Electronic Supplementary Information

Motor and track design, and nucleotide sequences (Figure S1)

Extra Data (Figure S2 – S6)

Motor and track design, and nucleotide sequences

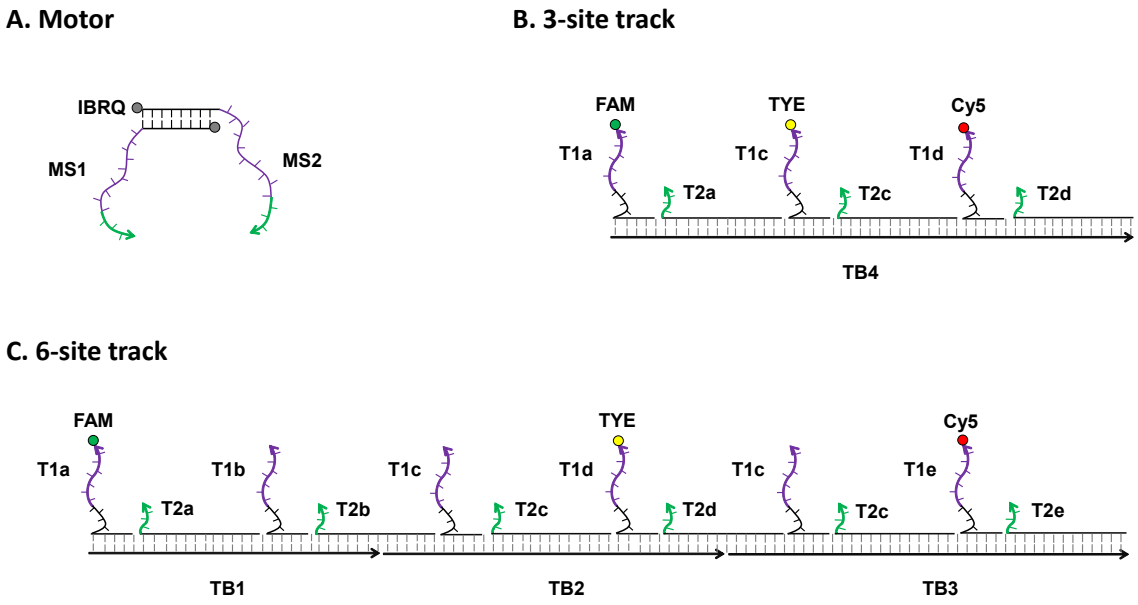


Figure S1. DNA design for motor and tracks.

The design for the DNA motor and tracks is illustrated in Figure S1, with all the constituent strands marked in the figure and their nucleotide sequences given below.

The two strands for the motors are
MS1 = 5′-IBRQ-M-D2-D1-3′ (20-14-6=40mer)
MS2 = 5′-IBRQ-M*-D2-D1-3′ (20-14-6=40mer)
with sequences
D1 = TAAACT
D2 = GCTGAGGGCTGAGG
M = TTACCATCTAGGTAGAGGCC

The 3-site track and the 6-site track are formed from the same set of strands. Four strands serve as templates for the duplex backbone of the tracks. These strands are

TB1 = 5′-D3-D4-D5-D6-3′ (15-45-15-25=100mer)

TB2 = 5′-D7-D8-D9-D10-D11-3′ (20-15-45-15-25=120mer)

TB3 = 5′-D12-D8-D9-D13-D14-3′ (20-15-45-15-45=140mer)

TB4 = 5′-D3-D4-D8-D9-D10-D11-D12-3′ (15-45-15-45-15-25-20=180mer)
with the sequences (from 5′ to 3′)

D3 = GATTTGCTGCTTTCC

D4 = ATTGAGCCTGTTTCTCTGCGCGACGTTCGCGGCGGCACTTACGGA

D5 =GCTAATTCGGTCTCG

D6 =CACTTACGGCCAATGCTTCGTTTCG -

D7 = TATCACCGACCGTCTTCTGC

D8 = CGTGTTTGTGCATCC

D9 = ATCTGGATTCTCCTGTCAGTTAGCTTTGGTGGTGTACCTTCTGCT

D10 = GTGGCAGTTGTAGTC

D11 = CTGAACGAAAACCTCCAGCGATTGG

D12 = CACATTGGCACTTCAGGGCT

D13 = AGAGCGTCACCTTCA

D14 = GTCAGTGCGTCCTGCTGATGTGCTCAGTATCACCGCCAGTTGTCG

The strands for binding sites are
T1a = 5′-D3* -S -D2*′-FAM-3′ (15-9-14=38mer)
T1b = 5′-D5*-S-D2*′-3′ (15-9-14=38mer)

T1c = 5′-D8*-S-D2*′-TYE-3′ (15-9-14=38mer; with TYE dye for 3-site track and without for 6-site track)
T1d = 5′-D10*-S-D2*′-Cy5-3′ (15-9-14=38mer; with Cy5 dye for 3-site track and with TYE dye instead for 6-site track)
T1e = 5′-D13*-S-D2*′-Cy5-3′ (15-9-14=38mer)

T2a = 5′-D4*-D1*-3′ (45-6=51mer)
T2b = 5′-D7*-D6*-D1*-3′ (20-25-6=51mer)
T2c = 5′-D9*-D1*-3′ (45-6=51mer)
T2d = 5′-D12*-D11*- D1*-3′ (20-25-6=51mer)
T2e = 5′-D14*- D1*-3′ (45-6=51mer)

The nucleotide segment S is a 9nt-long spacer as S = TTTTTTTTT. The nucleotide segments D1* – D14* have complementary sequences with D1 – D14, except for D2* mutated into D2*′ = CTTCAGCCTTCAGC (with two point mutations, marked by underlines, to avoid cutting by a nicking enzyme used for the original chemical motor).

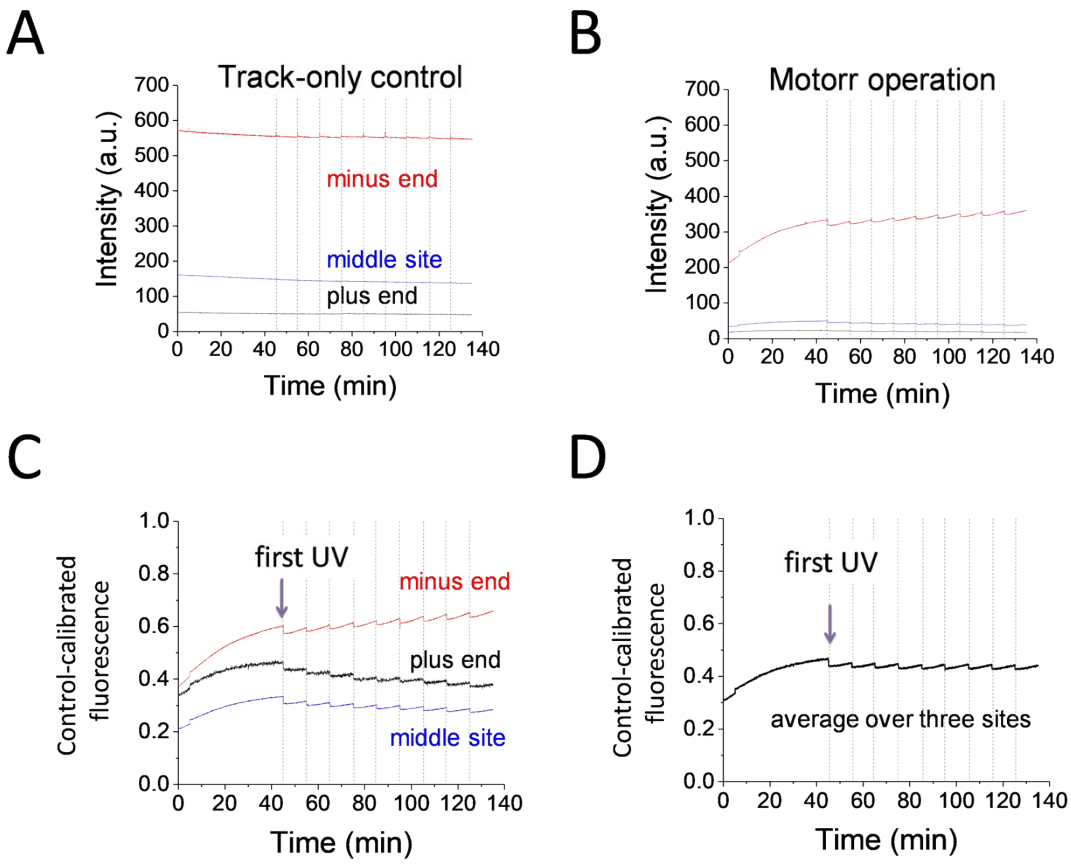


Figure S2. The raw fluorescence signals for the data shown in Fig. 2B (and Fig. 4A). Panel A is the raw fluorescence for the track-only control, and panel B is the raw fluorescence for the motor operation (experimental procedures explained in caption of Fig. 2). The time shown is before the adjustment of the first UV to time zero. Panel C is the control calibrated fluorescence, whilst Fig. 2 shows its change relative to the value immediately before the 1st UV (indicated in panel C here). Panel D shows the average of control-calibrated fluorescence over the three dyes. This average is virtually unchanged after the first UV (within ~ 1.6%), indicating a low probability for entire off-track derailment of the walker. Like Fig. 2, the time axis in each figure shows only the time covered by the visible light, with the time for applying each UV irradiation indicated by vertical dashed lines.

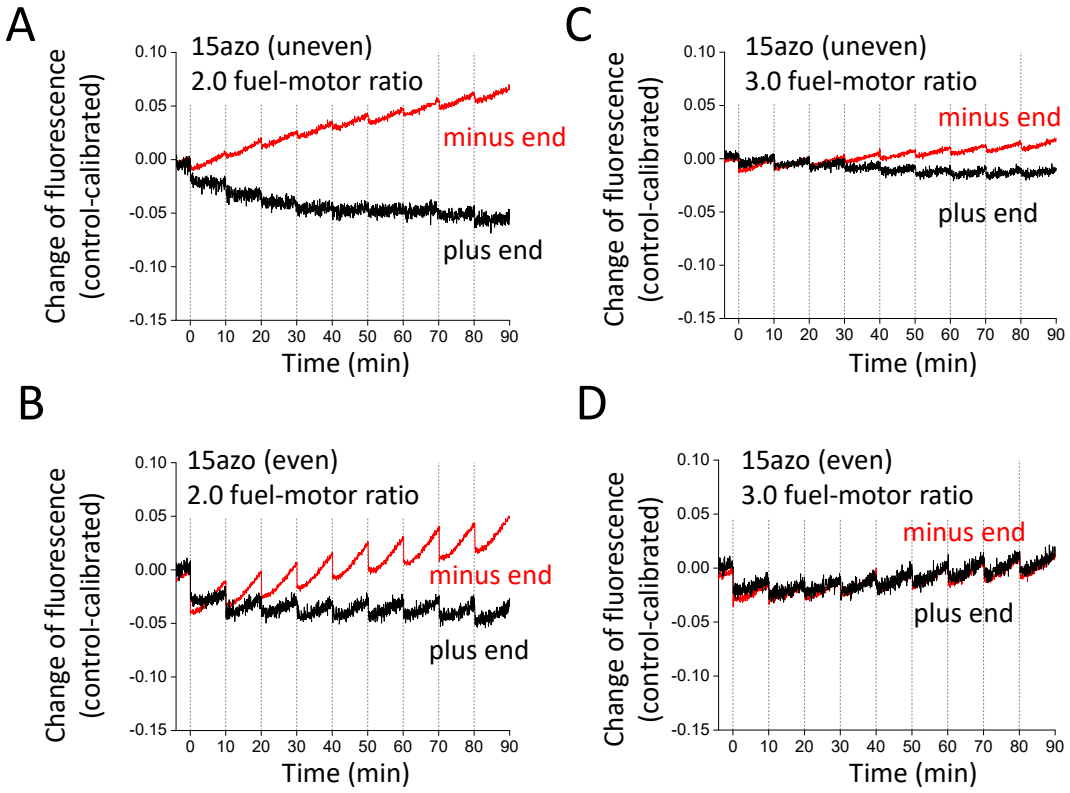


Figure S3. The walker's operation on the 3-site track in presence of the 15azo fuel analogs at fuel-motor ratio of 2 and 3. The experimental procedure is the same as for Figure 2. The time axis in each figure shows only the time covered by the visible light, with the time for applying each UV irradiation indicated by vertical dashed lines.

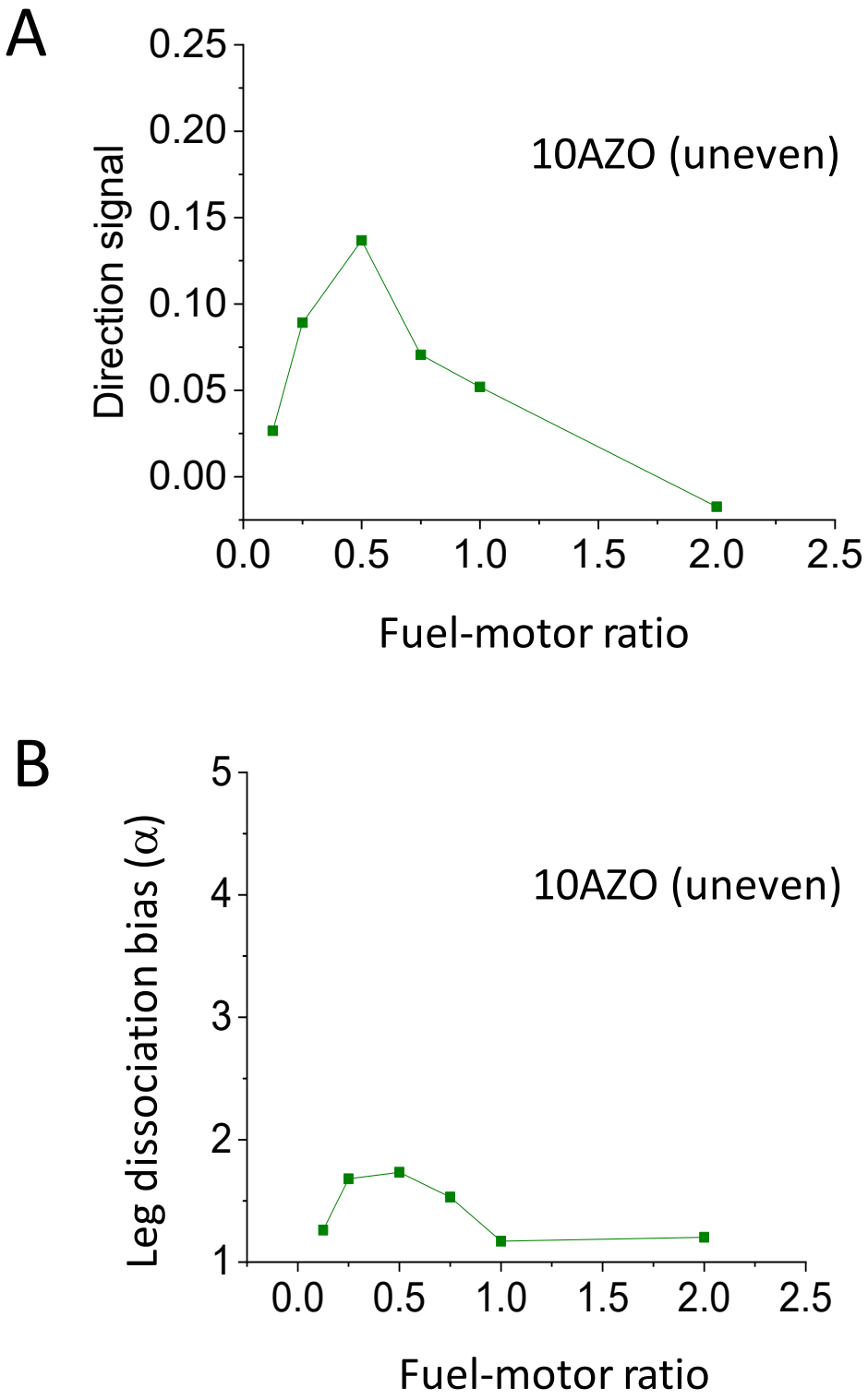
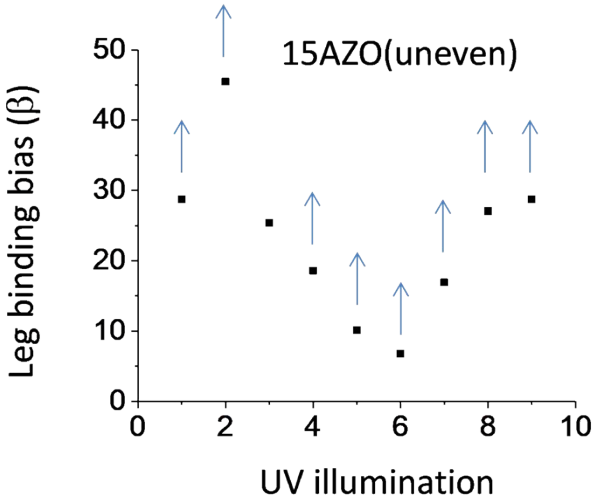
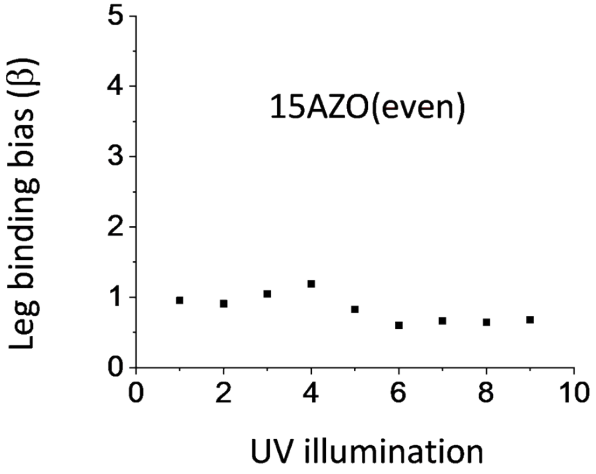


Figure S4. Direction signals (**A**) and leg dissociation bias (**B**) for the two 10-azo fuel analogs. The results are extracted using the same procedure as for Fig. 3C, D.

A


15AZO(uneven)
Leg binding bias (β)
UV illumination
0
10
20
30
40
50
0
2
4
6
8
10

B


15AZO(even)
Leg binding bias (β)
UV illumination
0
1
2
3
4
5
0
2
4
6
8
10

C

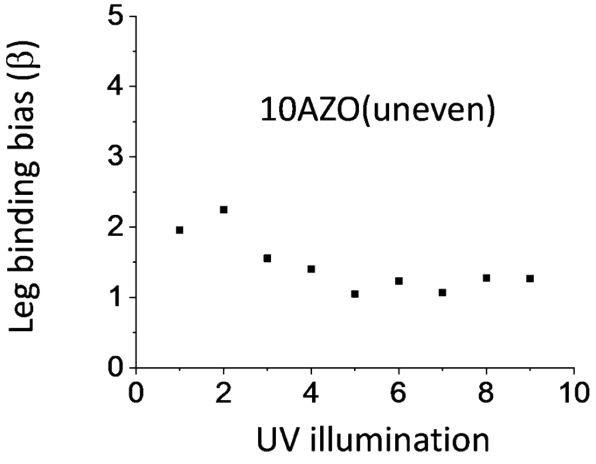

10AZO(uneven)
Leg binding bias (β)
UV illumination
0
1
2
3
4
5
0
2
4
6
8
10

Figure S5. The binding bias β extracted from the fluorescence data shown in Fig. 2 (for 1:1 fuel-motor ratio; using the method explained in Fig. 5A). The vertical arrows indicate data points that are obtained as a lower limit in case of near zero minus-end fluorescence drop by a UV illumination (i.e., $\Delta I_{down}(-) \sim 0$). Then the maximum of the drops upon the nine UV illuminations is used to estimate the lower limit for the bias, namely $\Delta I_{down}(+)/\text{maximum}[\Delta I_{down}(-)]$, which is below the real bias $\beta = \Delta I_{down}(+)/\Delta I_{down}(-)$.

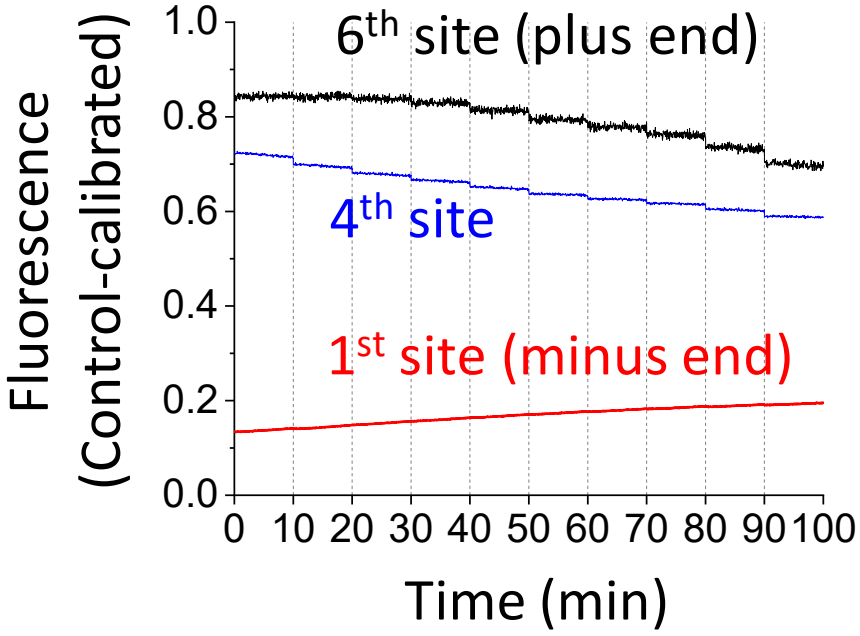


Figure S6. The raw control-calibrated fluorescence signals for Figure 5C. The data are from an experiment in which the walker is started from the minus-end site by a new method to assemble the walker and track (see Methods). The site-specific assembly is confirmed by the initial quenching, which is high for the minus-end site (1st site) but low for the plus-end site (6th site) and the intermediate 4th site. The time axis in each figure shows only the time covered by the visible light, with the time for applying each UV irradiation indicated by vertical dashed lines.

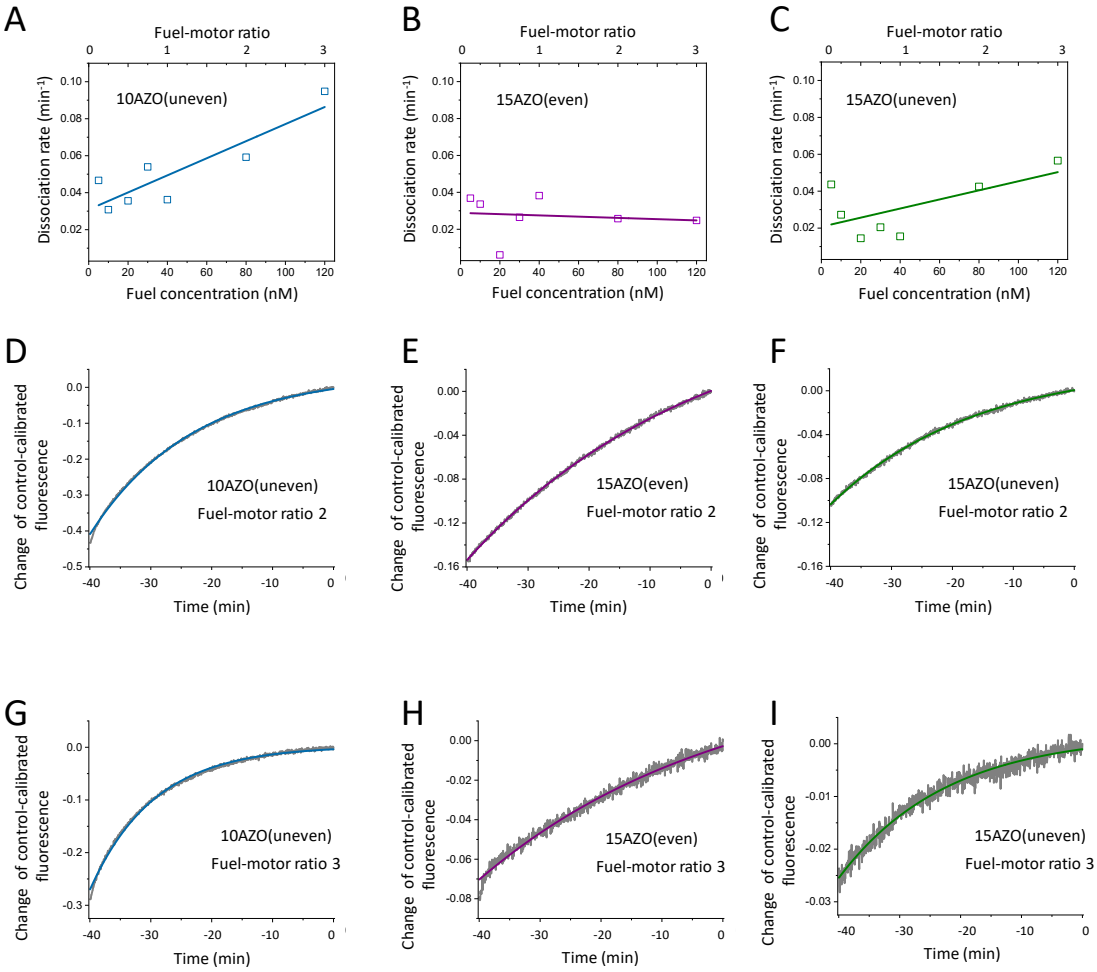


Figure S7. Dissociation rate constant (panels A, B, C) obtained from a single-exponential fitting to the minus-end fluorescence from the pre-operation fuel-mixing period (panels D – I, all fluorescence data collected from 3-site track, with the same time t = 0 for the first UV operation as for Fig. 5A, and with the same t = -40 minute as the time for adding the fuel analogs for leg dissociation). In panels A, B, C, the empty squares are the extracted rate constants and their linear regression is shown in straight lines. In panels D – I, the fluorescence signal is shown in grey color and the curves in cyan, purple and green are single-exponential fitting for different fuel analogs and fuel-motor ratios (indicated in figures). Shown here is the fitting by a single-exponential function (i.e., A – B×exp(-kt)) to the change of control-calibrated fluorescence data relative to the t = 0 value for sake of consistence with Fig. 5A. Repeating the same exponential fitting to the raw control-calibrated fluorescence data yields the same rate constants.