Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Single-Molecule Imaging of Telomerase Activity via Linear Plasmon Rulers

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Experimental Section

Reagents and Materials. Silver nitrate and epigallocatechin gallate (EGCG) were purchased from Sigma-Aldrich. Glycerol, polyvinylpyrrolidone (PVP), ammonium hydroxide, and ascorbic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Dulbecco's Modified Eagles Medium (DMEM), Leibovitz Medium (L-15), fetal bovine serum (FBS) and trypsin were purchased from Invitrogen. Telomerase kit was obtained from QiaoDu Biotech. Co. Ltd. (Shanghai, China). HeLa cells, MCF-7 cells, HepG2 cells, L-O2 cells, dNTPs and 1×PBS were obtained from KeyGen Biotech. Co. Ltd. (Nanjing, China). 1× CHAPS lysis buffer was purchased from EMD Millipore Corp. All other reagents were of analytical grade and were used without further purification. The water from Milli-Q (Millipore, Inc., Bedford, MA) was RNase-free by pretreated with diethylpyrocarbonate. The oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai China). Detailed DNA sequences were shown in Table S1.

Table S1. DNA Sequences Used in This Work

Table S1 chemically synthesized DNA sequences

DNA	Sequences (5′ to 3′)
TSP	SH-$(CH_2)_6$-AATCCGTCGAGCAGAGTT
anchor DNA (aDNA)	SH-$(CH_2)_6$-CCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACTCTGCTCGACGGATT
helper DNA (hDNA)	SH-$(CH_2)_6$-TTTTTT
complementary DNA	AGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG

Apparatus. Transmission electron micrographs were obtained on JEM-1011 and JEM-2100 transmission electron microscope (JEOL Ltd., Japan). Scanning electron micrographs were obtained on S-4800 scanning electron microscope（Hitachi, Japan). UV-vis absorption spectra were recorded using a UV-vis spectrophotometer (Nanodrop-2000C, Nanodrop, USA). The cell telomerase activity assay was performed by a Thermo Scientific Varioskan Flash (Thermo Fisher Scientific, USA).

Synthesis, modification and assembly of AgNPs.

The 44.1 nm AgNPs were synthesized using a seed-mediated method.[S1] In brief, a 50 mL glycerol-water mixture (40 vol% glycerol) was stirred (1200 rpm, 2.5 cm stirring bar) in a 100 mL flask and heated up to 95℃. After reaching the inner temperature, 9 mg silver nitrate, and 1 min later, 1 mL sodium citrate (3%) were added to the solvent. The reaction mixture was stirred for 1 h at 95℃. After the color of the mixture turned to dark-yellow, the colloidal solution was kept stirring at room temperature for another 15 min and then filtered through a 0.22 μm filter membrane. The seed AgNPs prepared by this modified Lee-Meisel synthesis have an average size of about 30.0 nm and the concentration of the seed AgNPs solution is calculated about 0.668 nM. To prepare 44.1 nm AgNPs, 138 mL water (Millipore, 18.2 MΩ), 23 mL glycerol and 0.58 g PVP were stirred (1200 rpm, 2.5 cm stirring bar) in a 250 mL beaker at RT, after which, 14.4 mL AgNPs seed solution was added into the mixture. The reaction was started 20 seconds later with the addition of 290 μL diamine silver complex (20 mg silver nitrate in 1 mL water plus 220 mL ammonium hydroxide 30%), together with 92 mL ascorbic acid solution (9.2 mg in 92 mL water). The growth process lasted for 1 h, reaching a final concentration of about 36 pM, and the particles were stabilized with 6 g PVP and stored in a plastic tube at 4 ℃. The size of the resulting AgNPs was determined using TEM.

DNA-modified AgNPs were prepared according to a modified literature procedure.[S2] To minimize multiple tether formation during hybridization of the Ag-ssDNA conjugates and avoid nonspecific adsorption, we introduced a helper DNA (hDNA) during the modification process. The hDNA:TSP(or aDNA):particle ratio was limited to 5000:25:1. The conjugation process was carried out as follows: the obtained AgNPs solution was firstly centrifuged to remove the excess PVP, and re-dispersed in water by sonication with a final concentration of 0.2 nM. 10 μL of hDNA (100 μM) and 5 μL of TSP or aDNA (1 μM) were incubated with 1 mL of above AgNPs solution (0.2 nM) for at least 18 h. To adjust the pH value and increase ionic strength of the resulting solution, 122 μL of 10 mM PBS was added to the solution and allowed to react for 6 h. Then 21 μL of 2 M NaCl was added to the solution and this procedure was repeated two times at the interval of 3 h such that the total NaCl concentration could be increased gradually. After an additional standing for at least 48 h, the nanoparticles were isolated by centrifugation for 15 min, 14 ℃, 3 times at 15000 rpm (Beckman Centrifuge, Allegra 64R, U.S.). The resulting AgNPs precipitate was washed, recentrifuged and re-dispersed in 0.1 M PBS, and stored at 4 ºC for further use.

The Ag plamon rulers were synthesized through the hybridization process between TSP and aDNA. Briefly, TSP-AgNPs solution was mixed with aDNA-AgNPs solution at a molar ratio of 1:1. After 12 h of incubation at room temperature, the mixture was centrifuged at 8,000 rpm for 20 min (4˚C), and the supernatant was discarded. The resulting Ag plamon rulers were washed and centrifuged twice and then re-dispersed in 200 μL PBS.

Dark-field Microscope (DFM) setup. The dark-field measurements were carried out on an inverted microscope (IX71, Olympus) equipped with a dark-field condenser ($0.8 < NA < 0.92$) and a 60X objective lens (NA 0.7). The sample slides were immobilized on a platform, and a 100 W halogen lamp provided white light source to excite the nanoparticles to generate plasmon resonance scattering light. The scattering light was collected by a true-color digital camera (Olympus DP80, Japan) to generate the dark-field color images, and was also splitted by a monochromator (Acton SP2358, PI, USA) which was equipped with a grating (grating density: 300 lines/mm; blazed wavelength: 500 nm) and recorded by an excelon EMCCD (400BR, PI, USA) to obtain the scattering spectrum. In this work, the scattering spectra of the nanoparticles were corrected by subtracting the background spectrum generated by the instrument itself.

Cell Culture and Telomerase Extract Preparation. Cells were cultured in DMEM medium supplemented with 10% fetal calf serum, and the cells were maintained at 37 ℃ in a humidified atmosphere (95% air and 5% CO_2). Cells were collected in the exponential phase of growth and washed twice with ice-cold sterile PBS; then 1×10^6 cells were resuspended in 200 μL of ice-cold CHAPS lysis buffer (10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 5 mM mercaptoethanol, 0.5% CHAPS, 10% glycerol). The lysate was incubated for 30 min on ice and centrifuged for 20 min (14000 rpm, 4 ℃) to pellet insoluble material. Then the cleaned lysate was carefully transferred to a 1.5 mL EP tube. The lysate was used immediately for telomerase assay or frozen at -80 ℃. For apoptosis assay, HeLa cells were treated with different concentrations of EGCG and cultured in 12-well plates for 48 h, and then dyed with acridine orange (AO) and ethidium bromide (EB), and finally sent for bright-field and fluorescence imaging to show the activity of cancer cells. The telomerase was extracted from EGCG-treated HeLa cells at the same way.

Telomerase kit detection. 40 μL of sample solution and 10 μL of biotinylated telomerase antibody solution (from the kit) or 50 μL of telomerase standard solution was added in the wells of ELISA plate, and incubated at 37 °C for 30min. Then solution in the wells was discarded, and the

wells were washed thoroughly with washing solution form the kit for 5 times. 50 μL solution of labeling reagent (from the kit) was added to each well, and the mixture was incubated at 37 °C for 60 min. Then the medium was removed and the wells were washed with washing solution for 5 times and patted dry. 50 μL of color development agent A and 50 μL of color development agent B (from the kit) were added to each well successively. After the plate was protected from light for 10~15 min at 37 °C, 50 μL of stop buffer was added to each well to stop the color reaction. The absorbance of each well was measured at 450 nm using a Thermo Scientific Varioskan Flash (Thermo Fisher Scientific, USA).

Telomerase Extension Reaction and Real-Time Optical Monitoring of the Extension of TSP. 10 μL of Ag plamon rulers was mixed with 1990 μL deionized water and then were dropped onto a glass microscope slide (22×40×0.1mm, ShiTai Co., Jiangsu, China). After 60 min incubation, the glass slide was washed with deionized water and dried with N_2 stream. For the *in vitro* monitoring of the extension of TSP, 200 μL of telomerase reaction solution which contained 10 μL telomerase extracts and 190 μL of telomerase reaction buffer (20 mM Tris-HCl buffer, pH 8.3, 1.5 mM $MgCl_2$, 0.63 mM KCl, 0.05% Tween 20, 1 mM EGTA, 0.1 mM dNTPs) was added onto the glass slide containing Ag plamon rulers and incubated for 2 h, during which, the scattering spectrum peak of Ag plamon ruler was recorded by measuring the scattering spectrum of individual Ag plamon ruler continuously. The Lorentzian algorithm was employed to fit the spectrum and to identify accurate peaks using OriginPro 8.0 software. For control experiments, telomerase extracts were pretreated at 95 ℃ for 20 min, and the next procedures were the same as above.

Numerical simulation. Three-dimensional full-field finite-difference time-domain (FDTD) (the package of Lumerical FDTD Solutions 8.15) was used to simulate the optical properties of Ag plasmon rulers. The refractive index of background was set as 1.33 and a total-field scattered-field source, ranging from 300 to 700 nm, was used to investigate the scattering properties of Ag plasmon rulers. All of the boundary conditions were all set as perfect and the meshing size used in the simulating regions was set as small as 0.4 nm. For simplicity, the contribution of DNA strands and glass slide were ignored. We performed FDTD simulation about Ag plasmon rulers with the interparticle distances set as 2 nm, 3 nm, 4 nm, 5 nm, 6 nm, 7 nm, 8 nm, 9 nm, 10 nm, 11 nm, 12 nm, 14 nm, 16 nm, 18 nm, 20 nm, 25 nm, 30 nm and 50 nm, respectively. The calculated scattering spectrum of Ag monomer was also given for comparison. The result of FDTD simulation was shown

in Figure 3A and Figure 3B. The fitted curve can be expressed as an Equation (1):

$$y = 572.12 - 53.63\left(1 - \exp\left(-\frac{x}{26.90}\right)\right) - 107.16\left(1 - \exp\left(-\frac{x}{2.72}\right)\right) \quad (1)$$

where y represents the scattering peak λmax (nm) and x represents the interparticle distance value S (nm). In consideration that theoretical simulation simplified the condition in experimental measurement and the AgNPs are not in a perfect round shape, distinctions between the results of simulation and experiment are reasonable.

Supporting Figures

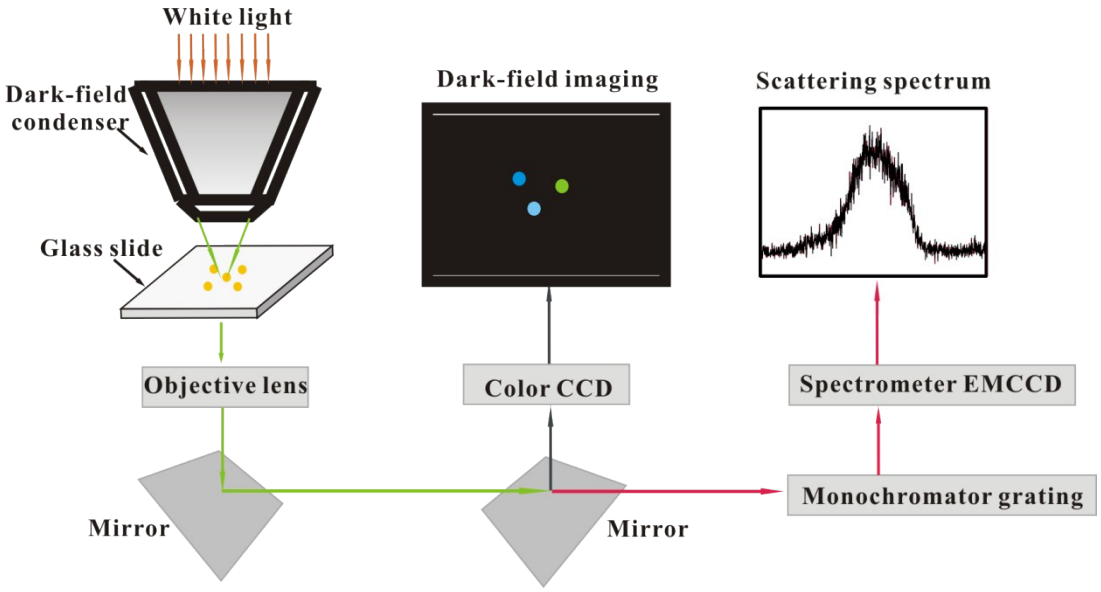

Figure S1. Illustration of the DFM and scattering spectrometer setup.

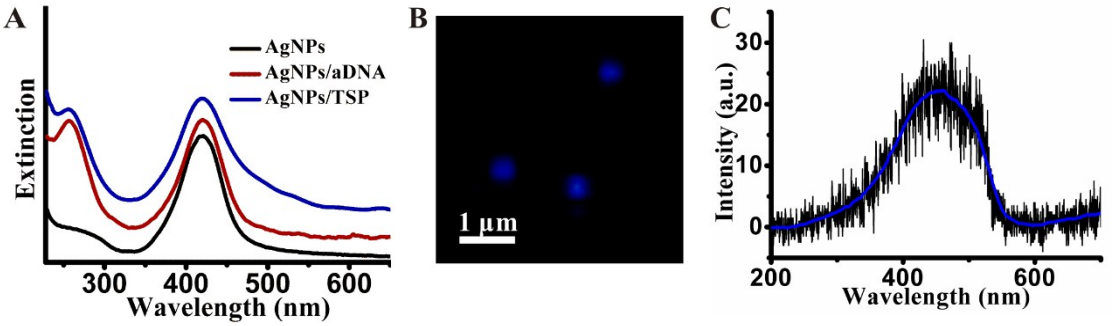

Figure S2. (A) UV-vis spectra of AgNPs (black line), AgNPs modified with aDNA (red line) and AgNPs modified with TSP (blue line). (B) Dark-field image of single AgNP modified with aDNA. (C) Typical scattering spectum of AgNP modified with aDNA.

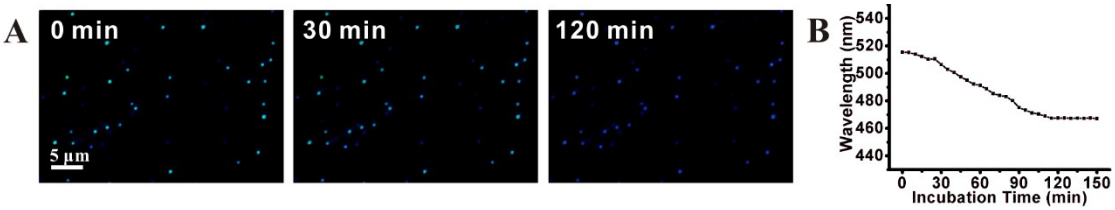

Figure S3. (A) DFM images of Ag PRs incubated with cell extracts and dNTPs at three different incubation times, including 0 min, 30 min, and 120 min. (B) Spectral position as a function of the incubation time after the addition of cell exacts and dNTPs.

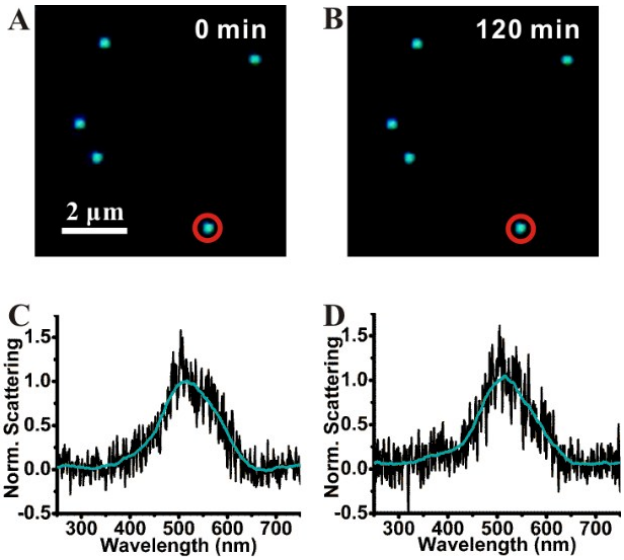

Figure S4. (A) and (B) DFM images of Ag dimers incubated with heat-inactivated cell extracts and dNTPs for 0 min and 120 min, respectively. (C) and (D) The scattering spectrum of the spot marked in red circle in D and E, respectively.

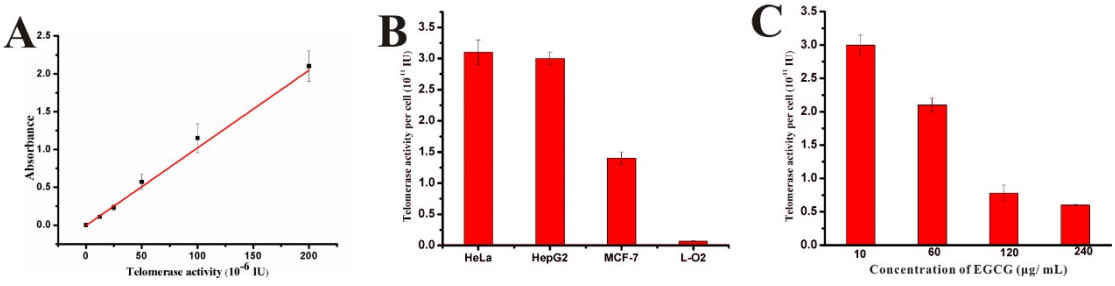

Figure S5. (A) Standard curve for in vitro detection of telomerase activity in solution with the ELISA kit. (B) Calculated average telomerase activity per cell (HeLa, HepG2, MCF-7 and L-O2 cells from left to right, respectively). (C) Calculated average telomerase activity per HeLa cell incubated with 10μg/ mL, 60 μg/ mL, 120 μg/ mL, 240 μg/ mL of EGCG, respectively.

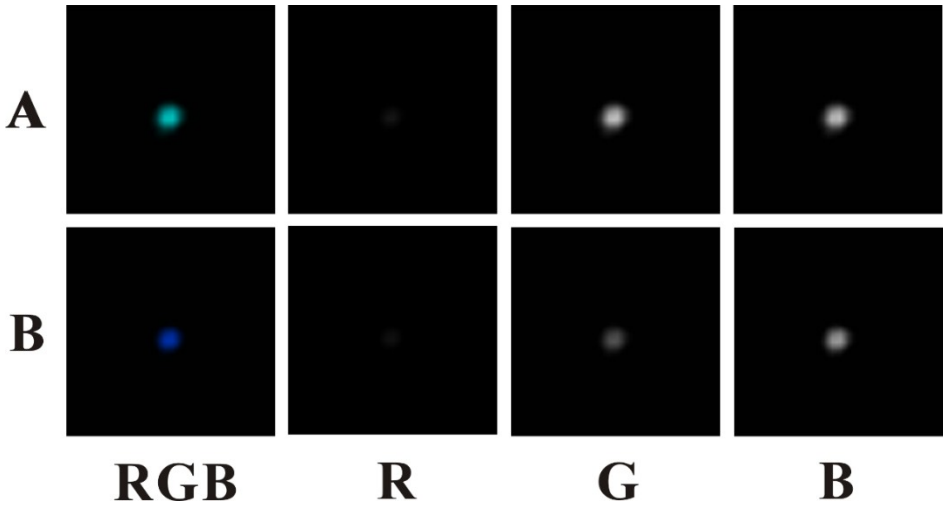

Figure S6. Dark-field images of PR before (A) and after (B) reaction with cell extracts and dNTPs. The images were further splitted into R, G and B channels, respectively.

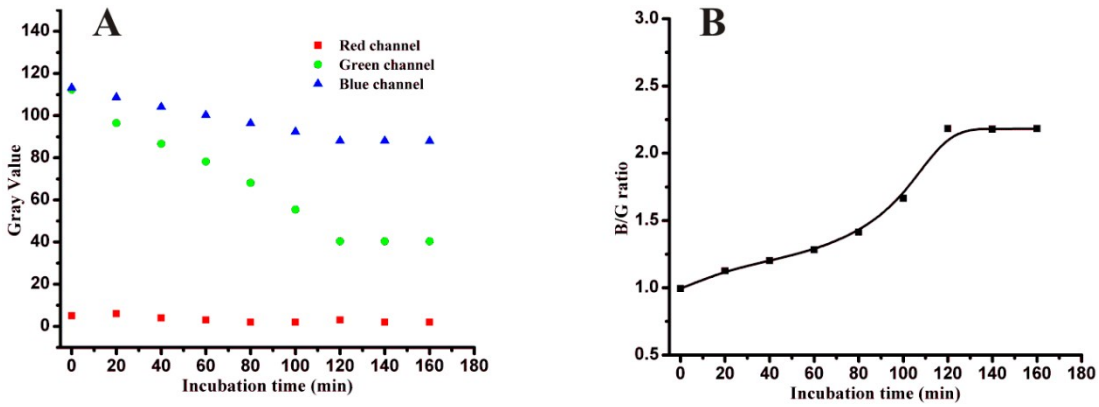

Figure S7. (A)Time-dependent changes of R, G and B gray values of one selected PR (Fig. 3) after introducing cell extracts (HeLa) and dNTPs. (B) Corresponding B/G ratio change with incubation time.

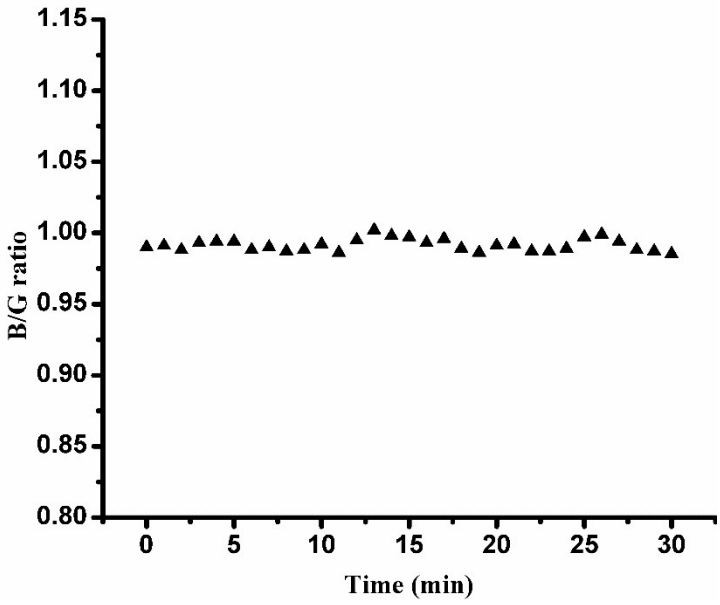

Figure S8. B/G ratio fluctuation of one selected PR in the absence of cell extracts and dNTPs.

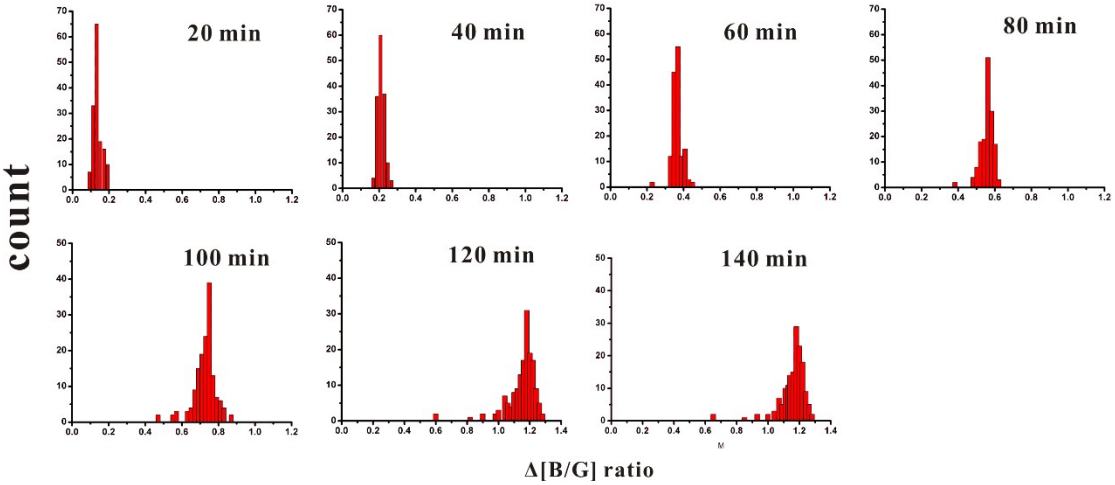

Figure S9. Δ[B/G] distribution of PRs incubated with cell extracts (HeLa cells) and dNTPs at different incubation times (20 min, 40 min, 60 min, 80 min, 100 min, 120 min, 140 min).

Surpporting Reference

(S1) Steinigeweg, D.; Schlucker, S. *Chem Commun (Camb)* **2012,** *48*, 8682-4.

(S2) Munro, C. H.; Smith, W. E.; Garner, M.; Clarkson, J.; White, P. C. *Langmuir* **1995,** *11*, 3712-3720.