## **Supporting Information**

## Bai et al. 10.1073/pnas.1215713109

## SI Text

The frequency-dependent fall-off of signal power in a cryo-EM reconstruction may be used to estimate the intrinsic variability in the underlying structure. After correcting for the modulation transfer function of the detector, a B-factor sharpening of approximately  $-1,000~\text{Å}^2$  gave a density map at the core of the object, as expected from the atomic model. This B-factor still comprises various components, among which errors in the rotational and translational assignments and intrinsic variability in the structure are assumed to be the most significant ones. From the tilt-pair validation plot in Fig. S4, we estimated the errors in the rotational assignments to be on the order of 2°. According to Henderson et al. (1) this estimate results in a B-factor component

 Henderson R, et al. (2011) Tilt-pair analysis of images from a range of different specimens in single-particle electron cryomicroscopy. J Mol Biol 413(5):1028–1046. of  $B_{rot} = (\Delta\Theta~D)^2/2200 = {\sim}400~{\rm Å}^2$ . On the basis of signal-tonoise considerations, we estimated the errors in the translational assignments to be on the order of  ${\sim}0.5$  pixel, or  ${\sim}1.8~{\rm Å}$ , leading to a B-factor component of  $B_{trans} = 8~\pi^2~(rmsd)^2 = 250~{\rm Å}^2$ . That then leaves a remaining B-factor component for the intrinsic structural variability (at the core of the object) of  $B_{structural} = 1,000 - 400 - 250 = 350~{\rm Å}^2$ , which corresponds to an rmsd in the atom positions of  ${\sim}2~{\rm Å}$ . The mentioned range of up to 3 Å in the main text is an attempt to reflect uncertainties in our B-factor estimation and in the empirical calculations related to the orientational errors. Additionally, as mentioned in the main text, the structural variability is larger at the periphery of the object than at its core.

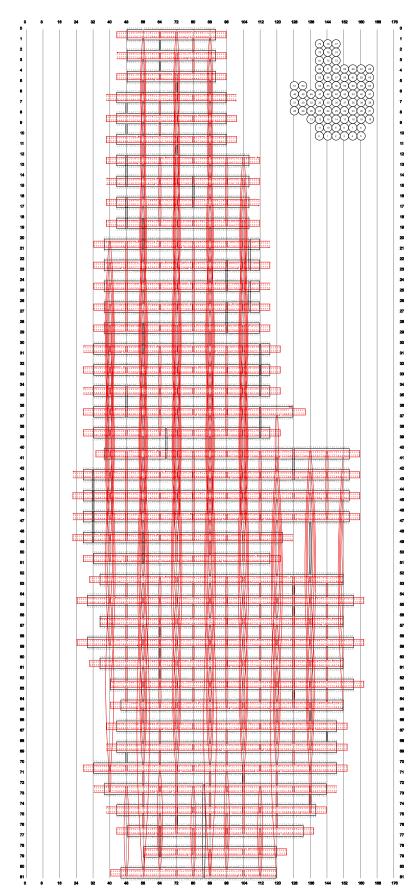


Fig. S1. caDNAno object design diagram. Scaffold strand path is depicted with black lines, and staple strands are shown as red lines.

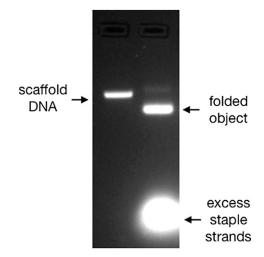


Fig. S2. Gel electrophoretic folding quality assessment. Photograph of an ethidium bromide-stained 2% agarose gel on which reaction products were electrophoresed.

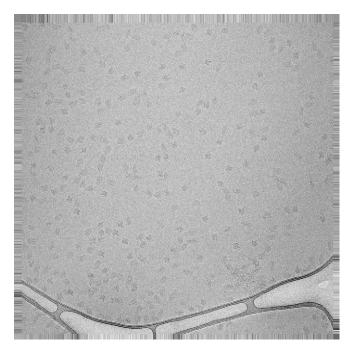


Fig. S3. Exemplary cryo-EM micrograph. The field of view is 1.45  $\mu m \times 1.45~\mu m.$ 

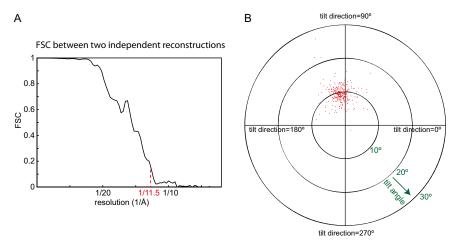


Fig. S4. Quality assessment of the cryo-EM map. (A) Fourier shell correlation (FSC) curve. (B) Tilt-pair validation plot. Note that 32 of the 342 particle pairs had tilt differences larger than 30° and are therefore not depicted.

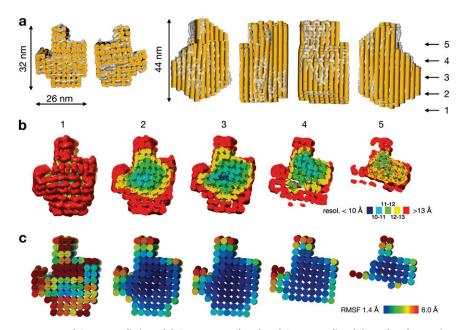


Fig. S5. Comparison of cryo-EM map and CanDo predictions. (A) Cryo-EM map (gray) and CanDo predicted shape (ocre) agree in overall dimensions and twist when assuming a reciprocal twist density of 10.44 bp/turn and an effective helical diameter of 2.6 nm. (B and C) The variation in resolution as estimated in the cryo-EM map (B) and the estimated root mean square fluctuation (rmsf) of the atoms in the shape prediction (C). Cross-sections are shown at five different slices (1–5) through the object, the positions of which are indicated with vertical arrows in A. Note that CanDo does not output a color scale bar for the estimated rmsf values. The scale bar shown reflects that colors are reported to range from dark blue (for an rmsf of 1.4 Å) to dark red (for an rmsf of 6 Å).

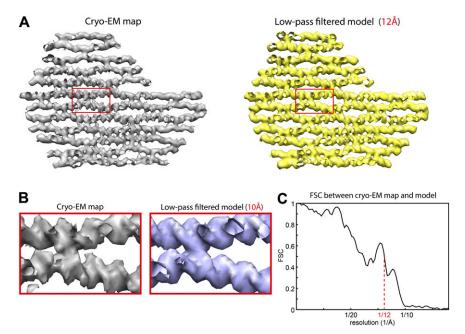


Fig. S6. Quality assessment of the pseudoatomic model. (A) Comparison of a slice (column 4) through the cryo-EM reconstruction and a 12-Å low-pass filtered map obtained from the pseudoatomic model. (B) As in A, but for a close-up of an area at the core of the object and a 10-Å low-pass filtered map obtained from the pseudoatomic model. (C) FSC between the cryo-EM map and the pseudoatomic model.

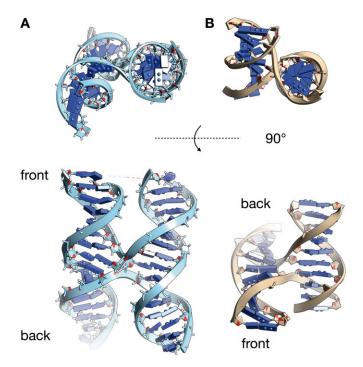


Fig. S7. Holliday junction conformation. (A) A typical Holliday junction in our structure (row 4, columns 4 and 5, base pairs 72–87). (B) Protein Data Bank ID code 1DCW. Both structures are depicted as ribbon-slab models. (*Upper*) The right helix in both junctions is pointing normally into the figure plane. (*Lower*) The right helix in both junctions is parallel to the figure plane.

Table S1. Sequence information

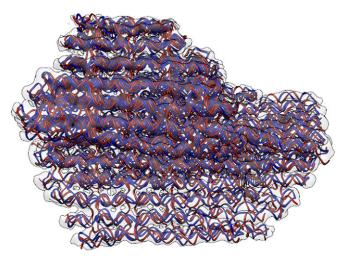
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3[43]	14[38]	TTTTTTTTTGGGTGAGACGGGCGAGTTTTTTTTTAAAGGGTCGTGCCGAGGATCCCCGGGTTTTT	67
3[78]	21[74]	AGCGAAATCGCCTGGCCCTTGCCCCAGAGTAAAATAACATCACTT	45
1[69]	16[86]	AAAATCAGCCCCGGCAGGCGAAAATCCTGACGCTGGTTGAGAGAGGG	48
5[62]	6[38]	GGGCCGTAAAGCACTAAATTTTTTTTTTTCGGAACCGGAAAGCCGGCGATTTTT	54
5[100]	8[82]	TTTTTAGCGGTCCTTTTTTTTTTTTTGATGGTGGTTCAATAGCCCTTGGGC	51
7[38]	22[54]	TTTTTACGTGGCAACAGCTGAGAAGTTTTCCTTTTTTTTT	56
3[81]	32[82]	GCGCGCGGGACGTGCTTCGCCGCTACAGCTTTCAATAGGAATTGCGG	48
9[90]	10[86]	AGGCGGTTTTTTTTTTTTTGCGTAGAGTTTTTTTTTTTATAGGGTTGAGTAAAGAACTT	58
10[52]	13[53]	AACCTAAGGGTTTTTTTTTAAGAAAGCGAAAGGTCACGCTAGCTCTTTTTTTT	67 48
10[85] 12[65]	30[86] 3[77]	GCGTTGAATGAGTGTAAAGTGTAATTGTTAAGGAAGATGATAATCATG	46 48
	32[106]	AACCCGCCGCCCCCTTTAATGAATCAGTTTGGCCTTATAAATCAAA TTTAACTCGCGTCTTTTTTTTTT	66
12[109] 13[54]	51[45]	CAAAACGTTAAAACTAGCTTGAGAGATCTGGAGCTCATTGAATCCCCCTCGAATCG	56
14[61]	10[53]	ACTCTAAGCTGCATTCCAGTCGCGTGAACCGTCTATCAGGGCGATGTTTTTTTT	67
1 <del>4</del> [01] 15[38]	33[53]	TTTTTTACCGGCGCGTAAACAACCCGAAATTTTTTAAAAATTCTGAGT	48
5[56]  5[62]	1[77]	CAGTGTAGCGGAGCGCGTAGAGCCCGAAATTTTTTAAAAATTCTGAGT	48
6[85]	44[86]	ATTTTATCCTCGTTCGTGCATCTGGTGTAGCACCAGCACTCAGAGCAA	48
6[111]	36[106]	TTTTTAACCAGAGGAATTTTTTTTTAAACGCTCCATCACCTCATTAG	48
8[64]	20[53]	GAGTAGAAGGTTGGGTAACGCCTTTTTTTTTTAGGGTGTTTTTTATCCA	48
18[93]	37[81]	TCTTTGATTAGTAAGAGTCTGTGGGCGATCCCAGGCAACAATAACCCA	48
20[52]	56[54]	GCTGGCAGCTTTCGCGCGAGCAACACCGCTCCTGATTTAGAACCTGA	46
20[61]	2[43]	ATTACGAATCAGTGCAGAATCCTGATTGCCTTCACCAGGTCGAGGTATCACCCAAATCAAGTTTTTT	67
	41[106]	TTTTTGCAACGCTAGTTTGACATATCTTTTTTTTTTTGGTCAGTTGGCACCG	52
21[48]	59[53]	GCGATTAATAGCTCAACTGGAAGTCTTTTGATCTATTATACCATCCTAGTCCTG	54
21[75]	60[86]	GCTGGTAATATCCGAATTGAGCCAATTCTAGGTCAGGAAAAAGATGC	47
22[53]	52[54]	ATCGGCCGGCGGATTCAGTATTGGCAAAGAGATGATTAACAATAA	48
22[65]	5[61]	GACTTCTGGTCGGTACGCAGGCCACCATTTAGAGCTTGACGGCTAAAG	48
22[77]	63[73]	CCAGTTCTGAGAGCTGTTTAGCATTGCATCATTAGAGAAA	40
23[46]	64[54]	GCCGAAAGGGTACGGTGTCATGTTTTGCGGATGGCTTAGAGCTT	44
23[78]	64[82]	AAGGTGCGGGGTTGATTCGAAGGTTACTTTAGGAGCACTAACGTTTTA	48
	21[116]	TTAGGAGGCCCAAATTAACCGTTTTTTTTTTTTGCAGCCATTTTTTT	47
24[69]	4[70]	AATGTGATGGGATACTGCAGGTACGGCCAGTTTTTCTTCTTCACCGGC	48
24[77]	60[78]	CAACATAATAAAGCGAAGATAATTGCACGTGATGATGGCATCGGAAGT	48
24[89]	0[80]	GCCAGGGCGCCGGAAGCAAGCTAACTTTTTTTTTTCACATTAAGTGTTTTTTTT	68
	42[106]	TTTTTTCAATCGTTGTACCAACCTAATTTTTTTTTAACATCGCCATTAAGATTTTCGAGCGG	63
26[57]	24[78]	GTTGGTCATAGCTGTTTCCTGTGTGAAAAGCCTGTTTTAACCAT	44
26[77]	56[78]	TATAAGCAAAAGGGGAAGCCTTTTTTTAATATTTCAATCATATGCGGG	48
26[97]	25[116]		55
27[90]	12[110]	CCATCACCAAAAACTCCGCTCACAATTCCACACTTTTTTT	40
28[80]	46[78]	GGCCAACAGATACGTGGTAATGCCGGGGAAGAAGG	35
28[105]	47[101]	CGATTAGTCTTTAATTTTTTTTTTTGCGCGAATATGATAACGGAACTGACGAGAAACACCAGTCAATA	68
30[61]	31[45]	CTATTTATGTCAATCCCTTCTGAAACAAGAGAATCGATCCTGAG	44
80[85]	51[73]	ATAAATCACAGACACCACATTCAACTAATGAT	32
31[27]	32[46]	TTTTTGGTCATTGGAATTTTTTTTTCGGTAATCGTAATATTTTGTGCAATGCTT	55
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1[91]	28[106]	TTTTTGAATGGCTACCAGTAAATTGGCAGATTCACCATTTTTTTT	53
32[45]	56[35]	TAGAACAATTACATAACAAACAATCATAATAGTACCGACAAAA	43
2[81]	51[93]	GATGAGAAAGGTAAATTGAAATCTACAAAAGAAGAGCAACACTATCAT	48
2[105]	58[110]	TATGTGAGTGAAGTTACAAGCCAACGATTTAACATAAATTGTAA	44
3[38]	45[41]	AATTATTTTTTTTAAATTCGCATTTCGGATTCACAGGCAATAGCATTAGG	52
3[54]	51[63]	AATTTAATCAGCTCATTAAATGTTTAATAAATATAAAGGAAT	42
5[27]	36[42]	TTTTTTAAATCATTCCTTTTTTTTTTGTGGGAACAAACTCAGGAAAATAGTAGTGAAAA	59
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86[93]	9[89]	AATGGTAGCGCCATATCGTAACAGAATCAGCACGTATAAG	40
86[105]	65[116]	ATAGACTTCAACCAGACCACCGCGCCTCCGGTATCTAACGAGCGTCT	47
37[58]	15[61]	GCAACATGAGGCGGTGACCGTAAGCGAGTACCACCA	36
37[82]	80[80]	GCAGCAGATTATCAAAAACAGATAGGCAGATTATACAAGACCTAAACTATATGTATCAATAG	62
7[122]	56[118]	CAAATATTTTTTTTTTTCAAACCCTCAATCATGCTGAACACCAGAAGAGGTTTAAAT	59
88[92]	7[100]	GAAAGAGAACAATGTTGGGAACCATCACGGATTAAAGTTGCAGCATTTTT	50
40[39]	21[47]	${\tt ATTTTTTTTTTTAAATATGCTTTTTTTTTAACTAAAGGGATTTTTTTT$	64
40[61]	14[62]	TTGCTCTTCATTCCCATTTGGGCGGCACCGCGACGACAGTAAAACGCG	48

Start	End	Sequence	Length (nt
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41[152]	60[146]	AACTTTTTTTTTAATTCGACAACTTTTAAAACATCGC	38
42[65]	18[65]	ATCAAAGCGGTATATTTTATATAACACCTCTTCGCTATCGGCCTTGCCT	49
42[105]		AACTTTGAGGTGCAGGGATTTCTTAATAATTTTTTAAAGTCAGATTTAT	48
43[22]	23[45]	TTTTTAAAATCAGGTCTTTGGCATCATTTTTTTTTTTTT	66
43[98] 43[131]	18[94]	CCCGAAACATTTCGGTAGATTTGCGCAACTATTACCGCTAGCAATACT	48 53
43[131] 43[152]	56[146]	CGAACGTTATTAATCGTATTATAAACAACTGAATTTTGTCGTCTTTCCAGACG GTTTTTTTTTT	38
43[132] 44[85]	71[73]	TTATTCGGAAACAGTTAGATTAAGACGCTGTTATAATTTAATGGGG	48
45[42]	62[40]	AAGGGTGTTTGGATATAGATAAATTTACGAGCATGTTTTTTTT	66
45[58]	26[58]	CATATCCAAAAGAAATTAGCAACGCAAGGAGTTAAATCTAAATT	44
45[142]	67[137]	AGATCATTTTTTCCATTACGCATAACGACAATGTAGAAAGGAG	44
45[152]	54[138]	ACATTTTTTTTTTCGGGAGAAACTCATTACCGTAATCTTGACAAGAACTGACCTTGTACAG	62
46[77]	24[70]	CTTGAGATGGTTTAAATTACCTTATTTCAAAATTAAGCTA	40
47[22]	33[37]	TTTTTACGAGACAAAATTCCTCATATTTTTTTTTTTTTT	42
47[94]	28[81]	AAAACGAGTAGCCGGAGAGTTCTAGCGAAAAAGCCTAAAAAGGGACATTCT	49
	17[111]		58
47[114]		TAACCTTCCCTTAGAATCCTTGCCAATCGCATATTTTAAGTACC	44
47[130] 47[153]		AATCGTCCGGATATAATAACGGACTGACCAGACGGTCAGTTACT	44
47[152] 48[34]	47[129] 30[27]	CAATTTTTTTTTATCAACGTAACAAAGCTGCTCATTCAGTA CTTTAAACTTTTTTTTTT	42 44
48[61]	26[78]	ACGCCAACCTGAAAGCGTAAGAAGATAGAACATATGTACCCCGGTTTG	44
	50[110]		54
50[109]		CAAAATAGCGAGAGGCTTTTGCGTTAATAATAGGAATAATA	41
51[46]	48[35]	TCGACTGGATAGCGTCCATTTTTTTTTATACTGCGAAATG	41
51[64]	12[66]	TACGAGGCCAGATACAAGGACGTTGAGAGGGTTAAAGATTCAAATATTAGCTCATTGCTGGC	62
51[74]	48[62]	AGTAAGTTTTGCCAGAGGGGGTAATAGTAATACCAGTCTA	40
51[94]	26[98]	AACCCTCGTTTTGAGATTAACGAACTATTCAACCCAGTCAAATTATTT	48
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52[65]	22[66]	GTTTTAGTATTACCTGAGAAAATTATAACAGAGGGTGCCACGTGAGGG	48
		AACATACAGTATAAAAATCGCGAATTGCGTAAAATACCATCTAAAGATGGAAATTCGCCA	60
52[125]		TAATTTTGCTTCTGTGAATAAGGCTTGCCCAACATTATTACTTTTT	46
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56[53]	72[32]	ATATAATACTAGAATGTGATAATTTTAACCCAAAGACAAAATTTTTTTT	70
56[77]	67[65]	CATTTTAAAGTACACAGCGATTCCCATGTATACCGAAGCCCTTTTTGA	48
56[93]	27[89]	GTAATTAATAAAGACAGAGGCGATAAAGCTTAATACTTCG	40
56[117]	81[105]	CGCCATCTCAACAGTTTCAAATAAGACAAAAAGACACCACGGAATAAT	48
		CAACTTTGAAAGAGGTTTTTTTTTACAGATGAACGGTCATCAAGA	46
57[35]	42[22]	GGTAAAGTAATTCGCTAATGCAGAACGCGCCTTTTTTTTT	71
		TAACGAAGGCACAGCCCTCATAGTTTTTTTTTTTTTAGCCCCACAAG	46
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	81[119] 47[113]		46 48
59[54]	81[55]	AACATGTTCATGTCCAGACTCATTTTAATAACGGTCACCGTCCGACATTC	50
60[77]	66[62]	CACCCTGTATCGGTAGGCTCCATTAGACGGTGTTTAACGTCAAAAATG	48
60[85]	70[82]	GGGATCCGAGGGTATTGACCCCACGGAGATCCCTCA	36
60[89]	24[90]	TTTAAGAGGAATATTCCTAATGAAAAGAACGAACATGGGCGCCCTGTA	48
		GATATAGATTTTGCAATCCTTTGATTTAGAAGTATTAGACTTTACATTAGTA	52
60[145]	57[151]	CCAAACGGGTAAAATTTTTTTTTTACGTAATGCCACTGCCGGAAC	46
61[98]	38[93]	GGTGAAGTTAAAGGGAATCATTGGAAGCAAGATTAGAGAATCAACAGTT	49
63[70]	20[62]	GCGTACCTTTTGAAATATTCTAAATATAATGCTGAACTCAAACT	44
63[74]	67[89]	GCCGTTGAACCTCCCAGCTACAATTTTATCCGATTTTTGAGAATTAAC	48
64[53]	68[38]	GAAGCCGTACCGCATTCCAAGAATAATCGGTAAGCAGATAGCCTTTTT	48
64[81]	69[93]	GCTTTATTTTCAAAAAATTATCAGCAGCTATCTCCGTAACACTGAGT	48
		AATTTCAACAGTTTCTTTTTTTTTAGCGGAGTGAGAAACAACAAC	46
65[54]	67[55]	TAGCAGCCTTTACAGTTTTTTTTTAGAGAATAACATA	38
65[98]	36[94]	CCAACGTCTAAGAACGCGAGGCAACTAATAACTCCAACGCGAACGACA	48

Table S1. Cont.

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66[61]	37[57]	AAAATAGTTGCTATCCTTATCACTCATCGAATAATATCGTCAGAAGCAATATAACT	56
66[105]	60[90]	CCCAATCCAAATAAGAAACTGAATCTAAAATCTCCATCGTAGCCGCTT	48
66[121]	37[121]	CACAGAGCCTAAGGAATTAGCAAATCTTCGGTCGGTTTTAATAAGAAACCCT	52
67[56]	45[57]	AAAACAGGAAGAAAAGCTGTCTTTATAAACAACAAGAAAATAATAAGAAC	50
67[66]	40[62]	AGCGCAAAAGGAGCTTTGCACCCGACTTGCGGGAGGTTTTAATTGCAA	48
67[90]	56[94]	TGAACAATAGCAATTTGCTTTCACTCATCTGCAACGGCAT	40
67[138]	66[122]	AGATAAGTAACTTTTTTTTTTGATCTAAAGTTTTTAGTTACAAAATAAA	48
68[135]	41[121]	AATTGAGTAATATCAGAACAACTAAATTTGCCCTGTATGGAGATATAGCA	50
69[38]	54[58]	TTTTTGAACACAGGGATAGCAAGCCCCACCACCGACGACAACCGAC	48
69[94]	47[93]	TTAACCGCCATTGTATCACATCTTCTATTCTTACGCGATAGCTACATA	48
69[114]	52[126]	CAACGCCTGTAGCAGTACTCAGCCGCGACCGAAAACTTTAGGGCTTAT	48
70[81]	79[77]	GATAAGACTCATAGACGGATATTCATGAGCCGCCGCCAGCATCGCCTC	48
71[74]	76[86]	CATGATACCGCCACGCACCATTACCATTAGTTTCATCGTAAACAGTGT	48
71[110]	75[117]	AGGTACCGCCACCAATGAAACCATCGAAGTTTGCCCTATT	40
71[122]	43[130]	GCCGTCGAGAGGGTCAGGCGCATGCTCCATATCATAAGTGAGGAAGGCGGAACAGCC	57
72[148]	71[121]	TTTTTACTCCTCAATTTTTTTTTTTAAGCAGTAGCGACAGAATCATAGCAGCATTAGGATGT	63
73[32]	55[37]	TTTTTCCAAGAAGGAATTTTTTTTTTACCGAGGATTAAATAAGAATTTTTTTT	64
73[62]	52[66]	ATGTGAATTAAATACCCAACCAGCGCTCCGGCTTAGGTTGGGAGAAGA	48
73[70]	63[69]	TACCTCAGAGAATAGGAAATACCAAGCTTTAATTCAGCAGCGGAACAA	48
74[89]	52[102]	GTCAAGGCCGCGTAGAAACTTATTACTTGTCACAAAATGCTGATGCAAAATAA	52
75[38]	77[61]	TTTTTTTTTTCATAATCCCTTGATATTTTTTTTTTTTTCACAAACAA	50
75[98]	61[97]	TGCCTATTAGCAAAGAAACGTCACCCTCAGCGTCACCAACTAAAACGA	48
75[118]	78[106]	ATGAGTGTACAGAGCCACTTTTTTTTTCACCCTCAGAGCCG	42
75[132]	60[134]	AAGTAGAGAAGGACCGTAATGTATCACCTTCCACAGACCAACCTAGTTGCGCCC	54
76[85]	75[97]	GCCTTGTCAGAGCCGCCACCCTCAGAACCGACCAGAACCC	40
77[62]	80[66]	AAACGATTGGAAAATCACGGTTGAGGAACCGATTGAGGGAGG	48
77[84]	81[101]	GAAAGCGCAGTCCGGGGTCATAATGCCCCACCACCACCAACATAAAGGTGGCA	50
77[122]	75[131]	CATACATGGCTTTTTTTTTTTTTGATGATACAGTCTGAAACATGA	46
	74[90]	CCCCACCCTCTGGTAATAAGTTTTAATCTGAATTTCAGACTGTAGCGC	48
79[56]	73[69]	CAGGTCAGACCGGAACTGACAGGACGGAACCACATTAAAGCACCAG	46
79[78]	73[61]	CCAGTAACAGGAGCCACCTCCTCATTGGTCATAGCCCCCTTAAGCAAA	48
80[65]	42[66]	TTAAAGAACTTTTGAAATCGCGAAACCGAGCCAGAAAGACAGCAATTC	48
80[79]	77[83]	AAAATTCAGAAGGTAAAAATTATTTGCCCGTAGCATTTTCAAAGCCAGAATG	52
81[102]		ACAGTTTATTGCAGTATGGTTAATTTTCGCCTGAACGCCAACTACAGAGGTTATCATCAG	60
81[106]	77[121]	ATAAAATTTTGCTCTTTCGGAACTTTAGCGTACCGTTCCAGTAAGCGT	48

The table lists the sequences of the 163 "staple" DNA oligonucleotides that form the object in addition to the M13mp18 scaffold DNA strand. "Start" and "End" refer to the location of 5' and 3' termini in the strand routing scheme (Fig. S1). The first number indicates the helix number; the second number (between square brackets) indicates the nt position within that helix.



Movie S1. The reconstructed cryo-EM density map (gray) is shown in gray, the pseudoatomic model in blue (scaffold strand) and red (staple strands).

## Movie S1