SUPPORTING INFORMATION:

In-situ monitoring of the influence of water on DNA radiation damage by near-ambient pressure X-ray photoelectron-spectroscopy

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1 Supplementary Methods

1.1 Experimental details of vacuum XPS measurements

The UHV-XPS measurements were done with an AXIS Ultra DLD photoelectron spectrometer (Kratos Analytical, Manchester, UK) with monochromatic Al Kα radiation (E = 1486.6 eV). The pressure was below 1 · 10⁻⁸ mbar. The electron emission angle was 0° and the source-to-analyzer angle was 60°. The binding energy scale of the instrument was calibrated following a Kratos analytical procedure which uses ISO 15472 binding energy data[3]. Spectra were taken by setting the instrument to the hybrid lens mode and the slot mode with a 300 × 700 µm² analysis area. Furthermore, the charge neutralizer was also used. During the vacuum measurements the measurement of C1s, O1s, N1s, and P2p regions took approximately 4525 s.

1.2 Experimental details of NAP XPS measurements

Laboratory Near-Ambient Pressure X-ray Photoelectron Spectroscopy (NAP XPS) measurements were done with an EnviroESCA (SPECS GmbH, Berlin, Germany).[1, 2] The monochromatic Al Kα x-ray source is separated from the measurement chamber by a silicon nitride window, and the hemispherical energy analyzer is under ultra-high vacuum (< 1×10⁻⁸ mbar) due to a three stage
differential pumping system between the analysis section and analyzer. The entrance aperture (nozzle) has a diameter of 300 µm and the usual working distance is 1-2 times the nozzle diameter. With this set-up, it is possible to measure gaseous, liquid as well as solid samples at pressures up to 50 mbar. For ambient pressure measurements DNA samples were inserted into the Envirotech and the pressure was slowly reduced below 14 mbar to allow residually dissolved gases to evaporate. During the NAP-XPS measurements of about 8 hours, the pressure was kept in the near-ambient pressure regime between 4 mbar-14 mbar. All survey spectra were acquired with a pass energy of 100 eV, a step size of 1.0 eV, and a dwell time of 0.1 seconds. High-resolution core-level spectra (O1s, N1s, C1s, and P2p) were recorded in fixed analyzer transmission (FAT) mode at pass energy of 50 eV, a step size of 0.2 eV, and a dwell time of 0.1 seconds. The binding energy scale of the instrument was calibrated according to ISO 15472.[3] The electron emission angle was 0° and the source-to-analyzer angle was 55°.

1.3 Analysis of the XPS data

Deconvolution of the XPS signals was performed with the Fityk software and a Levenberg-Marquardt algorithm from the MPFIT library.[4] A Shirley background was subtracted from all spectra. The BE values of the spectra measured under UHV conditions were charge corrected on the C1s BE at 285 eV. Under NAP conditions, no further correction was needed since sufficient charge compensation is provided by the surrounding gas. Additionally, five subsequently measured spectra were averaged for each analysis due to the lower signal-to-noise ratio under NAP conditions. The time dependent evolution and comparisons of the C1s, O2s, and N1s regions are normalized on the total integrated peak area of the P2p region at the same time. This is legitimate since the amount of phosphate groups, which are only present in the DNA backbone, can be assumed to be constant during the irradiation. This is based on the fact that the DNA backbone is covalently bound twice to the rest of the molecule and complex damage is a rare event for the doses applied in this study.[5, 6] The percentage of the intensity in the time dependent figures is given with respect to the total integrated peak area at the beginning of the irradiation. Voigt peaks with fixed shape of 0.3 (Lorentz-Gauss ratio) were fitted to the spectra based on the assignments from the literature given and summarized in table 1 of the main text. The peaks were constrained to a range of ±0.2 eV around their center positions within the same measurement series, either, UHV conditions, N2 or H2O atmosphere. Within each envelope the FWHM was constrained to ±0.05 eV (compare Tab. S1-S3). One exception was made for the case of the O1s spectra under water atmosphere. There, the gas peak of water around 535 eV binding energy was fitted with an independent FWHM. We note here, that in the case of the O1s and N1s spectra, a deconvolution with three peaks is a viable alternative to reduce the fitting residuals to some extent (compare Fig. S1 right). Thereby, the imides at around 399 eV, the amides and
amines connected to the ring-structure of the nucleobases (around 400 eV) are complemented by a third N1s peak (around 401 eV) which is sometimes assigned either to hydrogen bonded or protonated amine species, N-C=O, or base-substrate interaction.[7, 8, 9] Nevertheless, to enable a comparison with literature values from the referenced damage studies, we have chosen to stick to a deconvolution of the N1s signal into two Voigt peaks, as discussed in the main text. Since the argumentation related to N1s signal intensities in the main text is based on the evolution of the sum of the N1s under different conditions, a different peak deconvolution methodology would not alter the outcome here. The signal contribution from water in the O1s binding energy region was assigned, based on the work by Patel et al.[2] The relative uncertainties for peak area calculation under NAP conditions were estimated with a maximum value of 15 % using a matrix inversion approach.[10] Due to the better signal-to-noise ratio 5 % were achieved for the UHV measurements. An overview about spectra recorded under nitrogen atmosphere is given in Fig. S2. Peak fit parameters and results for all spectra at the beginning and end of the irradiations are summarized in Tab. S1-S3.

2 Particle Scattering Simulations

Particle scattering simulations were performed with the Geant4 10.5 framework and the Topas 3.3 interface. The g4em-livermore physics-list was enabled with a 1 nm particle cut length.[11, 12] During the simulation, multiple-scattering processes were disabled to increase the accuracy, and Auger, AugerCascade and Fluorescence calculations were enabled. $10^7$ primary photons with 1.487 keV primary energy were simulated. For each experimental setting, i.e. vacuum, nitrogen or water atmosphere, an independent simulation was performed. The vacuum was modeled with the standard “vaccum” settings, as included in Topas, NAP conditions with a temperature of 300 K, a pressure of 2000 Pa and a den-
Figure S2: XPS spectra under nitrogen atmosphere. C1s, O1s and N1s spectra at the beginning and end of the exposure. Additionally Voigt peak fits (blue), the sum (red) and fitting residuals (grey) are shown. For better visibility, the residuum was shifted towards lower y values.

Figure S3: Time evolution of the total area of the P2p signal used for the normalization procedure of the C1s, N1s and O1s time evolution as given in the main text. Error bars were determined using a matrix inversion approach, for details see the text.
Table S1: Voigt peak fitting parameter and results for the XPS spectra of DNA in vacuum. All values were rounded to the first decimal point.

| Spectra | Time | Center / eV | Area    | FWHM / eV |
|---------|------|-------------|---------|-----------|
| C1s     | Start| 285.1       | 12393.6 | 1.2       |
| C1s     | Start| 286.6       | 13927.3 | 1.2       |
| C1s     | Start| 287.8       | 6073.8  | 1.2       |
| C1s     | Start| 289.0       | 2119.6  | 1.2       |
| O1s     | Start| 530.9       | 23121.4 | 1.3       |
| O1s     | Start| 532.7       | 31430.1 | 1.4       |
| O1s     | Start| 535.8       | 2568.4  | 1.4       |
| N1s     | Start| 399.1       | 9837.7  | 1.3       |
| N1s     | Start| 400.5       | 10281.5 | 1.3       |
| C1s     | End  | 284.9       | 14276.2 | 1.2       |
| C1s     | End  | 286.4       | 12354.5 | 1.2       |
| C1s     | End  | 287.7       | 5685.5  | 1.2       |
| C1s     | End  | 288.9       | 2168.9  | 1.2       |
| O1s     | End  | 530.9       | 24530.0 | 1.4       |
| O1s     | End  | 532.6       | 24499.6 | 1.4       |
| O1s     | End  | 535.6       | 3014.7  | 1.4       |
| N1s     | End  | 398.9       | 9906.6  | 1.3       |
| N1s     | End  | 400.4       | 8899.6  | 1.3       |

Table S2: Voigt peak fitting parameter and results for the XPS spectra of DNA in nitrogen. All values were rounded to the first decimal point.

| Spectra | Time | Center / eV | Area    | FWHM / eV |
|---------|------|-------------|---------|-----------|
| C1s     | Start| 285.5       | 310.9   | 1.2       |
| C1s     | Start| 286.9       | 478.4   | 1.3       |
| C1s     | Start| 288.1       | 224.1   | 1.3       |
| C1s     | Start| 289.3       | 102.8   | 1.3       |
| O1s     | Start| 531.6       | 388.3   | 1.3       |
| O1s     | Start| 533.2       | 544.1   | 1.3       |
| N1s     | Start| 399.7       | 224.1   | 1.4       |
| N1s     | Start| 400.9       | 261.0   | 1.4       |
| C1s     | End  | 285.6       | 315.9   | 1.3       |
| C1s     | End  | 286.9       | 365.1   | 1.3       |
| C1s     | End  | 288.3       | 224.3   | 1.3       |
| C1s     | End  | 289.6       | 185.1   | 1.3       |
| O1s     | End  | 531.8       | 432.9   | 1.3       |
| O1s     | End  | 533.3       | 483.8   | 1.3       |
| N1s     | End  | 399.7       | 181.6   | 1.4       |
| N1s     | End  | 401.0       | 284.8   | 1.5       |
Table S3: Voigt peak fitting parameter and results for the XPS spectra of DNA in water. All values were rounded to the first decimal point.

| Spectra | Time | Center / eV | Area  | FWHM / eV |
|---------|------|-------------|-------|-----------|
| C1s     | Start| 285.2       | 833.7 | 1.1       |
| C1s     | Start| 286.6       | 973.1 | 1.1       |
| C1s     | Start| 287.9       | 498.3 | 1.1       |
| C1s     | Start| 289.1       | 187.2 | 1.1       |
| O1s     | Start| 531.4       | 1060.3| 1.3       |
| O1s     | Start| 532.9       | 2327.6| 1.3       |
| O1s     | Start| 535.4       | 2833.5| 0.7       |
| N1s     | Start| 399.4       | 499.2 | 1.3       |
| N1s     | Start| 400.6       | 619.3 | 1.3       |
| C1s     | End  | 285.2       | 784.1 | 1.1       |
| C1s     | End  | 286.5       | 333.0 | 1.1       |
| C1s     | End  | 287.9       | 266.9 | 1.1       |
| C1s     | End  | 289.1       | 164.1 | 1.1       |
| O1s     | End  | 531.4       | 1228.6| 1.3       |
| O1s     | End  | 532.9       | 1879.1| 1.3       |
| O1s     | End  | 535.5       | 1541.7| 0.8       |
| N1s     | End  | 399.4       | 213.4 | 1.2       |
| N1s     | End  | 400.5       | 336.8 | 1.3       |

A density of 22.5 $\mu g/cm^3$ for Nitrogen and a density of 14.4 $\mu g/cm^3$ for water, were used as a medium. The DNA was modeled with a density of 1.7 $g/cm^3$ with 50% GC content and no salts present. The surface layer was assumed to have 1 nm thickness of either water or nitrogen and a density of 1 $g/cm^3$. Dose and energy deposit were scored within a surface area of $10^4 nm^2$ and a depth of 10 nm for DNA. For the surface layer, the values were recorded for the depth of 1 nm and for the gas volume, within the last 99 nm before the sample. All values were determined by the dose, energy, charge and volume scorers as included in the Topas package. Results are summarized in Tab. S4.
Table S4: All values are given per $10^7$ primary photons entering the area of $10^4 \text{ nm}^2$ within the first 10 nm of DNA, after a passage through vacuum, water or nitrogen gas and a layer of 1 nm absorbed gas molecules on top of the DNA. Note the distinction between ionisation events caused by x-ray photons (photoelectric effect) and electrons (electron-electron scattering). For details see the text.

| Value                                      | Vacuum | Water | Nitrogen |
|--------------------------------------------|--------|-------|----------|
| X-ray ionizations in $9.9 \cdot 10^5 \text{nm}^3$ gas | 1      | 5     | 3        |
| X-ray ionizations in $10^4 \text{nm}^3$ absorbed gas | -      | 1445  | 1148     |
| Electron ionizations in $10^4 \text{nm}^3$ absorbed gas | -      | 80    | 56       |
| Netto e- flux in $10^4 \text{nm}^3$ absorbed gas | -      | 1021  | 796      |
| Energy deposit in $10^4 \text{nm}^3$ absorbed gas | -      | 1.27 MeV | 0.87 MeV |
| Dose in $10^4 \text{nm}^3$ absorbed gas | 5071 kGy | 3490 kGy |
| X-ray ionizations in $10^5 \text{nm}^3$ DNA | 17698  | 17652 | 17644    |
| Electron ionizations in $10^5 \text{nm}^3$ DNA | 7684   | 8009  | 7866     |
| Total e- produced $10^5 \text{nm}^3$ DNA | 25382  | 25661 | 25510    |
| Netto e- leaving the $10^5 \text{nm}^3$ DNA volume | 8299   | 7903  | 8027     |
| Thermalized e- in the $10^5 \text{nm}^3$ DNA volume | 17083  | 17362 | 17483    |
| Energy deposit in $10^5 \text{nm}^3$ DNA | 19.24 MeV | 19.67 MeV | 19.59 MeV |
| Dose in $10^5 \text{nm}^3$ DNA | 4533 kGy | 4634 kGy | 4616 kGy |

**Supplementary References**

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