

*Supplementary Information: “Rationally Designed DNA Looping Peptides Control DNA Topology,” by Gowetski, Kodis, and Kahn. Nucleic Acids Research, 2013.*

*Additional files provided in a zip archive: PDB files for models of LZD73 and LZD87 bound to DNA, PDB files for models of minicircle topoisomers formed from DNA loops, Microsoft Excel spreadsheet with the topoisomer distributions for the experiment of Figure 4.*

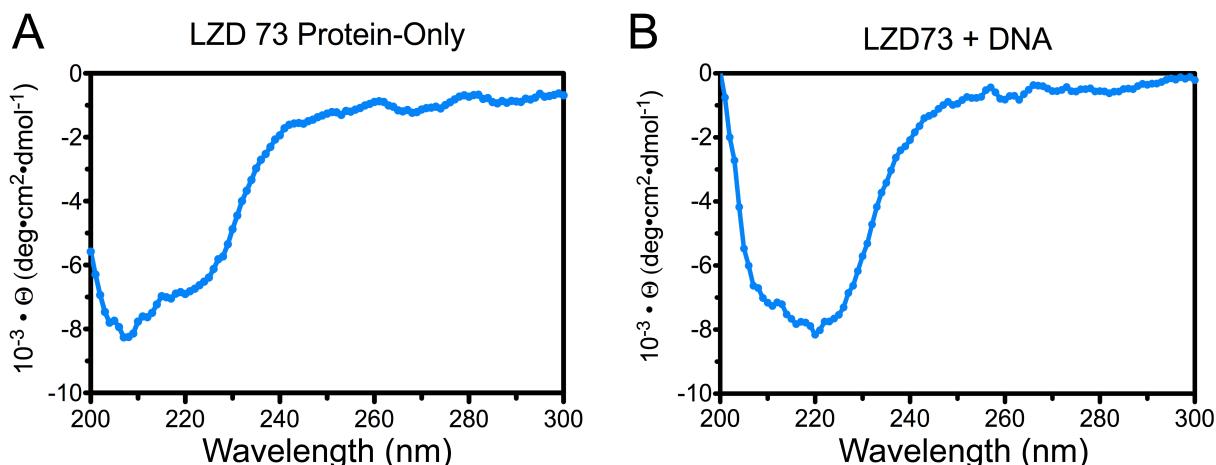
### SI Materials and Methods:

#### Protein Expression and Purification

The gene for LZD73 was synthesized commercially (BioMatrik Inc.) and was cloned into pRSET A (Invitrogen/Life Technologies) using BamHI and EcoRI restriction sites. The gene for LZD87 was cloned using standard methods by inserting an additional 42 bp (coding for 14 aa, two coiled coil turns) into the plasmid containing *Lzd73*. The peptides were expressed in *E. coli* BL21 DE3 (pLysS) cells, grown in LB media with ampicillin (100 µg/ml) and chloramphenicol (40 µg/ml) at 37 °C, induced with 0.5 mM IPTG during a 14 hour induction. Cells were lysed by French press in 10 mM MES pH 6.0, 0.5 M NaCl, 20 mM imidazole. The peptides were purified from the soluble supernatant by FPLC with a Co<sup>2+</sup>-charged HiTrap chelating affinity column (GE Healthcare) utilizing a 20-400 mM imidazole gradient. The peak elution fractions were concentrated using a Centricon Ultra 4000 (Millipore), and were buffer exchanged into a storage buffer of 10 mM Tris HCl pH 7.7, 150 mM NaCl, and 10 % glycerol using Biospin 6 columns (BioRad). The proteins were quantitated by UV absorption using an estimated ε(280 nm) = 8480 M<sup>-1</sup>cm<sup>-1</sup> from the ProtParam tool (ExPASy). Peptide molecular weight and purity (est. > 98 % based on Coomassie staining) was confirmed using SDS PAGE.

The expected α-helical folding of the coiled-coil was verified by circular dichroism (CD) as shown in Fig. S1.

**Supplementary Figure S1: CD analysis of the LZD73 peptide.** (A) LZD73 peptide alone (1.2 µM) in 20 mM Tris-HCl (pH 8.0 @ 25°C), 50 mM NaCl, 2 mM CaCl<sub>2</sub>. (B) LZD73 (1.2 µM) with 58mer CREB site dsDNA (1 µM). The two dips at 210 nm and 220 nm are indicative of α-helical folding. An increase in the amplitude of the 220 nm signal is observed upon DNA addition, consistent with additional folding of the basic region upon DNA binding.



**DNA Sequences Used in Cyclization Assays**

The DNA fragments below were made by PCR, subjected to XhoI digestion, and gel purified as described in the text. Each sequence contains one CREB site (5'-ATGACGTCAT-3') and one Inv-2 site (5' -GTCATATGAC-3'), which are highlighted, and two XhoI sites (5' -CTCGAG-3'), which are underlined.

Vx (448) 212

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AATGTGTGCCTGGCGATCTCGAGTGCAGCTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAAC  
TACCACTGGAGAACGAAAGTTGCGCGCCTGAAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGGATCCGAATTCTCCGGATCTGGCGTAATAGCGAAGAGGCCGCACCGATGCCCTTC  
CCAACAGTTGCGCAGCCTGAATGGCAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCG  
GGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTCG  
CTTCTCCCTTCTCGCCACGTTCGCCGGCTTCCCCGTCAAGCTCTAAATCGGGGGCT  
CCCTTAGGGTCCGATTAGTGCTTACGGCACCTGACCCAAAAAAACTTGATTAGGGTGAT  
GGTCACGTAGTGGCCATGCCCTGATAGACGGTTTCGCCCTTGACGTTGGAGTCCACGT  
TCTTAATAGTGGACTCTGTTCCAAACTGGAACAACACTCAACCTATCTCGCGTCAATGAC  
CAAGCTTGATCCGGCTGCTAACAAAGGCCGAAGGAAGCTGAGTTGGCTGCCACCGCTGAG  
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Vx (153) 414

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GAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGGATCCGAATTCTCCGGATCTGG  
CGTAATAGCGAAGAGGCCCGACCGATGCCCTCCAACAGTTGCGCAGCCTGAATGGCGAAT  
GGGACGCCCTGTAGCGCGCATTAGCGCGCGGGTGTGGTTACGGTCAATGACCAAG  
CTGAATTCGCGCTGACCTGGGAAATGTGCGCGAACCCCTATTGTTATTTTCTAAAT  
ACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAAATAATTGAAA  
AGGAAGAGTATGAGTATTCAACATTCCGTGCGCCCTATTCCTTTTGCGGCATTTTGCCC  
TTCCTGTTCTCGAGTTGCAGCTTT
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Vx (202) 414

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TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGGATCCGAATTCTCCGGATCTG  
GCGTAATAGCGAAGAGGCCCGACCGATGCCCTCCAACAGTTGCGCAGCCTGAATGGCGAA  
TGGGACGCCCTGTAGCGCGCATTAGCGCGCGGGTGTGGTTACGCGCAGCGTGAACCG  
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CCGCTCATGAGACAATAACCCTGATAAATGCTTCAAATAATTGAAAAGGAAGAGTATGAGTA  
TTCAACATTCCGTGCGCCCTATTCCTTTTGCGGCATTTTGCCTCCTGTTCTCGAGT  
TGCAGCTTT
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Vx (254) 414

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AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGATCCGATCCGAGCTGCTCTGAA  
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CTGGAAGACAAGGTGGAGGA~~ACTCGT~~GAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC  
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GCGTAATAGCGAAGAGGCCCGC~~ACCG~~ATGCCCTTCCAACAGTTGCGCAGCCTGAATGGCGAA  
TGGGACGCGCC~~CTGTAGCGCG~~CATTAAGCGCGGGGTGTGGTGGTTACGCGCAGCGT~~GACCG~~  
CTACACTTGCCAGCGCC~~CTAGCGCCG~~CTCCTTCGCTTCTTCCCTTCC~~TCTCGCCACGTT~~  
CGCCGGCTTCCCCGTCAAGCTCG~~CATATGACCAAG~~CTGAATTGCGC~~GCTGACCTCGGAAA~~  
TGTGCGCGGA~~ACCC~~CTATTGTTATTCTAA~~ATACATTCAA~~ATATGTATCCGCTCATGAGA  
CAATAAC~~CC~~CTGATAAA~~ATGCTCAATA~~ATATTGAAAAAGGAAGAGTATGAGTATTCAACATTCC  
GTGTCGCC~~CTTATTCC~~CTTGGCATTTCGCTTCTCGAGTTGCAGCTTT

Vx (310) 414

AATGTGTGCC~~TGGCG~~ATCTCGAGGAGGA~~TAA~~AGGATCGATGGGGATCCGATCCAGCTGCTCTGAA  
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TGAAGAAGCTGGTGGGTGA~~ACTGCAG~~ATGACGT~~CATGCGCGG~~ATCCGAATTCTCCGGATCTG  
GCGTAATAGCGAAGAGGCCCGC~~ACCG~~ATGCCCTTCCAACAGTTGCGCAGCCTGAATGGCGAA  
TGGGACGCGCC~~CTGTAGCGCG~~CATTAAGCGCGGGGTGTGGTGGTTACGCGCAGCGT~~GACCG~~  
CTACACTTGCCAGCGCC~~CTAGCGCCG~~CTCCTTCGCTTCTTCCCTTCC~~TCTCGCCACGTT~~  
CGCCGGCTTCCCCGTCAAGCT~~CTAAATCGGGG~~CTCC~~CTTAGGGTCCG~~ATTAGT~~GCTT~~TA  
CGGCACCTCGACCC~~CTGT~~CATATGACCAAGCTGAATTGCGC~~GCTGACCTCGGAA~~ATGTGCGCG  
GAACCC~~CTATTGTTATTCTAA~~ATACATTCAA~~ATATGTATCCGCTCATGAGACA~~ATAACC  
CTGATAAA~~ATGCTCAATA~~ATATTGAAAAAGGAAGAGTATGAGTATTCAACATTCCGTGTCGCC  
CTTATTCC~~CTTGGCATTTCGCTTCTCGAGTTGCAG~~CTTT

Vx (376) 414

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CTGGAAGACAAGGTGGAGGA~~ACTGCTGAG~~CAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC  
TGAAGAAGCTGGTGGGTGA~~ACTGCAG~~ATGACGT~~CATGCGCGG~~ATCCGAATTCTCCGGATCTG  
GCGTAATAGCGAAGAGGCCCGC~~ACCG~~ATGCCCTTCCAACAGTTGCGCAGCCTGAATGGCGAA  
TGGGACGCGCC~~CTGTAGCGCG~~CATTAAGCGCGGGGTGTGGTGGTTACGCGCAGCGT~~GACCG~~  
CTACACTTGCCAGCGCC~~CTAGCGCCG~~CTCCTTCGCTTCTTCCCTTCC~~TCTCGCCACGTT~~  
CGCCGGCTTCCCCGTCAAGCT~~CTAAATCGGGG~~CTCC~~CTTAGGGTCCG~~ATTAGT~~GCTT~~TA  
CGGCACCTCGACCC~~AAACTGATTAGGGT~~GATGGTCACGTAGTGGCCATGCC~~CTGATAGACGG~~  
AGACGGTTT~~TGCCC~~CTGT~~CATATGACCAAGCTGAATTGCGC~~GCTGACCTCGGAA~~ATGTGCG~~  
CGGAACCC~~CTATTGTTATTCTAA~~ATACATTCAA~~ATATGTATCCGCTCATGAGACA~~ATAA  
CCCTGATAAA~~ATGCTCAATA~~ATATTGAAAAAGGAAGAGTATGAGTATTCAACATTCCGTGTCG  
CCCTTATTCC~~CTTGGCATTTCGCTTCTCGAGTTGCAG~~CTTT

Vx (448) 414

AATGTGTGCC~~TGGCG~~ATCTCGAGGAGGA~~TAA~~AGGATCGATGGGGATCCGATCCAGATAAGGAT  
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CTACCACCTGGAGAACGAAGTTGCGCGC~~CTGAAGAAGCTGGTGGTGA~~ACTGCA~~GATGACGTCA~~  
~~TGCGCGCGG~~ATCCGAATTCTCCGG~~ATCTGGCG~~TAATAGCGAAGAGGCCCG~~CACCGATGCC~~CTT  
CCCAACAGTTGCGCAGCCTGA~~ATGGCGA~~ATGGGACGCGCC~~CTGTAGCGCGC~~ATTAA~~GCGCGC~~  
GGGTGTGGTGGTTACGCGCAGCGT~~GACCG~~CTACACTTGCCAGCGCC~~CTAGCGCCG~~CTC~~TT~~

GCTTCTCCCTCCTTCGCCACGTTGCCGGCTTCCCCGTCAAGCTCTAAATCGGGGGC  
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TGGTCACGTAGTGGGCCATGCCCTGATAGACGGTTTCGCCCTTGACGTTGGAGTCACG  
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Vx (435) 414

AATGTGTGCCTGGCGATCTGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA  
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Vx (438) 414

AATGTGTGCCTGGCGATCTGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA  
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GCGTAATAGCGAAGAGGCCCGCACCGATGCCCTCCAAACAGTTGCGCAGCCTGAATGGCGAA  
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Vx (440) 414

AATGTGTGCCTGGCGATCTGAGGAGGATAAGGATCGATGGGGATCCGATCCAGCTGCTCTGAA  
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GCGTAATAGCGAAGAGGCCCGCACCGATGCCCTCCAAACAGTTGCGCAGCCTGAATGGCGAA

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Vx (443) 414

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AATGTGCGCGAACCCCTATTGTTATTTCTAAATACATTCAAATATGTATCCGCTCATGA  
GACAATAACCCTGATAAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATT  
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Vx (445) 414

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GCGTAATAGCGAAGAGGCCCGCACCGATGCCCTCCAAACAGTTGCGCAGCCTGAATGGCGAA  
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Vx (450) 414

AATGTGTGCCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGATCCGATCCAGCTGCTCTGAA  
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TGAAGAACGCTGGTGGGTGAACTGCAGATGACGTCATGCGCGGATCCGAAATTCTCCGGATCTG

GCGTAATAGCGAAGAGGCCGCACCGATGCCCTCCAACAGTTGCGCAGCCTGAATGGCGAA  
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CGCCGGCTTCCCCGTCAAGCTCTAAATCGGGGCTCCCTTAGGGTCCGATTAGTGCCTTA  
CGGCACCTCGACCCAAAAACTTGATTAGGGTGTGGTCACGTAGTGGCCATGCCCTGAT  
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TGGAACAAACACTCAACCCTATAAGCTTAGTCATATGACAGCTGAATTGCGCCTGACCTCGGA  
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Vx (453) 414

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TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTATGCCCGGGATCCGAATTCTCCGGATCTG  
GCGTAATAGCGAAGAGGCCGCACCGATGCCCTCCAACAGTTGCGCAGCCTGAATGGCGAA  
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AGACGGTTTCGCCCTTGACGTTGGAGTCACGTTAAATAGTGGACTCTTGTCAAAC  
TGGAACAAACACTCAACCCTATAAGCTTAAGCGTCAATGACTGAATTGCGCCTGACCTCGGA  
AATGTGCGCGGAACCCCTATTGTTATTTCTAAATACATTCAAATATGTATCCGCTCATGA  
GACAATAACCTGATAATGCTTCAATAATATTGAAAAGGAAGAGTATGAGTATTCAACATTT  
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Vx (455) 414

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TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTATGCCCGGGATCCGAATTCTCCGGATCTG  
GCGTAATAGCGAAGAGGCCGCACCGATGCCCTCCAACAGTTGCGCAGCCTGAATGGCGAA  
TGGGACGCGCCCTGTAGCGCGCATTAAGCGCGGGGTGTGGTGGTTACGCGCAGCGTGACCG  
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CGCCGGCTTCCCCGTCAAGCTCTAAATCGGGGCTCCCTTAGGGTCCGATTAGTGCCTTA  
CGGCACCTCGACCCAAAAACTTGATTAGGGTGTGGTCACGTAGTGGCCATGCCCTGAT  
AGACGGTTTCGCCCTTGACGTTGGAGTCACGTTAAATAGTGGACTCTTGTCAAAC  
TGGAACAAACACTCAACCCTATAAGCTTAAGCGTCAATGACAATTGCGCCTGACCTCGGA  
AATGTGCGCGGAACCCCTATTGTTATTTCTAAATACATTCAAATATGTATCCGCTCATGA  
GACAATAACCTGATAATGCTTCAATAATATTGAAAAGGAAGAGTATGAGTATTCAACATTT  
CCGTGTGCCCTTATTCCCTTTGCGGCATTTGCCTCTCGAGTTGCAGCTTT

Vx (458) 414

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CTGGAAGACAAGGTGGAGGAACTGCTGAGCAAGAACTACCACCTGGAGAACGAAGTTGCGCGCC

TGAAGAAGCTGGTGGGTGAACTGCAGATGACGTCATGCCGCGGATCCGAATTCTCCGGATCTG  
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TGGGACGCCCTGTAGCGCGCATTAAGCGGGCGGGTGTGGTGGTACGCGCAGCGTGACCG  
CTACACTGCCAGGCCCTATGCCCGCTCCTTCGCTTCTTCCCTTCTGCCACGTT  
CGCCGGCTTCCCCGTCAAGCTCTAAATCGGGGCTCCCTTAGGGTCCGATTAGTGCCTTA  
CGGCACCTCGACCCAAAAACTTGATTAGGGTGTACGTAGTGGCCATGCCCTGAT  
AGACGGTTTTCGCCCTTGACGTTGGAGTCCACGTTAATAGTGGACTCTGTTCAAAC  
TGGAACACACTCAACCCTATAAGCTTAAGCTGAATGTCATATGACTCGCGCCTGACCTCGGA  
AATGTGCGCGAACCCCTATTGTTATTTCTAAATACATTCAAATATGTATCCGCTCATGA  
GACAATAACCCTGATAAATGCTCAATAATTGAAAAAGGAAGAGTATGAGTATTCAACATT  
CCGTGTCGCCCTATTCCCTTTGCGGCATTGCGCTTCTCGAGTTGCAGCTTT

**Primers used for PCR:**

XhoI sites (underlined) were introduced with a mismatched sequence on the primers.

Vx (448) 212 forward primer

5' -AATGTGTGCCTGGCGATCTCGAGCGCTGGAAAGACAAGGTGGAGGAAC-3'

Vx (448) 212 reverse primer

5' -AAAAGCTGCAACTCGAGGGTTATTGTCTCATGAGCG-3'

Vx (XXX) 414 forward primer

5' -AATGTGTGCCTGGCGATCTCGAGGAGGATAAGGATCGATGGGATCCGATCC-3'

Vx (XXX) 414 reverse primer

5' -AAAAGCTGCAACTCGAGAACAGGAAGGCAAAATGCCGC-3'