UK Redox Network

08 May 2025

Email: clare.hawkins@sund.ku.dk

Professor Clare Hawkins
Secretary General SFRR Europe

Dear Clare,

Re: Report – 2nd UK Redox Network Meeting 28 April 2025

Please find attached our report for the 2nd UK Redox Network meeting held at The Institute of Cancer Research on 28 April 2025. This meeting was coordinated by Dr Jörg Mansfeld (Institute for Cancer Research), Dr Olena Rudyk (King's College London) and Prof. Giovanni Mann (King's College London). It was aimed at extending the inaugural 1st UK Redox Biology Discussion Group meeting held in London on 18 June 2018 and followed the success of the 1st UK Redox Network Meeting held in London last year, on 17 April 2024. The meeting successfully brought together UK-based researchers in redox biology, achieving its aim of fostering collaboration and knowledge exchange, as confirmed by participant feedback.

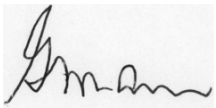
We attracted 73 registered delegates (England, Scotland, India and Italy) with 70 from different regions of the UK. Notably, 14 different UK academic institutions were represented (London, Surrey, Cambridge, Leicester, Birmingham, Liverpool, Newcastle, Dundee), including seven from London alone. At least 58 delegates, including the invited speakers, attended the meeting in person. A total of 5 invited delegates joined online. The speakers in our programme were invited to represent the different regions in the UK, and talks were given by established, early-career postdoctoral scientists and PhD students. In the morning/early afternoon, there were four invited talks from established principal investigators over the four themed sessions, including the plenary speaker, Prof. James Nathan from Cambridge, CITID, who gave the keynote lecture, and four more talks were selected from around 20 abstracts submitted that included postdocs and PhD students (see programme). Moreover, 14 posters were presented in person during the afternoon poster session. Professor Giovanni Mann concluded with a detailed outline of the benefits of becoming a member of SFRR Europe. We also had poster prizes selected by session chairs. The top four posters won a one-year membership for SFRR. The best poster, in addition, won the book "Oxygen", by the UCL researcher Nick Lane (Oxford Press).

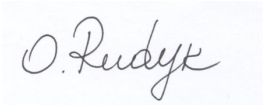
SFRR Europe outreach support will be used to reimburse costs for tea/coffee, lunch and light refreshments. This year, PCR Biosystems, based in London, kindly sponsored evening refreshments (pizza) accompanying the poster session – which has been a great success: discussion at posters continued until 7.30 pm when the meeting needed to close. In light of the total funding available we were not able to offer support towards travel costs for Early Career Researchers. Instead, by making sure that more than half of the presentation were given by early career (PhD and Postdoc) investigators and the focus in the poster session we fostered their in-person communication and networking.

It may be once again helpful if the delegates could be emailed to further outline the possibility of applying for institutional membership of SFRR Europe. If you, Clare, would like to contact the delegates, Jörg or Olena could email you their contact emails.

Should you require any further information, please do not hesitate to contact us.

With best wishes,


Giovanni E. Mann


Olena Rudyk

Jörg Mansfeld

UK Redox Network

GENERAL INFORMATION

Event: 'UK Redox Network' Meeting 2025 **Date:** Monday 28th April, 2025 **Time:** 9 am - 7 pm

Venue: The Institute of Cancer Research, 123 Old Brompton Road, South Kensington, London SW7 3RP

South Kensington station (Circle/District & Piccadilly underground lines); https://www.icr.ac.uk/contact-us

Format: In person talks, poster session & networking.
FREE to attend, registration https://forms.office.com/e/8Zt9Kq2dag

SUMMARY: This meeting brings together UK-based researchers with shared interests in redox biology to foster collaboration and knowledge exchange. Building on the success of the inaugural 2018 event, we aim to further strengthen the UK redox research community and expand engagement with SFRR-Europe. The programme features a plenary session, three oral communication sessions, an afternoon poster session, and flash talks, offering participants the opportunity to showcase their work. Attendees include PhD students, postdoctoral researchers, early- and mid-career investigators, and established leaders in redox biology. In addition to scientific presentations, the meeting provides a forum for strategic discussions on advancing future *UK Redox Network* initiatives and enhancing national coordination in redox biology research.

PROGRAMME

Plenary Speaker:

Professor James NATHAN, University of Cambridge

Invited Speakers:

Dr Elizabeth VEAL, Newcastle University

Dr Paraskevi (Pari) KRITSILIGKOU, University of Liverpool

Dr Joseph BURGOYNE, King's College London

Selected talks from Post-doctoral researchers:

Dr Dominic BYRNE, University of Liverpool

Dr Jan MILKOVIC, University of Cambridge

Dr Claudia LENNICKE, MRC Laboratory of Medical Sciences, London

Dr Daniel SIMOES de JESUS, Queen Mary University of London

Selected talks from PhD Students:

Miss Francesca AMORE, University of Florence/Queen Mary University of London

Mr Dejun ZHANG, University College London

Organisers & Chairs:

Jörg MANSFELD, Institute of Cancer Research (Organiser, Chair)

Olena RUDYK, King's College London (Organiser, Chair)

Giovanni MANN, King's College London (Organiser, Chair)

Helena COCHEMÉ, MRC Laboratory of Medical Sciences (Chair)

Sponsors: SFRR Europe, PCR Biosystems

UK Redox Network

'UK Redox Network' Meeting | Monday, April 28th, 2025

The Institute of Cancer Research
123 Old Brompton Road
London SW7 3RP

PCRBIOSYSTEMS
simplifying research

PROGRAMME

9.00 - 10.00	**Arrival- Registration, Tea/Coffee & Networking**
9.50 - 10.00	**Welcome & Introduction** (Jörg Mansfeld)
10.00 - 10.10	**Sponsor short talk by Dr Andrea Corr**, PCR Biosystems Ltd, London
10.10 - 10.45	**Plenary Talk. Redox and Metabolic Regulation** (Chair: Olena Rudyk)
	Professor James Nathan, University of Cambridge Lipoylation: a redox sensitive regulator of mitochondrial metabolism
10.45 - 11.45	**Session 1. Redox Regulation of Cell Signalling and Metabolism** (Chair: Giovanni Mann)
10.45 - 11.10	**Dr Elizabeth Veal**, Newcastle University Deciphering how peroxiredoxins affect redox signal transduction, adaptation and survival
11.10 - 11.30	**Dr Dominic Byrne**, University of Liverpool Aurora A regulation by reversible cysteine oxidation reveals evolutionarily conserved redox control of Ser/Thr protein kinase activity
11.30 - 11.45	**Miss Francesca Amore**, University of Florence/Queen Mary University of London The loss of SDHB enhances mitochondrial contact sites with the nucleus (NAM), leading to dysregulated cellular metabolism
11.45 - 12.00	**Coffee break**
12.00 - 13.05	**Session 2. Cutting Edge Redox Technologies** (Chair: Jörg Mansfeld)
12.00 - 12.25	**Dr Paraskevi Kritsiligkou**, University of Liverpool Utilising tethered biosensors to uncover intracellular redox heterogeneity
12.25 - 12.45	**Dr Jan Miljkovic**, University of Cambridge A novel mass spectrometry-based approach for quantifying protein and low molecular persulfides in biological systems
12.45 - 13.05	**Dr Claudia Lennicke**, MRC Laboratory of Medical Sciences, London OxiFly: Sex-specific redox networks in ageing Drosophila
13.05 - 14.00	**Lunch & Networking (Finger food and drinks at the back of lecture theatre)**

14.00 - 15.00	**Session 3. Redox-Modulating Strategies and Diseases** (Chair: Helena Cochemé)
14.00 - 14.25	**Dr Joseph Burgoyne**, King's College London Targeting thiol-dependent PKGI activation for therapy
14.25 - 14.45	**Dr Daniel Simoes de Jesus**, Queen Mary University of London KCTD12 redox state as a determinant and therapeutic target in pulmonary remodelling
14.45 - 15.00	**Mr Dejun Zhang**, University College London Regulation of DJ-1 by CoAlation in Parkinson's Disease
15.00 - 15.30	**Speed Poster talks (1 min)** (Moderators: Jörg Mansfeld and Olena Rudyk)
15.30 - 17.00	**Poster session with drinks and nibbles (in Cafeteria)**
17.00 – 17.10	**Concluding remarks & selection of the next organisers**
17.00 – 19.30	**Reception & Networking with PCR Bio-sponsored Pizza and drinks (in Cafeteria)**

End of Programme

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Organisers & Chairs:

Jörg MANSFELD, Institute of Cancer Research (Organiser, Chair)

Olena RUDYK, King's College London (Organiser, Chair)

Giovanni MANN, King's College London (Organiser, Chair)

Helena COCHEMÉ, MRC Laboratory of Medical Sciences (Chair)

Sponsors: SFRR Europe, PCR Biosystems

ORGANISER & CHAIR INFORMATION

Jörg MANSFELD (Institute of Cancer Research) *jorg.mansfeld@icr.ac.uk*

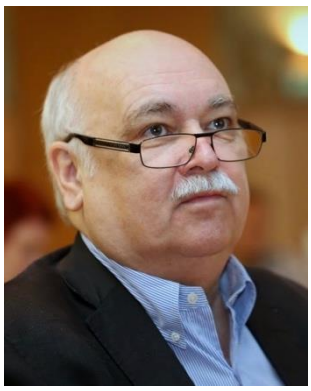
Organiser, Chair: Welcome & Introduction, Session 2. Cutting Edge Redox Technologies (12.00 - 13.05), Flash Poster Talks (15.00 - 15.30), Concluding Remarks

Biosketch: Jörg Mansfeld is a Reader of Protein Modifications and a Senior Cancer Research UK Research Fellow at Institute of Cancer Research. Jörg studied biology at the University of Konstanz in Germany, followed by a PhD in biochemistry at the ETH Zurich, Switzerland, and postdoctoral training in the laboratory of Jon Pines at the Wellcome Trust Gurdon Institute in Cambridge, UK. In 2013, he started his own laboratory at the Biotec Institute of the Technical University Dresden, Germany, investigating how the ubiquitin system controls the decision of cells to proliferate of not to proliferate. Since it became clear that ubiquitin enzymes and other components of the core cell cycle machinery can be targeted by reactive oxygen species his recent work aims to define the interplay between the cell cycle and redox systems, in particular how oxidation of cell cycle regulators affects cell proliferation and cell fate. He moved to the Institute of Cancer Research London (ICR) in 2020 and leads the Post-translational Modifications and Cell Proliferation team within the Cancer Biology Division.

Olena RUDYK (King's College London) *olena.rudyk@kcl.ac.uk*

Organiser, Chair: Plenary Talk. Redox and Metabolic Regulation (10.10 - 10.45), Flash Poster Talks (15.00 - 15.30), Concluding Remarks

Biosketch: Dr Olena Rudyk is a Senior Lecturer in Cardiovascular at King's College London (St Thomas' Hospital campus). She earned her PhD in 2007 from the Department of Blood Circulation at the Bogomoletz Institute of Physiology, Kyiv, Ukraine, followed by postdoctoral training at King's College London in the laboratory of Prof. Lucilla Poston and, later, Prof. Philip Eaton. In 2015, she obtained a British Heart Foundation Intermediate Basic Science Research Fellowship to establish her independent research programme focused on the role of redox-regulated kinases in cardiovascular disease. Her group has since developed a strong translational focus on pulmonary hypertension, aiming to uncover redox-sensitive and metabolic signalling pathways causing pulmonary vascular dysfunction and remodelling and identify new therapeutic targets for this and other hyperproliferative vascular diseases. Research in the Rudyk lab spans integrative biomolecular, cellular and genetic *in vitro*, *ex vivo* & *in vivo* approaches, utilising experimental disease models and genetically altered animals, and is complemented by advanced *in vivo* imaging techniques and clinical collaborations. Dr Rudyk has been a Pulmonary Vascular Research Institute member since 2017 and received the Butrous Foundation YIA in 2018 in recognition of her contributions to pulmonary hypertension research.



Giovanni MANN (King's College London) *giovanni.mann@kcl.ac.uk*

Organiser, Chair: Session 1. Redox Regulation of Cell Signalling and Metabolism (10.45 - 11.45), Concluding Remarks

Biosketch: Professor Giovanni Mann is Professor of Vascular Physiology at King's College London. He holds a BSc in Zoology from George Washington University and an MSc/PhD in Physiology from University College London. An internationally recognised leader in redox biology, he is the current President of the Society for Free Radical Research-International (SFRRI). He has held leadership roles in multiple scientific societies, including SFRR-Europe, The Physiological Society, and the European and British Microcirculation Societies. Prof Mann serves as Associate Editor of Physiological Reviews, Senior Reviews Editor for Free Radical Biology & Medicine, and Editor for Redox Biology. He has authored over 200 publications and organised more than 45 symposia at international conferences. His Vascular Biology Group investigates oxidative stress-mediated vascular dysfunction, focusing on Nrf2-regulated antioxidant gene expression in endothelial and smooth muscle cells. Current research explores redox signalling in ischaemic stroke, atherosclerosis, and gestational diabetes, including metallomic profiling in stroke models in collaboration with the London Metallomics Facility.

Helena COCHEMÉ (MRC Laboratory of Medical Sciences)
helena.cocheme@lms.mrc.ac.uk

Chair: Session 3. Redox-Modulating Strategies and Diseases (14.00 - 15.00)

Biosketch: Dr Helena Cochemé is a UKRI Investigator at the MRC Laboratory of Medical Sciences (LMS), where she leads the Redox Metabolism group. She is also an Honorary Senior Lecturer at Imperial College London. She received her PhD in Biochemistry from the University of Cambridge in 2006, at the MRC Mitochondrial Biology Unit in the group of Prof. Mike Murphy, where she first developed her interest in mitochondrial oxidative stress and redox biology. She started working with *Drosophila* in 2007 as a post-doc at the Institute of Healthy Ageing, UCL in the laboratory of Prof. Linda Partridge. As part of her postdoctoral work, she characterised the mitochondria-targeted H_2O_2 probe, MitoB, and demonstrated that mitochondrial H_2O_2 levels increase *in vivo* with age. She established her own independent group at the LMS in 2013, and was promoted to Programme Leader Track in 2018, and to Programme Leader in 2025. Research in the Cochemé lab is focused on the impact of redox signalling in ageing and metabolic disorders, mainly using *Drosophila* as an *in vivo* model system. She is particularly interested in dissecting the role of redox-responsive cysteines in regulating survival, by applying a combination of redox proteomic and genetic knock-in approaches. Ultimately, her work aims to uncover novel evolutionary conserved redox-sensitive targets and potential therapeutic strategies for health and longevity benefits.

SPEAKER INFORMATION

James NATHAN (Cambridge Institute of Therapeutic Immunology & Infectious Disease, CITIID) *jan333@cam.ac.uk*

Plenary Session Invited Speaker: Redox and Metabolic Regulation (10.10 - 10.45)

Biosketch: Professor James Nathan is Professor of Respiratory Medicine at the University of Cambridge. His research focuses on the cellular mechanisms of oxygen and metabolite sensing. James received his PhD from the University of Cambridge with Professor Paul Lehner, and undertook his postdoctoral training at Harvard University with Professor Alfred Goldberg. He was awarded a Wellcome Senior Clinical Research Fellowship in 2014, which was renewed in 2019. He was also awarded a Lister Fellowship Prize in 2017 for his work on oxygen and metabolite sensing. James' research goal has been to understand how oxygen and metabolic pathways interlink to shape cell function. These processes are of clear biological importance, but they are also highly relevant to the hypoxic or nutrient-poor environments of inflamed tissues and tumours. To interrogate these processes, his group were early proponents of human phenotypic mutagenesis screens, recognising that this would allow mapping cell function to distinct oxygen or metabolic signals. He focused on Hypoxia Inducible Factors (HIFs), the key mediators of oxygen sensing. His group discovered how HIFs recruit chromatin machinery, how metabolic signals can activate HIFs even when oxygen is present, and how lipoylation signals to the HIF pathway. Most recently, he has uncovered new HIF-independent oxygen-sensing responses, which allow cells to adapt quickly to fluctuating oxygen levels. Current and future research is focused on (1) how HIF-independent oxygen-sensing responses work, (2) the interplay between metabolism and immune effector function, and (3) how the lipoylation pathway can be targeted in disease contexts.

Title: Lipoylation: a redox sensitive regulator of mitochondrial metabolism

Abstract: The conjugation of lipoic acid to proteins, termed lipoylation, is an essential redox sensitive modification for mitochondrial function. However, despite lipoylation being described since the 1950's, the regulatory roles of lipoic acid are only just being appreciated. Using genetic approaches in human cells, we uncovered a regulatory role for lipoylation at the interface of oxygen-sensing and metabolism. I will discuss how we identified this role for lipoylation, and how it led to the discovery of a new lipoylation enzyme, ABHD11. I will also outline how these fundamental observations are important in disease states, and strategies we are pursuing to target lipoylation for therapeutic benefit.

Elizabeth VEAL (Newcastle University) *elizabeth.veal@newcastle.ac.uk*

Session 1 Invited Speaker. Redox Regulation of Cell Signal. & Metabolism (10.45 - 11.10)

Biosketch: Dr Veal began her career as a Wellcome Prize student in Malcolm Jackson's lab at Liverpool University investigating stem cell regulation in muscular dystrophy. This was followed by a two year postdoc in Grace Gill's lab at Harvard Medical School investigating the function of a new candidate tumour suppressor gene in regulating transcription. Following her return to the UK and a short career break caring for her two children she undertook a second postdoc in Brian Morgan's lab at Newcastle University. This introduced her to the power of yeast as a model system for answering fundamental biological questions, and the unexpected discovery that peroxiredoxins were important for responses to hydrogen peroxide. In 2004 she was awarded an MRC Career Development Award to start her independent group in Newcastle. This CDA also allowed her to spend 3 months in Keith Blackwell's lab in Boston. Here she began to use C. elegans to investigate the role that peroxiredoxins played in redox signalling, stress resistance and ageing in metazoans. Over the last 20 years Elizabeth Veal's group at Newcastle University have continued to exploit the complementary strengths of different model systems (fission yeast, worms and mammalian cells) to provide new mechanistic insight into how different oxidants/ROS levels initiate appropriate responses in various stress conditions.

Title: Redox-signalling mechanisms regulating growth, ageing and responses to stress

Abstract: Oxidative damage caused by ROS contributes to many diseases and is generally considered to be a hallmark of ageing. However, it is now widely appreciated that hydrogen peroxide is also used as signalling molecule, not only to initiate ROS-protective responses, but also to regulate cell division, differentiation and migration. The best-characterised examples suggest peroxide signals are transduced via the reversible oxidation of specific cysteines in targeted signalling proteins. However, there are still significant gaps in our understanding of which proteins are targeted and how oxidation affects their activity. An important question is how peroxide-signalling even possible when much more peroxide-reactive and abundant thiol peroxidases, such as peroxiredoxins, are present. Dr Veal will present an overview of recent work from her lab that provides new answers to these questions. This work also illustrates the important physiological role/s peroxiredoxins play in coordinating growth, division and protection against stress.

Paraskevi (Pari) KRITSILIGKOU (University of Liverpool) *pari@liverpool.ac.uk*

Session 2 Invited Speaker: Cutting Edge Redox Technologies (12.00 - 12.25)

Biosketch: Dr Paraskevi (Pari) Kritsiligkou obtained her degree in Biology from the University of Crete, working with Kostas Tokatlidis on the oxidative protein folding machinery in mitochondria. For her masters, she studied Biochemistry at the University of Oxford, working with Stuart Ferguson and Christina Redfield on the disulfide bond formation system in bacteria. She joined the Wellcome Trust-funded PhD program "The Dynamics of Cellular Pathways" at the University of Manchester, where she explored redox signalling cascades using tadpoles, cell lines and yeast as model organisms. During her PhD thesis with Chris Grant, Pari studied the role of redox regulation in organelle homeostasis. Her passion to uncover novel redox regulated cascades led her to the lab of Tobias Dick at the German Cancer Research Center in Heidelberg where she developed tethered biosensor platforms that reveal the extent of intracellular redox heterogeneity. In September 2024 Pari joined the Department of Biochemistry, Cell and Systems Biology at the University of Liverpool as a Tenure Track Fellow. In her lab, Pari will continue to explore the impact of intracellular redox microenvironments, with a strong focus on redox signalling events in the nucleus.

Title: Utilising tethered biosensors to uncover intracellular redox heterogeneity

Abstract: Genetically encoded redox biosensors have revolusionised the field of redox biology. They provided avenues to understand the dynamics of glutathione and hydrogen peroxide and identify new components involved in their regulation. By targeting these probes to cellular compartments such as the mitochondria, we were able to understand further cross-organellar communication and redox signalling. However, as redox signalling perhaps requires proximity to occur, highly localised oxidation events are unseen by the conventional, freely diffusible probes. To overcome this limitation we generated tethered biosensors, fusing genetically encoded redox probes at the C-terminus of every single open reading frame in S. cerevisiae. This approach allows us to monitor the redox environment around each individual protein. By exposing our biosensor libraries to different metabolic conditions, we were able to show that redox heterogeneity even within one compartment exists and that this is specific to the protein the probe is fused to.

Joseph BURGOYNE (King's College London) *joseph.r.burgoyne@kcl.ac.uk*

Session 3 Invited Talk: Redox-Modulating Strategies and Diseases (14.00 - 14.25)

Biosketch: Dr Joseph Burgoyne is a Reader in Cardiovascular Biology in the School of Cardiovascular and Metabolic Medicine & Sciences at King's College London. Dr Burgoyne received his PhD from the King's College London in 2008 and continued his postdoctoral studies in 2008-2011 as Sir Henry Wellcome Postdoctoral Fellow at King's College London and Boston University in the USA before obtaining a British Heart Foundation Intermediate Fellowship in 2014. His current main research interest is on identifying and characterising novel cysteine redox sensors within the cardiovascular system. To achieve this, his group employs an interdisciplinary approach that integrates bioinformatics, metabolic, proteomic, epigenetic, and molecular techniques, allowing the investigation of signalling pathways at both the cellular and systemic levels. Previous studies led to the discovery of a redox sensor in cGMP-dependent protein kinase (PKG) that directly regulates blood pressure (Burgoyne JR, Science, 2007). This important finding facilitated the development of a new class of antihypertensive compounds (Burgoyne JR, Hypertension, 2017; Prysyazhna O, Circulation, 2019) and uncovered a potential therapeutic strategy for treating HFpEF. Additionally, his group has identified a novel mechanism that links blood pressure regulation with cytosolic DNA sensing, a key player in sepsis pathophysiology, where it mediates hypotension and tissue hypoperfusion (Su J, Circulation, 2023). Beyond these findings, Dr Burgoyne's work has led to the identification of new redox sensors that regulate autophagy (Frudd K, Nature Communications, 2018), as well as other key processes that are currently under investigation.

Title: Targeting thiol-dependent PKGI activation for therapy

Abstract: Heart failure with preserved ejection fraction (HFpEF) is a highly prevalent condition associated with substantial morbidity and mortality, yet effective therapeutic options remain limited. Oxidation of cGMP-dependent protein kinase Iα (PKGIα) has been shown to enhance diastolic relaxation, a process impaired in HFpEF. To explore new therapeutic avenues, we evaluated natural compounds for their ability to oxidise and activate PKGIα. Through this approach, we identified urolithin A as a natural compound that improves diastolic function and limits cardiac remodeling in a preclinical HFpEF model by specifically targeting cysteine 42 of PKGIα. These findings were further validated in human engineered heart tissue, where urolithin A enhanced both relaxation and contraction kinetics. Collectively, our results highlight C42 in PKGIα as a promising therapeutic target and support the potential of urolithin A for the treatment of human HFpEF.

ATTENDEES (in person)

Name	Organisation
Ahluwalia, Amrita	Queen Mary University of London, WHRI
Alam, Sara	London Institute of Medical Sciences
Aldaz Casanova, Silvia	Babraham Institute
Amore, Francesca	Queen Mary University of London, WHRI
Arjun, Sapna	Queen Mary University of London, WHRI
Bharadwaj, Nihal	Biological E.Limited
Blower, Phil	King's College London
Burgoyne, Joseph	King's College London
Byrne, Dominic	University of Liverpool
Cho, Hyunju	Queen Mary University of London, WHRI
Cocheme, Helena	MRC Laboratory of Medical Sciences (LMS)
Coleman, Pierre	King's College London
Cook, Jacob	Aston University
Corr, Andrea	PCR Biosystems
Coupe, David	Institute of Cancer Research (ICR)
Cowley, Alexander	Institute of Cancer Research (ICR)
Crabtree, Mark	University of Surrey
Dayalan Naidu, Sharadha	University of Dundee
Durie, Emily	Institute of Cancer Research (ICR)
Featherston, Therese	Babraham Institute
Fernandez-Mosquera, Lorena	Queen Mary University of London, WHRI
Finch, Andy	Queen Mary University of London, Barts Cancer Institute
Fraser, Paul	King's College London
Gentier, Anouk	Queen Mary University of London
Gomes-Neto, Joana	Queen Mary University of London, WHRI
Green, Hannah	King's College London
Grice, Guinevere	University of Cambridge, Cambridge Infectious Diseases (CITIID)
Holbrook, Lisa	University of Surrey
Iqra, Sofina	Aston University
Jordon, Louise	University of Cambridge, Cambridge Stem Cell Institute
Kritsiligkou, Paraskevi	University of Liverpool
Kumar Chauhan, Anish	All India Institute of Medical Sciences New Delhi
Lai, Hinyuk	Institute of Cancer Research (ICR)
Leung, Jacky	Institute of Cancer Research (ICR)
Lennicke, Claudia	MRC Laboratory of Medical Sciences (LMS)
Lim, Wen Kin	Babraham Institute
Malanchuk, Oksana	University College London
Mann, Giovanni	King's College London
Manna, Suman	University College London
Mansfeld, Jörg	Institute of Cancer Research (ICR)
Marriott, Eloise	Queen Mary University of London, WHRI
Mehtani, Drshika	Babraham Institute
Miljkovic, Jan	University of Cambridge, MRC Mitochondrial Biology Unit
Nathan, James	University of Cambridge, Cambridge Stem Cell Institute
Pantoja, Yanisleidys	Aston University
Patel, Priyanka	University of Surrey
Peck, Barrie	Queen Mary University of London, Barts Cancer Institute

Rahman, Oishee	Babraham Institute
Rudyk, Olena	King's College London
Ryan, Panchal	Institute of Cancer Research (ICR)
Sateri, Kianoosh	Queen Mary University of London, Barts Cancer Institute
Sharpe, Hayley	Babraham Institute
Sidlauskaite, Eva	Queen Mary University of London, WHRI
Simoes de Jesus, Daniel	Queen Mary University of London, WHRI
Spickett, Corinne M.	Aston University
Su, Jie (Vivian)	King's College London
Switzer, Christopher	University of Leicester
Tangjitmungman, Monthon	University College London
Thomas, David	University of Cambridge
Torley, Elizabeth	Institute of Cancer Research (ICR)
Tuncay, Ahmet	Babraham Institute
Veal, Elizabeth	Newcastle University
Vuina, Karla	Institute of Cancer Research (ICR)
Wadsworth, Amber	Imperial College London
Wojdyla, Katarzyna	Babraham Institute
Wu, Pei	University of Cambridge, Cambridge Infectious Diseases (CITIID)
Wu, Xiaoqi	University College London
Yap, Vincent	MRC Laboratory of Medical Sciences (LMS)
Yong, Tanja	King's College London
Zhang, Dejun	University College London
Zhao, Yuhan (Tracy)	University College London
Zhou, Xuezhe	University College London

POSTER SESSION 15.30 - 17.00 *Indicates presenting author

Poster 1: Precision Persulfide Generation provides insight into the mitochondrial sulfane sulfur pool

*Suman Manna, Ragini Agrawal, Harinath Chakrapani and Amit Singh

University College London.

Poster 2: Covalent probe screening for a selective cysteine-based inhibitor of CDK4 to reverse pulmonary hypertension

*Hannah L. H. Green, Joana Cartaxo-Serralha, Hannah Knight, Chiara Naddeo, Miguel Mano, Olena Rudyk

School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, London, UK.

Poster 3: Redox regulation of the cell cycle protein PCNA

*Elizabeth Torley[1], Julia Vorhauser[1], Andreas Prestel[2], Birthe Brandt Kragelund[2], Joerg Mansfeld[1]

[1] Institute of Cancer Research, UK; [2] University of Copenhagen, Denmark.

Poster 4: ReDisulphID: a structural bioinformatic screen for identifying drug-targetable redox-regulated disulphide

*Pierre Coleman, Anna Laddach, Franca Fraternali, Joseph R Burgoyne

School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, London, UK.

Poster 5: Investigating the Role of Protein Cysteine Oxidation in Breast and Prostate Cancer

*Jacky Kieran Leung, Theo Roumeliotis, Jyoti Choudhary, and Jörg Mansfeld

Institute of Cancer Research, UK.

Poster 6: Investigating the redox regulation of G6PD in metabolism and survival

*Vincent Yap, Claudia Lennicke, Helena Cocheme

MRC Laboratory of Medical Sciences (LMS) & Imperial College London, London, UK.

Poster 7: Targeting Cysteine 42 in PKGIα Limits Diastolic Dysfunction in HFpEF

*Jie Su, Joseph R. Burgoyne

School of Cardiovascular and Metabolic Medicine & Sciences, King's College London; The British Heart Foundation Centre of Excellence, The Rayne Institute, St Thomas' Hospital, London, SE1 7EH, UK.

Poster 8: Pharmacologic and genetic activation of Nrf2 confers anti-fibrotic effects in liver fibrosis

*Sharadha Dayalan Naidu[1], John D. Hayes[1] and Albena T. Dinkova-Kostova[1,2]

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Poster 9: Identification of Redox Switches in the Cell Cycle Machinery Highlights p21 as a Redox-Regulated Cell Cycle Inhibitor

Julia Vorhauser[1,2], Theodoros I. Roumeliotis[1], Jacky K. Leung[1], *David Coupe[1], Lu Yu[1], Kristin Böhlig[3], André Nadler[3], Jyoti S. Choudhary[1], Jörg Mansfeld[1,2]

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Poster 10: Revitalising Regeneration: Boosting Pericytes for Next-Generation Healing

*Sofina Iqra, Jill Johnson

School of Biosciences, Aston University, Birmingham, UK.

Poster 11: A novel mass spectrometry-based approach for quantifying protein and low molecular persulfides in biological systems

*Jan Lj. Miljkovic[1], Nils Burger[1], Chak Shun Yu[1], Alexander H. Harkiss[2], Stefan Warrington[2], Stuart T. Caldwell[2], Scott A. Jones[1], Dunja Aksentijevic[4], Andrew M. James[1], Thomas Krieg[3], Richard C. Hartley[2] & Michael P. Murphy[1,3]

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Poster 12: Investigating lipid profiles and oxidised phospholipids in samples from patients with chronic venous leg ulcers

*Jacob Cook[1], Corinne Spickett1, Irundika Dias[2], Michael Wall[3]

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Poster 13: Mechano-regulation of redox balance at the plasma membrane

*Oishee Rahman, Silvia Aldaz Casanova, Hayley Sharpe

Signalling ISP, The Babraham Institute, Cambridge, UK.

Poster 14: CRISPR-dCas9 mediated overexpression of FAT1 gene in low endogenous FAT1 expressing glioma cells

*Chauhan Kumar Anish, Gupta Yakhlesh, Goswami Sanjeev, Kapoor Mani, Arora Manvi, Aimol Mona Lanu, Gupta Shruti, Chattopadhyay Parthaprasad, Sinha Subrata, Chosdol Kunzang

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Precision Persulfide Generation provides insight into the mitochondrial sulfane sulfur pool (poster presentation)

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Reactive sulfur species (RSS), such as Hydrogen sulfide (H2S), Persulfides (RS-SH), and Polysulfides (RSnH; n>2) are the major players in redox biology, which can mediate diverse signaling pathways and possess a plethora of exciting therapeutic opportunities. Nevertheless, the biological distinctions between different sulfur species are ignored because of the rapid interconversion that occurs among these species. 3-mercaptopyruvate sulphurtransferase (3-MPST) is one of the enzymatic systems that contribute to generating various sulfur species. MPST activity contributes to the cellular sulfane sulfur pool by producing low molecular weight persulfides and increased protein persulfidation within the cell. Within cells, the expression of MPST is higher in the mitochondrion and thus, MPST also plays a major role in enhancing the mitochondrial sulfane pool (MSS). Because of their instability, high reactivity, and scarcity of appropriate donors, persulfides remain difficult to study. Using a designed substrate for mitochondrial 3-MPST as well as cytoplasmic 3-MPST, which can specifically generate sulfane sulfur in these specific compartments, we examined the cellular responses in terms of redox balance and membrane potential. We discovered notable variations between the two species, supporting the importance of the location of the generation of sulfane sulfur. It has recently been determined that H2S plays a part in preventing the HIV-1 virus from emerging from latency. We specifically checked the activity of MSS and CSS, in controlling the virus reactivation. Surprisingly, we discovered that the presence of MSS boosted the virus's reactivation from latency. Our study opens new avenues of differential response to different sulfur species.

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Covalent probe screening for a selective cysteine-based inhibitor of CDK4 to reverse pulmonary hypertension (poster presentation)

*Hannah L. H. Green, Joana Cartaxo-Serralha, Hannah Knight, Chiara Naddeo, Miguel Mano, Olena Rudyk

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Pulmonary hypertension (PH) is a devastating disease with an unmet need for treatments to reverse vascular remodelling and alleviate pulmonary arterial pressure to prolong life. Hyperproliferation of pulmonary vascular cells is a key pathological component of PH, a process which is known to involve altered cellular redox balance and perturbation of cell cycle processes. Recently, we described a novel redox-regulated heterodimeric disulfide dimer between CDK4 C135 and Cyclin D C7/8, which acts to inhibit cyclin D-CDK4 kinase activity and attenuate pulmonary vascular cell proliferation. CDK4 C135 is a critical residue that is close to the kinase activation segment that permits catalysis and hence allosterically regulates its function. CDK4 C135 is indispensable for cyclin D-CDK4 kinase activity such that its oxidation, mutation or alkylation inhibits

phosphorylation of its main substrate retinoblastoma protein (Rb). In rodent models of PH, promoting the cyclin D-CDK4 disulfide in a non-selective manner by auranofin treatment, or inhibition of cyclin D-CDK4 activity by C135 mutation to alanine demonstrated that targeting this redox-switch may be therapeutically beneficial in PH. Our current work aims to identify a selective cysteine-based inhibitor targeting CDK4 C135 that could be further developed for this purpose. A high-throughput/high-content screening assay was designed and optimised to assess cyclin D-CDK4 activity by measuring phosphorylation of Rb (pRb) at position S780 in nuclei of human pulmonary artery smooth muscle cells. A library of 960 electrophilic covalent probes was screened for efficacy at decreasing pRb during an acute treatment of 1 hour (10-20mM). Several positive hits were identified which reproducibly decreased pRb to a similar extent as the FDA-approved breast cancer drug palbociclib which inhibits CDK4/6 *via* competitive binding to its ATP-binding pocket. Ongoing work aims to validate the specificity of hit compounds for CDK4 C135 using cell-based and *in vitro* kinase assays, determine dose-response relationships, and assess the ability of hit compounds to attenuate proliferation of pulmonary vascular cells before *in vivo* testing in experimental models of PH. It is hoped that this may contribute to a translational approach towards the development of a covalent allosteric inhibitor of CDK4 for the treatment of PH.

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Redox regulation of the cell cycle protein PCNA (poster presentation)

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Aberrations in both redox regulation and the cell cycle are common in cancers (Hanahan & Weinberg, 2000; Jorgenson et al., 2013); understanding of these aberrations is key to developing new cancer therapies. Our lab, along with others, have demonstrated two-way interplay between the cell cycle and redox regulation (Kirova et al., 2022), although much remains to be understood about the underlying molecular mechanisms. Crucially, very little is known about the interplay of redox regulation and the cell cycle in cancer.

I am investigating redox regulation of PCNA (Proliferating Cell Nuclear Antigen), a DNA clamp which shows variable oxidation throughout the cell cycle, and between cancer and non-cancer systems. Its many functions include DNA replication, DNA damage repair, chromatin assembly, and cell cycle regulation. These are largely mediated by recruitment of over 400 known PCNA-interacting proteins (PIPs), via a short, linear, unstructured motif (Horsfall et al., 2021). I hypothesise that the formation of a specific disulfide within PCNA (evidenced in around 50% of crystal structures) mediates a conformational change which modulates PIP binding. Depending on the PIPs implicated, this could have a variety of downstream effects on the cell cycle.

To this end, I am validating tools to manipulate PCNA oxidation state, both in cells and in vitro. This includes mutating cysteine residues to abolish their oxidation, targeting redox enzymes to PCNA, and direct treatment with oxidising or reducing agents. Subsequently, I can study the effect on PCNA structure and functions – as well as resulting cell cycle phenotypes – using techniques such as NMR, mass spectrometry, Western Blot, and SPOT binding assay.

For example, preliminary mass spectrometry and Western Blot analyses suggest that PCNA oxidation state affects its binding affinity to multiple PIPs, including DNA polymerase δ, and the licensing factor Cdt1 (as well as Cdt2, an E3 ubiquitin ligase which targets Cdt1 for degradation). I aim to establish the cell-based tools in both cancer (MCF7) and non-cancer (RPE-1, MCF10A) cell lines. By comparing the mechanisms and consequences of PCNA oxidation between these systems, I hope to inspire new avenues for cancer therapy.

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ReDisulphID: a structural bioinformatic screen for identifying drug-targetable redox-regulated disulphides (poster presentation)

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Redox modifications of thiols are critical regulators of protein function in both physiological and pathological contexts. These thiols offer promising targets for therapeutic intervention due to their reactivity with small-molecule electrophiles. In particular, thiols that form redox-active disulphide bonds are likely to have the highest therapeutic potential, given the critical role of these modification in regulating protein function. Redox disulphides regulate protein function by imposing structural constraints that significantly affect protein-protein interactions or enzymatic activity. Despite their therapeutic potential, there has been no broad identification of reversible redox-regulated disulphides due to limitations in their detection in complex biological systems.

To address this, we developed ReDisulphID, a bioinformatic tool that systematically identifies novel reversible redox-sensitive disulphides across mammalian proteins in the RCSB Protein Data Bank (RCSB PDB). By establishing characteristics of known redox-regulated disulphides, ReDisulphID assigns a redox-activity score to cysteine pairs in these protein structures. Through this approach, over 40,000 candidate disulphides were scored, identifying those most likely to exhibit redox sensitivity. This was combined with ligandability screens to pinpoint over 2,000 drug-targetable candidates.

Following its development, ReDisulphID facilitated the discovery of a previously unidentified redox sensor within PEPD, which plays a key role in regulating activation of genome guardian p53. Further investigation validated a compound capable of activating p53 by specifically targeting this novel redox sensor in PEPD. These results underscore the potential of ReDisulphID as a robust platform for identifying novel therapeutic targets through the discovery of druggable redox-active disulphide bonds.

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Investigating the Role of Protein Cysteine Oxidation in Breast and Prostate Cancer (poster presentation)

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Reactive oxygen species (ROS) and redox homeostasis are vital to the proper function of cells, and they are relevant throughout all stages of cancer progression. When there is oxidative stress, ROS reacts with cysteine thiols, which can modify proteins by altering their structure and function. However, the specific ways in which ROS can impact cancer cell function are not well understood. To investigate the role of cysteine oxidation in hormone-dependent breast and prostate cancer, we conducted a comprehensive proteome-wide analysis of cysteine oxidation in MCF7 breast cancer cells. We developed a differential alkylation method, which allowed us to identify thousands of cysteine residues that are reactive and can undergo reversible oxidative modification. Many of the sites identified are involved in crucial pathways for tumourigenesis, such as proliferation and cell death. We further examined the relationship between hormone signalling and cysteine sulfenylation in cell-based assays. Our findings support the relevance of protein cysteine oxidation in the context of hormone-dependent cancers, highlighting their importance in understanding the complexities of cancer progression.

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Investigating the redox regulation of G6PD in metabolism and survival (poster presentation)

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Glucose-6-phosphate dehydrogenase (G6PD) is a critical enzyme in cellular metabolism, playing a key role in the pentose phosphate pathway (PPP). By catalysing the conversion of glucose-6-phosphate into 6-phosphogluconolactone, G6PD is vital for redirecting glucose from glycolysis towards the PPP. G6PD also plays a pivotal role in the synthesis of NADPH, which is an essential cofactor for biosynthetic reactions and the maintenance of cellular redox balance. Interestingly, redox proteomic studies have shown that some G6PD cysteines are redox-sensitive under oxidising conditions, hinting at a role in dictating G6PD activity. Despite this, detailed mechanistic studies exploring the redox regulation of G6PD are notably lacking. In this study, we aim to elucidate the functional significance of cysteine residues in G6PD. Utilising HEK293T cells, we are investigating the role these cysteine residues play in modulating enzyme activity and function under varying redox and nutritional conditions. Furthermore, by using the CRISPR-Cas9 system, we will specifically modify these cysteine residues to assess their contribution to the redox regulation of G6PD. Our research will provide insights into how redox changes influence G6PD activity and its subsequent impact on cellular metabolism. Understanding these mechanisms is crucial, as dysregulation of G6PD has been implicated in various metabolic disorders and diseases.

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Targeting Cysteine 42 in PKGIα Limits Diastolic Dysfunction in HFpEF (poster presentation)

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Heart failure with preserved ejection fraction (HFpEF) is a prevalent condition with high morbidity and mortality, yet effective treatment options remain limited. To explore novel therapeutic strategies, we investigated the role of cysteine 42 (C42) in cGMP-dependent protein kinase Iα (PKGIα), a key regulator of vascular tone and diastolic function. Our study identified urolithin A as a potent activator of PKGIα through C42 targeting, significantly improving diastolic dysfunction and attenuating cardiac remodeling in a multi-hit HFpEF mouse model. Furthermore, urolithin A enhanced relaxation and contraction kinetics in engineered human heart tissue, underscoring its clinical relevance.

To elucidate its mechanism, we screened polyphenols, including urolithin A, for their ability to oxidise and activate PKGIα. This was assessed using recombinant protein assays, isolated rat aortic smooth muscle cells, and myography studies in mesenteric vessels. We further examined the effect of urolithin A on PKGIα-dependent phosphorylation of phospholamban (PLN) in neonatal rat ventricular myocytes (NRVMs). HFpEF was induced in wild-type and C42S PKGIα knock-in (KI) mice using a multi-hit approach, followed by treatment with either vehicle or urolithin A via oral gavage. Cardiac function was evaluated through echocardiography, and translational relevance was assessed in engineered human heart tissue.

Our findings revealed that quercetin, fisetin, and urolithin A activated PKGIα in a C42-dependent manner, promoting vasorelaxation, which was impaired in mesenteric vessels from C42S KI mice. In cardiomyocytes, urolithin A induced PKGIα-dependent phosphorylation of PLN. In the HFpEF mouse model, urolithin A reversed diastolic dysfunction and cardiac remodeling in wild-type mice but had no significant effect on KI mice, confirming the cardioprotective role that was dependent on C42 in PKGIα. Notably, urolithin A also improved relaxation and contraction kinetics in engineered human heart tissue, highlighting its potential for clinical application. These findings recognised urolithin A as a promising therapeutic option for HFpEF by enhancing thiol-dependent PKGIα activation. Its efficacy in preclinical models, coupled with a favorable safety profile and bioavailability, supports further clinical translation to address this challenging and underserved condition.

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Pharmacologic and genetic activation of Nrf2 confers anti-fibrotic effects in liver fibrosis (poster presentation)

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Chronic liver injury causes hepatic fibrosis, which if unresolved, leads to cirrhosis and ultimately liver failure requiring transplantation. Only one clinically-approved drug is available for the treatment of liver fibrosis,

highlighting an unmet clinical need. The hepatic stellate cells (HSCs), when transdifferentiated into proliferative myofibroblasts by highly potent cytokines such as transforming growth factor beta 1 (Tgfβ1), are the main cells responsible for liver fibrosis. It was previously shown that the electrophilic Nrf2 activator 4-octyl-itaconate decreased the levels of collagen and reactive oxygen species (ROS) in response to Tgfβ1 in systemic sclerosis patient-derived dermal fibroblasts. Thus, we hypothesized that Nrf2 activation in HSCs can counteract Tgfβ1 signaling, thereby inhibiting fibrogenesis. To activate Nrf2 pharmacologically, we used two types of Nrf2 activators, an electrophile and a non-electrophilic Keap1-Nrf2 protein-protein interaction inhibitor. Both Nrf2 activators decreased the Tgfβ1-induced expression of the pro-fibrotic markers collagen 1a1 and fibronectin. A global overview using high-resolution proteomics further revealed that pharmacological Nrf2 activation orchestrates a potent anti-fibrotic program. In search for potential mechanism(s), we considered that 4-OI has been shown to induce miR-29a, an Nrf2-regulated miRNA that suppresses collagen expression. Therefore, we performed small-RNA sequencing in HSCs to probe for small RNAs that could be regulated by Nrf2 in the context of hepatic fibrosis. Our results unveiled unique miRNA signatures induced by both types of Nrf2 activators in the absence or presence of Tgfβ1. We performed multiomics analysis, coupling our proteomics and miRNA sequencing to uncover the miRNA-target gene predictions and conducted functional enrichment analyses. Our findings support the development of miRNA mimics or Nrf2 activators as potential therapeutic agents to prevent and treat liver fibrosis.

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Identification of Redox Switches in the Cell Cycle Machinery Highlights p21 as a Redox-Regulated Cell Cycle Inhibitor (poster presentation)

Julia Vorhauser[1,2], Theodoros I. Roumeliotis[1], Jacky K. Leung[1], *David Coupe[1], Lu Yu[1], Kristin Böhlig[3], André Nadler[3], Jyoti S. Choudhary[1], Jörg Mansfeld[1,2]

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The cell cycle is tightly regulated by an internal control system that utilizes post translational modifications (PTMs) such as phosphorylation to regulate core cell cycle proteins. Besides this well-known control mechanism, we and others observed that the intracellular concentration of Reactive Oxygen Species (ROS) increases during the cell cycle and this increase is necessary for cell cycle progression[1–3]. Supporting this idea, recent studies show that ROS directly target core cell cycle proteins, such as CDK4, CDK2, and Aurora A, to regulate their activity and drive cell cycle progression[1,4–6]. Whether ROS affect other cell cycle proteins and serve as a general regulatory mechanism remains unclear.

Here, we performed redox proteomic analyses to identify cysteines of cell cycle proteins that are oxidized to sulfenic acids at different stages of the cell cycle. Our findings reveal that overall cysteine sulfenylation remain stable for most proteins, but a subset exhibits cell cycle stage-dependent oxidation. Among these is the CDK inhibitor p21, which is predominantly oxidized during G2 phase. Oxidation of a single cysteine residue (C41) on p21 during G2 determines proliferation. When C41 cannot be oxidized in G2, daughter cells inherit more p21, reducing proliferation and inducing senescence after irradiation. Mechanistically, C41 oxidation redirects p21 from CDK4 to CDK2. Since CDK2 promotes p21 degradation 7, this redirection slows p21 degradation and increases its inheritance. Based on these findings, we propose a model in which p21's oxidation state

regulates its binding to CDK4 or CDK2, modulating p21 levels. In this way p21 oxidation may serve as a sensor of cellular activity, with ROS driving p21 oxidation and facilitating cell cycle progression.

In summary, this work provides a quantitative, cell cycle-dependent redox proteome and demonstrates, using p21 as an example, how oxidation-induced changes in core cell cycle proteins can influence cell fate.

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Revitalising Regeneration: Boosting Pericytes for Next-Generation Healing (poster presentation)

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Background: Human placenta-derived pericytes (hPC-PL) are multipotent cells found along the microvasculature, playing key roles in angiogenesis, vascular integrity, tissue repair, and immune modulation. Their ability to differentiate into multiple cell lineages, such as osteoblasts, chondrocytes, and adipocytes, makes them promising candidates for regenerative medicine applications. This study aims to optimise culture conditions and develop specialized differentiation media to investigate the differentiation potential of hPC-PL. Additionally, comparisons with bone marrow-derived mesenchymal stem cells (MSCs) are being conducted to evaluate their relative regenerative efficacy.

Method: Culture conditions for human placenta-derived pericytes (hPC-PL) were optimised, and specialised media were developed to induce lineage-specific differentiation into osteoblasts, chondrocytes, and adipocytes. Differentiation was assessed over 21 days using visual inspection and histological staining methods: Alcian Blue staining for chondrogenic differentiation, Alizarin Red staining for osteogenic differentiation, and Oil Red O staining for adipogenic differentiation. Morphological changes and positive staining were used to evaluate lineage commitment and differentiation potential under each condition.

Results: Preliminary results demonstrated distinct morphological changes and early positive histological staining in hPC-PL cultures, suggesting successful differentiation into osteoblasts, chondrocytes, and adipocytes. Under their respective differentiation media, hPC-PL exhibited robust lineage-specific commitment, with responses comparable to those observed in bone marrow-derived MSCs. Further quantitative and molecular analyses are pending to confirm these findings.
Discussion: These preliminary results highlight the promising multipotency of hPC-PL and their potential for use in tissue engineering and regenerative therapies. Comparisons with MSCs suggest similar differentiation capabilities, but further investigation is needed to validate these observations. Future work will focus on transcription factor expression profiles to confirm lineage-specific differentiation at the molecular level, evaluate the influence of inflammatory mediators on differentiation potential, and conduct comprehensive comparative studies with MSCs. These insights will help identify key factors guiding pericyte differentiation, ultimately advancing their application in regenerative medicine.

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Investigating lipid profiles and oxidised phospholipids in samples from patients with chronic venous leg ulcers (poster presentation)

*Jacob Cook[1], Corinne Spickett1, Irundika Dias[2], Michael Wall[3]

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Venous leg ulcers (VLU) are a growing problem to both patients, who experience poor outcomes and quality of life, and healthcare systems, which are becoming increasingly overwhelmed with economic burden. The key pathophysiological role of inflammation in preventing healing is well understood through analysis of cytokines and MMPs, but a systematic review of the literature has shown that oxidative stress and biological effects of lipids have been considered in less detail. As inflammation can lead to oxidative stress, the aim of this study is to investigate levels of oxidised phospholipids (OxPL) and changes in lipid profiles in various sample types of chronic VLU. Biopsies from the centre and edge of the ulcer, plasma from the arm (systemic) and leg (local), and exudate samples, gathered using either filter paper or needle aspiration, were collected from patients with healing and non-healing chronic VLU. Using both untargeted and targeted LC-MS/MS techniques, the lipidome and OxPLs of each sample were analysed, respectively. IL-1β concentrations were also analysed by ELISA. Collecting exudate using filter paper was easier and more consistent than aspiration, due to some ulcers being inherently drier. OxPLs (PGPC, POVPC, PONPC, SGPC, SOVPC and SONPC) were successfully measured in all sample types. Significantly higher levels of IL-1β and OxPLs (all but SOVPC) were identified in some patients' biopsies from the centre of the ulcer, compared to the edge. Significant increases in plasma from the leg compared to the arm were only noticed for SOVPC in some patients. Overall, this early data demonstrates the feasibility to collect biopsies, exudate using filter paper, and plasma, and use these samples to identify lipid profiles and, more specifically, OxPLs using LC-MS/MS. This data also suggests the implications of oxidative stress in VLU chronicity, and that this may further feed into the inflammatory cycle.

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Mechano-regulation of redox balance at the plasma membrane (poster presentation)

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Cells regulate oxidant production to maintain balance between oxidative eustress and stress; and have harnessed Hydrogen Peroxide (H2O2) to be used in the immune system, signalling and metabolism. One way that H2O2 can be generated at the plasma membrane by an enzyme complex (NOX/DUOX) at the plasma membrane, where H2O2 is thought to re-enter the cell, and acts as a second messenger via aquaporins. Targets of intracellular H2O2-oxidation include Cysteine-based proteins, namely Protein Tyrosine Phosphatases (PTPs), which change conformation when oxidised by H2O2. This affects processes such as cell adhesion, migration and growth, and may also be perturbed in ageing, cancers and disease. However, the dynamics of H2O2 signalling and its roles as second messenger remain poorly defined; and historically reliance on non-specific dyes have been a limiting factor for the field. Regulation of H2O2 at the plasma membrane is also poorly understood.

HyPer7 is a Hydrogen Peroxide GFP-based biosensor, which is highly sensitive and undergoes a conformational change upon oxidation by H2O2 specifically, and results in a spectral shift, which is detected via live cell imaging. We have optimised a HyPer7 probe localised to the plasma membrane (PM-HyPer7), to investigate H2O2 spatiotemporal dynamics at the plasma membrane, where many of the PTPs are also localised. Our PM-HyPer7 biosensor was able to detect exogenous H2O2 (in nM range) in U2OS osteosarcoma cells, and the PM-HyPer7 can detect growth factor induced H2O2 when stimulated with growth factors such as Insulin and TGF-β. We have also developed a Drosophila melanogaster and knock-in mouse model expressing the biosensor, to investigate endogenous H2O2 dynamics in a range of cell types such as mouse embryonic fibroblasts and ovarian cells.

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CRISPR-dCas9 mediated overexpression of FAT1 gene in low endogenous FAT1 expressing glioma cells (poster presentation)

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Introduction: Advancements in genome editing technologies have opened new avenues for investigating the function of specific genes in cancer. The clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9) system has emerged as a powerful tool for precise manipulation of gene expression. In this study, we employed CRISPR-dCas9 technology to investigate the effects of FAT1 overexpression in low endogenous FAT1 expressing cancer-cells. The FAT1, atypical cadherin family, in cancer remains complex, with evidence suggesting both tumor-suppressive and oncogenic functions depending on the cellular context. In glioma FAT1 has been implicated as an oncogene.

Aim and Objectives: •To investigate the impact of CRISPR-dCas9-mediated overexpression of the FAT1 gene in cancer cells with low endogenous FAT1 expression. • To design FAT1 promoter specific single-guide RNAs (sgNAs) for targeted overexpression. • To use CRISPR-dCas9 system containing pLentiSAMV2 (sgRNA) and pLentiMPH (HSF-1, P65) along with packaging plasmids (pMD2.G& psPAX2). • To transduce Lentiviral into low-FAT1 expressing cell lines (T98G) and development of stable cell lines. • Verification of overexpression of FAT1 using quantitative PCR.

Material & Methods: pLentiSAMV2 (for cloning sgRNA) and pLentiMPH (HSF-1, P65) was used, accompanied by lentivirus packaging plasmids (pMD2.G & psPAX2). FAT1 promoter (-300bp to -200bp of TSS) targeting sgRNAs (sgRNA1/sgRNA2/sgRNA3) has been designed using online software 'ChopChop'. The sgRNAs were commercially synthesized and cloned into pLentiSAMV2 and sequenced. The sgRNA-cloned pLentiSAMV2, along with the packaging-plasmids, was co-transfected into HEK293T cells, for lentivirus production. The lentivirus collected and transduced into low FAT1-expressing T98G glioma cells, transduced cell lines were selected using selection antibiotic. The expression levels of FAT1 were assessed in both transduced and non-transduced low FAT1-expressing glioma T98G cells by q-PCR.

Results: T98G (glioma cell line) exhibited the lowest endogenous-FAT1 expression, and selected for FAT1 overexpression. sgRNAs were modified at 5'/3' ends, annealed and cloned into pLentiSAMV2-vector, transformed into E. Coli and confirmed by sequencing. Cloned LentiSAMV2 with 2nd generation packaging

plasmids were transfected in HEK293T cells. Virus collected from transfected HEK293T cells (BSL-2 bio-safety laminar flow hood) and stored at-800C. The virus particles (containing LentiMPH and LentiSAM v2) were tranduced into T98G cells. Transduced cells were selected with blasticidin+hygromycin. Stable clones developed were processed for expression of FAT1 by q-PCR. As compared to control, cells transfected with sgRNA3 cloned vector showed increase 2.5 fold-FAT1 expression. We further validated the FAT1 gene expression using luciferase reporter assay and upregulated.

Conclusion: We have successfully designed and cloned 3 FAT1 specific sgRNAs, used for lentivirus mediated transfection into T98G cells. sgRNA1 exhibited increased expression of FAT1 reflecting successful standardization of the CRISPR mediated overexpression. This system may further be implicated in studying the effect of gene overexpression on cellular function.

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Indicator study- Linking Lipid Oxidation to Outcomes in Drug-Coated Balloon Therapy for De Novo Coronary Artery Disease (not presenting)

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Coronary artery disease (CAD) remains one of the leading causes of morbidity and mortality worldwide. The development of Drug-Coated Balloon (DCB) therapy represents a promising advancement in the management of CAD, offering the potential to reduce restenosis and improve long-term patient outcomes. This therapy involves the delivery of antiproliferative drugs directly to the vascular wall during balloon angioplasty, aimed at preventing neointimal hyperplasia and reducing the likelihood of vessel reocclusion. Despite its clinical promise, the underlying mechanisms of DCB therapy and its long-term efficacy remain areas of active investigation.

This research aims to evaluate the impact of Drug-Coated Balloon therapy on plaque burden in patients with coronary artery disease, utilising advanced imaging and lipid profiling techniques. Specifically, the study investigates the effects of DCB therapy on lipid profiles and vascular remodelling in patients undergoing percutaneous coronary interventions (PCI). Our approach combines mass spectrometry-based lipidomics to analyse plasma samples and intracoronary ultrasound (IVUS) to assess plaque regression.
We hypothesise that DCB therapy will lead to favourable changes in lipid profiles and a reduction in plaque burden, contributing to improved clinical outcomes and enhanced patient quality of life. This study will provide novel insights into the clinical application of DCB therapy, informing future treatment strategies for CAD and potentially establishing new biomarkers for treatment efficacy.

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Aurora A regulation by reversible cysteine oxidation reveals evolutionarily conserved redox control of Ser/Thr protein kinase activity (oral presentation)

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Reactive oxygen species (ROS) are physiological mediators of cellular signaling and play potentially damaging roles in human diseases. In this study, we found that the catalytic activity of the Ser/Thr kinase Aurora A was inhibited by the oxidation of a conserved cysteine residue (Cys290) that lies adjacent to Thr288, a critical phosphorylation site in the activation segment. Cys is present at the equivalent position in ~100 human Ser/Thr kinases, a residue that we found was important not only for the activity of human Aurora A but also for that of fission yeast MAPK-activated kinase (Srk1) and PKA (Pka1). Moreover, the presence of this conserved Cys predicted biochemical redox sensitivity among a cohort of human CAMK, AGC, and AGC-like kinases. Thus, we predict that redox modulation of the conserved Cys290 of Aurora A may be an underappreciated regulatory mechanism that is widespread in eukaryotic Ser/Thr kinases. Given the key biological roles of these enzymes, these findings have implications for understanding physiological and pathological responses to ROS and highlight the importance of protein kinase regulation through multivalent modification of the activation segment.

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The loss of SDHB enhances mitochondrial contact sites with the nucleus (NAM), leading to dysregulated cellular metabolism (oral presentation)

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Introduction: Succinate dehydrogenase (SDH) plays a crucial role in the tricarboxylic acid cycle and the electron transport chain. Its four subunits (A-D) function as tumour suppressor genes associated with pheochromocytomas and paragangliomas (PPGL), but only mutations in the SDHB subunit elevate the risk of metastasis. Recent research has revealed that the mitochondrial translocator protein (TSPO) enhances pro-survival responses by facilitating contact sites between mitochondria and the nucleus, known as Nucleus Associated Mitochondria (NAM) 1,2. This study investigates the role of SDHB in metabolic and functional reprogramming in the human pheochromocytoma cell line hPheo1 3,4.

Results: Cells with SDHB mutations displayed a loss of SDH function, increased succinate levels, and reduced ATP levels compared to wild-type (WT) cells. These mutations also resulted in elevated intracellular lactate, indicating a metabolic shift consistent with the Warburg Effect, along with an increase in mitochondrial reactive oxygen species (ROS). Electron microscopy showed that SDHB knockout (KO) cells had less organized mitochondrial cristae and decreased distances between mitochondria and the nucleus. Western blot analysis indicated a positive correlation between TSPO levels and SDHB mutations. Immunofluorescence demonstrated heightened TSPO localization in the perinuclear area of mutated cells compared to WT cells. Proximity Ligation Assay (PLA) confirmed that TSPO plays a critical role in NAM formation, as there was a significant reduction in contact numbers following TSPO knockdown. Additionally, TSPO was involved in cholesterol trafficking and cell migration.

Conclusion: Our results reveal a link between metabolic reprogramming, functional changes, and NAM formation in SDHB-mutated cells.

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A novel mass spectrometry-based approach for quantifying protein and low molecular persulfides in biological systems (oral presentation)

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Hydrogen sulfide (H_2S) modifies low-molecular-weight (LMWSH) and protein thiols (PrSH), generating persulfides (RSS^-) that play key roles in antioxidant defence and enzyme activity. Despite their significance, their in vivo quantification remains a challenge. To address this, we developed a chemoselective method using a iodoacetamide derivative, TPP-IAM, which incorporates a trisphenylphosphonium (TPP) moiety. This approach traps sulfur species from low-molecular-weight and protein persulfides ($LMWSS^-$ and $PrSS^-$), followed by reduction and re-alkylation with IAM, converting them into diagnostic products for precise quantification via mass spectrometry. The positive charge of TPP moiety enhances ionization efficiency, enabling femtomolar sensitivity, while co-application of deuterated standards ensures absolute quantification across various biological matrices. By modifying key steps, this method also distinguishes sulfur derived from protein persulfides versus polysulfides. Using TPP-IAM, we also quantified endogenous glutathione persulfides and as well as free H_2S from normoxic and ischemic tissues in vivo. Application of this method to rodent tissues revealed low steady-state persulfide levels (0.1–0.2%), but H_2S exposure under oxidative stress significantly increased persulfide formation in mitochondria. These findings pave the way for elucidating the physiological, pharmacological, and pathological roles of persulfides.

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OxiFly: Sex-specific redox networks in ageing Drosophila (oral presentation)

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Redox regulation through reversible cysteine modification plays a central role in maintaining cellular homeostasis and function. Dysregulation of redox signalling is implicated in the ageing process. Across species, males generally exhibit shorter lifespans than females, but the mechanisms underlying these sex differences are not fully elucidated. To gain detailed molecular insight from a redox perspective, we performed in vivo redox proteomics across multiple body parts of male and female Drosophila throughout their lifespan. This 'OxiFly' dataset detected >20,000 unique cysteine-containing peptides on >6,700 unique proteins across all samples. This approach allowed us to identify tissue- and age-specific redox dynamics and reveal distinct, sex-biased networks of redox-sensitive proteins. These findings suggest differences in redox regulation between sexes that may contribute to differences in ageing trajectories.

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KCTD12 redox state as a determinant and therapeutic target in pulmonary remodelling (oral presentation)

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Pulmonary hypertension (PH) is a multifaceted and progressive disorder characterized by pulmonary artery remodelling, resulting in right heart failure and death. However, the lack of a comprehensive understanding of this condition has hindered the development of effective therapeutic interventions. Using redox proteomics, we identified potassium channel tetramerization domain containing 12 (KCTD12) as a protein that can change its thiol-disulfide redox state. KCTD12 has a basal disulfide bond that is reduced to the -SH state in isolated cells or tissues of mice subjected to hypoxia. Lungs and pulmonary arteries from idiopathic PH patients also showed this reductive accumulation of KCTD12-SH, which we speculated may be causal in the pulmonary remodelling observed in this condition because of its role in cancer cell proliferation. A structural review of the human KCTD12 pentamer indicated that the disulfide bond was likely between C300 (C302 in mice) and was corroborated by a plasmid C300A mutant that did not form this disulfide bond. KCTD12 C302A knock-in mice, which mimic the -SH reduced state, spontaneously developed pulmonary remodelling, increased right ventricular systolic pressure, and hypertrophy. Mechanistically, reduction of KCTD12 to the -SH state increased its interaction with the cell cycle enzyme CDK1, leading to dephosphorylation and activation, promoting cell proliferation. This, together with a decrease in oxygen consumption rate in cells from the C302A mutant mouse, indicates that reduction of KCTD12 drives carbon away from mitochondrial oxidation, rerouting it to anabolic processes as occurs in hyperproliferative cells. Therapeutically, adefovir dipivoxil, an approved drug for hepatitis B, prevented hypoxia-induced pulmonary remodelling by disrupting the KCTD12-CDK1 interaction and cell growth. DSDJ01, a molecule we identified

that selectively increased disulfide-KCTD12, also effectively protected against PH. In conclusion, the KCTD12 redox state is a determinant and a therapeutic target for PH.

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Regulation of DJ-1 by CoAlation in Parkinson's Disease (oral presentation)

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Coenzyme A (CoA) is an essential cofactor for cellular metabolism, gene expression regulation, and the biosynthesis of major cellular macromolecules. Recently, we found CoA can bind to cysteine residues via disulfide bond, this modification is termed CoAlation. CoAlation may play a protective role by shielding redox-sensitive cysteine residues during cellular responses to oxidative and metabolic stress. Disruptions in CoA synthesis and reduction of CoA level is known to cause neurodegeneration, namely Pantothenate kinase-associated neurodegeneration. Given the central role of oxidative stress in neurodegenerative conditions, CoA may exert neuroprotective effects in disorders such as Parkinson's Disease (PD). In the context of PD, the protein DJ-1 acts as an oxidative stress sensor, promoting antioxidant responses, cell survival, and suppressing apoptosis upon C106 oxidation. However, the oxidative stress in PD may over-oxidise C106 and its other two cysteine (C46 and C53), thereby inactivating DJ-1. We have found DJ-1 is CoAlated under oxidative stress at C46 and C53 using LC-MS analysis. This may protect DJ-1's cysteine from irreversible oxidation and/or modulate its function. Our in vitro and in vivo studies confirm DJ-1 CoAlation, and ongoing work is investigating its impact on DJ-1's glyoxalase activity. These findings provide novel insights into the protective role of CoAlation in oxidative stress and its potential therapeutic implications for neurodegenerative diseases.

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