EPR Based Estimation of Radiation-Induced Reactive Oxygen Species

Ken-ichiro Matsumoto,[1,2] Megumi Ueno,[1] Minako Nyui,[1] Yukihiro Ogawa,[1,2] Masato Kamibayashi,[1,2] Emiko Sekine-Suzuki,[1] Ikuo Nakanishi,[1] Kazunori Anzai,[1,3] and Toshihiko Ozawa[4]

1. Quantitative RedOx Sensing Team, Department of Basic Medical Sciences for Radiation Damages, National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology, Chiba 263-8555, Japan.
2. Graduate School of Advanced Integration Science, Chiba University, Chiba 263-8522, Japan
3. Division of Physical and Analytical Chemistry, Nihon Pharmaceutical University, Saitama 362-0806, Japan.
4. Division of Oxidative Stress Research, Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan.

National Institute of Radiological Sciences

RedOx Quantitative RedOx Sensing Team

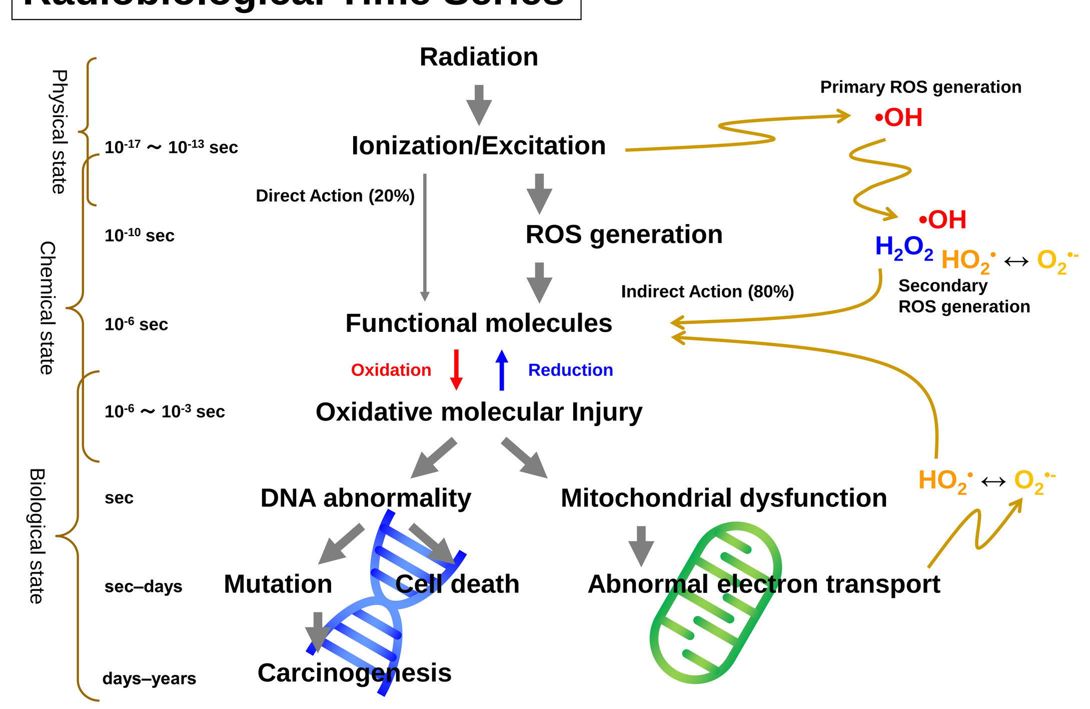
ABSTRACT

Generation of reactive oxygen species (ROS) is considered as essential trigger of biological effects of ionizing radiations, and may be deeply linked with the radiation quality.

Amounts of total oxidation reactions (i.e. oxidative free radical species, •OH and $HO_2^{\bullet}$), H_2O_2 generations, Oxygen consumptions, and •OH generations induced by X-ray, 20 keV/μm carbon beam, and 80 keV/μm carbon beam were estimated using EPR based techniques.

Total oxidation reactions were estimated as 3, 1.3, and 0.66 μmol/L/Gy, amount of H_2O_2 generations were 0.2, 0.57, and 0.35 μmol/L/Gy, oxygen consumptions were 0.4, 0.39, and 0.15 μmol/L/Gy for X-ray, 20 keV/μm and 80 keV/μm carbon beam, respectively. The ratio of H_2O_2 generation per oxygen consumption were increased with LET, and were 0.5, 1.46, 2.33 for X-ray, 20 keV/μm carbon beam, and 80 keV/μm carbon beam, respectively. The •OH generations expected to be localized on the track/range of the radiation beam/ray, and both sparse (≈ 3.3 mM) and very dense (> 1.7 M) •OH generations were suggested. Percentage of sparse •OH generation decreased with LET becoming higher.

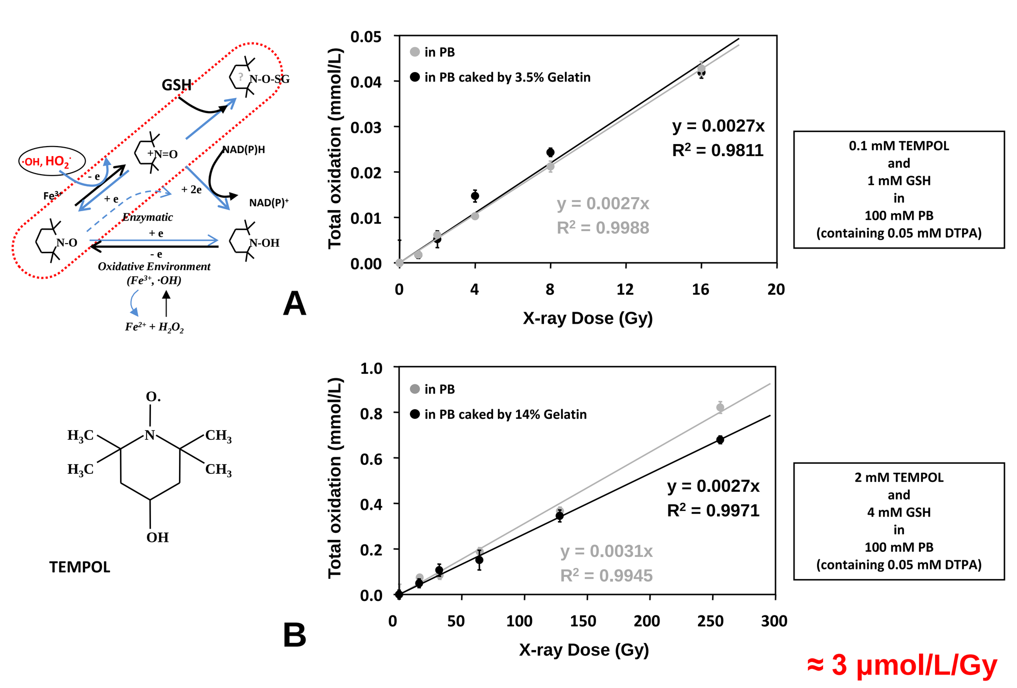
Radiobiological Time Series



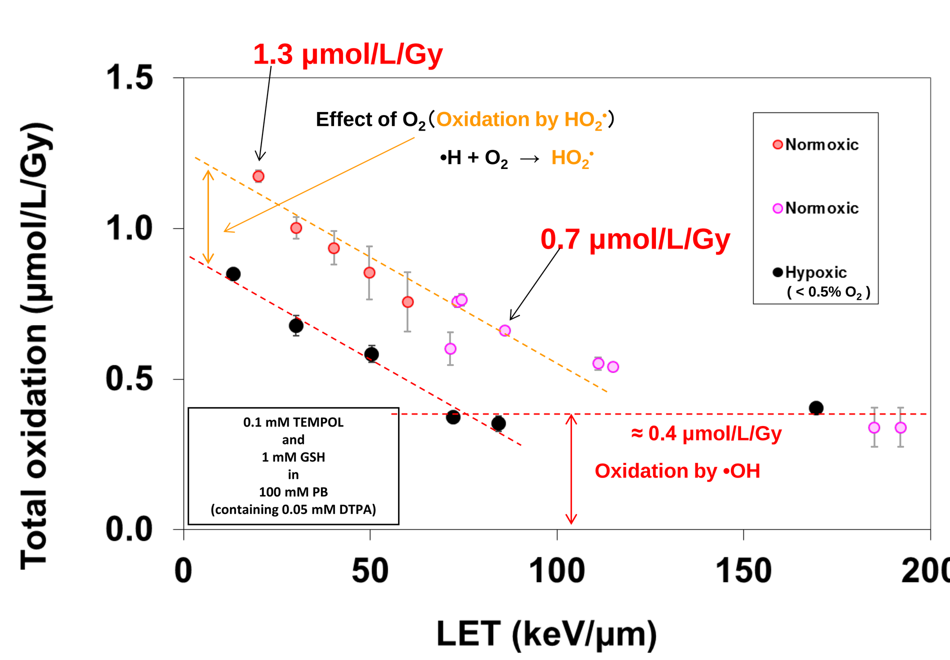
How much Oxidation can be occurred by radiation?

Total oxidation by •OH + $HO_2^{\bullet}$

(Highly oxidative free radical species)

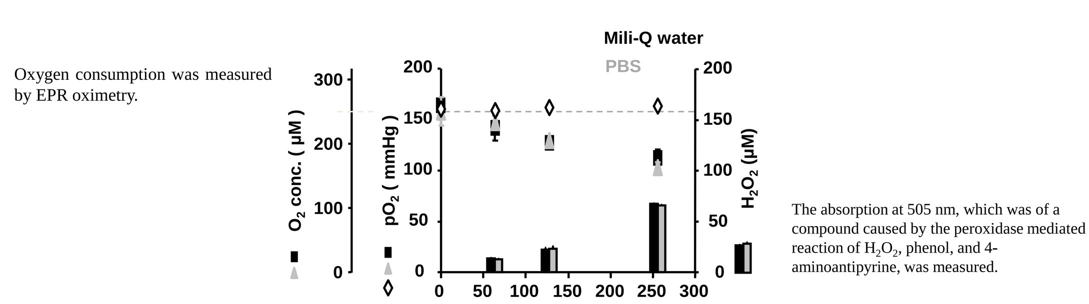


Total oxidation reaction (GSH-dependent reduction of TEMPOL) caused by X-ray

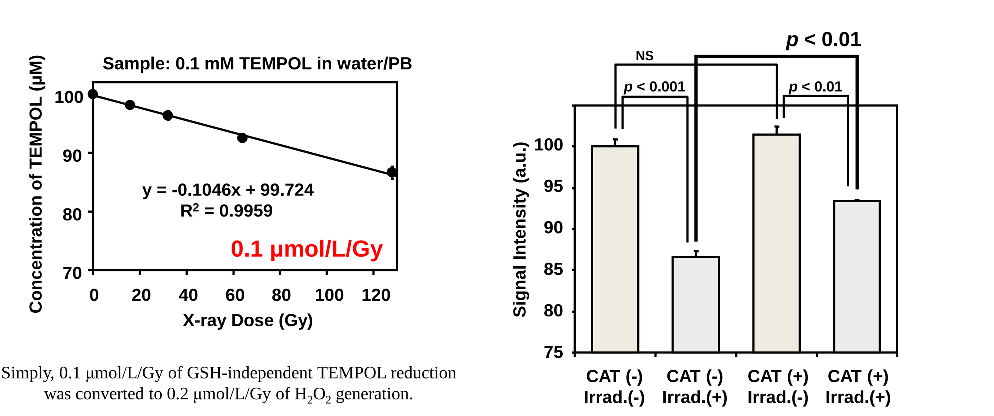


Total oxidation reaction by carbon-ion beam in normoxic and hypoxic conditions

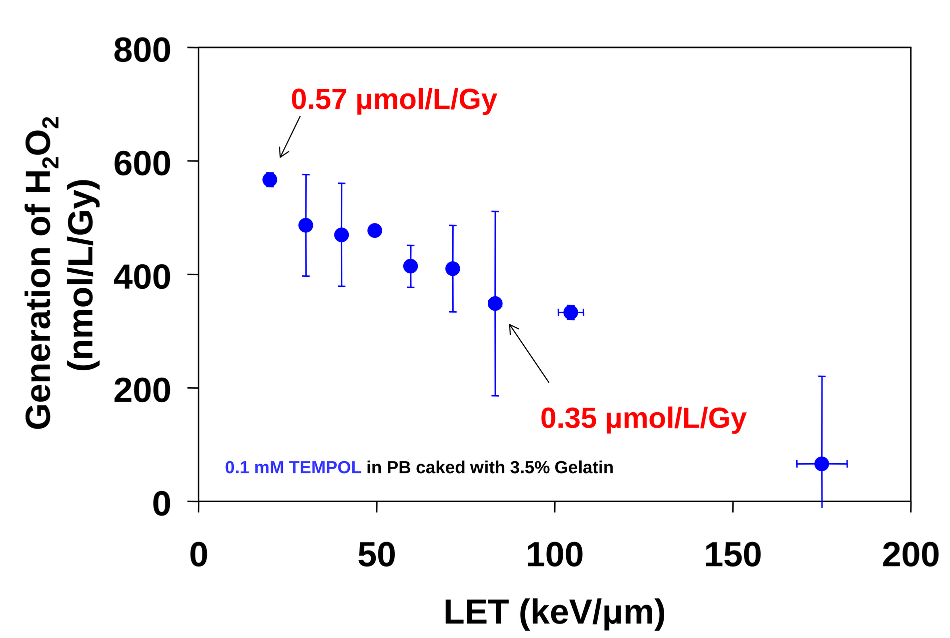
How much H_2O_2 can be generated by radiation?



Oxygen consumption and simultaneous H_2O_2 generation caused by X-ray

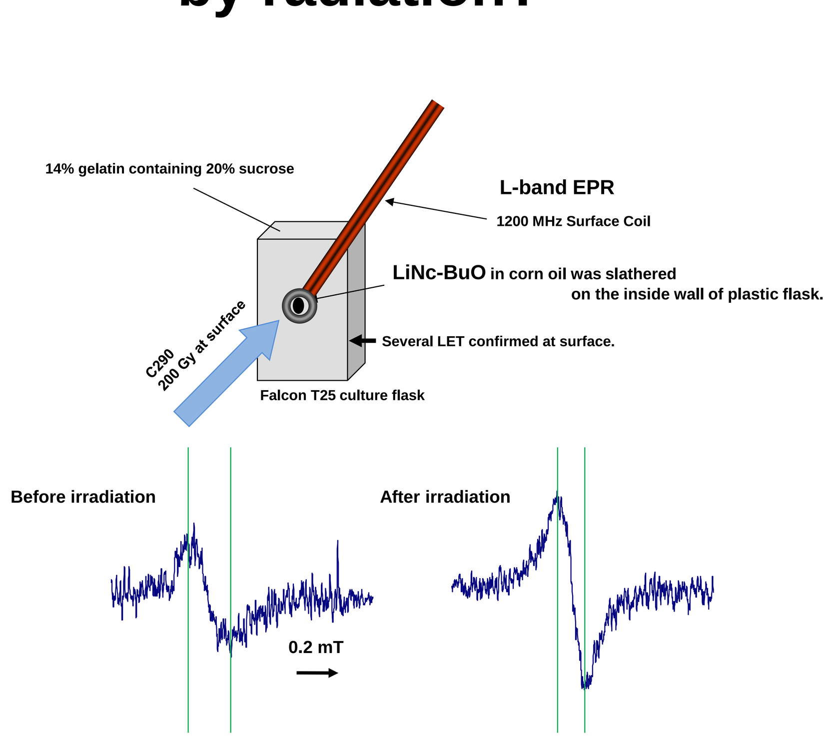


Estimation of X-ray induced H_2O_2 amount based on GSH-independent reduction of TEMPOL

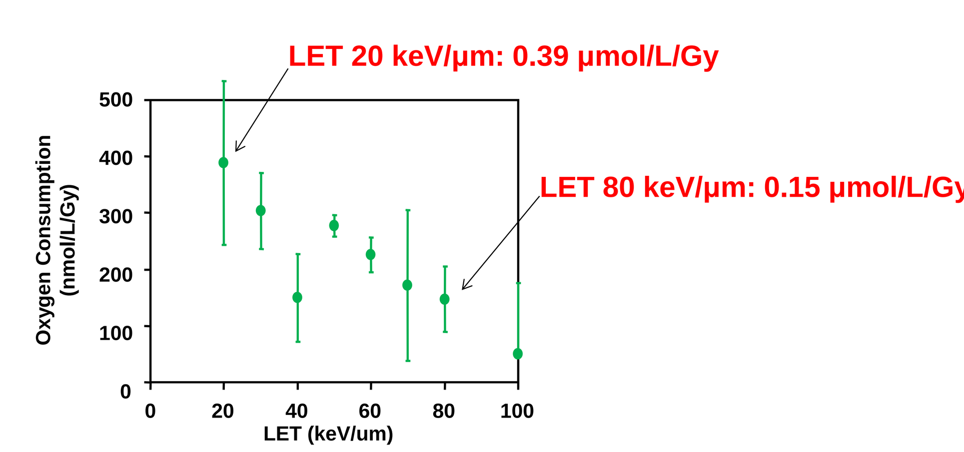


Amount of H_2O_2 generated by carbon-ion beam at an identical dose

How much O_2 can be consumed by radiation?

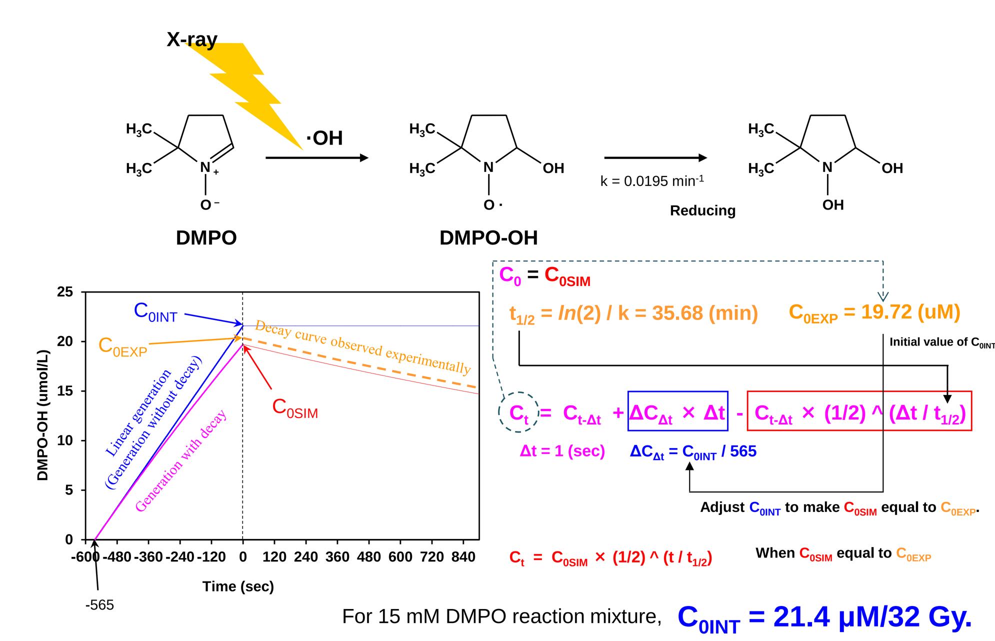


Measurement of oxygen consumption in gelatin sample using EPR oximetry technique

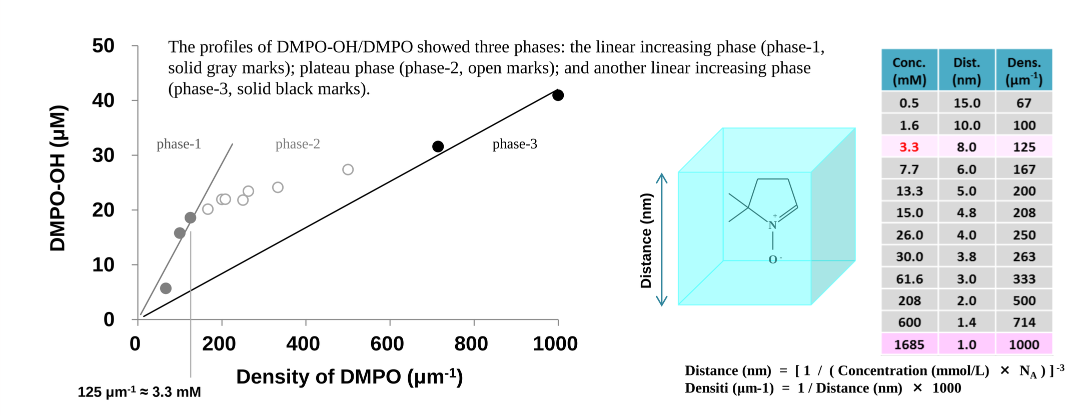


Oxygen consumption in gelatin sample irradiated by carbon-ion beam

How much •OH can be generated by radiation?

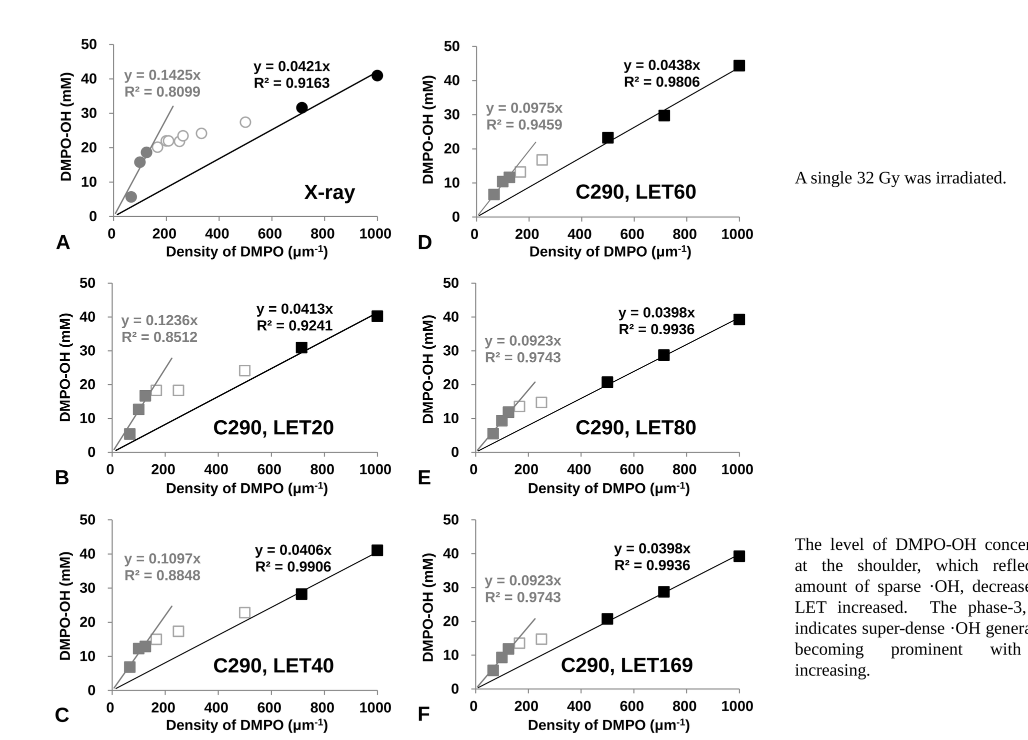


Estimation of net •OH amounts by correcting reduction of DMPO-OH, which had occurred during and after X-ray Irradiation.

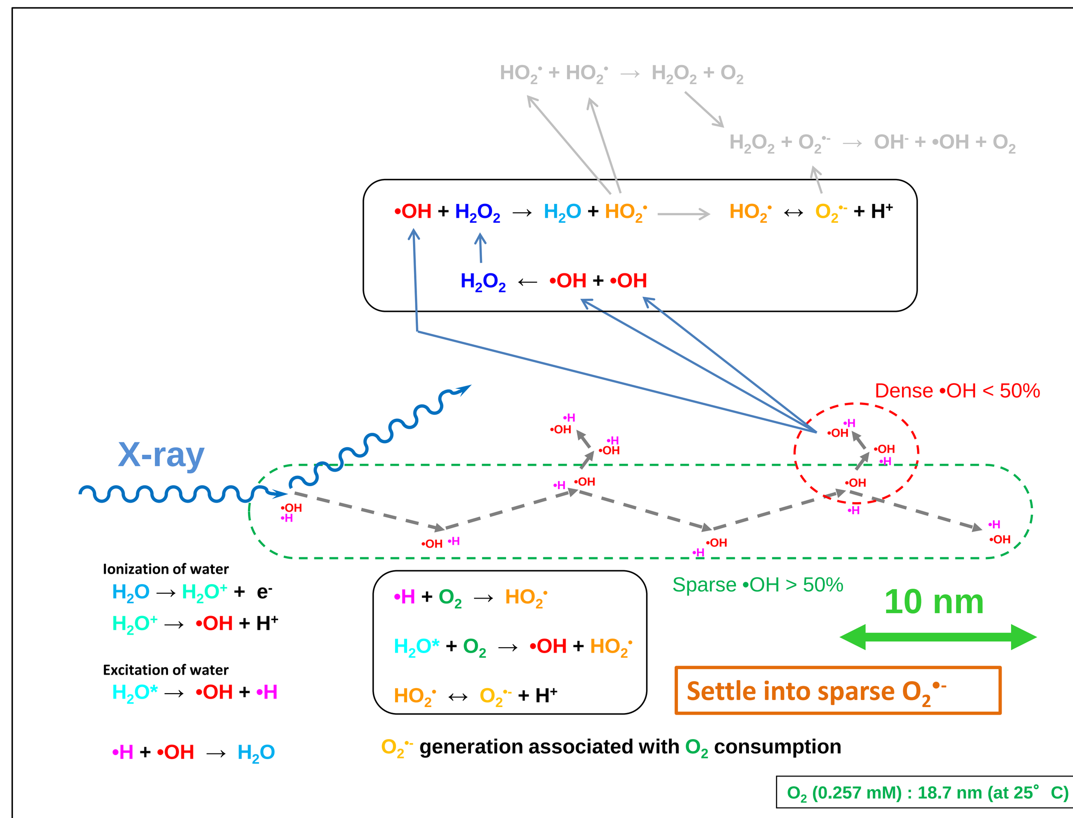


Linear increase through the origin indicates that the DMPO concentration is lower compared to local •OH concentration. The result suggests that two different densities of •OH generation, i.e., sparse and dense, existed in this reaction system.

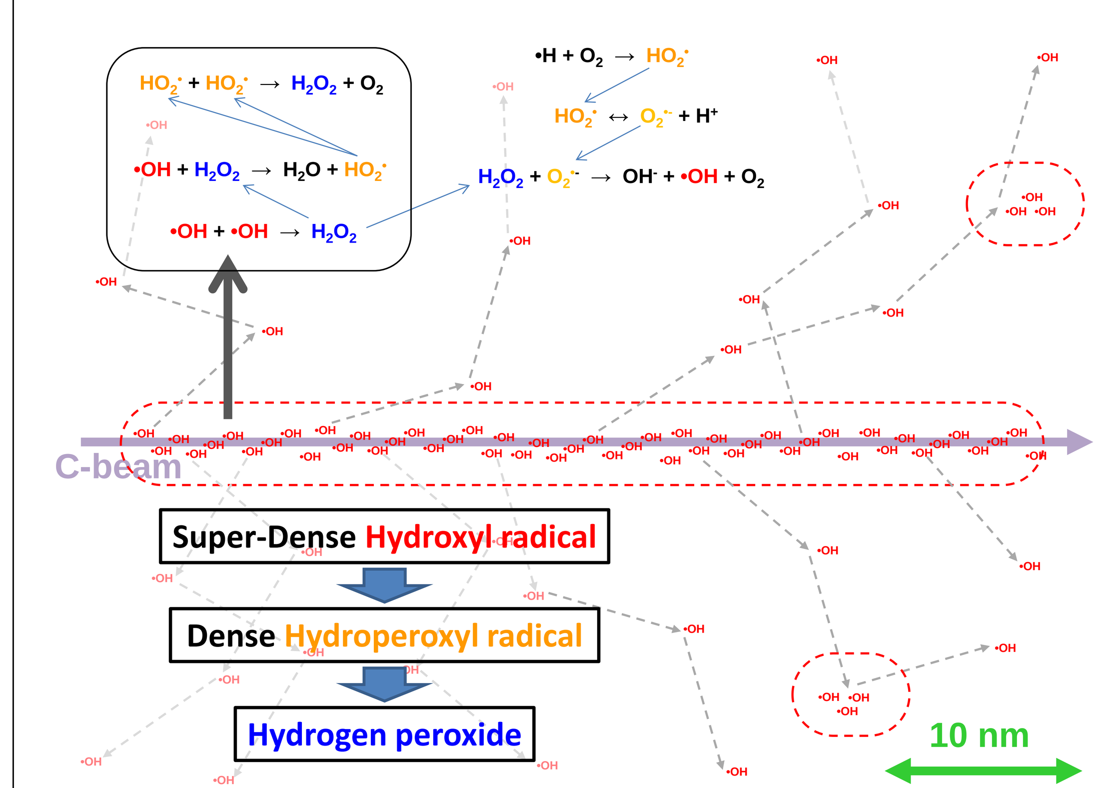
X-ray induced generation of DMPO-OH in reaction mixtures containing several different DMPO concentrations (densities)



Carbon beam induced generation of DMPO-OH in reaction mixtures containing several different DMPO concentrations (densities)



Prospective reactions of X-ray induced reactive species



Prospective reactions of carbon-ion beam induced reactive species

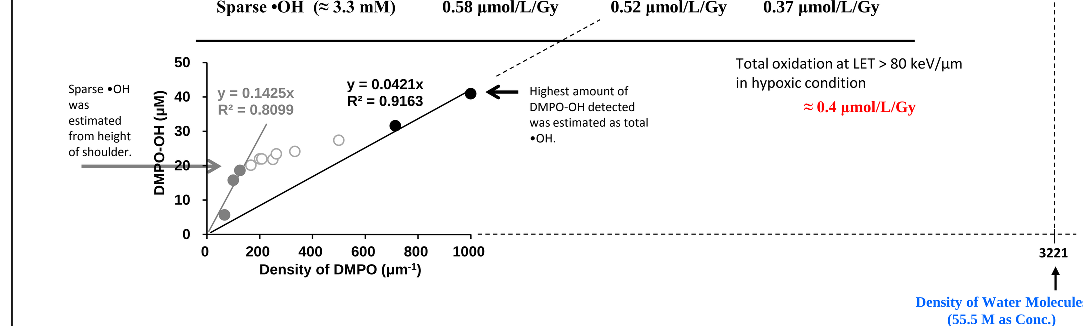
Table. ROS generation by X-ray and C-beam

	X-ray	Carbon ion beam 20 keV/μm	Carbon ion beam 80 keV/μm
Total Oxidation (involving $HO_2^{\bullet}$ and sparse •OH)	3 μmol/L/Gy	1.3 μmol/L/Gy	0.66 μmol/L/Gy
H_2O_2	0.2 μmol/L/Gy	0.57 μmol/L/Gy	0.35 μmol/L/Gy
(H_2O_2 / O_2 cons.)	(0.50)	(1.46)	(2.33)
Oxygen consumption	0.4 μmol/L/Gy	0.39 μmol/L/Gy	0.15 μmol/L/Gy
Total •OH	1.28 μmol/L/Gy	1.26 μmol/L/Gy	1.26 μmol/L/Gy
Sparse •OH (≈ 3.3 mM)	0.58 μmol/L/Gy	0.52 μmol/L/Gy	0.37 μmol/L/Gy

≈ 4 μmol/L/Gy

≈ 130 μM

The ratio of H_2O_2/O_2-consumption become larger for higher LET.



REFERENCES

1. Matsumoto K, Ueno M, Nakanishi I, Anzai K. Density of hydroxyl radicals generated in an aqueous solution by irradiating carbon-ion beam. *Chem. Pharm. Bull.* **63**, 1–5, 2015.
2. Matsumoto K, Aoki I, Nakanishi I, Matsumoto A, Nyui M, Endo K, Anzai K. Distribution of hydrogen peroxide-dependent reaction in a gelatin sample irradiated by carbon ion beam. *Magn. Reson. Medic. Sci.* **9**, 131–140, 2010.
3. Matsumoto K, Nagata K, Yamamoto H, Endo K, Anzai K, Aoki I. Visualization of free radical reactions in an aqueous sample irradiated by 290 MeV carbon beam. *Magn. Reson. Med.* **61**, 1033–1039, 2009.