nature portfolio

https://doi.org/10.1038/s41565-022-01080-w

Supplementary information

Chemical-to-mechanical molecular computation using DNA-based motors with onboard logic

In the format provided by the authors and unedited

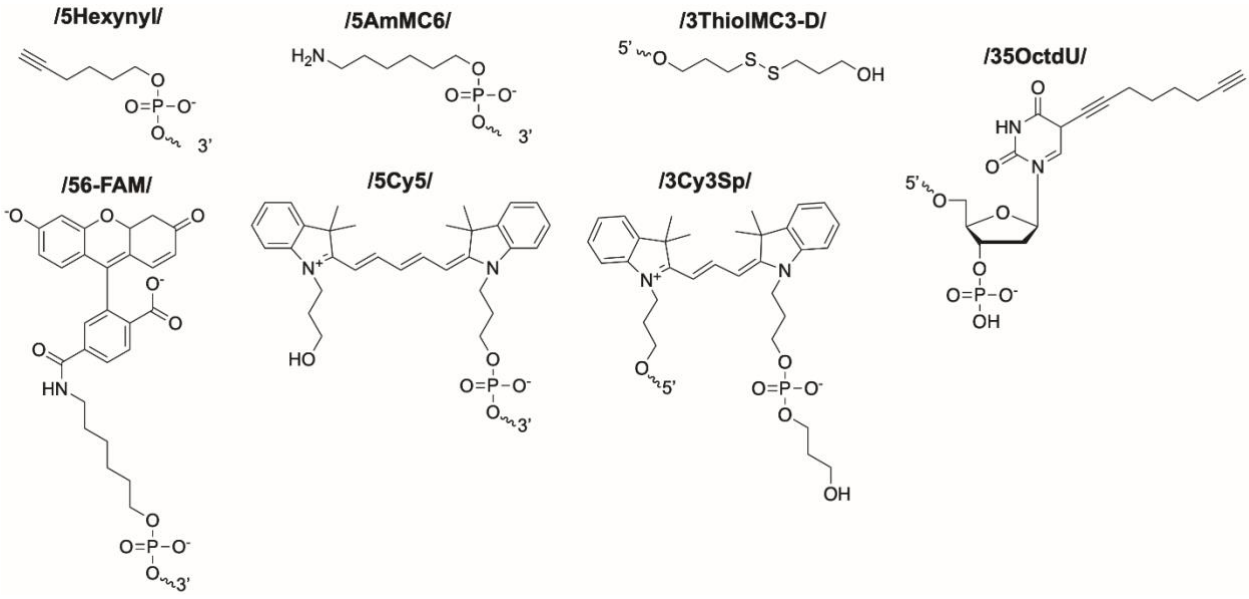
Chemical-to-mechanical molecular computation using DNA-based motors with onboard logic

Selma Piranej[1], Alisina Bazrafshan[1], Khalid Salaita[1,2*]

[1]Department of Chemistry, Emory University, Atlanta, GA 30322 (USA). [2]Wallace H. Coulter Department of Biomedical Engineering, George Institute of Technology and Emory University, Atlanta, GA 30322 (USA).
*email: ksalaita@emory.edu

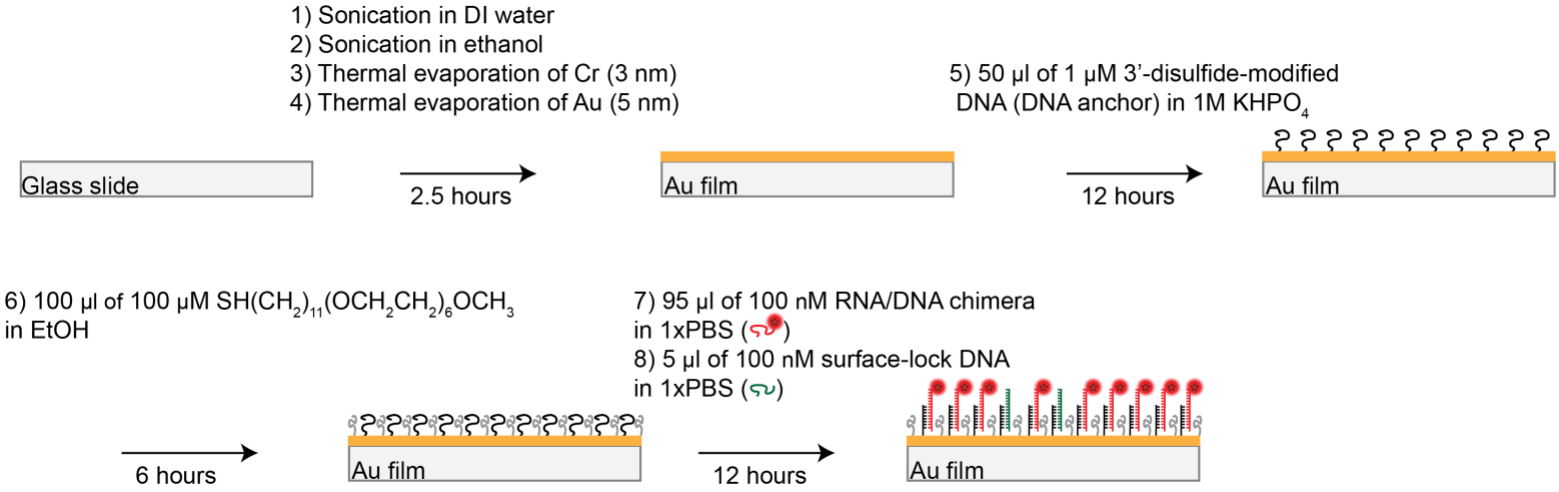
Supplementary Table 1: Oligonucleotide sequence design

ID	Sequences (5'-3')
DNA Anchor	/5AmMC6/GAGAGAGATGGGTGCTTTTTTTTTTTTTTT/3ThiolMC3-D/
RNA/DNA Chimera (F)	GCACCCATCTCTCTCCCCCCrCrUrGrUrGrArUrUrGrArUrUrArCrU /3Cy3Sp/
Guide DNA (G)	/5Hexynyl/TTTTTTTTTTTTTTTAGTAATCAATCACAG
Guide DNA complement (G*)	/56-FAM/CTGTGATTGATTACT
Surface-lock DNA (D*)	GCACCCATCTCTCTCACGTTACTGATT
Particle-lock DNA (C*)	CTCATAGCATACTCCCTTTTTTTTTTTTTT T/35OctdU/
Staple-lock DNA (CED)	/5Cy5/GGGAGTATGCTATGAGGCCATAACGCAATCAGTAACGT
Input A (C*E*)	CCGTTATCGCCTCATAGCATACTCCC
Input C (D*E*)	ACGTTACTGATTGCGTTATGGC
Particle-lock DNA (M*)	CACTTTGCATGTTCCATTTTTTTTTTTTTTT/35OctdU/
Staple-lock DNA (MND)	/56-FAM/TGGAACATGCAAAGTGACAGGCTAGAAATCAGTAACGT
Input B (M*N*)	TCTAGCCTGTCACTTTGCATGTTCCC
Particle-lock DNA (P*)	/5Cy5/GTATAAGTACCAGAGTCGCTACCTTGAATCAGTAACGT
Staple-lock DNA (PQD)	ACTCTGGTACTTATACTTTTTTTTTTTTTTT/35OctdU/
Input F (Q*D*)	CAAGGTAGCGACTCTGGTACTTATAC
Staple-lock DNA (CEDI)	/56FAM/CGGAGTATGCTATGAGGCCATAACGCAATCAGTAACGTCTCAGG CTAG
Anti-lock DNA (C*E*D*I*)	CTAGCCTGAGACGTTACTGATTGCGTTATGGCCTCATAGCATACTCCG
Staple-lock DNA (CEHD*N*M*X)	/56FAM/CGGAGTATGCTATGAGGCCATAACGCAGCAGTTATTGCACGTTA CTGATTTCTAGCCTGTCACTTTGCATGTTCCAGAATGCG
Input D (C*E*H*)	GCAATAACTGCT GCG TTA TGG CCT CAT AGC ATA CTC CG
Surface-lock DNA (H*)	GCACCCATCTCTCTCGCAATAACTGCT
Staple-lock DNA (X*MNDY)	/56FAM/CGCATTCTGGAACATGCAAAGTGACAGGCTAGAAATCAGTAACG TATTACTCGAT
Input E (D*N*M*)	ACGTTACTGATTTCTAGCCTGTCACTTTGCATGTTCCAGAATGCG



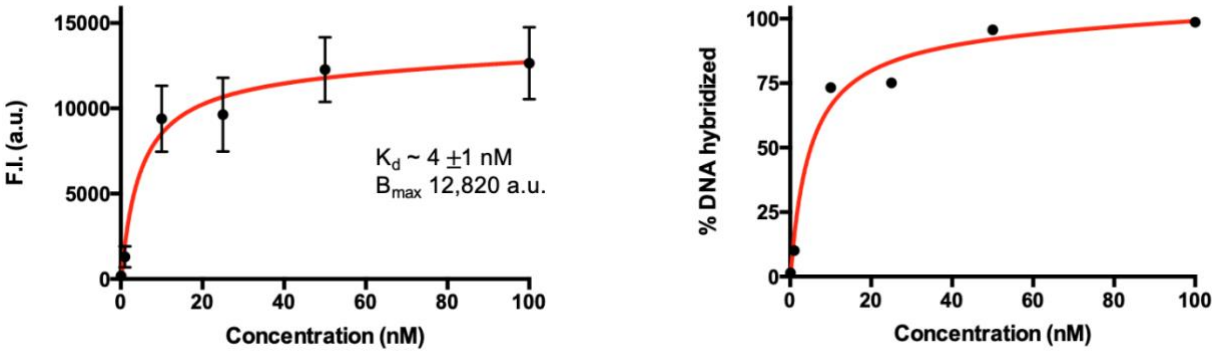
Supplementary Table 1. Table summarizing the sequences of oligonucleotides used in the design of DMOLs displayed in a 5' to 3' orientation. The sequences are color coded corresponding to each DMOL: in green are the sequences used for DMOL 1 and 3; purple DMOL 2; red DMOL 4; orange DMOL 5; blue DMOL 6. The 3' and 5' DNA and RNA modifications are indicated in the table and illustrated below it.

Supplementary Figure 1: Fabrication of chip surface



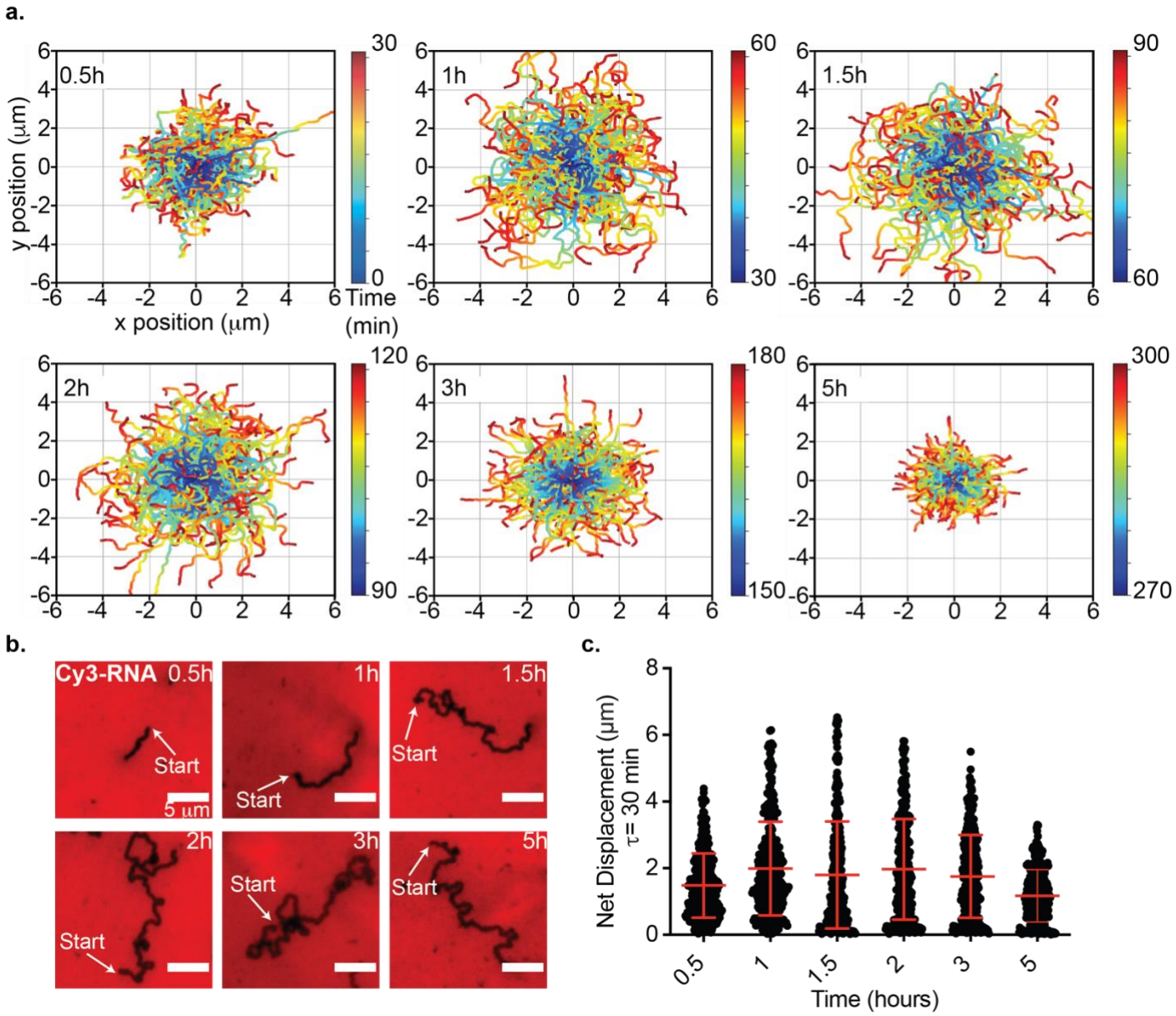
Supplementary Figure 1. Schematic of surface preparation steps used for preparing RNA monolayer surfaces along with the surface lock. Following thermal evaporation of 3 nm of Chromium (Cr) and 5 nm of gold (Au), the surface was treated with 1 µM of disulfide modified DNA anchor strand. After 12 hours of incubation, the surface was passivated with 100 µM thiolated polyethyleneglycol (PEG) for 6 hours. Then 95 nM of RNA/DNA chimera (Cy3-tagged) and 5 nM of surface-lock DNA were added to hybridize with the DNA anchor on the gold film to make a 5% surface-lock chip.

Supplementary Figure 2: Quantification of DMOL DNA hybridization efficiency



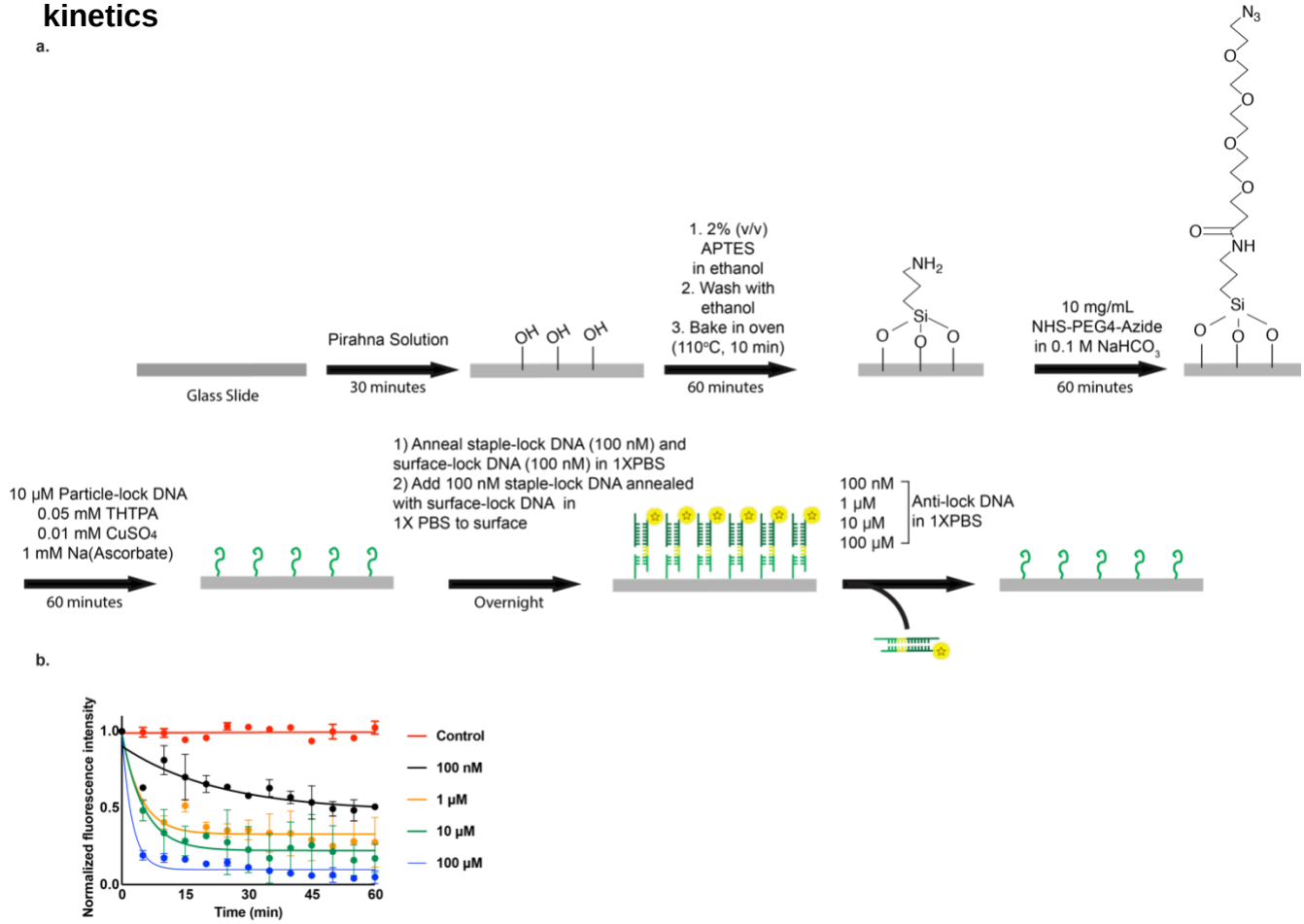
Supplementary Figure 2. The plot on the left shows DMOL fluorescence intensity as a function of concentration of complementary FAM-tagged oligonucleotide. DMOLs were prepared using 100% guide DNA (G) and then incubated overnight at room temperature with complementary G* oligonucleotide. We used 1xPBS for the hybridization. Afterwards, DMOLs were washed in 1xPBS and their fluorescence intensity was quantified using optical microscopy. The plot to the right is normalized such that the maximum intensity is set to 100% hybridization. The plot was fit to a standard binding isotherm using $\%hybridized = \frac{[L]}{[L]_0} + K_d$. Saturation is assumed at 100 nM which generated fluorescence intensity similar to the 50 nM incubation concentration. The fit produced a Kd ~ 4 ± 1 nM. Error bars represent the standard deviation from three independent measurements using *n* > 50 particles. This calibration plot was used to generate DMOLs with tunable surface density of oligonucleotides using hybridization.

Supplementary Figure 3: Analysis of DMOL motion over multi-hour time period



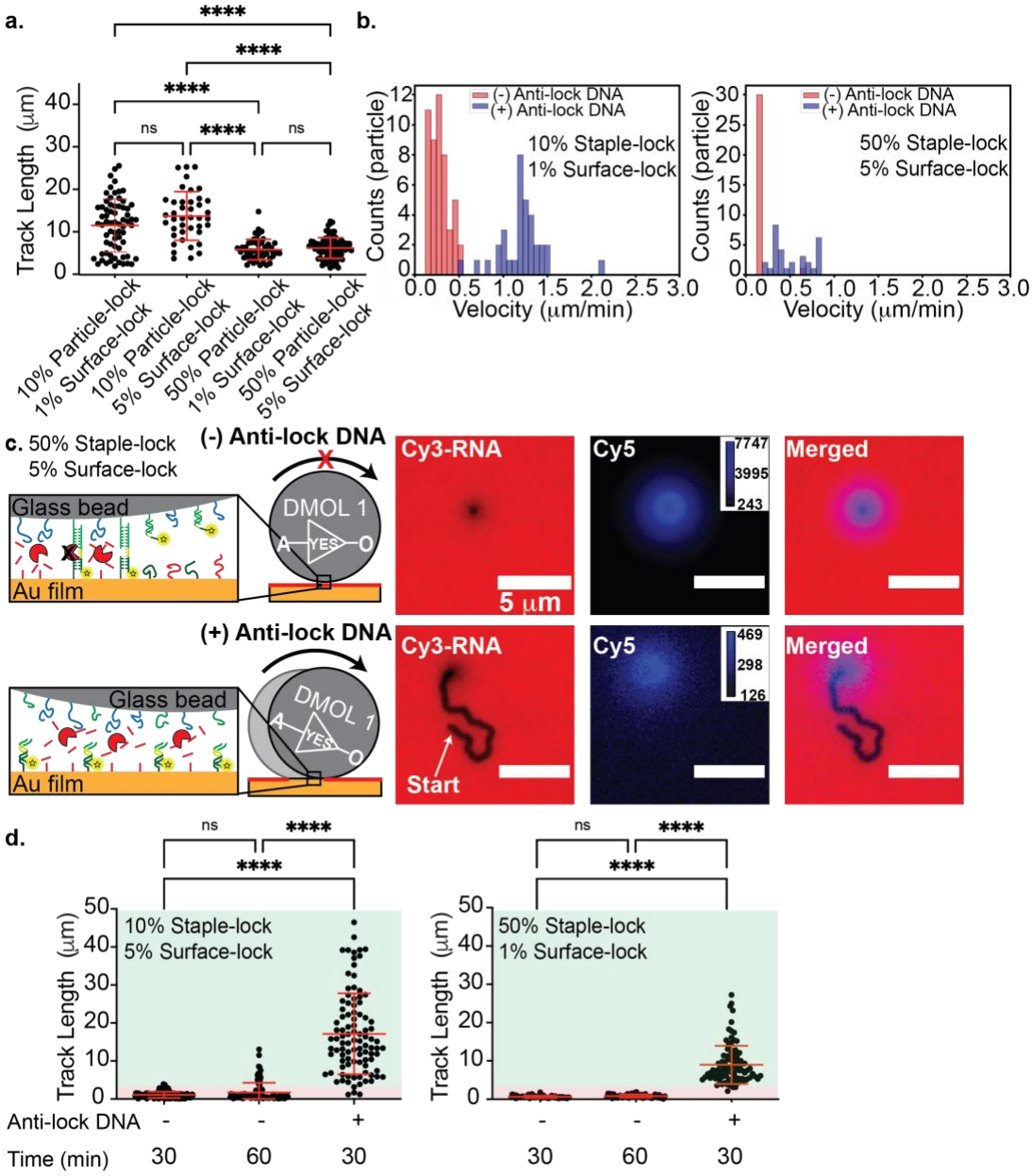
Supplementary Figure 3. a, Ensemble of DMOL trajectories plotted from the center of coordinates (0,0) taken at 30 min time intervals 0.5h, 1h, 1.5h, 2h, 3h, and 5h following RNase H addition. The DMOLs were functionalized with 50% particle lock (no staple-lock DNA) and the surface with 5% surface-lock DNA (95% RNA). Color indicates time from 0 to 30 mins. **b,** Representative Cy3 fluorescence images of depletion tracks from the same surface at t=0.5h, 1h, 1.5h, 2h, 3h, and 5h after RNase H addition. The tracks of individual DMOLs grow longer throughout the 5-hour period. **c,** Plots of net displacements (each taken at τ= 30 mins) following 0.5h, 1h, 1.5h, 2h, 3h, and 5h of RNase H addition. Even after 5h of RNase H addition, the DMOLs continue moving on the chip surface.

Supplementary Figure 4: Toehold mediated strand displacement reaction surface kinetics



Supplementary Figure 4. a, To perform these measurement, a glass slide was first treated with piranha solution then 2% (v/v) ATPES in ethanol. The glass slide was then baked in an oven before adding NHS-PEG4-Azide. Then a monolayer of alkyne particle-lock DNA (C*) was copper clicked to the surface. The Cy5-tagged CED staple-lock (100 nM) was annealed with the surface-lock DNA (100 nM) in 1x PBS. The annealed staple-lock and surface-lock DNA (D*) were then added to the surface through hybridization overnight. Different concentrations of anti-lock DNA strands (C*E*) were titrated with the substrate thus displacing the Cy5-tagged staple-lock (and hybridized surface-lock) from the surface. **b,** Kinetic plots showing decrease in fluorescence intensity which is indicative of anti-lock DNA binding to the toehold and displacing the substrate (Cy5-tagged) from the surface. Control was 1x PBS, no anti-lock DNA strand. Error bars represent the standard deviation in the average fluorescence intensity from at least 10 different regions across the surface. Assuming pseudo first-order binding kinetics, the data was fitted using an exponential curve fitting ($A(t) = A_0 \exp^{-kt} + c$) and a k_{obs} of 7×10^1 $M^{-1} \cdot s^{-1}$ was obtained with a calculated half-life of 100 s. Based on these results, we used 1 µM concentrations for all TMSD reactions.

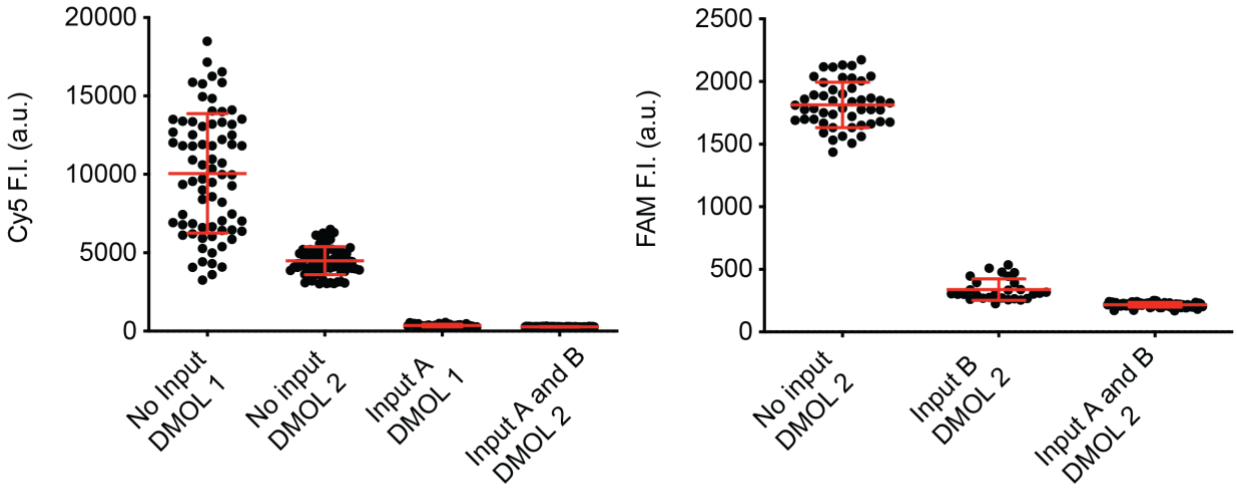
Supplementary Figure 5: Optimization of DMOL and surface design



Supplementary Figure 5. a, Plot of track lengths for DMOLs modified with 10% particle-lock DNA (no staple-lock), 1% surface-lock DNA; 10% particle-lock DNA, 5% surface-lock DNA; 50% particle-lock DNA, 1% surface-lock DNA; and 50% particle-lock DNA, 5% surface-lock DNA. Error bars correspond to standard deviation of *n*> 50 DMOLs from three independent experiments, **** indicates that p<0.0001. Note the track lengths for particles with 50% particle-lock are shorter because of the lower density of guide DNA (G) on these DMOLs. **b,** Average velocity of DMOLs modified with 10% staple-lock DNA, 1% surface-lock DNA (left) and 50% staple-lock DNA, 5% surface lock DNA (right) before and after the addition of anti-lock. The average velocity was calculated using particle tracking from BF acquisitions. As expected, the DMOLs with 10% staple-lock showed

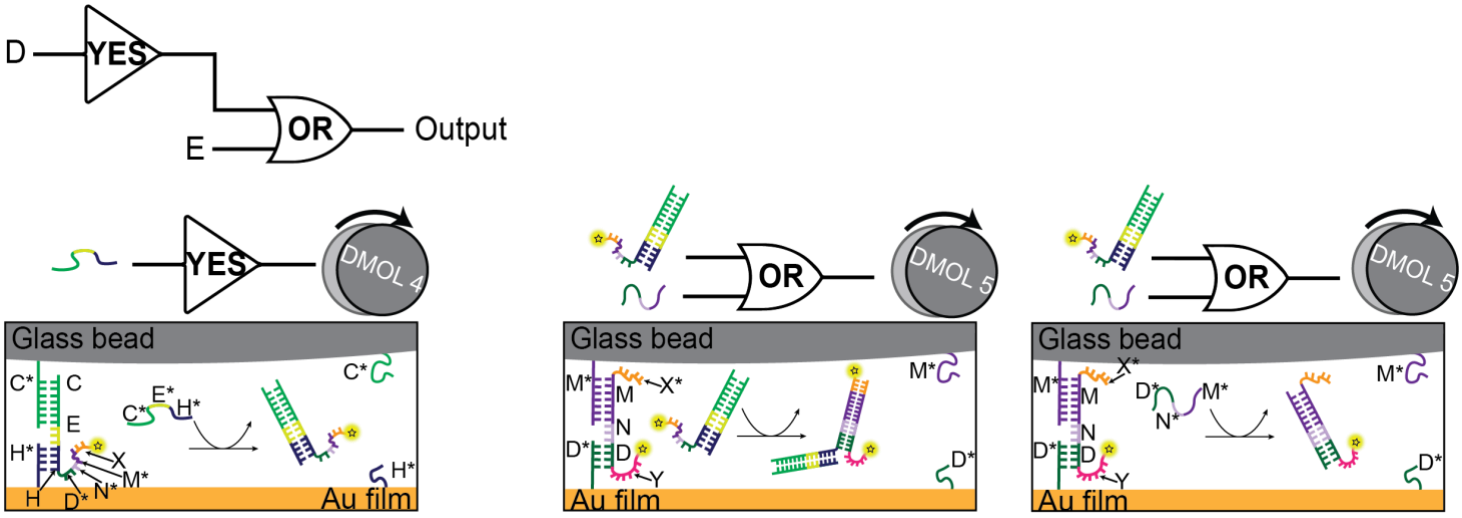
greater velocities compared to the 50% staple-lock DMOLs. **c,** Schematic of anti-lock triggering DMOL locomotion. The DMOL is modified with 50% staple-lock and the surface was modified with 5% surface-lock. Representative fluorescence images of DMOLs without (**top**) and with (**bottom**) anti-lock (1 μM) at t = 30 min after RNase H addition are shown. Note that the Cy3 depletion track and loss of Cy5 signal is observed after addition of the anti-lock. **d,** The plot on the left shows the measured track lengths for DMOLs modified with 10% staple-lock DNA and added to the chip with 5% surface-lock DNA. Track lengths are quantified 30 minutes and 60 minutes before the addition of anti-lock (-) as well as 30 minutes after addition (+). Similarly, the plot on the right shows the measured track lengths for DMOLs modified with 50% staple-lock DNA and added to the chip with 1% surface-lock DNA. Green region represents DMOLs with output=1 and red with output=0. Error bars show the standard deviation of n>80 DMOLs from three independent experiments, **** indicates that p<0.0001.

Supplementary Figure 6: Quantification of fluorophore-encoded DMOLs



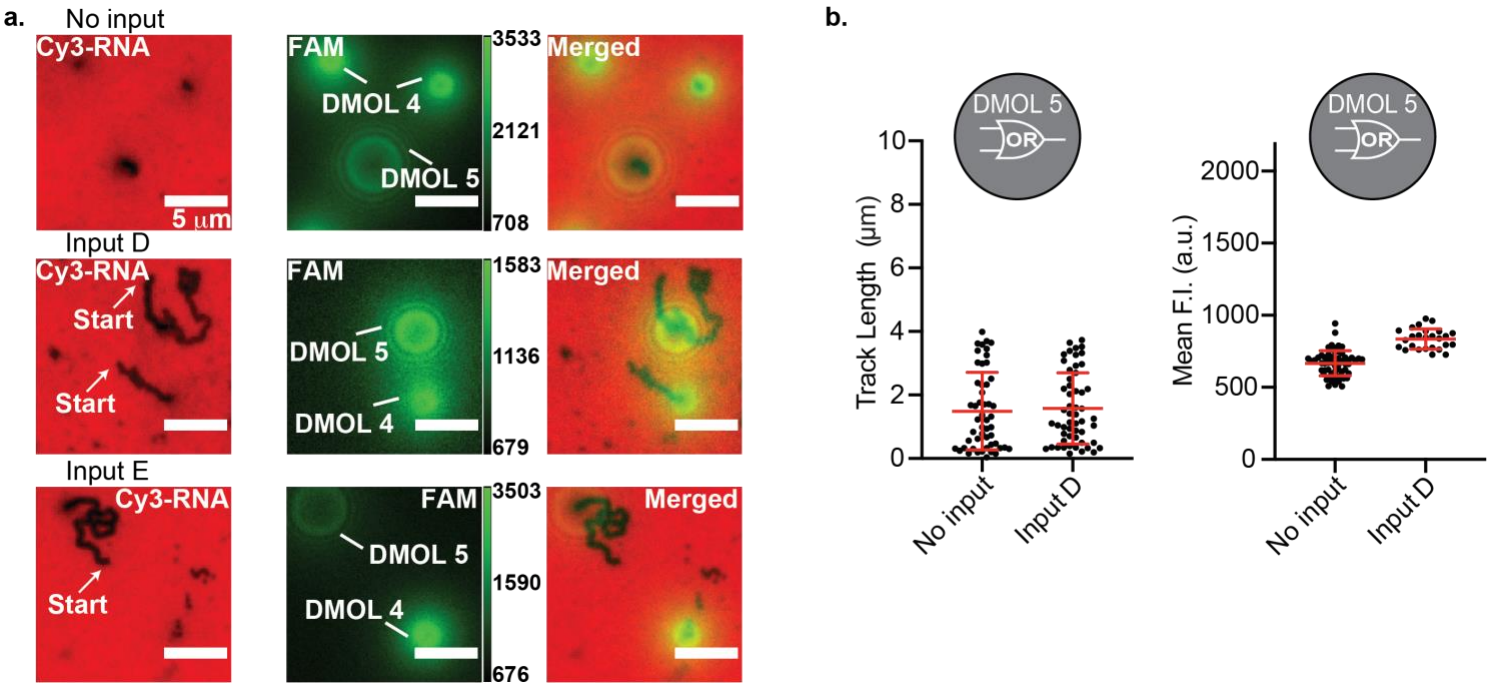
Supplementary Figure 6. The plot on the left shows the Cy5 fluorescence intensity of DMOL 1 and 2 after 30 minutes of no input, input A, and input A+B. The fluorescence intensity was obtained using a 100x oil objective in a fluorescence microscope (see methods). A higher fluorescence intensity is observed for DMOL 1 than DMOL 2 because DMOL 1 has a higher density of staple-lock CED (50%) than DMOL 2 (25%). A decrease in Cy5 fluorescence intensity is observed in both DMOLs 1 and 2 when input A was added as the locks functionalized with Cy5 are displaced from the particle. The plot on the right shows the FAM fluorescence intensity of DMOL 2 after 30 min of no input, input B, and input A+B. DMOL 1 was not included in this plot as it only has Cy5-tagged staple-lock. A decrease in FAM signal is observed when both inputs B and inputs A+B (simultaneously) are added as the MND lock is displaced from the particle through TMSD. Error bars show the standard deviation from at least n = 60 particles from three independent experiments.

Supplementary Figure 7: Reaction principle for cascading YES-OR logic gates



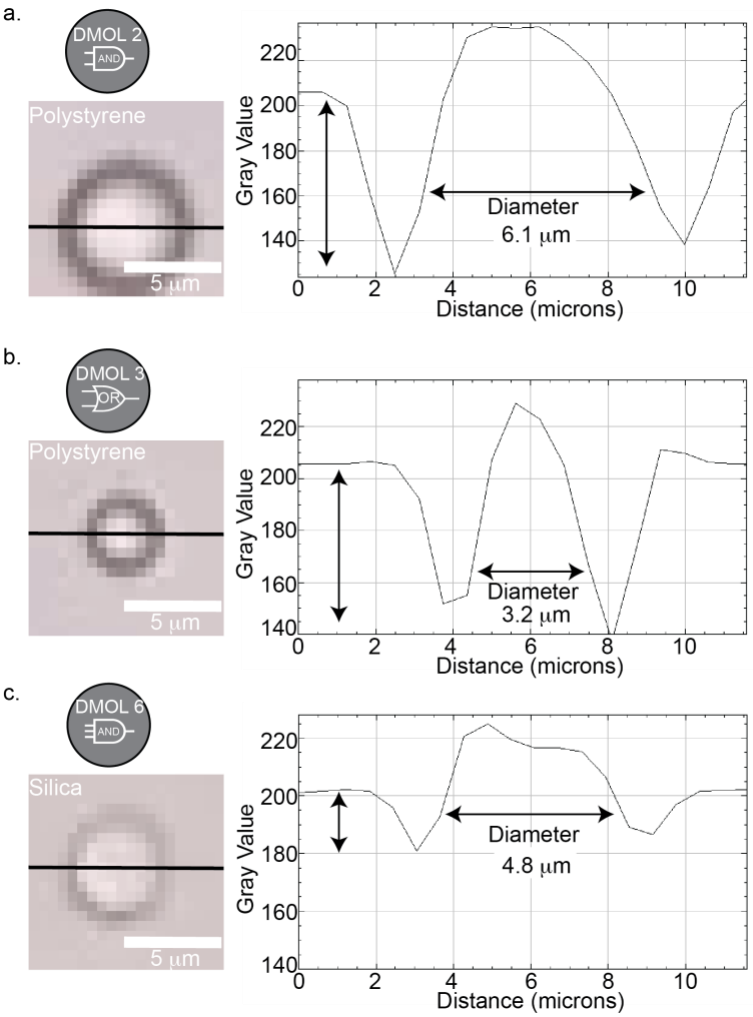
Supplementary Figure 7. Scheme of basic reaction principle of cascading YES and OR logic gates.

Supplementary Figure 8: Cascading YES-OR logic gates



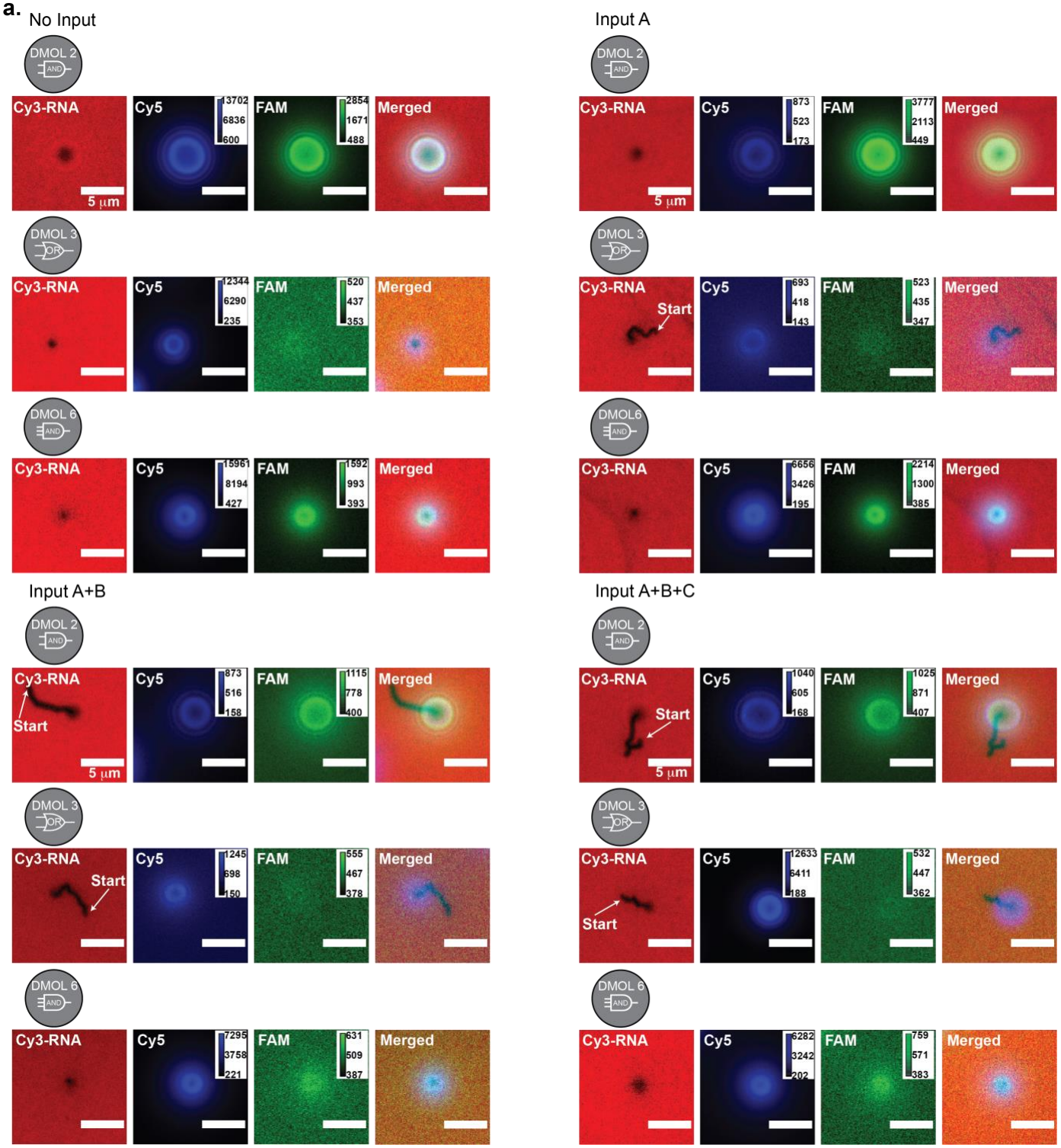
Supplementary Figure 8. a, Representative Cy3 and FAM fluorescence images along with the overlay of the two channels (at *t* = 30 minutes after RNase H addition). DMOL 4 is a 5 μm silica bead YES gate. DMOL 5 is 6 μm polystyrene OR gate. The fluorescence images show Cy3-tagged RNA and FAM fluorescently labelled particles treated with 3 conditions (inputs): no input, D only, and E only. **b,** Plots of track lengths (t= 30 minutes) for DMOL 5 (OR gate) responding to input D only. The plot on the right shows the FAM fluorescence intensity of DMOL 5 in the presence of input D. No decrease in track length or FAM fluorescence intensity is observed when input A is added compared to no input. The plotted fluorescence intensity values of the FAM labeled DMOL 5 were subtracted from the autofluorescence of the polystyrene beads in the FAM channel.

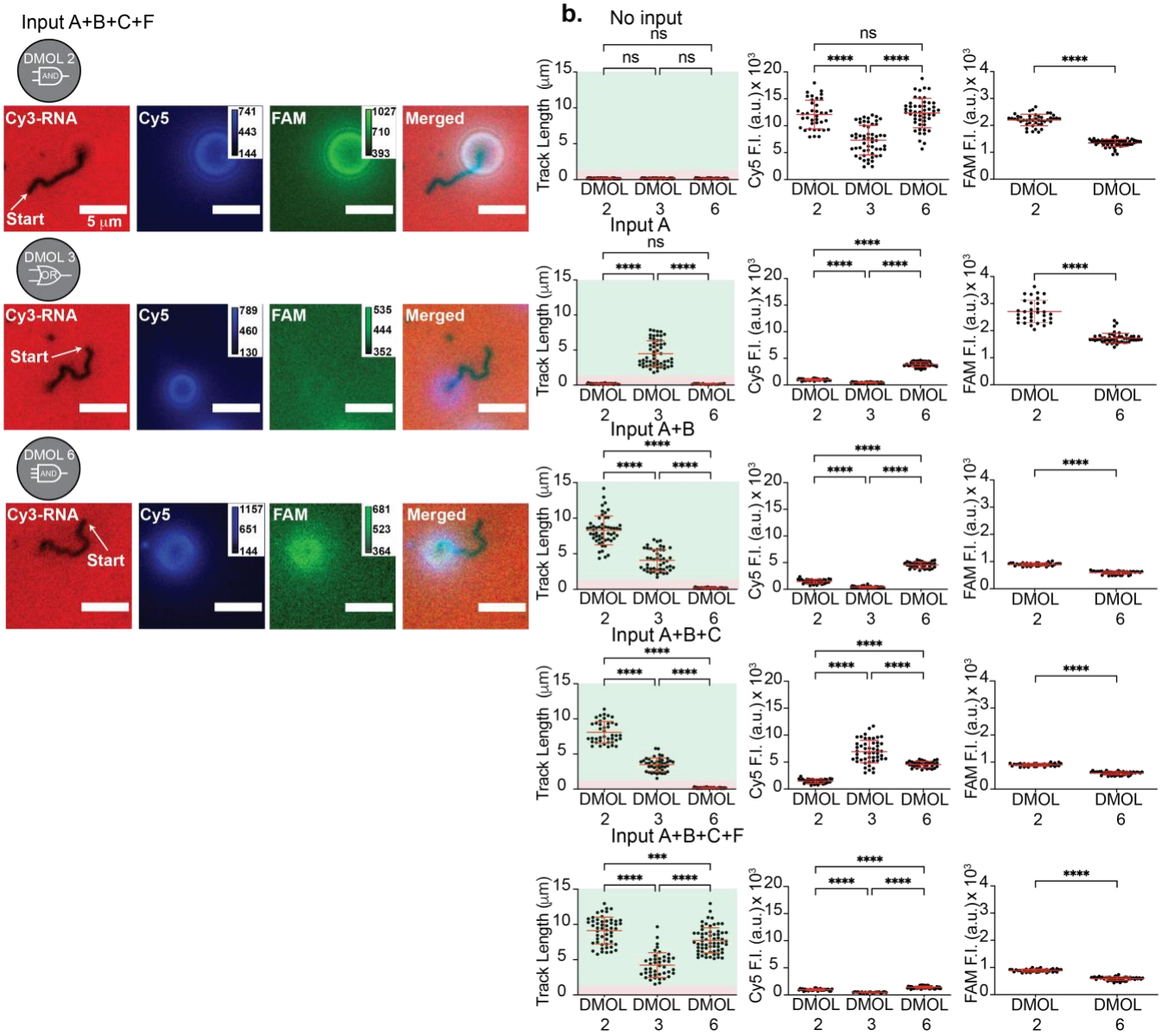
Supplementary Figure 9: Characterization of size and material encoded DMOLs



Supplementary Figure 9. a, Representative cellscope images of DMOL 2 (6 μm polystyrene); **b**, DMOL 3 (3 μm polystyrene); **c**, DMOL 6 (5 μm silica). The line scan shown in black was used to measure the diameter of each DMOL. The gray scale contrast (the difference between the background and the minimum at the bead perimeter was used to determine the material of the particles, as polystyrene generated greater contrast compared to silica. The polystyrene encoded DMOL 2 and 3 have greater contrast in gray values as denoted by their darker outer ring.

Supplementary Figure 10: Track length quantification of size and material encoded DMOLs





Supplementary Figure 10. a, Representative Cy3, Cy5, and FAM fluorescence images along with the overlay of the three channels (at *t* = 30 minutes after RNase H addition). DMOL 2 is a 6 μm polystyrene 2-input AND gate. DMOL 3 is 3 μm polystyrene OR gate. DMOL 6 is a 5 μm silica 3-input AND gate. The fluorescence images show Cy3-tagged RNA as well as the Cy5 and FAM fluorescently labelled particles treated with five (5) unique conditions (inputs): no input; A only; A+B; A+B+C; and A+B+C+F. **b,** Plots of track lengths (t= 30 minutes) for multiplexed DMOLs responding to 5 inputs. Green region represents DMOLs with output=1 and red with output=0. The plots on the right show the corresponding difference in Cy5 and FAM fluorescence intensity of the DMOLs. A decrease in Cy5 fluorescence intensity is observed when input A and input F are added as the locks functionalized with Cy5 are displaced from the particle. Loss of FAM fluorescence intensity is observed when input B is added, and input C did not lead to a decrease in Cy5 nor FAM fluorescence. Error bars show the standard deviation from n>40 DMOLs from three independent experiments. ns, ***, **** indicate not statistically significant, p= 0.0007, and p<0.0001.

Supplementary Movies

Supplementary Movie S1: Timelapse videos of Cy3 (red) and Cy5 (blue) fluorescence channels overlaid acquired at 5 s intervals for a duration of 15 mins. The video was acquired ~30 mins after RNase H addition using a 100x 1.49 NA objective. YES-gated DMOLs modified with 10% staple-lock CED are shown translocating on a 1% surface-lock D* chip after the addition of 1 μM anti-lock. Note that the Cy5 signal gradually bleaches over time. Scale bar is 10 μm.

Supplementary Movie S2: Timelapse videos of Cy3 (red), Cy5 (blue), and FAM (green) fluorescence channels overlaid acquired at 5 s intervals for a duration of 15 mins. The video was acquired ~30 mins after RNase H addition using a 100x 1.49 NA objective. AND-gated DMOLs modified with 50% staple-lock DNA (25% CED and 25% MND) are shown translocating on a 5% surface-lock D* chip after the addition of inputs A+B (1 μm each). Note that the Cy5 and FAM signals gradually bleach over time. Scale bar is 10 μm.

Supplementary Movie S3: Timelapse videos of Cy3 (red), Cy5 (blue), and FAM (green) fluorescence channels overlaid acquired at 5 s intervals for a duration of 15 mins. The video was acquired ~30 mins after RNase H addition using a 100x 1.49 NA objective. DMOL 1 (located at the top of the frame) modified with 50% staple-lock CED is shown translocating on a 5% surface-lock DNA chip after the addition of input A. DMOL 2 (located at the bottom of the frame) modified with 50% staple-lock (25% CED and 25% MND) is shown stalled on a 5% surface-lock D* chip after the addition of input A as it requires input A+B to unlock and translocate. Note that the Cy5 and FAM signals gradually bleach over time. Scale bar is 10 μm.

Supplementary Movie S4: Representative timelapse brightfield video acquired at 5 s intervals for a duration of 15 mins using cellscope. DMOLs 2 (6 μm polystyrene), 3 (3 μm polystyrene), and 6 (5 μm silica) are shown. DMOLs were added to a 5% surface-lock D* chip and introduced to input A which rescued motion of DMOL 3. Scale bar is 10 μm.

Supplementary Movie S5: Representative timelapse brightfield video acquired at 5 s intervals for a duration of 15 mins using cellscope. DMOLs 2 (6 μm polystyrene), 3 (3 μm polystyrene), and 6 (5 μm silica) are shown. DMOLs were added to a 5% surface-lock D* chip and introduced to input A+B which rescued motion of DMOLs 2 and 3. Scale bar is 10 μm.

Supplementary Movie S6: Representative timelapse brightfield video acquired at 5 s intervals for a duration of 15 mins using cellscope. DMOLs 2 (6 μm polystyrene), 3 (3 μm polystyrene), and 6 (5 μm silica) are shown. DMOLs were added to a 5% surface-lock D* chip and introduced to input A+B+C which rescued motion of DMOLs 2 and 3. Scale bar is 10 μm.

Supplementary Movie S7: Representative timelapse brightfield video acquired at 5 s intervals for a duration of 15 mins using cellscope. DMOLs 2 (6 μm polystyrene), 3 (3 μm polystyrene), and 6 (5 μm silica) are shown. DMOLs were added to a 5% surface-lock D* chip and introduced to input A+B+C+F which rescued motion of DMOLs 2, 3, and 6. Scale bar is 10 μm.