Online Supplement

1.Supplementary figures (Fig. S1-S7)

2.Nucleotide sequences for the origami rod and variations used in this study (Table S1)

3.Nucleotide sequences for the motor and variations used in this study (Table S2-S5)

1.Supplementary figures

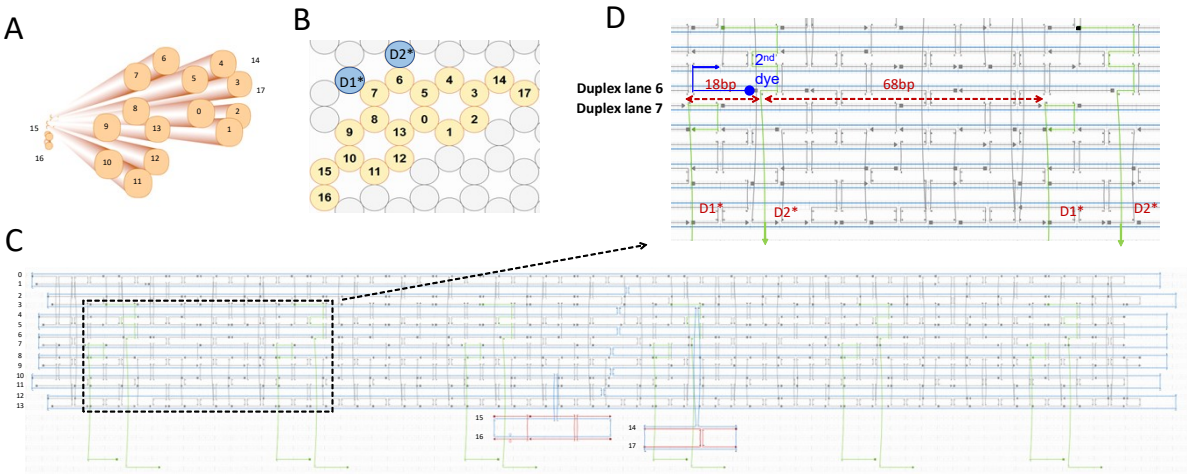

Fig. S1. Design of the origami rod (panel A for tilted view, panel B for the cross section), arrangement of D1* and D2* overhangs for the motor's track (panel C, for the 6-site track in Fig. 1E), and the track's minus end enlarged to show more details (panel D). All diagrams in this figure are generated using Cadnano software. The overhangs are extending from staple strands along the 6th and 7th duplex lanes (marked in all panels). The staple strands extending into D1* or D2* overhang are highlighted in green colour in panels C, D. Panel D also highlights in blue colour the staple strand that is tethered with the second dye for the minus-end bi-overhang site, namely the dye below the D2* overhang shown in Fig. 1B. The staple strand carrying the second dye for another bi-overhang site is chosen from similar location from the periodic origami lattice along the linear track.

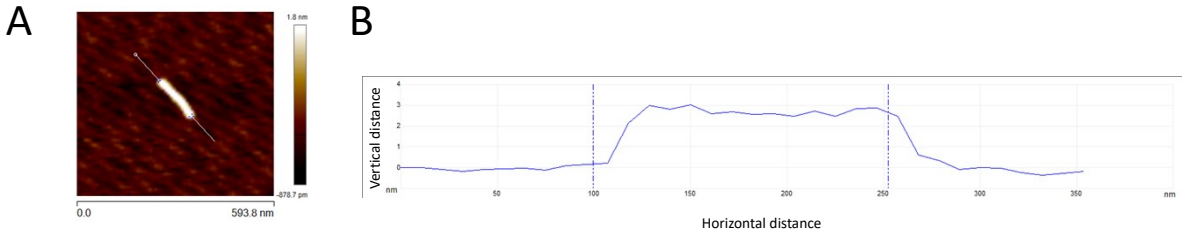

Fig. S2. AFM imaging of the origami rod. Panel A schematically illustrates a longitudinal scan, and panel B shows the resultant height profile.

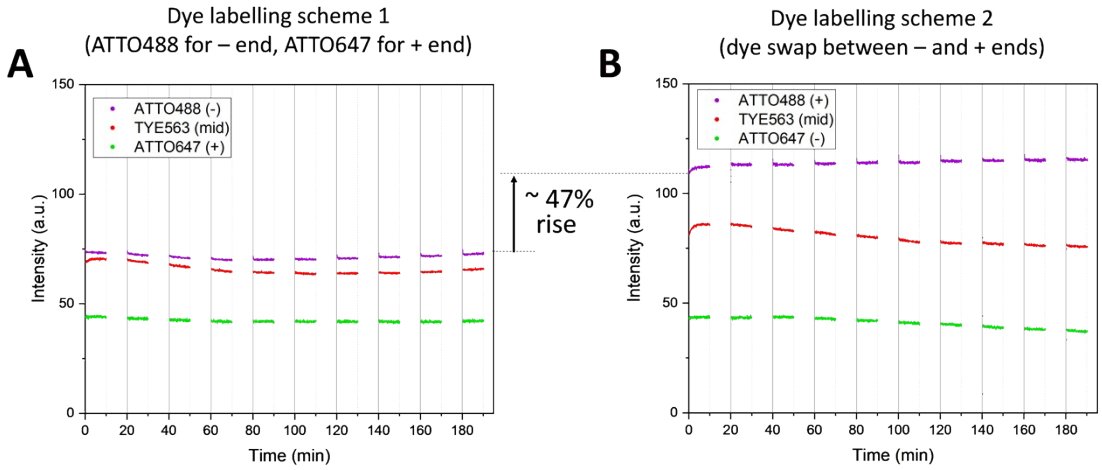

Fig. S3. **Fluorescence intensity of an origami track carrying the same three types of dyes but in two different labelling schemes (as indicated).** This is a track-only control experiment that involves no motor but still subjects the differently labelled tracks to the same 9 cycles of alternating UV-visible light irradiations designed for the motor's operation (explained in Fig. 3). The track used in this control is the same 5-site track used for the experiment presented in Fig. 4A. The track is illustrated in Fig. 2A (middle plot, two dyes of the same type per binding site, and with 6nt spacer below D1* overhangs). The fluorescence data in panels A and B are collected from the two differently labelled tracks in two separate fluorescence experiments, but using the same amount of track samples at the same concentration (8 nM). The indicated ~ 47% rise is calculated using the ATTO488 fluorescence at the start of light operation from the two experiments.

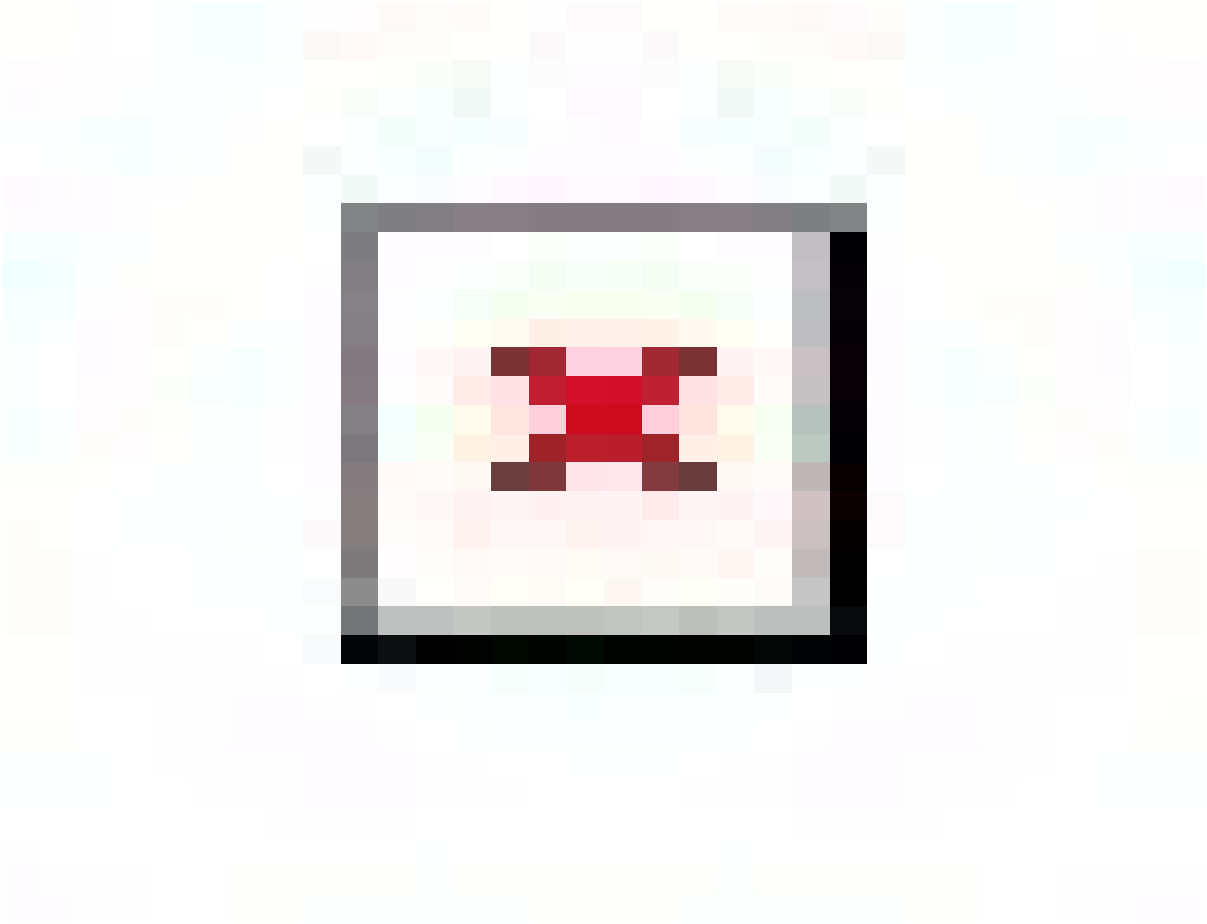

Fig. S4. Raw fluorescence data for the random-mix experiment shown in Fig. 3A, B (for the 3-site origami track in Fig. 2A). Panel A presents the fluorescence from a random motor-origami mix prepared for dye labelling scheme 1 (as indicated) under alternating UV-visible light operation (as explained in Fig. 3). The shown data are the fluorescence intensity normalized to the pre-operation intensity collected at the first visible light irradiation at time zero. Panel B presents the fluorescence collected during the motor-origami mixing process in preparation for the operation experiment in panel A. The data are again the normalized fluorescence intensity (to the pre-mix intensity of the origami at time zero, with the motor added from this time). Each data point is collected by a short visible light irradiation (10 seconds, with the shown data as the fluorescence average during this short period). The data thus have minimal photobleaching and reliably reflect the motor-origami binding during the mixing process. Panels C and D are the same as panels A, B, respectively, except for the same track now prepared for dye labelling scheme 2 (as indicated). Averaging the data in panels A and C according to the track's binding sites yields the data shown in Fig. 3A.

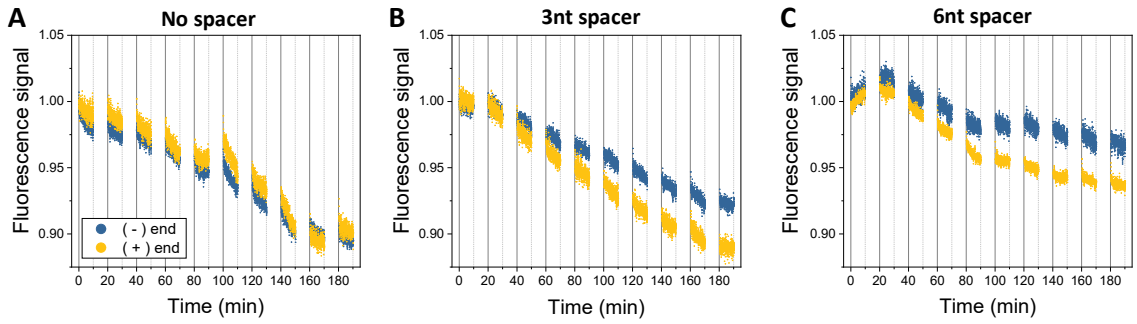

Fig. S5. Test of spacer length below the D1* overhang by a random-mix experiment using a simplified 3-site track (same as the one in Fig. 2A but with a single dye per site at the tip of D1*). The three panels are all the same as Fig. 3A execpt for the simpler track and different spacer lengths (as indicated).

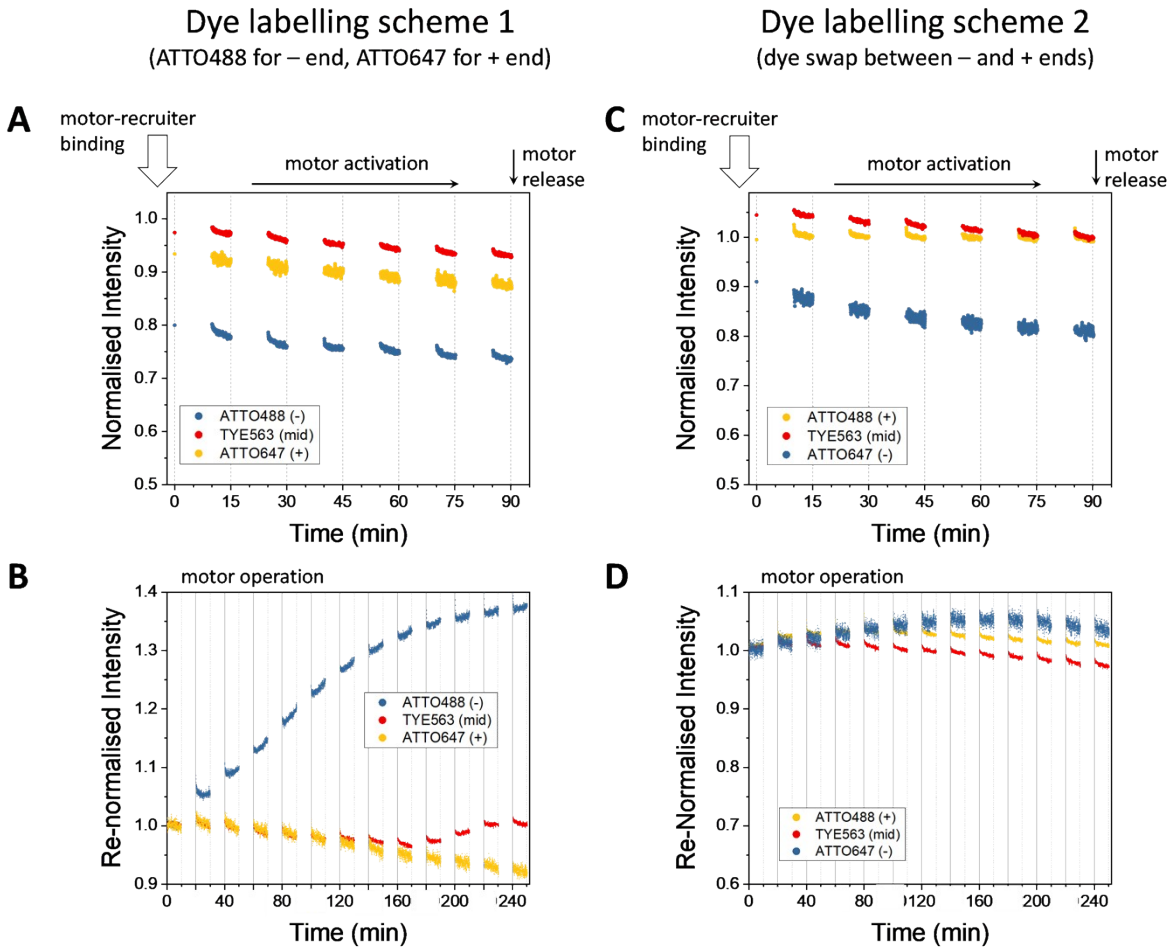

Fig. S6. Raw fluorescence data for the site-specific experiment shown in Fig. 4D (for the 6-site origami track in Fig. 2A; motor started from the minus-end site). Panel A presents the fluorescence during the motor's placement to the minus-end site of the 6-site track motor prepared for dye labelling scheme 1 (as indicated). The shown data are the normalized fluorescence intensity (to the origami-only intensity before the motor-origami mix and motor-recruiter binding). The time zero is set at the start of the UV-visible light irradiations that activate the motor's legs for track binding. Along the time axis, the durations without data are the UV irradiations and the durations with data are the visible light irradiations. The light irradiations are followed by adding the releasing strand to set the motor free from the recruiter. Panel B presents the fluorescence collected from the ensuing operation (again by alternating UV-visible light) of the site-specific motor-origami binding complex prepared by the experiment shown in panel A. The shown data are the fluorescence intensity normalized to the intensity collected at the first visible light irradiation (set as time zero for the operation experiment). Panels C and D are the same as panels A, B, respectively, except for the same origami track now prepared for dye labelling scheme 2 (as indicated). Averaging the data in panels B and D according to the track's binding sites yields the data shown in Fig. 4D.

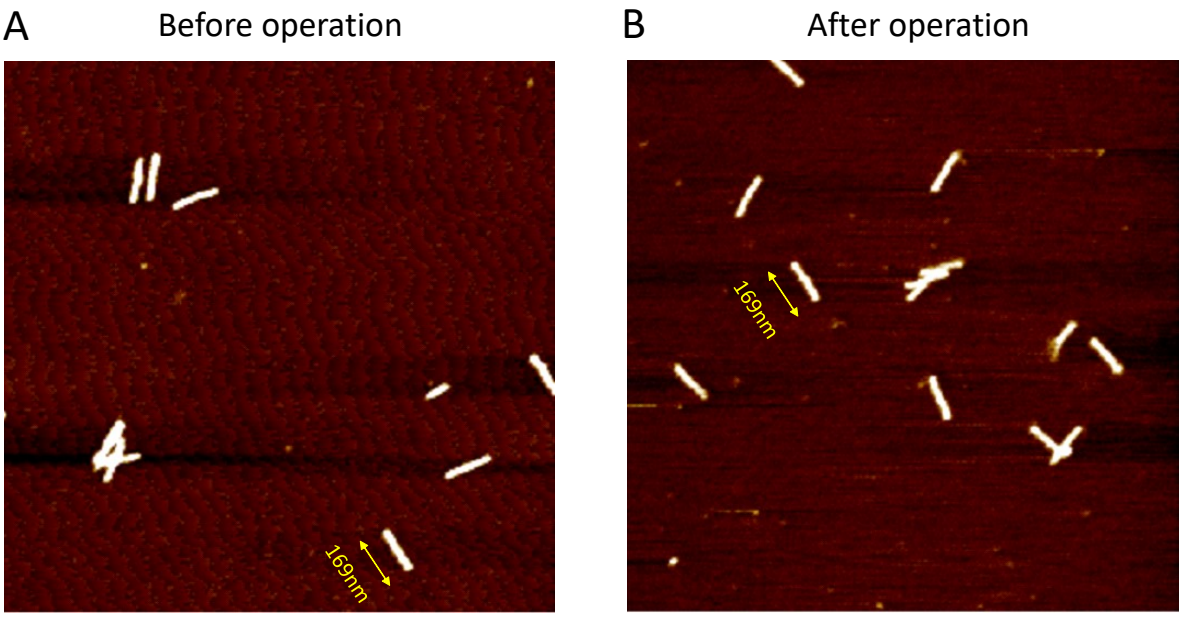

Fig. S7 AFM images of the origami rod (taken from the same batch of fabricated origami) before and after the typical light operation used in this study, namely 12 cycles of alternating UV-visible light irradiations (10 minutes per UV and 10 minutes per visible light). No apparent difference is observed between the origami images before and after the operation. The results suggest a negligible influence of the light operation in this study to the structural integrity of the DNA origami.

2.Nucleotide sequences for the origami rod and variations used in this study

Strand	Position	Sequence
staple-1	0[139]-2[140]	AAAGAAAAAACAATAACGGATTGAATTT
staple-2	0[146]-4[133]	GCGGAACGTTTTGATATCCCATCCTAAT
staple-3	0[181]-2[182]	ATTAATTCAAAATCGCGCAGACATAGCG
staple-4	0[202]-2[203]	TAAATCCTATTCATTTCAATTTTCCCTT
staple-5	0[223]-2[224]	AAACAATCAAAAGAAGATGATAATCGTC
staple-6	0[265]-2[266]	AATAGATTTACATTTAACAATTACATAA
staple-7	0[286]-13[300]	AACTAATGAATTACCTTATTACCGCCAGTTAGGAGCGCATAG
staple-8	0[293]-5[286]	CACTAACTGCATGCGTTTGGAACAGCCAGTAATAAATCCCAATCCAACT
staple-9	0[307]-2[308]	AATATCTCCATTGCAACAGGAGGCCTTG

staple-10	0[314]-4[301]	TATCTAATAGAGGAATAGGGTTGAGTGT
staple-11	0[335]-4[322]	TTGAAAGGCTCGAAAAATCAAAAGAATA
staple-12	0[377]-4[364]	TCAAACCATTGTTAATCCTGTTTGATGG
staple-13	0[391]-2[392]	CTGAACCCACCAGTCACACGATCCATCA
staple-14	0[398]-4[385]	CACCTTGCACACAAGGTTTGCCCCAGCA
staple-15	0[419]-4[406]	CAAATGAGCATAAATTGCAGCAAGCGGT
staple-16	0[426]-8[420]	CCAGCAGAGTAGTAAGGTCAT
staple-17	0[475]-2[476]	GGTGAGGGCACAGACAATATTTAAAGGG
staple-18	0[55]-2[56]	TGGATTAGTAAAACAGAAATAATGCAAA
staple-19	0[62]-4[49]	GATTGTTAATAGCAAAGTACCGCACTCA
staple-20	0[83]-4[70]	GCAATTCAGAAGGCTCCAAGAACGGGTA
staple-21	0[97]-2[98]	ATTATCACGTCAGATGAATATCCTCCGG
staple-22	1[25]-2[30]	CTACCATATCAAAATTAAAAGAACGCGAG
staple-23	1[75]-3[90]	AGATTTTCATATAACTTTTAATGGTTTGAA
staple-24	10[111]-8[98]	CACCCTCAGAACCGGAGCCACAGCGTCA
staple-25	10[118]-0[119]	GAACCGCGACGTTAGTAAATGCAGCAGCTATACCAAAGGAGC
staple-26	10[153]-8[140]	CACCGTACTCAGGACCGCCGCAGCAGCA
staple-27	10[160]-0[161]	GGTGTATACTTTCAACAGTTTCTTGCAGCGCCTGATTTGAGT
staple-28	10[174]-6[161]	TATAGCCCGGAATAGGTTGAGGTCACCAGAAACGCGTCAGAG
staple-29	10[181]-0[182]	ATATAAGGTGAGAATAGAAAGCGATATAATCCGCGGAACGTT
staple-30	10[195]-8[182]	CGTCGAGAGGGTTGTGGCCTTACCATTA
staple-31	10[237]-8[224]	TAGGATTAGCGGGGGAATGGACATTTGG
staple-32	10[258]-9[251]	TAGACTCCTCAAGAAATTTAC

staple-33	10[272]-8[266]	GAGTGTATCATACAATTATTC
staple-34	10[342]-6[329]	ATGGTCAATAACCTAGGCAAAATCAGAATCATTTTACGCCAG
staple-35	10[405]-7[400]	AACAGTTGATTCCCAAAAACACAAGAGATTTCATCAA
staple-36	10[454]-0[455]	GTTTTAAGTTTTAATTCGAGCCCACATTCTTTAATTATTAAC
staple-37	10[475]-0[476]	TATAATGCGAACCAGACCGGAATCAGTTTTATGCGAAACAGA
staple-38	10[90]-6[77]	CAGAGCCACCACCCCCCTCAGTTCATCGAGAACTGAATAGCA
staple-39	10[97]-0[98]	CCACCCTTAAAGTTTTGTCGTGAACGAGTCATCTTATTCCTG
staple-40	11[126]-9[125]	AATTTTCTACCGCCACCCTCACCACCAG
staple-41	11[133]-13[146]	TGTATGGGATTTTGCTTTTGCTACAACG
staple-42	11[196]-0[203]	TAAAGGAATTGCGACAACCATACTTAGCCTCGTAT
staple-43	11[210]-7[209]	ATAATAACAGTACCAGGCGGAAATAAATAAAATCACAATAGA
staple-44	11[217]-0[224]	TTTTTTCACGTTGATAGTTGCGACGGTCACTTTAC
staple-45	11[238]-0[245]	CAAAAAAAAGGCTCTCTTAAACGAACTGTTGAGGA
staple-46	11[25]-12[30]	GTCACCAGTACAAACTACATTAAACGGGT
staple-47	11[259]-0[266]	AGCCTTTAATTGTAAGCTTGCAGGACAGAGCCGTC
staple-48	11[280]-13[293]	GAATCGTCATAAATATAGCGTGACCAGG
staple-49	11[294]-10[280]	ATTCATTAGCTGAAAAGGTGGCATCAAT
staple-50	11[301]-13[314]	GAATCCCCCTCAAAATAGTAAGACCTTC
staple-51	11[322]-13[335]	AACAGTTCAGAAAAGAAGTTTTGACAAG
staple-52	11[357]-9[356]	AATCAGGACCATTAGATACATAAGCAAT
staple-53	11[364]-13[377]	TCTTTACCCTGACTAGACGACGCTGCTC
staple-54	11[378]-7[377]	ATTATAGCGAACGAGTAGATTTAAAGCTATCGTAATCTGGCC
staple-55	11[385]-13[398]	TCAGAAGCAAAGCGCTATCATGCTTGCC

staple-56	11[406]-13[419]	TCAAAAAGATTAAGCGAGGCACAGAACG
staple-57	11[42]-8[35]	CAACGCCGAACCCATGTACCGGAACCAGCATCTTT
staple-58	11[420]-6[413]	AGGAAGCTCTGGAAGTTTCATTACTTTTTGCCTGAGCGAGTATGGGAAG
staple-59	11[434]-9[440]	ACTTCAAATATCGCATATGCAACTAAAGTATTTCA
staple-60	11[462]-9[461]	TTCAAAGCTGTAGCTCAACATATTTTTA
staple-61	11[483]-7[484]	AGCAAACGCTTAATTGCTGAATTTAAATCTGATAATCACGTTGG
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staple-63	11[63]-8[56]	GACAGCCCAGGGATAGCAAGCCCGCCTCAGCCCCC
staple-64	11[70]-13[83]	CTCATAGTTAGCGTCAGAGGCCAAAAGA
staple-65	12[328]-10[308]	TGCCAGAGGGGGTATGCTTTATATATTTTCATTTG
staple-66	12[356]-0[350]	TAGCGAGTACCCAAGGTCAGT
staple-67	12[440]-4[427]	ACATAACCTTGAGATGCCACGGTGCCTACACCGCCTGGCCCT
staple-68	12[503]-0[497]	TTATTACCTCATTAACGAACC
staple-69	12[510]-10[497]	AACAACAATTAGAGCATTTTTGCGGATG
staple-70	13[105]-9[118]	TGACCCCTGCCTTTCACCCTCAGAGCCG
staple-71	13[112]-11[111]	CAGCGATGAAAGACAGCATCGCTTTCCA
staple-72	13[133]-12[126]	AACAAAGGGGATCGTCACCCT
staple-73	13[147]-9[160]	GAGATTTCATCGATCAGCATTGACAGGA
staple-74	13[154]-11[153]	GTATCATGGAGTTAAAGGCCGCTAAACA
staple-75	13[168]-9[181]	TAAATTGCGGAAACGCAGGTCAGACGAT
staple-76	13[175]-11[174]	TGTCGAATTCGGTCGCTGAGGCAGCGGA
staple-77	13[189]-10[196]	ACCTGCTCACCATTGATATTCACAAACATAAGTGC
staple-78	13[196]-11[195]	CCATGTTCGCCCACGCATAACGAACAAC

staple-79	13[210]-10[217]	CGGAACGAGCCAGCCCTCATTAAAGCCATTTTGCT
staple-80	13[217]-12[210]	AGGCGCAGCCGACAATGACAA
staple-81	13[231]-10[238]	AATCATACTTGAGCAAGCGCAGTCTCTGGAAGGAT
staple-82	13[238]-11[237]	AGGGAACCAGCTTGATACCGAAAATCTC
staple-83	13[252]-10[259]	ACCAACTGTGAATTCGTTCCAGTAAGCGCTGGTAA
staple-84	13[259]-11[258]	TTGAAAGTTTCGAGGTGAATTCAAAAGG
staple-85	13[273]-8[280]	ATGAACGGACGGAATGGCTTTTGATTCTACTAATATTTAAATTGTAATT
staple-86	13[280]-10[273]	GTGTACACCAATACTGTTATCTCGGTCGGATACAG
staple-87	13[301]-12[294]	GCTGGCTAATGTTTAGACTGG
staple-88	13[32]-4[26]	AATACGTAATGAATAATGTAGGAACAAGCAAGCCGTTTTT
staple-89	13[343]-11[342]	TATTCATAGGCTTTTGCAAAACGAGAAT
staple-90	13[357]-10[364]	ATCAACGATGTCAAAAAGCCTCAGAGCATAGTTTG
staple-91	13[385]-12[378]	GAATAAGAACCCTCGTTTACC
staple-92	13[427]-12[420]	AATTGGGGCCAAAAGGAATTA
staple-93	13[441]-9[454]	TGGTTTAGAGAGATACGCAAGGATAAAA
staple-94	13[462]-9[475]	CATTGTGCCGGAGAGAACCCTCATATAT
staple-95	13[469]-12[462]	AATTACCGAGATTTAGGAATA
staple-96	13[483]-11[503]	ATTTTAAGTTCTAGGCAATGCCTGAGTAGCTTAGATCCAACAGGTCAGG
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staple-104	2[181]-0[168]	ATAGCTTAGATTAACAAGTTATTAAAAG
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staple-111	2[440]-4[441]	AGTGTTTGCTGGCAAGTGTAGTGCCCTT
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staple-117	3[133]-13[132]	ACCGGAAATCAAAATCATAGGTCGGGAGCCACCAGAGCGCGA
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staple-120	3[203]-7[215]	AGCCAACGCTCAACACAACATACGCTAAAACAGGGAAATTC
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staple-127	3[329]-5[342]	AAATCGGAACCCTACCCTTATTTCGTAA
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staple-129	3[371]-7[383]	GGGAAAGCCGGCGAGGCGAAATCCGCTCACGCCAGTTCCTG
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staple-143	5[161]-2[154]	ATTAGTTAGTCCTGAACAAGAAAAAGCCAGAAGAG
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staple-161	6[160]-2[161]	GGTAATTAATCAAGAACATTAATTGCTTTGAATACGACGCTG
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staple-164	6[209]-5[202]	AAGCGCATTAGACGCCTGAAT
staple-165	6[244]-2[245]	GCAGCCTATTTGCCTTTAGAAACATCAAGAAAACAGTGAATA
staple-166	6[258]-8[259]	CAAAAATTTCAACCGATTGAGATTAAAG
staple-167	6[328]-2[329]	GGTTTTCGTACCGAGAATTGATCATGGAAATACCTAGTAGAA
staple-168	6[342]-8[343]	TTGGGTATTAACCAATAGGAATACCCCG
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staple-171	6[426]-8[427]	CAACTGTACAACCCGTCGGATGGCTATC
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staple-173	6[64]-5[55]	CCGAAGCCCTTTTTAACGCGCCC
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staple-179	7[273]-6[273]	AGGTAAATAACGTTAATATTTAGTGCCAAGATAAGAAACGAT
staple-180	7[28]-8[26]	AGTTACCTCATAATCA
staple-181	7[317]-13[328]	TGTTAAATCAGCAAGCCCCGTAATCT
staple-182	7[35]-6[26]	AGAAGGAAAGCAGATAGCCGAAC
staple-183	7[401]-11[405]	CATTAAATGTGAGAGTCTGGAAACACTAGTAAGAGCAACAGATTGCA
staple-184	7[485]-8[490]	TGTAGATGGGCGTTCAACC
staple-185	7[56]-13[76]	AATAATAACGGAATACCCAAAGCATTTTAAAGAGG
staple-186	7[98]-5[97]	CCTTATTAATAAGAGCAAGAAGTATTCT
staple-187	8[125]-6[112]	GACAGAACAAACGTAAGAATTGAGTTAA
staple-188	8[132]-10[133]	CAGTAGCAACCACCACCAGAGGGTTTAG
staple-189	8[251]-6[245]	ATCACCGGGCGACAGAAAATA
staple-190	8[300]-10[301]	GCAAATAGTAGTAGCATTAACGGGCGCG
staple-191	8[321]-10[322]	AAAAACAAAATCATACAGGCAGTTTAGC
staple-192	8[356]-6[343]	TCATATGCGCCATCGCAAGGCGATTAAG
staple-193	8[370]-12[357]	AACTAGCTAACAAAGATAAAAACCAAAA

staple-194	8[384]-10[385]	AACGGTAAAATCGGTTGTACCAATTCTG
staple-195	8[405]-10[406]	GAGCAAATTATGACCCTGTAATCCATAT
staple-196	8[440]-6[427]	CTACAAATCTCCGTCCATTCAGGCTGCG
staple-197	8[454]-12[441]	TATTTTTATTTCAACAACTAATGCAGAT
staple-198	8[461]-6[448]	GGGTAGCGATTGACAACCAGGCAAAGCG
staple-199	8[48]-10[49]	CGTTTGCAGCCACCACCGGAACCAATAG
staple-200	8[510]-9[517]	CCATCAAGGTAAAGATTCAAA
staple-201	8[517]-12[511]	CAAATCACAGGACGCTAACGG
staple-202	8[69]-10[70]	CGGTCATCCTCAGAGCCGCCATCATTTT
staple-203	9[28]-10[23]	ATCACCGTAACACTGAGTT
staple-204	9[308]-7[316]	ATCCAATGGAAGATTTAAATTTT
staple-205	9[336]-11[356]	GAATTAGCAAAATTTTCGCAAGACCATAAATCAAA
staple-206	9[420]-11[433]	GCGGGAGAAGCCTTTACGGTGCCGAAAG
staple-207	9[497]-6[497]	ATGTGTATATGATACATCGTATCGCACT
staple-208	14[337]-17[337]	TTTGGGGTCGAGGTGCCACCCAAATCAAGTTT
staple-209	15[229]-16[229]	CTGAAACATGAAAGTATATTTCGGAACCTATTATT
staple-210	15[245]-16[245]	TTAAGAGGCTGAAGTTTTAACAAACAGTTAATGCCCCCTGCC
staple-211	16[281]-15[281]	GTAACAGTGCCCGTATGGGGTCAGTGCCTTGA
staple-212	17[296]-14[296]	GGGCGATGGCCCACTACGTGAACCATCGCAAAGGGCGAAAAACCGTCTATCA
BS1-D2*	3[56]-6[80]	AATTTCATCTTCTGTTAAACCAGCAAATTCTTAGGTATATCTCCTTCTTAAAG
BS2-D2*	3[140]-6[164]	TCATAATTACTAGAAAAATAAAGCCTTAGAGCGGGTATATCTCCTTCTTAAAG
BS3-D2*	3[224]-6[248]	CTTAATTGAGAATCCTGTCCAGAGCCTATTACAGGTATATCTCCTTCTTAAAG
BS4-D2*	3[308]-6[332]	GGACTCCAACGTTAGCCCGAGTCCCCGGCCAGTGGTATATCTCCTTCTTAAAG
BS5-D2*	3[392]-6[416]	GAGAAAGGAAGGGACCACGCTCATACGAGGTGCGGTATATCTCCTTCTTAAAG

BS6-D2*	3[476]-6[500]	CGCCGCGCTTAATGGGGTGGTCGCTTTCGCTTTGGTATATCTCCTTCTTAAAG
BS1-D1*	7[61]-8[49]	GGAATG-[$(dT)_3$ or $(dT)_6$ spacer]-GGAAACGCTTATTAG
BS2-D1*	7[147]-8[133]	GGAATG-[$(dT)_3$ or $(dT)_6$ spacer]-TACATACACCGTAAT
BS3-D1*	7[231]-8[217]	GGAATG-[$(dT)_3$ or $(dT)_6$ spacer]-ATATGGTTGAATTAG
BS4-D1*	7[315]-8[301]	GGAATG-[$(dT)_3$ or $(dT)_6$ spacer]-AATTCGCATGTATAA
BS5-D1*	7[399]-8[385]	GGAATG-[$(dT)_3$ or $(dT)_6$ spacer]-TAGCCAGCATCGATG
BS6-D1*	7[483]-8[469]	GGAATG-[$(dT)_3$ or $(dT)_6$ spacer]-GGGATAGGATTAATG
BS2-2nd dye	6[148]-5[139]	CTAATATCAGAGAGATGCGGGAG
BS3-2nd dye	6[232]-5[223]	GAGAGAATAACATAAACGAGCGT
BS4-2nd dye	6[316]-5[307]	CACGACGTTGTAAAACTCGACTC
BS5-2nd dye	6[400]-5[391]	GGGCCTCTTCGCTATTACAATTC
BS6-2nd dye	6[484]-5[475]	CCGGCACCGCTTCTGGTTGCGCT

Table S1. Staple strands for the origami rod hosting the full 6-site track (based on the 7249nt-long M13mp18 scaffold). All sequences in the right column are listed from 5' to 3'. The staple position (middle column) refers to the Cadnano coordinates for each staple's start and end (the number before a bracket for the duplex name and the number in a bracket for the base position). Near the end of the table are the staples extending into the track's overhangs (highlighted in green in Fig. 1C) and the staples carrying the second dye for bi-overhang sites (highlighted in blue in Fig. S1D). These staple strands are named 'BSx' (left column) with 'x' as the number of binding sites counted from the minus end of the full 6-site track (as shown in Fig. 1E). BSx-D1* or BSx-D2* indicates a staple strand extending into D1* or D2* overhangs (D1* or D2* sequence underlined in the right column; BSx-D1* staples has a spacer as either TTT or TTTTTT). BSx-2nd dye indicates a non-overhang staple strand that may carry the second dye for a bi-overhang binding site (at 5' end of a BSx-2nd dye staple to appear below a D2* overhang on the origami, with the first dye at the 5' end of a BSx-D1* staple to appear at the tip of a D1* overhang). The origami hosting the 6-site track for the minus-end motor start (Fig. 2A, bottom plot) is fabricated using the BS1-D1* staple without its overhang segment (i.e., D1*-spacer), and using the BS2-D1* staple with its D1* segment mutated into the 17nt-long recruiter sequence (GAGAACCTGACGCAAGT) that is complementary to the tail sequence given in Table S3 or S4. The origami hosting the 5-site track for the mid-site motor start (Fig. 2A, middle plot) is fabricated using the two BS1 staples (i.e., BS1-D1*, BS1-D2*) without overhang segments, and using the BS4-D1* staple with its D1*-spacer segment mutated into the recruiter sequence. The 3-site track for random-mix operation (illustrated in Fig. 2A, upper plot) is fabricated using BS1, BS5 and BS6 staples without overhang segments.

3.Nucleotide sequences for motor and variations used in this study (Table S6-S9)

Strand	Sequence
Motor strand 1 (5′-D3-spacer-D2-D1-3′)	GAGTTACCATCTAGGTAGAG-AGTC- CTXTTXAAXGAXAGXGAXGAXTAXTAXCC-ATTCC - BHQ2
Motor strand 2 (5′-D3*-spacer-D2-D1-3′)	CTCTACCTAGATGGTAACTC-AGTC- CTXTTXAAXGAXAGXGAXGAXTAXTAXCC-ATTCC - BHQ2

Table S2. Motor used in random-mix experiments (shown from 5' to 3'). D2 and D1 segments in the motor's leg are underlined, with azobenzene moieties represented by 'X's.

Strand	Sequence
Inverted strand (3′-D3*-5′-5′-D4*-D3*-3′)	3′-CTCAATGGTAGATCCATCTC-5′-5′-ATGTCGGGGATTTCGTCACA- CTCTACCTAGATGGTAACTC-3'
Bridge-tail strand (5′-D4-spacer-tail-3′)	TGTGACGAAATCCCCGACA-TTTTT-ACTTGCGTCAGGTTCTC-ATGTCT
Leg strand as motor strand 1 in Table S2 (5′-D3-spacer-D2-D1-3′)	GAGTTACCATCTAGGTAGAG-AGTC- CTXTTXAAXGAXAGXGAXGAXTAXTAXCC-ATTCC - BHQ2

Table S3. Motor used in the first method for site-specific motor introduction (as schematically illustrated in Fig. 3B). The inverted strand contains an inverted base for reverse 5'-5' linkage. The bridge-tail strand has its tail segment underlined, with the part highlighted by red colour responsible for binding the recruiter, and with the part highlighted by cyan as the toehold for the releasing strand.

Strand	Sequence
Inverted strand with tail (3′-tail-$(dT)_4$ spacer-D3*-5′-5′-D4*-D3*-3′)	(3′) TCTGTA-CTCTTGGACTGCGTTCA-TTTT- CTCAATGGTAGATCCATCTC-5′-5′???-ATGTCGGGGATTTCGTCACA- CTCTACCTAGATGGTAACTC (3')
Bridge strand	TGTGACGAAATCCCCGACAT
Leg strand as motor strand 1 in Table S2 (5′-D3-spacer-D2-D1-3′)	GAGTTACCATCTAGGTAGAG-AGTC- CTXTTXAAXGAXAGXGAXGAXTAXTAXCC-ATTCC - BHQ2

Table S4. Motor used in the second method for site-specific motor introduction (as schematically illustrated in Fig. 3C). The inverted strand has its tail segment underlined, with the part highlighted by red colour responsible for binding the recruiter, and with the part highlighted by cyan as the toehold for the releasing strand.

Strand	Sequence
Protector strand 1	GGTATATCTCCTTCT
Protector strand 2	ATCTCCTTCTTAAAG
Protector strand 3	GGTATATCTCCTTCTTAAAG
Protector strand 4	GCAATGGCATATCGCCTTCGTAAAG
Releasing strand	AGACAT-GAGAACCTGACGCAAGT

Table S5. Protector strands and releasing strand used for site-specific motor introduction.