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CHUV

Impact of temporal structure of electron and proton and photon on G°(H2O2), G(H2O2), DNA damage and Zebrafish embryos

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Background

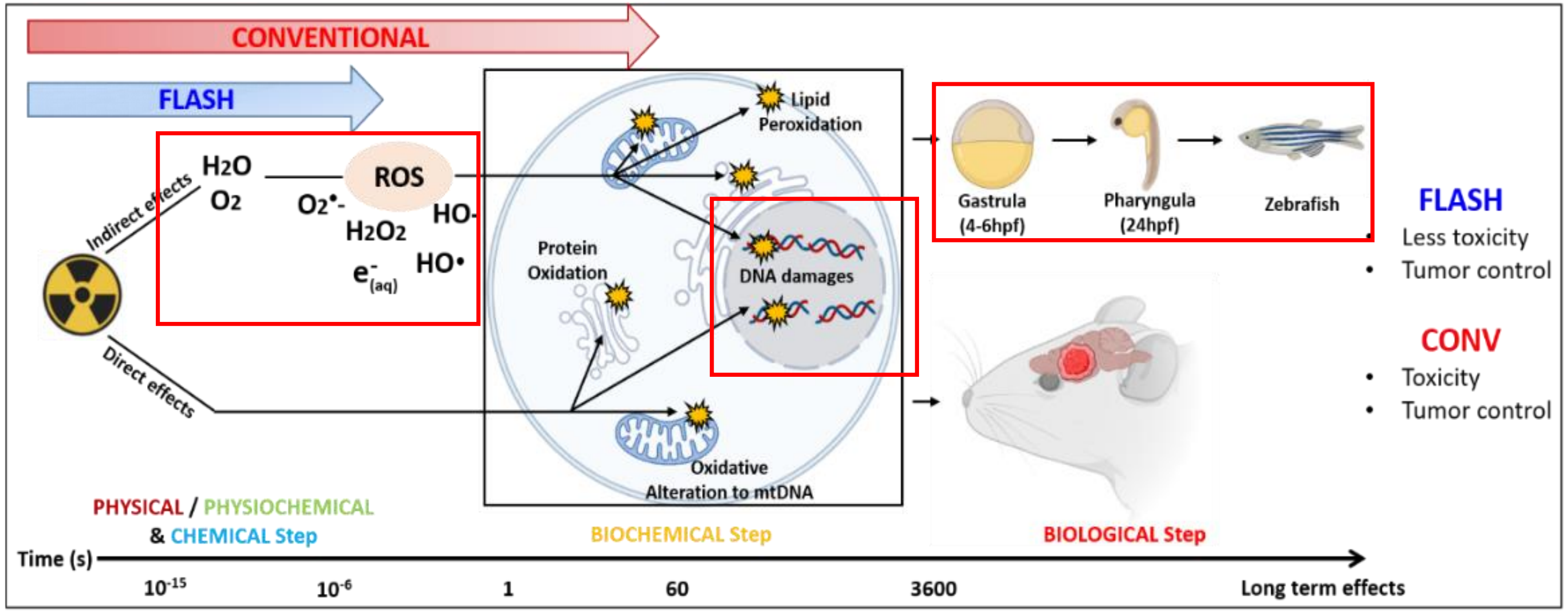
Ultra-high dose rate irradiation (UHDR-RT) vs Conventional irradiation (CONV-RT)

The FLASH biological effect: *In vivo* model Increase normal tissue tolerance and maintain tumor killing.

The biological FLASH effect was found with different particles with different temporal structure: Electron, proton and X-rays.

The FLASH effect might depend upon the early physico-chemcial events, biochemical events and biological outcomes

Aim

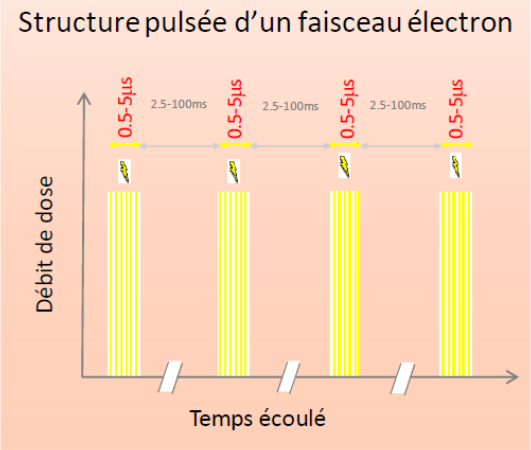


Water | Plasmids | Zebrafish embryos

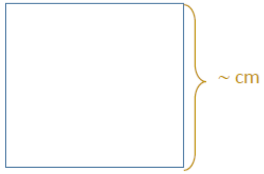
(Kacem et al, IJRB 2021)

Technology

Electron beam structure

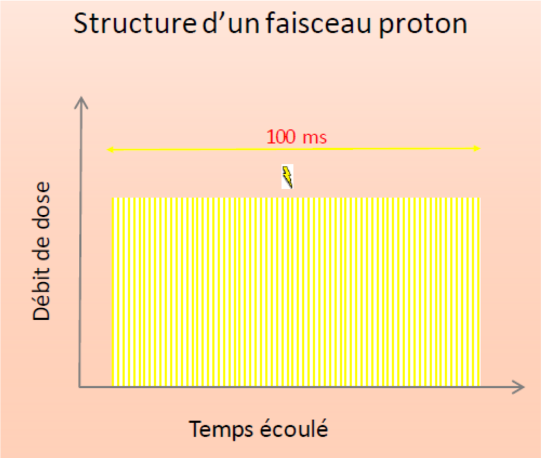


- 1 – 10 pulses
- Microstructure: 5000 bunches
- Pulse repetition frequency 10-250Hz

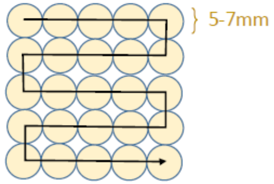


Beam	Electrons (e-RT6)	Transmission Protons (PSI)
Source	Accelerator	Cyclotron
Energy (MeV)	5.5	235
Beam structure	Pulsed	Quasi-Continuous
Conventional dose rate (Gy/s)	0.1	0.1-0.9
UHDR (Gy/s)	555 - 5.510^{6}	1260-1400

Proton beam structure



- 1 pulse
- Microstructure: 10^7 bunches
- Spot scanning (@1000Hz)



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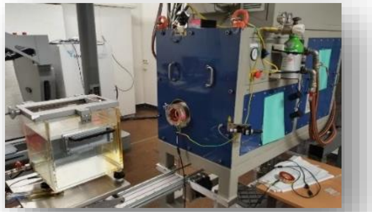

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Water radiolysis

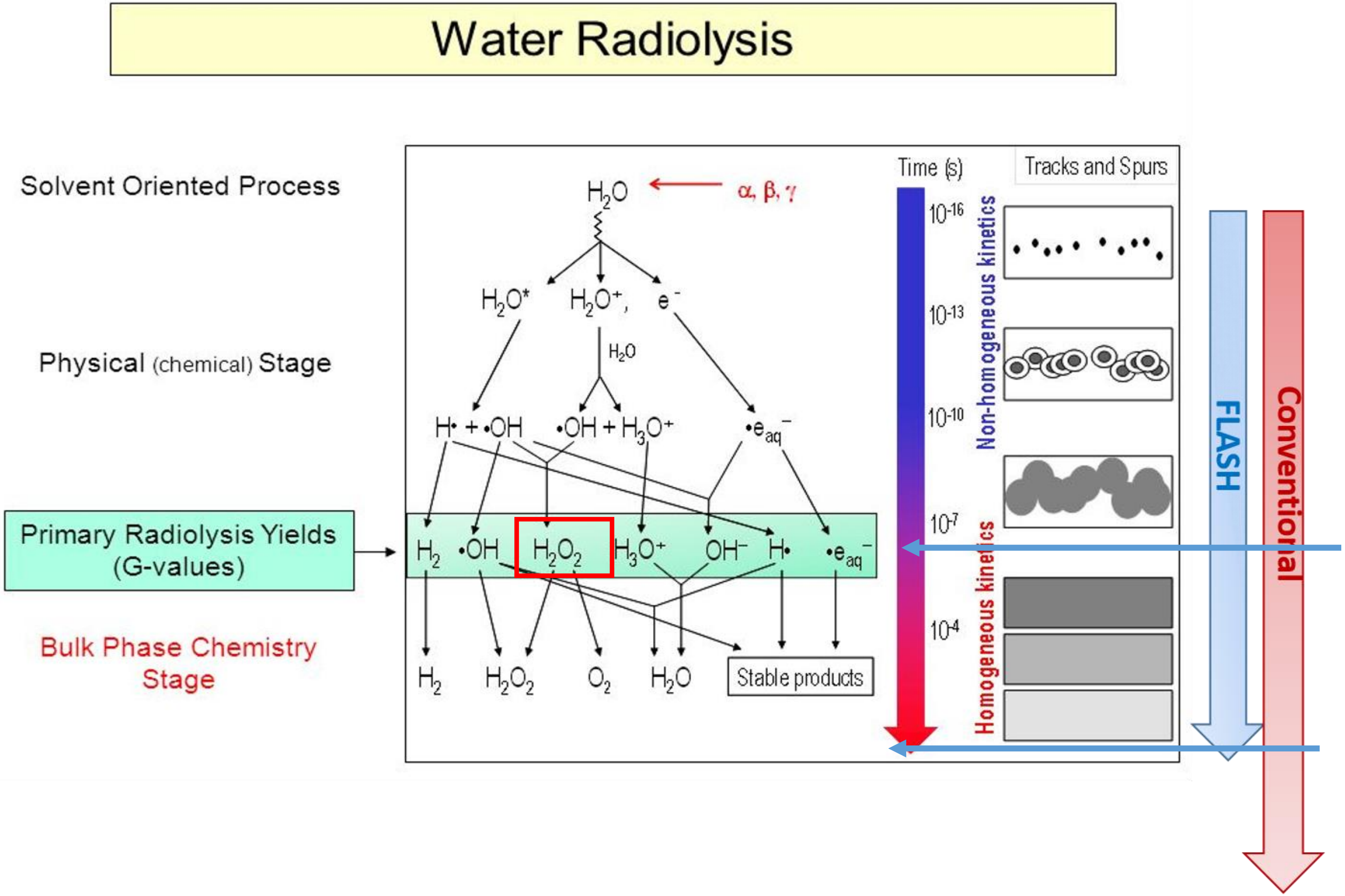
Water Radiolysis experiments

Radiolytic yield (G-value):

$$G(X) = \frac{\text{Number of species created (or destroyed)}}{\text{100 eV deposited energy}}$$

$$= \frac{\Delta[X](\frac{mol}{L})}{\Delta Dose(\frac{J}{kg}) \times \rho(\frac{kg}{L})}$$

Unit: (mol/J) or (molecules/100eV)



Initial chemistry measurements: 1 µs → start of homogeneous phase: initial yield: G°(H2O2)

Bulk chemistry measurements: min → Later time points/post diffusion yield: G(H2O2)

Experimental procedure

RT

Amplex Red Fluometric Assay

$G°(H_2O_2)$

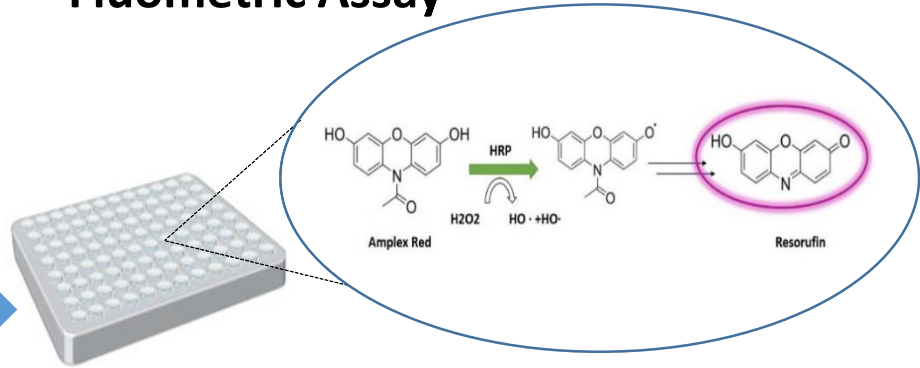
Milli-Q water + scavengers

(Biospherix hypoxia hood)

2mL Eppendorf tube at e-RT6

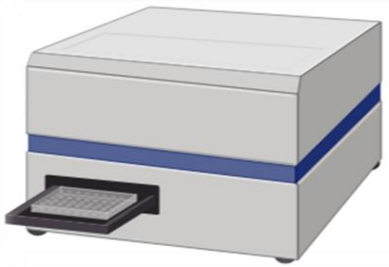
200µL PCR tube at PSI

225 kVp X-rays



30 min incubation in dark conditions

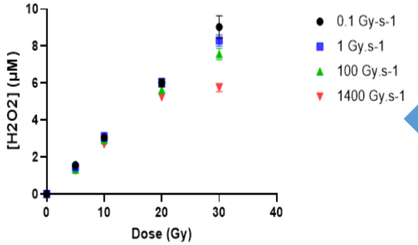
Fluorescence measurement using microplate reader



(Promega GloMax)

$G(H_2O_2)$

Milli-Q water



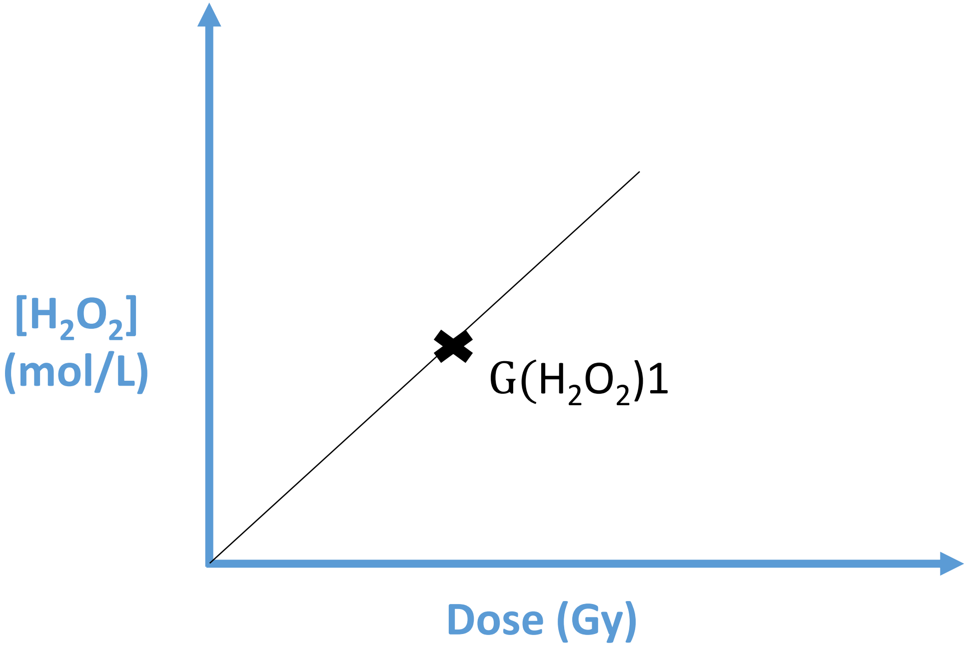
Savenging method to compute $G°(H_2O_2)$

$HO^\bullet + HO^\bullet \rightarrow H_2O_2$, $\quad k = 1.1 \times 10^{10}\ M^{-1}s^{-1}$

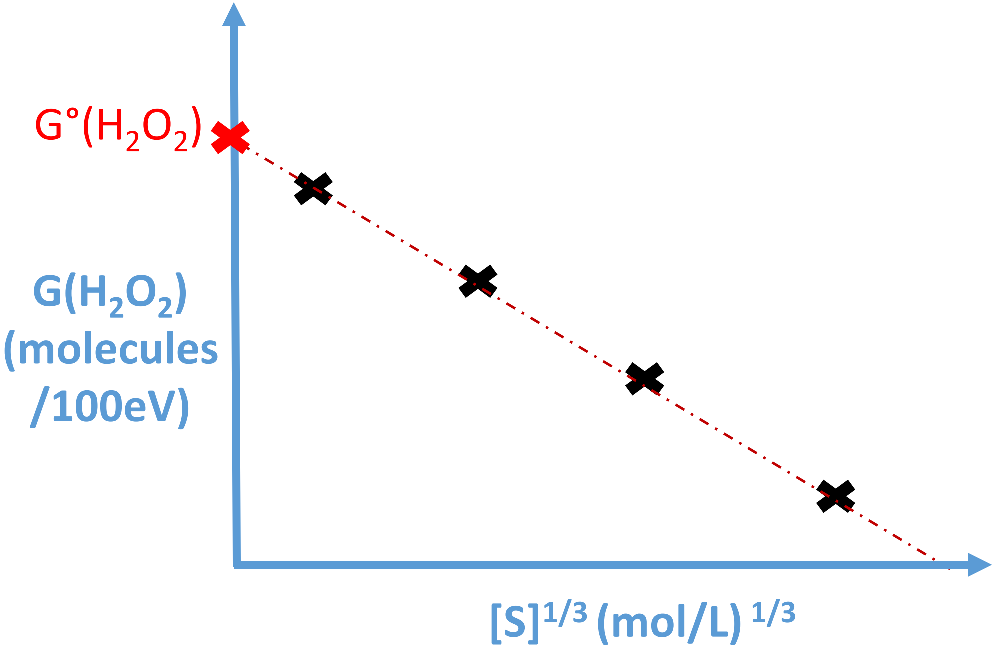
$HO^\bullet + S \rightarrow P$

↑ [S] ⟶ $t_S = \frac{1}{[S] \times k_S}$ ↓ ⟶ $HO^\bullet$ is scavenged at earlier time ⟶ $G(H_2O_2)$ ↓

Exemple: [S1]: $[H_2O_2] = G(H_2O_2)1 \times Dose$



$$G(H_2O_2) = G°(H_2O_2) - p \times \sqrt[3]{[S]}$$



(Sworski 1954)

Chemical system for scavenging method

Samples composition: N_2 saturated aqueous solutions (2% of using Hypoxia hood) containing various [$NaNO_2$] + 25mM [$NaNO_3$]

$NO_2^- + HO^\bullet \rightarrow NO^\bullet_2 + OH^-$, $k = 8 \times 10^9\ M^{-1}s^{-1}$

$HO^\bullet + HO^\bullet \rightarrow H_2O_2$, $k = 1.1 \times 10^{10}\ M^{-1}s^{-1}$

$H_2O_2 + HO^\bullet \rightarrow HO^\bullet_2 + H_2O$, $k = 2.7 \times 10^7\ M^{-1}s^{-1}$

[$NaNO_2$] = 10 μM
100 μM
1mM
10 mM
100mM

$NO_3^- + e^-_{(aq)} \rightarrow NO_3^{2-}$, $k = 9.7 \times 10^9\ M^{-1}s^{-1}$

$NO_3^- + H^\bullet \rightarrow HNO_3^-$, $k = 1.4 \times 10^6\ M^{-1}s^{-1}$

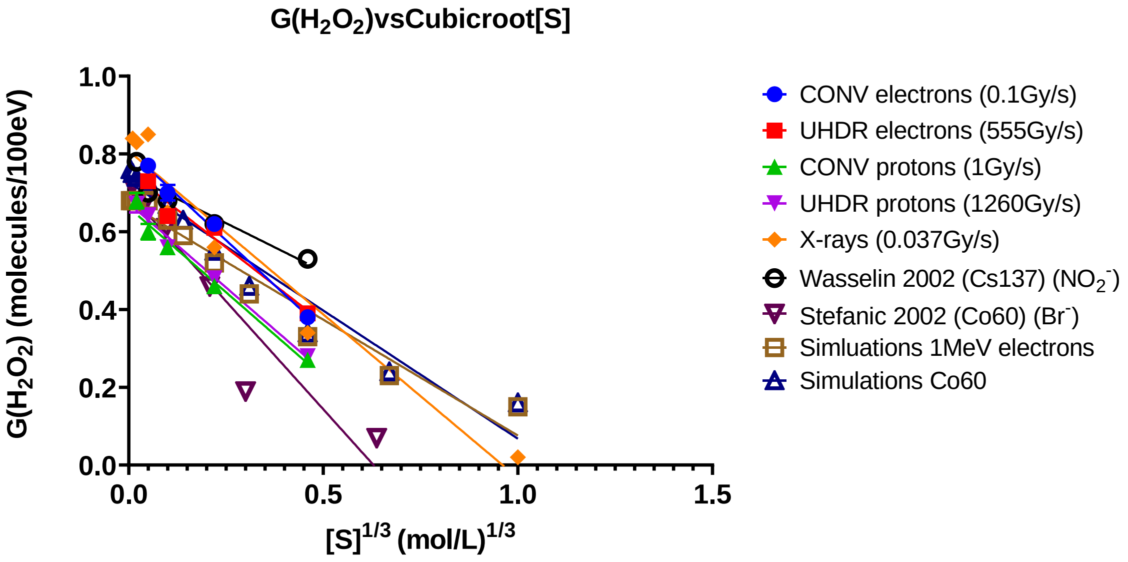
[$NaNO_3$] = 25 mM

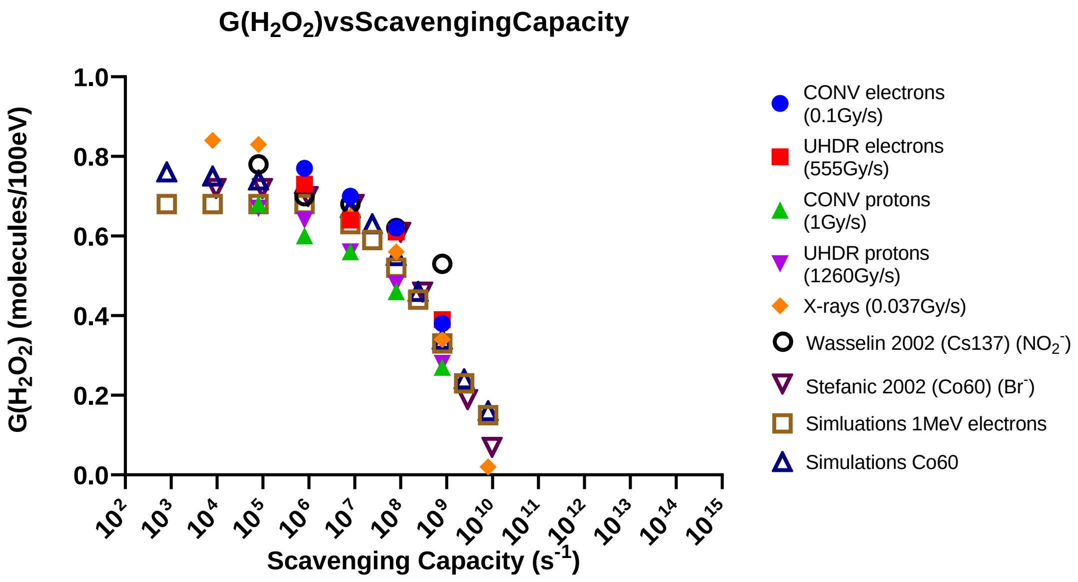
$H_2O_2 + e^-_{(aq)} \rightarrow HO^\bullet + OH^-$, $k = 1.1 \times 10^{10}\ M^{-1}s^{-1}$

$H_2O_2 + H^\bullet \rightarrow HO^\bullet + H_2O$, $k = 9 \times 10^7\ M^{-1}s^{-1}$

(Buxton 1988)

Results: G°(H2O2)



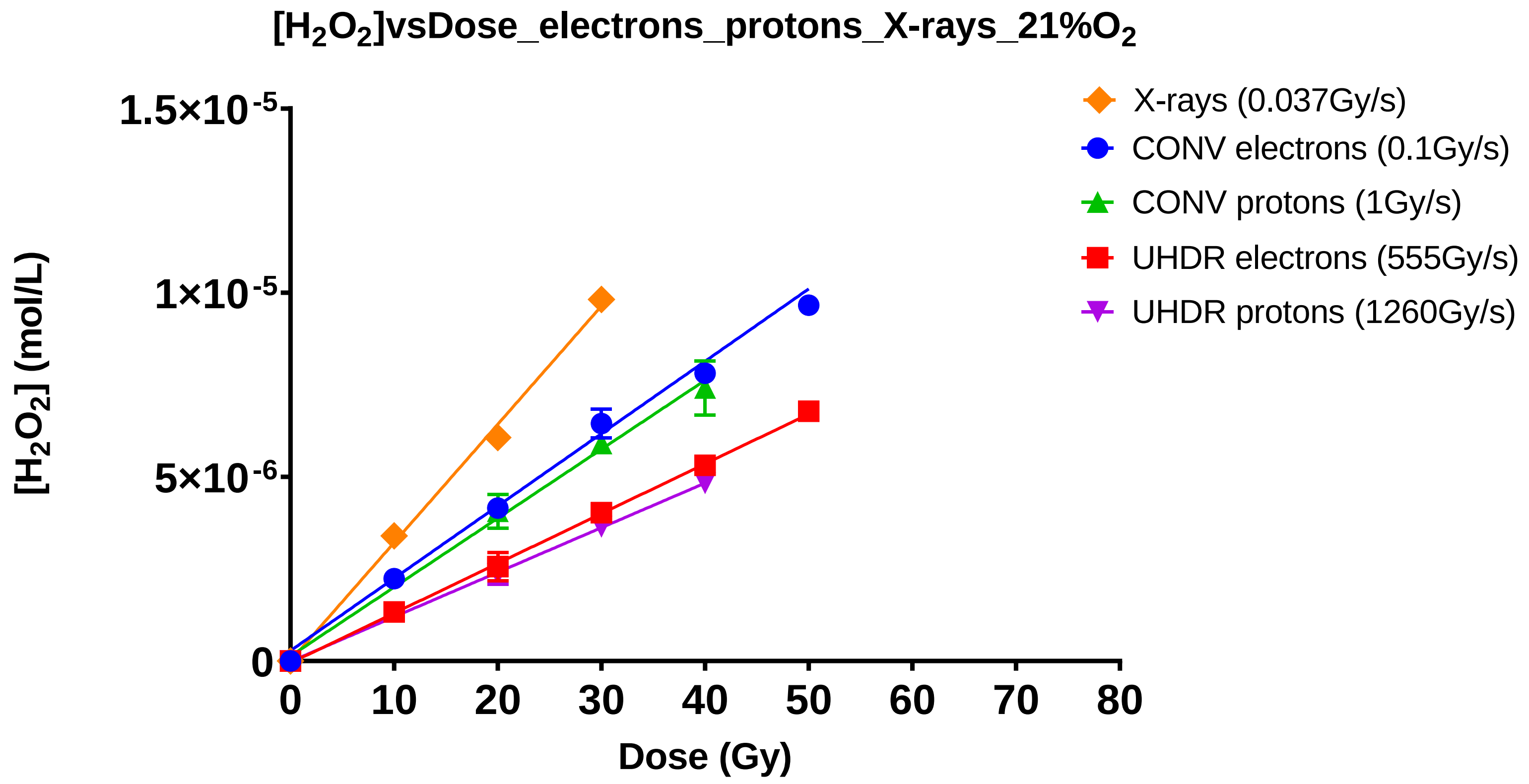


Hydrogen peroxide yield formed in aqueous solution in the presence of various scavenger concentrations (NO_2^- or Br^-) and constant NO_3^- from different beam sources, previous reported experimental results and simulations as a function of the cube root of the scavenger.

Hydrogen peroxide yields from different beams, previous reported data and simulations as a function of the scavenging capacity for $HO^{\bullet}$ radicals.

(Kacem et al, in preparation)

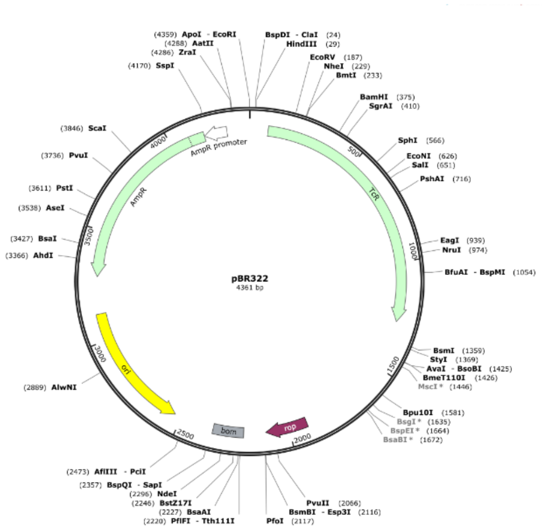
Results: G(H2O2)



DNA Damage

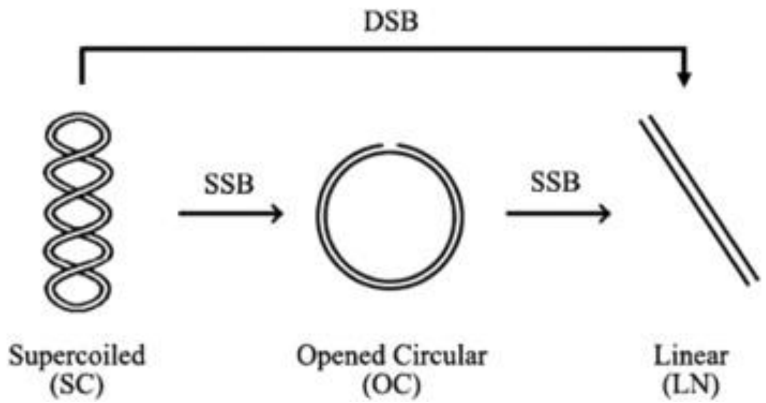
Experimental procedure

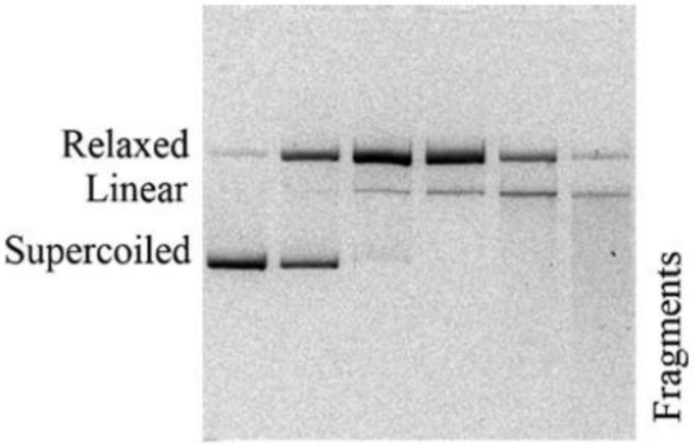
Plasmid DNA

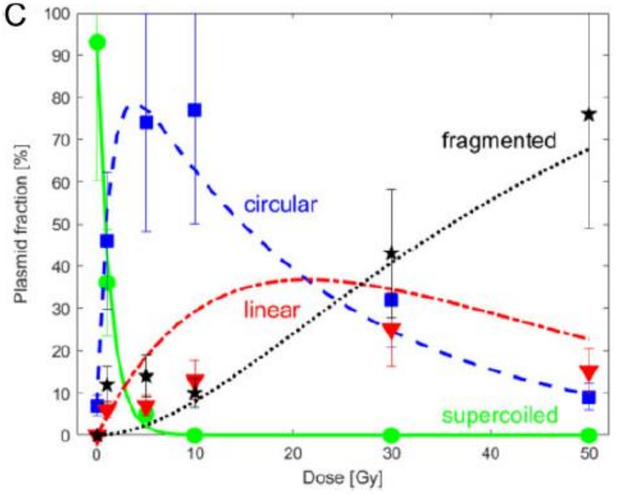


- Various spatial conformations
  - Linked to SSB and DSB
  - Electrophoretically separables
- Simple Model:

Absence of any repair process or interaction with other biomolecules



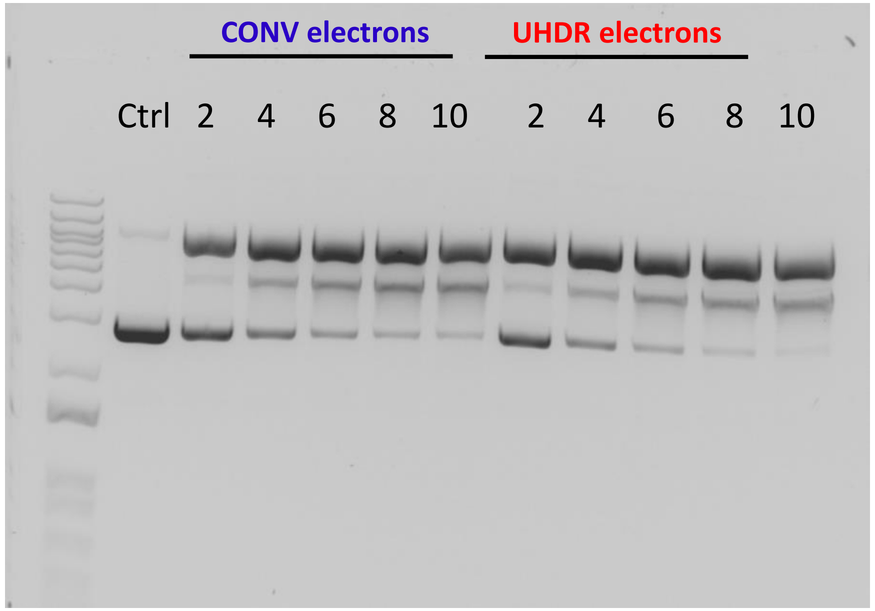


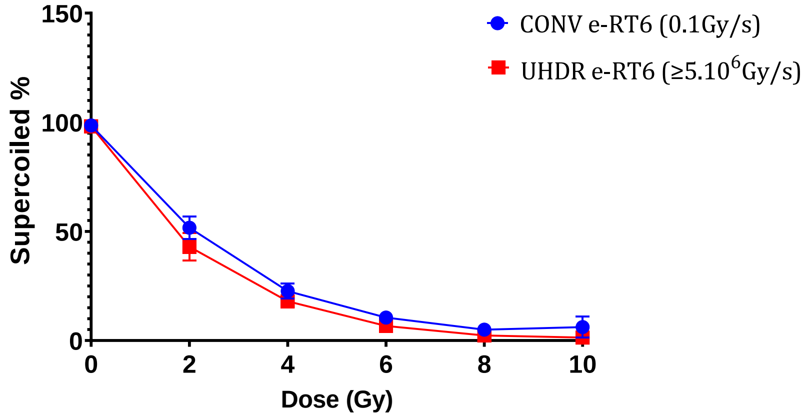


(Pachnerova Brabcova, 2019)

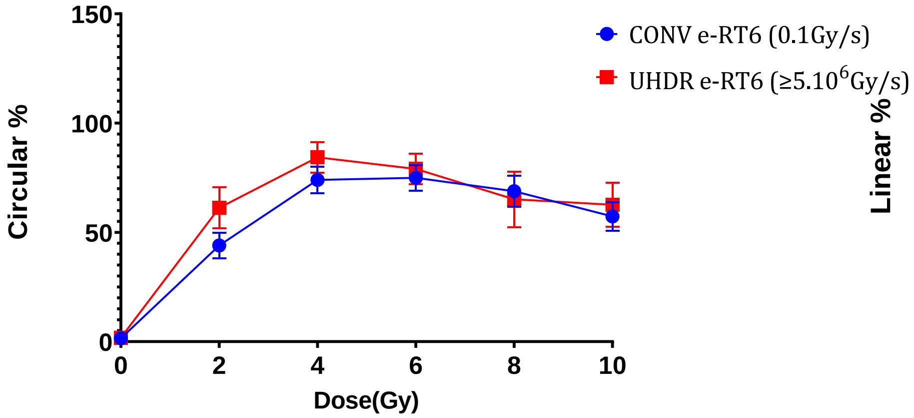
Results

e-RT6_5.5MeV_electrons_21%O2

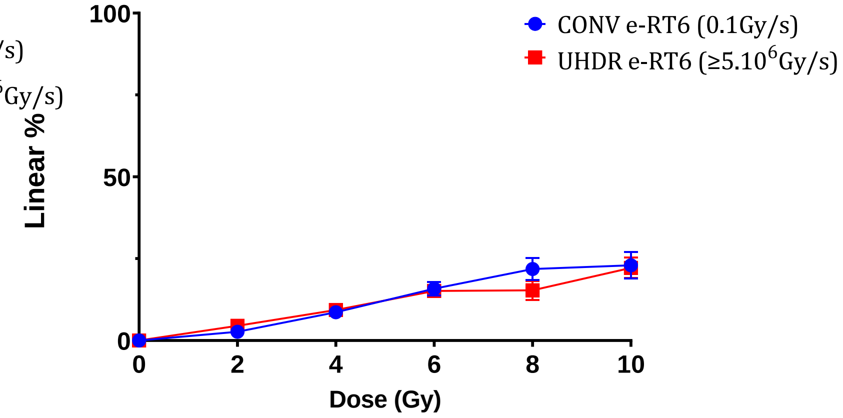




Supercoiled



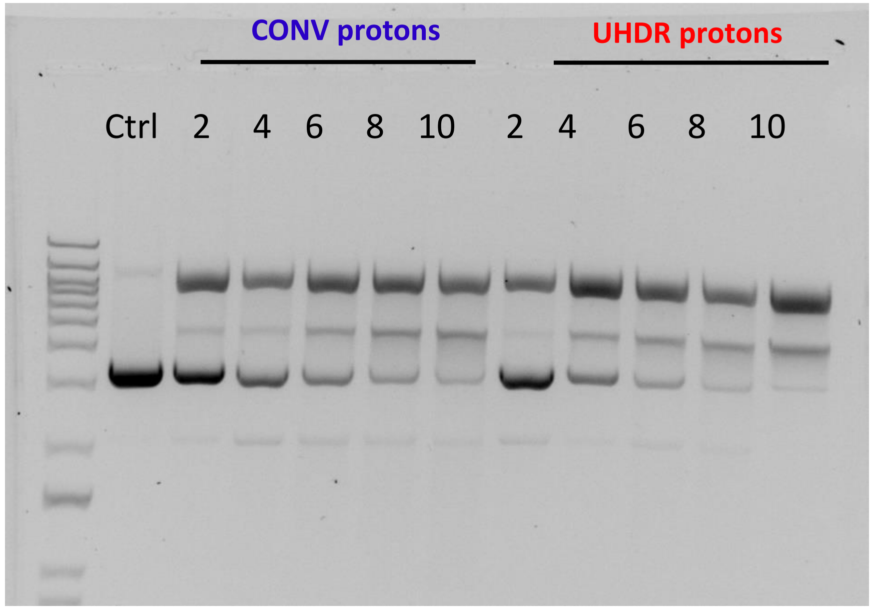
Circular

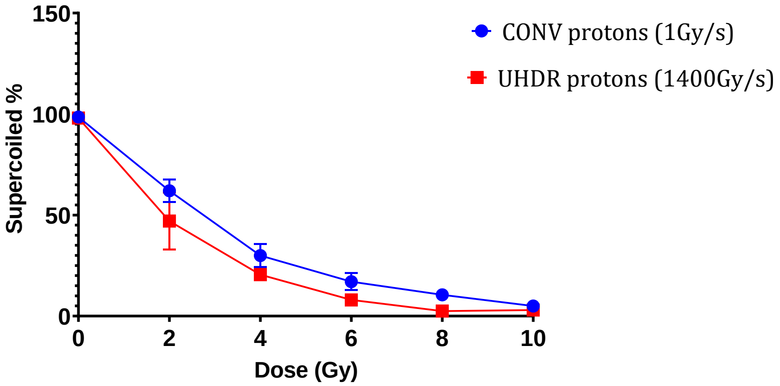


Linear

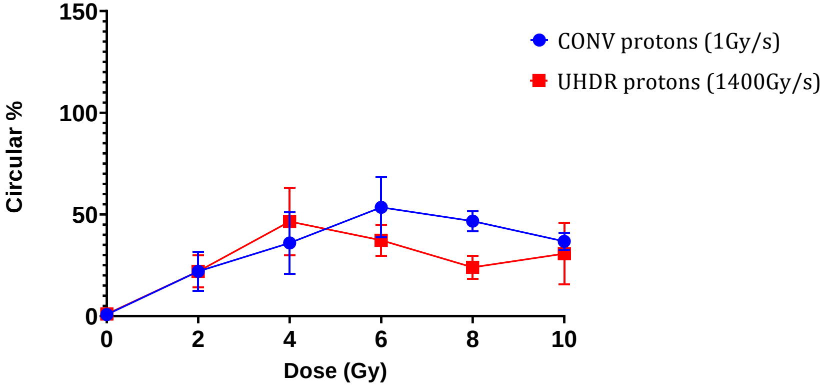
Results

PSI_235MeV_Transmission protons_21%O2

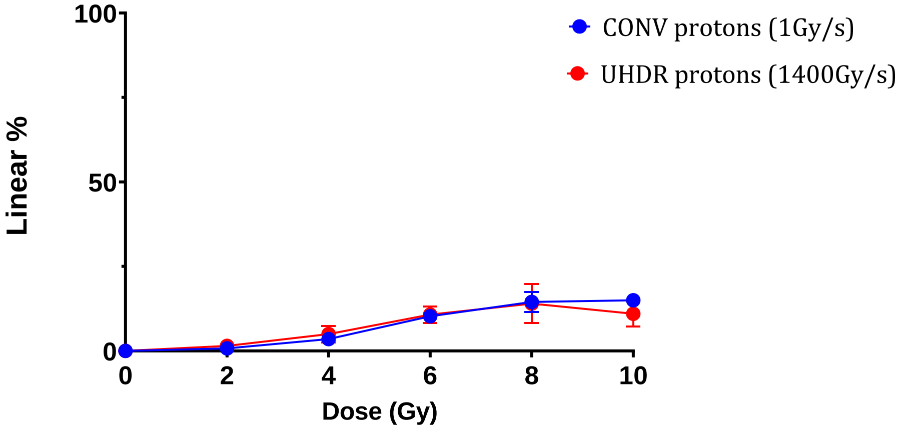




Supercoiled



Circular

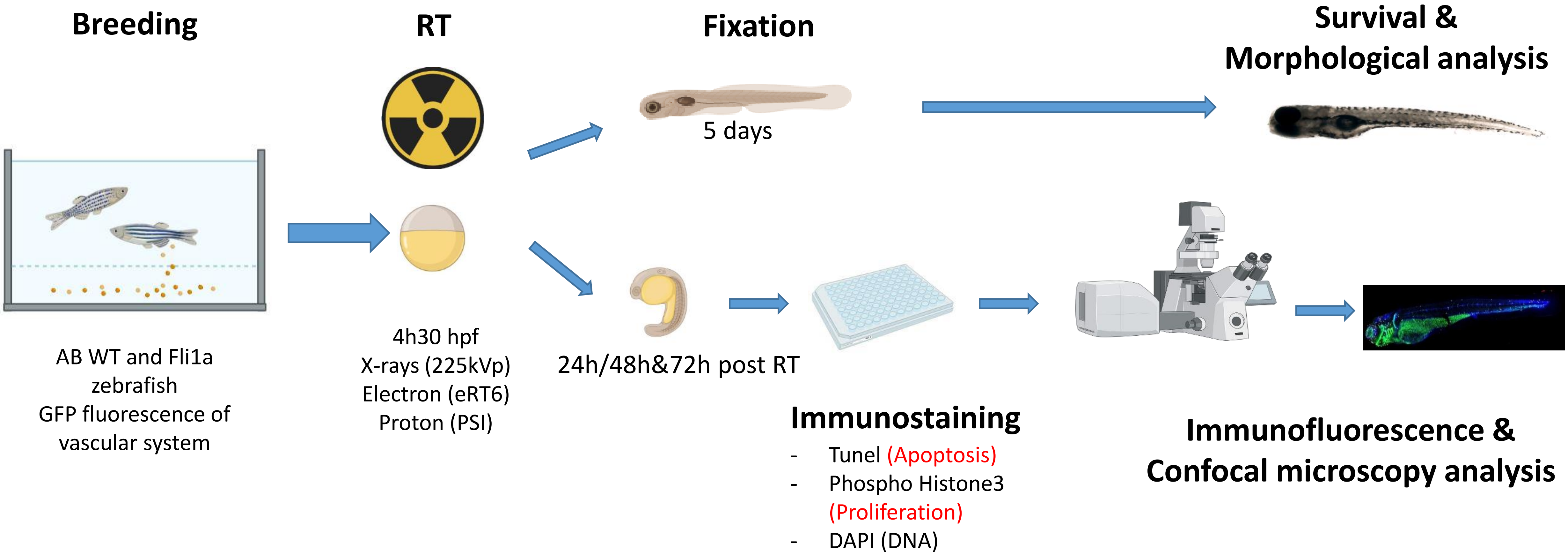


Linear

UHDR-RT results in similar DNA damage as CONV-RT in the plasmid model

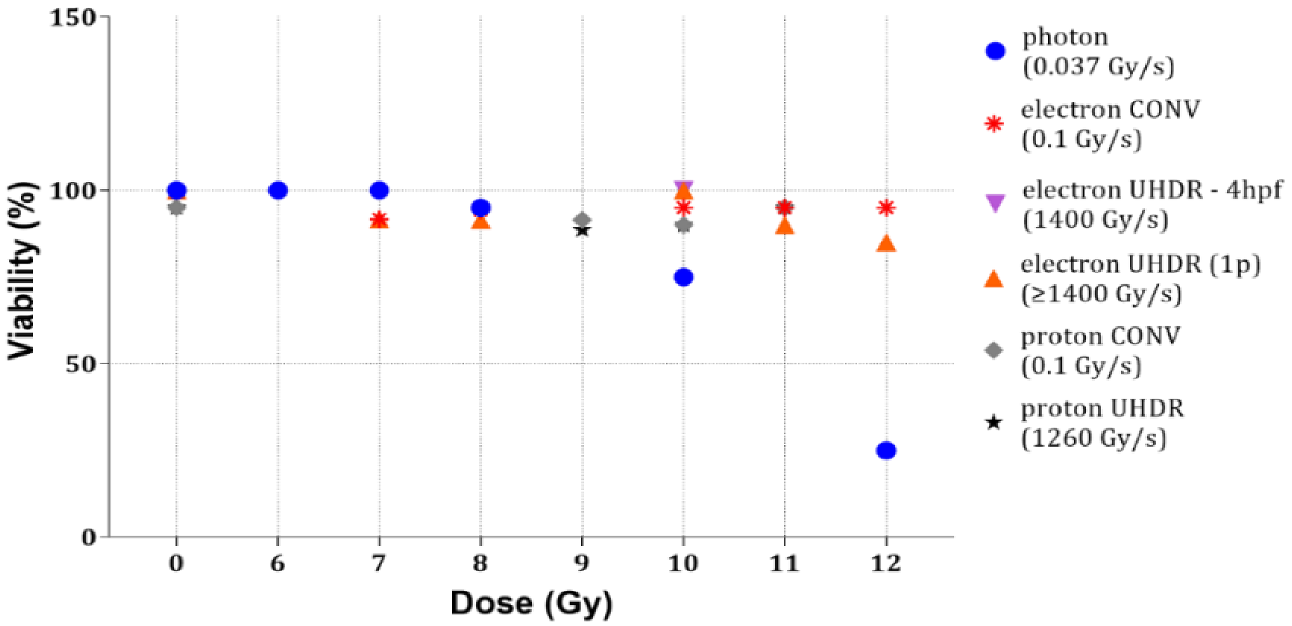
Zebrafish development

Experimental procedure



Results

Survival assessments of Zebrafish embryos

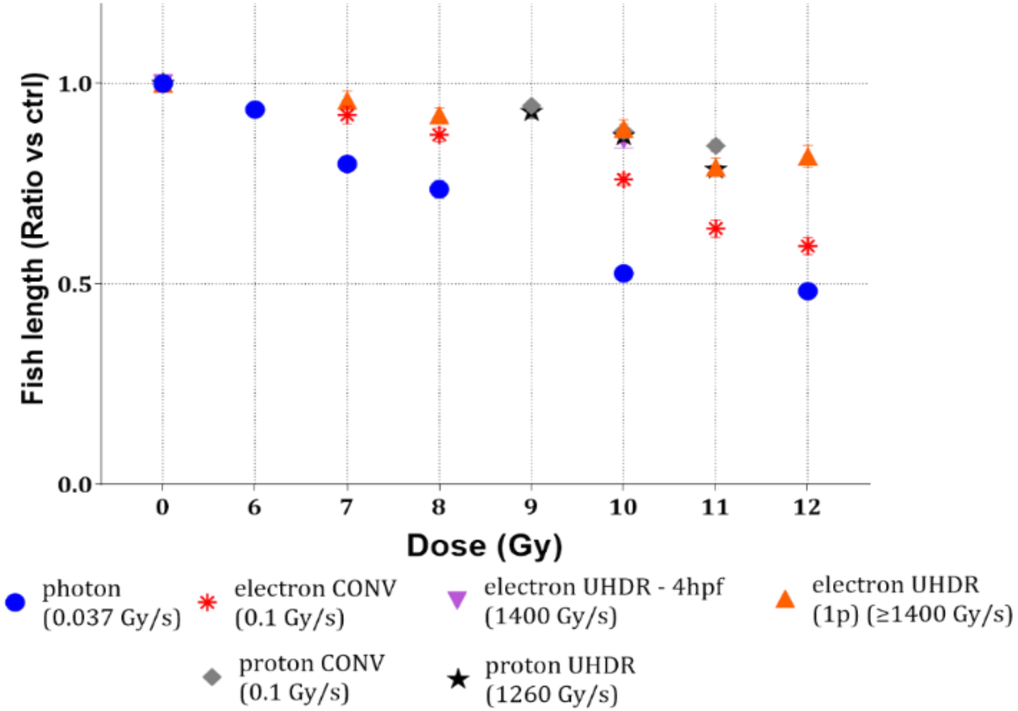


The FLASH sparing effect was found with electron at ≥1400 Gy/s and proton at 0.1 and 1260 Gy/s with a minimal impact on embryo survival and growth 5 days post-fertilization. Toxicity was found with 225 kV photon and electron beam at conventional dose rate.

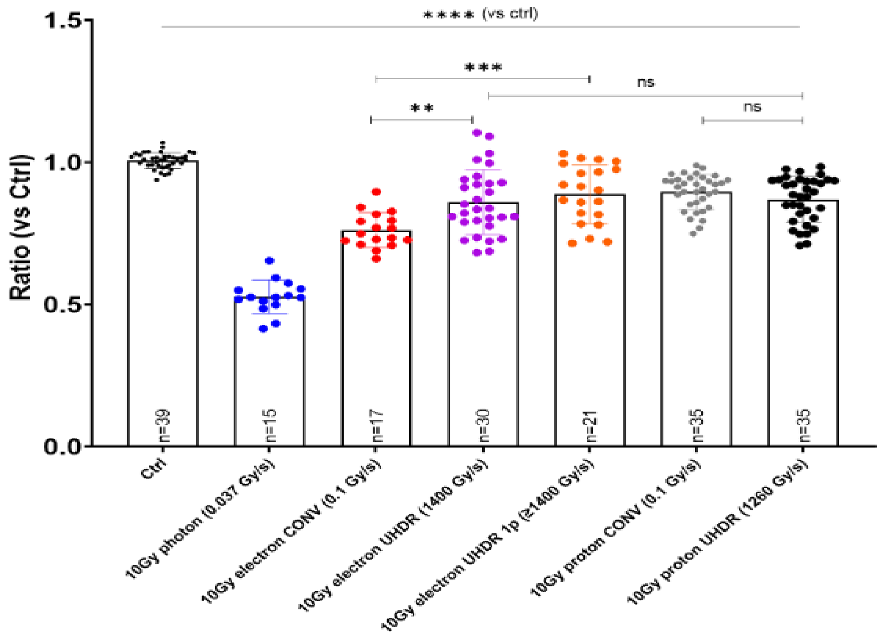
(Kacem et al, R&O 2022 Accepted)

Results

Fish length assess developmental retardation induced by irradiation at 5 days post-fertilization

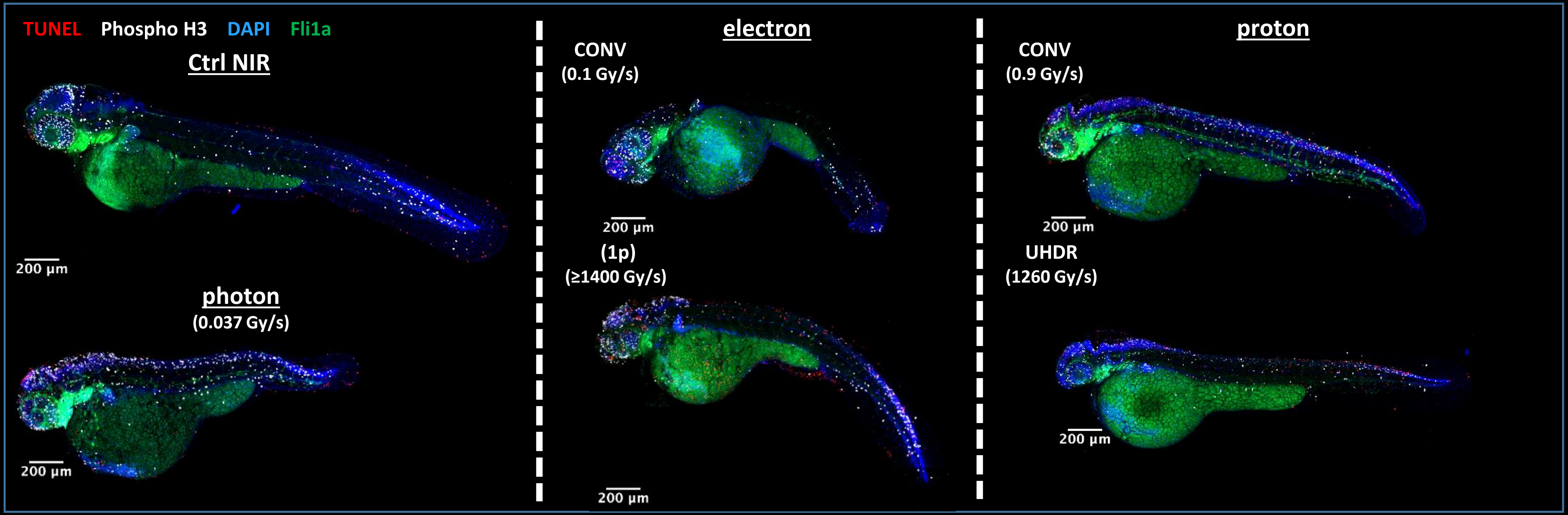


at 10Gy

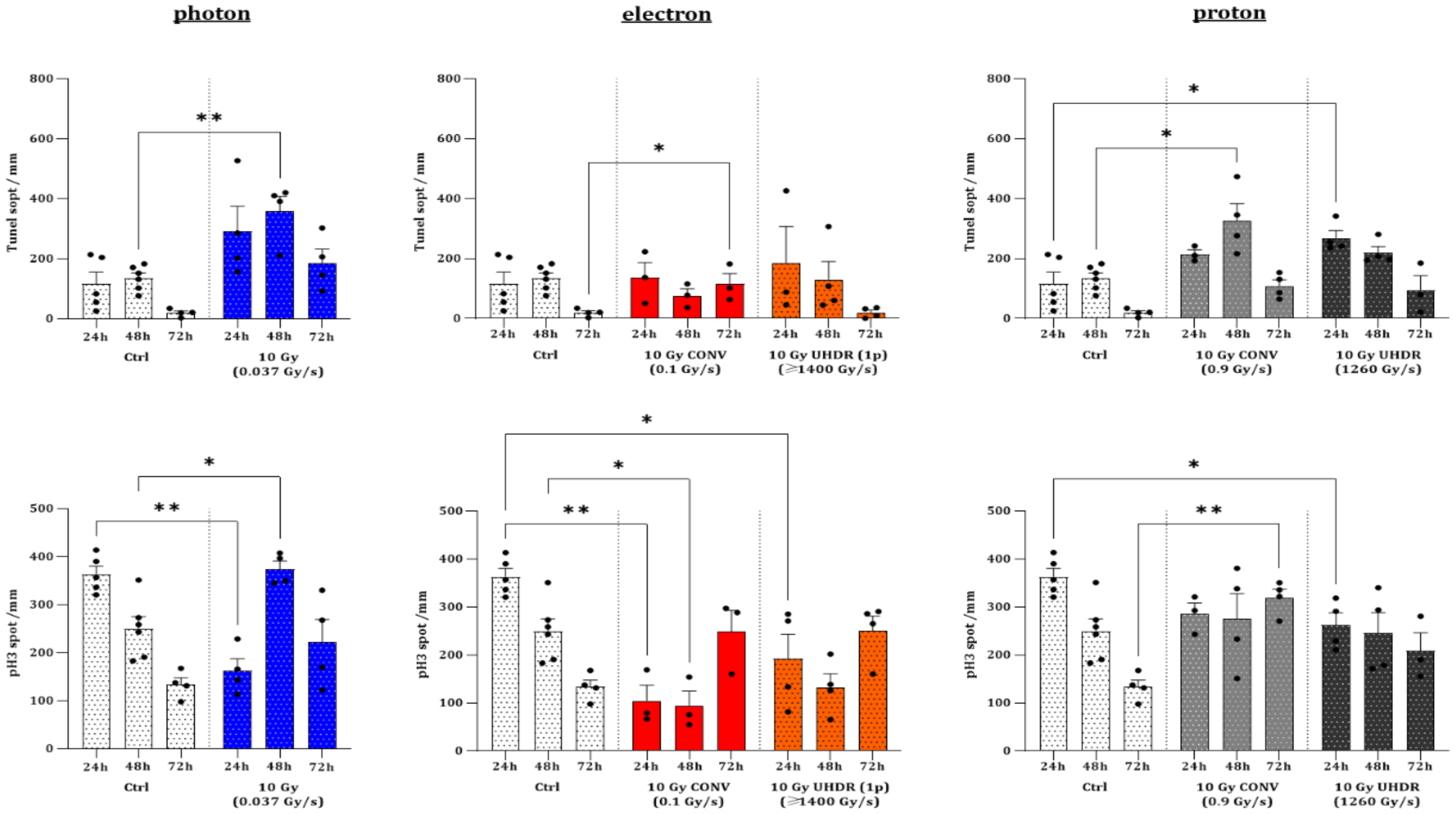


Developmental sparing effect was found at 5 days post fertilization induced by proton (conventional and UHDR) and electron UHDR. Whereas alteration in fish length was found with 225kV photon and CONV electron

10Gy at 48h post RT



at 10Gy



UHDR-electrons protected the development of ZF, protons at both dose rates were isoefficient at sparing ZF embryos

Conclusions

UHDR-RT does not impact early physico-chemical events

Differential production of H_2O_2 was found at UHDR compared to conventional irradiation

DNA damage in the plasmid model is similar at both dose rates with atmospheric conditions

Dose responses in ZF model were found for electron and proton beams whereas dose rate responses were found for the electron but not for the proton beam. Proton beams appeared to be protective at both dose rates.

Acknowledgments

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