NANOSCINTILLATORS-INDUCED PHOTODYNAMIC THERAPY : OVERCOMING LIGHT PENETRATION FOR TREATMENT OF DEEP LOCALIZED TUMORS

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7-8/09/2018 - Prague

Outline of the presentation

- What is Photodynamic therapy ?

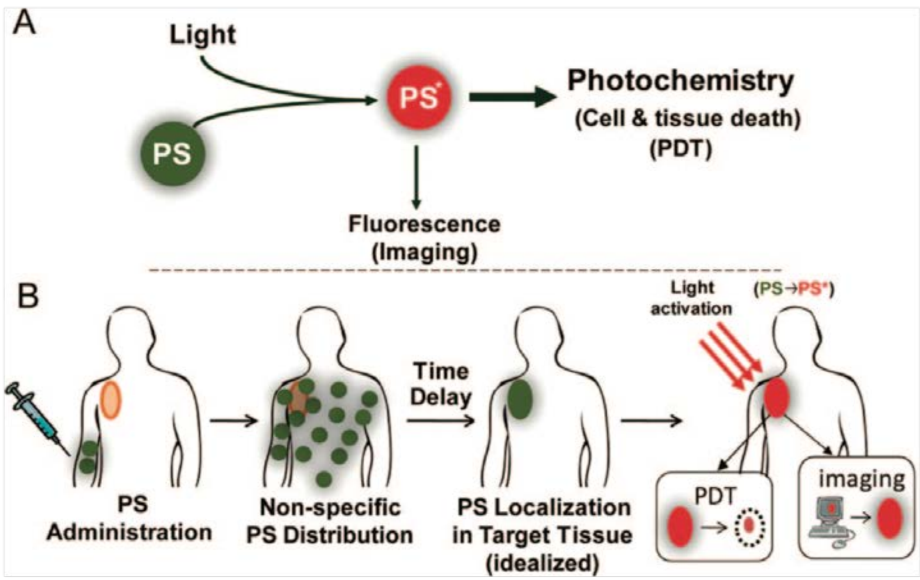
- How could nanoscintillators help overcoming some of PDT's limitations ?
  Some examples

- Where does the therapeutic effect come from ?

What is photodynamic therapy ?

Photodynamic therapy (PDT)

- Photochemistry-based modality used as cancer treatment
- Requires the combination of two non-toxic components: a **photosensitizer** and **light** **(+ oxygen)**

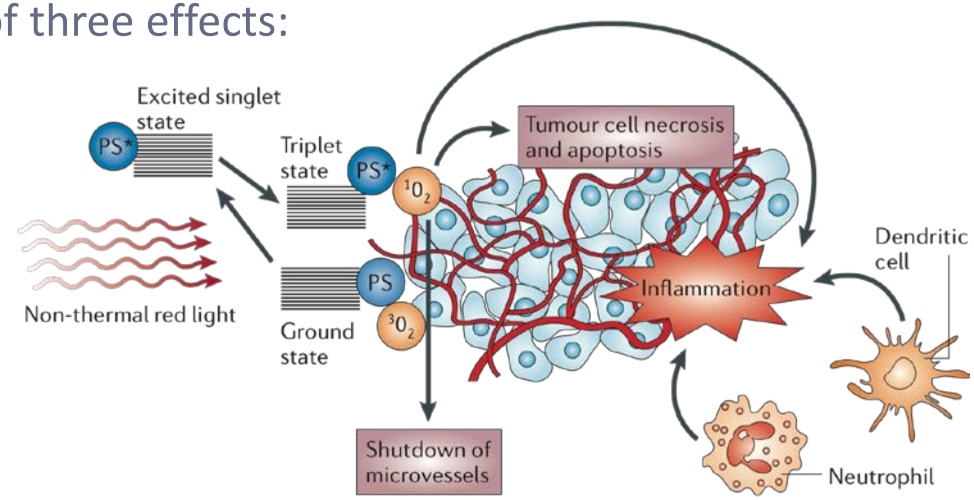


Celli et al., *Chem. Rev*. 110 **2010**

Dai et al., *Front Microbiol*. 3 **2012**

How does PDT induce tumor death ?

- Combination of three effects:



Castano et al., *Nat. Rev. Cancer* 6 **2006**

⇒ Necrotic and apoptotic cell death

⇒ Microvessels shutdown

⇒ Immune response

- Advantages of PDT:

✓ No dark toxicity

✓ Local effect ($r_{singlet\ oxygen}$ < 0.02 µm)

PDT in clinic

- Which targets ?

  - Superficial tumors:

e.g.: non-melanoma skin cancer

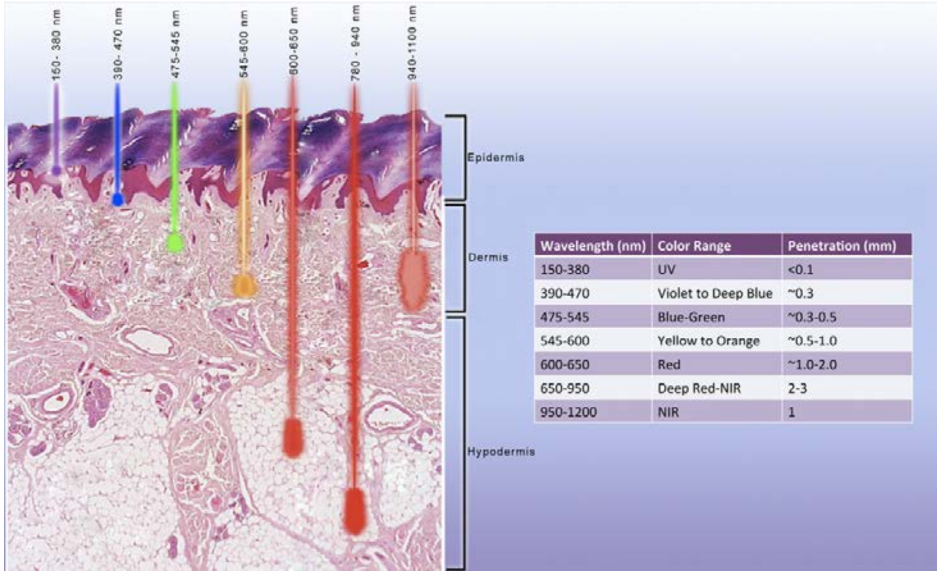
  - Endoscopically or easily accessible tumors:

e.g.: bladder, prostate, Head and Neck, Oesophagus cancers

  - Under investigation:

e.g.: Biliary tract, Glioma, Pancreatic cancer

One of the main limitation to PDT



Wavelength (nm)	Color Range	Penetration (mm)
150-380	UV	<0.1
390-470	Violet to Deep Blue	~0.3
475-545	Blue-Green	~0.3-0.5
545-600	Yellow to Orange	~0.5-1.0
600-650	Red	~1.0-2.0
650-950	Deep Red-NIR	2-3
950-1200	NIR	1

Avci et al., *Semin Cutan Med. Surg* 32:41-52 **2013**

- Limited penetration of light in tissues

  - Accessible tumors

  - Small tumors

Overcoming the light penetration issue: using more penetrating radiations

NIR radiations that lie in the optical windows

Penetrating X-rays

https://www3.epa.gov/radtown/medical-xrays.html

NIR excitation of the PS

2-photon excitation of the PS

➡ **Need for nanotransducers to locally convert penetrating radiations into visible light**

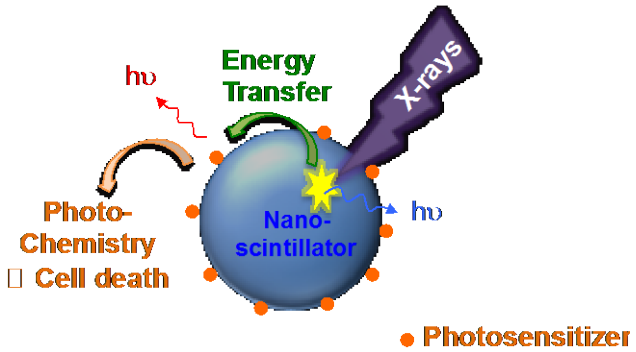
Up-converting nanoparticle

Nanoscintillator

Fan et al., *Chem. Soc. Rev.*, ***45***, 2016

Nanoscintillators and X-rays to overcome shallow penetration of light in tissues

Nanoscintillator induced PDT: The concept



- A three-steps process:
  1. The nanoscintillator absorb part of the X-ray radiation and down-convert the high-energy photons into visible light
  2. Energy is transferred from the nanoscintillator to the photosensitizer
  3. Excited photosensitizers induce photochemical reactions culminating in cell death

- For which pathology could this novel therapy be relevant ?
  - Pathologies for which RT is part of the standard of care
  - Pathologies for which PDT demonstrated promises
  - Deep-seeded or large tumor masses

Nanoscintillators-induced PDT: examples in the literature

Spectroscopic proof-of-concept study

Bulin et al. *J Phys Chem C,* **2013**

In vivo study

Chen et al. *Nano Letters*, **2015**

Modelisation and estimation of the maximum efficiency

Morgan et al. *Radiation Research*, 2009

Bulin et al. *Nanoscale,* 2015

Spectroscopic proof-of-concept study

THE JOURNAL OF PHYSICAL CHEMISTRY C

Article

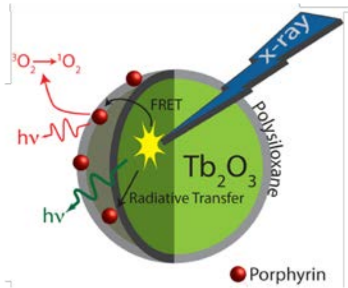
pubs.acs.org/JPCC

X-ray-Induced Singlet Oxygen Activation with Nanoscintillator-Coupled Porphyrins

Anne-Laure Bulin,[†,⊥] Charles Truillet,[†,⊥] Rima Chouikrat,[‡] François Lux,[†] Céline Frochot,[‡] David Amans,[†] Gilles Ledoux,[†] Olivier Tillement,[†] Pascal Perriat,[§] Muriel Barberi-Heyob,[∥] and Christophe Dujardin*[,†]

Nanoscintillators to induce deep tissue PDT - proof of concept

- The system we investigated: $Tb_2O_3@SiO_2$ nanoparticles conjugated to a porphryin



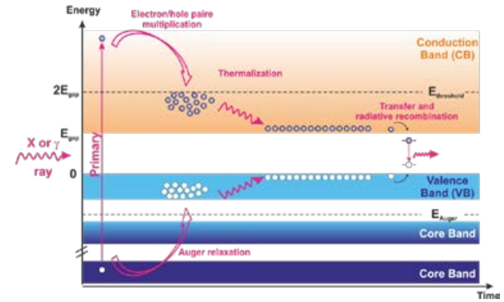
Porphyrin synthesis: group of C Frochot @ LRGP
Nanoparticle synthesis and conjugation: group of O Tillement @ ILM

- Proof of concept under X-ray irradiation:

➡ Existence of an energy transfer from the nanoscintillator to the photosensitizer upon X-rays irradiation

Characterization of the energy transfer

- UV excitation simulates the last step of the scintillation process

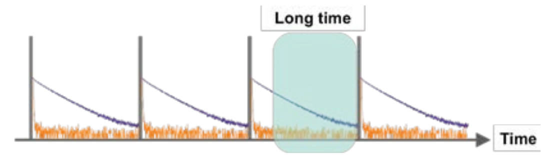


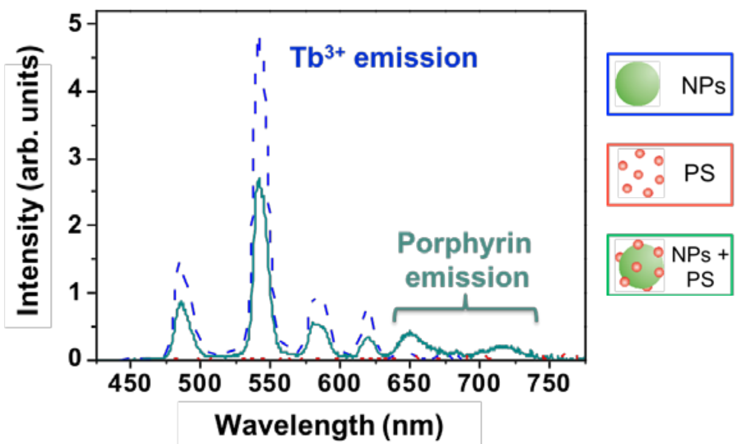
⇒ Allows time resolved spectroscopy

- How can we use time resolved spectroscopy to demonstrate the existence of a transfer ?

Characterization of the energy transfer

- Spectra mesured at “long times”





➡ Validation of the existence of an energy transfer

- Which type of transfer is it ?

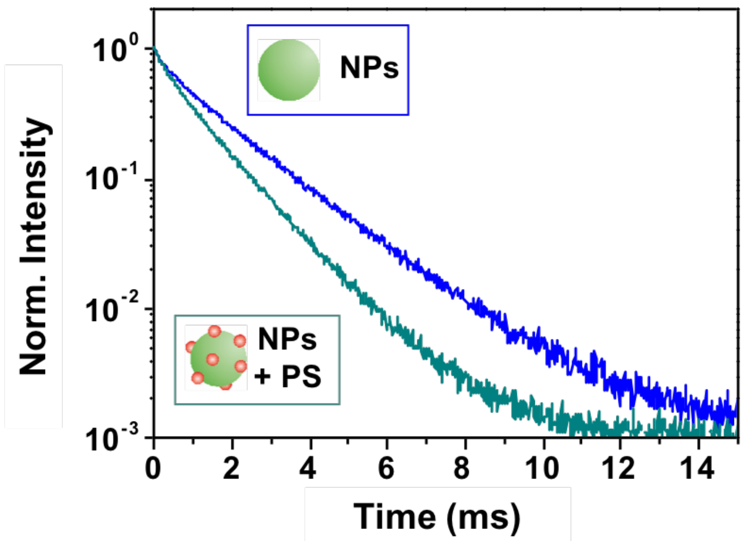
Radiativ transfer

Förster Resonant Energy Transfer (FRET)

Transfer type	Emitter’s decay
Radiative	→
FRET	↘

Characterization of the energy transfer

- Measurements of the decay times using a 300 nm excitation laser



➥ At least part of the energy transfer occurs as **FRET**

➥ The FRET transfer efficiency decreases as $1/r^6$

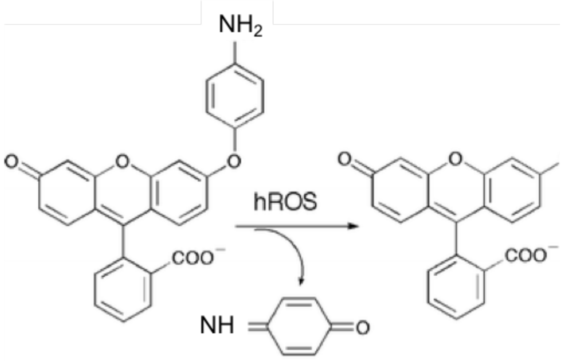
➥ **Emitter and acceptor have to be as close as possible**

(Small particles, short linkers, etc.)

Reactive oxygen species generated

- Using 2 chemical probes that are sensitive to singlet oxygen and other ROS

APF (3'-p-(aminophenyl) fluorescein)

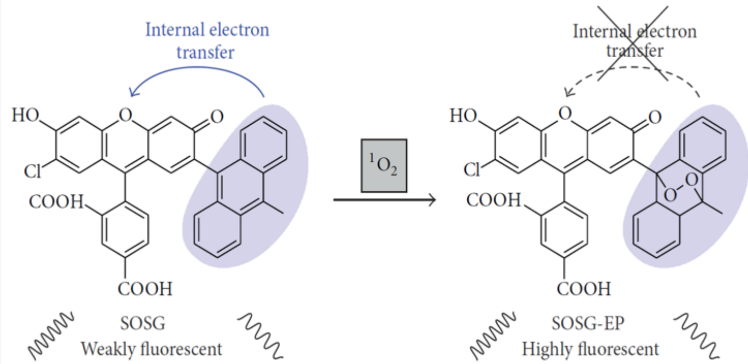


Price *et al, Photochem. Photobiol.*, **5**, 2009

⇒ Sensitive to both **•OH** and **$^1O_2$**

⇒ $^1O_2$ quenched by NaN_3

Sensor Green (SOSG)

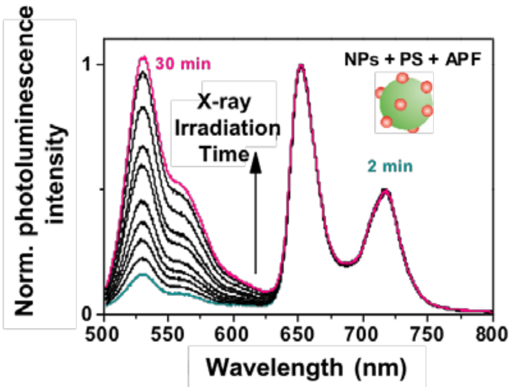


Kiesslich *et al, Biomed. Research International*, 2013

⇒ Sensitive only to **$^1O_2$**

Reactive oxygen species generated

- Results obtained with the APF



➥ Increase of the APF signal when the PS is conjugated onto the nanoscintillator

- What if we add NaN_3 ?

➥ The APF photoluminescence increase was due to the **generation of $^1O_2$**

Summary on the spectroscopic study

- X-ray-induced excitation of the porphyrin through energy transfer
- Characterization of the energy transfer – at least partially as FRET
- As a consequence of this energy transfer: generation of singlet oxygen

➥ Need to proceed to more biological experiments to validate the efficiency demonstrated in solution

In vitro and *in vivo* study reporting a beneficial effect of X-PDT

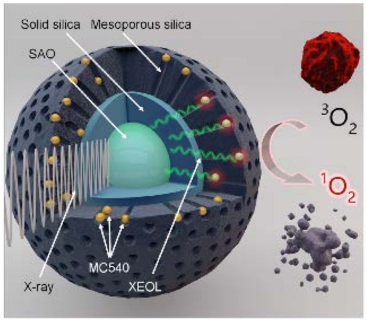
NANO LETTERS
Letter
pubs.acs.org/NanoLett

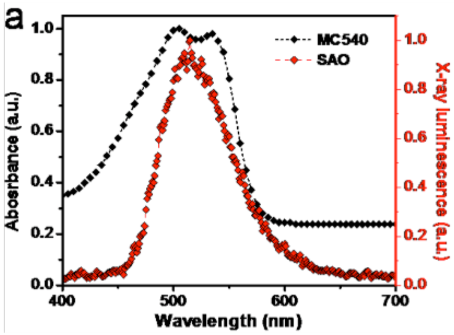
Nanoscintillator-Mediated X-ray Inducible Photodynamic Therapy for In Vivo Cancer Treatment

Hongmin Chen,[†,‡,§,∥] Geoffrey D. Wang,[†] Yen-Jun Chuang,[⊥] Zipeng Zhen,[†,‡] Xiaoyuan Chen,[∇] Paul Biddinger,[¶] Zhonglin Hao,[#] Feng Liu,[⊥] Baozhong Shen,[§,∥] Zhengwei Pan,[⊥] and Jin Xie*[,†,‡]

Nanoscintillator-induced deep tissue PDT *in vivo*: the nanoconjugate and its cellular toxicity

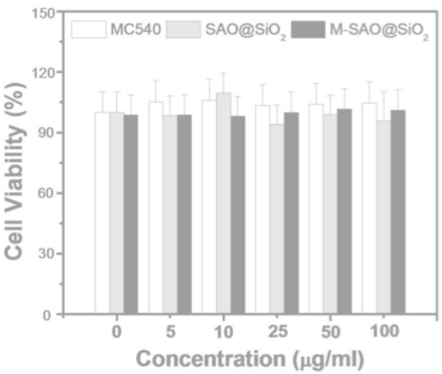
- The system we investigated: $SrAl_2O_4:Eu^{2+}$ nanoparticles conjugated to merocyanine 540



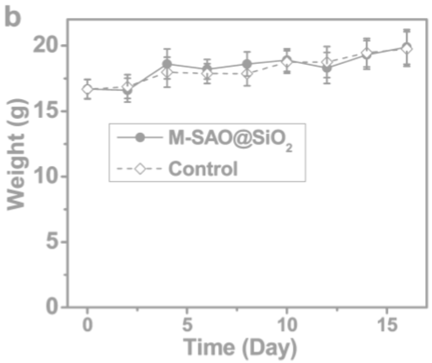


- Toxicity

In vitro
MTT assay (mitochondrial activity)
After 24h incubation



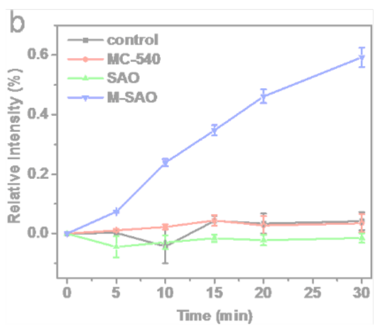
In vivo
Mice weight (n=5)
Intra-tumoral injection of 50µL
2.5 mg M-SAO@SiO_2/mL or PBS



Singlet oxygen production

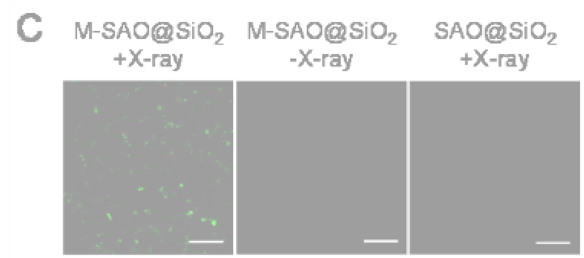
The change in fluorescence intensity (ex/em: 504/525nm) is related to the amount of $^1O_2$ that was generated

- In solution – upon X-ray irradiation

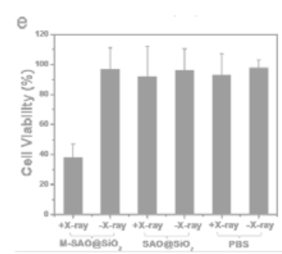


- *In vitro*

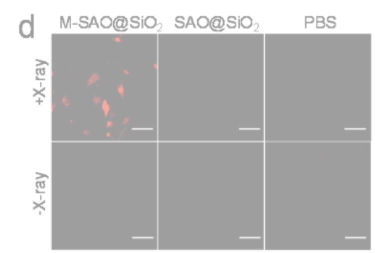
Singlet oxygen production



Induced cell death



MTT assay



Ethidium Bromide – confocal microscopy

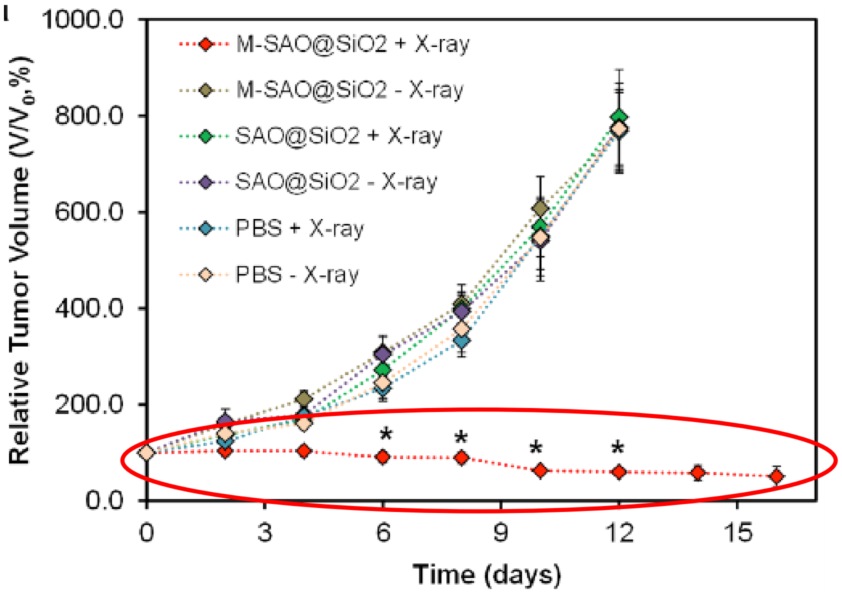
⇒ The Generation of $^1O_2$ requires the combination of SAO + MC540 + X-rays

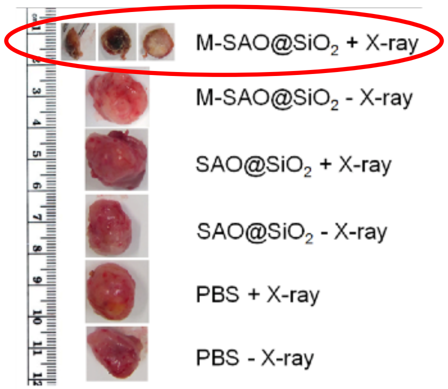
⇒ It is associated to a decrease of the cell viability

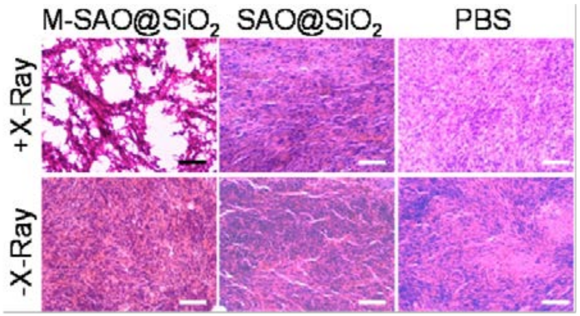
Demonstrating the efficiency *in vivo*

- Tumor volume calculated using:

  length x (width)^2/2







⇒ Confirmation of a strong effect when M-SAO nanoparticles are combined with X-rays

Estimating the maximum efficiency

RADIATION RESEARCH **171**, 236–244 (2009)
0033-7587/09 $15.00

Nanoscintillator Conjugates as Photodynamic Therapy-Based Radiosensitizers: Calculation of Required Physical Parameters

Nicole Y. Morgan,[a,1] Gabriela Kramer-Marek,[b,c] Paul D. Smith,[a] Kevin Camphausen[b] and Jacek Capala[b]

[a] Laboratory of Bioengineering and Physical Science, NIBIB, and [b] Radiation Oncology Branch, NCI, National Institutes of Health, Bethesda, Maryland 20892; and [c] A. Chelkowski Institute of Physics, University of Silesia, Uniwersytecka 4, 40-007 Katowice, Poland

Nanoscale

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Modelling energy deposition in nanoscintillators to predict the efficiency of the X-ray-induced photodynamic effect†

Anne-Laure Bulin,[a] Andrey Vasil'ev,[b] Andrei Belsky,[a] David Amans,[a] Gilles Ledoux[a] and Christophe Dujardin*[a]

Theoretical achievable efficiency – a first estimation

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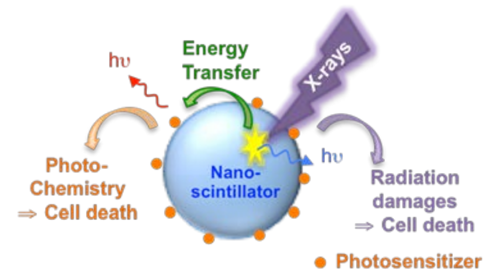


- How much singlet oxygen can be generated through energy transfer between nanoscintillator/photosensitizer ?

$$N(^1O_2) = \text{Absorption} \;.\; \text{Light Yield} \;.\; \text{Transfer} \;.\; \text{Efficiency}(^1O_2)$$

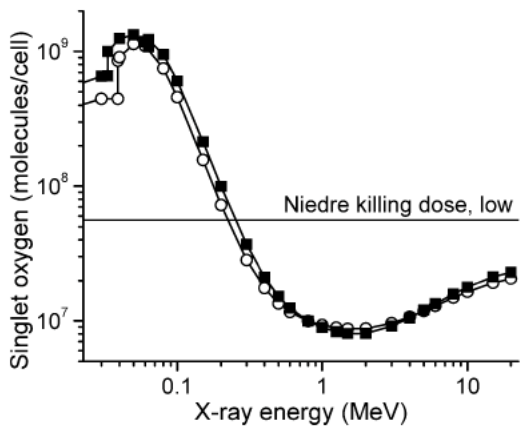
FIG. 1. The absorption of nanoparticle cores relative to an equal volume of soft tissue for LuI_3 (■) and LaF_3 (○). Absorption coefficients for atomic elements and soft tissue taken from ref. (*20*).

Theoretical achievable efficiency – a first estimation

- Number of singlet oxygen molecules generated in a 10μm-diameter cell

Parameters:

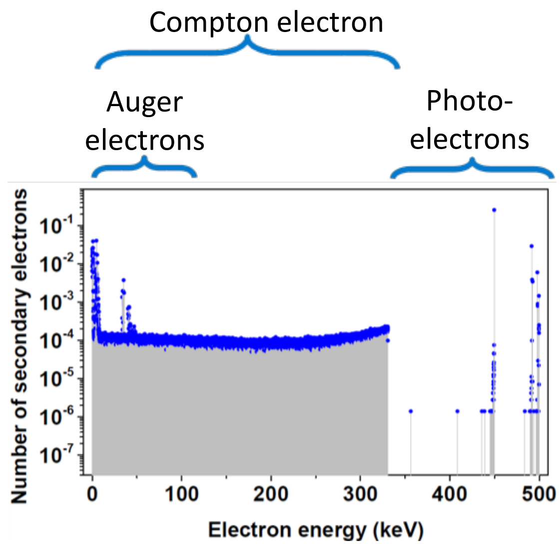
- dose: 60 Gy,
- nanoparticles occupy 5% of the cell volume
- Light yield: 0.5*(5.4.105 photons/MeV)
- ϕ_{FRET}= 0.75 and
- $\phi_{singlet\ oxygen}$=0.89

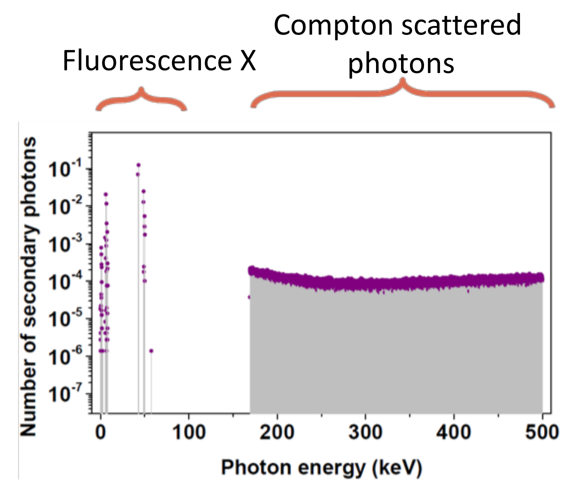


➥ Effect likely to be observed upon low-energy irradiation
Maximized around 50-80keV

Limitation of this first estimation

- Interaction of a 500keV photon in a scintillating material





➥ **It becomes crucial to consider the nano-size of the particles**

GEANT4 for the simulation of the energy deposited in nanoparticles

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Investigating the amount of singlet oxygen molecules that can be excited considering the geometry of the nanoparticles

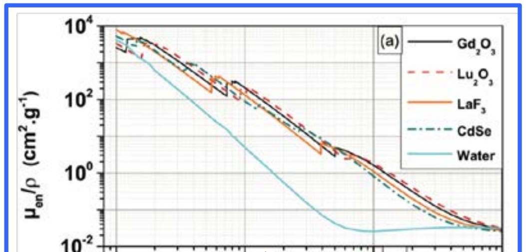
- GEANT4 (GEometry ANd Tracking): Monte Carlo toolkit to simulate particles/matter interactions

- A two steps program:

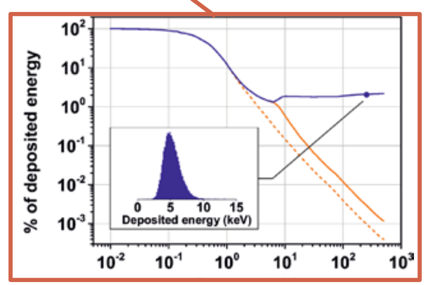
1. Establish the spectra of the secondary particles (electrons and photons) generated during the primary interaction
2. Quantify the fraction of energy deposited by each secondary particle within the nanoparticle

GEANT4 for the simulation of the energy deposited in nanoparticles

- Quantification of the energy deposited within Gd_2O_3 nanoparticles by an incoming X-ray photon of a given energy

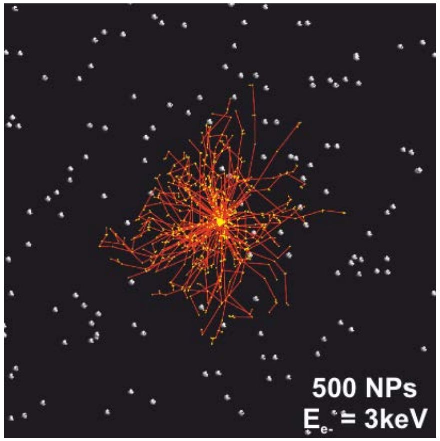


$$E_{\text{total}} = \sum_{k=\text{wat,Gd}} \beta_k \left(\sum_i S_k^{\text{e}}(E_\gamma, E_i)\, \eta_{\text{c}}^{\text{e}}(E_i) E_i + \sum_j S_k^{\text{p}}(E_\gamma, E_j)\, \eta_{\text{c}}^{\text{p}}(E_j) E_j \right)$$



$|_{\text{nano}}$ = Energy deposited in NP / Primary photon energy

GEANT4 for the simulation of the energy deposited in nanoparticles



Parameters of the program:

- Total tumor volume
- Occupation ratio

  C = Volume occupied by NPs / Total volume
- NPs characteristics (material – diameter)

➡ | nano

$$N_{^1O_2} = \eta_{nano} \times \Phi_{scinti} \times E_\gamma \times \Phi_{transfer} \times \Phi_\Delta$$

Regarding the therapeutic effect:

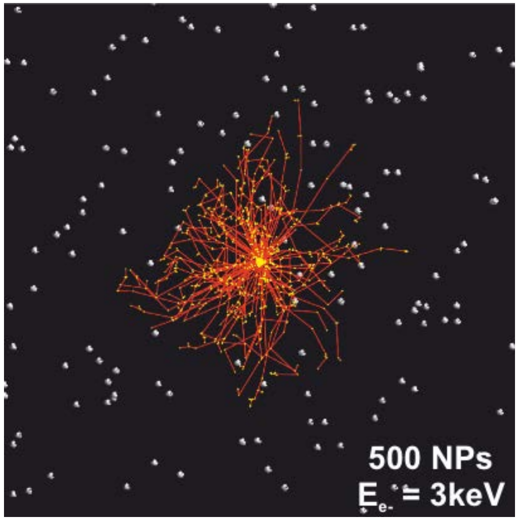
$\Rightarrow$ Energy deposited within the nanoparticle: likely to induce PDT

$\Rightarrow$ Energy deposited around the nanoparticle: local dose enhancement

GEANT4 – quantification of the total deposited energy – Therapeutic effect

- For one photon that interacts with the tumor volume (Gd_2O_3/water):

$C = 2 \cdot 10^{-3}$	10 nm		100 nm	
E_γ (keV)	Gd_2O_3	Water	Gd_2O_3	Water
100 keV	0.23	25.50	0.91	24.93
200 keV	0.30	49.67	0.79	49.20
300 keV	0.51	85.37	0.92	84.97
400 keV	0.79	127.45	1.13	127.10
500 keV	1.10	173.85	1.41	173.52
$C = 7 \cdot 10^{-3}$	10 nm		100 nm	
100 keV	0.95	38.49	2.12	37.36
200 keV	1.21	59.62	1.94	59.07
300 keV	1.84	90.99	2.47	90.55
400 keV	2.70	130.26	3.29	129.81
500 keV	3.71	174.93	4.28	174.29



- Therapeutic effect:

PDT activation:

$$N_{^1O_2} = \eta_{\text{nano}} \times \Phi_{\text{scinti}} \times E_\gamma \times \Phi_{\text{transfer}} \times \Phi_\Delta$$

44 $^1O_2$ molecules activated per 500 keV photon interacting with a tumor loaded with 10 nm NPs of Gd_2O_3 with C = 7.10^{-3}

Radiation Dose Enhancement:

For the same volume with water only:

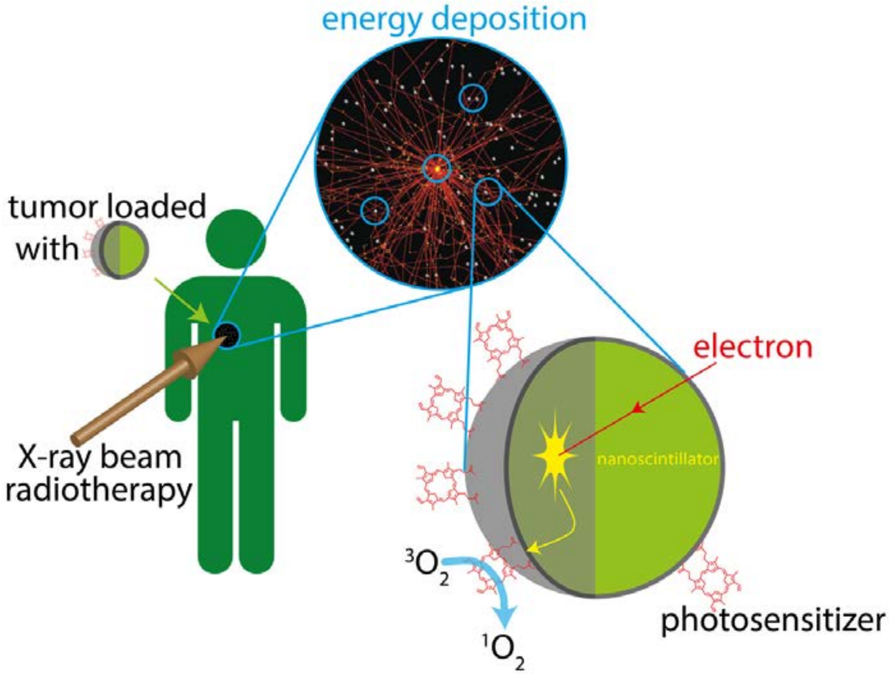
E_{dep} (100 keV) ≈ 15 keV

E_{dep} (500 keV) ≈ 173 keV

⇒ **Stronger effect for low energy photons**

A complex therapeutic combination

Deep-tissue PDT induced during radiation therapy: a combination of various effects



A-L Bulin and C Dujardin, *Atlas of Science* **2016**

Much more than "just" PDT !

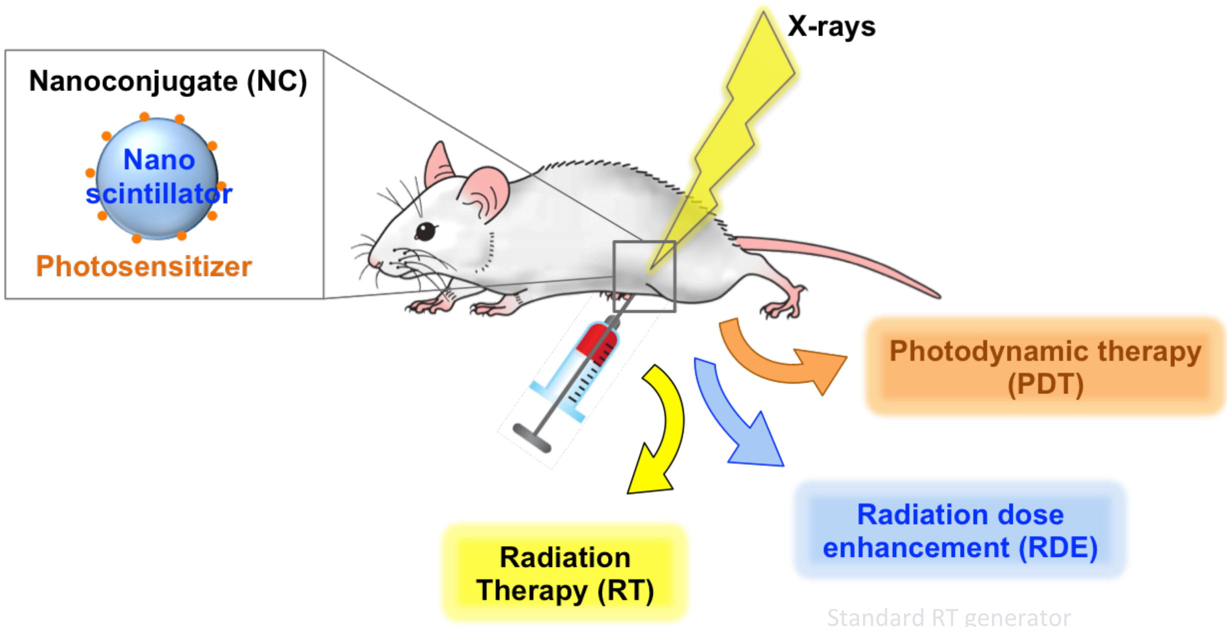
- Radiation therapy
- PDT
- Synergy between low dose PDT and radiation therapy ?
  A study to be published, biological perspective
- Radiation dose enhancement effect
  Bulin et al. O8-Mon
- UV-induced damages
  Muller et al. UV scintillating particles as radiosensitizer enhance cell killing after X-ray excitation *Radiotherapy and Oncology*, in press, **2018**

Nanoscintillators-induced PDT during (synchrotron) radiation therapy:

in vitro and *in vivo* investigations

- The ongoing program -

The ongoing program



Standard RT generator
vs
Synchrotron radiation

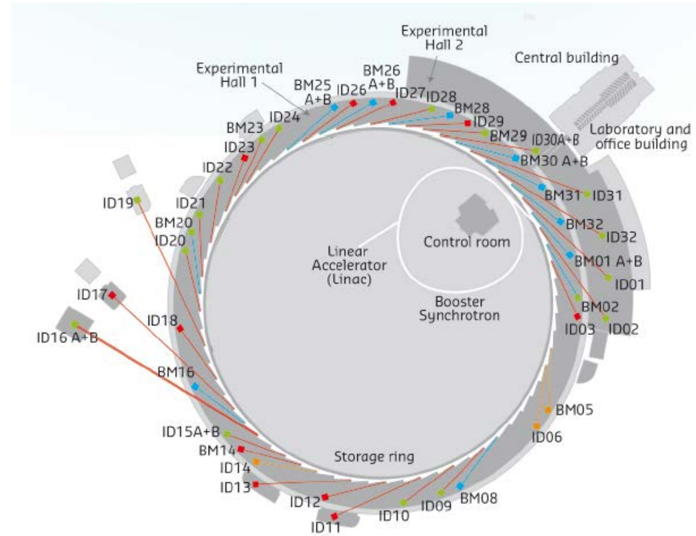
Targets:

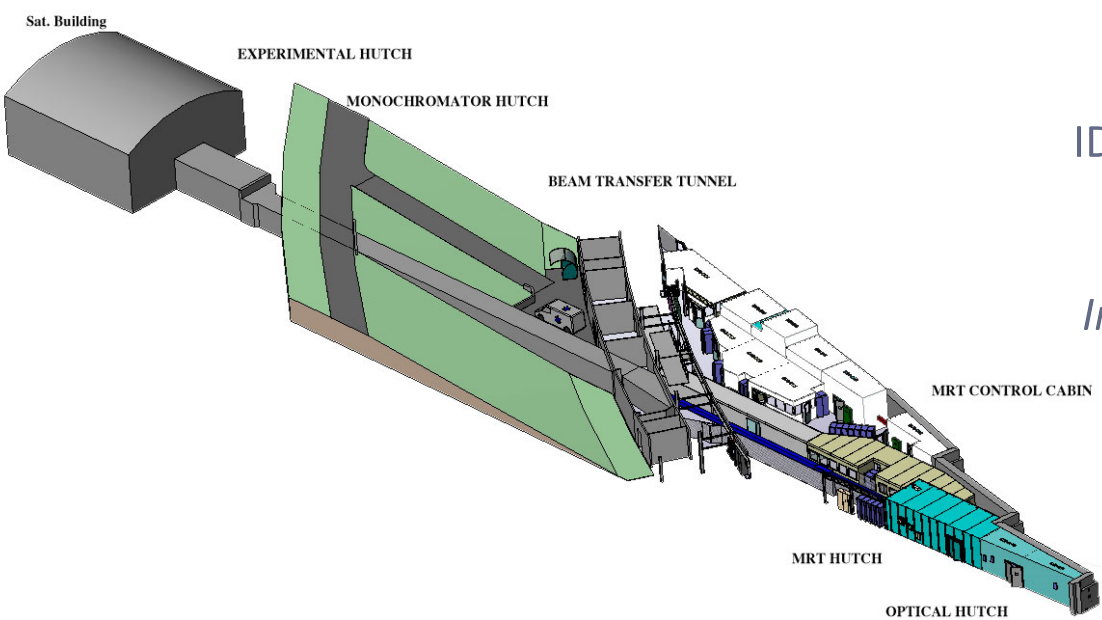
⇒ Glioblastoma

⇒ Ovarian cancer

http://www.hipec.coms

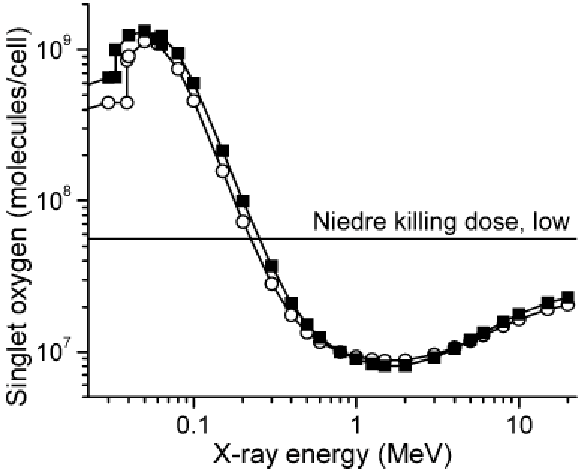
The ongoing study using synchrotron radiation (ESRF)

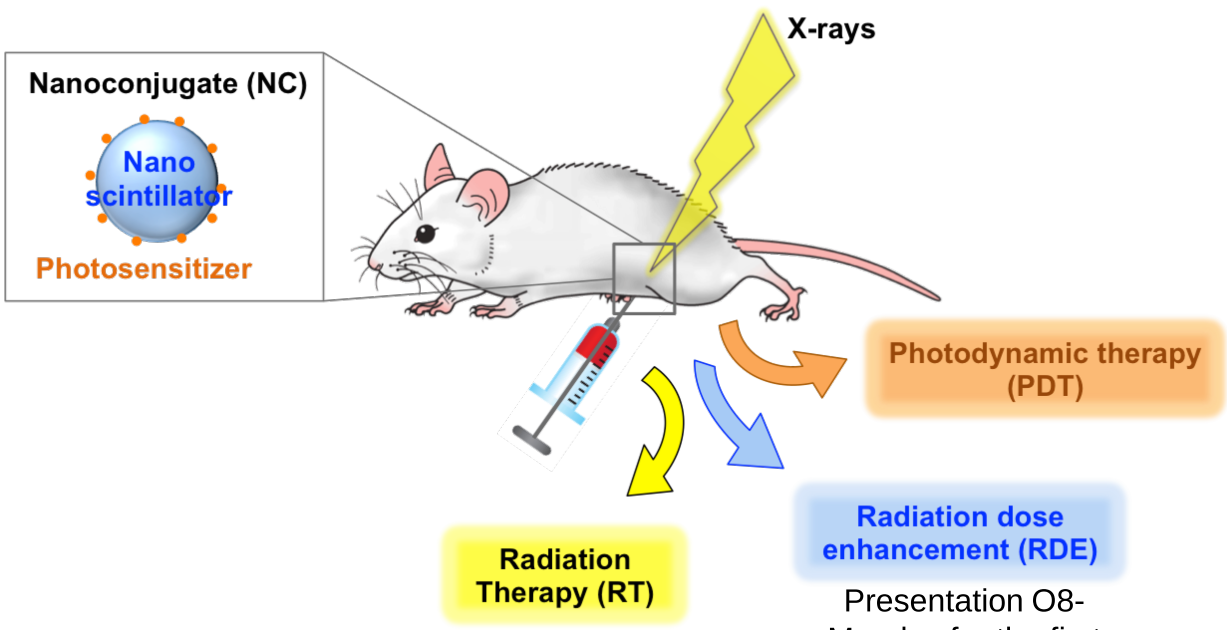




ID17 – Medical beamline
Monochromatic beam
25 – 100 keV
In vitro, *in vivo*, patients

The ongoing study using synchrotron radiation (ESRF)





Presentation O8-Monday for the first results

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Thank **you** for your attention!

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