BioScope Labs

A peek into *BioScope* Labs Consortium

"If free radicals are involved, EPR resolves"

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BioScope Labs

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Who are we?

University of Belgrade

Faculty of Physical Chemistry

www.ffh.bg.ac.rs

Part of the University of Belgrade

116 – years old tradition

BSc | MSc | PhD | studies
Students (500) | Teaching staff (40)

The influence on the Shanghai position of the University of Belgrade

According to the analysis of the impact of individual faculties on the achieved Shanghai position using the "PROMETHEE-GAIA" method, the Faculty of Physical Chemistry is on the first place according to the criteria: the number of citations per researcher, the number of citations and the number of papers per researcher, as well as in the final ranking by the PROMETHEE II method

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What is BioScope?

Laboratory for magnetic resonance

EPR
MRI
NMR

Laboratory for molecular spectrometry

UV/VIS
FTIR
RAMAN

Laboratory for nuclear spectrometry

α
β
γ

Laboratory for computational chemistry

GAUSSIAN
MCTDH
QTAIM

Laboratory for nonlinear dynamics

Oscillatory reactions
Modelling

Laboratory for bioelectrochemistry

Biosensors
Biomaterials
Redox lab

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What is EPR?

- **EPR** is a magnetic resonance technique that detects unpaired electrons in paramagnetic substances.
- Unpaired electrons occur in **free radicals** and many transition metals.
- Radicals can provoke damage to DNA, proteins, and cell membranes, and are related to many pathophysiological conditions.
- EPR is the **only technique** that unambiguously detects free radicals.
- EPR has number of applications:

- Medicine
- Pharmacy
- Biology
- Agriculture
- Food science
- Physiology
- Materials science
- Physics
- Chemistry
- Biochemistry

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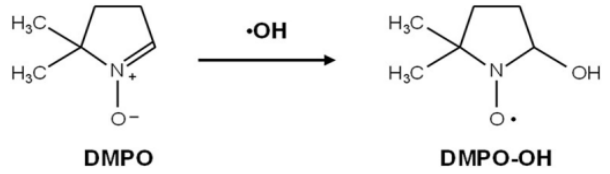
EPR methods

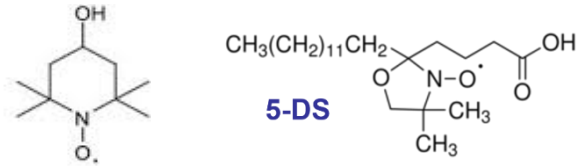
EPR spin-trapping

- Detecting short-lived radicals $^{\bullet}OH$, $^{\bullet}O_2^-$, $NO^{\bullet}$ … (oxidative stress, sepsis …)
- We always search for new ST formulations (e.g. for *in vivo* applications)



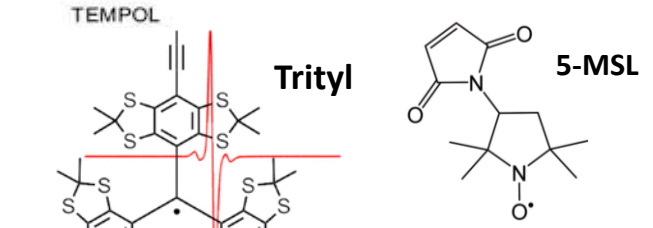
EPR spin-probing

- Detecting oxidative status (*in vitro/ex vivo/in vivo*)
- We use number of different spin-probes (cell membrane or BBB (im)permeable)



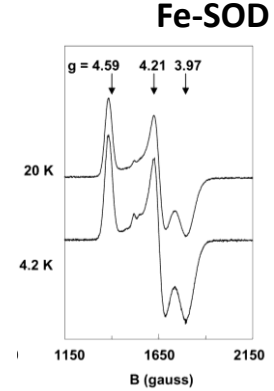
EPR spin-labeling

- Labeling proteins (to investigate conformational changes ...)
- Labeling membranes (cell membranes, liposomes ...)



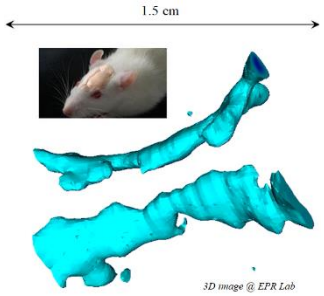
EPR oximetry

- To detect pO_2 *ex vivo/in vivo* (ischemia, post-radiation de&reoxigenation …)
- We use specialized spin-probes (LiPC, Trityl …)



EPR imaging

- 2D/3D/4D spectral-spatial distribution of oxidative status (*in vivo/ex vivo*)
- We use different spin-probes to localize image



EPR of metalloproteins

- Low T measurements (4K-77K) to detect oxidation state, ligands, coordination, intra & intermolecular interactions …

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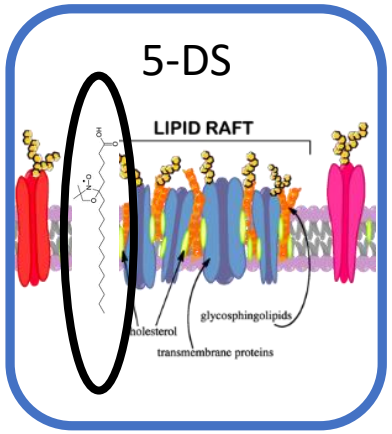
EPR in medicine

- **Selection of our current projects:**

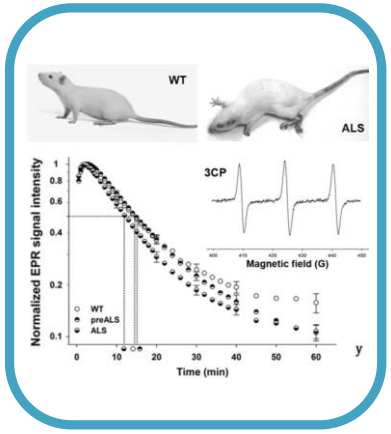
Biomarkers for Gaucher disease patients

- EPR spin-labeling of PBMC and erythrocytes membranes
- Therapy follow up



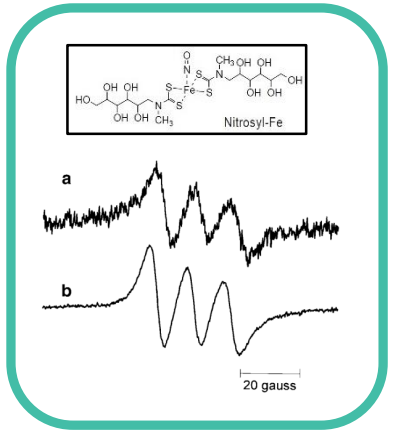
Investigating mechanisms in ALS & AD model

- Detecting BBB permeability *in vivo*
- EPR of metalloproteins *ex vivo*



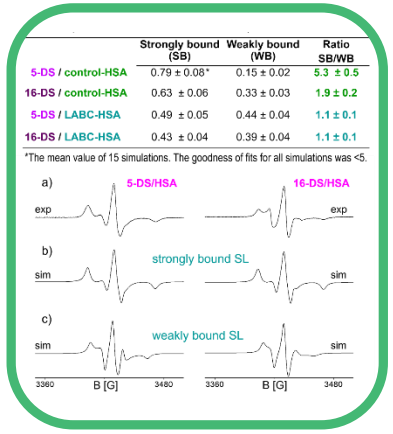
Detection of NO• in LPS induced septic shock

- NO• spin trapping *in vivo & ex vivo*
- NO• spin-adducts as MRI contrast agents



Spin-labeling of HSA as a biomarker for LABC

- Using spin-probes
- Calculating SB/WB ratio of EPR spectra components

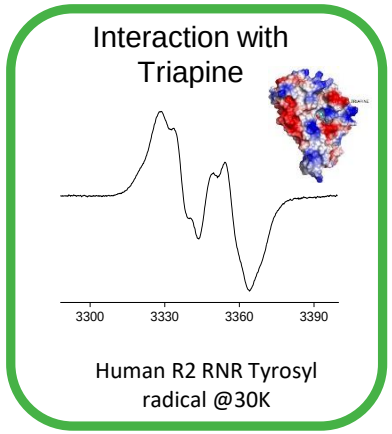


	Strongly bound (SB)	Weakly bound (WB)	Ratio SB/WB
5-DS / control-HSA	0.79 ± 0.08*	0.15 ± 0.02	5.3 ± 0.5
16-DS / control-HSA	0.63 ± 0.06	0.33 ± 0.03	1.9 ± 0.2
5-DS / LABC-HSA	0.49 ± 0.05	0.44 ± 0.04	1.1 ± 0.1
16-DS / LABC-HSA	0.43 ± 0.04	0.39 ± 0.04	1.1 ± 0.1

*The mean value of 15 simulations. The goodness of fits for all simulations was <5.

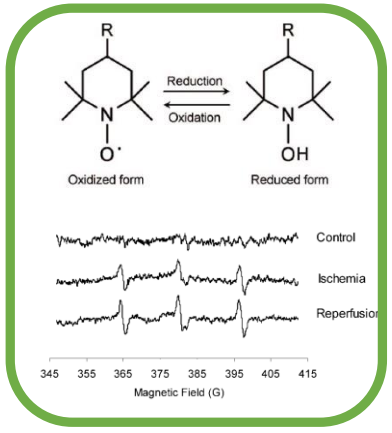
Testing efficacy of new anticancer drugs

- Ribonucleotide reductase (RNR) - target for anticancer drugs (Tyrosyl radical)



EPR for detecting ROS *in vivo*

- Middle cerebral artery occlusion (MCAO) rat
- ROS generated by cerebral ischemia and reperfusion



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EPR in pharmacy

- **Areas of interest where EPR spectroscopy is beneficial:**

EPR in pharmacology
- New smart drug delivery systems
- Antioxidants in cosmetics
- Radioprotectors
- MRI contrasts

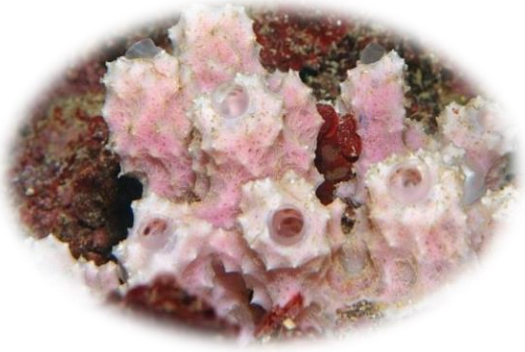
Detecting and evaluating degradation
- Photo
- Thermal
- Chemical degradation of APIs

Optimizing stability & shelf-life
- Degradation pathways
- Drug stability
- Antioxidant efficiency

Reaction monitoring
- Yield optimization
- Undestand react. mechanisms
- Process optimiz. and reaction monitoring

Sterilization processes
- Drug irradiation
- Degradation
- Structure of radicals
- Optimization of sterilization process

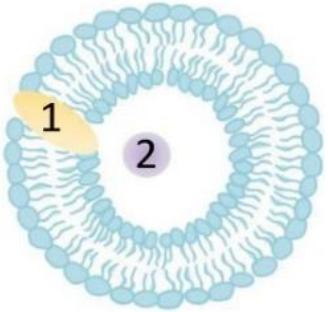
Paramagnetic impurity profiling
- Detecting impurities
- Traces of transition metals
- Drug degradation



Dysidea avara

What about water-insoluble antioxidants?

- Many most effective antioxidants are water-insoluble.
- How to evaluate the capacity of selected AOX to remove ROS/RNS?
- **BioScope**, using new liposome formulations, recently developed original experimental approach to solve this problem.
- Possibility to evaluate the effectiveness of selected AOX *in vivo*?



Journal of Liposome Research 2019.
DOI:10.1080/08982104.2019.1625378

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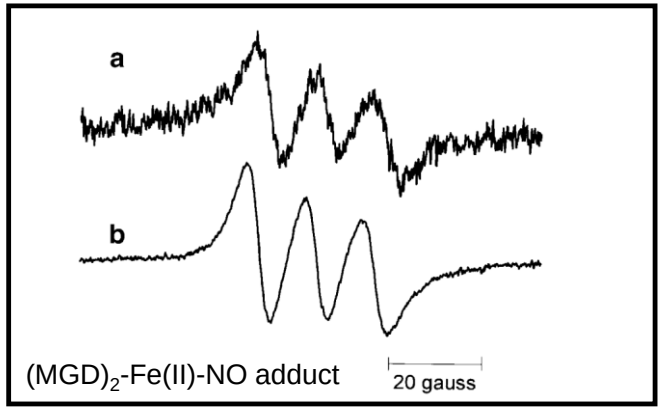
In vivo EPR

(L-band EPR spectroscopy & imaging)

Strategy 1 – trapping radicals *in vivo*

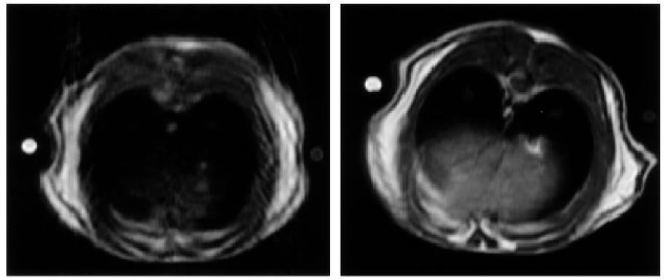
Inflammation - detecting distribution of $NO^{\bullet}$ *in vivo*, induced by lipopolysaccharide (LPS) septic shock in rat



a: In vivo L-band EPR spectrum of liver area.
b: Ex-vivo X-band EPR spectrum of liver.

Possibility to investigate *in vivo* the effectiveness of selected AOX to remove ROS/RNS

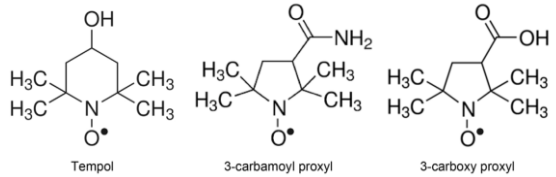
Spin-adducts are also MRI contrasts!

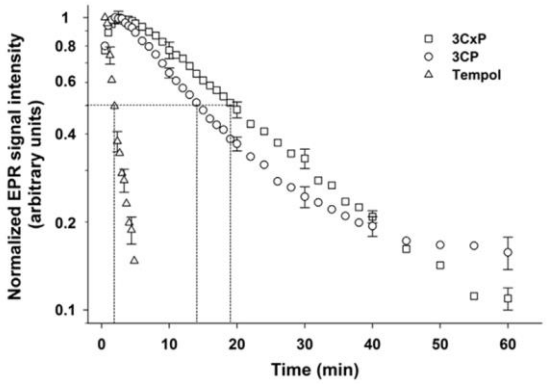


Transverse T1-weighted MR images in the axial plane of the liver area.
a: Control before MGD complex injection;
b: 60 min after the injection of MGD complex.

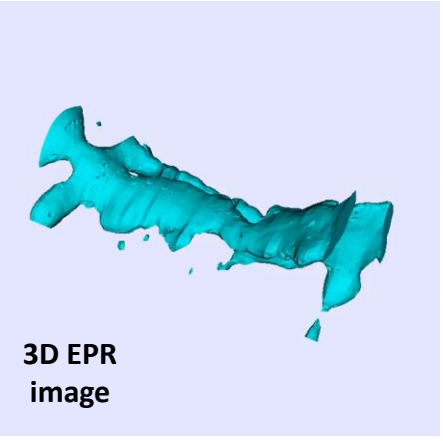
Strategy 2 – reducing stable radicals *in vivo*

Studying BBB permeability in ALS rat & AD mice model





The same strategy could be used to locate *in vivo* the level of ROS/RNS in specific organs



3D EPR image

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Sarching for partners

- **Our excellence, research equipment and methodology are unique in this part of Europe.**
- **We are always searching for new partners in making multilateral project proposals.**
- **Our students are well-known for strong scientific background and are open for new academic exchange programs.**
- **Our laboratories are open for workshops and training schools.**
- **We offer you help in the area of our expertise.**

Some of the projects proposals we are currently working on:

- **MAGLIDD (MAgnetic Glucose LIposome Drug Delivery) project:** To develop smart nanoscale delivery systems with functionalized surface and incorporated nano-magnetic component and selected drug. Specific accumulation at cancer sites allow target drug delivery and theranostics applications.
- **LAANI (Laboratory for Advanced Analysis of NeuroImages) project**: Dedicated to analysis of images obtained using conventional and advanced MRI techniques (3D T1, DTI, fMRI, dynamic contrast-enhanced MRI, etc.). Project strives to develop a multidisciplinary network for investigating various brain disorders and an outcome of disease treatment.
- **For more info please visit our site: www.bioscope.ffh.bg.ac.rs**

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