Planning 1st experiments on chemical and biological effects of PITZ beam

for PITZ run during KW 44-45 (4-11.11.2022)

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Zeuthen, 20.10.2022

HELMHOLTZ

DESY.

Education & research experience

Educational and Scientific Center (ESC) "Institute of Biology and Medicine", Taras Shevchenko National University (TSNU) of Kyiv, Ukraine

- 2011: Bachelor of Biology with Distinction (92/100 score)
- 2013: Master of Biochemistry with Distinction (97/100 score)

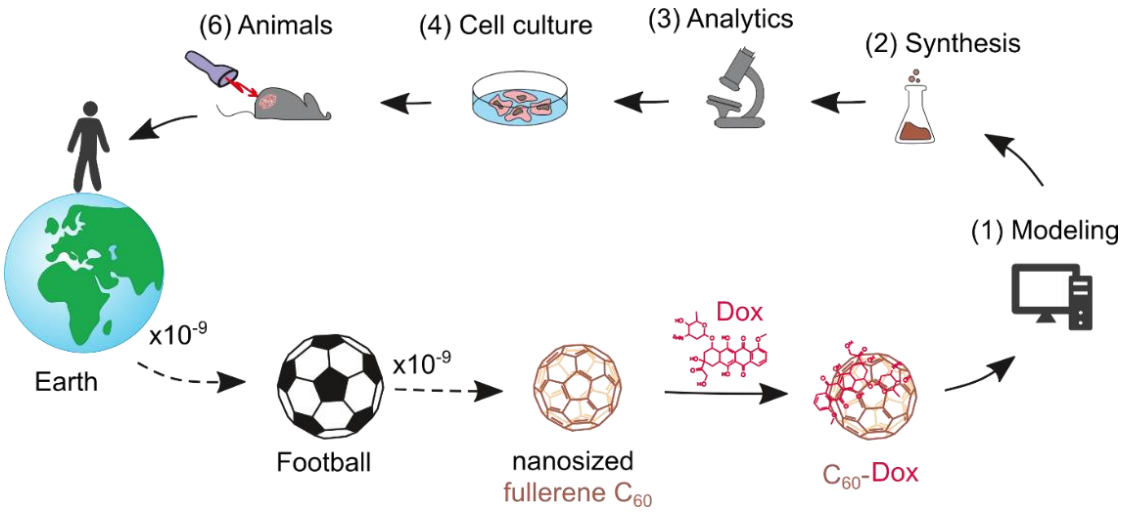
Faculty of Biology, University of Würzburg, Germany

- 2021: PhD in Biology with summa cum laude

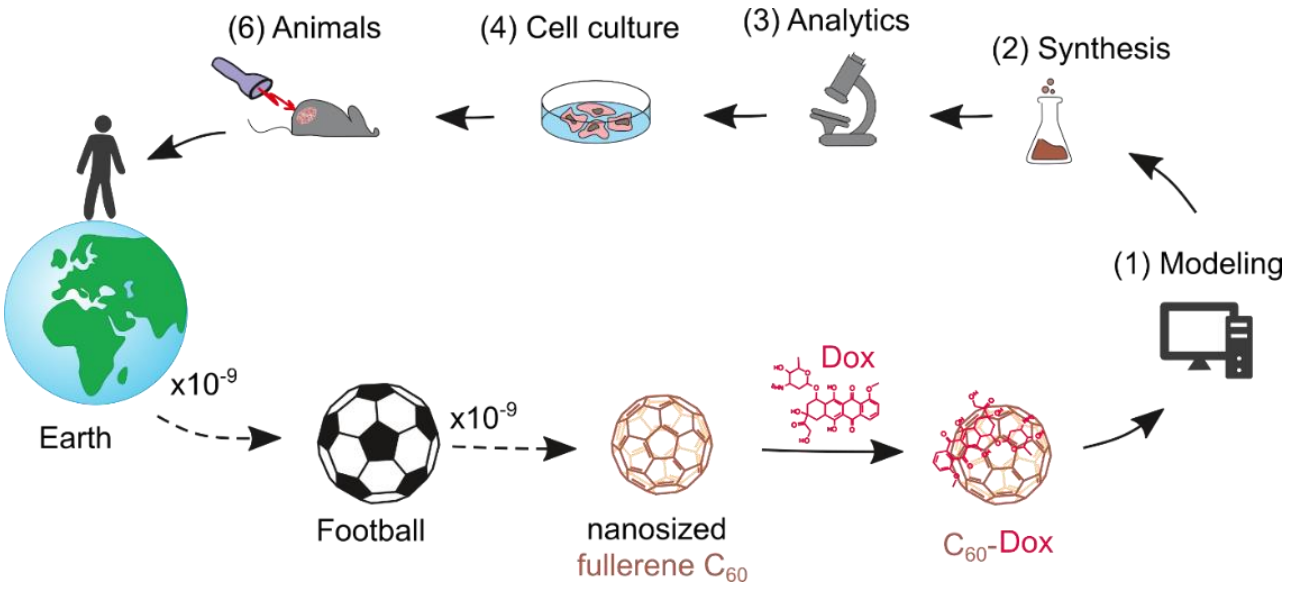
2011 – 2013: Bachelor and Master Student — *ESC "Institute of Biology and Medicine" TSNU Kyiv, Ukraine*

2021 – present: PhD Student, DAAD Scholar; Post-Doc, Junior Research Group Leader — *Molecular Biotechnology & Functional Genomics, Technical University of Applied Sciences Wildau, Germany*

Intradesciplinary background



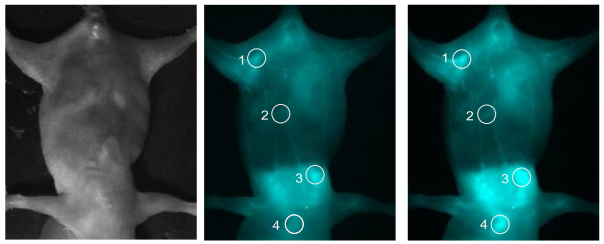
Intradesciplinary background



Multimodal synergistic cancer therapy

- Chemotherapy
- Photodynamic therapy
- Photothermal therapy
- Sonodynamic therapy
- Magnetic hyperthermia
- Radiation therapy

+ Imaging



R&D for Cancer Radiation Therapy (RT)

Conventional RT

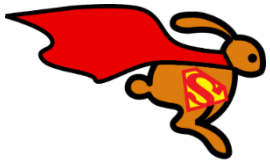
<5 Gy/min

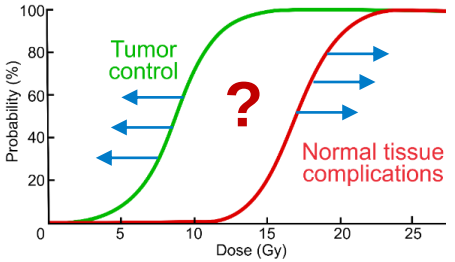
FLASH RT

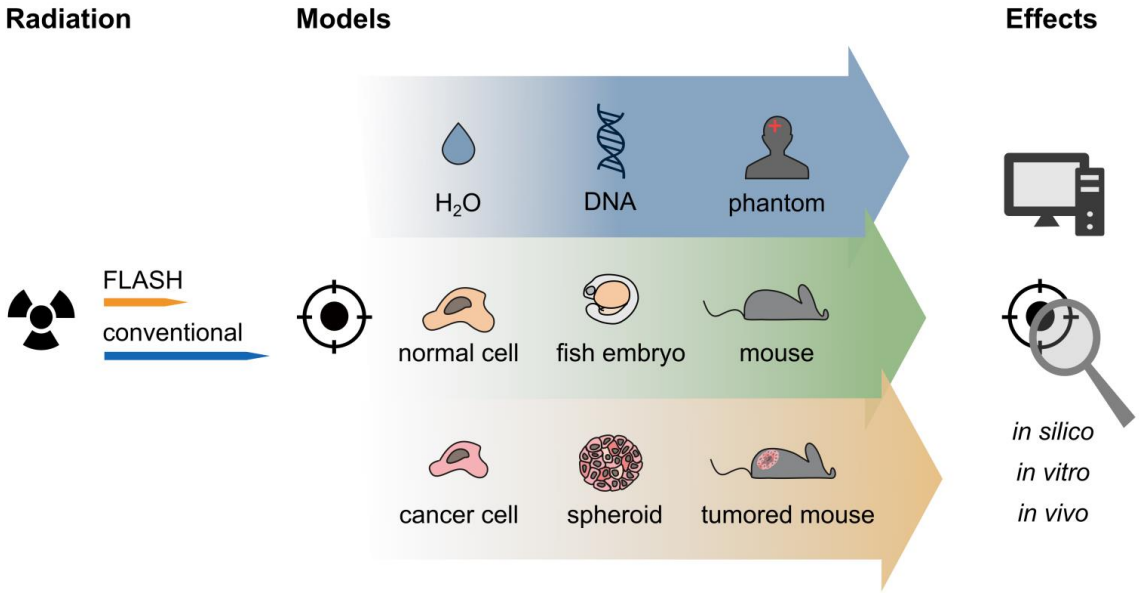
40-10^9 Gy/s

FLASH*lab*@PITZ

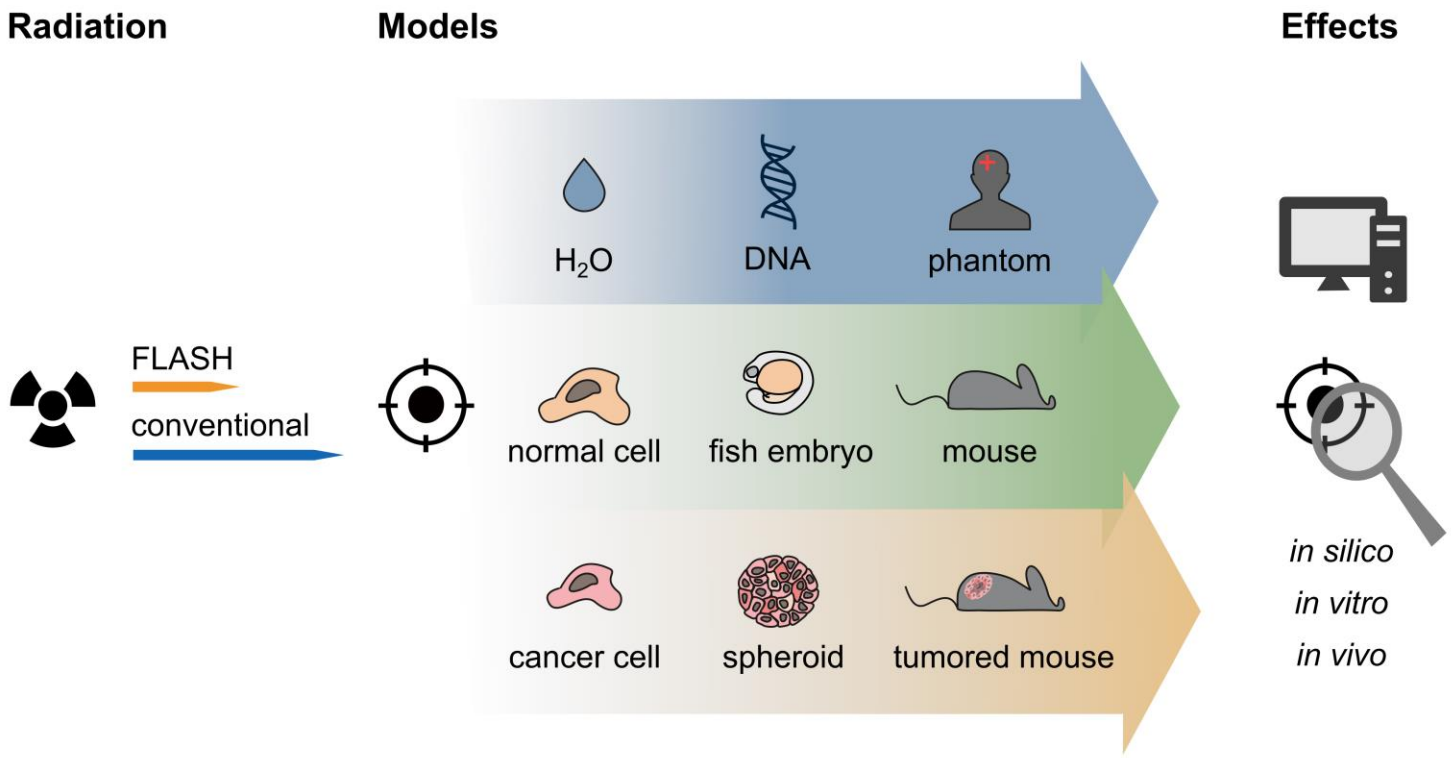
≤10^{14} Gy/s







R&D for Cancer Radiation Therapy (RT)



On behalf of the Organising Committee, we are delighted to confirm that your abstract has been selected for an **E-Poster Viewing.**

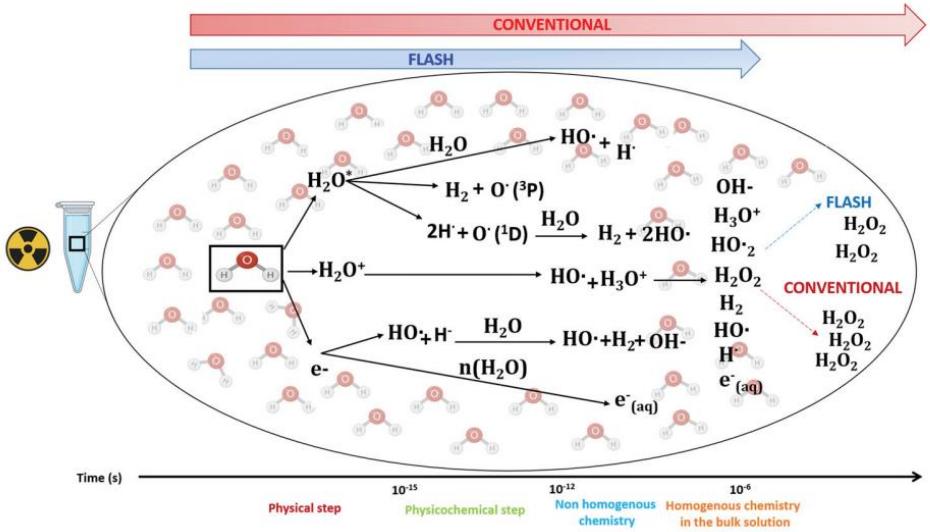
Abstract Number: 443
Abstract Title: CHEMICAL EFFECTS OF FLASHLAB@PITZ BEAM

Chemical effects of PITZ beam

H_2O_2 production measurements during water radiolysis

50/100 µL in
0.5 mL tube

h: 7/10 mm



Kacem et. al. 2022 DOI: 10.1080/09553002.2021.2004328

- Amplex® Red Hydrogen Peroxide/Peroxidase Assay Kit (Invitrogen)

Chemical effects of PITZ beam

H_2O_2 production measurements during water radiolysis

50/100 µL in 0.5 mL tube

h: 7/10 mm

- Amplex® Red Hydrogen Peroxide/Peroxidase Assay Kit (Invitrogen)
- One pretest measuring for checking timing

Dose:
0, 25, 50, 75, 100 Gy
Dose rate:
for conventional 10^{-2} Gy/s
for UHDR: 10^{2}, 10^{4}, 10^{6}, 10^{8} Gy/s

PITZ time:
With triplicates 63 samples – 9 samples per hour – 7 h
Repeated three times – 7 h at daytime (app. 6:00 am – 6:00 pm)

Challenges to solve

In H_2O_2 measurements:

? timing	Requirements	Plan
the reaction buffer (RB) should be added at the exact same time after irradiation for every sample	as fast as possible (200 s)	
after the RB addition, fluorescence measurements should be done at the exact same time point	30 min (up to 90 min)	• bring reader to DESY? (which dates?)

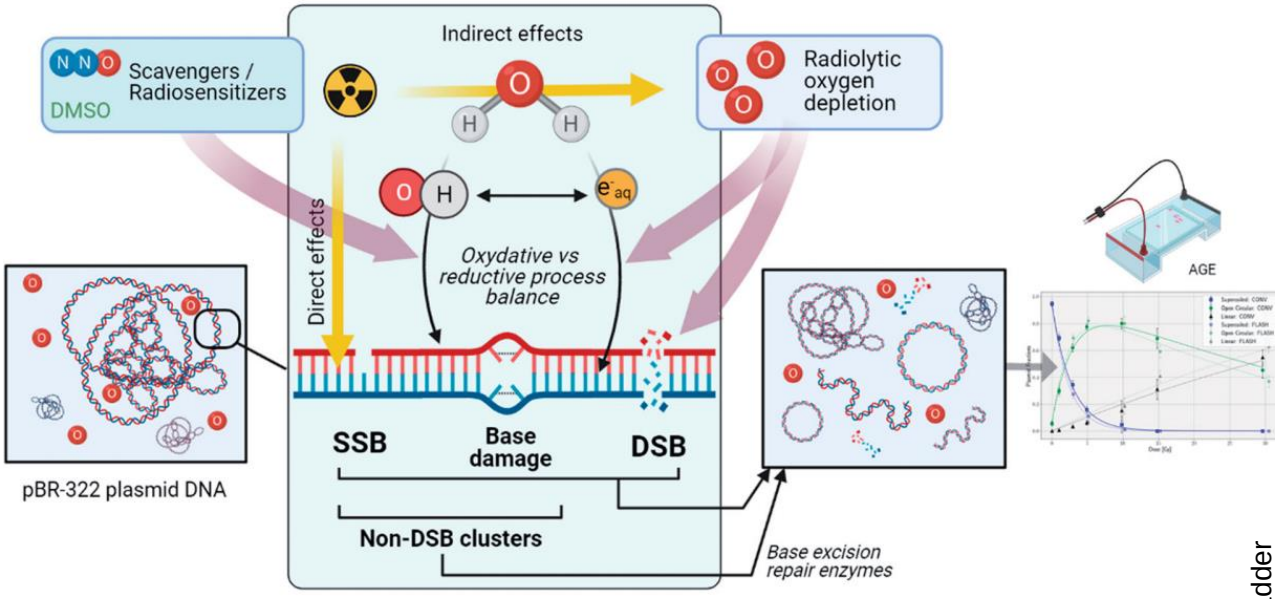
no positive control	to test assay	• make a calibration curve with stock H_2O_2

Biochemical effects of PITZ beam

DNA plasmid conformation

20 µL in
0.5 mL tube

h: 4.3 mm



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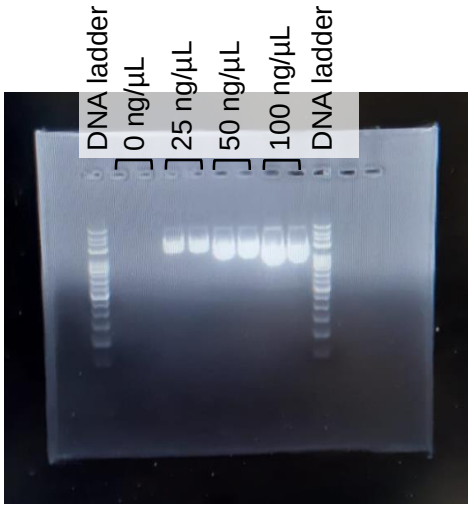
** Alternatively the set-up can be adjusted to 0.2 mL tube*



- Gel electrophoresis

Biochemical effects of PITZ beam

DNA plasmid conformation

20 µL in
0.5 mL tube

h: 4.3 mm

Dose:
0, 1, 2, 5, 10, 20, 30, 50 Gy
Dose rate:
for conventional 10^{-2} Gy/s
for UHDR: 10^{2}, 10^{4}, 10^{8} Gy/s

PITZ time:
With triplicates 66 samples – 10 samples per hour – 7 h
Repeated three times – 7 h at any time (samples should be stored at +4^{0}C)

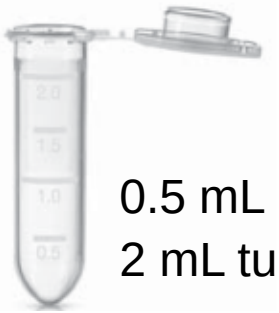
Challenges to solve

In DNA plasmid study:

? cooling	Requirements	Plan
samples should be cooled for storage	4^0C	

no positive control	to test assay	• test with UV light

Biological effects of PITZ beam??



0.5 mL in
2 mL tube

h: 11 mm
w: 10.5 mm

Cell survival after irradiation

- Cancer cell lines:

cervical carcinoma HeLa cells + lung adenocarcinoma A549 cells

- Normal cell lines:

fetal kidney HEK293 cells + fetal lung fibroblast HEL299 cells

Dose:
0, 2, 5, 10 Gy
Dose rate:
for conventional 10^{-2} Gy/s
for UHDR: 10^{2}, 10^{8} Gy/s

PITZ time:
With triplicates 30 samples per cell line – 3 h per cell line
Repeated min three times – 3 h per cell line at daytime (app. 6:00 am – 6:00 pm)

- Colony formation – classic method, shows proliferation activity of cells
- Cell viability – high-throughput method, shows metabolic activity of cells

Challenges to solve

In cell-based study:

? timing	Requirements	Plan
cell should be prepared in a week		• I can prepapre cells for every day – 8-11 Nov.
cells should be held in tubes as short as possible	a few hours are ok	• In 1 h 20 min I need to know when to prepare them and bring to PITZ from TH Wildau, • I will check longer times next week
colony formation assay	delivered reagents, 14 day-long analysis	• adapt the methodology • check one (two) cell lines • get extra hands

Challenges to solve

? timing vs. manpower	Plan
4-11 November – irradiation time + min 14 days for assays + min 10 days for analysis Deadline 28 November	• a lot of work in parallel → extra hands • **Yulia Komar**, Master student at Freie Uni / NTSU Kyiv – internship at TH Wildau / PITZ ? • **Aleksandar Radivoievych**, PhD student at TH Wildau – support in cell culture

Thank you for the attention!