Radiation Damage to DNA: From Initial Ionization Events to Final Damage Products

Samuel Ward, Amitava Adhikary & Michael D. Sevilla

Department of Chemistry, Oakland University, Rochester, MI, 48309

Purpose: Ionization of cellular DNA leads to a variety of electron and hole transfer processes that result in strand breaks, damaged bases or, in the case of the C5'-radical (C5'•), cyclization to form 8,5'-cyclopurine-2'-deoxynucleosides which can be difficult to repair. As the linear energy transfer (LET) of the radiation changes, so too do the radicals formed, subsequent chemistry and consequent final damage products and the difficulty of repair. According to track structure models,[1] in the high LET core of an ion-beam, ionization events are so densely clustered that charged (or, ion) radicals recombine at high rates driven by coulombic interactions even at 77 K while neutral radicals are more likely to escape recombination. Hence, in DNA, along the core of the ion-beam, neutral sugar radicals continue to accrue with more radiation while the yields of base radicals, which are either charged or come from charged precursors, quickly taper off. In the low LET penumbra, however, ionization events are sparsely distributed and both charged and neutral radicals can be found at 77 K (for a detailed discussion of the chemistry see "Results" here). Neutral sugar radicals are the precursors to strand breaks in DNA which lead to ionizing radiation-induced cellular death, mutations and aging.[2] The extent to which strand breakage and other base and sugar damage has occurred can be monitored via quantification of the final products. This work tests the hypothesis that LET, and, therefore, recombination at the Bragg peak is so high that maximum damage due to heavy ion-beam irradiation actually occurs just before the Bragg peak by quantifying radicals trapped at 77 K with electron paramagnetic resonance (EPR) spectroscopy and quantifying final base damage products with liquid and gas chromatography mass spectrometry (LC-MS/MS and GC-MS/MS) along the path of the beam in collaboration with Dr. Miral Dizdaroglu (NIST).[1]

Methods: D_2O (99.9 atom % D, Aldrich Chemical), *t*-butanol (Sigma Chemical), Salmon testes DNA (Type III, 42.7% G-C, Sigma Aldrich), human NTHL1 protein (Dr. Susan Wallace, University of Vermont) and human OGG1 protein (Dr. R. Stephen Lloyd, Health and Science University) were used as received.

A Varian Century Series X-band EPR spectrometer with an E-4531 dual cavity, 9 in. magnet and 200 mW klystron (9.3 GHz) was used for EPR employing Fremy's salt for field calibration.

LC-MS/MS was performed using a Thermo TSQ Altis Triple Stage Quadrupole MS/MS system with a Vanquish Flex Quaternary UHPLC LC-MS front-end system equipped with a diode array detector system (Thermo Fisher Scientific) and a Zorbax SB-Aq LC column (Agilent Technologies) with an Agilent Eclipse XDB-C8 guard column). For sample preparation and instrumental parameters, see the supporting information here.[1]

An in-house generated program was used to deconvolute irradiated DNA spectra and SRIM was used in conjunction with EPR, LC-MS/MS and GC-MS/MS results to determine Bragg peak location.

Ion-beam irradiation was done at the National Superconducting Cyclotron Laboratory at Michigan State University at 77K.

Results:

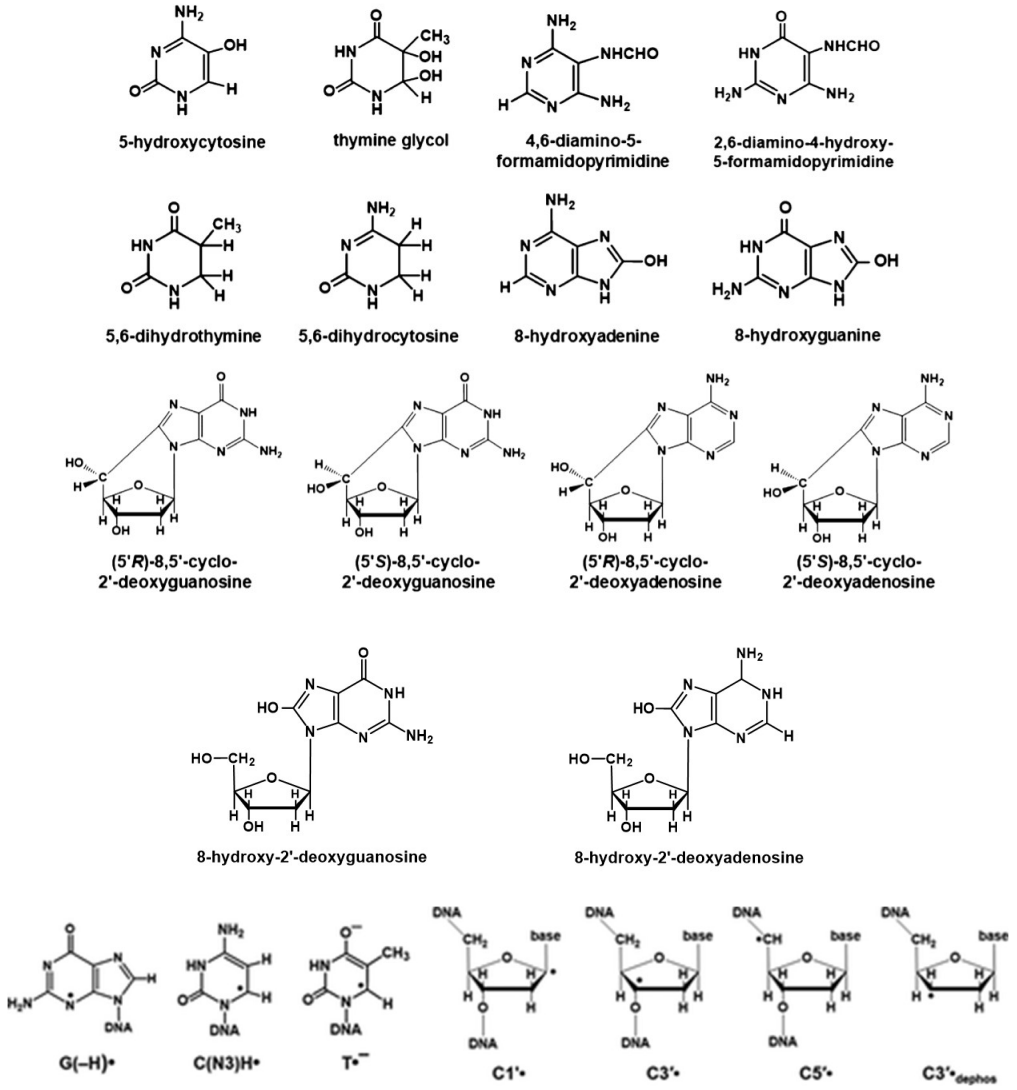


Figure 1. Radicals and structures considered in this work. All figures adapted from here.[1]

Figure 1 shows the base and sugar radicals present in irradiated DNA at 77 K and final DNA damage products quantified with LC-MS/MS and GC-MS/MS with isotope dilution. Figure 2 shows the total yields of radicals trapped at 77 K along a "packet" of DNA samples. The fifth sample along the beam track was only partially irradiated, placing the Bragg peak at approximately 5.2 mm into the packet. Figure 3 shows the dose response curve obtained after ^{22}Ne ion-beam irradiation; note that yield of charged radicals quickly tapers off while the yield of neutral radicals continues to increase with increased dose. Figure 4 shows the yields of damaged base products along the track in the same samples at room temperature; the yields of base damage products are highest just before the Bragg peak location.

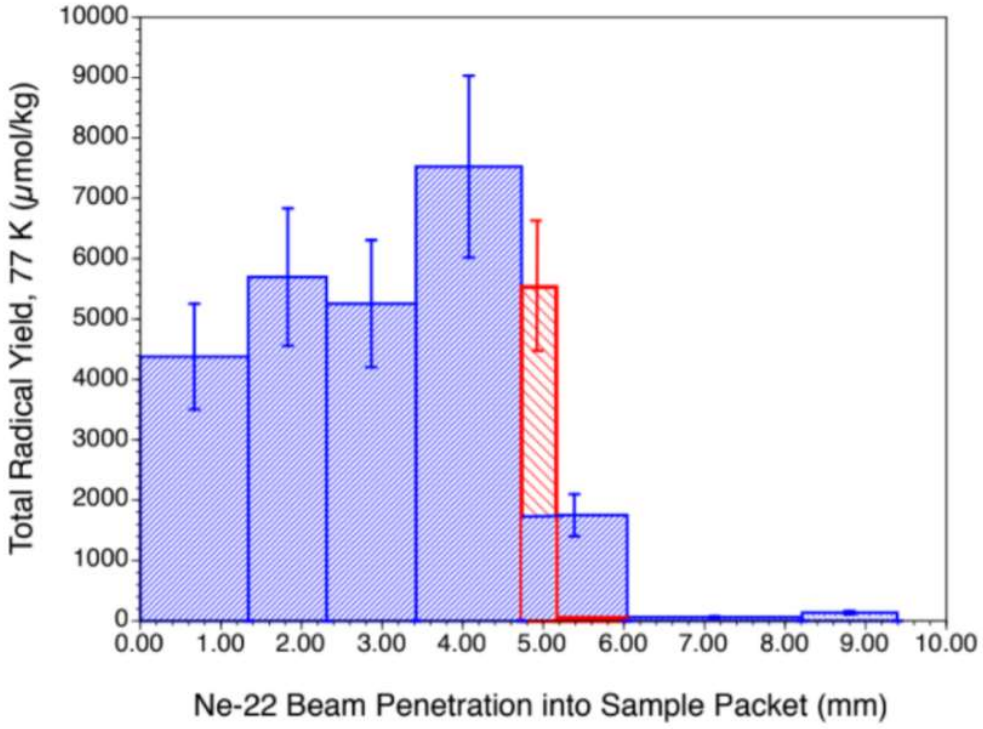


Figure 2. Total yields of radicals trapped at 77K in hydrated (Γ=12±3 H_2O/nucleotide) salmon testes DNA after irradiation with a ^{22}Ne ion-beam. Uncertainties represent the typical 20% variation seen in measuring radical yields.

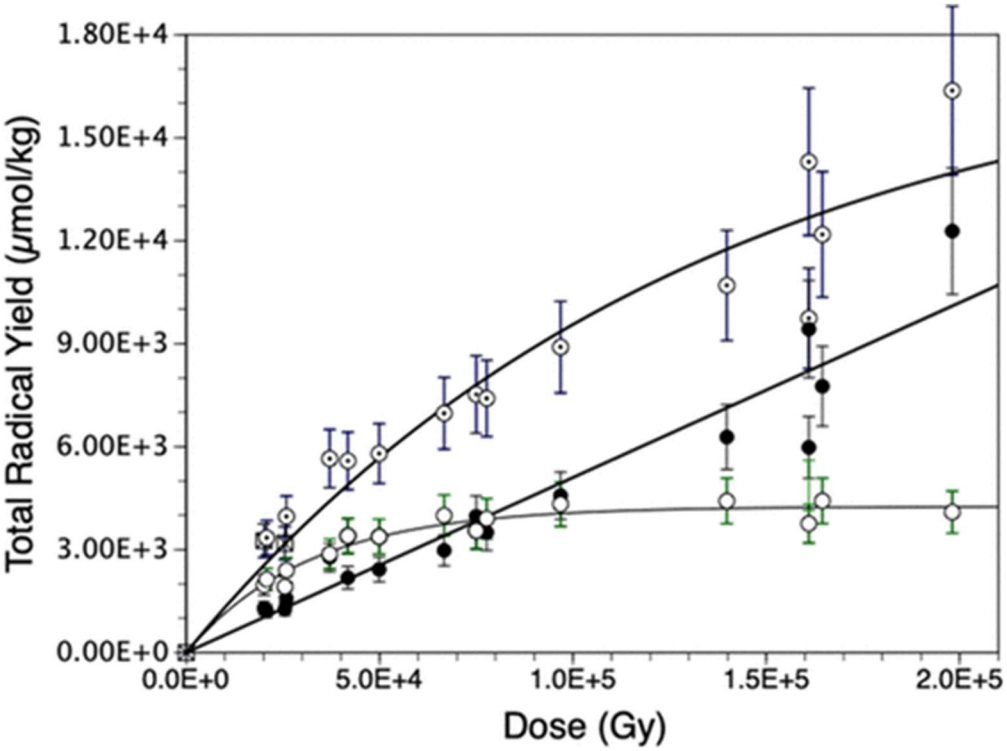
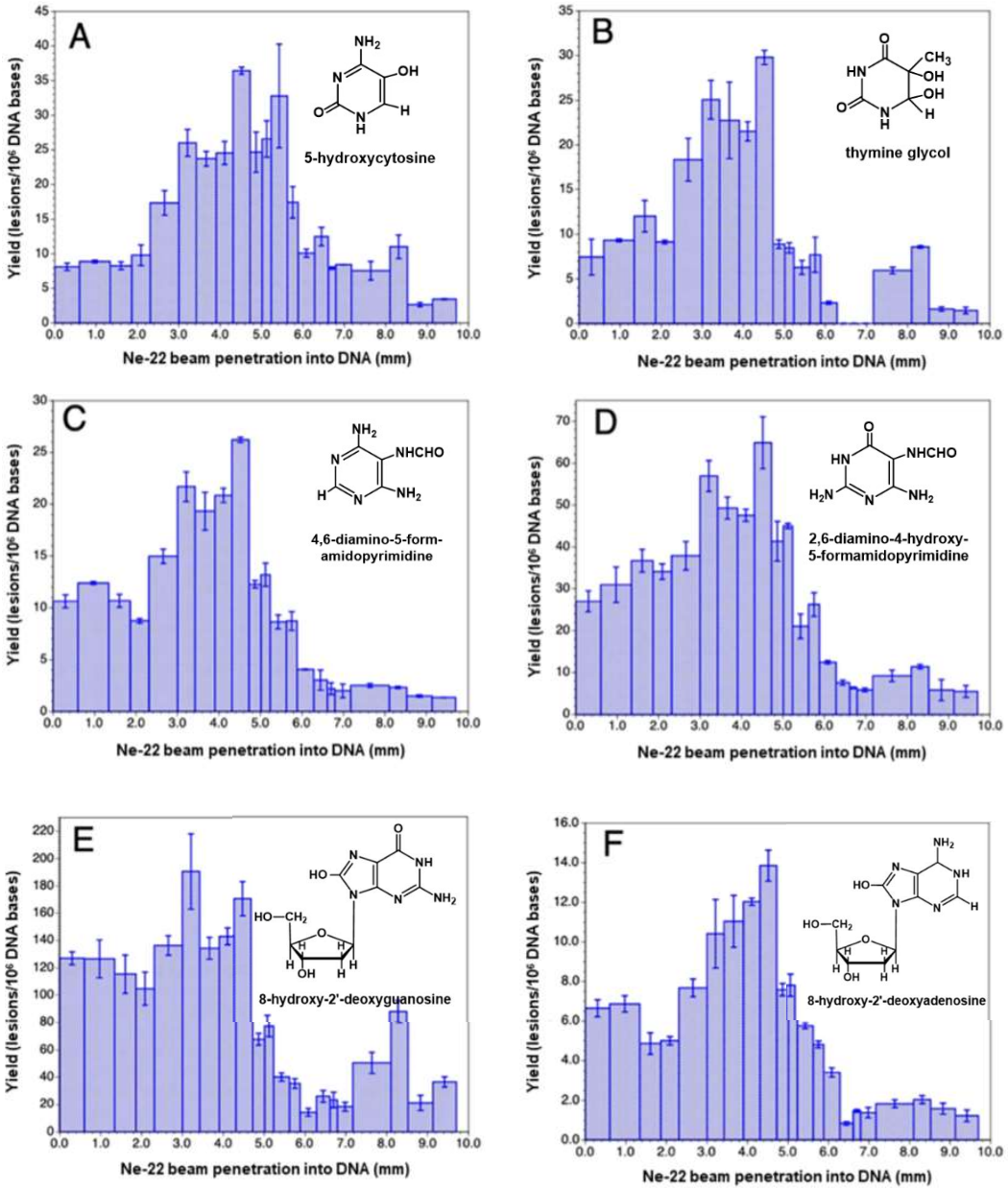


Figure 3. Dose response of trapped radicals. The upper curve (dotted circles) represents total radical yield, black circles indicate sugar radical yields, and open circles show the sum of base radicals. Uncertainties are estimates based on typical spin-counting variabilities.



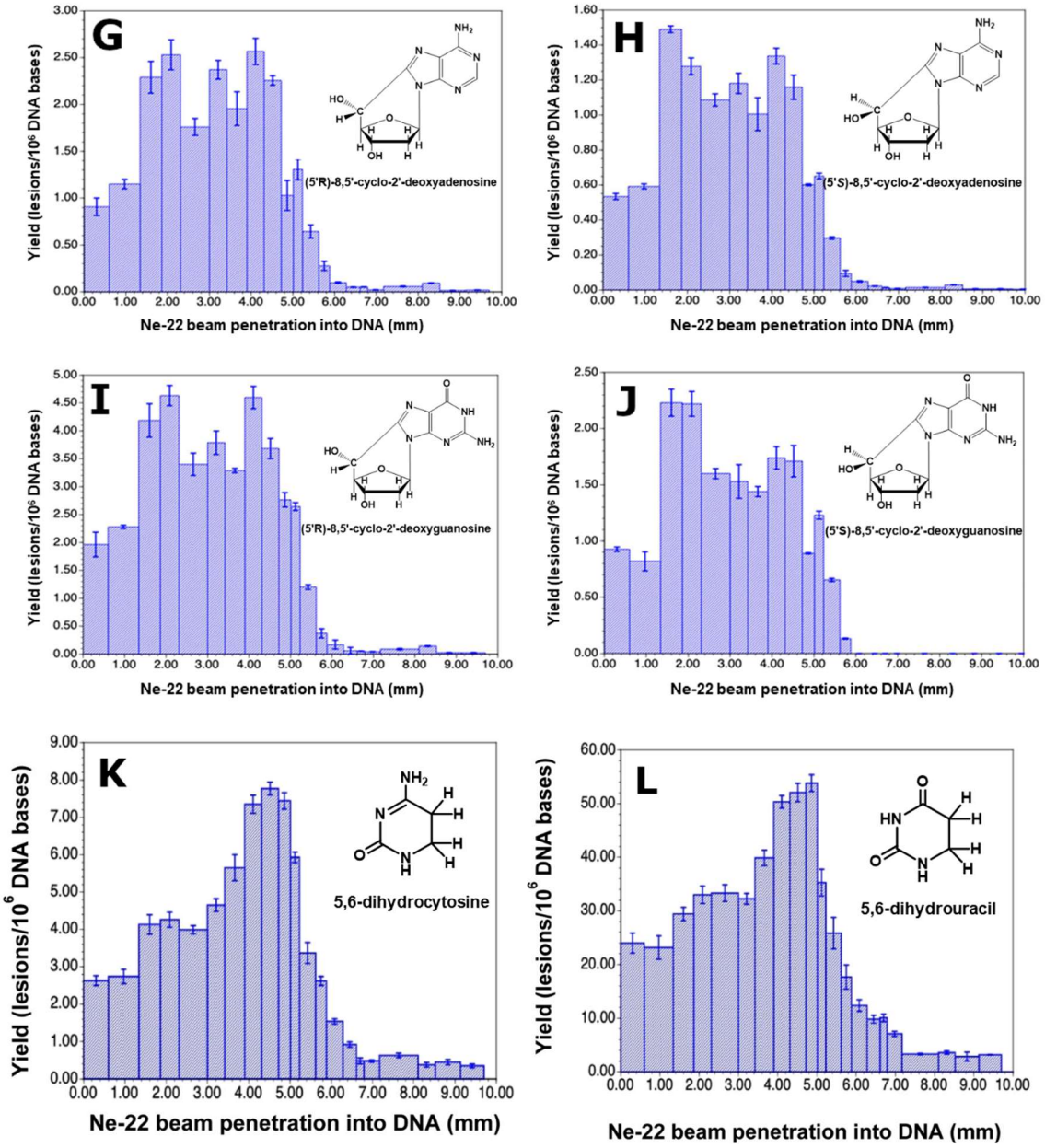


Figure 4. Yields of final damage products. Uncertainties are standard deviations.

Conclusions: Quantifying DNA-radicals trapped at 77 K after ^{22}Ne ion-beam irradiation has provided further proof that in the core of an ion-beam, recombinations occur at a much greater extent with the charged base radicals compared to neutral sugar radicals. However, at the location of highest LET, the Bragg peak, recombination is so likely that the location of maximum damage done to DNA by a heavy ion is just before the Bragg peak as evidenced by quantification of the radicals and the final DNA damage products with LC-MS/MS and GC-MS/MS.

Relevance to CIRMS: Understanding how ionizing radiation interacts with DNA is of utmost importance to designing new radiation therapies, especially ones involving radiosensitizers and/or radioprotectors. Combining both radical measurements (EPR) with product measurements using mass spectrometry (GC-

MS/MS and LC-MS/MS) to map the damage done to DNA from radicals at low temperature to products at room temperature in the same sample is also a novel combination of methods that helps add to that understanding. I plan to stay active in this area of research after I graduate. CIRMS gives me a unique opportunity to stay up to date on research involving types of ionizing radiation and measurements with its conferences and the discussions with experts I find there.

References:

1. Kant, M. *et al.,* "Ne-22 Ion-Beam Radiation Damage to DNA: From Initial Free Radical Formation to Resulting DNA-Base Damage," *ACS Omega* 6, 16600-16611 (2021).
2. Friedberg, E. C. *et al.,* "DNA Repair and Mutagenesis," *ASM Press, Washington DC* (2005).