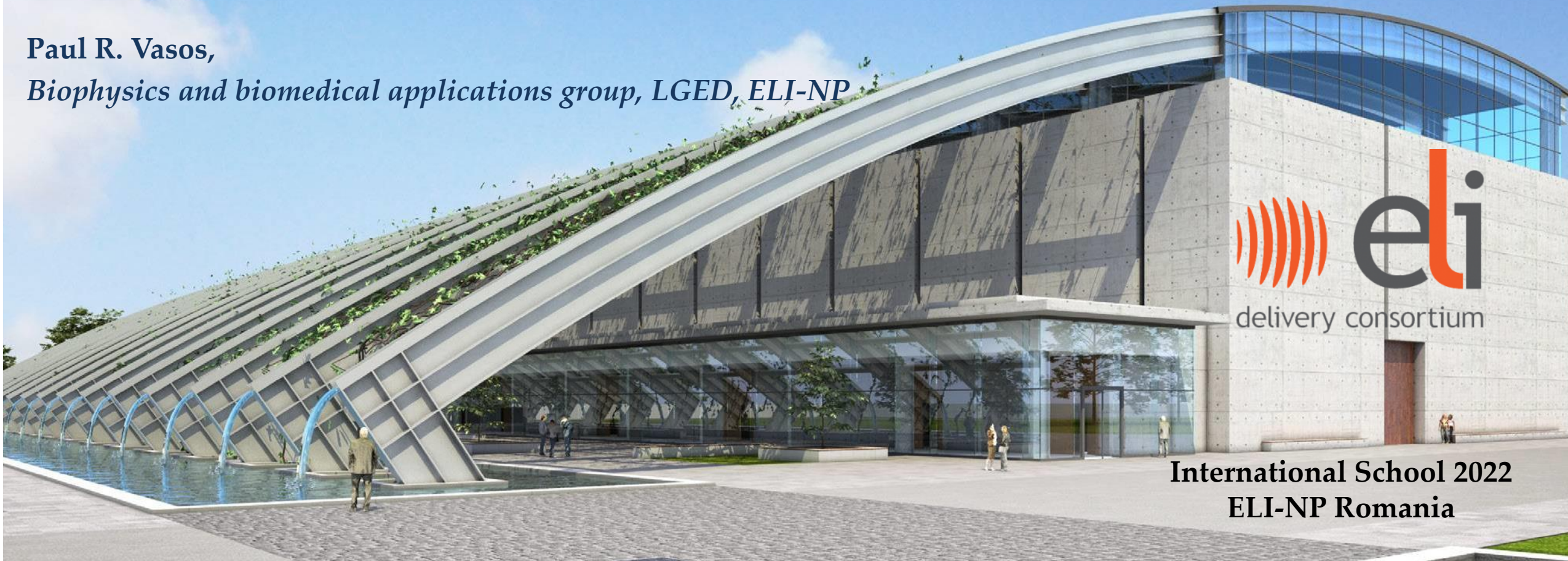
Operational Programme Competitiveness

Extreme Light Infrastructure – Nuclear Physics (ELI-NP) – Phase II
Project co-financed by the European Regional Development Fund

EUROPEAN UNION

Biomolecular effects of FLASH radiation

Paul R. Vasos,
Biophysics and biomedical applications group, LGED, ELI-NP



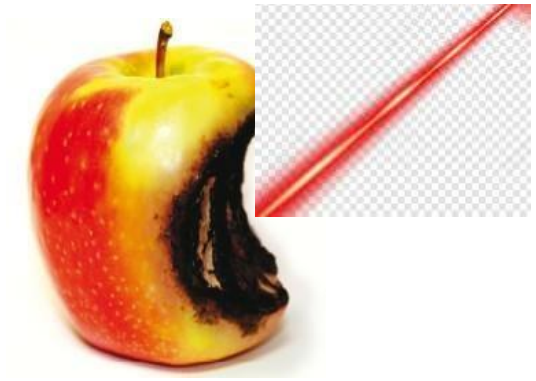
delivery consortium

International School 2022
ELI-NP Romania

Benefits of FLASH radiotherapy

CONVENTIONAL RADIOTHERAPY

FLASH



Focus in space and time

Montay- Gruel et al., PNAS, 2020
M-C Vozevin et al., Clin. Cancer Res., 2019

Asavei et al., Med. Phys. 2019

RADIOTHERAPY DESIDERATA

- EFFICIENCY:
Destroy enemy ships
(cancer cells)
- LACK OF TOXICITY:
while sparing ours
(healthy cells)

Ennemy ship: Corvus, bireme

Own ships: Syracusia

High-Power Lasers and Dose-Rates

ELI-NP ➡ **HIGH POWER = 10 PW**

$$\frac{Energy}{pulse\ time\ duration} = \frac{10\,J}{1\,fs\,(10^{-15}s)}$$

High-Power lasers = energy concentrated in time

(Donna Strickland & Gerald Mourou – Nobel Prize in physics)

Radiobiology ➡ **HIGH DOSE-RATE**

1 J / 1 kg = 1 Gy

$$\frac{Energy}{mass * time} = \frac{Gy}{ns}$$

MEDICAL PHYSICS
The International Journal of Medical Physics Research and Practice

Review Article | Open Access

Laser-driven radiation: Biomarkers for molecular imaging of high dose-rate effects

Theodor Asavei, Mariana Bobeica, Viorel Nastasa, Gina Manda, Florin Naftanaila, Ovidiu Bratu, Dan Mischianu, Mihail O. Cernaianu, Petru Ghenuche, Diana Savu, Dan Stutman, Kazuo A. Tanaka, Mihai Radu, Domenico Doria, Paul R. Vasos ... See fewer authors

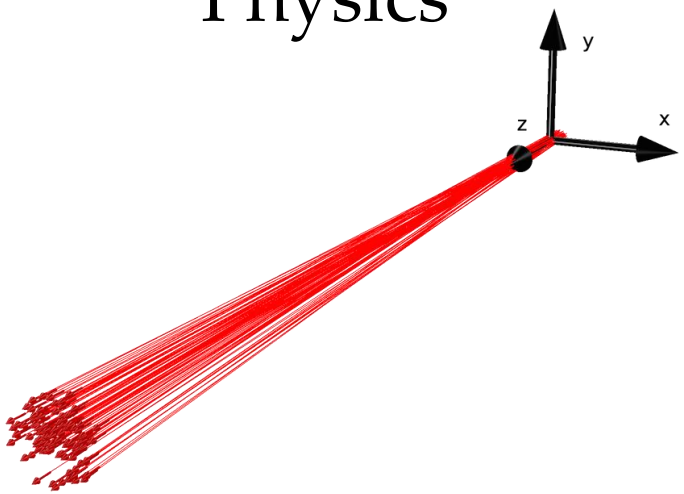
First published: 29 July 2019 | https://doi.org/10.1002/mp.13741

Interdisciplinary Problem Solving

INVOLVING KNOWLEDGE OF RADIATION, MOLECULES, SPECTROSCOPY

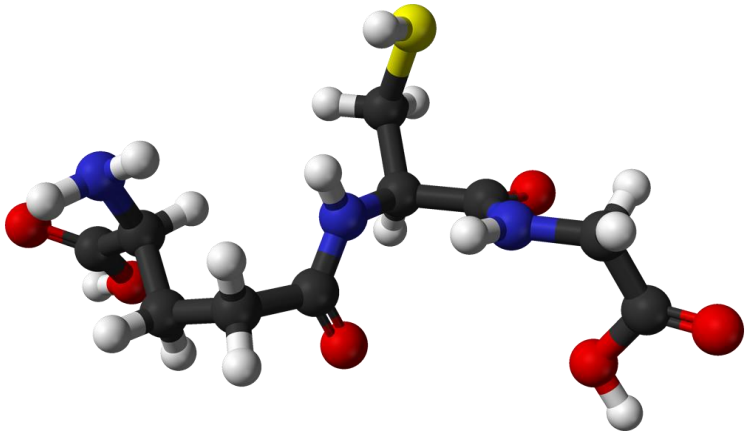
Radiation Dose-Rate versus Cellular Reactions Kinetics

Physics



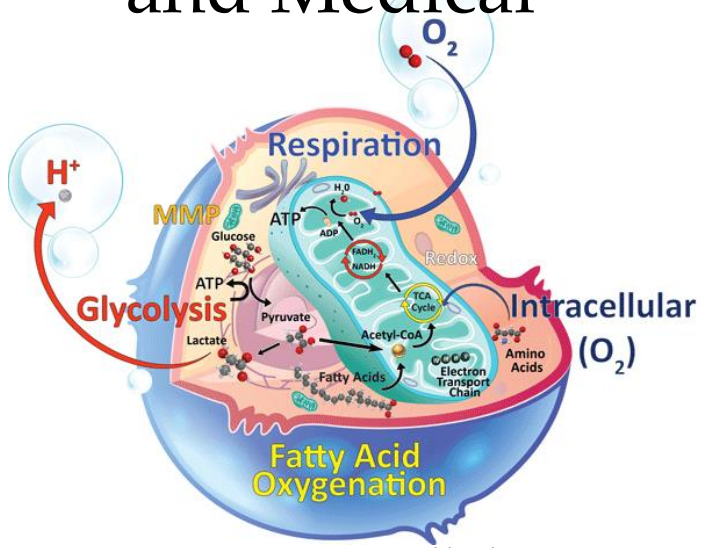
Particle beam characterization for FLASH technique

Biophysics and Biochemistry



Search for radiation-related biomarkers and means of detection

Biomedical and Medical

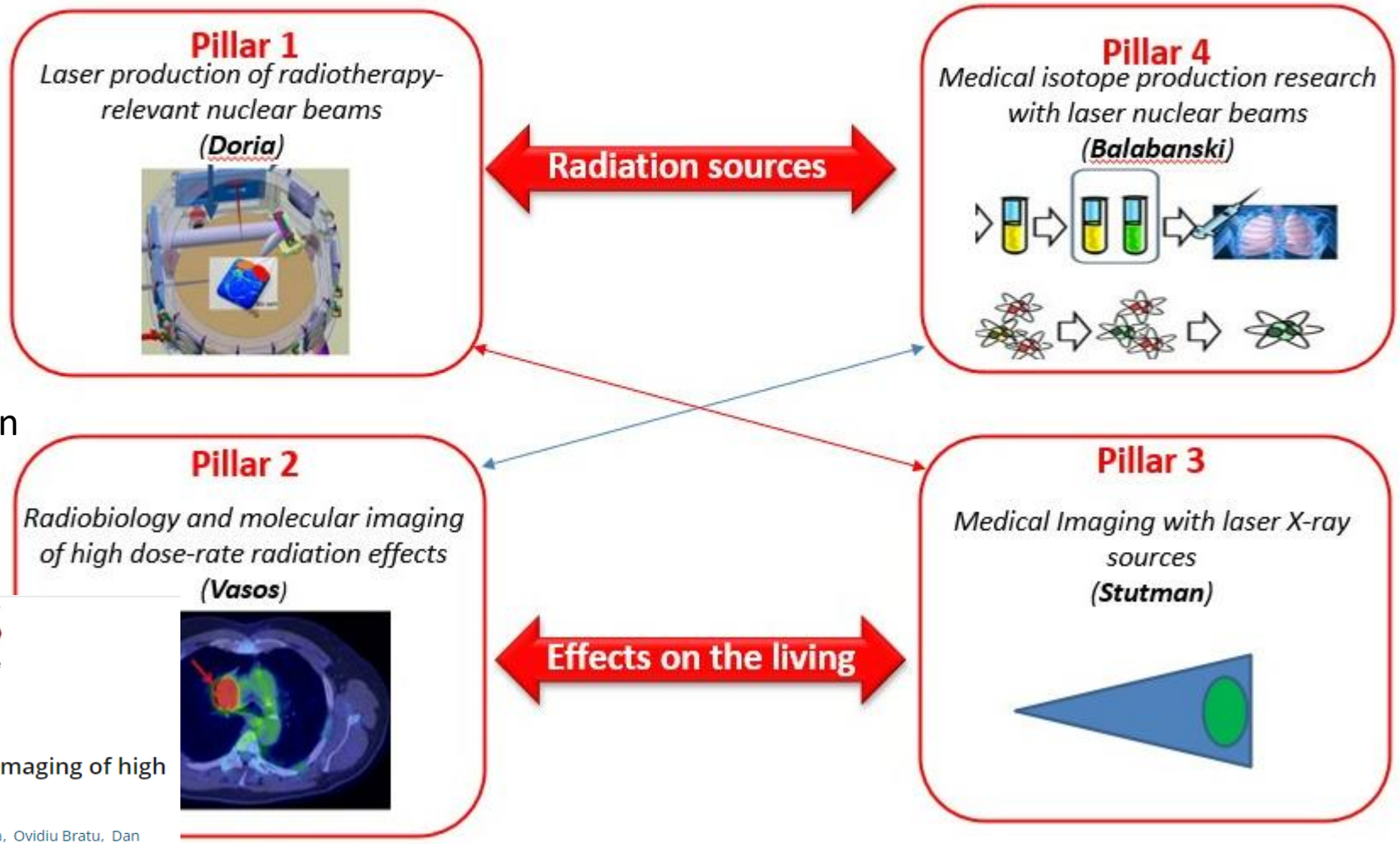


Investigate cellular response and behavior over time

ELI-NP Biomedical Program

ELI-NP Research Program with Complementary Pillars:

1. Laser-driven beams
2. Nuclear Magnetic Resonance (fast prediction of toxicity and efficiency with biomarkers)
3. X-rays
4. Isotopes and Positron Emission (PET-CT)



MEDICAL PHYSICS
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Laser-driven radiation: Biomarkers for molecular imaging of high dose-rate effects

Theodor Asavei, Mariana Bobeica, Viorel Nastasa, Gina Manda, Florin Naftanaila, Ovidiu Bratu, Dan Mischianu, Mihail O. Cernaianu, Petru Ghenuche, Diana Savu, Dan Stutman, Kazuo A. Tanaka, Mihai Radu, Domenico Doria, Paul R. Vasos ... See fewer authors

First published: 29 July 2019 | https://doi.org/10.1002/mp.13741

1. Premise

- Biomolecular effects of classical and laser-driven radiation
- FLASH: high and very high dose-rates, Gy/ms – Gy/ns regimes

2. Molecular biophysics

- Amino acids and H-bonds
- Proteins
- Membranes
- Free radicals and reactive oxygen species (ROS)

3. Experiments performed at ELI-NP and other sites

- Detection methods (chromatography, ESR, MS, NMR) with the clinics: Nuclear Magnetic Resonance
- Biomarkers of dose-rate detected by our group and clinical applications for follow up

4. Foreseen applications

- Pre-clinical tests, translation to the clinic

Paul Vasos

Conventional: 10 Gy in 4 minutes FLASH average 37 Gy/s, in Gy/us pulses

Effects on memory and brain structure **at 2-6 months**

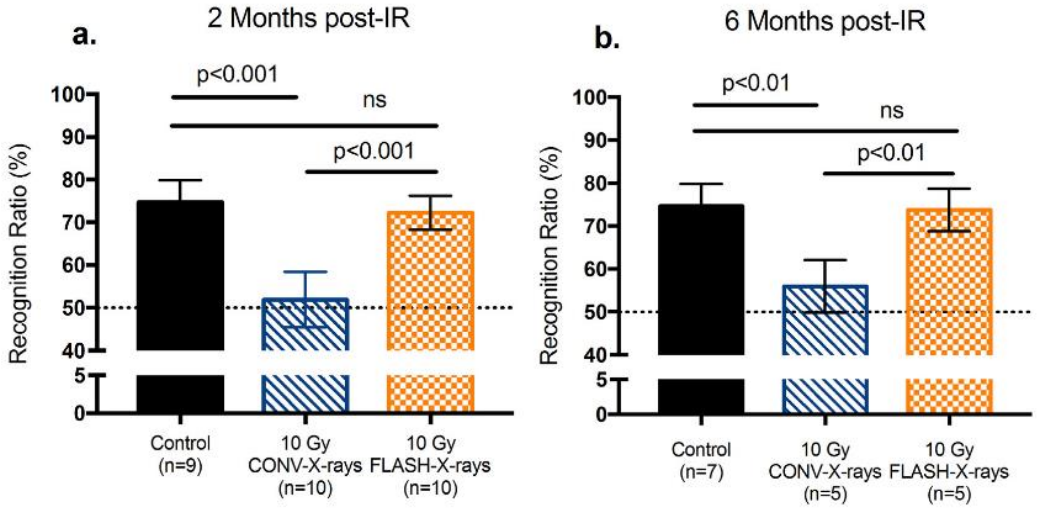


Fig. 1. Evaluation of the Recognition Ratio (RR) two (a) and six months (b) post-irradiation for groups of mice that received sham irradiation (Control) and 10 Gy WBI with FLASH-X-rays or with X-rays delivered at conventional dose-rate (CONV-X-rays). Bars represent mean values and whiskers the standard deviations.

P. Montay-Gruel et al. / Radiotherapy and Oncology 129 (2018) 582–588

beam (eRT6, [15]) could be reproduced with X-rays. Previous FLASH effect results were obtained with a pulsed electron beam delivering a dose-rate in the pulse of $4.5 \cdot 10^5$ Gy/s, corresponding to a mean dose-rate of 200 Gy/s [15]. Therefore, with the possibility to deliver a dose-rate in the slice of 12 000 Gy/s corresponding to a mean dose-rate of 37 Gy/s (FLASH-X-rays), the ESRF synchrotron facility was the ideal candidate to test this hypothesis. We used a broad beam, i.e. a flat beam of 50 μm without microbeam patterns. A dose of 10 Gy FLASH-X-rays was delivered to the whole brain of C57Bl/6J mice (<1 cm^3) with strict dosimetry recordings [16] by moving the head of the mice through the beam. Cognitive function and cellular brain toxicity were evaluated. Using a robust novel object recognition test and immunofluorescence assays, we observed an absence of radiation-induced memory-loss up to 6 months after irradiation, along with a better preservation of hippocampal cell-division and less radiation-induced scar astrogliosis compared to X-ray irradiation performed at a conventional dose-rate (0.05 Gy/s, Pxi Precision X-Ray), something which also irreversibly altered memory cognition in mice. These results were fully comparable with our previous results obtained with FLASH-electrons [7].

Materials and methods

Irradiation devices

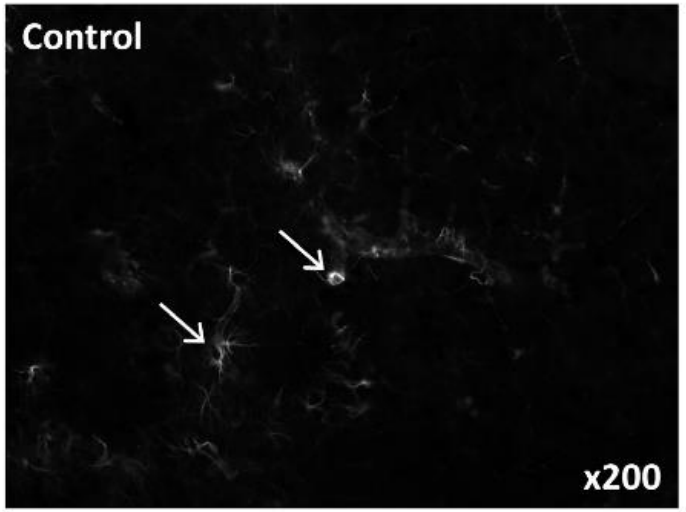
Irradiations were performed at the ID17 Biomedical Beamline of the ESRF (Grenoble, France). Conventional dose-rate irradiations were performed using a XRad 225Cx (Pxi Precision X-Ray) at the Lausanne University Hospital.

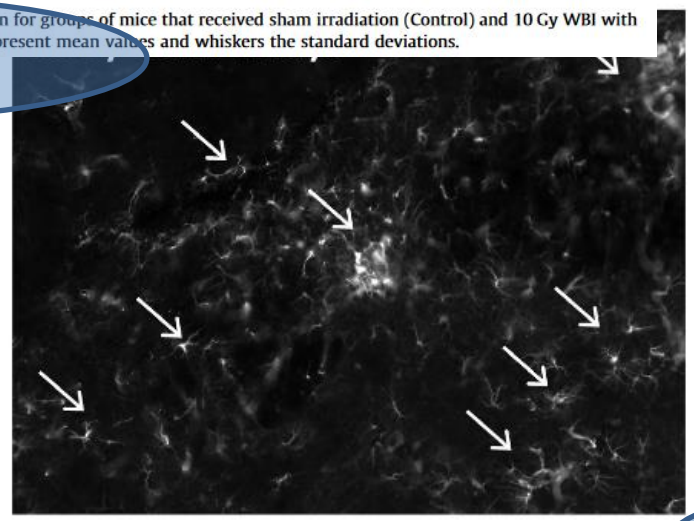
group) were purchased from CRL at the age of eight weeks. Animal experiments were approved by the Ethics Committee for Animal Experimentation of France and Switzerland and performed within institutional guidelines. All irradiations were performed under isoflurane anesthesia.

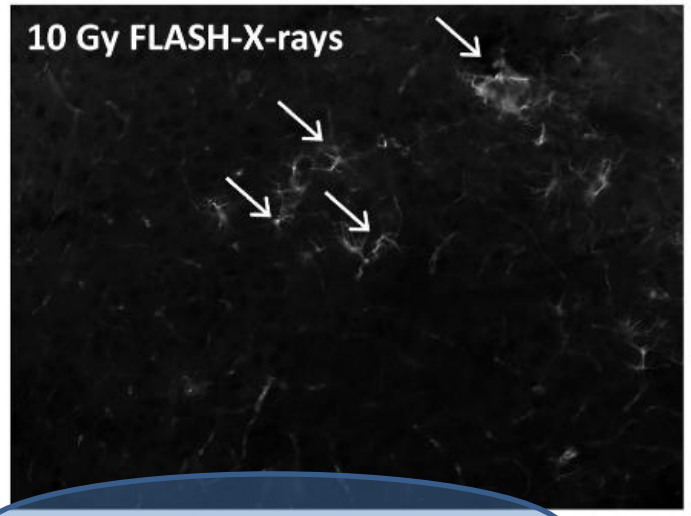
We delivered 10 Gy absorbed dose to water whole brain irradiation (WBI) at conventional dose-rate (CONV-X-rays, 0.05 Gy/s) using a 10 × 10 mm^2 field size, after fluoroscan imaging to position the mouse in order to avoid irradiating their eyes, mouth cavity, esophagus and trachea. Two horizontal opposed beams each delivering 5 Gy at 5 mm depth were irradiating the brain.

For 10 Gy WBI with FLASH-X-rays, the mice were anesthetized under isoflurane inhalation and irradiated under broad beam conditions. A horizontal slit height of 50 μm was selected to be able to adapt the speed of the MRT goniometer to scan the mouse vertically through the beam at speeds around 62 mm/s to cover a total field height of 17 mm diameter defined by a conformal mask placed 1 m upstream from the animals. A dose-rate in each 50 μm slice of 67 Gy/(s.mA) was measured at 2 cm depth using a Pinpoint ionization chamber (PTW, Ref. 31014). During the experiment, the machine current was 178 mA, leading to a dose-rate in the slice of about 12,000 Gy/s, corresponding to a mean dose-rate of 37 Gy/s for the delivery of 10 Gy to the whole mouse brain with the duration of 0.27 s.

Despite a difference in irradiation geometry between conventional dose-rate X-rays (10 × 10 mm^2 field size) and FLASH-X-rays (17 mm diameter), imaging performed before the irradiation ensured a proper mouse-positioning and the actual irradiation of the entire brain in both configurations.







ive astrogliosis assessment by GFAP immunostaining on brain striatum sections performed two months post whole brain irradiation on sham-irradiated mice
mice irradiated with 10 Gy WBI with FLASH-X-rays or with X-rays delivered at conventional dose-rate (CONV-X-rays). Arrows point at GFAP positive cells in the

Paul Vasos

First clinical application: FLASH RADIOTHERAPY

Radiotherapy and Oncology 139 (2019) 18–22

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

First in Human

Treatment of a first patient with FLASH-radiotherapy

Jean Bourhis [a,b,*], Wendy Jeanneret Sozzi [a], Patrik Gonçalves Jorge [a,b,c], Olivier Gaide [d], Claude Bailat [c], Fréderic Duclos [a], David Patin [a], Mahmut Ozsahin [a], François Bochud [c], Jean-François Germond [c], Raphaël Moeckli [c,1], Marie-Catherine Vozenin [a,b,1]

[a] Department of Radiation Oncology, Lausanne University Hospital and University of Lausanne; [b] Radiation Oncology Laboratory, Department of Radiation Oncology, Lausanne University Hospital and University of Lausanne; [c] Institute of Radiation Physics, Lausanne University Hospital and University of Lausanne; and [d] Department of Dermatology, Lausanne University Hospital and University of Lausanne, Switzerland

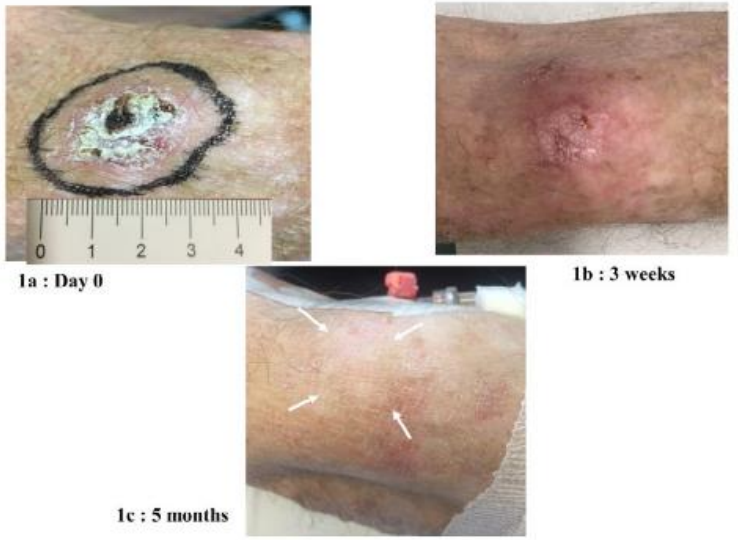


Fig. 1. Temporal evolution of the treated lesion: (a) before treatment; the limits of th PTV are delineated in black; (b) at 3 weeks, at the peak of skin reactions (grade 1 epithelitis NCI-CTCAE v 5.0); (c) at 5 months.

Healing:
4-5 months

Our patient had a long history of localized RT (110 different irradiations in about 10 years) and doses needed to control the lesions were typically 20–21 Gy in 6–10 fractions. Despite these relatively low total doses, the acute toxicity of these treatments was found to be relatively severe in the context of the particular skin frailty of this patient, with commonly 3–4 months for complete healing. Taking this into account, a dose of 15 Gy was proposed, high enough to trigger a FLASH effect and likely sufficient for obtaining a tumor control in this particular lymphoma. As extrapolated from our pre-clinical studies, the hypothesis was that the "equivalent FLASH dose for normal tissues" would be about 2/3 of the prescribed dose, i.e. around 10 Gy and the "equivalent-FLASH dose for the tumor" would be the real prescribed dose of 15 Gy [11,14]. Following this hypothesis, a single dose of "10-Gy-equivalent for normal tissues" was considered as feasible for this patient, although being likely at the upper acceptable limit given the previous history of severe acute skin reactions with fractionated 20–21 Gy.

The second key aspect in order to reproduce a FLASH effect in human normal tissues, was to use the parameters that were strongly correlated with a FLASH effect in pre-clinical studies *in vivo*. These data showed that the most relevant parameters were the combination of dose in the pulse ($\geq$1.5 Gy), dose-rate within the pulse ($\geq 10^6$ Gy), and overall irradiation time (<200 ms, but preferably less) [14]. Taking these considerations into account, the dose of 15 Gy was given in 10 pulses each of 1 μs, with a repetition rate of 100 Hz, which led to an overall treatment time of 90 ms. A 5-mm bolus was added so that the total depth covered by the 90% isodose was 1.3 cm, taking into account the thickness of the tumor.

Standard RT, ca 2 Gy/ session, 6-10 sessions
FLASH : 15 Gy in 200 ms, pulses of 1.5Gy/us

Paul Vasos

Acute Toxicity
as well as
Late Toxicity
to be considered

skin necrosis (7-9 months)
cats treated for
Facial tumours
maxilary bone necrosis
(3/7 FLASH
0/9 Standard of Care)

Editorial > Clin Cancer Res. 2022 Sep 1;28(17):3636-3638. doi: 10.1158/1078-0432.CCR-22-1255.

Shining a FLASHlight on Ultrahigh Dose-Rate Radiation and Possible Late Toxicity

Amit Maity [1 2 3], Constantinos Koumenis [1]

Affiliations + expand

PMID: 35736814 PMCID: PMC9444945 (available on 2023-03-01)
DOI: 10.1158/1078-0432.CCR-22-1255

Abstract

A recent study reported results from a clinical trial in cats and from experiments in mini-pigs in which a single dose of radiotherapy was delivered at ultrahigh dose rates (FLASH). There was acceptable acute toxicity; however, some animals suffered severe late toxicity, raising caution in the design of future trials. See related article by Rohrer Bley et al., p. 3814.

Trial registration: ClinicalTrials.gov NCT04986696 NCT04592887.

Ongoing : clinical trial with proton FLASH for metastasis control

Paul Vasos

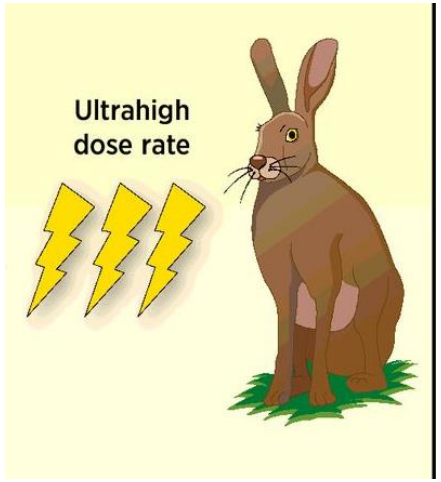
Clinical Trial Brief Report

Clinical Cancer Research

The Advantage of FLASH Radiotherapy Confirmed in Mini-pig and Cat-cancer Patients

Marie-Catherine Vozenin[1], Pauline De Fornel[2], Kristoffer Petersson[1,3], Vincent Favaudon[4], Maud Jaccard[1,3], Jean-François Germond[3], Benoit Petit[1], Marco Burki[5], Gisèle Ferrand[6], David Patin[3], Hanan Bouchaab[1], Mahmut Ozsahin[1,6], François Bochud[3], Claude Bailat[3], Patrick Devauchelle[2], and Jean Bourhis[1,6]

FLASH radiation ☺



36 weeks post radiation

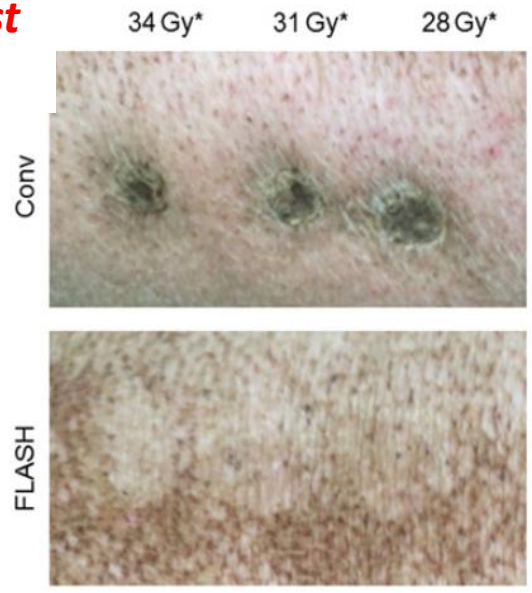
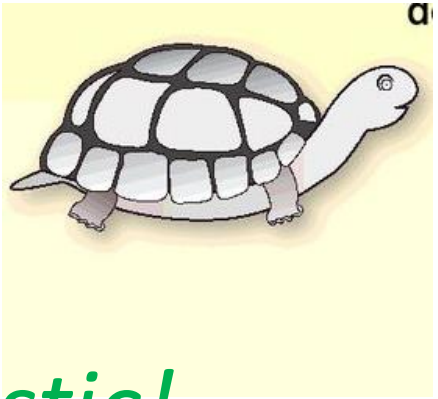
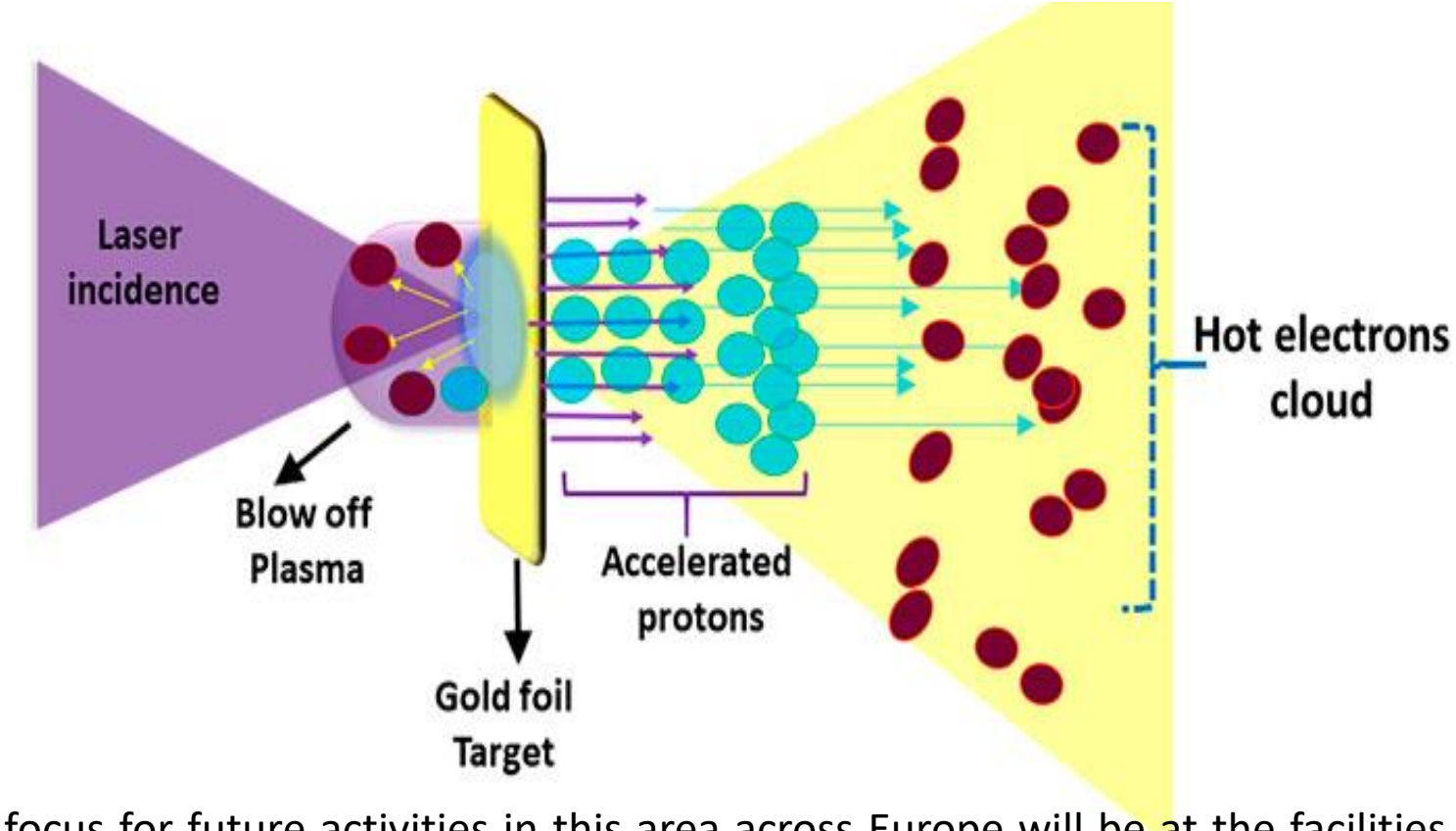


Figure 1.
A, Dose distributions calculated in XiO for the mini pig irradiation; a transversal slice reconstructed from the CT-scan showed beam apertures and dose distributions. **B,** Thirty-six weeks postradiotherapy, macroscopic visualization

LATE assessment of effects ☹



Need for fast diagnostic!
(Flash detection)



Front. Phys., 08 April 2021
Sec. Medical Physics and Imaging
https://doi.org/10.3389/fphy.2021.624963

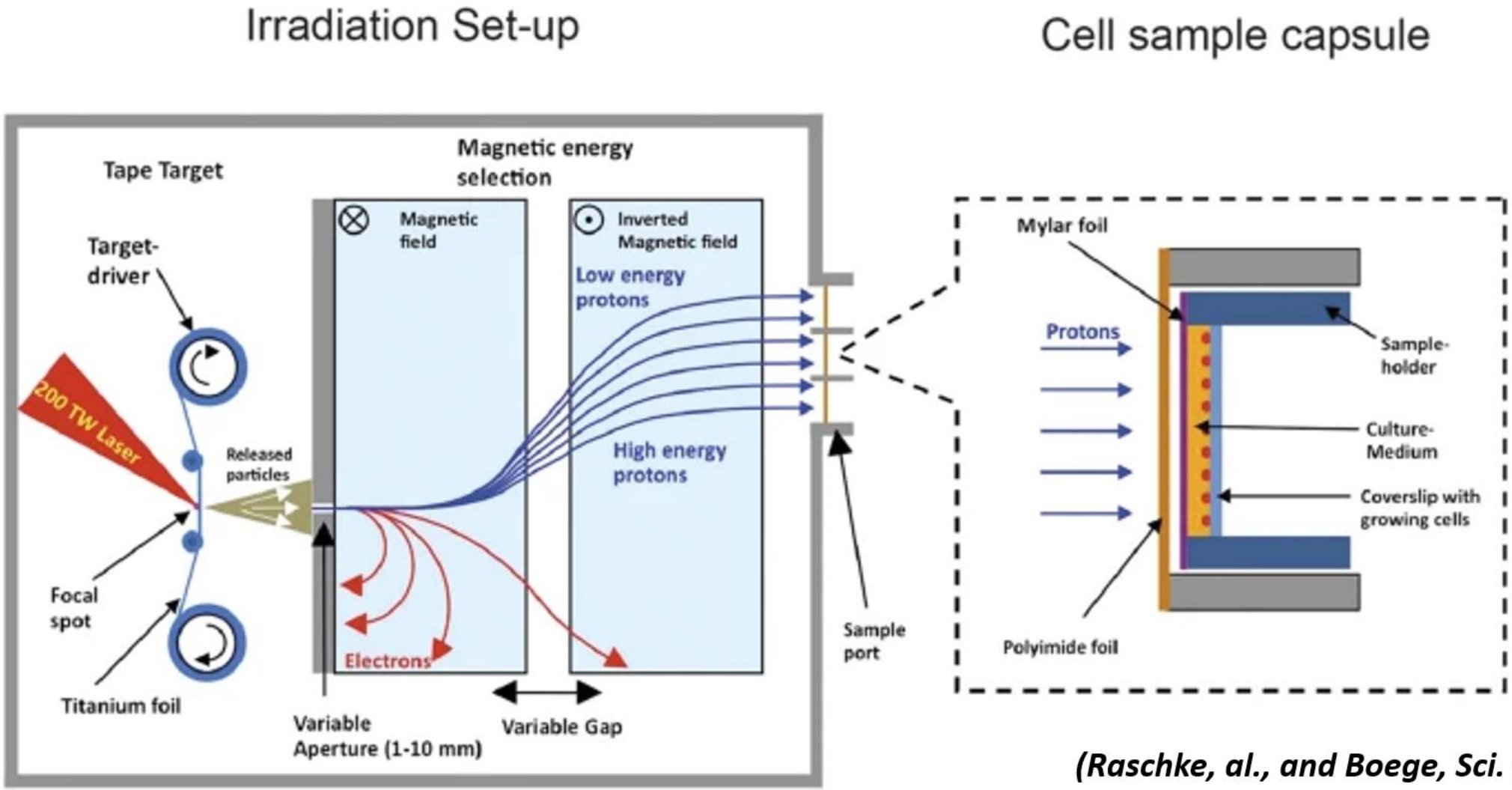
This article is part of the Research Topic
Applied Nuclear Physics at Accelerators
View all 57 Articles ›

Radiobiology Experiments With Ultra-high Dose Rate Laser-Driven Protons: Methodology and State-of-the-Art

Pankaj Chaudhary[1]*, Giuliana Milluzzo[2], Hamad Ahmed[2,3], Boris Odlozilik[2,4], Aaron McMurray[2], Kevin M. Prise[1]* and Marco Borghesi[2]*

A focus for future activities in this area across Europe will be at the facilities of the Extreme Light Infrastructure (ELI), particularly at ELI Beamlines (Czech Republic), where the Extreme Light Infrastructure Multidisciplinary Applications of Laser-Ion Acceleration (ELIMAIA) beam lines in Prague are being commissioned **ELI Nuclear Physics (ELI NP) Romania, is also planning an involvement in laser-driven ion radiobiology research**
(**Asavei, .., Doria* and Vasos*, Med. Phys. 201**9)
While hadrontherapy was highlighted as a key application for laser-driven protons at an early stage of the development of laser acceleration, it is clear that direct application of laser-driven beams remains challenging, and significant progress is still needed to match the parameters required for clinical particle therapy.

LASER-DRIVEN FLASH RADIOBIOLOGY

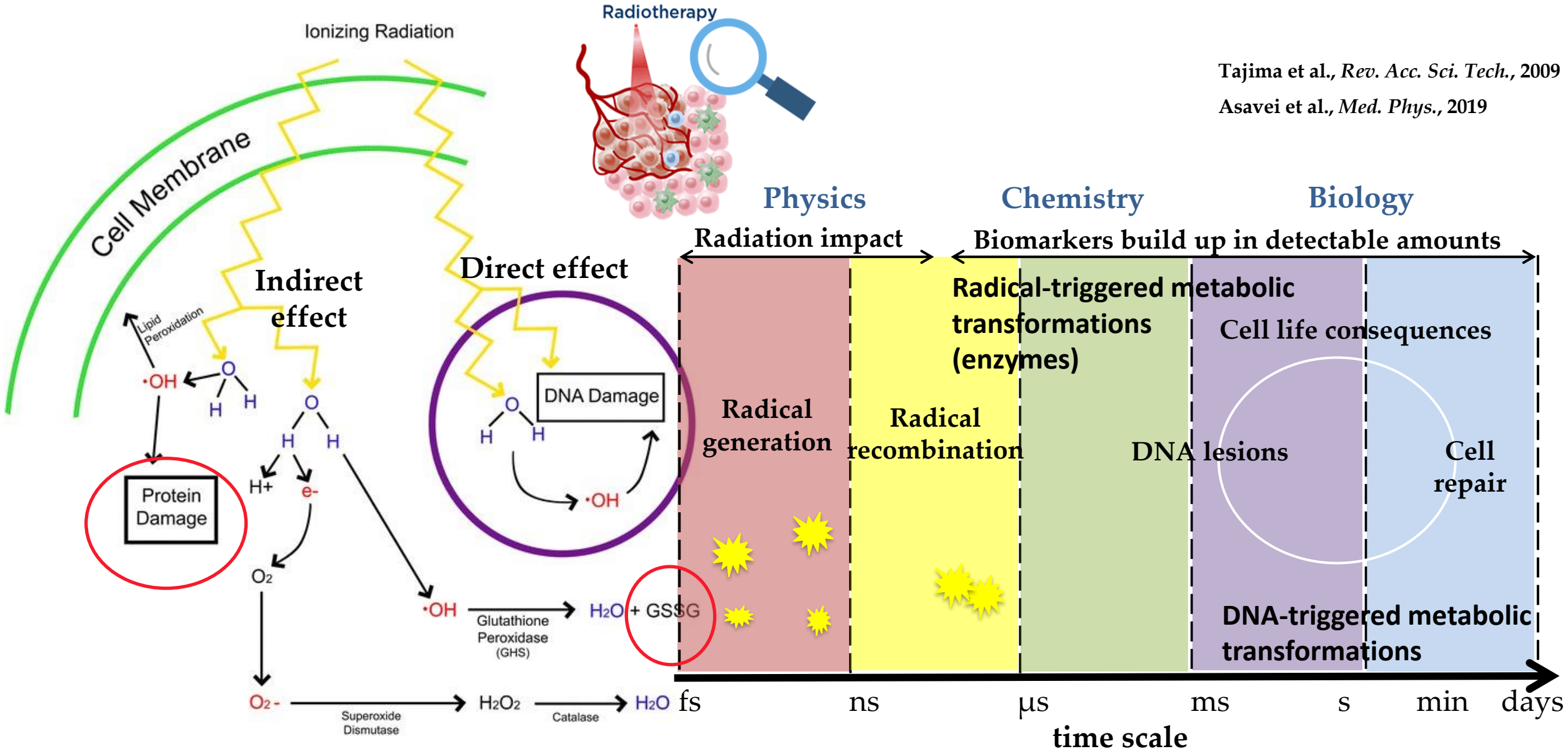


(Raschke, al., and Boege, Sci. Rep., 2016)

Molecular effect of radiation can be observed at different timescales

Tajima et al., *Rev. Acc. Sci. Tech.*, 2009

Asavei et al., *Med. Phys.*, 2019



Radiation dose-rate

- **time** is of the essence

high-power laser: ns compression and below for accelerated protons

>200 MeV protons expected at ELI-NP

Source Power P, pulse length τ_p	Average proton energy	Dose rate	Proton pulse duration	Reference
J-Karen *17 TW, 35 fs*	2.5 MeV	0.01 Gy/ns	15 ns	Minafra et al., Springer 2016
Draco *60 TW, 45 fs*	15 MeV	0.01 Gy/ns	2 ns	Zeil et al., Appl. Phys B., 2012
Arcturus *200 TW, 30 fs*	2.1 MeV	0.03 Gy/ns	1 ns	Raschke et al., Sci. Rep., 2016
Taranis *30 TW, 700 fs*	4.5 MeV	1 Gy/ns	1 ns	Doria et al., AIP Adv., 2012
Atlas *30 TW, 30 fs*	5.2 MeV	4.6 Gy/ns	1 ns	Bin et al., Appl. Phys Lett., 2012
Classical accelerators with doses applied on longer time scales				
Francis H. Burr cyclotron Proton Beam Therapy Center North East	230 MeV	Gy / min	200 ms	Schlegel et al., Berlin Heidelberg: Springer-Verlag, 2006
Hyogo Ion Beam Medical Center, Japan	70-230 MeV	Gy / min	400 ms	Yogo et al., Appl. Phys. Lett., 2009

Bolton, *Nucl. Instr. Meth. Phys Res. A*, **2016**

Tajima et al., *Rev. Acc. Sci. Tech.*, **2009**

classically accelerated protons

BIOMOLECULAR EFFECTS NEED TO BE STUDIED

At high dose rates, free radicals may recombine or the reactants of biochemical cascades may be saturated

(Raschke, Boege et al., 2016)

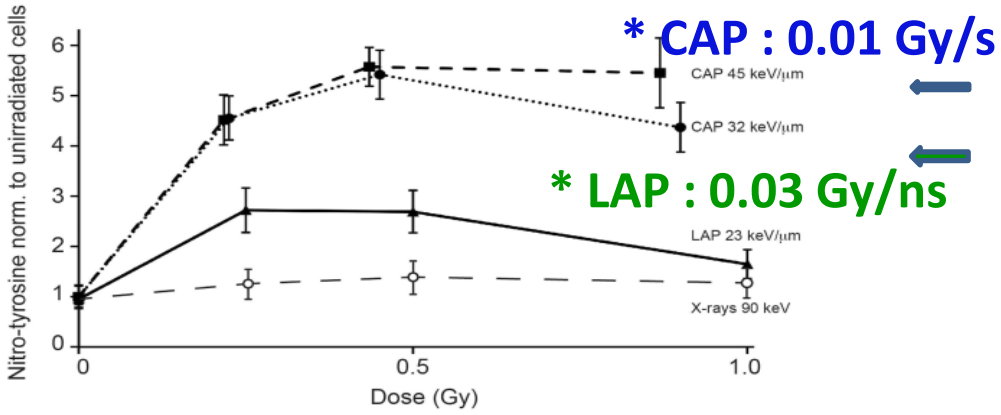
First-hand DNA strand breaks do not depend on radiation dose-rate.

(Bayart et al., 2019)

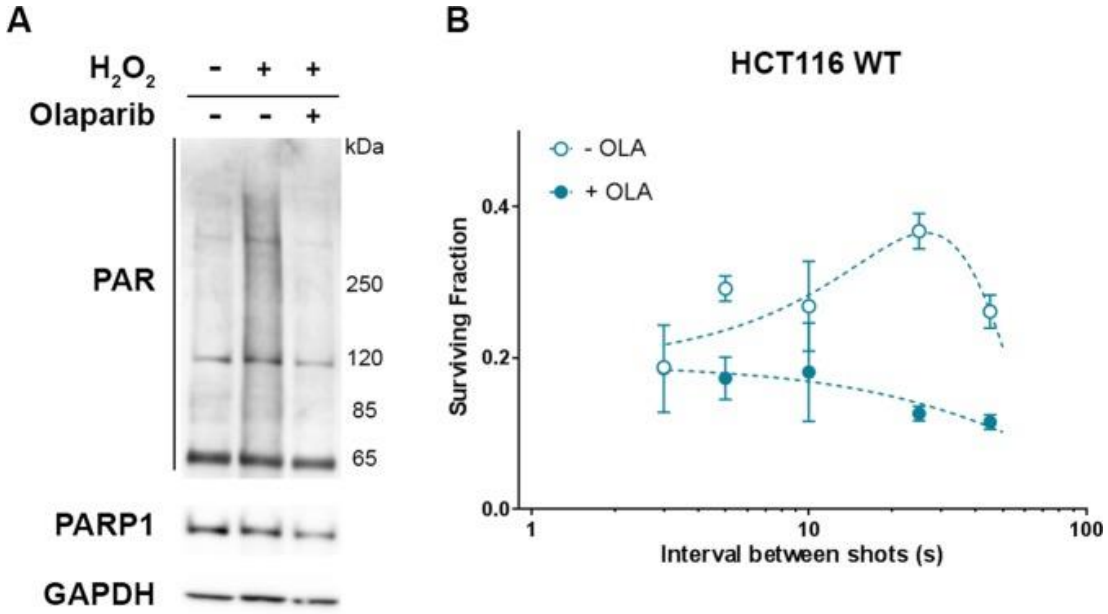
Free radicals, Reactive Species Produced

CAP: conventionally-accelerated protons

LAP: laser-accelerated protons



(Raschke, Boege et al., Sci. Rep., 2016)

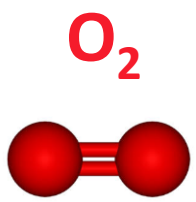


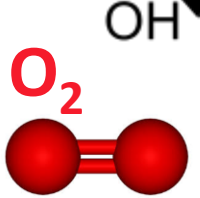
Bayart et al Sci. Rep. 2019

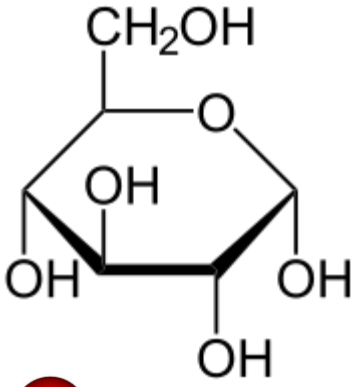
Paul Vasos

Warburg effect: metabolism kinetics linked to the influx of molecular resources distinguishes between cancer and normal cells

Normal cells: high-resource, high-throughput metabolism

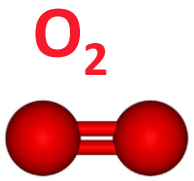


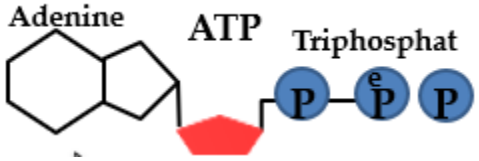


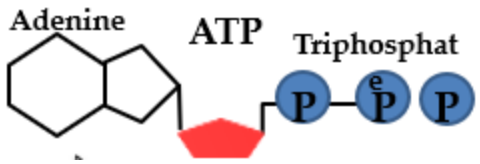


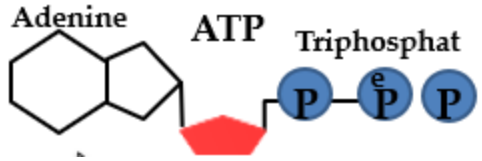
glucose

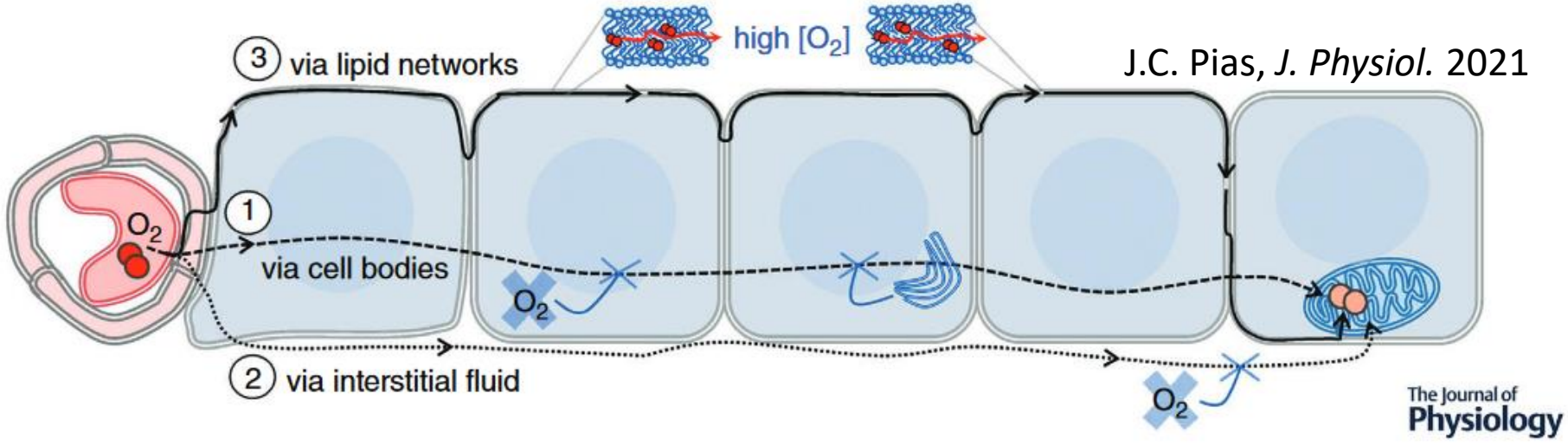
Cancer cells: low-resource, low-throughput metabolism





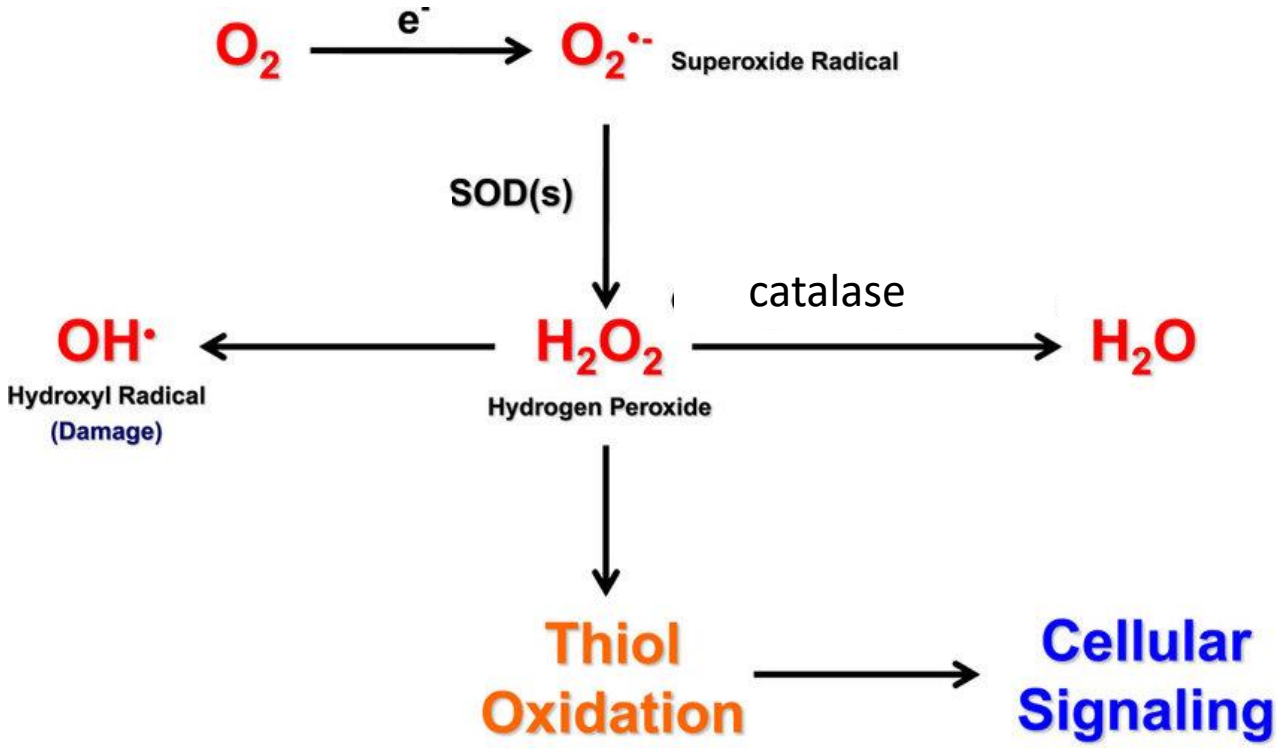


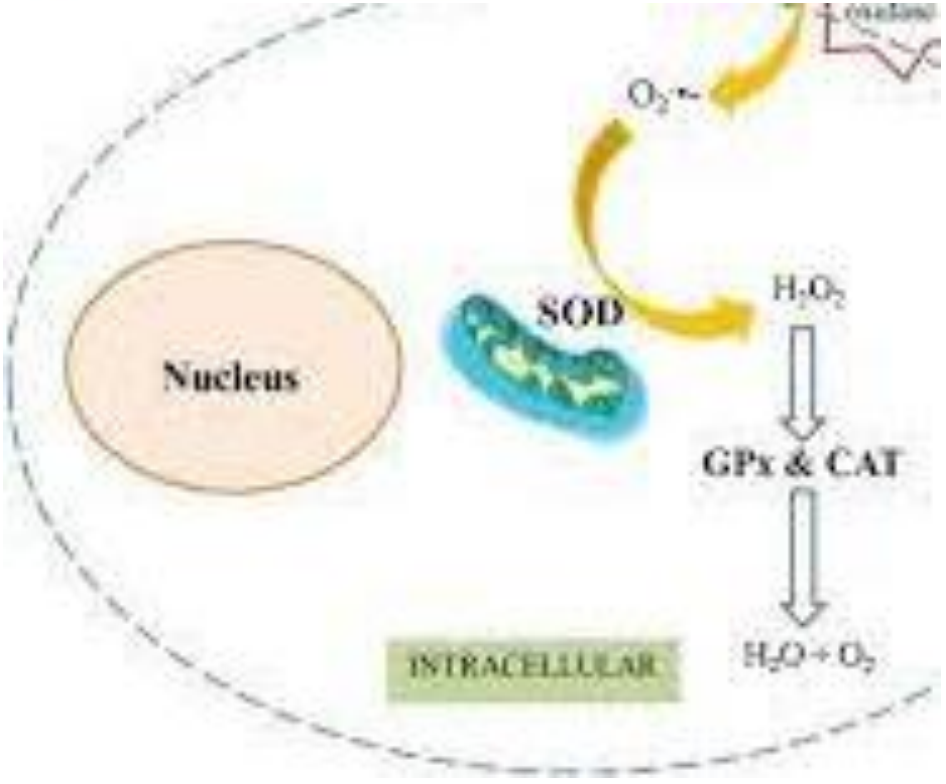




J.C. Pias, *J. Physiol.* 2021

Production free radicals:
Transport of relevant amounts
Enzyme activity rates:
Superoxide Dismutase (SOD) Catalase (CAT)





The biological mechanism responsible for the reduction in normal tissue toxicities following irradiation at FLASH dose rates is not currently understood, yet several non-mutually exclusive hypotheses have been proposed. Some researchers have suggested that the differential response between FLASH-RT and CONV-RT may be due to the radiochemical depletion of oxygen at ultra-high dose rates and subsequent radioresistance conferred to the irradiated tissue (32, 38, 39). It is widely accepted that hypoxic tissues are more radioresistant than well-oxygenated tissues. This is because in the presence of molecular oxygen there is fixation of indirect radiation-induced DNA damage. Indirect damage, the predominant mechanism by which low linear energy transfer (LET) radiation induces DNA damage, occurs when radiation results in the radiolysis of water molecules and the subsequent generation of free radicals. Free radicals are then incorporated into DNA, causing damage—yet this can be easily resolved. However, if a free radical reacts with molecular oxygen, this yields a peroxyl radical (HOO*, ROO*). Peroxyl radicals have the potential to induce permanent damage, and are therefore a more efficacious DNA damaging agent. Hence, a lack of oxygen in the immediate environment of a cell limits the extent of radiation-induced DNA damage

Review > Front Oncol. 2020 Jan 17;9:1563. doi: 10.3389/fonc.2019.01563. eCollection 2019.

Ultra-High Dose Rate (FLASH) Radiotherapy: Silver Bullet or Fool's Gold?

Joseph D Wilson [1], Ester M Hammond [1], Geoff S Higgins [1], Kristoffer Petersson [1 2]

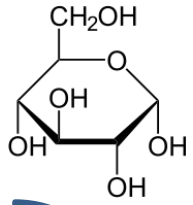
Paul Vasos

Hyperpolarised Magnetic Resonance Imaging

imaging metabolism kinetics
(time is of the essence)

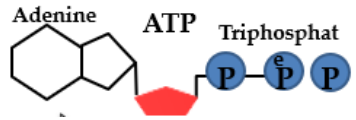
Structural imaging:
- are there many seeds?

Molecular Imaging:
- is it sweet? (sugar content)



Functional molecular imaging:
- is it turning sour? how fast?

$k \sim 1/t$



NEEDED : high sensitivity,
short time scale,
non-invasive

watermelon: timesnewroman.ro

Tissue shape

Tissue function characterisation within hours: *Functional molecular imaging*

- **Analysis of effects within months**

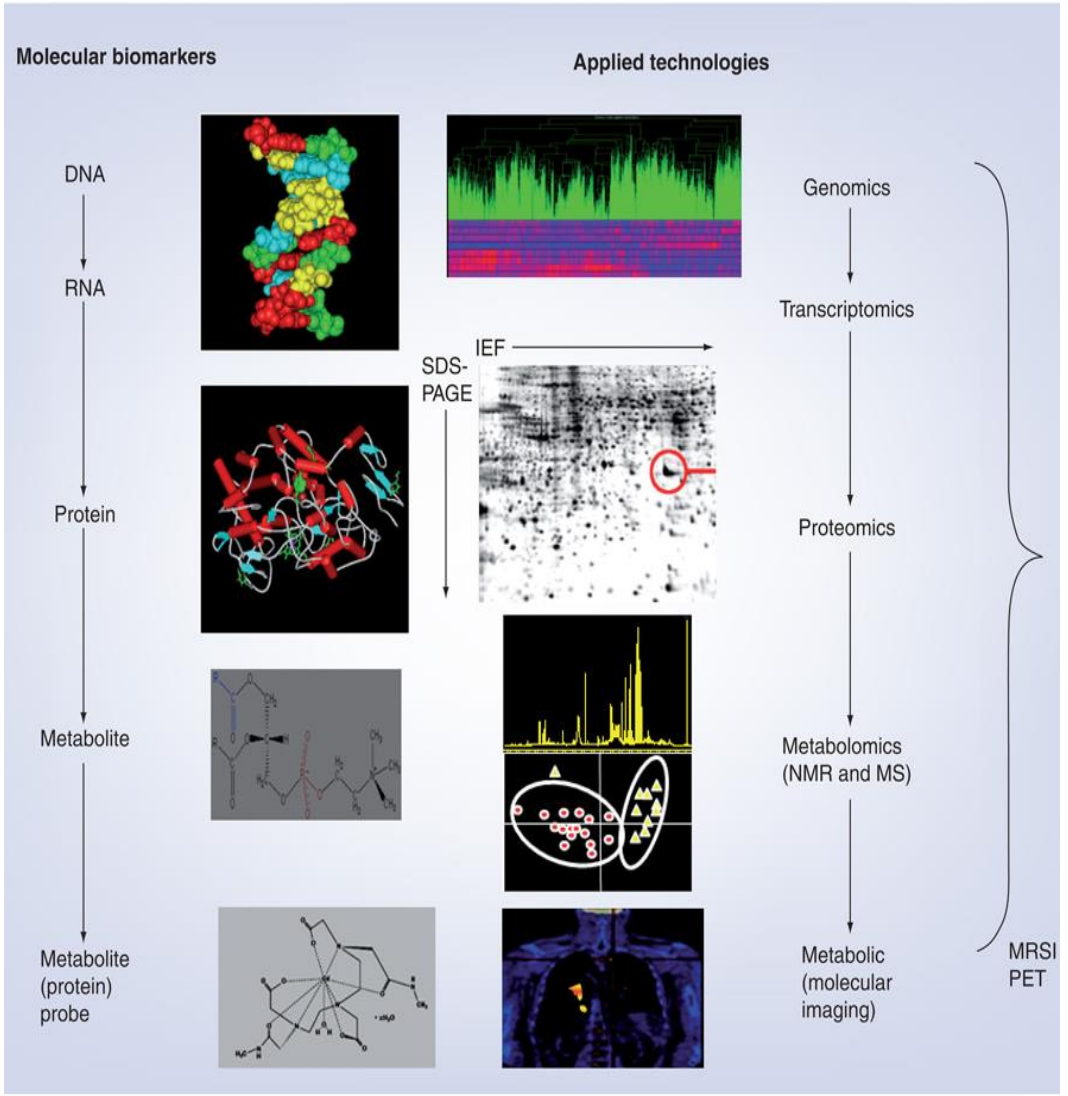
with structural imaging

Functional Magnetic Resonance

// in-vivo, in vitro

Do not require Lysis

Endpoint measurement



To follow:

How can radiation dose-rate effects be quantified?

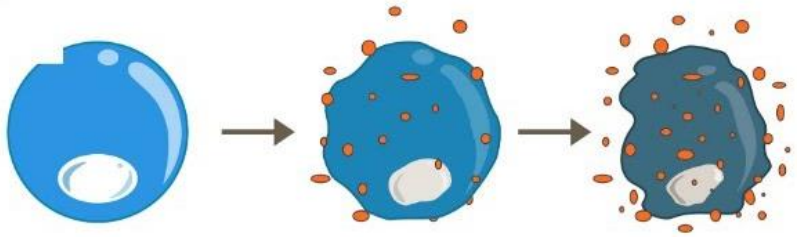
- Biomolecules inside cells (DNA, proteins, metabolites)
- Radiation fuel: oxygen and its availability, cell membrane permeability, creation of free radicals

- Molecular species carrying reactive electrons (**free radicals** and **reactive oxygen species**) formed by radiation

- Antioxidants that neutralize free radicals and reactive oxygen species

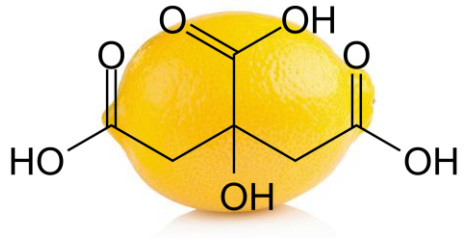
- How can we follow the rates (the time dependence) of the reactions involving the reactive molecules (free radicals, reactive oxygen species, other metabolites produced by cells in response) as a function of the rate of radiation delivery, as this will decide on radiation toxicity.

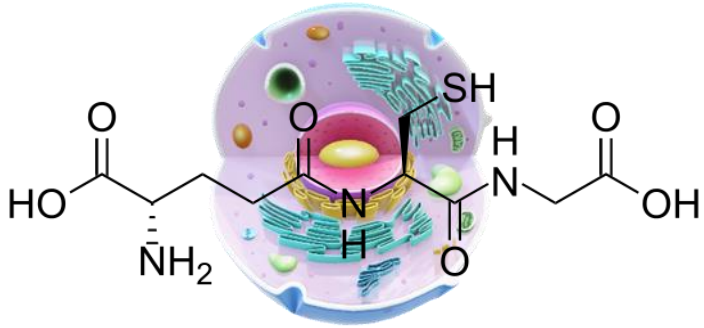
Oxidative stress leads to cell decay triggered by molecular processes



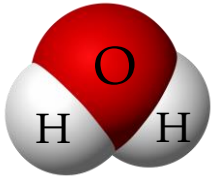
Free radicals,
Reactive Oxygen Species (ROS)

Antioxidants

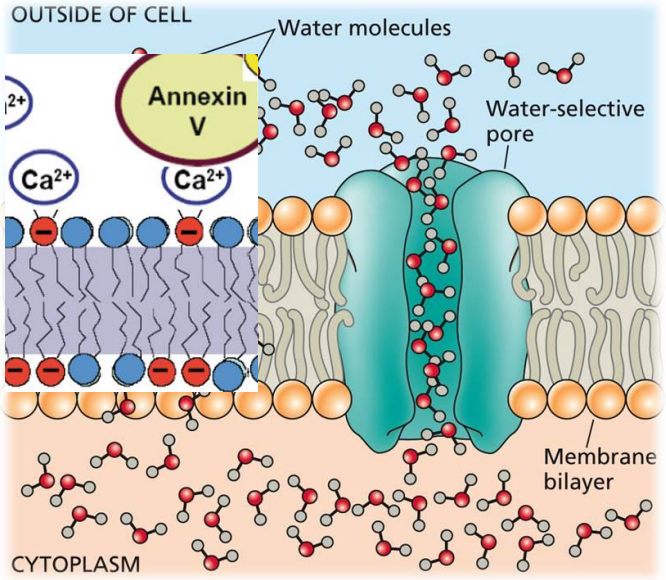


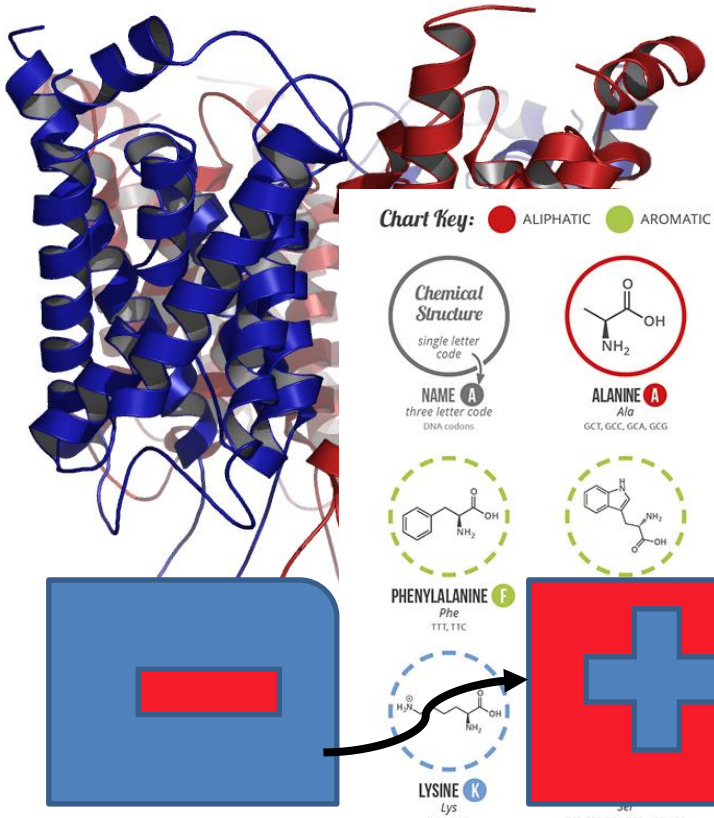


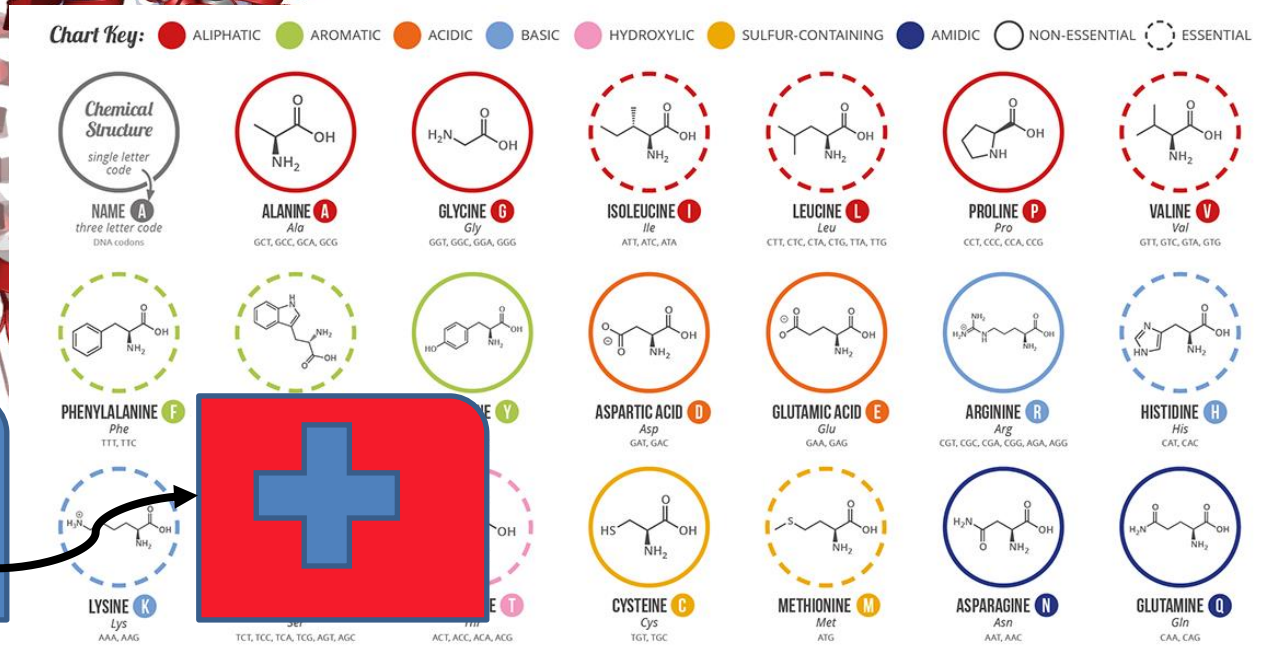
Molecular Complexity

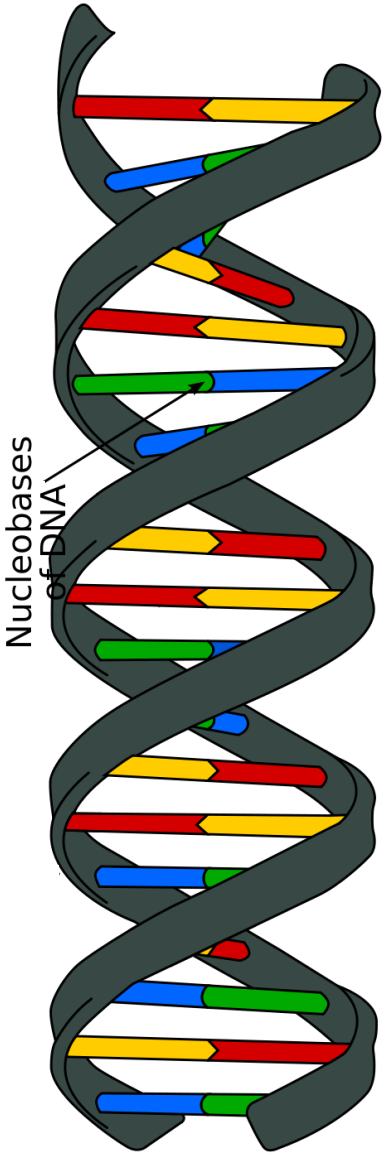


H_2O

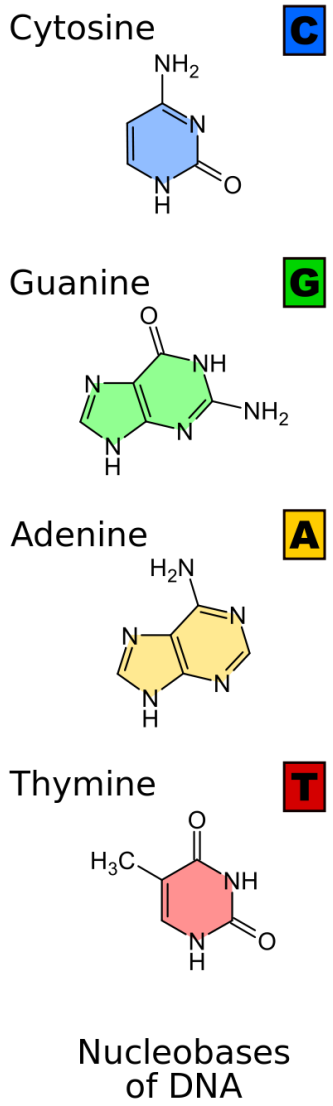




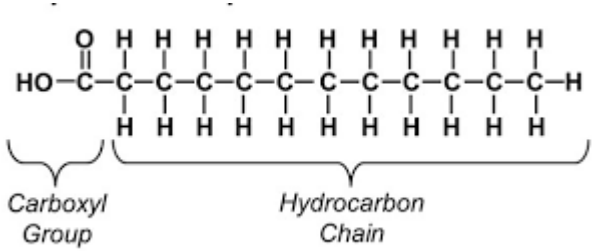




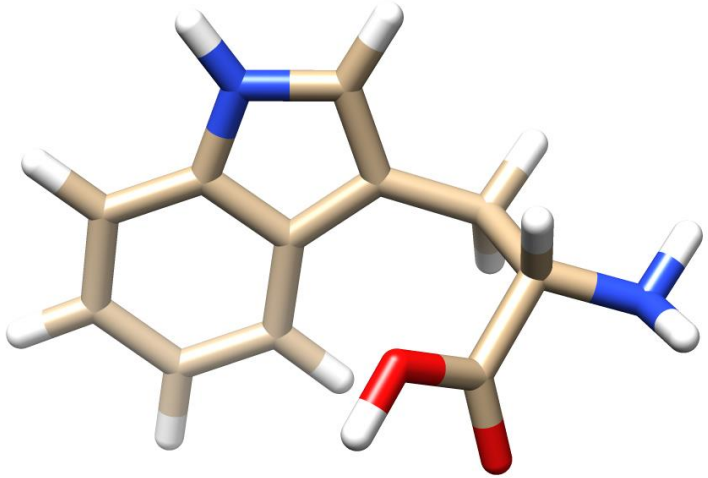
DNA
Deoxyribonucleic acid



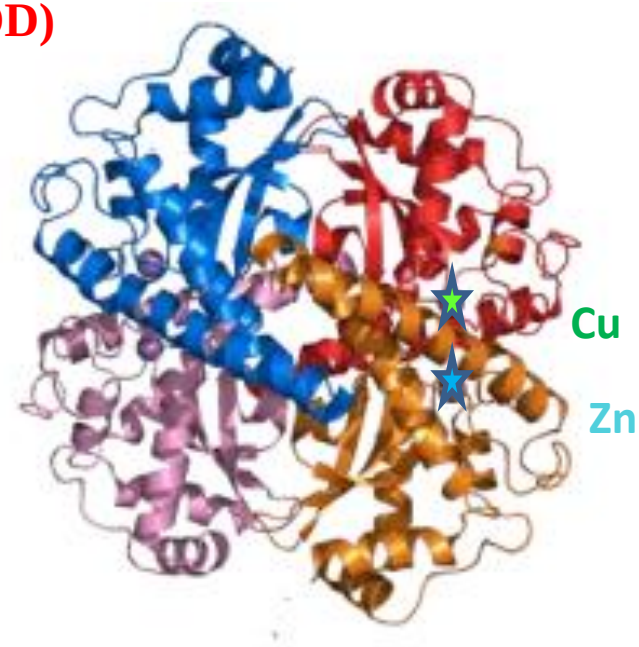
Simple, radiation-resistant molecule



Amino acid with aromatic side chains – Tryptophan



Proteins: Superoxide Dismutase (SOD)

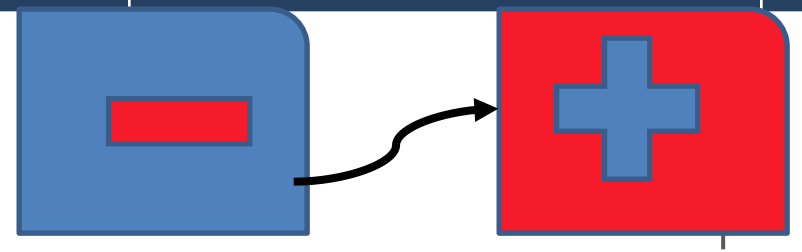


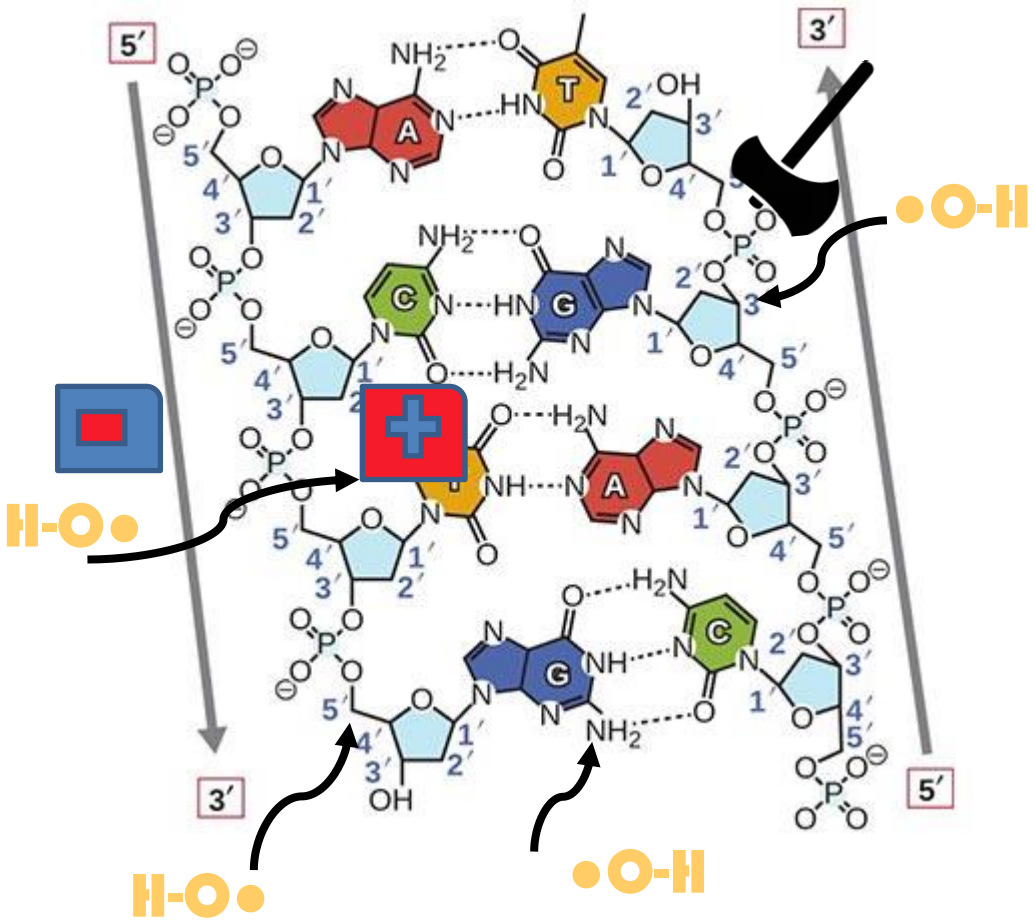
Molecular Complexity

Frailness when exposed to radiation

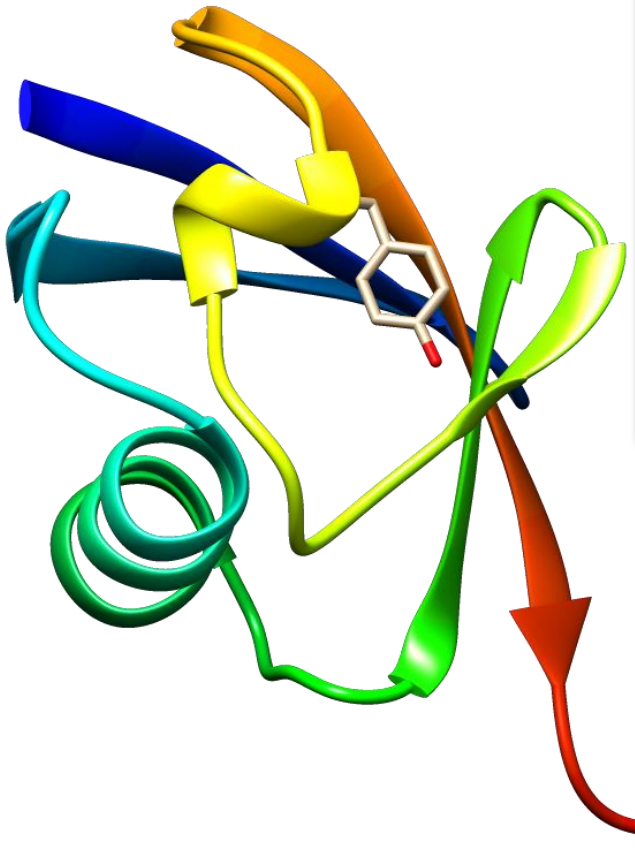
DNA Damage

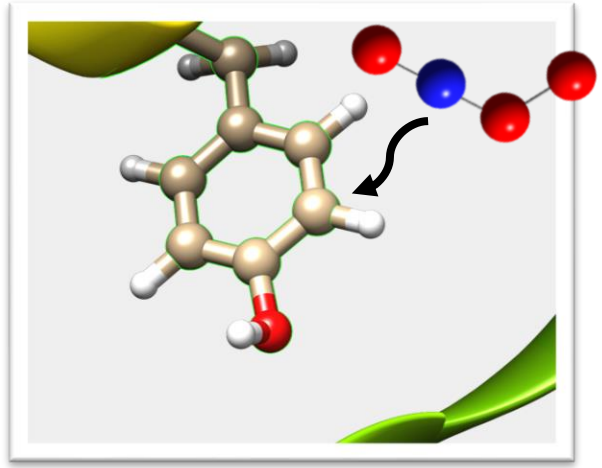
Protein Damage

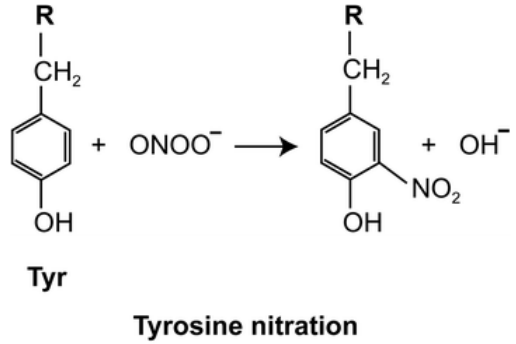




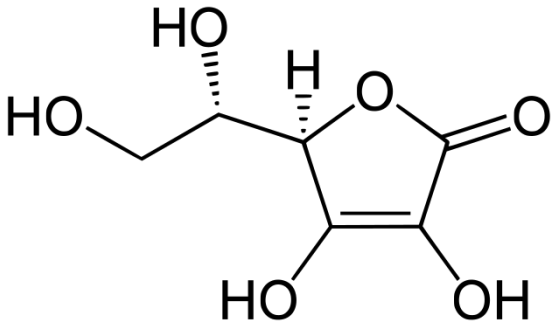
$$O_2^- \bullet + NO \longrightarrow ONOO^-$$



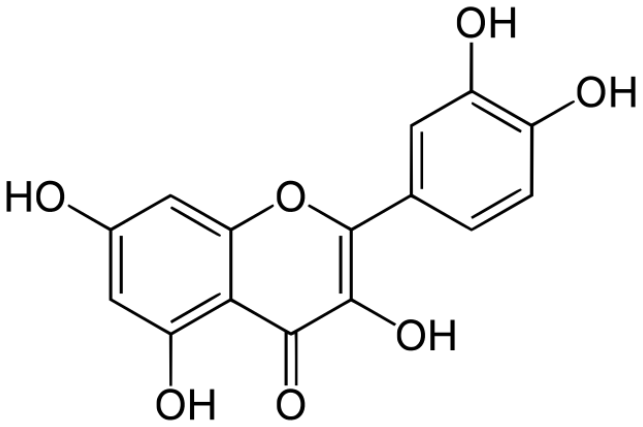




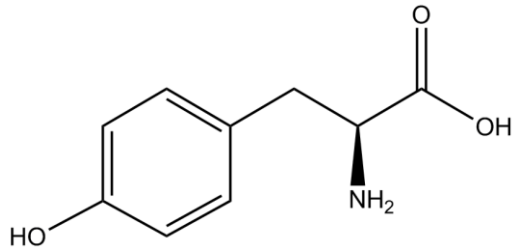
Antioxidants look similar to potential free radical targets



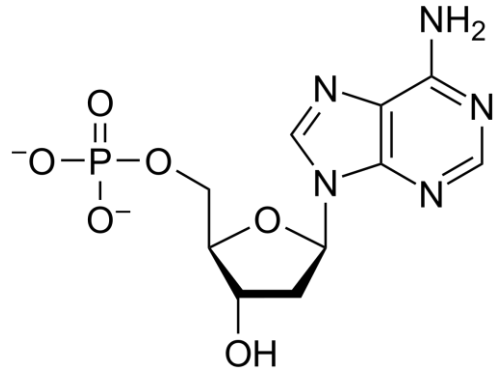
Vitamin C



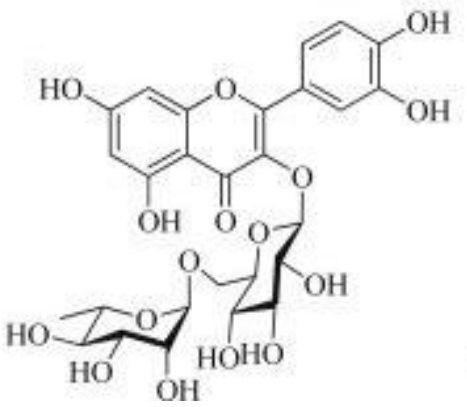
Quercitin from green tea

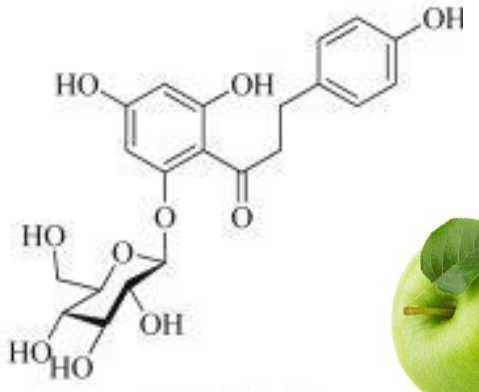


Aromatic amino acids



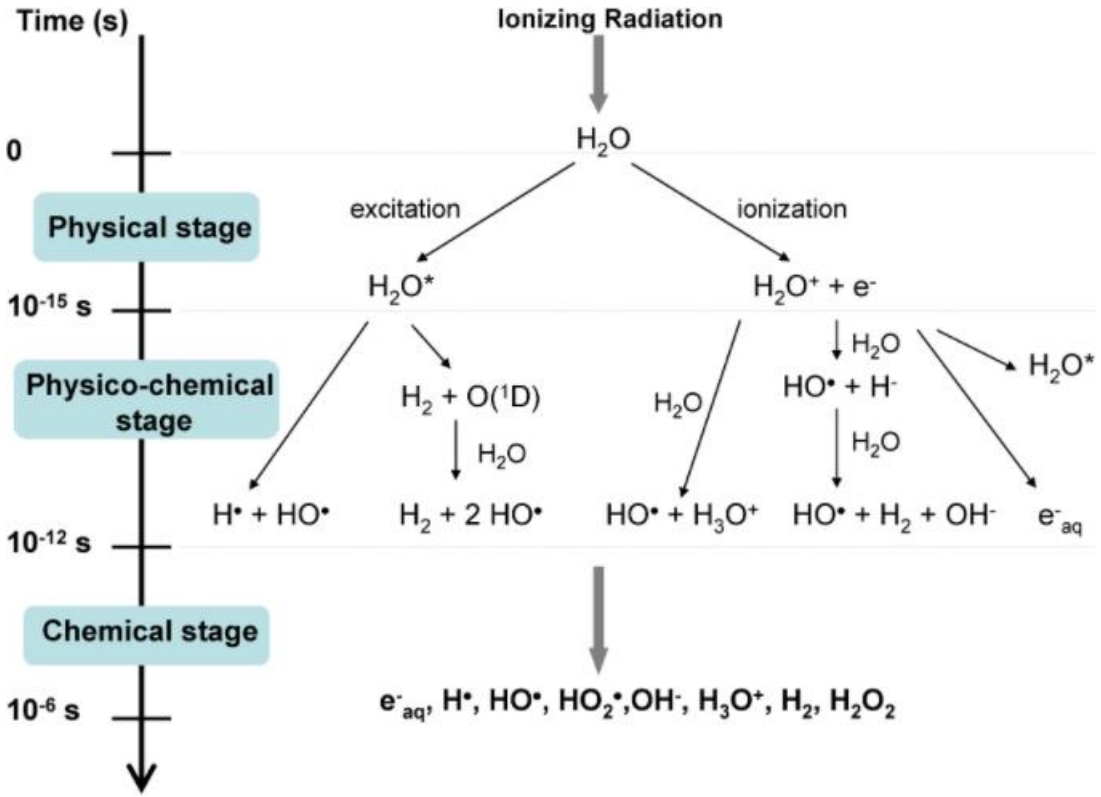
DNA nucleotides





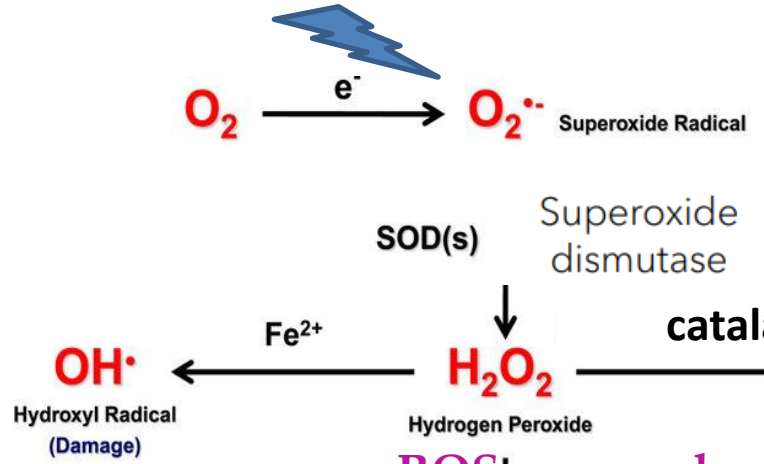
Polyphenols from apples

Effects of radiation



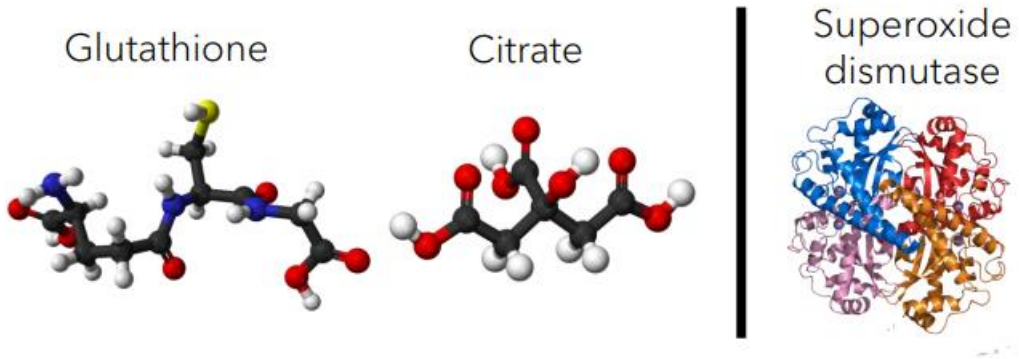
Free radicals, reactive oxygen species (ROS)

3 stages of water radiolysis – main reactions
S. Le Caër, Water, 2011



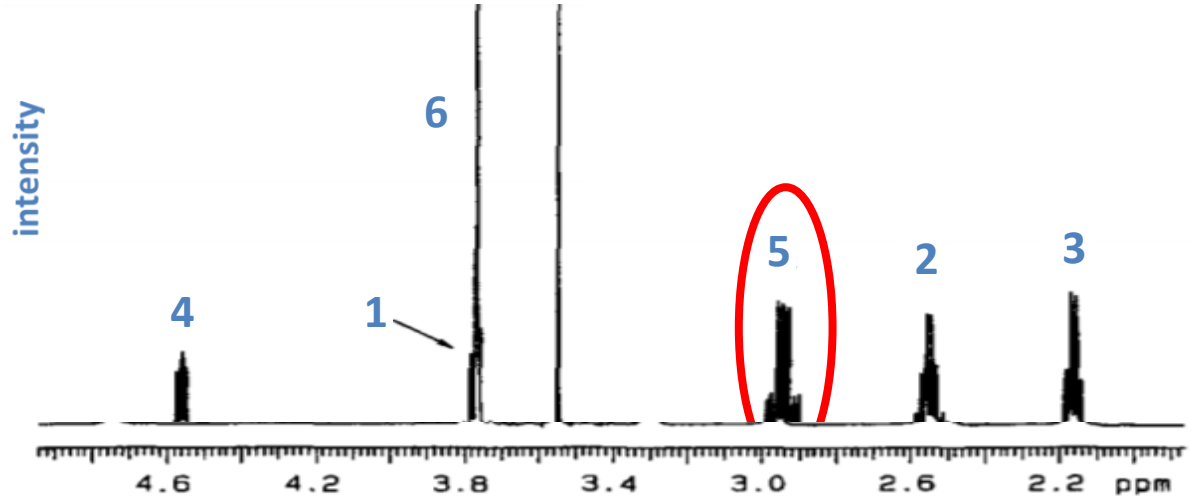
ROS: oxygen has e- ready to react – though not necessarily unpaired)

Quench them!



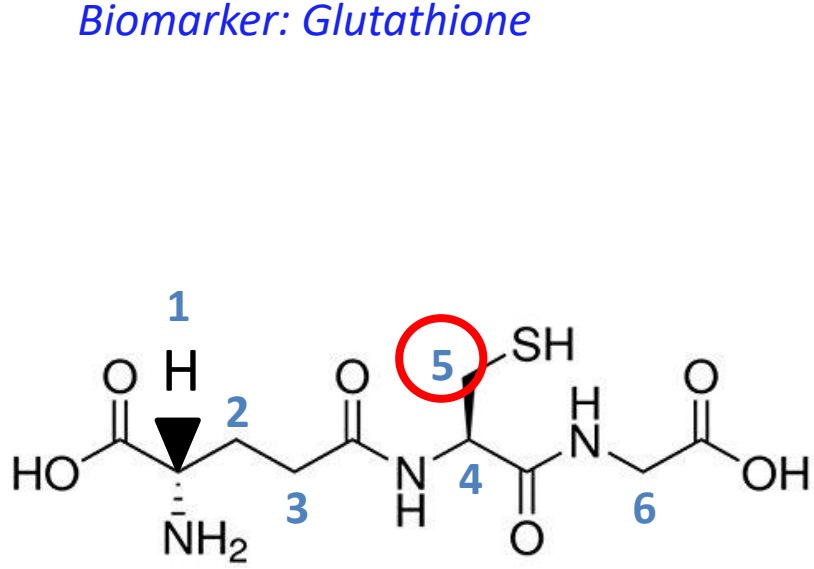
Paul Vasos

Nuclear Magnetic Resonance detection of metabolites
- atomic resolution: each atom of the molecule has its own signal



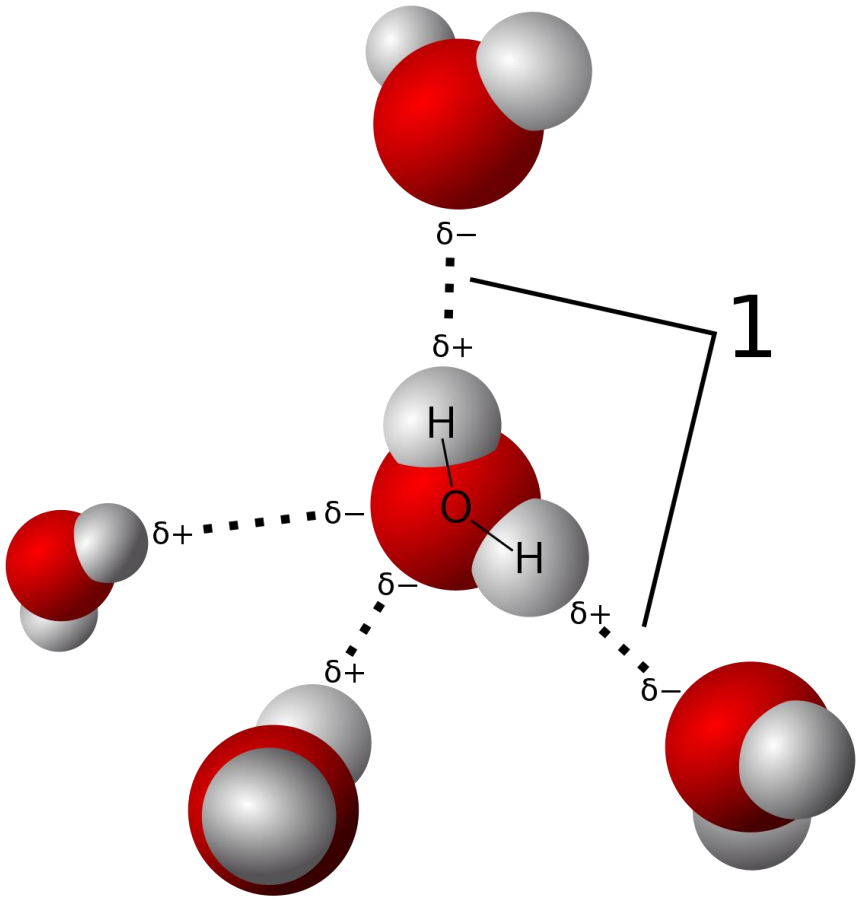
δ(^{1}H)/ppm (chemical shift) ~ υ(^{1}H)/Hz (resonance frequency in the magnetic field)

Biomarker: Glutathione



- atomic resolution: each atomic position of the molecule has its own signal
- enzymes typically modify molecular structure at one (reactive) site: the -(CH_2)-SH group here labelled '5'
- the enzyme activity can detected via the chemical shift of hydrogens at site 5
- enzyme activity can be quantified via the time-dependent variations in the *intensity* of the molecular signals

My name is Bond, H-Bond



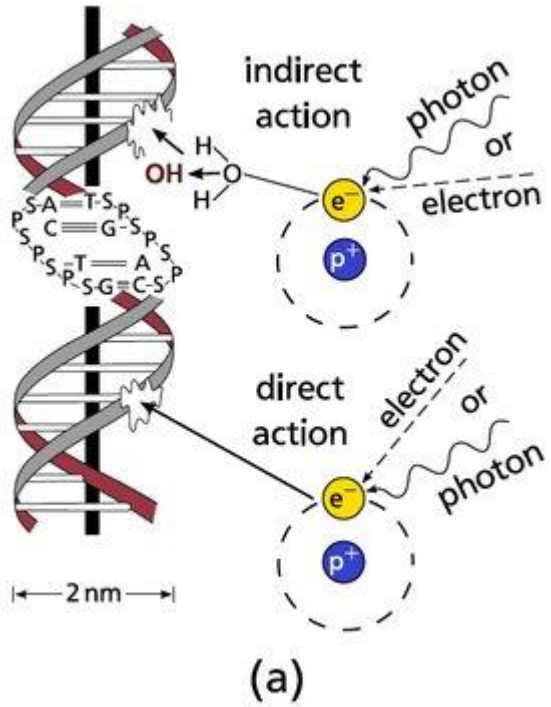
Hidrogen bonding in water

radiation impact on H-bonds

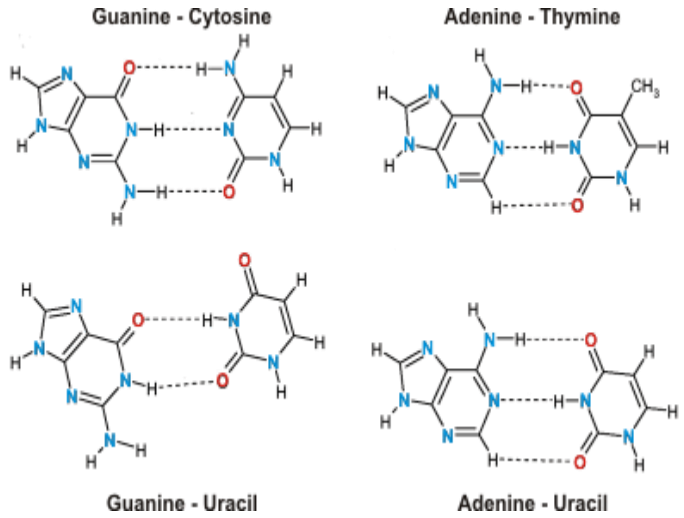
i) direct breaking (not dependent on dose-rate)

ii) breaking via free radicals (related to dose-rate)

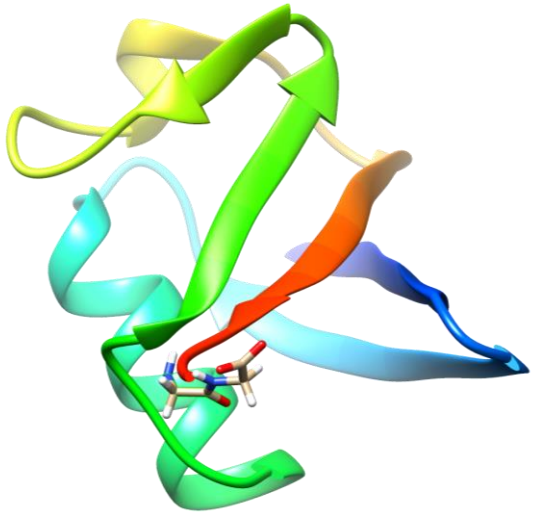
Single or double strand breaks



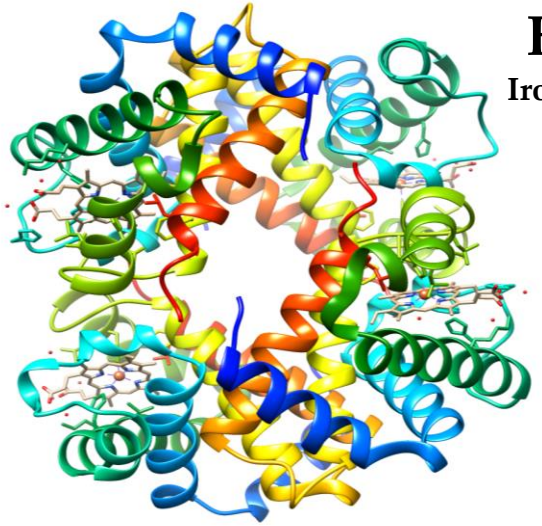
(a)



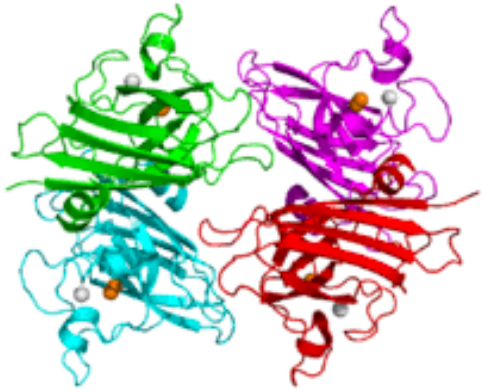
Proteins: what are they and how are they affected by radiation



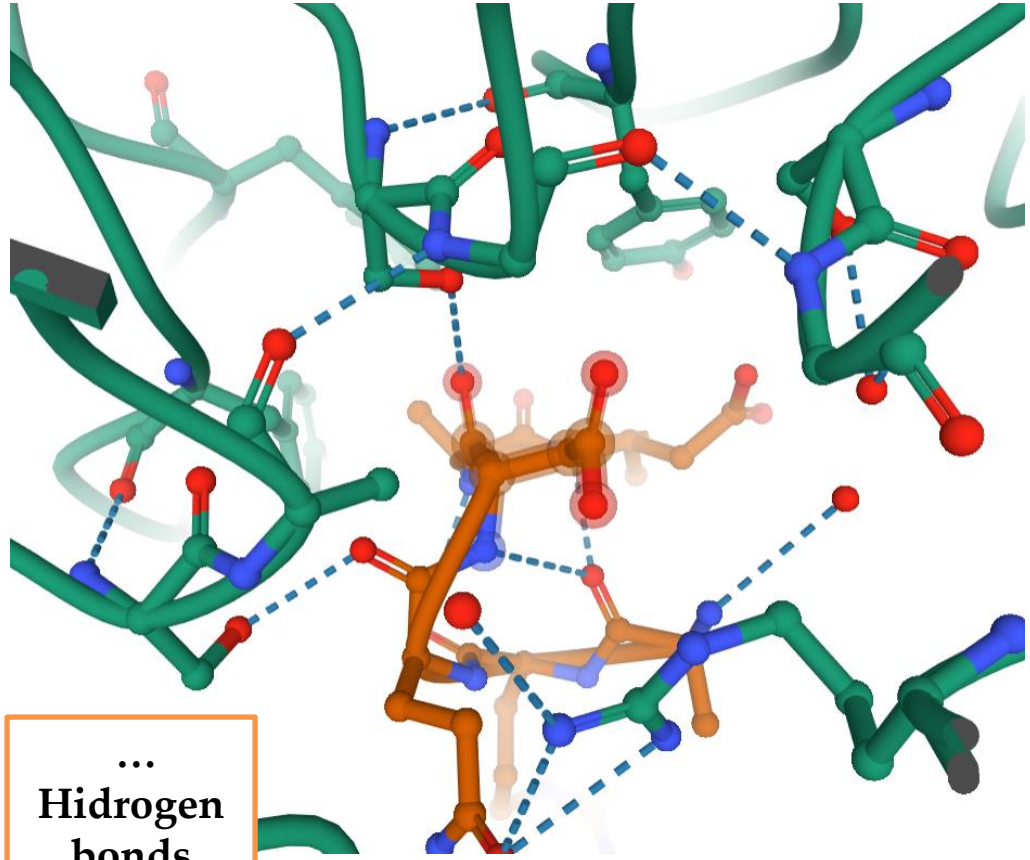
Ubiquitin
regulation of concentrations of proteins inside cell



Hemoglobin
Iron cofactor, O_2 binding



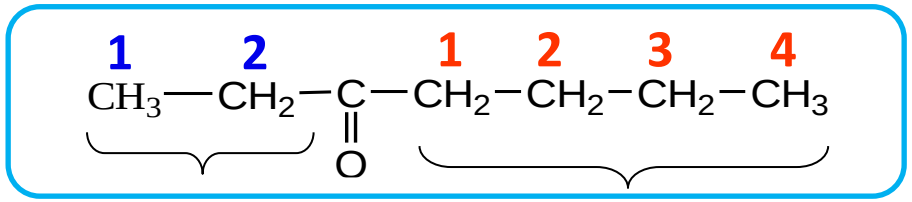
Superoxide Dismutase
oxidative stress, free radical metabolism

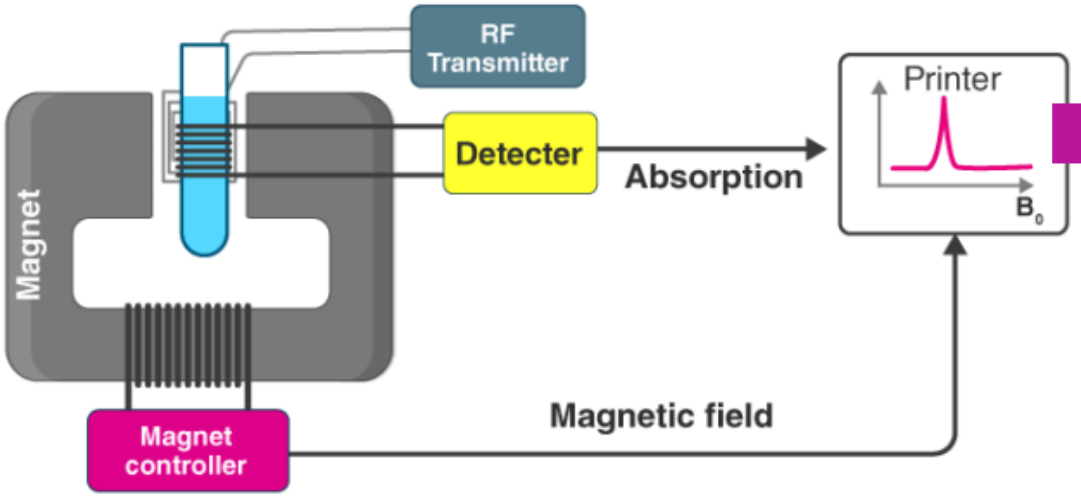


… Hidrogen bonds

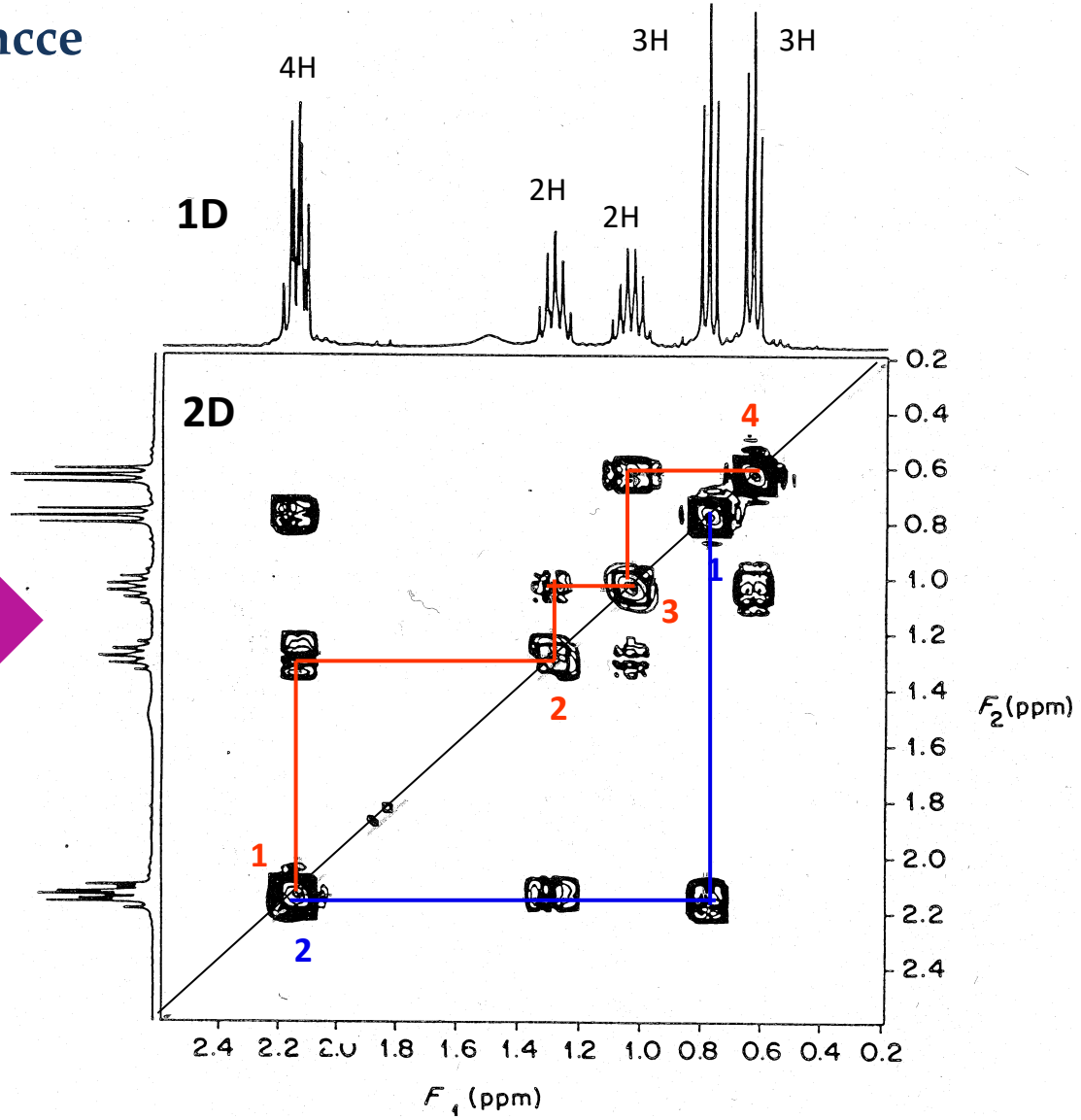
Slides: Dragana Dreghici, Florin Teleanu

2D (^{1}H-^{1}H) corelations in Nuclear Magnetic Resonancce – is there radiation damage?

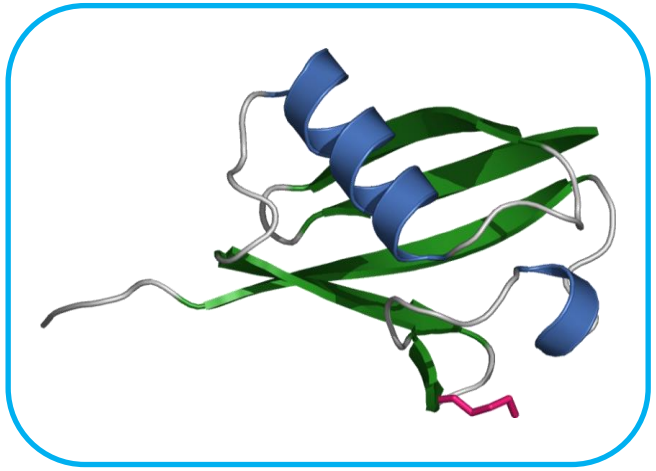


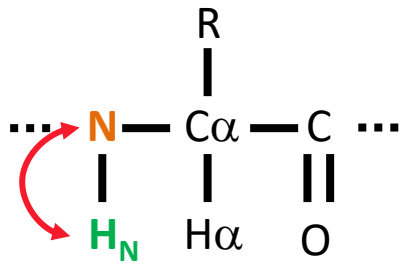


NMR Spectroscopy Instrumentation

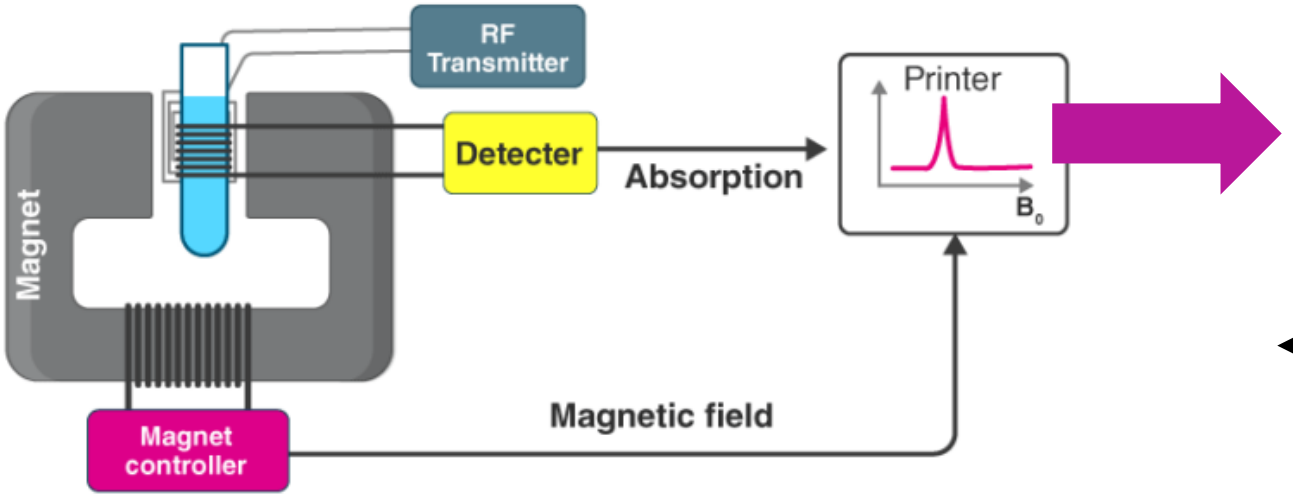


2D Nuclear Magnetic Resonancce correlations displaying the structure of proteins: Ubiquitin



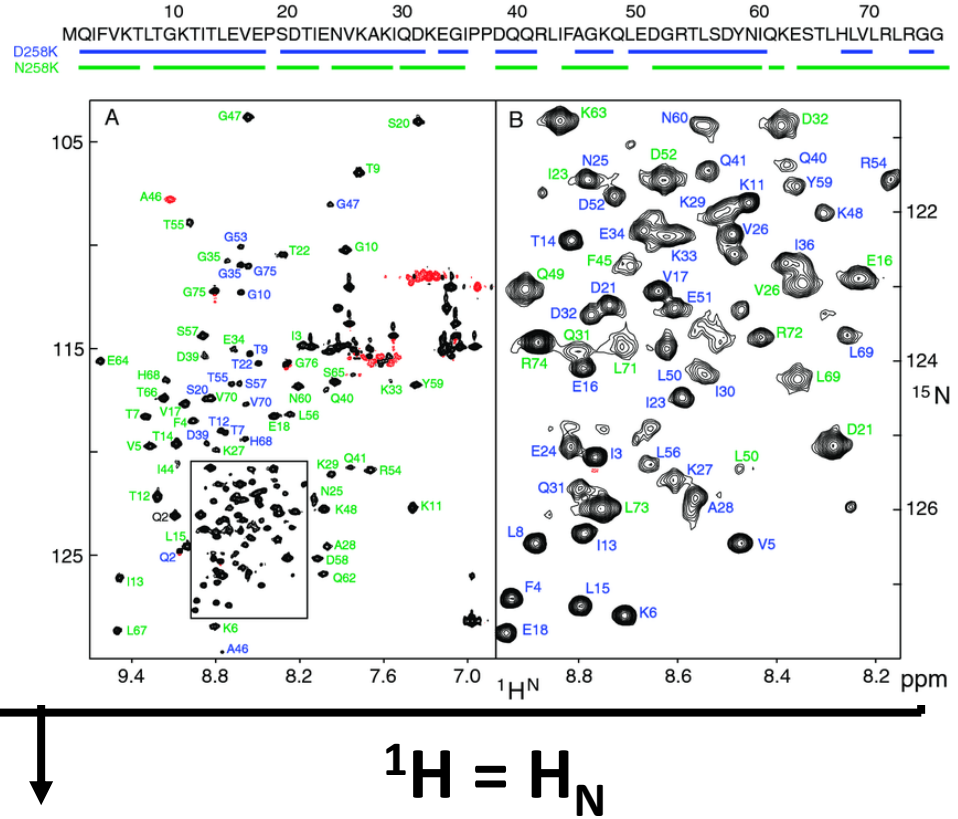


Side chain



NMR Spectroscopy Instrumentation

^{15}N

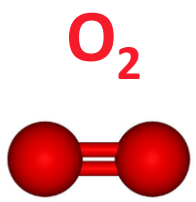


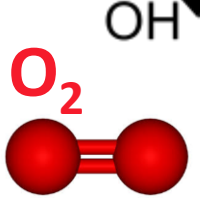
$^{1}H = H_N$

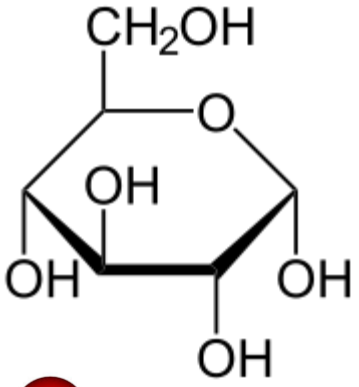
Paul Vasos

Warburg effect: metabolism kinetics linked to the influx of molecular resources distinguishes between cancer and normal cells

Normal cells: high-resource, high-throughput metabolism

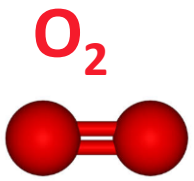


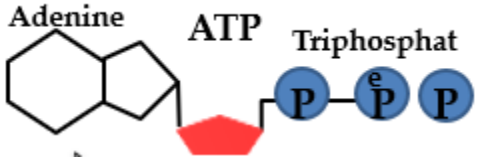


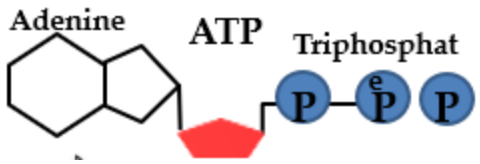


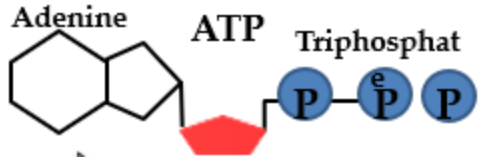
glucose

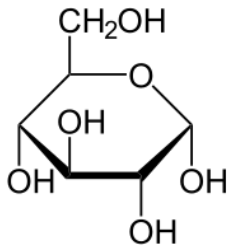
Cancer cells: low-resource, low-throughput metabolism











NADH+H NAD

Glucose PFK Pyruvate LDH **Lactate**

Cytosol

Anaerobic metabolism (Warburg)
(little O_2) – cancer cells

Membrane Transporter

Mitochondria

Pyruvate ⇄ Lactate (LDH) MCT

oxidation

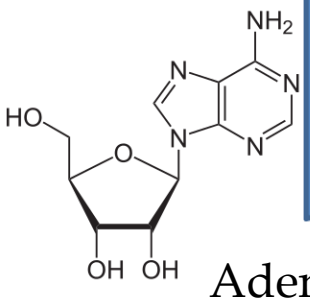
Phosphate dehydrogenase & Thiamine diphosphate

Acetyl CoA

Aerobic metabolism
(lots of O_2) – normal cells, glucose burns efficiently

Krebs Cycle → **ATP**

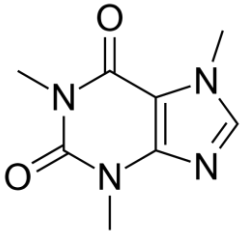
*PFK, LDH = enzymes



CELL RECEPTOR

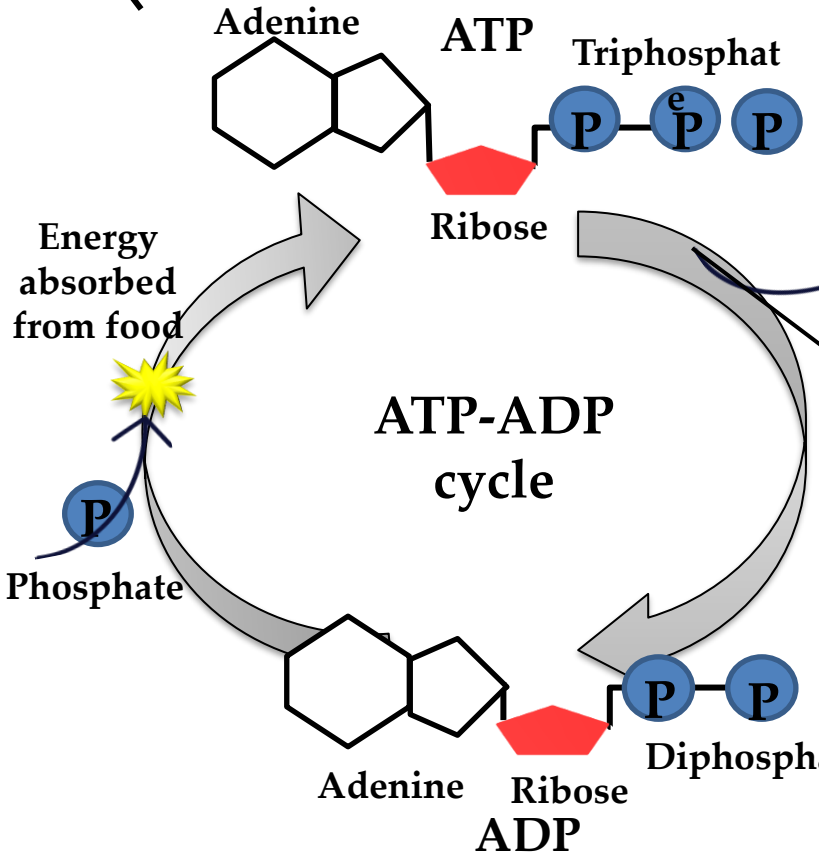
just got energy.
not tired, thanks!

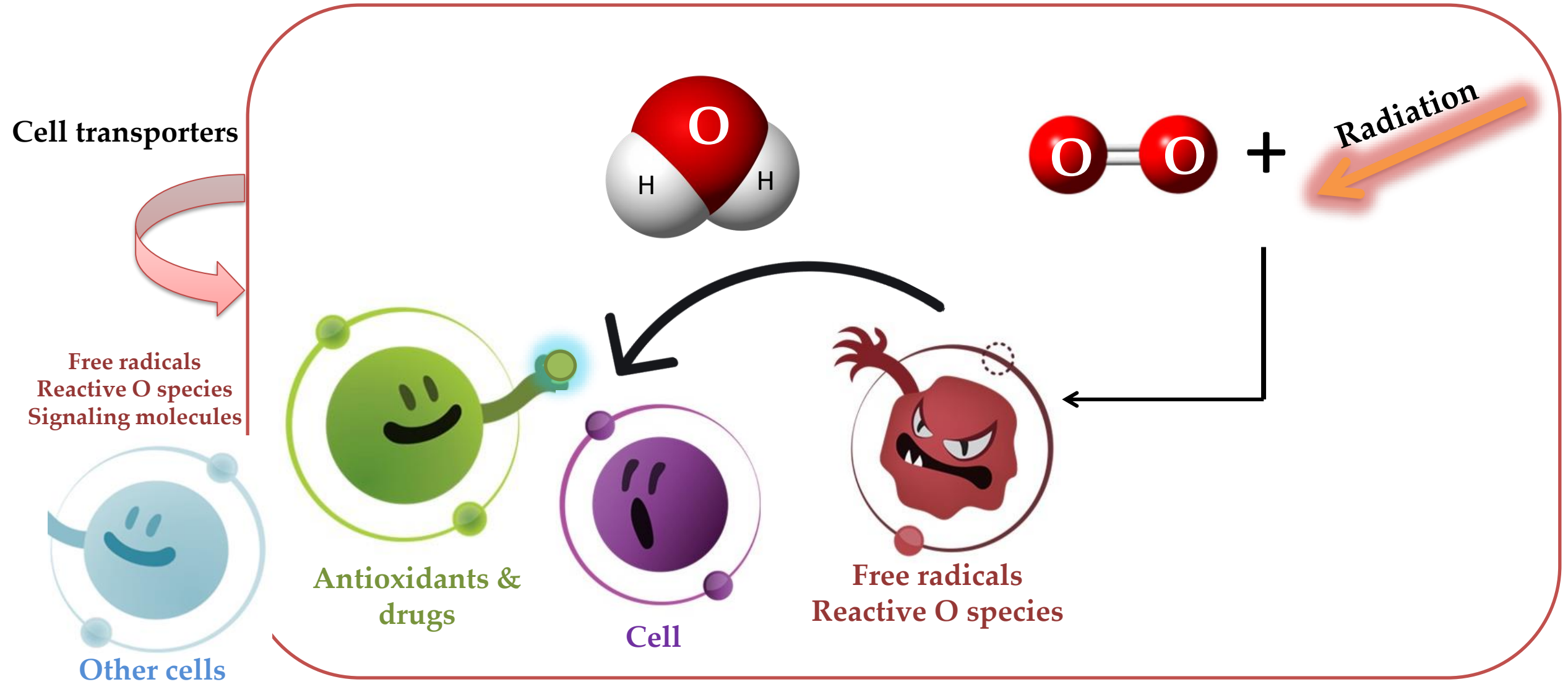
Adenosine



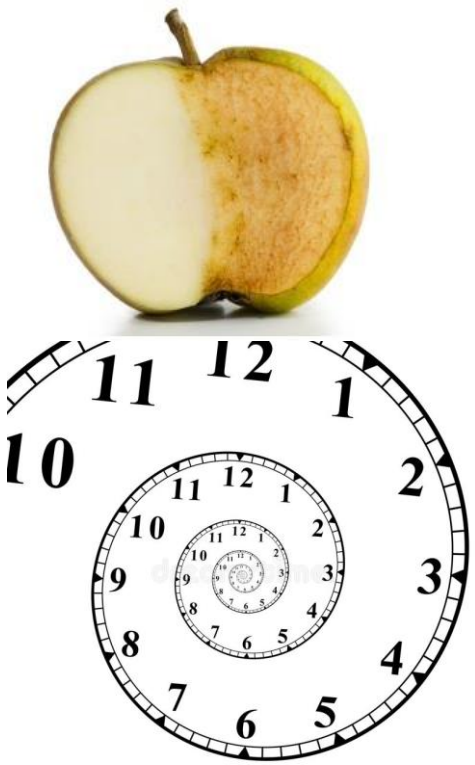
Caffeine

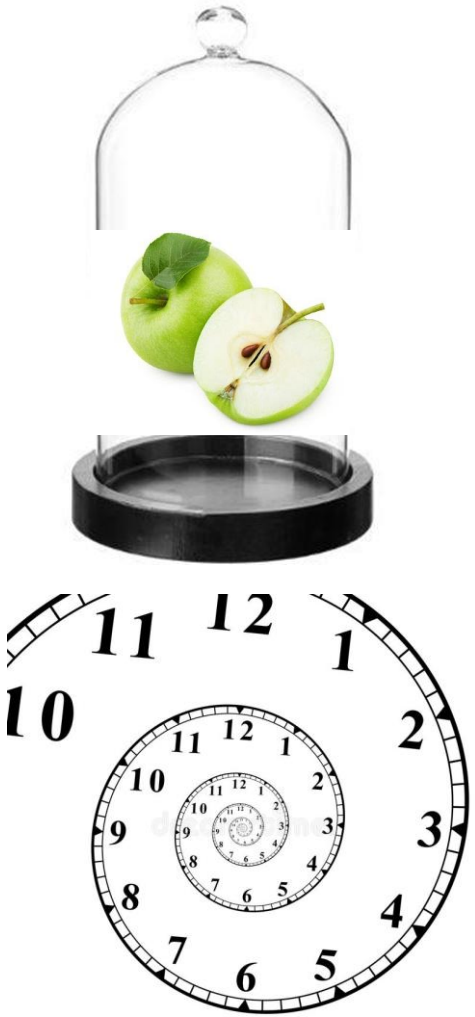
CELL RECEPTOR



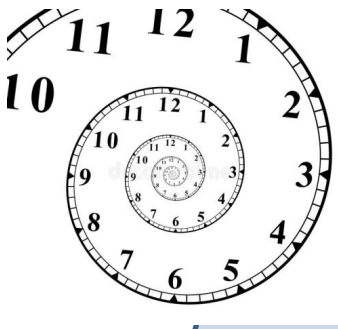


.. a (quite) simplified view of radiation effects

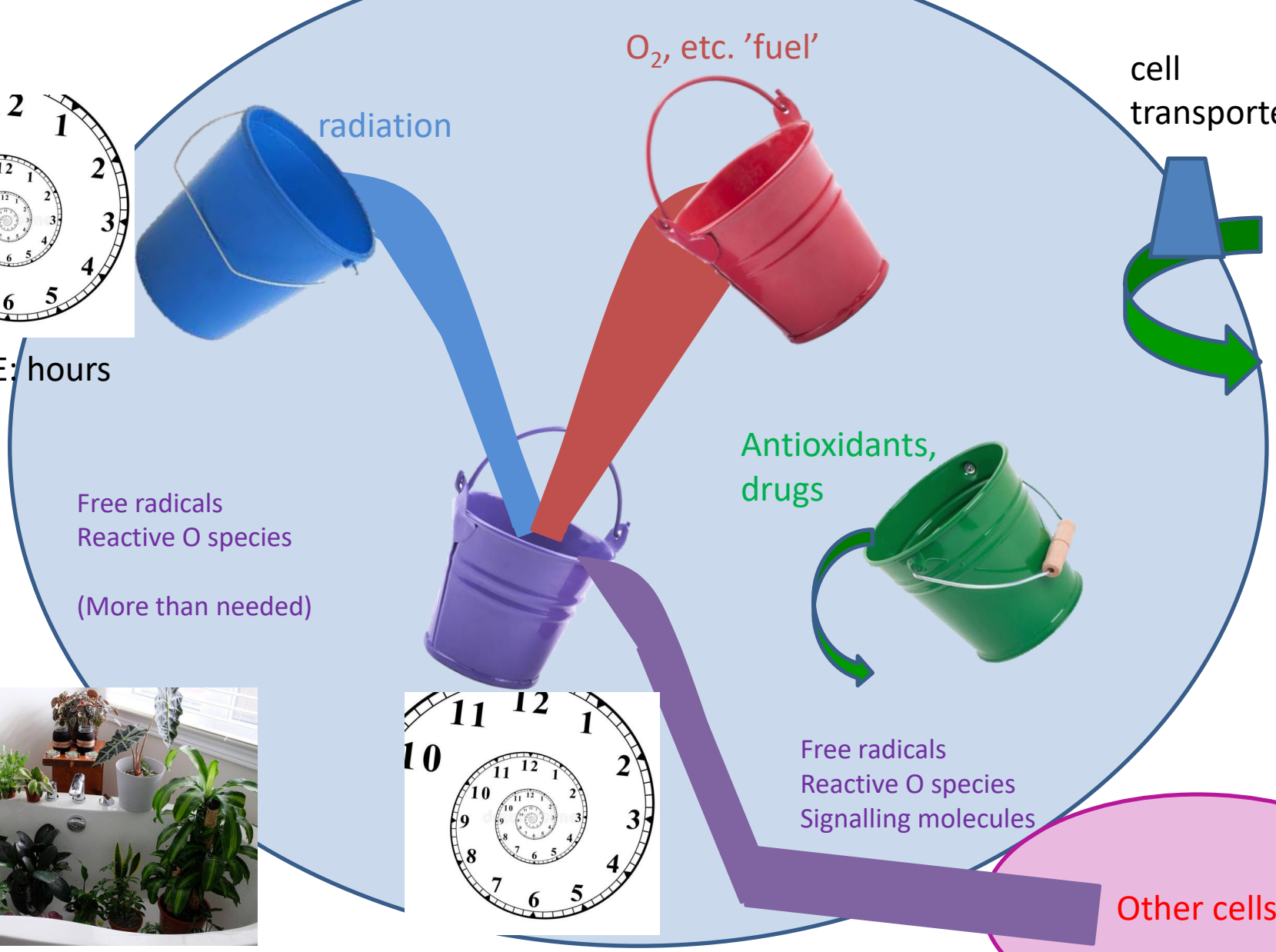




radiation effects:
a question of time
(and fuel)



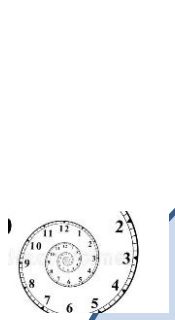
TIME: hours



i) CLASSICAL RADIATION DELIVERY

Sustained FREE RADICAL formation during a LONG time
Radiation→Toxicity route open

TOXICITY



TIME: ns-ms

radiation

O_2, etc. 'fuel'

cell transporters

ii) HIGH DOSE-RATE RADIATION DELIVERY

Limited FREE RADICAL formation during a SHORT time

Radiation→Toxicity route Clogged

Antioxidants, drugs

Antioxidants can hardly do anything in time
(e.g. acetyl cysteine, Clin Onc 2019)

RADIATION CAN OVERFLOW,
AS LONG AS REACTIVE SPECIES DO NOT

Free radicals
Reactive O species
Signalling molecules

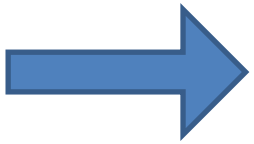
Other cells

Vozenin, Clin Onc 2019
FLASH-RT toxicity low in zebrafish
Independently of Antioxidant (n-acetyl Cys)

Home message

- Radiation effects depend on:
    - Radiation dose and dose-rate
    - Radiation fuel: oxygen and its availability
    - Biomolecules inside cells (DNA, proteins, small metabolites)
    - Cell membrane role: permeability, integrity
- Molecular species carrying reactive electrons (**free radicals** and **reactive oxygen species**) are formed by radiation and propagate its effects
- **Antioxidants** can neutralize free radicals and reactive oxygen species
- **The rates (the time dependence) of the reactions between molecules above compare to the rate of radiation delivery will decide on radiation toxicity.**

- **FLASH effectivenes likely to depend on cancer cell type (metabolic profile) and subject**



.. Experiments

To follow:

Experiments by which dose-rate effects were quantified:

- **Electron spin magnetic resonance (ESR) to detect free radicals (using 'spin traps')**
- Radiomics (metabolomics in a radiobiology context)
- Metabolic conversion rates

- Nuclear Magnetic Resonance biomarkers afford **in cell, *in vivo* and in clinics** to follow the rates (the time dependence) of the reactions involving the reactive molecules that trigger radiation toxicity and efficiency

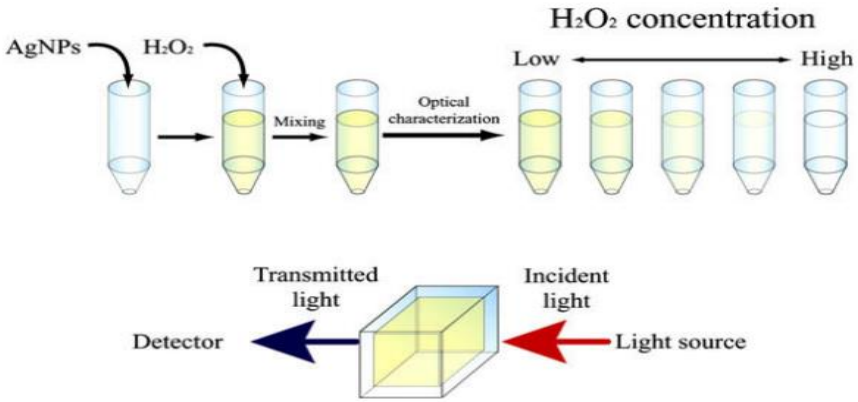
Biomarker detection

- how can we follow biomoleculecules

J. Borofsky – ‘Molecule Man’ (Los Angeles and Berlin)

Indirect detection

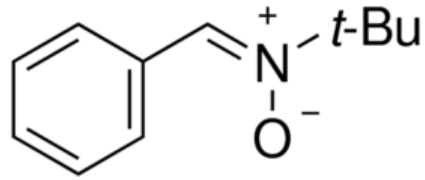
1) Radiation –generated free radicals in water are too short lived to be detected by spectroscopy
Solution: spin traps

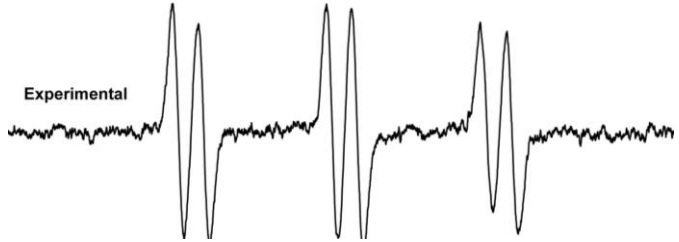


- H_2O_2 causes silver oxidation
- measuring the light absorption by Ag nanoparticles → ROS concentration

Tatsuro Endo, Yasuko Yanagida, Takeshi Hatsuzawa,
Quantitative determination of hydrogen peroxide using polymer coated Ag nanoparticles,
Measurement, Volume 41, Issue 9, 2008, Pages 1045-1053,

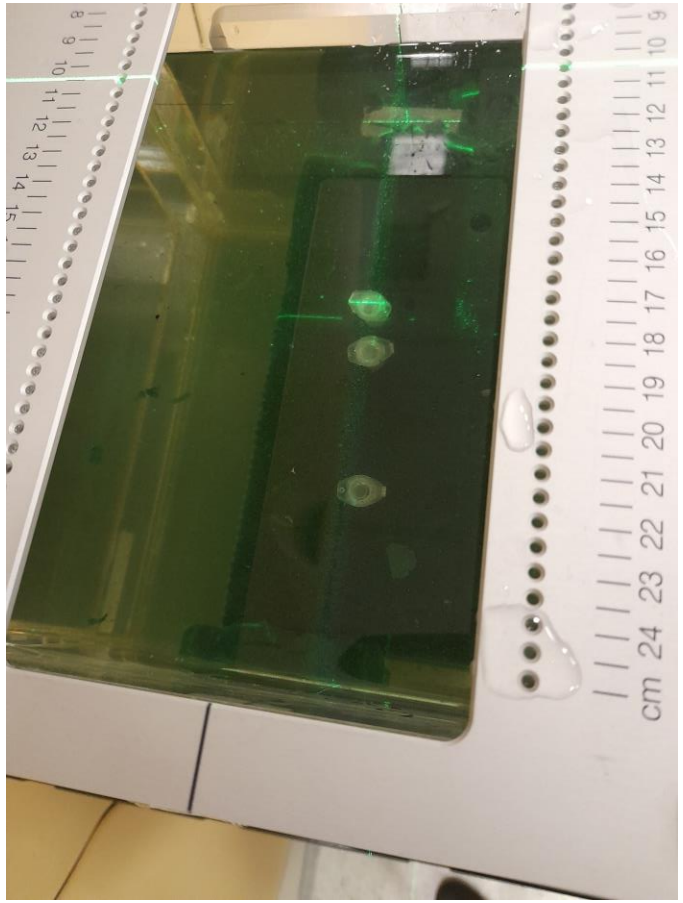
Quantitative determination of free radicals/ ROS using spin traps

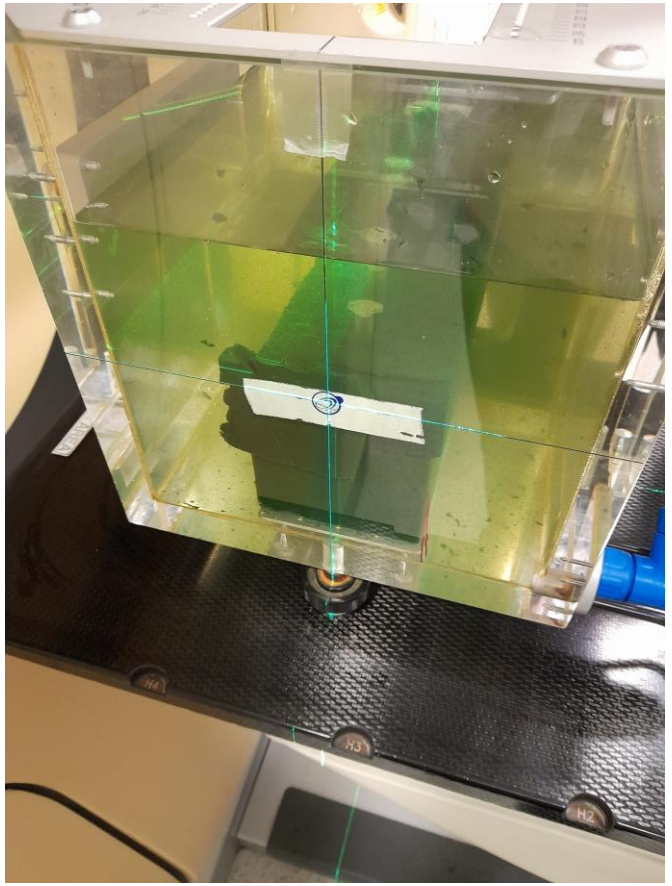


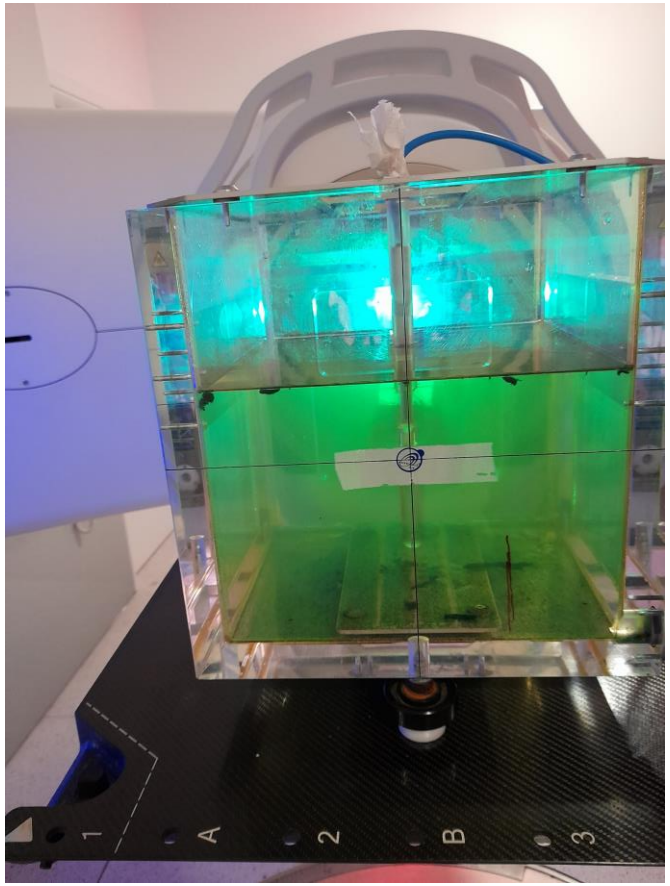


- *N-Tert-butyl-1-phenylmethanimine oxide (PBN)*
- Spin traps catch ROS molecules
- ROS identified with EPR spectroscopy

Collaboration Univ. of Bucharest , IFIN-HH



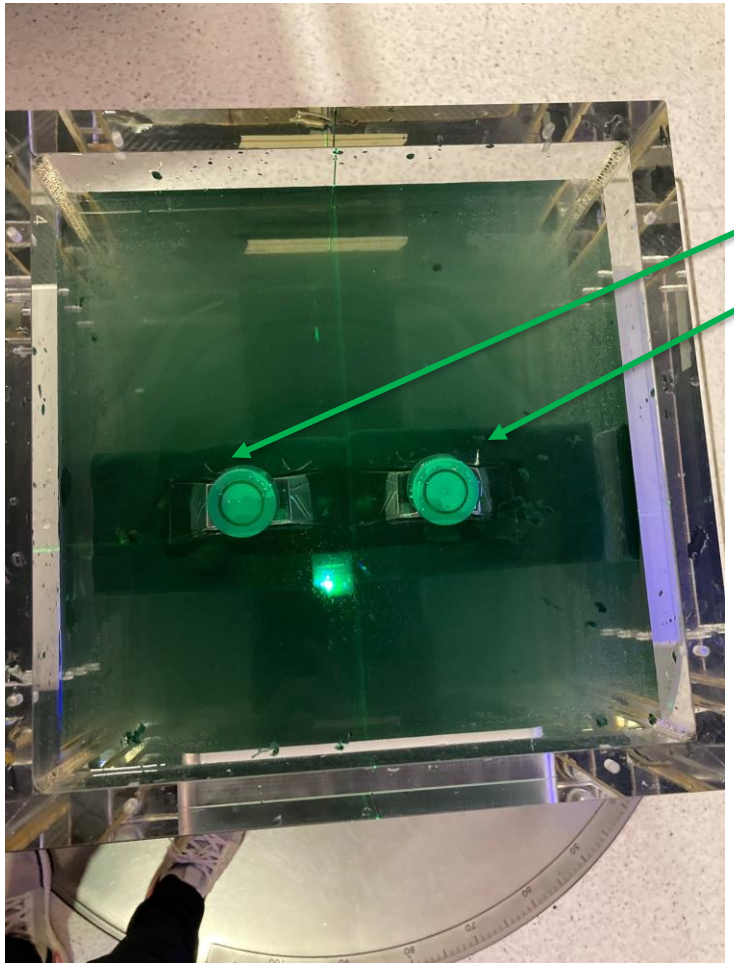




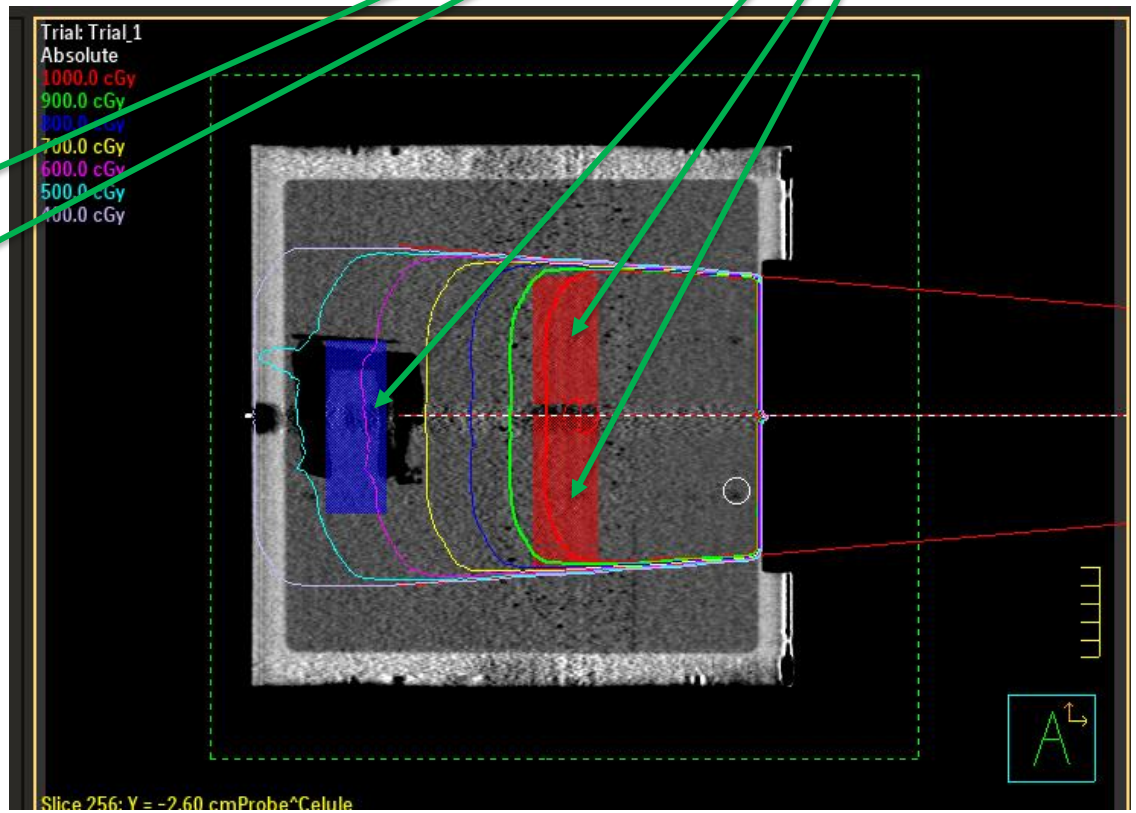
Amethyst Radiotherapy cell radiation in ‘phantom’ set-up

Amethyyst Radiotherapy

X-ray, 6 MV



flask with cells

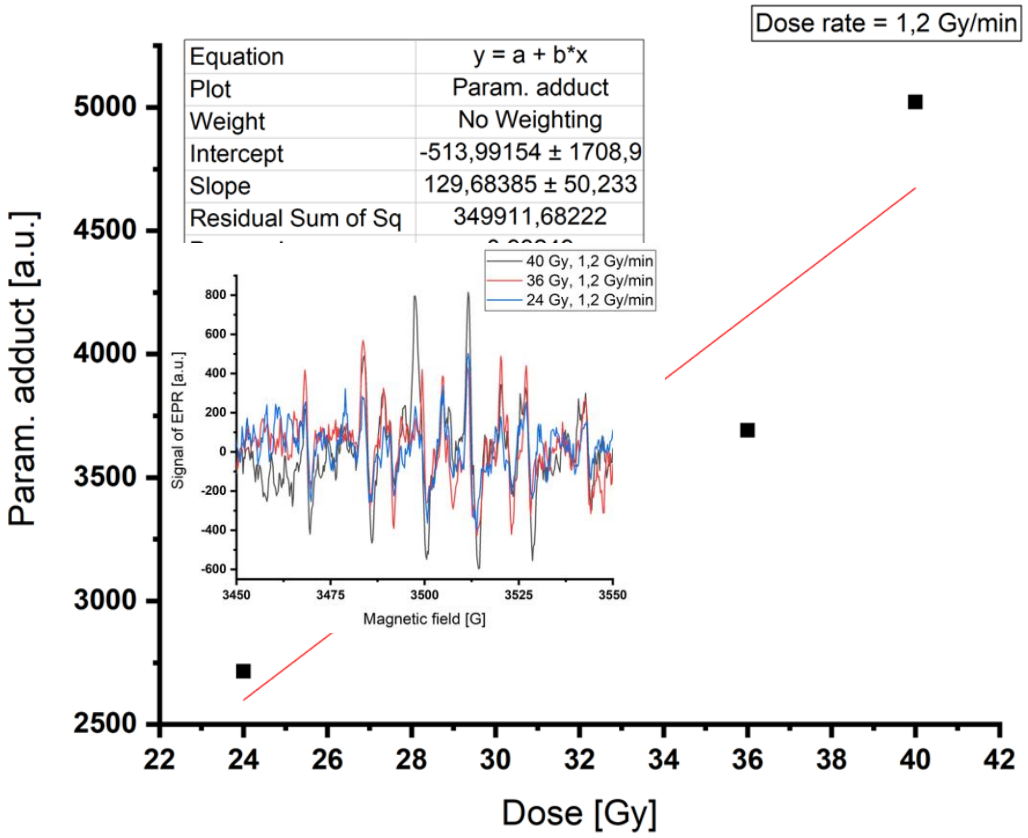


Isodose profiles

GENERATION OF FREE RADICAL WITH X RAYS

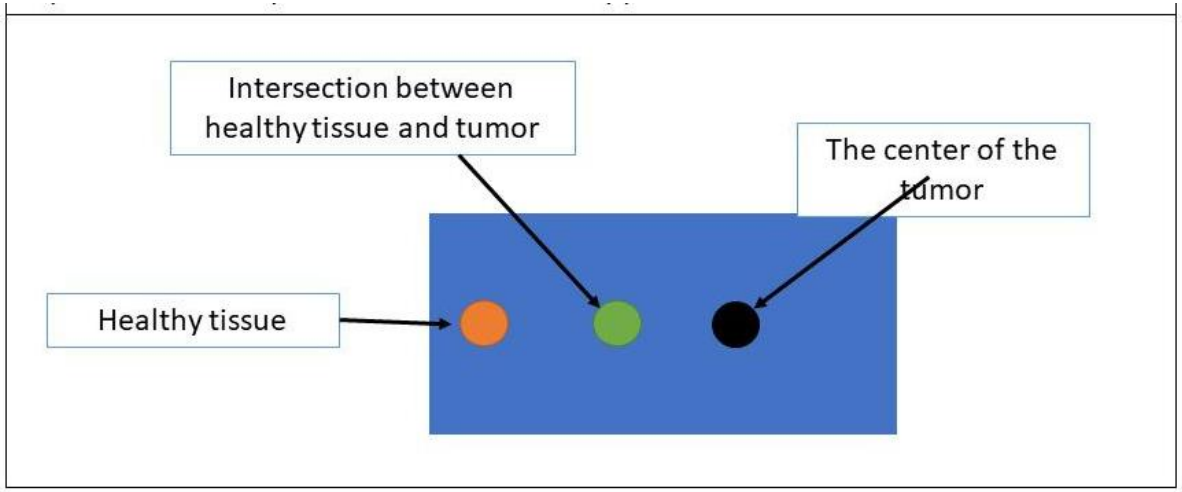
AMETHYST RADIOTHERAPY CENTER OTOPENI IRRADIATION

THE SIGNAL CAN BE CONFUSED WITH THE NOISE.



DEPENDENCE OF RELATIVE BMPO-OH ADDUCT ON DOSE

EXPERIMENTAL SETUP

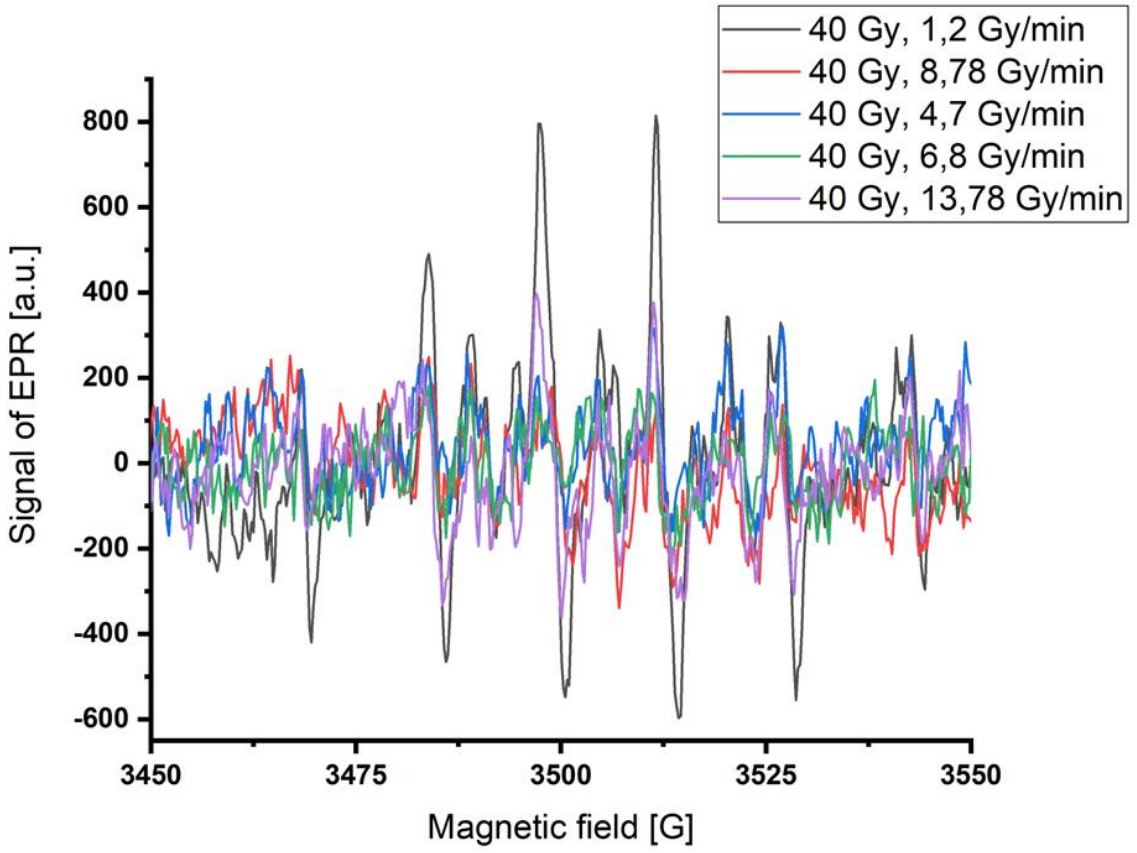


BMPO-OH ADDUCT FOR DIFFERENT POSITIONS OF THE SAMPLE(U = 6MV, Em=2 MeV, filter)

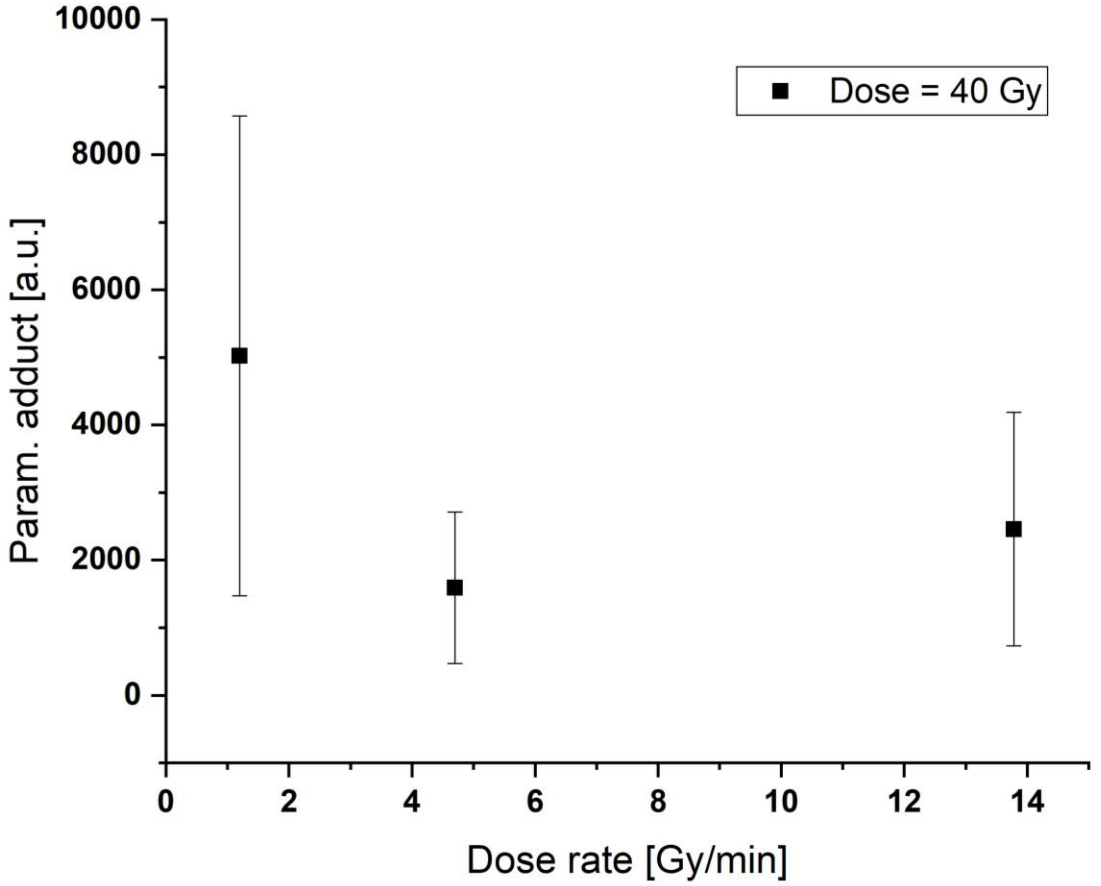
GENERATION OF FREE RADICALS VIA X-RAYS RADIATION

Amethyst Radiotherapy Center Otopeni irradiation

Conclusion: The amount of BMPO-OH adduct decreases with increasing dose-rate. The 40 Gy samples with dose-rates of 6.8 Gy/min and 8.78 Gy/min are much too noisy to be quantified.



BMPO-OH adduct dependence of dose-rate



Dependence of relative parameter of BMPO-OH adduct of dose-rate

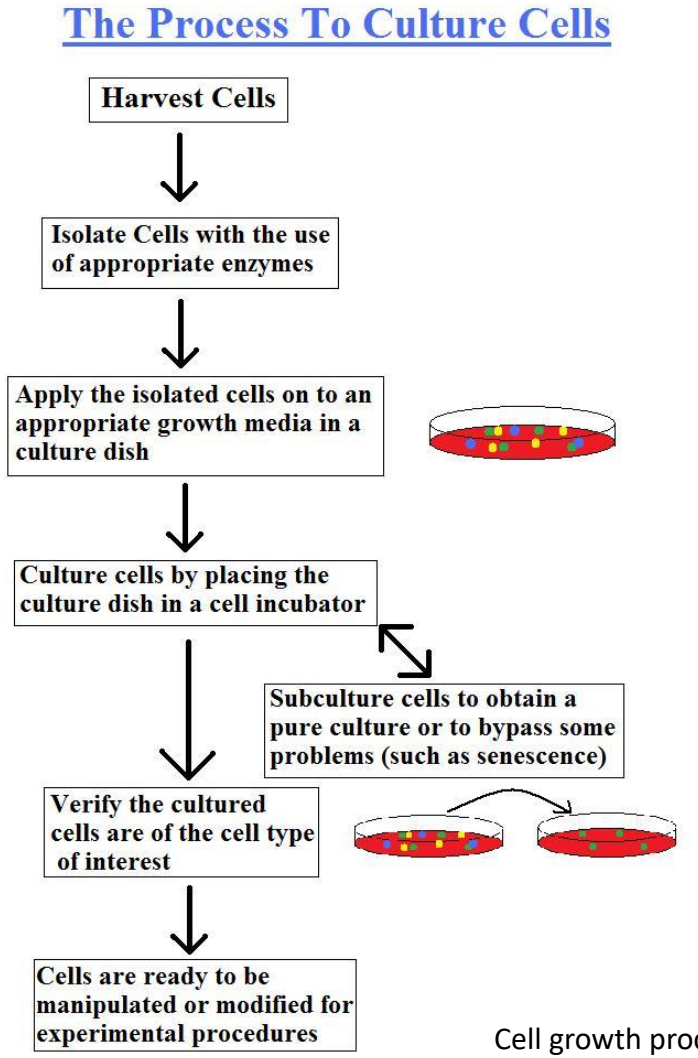
Cell Culture procedures and conditions

- Depends on cell types;
- The artificial environment for cell growth needs to have
  - essential nutrients: amino acids, carbohydrates, vitamins and minerals
  - growth factors
  - hormones
- cellular homeostasis.
- Apoxic and oxygen-rich conditions

(most convenient set-up: outside interaction chamber)

> Normal cells, Cancer cells, Cancer stem cells

Collaboration:
Gina Manda, Head of Radiobiology, Victor Babes Institute



Cell growth procedure

… observe (i.e., discuss a couple of papers concering the following options of assessing dose-rate radiation effects):

option 1) – *in situ* assessment (or in cell, without breaking cells)

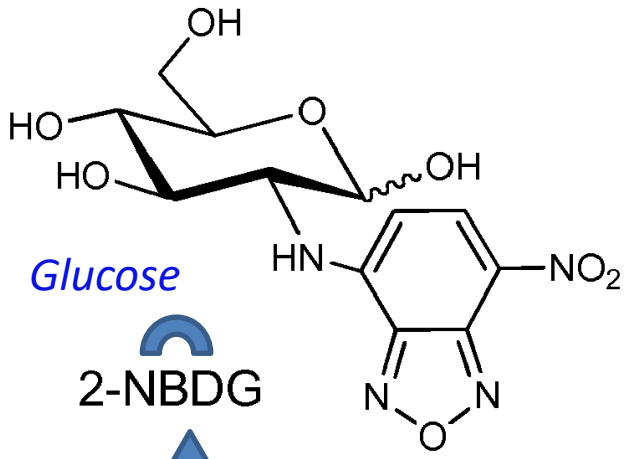
- allows optimisation of radiation protocol ‘on the go’, assessing (neo)adjuvant chemotherapy, etc.

option 2) – *ex situ* (or involving cell lysis)

- time elapsed after radiation is of the essence
-

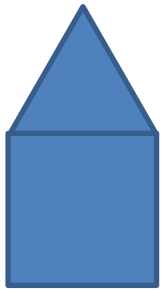
Biomarkers for Various Observation Methods

Biomarker - Visible



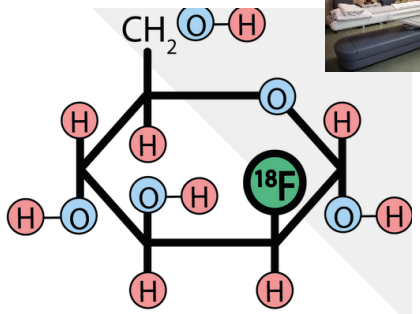
Glucose

2-NBDG



Biomarker – PET

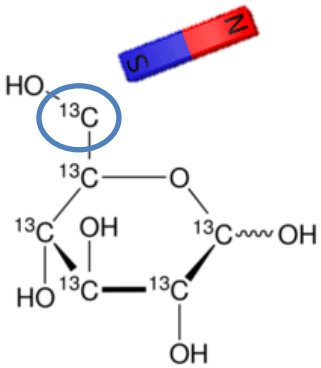
(Positron Emission Tomography)

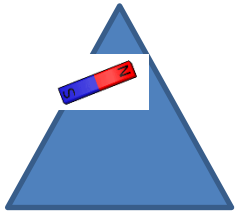


^{18}F - Glucose

Biomarker – MR

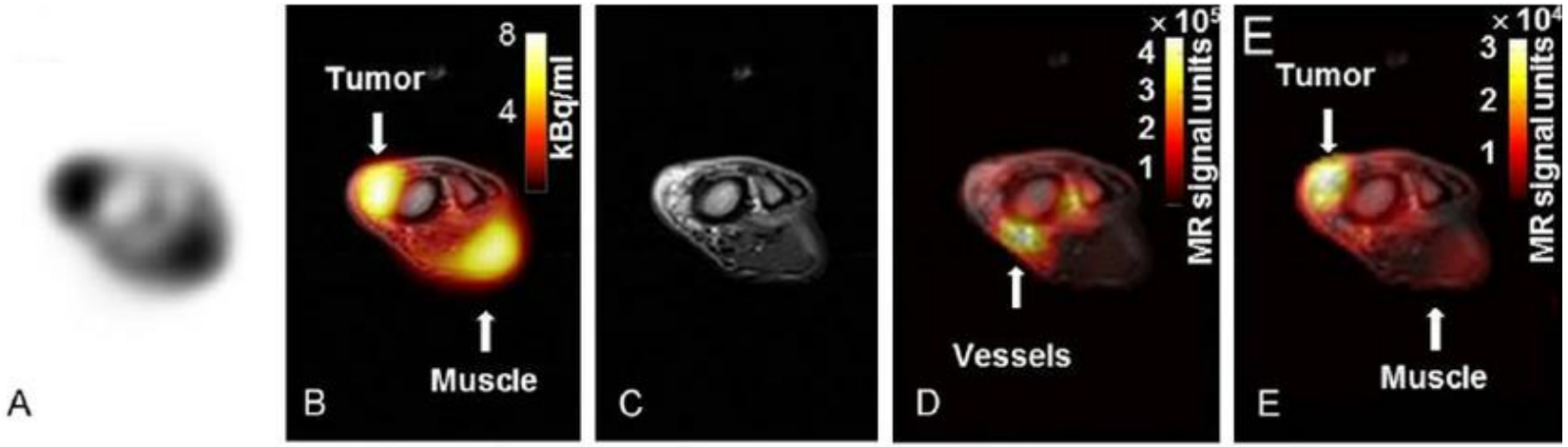
(Magnetic Resonance)





Current :PET-CT RADIOACTIVE ISOTOPE gold standard n diagnostic ('functional imaging')

Emerging: STABLE ISOTOPES Magnetic Resonance for cancer diagnostic



Henrik Gutte, Adam Espe Hansen, Helle Hjorth Johannesen, Andreas Ettrup Clemmensen , Jan Henrik Ardenkjær-Larsen, Carsten Haagen Nielsen, Andreas Kjær, The use of dynamic nuclear polarization 13C-pyruvate MRS in cancer, *Am J Nucl Med Mol Imaging* 5, 548-560, (2015)

Metabolic process RADIOACTIVE ISOTOPE

Positron Emission Tomography (PET) based on ^{18}F-glucose

ionising radiation

Water

^{1}H

MRI

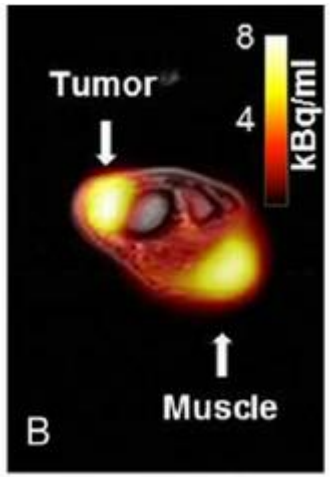
Metabolic imaging STABLE ISOTOPE

MRI-based:follows metabolic conversions of endogenous molecules

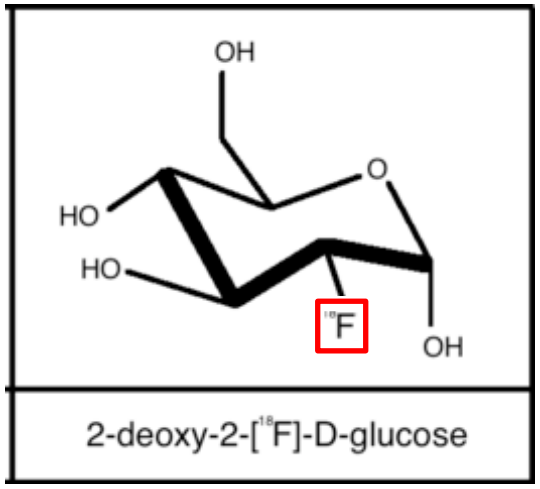
no ionising radiation

2) WITHOUT LYSING CELLS
Molecular Imaging, biomarkers with isotopes

Curent standard in diagnostic

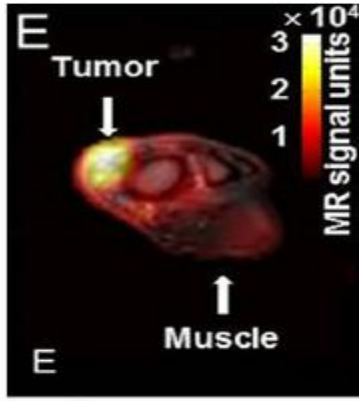


Positron Emission Tomography (PET)
based on ^{18}F-glucose



Gutte, Ardenkjaer-Larsen, et al., Am J Nucl Med Mol Imaging 2015

Emerging Diagnostic: Hyperpolarised Magnetic Resonance Imaging (MRI)



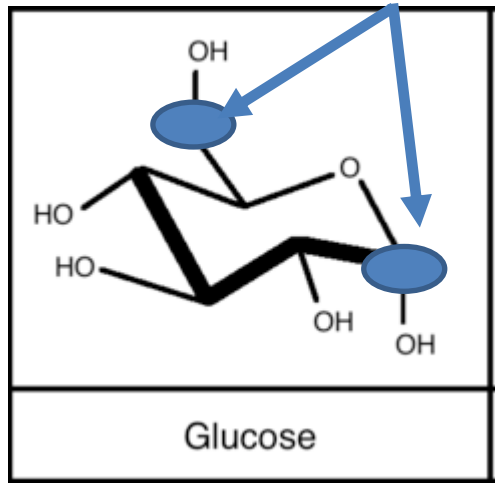
Now Possible

GE Healthcare
Clinical Imaging

– demonstrated since 2014

Interdisciplinary

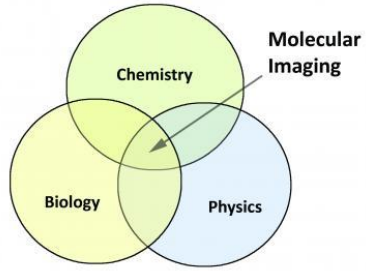
based on ^{13}C-glucose



Signal x 10'000

Safe

Use frequently for diagnostic



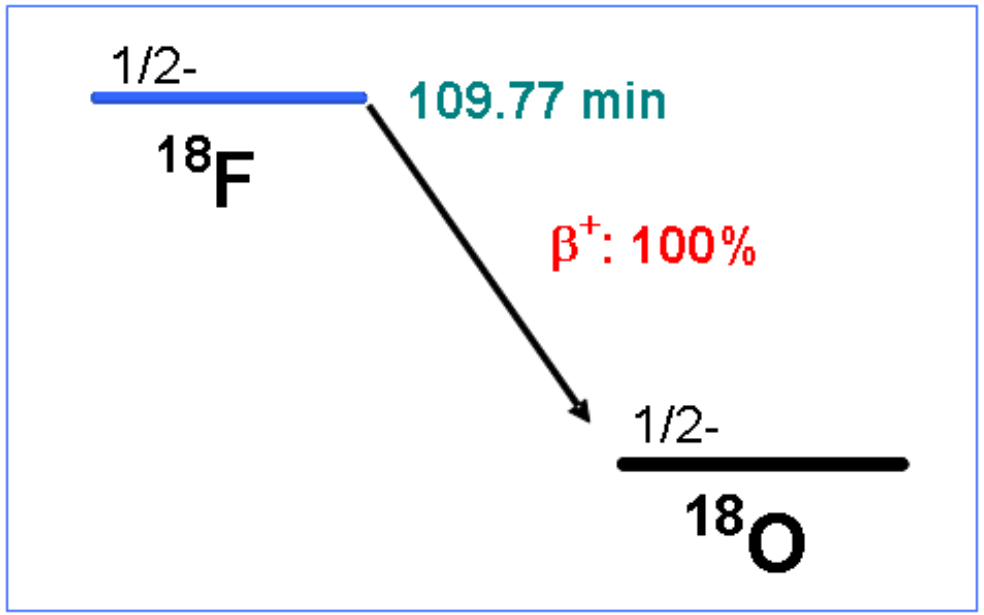
J.A. Ardenkjaer-Larsen et al., Proc. Nat. Acad. USA, 2003

P. Vasos et al., Proc. Nat. Acad. USA, 2010

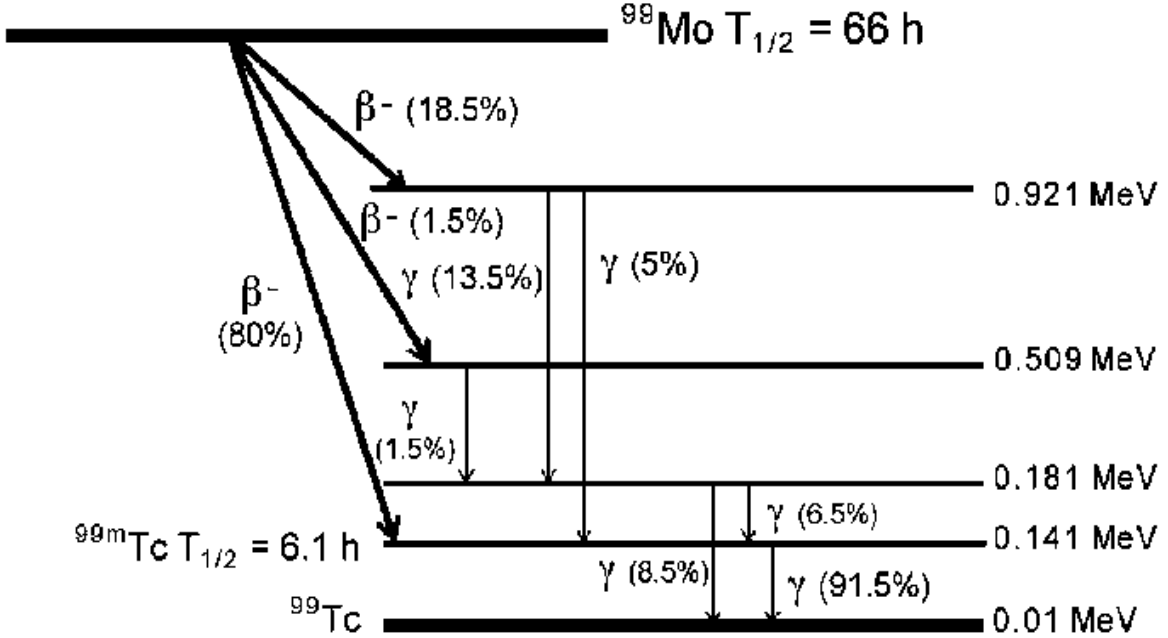
The issue with lifetimes in biomarkers labelled with decaying radiative or magnetic signal (PET or MRI)

The issue with lifetimes in biomarkers labelled with decaying radiative signal (PET)

^{18}F

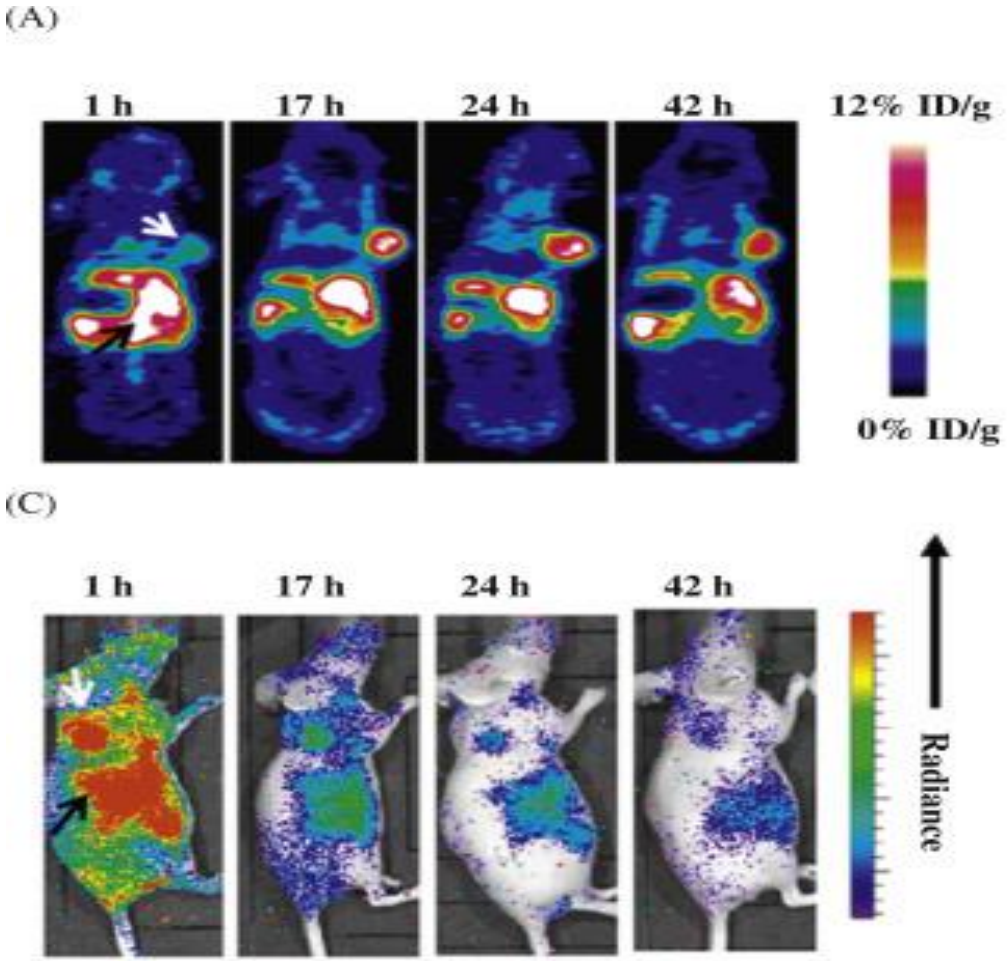


^{99m}Tc



Pillai M. et al Journal of Nuclear Medicine 2012

Lifetimes of different isotopes for (PET)



Representative Isotopes Suitable for PET Imaging and Their Physical Properties, *X. Sun et al, Acc. Chem. Res, 2015*

isotope	half-life
^{13}N	9.97 min
^{68}Ga	67.7 min
^{18}F	109.8 min
^{64}Cu	12.7 h
^{72}As	26 h
^{89}Zr	78.4 h

Paul Vasos

Hyperpolarised Magnetic Resonance Imaging

Structural imaging:
- are there many seeds?

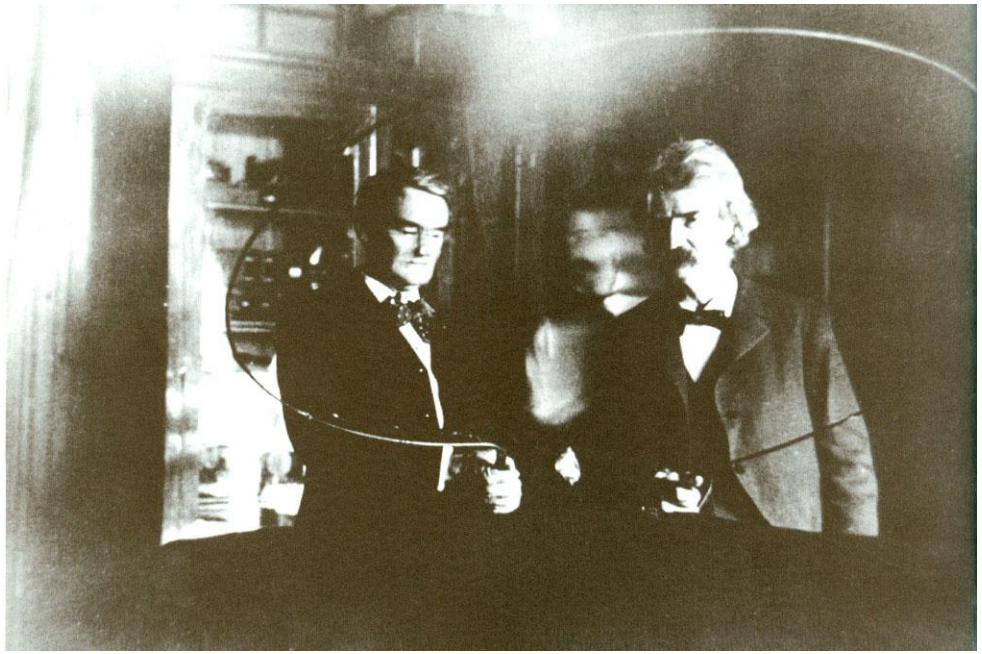
Molecular Imaging:
- is it sweet? (sugar content)

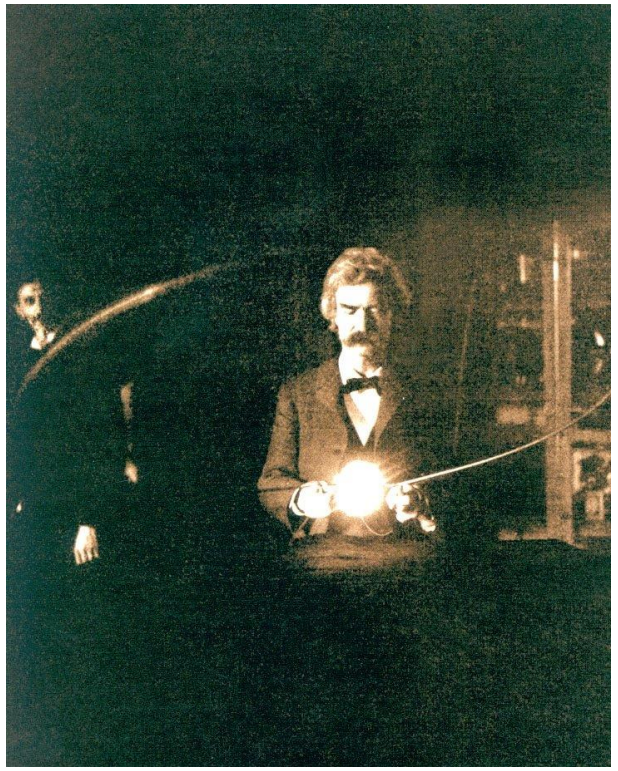
Functional molecular imaging:
- is it turning sour? how fast?

NEEDED : high sensitivity,
short time scale,
non-invasive

watermelon: timesnewroman.ro

Nuclear Magnetic Induction





Demonstration of an induction Coil

Nicola Tesla and Samuel Clemens (Mark Twain)

Radiation effects:

(unpaired e- spins)

- 1) Free radicals

(nuclear spins)

- 2) Biomolecular structure

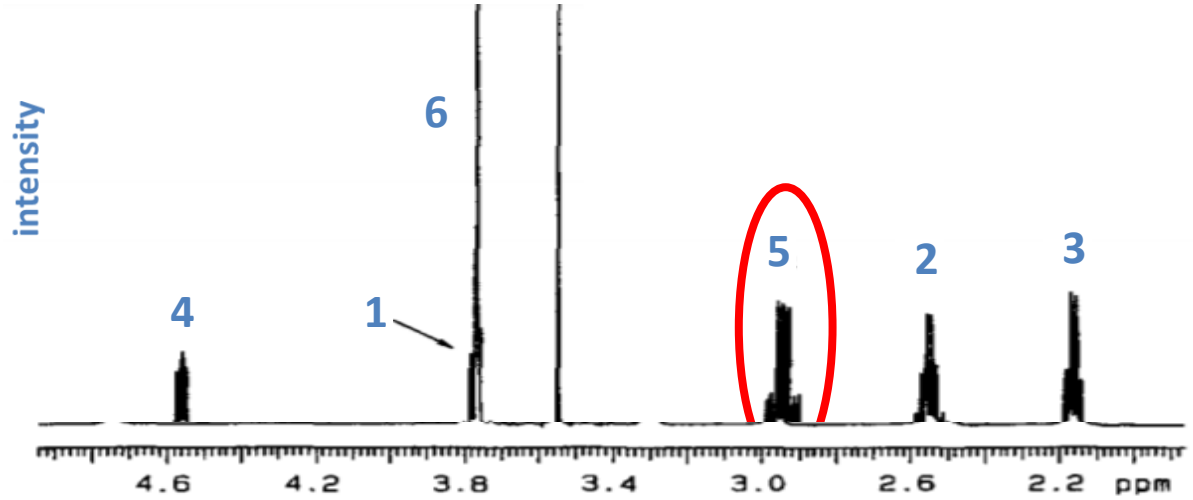
(unpaired e- from free radicals and nuclei via Dynamic Nuclear Polarisation – NMR)

- 3) Ensemble of small molecules within a cell (metabolomics)

- 4) Biomolecular transformations in cells

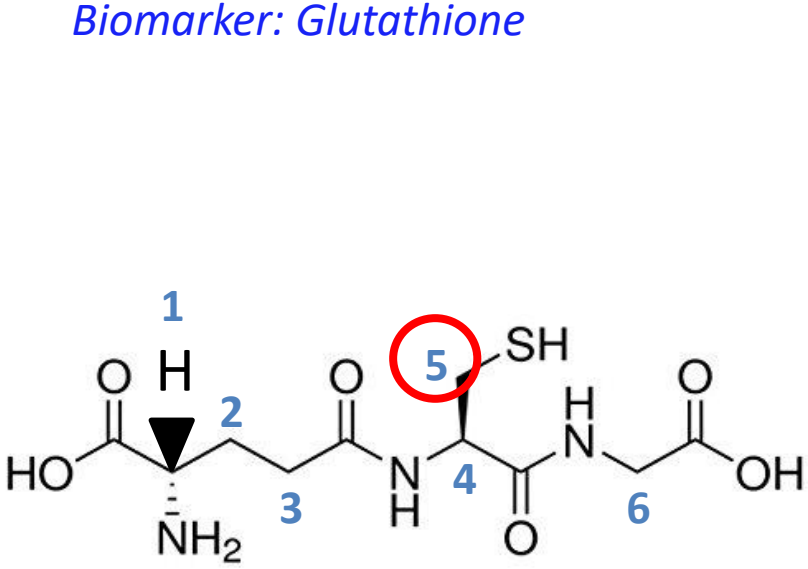
Paul Vasos

NMR detection of metabolites
- atomic resolution: each atom of the molecule has its own signal



δ(^{1}H)/ppm (chemical shift) ~ υ(^{1}H)/Hz (resonance frequency in the magnetic field)

Biomarker: Glutathione



- atomic resolution: each atomic position of the molecule has its own signal
- enzymes typically modify molecular structure at one (reactive) site: the -(CH_2)-SH group here labelled '5'
- the enzyme activity can detected via the chemical shift of hydrogens at site 5
- enzyme activity can be quantified via the time-dependent variations in the *intensity* of the molecular signals

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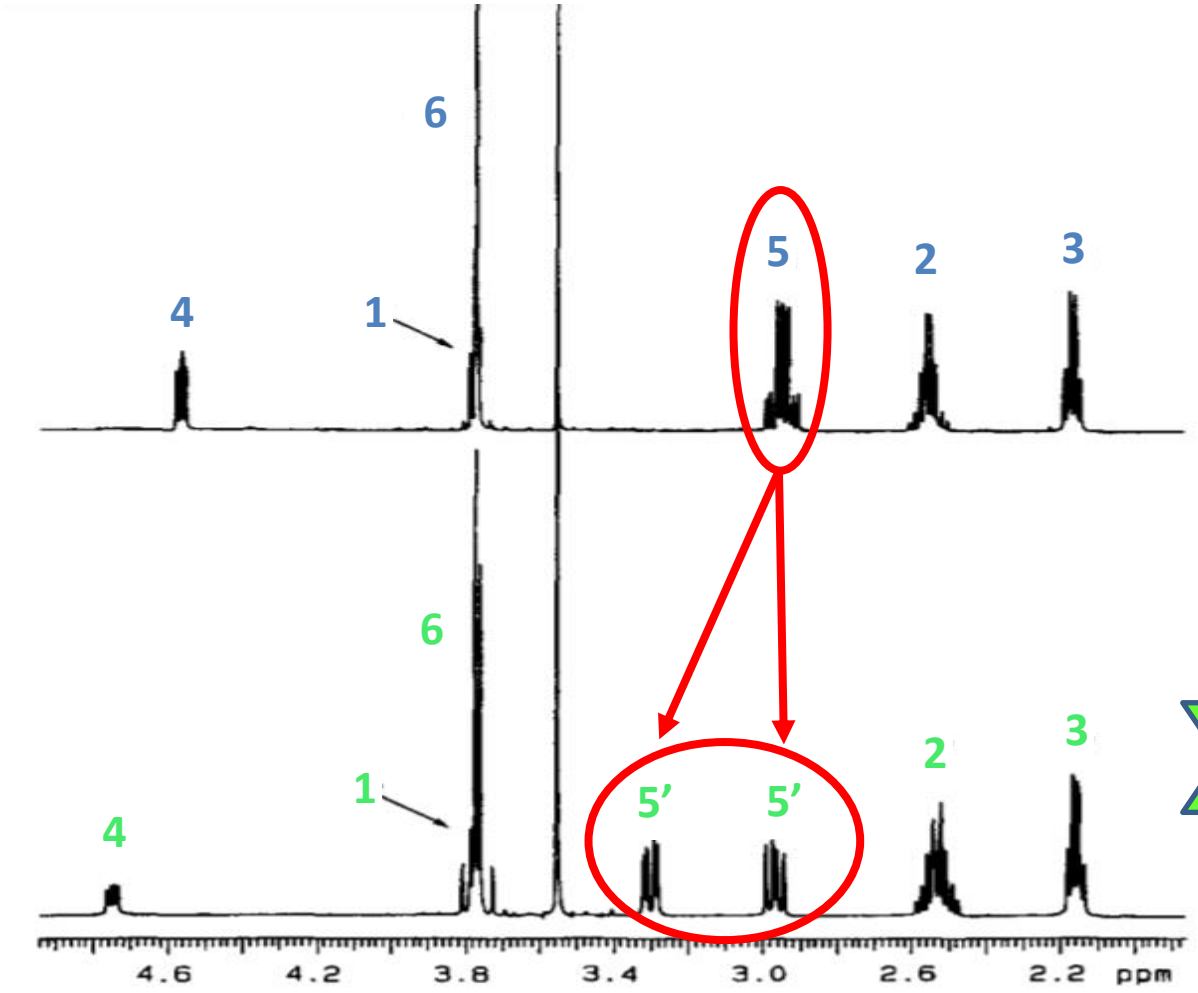
NMR detection of metabolites

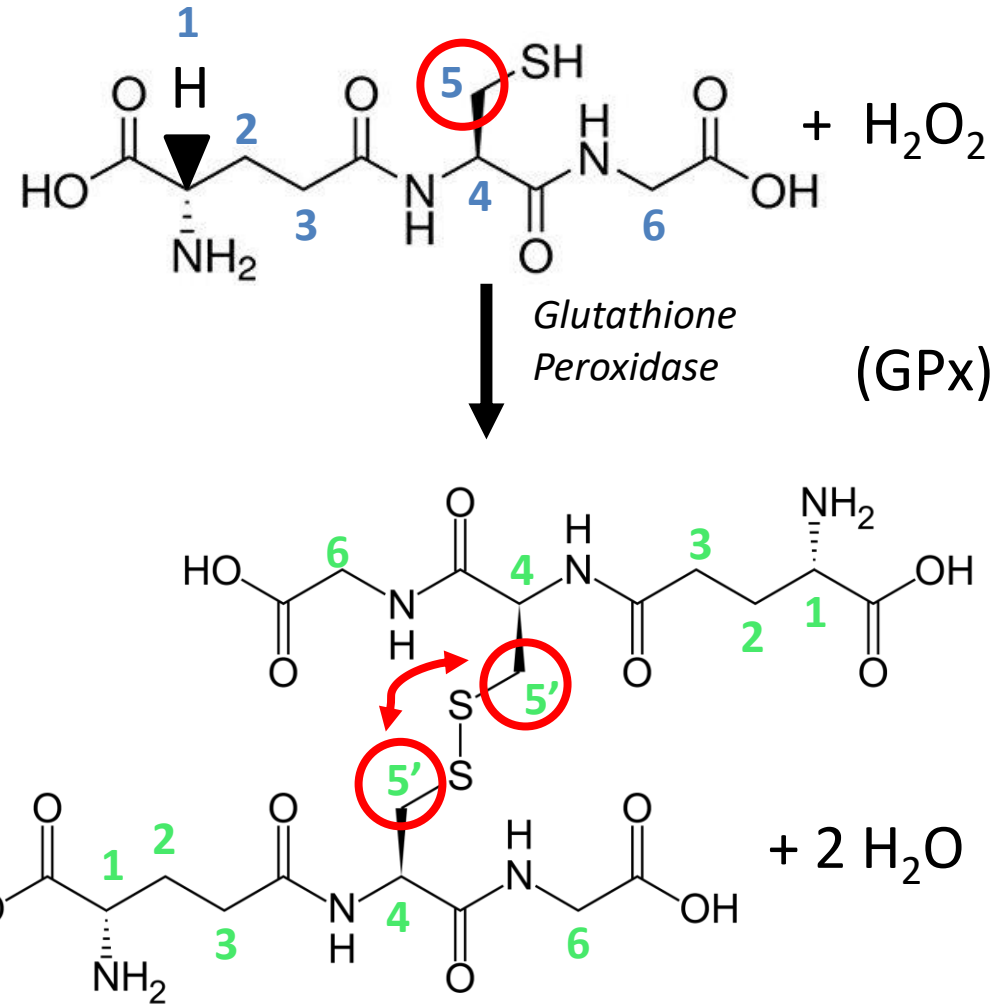


δ(¹H)/ppm (chemical shift) ~ υ(¹H)/Hz (resonance frequency in the magnetic field)

A

B

Biomarkers A, B



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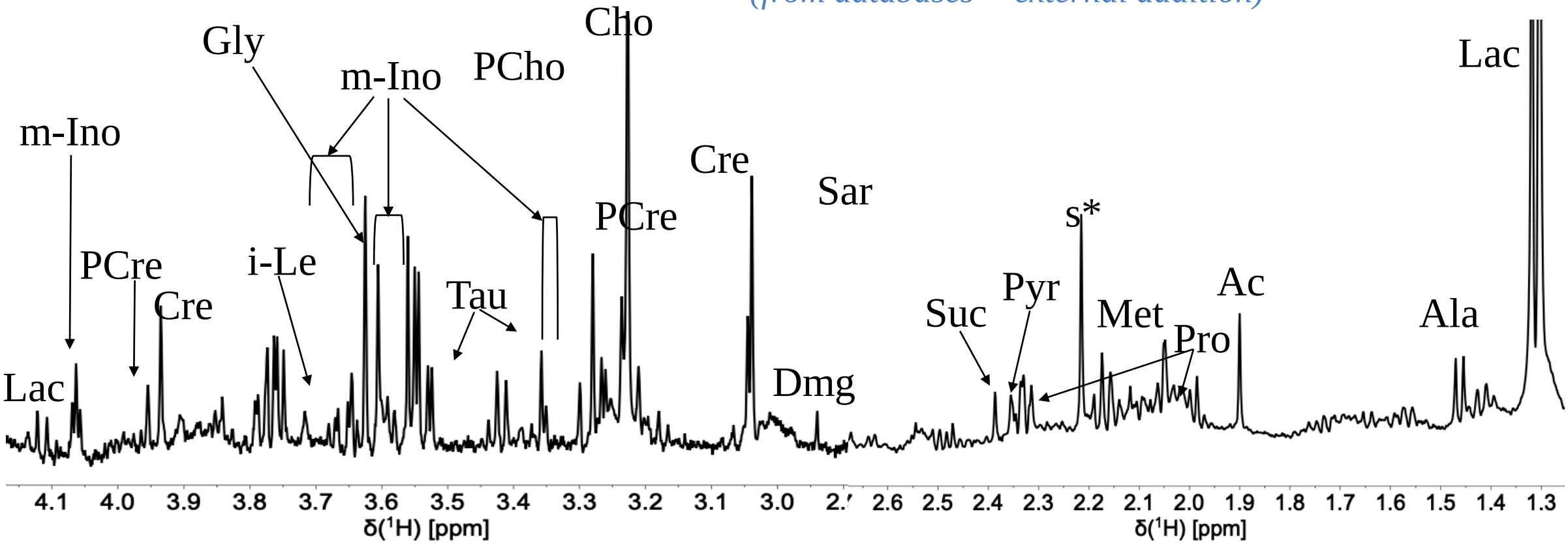
Identify metabolites in glioblastoma cells
(from databases + external addition)



1H NMR spectra of glioblastoma lysate recorded at 500 MHz. Exp. time 4.75 h, 1k scans.

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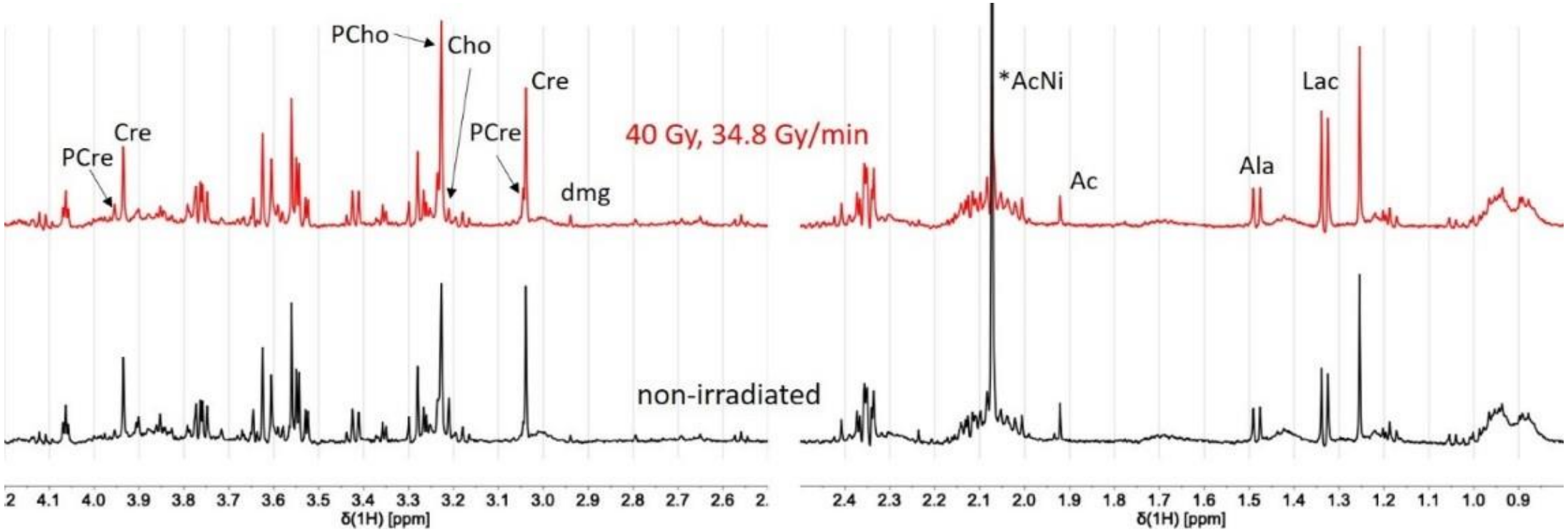
OUR EXPERIMENTS FOR DOSE-RATE DETECTION

NMR detection of metabolites (static 'metabolomics')

γ (60 Co source) irradiation

Human glioblastoma cell line U251 MG



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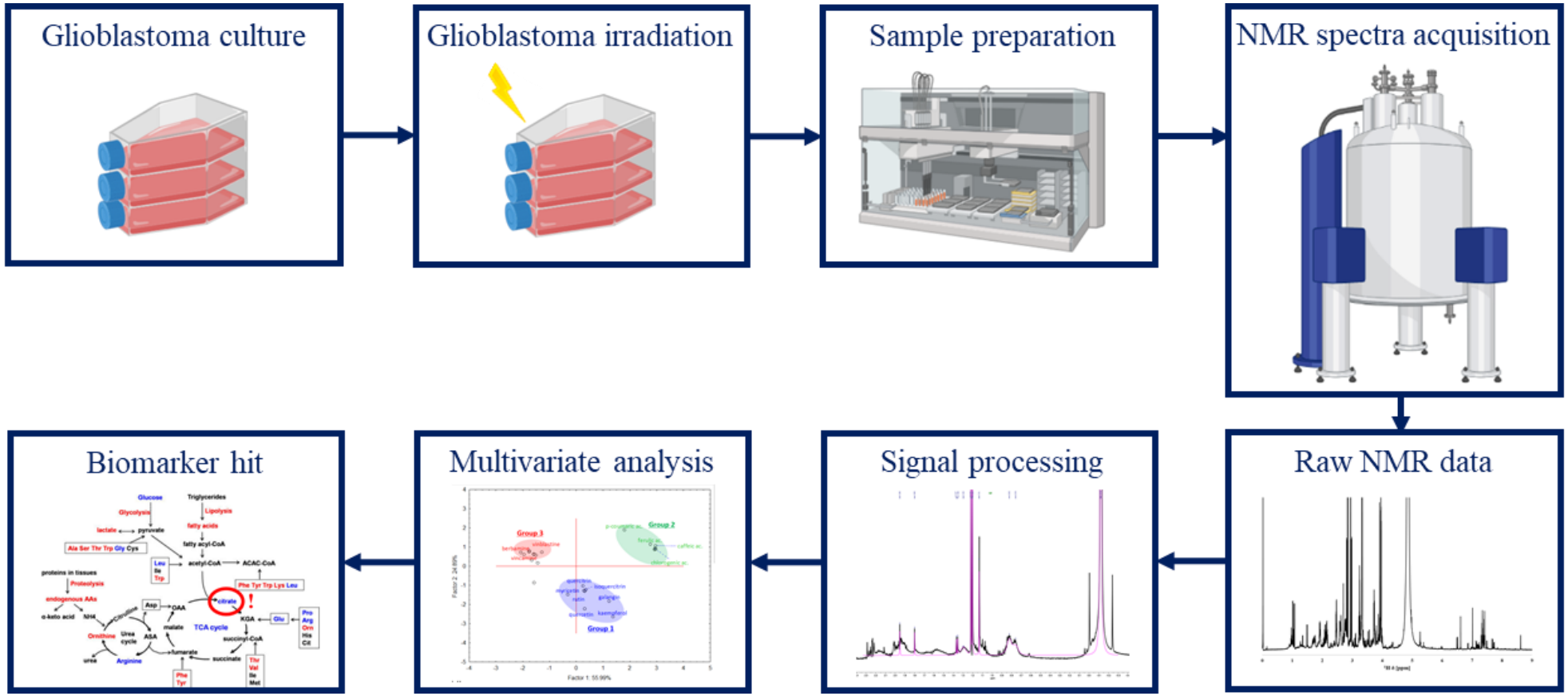
NMR workflow for metabolomics



- Optimise extraction protocol;
- Optimise # cells / probe;
- Optimise pulse sequence

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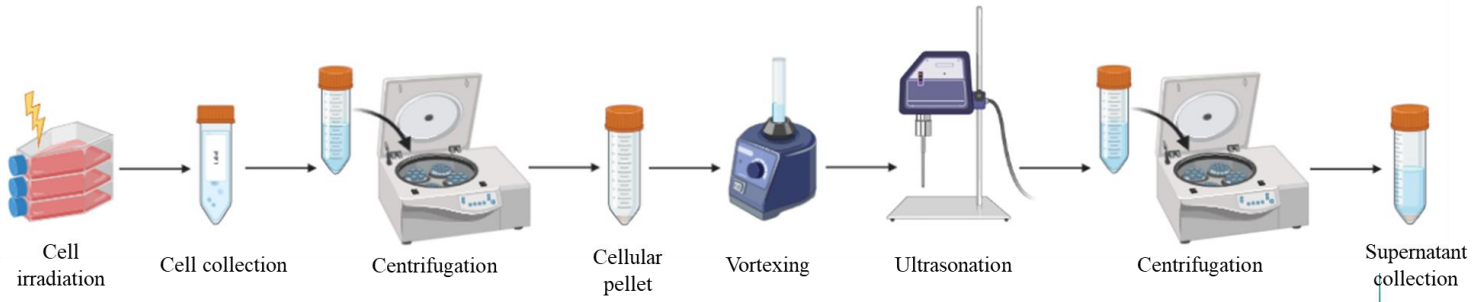
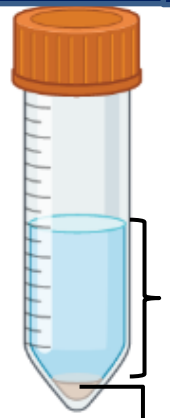
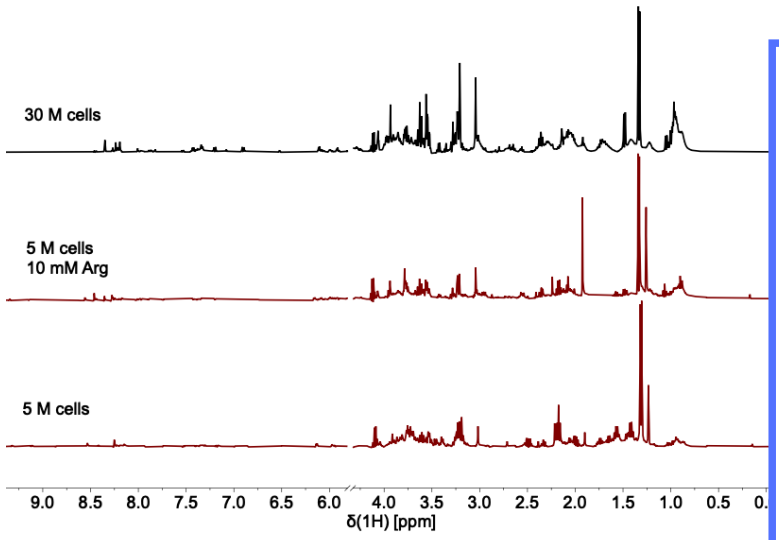
Optimise extraction protocol

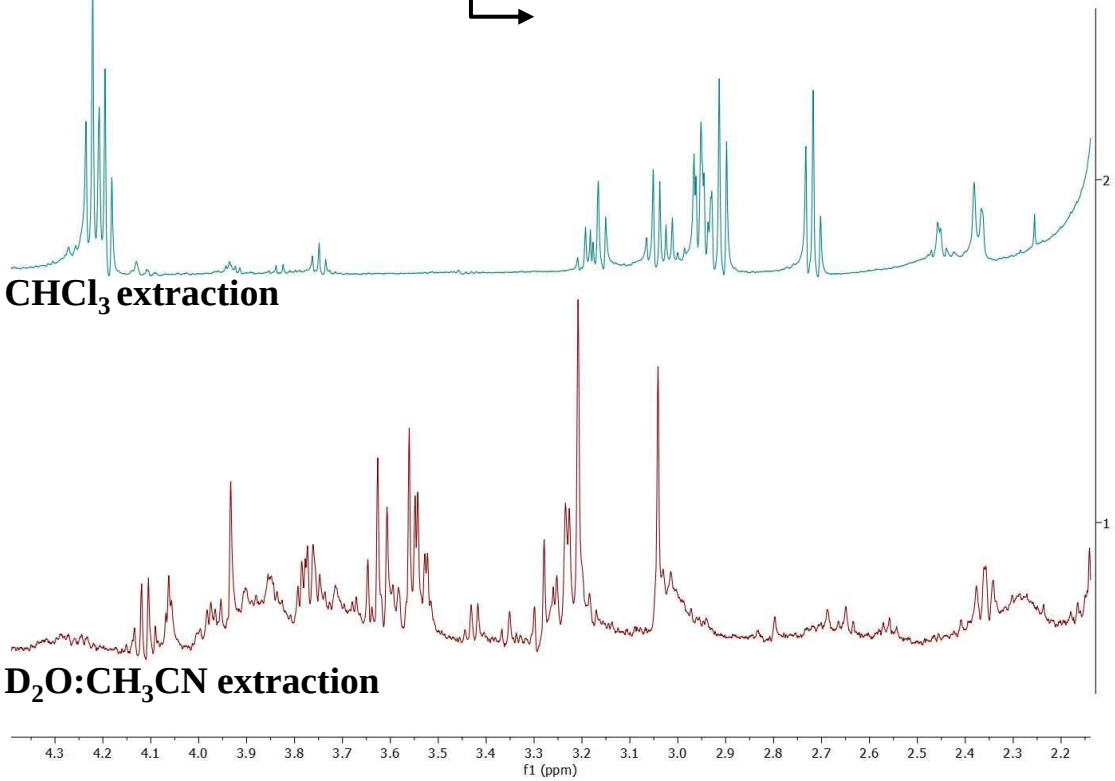




- D_2O:CH_3CN = 1:1
- D_2O:MetOH = 1:1
- 50 mM HPO_4^{2-} (pH = 7,4)
- CH3Cl

- Optimise cell # / probe → 5-10’000’000 cells at 500 MHz, *w/out hyperpolarisation*





Optimising Magnetic Resonance method: pulse sequence

- Water suppression pulse sequences
  - Single pulse
  - Perfect echo
  - CPMG-to reduce contributions from larger molecules (d1?)
  - NOESY1D

- Diffusion filtering pulse sequences
  - To enhance the signal from larger molecules and eliminate the contribution from smaller ones.

- Selective excitation pulse sequences
  - TOCSY1D-to identify a molecule by observing its multiplets signal after selecting one multiplet.

- Resultion enhancement pulse sequences
  - PSYCHE-pure shift, no multiplets

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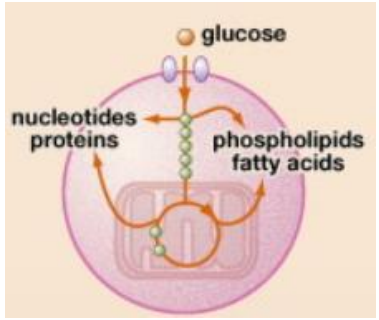
Warburg effect: metabolism kinetics linked to the influx of molecular resources distinguishes between cancer and normal cells

Normal cells: high-resource, high-throughput metabolism

Cancer cells: low-resource, low-throughput metabolism

Particularities of cancer cells → NMR and MRI biomarkers

Increased biosynthesis

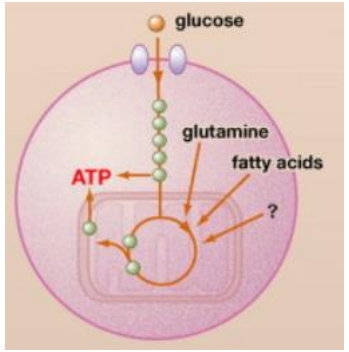


Warburg effect: cancer cells metabolize glucose by only glycolysis, even if they gain less ATP

hypoxia =>adapted transcriptional programme

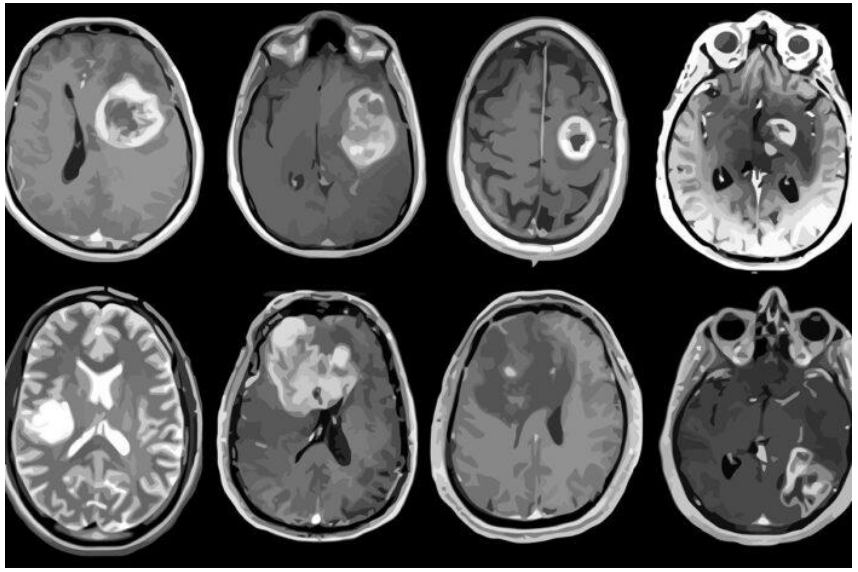
- Increased glucose consumption (→ use of fluorodeoxyglucose in positron emission tomography)
- Decreased oxydative phosphorylation
- Lactate overproduction

High energetic demand



Metabolite addiction:
Highly dependent on exogenous supply of aminoacids
Overexpression of aminoacid membrane transporters

Glioblastoma



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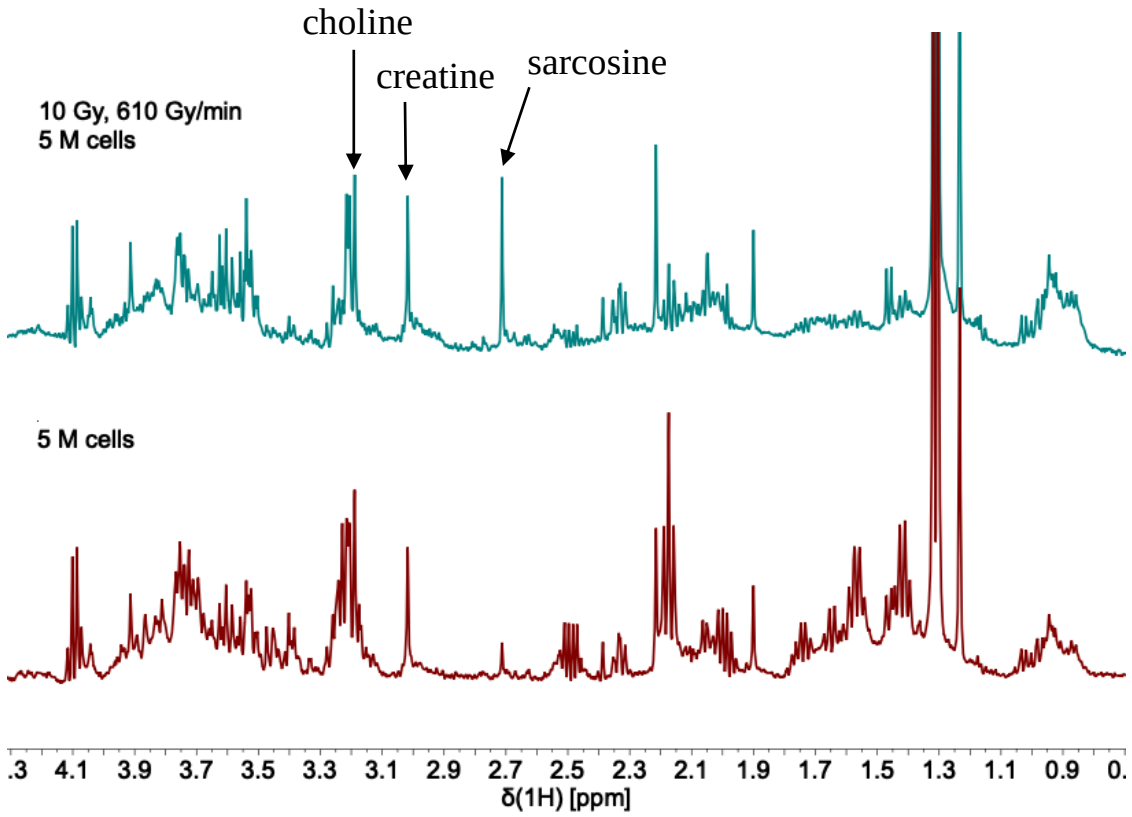
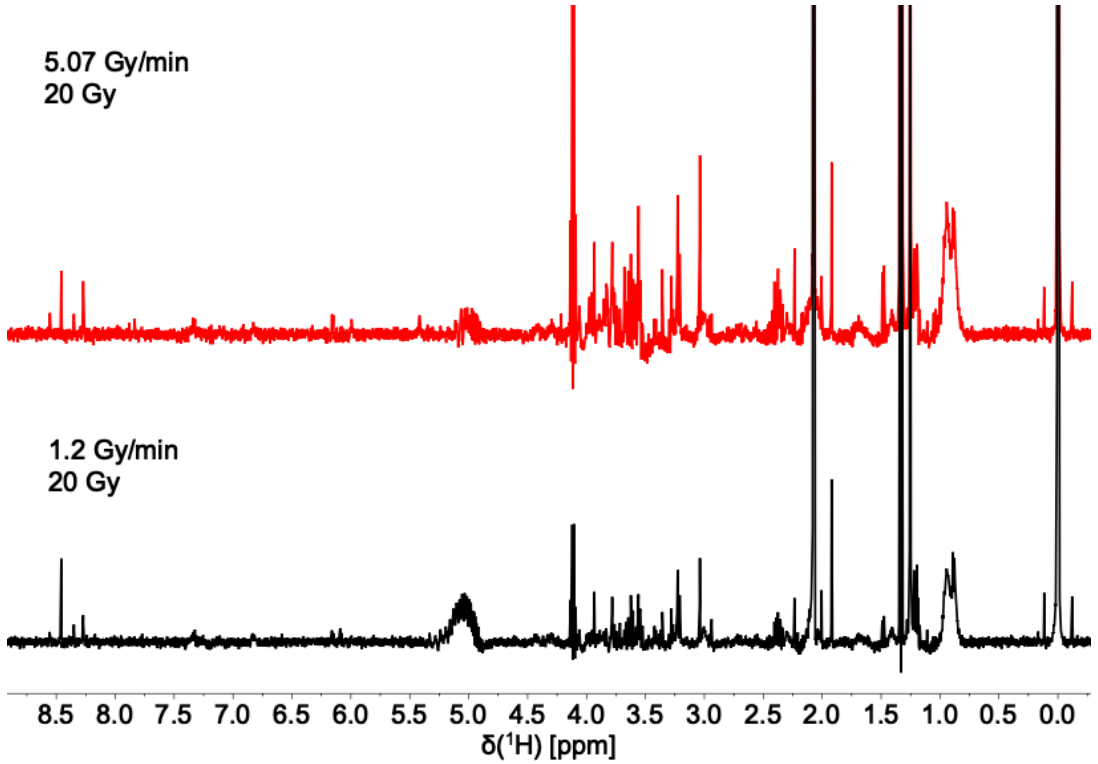
Changes in metabolite concentrations in glioblastoma cells upon irradiation





- modifications: metabolism or membrane changes following irradiation

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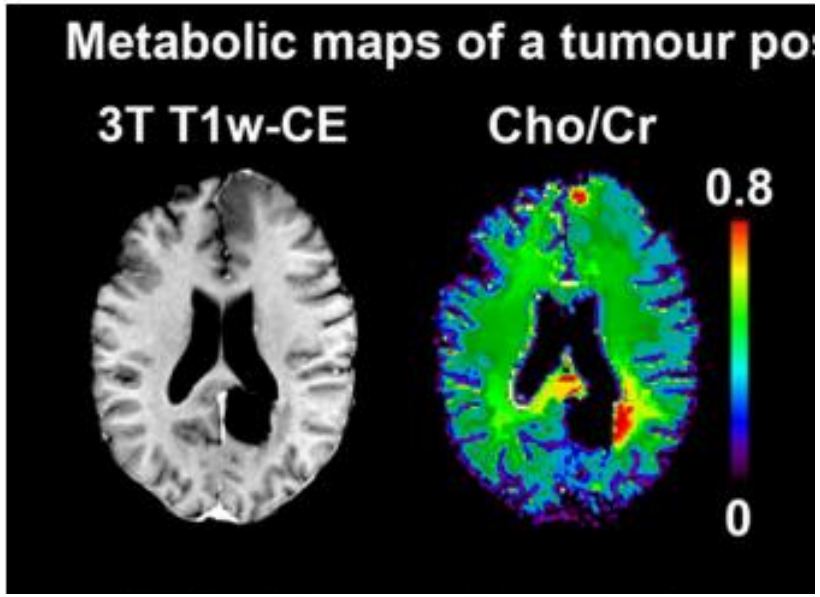
BIOMARKER: ratio of choline-to- creatine in cells [Cho]/[Cr]

High-Grade Glioma Treatment Response Monitoring Biomarkers: A Position Statement on the Evidence Supporting the Use of Advanced MRI Techniques in the Clinic, and the Latest Bench-to-Bedside Developments. Part 2:



Differentiating Radiation-Induced Necrosis from Recurrent Brain Tumor

In vivo:

Decreased Cho/Cr → lower chances of tumor recurrence

Radiotherapy effective → [Cho]/[Cr] decreases

Table 2. Summary of the functional outcomes among studies selected for meta-analysis.

First Author	Relative cerebral blood volume (Recurrent tumor vs. Necrosis)	Ratio of Cho/Cr (Recurrent tumor vs. Necrosis)
Prager (2015)[35]	1.81 (1.46, 2.58) vs. 1.015 (0.82, 1.46) †	NA
Alexiou (2014)[31]	6.71 (0.41) vs. 1.68 (0.42)	NA
Di Costanzo (2014)[32]	1.73 (0.56) vs. 0.86 (0.37)	2.12 (0.64) vs. 1.90 (0.32)
D'Souza (2014)[33]	3.01 (1.82) vs. 0.85 (0.34)	2.27 (0.59) vs. 1.26 (0.50)
Shin (2014)[34]	4.40 (3.07) vs. 2.08 (1.15)	NA
Huang (2011)[12]	2.49 (1.73) vs. 1.03 (0.23)	1.72 (1.10) vs. 1.34 (0.48)
Xu (2011)[11]	4.36 (1.98) vs. 1.28 (0.64)	NA
Matsusue (2010)[14]	3.33 (1.16) vs. 1.82 (0.80)	1.87 (0.39) vs. 1.11 (0.66)
Mitsuya (2010)[13]	3.5 (2.1–10)* vs. 1.0 (0.39–2.57)*	NA
Weybright (2005)[15]	NA	2.52 (1.66–4.26)^ vs. 1.57 (0.72–1.76)^
Rock (2002)[16]	NA	1.79 (0.79) vs. 0.89 (1.04)
Barajas (2009)[17]	2.38 (0.95) vs. 1.54 (0.92)	NA
Kamada (1997)[18]	NA	3.07 (0.23) vs. 2.07 (0.72)

PLOS ONE | DOI:10.1371/journal.pone.0141438 January 7, 2016

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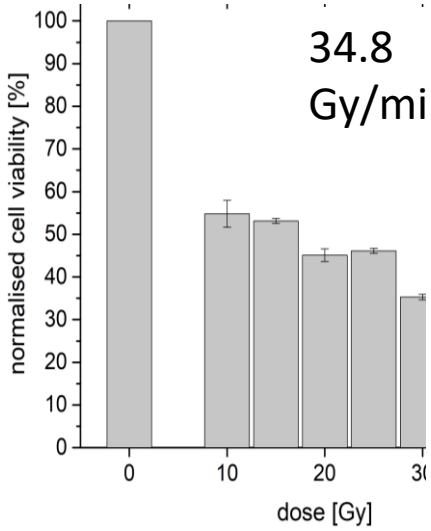
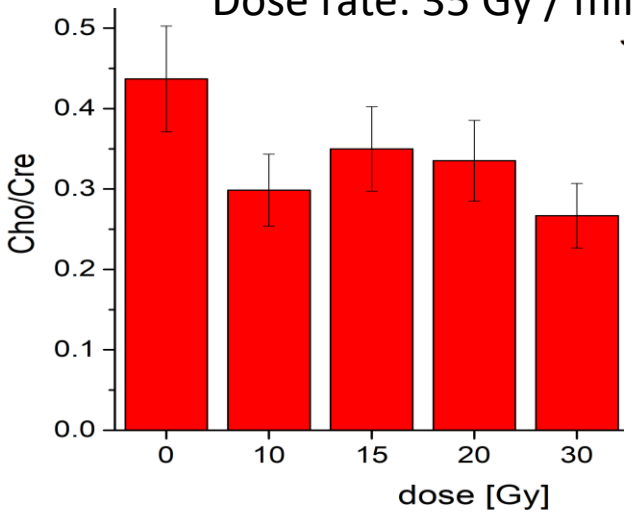
[Cho]/[Cr] biomarker Human glioblastoma cell line U251 MG

γ (^{60}Co source)
20 Gy total dose

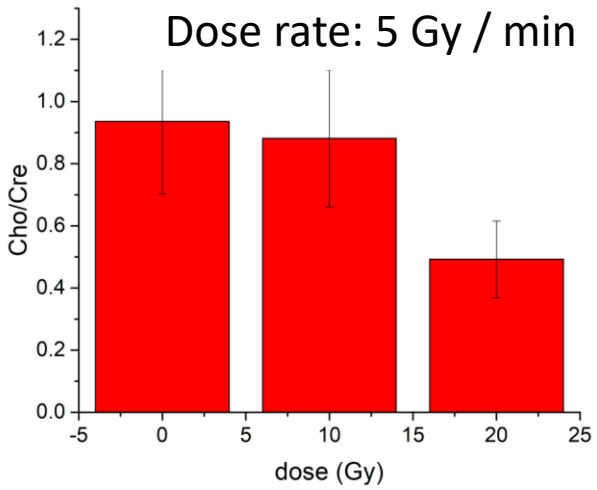
Dose Effect



Dose rate: 35 Gy / min

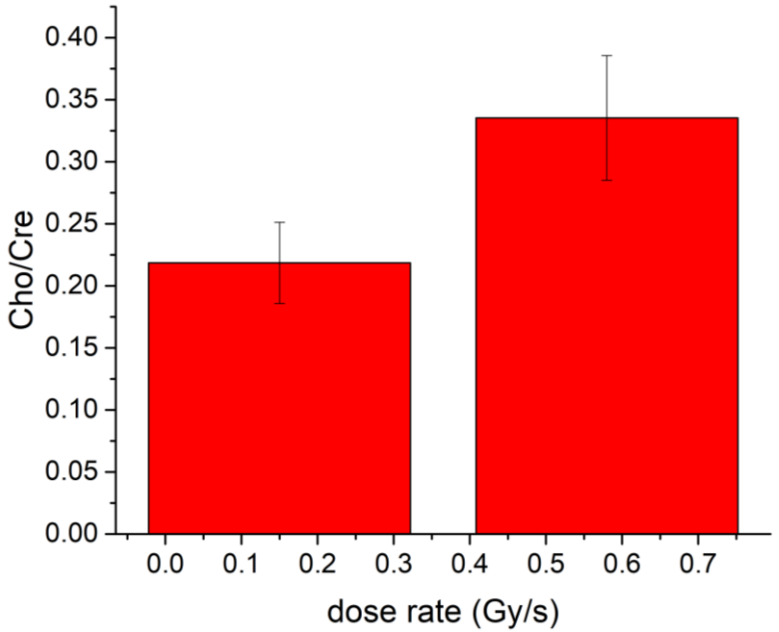


Dose rate: 5 Gy / min

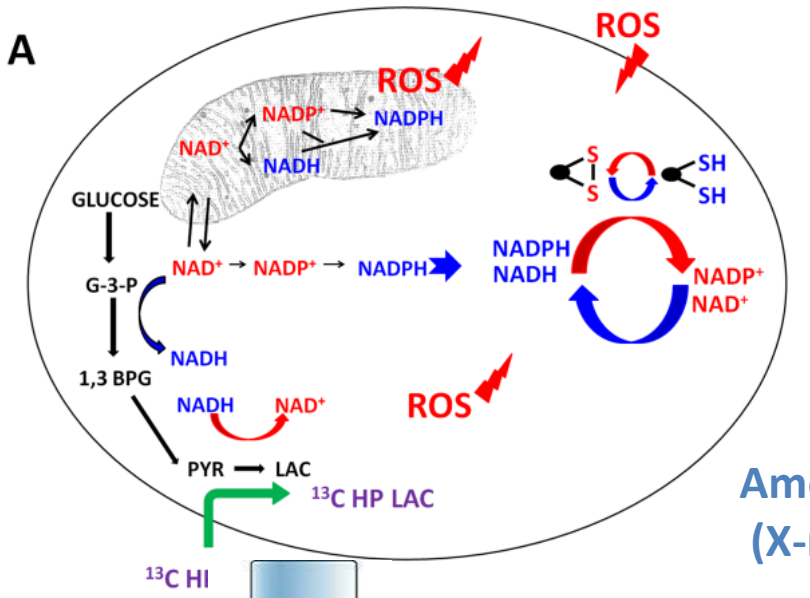


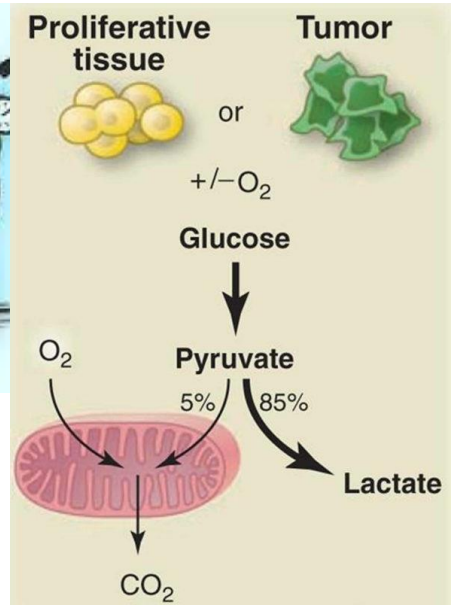
Amethyst Radiotherapy Centre, X-rays

Dose-rate effect

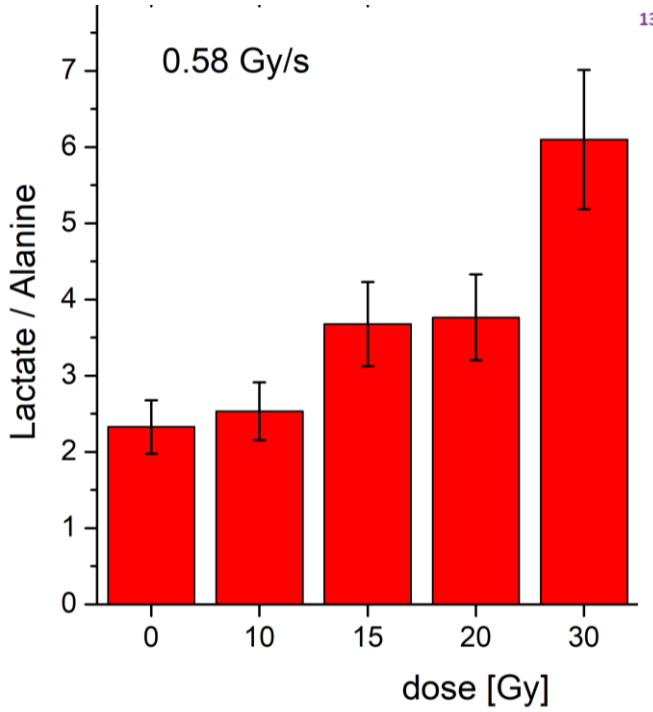


[Lac] as biomarker

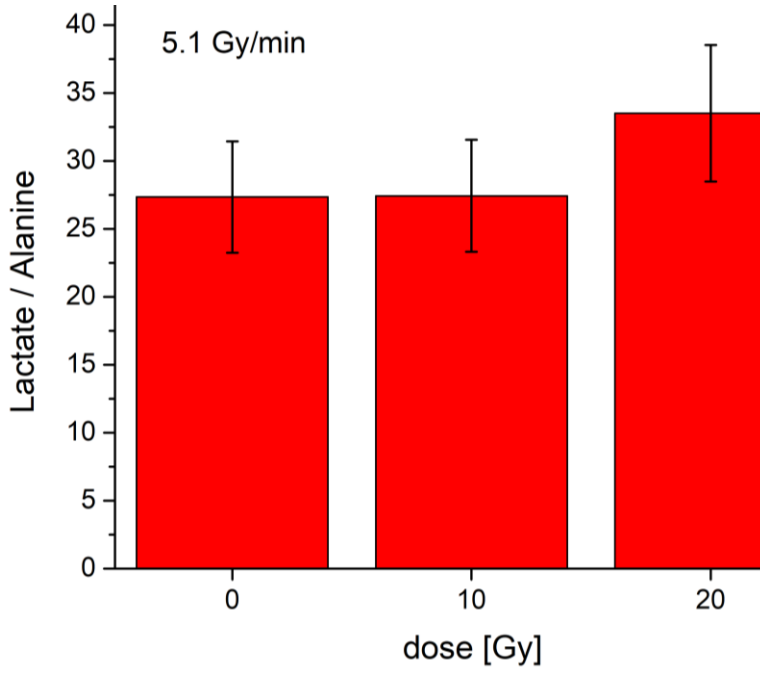




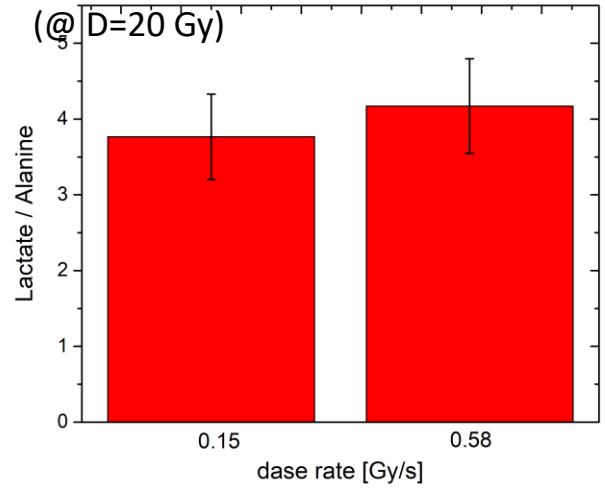
γ source (Co)

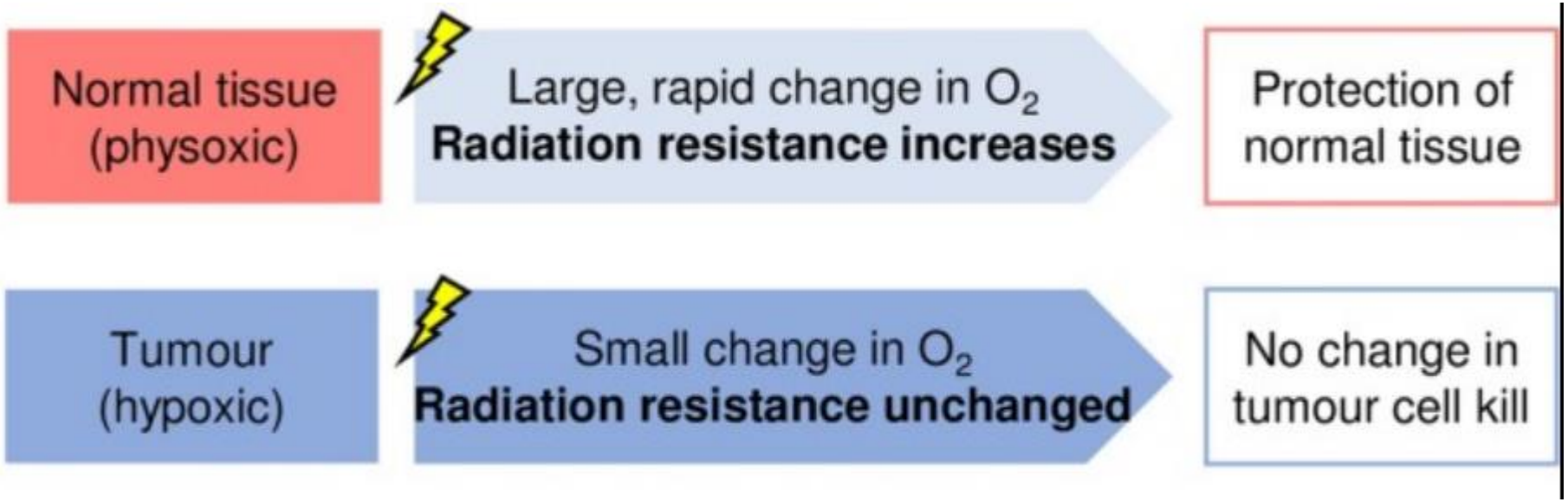


Amethyst Radiotherapy (X-ray)



Dose-rate variation by a factor 5 (@ D=20 Gy)





Nature 1959

Nature. 1959 May 23;183(4673):1450-1. doi: 10.1038/1831450a0.

Modification of the oxygen effect when bacteria are given large pulses of radiation

D L DEWEY, J W BOAG

Review > Front Oncol. 2020 Jan 17;9:1563. doi: 10.3389/fonc.2019.01563. eCollection 2019.

Ultra-High Dose Rate (FLASH) Radiotherapy: Silver Bullet or Fool's Gold?

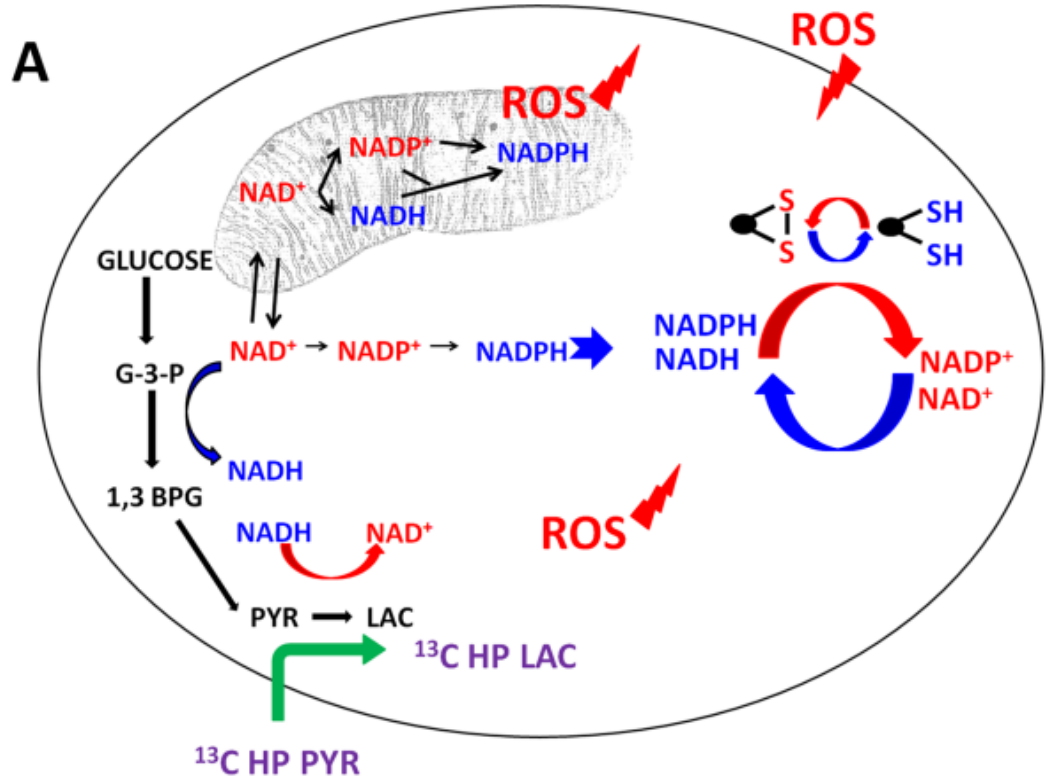
Joseph D Wilson [1], Ester M Hammond [1], Geoff S Higgins [1], Kristoffer Petersson [1 2]

Evaluate metabolic flux (kinetics) rather than accumulation of metabolites at endpoints: [Lac]/[Pyr]

HYPERPOLARISATION:

- decrease # cells
- Sufficient sensitivity for in-vivo
- Water → metabolites

From static evaluation of Metabolites
→
Metabolic Flux



Sandulache et al. PLoS One 2017

Radiobiology and Molecular Imaging - Molecular Markers (early response)

Step 1: **Biomarkers for cancer**
– Early monitoring necessary !

Especially at high dose-rate

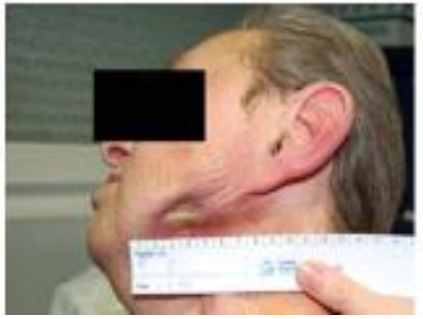
ELI-NP: high dose-rate radiation

Without early imaging

Step2:

Adapt to various cancer types
Towards personalized radiotherapy
using early monitoring

Effects of excessive dose without real-time monitoring



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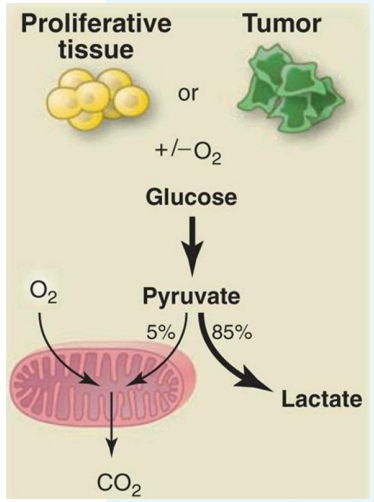
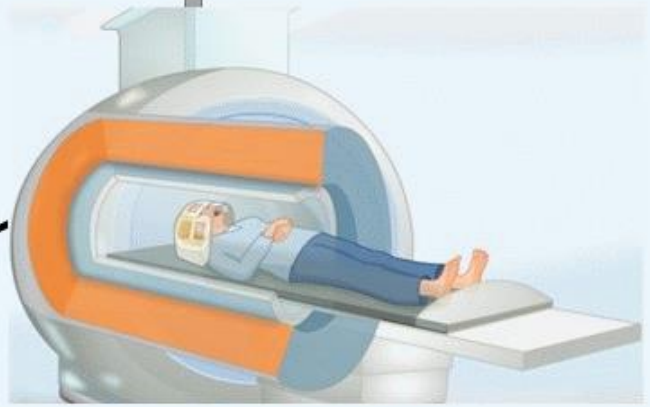
Currently employed clinical evaluation of radiation effects using hyperpolarised magnetic resonance

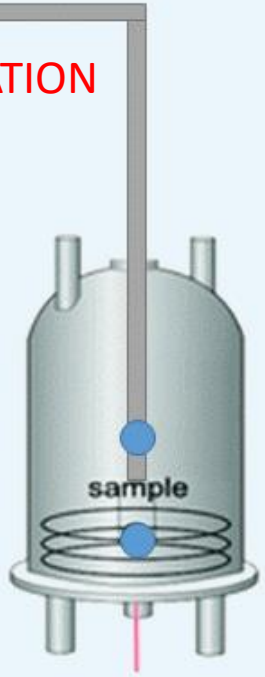
Dynamic Nuclear Polarisation (DNP) – Magnetic Resonance

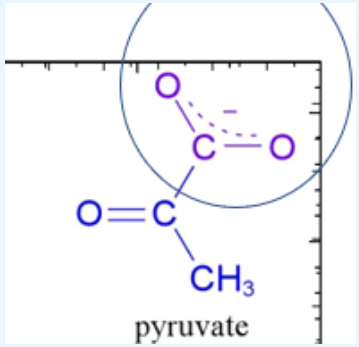


LONG LIFETIMES OF POLARISATION NEEDED !

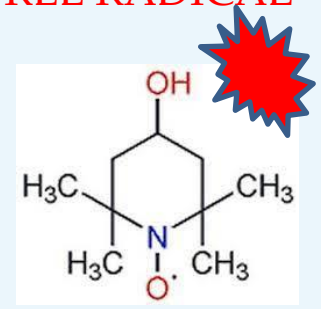


Analyse - MRI





Prepare Biomarker (hyperpolarized metabolite)
+
FREE RADICAL



Dynamic Nuclear Polarisation ("hyperpolarized") MRI : Ardenkjaer-Larsen et al., PNAS, 2003

Ahuja, Vasos, et al., ChemComm 2010, Vasos et al., PNAS, 2009

Translation to the clinic: **early cancer diagnostic** by molecular imaging

free radicals generate signal improvement

Nelson et al., Sci. Rep., 2013

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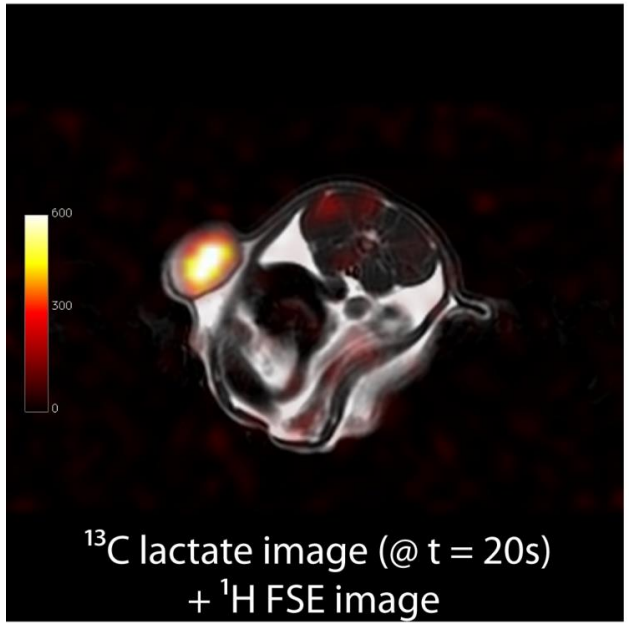
Early evaluation of radiotherapy using Hyperpolarised Magnetic Resonance via Dynamic Nuclear Polarisation (DNP)

Radiation



PLOS ONE

Probing Early Tumor Response to Radiation Therapy Using Hyperpolarized [1-^{13}C]pyruvate in MDA-MB-231 Xenografts

Albert P. Chen, William Chu, Yi-Ping Gu, Charles H. Cunnhingham

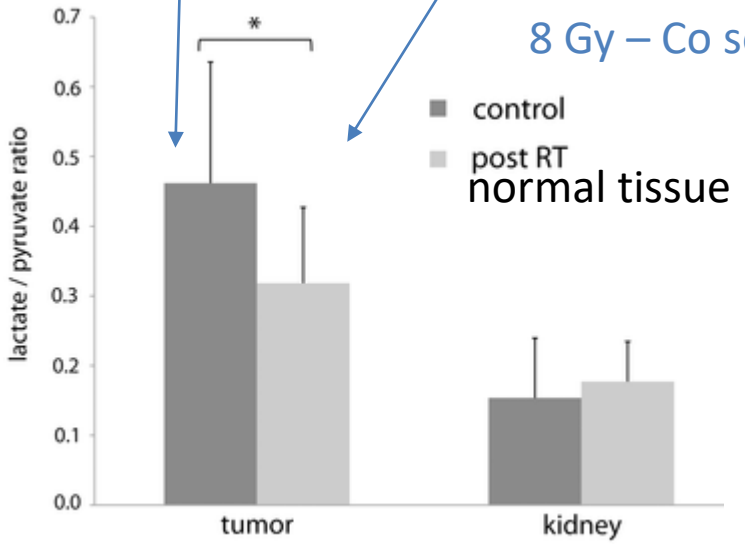
Published: February 12, 2013 • https://doi.org/10.1371/journal.pone.0056551

Biomarker: Pyr→Lac

Biomarker

hours after radiation

8 Gy – Co source



Probing Early Tumor Response to Radiation Therapy Using Hyperpolarized [1-^{13}C]pyruvate in MDA-MB-231 Xenografts

When each tumor reached approximately 1.5 cm in the largestdimension, the rat was either scanned as a control or treated withradiation. The average duration from tumor cell implantation toimaging was 48 days (stdev. = 11) for the control group and 51days (stdev. = 9) for the treatment group. For the radiationtreatment, the rats were anesthetized using a mixture of Ketamineand Xylazine at 7.5 mg and 1 mg per 100 g body weightrespectively. The tumors were exposed to ionizing radiation usinga model CP160 160-kVp x-ray system (Faxitron X-ray Corp.,Wheeling, IL, USA) [24]. Radiation treatments were given at adosage of 8 Gy to one side and another 8 Gy at the opposite sideof the tumor (lead shielding was used to protect the animal fromradiation exposure beyond the tumor). Tumors treated withradiation were scanned 96 hours after treatment. A total of 20animals were imaged (10 treated and 10 untreated) in this study

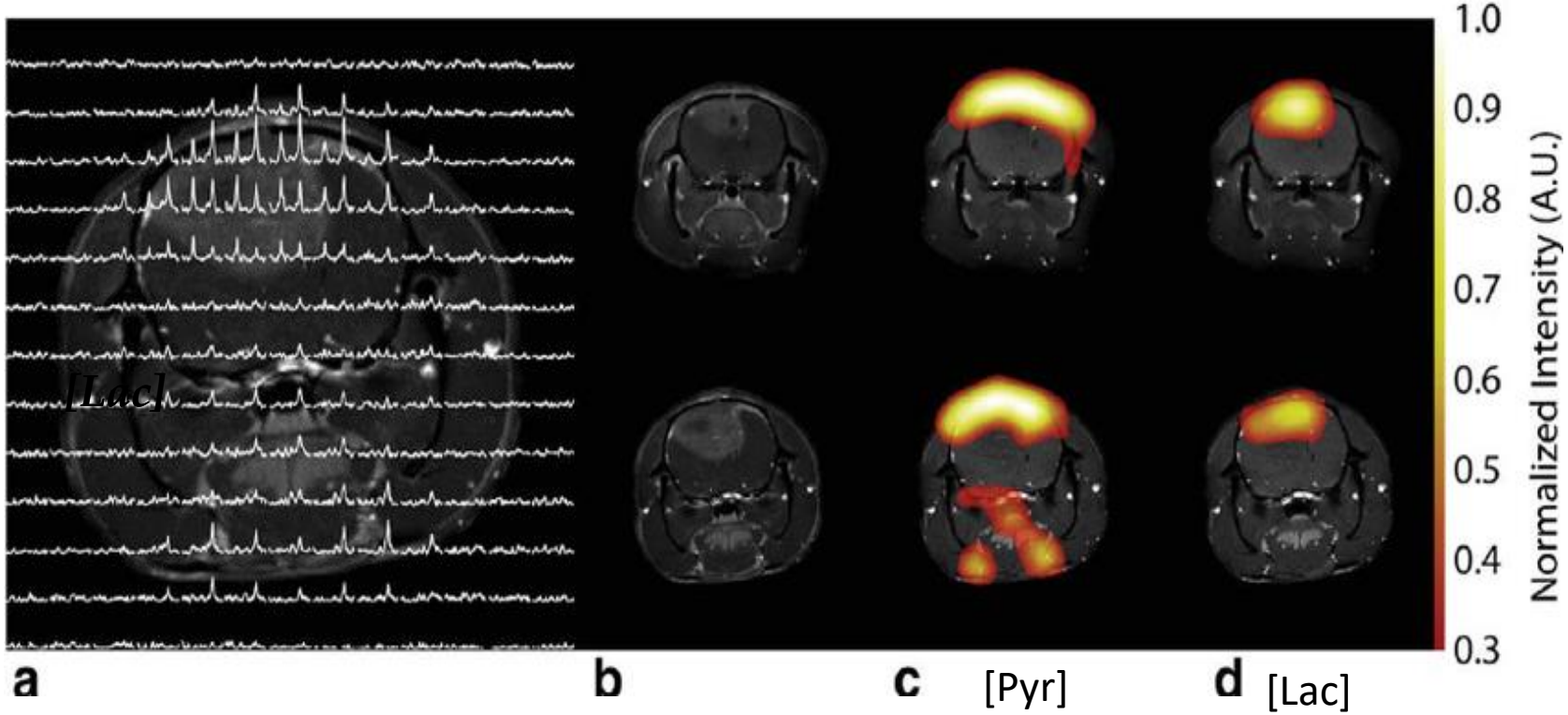
Parallel studies in-cells (NMR) and *in-vivo* (MRI) studies

Paul Vasos

Fast folow-up in Radiation Therapy: Pyr→Lac biomarker Hyperpolarised Magnetic Resonance in-vivo

Glioma

Implanted glioma tumour irradiation
The 60Co irradiator beam was collimated to produce an irradiation field of 16 cm by 5 cm. Further lead shielding covered the nose and neck. The whole brain was exposed to a dose of 15 Gy. Exposure was calibrated using thermoluminescent dosimeters embedded in the middle of 3.0 cm3 Lucite blocks (Total Plastics, Baltimore, MD), to approximate absorbed dose in the brain. These gave a dose rate of 153.6 6 2.4 rad/min in the unshielded areas, and 4.0 and 5.4 rad/min under the nose and body shields, respectively. The remaining four tumor-implanted animals did not receive radiotherapy and were used as controls.



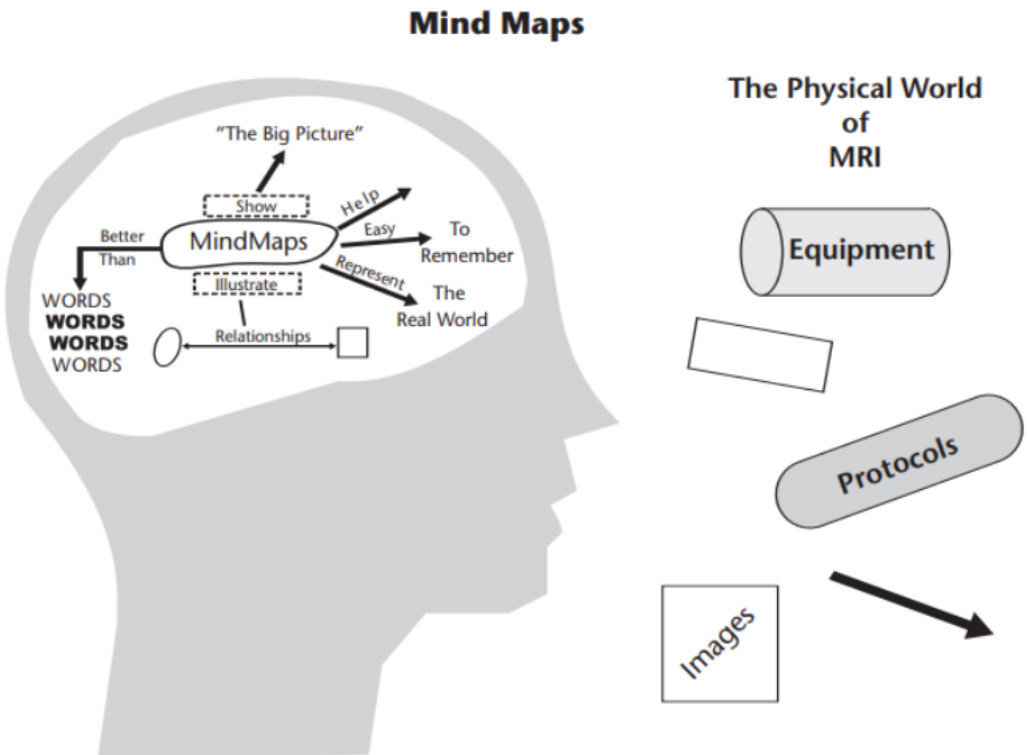
Before gamma radiation

96 h post [Lac] reduced

K. Brindle et al. Magn Res Med, 2014
Imaging of tumor in a rat model before and 96 hours after 15-Gy irradiation

tens of hours -post radiation -> early enough to stop toxic effects

What is MRI?



Perry Sprawls, Magnetic Resonance Imaging Principles, Methods and Techniques, 2000

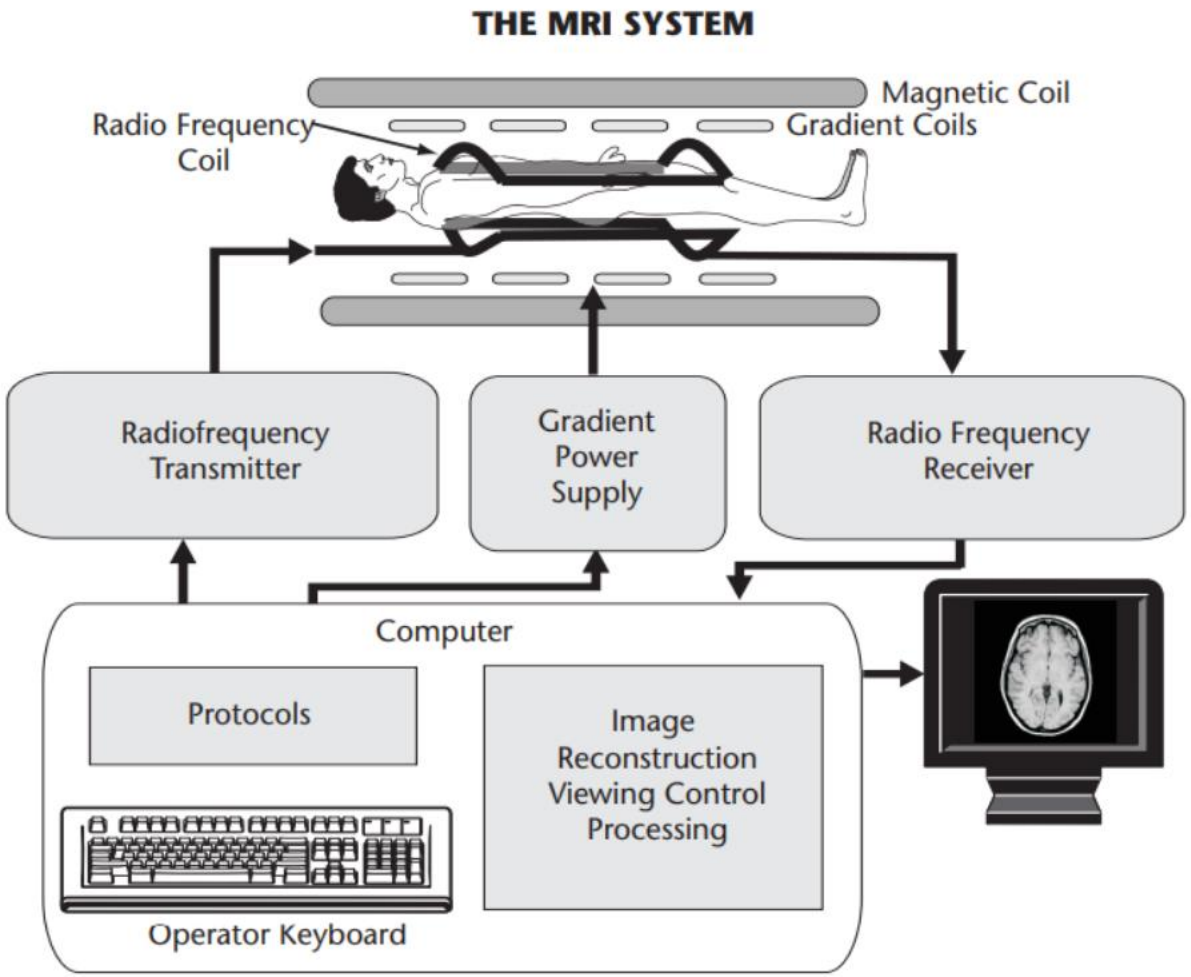
A technique used in radiology to form pictures of the anatomy and the physiological process of the body.

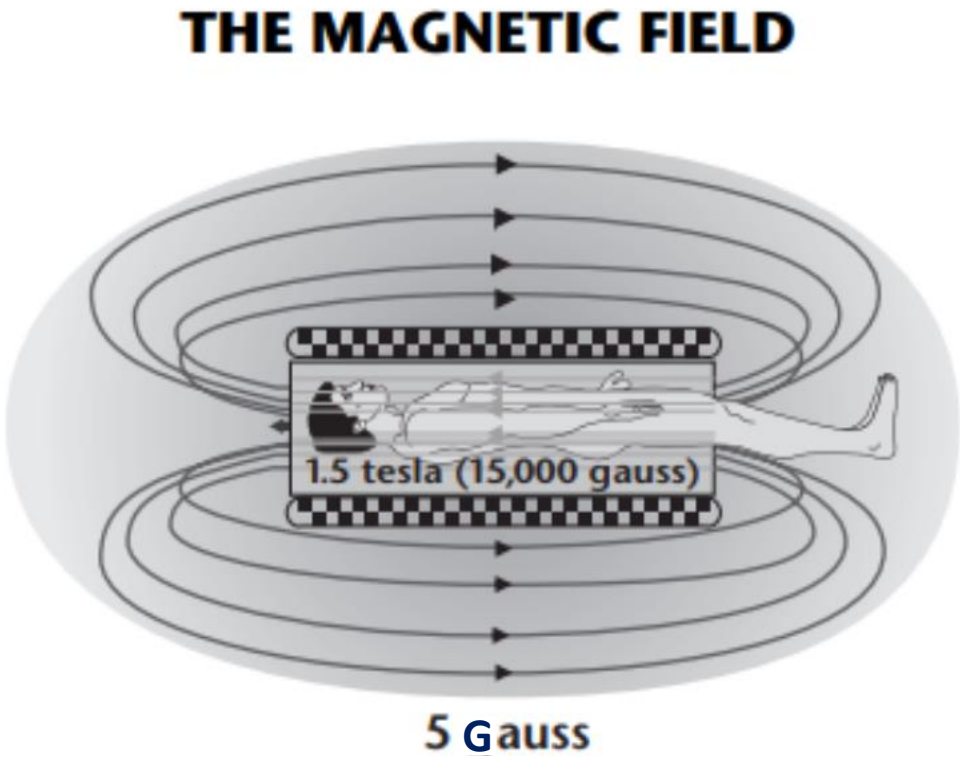
Is a non-invasive imaging technology

Is based on the principle of Nuclear Magnetic Resonance (NMR)

Certain atomic nuclei demonstrate the ability to absorb and re-emit radiofrequency energy when are placed in a magnetic field.

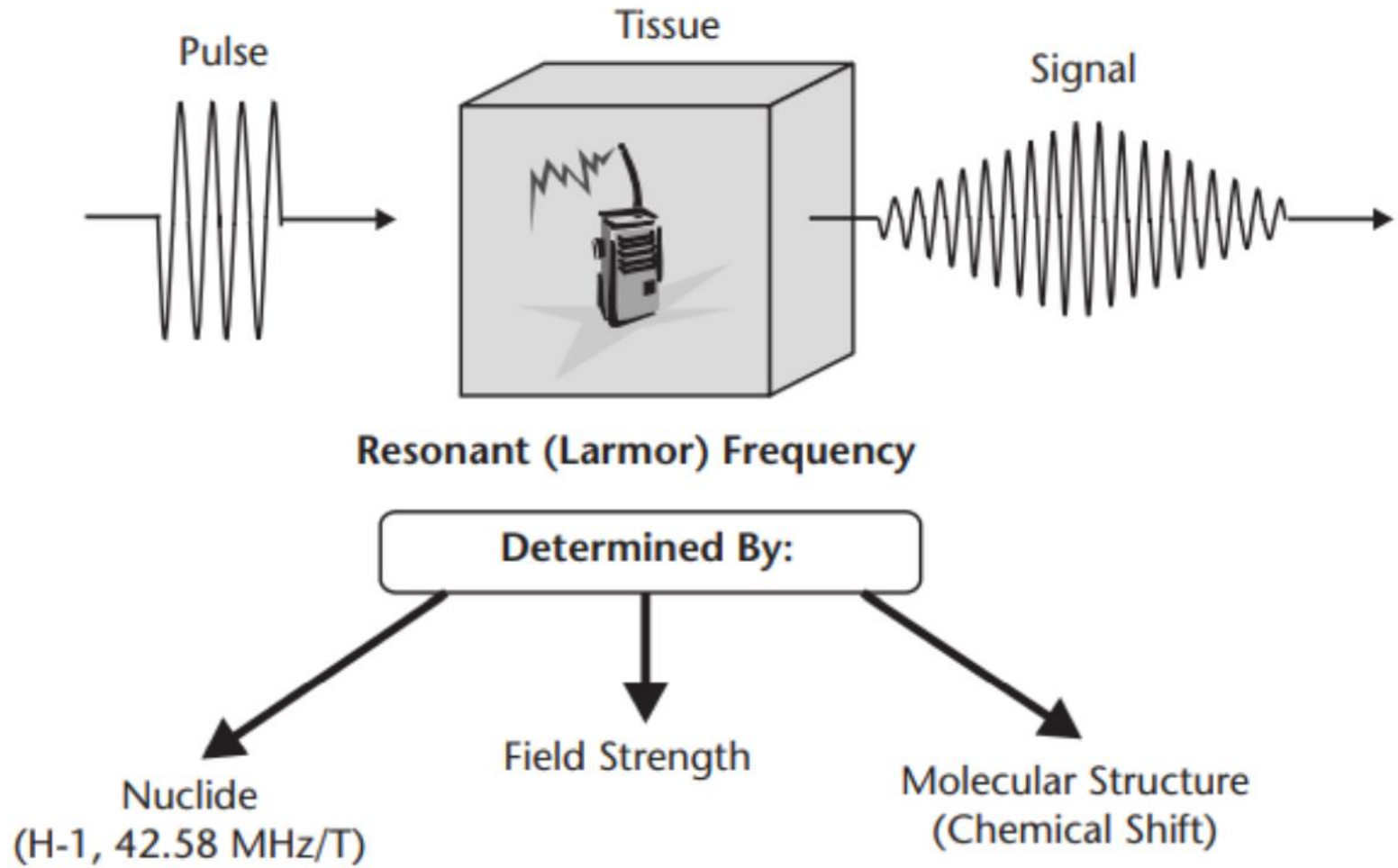
How does MRI work?



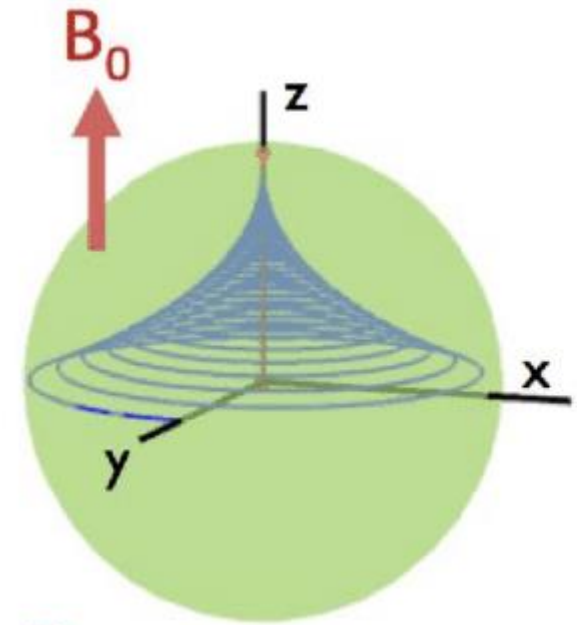


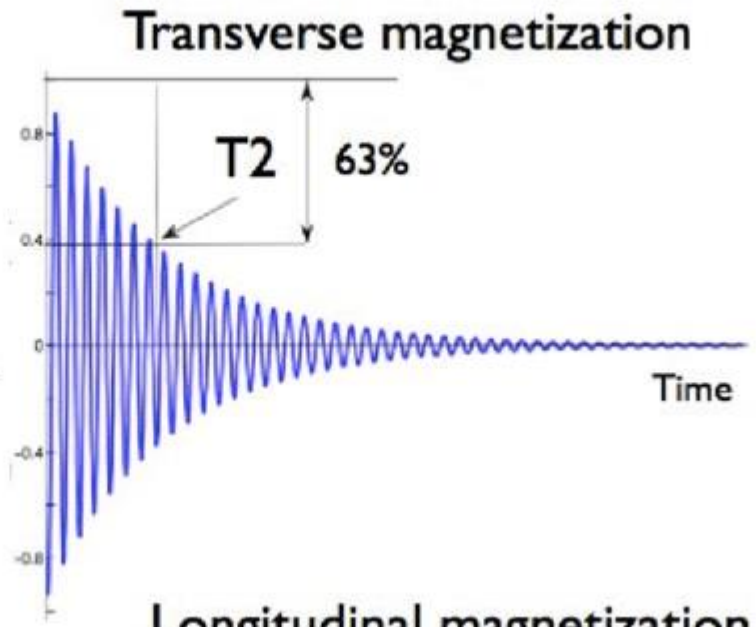
Perry Sprawls, Magnetic Resonance Imaging Principles, Methods and Techniques, 2000

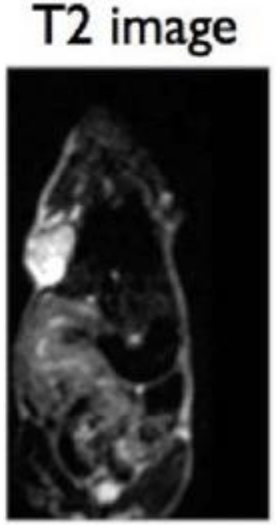
The concept of Nuclear Magnetic Resonance

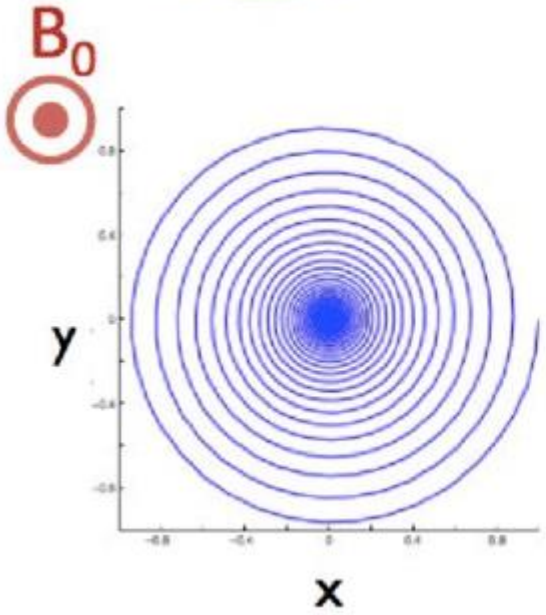


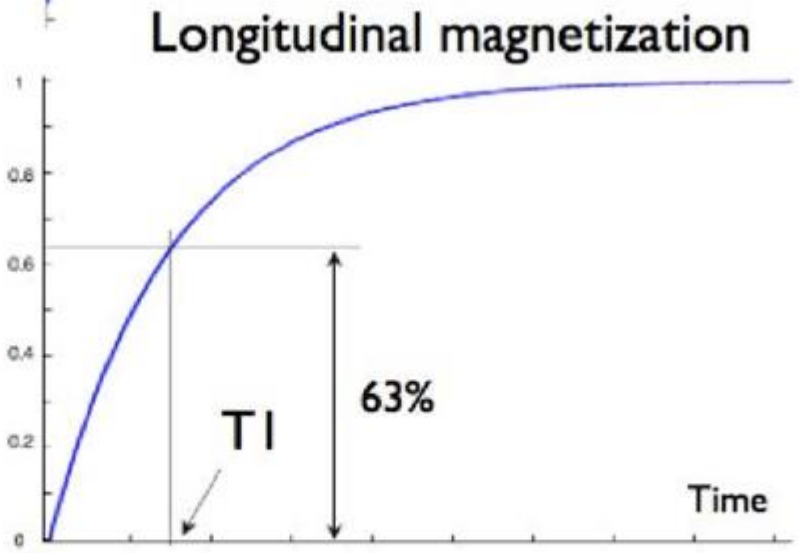
Perry Sprawls, Magnetic Resonance Imaging Principles, Methods and Techniques, 2000

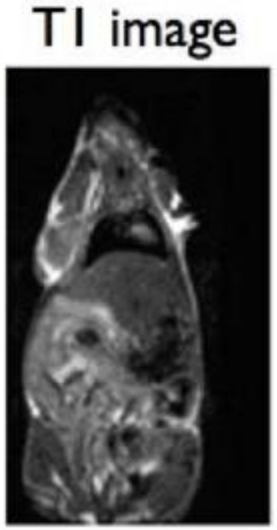






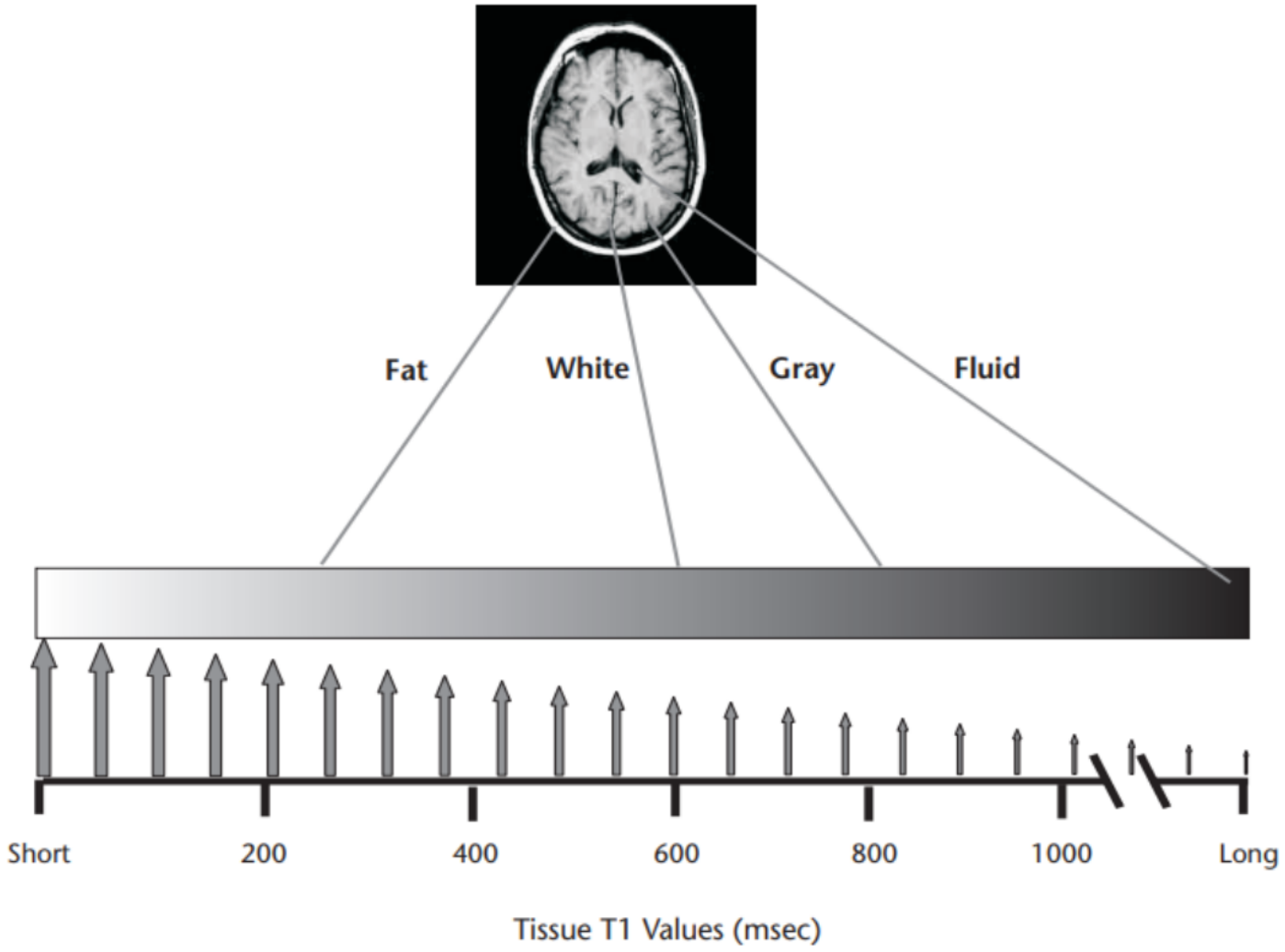






Wang G, Zhang X, Liu Y, Hu Z, Mei X, Uvdal K. Magneto--Fluorescent Nanoparticles with High--Intensity NIR Emission, T1 and T2 Weighted MR for Multimodal Specific Tumor Imaging. J Mater Chem B 2015

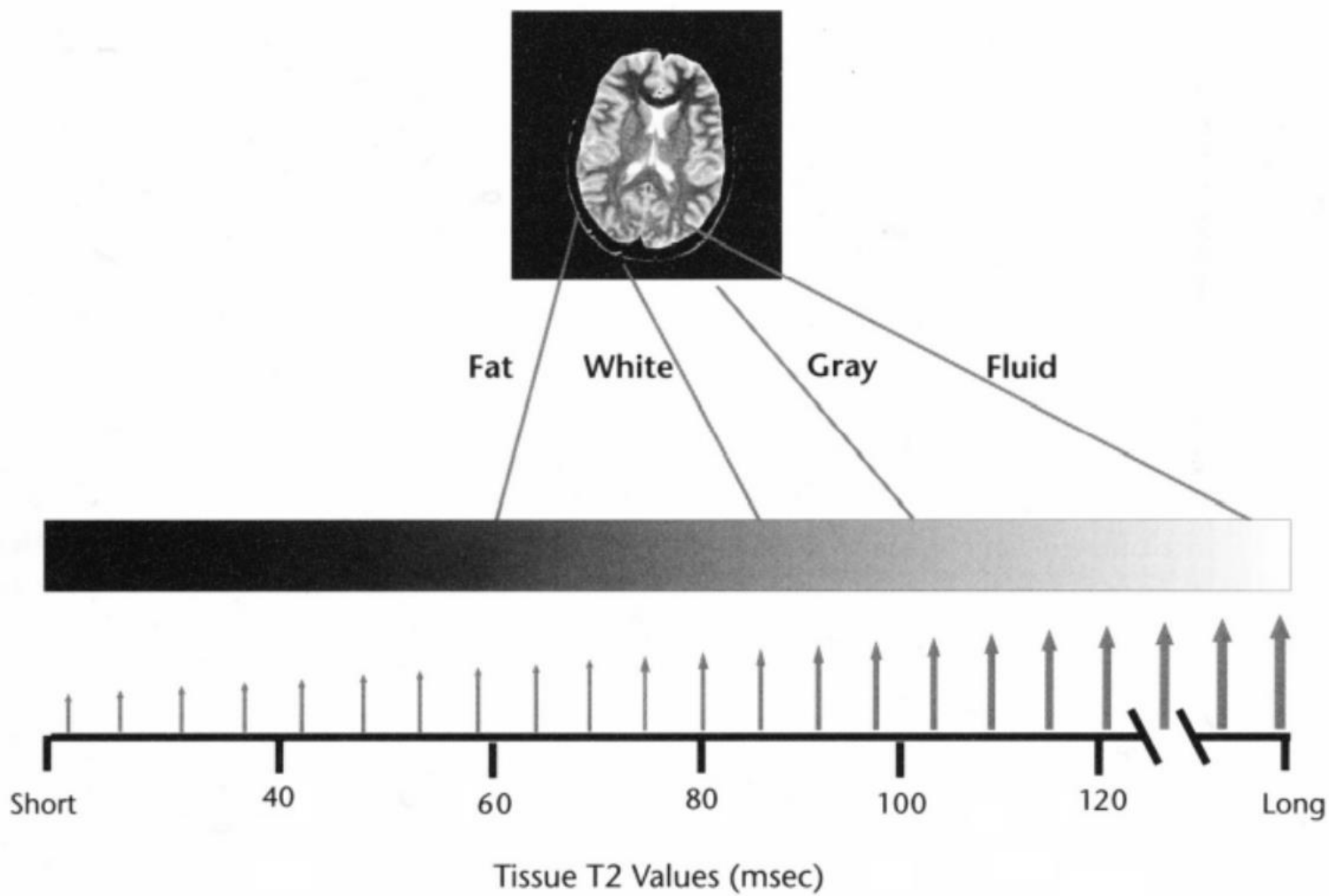
T1-WEIGHTED IMAGE



A T1 image showing the relationship of tissue brightness (signal intensity) to T1 values and level of magnetization during the longitudinal relaxation process.

Sensitive to O_2 and free radicals

T2 - image



A T2 image showing the relationship of tissue brightness (signal intensity) to T2 values

Sensitive to O_2 and free radicals

CT or MRI? ***Complementary !***

	Computerized tomography (**CT**) **scan**	Magnetic Resonance Imaging **(MRI)**
Principle of imaging	Absorbtion of X ray	Magnetic Resonance phenomen
Radiation exposure	Yes	No
Examination noise	Comparatively quiet	Noisy
Examination time	Short (5 – 10 min)	Long (15 – 30 min)
Used for	Bone fractures, tumors, cancer monitoring, finding internal bleeding	Joints, brain, wrists, ankles, breasts, heart, blood vessell

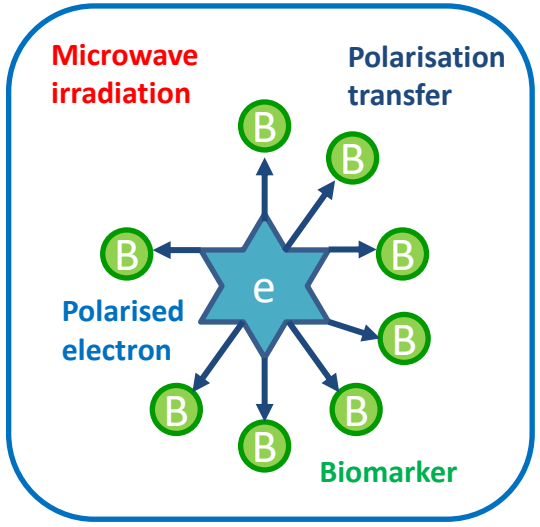
MRI main use: early diagnostic *based on function*
main shortcoming: low sensitivity

COMPLEX MIXTURES INSIDE CELLS

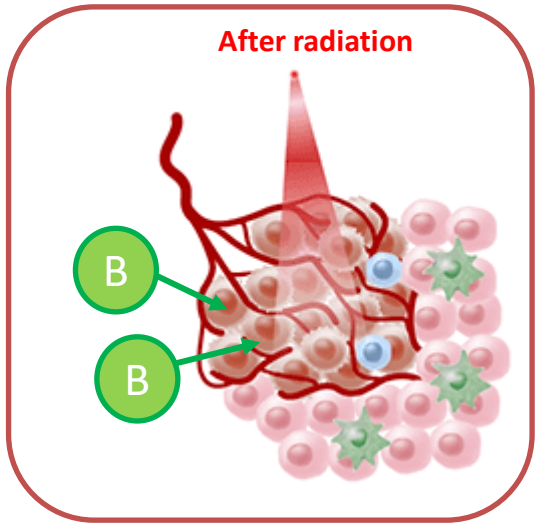
NMR instruments evolved to analyze complex samples: cells, lysates, etc.

- **Standard NMR cannot see inside cells within short times to follow radiation effects**

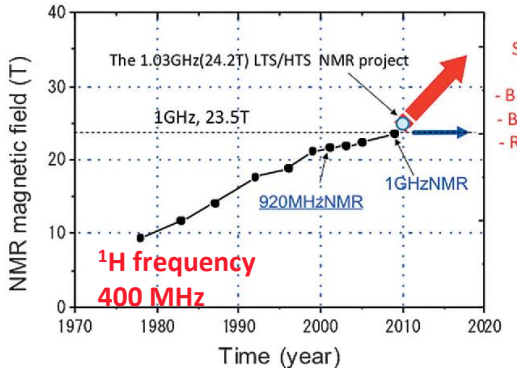
- **Solution: Dissolution Dynamic Nuclear Polarisation: signal enhancement by a factor 10'000 using e- in free radicals**



DNP polarisation transfer

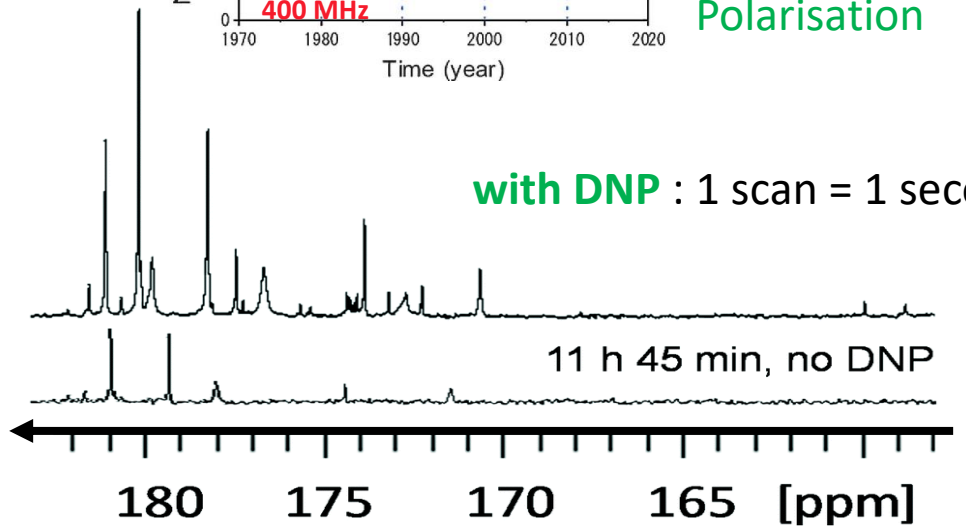


DNP polarisation transfer



State of the art:
1'000 MHz ^{1}H @ 2020

+ Dynamic Nuclear Polarisation



δ(^{13}C) ppm

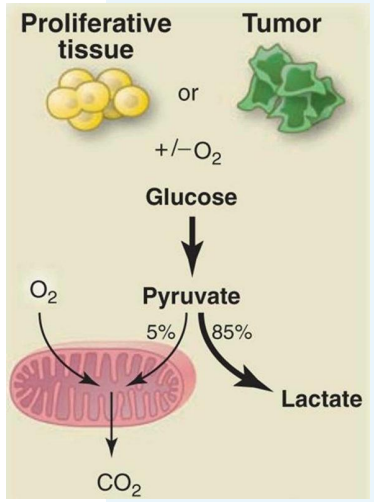
Analyst, 2020,145, 2457-2472

without DNP : 12 hours to record even at 700 MHz (proton spin resonance frequency) spectrometers

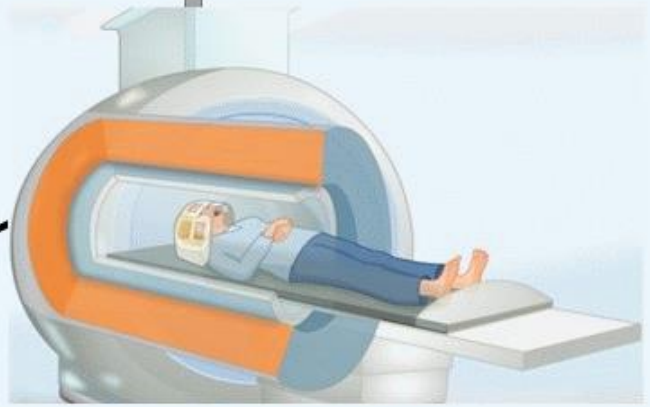
Paul Vasos

Currently employed clinical evaluation of radiation effects using hyperpolarised magnetic resonance

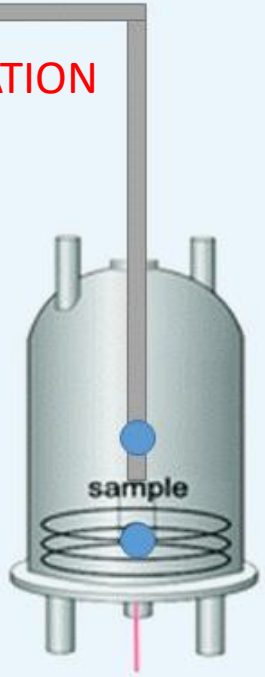
Dynamic Nuclear Polarisation (DNP) – Magnetic Resonance

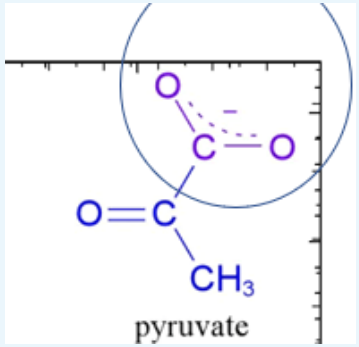


LONG LIFETIMES OF POLARISATION NEEDED !

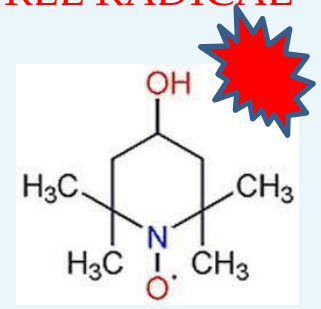


Analyse - MRI





Prepare Biomarker (hyperpolarized metabolite)
+
FREE RADICAL



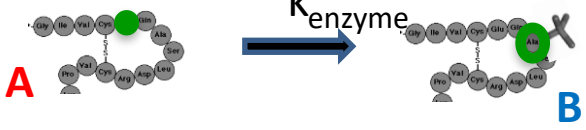
Dynamic Nuclear Polarisation ("hyperpolarized") MRI : Ardenkjaer-Larsen et al., PNAS, 2003

Ahuja, Vasos, et al., ChemComm 2010, Vasos et al., PNAS, 2009

Translation to the clinic: **early cancer diagnostic** by molecular imaging

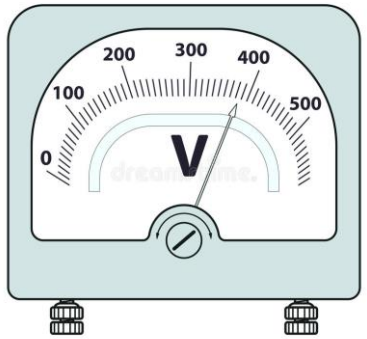
free radicals generate signal improvement

Nelson et al., Sci. Rep., 2013

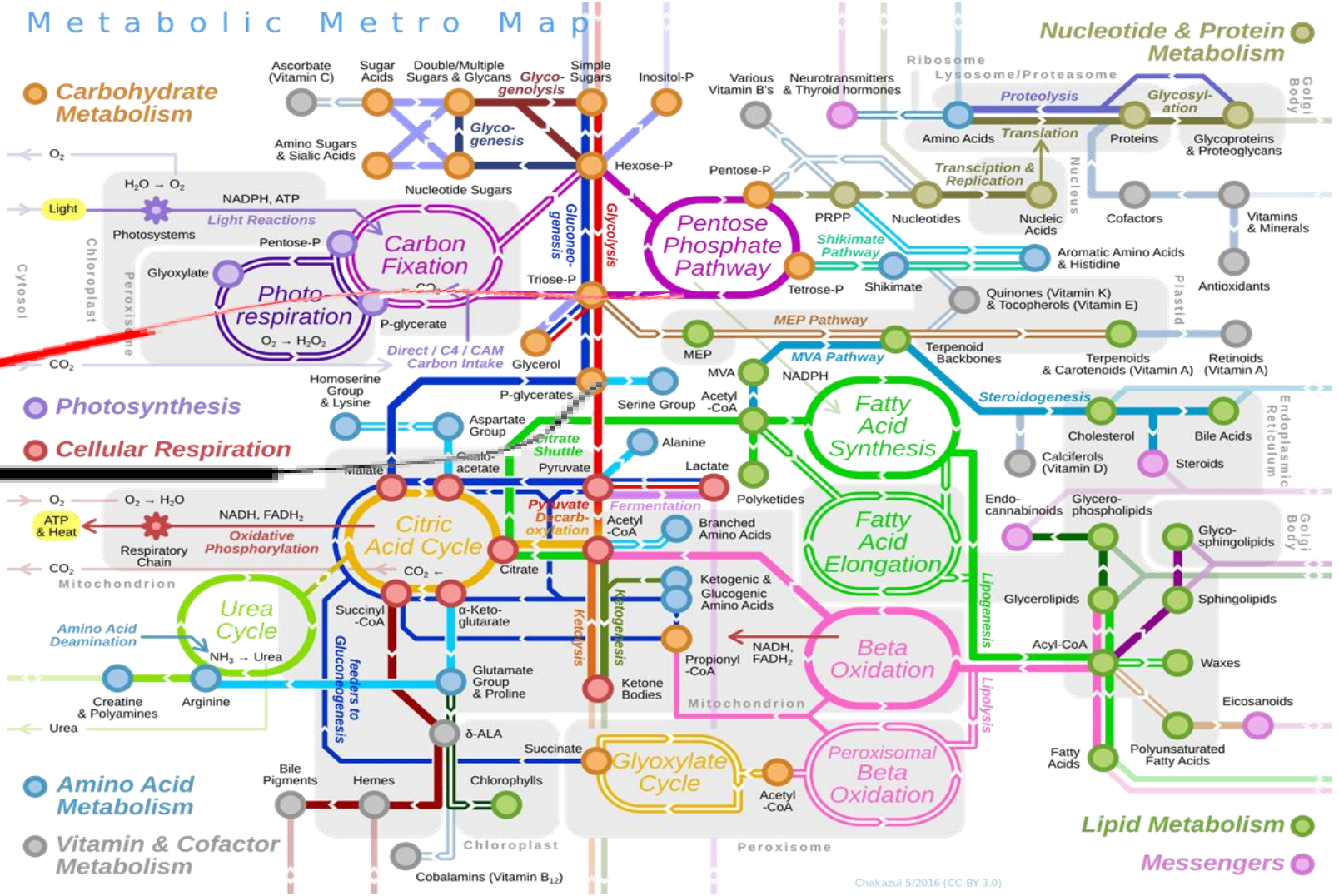


If metabolite transformations were the cell's circuitry

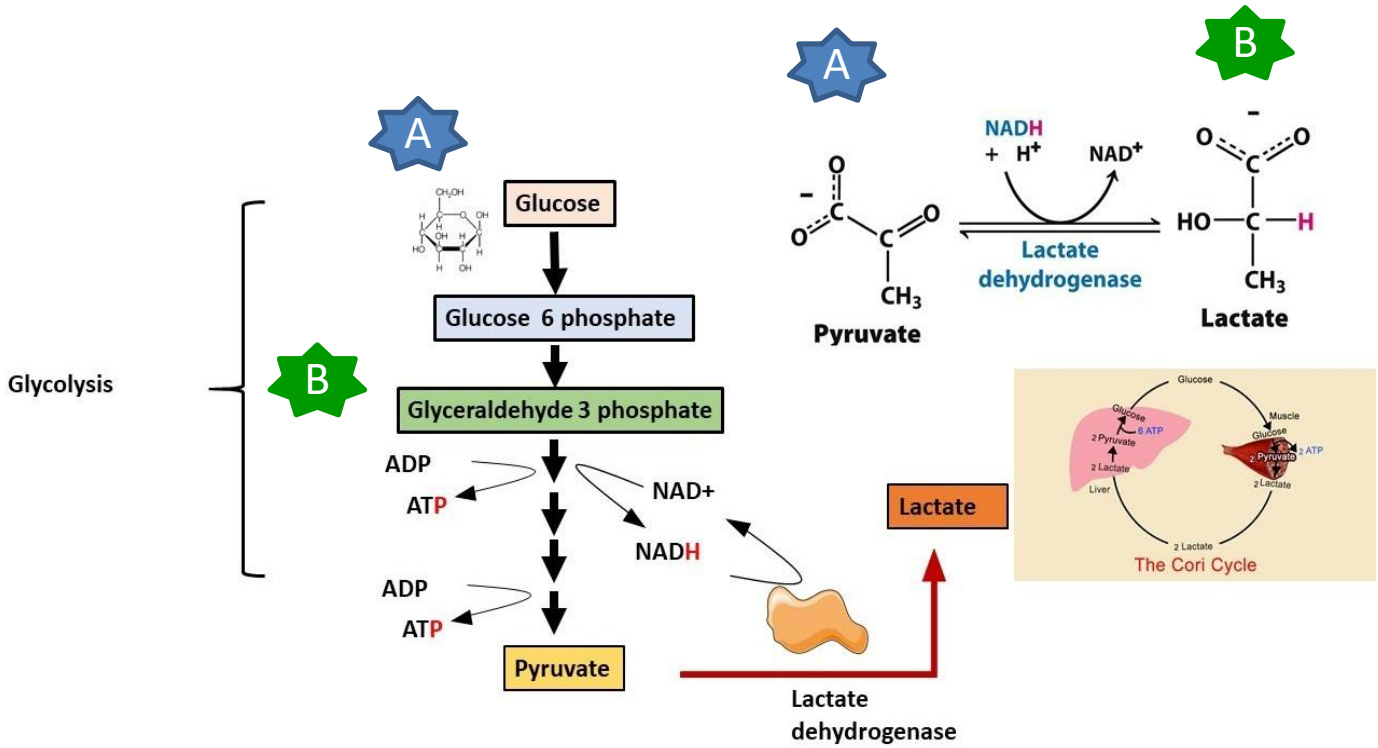
Detection of biomarkers



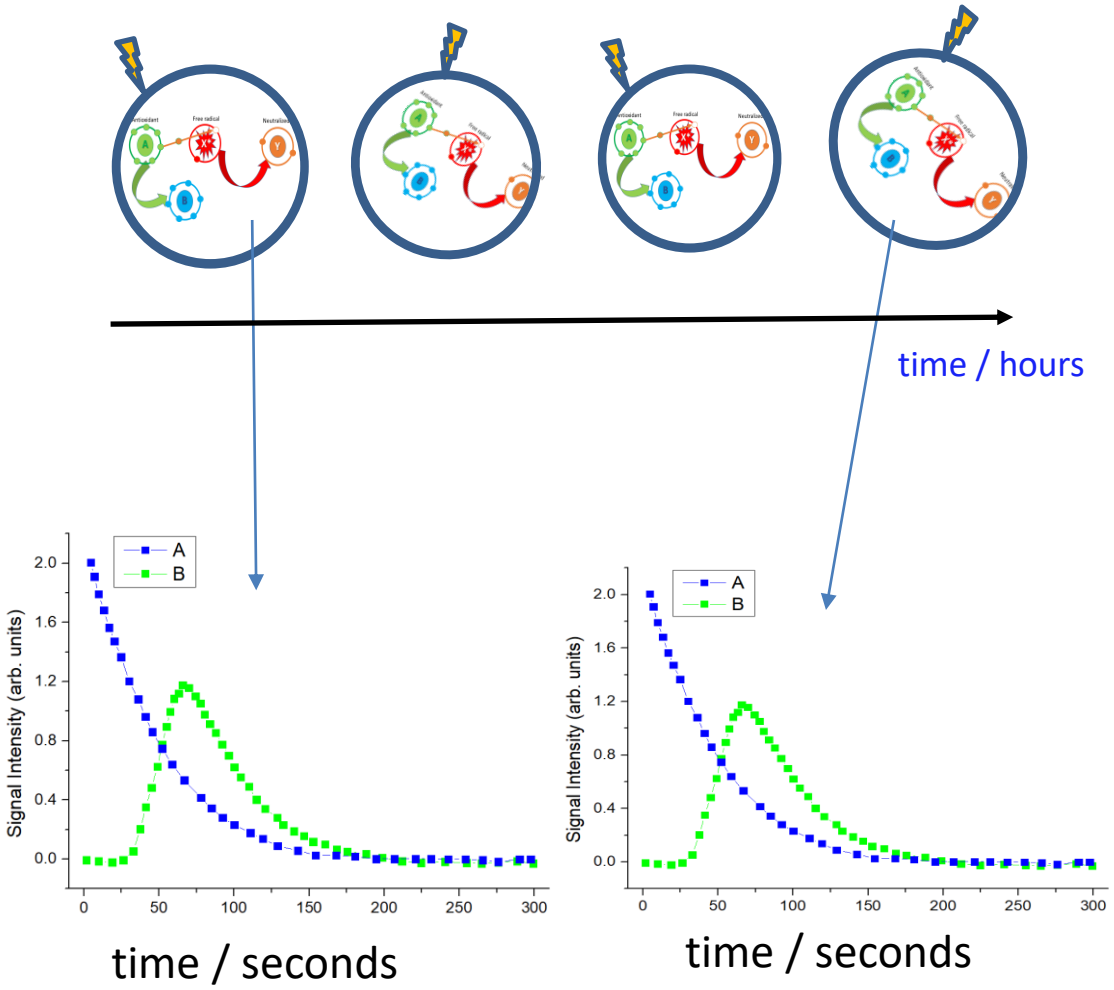
Signal = **concentration of metabolite A** / **concentration of metabolite B** (time)

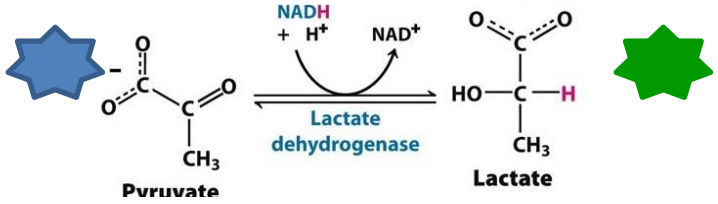


Biomarker pairs (A,B) to image radiation effects in real time: liquid-state DNP-NMR



Metabolites detected non-invasively by hyperpolarised magnetic resonance





PRECLINICAL AND CLINICAL IMAGING - Communication

Magnetic Resonance in Medicine 65:557–563 (2011)

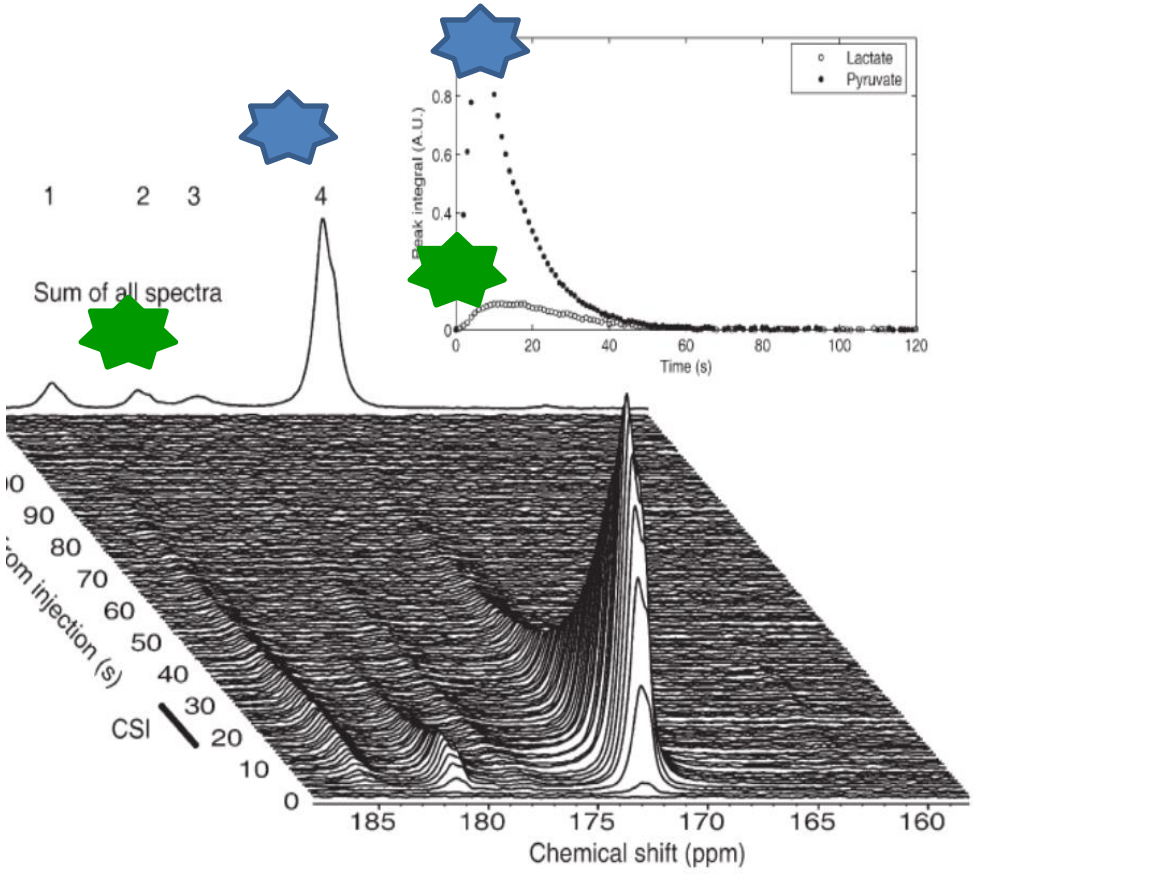
Detecting Response of Rat C6 Glioma Tumors to Radiotherapy Using Hyperpolarized [1-^{13}C]Pyruvate and ^{13}C Magnetic Resonance Spectroscopic Imaging

Sam E. Day,[1,3] Mikko I. Kettunen,[2,3] Murali Krishna Cherukuri,[4] James B. Mitchell,[4] Martin J. Lizak,[1] H. Douglas Morris,[1] Shingo Matsumoto,[4] Alan P. Koretsky,[1] and Kevin M. Brindle[2,3]*

We show here that hyperpolarized [1-^{13}C]pyruvate can be used to detect treatment response in a glioma tumor model; a tumor type where detection of response with ^{18}fluoro-2-deoxyglucose, using positron emission tomography, is limited by the high background signals from normal brain tissue. ^{13}C chemical shift images acquired following intravenous injection of hyperpolarized [1-^{13}C]pyruvate into rats with implanted C6 gliomas showed significant labeling of lactate within the tumors but comparatively low levels in surrounding brain. Labeled pyruvate was observed at high levels in blood vessels above the brain and from other major

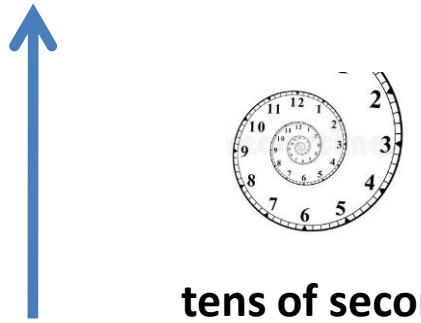
identify early treatment response (1). FDG-PET, however, is not effective in all tumor types and in brain tumors high uptake by surrounding brain tissue can mask high uptake by the tumor itself (2,3).

Glycolytic rate in human gliomas has been correlated with tumor lactate concentration, suggesting that detection of increased lactate concentration, for example, with ^{1}H MRS, might provide a similar diagnostic readout to that provided by FDG-PET in other tumor types (4,5).

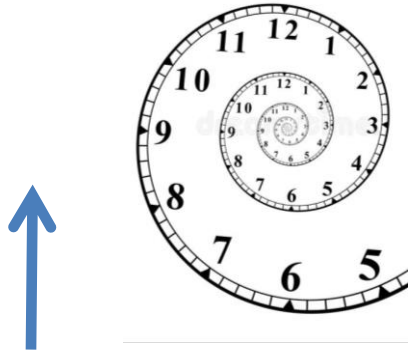


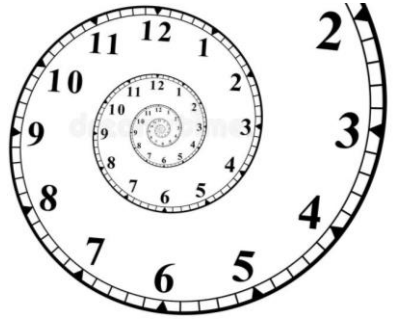
data acquired from a glioma-bearing rat. Spectra were acquired using a surface coil, following administration rized [1-^{13}C] pyruvate. The peaks in the summed spectrum are (1) lactate, 185 ppm (2) pyruvate hydrate, 181 and (4) pyruvate, 173 ppm. In some experiments a bicarbonate signal at ~162 ppm was also visible. The first

Spin memory : tiny nuclear magnets go a long way



tens of seconds





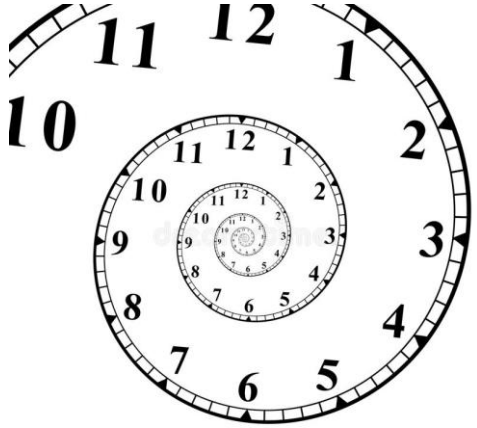
minutes

^{1}H ^{13}C ^{15}N

Magnetisation

Nuclear Singlet State

e.g. $^{1}H - ^{1}H$
$^{15}N - ^{15}N$

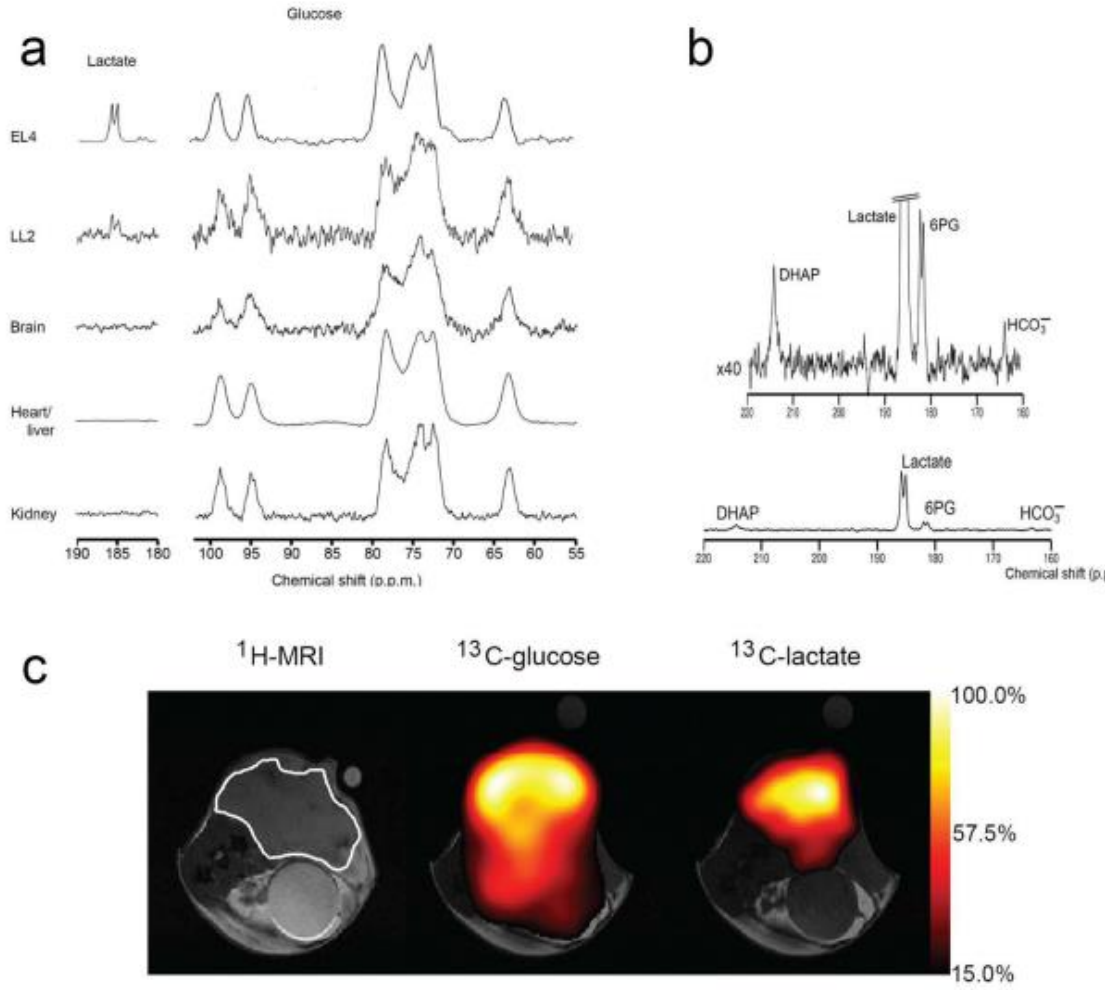


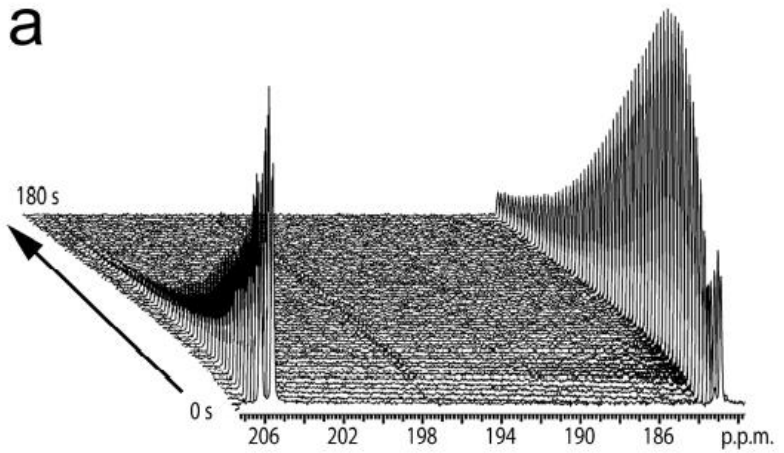
hours

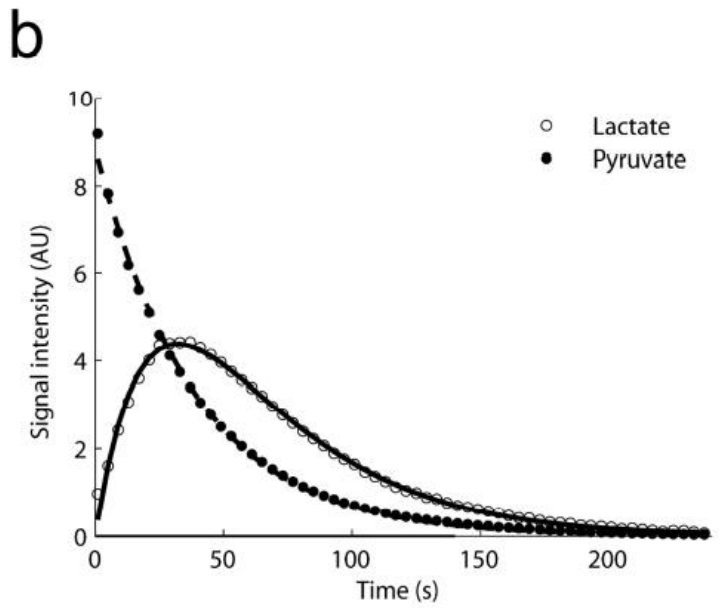
IMAGING METABOLISM WITH HYPERPOLARIZED ^{13}C-LABELED CELL SUBSTRATES

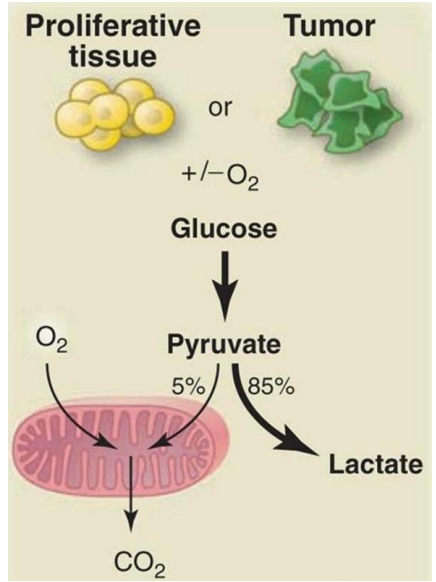
Kevin M. Brindle[1,2,3]

Glucose → Lactate & Pyruvate → Lactate biomarkers

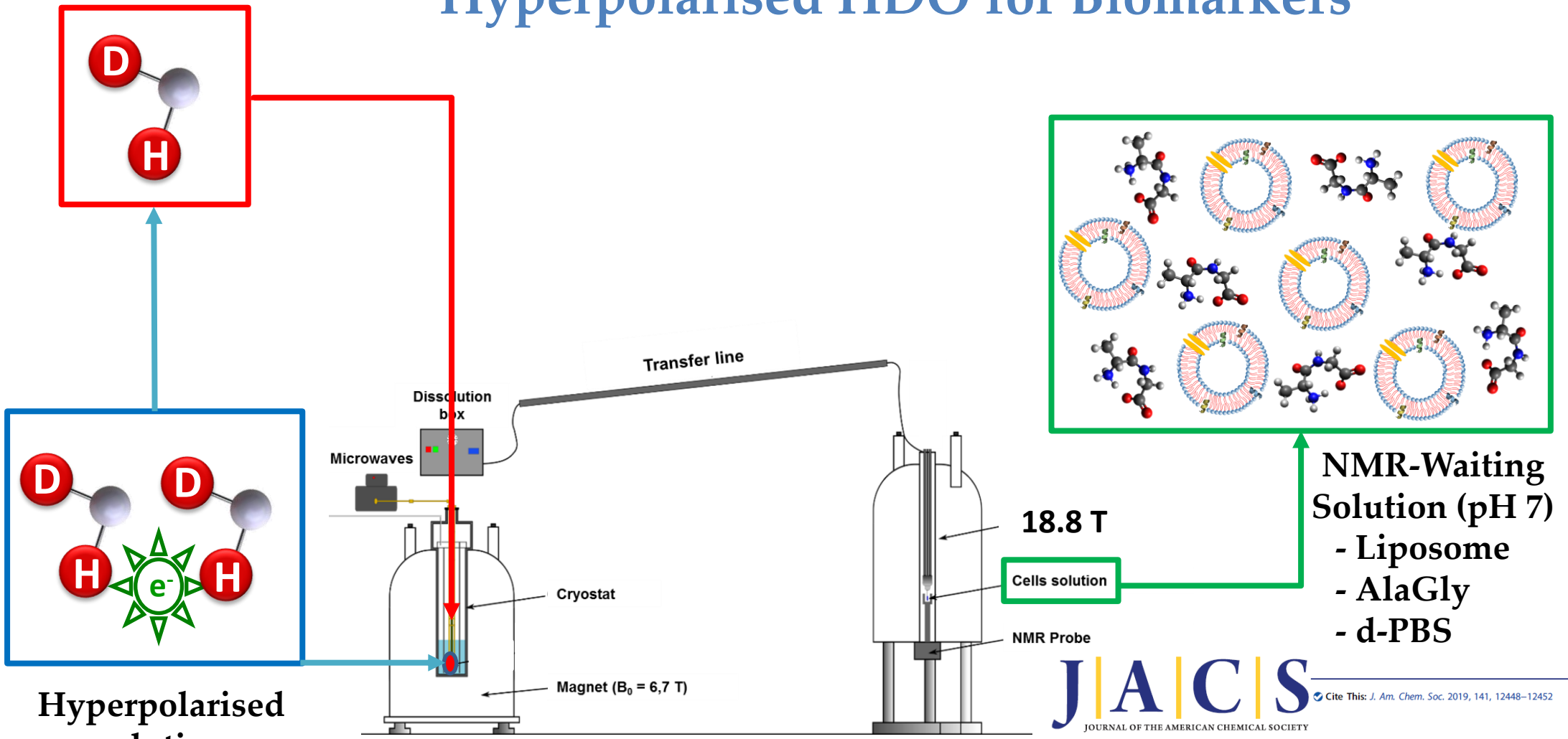








Hyperpolarised HDO for Biomarkers



Paul Vasos, Dennis Kurzbach, et al.

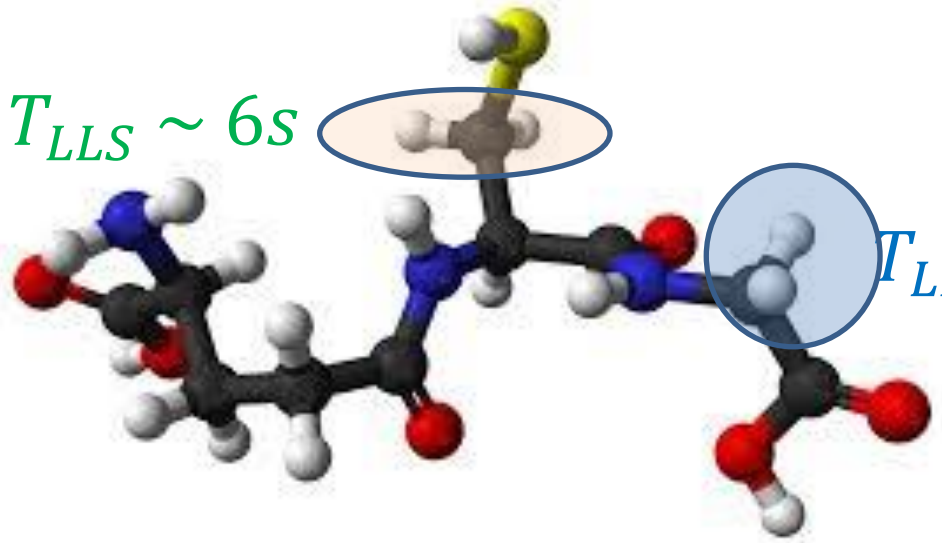
JACS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
Cite This: J. Am. Chem. Soc. 2019, 141, 12448−12452
pubs.acs.org/JACS

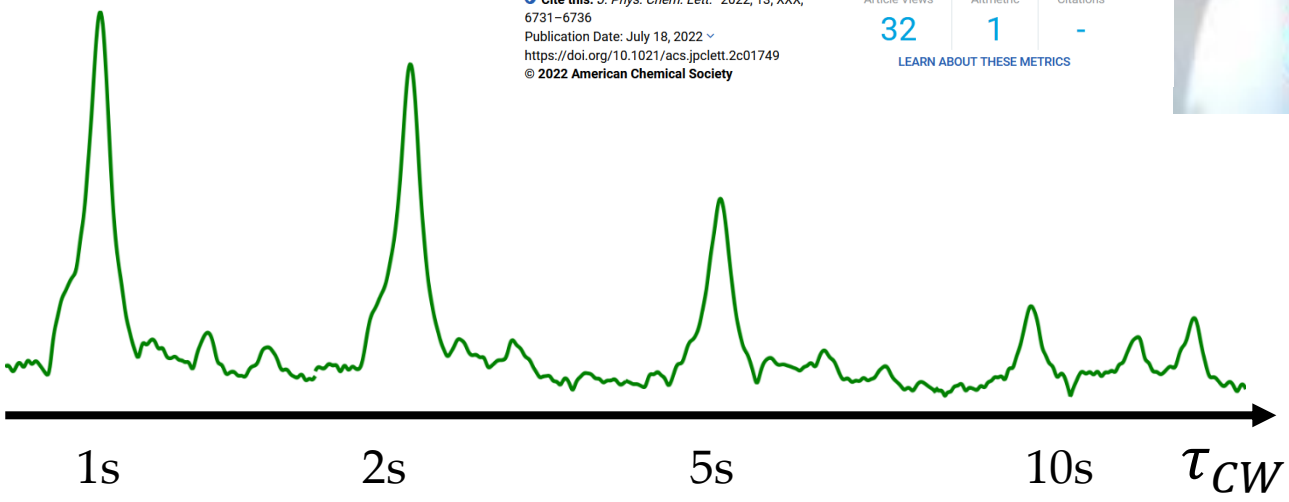
Hyperpolarized Water Enhances Two-Dimensional Proton NMR Correlations: A New Approach for Molecular Interactions

Aude Sadet,[†,#] Cristina Stavarache,[†,‡] Mihaela Bacalum,[§] Mihai Radu,[§] Geoffrey Bodenhausen,[∥] Dennis Kurzbach,[*,∥,⊥] and Paul R. Vasos[*,†,#]

Paul Vasos

Long-lived states (LLS) of ^{1}H singlets in glutathione





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Selective Excitation of Long-Lived Nuclear Spin States

Florin Teleanu, Adonis Lupulescu*, and Paul R. Vasos*

Cite this: *J. Phys. Chem. Lett.* 2022, 13, XXX, 6731−6736
Publication Date: July 18, 2022
https://doi.org/10.1021/acs.jpclett.2c01749
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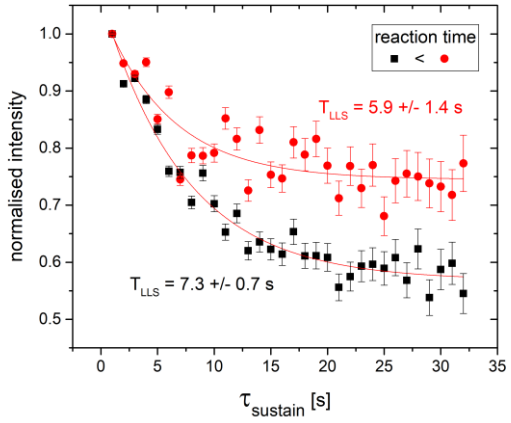
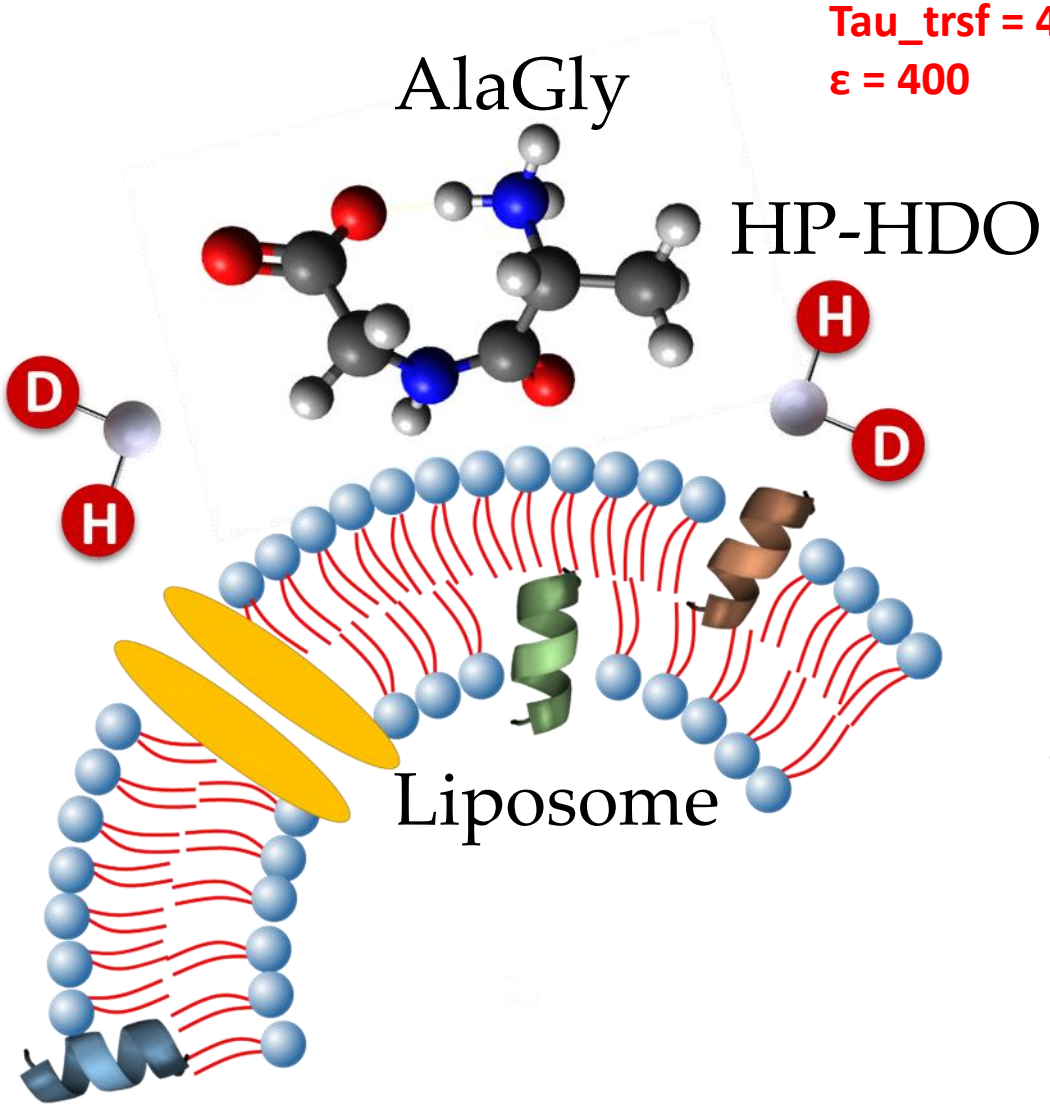
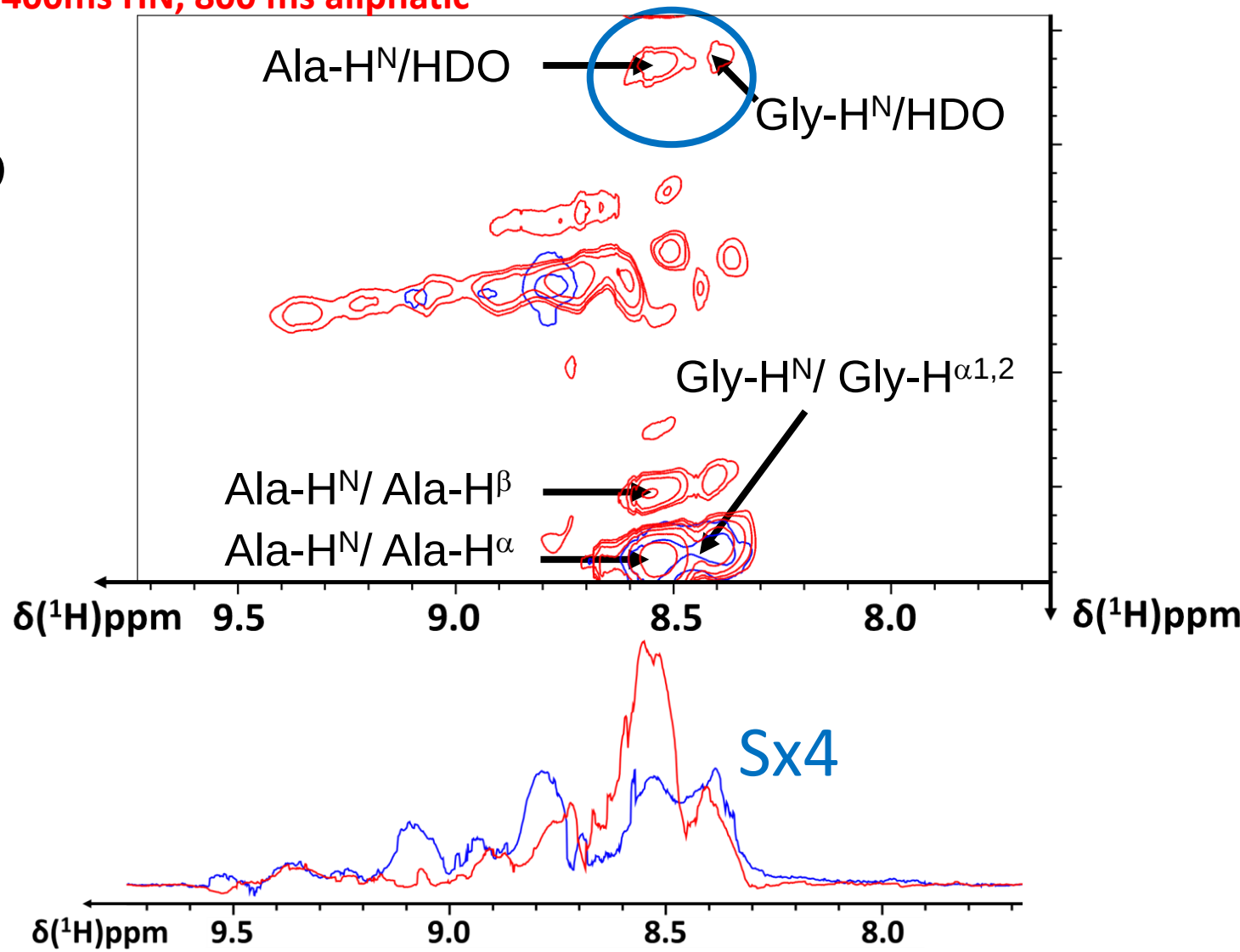
Stroboscopic detection of glutathione (GSH)



Acquisition time COSY-2D < 1 min
Tau_trsf = 400ms HN, 800 ms aliphatic
ε = 400

Time gain x 160'000





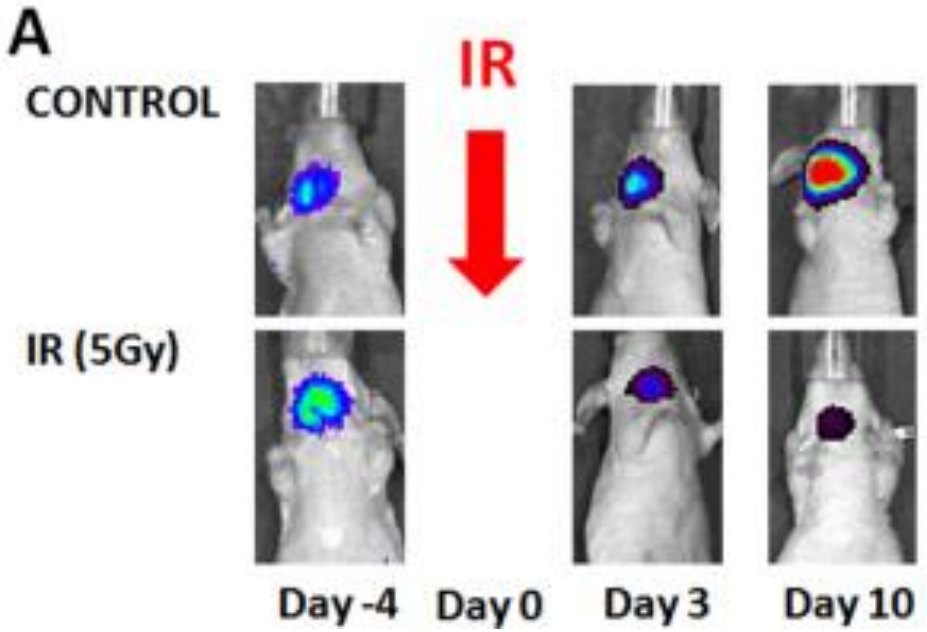
A. Sadet, al., M. Radu, D. Kurzbach, and P. Vasos,
J. Am. Chem. Soc. 2019

Paul Vasos

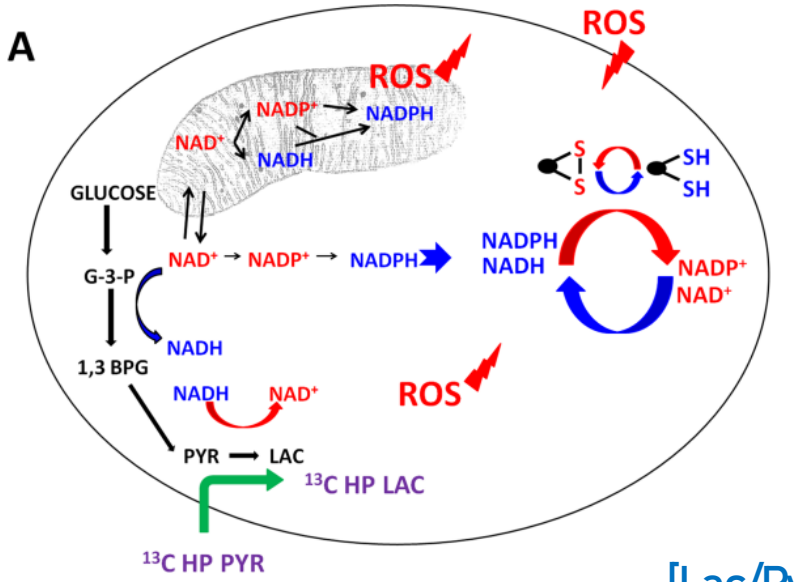
[LAC] – DYNAMIC: HYPERPOLARISED PYR → LAC

Evaluation of Hyperpolarized [1-^{13}C]-Pyruvate by Magnetic Resonance to Detect Ionizing Radiation Effects in Real Time

Vlad C. Sandulache[1,2], Yunyun Chen[2], Jaehyuk Lee[4], Ashley Rubinstein[6], Marc S. Ramirez[4], Heath D. Skinner[3], Christopher M. Walker[4], Michelle D. Williams[5], Ramesh Tailor[6], Laurence E. Court[6], James A. Bankson[4], Stephen Y. Lai[2,7]*



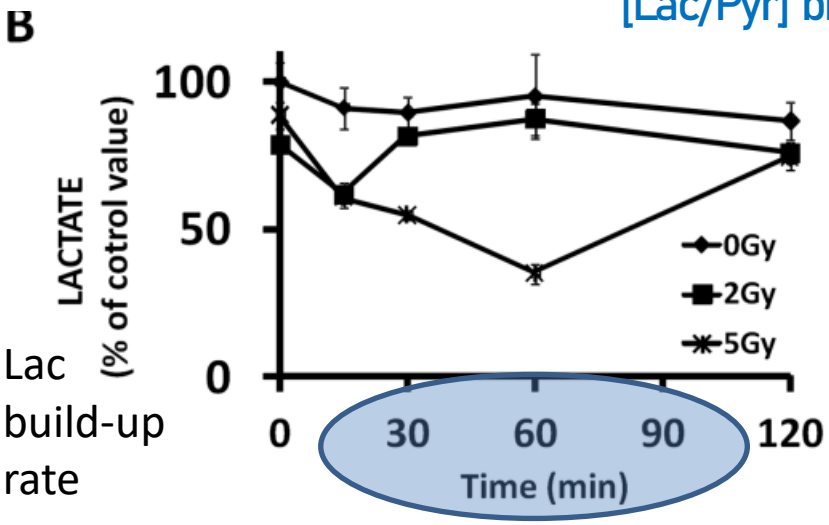
Oxidative stress: co-enzyme is depleted by **Glutamate** reactions



GSH

GS-SG

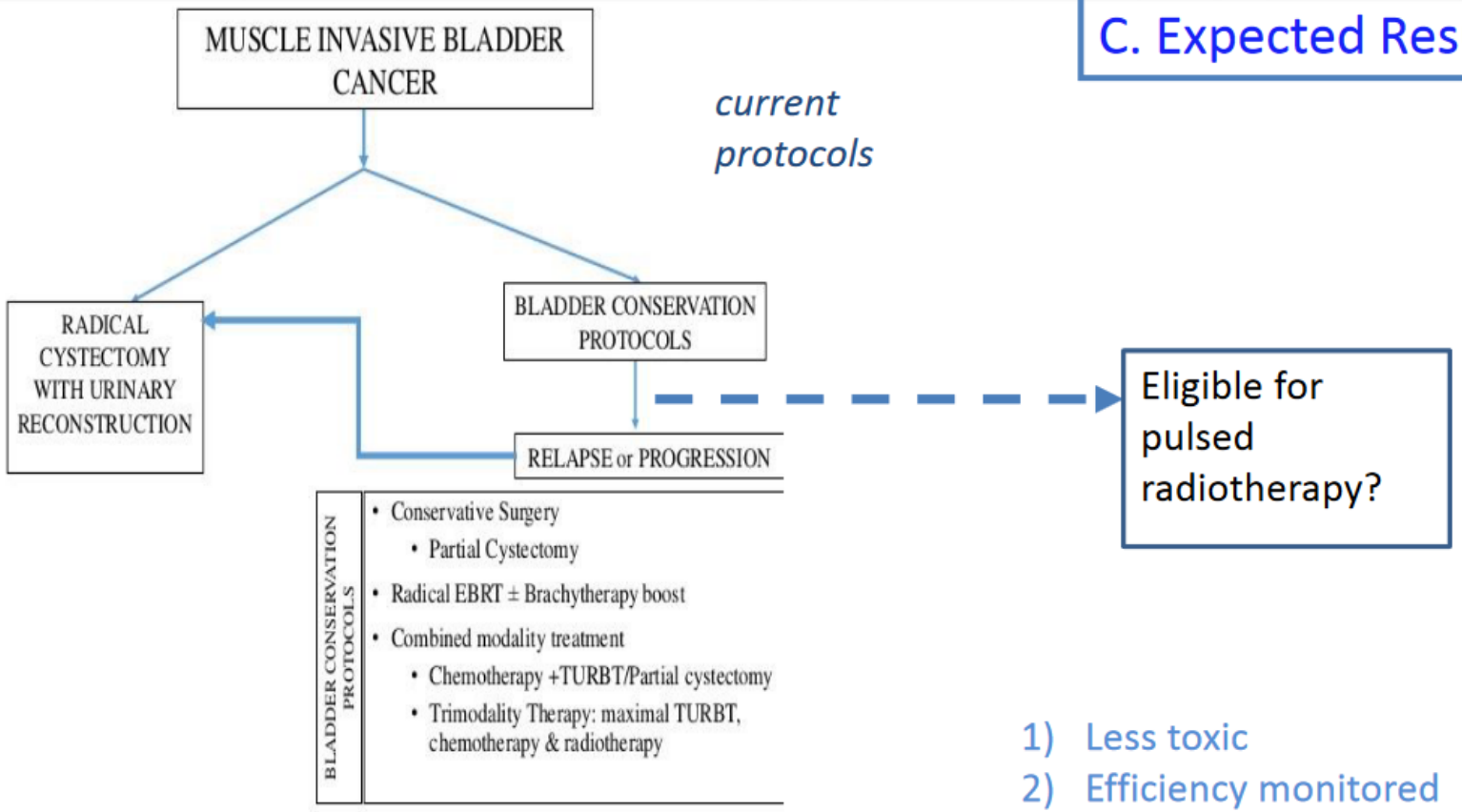
[Lac/Pyr] biomarker



Lac build-up rate

Towards pulsed radiotherapy being considered among first-line therapy options

C. Expected Results



1) Less toxic
2) Efficiency monitored

In real time

Delaying radical cystectomy for muscle-invasive bladder cancer
Chang et al., J. Urol. 2003

Current clinical practice guidelines on chemotherapy and radiotherapy for the treatment of non-metastatic muscle-invasive urothelial cancer: a systematic review and critical evaluation by the Hellenic Genito-Urinary Cancer Group (HGUCG).
Review article Zagouri F. et al. Crit Rev Oncol Hematol. 2015

3) Metabolomics

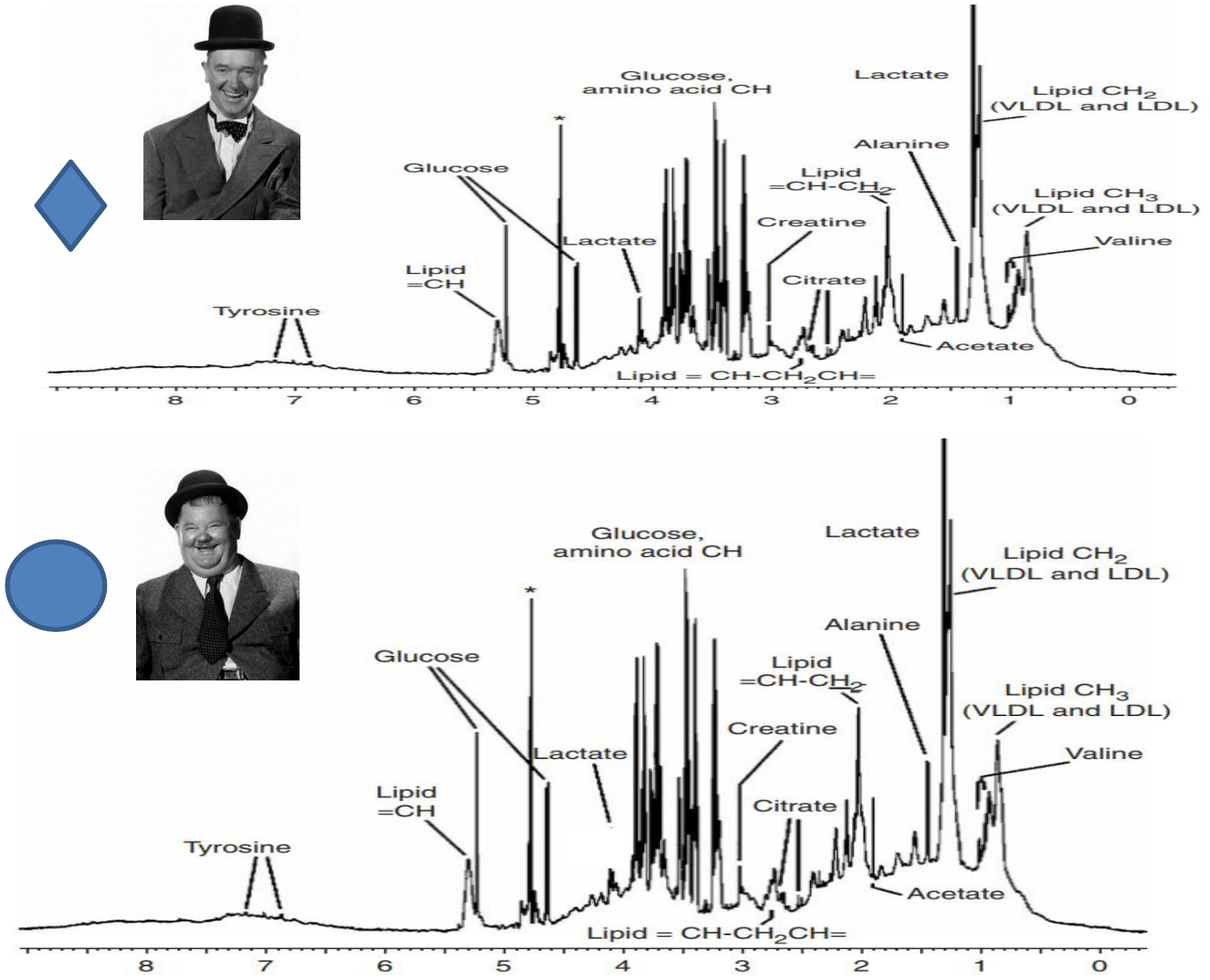
NMR spectrum of blood serum or cell lysate sample.

The spectrum shows signals of low molecular weight metabolites.

Some larger molecules such as lipoproteins, which feature broader signals, show up as well.

Concerted signal variations in several metabolites indicate effects of chemotherapy, radiotherapy, etc.

When such concerted spectral intensity effects are measured (at consistent chemical shifts) for samples from a majority individuals undergoing a treatment, an outlier sample indicates personalized variations in treatment response (and that an alternate treatment may be required for the individual).



Beckonert, O., Keun, H., Ebbels, T. et al.
Nat Protoc 2, 2692–2703 (2007)

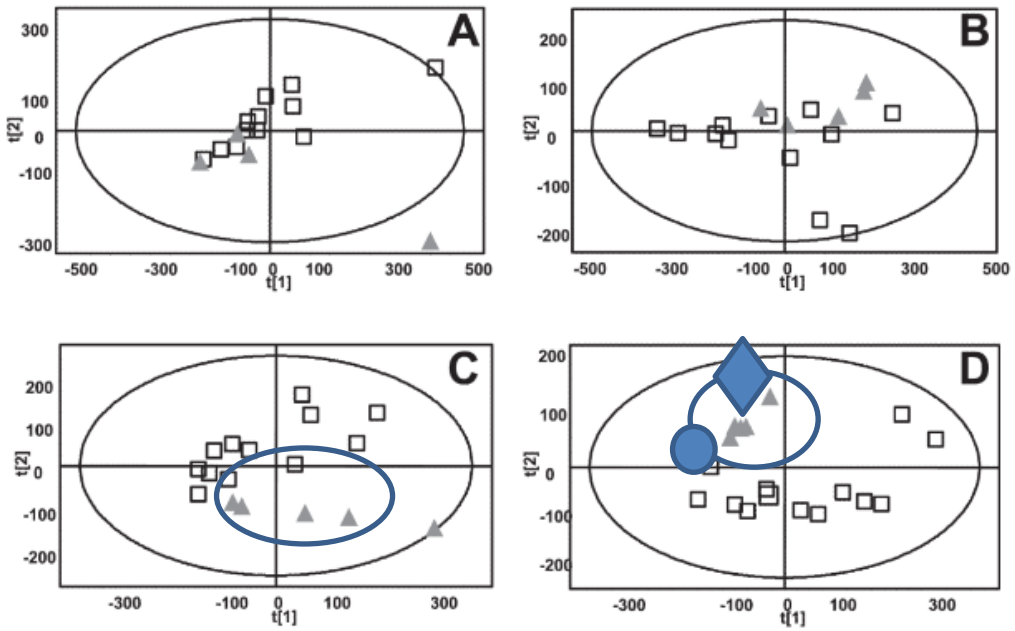
Personalised treatment RADIOMICS

Probes from subjects treated with irradiation

Probes from untreated subjects

1 Gy

6.5 Gy

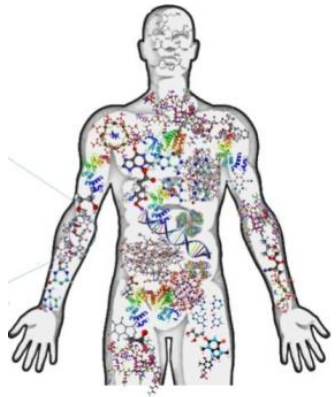


3.5 Gy

Radiotherapy likely to be toxic

Radiotherapy likely to be less toxic

8.5 Gy



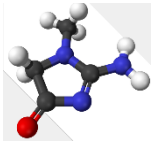
NMR metabolomics → biomarkers

Dose-rate effects yet to be explored by the same methods as the dose effect investigations

Johnson et al., Radiat Res 2012 ; 178(4): 328–340

Perspective: Identify personalized biomarkers transformations in cells

Biomarkers = metabolites concentrations



Biomarker [A] A

radiation

Patient I

B **Biomarker [B]**

Biomarker [A] A

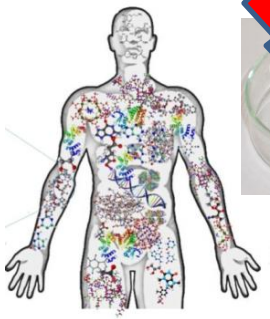
radiation

Patient II

Biomarker [C]

Biomarker

radiation



Use tissue probes

[?]

Testing Enzyme Function with Biomarkers

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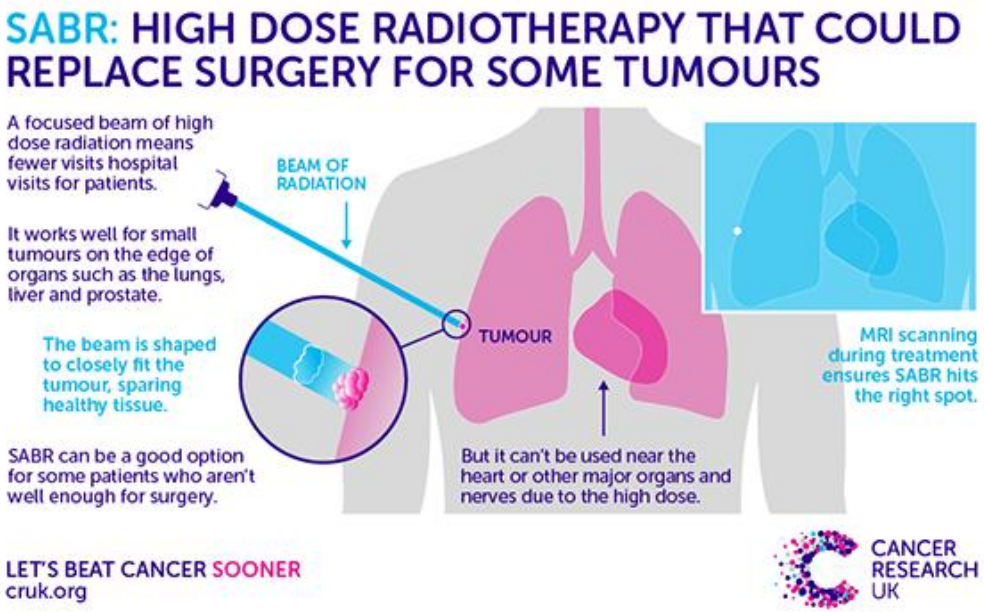
CCR Translations

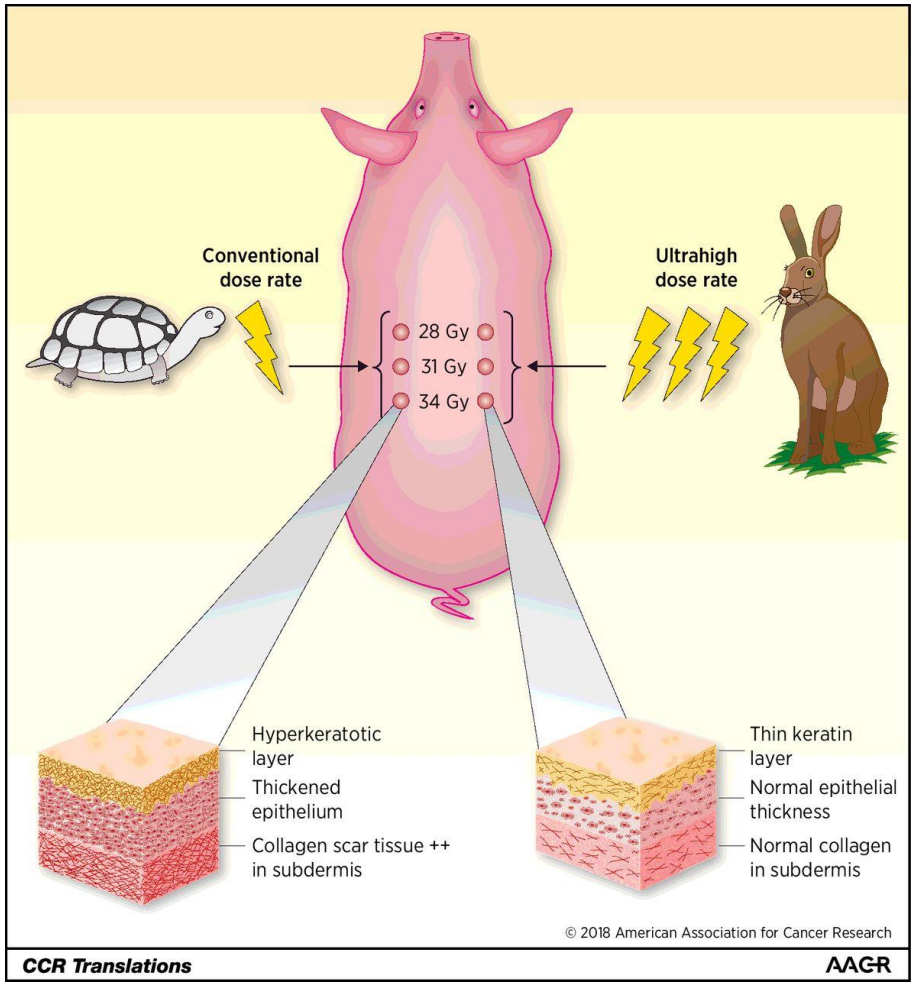
Ultrahigh Dose-rate Radiotherapy: Next Steps for FLASH-RT

Kevin J. Harrington

DOI: 10.1158/1078-0432.CCR-18-1796 Published January 2019 Check for updates



FAST MOLECULAR DIAGNOSTIC!



Home message

Experiments by which dose-rate effects are quantified:

- **Electron spin magnetic resonance (ESR) to detect free radicals**
  - **(using 'spin traps')**
- Radiomics (metabolomics in a radiobiology context):

  Detected Choline, Creatine, Lactate, Glutathione
- Metabolic conversion rates

- High dose-rate radiation (Gy/ns) driven by laser accelerators generates less radiation-related reactive species (toxicity) compared to low dose-rate radiation in abalysedglioblastoma cells

- **Magnetic resonance is able to pinpoint the metabolic signature of radiation protocol effectiveness and toxicity (via radiomics)**
- **and use this signature to follow treatment effects within hours**

Towards clinics:

- **Monitor toxicity and efficiency / cancer type**
- **Personalize (in the context of adjuvant therapies)**

Network & joint projects
UEFISCDI PED242, ELI-RO, PCE 545
Publications (program in Med. Phys. 2019)

ELI-NP LGED
& Biophysics and biomedical applications Team & lab

ELI-NP LDED

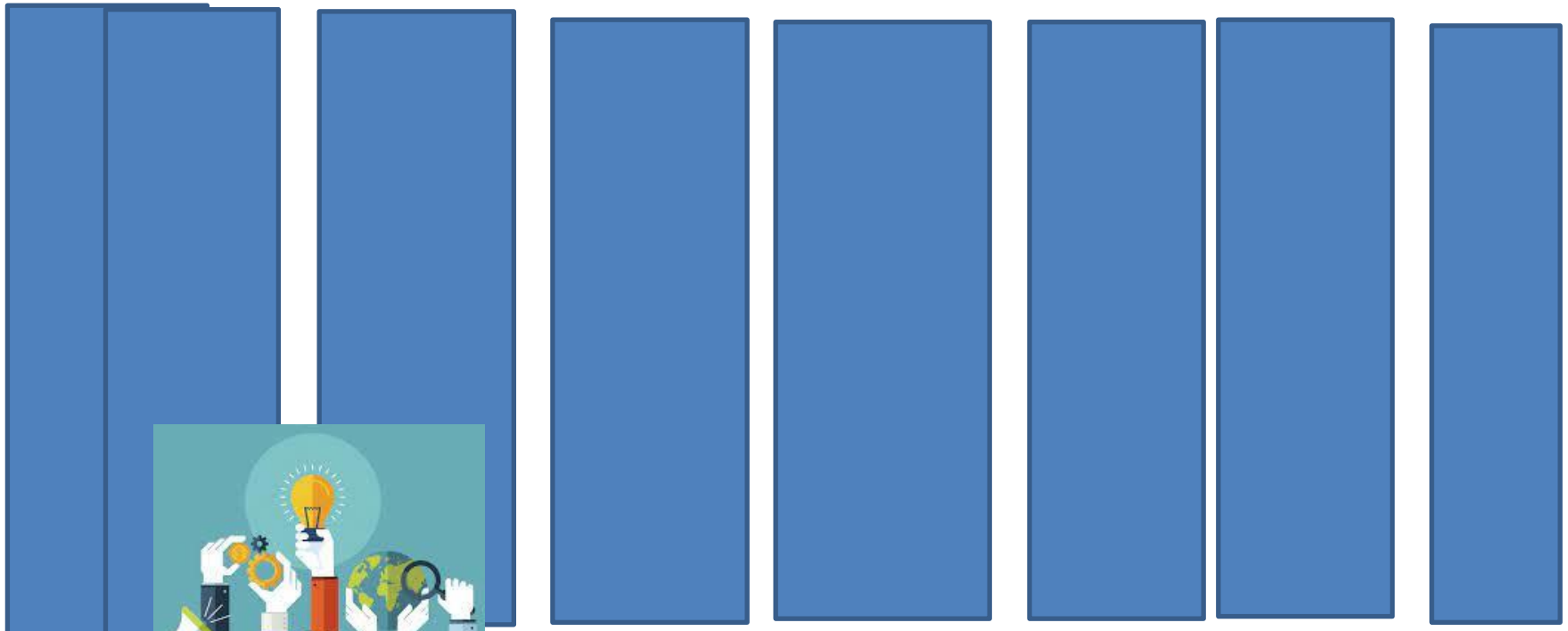
Optics & LSD

IFIN-HH

Amethyst Radiotherapy

Carol Davila Hospital

"Victor Babes" Research Inst for Pathology



Max Tishler,
Director and President of Merck Sharp
(synthesis of Cortisone, vitamin B12,
Streptomycin, penicillin, nicotinamide NAD)

National Medal for Science 1987

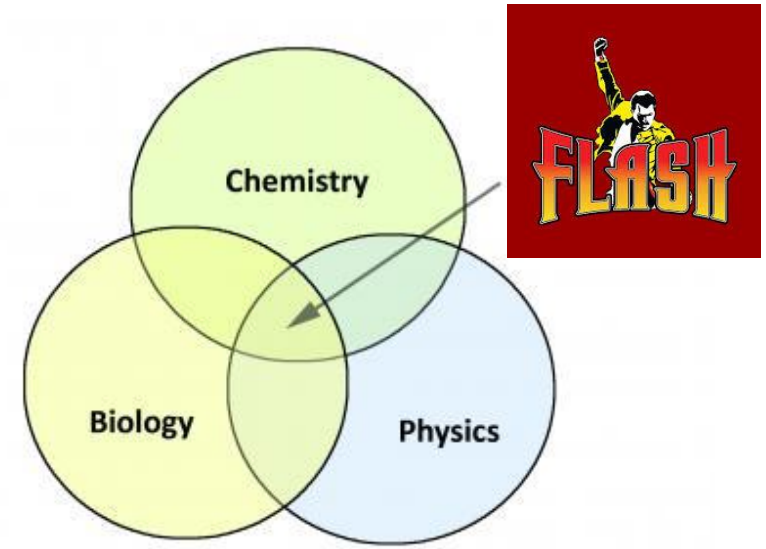
<<the national interest is best served if we never forget that directing science has not been and never will be as effective as letting it develop freely>>

The response of normal and tumor cells to ultra-high dose-rate radiation – a network biology approach

Conclusions and Perspectives

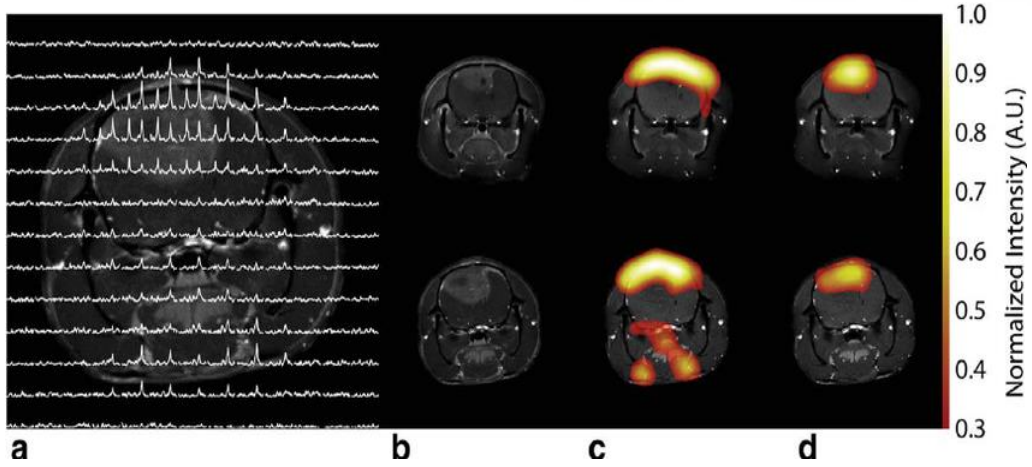
- Free-radical markers of FLASH radiation
- Metabolic markers: hyperpolarised metabolites

timely indicators of radiobiological effects



***In-vivo* detection of oxidative stress**

Early detection of free radicals and metabolites by hyperpolarised MRI



M. Rosen et al., NMR Biomed, 20018

LGED-Biophysics

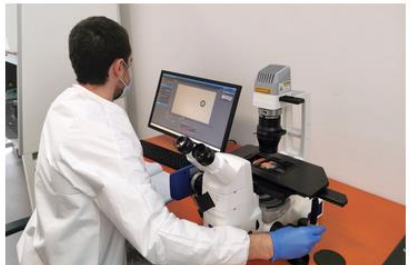
ELI-NP

Biophysics and Biomedical Applications

Laser System Department, ELI-NP	joint project
Victor Babes Institute	joint project & publications
Amethyst Radiotherapy	joint project & publications
CRUK Cambridge Institute	joint patents & publications
University of Vienna	joint paper

Eppur si muove!

On-going Research Highlighted Papers Laboratory



Projects (accepted projects)

+ UEFISCDI PED 545 /2021 with Amethyst Radiotherapy

- PN-III-P4-ID-PCE2020-2642 "Water hyperpolarization for radiation biomarker detection"
- ELI_09/01.10.2020 "CELLI - Advanced biological methods for the detection of normal and pre-leukemic cells' response after FLASH irradiation at ELI-NP"
- PN-III-P2-2.1-PED-2019-4212 "Molecular responses of irradiated cells with laser-generated particle beams at different doses and dose-rates"
- SGS-ERC-RO-NO-2019-0010 "Preliminary experiments for defining hyperpolarised magnetic resonance in radiobiology" collaboration with University of Cambridge
- PN-III-P1-1.1-PD2019-0778 "Propolis extracts effects on biomimetic lipid membranes" (PI B. Zorila, Supervision P. Vasos)

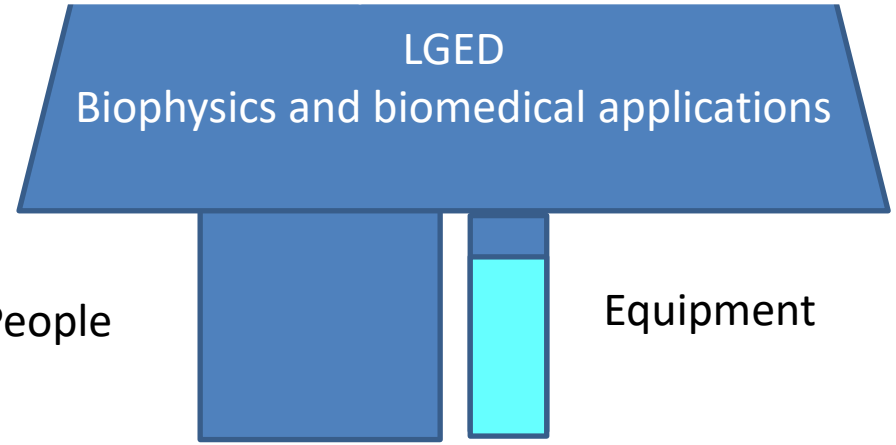
MEDICAL PHYSICS
The International Journal of Medical Physics Research and Practice

Review Article | Open Access

Laser-driven radiation: Biomarkers for molecular imaging of high dose-rate effects

Theodor Asavei, Mariana Bobeica, Viorel Nastasa, Gina Manda, Florin Naftanaila, Ovidiu Bratu, Dan Mischianu, Mihail O. Cernaianu, Petru Ghenuche, Diana Savu, Dan Stutman, Kazuo A. Tanaka, Mihai Radu, Domenico Doria, Paul R. Vasos ... See fewer authors

First published: 29 July 2019 | https://doi.org/10.1002/mp.13741



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ELI-NP

Biophysics and Biomedical Applications

Teleanu, Lupulescu*, and Vasos*, JPCL 2022

Eppur si muove!

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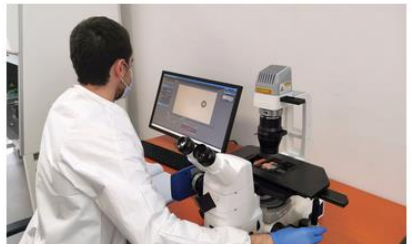

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Structural Instruments 2014-2020

Project co-financed by the European Regional Development Fund through the Competitiveness Operational Programme "Investing in Sustainable Development"

MEDICAL PHYSICS

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Hyperpolarized Water Enhances Two-Dimensional Proton NMR Correlations: A New Approach for Molecular Interactions

Aude Sadet, Cristina Stavarache, Mihaela Bacalum, Mihai Radu, Geoffrey Bodenhausen, Dennis Kurzbach*, and Paul R. Vasos*

Cite this: *J. Am. Chem. Soc.* 2019, 141, 32, 12448−12452
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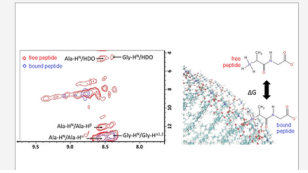
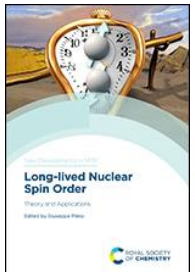
SUBJECTS: Redox reactions, Vesicles, Peptides and proteins, Magnetic properties, Polarization

Abstract

Protein and peptide interactions are characterized in the liquid state by multidimensional NMR spectroscopy experiments, which can take hours to record. We show that starting from hyperpolarized HDO, two-dimensional (2D) proton correlation maps of a peptide, either free in solution or interacting with liposomes, can be acquired in less than 60 s. In standard 2D NMR spectroscopy without hyperpolarization, the acquisition time required for similar spectral correlations is on the order of hours. This hyperpolarized experiment enables the identification of amino acids featuring solvent-interacting hydrogens and provides fast spectroscopic analysis of peptide conformers. Sensitivity-enhanced 2D proton correlation spectroscopy is a useful and straightforward tool for biochemistry and structural biology, as it does not recur to nitrogen-15 or carbon-13 isotope enrichment.





SCIENTIFIC REPORTS
nature research

Water hydrogen uptake in biomolecules detected via nuclear magnetic phosphorescence

Aude Sadet, Cristina Stavarache, Florin Teleanu & Paul R. Vasos

From the journal: Faraday Discussions

Hyperpolarised NMR to follow water proton transport through membrane channels *via* exchange with biomolecules

Viorel Nastasa, Cristina Stavarache, Anamaria Hanganu, Adina Coroaba, Alina Nicolescu, Calin Deleanu, Aude Sadet and Paul R. Vasos