Electronic Supplementary Material (ESI) for ChemComm.

Conformational enhancement of fidelity in toehold-sequestered DNA nanodevices

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Supplementary Data

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OxDNA SIMULATIONS

All toehold-sequestered devices were assembled using a Virtual Move Monte Carlo (VMMC) simulation that allowed the introduction of mutual traps between strands to reduce the computation time. A set particle is pulled toward its base-pairing complement at a predefined close-to-equilibrium distance. This pre-configuring step speeds up the assembly of the devices and requires minimal computing power. Simulations were interrupted once the structures were fully assembled. Molecular dynamics (MD) were performed on the resulting structures with the following parameters used in the input files for the simulation:

Key	Value
steps	$1e^8$
newtonian_steps	103
diff_coeff	2.5
thermostat	john
dt	0.005
verlet_skin	0.05

MATERIALS

All oligonucleotides were purchased from Integrated DNA Technologies, BVBA (Leuven, Belgium). DNA stock solutions were prepared at a concentration of a 100 µM in molecular biology grade water (5 Prime GmbH, Hilden, Germany).

Annealing and interrogation of the toehold-sequestered gates were performed in 1× TBE/Mg^{2+} buffer. A stock 10× TBE/Mg^{2+} buffer solution was prepared by adding magnesium chloride to 10× TBE buffer (Severn Biotech, Kidderminster, UK) to a concentration of 125 mM, and was then diluted as required.

DNA SEQUENCES

Internal toehold, n = 1

Toehold-sequestered device

CTGTCCTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATAATTT

CATTAAGCATTATTGAACGCAAATTATGAGGGAATAGGAACACACTTGGAAAGCAAATAGGACAG

GCGTTCAATAATGCTTAATGTATGGAGGAT

Inputs

$\mathbf{l}_a$ CTGTCCTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATAATTTGCGTTCAATA

$\mathbf{l}_b$ CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATAATTTGCGTTCAATAATGC

I_c CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATAATTTGCGTTCAATA

I_d CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATAATTTGCGTTCA

Internal toehold, n = 2

Toehold-sequestered device

CTGTCCTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGTG

CATTAAGCATTATTGAACGCCACGGATGAGGGAATAGGAACACACTTGGAAAGCAAATAGGACAG

GCGTTCAATAATGCTTAATGTATGGAGGAT

Inputs

I_a CTGTCCTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGTGGCGTTCAATA

I_b CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGTGGCGTTCAATAATGC

I_c CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGTGGCGTTCAATA

I_d CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGTGGCGTTCA

Internal toehold, n = 3

Toehold-sequestered device

CTGTCCTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGCG

CATTAAGCATTATTGAACGCCGCGGATGAGGGAATAGGAACACACTTGGAAAGCAAATAGGACAG

GCGTTCAATAATGCTTAATGTATGGAGGAT

Inputs

I_a CTGTCCTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGCGGCGTTCAATA

I_b CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGCGGCGTTCAATAATGC

I_c CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGCGGCGTTCAATA

I_d CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGCGGCGTTCA

External toehold, n = 1

Toehold-sequestered device

CTGTCCTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATAATTT

CATTAAGCATTATTGAACGCAAATTATGAGGGAATAGGAACACACTTGGAAAGCAAATAGGACAG

TAGGAGGTATGCGTTCAATAATGCTTAATG

Inputs

I_a CTGTCCTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATAATTTGCGTTCAATA

I_b CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATAATTTGCGTTCAATAATGC

I_c CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATAATTTGCGTTCAATA

I_d CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATAATTTGCGTTCA

External toehold, n = 2

Toehold-sequestered device

CTGTCCTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGTG

CATTAAGCATTATTGAACGCCACGGATGAGGGAATAGGAACACACTTGGAAAGCAAATAGGACAG

TAGGAGGTATGCGTTCAATAATGCTTAATG

Inputs

I_a CTGTCCTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGTGGCGTTCAATA

I_b CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGTGGCGTTCAATAATGC

I_c CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGTGGCGTTCAATA

I_d CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGTGGCGTTCA

External toehold, n = 3

Toehold-sequestered device

CTGTCCTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGCG

CATTAAGCATTATTGAACGCCGCGGATGAGGGAATAGGAACACACTTGGAAAGCAAATAGGACAG

TAGGAGGTATGCGTTCAATAATGCTTAATG

Inputs

I_a CTGTCCTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGCGGCGTTCAATA

I_b CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGCGGCGTTCAATAATGC

I_c CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGCGGCGTTCAATA

I_d CTATTTGCTTTCCAAGTGTGTTCCTATTCCCTCATCCGCGGCGTTCA

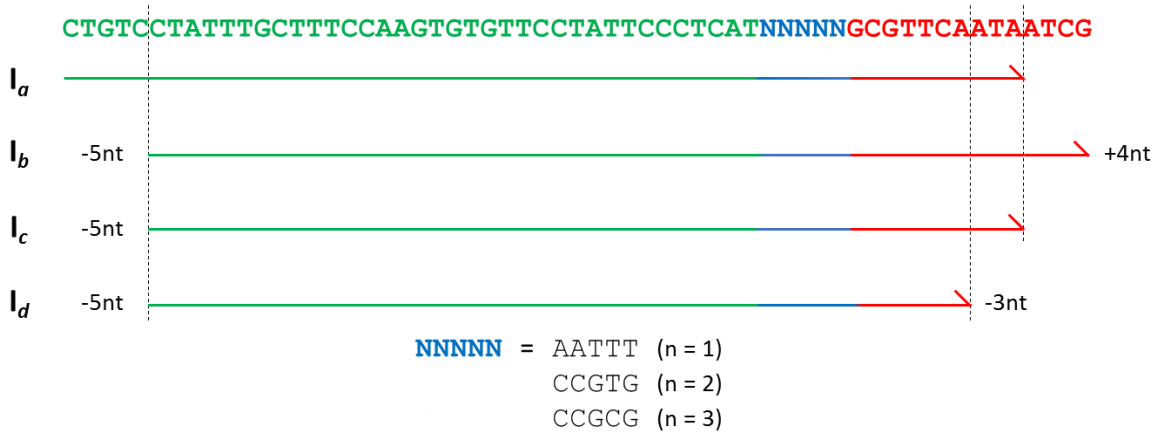

Figure S1. Comparison of the sequences of the various input strands used to interrogate the toehold-sequestered devices.

TOEHOLD BINDING ENERGIES

The binding energies of the toehold were calculated following two methods detailed in the Supporting information of supporting reference [1]. The difference in total standard free energy between an assembly where the spurious input is bound to the duplex by its toehold and the corresponding input-free assembly gives the binding energy of the toehold. The default calculation method makes use of a set of thermodynamic parameters for calculating the total standard free energy of DNA-based assemblies, which is both sequence- and motif- dependant. Separately, the toehold binding energies have been determined using NUPACK,[2] using the recommended "dangle=some" option. The toehold binding energies were derived from thermodynamic data computed from forced configurations of a given device, where the spurious strand is either bound or not bound. A corrective Protozanova[3] coaxial stacking term as well as a corrective factor that converts the energy from mole fractions to molar units were added to the predicted free energy of each complexes, as indicated in ref. [1]. The calculated toehold binding energies derived from both models are reported in Table S1.

Table S1. Predicted toehold binding energies (kcal/mol) obtained by calculation using the thermodynamic parameters described in the default model, and by computation using NUPACK.

		Default			**NUPACK (dangle=some)**		
	NNNNN =	AATTT	CCGTG	CCGCG	AATTT	CCGTG	CCGCG
Spurious input	I_a	-12.5	-13.8	-13.8	-13.0	-14.0	-14.0
	I_b	-19.4	-20.7	-20.7	-20.0	-21.0	-21.0
	I_c	-12.5	-13.8	-13.8	-13.0	-14.0	-14.0
	I_d	-9.9	-11.2	-11.2	-10.0	-11.1	-11.1

GATE FORMATION AND PURIFICATION

DNA sequences were purchased from IDT (Leuven, Belgium). The toehold-sequestered devices were assembled by combining equal amounts of the three component strands in 1× TBE/Mg^{2+} buffer. The resulting samples were annealed by heating to 90 °C for 5 minutes followed by slow cooling to 5 °C over 2 hours using a PCR Mastercycler (Eppendorf, Stevenage, UK). The gates were then incubated for 18 hours at room temperature with deactivated inputs lacking the toeholds required for toehold-mediated strand displacement. The assembled devices were subsequently purified by non-denaturing gel electrophoresis supplemented in 10% PAGE gels supplemented with 12.5 mM $MgCl_2$ in 1× TBE/Mg^{2+} buffer, and were run at 300 V for 37 minutes (room temperature and cooled down with an in-house fan system). Gels were stained with SYBR Gold (Life Technologies, Eugene, OR, USA). After ethanol extraction from gel bands, the DNA pellets were dissolved in 1× TBE/Mg^{2+} buffer. The concentrations of the DNA solutions were calculated by measuring their respective absorbance at 260 nm (NanoVue Plus, GE Healthcare Life Sciences, Uppsala, Sweden) and using the following formula to calculate their extinction coefficients:

$$E(ds, 260) = \sum_{i=1}^{3} E_i(ss, 260) \times [1 - (0.059 \times f_{GC} + 0.287 \times f_{AT})]$$

Here, E_i (*ss,* 260) is the calculated extinction coefficient of one component strand of the toehold-sequestered device at 260 nm; f_{GC} and f_{AT} are the fraction of G and C and the fraction of A and T in the sequence of the duplex section of the assembly, respectively.

ELECTROPHORETIC MOBILITY SHIFT ASSAY

All toehold-sequestered devices were subjected to electrophoresis through native and 6 M urea-enhanced 10% polyacrylamide gels supplemented with 12.5 mM $MgCl_2$. Gels were run at 300 V for 35 minutes. Relative mobilities were calculated by dividing the migration distances of bands corresponding to a toehold-sequestered device and a 55bp duplex used as internal standard.

EVALUATION OF LEAKAGE IN INTERNAL AND EXTERNAL TOEHOLD-SEQUESTERED DEVICES

All toehold-sequestered devices (following formation and purification as noted above) were incubated at a 5 μM concentration with 1.1 equivalents of the appropriate spurious input in 1× TBE/Mg^{2+} buffer. Samples were loaded on 12% polyacrylamide gels supplemented with 12.5 mM $MgCl_2$. Gels were run at constant 300 V in 1× TBE/Mg^{2+} buffer (32 minutes, room temperature and cooled down with an in-house fan system) and stained with SYBR Gold for 15 minutes. Gels were subsequently scanned on a Typhoon 9400 imager (Amersham Biosciences, Little Chalfont, UK). Gel band quantitation was performed using GelQuantNET (BiochemLabSolutions). The leakage yield was calculated as $\chi_n = [X_n] / ([X_n] + [D_n])$, where $[X_n]$ and $[D_n]$ are the band intensities of the leak-induced duplex product X_n and the

intact toehold-sequestered device [D_n], respectively. Experiments were repeated on four different gels to evaluate standard deviation for each dataset.

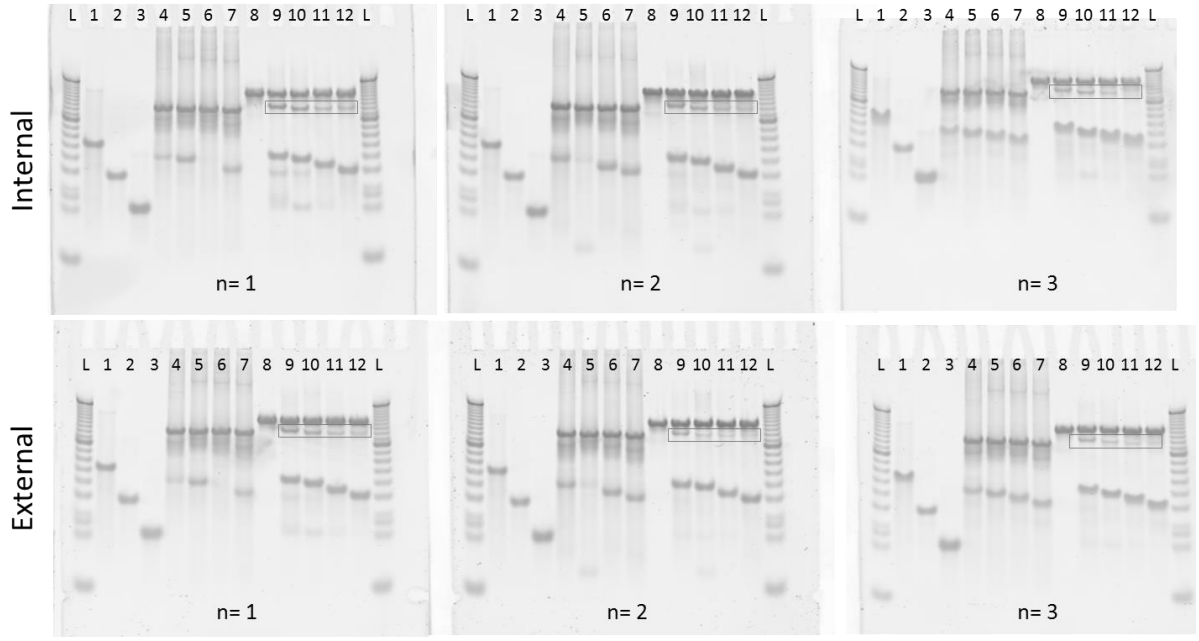

Figure S2. Native polyacrylamide gel electrophoresis for evaluating the robustness of the toehold-sequestered devices. Lane 1 to 3: Individual assembly strands. Lane 4 to 6: Duplex waste products (using I_a to I_d, from left to right). Lane 8: Control toehold-sequestered device. Lane 9 to 12: Toehold-sequestered devices incubated with the appropriate inputs I_a to I_d, from left to right. L: 5bp ladder.

Table S2. Leakage yield in internal toehold-sequestered devices.

Device	Input	Gel 1	Gel 2	Gel 3	Gel 4	Average	Standard deviation
n = 1	$\mathbf{I_a}$	0.29	0.29	0.24	0.30	0.28	0.028
	$\mathbf{I_b}$	0.25	0.25	0.25	0.26	0.25	0.0047
	$\mathbf{I_c}$	0.11	0.13	0.14	0.12	0.13	0.013
	$\mathbf{I_d}$	0.11	0.12	0.12	0.10	0.11	0.010
n = 2	$\mathbf{I_a}$	0.21	0.21	0.20	0.20	0.20	0.0045
	$\mathbf{I_b}$	0.16	0.15	0.16	0.17	0.16	0.0046
	$\mathbf{I_c}$	0.12	0.12	0.10	0.11	0.11	0.0089
	$\mathbf{I_d}$	0.11	0.11	0.092	0.092	0.10	0.011
n = 3	$\mathbf{I_a}$	0.13	0.11	0.18	0.16	0.14	0.033
	$\mathbf{I_b}$	0.13	0.10	0.17	0.17	0.14	0.032
	$\mathbf{I_c}$	0.045	0.029	0.091	0.092	0.064	0.032
	$\mathbf{I_d}$	0.0010	0.0018	0.0098	0.0027	0.0038	0.0041

Table S3. Leakage yield in external toehold-sequestered devices.

Device	Input	Gel 1	Gel 2	Gel 3	Gel 4	Average	Standard deviation
n = 1	$\mathbf{I_a}$	0.14	0.15	0.17	0.16	0.15	0.013
	$\mathbf{I_b}$	0.13	0.12	0.15	0.12	0.13	0.014
	$\mathbf{I_c}$	0.094	0.058	0.10	0.063	0.080	0.022
	$\mathbf{I_d}$	0.075	0.043	0.062	0.053	0.058	0.014
n = 2	$\mathbf{I_a}$	0.075	0.071	0.068	0.042	0.064	0.015
	$\mathbf{I_b}$	0.013	0.011	0.013	0.010	0.012	0.0015
	$\mathbf{I_c}$	0.0083	0.0076	0.0073	0.0051	0.0071	0.0014
	$\mathbf{I_d}$	0.0034	0.0058	0.0026	0.0040	0.0040	0.0014
n = 3	$\mathbf{I_a}$	0.046	0.057	0.052	0.049	0.051	0.0046
	$\mathbf{I_b}$	0.015	0.019	0.015	0.018	0.017	0.0021
	$\mathbf{I_c}$	0.0072	0.0067	0.0054	0.0061	0.0064	0.00077
	$\mathbf{I_d}$	0.0020	0.0045	0.0041	0.0043	0.0037	0.0012

SUPPORTING REFERENCES

[1] D. Y. Zhang and E. Winfree, *J. Am. Chem. Soc.*, 2009, **131**, 17303-17314.

[2] J. N. Zadeh, C. D. Steenberg, J. S. Bois, B. R. Wolfe, M. B. Pierce, A. R. Khan, R. M. Dirks and N. A. Pierce, *J. Comput. Chem.*, 2011, **32**, 170-173.

[3] E. Protozanova, P. Yakovchuk and M. D. Frank-Kamenetskii, *J. Mol. Biol.*, 2004, **342**, 775-785.