Investigating the Impact of Metalloporphyrins on DNA Damage During Electron Beam Irradiation

Ani Avetisyan

Molecular Physics Department,
Yerevan State University

Introduction

The interaction between ionizing radiation and biological systems has been extensively studied, providing insights into the DNA damage caused by X-rays, gamma rays, and energetic particles. However, the interaction between ultrashort electron beams and DNA molecules presents a unique set of challenges and opportunities.
These are topical problems of modern radiobiology and medicine, and they directly related to radiotherapy.

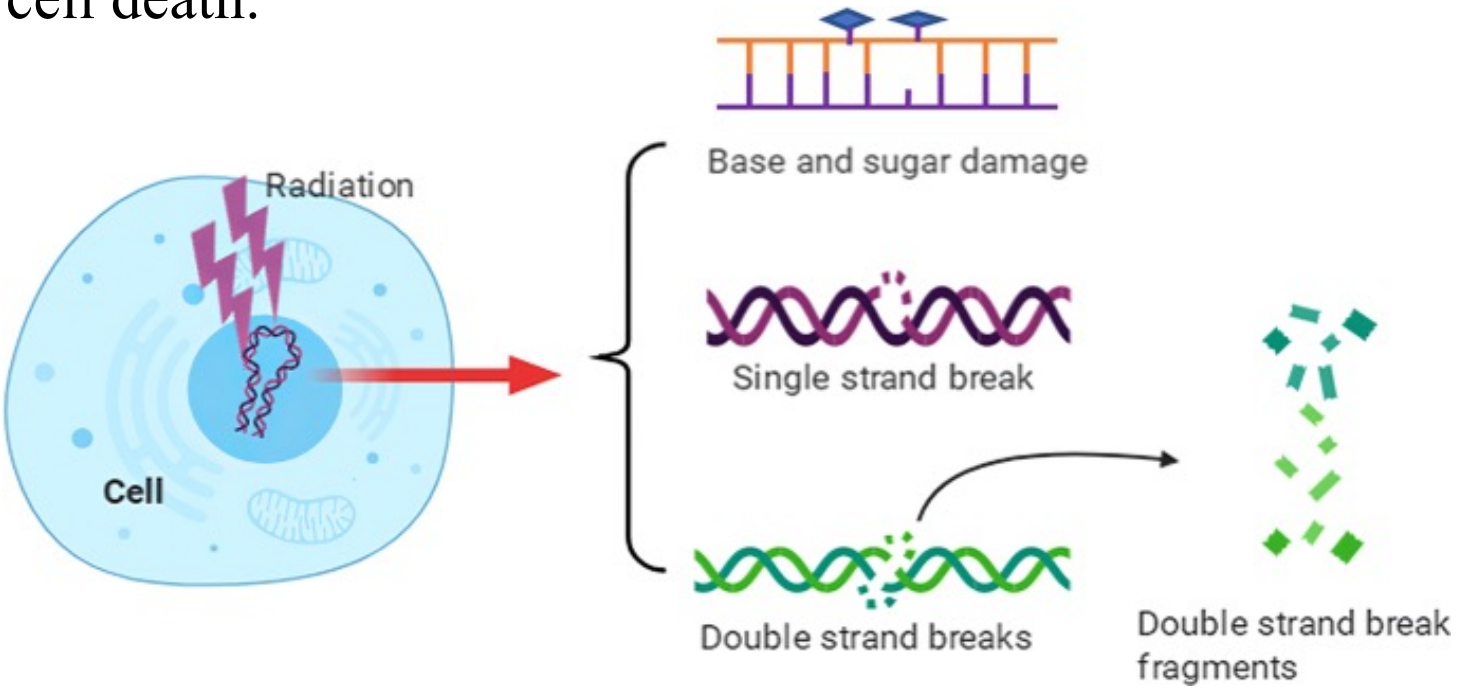
The effects of ultrashort electron beams on DNA integrity, structural alterations, and potential mutagenic outcomes have systematically been examined by multiple groups

What Is the Basis for Radiation Therapy?

Radiation therapy works by damaging the DNA of cells and destroys their ability to reproduce.

In general there are two types DNA damage makes to cell death.

Both normal and cancer cells can be affected by radiation, cancer cells have generally impaired ability to repair this damage, leading to cell death.

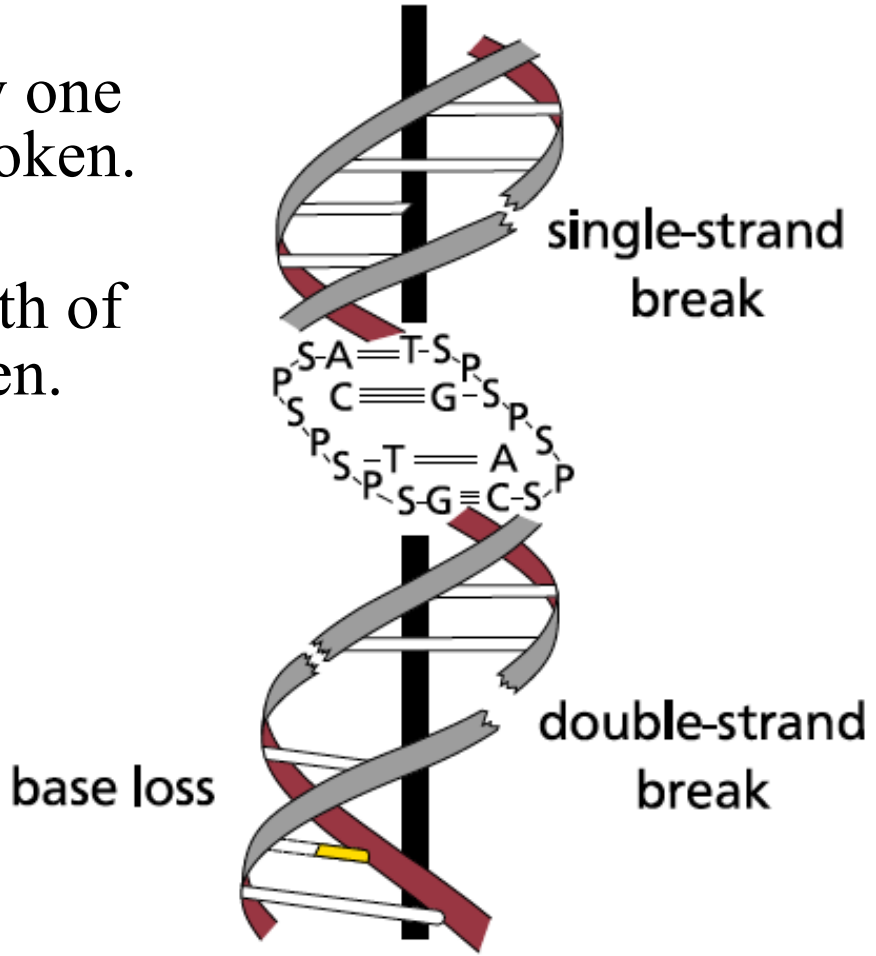


DNA damage

Two types of breaks in the sugar phosphate backbone can caused by ionizing radiation.

A single strand break occurs when only one of the sugar phosphate backbones is broken.

Double-strand breaks occurs when booth of the sugar phosphate backbones is broken.



Radiation therapy combination

In recent years, radiation therapy has been used in combination with other methods (chemotherapy, photodynamic therapy, etc.), which significantly increases the effectiveness of radiation therapy. Have been shown some compounds, such as cisplatin, which is widely used in chemotherapy, make radiation therapy more effective. So drug–radiotherapy combinations has clear potential to increase the effectiveness of radiotherapy and reduce side effects.

Interaction of porphyrins with DNA

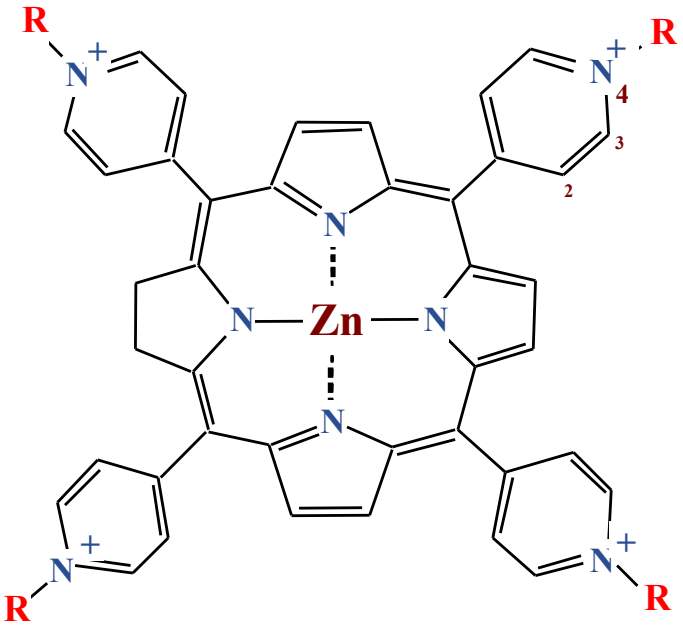
In recent years, in our laboratory we widely investigated the interaction of wiled class of biologicaly active combounds - cationic porphyrins with biopolymers, particularly with DNA Interaction of porphyrins and their derivatives with DNA has great interest due to their potential medical applications, for instance, as active compounds in fluorescence, radiological and magnetic resonance imaging of cancer detection, and as photosensitizers in photodynamic therapy of cancer.

The effect of ultrashort electron beams on DNA damage depend of dose was investigated from our group via spectroscopic, calorimetric methods:

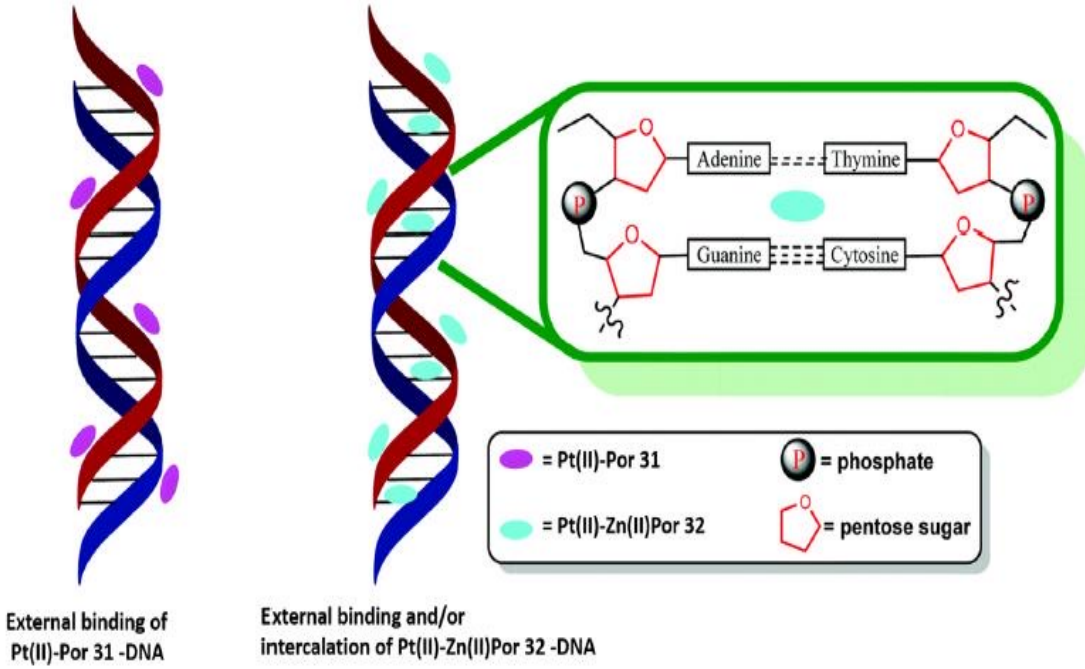
Schematic representation of the main types of porphyrin binding to DNA:

The porphyrins may associate with DNA in free binding modes

- intercalation
- external stacking interaction and external groove binding



R = CH_2-CH_2-OH TOEtPyP



External binding of Pt(II)-Por 31 -DNA

External binding and/or intercalation of Pt(II)-Zn(II)Por 32 -DNA

Main Idea

The goal of our investigations is to identify the structural changes that occur in the DNA molecule when irradiated with an electron beam, when the solution contains potentially antitumor compounds - porphyrins. These studies make it possible to determine the possible increase in the effect of radiation on DNA molecules. Also determine the optimal values of concentrations of compounds and radiation doses that can be used in radiation therapy.

Materials and Methods

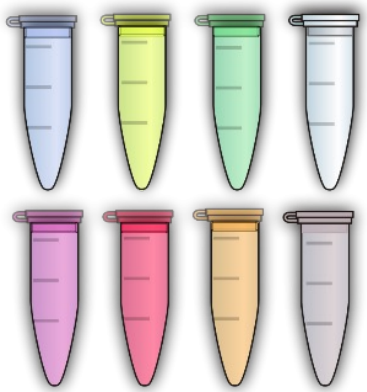
Ultra-pure DNA extracted from calf thymus which was characterized by low protein content (<0.1%), minimal RNA contamination (<0.2%), high molecular weight (> 30 MDa), and a GC content of 42%.

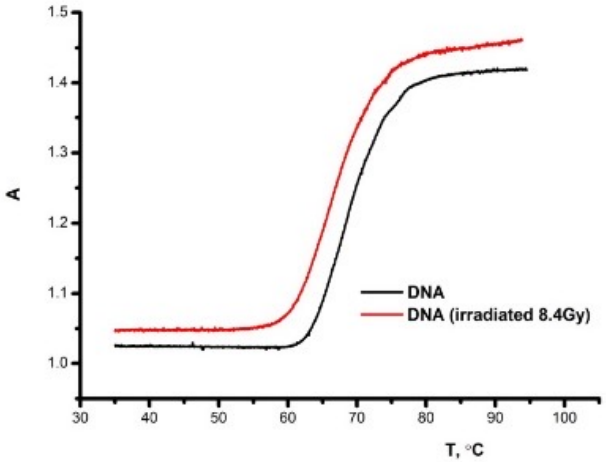
A stock solution of DNA was prepared in a 10^{-3} M NaCl solution at pH 7.0.

The concentration of DNA in base pairs was determined using an extinction coefficient $\varepsilon 260 = 1.31 \times 104\ M^{-1}\ cm^{-1}$.

DNA samples were irradiated using the AREAL (Advanced Research Electron Accelerator Laboratory) 2–5 MeV electron beam. AREAL utilizes high-frequency electromagnetic waves to accelerate electrons through a waveguide, making it a commonly employed treatment machine for external beam therapy. Currently the facility is able to provide ultra-short electron bunches with about 0.5 ps bunch length with a particle charge up to 800 pC.

Melting methods



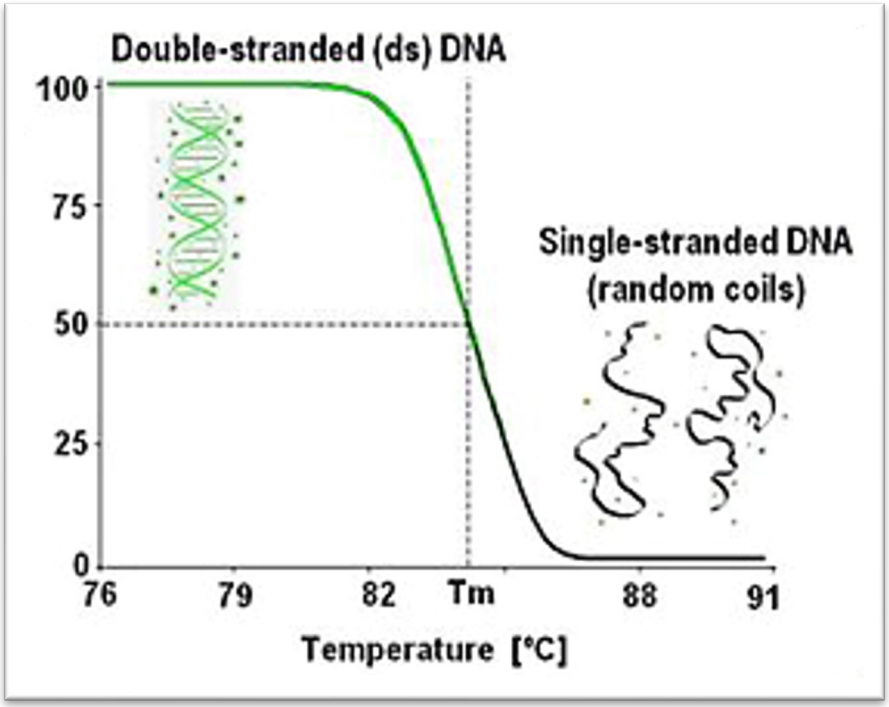


Melting curves, depicting the dependence of DNA absorbance(A) at 260 nm on temperature, were recorded using a Perkin Elmer Lambda 800 UV/VIS spectrophotometer with a heating rate of 1 °C/min. It is known, that the melting parameters (Tm and ΔT) of DNA is sensitive to the structure of double helix. All experimants were repeated at last three more time.

*A*min is the optical density at full helix, and
*A*max is the optical density at full coil,

Methodology

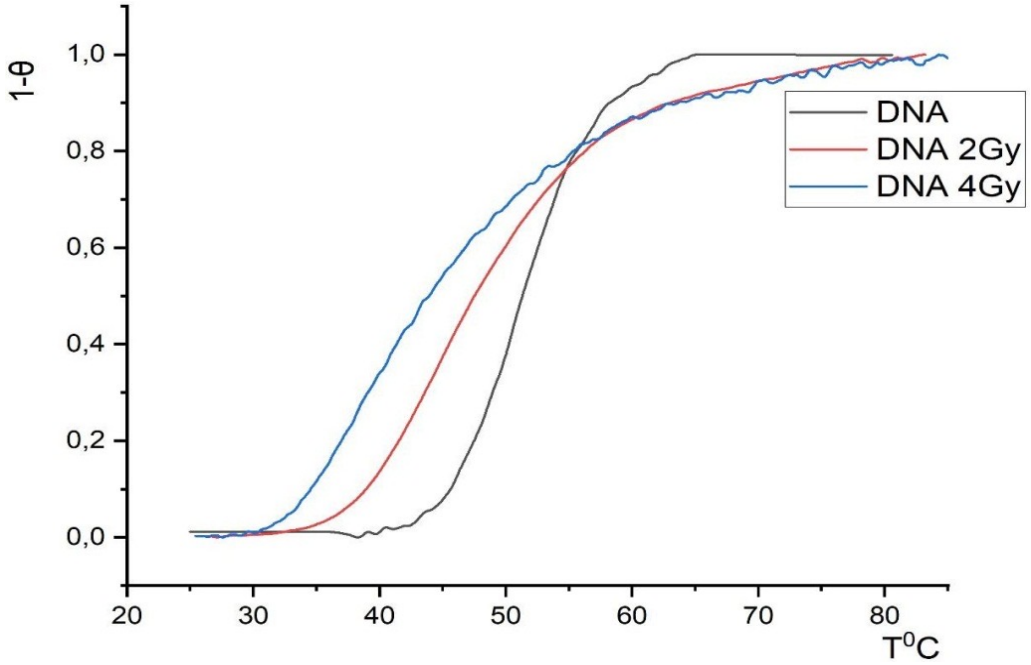
It is known, that the melting parameters (Tm and ΔT) of DNA is sensitive to the structure of double helix. Conclusion about the changes occurred in DNA can be made from melting parameters of the DNA ligand complexes. Therefore, it can be used as an indicator of strand breaks of DNA molecules after irradiation.



$$1 - q = (A - A_{min})/(A_{max} - A_{min})$$

q is the degree of helicity, 1 - q describes the number of monomers in the coil (melted) state.

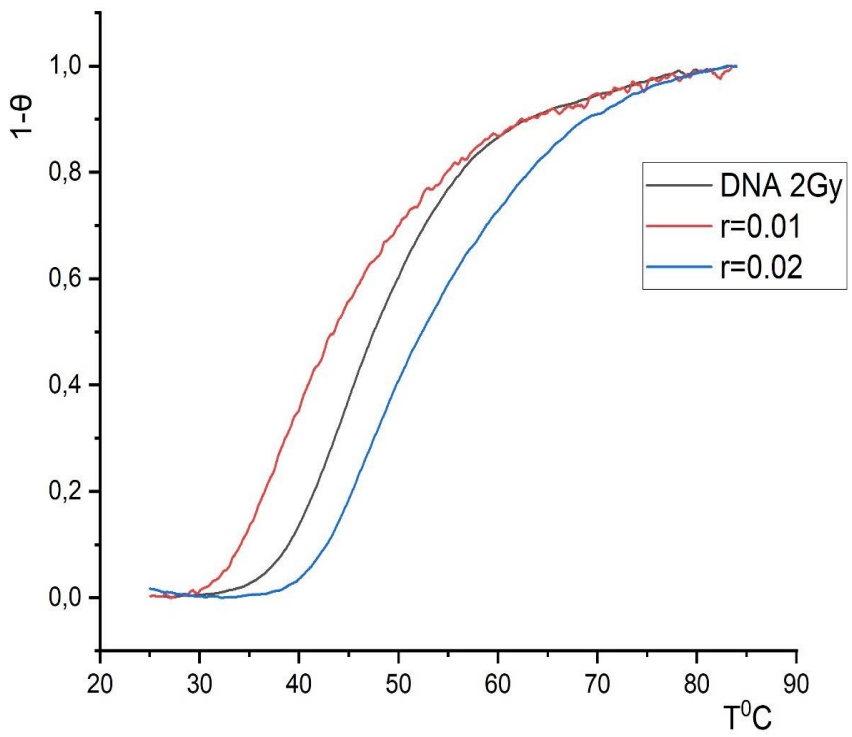
Results

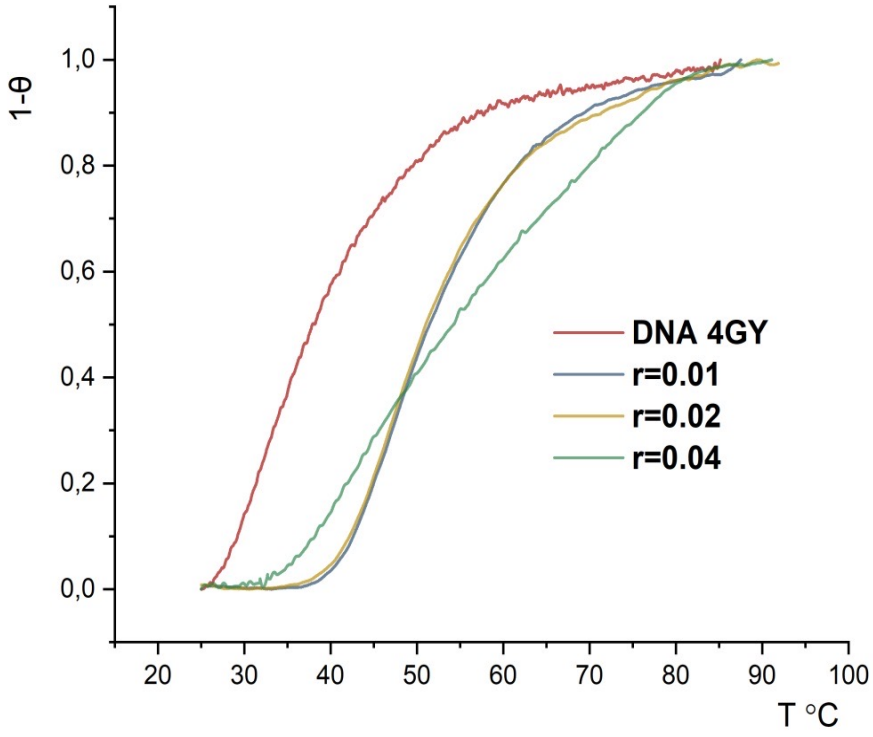


DNA	T_m (∘C)	ΔT (∘C)
DNA 0Gy	51.42	8.90
DNA 2Gy	47.56	16.76
DNA 4Gy	37.91	20.05

As can be seen from obtained data irradiation with electron beam leads to a decrease in the melting temperature, and this decrease increases depending on the dose of irradiation. These changes can be explained by the breaking hydrogen bonds in DNA.

Results





The normalized melting curves of DNA/ZnTOEPyP4 complexes at 2Gy and 4Gy
The relevant concentrations of DNA-porphyrin complexes equal 0.01; 0.02; 0.04 was used.

The melting parameters of DNA/ ZnTOEPyP4 complexes, at 2Gy and 4Gy irradiation dose, r = Cporph/CDNA

DNA/ZnTOETPyP4 $r=C_{porph}/C_{DNA}$	T_m (∘C)	ΔT (∘C)
r=0 (pure DNA) 2Gy	47.56	16.76
r=0.01	42.85	21.07
r=0.02	52.7	20.19

The small amount of ZnTOEPyP 4 porphyrin was enhanced of influence of radiation on DNA molecule

DNA/ZnTOETPyP4 $r=C_{porph}/C_{DNA}$	T_m (∘C)	ΔT (∘C)
r=0 (pure DNA) 4Gy	37.91	20.05
r=0.01	51.49	18.59
r=0.02	52.96	17.68
r=0.04	54.26	30.42

At a radiation of 4Gy dose, the melting temperature and melting interval are increases, It means that the 4Gy radiation causes more extensive DNA damage.

Summary

- It was shown that in the case of electron irradiation dose of 2Gy, hydrogen bonds of the DNA molecule are broken, and in the case of 4Gy, double strand breaks are likely to occur of the DNA chain.
- The small amount of ZnTOEPyP porphyrin was enhanced of influence of radiation on DNA molecule. This research is of paramount importance in radiation biology and medical applications and serves as a foundation for further investigations into the mechanisms underlying these intricate interactions.

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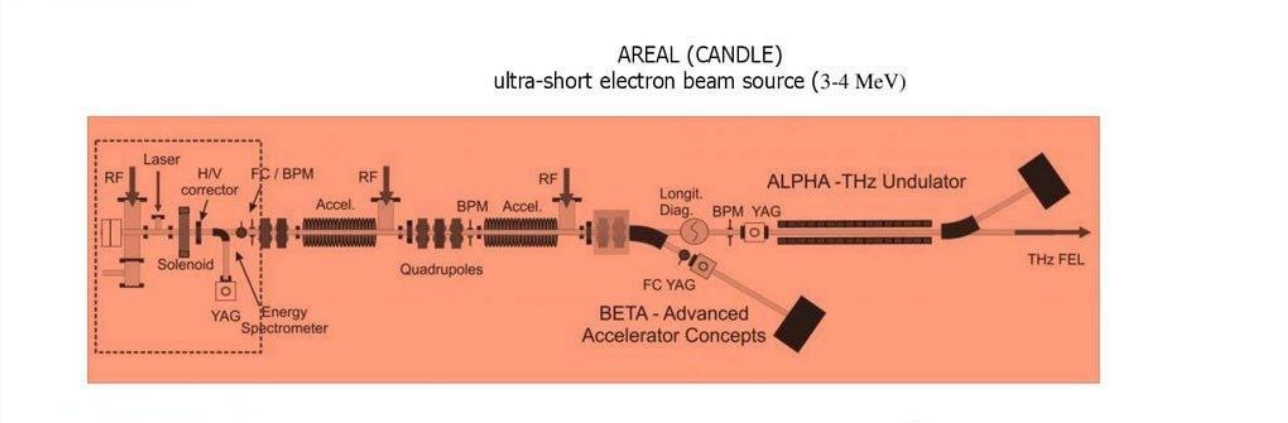
Thank you for attention!!!

5 MeV beam energy,
500 fs pulse duration and
2-50 Hz frequency.



The scheme of Advanced Research Electron Accelerator

$$D[Gy] = \frac{\varepsilon[ev] \times \Delta Q[c] \times R[Hz] \times T\,[s]}{m[kg]}$$

DNA samples were irradiated using the AREAL (Advanced Research Electron Accelerator Laboratory) 2–5 MeV electron beam. AREAL utilizes high-frequency electromagnetic waves to accelerate electrons through a waveguide, making it a commonly employed treatment machine for external beam therapy. Currently the facility is able to provide ultra-short electron bunches with about 0.5 ps bunch length with a particle charge up to 800 pC. For irradiation researches sub-picosecond long electron bunches with energies up to 5 MeV guarantee a short interaction time with the sample material, meanwhile delivering sufficient radiation dose due to bunch energy and hundreds of pico-Coulombs charge.