Supporting Information

Site-specific non-covalent interaction of the biopolymer poly(ADP-ribose) with the Werner syndrome protein regulates protein functions

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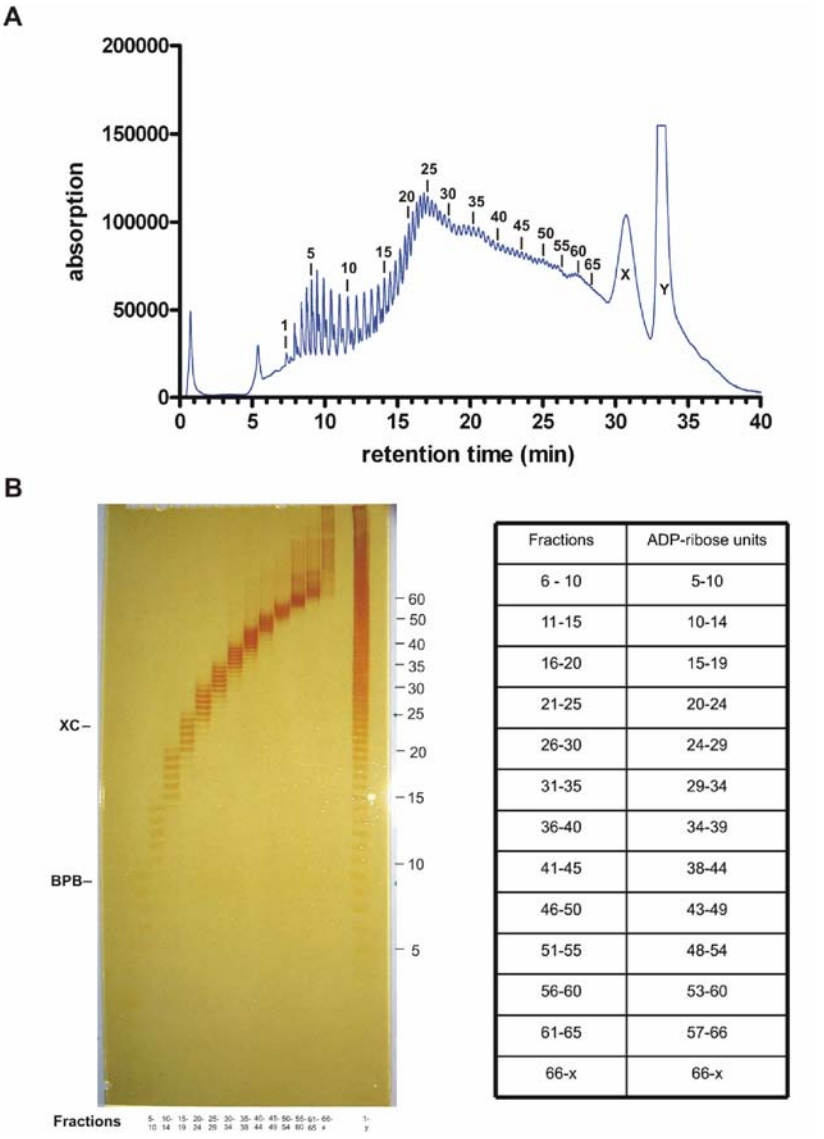
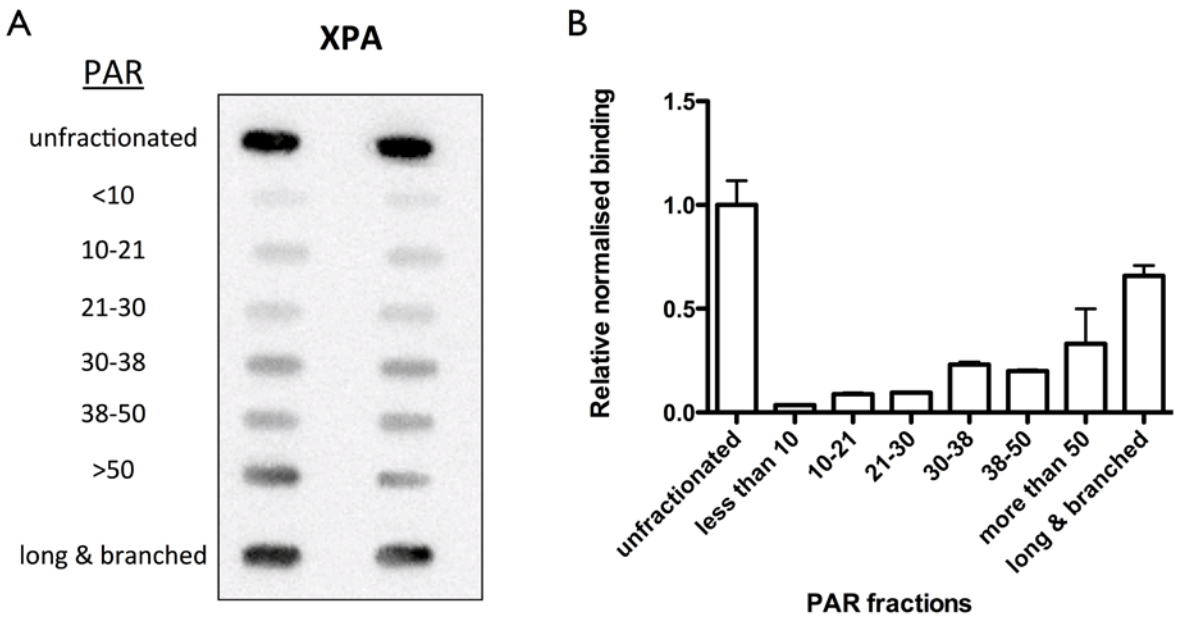
Supplementary Figure 1. Analytical HPLC fractionation of PAR and subsequent characterization of pooled fractions by sequencing gel analysis. A. 100 nmol of PAR was separated by anion exchange HPLC using a complex salt gradient. Peaks 1-65 are indicated. Peaks X and Y represent long PAR with high branching frequency. **B.** Fractionated PAR was analyzed on modified sequencing gels and silver staining. 100 pmol of each pooled fraction as well as 2 nmol of unfractionated PAR (1-Y) were separated by 15 % native PAGE followed by silver staining. Fraction numbers refer to the peak sequence obtained during HPLC. BPB migrates at the same position as an ADP-ribose octamer. BPB, bromphenol blue; XC, xylene cyanol.



Fractions	ADP-ribose units
6 - 10	5-10
11-15	10-14
16-20	15-19
21-25	20-24
26-30	24-29
31-35	29-34
36-40	34-39
41-45	38-44
46-50	43-49
51-55	48-54
56-60	53-60
61-65	57-66
66-x	66-x

Supplementary Figure 2. XPA binds longer PAR chains with higher affinity than short ones.

A. PAR overlay slot-blot evaluating the dependency of XPA-PAR binding on PAR chain length. XPA (15 pmol/slot) was immobilized on a nitrocellulose membrane (in duplicates) and incubated with 0.2 µM PAR of chain lengths as indicated. Removal of unspecific binding was ensured by high-stringency washing. Protein-bound PAR was detected using the anti-PAR monoclonal antibody 10H. **B.** Densitometric quantification of A, normalized to signals obtained from binding of unfractionated PAR.



Supplementary Figure 3. Confirmation of the inhibitory effect of PAR on WRN's exonuclease activity.

The impact of non-covalent PAR binding on WRN exonuclease activity was assessed by detection of a 5'-biotin-end-labeled oligonucleotide on a sequencing gel. **A.** WRN (40 nM) was pre-incubated with increasing concentrations of PAR as indicated. Exonuclease reaction was started by addition 300 fmol of a forked oligoduplex and carried out for 15 min. Following electrophoretic separation, products were immobilized on a nylon membrane, and detected via streptavidin-POD. A decrease in degradation of the oligonucleotide by increasing concentrations of PAR is observable. **B.** Densitometric evaluation from four independent experiments (means ± SEM). Curve was fitted using a sigmoidal dose-response curve with variable slope. Statistical analysis was performed with ANOVA followed by Dunnett's multiple comparison post-test. *** $p < 0.001$.