Supporting information

Inhibition of polymerase activity by pristine fullerene nanoparicles can be mitigated by abundant proteins

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Experimental Procedures

DNA template and primers: The sequence of ssDNA template (75 bases) is 5'-CCGCCTGATTAGCGATACTTACGTGAGCGTGCTGTCCCCTAAAGGTGATA CGTCACTTGAGCAAAATCACCTGCA-3', which was synthesized by Sangon Biological Engineering Technology and Services (Shanghai, China). The sequence of forward primer is 5'-CCGCCTGATTAGCGATACTT-3', and the sequence of reverse primer is 5'-TGCAGGTGATTTTGCTCAAG-3'.

***Real-time PCR*:** The real-time amplification and analysis of the template DNA was carried out using a Mx3005P QPCR Systems (Stratagene, USA). The real-time PCR reagents were from Brilliant SYBR QPCR Master Mix kit (Stratagene, USA). This 2 × master mix contained all of the reaction components except the template and primers. All the reagents were dispensed into a 0.2 mL 8-strip tube with a ultimate volume of 20 μL, containing 8 μL of 2× master mix, 1μL of 10 nM template, 0.5 μL of 10 μM forward primer, 0.5 μL of 10 μM reverse primer, and 10 μL of C_{60} solution. The subsequent temperature cycling program include 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, 40 cycles, and final extension at 72 °C for 10 min. A positive control (normal RT-PCR without C_{60} solution) and a negative control (normal RT-PCR without C_{60} solution and template) were included with each set of PCR experiments.

Gel electrophoresis of PCR products: The PCR products were analyzed by gel electrophoresis on 8% polyacrylamide gels. The running buffer was 1 × TBE (90 mM tris-borate buffer, 2 mM EDTA, pH 8.3). After running at 110V for 70 min, the gel was stained in ethidium bromide solution (0.5 μg/mL) for 20 min. The bands were visualized and imaged on a Syngene (Cambridge, UK) UV illuminator. The DNA markers were from 25 bp to 700 bp DNA fragments.

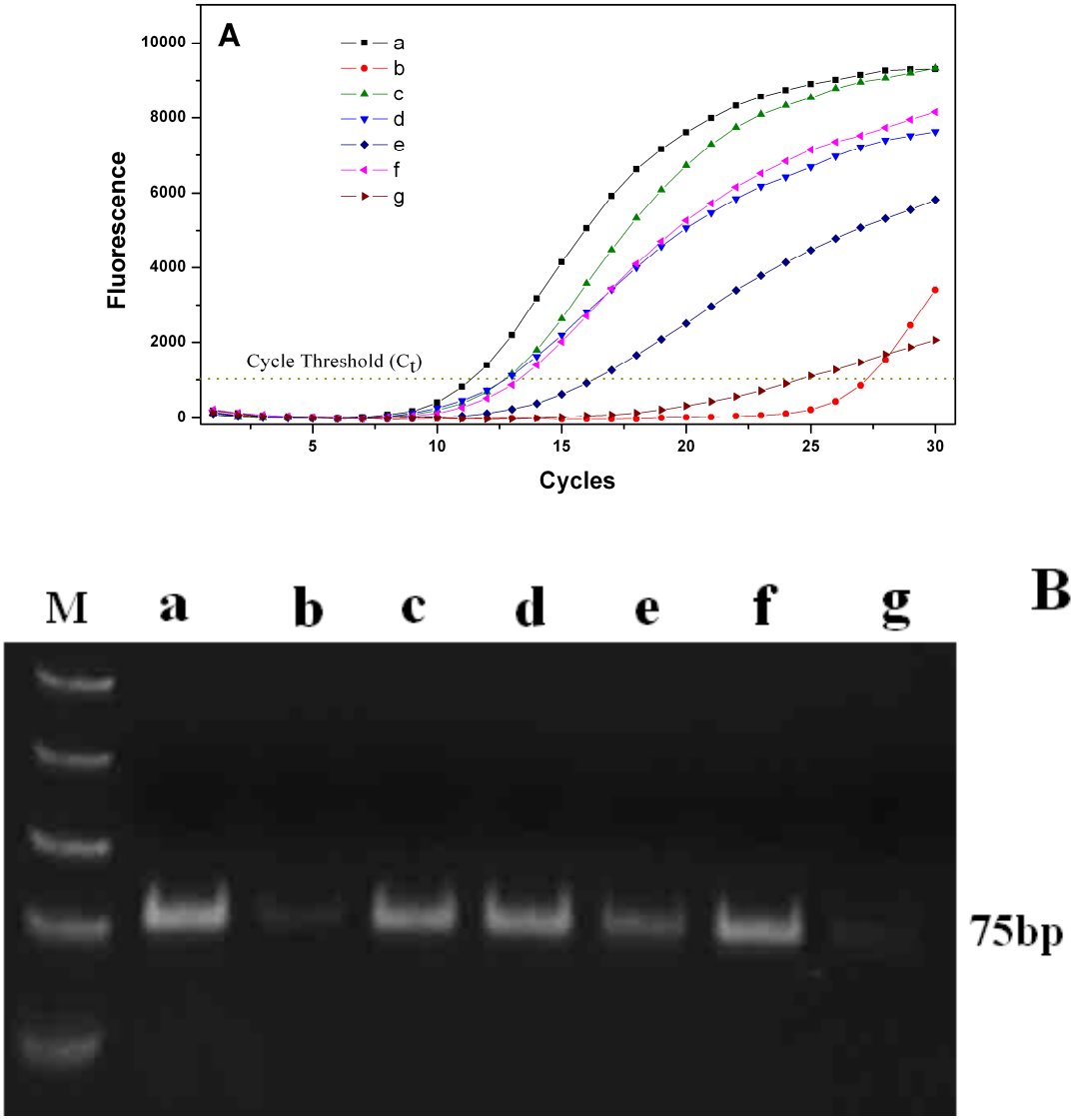

Fig. S1 The elimination of C_{60} induced PCR inhibition by addition of BSA and non-template ssDNA using Real-time PCR (**A**) and gel electrophoresis analysis (**B**). ***a***, positive control; ***b***, negative control; ***c***, BSA (0.1 mg/mL) added only; ***d***, ssDNA (5'-CTTCTGCCCGCCTCCTGG-3', ultimate concentration in solution was 1 μM) added only; ***e***, C_{60} (4.0 μg/mL) added only; ***f***; BSA (0.1 mg/mL) + C_{60} (4 μg/mL); ***g***, ssDNA (1 μM) + C_{60} (4.0μg/mL).

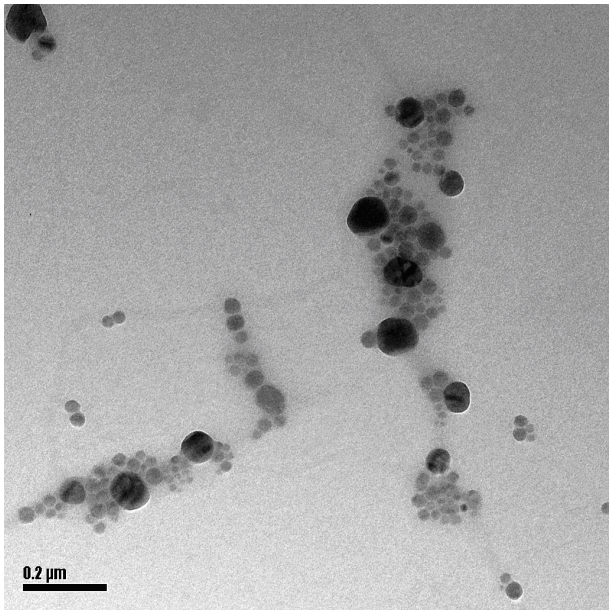

Fig. S2 TEM image of water-soluble C_{60}.

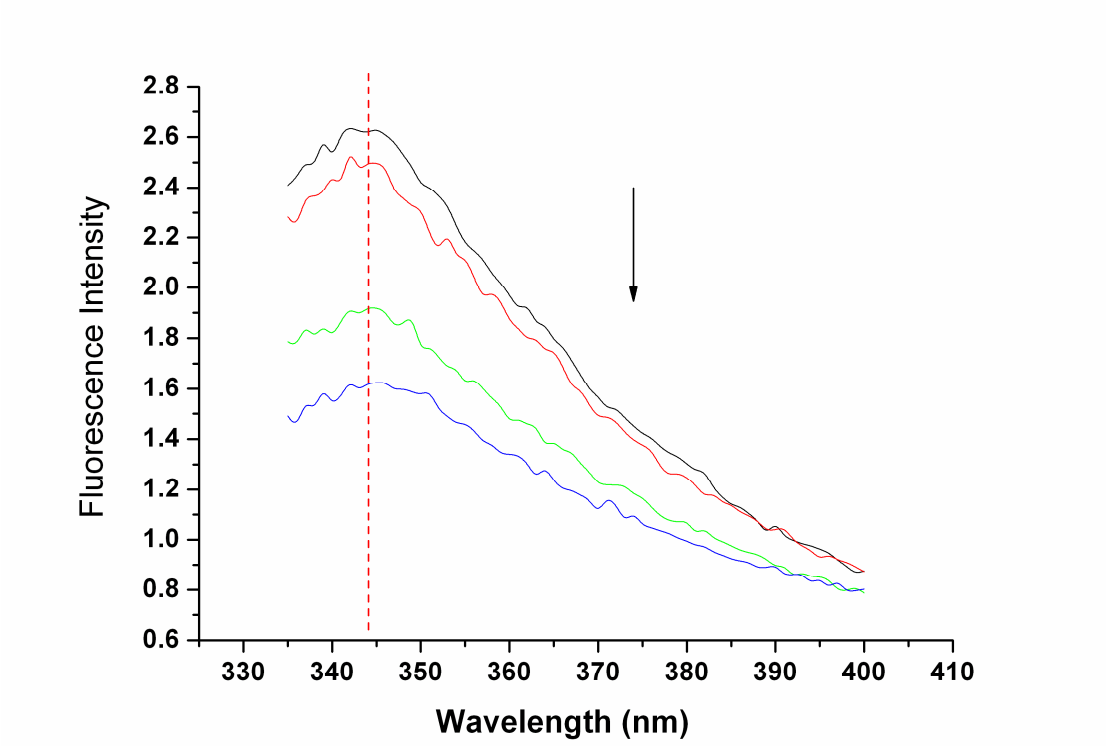

Fig. S3 Fluorescence quenching of the Taq polymerase in the presence of C_{60}. Polymerase: 0.6μL (5000 units/mL); Concentration of C_{60} (From up to down): 0, 1.41, 2.83, 5.66×10^{-6} mol/L; pH=7.4; ex=280 nm.

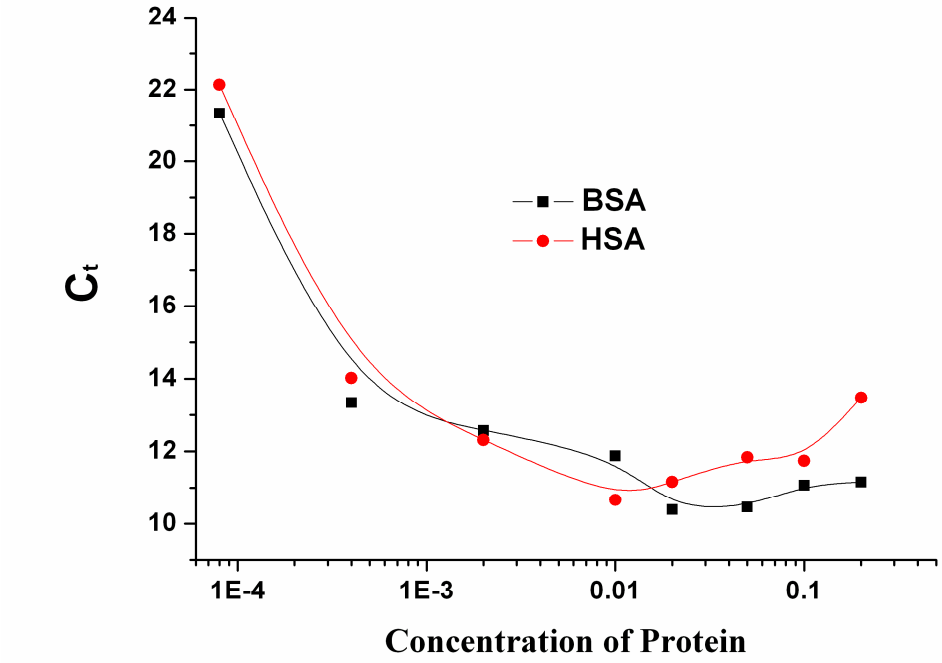

Fig. S4 The changes of real-time PCR C_t values obtained with addition of different BSA/HSA concentrations. The concentration of 75 nt DNA template is 0.25 nM. The concentration of C_{60} used for PCR inhibition is 5.0 μg/mL