

Supporting Information

The DNA Damaging Potential of Photoactivated P25

Titanium Dioxide Nanoparticles

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Supporting Figures:

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Figure S2: DNA base damage profile including positive control samples after (A) 3 h dark exposure, (B) 24 h dark exposure, (C) ambient light exposure or (D) UVA irradiation exposure.

Figure S3: 8-OH-deoxyguanosine and 8-OH-guanine analysis after 24 h dark exposure using LC/MS/MS and GC/MS/MS, respectively.

Supporting Materials and Methods:

- 1) Verification of TiO₂ NP Centrifugation Removal from TiO₂ NP/ct-DNA Samples Via ICP-OES
- 2) Enzymatic Digestion and GC/MS/MS Analysis of Exposure Samples
- 3) Isotope-Dilution LC/MS/MS Determination of 8-OH-deoxyguanosine in TiO₂ NP Dark Exposure Samples

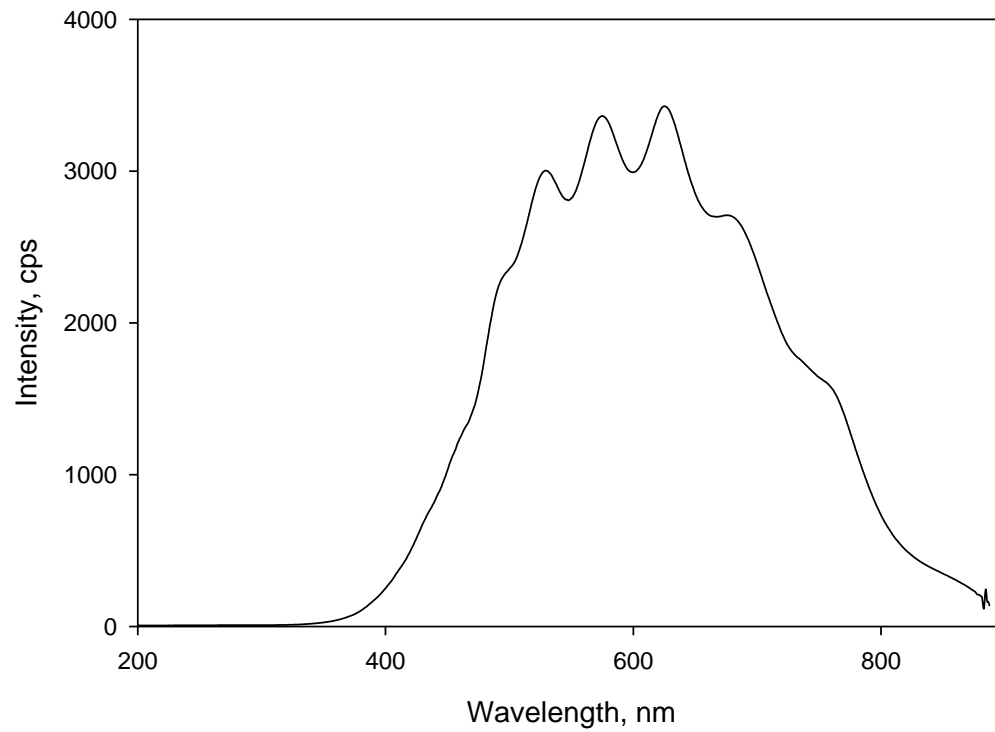


Figure S1. Spectral profile of the NIST calibrated halogen lamp.

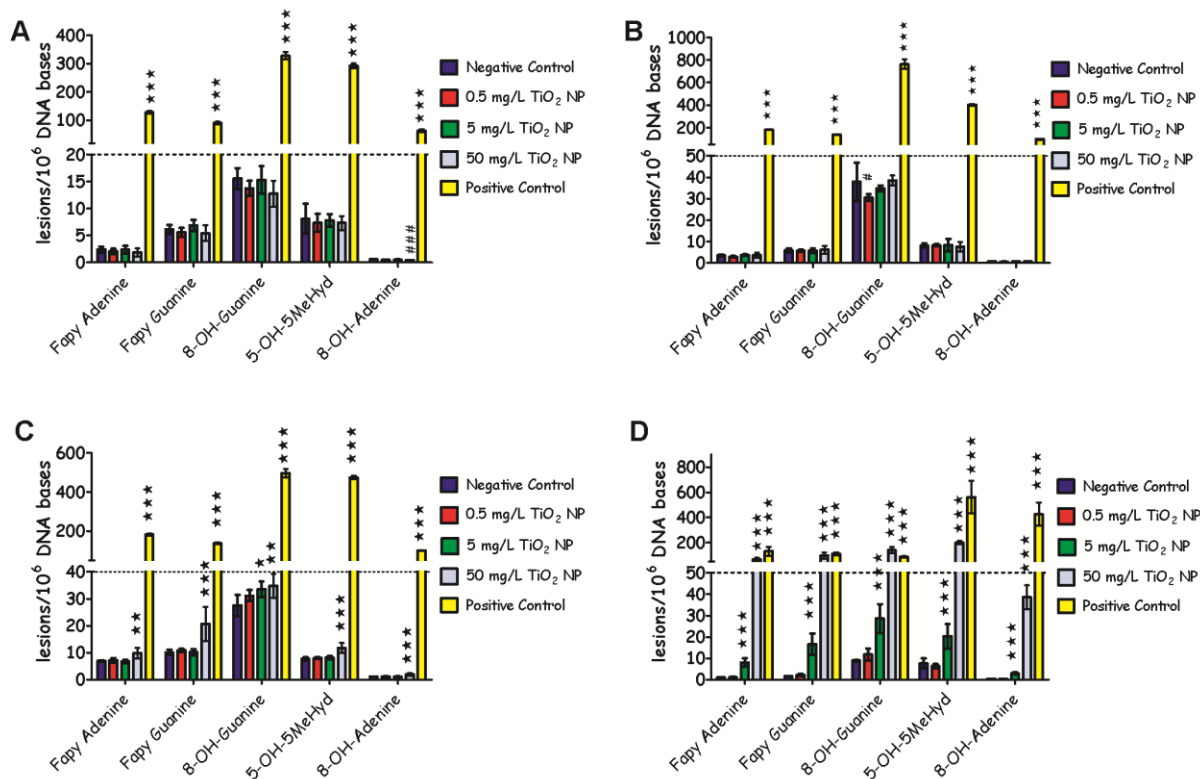


Figure S2. DNA base damage profile for ct-DNA exposed to increasing concentrations of P25 TiO₂ NPs (0.5 mg/L to 50 mg/L) after exposure (A) in the dark for 3 h, (B) in the dark for 24 h exposure, (C) using ambient lighting conditions (24 h; 14.5 kJ/m², 400 nm to 800 nm) and (D) using UVA irradiation conditions (30 min exposure; 10.0 kJ/m², 370 nm). Plot includes the responses from the positive controls (FeNTA damaged ct-DNA). Asterisks (*) and number signs (#) indicate statistically significant results compared to the control samples using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test. Asterisks and number signs correspond to significant increases and decreases, respectively, in the measured lesion levels. One, two, and three asterisks or number signs indicate $p < 0.05$, 0.01 , and 0.001 , respectively. All data points represent the mean of 5 to 6 independent measurements. Uncertainties are standard deviations.

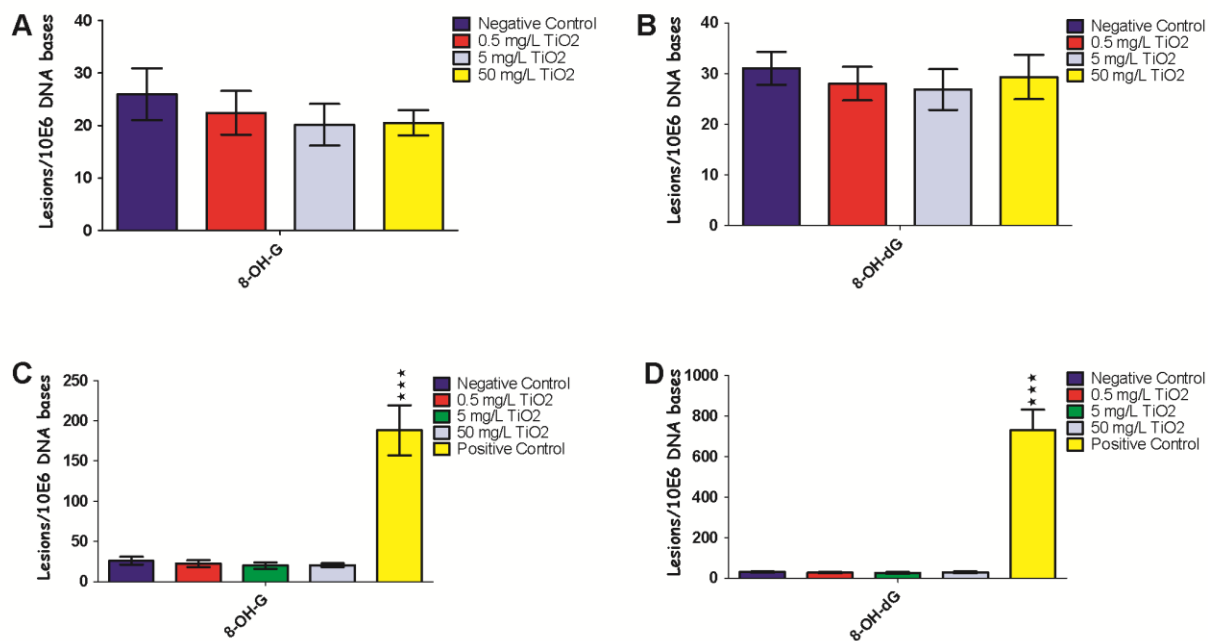


Figure S3. 8-OH-guanine (A, C) and 8-OH-deoxyguanosine (B, D) profiles for ct-DNA exposed to increasing concentrations of P25 TiO₂ NPs (0.5 mg/L to 50 mg/L) in the dark (24 h exposure). The plots are with (C, D) and without (A, B) the responses from the positive controls (FeNTA damaged ct-DNA). Asterisks (*) indicate statistically significant results (increases in lesion levels) compared to the control samples using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test. Three asterisks indicate $p < 0.001$. All data points represent the mean of 4 to 5 independent measurements. Uncertainties are standard deviations.

Verification of TiO₂ NP Centrifugation Removal from TiO₂ NP/ct-DNA Samples Via ICP-OES

An Optima 5300DV ICP-OES (PerkinElmer, CT, USA) was used to verify the removal efficiency of the TiO₂ NPs from the DNA samples after centrifugation. Prior to the analysis, one mL of concentrated hydrofluoric acid (AR Select, Mallinckrodt, USA) was added to ct-DNA samples amended with TiO₂ NPs. The samples were refluxed at 50 °C for 1 to 2 h, evaporated to near dryness and reconstituted with 0.2 mL of concentrated nitric acid (AR Select, Mallinckrodt, USA) and 10 mL of lab pure H₂O. The sample introduction system of the ICP-OES consisted of a MiraMist nebulizer and cyclonic spray chamber. Operating parameters were optimized for robust conditions, and a three point calibration curve was generated to determine the remaining percentage of TiO₂ NPs in the centrifuged DNA samples.

Enzymatic Digestion and GC/MS/MS Analysis of Exposure Samples

To prepare the samples for enzymatic digestion, samples were dissolved in a buffer consisting of 50 mmol/L sodium phosphate, 100 mmol/L potassium chloride, 1 mmol/L EDTA and 100 µmol/L dithiothreitol (pH 7.4). To this solution, 2 µg each of *E. coli* Fpg and EndoIII were added and each sample was digested at 37 °C for 1 h. The digestion was terminated with the addition of ice-cold absolute ethanol in combination with sample storage at – 20 °C. Samples were centrifuged at 14,000 g for 30 min, supernatant fractions containing the excised DNA lesions were transferred to glass vials and the solvent was evaporated under vacuum. Samples were solubilized in ddH₂O, lyophilized, and then trimethylsilylated using bis(trimethylsilyl)trifluoroacetamide / 1% trimethylchlorosilane in pyridine (120 °C for 30 min). Following derivatization, samples were analyzed by GC/MS/MS.

Oxidatively modified DNA base lesions in ct-DNA were identified and quantified using isotope-dilution GC/MS/MS in the multiple reaction monitoring (MRM) mode. This analysis was conducted based on modifications to a previously developed selected ion monitoring (SIM) mode gas chromatography/mass spectrometry (GC/MS) methodology.¹⁻³ Mass spectrometry analyses were performed on an Agilent 7000 series triple quadrupole GC/MS/MS system (Agilent Technologies) operated in positive ion mode with electron ionization. The modular system consisted of a 7693 autosampler, a 7890A GC oven and a 7000 series triple quadrupole mass analyzer set to widest resolution for MS1 and MS2. In the current MRM mode method, specific reaction transitions for five lesions (Figure 1), namely 4,6-diamino-5-formamidopyrimidine (Fapy adenine), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy guanine), 8-hydroxyguanine (8-OH-guanine), 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd) and 8-hydroxyadenine (8-OH-adenine) as well as for their stable isotopically-labeled analogues (Fapy adenine-¹³C, ¹⁵N₂, Fapy guanine-¹³C, ¹⁵N₂, 8-OH-guanine-¹⁵N₅, 5-OH-5-MeHyd-¹³C, ¹⁵N₂ and 8-OH-adenine-¹³C, ¹⁵N₂) were identified and optimized on the basis of the original SIM ions. The

isotopically-labeled lesion analogues function as internal standards (ISTDs) for lesion quantification. The relevant MRM mass transitions were: m/z 369 \rightarrow m/z 354 and m/z 372 \rightarrow m/z 357 for FapyAde / FapyAde- ^{13}C , $^{15}\text{N}_2$; m/z 457 \rightarrow m/z 442 and m/z 460 \rightarrow m/z 445 for FapyGua / FapyGua- ^{13}C , $^{15}\text{N}_2$; m/z 455 \rightarrow m/z 440 and m/z 460 \rightarrow m/z 445 for 8-OH-Gua / 8-OH-Gua- $^{15}\text{N}_5$; m/z 331 \rightarrow m/z 331 and m/z 334 \rightarrow m/z 334 for 5-OH-5-MeHyd / 5-OH-5-MeHyd- ^{13}C , $^{15}\text{N}_2$ and m/z 367 \rightarrow m/z 352 and m/z 370 \rightarrow m/z 355 for 8-OH-Ade / 8-OH-Ade- ^{13}C , $^{15}\text{N}_2$. Final results are reported in terms of the number of lesions quantified / 10^6 DNA bases.

Isotope-Dilution LC/MS/MS Determination of 8-OH-deoxyguanosine in TiO₂ NP Dark Exposure Samples

Determination of 8-OH-deoxyguanosine (8-OH-2'-deoxyguanosine or 8-OH-dG) was performed on an Agilent 1290 Infinity LC system coupled to a Thermo-Scientific TSQ Quantum Ultra AM triple quadrupole MS/MS system operated in positive-ion mode. The stable isotope-labeled internal standard, 8-OH-dG- $^{15}\text{N}_5$, was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Following TiO₂ NP exposure, the DNA samples (50 μg) were amended with a known mass of 8-OH-dG- $^{15}\text{N}_5$ and processed as previously described.^{4,5} The final samples were analyzed (20 μL injection on-column) using an Zorbax SB-Aq narrow-bore LC column (2.1 mm x 150 mm, 3.5 μm particle size) with an attached Agilent Eclipse XDB-C8 guard column (2.1 mm x 12.5 mm, 5 μm particle size). The column temperature was thermostatted at 40 °C. The gradient LC elution conditions were as follows (all solvent percentages are volume fractions): Mobile phase A = 2% acetonitrile in water; mobile phase B = 100 % acetonitrile; time program = 0.0 min, 98% A/2% B; 18.0 min, 80% A/20% B; 18.1 min, 0% 40/60% B; 20.0 min, 40% A/60% B; 20.1 min, 98% A/2% B; 25.0 min, 98% A/2% B; flow rate = 500 $\mu\text{L}/\text{min}$. MS data was only collected from 2 min to 4 min for each analysis; at all other times, the mobile phase was diverted to waste. Automated tuning and mass calibration of the MS/MS system in positive ion mode were performed by infusing a solution of polytyrosine - 1, 3, 6 and monitoring the specified protonated $[\text{M}+\text{H}]^+$ monomer (m/z 182), trimer (m/z 508) and hexamer (m/z 997) ions, respectively. Multiple-reaction-monitoring (MRM) was employed for the detection and quantification of 8-OH-dG using the following instrumental parameters: spray voltage = 3 kV; tube lens offsets = 91 V for Q1 and Q3; collision energy (unlabeled and labeled 8-OH-dG) = 17 V; vaporizer temperature = 270 °C; capillary temperature = 360 °C; sheath gas (nitrogen) pressure = 35 (arbitrary units); auxiliary gas (nitrogen) pressure = 30 (arbitrary units); collision gas (argon) pressure = 1.5 mTorr (1.99×10^{-4} kPa); scan width = 1.0 m/z and scan dwell time = 0.01 s. The relevant MRM mass transitions were m/z 284 \rightarrow m/z 168 and m/z 289 \rightarrow m/z 173 for unlabeled and labeled 8-OH-dG, respectively.

References

- (1) Dizdaroglu, M. (1984) The use of capillary gas-chromatography mass-spectrometry for identification of radiation-induced DNA base damage and DNA base amino acid cross links. *J. Chromatogr. A* 295, 103-121.
- (2) Dizdaroglu, M. (1985) Application of capillary gas-chromatography mass-spectrometry to chemical characterization of radiation-induced base damage of DNA : Implications for assessing DNA-repair processes. *Anal. Biochem.* 144, 593-603.
- (3) Dizdaroglu, M., Jaruga, P., Birincioglu, M., Rodriguez, H. (2002) Free radical-induced damage to DNA: mechanisms and measurement. *Free Radical Biol. Med.* 32, 1102-1115.
- (4) Jaruga, P., Birincioglu, M., Rodriguez, H., Dizdaroglu, M. (2002) Mass spectrometric assays for the tandem lesion 8,5'-cyclo-2'-deoxyguanosine in mammalian DNA. *Biochemistry* 41, 3703-3711.
- (5) Jaruga, P., Xiao, Y., Nelson, B. C., Dizdaroglu, M. (2009) Measurement of (5'R)- and (5'S)-8,5'-cyclo-2'-deoxyadenosines in DNA in vivo by liquid chromatography/isotope-dilution tandem mass spectrometry *Biochem. Biophys. Res. Commun.* 386, 656-660.