Supporting Information

Poly(ADP-ribose) polymerase-1 Activity Assay Based on the FRET between Cationic Conjugated Polymer and Supercharged Green Fluorescent Protein

Shiyun Tang, Zhou Nie,* Wang Li,* Daiqi Li, Yan Huang, Shouzhuo Yao
State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P. R. China.
Fax: +86-731-88821848; Tel: +86-731-88821626;
E-mail: niezhou.hnu@gmail.com, wli@hnu.edu.cn

Contents

1. Experimental details

1.1 Materials

The water soluble cationic conjugated polymer (CCP), poly(9,9-bis(6'-N,N,N-trimethylammonium)hexyl) fluorenylene phenylene (PF) was synthesized according to a reported method.[1,2] The supercharged green fluorescent protein (scGFP) was cloned and purified according to the protocol previously reported by our laboratory.[3] High-specific-activity PARP-1 and activated DNA were purchased from Trevigen (Gaithersburg, MD). Benzamide was purchased from Sigma-Aldrich (St. Louis, MO). HeLa cells were obtained from the Cell Bank of Xiangya Central Experiment Laboratory of Central South University (Changsha, China). All solutions were prepared using ultrapure water (18.25 MΩ·cm) from the Millipore Milli-Q system. All samples were illuminated at an excitation wavelength of 380 nm, and the fluorescence emission spectra were scanned from 400 to 600 nm in Tris-HCl buffer (50 mM, pH 7.5) at room temperature. Fluorescence spectra were recorded by fluorescence spectrophotometer F-4500.

1.2 Detection of Activity and Inhibition of PARP-1

The PARP-1 reaction solutions (10 μL) were composed of PARP-1, activated DNA (6 ng·μL^{-1}), NAD^+ (50 μM), DTT (1 mM), NaCl (100 mM), $MgCl_2$ (10 mM), and 50 mM Tris-HCl buffer (pH 8.0, 25 ℃). After incubation for 1 h at room temperature for PARP-1 catalyzed reaction, the pH value of the reaction solution was adjusted to 7.5, the activated DNA was digested by DNase I at 37 ℃ for 1 h. Then the resulting solution (20 μL) was added to the mixture of CCP and scGFP. The total volume was 100 μL, and the final concentration of CCP and scGFP were 74 nM in repeat units (RUs) and 5 nM, respectively. After incubation at room temperature for 1 min, fluorescence spectra were recorded at room temperature.

For PARP-1 inhibitor assay, the experiments were carried out using similar procedure as those for PARP-1 activity assay stated above, except for mixing the reaction solution with different concentrations of benzamide before the addition of PARP-1.

1.3 Analysis of poly(ADP-ribosyl)ated PARP-1 by capillary electrophoresis-laser-induced fluorescence (CE-LIF) method

For CE-LIF method (Figure S11), after PARP-1 (1.15 μM, 20 μL) was modified with poly(ADP-ribose), the solution was purified by tubular membrane ultrafiltration (10 kDa). After that, the protein was dissolved in 20 μL Na_2CO_3/$NaHCO_3$ buffer (50 mM, pH 9.0), then, FITC (20 μM, 10 μL) was added and reacted 8 hours at 4 ^oC. The solutions were injected at 0.5 psi and analyzed by CE-LIF. CE-LIF analysis was performed on a P/ACE MDQ system (Beckman, Fullerton, CA, USA), equipped with a 488 nm argon-ion laser and a photomultiplier tube (PMT) detector. The capillary: 75 μm i.d. × 375 μm o.d., total/effective length: 35/25 cm. Running buffer: Tris-Glycine

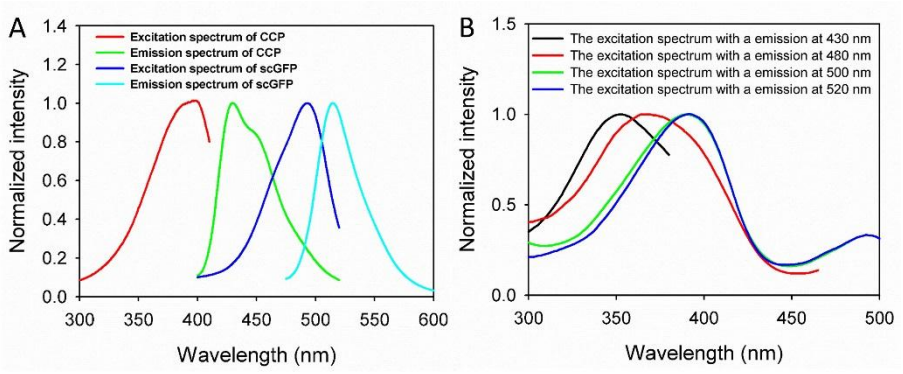
(20 mM Tris, 200 mM Glycine), pH 8.5. Separate voltage: 20 kV, reverse.

1.4 PARP-1 activity assay in the presence of diluted cells lysates, blood serum or human urine

HeLa cells (2×10^6 cells) were supplemented with 10% fetal bovine serum, Mimimum Eagle's essential medium (MEM) nonessential amino acid solution (0.1 mM), 1% insulin-transferrin-selenium A supplement, penicillin (100 $U\cdot mL^{-1}$), streptomycin (100 $mg\cdot mL^{-1}$), and amphotericin B (0.25 $mg\cdot mL^{-1}$). The cells were incubated under a humidified atmosphere containing 5% CO_2 at 37 ℃. The culture medium was replaced by Dulbecco's phosphate-buffered saline (DPBS). Then, Hella cells were removed by scraping and lysed in DPBS by sonication (200 W) for 2 s 60 times at an interval of 3 s each time. After that, the sample was centrifuged for 60 min at 22 000 rpm and 4 ℃, and the resulting supernatants were transferred to freezing tubes and stored at -20 ℃ for the experiments.

The cell lysates (2×10^5 $cells\cdot mL^{-1}$) were incubated with RNase I (0.5 $U\cdot\mu L^{-1}$) in Tris-acetate (20 mM, pH 8.0, 100 mM NaCl, 0.1 mM EDTA) at 37 ℃ for 2 h to remove the cellular total RNA. Then activated DNA (6 $ng\cdot\mu L^{-1}$), NAD^+ (50 μM), cell lysates (2×10^4 $cells\cdot mL^{-1}$), and different concentrations of PARP-1 were added to the reaction buffer (Tris-HCl buffer (50 mM, pH 8.0, 25 ℃), DTT (1 mM), NaCl (100 mM), $MgCl_2$ (10 mM)). After incubation for 1 h at room temperature for PARP-1 catalyzed reaction, the pH value of the reaction solution (10 μL) was adjusted to 7.5, then DNase I (6 U) was added to eliminate the cellular genomic DNA and the added active DNA, the solution (20 μL) was incubated at 37 °C for 60 min. Then the resulting solution (20 μL) was added to 80 μL of CCP (the final concentration of CCP was 74 nM in repeat units (RUs)) and scGFP (5 nM) mixture. After incubation at room temperature for 1 min, fluorescence spectra were recorded at room temperature.

The blood serum or human urine was diluted 10-folds with Tris-HCl (50 mM, pH 7.5), and 1 μL was added to the PARP-1 reaction solution. Except that, the other processes were similar with that of PARP-1 activity assay in the presence of cell lysates.



C

D/A pair	J ($M^{-1}cm^{-1}nm^4$)	R_0 (Å)	E (%)	R (Å)
CCP/scGFP	1.96×10^{15}	50.7	50.1	50.6

Figure S1. (A) The excitation and emission spectra of CCP and scGFP, respectively. (B) Excitation spectra of scGFP/poly(ADP-ribosyl)ated PARP-1/CCP complex monitored at different emission wavelengths. (C) The data summary of spectra overlap integral (J), Förster distance (R_0), energy transfer efficiency (E) and donor-acceptor distance (R).

According to a reported paper (*Chem. Commun.*, 2015, **51**, 4036-4039; *J. Am. Chem. Soc.*, 2003, **125**, 13306-13307), computation formula is as follows:

$$J(\lambda) = \int F_D(\lambda)\varepsilon_A(\lambda)\,\lambda^4 d\lambda$$

$$R_0^6 = (8.785 \times 10^{-5})k^2 Q_D J \eta^{-4}$$

$$E \propto \frac{1}{R^6}\int_0^{\infty} F_D(\lambda)\,\varepsilon_A(\lambda)\lambda^4 d\lambda$$

$$\mathrm{E} = \frac{R_0^6}{R_0^6 + R^6} = 1 - \frac{I_{DA}}{I_D}$$

Q_D is the quantum yield of donor, k^2 is the dipole orientation factor (assumed to be 2/3 based on random orientation distribution of donor and acceptor molecules), J is the overlap integral, F_D is the emission spectrum of donor, ε_A is the molar absorption coefficient of acceptor, η is the refractive index of the medium, R_0 is the Förster distance, R is the average donor-acceptor distance, I_{DA} is the fluorescence intensity of the donor in the presence of acceptor, and I_D is the fluorescence intensity of the donor in the absence of an acceptor.

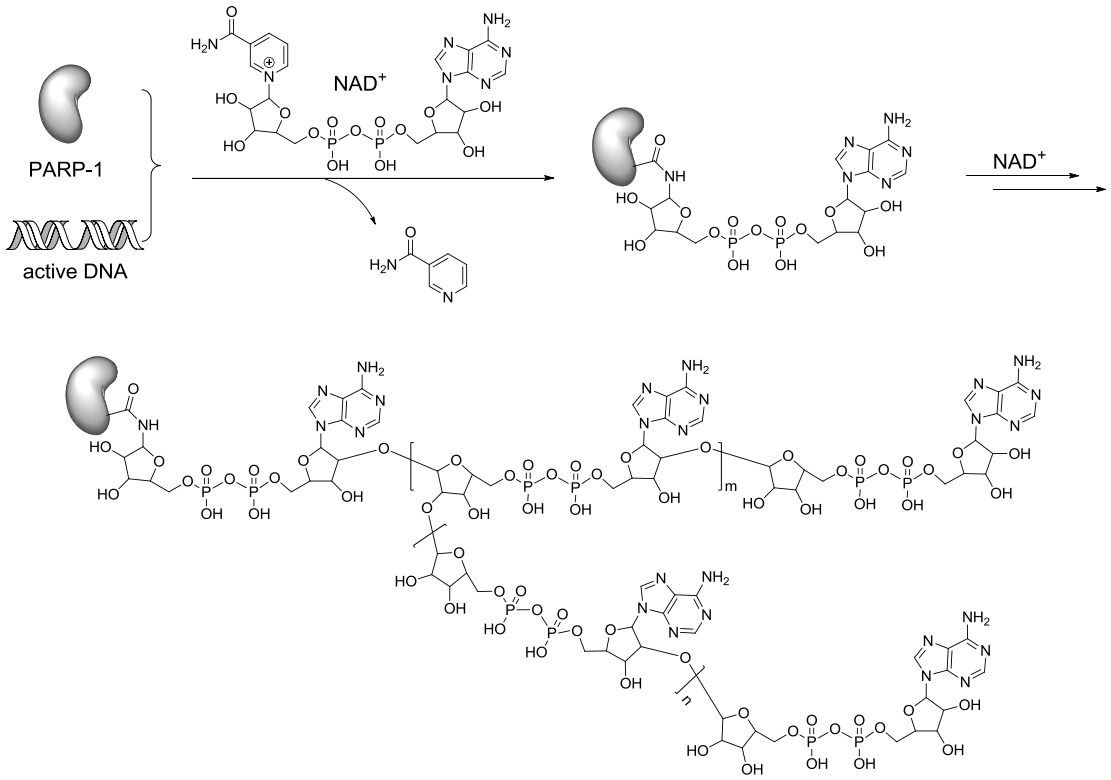


Figure S2. Schematic illustration of the automodification of PARP-1 with poly(ADP-ribose).

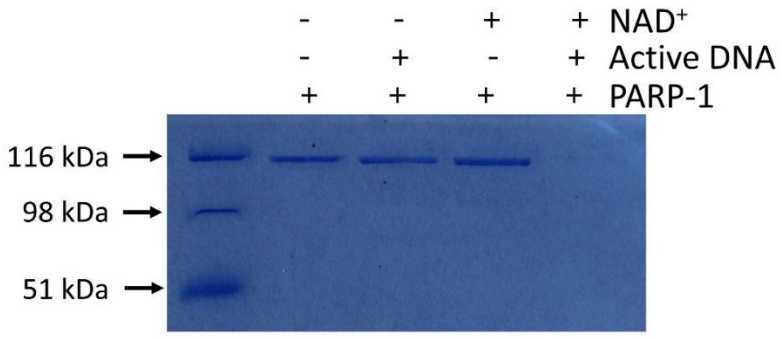


Figure S3. Polyacrylamide gel electrophoretic analysis of PARP-1 activity. The PARP-1 reaction solutions (10 μL) were composed of 50 μM NAD^+, 100 ng activated DNA, and 2 μg PARP-1, and the incubation time was 30 min at room temperature. Then 2 μL of 5X SDS-PAGE sample loading buffer was added, after that the solution was boiled in boiling water gently. Run the sample on a 4-12% SDS-PAGE gel, 10 $\mu L \cdot well^{-1}$. The gels were stained by coomassie blue.

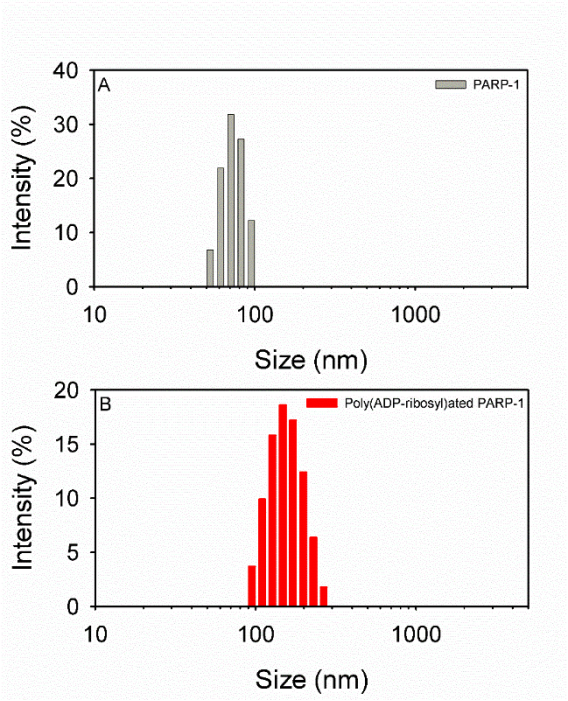


Figure S4. Hydrodynamic diameter analysis of (A) PARP-1 and (B) poly(ADP-ribosyl)ated PARP-1.

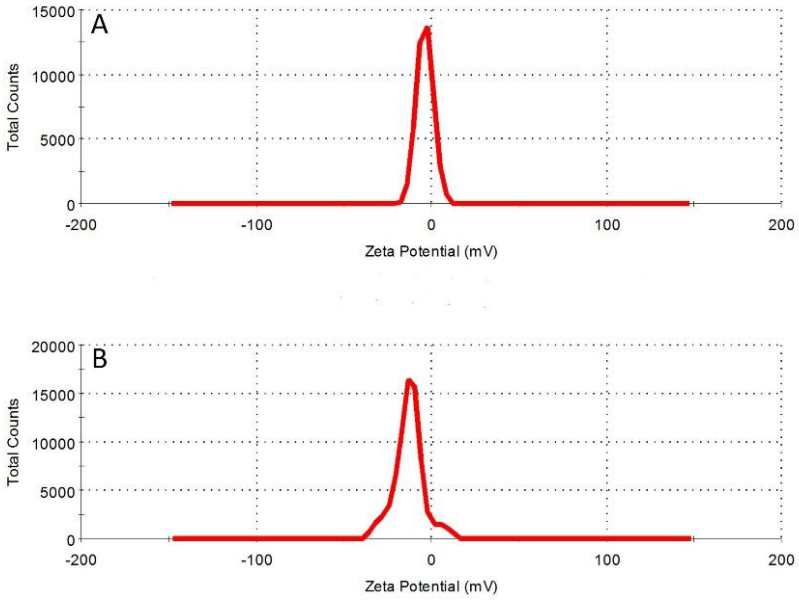


Figure S5. The zeta potential analysis of (A) PARP-1 and (B) poly(ADP-ribosyl)ated PARP-1.

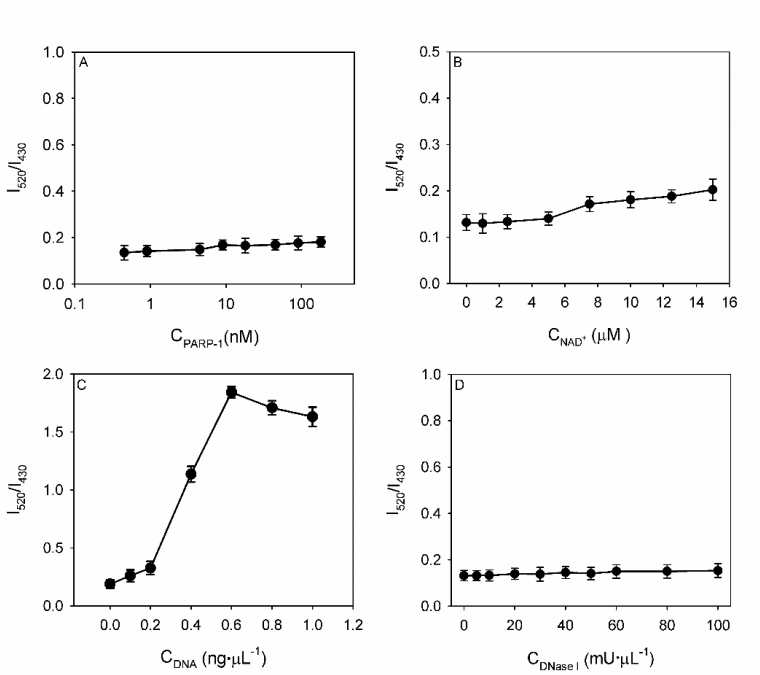


Figure S6. Effects of concentration of (A) PARP-1, (B) NAD^+, (C) active DNA, and (D) DNase I on the FRET ratio (I_{520}/I_{430}) between CCP and scGFP. Conditions: Different concentrations of (A) PARP-1, (B) NAD^+, (C) active DNA, and (D) DNase I were directly added to the mixture of scGFP and CCP (5 nM scGFP, 74 nM CCP (in RUs)), respectively. After incubation for 1 min at room temperature, fluorescence spectra were recorded at room temperature (excitation wavelength was 380 nm).

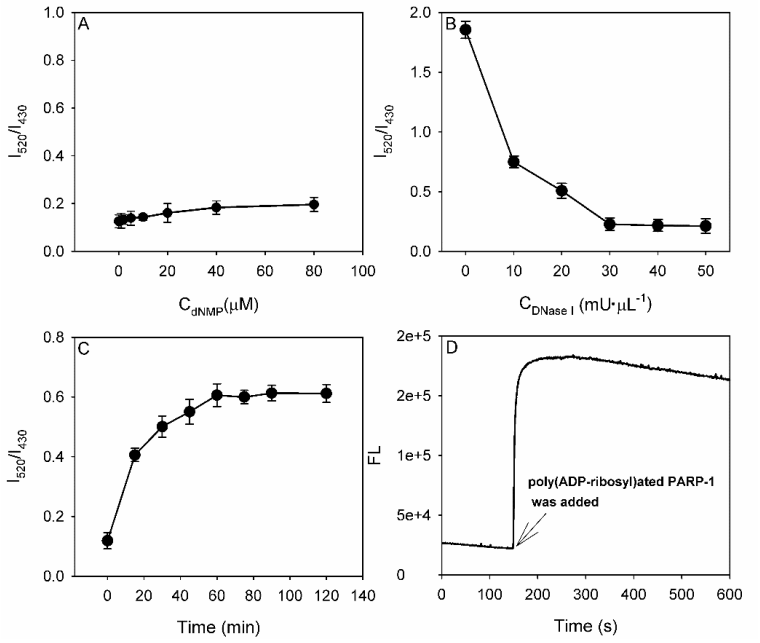


Figure S7. Effects of (A) dNMP concentration, (B) DNase I concentration, and (C) the reaction time of PARP-1 on the FRET ratio (I_{520}/I_{430}) of CCP and scGFP. (D) Time courses of PL response of the proposed sensor to the added poly(ADP-ribosyl)ated PARP-1. Conditions: 5 nM scGFP, 74 nM CCP

(in RUs), 5 μM NAD^+, 0.6 ng·μL^{-1} DNA, and 4.5 nM PARP-1.

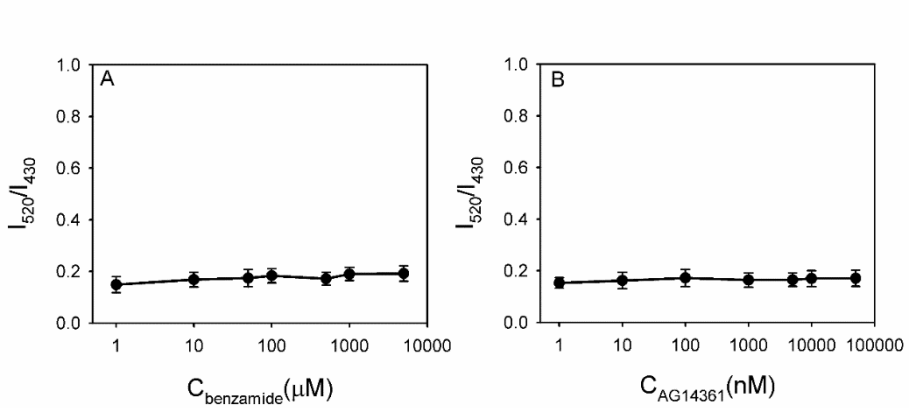


Figure S8. Effects of concentration of (A) benzamide and (B) AG14361 on the FRET ratio (I_{520}/I_{430}) of CCP and scGFP-based assay. Conditions: Different concentrations of (A) benzamide and (B) AG14361 were directly added to the mixture of scGFP and CCP (5 nM scGFP, 74 nM CCP (in RUs)), respectively. After incubation for 1 min at room temperature, fluorescence spectra were recorded at room temperature (excitation wavelength was 380 nm).

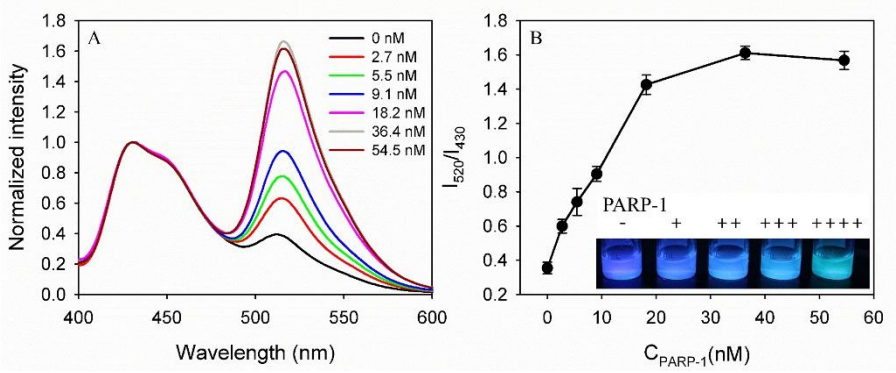


Figure S9. (A) Normalized fluorescence emission spectra and (B) FRET ratio (I_{520}/I_{430}) of the proposed sensor to probe the PARP activity in the presence of HeLa cells lysates. Inset of B: Photograph of samples with different PARP-1 concentrations (excitation wavelength was 365 nm).

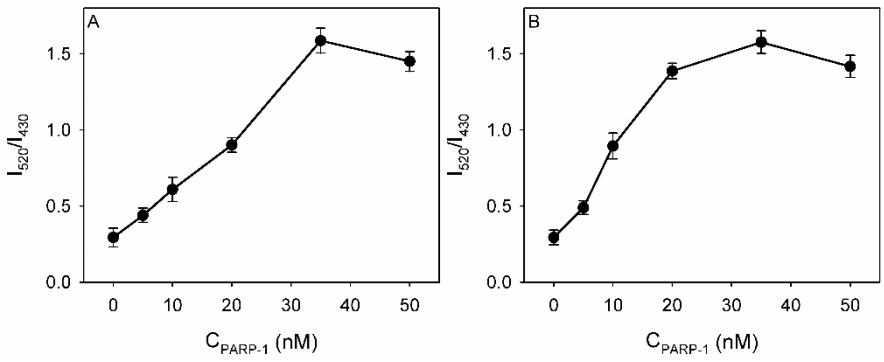


Figure S10. FRET ratio (I_{520}/I_{430}) of the proposed strategy to probe the PARP-1 activity in the presence of diluted blood serum (A), or diluted human urine (B).

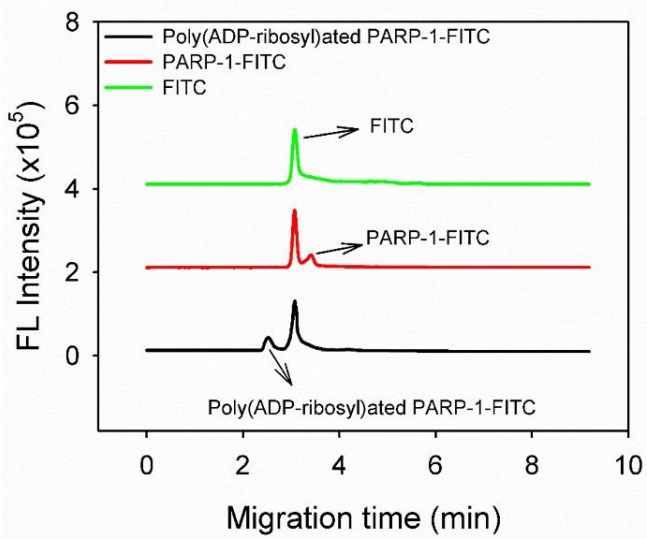


Figure S11. The analysis of FITC labeled PARP-1 and FITC labeled poly(ADP-ribosyl)ated PARP-1 by capillary electrophoresis-laser-induced fluorescence (CE-LIF) method.

Besides gel electrophoresis, dynamic light scattering and zeta potential, CE-LIF method was also employed to characterize the poly(ADP-ribosyl)ated PARP-1. We used FITC to label the original PARP-1 and the poly(ADP-ribosyl)ated PARP-1, and the resulting samples were analyzed by CE-LIF. Figure S11 shows the results of our preliminary experiments. The poly(ADP-ribosyl)ated PARP-1 can be clearly probed and the migration time of the poly(ADP-ribosyl)ated PARP-1 is shorter than that of unmodified PARP-1, suggesting that the charge-to-mass ratio of poly(ADP-ribosyl)ated PARP-1 is larger than that of PARP-1 and negatively charged ADP-riboses are grifted on PARP-1.

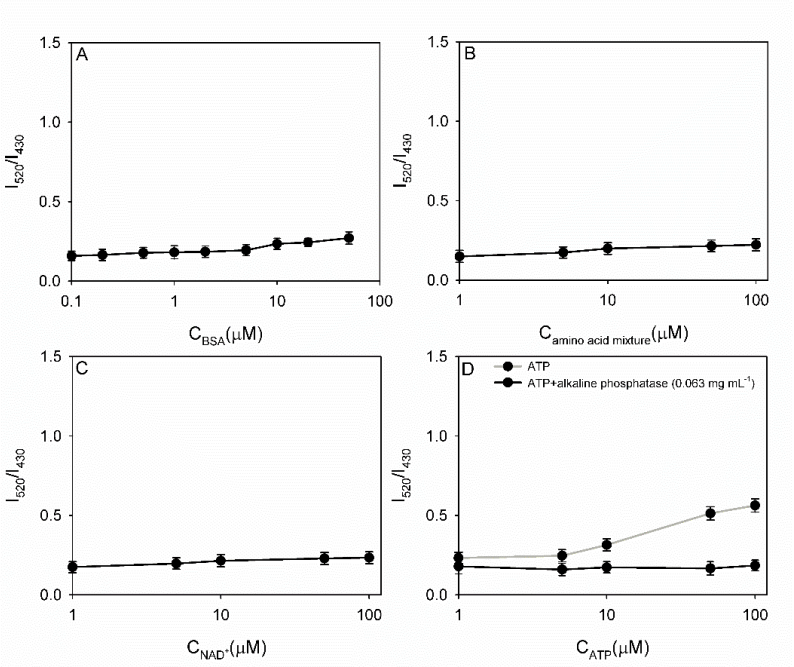


Figure S12. The effect of BSA (A), amino acid mixture (B), NAD^+ (C) and ATP (D) on the FRET between CCP and scGFP.

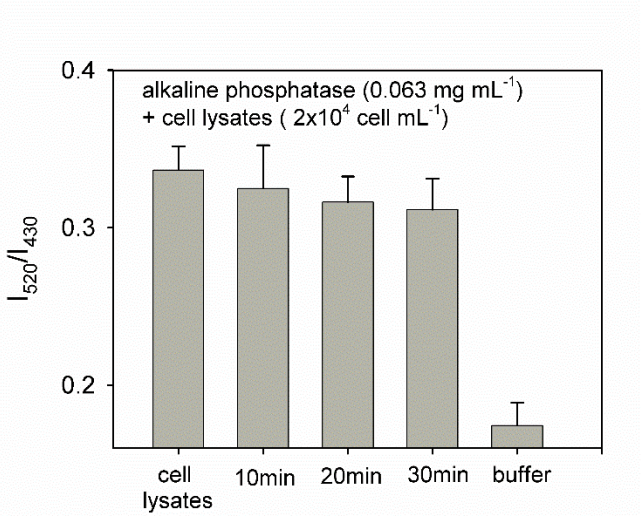


Figure S13. The background signals of the sample in the presence of diluted cells lysates treated with alkaline phosphatase. The sample of "buffer" is the sample without cell lysates.

Cell lysates system is a complex system containing various substances, such as nucleic acids, proteins, ions and small molecules, which are potential influence factors to the FRET system. Nucleic acids has adverse effect on the FRET system (Figure S6C), however, since in our original detection system, Deoxyribonuclease I (DNase I) and Ribonuclease (RNase) have been introduced, the effect of DNA (Figure S7B) or RNA can be avoided. The effect of dNMP, the product of digestion of DNA, on the FRET is ignorable (Figure S7A). Besides nucleic acid and the product of digestion, the effects of other substances, such as BSA (a kind of representative protein), amino acids mixture (Ser, Glu, Asp, Arg, Ala and Cys), NAD^+ and ATP (typical small molecules) on the FRET between CCP and scGFP were studied (Figure S12). For the BSA (Figure S12A), amino acids mixture (Figure S12B) and NAD^+ (Figure S12C), their effects on FRET are insignificant. For ATP, with the increase of the concentration of ATP (1 to 100 μM), the I_{520}/I_{430} values increase from 0.23 to 0.55 (Figure S12D), suggesting that ATP can affect the FRET between CCP and scGFP. Fortunately, the effects caused by ATP can be lowered by the assistance of alkaline phosphatase (Figure S12D). Meanwhile, the addition of alkaline phosphatase also can partially reduce the background of the sample in the presence of diluted cell lysates (Figure S13). Therefore, compared to that in ideal system, the slightly higher background signal in the presence of diluted cell lysates is probably caused by the undesirable electrostatic interaction between fluorescent probes (scGFP and CCP) and molecules with multiple negative charges such as ATP existing in the cell lysates.

Table S1. FRET involving PF (poly(9,9-bis(6'-N,N,N-trimethylammonium)hexyl)fluorenylene phenylene)

	Acceptor	**Target**	**Whether labeled**	**Literature**
1	fluorescein labeled DNA	DNA	labeled	*J. Am. Chem. Soc.*, 2003, **125**, 896-900
2	fluorescein labeled DNA	K^+	labeled	*J. Am. Chem. Soc.*, 2005, **127**, 12343-12346
3	fluorescein labeled DNA and EB	DNA	labeled	*Adv. Mater.*, 2006, **18**, 2105-2110
4	fluorescein labeled DNA	DNA	labeled	*J. Am. Chem. Soc.*, 2006, **128**, 6764-6765
5	fluorescein labeled biotin	Protein	labeled	*Macromol. Rapid Commun.*, 2006, **27**, 993–997
6	Thiazole Orange	DNA	labeled	*Macromol. Rapid Commun.*, 2007, **28**, 1804–1808
7	fluorescein labeled dGTP	single nucleotide polymorphisms	labeled	*J. Am. Chem. Soc.*, 2007, **129**, 4154-4155
8	fluorescein labeled DNA	nucleases and methyltransferases	labeled	*Adv. Mater.*, 2007, **19**, 3490-3495
9	fluorescein labeled DNA	DNA	labeled	*Langmuir*, 2008, **24**, 12138-12141
10	Texas Red labeled DNA, fluorescein labeled peptide	protease and nuclease	labeled	*Anal. Chem.*, 2009, **81**, 3731-3737
11	Thiazole Orange	single nucleotide polymorphisms	unlabeled	*Anal. Chem.*, 2009, **81**, 4099-4105
12	chromophore labeled DNA	nucleases	labeled	*Angew. Chem.*, 2009, **121**, 5420 -5425
13	fluorescein labeled DNA	cocaine	labeled	*J. Am. Chem. Soc.*, 2010, **132**, 1252-1254
14	Genefinder	DNase I, EcoR I, S1 nuclease	unlabeled	*Langmuir*, 2010, **26**, 4540-4545
15	fluorescein labeled DNA	methylation	labeled	*Nature Commun.*, 2012, **3**, 1-8
16	FITC labeled peptide	protein kinase (PKA)	labeled	*Analyst*, 2014, **139**, 4710–4716
17	scGFP	PARP-1	unlabeled	Our method

Table S2. Reported literatures on PARP-1 activity assay, inhibitors assessment

	Method	**Characteristic**	**Whether labeled**	**Whether radioactive**	**Interfacial or homogeneous**	**PARP-1 activity assay**	**Inhibitor**	**Literature**
1	ELISA	antibody	labeled	non-radioactive	interfacial	semiquantitative	Benzamide, IC_{50}=0.5μM	*Clin. Cancer Res.*, 1999, **5**, 1169-1172
2	biotin-labeled	Biotinylated NAD	labeled	non-radioactive	homogeneous	qualitative	-	*J. Histochem. Cytochem.* 2002, **50**, 91-98
3	radiolabeled NAD	^{13}C,^{15}N-Poly(ADP-ribose)	labeled	radioactive	homogeneous	semiquantitative	-	*J. Am. Chem. Soc.*, 2009, **131**, 14571-14578
4	fluorescence labeling	Clickable NAD Analogues	labeled	non-radioactive	homogeneous	semiquantitative	-	*J. Am. Chem. Soc.* 2010, **132**, 9363-9372
5	radiolabeled	^{14}C,^{32}P labeled NAD	labeled	radioactive	homogeneous	-	Benzamide, IC_{50}=22μM	*J. Biol. Chem.*, 1992, **267**, 1569-1575
6	radiolabeled	^{32}P-NAD, Biotinylated NAD	labeled	radioactive	homogeneous	-	Benzamide, IC_{50}=1.3μM	*Anal. Biochem.*, 2000, **282**, 24-28
7	radiolabeled	^{14}C labeled NAD	labeled	radioactive	homogeneous	-	AG14361, IC_{50}=21 nM	*Clin. Cancer Res.*, 2005, **11**, 8449-8457
8	Immunoblotting	antibody	labeled	non-radioactive	homogeneous	-	AG14361, IC_{50}=5 nM	*Nature Method*, 2013, **10**, 981-987
9	FRET	electrostatic interaction	unlabeled	non-radioactive	homogeneous	quantitative	Benzamide, IC_{50} = 51 μM, AG14361, IC_{50} = 42 nM	Our method

References

1. B. Liu, S. Wang, G. C. Bazan and A. Mikhailovsky, *J. Am. Chem. Soc.*, 2003, **125**, 13306-13307.
2. S. Tang, Y. Hu, Q. Shen, H. Fang, W. Li, Z. Nie and S. Yao, *Analyst*, 2014, **139**, 4710-4716.
3. C. Lei, Y. Huang, Z. Nie, J. Hu, L. Li, G. Lu, Y. Han and S. Yao, *Angew. Chem. Int. Ed.*, 2014, **53**, 8358-8362.