GATGCGAT	TAATGTGCTCAGTT	TTTGT	GGGTAGGGGGG	TTGGTT	IT AGA AGT AAG	GGTGCCTACCTA	TGCGTGC	ACCG TGAA
40	AGCAGCACAGAGG TCA	GATGTGCA	AGCAATCCGCTGTT	TTTGT	GGG TAGGG CGGC	TTGGTT	PTGGT TCGACG	CGGGAAGGCCTA	TGCGTGC	ACCGTGAA
41	AGCAGCACAGAGG TCA	GATGCGGT	GTTTCGAACCAATT	TTTGT	GGGTAGGGCGGC	TTGGTT	IT CACAGAGCG	CAGAGAGCCCTA	TGCGTGC	ACCG TGAA
42	AGCAGCACAGAGGTCA	GATGAGGG	CACTCAAACAGAAI	TTTGT	GGG TAGGG CGG C	TIGGTT	ITGATCCGGGA	CGATGGAGCCTA	IGCGIGC	ACCGIGAA
43	AGCAGCACAGAGGTCA	GATGGAGC	CTACAGCCAGCCCT	TTTGT	GGG AGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	TTGGTT	TGAAAAGCCA	GACAAAGGCCTA	TGCGTGC	ACCGTGAA
44	AGCAGCACAGAGGTCA	GATGTAAG	GGGGATACTGGAAT	TTTGT	GGG AGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	TTGGTT	TGCGGGAGCC	ACCTGTAACCTA	TGCGTGC	ACCGTGAA
45	AGCAGCACAGAGGTCA	GATGTGGG	AAGG TCTAACGGAT	TTTGT	GG AGGGGGGG	TTGGTT	TCGGGCAGAA	CACGAGGGCCTA	TGCGTGC	ACCGTGAA
46	AGCAGCACAGAGGTCA	GATGGGCA	GGTAATGATTIGT	TTTGT	GGG AGGGGGGGGG	TTGGTT	TGGAGGACTC	GAGCTTTTCCTA	TGCGTGC	ACCGTGAA
47	AGCAGCACAGAGGTCA	GATGGGGC	GCCAAGGGTGTCGT	TTTGT	GG TAGGG CGGC	TTGGTT	TTGTGGACAA)	AGTCCGAACCTA	TGCGTGC	ACCGTGAA
48	AGCAGCACAGAGGTCA	GATGCCCG	AACCTACATGGACT	TTTGT	GG TAGGG CGGC	TTGGTT	TAAGGGGTAA	CAAATTACCCTA	TGCGTGC	ACCGTGAA
49	AGCAGCACAGAGG TCA	GATGATGG	CGGGCCCTGTATAT	TTTGT	GGTAGGGGGG	TTGGTT	TGCAAACTGG	CAAATAGGCCTA	TGCGTGC	ACCGTGAA
50	AGCAGCACAGAGGTCA	GATGTGTA	GGATGAGCATACTT	TTTGT	GGG TAGGGCGGC	TTGGTT	ITCATAAACCG	CATCTCGACCTA	TGCGTGC	ACCGTGAA