Supporting Information

Nanopore-Based DNA Hard Drives for Rewritable and Secure Data Storage

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Materials and Methods

Fabrication of DNA-HD

The DNA HD was made by incubating the linearized M13mp18 single-stranded DNA (ssDNA) scaffold and short 38 bp oligonucleotides with designed positions replaced by those including ssDNA overhangs and DNA dumbbells (purchased from Integrated DNA Technologies with sequences listed Tables S1, S2, S3, S6 and S7).

The 7228 nt scaffold was linearized from M13mp18 ssDNA (7249 bases, N4040S, New England Biolabs) using the following protocol: 1) A 39 nt oligonucleotide (5'-TCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAATC-3') was hybridized to the M13mp18 ssDNA by mixing 40 µL M13mp18 ssDNA (250ng/µL), 8 µL 10x Cutsmart buffer (New England Biolabs), 2 µL the 39 nt oligonucleotide (100µM) and 28 µL deionized water. 2) The mixture was heated to 65 °C and linearly cooled down to 25 °C in a thermocycler over 40 minutes. 3) 1 µL of BamHI-HF and 1 µL EcoRI-HF (each 100000 units/ml, New England Biolabs) were added to the reaction mixture followed by incubation at 37 °C for 1 hour. 4) The DNA sample was then immediately purified using a Machery-Nagel NucleoSpin Gel and PCR Clean-up kit. 5) The concentration was measured using the NanoDrop and the sample was diluted to a concentration of 100 nM. The DNA HD was prepared using the following protocol: 1) 8 µL linearized M13mp18 ssDNA (100 nM), 20 µL oligonucleotide mixture (each oligo 200 nM), 4 µL 100 mM $MgCl_2$, 1.2 µL 100 mM Tris-HCl (pH=8), 10 mM EDTA and 6.8 µL deionized water were mixed. 2) The mixture was loaded in a thermocycler and heated to 70°C followed by a linear cooling ramp to 25 °C for 50 minutes. 3) After annealing, these excess oligonucleotides were removed using Amicon Ultra 100 kDa filters. One tube annealed as above was added to 460 µL of 10mM Tris-HCl (pH=8), 0.5 mM $MgCl_2$ and centrifuged at 9000 g for 10 minutes at 4°C. 460 µL more 10mM Tris-HCl (pH=8), 0.5 mM $MgCl_2$ was added and the sample centrifuged again for 10 minutes. The sample was then recovered by turning the filter upside down and centrifuging for 1 minute at 1000 g. This typically yielded ~25 µL at a concentration of ~30-50 ng/uL. 4) Solutions were immediately added after filtering to make the final salt concentration 10 mM Tris-HCl, 100 mM NaCl and 2 mM $MgCl_2$.

Nanopore measurement

Glass nanopores with diameters 14±3 nm (see characterization in a former study[1]) were fabricated by pulling quartz capillaries (outer diameter 0.5 mm and inner diameter 0.2 mm, Sutter Instrument) using a laser-heated pipette puller (P-2000, Sutter Instrument). The fabricated nanopores were assembled into a PDMS chip. The DNA sample was diluted in 4 M LiCl, 1×TE (pH=9.0) with the concentration of 0.2-1 nM and the solution was added to outside of the nanopore. An Axon Axopatch 200B amplifier (Molecular Devices) was used to apply a voltage 600 mV to drive the DNA through nanopores and measure the ionic current signal. The signal was filtered with an external Bessel filter (Frequency Devices) at 50 kHz and digitized at a 250 kHz sampling rate with a data card (PCI-6251, National Instruments). Data was collected and analysed using home-made LabVIEW algorithms.

Experimental Workflow

The workflow of the characterisation of the rewritable capability

We used a DNA-HD sample with ssDNA overhangs to start. Oligonucleotides and streptavidin were added to perform the writing and erasing. The resulting molecules were measured with nanopores.

1) Blank.

The DNA concentration of the blank sample was measured as 24 ng/μL (5.04 nM).

2) Writing of '00101'

We mixed the following samples and kept at room temperature for 1 h before nanopore measurement. The ratio of the concentration of the samples is 1 (ssDNA overhang): 4 (biotinylated oligonucleotide):16 (streptavidin).

DNA-HD – blank (5.04 nM): 10 μL

'00101' writing oligonucleotides (containing Oligonucleotides B3 and B5 at 200 nM): 1.01 μL

Monovalent streptavidin (200 nM): 8.08 μL

We name the resulting sample as DNA-HD - '00101' which has a DNA concentration of 2.64 nM.

3) Erasing of '00101'

We mixed the following samples and kept at room temperature for 1 h before nanopore measurement. The ratio of the concentration of the samples is 1 (ssDNA overhang): 8 (erasing oligonucleotide).

DNA-HD - '00101' (2.64 nM): 10 μL

'00101' erasing oligonucleotides (containing Oligonucleotides E3 and E5 at 200 nM): 1.06 μL

We name the resulting sample as DNA-HD – erased which has a DNA concentration of 2.39 nM.

4) Rewriting of '10100'

We mixed the following samples and kept at room temperature for 1 h before nanopore measurement. The ratio of the concentration of the samples is 1 (ssDNA overhang): 13 (erasing oligonucleotide): 52 (streptavidin)

DNA-HD – erased (2.39 nM): 5 μL

'10100' writing oligonucleotides (containing Oligonucleotides B1 and B3 at 200 nM): 0.78 μL

Monovalent streptavidin (200 nM): 6.2 μL

We name the resulting sample as DNA-HD - '10100' which has a DNA concentration of 1.00 nM.

Demonstration of writing 'CAMBRIDGE' – Erasing – rewriting 'CAVENDISH'

The experimental methods are the same as shown above with the corresponding oligonucleotides added in each step and the sample measured with nanopores.

Decoding the data encrypted in DNA-HDs

We prepared the DNA-HD samples encoded with 'S', 'H', 'A', 'N', 'N', 'O' and 'N' with the addresses '0', '1', '2', '3', '4', '5' and '6' respectively. The concentrations are 6.72 nM, 6.51 nM, 5.04 nM, 6.93 nM, 5.88 nM, 7.35 nM and 6.09 respectively. Here we use the ratio of the concentration of the samples 1 (ssDNA overhang): 10 (biotinylated oligonucleotide): 40 (streptavidin) to have high binding efficiency. We mixed the following samples and kept at room temperature for 1 h before nanopore measurement.

1.09 µL, 1.13 µL, 1.46 µL, 1.06 µL, 1.25 µL, 1 µL and 1.21 µL of the seven samples.

2.58 µL 200 nM B5 (sequence shown below), 2.58 µL 200 nM B3 (sequence shown below)

1.5 µL 100 mM $MgCl_2$

4.13 µL 1 µM monovalent streptavidin.

Figure S1.

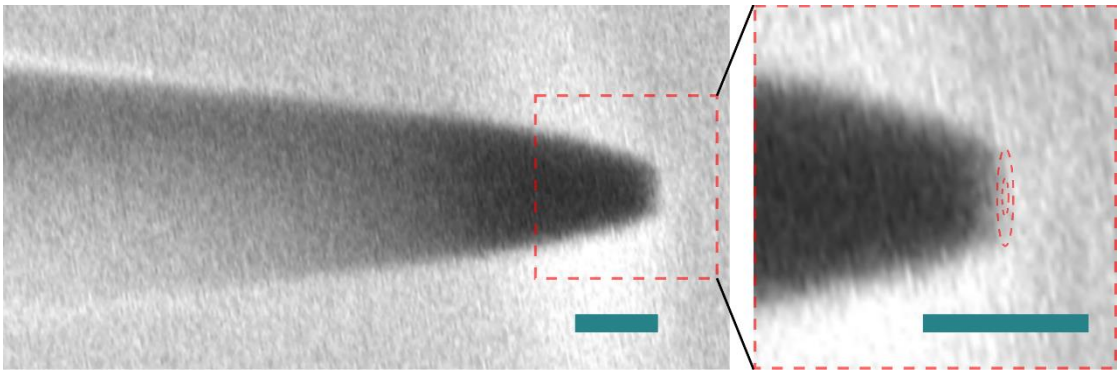

Figure S1. An example of the Scanning Electron Microscope (SEM) image of the nanopore. The image shows the outline of the glass capillary. The inner diameter estimated from the outer diameter. The scale bar is 50 nm. More details on the characterization are given in a previous study.[1]

Figure S2.

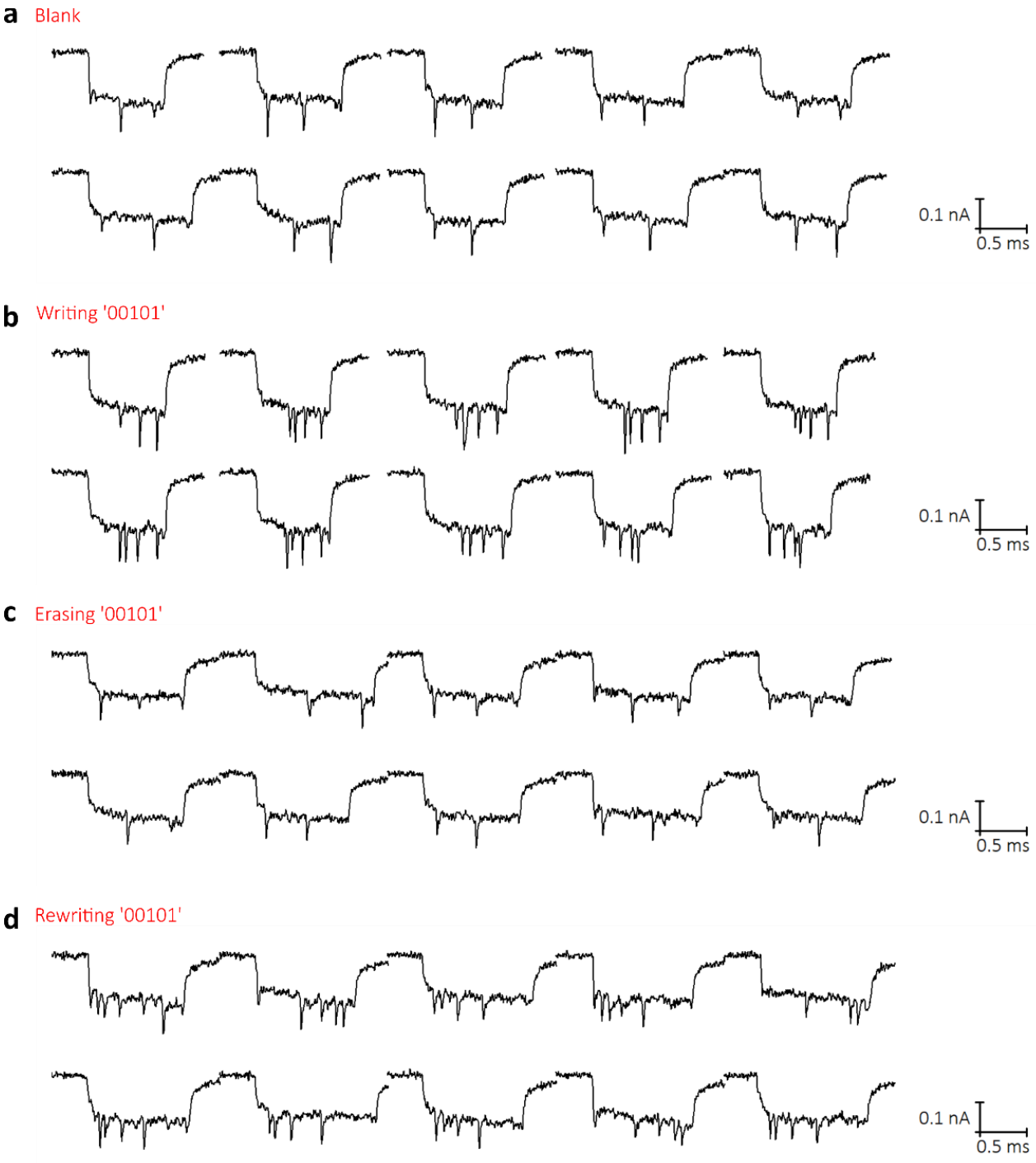

Figure S2. Example events from nanopore measurement during the writing '00101'- erasing – rewriting '10100' process. Events in the four stages are shown in (a)-(d).

Figure S3.

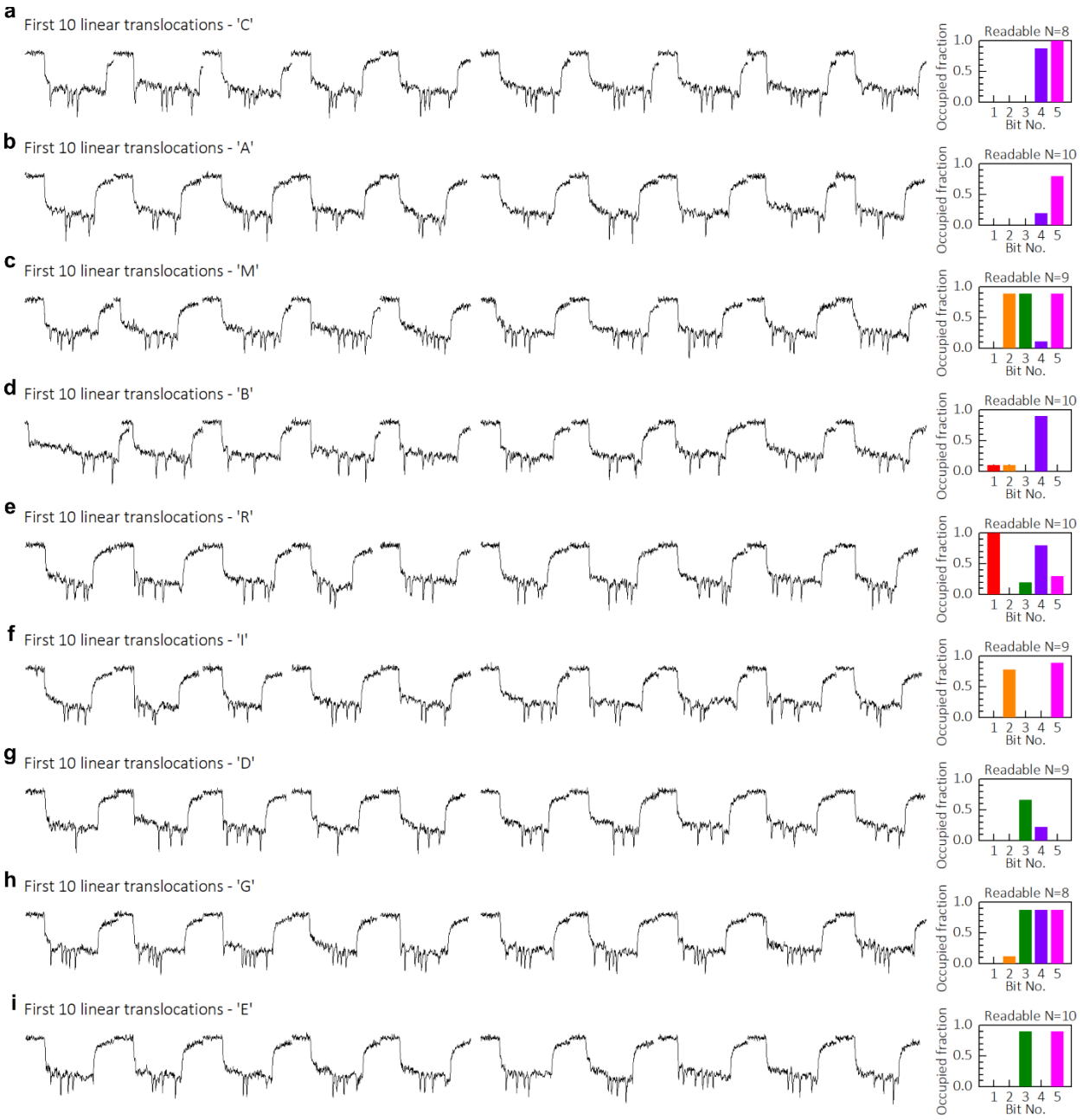

Figure S3. Nanopore data for the DNA-HDs written with 'CAMBRIDGE'. The first 10 unfolded translocation events and occupied fractions are shown in (a)-(i). In the histogram, we only included the events ('Readable N') with verified correct REF signals.

Figure S4.

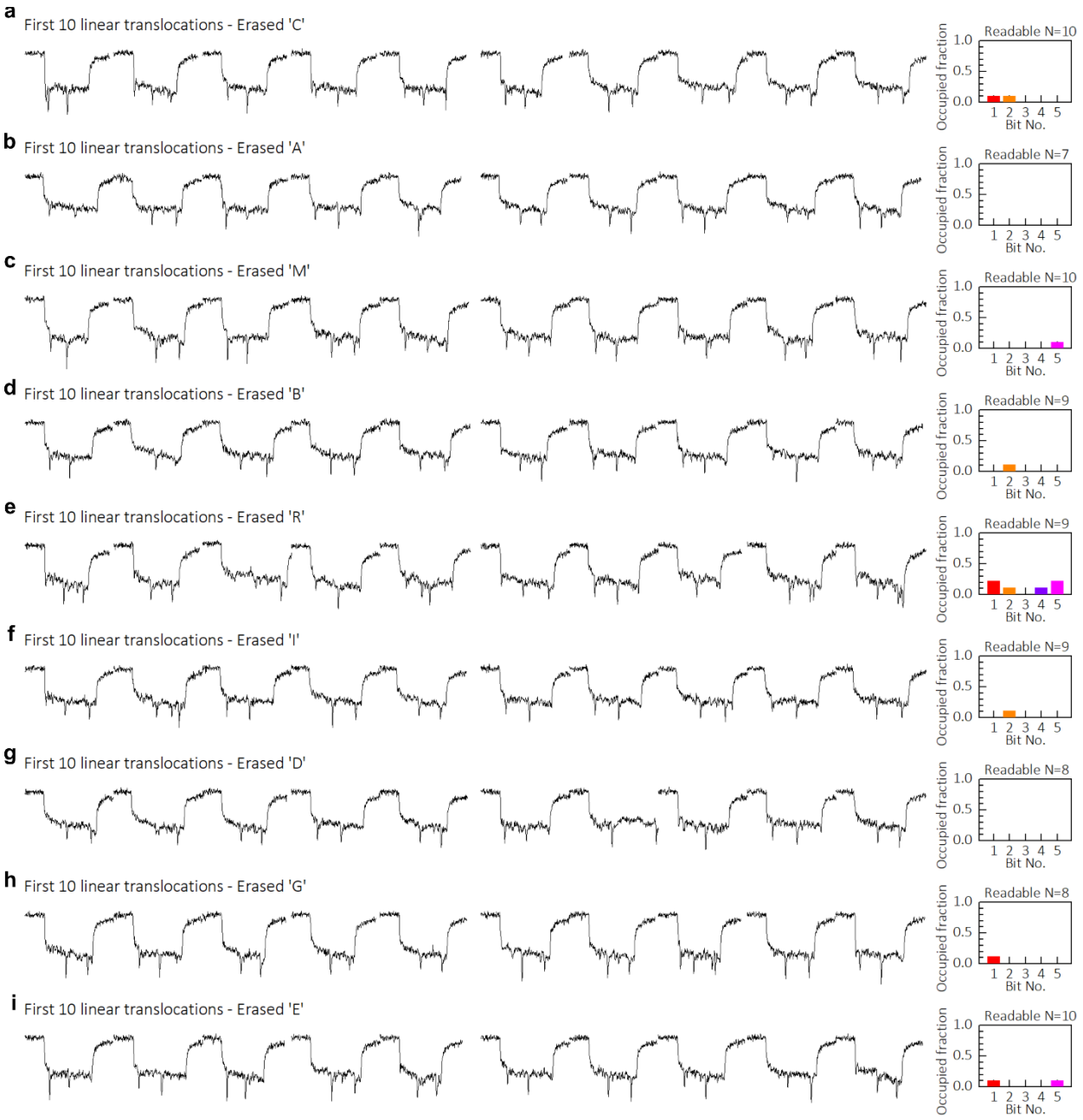

Figure S4. Nanopore data for the erased DNA-HDs. The first 10 unfolded translocation events and occupied fractions are shown in (a)-(i). In the histogram, we only included the events ('Readable N') with verified correct REF signals.

Figure S5.

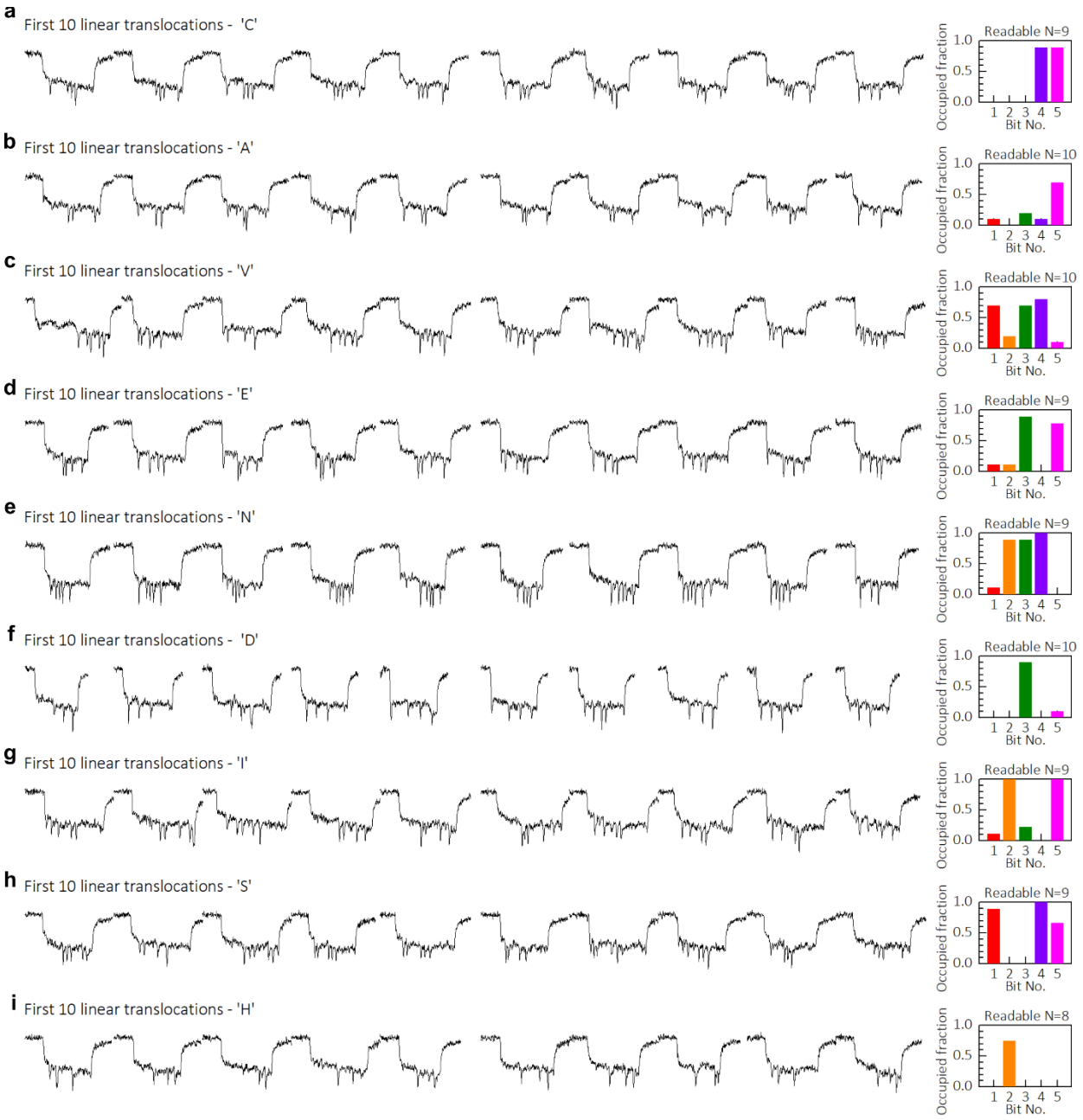

Figure S5. Nanopore data for the DNA-HDs rewritten with 'CAVENDISH'. The first 10 unfolded translocation events and occupied fractions are shown in (a)-(i). In the histogram, we only included the events ('Readable N') with verified correct REF signals.

Figure S6.

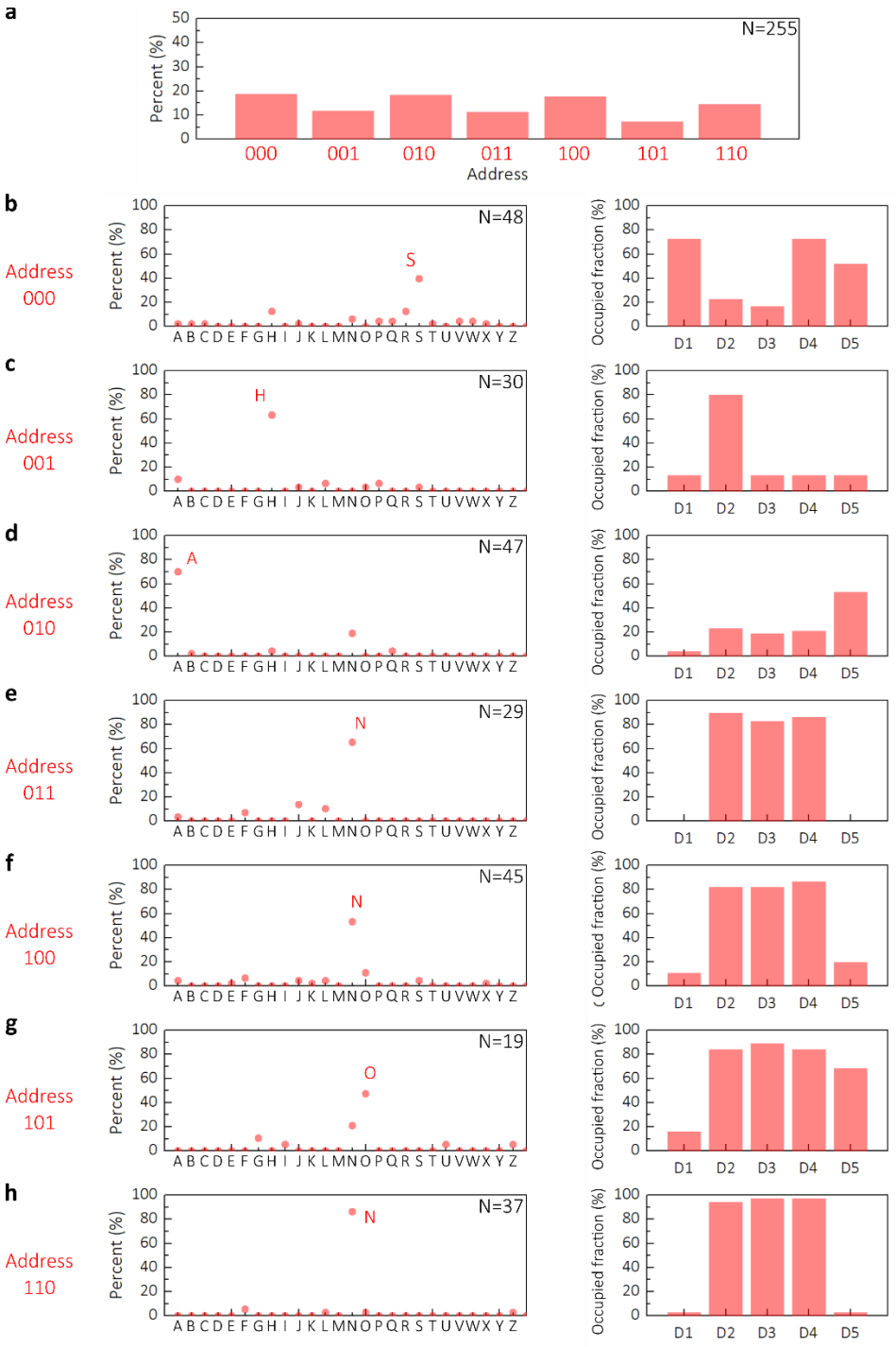

Fig S6. Correct decoding the information with the address and data keys. (a) Per cent of events assigned to addresses 000-100. N is the event number. (b)-(h) show the letter (left) decoded at each address and the occupied fractions at the five data sites.

Figure S7.

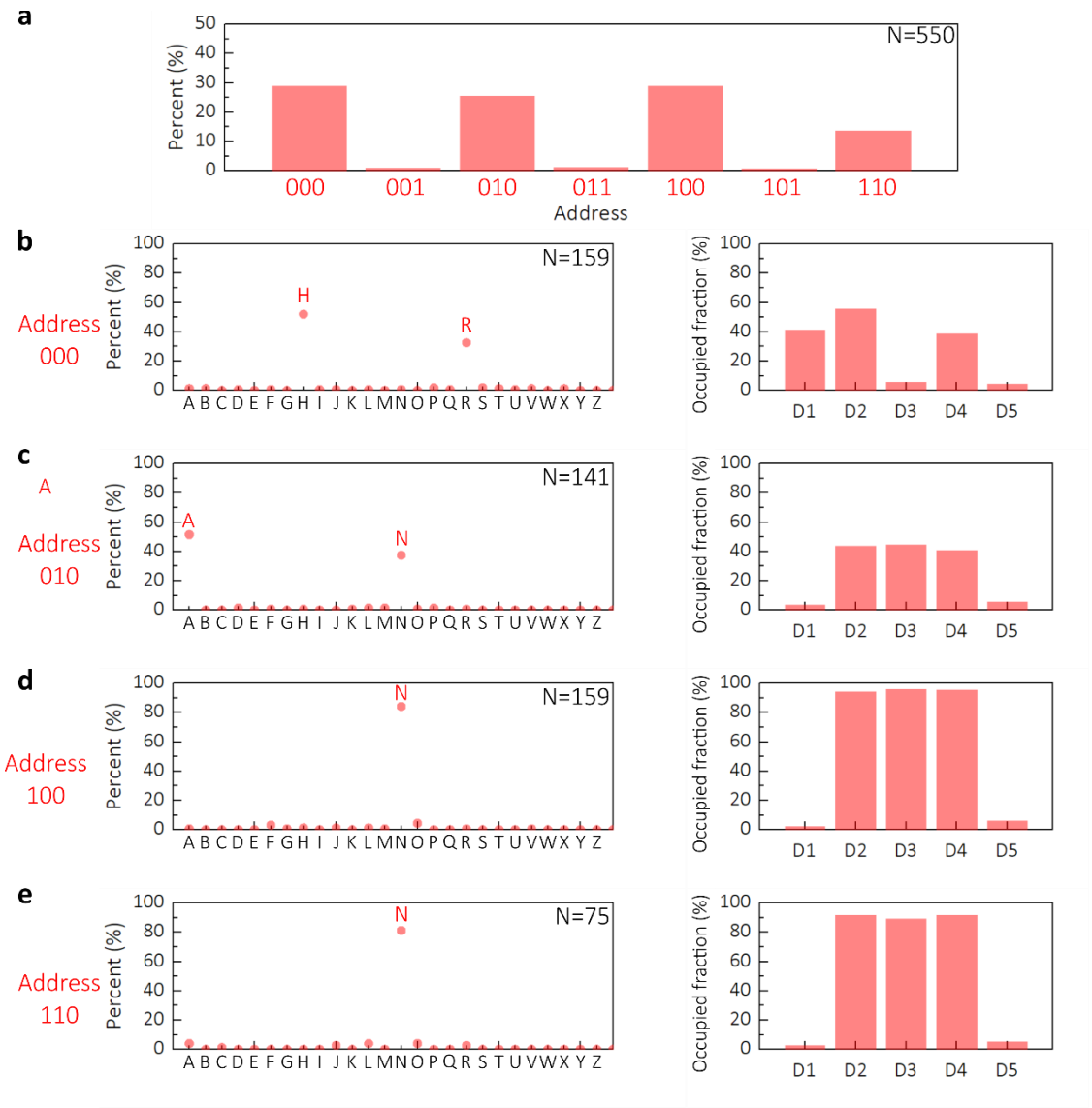

Figure S7. Wrong information decoded without keys. (a) Per cent of events assigned to addresses 000-100. N is the event number. (b)-(e) show the letter (left) decoded at each address and the occupied fractions at the five data sites. Here we only had four addresses because the third address site was unrevealed so it was always decoded as '0'.

Table S1.

Oligo No.	Sequence	Oligo No.	Sequence
1	TTTTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATC	96	CTTGAGCCATTTGGGAATTAGAGCCAGCAAAATCACCA
2	CGCTCACAATTCCACACAACATACGAGCCGGAAGCATA	97	GTAGCACCATTACCATTAGCAAGGCCGGAAACGTCACC
3	AAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACT	98	AATGAAACCATCGATAGCAGCACCGTAATTCAGTAGCGA
4	CACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGT	99	CAGAATCAAGTTTGCCTTTAGCGTCAGACTGTAGCGCG
5	CGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGC	100	TTTTCATCGGCATTTTCGGTCATAGCCCCCTTATTAGC
6	CAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCCA	101	GTTTGCCATCTTTTCATAATCAAAATCACCGGAACCAG
7	GGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGC	102	AGCCACCACCGGAACCGCCTCCCTCAGAGCCGCCACCC
8	TGATTGCCCTTCACCGCCTGGCCCTGAGAGAGTTGCAG	103	TCAGAACCGCCACCCTCAGAGCCACCACCCTCAGAGCC
9	CAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAAT	104	GCCACCAGAACCACCACCAGAGCCGCCGCCAGCATTGA
10	CCTGTTTGATGGTGGTTCCGAAATCGGCAAAATCCCTT	105	CAGGAGGTTGAGGCAGGTCAGACGATTGGCCTTGATAT
11	ATAAATCAAAAGAATAGCCCGAGATAGGGTTGAGTGTT	106	TCACAAACAAATAAATCCTCATTAAAGCCAGAATGGAA
12	GTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGT	107	AGCGCAGTCTCTGAATTTACCGTTCCAGTAAGCGTCAT
13	GGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGG	108	ACATGGCTTTTGATGATACAGGAGTGTACTGGTAATAA
14	GCGATGGCCCACTACGTGAACCATCACCCAAATCAAGT	109	GTTTTAACGGGGTCAGTGCCTTGAGTAACAGTGCCCGT
15	TTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAA	110	ATAAACAGTTAATGCCCCCTGCCTATTTCGGAACCTAT
16	CCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAA	111	TATTCTGAAACATGAAAGTATTAAGAGGCTGAGACTCC
17	AGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCG	112	TCAAGAGAAGGATTAGGATTAGCGGGGTTTTGCTCAGT
18	AAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGT	113	ACCAGGCGGATAAGTGCCGTCGAGAGGGTTGATATAAG
19	CACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATG	114	TATAGCCCGGAATAGGTGTATCACCGTACTCAGGAGGT
20	CGCCGCTACAGGGCGCGTACTATGGTTGCTTTGACGAG	115	TTAGTACCGCCACCCTCAGAACCGCCACCCTCAGAACC
21	CACGTATAACGTGCTTTCCTCGTTAGAATCAGAGCGGG	116	GCCACCCTCAGAGCCACCACCCTCATTTTCAGGGATAG
22	AGCTAAACAGGAGGCCGATTAAAGGGATTTTAGACAGG	117	CAAGCCCAATAGGAACCCATGTACCGTAACACTGAGTT
23	AACGGTACGCCAGAATCCTGAGAAGTGTTTTTATAATC	118	TCGTCACCAGTACAAACTACAACGCCTGTAGCATTCCA
24	AGTGAGGCCACCGAGTAAAAGAGTCTGTCCATCACGCA	119	CAGACAGCCCTCATAGTTAGCGTAACGATCTAAAGTTT
25	AATTAACCGTTGTAGCAATACTTCTTTGATTAGTAATA	120	TGTCGTCTTTCCAGACGTTAGTAAATGAATTTTCTGTA
26	ACATCACTTGCCTGAGTAGAAGAACTCAAACTATCGGC	121	TGGGATTTTGCTAAACAACTTTCAACAGTTTCAGCGGA
27	CTTGCTGGTAATATCCAGAACAATATTACCGCCAGCCA	122	GTGAGAATAGAAAGGAACAACTAAAGGAATTGCGAATA
28	TTGCAACAGGAAAAACGCTCATGGAAATACCTACATTT	123	ATAATTTTTTCACGTTGAAAATCTCCAAAAAAAAGGCT
29	TGACGCTCAATCGTCTGAAATGGATTATTTACATTGGC	124	CCAAAAGGAGCCTTTAATTGTATCGGTTTATCAGCTTG
30	AGATTCACCAGTCACACGACCAGTAATAAAAGGGACAT	125	CTTTCGAGGTGAATTTCTTAAACAGCTTGATACCGATA
31	TCTGGCCAACAGAGATAGAACCCTTCTGACCTGAAAGC	126	GTTGCGCCGACAATGACAACAACCATCGCCCACGCATA
32	GTAAGAATACGTGGCACAGACAATATTTTTGAATGGCT	127	ACCGATATATTCGGTCGCTGAGGCTTGCAGGGAGTTAA
33	ATTAGTCTTTAATGCGCGAACTGATAGCCCTAAAACAT	128	AGGCCGCTTTTGCGGGATCGTCACCCTCAGCAGCGAAA
34	CGCCATTAAAAATACCGAACGAACCACCAGCAGAAGAT	129	GACAGCATCGGAACGAGGGTAGCAACGGCTACAGAGGC
35	AAAACAGAGGTGAGGCGGTCAGTATTAACACCGCCTGC	130	TTTGAGGACTAAAGACTTTTTCATGAGGAAGTTTCCAT
36	AACAGTGCCACGCTGAGAGCCAGCAGCAAATGAAAAAT	131	TAAACGGGTAAAATACGTAATGCCACTACGAAGGCACC
37	CTAAAGCATCACCTTGCTGAACCTCAAATATCAAACCC	132	AACCTAAAACGAAAGAGGCAAAAGAATACACTAAAACA
38	TCAATCAATATCTGGTCAGTTGGCAAATCAACAGTTGA	133	CTCATCTTTGACCCCCAGCGATTATACCAAGCGCGAAA
39	AAGGAATTGAGGAAGGTTATCTAAAATATCTTTAGGAG	134	CAAAGTACAACGGAGATTTGTATCATCGCCTGATAAAT
40	CACTAACAACTAATAGATTAGAGCCGTCAATAGATAAT	135	TGTGTCGAAATCCGCGACCTGCTCCATGTTACTTAGCC
41	ACATTTGAGGATTTAGAAGTATTAGACTTTACAAACAA	136	GGAACGAGGCGCAGACGGTCAATCATAAGGGAACCGAA
42	TTCGACAACTCGTATTAAATCCTTTGCCCGAACGTTAT	137	CTGACCAACTTTGAAAGAGGACAGATGAACGGTGTACA
43	TAATTTTAAAAGTTTGAGTAACATTATCATTTTGCGGA	138	GACCAGGCGCATAGGCTGGCTGACCTTCATCAAGAGTA
44	ACAAAGAAACCACCAGAAGGAGCGGAATTATCATCATA	139	ATCTTGACAAGAACCGGATATTCATTACCCAAATCAAC
45	TTCCTGATTATCAGATGATGGCAATTCATCAATATAAT	140	GTAACAAAGCTGCTCATTCAGTGAATAAGGCTTGCCCT
46	CCTGATTGTTTGGATTATACTTCTGAATAATGGAAGGG	141	GACGAGAAACACCAGAACGAGTAGTAAATTGGGCTTGA
47	TTAGAACCTACCATATCAAAATTATTTGCACGTAAAAC	142	GATGGTTTAATTTCAACTTTAATCATTGTGAATTACCT
48	AGAAATAAAGAAATTGCGTAGATTTTCAGGTTTAACGT	143	TATGCGATTTTAAGAACTGGCTCATTATACCAGTCAGG
49	CAGATGAATATACAGTAACAGTACCTTTTACATCGGGA	144	ACGTTGGGAAGAAAAATCTACGTTAATAAAACGAACTA
50	GAAACAATAACGGATTCGCCTGATTGCTTTGAATACCA	145	ACGGAACAACATTATTACAGGTAGAAAGATTCATCAGT
51	AGTTACAAAATCGCGCAGAGGCGAATTATTCATTTCAA	146	TGAGATTTAGGAATACCACATTCAACTAATGCAGATAC
52	TTACCTGAGCAAAAGAAGATGATGAAACAAACATCAAG	147	ATAACGCCAAAAGGAATTACGAGGCATAGTAAGAGCAA
53	AAAACAAAATTAATTACATTTAACAATTTCATTTGAAT	148	CACTATCATAACCCTCGTTTACCAGACGACGATAAAAA
54	TACCTTTTTTAATGGAAACAGTACATAAATCAATATAT	149	CCAAAATAGCGAGAGGCTTTTGCAAAAGAAGTTTTGCC
55	GTGAGTGAATAACCTTGCTTCTGTAAATCGTCGCTATT	150	AGAGGGGGTAATAGTAAAATGTTTAGACTGGATAGCGT
56	AATTAATTTTCCCTTAGAATCCTTGAAAACATAGCGAT	151	CCAATACTGCGGAATCGTCATAAATATTCATTGAATCC
57	AGCTTAGATTAAGACGCTGAGAAGAGTCAATAGTGAAT	152	CCCTCAAATGCTTTAAACAGTTCAGAAAACGAGAATGA
58	TTATCAAAATCATAGGTCTGAGAGACTACCTTTTTAAC	153	CCATAAATCAAAAATCAGGTCTTTACCCTGACTATTAT
59	CTCCGGCTTAGGTTGGGTTATATAACTATATGTAAATG	154	AGTCAGAAGCAAAGCGGATTGCATCAAAAAGATTAAGA
60	CTGATGCAAATCCAATCGCAAGACAAAGAACGCGAGAA	155	GGAAGCCCGAAAGACTTCAAATATCGCGTTTTAATTCG
61	AACTTTTTCAAATATATTTTAGTTAATTTCATCTTCTG	156	AGCTTCAAAGCGAACCAGACCGGAAGCAAACTCCAACA
62	ACCTAAATTTAATGGTTTGAAATACCGACCGTGTGATA	157	GGTCAGGATTAGAGAGTACCTTTAATTGCTCCTTTTGA
63	AATAAGGCGTTAAATAAGAATAAACACCGGAATCATAA	158	TAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATTG
64	TTACTAGAAAAAGCCTGTTTAGTATCATATGCGTTATA	159	CTGAATATAATGCTGTAGCTCAACATGTTTTAAATATG
65	CAAATTCTTACCAGTATAAAGCCAACGCTCAACAGTAG	160	CAACTAAAGTACGGTGTCTGGAAGTTTCATTCCATATA
66	GGCTTAATTGAGAATCGCCATATTTAACAACGCCAACA	161	ACAGTTGATTCCCAATTCTGCGAACGAGTAGATTTAGT
67	TGTAATTTAGGCAGAGGCATTTTCGAGCCAGTAATAAG	162	TTGACCATTAGATACATTTCGCAAATGGTCAATAACCT

68	AGAATATAAAGTACCGACAAAAGGTAAAGTAATTCTGT	163	GTTTAGCTATATTTTCATTTGGGGCGCGAGCTGAAAAG
69	CCAGACGACGACAATAAACAACATGTTCAGCTAATGCA	164	GTGGCATCAATTCTACTAATAGTAGTAGCATTAACATC
70	GAACGCGCCTGTTTATCAACAATAGATAAGTCCTGAAC	165	CAATAAATCATACAGGCAAGGCAAAGAATTAGCAAAAT
71	AAGAAAAATAATATCCCATCCTAATTTACGAGCATGTA	166	TAAGCAATAAAGCCTCAGAGCATAAAGCTAAATCGGTT
72	GAAACCAATCAATAATCGGCTGTCTTTCCTTATCATTC	167	GTACCAAAAACATTATGACCCTGTAATACTTTTGCGGG
73	CAAGAACGGGTATTAAACCAAGTACCGCACTCATCGAG	168	AGAAGCCTTTATTTCAACGCAAGGATAAAAATTTTTAG
74	AACAAGCAAGCCGTTTTTATTTTCATCGTAGGAATCAT	169	AACCCTCATATATTTTAAATGCAATGCCTGAGTAATGT
75	TACCGCGCCCAATAGCAAGCAAATCAGATATAGAAGGC	170	GTAGGTAAAGATTCAAAAGGGTGAGAAAGGCCGGAGAC
76	TTATCCGGTATTCTAAGAACGCGAGGCGTTTTAGCGAA	171	AGTCAAATCACCATCAATATGATATTCAACCGTTCTAG
77	CCTCCCGACTTGCGGGAGGTTTTGAAGCCTTAAATCAA	172	CTGATAAATTAATGCCGGAGAGGGTAGCTATTTTTGAG
78	GATTAGTTGCTATTTTGCACCCAGCTACAATTTTATCC	173	AGATCTACAAAGGCTATCAGGTCATTGCCTGAGAGTCT
79	TGAATCTTACCAACGCTAACGAGCGTCTTTCCAGAGCC	174	GGAGCAAACAAGAGAATCGATGAACGGTAATCGTAAAA
80	TAATTTGCCAGTTACAAAATAAACAGCCATATTATTTA	175	CTAGCATGTCAATCATATGTACCCCGGTTGATAATCAG
81	TCCCAATCCAAATAAGAAACGATTTTTTGTTTAACGTC	176	AAAAGCCCCAAAAACAGGAAGATTGTATAAGCAAATAT
82	AAAAATGAAAATAGCAGCCTTTACAGAGAGAATAACAT	177	TTAAATTGTAAACGTTAATATTTTGTTAAAATTCGCAT
83	AAAAACAGGGAAGCGCATTAGACGGGAGAATTAACTGA	178	TAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAG
84	ACACCCTGAACAAAGTCAGAGGGTAATTGAGCGCTAAT	179	GAACGCCATCAAAAATAATTCGCGTCTGGCCTTCCTGT
85	ATCAGAGAGATAACCCACAAGAATTGAGTTAAGCCCAA	180	AGCCAGCTTTCATCAACATTAAATGTGAGCGAGTAACA
86	TAATAAGAGCAAGAAACAATGAAATAGCAATAGCTATC	181	ACCCGTCGGATTCTCCGTGGGAACAAACGGCGGATTGA
87	TTACCGAAGCCCTTTTTAAGAAAAGTAAGCAGATAGCC	182	CCGTAATGGGATAGGTCACGTTGGTGTAGATGGGCGCA
88	GAACAAAGTTACCAGAAGGAAACCGAGGAAACGCAATA	183	TCGTAACCGTGCATCTGCCAGTTTGAGGGGACGACGAC
89	ATAACGGAATACCCAAAAGAACTGGCATGATTAAGACT	184	AGTATCGGCCTCAGGAAGATCGCACTCCAGCCAGCTTT
90	CCTTATTACGCAGTATGTTAGCAAACGTAGAAAATACA	185	CCGGCACCGCTTCTGGTGCCGGAAACCAGGCAAAGCGC
91	TACATAAAGGTGGCAACATATAAAAGAAACGCAAAGAC	186	CATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCG
92	ACCACGGAATAAGTTTATTTTGTCACAATCAATAGAAA	187	ATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGA
93	ATTCATATGGTTTACCAGCGCCAAAGACAAAAGGGCGA	188	AAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACG
94	CATTCAACCGATTGAGGGAGGGAAGGTAAATATTGACG	189	CCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGC
95	GAAATTATTCATTAAAGGTGAATTATCACCGTCACCGA	190	CAGTGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCTTTT

Table S1. Sequences of the 190 staples complementary to the scaffold. The length of each oligonucleotide is 38 nt except for the 46 nt ends.

Table S2.

Site	Sequence	To replace Oligo Nos
REF1	ACATCACTTGCCTGAGTAGA	26-30
	AGAACTCAAATCCTCTTTTGAGGAACAAGTTTTCTTGTCTATCGGCCT	
	TGCTGGTAATTCCTCTTTTGAGGAACAAGTTTTCTTGTATCCAGAACA	
	ATATTACCGCTCCTCTTTTGAGGAACAAGTTTTCTTGTCAGCCATTGC	
	AACAGGAAAATCCTCTTTTGAGGAACAAGTTTTCTTGTACGCTCATGG	
	AAATACCTACTCCTCTTTTGAGGAACAAGTTTTCTTGTATTTTGACGC	
	TCAATCGTCTTCCTCTTTTGAGGAACAAGTTTTCTTGTGAAATGGATT	
	ATTTACATTGGCAGATTCAC	
	CAGTCACACGACCAGTAATAAAAGGGACAT	
D1	CTCCATTTCCCTTTCATTCTTT TTCGACAACTCGTATTAAATCCTTTGCCCGAACGTTAT	42
	AGAATGAAAG	
D2	CTCATATCTTCCTATCCTAC TT GTGAGTGAATAACCTTGCTTCTGTAAATCGTCGCTATT	55
	GTAGGATAGG	
D3	CAACCATCACATCACCAACA TT AGAATATAAAGTACCGACAAAAGGTAAAGTAATTCTGT	68
	TGTTGGTGAT	
D4	ACCCAAATCTCTGATCTTAC TT TCCCAATCCAAATAAGAAACGATTTTTTGTTTAACGTC	81
	GTAAGATCAG	
D5	CTATATACTACCTAATACTC TT CATTCAACCGATTGAGGGAGGGAAGGTAAATATTGACG	94
	GAGTATTAGG	
REF2	TCACAAACAAATAAATCCTCATTAAAGCCAGAATGGAAAGCGCAGTCTCTGAATTT	106-112
	ACCGTTCCAGTAAGCGTCAT	
	ACATGGCTTTTCCTCTTTTGAGGAACAAGTTTTCTTGTTGATGATACA	
	GGAGTGTACTTCCTCTTTTGAGGAACAAGTTTTCTTGTGGTAATAAGT	
	TTTAACGGGGTCCTCTTTTGAGGAACAAGTTTTCTTGTTCAGTGCCTT	
	GAGTAACAGTTCCTCTTTTGAGGAACAAGTTTTCTTGTGCCCGTATAA	
	ACAGTTAATGTCCTCTTTTGAGGAACAAGTTTTCTTGTCCCCCTGCCT	
	ATTTCGGAACTCCTCTTTTGAGGAACAAGTTTTCTTGTCTATTATTCT	
	GAAACATGAAAGTATTAAGA	
	GGCTGAGACTCCTCAAGAGAAGGATTAGGATTAGCGGGGTTTTGCTCAGT	

Table S2. DNA sequences for the design of the rewritable DNA-HD.

Table S3.

Name	Sequence
B1	Biotin-TTTTTTT AGAATGAAAGGGAAATGGAG GAGTGAG
B2	Biotin-TTTTTTT GTAGGATAGGAAGATATGAG GGTATGG
B3	Biotin-TTTTTTT TGTTGGTGATGTGATGGTTG AGGAGTG
B4	Biotin-TTTTTTT GTAAGATCAGAGATTTGGGT GTAAGGT
B5	Biotin-TTTTTTT GAGTATTAGGTAGTATATAG TGTAGTG
E1	CTCACTC CTCCATTTCCCTTTCATTCT
E2	CCATACC CTCATATCTTCCTATCCTAC
E3	CACTCCT CAACCATCACATCACCAACA
E4	ACCTTAC ACCCAAATCTCTGATCTTAC
E5	CACTACA CTATATACTACCTAATACTC

Table S3. Sequences of the oligonucleotides for wiring and erasing data on the rewritable DNA-HD. B1-B5 are biotinylated oligonucleotides that can bind to the overhangs at D1-D5. E1-E5 are oligonucleotides that can bind to B1-B5 to remove them from the DNA-HD using strand displacement reactions.

Table S4.

No.	Pore name	Stage	Encoded information	Total event No.	Unfolded event No.	Readable event No.	Bit 4 occupied No.	Bit 3 occupied No.	Bit 2 occupied No.	Bit 1 occupied No.	Bit 0 occupied No.
1	W&E_1	0 (Blank)	00000	693	119	**111**	**3**	**1**	**1**	**5**	**5**
2	W&E_2	0 (Blank)	00000	280	49	**42**	**0**	**1**	**2**	**1**	**2**
3	W&E_3	0 (Blank)	00000	289	57	**50**	**3**	**4**	**6**	**3**	**3**
4	W&E_4	1 (1st write)	00101	478	101	**96**	**3**	**5**	**85**	**6**	**84**
5	W&E_5	1 (1st write)	00101	440	80	**73**	**4**	**4**	**66**	**7**	**64**
6	W&E_6	1 (1st write)	00101	327	80	**75**	**2**	**3**	**65**	**7**	**70**
7	W&E_7	2 (Erase)	00000	373	67	**61**	**7**	**1**	**2**	**2**	**4**
8	W&E_8	2 (Erase)	00000	354	75	**63**	**2**	**1**	**4**	**0**	**5**
9	W&E_9	2 (Erase)	00000	303	49	**46**	**2**	**0**	**2**	**0**	**3**
10	W&E_10	3 (2nd write)	10100	192	41	**35**	**31**	**2**	**28**	**0**	**4**
11	W&E_11	3 (2nd write)	10100	338	72	**61**	**48**	**7**	**47**	**3**	**6**
12	W&E_12	3 (2nd write)	10100	378	69	**61**	**52**	**6**	**51**	**3**	**5**

Table S4. Statistics of the measurement for the characterization of the writing and erasing (Blank-‘00101’-Erased-‘10100’).

Table S5.

No.	Pore name	Stage	Encoded information	Unfolded events used	Readable event No.	Bit 4 occupied No.	Bit 3 occupied No.	Bit 2 occupied No.	Bit 1 occupied No.	Bit 0 occupied No.
1	Letter_11	1 (1st write)	C (00011)	10	**8**	**0**	**0**	**0**	**7**	**8**
2	Letter_12	1 (1st write)	A (00001)	10	**10**	**0**	**0**	**0**	**2**	**8**
3	Letter_13	1 (1st write)	M (01101)	10	**9**	**0**	**8**	**8**	**1**	**8**
4	Letter_14	1 (1st write)	B (00010)	10	**10**	**1**	**1**	**0**	**9**	**0**
5	Letter_15	1 (1st write)	R (10010)	10	**10**	**10**	**0**	**2**	**8**	**3**
6	Letter_16	1 (1st write)	I (01001)	10	**9**	**0**	**7**	**0**	**0**	**8**
7	Letter_17	1 (1st write)	D (00100)	10	**9**	**0**	**0**	**6**	**2**	**0**
8	Letter_18	1 (1st write)	G (00111)	10	**8**	**0**	**1**	**7**	**7**	**7**
9	Letter_19	1 (1st write)	E (00101)	10	**10**	**0**	**0**	**9**	**0**	**9**
10	Letter_21	2 (Erase)	00000	10	**10**	**1**	**1**	**0**	**0**	**0**
11	Letter_22	2 (Erase)	00000	10	**7**	**0**	**0**	**0**	**0**	**0**
12	Letter_23	2 (Erase)	00000	10	**10**	**0**	**0**	**0**	**0**	**1**
13	Letter_24	2 (Erase)	00000	10	**9**	**0**	**1**	**0**	**0**	**0**
14	Letter_25	2 (Erase)	00000	10	**9**	**2**	**1**	**0**	**1**	**2**
15	Letter_26	2 (Erase)	00000	10	**9**	**0**	**1**	**0**	**0**	**0**
16	Letter_27	2 (Erase)	00000	10	**8**	**0**	**0**	**0**	**0**	**0**
17	Letter_28	2 (Erase)	00000	10	**8**	**1**	**0**	**0**	**0**	**0**
18	Letter_29	2 (Erase)	00000	10	**10**	**1**	**0**	**0**	**0**	**1**
19	Letter_31	3 (2nd write)	C (00011)	10	**9**	**0**	**0**	**0**	**8**	**8**
20	Letter_32	3 (2nd write)	A (00001)	10	**10**	**1**	**0**	**2**	**1**	**7**
21	Letter_33	3 (2nd write)	V (10110)	10	**10**	**7**	**2**	**7**	**8**	**1**
22	Letter_34	3 (2nd write)	E (00101)	10	**9**	**1**	**1**	**8**	**0**	**7**
23	Letter_35	3 (2nd write)	N (01110)	10	**9**	**1**	**8**	**8**	**9**	**0**
24	Letter_36	3 (2nd write)	D (00100)	10	**10**	**0**	**0**	**9**	**0**	**1**
25	Letter_37	3 (2nd write)	I (01001)	10	**9**	**1**	**9**	**2**	**0**	**9**
26	Letter_38	3 (2nd write)	S (10011)	10	**9**	**8**	**0**	**0**	**9**	**6**
27	Letter_39	3 (2nd write)	H (01000)	10	**8**	**0**	**6**	**0**	**0**	**0**

Table S5. Statistics of the measurement for word storage ('CAMBRIDGE' – Erased - 'CAVENDISH').

Table S6.

Site	Sequence	To replace Oligo Nos
REF1	ACATCACTTGTCCTCTTTTGAGGAACAAGTTTTCTTGTCCTGAGTAGA	26-30
	AGAACTCAAATCCTCTTTTGAGGAACAAGTTTTCTTGTCTATCGGCCT	
	TGCTGGTAATTCCTCTTTTGAGGAACAAGTTTTCTTGTATCCAGAACA	
	ATATTACCGCTCCTCTTTTGAGGAACAAGTTTTCTTGTCAGCCATTGC	
	AACAGGAAAATCCTCTTTTGAGGAACAAGTTTTCTTGTACGCTCATGG	
	AAATACCTACTCCTCTTTTGAGGAACAAGTTTTCTTGTATTTTGACGC	
	TCAATCGTCTTCCTCTTTTGAGGAACAAGTTTTCTTGTGAAATGGATT	
	ATTTACATTGTCCTCTTTTGAGGAACAAGTTTTCTTGTGCAGATTCAC	
	CAGTCACACGACCAGTAATAAAAGGGACAT	
REF2	TGAATCTTACCAACGCTAACGAGCGTCTTTCCAGAGCCTAATTTGCCAGT	79-85
	TACAAAATAAACAGCCATAT	
	TATTTATCCCTCCTCTTTTGAGGAACAAGTTTTCTTGTAATCCAAATA	
	AGAAACGATTTCCTCTTTTGAGGAACAAGTTTTCTTGTTTTTGTTTAA	
	CGTCAAAAATTCCTCTTTTGAGGAACAAGTTTTCTTGTGAAAATAGCA	
	GCCTTTACAGTCCTCTTTTGAGGAACAAGTTTTCTTGTAGAGAATAAC	
	ATAAAAACAGTCCTCTTTTGAGGAACAAGTTTTCTTGTGGAAGCGCAT	
	TAGACGGGAGTCCTCTTTTGAGGAACAAGTTTTCTTGTAATTAACTGA	
	ACACCCTGAACAAAGTCAGA	
	GGGTAATTGAGCGCTAATATCAGAGAGATAACCCACAAGAATTGAGTTAAGCCCAA	
REF3	ACAGTTGATTCCCAATTCTGCGAACGAGTA	161-165
	GATTTAGTTTGACCATTAGA	
	TACATTTCGCTCCTCTTTTGAGGAACAAGTTTTCTTGTAAATGGTCAA	
	TAACCTGTTTTCCTCTTTTGAGGAACAAGTTTTCTTGTAGCTATATTT	
	TCATTTGGGGTCCTCTTTTGAGGAACAAGTTTTCTTGTCGCGAGCTGA	
	AAAGGTGGCATCCTCTTTTGAGGAACAAGTTTTCTTGTTCAATTCTAC	
	TAATAGTAGTTCCTCTTTTGAGGAACAAGTTTTCTTGTAGCATTAACA	
	TCCAATAAATTCCTCTTTTGAGGAACAAGTTTTCTTGTCATACAGGCA	
	AGGCAAAGAATTAGCAAAAT	
REF4	CATTCGCCATTCAGGCTGCGCAACTGTTGGGAAG	186-190
	GGCGATCGGTTCCTCTTTTGAGGAACAAGTTTTCTTGTGCGGGCCTCT	
	TCGCTATTACTCCTCTTTTGAGGAACAAGTTTTCTTGTGCCAGCTGGC	
	GAAAGGGGGATCCTCTTTTGAGGAACAAGTTTTCTTGTTGTGCTGCAA	
	GGCGATTAAGTCCTCTTTTGAGGAACAAGTTTTCTTGTTTGGGTAACG	
	CCAGGGTTTTTCCTCTTTTGAGGAACAAGTTTTCTTGTCCCAGTCACG	
	ACGTTGTAAATCCTCTTTTGAGGAACAAGTTTTCTTGTACGACGGCCA	
	GTGCCAAGCTTCCTCTTTTGAGGAACAAGTTTTCTTGTTGCATGCCTG	
	CAGGTCGACTTCCTCTTTTGAGGAACAAGTTTTCTTGTCTAGAGGATCTTTT	

Table S6. Sequences of the oligonucleotides for forming the dumbbells as REFs on the DNA-HD. Each group consists of 6 DNA dumbbells except for REF4 with 8 DNA dumbbells.

Table S7.

Site	Sequences for '1'		Sequences for '0'
A1	CACTAACAACTAATTCCTCTTTTGAGGAACAAGTTTTCTTGTAGATTAGAGC	To replace Oligos 40-43	Oligos 40-43
	CGTCAATAGATCCTCTTTTGAGGAACAAGTTTTCTTGTTAATACATTT		
	GAGGATTTAGTCCTCTTTTGAGGAACAAGTTTTCTTGTAAGTATTAGA		
	CTTTACAAACTCCTCTTTTGAGGAACAAGTTTTCTTGTAATTCGACAA		
	CTCGTATTAATCCTCTTTTGAGGAACAAGTTTTCTTGTATCCTTTGCC		
	CGAACGTTATTCCTCTTTTGAGGAACAAGTTTTCTTGTTAATTTTAAA		
	AGTTTGAGTAACATTATCATTTTGCGGA		
A2	TTACCTGAGCAAAAGAAGATGATGAAACAAACATCAAGAAAACA	To replace Oligos 52-57	Oligos 52-57
	AAATTAATTACATTTAACAA		
	TTTCATTTGATCCTCTTTTGAGGAACAAGTTTTCTTGTATTACCTTTT		
	TTAATGGAAATCCTCTTTTGAGGAACAAGTTTTCTTGTCAGTACATAA		
	ATCAATATATTCCTCTTTTGAGGAACAAGTTTTCTTGTGTGAGTGAAT		
	AACCTTGCTTTCCTCTTTTGAGGAACAAGTTTTCTTGTCTGTAAATCG		
	TCGCTATTAATCCTCTTTTGAGGAACAAGTTTTCTTGTTTAATTTTCC		
	CTTAGAATCCTCCTCTTTTGAGGAACAAGTTTTCTTGTTTGAAAACAT		
	AGCGATAGCTTAGATTAAGA		
	CGCTGAGAAGAGTCAATAGTGAAT		
A3	CTATATACTACCTAATACTCTTCCAGACGACGACAATAAACAACATGTTCAGCTAATGCA	To replace Oligo 69	Oligo 69
D1	CATTCAACCGATTGAGGGAGGGAAGGTCCTCTTTTGAGGAACAAGTTTTCTTGTTAAATATTGA	To replace Oligos 94-97	Oligos 94-97
	CGGAAATTATTCCTCTTTTGAGGAACAAGTTTTCTTGTTCATTAAAGG		
	TGAATTATCATCCTCTTTTGAGGAACAAGTTTTCTTGTCCGTCACCGA		
	CTTGAGCCATTCCTCTTTTGAGGAACAAGTTTTCTTGTTTGGGAATTA		
	GAGCCAGCAATCCTCTTTTGAGGAACAAGTTTTCTTGTAATCACCAGT		
	AGCACCATTATCCTCTTTTGAGGAACAAGTTTTCTTGTCCATTAGCAAGGCCGGAAACGTCACC		
D2	TCACAAACAAATAAATCCTCATTAAAGCCAGAATGGAAAGCGCAGTCTCTGAATTT	To replace Oligos 106-112	Oligos 106-112
	ACCGTTCCAGTAAGCGTCAT		
	ACATGGCTTTTCCTCTTTTGAGGAACAAGTTTTCTTGTTGATGATACA		
	GGAGTGTACTTCCTCTTTTGAGGAACAAGTTTTCTTGTGGTAATAAGT		
	TTTAACGGGGTCCTCTTTTGAGGAACAAGTTTTCTTGTTCAGTGCCTT		
	GAGTAACAGTTCCTCTTTTGAGGAACAAGTTTTCTTGTGCCCGTATAA		
	ACAGTTAATGTCCTCTTTTGAGGAACAAGTTTTCTTGTCCCCCTGCCT		
	ATTTCGGAACTCCTCTTTTGAGGAACAAGTTTTCTTGTCTATTATTCT		
	GAAACATGAAAGTATTAAGA		
	GGCTGAGACTCCTCAAGAGAAGGATTAGGATTAGCGGGGTTTTGCTCAGT		
D3	TGGGATTTTGCTAAACAACTTT	To replace Oligos 121-124	Oligos 121-124
	CAACAGTTTCTCCTCTTTTGAGGAACAAGTTTTCTTGTAGCGGAGTGA		
	GAATAGAAAGTCCTCTTTTGAGGAACAAGTTTTCTTGTGAACAACTAA		
	AGGAATTGCGTCCTCTTTTGAGGAACAAGTTTTCTTGTAATAATAATT		
	TTTTCACGTTTCCTCTTTTGAGGAACAAGTTTTCTTGTGAAAATCTCC		
	AAAAAAAAGGTCCTCTTTTGAGGAACAAGTTTTCTTGTCTCCAAAAGG		
	AGCCTTTAATTCCTCTTTTGAGGAACAAGTTTTCTTGTTGTATCGGTTTATCAGCTTG		
D4	CAAAGTACAACGGAGATTTGTATC	To replace Oligos 134-139	Oligos 134-139
	ATCGCCTGATAAATTGTGTC		
	GAAATCCGCGTCCTCTTTTGAGGAACAAGTTTTCTTGTACCTGCTCCA		
	TGTTACTTAGTCCTCTTTTGAGGAACAAGTTTTCTTGTCCGGAACGAG		
	GCGCAGACGGTCCTCTTTTGAGGAACAAGTTTTCTTGTTCAATCATAA		
	GGGAACCGAATCCTCTTTTGAGGAACAAGTTTTCTTGTCTGACCAACT		
	TTGAAAGAGGTCCTCTTTTGAGGAACAAGTTTTCTTGTACAGATGAAC		
	GGTGTACAGATCCTCTTTTGAGGAACAAGTTTTCTTGTCCAGGCGCAT		
	AGGCTGGCTGACCTTCATCA		
	AGAGTAATCTTGACAAGAACCGGATATTCATTACCCAAATCAAC		
D5	CAACCATCACATCACCAACATTAGAGGGGGTAATAGTAAAATGTTTAGACTGGATAGCGT	To replace Oligos 150	Oligo 150

Table S7. Sequences of the oligonucleotides for forming the address and data sites on the DNA-HD.

REFERENCES

(1) Bell, N. A.; Keyser, U. F. Digitally encoded DNA nanostructures for multiplexed, single-molecule protein sensing with nanopores. *Nat. Nanotechnol.* **2016**, *11* (7), 645.