International hybrid Conference on

Oxygen Binding and Sensing Proteins

ROME, 6-9 SEPTEMBER 2022

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O2BiP 2022

BOOK OF ABSTRACTS

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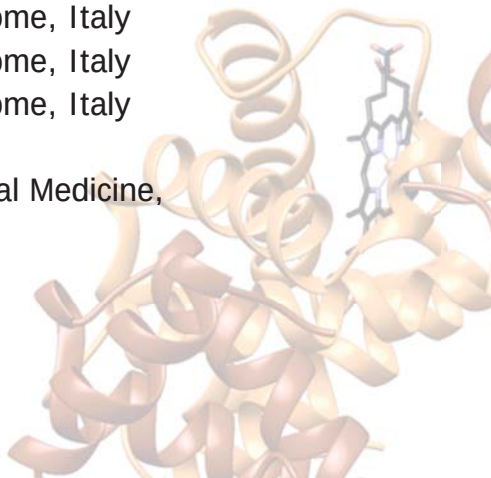

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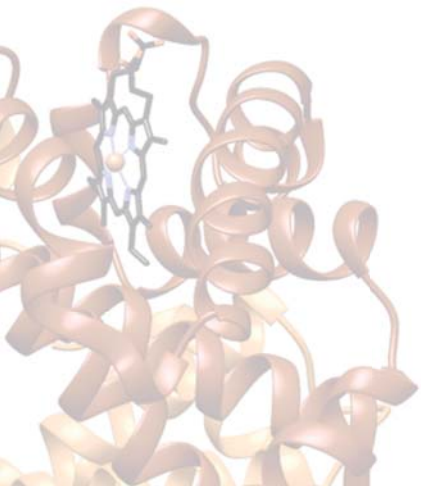

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Programme

Tuesday, September 6th

15:00-18:00 REGISTRATION

18:00-18:10 WELCOME

18:10-19:00 Session OPENING LECTURE: Thorsten Burmester and Thomas Hankeln - 20th Anniversary of Neuroglobin

18:10 *Thorsten Burmester* and *Thomas Hankeln*
Neuroglobin at the age of 22: finally reaching adulthood?
PRESENTER: *Thorsten Burmester*

19:00-21:00 Welcome Cocktail

Wednesday, September 7th

09:29-12:50 Session 1: BACTERIAL, PLANT AND INVERTEBRATE HEME-PROTEINS: STRUCTURE, FUNCTION AND EVOLUTION

CHAIRS:
F. Javier Luque, *Giulietta Smulevich* and *Martino Bolognesi*

09:30-10:50 Session Invited Speaker

09:30 *Markéta Martínková*
The structure and function relationships of heme-containing oxygen sensors

10:10 *Robert Clubb*
Good Eating: How Bacterial Pathogens Acquire Iron from Human Hemoglobin

10:50-11:10 Coffee Break

11:10-12:50 Session Oral Presentations

11:10 *Carina Osterhof*, *Ruben Petry*, *Annika Bast*, *Andreas Prothmann* and *Thomas Hankeln*
CRISPR-Cas9 mediated knockout of glob1 in Drosophila melanogaster
PRESENTER: *Carina Osterhof*

11:30 *Tim Loier*, *Patrick Laurent* and *Bart Braeckman*
Caenorhabditis elegans globin-3 functions in behaviour and reproduction
PRESENTER: *Tim Loier*

11:50 *Dima Svistunenko*, *Jacob Pullin*, *Justin Bradley*, *Geoffrey Moore*, *Nick Le Brun* and *Michael Wilson*
Two pathways of electron transfer from the haem in Escherichia coli bacterioferritin: iron mobilisation or antioxidant defence against H2O2?
PRESENTER: *Dima Svistunenko*

12:10 *Martina R. Nastasi*, *Vitaly B. Borisov* and *Elena Forte*
The non-canonical function of Cytochrome bd-II oxidase from Escherichia coli: a new peroxide scavenging enzyme
PRESENTER: *Martina R. Nastasi*

12:30 *Luciana Capece*, *Laia Julió Plana*, *Jaime E. Martinez Grundman*, *Dario A. Estrin* and *Juliette T. J. Lecomte*
Hexacoordination in the algal hemoglobin THB1: A combined computer simulation and experimental study
PRESENTER: *Luciana Capece*

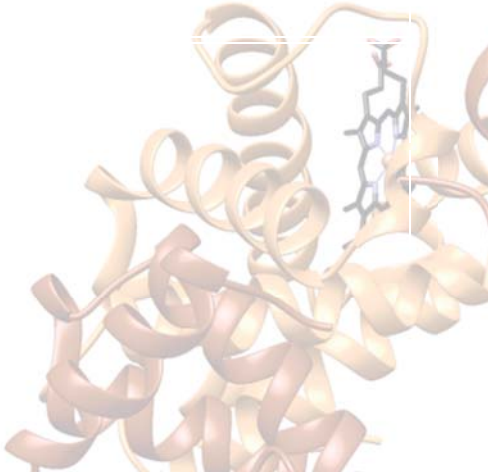
12:50-14:30 Lunch Break

12:50-14:30 Session Poster

Mark Shepherd
Drug repurposing approaches to target bacterial cytochrome bd oxidases

Ryan Sturms and *Max Brown*
Structural Determinants of Oxygen Transport in Phytoglobins
PRESENTER: *Ryan Sturms*

Sydney Dvorak and *Ryan Sturms*
Nitrite and Hydroxylamine Reduction by Bryophyte Hemoglobin
PRESENTER: *Sydney Dvorak*



Michelle Balling, *Carina Osterhof*, *Anne Bicker* and *Thomas Hankeln*
Myoglobin gene expression in breast and prostate cancer at single-cell resolution
PRESENTER: *Michelle Balling*

Caleb Northam, *Michael Berenbrink* and *Kevin Campbell*
Reductions in hemoglobin buffering capacity increase O2 delivery in pre-and post-natal high metabolic-rate birds and mammals
PRESENTER: *Kevin Campbell*

Alessandra Pesce, *Sylvia Dewilde*, *Luc Moens*, *Martino Bolognesi* and *Marco Nardini*
High-resolution crystal structure of the hexacoordinated nerve Hemoglobin of the bivalve mollusc Spisula solidissima
PRESENTER: *Alessandra Pesce*

Giovanna De Simone, *Francesca Bacigalupo*, *Andrea Pasquadibisceglie*, *Marianna Caterino*, *Margherita Ruoppolo*, *Paolo Ascenzi* and *Alessandra di Masi*
Nitrobindins: a new family of heme-based sensors
PRESENTER: *Giovanna De Simone*

Rasmus Hejlesen, *Kasper Kjær-Sørensen*, *Claus Oxvig* and *Angela Fago*
Generating myoglobin knockout and knockin zebrafish to study the role of myoglobin in metabolic rate and respiration
PRESENTER: *Rasmus Hejlesen*

Giovanna Bastari, *Virginia Solar Fernandez*, *Maria Marino* and *Marco Fiocchetti*
EXTRACELLULAR NEUROGLOBIN IN THE NEURONAL TRANSMISSION OF CELL RESILIENCE TO OXIDATIVE AND MITOCHONDRIAL STRESS
PRESENTER: *Giovanna Bastari*

14:29-17:30 Session 2: VERTEBRATE HEME-PROTEINS: STRUCTURE, FUNCTION AND EVOLUTION
CHAIRS:
Angela Fago, *Massimo Coletta* and *Dario Estrin*

14:30-15:10 Session Invited Speaker

14:30 *Christian Damsgaard*
Evolutionary interactions between hemoglobin function and vision in vertebrates

15:10 *Paola Corti*
Assessing novel functions of cellular heme globins using CRISPr/Cas9 knock-out in zebrafish models.

15:50-16:10 Coffee Break

16:10-17:30 Session Oral Presentations

16:10 *Michael Berenbrink* and *Kevin L. Campbell*
Myoglobin as a sulphur store during the catastrophic feather moult of penguins
PRESENTER: *Michael Berenbrink*

16:30 *Hans Malte* and *Gunnar Lykkeboe*
How the Bohr effect shapes the oxygen equilibrium curve and why that leads to an underestimation of it importance for gas exchange
PRESENTER: *Hans Malte*

16:50 *Brandon Reeder*, *Giuseppe Deganutti*, *John Ukeri*, *Silvia Atanasio*, *Dimitri Svistunenko*, *Christopher Ronchetti*, *Juan Carlos Mobarec*, *Marten Vos*, *Michael Wilson* and *Christopher Reynolds*
Expression and characterization of a stable form of the circularly permuted globin domain of human androglobin
PRESENTER: *Brandon Reeder*

17:10 *Antonio Tsuneshige*
Stepwise Reconstruction of Hemoglobin From Its Subunits

20:15-21:15 Journeys through Ancient Rome: Forum of Augustus (optional)

Thursday, September 8th

09:29-12:30 Session 3: HEME-BASED SENSORS

CHAIRS:

Alessandra Pesce, Alessandra DiMasi and Mark Shepherd

09:30-10:50 Session Invited Speaker

09:30 Giovanna De Simone

Nitrobindins: heme-based sensors evolutionarily conserved

10:10 Mark Shepherd

Evolution of oxygen-dependent respiratory complexes and drug repurposing approaches to target the cytochrome bd quinol:oxygen oxidoreductases

10:50-11:10 Coffee Break

11:10-12:30 Session Oral Presentations

11:10 Jakub Vavra, Artur Sergunin, Toru Shimizu and Marketa Martinkova

Kinetic analysis of the heme regulated inhibitor in the presence of various nucleotide triphosphates and sulphur species

PRESENTER: Jakub Vavra

11:30 Kenichi Kitanishi

Characterization of a Cobalt Porphyrin-substituted Globin-coupled Oxygen Sensor

11:50 Juan Cruz Palermo, Melisa Carllinni Colombo, Jonathan Alexis Semelak, Fernando Martin Boubeta, Dario Ariel Estrin and Sara Elizabeth Bari

Sulfide-mediated autocatalytic reduction of metmyoglobin

PRESENTER: Juan Cruz Palermo

12:10 Matthew Dent, Anthony DeMartino, Kaitlin Bocian, Jason Rose, Jesus Tejero and Mark Gladwin

Elucidating ligand binding selectivity in the heme-dependent, carbon monoxide-sensing transcription factor PxRcoM-1

PRESENTER: Matthew Dent

12:30-14:30 Lunch Break

12:30-14:30 Session Poster

Zainab Hafideddine, Niels Van Brempt, Tim Loier, Roberta Sgammato, Roy Aerts, Christian Johannessen, Wouter Herrebout, Bart Braeckman, Dietmar Hammerschmid, Luc Moens, Sylvia Dewilde and Sabine Van Doorslaer

Biophysical characterization of the cysteine-rich globin-3 from Caenorhabditis elegans

PRESENTER: Luc Moens

Michel Seiwert, Flores Kneilmann, Kim Klein and Thomas Hankeln

Phylogenetic conservation of Globin Y expression sites

Andrea Pasquadibisceglie, Giovanna De Simone, Alessandra di Masi, Andresa Messias da Silva, Mauro Bringas, Dario A. Estrin, Paolo Ascenzi and Fabio Polticelli

Computational study of ligand binding to Nitrobindins

PRESENTER: Andrea Pasquadibisceglie

Giovanna De Simone, Federico Sebastiani, Roberta Toti, Alessandra Pesce, Alessandra di Masi, Giulietta Smulevitch, Massimo Coletta and Paolo Ascenzi

Structural and functional characterization of Danio rerio nitrobindin.

PRESENTER: Giovanna De Simone

Brandon Reeder, Michelle Simons, Miranda Melis, Daniel Hofmaenner, Michael Wilson, Chris Cooper, Alex Dyson and Mervyn Singer

Using genetic engineering to target the toxic effects of hemoglobin for a new generation of hemoglobin -based oxygen carrier

PRESENTER: Brandon Reeder

Jeffrey Asaju, Michael Wilson, Christopher Reynolds and Brandon Reeder
Expression and characterization of N-terminal calpain-like domain of human androglobin
PRESENTER: Jeffrey Asaju

Juan Cruz Palermo, Melisa Carllinni Colombo, Magalí Scocozza, Daniel H. Murgida, Fernando M. Boubeta, Darío A. Estrin and Sara E. Bari
Disulfane-mediated reduction of metmyoglobin
PRESENTER: Sara E. Bari

Kajal Yadav, Mohd. Asim Khan and Suman Kundu
Structural and Functional Characterization of Recombinant Human Hemoglobin as an Artificial Oxygen Carrier
PRESENTER: Kajal Yadav

Jordi Zamarreño Beas, Marco Videira, Joana Batista and Ligia Saraiva
Role of haem biosynthesis in Gram-negative pathogens
PRESENTER: Jordi Zamarreño Beas

Samantha Henry, Calum Webster and Mark Shepherd
Drug repurposing approaches to target bacterial cytochrome bd oxidases
PRESENTER: Samantha Henry

Katerina Barmpidi, Elnaz Aledavood, Federico Issoglio, Dario A. Estrín, Carolina Estarellas and F. Javier Luque
Targeting a putative reductase partner of the truncated hemoglobin N in Mycobacterium tuberculosis
PRESENTER: Katerina Barmpidi

14:29-17:30 Session 4: HYPOXIA RESPONSE AND ADAPTATION - a Tribute to Guido di Prisco
CHAIRS:
Jay Storz, Thomas Hankeln and Cinzia Verde

14:30-15:50 Session Invited Speaker

14:30 Jacob Daane and H William Detrich
Global paleoclimate change and the loss of erythrocytes by Antarctic icefishes
PRESENTER: H William Detrich

15:10 Graham Scott
Physiological implications of haemoglobin evolution in high-altitude deer mice

15:50-16:10 Coffee Break

16:10-17:30 Session Oral Presentations

16:10 Alena Krüger, Julia Orth, Andrej Fabrizius and Thorsten Burmester
Overexpression of neuroglobin improves neuronal cell survival in stress experiments
PRESENTER: Alena Krüger

16:30 Annette Schlosser, Kathrin Helfenrath, Thorsten Burmester and Andrej Fabrizius
Cytoglobin 1 knockout causes age-dependent degenerative changes and stress response in Danio rerio
PRESENTER: Annette Schlosser

16:50 Daniela Giordano, Alessandra Pesce, Stefano Bruno, Sylvia Dewilde, Paolo Ascenzi, Francisco Luque, Martino Bolognesi, Cristiano Viappiani, Guido di Prisco and Cinzia Verde
Structural and functional characterization of globins in the Antarctic fish
PRESENTER: Daniela Giordano

17:10 Gesa Poetzsch, Luca Jelacic, Yukiko Gaudreault and Thomas Hankeln
The adaptation of the Nannospalax galili transcriptome to life under hypoxia – a key to longevity?
PRESENTER: Gesa Poetzsch

-23:00 Private guided tour of Papal Basilica St. Paul Outside-The-Walls and social dinner (optional)

Friday, September 9th

View this program: with ssession overviewtalk overview

09:29-12:30 Session 5: HEME-PROTEINS IN HEALTH AND DISEASES

CHAIRS:

Alberto Boffi, *Beatrice Vallone* and *David Hoogewijs*

09:30-10:50 Session Invited Speaker

09:30 *Michael Wilson* and *Brandon Reeder*
The pathophysiological consequences of the peroxidatic activities of oxygen binding proteins
PRESENTER: *Michael Wilson*

10:10 *Chandrasekhar Natarajan*, *Anthony Signore*, *Naim Bautista*, *Hoffmann Federico*, *Jeremy Tame*, *Hideaki Moriyama*, *Angela Fago* and *Jay Storz*
Evolution and molecular basis of a novel allosteric property of crocodilian hemoglobin
PRESENTER: *Jay Storz*

10:50-11:10 Coffee Break

11:10-12:30 Session Oral Presentations

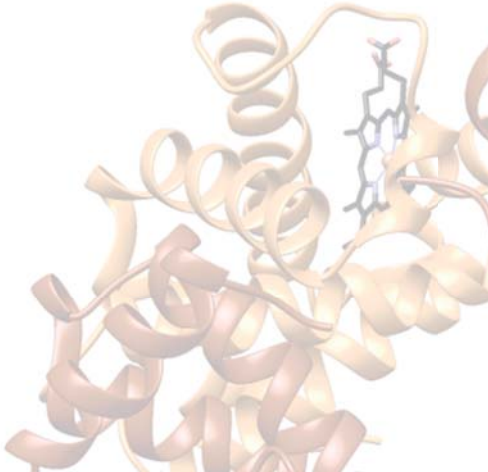
11:10 *Le Thuy*, *Vu Ngoc Hieu*, *Hoang Hai* and *Norifumi Kawada*
Anti-fibrotic Capacity of Extracellular Globins via Scavenging Reactive Oxygen Species
PRESENTER: *Le Thuy*

11:30 *Anna Keppner*, *Saar Adriaensen*, *Darko Maric*, *Miguel Correia*, *Teng Wei Koay*, *Carina Osterhof*, *Thomas Hankeln* and *David Hoogewijs*
Androglobin, a chimeric mammalian globin, is associated with ciliogenesis
PRESENTER: *Anna Keppner*

11:50 *Mostafa Aboouf*, *Julia Armbruster*, *Max Gassmann*, *Glen Kristiansen*, *Anne Bicker*, *Tom Hankeln*, *Hao Zhu* and *Thomas Gorr*
Wide ranging roles of myoglobin in breast epithelia: from shuttling fatty acids to delimiting the malignant transformation of cells
PRESENTER: *Thomas Gorr*

12:10 *Stefania Abbruzzetti*, *Cristiano Viappiani*, *Pietro Delcanale*, *Stefano Bruno*, *Montserrat Agut*, *Santi Nonell*, *Massimiliano Tognolini*, *Carmine Giorgio*, *Paolo Bianchini* and *Alberto Diaspro*
Targeted photodynamic therapy using heme proteins
PRESENTER: *Stefania Abbruzzetti*

12:30-14:30 Closure of the Meeting and Concluding Remarks



Opening Lecture

Neuroglobin at the age of 22: finally reaching adulthood?

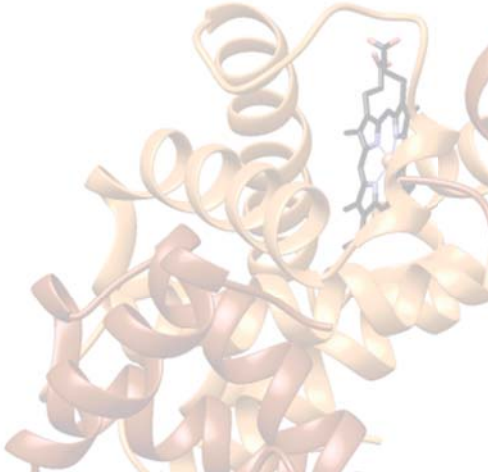
Thorsten Burmester[1] & Thomas Hankeln[2]

[1]Faculty of Biology, Molecular Animal Physiology, University of Hamburg, Hamburg Germany
[2]Facuöty of Biology, Institute of Organismic and Molecular Evolution, Molecular Genetics and Genome Analysis, Johannes Gutenberg University Mainz, Mainz, Germany

Identified more than 20 years ago in the growing amount of mammalian genome data, neuroglobin (Ngb) was the first „novel“ member of the metazoan globin gene family besides the well-known hemo- and myoglobins. Its discovery started a world-wide and successful search for additional members of the globin family with novel expression sites and functions, namely cytoglobin, androglobin and globin X. Importantly, the Ngb discovery initiated a multitude of successful (and pleasant!) scientific collaborations between workers in the globin field and beyond. Ironically, despite its early identification in 1999 and its evolutionary conservation along large parts of the metazoan phylogenetic tree, the molecular function(s) of Ngb in vivo are not yet entirely understood. With more than 740 citations in PubMed, it is meanwhile difficult, if not impossible to review all aspects of Ngb biology. We will therefore focus on aspects of Ngb research which may guide future research on this gene/protein, with the aim to ultimately promote this still somewhat adolescent (globin) family member to a respected (i.e. textbook-ready) adult.

SESSION 1

Bacterial, plant and invertebrate heme-proteins: structure, function and evolution



Invited Speaker

The structure and function relationships of heme-containing oxygen sensors

Markéta Martínková

Charles University, Faculty of Science, Department of Biochemistry, Hlavova 2030/8, Prague 2, 128 00 Czech Republic,
marketa.martinkova@natur.cuni.cz

Keywords: heme-containing sensor proteins, oxygen sensors, signal transduction, histidine kinase

In heme-containing oxygen-sensing proteins, heme acts as the sensing site for binding oxygen molecules and indirectly regulates many physiological functions, including the activities of protein kinases, in response to O2 availability. Conceptually, these proteins are always composed of at least two domains: one is a sensing domain (heme-based oxygen sensing), and the other is a functional domain. However, the structure-function relationship and mechanisms of communication between these domains have not been fully understood.

Therefore, we selected several model systems, including a globin-coupled histidine kinase, AfGcHK, to study the signal transduction in these hemoproteins. The AfGcHK is a part of the two-component signal transduction system from the soil bacterium Anaeromyxobacter sp. Fw109-5. Once the O2 (as the first signal) binds to the heme iron complex in the sensor domain of AfGcHK, the functional domain is stimulated, leading to autophosphorylation at a conserved His residue in the functional domain. The phosphate group of phosphorylated AfGcHK is transferred to the cognate response regulator.

Since most heme-based oxygen sensors form very flexible structures, studying them in their full-length, wild-type forms is challenging. However, only such approaches to studying the full-length proteins can reveal the signal transduction mechanism between the sensing and function domains. Thus, the detailed enzyme kinetic study was combined with the hydrogen-deuterium exchange experiments associated with mass spectrometry. It was suggested that the dimerization interface of the sensing domain is essential for signal transmission from the sensing domain to the functional domain. All data together will be discussed to illustrate the signal transduction mechanism in the model system.

Supported by the grant 8F20011 from The Ministry of Education, Youth and Sports.

Invited Speaker

Good Eating:
How Bacterial Pathogens Acquire Iron from Human Hemoglobin

Brendan Mahoney[1], Kat Ellis-Guardiola[1], Joseph Clayton[2], Jeff Wereszczynski[2] and Robert T. Clubb[1]

[1]UCLA Department of Chemistry and Biochemistry, Los Angeles, CA; [2]Illinois Institute of Technology, Chicago, IL (USA)

Iron is an essential micronutrient that is required by bacterial pathogens to proliferate and cause disease. During infections, microbial pathogens forage iron from human hemoglobin (Hb), as it contains ~70% of the human body's total iron content in the form of heme. Bacteria access Hb when it is released from erythrocytes that have either spontaneously ruptured or been lysed by bacterial cytotoxins. Here, I discuss my laboratory's recent efforts to understand how pathogenic Staphylococcus aureus and Streptococcus pyogenes bacteria harvest iron from Hb. These microbes display receptors that remove heme from Hb despite its pico-molar affinity for this ligand. Using a combination of structural, biophysical and computational methods we show that these pathogens harvest heme using distinct mechanisms. In S. aureus, the surface displayed IsdB/IsdH receptors actively extract heme by distorting Hb's F-helix, accelerating heme release more than a 1,000-fold by reducing the energy needed to rupture the axial HisF8 Nϵ-Fe^{3+} bond. Directed inter-domain motions within the receptor play a critical role in the extraction process by transiently positioning the heme extraction unit near the F-helix. In contrast, recent studies of the Hb receptor from S. pyogenes, called Shr, reveal that this microbe employs a distinct molecular solution to capture Hb's heme. New structural data indicate that it preferentially recognizes the heme-loaded form of Hb by directly binding to heme's exposed propionate groups. Based on native mass spectrometry, SAXS, and heme transfer measurements we show that Shr initially removes heme from the β subunits in Hb. We propose that the receptor transiently unlatches from the β subunits to facilitate heme release and capture by the receptor's heme binding domain. Collectively, the results of this work provide new insight into how microbial pathogens exploit metal containing oxygen binding and sensing proteins as nutrient sources.

Oral Presentation

CRISPR-Cas9 mediated KO of glob1 in Drosophila melanogaster

Carina Osterhof*[1], Ruben Petry[1], Annika Bast[1], Andreas Prothmann[1] and Thomas Hankeln[1]

[1]Molecular Genetics & Genome Analysis, iOME, Johannes-Gutenberg-University Mainz, Germany,
*email:caosterh@uni-mainz.de

Globins are phylogenetically ancient proteins and thus also belong to the standard gene repertoire of arthropods. Three ancestral globin lineages, termed HbL (hemoglobin-like), GbX (globin X) and GbXL (globin X-like) have been defined in arthropod genomes, but arthropod taxa differ in the presence and copy number of these gene lineages. Brachyceran Diptera, for example, lack the GbX and GbXL lineages, but harbour different numbers of globins of the HbL class. The model species *Drosophila melanogaster* (Dmel) contains a "major" HbL copy named glob1 plus two other gene duplicates, glob2 and 3, which both are exclusively expressed in testis tissue.

Dmel glob1 is found in all developmental stages, with particularly high amounts in fat body and trachea. Our reanalysis of expression levels and gene structure revealed that glob1 is expressed in two different modes: one promotor conveys ubiquitous, but low expression whereas activation of the second one often results in high levels of mRNA. Recent studies on three different types of experimental *knockdown* systems suggested an unexpected variability of glob 1 deficiency phenotypes, ranging from a reduction in lifespan under hypoxia or even normoxia to severe developmental defects. However, residual expression levels, potential off target effects and the influence from the two different transcription start sites probably complicated the interpretation of these results. We therefore established a complete genetic knockout of *Dmel* glob1 via CRISPR/Cas9. Preliminary transcriptomic and phenotypic analyses confirm developmental defects, but to a much lesser extent than described in earlier studies. In contrast, we found no evidence for a reduced life span under normoxic conditions. These results demonstrate the need for a true genetic KO system for functional analysis of phenotypical defects. Future studies with a focus on high glob1-expressing organs such as the fat body may shed light on the physiological function of glob1.

Oral Presentation

Caenorhabditis elegans globin-3 functions in behaviour and reproduction

Loier, T.*[1], Laurent, P.[2], Braeckman, B.P. °[1]

*lead presenter; ° corresponding author

[1] Laboratory for Aging Physiology and Molecular Evolution, Ghent University, BELGIUM, Bart.Braeckman@UGent.be

[2] Laboratory for Neurophysiology, ULB Neuroscience Institute (UNI), Brussels, BELGIUM, patrick.laurent@ulb.be

Keywords: *Caenorhabditis elegans*, globin 3, *glb-3*, neuron, reproduction

The genome of the nematode model organism *Caenorhabditis elegans* encodes 34 globins, most of which have not been functionally characterized yet. Globin 3 is a small, cysteine-rich, *C. elegans* globin that is predicted to be spliced in two isoforms (210 and 282 AA respectively). It is expressed predominantly in a specific set of neurons, the somatic gonad, and vulval muscles. This expression pattern agrees with the abnormal behaviour and sterility of the homozygous mutant.

In the *glb-3* knockout mutant, locomotion is heavily impaired with reduced speed and exaggerated head curvatures as major hallmarks, hinting at dysregulation of motor neurons. Also, pharyngeal pumping is reduced by about 50%, which links to the presence of GLB-3 in pharyngeal neurons in wild-type worms. *glb-3* mutants are less responsive to organic attractants but show a normal response to O_2 and CO_2. Although impaired food sensing has been linked to longevity, *glb-3* mutants have a normal lifespan.

Mutation of *glb-3* causes complete sterility resulting from severe underdevelopment and malformation of the gonad. In the oviduct, the number of oocytes is often reduced in number and size, while the terminal oocyte often appears bloated.

The overall expression pattern as well as the protein structure of GLB-3 resembles that of GLB-12, a known superoxide generator that works in concert with superoxide dismutase to signal to the germline. For *glb-3*, we found a mild interaction with the mitochondrial *sod-2* and *sod-3* in the neuronal phenotypes (pharyngeal pumping rate and chemosensing, respectively).

In summary, GLB-3 is likely a redox active protein that optimizes the activity of a specific set of neurons and regulates oocyte maturation in the gonads of *C. elegans*.

Oral Presentation

Two pathways of electron transfer from the haem in Escherichia coli bacterioferritin: iron mobilisation or antioxidant defence against H_2O_2?

Svistunenko, D.A.*°[1], Pullin, J.[2], Bradley, J.M.[3], Moore, G.R.[3], Le Brun, N.E.[3], Wilson, M.T.°[1]

* lead presenter; ° corresponding author

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Keywords: bacterioferritin, haem, heme, ferroxidase centre, electron transfer, H_2O_2, antioxidant

Ferritins play the key role in iron metabolism in practically all life forms. Typically made up of 24 subunits, one ferritin molecule takes a shape of a sphere with a hollow cavity. Ferritins oxidise soluble Fe^{2+} to poorly soluble Fe^{3+} and deposit the ferric iron in the form of a mineral inside the cavity (up to several thousand iron atoms). Such ubiquitous iron storage proteins seems to be an evolutionary response of the contrariety of the high importance of iron for life and its scarce availability.

Bacterioferritins are distinct type of ferritins that contain haem prosthetic groups – at the interface of two subunits. Bacterioferritin from Escherichia coli (EcBfr) is a homo-24-mer, each subunits containing a di-iron motif called ferroxidase centre (FC). Thus, there are two distinct iron motifs in EcBfr – each molecule contains 24 FCs and up to 12 haem groups. It appear that the two motifs play roles in two opposing processes: the FC is where O_2 binds and oxidises iron in the mineralisation process; whereas the haem, when reduced, can provide an electron via an electron transfer (ET) pathway to the mineral core thus enabling iron reduction and mobilisation for cell's needs.

We have found another ET pathway from reduced haem - to the FC.[1] We also found that iron oxidation at FC by H_2O_2 is 1000-fold faster than by O_2.[2] Taken together these two findings allow hypothesis that primary function of EcBfr is antioxidant defence against H_2O_2, not iron sequestering, and that FC acts as an active site of a true enzyme exhibiting redox cycling while relaying electrons from the haem to H_2O_2 thus reducing it to water and thereby providing an antioxidant defence.

1. Pullin et al., (2021), Angew Chem Int Ed Engl 60, 8376-8379.
2. Pullin et al., (2021), Angew Chem Int Ed Engl 60, 8361-8369.

Oral Presentation

The non-canonical function of Cytochrome bd-II oxidase from Escherichia coli: a new peroxide scavenging enzyme

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Keywords: respiratory chain, terminal oxidase, heme, reactive oxygen species

The Escherichia coli respiratory chain contains three terminal oxidases that catalyze the reduction of O_2 to $2H_2O$ to generate ATP: a heme copper cytochrome bo_3 and two bd-type oxidases, bd-I and bd-II containing the low-spin heme b and the two high-spin hemes b and d. E. coli bd-II is relatively poorly characterized and its function less clear [1], but it seems to be involved in different physiological processes compared to the other oxidases as its genes are expressed at different environmental and O_2 conditions. Remarkably, in the presence of H_2O_2, bd-II provides a fitness advantage during anaerobic growth to E. coli in the inflamed murine intestine and to Salmonella in the streptomycin-treated gut [2]. Therefore, we investigated whether of E. coli bd-II plays a role in H_2O_2 metabolism and tolerance, in addition to its function in bioenergetics. Polarographic O_2 measurements have shown that preparations of the untagged bd-II oxidase from E. coli strain decomposes H_2O_2 to O_2 with a high rate by producing half a mole of O_2 per mole of H_2O_2. Such catalase activity is insensitive to N-ethylmaleimide (the sulfhydryl binding compound), antimycin A (a ligand to a quinol binding site) and CO that binds to the reduced heme d. In contrast, the catalase reaction is inhibited by cyanide (IC =4.5 0.5 μM), azide and it vanishes when cytochrome bd-II is converted into the fully reduced state or inactivated by thermal denaturation, suggesting a role of heme-b in the hydrogen peroxide scavenging activity. The ability of cytochrome bd-II to detoxify H_2O_2 could play a role in bacterial physiology by conferring resistance to the peroxide-mediated stress.

[1] Grauel et al., (2021) Nat. Commun., 12:6498

[2] Borisov et al., (2021) Antioxid. Redox Signal.34, 16: 1280-1318

Oral Presentation

Hexacoordination in the algal hemoglobin THB1: A combined computer simulation and experimental study

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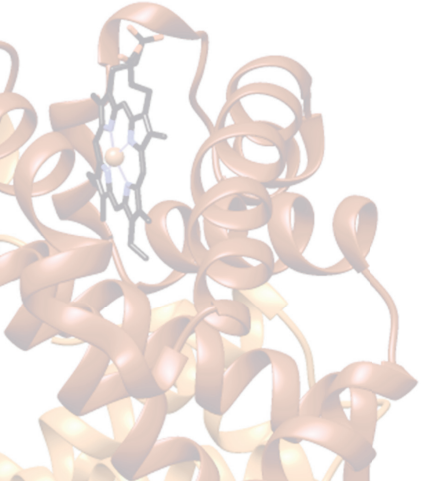
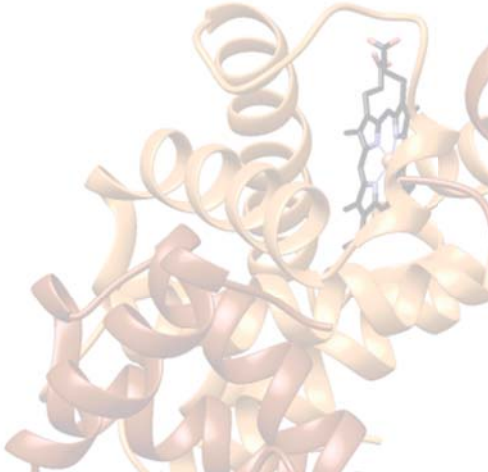
Keywords: Hexacoordination; Lysine ionization; Molecular dynamics; QM/MM calculation; THB1; Truncated hemoglobins.

THB1 is a monomeric truncated hemoglobin from the green alga Chlamydomonas reinhardtii. In the absence of exogenous ligands and at neutral pH, the heme group of THB1 is coordinated by two protein residues, Lys53 and His77. THB1 is thought to function as a nitric oxide dioxygenase, and the distal binding of O_2 requires the cleavage of the Fe-Lys53 bond accompanied by protonation and expulsion of the lysine from the heme cavity into the solvent. Nuclear magnetic resonance spectroscopy and crystallographic data have provided dynamic and structural insights of the process, but the details of the mechanism were not fully elucidated. We applied a combination of computer simulations and site-directed mutagenesis experiments to shed light on this issue. Molecular dynamics simulations and hybrid quantum mechanics/molecular mechanics calculations were performed to explore the nature of the transition between the decoordinated and lysine-bound states of the ferrous heme in THB1. Lys49 and Arg52, which form ionic interactions with the heme propionates in the X-ray structure of lysine-bound THB1, were observed to assist in maintaining Lys53 inside the protein cavity and play a key role in the transition. Lys49Ala, Arg52Ala and Lys49Ala/Arg52Ala THB1 variants were prepared, and the consequences of the replacements on the Lys (de)coordination equilibrium were characterized experimentally. Additionally, the "Lys-off" X-ray structure, represented by the cyanide adduct of the Fe(III) protein, allowed to hypothesize that interactions that differ between the known "Lys-on" structure and the Lys-off structure participate in the control of Lys53 affinity for the heme iron. The results reinforced the dynamic role of protein-propionate interactions and strongly suggested that cleavage of the Fe-Lys53 bond and ensuing conformational rearrangement is facilitated by protonation of the amino group inside the distal cavity.



SESSION 2

Vertebrate heme-proteins: structure, function and evolution



Invited Speaker

Evolutionary interactions between hemoglobin function and vision in vertebrates

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Keywords: hemoglobin, evolution, oxygen secretion, vision, retina

The light-absorbing retina within the eye has an exceptionally high oxygen demand, which imposes two conflicting needs: high rates of blood perfusion and an unobstructed light path devoid of blood vessels. Improved oxygen release from hemoglobin via the Root effect represents a physiological solution to improve the oxygen supply to the retina without increasing the density of light-scattering blood vessels. In this presentation, I will 1) present a new molecular mechanism underlying Root effect-mediated oxygen supply to the retina, 2) show how the evolutionary origin of this mechanism supported the evolution of sharp vision in the ray-finned fishes, and 3) discuss how biochemical constraints in hemoglobin of terrestrial vertebrates led to fundamentally different respiratory mechanisms for retinal oxygen supply in sharp-sighted tetrapods.

Invited Speaker

Assessing novel functions of cellular heme globins using CRISPr/Cas9 knock-out in zebrafish models.

Paola Corti

The globin proteins are found throughout evolution in all kinds of organisms and their function primarily depends on their heme active site. Based on its redox status, the iron heme can bind different ligands or can favor electron transfer reactions to exert different functions such as decomposition or production of NO, detoxification from reactive oxygen species or intracellular signaling. These biological functions are relevant in many systems and presumably have some implications in development and tissue regeneration. Our goal is to understand the role of globins in biology and we use the zebrafish as model to unravel the function of globins during development and heart regeneration. We analyzed transcript and protein levels and found that myoglobin, cytoglobin1 and cytoglobin2 are expressed in the adult heart and in the embryos in different cell types. We generated the zebrafish knock-out using CRISPr/Cas9 mutagenesis and discovered that myoglobin and cytoglobin1 are important during heart regeneration while cytoglobin2 appears to be involved in heart development. The advent of genome sequencing resulted in the discovery of a host of previously unidentified globin proteins expressed outside of red blood cells and skeletal muscle, changing the paradigm through which we understand this well studied family of proteins. While heme globins such as myoglobin and cytoglobin are highly conserved in vertebrates, their physiological functions have been unknown, largely related to inconsistent and mild to absent observed phenotypes in mice. Here we present valuable zebrafish models, amenable of genetic manipulations, useful to potentially filling the gap in knowledge on the role of globins in biological systems.

Oral Presentation

Myoglobin as a sulphur store during the catastrophic feather moult of penguins

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Keywords: myoglobin, penguins, environmental adaptation

The annual feather moult in penguins is critically important for survival and reproduction and a recognised energetic bottleneck, because penguins cannot enter water and hunt for several weeks during this time and need to renew their whole plumage from internal stores in what is known as a catastrophic feather moult.

Feathers consist of more than 90% proteins that are exceptionally rich in the sulphur (S)-containing amino acid cysteine, enabling crosslinked disulphide bridges for structural stability. Because of a low average S content of non-feather proteins, an exceptionally high amount of body protein needs to be broken down to provide S for the complete renewal of the feather coat.

Pre-moult 'fattening' of penguins includes a built-up of pectoral muscle mass with high levels of O_2-storing myoglobin (Mb) that supports the prolonged breath-hold feeding dives of these animals and is subsequently broken down during moult fast. Here we test for a novel role of Mb as S store that is recycled during catastrophic feather moult by analysing Mb primary structures of all extant penguin species obtained from feather DNA extracts and recently released whole genome sequences. We found a 2- to 3.5-fold higher S content in penguin compared to other avian or non-avian Mbs. Mb S content was negatively correlated with body mass, consistent with a lower surface area to volume ratio in larger birds and hence lower relative feather mass. Ancestral reconstructions of maximum Mb concentration and specific S content revealed concurrent increases already in the last common ancestor of extant penguins, suggesting a unique co-option of O_2-storing Mb as a S store as long as 16-20 million years ago.

Oral Presentation

How the Bohr effect shapes the oxygen equilibrium curve and why that leads to an underestimation of it importance for gas exchange

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Keywords: Bohr effect, Haldane effect, Oxygen affinity, Gas exchange

Despite the fact that the Bohr and Haldane effects are of equal size at the molecular level due to their thermodynamic linkage, the influence of the Bohr effect on the delivery of blood borne oxygen to the respiring tissues has been deemed secondary to the influence of the Haldane effect on the uptake of carbon dioxide by the blood. Here we show that this is wrong. Using a simple two-ligand, two-state formulation we modelled the simultaneous oxygen and proton binding to hemoglobin as well as the resulting acid-base changes of the surrounding solution. When the Bohr effect is blocked in this model system, we see a dramatic increase in the oxygen affinity, with a fall in oxygen half saturation pressure (P_{50}) from 27 to 6mmHg. We also show that the P_{50} and the Bohr factor are not independent but directly related. Thus, changes in hemoglobin structure that lead to changes in the Bohr factor will inevitably also change hemoglobin oxygen affinity. The physiological importance of the Bohr effect on oxygen unloading cannot be assessed by comparing oxygen equilibrium curves measured in the lab at different constant Pco_2 or pH because each of these curves are already shaped by the Bohr/Haldane effect.

Oral Presentation

Expression and characterization of a stable form of the circularly permuted globin domain of human androglobin

Brandon J. Reeder[1], Giuseppe Deganutti[1,3], John Ukeri[1], Silvia Atanasio[1], Dimitri A. Svistunenko[1], Christopher Ronchetti[1], Juan Carlos Mobarec[1], Marten H. Vos[2], Michael T. Wilson[1], and Christopher A. Reynolds[1,3]

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Keywords: Androglobin, protein expression, molecular dynamics, homology modelling, disulfide

Androglobin, is a multi-domain hemoglobin with a circularly permuted globin domain which is split by an IQ calmodulin binding motif. Expression of this 190 kDa protein *in vivo* is correlated with overexpression of FOXJ1, a crucial transcription factor of ciliogenesis(1). Since its initial discovery in 2012, efforts to generate and study a stable form of the circularly permuted globin by recombinant expression have proven unsuccessful. Using a remote homologue alignment method, molecular modelling and molecular dynamics, we identified the alignment to other hemoglobins. Validation of our proposed alternative helix alignment lies, at least in part, in the generation of a stable recombinant form of the globin domain protein, which we have characterized. As expressed, the heme iron is hexacoordinate in the ferrous form but partially pentacoordinate in the ferric form, similar to that observed for some other globins such as cytoglobin. A disulfide bond is also detected in a corresponding position to that observed in neuroglobin, stabilizing the heme binding and significantly affecting the reactivity with some ligands. This opens up future research into examining the behavior of the androglobin *in vitro* and hence its potential mechanism of action in ciliogenesis and spermatogenesis.

(1) Koay, T. W.; Osterhof, C.; Orlando, I. M. C.; Keppner, A.; Andre, D.; Yousefian, S.; Suarez Alonso, M.; Correia, M.; Markworth, R.; Schodel, J.; Hankeln, T.; Hoogewijs, D., Androglobin gene expression patterns and FOXJ1-dependent regulation indicate its functional association with ciliogenesis. J. Biol. Chem. 2021, 296, 100291.

Oral Presentation

Stepwise Reconstruction of Hemoglobin from Its Subunits − An Insight Into the Origin of Cooperativity –

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Keywords: hemoglobin, origin of cooperativity, reconstruction from subunits

In the present study, we have attempted to reconstruct step-by-step the tetrameric human hemoglobin (Hb) of the form $\alpha_2\beta_2$, by the controlled association of its isolated α- and β-subunits, and trace the origin of cooperativity. Normally, β-units form a tetramer of the form β_4, showing no cooperativity. Alkylation of its βCys112 with iodoacetamide or *N*-acetyl succinimide, produced monomers. Alkylation of βCys93 did not prevent association into oligomers. However, while alkylation of this residue with iodoacetamide did not affect the affinity for oxygen, alkylation with *N*-ethyl maleimide clearly reduced its affinity for oxygen ten times.

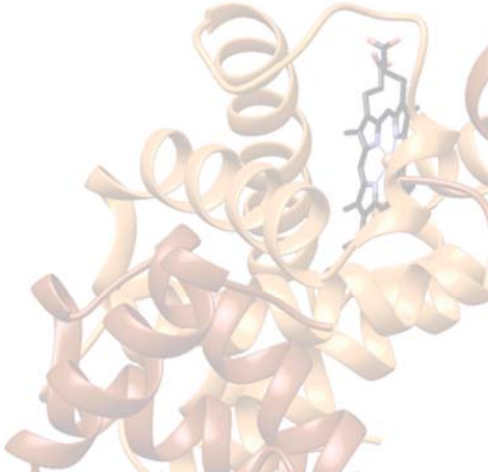
Alpha- and β-semiHbs are dimeric forms in which a heme-containing α- or β-subunit is attached to its complementary heme-depleted subunit (i.e., apo subunit), respectively. They do not show cooperativity, but their oxygen affinities are intermediate to the affinity for the first and last oxygen in native tetrameric Hb, indicating that the formation of the α-β interface modulates the affinities for oxygen of the isolated subunits.

Strikingly, when the residue βCys112 in these semihemoglobins were alkylated with bulky moieties, such as thiopyridyls, their oxygen affinity showed cooperativity and resembled that of a system with two oxygen-binding sites. Tetramer formation was confirmed by size-exclusion chromatography, indicating that the cross-talk between both the two heme-carrying β-subunits was indeed established, irrespective of the fact that both the α-subunits lacked heme. Moreover, chemical modification of the β Cys112, which is located in the α-β interface seem to play a pivotal role in the cooperativity involving both the β-subunits.

The binding for the first and second oxygen molecules showed affinities that were lower and higher than that of the monotonical unmodified semiHb, respectively.

SESSION 3

Heme-based sensors



Invited Speaker

Nitrobindins: heme-based sensors evolutionarily conserved

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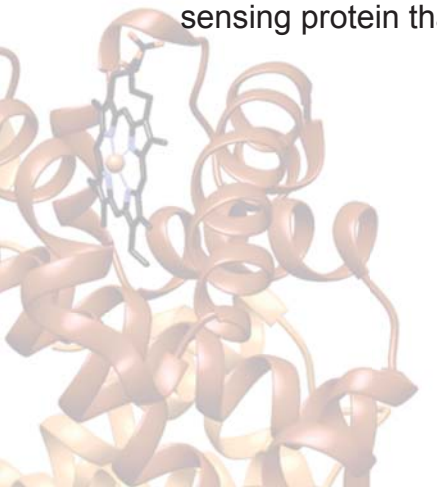
Keywords: Nitrobindins, heme-based sensors, reactive nitrogen species detoxification, NO scavenging.

Nitrobindins (Nbs) form a new class of evolutionary conserved heme-proteins characterized by a 10-stranded anti-parallel β-barrel fold. In Nbs, the heme-Fe atom is coordinated to a proximal His residue and is stable in the ferric form. Although the physiological role(s) of Nbs are still unclear, it has been postulated that they are involved in both NO/O2 and reactive nitrogen and oxygens species (RNS and ROS, respectively) metabolism1,2,3. To date, Mycobacterium tuberculosis Nb (Mt-Nb), Arabidopsis thaliana Nb (At-Nb), Homo sapiens Nb (Hs-Nb), and Danio rerio Nb (Dr-Nb) have been characterized by our research group from both the structural and functional viewpoints1,2,4,5. Interestingly, while Mt-Nb, At-Nb and Dr-Nb are single-domain proteins, Hs-Nb has been described as a domain of the human protein named THAP4[2,4]. THAP4 is composed of an N terminal modified zinc finger domain able to bind DNA and a C terminal Nb domain2,5,6. To define the role of THAP4 in RNS detoxification, silencing experiments have been performed in breast cancer MCF-7 human cells, in which the expression levels of THAP-4 results higher compared to other human cell lines. Besides, transcriptomic analyses have been performed in both wild-type and silenced cells after treatment with the NO-donor DEA NONOate. In addition, the THAP4 interactome has been retrieved using BioGRID and IntAct molecular interaction databases [7,8]. Finally, the Gene Ontology Resource and the web server QuickGo[9] have been used to investigate the biological processes involving THAP4. Overall, data obtained suggest an evolutionary conserved structure and anti-oxidant function of Nbs, and highlight a possible role of human THAP4 as a sensing protein that couples the heme-based Nb reactivity with gene transcription.



Invited Speaker

Evolution of oxygen-dependent respiratory complexes and drug repurposing approaches to target the cytochrome *bd* quinol:oxygen oxidoreductases

Mark Shepherd

School of Biosciences, University of Kent

The great oxidation event that led to an abundance of oxygen in the Earth's atmosphere delivered an attractive alternative as a respiratory electron acceptor, which has clearly had a dramatic impact upon the evolution of electron transport components. The first part of this talk will touch upon the evolution of oxygen-binding respiratory oxidoreductases and highlight the diversity of these protein complexes.

The second part of the talk will focus on Cytochrome *bd* complexes, which are found exclusively in prokaryotes and generate a proton motive force by coupling quinol oxidation to the reduction of dioxygen. Previous work has demonstrated that cytochrome *bd* complexes are important during infection for a variety of bacterial pathogens, including *E. coli* and *M. tuberculosis*, demonstrating their potential as drug targets. Herein, *in silico* tools were used to screen a library of approved drugs for their ability to inhibit cytochrome *bd*-I from *E. coli*. In order to investigate the efficacy and specificity of the top hits, mutant strains of *E. coli* that express either cytochrome *bd*-I or cytochrome *bo*' as the sole respiratory oxidase were used as a test system, and the expected haem signals of these respiratory oxidases were confirmed for these strains using difference spectroscopy. Membranes were isolated from these strains, and candidate drugs from the *in silico* analyses were tested for their ability to inhibit oxygen consumption by cytochrome *bd*-I and cytochrome *bo*' using an oxygen electrode. Selected drugs were identified as potent inhibitors of cytochrome *bd*-I, and further work has been undertaken to aid our understanding of their mechanisms of action and potential for broader applications in antimicrobial chemotherapy.

Oral Presentation

Kinetic analysis of the heme regulated inhibitor in the presence of various nucleotide triphosphates and sulphur species

Jakub Vavra*°[1], Artur Sergunin[1], Toru Shimizu[1], Marketa Martinkova[1]

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Keywords: heme sensor proteins, heme regulated inhibitor, sulphur species, nucleotide triphosphates

Heme sensor proteins detect changes of either heme (so called heme-responsive sensor proteins) or gas signalling molecules (so called heme-based gas sensor proteins) concentration in many cell types and subsequently modulate their specific functions such as enzymatic activity or DNA binding. Hence, these proteins are intensively discussed as potential therapeutic targets in many pathological processes including bacterial infections or cancer [Shimizu T. et al 2019. Chem. Soc. Rev. 48:5624-5657].

Specifically, heme regulated inhibitor (HRI) has been chosen as a model heme-responsive sensor protein in our study. It is a kinase of eukaryotic initiation factor 2α and therefore affects protein synthesis throughout this mechanism. If heme molecule interacts with HRI, the kinase activity is inhibited. Recently, we have focused on the kinetic analysis of HRI in the presence of various nucleotide triphosphates (NTPs) as their concentration in cells could be modulated as a result of cancer associated processes [Shugar D. 1996. Acta Biochim. Pol. 43:9-24]. In addition to that, HRI has been reported as a potential nitrogen monoxide sensor in cells [Martinkova M. et al 2007. FEBS Lett. 581:4109-4114]. Furthermore, various sulphur species (mostly hydrogen sulfide) has been recently discussed as potential signalling molecules [Kimura H. 2015. Antioxid. Redox Signal. 22:362-375], so we have studied their effect on the kinase activity of HRI.

The effect of HRI incubation with various NTPs as well as selected sulphur species on its kinase activity will be discussed. The results of this study will broaden our knowledge about heme sensor proteins mechanism of function as well as their importance in pathological processes including cancer.

Supported by the grant 158120 from the Grant Agency of Charles University.

CharacterizationofaCobaltPorphyrin-substitutedGlobin-coupledOxygenSensor

Oral Presentation

Characterization of a Cobalt Porphyrin-substituted Globin-coupled Oxygen Sensor

Kenichi Kitanishi*°[1]

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Keywords: heme, globin, oxygen sensor, cobalt porphyrin, histidine kinase

Globin-coupled histidine kinase from Anaeromyxobacter sp. Fw109-5 (AfGcHK) is an oxygen sensor enzyme in which oxygen binding to Fe(II) heme in the globin sensor domain substantially enhances its autophosphorylation activity. Here, we reconstituted AfGcHK with cobalt protoporphyrin IX (Co-AfGcHK) in place of heme (Fe-AfGcHK) and characterized spectral and catalytic properties of the full-length proteins. Spectroscopic analyses indicated that Co(III) and Co(II)-O_2 complexes were in a 6- coordinated low-spin state in Co-AfGcHK, like Fe(III) and Fe(II)-O_2 complexes of Fe-AfGcHK. Although both Fe(II) and Co(II) complexes were in a 5-coordinated state, Fe(II) and Co(II) complexes were in high-spin and low-spin states, respectively. The autophosphorylation activity of Co(III) and Co(II)-O_2 complexes of Co-AfGcHK were fully active, whereas that of the Co(II) complex was moderately active. This contrasts with Fe-AfGcHK, where Fe(III) and Fe(II)-O_2 complexes were fully active and the Fe(II) complex was inactive. Collectively, activity data and coordination structures of Fe-AfGcHK and Co-AfGcHK indicate that all fully active forms were in a 6-coordinated low-spin state, whereas the inactive form was in a 5-coordinated high-spin state. The 5-coordinated low-spin complex was moderately active—a novel finding of this study. These results suggest that the catalytic activity of AfGcHK is regulated by its heme coordination structure, especially the spin state of its heme iron. This study presents the first successful preparation and characterization of a cobalt porphyrin-substituted globin-coupled oxygen sensor enzyme, and may lead to a better understanding of the molecular mechanisms of catalytic regulation in this family.

Ref. Kitanishi, K. et al. (2021) ACS Omega 6, 34912-34919.

Oral Presentation

Sulfide-mediated autocatalytic reduction of metmyoglobin

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KEYWORDS. myoglobin, hydrogen sulfide, disulfane, sulfanyl radical, disulfanuidyl radical

The coordination of hydrogen sulfide, H2S, to ferric hemeproteins has been reported in more than 40 examples, an d forms moderately stable hexacoordinated low spin complexes, [FeIII(SH-)].1 The metal centered reduction has been reported in some cases, with varying timescales. Subsequent aerobic reactivity of the metmyoglobin complex, MbFeIII(SH-) has been reported and yields myoglobin, MbFeII, with the concomitant formation of thiosulfate, sulfite and polysulfides.2 Combining kineti c an d spectroscopic methods, we proposed a mechanism for the reduction of MbFeIII by excess sulfide, under argon atmosphere. Asymmetric S-shaped time-traces for the formation of MbFeII, under varying sulfide concentrations or pH were observed, pointing to an autocatalytic behavior. Further analysis of the time-traces at selected times revealed a secondary sigmoidal dependence on the initial concentration of sulfide, suggesting a full time-dose response. The slow initial phase is suggested to depend on the resonant form FeII(SH*) of the starting complex, yielding minor quantities of MbFeII and sulfanyl radical, SH*. The overall rate of the reaction is augmented with increasing pH, pointing to hydrosulfide, SH-, as a critical species in the early steps. We propose the intermediacy of the disulfanuidyl radical anion, HSS*2-, promoted under alkaline conditions by reaction of HS- and HS*, as a source of disulfane (HSS-).3 Significantly, the formation of MbFeII after the addition of HSS- to MbFeIII(SH-) is faster than the isolated addition of HS- or HSS- to MbFeIII(H2O), suggesting a synergistic effect, and pointing to HSS- as a key species in the steep increase of the reduction rate of MbFeIII by sulfide. Kinetic simulations of the sigmoidal traces assisted the evaluation of the proposed mechanism. The process has been termed reductive sulfhydration, after the well described reductive nitrosylation ; this denomination should be discussed, as ferrous forms do not form stable complexes with sulfide.

References

(1) Boubeta, F. et al. Antioxid Redox Signal. 2020, 32(4), 247-257 (10.1089/ars.2019.7878).

(2) Bostelaar, T. et al. J Am Chem Soc. 2016, 138 (27), 8476–8488 (10.1021/jacs.6b03456).

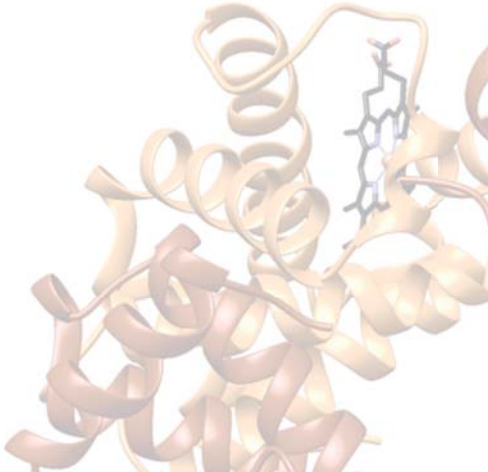
(3) Mills, G. et al. J Phys Chem. 1987, 91 (6), 1590–1596 (10.1021/j100290a060).

Oral Presentation

Elucidating ligand binding selectivity in the heme-dependent, carbon monoxide-sensing transcription factor PxRcoM-1

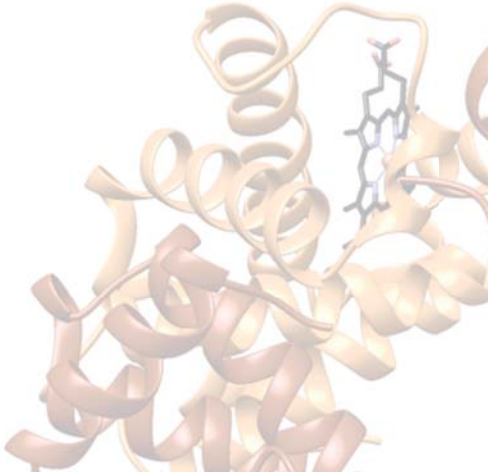
Matthew R. Dent, Anthony W. DeMartino, Kaitlin A. Bocian, Jason J. Rose, Jesus Tejero, Mark T. Gladwin

Carbon monoxide (CO) is an important signaling molecule that has been implicated in physiological processes ranging from inflammation response to cellular proliferation to circadian regulation. Given that CO is chemically inert under most physiological conditions, hemoproteins, which exhibit a strong affinity for CO, represent a class of likely protein targets for CO signaling. Importantly, hemoproteins involved in CO signaling must selectively respond to CO in the presence other heme-binding small molecules, such as oxygen (O_2) and nitric oxide (NO). To better understand CO selectivity in biological signaling, we examined the CO-sensing transcription factor, RcoM (regulator of CO metabolism), from the soil bacterium Paraburkholderia xenovorans. The RcoM heme, which is constitutively coordinated by His74, undergoes a unique redox-mediated ligand switching mechanism on the opposite heme face: Cys94, which binds to Fe(III) heme, is replaced by Met104 upon heme reduction. This weakly bound Met is replaced by CO, which exhibits nanomolar affinity for the RcoM Fe(II) heme. Using electronic absorption spectroscopy, we characterized ligand binding and chemical reactivity in variants of the PxRcoM-1 ortholog bearing alterations to distal heme pocket residues Cys94, Met104, and Met105. We studied heme pocket alterations in full-length PxRcoM-1 and in truncates bearing the C-terminal, heme-binding Per-Arnt-Sim (PAS) domain. Given that RcoM exhibits remarkably high affinity for CO, we directly quantified CO binding equilibrium constants (K_{CO}) through a competition assay using a well-characterized, high-affinity variant of human neuroglobin (Ngb-H64Q-CCC). In general, heme-binding truncates exhibited a more open heme pocket than full-length variants, as evidenced by larger ligand binding equilibrium constants and faster nitrite reduction rates. The influence of alterations to heme pocket residues on ligand binding affinities will be discussed in detail.



SESSION 4

Response and adaptation
a tribute to Guido Di Prisco



Invited Speaker

Global paleoclimate change and the loss of erythrocytes by Antarctic icefishes

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Keywords: notothenioid, channichthyid, erythropoiesis, erythroid gene regulatory regions

Understanding adaptation to environmental change is of fundamental importance to predicting the evolvability of species in the Anthropocene. Antarctic icefishes (Channichthyidae) lost the ability to produce red blood cells as the Southern Ocean (SO) cooled and dissolved oxygen concentrations rose, providing a test case for analyzing the evolutionary genomic responses to environmental change and the potential for species resilience as the SO now warms. By integrating paleoclimate records with an extensive phylogenomic dataset, we demonstrate relaxation of purifying selection across erythrocyte-associated genetic regions following a rapid decline in global temperatures and the formation of stable ice sheets. Acceleration of variation in erythrocyte-associated regions continues in modern Antarctic notothenioids, including red-blooded species. For example, we detected predicted deleterious variation in the beta-spectrin gene of red-blooded dragonfishes, one of which has spherocytic erythrocytes like those observed in humans with mutations in this gene. Despite loss-of-function mutations in a few key erythrocyte-specific genes, we show that most of the erythroid genetic toolkit has been maintained in icefishes. Interestingly, there is a bias in the accumulation of drift in putative gene-regulatory regions flanking genes expressed late in erythropoiesis. Together, results indicate that erythropoiesis in icefishes is blocked late in erythrocyte differentiation, consistent with the presence of proerythroblasts in icefishes. Our results provide a comprehensive phylogenomic perspective of the genetic changes in icefishes that led to loss of erythrocytes and a framework for understanding the potential for their adaptive resilience as the SO warms. Supported by US NSF PLR-1444167 (H.W.D.) and OPP- 1955368 (JD, HWD).

Invited Speaker

Physiological implications of haemoglobin evolution in high-altitude deer mice

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Keywords: oxygen transport pathway, aerobic capacity, thermogenesis, hypoxia

The hypoxic and cold environment at high altitudes requires that endotherms sustain high rates of O_2 consumption for thermogenesis and locomotion while facing diminished O_2 availability. Haemoglobin (Hb) evolution has contributed to high-altitude adaptation in many high-altitude mammals and birds, but the physiological significance of changes in Hb gene sequence and protein function has remained largely unresolved. I will discuss our research on this issue in the deer mouse (*Peromyscus maniculatus*). Deer mice exhibit the broadest elevational distribution of any North American mammal, and high-altitude populations have evolved increased Hb-O_2 affinity. I will show that the physiological significance of this evolved change in Hb function on aerobic capacity was likely contingent upon antecedent changes at other steps in the O_2 transport pathway (e.g., O_2 diffusing capacity of active tissues). I will also show that variation in Hb genes has other unanticipated effects on respiratory physiology, beyond the canonical function of this protein in circulatory O_2 transport. Our work highlights the importance of integrative approaches that maintain a wide field of vision for uncovering the adaptive significance of haemoglobin evolution.

Oral Presentation

Overexpression of neuroglobin improves neuronal cell survival in stress experiments

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Neuroglobin is part of the globin superfamily and is expressed in the central and peripheral nervous system of every vertebrate class. It is presumed to have a conserved and important physiological function. A wide range of potential functions has been described, including a protective role in neurons in hypoxic and oxidative stress related insults. However, the underlying mechanisms of neuroprotection of neuroglobin remain poorly understood. Here, we have investigated the function of mouse and zebrafish neuroglobin as well as mouse myoglobin in a murine neuronal cell culture (HN33) under hypoxia (1% O_2) and oxidative stress (0.5 µM H_2O_2). Transfection of mouse neurons with all three globins resulted in reduced Caspase activity in normoxia, hypoxia and, most significantly, under oxidative stress compared to the mock control. Cell viability, measured via CellTiter-Glo assay showed higher viability of cells transfected with zebrafish neuroglobin and mouse myoglobin under hypoxia and of mouse neuroglobin cells under ROS stress. To investigate the cellular effects of globin overexpression, we analyzed the transcriptomes of the transfected HN33 cells after treatment with normoxia, hypoxia and ROS stress. Transfection of mouse and zebrafish neuroglobin resulted in upregulation of genes regulating neuronal cell death, response to oxygen levels and glycolytic processes in normoxic conditions. Mock and myoglobin transfected cells showed a conserved cellular response to hypoxia with upregulation of glycolytic genes. Little regulation occurred in neuroglobin transfected cells after subjecting them to hypoxia. In addition, no significant regulation was found between cells transfected with mouse neuroglobin in normoxic conditions and after ROS treatment. This indicates that overexpression of neuroglobin but not myoglobin results in a general cellular answer, enhancing cell survival in hypoxic and oxidative stress conditions without high levels of regulation of gene expression.

Oral Presentation

Cytoglobin 1 knockout causes age-dependent degenerative changes and stress response in Danio rerio

Annette Schlosser*°[1], Kathrin Helfenrath[1], Thorsten Burmester[1] and Andrej Fabrizius[1]

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Keywords: cytoglobin 1, zebrafish, CRISPR/Cas9 knockout, RNA-seq, mRNA expression, hypoxia

In literature, there are many studies on vertebrate cytoglobin, but the properties of non- mammalian cytoglobins are largely uncharacterized. As most teleost fish, zebrafish harbor two cytoglobin paralogs, cytoglobin 1 and cytoglobin 2. Dre-cygb1 is expressed ubiquitously in most zebrafish tissues, whereas Dre-cygb2 is highly expressed in neuronal tissue. Here we describe the effects of CRISPR/Cas9 induced cygb1 deficiency on zebrafish physiology and development. We report enhanced occurrence of age-dependent degenerative changes in adult cygb1$^{-/-}$, mainly weight loss phenotype accompanied by surface respiration in male zebrafish. Transcriptome analysis revealed both tissue specific and global knockout effects. In brain and liver the lipid metabolism, hypoxic response (without stress) and inflammation pathways were dysregulated. In the cygb1$^{-/-}$ liver apolipoprotein D (apoda.2) was strongly upregulated. Apoda.2 is related with aging processes and might function as a “good Samaritan” helping cells to cope with oxidative stress. In the cygb1$^{-/-}$ brain we found enhanced expression of hemoglobin alpha and beta (hbaa1; hbba1) and glutathione peroxidase (gpx1a), that points to a local hypoxic adaptation. Further, we conducted hypoxia experiments in adult WT and in cygb1$^{-/-}$ larvae (33hpf) to evaluate the effect of the knockout during development. Cygb1$^{-/-}$ larvae were more susceptible to hypoxia (~0.7 kPa) since enolase 1 (eno1), heatshock protein 27 (hsp27) and vascular endothelial growth factor (vegfa) expression was enhanced. Additionally, cygb1$^{-/-}$ larvae strongly downregulated protein translation during hypoxia. The penta- coordination of cygb1 and its slow autoxidation rate together with our results might point to a role for cygb1 as an intracellular oxygen carrier protein in zebrafish.

Oral Presentation

Structural and functional characterization of globins in the Antarctic fish

Giordano, D.*[1], Pesce, A.[2], Bruno, S.[3], Dewilde, S.[4], Ascenzi, P.[5], Luque, F.J.[6], Bolognesi, M.[7], Viappiani, C.[8], di Prisco, G.[1], Verde, C.[1°]

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Keywords: Antarctic fish, myoglobin, neuroglobin, cytoglobin

In the freezing waters of the Southern Ocean, the dominant suborder of Antarctic teleosts, Notothenioidei, have developed unique adaptations to cope with cold, including, at the extreme, the loss of hemoglobin in icefish. As a consequence, icefish are thought to be the most vulnerable of the Antarctic fish species to ongoing ocean warming.

Notothenioidei are one of the most interesting models to study how low temperature and high oxygen concentrations, the main environmental stressors over millions of years, have affected oxygen supply and delivery in Antarctic marine environments. Some icefish also fail to express myoglobin but all appear to retain neuroglobin, cytoglobin-1, cytoglobin-2, and globin-X. Temperature up-regulates globin expression more effectively in white-blooded than in red-blooded fish while hypoxia strongly up-regulates globins in red-blooded fish, particularly in the gills, suggesting that globins function as regulators of temperature and hypoxia tolerance.

The structural and functional properties of neuroglobins and cytoglobins of Antarctic notothenioids have been studied and their adaptive features have been inferred from comparisons with human proteins. Similar to the human proteins, Antarctic fish neuroglobin, cytoglobin-1 and cytoglobin-2 can reversibly bind oxygen and carbon monoxide in the Fe^{2+} form, and show six-coordination by distal His in the absence of exogenous ligands.

The 3D structure of *Dissostichus mawsoni* cytoglobin-1 has been determined through X-ray crystallography at 3 Å resolution (data collected at ESRF, Grenoble, France).

At the light of a remarkable 3D-structure conservation, the observed differences in ligand-binding kinetics may reflect specific features in the dynamics of the heme distal region and protein matrix cavities, suggesting adaptation to functional requirements posed by the cold environment.

Oral Presentation

The adaptation of the Nannospalax galili transcriptome to life under hypoxia – a key to longevity?

Poetzsch, G. *°[1,3], Jelacic. L[1], Gaudreault. Y [2], Hankeln, T. [1]

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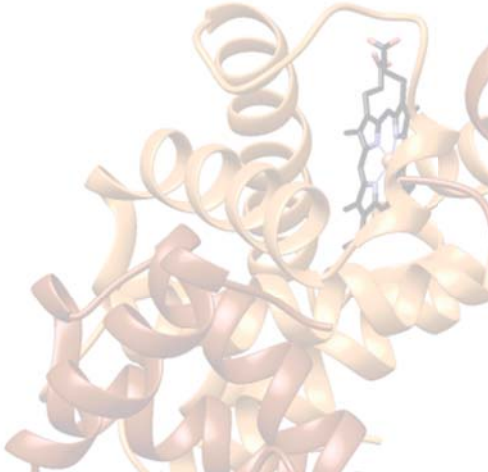
Hypoxia adaptation, Cancer, Longevity, Transcriptomics, Generegulation

The subterranean blind mole rat Nannospalax galili, which populates underground burrows, is adapted to a temporary lack of oxygen (hypoxia), even surviving ~3% O_2 for 14h. The adaptation to hypoxia is accompanied by an enhanced haematocrit and increased concentrations of haemoglobin. Additionally, elevated mRNA- and protein level for other oxygen-binding proteins such as myoglobin, neuroglobin and cytoglobin compared to rat have been detected in various Spalax tissues.
One of the mole rats most interesting features is its extended lifespan. Spalax become five to seven times older than its close relatives, mouse and rat, without displaying clear signs of ageing or ageing-related disorders. We hypothesize that the longevity phenotype in Spalax, which is paralleled by a resistance against tumor formation, might be mechanistically linked to the hypoxia adaptation. To infer Spalax-specific gene regulation patterns, we produced RNA-Seq data of liver, kidney and spleen heart from Spalax individuals subjected to 6% O_2 or normoxia and compared these data to the hypoxia-sensitive rat.
In all three organs, we observed a significantly higher hypoxia-induced transcriptional stress response in the rat. In Spalax organs, on the other hand, we detected constitutively different, often elevated mRNA levels in comparison to rat. These constitutively increased transcript levels may enable Spalax to react faster to acute changes in the oxygen level of its habitat, alleviating the need to regulate response genes after the onset of stress. The body-wide transcriptome differences that were detected between Spalax and rat involve many pathways associated with genome stability maintenance and DNA repair, suggesting an explanation for the extraordinary lifespan of Spalax. In the future, it will be interesting to link the differential gene expression patterns to epigenomic changes which may orchestrate the Spalax-specific transcription.

SESSION 5

Heme-proteins in health and diseases



Invited Speaker

The pathophysiological consequences of the peroxidatic activities of oxygen binding proteins

Michael T. Wilson* and Brandon J. Reeder

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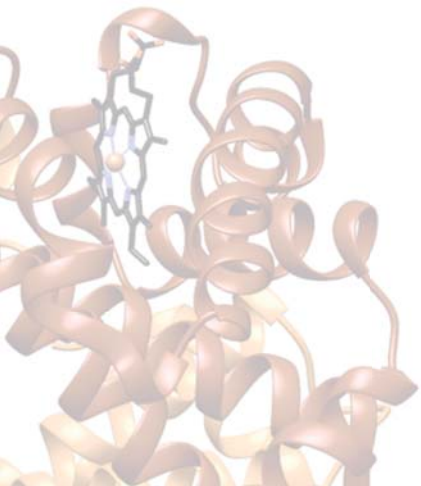
All oxygen binding heme proteins possess enzymic activities. Some of these are reported to have physiological roles (e.g. NO dioxygenases, nitrite reductases), while others are pathophysiological in their consequences. We discuss here the most important of these, which is the peroxidatic activity initiated by the reaction of the heme group with hydrogen peroxide that yields a highly oxidizing oxy-ferryl species and a free radical. Both of these, individually or in combination, can oxidize biological molecules leading to cellular oxidative stress. The evidence that such reactions take place *in vivo* comes from detection of a number of biomarkers and will be reviewed. These reactions can take place within the cellular environment but because the cellular antioxidative defence systems are efficient the extent to which they occur is limited. Once, however, myoglobin or hemoglobin escape their cellular environments and are away from these defences the picture is very different. Following crush injuries that release myoglobin into the blood stream and consequently the kidney (rhabdomyolysis) or sub-arachnoid haemorrhage that allows hemoglobin entry to the cerebral spinal fluid, oxidation of lipids take place to form potent vasoconstrictors e.g. isoprostanes or neuroprostanes. In the course of catalysing these reactions some of the heme proteins themselves are modified forming a covalent bond between the heme group and the protein. The presence of these species provide biomarkers that are unequivocal evidence for the peroxidatic activity. How the pathological consequences flow from these reactions and how therapeutic interventions can be devised to defend against them will be reviewed. The lessons learnt from these studies have informed the design of the next generation of hemoglobin based “blood substitutes” and will also be discussed.



Invited Speaker

Evolution and molecular basis of a novel allosteric property of crocodilian hemoglobin

Natarajan, C.[1], Signore, A.V.[1], Bautista, N.M.[1], Hoffmann, F.G.[2,3], Tame, J.R.H.[4], Moriyama, H.[1], Fago, A.[5], Storz, J.F.*[o1]

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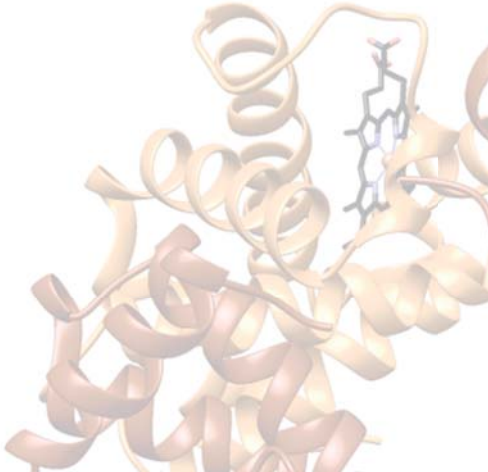
Keywords: allostery, biochemical adaptation, protein evolution

How do novel protein functions evolve in an incremental, step-by-step fashion, and to what extent is the gain of new function mechanistically coupled with the loss of ancestral function? These are fundamental questions in molecular evolution and they can be addressed with a protein-engineering approach that permits the identification and functional characterization of causative mutations. Here we report discoveries regarding the molecular basis of a key physiological innovation during vertebrate evolution. We used ancestral protein resurrection in conjunction with site-directed mutagenesis experiments to dissect the molecular basis of a unique allosteric property of crocodilian hemoglobin (Hb). Among vertebrate Hbs, crocodilian Hb is unique in that its O_2-affinity is primarily modulated by bicarbonate ions rather than organic phosphates such as ATP. The mechanistic basis of this unique mode of allosteric regulatory control has remained a mystery. We report new findings regarding the specific substitutions responsible for the gain and loss of different allosteric interactions and the biophysical mechanisms by which they exert their functional effects.



Oral Presentation

Anti-fibrotic Capacity of Extracellular Globins via Scavenging Reactive Oxygen Species

Le Thi Thanh Thuy*[1], Vu Ngoc Hieu[1], Hoang Hai[1], and Norifumi Kawada°[1]

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Anti-fibrotic therapy remains an unmet medical need in human chronic liver diseases. Recently, Cytoglobin (CYGB) was reported to inhibit hepatic stellate cells (HSCs) activation and their collagen production. We aim to study anti-fibrotic property of 4 human globin-proteins including tetramer Hemoglobin (HB), monomer-Myoglobin (MB), -CYGB, and -Neuroglobin (NG).

We produced recombinant human CYGB and NG. MB and HB were from commercials. IC_{50} values of ROS-scavenging activity of human HB, MB, CYGB and NG were measured. The bio-distribution of globin-proteins after *in vitro* and *in vivo* administration was traced. Cellular fractionation revealed that extracellularly added MB, NG, and CYGB, but not HB, penetrated human HSCs (HHSteCs) and located in membrane, cytoplasm and cytoskeletal fractions. Except HB, others scavenged reactive oxygen species generated spontaneously or stimulated by H_2O_2 or transforming growth factor β1 in HHSteCs and reduced collagen 1A1 production via suppressing its promoter activity. RNA-seq analysis of MB, NG and CYGB-treated HHSteCs revealed the common downregulation of extracellular matrix-encoding and fibrosis-related genes, and the upregulation of antioxidant genes or inactivated markers of HSCs including GATA, EST2, and PPARγ. Disruption of disulfide bond in NG decreased heme activity, superoxide-scavenging activity, and collagen inhibition capacity. Six weeks of CCl_4 treatment in mice induces collagen deposition indicated by Sirius Red and Fast Green staining, αSMA expression, CD68 macrophage infiltration, and neutrophil populations. Intravenously injected MB, NGB, or CYGB at 1mg/kg BW, twice a week for last 4 weeks of CCl_4, exhibited the clear attenuation of these manifestations.

Conclusion: These findings revealed an unexpected and profound role for MB, NG and CYGB in maintaining HSCs in deactivated status and protect the mouse liver against cirrhosis, proposing the globin therapy as a new strategy to combat fibrotic liver disease.

Oral Presentation

Androglobin, a chimeric mammalian globin, is associated with ciliogenesis

Keppner, Anna1, Adriaensen, Saar1,2, Maric, Darko1, Correia, Miguel1, Koay, Teng Wei1, Osterhof, Carina3, Hankeln, Tom3, Hoogewijs, David1

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Androglobin (Adgb), the youngest mammalian globin family member, owns its name to its predominant expression in testis. Adgb displays a unique chimeric domain structure among the globin family, consisting of an atypical permutated globin domain that is split into two parts by a calmodulin (CaM)-binding motif, an N-terminal calpain- like protease domain and a long C-terminal tail of yet unknown function. Here we will provide an overview of recent observations supporting a function of Adgb in ciliogenesis. Adgb is transcriptionally regulated by the master regulator of ciliogenesis FOXJ1, whereby its transcription not only relies on its promoter, but also on a distal enhancer region. Moreover, scRNAseq revealed expression of Adgb specifically in ciliated cells of the lungs, brain and female reproductive tract. Our data suggest a specific role for the isolated globin domain, likely resulting from auto-proteolytic cleavage, in the ciliary basis. Accordingly, Adgb KO mice develop a ciliopathy termed primary ciliary dyskinesia (PCD) including male infertility due to abnormal sperm flagellum formation, hydrocephalus, mucus accumulations in sinus and lungs, congenital heart defects, polycystic kidneys and rare cases of laterality defects. We found a ciliopathy-associated septin family member as specific Adgb interactor. In vitro data suggest that Adgb contributes to septin proteolysis in a CaM-dependent manner with most efficient CaM binding to Adgb upon isolation of the globin domain. Human PCD is also marked by airway oxidative stress and reduced nasal NO levels, which is commonly used as diagnostic approach, but with still unknown origin. Adgb thus represents an excellent candidate gene which may link ciliogenesis with airway ROS/RNS detoxification. Primary mouse tracheal epithelial cells from control and KO mice are currently exploited to further study the function of Adgb in ciliogenesis, and how this enigmatic globin interconnects oxygen physiology and ciliary function.

Oral Presentation

Wide ranging roles of myoglobin in breast epithelia: from shuttling fatty acids to delimiting the malignant transformation of cells

Mostafa A. Aboouf[1,2], Julia Armbruster[1], Max Gassmann[1], Glen Kristiansen[3], Anne Bicker[4], Thomas Hankeln[4], Hao Zhu[5], Thomas A. Gorr[1]

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High-level expressed myoglobin (MB) is known to deliver oxygen in striated muscles. MB exists also at low levels in mammary epithelial cells, specifically in the lipid- secreting inner luminal cells of the milk duct epithelium where, however, MB's function is unclear. Oxygenated MB is able to bind long-chain fatty acids (FAs) in vitro. Ablation of the MB gene in the heart and brown adipose tissue (reference below) switches substrate utilization from highly aerobic FA ß-oxidation to relatively O2-sparing glucose oxidation. We now utilized mice and human breast cancer cells with targeted MB ablation to examine MB's impact, and of its oxygenation status, on FA metabolism in mouse milk and mammary epithelia. MB expression shifted FA composition toward more saturated and shorter FA's in milk and cells. Presence of MB also increased cytoplasmic FA solubility under normoxia and FA deposition to lipid droplets under severe hypoxia.
Since MB frequently occurs in mammary carcinoma, we further studied the globin´s role in breast cancer development and progression in vivo. We, thus, crossed PyMT and WapCreTRP53flox mouse models that both develop spontaneous breast cancer, yet differ in onset and tumor grade/type, with MB knock out mice. While the loss of MB in WapCre;Trp53flox mice had no effect on tumor development, it decreased tumor growth but increased tissue hypoxia and the number of lung metastases in PyMT mice. Furthermore, Doxorubicin therapy prevented the stronger metastatic propensity of MB- deficient tumors in PyMT mice.
Together, MB emerges as an intracellular O2-dependent shuttle of oxidizable FA substrates in normal and malignant breast epithelia. Regarding breast cancer, we propose that determining the expression level of MB in malignant breast cancer biopsies will improve tumor stratification and outcome prediction. MB-targeted therapeutics in combination with standard strategy may offer a novel intervention concept for advanced metastatic breast cancer.

Reference: Aboouf, M.A. et al. « Myoglobin, expressed in brown adipose tissue of mice, regulates the content and activity of mitochondria and lipid droplets « ; Biochim Biophys Acta Mol Cell Biol Lipids. 2021 Dec;1866(12):159026.

Oral Presentation

Targeted photodynamic therapy using heme proteins

Abbruzzetti, S*°[1], Viappiani, C. °[1], Delcanale, P. [1], Bruno, S. [2], Agut, M. [3], Nonell, S. [3], Tognolini, M. [2], Giorgio, C. [2], Bianchini, P. [4,5], Diaspro, A. [4,5]

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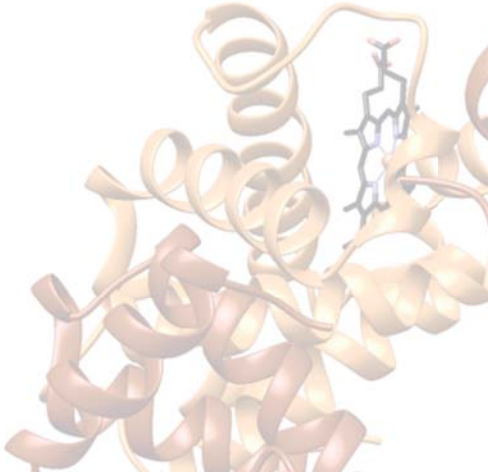
Keywords: photodynamic therapy, heme-proteins, Zn-myoglobin, singlet oxygen

Photodynamic therapy (PDT) is a clinically approved methodology able to kill malignant cells and pathogens. Its efficacy relies on the cytotoxic effects of reactive oxygen species, in particular singlet oxygen, formed by excitation of a photoactivable molecule, called photosensitizer (PS), with visible light in presence of molecular oxygen. PDT is considered a promising alternative treatment, and its efficacy of the PDT against antibiotic-resistant bacteria has drawn a renewed attention. Essential properties that an effective photoactive system for PDT must possess, are high bioavalaibility of the PS, and targeting capability of the developed construct. Two important issues must be met. First, PSs are usually not water soluble molecules. Second, the lifetime of singlet oxygen is around 3.5 s, thus limiting its action radius to approximately 200 nm. Can heme-proteins be of help?

Using proteins to solubilize PSs is an interesting but not widely exploited tool. We proposed Zinc substituted-myoglobin (ZnMb) as a promising system that combines a high quatum yield of production of singlet oxygen thanks to substitution of Fe(II) with Zn(II) at the center of porphyrinic ring (0.9), and the biocompatibility of the protein scaffold. The efficacy of this system has been proved on S. aureus with a decrease up to 99.99% in CFU. Due to the fact that ZnMb forms naturally during nitrate-free ham aging, this protein may represents a tool for Gram-positive bacteria decontamination of food.

However, the efficacy of a construct for PDT is strongly dependent on its ability to target specific receptors on the plasma membrane of cancer cells or on the bacterial wall. To address this issue, we fused the gene of human myoglobin to a short peptide that targets a receptor overexpressed in some tumor cells. Preliminary experiments demonstrate that the chimeric construct binds prostate cancer cells, and the presence of the targeting agent is essential to obtain this interaction.

POSTERS



Biophysical characterization of the cysteine-rich globin-3 from *Caenorhabditis elegans*

Hafideddine, Z.[1,2], Van Brempt, N.[1], Loier, T.[3], Sgammato, R.[4], Aerts, R.[4], Johannessen, C.[4], Herrebout, W.[4], Braeckman, B.P.[3], Hammerschmid, D.[2], Moens, L.*[2], Dewilde, S.[2,†], Van Doorslaer, S.°[1]

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Keywords: *Caenorhabditis elegans*, globin 3, spectroscopy, redox protein

The popular genetic model organism *Caenorhabditis elegans* (*C. elegans*) encodes 34 globins, whereby the few that are well-characterized show divergent properties besides the typical oxygen carrier function. Here, we present a biophysical characterization of *C. elegans* globin-3 (GLB-3) using a multitude of techniques. GLB-3 is predicted to exist in two isoforms and is expressed in the reproductive and nervous system.

The presented spectroscopic analysis reveals that GLB-3 exists as a bis-histidyl ligated low-spin form both in the ferrous and ferric state. Unlike other globins, GLB-3 is not capable of reacting with H_2O_2, H_2S, and nitrite. Site-directed point mutation of the distal histidine to an alanine resulted in nitrite reductase activity, implying the strong distal histidine affinity to the heme iron in the wild-type globin. Intriguingly, not only does GLB-3 contain a high number of cysteine residues, it is also highly stable under harsh (pH = 2) conditions. Redox potentiometric measurements reveal a positive redox potential (+8 ± 19 mV vs. SHE) similar to other heme proteins. Based on our study and a detailed comparison with other globins, we postulate an electron transfer function for GLB-3.

Phylogenetic conservation of Globin Y expression sites

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Globin Y (GbY), one of the “new members” of the vertebrate globin family, has been documented in the genomes of lobe-finned and cartilaginous fish, amphibians, several reptiles and in the monotreme platypus, but not in most bony fish, birds, or higher mammals.
Until now, there is only few data on the physicochemical properties of GbY, and a reliable hypothesis about its physiological function is still missing. The knowledge of main sites of expression may help to understand its function.
Here we analyzed the expression pattern of GbY in the kidney of the frog *Xenopus laevis* and the "living fossile" *Lepisosteus oculatus,* an ancient fish with a primitive lung belonging to the Neopterygii, via publicly available RNA-Seq data, immunofluorescence and mRNA *in situ* hybridization.

RNA-Seq and qPCR data pointed to a very high GbY mRNA expression in the adult kidney of both species. Subsequent immunofluorescence combined with mRNA *in situ* hybridizations showed an allocation of GbY in the proximal tubules covered with microvilli. Only in X. laevis, but not the fish, GbY was localized in the inter-renal, possibly steroidogenic cells, which have a very high fat content. In those inter-renal cells, the GbY signals are in close proximity to lipid droplets, always surrounded by serotonin-positive chromaffine cells.
We hypothesize that the observed difference in the distribution of GbY expression in adult kidneys of fish and amphibia may hint at two distinct functions of GbY, of which the role in the proximal tubules is conserved. The association of GbY with lipid droplets in inter-renal cells could imply a role in fatty acid metabolism, like it was suggested for myoglobin and cytoglobin.

Computational study of the protein and solvent dynamics in Nitrobindins

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Keywords: Nitrobindins, heme, reactivity, molecular dynamics

Recently, a new class of all-β-barrel heme-proteins, named nitrobindins (Nbs), has been identified along the evolutionary ladder (De Simone et al., 2016). Nbs are characterized by a stable solvent-exposed heme-Fe(III) atom, coordinated by a proximal His residue. To date, the physiological role of Nbs has not been understood. However, *in vitro* evidence suggest that ferric Nbs from *Arabidopsis thaliana* (*At*-Nb), *Mycobacterium tuberculosis* (*Mt*-Nb) and *Homo sapiens* (*Hs*-Nb) are able to catalyze the conversion of peroxynitrite to nitrate (De Simone et al., 2018, 2020). Noteworthy, in both *Mt*-Nb (PDB ID: 6R3W; De Simone et al., 2020) and *At*-Nb (PDB ID: 3EMM; Bianchetti et al., 2010) crystal structures, a water molecule is coordinated to the heme- Fe(III) atom forming a 6-coordinate High Spin (6cHS) His-Fe-H_2O. Although nuclear magnetic relaxation dispersion (NMRD) profiles showed a fast exchange between coordinated and bulk water, the reactivity of *Mt*-Nb and *Hs*-Nb appears to be modulated by residues of the heme distal pocket (De Simone et al., 2020). In order to investigate the solvent-mediated interactions between the heme distal pocket and the heme-Fe atom, we performed classical molecular dynamics simulations. The present computational approach allowed us to observe the system's dynamics, trying to understand how the solvent and the heme pocket could influence the Nb function. In addition, steered molecular dynamics simulations were performed to obtain the free energy profiles for ligand migration (Capece et al., 2013).

References

Capece, L., Boechi, L., Perissinotti, L.L., Arroyo-Mañez, P., Bikiel, D.E., Smulevich, G., Marti, M.A., and Estrin, D.A. (2013). Small ligand–globin interactions: Reviewing lessons derived from computer simulation. Biochim. Biophys. Acta - Proteins Proteomics *1834*, 1722–1738.

De Simone, G., Ascenzi, P., and Polticelli, F. (2016). Nitrobindin: An Ubiquitous Family of All β-Barrel Heme-proteins. IUBMB Life *68*, 423–428.

De Simone, G., di Masi, A., Polticelli, F., and Ascenzi, P. (2018). Human nitrobindin: the first example of an all-β-barrel ferric heme-protein that catalyzes peroxynitrite detoxification. FEBS Open Bio *8*, 2002-2010.

De Simone, G., Di Masi, A., Vita, G.M., Polticelli, F., Pesce, A., Nardini, M., Bolognesi, M., Ciaccio, C., Coletta, M., Turilli, E.S., et al. (2020). Mycobacterial and Human Nitrobindins: Structure and Function. Https://Home.Liebertpub.Com/Ars *33*, 229–246.

Structural and functional characterization of *Danio rerio* nitrobindin.

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Keywords: Nitrobindins; heme; reactivity; structure.

Nitrobindins (Nbs) are all-β-barrel heme-proteins present in prokaryotes and eukaryotes. Although the physiological role(s) of Nbs are still unclear, it has been postulated that they are involved in the NO/O_2 metabolism. The high reactivity of Nbs towards reactive nitrogen and oxygen species reflects the solvent exposure of the metal center [1,2,3]. To date, *Arabidopsis thaliana* Nb (*At*-Nb), *Mycobacterium tuberculosis* Nb (*Mt*-Nb), and *Homo sapiens* Nb (*Hs*-Nb) have been characterized from both structural and functional viewpoints [1,2,4,5]. To study Nb function(s), *Danio rerio* Nb (*Dr*-Nb) has been expressed, purified, and spectroscopically characterized. Around neutrality, the UV-Vis and Resonance Raman (RR) spectra of ferric *Dr*-Nb display a mixture of a 5cHS and a 6cHS aquo species. Similarly to *Mt*-Nb and *Hs*-Nb, the ferrous form is mainly 5cHS, characterized by the same Fe-proximal His bond strength. Furthermore, both UV-Vis and EPR spectroscopies indicate that the heme-Fe(II) atom of *Dr*-Nb(II)-NO is mostly five-coordinated. Kinetics of *Dr*-Nb(II) nitrosylation are likely impaired by the crowded network of water molecules which shields the heme pocket. On the other hand, kinetics of *Dr*-Nb(II)-NO denitrosylation may reflect an easy way out for the ligand into the outer solvent. Ongoing studies involving the RR spectroscopy of the CO complexes, the kinetics of CO binding, and the resolution of *Dr*-Nb three-dimensional structure will allow us to understand the structure-function relationships of fish Nb.

References

[1] C.M. Bianchetti, G.C. Blouin, E. Bitto, J.S. Olson, G.N Phillips Jr. Proteins (2010), 78, 917-931.
[2] C.M.Bianchetti, C.A. Bingman, G.N. Phillips Jr. Proteins (2011), 79, 1337-1341.
[3] G. De Simone, P. Ascenzi, F. Polticelli F. IUBMB Life (2016), 68, 423-428.
[4] G. De Simone, A. di Masi, F. Polticelli, P. Ascenzi. FEBS Open Bio (2018), 8, 2002-2010.
[5] G. De Simone, A. di Masi, G.M. Vita, F. Polticelli, A. Pesce, et al. Antioxid Redox Signal (2020), 33, 229-246.

Using genetic engineering to target the toxic effects of hemoglobin for a new generation of hemoglobin-based oxygen carrier

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There is a significant clinical need for a synthetic oxygen therapeutic / blood substitute that is both long-lasting and sterile. Hemoglobin-based oxygen carriers (HBOCs) have been a focus of numerous studies and development as a potential artificial oxygen therapeutic / blood substitute. However, previous attempts have met with many failures seen during clinical trials due to observed adverse effects and toxicities. We have utilised genetic engineering to reengineer the hemoglobin molecule, adding numerous features, whilst keeping a focus on protein and heme stability. Using fetal hemoglobin as a template has made the protein much more stable with lower rates of autoxidation and heme loss. A decrease in nitric oxide dioxygenase activity to limit the nitric oxide scavenging capacity of the HBOC was achieved by using the mutations reported previously by the Olson group. Several new technologies have been added to our HBOC: The addition of through-protein electron transfer pathway to enhance the reaction of ferric and ferryl oxidation states with plasma antioxidants such as ascorbate and urate. Furthermore, we have added a mutation to generate a homogeneously PEGylated HBOC that does not significantly disrupt the allosteric oxygen binding of the HBOC.

We have completed a scalable manufacturing process and are currently conducting focused pre-clinical studies to specifically identify potential toxic effects of our HBOC on various tissues including myocardial and renal tissue. This approach seeks to de-risk future full pre-clinical and Phase I/II clinical trials. Initial clinical targets for the product will be as an oxygen therapeutic, potentially treating patients with stroke and other thromboses, sickle cell crisis, trauma, those with impaired immune systems and numerous other potential roles.

Expression and characterization of N-terminal calpain-like domain of human androglobin

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Keywords: Androglobin, calpain, cysteine protease, protein expression

Androglobin, a multi-domain hemoglobin of the metazoans, has been proposed to play a role in the formation of motile cilia during ciliogenesis and spermatogenesis. The 190 kDa human protein has several domains, including a central circularly permuted globin domain, an N-terminal region containing a calpain-like region and a largely disordered C-terminal region containing sequences for a coiled-coil region, and nuclear localization signal and endoplasmic reticulum retention signal. Previous studies of the expression of the full-length androglobin have reported potential auto-proteolytic activity of the calpain domain (1). The active site of calpain proteases typically contain a cysteine-histidine-asparagine catalytic triad. Examination of the sequence homology of the calpain domain of androglobin with human calpain-7 has identified a partially conserved candidate for the active site cysteine (2). However, the calpain-7 active site histidine and aspartate residues did not show equivalent residues in sequence alignments to androglobin sequences. Nonetheless, a number of conserved residues for the histidine and asparagine are present that could present candidates for these active site residues. Here we examine the recombinant expression of the N-terminal domain and its auto-proteolytic activity. We have also examined potential candidates for the active site residues for their effect on auto-proteolytic activity through site-directed mutagenesis.

(1) Bracke, A.; Hoogewijs, D.; Dewilde, S., Exploring three different expression systems for recombinant expression of globins: Escherichia coli, Pichia pastoris and Spodoptera frugiperda. Anal. Biochem. 2018, 543, 62-70.

(2) Hoogewijs, D.; Ebner, B.; Germani, F.; Hoffmann, F. G.; Fabrizius, A.; Moens, L.; Burmester, T.; Dewilde, S.; Storz, J. F.; Vinogradov, S. N.; Hankeln, T., Androglobin: a chimeric globin in metazoans that is preferentially expressed in Mammalian testes. Mol. Biol. Evol. 2012, 29 (4), 1105-14.

Disulfane-mediated reduction of metmyoglobin

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KEYWORDS. myoglobin, disulfane, reactive sulfur species

Dihydrogen disulfane, H_2S_2, is an endogenous species that has been reported as effector of the biochemical activity of hydrogen sulfide, H_2S, in certain tissues.[1] The metal centered reduction of the heme protein indoleamine 2,3 dioxygenase by disodium disulfane, Na_2S_2, leading to a potent activation of the enzyme, precedes and highlights the interest of heme proteins as biochemical targets of disulfane species.[2] Herein, we focused on the reactivity of H_2S_2, or the conjugated base HSS^-, towards metmyoglobin, $MbFe^{III}$.

The reaction of excess Na_2S_2 with $MbFe^{III}$, was studied under argon atmosphere at 25ºC. A fast biexponential formation of $MbFe^{II}$ was observed by UV-Vis spectroscopy in the interval 6<pH<8, with varying concentrations of disulfane, suggesting the one-electron oxidation of HSS^-, forming the disulfanyl radical HSS^* along with $MbFe^{II}$.

The intermediacy of a coordination complex, with UV-Vis features similar to that of the characterized $[MbFe^{III}(SH^-)]$ complex,[3] was detected at the more acidic pH evaluated. The formation of a hexacoordinated low spin complex was confirmed by resonance Raman spectroscopy at 10ºC. As control experiments of the sulfide- mediated reduction $MbFe^{III}$ reveal a hysteretic behavior, the participation of contaminant H_2S/HS^- is disregarded, suggesting the formation of a $[MbFe^{III}(SSH^-)]$ complex.

The spectroscopic detection of higher polysulfides HS_nH (2<n<8) in the final reaction mixture, may be not only a consequence of the dimerization of HSS*, but also the result of the chemistry of disulfane, that characterizes its aqueous solutions.[3]

References

(1) Kimura, H. *Neurochem. Internat.* **2013,** *63 (5)*, 492–497 (10.1016/j.neuint.2013.09.003).
(2) Nelp, N.T. et al. *J Am Chem Soc.* **2019**, *141*, 15288-15300 (10.1021/jacs.9b07338).
(3) Bogdándi, V. et al. *Br J Pharmacol*. **2019,** *176(4), 646-670* (10.1111/bph.14394).

Structural and Functional Characterization of Recombinant Human Hemoglobin as an Artificial Oxygen Carrier

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Keywords: Blood substitute, Recombinant hemoglobin, Heme stability, Artificial oxygen carriers, Moderate oxygen affinity, Low autooxidation rate

More than two-thirds of the world does not have adequate blood supplies. Moreover, conventional red blood cells have a limited shelf life. Therefore, Hemoglobin based oxygen carriers (HBOCs) were developed as alternatives to blood transfusion, and used as oxygen therapeutics in ischemic conditions. These HBOCs should have high heme affinity, low autooxidation rate, high shelf life and high apoglobin stability. We can use site-directed mutagenesis to change amino acids surrounding the heme pocket in recombinant human hemoglobin in order to enhance heme stability based on the unprecedented heme stability of *Synechocystis* hemoglobin, which was successfully engineered in myoglobin. We have analyzed heme retention ability, autooxidation rates and oxygen binding properties of some of the mutants, of which one was significantly stable with retarded heme loss. Since these rHbs are expressed in *E.coli,* these might confer immunogenic problems when administered in animal or human subjects; therefore, it is highly desirable to employ sensitive and selective detection of lipopolysaccharides in recombinant hemoglobin solutions, which must be subsequently depleted.

Role of haem biosynthesis in Gram-negative pathogens

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Keywords: Haem biosynthesis, Gram-negative pathogens, Haem transfer

Campylobacter jejuni is the major cause of gastroenteritis in the world and responsible for ~1/4 of diarrhoeal diseases in the world. Despite the increasing number of infections caused by C. jejuni, the molecular mechanisms involved in pathogen's survival within the host are still poorly understood.

Haem biosynthesis plays a crucial role in pathogen´s physiology as it ensures the formation of several essential haem-binding proteins/enzymes. Indeed, haem cofactor is responsible for the function of several key cellular processes such as, respiration, signaling and oxidative stress detoxification. Therefore, most prokaryotes, synthesize haem endogenously via specific Haem Synthesis Pathways (HSP). All the so far known HSP begin with the universal tetrapyrrole precursor δ-aminolaevulinic acid (ALA) and uses a cascade of enzymes to finally produce haem. The best-known pathway, currently named the protoporphyrin dependent pathway (PPD), involves at least eight enzymes to go from ALA to haem and is mostly present in Gram-negative bacteria. Our group participated in the discovery of two other distinct pathways, namely the sirohaem dependent pathway and the coproporphyrin dependent pathway (1–4). That are present mainly in sulphate-reducing bacteria and Gram-positive pathogens, respectively.

We described our data on the elucidation of which pathway does C. jejuni uses to synthesise haem. We performed the biochemical characterization of the enzymes involved in this pathway. Moreover, we show results on the identification of a novel putative haem chaperon encoded in C. jejuni.

1. Videira MAM, Lobo SAL, Sousa FL, Saraiva LM. FEBS J. 287(8):1537. (2020)
2. Videira MAM et al. Mol. Microbiol. 109(3):385. (2018)
3. Lobo SAL et al. Mol. Microbiol. 97(3):472. (2015)
4. Bali S et al. Proc Natl Acad Sci U S A. 108(45):18260. (2011)

Drug repurposing approaches to target bacterial cytochrome bd Oxidases

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Cytochrome bd complexes are terminal respiratory oxidases found exclusively in the aerobic respiratory chains of prokaryotes that generate a proton motive force by coupling quinol oxidation to the reduction of dioxygen. Previous work has demonstrated that cytochrome bd complexes are important during infection for a variety of bacterial pathogens, including E. coli and M. tuberculosis, demonstrating their potential as drug targets. Herein, in silico tools were used to screen a library of approved drugs for their ability to inhibit cytochrome bd-I from E. coli. In order to investigate the efficacy and specificity of the top hits, mutant strains of E. coli that express either cytochrome bd-I or cytochrome bo' as the sole respiratory oxidase were used as a test system, and the expected spectral signals of these respiratory oxidases were confirmed for these strains using difference spectroscopy. Membranes were isolated from these strains, and candidate drugs from the in silico analyses were tested for their ability to inhibit oxygen consumption by cytochrome bd-I or cytochrome bo' using an oxygen electrode. Selected drugs were identified as inhibitors of cytochrome bd-I, and further work has been undertaken to aid our understanding of their mechanisms of action and potential for broader applications in antimicrobial chemotherapy.

Targeting a putative reductase partner of the truncated hemoglobin N in Mycobacterium tuberculosis

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Keywords: Nitric oxide detoxification, truncated hemoglobin, reductase, drug design

Tuberculosis is amongst the major worldwide health threats. Besides the spread of the disease, multidrug-resistant strains of Mycobacterium tuberculosis (Mt) have emerged. As a consequence, treatment failure is, unfortunately, becoming more usual and there is urgency for disclosing innovative therapeutic strategies, which has lead to renewed screenings for antimycobacterial substances and the identification of novel targets and mechanisms of action.

Truncated hemoglobin, tHbN, of Mt protects its host from the toxic effects of nitric oxide (NO) due to its potent O_2-dependent NO dioxygenase (NOD) activity. This protein converts NO produced by macrophages into the harmless nitrate anion. Based on the studies of our research group about the structure of tHbN and the migration of NO and O_2, protein tunnel system composed of short and long branches facilitates ligand entry to the distal heme site. On the other hand, the oxyferrous heme interacts with NO to make nitrate and ferric heme, and subsequently a reductase partner would be needed to recover the ferrous state, thus enabling the protein to start the cycle again.

This communication reports the efforts carried out in the search of putative reductase partners, which to the best of our knowledge has not been identified yet. A careful bioinformatics analysis led to a selected reductase candidate, whose relevance for the survival of the bacillus has been experimentally confirmed. Our work has included building up a 3D homology model of the reductase as well as of the complex formed with tHbN, which has been subsequently refined by extended Molecular Dynamics simulations. Furthermore, calculations have been performed to estimate the feasibility of the electron transfer process between reductase and tHbN. Finally, the 3D model has enabled to disclose druggable pockets that might mediate the inhibitory NOD activity.

Drug repurposing approaches to target bacterial cytochrome *bd* oxidases

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Cytochrome *bd* complexes are terminal respiratory oxidases found exclusively in the aerobic respiratory chains of prokaryotes that generate a proton motive force by coupling quinol oxidation to the reduction of dioxygen. Previous work has demonstrated that cytochrome *bd* complexes are important during infection for a variety of bacterial pathogens, including *E. coli* and *M. tuberculosis*, demonstrating their potential as drug targets. Herein, *in silico* tools were used to screen a library of approved drugs for their ability to inhibit cytochrome *bd*-I from *E. coli*. In order to investigate the efficacy and specificity of the top hits, mutant strains of *E. coli* that express either cytochrome *bd*-I or cytochrome *bo*' as the sole respiratory oxidase were used as a test system, and the expected haem signals of these respiratory oxidases were confirmed for these strains using difference spectroscopy. Membranes were isolated from these strains, and candidate drugs from the *in silico* analyses were tested for their ability to inhibit oxygen consumption by cytochrome *bd*-I and cytochrome *bo*' using an oxygen electrode. Selected drugs were identified as potent inhibitors of cytochrome *bd*-I, and further work has been undertaken to aid our understanding of their mechanisms of action and potential for broader applications in antimicrobial chemotherapy.

Structural Determinants of Oxygen Transport in Phytoglobins

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Keywords: Phytoglobins, Oxygen transport, Hexacoordination, Site-directed mutagenesis

Symbiotic hemoglobins typically display features associated with oxygen transport. These features include: pentacoordinate heme irons, high concentrations, modest oxygen affinity dictated by a rapid on rate and a corresponding fast off rate. Phytoglobins, previously referred to as class 1 non-symbiotic hemoglobins, differ in each of these characteristics typical of oxygen transporting globins. Prior studies have shown that the oxygen transport ability of hemoglobin in plants evolved in at least two convergent pathways, one from the class 2 globins, and a unique pathway found in phytoglobins from two plant species whose proteins share 93 percent identity. Phytoglobin from the plant *Parasponia andersonii* is a typical oxygen transport globin while the phytoglobin from *Trema tomentosa* has hexacoordinate heme iron and a low oxygen dissociation rate, both characteristics of a poorer oxygen transporter. Determining which amino acid substitutions between Trema and para phytoglobins lead to changes in oxygen dissociation rate can lead to a better understanding of how globin structure relates to physiological function. This work shows that site directed mutants near the distal histidine and CD corners of Trema phytoglobin affect both bis-histidyl hexacoordination and oxygen dissociation rates. A single mutation near the distal histidine (M72T) shows an oxygen dissociation rate 3-5 times faster than the WT protein and a double mutant of M72T and a mutation near the protein's CD corner (I59V) showed a tenfold increase in dissociation rate. These changes are indicative of a protein with more oxygen transporting ability, as observed in wild-type Para Hb. These results begin to address the complex relationships observed between globin structure, bis-histidyl hexacoordination, and physiological function.

Nitrite and Hydroxylamine Reduction by Bryophyte Hemoglobin

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Keywords: Bryophyte, Phytoglobins, Nitrogen metabolism, Nitrite, Hydroxylamine, Ammonium

During periods of hypoxic stress, like those caused by soil flooding, harmful amounts of nitrite and nitric oxide accumulate in cells. It has been shown that during these events, phytoglobins as well as hemoglobins found in bryophytes are upregulated. Previous studies have shown that phytoglobins from vascular plants are able to reduce nitrite and hydroxylamine. This opens a possible anaerobic respiration mechanism with ammonium as a final product that may increase plant survival and nitrogen utilization. Previous phylogenetic studies have shown that a common ancestral globin predates modern vascular plant hemoglobins, including those found in the phytoglobin class as well as the class 2 hemoglobins and the later evolved leghemoglobins which are involved in oxygen transport. Hemoglobin from the moss, *Physcomitrella patens,* is a representative member of this ancestral protein family and little work has been done to probe its function. Here we demonstrate through spectrophotometric studies that hemoglobin from *Physcomitrella patens* efficiently reduces both nitrite and hydroxylamine at rates comparable to or faster than phytoglobins. These results will advance our understanding of the reductive reactions that hemoglobins are able to catalyze in support of nitrogen metabolism. Further, by characterizing primordial globins in this manner, we can gain further insight into the evolution of physiological functions in the globin superfamily.

Myoglobin gene expression in breast and prostate cancer at single-cell resolution

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Myoglobin (Mb) is primarily expressed in mitochondria-rich myocytes where it fulfils a prime role in oxygen storage and intracellular transport, but it also has additional non-classical functions such as the detoxification of nitric oxide or reactive oxygen species. Mb expression has also been reported for brown fat, where Mb probably affects cellular metabolism via its fatty-acid binding properties. In addition, Mb is expressed in secretory cells of mammalian epithelia and in several human tumour entities. Importantly, previous studies have associated expression of Mb with favourable prognosis in both breast and prostate cancer.

To study the role of Mb in a cancer context, we evaluated cell type-specificity of Mb expression and its influence on transcriptome-wide gene expression at single-cell resolution, making use of publicly available single-cell RNA-sequencing (scRNA-seq) expression data of breast and prostate cancer patients.

Cluster analysis revealed strong association of Mb expression with the (mature) luminal cell-type in both breast and prostate cancer. Across mature luminal cells in HER2-positive breast cancer profiles, we discovered Mb-associated transcriptional heterogeneities. A particular cell population displaying high expression of proliferation marker MKI67 exhibited lower on average expression of Mb, suggesting an association of Mb expression with the differentiation status of luminal cells in cancerous breast tissue. Differential gene expression and gene ontology analyses were employed to further dissect transcriptional heterogeneity linked to Mb expression. The results suggest an association of Mb expression with secretory properties in breast and prostate cancer cells, confirming the previously documented correlation of Mb expression with the luminal cell-type. Thus, we conclude that Mb transcripts may be considered a molecular marker of better differentiated and secretory mature luminal cell-type in both breast and prostate cancer.

Reductions in hemoglobin buffering capacity increase O_2 delivery in pre-and post-natal high metabolic-rate birds and mammals

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Keywords: hemoglobin, specific-buffer capacity, convergent evolution, endotherm

The oxygenation-linked binding and release of protons to surface-exposed histidine residues of vertebrate hemoglobin (Hb) is an important contributor to O_2 uptake and delivery (via the Bohr effect) and blood acid-base balance. We tested the premise that reductions in titratable histidine content enhance O_2 delivery in lineages of small endothermic birds and mammals, which have basal mass-specific O_2 requirements that are ~75-fold higher than the largest terrestrial species. Specifically, we hypothesized that reductions in specific Hb buffer value (βHb) should produce an exaggerated reduction in red blood cell pH for a given acid (CO_2) load, which then through the Bohr effect would augment O_2 offloading to metabolically active tissues while safeguarding O_2 uptake at the lungs. Consistent with our expectations, calculated βHb values for 369 avian and 449 mammalian species based on the primary structures of their component globin chains revealed strong (18-47%) independent reductions in predicted βHb in five clades (hummingbirds, passerines, shrews, bats and afroinsectivorans) relative to the phylogenetic mean of their respective classes. Notably, convergent replacements of histidine residues at five positions largely underlie reductions in βHb in these high metabolic rate clades. Theoretical modelling employing measured βHb in shrews (which is 46% lower than in adult human Hb) suggests this trait alone increases O_2 offloading by ~42% per transit through the systemic capillaries at rest. Reductions in β Hb should also facilitate an elevated hematocrit (a common adaptation in small endothermic clades) that further increase O_2 carrying capacity/delivery. Finally, since the red blood cells of embryonic birds and most fetal mammals contain the same hemoglobin isoforms as found in adults, evolutionary reductions in βHb are expected to increase maternal-fetal gas exchange (mammals), while aiding systemic O_2 offloading in both prenatal birds and mammals.

High-resolution crystal structure of the hexacoordinated nerve Hemoglobin of the bivalve mollusc *Spisula solidissima*

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[#] In memory of our friend and colleague Prof. Sylvia Dewilde

Keywords: nerve globin, heme iron hexacoordination, invertebrate globin, crystal structure

Members of the hemoglobin (Hb) superfamily are present in nerve tissue of several vertebrate and invertebrate species. In invertebrates they have a hexa- or pentacoordinate heme iron, are mostly expressed at high levels (mM), and have been suggested to have a myoglobin-like function. The native Hb of the surf clam *Spisula solidissima* (SsHb), composed of 162 amino acids, was previously characterized as a hexacoordinate heme iron protein by UV-visible and resonance Raman spectroscopy. Furthermore, kinetic and equilibrium measurements showed a moderate oxygen affinity, with P50 ~0.6 torr, and no cooperativity. Phylogenetic analysis demonstrated a clustering of the *S. solidissima* nerve Hb with mollusc Hbs and myoglobins, but not with the vertebrate neuroglobins. Here, we present the high-resolution (1.7 Å) crystal stucture of SsHb and we describe its properties within the hexacoordinated Hb family.

Nitrobindins: a new family of heme-based sensors

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Keywords: Nitrobindins, heme-based sensors, reactive nitrogen species detoxification, NO scavenging.

Nitrobindins (Nbs) form a new class of evolutionary conserved heme-proteins characterized by a 10-stranded anti-parallel β-barrel fold. In Nbs, the heme-Fe atom is coordinated to a proximal His residue and is stably in the ferric form. The high reactivity of Nbs towards reactive nitrogen and oxygen species (RNS and ROS, respectively) reflect the highly solvent exposure of the metal center [1,2,3]. Interestingly, while *Mt*-Nb and *At*-Nb are single-domain proteins, *Hs*-Nb has been described as a domain of the human protein named THAP4, whose function is still unknown [2,4]. THAP4 is composed of an *N*-terminal modified zinc finger domain that binds DNA and a *C*-terminal Nb domain [2,5,6]. Here, we aim at shedding light of THAP4 role in human cells. First, its expression levels and cellular localization have been analyzed in several human cell lines (*i.e.*, human embryonic kidney (HEK293), human breast cancer (MCF7), human neuroblastoma (SH-SY5Y), and human glioblastoma (U251MG). We found that THAP4 is mainly expressed in HEK293, MCF7, and SH-SY5Y cell lines and localized into the nucleus. To define THAP4 role in RNS detoxification, transcriptomic analyses were performed after cells treatment with either the peroxynitrite-donor SIN-1 or the NO-donor DEA NONOate. In addition, THAP4 interactors have been identified by mass-spectrometry and the THAP4 interactome has been retrieved using BioGRID and IntAct molecular interaction databases [7,8]. Finally, the Gene Ontology Resource and the web server QuickGo[9] have been used to investigate the biological processes involving THAP4. Overall, data obtained suggest an evolutionary conserved structure and anti-oxidant function of Nbs, and highlight a possible role of human THAP4 as a sensing protein that couples the heme-based Nb reactivity with gene transcription.

References

[1] C.M. Bianchetti, G.C. Blouin, E. Bitto, J.S. Olson, G.N Phillips Jr. Proteins (2010), 78, 917-931. [2] C.M.Bianchetti, C.A. Bingman, G.N. Phillips Jr. Proteins (2011), 79, 1337-1341.
[3] G. De Simone, P. Ascenzi, F. Polticelli F. IUBMB Life (2016), 68, 423-428.
[4] G. De Simone, A. di Masi, F. Polticelli, P. Ascenzi. FEBS Open Bio (2018), 8, 2002-2010.
[5] G. De Simone, A. di Masi, G.M. Vita, F. Polticelli, A. Pesce, et al. Antioxid Redox Signal (2020), 33, 229-246.
[6] G. De Simone, A. di Masi, A. Pesce, M. Bolognesi, C. Ciaccio, et al. Int J Mol Sci (2021) 22, 1674- 1700.
[7] R. Oughtred, J. Rust, C. Chang, B.J. Breitkreutz, C. Stark, A. Willems, et al. Protein Sci (2021) 30, 187-200.
[8] S. Orchard, M. Ammari, B. Aranda, L. Breuza, L. Briganti,et al. Nucleic Acids Res. (2014) 42, D358- D363.
[9] D. Binns, E. Dimmer, R. Huntley, D. Barrell, C. O'Donovan, R. Apweiler. Bioinformatics (2009) 25, 3045-3046.

Generating myoglobin knockout and knockin zebrafish to study the role of myoglobin in metabolic rate and respiration

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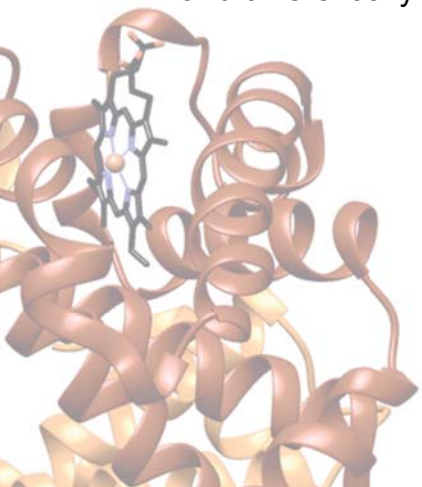
Keywords: myoglobin (Mb), CRISPR-Cas, knockout (KO), knockin (KI), zebrafish, genetic compensation

Myoglobin (Mb) is a carrier and shuttle of oxygen in the heart of vertebrates. We intend to investigate how Mb content and its affinity for O_2 and H_2S affect whole animal metabolic rate and heart mitochondrial respiration by generating a knockout (KO) and knockin (KI) of the myoglobin gene (mb) in zebrafish models using the CRISPR-Cas9 gene editing system.

The approach chosen for the KO relies on co-injecting 4 guide RNAs (gRNAs) together with Cas9 mRNA in 1-cell zebrafish embryos. This "shotgun" approach is intended to generate a large inframe deletion in the mb gene. The reason we are straying from the mainstream approach of introducing an early frameshift, and the resulting premature termination codon (PTC), is to avoid the upregulation of homologous genes through genetic compensation.

In the KI model, we want to generate a missense mutation of the distal histidine (E7) to a glutamine to alter Mb heme ligand affinity. To generate the KI we are injecting a 300 nucleotide long single stranded DNA template carrying the mutation, Cas9 mRNA and a single gRNA in 1-cell embryos. The gRNA together with Cas9 will introduce a double stranded break and the single stranded donor template will hopefully be utilized for homology directed repair.

We plan to utilize Oroboros instruments, a intermittent-flow respirometry system and transcriptomics to respectively investigate mitochondrial respiration, metabolic rate and differentially expressed genes in adult KO, KI, and wildtype zebrafish.



Extracellular neuroglobin in the neuronal transmission of cell resilience to oxidative and mitochondrial stress

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Keywords: Neuroglobin, Neuroprotection, Cellular Stress, Cell-Cell Comunication

Neuroglobin (NGB) is a stress sensor protein, that exerts, when overexpressed, cytoprotective effects against oxidative stress and neurodegeneration. We defined that the activation of Estrogen Receptor (ER)-mediated pathways, induced by 17 - estradiol (E2) and exogenous ligands (resveratrol - Res), is pivotal for NGB- upregulation and its cell-autonomous effect in promoting cell survival. Recent data have also indicated that NGB could exist at the extracellular level, opening to the possibility of a new functional role of exogenous NGB in the nervous system.
Following this evidence, we investigated the possibility of extracellular NGB release in the presence of NGB inducers and its possible role outside cells. Data indicate that oxidative stress (H2O2) exposure and the activation of ER promote NGB secretion by neuron-derived cells (SH-SY-5Y) through the exosomal and non- exosomal pathways, suggesting a paracrine function of the protein.
To strengthen this idea, we evaluated the effect of cell-derived extracellular NGB, by using conditioned media (CM) and exosomes derived from wild type (WT) and NGB overexpressing cells (NGB-HA). Obtained results demonstrated that NGB- enriched CM, collected by NGB-HA cells, prevent the early mitochondrial fragmentation and, in turn, reduce apoptotic cell death in SH-SY-5Y cells after oxidative stress treatment or mitochondrial toxicity induced by 3-nitropropionic acid (3NP). Furthermore, we observed a similar anti-apoptotic effect by using the NGB- enriched exosomal or the exosome-deprived CM fractions, suggesting that NGB release as free protein or through extracellular vesicles can be neuroprotective, independently.
Altogether, obtained results strengthen the idea that NGB could operate in the extracellular compartment as a transmission factor in non-cell-autonomous mechanisms of neuroprotection, opening to the possibility of exogenous NGB as a new targetable neurotrophic protein in neurodegenerative disease.