Report on SFRRI-Supported Conference/Meeting

Name of Conference: Thiol-based redox switches: From chemistry to physiology and pathology

Location of Conference: Sant Feliu de Guixols, Girona, Spain

Date of Conference: 13-18 September 2025

Use of funds granted (1,000 characters)
Describe use (Note, the funds granted must only be used for the indicated purposes)
Support travel and reduce registration fees for young researchers and students

Acknowledgement of SFRRI support
Confirm acknowledgement of SFRRI support (Note, SFRRI support must be acknowledged in the printed program, book of abstracts, welcome board, materials, bags, etc. and electronic media, *e.g.*, meeting website and email promotions)

Printed Program	☐
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Meeting Website	☒
Email Promotions	☐
Others	☒ (If ticked, please provide details) in opening and closing talks by the orgenizers

Scientific report (3,500 characters)
Including number of delegates, main themes, major breakthroughs, etc.

The conference brought together 158 participants (85 PhD students and postdocs, and 73 senior scientists and PIs) representing diverse fields including chemistry, cell biology, cancer research, biochemistry, microbiology, and physiology.

The participants comprised 81 women and 77 men, with the majority from Europe (116). Additional participants included 14 from Asia (China, Japan, India), 13 from South America (Brazil, Uruguay, Argentina), 7 from Israel, 7 from North America (USA and Mexico), and 2 from New Zealand.

The scientific program integrated multiple aspects of redox biology: from imaging and quantitative methodologies to measure redox status in cells, proteins, and RNA to elucidation of biological pathways regulated by oxidants and reductants; and their impact on development, pathologies and longevity.

The conference featured a wide range of talks by established and early-career PIs, as well as PhD students and postdocs. In total, we had 58 talks deleivered by 30 invited speakers and 28 selected from abstracts speakers. Topics included: Structural biology and organization of redox-switch proteins;

Selenocysteine-containing proteins and their biological pathways; Ferroptosis; Polysulfidation and its role in signaling and stress response; Redox biology in cellular organelles (ER, mitochondria, nucleus) and inter-organelle crosstalk; Redox regulation of proteostasis, the cell cycle, proliferation, differentiation, cancer, and aging; Interplay between redox homeostasis and metabolism in health and disease; Redox regulation in microorganisms (bacteria, yeast, viruses) and the role of thiol-switch proteins in host–pathogen interactions; Understanding the beneficial role of oxidation in adaptation to stress response, development, and longevity.

A central aim of the meeting was to bridge chemistry and biology, highlighting novel methodologies in redox chemistry that can be applied to biological systems, and introducing biological questions that can inspire chemists to develop new tools. Among the open questions discussed were:

• What is the origin of oxidation in different organelles?
• How do changes in redox status during early development positively affect longevity and proliferation?
• What are the key players in the crosstalk between metabolism, the cell cycle, and redox homeostasis in health and disease?
• What are the structures and functions of complexes formed by low-molecular-weight antioxidants and oxidants, and how are they transferred across cells and tissues?
• How can profiles of redox-dependent post-translational modifications be defined, and how might they be translated into clinical applications?

With support from SFRII and other sponsors, we managed to reduce registration fees, provide travel grants to students and young PIs, and award 6 poster prizes and 3 talk awards to students and postdocs.

The talks, poster sessions, multiple networking events stimulated numerous and fascinating discussions across disciplines and career stages, generating new collaborations, exchanges of ideas, and formulation of new theories. It was clearly evident (and repeatedly mentioned by students and PIs) that the atmosphere encouraged interaction and initiated many new connections between students, postodocs and PIs. Several initiatives emerged to establish long-term frameworks for collaboration, such as COST actions and “student redox clubs,” aimed at fostering continued exchange and cooperation in the field.

Meeting Program and Book of Abstracts
Attach as separate files (in pdf format)

Person responsible for this Report
Name: Dana Reichmann Role in SFRRI-supported Conference: Main orgenizer
Affiliation: The Hebrew
University of Jerusalem, Israel

Date: 30/9/2025 Signature: D. Reichmann

Email this report and attachments to:
The Secretary-General, SFRRI: Professor Patricia Oteiza (poteiza@ucdavis.edu)

EMBO
Workshop

ABSTRACT BOOK

Thiol-based redox switches: From chemistry to physiology and pathology

13 – 18 September 2025

CONTENT

ABOUT THE WORKSHOP

Redox reactions have been essential for the emergence of life on Earth, and diverse strategies have evolved for responding to various cellular redox species. These mechanisms are still of vital importance in the physiology and pathology of present-day organisms (from bacteria to men).

At the core of these mechanisms lie functionally and structurally distinct thiol-switch proteins, utilizing reversible, site-specific oxidation that regulates their activity, downstream signaling, and biological processes.

This EMBO Workshop will bring together redox chemists and cell biologists to exchange knowledge in thiol-redox biology and start new collaborations to uncover the relevance of thiol oxidation for organismal physiology and pathology.

ABOUT EMBO COURSES AND WORKSHOPS

EMBO Courses and Workshops are selected for their excellent scientific quality and timelines, provision of good networking activities for all participants and speaker gender diversity (at least 40% of speakers must be from the underrepresented gender).

Organisers are encouraged to implement measures to make the meeting environmentally more sustainable.

ORGANIZERS

Dana Reichmann
Dept. of Biological Chemistry,
The Hebrew University of Jerusalem,
Israel

Tobias Dansen
University Medical Center Utrecht,
Center for Molecular Medicine,
Utrecht, Netherlands

SPEAKERS

- **Haike Antelmann,** Institute of Biology-Microbiology, Freie Universität Berlin, Berlin, Germany
- **Tslil Ast,** Biomolecular Sciences Dept., Weizmann Institute of Science, Rehovot, Israel
- **Keriann Backus,** Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA, USA
- **Liron Bar-Peled,** Krantz Family Center for Cancer Research, Massachusetts General Hospital, Boston, MA, USA
- **Daphne Bazopoulou,** Department of Biology, University of Crete, Heraklion, Greece
- **Vsevolod Belousov,** Federal Center of Brain Research and Neurotechnologies, FMBA, Moscow, Russia
- **Helena Cochemé,** MRC Laboratory of Medical Sciences (LMS), London, UK
- **Jean-François Collet,** de Duve Institute, Université Catholique de Louvain, Brussels, Belgium
- **Marcus Conrad,** Institute of Metabolism and Cell Death, Molecular Target and Therapeutics Center, Helmholtz Munich, Neuherberg, Germany
- **Jan-Ulrik Dahl,** Microbiology, School of Biological Sciences, Illinois State University, USA
- **Tobias Dick,** Division of Redox Regulation, DKFZZMBH Alliance, German Cancer Research Center (DKFZ), Heidelberg, Germany
- **José Antonio Enríquez,** Centro de investigaciones Cardiovasculares (CNIC) & Centro de investigaciones Biomédicas en Red (CIBERFES), Madrid, Spain
- **Deborah Fass,** Department of Chemical and Structural Biology, Weizmann Institute of Science, Rehovot, Israel
- **Ivan Gout,** Department of Structural and Molecular Biology, University College London, United Kingdom
- **Elena Hidalgo,** Oxidative Stress and Cell Cycle Group, Universitat Pompeu Fabra, Barcelona, Spain
- **Marianne Ilbert,** CNRS, Aix-Marseille University, Bioenergetic and Protein Engineering Laboratory, BIP UMR 7281, Mediterranean Institute of Microbiology, Marseille, France
- **Ursula Jakob,** Department of Molecular, Cellular, and Developmental Biology, University of Michigan, USA
- **Paraskevi Kritsiligkou,** University of Liverpool, United Kingdom
- **Lars Leichert,** Ruhr University Bochum, Institute of Biochemistry and Pathobiochemistry, Microbial Biochemistry, Bochum, Germany
- **Roland Lill,** Institut für Zytobiologie, Philipps-Universität Marburg, Germany
- **Brian McDonagh,** Discipline of Physiology, School of Medicine, University of Galway, Ireland
- **Iria Medraño-Fernández,** Neuroscience and Biomedical Science, Universidad Carlos III, Madrid, Spain
- **Norman Metanis,** Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel
- **Bruce Morgan,** Zentrum für Human- und Molekularbiologie (ZHMB), Universität des Saarlandes, Biochemie Campus, Saarbrücken, Germany
- **Dejana Mokranjac,** Biocenter-Department of Cell Biology, LMU Munich, Munich, Germany
- **Rafael Radi,** Department of Biochemistry, Universidad de la República, Uruguay
- **Jan Riemer,** Institute of Biochemistry, University of Cologne, Germany
- **Sharon Ruthstein,** Department of Chemistry and the Institute of Nanotechnology and Advanced Materials (BINA), Bar-Ilan University, Ramat-Gan, Israel
- **Carolyn Sevier,** Department of Molecular Medicine, College of Veterinary Medicine, Cornell, Ithaca, NY, USA
- **Kai Tittmann,** Department of Molecular Enzymology, Göttingen Center of Molecular Biosciences, Georg-August University Göttingen, Germany

PROGRAMME

Day 1 | 13 September 2025

14:00-18:30	Arrival and Registration
18:30-18:45	**OPENING AND WELCOME** Dana Reichmann and Tobias Dansen
18:45-19:30	**KEYNOTE LECTURE: ROLAND LILL** Chair: Tobias Dansen *The crosstalk of eukaryotic iron-sulfur protein biogenesis and thiol redox biology*

Day 2 | 14 September 2025

SESSION 1: NEW TOOLS AND TECHNIQUES FOR REDOX BIOLOGY
Chair: Armindo Salvador

09:00-09:30	*Heterooligomerisation: a new paradigm in peroxiredoxin biology* Bruce Morgan
09:30-9:50	*CyReB, a biosensor dedicated to the dynamic monitoring of the intra cellular cysteine pool* Jérémy Couturier
9:50-10:10	*Genetically-encoded biosensors to monitor cysteine and taurine in living cells* Brandán Pedre Pérez
10:10-10:40	*Modeling neugodegeneration using redox chemogenetics* Vsevolod Belousov
10:40-11:10	Identification of Druggable and Redox Vulnerabilities in Cancer Liron Bar-Peled
11:10-11:40	Coffee break

PROGRAMME

Day 2 | 14 September 2025

11:40-12:10	*Redox proteomic approaches to delineate the impact of cell stress* Keriann Backus
12:10-12:30	*Bio-chalogenides production and functions in human vascular endo thelium* Takaaki Akaike
12:30-12:50	*The cellular Trx/TrxR system as a stress integration hub for tumour-selective drug release* Julia Thorn-Seshold
12:50-13:10	*ROS induce hormesis in a localization- and p53-dependent manner* Janneke Keijer
13:10-13:30	*H2Switch: A Chemogenetic Approach for Intracellular Hydrogen Sulfi de Modulation* Asal Ghaffari Zaki
13:30-15:30	**Lunch and Free Time**

SESSION 2: STRUCTURE-FUNCTION RELATIONSHIPS IN REDOX PROTEINS

Chair: Christopher Horst Lillig

15:30-16:00	*Thiol Redox Switches in Intestinal Innate Immunity* Deborah Fass
16:00-16:20	*Integrating low-resolution biophysical approaches and computational simulations to characterize disulfide roles in protein conformational landscapes* Ari Zeida
16:20-16:40	*Prediction of redox sensitive disordered regions* Gabor Erdos
16:40-17:10	*Knockin' on redox doors: the ins and outs of peroxiporin fluxes* Iria Medraño-Fernández
17:10-17:40	**Coffee break**

PROGRAMME

SESSION 3: SELENOCYSTEINES: DUAL ROLES AS ESSENTIAL TOOLS AND ACTIVE PLAYERS IN BIOLOGICAL SYSTEMS

Chair: Dana Reichmann

17:40-18:10	*Ferroptosis as the root cause of neurodegenerative disease* Marcus Conrad
18:10-18:30	*Metabolic modulation of Selenite toxicity* Inés Garcia de Oya
18:30-19:00	*Chemical Modification at Cysteine and Selenocysteine in Peptides and Proteins* Norman Metanis
19:00-19:30	**FLASH TALKS SESSION 1 /** ***POSTERS P01-P045***
19:30-20:30	**Dinner**
20:30-22:30	**POSTER SESSION 1 WITH DRINKS /** ***POSTERS P01-P045***

Day 3 | 15 September 2025

SESSION 4: BEYOND THE ORDINARY: INVESTIGATING NOVEL THIOL MODIFICATIONS AND REGULATORY FUNCTIONS

Chair: Deborah Fass

9:00-9:30	*Cysteine-lysine redox switches - an update* Kai Tittman
9:30-10:00	TBA TBA
10:00-10:20	*Persulfidation loss leads to cysteine hyperoxidation with impairment of proteasome function.* Martín Hugo
10:20-10:50	*Understanding the role of per- and polysulfides in cytoprotection* Tobias Dick
10:50-11:10	*Modulating Endogenous Hydropersulfides to Promote Antioxidant Response, Inhibit Ferroptosis* Harinath Chakrapani
11:10-11:40	**Coffee break**

PROGRAMME

Day 3 | 15 September 2025

11:40-12:10	*Redox control of mitochondrial translation* Tslil Ast
12:10-12:30	*Mammalian cells contain elemental sulfur that protects them against ferroptosis* Uladzimir Barayeu
12:30-12:50	*Coupling RNA processing pathways with Fe-S cluster distribution and redox homeostasis drives (patho)biochemistry of PYROXD1* Igor Asanovic
12:50-13:20	*The peroxide-carbon dioxide interplay in the biochemistry of thiol redox switches* Rafael Radi
13:40-15:20	**Lunch and Free Time**

SESSION 5: REDOX CONTROL OF PROTEOSTASIS

Chair: Aeid Igbaria

15:20-15:50	*Redox homeostasis and protein quality control* Carolyn Sevier
15:50-16:10	*Metal-Responsive Up-regulation of Bifunctional Disulfides for Suppressing Protein Misfolding and Promoting Oxidative Folding* Takahiro Muraoka
16:10-16:30	*Small molecule-mediated inhibition of the oxidoreductase ERO1A restrains aggressive breast cancer by impairing VEGF and PD-L1 in the tumor microenvironment* Ester Zito
16:30-16:50	*Ca2+-driven PDIA6 condensate formation to ensure oxidative proinsulin folding* Masaki Okumura
16:50-17:20	**Coffee break**
17:20-17:50	*Organizing a compartment by redox - the mitochondrial intermembrane space* Jan Riemer

PROGRAMME

17:50-18:20	*Peroxiredoxin dependent mitochondrial remodelling in C. elegans* Brian McDonagh
18:20-18:50	*A novel pathway for oxidation and assembly of mitochondrial inner membrane proteins* Dijana Morkanjae
18:50-19:30	**FLASH TALKS SESSION 2 /** *POSTERS P46-P095*
19:30-20:30	**Dinner**
20:30-22:00	**POSTER SESSION 2 WITH DRINKS /** *POSTERS P46-P095*

Day 4 | 16 September 2025

SESSION 6: REDOX BIOLOGY OF MICROORGANISMS AND ITS ROLE IN MICROBE-HOST INTERACTIONS

Chair: Jean-Francois Collet

9:00-9:30	*Redox-sensing mechanisms of quinones in Staphylococcus aureus* Haike Antelmann
9:30-9:50	*Glutathione beyond the cytosol – Comprehensive elucidation of glutathione import and its role in the periplasm of Escherichia coli* Lisa Roxanne Knoke
9:50-10:10	*Emerging roles of Prx6-type enzymes in H2S and persulfide metabolism* Laura Leiskau
10:10-10:40	*Respiratory metabolism, mitochondrial efficiency and ROS production: key drivers of longevity* Elena Hidalgo
10:40-11:10	*Utilising tethered biosensors to uncover intracellular redox heterogeneity* Paraskevi Kritsiligkou
11:10-11:40	Coffee break

PROGRAMME

Day 4 | 16 September 2025

SESSION 6: REDOX BIOLOGY OF MICROORGANISMS AND ITS ROLE IN MICROBE-HOST INTERACTIONS

Chair: Marianne Ilbert

11:40-12:10	*Redox switches in the interaction of immune cells and bacteria* Lars Leichert
12:10-12:30	*Neutrophil-derived ROS as a source of mutagenesis in cancer* Wytze den Toom
12:30-13:00	*Response and defense mechanisms of bacterial pathogens towards reactive oxygen and chlorine species* Jan-Ulrik Dahl
13:00-13:30	*How the assembly of the bacterial envelope depends on cysteine residues* Jean-Francois Collet
13:30-13:50	*Differential Role of Sulphur Species in Regulating HIV-1 Latency* Ragini Agrawal
13:50-15:00	**Lunch**
15:00-18:30	**Social Activity / Free Time**
18:30-19:30	**NETWORKING EVENT FOR YOUNG INVESTIGATORS** Chair: Tobias Dick and Sharon Ruthstein
19:30-21:00	**Dinner**

PROGRAMME

Day 5 | 17 September 2025

SESSION 7: BRIDGING METABOLISM, REDOX REGULATION AND CELL PROLIFERATION IN HEALTH AND DISEASE

Chair: Tobias Dansen

9:00-9:30	*From molecular level knowledge on the cellular copper uptake to the development of 64Cu-based radiotracer* Sharon Ruthstein
9:30-9:50	*Copper based Chalcogenide Quantum Dots for Neuron stimulation, Controlled ROS Signalling, and Neuroprotection against Ferroptosis* Sharadrao Vhanalkar
9:50-10:20	*Copper Impacts Proteostasis: A Closer Look* Marianne Ilbert
10:20-10:40	*Reductive stress by thiol antioxidants activates the hypoxia response pathway* Jogender Singh
10:40-11:00	*Oxidation of Human Glutamine Synthetase Thiols Parallels Enzyme Inactivation and Promotes Aggregation* Silvina Bartesaghi
11:00-11:30	**Coffee break**

SESSION 7

Chair: Nikolas Gunkel

11:30-12:00	*Coenzyme A biology, but not as we know it* Ivan Gout
12:00-12:20	*NAD(P)H sensing regulates the fate of enteroendocrine cells* Bohdana Rovenko
12:20-12:40	*Redox control of intestinal organoids in homeostasis and development* Silvia Torchio
12:40-13:00	*When hydrogen peroxide helps or harms: micromolar cytosolic thres holds in cultured human cells* Olga Lyublinskaya
13:00-13:30	*Redox signals from the mitochondrial electron transport chain* José Antonio Enríquez
13:30-15:30	**Lunch and Free Time**

PROGRAMME

Day 5 | 17 September 2025

SESSION 8: REDOX REGULATION IN AGEING
Chair: Dejana Mokranjac

15:30-16:00	*Redox signalling in ageing* Helena Cocheme
16:00-16:20	*Peroxiredoxins as markers of redox homeostasis in human blood cells during ageing* Mark Hampton
16:20-16:40	*Patient derived fibroblasts as a novel model to study redox related alterations in tauopathies* Mariana Holubiec
16:40-17:10	*Early-in-life redox-regulation of health and longevity* Daphne Bazopoulou
17:10-17:40	**Coffee break**
17:40-18:25	*Keynote lecture: Redox Regulated HIstone Modifications - How Transient ROS Events Turn into Long-Lasting Changes* Ursula Jakob
18:25-18:40	**CLOSING REMARKS AND PRIZES** Dana Reichmann and Tobias Dansen
19:30-21:30	**Gala Dinner**

Day 6 | 18 September 2025

7:00-10:00	**Breakfast and departure** Scheduled transfer services: Departures are at 7:00 AM and 10:00 AM.

INVITED SPEAKERS

** Abstracts are listed in alphabetical order*

Redox-sensing of quinones by the QsrR and MhqR repressors in Staphylococcus aureus

Thao Thi-Phuong Nguyen[1#], Paul Weiland[2,3#], Vu Van Loi[1], Stephan Kiontke[2], Victor Zegarra Leon[2], Antonia Kern[2], Agnieszka K. Bronowska[4], Daniil Baranov[4], Verena Nadin Fritsch[1], Gert Bange[2,5], Haike Antelmann[1*]

#contributed equally to this work; *corresponding author

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[2] *Center for Synthetic Microbiology (SYNMIKRO) and Department of Chemistry, Philipps-University Marburg, D-35043 Marburg, Germany*

[3] *Center for Tumor Biology and Immunology, Department of Medicine, Philipps-University Marburg, Marburg, Germany*

[4] *Newcastle University Centre for Biomedical Engineering SNES - Chemistry, Bedson Building, Newcastle University, NE1 7RU Newcastle upon Tyne*

[5] *Max-Planck Institute for Terrestrial Microbiology, Marburg, Germany*

During infection, Staphylococcus aureus is exposed to reactive electrophilic species, such as quinones and aldehydes as well as redox-active antimicrobial compounds. The quinone response is controlled by a complex network of quinone reductases and ring cleavage dioxygenases governed by the MarR-type repressors QsrR and MhqR, which confer resistance towards quinones and antimicrobials, such as rifampicin, ciprofloxacin, and pyocyanin.

The QsrR repressor was characterized as redox-sensing repressor, which senses methylhydroquinone (MHQ) via a thiol-switch mechanism, involving intersubunit disulfide formation between the conserved Cys4 and the Cys29 or Cys32 residues. However, the quinone-sensing mechanism of the MhqR repressor was unknown thus far. We hypothesized that quinones bind to a ligand-binding pocket of MhqR, leading to its inactivation and derepression of transcription of the mhqRED operon for quinone detoxification. Thus, we resolved the crystal structures of apo-MhqR and the MHQ-bound MhqR complex. In addition, AlphaFold3 and MD simulations were used to model the predicted structure of MhqR in complex with its operator DNA. In the DNA-bound MhqR state, we identified an allosteric loop, which formed an extended helix α4 for optimal DNA binding. Key residues for MHQ interaction were identified as F11, F39, E43 and H111, forming the hydrophobic ligand binding pocket of MhqR. MHQ binding prevented the formation of the extended helix α4 in the allosteric loop, which adopted a coiled conformation, incompatible for DNA binding. Using mutational analyses, we validated that F11, F39 and H111 are required for MHQ sensing in vivo, whereas critical residues of the allosteric loop were essential for DNA binding in vivo. In conclusion, our structure-guided modelling and mutational analyses identified a quinone-binding pocket of MhqR and the mechanism of MhqR inactivation, which involves local structural rearrangements of an allosteric loop to prevent DNA interactions.

Redox control of mitochondrial translation

Ast Tslil

Biomolecular Sciences Dept., Weizmann Institute of Science, Rehovot, Israel

Friedreich's ataxia (FA), the most prevalent monogenic mitochondrial disorder, arises from deficiency of frataxin (FXN), a critical iron-sulfur (Fe-S) cluster biogenesis factor. To elucidate the cellular consequences of frataxin depletion, we integrated proteomic profiling with genetic interaction mapping of FXN edited cells. This approach uncovered impaired mitochondrial translation as a key downstream consequence of FXN loss, with the methyltransferase-like protein METTL17 emerging as a critical mediator. Through comparative genomics, site-directed mutagenesis, biochemical reconstitution, and cryo-EM structural analysis, we demonstrate that METTL17 associates with the mitoribosomal small subunit during late-stage assembly and harbors a previously uncharacterized [Fe4S4]2+ cluster that is indispensable for its mitoribosomal stability. This Fe-S cluster renders METTL17 sensitive to its redox environment, depleting the levels of this assembly factor when respiratory chain activity is perturbed. Remarkably, METTL17 overexpression selectively rescued mitochondrial translation and bioenergetic capacity in FXN-null cells while failing to restore cellular proliferation. These findings support a model whereby METTL17 functions as a Fe-S cluster-dependent checkpoint mechanism, coupling mitochondrial protein synthesis to cellular iron-sulfur cluster availability and thereby ensuring coordinated assembly of Fe-S cluster-rich OXPHOS complexes only under conditions of adequate cluster supply.

Identification of Druggable and Redox Vulnerabilities in Cancer

Liron Bar-Peled, PhD

Krantz Family Center for Cancer Research, Massachusetts General Hospital, Boston, MA, USA

Reactive oxygen species (ROS) underlie human pathologies including cancer and neurodegeneration. However, the proteins which sense ROS levels and regulate their production through their cysteines remain ill defined. Systematic base-editor and computational screens revealed cysteines in VPS35–a Retromer trafficking complex member, when mutated phenocopy inhibition of mitochondrial translation. We find that VPS35 underlies a reactive metabolite-sensing pathway that lowers mitochondrial translation to decrease ROS levels. Intracellular H2O2 oxidizes cysteines within VPS35, resulting in Retromer dissociation from endosomal membranes and subsequent plasma membrane remodeling. We demonstrate that plasma membrane localization of Retromer substrate SLC7A1 is required to sustain mitochondrial translation. Furthermore, lowering VPS35 levels or oxidation of its ROS-sensing cysteines confers resistance to ROS-generating chemotherapies including cisplatin in ovarian cancer models. Thus, we identify that intracellular ROS levels are communicated to the plasma membrane through VPS35 to regulate mitochondrial translation, connecting cytosolic ROS sensing to mitochondrial ROS production.

Early-in-life redox-regulation of health and longevity

Daphne Bazopoulou

Department of Biology, University of Crete, Heraklion, Greece

Reactive oxygen species (ROS) are widely recognized as key regulators of cellular signaling, playing a role in maintaining homeostasis and promoting organismal survival. Previous studies showed that a mild elevation in ROS which occurs during C. elegans development can lead to adaptation to external perturbations and increased resilience to age-dependent cellular decline. We now show that early in life redox-altering events 1) are an integral part of known longevity-promoting pathways; 2) can strengthen immune defenses and 3) even trigger beneficial heritable changes. As we further investigate these redox-sensitive events, we highlight the importance of developmental ROS as universally relevant pro-health and pro-longevity signals, with the clear potential to identify early-life stage interventions that can positively affect long term organismal fitness.

How the assembly of the bacterial envelope depends on cysteine residues

Jean-Francois Collet

de Duve Institute, Université Catholique de Louvain, Brussels, Belgium

The outer membrane of Gram-negative bacteria is physically tethered to the underlying peptidoglycan, a connection long thought to be purely structural. Our recent work reveals that this attachment enables the buildup of periplasmic turgor, a mechanical force that is essential to protect bacteria from osmotic challenges. I will show that this envelope connectivity is not static but subject to redox regulation, uncovering a new layer of control in how bacteria adapt their envelope architecture to environmental stress.

Ferroptosis as the root cause of neurodegenerative disease

Marcus Conrad

Institute of Metabolism and Cell Death, Molecular Target and Therapeutics Center, Helmholtz Munich, Neuherberg, Germany

Selenium-dependent glutathione peroxidase 4 (GPX4) is the guardian of ferroptosis due to its unique role in reducing peroxides in lipid bilayers to their corresponding alcohols. The genetic deletion of Gpx4 in mice leads to early embryonic death at the gastrulation stage, while biallelic deleterious variants of GPX4 are associated with Sedaghatian-type spondylometaphyseal dysplasia (SSMD), an ultra-rare congenital disorder. SSMD is typically fatal within days of birth and is characterised by severe chondrodysplasia, delayed epiphyseal ossification, irregular iliac crests, severe hypotonia and cardiorespiratory issues. Additionally, affected patients exhibit severe central nervous system defects, including mental, behavioural and motor impairments. To investigate the molecular and structural role of GPX4 in ferroptosis and neuroprotection, we conducted an extensive study on a missense variant (p.R152H) recently identified in SSMD patients. These studies not only elucidated a unique structural mechanism by which GPX4 prevents ferroptosis but also uncovered yet-unrecognized cellular death processes underlying neurodegeneration in humans. Our findings should therefore spur future efforts to develop ferroptosis-targeted therapies, with potential applications for treating neurodegenerative diseases and beyond.

Response and defense mechanisms of bacterial pathogens towards reactive oxygen and chlorine species

Jan-Ulrik Dahl

Microbiology, School of Biological Sciences, Illinois State University, USA

Reactive oxygen and chlorine species (RO/CS) are ubiquitous, and organisms have likely used them to their evolutionary advantage from the time that oxygen became abundant in the atmosphere. In fact, oxidative stress is not always a bad thing given that it is generated by the human host to control the bacterial population. For example, neutrophils and macrophages produce high levels of RO/CS to kill phagocytized microbes during oxidative burst. The major oxidant generated during phagocytosis is hypochlorous acid (HOCl), which is also the active ingredient of household bleach, one of the most widely used disinfectants in domestic, industrial, and medical settings. HOCl is extremely reactive and bactericidal damaging many cellular components including proteins, DNA, lipids, and cofactors even at low micromolar concentrations. Likewise, exposure to several antibiotics has been associated with RO/CS formation in bacteria. However, bacteria have also evolved mechanisms to counter the detrimental effects of RO/CS, although we know very little about how inflammationassociated bacteria sense and defend RO/CS-stress. The bacterial response and defense strategies are expected to be critical for their ability to survive immune cell attack and/or antimicrobial treatment and therefore likely shape how bacteria and their host interact.

The long-term goal of my lab is to understand the RO/CS stress responses in medically relevant bacteria anddefine the role of these defense strategies during colonization and pathogenesis. We utilize biochemical, genetic, transcriptomic, and systems biology approaches to identify and characterize novel molecular mechanisms that bacteria employ to protect themselves from RO/CS-mediated damage. I will share our latest data on a novel defense system important for bacterial survival during HOCl stress, which we hypothesize links RO/CS resistance to motility and host cell invasion. Moreover, I will share how polyphosphate, a universally conserved stress defense system, protects bacteria from RO/CS-generating membrane-targeting antibiotics, exploring a novel and uncharacterized phenotype for this biopolymer. Identifying, characterizing, and targeting RO/CSstress defense systems could potentially lead to novel microbe-targeted treatment options that help to increase the body's own capacity to fight infectious diseases.

Redox signals from the mitochondrial electron transport chain

José Antonio ENRÍQUEZ

Centro de investigaciones Cardiovasculares (CNIC) & Centro de investigaciones Biomédicas en Red (CIBERFES), Madrid, Spain

The mitochondrial electron transport chain (ETC) serves not only as an energy production system but also as a critical source of redox signals that regulate cellular homeostasis. Those include the control of NAD+/NADH, CoQ/CoQH2, that have incidence on the production superoxide and peroxide by complex I. Mitochondrial Na+ acts as a novel second messenger that regulates inner mitochondrial membrane fluidity and reactive oxygen species (ROS) production, particularly by complex III. The control of mitochondrial Na+ is controlled by Na+/C2+ (NCLX) and Na+/H+ (complex I) antiporters. Na+ behaves as a dual modulator of mitochondrial ROS production - functioning as a positive modulator of forward mitochondrial ROS production by complex III while potentially serving as a negative modulator of reverse electron transfer by complex I. This duality depends critically on the bioenergetic status, cation and redox contexts, and can lead to either cellular adaptations or cell death. Finally, the ability of respiratory complexes to superassembly modulate the production of ROS by the electron transport chain and regulate the fuel preference of the ETC.

Thiol Redox Switches in Intestinal Innate Immunity

Deborah Fass

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The extensive tissue surfaces exposed to the intestinal lumen are protected by a combination of innate and adaptive immune mechanisms. Mucus secreted from goblet cells along the villi and crypts of the intestinal epithelium provides one level of innate immunity by binding pathogens and particles, physically shielding the cell surface. Mucus is composed of glycopeptides called “mucins,” which self-assemble into disulfide-bonded polymers through a stepwise mechanism involving a thiol-disulfide rearrangement while the mucins are trafficked along the secretory pathway. Structural studies of mucins have revealed the starting and ending states of this rearrangement, but how the switch is initiated is still poorly understood. Another branch of innate immunity in the gut consists of anti-microbial proteins, secreted from Paneth cells at the base of the intestinal crypts. These proteins are small, cationic, disulfide-bonded, and in many cases have direct bactericidal activity by damaging cell membranes. One human version, human defensin 6 (HD6), was proposed to function instead by forming “nanonets” that entrap bacteria and prevent them from penetrating into the tissues. We recently found that HD6 uses diet-derived antioxidant molecules to self-assemble into filaments. Moreover, we observe that, at physiological protein concentrations, HD6 forms an impenetrable gel rather than a loose net. It has been proposed that HD6 gains direct bactericidal activity upon reduction of its disulfide bonds, but the physiological relevance of this observation depends on the actual reducing potential of the intestinal lumen and the sensitivity of the gelled and free forms of HD6 to natural reductants. Overall, the intestinal lumen is a hugely complex biochemical environment in which multiple polymeric or filamentous molecules engage in thiol-based redox switches or other redox-related reactions to protect sensitive and active absorptive tissue.

Coenzyme A biology, but not as we know it

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Coenzyme A (CoA) is an essential cofactor in all living cells. CoA and its thioesters participate in diverse anabolic and catabolic pathways, allosteric interactions, biosynthesis of neurotransmitters and the regulation of gene expression. Deregulation of CoA biosynthesis in animal models and inborn mutations in human genes involved in the CoA biosynthetic pathway have been associated with neurodegeneration and cardiomyopathy.

We have recently discovered the antioxidant function of CoA, involving covalent protein modification by this cofactor and termed it CoAlation. To discover and study protein CoAlation and the antioxidant function of CoA, we have developed several novel reagents and methodologies, including: (a) anti-CoA mAb, which specifically recognize CoA in ELISA, WB, IP and IHC; (b) a robust mass spectrometry-based methodology for the identification of CoAlated proteins; and (c) efficient in vitro CoAlation and deCoAlation assays. They have been employed to demonstrate that protein CoAlation is a reversible and widespread post-translational modification induced by oxidizing agents and metabolic stress in cells, tissues and model organisms. To date, we have identified more than 2300 CoAlated proteins and showed that CoAlation modulates the activity and subcellular localization of modified proteins. It can also protect oxidized cysteine residues from overoxidation and induce significant conformational changes. Based on these findings, we propose that under physiological conditions CoA functions as a key metabolic cofactor but acts as an antioxidant in cellular response to oxidative or metabolic stress. The pattern of protein CoAlation has been examined in human pathologies associated with oxidative stress, including neurodegeneration and cancer. Recent advances on this emerging topic of redox regulation will be presented.

Respiratory metabolism, mitochondrial efficiency and ROS production: key drivers of longevity

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Although calorie restriction and enhanced mitochondrial respiration are associated with lifespan extension, the molecular bases between mitochondrial activity and longevity remain complex and context-dependent. We have investigated some of these bases by characterizing environmental interventions and longevity mutants affecting longevity in fission yeast. Characterization of the long-lived Δpka1 mutant revealed enhanced reliance on mitochondrial respiration, increased mitophagy, and a constitutively active stress response. Transcriptomic and metabolomic profiling of Δpka1 cells showed a shift toward a calorie restriction-like metabolic state. Lifespan extension in Δpka1 depended on mitochondrial morphology, cristae structure, and controlled H_2O_2 signaling. To test the importance of mitochondrial architecture in mitochondrial efficiency and cellular longevity, we will show the effect of disrupting mitochondrial cristae and respiratory supercomplex formation in Δpka1. In parallel, we have investigated whether physiological fluxes of hydrogen peroxide (H_2O_2) of mitochondrial origin can rule aging-promoting effects. We quantified H_2O_2 fluxes emanating from the mitochondria and their cellular consequences. Using genetically encoded H_2O_2 biosensors (HyPer7) and localized expression of D-amino acid oxidase (Dao1) to generate peroxide fluxes, we demonstrated that mitochondrial-derived H_2O_2 can reach other compartments and modulate gene expression. Low levels of mitochondrial H_2O_2 activated antioxidant defenses and promoted longevity, whereas higher concentrations impaired mitochondrial integrity and respiration. These findings highlight the dual role of mitochondrial H_2O_2 as both a beneficial signal and a damaging agent, depending on concentration.

Copper Impacts Proteostasis: A Closer Look

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Copper (Cu) is an essential micronutrient, required for numerous biological processes. However, at elevated concentrations it becomes highly toxic to cells through multiple mechanisms, making it a potent antimicrobial agent.

Copper toxicity is classically attributed to ROS generation and protein mismetalation. We have additionally demonstrated that Cu can severely disrupt proteostasis through a ROS-independent mechanism. Binding of Cu to high-affinity residues such as cysteine and histidine appears to destabilize native protein conformations. Under these conditions, we demonstrated that molecular chaperones play a critical role, with some even activated by copper.

We also observed that bacterial killing by copper requires very high concentrations in LB medium, whereas much lower concentrations are sufficient in minimal medium. This difference is due to copper chelation by components of rich media, which reduces Cu antimicrobial efficiency. This prompted us to investigate copper–ligand (Cu–L) complexes, which do not react with medium components, can cross bacterial membranes, and efficiently kill bacteria by yet unknown mechanisms.

We are currently characterizing two distinct Cu–L complexes, using complementary approaches to elucidate their mode of action and assess their potential as enhanced antimicrobial agents in complex environments.

KEYWORDS: Copper stress; proteostasis; protein aggregation; molecular chaperone; bacteria

Utilising tethered biosensors to uncover intracellular redox heterogeneity

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Genetically encoded fluorescent biosensors have transformed the field of redox biology, offering valuable insights into the dynamics of glutathione and hydrogen peroxide, as well as uncovering novel regulatory components of intracellular pathways. By directing these probes to specific cellular compartments, such as mitochondria, researchers have gained a deeper understanding of cross-organellar communication and redox signaling pathways. However, since redox signaling likely depends on spatial proximity, conventional freely diffusible probes perhaps fail to capture highly localized oxidation events. To addres this limitation, we developed tethered biosensors by fusing genetically encoded redox probes to the C-terminus of every open reading frame in S. cerevisiae. This innovative approach enables the monitoring of redox dynamics in the immediate vicinity of individual proteins. By subjecting our biosensor libraries to growth on varying metabolic and stress conditions, we demonstrated the existence of redoxheterogeneity even within a membrane bound compartment—an effect that isspecific to the protein to which the probe is attached to.

Redox switches in the interaction of immune cells and bacteria

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Hypochlorous acid (HOCl), a highly reactive oxidant generated in the phagolysosome of professional phagocytes, is one of the main factors in the killing of phagocytized pathogens. It oxidizes thiols but also affects amino groups: HOCl-induced N-chlorination of positively charged amino acids activates the chaperone function of RidA and, presumably, of other E. coli proteins as well, such as CnoX. This activation can be reversed by the thiol-disulfide oxidoreductase TrxA, indicating a novel thioredoxin-based reduction mechanism of N chlorinated proteins, interconnecting N-chlorination with major thiol-based redox switch mechanisms. Here, we investigate the nature of the TrxA interaction with N-chlorinated proteins and model monochloramines. N-chloramine reduction by the thioredoxin system is NADPH-dependent and relies on TrxA's CXXC motif. Using catalytic amounts of TrxA and its C35S variant together with TrxB and NADPH, we demonstrate that the resolving cysteine C35 is required for inactivation of RidA's chaperone function. Using a model chloramine, we detected rapid formation of covalently linked chloramine-TrxAC35S adducts at low pH values. However, at neutral pH, chloramines do not form covalent intermediates but lead to overoxidation of TrxAC35S's sole cysteine to cysteine sulfonic acid. Using model proteins, HOCl and N-chloramines, we further study the interaction between immune cell-generated chlorine species and protein thiol switches.

The mechanism of eukaryotic iron-sulfur protein biogenesis and its crosstalk to thiol redox biology

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Iron-sulfur (Fe-S) proteins play crucial roles in numerous important cellular processes including respiration, metabolism, genome maintenance, protein translation and antiviral response. The synthesis of Fe S clusters and their insertion into apoproteins in (non-green) eukaryotes is a multifaceted process involving over 30 proteins located in mitochondria and cytosol. The process is tightly connected to thiol redox biology, because i) Fe-S clusters are both synthesized and terminally bound by redox-sensitive cysteinyl thiols, ii) the biogenesis of Fe-S clusters requires both glutathione and glutaredoxins, and iii) Fe-S clusters can be damaged by oxidative conditions. Studies over more than 25 years have shown that the biogenesis of mitochondrial Fe-S proteins is orchestrated by the iron-sulfur cluster assembly (ISC) machinery which was inherited from bacteria during evolution (Braymer et al, 2021; Lill, 2020; Lill & Freibert, 2020). Cytosolic and nuclear Fe-S protein assembly also relies on the function of this machinery, yet additionally requires the mitochondrial ABC exporter ABCB7, glutathione, and the cytosolic iron-sulfur protein assembly (CIA) machinery (Paul & Lill, 2015). A combination of in vivo and in vitro studies have generated a good understanding of the general outline of the sequential steps of Fe-S protein biogenesis. Currently, the detailed molecular mechanisms underlying the individual reaction steps are investigated by using cell biological, biochemical, spectroscopic, and structural approaches. In my seminar, I will present some of our recent structural, spectroscopic, and biochemical insights into the molecular mechanisms underlying [2Fe-2S] cluster assembly in mitochondria. These mechanistic insights also help advancing our molecular understanding of the biochemical consequences of numerous "Fe-S diseases" linked to mutations in almost any of the ISC genes (Lill & Freibert, 2020). In particular, our studies suggest a molecular mechanism for the function of frataxin, a protein functionally impaired in Friedreich's ataxia, in the mobilization of sulfur during Fe-S cluster biogenesis. Finally, I will highlight recent collaborative studies elucidating the molecular foundation of D-cysteine toxicity for certain cancer cells.

Reviews:

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Knockin' on redox doors: the ins and outs of peroxiporin fluxes

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Redox microdomains are signaling hotspots in redox biology. Due to the physical separation of major producers and targets by lipid bilayers, the channels that distribute the messengers between compartments are key candidates for their formation and maintenance. Here, we present evidence for a topologically restrained oxidative shield surrounding the endoplasmic reticulum (ER). Using targeted probes, we characterized the ER-proximal cytosolic environment and found it to be strikingly more oxidative than the bulk cytosol. We further demonstrate that this oxidative shield is dependent on the H_2O_2 flux mediated by the peroxiporin AQP11, which constitutively channels the messenger from inside the ER. Compromising oxidative folding by silencing its main oxidase, Ero1alpha, increases the oxidative power of the region, suggesting that the wealth of the ER folding machinery can be translated into signals that promote adaptation in the cytosol. Accordingly, we identified the transcription factor HIF-1α as a key target. Altogether, our data reveal the existence of an extremely oxidative cytosolic microdomain downstream of AQP11 that, endowed with a tunable area of influence, serves as a communication hub between organelles.

Chemical Modification at Cysteine and Selenocysteine in Peptides and Proteins.

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The development of chemoselective methods for site-specific protein modification continues to expand the chemical biology toolbox.[1] In our early work, we introduced a strategy for the selective modification of selenocysteine (Sec) residues using a copper-catalyzed radical reaction with hydrazine substrates,[2] enabling facile and efficient conjugation in peptides and proteins under mild aqueous conditions. Building on this approach, we later established a general and versatile ynamide-based platform for cysteine (Cys) modification,[3] providing access to stable and functional protein conjugates with broad applicability in chemical biology. Most recently, we advanced this field by designing a late-stage aromatic C–H functionalization strategy that allows direct copper-mediated bioconjugation at Cys and Sec residues.[4] This approach enables efficient stapling, cross-linking, and modification with natural products or drug-like molecules, significantly broadening the scope of peptide and protein functionalization. Together, these contributions establish a coherent trajectory of innovation in selective protein bioconjugation, opening new avenues for probing and controlling protein function. In this lecture we will discuss these advances in the field.

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Heterooligomerisation: a new paradigm in peroxiredoxin biology

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Peroxiredoxins (Prxs) are highly conserved thiol peroxidases central to peroxide detoxification, redox signaling, and chaperone activity. Eukaryotic cells often express multiple Prx1/AhpC-type isoforms within the same compartment, yet these were long thought to assemble only as homooligomers. We show that heterooligomerization is, in fact, a conserved and functionally important feature of eukaryotic Prx1/AhpC-type peroxiredoxins. In yeast, oxidative stress promotes the formation of Tsa1–Tsa2 heterodecamers, where Tsa2 incorporation stabilizes the decameric state. Similar heterooligomerization occurs in human (PRDX1/2), plant, and parasite peroxiredoxins, where it modulates oligomeric state and structural stability. These findings overturn the paradigm of strict Prx homooligomerization and identify heterooligomer formation as a novel mechanism contributing to redox regulation and stress adaptation.

A novel pathway for oxidation and assembly of mitochondrial inner membrane proteins

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Import and assembly of mitochondrial proteins into multimeric complexes are essential for cellular function. Yet, many steps of these processes and the proteins involved remain unknown. Using yeast Saccharomyces cerevisiae as a model system, we recently identified a novel pathway for disulfide bond formation and assembly of mitochondrial inner membrane (IM) proteins. Dbi1, a previously uncharacterized IM protein, interacts with an unassembled pool of Tim17, the central subunit of the presequence translocase of the IM, and is upregulated in cells with increased levels of unassembled Tim17. In the absence of Dbi1, conformation of the presequence translocase is affected and stability of Tim17 is reduced. Furthermore, Dbi1, through its conserved CxxC motif, is involved in formation of the disulfide bond in Tim17 in a manner independent of the disulfide-relay system, the major oxidation-driven protein import pathway into mitochondria. The substrate spectrum of Dbi1 is not limited to Tim17 but includes at least two more IM proteins, Tim22 and Cox20. The experiments that led to the identification of this pathway and the future directions will be discussed.

The peroxide-carbon dioxide interplay in the biochemistry of thiol redox switches

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Humans produce approximately 1kg of CO2 per person per day, mainly because of oxidative decarboxylation reactions of intermediary metabolism. Once formed, CO2 is rapidly hydrated and equilibrated with HCO3- typically via a carbonic anhydrase-catalyzed reaction. We have recently examined that CO2 can enhance or redirect the reactivity of peroxides, modulating the velocity, extent and type of one- and two-electron oxidation reactions mediated by hydrogen peroxide (H2O2) and peroxynitrite (ONOO-/ONOOH) (1). In this presentation, I will further advance on how CO2 could influence the sensitivity of redox switches to peroxides, including that of glyceraldehyde-3-phosphate dehydrogenase (2, 3). Potential mechanisms of CO2-dependent redox signaling that include the intermediacy of peroxymonocarbonate (HCO4-) will be presented and scrutinized. The discovery of "CO2-sensitive redox switches" supports the concept that metabolism-mediated fluctuations in cellular steady-state concentrations of CO2 can be coupled with peroxide signaling.

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Organizing a compartment by redox - the mitochondrial intermembrane space

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The mitochondrial intermembrane space (IMS) is a narrow compartment between the inner and outer mitochondrial membranes. The IMS hosts a specialized oxidative protein-folding pathway called the mitochondrial disulfide relay system, which ensures the proper folding and retention of many IMS proteins. Its key players in human cells, MIA40, ALR and AIFM1 directly connect protein biogenesis to mitochondrial redox status, on the one hand by the need to transfer electrons from disulfide formation into complex IV, and on the other hand by sensing the overall IMS redox status during oxidative proteins folding. In my presentation, I will discuss our latest findings on novel substrates of the pathway, and the sensing and regulation of metabolism by members of the disulfide relay.

From molecular level knowledge on the cellular copper uptake to the development of 64Cu-based radiotracer

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In the last couple of years, my lab has been exploring the cellular copper cycle in eukaryotic and prokaryotic systems using various biophysical and biochemical methods. Dysfunction of the copper regulation system can lead to neurological diseases, cancer, and cell death, it is essential to understand every little detail in the copper cycle to be able to control it according to specific needs.

Ctr1 is a high affinity copper transporter, is a homotrimer integral membrane protein that provides the main route for cellular copper uptake. Together with a sophisticated copper transport system, Ctr1 regulates Cu(I) metabolism in eukaryotes. Despite its pivotal role in normal cell function, the molecular mechanism of copper uptake and transport via Ctr1 remains elusive. Using electron paramagnetic resonance (EPR), UV-visible spectroscopy, and all-atom simulations, we resolved the Cu(II) and Cu(I) binding sites to full-length human Ctr1 (hCtr1), and elucidated how metal binding at multiple distinct sites affects the hCtr1 conformational dynamics *in-vitro* and *in-cell* [1-5]. The molecular level knowledge gained on this transporter, was then used to develop a novel ^{64}Cu(II)-based radiotracer for detection of hypoxic conditions in tumors. This radiotracer integrates into the cellular copper cycle and presents high affinity towards Ctr1, allowing active uptake by the cell. *In-vitro* as well as *in-vivo* PET-MRI imaging experiments on mice with breast cancer tumors showed high tumor to muscle (T/M) ratio with clearly delineated tumor boundaries. As compared with the ^{64}Cu-ATSM and ^{18}F-FDG tracers, our radiotracer offered a superior T/M ratio and could clearly report on the tumor state, indicating high sensitivity to hypoxic tissues. This study illustrates the importance of molecular level knowledge on the chemical and biological cellular mechanism for the development of the next generation of diagnostic and therapeutic compounds.

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Redox homeostasis and protein quality control

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The endoplasmic reticulum (ER) is responsible for folding and processing roughly one-third of the eukaryotic proteome. The unfolded protein response (UPR) plays a critical role in maintaining the ER folding capacity. The UPR senses changes in the ER environment and initiates a compensatory adaptive or apoptotic response. We previously reported that overly oxidizing ER conditions are exacerbated by a loss of functional UPR signaling. Specifically, we found that a strain lacking the UPR sensor Ire1 (ire1Δ) shows a pronounced growth inhibition when overly oxidizing conditions are induced within the ER lumen. This observation suggests that activation of the UPR helps to maintain or buffer changes associated with an overly oxidizing ER. Our original expectation was that the extensive network of genes upregulated upon UPR activation work together to counteract any disruption in proteostasis. However, our recent work suggests that the negative impact for a loss of UPR signaling under overly oxidizing conditions relates primarily to a decrease in the levels of the Hsp70 molecular chaperone BiP (Kar2), which impedes the outcomes associated with BiP oxidation. We trace a central role for modification of the conserved BiP (Kar2) cysteine in the maintenance of ER homeostasis, and show that cells unable to signal redox imbalance through BiP (Kar2) modification are more sensitized to an overly oxidizing cellular environment than cells lacking a functional UPR. Our work also reveals a new layer of communication between the UPR and the heat shock response (HSR), which we suggest coordinate to maintain ER redox homeostasis.

Lysine-cysteine redox switches in proteins - An update

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Modifications of cysteine residues in redox-sensitive proteins are key to redox signaling and stress response in all organisms with disulfide bridges between two proximal cysteines being a prevalent form. We recently discovered a novel type of redox switch that comprises lysine and cysteine residues covalently linked by a nitrogen–oxygen–sulfur (NOS) bridge. Our systematic survey of the whole protein structure database disclosed that NOS bridges are ubiquitous redox switches in proteins of all domains of life and are found in numerous structural motifs and chemical variants. In several instances, lysines are observed in simultaneous covalent linkage with two cysteines, forming a sulfur–oxygen–nitrogen–oxygen–sulfur (SONOS) bridge with a trivalent nitrogen, which constitutes an unusual native branching cross-link. In many proteins, the NOS switch contains a functionally essential lysine with direct roles in enzyme catalysis or binding of substrates, DNA or effectors, linking lysine chemistry and redox biology as a novel regulatory principle. NOS/SONOS switches are frequently found in proteins from human and plant pathogens, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and also in many human proteins with established roles in gene expression, redox signaling and homeostasis in physiological and pathophysiological conditions. I will discuss the chemical and biological implications of the novel redox switches.

ORAL COMMUNICATIONS

** Abstracts are listed in alphabetical order*

Differential Role of Sulphur Species in Regulating HIV-1 Latency

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Understanding the mechanism of HIV-1 latency establishment and maintenance is important. Previous studies from our laboratory suggests that hydrogen sulfide metabolism and protein S-persulfidation are pivotal in modulating the HIV-1 latent reservoir. However, molecular players involved in regulating dynamic changes in sulfide-persulfides levels during virus infection remains poorly characterized. In this study, we utilized well-established HIV-1 latency and infection models to investigate the role of H_2S/persulfide biogenesis pathways using targeted chemical and genetic perturbations. Quantitative analysis of sulfide species revealed a striking induction of S-persulfide levels concomitant with upregulation of MPST, a key persulfide-generating enzyme, during viral reactivation. Conversely, H_2S concentrations declined upon reactivation, suggesting distinct roles for these redox-active sulfur species. Pharmacological activation of MPST potentiated HIV-1 reactivation, while its inhibition via I3MT-3 suppressed viral replication. Stabilization of persulfides using the thioredoxin reductase inhibitor Auranofin further augmented viral load, supporting a pro-viral role of S-persulfidation. Mechanistically, persulfidation enhanced the activity of HIV-1 Tat, a redox-sensitive transactivator essential for viral transcription. Proteomic profiling via LC-MS identified novel host transcription factors undergoing persulfidation during reactivation, implicating them in latency control. Together, our findings reveal a novel redox regulatory axis wherein H_2S promotes latency maintenance, while persulfides act as molecular switches to trigger reactivation. This study underscores the therapeutic potential of targeting sulfur metabolic pathways to manipulate HIV-1 latency and supports the development of redox-based latency-reversing strategies.

Bio-chalogenides production and functions in human vascular endothelium

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Biological chalcogenides like sulfur and selenium are now identified to be a crucial component of chalcogen super-complex that are highly physiological relevant in various organisms. This discovery may open up a brand-new venue of redox biology, which is governed by bio-chalcogenides that are mostly consisted of supersulfides, i.e., sulfur-catenated molecular species. Supersulfides mediate many physiological functions, such as antioxidant activity and mitochondrial energy metabolism. In fact, we recently discovered that nitric oxide synthase (NOS) catalyzes the catenation by using polysulfides, leading to the formation of cyclo-octasulfur (S_8) in mammals. In this study, we analyzed the activation mechanism of supersulfides and the production of S_8 using human endothelium that expresses endothelial NOS (eNOS).

We first developed a mass spectrometry-based technology to detect and quantify S_8, by which endogenous S_8 levels in human umbilical vein endothelial cells (HUVECs) were determined. HUVECs showed appreciable S8 formation, where S_8 was significantly increased when treated with the Ca^{2+} ionophore A23187 to activate eNOS. Notably, eNOS colocalized with lipid droplets in HUVECs under baseline conditions, which correlated well to increased S_8 production in HUVECs. The other preliminary investigation showed a unique bio-chalcogenides containing selenium was also identified in such a super-complex.

Our study reveals that S_8 is physiologically produced by eNOS and accumulates in lipid droplets in HUVECs, suggesting that S_8 may play a novel role in lipid metabolism, e.g., regulation of lipid peroxidation and redox cell signaling. The pharmacological intervention and medicinal manipulation of bio-chalcogenides may benefit prevention and regulation of disease pathogenesis.

Coupling RNA processing pathways with Fe-S cluster distribution and redox homeostasis drives (patho)biochemistry of PYROXD1

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Transfer RNAs rely on a plethora of biochemical maturation steps to gain functionality. While sequential reactions require orchestration, coupling between seemingly distinct processes is puzzling. Not a single tRNA molecule undergoes both splicing and thiolation, yet cancer cells display striking co-dependency on the corresponding enzymes.

Here, we show that the flavoprotein PYROXD1 binds NFS1, cysteine desulphurase that provides sulphur for tRNA thiolation; tRNA-LC (tRNA ligase complex), critical for pre-tRNA splicing; and CIA-TC (cytosolic Fe-S assembly targeting complex), which delivers Fe-S clusters to multiple RNA modifying enzymes.

PYROXD1 protects both the tRNA-LC and NFS1. Binding of PYROXD1 sterically prevents Cu2+- and H2O2-mediated oxidative inactivation of the tRNA-LC, the primary target of a new redox-dependent degradation pathway. Alternatively, PYROXD1 binds NFS1 and stabilises it against aggregation under nucleo-cytosolic pH conditions, sustaining tRNA thiolation and MoCo biosynthesis. Interplay with the CIA-TC, impaired in a pathogenic PYROXD1 variant, is puzzling. PYROXD1 may acquire an Fe-S cluster for its antioxidative activity – or relay it to the tRNA-LC, whose activity drops upon silencing of the CIA-TC. This would turn the tRNA-LC into a novel Fe-S enzyme.

These interactions are however not constitutive. PYROXD1 favours the tRNA-LC to NFS1 upon binding NADPH, while H2O2 and metals react with cysteine residues to sequestrate PYROXD1 in large polymeric forms. Variants defective in these switches cause myopathies in humans.

These data establish PYROXD1 as a command centre that sustains and navigates seemingly disparate RNA processing pathways, resolving their cellular co-dependencies and uncovering their roles in PYROXD1-associated genetic disorders.

Mammalian cells contain elemental sulfur that protects them against ferroptosis

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Elemental sulfur is an evolutionarily ancient metabolite. It is well known to be utilized by bacteria, archaea and algae, as well as, to lesser extent, by plants. However, its generation, storage, or utilization in animals has remained unknown until now. Here we demonstrate the existence and functional role of elemental sulfur in the form of the most stable allotrope S8 in mammalian cells. We find that S8 accumulates at millimolar concentrations within mitochondrial membranes and in lipid droplets in both mouse and human cells. Furthermore, we identify lipid droplet-associated nitric oxide synthase as a previously unrecognized source of S8. Notably, endogenous levels of S8 are markedly elevated in cancerous versus normal breast adipose tissue. Functionally, we show that accumulation of S8 in lipid droplets limits lipid peroxidation, thereby protecting cells from ferroptosis. Therapeutic injections of solubilized S8 prevents lipid peroxidation in a mouse model of osteoarthritis. In summary, our findings reveal a previously unrecognized mammalian S8 pool that serves as a defense mechanism against oxidative membrane damage.

Oxidation of Human Glutamine Synthetase Thiols Parallels Enzyme Inactivation and Promotes Aggregation

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Glutamine synthetase (GS) is a key metabolic enzyme that catalyzes the ATP-dependent synthesis of glutamine from glutamate and ammonia. It is highly expressed in astrocytes, playing a major role in the sustaining the glutamate-glutamine cycle at glutamatergic synapses. Changes in levels /activity can result in astroglial dysfunction and neuronal death. Several studies have related the loss of GS activity with neurodegenerative disorders, such as Alzheimer's Disease, being found a correlation between enzyme inactivation and oxidative modifications.

Previously, we found that recombinant human GS (HsGS) is susceptible to oxidative inactivation and aggregation by peroxynitrite (ONOO-), which induced post-translational modification of several residues including tyrosine, tryptophan, methionine and cysteine. Although the direct involvement of each modification could not be addressed, data suggested that cysteine modification could be mediating HsGS-aggregation.

Herein, we performed in vitro studies with purified-HsGS exposed to several oxidants under different experimental conditions. We observed that ONOO--induced protein aggregation was prevented by incubation of ONOO--treated HsGS with thiol reducing agents. Furthermore, exposure of HsGS to hydrogen peroxide (H2O2) or diamide induced protein aggregation, along with thiol oxidation and a subsequent time-dependent loss of enzyme activity. Besides, we observed that ligand binding (MgATP) significantly prevented H2O2-induced protein aggregation and loss of activity while decreasing the degree of cysteine oxidation and increasing protein thermal stability.

Altogether, these results indicate that modification of cysteine residues parallels HsGS inactivation and promotes its aggregation in vitro and this process can be prevented through binding of MgATP, which stabilizes HsGS and protects from thiol oxidation.

Modulating Endogenous Hydropersulfides to Promote Antioxidant Response, Inhibit Ferroptosis

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Hydrogen sulfide (H2S) and hydropersulfides (RS-SH) have diverse roles and protect cells from oxidative stress and inflammation. Recently, hydropersulfides have been found to protect cells from ferroptosis, which is a type of programmed cell death dependent on iron and characterized by an increase in iron-dependent accumulation of oxidatively damaged phospholipids leading to lipid peroxidation, membrane rupture, and eventually cell damage and death. 3-Mercaptopyruvate sulfurtransferase (3-MST) is an endogenous antioxidant enzyme that generates hydropersulfides. Our lab developed a series of artificial substrates for 3-MST, and these small molecules were shown to promote endogenous antioxidant response through the generation of hydropersulfides. We expanded this approach through a computational structure-guided series of artificial substrates for 3-MST. These novel compounds were found to enhance hydropersulfides, reduce neuroinflammation in an animal model, and protected cells from ferroptosis. Hence, we use the cell's own biosynthetic machinery to inhibit ferroptosis, underscoring the importance of precisely modulating biological sulfur transfer. Due to the involvement of ferroptosis in several degenerative conditions, our data lays the foundation for new therapeutic interventions that involve enhancement of hydropersulfides.

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CyReB, a biosensor dedicated to the dynamic monitoring of the intracellular cysteine pool

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Over the last two decades, the development of fluorescent probes has transformed the way we measure physiological parameters in intact cells and has helped to answer important questions in the field of redox biology. We developed a genetically encoded biosensor called CyReB to monitor intracellular cysteine in real time. This biosensor exploits the ability of a particular bacterial chimeric cysteine desulfurase to promote the oxidation of reduction-oxidation-sensitive green fluorescent protein 2 in the presence of cysteine. The specificity, sensitivity, and the oxidation-reduction dynamics of CyReB were first investigated in vitro before its in vivo functionality was confirmed by expressing CyReB in Escherichia coli and Saccharomyces cerevisiae cells. Expressing CyReB and an inactive version of it in wild-type and various mutant strains of Escherichia coli showed that this sensor could be used to monitor intracellular cysteine dynamics, particularly in the context of the cysteine-cystine shuttle system. This work demonstrates how using this cysteine biosensor could provide new insights into the metabolism of cysteine and cysteine-related pathways in various model organisms.

Neutrophil-derived ROS as a source of mutagenesis in cancer

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Mutations in chromosomal DNA caused by oxidative lesions, such as 8-oxoguanine, accumulate during aging and are common in various tumor types. However, what source of reactive oxygen species (ROS) causes these mutations is unclear. Mitochondrial respiration-derived ROS were long assumed to be the main driver of chromosomal oxidative DNA damage, but recent work has shown that ROS from mitochondria do not diffuse into the nucleus, and are unlikely to contribute to oxidative DNA mutations. Neutrophils are innate immune cells that produce a burst of ROS in response to pathogens. We hypothesised that this burst of ROS could be sufficiently strong to overwhelm the cellular antioxidant system and cause chromosomal DNA damage. We have set up a novel co-culture system, combining neutrophils and retinal pigment epithelial (RPE) cells with genetically-encoded hydrogen peroxide sensor, HyPer7. Neutrophils are activated upon binding to IgG-opsonized RPE cells, resulting in oxidation of nuclear HyPer7 and activation of the DNA damage response. Although neutrophils preferably accumulate around IgG-opsonized cells in mixed cultures, HyPer7 oxidation also occurs in adjacent non-opsonized cells. We therefore conclude that neutrophils could in principle cause DNA mutations in neighbouring cells. This could contribute to tumor initiation in response to immune activation by for instance infection, but also stimulate tumor evolution in neoantigen-presenting tumors, which are often infiltrated by neutrophils.

Prediction of redox sensitive disordered regions

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The discovery that many proteins function without adopting a stable three-dimensional structure has challenged the classical structure-function paradigm in molecular biology. Intrinsically disordered proteins (IDPs) and regions (IDRs) play central roles in cellular regulation through conformational flexibility, particularly in response to environmental cues such as pH and redox changes. Despite their biological importance, the systematic identification of redox-sensitive IDRs remains a major bottleneck due to the lack of reliable predictive tools and challenging experimental measurements.

In previous work, we developed the first computational method specifically designed to detect redox-sensitive disordered regions, leveraging a biophysically grounded energy-based force field. This method compared disorder profiles of native protein sequences with cysteine-to-serine mutants, interpreting large differences as indicators of redox responsiveness. While promising, its broader application was constrained by limited training data and computational capacity.

Here, we propose a next-generation redox-sensitivity predictor based on a reformulated force field integrated into a graph-based transformer message passing neural network (trMPNN) framework. This architecture allows for GPU-accelerated training on large datasets, models residue-level contacts directly, and scales efficiently in both memory and performance.

The new model incorporates recent experimental advances, including high-resolution redox proteomics, to enable the accurate identification of redox-sensitive IDRs across proteomes. By combining mechanistic interpretability with modern deep learning capabilities, our approach aims to provide a scalable and robust tool for uncovering redox-regulated protein regions, supporting the discovery of novel regulatory mechanisms and therapeutic targets.

Metabolic modulation of Selenite toxicity

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Selenium is an essential trace element which, when provided in its oxidized forms such as sodium selenite (Se4+), has been shown to be highly toxic to mammals. However, little is known about the interaction between selenite and other metabolites, such as hydrogen sulfide (H2S), within cells. In the body, H2S acts as a gaseous signaling molecule involved in mitochondrial function, human disease, cancer and ageing. We used budding yeast to study the impact of H2S on selenite toxicity at the genetic and molecular levels. Interestingly, we found that selenite toxicity correlates with the amount of endogenous H2S production. Yeast cells that produce more H2S are highly sensitive to selenite treatment, while cells that produce less H2S are more resistant.

Based on these initial observations, we further assessed the impact of H2S on DNA damage formation and signaling. Our results indicate that H2S strongly promotes selenite-induced DNA damage, as determined by the formation of DNA repair centers and histone H2A phosphorylation, which is an indicator of DNA breakage. Furthermore, selenite treatment activates the DNA damage response (DDR) in H2S producing cells, as determined by Rad53 (hCHK2) phosphorylation. We are therefore considering the possibility that H2S could act as a Trojan horse in the treatment of tumors with elevated H2S levels using selenite.

H_2Switch: A Chemogenetic Approach for Intracellular Hydrogen Sulfide Modulation

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Chemogenetics enables precise control of cellular processes through the use of engineered enzymes or receptors that respond exclusively to exogenously supplied, biologically inert substrates. This strategy offers unmatched spatial and temporal precision for manipulating complex biological signaling. Hydrogen sulfide (H_2S) is now recognized as a key signaling molecule involved in energy metabolism, vascular regulation, cancer progression, and neurodegeneration. While significant advances have been made in H_2S detection using fluorescent and genetically encoded biosensors, tools that allow selective and controllable intracellular H_2S production remain limited.

In this study, we introduce a substrate-based chemogenetic system for intracellular H_2S generation. Salmonella typhimurium D-cysteine desulfhydrase (stDCyD) converts D-cysteine into H_2S (1), offering efficient, tunable, and artifact-free elevation of intracellular H_2S levels in vitro. Furthermore, we previously repurposed stDCyD for intracellular acidification in our pH-Control system (2), highlighting the modular potential of this bacterial enzyme for dual-function chemogenetic applications. Together, our work establishes stDCyD as a highly versatile chemogenetic actuator for investigating the multifaceted roles of H_2S and pH in cellular physiology and pathophysiology. This system expands the current chemogenetic repertoire and offers a robust platform for dissecting complex redox and metabolic signaling networks with unprecedented precision.

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Peroxiredoxins as markers of redox homeostasis in human blood cells during ageing

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Oxidative stress and mitochondrial dysfunction are proposed to play prominent roles in the biology of ageing. Investigations can be confounded, however, by metabolic disturbances that occur as a consequence of age-related disease. We have reported an increase in circulating mitochondrial and oxidative stress biomarkers in faster-ageing people during mid-life, prior to the emergence of age-related disease. To examine alterations in cellular redox homeostasis, we measured the redox state of endogenous peroxiredoxins in blood cells from the same individuals. There was a weak association between oxidised mitochondrial peroxiredoxin 3 in platelets and the pace-of-ageing. Of most interest was erythrocyte peroxiredoxin 2. While the majority (>95%) of peroxiredoxin 2 was present in its reduced form in circulating erythrocytes, it was rapidly oxidised following addition of a bolus of hydrogen peroxide to blood. In stark contrast to other cells, erythrocyte peroxiredoxin 2 only slowly returned to its basal state. The rate of reduction varied considerably between individuals, and there was a significant relationship with their pace-of-ageing. More research is required to determine exactly how ageing impacts erythrocyte thiol reductive mechanisms, and what might be the relevance for other cells.

Patient derived fibroblasts as a novel model to study redox related alterations in tauopathies

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Progressive supranuclear palsy (PSP), a neurodegenerative tauopathy distinguished by pathological tau protein aggregation and accumulation is also characterized by oxidative distress and mitochondrial dysfunction. Tau is mainly expressed in neurons and astrocytes playing key roles in cytoskeleton stability, mitochondrial functions and gene expression. We propose that mitochondrial alterations coupled with redox disbalances are key molecular hallmarks determining the development of tauopathies, however it remains elusive whether other cell types can be used as disease models. We used fibroblasts from patients diagnosed with PSP and control donors (CTL), belonging to our recently created tau-Latin American fibroblast repository, to address significant gaps in the research of tauopathies related to the interaction between tau, mitochondrial homeostasis and redox regulation.

Preliminary analysis revealed that PSP fibroblasts present increased levels of tau and phosphorylated tau coupled with decreased colocalization of tau-tubulin, indicating a loss of tau interaction with microtubules. Regarding mitochondrial homeostasis and redox regulation, PSP cells showed decreased mitotracker staining, suggesting changes in mitochondrial membrane polarization. Furthermore, hydrogen peroxide levels within mitochondria showed slight, although not significant, increases when measured using the Hyper7 probe. Interestingly, PSP fibroblasts showed decreased levels of Trx1 and Trx2 redoxins. These results suggest that both mitochondrial homeostasis as well as thiol signaling are being altered in PSP derived fibroblasts. We show that these cells can be used as an innovative model to study the molecular mechanisms underlying neurodegenerative diseases while they could also be used as a simple and less-invasive diagnostic and/or prognostic tool for these pathologies.

Persulfidation loss leads to cysteine hyperoxidation with impairment of proteasome function.

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Proteasome is a highly sophisticated protease complex responsible for degradation of many intracellular proteins, ensuring protein homeostasis. In this research, we focused on addressing the role of thiol oxidation, in particular protein persulfidation and sulfonylation, on proteasome activity using immortalized striatal precursor cells (STHdh Q7/Q7 and STHdh Q111/Q111) and mouse embryonic fibroblasts (WT and CSE-/-). Persulfidome analysis showed that persulfidation of several 26S proteasomal subunits was significantly lower in Q111/Q111 compared to Q7/Q7 cellls. This pattern was also observed in many other pathways, and the trend was reversed when cells were previously treated with H2S donor. In our models, H2O2 treatment decreased proteasome activity dose-dependently; an effect that H2S could partially prevent. CSE-/- MEFs, or Q7/Q7 cells treated with CSE siRNA, both had lower basal chymotrypsin (but not trypsin or papain)-like proteasome activity when compared to the wild type cells. Additionally, Q111/Q111 cells, known to have barely detectable CSE levels, also presented lower basal chymotrypsin-like activity when compared to Q7/Q7 cells. In all circumstances, the administration of the strong reducing agent DTT resulted in higher proteasome activity, but in case of cells lacking CSE this recovery was barely detectable suggesting that part of the proteasome exists in hyperoxidized state that cannot be recovered. Immunoprecipitation of beta-5 subunits from Q111/Q111, but not from Q7/Q7 cells, led to the detection of cysteine 52 overoxidized to sulfonic acid (C55-SO3H). Together these results suggest that age-induced decline of proteasome persulfidation could contribute to proteotoxic stress and pathologies caused by it.

ROS induce hormesis in a localization- and p53-dependent manner

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"What doesn't kill you makes you stronger" is a famous statement by philosopher Friedrich Nietzsche. This statement seems to hold up in biology, and it has been shown that low levels of a stressor can provide resistance to high levels of that stressor and can even extend lifespan in model organisms. The adaptive response invoked by a low dose of a stressor resulting in resistance is known as hormesis. Mild oxidative stress ensuing from Reactive Oxygen Species (e.g. H_2O_2) derived from mitochondria has been implicated in hormesis, but the presented evidence for this mito-hormesis is circumstantial.

We used ectopic D-amino acid oxidase expression to mimic H_2O_2 production at the nucleosome or released by mitochondria, and investigated whether this can induce a hormetic response. Surprisingly, whereas mitochondrial H_2O_2 release did not result in hormesis, sublethal H_2O_2 production at the nucleosomes provided p53-dependent resistance to a subsequent high dose of H_2O_2 . We are currently investigating what mechanisms downstream of p53 are responsible for this protection.

Glutathione beyond the cytosol – Comprehensive elucidation of glutathione import and its role in the periplasm of Escherichia coli

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Glutathione is found in a wide variety of biological systems, ranging from bacteria to eukaryotes. As a redox couple, consisting of reduced glutathione (GSH) and its oxidized form, glutathione disulfide (GSSG), it is crucial for maintaining the cellular redox balance. Here we use the glutathione-measuring Grx1-roGFP2 to comprehensively elucidate import of extracellular glutathione into the cytoplasm of the model organism Escherichia coli. The elimination of only two ATP-Binding Cassette (ABC) transporters, Gsi and Opp, completely abrogated glutathione import into E. coli's cytoplasm, both in its reduced and oxidized form. The lack of only one of them, Gsi, completely prevented import of GSSG, while the lack of the other, Opp, substantially retarded uptake of reduced glutathione (GSH).

To assess the in vivo thiol homeostasis imposed onto protein thiol disulfide pairs in the periplasm, a compartment more oxidizing than the cytosol, we targeted roGFP (roGFP2 and roGFP-iL) probes to E. coli's periplasm. Analyzing the probe's oxidation state revealed that deletion of DsbA, naturally introducing disulfide bonds in extracytosolic proteins, shifted the periplasmic steady state thiol-redox potential to more reducing conditions. In ΔdsbA, roGFP2 reoxidation after a reductive pulse was significantly slower and could be fully restored by exogenous addition of GSSG. Concomitantly, GSSG rescued activity of the native DsbA substrate PhoA when DsbA was missing while enhancing PhoA activity in the WT. In line, GSH-deficient cells showed a more reducing periplasm, and performed worse in oxidative folding of PhoA. Together, this suggests the presence of an auxiliary, glutathione-dependent periplasmic thiol-oxidation system.

Emerging roles of Prx6-type enzymes in H2S and persulfide metabolism

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The absence of a universal reducing agent distinguishes the Prx6-type subfamily of peroxiredoxins from the structurally similar Prx1-type subfamily. Here, we identified the hydrosulfide ion, HS−, as a highly reactive electron donor for Prx6-type enzymes. Stopped-flow kinetic measurements revealed a rapid reduction for oxidized PfPrx6 from Plasmodium falciparum and human PrxVI with second-order rate constants > 108 M-1s-1. Mass spectrometry confirmed the formation of a protein-hydropersulfide intermediate, which cannot be reduced by thioredoxin, glutaredoxin or glutathione but reacts with a second molecule of HS− (k ≈ 104 M-1s-1). Furthermore, HEK293 cells without PrxVI are more tolerant towards exogenous Na2S. The establishment of a steady-state kinetic assay, the potential catalytic formation of H2S2, the persulfide state of Prx6-knockout cells, and the suitability of Prx6-type enzymes as H2S sensors are currently under investigation. Our results suggest that Prx6-type enzymes link hydroperoxide and H2S metabolism, pointing to a previously unrecognized role of these enzymes in persulfide generation and sulfur-based redox signaling.

When hydrogen peroxide helps or harms: Micromolar cytosolic thresholds in cultured human cells

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H_2O_2 is a central redox signaling molecule that, at low to moderate levels, regulates key cellular processes such as proliferation, migration, and differentiation. At higher concentrations, however, H_2O_2 contributes to oxidative stress and cellular damage. Despite this established dual role, the precise intracellular thresholds that separate regulatory from harmful effects have remained unclear.

To address this, we used a previously developed [1] HyPer-based method to monitor cytoplasmic and nuclear H_2O_2 concentrations in both normal and tumor-derived human cell cultures exposed to varying levels of extracellular H_2O_2. We found that enhanced cell migration, without impairing proliferation or DNA integrity, occurs when intracellular H_2O_2 levels remain within the sub-micromolar range. At these concentrations, peroxidase activity peaks and is regulated primarily through the thioredoxin (Trx) pathway.

Once H_2O_2 levels exceed this micromolar threshold, Trx-dependent peroxidase activity declines, leading to DNA damage and cell cycle arrest. These results not only clarify the redox conditions under which H_2O_2 serves as a signaling molecule versus a stressor, but also highlight the critical role of the Trx system in maintaining genomic stability. Moreover, our findings indicate that the physiological concentrations of peroxide may be significantly higher than the sub-nanomolar and nanomolar ranges reported in previous studies using HyPer. These studies relied on an incorrect rate constant for the oxidation of HyPer, which may have led to inaccurate results.

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Metal-Responsive Up-regulation of Bifunctional Disulfides for Suppressing Protein Misfolding and Promoting Oxidative Folding

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The stress-responsive up-regulation process is a sophisticated biological response to maintain cellular homeostasis. In intracellular anti-oxidant systems, the expression level of oxidoreductases is up-regulated under oxidative stress, mitigating oxidative damage on biomolecules and enhancing protein folding capacity. Herein, inspired by the biological system, we developed a synthetic folding promotor whose reactivity is up-regulated under stress conditions. We conjugated two metal-binding 1,4,7,11-tetraazacyclodecane (cyclam) ligands and a redox-active disulfide to obtain cyclam-SS, whose reactivity can be enhanced under metal-induced stress. Metal coordination increased the redox potential of cyclam-SS, activating it as an oxidant. While Cu(II) ions severely hampered the oxidative folding of substrate polypeptides, cyclam-SS exhibited bifunctional folding-promoting properties, (i) suppressing Cu(II)-mediated misfolding and aggregation, and (ii) harnessing Cu(II) to enhance oxidative folding. Cyclam-SS was also useful for disulfide-bond formation to promote oxidative folding of pharmaceutical and pathological proteins, as demonstrated with proinsulin and superoxide dismutase 1 (SOD1). Furthermore, cyclam-SS protected cultured cells from copper-induced stress. Thus, we demonstrated the induction of the stress-responsive up-regulation process by a bifunctional folding promotor controlling the folding status of biologically important proteins under metal-induced stress. The strategy of “stress-responsive up-regulation” could aid the development of novel synthetic materials for treating intracellular stress and related disorders.

Ca2+-driven PDIA6 condensate formation to ensure oxidative proinsulin folding

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All eukaryotic cells contain an endoplasmic reticulum (ER), where plays roles in protein quality control and Ca2+ storage, suggesting that homeostatic plasticity mechanisms are in place to ensure high fidelity for maintaining protein homeostasis. Herein, we show that Ca2+ triggers the phase separation of P5, one of PDI family. In contrast to the droplet formation mechanism and sequential amyloidogenesis observed for low-complexity domains, our results indicated that transient but specific electrostatic interactions between two thioredoxin-like domains establish pivotal role in the droplet formation. Ca2+-driven droplet condenses proinsulin to increase their local concentration, resulting in the enhancement of proinsulin folding efficiency by inhibiting proinsulin aggregation and catalyzing oxidative folding 28-fold faster than enzymatic activity. Our findings shed light on the Ca2+-mediated proteostasis cascade that occurs in the ER and paves the way to protein folding paradigm as a protein quality control granule.

Genetically-encoded biosensors to monitor cysteine and taurine in living cells

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A major limitation in thiol redox biology is the lack of tools to monitor key sulfur-containing metabolites in real time and within the native cellular context. We address this challenge by developing genetically-encoded fluorescent biosensors for two biologically essential sulfur metabolites: cysteine and taurine.

Cysteine is the limiting precursor of glutathione, coenzyme A, iron–sulfur clusters, and sulfane sulfur species. Its limited availability, especially in some tumor cells, can trigger ferroptosis. Our ratiometric cysteine biosensors are selective, pH-stable, and span the physiological concentration range, with an exceptionally high dynamic range. They allow in situ quantification in living cells and tissues and are responsive to cysteine-depleting treatments such as hydrogen peroxide and erastin.

Taurine is one of the most abundant amino acids in mammals and plays roles in osmoregulation, mitochondrial function, and healthy aging. Despite its abundance, taurine metabolism at the subcellular level remains poorly understood. We developed a series of taurine biosensors optimized for high intracellular concentrations. We have also demonstrated its functionality and responsiveness in vivo via static and dynamic taurine measurements in mitochondria and peroxisomes, subcellular organelles implicated in taurine metabolism.

These sensors open the door to a new generation of studies on sulfur metabolism, redox regulation, and aging—across scales from subcellular compartments to whole organisms.

NAD(P)H sensing regulates the fate of enteroendocrine cells

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Sugars are essential macronutrients for sustaining life. Cells read and integrate signals about the presence of sugars through specific sugar sensing protein networks. What are precise components of these sugar sensing networks and which metabolic signals activate them is not well understood. Here, we identify nicotinamide redox cofactors as secondary messengers in the sugar-induced transcriptional network in the Drosophila intestine. Dynamic changes in NAD(P)H levels are sensed locally in the hormone-producing intestinal enteroendocrine (EE) cells through transcription cofactor C-terminal binding protein (CtBP). CtBP acts at the interface between nutrient and redox sensing and fate regulation of EE cells, controlling organismal energy metabolism and survival on a high sugar diet. Mechanistically, CtBP binds to EE cell fate regulator Prospero through a conserved binding motif, and controls expression of genes that regulate EE cell fate and size. Collectively, this study introduces a modality where local changes in cellular redox state serve as an instructive signal to globally control organismal homeostasis.

Reductive stress by thiol antioxidants activates the hypoxia response pathway

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Reductive stress, caused by an excess of antioxidants, is linked with several pathological conditions, including cancer and neurodegenerative diseases. However, the effects of reductive stress on cellular physiology remain poorly characterized. Using the nematode model Caenorhabditis elegans, we showed that thiol antioxidants such as dithiothreitol (DTT) and β-mercaptoethanol modulate the methionine–homocysteine cycle by upregulating an S-adenosylmethionine-dependent methyltransferase, rips-1 (Gokul and Singh, 2022). We carried out genetic screens to understand how thiol antioxidants resulted in the upregulation of rips-1. We discovered that rips-1 is highly upregulated in mutants that have constitutive activation of the hypoxia response pathway. On the other hand, thiol antioxidants-mediated upregulation of rips-1 is fully blocked in mutants defective in hypoxia response. We demonstrated that thiol stress activates the hypoxia response pathway (Ravi et al., 2023). The activation of the hypoxia response pathway by thiol stress is conserved in human cells. The hypoxia response pathway enhances thiol toxicity via rips-1 expression and confers protection against thiol toxicity via rips-1-independent mechanisms. Our ongoing studies explore the players downstream of the hypoxia response pathway that protect from thiol toxicity. Our studies reveal an intriguing interaction between thiol-mediated reductive stress and the hypoxia response pathway.

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The cellular Trx/TrxR system as a stress integration hub for tumour-selective drug release

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Solid tumours contain metabolically and biochemically diverse microenvironments. To effectively treat whole tumours of any type, we need to target common features of these microenvironments that are molecularly distinct from healthy tissue. The antioxidant microenvironment of solid tumours has been of interest for basic and translational research for decades The thioredoxin system is one of two central cellular antioxidant systems, that continuously drives vital cellular functions but is also a stress-responsive safety net needed to keep cells alive under pathological conditions. We developed bicyclic dichalcogenides that are highly reduction-resistant but are capable of being efficiently reduced by high turnover rates in the cellular thioredoxin system [1]. We report the corresponding series of duocarmycin prodrugs, that are the first prodrugs to aim at tumour-selective activation through dichalcogenide reduction [2]. We established tolerability in vivo, indicating low systemic release and generated promising initial results in murine tumour models indicating effective tumoral drug release. This work introduces a chemically novel class of prodrugs against a previously unaddressed manifold of reductases with the potential to integrate multiple cellular stresses and therefore serve as a new platform strategy for specific bio-reduction-based release, with applications in basic and translational research on cancer and inflammation.

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Redox control of intestinal organoids in homeostasis and development

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Reactive oxygen species (ROS) are important signal transducers in several stem cells: in some tissues, like the developing Zebrafish retina, ROS control the balance between stem cell expansion and differentiation.

Based on these findings, we characterized the potential role of ROS in regulating the transition from fetal progenitors to adult stem cells in the mouse intestine, and in further controlling the choice between stem cell self-renewal and differentiation at homeostasis.

We found, using two distinct ROS sensors, that intestinal stem cells are enriched in ROS in adult intestinal organoids, whereas terminally differentiated lineages show low ROS levels. We next probed the functional role of ROS in organoids: increased ROS trigger an expansion of both proliferative and Paneth cells, with enterocytes loss; in contrast, quenching ROS levels pushed proliferative cells into enterocyte differentiation. In addition, when we stimulated with ROS Paneth cell-enriched organoids, we detected re-entry into cell cycle of some terminally-differentiated Paneth cells, suggesting dedifferentiation.

To define how ROS heterogeneity is established throughout intestinal development, we derived intestinal organoids from different mouse embryonic stages. Fetal organoids are made of homogeneously cycling progenitors, and accordingly they presented uniform high ROS levels. However, pulses of high ROS triggered symmetry breaking of fetal organoids, which acquired adult cell types and architecture.

Overall, we show that ROS levels control cell fate decisions in mouse intestinal stem cells and trigger mechanisms of plasticity and regeneration; in parallel, ROS contribute to the fetal-to-adult transition during intestinal development and the establishment of the adult stem cell pool.

Integrating low-resolution biophysical approaches and computational simulations to characterize disulfide roles in protein conformational landscapes

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Disulfide bonds are crucial for protein structure, being able to serve a purely structural function or by having functional roles, among which we find catalytic or allosteric disulfide bonds. Their formation/reduction is a well-known process involved in the enzymatic cycle of several enzymes that play catalytic and/or regulatory activities. Structurally, the process involves short- or long-range conformational changes, with a variety of consequences to protein stability and function (1).

Aimed to characterize such processes in terms of structure, dynamics and thermodynamic properties, here we integrate biophysical low-resolution techniques like circular dichroism and tryptophan fluorescence lifetime, together with several advanced sampling computer simulation techniques such as: accelerated molecular dynamics (aMD) (2), weighted ensemble (WE) and structure-based model molecular dynamics (SBM-MD) (3). Using methionine sulfoxide reductase as a model enzyme, we were able to describe the energetic landscape associated with redox changes, along with estimations of important thermodynamic properties during such processes. Results suggest that this hybrid theoretical-experimental approach is suited to fully understand these redox processes, setting a workflow to predict the impact of disulfide formation/reduction in proteins.

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Small molecule-mediated inhibition of the oxidoreductase ERO1A restrains aggressive breast cancer by impairing VEGF and PD-L1 in the tumor microenvironment

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ERO1A aids protein folding by acting as a protein disulfide oxidase, and under cancer-related hypoxia conditions, it favors the folding of angiogenic VEGFA, leading tumor cells to thrive and spread. The upregulation of ERO1A in cancer cells, oppositely to the dispensability of ERO1A activity in healthy cells, renders ERO1A a perfect target for cancer therapy. Here, we report the upregulation of ERO1A in a cohort of aggressive triple-negative breast cancer (TNBC) patients in which ERO1A levels correlate with a higher risk of breast tumor recurrence and metastatic spread. For ERO1A target validation and therapy in TNBC, we designed new ERO1A inhibitors in a structure-activity campaign of the prototype EN460. Cell-based screenings showed that the presence of the Micheal acceptor in the compound is necessary to engage the cysteine 397 of ERO1A but not sufficient to set out the inhibitory effect on ERO1A. Indeed, the ERO1 inhibitor must adopt a non-coplanar rearrangement within the ERO1A binding site. I2 and I3, two new EN460 analogs with different phenyl-substituted moieties, efficiently inhibited ERO1A, blunting VEGFA secretion. Accordingly, in vitro assays to measure ERO1A engagement and inhibition confirmed that I2 and I3 bind ERO1A and restrain its activity with a IC50 in a low micromolar range. EN460, I2 and I3 triggered breast cancer cytotoxicity while specifically inhibiting ERO1A in a dose-dependent manner. I2 more efficiently impaired cancer-relevant features such as VEGFA and PD-L1. Thus, small molecule-mediated ERO1A pharmacological inhibition is feasible and promises to lead to effective therapy for the still incurable TNBC.

POSTERS

POSTER 01

The Role of Redox Systems in Ferroptosis Pathway in colorectal cancer cell line HCT116

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Thiol-based redox systems are key regulators of cellular responses to oxidative stress, affecting both, physiological signalling and pathological processes. In this study, we explored the impact of oxidative stress induced by erastin (10 μM) on redox homeostasis in HCT116 wild-type (WT) and GPX4 knockout (KO) cells lacking a gene involved in redox regulation.

Following erastin treatment, both WT and KO cell lines exhibited a significant increase in reactive oxygen species (ROS) levels, as measured by DCF fluorescence, confirming effective oxidative stress induction. Assessment of total glutathione levels revealed a marked decrease in both cell lines, with KO cells displaying overall lower glutathione content, suggesting impaired antioxidant capacity in the knockout background.

We also investigated the expression of glutathione reductase (GSR), a key enzyme in maintaining glutathione recycling, in its reduced form. In WT cells, GSR expression increased in response to erastin, indicating a compensatory up-regulation of the antioxidant system. In contrast, KO cells showed a decrease in GSR levels following treatment, suggesting a disrupted redox response mechanism. Similarly, thioredoxin (Trx), another major thiol-based antioxidant, was upregulated in WT cells upon stress induction, while in KO cells its levels remained unchanged and similar to control levels.

Our results highlight the differential activation of thiol-based redox systems in WT versus KO cells and underscore the importance of the deleted gene in orchestrating adaptive antioxidant responses. This study provides insights into the complexity of redox regulation under stress conditions.

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POSTER 02

SUBSTITUTION OF PEROXIDATIC AND RESOLVING CYSTEINE FOR SELENOCYSTEINE GREATLY ENHANCES THE CATALYTIC ACTIVITY OF HUMAN PEROXIREDOXIN 2

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Human Peroxiredoxin 2 (Prx2) is an important hydrogen peroxide scavenger and regulates signaling processes via controlling the intracellular H2O2 level. It is mainly located in the cytosol as a homodimer in a head to tail orientation, but readily forms decamers or larger oligomers. Prx2 belongs to the 2-Cys peroxiredoxin family containing two active site cysteines, peroxidatic (CP) and resolving (CR) residues. In the catalytic cycle of Prx2, the CP is oxidized to sulfenic acid (CP-SOH). The CP is also sensitive to overoxidation. In the resolving step the sulfenic acid condenses with the CR of the opposing monomer of the functional dimer. The CP-S-S-CR disulfide is reduced back to thiol form by the thioredoxin or glutathione system.

In a few bacterial species, there are isoforms of peroxiredoxins with selenocysteine (Sec, U) replacing the CP residue. Sec is generally known as a more reactive amino acid than Cys, and also more resistant to overoxidation. Thus, we here investigated if a CP-to-UP substitution in Prx2 would alter its enzymatic properties, which could shed light upon the evolutionary or functional constraints that govern the choice of Cys over Sec in nature. Expressing, purifying and characterizing recombinant human Prx2 C51U, Prx2 C172U and Prx2 C51,172U we found that the selenoprotein variants are several orders of magnitude more active peroxidases than the wild-type enzyme.

The explanation for why a less active cysteine variant of human peroxiredoxin evolved over time is likely related to the enzyme's role in redox regulation rather than its peroxidase activity.

POSTER 03

Peroxiredoxin heterooligomerisation: investigating assembly mechanism and functional relevance

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Peroxiredoxins (Prx) are among the most abundant cellular proteins. Prx1/AhpC-type Prxs exist in an equilibrium between different oligomeric states including dimers, decamers, and higher molecular weight structures, which may be coupled to functional plasticity of these proteins. Interestingly, more than 80% of eukaryotes harbor two or more Prx1/AhpC-type Prx within at least one subcellular compartment. These different Prx isoforms have been thought to exclusively form homo-oligomeric complexes. However, our recent research shows that different Prx1/AhpC-type Prx from diverse eukaryotes readily assemble into heterodimers and heterodecamers.

Here, using in vitro, E.coli and yeast-based assays we studied the requirements for yeast Tsa1–Tsa2 heterooligomer formation. Surprisingly, we found that Tsa1–Tsa2 heterooligomers only form when Tsa1 and Tsa2 are expressed at the same time in both yeast cells and in E.coli. We have been unable to reconstitute heterooligomer formation by mixing of recombinant Tsa1 and Tsa2, by mixing of yeast lysates containing either Tsa1 or Tsa2, or by sequential expression of both proteins in E.coli. This is possibly related to the stability of the Tsa2 decamer, which may preclude subunit exchange.

The formation of Tsa1-GFP 'puncta' has previously been observed in response to H2O2, heat stress and upon zinc depletion and suggested to be related to protein disaggregation. We find a possible role for TSA2 expression and consequent heterooligomer formation in regulating puncta formation and persistence. Future work will be focused on further elucidating the functional relevance of heterooligomer formation and translating our findings to other organisms.

POSTER 04

Modulation of Reactive Carbonyl Species Detoxifying Enzymes by Thiols

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Oxidative stress (OS) is a major factor in several disorders, including carcinogenesis. OS is typically characterized by increased oxidant species coupled with reduced intracellular antioxidant defences, leading to lipid peroxidation and subsequent production of reactive carbonyl species (RCS), detoxified by structurally and functionally different enzymes such as AKR1B10, AKR1C3, AKR1B1 and ALDH1A1.

Under oxidative conditions, irreversible oxidation of protein cysteines is prevented by the formation of mixed disulfides between protein cysteines and free thiols, such as glutathione. Moreover, several studies have highlighted the relevance of glutathionylation in cancer development, revealing that the pattern of glutathionylated proteins markedly differs between physiological and pathological conditions (1). Given that glutathionylation can modulate enzyme functioning, understanding its effect on key RCS-detoxifying enzymes could provide valuable insights into redox regulation.

To assess the influence of glutathionylation on enzyme activity, in vitro assays were conducted on human recombinant purified proteins, using each enzyme's physiological RCS substrate. Reversibility of glutathionylation and changes in inhibitor sensitivity were evaluated. To extend these findings to a cellular context, cancer cell lines were treated with oxidative agents or chemotherapeutic drugs, and redox markers were analyzed to link enzyme modifications with potential glutathionylation-dependent regulatory mechanisms affecting redox balance and drug response.

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POSTER 05

Conformational dynamics and glutathione-binding in the active sites of class I glutaredoxins

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Class I glutaredoxins are crucial for various redox-signaling events and as electron donor for metabolic enzymes. The proteins catalyze reversible thiol-disulfide reactions, e.g., (de-) glutathionylation. Based on the analysis of all Grx structures deposited in the protein data bank, we proposed that a specific switch between two conformations in the active site loop controls their reaction cycles. We analyzed the conformational dynamics and glutathione binding in the active site sites of four model Grxs: A. thaliana GrxC5, E. coli Grx1, and human Grx1 and Grx2. Utilizing the fluorescence of both naturally present and engineered tryptophan residues, mutants of both catalytic Cys residues, as well as residues proposed to take part in GSH binding, we were able to: (1.) Confirm the dynamic switch in the active site through the proteins' full redox cycle. (2.) Comprehensibly analyzed all reactions with GSH/GSSG, including all binding events. Our results help to understand the different activities of class I and II glutaredoxins. The quantitative results allow us to model the redox and GSH-binding states of the proteins in vivo under different conditions. For instance, we can model the conditions under which human Grx2 is activated during ferroptosis. Our results also imply a stable pool of dithiol Grxs in the mixed disulfide form with GSH under normal physiological conditions. Our study reveals that a dynamic conformational switch in the active site of glutaredoxin oxidoreductases regulates their redox cycle and glutathione binding, providing mechanistic insight into their diverse activities and enabling predictive modeling of their physiological roles.

POSTER 06

Unbiased proteome-wide glutathione interactome screen

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Glutathione (γ-glutamyl-cysteinyl-glycine, GSH) is a tripeptide found in all mammalian cells as the most abundant intracellular thiol-based antioxidant. As a redox mediator, GSH is essential for maintaining the cellular redox potential. It also acts as a reactive oxygen species (ROS) scavenger, reducing peroxides and free radicals. Furthermore, GSH is crucial for protein folding by supporting the formation of disulfide bonds through mixed disulfides and maintaining the redox balance. As part of the phase II detoxification system, GSH removes electrophiles and xenobiotics. [1]

Despite these well-studied properties, an unbiased proteome-wide interactome screen to discover unknown interaction partners, binding motifs, and potentially new functions of GSH remains to be conducted.

Protein-ligand interactions release free Gibbs energy, which increases the melting temperature compared to the free ligand. This phenomenon is used in the temperature-based proteome integral solubility alteration assay (PISA T). As the temperature increases, non-interacting proteins are gradually removed from the solution as a function of their binding strength to the ligand. [2]

Here, we present a PISA T study with GSH using cell lysates. In this proteome-wide unbiased study, we validated known interaction partners and identified new potential ones after controlling for structural features and GSH's reducing property.

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POSTER 07

DpCoA CaptureSeq: Specific biotinylation and enrichment of dpCoA-RNA

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Dephospho-Coenzyme A (dpCoA) has been found in the recent years to be one of several non-canonical cap structures at the 5' end of RNA. [1] The potential roles of this cap in gene regulation or signalling are currently unknown. In order to decipher its function, the identification of dpCoA-capped RNA sequences is crucial. Previous tagging and sequencing protocols based on the maleimide-thiol reaction were unable to reliably identify dpCoA-RNAs, possibly due to side reactions with other thiol modifications. [2,3]

To overcome this problem, we tested a novel approach utilizing a specific chemoenzymatic modification step to biotinylate dpCoA-capped RNAs. The biotinylated RNA is then enriched on streptavidin beads and used for reverse transcription, yielding cDNA, which can be used for sequencing experiments. In experiments with in vitro prepared RNAs, this method displayed a clear specificity for dpCoA capping. We are currently transferring the method to a protocol for the use on total RNA of bacterial and eukaryotic origin, taking in vitro prepared dpCoA- and uncapped RNAs as internal standards. Transcripts identified as dpCoA-capped in this way could provide insight into the biological significance of this modification and form the basis for further studies on it.

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POSTER 08

Elucidating structure-function relationships in Class I glutaredoxins

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Glutaredoxins are ubiquitous members of the thioredoxin superfamily and play a central role in the cellular antioxidative defence. They are divided into two classes based on conserved structural elements and functional differences: Class I glutaredoxins, typically dithiol, act as glutathione-dependent thiol-disulfide oxidoreductases, while Class II glutaredoxins are monothiol and are involved in iron-sulfur cluster sensing and assembly. Despite their shared highly conserved thioredoxin-like fold, many questions remain regarding which structural motifs are responsible for the distinct functional activities of different glutaredoxins.

In this study, we developed novel assays to investigate structure-function relationships and monitor enzyme activity, with distinct physiological substrates, inside living cells. Consistent with previous studies, we found that several highly conserved structural motifs contribute to the enzymatic activity of glutaredoxins. We observed similar activity among all glutaredoxins studied, and mutants thereof, with both cystine and glutathione disulfide as substrates. Interestingly, we found that the relative importance of different structural motifs varied in different glutaredoxins. For example, whilst exchange of the loop region at the N-terminal side of the active site with a Class II loop completely abolished enzymatic activity in human GLRX2 or Saccharomyces cerevisiae Grx7, it had no effect on the enzymatic activity in human GLRX1. Our results support the conclusion that the functional differences between Class I and Class II glutaredoxins depend on conserved variations in multiple structural motifs that show different relative importance in different glutaredoxin isoforms.

POSTER 09

Developing novel and improved redox-sensitive red fluorescent protein-based probes

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Redox processes are fundamental to all living organisms. Maintaining redox homeostasis is critical for numerous cellular functions including signaling, proliferation, immune responses, and protein folding. This balance is tightly regulated by multiple cellular systems, and its disruption can result in oxidative stress and cell death. GFP-based redox probes have become invaluable tools for specific monitoring of intracellular redox dynamics with subcellular resolution. In principle, RFP-based redox probes offer several advantages including reduced phototoxicity, deeper tissue penetration, lower autofluorescence, and the possibility for multiparametric imaging together with current GFP-based sensors. However, existing RFP-based redox sensors are limited by low molecular brightness, strongly negative redox midpoint potentials, high pH sensitivity, and a lack of options for ratiometric imaging,

To address the limitations of current redox-sensitive RFPs, we are employing a combination of directed molecular evolution and rational engineering approaches. Using mScarlet-I3 - a state-of-the-art monomeric RFP characterized by high brightness, low pK_a, and rapid maturation - as a scaffold, we first introduced cysteine residues on the surface of the β-barrel, adjacent to the chromophore, to allow for modulation of its fluorescence properties upon oxidation/reduction. Subsequently, we developed an E. coli-based screening platform to assess mutants generated in multiple rounds of directed evolution, resulting in the generation of ro-mScarlet-I3 variants with enhanced fluorescence dynamic range, whilst preserving a high brightness. Future work will focus on modulation of the probe redox midpoint potential and exploring options for developing variants permissive for ratiometric imaging.

POSTER 10

The Role of Redox Systems in Ferroptosis Pathway in HaCaT Keratinocytes – A Comparative Study of WT and GPX4-KO Lines

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Ferroptosis is a regulated form of cell death driven by iron-dependent accumulation of reactive oxygen species (ROS) and lipid peroxidation, with a critical role for redox homeostasis. This study aimed to assess redox responses and antioxidant pathway activation in human HaCaT keratinocytes,wild-type (WT) and GPX4 knockout (KO) line,under ferroptosis stimulation.

Ferroptosis was induced by erastin exposition, at two doses, of 5 and 10 μM. Elevation of ROS levels was observed in WT cells, while GPX4 KO cells maintained ROS levels comparable to untreated control. Despite this, total glutathione content in the GPX4 KO line dropped below control levels, indicating redox imbalance independent of ROS accumulation.

Gene expression analysis revealed that ferroptosis induced an up-regulation of NRF2, TRX, and GPX4 in WT cells, suggesting activation of compensatory antioxidant responses. In contrast, GPX4 KO cells showed increased expression of NRF2 and TXNRD, accompanied by a decrease in TRX levels and a complete absence of GPX4 expression. These findings suggest a compensatory shift toward the thioredoxin pathway in the absence of GPX4 activity.

Our results highlight the complexity of redox regulation in skin cells under ferroptotic stress and underscore the pivotal roles of the NRF2 axis and TRX/TXNRD system when the glutathione-dependent GPX4 pathway is disrupted.

Aknowlegments: The work was co-financing of Project Based Learning - PBL in accordance with the Regulations No. 54/2020 and 55/2020 of the Rector of the Silesian University of Technology of March 13, 2020.

POSTER 11

Controlled Mitochondrial H2O2 Fluxes Regulate Gene Expression and Cell Fitness in Fission Yeast

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Reactive oxygen species (ROS) are byproducts of normal cellular metabolism, especially during mitochondrial respiration, and play both beneficial and harmful roles in biological systems depending on their concentrations. Using Schizosaccharomyces pombe, we have studied how oxidative eustress can have repercussions in cell's fitness and aging. We generated a combination of strains expressing generators and biosensors of hydrogen peroxide (H_2O_2). Particularly, generation of mitochondria-to-cytosol peroxide waves was accomplished through the localized over-expression of D- amino oxidase (Dao1) from S. pombe. Dao1 catalyzes the oxidative deamination of D-amino acids, generating H_2O_2 as a byproduct (1). When D-amino acids are added in low doses to MTS-Dao1 expressing cells, these H_2O_2 generated in the mitochondria promotes stress adaptation and lifespan extension. We also performed experiments blocking H_2O_2 fluxes with the mitochondrial co-expression of Dao1 with scavengers such as thioredoxin peroxidase (Tpx1) and catalase (Ctt1), in order to study how the blockage of H_2O_2 waves impacts the previous results obtained. We also tested the relevance of signaling cascades triggered by ROS, by knocking out atf1 and pap1 genes at the same time that we over-expressed MTS-Dao1 in the matrix and how this affects the core environmental stress response genes regulation.

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POSTER 12

Iron-deplete diet enhances Caenorhabditis elegans lifespan via oxidative stress response pathways

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Gut microbes play a crucial role in modulating host lifespan. However, the microbial factors that influence host longevity and their mechanisms of action remain poorly understood. Using the expression of Caenorhabditis elegans FAT-7, a stearoyl-CoA 9-desaturase, as a proxy for lifespan modulation, we conduct a genome-wide bacterial mutant screen and identify 26 Escherichia coli mutants that enhance host lifespan. Transcriptomic and biochemical analyses reveal that these mutant diets induce oxidative stress and activate the mitochondrial unfolded protein response (UPRmt). Lifespan extension requires the oxidative stress response regulators SKN-1, SEK-1, and HLH-30. Mechanistically, these effects are linked to reduced iron availability, as iron supplementation restores FAT-7 expression, suppresses UPRmt activation, and abolishes lifespan extension (Das et al., 2025). Iron chelation mimics the pro-longevity effects of the mutant diets, highlighting dietary iron as a key modulator of aging. Since low iron can reduce iron-sulfur (Fe-S) cluster biogenesis, we propose that impaired Fe-S cluster assembly underlies the observed phenotypes. Consistent with this hypothesis, RNAi-mediated knockdown of iscu-1, a key Fe-S cluster assembly protein, phenocopies the effect of mutant diets. Our findings reveal a bacterial-host metabolic axis that links iron homeostasis, oxidative stress, and longevity in C. elegans.

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POSTER 13

The hexameric atypical thioredoxin from poplar, DCC1, possesses a redox holdase activity

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Photosynthetic organisms contain more than 150 proteins belonging to the thioredoxin (TRX) superfamily. Many possess one or two redox-active cysteines and a characteristic cis-proline at defined positions, along with additional domains or secondary structures. We have identified a new set of TRX-like proteins in photosynthetic organisms, with unknown functions (1). Among these, phylogenetic studies have identified a distinct family called DCC, characterized by a conserved DXXCXLC motif and a specific C-terminal extension. We have performed the biochemical and structural characterization of DCC1 from poplar and Arabidopsis, one of the three DCC proteins in terrestrial plants. Our results indicate that full-length recombinant DCC1 proteins form primarily high order oligomers, which in the case of poplar DCC1 are hexamers as assessed by light scattering and cryo-EM experiments (2). This multimeric conformation correlates with the existence of redox-dependent holdase activity while poor oxidoreductase activity have been detected. Indeed, truncated versions of DCC1 devoid of the C-terminal extensions have lost holdase activity. Moreover, truncated and mutated versions mimicking the classical active site motifs present in typical TRX/GRX did not acquire conventional oxidoreductase activity, despite the cysteines are reactive and form an intramolecular disulfide bridge. These results point to the existence of redox chaperones (chaperedoxins) belonging to the TRX superfamily in plant organelles.

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POSTER 14

A redox-dependent interaction between Protein Disulfide Isomerase-A1 (PDIA1) and Guanine Nucleotide Dissociation Inhibitor-α (Rho GDIα)

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Rho GTPases and redox processes converge to regulate cytoskeleton architecture and cell plasticity, but underlying mechanisms are unclear. RhoGDIs regulate spatial Rho GTPase activation and directional cell migration. We previously described fine-tuning of cytoskeleton regulation and directional migration by PDIA1, a dithiol redox chaperone from the thioredoxin superfamily having canonical roles in redox protein folding in endoplasmic reticulum. PDIA1 can also translocate extracellularly or, as we showed recently, to the cytosol. We found that RhoGDI family genes form highly conserved microsynteny clusters with PDI family genes, while co-localization and proximity ligation assays support PDIA1/RhoGDIα association in vascular cells. We hypothesize that PDIA1/RhoGDIα interaction is a converging redox-dependent hub of Rho GTPase and cytoskeletal regulation. Here we further advance into the molecular details of this interaction. Using purified proteins, direct PDIA1/RhoGDIα interaction was suggested by pull-down and ELISA assays, showing preference for thiol-oxidized PDIA1. To corroborate these data and identify interaction epitopes, we performed NMR spectroscopy with oxidized/reduced PDIA1 incubated with 15N-enriched-RhoGDIα, demonstrating that both redox states of PDIA1 interact with RhoGDIα, but affinity/interaction epitopes depict more stable interaction with oxidized PDIA1. Furthermore, ELISA assays with isolated PDIA1-domains indicate that the b-b' domains are preferential interaction sites, in line with their reported enhanced accessibility in oxidized PDIA1, which displays an open conformation. Computational molecular modeling based on such NMR results supports the predominance of interacting residues from PDIA1 a' and b' domains. The PDIA1/RhoGDIα interaction may provide a novel regulatory level to understand Rho GTPase regulation and downstream signaling.

POSTER 16

Development of novel subfamilies of NAPstar NADPH/NADP+ redox state sensors

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The redox coenzyme nicotinamide adenine dinucleotide phosphate (NADPH and NADP+) is central to cellular metabolism, providing most electrons required for anabolism and the reduction of thiol-dependent redox enzymes. To explore the real-time dynamics of this redox couple in vivo, we developed NAPstars, a family of genetically encoded fluorescent NADPH/NADP+ ratio sensors. NAPstars are based on two, typically identical, copies of an engineered version of the NADH-binding bacterial repressor REX and a circularly permuted GFP T-Sapphire (cpTS) reporting changes in the NADP redox state. Despite a 10-fold range of KD(NADPH) values (1–12 µM), the probes are nearly saturated with NADPH for example in the plant chloroplast. To enhance control over probe properties, including KD(NADPH), and expand their utility across different cell types or compartments, we developed novel NAPstar variants by independently mutating each REX domain. This mixed-domain NAPstar subfamily exhibited a wider range of KD(NADPH) from 14–80 µM.

NAPstars usually contain a C-terminally fused mCherry to allow for normalization of cpTS fluorescence on expression levels. We replaced mCherry with the long-stokes shift mBeRFP to generate a novel subfamily of NAPstars suitable for multiparametric imaging with other RFP-based redox sensors. The resulting constructs retain similar KD(NADPH) values coupled with enhanced specificity towards NADP. As a proof of principle, we show that NAPstar-mBeRFP variants can be used simultaneously with HyperRed in plant and mammalian cells. Finally, we are working to generate fully red NAPstars by exchanging cpTS with a circularly permuted RFP to allow for concomitant use with common GFP-based probes.

POSTER 17

A novel prodrugs with enhanced radiosensitizing activity for potentially clinical usage against cancer cell lines.

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One of the primary cellular defense mechanisms against oxidative stress is the glutathione shield. Glutathione (GSH), a tripeptide composed of glutamate, cysteine, and glycine, is the most abundant intracellular thiol and acts as a major electron donor, maintaining redox homeostasis. Its antioxidant properties help preserve cellular integrity and functionality. Enzymes such as glutathione peroxidase (GPx), glutathione reductase (Grx), and glutathione transferase (GST) enable glutathione cycling reactions, forming an integral part of this defense system. In oxidative environments, two GSH molecules can form glutathione disulfide (GSSG) via a disulfide bridge (-S–S-), especially after neutralizing of free radicals, often generated in mitochondrial complexes I and III of the electron transport chain or under the external oxidative stress stimuli.

In this context, KC29, a novel compound under investigation, is designed to function analogously to glutathione and exhibit affinity for glutathione-binding proteins. The potential radiosensitizing effect of KC29 was evaluated in vitro using SSC25, BEAS-2B, and SH-SY5Y cell lines.

Studies involving nitroimidazole derivatives, such as metronidazole, demonstrated increased oxidative stress and a corresponding rise in total GSH/GSSG levels, suggesting a feedback activation of antioxidant defenses. Despite, no significant reduction in viability or mitochondrial potential, a decrease in mitochondrial mass after irradiation indicates a cell cycle blockade effect.

Together, the interplay of ROS generation, glutathione-mediated defense, and synthetic radiosensitizers like KC29 and metronidazole provides a foundation for developing more effective and selective cancer therapies through redox-based mechanisms.

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POSTER 19

NADPH-dependent oxidation of different target proteins through a MICAL1-Prx1 redox relay

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The semaphorin (Sema) signalling pathway controls the formation of axonal networks, and thereby neuronal development and cell migration, through the regulation of cytoskeletal dynamics. CRMP2, one part of the Sema signalling pathway, is regulated by post-translational modifications including a dithiol-disulfide switch. The reduction of CRMP2s redox switch by its specific reductase Grx2c was established [1], the signal-induced oxidation however remains to be elucidated. MICAL proteins are FAD-dependent monooxygenases that act as effector proteins in cell signalling cascades, i.e. in the Sema pathway. Activated MICAL can produce H2O2 [2], however a direct oxidation of specific methionyl residues in beta-actin has also been described [3].

Here, we present the specific oxidation of CRMP2 through a redox relay involving MICAL1 and the peroxidase Prx1 as specific transducers. Using electrons provided by NADPH and molecular oxygen, MICAL produces hydrogen peroxide that specifically oxidises Prx1 through direct interactions. Subsequently Prx1 oxidises CRMP2. The loss of any component of this redox relay dysregulates neurite outgrowth.

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POSTER 20

Reconsidering Inertness: Neuronal Suppression by D-Amino Acids in the Absence of DAAO Expression

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Hydrogen peroxide (H_2O_2) is one of the most extensively studied reactive oxygen species (ROS) in biology, as both its deficiency and excess can disrupt physiological processes. While a certain concentration is required for its function as a signaling molecule in redox-regulated pathways, elevated levels lead to oxidative stress and contribute to various pathologies. To achieve spatial and temporal control over H_2O_2 production in specific cells and subcellular compartments, recombinant yeast-derived D-amino acid oxidase (DAAO), together with its D-amino acid (D-aa) substrates, has recently emerged as a novel chemogenetic tool (Erdogan et al., 2021). For neuronal applications, D-norvaline is preferred over the commonly used D-alanine, which has been shown to interfere with neuronal activity (Kalinichenko et al., 2023).

In this study, we found that D-norvaline reduced neuronal calcium activity in rat primary cortical neuron–astrocyte co-cultures, even without DAAO expression. Neuronal activity was quantified in GCaMP8s-expressing neurons by analyzing calcium transients, including peak frequency and amplitude. Moreover, other D-aa's, previously considered biologically inactive, also significantly suppressed neuronal activity (Seckler & Jewis, 2020). As expected, D-aspartate, an NMDA receptor agonist, increased neuronal activity, while L-alanine, used as a negative control, had no significant effect.

Our findings indicate that D-aa's previously considered to be inert may suppress neuronal activity. This highlights the importance of compound selection and dose optimization in neuron-specific and in vivo applications of the DAAO chemogenetic tool for studying H_2O_2, where systemic effects may also arise. Experiments are ongoing to understand to underlaying mechanisms by which D-aa suppress neuronal activity.

POSTER 21

Studying the interdependence between respiratory efficiency, cysteine biosynthesis, and H□S accumulation in fission yeast

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Hydrogen sulfide (H_2S) interacts with the mitochondrial electron transport chain (ETC) in a dual manner: at physiological concentrations, it supports ATP production by feeding electrons to ubiquinone via the sulfide:quinone oxidoreductase (SQOR); conversely, at toxic levels, it inhibits complex IV, blocking respiration.

Unlike other yeasts, the fission yeast Schizosaccharomyces pombe possesses both an active mitochondrial metabolism similar to human cells and the enzyme SQOR (encoded by hmt2), making it a valuable model to study H_2S effects on mitochondrial function.

In this work, we examine the metabolic and toxicological roles of H_2S. We characterize the cysteine/methionine biosynthetic pathway, and show that mitochondrial, but not cytosolic, sulfide detoxification is essential for maintaining respiratory competence. Disruption of the mitochondrial sulfide scavenging leads to elevated H2S levels, which impairs respiration and downregulates ETC genes. Notably, the respiratory deficiency observed in sulfide-accumulating mutants is reversed by genetically abolishing endogenous H_2S production.

Although sulfide levels vary across strains, only those with high sulfide accumulation exhibit respiratory defects, suggesting a signaling role for H_2S. To further explore this, we performed a genome-wide screen using a S. pombe deletion library to identify mutants with elevated H_2S levels. This screen reveals not only factors essential for mitochondrial respiration but also genes involved in transcriptional regulation and chromatin organization.

In conclusion, our findings demonstrate that H_2S accumulation is both a cause and a consequence of mitochondrial dysfunction. These results support the potential of H_2S as a biomarker for mitochondrial impairment and as a modulator of redox-dependent signaling pathways.

POSTER 22

Patrx2: a paradoxal oxidoreductase implicated in alginate biofilm formation in Pseudomonas aeruginosa

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Bacteria maintain a reducing cytoplasmic environment in which abnormally oxidized cysteines are restored by thiol-disulfide oxidoreductase (TDOR) systems. In the Gram-negative opportunistic pathogen Pseudomonas aeruginosa, we identified a cytoplasmic TDOR we named Patrx2, featuring an unusual active site motif (CGHC) typically found in eukaryotic protein disulfide isomerases (PDIs). Based on our in vitro analyses, Patrx2 functions as a cytoplasmic disulfide isomerase with a notably high redox potential at its active-site cysteines, determined by NMR to be –172 mV. It exhibits modest disulfide reductase activity and substantial oxidase activity. To understand how disulfide isomerase or oxidase activity can occur in the reducing environment of the P. aeruginosa cytoplasm, we explored the expression conditions of patrx2 using a transposon mutagenesis library. Our results reveal that patrx2 expression is induced in response to envelope stress via the alternative sigma factor AlgU. Notably, AlgU is overexpressed in clinical isolates that form alginate-rich biofilms, known as “mucoid variants.” We quantified alginate production in these mucoid P. aeruginosa strains and found that a catalytic mutant of Patrx2 secreted three times less alginate than the wild-type mucoid strain. Current investigations aim to identify potential substrates of Patrx2. Preliminary data suggest that it may catalyze disulfide bond formation in the first enzyme of the alginate biosynthesis pathway. Collectively, our findings challenge the prevailing view that the bacterial cytoplasm only supports disulfide reductase activity and highlight the role of redox regulation in mucoid biofilm formation in P. aeruginosa.

POSTER 23

Understanding redox-triggered amyloid fibril formation of tumour suppressor p16INK4a

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p16INK4a (p16) is a tumour suppressor protein that regulates cell cycle progression by binding to and inhibiting the cyclin-dependent kinases 4 and 6 (CDK4/6). p16 is a 16 kDa, α-helical protein with a single solvent-accessible cysteine residue located on the surface facing away from the CDK4/6 binding site. Our group discovered that this single cysteine in p16 can, under relatively mild oxidizing conditions, form disulfide-dependent homodimers. This dimerization leads to subsequent structural rearrangement into amyloid fibrils. In this amyloid fibril state p16 is unable to perform its normal function as a CDK4/6 inhibitor. This is the first instance of a thiol-based redox switch protein triggering the formation of amyloid fibrils.

To unravel the mechanism of p16 amyloid fibril formation and understand how p16 is inactivated in cancer, we have screened cancer-associated single-residue mutations. These variants have been characterized using thioflavin-T aggregation assays, SDS-PAGE analysis of dimerization, and electron microscopy. Intriguingly, almost all cancer-associated mutations result in a rapid increase in the rate of dimer and/or fibril formation. Combined with an investigation of mutations that stabilize the monomeric structure, our detailed analysis has shed light on the key influences in p16 dimerization and amyloid fibril formation. These exciting findings will be applied to investigate the short-lived dimer species and disulfide-stabilized structure of p16 amyloid fibrils. Overall, this work provides fascinating insights into the mechanism by which these unique oxidation-triggered aggregate species form and their role in pathogenic processes.

POSTER 24

Differential KEAP1/NRF2 mediated signaling widens the therapeutic window of redox-targeting drugs in SCLC therapy

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DKFZ

Small cell lung cancer (SCLC) patients frequently experience a remarkable response to first-line therapy. Follow up maintenance treatments aim to control residual tumor cells, but generally fail due to cross-resistance, inefficient targeting of tumor vulnerabilities, or dose-limiting toxicity, resulting in relapse and disease progression. Here, we show that SCLC cells, similar to their cells of origin, pulmonary neuroendocrine cells (PNECs), exhibit low activity in pathways protecting against reactive oxygen species (ROS). When exposed to a novel thioredoxin reductase 1 (TXNRD1) inhibitor, these cells quickly exhaust their ROS-scavenging capacity, regardless of their molecular subtype or resistance to first-line therapy. Importantly, unlike non-cancerous cells, SCLC cells cannot adapt to drug-induced ROS stress due to the suppression of ROS defense mechanisms by multiple layers of epigenetic and transcriptional regulation. By exploiting this difference in oxidative stress management, we safely increased the therapeutic dose of TXNRD1 inhibitors in vivo by pharmacological activation of the NRF2 stress response pathway. This resulted in improved tumor control without added toxicity to healthy tissues. These findings underscore the therapeutic potential of TXNRD1 inhibitors for maintenance therapy in SCLC.

POSTER 25

Thioredoxin-dependent pathway as a key line of antioxidant defense in human cells under mild oxidative stress conditions

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In this study, we used the genetically encoded biosensor HyPer to quantify the peroxidase activity under exogenous H_2O_2-induced oxidative stress of varying intensity in human myeloid leukemia K562 cells by utilizing the method developed earlier (Zenin et al., 2022). We have identified two different stress regimes: under mild stress, peroxidase activity is maximal but gradually decreases with increasing H_2O_2 concentration; under moderate stress, it is exhausted and no longer responds to further increases in H_2O_2. Inhibitor analysis revealed that the antioxidant response under mild oxidative stress conditions is primarily regulated by the Trx-dependent pathway, while the moderate stress mode is characterized by the predominant role of the GSH-dependent system in H_2O_2 detoxification. At the next step, we tested whether the decrease in peroxidase activity under mild stress was caused by the oxidative inactivation of Prx, Trx-dependent peroxidases. However, siRNA-mediated knockdown of Prx1/2 not only failed to reduce peroxidase activity but also significantly increased Trx-dependent reductase activity in cells – likely due to an increased pool of reduced Trx, which is no longer consumed for Prx reduction. Our findings indicate that while the Trx pathway regulates the primary antioxidant defense under mild oxidative stress conditions in human cells, Prx are not the main actors. Notably, our data are consistent with and expand upon findings highlighting the role of Trx-dependent redox-active proteome in peroxide scavenging previously reported in yeast models (Tomalin et al., 2016).

This work was supported by Russian Science Foundation (Grant#21-74-20178-P).

POSTER 26

PYROXD1 is a new interactor of the CIA-targeting complex: client, partner, or both?

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Iron-sulfur clusters (ISCs) are cofactors in a large number of essential proteins. Their biogenesis and maturation involve several machineries in both mitochondria and cytosol. The cytosolic ISC assembly targeting complex (CIA-TC) delivers ISCs to proteins with crucial roles in RNA and DNA metabolism. Little is known about the mechanisms regulating the CIA-TC and many potential client proteins remain undiscovered.

We uncover the essential oxidoreductase PYROXD1, previously described by our laboratory in the context of pre-tRNA splicing, as a new interactor of the CIA-TC. The interaction is stable and can be reconstituted in vitro. Critically, a myopathy-causing variant displaying no other phenotype of PYROXD1 abrogates this interaction.

We propose two alternative hypotheses for the biological role of this interplay. We are performing 55Fe incorporation, in vitro reconstitution and anaerobic purification to test if the CIA-TC inserts an ISC into PYROXD1. This is supported by the presence of metal-binding residues and a LYR-like motif in PYROXD1, and could directly affect its known properties: FAD-mediated NAD(P)H activity and thiol-based polymerization. We are also exploring if the ISC could provide PYROXD1 new properties, ultimately explaining the reported anti-oxidative function. Alternatively, we are testing whether PYROXD1 may facilitate the delivery of ISCs to potential new targets of the CIA-TC including tRNA-LC, whose activity decreases upon silencing of the CIA-TC.

Together, these data bring ISCs to the forefront of PYROXD1 biology, implicating the CIA-TC in pre-tRNA splicing and revealing a new molecular phenotype in the myopathies caused by PYROXD1 variants.

POSTER 27

Redox-Dependent ER-to-Cytosol Protein Reflux Drives Spatial Reprogramming of Protein Function

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Interorganellar spatial redistribution of proteins represents a critical yet understudied aspect of eukaryotic proteostasis. This dynamic process enables proteins to acquire new functions depending on their subcellular localization, allowing cells to adapt to diverse physiological and pathological contexts, including cancer, neurodegeneration, and viral infection[1, 2]. Despite its emerging significance, the molecular mechanisms driving protein relocalization and the resulting functional consequences remain incompletely understood. Here, we identify a redox-dependent mechanism of ER-to-cytosol protein reflux (ERCYS) that facilitates spatial and functional reprogramming of proteins under stress conditions. During ER and genotoxic stress, the ER selectively refluxes oxidatively damaged proteins into the cytosol and, in some cases, out of the cell. Refluxed proteins are recognized by the cytosolic co-chaperone SGTA via a redox-sensitive interaction dependent on its cysteine 153 residue[3]. SGTA subsequently mediates the transfer of these proteins to new cytosolic partners, resulting in altered—either gained or lost—functional states within the recipient compartment. Together, these findings define a novel redox-regulated pathway of interorganellar protein trafficking and functional reassignment, revealing an unrecognized layer of proteostatic regulation that enables cellular adaptation to stress.

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POSTER 28

Acquisition of a senescence phenotype and rejuvenation alter antioxidant activity in human mesenchymal stem cells

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Alteration of redox homeostasis has always been associated with the development of cellular senescence. However, existing data, especially those related to changes in the activity of the antioxidant defense system, remain controversial. In this study, we applied newly developed methods based on the genetically encoded hydrogen peroxide sensor HyPer, which allows quantitative assessment of peroxidase and disulfide reductase activity under conditions of H_2O_2-induced oxidative stress in living cells. Using these methods, we found that disulfide reductase activity is decreased, while peroxidase activity is increased in senescent compared to young human mesenchymal stem cells in both replicative and stress-induced senescence cell culture models. The results obtained partly contradict the existing hypothesis of a weakened antioxidant defense in senescent cells. A partial reprogramming approach – short-term overexpression of the pluripotent Yamanaka factors – enabled the rejuvenation of senescent cells and the restoration of their disulfide reductase activity to levels typical of young cells, but had little impact on peroxidase activity. The shifts in antioxidant activity observed in senescent cells showed little correlation with changes in the expression of genes related to the corresponding antioxidant enzymatic pathways. So, we propose that higher-level regulatory mechanisms may affect the redox metabolism in senescent cells – possibly related to a reduced rate of thiol oxidation caused by acidification of the intracellular milieu accompanying the development of cellular senescence. The work was supported by the Russian Science Foundation (grant # 21-74-20178 Prolongation).

POSTER 29

Mitophagy acts as a compensatory mechanism to ensure cell survival in the absence of PRDX1-dependent mitochondrial redox protection.

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Hydrogen peroxide (H2O2) has gained interest not just as a cellular by-product causing oxidative stress, but also as a key player in cellular redox signaling across cellular compartments. Mitochondria are considered one of the main cellular sources of H2O2, and although several regulatory factors have been identified, the underlying molecular mechanism remains poorly understood. In this study, we used the genetically encoded sensor HyPer7 to monitor real-time H2O2 fluctuations in both the cytosol and the mitochondrial matrix of HEK293 cells with deletions of specific peroxiredoxins (PRDXs), key enzymes involved in H2O2 detoxification. We demonstrate that the cytosolic localized PRDX1 is the main reducer of cellular H2O2, with its influences extending into the mitochondrial matrix. Surprisingly, mitochondrial-localized PRDXs did not exhibit this regulatory effect within the mitochondrial matrix. This was observed by treating cells with a low exogenous H2O2 bolus, as well as using a chemogenetic system (mtDAO) to induce mitochondrial H2O2 production. A mitochondria- and metabolism-focused CRISPR/Cas9 screen in cells lacking both cytosolic PRDXs (PRDX1/2) revealed genetic factors involved in the handling of mitochondrial-produced H2O2. Among these, a mitophagy regulator was identified. Taken together, our data reveal that the cytosolic PRDX1 influences the mitochondrial redox homeostasis and suggest that mitophagy functions as a compensatory mechanism when cytosolic buffering of the mitochondrial-produced H2O2 is impaired.

POSTER 30

BCAT1/APE1 dependent regulation of glioblastoma phenotypic plasticity

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Glioblastoma (GB) is the most common primary malignant brain tumour in adults. A major contributor to treatment resistance and aggressive recurrence is the phenotypic plasticity seen in GB cells. This allows GB cells to transition between different cellular states in response to therapeutic stress, and is influenced by genetic, epigenetic and microenvironmental cues.

Branched-chain amino acid transaminase 1 (BCAT1) is overexpressed in GB where it plays a role in maintaining the cells in an undifferentiated, mesenchymal-like state (1). It was also identified that BCAT1 has a CXXC redox motif which is necessary for the maintenance of mitotic fidelity (Francois et al., 2022). However, the redox signalling of BCAT1 through this motif had not yet been investigated.

Through IP-MS experiments, the redox factor AP-endonuclease 1 (APE1) was identified as an interacting partner of BCAT1. APE1 is overexpressed in several cancers where it functions as a redox switch to activate oncogenic transcription factors. Using a thiol-disulphide exchange assay, we confirmed the participation of BCAT1 and APE1 in a redox exchange. The BCAT1/APE1 interaction will be further studied using redox-based assays and mutant proteins. In addition, the BCAT1/APE1 dependent regulation of GB phenotypic plasticity is also being studied in GB cell lines and iPSC-based 2D and 3D models.

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POSTER 31

Redox-dependent urmylation of human peroxiredoxin PRDX5 in yeast

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Post-translational modifications by ubiquitin and ubiquitin-like (Ubl) proteins play vital roles in numerous cellular processes. Regarding ubiquitin, such ubiquitination is essential for intracellular protein degradation and various non-proteolytic processes. Ubiquitin-related modifier 1 (Urm1) is a special member of the eukaryotic Ubl family that requires C-terminal sulfur-activation by thiocarboxylation. It attaches to target proteins under oxidative stress in a process called urmylation and also provides sulfur for tRNA thiolation. Based on recent in vitro findings, Urm1 may also transfer sulfur to redox active thiols of proteins during urmylation, resulting in their persulfidation (EMBO J. 2022;41(20):e111318). Protein persulfidation is a post-translational modification associated with neurodegenerative diseases. Its cellular benefit may be the protection of catalytic thiols from irreversible hyperoxidation (Cell Metab. 2019;30(6):1152-1170.e13). Hence, it is crucial to investigate this non-ubiquitin-like function of Urm1 in vivo. To date, redox-dependent urmylation of the yeast peroxiredoxin Ahp1 is well characterized (Redox Biol. 2020;30:101438). However, yeast only shows mild phenotypes for urmylation deficiency and might not be adequate to determine the true potential of urmylation-mediated persulfidation. We wanted to transfer our knowledge to the human ortholog of Ahp1, PRDX5, to initiate further human studies. Our research questions were: (i) can PRDX5 compensate for the loss of Ahp1 and (ii) is PRDX5 urmylated in yeast? Interestingly, heterologous expression of PRDX5 rescued Ahp1-deficient cells when treated with oxidants. Moreover, we indeed found urmylation of the human peroxiredoxin by yeast Urm1. Thus, we are now using our well-established assays to further elucidate the details of PRDX5 urmylation in yeast.

POSTER 32

Exploring a role of mitochondrial thioredoxin-like protein in ROS-driven cellular differentiation

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Reactive oxygen species (ROS) are key signaling molecules that influence diverse cellular processes through redox-mediated protein modifications. Hydrogen peroxide, in particular, relays mitochondrial signals that alter cellular functions. However, the mechanism by which mitochondrial ROS transmit signals to specific targets remains poorly understood.

In Trypanosoma brucei, a unicellular parasite, ROS signaling is crucial for differentiation within the tsetse fly host. Using T. brucei as a model, we investigate the redox relay pathway linking mitochondrial ROS production to cellular response. Among the ROS scavenger proteins, a mitochondrial outer membrane thioredoxin-like protein (Trx5780) exhibited increased expression during ROS-driven differentiation, suggesting a role in redox signaling. This protein possesses a typical thioredoxin-fold domain and is predicted to face the cytoplasm.

Deletion of Trx5780 impaired several differentiation hallmarks, including mitochondrial membrane potential, endocytosis rates, and expression of life cycle-specific surface proteins. The carboxy-H_2DCF probe revealed lower cytosolic ROS levels during differentiation of Trx5780 knockout parasites. The dysregulation of these differentiation markers demonstrates a role for Trx5780 in parasite programmed differentiation.

To assess Trx5780's redox state and function, we are employing non-reducing SDS-PAGE, insulin reduction assays with recombinant Trx5780, and characterizing its topology and oligomeric state in the mitochondrial outer membrane. Additionally, TurboID-based proximity labeling will help identify transient interactions, possibly identifying protein targets in the redox signaling pathway. Altogether, this will provide deeper insight into the role of Trx5780 in T. brucei ROS-driven differentiation.

POSTER 33

Mechanistic insights into the redox-driven inactivation of viruses by extracellular protein disulfide isomerase

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Protein disulfide isomerase (PDI) catalyzes the formation of native disulfide bonds during client protein folding in the mammalian endoplasmic reticulum. However, PDI can also localize extracellularly, where its function remains poorly understood. Here, we identify a redox-driven antiviral role for extracellular PDI against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). PDI efficiently catalyzes the reductive unfolding of the receptor-binding domain (RBD) of the viral spike protein by targeting all four disulfide bonds. Structural and biophysical analyses reveal that the RBD binds PDI with higher affinity than ACE2, the physiological viral receptor. In human bronchial organoid cells, extracellular PDI inhibits SARS-CoV-2 infection, and this antiviral activity extends to Delta and Omicron variants as well as to human coronavirus 229E (HCoV-229E). In this workshop, we will discuss a redox-based defense mechanism that inactivates coronaviruses through the reductive unfolding of spike proteins.

POSTER 34

LSD1 – At the Intersection of Epigenetic Modulation and Redox Regulation

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Redox regulation plays a fundamental role in neural differentiation, yet its integration into epigenetic control remains poorly understood. LSD1, a flavin-dependent histone demethylase, may exemplify this intersection by generating hydrogen peroxide (H_2O_2) during chromatin remodeling, thereby potentially regulating redox-related processes within the nucleus.

This project identifies LSD1 as both a nuclear oxidase and a modulator of neural stem/progenitor cell (NSPC) differentiation. We demonstrate that LSD1 inhibition or loss selectively impairs oligodendrocyte maturation and decreases the amount of oxidized nuclear proteins. Mass spectrometry reveals condition-specific changes in LSD1's interactome, while redox proteomics uncovers inhibitor-induced modifications in its cysteine residues.

Using the HyPer7 sensor, we monitor nuclear H_2O_2 formation. Furthermore, we examine LSD1's interactions with other histone modulators (e.g., Sirt1), non-histone targets (e.g., p53), and assess redox-sensitive protein complexes and DNA integrity. To delineate redox-signaling pathways, we identify thiol switches and H_2O_2-modified residues, complemented by cellular/animal models deficient for oxidoreductases of the thioredoxin family.

This work defines LSD1 as a central redox regulator and identifies nuclear oxidative mechanisms with potential clinical relevance. LSD1's dual role as an epigenetic modifier and redox regulator positions it as a promising therapeutic target in both cancer and neurodegeneration, underscoring the need for deeper insights into its intricate mechanism and multifaceted functions.

POSTER 35

Caloric restriction and its interplay with redox signaling in lifespan and healthspan

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Reactive oxygen species (ROS) are key signaling molecules that regulate stress responses and promote survival. We previously showed that a transient ROS increase during C. elegans development modulate the COMPASS complex, reduce its H3K4 methyltransferase activity and increase stress responses and lifespan (Bazopoulou et. al, 2019). We now sought to explore the role of ROS as a master signal in lifespan determination and its interplay with caloric restriction (CR), focusing on how the redox-regulated COMPASS and small RNA pathways mediate transgenerational effects in this context.

Bazopoulou, D., Knoefler, D., Zheng, Y., Ulrich, K., Oleson, B. J., Xie, L., Kim, M., Kaufmann, A., Lee, Y. T., Dou, Y., Chen, Y., Quan, S., & Jakob, U. (2019). Developmental ROS individualizes organismal stress resistance and lifespan. Nature, 576(7786), 301–305. https://doi.org/10.1038/s41586-019-1814-y

POSTER 36

The Host-Microbe Redox interplay and its role in Aging and Neurodegeneration

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Reactive Oxygen Species (ROS) are produced at host barrier sites in response to bacterial contact in every eukaryote host studied. Those highly reactive molecules play a dual role: excessive ROS induce oxidative stress and, therefore, contribute to human diseases, while low levels of ROS act as signalling molecules and regulate biological processes physiologically. Previous studies showed that treatments that elevate endogenous ROS during developmental stages have life-altering effects on C. elegans i.e., in lifespan and neuroprotection (1,2). We now find that dietary bacteria are important for these redox-altering treatments to elicit their effects in the C. elegans host. We also show that the timing of application and redox state of the dietary bacteria are both critical factors. These findings will advance our understanding on host-bacteria interactions and, in particular, on redox-regulated events triggered by dietary bacteria that impact the health and lifespan of the host.

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POSTER 37

TRC40 as a redox-regulated chaperone in cellular defense against oxidative unfolding stress

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Oxidative stress leads to ATP depletion, impairing ATP-dependent chaperones and proteasomal function, thereby promoting protein misfolding and aggregation. We identified TRC40, the mammalian homolog of yeast Get3, as a redoxregulated chaperone that transitions from an ATP-dependent targeting factor to an ATP-independent holdase upon thiol oxidation. Our in vitro data show that oxidized TRC40 forms chaperone-active tetramers and high-molecular-weight complexes that prevent protein aggregation. The switch is fine-tuned by the nucleotide-binding state of TRC40. In cells, TRC40 forms highly dynamic foci in response to ATP-depleting oxidative stress, which co-localize with Hsp70, suggesting a role in managing misfolded proteins. TRC40 depletion results in impaired recovery from hydrogen peroxide-induced oxidative stress and an accumulation of misfolded and unfolded proteins. We found that TRC40-dependent clearance of these proteins relies on autophagy. Consistently, TRC40 levels decrease during stress recovery, an effect blocked by autophagy inhibition. Our findings highlight TRC40 as a critical component of the proteostasis network, bridging oxidative stress response and autophagy-mediated protein clearance to support cellular recovery from proteotoxic conditions.

POSTER 38

Emulating free radical reactions in lipid peroxidation via machine learning

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Lipid peroxidation encompasses a variety of complex chemical reactions caused by the reactive oxygen species (ROS) induced oxidation of unsaturated fatty acids in biomembranes and the resulting radical chain reactions. These processes are known to result in cell damage and cell death by Ferroptosis. However, due to the complex membrane environment and multitude of possible redox reactions, many aspects of the radical progression remain unknown. We recently developed a hybrid kinetic Monte Carlo / Molecular Dynamics (KIMMDY) reaction emulator that incorporates such chemistry into classical molecular simulations in a highly efficient and yet accurate manner [1]. As of now, KIMMDY can simulate the radical chemistry of chemical bond scissions and hydrogen atom transfer reactions within stretched collagen. KIMMDY chooses reactions based on their rates, which are predicted by machine learning models trained on thousands of quantum chemical calculations [2]. Now, we expanded KIMMDY to emulate reactions associated with lipid peroxidation. By training a graph neural network on new datasets comprising structures and energy barriers specifically generated for lipid peroxidation, we aim to predict these reactions and simulate the associated mechanisms using KIMMDY. The same methods could simultaneously be used to assess and predict the effectiveness of antioxidants in various systems and allow for the subsequent generative design of novel molecules.

1: https://doi.org/10.1021/acs.jctc.9b00786

2: https://doi.org/10.26434/chemrxiv-2023-7hntk

POSTER 39

The role of the glutaredoxin system in redox regulation of the stress-sensing chaperone Get3

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Stress-sensing chaperones serve as fast-acting and highly dynamic components of the proteostasis network to prevent protein aggregation upon sudden environmental changes. In yeast, the oxidative activation of the cytosolic protein Get3 as an ATP-independent chaperone protects cells against ATP-depleting stress conditions, such as oxidative stress and glucose deprivation.

When cellular ATP levels drop, Get3 shifts to its nucleotide-free, open conformation, which promotes the reversible oxidation of its CXC motif, causing partial unfolding and the formation of chaperone-active oligomers. Oxidative chaperone activation can be also induced via transient S-glutathionylation. Intriguingly, we identified glutaredoxin-1 (Grx1), which catalyzes protein (de)glutathionylation, as an interaction partner of chaperone-active Get3. Our initial findings suggest that Grx1 catalyzes S-glutathionylation of Get3 and hence, promotes its oxidative activation. We further hypothesize that the glutathione/glutaredoxin system plays a crucial role in the reductive inactivation of the chaperone function, which is essential to release bound client proteins during stress recovery.

POSTER 40

Detection and effects of N-chloramines in thiol oxidation during host pathogen interaction

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In humans, neutrophils represent the first line of defense against bacterial pathogens. Invading bacteria are eliminated by neutrophils through a process called phagocytosis, during which the pathogens are exposed to a cocktail of reactive oxidants. One of the most potent of these is hypochlorous acid (HOCl), which is produced by the enzyme myeloperoxidase (MPO) in the phagolysosome. HOCl primarily oxidizes thiol groups in proteins but can also react with free amines to form protein N-chloramines, taurine-N-chloramine, or monochloramine.

Protein N-chlorination often leads to a loss of protein function; however, some proteins acquire holdase-like chaperone activity through this modification. To better understand the specific roles of different chlorinating species, we compared their reactivity in inducing N-chlorination and thiol oxidation. We found that these compounds have very different and sometimes opposing chlorination and oxidation efficiencies for thiols and lysines.

Despite the clear effect of N-chlorination on protein function, there are currently no strategies to study the extent of N-chloramines in vivo. We have recently shown that protein N-chlorination can be detected in vitro using the DANSyl derivative DANSyl sulfinic acid ($DANSO_2H$). To advance insights into the role of N-chlorination in host-pathogen interactions, we aim to harness this chemistry in combination with isotopic labeling to establish a robust, quantifiable labeling protocol to detect N-chloramines in vitro and in vivo. The labeling degree was quantified through in-gel fluorescence measurements of the DANSyl products and confirmed by mass spectrometry analysis.

POSTER 41

The differential substrate specificity and catalytic mechanisms of glutaredoxins – From structural principles to physiological consequences

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Glutaredoxins catalyze the glutathione-dependent reduction of protein disulfides and reversible glutathionylation of thiols, using either a mono- or a dithiol mechanism with one or two cysteine residues. Contrary to the well-characterized reduction of glutathione disulfides, the mechanisms by which glutaredoxins reduce non-glutathione disulfides, as well as the factors that determine target substrates and the differential GSH-specificity of glutaredoxins, remain to be deciphered. In this work, we used stopped-flow kinetic measurements, roGFP2-based in cellulo assays, and molecular docking simulations to scrutinize the reduction of non-glutathione disulfides and the glutathionylation of different thiols by glutaredoxins. Our comprehensive characterization of the glutaredoxin-catalyzed reduction of non-glutathione disulfides revealed three different reaction mechanisms that are dictated by the substrate structure. Furthermore, we show how glutaredoxins efficiently glutathionylate thiols and disulfides and identify the dithiol mechanism as the determinant factor for the differential GSH-specificity of glutaredoxins compared to other thiols. Our results elucidate fundamental principles of glutaredoxin catalysis that explain how enzyme-substrate interactions and intrinsic structural features of each enzyme species balance efficient turnover with substrate specificity and inhibition. Together, these results unveil mechanisms, that may enable the reduction of GSSG independent of glutathione reductase, a crosstalk between thioredoxins and glutaredoxins, and the equilibration of thiol redox couples beyond GSH/GSSG, with potential implications for glutaredoxin-based redox sensing.

POSTER 42

Redox Regulation of Unconventional FGF2 Secretion

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Fibroblast growth factor (FGF2) is a secretory protein that plays a critical role in cell proliferation, inflammation as well as angiogenesis and is closely linked to tumor development, progression and chemotherapy resistance.

The secretion of FGF2 happens in a highly unusual manner: FGF2 binds to the phosphoinositide PI(4,5) P2 on the intracellular side of the plasma membrane (PM), triggering its oligomerization, which finally leads to the formation of a toroidal pore, through which FGF2 directly translocates to the extracellular space. Recently, it was revealed that the prerequisite for FGF2 oligomerization and therefore secretion is the oxidation of a specific surface-exposed cysteine residue (C95), resulting in the formation of disulfide-bridged dimers.

However, the mechanism of FGF2 oxidation and the factors involved in its redox regulation remain unknown. This project therefore investigates the mode of FGF2 oxidation, and aims to identify the electron acceptor, local redox environment and reducing enzymes that potentially counteract oxidation. This investigation will be taken as a starting point to determine potential targets for the inhibition of FGF2 secretion in oncogenic contexts. Further, we aim to identify the essential features of FGF2 that enable its oxidation, which could help us to identify the underlying basic mechanism of membrane-supported oxidation.

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POSTER 43

A single cysteine mutation in Drosophila FOXO extends lifespan

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Ageing is a major risk factor for many diseases, including metabolic disorders like diabetes. Human longevity and healthy ageing are influenced by both environmental and genetic factors. The insulin/insulin-like growth factor signalling (IIS) pathway, a key regulator of metabolism, is strongly implicated in survival. Downregulation of IIS leads to lifespan extension, with evolutionary conservation across model systems. Forkhead box class O (FOXO) transcription factors are major downstream effectors of the IIS cascade, responding to the nutritional status of an organism, and are negatively regulated by AKT. FOXOs are involved in important cellular processes, including cell cycle regulation, apoptosis, growth and resistance to ROS. Studies in animal models and human cohorts have also shown that FOXO transcription factors are important regulators of lifespan. Drosophila has a single isoform, dFOXO, containing 9 cysteine residues of which 2 are highly conserved across species, including humans. To investigate the redox regulation of dFOXO in Drosophila, we generated a comprehensive toolkit of Drosophila knock-in mutants, where each Cys residue is mutated individually, to interrogate their contribution to redox signalling in vivo. We performed detailed phenotypic, molecular and transcriptomic profiling, and show that mutating a single cysteine in dFOXO was sufficient to extend Drosophila lifespan. These long-lived mutant flies were resistant to several modes of redox stress, but sensitive to starvation. Furthermore, RNASeq analysis revealed a unique transcriptomic signature in the long-lived mutant. Overall, this study highlights the importance of redox signalling, at the level of a single cysteine residue, in regulating metabolism and lifespan.

POSTER 44

Thioredoxin-like protein-1 (TXNL1) is associated with p62 and Nrf2 pathways upon oxidative stress

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Thioredoxin-like protein-1 (TXNL1; also known as thioredoxin-related protein of 32 kDa, TRP32) is ubiquitously expressed in eukaryotes but has yet essentially unknown roles in relation to cellular redox homeostasis. Recently, we have demonstrated that TXNL1 has dual functions as a thioredoxin-like reductase and also an ATP- and redox-independent chaperone. Here, we found that treatment with auranofin (AF), an FDA-approved thioredoxin reductase inhibitor and the strong activator of transcription factor Nrf2, very rapidly (within hours) downregulates TXNL1 in a time- and dose-dependent manner, while AF had no such effects on thioredoxin 1 (Trx1, TXN1) protein levels. Pre-treatment of A549 cells with proteasome inhibitors (Bortezomib/MG132) reversed the effect of AF on TXNL1 levels, but a ubiquitin activating enzyme inhibitor (TAK-243) did not, suggesting that TXNL1 is degraded via ubiquitin-independent proteasomal manner. Interestingly, CRISPR-Cas9 knockout of TXNL1 in 293T cells resulted in a mild accumulation of poly-ubiquinated proteins and significant decrease of p62 levels and its monomer compared to WT-cells under non-reducing condition, indicating increased p62 aggregation and/or sequestration in the absence of TXNL1. Moreover, TXNL1-KO cells showed a higher basal level of Nrf2 activation, but it was stabilized upon AF treatment compared to WT-cells. Taken together, these results suggest that TXNL1 is involved in regulation of p62 and is a major target in AF-triggered proteasomal degradation, possibly providing a functional link between Nrf2 and the ubiquitin–proteasome system in responses to oxidative stress.

POSTER 45

2-Mercapto Malonates Generate Hydropersulfides with No Trace Left Behind

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3-Mercaptopyruvate (3-MP) is a byproduct of cysteine metabolism, and is a precursor to hydropersulfides (RS-SH), as a substrate for 3-mercaptopyruvate sulfurtransferase (3-MST). 3-MP through the cleavage of a C-S bond produces the stable enolate of pyruvate as the product and a persulfide of 3-MST. The resulting 3-MST persulfide can transfer sulfur to glutathione (GSH) or a cysteine residue of protein, which acts as a 'redox switch' (S-sulfhydration).[1] Hydropersulfides can directly, through inhibition of lipid peroxidation, and indirectly through mitigation of inflammation, promote antioxidant response and protect cells from oxidative stress.[2] Malonate is an endogenous component of fatty acid metabolism, and similar to pyruvate can produce a stable enolate. Taking this cue, we designed 2-mercaptomalonate (2-MM) as a candidate for hydropersulfide (RS-SH) generation. We synthesized a library of 2-MM diesters, and we find that these compounds, under ambient physiological pH, directly transfer a sulfhydryl group (SH) to GSH to form glutathione persulfide (GS-SH), along with malonate in a nearly quantitative yield. These esters are known to be hydrolysed to malonic acid.[3] We also demonstrate that these compounds are substrates for 3-MST, providing evolutionary insights into sulfur transfer mechanisms. Taken together, 2-MM diesters are excellent candidates for GS-SH generation along with metabolically integrated byproducts, leaving no trace behind during hydropersulfide generation.

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POSTER 46

Heterooligomerization drives structural plasticity of eukaryotic peroxiredoxins

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Peroxiredoxins (Prdxs) are highly conserved thiol peroxidases essential for peroxide etoxification, redox signaling, and chaperone activity. Prdx1/AhpC-type are found throughout the eukaryotic kingdom in a dimer-decamer equilibrium, where multiple isoforms frequently

coexist within the same cell and even in the same subcellular compartment. Until now, Prdxs are believed to form exclusively homooligomeric structures. However, in the last years, it has been reported that the genetically encoded roGFP2-Tsa2ΔCR probe forms enzymatically active

heterooligomeric complexes with endogenous Tsa1 in yeast. More recently, human Prdx1 and

Prdx2 were also shown to form heterooligomers in vitro. However, further biophysicalcharacterization of this new assemblies is lacking in order to unravel the mechanisms behind this phenomenon. Here, we show that cytosolic yeast Prdxs (Tsa1 and Tsa2) can assemble into heterooligomers with a wide range of different subunit stoichiometries, even forming disulfidelinked Tsa1-Tsa2 dimers. Furthermore, we demonstrate that the incorporation of Tsa2 subunits stabilizes the decameric state of the heterooligomers. This study raises a new question: Is Prdx heterooligomerization merely a rare artifact seen under specialized experimental conditions, or does it reflect a broader, biologically important feature of eukaryotic Prdx1/AhpC-type Prdxs?

POSTER 47

Conservation and mechanism of the methionine reductase C family

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Oxidation of methionine to methionine sulfoxide (MSO) is reversed by enzymes known as methionine sulfoxide reductases (Msr), a family of six evolutionarily-unrelated proteins that converge on this activity. Thiol-dependent MsrA, B and C rely on a nucleophilic attack of a catalytic cysteine on the sulfoxide, forming a sulfenic acid that's resolved by thiol:disulfide exchanges. However, MsrC mechanism is still puzzling, partly because there're at least two families with different conserved residues. Aiming to solve this, we combine in vitro and in vivo approaches. We first explore the conservation and sequence features of the msrC gene family, a subset of GAF-containing proteins conserved in most prokaryotes, and few eukaryotes. We then study the recombinant protein and mutants biophysically, and characterize the reaction with MSO by NMR, fluorescence spectroscopy and kinetics. Using an in-vivo system that links Msr activity to bacterial survival, we study the mechanism of E. coli MsrC and representatives from several other species. Surprisingly, MsrC is active even if only the catalytic cysteine remains, suggesting a monothiol mechanism. Hence, we hypothesize that MsrC may rely on GSH instead of Trx, as it's the normal case for MsrA and B. To test that, we performed in vitro and in vivo experiments, using genetically engineered strains devodied of trxB or gor, in combination with MS and kinetics. Combining experimental evidence and biomolecular simulations we propose that MsrC work with Trx and GSH systems, something previously unknown, and rely on a single cysteine.

POSTER 48

Disulfide bridge-dependent dimerization triggers FGF2 membrane translocation into the extracellular space

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Fibroblast Growth Factor 2 (FGF2) is an unconventionally secreted protein transported into the extracellular space by direct translocation across the plasma membrane. This process is initiated by FGF2 recruitment at the inner plasma membrane leaflet mediated by the alpha1 subunit of the Na,K-ATPase. Subsequently, FGF2 forms oligomers in a PI(4,5)P2-dependent manner that trigger the opening of a lipidic membrane pore through which FGF2 translocates across the membrane. At the outer leaflet, FGF2 is captured by the heparan sulfate chains of Glypican-1 (GPC1), resulting in FGF2 translocation into the extracellular space. The building blocks for the formation of pore-forming FGF2 oligomers are C95-C95 disulfide-linked homodimers that are formed at the inner leaflet of the plasma membrane. Variant forms of FGF2 lacking C95 do not oligomerize, do not form membrane pores and, therefore, are not secreted from cells. Based on atomistic molecular dynamics simulations, cross-linking mass spectrometry and cryo-EM analyses, the molecular interface in FGF2 was identified that brings C95 residues into close proximity, promoting oxidative dimerization. Recent insights on the molecular mechanism of membrane-dependent, oxidative FGF2 dimerization will be presented.

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POSTER 49

Predicting protein structures with redox-dependent cysteine modifications

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Cysteine residues are central to redox regulation, undergoing diverse oxidative and sulfur-based modifications that can profoundly alter protein structure and function. However, current structure-prediction pipelines seldom account for these states, leaving an important gap in our understanding of redox-driven conformational dynamics.

In this work, I demonstrate how AlphaFold3 can be adapted to model protein structures that explicitly incorporate redox-relevant cysteine chemistries. By introducing custom inputs that approximate realistic redox conditions, we obtain structural predictions that reveal how such modifications remodel local microenvironments, rewire interaction networks, and influence global protein folds.

As a case study, I will present predictions for the H_2O_2 biosensor **HyPer7**, highlighting how alternative cysteine oxidation states—including sulfenic acid, persulfidation, glutathionylation, and disulfide formation—shape its conformational landscape.

These findings establish AlphaFold3 as a versatile framework for predicting redox-modulated conformations, providing guidance for experimental studies and new opportunities to explore protein regulation through predictive structural modeling.

Acknowledgements

I am grateful to Daria Ezeriņa and Tobias Dick for insightful discussions and ideas on potential disulfide-bond formation in HyPer7. I also thank Dirk Reiter for maintaining the GPU servers, updating the software, and managing our data infrastructure.

POSTER 50

Discovery of an epigenetically controlled Lipid Oxygen Radical Defense pathway

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Membrane phospholipids are highly susceptible to oxidative radicals, and uncontrolled lipid peroxidation can compromise cell viability. While studying the transcriptional regulation of ZDHHC20, an S-acyltransferase that conjugates fatty acids to viral proteins, and GPX4, the major protective enzyme against lipid peroxidation, we discovered the Lipid Oxygen Radical Defense (LORD) pathway. This epigenetically controlled stress response protects cells from the detrimental effects of lipid peroxide accumulation.

Under normal conditions, ZNF354A acts as a master transcriptional regulator, repressing antioxidant defense genes via a complex that includes the epigenetic regulator KAP1, histone methyltransferase SETDB1, and the activator ATF2. Upon lipid peroxide accumulation, p38- and JNK-dependent phosphorylation of these complex members triggers the dissociation of ZNF354A, releasing it from specific DNA loci and enabling the expression of genes involved in antioxidant defenses and innate immune regulation.

Our analysis of ZDHHC20 transcription control reveals that this circuit involves the cooperation of ATF2 with several transcription factors, including FOXA1, SP1, and the global redox regulator NRF2. Our study highlights the LORD pathway as a critical regulatory axis for understanding lipid peroxidation-related disorders, providing new insights into cellular defense mechanisms against lipid damage.

POSTER 51

S-Acylation Dysregulation in Muscular Dystrophy

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Duchenne muscular dystrophy (DMD), the most common childhood myopathy (1: 3500 male births) causes early, progressive muscle wasting that ends in loss of ambulation and premature death, and still has no cure. Protein lipidation—especially S-acylation—is emerging as a disease driver, but its role in skeletal muscle and DMD remains undefined. Protein S-acylation involves the covalent attachment of medium/long-chain fatty acid (most frequently palmitate) to specific Cys residues, thanks to the activity of 23 ZDHHC acyl-transferases, whereas several acyl protein thioesterases mediate the removal. We found that expression of ZDHHC enzymes is markedly dysregulated in both mdx mouse skeletal muscle—a preclinical model of DMD—and in muscle biopsies from DMD patients.

We then optimized and validated a quantitative palmitoyl-proteomics pipeline—integrating Acyl-RAC enrichment with high-resolution LC-MS/MS—to comprehensively profile the skeletal-muscle palmitome, uncovering numerous disease-relevant proteins whose S-acylation is differentially regulated in dystrophic muscle. Altogether, our findings establish S-acylation as a pivotal mechanistic axis in muscular dystrophy pathogenesis and provide a framework for discovering new druggable targets.

POSTER 52

Monitoring Golgi redox potential in mammalian cells under hypoxia: a comparison between non-cancerous and cancerous cells

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N-glycosylation defines pharmacokinetics and pharmacodynamic properties in therapeutic glycoproteins, and its deregulation helps cancer progression. A recent study shows the importance of Golgi's redox environment in the activity of alpha-2,6-sialyltransferase (ST6Gal-I), a key enzyme in the N-glycosylation. Hypoxia affects protein N-glycosylation, although the mechanisms are unclear. We hypothesize hypoxia affects the Golgi's redox potential, as has been observed for other organelles, leading to changes in N-glycosylation. Hypoxia can arise both in large scale cell cultures and in tumours. We studied the Golgi's redox potential and its response to hypoxia in CHO and HeLa cells, which are used in the production of therapeutic glycoproteins and in medical research, respectively. We used the redox sensor roGFP1-iL fused to the transmembrane domain of the α-2,3-sialyltransferase (ST3Gal IV) for Golgi localization. CHO and HeLa cells expressing Golgi-roGFP1-iL were kept in normal conditions or in hypoxia for 48 hours. Under hypoxia, we found the Golgi's redox potential became more reducing in CHO cells, while it became less reducing in HeLa cells. Also, only in CHO cells, the viability decreased to ≈ 84%. Transcriptomic analysis showed a profound response of CHO cells under stress produced by hypoxia, totally different to HeLa cells. Genes related to TFE3 pathway were overexpressed only in CHO cells, suggesting a stress in Golgi. This discovery advances our comprehension about how Golgi works, and it can be useful to improve therapeutic protein quality as well as in search of diagnostic and therapeutic tools for cancer.

POSTER 53

PYROXD1 acts as a chaperone of nuclear-cytosolic NFS1 to sustain tRNA thiolation and MoCo biosynthesis

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Sulphur is an essential element required for enzyme catalysis, redox regulation, and iron-sulphur cluster formation. Mobilisation of sulphur from cysteine by NFS1 is a key step in generating multiple sulphur-containing biomolecules. Despite its well-characterized mitochondrial role, one aspect of NFS1 biology remains disputed: potential roles of NFS1 outside of mitochondria.

We reveal a new factor in the biology of NFS1: PYROXD1, a flavoprotein that our lab characterized as the guardian of the tRNA ligase complex against oxidative inactivation. Here, we show that PYROXD1 interacts with NFS1 at pH 7.4, typical for nuclear-cytosolic compartments. This interaction prevents aggregation of NFS1, which does not occur at more alkaline pH, typical of mitochondria. We then show that traditional cell fractionation methods have underestimated the levels of nuclear-cytosolic NFS1 due to aggregation-stimulating buffer conditions. Finally, depletion of PYROXD1 from cells causes co-depletion of nuclear-cytosolic NFS1, establishing PYROXD1 as a chaperone of nuclear-cytosolic NFS1.

Before tackling whether stabilisation of NFS1 by PYROXD1 has an effect in cells, we are first addressing functions of the extramitochondrial NFS1 isoform by generating and characterizing the first cell lines with replacements in the start codon initiating the translation of this isoform. However, our observation that tRNA thiolation and molybdenum cofactor biosynthesis are specifically affected by depletion of PYROXD1 implies that extramitochondrial NFS1 plays a primary role in these processes.

We propose that PYROXD1 is a necessary factor for the stability and functionality of the extramitochondrial NFS1 isoform, thus playing a constitutive role in cellular sulphur distribution.

POSTER 54

Development of proteome analysis for bio-chalcogenide-modified proteins

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[Background]

Supersulfides are sulfur-catenated molecular species that are involved in various physiological events such as mitochondrial energy metabolism and redox signaling, etc. Our recent study indicates that not only supersulfides but also other chalcogenides like selenium are present not only as low-molecular-weight metabolites but also in protein modifications. These bio-chalcogenides therefore may play an important role in regulating the protein function. In this context, the proteomics for bio-chalcogenide protein modification is thought to be essential for thoroughly understanding the redox signaling and metabolism in various organisms that we are pursuing.

[Methods and Results]

Recently, we developed a mass spectrometry-based proteome analysis for the proteins modified by bio-chalcogenides. In this method, the level of chalcogenides modification at each cysteine residue is examined in a quantitative manner with the protein treated via alkylation by b-(4-hydroxyphenyl) ethyl iodoacetamide (HPE-IAM) followed by trypsin digestion. We could thereby determine the percentage of various chalcogenides modifications (e.g., CysS-SH, CysS-SeH) on cysteine residues of various proteins. Also, herein, a new biotin switch assay with use of an HPE-IAM probe recently developed was established successfully.

[Conclusion]

In this study, we revealed that bio-chalcogenides are bound in diverse forms of modification on cysteine residues of proteins. Further analysis in combination with this method with the HPE-IAM-based chalcogenides metabolome will further elucidate the exact physiological significance of these brand-new protein modifications.

POSTER 55

PRDX6 depletion disrupts lipid homeostasis and trafficking increasing ferroptosis sensitivity in hepatocarcinoma cells and Caenorhabditis elegans models

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Peroxiredoxin 6 (PRDX6) is a multifunctional enzyme provided with peroxidase, phospholipase A2 and lysophosphatidilcoline acyltransferase activities, being involved, among other processes, in phospholipid peroxide repair and metabolism. In this work we report a comparative study between SNU475 cell lines with or without PRDX6 at the level of global lipid composition and lipid-related cellular processes.

Human hepatocarcinoma SNU475 cells lacking PRDX6 have shown modified lipid composition and lipid-related cellular processes presenting a general decrease in all kinds of lipids. Among these modifications, the formation of lipid droplets accumulating various polyunsaturated fatty acids (PUFA) and PUFA-containing triacylglycerols has been described,

indicating an altered fatty acid flux in absence of PRDX6. An increment in arachidonic acid containing phosphatidylcholines was also observed, suggesting a preference of the PLA2 activity of this enzyme for these AA-storing glycerophospholipids.

SNU475-KO cells also showed increased total lipid peroxides and enhanced sensitivity to ferroptosis, leading to changes in morphology and survival of SNU475-KO cells, which could be explained by an alteration in plasmalogen homeostasis. Both effects were also observed in a PRDX6 lacking Caenorhabditis elegans model under ferroptosis induction by diethyl maleate, supporting PRDX6's role in these processes.

These results show that all three enzymatic activities of PRDX6 contribute to the role of this enzyme in a variety of cellular processes, from membrane phospholipid remodelling and functional diversity of glycerophospholipids to the fate of lipid peroxides and modulation of AA levels. These contributions explain the complexity of the changes that loss of PRDX6 exerts on cellular functionality.

POSTER 56

Therapeutic potential of hydrogen sulfide: preventing diabetic liver damage via ferroptosis inhibition

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We have recently shown that ferroptosis contributes to diabetes-related liver damage and suggested that it could be used as a new target for the treatment of this diabetic complication. While recent studies suggest that H_2S and its related sulfur-reactive metabolites (RSS) protect cells from ferroptosis, their role in preventing diabetes-related liver injury is unknown. Here, we investigated whether targeting ferroptosis with donors of H_2S (GYY4137), polysulfides (Na2S4) and persulfides (cysteine-S3, Cys-S3) could have antidiabetic effects and alleviate diabetic liver damage.

The results showed that H_2S/RSS donors, especially GYY417 and Cys-S3, attenuated ferroptotic phenotype in the liver of diabetic rats, seen through: increased accumulation of pro-oxidative parameters (liable iron, lipofuscin and lipid peroxides); inactivation of Nrf2 and GSH-related antioxidative defense parameters (GPX4, GCLC, GSS); decreased expression of iron sequestering proteins (ferritin and ferroportin); increased expression of key factor for ferroptosis. Observed mitigation of ferroptotic events in diabetic liver by H2S/RSS donors culminated in strongly improved functional state of hepatocytes, as evidenced by improved biochemical parameters related to liver injury (ALT and AST), reduced hepatocyte size, binucleation and liver fibrosis, and were similar to the effects of the ferroptosis inhibitor liproxtatin-1. In addition, H_2S/RSS donors restored diabetes-altered expression of the predominant enzyme for H_2S synthesis in the liver, CSE, indicating involvement of H_2S biosynthetic pathways in the observed antiferroptotic/antidiabetic effects of the H_2S/RSS donors.

The present study clearly demonstrates the strong antiferroptotic potential of H_2S/RSS donors in diabetic liver complications and suggest a new approach for the prevention/treatment of this pathology.

POSTER 57

Mitochondrial ROS promotes cellular differentiation of the unicellular parasite Trypanosoma brucei

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Cellular differentiation requires elaborate intracellular communication. Before committing to a new cell fate, it is imperative for the energy producing mitochondria to communicate their bioenergetic status to the rest of the cell. One mechanism for delivering this message is the redox signaling pathway, which employs ROS as second messengers to modify redox sensitive targets.

We utilize the extracellular parasite Trypanosoma brucei to investigate the role of physiological levels of ROS during cellular differentiation. The unicellular T. brucei is an elegant model for these purposes because it harbors a single mitochondrion that undergoes extensive metabolic rewiring and ultrastructure remodeling during the parasite's complex life cycle between a mammalian host and its insect vector, the tsetse fly. As it traverses through the digestive tract of the tsetse fly, it undergoes four distinct life cycle stages. Previously, we have demonstrated that the heterologous expression of catalase interferes with this programmed differentiation, indicating an integral role of ROS.

Here we present evidence that the over expression of an endogenous mitochondrial super oxide dismutase or the heterologous expression of a mitochondrial targeted catalase further impedes parasite differentiation, decreases ROS and alters mitochondrial physiology. However, when we inadvertently increase both mitochondrial and cellular ROS, we observe a more thorough and accelerated rate of parasite differentiation. We are currently employing Hyper7 localized in various submitochondrial compartments to decipher when and where H2O2 might contribute to redox signaling during differentiation. Finally, we will also implement redox-sensitive GFP-based probes to elucidate the general redox state of the parasite.

POSTER 58

Genetically-encoded photosensitizers as a tool to elucidate the role and regulation of subcellular site-specific lipid peroxidation in ferroptosis

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Ferroptosis is a recently described form of non-apoptotic cell death with potentially relevant for neurodegenerative diseases, ischemia-reperfusion injury and cancer therapy. It differentiates itself from other forms of immunogenic cell death, in that it is driven by the accumulation of lipid hydroperoxides. Lipid peroxidation (LPO), once out of control, permeabilized the plasma membrane, triggering osmotic cell death. To prevent ferroptosis, the cell employs various anti-ferroptotic enzymes and pathways including GPX4, FSP1 or the recently described low molecular weight hydropersulfides. However, how these cellular pathways are regulated and employed remains elusive as most ferroptosis induction strategies rely on small molecules, which act with limited subcellular specificity. Singlet-oxygen producing proteins (SOPP) catalyze a type II photosensitizing reaction that produces singlet oxygen with spatiotemporal control. By doing so in proximity to a site-specific membrane, the lipid-peroxide radical chain reaction can be initiated, resulting in ferroptotic stress or even programmed cell death. Our aim is to use this novel tool, to study the wave-like propagation of lipid peroxidation between organelles. Furthermore, we are interested in the role of individual anti-ferroptotic pathways in protecting particular membranes.

POSTER 59

Biochemical analysis of a Prx5-group peroxiredoxin-glutaredoxin hybrid protein expressed by Haemophilus parainfluenzae species within the oral microbiome

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The Prx5 subgroup of peroxiredoxins (Prxs) is the most diverse among six defined subgroups regarding reductive recycling mechanisms and the location of the resolving cysteine, if any. A subset of these, found in Haemophilus influenzae and other bacterial pathogens (including Yersinia pestis, Neisseria meningitidis and Vibrio cholerae), are fused at their C-terminus with a glutaredoxin (Grx) domain homologous to E. coli Grx3 (designated as PdgX). Together, these domains catalyze the glutathione-dependent reduction of peroxides. H. influenzae and other related species, including H. parainfluenzae, form biofilms in vivo containing PdgX. Bioinformatics analysis of oral microbiome species supports a protective role for H. parainfluenzae in humans to combat oxidative stress associated with chemoradiation therapy-induced oral mucositis, a harmful side effect of head and neck cancer treatments. To analyze the biochemical properties of HpPdgX, we have expressed and purified the intact PdgX protein and isolated Prx and Grx domains from E. coli. This has enabled us to assess peroxide specificity of the multi-domain enzyme and biochemical properties of the individual domains. Glutathione-dependent peroxidase activity was also reconstituted using individual Prx and Grx domains. A model accounting for activities of intact HpPdgX protein and individual domains has been developed. Further work on variants of these proteins will be informed by the analysis of specific PdgX sequences from H. parainfluenzae isolates and their correlation with severity of oral mucositis after treatment to evaluate protective activity of variants, and their contribution to oral cavity biofilm formation.

POSTER 60

Identification of S-glutathionylated cysteines by bioorthogonal labeling

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Oxidative cysteine modifications, known to alter protein function, may act as redox-dependent switches involved in regulating signaling pathways. One of such modifications is glutathionylation, which plays an important role in preventing proteins from further oxidation and is implicated in various signaling processes in health and disease. Therefore, it is interesting, to identify and characterize the dynamics of protein S-glutathionylation in a global manner. For this, several methods have been proposed including direct detection and enzymatic based switch-enrichments techniques. We adopted a method based on expressing a glutathione synthetase mutant (GS M4) in LN18 glioblastoma cells as well as in the endometrial epithelial cell line12Z, which incorporates azido-functionalized alanine (3ALA) instead of glycine into newly synthetized glutathione like molecules. This enables the detection and enrichment of proteins respectively cysteines modified by the functionalized glutathione derivative. Here, we show a proof of principle, that redox-dependent S-Glutathionylated changes can be monitored with the used system and first biological implications.

POSTER 61

Mechano-regulation of redox balance at the plasma membrane

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Cells maintain a tight balance of oxidants and reductants to sustain redox eustress, and have harnessed oxidants like hydrogen peroxide (H2O2) in processes such as cell migration, and in the immune system. One source of oxidation at the plasma membrane occurs via NOX/DUOX enzyme complexes, to generate H2O2, which can re-enter cells via aquaporins, and acts as a second messenger. However, the regulation of redox balance here remains poorly defined. The plasma membrane itself is dynamic and subject to various mechanical stresses such as shear stress and substrate stiffness. Mechano-regulation is often impaired in cancer and ageing, and redox imbalance is similarly implicated in these conditions. Despite many of the redox machinery being linked to mechano-sensitive processes, it remains poorly understood whether redox balance is affected by broader mechano-regulation. We hypothesise that the redox balance at the plasma membrane is subject to mechano-regulation. HyPer7 is a GFP-based H2O2 biosensor, which undergoes a conformational change upon oxidation, resulting in a spectral shift, detectable via live cell imaging. We have optimised a HyPer7 biosensor localised to the plasma membrane (PM-HyPer7) to address how mechano-regulation affects redox balance at the plasma membrane. Our PM-HyPer7 biosensor was able to detect exogenous H2O2 (in nM range) in U2OS osteosarcoma cells, and the PM-HyPer7 can detect induced oxidation when stimulated with growth factors like TGF-β. We have also developed a Drosophila melanogaster and knock-in mouse model for PM-HyPer7, to investigate redox dynamics in a range of cell types such as mouse embryonic fibroblasts and ovarian cells.

POSTER 62

Glucose-Responsive "Smart" Insulin system

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Insulin is a hormone that regulates blood glucose levels in mammals. The insulin polypeptide consists of 51 amino acid residues, divided into two chains: the B chain (30 residues) and the A chain (21 residues). The native structure of insulin contains three α-helices, stabilized by two intermolecular disulfide bridges (A7-B7 and A20-B19) and one intrachain bridge (A6-A11). The formation of these disulfide bonds is crucial for maintaining the structure, stability, and receptor binding activity of insulin.

Recent advancements in research have led to the development of "smart" insulin, also known as glucose-responsive insulin (GRI). This innovative insulin variant uses modified insulin analogues, where its activity and bioavailability in the human body are directly dependent on blood glucose levels, eliminating the need for external monitoring.

The activity of insulin is derived from its binding to the insulin receptor (IR) through several key residues, including the C-terminal of the B chain. Based on this, we propose the synthesis of a modified insulin system, termed "GRI," which blocks the C-terminal of the insulin B chain. This modification is expected to impact the binding of the modified insulin to the IR, and subsequently deactivate it. Our suggested modification is position B26 in insulin, where a cleavable glucose linker, 3-Fluoro-Phenylboronic acid (3F-PBA), is conjugated to Dopa at this position (normally Tyrosine in insulin). Under low glucose conditions, this modification renders the insulin analogue inactive. However, in the presence of high glucose concentration, glucose binds to the cleavable linker, triggering the release of active insulin to respond to the elevated glucose levels.

However, the challenges associated with the chemical synthesis of insulin, particularly the difficulties in the combination reaction between the isolated A and B chains (which results in low yields), make insulin and its analogues labor-intensive targets for total chemical synthesis using Fmoc-SPPS. Additionally, incorporating modifications such as Dopa at position B26 presents significant challenges, as Dopa is highly sensitive to various external factors, leading to issues with aggregation, polymerization, and oxidation when unprotected. These challenges make insulin semi-synthesis, using trypsin as a catalyst in enzymatic reactions followed by cleavage and coupling, a more effective alternative for preparing insulin and its modified analogues.

POSTER 63

Thiol reductive stress activates the hypoxia response pathway

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Thiol-mediated redox regulation is critical for cellular homeostasis, yet the physiological consequences of thiol reductive stress are not completely understood. This study reveals a novel interplay between thiol reductive stress and the hypoxia response pathway, challenging the paradigm that thiol antioxidants like dithiothreitol (DTT) act solely on the endoplasmic reticulum (ER). Using Caenorhabditis elegans, we demonstrate that DTT-induced reductive stress activates hypoxia-inducible factor 1 (HIF-1), and hypoxia-response genes. Activation of HIF-1 is required for upregulation of S-adenosylmethionine (SAM) dependent methyltransferase, rips-1 which converts SAM into S-adenosylhomocysteine and causes toxicity. Genetic screens identified cysl-1 as essential for this activation, linking thiol reductive stress to hydrogen sulfide production, a known HIF-1 modulator. The hypoxia pathway exhibits dual roles: while enhancing rips-1 dependent toxicity at moderate DTT levels, it confers protection against severe thiol stress through rips-1-independent mechanisms[1]. Strikingly, activation of hypoxia response pathway is conserved in human cells, as DTT induces expression of hypoxia response pathway genes and cellular milieus. Our findings redefine thiol antioxidants as multifaceted stressors, linking redox imbalance to hypoxia signaling, with implications for pathologies linked to reductive stress. This work emphasizes the complexity of thiol redox switches in cellular adaptation and highlights their potential as therapeutic targets in redox-associated diseases.

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POSTER 64

Glutaredoxin-catalyzed reduction of GSNO and glutathione polysulfides

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Class I glutaredoxins (Grxs) are small oxidoreductases that catalyze the reversible reduction of protein disulfides or low-molecular-weight disulfides using glutathione (GSH) as an electron donor. While the Grx-catalyzed reduction of several protein or low-molecular-weight glutathione disulfides is well established, the reactivity of Grx with emerging non-standard substrate candidates such as glutathione trisulfide (GSSSG), glutathione hydropersulfide (GSSH) or S-nitrosoglutathione (GSNO) remains to be characterized. Here, we investigated the kinetics and mechanism of the Grx-catalyzed reduction of these substrate candidates. Our results demonstrate that GSSSG is a particularly efficient Grx substrate, undergoing rapid reduction with a rate constant ≥ 10^7 M^−1 s^−1, GSSH is reduced with a rate-constant ≥ 10^6 M^−1 s^−1, whereas GSNO reduction is much slower. The potential release of H_2S or HNO during these reactions is currently under investigation. In summary, our data demonstrate an efficient turnover of GSSSG, GSSH and GSNO as non-standard glutathione substrates suggesting novel physiological roles of Grx for polysulfide metabolism and NO redox signaling.

POSTER 65

Spatial localization and scaffold proteins enable sensitive and site-specific H2O2 signaling through peroxiredoxin-mediated redox relays

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In the cytosol of human cells, 2-Cys peroxiredoxins (Prdx1/2) confine H2O2 to microdomains, and upon oxidation to sulfenic or disulfide forms they can also relay oxidizing equivalents to other thiol proteins. We explored the factors determining the sensitivity and specificity of H2O2 signaling in this context using reaction-diffusion models of the cytosolic Prdx1/2-Trx1 system and associated redox relays. The results show: (i) Disulfide and sulfenic forms of both peroxiredoxins, and Trx1 disulfide are spatially localized to the neighborhood of H2O2 sources; and these Prdx1 species are more localized than Prdx2's. (ii) Scaffold proteins (ScP) can improve redox relays' H2O2 detection limits by recruiting both Prdx and regulation targets to H2O2 sources. (iii) The system's input dynamic range is primarily controlled by ScP-target binding affinity, potentially enabling efficient target oxidation at low overall Prdx oxidation. (iv) Site-specific ScP allow H2O2 released to the cytosol at distinct sites to independently regulate distinct processes.

Altogether, this work provides mechanistic insights into H2O2 signaling specificity, highlighting the crucial role of spatial organization and scaffold proteins in cellular redox regulation.

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POSTER 66

Structural and dynamical characterization of the FF-LU conformational transition in peroxiredoxins

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Peroxiredoxins (Prx) are a family of antioxidant enzymes that detoxify organic hydroperoxides found across all domains of life which are classified based on the number and position of cysteines in their structure. A well-studied example is the Escherichia coli thiol peroxidase (EcTpx), which contains a peroxidatic cysteine (CP) and a resolving cysteine (CR). During catalysis, CP forms a sulfenic acid intermediate that is resolved by CR, generating an intramolecular disulfide bond. This disulfide is then reduced by the thioredoxin system, allowing the enzyme to enter a new catalytic cycle. In its reduced state, CP resides at the end of an alpha helix configuring what is known as the fully folded (FF) conformation. Upon disulfide formation, this helix partially unfolds, adopting a locally unfolded (LU) conformation. This structural shift is part of the catalytic cycle, but it remains unclear whether the reduced enzyme may also displays a conformational equilibrium between these two states, and more importantly, how the redox status affects the transition.

Here we employed a combination of several physics-based advanced sampling computer simulation techniques such as weighted ensemble (WE) and structure-based model molecular dynamics (SBM-MD), together with AI-based structure ensemble prediction approaches, to fully characterize the structural, dynamic, and thermodynamic properties of this intriguing redox-associated conformational change.

POSTER 66

Mechanism of glutathionylation of the active site thiols of peroxiredoxin 2

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Peroxiredoxin 2 (Prdx2) is rapidly glutathionylated, with glutaredoxin-catalyzed deglutathionylation providing an alternative reduction pathway to the thioredoxin/thioredoxin reductase system (Peskin et al., JBC 216,3053,2016). New kinetic analyses using stopped-flow, SDS-PAGE, and mass spectrometry-based product characterization clarified the glutathionylation mechanism. Kinetic modeling indicates that Prdx2 disulfide (Prdx2-SS) reacts with millimolar glutathione (GSH) in seconds to minutes, initially at one active site, generating glutathionylated one-disulfide-linked-dimers. Further exchange with GSH leads to glutathionylation at the peroxidatic (CP, predominantly) and resolving (CR) cysteines. Thiol-disulfide-exchange-mediated glutathionylation and deglutathionylation proceed with k=1.5 $M^{-1}s^{-1}$ and k=0.021 s^{-1}, respectively. Similar reactions occur at the second active site. GSH reacts with wild-type Prdx2 CP sulfenic acid (CP-SOH) (k=10 $M^{-1}s^{-1}$) 8–30-fold slower than for CR->Ser/Trp/Asp mutants, too slow to outcompete intramolecular condensation. Consequently, reaction of reduced Prdx2 with H_2O_2 in presence of GSH yields Prdx-SS as the predominant primary product, glutathionylation then following through exchange. However, glutathionylation at CR in presence of H2O2 promotes CP-SOH condensation with GSH, forming diglutathionylated species and inhibiting hyperoxidation. This displaces equilibrium and accelerates Prdx2 conversion into monomers. Ongoing analyses are probing why the reaction of GSH with wild-type CP-SOH is slow. These findings help understanding the mechanism of relays between Prdx2 and other thiol proteins.

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POSTER 68

Exploring the regulation and function of protein persulfidation with a new quantitative assay

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Protein per-/polysulfidation has emerged as an ubiquitous and dynamic post-translational modification. However, technical difficulties have limited its detection and therefore the study of its origins and functions. Here we present a novel method for the robust quantification of overall protein per-/polysulfidation levels in biological samples. Using this approach, we confirm that 1-5% of all protein thiols are per-/polysulfidated, depending on the organism and cell type. We identify enzymes and metabolites that contribute to endogenous protein per/polysulfidation. We demonstrate that elevated protein persulfidation correlates with enhanced ferroptosis resistance in subtypes of neuroblastoma and lung cancer. Finally, we show that enhanced generation of free radicals leads to enhanced protein persulfidation. Further evidence suggests that protein persulfidation contributes to the protection of cells against irreversible radical damage.

POSTER 69

Cross-validation of lipid peroxidation simulations with liposomal systems

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Ferroptosis is a form of cell death driven by the peroxidation of polyunsaturated fatty acids, ultimately resulting in membrane rupture. Given its involvement in neurodegenerative diseases and cancer, understanding its underlying mechanisms is crucial for advancing the development of therapeutic strategies. However, the rapid and uncontrolled nature of radical-driven lipid peroxidation makes ferroptosis particularly challenging to study through experimental means alone. To address this, we recently developed a hybrid kinetic Monte Carlo / Molecular Dynamics (KIMMDY) reaction emulator that incorporates such chemistry into classical molecular simulations in a highly efficient yet accurate manner. KIMMDY chooses reactions based on their rates, which are predicted by machine learning models trained on thousands of quantum chemical calculations [1]. To validate these simulations, we are establishing an experimental platform using large unilamellar liposomes that mimic the lipid composition of biological membranes. Here, lipid peroxidation is induced by a radical initiator (DTUN) and monitored in real time using the fluorescent dye Bodipy C11. One key question we aim to address is whether the rate of lipid peroxidation differs between the outer and inner leaflets of the cell membrane, which will be compared to KIMMDY predictions. With our setup, we can further attempt to modulate the kinetics of lipid peroxidation by varying the membrane composition or adding low-molecular-weight components such as pro- and anti-oxidants. Gained results can be cross-validated with simulation data and could guide the rational design of membrane systems that are either exceptionally resistant or deliberately sensitive to lipidoxidationdriven ferroptosis.

1: https://doi.org/10.26434/chemrxiv-2023-7hntk

POSTER 70

Metabolic Dysfunction-Associated Steatohepatitis is characterized by reduced H2S-producing enzyme levels and protein persulfidation

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Metabolic dysfunction-associated steatotic liver disease (MASLD), a progressive disease driven by obesity-related oxidative stress, leading to hepatocellular damage and impaired protein function. Hydrogen sulfide (H_2S), a signaling molecule produced predominantly in hepatocytes, has emerged as a key regulator of redox homeostasis through the post-translational modification of protein cysteines via persulfidation (PSSH). However, its role in MASLD remain unclear. We demonstrate that H_2S-producing enzymes (CBS, MPST) are downregulated in both human and mouse livers with steatohepatitis and fibrosis, correlating with reduced global PSSH levels. Using dimedone-switch mass spectrometry in advanced MASLD models, we identified paradoxical increased PSSH on specific protein despite systemic H2S declines. Notably, protein tyrosine phosphatases (PTPs) and redox regulators including peroxiredoxins and thioredoxins exhibited enhanced PSSH, suggesting a targeted adaptive mechanism to counteract heightened oxidative stress in MASH. To study the role of CBS in regulating liver persulfidome, we analyzed hepatic-specific CBS knockout mice under obesogenic diet. Although these mice displayed aggravated liver dysfunction and oxidative damage, total PSSH levels were only modestly reduced, without compensatory upregulation of other H_2S-producing enzymes. This suggests that the liver may sustain persulfidation through adaptive sulfur flux redistribution or non-enzymatic mechanisms under metabolic stress, although this adaptability may lost in advanced disease. Our findings demonstrate that impaired H_2S production disrupts protective persulfidation networks, while selective preservation of PSSH in redox-sensitive proteins reveals a compensatory response to progressive liver damage. Our work establishes dysregulated persulfidation as central features of MASLD pathophysiology, highlighting its therapeutic potential in restoring redox homeostasis during chronic liver disease.

POSTER 71

Mechanistic Insights into the Specificity of Peroxiredoxins Redox Signaling Cascades

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Peroxiredoxins (Prxs) are essential thiol peroxidases involved in oxidative stress response and redox signaling. Upon oxidation, certain Prxs can engage in thiol-disulfide exchange reactions, transferring oxidizing equivalents to downstream signaling proteins. Notably, it has been shown that H_2O_2 induces Prx2 to form a redox relay with STAT3, blocking its nuclear translocation, while Prx1 promotes ASK1 oxidation, triggering p38 phosphorylation and apoptosis[1,2]. Previous studies from our group demonstrated that urate hydroperoxide (HOOU), a by-product of uric acid oxidation, reacts rapidly (~10^6M-1.s-1) with Prx1 and Prx2[3]. Given our findings that suggest Prxs as primary cytosolic targets of HOOU-mediated oxidation, we sought to determine whether these peroxiredoxins mediate redox relays with redox-regulated proteins in differentiated macrophages (dTHP-1 cell lineage). Our results revealed that both Prx1 and Prx2 rapidly oxidize within 2 minutes of exposure to either HOOU or H_2O_2, with the effect diminishing after 10 minutes. However, a key difference between the two oxidants was observed in the formation of high-molecular-weight (HMW) structures. Specifically, H_2O_2 treatment induced significant formation of HMW structures, likely representing heterodimers with redox-targeted proteins. In contrast, HOOU exposure failed to induce such structures at any time point, suggesting distinct oxidative interaction dynamics. Additionally, our investigation of the thioredoxin system revealed further differences in redox responses between H_2O_2 and HOOU. The thioredoxin reductase/thioredoxin (TrxR1/Trx1) reduction system displayed a distinct 66kDa TrxR1 band exclusively in H_2O_2-treated samples, which was absent in HOOU-treated conditions. These findings suggest that different oxidants may elicit differential redox signaling pathways after Prx1 and Prx2 oxidation.

POSTER 72

Riboflavin metabolism shapes FSP1-driven ferroptosis resistance

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Ferroptosis – a form of cell death characterized by iron-dependent lipid peroxidation – has gained significant attention as a potential target for eradicating therapy-resistant tumors. Recent studies have identified the flavoprotein “ferroptosis suppressor protein 1” (FSP1) as a crucial regulator of ferroptosis, alongside glutathione peroxidase-4 (GPX4). FSP1, an NADP(H)-dependent oxidoreductase enzyme, plays a protective role by reducing lipophilic radical-trapping molecules (such as ubiquinone, vitamin E, and vitamin K) to prevent uncontrolled lipid peroxidation. In the present work, we employed CRISPR-Cas9-based screens using an FSP1-dependent cellular model to identify novel metabolic pathways essential for FSP1 function. Our findings establish riboflavin (vitamin B_2) as a key modulator of the FSP1-antioxidant recycling pathway and reveal a previously unrecognized link between riboflavin metabolism and ferroptosis. Furthermore, we propose a rational strategy to target these pathways and sensitize cancer cells to ferroptosis, with implications for understanding the interaction of nutrients and their contributions to cellular antioxidant capacity.

POSTER 73

RedOx Regulation of Liquid-Liquid Phase Separation of Proteins Involved in ALS

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Stress granules (SGs) are transient, membraneless subcellular compartments consisting of mRNA, RNA-binding proteins and other proteins. In response to stress, they form dynamically through liquid-liquid phase separation (LLPS) as a key cellular defense mechanism. Dysregulation of SG dynamics and LLPS is linked to neurodegenerative diseases, such as amyotrophic lateral sclerosis and frontotemporal dementia, where persistent SGs can mature into pathogenic solid-state aggregates. The RNA-binding proteins TIA1 and G3BP1 play central roles in SG formation. Although dysregulated reactive oxygen species (ROS) are known to affect cellular stress responses, their precise influence on LLPS dynamics, SG transition to solid aggregates, and the specific functions of TIA1 and G3BP1 remain poorly understood. This project investigates how oxidation conditions affect TIA1 and G3BP1, droplet formation, and aggregation, using fluorescence microscopy, mass spectrometry, and turbidity measurements. By lucidating the redox-dependent regulation of SG dynamics, this research seeks to advance our understanding of cellular stress responses and their contribution to neurodegenerative disease pathology.

POSTER 74

Decoding AQPs-mediated redox signaling in a 2D keratinocyte model of wound-like repair

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Efficient and timely wound closure is essential for maintaining skin integrity and preventing infection, inflammation, and chronic tissue damage. This complex process requires the precise coordination of keratinocytes' behaviors such as migration, proliferation, and remodeling. It is now widely accepted that redox signaling—particularly mediated by hydrogen peroxide (H_2O_2)—is a key regulatory mechanism orchestrating these events. As a central signaling molecule, H_2O_2 modulates various aspects of keratinocyte function. Although its role in cell migration and tissue regeneration has been demonstrated in animal models such as zebrafish, how redox signaling dynamically operates during wound repair in human skin remains largely unexplored.

To address this gap, we developed a human keratinocyte model using a homogeneous progenitor-like population derived from HaCaT cells, stably expressing the genetically encoded HyPer7 probe to monitor cytosolic H_2O_2 in real time. Live-cell imaging of mechanically induced injury revealed dynamic and spatially coordinated fluctuations in H_2O_2 levels, which correlated with distinct cellular processes during wound closure. Furthermore, targeted modulation of two well-characterized peroxiporins, AQP3 and AQP8, altered intracellular oxidative states and significantly impacted keratinocyte proliferation and migration.

Our findings provide new insights into how finely tuned redox signaling governs cellular responses during human skin repair. These results not only enhance our understanding of epidermal homeostasis but also underscore redox regulation as a potential therapeutic target for improving wound healing and treating skin pathologies.

POSTER 75

The effect of hydrogen (per)sulfide donors on β cells ferroptosis in diabetic conditions

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Hydrogen sulfide (H2S) is an important gassotransmiter which level is reduced in diabetic state. Although its donors represent an innovative approach in the treatment of some diabetes-related pathologies, its role in the regulation of function/destiny of β-cells has not been elucidated. We examined the effects of slow releasing H2S donor (GYY4132), polysulfide (Na2S4) and persulfide (Cys-3S) donors on β-cells ferroptosis under diabetic conditions in vitro and in vivo.

Cys-3S had positive effects on diabetes-induced proferroptotic events (accumulation of iron and lipid peroxides and failure of the antiferroptotic axis, Nrf2/xCT/GPX4/GSH) in pancreatic islets in vivo, which was reflected in the maintenance of the functional population of β-cells, serum glucose level and decrease in diabetes incidence. In parallel, the expression of CSE in pancreatic islets was significantly upregulated by Cys-3S treatment. The results from in vitro study confirm its strong antiferroptotic potential: Cys-3S prevented high glucose- and RSL-3-induced β-cell death in similar manner as established ferroptosis inhibitor Fer-1. Na2S4 also acted beneficially on the ferroptotic parameters and pancreatic CSE level, but failed to maintain β-cell population and stabilize hyperglycemia in diabetic animals. The effects of GYY4137 on diabetes-induced changes in systemic or tissue ferroptotic parameters were less pronounced and complete.

Although both donors of polysulfide and persulfide ameliorated diabetes-induced ferroptotic phenotype of β-cells and disturbances in H2S biosynthetic pathways, only in the case of Cys-3S antidiabetic effect and better metabolic control was achieved. This qualifies direct donors of persulfides as a effective approach in the prevention of β-cells death and diabetes development.

POSTER 76

Ir(relevance) of H2O2 for the survival of Plasmodium falciparum and the mode of action of antimalarial drugs

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The blood stages of the malaria parasite Plasmodium falciparum multiply in human erythrocytes, which are thought to provide a pro-oxidative environment due to their hemoglobin and oxygen content. This environment and the liberation of heme in the parasite's specialized lysosome were suggested to cause oxidative stress. Furthermore, oxidative stress was hypothesized to play a major role for the mode of action of antimalarial drugs that target, for example, heme detoxification or the mitochondrial electron transport chain. Here, we analyzed the relevance of H2O2 for parasite survival and the mode of action of antimalarial drugs. Decreasing or increasing the local intracellular H2O2 concentration with titratable targeted heterologous catalase or D-amino acid oxidase revealed compartment-specific growth effects. In contrast, manipulation of the endogenous H2O2 concentration had no or only minor effects on the IC50 values of antimalarial drugs. In summary, while balanced compartment-specific H2O2 concentrations are crucial for parasite survival, H2O2 does not play a major role in the mode of action of a variety of antimalarial drugs contrary to frequently cited hypotheses.

POSTER 77

Limitations of the fluorescent protein sensor roGFP2 for monitoring dehydroascorbate levels and dehydroascorbate reductase redox state

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Dehydroascorbate reductases (DHARs) are enzymes of the glutathione S-transferase (GST) superfamily that play a central role in the ascorbate–glutathione pathway by catalyzing the glutathione (GSH)-dependent reduction of dehydroascorbate (DHA) to ascorbate. This reaction is important for maintaining a reduced ascorbate pool, ensuring efficient detoxification of hydrogen peroxide (H_2O_2) by ascorbate peroxidases (APXs) (1).

The development of roGFPs has, over the past decades, enabled the monitoring of both oxidation and reduction of target proteins (2), as well as the measurement of cellular redox potentials or levels of small molecules (3). Hence, we employed the redox-sensitive fluorescent protein sensor roGFP2 to investigate whether the redox dynamics of dehydroascorbate and DHARs—specifically the DHAR2 isoform from Populus trichocarpa (PtDHAR2), can be used. However, what initially appeared to be a straightforward reaction to monitor, turned out to be unexpectedly complex. DHA, the reaction substrate, was found to directly oxidize roGFP2, preventing us from specifically tracking the target enzyme. This prompted us to elucidate the mechanism underlying DHA-induced roGFP2 oxidation in vitro using mutants for both redox-active cysteine residues (Cys147 and Cys204) of roGFP2.

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POSTER 78

Reduction of fatty acid hydroperoxides by human peroxiredoxin 5

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Human peroxiredoxin 5 (Prdx5) is a thiol-dependent peroxidase expressed in different cell compartments that play key roles in physiopatological processes. Prdx5 is highly resistant to hyperoxidation compared to other 2 Cys Prdxs, although it was detected in hyperoxidized form in hearts from ischemia-reperfusion animal models1. Organic hydroperoxides oxidize Prdx5 with rate constants in the 10e6-10e7 M-1s-1 range2. Still, the reaction with fatty acid hydroperoxides (FA-OOH), either free or forming part of more complex lipids of biological membranes, was unexplored. In this work, we investigate the reaction between reduced Prdx5 and FA-OOH derived from linoleic and arachidonic acids. Prdx5 reduced free FA-OOH with a stoichiometry of 1:1. Furthermore, mass spectrometry determinations indicated that FA-OOH oxidizes Prdx5 to the disulfide state without causing hyperoxidation. Adding FA-OOH to reduced Prdx5 caused a rapid increase in the intrinsic fluorescence intensity of the enzyme, consistent with its oxidation. The plot of the observed rate constants of fluorescence change versus FA-OOH concentration indicated a linear relationship, reflecting a bimolecular process with a rate constant of ~10e6 M-1s-1. The plots consistently showed offsets of ~ 10 s-1, which we interpret as k-1, the rate constant of the dissociation of the ES complex. Although FA-OOH addition caused no Prdx5 hyperoxidation under non-catalytic conditions, it caused Prdx5 inactivation during catalysis. The mechanism of such inactivation is currently being explored.

1 Paulech et al, Molecular & Cellular Proteomics, 2015, 14, 609-620.

2 Trujillo et al, Archives of Biochemistry and Biophysics, 2007, 67, 95-106.

POSTER 79

Kinetics and mechanisms of mitochondrial peroxiredoxin 3 thiolation

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Peroxiredoxin 3 (Prdx3) is a thiol-dependent peroxidase that catalyzes the reduction of different hydroperoxides. It is expressed in the mitochondrial matrix and intermembrane space and can be reduced by thioredoxin 1(Trx1), thioredoxin 2(Trx2) and glutaredoxin 2(Grx2)(1). The enzyme has been detected forming mixed disulfides with glutathione (GSH) and with coenzyme A (CoA) in cellular or animal models of oxidative stress. However, the mechanisms and kinetics of the reactions of Prdx3 with glutathione and CoA, low molecular weight thiols present in the mitochondria matrix at mM concentrations, are still unknown, although the kinetics and mechanisms of glutathionylation have recently been reported for other peroxiredoxins(2).

Peroxiredoxin 3 oxidized to sulfenic acid (Prdx3-SOH) reacted with GSH and with CoA with rate constants of 64 M-1s-1 at pH 7.8 and 1,173 M-1s-1 at pH 7.4, respectively. Furthermore, GSH and CoA reacted with the disulfide form of Prdx3 to form thiolated forms of Prdx3. The mixed disulfides with the corresponding thiols were detected immunochemically and by mass spectrometry. Grx2 reduced the glutathionylated form of Prdx3, while GSH plus Grx2 catalyzed the reduction of Prxd3. Although some thioredoxins and thioredoxin-related proteins were reported to reduce proteinS-SCoA mixed disulfides(3), Trx1 did not reduce Prdx3S-SCoA. CoA inhibited NADPH oxidation in a coupled assay using thioredoxin reductase, Trx1, Prdx3 and 50 μM H_2O_2 and protected against Prdx3 hyperoxidation at higher H_2O_2 levels. Thus, Prdx3 thiolation represents a kinetically feasible mechanism to modulate Prdx3 activity.

(1) Hanschmann EM, et al.JBC.2010.

(2) Peskin A, et al.JBC.2025

(3) Tossounian MA, et al.Antioxidants.2023

POSTER 80

Catalytic and structural consequences of thiol oxidation in human GAPDH

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is defined as the glycolytic lynchpin, as it is involved in the "beneficial" stage of glycolysis where NADH and ATP are synthesized. The enzyme behaves as a homotetramer that catalyzes the reversible conversion of 1,3-bisphosphoglycerate from glyceraldehyde-3-phosphate (GAP) in the presence of NAD+ to yield NADH. The active site architecture involves a catalytic cysteine residue (C152, human) which is crucial for the enzymatic activity. C152 has been proven to be oxidized by hydrogen peroxide (H2O2), forming a sulfenic acid (CysSOH) and inhibiting catalysis. Formation of CysSOH in GAPDH C152 along with other oxidative posttranslational modifications (glutathionylation, persulfidation, disulfide bond formation and nitroalkylation among others), deviate the enzyme from its canonical to moonlighting functions, thus expanding its biological effects. It was recently shown that H2O2 oxidation of GAPDH reroutes the metabolic flux from glycolysis to the pentose phosphate pathway yielding an increase in NADPH production1. More recently, it was proposed that CO2 enhances H2O2-dependent GAPDH oxidation, the mechanism of which remains undefined2. Our work is aimed to assess the kinetics and the extent of oxidation of human GAPDH (HsGAPDH) cysteines by H2O2 and its CO2 adduct, peroxymonocarbonate HCO4-, to better understand how this thiol dependent metabolic switch operates in the context of peroxide signaling in energy metabolism. Additionally, we are assessing the effect of cysteine oxidation on both the catalytic properties and structural dynamics of the HsGAPDH homotetramer, using in vitro and in silico approaches, to elucidate how redox modifications change the enzyme structure and function.

POSTER 81

IRONING OUT CANCER: TARGETING MEDULLOBLASTOMA'S METABOLIC DEPENDENCY

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Iron plays an essential role in normal neurodevelopment, contributing to processes such as myelination, neurotransmitter synthesis, and mitochondrial function. During early brain development, the demand for iron is particularly elevated, and disruptions in iron homeostasis can result in long-lasting neurodevelopmental impairments. Emerging evidence suggests that the molecular pathways regulating iron metabolism in the developing brain are co-opted by embryonic tumors to fulfill their elevated iron requirements, which are critical for rapid proliferation, survival, and resistance to therapy. Ferritin, the major intracellular iron storage protein, plays a critical role in maintaining iron homeostasis by buffering labile iron and preventing iron-induced oxidative stress. However, beyond its traditional role as a passive iron reservoir, ferritin is increasingly recognized as a dynamic regulator of tumor biology.

Here the emerging roles of ferritin in cancer, with a particular focus on its function in regulating ferroptosis—an iron-dependent, non-apoptotic form of cell death driven by lipid peroxidation, will be discussed. Using medulloblastoma as a model of embryonic brain tumor, we demonstrate that genetic ablation of the ferritin heavy chain (FTH) sensitizes tumor cells to ferroptosis and impairs tumor growth in vivo. Furthermore, we uncover a ferroptosis-independent mechanism of iron toxicity induced by high-dose vitamin C, which leads to rapid ferritin degradation and catastrophic iron release, culminating in cell death that is preventable only by iron chelation.

These findings establish ferritin as a critical regulator of iron metabolism and tumor cell survival, revealing mechanistic vulnerabilities that tumors exploit for growth and therapeutic resistance.

POSTER 82

Thiol-Based Crosstalk between Nrf2 Signaling and H2S Pathways in Response to Citrus Flavanones

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Age-associated redox imbalance involves complex regulation of transcriptional and post-translational processes. Among these, the crosstalk between Nrf2 signaling and protein persulfidation (PSSH) emerges as a critical axis of thiol-based redox control. Citrus flavanones, naringenin (NAR), and hesperetin (HES) show strong antioxidant properties and redox regulatory potential. We investigated how NAR and HES modulate hepatic thiol redox pathways in a model of natural old age (24-month-old Wistar rats). Flavanones were administered orally (15 mg/kg body weight, for 28 days), and redox-related targets were evaluated on gene and protein levels. Our results show coordinated regulation of the Nrf2–Keap1 system and thioredoxin machineries (Trx1, Trx2, TrxR1), indicating a transcriptionally driven reinforcement of antioxidant buffering. PSSH levels and the expression of H_2S-producing (CBS, CSE, MST) and -catabolizing enzymes (SQR, TST) analysis showed a networked response linking Nrf2 activation and H_2S metabolism. Considering the ability of persulfidation to modify Keap1 cysteines, a shift in H_2S enzyme expression may facilitate Nrf2 release and nuclear translocation after NAR. In addition, Nrf2 and TrxR1 exhibited opposing expression after NAR and HES treatment, suggesting the necessity for fine-tuning regulation in the aged hepatic redox milieu. Our data support a model in which dietary flavanones engage thiol-based networks through interlinked regulation of Nrf2, TrxR1, and persulfidation machinery. The therapeutic relevance of nutritional redox modulators in maintaining hepatic redox resilience during aging through fine-tuned transcriptional and post-translational thiol signaling needs to be further evaluated regarding health and lifespan extension.

POSTER 83

Oxidative stress sensor PDIA6 condensates in the ER

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PDIA6, an endoplasmic reticulum (ER)-resident disulfide isomerase and molecular chaperone, condensate is essential for insulin secretion by accelerating the oxidative proinsulin folding and suppressing the aggregation. Here, we show that reactive oxygen species, hydrogen peroxide and nitric oxide, triggers the PDIA6 granule formation and modulates its fluidity inside granule in the endoplasmic reticulum. The underlying mechanism was found to be modulations of both its conformational dynamics at single molecule level and the condensate formation by the the redox status of the active site in the first a0 domain. We further show that the overexpression, which promotes the granules formation, suppresses apoptosis and prolongs the lifespan, thereby paving the way towards understanding the protein quality control granule-associated apoptosis.

POSTER 84

An Unexpected Turn: Discovering a Novel Thiol RNA Modification in the Search for Carbohydrate-Modified RNA

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Among the more than 170 co- and post-transcriptional RNA modifications, several contain sulfur in the form of thioether or thioketone groups. In contrast, thiol groups are rare and have so far only been identified in the form of a noncanonical Coenzyme A cap. Thiols possess unique chemical reactivity and can be selectively targeted using molecular probes containing Michael acceptors or haloalkyl groups. However, this reactivity can also interfere with established biochemical methods – such as click chemistry or metabolic labeling with peracetylated carbohydrate reporters – potentially causing false-positive results.

In this work, we describe how an attempt to detect carbohydrate-modified RNA in E. coli took an unexpected turn, leading to the discovery of a novel thiol modification specific to the asparagine tRNA. This tRNA can be selectively labeled with a fluorophore using thiol-specific maleimide conjugation. Further localization of the modification site was achieved by cleavage with sequence-specific RNA-cleaving DNAzymes, pinpointing the modification to the wobble position. This position is typically occupied by the hypermodified nucleoside queuosine (Q), and analysis of RNA from Q-deficient knockout mutants suggests the presence of a previously unknown thiol modification of Q.

To confirm this, we analyzed isolated asparagine tRNA, digested to single nucleosides, by tandem mass spectrometry. This revealed a novel Q derivative that can be metabolically labeled with 34S, is sensitive to reducing agents and can be alkylated with iodoacetamide.

Guided by a side reaction with a carbohydrate reporter, we therefore identified a novel thiol-containing Q derivative—one of few known thiol modifications in RNA.

POSTER 85

Glutathione vs. Thioredoxin: Divergent Redox Responses to Ferroptosis in Normal Human Fibroblast and Melanoma Cells

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Introduction:

Ferroptosis, a regulated cell death driven by iron accumulation and lipid peroxidation, is implicated in skin diseases like melanoma. This study compares ferroptosis regulation in two human skin-derived cell lines: melanoma (Lu1205) and normal dermal fibroblasts (NHDF).

Materials & Methods:

Ferroptosis was induced using Erastin (5, 10 μM). After 24 hours, cell viability (MTT assay), total glutathione (GSH), reactive oxygen species (ROS), and lipid peroxidation were assessed. RT-qPCR evaluated expression of genes involved in antioxidant defense, lipid metabolism, and iron homeostasis.

Results:

Erastin reduced viability by up to 60% in NHDF and 30% in Lu1205 cells. In NHDF, glutathione increased and ROS decreased dose-dependently. Lu1205 showed increased ROS and lipid peroxidation but decreased glutathione. In NHDF, antioxidant genes (GPX4, FSP1) and lipid metabolism gene ACSL4 were upregulated. FTMT increased with dose, while IRP2 and TFRC moderately increased. In Lu1205, ACSL4, TFRC, and IRP2 expression increased in a dose-dependent manner, while FTMT decreased. GPX4 was slightly upregulated, FSP1 increased only at 10 μM, and TRX showed dose-dependent upregulation.

Conclusion:

In fibroblasts, ferroptosis triggers a glutathione-based antioxidant response. In melanoma cells, where glutathione declined, the thioredoxin system may dominate redox regulation. This indicates differential antioxidant engagement in healthy versus cancerous skin cells under ferroptotic stress.

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GPX4–Glutathione Peroxidase 4

FSP1–Ferroptosis Suppressor Protein 1

ACSL4–Acyl-CoA Synthetase Long Chain Family Member 4

FTMT–Mitochondrial Ferritin

IRP2–Iron Responsive Element Binding Protein 2

TFRC–Transferrin Receptor

TRX–Thioredoxin

POSTER 86

Single-Cell Analysis Reveals Cell-Specific Patterns and Spatiotemporal Regulation of Nuclear Redox State

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The redox state, defined as the balance between oxidized and reduced molecular species, is a fundamental aspect of cellular metabolism, impacting energy production, signal transduction, and protein function. While extensive studies have explored redox regulation in cellular physiology, post-embryonic development, and aging, the dynamics and regulation of redox states during embryogenesis remain poorly understood. To address this gap, we performed single-cell, high-temporal-resolution stoichiometry of redox states in C. elegans early embryogenesis. Using genetically encoded biosensors and live imaging, we mapped glutathione redox potential and H2O2 level in individual nuclei with a ubiquitously expressed Grx1-roGFP2 and HyPer7 fluorescence biosensor, respectively. This approach enabled us to generate a comprehensive, minute-by-minute map of nuclear redox dynamics across every early cell. This atlas reveals highly spatiotemporally specific redox patterns, with distinct cell types displaying characteristic redox states that undergo temporal transitions to achieve their specific states. Notably, these cellular redox states can be accurately predicted by the combinatorial expression of oxidoreductase genes, which displayed strong developmental specificity in their expression and associated metabolic pathways. Additionally, our functional genomic analysis prioritized over 20 transcription factors that preferentially target oxidoreductase gene promoters or are functionally linked to these enzymes, laying a foundation for future mechanistic studies. Together, our work presents a cellular-resolution redox atlas of embryogenesis in a metazoan species and provides insights into the spatiotemporal dynamics and regulation of redox states.

POSTER 87

Chemical Reactivity of Protein and Low-Molecular-Weight Persulfides with (Anti) oxidants

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Persulfides (RSSH) are reactive sulfur species implicated in redox regulation and antioxidant defense, yet their detailed

chemical reactivity remains underexplored.

In this study, we investigated the interactions of persulfides with electrophiles, nucleophiles, and free radicals to elucidate

their potential biochemical roles. For that, we used the well-characterized protein persulfide Trx1 [Schilling, Barayeu et al., 2022]. The commonly used GPX4 inhibitor RSL3 is an electrophile that typically reacts with the selenocysteine residue in the active site of GPX4. In our system, RSL3 selectively reacted with persulfides but not with thiols, highlighting the uniquenucleophilicity of the persulfide. In contrast, S-nitrosoglutathione (GSNO) reacted with both persulfides and thiols,forming trisulfide (-SSSG) and disulfide (-SSG) adducts, respectively.

Reaction of persulfides with the model free radical $ABTS^{+\bullet}$ resulted in a variety of oxidized adducts. Most notably, this reaction promoted protein dimerization, likely via polysulfide bond formation through recombination of perthiyl radicals (RSS•).

Complementary experiments using low-molecular-weight persulfides (Na_2S_4) and the free radical scavenger Trolox revealed unexpected rapid reactivity, converting persulfides to hydrogen sulfide (H_2S) and elemental sulfur (S_8). This reaction is further investigated in cells as a potential strategy to deplete endogenous persulfides, thereby controlling their intracellular levels.

Collectively, these findings highlight the chemical versatility of persulfides and their dual role as both reactiveintermediates and protective species. Our study provides new insights into persulfide-mediated redox chemistry in

biological systems and lays the groundwork for future cellular studies of the persulfide modulators.

1. DOI: 10.1002/anie.202203684

POSTER 88

A putative redox metabolic switch controls G1/S transition in Saccharomyces Cerevisiae

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The Yeast Metabolic Cycle (YMC) is a phenomenon of sustainable population-wide synchronized metabolic state oscillations that arise spontaneously in Saccharomyces Cerevisiae cells grown under defined chemostat-based conditions. These metabolic oscillations are tightly coupled with the Cell Division Cycle (CDC), making the YMC an excellent model for investigating the relationship between metabolite and metabolic state dynamics and cell cycle progression.

Previously we employed genetically-encoded fluorescence sensors to reveal cell cycle coupled redox metabolite dynamics in the YMC. Furthermore, our previous research indicated that reduction of one or more cellular disulfides is an essential prerequisite for driving G1/S transition. Recent microfluidics-based experiments with yeast cells expressing fluorescent redox sensors also revealed cell cycle-dependent redox oscillations consistent with our fermentor-based observations. These results indicate that the YMC represents a population synchronization of cell cycle coupled metabolic oscillations that occur on a single-cell level.

We now demonstrate that the thiol oxidant diamide significantly delays the G1/S transition, with the extent of the delay strongly dependent on the YMC phase at the time of its addition. Furthermore, we find that the phase-dependent impact of diamide addition is highly dependent upon the activity of the Protein Kinase A (PKA) signaling pathway. Based upon our observations we propose a model in which oscillatory metabolic state, via redox regulation of PKA activity, controls cell growth and division.

POSTER 89

Emerging Impact of Citrus Flavanones on Protein Persulfidation and Cardiovascular Redox Regulation in Old-aged Rats

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Protein persulfidation (PSSH) sheds a new light on all physiological functions and imposes reconsideration of current interpretations of metabolic processes and signaling networks. This evolutionarily conserved posttranslational modification of cysteine residues, where the thiol group (-SH) is converted to persulfide (-SSH), is essential in preventing hyperoxidation of proteins and loss of their function. The protective pools of intracellular PSSH decrease with age, and this process is correlated with the loss of protein expression of H2S-producing enzymes. Citrus flavanones are well recognized as a potent and promising antioxidant bioactive compound which promotes health and longevity.

Our study investigated oscillations of PSSH in the heart of 24-month-old male Wistar rats supplemented with citrus flavanones. Nutritionally relevant doses of hesperetin, naringenin, or lemon extract were applied orally, once a day, for four weeks. A novel dimedone-based method for persulfide detection was used, while the protein expressions of H2S-producing enzymes were evaluated by Western blot. Citrus flavanones notably modulated levels of protein persulfidation in the hearts of old rats, leading to a significant increase of PSSH when compared to corresponding controls. Furthermore, detected alterations were accompanied by differences in the expression levels of H2S-producing enzymes. Observed changes demonstrated promising effects of citrus flavanones as a potent bioactive in regulating PSSH levels in the hearts of old rats, regardless of proven PSSH decline induced by aging. Understanding more fully the ties between the aging and PSSH will offer a deeper insight into cardiovascular redox regulation and identify novel therapeutic strategies to enhance healthspan and lifespan.

POSTER 90

Thiol-based conjugation of yeast peroxiredoxin Ahp1 by an archaeal Urm1-like modifier

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Alkylhydroperoxide reductase 1 (Ahp1) is a typical 2-Cys-peroxiredoxin in budding yeast Saccharomyces cerevisiae. Homodimers of Ahp1 detoxify peroxides upon oxidation by forming intersubunit disulfides between peroxidatic and resolving cysteines. The thioredoxin system reduces these disulfides to repair Ahp1 for another cycle of peroxide detoxification. Beside its role in detoxification, Ahp1 is a bona fide target for ubiquitin-like urmylation (Redox Biol 2020;30: 101438). The latter refers to lysine-directed modification by Urm1 (ubiquitin-related modifier 1), which is triggered by oxidative stress.

Urm1 is a bifunctional protein that following sulfur-dependent activation, either transfers sulfur onto tRNA for anticodon thiolation or conjugates to target proteins in a ubiquitin-like fashion. It was shown in vitro that during urmylation, Urm1 transfers sulfur to the peroxydatic cysteine of Ahp1 to form a persulfide (EMBO J. 2022;41(20): e111318) may protect the peroxiredoxin against damaging overoxidation.

An archaeal Urm1-like modifier, which conjugates to target proteins, also occurs in Sulfolobus acidoaldarius (Nat Commun. 2015;6: 8163). Based on its structural similarity to yeast Urm1, we expressed the archaeal Urm1 protein in URM1 deficient strains of S. cerevisiae and investigated its ability to conjugate to Ahp1. We find that archaeal Urm1 needs sulfur-activation to attach to Ahp1. Additionally, Ahp1 mutants lacking the critical cysteine for peroxiredoxin activity, are not modified by the Urm1 counterpart, strongly suggesting that the Urm1-like conjugation reaction is dependent on cysteine-thiol groups in Ahp1. Our data reveal that substrate specificity, redox requirements and sulfur activation are shared between Ahp1 conjugation by yeast Urm1 and an archaeal Urm1-like modifier.

POSTER 91

ROS-driven peptide reprogramming: hypohalous acids (HOX) as immune modulators in neutrophils

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Neutrophils are rapid-response immune cells that eliminate microbes through reactive oxygen species (ROS). Central to this defense is myeloperoxidase, which converts ROS into highly reactive hypohalous acids (HOX). Within the phagosome, HOX can reach concentrations up to 200 mM, driving a potent antimicrobial assault to pathogens. Yet beyond their established microbicidal role, the contribution of these reactive species to immune modulation remains poorly understood.

Here, we reveal a novel ROS-dependent mechanism by which HOX modify the antimicrobial peptides α-defensin 1–3 (HNPs) during neutrophil activation. Specifically, we show that HOX selectively halogenate tyrosine residues, chlorinating tyrosine 21 and iodinating tyrosine 16. These modifications occur in infected patients and Cystic Fibrosis lungs, and are conserved in rat neutrophils. Structural and biophysical analyses demonstrate that halogenation increases HNPs hydrophobicity without altering their native structure. Importantly, halogenated HNPs acquire enhanced immunomodulatory activity, inducing the expression of specific chemokines in immune cells, as confirmed by transcriptional and in vivo studies.

Altogether, our findings identify a previously unrecognized mechanism linking ROS-derived hypohalous acids to immune signaling. By chemically reprogramming antimicrobial peptides into potent immunomodulators, neutrophils extend the functional repertoire of ROS beyond pathogen killing, with important implications for host defense, inflammation, and ROS-mediated immune regulation.

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