

## Supporting Information to

### **DNA origami nanopores for controlling DNA translocation**

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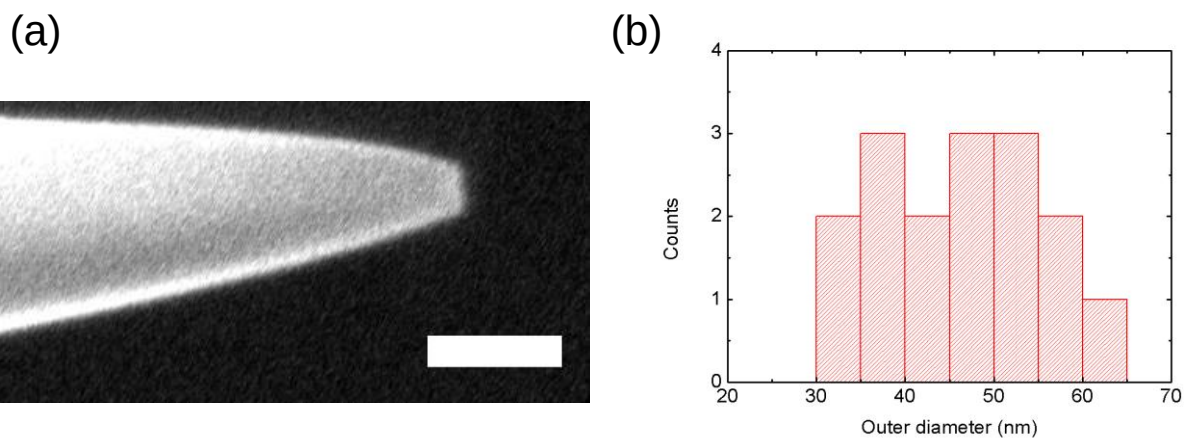
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### 1. SEM image of a representative bare nanocapillary

Uncoated glass nanocapillaries were imaged with a Philips XL30 FEGSEM. The acceleration voltage was 2 kV.



**Figure S1.** (a) Scanning electron microscopy image shows the conical shape of a glass nanocapillary. Scale bar = 100 nm. (b) Histogram of the outer diameter of 16 nanopores observed by SEM images. An outer diameter of mean 46 nm and standard deviation 9 nm was measured.

## 2. Schematic representation of the devices containing the glass nanocapillaries

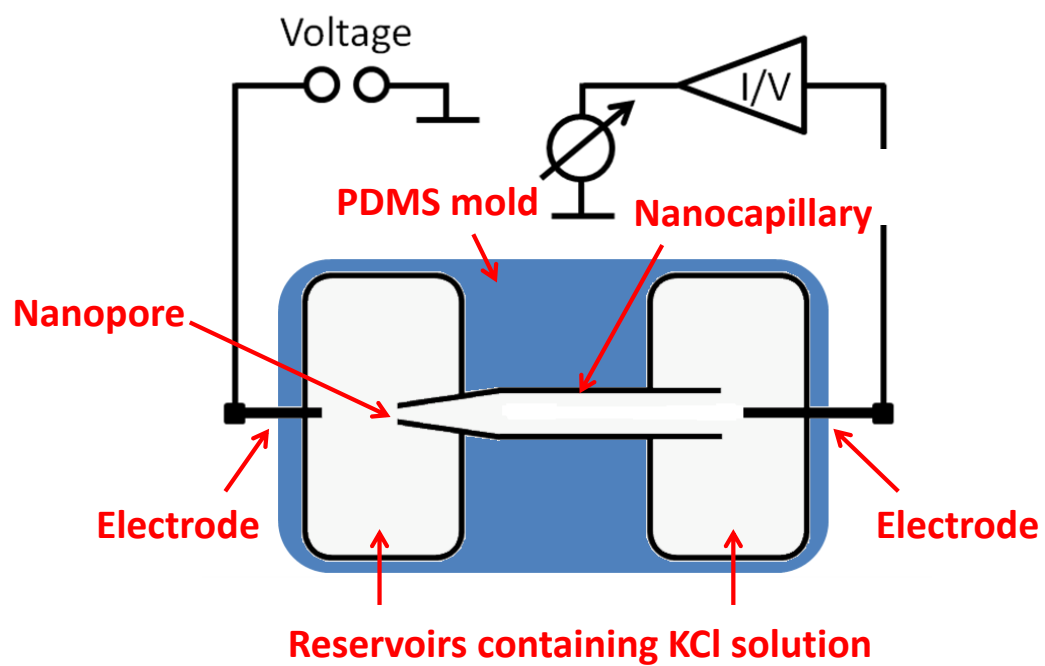
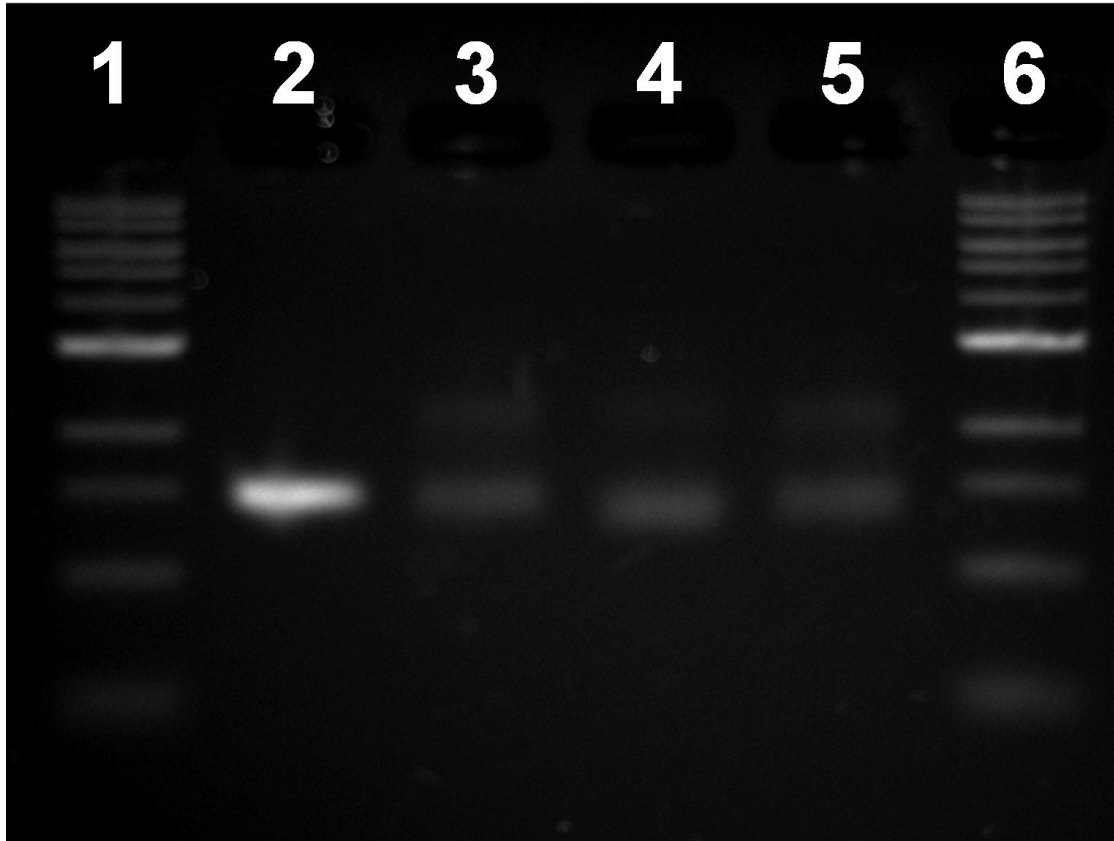


Figure S2. Device schematic

### 3. Agarose gel analysis of purified DNA origami structures

A bright and narrow band which moves similarly to the M13mp18 scaffold is observed for all DNA origami structures. This feature indicates well folded structures.



**Figure S3.** Agarose gel analysis of purified DNA origami structures. Structures were electrophoresed in 11 mM  $\text{MgCl}_2$  solution buffered with  $0.5 \times \text{TBE}$ .

1 and 6 = 1 kb DNA Ladder.

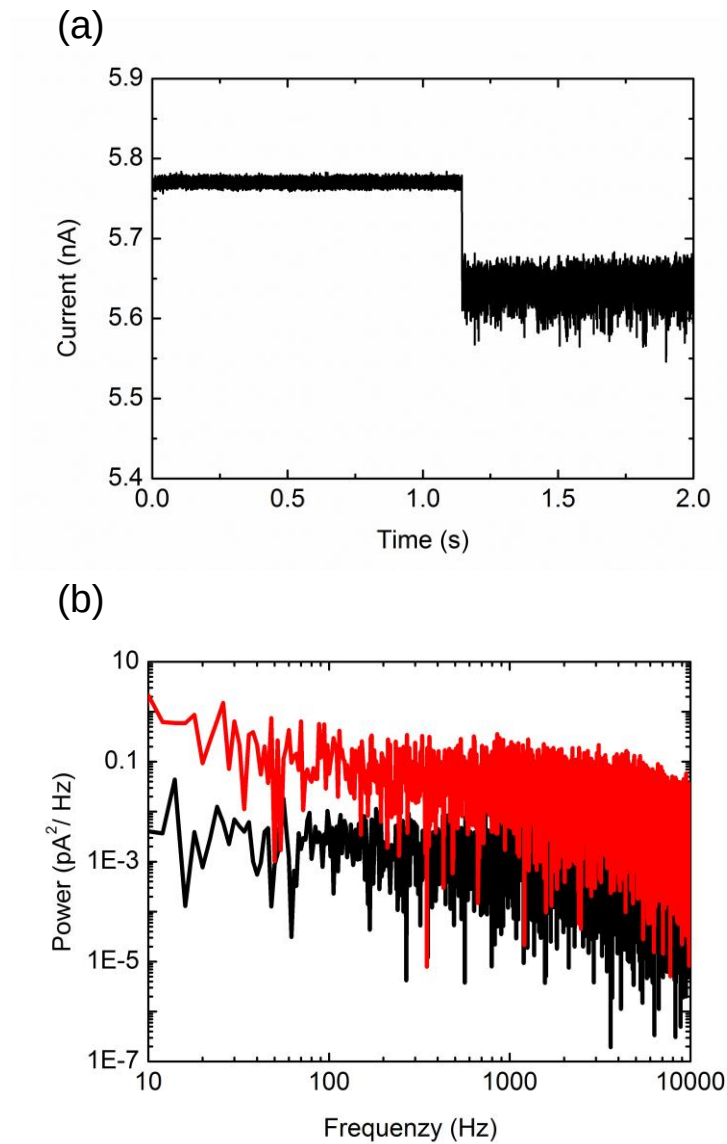
2 = M13mp18 scaffold.

3 = 14 nm Cy3 fluorescently labelled DNA origami structure.

4 = 5 nm DNA origami structure.

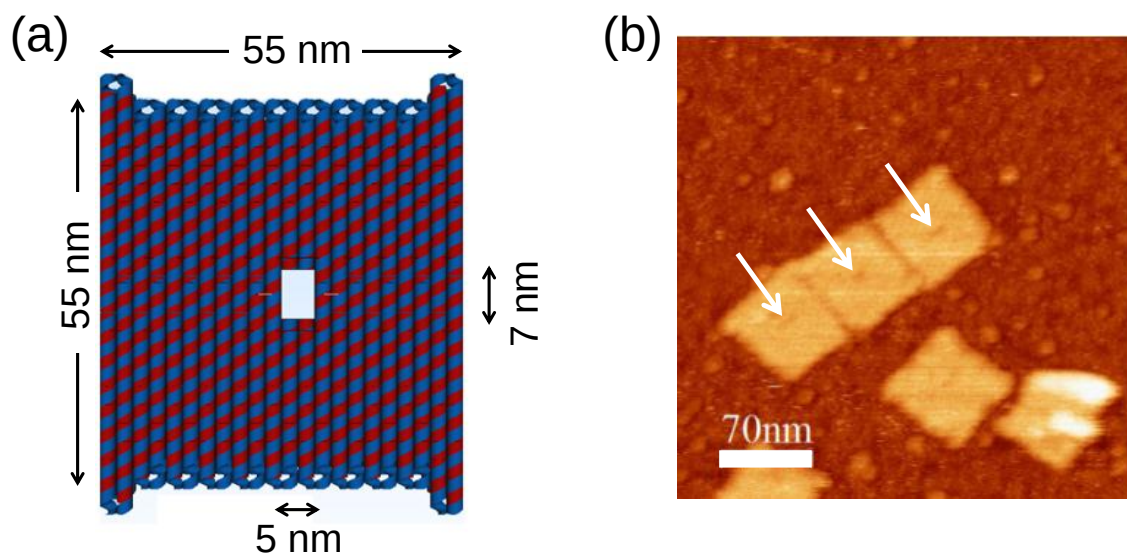
5 = 14 nm DNA origami structure.

#### 4. Ionic current noise in bare nanocapillary and hybrid nanopore



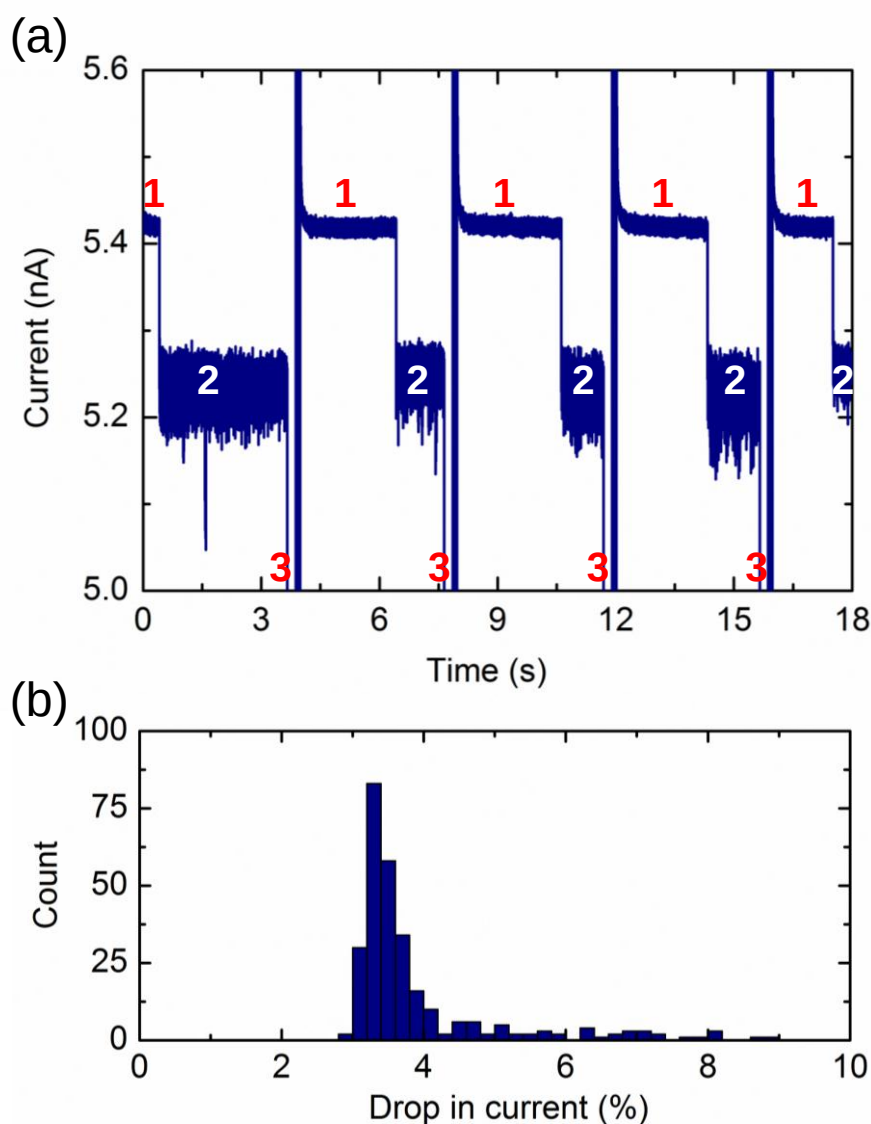
**Figure S4.** a) Ionic current trace recorded for one of the 14 nm pore DNA origami trappings. b) A comparison of the current power spectral density of a bare nanocapillary (black) and its corresponding hybrid one (red). Both correspond to 0.5 s of the ionic trace before (black) and after trapping (red). A significant enhancement in 1/f type low-frequency noise is observed in the hybrid nanopore, as expected due to the fluctuations produced by the DNA origami structure.

## 5. Schematic representation and AFM image of '5 nm' DNA origami structure



**Figure S5.** a) Schematic representation of the DNA origami structure with a central 5 nm x 7 nm pore. b) Topographic AFM image of assembled DNA origami structures deposited on mica and imaged in solution. The pores are indicated by white arrows.

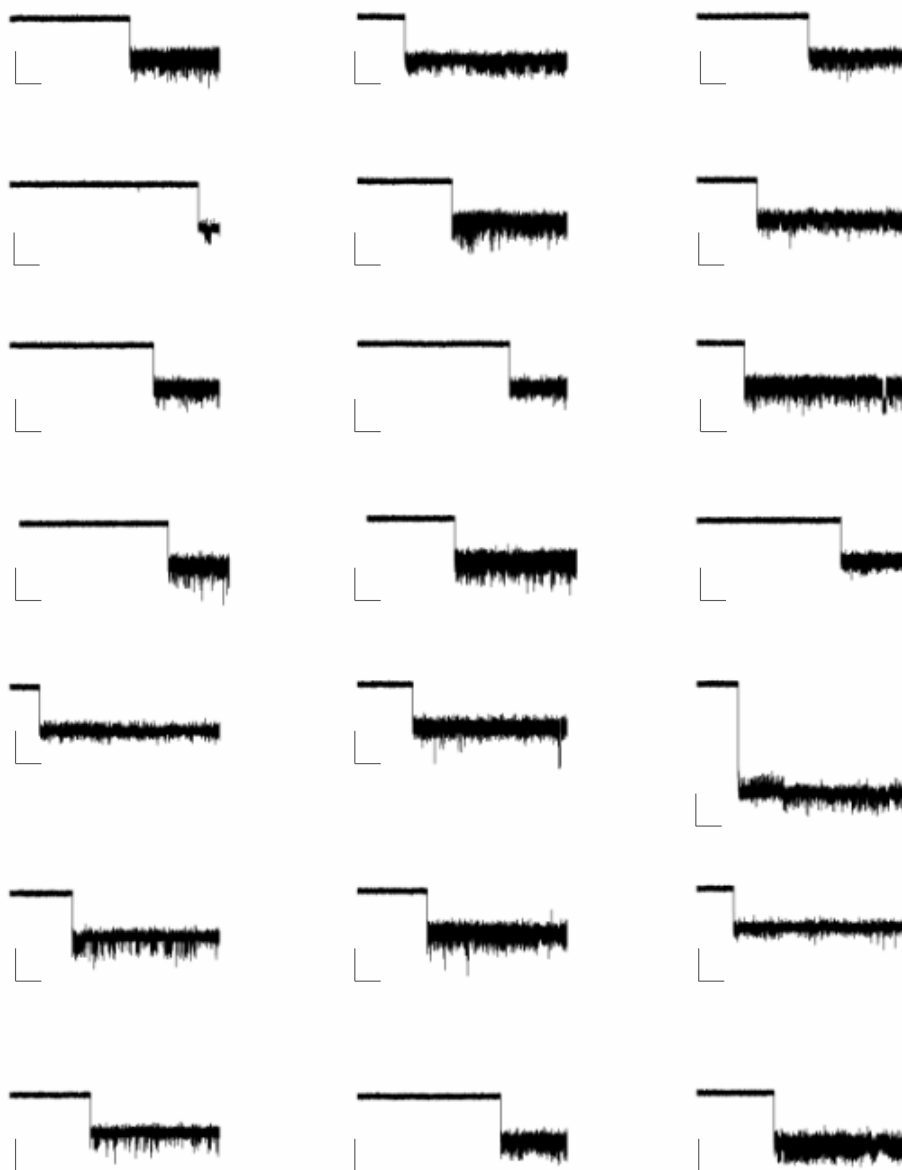
**6. Reversible and repeated trapping and ejection of DNA origami (5 nm design) on the glass nanocapillary and histogram of the percentage drop in ionic current.**



**Figure S6.** a) Ionic current trace showing reversible and repeated trapping and ejection of DNA origami on the glass nanocapillary ('5 nm' design). 1: Ionic current at 300 mV in the bare nanocapillary; 2: Ionic current at 300 mV once the DNA origami has been trapped; 3: Ionic current upon applying negative voltage. b) Histogram of the percentage drop in ionic current produced for 302 origami trappings on one individual nanocapillary.

## 7. Ionic current traces showing DNA origami trapping

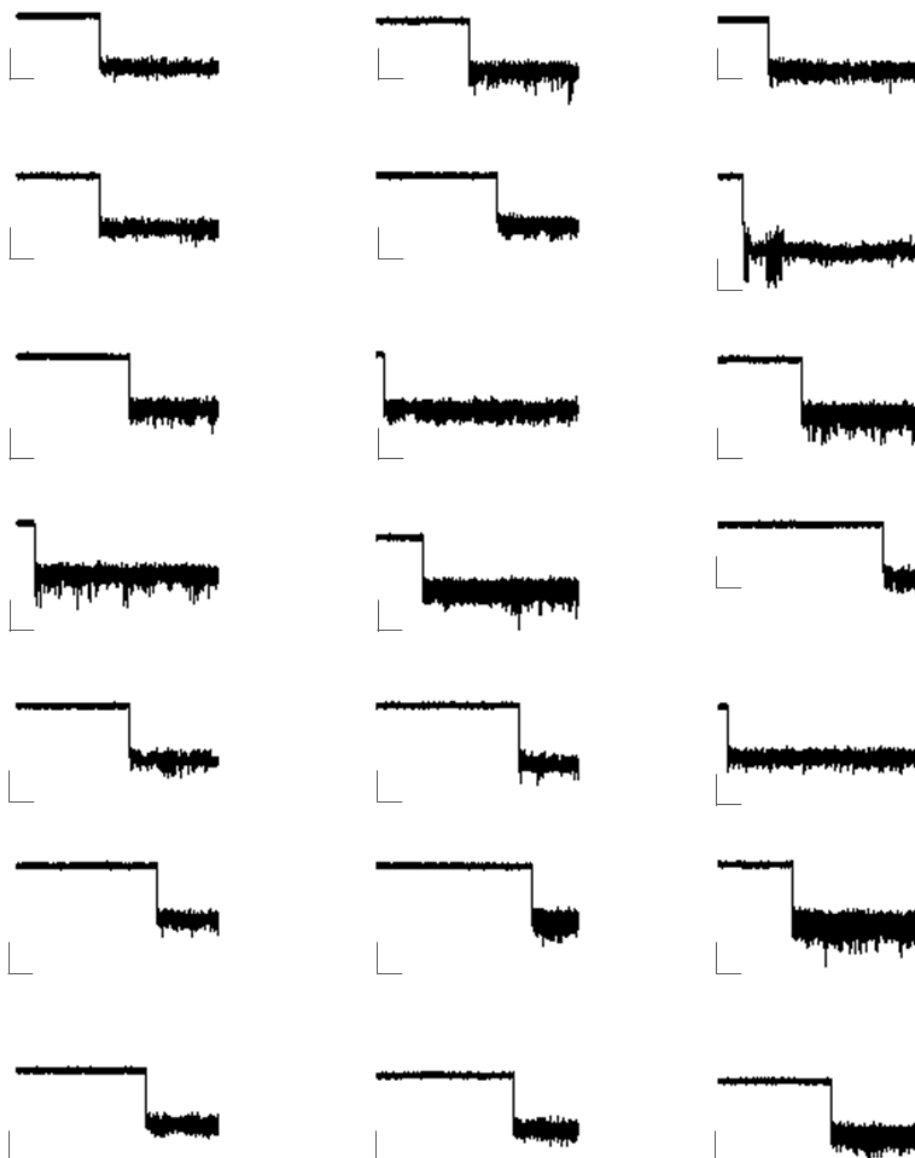
a) 14 nm design



**Figure S7.** Ionic current traces showing 14 nm pore DNA origami trappings. These traces are some examples of the 352 trappings recorded for the experiment cited in the main text (Figure 1e). Scale bar: Y axis is ionic current (100 pA) and X axis is duration (0.25 s).

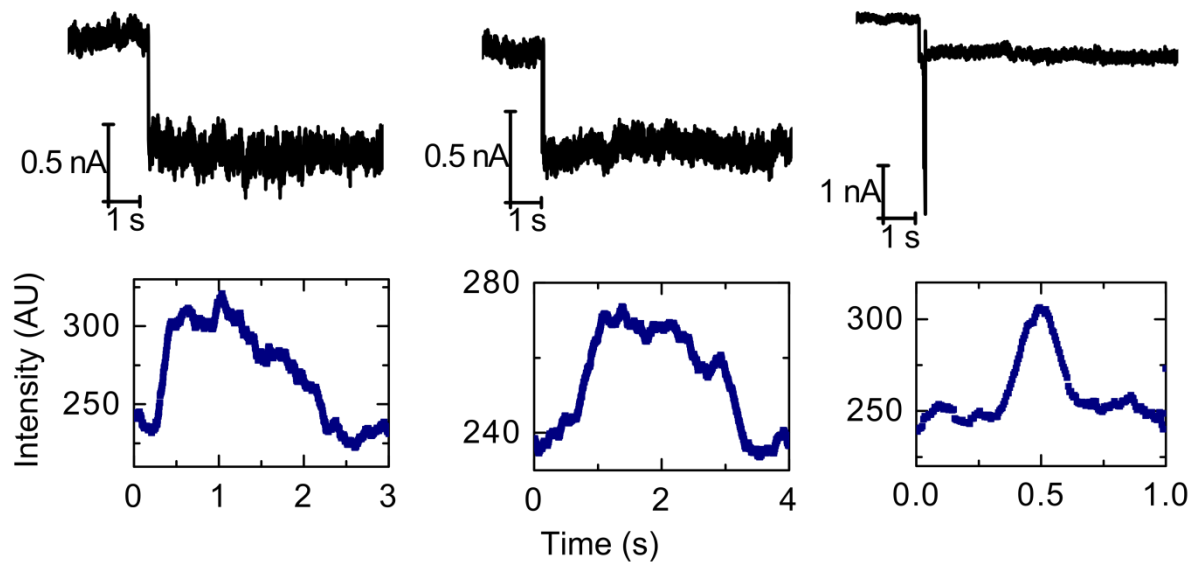


b) 5 nm design



**Figure S8.** Ionic current traces showing ‘5 nm’DNA origami trappings. These traces are some examples of the 302 trappings recorded for this experiment. Scale bar: Y axis is ionic current (100 pA) and X axis is duration (0.25 s).

c) 14 nm design labelled with Cy3



**Figure S9.** Ionic current traces (top) showing 14 nm labelled with Cy3 DNA origami nanopore trappings. The fluorescence intensity at the nanocapillary tip corresponding to each insertion is shown (below). Photobleaching occurs on a consistent timescale across insertions.

## 8. Staple strands sequences and scaffold-staple layout for each DNA origami structure

### a) 14 nm design

| Start   | End     | Sequence                                      | Length |
|---------|---------|-----------------------------------------------|--------|
| 0[79]   | 45[71]  | AAATCAAGTTTTTTGGGCGAGAAAGGAGTGTAAATAAATCC     | 40     |
| 0[103]  | 45[103] | GCCCACTACGGGCGCTGTAAGCGTGCAGTCTC              | 32     |
| 0[143]  | 45[135] | TCCAACGTCAAAGGGCCGAAATCCGCCACCCAGAGCCA        | 38     |
| 0[175]  | 45[167] | GAACAAGAGTCCACTAGAAATAGCCGGTGTATCAGCCCAAT     | 40     |
| 1[56]   | 47[71]  | CGAACGTGGGTGCGAGGTTAATGCCCCCTGCCT             | 32     |
| 1[88]   | 47[103] | AAAGGAGCGTGAACCTTCTGAAACATGAAA                | 30     |
| 1[112]  | 47[135] | GGCAAGATGGTGGTTCGAAAAACCACTCCTCAAGAGAAG       | 39     |
| 1[152]  | 47[167] | AATCAAAATTAAAGAAGTTTTGCTCAGTACCA              | 32     |
| 2[71]   | 43[71]  | AATGCGCCCTAAACAGCACCAGAGACCACCGG              | 32     |
| 2[103]  | 43[103] | GCTGCGCGACAGGAACCCACCACCCCTCAGA               | 32     |
| 2[135]  | 43[135] | GCAGGCGAAGAGGCGTAGTTAGCCGTTAGT                | 30     |
| 2[167]  | 43[167] | TGCAGCAAGTTTTTCTAACGCCTGTGCTAAAC              | 32     |
| 3[56]   | 46[56]  | AGCGGGAGGCTACAGGACAAACAACCTGGTAAT             | 32     |
| 3[88]   | 46[88]  | ATTTTAGTAACCACCGGAAAGCCATACATG                | 30     |
| 3[112]  | 46[120] | AGAATAACGCGCGGGGAAATCCTGCCACCCTCTCAGAACC      | 40     |
| 3[152]  | 46[152] | CCAGGGTGGCGGTCCAGGATAGCAACCGTACT              | 32     |
| 4[71]   | 41[71]  | GTCTGTCCAATATCGGGCATTTTCCAATGAA               | 32     |
| 4[103]  | 41[103] | TTTATAATAATATTACCTTTAGCGCAGTAGCG              | 32     |
| 4[135]  | 41[135] | CTGCATTACAATTCCAATCTCCAATCGGTT                | 30     |
| 4[167]  | 41[167] | CGCTTTCCAAAGTGTAAGTTGCGTCTTAAA                | 32     |
| 5[56]   | 44[56]  | AGAACTCAATCACGCACCAGAGCCCCGCCGCC              | 32     |
| 5[88]   | 44[88]  | CCAGAACCAGTGAGGGCCGCCACTCAGAGC                | 30     |
| 5[112]  | 44[120] | ATTGCTTATCCGCTCAATGAATCGTTTCCAGAGTAACGAT      | 40     |
| 5[152]  | 44[152] | GGAAGCATAGTCGGGATGGGATTTTAGCATTC              | 32     |
| 6[71]   | 39[71]  | TCGTCTGAAGAACCCTTGAGCCATAAGGGCGA              | 32     |
| 6[103]  | 39[111] | CTCATGGACGTGGCACCATTAAAGAGGTAAATATTGACGG      | 40     |
| 6[135]  | 39[135] | TGTTTCCTTGGGTAATGCAGGGACAGCAGC                | 30     |
| 6[167]  | 39[167] | CCGAGCTCGTTGTAAACGCATAACAGCAACGG              | 32     |
| 7[56]   | 42[56]  | ACAGAGATAATGGATTAAACGTCACGGTCATA              | 32     |
| 7[88]   | 42[88]  | AAGAATAAATACCTACCGTAATTCAGACTG                | 30     |
| 7[112]  | 42[120] | TTTTTGGCGATTAAAGTGTGTGAAATTAATTGTAAAAAAG      | 40     |
| 7[152]  | 42[152] | GTCACGACGAATTTCGTAGGTGAATAATAATAA             | 32     |
| 8[71]   | 37[71]  | ATCGCCATGTGCCACGGACACCACTAGAAAAT              | 32     |
| 8[95]   | 40[88]  | GCGCGAACGGAGGGAGTGAATTA                       | 23     |
| 8[135]  | 40[120] | AAAGGGGGATGTGCTGGTCACCCTGTAAAGG               | 32     |
| 8[167]  | 37[167] | GCGGGCCTTGCCGGAATAAACGGGAGAATACA              | 32     |
| 9[56]   | 40[56]  | CTGCAACATAAAAAATAAAAGACAATTGGGAAT             | 32     |
| 9[139]  | 40[152] | GCTTTCGGCACCGCTTCTGGCTTCGCTAACGAGGGTCGATATAT  | 45     |
| 10[84]  | 35[71]  | GCAAATGAAAAATCTAAAGCATTGAGGAACAATAATATAAGCAGA | 45     |
| 10[167] | 35[167] | TCGGCCTCGTCACGTTAAGTACAACATGTTAC              | 32     |
| 11[56]  | 38[56]  | GAAAGGAATCACCTTGGCAAACGGGAATAA                | 30     |

|         |         |                                                |    |
|---------|---------|------------------------------------------------|----|
| 11[139] | 38[152] | GGATTGACCGTAATGGGATAGAGGAAGATAGGCAAATAAAATA    | 43 |
| 12[84]  | 33[71]  | ATCTTTAGGAGCACTAACAACTCGTATTAGCCCAATTAATTGAG   | 45 |
| 12[167] | 33[167] | CCGTCGGAAAAATAATTCCAACCTTTTCATCAAG             | 32 |
| 13[56]  | 36[56]  | TTCGACAATAATAGATAGAAAAGACGGAAT                 | 30 |
| 13[139] | 36[152] | AACCAATAGGAACGCCATCAATTCTCCGTCCTGCTCCGGAGAT    | 43 |
| 14[84]  | 31[71]  | GAACGTTATTAATTTTAAAAAGTCAGATGAAGAATAACATAAACAG | 45 |
| 14[167] | 31[167] | TAAATTTTAAAAACAGGAAGCTGCTTTACCTTA              | 32 |
| 15[56]  | 34[56]  | CCTGATTATTTGAGTACAGAGGGAATAAGA                 | 30 |
| 15[96]  | 31[103] | GATTGTTTGTCAAAAAATAAGAAA                       | 24 |
| 15[120] | 31[135] | CAATCATATGTACCCTGACGAGAATGGTTT                 | 30 |
| 15[152] | 34[152] | AAGCCCCATGTTAAATCTGACCTGAAAGAG                 | 30 |
| 16[71]  | 29[71]  | CGTAAAACGAGAAACAAGCTACAATAAGAAC                | 32 |
| 16[103] | 29[103] | GTTAGAAGTGAATACCTTAAATCACGACTTGC               | 32 |
| 16[135] | 29[135] | GGTAATCGCATCAATACAACATTTAGGAAT                 | 30 |
| 16[167] | 29[167] | GAGTCTGGTAAATTAAAAAATCTAATAACGCC               | 32 |
| 17[56]  | 32[56]  | TACATCGGAGAAATAAGTTACAAAATAAAAAAC              | 32 |
| 17[88]  | 32[88]  | ATTGCTTCTACCATAAATCCAATGAAAATA                 | 30 |
| 17[112] | 32[120] | AAATCAGTCAAATCACTAAAAGTAGGCTTGAGAACACCAG       | 40 |
| 17[152] | 32[152] | CTAGCTGAAGCAAACAATTGTGAACATTCACT               | 32 |
| 18[71]  | 27[71]  | ATGATGAAATAAATCATTATTTTCAATCAATA               | 32 |
| 18[103] | 27[103] | TTATTCATCTTCTGTAAACCGCACTCCAAGAAC              | 32 |
| 18[135] | 27[135] | GGTGAGAAAAAAACAAGAGGCTTGTAAT                   | 30 |
| 18[167] | 27[167] | CCTGAGTAGGAGAAGCTTACCAGACTGCGGAA               | 32 |
| 19[56]  | 30[56]  | AACAGTACACAAACATCCGGTATTTTTTATCC               | 32 |
| 19[88]  | 30[88]  | AACCTTGTTCAATTAACCTCCAGATTAGT                  | 30 |
| 19[112] | 30[120] | CTATTTTCGGTTGTACCAGGCCGGATTGAGATTATTACAGG      | 40 |
| 19[152] | 30[152] | CTTTTTCGATGTGTAGGCAGATACCGTTAATA               | 32 |
| 20[71]  | 25[71]  | GATTAAGATTGGGTTAGTTTATCAAGAATATA               | 32 |
| 20[103] | 25[103] | TTAGAATCGCAAATCCACAACATGATTCTGTC               | 32 |
| 20[135] | 25[135] | CTCAGAGCTGTTTAGGGTCTTTATGCATCA                 | 30 |
| 20[167] | 25[167] | GCAAAGAACTGAAAAGGAAAACGAGACTTCAA               | 32 |
| 21[56]  | 28[56]  | GGCTTAGGCGCTGAGATAGAAACCATCGTAGG               | 32 |
| 21[88]  | 28[88]  | TGCTGATCTTGAAAATATCATTTCATCGAGA                | 30 |
| 21[112] | 28[120] | GACAAGGTCAATAACCATAAAGCTGGGTAATATTGCAAAA       | 40 |
| 21[152] | 28[152] | GGCGCGAGTTAGCAAAGTCCAATACGACGATA               | 32 |
| 22[71]  | 24[56]  | CTTCTGACTTACTAGATAATTGAGAATCGCCA               | 32 |
| 22[103] | 23[111] | ACTTTTCCGTTATACAAATTTTT                        | 24 |
| 22[135] | 24[128] | TTAGATACTTAATTGAATTGCTC                        | 23 |
| 22[167] | 24[160] | ATTCTGCGATGTTTTACTCCAACA                       | 24 |
| 23[56]  | 26[56]  | AATCATAACTAAATTTGTAATAAGACAATAGA               | 32 |
| 23[88]  | 26[88]  | TCATATGAAATATATTAAAGTATTCAGCTA                 | 30 |
| 23[112] | 26[120] | GCGGATGGCTTAGAGCATTTTCGCAAAGCGGATCCCTGACT      | 40 |
| 23[144] | 26[152] | AATGCTGTAGCTCAACAACGAGTAGCCCGAAAGAATGACC       | 40 |
| 24[55]  | 21[55]  | TATTTAACTCGAGCCAAATGGTTTTAACCTCC               | 32 |
| 24[95]  | 21[87]  | AGCCAACGCTCAACAACAAAAGGTTTAGTTTATGTA           | 38 |
| 24[127] | 22[104] | CTTTTGATAAGAGGTCCAGAAGCAAATAGAACGCGAGAAA       | 40 |

|         |         |                                              |    |
|---------|---------|----------------------------------------------|----|
| 24[159] | 21[151] | GGTCAGGATTAGAGAAAAGAGGAAGATTTAGTCATTTGG      | 38 |
| 24[186] | 21[191] | GAACCAGACCGTTTTAATTTAACAGTTAATTCTACTAATAGTA  | 43 |
| 25[37]  | 23[55]  | CAGAGGCATTTAACGCCAATAAGAATAAACACCCGG         | 35 |
| 25[72]  | 23[87]  | AAGTACCGGTAGGGCTAAAAGCCTGTTTAGTA             | 32 |
| 25[104] | 24[96]  | CAGACGACATTCTTACCAGTATAA                     | 24 |
| 25[136] | 23[143] | AAAAGATTGTACCTTTCTGAATAT                     | 24 |
| 25[168] | 23[191] | ATATCGCGGAAGCAAAAATATGCAACTAAAGTACGGTGTC     | 40 |
| 26[55]  | 19[55]  | TAAGTCCTCGAGCATGAGAGTCAATTAATGGA             | 32 |
| 26[87]  | 19[87]  | ATGCAGATCTTTCCTCATAGCGGAGTGAAT               | 30 |
| 26[119] | 20[104] | ATTATAGTGCCAGAGGAAAAATTAATTTTCCC             | 32 |
| 26[151] | 19[151] | ATAAATCTGGATAGCATTAAAGCCTGTAATA              | 30 |
| 26[186] | 19[191] | AAATGCTTTAAAAATATTCAAAATCATACAACGCAAGGATAAAA | 43 |
| 27[37]  | 22[33]  | ATCCTAATTTAGAACAAGATACCTTTTGAAATACCGACCGTG   | 42 |
| 27[72]  | 22[72]  | ATCGGCTGACGCGCCTTATAACTAAATTTTCAT            | 32 |
| 27[104] | 21[111] | GGGTATTAGACAATAAAATCGCAA                     | 24 |
| 27[136] | 22[136] | GTTTAGACAAAAATCACTATATTTTTTGACCA             | 32 |
| 27[168] | 22[168] | TCGTCATAACAGTTCAGTGGCATCGATTCCCA             | 32 |
| 28[55]  | 17[55]  | AATCATTAAGGCTTATCAAGAAAAGTACCTTT             | 32 |
| 28[87]  | 17[87]  | ACAAGCATTTTAGCGCCTGAGCTTCGCCTG               | 30 |
| 28[119] | 18[104] | GAAGTTTTTTCATCAGGACGCGCAGAGGCGAA             | 32 |
| 28[151] | 17[151] | AAAACCACAACATAATGTAAAGACAACCGTT              | 30 |
| 28[186] | 17[191] | ACACTATCATATTACGAGGTATTTTAAAGGGTAGCTATTTTTG  | 43 |
| 29[37]  | 20[33]  | TCAGATATAGACCGCGCCCTACCTTTTAGTGAATTTATCAA    | 42 |
| 29[72]  | 20[72]  | GCGAGGCGAGCCGTTTATATATGTATAGCTTA             | 32 |
| 29[104] | 19[111] | GGGAGGTAAACCAAGTAATCGTCG                     | 24 |
| 29[136] | 20[136] | ACCACATTAAATAGCGTTATGACCAATAAAGC             | 32 |
| 29[168] | 20[168] | AAAAGGAAACCCCTCGTCTTTATTTTCAGGCAAG           | 32 |
| 30[55]  | 15[55]  | TGAATCTTATTTGCCAAGAAATTGATCATATT             | 32 |
| 30[87]  | 15[95]  | TGCTATTTTTATCCCTCAAAATCATCAATATAATCCT        | 37 |
| 30[119] | 16[104] | TAGAAAGAGTAAATTGGCAGAATAATGGAAGG             | 32 |
| 30[151] | 15[151] | AAACGAACTTTAATCAGAGAATAATCAGAA               | 30 |
| 30[186] | 15[191] | AGTCAGGACGTTAAGAACTTCAGGTCAATAAGCAAATATTTAA  | 43 |
| 31[37]  | 18[33]  | TCCAGAGCCTAACCAACGCCAGTAACACAAAATTAATTACAT   | 42 |
| 31[72]  | 18[72]  | CCATATTATTGCACCCATAACGGAAAAAGAAG             | 32 |
| 31[104] | 17[111] | CGATTTTTTTGAAGCCAAGTTACA                     | 24 |
| 31[136] | 18[136] | AATTTCAACTAACGGAATGATATTTTCAAAAAG            | 32 |
| 31[168] | 18[168] | TGCGATTTTGGAAGATGCCGAGATGCAATG               | 32 |
| 32[55]  | 13[55]  | AGGGAAGCAACAAAAGTACATTATCACAAACAA            | 32 |
| 32[87]  | 33[90]  | GCAGCCTTCAGAGAGATA                           | 18 |
| 32[119] | 15[119] | AACGAGTATGTTTAACGGATTATACTTCTTGT             | 32 |
| 32[151] | 14[139] | GAATAAGTAGGCTGGCAGCTCATTTTTT                 | 28 |
| 32[186] | 13[191] | TACCCAAATCATGACAAGATTGTTAAAGCCTTCCTGTAGCCAG  | 43 |
| 33[37]  | 16[33]  | TGAACACCCTGGCATTAGAGAATTATCCGTAGATTTTCAGGT   | 42 |
| 33[72]  | 16[72]  | CGCTAATATTACAGAGTGGCAATTTATTTGCA             | 32 |
| 33[133] | 16[136] | GACCAGGCGCAGCTTGCCCCGTTGATCGATGAAC           | 35 |
| 33[168] | 16[168] | AGTAATCTACGTAACAAAGATTGTTTGCCTGA             | 32 |

|         |         |                                             |    |
|---------|---------|---------------------------------------------|----|
| 34[55]  | 11[55]  | GCAAGAAACCTTTTTATAGAGCCGCAACAGTT            | 32 |
| 34[90]  | 35[90]  | ACCCACAAGAACAAAGTTACCA                      | 22 |
| 34[151] | 12[139] | GACAGATGATCCGCGAGGGAACAAACGGC               | 29 |
| 34[186] | 11[191] | CATAAGGGAACAACGAGGCGTGAGCGATGGGCGCATCGTAACC | 43 |
| 35[37]  | 14[33]  | CTTACCGAAGCCAATGAAATAGACTTTATTTTGCGGAACAAA  | 42 |
| 35[72]  | 13[84]  | TAGCCGAATTGAGTTAAAATCCTTTGCCC               | 29 |
| 35[133] | 34[133] | ATTGTGTCGAAAACGGTGTACA                      | 22 |
| 35[168] | 14[168] | TTAGCCGGCGAACTGACGCGTCTGATTTCGCAT           | 32 |
| 36[55]  | 9[55]   | ACCCAAAAGTATGTTACTGAACCTAACACCGC            | 32 |
| 36[90]  | 37[90]  | GAAGGAAACCGAAAGGTGGCAA                      | 22 |
| 36[151] | 10[139] | TTGTATCAAACGAAAGCGCACTCCAGCCA               | 29 |
| 36[186] | 9[191]  | TTATACCAAGCCTCATCTTGGGGACGAAAGCGCCATTCGCCAT | 43 |
| 37[37]  | 12[33]  | CTTATTACGCAGAACTGGCTGGCAAATTCAATAGATAATACA  | 42 |
| 37[72]  | 11[84]  | ACATACATAGGAAACGGGTATCTAAAAAT               | 29 |
| 37[133] | 36[133] | CACCAACCTAATCGCCTGATAA                      | 22 |
| 37[168] | 12[168] | CTAAAACAGCGAAACAGGTGTAGAGTAACAAC            | 32 |
| 38[55]  | 7[55]   | GTTTATTTCCAGCGCCCCGAACGATCTGGCCA            | 32 |
| 38[90]  | 7[87]   | CATATAAAAGACGATTGAGTGATAGCGAAAGCGT          | 34 |
| 38[151] | 7[151]  | CGTAATGCGCATCGGATTACGCCTTTTCCCA             | 31 |
| 38[186] | 7[190]  | TTCATGAGGAAGCTTTGAGGGGAAGGGCAGTGCCAAGCTTGC  | 42 |
| 39[37]  | 10[33]  | CATATGGTTTATGTCACAATCAGTATTCAAATATCAAACCCT  | 42 |
| 39[72]  | 9[84]   | CATTCAACAACGCAAACTGAGAGCCAGCA               | 29 |
| 39[112] | 8[96]   | GCGGGATCCAAGAATGGCTATTAGTCTTTAAT            | 32 |
| 39[136] | 38[133] | GAAAGACACACTACGAAGG                         | 19 |
| 39[168] | 10[168] | CTACAGAGGTTTCCATACCAGGCACGACAGTA            | 32 |
| 40[55]  | 5[55]   | TAGAGCCAAAGGCCGATTTACATTGAGTAGA             | 32 |
| 40[87]  | 5[87]   | TCACCGTTAGCAGCACATTTTGGGTAATAT              | 30 |
| 40[119] | 6[104]  | CCGCTTTTAGGAGCCTTTGAACAGGAAAAACG            | 32 |
| 40[151] | 5[151]  | TCGGTCGTGCTTTTCAATCATGTACGAGCC              | 30 |
| 40[186] | 5[190]  | TGACAACAACCTACCGATAAGAGGATCGGTGCCTAATGAGTG  | 42 |
| 41[37]  | 8[33]   | TTACCATTAGCGCAAAATCAGGGACATACCACCAGCAGAAGA  | 42 |
| 41[72]  | 8[72]   | ACCATCGACACCGACTTCTGACCTCCTAAAAAC           | 32 |
| 41[104] | 7[111]  | ACAGAATCAAATTATTAGACAATA                    | 24 |
| 41[136] | 8[136]  | TATCAGCTCTGAGGCTCGCCAGGGAGCTGGCG            | 32 |
| 41[168] | 8[168]  | CAGCTTGAATCGCCCAACGACGGCCGATCGGT            | 32 |
| 42[55]  | 3[55]   | GCCCCCTTACCCGGAATAAACCAGAATCAG              | 32 |
| 42[87]  | 3[87]   | TAGCGCGCCCTCAGACCACCGATTAAAGGG              | 30 |
| 42[119] | 4[104]  | GCTCCAAATTGTCGTGCGCCCTGAGAAGTGTT            | 32 |
| 42[151] | 3[151]  | TTTTTCTTTCTGTAAACCTGTATTGGGCG               | 30 |
| 42[186] | 3[190]  | TAGAAAGGAACACAGTTTCCGTTGCGCGTGAGACGGGCAACA  | 42 |
| 43[37]  | 6[33]   | ATAATCAAAATATTAGCGTCACTTGCTTGGCAGATTACCCAG  | 42 |
| 43[72]  | 6[72]   | AACCGCCTTTTTCATCGCCTTGCTACGCTCAA            | 32 |
| 43[104] | 5[111]  | ACCGCCACAAGTTTGCCGCCAGCC                    | 24 |
| 43[136] | 6[136]  | AAATGAATACGTTGAAACACAACAGTCATAGC            | 32 |
| 43[168] | 6[168]  | AACTTTCAAATAAAGAAGCCTGGCCCCGGTA             | 32 |
| 44[55]  | 1[55]   | AGCATTGATGATATTTCGCGCGTACAAAGCCGG           | 32 |

|         |        |                                            |    |
|---------|--------|--------------------------------------------|----|
| 44[87]  | 1[87]  | CGCCACCGCCAGAATACACCCGAGAAAGCG             | 30 |
| 44[119] | 2[104] | CTAAAGTTCAGAACCGTTTGTGTAGCGGTCAC           | 32 |
| 44[151] | 1[151] | CACAGACATTTTCAGCGCTGGTCCCTTATA             | 30 |
| 44[186] | 1[183] | CGTCACCAGTAATGTACCGCTGGCCCTGGTTGAGT        | 35 |
| 45[37]  | 4[33]  | ACGATTGGCCTCAGGAGGTTCTCGTTGTTGTAGCAATACTT  | 42 |
| 45[72]  | 4[72]  | TCATTA AAAAGAACACGAGGCCGAGTAAAAGA          | 32 |
| 45[104] | 3[111] | TGAATTTACCTCAGAGGGTACGCC                   | 24 |
| 45[136] | 4[136] | CCACCCTCAGCCCTCAGTTTGCGTCGTGCCAG           | 32 |
| 45[168] | 4[168] | AGGAACCCCAAACACTTTTCACCATCACTGCC           | 32 |
| 46[55]  | 0[41]  | AAGTTTTAATAAACAGTGCCGTAAAGCACTA            | 31 |
| 46[87]  | 0[80]  | GCTTTTGACCTATTAATCACCC                     | 22 |
| 46[119] | 0[104] | GCCACCCTAGGCTGAGGTCTATCAGGGCGATG           | 32 |
| 46[151] | 0[144] | CAGGAGGTTAGCGGGCGTGAC                      | 22 |
| 46[186] | 0[176] | ATAAGTATAGCAGTGCCGTCCAGTTTG                | 27 |
| 47[37]  | 2[33]  | ACAGTGCCCGTACGGGGTCTGACGGGGTATGGTTGCTTTGAC | 42 |
| 47[72]  | 2[72]  | ATTTCCGAATGATACAGGAAGGGACCGCGCTT           | 32 |
| 47[104] | 1[111] | GTATTAAGCCGTTCCAAGGGCGCT                   | 24 |
| 47[136] | 2[136] | GATTAGGATTTAGTACGGCAAAATTTGCCCCA           | 32 |
| 47[168] | 2[168] | GGCGGATACCGGAATACGAGATAGGAGAGAGT           | 32 |

## b) 5 nm design

| Start  | End     | Sequence                                 | Length |
|--------|---------|------------------------------------------|--------|
| 0[47]  | 42[27]  | TAAAGCACTAAATCGGGCCGGCGACGCCACCAGAACC    | 37     |
| 0[71]  | 41[71]  | AAGTTTTTAAGCGAAAACCGCCACTTAGCGTT         | 32     |
| 0[103] | 41[103] | ATGGCCCAAAGTGTAGAACCGCCTCACCGGAA         | 32     |
| 0[143] | 41[135] | GACTCCAACGTCAAAGCGCTGGTTATTATTCTTCAAGAGA | 40     |
| 0[175] | 41[167] | TTGGAACAAGAGTCCATTTGATGGCAGTTAATGCTCAGTA | 40     |
| 0[205] | 42[184] | GCCCGAGATAGGGTTTATAAATGTCAGTGCCTTGAGTA   | 38     |
| 1[21]  | 43[39]  | TGACGGGGAAAAACCTAAGCCGCCGCCAGCATTG       | 35     |
| 1[56]  | 43[63]  | AGGGAAGATGGGGTCGGGTCAGAC                 | 24     |
| 1[88]  | 43[96]  | GCGCTGGCCTACGTGAAAATAAATC                | 25     |
| 1[120] | 43[127] | GCGGTCCAGGCGAAAACGCAGTCT                 | 24     |
| 1[152] | 43[159] | AAATCCTGCTATTAATCATACA                   | 22     |
| 1[184] | 43[196] | AAAATCCCTGAGTGTTACTGGTAATAAGT            | 29     |
| 2[71]  | 39[71]  | TAATGCGCGCTAAACAACAGAATCCAAAATCA         | 32     |
| 2[103] | 39[103] | CGCTGCGCGACAGGAAACCATCGAAAGGCCGG         | 32     |
| 2[135] | 39[135] | CTGATTGCTGCCCGCTTACCGCCACTCAGAGC         | 32     |
| 2[167] | 39[167] | TTTTTCTTCCAGCTGCATAGGTGTGCAAGCCC         | 32     |
| 3[56]  | 42[56]  | GAGCGGGACGCTACAGCCCCCTTACCTCAGAG         | 32     |
| 3[88]  | 42[88]  | GGATTTTAGTAACCACATCAAAATCCCTCAGA         | 32     |
| 3[120] | 42[120] | GCGCTCACCCCTTACCGAGACTCCGAAACATG         | 32     |
| 3[152] | 42[152] | CTGTCGTGTTACACAGGGGGTTTTGCCCCCTG         | 32     |
| 4[71]  | 37[71]  | GTGAGGCCTTGCCCTGAATTGACGGGTCACAAT        | 32     |

|         |         |                                   |    |
|---------|---------|-----------------------------------|----|
| 4[103]  | 37[103] | GCCAGAATCTTGCTGGCATTCAACCCAGCGCC  | 32 |
| 4[135]  | 37[135] | CTGGGGTGTCGACTCTTCATAGTTAGACGTTA  | 32 |
| 4[167]  | 37[167] | ACATACGACGAATTCGTACAACGCTTTTGCTA  | 32 |
| 5[56]   | 40[56]  | AACATCACACCGAGTAAGAGCCAGAAGTTTGC  | 32 |
| 5[88]   | 40[88]  | CTATCGGCCCTGAGAACCATTAGCTAGCAGCA  | 32 |
| 5[120]  | 40[120] | CCTGCAGGCCTAATGACCGCCACCCCTCAGA   | 32 |
| 5[152]  | 40[152] | ACCGAGCTGCCGGAAGCAGGGATAATCACCGT  | 32 |
| 6[71]   | 35[71]  | AGGAAAAAACACGACCCGTAGAAAAAGGAAAC  | 32 |
| 6[103]  | 35[103] | CCAGAACACAACAGAGAAGACTCCATACCCAA  | 32 |
| 6[135]  | 35[135] | GTCACGACCATTGCGCGAAAATCTTGTATCGG  | 32 |
| 6[167]  | 35[167] | GATTAAGTAAGGGCGAAAGGAATTAATTTCTT  | 32 |
| 7[56]   | 38[56]  | CACCAGTCCGCTCATGTTTATTTTAAATTATT  | 32 |
| 7[88]   | 38[88]  | ATTCTGGCATATTACCATGGTTTACGATTGAG  | 32 |
| 7[120]  | 38[120] | CAAAGCGCGTTGTAAAGTCTTTCCAGCGTAAC  | 32 |
| 7[152]  | 38[152] | CTGTTGGGTGGGTAACGTATGGGACTGTAGCA  | 32 |
| 8[71]   | 33[71]  | GCACAGACCGAACCAGAAATAGCGCTAAT     | 29 |
| 8[127]  | 36[120] | CTTTCCGGCCTTTAATCCAAAAA           | 24 |
| 8[135]  | 34[123] | CCAGCCAGCTCCGTGGGCTTGCAGGGAGT     | 29 |
| 8[167]  | 33[167] | ACGACAGTATGGGATACCACGCATCGAAAGAC  | 32 |
| 9[56]   | 36[56]  | ATACCGAAAATATTTTGTACCAGATACATAC   | 32 |
| 9[88]   | 36[88]  | CAGAGGTGACCTGAAAATAACGGATTATTACG  | 32 |
| 9[152]  | 36[152] | GACCGTAATCGGCCTTCGAGGTGGCGAATAA   | 31 |
| 10[71]  | 31[71]  | GCAACAGATCAATATCATAAAAAGCCATATT   | 31 |
| 10[135] | 31[135] | GTCGGATTAAATTCGCGGTAAAATACGAAAGA  | 32 |
| 10[167] | 31[167] | ATCAACATCATTTTTTGGACTTTTTTCATCTTT | 32 |
| 11[56]  | 34[56]  | AACCCTCATGCCACGCTAATTGAGCAATAGCT  | 32 |
| 11[88]  | 33[100] | ATCAACAGAGGCGGTCACAAGAATTGAGT     | 29 |
| 11[96]  | 31[103] | TTGAAAGGATGAAAATACGATTTT          | 24 |
| 11[152] | 34[152] | AATCAGCTTAAATGTTCAGCAGAACCGAT     | 29 |
| 12[71]  | 29[71]  | TAACAACTTCGTATTAATTTTATCCGCGAGGC  | 32 |
| 12[103] | 29[103] | GGAAGGTTATTTTAAAAAGATTAGCGGGAGGT  | 32 |
| 12[135] | 29[135] | GGAAGATTATCTACAACGCGACCTACGGTCAA  | 32 |
| 12[167] | 29[167] | CCGGTTGAAGTCTGGAGTATCATCACTTTGAA  | 32 |
| 13[56]  | 32[56]  | TCGACAACAATAGATTAATAAACACAGGGAAG  | 32 |
| 13[88]  | 32[88]  | CGTTATTAATCTAAAAAATAAGAAAGCAGCCT  | 32 |
| 13[120] | 32[120] | TTTGAGAGGTATAAGCAACCTAAAACGTAATG  | 32 |
| 13[152] | 32[152] | TGCCTGAGTAATCAGATAAAACACCATGAGGA  | 32 |
| 14[71]  | 27[71]  | CCACCAGATATACTTCCATCGTAGAATCGGCT  | 32 |
| 14[103] | 27[103] | AGTAACATTACCATATTCATCGAGCGGGTATT  | 32 |
| 14[135] | 27[135] | ACCGTTCTCAACGCAATATTCATTGTGAATAA  | 32 |
| 14[167] | 27[167] | GACAGTCAATATATTTTTCATCAAAGAACGAG  | 32 |
| 15[56]  | 30[56]  | GTTTGGATAGGAGCGGTCTAAGAACTGAATCT  | 32 |
| 15[88]  | 30[88]  | TTAGAACCTATCATTTCCGACTTGTTGCTATT  | 32 |
| 15[120] | 30[120] | CTTTATTTAGCTGATAAGGCGCAGGCTCCATG  | 32 |
| 15[152] | 30[152] | GAACCCTCAATCACCAACTGACCAGCCTGATA  | 32 |
| 16[71]  | 25[71]  | GCGTAGATGATTGCTTAACAATAGAAAGTACC  | 32 |



|         |         |                                                   |    |
|---------|---------|---------------------------------------------------|----|
| 16[103] | 25[103] | TATTTGCAGCAGAGGCGTTCAGCTCCAGACGA                  | 32 |
| 16[135] | 25[135] | ACCAAAAACGAGCTGATTATACCATAAAACGA                  | 32 |
| 16[167] | 25[167] | AAGCCTCATAGTAGTAATTACCTTGGTAGAAA                  | 32 |
| 17[56]  | 28[56]  | ATTCGCCTTTTCAGGTCAATCAATGAATCATT                  | 32 |
| 17[88]  | 28[88]  | AAAATCGCCGTAAAACCTCCAAGAAAAACAAGCA                | 32 |
| 17[120] | 28[120] | TTGGGGCGCATTATGACTCATTCAACCCAAAT                  | 32 |
| 17[152] | 28[152] | TCTACTAAGAGCATAAGAAACACCGAGTAATC                  | 32 |
| 18[71]  | 23[71]  | ATGAAACAAATCAATAGAATCGCCTACTAGAA                  | 32 |
| 18[103] | 22[104] | TTCATTTCTGTAAATAAGCCAACGTTATACAAATTCTTAATCCAAAG   | 48 |
| 18[135] | 23[135] | CATTTTCGCCTGAATATAAGAGCAATAAAAAACC                | 32 |
| 18[167] | 23[167] | GAACGAGTAAATATGCCATAACGCAAGAAGTT                  | 32 |
| 19[56]  | 26[56]  | AGTACATAAACATCAAGAGAATATATAAGTCC                  | 32 |
| 19[88]  | 26[88]  | CCTTGCTTAATTACCTAATTCTGTAATGCAGA                  | 32 |
| 19[120] | 26[119] | CTTAATTGAAATGGTCTACGTAAAGTCAGGACG                 | 33 |
| 19[152] | 26[152] | CATGTTTTAGATTTAGTTATTACAATGCGATT                  | 32 |
| 20[71]  | 22[56]  | AACATAGCTTATATAAGTTAATTTTCATCTTCT                 | 32 |
| 20[103] | 21[119] | TATTAATTTCCAATCGCAAGAAAAAATCAGGT                  | 32 |
| 20[135] | 22[119] | TGCTCCTTTGACTATTTTCAGAAAACGAGAATG                 | 33 |
| 20[167] | 22[152] | CAACAGGTCATCAAAATCATTGAATCCCCCTC                  | 32 |
| 20[202] | 22[184] | CGAGCTTCAAATTCAAATAAGCGTCCAATACTGCG               | 35 |
| 21[24]  | 22[26]  | GTCTGAGAGACTACCTTTTGAAATACCGAC                    | 30 |
| 21[56]  | 24[56]  | AGGTTGGGGATAGCTTATCATAATATATTTAA                  | 32 |
| 21[88]  | 24[88]  | GATGCAAAAATTTTCCTCATATGCGCTCAACA                  | 32 |
| 21[120] | 24[119] | CTTTACCCTTGATAAGGACGACGACACTATCAT                 | 33 |
| 21[152] | 24[152] | GCGGATTGCAGGATTATTTTGCAACAAAAGGA                  | 32 |
| 21[184] | 23[196] | CGAAAGACGCGAACCATAGTAAAATGTTT                     | 29 |
| 22[55]  | 19[55]  | GACCTAAAACACCGGAAGATTAAGATGGAAAC                  | 32 |
| 22[103] | 19[87]  | AACGCGAGAAAACCTTTTTCAAATTTTAGTACTTAGAATGAATAA     | 44 |
| 22[118] | 20[104] | ACCATAAGTTTACCAAGGTCATTTTTGCCGC                   | 31 |
| 22[151] | 19[151] | AAATGCTGAGAGGCGAGAGTAAGCTCAA                      | 28 |
| 22[183] | 19[191] | GAATCGTCGGGGGTAAGACCGGAATACGGTGTCTGGAAGT          | 40 |
| 23[27]  | 21[55]  | TAAGGCGTTAAATAAGAATAATTTAATGGTTTTAACCTCCGGCTT     | 45 |
| 23[72]  | 21[87]  | AAAGCCTGATATTTTACTATATGTAAATGCT                   | 31 |
| 23[136] | 21[151] | AAAATAGCTTAAACAGATAGTCAGAAGCAAA                   | 31 |
| 23[168] | 21[183] | TTGCCAGAATAAATATAGATTAAGAGGAAGCC                  | 32 |
| 24[55]  | 17[55]  | CAACGCCAAGTAATAAGAAAACAAAATAACGG                  | 32 |
| 24[87]  | 17[87]  | GTAGGGCGTAAAGTGAGCAAAAAGTTAC                      | 28 |
| 24[118] | 18[104] | AACCTCGAAAAATCAATAACCTGTTTATTA                    | 31 |
| 24[151] | 17[151] | ATTACGAAACAACATTTGACCCATCAAT                      | 28 |
| 24[187] | 17[191] | ACATTCAACTAAAGTTGAGATGATTCCCATCCAATAAATCATAC      | 44 |
| 25[38]  | 20[24]  | TTTTCGAGCCACATGTAACTTTTTAACGCTGAGAAGAGTCAATAGTGAA | 50 |
| 25[72]  | 20[72]  | GACAAAAGTTAATTGATATGTGAGTCCTTGAA                  | 32 |
| 25[104] | 19[119] | CGACAATACCAGTATACGTGGATGGCTTAGAG                  | 32 |
| 25[136] | 20[136] | ACTAACGGGGCATAGTAATGCTGTCCTTTAAT                  | 32 |
| 25[168] | 20[168] | GATTCATCTGCAGATAAACTAAAGGCAAACTC                  | 32 |
| 26[55]  | 15[55]  | TGAACAAGGTAGAACTTAACGTCTCCTGATT                   | 32 |

|         |         |                                                |    |
|---------|---------|------------------------------------------------|----|
| 26[87]  | 15[87]  | ACGCGCCTTATCATAGAAATAGGAAGGG                   | 28 |
| 26[118] | 16[104] | TTGGGAACAAAGCTGCCCTGTAATACTTAAT                | 31 |
| 26[151] | 15[151] | TTAAGAACTGACGAAGCTAAAATTTT                     | 28 |
| 26[187] | 15[191] | TTCAACTTTAATTGGGCTTGGCAAATTATGCCTGAGTAATGTG    | 44 |
| 27[38]  | 18[34]  | TTACGAGCATAAAAATAAGGAGAAACAATTAATTACATTT       | 40 |
| 27[72]  | 18[72]  | GTCTTTCCTGTTTATCTGAATACCAGAAGATG               | 32 |
| 27[104] | 17[119] | AAACCAAGAACAACATGAAGCTATATTTTCAT               | 32 |
| 27[136] | 18[136] | GGCTTGCCCTGGCTCAAAAGGTGGATTAGATA               | 32 |
| 27[168] | 18[168] | TAGTAAATCATTGTGAGCATTAAACAATTCTGC              | 32 |
| 28[55]  | 13[55]  | ACCGCGCCTCCGGTATAATTATCACAAACAAT               | 32 |
| 28[87]  | 13[87]  | AGCCGTTGAACCTCTGCGGAAGCCCCGAA                  | 28 |
| 28[119] | 14[104] | CAACGTAACCGGAACGAATTAATGCCGGATTG               | 32 |
| 28[151] | 13[151] | TTGACAAGAACCGATCAATATAGGTCAT                   | 28 |
| 28[187] | 13[191] | GGCGCATAGGCTGATGAACGGGTGAGAAGAGAATCGATGAACGG   | 44 |
| 29[38]  | 16[34]  | AGAAGGCTTACAATAGCACAAATATAAAGATGAATATACAG      | 40 |
| 29[72]  | 16[72]  | GTTTTAGCTTTATTTTGAATAATAAGAAATT                | 32 |
| 29[104] | 15[119] | TTTGAAGCTACCGCACCAATTGCGGGAGAAGC               | 32 |
| 29[136] | 16[136] | TCATAAGGGAACCGGAGGATAAAAATCGGTTGT              | 32 |
| 29[168] | 16[168] | AGAGGACAGGCTGACCTAAATGCAAAGCAATA               | 32 |
| 30[55]  | 11[55]  | TACCAACGAGTTACAAAGAGCCGTAAATATCA               | 32 |
| 30[87]  | 11[87]  | TTGCACCCAATCCATATCTTTTTGGCAA                   | 28 |
| 30[119] | 11[127] | TTACTTAGAAGGCACCAAATATTTTCGTTAATATTTTGTTA      | 40 |
| 30[151] | 11[151] | AATTGTGAATACACAAAGCCCTTTGTGA                   | 28 |
| 30[186] | 11[191] | CAAAGTACAACGCGATTATGTCAATCAGGAACGCCATCAAAAA    | 43 |
| 31[38]  | 14[34]  | CTAATTTGCCCTAACGAGAGACTTTATCATATTCCTGATT       | 40 |
| 31[72]  | 14[72]  | ATTTATCCCAGCTACAAATCCTTTCAAAGAAA               | 32 |
| 31[104] | 13[119] | TTGTTTAACTTAAATCAGTGAGGGTAGCTATT               | 32 |
| 31[136] | 14[136] | GGCAAAAGTCGAAATCAGGCTATCGATATTCA               | 32 |
| 31[168] | 14[168] | GACCCCCAGGAGATTTGCAAACAAAGGCCGGA               | 32 |
| 32[55]  | 9[55]   | CGCATTAGTCAGAGGGTGAGAGCCCATTAAAA               | 32 |
| 32[87]  | 9[87]   | TTACAGAGATAACCCAGTATTAAAGATAAAA                | 31 |
| 32[119] | 12[104] | CCACTACGCGTCAAAAAATGTAAAAAATTTGA               | 32 |
| 32[151] | 9[151]  | AGTTTCCCGTCACCCGAGCGAGTGCGGATT                 | 31 |
| 32[186] | 9[191]  | AGAGGCTTTGAAACGAGGGCCTGTAGCTGGTGTAGATGGGCGC    | 43 |
| 33[38]  | 12[34]  | CTGAACAAAGACGGGAGATGAACCTCCAATAGATAATACA       | 40 |
| 33[72]  | 12[72]  | ATCAGAGAGAGAATAACTGGTCAGAGGAGCAC               | 32 |
| 33[123] | 12[136] | TAAAGGCCGCTTTTTCGGGATATTAAACGATTAAATTCAAAAAACA | 45 |
| 33[168] | 12[168] | AGCATCGGGGACTAAAAACCAATATATGTACC               | 32 |
| 34[55]  | 7[55]   | ATCTTACCCGAACAAATGAATGGCGGCAGATT               | 32 |
| 34[100] | 7[87]   | TAAGCCCAATAATAAGAGCAACGCAATAGCGTAAGAAGGGAC     | 42 |
| 34[151] | 7[151]  | ATATTGCGCTTGCTTCAGGAAGTGCGCAA                  | 29 |
| 34[186] | 7[191]  | CAATGACAACATGATACCGCCAGTTTGGGCCTCTTCGCTATTA    | 43 |
| 35[38]  | 10[34]  | AGCAGATAGCGAAGCCCTAACATCGCAGCAGCAAAATGAAA      | 40 |
| 35[72]  | 10[72]  | CGAGGAAAGAAACAATCCAGCAGACACCGCCT               | 32 |
| 35[104] | 7[119]  | AAGAACTGAAAAGGAGCACCGCTTAAACCAGG               | 32 |
| 35[136] | 10[136] | TTTATCAGTCGCTGAGGAACAAACAACAACCC               | 32 |

|         |         |                                             |    |
|---------|---------|---------------------------------------------|----|
| 35[168] | 10[168] | AAACAGCTACCATCGCGGTCACGTCAGCTTTC            | 32 |
| 36[55]  | 5[55]   | ATAAAGGTGGAATAAGGAAATACCTTAGTAAT            | 32 |
| 36[87]  | 5[87]   | CAGTATGAATTCATGCCAGCCACTCAAA                | 28 |
| 36[119] | 6[104]  | AAGGCTCCGTTTTGTGCACGACGGCCAGTGTAT           | 32 |
| 36[151] | 5[151]  | TAATTTTATTTTCTGCCAGGGCCCGGGT                | 28 |
| 36[186] | 5[191]  | GAATAGAAAGGTCAACAGTGGATGTGCGTCATAGCTGTTTCCT | 43 |
| 37[38]  | 8[34]   | AAGACACCACGGCAACATTTTACATTTATTAGTCTTTAAT    | 40 |
| 37[72]  | 8[72]   | CAATAGAAATTAGCAAAAGTAATAAAATACGTG           | 32 |
| 37[104] | 8[96]   | AAAGACAAGCATGATTATAGCCGGCTGGTGAACCCCTTCTG   | 40 |
| 37[136] | 8[136]  | GTAAATGATTACGTTATTCAGGCATCGCACT             | 32 |
| 37[168] | 8[168]  | AACAACCTTAACAACATATCGGTGCGAGGGGACG          | 32 |
| 38[55]  | 3[55]   | CATTAAAGTGGGAATTAAAGAGTCTAGAATCA            | 32 |
| 38[87]  | 3[87]   | GGAGGGAACCATTAGTGTTTTATTAAAG                | 28 |
| 38[119] | 4[104]  | GATCTAAACCTCAGAAGTGAGCTAACTCATAC            | 32 |
| 38[151] | 3[151]  | TTCCACATCATTTTCATAAAGGGGAAAC                | 28 |
| 38[186] | 3[191]  | TTTCGTCACCACCCATGTACTCACAATATCGGCCAACGCGCGG | 43 |
| 39[38]  | 6[34]   | TTGAGCCATTGTGAATTATTCTTTGATACATTTTGACGCT    | 40 |
| 39[72]  | 6[72]   | CCAGTAGCAGGTAAATGTAGAAGAATTGCAAC            | 32 |
| 39[104] | 5[119]  | AAACGTCAAAGGGCGATAACCAAGCTTGCAATG           | 32 |
| 39[136] | 6[136]  | CACCACCCGACAGCCAGAGGATCTTTTCCCA             | 32 |
| 39[168] | 6[168]  | AATAGGAAGTACAACTAATCATGTGCAAGGC             | 32 |
| 40[55]  | 1[55]   | CTTTAGCGGGTCATAGGGCGCGTAAGAAAGGA            | 32 |
| 40[87]  | 1[87]   | CCGTAATTTTCATACACACCCCGCTAGG                | 28 |
| 40[119] | 2[104]  | ACCGCCACAAGAGGCTGCCTGGCCCTGAGTCA            | 32 |
| 40[151] | 1[151]  | ACTCAGGGATTAGCTGAGACGCAGGCGA                | 28 |
| 40[186] | 1[183]  | GATATAAGTATATAAGTGCTTGGGCGCAAATCGGC         | 35 |
| 41[38]  | 4[34]   | CGGCATTTTCTCAGACTGTTTCCTCGTTGTCCATCACGCAA   | 40 |
| 41[72]  | 4[72]   | TGCCATCTCAGTAGCGGGAGGCCGTATAATCA            | 32 |
| 41[104] | 3[119]  | CCAGAGCCCCAATGAACGGCATTAAATTGCGTT           | 32 |
| 41[136] | 4[136]  | AGGATTAGAGGTTTAGTTCCAGTCTGTAAAGC            | 32 |
| 41[168] | 4[168]  | CCAGGCGGAGCCCGGAATTAATGATCCACACA            | 32 |
| 42[55]  | 0[48]   | CCACCACCTTGAGGCAAGGTGCCG                    | 24 |
| 42[87]  | 0[72]   | GCCGCCATCACAAACACCATCACCCAAATC              | 30 |
| 42[119] | 0[104]  | AAAGTATTATGGAAGACCGTCTATCAGGGCG             | 32 |
| 42[151] | 0[144]  | CCTATTTAGTAAGCGAGAACGTG                     | 23 |
| 42[183] | 0[176]  | ACAGTGCCAGGAGTGTTCCAGT                      | 24 |
| 43[40]  | 2[34]   | ACAGGAGGCTCAGAGCACGTGGCGCTATGGTTGCTTTG      | 38 |
| 43[64]  | 2[72]   | GATTGGCCTTGATATCCCTCAGAGGAGCGGGGCCGCGCT     | 39 |
| 43[97]  | 1[119]  | CTCATTAAAGCCAGAACCACCGCGGAGAGTTGCAGCAA      | 39 |
| 43[128] | 2[136]  | CTGAATTTACCGTTCCCGGAACCTTGCCCCAGGGCAACAG    | 40 |
| 43[160] | 2[168]  | TGGCTTTTGATGATACCGTATAAATGGTTCCGCAGGGTGG    | 40 |

### c) 14 nm design labelled with Cy3

The following staples were modified at the 3' end with a Cy 3 label. The start and end positions correspond to the original unmodified staple shown in S9a.

| Start   | End     | Sequence                                         | Length |
|---------|---------|--------------------------------------------------|--------|
| 26[55]  | 19[55]  | TAAGTCCTCGAGCATGAGAGTCAATTAATGGAT <b>TTT-Cy3</b> | 32     |
| 27[168] | 22[168] | TCGTCATAACAGTTCAGTGGCATCGATTCCCAT <b>TTT-Cy3</b> | 32     |
| 34[55]  | 11[55]  | GCAAGAAACCTTTTTATAGAGCCGCAACAGT <b>TTT-Cy3</b>   | 32     |
| 35[168] | 14[168] | TTAGCCGGCGAACTGACGCGTCTGATTTCGCAT <b>TTT-Cy3</b> | 32     |
| 44[55]  | 1[55]   | AGCATTGATGATATTCGCGCGTACAAAGCCGG <b>TTT-Cy3</b>  | 32     |
| 45[168] | 4[168]  | AGGAACCCCAAACTACTTTCACCATCACTGCCT <b>TTT-Cy3</b> | 32     |

### d) 14 nm design modified with the ssDNA overhangs.

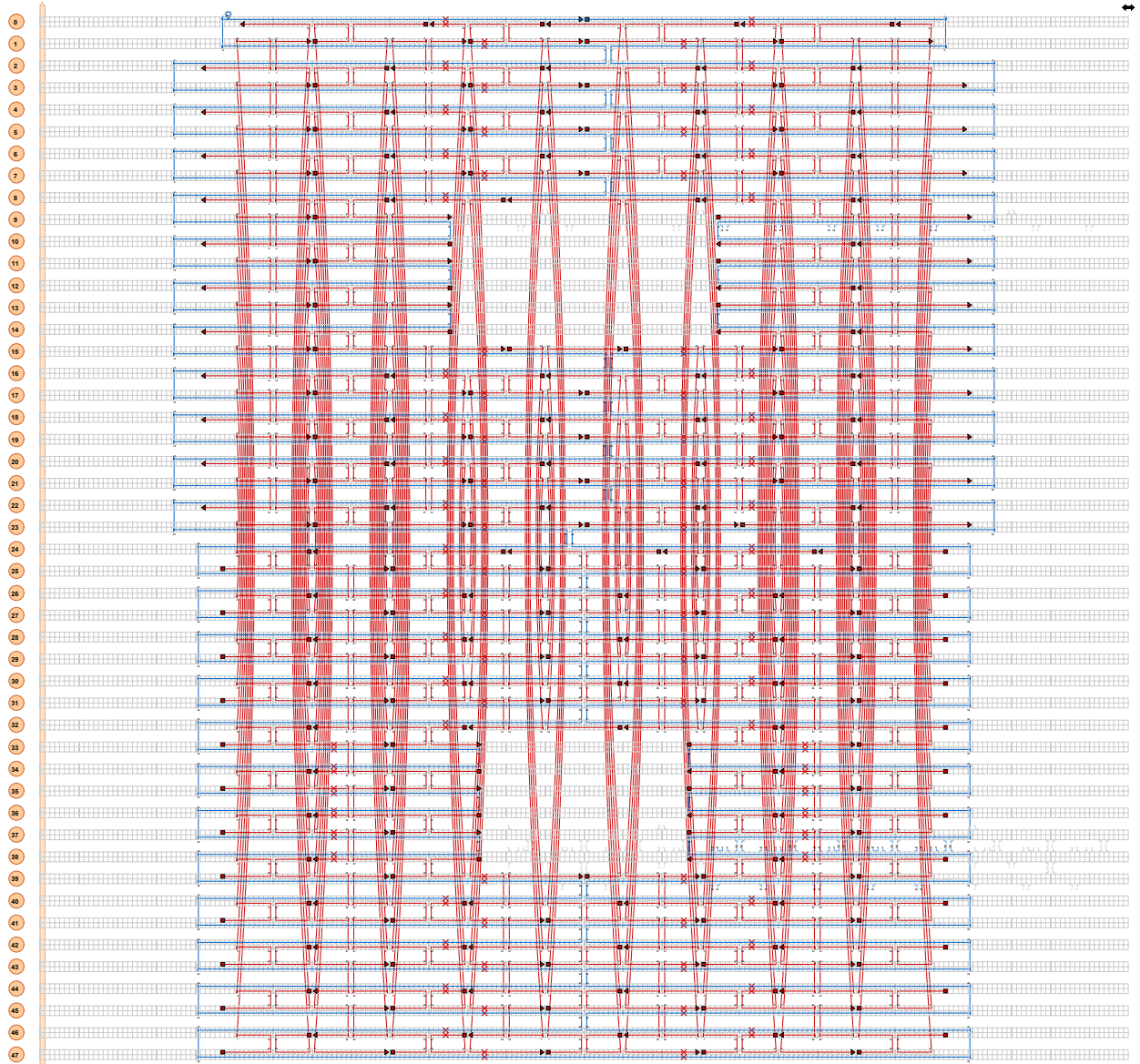
The following staples incorporated the ssDNA overhangs at the 3' end (shown in bold). The start and end positions correspond to the original unmodified staple shown in S9a.

| Overhang | Start   | End     | Sequence                                      | Length |
|----------|---------|---------|-----------------------------------------------|--------|
| A        | 34[151] | 12[139] | GACAGATGATCCGCGAGGGAACAAACGGCT <b>TTTATAT</b> | 36     |
| B        | 37[72]  | 11[84]  | ACATACATAGGAAACGGGTATCTAAAAT <b>GGGCGCG</b>   | 36     |
| C        | 34[151] | 12[139] | GACAGATGATCCGCGAGGGAACAAACGGCT <b>TTTAT</b>   | 34     |

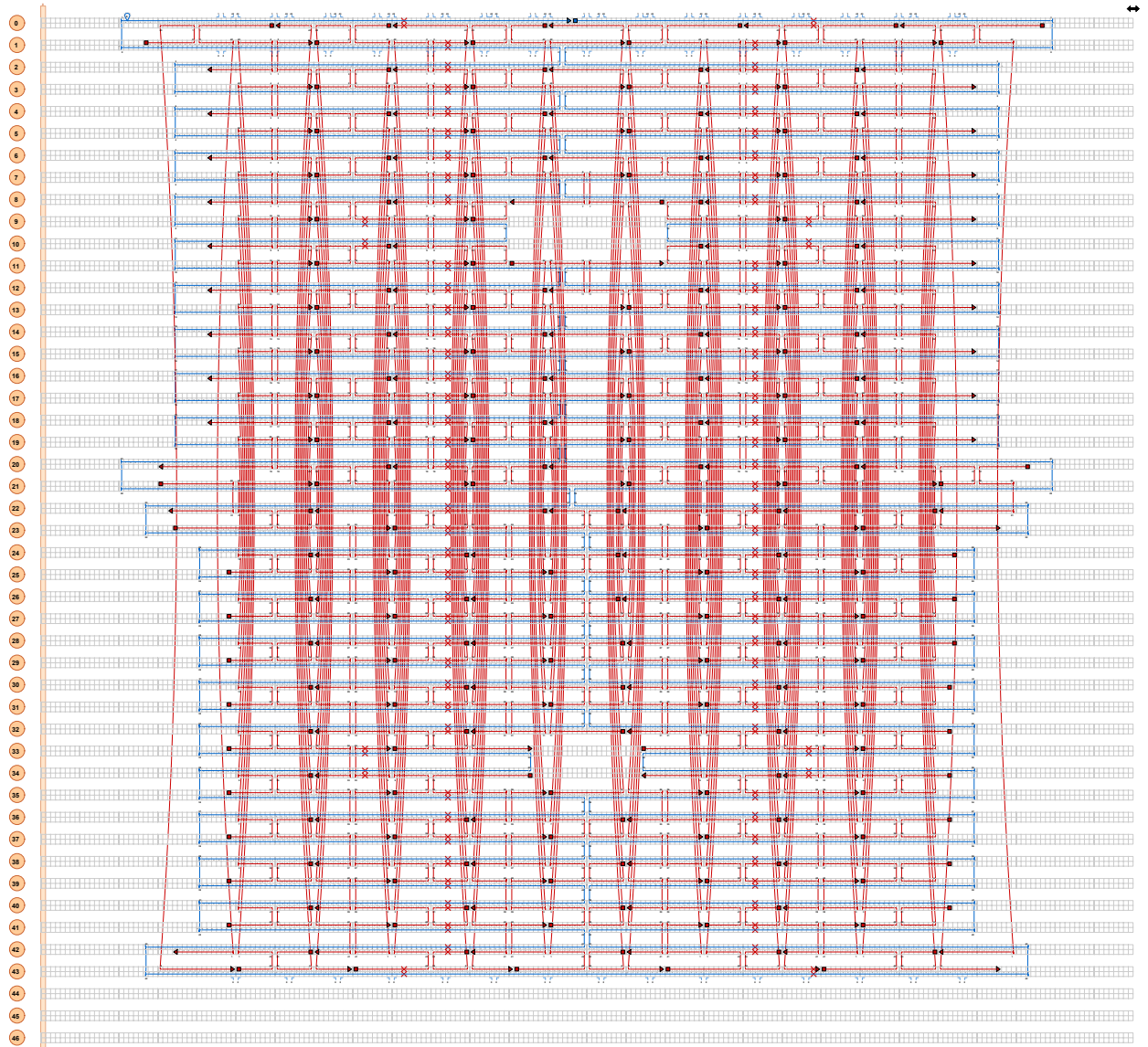
The sequences of the five preys are shown in the following table. The complementary overhang for each prey is indicated.

| Overhang | Sequence                                | Length |
|----------|-----------------------------------------|--------|
| A        | [T] <sub>23</sub> ATAT[T] <sub>23</sub> | 50     |
| B        | [T] <sub>24</sub> GCG[T] <sub>23</sub>  | 50     |
| B        | [T] <sub>23</sub> CGCG[T] <sub>23</sub> | 50     |
| C        | [T] <sub>24</sub> AT[T] <sub>24</sub>   | 50     |

# 14 nm pore design



## 5 nm pore design



## **9. Video showing ionic current and single molecule fluorescence detection of DNA origami nanopore trapping**

**Video 1.** We demonstrate a clear correspondence between the drop in ionic current (shown in Figure 2b) and the single molecule fluorescence detection of trapping at the nanocapillary tip. Fluorescence imaging is performed at 200 fps and the ionic current is acquired at 100 kHz. The two are synchronised at 100 fps. The presence of the origami nanopore at the nanocapillary tip is indicated by the arrow. Scale bar in the ionic current animation: *Y* axis is ionic current (1 nA) and *X* axis is time (1 s).