Exploring the speed limit of toehold exchange with a cartwheeling DNA acrobat

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SUPPLEMENTARY INFORMATION

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<u>Supplementary Note 1</u>

Kinetic Modelling of Walker Stepping in a 3-Foothold System

Stepping by a walker with toehold domain length $a \in \{5, 6, 7, 8\}$ and branch migration domain length $b \in \{6, 13, 20\}$ was numerically simulated at single-base resolution according the reaction scheme shown in Supplementary Fig. 5a, using a version of the Gillespie algorithm[1] implemented in MATLAB. The scheme shown in Supplementary Fig. 5a depicts a system with $b = 13$; different values of b will result in a different number of states B_β, where $\beta \in \{0, 1...b\}$ indicates the number of base pairs that have been displaced in the branch migration process. States S_1, S_2, and S_1' represent states in which the walker is bound only to foothold F_1, F_2, or F_1', respectively. Thus, each reaction scheme has $2(b + 1) + 3 = 2b + 5$ total states, indicated by $i \in \{1, 2...2b + 5\}$.

The simulation algorithm consists of the following steps:

1. Choose a random starting state $i \in \{1, 2...2b+5\}$
2. Calculate the wait time Δt until the next reaction by drawing a random number from the exponential distribution with mean value τ_i calculated as

$$\tau_i = \frac{1}{k_{i \to i+1} + k_{i \to i-1}} \quad (1)$$

where $k_{i \to i+1}$ and $k_{i \to i-1}$ are the rate constants for transition to state $i + 1$ and $i - 1$, respectively.

3. Determine the probability $P_{i \to i+1}$ of a transition to state $i + 1$ according to

$$P_{i \to i+1} = \frac{k_{i \to i+1}}{k_{i \to i+1} + k_{i \to i-1}} \quad (2)$$

4. Choose a random number p from a uniform distribution over the interval [0,1]; if $p \leq P_{i \to i+1}$, transition to state $i + 1$; otherwise, transition to state $i - 1$.
5. Repeat steps 2-4 until the simulation end time is reached.

Each type of walker was simulated for a total of 1000 seconds of simulation time, and its stepping lifetime estimated as the total simulation time divided by the number of times the trajectory crossed between S_{1+2} and $S_{2+1'}$. Note that a trajectory can enter the middle state with toehold a dissociated (*e.g.,* state $i = 16$ in Supplementary Fig. 5a) without taking a step to the next foothold; it may instead (with 50% probability in our initial model) return to its original foothold, in which case the event will not be counted as a step. Note also that we make the simplifying assumption that toeholds can only dissociate from the terminal states, *e.g.,* B_0 and B_{13} for $b = 13$.

Rate constants were chosen as follows. The second-order binding rate constant for toeholds of all lengths was estimated as 3.0×10^6 M^{-1}s^{-1} on the basis of prior single-molecule[1] and ensemble[2,3] measurements and theoretical treatments of toehold-mediated strand displacement. The local effective concentration of the toehold was estimated as ~100 μM by analogy to a similar system studied previously by smFRET[4], implying that the pseudo-first order rate constant of toehold binding $k'_{bind} = (3.0 \times 10^6 \ M^{-1}s^{-1})(10^{-4}M) = 300 \ s^{-1}$. A branch migration rate constant of $k_{bm} = 10000 \ s^{-1}$ was chosen to reflect literature on the rate of three-way branch migration in DNA[3,5]; this also provided a close match between the timescales of the autocorrelation function of the state number in simulations of walker $W_{8\_13\_8}$ (Supplementary Fig. 5b) and the cross-correlation function in single-molecule FRET measurements of walker $W_{8\_13\_8}$ on a 2-Foothold DNA tile (12 ms; see Figure 1). Based on previous estimations that initiation of branch migration incurs a penalty of 2 kcal/mol (in addition to a free energy barrier of ~5.3 kcal/mol for branch migration itself)[3], initiation of branch migration was assigned an approximately 7-fold slower rate
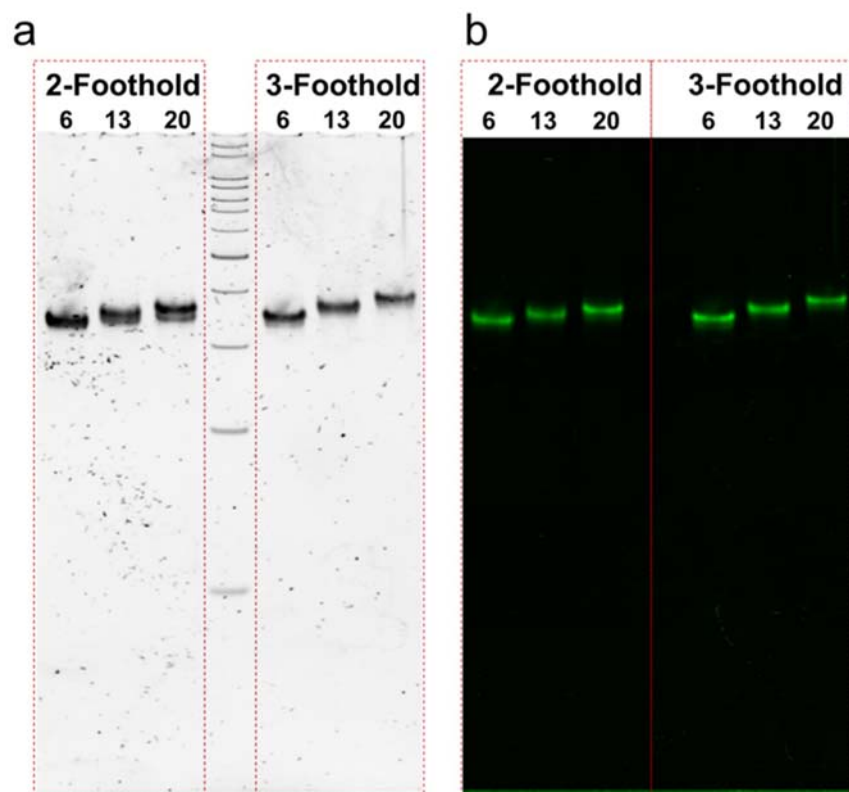
constant of $k_{bmi} = 1400 \ s^{-1}$. Finally, as a starting point, rate constants of dissociation for short oligonucleotides reported by Dupuis *et al.*[1] were used to construct the approximate empirical relationship $k_{dissoc} = (3 \times 10^6 \ s^{-1})e^{-2.031a}$, which was used to calculate k_{dissoc} for different values of a.

Given the approximate nature of this model, and the lack of any explicit treatment of the geometry or dynamics of the footholds and substrate, it recapitulates the experimentally observed stepping behaviour surprisingly well (Supplementary Fig. 5c), predicting the predominance of hybrid states S_{1+2} and $S_{2+1'}$ for all walkers, and qualitatively predicting that stepping rate will increase as the toehold length is decreased. Moreover, the stepping rates predicted for 8- and 7-nt toeholds are quantitatively similar to the experimental values (Supplementary Fig. 5d). However, there is a significant discrepancy with the experimentally determined stepping rate for $W_{6\_13\_6}$ and $W_{5\_13\_5}$, which are much slower than our model predicts, suggesting that an influence other than toehold dissociation is limiting the apparent stepping rate of $W_{5\_13\_5}$ in our smFRET measurements. For instance, binding of the dissociated toehold D_A to foothold F_1' may be slower than to foothold F_1, e.g., due to a slightly different distance between F_2 and F_1' compared to that between F_1 and F_2. This is consistent with the fact that the high-FRET states for all walkers in the $W_{x\_13\_x}$ series exhibit significantly longer median lifetimes than the low-FRET states (Figure 2n). In this interpretation, the fact that the asymmetry between lifetimes in the high- and low-FRET states increases as the toehold length is decreased from 8 to 5 nucleotides might be due to the progressive shortening of the walker-foothold duplex as the size of the toehold is decreased (while the toehold sequence of the foothold does not change in length, an increasing fraction of it becomes single-stranded as the walker toehold decreases in length, resulting in a conformation that is predicted to be more coiled up and resistant to extension for entropic reasons[6], and hence a shorter overall reach for the walker). Thus, as the walker-foothold duplex and the walker's reach shorten, the hypothesized asymmetry between the F_1-F_2 and F_2-F_1' distances may exert a stronger influence on stepping kinetics. Such an asymmetry is plausible, given that strand polarity considerations predict that footholds F_1 and F_2 emerge from the tile surface in orientations pointing somewhat toward one another, whereas foothold F_1' is predicted to emerge pointing somewhat away from F_2 (Supplementary Fig. 5). Intriguingly, the DNA origami scaffold results in a reversal of the FRET state bias compared to the DNA tile (compare Fig. 2n and Supplementary Fig. 9g), suggesting that biases can indeed be imposed by subtle structural details of the scaffold.

To explore the potential of biased binding of one toehold over the other to reproduce the slower-than-expected apparent stepping rates of $W_{6\_13\_6}$ and $W_{5\_13\_5}$ in our smFRET analysis, we introduced a preference for binding one of the footholds (F_1') over the other (F_1) that varied as a function of toehold length. In these simulations, the rate constant for binding F_1, $k_{bind}',_{F1}$, was obtained by dividing the rate constant for binding F_1' ($k_{bind}',_{F1'}$) by a parameter r that was dependent on toehold length: $k_{bind}',_{F1} = k_{bind}',_{F1'}/r$, where $r = 1, 1.5, 3,$ and 10 for $W_{6\_13\_6}, W_{6\_13\_6}, W_{6\_13\_6}$, and $W_{6\_13\_6}$, respectively (to reflect the increased bias in FRET dwell time as the toehold length decreases). To simulate the time resolution of most of our smFRET measurements, trajectories were binned in 100 ms intervals, with each bin consisting of a time-weighted average of all states occurring in that time interval. This had the effect of reducing or eliminating very brief dwell times, giving the appearance of longer average dwell times (Supplementary Fig. 5e). Finally, the trajectories were fit by hidden Markov modelling in the same manner as our experimental smFRET data to determine the apparent stepping lifetime. Intriguingly, the same deviation from an exponential dependence on toehold length is observed as for our experimental data (Supplementary Fig. 5d), suggesting that at least part of the reason the apparent stepping rates of $W_{6\_13\_6}$ and $W_{5\_13\_5}$ are slower than expected is the combination of FRET bias and limited time resolution.

The simulations also predict that stepping rate will decrease linearly as b increases (Supplementary Fig. 5f), since the walker's toehold can only dissociate from its complement in a small fraction of branch migration states (in our

simplified model, only from the very terminal states B_0 and B_b). As the number of branch migration states increases, the fraction of these states compatible with toehold dissociation, and hence stepping, will decrease. However, this is in direct contradiction with the experimental results shown in Fig. 2o, which suggest *slower* stepping for $b > 1$ shortest than for longer D_B. Again, we interpret this discrepancy as arising from aspects of tile geometry – such as the match between the length of the walker-foothold duplex and the foothold spacing, with consequences for local effective concentrations and tension within single-stranded components of the system – that are not captured by our kinetic model, and are at present difficult to determine experimentally with sufficient accuracy to be useful in the model.

Supplementary Fig. 1 | Design of cartwheeling walker and DNA tile. a, *2-Foothold* system foothold strands F_1 and F_2 are 5′ and 3′ extensions of ssDNA strands within the 4-helix tile (grey cylinders). **b,** *3-Foothold* system with $F_1′$ having same sequence as F_1. **c,** The walker W is a single-stranded DNA oligonucleotide comprising a 13-nucleotide branch migration domain D_B (coloured black) flanked by two 5- to 8-nucleotide toehold domains D_A (coloured red) and D_C (coloured orange) with distinct sequences.

Supplementary Fig. 2 | 5% Native PAGE characterization of *2-Foothold* and *3-Foothold* DNA tile systems. a, 5% native PAGE with SYBR Green stain of different tile constructs used in the paper. **b,** Fluorescence gel characterization of Cy3-labeled tile. The number above each lane (6, 13, 20) represents the number of nucleotides in the middle domain ($\overline{D_B}$) of each foothold strand.
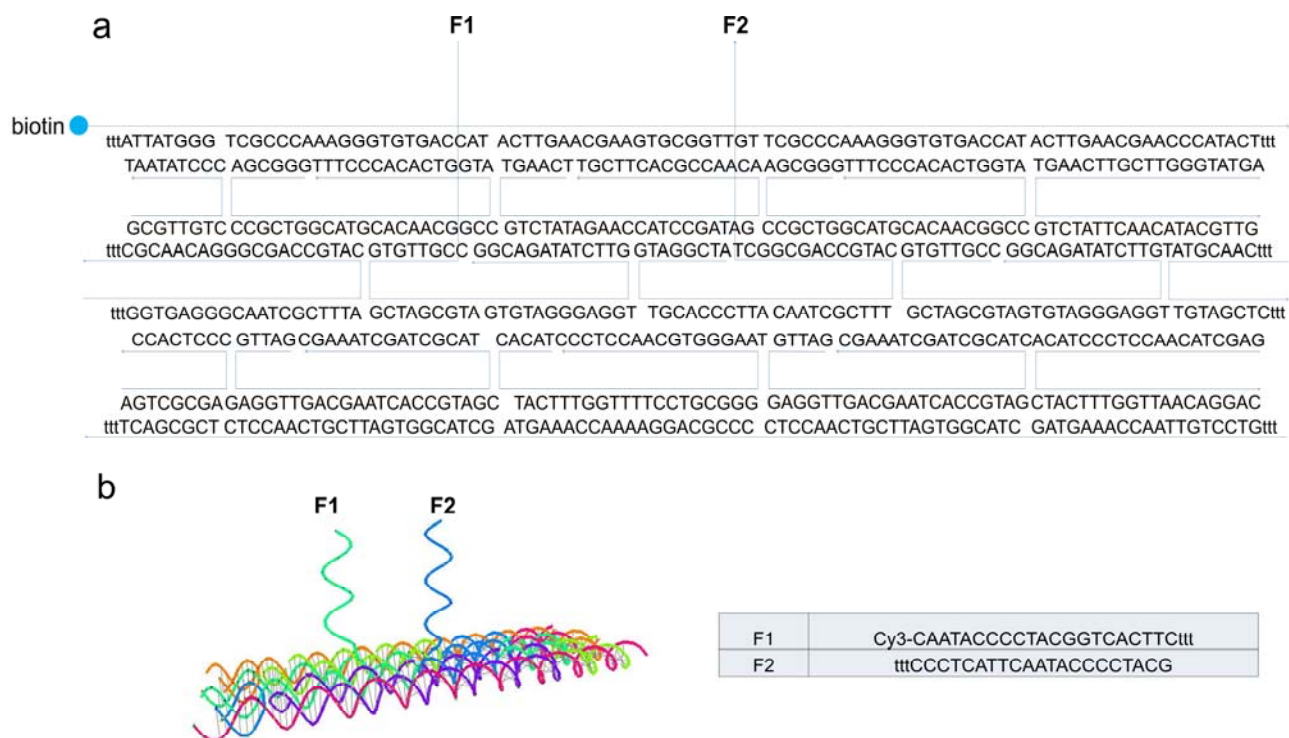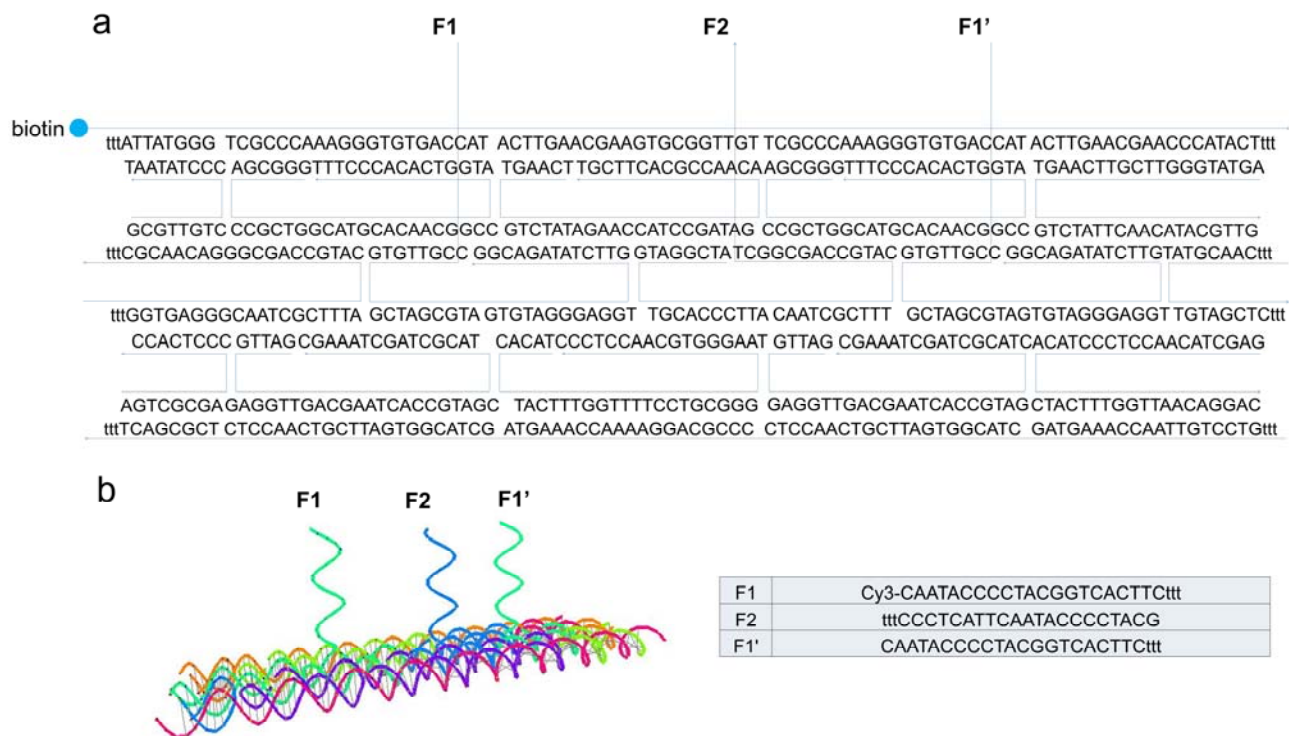
Supplementary Fig. 3 | DNA sequence design for 4HX tile with *2-Foothold*. a, The structure incorporates two ssDNAs as the two footholds, F1 (5'-CAATACCCCTACGGTCACTTC) and F2 (CCCTCATTCAATACCCCTACG-3'). The distance between 2 footholds are designed to be 7 nm and facing the same side of 4HX tile. **b,** Computer modelling (Tiamat) of DNA nanostructure and the detailed sequence and labelling strategy of T1 and T2. Cy3 dye is labelled at 5' of F1 with 2 T bases as spacer. For both F1 and F2, A single-stranded 3T spacer was added between the foothold and the tile to allow for flexibility.
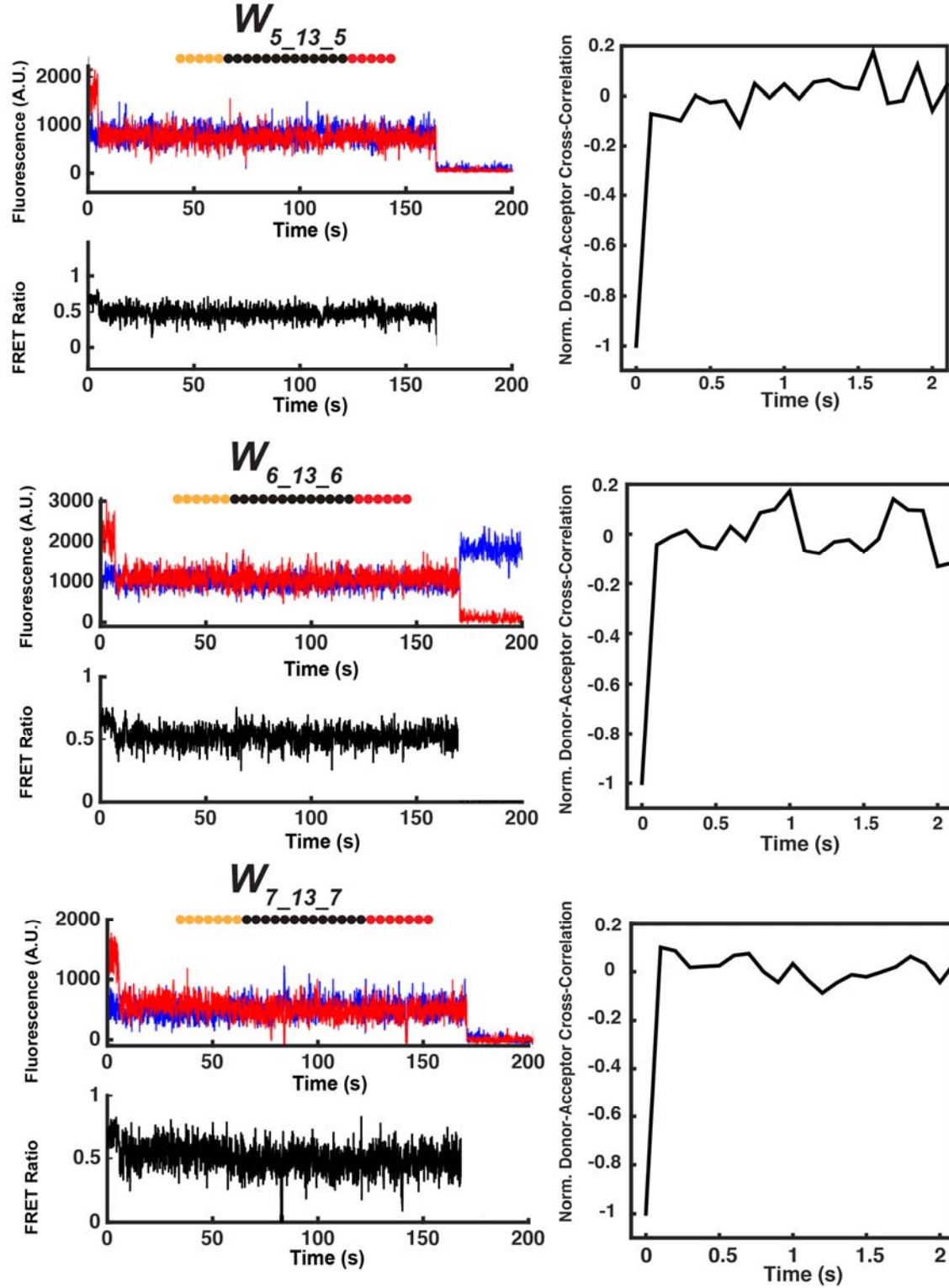
Supplementary Fig. 4 | Evidence of rapid FRET dynamics for $W_{5\_13\_5}$, $W_{6\_13\_6}$, $W_{7\_13\_7}$ on *2-Foothold* DNA tile. Rapid anti-correlated fluctuations in Cy3 (blue) and Cy5 (red) fluorescence intensity for a single walker-tile complex, suggestive of branch migration in hybrid state S_{1+2}.
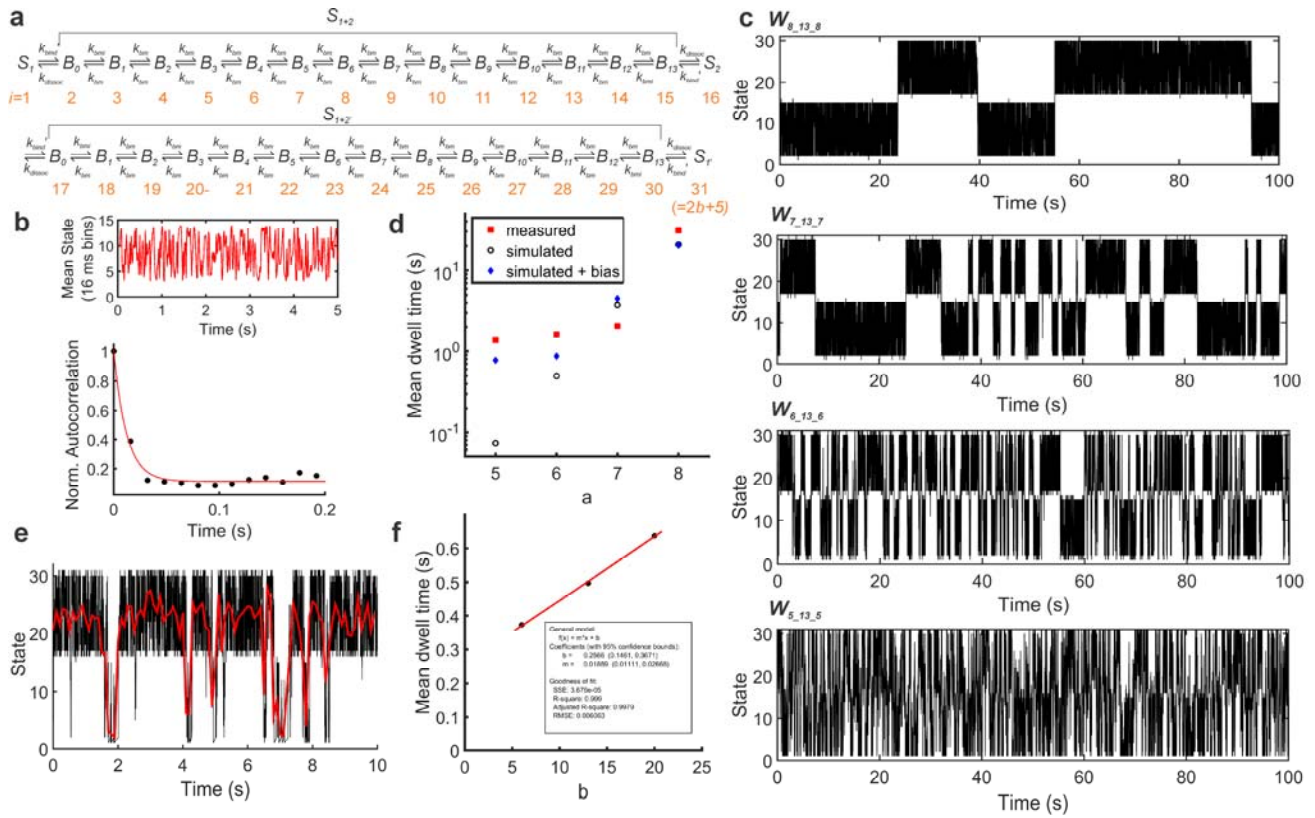
Supplementary Fig. 5 | Monte Carlo simulation of cartwheeling DNA walkers in a 3-foothold system. a, Scheme for kinetic modelling of 3-foothold system (b = 13 nucleotides in the depicted scheme). See Supplementary Note 1 for details regarding the model. **b,** (top) Representative portion of a simulated trajectory of $W_{8\_13\_8}$ in a 3-foothold system, zoomed in to show the rapid fluctuations among branch migration states. State values in this plot are binned to a time resolution of 16 ms to match the time resolution of donor-acceptor anticorrelation measurements (see Fig. 1, main text). (bottom) Exponential fit to the normalized autocorrelation function of the time-binned trajectory shown at the top. The lifetime of the exponential fit is 12.1 ms (95% confidence interval: [9.6, 14.6 ms]). **c,** Representative state vs. time trajectories for simulated walkers with b = 13 and toehold length a varying from 5 to 8 nucleotides. Rapid fluctuations among branch migration intermediates are punctuated by rare toehold dissociation and stepping events, which become more frequent as a decreases. **d,** Mean stepping dwell time of simulated trajectories (black filled circles) with b = 13 and varying a, as compared to the experimentally determined values (red squares) and simulated trajectories incorporating a toehold length-dependent bias towards one FRET state (blue diamonds). **e,** Simulated trajectory of $W_{5\_13\_5}$ with a 10-fold bias towards binding one foothold (black), along with a time-binned version of the same trajectory (red). The time binning in the red trajectory is 100 ms, to match the time resolution of smFRET measurements. **f,** Mean stepping dwell time of simulated trajectories with varying b and constant a (=6). The trend is well fit by a linear function (R^2 > 0.99).

Supplementary Fig. 6 | DNA sequence design for 4HX tile with *3-Foothold*. a, The structure incorporates 3 ssDNAs as the three footholds, F1, F1' (5'-CAATACCCCTACGGTCACTTC) and F2 (CCCTCATTCAATACCCCTACG-3'). The distance between each two footholds are designed to be 7 m and facing the same side of 4HX tile. **b,** Computer modelling (Tiamat) of DNA nanostructure and the designed sequence of footholds F1, F1' and F2. Cy3 dye is labelled at 5' of F1 with 2 T bases as spacer. For all three footholds, a single-stranded 3T spacer was added between the foothold and the tile to induce flexibility.
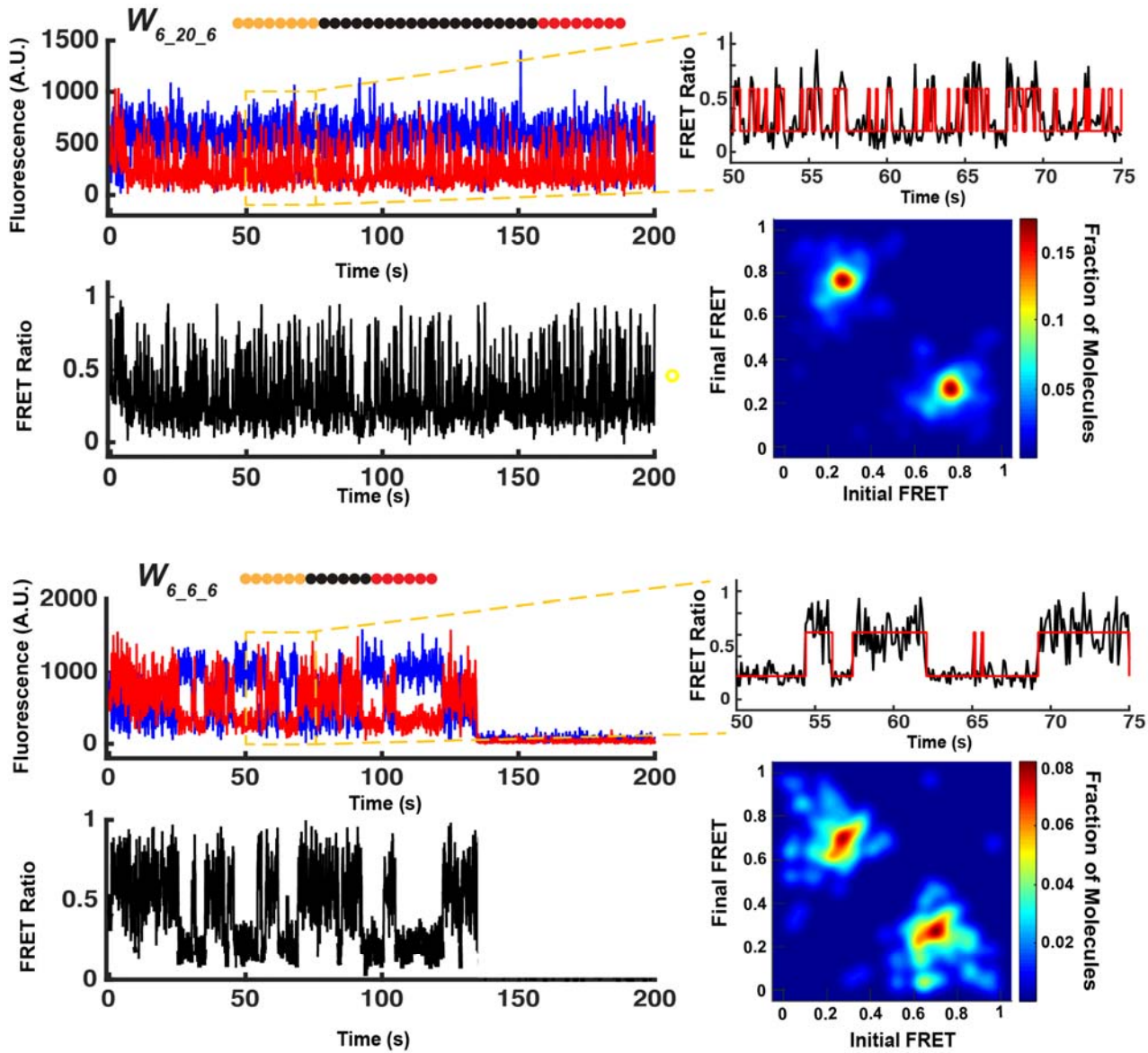
Supplementary Fig. 7 | Single-molecule FRET characterization of $W_{6\_20\_6}$ and $W_{6\_6\_6}$ on *3-Foothold* DNA tile. Representative smFRET trajectories of $W_{6\_20\_6}$ and $W_{6\_6\_6}$ on *3-Foothold* DNA tile are shown with Cy3 fluorescence in blue and Cy5 fluorescence in red. Zoomed-in trajectories (upper-right corner of each panel) show FRET transitions for 25-s segments in greater detail. Transition occupancy density plots (TODPs, lower-right corner of each panel) show the most frequently observed FRET transitions across all molecules.

a

b
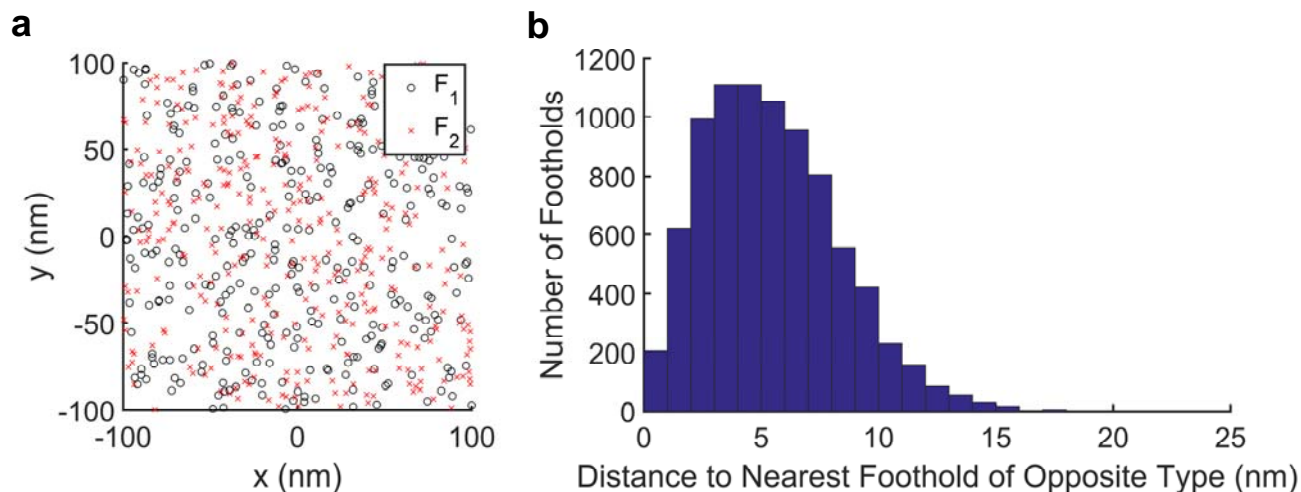
Supplementary Fig. 8| Simulated distributions and distances to nearest neighbour footholds on 2D surfaces.
a, Representative 200 nm × 200 nm region showing randomly distributed footholds F_1 and F_2. **b,** Histogram of predicted distances to nearest-neighbour footholds of the opposite type within a $(1000 \text{ nm})^2$ region containing 8350 randomly distributed copies each of F_1 and F_2. Foothold positions are assumed to be independent of all other footholds.

a

b

c

100 nm

d

e

f

g

Initial FRET

Final FRET

Fraction of Molecules

3-Foothold DNA Origami Lifetime Range (s)

high-FRET
low-FRET
mean
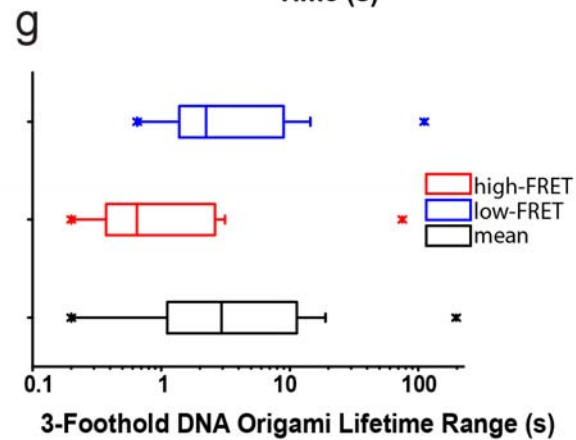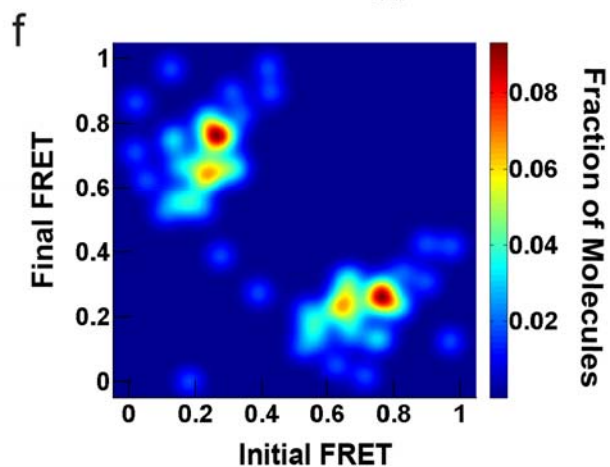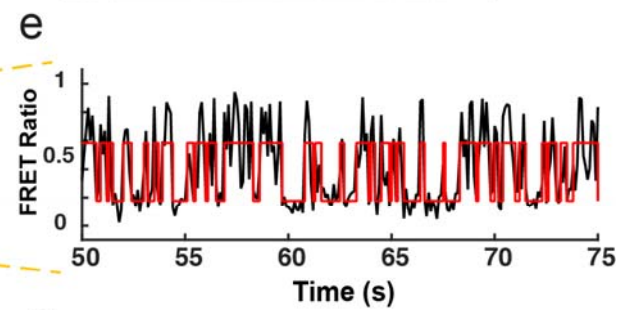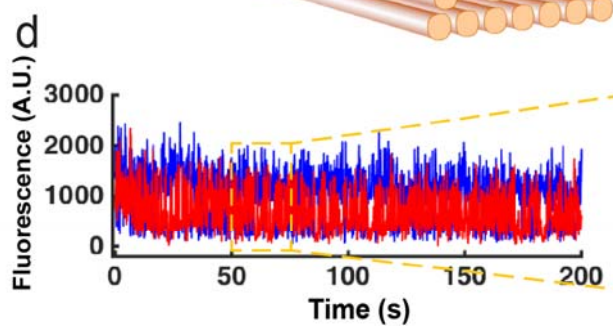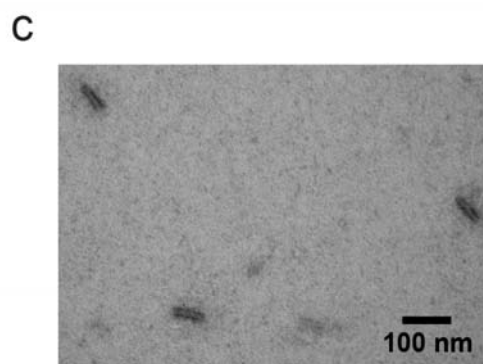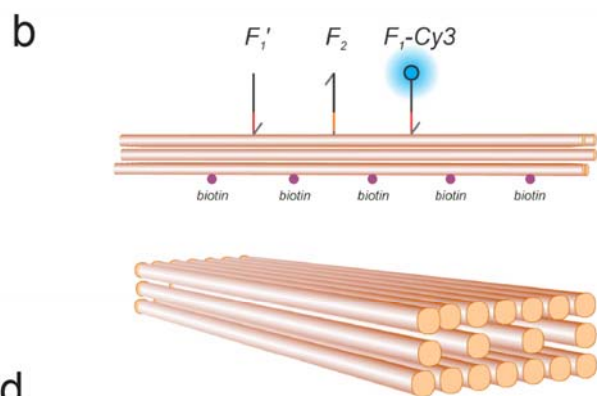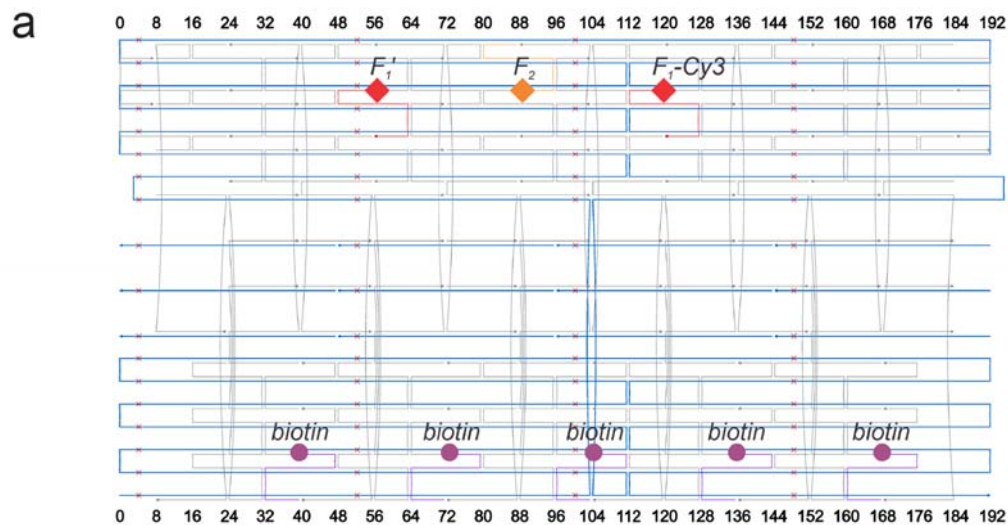
Supplementary Fig. 9 | Single-molecule FRET characterization of $W_{6\_13\_6}$ on *3-Foothold* DNA origami. a, caDNAno scaffold routing diagram for *3-Foothold* DNA origami, showing positions of footholds and biotins used for anchoring to the imaging surface for TIRF. **b,** Cartoon schematics of *3-Foothold* DNA origami, including a side view of foothold and biotin positions (top) and a perspective view of the underlying nanostructure (bottom). The distance between adjacent footholds is predicted to be ~10.5 nm. **c,** TEM characterization of *3-Foothold* DNA origami. **d,** A representative single-molecule FRET trajectory of $W_{6\_13\_6}$ on *3-Foothold* DNA origami. Cy3 fluorescence is shown in blue, while Cy5 fluorescence is shown in red. **e,** Zoomed-in trajectories showing FRET transitions for 25-s segments in **d**. **f,** Transition occupancy density plots (TODPs) illustrating the most common FRET transitions. **g,** Box-and-whisker plot of stepping kinetics in the high- and low-FRET states for $W_{6\_13\_6}$ on *3-Foothold* DNA Origami.
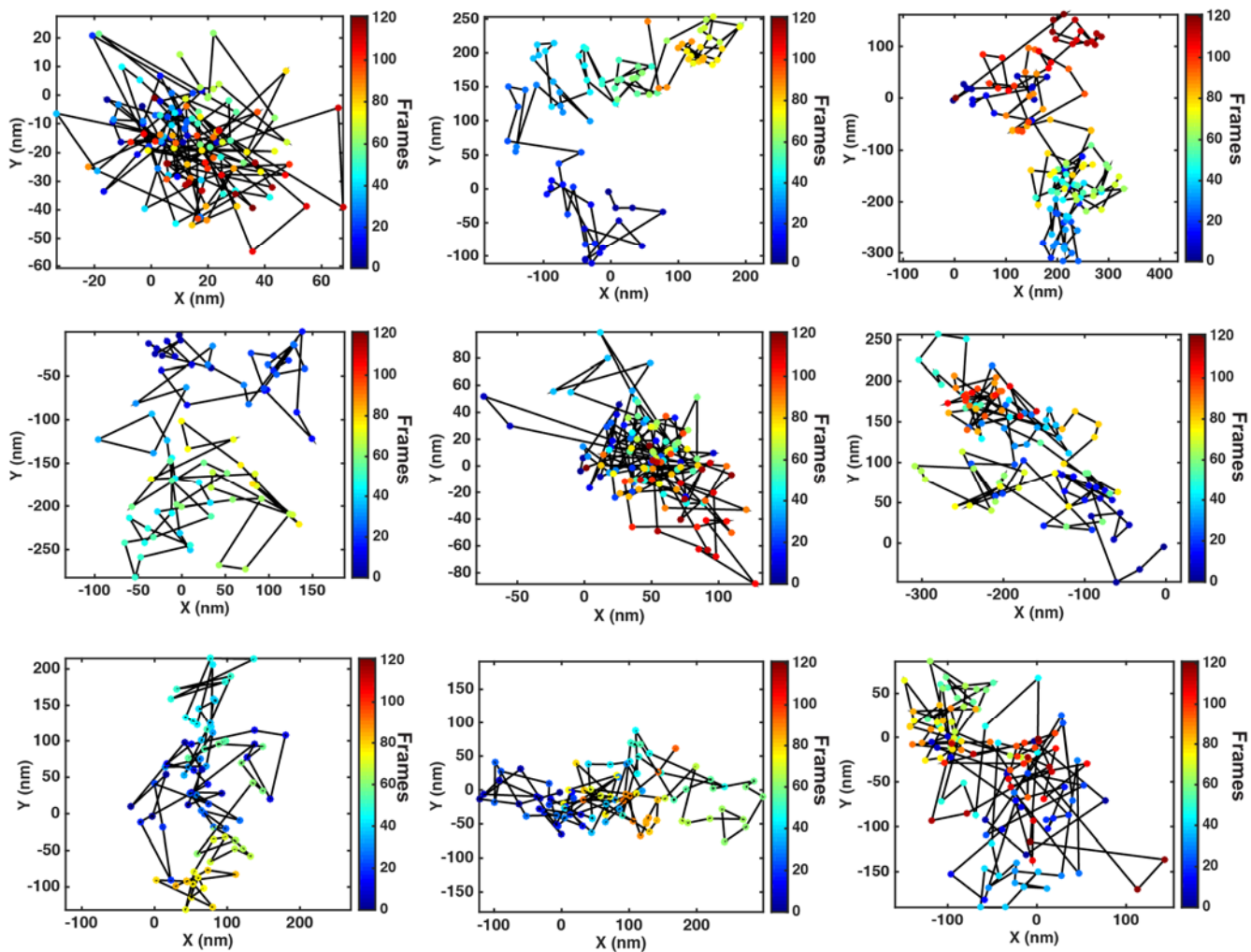
Supplementary Fig. 10 | Representative 2D particle tracking trajectories of $W_{6\_13\_6}$ on surface coated with F_1 and F_2. One frame was acquired every 30 s.
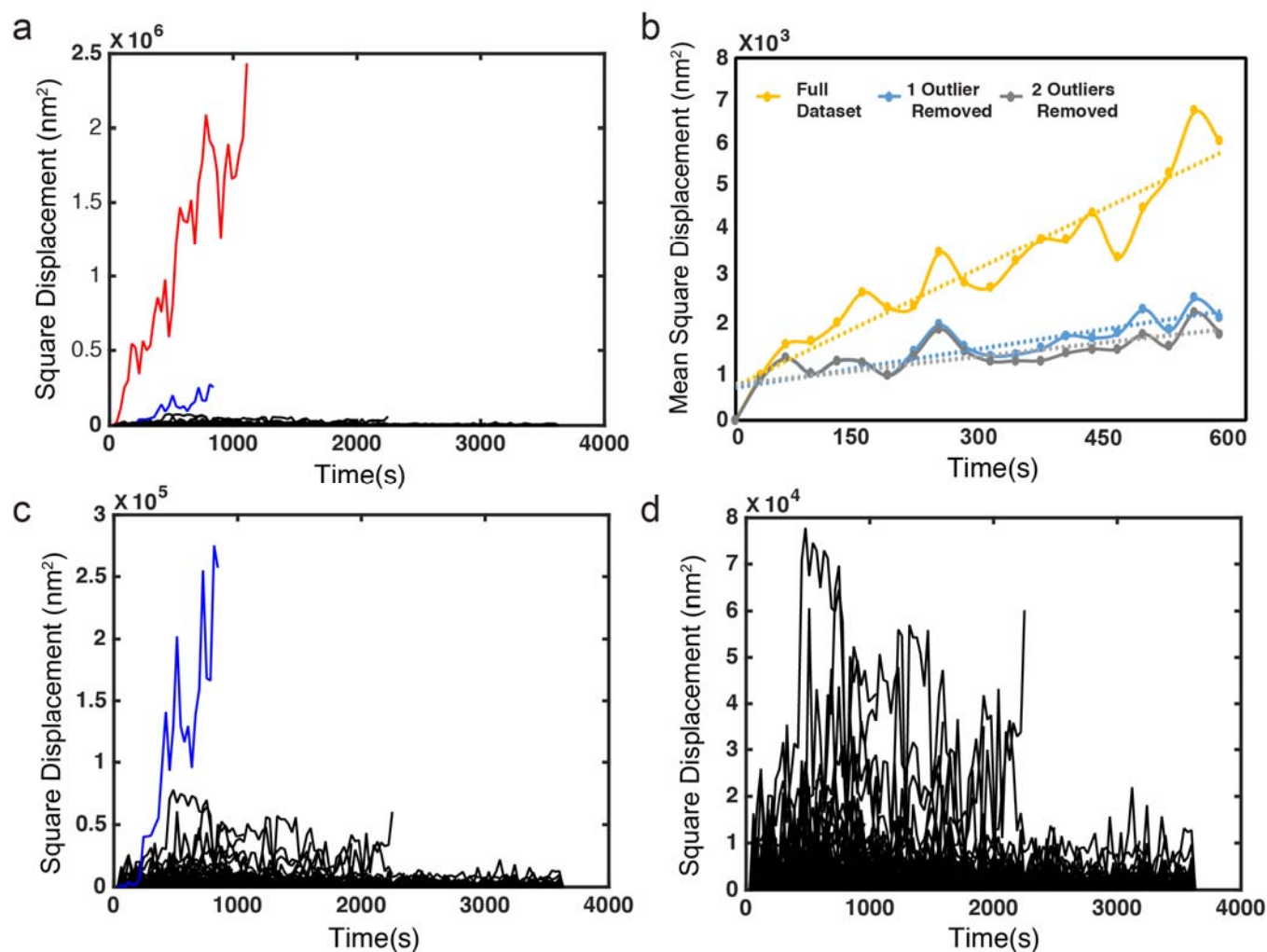
Supplementary Fig. 11 |MSD comparison for $W_{8\_13\_8}$. a, Square displacement for all trajectories (353 molecules). The extremely fast-moving outlier trajectory is highlighted in red. **b,** MSD comparison between all trajectories (n = 353 trajectories, yellow line), without the single fast-moving trajectory (n = 352 trajectories, blue line) and without the second fastest moving trajectory (n=351 trajectories, grey line). Dotted lines indicate linear regression fits to the data, resulting in calculated 2D diffusion coefficient estimates of 2.2 nm²/s (yellow line), 0.7 nm²/s (blue line), and 0.5 nm²/s (grey line). Thus, removal of the fastest-moving trajectory reduces the apparent diffusion coefficient >3-fold, suggesting that this particle is diffusing by a different mechanism and justifying its removal from the MSD calculation. However, removal of the second-fastest trajectory only reduces the apparent diffusion coefficient by a factor of <0.3, so we conservatively include it in MSD calculations. **c,** Square displacement for all remaining trajectories after the fastest-moving outlier (red in **a**) has been removed. **d,** Square displacement for all remaining trajectories after the second-fastest-moving outlier (blue in **a** and **c**) is also removed.
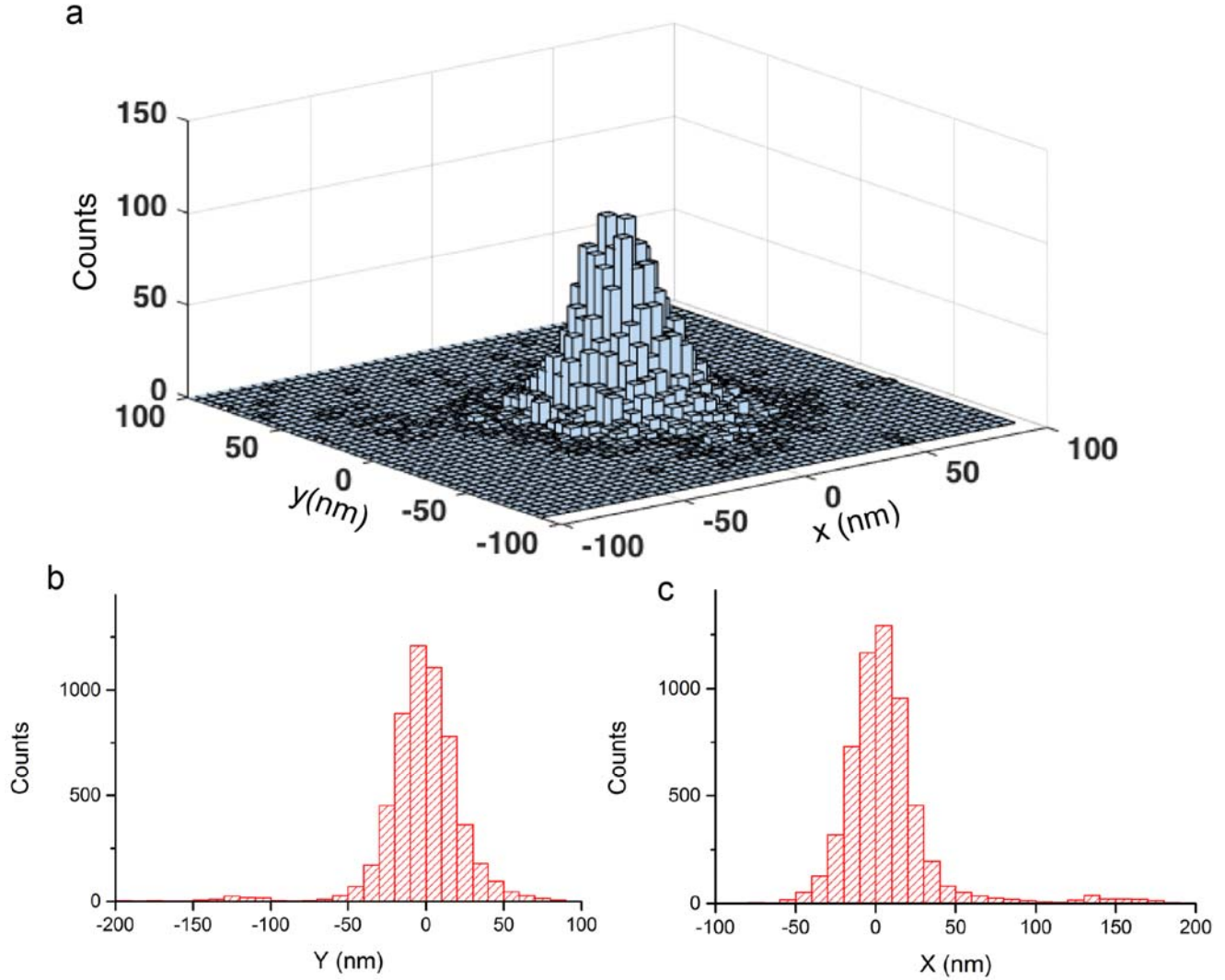
Supplementary Fig. 12| Position distribution of $W_{6\_13\_6}$ on a surface bearing only one foothold type (F_1) during 10 min of single-particle tracking. No walking is expected to occur on this control surface. **a,** 2D histogram showing the distribution of apparent x-y positions of all walkers (n=107) relative to their starting positions (0,0) over 10 min of observation. **b, c,** Histograms of walker coordinates in the x-(**b**) and y-(**c**)directions. The standard deviations of these coordinates (σ_x = 16.4 nm, σ_y = 15.2 nm) represent the approximate precision of localization in particle tracking experiments.

Supplementary Table 1 | Staple sequences for the *3-Foothold* DNA origami design

Name	Sequence (5′→3′)
Oligo0	AGGTTTAGTACCGCCATGAGTTTCGTCACCAGTTTTCCAATC
Oligo1	GTATAAACAGTTAATGTGCGAATAATAATTTTTTTTCCAATC
Oligo2	CAGGAGGTTGAGGCAGAGGGAGTTAAAGGCCGTTTTCCAATC
Oligo3	TTCATCGGCATTTTCGTACACTAAAACACTCATTTTCCAATC
Oligo4	TTATTCATTAAAGGTGATGAACGGTGTACAGATTTTCCAATC
Oligo5	TACGCAGTATGTTAGCTCATTGTGAATTACCTTTTTCCAATC
Oligo6	GATAACCCACAAGAATGAGGCATAGTAAGAGCTTTTCCAATC
Oligo7	GTTACAAAATAAACAGAGTTCAGAAAACGAGATTTTCCAATC
Oligo8	TAGCAAGCAAATCAGATACCTTTAATTGCTCCTTTTCCAATC
Oligo9	ATCAACAATAGATAAGCATTTCGCAAATGGTCTTTTCCAATC
Oligo10	TAAAGCCAACGCTCAATTATGACCCTGTAATATTTTCCAATC
Oligo11	CAAGACAAAGAACGCGAATGCCGGAGAGGGTATTTTCCAATC
Oligo12	CTGTAAATCGTCGCTATAAACGTTAATATTTTTTTTCCAATC
Oligo13	ATTGCTTTGAATACCATGGGATAGGTCACGTTTTTTCCAATC
Oligo14	TTCATCAATATAATCCGTGCGGGCCTCTTCGCTTTTCCAATC
Oligo15	TCAATAGATAATACATTGGCTAGTACCCGTATTTTTCCAATC
Oligo16	CACCGCCTGCAACAGTCCGCTTTCCAGTCGGGTTTTCCAATC
Oligo17	AGGGACATTCTGGCCACAGCAGGCGAAAATCCTTTTCCAATC
Oligo18	TACAAACTACAACGCCTATCACCGTACTCAGGTTATCCATTC
Oligo19	TTCACGTTGAAAATCTTTGAGTAACAGTGCCCTTATCCATTC
Oligo20	CTTTTGCGGGATCGTCCCGCCGCCAGCATTGATTATCCATTC
Oligo21	TCTTTGACCCCCAGCGCAGACTGTAGCGCGTTTTATCCATTC
Oligo22	CCAGGCGCATAGGCTGTAAATATTGACGGAAATTATCCATTC
Oligo23	TATGCGATTTTAAGAAGATTAAGACTCCTTATTTATCCATTC
Oligo24	AACACTATCATAACCCGCGCTAATATCAGAGATTATCCATTC
Oligo25	ATGACCATAAATCAAAAGAGCCTAATTTGCCATTATCCATTC
Oligo26	TTTTGATAAGAGGTCATCATTACCGCGCCCAATTATCCATTC
Oligo27	AATAACCTGTTTAGCTCAGAACGCGCCTGTTTTTATCCATTC
Oligo28	CTTTTGCGGGAGAAGCCAAATTCTTACCAGTATTATCCATTC
Oligo29	GCTATTTTTGAGAGATGATGCAAATCCAATCGTTATCCATTC
Oligo30	GTTAAAATTCGCATTAGTGAATAACCTTGCTTTTATCCATTC
Oligo31	GGTGTAGATGGGCGCAATAACGGATTCGCCTGTTATCCATTC
Oligo32	TATTACGCCAGCTGGCTATCAGATGATGGCAATTATCCATTC
Oligo33	AAGGATCCCCGGGTACTAATAGATTAGAGCCGTTATCCATTC
Oligo34	AAACCTGTCGTGCCAGAGGCGGTCAGTATTAATTATCCATTC
Oligo35	TGTTTGATGGTGGTTCCACGACCAGTAATAAATTATCCATTC
Oligo36	CCCTCAGAACCGCCACAAGCCCAATAGGAACCTTTTCATACC
Oligo37	GGAACCTATTATTCTGAGTGAGAATAGAAAGGTTTTCATACC
Oligo38	TTGATATTCACAAACAATAACCGATATATTCGTTTTCATACC
Oligo39	TTAGCGTTTGCCATCTGCACCAACCTAAAACGTTTTCATACC
Oligo40	CGACTTGAGCCATTTGAACCGAACTGACCAACTTTTCATACC
Oligo41	ATACATAAAGGTGGCATGGGCTTGAGATGGTTTTTTCATACC
Oligo42	AATAAGAGCAAGAAACAATGCAGATACATAACTTTTCATACC
Oligo43	CAATCCAAATAAGAAAATTCATTGAATCCCCCTTTTCATACC
Oligo44	CGGTATTCTAAGAACGAAGCAAACTCCAACAGTTTTCATACC
Oligo45	ATAATATCCCATCCTAGAACGAGTAGATTTAGTTTTCATACC
Oligo46	GAGAATCGCCATATTTGCATAAAGCTAAATCGTTTTCATACC
Oligo47	ATATATTTTAGTTAATTATGATATTCAACCGTTTTTCATACC
Oligo48	TTAGAATCCTTGAAAAAGGAAGATTGTATAAGTTTTCATACC
Oligo49	CAGAGGCGAATTATTCTCCGTGGGAACAAACGTTTTCATACC
Oligo50	TACTTCTGAATAATGGCAGGCTGCGCAACTGTTTTTCATACC
Oligo51	TATTAGACTTTACAAACGAGGCAAGTCCGCTATTTTCATACC
Oligo52	AGCAGCAAATGAAAAATAACTCACATTAATTGTTTTCATACC
Oligo53	TTCTGACCTGAAAGCGGTTGCAGCAAGCGGTCTTTTCATACC
Oligo54	CATGTACCGTAACACCCCTCAGAACCGCCATTTTCATCAC
Oligo55	AACAACTAAAGGAATCCCCCTGCCTATTTCTTTTCATCAC
Oligo56	GTCGCTGAGGCTTGCGTCAGACGATTGGCCTTTTCATCAC
Oligo57	AAAGAGGCAAAAGAAGTCATAGCCCCCTTATTTTCATCAC

Oligo58	TTTGAAAGAGGACAGAATTATCACCGTCACTTTTCATCAC
Oligo59	TAATTTCAACTTTAAAAACGTAGAAAATACTTTTCATCAC
Oligo60	GCCAAAAGGAATTACTGAGTTAAGCCCAATTTTTCATCAC
Oligo61	TCAAATGCTTTAAACCCATATTATTTATCCTTTTCATCAC
Oligo62	GTCAGGATTAGAGAGTATAGAAGGCTTATCTTTTCATCAC
Oligo63	TTTGACCATTAGATATCCTGAACAAGAAAATTTTCATCAC
Oligo64	GTTGTACCAAAAACACAGTAGGGCTTAATTTTTTCATCAC
Oligo65	TCTAGCTGATAAATTAGAAAACTTTTTCAATTTTCATCAC
Oligo66	CAAATATTTAAATTGTTAATTAATTTTCCCTTTTCATCAC
Oligo67	GCGGATTGACCGTAAAGTTACAAAATCGCGTTTTCATCAC
Oligo68	TGGGAAGGGCGATCGTGATTGTTTGGATTATTTTCATCAC
Oligo69	GCGACCGTATACGCATTGAGGATTTAGAAGTTTTCATCAC
Oligo70	CGTTGCGCTCACTGCGCCACGCTGAGAGCCTTTTCATCAC
Oligo71	CACGCTGGTTTGCCCACAGAGATAGAACCCTTTTCATCAC
Oligo72	ATAAGTGCCGTCGAGAGCGTAACGATCTAAAGTTTTCTACAC
Oligo73	GATGATACAGGAGTGTTTGTATCGGTTTATCATTTTCTACAC
Oligo74	CCTCAGAGCCACCACCAGGGTAGCAACGGCTATTTTCTACAC
Oligo75	AGCAGCACCGTAATCAAGATTTGTATCATCGCTTTTCTACAC
Oligo76	GCGCCAAAGACAAAAGAACCGGATATTCATTATTTTCTACAC
Oligo77	AAACCGAGGAAACGCAAGAAAAATCTACGTTATTTTCTACAC
Oligo78	ACGGGAGAATTAACTGAGCGAGAGGCTTTTGCTTTTCTACAC
Oligo79	GCTACAATTTTATCCTGAAGCAAAGCGGATTGTTTTCTACAC
Oligo80	CGCACTCATCGAGAACAATATAATGCTGTAGCTTTTCTACAC
Oligo81	AGTAATTCTGTCCAGATGGCATCAATTCTACTTTTTCTACAC
Oligo82	GAATCATAATTACTAGGAACCCTCATATATTTTTTTCTACAC
Oligo83	TTTTAACCTCCGGCTTTCTGGAGCAAACAAGATTTTCTACAC
Oligo84	TGAATTACCTTTTTTAAATAGGAACGCCATCATTTTCTACAC
Oligo85	CGTCAGATGAATATACCGACGACAGTATCGGCTTTTCTACAC
Oligo86	AACAAAGAAACCACCATGGGTAACGCCAGGGTTTTTCTACAC
Oligo87	AGGAATTGAGGAAGGTGTTTCCTGTGTGAAATTTTTCTACAC
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Oligo90	TTTTGTCGTCTTTCCCTCAGTACCAGGCGGTTATCTTCCA
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Oligo92	CAGAGGCTTTGAGGACCTCAGAACCGCCACTTATCTTCCA
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Oligo94	CCCAAATCAACGTAATCATATGGTTTACCATTATCTTCCA
Oligo95	ATAAAACGAACTAACAAAGTTACCAGAAGGTTATCTTCCA
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Oligo98	TCAACATGTTTTAAATATTAAACCAAGTACTTATCTTCCA
Oligo99	AATAGTAGTAGCATTACCGACAAAAGGTAATTATCTTCCA
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Oligo102	AAAATAATTCGCGTCTTTAACAATTTCATTTTATCTTCCA
Oligo103	CTCAGGAAGATCGCAGATTTTCAGGTTTAATTATCTTCCA
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Oligo105	TGTTATCCGCTCACAAAATCAACAGTTGAATTATCTTCCA
Oligo106	TGGGCGCCAGGGTGGGCCCTAAAACATCGCTTATCTTCCA
Oligo107	GGTTGAGTGTTGTTCACCTACATTTTGACGTTATCTTCCA
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Oligo110	CCCTCAGAGCCGCCACCTAAAGACTTTTTCATTTTTACCCAT
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Oligo145	AGCCAGAATGGAAAGCGCCGACAATGACAA
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Oligo149	CTATCTTACCGAAGCCAGTTGAGATTTAGG
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Oligo239	AATCACCAAAAAACATTATGACCCAGCTAAAT
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Oligo244	CCAGGGTGAAAGTGTAAAGCCTGGTCGTAATCCTGCTTCCCTACGCT
Oligo245	TTTGCGTATTGGGCGGTTGCAGCAAGCGGTGTTGAGTGTTGTTCC
Oligo246	GCGCGGGGAGCTAACTCACATTAAGTATAAGGCAAATTCAGATGACTC
Oligo247	TAATGAATCGGCCAACCAGCAGGCGAAAATCCCCTTATAAATCAAAAG
Oligo248	TCGTGCCGCCCGCTTTCCAGTCGCTAGCGAGTGCAGAAAGGCTGTC
Oligo249	CGGTTGTAGAAACCTGCAAATGGTCAATAACCGAAGGCACATACATTT
Oligo250	GAGCATAATGTAATACTTTTGCGGGGTGAGAAGCCGCCCAACTGAGG
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Oligo252	AGAATTAGCAACGCAAGGATAAAACCTGAGTAGGTGCATAAACGCAAC
Oligo253	CATACAGGCAAGGCAAAATTCTACTAATAGTAAGGACTAAAGACTTTT
Oligo254	CTGATTGCCCTTCACCCCACTATTAAAGAACG
Oligo255	TAACGCCATATCATAACCCTCGTTAAAACGAGAGGTCTGGACGCTACA

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Oligo278	GAACGAGGACCTAAAACGAAAGAGGCCACTACTGTTTAGCTATATTTT
Oligo279	AATCCGCAAACACTCATCTTTGAAGTTTCCCTGAAAAGGTGGCATC
Oligo312	ATGGGATACGTGCATCTGCCAGTTAGCTGGCG
Oligo313	CTCCGTGGTATCGGCCTCAGGACGATCGGT
Oligo314_T2	TTAAATGTAGCTTTCCGGCACCGCCGCCATTCttttttCCCTCATTCAATACCCCTACG
Oligo315	CTGGCCTTGGTTGATAATCAGAAAGTCAATCA
Oligo316	CAATAGGAGATTGTATAAGCAAAATCGATG
Oligo317	AAAGGGGGCAGGGTTTTCCCAGTCAGCTGTTT
Oligo318_T1'	CAATACCCCTACGGTCACTTCttttttGCGGGCCACGGCCAGTGCCAAGGTACCGAG
Oligo319	AGGCTGCGTCGACTCTAGACCTTTCGCATGGC
Oligo320_T1_Cy3	/5Cy3/CAATACCCCTACGGTCACTTCttttttTATGTACCAATATGATATTCAACCGACAGTCA
Oligo321	AACGGTATAATGCCGGAGAGGGGTAAAGAT
Oligo324	GGAAGCAGTTTTTAACCCAGGCTTGTTTGTCGTATGTGGAACGGCCT
Oligo325	CATCCGCCGAGTCATCTGAATTTGCGTGCTACAGAATTGAAGCGTAG
Oligo326	TGGGCGGCTCGATTCCATAATCAAGACAGCCTTTCTGCACCACTAAT
Oligo327	TACTCAAAGTTGCGTTTATGCACCGGTTCATTGACGTAGCCCTCAGT
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Oligo333	CAAGCGGGTGAAAATGAGCGTCCCCTAGTCCACAACTCTTGTAGAAG
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Oligo361	CCTTTAATCCGAAAGACTTCAAATGTCATAAA
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Oligo363	CCATGTTAAGGGAACCGAACTGACTACCCAAA
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Oligo366	AATAGCCCACAGTTGATTCCCAATAGAGAGTAttttt/3bio/
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Oligo368	TACGTAATGCAAAAGAATACACTAGACCTGCTttttt/3bio/
Oligo369	TCATGAGGACCCCCAGCGATTATATCATCGCCttttt/3bio/

Supplementary References

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