Supplemental Section

Energetically Biased DNA Motor Containing a Thermodynamically Stable Partial Strand Displacements State

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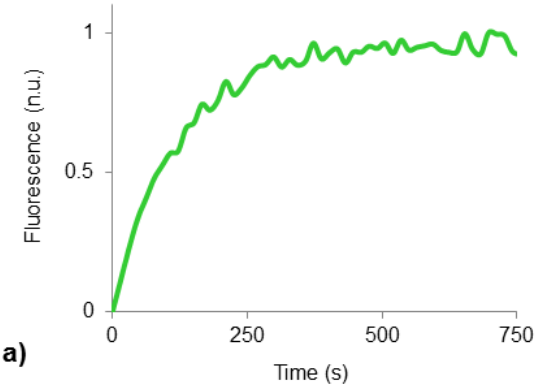
Table S1. Domain Sequences

Domain	Sequence
g	5'-**ACCTA CTCAA CCATA CATCA CTCTA CCTCA** aacac tcgaa t*CTCC CAAAT ACTAA GCTGT TCAAC TCACC*-3'
g″	5'- CCTCC AGTTC CAAGT ACCAC CATCA **CACCT ACTCA ACCAT ACATC ACTCT ACCTC A**aaca ctcga at*CTC CCAAA TACTA AGCTG TTCAA CTCAC C*-3'
j	5'- *TTCTG GGGAG TGGGA GATGt TGGCT AGAT TACGG TA*taa **TGAGG TAGAG TGATG TATGG TTGAG TAGGT**–3'
j′	5'- TTCTG GGGAG TGGGA GATGt TGGCT AGATT TACGG TAtaa **TIAII TAIAI TIATI TATII TTIAI TAIIT**–3'
j″	5'- *TTCTG GGGAG TGGGA GATGt TGGCT AGAT TACGG TA*taa **TGAGG TAGAG TGATG TATGG TTGAG TAGGT** GTGAT GGTGG TACTT GGAAC TGGAG G –3'
j‴	5'- *TTCTG GGGAG TGGGA GATGt TGGCT AGATT TACGG TA*taa **TIAII TAIAI TIATI TATII TTIAI TAIIT** GTGAT GGTGG TACTT GGAAC TGGAG G–3'
h	5'-*CGCAA TCCAC CGATC ATCCG CAATC C*aaat ct**CCC AACCA CAACA AACCA AACCA ACAAC AAACA ACACC** ACTAT GGCGC ATGCT CTAAC TATGG C-3'
k	5′-GCCAT AGTTA GAGCA TGCGC CATAG T**IITI TTITT TITTI TTIIT TTIIT TTITT ITIIT TIII**t ctttt CCGAA TGCAG CTGCC ATTCC GAATG C-3'
α_1	5'- *GGTGA GTTGA ACAGC TTAGT ATTTG GGAGA* /IbRQ/-3'
α_2	5'-/TEX/*ATACC GTAAA TCTAG CCAAC ATCTC CCACT CCCCA GAA*-3'
α_3	5'- *GCATT CGGAA TGGCA GCTGC ATTCG GT*/FAM/-3'
α_4	5'-/IbFQ/*GGATT GCGGA TGATC GGTGG ATTGC G* -3'
$f_{(10,30)}$	5′-ttcgagtgtt **TGAGG TAGAG TGATG TAT GG TTGAG TAGGT**-3′
$f_{(8,30)}$	5′-cgagtgtt **TGAGG TAGAG TGATG TAT GG TTGAG TAGGT**-3′
$f_{(7,30)}$	5′-gagtgtt **TGAGG TAGAG TGATG TAT GG TTGAG TAGGT**-3′
$f_{(6,30)}$	5′- agtgtt **TGAGG TAGAG TGATG TAT GG TTGAG TAGGT**-3′
$f_{(5,30)}$	5′- gtgtt **TGAGG TAGAG TGATG TAT GG TTGAG TAGGT**-3′
$f_{(4,30)}$	5′-tgtt **TGAGG TAGAG TGATG TAT GG TTGAG TAGGT**-3′
$f_{(3,30)}$	5′-gtt **TGAGG TAGAG TGATG TAT GG TTGAG TAGGT**-3′
$f_{(2,30)}$	5′-tt **TGAGG TAGAG TGATG TAT GG TTGAG TAGGT** -3′
$f_{(1,30)}$	5′-t **TGAGG TAGAG TGATG TAT GG TTGAG TAGGT**-3′
$f_{(0,30)}$	5′-**TGAGG TAGAG TGATG TAT GG TTGAG TAGGT**-3′

f_2	5'-agaag taagt ag**GGT GTTGT TTGTT GTTGG TTTGG TTTGT TGTGG TTGGG** agatt tggat tg**aag tgagc gtaa -**3'
f_2*	5'-/Cy5/agaag taagt ag**GGT GTTGT TTGTT GTTGG TTTGG TTTGT TGTGG TTGGG** agatt tggat tg**aag tgagc gtaa -**3'
f_{c1}	5'-**ACAAC AAACA ACACC ctact tactt ct -**3'
f_{c2}	5'-**ttacg ctcac tt**caa tccaa atct**C CCAAC CACAA CAAA**-3'

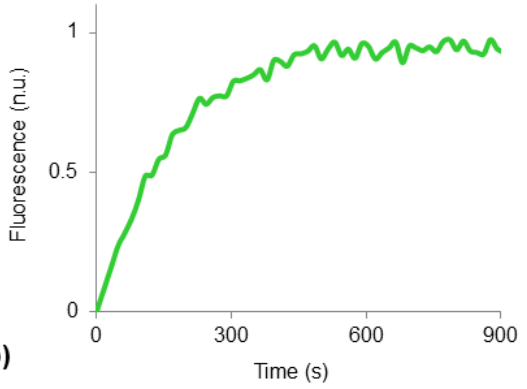
Opening toehold domains are in lower case, closing domains are in bold lower case, branch migration domains are bold upper case and hinges are underlined. IbFQ = Iowa black FQ quencher, Fam = fluorescein, TEX = Texas red 615 and IbRQ = Iowa black RQ quencher.

$n = 10$, $[S] = 3\ nM$, $[f] = 30\ nM$, $k_f = 228000\ M^{-1}s^{-1}$



a)

$n = 8$, $[S] = 3\ nM$, $[f] = 30\ nM$, $k_f = 188000\ M^{-1}s^{-1}$



b)

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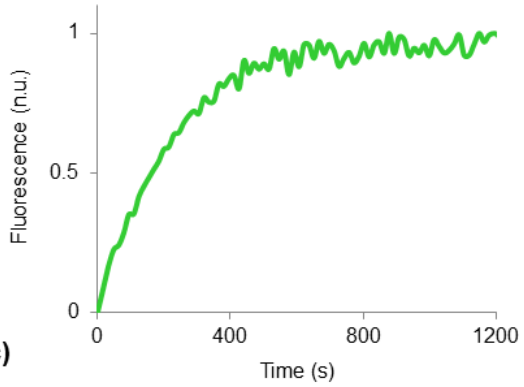
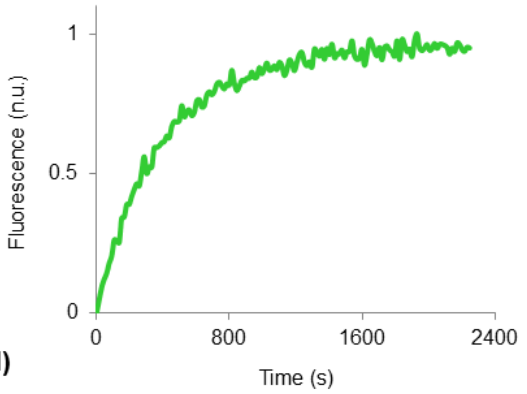


c)

$n = 6$, $[S] = 3\ nM$, $[f] = 30\ nM$, $k_f = 102000\ M^{-1}s^{-1}$



d)

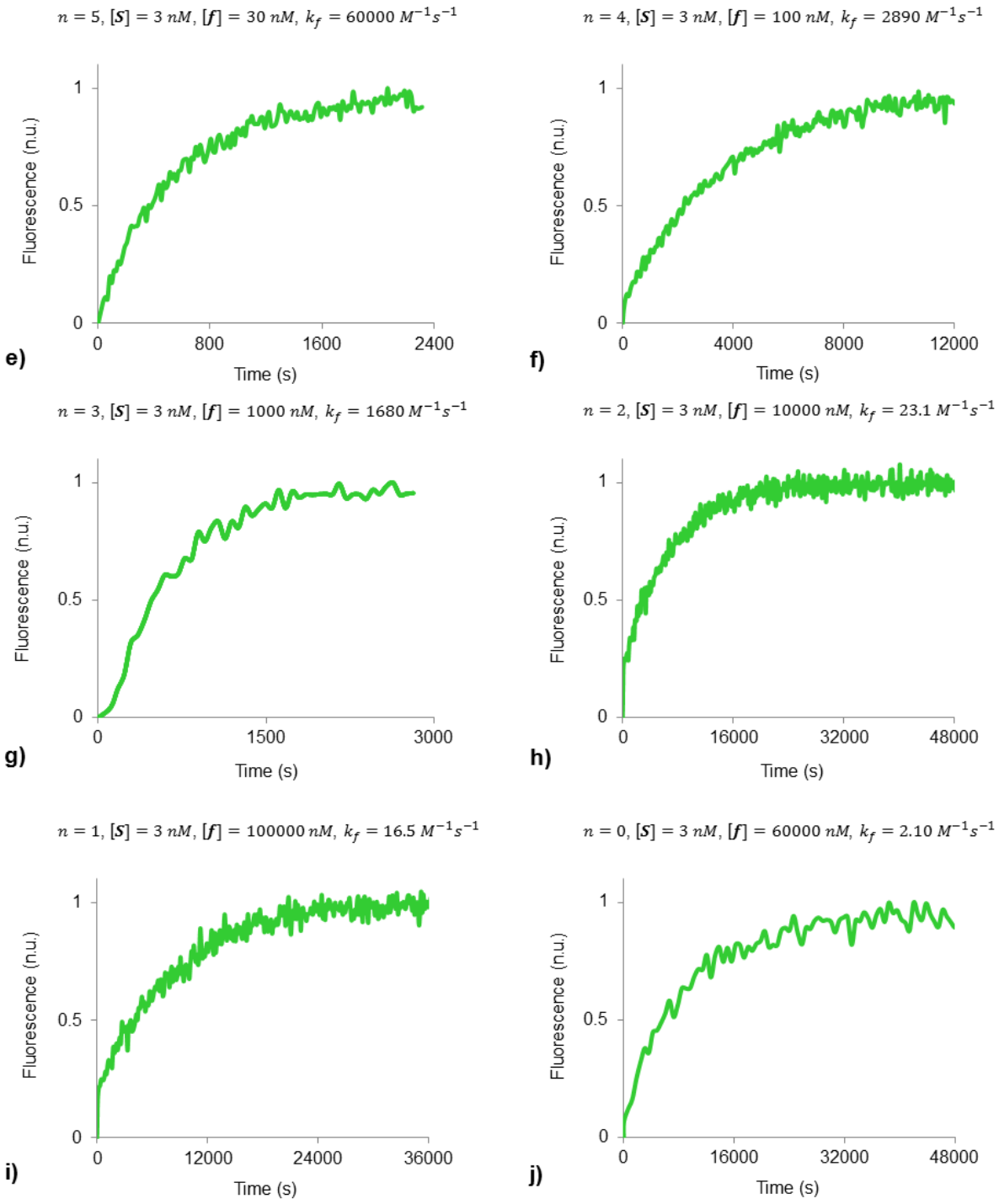
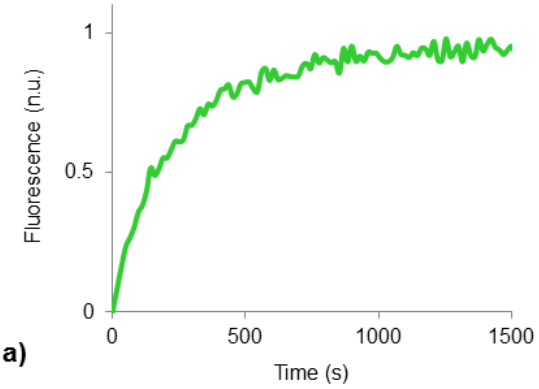


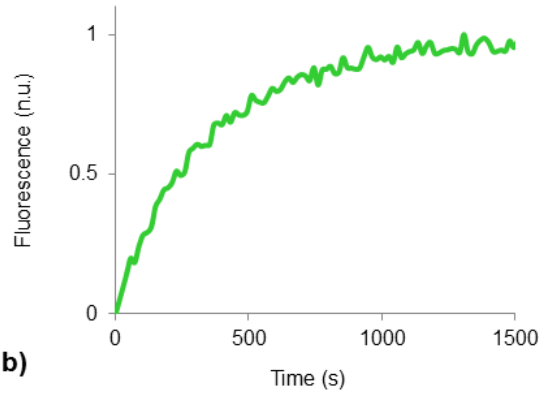
Figure S1. Toehold-mediated strand displacement measured by time-lapsed fluorescence of device ***S*** invaded by fuel strand $\boldsymbol{f}(b, n)$ at 41 ℃ with the indicated concentrations.

$n = 10$, $[S] = 3\ nM$, $[f] = 30\ nM$, $k_f = 121000\ M^{-1}s^{-1}$



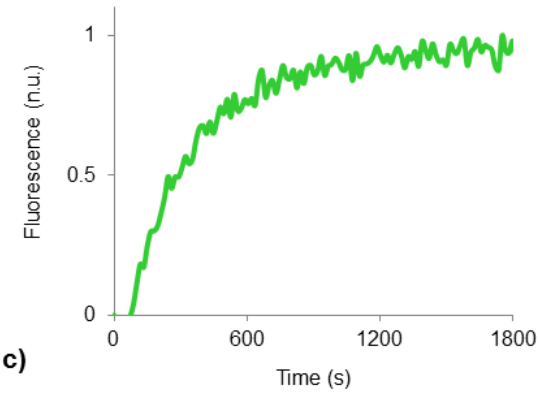
a)

$n = 8$, $[S] = 3\ nM$, $[f] = 30\ nM$, $k_f = 96100\ M^{-1}s^{-1}$



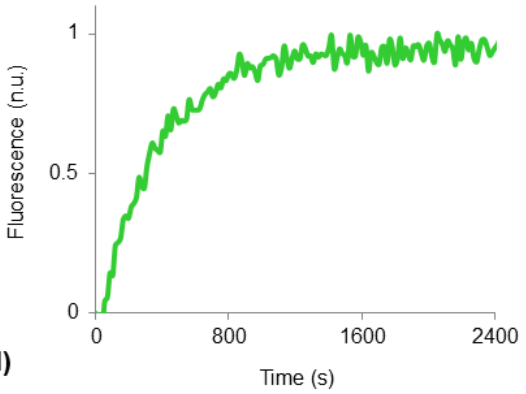
b)

$n = 7$, $[S] = 3\ nM$, $[f] = 30\ nM$, $k_f = 80600\ M^{-1}s^{-1}$



c)

$n = 6$, $[S] = 3\ nM$, $[f] = 30\ nM$, $k_f = 77100\ M^{-1}s^{-1}$



d)

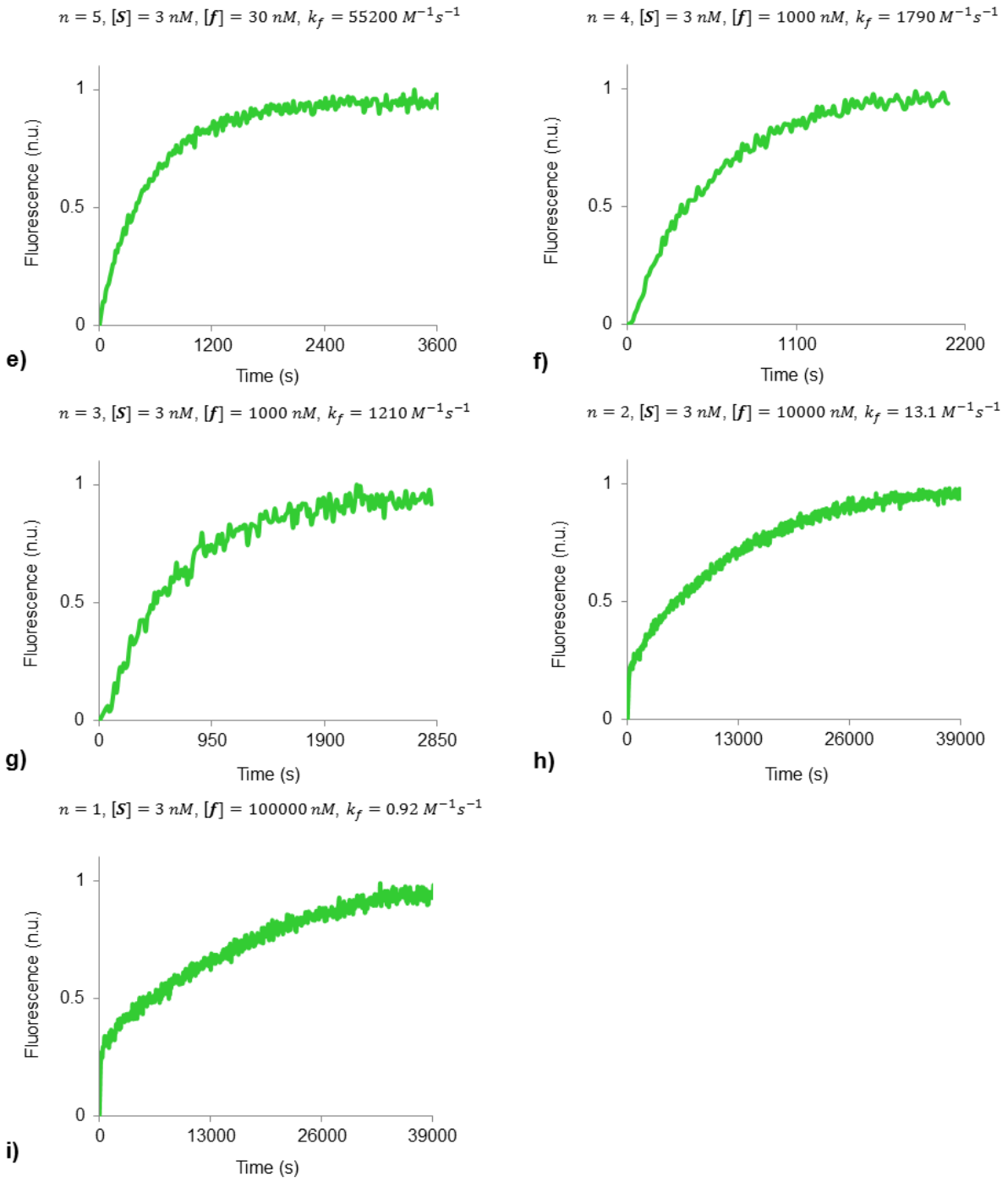


Figure S2. Toehold-mediated strand displacement measured by time-lapsed fluorescence of device ***S*** invaded by fuel strand $\boldsymbol{f}(b, n)$ at 25 ℃ with the indicated concentrations.

Unbiased DNA motor Devices.

Branch migration is assumed to be random walk process mediated by the hybridization between the toeholds on the fuel strands and their complements on the devices.[1] Thus, branch migration can only occur when the extending fuel strands are associated to the device. At thermodynamic equilibrium, the fraction of devices associated with the toeholds on the extending strands can be approximated by θ (neglecting the branch migration domain).[2]

$$\theta = 1 - \left(\frac{K([U]-[e])-1}{2K[U]}\right) + \frac{\sqrt{K^2([e]-[U])^2+2K([e]+[U])+1}}{2K[U]}$$

where [e] and [U] are the molar concentrations of the extending fuel stands and the unbiased DNA motor devices, respectively. The equilibrium constant $K = e^{-\Delta G/RT}$ where $\Delta G = \Delta H - T\Delta S$. The thermodynamic parameters ΔH (enthalpy) and ΔS (entropy) were found using the nearest neighbor model, including only the bases in the toehold.[3] T is temperature and R is the ideal gas constant. The function θ represents the fraction of devices eligible to compete in branch migration. Branch migration is iso-energetic. Thus, every occupied state of branch migration leaves the system at the same energy. The maximization of entropy guaranties that every energy state of the system with the same energy will be equally occupied.[4] If the toeholds are sufficiently long they will function as a hinge. At equilibrium, it is reasonable to assume energetically stable completion states do not exist. However, the branch migration allows many states in the system to be populated in the system. This is expected to be a dynamic equilibrium state.[1]

The unbiased devices required super saturating with the extending fuel strands in order for the populations to reach significant fluorescence intensities. The devices were saturated with 800-6400 times more extending fuel strands than devices. The fluorescence from a system of unbiased DNA motor devices (with a concentration of 100 nM) was measured at various temperatures between 4° - 41° C. The values predicted by θ (after correcting for the temperature dependence of the fluorophores) and the experimental results are plotted together (Fig. S3).

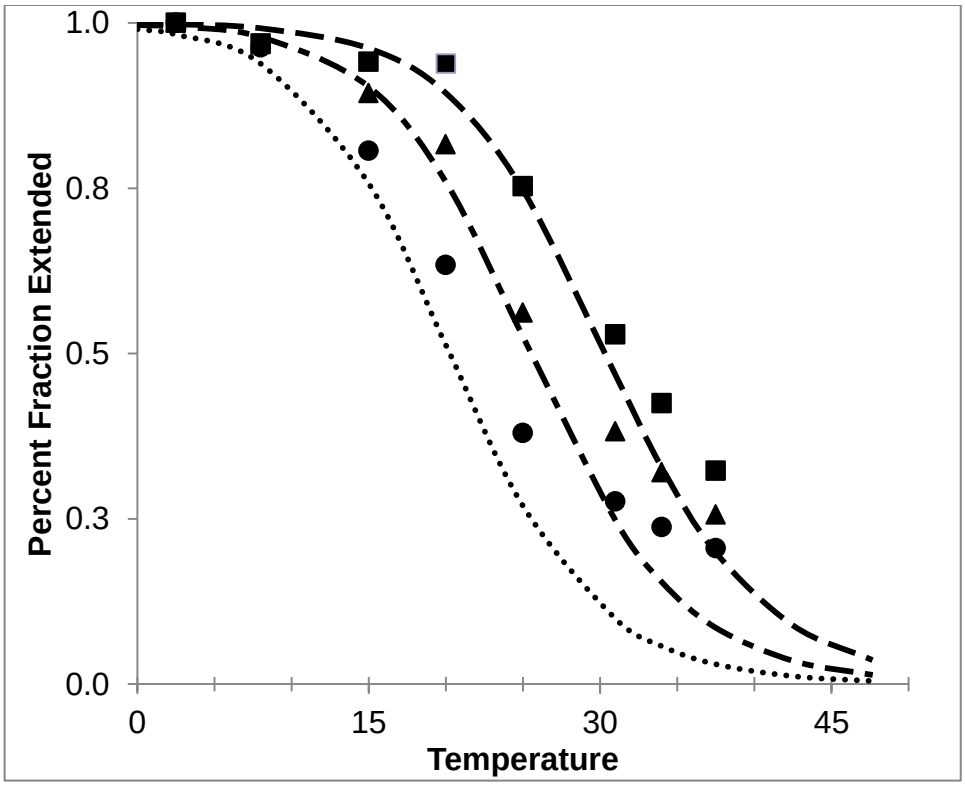


Figure S3. Extending behavior of the unbiased DNA motor device. The efficacy of an unbiased DNA motor device (containing hinges H) with a not 6 nt toehold built into the arm was shown to be dominated by the energy in the toehold alone. The example demonstrated the inefficiency of bungee type device without a thermodynamically stable partial stand displacement state. The devices were suspended with 100 nM concentration prior to being inundated with extended fuel strands with concentrations of 800× (●), 2400× (▲)and 6400× (■). The dotted lines are the uncorrected theta function.

Calculation and detailed discussion of kinetic rates.

The rate constants (k) for the opening and closing of the DNA motor devices were calculated in Matlab. The modeling was performed utilizing the function "lsqcurvefit" for least squares fitting of the parameters. Due to the stiff nature of the kinetics data and equations, integration of the differential equations was carried out using "ode23s". For curve fitting, the data was scaled from 0 to 1 with 0 relating to the fully quenched state (contracted state) and 1 to maximum observed fluorescence when all the DNA motor devices are extended.

The opening of the DNA motor devices from the contracted to the extended state was modeled as a second order reaction between the contracted motor (*CM*) and the extending strand ($\boldsymbol{f_2}$) to produce a fluorescent extended motor (*EM*) as represented by:

$$[\boldsymbol{f_2}] + [CM] \xrightarrow{k} [EM]$$

The standard second order kinetics equation was utilized for least squares fitting:

$$\frac{d[EM]}{dt} = k[\boldsymbol{f_2}][CM]$$

The concentration of extending strand ($[\boldsymbol{f_2}]$) and contracted motors ($[CM]$) can be approximated utilizing the fluorescence data using the following relations.

$$[CM] = 1 - [EM]$$

$$[\boldsymbol{f_2}] = [\boldsymbol{f_2}]_0 - [EM]$$

Where $[\boldsymbol{f_2}]_0$ is the concentration of extending strand added to the reaction vessel.

When the motor extension did not run to completion (as determined by the fluorescence not reaching the maximum fluorescence observed when all strands are extended), the reaction was treated as being reversible. This was observed for the inosine substitution motor extension experiments (Fig. 3a). In this case, it was assumed that the weak portion ($\boldsymbol{\mu}$) on the motor displaced the extending strand.

$$[\boldsymbol{f_2}] + [CM] \rightleftharpoons [EM] + [\mu]$$

The concentration of the weak portion (μ) was approximated by its local concentration (≈160 μM = 1600 X). The kinetics equation then becomes:

$$\frac{d[EM]}{dt} = k_F[\boldsymbol{f_2}][CM] - k_R[EM][\mu]$$

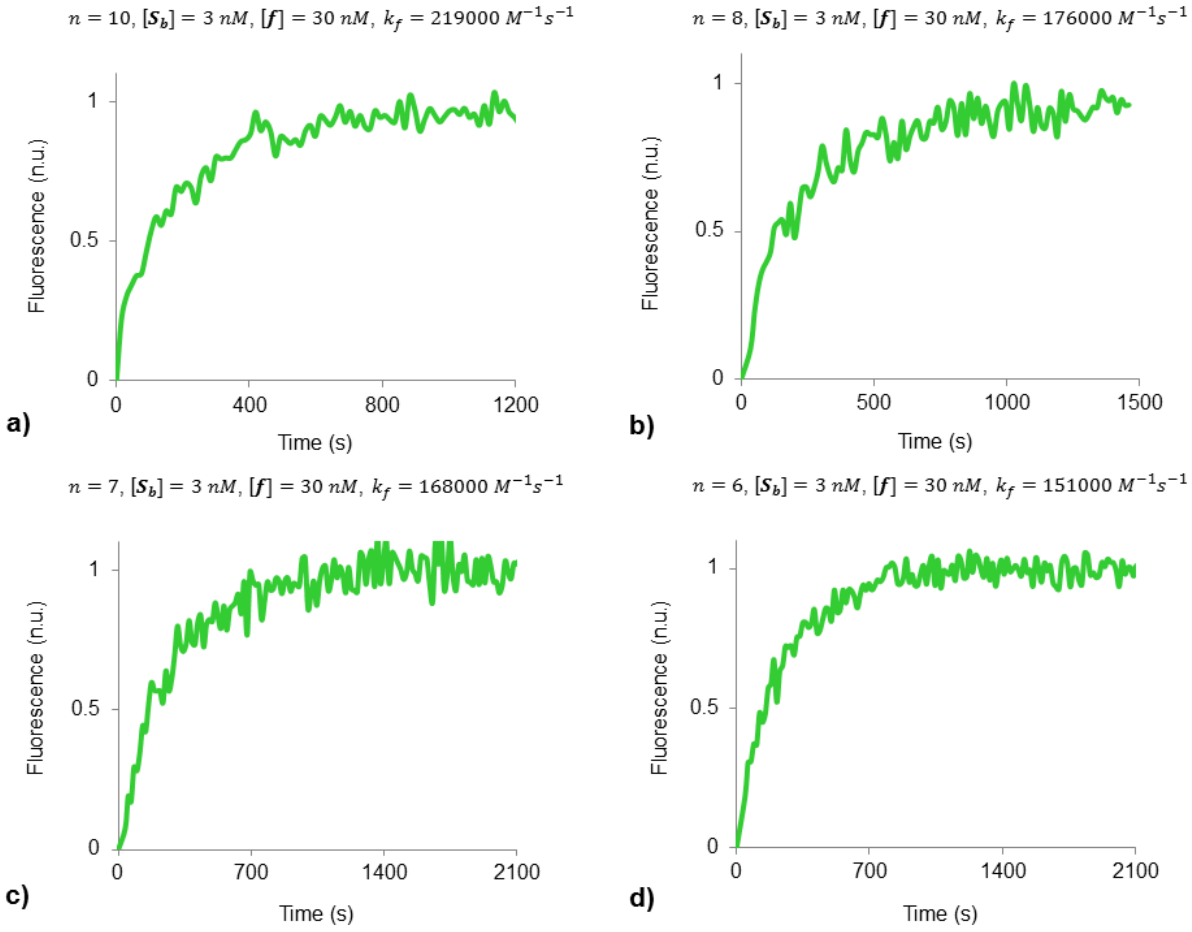
Closing of motors from extended to the contracted state was modeled as either a reversible second order or third order reaction depending on whether 1 or 2 contracting strands (f_{c1} and f_{c2}) were used to remove the extending strand from the motor device. The fluorescence decreases as a result of the addition of the contracting strands, however, adding excess contracting strands does not result in the contraction of all of the devices (Figure 2) indicating that removal of the $\boldsymbol{f_2}$ is a reversible process. The contracting strand of the motor was modeled as a reversible reaction. The resulting equation becomes:

$$\frac{d[EM]}{dt} = -k_F[EM][f_C] + k_R[CM][f_2 f_C]$$

In the models, it was assumed that free extending strands would bind quickly with free contracting strands reducing the effective concentration of the free contracting strands. The concentrations of the unbound and bound contracting strands were approximated as:

$$[f_C] = [f_C]_0 - [f_2]$$

$$[f_2 f_C] = [f_2]$$


$n = 10$, $[S_b] = 3\ nM$, $[f] = 30\ nM$, $k_f = 219000\ M^{-1}s^{-1}$
Fluorescence (n.u.)
Time (s)
0
0.5
1
0
400
800
1200
a)
$n = 8$, $[S_b] = 3\ nM$, $[f] = 30\ nM$, $k_f = 176000\ M^{-1}s^{-1}$
Fluorescence (n.u.)
Time (s)
0
0.5
1
0
500
1000
1500
b)
$n = 7$, $[S_b] = 3\ nM$, $[f] = 30\ nM$, $k_f = 168000\ M^{-1}s^{-1}$
Fluorescence (n.u.)
Time (s)
0
0.5
1
0
700
1400
2100
c)
$n = 6$, $[S_b] = 3\ nM$, $[f] = 30\ nM$, $k_f = 151000\ M^{-1}s^{-1}$
Fluorescence (n.u.)
Time (s)
0
0.5
1
0
700
1400
2100
d)

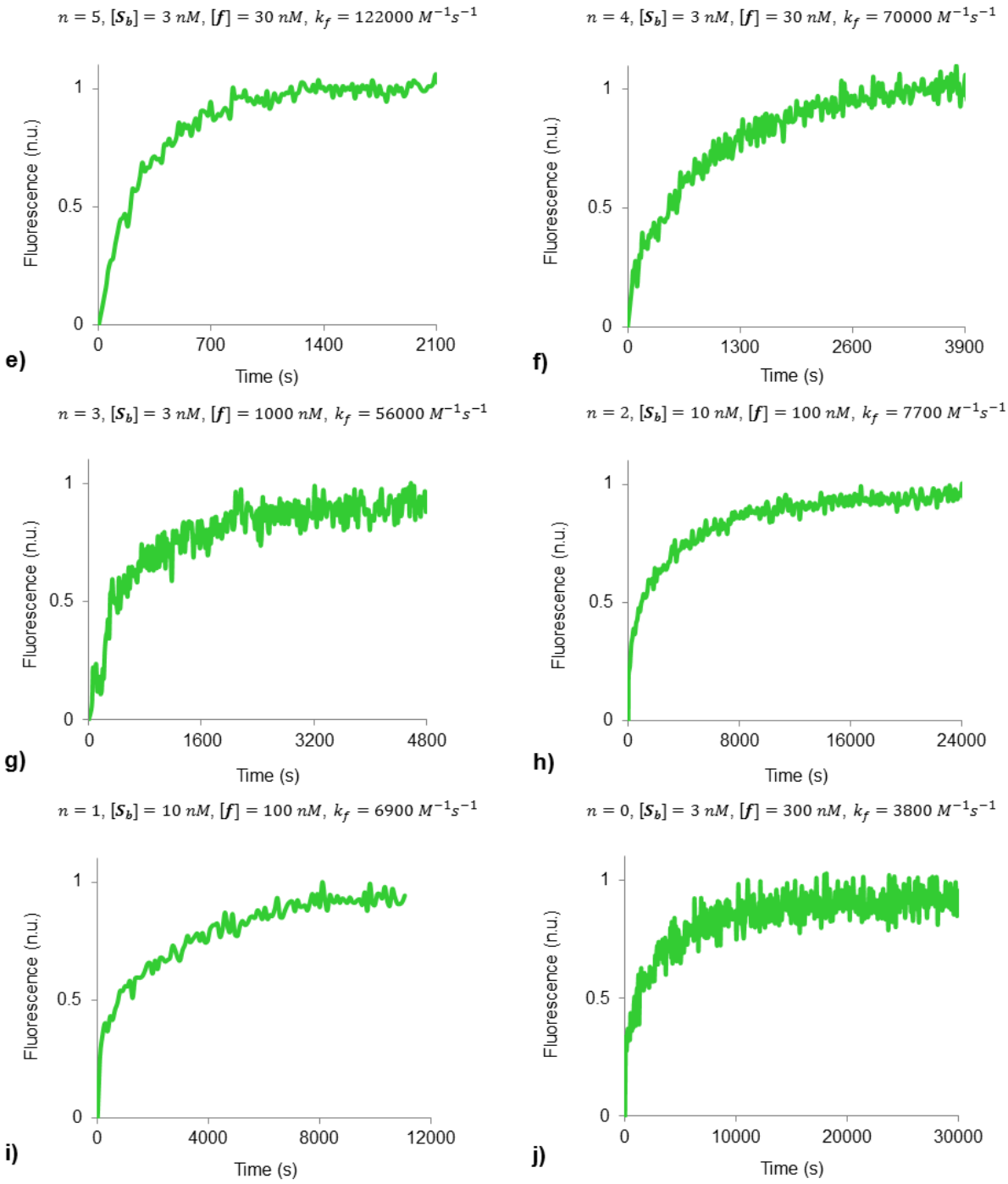
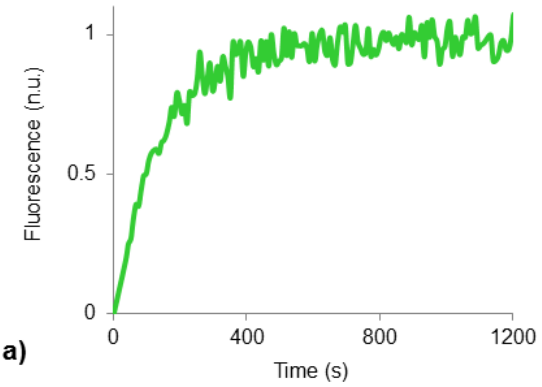


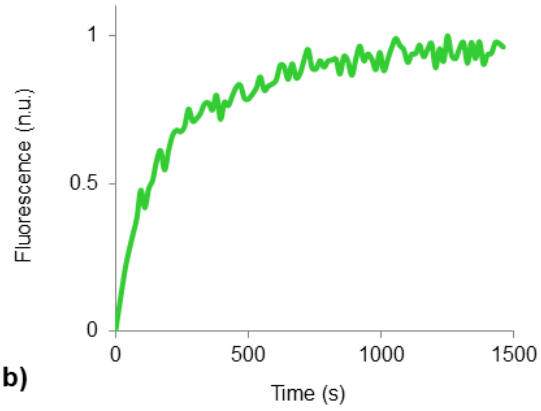
Figure S4. Toehold-mediated strand displacement measured by time-lapsed fluorescence of device $\boldsymbol{S_b}$ invaded by fuel strand $\boldsymbol{f}(b, n)$ at 25 ℃ with the indicated concentrations.

$n = 10$, $[\boldsymbol{M_b}] = 3\ nM$, $[\boldsymbol{f}] = 30\ nM$, $k_f = 231000\ M^{-1}s^{-1}$



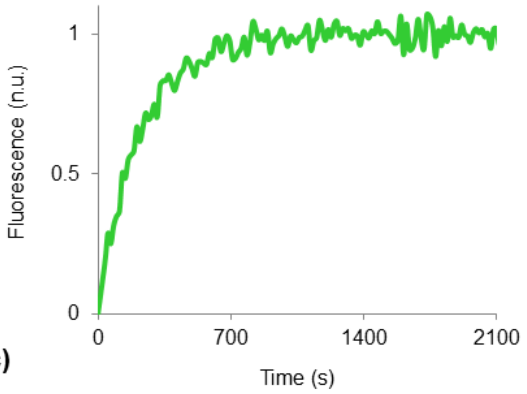
a)

$n = 8$, $[\boldsymbol{M_b}] = 3\ nM$, $[\boldsymbol{f}] = 30\ nM$, $k_f = 176000\ M^{-1}s^{-1}$



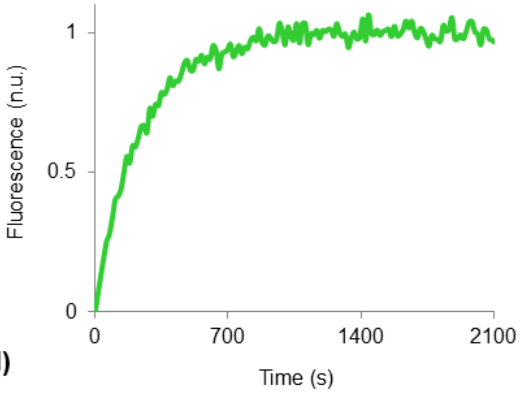
b)

$n = 7$, $[\boldsymbol{M_b}] = 3\ nM$, $[\boldsymbol{f}] = 30\ nM$, $k_f = 162000\ M^{-1}s^{-1}$



c)

$n = 6$, $[\boldsymbol{M_b}] = 3\ nM$, $[\boldsymbol{f}] = 30\ nM$, $k_f = 154000\ M^{-1}s^{-1}$



d)

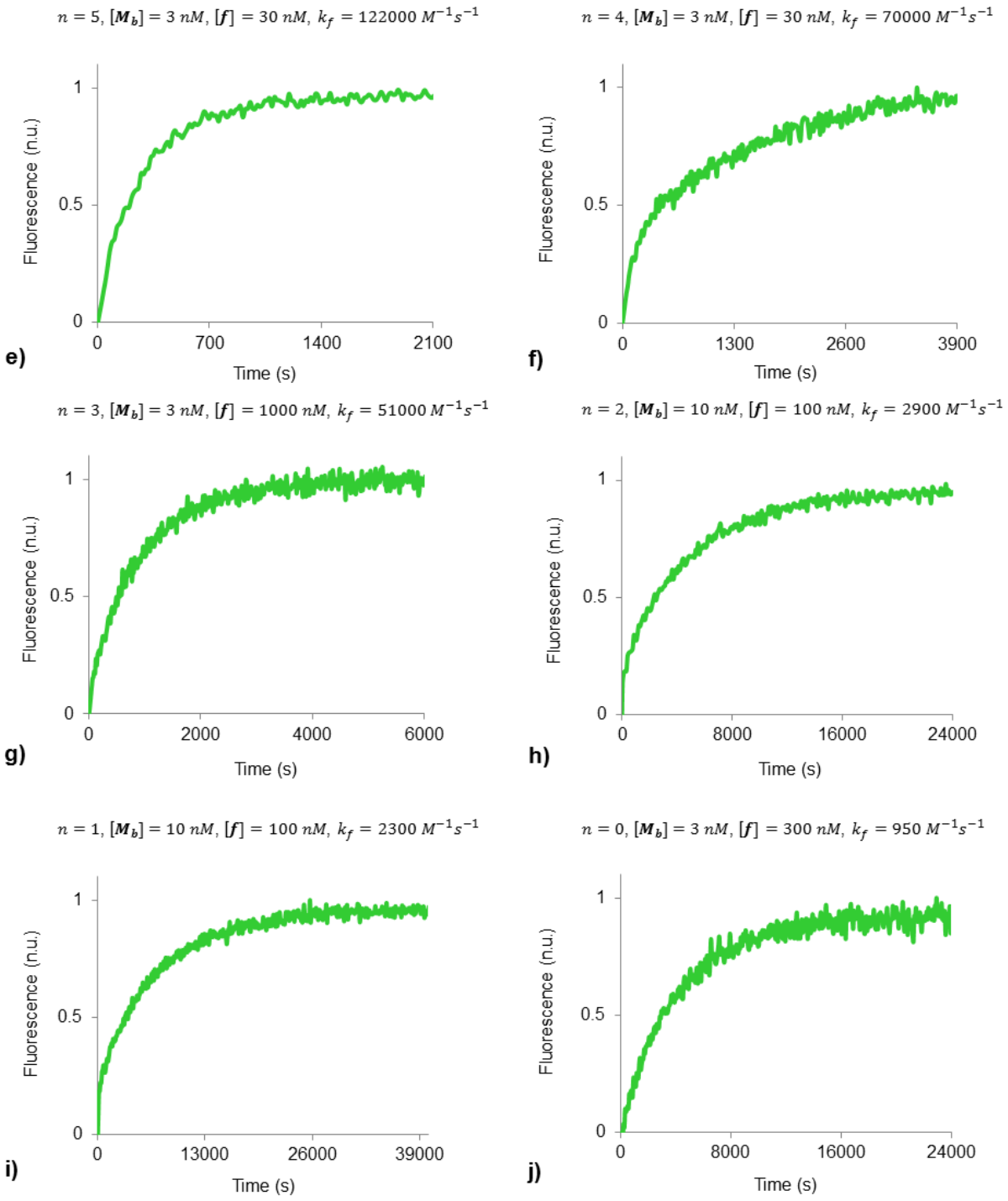


Figure S5. Toehold-mediated strand displacement measured by time-lapsed fluorescence of device $\boldsymbol{M_b}$ invaded by fuel strand $\boldsymbol{f}(b, n)$ at 25 ℃ with the indicated concentrations.

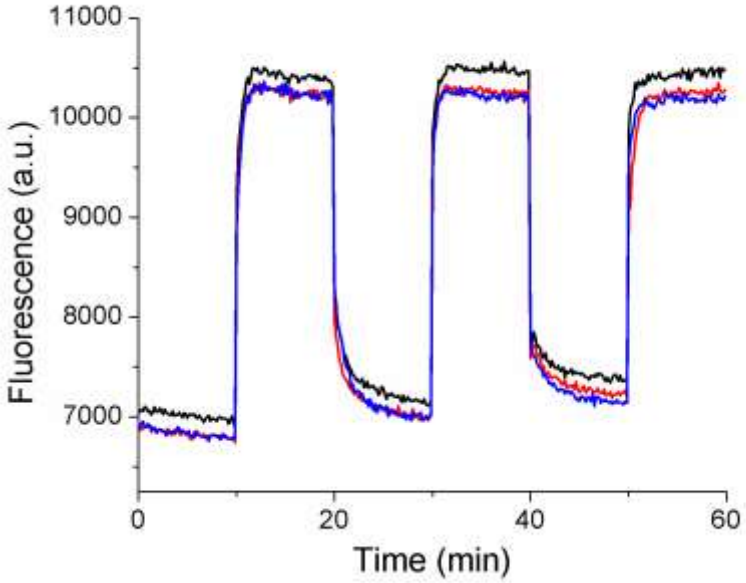


Figure S6. Time-lapse fluorescence cycles from $\boldsymbol{M_{b2}}$ at 37°C using raw data.

REFERENCES

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(2) Owczarzy, R., Melting temperatures of nucleic acids: Discrepancies in analysis. *Biophys. Chem.* 2005, 117, (3), 207-215.

(3) Xia, T. B.; SantaLucia, J.; Burkard, M. E.; Kierzek, R.; Schroeder, S. J.; Jiao, X. Q.; Cox, C.; Turner, D. H., Thermodynamic parameters for an expanded nearest-neighbor model for formation of RNA duplexes with Watson-Crick base pairs. *Biochemistry* 1998, 37, (42), 14719-14735.

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