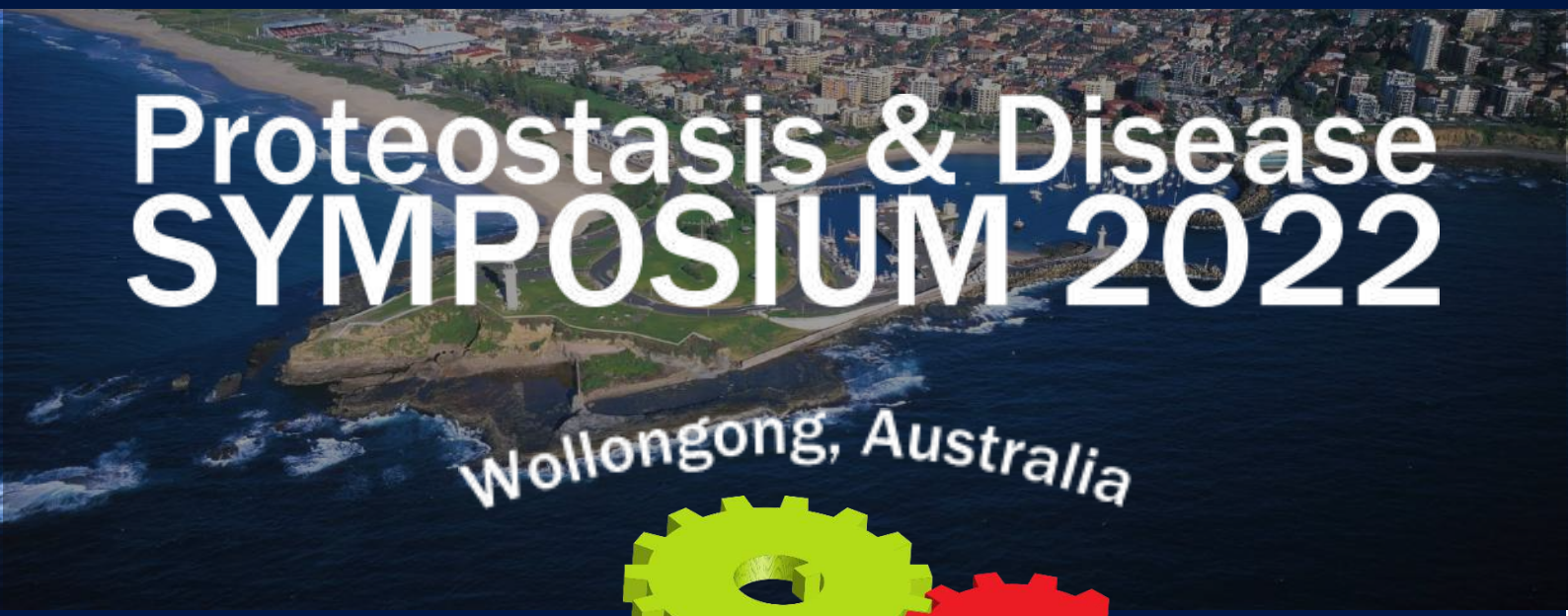

Information Booklet

Welcome

Whether you are joining us in sunny, coastal Wollongong or virtually, we are excited to welcome you to the 4th Proteostasis and Disease Symposium.

Proteostasis encompasses all those processes that act to maintain the correct levels and function of proteins in living systems. The Symposium will focus on proteostasis function and dysfunction, particularly in disease settings, and features presentations from a range of experts from around the globe.

We hope you enjoy the Symposium!

Contents

General Information

Disclaimer: The Proteostasis & Disease Symposium reserves the right to amend or alter any advertised details relating to dates, program, and speakers, if necessary, without notice, as a result of circumstances beyond their control. All attempts have been made to keep changes to an absolute minimum.

Policies and Procedures: Please familiarise yourself with the Code of Conduct, Social Media Guidelines, COVID Management Policy and Reporting Procedures available either online or at the end of this booklet.

WHOVA online platform: You can view all live-streamed sessions, find up-to-date program information, connect with delegates, and pose questions to the speakers during the live Q&A all using our online WHOVA platform. Log in via WHOVA on the web or download the mobile app! Check out the Getting Started guide for more information or speak to the registration desk.

Wi-Fi internet access: Complimentary wireless internet access is available for the duration of the Symposium. Please ensure Wi-Fi is activated on your device, then select Novotel Conference Network. Click on your internet browser after which you will be re-directed to a login page: enter the access code on the voucher provided, accept the terms and conditions then click 'Continue'. Happy surfing, streaming & liking!

Mobile Phones: As a courtesy to other delegates, please ensure that all mobile phones are switched off or put on silent mode during all sessions and social functions at the Symposium.

Name Badges: All delegates, speakers and exhibitors will be provided with a name badge, which must be worn at all times within the Symposium venue, and required for access to all Symposium sessions, catering and social functions.

Photographs, Videos and Recording of Sessions: Delegates are not permitted to use any type of camera or recording device at any of the sessions unless written permission has been obtained from the relevant speaker. Where permission has been granted by speakers to the Symposium committee, sessions will be recorded by the Symposium committee and access provided to all delegates via the WHOVA platform for a period of two weeks following the conclusion of the symposium.

Poster Session: Posters will be available for viewing for the duration of the Symposium. Posters will be attended by their presenting author during the Poster Session, held during the Welcome drinks function on Monday 21st November. Those presenting posters are asked to be present at their poster for the duration of this session.

Presenters and Session Chairs: All Speakers are asked to provide their presentation on USB to the Registration Desk at least half an hour before their session commences. All presentations will be loaded to the Symposium laptop – there will be PC and Mac available. An audio-visual technician will be present throughout the Symposium. Speakers are asked to introduce themselves to their session Chair during the break before their session – at least ten minutes before the session, if possible - to familiarise themselves with the equipment and check that presentations are working.

Quiz and Win: Find the quiz questions in your registration pack. Visit the exhibitors to find the answers. First prizes drawn at the close of Monday's sessions and final prize drawn at close of Symposium on Wednesday afternoon. Drop your answer sheet into the Registration Desk by 4.00 pm Monday to be eligible for first prize draw and retrieve your answer sheet to keep collecting answers for the final prize draw on Wednesday (you have until 1.30 pm Wednesday to drop your answers to the Registration Desk). All answers need to be complete and correct to be eligible to win a prize.

Getting Around in Wollongong: A FREE Shuttle bus service, the Gong Shuttle, operates in Wollongong incorporating the city centre, beach, University and train station. There is a shuttle stop outside the Novotel hotel. For more information visit: http://www.visitwollongong.com.au/find-your-way/how-to-get-around-once-youre-here

Registration Desk/Contacts: Please direct all enquiries to the staff at the Registration Desk. The Registration Desk will be open:

Monday 9:00 am – 6:30 pm

Tuesday 8:00 am – 3:30 pm

Wednesday 8:00 am – 2:00 pm

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Social Program

Welcome Reception: "Drinks on the Deck", Monday 6:00 pm – 8:00 pm

The Deck, Novotel Wollongong Feel the ocean breeze, breathe the fresh air, hear the waves crashing on the shore – relax with a drink and canapés on The Deck, overlooking the Pacific Ocean.

Dress – Smart Casual; Attendance is included in the registration fee.

Symposium Dinner: "Dinner on the Beach", Tuesday 6:30 pm, Northbeach Pavilion

Northbeach Pavilion Restaurant is located inside the Heritage Listed 1930's North Beach Bathers Pavilion at picturesque North Wollongong Beach. Join us seaside for a relaxing, casual night with a great menu. The Restaurant is located directly across the road from the Novotel. Cross the road to the beach, down the stairs to the right, the restaurant is in the south side of the surf pavilion.

Dress – Smart Casual; Attendance is in addition to the registration fee.

Free Time: Tuesday Afternoon 3:30 pm – 6:30 pm

Feeling adventurous? Here are some local activities to make the most of your free afternoon!

Cycling the Blue Mile Boardwalk

Cycling is one of the activities that lie at the heart of Wollongong. With over 60km of on-road and off-road cycle ways in and around Wollongong, there are plenty of tracks to explore and enjoy. The Blue Mile boardwalk stretches along the Wollongong harbour and foreshore and enables you to take in the magnificent view of the South Pacific Ocean and stop for a coffee or casual lunch at one of the many seaside dining options. Bikes are available for hire at the Novotel. Payment can be easily made via credit card at the bike station and helmets are available from the Novotel Reception.

Surf Lessons at Wollongong City Beach

Pines Surfing Academy has 2-hour group surfing lessons daily at Wollongong City Beach. These lessons are for surfers of all levels and abilities. It doesn't matter if it is your first-time surfing or if you have been surfing for years.

Cost is from $50. Visit http://www.pinessurfingacademy.com.au/

Tandem Skydive over North Wollongong Beach

Skydive the Beach offers tandem skydiving from Australia's highest altitude of up to 15,000ft, directly over North Wollongong beach. Experience the ultimate thrill of freefall at over 200km/hr for up to 65 seconds, and then float under canopy for 5-7 minutes soaking up the spectacular views of Wollongong and the Southern Sydney beaches.

Cost is from $399 per person. Visit the website for more information: https://www.skydive.com.au/skydive/Wollongong

Academic Program

Day 1: Monday November 21st		
9:00 AM – 10:30 AM	**Registration and Morning Tea**	
10:45 AM – 11:00 AM	**Symposium Opening & Welcome**	*Symposium Co-Chairs*
11:00 AM – 1:00 PM	**Session 1: Proteostasis and Disease**	*Chair: Lezanne Ooi*

Chris Dobson commemorative lecture
Michele Vendruscolo - University of Cambridge, UK
1.1: *Kinetics-Based Drug Discovery for Alzheimer's Disease*

Albert Lee - Macquarie University, Australia
1.2: *Identifying the Tau aggresome in progressive supranuclear palsy by in situ biotinylation by antibody recognition (BAR) mass spectrometry*

John Carver - Australian National University, Australia
1.3: *Proteostasis in the eye lens*

Rachelle Balez - University of Wollongong, Australia
1.4: *Increased neuronal nitric oxide synthase in Alzheimer's disease mediates aberrant glutamatergic calcium responses*

Lou Fourriere-Chea - University of Melbourne, Australia
1.5: *RUSHing to understand the segregation of the membrane cargoes BACE1 and amyloid precursor protein (APP) along the secretory pathway.*

1:00 PM – 2:00 PM	**Lunch**	
2:00 PM – 4:00 PM	**Session 2: Neurodegeneration and Proteostasis**	*Chair: Luke McAlary*

Kay Double - University of Sydney, Australia
2.1: *When a good protein goes bad: superoxide dismutase 1 and neurodegeneration*

Victoria Lawson - University of Melbourne, Australia
2.2: *Neurological impact of COVID-19 in a mouse of model of SARS-CoV-2 infection*

Adam Walker - University of Queensland, Australia
2.3: *Identification of modulators of TDP-43 aggregation and toxicity using genome-wide CRISPR knockout screens*

	Erika Gyengesi - Western Sydney University, Australia 2.4: *The contribution of glial reactivity to aging and neurodegenerative disorders: from cellular modifications to possible therapeutic approaches* Michael Heneka – University of Luxembourg, Luxembourg 2.5: *Inflammatory causes for protein aggregation*	
4:00 PM – 4:30 PM	**Afternoon Tea**	
4:30 PM – 6:00 PM	**Session 3: Flash Talks**	*Chair: Dezerae Cox*
6:00 PM – 8:00 PM	**Welcome reception "Drinks on the Deck"** *Includes the Poster Session from 6:00-7:00 pm*	

Day 2: Tuesday November 22nd

8:30 AM – 10:30 AM	**Session 4: Protein folding, misfolding and chaperones I**	*Chair: Isabella Lambert-Smith*

Benedetta Bolognesi - Institute for Bioengineering of Catalonia, Spain
4.1: *Deep indel mutagenesis to understand amyloid aggregation*

Blagojce Jovcevski - Adelaide University, Australia
4.2: *Unraveling the aggregation dynamics of amyloid fibril-forming proteins and their interactions with molecular chaperones using native MS-based approaches*

Jeremy Lum - University of Wollongong, Australia
4.3: *A polytherapy approach for promoting maturation of ALS-associated SOD1 mutations*

Aidan Grosas - University of Wollongong, Australia
4.4: *Structural, Functional, and Mechanistic Basis for the Oligomerisation of the Major Eye Lens Protein βB2-crystallin*

Michael Griffin - University of Melbourne, Australia
4.5: *Unravelling the activities and active sites of small heat-shock proteins*

10:30 AM – 11:00 AM	**Morning Tea**

11:00 AM – 1:00 PM	**Session 5: Protein folding, misfolding and chaperones II**	*Chair: Heath Ecroyd*

Ursula Jakob - University of Michigan, USA
5.1: *Early Life ROS as Modulator of Lifespan and Age-Associated Diseases*

Danny Hatters - University of Melbourne, Australia
5.2: *Sequence grammar underlying unfolding and phase separation of globular proteins*

Dezerae Cox - University of Cambridge, UK
5.3: *Evaluating models of Motor Neuron Disease molecule by molecule.*

Nicholas Marzano - University of Wollongong, Australia
5.4: *Real-time single-molecule observation of chaperone-assisted protein folding*

Nadinath Nillegoda - Monash University, Australia
5.5: *Regulation of protein disaggregation in mammalian cells*

1:00 PM – 2:00 PM	**Lunch**

2:00 PM – 3:30 PM	**Session 6: Early-Career Researcher Session**	*Chairs: Danny Hatters & Victoria Lawson*

Nicole Miles - University of Wollongong, Australia
6.1: *Assessing FDA-approved drugs as potential therapeutics in cell and C. elegans models of motor neurone disease*

Pierre Hofstee - University of Wollongong, Australia
6.2: *Investigating the association of protein aggregation with preeclampsia and postpartum*

Michelle Newbery - University of Wollongong, Australia
6.3: *Investigating the role of amyotrophic lateral sclerosis mutations on skeletal muscle cell function and susceptibility to proteasome inhibition*

Helena Targa Dias Anastacio - University of Wollongong, Australia
6.4: *Elevated Ca2+ signalling of AMPA receptors in induced pluripotent stem cell derived Alzheimer's disease neurons*

Thomas Walker - University of Wollongong, Australia
6.5: *Use with caution: translation-inhibiting antibiotics upregulate cellular stress response pathways*

Lauren Rice - University of Wollongong, Australia
6.6: *Observation of small heat shock protein chaperone activity using a single molecule photobleaching approach*

Simon Maksour - University of Wollongong, Australia
6.7: *CoREST3 expression is decreased in Alzheimer's disease and gene knockdown significantly increases HDAC2 expression in cortical neurons derived from human pluripotent stem cells*

Dzung Do-Ha - University of Wollongong, Australia
6.8: *Cellular crosstalk between ALS astrocytes and motor neurons diminishes neuronal firing*

Yasith Mathangasinghe - Monash University, Australia
6.9: *Cellular aging impedes stress-activation of a crucial JDP-Hsp70 protein disaggregase*

6:30 PM onwards	**Conference dinner: "Dinner on the Beach"**	*Northbeach Pavillion*

Day 3: Wednesday November 23rd

9:00 AM – 11:00 AM	**Session 7: Protein trafficking & degradation**	*Chair: Nick Geraghty*

Daniel Finley - Harvard Medical School, USA
7.1: *Global remodeling of the proteome in terminal differentiation*

Stephanie Rayner - Macquarie University, Australia
7.2: *Cyclin F influences the proteostasis of TDP-43*

Emma-Jayne Proctor - University of Wollongong, Australia
7.3: *Lactoferrin is an amyloid-specific extracellular chaperone*

Isabella Lambert-Smith - University of Wollongong, Australia
7.4: *Increased levels of UBA1 protect against mutant SOD1 toxicity*

Julia Pagan - University of Queensland, Australia
7.5: *FBXL4 mitochondrial DNA depletion syndrome protein mediates the degradation of BNIP3 and NIX mitophagy receptors to antagonize mitophagy*

11:00 AM – 11:30 AM	**Morning Tea**

11:30 AM – 1:30 PM	**Session 8: Protein folding, misfolding and chaperones II**	*Chair: Mark Wilson*

Yuji Goto - Osaka University, Japan
8.1: *Linking protein folding and misfolding under macromolecular crowding*

Emily Wires - NIH, USA
8.2: *Perturbations to endoplasmic reticulum proteostasis associated with neuronal loss in neurological disease models*

Shouxiang Zhang - La Trobe University, Australia
8.3: *Chemical Guided Global Analysis of Unfolded Proteins In Cells*

Nicholas Geraghty - University of Wollongong, Australia
8.4: *A high-throughput flow cytometry drug screen to discover new treatments for motor neurone disease*

Massimo Hilliard - University of Queensland, Australia
8.5: *OSP-1 protects neurons from oxidative stress by remodelling the endoplasmic reticulum and modulating autophagy*

1:30 PM – 1:45 PM	**Symposium Closing & Farewell**	*Symposium Co-Chairs*
1:45 PM onwards	**Departure Lunch**	

Poster Presentations

P1: *Prion-like amplification of misfolded polyglutamine protein monomer which exerts toxicity*
Daisaku Ozawa

P2: *Amyotrophic lateral sclerosis and inflammatory signalling alter expression of CD99 in astrocytes*
Liam Robinson

P3: *Knockdown of the transcriptional corepressor CoREST3 increases cell viability in the human C20 microglial cell line*
Calista Turner

P4: *A bioinformatic analysis of gene expression in induced pluripotent stem cell-derived skeletal muscle cells from sporadic ALS patients*
Charles Dodds

P5: *N-terminal amyloidogenic regions in SOD1 modulate its aggregation in living cells*
Luke McAlary

P6: *The flexible residual structure of acid-denatured ?2-microglobulin is relevant to an ordered fibril morphology*
Kazumasa Sakurai

P7: *Macrophages in the reticuloendothelial system inhibit early induction stages of mouse apolipoprotein A-II amyloidosis*
Hiroki Miyahara

P8: *Macromolecular crowding and supersaturation protect hemodialysis patients from the onset of dialysis-related amyloidosis*
Kichitaro Nakajima

P9: *Reducing the size of tags in proximity-based labeling interactors of disease-associated aggregate proteins*
Ruohua Lyu

P10: *Study on ultrasonic intensity and frequency dependences of seed-dependent amyloid fibril formation of ?2-microglobulin*
Kakeru Hanada

P11: *Age-related amyloid deposition in C57BL/6 mice: Pathological findings and characterization of the renal damage*
Jian Dai

P12: *Local conformation of intrinsically disordered regions of Sup35 produces temperature sensitivity of liquid-liquid phase separation*
Yumiko Ohhashi

P13: *Mechanisms of polyphosphate-induced amyloid formation under ultrasonication*
Keiichi Yamaguchi

P14: *Developing novel small molecule ligands of TDP-43*
Alison Cheng

P15: *Biophysical analysis of the amyloid fibril formation by serum amyloid A*
Taishi Okunishi

P16: *Inhibitory effect of fucoidan on the fibril formation by amyloid-beta peptides*
Masaru Hoshino

P17: *Amyloid fibril formation of transthyretin induced by fragmentation*
Keisuke Yuzu

P18: *Formation of two types of tau oligomers depending on redox conditions*
Ayumi Masui

P19: *Disclosing the Relation Between Protein Quality Control and Cell Cycle Using Chemical Probes*
Jiamin Zhao

P20: *Decoding the determinants driving phase separation of unfolded proteins*
Samin Zadeh

P21: *Analysis of Fluorescent Molecular Rotors as Probes for Protein Liquid-Liquid Phase Separation*
Adam Turner

P22: *Characterization of Stress Granule Formation in U2OS Cells Shows Protein-Dependent and Stress-Dependent Effects on Their Formation*
William Matinca

P23: *Development of General Fluorescence-Based Quantification Method for Study of In-Vitro, Cellular Proteostasis Polarity and Polarity Response*
Tze cin Owyong

P24: *Small molecule fluorescent reporters for autophagy*
Siyang Ding

P25: *Conformational transitions of amyloid fibrils during cross-seeding*
Eri Chatani

P26: *The chaperone function of ?B-crystallin through the formation of a complex with the amyloid precursor of the insulin B-chain*
Yuki Kokuo

P27: *DNAJB chaperones suppress destabilised protein aggregation via a region distinct from that used to inhibit amyloidogenesis*
Shannon Mcmahon

P28: *NMR characterization of the amyloidogenic interaction motifs in the host adapter protein, RIPK3, and the viral inhibitor of necroptosis, M45*
Nikhil Varghese

P29: *RaPID discovery and characterisation of RIPK3 RHIM-binding peptides*
Jessica Buchanan

P30: *Towards improved transthyretin amyloidosis detection*
Joanna New

P31: *Investigating the effect of SMOC1 on AB amyloid fibril formation*
Caitlin Johnston

P32: *Spontaneous protein-protein crosslinking of long-lived proteins*
Michael Friedrich

P33: *Characterising the localisation of small heat shock proteins binding to ?-synuclein fibrils*
Nicola Auld

P34: *CuATSM improves motor function in a mouse model expressing human wildtype SOD1 and decreased intracellular copper*
Connor Karozis

P35: *Solubility and foci formation of sequestosome-1/p62 are regulated by SCFcyclin F complex ubiquitylation as an early pathomechanism in ALS*
Jennilee Davidson

P36: *Investigating mechanisms of molecular chaperone function at the single-molecule level*
Bailey Skewes

P37: *Cells overexpressing ALS-associated SOD1 variants are differentially susceptible to CuATSM-associated toxicity*
Mikayla Brown

P38: *A copper chaperone-mimetic polytherapy for SOD1-associated amyotrophic lateral sclerosis*
Victoria Shephard

P39: *Chemical Guided Global Analysis of Unfolded Proteins In Cells*
Shouxiang Zhang

Abstracts

Abstract 1.1

Kinetics-Based Drug Discovery for Alzheimer's Disease

Michele Vendruscolo

Centre for Misfolding Diseases, Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, UK

The phenomenon of protein misfolding and aggregation is associated with a wide range of human disorders, including Alzheimer's and Parkinson's diseases. A central role in these conditions is played by protein misfolded oligomers, which are among the most cytotoxic products resulting from the process of protein aggregation. It has been very challenging, however, to target these oligomers with therapeutic compounds, because of their dynamic and transient nature. To overcome this problem, I will describe a kinetic-based approach, which enables the discovery and systematic optimization of compounds that reduce the number of oligomers produced during an aggregation reaction. I will illustrate this strategy for the amyloid beta peptide, which is closely linked to Alzheimer's disease. As this strategy is general, it can be applied to oligomers of any protein in drug discovery programmes.

Abstract 1.2

Identifying the Tau aggresome in progressive supranuclear palsy by in situ biotinylation by antibody recognition (BAR) mass spectrometry.

Rowan Radford [1], Stephanie Rayner [1], Paulina Szwaja [1], Livia Rosa-Fernandes [1], Flora Cheng [1], Marco Morsch[1], Tianyi Zhu [2], Jocelyn Widagdo [2], Victor Anggono [2], Dean Pountney [3], Roger Chung [1], Albert Lee [1]

[1] Centre for MND Research, Macquarie Medical School, Macquarie University, North Ryde 2109.; [2] Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, QLD, Australia; [3] School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, QLD, Australia.

The pathological feature of many neurodegenerative diseases is the presence of protein inclusions that features a key protein-of-interest (POI) (e.g., TDP-43 in motor neuron disease and tau in progressive supranuclear palsy [PSP]) that is confirmed by pathology post-mortem. However, one of the most fundamental questions that arises is 'what are the unique proteins that make up the composition of these aggregates?' and 'what are their biological role(s) that presumably seed events that lead to neuronal degeneration?' Presently, the classical approaches that extract inclusions from tissue have some technical limitations – the most relevant being that they are highly disruptive and risk losing some of the true composition of the aggregates.

We have developed and optimized a workflow to overcome these shortcomings and enabled us to characterize the protein composition of protein aggregates by unbiased and semi-quantitative proteomics. Our Biotinylation by Antigen Recognition (BAR) workflow uses the same principles as immunohistochemistry but instead covalently labels proteins with biotin that are within proximity to the POI. We demonstrated proteomic profiling of phosphorylated-Tau (p-Tau) inclusions from PSP post-mortem tissue. Our data revealed novel insight into the complex molecular composition of these p-Tau-disease inclusions and allowed us to predict how these inclusions form and how they trigger multiple molecular signaling cascades leading to neurodegeneration. By understanding the different proteins in POI-positive aggregates ("aggresome") at various stages of disease pathogenesis will enable us to determine the proteomic signatures that represent the multiple and converging mechanisms that are precursor molecular events that lead to protein aggregation.

Abstract 1.3

Proteostasis in the eye lens

John A. Carver

Research School of Chemistry, The Australian National University, Action, ACT 2601, Australia

The mammalian eye lens is a unique organ. It is avascular. It is composed of lens fibre cells that contain mostly protein, the crystallins, at a very high concentration (up to 300-400 mg/mL in the centre (nucleus) of the lens). There are three types of crystallins: alpha, beta and gamma. The alpha-crystallins are small heat-shock molecular chaperones and the beta- and gamma-crystallins are related, structural beta-sheet proteins. In the lens, crystallins are arranged in a well-ordered, supra-molecular array that enables refraction of light and lens transparency to ensure proper focussing of light on to the retina. Lens fibre cells have lost their organelles and are essentially metabolically inactive. They have no protein turnover meaning that crystallins are long-lived proteins and crystallins in the nucleus are as old as the individual. By necessity, therefore, crystallins are highly stable proteins. Standard mechanisms of proteostasis cannot therefore operate within lens fibre cells.

With age, many post-translational modifications (PTMs) occur to lens crystallins, including deamidation, racemization, phosphorylation, cross-linking and truncation. Potentially, these PTMs may lead to crystallin unfolding, destabilisation, misfolding and aggregation resulting in lens opacification, i.e. cataract formation. However, many of these PTMs are benign with respect to crystallin stability and solubility and therefore are not necessarily deleterious. In contrast to the prevailing view that crystallin PTMs are detrimental to crystallin structure and function, we propose that lens crystallin solubility with age is maintained by complementary and synergistic interactions of the modified crystallins, coupled with the chaperone action of the alpha-crystallins.

Abstract 1.4

Increased neuronal nitric oxide synthase in Alzheimer's disease mediates aberrant glutamatergic calcium responses

Rachelle Balez [1,2], Claire H. Stevens [1,2], Kerstin Lenk [3,4,5], Greg Sutherland [6], Lezanne Ooi [1,2]

[1] Illawarra Health and Medical Research Institute, University of Wollongong, NSW, Australia; [2] School of Chemistry and Molecular Bioscience, University of Wollongong, NSW, Australia; [3] Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland; [4] Institute of Neural Engineering, Graz University of Technology, Graz, Austria; [5] BioTechMed, Graz, Austria ; [6] Charles Perkins Centre, University of Sydney, NSW, Australia

Nitric oxide (NO), produced by the calcium (Ca2+) activated enzyme neuronal nitric oxide synthase (nNOS), is a second messenger and modulates glutamatergic Ca2+ signaling in neurons. Aberrant Ca2+ signaling occurs during sporadic Alzheimer's disease (sAD), however, the contribution of nNOS activity and NO to this phenotype is not well understood. The aim of this study was to quantify nNOS expression and nitrite, as a downstream marker of NO, in sAD neurons and assess the impact of inhibiting nNOS activity or scavenging NO on neuronal Ca2+ signaling. Quantification of nNOS by western blotting showed a significant increase in nNOS amount in both human post-mortem tissue and induced pluripotent stem cell (iPSC) derived neurons, from sAD donors. This was in conjunction with significantly elevated levels of nitrite in sAD iPSC-derived neurons, quantified by Griess assay. Live cell Ca2+ imaging demonstrated that inhibition of nNOS activity, or scavenging of NO, significantly decreased the glutamatergic Ca2+ response in healthy iPSC-derived neurons. This modulatory effect was lost in the sAD neurons, however, there was a decrease in the proportion of spontaneously signaling neurons, suggesting pathogenic modification of signaling receptors. In conclusion, this study reports an increase in nNOS amount during sAD and demonstrated that NO strengthens the Ca2+ response to glutamate in healthy neurons, while dampening the response and contributing to spontaneous Ca2+ signaling during sAD. This highlights the dual role of NO as both neuroprotective and neuropathogenic, where during the progression of sAD it may contribute to the breakdown of neuronal Ca 2+ signaling.

Abstract 1.5

RUSHing to understand the segregation of the membrane cargoes BACE1 and amyloid precursor protein (APP) along the secretory pathway

Lou Fourriere [1], Jingqi Wang [1], Paul A. Gleeson [1].

[1] The Department of Biochemistry and Pharmacology and Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria 3010

Alteration of the membrane trafficking can lead to neurodegenerative diseases such as Alzheimer's disease (AD). Accumulation of amyloid plaques in the brain is a hallmark of AD. Amyloid plaques are formed by the aggregation of amyloid β peptide (Aβ) generated after a proteolytic processing of the amyloid precursor protein (APP) by the protease ß-secretase (BACE1). Defining the intracellular trafficking of newly synthesized BACE1 and APP is required to understand the regulation of Aβ and the development of AD and design novel therapeutics.

We combined cell biology techniques, super-resolution, and the Retention Using Selective Hooks (RUSH) system, to investigate the trafficking of newly synthesized BACE1 and APP and APP processing in HeLa cells and primary mouse neurons. We observed that (1) APP and BACE1 are sorted into different post-Golgi transport pathways, (2) APP and BACE1 are segregated in the cis-Golgi and throughout the Golgi stack, (3) differences in the trafficking and processing of wild-type APP and two disease-related APP mutants along the secretory pathway. These findings reveal key segregation of membrane cargoes in the early Golgi and the importance of BACE1 and APP segregation to limit APP processing and Aβ production.

Abstract 2.1

When a good protein goes bad: superoxide dismutase 1 and neurodegeneration

Kay L. Double

Brain and Mind Centre and School of Medical Sciences Neuroscience, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW 2006, Australia

The development of aberrant forms of endogenous proteins is linked to neurodegeneration in a range of disorders. We described the presence of structurally-disordered, deposited superoxide dismutase 1 (SOD1) restricted to degenerating regions of the Parkinson disease brain. Further we showed that deposited SOD1 in Parkinson disease appears to be abnormally metalated. While misfolded, mutant SOD1 protein has been well-described to be linked to motor neuron death in some forms of familial amyotrophic lateral sclerosis, in Parkinson disease aberrant SOD1 is wildtype protein. We subsequently showed that structurally-disordered, mismetallated wildtype SOD1 is found in the degenerating ventral spinal cord in sporadic ALS. We hypothese that, in a copper-deficient environment, post-translational maturation of mutant or wildtype SOD1 is altered, resulting in structurally-disordered, aggerating protein with a toxic gain-of-function. Development of a novel mouse strain expressing human wildtype SOD1 protein in a copper-deficient brain resulted in age-associated deposition of misfolded SOD1 and dopamine cell death in the substantia nigra. Our data suggest aberrant SOD1 can form via shared pathways in several neurological disorders and thus represents a promising target for development of disease modifying treatments.

Abstract 2.2

Neurological impact of COVID-19 in a mouse of model of SARS-CoV-2 infection

Lawson VA [1,2], Daymond C [1], Fortunato de Miranda N [1], Smith T [1], Ong J [1], Purcell D [1] and McAuley [1].

[1] Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, 792 Elizabeth St, Melbourne, Victoria, Australia ; [2] Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, VIC, 3010, Australia

Understanding the pathogenesis of acute and chronic (long) neurological symptoms of COVID-19 is essential to developing effective interventions. Mouse models of infection can be used to assess neural invasion and pathology of acute viral infection and study the long-term health outcomes. We have used a naturally mouse tropic variant of SARS-CoV-2 to infect wildtype mice to assess the acute and long-term neurological impact of COVID-19.

Following intranasal infection with VIC2089(N501Y) adult (10 week) and aged (6 month) wildtype mice develop a productive respiratory infection with virus detected in lungs on days 1 and 3 post infection and cleared by 7 days post infection.

Pathology consistent with a vacuolating encephalitis was detected in the brain of adult and aged mice during acute respiratory infection which persisted for at least 14 days post inoculation and correlated with increased immunoreactivity of astrocytes to GFAP.

We will report the effect of SARS-CoV-2 infection associated neuropathology on proteins associated with neurodegeneration.

This model of SARS-CoV-2 associated neuropathogenesis can be used to identify and test therapeutic targets that may prevent the acute and chronic neurological consequences of COVID-19.

Abstract 2.3

Identification of modulators of TDP-43 aggregation and toxicity using genome-wide CRISPR knockout screens

Adam Walker [1], Jun Xu [2], Karla J Cowley [3], Jun Ma [2], Sohye Yoon [2], Kaylene J Simpson [3,4], Rebecca San Gil [1]

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Cytoplasmic aggregation of TAR DNA-binding protein-43 (TDP-43) within neurons is strongly correlated with neurodegeneration in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). However, the molecular pathways that control TDP-43 aggregation and associated toxicity remain unknown. We therefore aimed to identify key genes and biological processes that modulate TDP-43 aggregation and toxicity using a pooled genome-wide CRISPR knockout screen. Analysis of the knockout screen sequencing data revealed seven distinct groups of 'TDP-43 modifier genes' with functionally distinct roles, including inhibiting or enhancing TDP-43 aggregation and/or protecting against or sensitising to TDP-43 toxicity. Gene knockouts that modulated TDP-43 toxicity were enriched with genes associated with pathways including endoplasmic reticulum to cytosol transport and autonomic nervous system development. Gene knockouts that modulated cytoplasmic TDP-43 aggregation were enriched in genes associated with pathways including neuron projection organisation and response to interferon-beta. Using a combination of analysis algorithms for genome-wide CRISPR knockout data (e.g. MAGeCK) and gene ontology pathways (WebGestalt), we short-listed 323 genes for secondary prioritisation as potential targets that decrease TDP-43 aggregation and/or enhance neuronal survival. We developed and applied analytical pipelines to identify top-priority gene candidates amongst these 323 short-listed candidates, using independent arrayed CRISPR knockout combined with multi-parametric high-content imaging. These validated targets now progress for further investigation in induced pluripotent stem cell neurons, transgenic TDP-43 mice, and human ALS and FTD autopsy tissues. These findings reveal potential avenues for therapeutic interventions aimed at protecting neurons against TDP-43 pathology for the treatment of ALS and FTD.

Abstract 2.4

The contribution of glial reactivity to aging and neurodegenerative disorders: from cellular modifications to possible therapeutic approaches

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Pathological aging, neuronal senescence, involving chronic oxidative and inflammatory stress increase the risk of cognitive degenerative diseases such as dementia, including Alzheimer's disease (AD). Inflammatory stress likely contributes to unregulated aberrant activation of innate immune responses of the brain termed "neuroinflammation". Augmented glial activation, accompanied by increased levels of pro-inflammatory cytokines such as IL-6 and TNF-α are biomarkers of aging and age-related diseases and have been shown to contribute to neurodegeneration. Since most mouse models of neurodegenerative diseases lack the chronic inflammatory aspect, we used the GFAP-IL6 mouse model in which interleukin-6 is expressed under the GFAP promoter, resulting in the constant release of IL-6 by astrocytes, triggering a circle of progressive inflammatory events throughout the lifespan of the animals. We investigated the behavioral, anatomical, physiological, and molecular features of aging with chronic glial reactivity during the aging process, and tested whether these can be controlled by natural anti-inflammatory agents, such as highly bioavailable curcumin preparations. We quantitatively analyzed various areas of the brain using unbiased stereology and qPCR, including the cerebellum, the hippocampus, and the basal forebrain cholinergic system, correlating anatomical and histological with behavioral phenotypes and cellular activity using in vitro electrophysiological recordings. Our findings demonstrated a potential direct effect of chronic glial reactivity on glial and neuronal cell numbers, altered cellular morphology, and declining cellular function resulting in both cognitive and motor function decline. We also found that both short- and longer-term treatment with modified highly bioavailable curcumin could restore neurodegenerative impairments associated with pathological aging.

Abstract 2.5

Inflammatory causes for protein aggregation

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Multiplexed assays of variant effects (MAVEs) guide clinical variant interpretation and reveal disease mechanisms. To date, MAVEs have focussed on a single mutation type - amino acid (AA) substitutions - despite the diversity of coding variants that cause disease. We have recently used Deep Indel Mutagenesis (DIM) to generate the first comprehensive atlas of diverse variant effects for a disease protein, the amyloid beta (Aß) peptide that aggregates into fibrils in Alzheimer's disease (AD) and is mutated in familial AD (fAD). We combined DIM to a selection method that reports on the rate of amyloid nucleation, i.e. the mechanism by which protein fibrils form in the first place. The resulting comprehensive atlas identifies known fAD mutations and reveals that many variants beyond substitutions accelerate Aß aggregation and are likely to be pathogenic. Truncations, substitutions, insertions, single- and internal multi-AA deletions differ in their propensity to enhance or impair aggregation, but we identify likely pathogenic variants in all classes of mutations. Overall, mutations that increase the propensity of the peptide to aggregate into amyloid fibrils are highly enriched in the polar N-terminus of Aß, a region which remains unstructured in mature Aβ fibrils and - as a result - has been largely under-studied. This first comparative atlas highlights the importance of including diverse mutation types in MAVEs, while providing important mechanistic insights into amyloid nucleation.

Abstract 4.1

Deep indel mutagenesis to understand amyloid aggregation

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Multiplexed assays of variant effects (MAVEs) guide clinical variant interpretation and reveal disease mechanisms. To date, MAVEs have focussed on a single mutation type - amino acid (AA) substitutions - despite the diversity of coding variants that cause disease. We have recently used Deep Indel Mutagenesis (DIM) to generate the first comprehensive atlas of diverse variant effects for a disease protein, the amyloid beta (Aß) peptide that aggregates into fibrils in Alzheimer's disease (AD) and is mutated in familial AD (fAD). We combined DIM to a selection method that reports on the rate of amyloid nucleation, i.e. the mechanism by which protein fibrils form in the first place. The resulting comprehensive atlas identifies known fAD mutations and reveals that many variants beyond substitutions accelerate Aß aggregation and are likely to be pathogenic. Truncations, substitutions, insertions, single- and internal multi-AA deletions differ in their propensity to enhance or impair aggregation, but we identify likely pathogenic variants in all classes of mutations. Overall, mutations that increase the propensity of the peptide to aggregate into amyloid fibrils are highly enriched in the polar N-terminus of Aß, a region which remains unstructured in mature Aβ fibrils and - as a result - has been largely under-studied. This first comparative atlas highlights the importance of including diverse mutation types in MAVEs, while providing important mechanistic insights into amyloid nucleation.

Abstract 4.2

Unraveling the aggregation dynamics of amyloid fibril-forming proteins and their interactions with molecular chaperones using native MS-based approaches

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The pathogenesis of neurodegenerative diseases such as Parkinson's disease and amyotrophic lateral sclerosis is believed to be caused by deficits in proteostasis. The formation of amyloid fibrils is preceded by the recruitment of misfolding monomers to form soluble oligomers, which are thought to be a major toxic component in cells, in part through damage of lipid membranes. However, very little structural and dynamic information is known about these oligomers in terms of toxicity and their interaction with lipids. A range of biophysical techniques, primarily native MS, have been used to elucidate the structure and dynamics of amyloid-forming proteins and their interaction with model membrane systems of varying lipid composition, as well as small molecule inhibitors and chaperone proteins. We provide structural and mechanistic insights into the effects of two lipid membrane systems (anionic and zwitterionic) on the inhibition of Aβ40 and α-synuclein fibrilization in the presence of two well-known polyphenol inhibitors (-)-epigallocatechin gallate and resveratrol. Further mechanistic understanding of how other phenolics from exotic sources prevent α-synuclein fibrilization at distinct phases of aggregation was examined by ion-mobility MS. Finally, this approach, in combination with collision-induced unfolding, was used to examine the interaction between the molecular chaperone β-casein with α-lactalbumin, a model protein that undergoes both amorphous and fibrillar aggregation. Together, these works highlight how native MS-based approaches can be utilised to provide insights into the conformational properties of aggregation-prone proteins as well as examine challenging transient and dynamic interactions that are responsible for protein aggregation and maintaining protein homeostasis.

Abstract 4.3

A polytherapy approach for promoting maturation of ALS-associated SOD1 mutations

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CuATSM has repeatedly shown to be a favourable therapeutic avenue in amyotrophic lateral sclerosis (ALS) mouse models, most notably familial SOD1 ALS models. Encouragingly, on-going Phase II/III clinical trials also report promising results. We have shown CuATSM stabilises SOD1mut protein, reducing SOD1 misfolding and aggregation associated with ALS. We have identified two additional FDA-approved small molecules, ebselen and telbivudine that may aid in reducing SOD1mut misfolding and target alternate pathways thought to contribute to sporadic ALS, including ferroptosis and HERV-K activation. Here, we aimed to investigate if CuATSM, ebselen and telbivudine polytherapy could improve on the therapeutic efficacy of CuATSM treatment alone for the treatment of ALS. Using a 3D checkerboard approach and automated microscope analysis, polytherapy treatment effectively reduced inclusion formation and increased cell survival of NSC-34 cells expressing SOD1mut, more than any one compound alone. All three compounds were present within brain tissue following acute polytherapy administration (50 mg/kg/day CuATSM, 100 mg/kg/day ebselen and 150 mg/kg/day telbivudine; oral) in C57BL/6 mice. Furthermore, safety and tolerability study, showed polytherapy administration was well-tolerated in C57BL/6 mice following 30 day continuous treatment. In addition, polytherapy treatment in SOD1G93A mice delayed disease onset, slowed disease progression and motor impairments, and increased survival, compared to vehicle- or CuATSM-treated mice. These findings support CuATSM, ebselen and telbivudine polytherapy for the treatment of familial SOD1 ALS and is more therapeutically advantageous than CuATSM alone.

Abstract 4.4

Structural, Functional, and Mechanistic Basis for the Oligomerisation of the Major Eye Lens Protein βB2-crystallin

Aidan B. Grosas, Jeeeun Shin, David C. Thorn, Henry W. Orton, Mithun C. Mahawaththa, Martyna M. Judd, Li Lynn Tan, Joe A. Kaczmarski, Nicholas Cox, Thomas Huber, Gottfried Otting, Colin J. Jackson, John A. Carver

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Crystallin proteins in the translationally quiescent lens fibre cells must maintain their structure and soluble properties over a lifetime to maintain normal vision. However, environmental and chemical insults to the crystallins can lead to predominantly amorphous aggregation pathologically characterised as age-related cataract. βB2-crystallin is the principal β-crystallin isotype in the mammalian lens and has several oligomeric forms. Curiously, β-crystallin oligomers shift from dimers to tetramers with age, and homo-tetrameric βB2-crystallin is a major species. Given the relevance to age-related cataract, we investigated the formation of βB2-crystallin tetramer from its stable dimeric form and characterised its structure, conformation and biophysical properties. We used SEC to identify and interrogate the low pH, high salt conditions required to form tetramer from dimer. XRD allowed us to solve the tetrameric structure and identify pivotal interfacial ion-pairs that explain the conditions of tetramer formation. We used EPR-DEER, IM-MS and SEC-SAXS to study a large conformational rearrangement from a compact 'face-en-face' dimeric conformer to a domain-swapped tetramer, managing to also detect a domain-swapped dimer as a conformational intermediate. As a result of this oligomerisation and conformational change, tetrameric βB2-crystallin has reduced terminal extension flexibility as indicated by NMR and MD simulations, while several spectroscopic experiments showed the tetramer has an increased stability of ~12C and no aggregation with time at 60C, relative to the dimeric form. Our results allow us to propose a mechanism by which βB2-crystallin oligomerisation increases the stability of the lens proteome to stave off the formation of age-related cataract.

Abstract 4.5

Unravelling the activities and active sites of small heat-shock proteins

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Small heat-shock proteins (sHSPs) are ubiquitous molecular chaperones that inhibit protein misfolding and aggregation, and thereby help protect against proteostasis failure. The complex structure-function relationships of sHSPs remain elusive, partly due to their tendency to populate polydisperse ensembles of multimers that readily exchange small oligomers. sHSPs comprise a conserved α-crystallin domain (ACD) flanked by variable N- and C-terminal regions (NTR; CTR). The ACD forms dimers via antiparallel pairing of an extended β-strand, creating a shared β-sheet between the two subunits, while the NTR and CTR mediate formation of higher order oligomers. We have delineated contributions of the three regions of sHSPs to chaperone function during amyloid formation, using human αB-crystallin (αB-C) and heat-shock 27 kDa protein (Hsp27) as exemplar sHSPs. Our data show that the NTR is important for delaying amyloid fibril nucleation and for disaggregating mature apoC-II fibrils. The terminal regions are also required for stable fibril binding and for mediating lateral fibril-fibril association, which sequesters pre-formed fibrils into large aggregates and may be cytoprotective. We have also defined interactions of the isolated ACD with amyloid fibrils. Using NMR, we observed an ACD-fibril interaction that is preferential for fibril ends. Further biophysical and mutagenic analysis showed that the primary fibril-binding species is monomeric, likely utilising the monomer-monomer interface as a fibril binding site. Our results highlight the interplay of the various regions of sHSPs and the importance of the dynamic nature of sHSP assemblies in their function.

Abstract 5.1

Early Life ROS as Modulator of Lifespan and Age-Associated Diseases

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Stochasticity, a fundamental property of biology, is particularly prevalent in aging. Even genetically identical animals, when grown under the same environmental conditions, show large lifespan variations and seemingly random patterns of aging-related pathologies. Our research shows that reactive oxygen species (ROS) serve as naturally occurring stochasticity factors, which individualize biological processes by persistently altering the epigenetic landscape. Our studies in the aging model organism Caenorhabditis elegans revealed that inter-individual differences in developmental ROS-levels contribute to the observed variations in stress resistance and lifespan. We identified the underlying mechanism by demonstrating that the highly conserved histone modifying complex COMPASS is oxidation sensitive, and that the level of K4-trimethylated histone H3 is directly connected to the redox environment of the cell. To our knowledge, these studies provide the first demonstration of a redox-regulated histone methylation event known to biology. We now demonstrate that transient accumulation of ROS during development protects organisms against amyloid toxicity later in life, and reveal the cellular pathway by which downregulation of H3K4me3 exerts its protective and lifespan extending effects. In summary, our studies provide insights into the role of ROS as important stochasticity factors and the role of redox-sensitive epigenetic modifiers as downstream modulators that are responsible for imprinting variability and individualizing health span and longevity in organisms.

Abstract 5.2

Sequence grammar underlying unfolding and phase separation of globular proteins

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Aberrant phase separation of globular proteins is associated with many diseases. Here, we use a model protein system to understand how unfolded states of globular proteins drive phase separation and the formation of unfolded protein deposits (UPODs). We find that for UPODs to form, the concentrations of unfolded molecules must be above a threshold value. Additionally, unfolded molecules must possess appropriate sequence grammars to drive phase separation. While UPODs recruit molecular chaperones, their compositional profiles are also influenced by synergistic physicochemical interactions governed by the sequence grammars of unfolded proteins and of cellular proteins. Overall, the driving forces for phase separation and the compositional profiles of UPODs are governed by the sequence grammars of unfolded proteins. Our studies highlight the need for uncovering the sequence grammars of unfolded proteins that drive UPOD formation and cause gain-of-function interactions whereby proteins are aberrantly recruited into UPODs.

Abstract 5.3

Evaluating models of Motor Neuron Disease molecule by molecule.

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Inappropriate protein aggregation is a central molecular signature of many neurodegenerative diseases, including Alzheimer's and Motor Neuron (MND) diseases. However, decades of research on protein aggregates have not yet determined the cause-effect relationship and overall role of aggregates in disease pathology and progression. This is in part due to the low abundance and high heterogeneity of aggregate species which has made their quantitation and study challenging. As a consequence, it remains unclear how well current experimental models of MND recapitulate true disease phenomena. Here, we report a novel single-molecule immunoassay for sensitive and specific detection of small soluble oligomeric aggregates. Using the MND-associated aggregation-prone protein TAR DNA-binding protein 43 (TDP-43), we show this assay can detect and characterise aggregate particles ranging from recombinant TDP-43 to complex whole-proteome mixtures. We demonstrate the application of this assay to comparing patient-derived tissue extracts and induced pluripotent stem cell models. Surprisingly, we did not observe consistent differences in the number of aggregate particles extracted from disease-derived tissues compared with age-matched controls. However, we find differences in the physicochemical properties of these aggregates. This knowledge contributes to the growing body of research concerning the fundamental biology underlying MND and may improve our understanding of the relationship between protein aggregation and disease progression to inform future early diagnosis efforts. In addition, we hope providing a tool for quantitative evaluation of existing cellular models will empower researchers to select the most appropriate model system when investigating aggregate pathology, paving the way for more robust early-stage therapeutic research.

Abstract 5.4

Real-time single-molecule observation of chaperone-assisted protein folding

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The maintenance and regulation of protein homeostasis is heavily reliant on the Hsp40/Hsp70 system of molecular chaperones, which play critical roles in de novo protein folding and the refolding of misfolded proteins. However, due to the dynamic and heterogeneous nature by which these chaperones interact with their co-chaperones and clients, questions remain regarding the precise molecular mechanisms by which Hsp70 affects the conformation of their clients and assists in their refolding. To address this, we have developed the chaperone client protein, firefly luciferase (Fluc), such that its conformation can be monitored temporally using single-molecule fluorescence resonance energy transfer (smFRET) and total internal reflection fluorescence (TIRF) microscopy. For the first time, the conformation of individual client proteins as they are being folded by the bacterial or human Hsp70 chaperone machinery (i.e., Hsp40, Hsp70 and a nucleotide-exchange factor) was monitored in real time. smFRET results revealed that Hsp70 binds to clients and conformationally expands them via an entropic pulling mechanism, thereby resolving misfolded states, which provides an opportunity for correct folding upon chaperone release. Moreover, we demonstrate that multiple cycles of chaperone binding-and-release to a single misfolded client protein is conducive for efficient refolding. Crucially, the temporal observation of the chaperone-assisted refolding process enabled key kinetic details to be determined that are typically inaccessible using other approaches. Thus, the developed Fluc protein-folding sensor is an ideal tool and represents an exciting platform for further single-molecule investigations of chaperone function.

Abstract 5.5

Regulation of protein disaggregation in mammalian cells

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Cells have evolved multi-tiered cellular quality control systems that tightly monitor protein folding and damage to minimize protein aggregation. Under acute and chronic proteotoxic stresses, these systems overload and results in accumulation of toxic protein aggregates [1]. Decreased cellular capacity in aggregate clearance manifests in cellular deterioration, aging and disease. Human cells possess an Hsp70-based disaggregase system to resolve a wide range of protein aggregates [2, 3]. How these disaggregases are assembled and regulated in mammals remain unclear. We report the identification of a protein quality control (PQC) pathway dedicated to boosting protein disaggregation activity in human cells recovering from heat stress. This pathway, termed the stress-induced protein disaggregase activation pathway (siDAP), selectively induces a ternary DNAJA1+DNAJB1-Hsp70 disaggregase. The Hsp70 disaggregase functions sequentially with VCP to resolve different populations of heat-induced aggregates in temporally distinct aggregate clearance phases. Strikingly, the assembly of this disaggregase is compromised in cells undergoing replicative aging. However, these cells still retain a fully functional HSR, UPRER and proteasome activity making DNAJA1+DNAJB1-Hsp70 disaggregase one of the earliest PQC machines to collapse in aging cells.

1. Morimoto RI. The heat shock response: systems biology of proteotoxic stress in aging and disease. Cold Spring Harb Symp Quant Biol. 2011; 76:91-9.
2. Nillegoda NB et al. Crucial HSP70 co-chaperone complex unlocks metazoan protein disaggregation. Nature. 2015; 524(7564):247-51.
3. Wentink AS et al. Molecular dissection of amyloid disaggregation by human HSP70. Nature. 2020; 587(7834):483-488.

Abstract 6.1

Assessing FDA-approved drugs as potential therapeutics in cell and C. elegans models of motor neurone disease

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Background: The aggregation of misfolded proteins to form inclusions in the cytoplasm of neuronal and glial cells is a hallmark of MND. TDP-43 is found within inclusions in ~ 97% of sporadic MND cases and is associated with neuronal death. Overexpression of TDP-43 in motor neurone-like cells and C. elegans provides simple MND models which can be used to identify and characterise potential therapeutic compounds. Purpose: To evaluate FDA-approved drugs previously identified as 'hits' in a cell-based drug screen, to select the best candidates to progress to animal models. Methodology: A modified Flow Cytometry of Inclusions and Trafficking (FloIT) assay (1) was used to evaluate the effect of drugs on the numbers of TDP-43 inclusions. Effects on cell viability were also assessed in MTS assays. A C. elegans MND model with a paralysis phenotype will be treated with drugs to assess if mobility is improved. Results: Three FDA-approved 'hit' compounds provided ~ 40% reduction in the numbers of TDP-43 inclusions, and increased cell viability. Compound A produced these effects at 0.5 µM, compounds B and C produced similar effects at 5 and 10 µM. A C. elegans mobility assay is being optimised; preliminary data shows a TDP-43-expressing strain has decreased mobility compared to the wildtype strain. Conclusion: Compound A, B and C may be of interest therapeutically as they decrease TDP-43 inclusions and increase cell viability in a MND cell model. Compounds that also increase mobility in the C. elegans model will be progressed into zebrafish and mouse models of MND.

1. Whiten, D. R., San Gil, R., McAlary, L., Yerbury, J. J., Ecroyd, H. & Wilson, M. R. (2016). Rapid flow cytometric measurement of protein inclusions and nuclear trafficking. Scientific Reports, 6 31138-1 - 31138-9

Abstract 6.2

Investigating the association of protein aggregation with preeclampsia and postpartum

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Background Preeclampsia is the world's most common hypertensive disorder of pregnancy. Currently, early diagnosis of preeclampsia remains rudimentary, with manifestation of late clinical signs – such as proteinuria and hypertension – the only defined characteristics. This study aimed to determine whether the presence of protein aggregates within urine of preeclamptic women can be used to identify preeclampsia, and whether these protein aggregates continue to be found postpartum. Methods Women were recruited from Lyell McEwin Hospital, Adelaide. Urine samples were collected during pregnancy and 6-months postpartum from women with uncomplicated pregnancies (n = 48) and women with pregnancies complicated by preeclampsia (n = 42). Urine levels of protein aggregates were determined using urine congophilia (Congo Red Dot blot test). Urine total protein and creatinine levels were examined and correlated. Results Preeclamptic women exhibited increased urine congophilia ($P < 0.001$) and total protein ($P < 0.05$) during pregnancy; there was no correlation between urine congophilia and total protein. Although protein aggregates were no longer detected 6-months postpartum in preeclamptic women, total protein remained elevated ($P < 0.05$). Serum creatinine was also higher in preeclamptic women during pregnancy ($P < 0.001$), concomitant with a rise in urine creatinine (PPE <0.05) within the preeclamptic group. Congruently, blood pressure of preeclamptic women was significantly higher during pregnancy ($P < 0.0001$) and postpartum ($P < 0.001$). Conclusion The presence of urine congophilia during pregnancy is correlated to preeclampsia, does not continue postpartum, and may provide an early screening tool prior to clinical manifestation of typical signs of preeclampsia.

Abstract 6.3

Investigating the role of amyotrophic lateral sclerosis mutations on skeletal muscle cell function and susceptibility to proteasome inhibition

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterised by progressive motor neuron degeneration and skeletal muscle (SkM) wasting. Despite the multisystemic nature of the disease there is very little research investigating the role of SkM in ALS. This study investigates SkM function and susceptibility to proteasome stress during various stages of muscle development to investigate the effect of ALS mutations on SkM differentiation and function. Myogenic precursors, myoblasts and myocytes were differentiated from induced pluripotent stem cell (iPSC) lines bearing familial ALS mutations (CCNFS621G/WT and SOD1E101G) and controls using a growth factor-based differentiation protocol. Calcium flux assays conducted using fura-2 AM and the Flexstation 3 showed the differentiation of acetylcholine responsive myoblasts and myocytes from all iPSC lines. Following treatment of myogenic precursors with MG132 (0.001 – 3 µM concentration range, 16 h treatment) a dose dependent decrease in cell viability was observed in all cell lines. Additionally, myogenic precursors that were differentiated from cell lines bearing ALS mutations were found to have significantly reduced cell viability compared to controls when treated with 0.1 µM MG132 for 16 h. However, when myoblasts were treated with the same concentrations of MG132 no dose dependent decrease in cell viability was observed. Myoblasts were treated with increased concentrations 0.01 – 30 µM of MG132 for 48 h and a dose dependent reduction in cell viability was observed with no significant differences between disease and control line viability. In conclusion, these results suggest that myogenic precursors bearing ALS mutations may be more susceptible to proteasome stress than control myogenic precursors. Moreover, the results suggest that myogenic precursor cells are more dependent upon the ubiquitin proteasome system than myoblasts as indicated by increased sensitivity to MG132 treatment.

Abstract 6.4

Elevated Ca2+ signalling of AMPA receptors in induced pluripotent stem cell derived Alzheimer's disease neurons

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Alzheimer's disease (AD) is the most common neurodegenerative disorder in the world but unfortunately, there is still no cure available and current treatments mainly focus on alleviating symptoms but do not stop the progression of the disease. Clinical and pre-clinical studies have shown cortical hyperactivity in early stages of the disease before there is a switch to neuronal hypoactivity and neuronal cell death. However, the cellular mechanisms underlying these functional changes in AD neurons remain unclear. We hypothesised that ionotropic glutamate receptors contribute to this hyperexcitable phenotype in cortical neurons from Alzheimer's patients. To address this, we generated induced pluripotent stem cell derived cortical excitatory neurons from familial Alzheimer's disease patients (PSEN1A246E and PSEN1S290C), their relevant isogenic controls and healthy controls. After 35 days in culture, the neurons were loaded with the ratiometric dye Ca2+ indicator fura-2-AM and treated with either 100 µM glutamate, NMDA, AMPA or kainate. The Ca2+ response to each agonist was measured using the 340/380 fluorescence excitation ratio. AD neurons showed increased Ca2+ responses to AMPA (1.7 ± 0.1) compared to their isogenic controls (1.3 ± 0.08 SEM) ($p<0.05$). No significant differences were observed with glutamate, NMDA or kainate treatment. Further work is addressing the mechanisms underlying AMPA regulation in AD neurons. These results suggest that AMPA receptors but not NMDA or kainate receptors are involved in increased Ca2+ signalling in cortical excitatory neurons in familial AD.

Abstract 6.5

Use with caution: translation-inhibiting antibiotics upregulate cellular stress response pathways

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Antibiotics are frequently used in culture of human cell lines, as a preventative measure against bacterial contamination, for selection of stable cell lines, and in the use of inducible protein expression systems. Many of the antibiotics used in these applications inhibit protein synthesis by blocking progression of the ribosome along its bound mRNA. Recent evidence reporting on the effect of translation-inhibiting antibiotics in eukaryotic cells has revealed multivalent binding interactions with the eukaryote ribosome, as well as changes to gene expression and regulation. Here, we sought to comprehensively assess the effect of range of antibiotics in cultured human bone osteosarcoma epithelial (U2OS) cells. We found that the antibiotics used were differentially inhibitory to cell growth across a broad range of concentrations. When exposed to therapeutic concentrations of antibiotics, U2OS cells were observed to upregulate key markers of the heat shock response (HSR), the unfolded protein response (UPR) and the integrated stress response (ISR) and assembled cytoplasmic stress granules more readily upon oxidative stress and heat stress. An upregulation in the ubiquitination of proteins was also detected. Collectively, these results highlight the need for caution when culturing human cells in the presence of translation-inhibiting antibiotics, particularly in research investigating cellular stress responses.

Abstract 6.6

Observation of small heat shock protein chaperone activity using a single molecule photobleaching approach

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Small heat shock proteins (sHsp) are a class of molecular chaperones that are upregulated in response to cellular stress to prevent the aberrant aggregation of misfolded proteins and thus the progression of diseases associated with protein aggregation, such as Parkinson's and Alzheimer's disease. Although it's widely suggested that sHsps prevent aggregation by binding to misfolded, yet folding-competent, client proteins, their dynamic and heterogeneous nature has previously meant that mechanistic details surrounding this function remains unclear. Therefore, the interactions between sHsps and their clients are ideally suited to investigation using single molecule techniques. We have exploited total internal reflection fluorescence microscopy to monitor the photobleaching of single fluorophores attached to the model aggregation-prone client proteins firefly luciferase (FLUC), rhodanese, and chloride intracellular channel 1 protein (CLIC1), and the sHsps αB-crystallin (αB-c) and Hsp27. In doing so, we were able to monitor the formation of client-sHsp complexes and record temporal changes in the stoichiometries within these complexes. We found that small (monomeric or dimeric) forms of αB-c and Hsp27 bind to misfolded clients early during aggregation, resulting in the formation of soluble client-sHsp complexes. Furthermore, stoichiometric analysis revealed that αB-c accumulates onto existing client-sHsp complexes to form larger species, whereas Hsp27 does not, which suggests Hsp27 may interact transiently with misfolded clients to prevent their aggregation. Elucidating these mechanisms of sHsp function is crucial to our understanding of how they maintain proteostasis, and could not be determined using conventional ensemble averaging approaches.

Abstract 6.7

CoREST3 expression is decreased in Alzheimer's disease and gene knockdown significantly increases HDAC2 expression in cortical neurons derived from human pluripotent stem cells

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Alzheimer's disease (AD) is a devastating neurodegenerative condition that results in the gradual decline of cognition and memory. The underlying molecular mechanisms that contribute to the cause and progression of neurodegeneration remain largely unknown. The epigenetic regulator, histone deacetylase 2 (HDAC2), has been shown to be upregulated in AD and disrupt neuronal dynamics. The transcriptional repressor, REST corepressor 3 (CoREST3) has a potential binding site in HDAC2 and therefore CoREST3 may regulate HDAC2 expression. Therefore, this study aimed to examine CoREST3 expression in neurodegeneration using post-mortem AD tissue and identify whether CoREST3 regulates HDAC2 gene expression in neurons derived from human pluripotent stem cell (hPSC) models. RCOR3 (encoding CoREST3) expression was shown to significantly increase during differentiation of glutamatergic cortical, GABAergic ventral forebrain neurons and mature functional NGN2 induced neurons from hPSCs, consistent with the notion that CoREST3 is functional in healthy neurons. CoREST3 expression was analysed in an AD post-mortem tissue of four brain regions that are affected with varying severity via Western blot analysis. The brain regions, in order of decreasing severity, were the superior temporal gyrus (STG), inferior temporal gyrus (ITG), precuneus (PRE) and primary visual cortex (PVC) (n = 23 AD, n = 18 matched controls). Neuronal counts showed a significant reduction only in the STG. CoREST3 protein levels were significantly decreased by ~ 30 % in the STG, ITG and PRE ($p < 0.05$), suggesting changes in CoREST3 are not solely dependent on neuronal loss. CoREST3 shRNA knockdown in hPSC-derived cortical neurons resulted in a significant increase in HDAC2 expression. Together these data suggest that CoREST3 may play neuroprotective role through regulating HDAC2 expression, with this pathway downregulated in AD. Better understanding the regulatory networks of the brain will deepen our understanding of the biological basis of neurodegeneration.

Abstract 6.8

Cellular crosstalk between ALS astrocytes and motor neurons diminishes neuronal firing

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease leading to progressive paralysis. One of the earliest clinical observations in ALS patients is hyperexcitability of motor neurons in the cortex and spinal cord prior to hypoexcitability and deterioration of motor neuron function. The mechanisms that underlie the alterations in electrical signalling of ALS motor neurons throughout disease progression are not yet fully understood. Our previous work has demonstrated that ALS-causing mutations, CCNF S621G or C9ORF72 exp, increase neuronal excitability in induced pluripotent stem cell (iPSC)-derived motor neurons. However, as astrocytes are known to be impaired in ALS, the aim of this study was to further investigate how co-culture of ALS astrocytes with motor neurons affects motor neuron excitability and function. Whole-cell patch clamping was used to assess electrophysiological properties of co-cultured motor neurons. The addition of CCNF S621G or C9ORF72 exp astrocytes caused the loss of neuronal firing in ALS and control motor neurons, in some cases completely abolishing repetitive neuronal firing. Moreover, Na + and K+ currents, which govern neuronal excitability, were reduced by up to 55% and 30% ($P \leq 0.001$), respectively, in both ALS and control motor neurons co-cultured with ALS astrocytes. This shows that ALS astrocytes impair firing in ALS patient and control motor neurons. The findings highlight that the cellular crosstalk between astrocytes and motor neurons plays a significant role in altering intrinsic neuronal excitability, which could impact ALS progression. Thus, ALS astrocytes may be involved in the transition from motor neuron hyperexcitability to hypoexcitability.

Abstract 6.9

Cellular aging impedes stress-activation of a crucial JDP-Hsp70 protein disaggregase

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Proteins misfold and aggregate in cells upon exposure to proteotoxic stresses such as heat shock, oxidative stress and the presence of genetic mutations, and exert cytotoxicity in multiple gain-of-toxicity pathways [1]. Aggregation of proteins is also accelerated due to cellular ageing where protein quality control (PQC) pathways that suppress protein aggregation gradually become dysfunctional [1]. In multicellular organisms, the Hsp70-based disaggregases play a critical role in maintaining protein homeostasis by solubilizing protein aggregates [2]. However, the regulation of these disaggregases in cells remained unclear. Using a cutting-edge in situ protein-protein interaction detection assay, we identified a ternary DNAJA1+DNAJB1-Hsp70 disaggregase in human cells, whose activity is selectively boosted by a protein quality control pathway termed the stress-induced disaggregase activation pathway (siDAP) during heat stress recovery [3]. The disruption of this disaggregase results in the persistence of heat-induced aggregates and subsequently compromises cellular fitness. siDAP rescues and also help degrade aggregated proteins in corporation with the ubiquitin proteasomal system. Strikingly, the activation of the DNAJA1+DNAJB1-Hsp70 disaggregase is severely impeded in the early stages of replicative ageing, while the heat shock response is entirely active. Taken together, our findings show that siDAP is a dedicated PQC pathway that induces protein disaggregation activity to help human cells recover from proteotoxic stress, and it is one of the earliest PQC pathways that fail during cellular ageing.

1. Labbadia, J and Morimoto, RI (2015) Annu Rev Biochem 84, 435-468
2. Nillegoda, NB et al. (2015) Nature 524, 247-251
3. Mathangasinghe, Y et al. (2022) BioRxiv

Abstract 7.1

Global remodeling of the proteome in terminal differentiation

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Erythroid progenitors differentiate within a few days into erythrocytes with a specialized proteome, in which a remarkable 98% of soluble protein is hemoglobin. During the reticulocyte stage, mitochondria, ER, cytoskeleton, and ribosomes are all eliminated. The mechanisms underlying these events are poorly understood. Applying multiplexed quantitative proteomics, we previously showed that the ubiquitinating enzyme UBE2O remodels the proteome by catalyzing degradation of ribosomes. Recently, using mouse models, we have studied how other components of the ubiquitin-proteasome system that are highly induced in reticulocytes promote degradation of multiple proteins that are otherwise stable for the most part. In particular, the TRIM10 and TRIM58 ubiquitin ligases, together with the ubiquitin-like (UBL) protein TBCEL, drive replacement of the cytoskeleton. While TRIM10 and TRIM58 target dynein, dynactin, actin crosslinkers, centrosomal components, and nonmuscle myosin, TBCEL apparently targets only alpha and beta tubulin with any efficiency, and is the first known specificity factor for tubulin degradation. Crystallographic studies have provided a working model for how TBCEL drives tubulin degradation. UBE2H, together with its cognate E3, the GID complex, drives the elimination of 39 proteins that regulate mRNA, including many components of P-bodies and stress granules. Presumably as a consequence, numerous mRNAs are also stabilized in Ube2h mutants. In summary, upregulation of specific components of the UPS during terminal erythropoiesis triggers the elimination of independent components of the cell on a vast scale, eliminating many of the basic functionalities of cell biology. Our work indicates a new capacity of the UPS–developmentally controlled, global proteomic remodeling.

Abstract 7.2

Cyclin F influences the proteostasis of TDP-43

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Background: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are neurodegenerative diseases characterised by the loss of upper and lower motor neurons of the central nervous system. A key pathological feature of these diseases is the accumulation of cytoplasmic TDP-43 aggregates in affected neurons, raising the question of how these aggregates have formed.

In previous studies, we found that mutations in CCNF are causative of ALS/FTD. CCNF encodes cyclin F, a component of a ubiquitin ligase that tags substrates for degradation via the ubiquitin proteasome pathway. Notably, we also found skein-like TDP-43 inclusions within motor neurons of a patient carrying a CCNF mutation.

Objective: Given that TDP-43 aggregates occurred in a patient with a CCNF mutation, we further investigated the relationship between cyclin F and TDP-43.

Methodology and Results: Using immunoprecipitations, in vitro ubiquitylation assays, immunofluorescence imaging, immunocytochemistry, immunoblotting and flow cytometry we found that cyclin F can help tag TDP-43 with poly-ubiquitin chains - a process which is impaired when cyclin F carries an ALS/FTD-causing mutation. Furthermore, we found that this impairment leads to the accumulation of polyubiquitylated TDP-43 in the cytoplasm of neuron-like cells.

Conclusion: This study demonstrates a ubiquitylation mechanism for TDP-43, revealing important insights into the regulation of TDP-43 turnover and clues towards understanding the molecular origins of the ubiquitylated TDP-43 inclusions that are hallmark feature of disease.

Abstract 7.3

Lactoferrin is an amyloid-specific extracellular chaperone

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Lactoferrin (LF) is an 80 kDa protein present in numerous human body fluids including blood, milk, saliva, and tears. While best known for its antimicrobial activity, plasma LF levels decrease during neurodegenerative diseases associated with dysfunctions of proteostasis, such as Alzheimer's disease and Parkinson's disease [1, 2]. We report that LF inhibits the aggregation of amyloid-β (Aβ) and coiled-coil beta peptide (ccβω) to form amyloid fibrils and protects murine motor neurone-like NSC-34 cells from amyloid cytotoxicity in vitro. Notably, however, LF does not inhibit the amorphous aggregation of either citrate synthase (CS) or creatine phosphokinase (CPK), suggesting that its chaperone activity is amyloid-specific. This type of chaperone activity is like that recently described for neuroserpin and transthyretin [3], as well for C1r, C1s and prothrombin [4]. Lactoferrin is thus the most recently identified member of the expanding family of extracellular chaperones and this activity may play important physiological roles in the eye. The presence of an amyloid-specific chaperone in the eye may act to protect from the accumulation of Aβ in the retina that is associated with Alzheimer's disease, glaucoma, and age-related macular degeneration [5].

1. González-Sánchez, M., et al., Decreased salivary lactoferrin levels are specific to Alzheimer's disease. EBioMedicine, 2020. 57: p. 102834.
2. Grau, A.J., et al., Assessment of plasma lactoferrin in Parkinson's disease. Movement Disorders, 2001. 16(1): p. 131-134.
3. West, J., et al., Neuroserpin and transthyretin are extracellular chaperones that preferentially inhibit amyloid formation. Sci Adv, 2021. 7(50): p. eabf7606.
4. Geraghty, N.J., et al., Expanding the family of extracellular chaperones: Identification of human plasma proteins with chaperone activity. Protein Science, 2021. 30(11): p. 2272-2286.
5. Wang, L. and X. Mao, Role of Retinal Amyloid-β in Neurodegenerative Diseases: Overlapping Mechanisms and Emerging Clinical Applications. International Journal of Molecular Sciences, 2021. 22(5): p. 2360.

Abstract 7.4

Increased levels of UBA1 protect against mutant SOD1 toxicity

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Ubiquitylated protein inclusions are universally present in post-mortem motor neurons of amyotrophic lateral sclerosis (ALS) patients. We have demonstrated that ubiquitin homeostasis is altered in a cell model of ALS caused by the A4V mutation in SOD1 (SOD1-A4V) [1]. In NSC-34 cells containing SOD1-A4V aggregates, this was accompanied by dysfunction of the ubiquitin-proteasome system (UPS). This suggests that SOD1-A4V causes toxicity by disrupting UPS function and the homeostasis of cellular ubiquitin pools. We aimed to examine the effects of augmenting UPS components in models of SOD1-A4V linked ALS. To do this, yeast strains expressing wild-type (WT), SOD1-A4V, or the backbone vector were crossed with a GFP-fusion yeast library [2]. A key hit from the SOD1-A4V yeast screen was then examined in NSC-34 cells.

Yeast expressing SOD1-A4V had upregulated levels of key UPS components, including UBA1, which is implicated in spinal muscular atrophy. Interestingly, the expression of SOD1-A4V in yeast caused no toxicity, and there was no formation of SOD1-A4V aggregates. In contrast, SOD1-A4V expression in NSC-34 cells caused striking toxicity, accompanied by the accumulation of numerous large inclusions containing SOD1-A4V. Co-overexpression of catalytically-active WT UBA1 with SOD1-A4V appeared to buffer against SOD1-A4V toxicity, with the cell population growth rate greater than that of cells co-overexpressing catalytically inactive UBA1-C632S ($p = 0.0020$) or mCherry alone ($p = 0.0003$). Increasing the levels of UBA1-WT had no impact on the overall numbers of SOD1-A4V aggregates that formed in cells.

In ALS patient motor neurons, the UPS is subject to the heavy load of misfolded proteins and genetic mutations. These insults likely divert UPS components, causing dysregulation of normal activity. We propose that increasing UBA1-WT levels may have contributed to enhancement in the capacity of cells to ubiquitylate other proteins, restoring normal activity of downstream ubiquitin-dependent processes, ameliorating SOD1-A4V toxicity. This hypothesised mechanistic pathway, however, remains to be explored through further investigation.

1. Farrawell, N E, Lambert-Smith, I A, Mitchell, K et al. Journal of Cell Science 2018; doi: 10.1242/jcs.209122
2. Huh, W. K., Falvo, J. V., Gerke, L. C. et al Nature 2006; 425: 686-691

Abstract 7.5

FBXL4 mitochondrial DNA depletion syndrome protein mediates the degradation of BNIP3 and NIX mitophagy receptors to antagonize mitophagy

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Cells critically depend upon a balanced pool of healthy mitochondria and will selectively remove excessive or damaged mitochondria through mitophagy, a specialized form of autophagy. Mitophagy is induced in response to diverse conditions, including hypoxia, cellular differentiation, and mitochondrial damage. However, the mechanisms by which cells maintain appropriate mitochondrial content by sensing and removing excessive mitochondria under steady-state conditions is little understood. Here, we report that SCFFBXL4, an SKP1/CUL1/F-box protein ubiquitin ligase complex, constitutively localizes to the mitochondrial outer membrane in unstressed cells and mediates the ubiquitylation and degradation of the mitophagy receptors BNIP3 and NIX to suppress mitophagy. We demonstrate that, unlike wild-type FBXL4, pathogenic variants of FBXL4 that cause encephalopathic mtDNA depletion syndrome, do not efficiently interact with the core SCF ubiquitin ligase machinery or mediate the degradation of BNIP3 and NIX. Thus, we reveal a molecular mechanism controlling BNIP3 and NIX stability that actively restricts mitophagy in basal conditions and propose that mutations in FBXL4 contribute to encephalopathic mtDNA depletion syndrome through excessive mitophagy mediated by BNIP3 and NIX hyperaccumulation.

Abstract 8.1

Linking protein folding and misfolding under macromolecular crowding

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The thermodynamic hypothesis of protein folding, known as the "Anfinsen's dogma" states that the native structure of a protein represents a free energy minimum determined by the amino acid sequence. However, inconsistent with the Anfinsen's dogma, proteins misfold to form amyloid fibrils. Here, we present a general concept for the link between folding and misfolding (1). We show that folding and amyloid formation are separated by the supersaturation barrier of a protein and that the breakdown of supersaturation links the Anfinsen's intramolecular folding universe and the intermolecular misfolding universe. To clarify the role of supersaturation in vivo, we studied the mechanism of dialysis-related amyloidosis (DRA), a serious complication among long-term hemodialysis patients caused by amyloid fibrils of β2-microglobulin (β2m) (2). Although high serum β2m levels and a long dialysis vintage are the primary and secondary risk factors for the onset of DRA, respectively, patients with these do not always develop DRA, indicating that there are additional risk factors. We found that serum albumin prevented amyloid fibril formation based on macromolecular crowding effects, and that the decreased serum albumin concentration in dialysis patients is a tertiary risk factor for the onset of DRA. The model was constructed assuming accumulative effects of three risk factors and may be useful for predicting the onset of DRA.

1. Noji, M. et al. Commun. Biol. 4, 120 (2021).
2. Nakajima, K. et al. bioRxiv doi: https://doi.org/10.1101/2022.02.01.478730

Abstract 8.2

Perturbations to endoplasmic reticulum proteostasis associated with neuronal loss in neurological disease models

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Neurological impairments caused by disease or environmental factors are manifestations of impaired cellular functions, including proteostasis. Under homeostatic conditions, the endoplasmic reticulum (ER) contains resident proteins that carry out intrinsic cellular functions such as protein processing and trafficking, lipid and carbohydrate metabolism, and drug detoxification. The localization of these proteins to the ER depends on the maintenance of a steep calcium gradient with ER luminal calcium concentrations being much greater than those in the cytoplasm. Our lab has found that depletion of ER calcium triggers a mass redistribution of normally ER resident proteins into the extracellular space, a phenomenon called ER exodosis. Here, we identify glutamate excitotoxicity, hyperthermia, and MDMA exposure as triggers of neuronal ER exodosis and explore ER exodosis as an underlying mechanism of disease. We utilize an exogenous reporter of ER exodosis and establish a panel of endogenous biomarkers of ER exodosis that can be used in vitro and in vivo. We demonstrate two therapeutic strategies to combat ER exodosis and improve cellular proteostasis in disease states and investigate how ER exodosis impacts neuronal function, neuroinflammation, and cell death. Overall, we provide new insight into disease-associated neuronal dysfunction and discuss potential therapeutic options for relevant neurological disorders.

Abstract 8.3

Chemical Guided Global Analysis of Unfolded Proteins In Cells

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Changes in protein folding can affect protein function. Tools to study protein unfoldedness especially in the cellular context, however, are limited. We present here a suite of chemical probes that uniquely target on cysteines in a hydrophobic environment and display a fluorescence turn-on effect selectively on proteins with cysteine exposure on unfolded proteins in cells. Through proteomic approach, we identify both basal unfolded proteins and proteins that change their foldedness under proteostatic stress and in cells derived from Parkinson's disease (PD) patients. By incorporating an environmentally sensitive fluorophore, we develop a method to quantify subcellular polarity change in response to protein unfolding, from which we find the unfolded proteome experiences a more hydrophilic environment only in the nucleus across all the stress conditions. Altogether, these chemical probes can be useful tools for the study of protein unfolding in their native context in cells.

1. Chen et al., Nature Communication, 2017, 8, 474
2. Weerapana et al, Nature, 2010, 7325, 468
3. Yasmin, Nature Methods, 2012, 9, 467

Abstract 8.4

A high-throughput flow cytometry drug screen to discover new treatments for motor neurone disease.

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A pathological hallmark of MND is the accumulation of insoluble misfolded protein aggregates within neurones and glia in the spinal cord and/or brain of affected individuals. Accumulation and aggregation of cytoplasmic TAR-DNA binding protein of 43 kDa (TDP-43) is associated with neurone loss, and ~ 97% of sporadic MND cases feature protein inclusions enriched in TDP-43. However, current therapies do not target proteostasis and provide very limited benefits. We aimed to rapidly screen compounds from chemical libraries and extracts of native Australian flora/fauna for potential therapeutic activity using a novel flow cytometric assay (FloIT [1, 2]) adapted to suit a 96-well plate based high-throughput screening platform. This FloIT assay enumerates inclusions and nuclei in lysates of motor neuron-like NSC34 cells transfected to over-express dNLS-TDP-43-GFP (lacking the nuclear localisation signal causing cytoplasmic accumulation). Using this platform, we have screened thousands of small molecules from Compounds Australia chemical libraries, including ~ 4,000 FDA approved drugs, 40,000 NatureBank fractions (of ~ 120,000 natural fractions derived from Australian flora and fauna), and 10,000 Open Scaffold compounds (of ~ 35,000). Three NatureBank fractions reduced the numbers of TDP-43 inclusions by up to 70%, and the active compound(s) are currently being isolated and identified. These compounds will subsequently be tested in C. elegans (worm), zebrafish and murine models of MND. This screening platform rapidly identifies potential drug candidates, which will be tested in a variety of both cell and animal models.

1. Whiten DR, San Gil R, McAlary L, Yerbury JJ, Ecroyd H, Wilson MR. Rapid flow cytometric measurement of protein inclusions and nuclear trafficking. Sci Rep. 2016;6(1):31138.
2. Zeineddine R, Whiten DR, Farrawell NE, McAlary L, Hanspal MA, Kumita JR, et al. Flow cytometric measurement of the cellular propagation of TDP-43 aggregation. Prion. 2017;11(3):195-204.

Abstract 8.5

OSP-1 protects neurons from oxidative stress by remodelling the endoplasmic reticulum and modulating autophagy.

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Oxidative stress, caused by accumulation of reactive oxygen species (ROS), is a pathological factor in several incurable neurodegenerative conditions. To identify molecules involved in the neuronal response to ROS damage in vivo, we used C. elegans expressing KillerRed, a red fluorophore that generates ROS upon illumination with green light, in selected neurons. In an unbiased forward genetic screen, we isolated a mutant strain with increased neurodegeneration and identified the causative mutation to be in a previously uncharacterised gene that we have named oxidative stress protective 1 (osp-1). We show that OSP-1 protects C. elegans as well as mammalian neurons from oxidative damage in a cell-autonomous fashion, and localises to the endoplasmic reticulum (ER), an organelle that plays a central role in the cellular response to stress. Interestingly, overexpression of OSP-1 remodels the ER and affects the positioning of lysosomes, cellular organelles involved in protein degradation and part of the autophagy/lysosomal pathway implicated in neurodegeneration. Our results point to a role of OSP-1 in modulating autophagy, with this type of neuronal death triggered by oxidative damage.

Abstract P1

Prion-like amplification of misfolded polyglutamine protein monomer which exerts toxicity

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PURPOSE: Prion-like amplification and propagation of misfolded proteins in many neurodegenerative diseases are thought to occur by transmission of abnormal conformation between the disease-related proteins. The misfolded proteins are also thought to lead to neuronal toxicity and neurodegeneration. However, the mechanism by which misfolded proteins are amplified remains unclear. Moreover, whether the newly formed misfolded proteins exert toxicity is unknown. Here, we examined the amplification mechanism and toxicity of misfolded polyglutamine protein monomer. METHODS: We used thioredoxin-polyglutamine (thio-polyQ) which converts from normal/alpha-helix to abnormal/beta-sheet structure in the monomeric state. To assess the formation of thio-polyQ beta-sheet monomer, we performed native-PAGE, circular dichroism spectroscopy and ultracentrifugation analysis. We then performed cell and C. elegans experiments to assess the toxicity of thio-polyQ beta-sheet monomer. RESULTS: We revealed that the amplification of thio-polyQ beta-sheet monomer occurs by prion-like conformational transmission between the polyglutamine protein monomers. Moreover, we found that the newly formed thio-polyQ beta-sheet monomer exerts toxicity in cell and C. elegans. CONCLUSIONS: We provide, for the first time, experimental evidence for the amplification of misfolded protein in the prion hypothesis proposed by Prusiner. Because the newly formed misfolded monomer is toxic, we propose that preventing the formation of toxic misfolded monomer is a promising target for treatment of the neurodegenerative diseases.

Abstract P2

Amyotrophic lateral sclerosis and inflammatory signalling alter expression of CD99 in astrocytes

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CCNFS621G is a genetic mutation linked with familial and sporadic amyotrophic lateral sclerosis (ALS), a devastating neurodegenerative disease affecting the motor system. Astrocytes are a supporting cell type in the brain that have long been linked with ALS progression and pathogenesis. At present, the implications of the CCNFS621G mutation in astrocytes are largely unknown. By proteomic analysis of mixed cultures of CCNFS621G motor neurons and astrocytes, we identified increased expression of the cluster of differentiation 99 (CD99) protein in ALS cells, a finding that was confirmed in sporadic ALS post mortem tissue. To further investigate the regulation of CD99 expression in ALS astrocytes we used induced pluripotent stem cells (iPSCs) from an ALS patient (CCNFS621G/wt) and an isogenic control (CCNFwt/wt). Astrocytes were generated by lentiviral transduction of SRY-box transcription factor 9 (SOX-9) and nuclear factor I B (NFIB). Immunocytochemistry indicated expression of key astrocyte markers in the majority of cells, with SOX-9 recorded in 99.18% of healthy (CCNFwt/wt) cells, and in 99.63% of diseased (CCNFS621G/wt) cells; and glial fibrillary acidic protein (GFAP) recorded in 81.15% of CCNFwt/wt cells and 78.95% of CCNFS621G/wt cells. To assess the downstream effects of inflammatory stimulation, astrocytes were treated with tumour necrosis factor alpha (TNF-α) and interleukin-1beta (IL-1β). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis confirmed upregulation of the inflammatory marker, IL-6, in both CCNFwt/wt and CCNFS621G/wt cells following inflammatory stimulation. Inflammatory stimulation led to significant downregulation of astrocyte markers in both cell lines. Meanwhile, expression of CD99 was significantly downregulated in control CCNFwt/wt astrocytes following inflammatory treatment, however, this regulation was lost in ALS CCNFS621G/wt cells. Together, this data provides evidence that CD99 expression is altered in CCNFS621G and sporadic ALS and during inflammatory activation of astrocytes. The role of CD99 in inflammatory signalling in astrocytes and its role in ALS thus requires further investigation.

Abstract P3

Knockdown of the transcriptional corepressor CoREST3 increases cell viability in the human C20 microglial cell line

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Neuroinflammation is a process whereby the brain responds to foreign pathogens or injury, mediated by the release of inflammatory factors and increased proliferation of microglia. The repressor element 1 silencing transcription factor (REST) is a regulator of neurogenesis and neuroprotection and functions through corepressor recruitment. Consequently, the dysregulation of REST has been implicated in neurodegenerative diseases, including Alzheimer's Disease. REST functions by recruiting corepressors, including the CoREST family (CoREST1, CoREST2 and CoREST3), although the role of CoREST3 is largely unexplored. Based on the identification of unique CoREST3 binding sites in microglial-specific target genes, we hypothesise that CoREST3 plays a role in neuroinflammation. Therefore, this study aimed to utilise the human C20 microglial cell line, shRNA silencing and an inducible overexpression system to examine the role of CoREST3 in microglia. The human C20 microglia line expressed human microglial and hematopoietic markers, including CD11b, CD43, CD45, CX3CR1, and IBA1 by immunocytochemistry and flow cytometry. The knockdown of RCOR3 significantly increased cell viability in the microglial cells, compared to the scrambled control, shown via a resazurin assay. However, overexpression of RCOR3 did not significantly change cell viability. These findings are suggestive of a potential role for CoREST3 in regulating microglial proliferation, requiring further analysis. Understanding the molecular mechanisms that regulate microglial function will provide valuable insight into neuroinflammation.

Abstract P4

A bioinformatic analysis of gene expression in induced pluripotent stem cell-derived skeletal muscle cells from sporadic ALS patients.

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Background: Amyotrophic lateral sclerosis (ALS) is a heterogenous neurodegenerative condition, for which there is no cure. ALS is caused by the death of motor neurons and the consequent loss of neuronal signalling from the motor cortex and spinal cord to skeletal muscle cells. Identifying changes in skeletal muscle cells is therefore a key area of ALS pathology research.

Experimental Approach: Induced pluripotent stem cell (iPSC)-derived skeletal muscle cells were cultured from patients with sporadic ALS (sALS) and healthy individuals. RNA sequence data for three myogenic stages of development (myogenic precursors, myoblasts and myocytes) were provided for bioinformatic analysis and sample clustering revealed dysregulated genes in ALS vs control.

Key Results and Implications: Clustering analysis of the RNA sequence data produced stage-specific lists of genes that demonstrated statistically robust dysregulation in sALS vs control samples (lfcTheshold±1; $P<0.05$; $FDR<0.05$). This yielded 71 up- and 220 down-regulated genes in myogenic precursors, 12 up- and 31 down-regulated genes in myoblasts, and 21 up- and 28 down-regulated genes in myocytes. Functional analysis generated a map of nodes linked according to the interrelatedness of their cellular functions. A shortlist of candidate genes was developed based on the statistical and functional validity of the dysregulated gene cohort. While experimental validation of the bioinformatic results is required, the preliminary results indicate that key genes found to be dysregulated in the sALS cells may offer insights into the pathology of ALS.

Abstract P5

N-terminal amyloidogenic regions in SOD1 modulate its aggregation in living cells

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Mutations in superoxide dismutase 1 (SOD1) result in misfolding of the protein and the eventual development of amyotrophic lateral sclerosis(ALS). SOD1-associated ALS shows SOD1-positive inclusions in the motor neurons of patients, indicating that aggregation may play a role in disease. SOD1 (153 amino acids) has over 160 ALS-associated mutations that can occur throughout the sequence, suggesting that there are common site(s) important for aggregation.

We scanned the SOD1 sequence for amyloidogenic segments using multiple bioinformatic tools, finding 7 amyloidogenic segments. We then introduced proline mutations into the sequence to ablate predicted amyloid propensity. The mutants that reduced amyloid-forming propensity in each segment were generated on a SOD1-G85R-AcGFP plasmid for the transfection and expression in living cells. Using advanced image analysis with machine learning, we determined that prolines in beta-strands 2 and 3 substantially reduced the formation of inclusions in living cells as well as soluble oligomers. Co-expression of SOD1-G85R-tdTomato (no proline mutations) with proline mutant SOD1-G85R-AcGFP showed a substantial reduction in the colocalization of beta strand 2 and 3 mutants to SOD1-G85R-TdTomato inclusions. Fluorescence recovery after photobleaching (FRAP) analysis of both TdTomato and AcGFP in inclusions shows that the colocalized SOD1-G85R-AcGFP is highly mobile and rapidly recovers if it contains proline mutations in either beta strand 2 or 3.

Collectively, this research suggests that beta strands 2 and 3 of SOD1 are important for the intracellular aggregation and templating that governs the formation of inclusions.

Abstract P6

The "flexible" residual structure of acid-denatured β2-microglobulin is relevant to an ordered fibril morphology

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β2-Microglobulin (β2m) forms amyloid fibrils in vitro under acidic conditions. Under these conditions, the residual structure of acid-denatured β2m is relevant to seeding and fibril extension processes. SS bond-oxidized β2m has been shown to form rigid, ordered fibrils, whereas SS bond-reduced β2m forms curvy, less-ordered fibrils. These findings suggest that the presence of an SS bond affects the residual structure of the monomer, which subsequently influences the fibril morphology. To clarify this process, we herein performed NMR experiments. The results obtained revealed that oxidized β2m contained residual structures not only at the central, hydrophobic region but also at the N- and C-termini. Interestingly, the residual structures at the termini were flexible. On the other hand, the residual structure of the reduced form was localized and other regions had a random coil structure. These results indicate that acid-denatured β2m has variable conformations. Most conformations in the ensemble cannot participate in fibril formation because their central, hydrophobic residues are covered by terminal regions. However, when these hydrophobic residues are exposed, polypeptides competently form an ordered fibril. This conformational selection phase may be needed for the ordered assembly of amyloid fibrils.

Abstract P7

Macrophages in the reticuloendothelial system inhibit early induction stages of mouse apolipoprotein A-II amyloidosis

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Amyloidosis refers to a group of degenerative diseases that are characterized by the deposition of misfolded protein fibrils in various organs. Deposited amyloid may be removed by a phagocyte-dependent innate immune system; however, the precise mechanisms during disease progression remain unclear. We herein investigated the properties of macrophages that contribute to amyloid degradation and disease progression using inducible apolipoprotein A-II amyloidosis model mice. Intravenously injected AApoAII amyloid was efficiently engulfed by reticuloendothelial macrophages in the liver and spleen and disappeared by 24 h. While cultured murine macrophages degraded AApoAII via the endosomal-lysosomal pathway, AApoAII fibrils reduced cell viability and phagocytic capacity. Furthermore, the depletion of reticuloendothelial macrophages prior to the induction of AApoAII markedly increased hepatic and splenic AApoAII deposition. These results highlight the physiological role of reticuloendothelial macrophages in the early stages of pathogenesis and suggest the maintenance of phagocytic integrity as a therapeutic strategy to inhibit disease progression.

Abstract P8

Macromolecular crowding and supersaturation protect hemodialysis patients from the onset of dialysis-related amyloidosis

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Dialysis-related amyloidosis (DRA) is specific for long-term dialysis patients and is caused by amyloid fibrils of β2-microglobulin (β2m). Previous studies revealed that primary and secondary risk factors for the onset of DRA are an abnormal increase in the serum concentration of β2m monomer during long-term dialysis treatment and a long dialysis vintage, respectively. However, patients with these risk factors do not always develop DRA, implying the existence of unknown risk factors. In this study (1), amyloid fibril formation of β2m was studied in a crowded milieu made by adding human sera to sample solutions to investigate the unknown risk factors for the onset of DRA. We used over 100 sera collected from dialysis patients and healthy controls for statistical analysis. Amyloid fibril formation of β2m monomer solution was induced by an ultrasonic instrument, HANABI-2000, which we originally developed. The experimental results using HANABI-2000 and physicochemical analyses showed that serum albumin, the most abundant protein in a serum milieu, inhibited amyloid fibril formation through weak nonspecific interaction with β2m native monomers. This fact indicates that serum albumin plays an important role in keeping the proteostasis network in vivo and that the decrease in the serum concentration of serum albumin, which is a typical symptom of long-term dialysis patients, facilitates amyloid fibril formation in a serum milieu, being one of the risk factors for the onset of DRA.

1. Nakajima, K. et al. “Macromolecular crowding and supersaturation protect hemodialysis patients from the onset of dialysis-related amyloidosis.” Nat. Commun. 13, 5689 (2022).

Abstract P9

Reducing the size of tags in proximity-based labeling interactors of disease-associated aggregate proteins

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Proximity labelling by biotinylation is widely used for charactering protein-protein interaction in cells. One drawback of the established approaches is the requirement for the protein of interest (POI) to be tagged with an enzyme directing biotinylation such as biotin ligase or ascorbate peroxidase APEX2. The fusion tag, which is at least 19.7kDa (for the UltraID miniaturized variant) may sterically interfere with the POI function or have limited effectivity to define interactors of nascent proteins prior to the folding of the biotinylating enzyme. We are developing strategies to overcome both limitations by combining proximity labeling methods TurboID and APEX2 with an epitope targeting approach called SunTag system, which uses 10-24 epitope modules comprising nineteen amino acids (2.3kDa). SunTag recruits biotin ligase (TurboID) or ascorbate peroxidase (APEX2) fused to an anti-SunTag antibody that is co-expressed with SunTag fused POI in mammalian cells. We have validated this approach with human lamin A (lamin) protein, which has been previously used to establish the BioID methodology and found that a single SunTag epitope module gave excellent coverage of the known interactors of lamin and potentially novel ones. We are now applying this SunTag-mediated proximity labelling method to identify novel interactors of disease-associated aggregate proteins.

Abstract P10

Study on ultrasonic intensity and frequency dependences of seed-dependent amyloid fibril formation of β2-microglobulin.

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Detection of amyloid-fibril seeds in biological fluids has attracted attention as an early diagnosis method for amyloidoses. Previous research succeeded in distinguishing a type of amyloidosis based on the detection of amyloid seeds in cerebrospinal fluids using the protein-misfolding-cyclic-amplification method, which amplifies the seeds by shaking sample solutions. Meanwhile, our research group has demonstrated that ultrasonic irradiation is an effective method for amyloid-fibril assays. Ultrasonic irradiation to solutions of amyloidogenic proteins highly accelerates amyloid fibril formation and sensitively detects the seeds compared to the shaking method, owing to the effects of ultrasonic cavitation (1). Theoretically, it is expected that amyloid fibril formation under ultrasonic irradiation strongly depends on the intensity and frequency of ultrasound. Indeed, we reported that the spontaneous formation of amyloid fibrils, where amyloid fibrils are formed by primary nucleation from monomers, depends on ultrasonic irradiation conditions. However, seed-dependent amyloid formation, where amyloid fibrils are formed by elongation of pre-existing seeds without primary nucleation, has not been studied systematically. In this study, we investigate ultrasonic intensity and frequency dependences of seed-dependent amyloid formation of β2-microglobulin, the causative protein of dialysis-related amyloidosis, using an ultrasonic reactor originally developed in this study. The experimental results show that the detection sensitivity of seeds is improved under ultrasonic irradiation with a frequency of 26 kHz compared to other frequencies. These findings provide insight into the application of the ultrasonic method to the early diagnosis of amyloidoses.

1. Nakajima, K. et al. Ultrasonics Sonochemistry, 73, 105508 (2021)

Abstract P11

Age-related amyloid deposition in C57BL/6 mice: Pathological findings and characterization of the renal damage

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Background: The C57BL/6 mouse strain is the most widely used for studying aging mechanisms and age-related diseases. However, little attention has been paid to age-related amyloid deposition, which can damage some organs in mice. It was recently reported that spontaneous amyloidosis commonly occurs in the aged C57BL/6 strain1, but the pathophysiological and biochemical characterizations of amyloidosis in C57BL/6 mice have not been clarified. We have identified the amyloid protein associated with mouse senile systemic amyloidosis found in the senescence-accelerated mouse strain (SAMP1) as Apolipoprotein A-II (ApoA-II), which is the second most abundant protein constituent of high-density lipoprotein2. We have revealed that AApoAII amyloidosis occurs with aging in C57BL/6 and SAMP1 mouse strains.

Objective: Although biomedical researchers have been using C57BL/6 mice as a standard model for basic aging research, the onset of spontaneous amyloidosis and the effects of amyloid deposition on various organ functions in aged mice may cause inaccurate analysis of experimental data. The present study investigated the age-related prevalence of amyloidosis in C57BL/6 mice, the type of amyloid protein, and the effects of amyloid deposition on organ functions.

Material & Methods: The C57BL/6 mice were purchased from the Jackson Laboratory Japan, Inc. At 12-86 weeks old, we examined the degree of amyloid deposition in multiple organs and identified the amyloid protein by immunohistochemistry, immunoblotting, and mass spectrometry. In addition, we induced amyloidosis by injecting amyloid fibrils extracted from aged mice into the tail vein of young mice and evaluated amyloid-induced impairment of organ function at 40 weeks old to distinguish between the effects of aging and amyloid deposition.

Results: 1. Amyloid deposits were first observed in the small intestine and tongue between 36-40 weeks old and gradually expanded to multiple organs, except for brain tissue, with increasing age. Mice over 70 weeks old exhibited moderate-to-severe amyloid deposition in multiple organs, including the heart, kidney, intestine, tongue, stomach, lungs, and skin. 2. The results obtained from immunohistochemistry, immunoblotting, and mass spectrometry suggest that the amyloid protein of the spontaneous amyloidosis occurring in aged C57BL/6 mice was ApoA-II and excluded the possibility of other protein deposits such as Serum amyloid A (SAA), Apolipoprotein A-I (ApoA-I), Transthyretin (TTR), and Apolipoprotein A-IV (ApoA-IV). 3. Amyloid deposition caused impairment of renal function in mice exhibiting significant proteinuria, and electron microscopical and Immunofluorescent analysis results suggest that amyloid deposition damaged the intra-glomerular mesangial cells and podocytes.

Summary & Conclusion: We found AApoAII mouse senile amyloidosis in SAMP1 mice with type C amyloidogenic ApoA-II. C57BL/6 mice have type A ApoA-II, and we detected AApoAII amyloidosis in the tissues of all C57BL/6 mice aged over 40 weeks. Our results strongly suggest that deposition of ApoA-II as amyloid fibrils is the leading cause of spontaneous amyloidosis in C57BL/6 mice. This research suggests that aged C57BL/6 mice have a high prevalence of renal dysfunction associated with severe amyloid deposition in the glomerulus of amyloidosis-induced mice (40 weeks old) comparable to renal dysfunction in old mice (80 weeks old). We will investigate the deleterious effects of amyloid deposition in other organs in subsequent work.

1. Christina Pettan-Brewer et al, Pathobiol Aging Age Relat Dis, 2011; 1: 10.3402/pba.v1i0.7202.
2. Higuchi K et al. J Biol Chem. 261, 27, 25 12834-12840, 1986.

Abstract P12

Local conformation of intrinsically disordered regions of Sup35 produces temperature sensitivity of liquid-liquid phase separation

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Recent studies have revealed that amyloid fibrils and aggregations of many proteins involved in human neurodegenerative diseases are formed via liquid-liquid phase separation of the causative proteins. Therefore, to avoid amyloid formation, it is required intracellular droplets be formed quickly when necessary and dissociated quickly after completing their task, in response to changes in the environment. In this study, we investigated the molecular mechanism of temperature sensitivity of LLPS droplet formation using the N-terminal intrinsically disordered region (Sup35NM) of the translation termination factor Sup35 from the budding yeast S. cerevisiae. The droplet formation of Sup35NM is highly sensitive to temperature, and the amount of droplet changes vastly within a narrow temperature range. From experiments with several partial deletion mutants, we found that the temperature sensitivity of Sup35NM droplet formation derived from a region with a local conformation in the N-terminal domain. Then, we prepared mutants with alanine or phenylalanine substituted for tyrosine in the local conformation region and observed changes in temperature sensitivity. As a result, both mutants showed decreased temperature sensitivity, indicating that tyrosine residues are deeply involved in temperature sensitivity. Blue-native PAGE revealed that the local conformation of the alanine mutant was destructed and the molecules were extending, suggesting that the clustering of the tyrosine side chain is related to the acquisition of temperature sensitivity. On the other hand, the phenylalanine mutant maintained the local conformation but was more compact than the wild-type Sup35NM. Microscopic observation revealed that the phenylalanine mutant gelled immediately after droplet formation, resulting in temperature resistance. The gelation of the droplet is considered to be caused by the enhancement of the intra- and intermolecular interaction due to the substitution of phenylalanine for tyrosine.

Abstract P13

Mechanisms of polyphosphate-induced amyloid formation under ultrasonication

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Much effort has been devoted to elucidate mechanisms of amyloid formation using various kinds of additives, such as salts, metals, detergents and biopolymers, etc. We here focused on the effects of polyphosphate (polyP) on amyloid formation of β2-microblobulin (β2m)1) and α-synuclein (αSyn)2). PolyP, consisting of up to 1,000 phosphoric anhydride bond-linked phosphate monomers, is one of the most ancient, enigmatic and negatively charged molecules in biology. The result of amyloid experiment under the ultrasonication, polyP remarkably accelerated the amyloid formation of β2m and αSyn, respectively, depending on the chain length of polyP at quite low concentrations, comparable with the concentration in vivo. Amyloid formation of both β2m and αSyn could be induced by the counter anion-binding and the preferential hydration at relatively lower and higher concentrations of polyP, respectively, in the concentration-dependent distinct manners. These bimodal concentration-dependent effects were also observed in the salt- and heparin-induced amyloid formation. The protein monomer tends to form compact conformation through intramolecular interactions upon shielding the charge repulsions or the preferential hydration by the anions including polyP. Although these interactions are similar to those stabilizing the native state, they simultaneously induce amyloid formation through intermolecular interactions with decreasing the solubility and breakdown of the supersaturation.

1. Zhang, C., et al., PNAS 116, 12833-12838 (2019).
2. Yamaguchi, K., et al., JBC 296, 100510 (2021).

Abstract P14

Developing novel small molecule ligands of TDP-43

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The TAR DNA-binding protein 43 (TDP-43) is a DNA/RNA binding protein involved in RNA transcription and translation.1 Accumulation of intracellular TDP-43 inclusions is a pathological hallmark in some patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). To date, there are no disease-altering treatments for these diseases. The pathological overlap of TDP-43 in both diseases makes it an attractive target for therapeutic intervention and PET ligand development. Over the years, to assist in development of such ligands, several studies have attempted to purify full length TDP-43 to a reasonable yield, but limitations in reproducibility have been reported.2 A novel protocol was developed to express and purify full length TDP-43 with a hexa-histidine, ubiquitin fusion tag (His-Ubiq-TDP-43). Following removal of the His-Ubiq tag, characterisation using circular dichroism indicated significant □-helical content. His-Ubiq-TDP-43 was used to perform an amplified luminescent proximity homogeneous assay (AlphaScreen®), in order to identify compounds that interfered with RNA binding to the RNA-recognition motifs (RRMs) of TDP-43. From a hit-library of 8 compounds, we identified compound 1 that appeared to occupy two binding sites. Fitting to a two-site fit model($p<0.001$) suggested binding affinities (Ki) of 78.1 nM (pKi = -7.10 ± 0.29 M) at the high-affinity site and 85.8 □M (pKi = -4.06 ± 0.45 M) at the low-affinity site. We also identified compound 2 that occupied one binding site, with Ki of 1.68 □M (pKi of -5.77 ± 0.23 M). Our findings have identified novel small molecules that target DNA-protein interactions of TDP-43 and can act as leads for further iterative drug discovery, with the ultimate aim of producing therapeutics and PET ligands for use in ALS and FTD.

1. Neumann, M.; Sampathu, D. M.; Kwong, L. K.; Truax, A. C.et al., Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006, 314 (5796), 130-133.
2. Vega, M. V.; Nigro, A.; Luti, S.; Capitini, C.et al., Isolation and characterization of soluble human full-length TDP-43 associated with neurodegeneration. The FASEB Journal 2019, 33 (10), 10780-10793.

Abstract P15

Biophysical analysis of the amyloid fibril formation by serum amyloid A

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The formation and deposition of amyloid fibrils by serum amyloid A (SAA) protein in multiple organs causes an intractable disease, systemic amyloidosis, in humans and animals. SAA protein is known to be an acute phase protein whose serum levels rise dramatically under the action of inflammatory cytokines. Although the elevated serum levels are considered to one of the key factors for the AA amyloidosis, the details of molecular mechanisms of fibril formation by SAA protein are unclear. In this study, we first analyzed the secondary structure of SAA1 protein by far-UV CD spectroscopy. The spectrum showed typical double minima at 222 and 208 nm, suggesting that the protein assumed mostly α-helical conformations. However, the α-helical structure was very unstable and present only at 4 °C, and the protein was thermally denatured by increasing temperature to 37 °C. We also analyzed the fibril formation by monitoring fluorescence of thioflavin T (ThT), and found that spontaneous fibrillation was observed only at 37 °C but not at 4 °C. Furthermore, the addition of small amounts of preformed aggregates (seed) resulted in disappearance of a long lag-period for amyloid formation at 37 °C. On the other hand, the addition of the seed into monomeric protein solution did not promote aggregation at 4 °C. Based on these observations, we suggest that partial denaturation is a key step for the amyloid fibril formation by SAA1 protein.

Abstract P16

Inhibitory effect of fucoidan on the fibril formation by amyloid-beta peptides

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The conversion of a soluble, non-toxic molecule to a beta-sheet rich structure is a hallmark of amyloidoses including Alzheimer's disease. The major component of amyloid fibrils deposited in AD brains is amyloid beta-peptide (Abeta), which is mainly composed of 40-42 amino acid residues. Whereas growing evidence indicates that the Abeta amyloid fibrils consist of in-register parallel beta-sheets, little is known about the mechanisms by which soluble monomeric peptides form insoluble fibrillar structures. Fucoidan represents a collective word for sulfonated polysaccharide mainly composed of L-fucose (6-deoxy galactose). It is contained and extracted from slimy components of brown algae (seaweeds), and has been known to possess various bioactivities such as antitumor, antivirus, anticoagulant activities. Here, we report that one kind of fucoidan effectively inhibits the amyloid fibril formation by Abeta(1–40) peptides. A detailed analysis by thioflavin T fluorescence and solution state NMR suggested that fucoidan prevents the initial "nucleation" step of fibrillation process by Abeta molecules.

Abstract P17

Amyloid fibril formation of transthyretin induced by fragmentation

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Transthyretin (TTR) is a plasma protein that functions as a transporter of thyroxine and retinol. On the other hand, TTR is often observed as amyloid deposition in tissues, which is known to be associated with the pathogenesis of serious diseases such as familial amyloid polyneuropathy and senile systemic amyloidosis. However, the fibrillation mechanism of TTR has not been clarified because TTR is very stable as a homotetramer under physiological conditions and thus it is difficult to reproduce the fibrillation in vitro. Recently, fragmentation of TTR, represented by the N-terminal truncated TTR49-127 fragment produced by proteolytic cleavage at the carboxylic side of the residue K48, has attracted attention as a factor that facilitates the amyloid fibril formation of TTR. In this study, we investigated the fibrillation of various TTR fragments. We first found that the TTR49-127 fragment forms amyloid fibrils under shaking conditions at neutral pH, which showed high thioflavin T fluorescence intensity, a needle-like morphology, and a typical circular dichroism spectral shape assigned to β-sheet structure. In addition, we cleaved the full-length TTR with lysyl endopeptidase and purified various short TTR peptides to identify the amyloid core region of TTR. We found that TTR16-36 and TTR81-126/127 peptides formed fibrillar aggregates, suggesting that these regions are the amyloid core sequence of TTR. These results suggest that fragmentation is a key molecular mechanism for amyloid fibril formation of TTR.

Abstract P18

Formation of two types of tau oligomers depending on redox conditions

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Tau protein, which belongs to the family of microtubule-associated proteins, is involved in neurodegenerative diseases by forming amyloid fibrils in cells. The 2N4R tau has two cysteine residues C291 and C322, and the change in redox potential in cells may cause the formation of inter- and intramolecular disulfide bonds and then influence aggregation behavior. However, the details are not clear. Here, we have investigated the effect of disulfide bonds on the formation of tau oligomers by using dynamic light scattering (DLS) to follow the assembly process in the early stage of the reaction in vitro. The amyloid fibrils formation of tau was initiated by the addition of heparin, and early aggregation was monitored under various redox conditions. When the behavior of the size development of aggregates was tracked by DLS measurements, it showed significant difference depending on redox conditions. Under reduced conditions, amyloid fibrils with fibrous morphology and seeding ability were formed as the end products. In contrast, under oxidized conditions, granular oligomers and short, rod-shaped non-amyloid aggregates were observed. These results suggest that there are two pathways for oligomer formation depending on the presence or absence of intermolecular disulfide bonds, and the preference of two pathways depends on redox potential. In this presentation, we will report the mechanism of tau assembly prior to the formation of amyloid fibrils and the properties of the two oligomers.

Abstract P19

Disclosing the Relation Between Protein Quality Control and Cell Cycle Using Chemical Probes

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Cellular transcriptional mistakes and translational failures might cause various unrepairable stresses and further lead to permanent cellular damage. Permanent cellular damage eventually results in the commencement of protein aggregation-related diseases. Neurodegenerative diseases are found to associated with misfolded proteins accumulation, manifests the native functions lost and aggregation tendency in cells. Many cellular mechanisms involve in maintaining the homeostasis of protein by reducing or eliminating toxic protein aggregates. The failure of these processes can result in cell damage that leads to the onset of disease. Our home-made multifunctional probe, NTPAN-MI, becomes fluorescent when it specifically reacts with the free thiol on Cys residues, then quantifying of unfolded proteins becomes applicable. NTPAN-MI performs a fluorescence spectral shift in response to chemical polarity environment upon reacting with Cys. We mapped the chemical polarity environment of the labeled unfolded proteins in the cellular environment with subcellular resolution using the spectral profile. We further apply NTPAN-MI in conjunct with the commercial protein aggresome dye PROTEOSTAT and another home-made probe TPE-MI to investigate protein unfolding and stress response during different phases of the cell cycles. Interestingly, spectral phasor analysis results indicated DNA content in nucleus effects the amount of unfolded proteome formation. Furthermore, quantifying unfolded proteome amount proves that cells in different cell-cycle phases will respond differently to heat shock stress, which can promote the formation of unfolded protein. Further analysis of the nucleus and cytoplasmic unfolded proteins will be conducted, which can provide us more details to disclose the mystery of proteostasis in cell cycle.

Abstract P20

Decoding the determinants driving phase separation of unfolded proteins

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At least 8 globular proteins form aggregates in a range of diseases upon mutations that destabilize the folded state. The mechanisms directing aggregation of globular proteins, and how they mediate specificity of co-aggregation of other endogenous proteins, including chaperones, remains incompletely understood. We have co-opted prokaryotic protein barnase as template to explore these mechanisms as it offers an orthogonal format for enabling a focus on the fundamental protein-sequence determinants. Using a phase separation model of aggregation, we have identified two properties that are requisite to aggregation. One is the protein must be in an unfolded state to phase separate, and the second is that phase separation is dependent on the pattern of residues that act as stickers, which are associative motifs driving phase separation, and residues that act as spacers, which are motifs that intersperse stickers and modulate the property of the aggregate phase. To better understand how the patterning of stickers and spacers governs aggregation and influences co-aggregation with endogenous proteins, we plan to undertake deep mutational analysis of barnase. Here, we describe the workplan and results to date.

Abstract P21

Analysis of Fluorescent Molecular Rotors as Probes for Protein Liquid-Liquid Phase Separation

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A growing area of interest in research is the understanding of proteins and nucleic acids in cells undergoing liquid-liquid phase separation (LLPS). Protein phase separation is the de-mixing of liquid environments and is important for biological processes in cells. LLPS is also associated with pathological aggregation in neurological disorders such as Amyotrophic Lateral Sclerosis. An important biophysical parameter in LLPS is viscosity. Techniques to measure viscosity are currently laborious, and more accessible tools would be ideal. A possible development is the use of Fluorescent molecular rotors (FMRs), a specialised fluorescent molecule that acts as a viscosity probe in media. FMRs have bond rotations that hinder due to viscous environments, which impacts photophysical parameters such as fluorescent intensity, fluorescence lifetime, and their diffusion coefficient. This study assesses fluorescent molecular rotors as a probe for liquid-liquid phase separation dynamics. Selected FMRs including sulforhodamine B, DiSC2 (3), DiSC2 (5), and Thiazole Orange all exhibited increased fluorescent intensity and fluorescence lifetime in various concentrations of glycerol. Selected FMRs were combined in protein phase separated solutions of DDX4, hnRNPA2-WT and D290V, and Ubiqulin2-WT and T487I. FMR photophysical properties were impacted by phase separated droplets and displayed significant increases to fluorescence lifetime between Ubiquilin variants. Some FMRs displayed unique results, meaning further interactions could possibly be occurring. While information is still preliminary, this study displays that FMRs could possibly be a viable and more accessible tool to measure liquid-liquid phase separation dynamics, with further research required before use in cellular systems and disease models.

Abstract P22

Characterization of Stress Granule Formation in U2OS Cells Shows Protein-Dependent and Stress- Dependent Effects on Their Formation

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Stress granules are cytoplasmic micrometre-sized biomolecular condensates composed of RNA and proteins, they arrest and protect translational machinery during phases of acute cellular stress and are critical for cellular health. Stress granules have recently gained increased attention due to their involvement in some neurodegenerative diseases such as amyotrophic lateral sclerosis. The most common method to induce stress granules in vitro is by incubation of cells with sodium arsenite, however, heat-shock and hyperosmolarity are also used. We found each condition successfully triggers stress granule formation that manifest as unique phenotypes that can be quantified through the construction of powerful image analysis pipelines. Moreover, cell systems burdened by misfolded protein aggregates are shown to have impaired proteostasis mechanisms of which many are critical to stress granule regulation. Although some work has been done previously, it is currently unclear how accumulated misfolded protein in combination with each of these stresses affects the process of stress granule formation. Through application of oxidative, heat, and osmotic stress to a model cell line (U2OS) transfected with model aggregating proteins we identify that granule response to stress was underwhelming when compared to stressed, non-transfected controls. In combination with the use of a high throughput image analysis pipeline, we have successfully characterised differences in stress granule size, number and localisation between stress conditions and speculate that misfolded proteins may be saturating critical stress granule regulatory mechanisms.

Abstract P23

Development of General Fluorescence-Based Quantification Method for Study of In-Vitro, Cellular Proteostasis Polarity and Polarity Response

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The intracellular environment is very complex, with a multitude of biological processes concurrently occurring in distinct chemical environments. Organisation and control over these biological processes can be achieved by spatial discretisation, with diffusion of molecules in these compartments playing a further role in these processes. Many compartmentalised organelles rely on a membrane for separation, with some examples including the nucleus, mitochondria and endoplasmic reticulum, among others. Building upon previous work [1], we develop a new fluorescence-based polarity probe (FLAM) and in conjunction with spectral phasor analysis, show polarity changes in cells undergoing proteostasis stress. The versatility of FLAM is shown as we recently applied the dye for identification and characterisation of phase seperated protein droplets.

1. Angew. Chem. Int. Ed. 2019, 59: 10129–10135.

Abstract P24

Small molecule fluorescent reporters for autophagy

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Autophagy is an evolutionarily conserved survival mechanism in living organisms during which intracellular components ('cargo') are identified and delivered to lysosomes for degradation. Basal autophagy plays a vital role in maintaining cellular homeostasis; dysregulation of autophagy has been found to associate with diseases ranging from neurodegeneration, early stage of cancers, cardiovascular disease to infectious disease, with different stages of autophagy being impaired. Research efforts have identified multiple molecular targets to rectify autophagy with the promise for therapeutic intervention. Modulators that enhance or inhibit autophagic activities have largely contributed to mechanistic understanding of autophagy and its implications in diseases. As autophagy is a multi-step process, it is important to identify and assess which stage of autophagy is affected by a modulator, to quantify the extent of the regulation and to draw a clear mechanistic picture. However, tools that allow real-time monitoring of the dynamics of autophagy, especially with quantitative readout, are still scarce and highly desirable. Conventional methods involving the expression of fluorescent protein tagged autophagy markers require tedious and complexed transfection procedures and are not applicable in clinical samples. Here, we design and synthesize a series of fluorescent chemical probes that is highly specific to autophagy. Also, the introduction of a pH-sensitive fluorophore into the molecular design enables the discrimination between autophagosomes and acidic autolysosomes. These tailormade probes has been proven to be applicable in live cells and in vivo under conditions that induce/inhibit autophagy, which was validated with standard autophagy methods. Protocols for quantitative analysis of cellular autophagic flux have been established. In summary, our new probes provide a robust tool to quantify the transient autophagy flux in situ, highlighting its great potential in autophagy studies.

Abstract P25

Conformational transitions of amyloid fibrils during cross-seeding

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Amyloid fibrils are aberrant protein aggregates associated with many amyloidoses and neurodegenerative diseases. It has been proposed that various structures of amyloid fibrils, i.e., amyloid polymorphs, serve as a molecular basis of different physiological properties such as cytotoxicity and transmissibility. The diverse structures further propagate by seed-dependent growth, and in addition to self-seeding, cross-seeding also takes place and it seems deeply involved in the progression of several diseases such as Alzheimer's disease and prion disease. However, much remains unclear how amyloid structure propagates in the cross-seeding reactions. Here, we have investigated the preservation or change in fibril structure in the cross-seeding of human and bovine insulin. We previously discovered that iodine staining showed various colors depending on amyloid polymorphs, which we used for identifying amyloid structures in this study. The iodine-stained human and bovine insulin amyloid fibrils showed different UV-Vis absorption spectra in visible region, suggesting different fibril structures. When cross-seeding was performed using these two types of insulin amyloid fibrils as seeds, the absorption spectral patterns gradually changed and finally converged to different ones, in contrast to the robust preservation of each intrinsic spectral patterns in self-seeding. Moreover, a new fibril structure with distinct cytotoxicity was formed when human insulin was cross-seeded with bovine insulin seeds. These observations suggest that cross-seeding tends to bring various structures of amyloid fibrils and cause changes in pathology.

Abstract P26

The chaperone function of αB-crystallin through the formation of a complex with the amyloid precursor of the insulin B-chain

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Amyloid fibrils are abnormal protein aggregates associated with many serious diseases, such as Alzheimer's and Parkinson's diseases. A cellular strategy for preventing protein aggregation is the expression of small heat shock proteins (sHsps) in response to cellular stress, which act as molecular chaperones to rescue unfolding proteins in an ATP-independent manner. αB-crystallin (αB-C) is a typical mammalian sHsp. There have been many observations showing its inhibitory effects against fibrillation in vitro. However, the mechanism of this inhibition remains to be fully elucidated. In this study, we analyzed the mechanism by which αB-C inhibits fibrillation using human insulin B-chain as a substrate. B-chain is an excellent model of amyloidogenic proteins, as it forms amyloid precursors prior to amyloid nucleation and the reaction processes are also well characterized. When B-chain aggregation was observed in the presence of αB-C, αB-C formed a complex with the amyloid precursor of the B-chain and inhibited its growth in a concentration-dependent manner. The complex maintained structural stability against ultrasonication-induced amyloid nucleation. Small-angle X-ray scattering and transmission electron microscopy showed that the complex was shaped like a filament and tended to become shorter and thicker as the percentage of αB-C increased. Taken together, it is suggested that αB-C inhibits the formation of B-chain amyloid fibrils effectively by suppressing amyloid nucleation via interacting with amyloid precursors to stabilize them and prevent their further conformational change and polymerization.

Abstract P27

DNAJB chaperones suppress destabilised protein aggregation via a region distinct from that used to inhibit amyloidogenesis

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The maintenance of cellular protein homeostasis (proteostasis) is dependent upon a complex network of molecular chaperones, degradation machinery and other regulatory factors. Disturbances to proteostasis can lead to protein aggregation and inclusion formation, processes associated with a variety of neurodegenerative disorders. DNAJBs are molecular chaperones which have been identified as potent suppressors of disease-related protein aggregation. In this work, a destabilised isoform of firefly luciferase (R188Q/R261Q Fluc; FlucDM) was overexpressed in cells to assess the capacity of DNAJBs to inhibit inclusion formation. Co-expression of all DNAJBs tested significantly inhibited the intracellular aggregation of FlucDM. Moreover, we show that DNAJBs suppress aggregation by supporting the Hsp70-dependent degradation of FlucDM via the proteasome. The serine-rich stretch in DNAJB6 and DNAJB8, essential for preventing fibrillar aggregation, is not involved in the suppression of FlucDM inclusion formation. Conversely, deletion of the C-terminal TTK-LKS motif in DNAJB6 and DNAJB8, a region not required to suppress polyQ aggregation, abolished its ability to inhibit inclusion formation by FlucDM. Thus, our data suggest that DNAJB6 and DNAJB8 possess two distinct regions for binding substrates, one that is responsible for binding β-hairpins that form during amyloid formation and another that interacts with exposed hydrophobic patches in aggregation-prone clients. These findings highlight the potential of specifically targeting DNAJB chaperone action in the context of protein aggregation associated with disease.

Abstract P28

NMR characterization of the amyloidogenic interaction motifs in the host adapter protein, RIPK3, and the viral inhibitor of necroptosis, M45

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Necroptosis is a form of programmed cell death that functions to commit cells to death during viral infections when apoptosis pathways are inhibited [1]. Necroptosis relies on the formation of a functional amyloid signalling complex comprised of the receptor interacting protein kinase 3 (RIPK3) and other host adapter proteins [2, 3]. These structured protein assemblies are stabilised by interactions between RIP Homotypic Interaction Motifs (RHIMs) in the component proteins [3]. Previous structural studies of RIPK3 through ssNMR and Cryo-EM have revealed the structure of the amyloid core in both heteromeric assemblies with RIPK1, and homomeric RIPK3 assemblies [3, 4, 5]. The work presented here expands on the existing knowledge of the RHIM interactions of RIPK3 by probing the dynamics of homomeric RIPK3 assembly through solution NMR. The results presented here establish that the RHIM of RIPK3 is disordered in solution, although predicted to be structured, and exchange dynamics between free and amyloid-bound RIPK3 monomers identify a 20-residue stretch outside the RHIM as being important in monomer recruitment. This region has not been observed in previous cryo-EM or ssNMR structural models of RIPK3 assemblies. The murine cytomegalovirus is known to encode a RHIM-containing protein, M45, which can interact with RIPK3 and enable viral evasion of necroptosis [6, 7]. This work also utilises solution NMR to characterise the dynamics of homomeric assembly of viral M45 protein, further expanding our understanding of viral RHIM-mediated interactions that underpin the viral inhibition of necroptosis.

1. Kaiser WJ, Upton JW, and Mocarski ES, Curr Opin Virol 3: 296-306 (2013).
2. Li J, McQuade T, Siemer AB, Napetschnig J, Moriwaki K, Hsiao YS, et al. Cell 150: 339-350 (2012).
3. Mompean M, Li W, Li J, Laage S, Siemer AB, Bozkurt G, et al., Cell 173: 1244-1253 (2018).
4. Wu X, Ma Y, Zhao K, Zhang J, Sun Y, Li Y, et al., Proc Natl Acad Sci U S A 118: 1-10 (2021).
5. Wu XL, Hu H, Dong XQ, Zhang J, Wang J, Schwieters CD, et al., Nat Commun 12: 1627 (2021).
6. Upton JW, Kaiser WJ, and Mocarski ES, J Biol Chem 283: 16966-70 (2008).
7. Pham CL, Shanmugam N, Strange M, O'Carroll A, Brown JW, Sierecki E, et al., EMBO Rep 20: (2019).

Abstract P29

RaPID discovery and characterisation of RIPK3 RHIM-binding peptides

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Organisms use multiple forms of programmed cell death to protect against invading pathogens, including apoptosis and necroptosis. Necroptosis is highly immunogenic and causes lytic cell death. Dysregulation of necroptosis has been implicated in multiple disease states, including inflammatory bowel disease and ischaemic injury after stroke. Central to necroptosis is a functional amyloid complex referred to as the necrosome, which is comprised largely of the protein RIPK3. The necrosome forms when the ~18 residue receptor homotypic interaction motif (RHIM) within RIPK3 adopts an amyloid cross-β structure and recruits additional monomers. Oligomerisation of RIPK3 results in autophosphorylation of this kinase and subsequent phosphorylation of downstream effector proteins, causing lytic cell death. There are currently no small molecules available that selectively bind to the amyloidogenic RHIM region of RIPK3. Here, we have aimed to identify cyclic peptides that bind with high specificity to the RIPK3 RHIM region. The RIPK3 RHIM region was used as the target for screening against a cyclic peptide library in a selection known as random non-standard peptide integrated discovery (RaPID). From this screen, several novel cyclic peptide binders were identified. These peptides therefore have the potential to bind specifically to the RIPK3 RHIM amyloid fibril interface and may have use as diagnostic tools to detect RIPK3 functional amyloid formation. Further characterisation has revealed that these peptides independently form assemblies that display amyloid-like properties. Investigation of peptide:RIPK3 RHIM binding is underway using fluorescent colocalization studies, transmission electron microscopy and label-free assays utilising RIPK3 autofluorescence.

Abstract P30

Towards improved transthyretin amyloidosis detection

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Aggregation of the serum protein transthyretin into an insoluble, fibrillar form known as amyloid is reported as the cause of life-threatening transthyretin amyloidosis (ATTR). Elucidation of the pathogenic mechanisms involved in ATTR has led to the development of disease-modifying therapies that significantly slow ATTR progression and improve survival rates. However, symptomatic variety and overlap with other conditions means misdiagnosis is common, delaying effective treatment. Current diagnostic methods such as histological identification and genetic testing, whilst effective in many regards, have shortcomings that could be mitigated with high specificity binders carrying fluorescent, radionucleotide or other ligands compatible with patient screening. Advances in developing protein-motif specific binders raise the possibility of discovering TTR-amyloid specific probes but requires the optimisation of in vitro TTR amyloid fibril production. Therefore, this work aims to develop a robust in vitro system for producing TTR amyloid fibrils with the hallmarks of ATTR. Wild-type and mutant variants of TTR have been cloned into bacterial expression vectors to determine which construct has superior expression and ease of purification. The ability of purified TTR to form amyloid fibrils is also assessed. TTR amyloid formation in vitro is triggered by proteolytic cleavage between residues 48 and 49, releasing a highly amyloidogenic product. Once formed, electron microscopy and protease digestion are used to characterise amyloid morphology. These characterisation methods will be applied to compare in vitro-produced and ex vivo TTR fibrils and to assess the binding of potential TTR-specific probes.

Abstract P31

Investigating the effect of SMOC1 on AB amyloid fibril formation

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Amyloid plaques are a neuropathological hallmark of Alzheimer's disease (AD) and primarily consist of the peptide amyloid beta (AB) in fibrillar and oligomeric forms. However, these plaques also contain hundreds of other proteins. Recent proteomic studies have identified SMOC1 (secreted modular calcium-binding protein 1) as a novel protein that is strongly up-regulated in AD and highly enriched in amyloid plaques [1]. Whilst SMOC1 has been shown to be involved in many processes including glucose homeostasis, angiogenesis and ocular and limb development, the mechanistic role of SMOC1 in AD is currently unknown. Furthermore, it is not known if SMOC1 interacts with AB during the process of fibril formation or after deposition within the plaques. To investigate if SMOC1 affects the formation of AB fibrils we have characterised the interaction between recombinant SMOC1 and AB42 in vitro using Thioflavin T assays and electron microscopy. Utilising these techniques, we have observed that the presence of SMOC1 effects the aggregation kinetics of AB42 and the morphology of the resulting amyloid fibrils. Overall, these preliminary data suggest that SMOC1 may influence the formation of AB fibrils during the process of plaque formation, thereby resulting in SMOC1 also being sequestered into these protein deposits.

1. Drummond, E., et al., The amyloid plaque proteome in early onset Alzheimer's disease and Down syndrome. Acta Neuropathologica Communications, 2022. 10(1): p. 1-24.

Abstract P32

Spontaneous protein-protein crosslinking of long-lived proteins

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Background: Throughout the body, long-lived cells are more common than previously thought. These post-mitotic cells and some of the components within them are present for life. Over the course of decades these cellular components can break down. This deterioration in particular with long-lived proteins (LLPs) is often spontaneously occurring at intrinsically unstable amino acids. Much of our knowledge of these processes has come from the human lens due to its unique architecture leading to proteins that are life-long. It is from these studies we know the common posttranslational modifications of LLPs are racemisation, isomerisation, deamidation, truncation and cross-linking. While many of these processes are well characterised, spontaneous protein-protein crosslinking is currently poorly understood. Purpose: To identify the novel processes that spontaneous protein-protein crosslinks can form in long-lived proteins. Methods: Proteomics was used to characterise novel crosslinking sites in humans lenses with age and cataract. Once sites of crosslinking were determined synthetic peptides were used to elucidate the mechanisms of spontaneous protein-protein crosslinking. Results: Numerous sites of crosslinking were found in the human lens with age occurring via five distinct spontaneous crosslinking mechanisms occurring at Asn/Asp, Gln/Glu , Ser, Thr and Cys. Cataract lenses displayed a higher degree of crosslinking in comparison to controls. Conclusion: We identified five distinct novel crosslinking mechanisms that occur in LPPs. These protein-protein crosslinks are common in the human lens and has implications in cataract.

Abstract P33

Characterising the localisation of small heat shock proteins binding to α-synuclein fibrils

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As an intrinsically disordered protein, α-synuclein is prone to aggregation into amyloid fibrils a hallmark of Parkinson's disease. A network of proteins known as the molecular chaperones are able to prevent protein aggregation into fibrils. In particular, the small heat-shock proteins (sHsps) αB-crystallin and Hsp27 are able to inhibit α-synuclein fibril elongation and stably bind to mature α-synuclein fibrils. It has been hypothesised that one mechanism by which sHsps inhibit fibril elongation involves the preferential binding of sHsps to the ends of fibrils, preventing monomer association at these points. In addition, the Hsp70 system of molecular chaperones are capable of disaggregating α-synuclein fibrils. Hsp70 and its co-chaperone Hsp40 recognise and bind to α-synuclein fibrils at high density during this disaggregation. This disaggregation process is also hypothesised to begin at fibril ends. To develop a framework to investigate preferential binding to fibril ends, computational analysis was conducted to investigate if sHsp isoforms (αB-crystallin, wild-type Hsp27 and a phosphomimetic mutant of Hsp27) preferentially bind to mature α-synuclein fibril ends from TIRF microscopy images. This involved using the Ridge Detection plugin on ImageJ to identify and measure the length of fibrils. A custom-written ImageJ macro was developed to identify chaperones that were colocalised to fibrils and a custom-written R script was used to identify whether individual chaperones were bound to the "end" or "middle" regions of fibrils. We will report on any preferential binding to fibril ends by αB-crystallin, Hsp27, Hsp273D (a phosphomimetic mutant of Hsp27 that exists primarily as dimers) and DNAJB1 (a metazoan Hsp40).

Abstract P34

CuATSM improves motor function in a mouse model expressing human wildtype SOD1 and decreased intracellular copper

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Our group has previously identified that aggregates of wildtype cuproprotein superoxide dismutase 1 (SOD1) occur in degenerating regions of Parkinson's disease (PD) brains, and that these aggregates are associated with a reduction in copper required for SOD1 maturation. Our group developed and validated a novel mouse model, termed hSOD1WT/Ctr1+/-, that recapitulates increased human wildtype-SOD1 aggregation and reduced brain copper. In this study we investigated whether these changes affected motor performance, and if any motor dysfunction could be attenuated by copper treatment. hSOD1WT/Ctr1+/- and wildtype control C57BL/6 mice were orally administered a blood-brain-barrier permeable copper delivery drug diacetyl-bis(4-methylthiosemicarbazone)-copperII (Cu-ATSM), or vehicle, daily for three-months. Ctr1+/- and hSOD1WT control mice results were also reported. The effect of treatment on motor performance was recorded using a battery of behavioural tests. Compared to wildtype mice, hSOD1WT/Ctr1+/- mice showed significant motor impairment on the balance beam (3-fold slower traversing the beam, $p<0.001$, and 16-fold greater paw slips, $p<0.001$). hSOD1WT/Ctr1+/- mice also had reduced grip strength (20% decrease compared to wildtype, $p=0.033$). The genetic control hSOD1WT also experienced motor impairment which was less severe than hSOD1WT/Ctr1+/- mice (2-fold increased balance beam latency, $p=0.001$, and 27% reduced grip strength, $p=0.005$) while Ctr1+/- mice showed no abnormal phenotype. hSOD1WT/Ctr1+/- mice treated with Cu-ATSM (15mg/kg, 3-months) showed significant improvement on the balance beam compared to vehicle treated mice (55% reduced latency, $p=0.004$, 52% reduced paw slips, $p=0.004$). These results support our hypothesis that reduced copper is necessary for development of wildtype-SOD1 proteinopathy and may underly motor dysfunction in PD.

Abstract P35

Solubility and foci formation of sequestosome-1/p62 are regulated by SCFcyclin F complex ubiquitylation as an early pathomechanism in ALS

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Emerging evidence indicates that the formation of sequestosome-1/p62 (p62) foci is a preceding step to protein clearance, and that the biochemical properties of p62 regulate p62 foci formation under physiological conditions. In this study, we investigated how the mechanistic regulation of p62 may contribute to ALS pathogenesis using cyclin F-linked ALS as a model. To investigate this, we used a combination of ubiquitylation assays, co-immunoprecipitations, solubility assays, mass spectrometry, immunoblot and immunofluorescence analysis. We identified that p62 was an ubiquitylation substrate of the E3 ligase SCFcyclin F complex and found that cyclin F regulated p62 solubility via site-specific ubiquitylation. Intriguingly, cyclin F WT expression promoted p62 foci formation, which corresponded to increased insoluble p62 levels in neuronal-like cells and patient derived fibroblasts. The ALS-linked mutant cyclin F p.S621G aberrantly ubiquitylated p62 in vitro and in vivo, dysregulated the amount of insoluble p62 and disrupted p62 foci formation. Our data suggest that selective ubiquitylation of p62 by cyclin F may facilitate the biochemical properties required to regulate the solubility of p62 leading up to its function in protein clearance and implicates the differential ubiquitylation of p62 as an early pathogenic mechanism of ALS.

Abstract P36

Investigating mechanisms of molecular chaperone function at the single-molecule level

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The Hsp70 chaperone system performs a critical function in the maintenance of protein homeostasis and cellular health. However, many of the molecular mechanisms that underpin this activity have yet to be well characterised. This study sought to interrogate these processes through the use of single-molecule Fluorescence Resonance Energy Transfer (smFRET) experiments within a Total Internal Reflection Fluorescence (TIRF) microscopy framework. A mutant form of firefly luciferase (FlucIDS) that can be site-specifically labelled with fluorophores was employed for use as a model client protein for refolding via the bacterial Hsp70 system (DnaK, DnaJ, GrpE). This study was able to demonstrate that Hsp70-mediated refolding of FlucIDS is largely driven by conformational expansion of the misfolded protein by DnaK; a mechanism only possible also in the presence of co-chaperone DnaJ. In addition to stimulating ATP-hydrolysis, DnaJ was also shown to exhibit conformational remodelling of the misfolded FlucIDS structure prior to binding of DnaK for further unfolding of the client. Addition of the nucleotide exchange factor (NEF) GrpE was required for a loss of this globally expanded state and allowed for successful refolding of FlucIDS to be observed. The results of this study represent an exciting avenue for investigating the mechanisms behind the action of molecular chaperones via single-molecule fluorescence techniques.

Abstract P37

Cells overexpressing ALS-associated SOD1 variants are differentially susceptible to CuATSM-associated toxicity

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Introduction: CuATSM has been proven to be neuroprotective in both cell and mouse models and is currently being investigated in Phase II/III clinical trials for the treatment of ALS, with promising results. However, we have found CuATSM-associated toxicity in SOD1G93A mice when treated with higher doses. CuATSM can act to stabilise mutant SOD1 protein, although our recent in vitro work demonstrated CuATSM only showed therapeutic efficacy for wild-type-like (WTL) SOD1 mutations and not metal-binding-region (MBR) mutations, suggesting genotype-specific effects.

Objective/Aims: Given the apparent variability of toxicity in mice, we aimed to examine the link between inherent SOD1 mutant toxicity and CuATSM-associated toxicity. Methods: NSC-34 cells were transiently transfected with 11 different SOD1-EGFP genotypes with various levels of toxicity before treatment with a range of CuATSM concentrations (0 – 10 uM), and examination via time-lapse microscopy.

Results: Our results identified variations of peak efficacious CuATSM concentration across WTL mutants (V148G > A4V, G37R > G93A > E100G > C6G > D90A). Furthermore, the no-observed-adverse-effect level and calculated relative LC50 were also genotype-specific, suggesting CuATSM-associated toxicity depends upon the Cu requirements of the cell given the particular SOD1 mutation expressed.

Conclusion: While CuATSM has been identified as a promising therapeutic for the treatment of SOD1-fALS, our results suggest that this therapeutic efficacy is genotype-specific. These results therefore emphasise the importance of a personalised approach to enhance the level of efficacy achieved when treating fALS in humans

Abstract P38

A copper chaperone-mimetic polytherapy for SOD1-associated amyotrophic lateral sclerosis

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Introduction: Mutations in SOD1 reduce protein folding stability via disruption of metal binding and/or disulfide formation, resulting in misfolding, aggregation, and ultimately cellular toxicity. A great deal of effort has focused on preventing the misfolding and aggregation of SOD1 as a potential therapy for ALS; however, the results have been mixed. Here, we utilize a small-molecule polytherapy of CuATSM and ebselen to mimic the metal delivery and disulfide bond promoting activity of the cellular chaperone of SOD1, the "copper chaperone for SOD1." Objective/Aims: Examine the efficacy of potential therapeutics targeting folding stability of fALS associated SOD1 proteins expressed in NSC34 cells. Methods: Microscopy and automated image analysis were used to assess changes in aggregation propensity of fALS associated mutant SOD1 in NSC34 cells in response to ebselen and CuATSM treatments. Zymography and immunoblotting were further used to assess changes to dismutase activity and disulfide presence within fALS SOD1 samples. Results: Polytherapy utilizing CuATSM and ebselen was able to reduce aggregation formation of both metal binding and wild type like SOD1 mutants in NSC34 cells. Additionally, combination treatment of CuATSM and ebselen was shown to be effective at rescuing SOD1 ALS-associated mutant folding. Conclusion: Results indicate that targeting folding stability of mutant SOD1 proteins via polytherapy allows for improved folding/maturation as well as reduced aggregation formation in NSC34 cells.

Policies & Procedures

Code of Conduct

We are committed to providing a safe, productive, and welcoming environment for everyone, regardless of gender, sexual orientation, (dis)ability, physical appearance, body size, race, nationality or religion. All Symposium participants are expected to abide by the following Code of Conduct, including, but not limited to, attendees, speakers, volunteers, exhibitors, staff and service providers.

Expected Behaviour

By participating in the Symposium, you agree to:

- be considerate, respectful, and collaborative with others.
- communicate openly, critiquing ideas rather than individuals.
- value and encourage a diversity of views and opinions
- avoid personal attacks or insults directed toward other participants.
- be mindful of your surroundings and fellow participants, reporting any issues immediately.
- respect the rules and policies of the meeting venue
- adhere to the Social Media Guidelines and COVID management policy

Unacceptable Behaviour

Physical or verbal abuse, harassment, intimidation, or discrimination in any form will not be tolerated. Harassment includes, but is not limited to:

- sustained disruption of talks or other events
- inappropriate physical contact, sexual attention or innuendo
- deliberate intimidation or stalking
- photography or recording of an individual without consent
- offensive comments related to gender, sexual orientation, disability, physical appearance, body size, race, or religion.

In addition, recording or photography of another individual's presentation without explicit consent is not permitted. Please refer to the following Social Media Guidelines for more detailed information.

Social Media Guidelines

We encourage you to engage with social media before, during, and after the Symposium as a means to share information and build networks with other attendees, as well as the broader community. However, please be mindful of the following guidelines:

- Attendees are welcome to blog, post or tweet freely about what they hear, learn and experience at the Symposium however attendees must refrain from sharing when the speaker explicitly requests you do so. The content of talks are considered shareable by default, but speakers may request that specific details or slides are not shared using the "Please don't tweet" logo. Versions of this logo are available for speakers to download (here) and will be provided in sticker format for poster presentations during the Symposium.
- The use of photographic, video, or other types of recording device is strictly prohibited in all oral and poster sessions displaying the "Please don't tweet" logo. Likewise, it is strictly prohibited to post photos, images or video recordings from these sessions to any online platform, including but not limited to social media, blogs, personal web pages, etc.
- Attendees must not capture, transmit, or re-distribute data presented by others at the Symposium. This may preclude subsequent publication of the data in a scientific journal. Please be respectful of journal embargo policies and do not jeopardize the work of your colleagues!
- All online communication should be respectful and considerate towards others, and constructive criticism should be directed at ideas, not individuals. Do not engage in rudeness, slander, or personal attacks.

Remember to tag the Symposium on Facebook, Twitter and Instagram using the #ozproteostasis2022 tag, and be sure to follow us @ProteostasisAU on Twitter!

Diversity, Balance and Inclusion Statement

The Symposium Committee recognize that some sections of the scientific community are often under-represented in conference programs and attendance, and that this adversely affects the community's long-term diversity goals. Our policies were therefore developed to promote equality in all aspects of the Symposium.

The program chairs will ensure that the scientific program is of the highest quality and encompasses speakers representing the broad diversity of our community without bias in regards to gender identity or expression, sexual orientation, race, religion, geography, (dis)abilities, or nation of origin. In addition, we commit to gender parity with respect to committee members, invited international, national and selected speakers, and session chairs.

To aid this commitment, the Symposium will support a family-friendly environment by providing free registration for children and carer attendees. Family space will be provided and children will be allowed in sessions.

We are also pleased to report that the organising committee is composed of 50% female researchers spanning early to established career stages. We will report statistics detailing the geographic distribution, career stage and gender balance of the speakers, session chairs, and attendees on our website immediately following the Symposium.

COVID-19 Management Policy

The 2022 edition of the Proteostasis and Disease Symposium is intended to operate as a hybrid event, with both in-person and virtual components. We have worked hard to develop a unique experience for both components of the conference for all our attendees and sponsors, and are committed to delivering an engaging experience irrespective of the final format.

Due to the ongoing uncertainty associated with the COVID-19 pandemic, we have developed this COVID management plan to ensure all attendees are able to engage with the Symposium safely. In the event that changes to local COVID restrictions force us to alter the Symposium format, registered attendees will be notified via email in addition to general updates being provided via the Symposium website. We will endeavour to maintain timely updates of any information as it is announced, however attendees are also advised to monitor the relevant public health information services as outlined below. In addition, international attendees should ensure they meet Australian Government border entry requirements before embarking on travel to the Symposium.

COVID-safe plan for the in-person event

For those attending in person, we request that you consider continuing to wear a mask or other face covering, and maintain social distancing where possible to prevent the spread of COVID-19. All attendees are required to follow NSW Health guidelines during the event. In addition, as part of the University of Wollongong's ongoing response to the pandemic and to minimise the risk of COVID-19 to the broader community, COVID-19 vaccination is recommended for anyone attending or undertaking University activities in third-party or public settings. You can view a copy of the University of Wollongong's COVID-19 response plan along with relevant resources.

Attendees are asked to be diligent in monitoring for symptoms of COVID-19. If you experience ANY symptoms, are required to isolate or quarantine for any reason, or are awaiting test results, DO NOT attend the in-person Symposium. In each of these circumstances, it is essential that you follow the current guidance provided by NSW Health or your local health authority.

If you are experiencing any disruption to your ability to attend in person due to COVID-19 infection, get in touch with us as soon as possible at proteostasis-symposium@uow.edu.au and we will convert your registration to a virtual attendee.

Reporting Procedure

If you are the subject of unacceptable behaviour or have witnessed any such behaviour, please immediately notify a committee member on-site or by emailing your concern to proteostasis-symposium@uow.edu.au. All reports will be handled in a strictly confidential manner.

Anyone experiencing or witnessing behaviour that constitutes an immediate or serious threat to public safety is advised to contact triple-zero (000) and/or locate a venue phone to contact security.

Anyone requested to cease unacceptable behaviour will be expected to comply immediately. Security may take any action deemed necessary and appropriate, including immediate removal of individuals from the Symposium without warning or refund. The committee reserves the right to prohibit attendance at any future meeting by anyone violating this code of conduct.

We also welcome any feedback regarding the above statements, policies and procedures, which were adapted from various resources. Please contact proteostasis-symposium@uow.edu.au with any questions, concerns or suggestions.