Supplementary Materials

Supplementary Table 1. The fractional occupancies (O_i) of the PARP1(2) on DNA*.

	PARP1		**PARP2**	
	1200-bp	Nicked 1200-bp	1200-bp	Nicked 1200-bp
[P] (nM) [DNA] (nM) $n_{Fragment}$ $n_{Complex,Und}$ $n_{Complex,Ends}$ $n_{Complex,Nick}$	3.5 1.5 138 55 35	3.5 1.5 210 57 39 61	3.5 1.5 326 35 24	3.5 1.5 189 17 8 79
$O_{fragment}$	0.65±5.3%	0.75±7.2%	0.18±3.8%	0.55±5.3%
O_{End}	0.126±5.4%		0.036±7.1	
O_{Und}	$0.4 \cdot 10^{-3}$±9.3%	$0.30 \cdot 10^{-3}$±5.1%	$0.11 \cdot 10^{-3}$±6.0%	$0.10 \cdot 10^{-3}$±4.4%
O_{Nick}		0.29±8.3%		0.42±9.2%

*[P] and [DNA] are the concentration of proteins and DNA in solution, (n)-numbers of DNA fragments and complexes. The fractional occupancy of the DNA fragment ($O_{Fragment}$), the apparent fractional occupancy of DNA termini (O_{End}), nick site (O_{nick}) and of undamaged DNA (O_{Und}) were determined as described by Yang et al. (*Nucleic Acids Res., 2005, 33, 4322-4334*).

Supplementary Table 2. DNA fractional occupancies (O_i) of PARylated PARP1 on nicked 1200-bp DNA and binding dissociation constants of the modified proteins*.

	PARylated PARP1	PARylated PARP2
	Nicked 1200-bp	Nicked 1200-bp
[P] (nM)	3.5	3.5
[DNA] (nM)	1.5	1.5
$n_{Fragment}$	1618	1486
$n_{Complex,Und}$	43	3
$n_{Complex,Ends}$	32	20
$n_{Complex,Nick}$	19	32
$O_{fragment}$	0.055	0.037
O_{End}	0.01	0.006
O_{Und}	$0.03 \cdot 10^{-3}$	$0.0023 \cdot 10^{-3}$
O_{Nick}	0.011	0.021
$K_{d,Nick}$ (μM)	~0.3	~0.166
$K_{d,End}$ (μM)	~0.3344	~0.588
$K_{d,Und}$ (μM)	~125	~14281
$K_{d, AFM\ Macro}$** (μM)	~0.052	~0.117

*[P] and [DNA] are the concentration of proteins and DNA in solution, (n)-numbers of DNA fragments and complexes. The fractional occupancy of the DNA fragment ($O_{Fragment}$), the apparent fractional occupancy of DNA termini (O_{End}), nick site (O_{nick}) and of nonspecific binding sites (O_{Und}) were determined as described by Yang et al. (Nucleic Acids Res., 2005, 33, 4322-4334). K_d - site-specific binding affinities. **Composite macroscopic binding constants of PARylated PARP1(2) to nicked 1200-bp DNA fragments determined as described by Yang et al. (*Nucleic Acids Res., 2005, 33, 4322-4334*).

Supplementary Figure Legends

Supplementary Figure S1. AFM analysis of PARP1(2) position on the DNA**.** The protein–DNA complexes detected by AFM were categorized according to whether they were located at the ends (**A**), on the undamaged DNA (B) or at the nick site (**C**).

(**D**) Schematic view of the long DNA substrates used for the experiments in this study: 1200-bp DNA fragment with blunt ends and 1200-bp DNA fragment containing an SSB in the middle of the chain after action of the nicking endonuclease, Nb-Bsm1.

(**E**) AFM images showing PARP1 bound to DNA ends and nick. Z scale: 7 nm.

(**F**) The average contour length of complexed DNA with PARP1 (l_e) and PARP2 (l_n) ($n_{molecules}$ = 40 for each PARP). The interval of DNA length equal to 40 nm was selected on the basis of the mean ± SD contour length of complexed DNA (E)

Supplementary Figure S2. Volumes of PARP1 and PARP2 calculated from AFM images (Figure 1A). PARP1 (2 nM) **(A)** or PARP2 (2 nM) **(B)** were incubated in the presence of 5 mM Pu^{2+} (number of molecules analyzed, 72 for PARP1 and 65 for PARP2) or 50 μM Spd^{3+} (number of molecules analyzed, 140 for PARP1 and 76 for PARP2). The molecular volume of PARP1 and PARP2 was determined from particle dimensions derived from AFM images. The height and half-height diameters were measured from cross-sections of each particle. The molecular volume of the protein particles was determined on the basis of particle dimensions derived from AFM images using the following equation:

$$V = \frac{\pi h}{6}(3r^2 + h^2)$$

Where *h* is the particle height and *r* is the radius at half height (*Schneider S.W. et al., Pflugers Arch. 1998, 435, 362-367*).

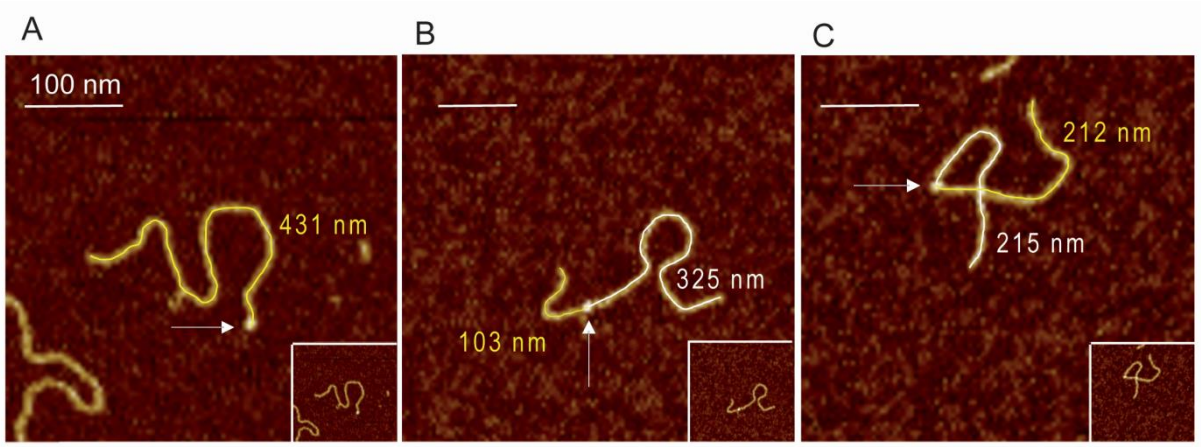
Supplementary Figure S3. The formation of circular form plasmid after treatment of pBR with the site-specific nicking endonuclease (Nb.BsmI). AFM images of pBR before (A) and after (B) Nb-Bsm1 treatment. Scale bar: 1 μm, Z scale: 7 nm. (C) Agarose-based gel assay of pBR before (lane 1) and after (lane 2) Nb-Bsm1 treatment.

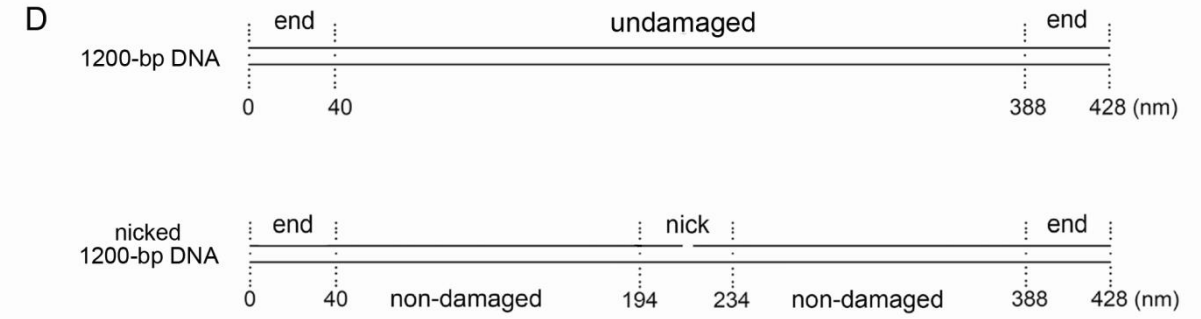
Supplementary Figure S4. Analysis of the binding of PARP1 and PARP2 to nicked circular pBR. (A) Binding of PARP1 or PARP2 to nicked pBR analyzed by AFM. White arrows indicate binding of PARP1(2) to DNA. Scale bar: 200 nm, Z scale: 7 nm. PARP1 (3.5 nM) or PARP2 (3.5 nM) and 0.35 nM nicked pBR322 were incubated in AFM deposition buffer (12.5 mM Hepes, pH 8.0, 12.5 mM KCl, 1 mM DTT) on ice for 1-5 min. Just before sample deposition, to adsorb DNA-PARP1 or DNA-PARP2 complexes, Pu^{2+} was added to the solution to final concentrations of 5 mM. (B) Fluorescence measurements of PARP1 or PARP2 binding to nicked pBR. The reaction mixtures containing fluorescein-labelled protein PARP1 or PARP2 were titrated with increasing amounts of nicked pBR. Fluorescence titration experiments were performed by adding increasing amounts of nicked pBR to a fixed concentration of protein **(**20 nM for PARP1-fluorescein and 90 nM for PARP2-fluorescein) in buffer containing 12.5 mM Hepes-KOH (pH 8.0), 12.5 mM KCl and 1 mM DTT. Bars indicate the standard error of three independent experiments.

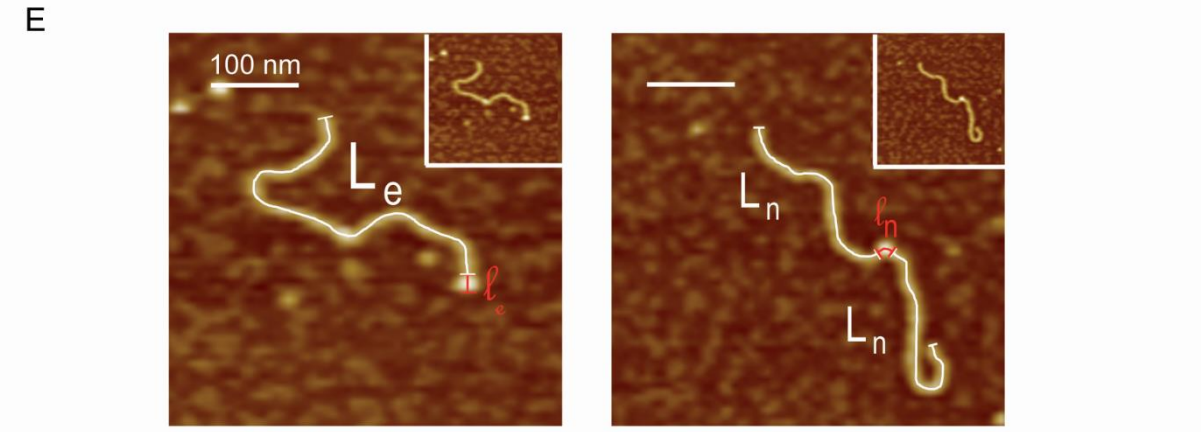
Supplementary Figure S5. AFM images of PARP1 or PARP2 incubated with NAD^+ show that these proteins do not form detectable amount of PARylated proteins in the absence of DNA substrate. PARP1 (2 nM) or PARP2 (2 nM) was incubated in AFM deposition buffer (12.5 mM Hepes, pH 8.0, 12.5 mM KCl, 1 mM DTT) at 37°C for PARP1 and on ice for PARP2 for 60 min in the presence of 100 μM NAD^+, then deposited on mica surfaces in the presence of 5 mM Pu^{2+}. Scale bar: 1 μm; Z scale: 7 nm.

Supplementary Figure S6. Time course showing the level of PARP2 auto-modification in the presence of 1200-bp DNA (lanes 1-4) or nicked 1200-bp DNA at 37°C (lanes 5-8) and at 0°C (lanes 9-12). The reaction mixture (30 µl) contained 12.5 mM Hepes-KOH, pH 8.0, 12.5 mM KCl, 1 mM DTT, 25 nM 1200-bp DNA with or without nick, 100 µM NAD^+ + 0.5 µCi [^{32}P]NAD^+, and 25 nM PARP2. The reaction mixtures were incubated at 37 or 0°C, and aliquots (6 µl) were taken at 30, 60, 90 and 120 min. The reactions were stopped by adding SDS-sample buffer and heating for 5 min at 90ºC. The reaction mixtures were analyzed by 10% SDS-PAGE with subsequent phosphorimaging.

Supplementary Figure S7. Analysis of protein-free ADP-ribose polymers synthesized by PARP1 (A) and PARP2 (B) in the presence of 1200-bp DNA, nicked 1200-bp DNA or super coiled pBR. The reaction mixtures (50 µl) contained 12.5 mM Hepes-KOH, pH 8.0, 12.5 mM KCl, 1 mM DTT, 13 nM 1200-pb DNA or nicked 1200-bp DNA, 35 nM PARP1 or PARP2, 100 µM NAD^+ + 0.5 µCi [^{32}P]NAD^+, and 10 mM $MgCl_2$ for PARP1 auto-modification. The reactions were incubated for 120 min at 37°C or on ice for PARP1 and PARP2, respectively. The reaction was stopped by addition of 50 µl ice-cold 20% TCA. The proteins thus precipitated were collected by centrifugation at 16000*g* for 30 min at 4°C. After removal of the supernatant, 50 µl of 0.2 M NaOH and 20 mM EDTA were added to the pellet and incubated for 120 min at 60°C to cleave the PAR polymer from the PARPs (*Alvarez-Gonzalez R., Jacobson M.K, Methods Mol. Biol., 2011, 780, 35-46).* NaOH was neutralized with 0.1 N HCl. The samples were extracted with phenol/$CHCl_3$/isoamyl alcohol. Then PAR polymers were precipitated with ethanol overnight at -80°C. Following centrifugation the PAR polymer pellets were dried, resuspended in 20 µl RNA-loading dye (50% formamide, 20 mM EDTA, 0.01% SDS, xylene cyanol (XC), and bromophenol blue (BFB), heated at 55°C for 15 min and separated by electrophoresis in a 10% polyacrylamide gel containing 8 M urea in 89 mM Tris-HCl, 89 mM boric acid and 2 mM EDTA, pH 8.8. The gels were dried and subjected to phosphorimaging for quantification using Molecular Imager/Quantity One software (Bio-Rad, USA).







F Table S1. The avarage contour length of PARP1 and PARP2 bound to DNA.

	L_e	ℓ_e	L_n	ℓ_n
PARP1	405 ± 15	26.5 ± 7.9	204 ± 11	27.5 ± 6.4
PARP2	402 ± 18	29.2 ± 5.8	202 ± 12	26.0 ± 7.5

Figure S1

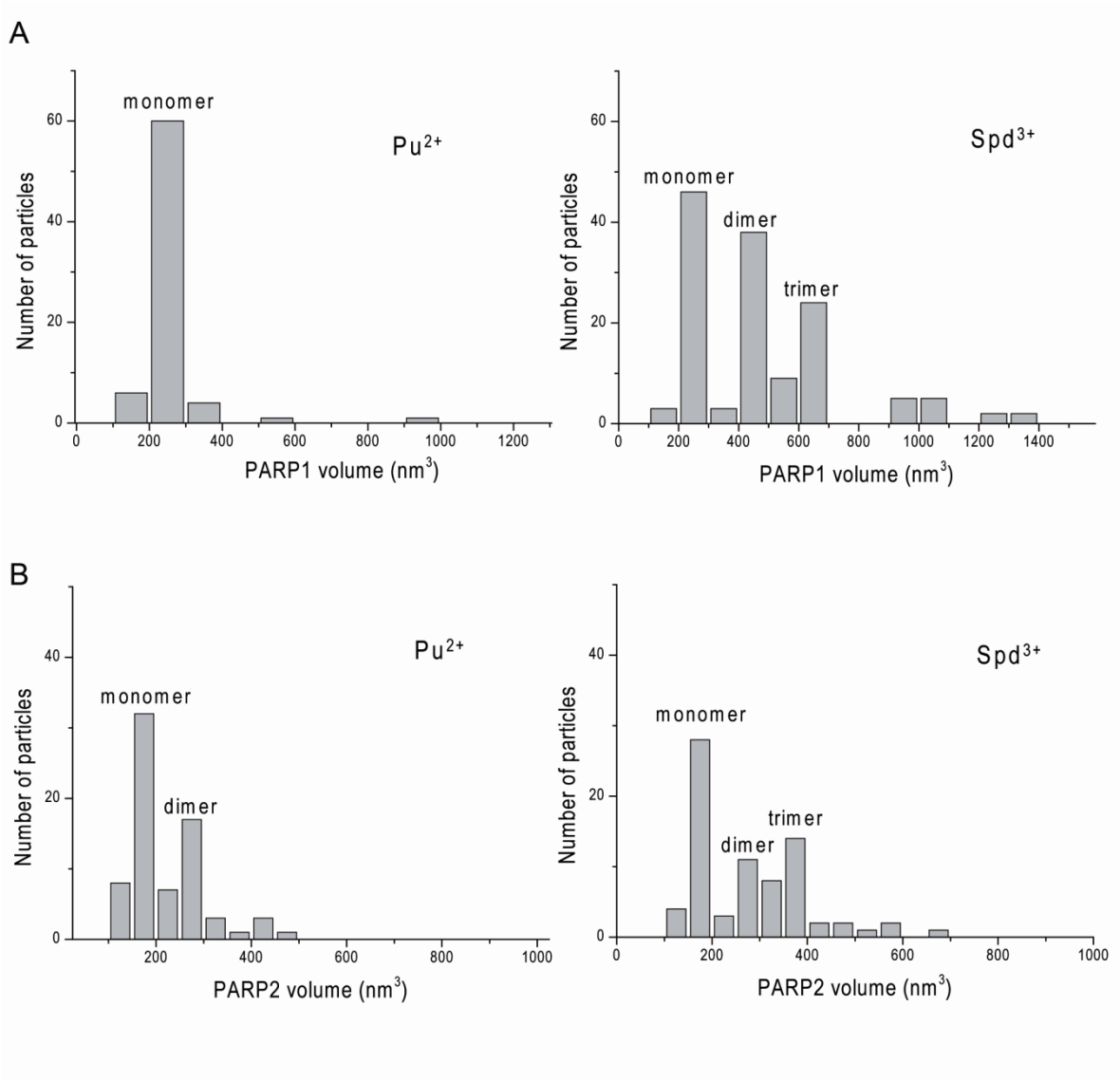


Figure S2

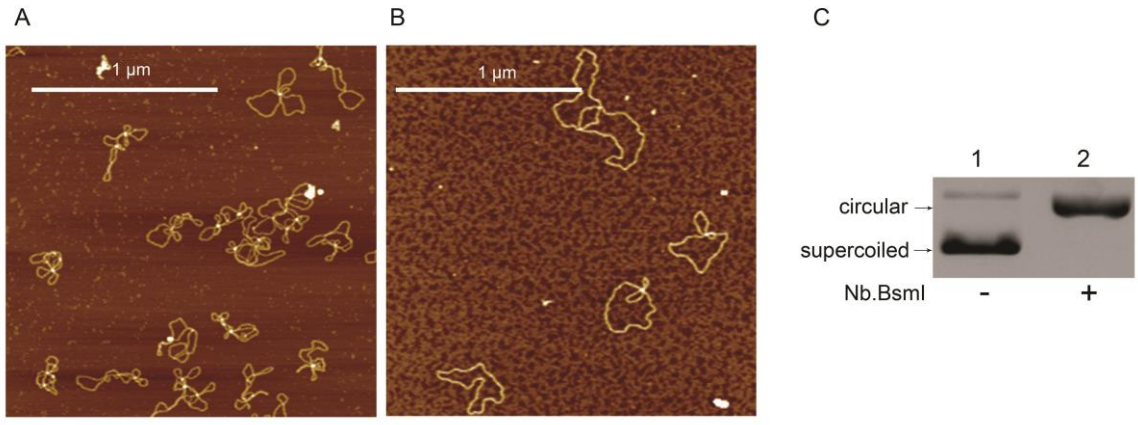


Figure S3

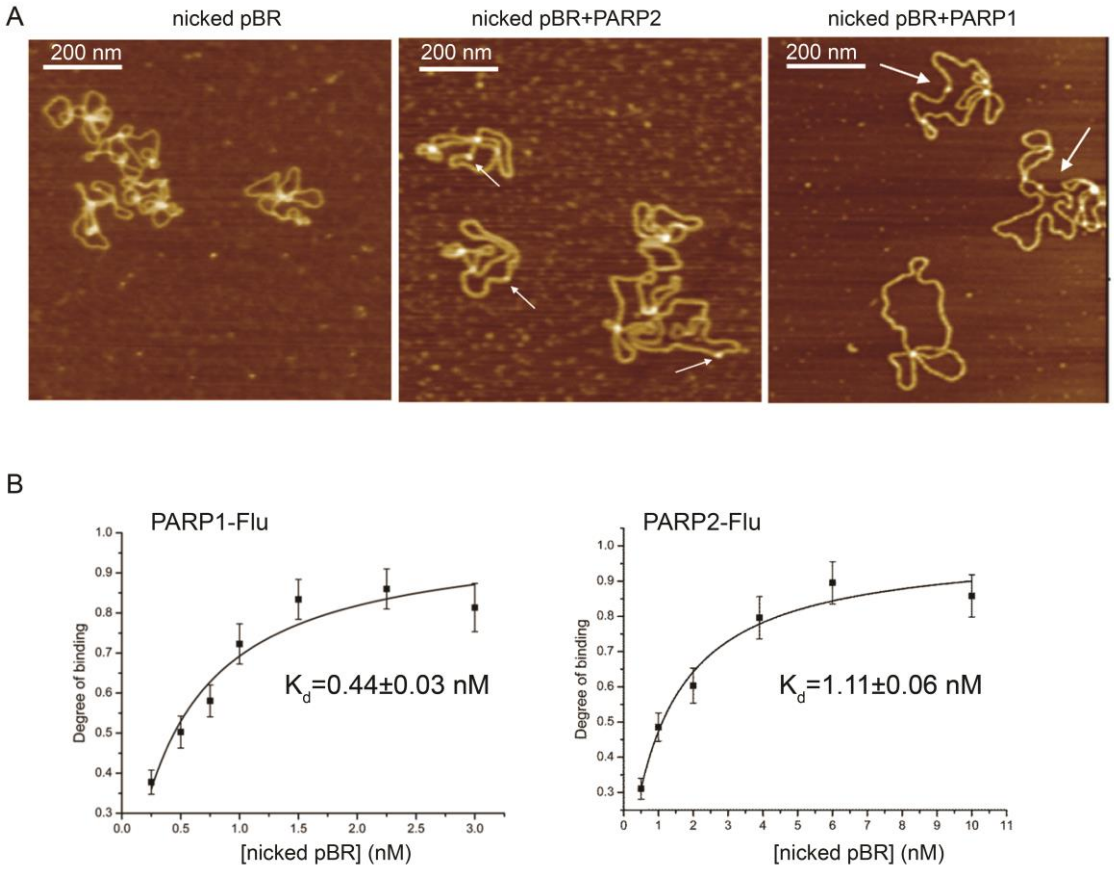


Figure S4

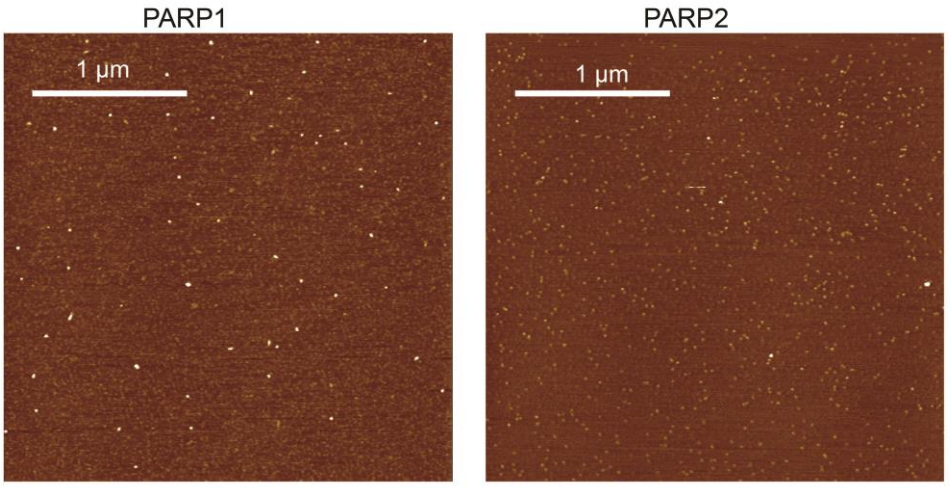


Figure S5

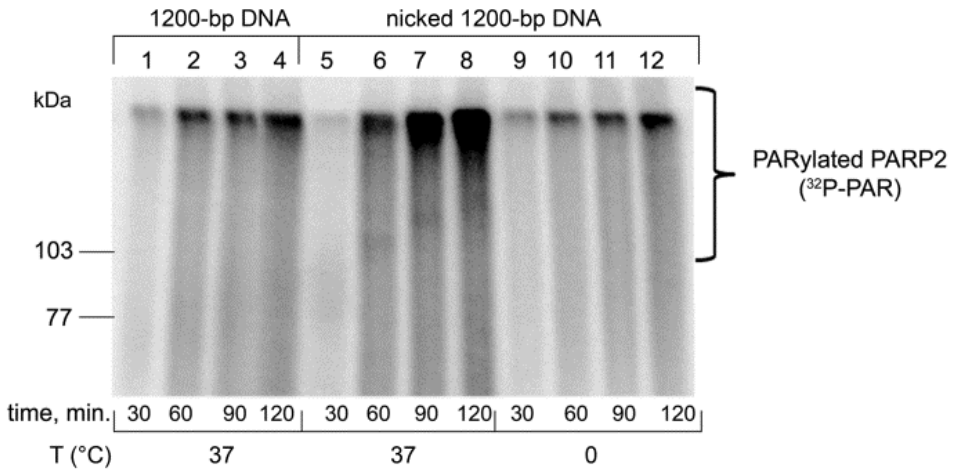

1200-bp DNA
nicked 1200-bp DNA
1 2 3 4 5 6 7 8 9 10 11 12
kDa
103
77
PARylated PARP2
(32P-PAR)
time, min.
30 60 90 120 30 60 90 120 30 60 90 120
T (°C)
37
37
0

Figure S6

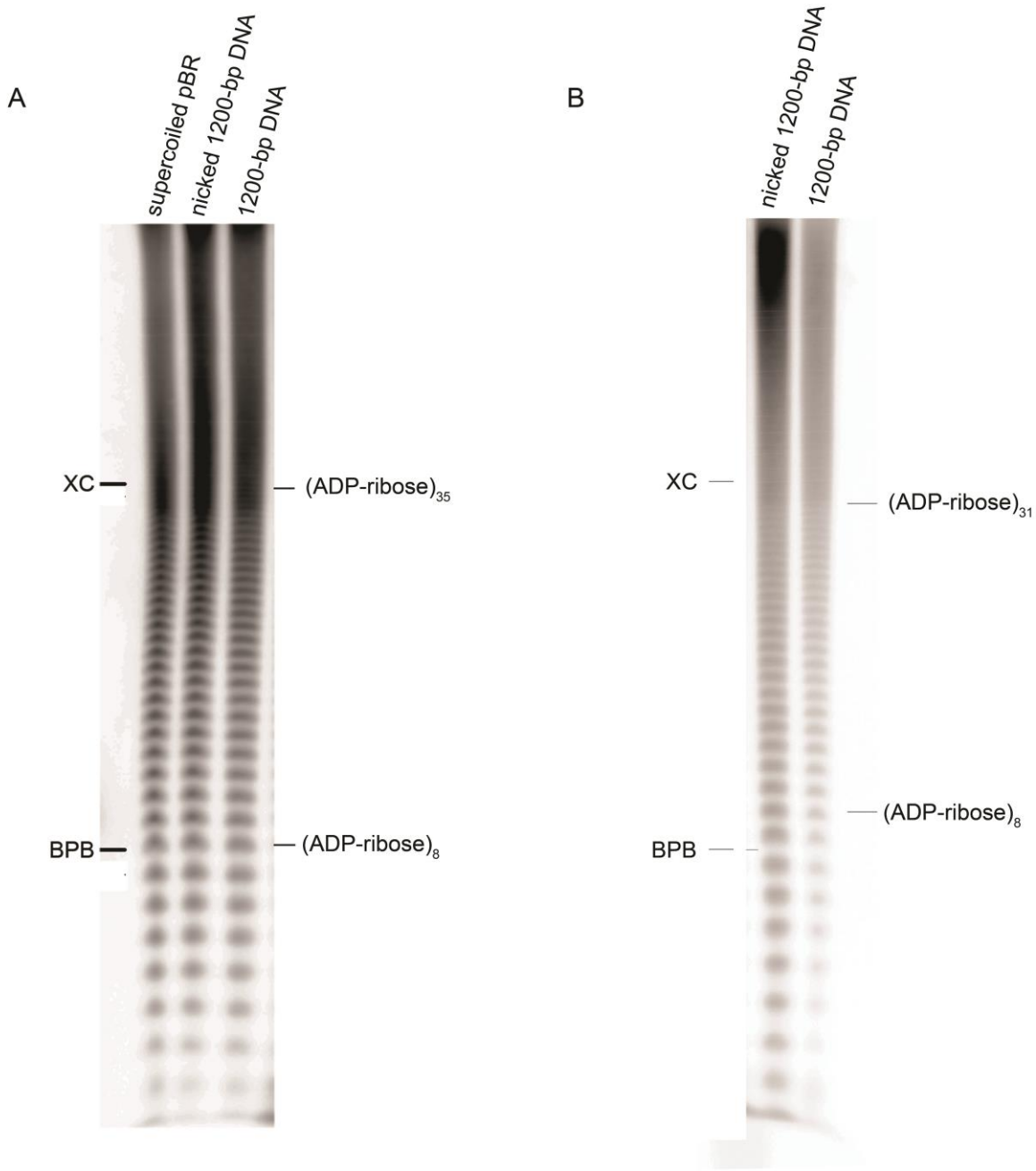

A
supercoiled pBR
nicked 1200-bp DNA
1200-bp DNA
XC
BPB
(ADP-ribose)35
(ADP-ribose)8
B
nicked 1200-bp DNA
1200-bp DNA
XC
BPB
(ADP-ribose)31
(ADP-ribose)8

Figure S7