Supporting information

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

Song Maoyong, Jiang Guibin, Yin Junfa, Wang Hailin*

State Key Laboratory of Environmental Chemistry and Ecotoxicology Research
Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China,

100085

*corresponding author: hlwang@rcees.ac.cn
**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 C₆₀ 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 C₆₀ solution) and a negative control (normal RT-PCR without C₆₀ 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 C₆₀ 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’-CTTCTGCCCGCCTCTGG-3’, ultimate concentration in solution was 1 µM) added only;  

_e_, C₆₀ (4.0 µg/mL) added only;  

_f_, BSA (0.1 mg/mL) + C₆₀ (4 µg/mL);  

_g_, ssDNA (1 µM) + C₆₀ (4.0µg/mL).
Fig. S2 TEM image of water-soluble C$_{60}$.
**Fig. S3** Fluorescence quenching of the Taq polymerase in the presence of C₆₀.

Polymerase: 0.6µL (5000 units/mL); Concentration of C₆₀ (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.