Supporting Text

Inserts of Plasmids pPA2-24 (cloned into the *Eco*RI/*Hin*dIII site of pUC19).

Legend: light gray boxes, target site for pcPNAI;

dark gray boxes, target site for pcPNAs II and III;

underlined sequences, conserved flanking regions.

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pPA2
5′-AATTCACGACGTAGGATATCGAATTGATCAATTGCATGTTTGAAGCAGATCGTAAGCTGTC
       GTGCTGCATCCTATAGCTTAACTAGTTAACGTACAAACTTCGTCTAGCATTCGACAGTCGA-5′

pPA4
5′-AATTACGACGTAGGATATCGAATTGATCAATTCTGCATGTTTGAAGCAGATCGTAAGCTGT
       TGCTGCATCCTATAGCTTAACTAGTTAAGACGTACAAACTTCGTCTAGCATTCGACATCGA-5′

pPA6
5′-AATTCGACGTAGGATATCGAATTGATCAATTGCCTGCATGTTTGAAGCAGATCGTAAGCTG
       GCTGCATCCTATAGCTTAACTAGTTAACGGACGTACAAACTTCGTCTAGCATTCGACTCGA-5′

pPA8
5′-AATTGACGTAGGATATCGAATTGATCAATTGCTCCTGCATGTTTGAAGCAGATCGTAAGCT
       CTGCATCCTATAGCTTAACTAGTTAACGAGGACGTACAAACTTCGTCTAGCATTCGATCGA-5′

pPA10
5′-AATTACGTAGGATATCGAATTGATCAATTGCAGTCCTGCATGTTTGAAGCAGATCGTAAGC
       TGCATCCTATAGCTTAACTAGTTAACGTCAGGACGTACAAACTTCGTCTAGCATTCGTCGA-5′

pPA12
5′-AATTCGTAGGATATCGAATTGATCAATTGCAGACTCCTGCATGTTTGAAGCAGATCGTAAG
       GCATCCTATAGCTTAACTAGTTAACGTCTGAGGACGTACAAACTTCGTCTAGCATTCTCGA-5′

pPA14
5′-AATTGTAGGATATCGAATTGATCAATTGCAGAGTCTCCTGCATGTTTGAAGCAGATCGTAA
       CATCCTATAGCTTAACTAGTTAACGTCTCAGAGGACGTACAAACTTCGTCTAGCATTTCGA-5′

pPA16
5′-AATTTAGGATATCGAATTGATCAATTGCAGAGTCGATCCTGCATGTTTGAAGCAGATCGTA
       ATCCTATAGCTTAACTAGTTAACGTCTCAGCTAGGACGTACAAACTTCGTCTAGCATTCGA-5′

pPA18
5′-AATTAGGATATCGAATTGATCAATTGCAGAGTCACGATCCTGCATGTTTGAAGCAGATCGT
       TCCTATAGCTTAACTAGTTAACGTCTCAGTGCTAGGACGTACAAACTTCGTCTAGCATCGA-5′

pPA20
5′-AATTGGATATCGAATTGATCAATTGCAGAGTCCAACGATCCTGCATGTTTGAAGCAGATCG
       CCTATAGCTTAACTAGTTAACGTCTCAGGTTGCTAGGACGTACAAACTTCGTCTAGCTCGA-5′

pPA22
5′-AATTGATATCGAATTGATCAATTGCAGAGTCCATGACGATCCTGCATGTTTGAAGCAGATC
       CTATAGCTTAACTAGTTAACGTCTCAGGTACTGCTAGGACGTACAAACTTCGTCTAGTCGA-5′

pPA24
5′-AATTATATCGAATTGATCAATTGCAGAGTCCATTCGACGATCCTGCATGTTTGAAGCAGAT
       TATAGCTTAACTAGTTAACGTCTCAGGTAAGCTGCTAGGACGTACAAACTTCGTCTATCGA-5′
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PCR Amplification of Different Regions of Plasmids pPA2-24. The following primer pairs were used (pPA locations are given in parentheses):

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A) PrA-F (s404-427 for pPA10, s401-424 for pPA16): 5'-TAGGATATCGAATTGATCAATTGC
   PrA-R (as634-654): 5'-GATTCATTAATGCAGCTGGCA

B) PrB-F (s376-396):  5'-GTTGTAAAACGACGGCCAGTG
   PrB-R (as609-626): 5'-TTCCCGACTGGAAAGCGG

C) PrC-F (s355-374):  5'-CAGGGTTTTCCCAGTCACGA
   PrC-R (as583-605): 5'-GTGAGCGCAACGCAATTAATGTG

D) PrD-F (s315-334):  5'-AAAGGGGGATGTGCTGCAAG
   PrD-R (as548-565): 5'-GGCACCCCAGGCTTTACA

E) PrE-F (307-323):   5'-AGCTGGCGAAAGGGGGA
   PrE-R(as535-557):  5'-AGGCTTTACACTTTATGCTTCCG

F) PrF-F (290-309):   5'-CTCTTCGCTATTACGCCAGC
   PrF-R (as521-540): 5'-CTTCCGGCTCGTATGTTGTG

G) PrG-F (s275-289):  5'-GCGATCGGTGCGGGC
   PrG-R (as501-525): 5'-TTGTGTGGAATTGTGAGCGGATAAC

H) PrH-F (s235-252):  5'-GGCGCCATTCGCCATTCA
   PrH-R (as461-485): 5'-ACAGCTATGACCATGATTACGCCAA

I) PrI-F (s203-220): 5'- GCACAGATGCGTAAGGAG
   PrI-R (as433-454 for pPA10, as436-457 for pPA16): 5'-TACGATCTGCTTCAAACATGCA
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PCR Conditions. PCRs from 50-100 ng plasmid were performed in 100-µl reaction volumes containing 1× Pfu buffer (20 mM Tris•HCl (pH 8.8), 2 mM $MgSO_4$, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, and 100 µg/ml BSA), 200 µM each dNTP, 0.5 µM each of two primers, and 5.0 units of *Pfu* DNA polymerase (Stratagene). Amplification was typically carried out with an initial denaturation step at 94°C for 60 s, followed by 36 cycles of denaturation at 94°C for 60 s, primer annealing at 62°C for 30 s, and extension at 72°C for 30 s. The last cycle was followed by an extension step at 72°C for 10 min.

PCR Products for Phasing Analysis. Each plasmid (pPA2-24) was amplified by PCR using primer pair E.

PCR Products for CPA. Plasmids pPA10 and pPA16 were amplified by using primer pairs A–I, respectively.

Determination of Bend Angles by CPA. Bend angles were determined from plots of the relative mobility of pcPNA-bound DNA fragments against the bend location *L*. Data points were fitted to a second order polynomial function according to the algorithm by Ferrari *et al.* (1):

$$R_{\text{bound}}/R_{\text{free}} = aL^2 - bL + c$$

From the determination of the parameters *a*, *b*, and *c*, the angle θ is obtained by either of the following equations

$$\cos(\theta) = (a/2c) - 1$$

$$\cos(\theta) = (b/2c) - 1$$

yielding the bending angle $\alpha = 180^{\circ} - \theta$. Good quadratic fits (average $r^2 = 0.982$) were obtained in all cases studied, and the two values of θ for each fit were identical, within the precision of the method ($\approx 2^{\circ}$).

Bend angles of pcPNA–DNA complexes were also calculated by using the algorithm by Kerppola and Curran (2). Here, data points are connected by the best fit of a cosine function in the plot of the relative mobility against the bend location. The bend angle is then derived from the following equation:

$$A_{CP} = 1 - \cos(k\alpha/2)$$

where A_{CP} is the amplitude of the circular permutation function and *k* is a coefficient that was introduced to adjust for electrophoretic conditions. For 5% nondenaturing PAGE, which we used here, *k* values of 1.00 and 1.02 were reported, while with higher

percentage gels slightly higher values between 1.06 and 1.10 were obtained (2-7). Therefore, we used a *k* value of 1.0 for our calculations. Excellent fits of the experimental data to a cosine function with a mean value of 0.995 for r^2 were obtained.

One assumption of the used algorithms, which are based on the reptation model describing the migration of DNA chains through a gel (8, 9), is that the elastic force constant does not vary substantially within a particular set of probes. Measuring the bending angle as a function of polyacrylamide concentration checks this assumption (1). Indeed, we found the measured mean bending angle values for pcPNA–DNA complexes to be only slightly dependent on the percentage of the polyacrylamide gels, in a manner similar as has been reported for A-tracts (10), thus validating the use of the CPA in our case.

Note that the permutation analysis data demonstrate the absence of a significant intrinsic bend within the free DNA fragments, as the electrophoretic mobility of lower bands (Fig. 3 *A–D* in the main text) remains unchanged. EM data of unbound DNA fragments support this conclusion.

Construct for the Assembly of DNA Minicircles. We opted to obtain the monomer duplex not directly from annealing of two 85-nt long oligonucleotides, but from a longer precursor duplex that was cleaved close to both termini by a type IIs restriction enzyme (*Bbs*I). Advantages of this method are that the length of the cleaved duplex is exactly defined, with all 5' ends phosphorylated, and that larger quantities of the duplex can be obtained through amplification of its precursor by means of PCR.

Oligo 99-1: 5′-AGCGCGTTGACGTCTTCGAGATGAAGTATGATATCGAATTGATCAATTGCTGCATGTTTGAAG-CAGATCCTAAGCAGTACAGGAAGACAGGTTGCGCAG

Oligo 99-2: 5′-CTGCGCAACCTGTCTTCCTGTACTGCTTAGGATCTGCTTCAAACATGCAGCAATTGATCAATT-CGATATCATACTTCATCTCGAAGACGTCAACGCGCT

Annealing of oligonucleotides 99-1 and 99-2 to duplex 99:

5′-N_4-NGTTGNNGTCTTC-N_{21}-**TTGATCAA**TTGCT**GCATGTTTGA**-N_{21}-GAAGACNNGTTGN-N_4

3′-N_4-NCAACNNCAGAAG-N_{21}-**AACTAGTT**AACGA**CGTACAAACT**-N_{21}-CTTCTGNNCAACN-N_4

(Shaded boxes: target sites for pcPNAs; underlined sequence: recognition sequence for *Bbs*I)

The two oligonucleotides were annealed at a ratio of 1:1 (10 μM final concentration) by heating for 3 min at 95°C, followed by cooling to 16°C at a rate of 1°C per min.

The resulting 99-bp DNA duplex (1 μM) was then incubated for 3 h at 37°C with 40 units of *Bbs*I in 200 μl of 1× NEBuffer 2 (New England Biolabs), and the DNA was isolated (the short fragments cleaved at both termini were removed by gel filtration), yielding the DNA monomer used for minicircle formation:

5′-pGTTGNNGTCTTC-N_{21}-**TTGATCAA**TTGCT**GCATGTTTGA**-N_{21}-GAAGACNN
3′-NNCAGAAG-N_{21}-**AACTAGTT**AACGA**CGTACAAACT**-N_{21}-CTTCTGNNCAACp

Samples of this DNA duplex in the absence or presence of pcPNAs were analyzed by 10% nondenaturing PAGE.

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