Supporting Information

Ne-22 Ion-Beam Radiation Damage to DNA: From Initial Free Radical Formation to Resulting DNA-Base Damage

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Experimental

Materials. Following our previous works,[5-7,14-17,19-21] Salmon testes DNA (Type III, 57.3 % A-T, 42.7 % G-C) and 99.9 % D atom D_2O were purchased from Sigma Aldrich and used without further purification. Human NTHL1 protein and human OGG1 protein were gifts from Dr. Susan Wallace of the University of Vermont and from Dr. R. Stephen Lloyd of the Health and Science University, Portland, OR, respectively.

DNA Sample Preparation: Salmon testes DNA as received from Sigma-Aldrich is hydrated to Γ = *ca*. 5 H_2O/nucleotide. For the sample preparation, it is further hydrated to $\Gamma = 12\pm3$ H_2O/nucleotide by equilibration over a saturated $NaCl/H_2O$ or $NaCl/D_2O$ solution for two weeks under a N_2 atmosphere. As in our previous work, 50 mg to 75 mg of the hydrated DNA is pressed (in air) into rectangular parallelepiped blocks of ca. 10 mm x 4 mm x 1 mm dimensions using an aluminum dye and press.[14-17,19-21] Samples are then rehydrated for a few weeks. Approximately 7 to 9 individual parallelepiped samples are then assembled into a sample packet as shown in Figure 2 and placed in a plexiglass sample packet holder for irradiation.

Rehydration is done once more to ensure that these samples stay hydrated at $\Gamma = 12\pm3$ H_2O/nucleotide. As mentioned earlier, all hydration steps are done using a saturated $NaCl/H_2O$ solution with an N_2 atmosphere. Before irradiation, the plexiglass sample holders containing the DNA samples are rapidly plunged into liquid N_2 (77 K) for transportation to the National Superconducting Cyclotron Laboratory (NSCL) at Michigan State University, East Lansing. After Ne-22 ion-beam irradiation and ESR analysis at 77 K, the samples are warmed to room temperature and sliced by hand with a razor blade to dimensions of *ca.* 10 mm x 4 mm x (0.3 to 0.5) mm for product analysis. In this work, seven samples were subjected to ESR measurements, and thinner samples used for product analysis (vide infra). It is not possible to produce exact parallelepiped shaped samples for product analysis by hand slicing. The average depth of the thinner samples is calculated by using the mass of the thinner sample compared to the mass and measured depth of the larger sample from which it was cut.

Ne-22 ion-beam and γ-irradiation: DNA samples were irradiated at 77 K at the National Superconducting Cyclotron Laboratory of the Michigan State University using the Coupled Cyclotron Facility. The Ne-22 beam had an energy of 1.514 GeV at the exit of the fragment separator, which was used for the energy

selection of the degraded primary beam. After passing through a 75 μm zirconium window, 433 mm of air and 28 mm of Styrofoam, the nominal energy of the beam at the front of and before it enters a sample packet was calculated by LISE++ [22] to be 1.363 GeV. However, the depth of formation of color centers in the plexiglass sample packet holders and the measured yields of products in the sample packets themselves both indicate that the actual energy at the sample packet entrance is ca. 1.14±0.05 GeV. An example of the plexiglass color center is shown in Figure S1. The average depth that the Ne-22 beam penetrates into the plexiglass is determined to be 4.94 mm. Using TRIM and this penetration depth, the energy of the beam at the front of the sample holder is found to be 1.18 GeV. Infiltration of liquid nitrogen in front of the sample packets, and, perhaps, between individual samples within the packet, likely accounts for the additional energy loss from 1.363 GeV and 1.18 GeV to the final value (1.14 GeV) cited above. At the energy of the beam, 1.1 mm of liquid nitrogen causes an energy loss of ca. 0.10 GeV. The blue arrows in Figure S2 specify the darkest region of color in the color center development and is indicative of the Bragg peak. A faint halo of color formation is visible beyond the Bragg peak. This is likely due to beam fragmentation. Beam range straggling may also account for some variation in depth.

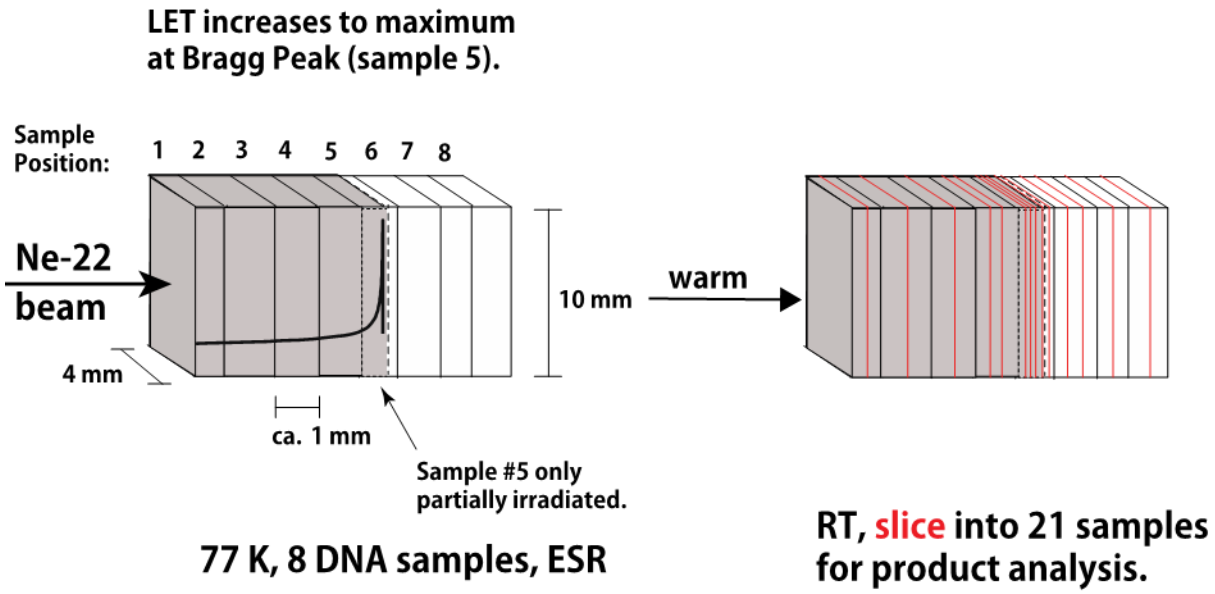

Figure S1. Depiction of sample packet containing eight DNA samples. Each sample has dimensions of *ca.* 4 mm x 10 mm x 1 mm. The Ne-22 beam is shown as stopping in the fifth sample in this packet, leaving sample #5 partially irradiated and three samples unirradiated. After warming to room temperature (RT), all of the samples are sliced into thinner slices to get better than 1 mm resolution for product

analysis. In this case, 21 samples result for after this procedure. Note that beam fragmentation (ca. 3%) results in some dose beyond the Bragg peak.

Sample doses were calculated using the layer feature of the Transport of Ions in Matter (TRIM) code[23], beam parameters and duration of irradiation. Each small DNA sample is treated as a layer in TRIM using its depth and density as input, and the EXYZ output of the program is used to determine the energy deposited in each sample. The dose, in Gy, in each sample is calculated using equation S1[17]:

$$\text{dose} = \frac{(\text{ion/s})(\text{time})(\text{energy deposited/ion})(\text{fraction beam intercepted by sample})}{\text{mass sample (kg)}} = \frac{(\text{I/Z})(\text{No})(\text{t})(\text{E})(\text{f})}{(\mathcal{F})(\text{m})} \quad \text{(S1)},$$

where I = ion current in electrical amps, Z = ion charge (10+) of the fully stripped ion, for which the ion current in electrical amps is determined, No = Avogadro's number, t = irradiation time in seconds, E = energy deposited in sample from the TRIM code in joules, f = fraction of beam spot area intercepted by sample area, F = faraday constant, and m = mass of the sample (kg). Typical values in this experiment were, I = 6.8 x 10^{-9} A, n = 10+, t = 300 s, Energy deposited in sample per ion (from TRIM) = 0.149 GeV (2.39 x 10^{-11} J), f = 40.2 mm^2/377 mm^2 = 0.107, m = 43.0 mg. These data result in a dose of 7.57 x 10^4 Gy. The LET (in keV/μm) is calculated using the keV deposited per sample divided by the depth of the sample. Since the samples are somewhat irregular in depth, LETs are estimated to have ±20 % uncertainty. A typical dose rate was 252 Gy/s. Both the dose and LET increase as the beam penetrates into the DNA sample packet (Figure S1) varying from 62.8 kGy in the first sample to *ca.* 362 kGy for samples near the Bragg peak. Accurate determination of the dose for the samples at the end of the beam path is difficult since it depends on the somewhat uncertain degree to which the beam penetrates into the last irradiated sample; that is, as the beam stops in the last irradiated sample, a fraction of the sample mass may remain unirradiated. Due to the limited range of the ion-beam, only the first 4 ESR samples in each sample packet were actually completely irradiated. Fragmentation in the relatively low energy beam used is estimated by LISE++ to be 3.4 % using the EPAX 3.1a empirical model for production cross sections.[24] Thus, a fairly sharp Bragg peak should result, and, in addition, a large majority of the damage observed is done by the primary beam. For γ-irradiation, hydrated salmon testes DNA samples (Γ = 12±3 H_2O/nucleotide) were kept under a N_2-atmosphere and under H_2O vapor in a closed vial. This vial was γ-irradiated (absorbed dose = 20 kGy) at room temperature using a 109-GR9 ^{60}Co γ-irradiator (J. L. Shepherd & Associates).

Electron Spin Resonance Spectroscopy and Computer Analyses; A Varian Century Series X-band (9.3 GHz) ESR spectrometer with an E-4531 dual cavity, 22.9 cm magnet, and 200 mW Klystron was used, and Fremy's salt [g = 2.0056, A_N = 13.09 G] was employed for the field calibration. All ESR spectra were recorded at 77 K and at 41 dB (16 μW). Spectral recording is done at 77 K because the relevant free radicals decay as recombination reactions occur at higher temperatures.[14-17,19-21] The composite ESR spectra, each resulting from at least seven radicals (Figure 2 in the main manuscript), of 14 irradiated samples of Ne-22 irradiated DNA, hydrated with D_2O instead of H_2O, were deconvoluted using four benchmark spectra of DNA radicals. These were: (1) $G(-H)^{\bullet}/G^{\bullet+}$ ($G(N1-H)^{\bullet}$:$C(N3H^{+})$), (2) $C(N3)H^{\bullet}$, (3) $T^{\bullet-}$, (4) $dR^{\bullet} = \Sigma(C1^{\bullet\prime}+C3^{\bullet\prime}+ C5^{\bullet\prime}+ C3^{\bullet\prime}_{dephos})$[3,13,17] in order to obtain the dose response curves shown below and to determine the fractional amount of these radicals in the experimentally recorded spectra of the irradiated DNA.

Analysis was done using the programs ESRADSUB and ESRPLAY, written in our laboratory. Dose response data often tends to a plateau at higher doses. Such data is fit to equation S2 which accounts for the fact that pre-existing radicals may be destroyed by further radiation.[15-17]

$$Y = \left(G/_k\right) * (1 - \exp(-k * d)) \quad \text{(S2)}$$

In equation S2, Y = yield of trapped radicals (μmol/kg), d = dose (Gy), G = yield of trapped radicals in μmol/J, k = the destruction constant (Gy^{-1}).[15-17]

Measurement of DNA lesions: Ne-22 ion-irradiated or γ-irradiated DNA samples were dissolved in water at 4 °C for 18 h. The concentration of DNA in each sample was measured by absorption spectrophotometry at 260 nm (absorbance of 1 corresponds to 50 μg of DNA/mL). Subsequently, 9 replicates of 50 μg aliquots of each Ne-22 ion-irradiated DNA sample were dried in a SpeedVac under vacuum, and then stored at 4 °C until use. GC-MS/MS with isotope-dilution was used to identify and quantify DNA base lesions 5-hydroxycytosine (5-OH-Cyt), thymine glycol (ThyGly), 4,6-diamino-5-formamidopyrimidine (FapyAde), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) in a set of DNA samples with 3 replicates. For this purpose, the triplicates of dried DNA samples (50 μg each) were supplemented with the aliquots of the stable isotope-labeled analogues of these compounds, i.e., 5-OH-Cyt-^{13}C,$^{15}N_2$, ThyGly-$^{2}H_4$, FapyAde-^{13}C,$^{15}N_2$, and FapyGua-^{13}C,$^{15}N_2$ as internal standards, which are a part of the NIST (*National Institute of Standards and Technology) Standard Reference Material 2396 Oxidative DNA Damage Mass Spectrometry Standards (NIST SRM 2396)*.[25] The samples were dried in a SpeedVac under vacuum, and then dissolved in 50 μL of an incubation buffer consisting of 50 mmol/L

phosphate buffer (pH 7.4), 100 mmol/L KCl, 1 mmol/L ethylenediaminetetraacetic acid (EDTA), and 0.1 mmol/L dithiothreitol. Subsequently, they were incubated with 1 μg of human NTHL1 protein and 1 μg of human OGG1 protein at 37 °C for 1 h to release the modified DNA bases from DNA. These DNA glycosylases are specific for the efficient removal of the aforementioned DNA base lesions from DNA.[26] An aliquot of 200 μL ethanol was added to precipitate DNA and to stop the reaction. After centrifugation, the supernatant fractions were separated and lyophilized as described.[27] An aliquot of 60 μL of a mixture of nitrogen-bubbled bis(trimethylsilyl)trifluoroacetic acid [containing trimethylchlorosilane (1%; v/v)] (BSTFA) and pyridine (1:1, v/v) was added to lyophilized supernatant fractions. Samples were vortexed and purged individually with ultrahigh-purity nitrogen, tightly sealed under nitrogen with Teflon-coated septa, and then heated at 120 °C for 30 min. After cooling, the clear supernatant fractions were removed and placed in vials used for injection of samples onto the GC column. Vials are purged with ultrahigh-purity nitrogen and tightly sealed with Teflon-coated septa. Aliquots (4 μL of derivatized samples were analyzed by GC-MS/MS with split-mode injection and a split ratio of 10 to 1 using multiple reaction monitoring (MRM) as described previously.[9,27,28] The following mass/charge (*m/z*) transitions were used : *m/z* 343→ *m/z* 342 and *m/z* 346→ *m/z* 345 for 5-OH-Cyt and 5-OH-Cyt-$^{13}C,^{15}N_2$, respectively; *m/z* 448→ *m/z* 259 and *m/z* 452→ *m/z* 262 for ThyGly and ThyGly-$^{2}H_4$, respectively; *m/z* 369→ *m/z* 368 and *m/z* 372→ *m/z* 371 for FapyAde and FapyAde-$^{13}C,^{15}N_2$, respectively; *m/z* 457 → *m/z* 368 and *m/z* 460 → *m/z* 371 for FapyGua and FapyGua-$^{13}C,^{15}N_2$, respectively. These *m/z* transitions are based on the known mass spectra of the trimethylsilyl derivatives of these compounds and their fragmentation patterns.[29,30]

A second set of the triplicates of DNA samples (50 μg each) was used for the measurement of (5'*R*)-8,5′-cyclo-2′-deoxyadenosine (*R*-cdA), (5'*S*)-8,5′-cyclo-2′-deoxyadenosine (*S*-cdA), (5'*R*)-8,5′-cyclo-2′-deoxyguanosine (*R*-cdG) and (5'*S*)-cyclo-2′-deoxyguanosine (*S*-cdG), 8-hydroxyadenine (8-OH-Ade) as its 2′-deoxynucleoside, i.e., 8-hydroxy-2′-deoxyadenosine (8-OH-dA) and 8-hydroxyguanine (8-OH-Gua) as its 2′-deoxynucleoside, i.e., 8-hydroxy-2′-deoxyguanosine (8-OH-dG). DNA samples were supplemented with the aliquots of the stable isotope-labeled analogues of these compounds, i.e., *R*-cdA-$^{15}N_5$, *S*-cdA-$^{15}N_5$, *S*-cdG-$^{15}N_5$, *R*-cdG-$^{15}N_5$, *S*-cdA-$^{15}N_5$, 8-OH-dG-$^{15}N_5$, and 8-OH-dA-$^{15}N_5$. 8-OH-dG-$^{15}N_5$ is a part of the aforementioned NIST SRM 2396. The other $^{15}N_5$-labeled compounds were synthesized and isolated as described.[31,32] The samples were dried in a SpeedVac under vacuum and then dissolved in 50 μL of 10 mmol/L Tris-HCl solution (pH 7.5) containing 45 mmol/L $ZnCl_2$, supplemented with 2.5 μL of 1 mol/L sodium acetate (final pH 6.0). Aliquots of nuclease P1 (2 U), snake venom phosphodiesterase (0.004 U) and alkaline phosphatase (16 U) were added and the samples were incubated at 37 °C for 24 h. The samples were then filtered using Nanosep® 3K Omega ultrafiltration tubes with a molecular mass cutoff of 3 kDa (Pall Corporation, Port Washington, New York) by centrifugation at

12000 g for 30 min. The aliquots (40 µL) of the samples were analyzed by LC-MS/MS using a Thermo TSQ Altis Triple Stage Quadrupole MS/MS system with a Vanquish Flex Quarternary UHPLC LC-MS front-end system equipped with a diode array detector (Thermo Fisher Scientific). A Zorbax SB-Aq LC column (2.1 mm x 150 mm, 3.5 µm particle size) (Agilent Technologies, Wilmington, DE) with an attached Agilent Eclipse XDB-C8 guard column (2.1 mm x 12.5 mm, 5 µm particle size) was used. In all instances, the autosampler and column temperature were kept at 6 °C and 40 °C, respectively. Mobile phase A was a mixture of water (98 %) and acetonitrile (2 %), and mobile phase B was acetonitrile, both containing 0.1 % formic acid (v/v). A gradient analysis of 0 % B/min to 24 % of B/min in 10 min was used with a flow rate of 0.5 mL/min. After 10 min, B was increased to 90 % in 0.5 min and kept at this level for 5 min and then another 15 min at 0 % to equilibrate the column. The total analysis time was 30 min. The following MS/MS parameters were used for all measurements: spray voltage = 3.5 kV; tube lens offsets = 89 V for Q1 and Q3; vaporizer temperature = 275 °C; ion transfer tube temperature = 325 °C; sheath gas (nitrogen) pressure = 50 (arbitrary units); auxiliary gas (nitrogen) pressure = 10 (arbitrary units); sweep gas 2 (arbitrary units); collision gas (argon) pressure = 2.67×10^{-5} Pa (2 mTorr). Selected reaction monitoring (SRM) data were acquired in the positive ionization mode at a mass range of *m/z* 100 to *m/z* 1500 with scan width m/z 2.000 and scan time 0.10 s. SRM scans were performed with the *m/z* transitions *m/z* 250 → *m/z* 164 for R-cdA and S-cdA, *m/z* 255 → m/z 169 for *R*-cdA-$^{15}N_5$ and *S*-cdA-$^{15}N_5$, *m/z* 266 → *m/z* 180 for *R*-cdG and *S*-cdG, and *m/z* 271 → *m/z* 185 for *R*-cdG-$^{15}N_5$ and *S*-cdG-$^{15}N_5$, *m/z* 284 → *m/z* 168 for 8-OH-dG, *m/z* 289 → *m/z* 173 for 8-OH-dG-$^{15}N_5$, *m/z* 268 → *m/z* 152 for 8-OH-dA and *m/z* 273 → *m/z* 157 for 8-OH-dA-$^{15}N_5$. These *m/z* transitions are based on the known mass spectra of these compounds and their fragmentation patterns.[12,31,33,34] The optimal (maximum) collision energies were determined by separately infusing a solution of these compounds directly into the ion source of the MS/MS. The maximum collision energy for each of these compounds was found to be 17 V for *R*-cdA, *S*-cdA, *R*-cdG and *S*-cdG, 13 V for 8-OH-dG and 15 V for 8-OH-dA.

A third set of triplicates of DNA samples (50 µg each) was used for the measurement of 5,6-dihydrothymine (5,6-diHThy), 5,6-dihydrocytosine (5,6-diHCyt) and 5,6-dihydrouracil (5,6-diHUra), which is produced by deamination of 5,6-diHCyt under acidic conditions. DNA samples were supplemented with the aliquots of the stable isotope-labeled analogues of 5,6-diHThy and 5,6-diHUra, i.e., 5,6-diHThy-^{13}C,$^{15}N_2$ and 5,6-diHUra -^{13}C,$^{15}N_2$, which were synthesized as described previously.[35] A stable isotope-labeled analogue of 5,6-diHCyt was not available. 5,6-diHUra-^{13}C,$^{15}N_2$ was also used as an internal standard for the quantification of 5,6-diHCyt because the trimethylsilyl derivatives of these compounds have similar mass spectra in terms of their typical ions and their intensities.[36] Subsequently, the samples were hydrolyzed using 100 µL of formic acid (60 %) at 90 °C for 30 min in evacuated and

sealed tubes. Hydrolyzed samples were lyophilized and trimethylsilylated as described,[25] and then analyzed by GC-MS/MS using MRM. The following *m/z* transitions were used: *m/z* 272 → *m/z* 271 and *m/z* 275→ *m/z* 274 for 5,6-diHThy and 5,6-diHThy-^{13}C,$^{15}N_2$, respectively, *m/z* 258 → *m/z* 257 and *m/z* 261→ *m/z* 260 for 5,6-diHUra and 5,6-diHUra-^{13}C,$^{15}N_2$, respectively, and *m/z* 257→ *m/z* 256 for 5,6-diHCyt on the basis of their known mass spectra.[29,36]

Calf thymus DNA was dissolved in 10 mM sodium phosphate buffer (pH 7.4) (0.3 mg/mL) at 4 ºC. Then, it was bubbled with N_2O and irradiated γ-rays in a ^{60}Co γ-source at a dose of 10 Gy. Irradiated DNA sample was dialyzed against water for 18 h at 4 ºC as described.[37] Two sets of triplicates with 50 µg aliquots of DNA were dried in a SpeedVac. The samples were then analyzed by GC-MS/MS or LC-MS/MS as above.

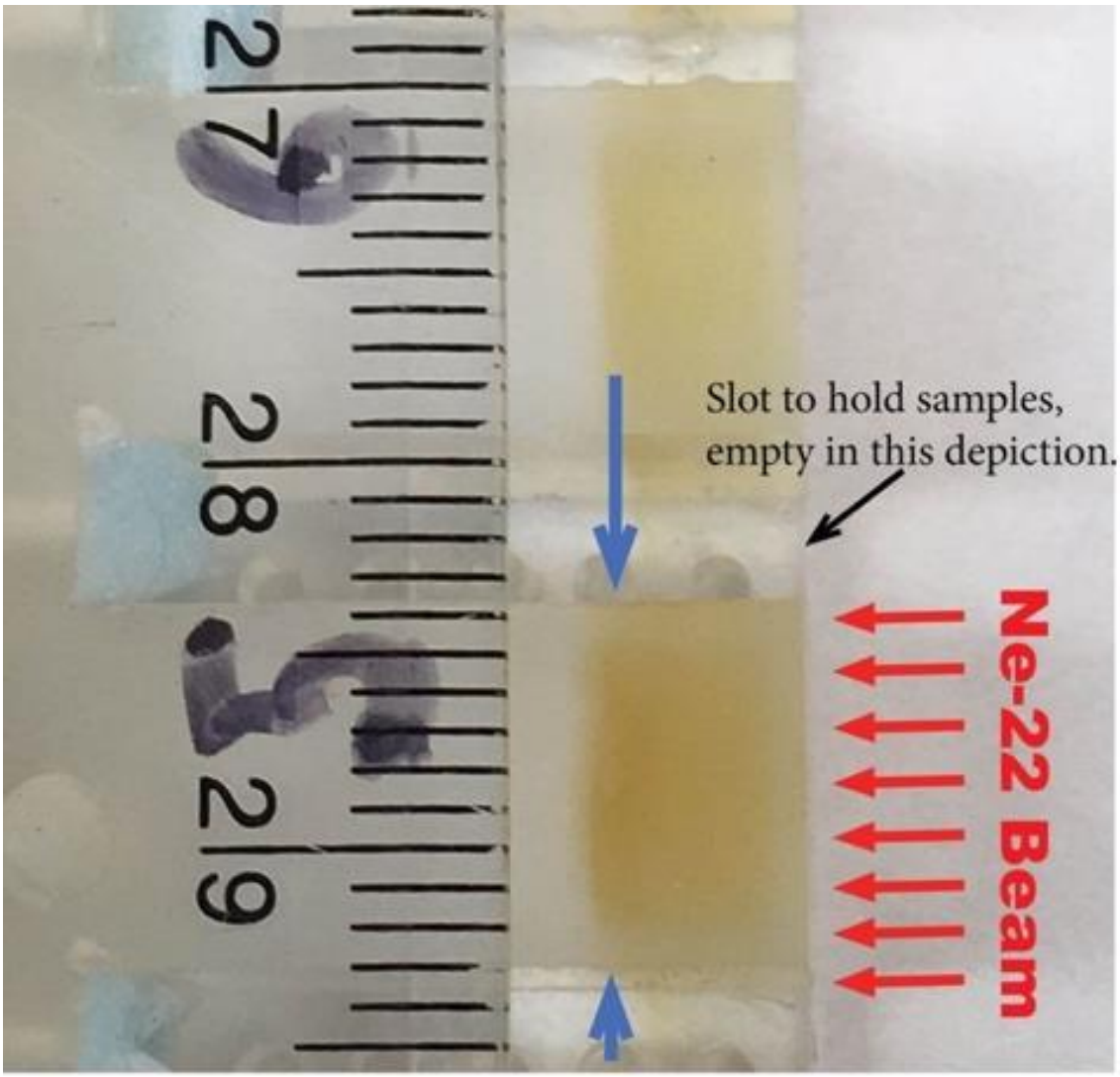

Figure S2. Photograph of plexiglass sample holder, after warming to room temperature, with a color center formed due to Ne-22 ion-beam irradiation. At 77 K, the color center is dark black. The darker color visible between the blue arrows is indicative of the Bragg peak. A slight halo exists beyond the Bragg peak, indicating that a small fraction of ions or fragments has a range that extends beyond the Bragg peak.

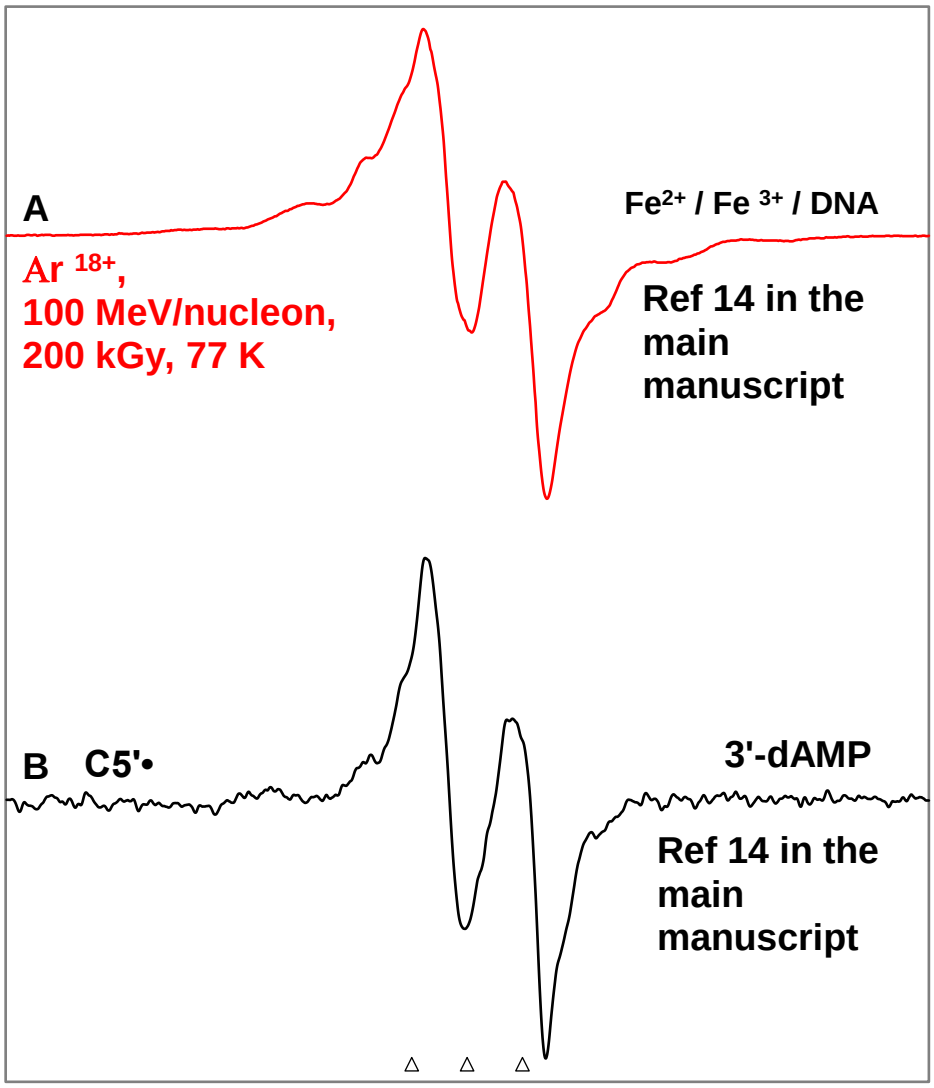

Figure S3. Benchmark Spectrum used in ESR spectra deconvolution. (A) Benchmark ESR spectrum resulting from the four neutral sugar radicals, ΣdR• = Σ(C1′• + C2′• + C3′• + C3′$_{dephos}$•)**.** (B) Benchmark spectrum resulting from C5′•. In ion beam irradiated samples, the C5'• benchmark in (B) accounts for ca. 25 % of the spectrum in (A). The two spectral intensities have been adjusted so that the peak-to-peak heights are the same. (Reprinted (Adapted or Reprinted in part) with permission from A. Adhikary, D. Becker, B.J. Palmer, A.N. Heizer and M. D. Sevilla, Direct Formation of the C5′-Radical in the Sugar−Phosphate Backbone of DNA by High-Energy Radiation. *J. Phys. Chem. B*, **2012**, *116*, 5900−5906) Copyright [2012], American Chemical Society), see Ref. 14 in the main manuscript.

Table S1. Yields of 8,5′-cyclopurine-2′-deoxynucleosides in Ne-22 irradiated DNA.[a]

Dose[b] (kGy)	LET[b] (keV/μm)	*R*-cdA[c]	*S*-cdA[c]	*R*-cdG[c]	*S*-cdG[c]	*R*/*S* (cdA)	*R*/*S* (cdG)
62.8	107	0.91±0.19	0.534±0.034	1.96±0.44	0.93±0.04	1.70	2.12
66.0	109	1.15±0.10	0.592±0.028	2.28±0.06	0.82±0.15	1.94	2.77
69.5	163	2.29±0.33	1.49±0.04	4.19±0.59	2.23±0.24	1.54	1.88
77.5	182	2.53±0.31	1.28± 0.10	4.63±0.35	2.22±0.22	1.98	2.08
81.7	151	1.76±0.18	1.09± 0.07	3.40±0.39	1.60±0.09	1.62	2.13
93.0	171	2.37±0.19	1.18±(0.11	3.79±0.41	1.53±0.29	2.01	2.48
102	221	1.95±0.35	1.00±(0.19	3.29±0.08	1.44±0.09	1.95	2.29
120	261	2.57±0.27	1.34± 0.09	4.60±0.39	1.74±0.19	1.92	2.64
151	327	2.26±0.10	1.16±0.14	3.68±0.36	1.71±0.27	1.95	2.15
226	472	1.03±0.32	0.60±0.01	2.76±0.25	0.89±0.01	1.71	3.11
362[d]	764[d]	1.31±0.22	0.65±0.032	2.64±0.14	1.23±0.07	2.01	2.15
					Average =	1.85±0.17	2.35±0.37

[a]Yields are shown only for those samples which are believed to have been within the beam range and were therefore irradiated and are listed in order by position in the sample packet. Please consult Figures S1 and S2 for yields of post Bragg peak samples.
[b]Absolute error in dose and LET for most samples is estimated to be ±15 %. All doses were calculated for this packet with the assumptions that the ion range was 5.20 mm, with beam energy 1.14 GeV. The ion range cannot be precisely measured in this experiment.
[c]All yields are expressed as lesions/10^6 DNA bases. Uncertainties are standard deviations.
[d]Dose and LET in the last sample irradiated are poorly characterized and subject to very large uncertainties, since they both depend on the location of the Bragg peak in the sample, i.e., the range of the ion, and this can be only approximately determined in this experiment.

Table S2. Yields of 8,5′-cyclopurine-2′-deoxynucleosides in DNA γ-irradiated at room temperature in aqueous solution or in hydrated solid state.

Dose	State	*R*-cdA[a]	*S*-cdA[a]	*R*-cdG[a]	*S*-cdG[a]	*R*/S (cdA)	*R*/S (cdG)
10 Gy	Aqueous	3.54±0.04	3.96±0.26	2.77±0.03	3.82±0.12	0.90	0.73
20 kGy	Hydrated	0.138±0.006	0.066±0.004	0.157±0.010	0.030±0.002	2.10	5.30

[a]All yields are expressed as lesions/10^6 bases. Uncertainties are standard deviations.

Table S3. Yields of DNA base lesions in Ne-22 irradiated DNA[a]

Dose[b] (kGy)	LET[b] (keV/µm)	5-OH-Cyt[c]	ThyGly[c]	FapyAde[c]	8-OH-Ade[c,d]	FapyGua[c]	8-OH-Gua[c,d]	5,6-diHThy[c]	5,6-diHCyt[c]
62.8	107	8.12±0.53	7.43±2.00	10.64±0.63	6.65±0.43	6.65±0.43	127.05±4.69	2.63±0.13	23.99±1.86
66.0	109	8.88±0.19	9.31±0.15	12.40±0.14	6.87±0.41	30.98±4.22	126.64±13.85	2.74±0.19	23.16±2.18
69.5	163	8.26±0.56	12.02± 1.77	10.69±0.62	4.87±0.54	36.72±2.71	115.37±14.09	4.13±0.26	29.42±1.23
77.5	182	9.79±1.48	9.13±0.20	8.76±0.21	5.01±0.21	34.11±1.85	104.84±12.10	4.26±0.20	33.00±1.62
81.7	151	17.35±1.77	18.34±2.39	14.98±0.69	7.68±0.45	37.90±3.41	136.43±7.12	3.99±0.11	33.32±1.55
93.0	171	26.01±1.94	25.07±2.17	21.69±1.43	10.41±1.73	56.94±3.69	190.57±27.58	4.65±0.17	32.26±1.00
102	221	23.73±1.07	22.75±4.28	19.33±1.83	11.04±1.31	49.30±2.62	134.39±8.01	5.65±0.35	39.88±1.44
120	261	24.56±1.67	21.52±1.07	20.85±0.68	12.03±0.18	47.55±1.46	143.09±6.16	7.35±0.24	50.31±1.17
151	327	36.44±0.51	29.80±0.79	26.21±0.24	13.85±0.78	64.90±0.20	170.21±12.41	7.77±0.17	52.06±1.71
226	472	24.68±2.91	8.88±0.47	12.28±0.36	7.59±0.31	41.35±4.73	57.82±24.97	7.44±0.22	53.81±1.56
362[e]	764[e]	26.59±2.63	8.47±0.58	13.20±1.12	7.80±0.56	45.01±0.62	77.40±7.67	5.93±0.14	35.28±2.50

[a]Yields are shown only for those samples that are within the beam range and were therefore irradiated and are listed in order by position in the sample packet. Please consult Figure 10 for yields of post Bragg peak samples.

[b]Absolute error in dose and LET for most samples is estimated to be ±15%. All doses were calculated for this packet with the assumption that the ion range was 5.20 mm, with beam energy 1.14 GeV. The ion range cannot be precisely measured in this experiment.

[c]All yields are expressed as lesions/10^6 bases. Uncertainties are standard deviations.

[d]8-OH Ade and 8-OH-Gua were measured as their 2'-deoxynucleosides, i.e., 8-OH-dA and 8-OH-dG, respectively.

[e]Dose and LET in the last sample irradiated are poorly characterized and subject to very large uncertainties, since they both depend on the location of the Bragg peak in the sample, i.e. the range of the ion, and this can be only approximately determined in this experiment.

Table S4. Yields of DNA base lesions in γ-irradiated at room temperature in aqueous solution or in hydrated solid state.[a]

Dose	State	5-OH-Cyt	ThyGly	FapyAde	8-OH-Ade[b]	FapyGua	8-OH-Gua[b]
10 Gy	Aqueous	30.91±5.52	32.18±2.07	20.30±1.89	17.14±2.63	100.3±1.9	298.0±17.2
20 kGy	Hydrated	2.30±0.19	10.73±0.54	26.25±0.96	31.76±0.55	74.50±2.20	290.0±8.3

[a]All yields are expressed as lesions/10^6 bases. Uncertainties are standard deviations.
[b]8-OH Ade and 8-OH-Gua were measured as their 2'-deoxynucleosides, i.e., 8-OH-dA and 8-OH-dG, respectively.

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