VHEE2020
5-7 October 2020

Oxygen depletion and H_2O_2 yields in water after UHDR irradiations with electrons

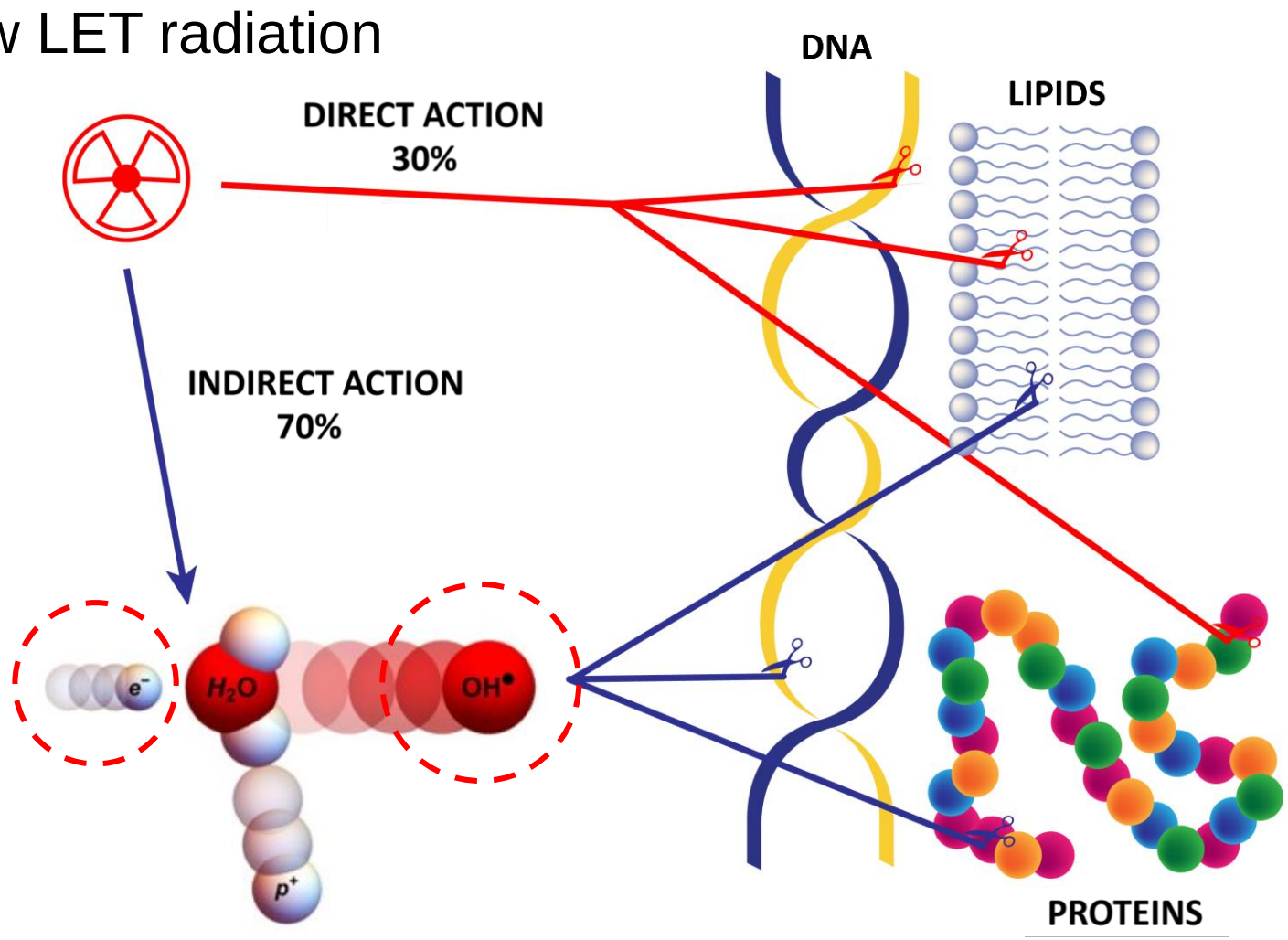
Veljko Grilj, IRA/CHUV

FLASH chemistry:

Two hypothesis proposed to explain the FLASH effect

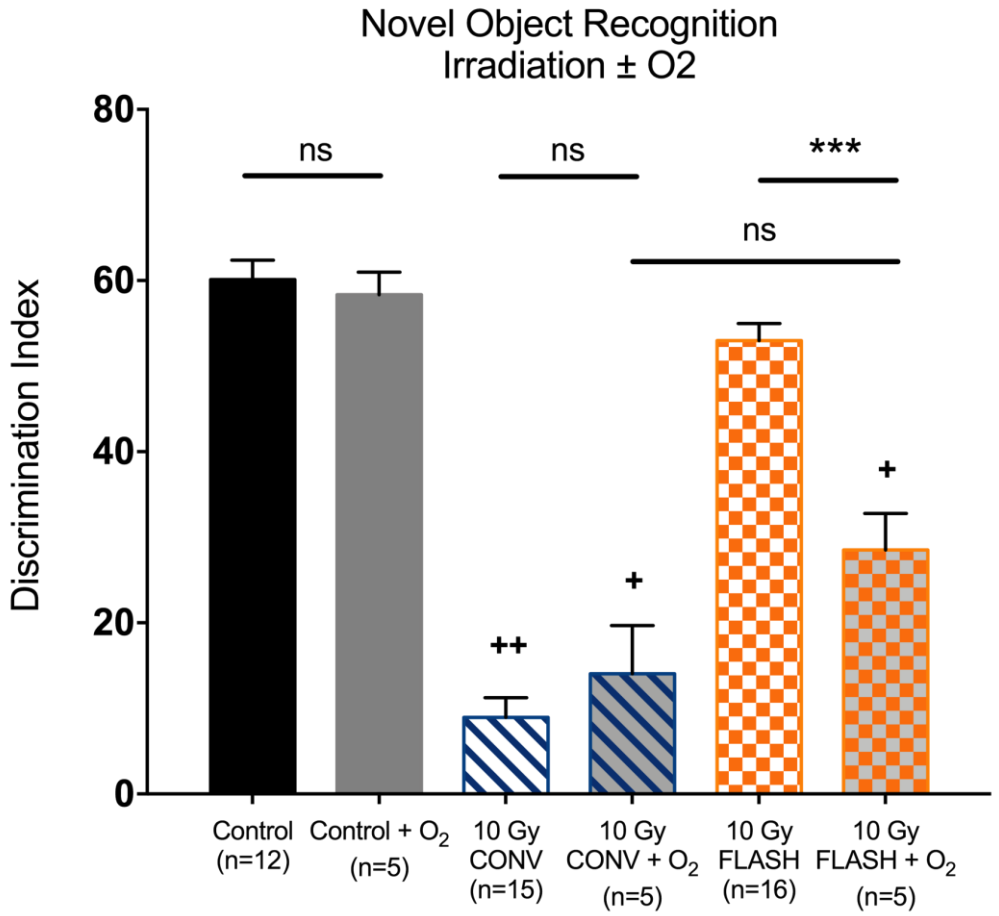
Radiolytic oxygen depletion

Reduced yield of free radicals

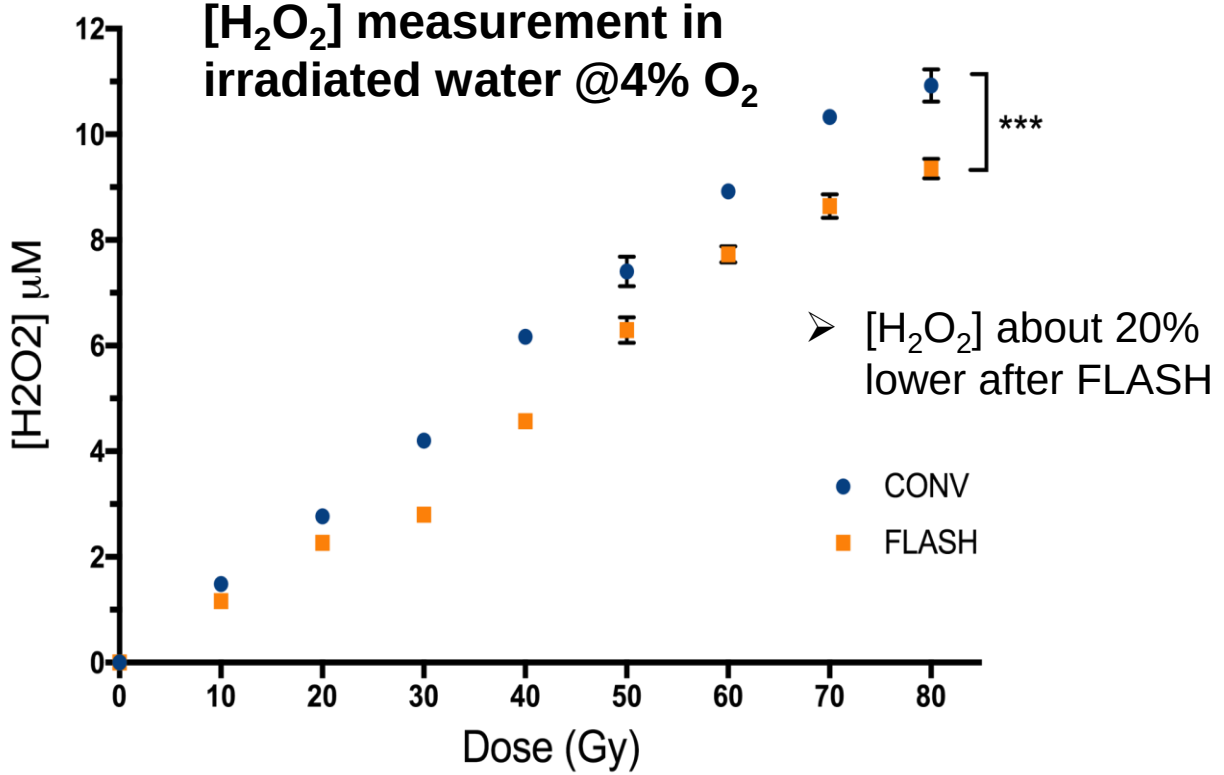


Published data:
In support of the two hypothesis

- Oxygen plays important role



- Lower $[H_2O_2]$ after FLASH
  - reduced yield of •OH?
  - radical recombination, increased H_2O_2 removal?



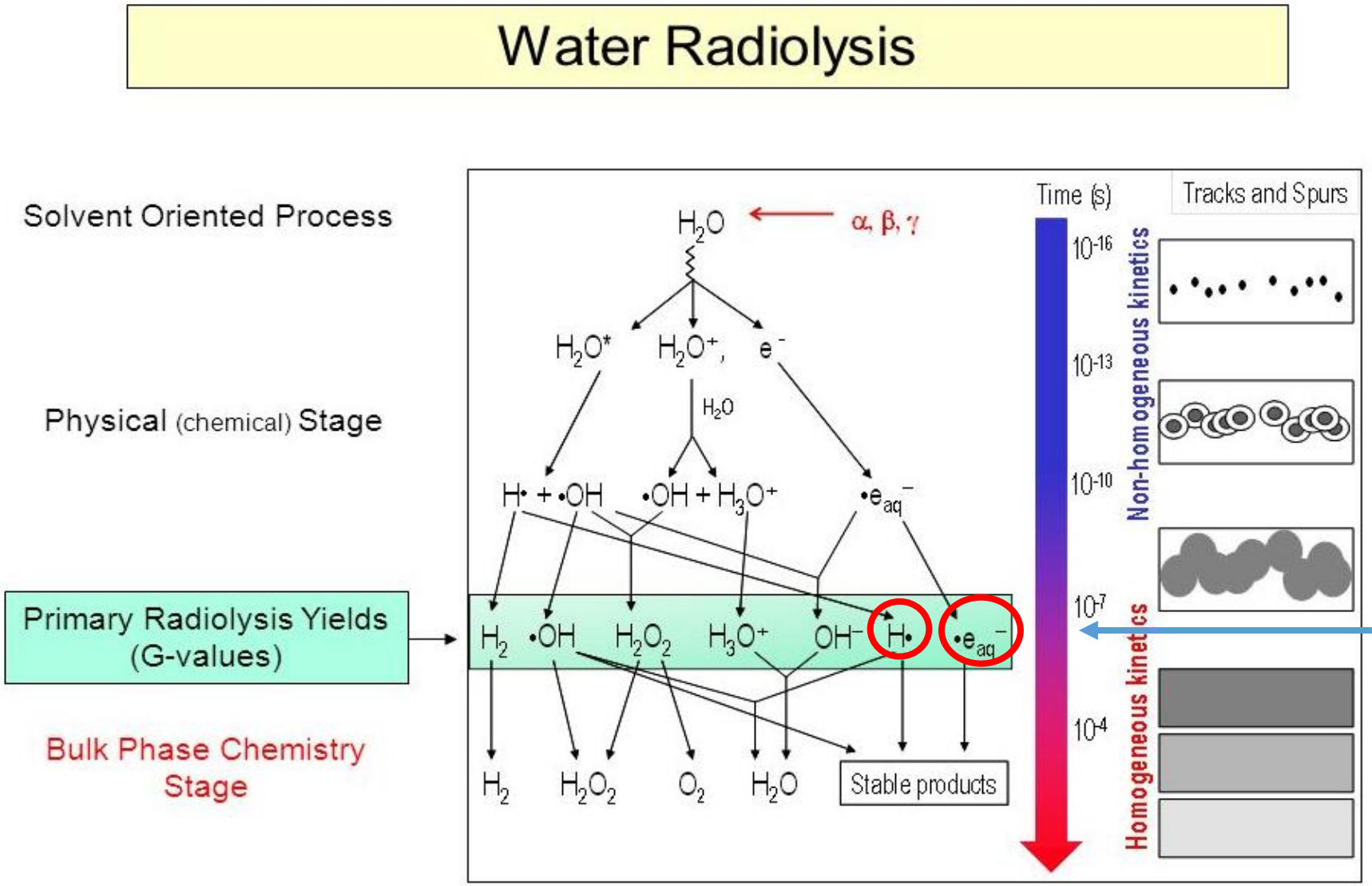
$[H_2O_2]$ measurement in irradiated water @4% O_2

- $[H_2O_2]$ about 20% lower after FLASH

Montay-Gruel et al, PNAS, 2019

Radiolytic Oxygen Depeletion in Water

Water radiolysis:
Depleting dissolved oxygen from medium



$O_2 + e(aq) \rightarrow O_2^{\bullet -}$

$k = 1.9 \times 10^{10}\ M^{-1}s^{-1}$

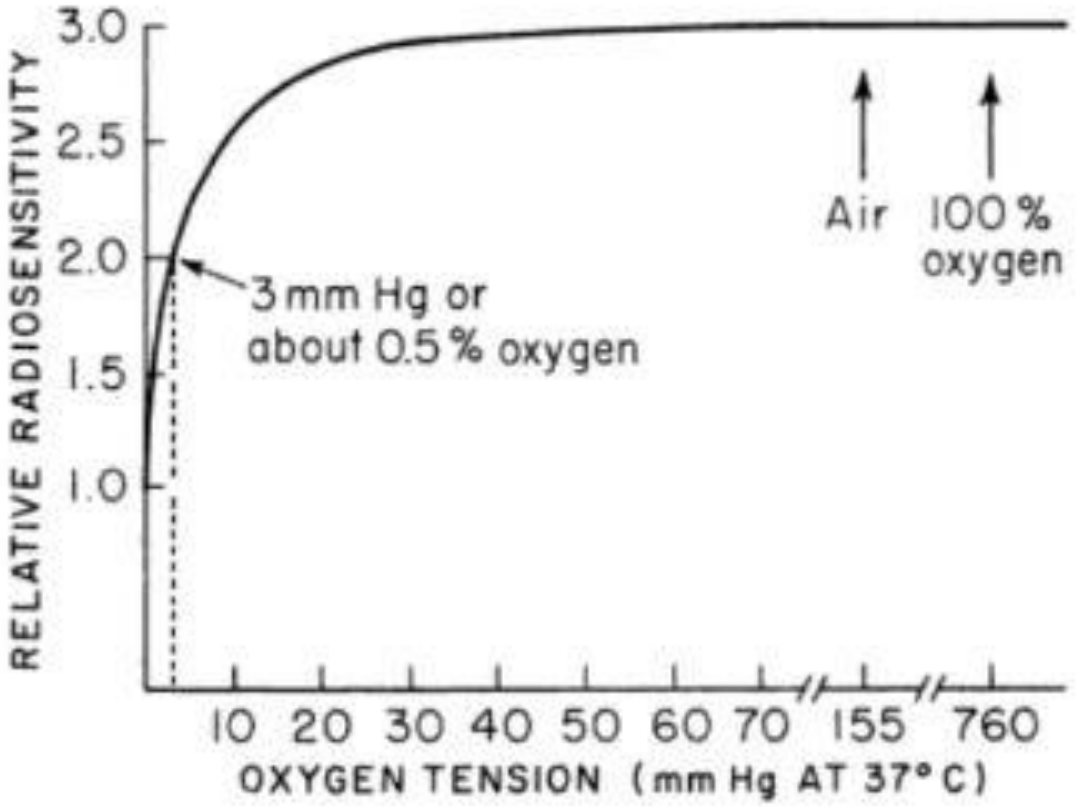
$O_2 + H^{\bullet} \rightarrow HO_2^{\bullet}$

$k = 2.0 \times 10^{10}\ M^{-1}s^{-1}$

1 µs → start of homogeneous phase

Oxygen depletion hypothesis
Can it explain the FLASH effect?

- FLASH radiation depletes oxygen quickly with NO time for reoxygenation
- Oxygen works as radio-sensitizer (oxygen fixation and/or increased yields of ROS)



*OER curve taken from E. Hall's book

Oxygen Enhancement ratio:

$$OER = \frac{\text{dose in anoxic conditions}}{\text{dose in oxic conditions}}$$

for the same biological outcome

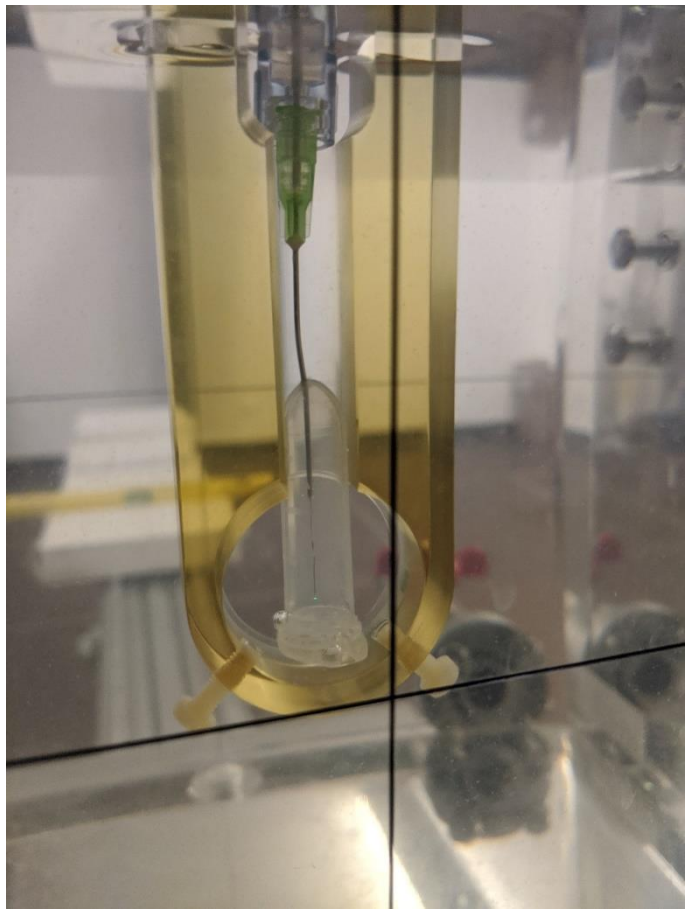
Oxygen depletion rates
What can be found in the literature?

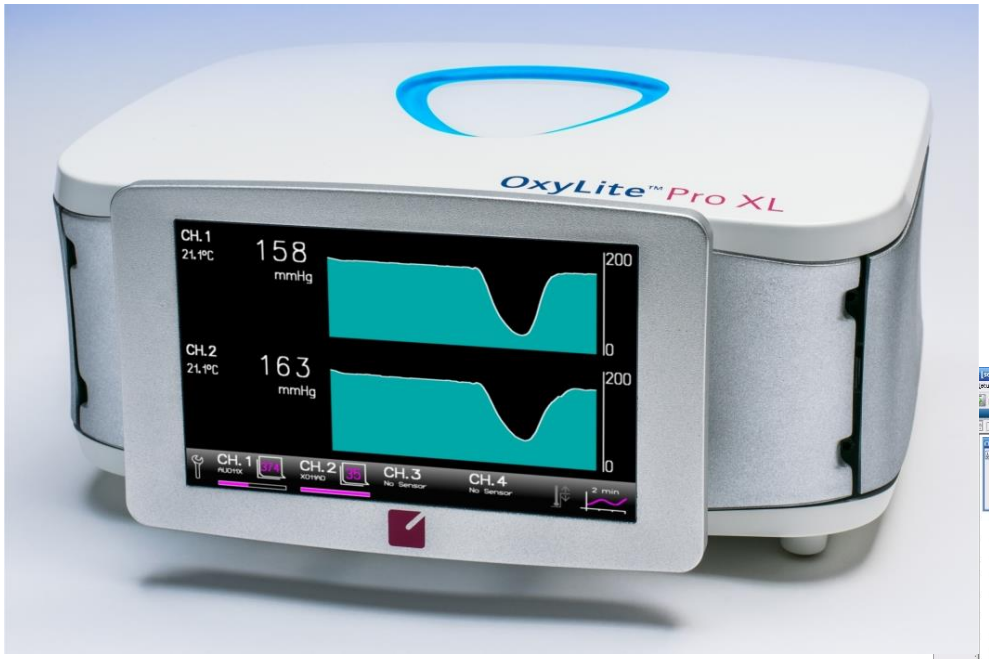
Year	Lead author	Paper type	O_2 depletion per 100 Gy
1949	Day M.J.	experiment	3.3%
1969	Evans N.T.S.	experiment	2.6%
1974	Weiss H.	experiment	2.6%
1975	Ling C.	modelling	2.6%
1986	Michaels H.B.	experiment	3.3%
2019	Pratx	modelling	5.5%
2020	Boscolo D.	modelling	2.4%
2020	Petersson K.	modelling	5% and 10%
2020	Zhou S.	modelling	2.6%
2020	Hu A.	modelling	3.7%
2020	Labarbe R. (IBA)	modelling	2.2%

Experiment:
The setup for measuring O_2

Beam: 6MeV electrons
Irradiator: Oriatron eRT6

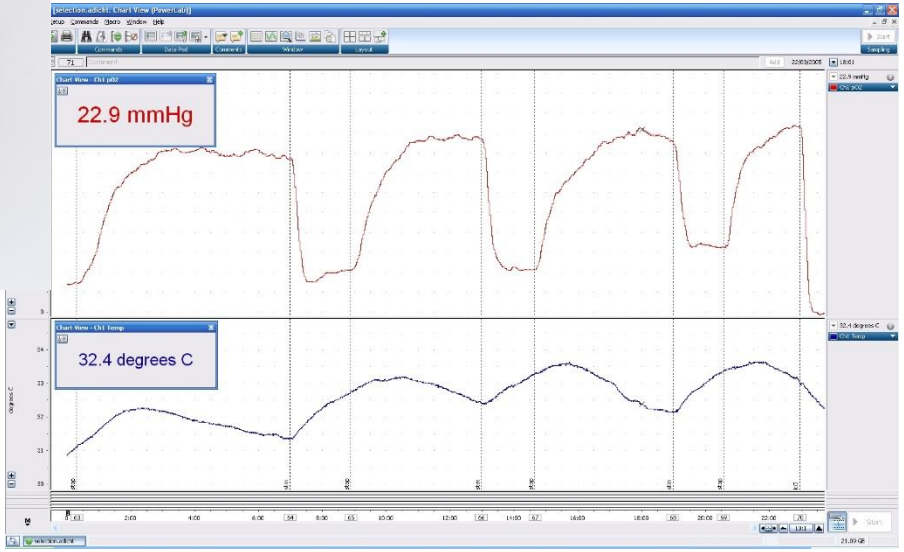
Oxygen sensor



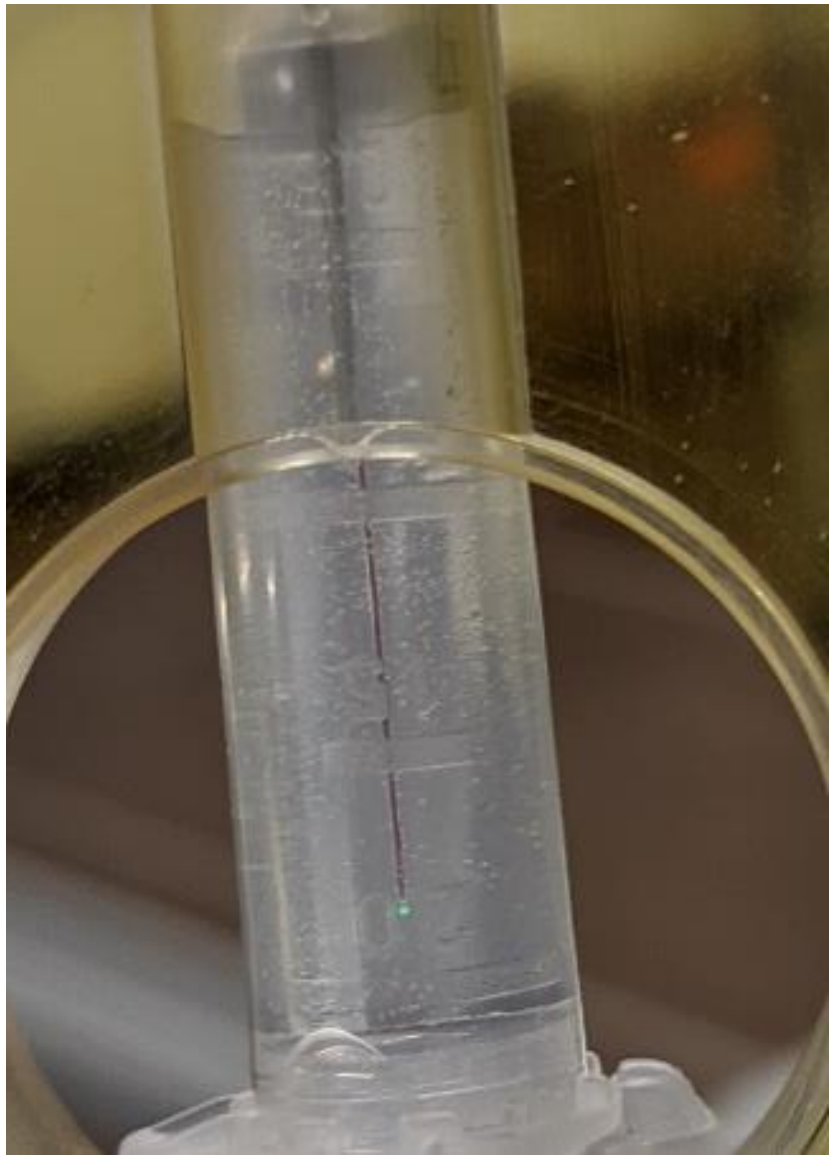


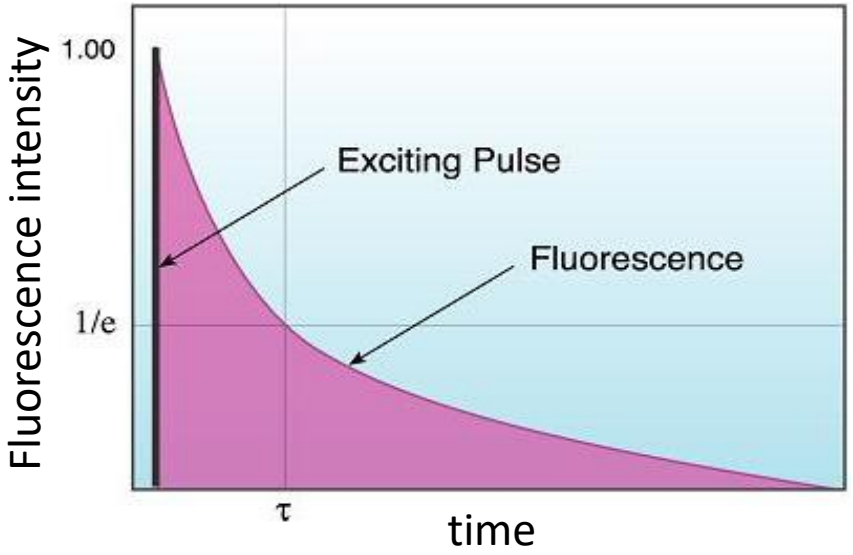
OxyLite Pro XL monitor

LabChart software



The principle of operation: Oxygen quenched fluorescence lifetime

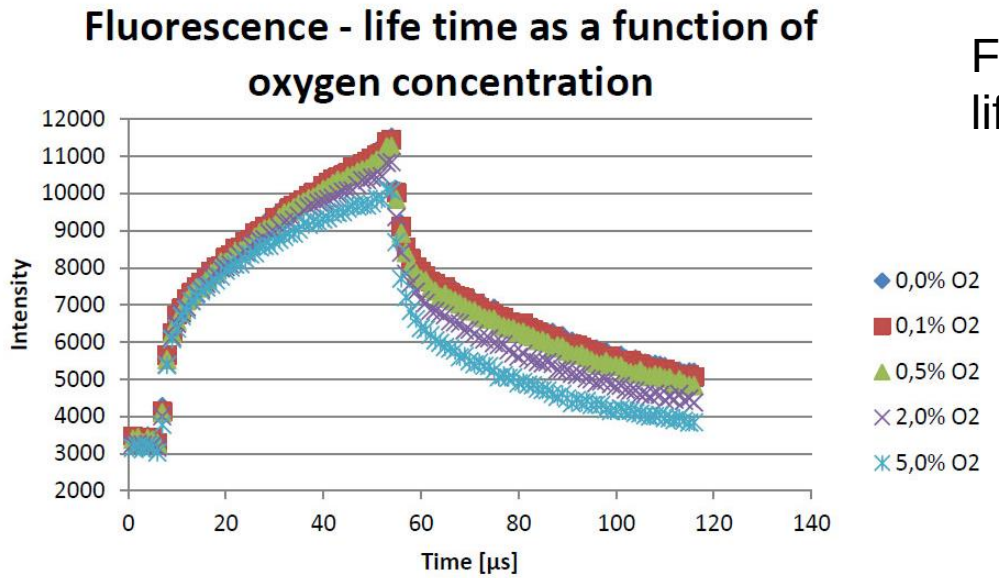




Oxygen concentration ↑

↓

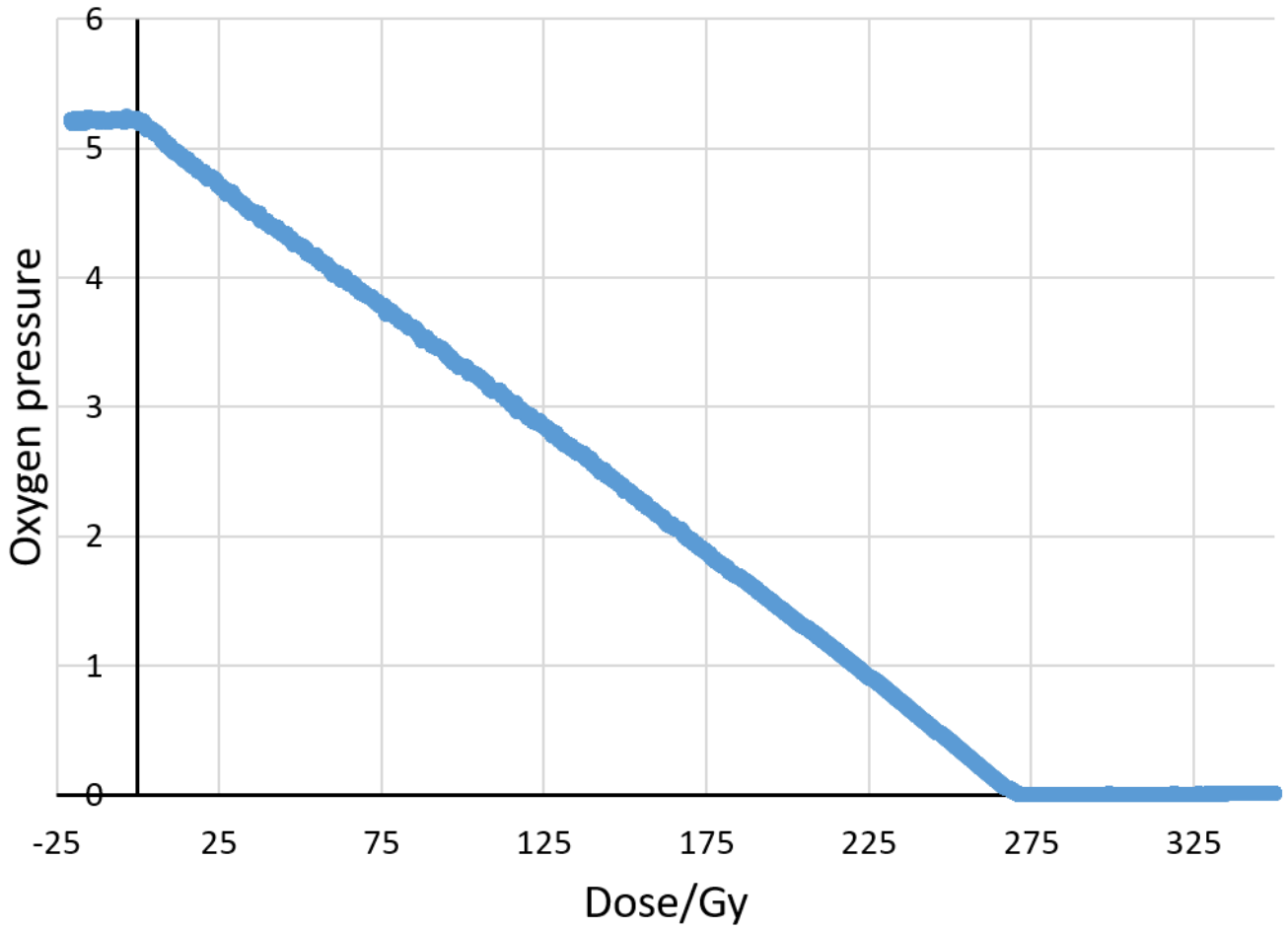
Fluorescence lifetime ↓



Oxygen depletion in water by CONV radiation (7 Gy/min)

Starting oxygen pressure = 5%

*1% O_2 = 7.6 mmHg

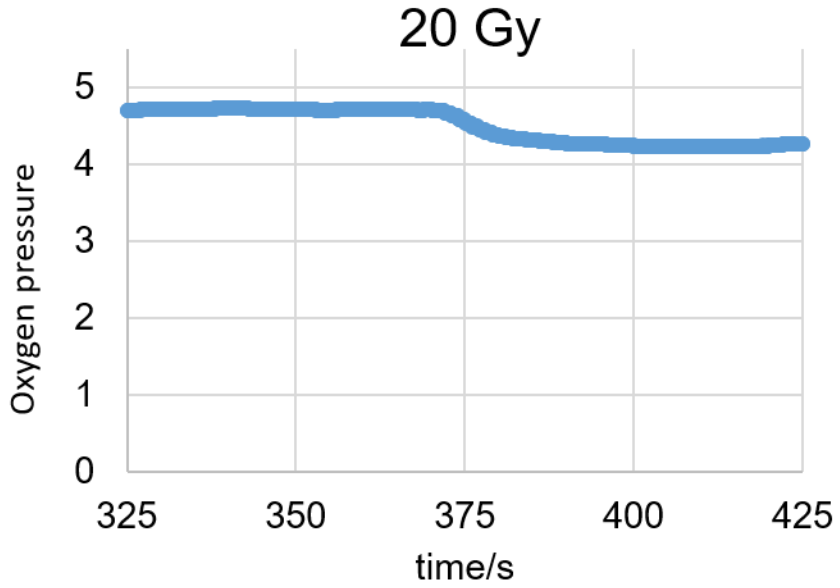


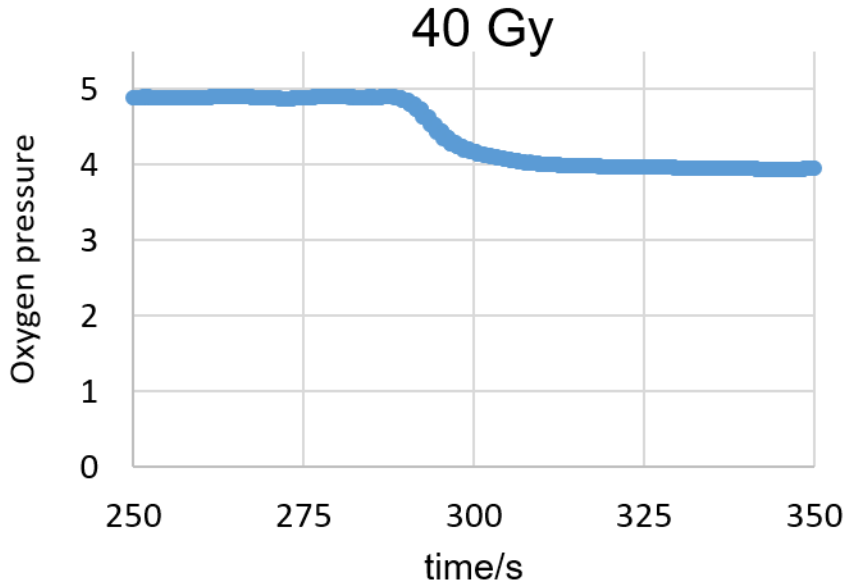
Depletion rate: 2.2% O_2 per 100 Gy

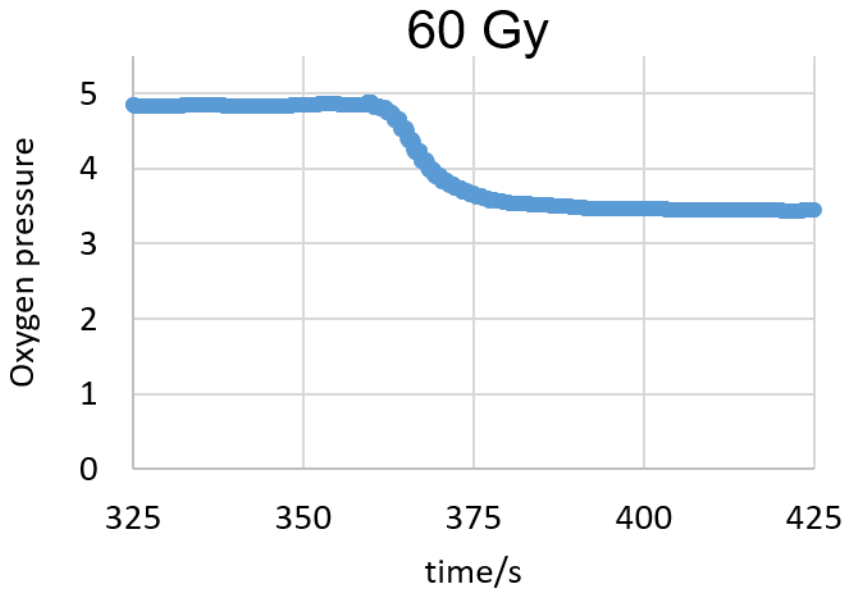
- No dependance on the starting O_2 pressure

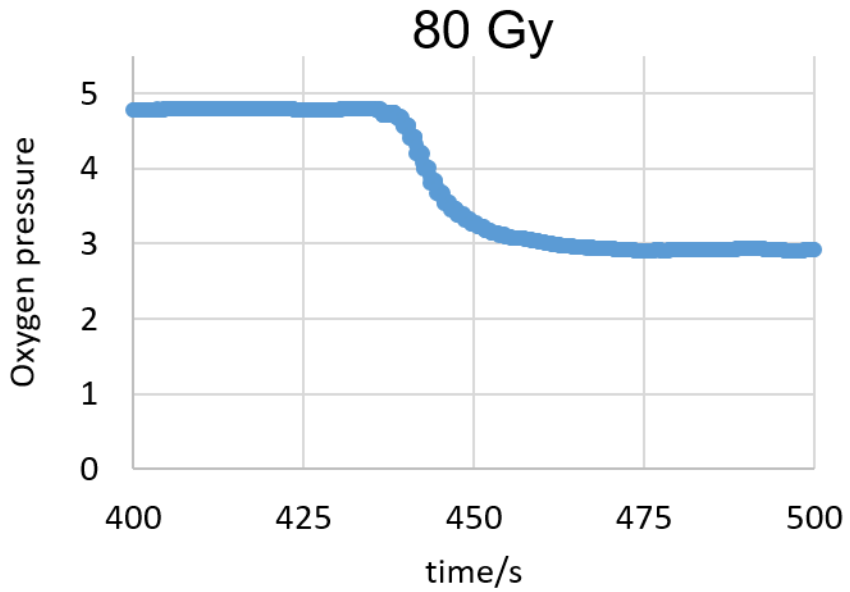
Oxygen depletion in water by FLASH radiation (>500 Gy/s)

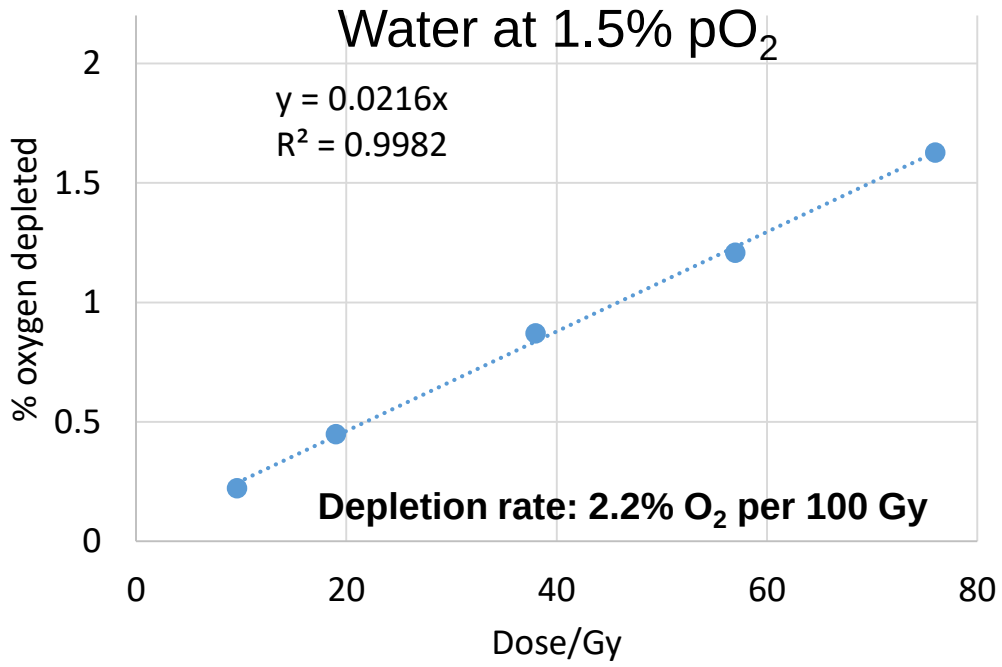
Starting oxygen pressure = 5%

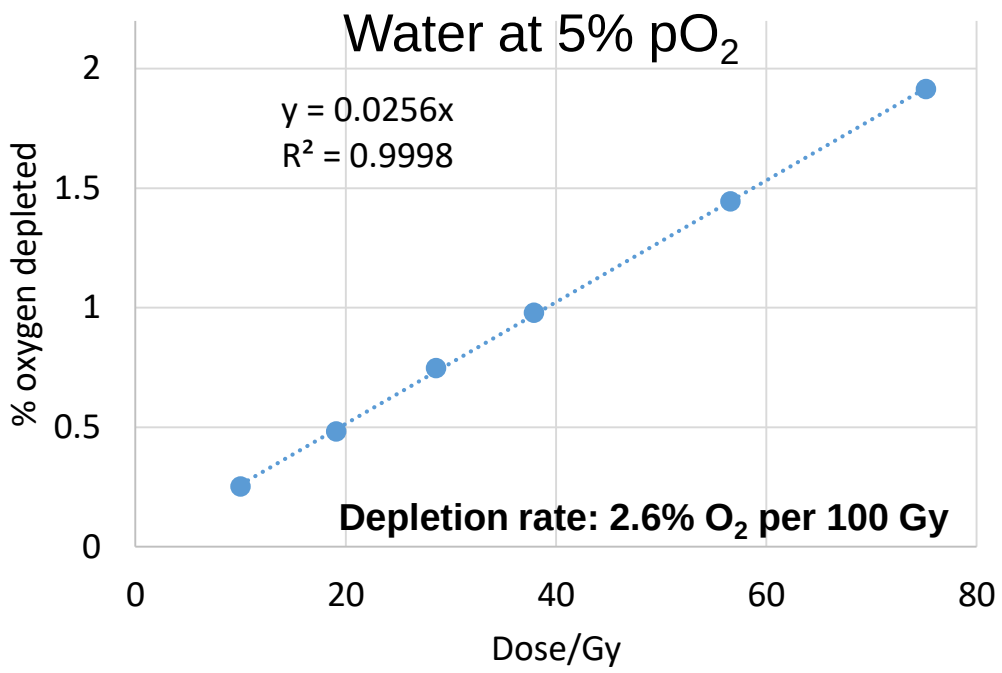


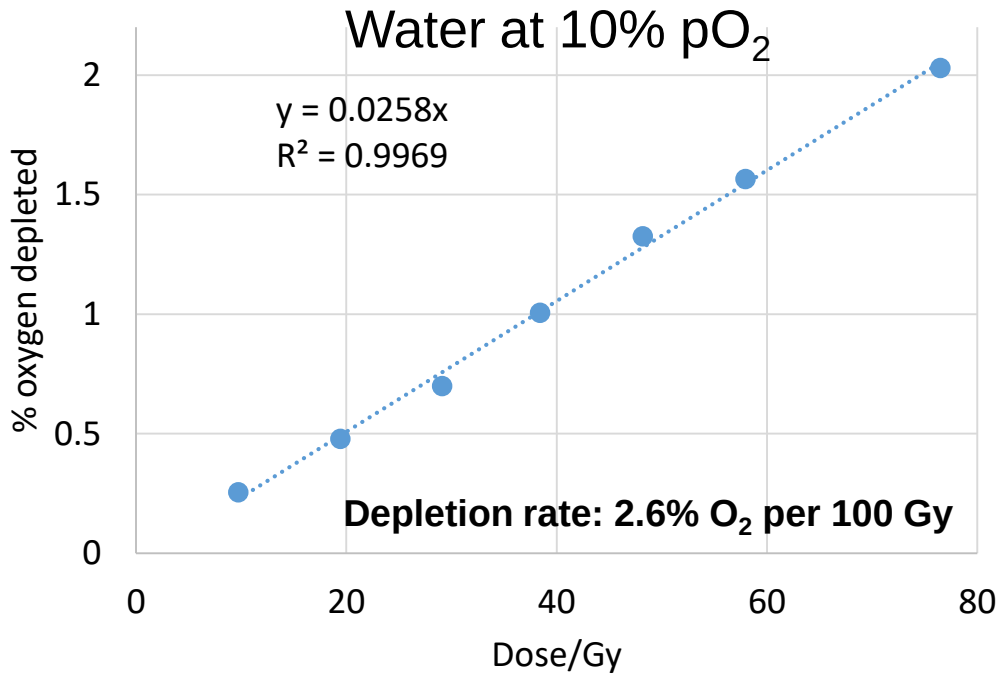


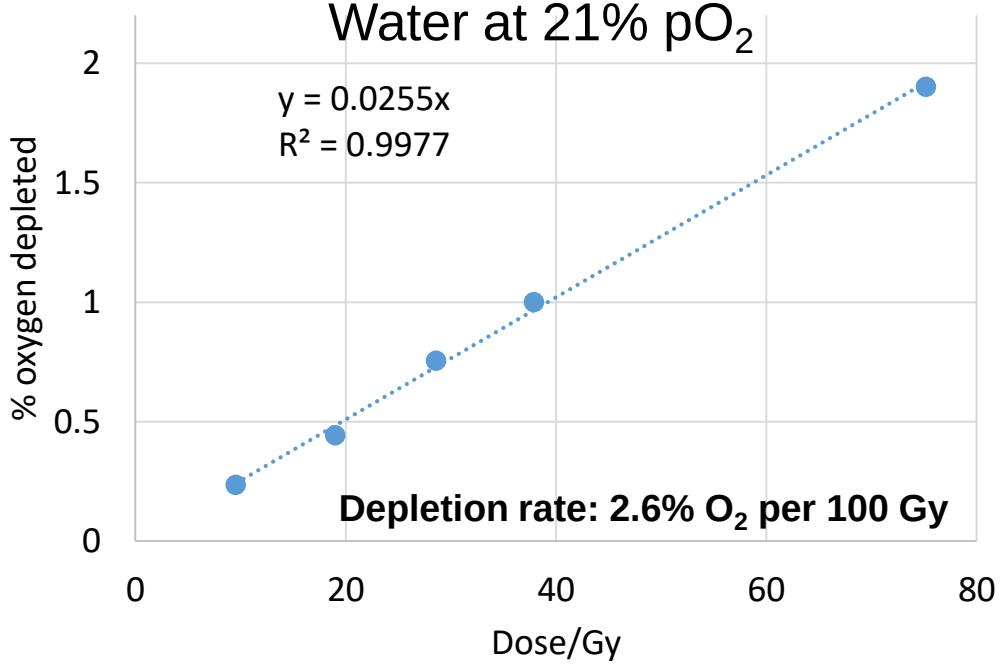




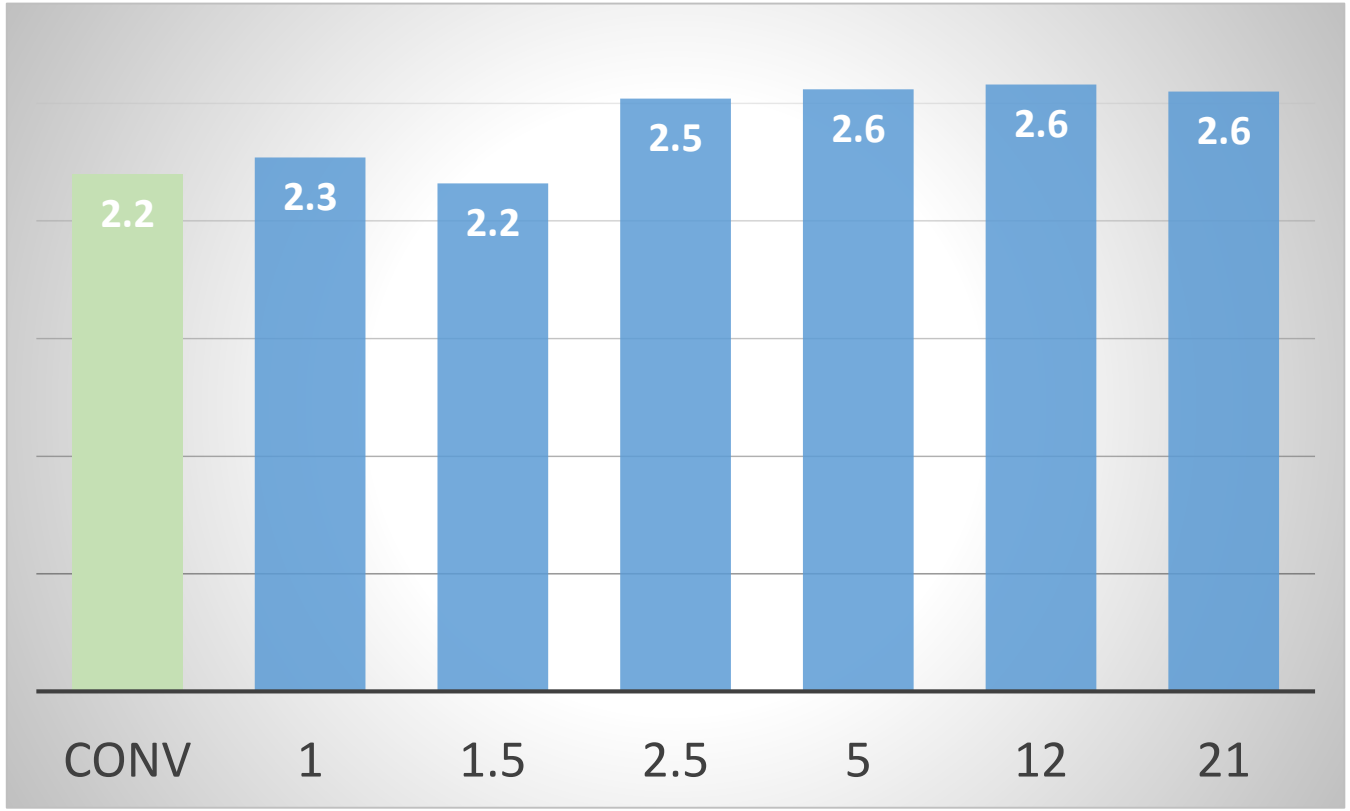

Water at 1.5% pO2
y = 0.0216x
R² = 0.9982
Depletion rate: 2.2% O2 per 100 Gy
% oxygen depleted
Dose/Gy
0
0.5
1
1.5
2
0
20
40
60
80


Water at 5% pO2
y = 0.0256x
R² = 0.9998
Depletion rate: 2.6% O2 per 100 Gy
% oxygen depleted
Dose/Gy
0
0.5
1
1.5
2
0
20
40
60
80


Water at 10% pO2
y = 0.0258x
R² = 0.9969
Depletion rate: 2.6% O2 per 100 Gy
% oxygen depleted
Dose/Gy
0
0.5
1
1.5
2
0
20
40
60
80


Water at 21% pO2
y = 0.0255x
R² = 0.9977
Depletion rate: 2.6% O2 per 100 Gy
% oxygen depleted
Dose/Gy
0
0.5
1
1.5
2
0
20
40
60
80

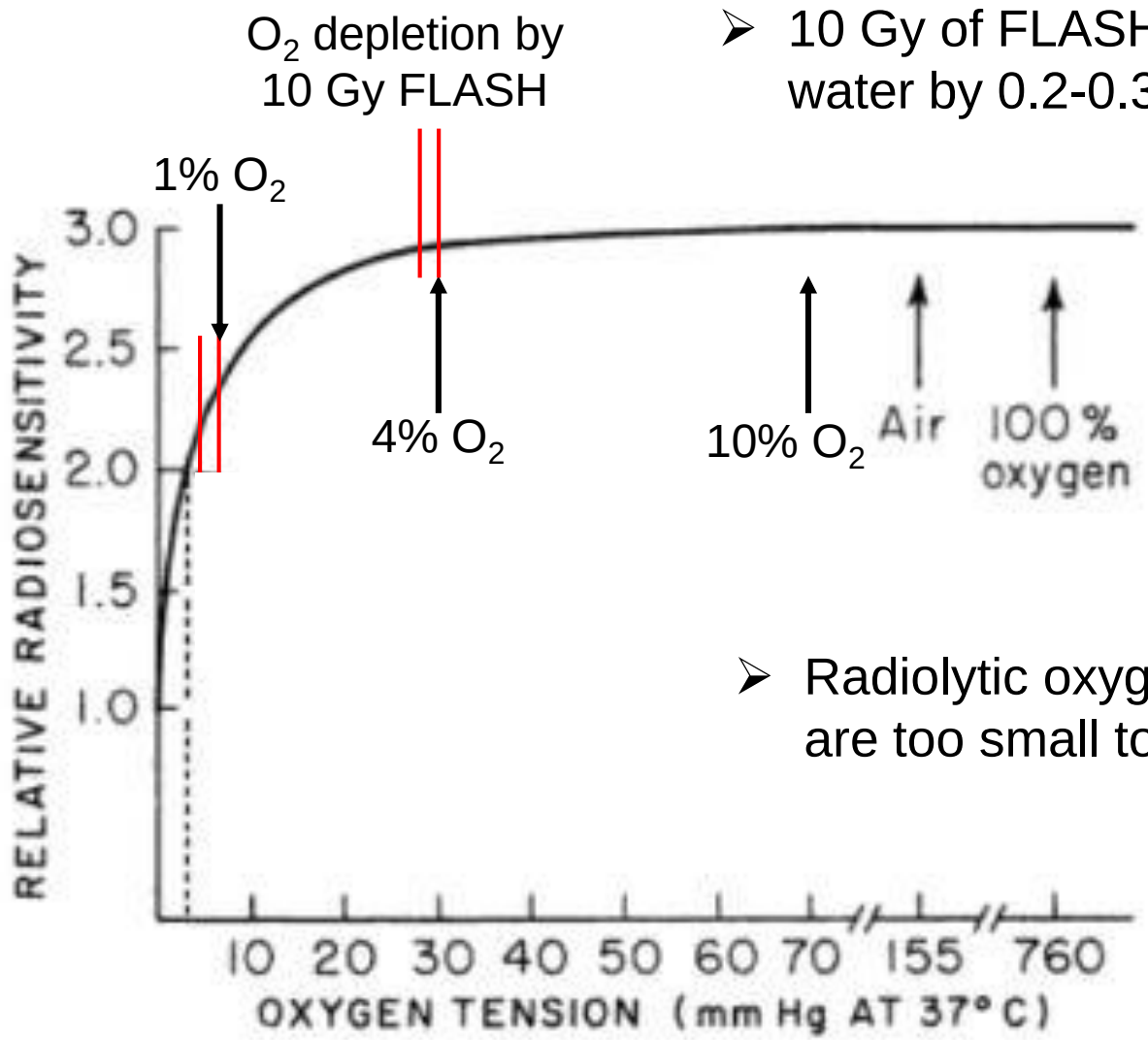
Oxygen depletion in water by FLASH and Conv
Summary of depletion rates



Radiation	% O_2 depleted per 100 Gy
CONV, 7 Gy/min	2.2
CONV, 14 Gy/min	2.2
FLASH 1%	2.3
FLASH 1.5%	2.2
FLASH 2.5%	2.5
FLASH 5%	2.6
FLASH 10 %	2.6
FLASH 21%	2.6

- Depletion rates seem to be too low for significant biological impact
- FLASH depletes about 20% more O_2 than Conv for pO_2 >2%

Oxygen depletion hypothesis:
In light of experimental results from pure water



- 10 Gy of FLASH reduces pO_2 in water by 0.2-0.3%

- Radiolytic oxygen depletion yields in water are too small to account for the FLASH effect

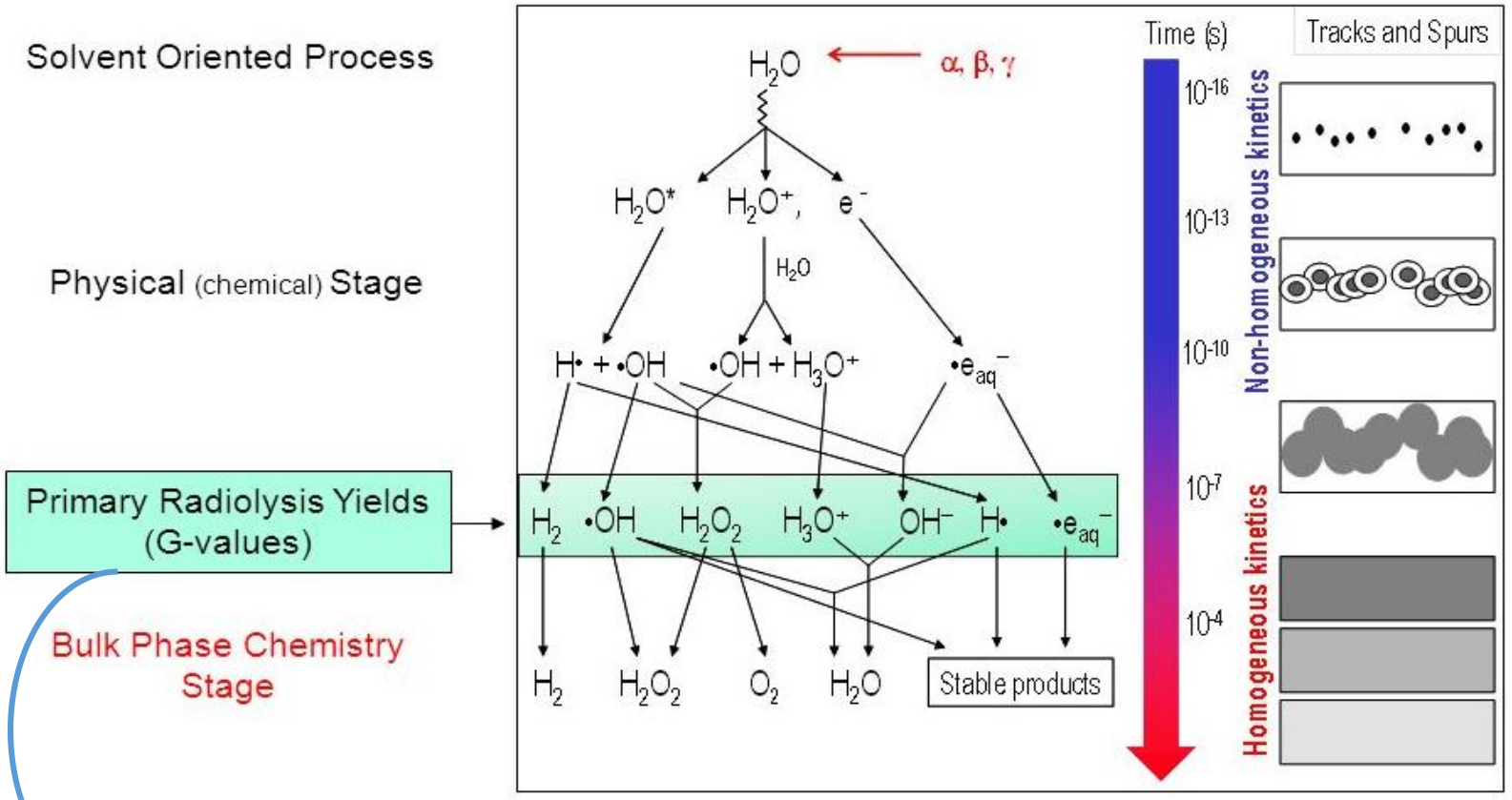
Houda Kacem

Radiolytic Yield of H2O2 in Water during Conv and FLASH Irradiation

Primary radiolytic yields

$G_0(H_2O_2)$

Water Radiolysis



$G_0(H_2O_2)$ determined at the beginning of the homogeneous phase

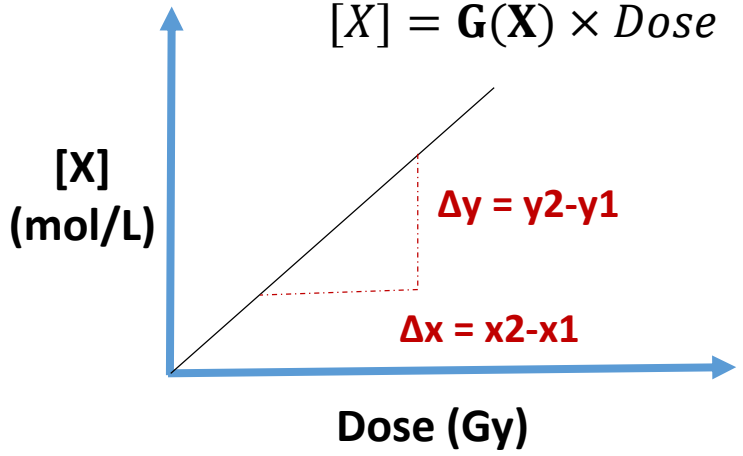
Radiolytic yield (G-value):

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

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

Measurement of G-values:

$$[X] = \mathbf{G}(\mathbf{X}) \times Dose$$



Hydrogen peroxide reactions in water radiolysis
Rate constants

- Production:

$\bullet OH + \bullet OH \rightarrow H_2O_2$, $k = 0.6 \times 10^{10}\ M^{-1}s^{-1}$

- Removal:

$H_2O_2 + \bullet OH \rightarrow \bullet HO_2 + H_2O$, $k = 2.7 \times 10^{7}\ M^{-1}s^{-1}$

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

$H_2O_2 + \bullet H \rightarrow \bullet OH + H_2O$, $k = 9.0 \times 10^{7}\ M^{-1}s^{-1}$

- **Need to introduce <u>scavengers</u>:** Chemical agents to protect the formation of H_2O_2 by scavenging the production of free radicals.

Empirical model to determine the $G_0(H_2O_2)$: The scavenging method

•OH + •OH $\rightarrow \mathbf{H_2O_2}$, $k = 0.6 \times \mathbf{10^{10}}\ \boldsymbol{M^{-1}s^{-1}}$

•OH + S → P

[S] ↑ → $t_s = \frac{1}{[S] \times k_s}$ ↓ → •OH is scavenged at earlier time → $G(H_2O_2)$ ↓

[S1] < [S2] < [S3] < [S4]

$\mathbf{G1}(H_2O_2) > \mathbf{G2}(H_2O_2) > \mathbf{G3}(H_2O_2) > \mathbf{G4}(H_2O_2)$

*Sworski, T. J. Yields of Hydrogen Peroxide in the Decomposition of Water by Cobalt γ-Radiation. I. Effect of Bromide Ion. *J. Am. Chem. Soc.* **76**, 4687–4692 (1954). Wasselin-Trupin, V., **Baldacchino, G., Bouffard, S. & Hickel, B. Hydrogen peroxide yields in water radiolysis by high-energy ion beams at constant LET. *Radiation Physics and Chemistry* **65**, 53–61 (2002).

Experimental conditions:
Sample composition

$[NaNO_2]$ = 1 / 5 / 10 / 20 & 40 µM

$[NaNO_3]$ = 1 mM

- Aqueous samples of various $[NaNO_2]$ + fixed $[NaNO_3]$ saturated at 1-2% O2 (Hypoxia hood)
  - To scavenge •OH:

$$NO_2^- + \bullet OH \rightarrow NO_2\bullet + OH^-, \quad k = 0.8 \times 10^{10}\ M^{-1}s^{-1}$$

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

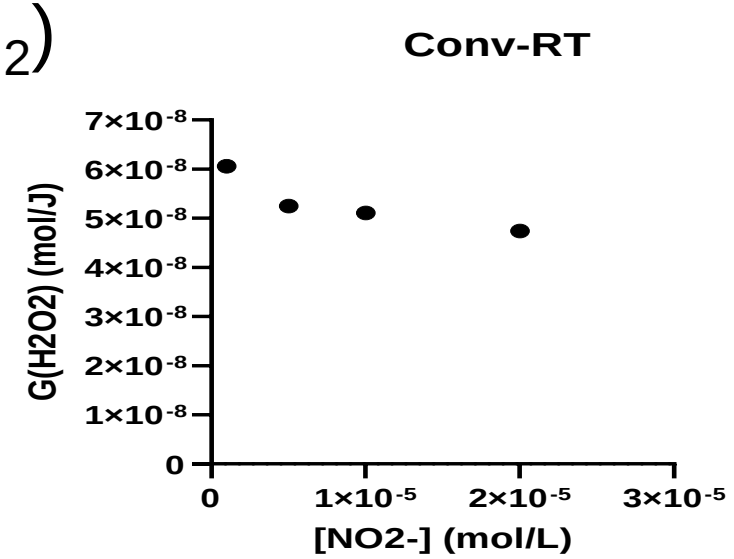
$$H_2O_2 + \bullet OH \rightarrow \bullet HO_2 + H_2O, \quad k = 2.7 \times 10^{7}\ M^{-1}s^{-1}$$

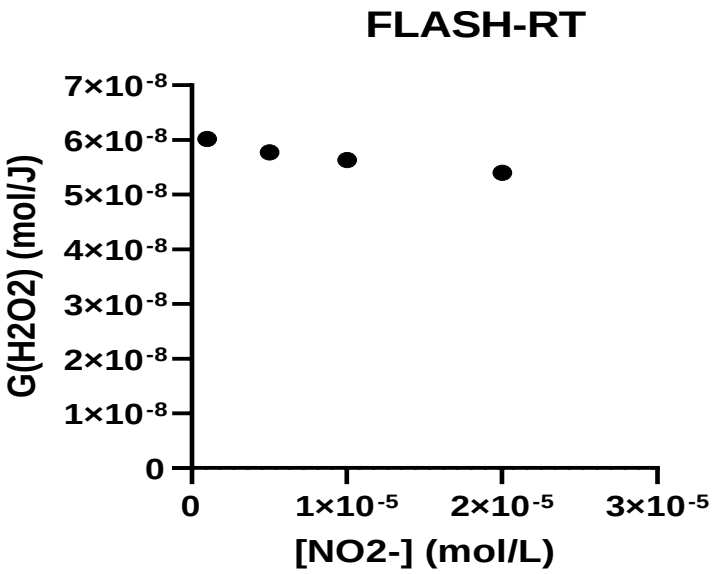
  - To scavenge e(aq):

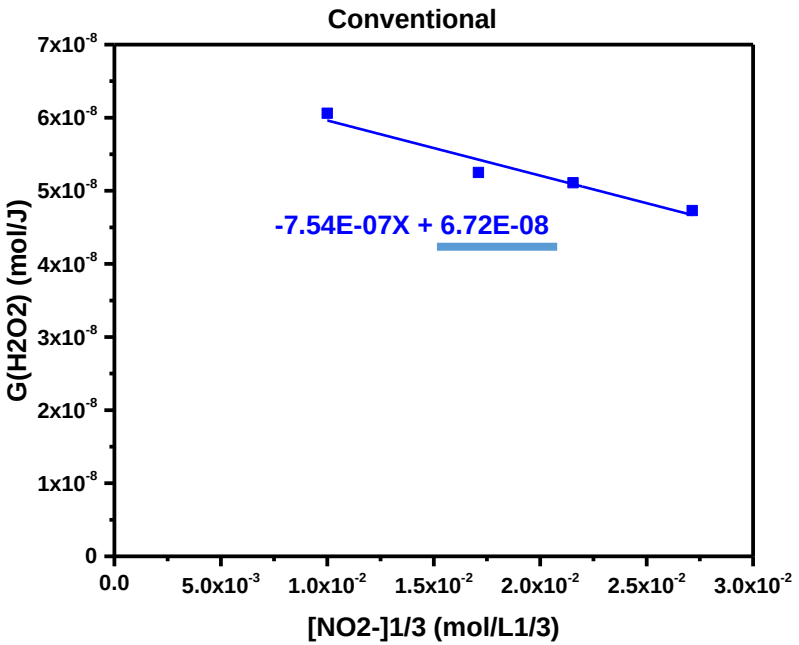
$$NO_3^- + e(aq)^- \rightarrow NO^{2-}_3, \quad k = 1 \times 10^{10}\ M^{-1}s^{-1}$$

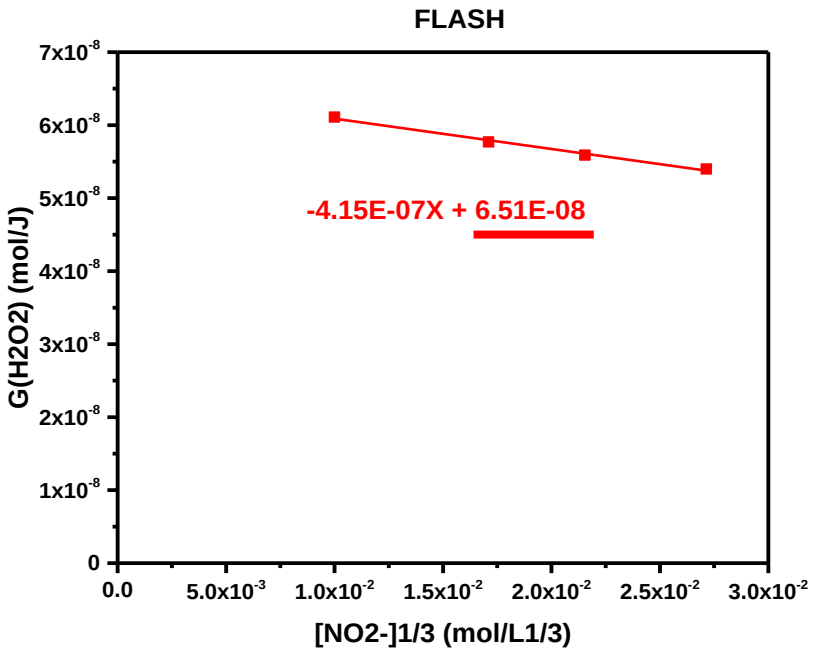
$$H_2O_2 + e(aq^-) \rightarrow \bullet OH + OH^-, \quad k = 1.1 \times 10^{10}\ M^{-1}s^{-1}$$

Results
$G_0(H_2O_2)$









Conclusions:
No indication of reduced primary radical yield after FLASH

- $G(H_2O_2)$ values with FLASH less sensitive to scavenger concentration compared to Conv
- $G_0(H_2O_2)$ is similar for FLASH and Conv modalities.

	FLASH	**Conv**
$G_0(H_2O_2)$ (mol/J)	0.651 ± 0.002	0.672 ± 0.002

3% diffrence

- No support found for lower primary radical production with FLASH
- Lower yields of H_2O_2 after FLASH at 4% O_2 explained by higher O_2 depletion rate (similar difference of 20% in both cases)?

Radio-Oncology lab

MC Vozenin
P Montay-Gruel
B Petit
J Ollivier
I Petridis
H Kacem
P Barrera
N Cherbuin
R Leavitt
V To
A Almeida

Radiation-Oncology

J Bourhis
W Jeanneret
M Ozsahin

Surgery
N Demartines
D Clerc
C Simon
K Lambercy

IRA

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C Bailat
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R Moeckli
T Buchillier
M Gondre

K Petersson
M Jaccard

Charles Limoli

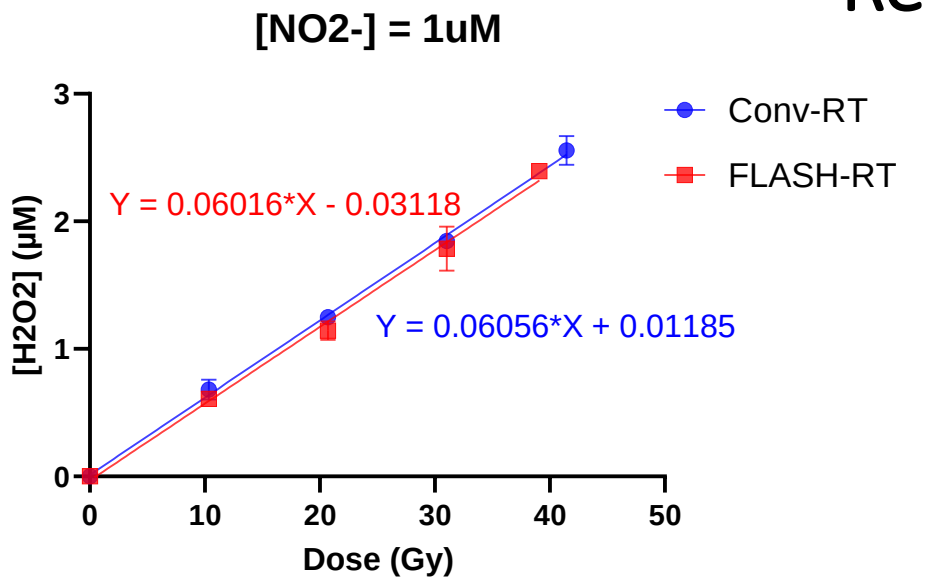
Doug Spitz

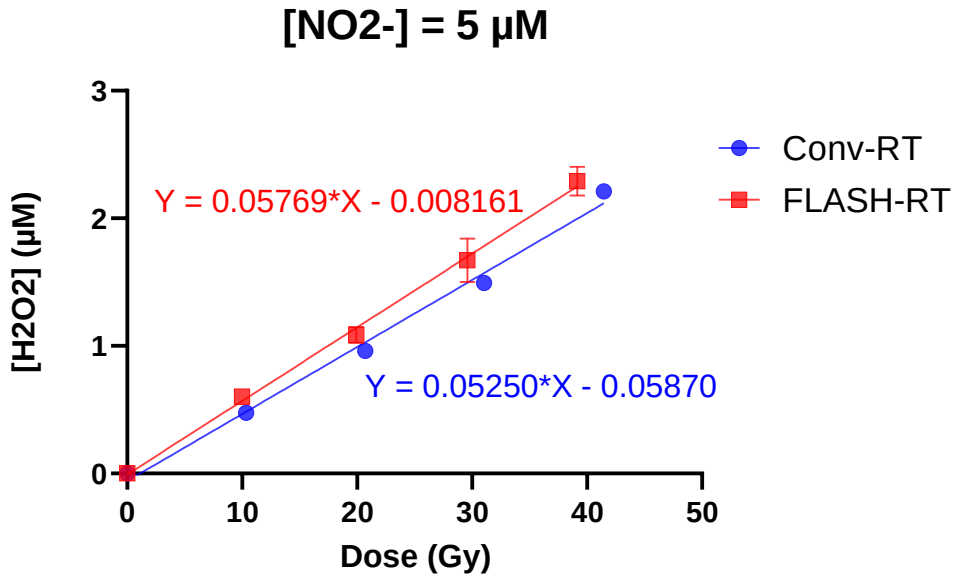
Animal Facility of Epalinges

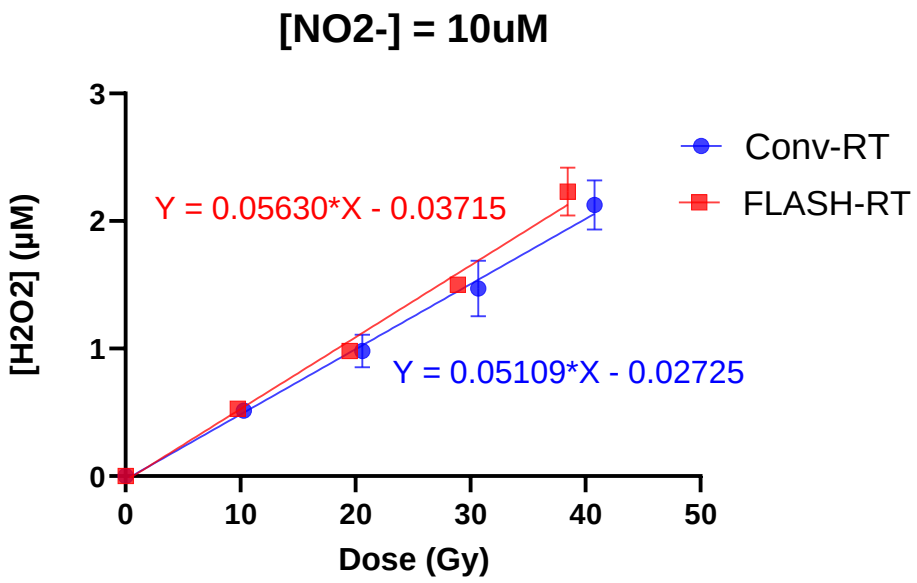
Fond'action
FNS N°31003A_156892
FNS/ANR CR32I3L_156924
ISREC Foundation thank to Biletema donation
PO1CA244091

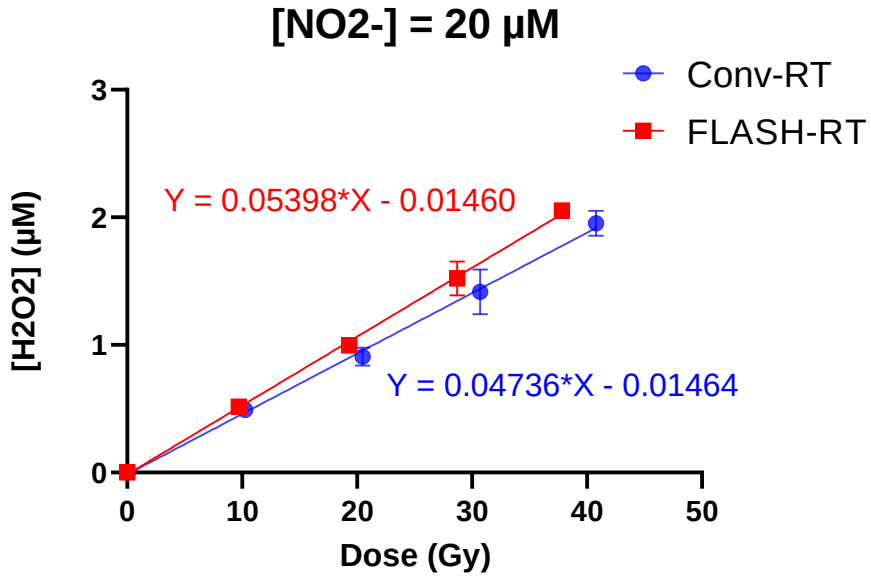
Thank You

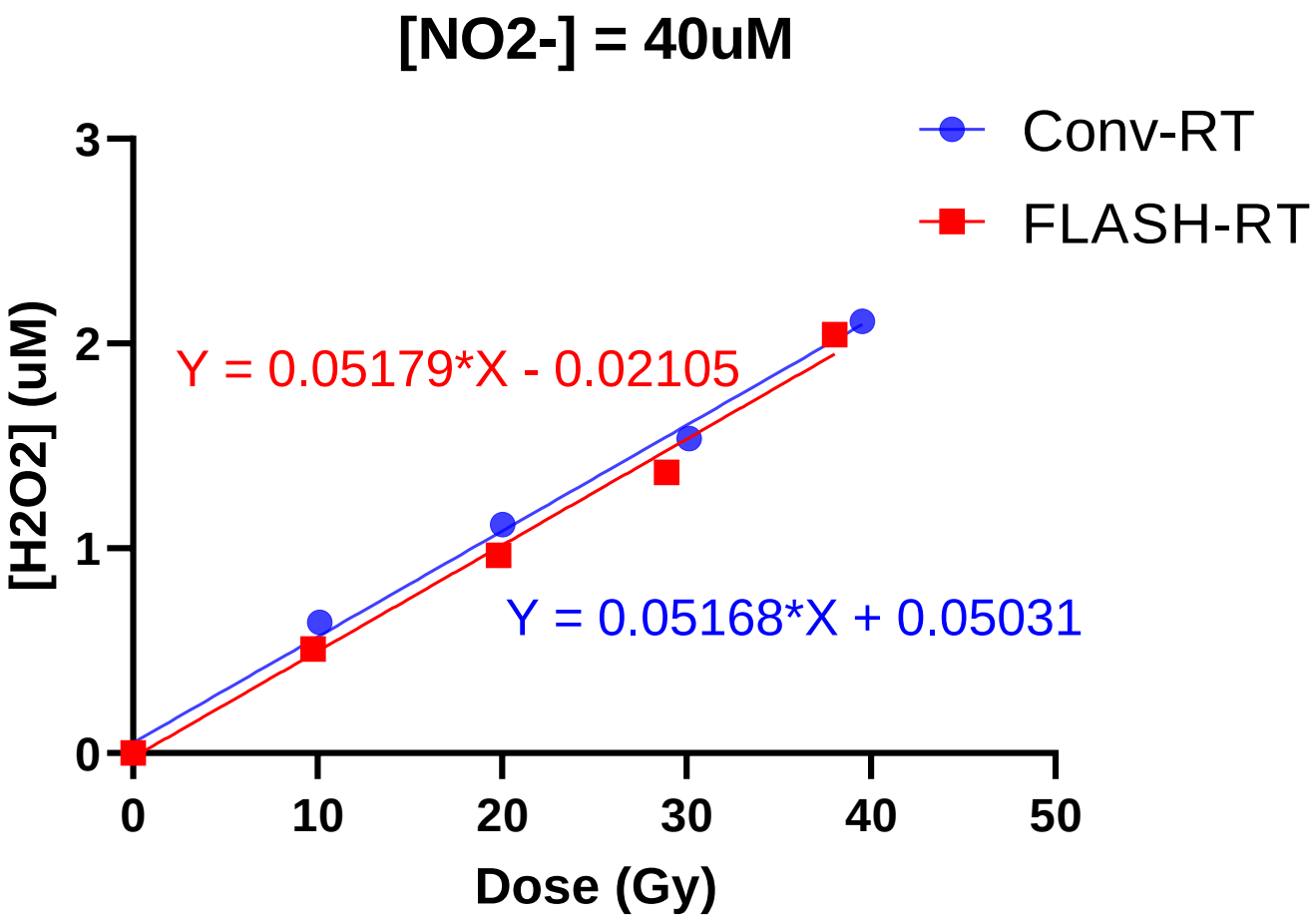
Results


[NO2-] = 1uM
Conv-RT
FLASH-RT
Y = 0.06016*X - 0.03118
Y = 0.06056*X + 0.01185
[H2O2] (μM)
Dose (Gy)
0
1
2
3
0
10
20
30
40
50


[NO2-] = 5 μM
Conv-RT
FLASH-RT
Y = 0.05769*X - 0.008161
Y = 0.05250*X - 0.05870
[H2O2] (μM)
Dose (Gy)
0
1
2
3
0
10
20
30
40
50


[NO2-] = 10uM
Conv-RT
FLASH-RT
Y = 0.05630*X - 0.03715
Y = 0.05109*X - 0.02725
[H2O2] (μM)
Dose (Gy)
0
1
2
3
0
10
20
30
40
50


[NO2-] = 20 μM
Conv-RT
FLASH-RT
Y = 0.05398*X - 0.01460
Y = 0.04736*X - 0.01464
[H2O2] (μM)
Dose (Gy)
0
1
2
3
0
10
20
30
40
50


[NO2-] = 40uM
Conv-RT
FLASH-RT
Y = 0.05179*X - 0.02105
Y = 0.05168*X + 0.05031
[H2O2] (uM)
Dose (Gy)
0
1
2
3
0
10
20
30
40
50

Irradiation parameters

Modality	Dose [Gy]	GT [V]	f [Hz]	w [µs]	N pulse	Irr. Time [s]	SSD [m]	Mean dose rate [Gy/s]	Instantaneous dose rate [Gy/s]	Dose per pulse [Gy]
FLASH	10	300	100	1.95	2	0.01	0.46	1000	2.56E+06	5
	20				4	0.03		667		
	30				6	0.05		600		
	40				8	0.07		571		
CONV	10	100	10	1	860	85.9	0.6	0.12	1.16E+04	0.012
	20				1720	171.9				
	30				2580	257.9				
	40				3440	343.9				

*Mean values of five experiments

Water Radiolysis

Solvent Oriented Process

Physical (chemical) Stage

Primary Radiolysis Yields (G-values)

Bulk Phase Chemistry Stage