

Electronic Supplementary Information

for manuscript entitled

"A Four-Helix Bundle DNA Nanostructure with Binding Pockets for Pyrimidine Nucleotides"

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1. Other folding motifs tested

Given below are results from other, seemingly similar four-helix bundle motifs that were tested during our screen. Conditions for annealing and native PAGE analysis were those given in the Experimental Part of the main paper. A listing of the sequences is provided in Chapter 7 of this ESI.

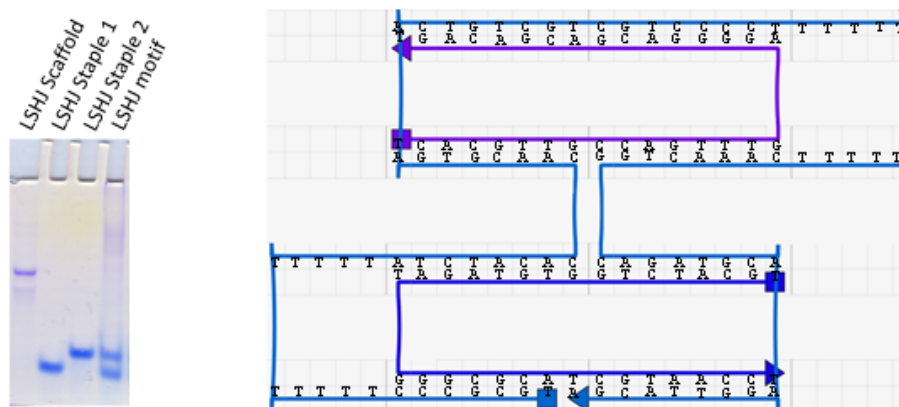


Figure S1. Native PAGE gel (left) and CaDNAo scheme (right) of LSHJ motif. The lack of a defined product band suggests that no proper folding of the structure occurred.

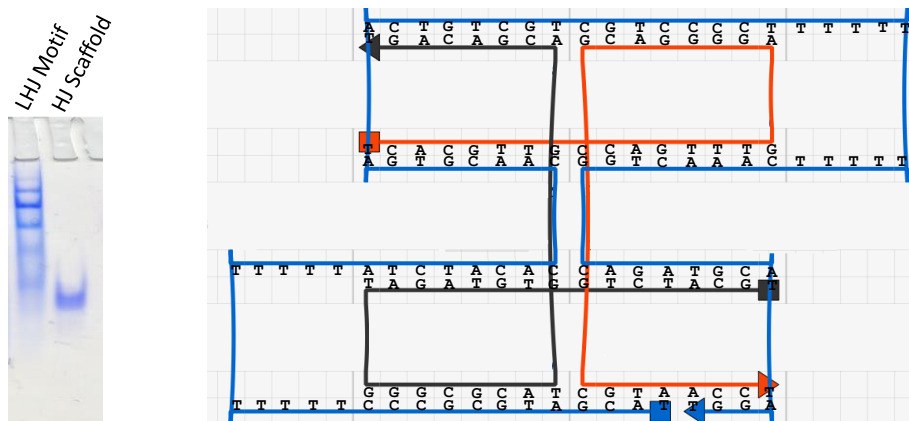


Figure S2. Native PAGE gel (left) and CaDNAo scheme (right) of LHJ motif. This gel shows several bands for higher molecular weight species, suggesting oligomerization during annealing.

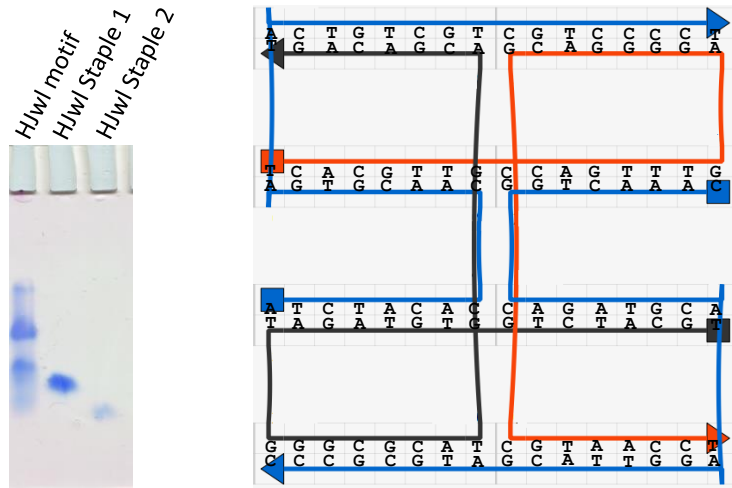


Figure 3. Native PAGE gel (left) and CaDNAno scheme (right) of HJwl motif. This motif does not fold in high yield and the gel shows a second band indicative of dimerization during annealing.

2. Native and denaturing PAGE gels of 4HB motifs

Shown below are gels for different versions of the 4HB family of motifs. For conditions, see the legend of Figure 3a/b, where the corresponding data is shown for 4HBt.

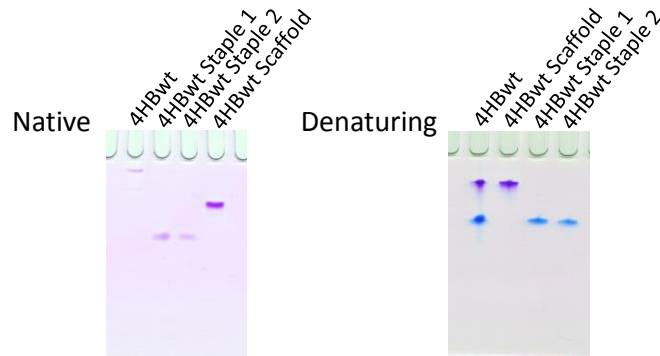


Figure 4. Native PAGE gel (left) and denaturing PAGE gel (right) for 4HBwt.

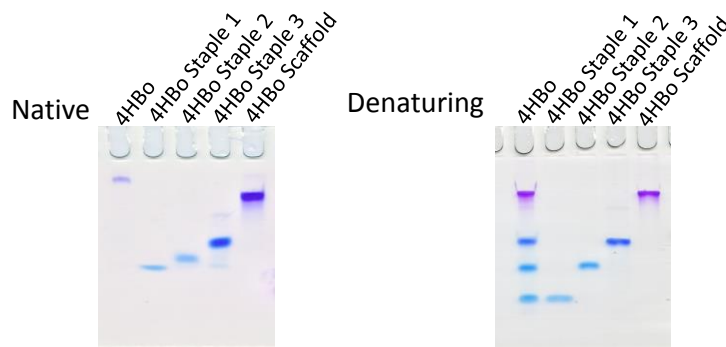


Figure 5. Native PAGE gel (left) and denaturing PAGE gel (right) for 4HB0.

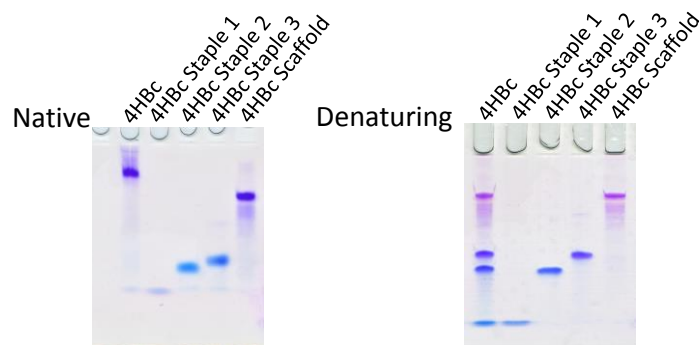


Figure 6. Native PAGE gel (left) and denaturing PAGE gel (right) for 4HBc.

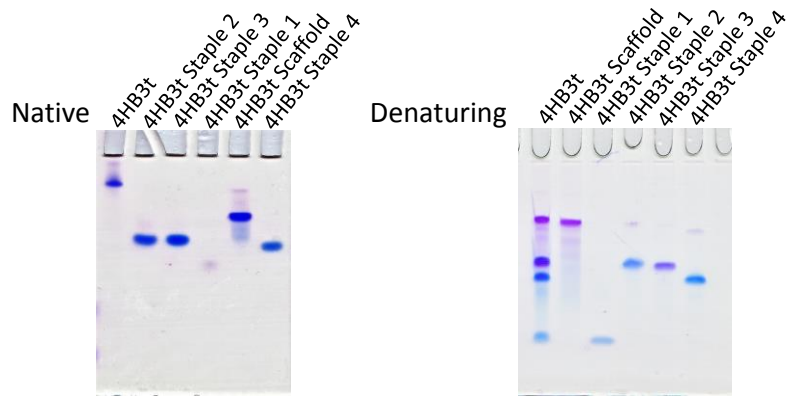


Figure 7. Native PAGE gel (left) and denaturing PAGE gel (right) for 4HB3t.

3. HPLC analysis of 4HBc

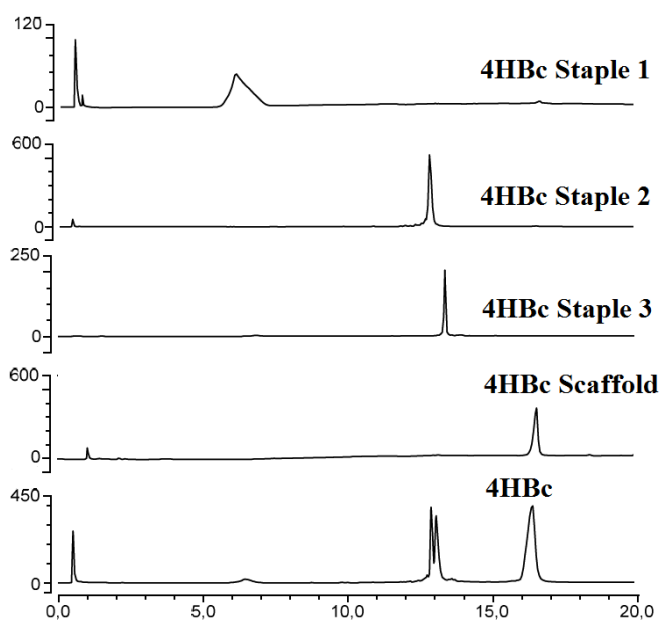


Figure 8. RP-HPLC traces of 4HBc, 4HBc Scaffold and 4HBc staples showing the composition of the assembly as designed. Conditions were identical to those given in the legend to Figure 3c.

4. Additional melting curves

Shown below are melting curves for the different motifs of the 4HB family, except for 4HBt, for which the equivalent data is shown in Figure 4b.

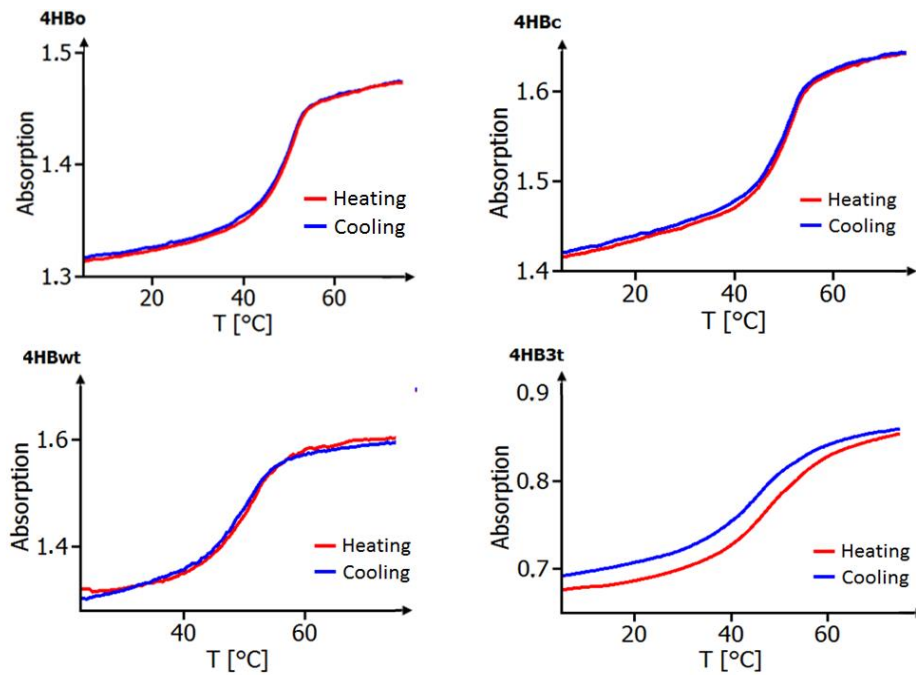


Figure 9. UV-Melting curves of 4HB motifs with or without binding site. For conditions see the legend to Figure 4b in the main paper.

5. UV-Spectra from binding assays

Shown below are representative UV-spectra from equilibrium filtration assays performed to determine dissociation constants of complexes between nucleoside/nucleotide ligands and DNA motifs. Binding was determined from the amount of ligand retained on the membrane in the presence of a binding motif. The spectra of eluates from filtration of solutions containing the respective motif (red line) or the control filtration lacking it (black line) are shown in each case.

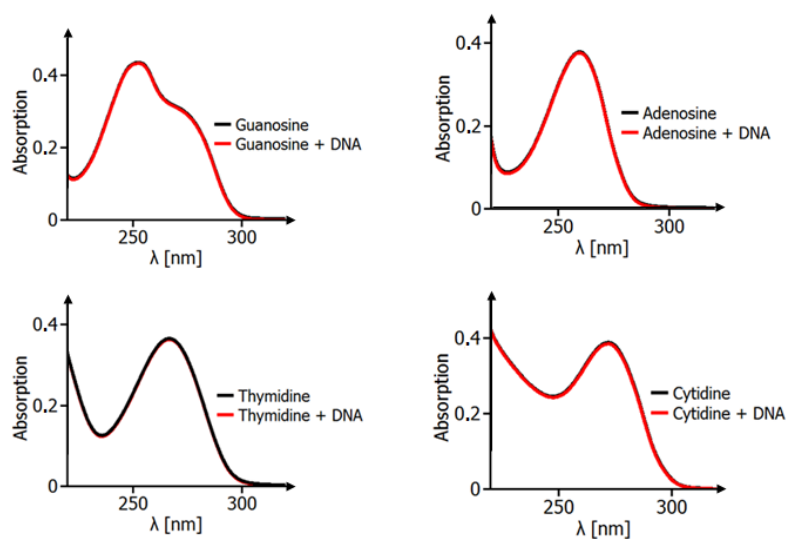


Figure S10. UV spectra from filtration assays with 4HBwt and deoxynucleoside ligands in Tris/EDTA buffer. Red curves are spectra of the filtrate of the solution containing the complex and black curves are spectra from the control solution containing just the ligand.

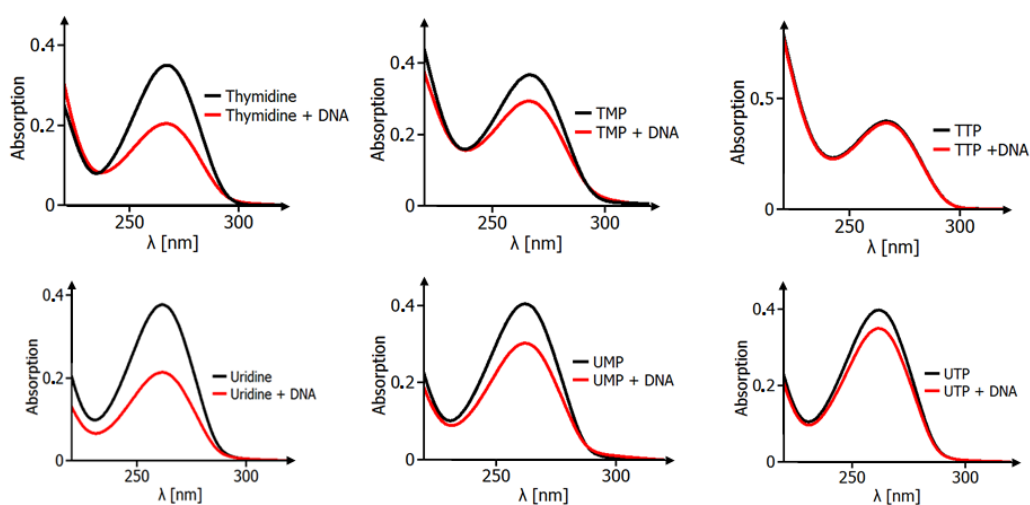


Figure S11. UV spectra from filtration assays with 4HBt and thymidine/uridine ligands in Tris/EDTA buffer.

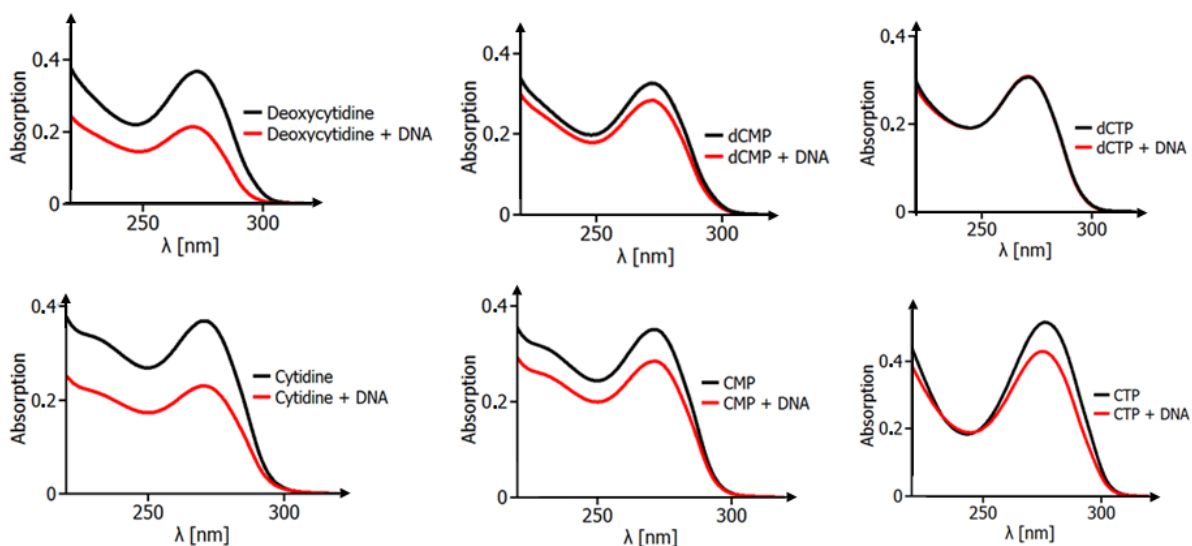


Figure S12. UV spectra from filtration assays with 4HBc and deoxycytidine/cytidine ligands in Tris/EDTA buffer.

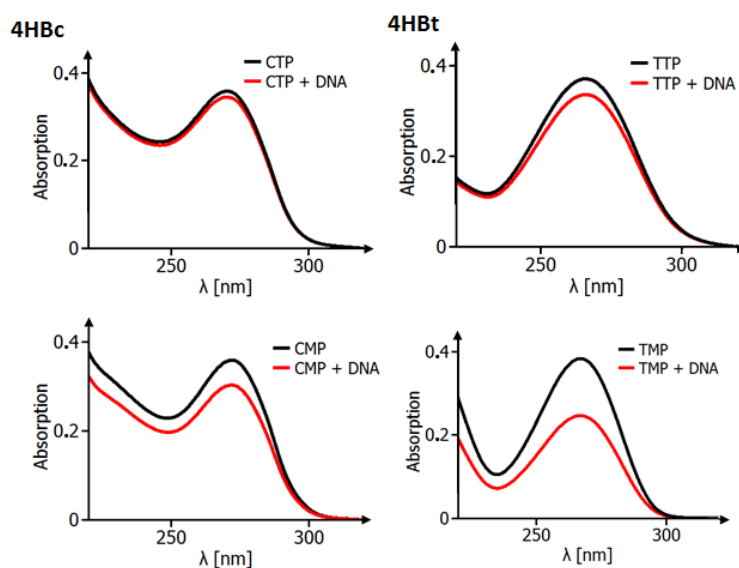


Figure S13. UV spectra from filtration assays with 4HBc (left) and cytidine/CMP or 4HBt (right) and thymidine/TMP in 'Binding Buffer' containing Ca^{2+} .

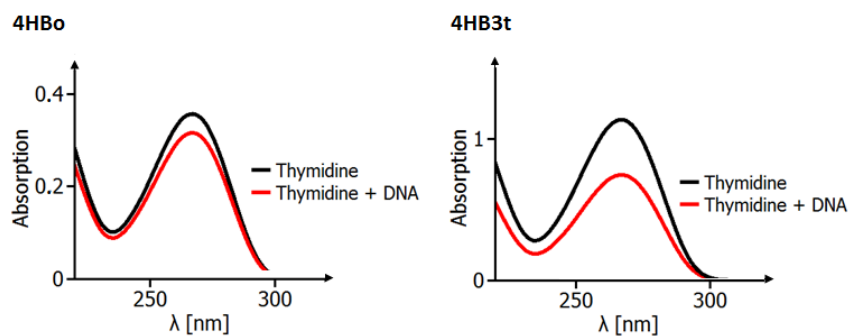


Figure S14. UV spectra from filtration assays with 4HBo (left) or 4HB3t (right) and thymidine in Tris/EDTA buffer.

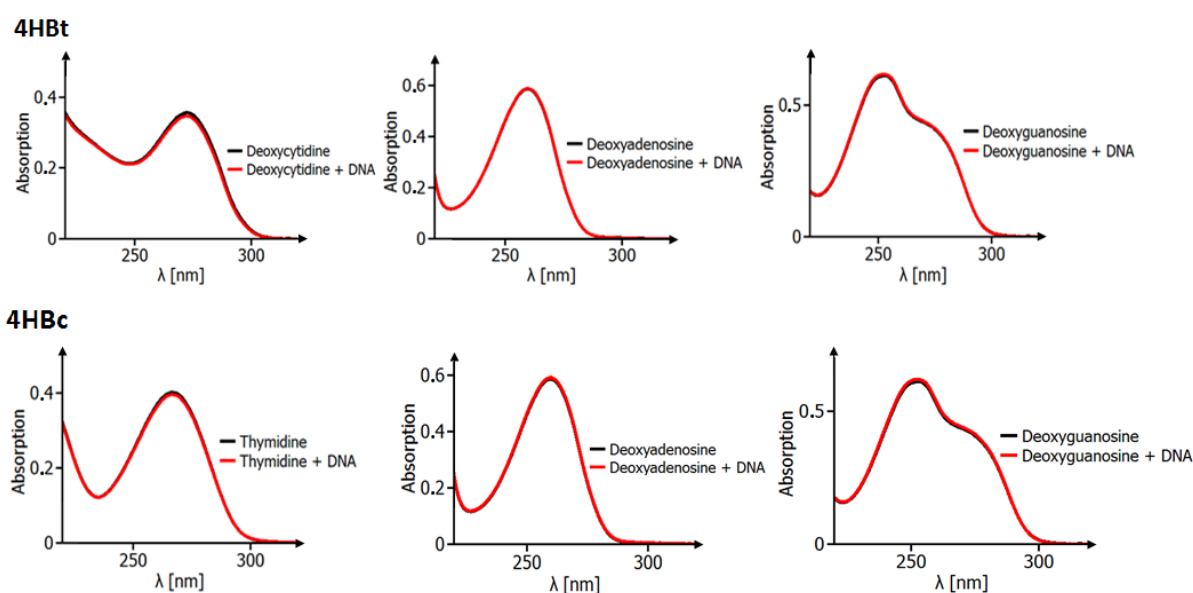


Figure S15. UV spectra from filtration assays with 4HBt (upper site) or 4HBc (lower site) and non canonical nucleosides as ligand in Tris/EDTA buffer.

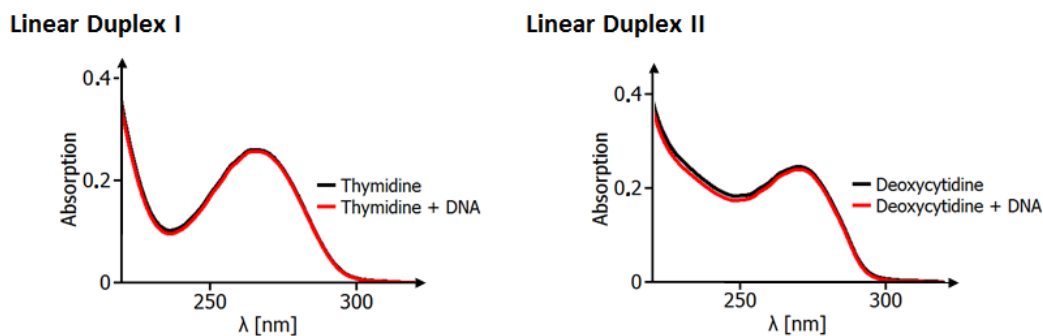


Figure S16. UV spectra from filtration assays with Linear Duplex I and thymidine (left) or Linear Duplex II and deoxycytidine (right) in Tris/EDTA buffer.

Table S1. Dissociation constants for complexes of four-helix motifs and cyclic nucleoside phosphates.^a

Motif	Ligand	K_d [μM]
4HBt	cTMP	130
4HBt	2',3'-cUMP	100
4HBt	3',5'-cUMP	115
4HBc	2',3'-cCMP	115
4HBc	3',5'-cCMP	130

^a Determined by equilibrium filtration and UV-absorbance of eluates at 40 μM concentration of both ligand and motif in Tris/EDTA buffer: 5 mM Tris, 1 mM EDTA, 20 mM MgCl₂, pH 8.2.

6. Additional TEM images

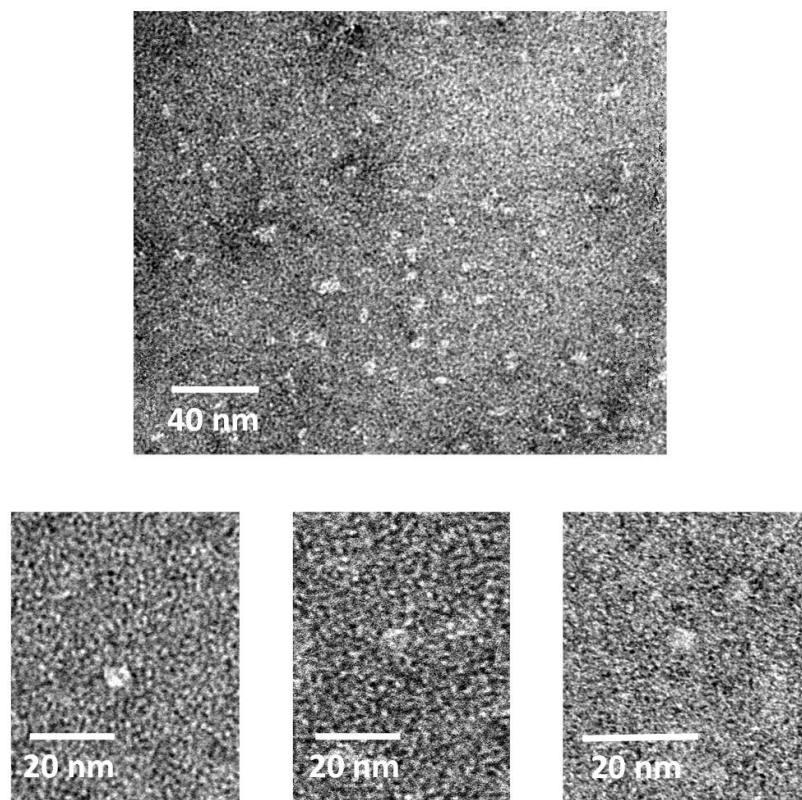


Figure S17. TEM images of 4HBt, stained with uranyl formate at two different magnifications with respective scale bars. See Figure 4a of the main paper for other TEM images measured under the same conditions.

7. Oligonucleotide Sequences

LSHJ Scaffold: 5'-TGCGCCCTTTTTTTTTTATCTACACCAACGTGAACTGTCGTCGTC
CCCTTT TTTTTTTTCAAACCTGGCAGATGCAAGGTTACGA-3'

LSHJ Staple 1: 5'-TCACGTTGCCAGTTTGAGGGGACGACGACAGT-3'

LSHJ Staple 2: 5'-TGCATCTGGTGTAGATGGGCGCATCGTAACCT-3'

LHJ Scaffold: 5'-TACGATGCGCCCTTTTTTTTTTATCTACACCAACGTGAACTGTCGT
CGTCCCCT TTTTTTTTCAAACCTGGCAGATGCAAGGT-3'

LHJ Staple 1: 5'-TGCATCTGGTGTAGATGGGCGCATAACGACAGT-3'

LHJ Staple 2: 5'-TCACGTTGCCAGTTTGAGGGGACGCGTAACCT-3'

HJwl 'Scaffold 1': 5'-ATCTACACCAACGTGAACTGTCGTCGTCGCCCT-3'

HJwl 'Scaffold 2': 5'-CAAACCTGGCAGATGCAAGGTTACGATGCGCCC-3'

HJwl Staple 1: 5'-TGCATCTGGTGTAGATGGGCGCATAACGACAGT-3'

HJwl Staple 2: 5'-TCACGTTGCCAGTTTGAGGGGACGCGTAACCT-3'

4HBwt Scaffold 5'-TTCCCATTTTTTTTTTCGGAGAATCCGACGGGTTTTTTTTTTTT
TTTTATAACTCATAACATTGTAGCGATGAAGATGAAAGATT
TTTTGGCTACAGGA-3'

4HBwt Staple 1: 5'-CCCGTCGGTGAGTTATTCTTTCATCTTCATCGCT -3'

4HBwt Staple 2: 5'-AATGTGTAATTCTCCGTGGGAATCCTGTAGCC-3'

4HBt Scaffold: 5'-TTCCCATTTTTTTTTTCGGAGAATCCGACGGGTTTTTTTTTTTT
TTTTATAACTCATAACATTGTAGCGATGAAGATGAAAGATT
TTTTGGCTACAGGA-3'

4HBt Staple 1: CATCGCT

4HBt Staple 2: 5'-CCCGTCGGTGAGTTATTCTTTCATCT-3'

4HBt Staple 3: 5'-AATGTGTAATTCTCCGTGGGAATCCTGTAGCC-3'

4HBc Scaffold: 5'-TTCCCATTTTTTTTTTCGGAGAATCCGACGGGTTTTTTTTTTTT
TTTTATAACTCATAACATTGTAGCGATGAAGATGAAAGATT
TTTTGGCTACAGGA-3'

4HBc Staple 1: 5'-ATCGCT-3'

4HBc Staple 2: 5'-CCCGTCGGTGAGTTATTCTTTCATCTT-3'
4HBc Staple 3: 5'-AATGTGTAATTCTCCGTGGGAATCCTGTAGCC-3'

4HBo Scaffold: 5'-TTCCCATTTTTTTTTTTCGGAGAATCCGACGGGTTTTTTTTTTTT
TTTTATAACTCATACACATTGTAGCGATGAAGATGAAAGATT
TTTTGGCTACAGGA-3'

4HBo Staple 1: 5'-TCATCTTCATCGCT-3'
4HBo Staple 2: 5'-CCCGTCGGTGAGTTATTCT -3'
4HBo Staple 3: 5'-AATGTGTAATTCTCCGTGGGAATCCTGTAGCC-3'

4HB3t Scaffold: 5'-GGTTTTTTTTTTTTTTTTATAACTCATACACATTTAATGTTGATGA
AAGATTTTTTGGCTACAGGA-3'

4HB3t Staple 1: 5'-CAACATTA-3'
4HB3t Staple 2: 5'-AATGTGTAATTCTCCGTGGGACTCCTGTAGCC-3'
4HB3t Staple 3: 5'-AAAAAAAACCGAGTCGGTGAGTTATTCTTTCA-3'
4HB3t Staple 4: 5'-CCCATTTTTTTTTTTCGGAGAATCCGAC-3'

Linear Duplex I

Strand 1: 5'- AGCGATGAAGATGAAAGA-3'
Strand 2: 5'-TCGCTACT-3'
Strand 3: 5'-CTACTTTCT-3'

Linear Duplex II

Strand 1: 5'-AGCGATGAAGATGAAAGA-3'
Strand 2: 5'-TCGCTACTT-3'
Strand 3: 5'-TACTTTCT-3'