# Supporting Information

# Expanding Molecular Logic Capabilities in DNA-Scaffolded

MultiFRET Triads

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Phosphoramidites:
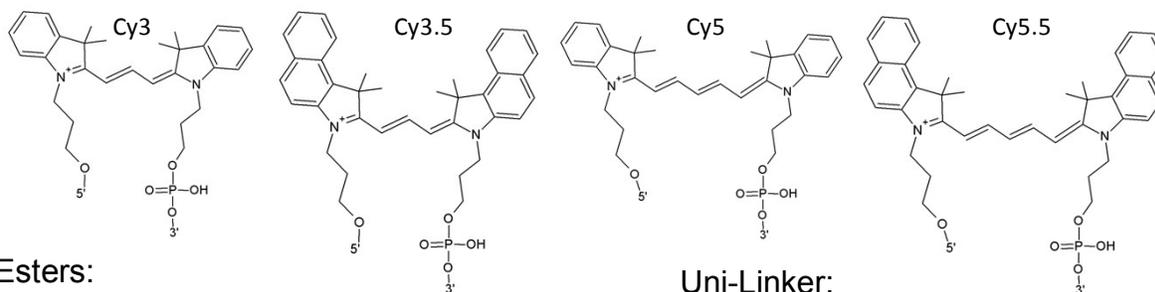
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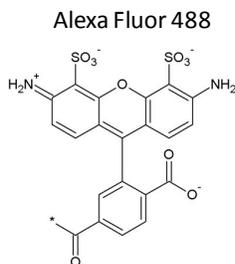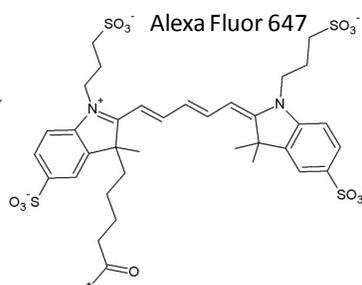
Uni-Linker:
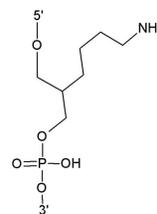

Figure S1. Chemical structures of attached dyes

Optimization of loop and trigger strands

	Trig1	Trig2	Trig3
Loop1	--	0.21	0.34
Loop2	0.27	--	0.39
Loop3	0.06	1.9	--

Table S1 - Off-target binding probabilities of LoopLink structure, in percent (assuming 500 nM concentration).
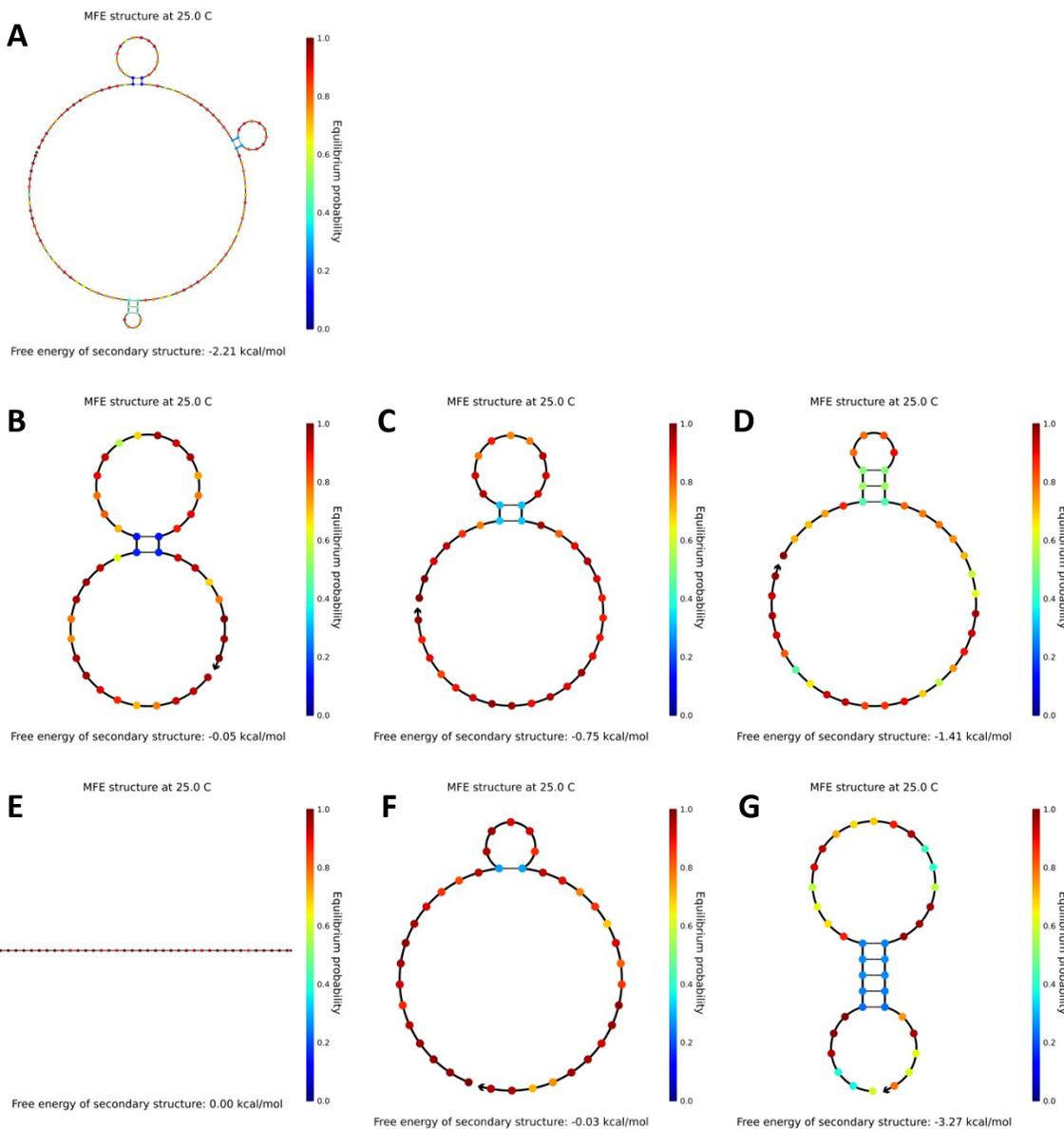
Figure S2. Structural optimization of LoopLink structure. Panels show secondary structure of various elements, optimized in NUPACK. (A) All loops as a single strand, best approximating potential intramolecular interactions of the linked strands within the switch structure. Individual strands were also modeled: (B) Loop1 (C) Loop2 (D) Loop3 (E) Trigger 1 (F) Trigger 2 (G) Trigger 3.
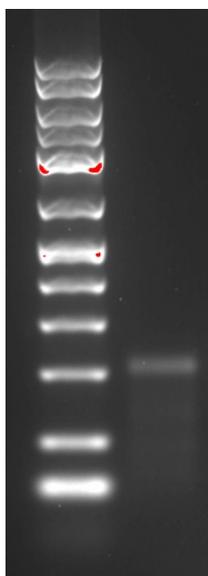
Figure S3. 2% agarose gel of the Dye-in-Loop switch structure. Gel analysis estimated 63% formation efficiency based on overall band intensity.
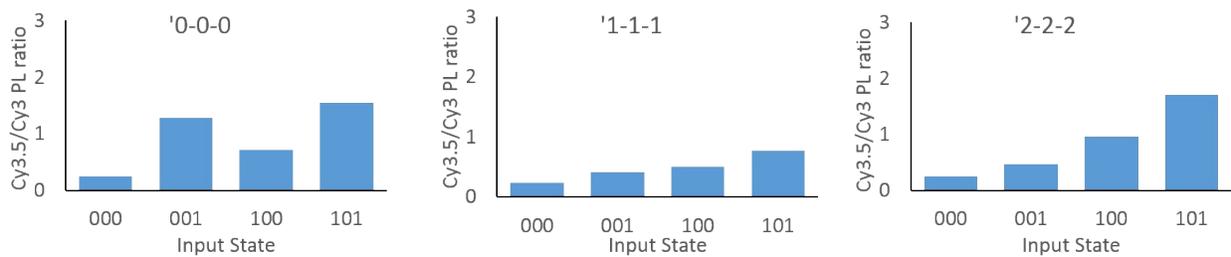
Figure S4. Output ratios for the 0-00-0, 1-1-1 and 2-2-2 spacing used in Figure 4. Thresholds shown in Fig 4F for the 0-0-0 structure is taken from the difference between the 000 and the 100 for the OR gate and between 001 and 101 for the AND gate. For all others the OR gate threshold is between 000 and 001 and the AND gate threshold is between 100 and101.

Table S2. DNA Sequences - Removable Linker

| Name	Sequence	Modification	Tm (1X TAE 12.5 mM MgCl$_2$)	Source
Arm 1	GGTTCAGCCGCAATCCGGCACAGCTATAATAA*GAGTTTGATGAAATGGAGCGGACGTGAGATGG	Internal Cy3	79.0	IDT
Arm2	GCAAGACTCGTGCTCAGGTCCTAAGTTGGTTCT*ACCGCATGGTATATGGGGCTTACGGTGGTGCG	Internal Cy3.5 or Unilinker + AF488	79.7	Operon
Arm 3	GGATCAGAGCTGGACGGGAGCCTATCGGGTAG*TTATGTTGTTCGCTGGTGTTACTGCATCCAGG	Internal Cy5 or Unilinker + AF647	79.3	IDT
Cap 1	CCATCTCACGTCCGCTGGATTGCGGCTGAACC		76.7	IDT
Cap 2	CGCACCACCGTAAGCCTGAGCACGAGTCTTGC		76.5	IDT
Cap 3	CCTGGATGCAGTAACACGTCCAGCTCTGATCC		73.9	IDT
Link1 0 base spacing	GCCGGAGACCATATACCATGCGGTTTATTATAGCTGTGCC		75.0	IDT
Link1 1 base spacing	GCCGGAGACCATATACCATGCGGTATTATTATAGCTGTGCC		74.6	IDT
Link1 2 base spacing	GCCGGAGACCATATACCATGCGGTAATTATTATAGCTGTGCC		74.7	IDT
Link1 3 base spacing	GCCGGAGACCATATACCATGCGGTAAATTATTATAGCTGTGCC		74.7	IDT
Link1 6 base spacing	GCCGGAGACCATATACCATGCGGTAAAAAATTATTATAGCTGTGCC		75.0	IDT
Link1 9 base spacing	GCCGGAGACCATATACCATGCGGTAAAAAAAAATTATTATAGCTGTGCC		75.2	IDT
Link2 0 base spacing	CCTGTACGCCAGCGAACAACATAAGAACCAACTTAGGACC		75.1	IDT
Link2 1 base spacing	CCTGTACGCCAGCGAACAACATAATGAACCAACTTAGGACC		75.2	IDT

Link2 **2 base spacing**	CCTGTACGCCAGCGAACAACATAATAGAACCAACTTAGGACC	74.8	IDT
Link2 **3 base spacing**	CCTGTACGCCAGCGAACAACATAATAAGAACCAACTTAGGACC	74.8	IDT
Link2 **6 base spacing**	CCTGTACGCCAGCGAACAACATAATAATAAGAACCAACTTAGGACC	74.6	IDT
Link2 **9base spacing**	CCTGTACGCCAGCGAACAACATAATAATAATAAGAACCAACTTAGGACC	74.5	IDT
Link3 **0 base spacing**	GATACGGACCATTTCATCAAACTCCTACCCGATAGGCTCC	74.4	IDT
Link3 **1 base spacing**	GATACGGACCATTTCATCAAACTCACTACCCGATAGGCTCC	74.3	IDT
Link3 **2 base spacing**	GATACGGACCATTTCATCAAACTCAACTACCCGATAGGCTCC	74.4	IDT
Link3 **3 base spacing**	GATACGGACCATTTCATCAAACTCAAACTACCCGATAGGCTCC	74.5	IDT
Link3 **6 base spacing**	GATACGGACCATTTCATCAAACTCAAATAAACTACCCGATAGGCTCC	74.4	IDT
Link3 **9 base spacing**	GATACGGACCATTTCATCAAACTCAAATAAATTCTACCCGATAGGCTCC	74.4	IDT

*in sequence indicates modifier placement

Table S3. DNA Sequences - Looped Linker

Name	Sequence	Modification	Tm (1X TAE 12.5 mM MgCl$_2$)	Source
Arm 1	GGTTCAGCCGCAATCCGGCACAGCTATAATAA*GAGTTTGATGAAATGGAGCGGACGTGAGATGG	Internal Cy3	79.0	IDT
Arm2	GCAAGACTCGTGCTCAGGTCCTAAGTTGGTTCT*ACCGCATGGTATATGGGGCTTACGGTGGTGCG	Internal Cy3.5	79.7	Operon
Arm 3	GGATCAGAGCTGGACGGGAGCCTATCGGGTAG*TTATGTTGTTCGCTGGTGTTACTGCATCCAGG	Internal Cy5	79.3	IDT
Cap 1	CCATCTCACGTCCGCTGGATTGCGGCTGAACC		76.7	IDT
Cap 2	CGCACCACCGTAAGCCTGAGCACGAGTCTTGC		76.5	IDT
Cap 3	CCTGGATGCAGTAACACGTCCAGCTCTGATCC		73.9	IDT
Loop Link1	CCATATACCATGCGGTTTAGTTAGAGTGAGACATAAAGCAAAGTTAGTGAGTGAGATTATTATAGCTGTGCC		76.9	IDT
Trigger 1	TCTCACTCTAACTAATCTCACTCACTAACTCATCACAA		70.9	IDT
Comp 1	TCACTCACTAACTTTGCTTTATGTCTCACTCTAACT		70.7	IDT
Loop Link2	CCAGCGAACAACATAAGTATAGGGTTAAGGGTTCCATTTCAGTTTCATTTCAAGTAAGAACCAACTTAGGACC		77.6	IDT
Trigger 2	CCCTTAACCCTATACTACTTGAAATGAAACGCCCTTAT		71.8	IDT
Comp 2	CTTGAAATGAAACTGAAATGGAACCCTTAACCCTAT		70.9	IDT
Loop Link3	CCATTTCATCAAACTCGGATCAGATTTATCTTAGTGTTAGGTGTAGCAGGCATAGTCTACCCGATAGGCTCC		77.6	IDT
Trigger 3	AGATAAATCTGATCCACTATGCCTGCTACAGATACGGA		72.8	IDT
Comp 3	TATGCCTGCTACACCTAACACTAAGATAAATCTGAT		70.5	IDT

*in sequence indicates modifier placement

Table S4. DNA Sequences – Dye-in-Loop

Name	Sequence	Modification	Tm (1X TAE 12.5 mM MgCl$_2$)	Source
Arm 1	GGTTCAGCCGCAATCCGGCACAGCTATAATAA*GAGTTTGATGAAATGGAGCGGACGTGAGATGG	Internal Cy3	79.0	IDT
Arm2	GCAAGACTCGTGCTCAGGTCCTAAGTTGGTTCT*ACCGCATGGTATATGGGGCTTACGGTGGTGCG	Internal Cy3.5	79.7	Operon
Arm 3	GGATCAGAGCTGGACGGGAGCCTATCGGGTAG*TTATGTTGTTCGCTGGTGTTACTGCATCCAGG	Internal Cy5	79.3	IDT
Cap 1	CCATCTCACGTCCGCTTGAGCACGAGTCTTGC		75.3	IDT
Cap 2	CGCACCACCGTAAGCCCGTCCAGCTCTGATCC		77.1	IDT
Cap 3	CCTGGATGCAGTAACAGGATTGCGGCTGAACC		74.8	IDT
Trigger 1	CCATTTCATCAAACTCGAACCAACTTAGGACC		70.5	IDT
Comp 1	TTCATCAAACTCATTATTATAGCTG		61.4	IDT
Trigger 2	CCATATACCATGCGGTCTACCCGATAGGCTCC		73.2	IDT
Comp 2	ATACCATGCGGTAGAACCAACTTAG		66.8	IDT
Trigger 3	CCAGCGAACAACATAATTATTATAGCTGTGCC		69.7	IDT
Comp 3	CGAACAACATAAACTACCCGATAGG		65.4	IDT

*in sequence indicates modifier placement

Table S5. Fluorophore photophysical and FRET properties.

Fluorophores	Quantum yield	Extinction coefficient $(M^{-1}cm^{-1})$	λ_{max} absorption	λ_{max} emission	R_0 in Å / $J(\lambda)$ in $cm^3\,M^{-1}$				
					AF488	Cy3	Cy3.5	Cy5	A647
AF488	0.39	71,000	495 nm	519 nm	$46/1.23e^{-13}$	$61/6.9e^{-13}$	$59/5.93e^{-13}$	$49/1.9e^{-13}$	$47/1.42e^{-13}$
Cy3	0.15	150,000	550 nm	570 nm	---	$47/3.68e^{-13}$	$53/8.01e^{-13}$	$54/9.37e^{-13}$	$53/7.83e^{-13}$
Cy3.5	0.15	150,000	581 nm	596 nm	---	---	$44/2.70e^{-13}$	$60/1.69e^{-12}$	$59/1.58e^{-12}$
Cy5	0.28	250,000	649 nm	670 nm	---	---	---	$65/1.39e^{-12}$	---
AF647	0.33	190,000	675 nm	694 nm	---	---	---		$65/1.17e_{-12}$