Supporting Information:

Enzymatic Synthesis and Structural Characterization of ^{13}C, ^{15}N - Poly(ADP-ribose)

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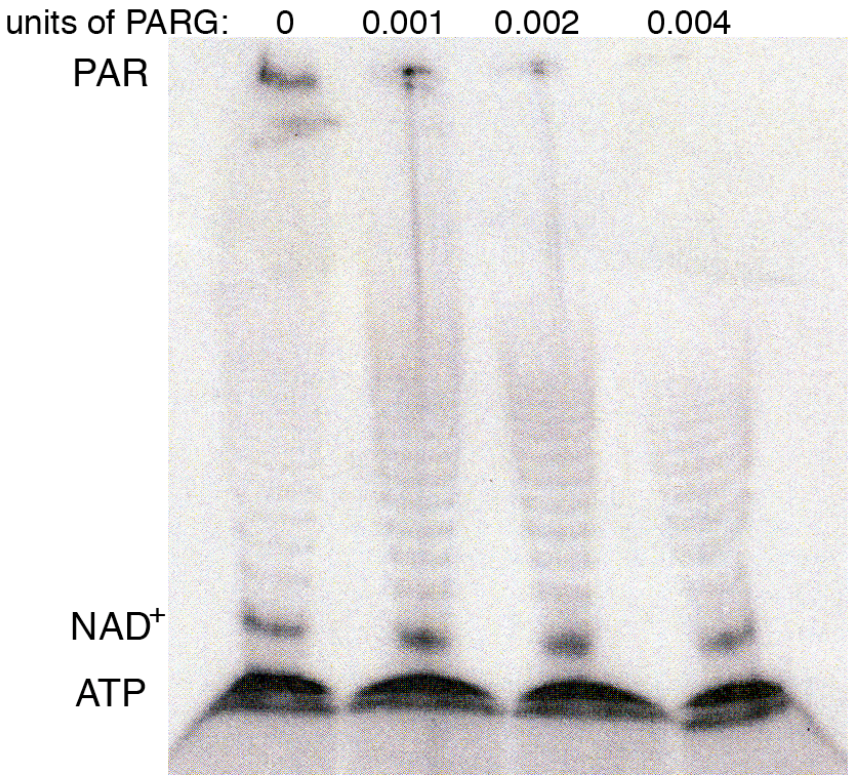
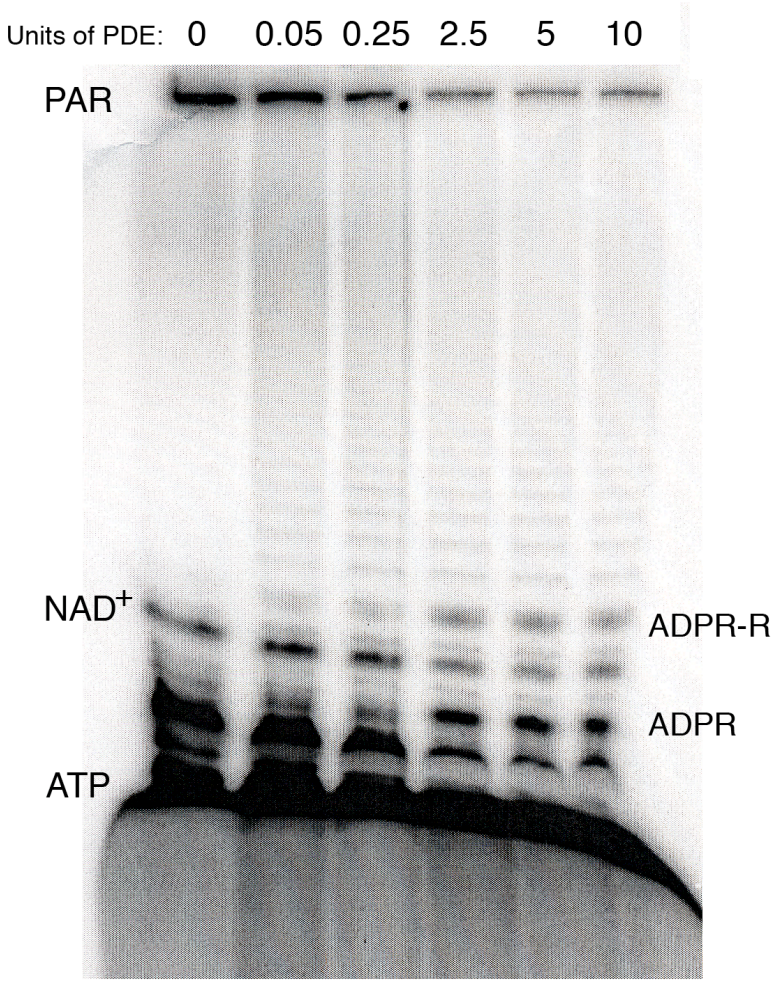
Supplementary Table 1

Poly (ADP-ribose)	
UV titration	Mg^{++}: 0 - 5 equivalents pH range: 2 - 11
Thermal Denaturation	0.1 M NaCl (pH: 4.5, 6.5, 7.5, 10.5) 0.1 M NaCl, 20 mM $MgCl_2$ (pH 4.5, 6.5, 7.5, 10.5) 0.5 M NaCl, pH 7.5 (25, 50, 100, 150, 200 mM $MgCl_2$) pH 7.5 (0.1, 0.5, 1, 2, 4 M NaCl)
NMR Experiments	1D ^1H pH 6.5 (0.15, 4 M NaCl) 2D NOESY* 2D COSY 2D TOCSY 1D ^1H Mg^{++} titration 2D ^1H-^{13}C NOESY 2D ^1H-^{13}C HSQC 2D ^1H-^{15}N HSQC
ADP-ribose	
NMR Experiments	1D ^1H pH 6.5 (0.15, 4 M NaCl) 2D ROESY 2D Natural Abundance ^1H-^{13}C HSQC
Poly-Adenosine	
Thermal Denaturation	0.1 M NaCl (pH: 4.5, 6.5, 7.5, 10.5) 0.1 M NaCl, 20 mM $MgCl_2$ (pH 4.5, 6.5, 7.5, 10.5)
NMR Experiments	1D ^1H 2D NOESY

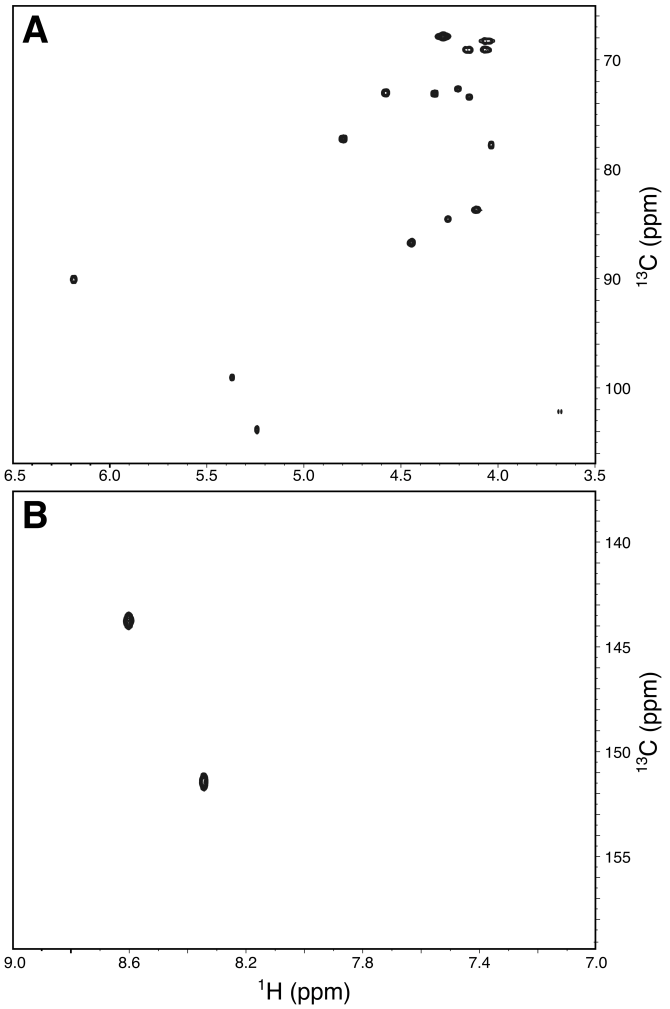
*All NMR experiments were conducted at pH 6.5 with 0.15 M NaCl unless otherwise noted.



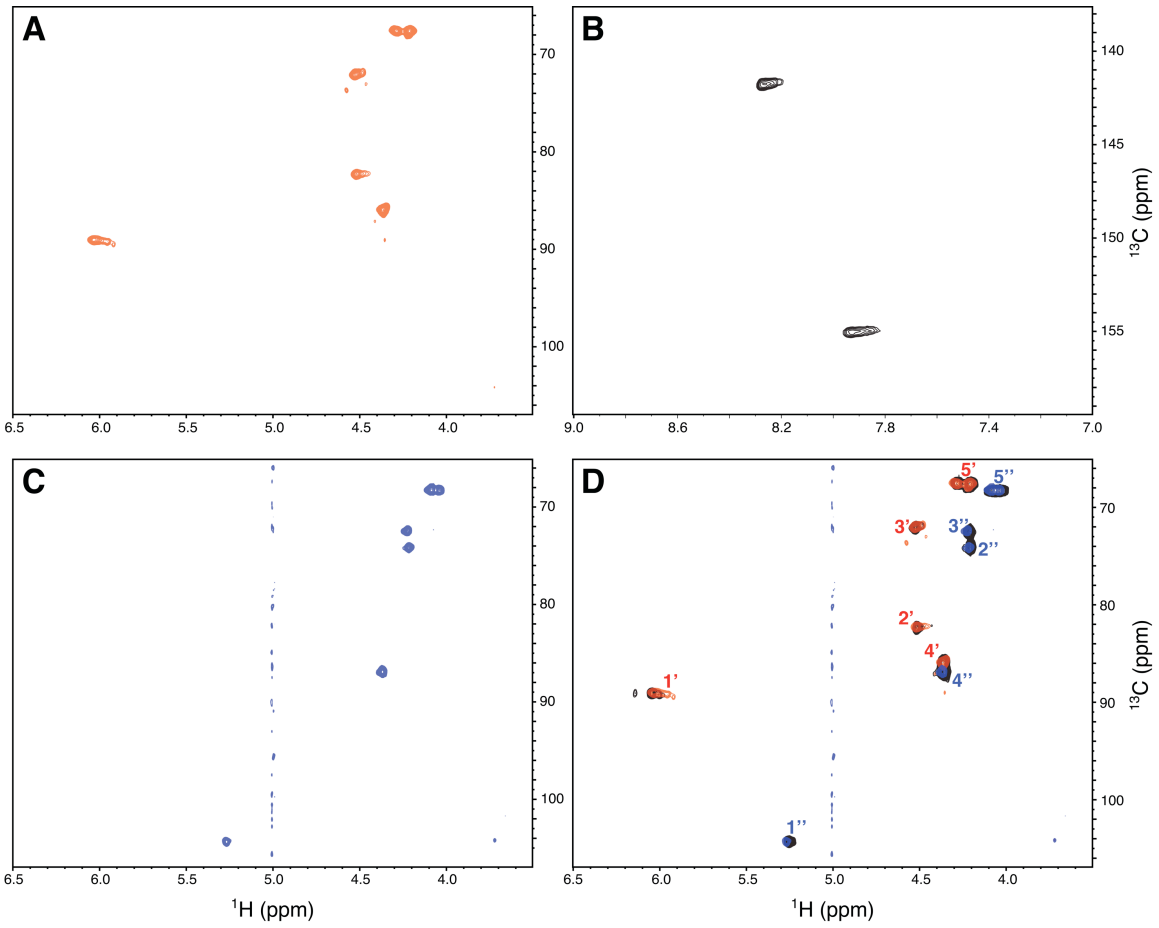
Supplementary Figure 1: Limited digest of PAR by poly(ADP-ribose) glycohydrolase (PARG). Denaturing 20% polyacrylamide shows crude ^{32}P labeled PAR synthesis digested with specified amounts of PARG overnight at 25°C.



Supplementary Figure 2: Limited digest of PAR by phosphodiesterase (PDE). Denaturing 20% polyacrylamide shows a crude ^{32}P-labeled PAR synthesis digested with specified amounts of PDE for 5 min at 25°C. The presence of two digestion products, ADP-ribose (ADPR) and ADP-ribose-ribose (ADPR-R), suggests that branched linkages are present at a low but detectable level.



Supplementary Figure 3: Natural Abundance ^{1}H-^{13}C-HSQC of ADP-ribose.
Spectra of ADP-ribose monomer show the ribose region (A) and adenine base region (B). The additional peaks observed in the ribose spectrum are associated with the different anomeric forms (α, β) that the ribose moiety can adopt.



Supplementary Figure 4: The two ribose subunits of PAR can be individually labeled. Carbon HSQC spectra of poly(ADP-ribose) samples that were labeled with U-^{13}C, ^{15}N-ATP (A,B) or U-^{13}C-glucose (C). Only those resonances within the adenine conjugated ribose or the unconjugated ribose are labeled, respectively. (D) Overlay of the two ribose spectra (A,C) yields the spectrum obtained from the ^{13}C, ^{15}N- PAR sample (Figure 3A, main text).