

Measurements of Reactive Oxygen Species production induced by Gold Nanoparticles in Radiotherapy protocols

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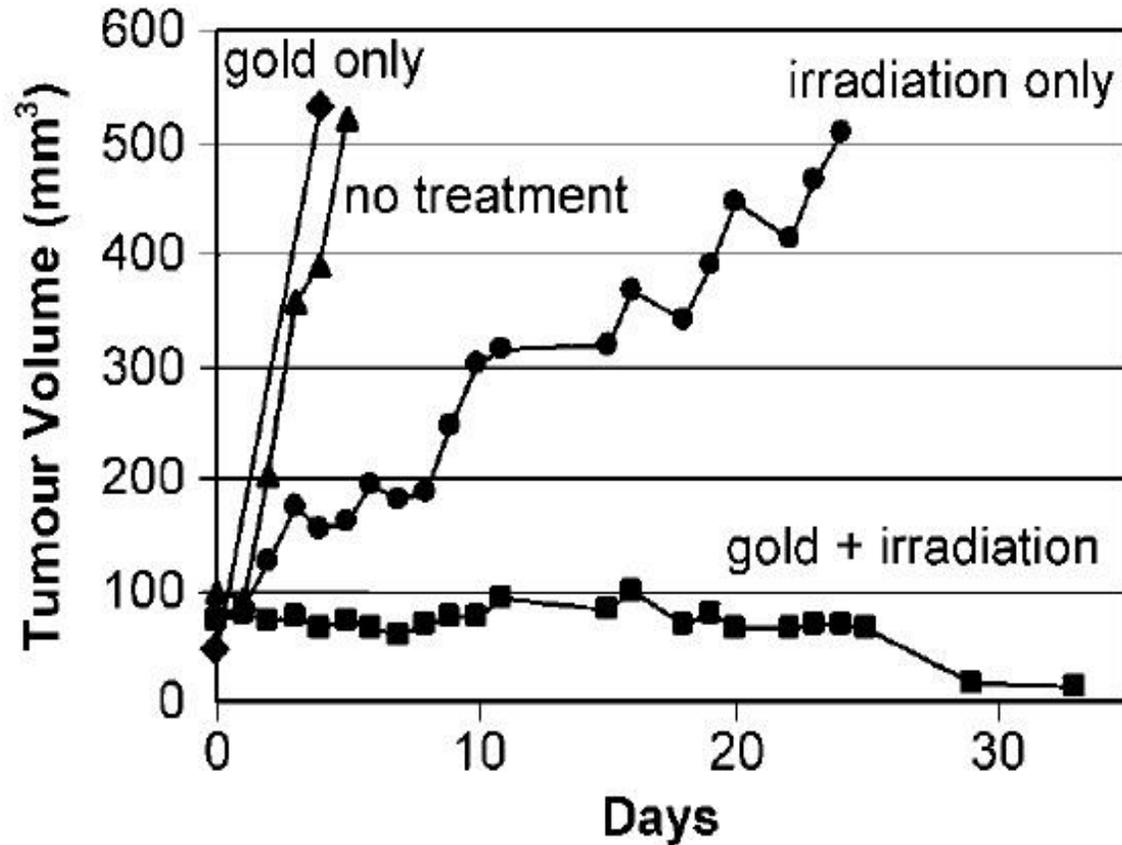
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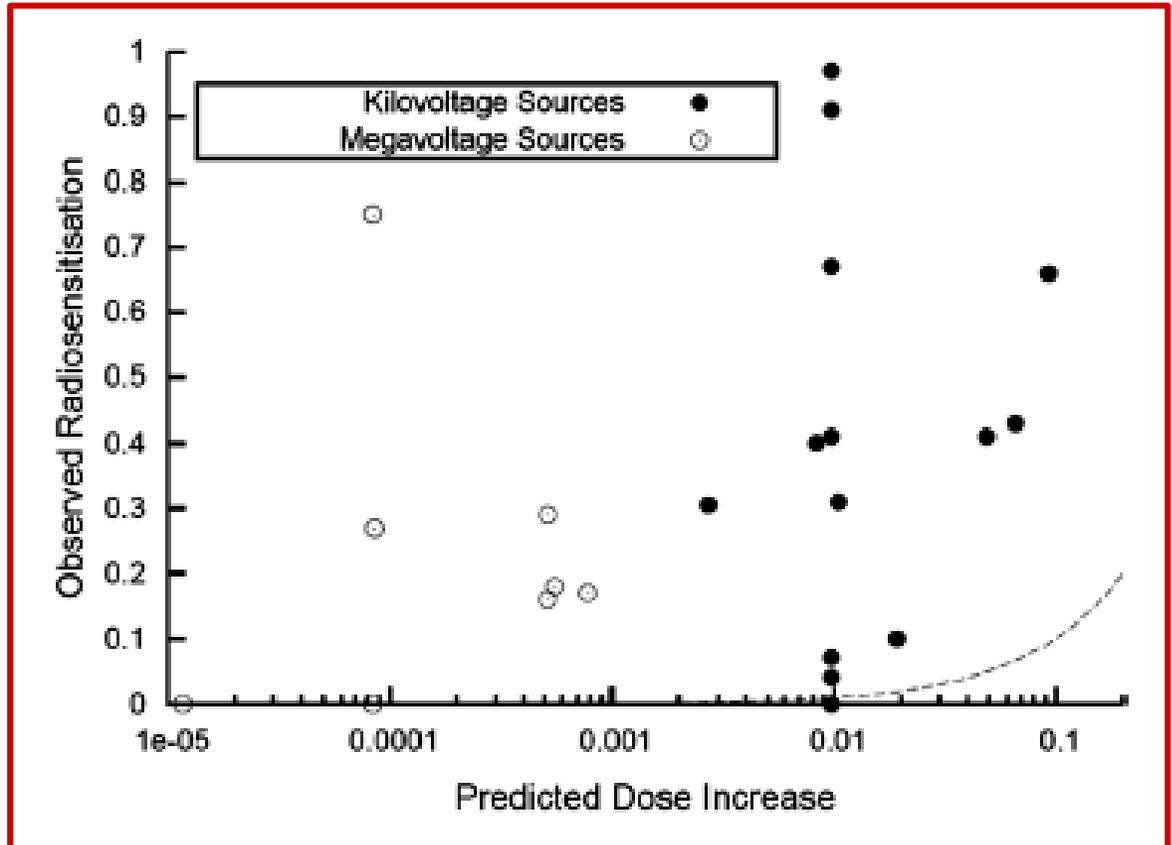
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GNP as radiosensitizers?



The use of gold nanoparticles to enhance radiotherapy in mice.
James F Hainfeld, Daniel N Slatkin and Henry M Smilowitz



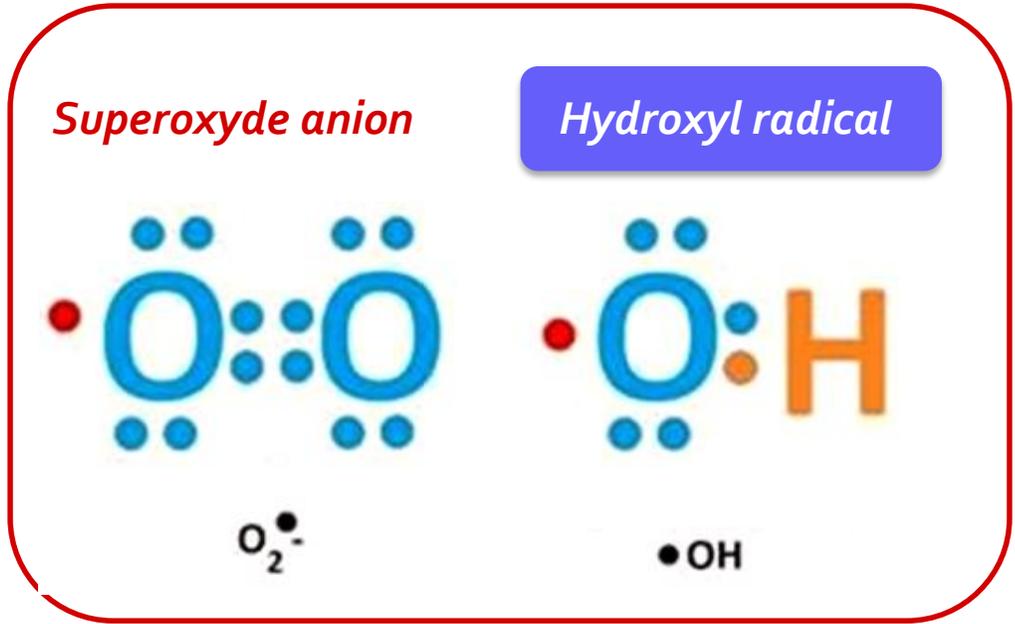
Karl T Butterworth, Stephen J McMahon, Fred J Currell, and Kevin M Prise. *Physical basis and biological mechanisms of gold nanoparticle radiosensitization*

GNP-induced Reactive Oxygen Species?

The *bio-chemical* mechanisms of GNP-induced radiosensitization are not fully understood

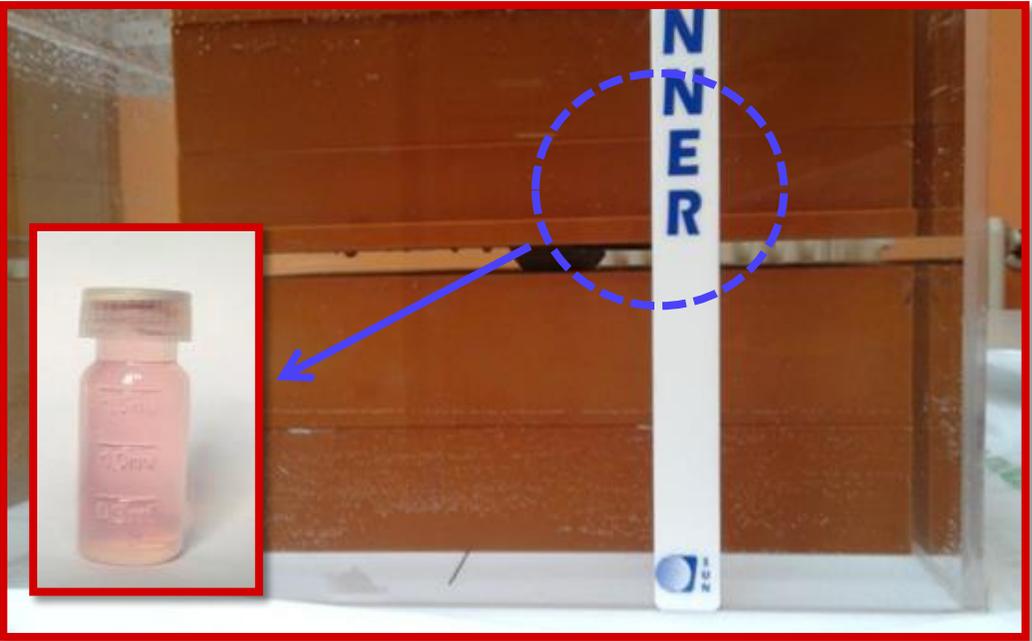


Increased ROS production?



Measured ROS

Reactive Oxygen Species Measurements



Samples containing GNPs in PBS solution
at physiological pH

pH \approx 7.35

What protocol?

Fluorescent Probes

Molecules that interact with specific ROS and thereby change their fluorescent properties

- (relatively) simple
- sensitive
- Stable
- reproducible

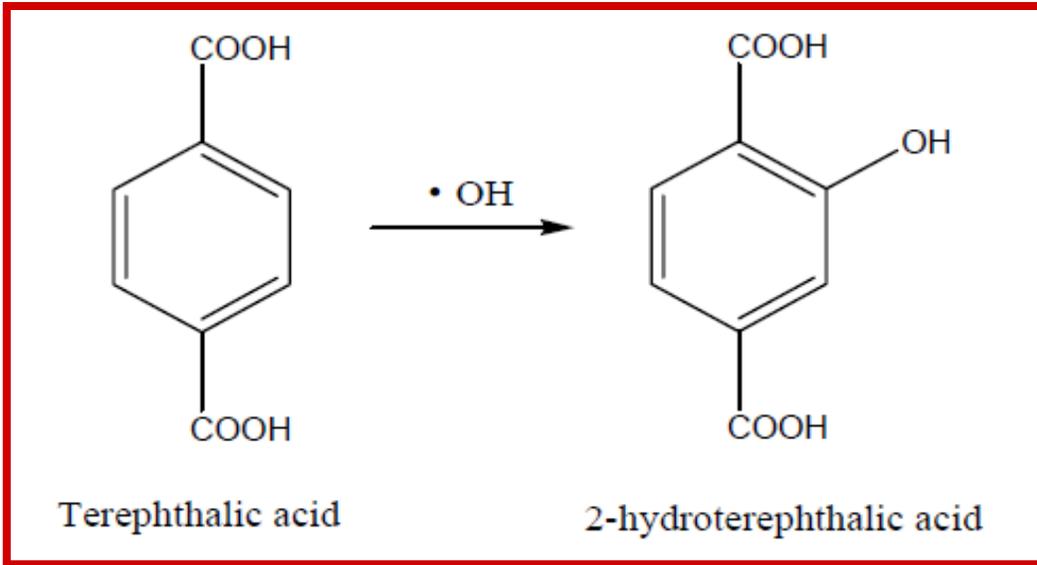
Spectrofluorometer

High sensitivity measurements of the fluorescent spectrum of samples



The selected probe: Terephthalate (TPA)

TPA quenching reaction



when interacting with the **Hydroxyl radical** it generates **HTPA (di-hydroxyl-terephthalate)**, which is fluorescent

$$\lambda_{excitation} = 310 \text{ nm}$$

$$\lambda_{emission} = 430 \text{ nm}$$

No papers in literature in which TPA is used in the foreseen experimental conditions

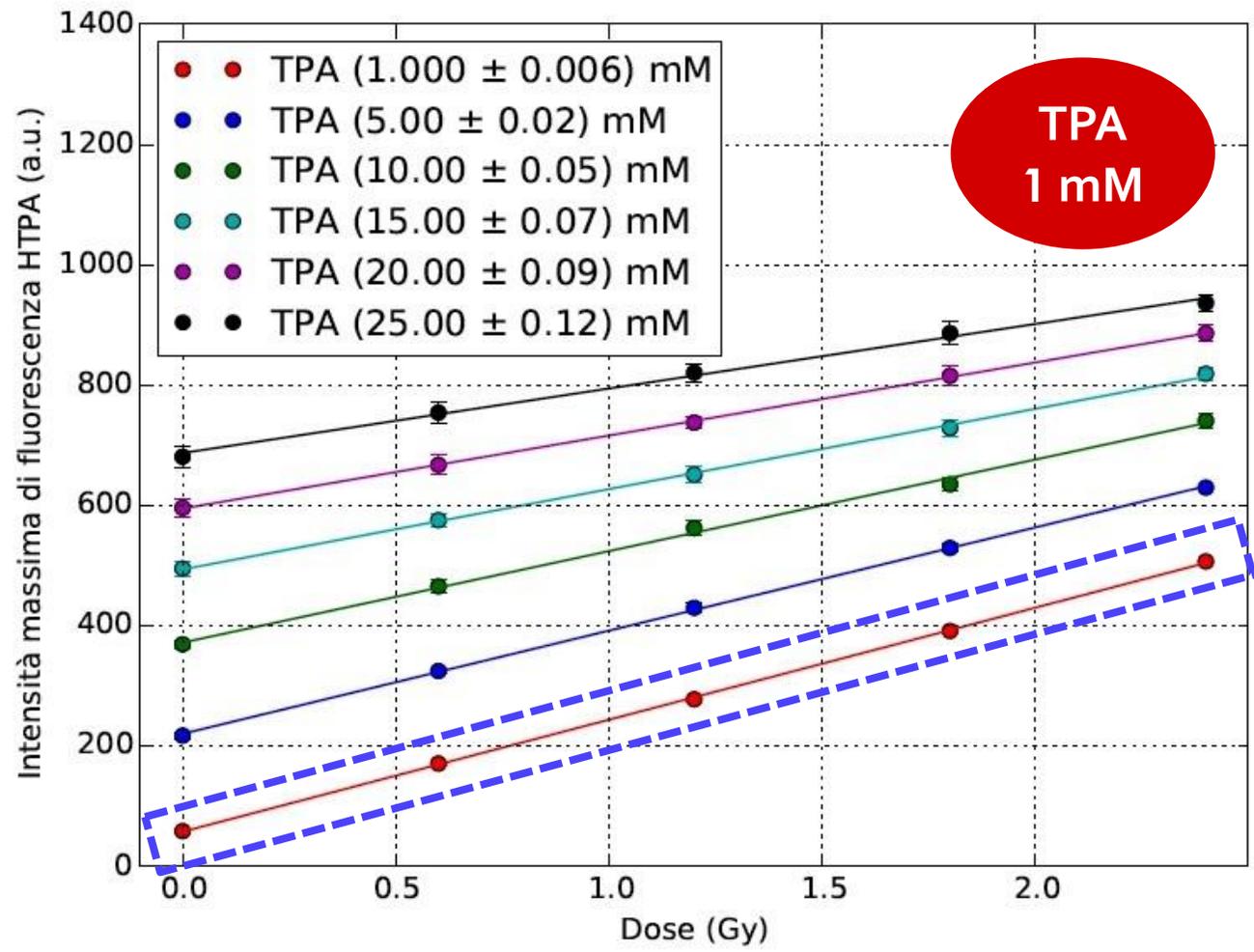
A protocol must be set up

What is the optimal TPA concentration?

- Sensitivity
- No saturation

Irradiation (with 6 MV photons) of PBS samples containing TPA in different concentration

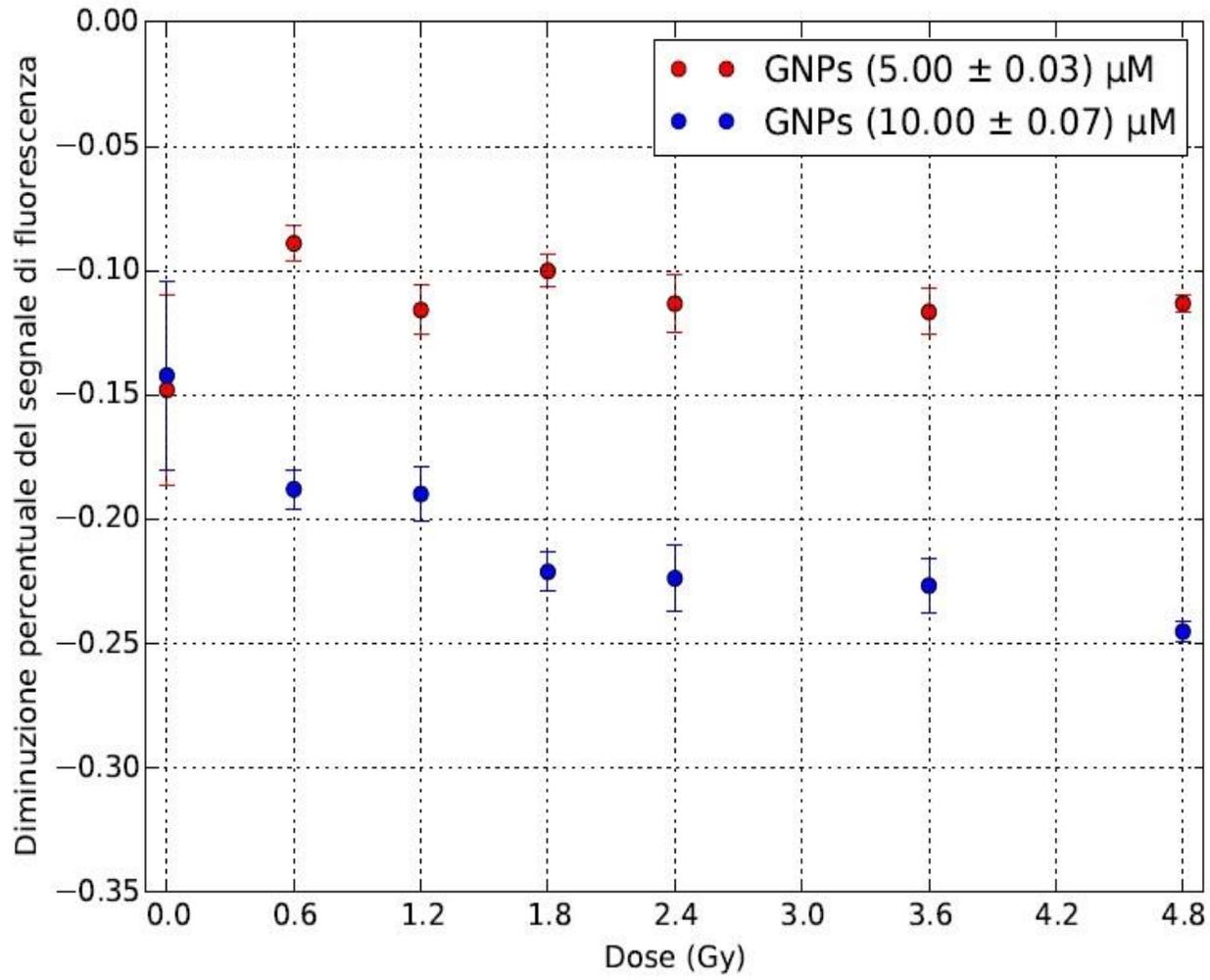
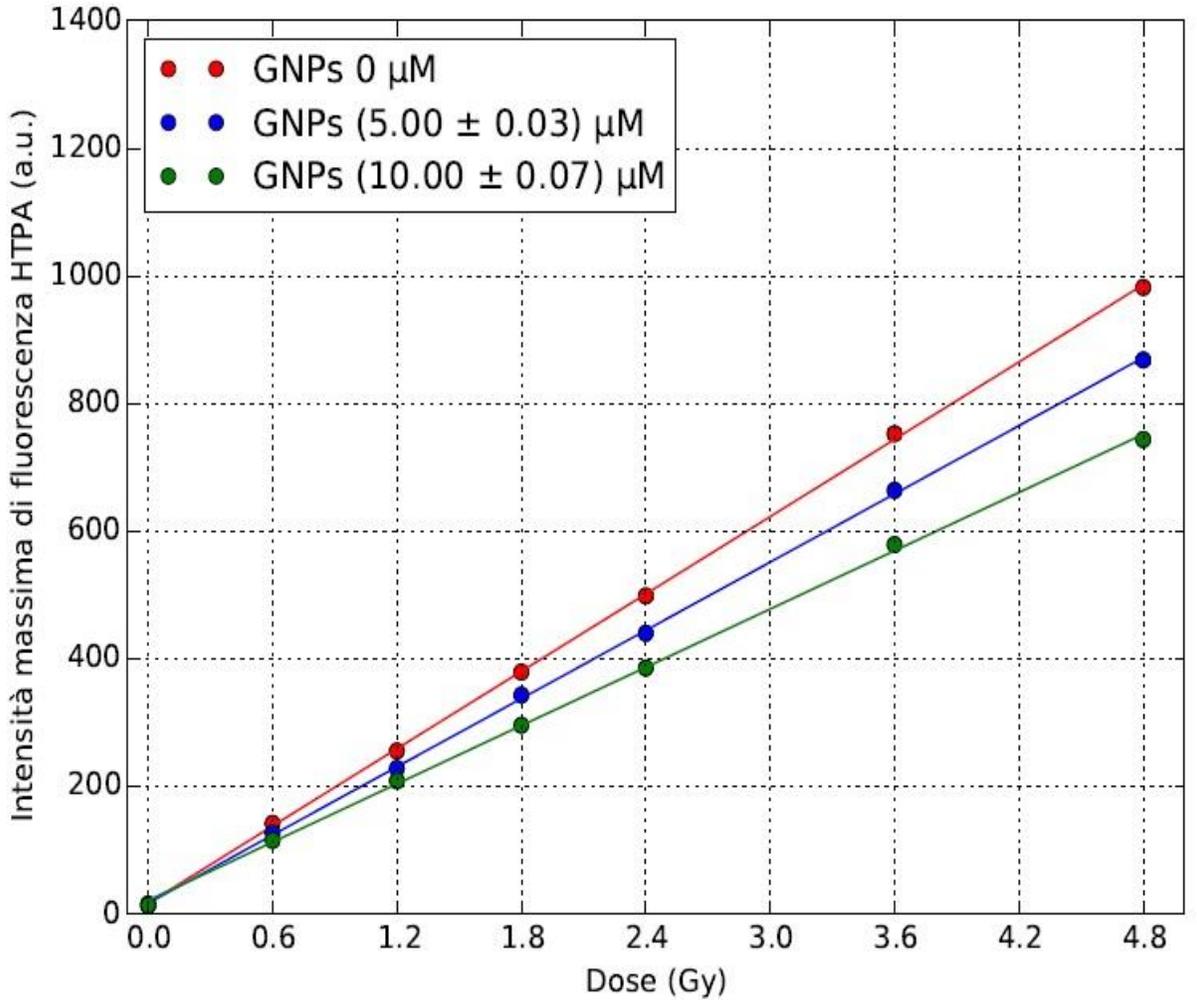
Conc TPA (mM)	Slope (a.u. nm ⁻¹)
→ 1.000 ± 0.006	186 ± 1
5.00 ± 0.02	171 ± 2
10.00 ± 0.05	150 ± 7
15.00 ± 0.07	134 ± 3
20.00 ± 0.09	122 ± 2
25.00 ± 0.12	106 ± 4



Results with GNP (d=20 nm) and 6 MV photons

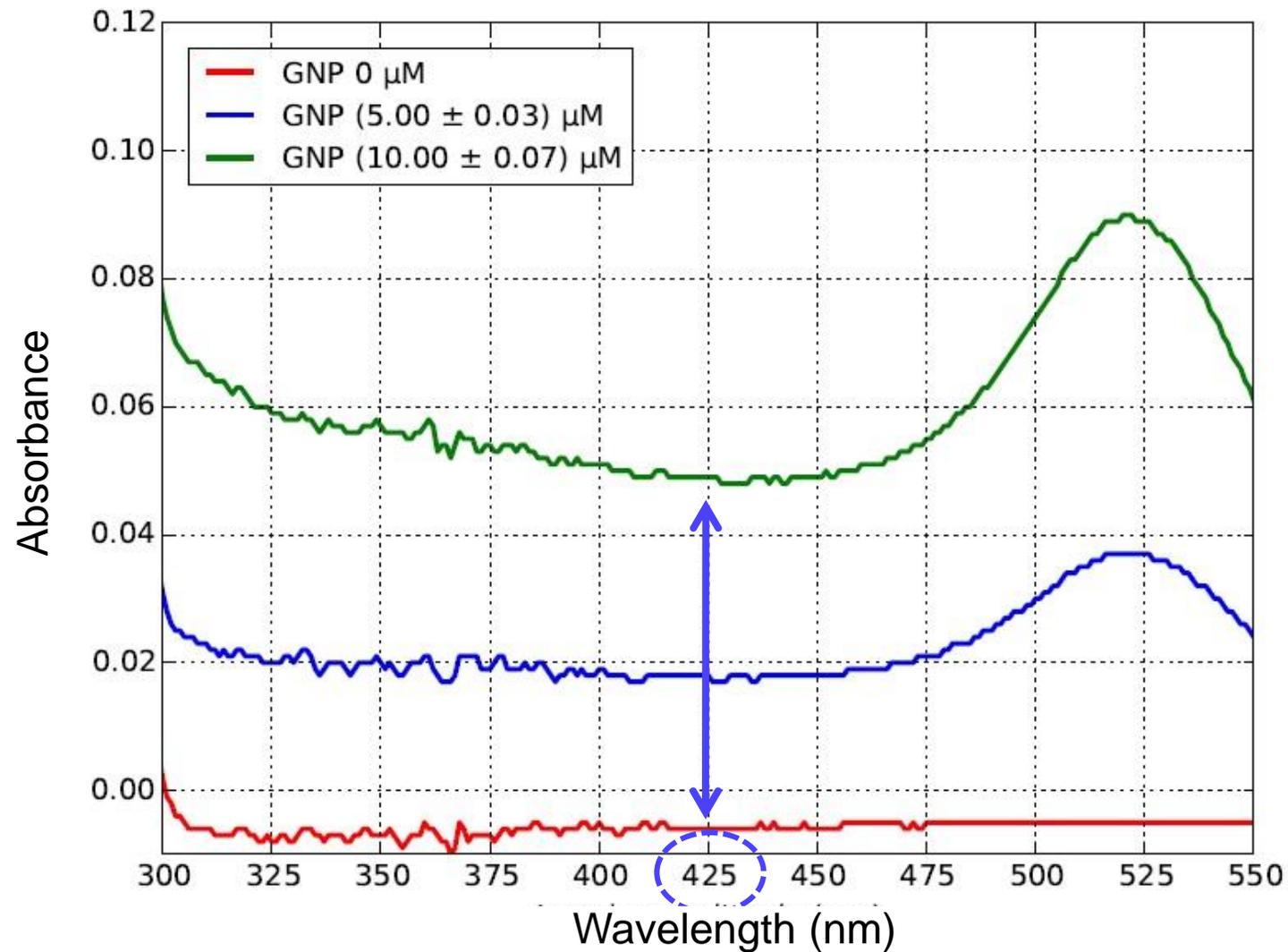
GNP cannot cause a reduction in ROS production! \Rightarrow

What correction factors should be applied to measurements?



GNP Absorption?

$$K_{abs} 5 \mu M = (0.024 \pm 0.001) \quad K_{abs} 10 \mu M = (0.054 \pm 0.001)$$



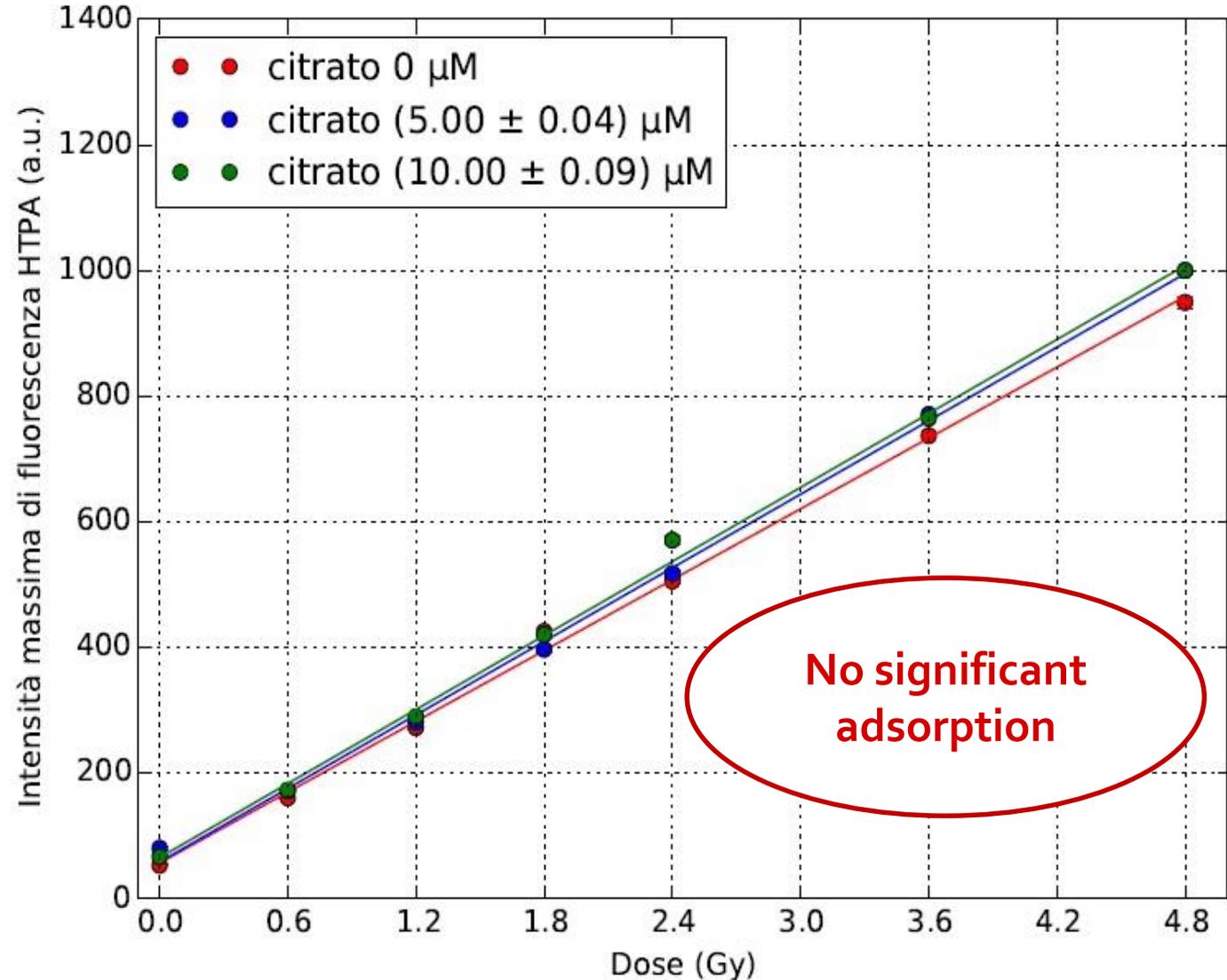
Adsorption between citrate and TPA?

Citrate: negative charge

Possible chemical interaction between the GNP citrate shell and the TPA

How to evaluate the effect?

Irradiation (with 6 MV photons) of samples only containing citrate (0,5,10 μM) and TPA



No significant adsorption

Integrated correction (d=20 nm)

The fluorometer response depends on the EM slit opening – and so does its dynamic range

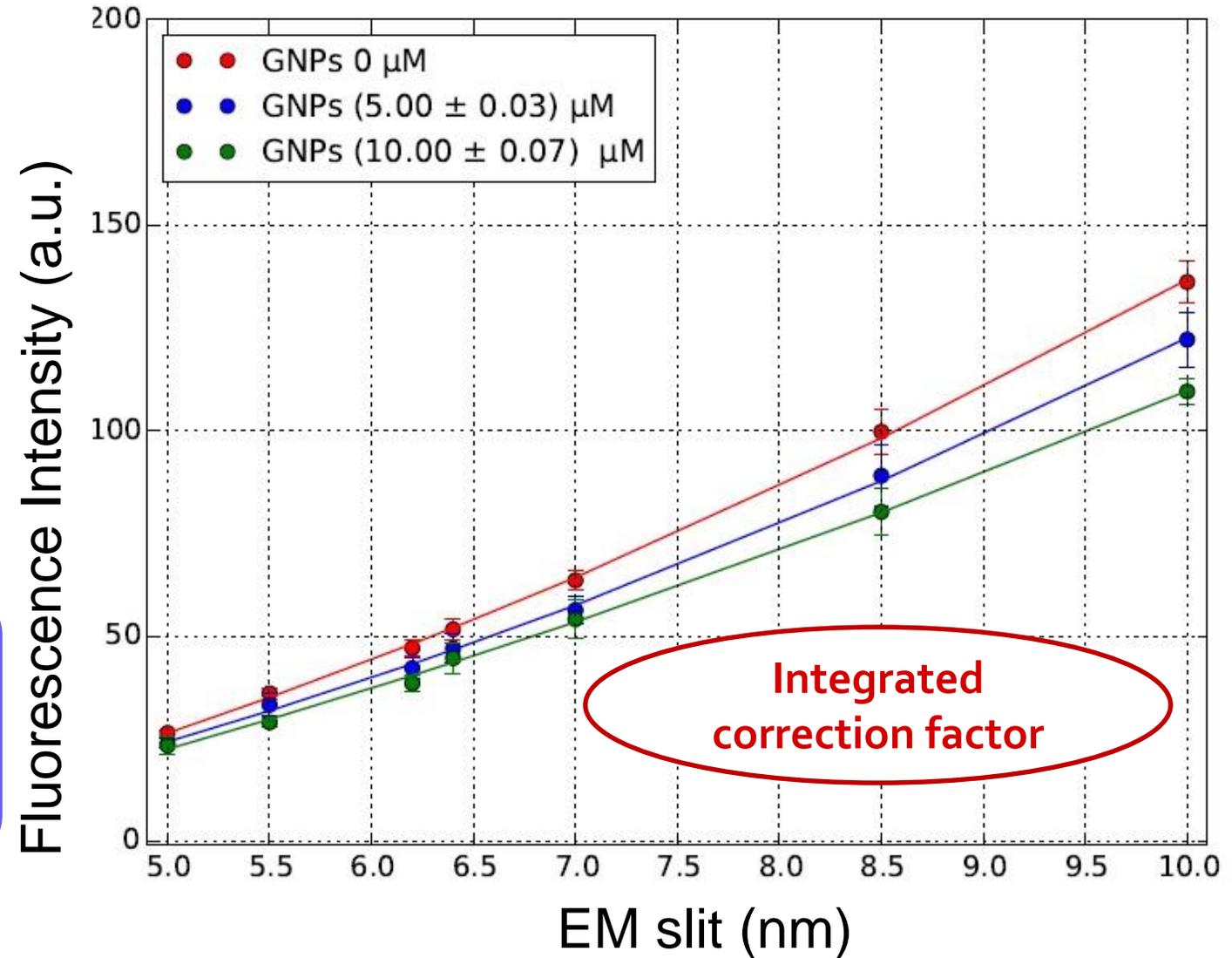
EM slit

Tune the amount of fluorescent light that generates the signal

How to quantify the correction?

Samples containing TPA and GNPs at different concentrations (0,5,10 μM) without irradiation must overlap

$$C_{5 \mu M}(6.4 \text{ nm}) = 0.10 \pm 0.04 \quad C_{10 \mu M}(6.4 \text{ nm}) = 0.16 \pm 0.05$$



Results with $d=20$ nm GNPs

Irradiated Samples

TPA

(1.000 ± 0.006) mM

GNP

(20 ± 2) nm average diameter

$0 \mu\text{M}$, $(5.00 \pm 0.03) \mu\text{M}$, $(10.00 \pm 0.07) \mu\text{M}$

PBS

pH = 7.35

Radiation

Photons

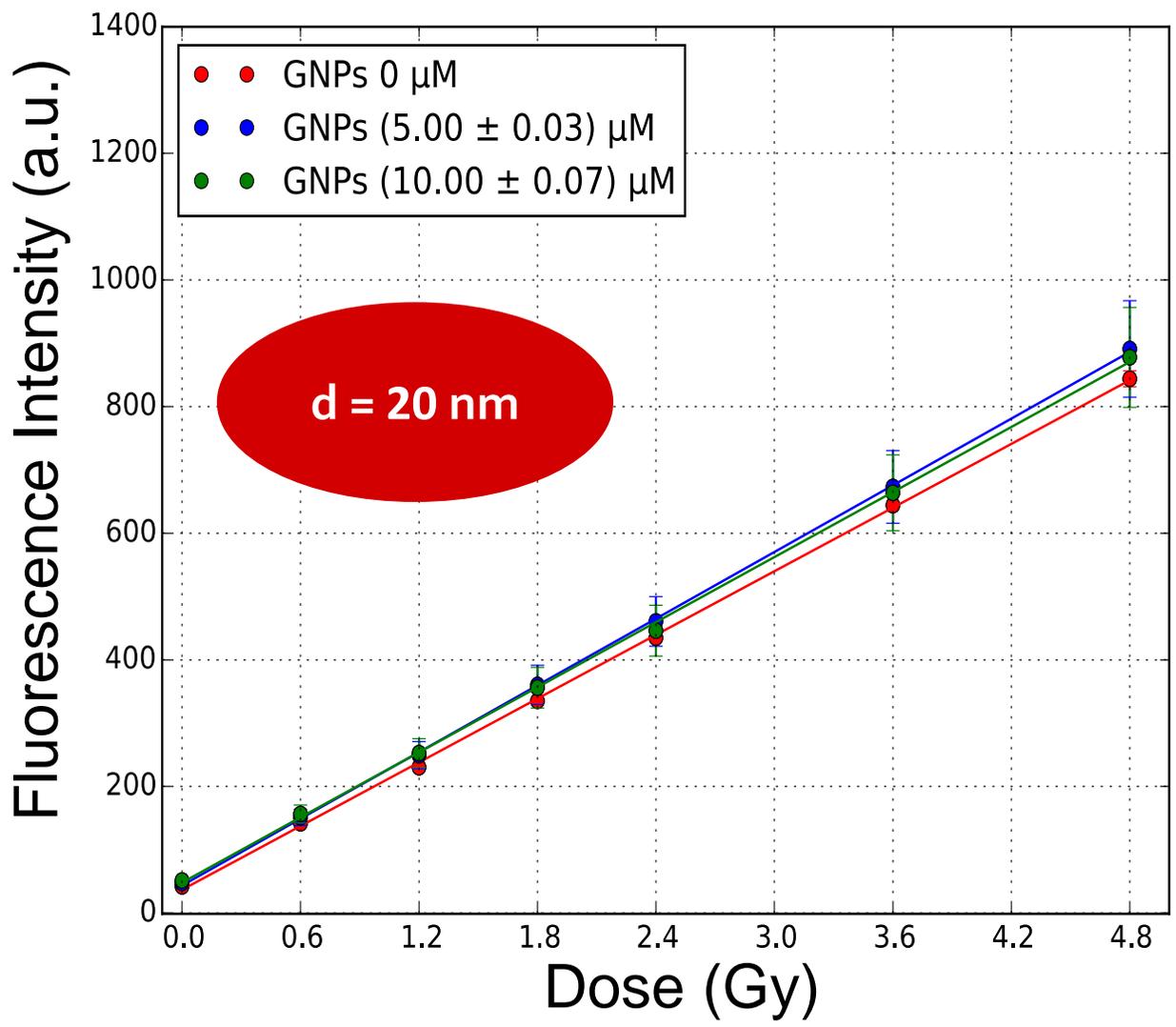
Energy = 6, 15 MV

Electrons

Energy = 6, 12, 15 MeV

Raw results were corrected with the integrated correction factors, as previously evaluated

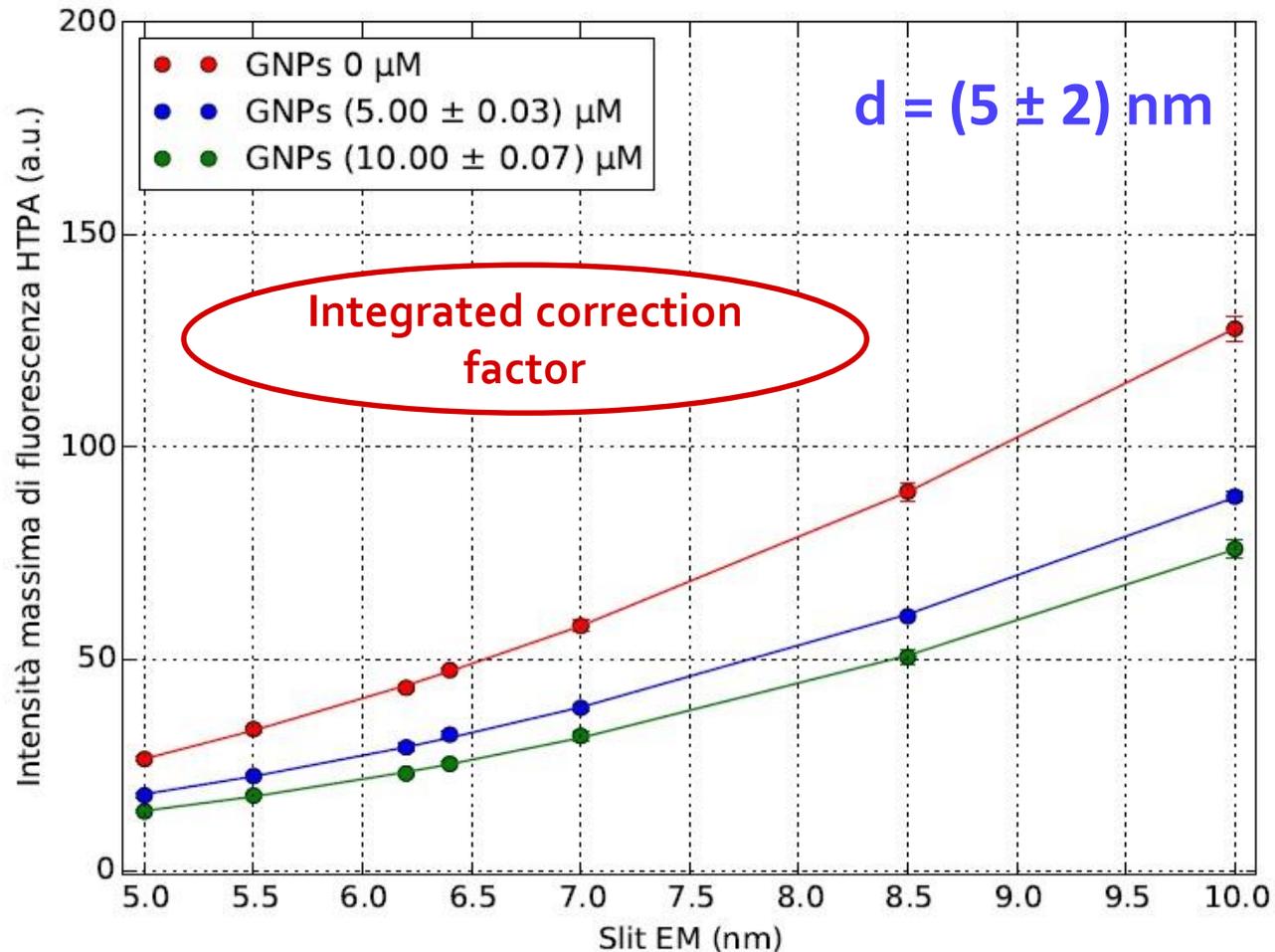
Fluorescence Intensity vs Dose vs [GNP], 6 MV photons



ROS production depends linearly on the dose
No significant dependence on the radiation type

**Slight, if significant,
 GNP-induced increase in OH⁻ production**

Is there any dependence on the GNP size?



Measurements

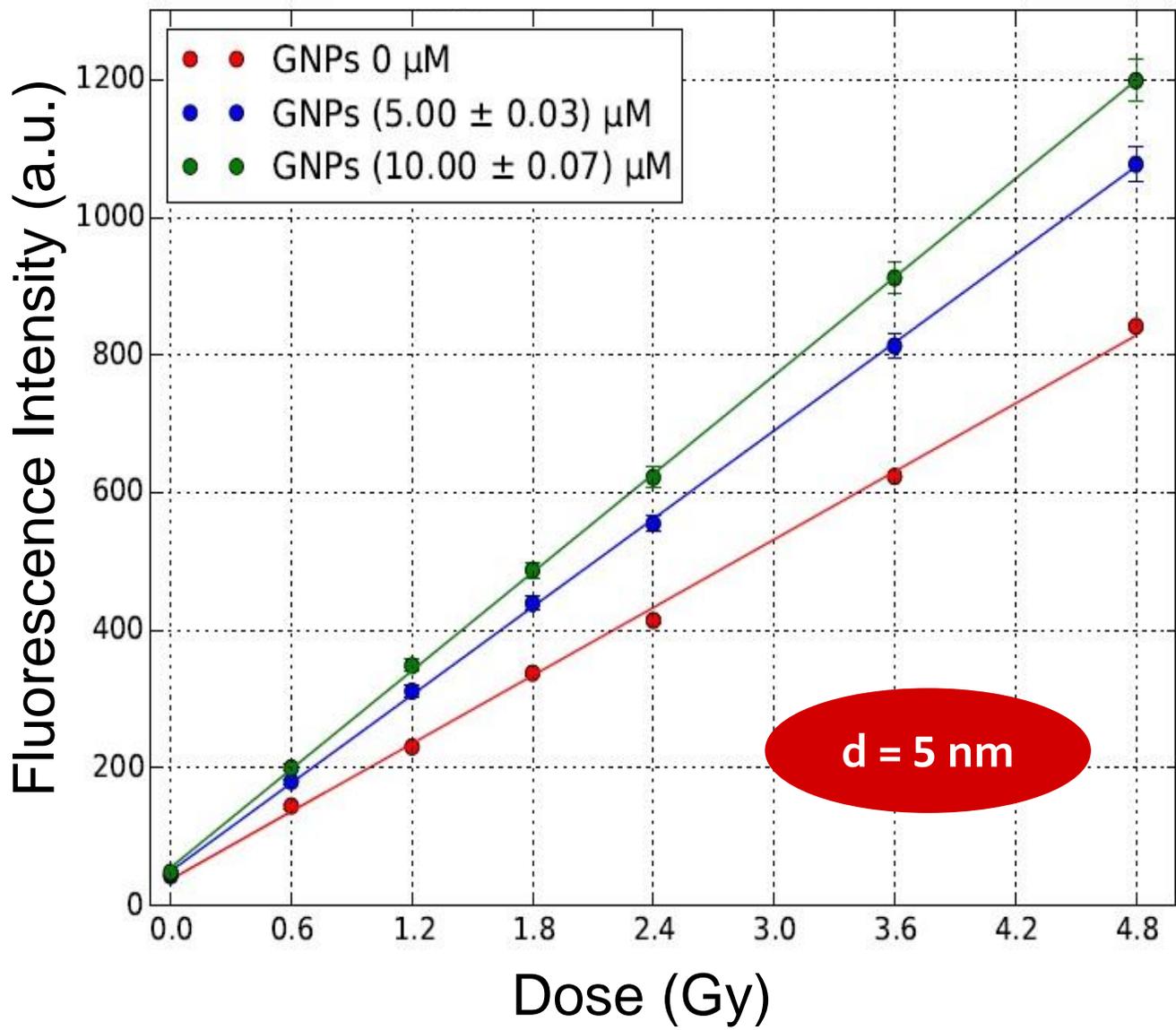
Irradiation with:
6 and 15 MV photons

TPA (1.000 ± 0.006) mM

GNP $d = (5 \pm 2)$ nm average diameter
 0μ M, 5μ M, 10μ M

PBS, pH = 7.35

6, 15 MV photons - d=5 nm GNPs



6 MV photons

$d = (5 \pm 2) \text{ nm}$

GNP (5.00 ± 0.03) μM : 0.21 ± 0.04
GNP (10.00 ± 0.07) μM : 0.28 ± 0.04

$d = (20 \pm 2) \text{ nm}$

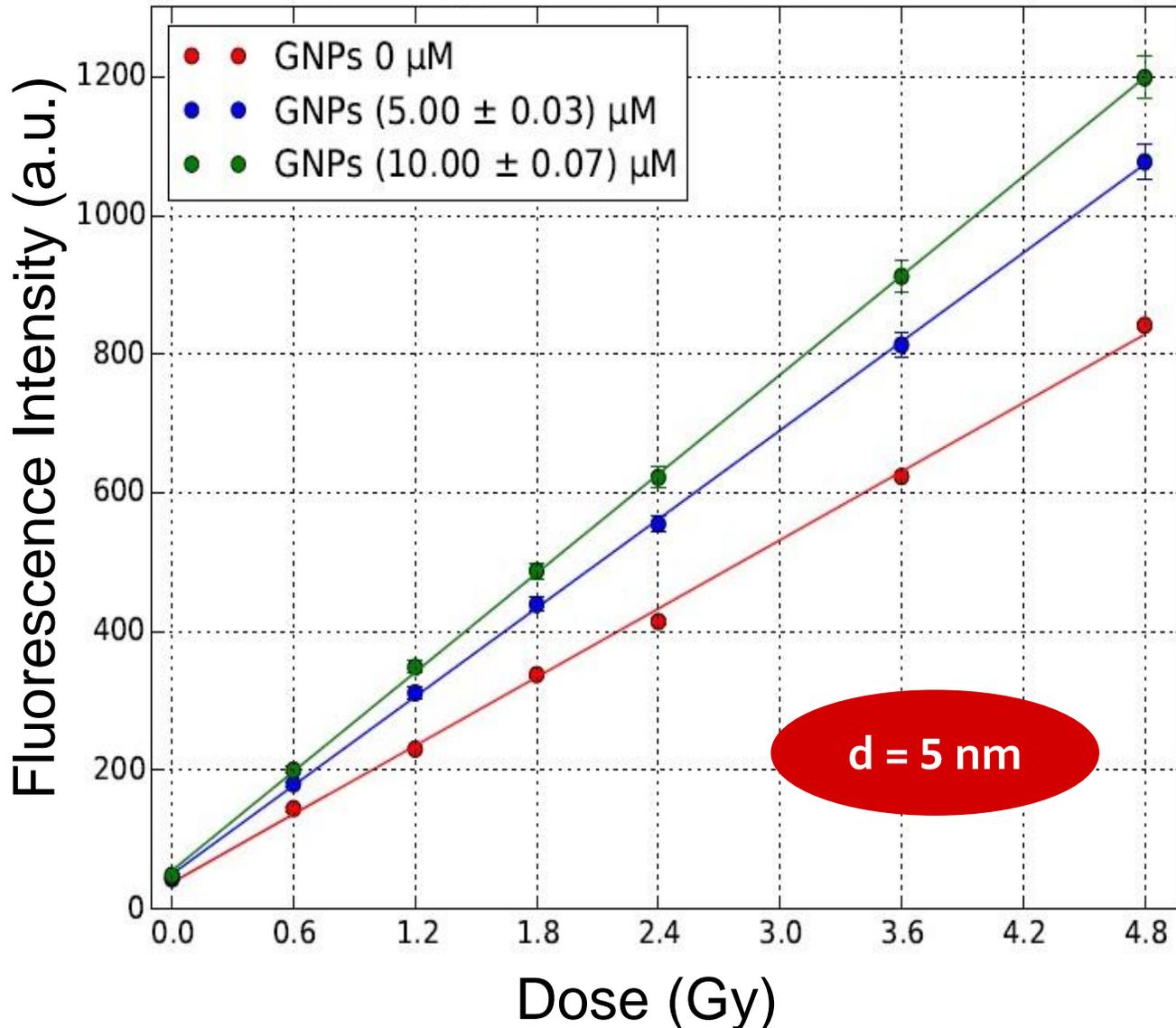
GNP (5.00 ± 0.03) μM : 0.08 ± 0.05
GNP (10.00 ± 0.07) μM : 0.09 ± 0.05

15 MV photons

$d = (5 \pm 2) \text{ nm}$

GNP (5.00 ± 0.03) μM : 0.32 ± 0.05
GNP (10.00 ± 0.07) μM : 0.47 ± 0.05

*Increase in OH⁻ production,
 Roughly constant with the dose*



There seems to be a GNP-induced, size dependent increase in OH⁻ production in clinical like conditions

Results with 5 nm and 20 nm GNP are consistent with a surface/volume effect

Next steps:

- measure the effect of GNP with $d = 10 \text{ nm}$ and $d = 3 \text{ nm}$
- measure the effect of hadron beams