SUPPORTING INFORMATION

A Triangular Three-Dye DNA Switch Capable
of Reconfigurable Molecular Logic

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Gel electrophoresis. DNA was loaded into a 2% agarose gel buffered with 1× TBE buffer (89 mM Tris-borate, 89 mM boric acid, 2 mM EDTA pH 8.3) in TBE running buffer and prestained with gel red intercalating dye (Biotium). The gel was run at 5V/cm for 2 hours on an ice bath. The DNA bands were visualized on a BioRad ChemiDoc XRS+ imaging system.

Assembly Efficiency. Gel electropherograms demonstrate that both 2.5×PBS and 1×Tris acetate EDTA (TAE) supplemented with 12.5 mM $MgCl_2$ display sufficient charge screening for efficient DNA hybridization as evidenced by the bands that appear between 200 to 300 base pairs in both buffer conditions (Figure 2C). The fully formed structure should migrate at a size corresponding to 220-250 base pairs based on just molecular weight alone which is what is indeed observed. However, refining this estimate is complicated by 3-dimensional shape, and full formation was therefore verified by comparison to partially formed structures as shown in the SI. Using image analysis to derive a quantitative comparison of the electropherograms in Figure 2C, the estimated yield for the core structure formed in PBS was around 65%, while the same structure in TAE with Mg^{2+} had a yield of close to 80%. As the switch structure is more efficiently formed when 1×TAE with $MgCl_2$ is used, this buffer was employed in all subsequent work.
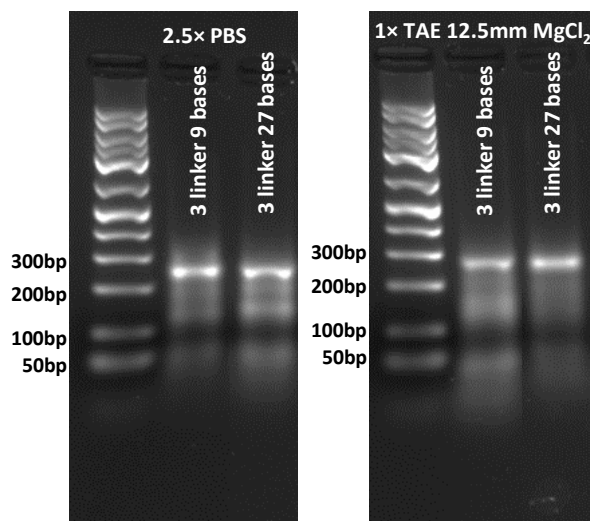
Figure S1. Gel electropherograms characterizing the switch assembly formation. Both 2.5×PBS and TAE supplemented with 12.5 mM $MgCl_2$ were assayed as assembly buffers to assure good formation while minimizing non-specific interactions. Samples were then separated in 2% agarose gels. Both buffers are suitable, but TAE with $MgCl_2$ was used for its slightly improved performance.
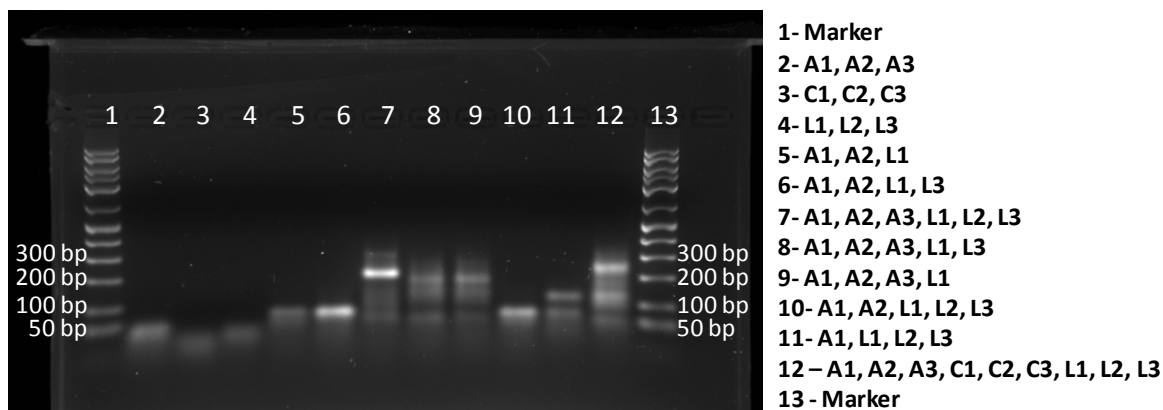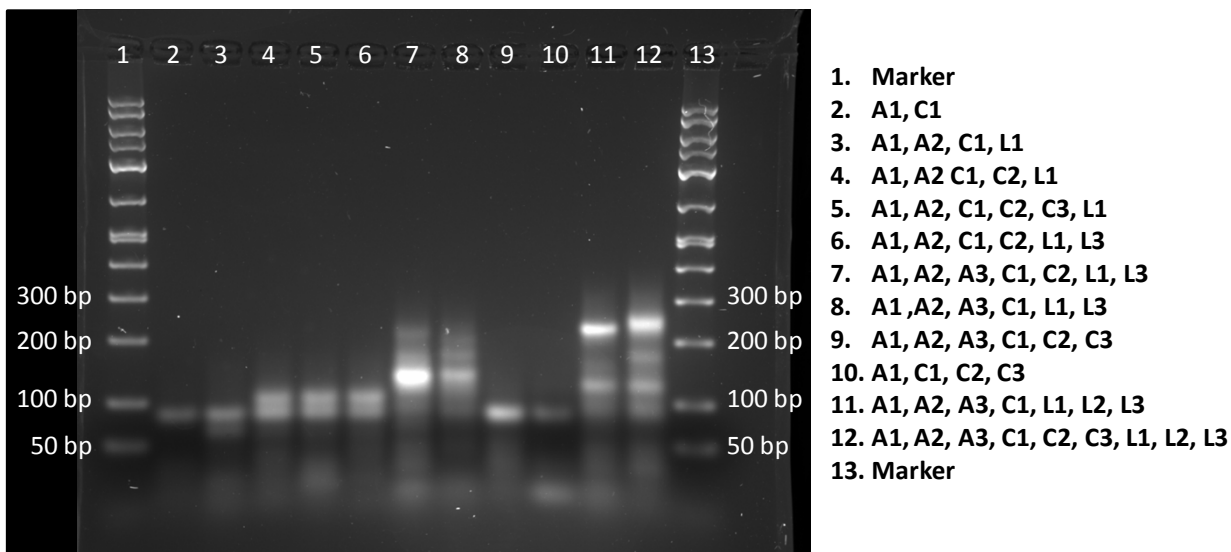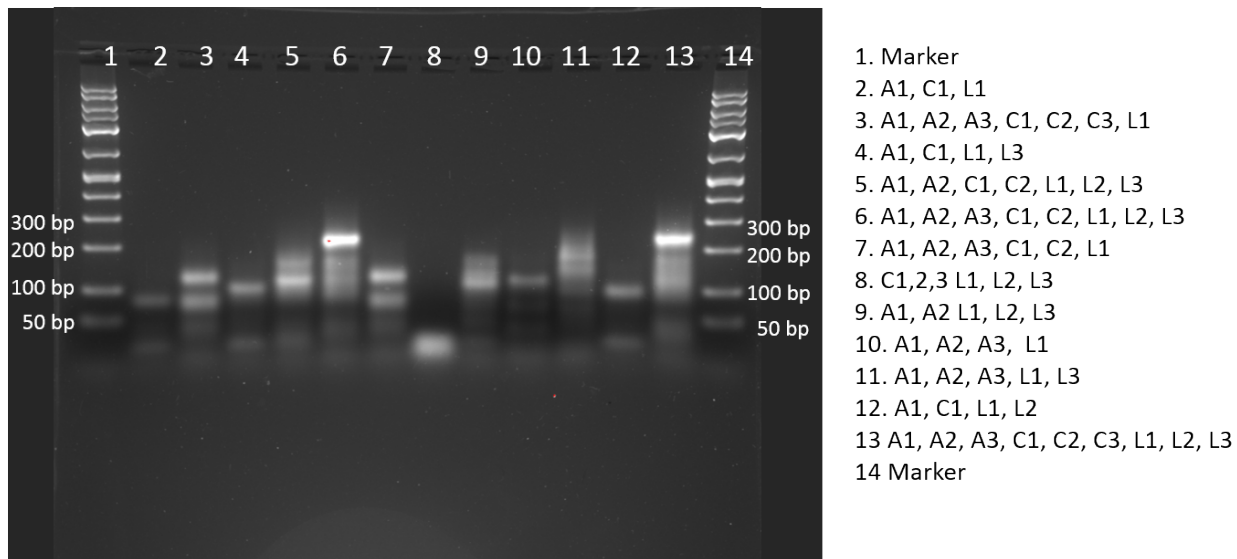
Figure S2. Agarose gel of the control structures showing intentional malformation and partial formations used to verify full assembly and assembly efficiency. A = Arm, C = Cap and L = Linker.

Single Input Logic. We begin evaluating the potential of the DNA scaffold system by creating single input logic gates, *i.e.*, that process one input to produce a meaningful output. In particular, we devised a simple yes/no buffer gate where the presence or absence of an input produces an ON/1 or OFF/0 response, respectively, see Figure S3, with the input provided by a DNA linker strand (9 base ssDNA linker). Three distinct buffer gates were created by including (or excluding) the 9 base ssDNA linker between the Cy3 and Cy3.5 (L1 switch), the Cy3.5 and Cy5 (L2 switch), or the Cy3 and Cy5 (L3 switch), Figure S3i. These three structures are formed such that each state, whether ON or OFF, is annealed separately. The corresponding PL spectra for the L1, L2, and L3 switches are also displayed in Figure S3i (bottom) with the PL shown for each in the absence (yellow spectra) and presence of the linker (blue spectra). These PL spectra are converted into a Boolean logic output by quantizing the ratio of the PL peak height of the FRET acceptor dye to that of the donor (Figure S3iii). In the case of the L2 and L3 switches, a PL ratio threshold of 0.2 was established so that PL peak height ratios above 0.2 are converted to ON/1 outputs while those below become OFF/0. For the L1 switch the PL ratio threshold was set to 0.5 in order to compensate for direct excitation of the Cy3.5 dye. This latter adjustment highlights an important underlying point about such multi-dye constructs and FRET, namely that some donor-acceptor combinations will be more susceptible to direct acceptor excitation or more efficient than others at the same nominal distance, and the assignment of thresholds needs to consider this and not remain static assuming one value will suffice for all.

Figure S3. Two-arm single input device. i) Schematic showing the formation of the three different one linker (input) structures along with respective spectra for each when the linker is present (blue) or not (yellow). **ii)** These spectra correspond to a buffer gate that reflects the yes/no (ON/1 or OFF/0) status of the 9 base linker. **iii)** Shows the output signal and assigned output state for each structure. Note, that due to the close spectral overlap of Cy3 and Cy3.5, the designated threshold must be set higher than the other two dye sets. Error bars represent the standard deviation of at least n = 3 experiments.

Table SI. Fluorophore photophysical and FRET properties.

Fluorophores	Quantum yield	Extinction coefficient $(M^{-1}cm^{-1})$	λ_{max} absorption	λ_{max} emission	$^1R_0$ in Å / $J(\lambda)$ in cm^3 M^{-1}		
					Cy3	Cy3.5	Cy5
Cy3	0.15	150,000	550 nm	570 nm	47 / 3.68e^{-13}	53 / 8.01e^{-13}	54 / 9.37e^{-13}
Cy3.5	0.15	150,000	581 nm	596 nm	---	44 / 2.70e^{-13}	60 / 1.69e^{-12}
Cy5	0.28	250,000	649 nm	670 nm	---	---	65 / 1.39e^{-12}

$^1R_0$ and $J(\lambda)$ values are averages calculated from the spectra of all dye-labeled DNA used in this study.

Table SII. DNA sequences

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
Link1 **9 base spacing**	GCCGGAGACCATATACCATGCGGTAAAAAAAATTATTATAGCTGTGCC	75.2	IDT
Link1 **18 base spacing**	GCCGGAGACCATATACCATGCGGTAAAAAAAAAAAAAAAATTATTATAGCTGTGCC	76.0	IDT
Link1 **27 base spacing**	GCCGGAGACCATATACCATGCGGTAAAAAAAAAAAAAAAAAAAAAATTATTATAGCTGTGCC	76.9	IDT
Link2 **9 base spacing**	CCTGTACGCCAGCGAACAACATAATAATAATAAGAACCAACTTAGGACC	74.5	IDT
Link2 **18 base spacing**	CCTGTACGCCAGCGAACAACATAATAATAATAATAATAAGAACCAACTTAGGACC	74.4	IDT
Link2 **27 base spacing**	CCTGTACGCCAGCGAACAACATAATAATAATAATAATAATAATAATAAGAACCAACTTAGGACC	74.7	IDT
Link3 **9 base spacing**	GATACGGACCATTTCATCAAACTCAAATAAATTCTACCCGATAGGCTCC	74.4	IDT
Link3 **18 base spacing**	GATACGGACCATTTCATCAAACTCAAATAAATTAAATAAATTCTACCCGATAGGCTCC	74.7	IDT
Link3 **27 base spacing**	GATACGGACCATTTCATCAAACTCAAATAAATTAAATAAATTAAATAAATTCTACCCGATAGGCTCC	75.2	IDT
Link1 **9 base comp**	GGCACAGCTATAATAATTTTTTTTTACCGCATGGTATATGGTCTCCGGC	75.2	IDT
Link1 **18 base comp**	GGCACAGCTATAATAATTTTTTTTTTTTTTTTTTACCGCATGGTATATGGTCTCCGGC	76.0	IDT
Link1 **27 base comp**	GGCACAGCTATAATAATTTTTTTTTTTTTTTTTTTTTTTTTTTACCGCATGGTATATGGTCTCCGGC	76.9	IDT
Link2 **9 base comp**	GGTCCTAAGTTGGTTCTTATTATTATTATGTTGTTCGCTGGCGTACAGG	74.5	IDT
Link2 **18 base comp**	GGTCCTAAGTTGGTTCTTATTATTATTATTATTATTATGTTGTTCGCTGGCGTACAGG	74.4	IDT

Link2 **27 base comp**	GGTCCTAAGTTGGTTCTTATTATTATTATTATTATT ATTATTATTATGTTGTTCGCTGGCGTACAGG	74.7	IDT
Link3 **9 base comp**	GGAGCCTATCGGGTAGAATTTATTTGAGTTTGATG AAATGGTCCGTATC	74.4	IDT
Link3 **18 base comp**	GGAGCCTATCGGGTAGAATTTATTTAATTTATTTGA GTTTGATGAAATGGTCCGTATC	74.7	IDT
Link3 **27 base comp**	GGAGCCTATCGGGTAGAATTTATTTAATTTATTTAA TTTATTTGAGTTTGATGAAATGGTCCGTATC	75.2	IDT

*in sequence indicates modifier placement

Table SIII. Data averages and standard deviations for the three linker logic.

Permutation			Average			Std. Dev.		
L1	**L2**	**L3**	**606/564**	**664/606**	**664/564**	**606/564**	**664/606**	**664/564**
0	0	0	0.337	0.098	0.033	0.045	0.021	0.007
0	0	27	0.466	0.780	0.367	0.073	0.077	0.084
0	9	0	0.199	0.775	0.153	0.024	0.084	0.007
0	9	27	0.271	1.859	0.503	0.013	0.075	0.012
9	0	0	0.864	0.069	0.059	0.104	0.013	0.011
9	0	27	1.002	0.527	0.530	0.050	0.066	0.080
9	9	0	0.591	1.114	0.663	0.050	0.165	0.145
9	9	27	0.449	1.867	0.850	0.070	0.180	0.209