Electronic Supplementary Material (ESI) for Chemical Communications
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Supplementary Information

Precise engineering and visualization of signs and magnitudes of DNA writhe on basis of PNA invasion

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EXTENDED EXPERIMENTAL PROCEDURES

Reagents: Taq DNA Polymerase, T4 DNA ligase, Nuclease BAL-31 and SacI were purchased from New England Biolabs (Ipswich, MA). All Plasmid DNA were provided by Generay Biotech (Shanghai, China).

Polymerase chain reaction: Polymerase chain reaction was carried out following standard procedures with Taq DNA Polymerase. Primers for liner DNA 1: ssODN-1 (5'-3': CCGAGCTCCCGTAATACGACTCACTTA) and ssODN-2 (5'-3': TCGTTTGGTATGGCTTCATT). Primers for linear DNA 2: ssODN-3 (5'-3': GTGGATCCTCGTCGCAAAAC) and ssODN-4 (5'-3': CCGAGCTCAGCGCGCAATTAACCCTCAC).

Reactions of SacI with Linear DNA : A solution containing 10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, linear DNA (500 ng) and 10 U SacI was incubated at 37 ℃ for 1 hr. The reaction products were further analyzed using agarose electrophoresis (2%).

Preparations of circular DNA: A 50 μl solution containing 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM ATP, 10 mM dithiothreitol, 500 ng linear DNA with cohesive ends and 20 U T4 DNA ligase was incubated at 16 ℃ for 8 hrs. The obtained circular DNA products were further analyzed using agarose electrophoresis (2%).

PNA invasion: In the binding reactions of PNA 1 to target sites, the PNA concentration was kept at a large excess over the DNA concentration, and the binding was performed at 37 ℃ for 6 hr in 10mM Sodium-Phosphate Buffer (PH = 6.9).

AFM studies: Immobilization of DNA samples on micas were carried out following the previously reported procedures.[S1,S2] AFM images were obtained in Tapping Mode™ on a

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Multimode^{TM} AFM (Veeco, Santa Barbara, CA) in connection with a Nanoscope V^{TM} controller. Antimony (n) doped Si cantilevers with nominal spring constants between 20 and 80 N/m were selected. Scan frequency was 1.9 Hz per line and the modulation amplitude was in a nanometer range. All DNA sample determinations were carried out in air at room temperature.

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DETAILED LEGEND OF FIGURE 2:

(C) Illustration of molecular structure of PNA 1. PNAs are written from the N terminus to the C terminus using normal peptide conventions: H is a free amino group; NH2 is a terminal carboxamide; Lys is the lysine residue; J denotes pseudoisocytosine and eg1 denotes the linker unit, 8-amino-3,6-dioxaoctanoic acid.

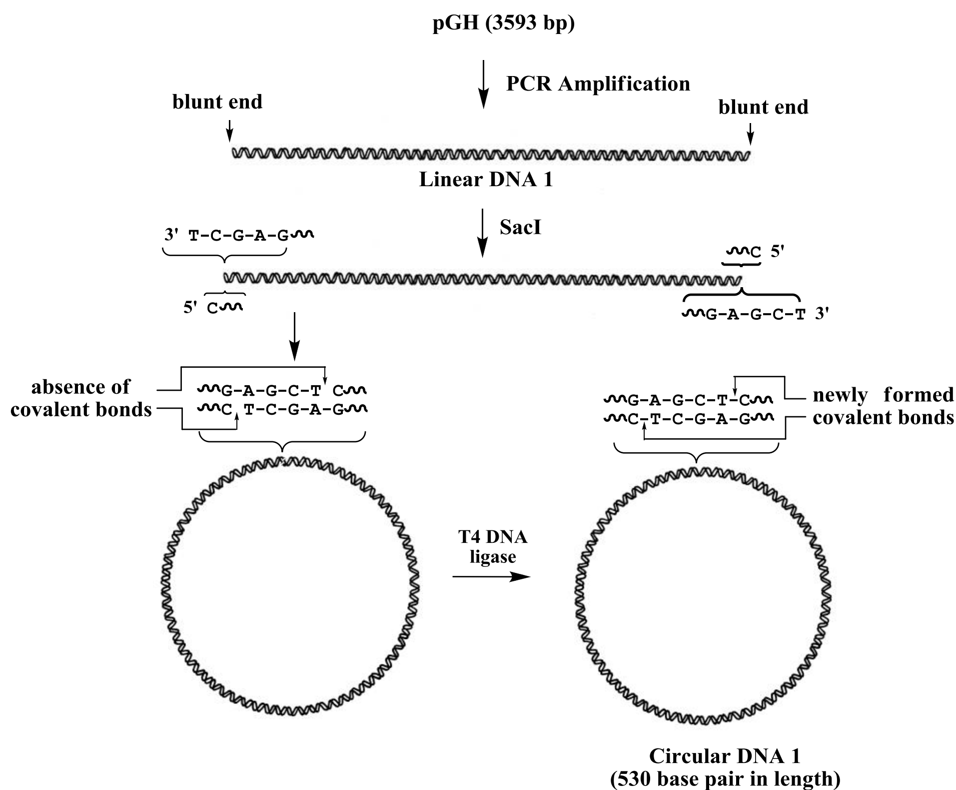
DETAILED LEGEND OF FIGURE 3:

(B) AFM image of negatively supercoiled structure with writhe number of -2 (scale bar 100 nm): I) height image; II) amplitude and III) 3D image showing the ring crossing path more clearly; IV) section image analysis of the self-crossing.

DETAILED LEGEND OF FIGURE 4:

(B) AFM image of positively supercoiled structure with writhe number of +1 (scale bar 100 nm): I) height image; II) amplitude and III) 3D image showing the ring crossing path more clearly; IV) section image analysis of the self-crossing.

A



B

```
5' CCGAGCTCCCGTAATACGACTCACTTAAGGCCTTGACTAGAGGGTACCAACCTAGGTATCTAGAACCGGTCTCGAGCCATAACTTCGTATAG
3' GGCTCGAGGGCATTATGCTGAGTGAATTCCGGAACTGATCTCCCATGGTTGGATCCATAGATCTTGGCCAGAGCTCGGTATTGAAGCATATC

   CATACATTATACGAAGTTATATAAGCTGTCAAACATGAGAATTCTTGTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGT
   GTATGTAATATGCTTCAATATATTCGACAGTTTGTACTCTTAAGAACAATATCCAATTACAGTACTATTATTACCAAAGAATCTGCAGTCCA

   GGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTG
   CCGTGAAAAGCCCCTTTACACGCGCCTTGGGGATAAACAAATAAAAAGATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGAC

   ATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTC
   TATTTACGAAGTTATTATAACTTTTTCCTTCTCATACTCATAAGTTGTAAAGGCACAGCGGGAATAAGGGAAAAAACGCCGTAAAACGGAAG

   CTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAAC
   GACAAAAACGAGTGGGTCTTTGCGACCACTTTCATTTTCTACGACTTCTAGTCAACCCACGTGCTCACCCAATGTAGCTTGACCTAGAGTTG

   AGCGGTAAGTTAAGCTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGAAGGAGAGAAGAGCTGGAGCTCAATGAAGCCATACC
   TCGCCATTCAATTCGAAAAACGTGTTGTACCCCCTAGTACATTGAGCGGAACTAGCTTCCTCTCTTCTCGACCTCGAGTTACTTCGGTATGG

   AAACGA 3'
   TTTGCT 5'
```

Linear DNA 1*

* This linear DNA 1 was obtained by PCR amplification from vector pGH (3593 bp).
The duplex segment highlighted in red is designed PNA binding site.

C

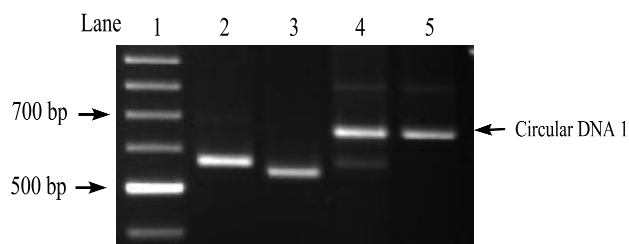


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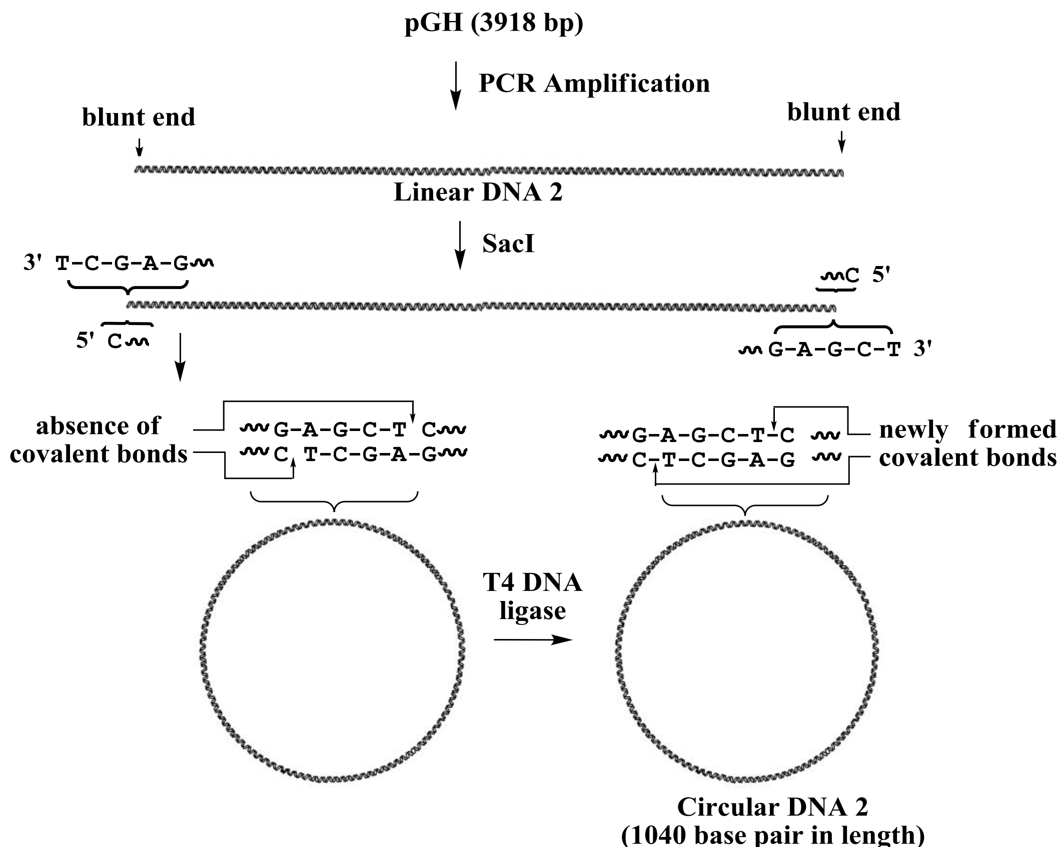
Fig. S1 Synthesis and confirmation of Circular DNA 1 (530 bp in length) from Linear DNA 1 (558 bp in length).

(A) Diagrammatic illustration of synthesis of Circular DNA 1 from Plasmid DNA (pGH (3593 bp)); (B) Nucleotide sequences of Linear DNA 1 (PCR product); (C) Agarose gel electrophoretic analysis of DNA products; Lane 1: Molecular weight markers; Lane 2: Linear DNA 1 obtained by PCR amplification; Lane 3: Digested product of Linear DNA 1 by SacI; Lane 4: Circular DNA (Circular DNA 1) promoted by T4 DNA Ligase; Lane 5: reaction mixture of ligase reaction followed by Nuclease BAL-31 hydrolysis

A



B

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5' GTGGATCCTCGTCGCAAAACGAGCTCCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTCCGTAATA
3' CACCTAGGAGCAGCGTTTTGCTCGAGGCTAATTCAACCCATTGCGGTCCCAAAAGGGTCAGTGCTGCAACATTTTGCTGCCGGTCAGGCATTAT

   CGGCTCACTTAAGGCCTTGACTAGAGGGTACCAACCTAGGTATCTAGAACCGGTCTCGAGCCATAACTTCGTATAGCATACATTATACGAAGTT
   GCCGAGTGAATTCCGGAACTGATCTCCCATGGTTGGATCCATAGATCTTGGCCAGAGCTCGGTATTGAAGCATATCGTATGTAATATGCTTCAA

   ATATAAGCTGTCAAACATGAAACCTCTTGTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGT
   TATATTCGACAGTTTGTACTTTGGAGAACAATATCCAATTACAGTACTATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCCTTTACA

   GCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATAATAACCCTGATAAATGCTTCAATAATATTGAAAAAG
   CGCGCCTTGGGGATAAACAAATAAAAAGATTTATGTAAGTTTATACATAGGCGAGTATTATTGGGACTATTTACGAAGTTATTATAACTTTTTC

   GAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCTCTCCCTATAGTGAGTCGTATTAATACCCTCAGCTTCACCCAT
   CTTCTCATACTCATAAGTTGTAAAGGCACAGCGGGAATAAGGGAAAAAACGAGAGGGATATCACTCAGCATAATTATGGGAGTCGAAGTGGGTA

   GAGAAGATTGACATCACATAAACTATTCATACAGGATAATTGGGAGGCTTTATTGAAAGCCCACTCACTGATTAACGGGCCTTCCTGTTTTTGC
   CTCTTCTAACTGTAGTGTATTTGATAAGTATGTCCTATTAACCCTCCGAAATAACTTTCGGGTGAGTGACTAATTGCCCGGAAGGACAAAAACG

   TCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGTTA
   AGTGGGTCTTTGCGACCACTTTCATTTTCTACGACTTCTAGTCAACCCACGTGCTCACCCAATGTAGCTTGACCTAGAGTTGTCGCCATTCAAT

   AGCTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGAAGGAGAGAATCCAAGAGAGGAATAGCTCTCCTTTTGAGGTGTTGCTCAA
   TCGAAAAACGTGTTGTACCCCCTAGTACATTGAGCGGAACTAGCTTCCTCTCTTAGGTTCTCTCCTTATCGAGAGGAAAACTCCACAACGAGTT

   TGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGTGATTCCTCGAGCCATAACTTCGTATAGCATACATTATACGAAGTTATCC
   ACTTCGGTATGGTTTGCTGCTCGCACTGTGGTGCTACGGACGTCACTAAGGAGCTCGGTATTGAAGCATATCGTATGTAATATGCTTCAATAGG

   ATGGACTAGTGTATTACGTAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGA
   TACCTGATCACATAATGCATCGAACCGCATTAGTACCAGTATCGACAAAGGACACACTTTAACAATAGGCGAGTGTTAAGGTGTGTTGTATGCT

   GCCGGAAGCATAAAGTGTAAAGCCTGGGGGAATTCGGGGTTAACCATGGATCCGGGGGATATCACGTGAAGCTTGCAAGCTCCAGCTTTTGTTC
   CGGCCTTCGTATTTCACATTTCGGACCCCCTTAAGCCCCAATTGGTACCTAGGCCCCCTATAGTGCACTTCGAACGTTCGAGGTCGAAAACAAG

   CCTTTAGTGAGGGTTAATTGCGCGCTGAGCTCGG 3'
   GGAAATCACTCCCAATTAACGCGCGACTCGAGCC 5'
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Linear DNA 2*

* This linear DNA 2 was obtained by PCR amplification from vector pGH (3918 bp).
The duplex segments highlighted in red are designed PNA binding sites.

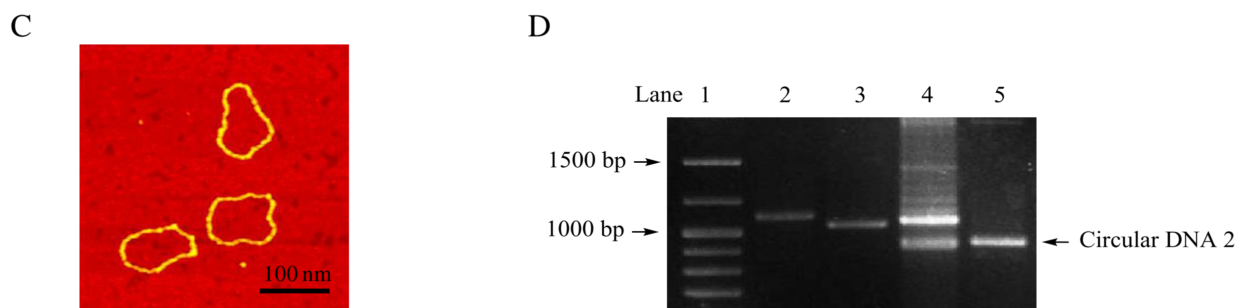

Fig. S2 Synthesis and confirmation of Circular DNA 2 (1040 bp in length) from Linear DNA 1 (1068 bp in length).

(A) Diagrammatic illustration of synthesis of Circular DNA 2 from Plasmid DNA (pGH (3918 bp)); (B) Nucleotide sequences of Linear DNA 2 (PCR product); (C) AFM images of Circular DNA 2 (scale bar 100 nm). (D) Agarose gel electrophoretic analysis of DNA products. Lane 1: Molecular weight markers; Lane 2: Linear DNA 2 obtained by PCR amplification; Lane 3: Digested product of Linear DNA 2 by SacI; Lane 4: Circular DNA (Circular DNA 2) promoted by T4 DNA Ligase; Lane 5: reaction mixture of ligase reaction followed by Nuclease BAL-31 hydrolysis

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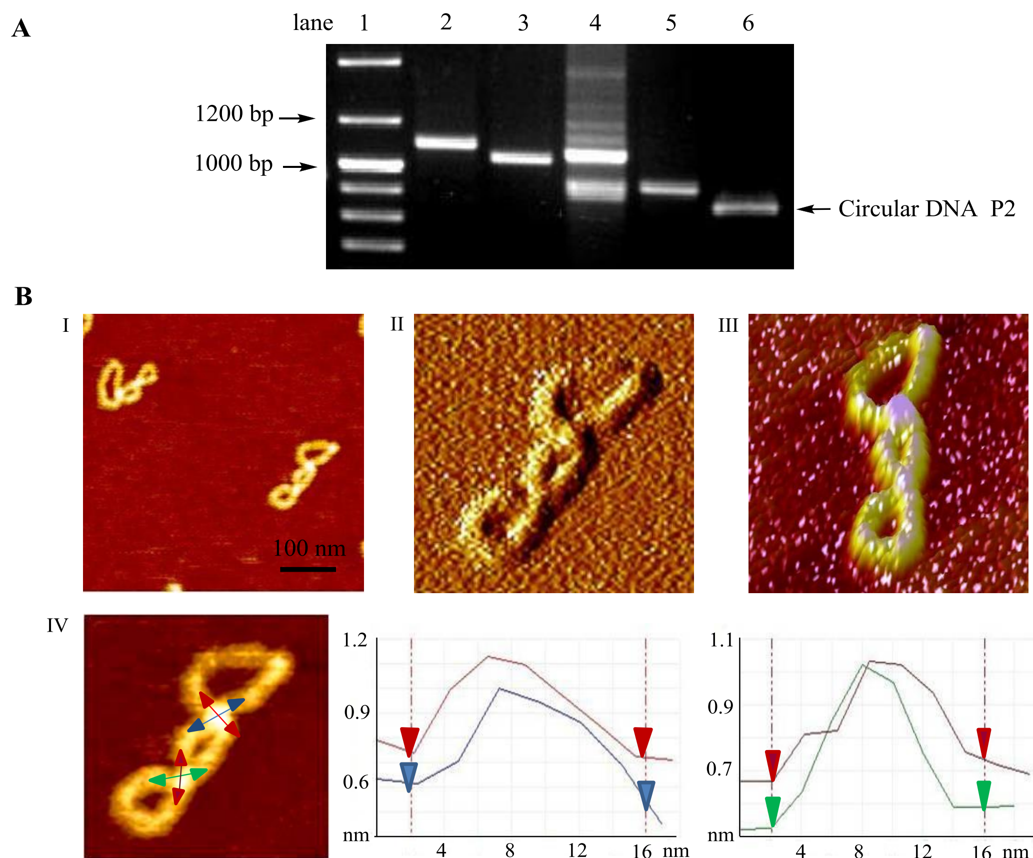

Fig. S3 Engineering of positive DNA supercoil with writhe number of +2 (Circular DNA P2).

(A) Agarose gel electrophoretic analysis of DNA products. Lane 1: Molecular weight markers; Lane 2: Linear DNA 2 obtained by PCR amplification; Lane 3: Digested product of Linear DNA 2 by SacI; Lane 4: Circular DNA (Circular DNA 2) promoted by T4 DNA Ligase; Lane 5: Reaction mixture of ligase reaction followed by Nuclease BAL-31 hydrolysis; Lane 6: PNA-containing circular DNA P2 produced upon the invasion of PNA 1; (B) AFM image of positively supercoiled structure with writhe number of +2 (scale bar 100 nm): I) height image; II) amplitude and III) 3D image showing the ring crossing path more clearly; IV) section image analysis of the self-crossing.

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Table S1. Statistical data of DNA molecules examined using AFM and their measurement errors

	Total number of DNA molecules measured	Height of duplex DNA molecules (nm)	Height of ring crossing (nm)	Number of DNA molecules with no self-crossing	Number of DNA molecules with one self-crossing	Number of DNA molecules with two self-crossings	Number of DNA molecules identified as positive supercoils	Number of DNA molecules identified as negative supercoils	Identifiable rate of self-crossing
Circular DNA 1	51	0.7 ± 0.1	—	51	0	0	—	—	—
Circular DNA N1	61	0.8 ± 0.1	1.3 ± 0.2	16	45	0	—	37	82% (37/45)
Circular DNA P1	69	0.6 ± 0.1	1.0 ± 0.3	17	52	0	35	—	67% (35/52)
Circular DNA 2	57	0.7 ± 0.1	—	55	2	0	—	—	—
Circular DNA N2	68	0.7 ± 0.1	1.2 ± 0.2	1	5	42	—	30	71% (30/42)
Circular DNA P2	65	0.7 ± 0.2	1.1 ± 0.2	2	8	40	29	—	73% (29/40)

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2 Y. L. Lyubchenko, A. A. Gall, L. S. Shlyakhtenko, R. E. Harrington, B. L. Jacobs, P. I. Oden and S. M. Lindsay, *J. Biomolec. Struct. Dyn.*, 1992, **9**, 589-606.