Symposium on Biophysics for Biology and Medicine

April 10-11th, 2024

Marseille

Balaraman Kalyanaraman : Docteur Honoris Causa 2024

Aix Marseille University

Welcome to the Symposium on Biophysics for Biology and Medicine

Dear Colleagues,

Welcome to the Symposium, the International Conference on Biophysics for Biology and Medicine. Outstanding scientists will review progress in redox biology, cancer, neurodegenerative diseases and cover selected topics of biomedical applications of advanced EPR spectroscopy and imaging, such as metal proteins and macromolecules, development and use of new spin labels, spin traps and spin probes for EPR and dynamic nuclear polarization, oxidative damage, oximetry, biophysics and clinical applications.

The meeting should provide great opportunities for sharing new ideas and research experience. The sessions will include lectures, plenary conferences and talks.

The conference is organized at the Hotel Dieu intercontinental located in the heart of Marseille. In addition to exciting sessions and lively discussions, do not miss the gala dinner organized on April 10th and Raman's anniversary on April 12th to enjoy together French cooking, Vieux Port view and champagne.

We are grateful to the sponsors who have provided financial support to help us in the organization of this international conference.

We wish you a very stimulating meeting. Enjoy the conference and Marseille!

The organizers

Micael Hardy
Aix-Marseille University

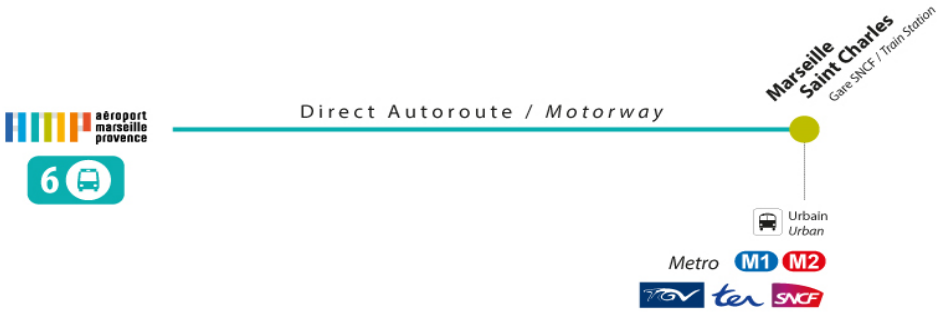
Olivier Ouari
Aix-Marseille University

Hakim Karoui
Aix-Marseille University

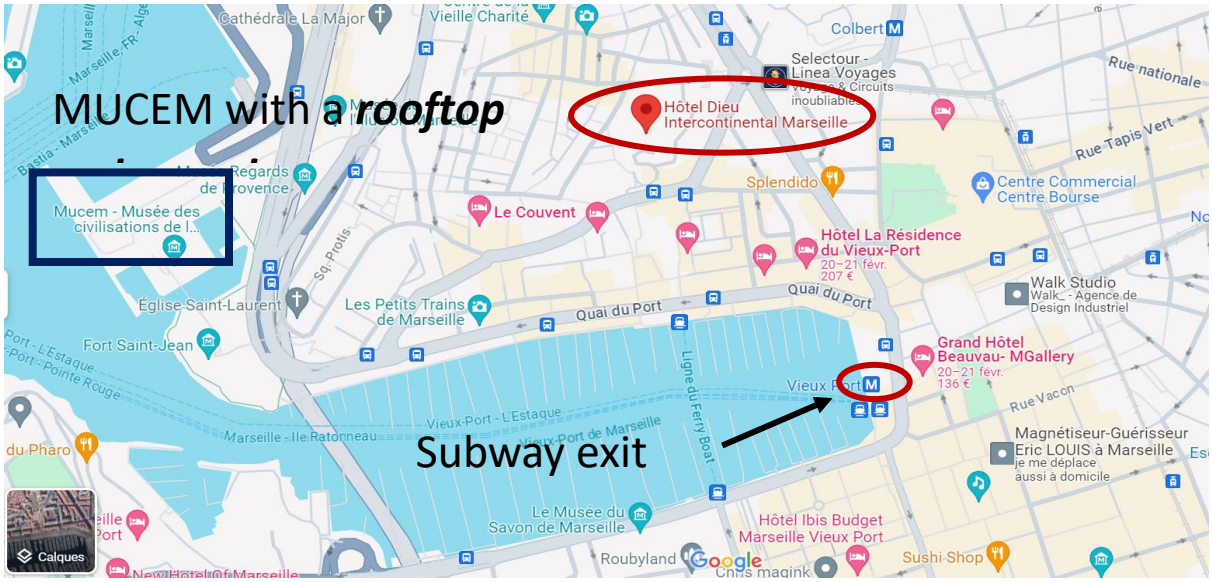
Direction to the symposium

The conference is held at the hotel Dieu Intercontinental, 1 Pl. Daviel, 13002 Marseille 45, rue des Saints Pères (cf. map). Access with public transportations:

At your arrival at Marseille airport (Marseille Provence Airport)
-The fastest way to reach your hotel (Intercontinental or other hotel located in the downtown area) is to take a **taxi**.
-There is a possibility to take a **Bus** which goes from the Airport to **Marseille St Charles Station and Marseille city center (motorway).** From the airport, there is a bus every 20 min.
https://www.marseille-airport.com/access-car-parks/access/bus/marseille-st-charles-station



At Saint Charles train station, there is a direct access to the subway station. (https://www.rtm.fr/en). Take the M1 metro (direction La Fourragère) and get out to **VIEUX PORT station**. Take the QUAI DES BELGES exit and you will be on the Vieux Port big place.



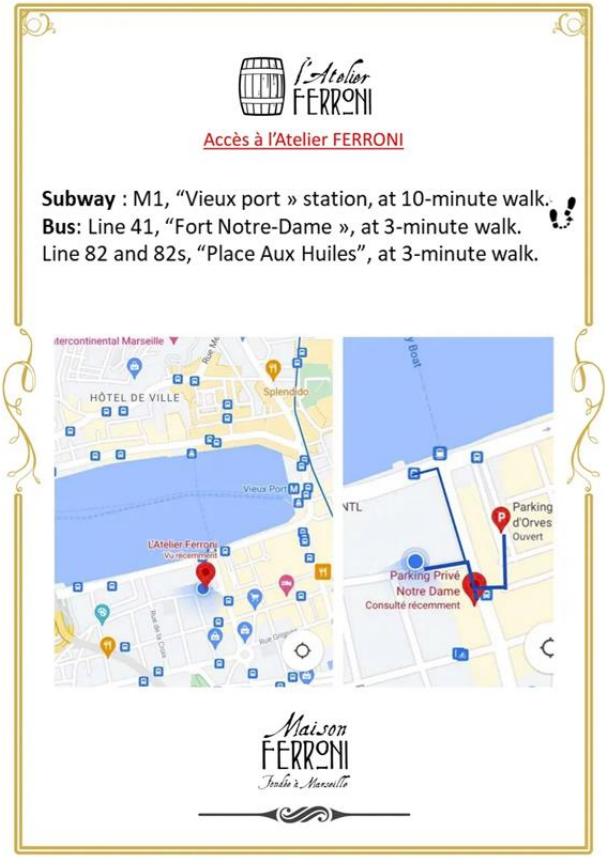
Hôtel Dieu Intercontinental

Direction to the Gala dinner (Wednesday, April 10 nd)

The gala dinner will take place at the Intercontinental Hotel at the “escaliers Monumentaux”

Direction to Atelier Ferroni dinner (Friday, April 12nd)

We have privatized a “rum workshop” from the company Ferroni. **https://carrynation.fr/bar/atelier**
The place is located at 8, rue Neuve Sainte Catherine - 13007 Marseille (10 min walk from the hotel).



Sponsors

Institut Chimie Radicalaire

Aix*Marseille université
Socialement engagée

International Research Program
SuperO2

Scientific Program

Wednesday, April 10 nd

8.30 – 8.50	Registration
8.50 – 9.00	Welcome words
9.00 – 11.00	***Session 1: Redox biology, cancer and neurodegeneration*** Chair: **Olivier Ouari**
9.00 – 9.45	**Plenary - Nitrogen oxide-derived anti-inflammatory lipid signaling mediators** **New drug candidates?** *Bruce Freeman* (Pittsburgh, USA)
10.00 – 10.30	**Implications of SPR mimetics to BH4-deficiency and pain management** *Jeannette Vasquez-Vivar* (Milwaukee, USA)
10.30 – 11.00	Coffee break
11.00 – 11.30	**Mitigating gut microbial degradation of levodopa and enhancing dopamine in the brain: Implications in Parkinson's disease** *Gang Cheng* (Milwaukee, USA)
11.30 – 12.30	***Session 2: EPR spectroscopy for structural biology*** Chair: : **Valérie Belle**
11.30 – 12.00	**Electroformation of giant unilamellar vesicles from moist lipid films formed by vesicle fusion on the surface of an electrode with emphasis on membranes with high cholesterol content** *Marija Raguz* (Split, Croatia)
12.00-12.30	**In-cell EPR: exploring protein structural dynamics inside cells by SDSL-EPR** *Elisabetta Mileo* (Marseille, France)
12.30-14h30	Lunch
14.30 – 16.30	***Session 3: Structure / function by DNP MRI and NMR*** Chair: **Elisabetta Mileo**
14.30 – 15.00	**Structural insights from NMR of protein-lipid assemblies in degenerative diseases** *Francesca M. Marassi* (Milwaukee, USA)
15.00 – 15.30	**Hyperpolarized ^{13}C NMR probes to assess the activity of the mitochondrial TCA cycle** *Zoltan Kovacs* (Dallas, USA)
15.30 – 16.00	Coffee break
16.00 – 17.00	***Session 4: Photoreactivity, phototoxicity in Krakow city !***

	Chair: **Jacek Zielonka**
16.00 – 16.30	**Photoreactivity and phototoxicity of fine particulate matter from air pollutants** *Tadeusz Sarna* (Krakow, Poland)
16.30 – 17.00	*Ravinder J. Singh* (Rochester, USA)
19h30	**Gala dinner**

Thursday, April 11 nd

9.00 – 10.00	***Session 5: in vivo Imaging and Oximetry*** Chair: **Periannan Kuppusamy**
9.00 – 9.30	**EPR Oximetry: Capillary to Clinic** *Periannan Kuppusamy* (Dartmouth, USA)
9h.30 – 10.00	**An autocatalytic mechanism for the reaction between S-nitrosoglutathione and hydrogen sulfide** *Neil Hogg* (Milwaukee, USA)
10.00 – 10.30	Coffee break
10.30 – 12.00	***Session 6: Spin labels, spin traps, spin probes for EPR*** Chair: **Micael Hardy**
10.30 – 11.00	**Fluorogenic cyclization of phenyl-type radicals for specific detection of peroxynitrite** *Jacek Zielonka* (Milwaukee, USA)
11.00 – 11.30	**The reaction of glutathione-derived dinitrosyl iron complexes and superoxide radical anion - the detection and quantitation of peroxynitrite** *Adam Sikora* (Lodz, Poland)
11.30 – 12.00	**Unveiling the Therapeutic Frontiers of Targeted Nitroxides** *Marcos Lopez* (Puerto Rico, USA)
12.00	Lunch

Oral presentations

Redox biology, cancer and neurodegeneration

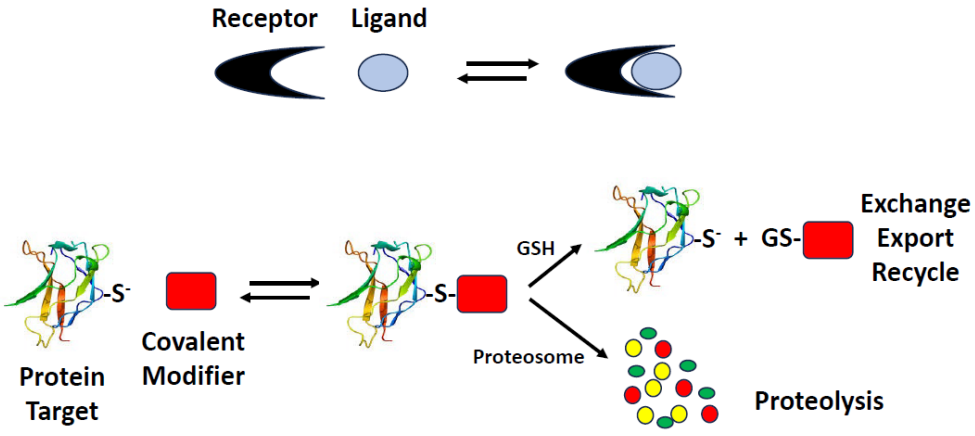
Nitrogen oxide-derived anti-inflammatory lipid signaling mediators
New drug candidates?

Bruce Freeman.[1]

[1]*Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh Pennsylvania, USA.*

A broad array of nitrogen oxide (NOx) and oxygen-derived oxidizing, nitrosating and nitrating species are generated endogenously during metabolism and inflammation. Nitric oxide ($^{\bullet}NO$), nitrite (NO_2^-) and nitrate (NO_3^-) are readily transformed by metabolic and inflammatory **red**uction-**ox**idation (redox) reactions to yield an array of reactive species that can mediate toxic and adaptive signaling responses, depending on concentration. For example, unsaturated fatty acid reaction with $^{\bullet}NO_2$ produces fatty acid nitroalkene and nitro-nitrate derivatives (NO_2-FA). These species are conferred with an electrophilic character that supports *kinetically rapid and reversible adduction of nucleophilic amino acids, predominantly cysteine (Cys)*. Preclinical and clinical data affirms that NO_2-FA at endogenous and therapeutic concentrations react with the "hyper-reactive Cys proteome". These covalent reactions broadly impact cell and organ function by altering the structure/function of ~75-150 hyper-reactive protein targets that evolved to mediate adaptive signaling responses. This includes redox-sensitive transcription factors, protein chaperones and enzymes regulating stress responses and tissue repair.

Receptor-Ligand versus Covalent Modifier Signaling



Downstream responses include inhibition of cytokine expression, improved oxidant-antioxidant "balance", enhanced mitochondrial and metabolic function. These concerted actions will suppress inflammation and limit pathologic cell proliferation, thus potentially providing clinical benefit. This presentation will also summarize a) Phase 1 and 2 clinical trial results for orally-administered electrophilic 10-nitro-octadec-9-enoic acid (nitro-oleate, CP-6) in renal and pulmonary disease patients and b) recent discoveries of how small molecule nitroalkenes can limit other pathologic cell proliferation responses.

Implications of SPR mimetics to BH4-deficiency and pain management

Jeannette Vasquez-Vivar,[1] James Woodcock,[1] Steven Traeger,[1] Zhongjie Shi,[2] Sidhartha Tan.[2]

[1]*Department of Biophysics, Redox Biology Program, Medical College of Wisconsin, Milwaukee, Wisconsin 53226.*

[2]*Department of Pediatrics Wayne State University, Detroit, Michigan 48201.*

Sepiapterin reductase (SPR) is a NADPH-dependent enzyme in the tetrahydrobiopterin synthetic pathway. Biallelic SPR deficiency (SPRD) leads to intellectual disabilities and motor disorders. Still, it is distinctive because it does not cause hyperphenylalaninemia, which is generally present in other deficiencies. It is thought that carbonyl and aldo-keto reductases (CR, AKR) mimicking SPR activity explain this finding, although their efficiencies in vivo remain unclear. Recent human genome-wide association studies have indicated a role for the BH4 pathway in neuropathic chronic pain, a condition caused by injury to the somatosensory system. Since both CR and AKRs could represent a therapeutic approach for SPRD and could be an unrealized target of pain therapies focused on inhibiting SPR, we sought to better characterize the role of AKRs in the BH4 pathway. We generated homozygous knockout (SPR-KO) HEK cells targeting SPR exon 2, a frequent mutation locus of human SPRD, using CRISPR/Cas9. The biallelic SPR mutation was confirmed by Sanger sequencing, and the absence of SPR protein expression was confirmed by Western blot. Activity assays showed the total loss of SPR activity, yet 18% of the basal wild-type BH4 persisted. The pattern of BH2 depletion in SPR-KO indicated that at least some of the cellular BH2 arises from the biosynthetic pathway. The overexpression of the human AKR1C3 isoform in SPR-KO cells, in direct contradiction to previous in vitro studies, failed to bring BH4 to wild-type levels. A 21% increase in BH4 was found in clones expressing AKR1C3 SPR-KO cells. Also, no significant BH4 changes were found in wild-type cells overexpressing AKR1C3. We confirmed that AKR1B1 and CR are expressed in HEK cells, although CR is expressed at exceedingly low levels. The impact of SPR/BH4 insufficiency and SPR mimetics on cellular functions and their possible role in inflammatory conditions will be discussed.

Research reported in this work was supported by the National Institute of Neurological Disorders and Stroke of the National Institutes of Health under award numbers R01NS1149972 to ST, R01NS117146 to ST, JVV, XJ.

Mitigating gut microbial degradation of levodopa and enhancing dopamine in the brain: Implications in Parkinson's disease

Gang Cheng,[1] Micael Hardy,[2] Cecilia J. Hillard,[3] Jimmy B. Feix,[1] Balaraman Kalyanaraman.[1*]

[1]*Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI 53226, United States.*
[2]*Aix-Marseille Univ, CNRS, ICR, UMR 7273, Marseille 13013, France.*
[3]*Department of Pharmacology and Toxicology and Neuroscience Research Center, Medical College of Wisconsin, Milwaukee, WI 53226, United States.*

Levodopa is used to manage Parkinson's disease symptoms. As Parkinson's disease progresses, patients require increased doses of levodopa, causing undesirable side effects. Additionally, the oral bioavailability of levodopa decreases in Parkinson's disease patients due to the increased metabolism of levodopa to dopamine by gut bacteria, *Enterococcus faecalis*, resulting in decreased neuronal uptake and dopamine formation. Parkinson's disease patients have varying levels of these bacteria. Thus, decreasing bacterial metabolism is a promising therapeutic approach to enhance the bioavailability of levodopa in the brain. Mito-*ortho*-HNK, formed by modification of a naturally occurring molecule such as honokiol conjugated to a triphenylphosphonium moiety, mitigated metabolism of levodopa—alone or combined with carbidopa—to dopamine. Mito-*ortho*-HNK suppressed the growth of *E. faecalis*, decreased dopamine levels in the gut, and increased dopamine levels in the brain. One way to enhance the bioavailability of levodopa is to mitigate its gut metabolism to dopamine using Mito-*ortho*-HNK and analogs.

Keywords: microbiome, mitochondria-targeted drugs, bioenergetics, levodopa, TPP^+-based drugs, Parkinson's disease.

EPR Spectroscopy for structural biology

Electroformation of giant unilamellar vesicles from moist lipid films formed by vesicle fusion on the surface of an electrode with emphasis on membranes with high cholesterol content

M. Raguz,[1] I. Mardešić [1] Z. Boban.[1]

[1]*University of Split School of Medicine, Department of medical physics and biophysics, Split, Croatia.*

Artificial models that are often used to mimic cell membranes are giant unilamellar vesicles (GUVs). They are most commonly produced using the electroformation method. The traditional protocol involves a step in which the organic solvent is completely evaporated, forming a dry lipid film. This leads to an artifactual demixing of cholesterol (Chol) in the form of anhydrous crystals. These crystals do not participate in the formation of the lipid bilayer, which leads to a decrease in the Chol concentration in the bilayer compared to the initial lipid solution. We propose a novel protocol that bypasses the dry lipid film phase by combining of rapid solvent exchange (RSE), extrusion, plasma cleaning and spin-coating to produce GUVs from moist lipid films. We tested the efficiency of the protocol with Chol/phosphatidylcholine (POPC) lipid mixtures with a mixing ratio between 0 and 2.5. The most reproducible results were obtained when the duration of vesicle spin-coating was 30 s and large unilamellar vesicles (LUVs) were extruded through 100 nm membrane pores. The reduction in vesicle size was about 40% for all vesicles with a Chol/POPC mixing ratio above 1.5 compared to the pure POPC bilayer (Figure 1). We believe that this new, improved electroformation protocol will allow us to successfully study the physical properties, lateral organization, and domain formation of membranes with very high Chol content such as the plasma membranes of the eye lens fiber cells. The elimination of organic solutions by the RSE method and the plasma cleaning of the electrode have proven to be advantageous for the preparation of GUVs with charged lipids and buffer solutions.

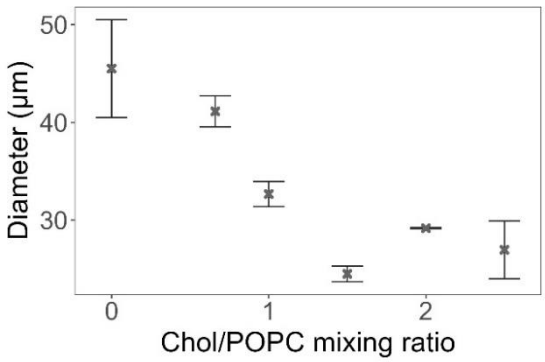


Figure 1. GUVs sizes for different Chol/POPC mixing ratios.

In-cell EPR:
exploring protein structural dynamics inside cells by SDSL-EPR

A. Pierro,[1] A. Bonucci,[2] B. Zambelli,[3] O. Ouari,[4] A. Magalon[5], V. Belle[2], E. Mileo.[2]

[1]*Department of Chemistry, University of Konstanz, Konstanz, Germany.*
[2]*Aix Marseille Univ, CNRS, BIP, Marseille, France.*
[3]*Laboratory of Bio-Inorganic Chemistry, University of Bologna, Italy.*
[4]*Aix Marseille Univ, CNRS, ICR, Marseille, France.*
[5,6]*Aix Marseille Univ, CNRS, LCB UMR7283, Marseille, France.*

Understanding how the intracellular medium modulates protein structural dynamics and protein-protein interactions is an intriguing but required topic scientists search to address by studying biomolecules in their native environment. As the cellular environment cannot be reproduced in vitro, investigation of biomolecules directly inside cells has attracted a growing interest in the past decade. Indeed, efforts in magnetic resonance spectroscopies have enabled important improvements in the study of structural dynamics directly in the cellular context.

Among magnetic resonances approaches, site-directed spin labeling coupled to electron paramagnetic resonance spectroscopy (SDSL-EPR) has demonstrated to be one of the powerful approaches to study structural properties of biomolecules [1]. In particular, nitroxide-based SDSL-EPR couples the benefits of high sensitivity and the lack of size constraints for the biomolecule of interest with the ability to study protein structural transitions and interactions at physiological temperature. [2-3]

In this lecture, I will introduce the basic principles of *in-cell* EPR spectroscopy of proteins. I will discuss the main technical problems limiting the application of the method. In parallel, I will overview recent developments and highlight the main future research directions in the field. [3-4-5]

References:

[1] - A. Bonucci, O. Ouari, B. Guigliarelli, V. Belle, E. Mileo, *ChemBioChem* **2020**, 21, 451-460.

[2] - G. Karthikeyan, A. Bonucci, G. Casano, G. Gerbaud, S. Abel, V. Thomé, L. Kodjabachian, A. Magalon, B. Guigliarelli, V. Belle, O. Ouari, E. Mileo, *Angew. Chem. Int. Ed.* **2018**, 130, 1380-1384.

[3] – A. Pierro, A. Bonucci, D. Normanno, M. Ansaldi, E. Etienne, G. Gerbaud, E. Pilet, B. Guigliarelli, O. Ouari, A. Magalon, V. Belle, E. Mileo, *Chem. – Eur. J.* **2022***,* 28, e202202249.

[4] – A. Pierro, K. C. Tamburrini, H. Leguenno, E. Etienne, G. Gerbaud, B. Guigliarelli, V. Belle, B. Zambelli, E. Mileo, *iScience, 2023*, 26 (*10*), 107855*.*

[5]- Y. Ben-Ishay, Y. Barak, O. Ouari, A. Pierro, E. Mileo, X.-C.-Su and D. Goldfarb, *Protein Science, **2024**, in press.*

Structure / function by DNP MRI and NMR

Structural insights from NMR of protein-lipid assemblies in degenerative diseases

Francesca M. Marassi, Rajlaxmi Panigrahi, Kyungsoo Shin, Gopinath Tata, Nicholas Wood.

Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Medical College of Wisconsin, Milwaukee, WI 53226, United States.

Elucidating the structure and activity of proteins in their native environment is a fundamental goal of structural biology. Here we show that nuclear magnetic resonance (NMR) spectroscopy is ideally suited for this task. Using NMR experiments with both soluble and solid-state samples we explore proteins in complex biomolecular assemblies, including microbial membranes and calcified protein-lipid deposits. NMR studies of bacterial samples yield insights about the native structure and function of virulence proteins and set the stage for probing their interactions with the complex milieu of immune response proteins present in human serum. Similarly, the ability to focus on calcified protein-lipid assemblies with NMR sheds light on the calcified depositions that accumulate in the aging eye and brain in degenerative diseases such as agerelated macular degeneration and Alzheimer's disease. These deposits are rich in blood proteins, lipids and hydroxyapatite, the stable, mineralized form of hydroxylated calcium phosphate that makes up the bulk of bone and teeth, but the process of deposit formation is poorly understood. We show that detailed structural and functional information can be obtained from solid-state NMR and solution NMR with complex native or native-like samples.

Hyperpolarized ^{13}C NMR probes to assess the activity of the mitochondrial TCA cycle

M. Huynh,[1] J. Singh,[1] Z. Erfani,[1] E. H. Suh,[1,2] J. Chen,[1] J. M. Park,[1] and Z. Kovacs.[1]

[1] *Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, TX, USA.*
[2] *Department of Pharmaceutical Sciences, HSC College of Pharmacy, The University of North Texas Health Science Center, Fort Worth, TX USA.*

The mitochondrial TCA cycle is a central metabolic pathway for energy production and biosynthesis. The goal of this project was to develop hyperpolarized (HP)-^{13}C labeled probes that can report TCA cycle activity in vivo in real-time. We synthesized [2-^{13}C, 3-$^{2}H_3$]pyruvate and diethyl [1,2-$^{13}C_2$]-2-ketoglutarate as HP-^{13}C MRS probes for the TCA cycle. The C2 carbon of pyruvate enters the TCA cycle as acetyl-CoA and appears in downstream metabolites such as [5-^{13}C]glutamate. 2-Ketoglutarate in its cell permeable ester form is taken up by cells, hydrolyzed by esterases and enters the cycle as ketoglutarate and is decarboxylated to [^{13}C]bicarbonate by ketoglutarate dehydrogenase. The probes were hyperpolarized by dynamic nuclear polarization and administered into Wistar rats via tail vein injection. In vivo ^{13}C MRS was performed at 3 T with a ^{13}C surface coil placed over the liver. As expected, HP-[2-^{13}C, 3-$^{2}H_3$]pyruvate produced large amounts of lactate and alanine and several metabolites of the glycolysis / gluconeogenesis pathway via pyruvate carboxylation. However, only a weak signal [5-^{13}C]glutamate was observed. On the other hand, diethyl [1,2-$^{13}C_2$]-2-ketoglutarate produced easily detectable HP-[^{13}C]bicarbonate via ketoglutarate dehydrogenase in the liver reflecting flux through the TCA cycle. Thus, diethyl [1,2-$^{13}C_2$]-2-ketoglutarate is a better probe for monitoring TCA cycle activity than [2-^{13}C, 3-$^{2}H_3$]pyruvate. This is likely due to substrate competition as other sources of acetyl-CoA can contribute to TCA flux competing with the probe.

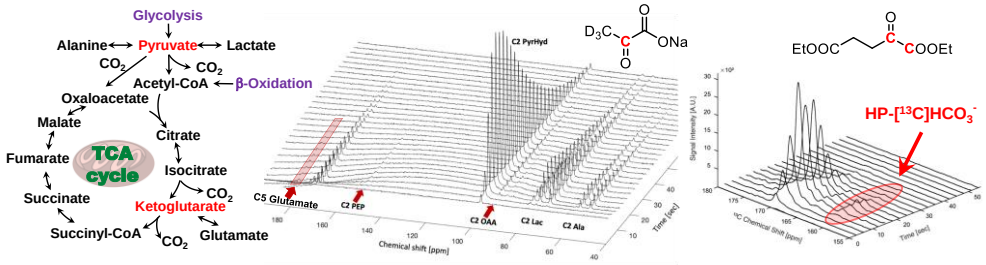


Figure 1. The TCA cycle (left), the downstream metabolites of HP-[2-^{13}C, 3-$^{2}H_3$]pyruvate (middle) and the formation of HP-[^{13}C]bicarbonate from HP diethyl [1,2-$^{13}C_2$]-2-ketoglutarate in vivo (right).

Photoreactivity, phototoxicity in Krakow city !

Photoreactivity and phototoxicity of fine particulate matter from air pollutants

K. Mokrzynski,[1] O. Krzysztynska-Kuleta,[1] M. Zawrotniak,[2] M. Sarna,[1] M. Wojtala,[1] D. Wnuk[3] and T. Sarna.[1]

[1]Jagiellonian University, [1]Department of Biophysics.
[2]Department of Comparative Biochemistry and Bioanalytics.
[3]Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland.

Although human skin efficiently protects the body against harmful effects of environmental factors, excessive exposure of the skin to certain air pollutants, such as fine particulate matter (PM), could lead to oxidative stress and result in pathological conditions. It has been reported that $PM_{2.5}$ containing particles with aerodynamic diameter smaller than 2.5 μm could not only penetrate through barrier-disrupted skin, but also induce skin barrier dysfunction (1). Because typical constituents of PM exhibit photochemical activity, their harmful effects could be intensified by solar radiation.

This paper will review a recent study aimed at determining phototoxicity of $PM_{2.5}$ collected in the Krakow area in the four seasons (2). The effect of different $PM_{2.5}$ on selected parameters of cultured human keratinocytes, subjected to irradiation with light derived from a solar simulator, were measured by standard cell and molecular biology techniques. Photoreactivity of the studied $PM_{2.5}$, particularly their efficiency to photogenerate singlet oxygen and free radicals were determined by time-resolved singlet oxygen phosphorescence and EPR-spin trapping, respectively.

Our data showed that the highest phototoxicity and photoreactivity exhibited $PM_{2.5}$, collected from air pollutants in the winter season. This, in part, could be due to the fact that increased coal burning occurs in the Krakow area in the winter season. As a result, the winter season ambient particles are likely to contain substantial amount of photoreactive aromatic hydrocarbons. In a follow up study, we also examined if selected antioxidants could protect HaCaT cells from phototoxicity mediated by $PM_{2.5}$ (3). The obtained data revealed that L-ascorbic acid and resveratrol, especially when used in combination, could serve as protective agents against adverse effects of $PM_{2.5}$ both dark and light in an in vitro model of human skin.

Literature

1. *Liao, Z. et al., Toxicol Rep 2020, 7, 1-9*
2. *Mokrzynski, K. et al., Int J Mol Sci 2021, 22, 10645, 1-20*
3. *Mokrzynski, K. et al., Photochem Photobiol 2023, 99: 983–992*

In vivo Imaging and Oximetry

EPR Oximetry: Capillary to Clinic

P. Kuppusamy.[1]

[1]*Geisel School of Medicine, Dartmouth College, Lebanon, NH 03756, USA*.

Knowledge of tissue oxygen levels holds paramount importance to understand the mechanism of several pathophysiological disorders and to develop treatment strategies to mitigate disease progression. This would require methods capable of quantifying the level of tissue oxygen with good reliability and accuracy in the clinical setting. Electron paramagnetic resonance (EPR) spectroscopy is capable of measuring tissue oxygen levels using injected (soluble) or implanted (solid) paramagnetic probes as oxygen-sensors. The principle of EPR oximetry is based on the paramagnetic property of molecular oxygen, which can shorten spin-spin relaxation time (T2) of EPR spin-probes leading to EPR line-broadening. The effect of oxygen on EPR spectrum was first observed in DPPH solutions by Deguchi in 1960 and later in nitroxide solutions by others (Edelstein, et. al., 1964; Povich, 1975; Backer, et. al., 1977). The practical application of this phenomenon, termed as 'spin label oximetry', was developed by Hyde and others in the 1980s for studying oxygen transport and metabolism in cell systems. The developments of low-frequency EPR instrumentation and probes during 1990s and later, have enabled the use EPR oximetry for a variety of *in vivo* applications such as isolated functioning organs (e.g., heart), intact animals, and humans. Over the years, the EPR oximetry technology has continuously evolved, with respect to both the probes and methods, to make reliable, accurate, and repeated measurements in living systems under minimally invasive conditions. EPR oximetry applications have been rapidly expanding to include measuring myocardial tissue oxygen for assessing the extent of myocardial injury, chronic wound healing, ischemic stroke, and monitoring tumor oxygenation levels for enhancing cancer-treatment efficacy in animal models and cancer patients. This presentation will provide an overview of the principle of EPR oximetry, available methods/probes, and applications from *in vitro* studies in capillaries to *in vivo* measurements in cancer patients in the clinic.

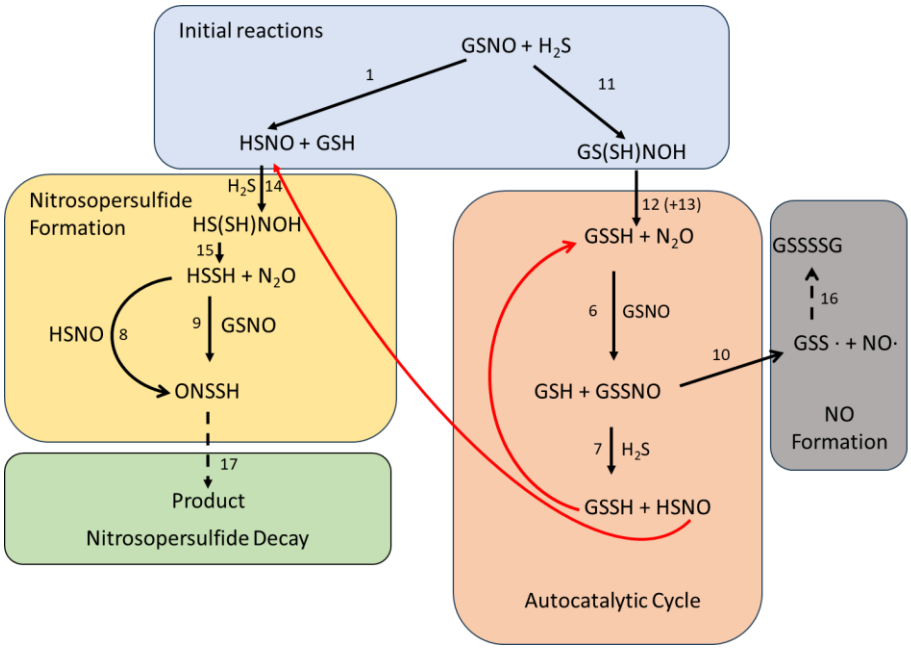
Acknowledgments: The work was supported by NIH grant funding R01 EB004031 and R01 CA269234.

An Autocatalytic Mechanism for the Reaction between S-Nitrosoglutathione and Hydrogen Sulfide

Neil Hogg

Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI.

Small-molecule gaseous signaling agents, that have been collectively referred to as gasotransmitters, include nitric oxide (NO), hydrogen sulfide (H_2S) and carbon monoxide. There is evidence that these molecules are able to work together and, in some circumstances, may act synergistically. For this reason, there has been significant interest in understanding the biological chemistry of interactions between gasotransmitters. Studies of the reaction between NO and H_2S uncovered the synthesis of a yellow-colored molecule with an absorbance maximum at 402 nm. While there has been some controversy regarding the chemical nature of this molecule, it has now been well established to be nitrosopersulfide ($ONSS^-$). Nitrosopersulfide can be generated in good yield from the reaction between H_2S and nitrosothiols such as S-nitrosoglutathione (GSNO). Although this reaction has been know for several decades, no viable mechanism has been established. The reaction kinetics indicate significant sigmoidal behavior indicating possible autocatalysis. We will present a mechanism (see scheme) that contains all the salient features of the reaction kinetics and product formation, and that fits well to experimental kinetic data, and will also discuss possible biological significance of this mechanism.



Spin labels, spin traps, spin probes for EPR

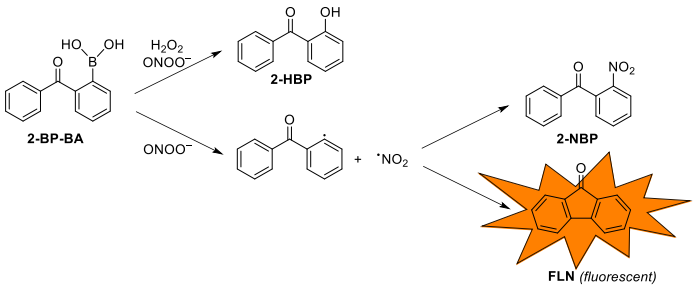
Fluorogenic cyclization of phenyl-type radicals for specific detection of peroxynitrite

A. Grzelakowska,[1,2] R. Podsiadly,[1] J. Zielonka.[2]

[1]*Institute of Polymer and Dye Technology, Lodz University of Technology, Poland.*
[2]*Department of Biophysics, Medical College of Wisconsin, Milwaukee, United States.*

Peroxynitrite ($ONOO^-$), a biological oxidizing and nitrating species responsible for posttranslational modification of cellular proteins, has been implicated in numerous pathologies carrying an inflammatory component. Specific detection of $ONOO^-$ in biological systems remains a challenge and boronates are regarded as a most promising class of probes for the detection and quantitation of $ONOO^-$. However, boronates can be oxidized not only by $ONOO^-$, but also by several other biologically relevant oxidants, including hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). Oxidation of boronate probes by $ONOO^-$ results in the formation of minor, $ONOO^-$-specific products via a reaction pathway involving a phenyl radical-type intermediate, in addition to the major, phenolic product.[1]

Here, we report a new approach for specific detection of $ONOO^-$, based on fluorogenic cyclization of the phenyl type radical formed during oxidation of a boronate probe by $ONOO^-$, with the production of a fluorescent product. We characterized the kinetics and stoichiometry of the reaction of benzophenone-2-boronic acid (2-BP-BA) with $ONOO^-$ and identified 2-hydroxybenzophenone (2-HBP) as the major product and fluorescent fluorenone (FLN) and 2-nitrobenzophenone (2-NBP) as the minor, $ONOO^-$-specific products (Scheme 1). Neither hydrogen peroxide alone nor in the presence of myeloperoxidase and nitrite produce FLN or 2-NBP. Moreover, FLN is formed both from bolus $ONOO^-$ and from in situ generated $ONOO^-$, produced during the decomposition of SIN-1, a thermal source of $O_2^{\bullet-}$ and $^{\bullet}NO$.



Scheme 1. Products detected upon oxidation of 2-BP-BA by H_2O_2 vs. $ONOO^-$

As fluorenone can be selectively detected using fluorescence spectroscopy, the observed reaction provides a model for the development of next generation probes for $ONOO^-$, for non-invasive, fluorescence-based specific detection of $ONOO^-$.

---

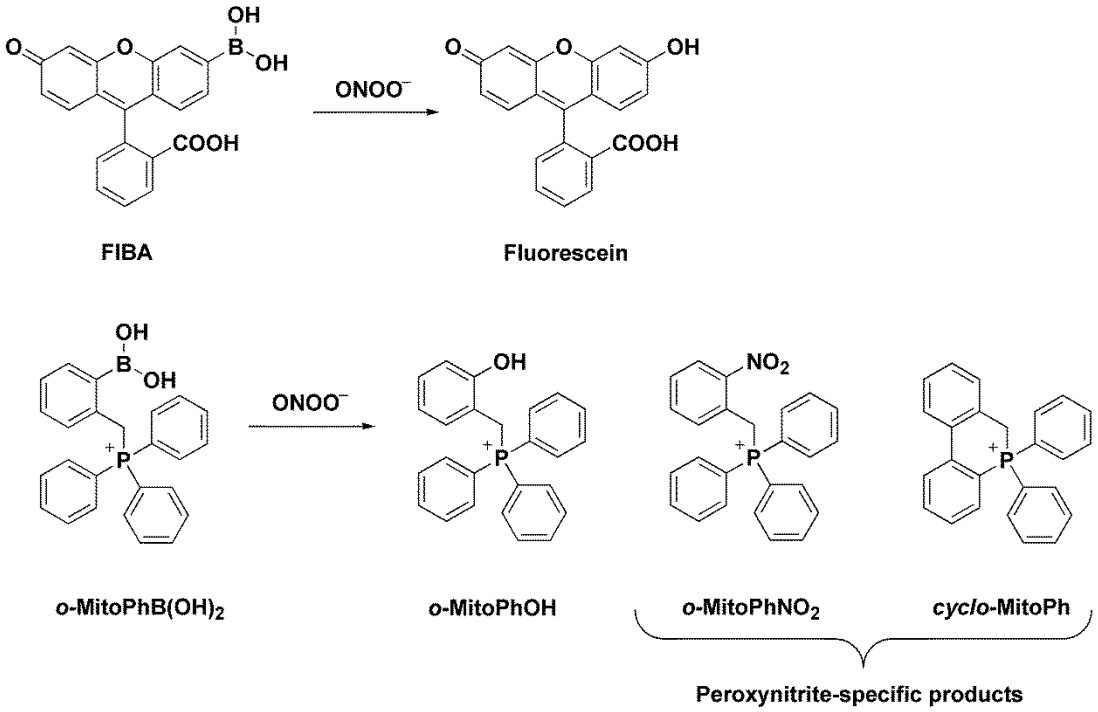
[1]Sikora A. *et al.* Boronate-based probes for biological oxidants: A novel class of molecular tools for redox biology. *Front. Chem.* 2020; 8:580899. doi: 10.3389/fchem.2020.580899

The reaction of glutathione-derived dinitrosyl iron complexes and superoxide radical anion - the detection and quantitation of peroxynitrite

M. Rola,[1] A. Artelska,[1] J. Zielonka,[2] R. Michalski,[1] A. Sikora.[1]

[1]*Institute of Applied Radiation Chemistry, Lodz University of Technology, Lodz, Poland.*
[2]*Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI, United States.*

Nitric oxide produced in cells reacts with iron ions and thiols to form the so-called dinitrosyl iron complexes (**DNIC**). It is assumed that **DNICs** are important products of the cellular metabolism of nitric oxide, constituting its reservoir. Despite this, the biological chemistry of **DNICs** is not well understood, and little is known about the mechanism of their formation and degradation. An important reaction of nitric oxide is its interaction with the superoxide radical anion ($\mathbf{O_2^{\bullet-}}$), leading to the formation of peroxynitrite ($\mathbf{ONOO^-}$). Here, we present the results of our study on the detection and quantification of peroxynitrite formed in the reaction of glutathione-derived **DNIC** and superoxide radical anion. Boronate probes **FlBA** and ***o*-MitoPBA** were employed for the detection of peroxynitrite and its quantitative determination. We compared the yield of peroxynitrite generation in the **DNIC**/$\mathbf{O_2^{\bullet-}}$ system with the efficiency of its production in the **GSNO**/$\mathbf{O_2^{\bullet-}}$ system. The effects of **SOD** and catalase were also investigated.



Unveiling the Therapeutic Frontiers of Targeted Nitroxides

KM Vega-Escobar,[2] K. Valentín-Meléndez, [2, 3] S. Sanabria-Barrera,[4]
O. Ouari,[5] M. Hardy,[5] and M. Lopez.[1, 2]

[1] *Research Institute, Puerto Rico Science, Technology, and Research Trust, San Juan, Puerto Rico.*
[2] *Department of Chemistry and NIH-URISE Program, University of Puerto Rico at Humacao, Humacao, Puerto Rico.*
[3] *Department of Biology, University of Puerto Rico at Humacao, Humacao, Puerto Rico.*
[4] *Innovation and Technology Development, Fundación Cardiovascular de Colombia, Floridablanca, Colombia.*
[5] *Aix Marseille Univ, CNRS, ICR, Equipe SREP, Marseille, France.*

Nitroxides have evolved from their well-established role as potent antioxidants and superoxide dismutase (SOD) mimetics to become critical players in experimental therapeutics. Here, we highlight the delves into the strategic targeting of nitroxides to mitochondria, a critical evolution that has significantly broadened their therapeutic horizons beyond their antioxidant activity. Once celebrated primarily for their capacity to neutralize reactive oxygen species, nitroxides have revealed their prowess as versatile therapeutic agents.

Mitochondria-targeted nitroxides like Mito-CP and Mito-SG1 have emerged as a groundbreaking strategy, addressing the intricate challenges of mitochondrial-derived free radicals. This targeting endeavor has reinforced the traditional antioxidant paradigm and unveiled a spectrum of novel therapeutic potentials in cancer, neuroscience, and cardiometabolic diseases. These include modulation of cell signaling pathways, selective inhibition of mitochondrial functions, and initiating protective mechanisms against cellular stress and damage.

The transition from general antioxidants to mitochondria-specific interventions marks a significant milestone, reflecting a deeper understanding of cellular oxidative processes and the critical role of mitochondria in health and disease. Drawing from the contributions of researchers like our mentor **Balaraman Kalyanaraman**, here we showcase the multifaceted therapeutic applications of targeted nitroxides, from cancer and neuroprotection to cardiovascular diseases, positioning them at the forefront of therapeutic innovation. The strategic targeting of nitroxides represents a paradigm shift from conventional antioxidant applications to a multifaceted therapeutic approach. This evolution highlights the potential of targeted nitroxides to navigate the complex interplay between oxidative stress and mitochondrial dysfunction, offering promising avenues for developing targeted therapeutic interventions.

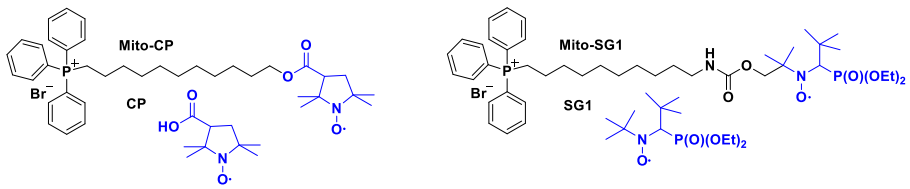


Figure 1 *– Structures of Mito-CP and Mito-SG1 nitroxides*

Enjoying Marseille

Restaurants

Around the Intercontinental Hotel, there is the ***Panier*** neighborhood

You will find places for lunch and dinner.

"**La VIEILLE PELLE"** Pizza, Italian cuisine. One of the oldest restaurants in this district

Le Tribeca Pizza and Mediterranean food 200 quai du Vieux Port (5 min walking distance from Hotel Intercontinental)

"***Entre Ciel et Mer***" seafood, cheeses, charcuteries

https://www.entre-terre-et-mer-marseille.com/

And the menu

https://www.entre-terre-et-mer-marseille.com/carte-entre-terre-et-mer-restaurant-fruits-de-mer-marseille

« **Le Barrio** »

Peruvian fusion cuisine

https://www.barrio28.fr/

And the menu

https://www.barrio28.fr/carte

If you on the other side of the Vieux Port, you will find an Italian-style piazza, the ***COURS ESTIENNE D'ORVES*** with places to eat.

"**Les ARCENAULX**" French cuisine, great wines

https://www.les-arcenaulx.com/

And example of menus

https://www.les-arcenaulx.com/le-restaurant/la-carte/

For the meat lovers, "La ***Côte de Boeuf*"**, great meat, outstanding wine selection

https://www.lacotedeboeuf.net/la-carte-la-cote-de-boeuf-restaurant-marseille

Wine

https://www.lacotedeboeuf.net/cave-la-cote-de-boeuf-restaurant-marseille

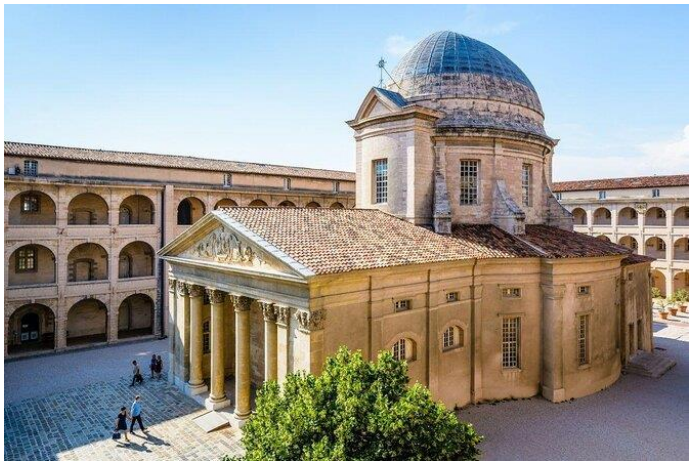
To visit (nearby)

MUCEM (museum) and the **Fort Saint Jean** (bridge)

Grotte Cosquer

https://www.grotte-cosquer.com/

La Vieille Charité (church and museum) dans le Panier



Cathédrale La Major

Le Pharo (on the other side of the Vieux Port)

Further

La Corniche Kennedy

Le Vallon des Auffes

Malmousque

Or the boat trip

https://www.croisieres-marseille-calanques.com/en/creeks-and-if-castle-visit/our-boat-tours

Much further : **les Calanques : les Goudes**