Divalent and multivalent cations control liquid-like assembly of poly(ADP-ribosyl)ated PARP1 into multimolecular associates *in vitro*

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Supplementary Information

Supplementary Tables

Supplementary Table 1. The hydrodynamic radius (R_h, nm) for PARylated PARP1 as determined in the presence of different concentrations of Mg^{2+}, Mn^{2+}, Ca^{2+}, Spn^{4+}, Put^{2+} and Spd^{3+}. R_h is the average R_h value estimated from at least three DLS experiments.

Cations (mM)	R_h (nm)			
	Before PARylation* (PARP1 + DNA)	After PARylation** (PARP1+ DNA + NAD+)	After addition of cations to PARylated PARP1***	After addition of cation and then EDTA****
Mg^{2+}				
0	8.2 ± 0.9	14 ± 2.0	-	-
5	-	-	14 ± 2.0	-
10	-	-	14 ± 2.0/**699 ± 63**	-
15	-	-	**773 ± 108**	-
15 mM + 30 mM EDTA	-	-	-	17 ± 4.0
Mn^{2+}				
0	7.1 ± 0.2	12 ± 2	-	-
0.5	-	-	12 ± 2	-
1	-	-	12 ± 1	-
2	-	-	**747 ± 149**	-
2 mM + 5 mM EDTA	-	-	-	15 ± 2
Ca^{2+}				
0	7.1 ± 0.4	13 ± 2	-	-
1	-	-	14 ± 1	-
2	-	-	14 ± 1	-
4	-	-	13 ± 2	-
6	-	-	14 ± 2	-
8	-	-	**1198 ± 85**	-
8 mM + 20 mM EDTA	-	-	-	18 ± 2
Put^{2+}				
0	7.7 ± 0.4	14 ± 2.0	-	-
1	-	-	15 ± 2	-
2	-	-	16 ± 2	-
4	-	-	17 ± 3	-
5	-	-	16 ± 2	-
7	-	-	19 ± 4	
9	-	-	18 ± 3	-
11	-	-	19 ± 4	-
13	-	-	17 ± 3	-
17	-	-	17 ± 2	-
21	-	-	17 ± 3	-
25	-	-	17 ± 2	-
60	-	-	18 ± 2	-
Spd^{3+}				-
0	7.1 ± 0.3	16 ± 3	-	-
1	-	-	17 ± 2	-
2	-	-	19 ± 2	-
3	-	-	18 ± 3	-
5	-	-	18 ± 2	-
7	-	-	17 ± 2	-
9	-	-	**714 ± 55**	

Spn^{4+}	7.1 ± 0.3	12.5 ± 1.2	-	
0	-	-		-
0.01	-	-	15.4 ± 1.1	-
0.06	-	-	15.3 ± 1.2	-
0.11	-	-	15.2 ± 0.9	-
0.36	-	-	16.4 ± 1.4	-
0.6	-	-	15.6 ± 0.8	-
0.86	-	-	15.7 ± 0.9	-
1.0	-	-	15.1 ± 0.7	-
3.5			**730 ± 130**	-

* R_h measurement was performed after incubation of 2.5 µM PARP1 with 2.5 µM DNA-gap for 1 min at 30 °C.
** R_h measurement was performed after the PARP1 PARylation in the absence of cations for 30 min at 30°C.
*** R_h measurement was performed after addition of cations to PARylated PARP1.
**** Rh measurement was performed after incubation of PARylated PARP1 with cations at the maximum concentration, followed by the addition of EDTA.
Particle sizes corresponding to liquid-like assembly of PARylated PARP1 in the presence of cations are shown in bold.

Supplementary Table 2. The hydrodynamic radius (R_h, nm) for protein-free PAR as determined in the presence of different concentrations of Mg^{2+}, Mn^{2+}, Ca^{2+}, Spn^{4+}, Put^{2+} and Spd^{3+}. R_h is the average R_h value estimated from at least three DLS experiments.

Cations (mM)	R_h (nm)		
	Before addition of cations*	After addition of cations**	After addition of cations and then EDTA
Mg^{2+}			
0	12 ± 5	-	-
1	-	8 ± 3	-
3	-	8 ± 4	-
5	-	6 ± 3	-
7	-	7 ± 5	-
9	-	15 ± 5	-
11	-	**143 ± 18**	-
13	-	**211 ± 22**	-
17	-	**247 ± 39**	-
20	-	**316 ± 18**	-
30	-	**346 ± 51**	-
60 mM + mM EDTA	-	-	46 ± 20
Mn^{2+}			
0	11 ± 5	-	-
1	-	15 ± 5	-
2	-	13 ± 4	-
3	-	**75 ± 10**	-
4	-	**150 ± 11**	-
6	-	**241 ± 42**	-
8	-	**312 ± 31**	-
10	-	**440 ± 88**	-
10 mM + 20 mM EDTA	-	-	10± 2
Ca^{2+}			
0	10 ± 6	-	-
3	-	7.8 ± 0.7	-
6	-	14 ± 5	-
9	-	**147 ± 27**	-
15	-	**239 ± 21**	-
21	-	**307 ± 30**	-
21 mM + 40 mM EDTA	-	-	7 ± 3
Spn^{4+}			
	5.4± 0.3	-	-
1	-	7.8 ± 1.5	-
2	-	**159 ± 40**	-
3	-	**247 ± 79**	-
4	-	**334 ± 36**	-
5	-	**321 ± 56**	-
7	-	7 ± 4	-
9	-	6 ± 4	-

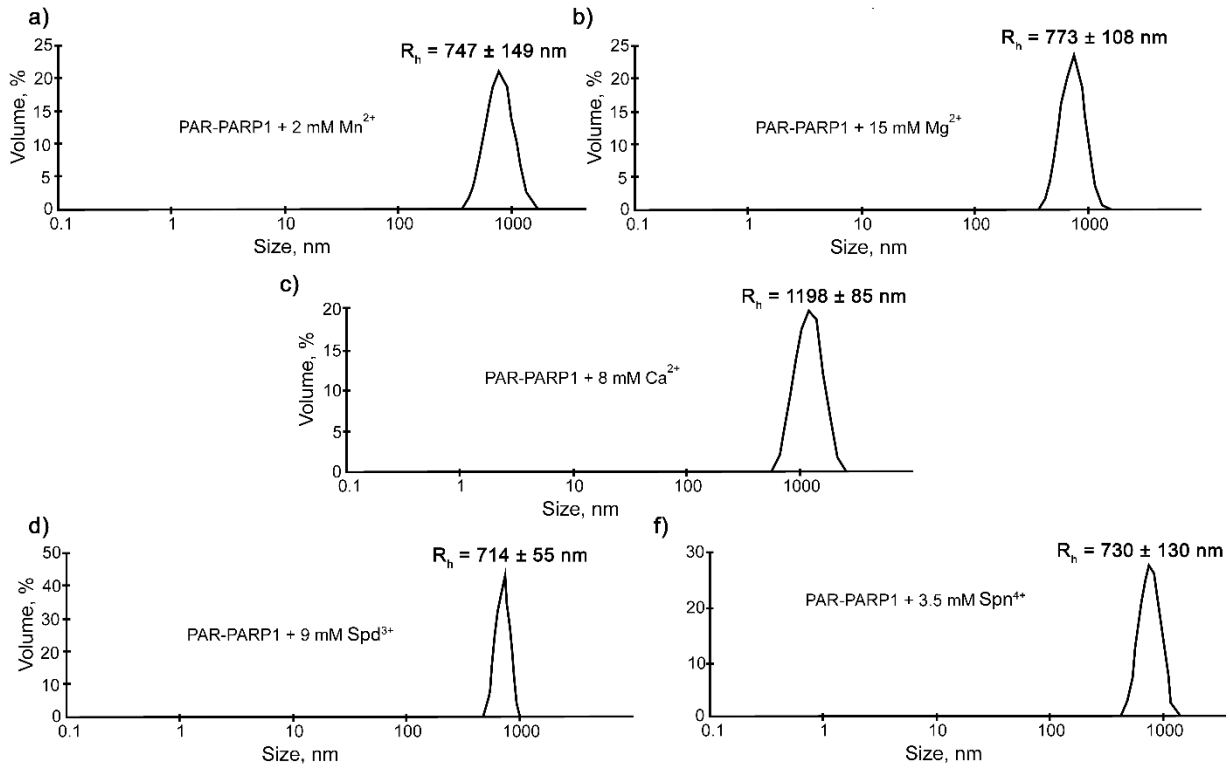
* R_h measurement was performed after incubation of 50 µM ADP-ribose(PAR) for 1 min at 30 °C.
** R_h measurement was performed after addition of cations to PAR.
Particle sizes corresponding to liquid-like assembly of PAR in the presence of cations are shown in bold.

Supplementary Table 3. The hydrodynamic radius (R_h, nm) for PARylated PARP1 as determined when its modifications proceeds in the presence of 3 mM Mn^{2+}, 10 mM Mg^{2+},10 mM Ca^{2+}, 13 mM Spd^{3+} or Spn^{4+}. R_h is the average R_h value estimated from at least three DLS experiments.

Cations	R_h (nm)	
	After PARP1 PARylation	After addition of EDTA
3 mM Mn^{2+} + 6 mM EDTA	1143 ± 86 -	- 11 ± 1
10 mM Ca^{2+} + 20 mM EDTA	930 ± 104 -	- 11 ± 1
10 mM Mg^{2+} + 20 mM EDTA	1037 ± 112 -	- 21 ± 3
13 mM Spd^{3+}	744 ± 56	-
3 mM Spn^{4+}	700 ± 72	-

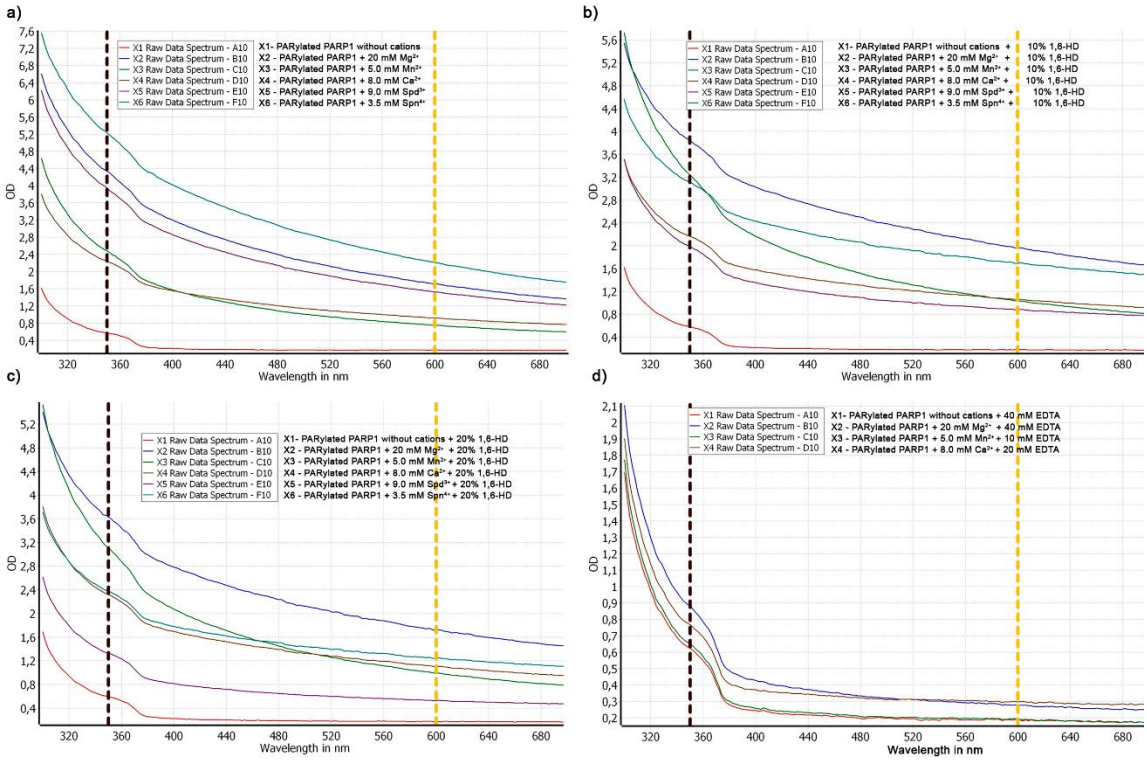
Supplementary Figures

Supplementary Figure 1. Typical volume-weighted size distributions for PARylated PARP1 (PAR-PARP1) after addition of Mn^{2+} (a), Mg^{2+}(b), Ca^{2+} (c), Spd^{3+} (d) or Spn^{4+} (f).



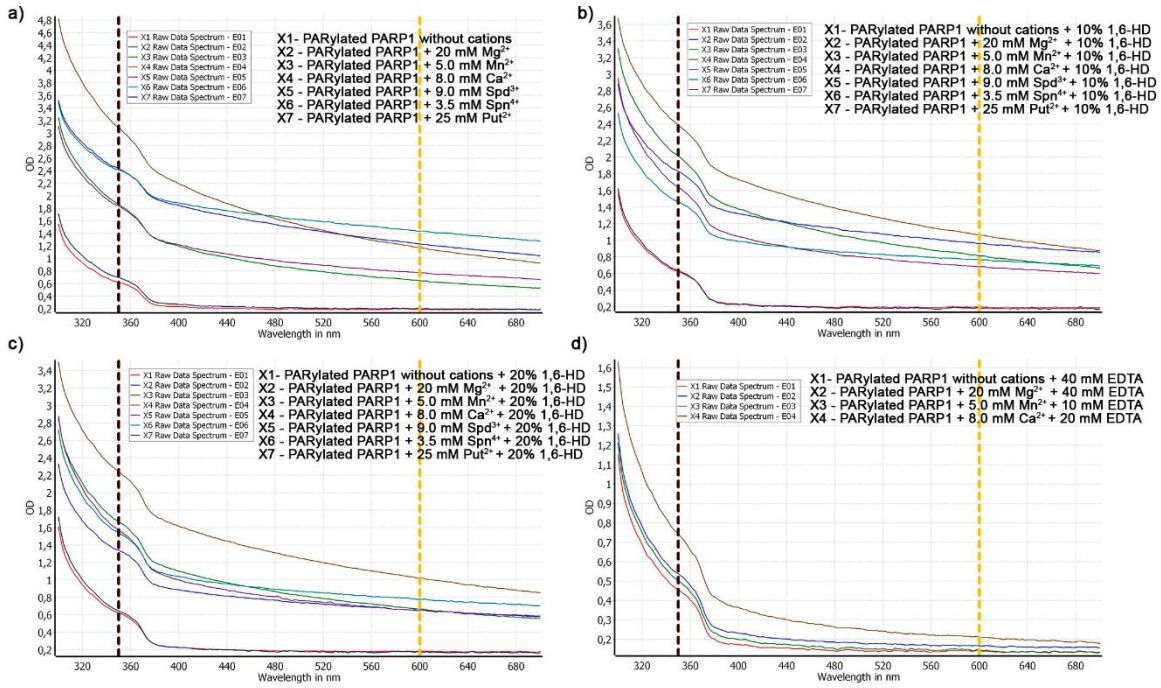
The R_h values were measured directly after incubation of 2.5 μM PARP1 with 2.5 μM DNA and 1 mM NAD^+ (when the size growth reached the plateau) for 30 min at 30°C followed by the addition of Mn^{2+} up to 2mM (**a**), Mg^{2+} up to 15 mM (**b**), Ca^{2+} up to 8 mM (**c**), Spd^{3+} up to 9 mM (**d**) or Spn^{4+} up to 3.5. mM (**f**). The profiles were obtained by means of experimental autocorrelation functions in the Zetasizer Nano ZS software. The average hydrodynamic radii (R_h) computed from the distributions are presented as well. R_h is the average R_h value estimated from at least three DLS experiments.

Supplementary Figure 2. Liquid-like assembly of PARylated PARP1 in the presence of olaparib and cations monitored by turbidity measurement.

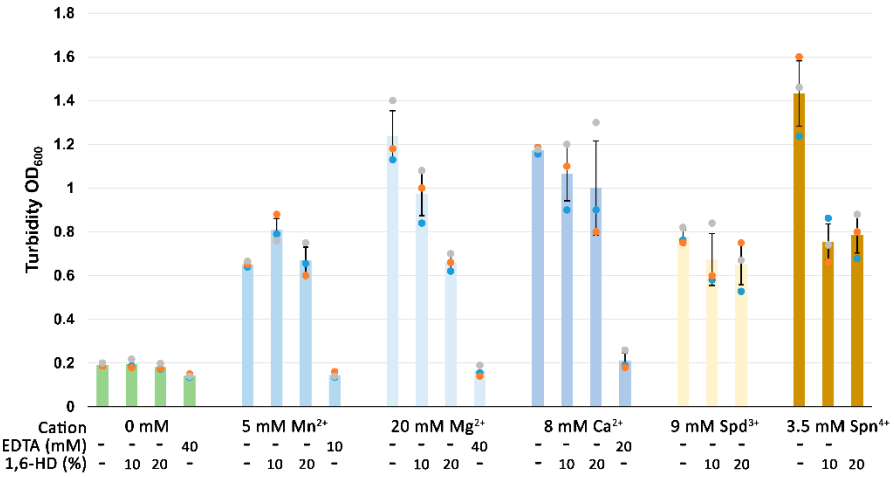


a) Absorbance spectra of PARylated PARP1 in the absence (red line) or presence of cations. 2.5 μM PARP1 was incubated with 2 μM DNA-gap and 1 mM NAD^+ at 30°C for 30 min. The reactions were stopped by the addition of olaparib to a final concentration of 250 μM. After that, the reactions were supplemented with Mg^{2+} up to 20 mM (blue line), Mn^{2+} up to 5 mM (green line), Ca^{2+}up to 8 mM (brown line), Spd^{3+} up to 9 mM (purple line), Spn^{4+} up to 3.5 mM (turquoise line).
b) Absorbance spectra of PARylated PARP1 in the absence (red line) or presence of cations after adding of 1,6-hexandiol up to 10%.
c) Absorbance spectra of PARylated PARP1 in the absence (red line) or presence of cations after adding of 1,6-hexandiol up to 20%.
d) Absorbance spectra of PARylated PARP1 in the absence (red line) or presence of Me^{2+} cations after adding of EDTA up to 10, 20 or 40 mM, as indicated.
The absorbance of the protein solution was recorded from 300 to 700 nm in a 96-well non-binding black microplates of transparent bottom (Corning 3881), using a POLARstar Optima multidetection microplate reader (BMG Labtech, Offenburg, Germany). From left to right, two vertical dash lines indicate the position of 350 and 600 nm wavelengths, respectively. From left to right, two vertical dashed lines indicate the position of 350 and 600 nm wavelengths, respectively.

Supplementary Figure 3. Liquid-like assembly of PARylated PARP1 in the absence of olaparib and presence of cations monitored by turbidity measurement.



e)

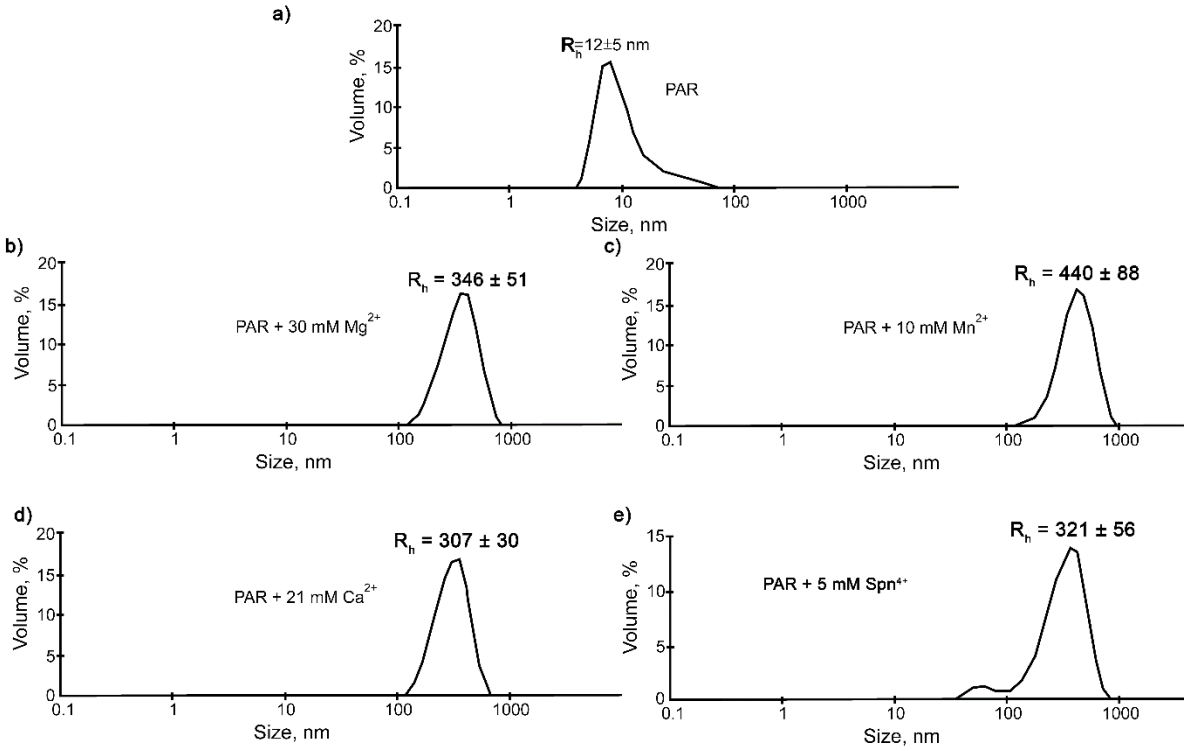


a) Absorbance spectra of PARylated PARP1 in the absence (red line) or presence of cations. 2.5 μM PARP1 was incubated with 2 μM DNA-gap and 1 mM NAD^+ at 30°C for 30 min. After that, the reactions were supplemented with Mg^{2+} up to 20 mM (blue line), Mn^{2+} up to 5 mM (green line), Ca^{2+} up to 8 mM (brown line), Spd^{3+} up to 9 mM (purple line), Spn^{4+} up to 3.5 mM (turquoise line) or Put^{2+} up to 25 mM (deep blue line).**b)** Absorbance spectra of PARylated PARP1 in the absence (red line) or presence of cations after adding of 1,6-hexandiol up to 10%. **c)** Absorbance spectra of PARylated PARP1 in the absence (red line) or presence of cations after adding of 1,6-hexandiol up to 20%. **d)** Absorbance spectra of PARylated PARP1 in the absence (red line) or presence of Me^{2+} cations after adding of EDTA up to 10, 20 or 40 mM, as indicated. The absorbance of the protein solution was recorded from 300 to 700 nm in a 96-well non-binding black microplates of transparent bottom (Corning 3881), using a POLARstar Optima multidetection microplate reader (BMG Labtech, Offenburg, Germany). From left to right, two vertical dash lines indicate the position of 350 and 600 nm wavelengths, respectively. **e)** Histograms present turbidity (optical density [OD] at 600 nm) of PARylated PARP1 in the absence (green bars) or presence of cations, cations and 10% of 1,6-hexandiol (1,6-HD), cations and 20% of 1,6-hexandiol (1,6-HD), or cations and EDTA as indicated (the mean ± SD of three independent measurements) (panel a, b, c, d).

For analysis of the turbidity of PARylated PARP1 solutions after its modification, 2.5 μM PARP1 was incubated with 2 μM DNA-gap in a buffer consisting of 140 mM NaCl, 20 mM HEPES-NaOH pH 7.5, 1 mM DTT, and 1 mM NAD^+ at 30°C for 40 min. After that, the reactions were supplemented with 5 mM Mn^{2+}, 20 mM Mg^{2+}, 8 mM Ca^{2+}, 9 mM Spd^{3+}, or 3.5 mM Spn^{4+}, and an absorption spectrum of the solutions was recorded. Then, either 1,6-

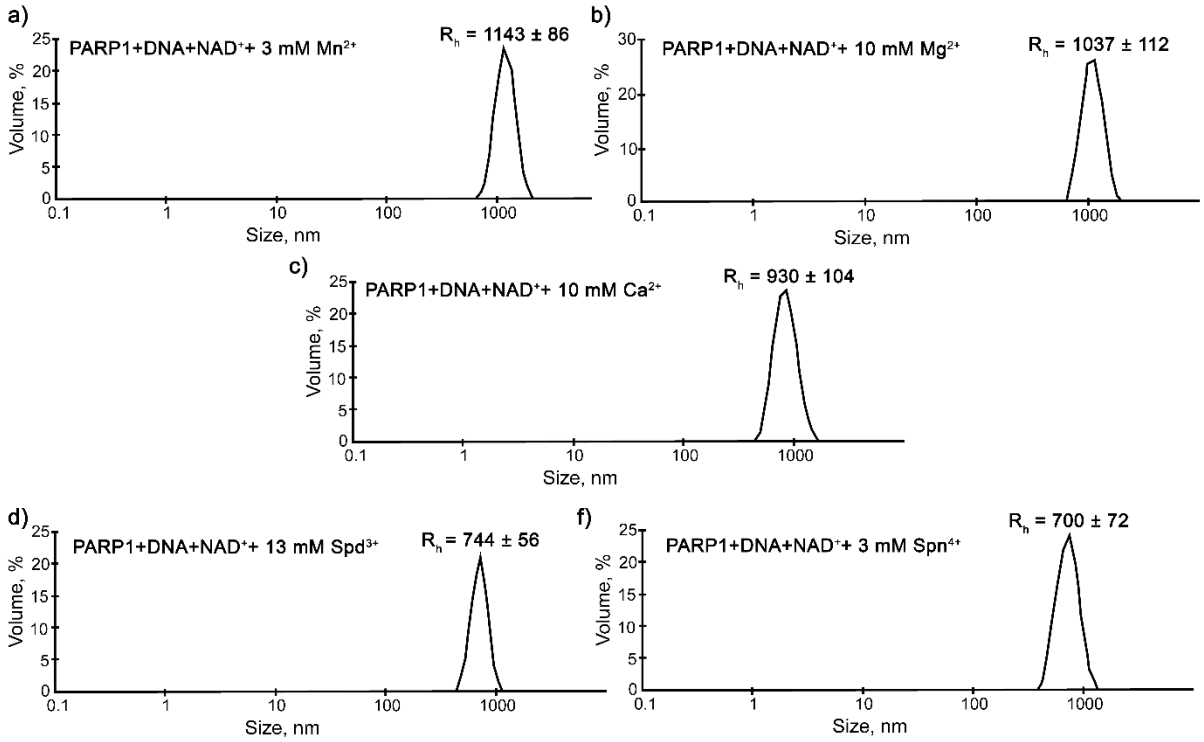
hexandiol or EDTA was added as indicated in a figure legend, and the absorption spectrum of the solutions was recorded again.

Supplementary Figure 4. Typical volume-weighted size distributions for protein-free PAR before (a) and after addition of Mg^{2+} (b), Mn^{2+}(c), Ca^{2+} (d) or Spn^{4+} (e).



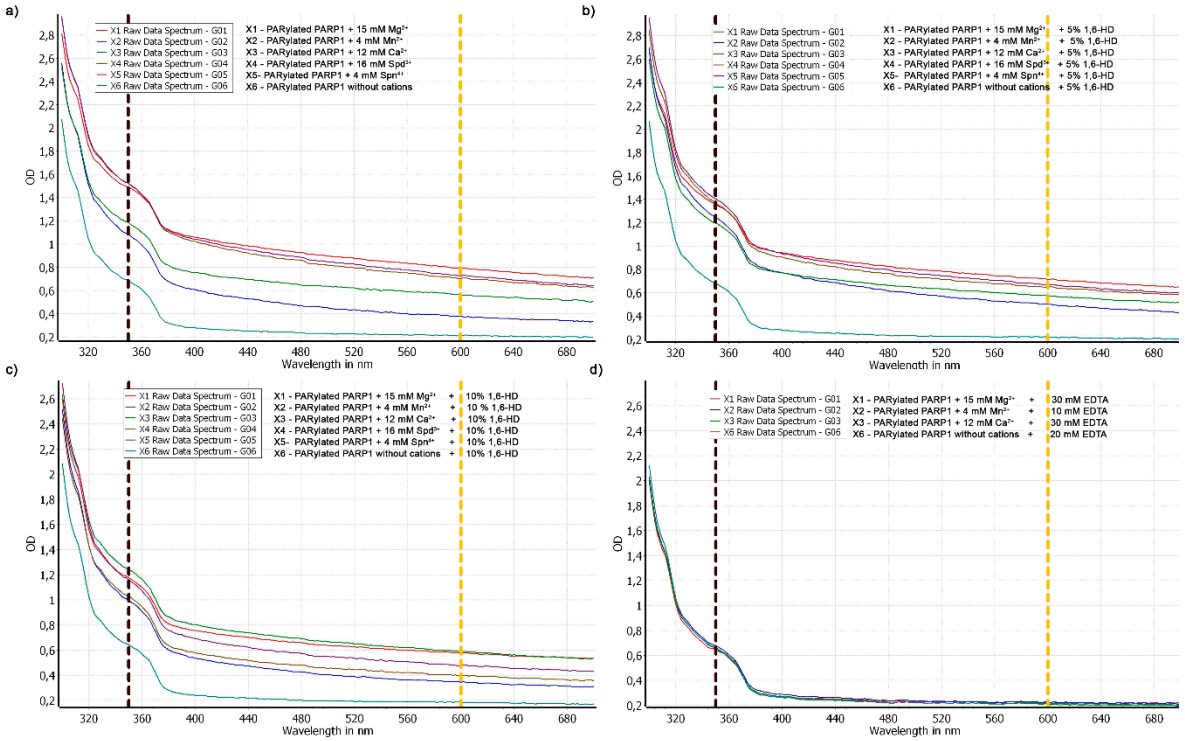
The R_h values were measured directly after incubation of 50 μM ADP-ribose (PAR) in the buffer consisting of 200 mM NaCl, 20 mM Hepes, pH 7.5 in the absence (**a**) or presence of 10 mM Mn^{2+} (**c**), 30 mM Mg^{2+} (**b**), 21 mM Ca^{2+} (**d**) or 5 mM Spn^{4+} (**e**). The profiles were obtained by means of experimental autocorrelation functions in the Zetasizer Nano ZS software. The average hydrodynamic radii (R_h) computed from the distributions are presented as well. R_h is the average R_h value estimated from at least three DLS experiments.

Supplementary Figure 5. Typical volume-weighted size distributions for PARP1 during PARylation in the presence of 2 mM Mn^{2+}(a), 10 Mg^{2+} (b), 10 mM Ca^{2+} (c), 13 mM Spd^{3+} (d) or 3 mM Spn^{4+} (f).



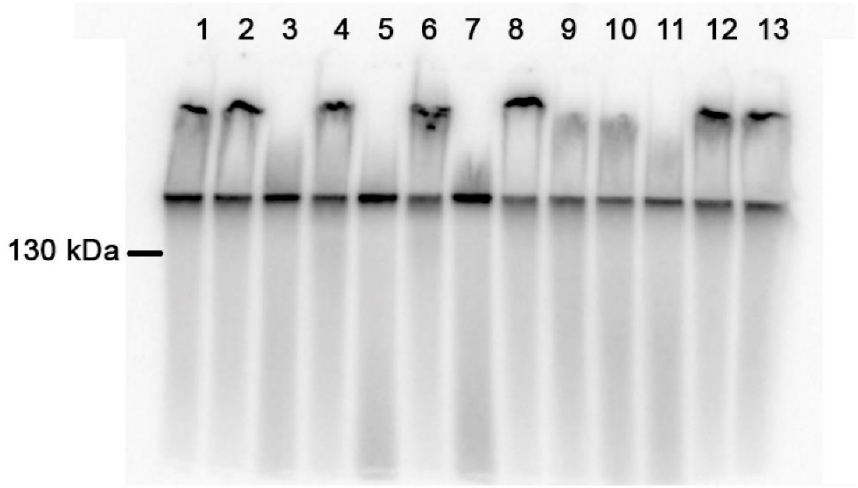
The R_h values were measured directly after incubation of 2.5 μM PARP1 with 2.5 μM DNA and 1 mM NAD^+ in the presence of cations as indicated for 30 min at 30°C. The profiles were obtained by means of experimental autocorrelation functions in the Zetasizer Nano ZS software. The average hydrodynamic radii (R_h) computed from the distributions are presented as well. R_h is the average R_h value estimated from at least three DLS experiments.

Supplementary Figure 6. Liquid-like assembly of PARP1 during its PARylation in the presence of cations monitored by turbidity measurement.



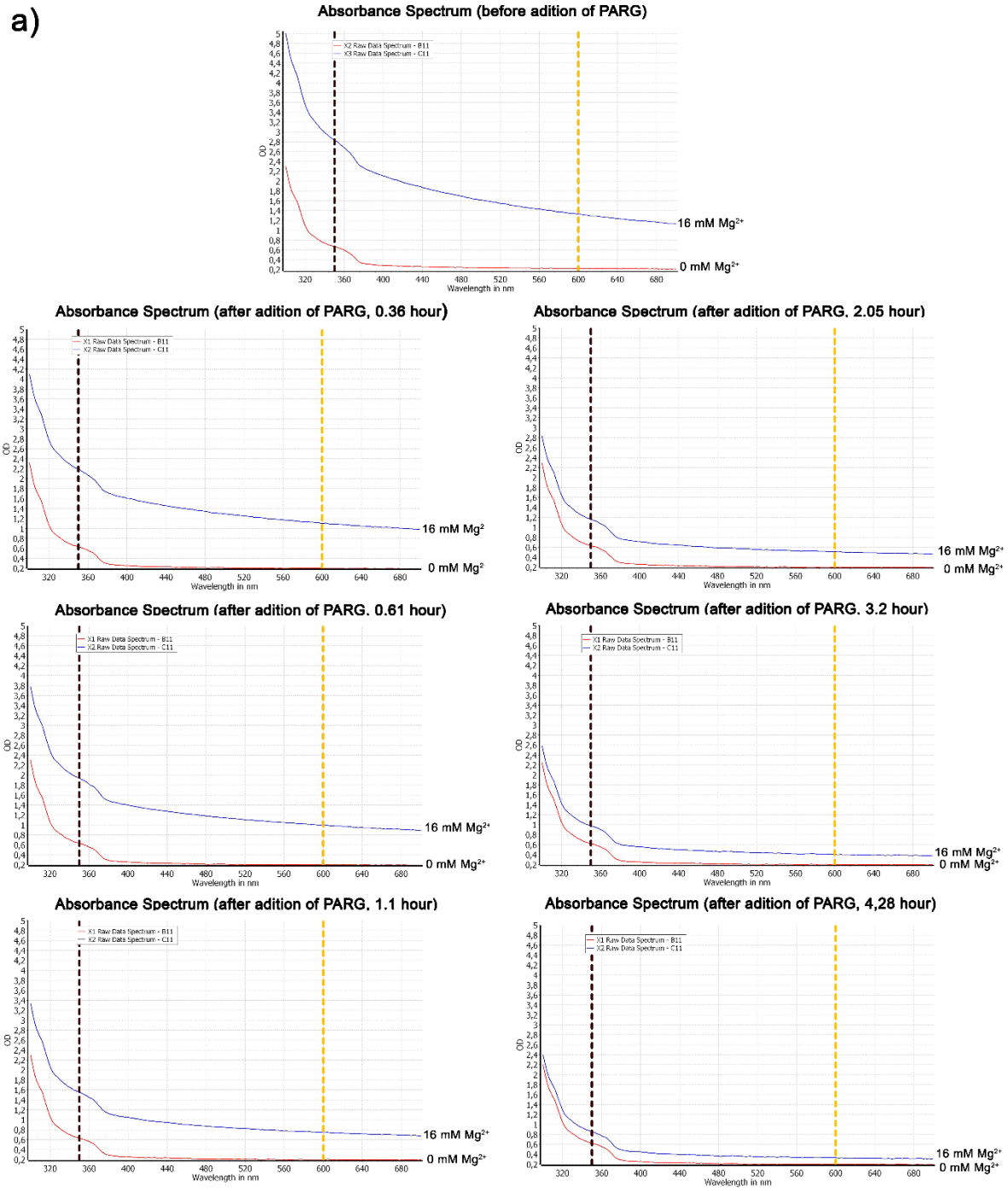
a) Absorbance spectra of PARylated PARP1 in the absence (turquoise line) or presence of cations. 2.5 μM PARP1 was incubated with 2 μM DNA-gap and 1 mM NAD^+ in the presence of 15 mM Mg^{2+} (red line), 4 mM Mn^{2+} (deep blue line), 12 mM Ca^{2+} (green line), 16 mM Spd^{3+} (brown line), 4 mM Spn^{4+} (violet line) at 30°C for 40 min. After that, the adsorption spectrum of the solutions was recorded.
b) Absorbance spectra of PARylated PARP1 in the absence (turquoise line) or presence of cations after adding of 1,6-hexandiol up to 5%.
c) Absorbance spectra of PARylated PARP1 in the absence (turquoise line) or presence of cations after adding of 1,6-hexandiol up to 10%.
d) Absorbance spectra of PARylated PARP1 in the absence (turquoise line) or presence of Me^{2+} cations after adding of EDTA up to 10 or 30 mM as indicated.
The absorbance of the protein solution was recorded from 300 to 700 nm in a 96-well non-binding black microplates of transparent bottom (Corning 3881), using a POLARstar Optima multidetection microplate reader (BMG Labtech, Offenburg, Germany). From left to right, two vertical dash lines indicate the position of 350 and 600 nm wavelengths, respectively.

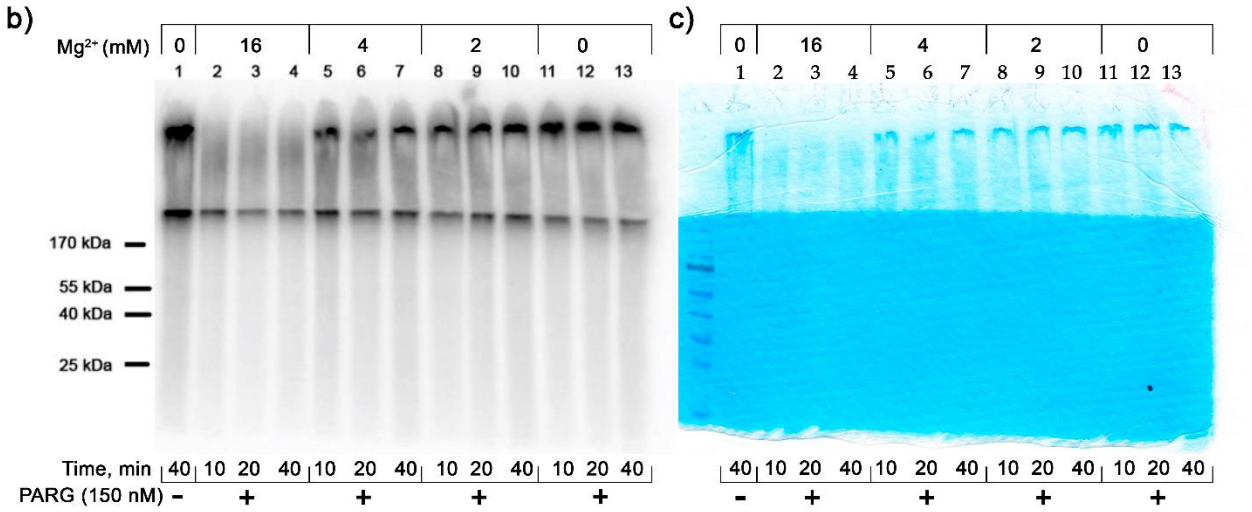
Supplementary Figure 7. PARP1 PARylation detected by SDS-PAGE and subsequent phosphorimaging.



The reaction mixtures were composed of 2.5 μM PARP1, 2 μM DNA-gap, 1 mM NAD^+ ([^{32}P]NAD+, 2.5 μCi/50 μl), and without cations (lane 1) or with 2 mM Mg^{2+} (lane 2), 15 mM Mg^{2+} (lane 3), 2 mM Mn^{2+} (lane 4), 4 mM Mn^{2+} (lane 5), 2 mM Ca^{2+} (lane 6), 12 mM Ca^{2+} (lane 7), 2 mM Spd^{3+} (lane 8), 9 mM Spd^{3+} (lane 9), 2 mM Spn^{4+} (lane 10), 3.5 mM Spn^{4+} (lane 11), 2 mM Put^{2+} (lane 12), 8 mM Put^{2+} (lane 13).

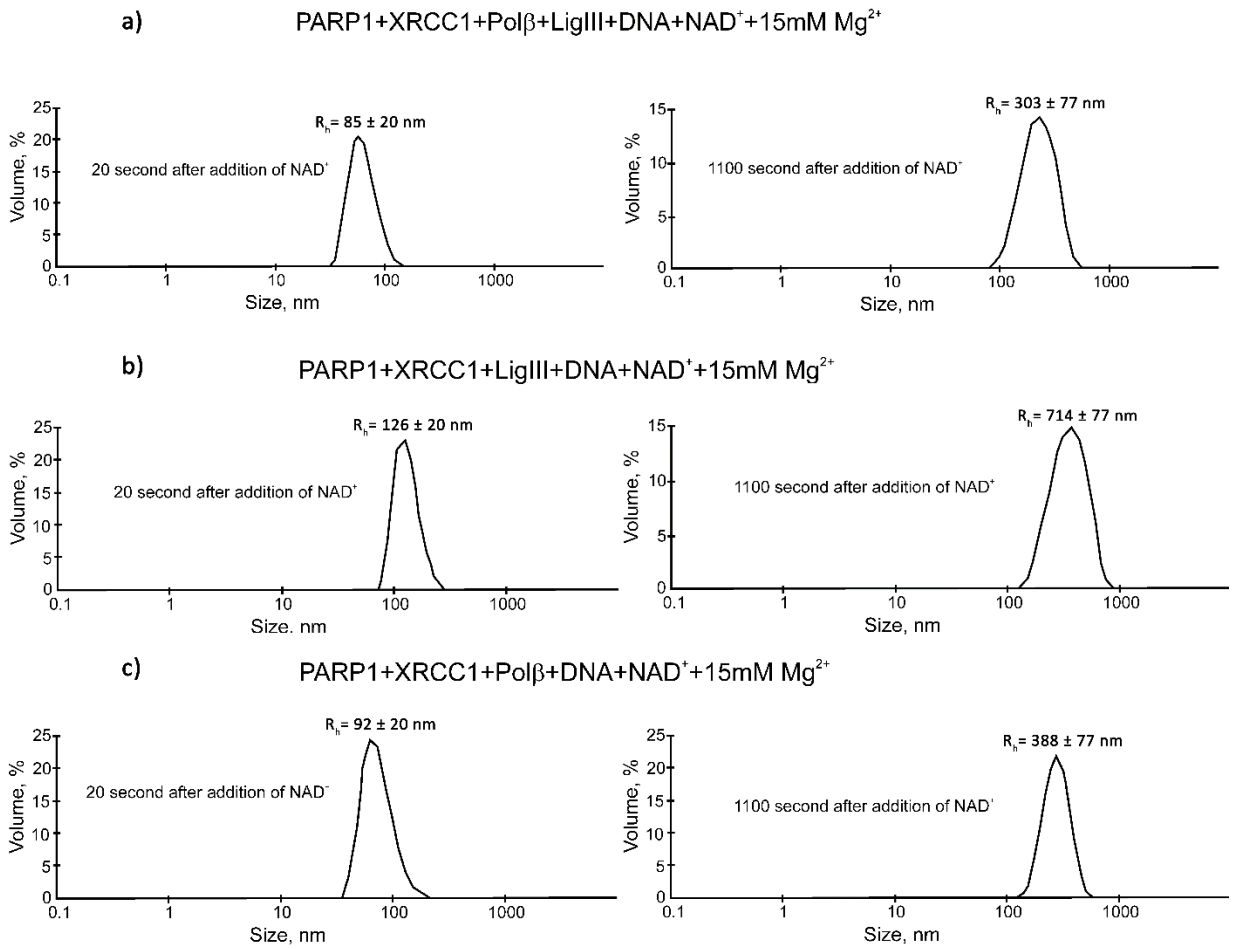
Supplementary Figure 8. Mg^{2+}-dependent liquid-like assembly of PARylated PARP1 leads to stimulation of PARG activity.





a) Absorbance spectra of PARylated PARP1 in the absence (red line) or presence of 16 mM Mg^{2+} (deep blue line) before and after addition of PARG. 2.5 μM PARP1 was incubated with 2 μM DNA-gap and 1 mM NAD+ at 30°C for 30 min, after the reactions were stopped by the addition of olaparib to a final concentration of 200 μM. Then, PARG to the final concentration of 150 nM and Mg^{2+} to the final concentration of 16 mM was added as indicated in a figure legend, and the absorption spectrum of the solutions was recorded again during 0.6 - 4.5 hours. Degradation of PAR attached to PARP1 by PARG detected by SDS-PAGE and subsequent phosphorimaging (**b**) and Coomassie blue staining (**c**). For the analysis of the degradation of PARP1-bound PAR by PARG, 2.5 μM PARP1 was incubated with 2 μM DNA-gap in a buffer consisting of 140 mM NaCl, 20 mM HEPES-NaOH pH 7.5, 1 mM DTT, and 1 mM NAD^+, [^{32}P]NAD^+ (7.5 μCi/50 μl) at 30°C for 30 min. After that, the reactions were stopped by addition of olaparib to the final concentration of 200 μM. Next, 2, 4 or 16 mM Mg^{2+} was added to the samples, the samples were equilibrated for 5 min, and then PARG (lanes 2-13) was added to the final concentration of 150 nM followed by incubation for 10-40 min at 30°C. Bands of [^{32}P]PAR-labeled PARP1 were quantified by means of the Quantity One Basic software (Bio Rad). The relative level of PARylated PARP1 hydrolysis in the presence of PARG was normalized to the level of PARP1 automodification observed after 40 min in the absence of cations and PARG (panel (b), first line).

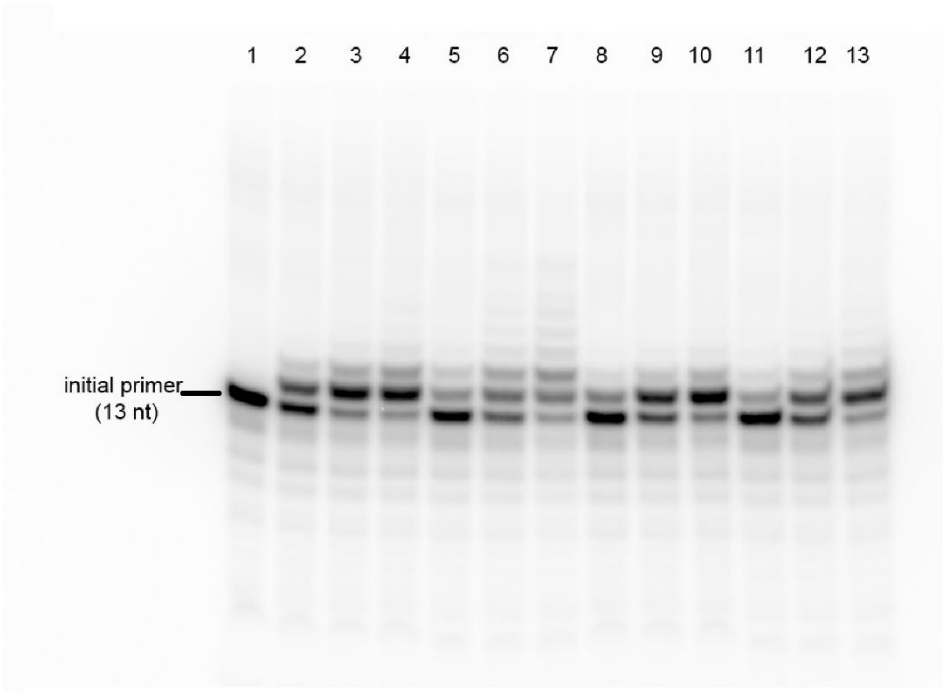
Supplementary Figure 9. Typical volume-weighted size distributions for mixture of repair protein and PARP1 during PARylation in the presence of NAD$^+$ after 20 and 1100 second incubation.



Hydrodynamic radii (R_h) of PARP1, XRCC1, and Pol β (**c**), PARP1, XRCC1, and Lig III (**b**) and PARP1, XRCC1, Pol β and Lig III (**a**) mixtures under conditions of PARP1 activation and liquid-like assembly, i.e., in the presence of DNA, NAD$^+$ and 15 mM Mg^{2+}.

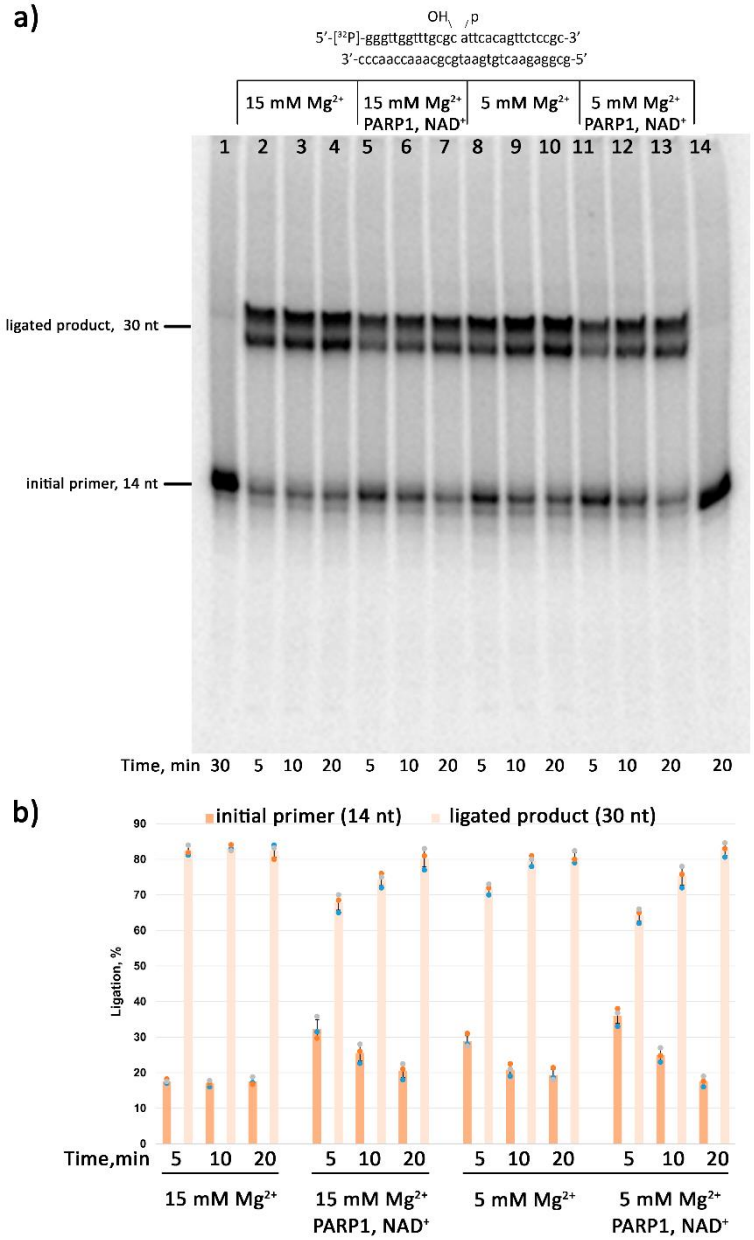
The hydrodynamic size was determined in reaction mixtures consisting of (**c**) 2.5 μM PARP1, 0.1 μM XRCC1, 0.1 μM Pol β, 1 μM DNA, 1 mM NAD$^+$, and 15 mM Mg2+; (**b**) 2.5 μM PARP1, 0.1 μM XRCC1, 0.1 μM Lig III, 1 μM DNA, 1 mM NAD+, and 15 mM Mg^{2+}; (**a**) 2.5 μM PARP1, 0.1 μM XRCC1, 0.1 μM Pol β, 0.1 μM Lig III, 1 μM DNA, 1 mM NAD+, and 15 mM Mg^{2+}. The R_h values were measured directly after 20 and 1100 s incubation of PARP1 with BER proteins in the presence of DNA and NAD$^+$. R_h is the average calculated from at least three independent experiments

Supplementary Figure 10. The DNA synthesis catalyzed by Pol β.



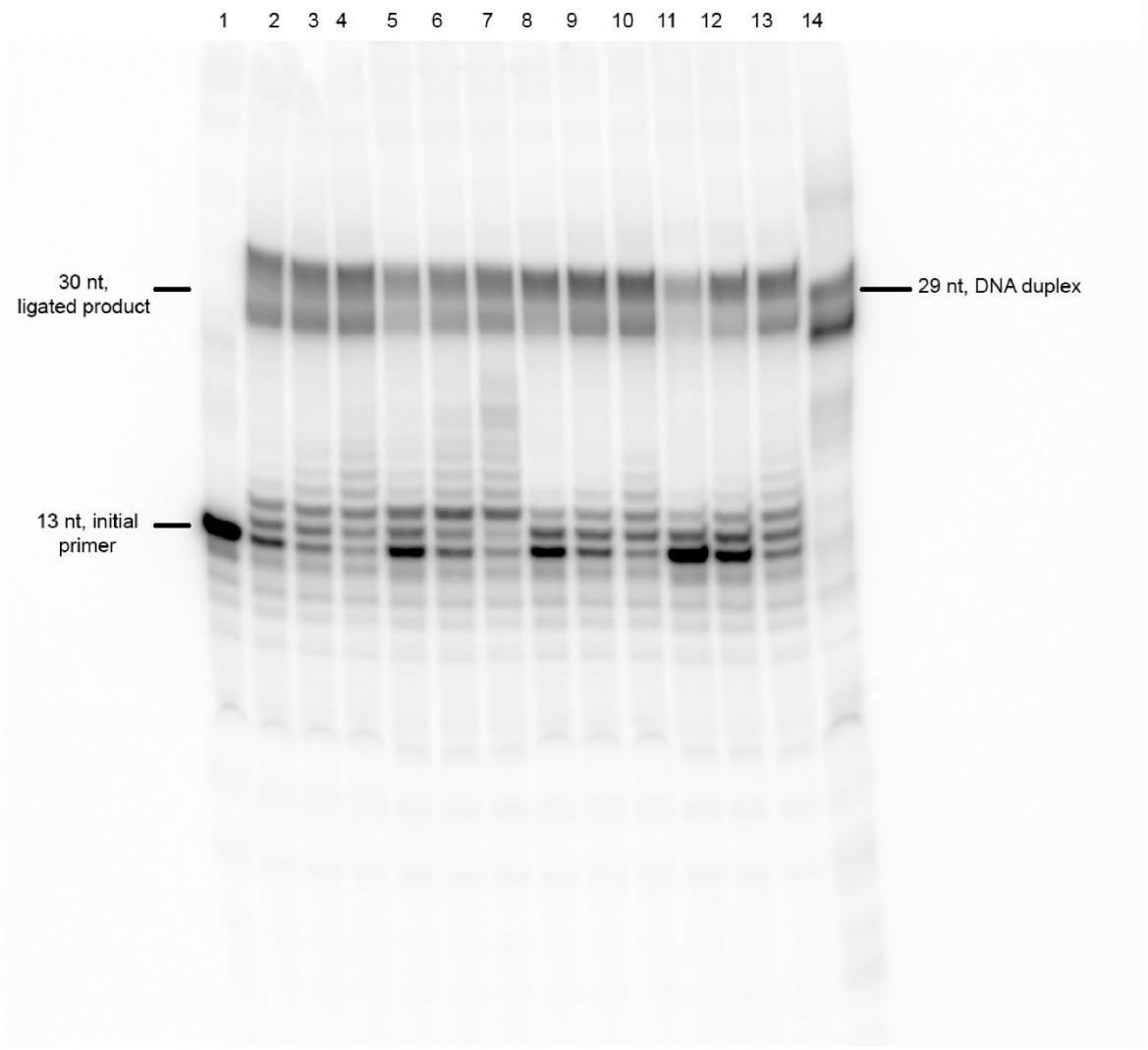
The DNA synthesis catalyzed by Pol β was carried out in reaction mixtures consisting of 2.0 μM 5'-^{32}P-labeled DNA-gap, 0.1 μM Pol β, 0.1 μM XRCC1, 100 μM four dNTPs, 1 mM NAD+, and 5 mM Mg^{2+} (lanes 8-13) or 15 mM Mg^{2+} (lanes 2-7) in the absence of 2.5 μM PARP1 (lanes 2-4, 8-10) or presence of 2.5 μM PARP1 (lanes 5-7, 11-13) for 5–30 min at 30°C. The products were separated by denaturing electrophoresis in a 20% denaturing (7 M urea) polyacrylamide gel electrophoresis (PAGE). The gels were dried and subjected to phosphoimaging for quantification using a Typhoon FLA9500 imager (GE Healthcare, UK) and software (Quantity One, Bio-Rad, USA).

Supplementary Figure 11. Mg^{2+}-dependent liquid-like self-assembly of PARylated PARP1 does not affect Lig III activity.



a) The nick sealing catalyzed by Lig III was implemented in reaction mixtures consisting of 2.0 μM 5′-^{32}P-labeled DNA duplex containing a nick (DNA-nick), 0.1 μM Lig III, 0.1 μM XRCC1, 1 mM ATP, 1 mM NAD^+ (lanes 5-7, 11-13), and 5 (lanes 8-13) or 15 mM Mg^{2+} (lanes 2-7) in the absence or presence of 2.5 μM PARP1, as indicated, for 5–20 min at 30°C. Schematic representation of the DNA substrate is provided at the top of the gel.
b) Histograms of quantification of the ligation product (%) from panel (a). The yield of the products was calculated as the percentage of the total radioactivity of all the ^{32}P-containing bands DNA in a lane and represents the mean ± SD of three independent measurements; the SD did not exceed 12% of total counts.

Supplementary Figure 12. The Pol β – catalyzed DNA synthesis coupled with ligation.



The Pol β – catalyzed DNA synthesis coupled with ligation was carried out in reaction mixtures consisting of 2.0 μM 5'-^{32}P-labeled DNA-gap, 0.1 μM Pol β, 0.1 μM XRCC1, 0.1 μM Lig III, 100 μM four dNTPs, 1 mM ATP, 1 mM NAD^+, and 5 mM Mg^{2+} (lanes 8-13) or 15 mM Mg^{2+} (lanes 2-7) in the absence of 2.5 μM PARP1 (lanes 2-4, 8-10) or presence of 2.5 μM PARP1 (lanes 5-7, 11-13) for 5–20 min at 30°C. The products were separated by denaturing electrophoresis in a 20% denaturing (7 M urea) polyacrylamide gel electrophoresis (PAGE). The gels were dried and subjected to phosphoimaging for quantification using a Typhoon FLA9500 imager (GE Healthcare, UK) and software (Quantity One, Bio-Rad, USA).