

Supplementary information

**Design, assembly, and characterization of
membrane-spanning DNA nanopores**

In the format provided by the
authors and unedited

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Supplementary Table 1: DNA oligonucleotide sequences of nanobarrel 6HB (J. R. Burns, *et al.*, *Nat. Nanotechnol.*, 2016, **11**, 152-158). All strands can be modified with a cholesterol lipid anchor on the 3' end via a tri(ethylene glycol) (TEG) linker.

Strand	Sequence (5' to 3')
1	AGCGAACGTGGATTTTGTCCGACATCGGCAAGCTCCCTTTTTTCGACTATT
2	CCGATGTCCGACTTTTACACGATCTTCGCCTGCTGGGTTTTGGGAGCTTG
3	CGAAGATCGTGTTTTTCCACAGTTGATTGCCCTTCACTTTTCCCAGCAGG
4	AATCAACTGTGGTTTTTCTCACTGGTGATTAGAATGCTTTTGTGAAGGGC
5	TCACCAGTGAGATTTTTGTTCGTACCAGGTGCATGGATTTTTGCATTCTAA
6	CCTGGTACGACATTTTTCCACGTTTCGCTAATAGTCGATTTTATCCATGCA

Supplementary Table 2: Oligonucleotide sequences of the 6HB nanopore (J. R. Burns, *et al.*, *Angew. Chem. Int. Ed.*, 2014, **53**, 12466-12470). All DNA sequences are listed from the 5' to the 3' terminus. * indicates locations of possible phosphorothioate (PPT) modifications.

Strand	Sequence (5' to 3')
1	ACA*G*G*A*T*T*TTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTTTTTGG CTATTCTTTTGATTTATAAGGGATTTT GCCGA*T*T*T*C*G*GAA
2	CAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATC*A*G*C*T*G*TTGTTTTCA A*C*A*G*C*A*T*C*C*TGTTTC*C*G*A*A*A*TCGGCATTAAAG*A*C*CAGCTG
3	TCT*C*A*C*T*G*GTGAAAAGAAAACCACCTGGCGCCCAATACGCTTTTTTC CCCGCGCGTTGGCCGATTCATTAATGCAGCTG*G*C*A*C*G*ACA
4	GGCGAA*A*T*GATTGCTTTCAC*C*A*G*T*G*AGATGT*C*G*T*G*A*C*G*T*GGA TTTTTCC*A*C*G*T*T*CTTTAATAGTGGACTCTTGTTCCAAACTGGAACA
5	TGTTCCAAATAGCCAAGCGGTCCACGCTCCCTGAGGGGCGCCAGGGTGGGA ATCGGACAAGAGTCCACTAAAATCCCCCAGCA
6	CATTAATTTTTTCTCCTTCACCGCCTGGGGTTTGCTTATAAATCAAAGGTTTG GACCAACGCGCGGGGAGCGTATTAGAGTTG

Supplementary Table 3: Oligonucleotide sequences of the 72HB DNA origami pore (T. Diederichs, et al., Nat. Comm., 2019, 10, 5018). Sequences of DNA staple, adaptor, and cholesterol-modified anchor oligonucleotides. All DNA sequences are from the 5' to the 3' terminus. The names of staple and adaptor oligonucleotides indicate their position within the 2D DNA map of the pore (see source paper, Figure S2) described as helix and base pair coordinate for the strands' 3' terminus. The name of two cholesterol-modified anchor strands indicates whether the cholesterol-TEG modification is attached to the 5' or 3' terminus. Adaptor strands have two sequence parts which hybridize either to the DNA staple strands or the cholesterol-modified anchor oligonucleotides (highlighted in bold). Adaptors that carry the latter sequence part at the 5'-end hybridize to 3'-cholesterol anchor oligonucleotides. Conversely, adaptors with the sequence part at the 3'-terminus hybridize to 5'-cholesterol anchor oligonucleotides. Adapter strand 21[64] features both a 5'- and a 3'-terminal hybridization segment.

Name	Sequence
	Staple strands
47[136]	GGAACAAATCATATATCCCACAAGCTTACCGAAGCTTGATTTCCGGTCCG
71[156]	AAATGTTACAAAATCGCGCAAAA
35[125]	TAACACCACCAGAGCCACC
44[190]	GAAGGGTTAGAACCTTATACTTCTGAATAA
45[115]	CATTAAGTAAGCATGAGCGCTCCCTGAACTCTGG
0[102]	CCAAGCGTTGAGCCAAGGTGAATGTCA
33[109]	GTGCATTAATTAGCTCGAATTCGTAATC
52[111]	AAAATTCGAACCAATACTCCCGACAAAGCACTC
62[135]	ACGGCCAGTAGCTGTTTCCTGTGTGAAATTGTTAT
65[149]	CTCAAGTGTAAGAATCATAACCGAGTAAAAGAACG
22[90]	TAAGAAGAAAATCTACAAAGCCGGAGACAGTCAAATCAC
4[94]	GGCTCCATTAATTGTCTGAAC
34[164]	TATTTTCAGAGCGGATGGTTGCTTTGACGAACGCT
32[122]	GGCTGAATTTAGCCTTGAGT
44[106]	AAAGTTACCAGATAAAAGAGGACTAAAGCGATTATA
48[156]	TGAGTCAGAAGGAGCGGAATTATCATCATATATAATCAGC
57[131]	CGCACTTCCAAGAAGATAAGTGTATAGCCGGATTAGGA
31[125]	AACAACATGTTCAAGAGAACGGAATAGG
25[133]	GATAATTTGCCTTGCTAAAGCGAATAAT
0[82]	AACTAGCAACGGCTACAGAGTC
10[118]	AACAGTGAGACTCCTCAGCTAATGCAGATAAGGCT
56[166]	AGAACCCCCAGTCACACCAATCAATCCT
70[130]	AGAACGTGGACATCAAGTTTTCAATTATTGCTCCTGCT
13[128]	ACCGGAACCAGTAGCGTAATT
31[112]	ATTTAACAAGCTGGCGAACTGTTGGGAAGGGCCGG
20[94]	AGAACTGATAAAGCTAAAGGGTGAGA
2[170]	AACCATCGCCCCACTACGACTTATTACACCAGCGC
43[141]	TTTATTTTCGCAATCAATAGGAGGGAGGGCACC
27[113]	ATCGAGAAGATGGGCGGAACAAACGGCGGATTGAC
10[83]	TATTTTCGGATAATCTTGACAATTATAT
62[114]	TGCGGGTTTTCCAGTCACATTACGCCACGCC
12[167]	CCACCCTCAAGACAAAGAGTC

24[122] CAAATAAGAAAATCGTAAAAACAAGAGAATC
25[157] CTAAGCATCACAATATCTGGTCAGTTGCACTAACGCAGCCTTTACAG
19[141] GAACGTAGAAATTAACGGGAATACT
52[193] CCACGCTGAGAAAGGAATTG
28[175] ATGTAGAAACGACCAGAATACCTACATTTTGATTA
1[120] CATGAGGATGCAGGGACGAGG
66[132] AACGCGCGGGGAGGCAACAGCCTACCTTTGTGCG
26[114] AACGGAACGCCGCCAGCTTT
56[185] CATTCTGGCCTCTTTAAT
20[111] GATAGCCGATCAGCTTCCGCTT
35[157] CGCAGAGCCACGCCACCCTCAGAACCGACCAA
13[141] CCCTCATAGGTAAATGCTGAACAATCGGCC
52[143] AACCTCAACCACCAGCGTATTCTATATTTTCACCT
32[90] TGCCTCATTAAATGCCCCCTGCC
19[125] AAATTAAGAAATGACCCTGTAATA
41[115] GTAGCGGAAATTAACGGAATAGACCCCCAGACTTTTT
64[164] AGCCGGAAGCATAGTTAGAATTAGTTAATCCAAT
57[108] GACAGCCGGAACCAGGCAAAGCGC
39[125] GCAATATTGACTCAACATGTTTTAAATATGCA
53[164] GGCGGTGAGTATTAACACCGGCGCGAA
31[88] GAATCGTCTGACTGGATAAACATGATAGTAC
4[146] CTAGTCAAAAACGTCTTTCCCCTCAATCCTTGCTG
7[57] ATGAACGGTGTACAGACTTTGA
37[133] AATTCAATATAAAAATCGG
1[155] AATGCACGCATAAAGAAGCTGGAAATAGCATATTTCAAAA
22[122] ACAAATATCAGTAATGCCGATTCAACCGTTCTAGC
30[138] CGACGGCTGGTAATATCCAGAACAATACGCTCAATAAT
54[110] CATCAACTCTCCGTGGCATCGTAACCGTGCATCTGC
14[162] TGATAGCGATAAATTACCTTAGCCCGAAGTGTTGTTCCAGTTTCACT
46[120] TGAGTAATGAATATGATGAGAGGGTA
2[135] CTGAGGCTAGTTTCCAATACATACTTGTCAAAAT
9[104] AGAGGCTGCCCGTATATCAGCCAT
45[133] CTTTTGCGGAAATAAAGATAACG
8[89] CGCCACCCTCAGAACAGGCGCATAGGTTTCATCAAGAGACCTATTATT
53[96] CATTTTTTCATTAAATAAAGGAATGAGAT
4[114] TTGCAATCCAAAATAAACAGAAGATTGATTTTGT
54[167] CTA AACACGCGCCCAAATCAGAT
42[190] GAGACCCAATTCTGCAGTACCTTTTACATC
61[91] CGATTAAGTTGGGAATCCCCCTGACCATAAATCA
47[108] CATCTGTAGGTAAGGGTAAT
0[170] CACCAACCTAAGAAACGTC
32[170] ATGAGCAATACAGTGTTTTTATAATCACACAATTCACG
50[132] CGGTACGATTTTTGAGAATTATCTTAAACAGCCC
12[110] CCAGCATTGAAAGCCGTAAATCAGGTCTTGCCCGCTTGGGCGCCAGGG
38[178] AATTCATTTGGCTTAGATGAAA
37[109] TTCCGGTCCACTTCACCAGT

10[164] CAGGAGTGTACTGGTAATAAGGGTTTTGCTCTGT
8[107] ACTCTAGACGCGCCTGTTTACTTCTGGTGTATCGG
15[141] ATTTACATAAATTTCCCTTCAACCGCCTGGC
68[171] GCGACCACACCCGCCGCGCTCTACAGGG
24[106] ATTTTGCGGGACTCATAGTCCACCACCCCGT
37[89] AAATATCTAGCGCGTTCACCGACCGAAA
29[155] ATCCCATAATCGGCCGTAACAATAGAAGGCCAG
16[170] AGACAAAAGGGAAAATTAATAAC
64[133] GCCTAATGAGTGAGGTCGTGCCGGTTTGAATTATA
24[98] TTAGACGTTAGCAAAAAAAAA
60[167] GAGTAGAAACCGTTGTCTGTTATACAAAAAGCCATA
13[109] CACCGGCAAAAAGATTAAG
17[125] ATTATAAAGGTACATCCAATAAATTGCGTAGA
15[69] TGCTCATTCAAGTGAATAAGTTTCATCGGCATTTTCGGTCACAACG
48[193] TAAATCCTTTTTATCAGA
6[129] AGCATTCCACATGGGATTAGTTA
18[148] GCAAATACCCAAACCGATATAACCGATAGAACAA
67[113] GAGACGGAGGCGGTTAGGTTGGGATACCGACGCAG
48[110] GCTATTTCTGAGAGAAAGTCAGATTTA
49[139] TAATATTAGACGGTGTTTAACAAGGAATTCAACTTTC
12[82] CAGACGATTGGCCTTGCATTA
0[134] AAAACACCACCAGTAGAAGGTAAAAAGAAGATTATTCAT
6[94] GATCTAATCTTTCCAGGAATACCGAAAGATTCCGG
59[91] CATTGCCATTCAAATGTTTAATAAATATAACAGTTAAGCCAGAATGAC
50[183] TAAATATCTTTAGGAGGCAAATCAACAGTTG
71[87] GGGCGATGGTTTTGCGGATG
4[162] GAATAGAAAGGTTGCGCCGAAAACAGGTAAGCCCAGTT
18[90] TGGTGGCATCATAAAGCCTCAG
40[190] AACGGATTCGCCTGAAACAGTTGATTAACA
37[149] CCTTATCAAATCAGAGCCCACCCTCAG
69[135] CAAAATCCCTTATAAATGGCGCTGGAGAAT
52[183] AGCCAGCAGCAAATGAAAAATCTACAATTTTATACCAACG
43[157] CAGTTGCACGTAAAACAGAGAAGCCTTATAGC
50[143] CCGTCAATATATCAAACAGAGCCTTAGTTGCTACT
29[165] AATTTACGAGCAGTACCGAAGCCATTGACTTGCCT
31[157] CAGAAAGTAATTCAGTACCAGGCG
59[108] GCGCAAAGGGGGATGTGCTGCAAGG
13[62] CAAATTAGCCCCCTTATTAGCGTTTGCAGGT
55[150] AGACAATATTTTTGAATG
39[100] GGATGAGGTCATCCCACTACGTGA
20[130] TTTTCGCAATAAAATCATACAGGCAAGGCAAAGAATAAA
50[91] TTGAGGCTATCAGGTCATTGTTGAGAGAATCTA
29[88] TTGCCAGACGAGAGGCACCGCCACATAGTAAGTAA
68[158] GCGCTAGCAAAAAGAATTTTTAATAAGAAAACCGAC
26[100] GAGGCCCTCAGAGTAGCGTAAC
41[147] GTACGAACCGATTAATAATTCAAGGCAAAAAGTAAATACGT

46[156] ATTTTTAGAACCCGAAACCACAACATTATCATTTTTGCAGA
23[157] ATAACTAATAGATTTAGAAGTATTAGTTTTAAAAATAATAAG
30[146] CCAAGAAAAATCGTCTGAAATGGAAGCCAGCTTTTCGAT
33[131] AGAATAATTTAATAGCTGCATTAATGGTGCTTTC
19[93] CTTATGCGAAATTAAGCAAATTCTACTA
48[175] GTTATTAACCTTTACAAACAATTC
34[138] CTAAACACCCGAGCCTGGGGT
36[178] GACGCTGAGAAGAACGCGACGCGT
29[101] TAAAGGAGGTTAAGTATTA
49[133] GATTGATAAATAGAGATAATTTAAATGCAATGCC
70[170] TTGGATAGCCGGCGAACGTGAAGGGAA
42[132] GGTCAATAACCTGTTTAGCTATAGCATTAGGCAA
67[157] GCGAGTGAATTTGAAAACAAACCATCGCAAGG
11[116] TCTCTTAATTGAGAATCTTGTAACGCCACTGCAGGTGCGACTCTAGAGGA
23[109] TCCAAAATCTCTAAATGAA
12[99] AGGAAAGCGGATTGCATCTTTGCGTATTTCCAGTC
3[64] TTGTGTCGAAATCCGCAAAGACAGCATATAAAGCCTGCGG
36[162] AATCGTAACCAAAGGAGCGG
58[124] CACCGTCAACAATACGGGTATCAGGGATAAGGCG
63[121] ATGGTCATGCCAAGCGGCGTTAAAGTAG
2[92] ACCCTCAGCAGCGGACAGCCTAAAGG
71[108] ACCATCACCCAATCCAACGTACCTTTAAAATAA
67[96] TTTTCTTGCTGGTTTGCC
43[105] ATAGTAGTATTTTCAAAGACACCTTCATTAATTTG
8[164] GATAAGTGCCGTCGAGAGGGTCCCATGTACTGTC
57[91] CAGTTTGAGGGGACAAAATAGGGGGG
16[155] ATTCGTGTCTGGTTTGACCATTAGATACTCAGGTTTTTA
44[167] AAAATTATATGAATATACAGTAACGAACGAGTCAA
46[190] GATGGCAATTCATCAATTCCTGAGCCCGAAC
1[72] AACGAGGGGGAGATTTGTATCATCAAAGC
18[114] CATAAGGAAACGTAAAGGGCTTTTCGATCACG
42[167] AGATTTAGAAGTTTCATTCC
11[133] CCAACCAGAACCTATATGTCTGAGAGATGATTGCC
40[162] CAACGGAACCCTAAAGGGAGCCCCGAGACGGGGA
54[122] GTAATCAAAAATAATTCGCAATTGTAAATCAA
0[90] CAACGAGTAGTAAATTGCATTTGGGGCAATTGCTG
8[118] TGTATCACTCATTTTTAAACCAAGTACCAACCGAC
50[193] GAAGGTTATCGACAACCTCGT
6[140] ACAACAATAGGAATGATATAACCTGAACAGACGA
68[131] CCTGAGAGAGTTTGGTTCCGTGTGAGTGCCTGA
15[108] GGAATGCAGCTTCAAAGCGAGCAGGCGAGAAAAACCGTCTATCA
4[126] AATTTTTGGTGAATTAATGA
5[112] TTTTCTGTAGACAGCCGGTTTTGATAGCG
15[157] CCGAACGAAAGTATGGTTTGCAGTATGAACGT
55[132] CGTAGTCTGGCCGCCGTTTTAGAACGCGGCAAGCCCGCCTGT
63[155] CCGCTGTGAGGCCATTACTAGAAATTCCTTGCTTTTGATGATA

41[172] ATATTTGCTTTGTTACATTTAAGGG
21[101] CGTAAGATTCAAATCGGTTGTACCAATAGCA
71[128] TTTTGGGGTTCGAGGTGCCGTAAAGGGAACAAGCAAACATCGGAAA
5[144] AACAGTTTCAGTACAAATTTTGCACCTTATCCGAGA
57[119] CCTCAGGAAGTTGGTGTACAAGCAATTCCT
59[163] CCGCCCAAAGGTTAATAAGAGAATATAAATCAT
20[146] TATAATTGAGTGAAGCGCACATTTGAGGATTAGAG
46[167] CTGGGATACGCAAATTGTTTGGATACCATATCAGA
60[185] TAATAACATCCAACAGGAAA
56[154] ACCATCATTACTCGCCATTAACAGAGGTGA
22[172] AGAGAATAACATAACAATGACAAACAATGCATGATTA
61[140] TCTGTCCATCACGCAAATTAGAACTCAACGAGC
37[125] TATACAGAATCAGCAAATTCATCTTTAGTTT
41[104] AATATAATTTTGATAATAGAGAGTCAAAGGGCAAATCCTGT
62[185] AATCCTGAGATTCTTTGATT
34[148] ATCTTCATACATGACCAGTATGGCATTTTACTATC
67[136] CTTCAGTGTAGCGGTCCGACGTATATGCAAATTTTC
34[178] AACTTTTTCAATGTTTAGTACAAACATCACACGGAACGGTACGCC
60[145] GGCCTTGGCCTCTTCGCTGACGTTGTCGCTCAACATA
66[170] ACTGAGCTAAACAGGAGGCGATTTTAG
53[147] AGATAAAAAATACCGAACGAAATGATACGTGGCAC
63[91] TCCCCGGGTACCGGCGTTGCGCTC
38[114] CCTTTAGAGCCAAGTTTGCCTTTAGCGAAAAT
28[146] TTTTCGTAGGATGAAAGCGTAAGAGGATAGGTCACGAT
27[93] CAGTGAGCGAGAATCAGCT
28[140] TATCATCCTTATTTACATTGGCAGATTCATTCTG
54[176] CTGATAGCCGCTATTAGAACAGAGAT
65[111] GGGAAACCTCTAACTCAATAAATAATTGCA
50[98] ACCCATCAGTTTAATAAAAATCGGTTTAACATTTTA
6[162] AGTTTCGTCACCAGCGGAGCTAACGAGTGAAA
13[96] TCATAATCTCAGACTGGCGTTTTAATTCG
26[122] TTTAGCCTTAAACGTTAATTATAAGCAAATATTTAGAA
41[141] ACTGAGGCGAATGATGAAAAGTCCACTATTAA
6[64] AAGAGGACAGGCGCAGACGGTCAATTACTTAGCCGGAACGA
12[142] AGCCGCCGTAAGCGTCTGAC
55[88] TAACAACCCGTCGGATATTAATGACGACGATTAC
5[80] AACTGTCGAGTTTCGACAG
49[117] AGCACTAGCATGTCAATCAAAAAACAGGCCAT
58[185] CGCTCATGGATAATAAAAGG
59[131] CGGTGCAATAAACATGTAATTGGGTCAGTCCGTT
16[90] CCGTAGAGCTTGCGAGCTGAAA
9[137] TTAGCGGTTTTAACGTAGGCAGAAAAGCCAAAAG
69[113] TTGATGGGCAGCAAGTGTAATCTTAAC
38[146] CAGACCATTAGATAGCAGCACCGTAATCGCCT
25[91] CGCCATTTTGTATAATCAGAAAAGCCCCTATGT
2[105] TTGCGGGATCGGCTTTGAAACGGGCTTGAGA

36[114] CTCCGGAACCAGAGCCGCGG
Adaptor strands
33[64] **CTCCGTCTATC**TTTTTGTTCAGAAAACGAGAATCAAA
39[64] **CTCCGTCTATC**TTTTTTTTCAAACCTCCAACAGTATCA
25[64] **CTCCGTCTATC**TTATGACTGACCAACCCGCCA
27[64] **CTCCGTCTATC**TTCATAACCCTCGTTTAC
31[64] **CTCCGTCTATC**TTTTTATACTGCG
17[64] **CTCCGTCTATC**AGAAACACCAGAAAGTAC
19[64] **CTCCGTCTATC**TTAATCATTGTGAATTAC
29[64] **CTCCGTCTATC**TTTTTAAGAAGTT
37[64] **CTCCGTCTATC**TTTTTTTTGACTTC
35[64] **CTCCGTCTATC**TTTTTTTTATAGTCAGAAGCAGGTTGAGGCCATCTTT
23[64] **CTCCGTCTATC**TTTATCTCCATGTCATAAGGG
21[64] **CTCCGTCTATC**GTGAGGACGTTGGCGGAACAACATTT**GCTACGTCAGC**
65[93] ACTTACCCTGACTATTTTTTTTT**GCTACGTCAGC**
4[82] CTGTACAGGTAACATTCAACTATT**GCTACGTCAGC**
43[85] AGGTTTAATTTCAACT**GCTACGTCAGC**
45[85] AGCGCTCATTATACCAG**GCTACGTCAGC**
41[85] GCTGCTTGCCCTGACGG**GCTACGTCAGC**
9[85] CTGAGCGTCCATTTTT**GCTACGTCAGC**
7[80] CCCTCAGATTTTGCAATTTTT**GCTACGTCAGC**
11[71] TCACAAACAAATAAATCTTTAAACATTTTT**GCTACGTCAGC**
25[83] ATACAAGCAACACTATTT**GCTACGTCAGC**
67[93] TGGAGGAAGCCCGAAATTTTTTTTT**GCTACGTCAGC**
69[93] CCAACCAGACCGGAAGTTTTTTTT**GCTACGTCAGC**

Anchor strands

Cholesterol

3' GATAGACGGAG-TEG-Chol

Cholesterol

5' Chol-TEG-GCTGACGTAGC